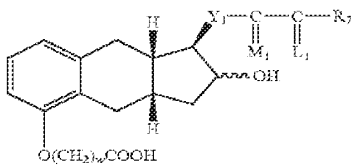
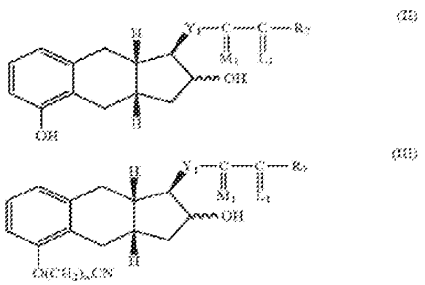
Exhibit G

Invalidity Claim Chart for the '393 Patent

Claim Language	Invalidity Contentions Exemplary Prior Art Disclosures
1. A product comprising a compound of formula I  or a pharmaceutically acceptable salt thereof, wherein said product is prepared by a process comprising	'117 Patent, 20:10-21:12. U.S. Patent Publication No. 2005/0085540 (April 2005), figures 15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024] Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil), J. Org. Chemistry., 69(6), 1890-1902 (2004). E.g. abstact, page 1892 (compound 7), page 1902. Lin et al., Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Org. Chemistry, 52,5594-5601 (1987). E.g. page 5595 Aristoff et al., Total Synthesis of a Novel Antiulcer Agent via a Modification of the Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Am. Chem., 107, 7967-7974 (1985). E.g. page 7971 McManus et al., Tetrazole Analogs of Plant Auxins, J. Org. Chemistry, 24, 1464-1467 (1959). E.g. pages 1465-1467. Arumugan et al., A New Purification Process for Pharmaceutical and Chemical Industries, Organic Process Research & Development 2005, 9, 319-320 (2005). E.g. page 319.

	Yu et al., Novel Synthetic Route of a Pivotal Intermediate for the Synthesis of 1â-Methyl Carbapenem Antibiotics, Organic Process Research & Development, 10, 829-832 (2006). E.g. page 832. Monson, ADVANCED ORGANIC SYNTHESIS, METHODS AND TECHNIQUES, 178-188 (1971). E.g. pages 181-183, 185 Harwood, EXPERIMENTAL ORGANIC CHEMISTRY: PRINCIPLES AND PRACTICE, 127-134 (1989). E.g. pages 127-134. Eliel, STEREOCHEMISTRY OF ORGANIC COMPOUNDS, 322-325 (1994). E.g. page 322. Jones, ORGANIC CHEMISTRY, 153-155 (2nd ed. 2000). E.g. pages 153-155. Sorrell, ORGANIC CHEMISTRY, 755-758 (1999). E.g. pages 755-757. Pavia, INTRODUCTION TO ORGANIC LABORATORY TECHNIQUES, 648 (1998). E.g. page 648. Priscinzano, Piperidine Analogues of 1-[2-[Bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine (GBR 12909): High Affinity Ligands for the Dopamine Transporter, J. Med. Chem., 45, 4371-4374 (2002). E.g. pages 4371-4374. Ohno, Development of Dual-Acting Benzofurans for Thromboxane A2 Receptor Antagonist and Prostacyclin Receptor Agonist: Synthesis, Structure-Activity Relationship, and Evaluation of Benzofuran Derivatives, J. Med. Chem., 48, 5279-5294 (2005). E.g. pages 5279-5294, compound 7.

	Burk, An Enantioselective Synthesis of (S)-(+)-3-Aminomethyl-5-methylhexanoic Acid via Asymmetric Hydrogenation, J. Org. Chem., 68, 5731-5734 (2003). E.g. pages 5731-34. The 2005 Physicians' Desk Reference for Bicillin® L-A (penicillin G benzathine suspension)
(a) alkylating a compound of structure II with an alkylating agent to produce a compound of formula III,  wherein w=1, 2, or 3; Y_1 is trans-CH=CH—, cis-CH=CH—, —$CH_2(CH_2)_m$—, or —C≡C—; m is 1, 2, or 3; R_7 is (1) —C_pH_{2p}—CH_3, wherein p is an integer from 1 to 5, inclusive, (2) phenoxy optionally substituted by one, two or three chloro, fluoro, trifluoromethyl, (C_1-C_3) alkyl, or (C_1-C_3)alkoxy, with the proviso that not more than two substituents are other than alkyl, with the proviso that R_7 is phenoxy or substituted phenoxy, only when R_3 and R_4 are hydrogen or methyl, being the same or different, (3) phenyl, benzyl, phenylethyl, or phenylpropyl optionally substituted on the aromatic ring by one, two or three chloro, fluoro, trifluoromethyl, (C_1-C_3)alkyl, or (C_1-C_3)alkoxy, with the	'117 Patent, 20:10-21:12. U.S. Patent Publication No. 2005/0085540 (April 2005), figures 15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024] Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil), J. Org. Chemistry., 69(6), 1890-1902 (2004). E.g. abstact, page 1892 (compound 7), page 1902. Lin et al., Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Org. Chemistry, 52,5594-5601 (1987). E.g. page 5595 Aristoff et al., Total Synthesis of a Novel Antiulcer Agent via a Modification of the Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Am. Chem., 107, 7967-7974 (1985). E.g. page 7971 McManus et al., Tetrazole Analogs of Plant Auxins, J. Org. Chemistry, 24, 1464-1467 (1959). E.g. pages 1465-1467.

proviso that not more than two substituents are other than alkyl, (4) cis-$CH_=CH_-CH_2-CH_3$, (5) $-(CH_2)_2-CH(OH)-CH_3$, or (6) $-(CH_2)_3-CH=C(CH_3)_2$; $-C(L_1)-R_7$ taken together is (1) (C_4-C_7)cycloalkyl optionally substituted by 1 to 3 (C_1-C_5)alkyl; (2) 2-(2-furyl)ethyl, (3) 2-(3-thienyl)ethoxy, or (4) 3-thienyloxymethyl; M_1 is α-OH:β-R_5 or α-R_5β-OH or α-OR_1:β-R_5 or α-R_5:β-OR_2, wherein R_5 is hydrogen or methyl, R_2 is an alcohol protecting group, and L_1 is α-R_3:β-R_4, α-R_4:β-R_3, or a mixture of α-R_3:β-R_4 and α-R_4:β-R_3, wherein R_3 and R_4 are hydrogen, methyl, or fluoro, being the same or different, with the proviso that one of R_3 and R_4 is fluoro only when the other is hydrogen or fluoro,	Arumugan et al., A New Purification Process for Pharmaceutical and Chemical Industries, Organic Process Research & Development 2005, 9, 319-320 (2005). E.g. page 319. Yu et al., Novel Synthetic Route of a Pivotal Intermediate for the Synthesis of 1â-Methyl Carbapenem Antibiotics, Organic Process Research & Development, 10, 829-832 (2006). E.g. page 832. Monson, ADVANCED ORGANIC SYNTHESIS, METHODS AND TECHNIQUES, 178-188 (1971). E.g. pages 181-183, 185 Harwood, EXPERIMENTAL ORGANIC CHEMISTRY: PRINCIPLES AND PRACTICE, 127-134 (1989). E.g. pages 127-134. Eliel, STEREOCHEMISTRY OF ORGANIC COMPOUNDS, 322-325 (1994). E.g. page 322. Jones, ORGANIC CHEMISTRY, 153-155 (2nd ed. 2000). E.g. pages 153-155. Sorrell, ORGANIC CHEMISTRY, 755-758 (1999). E.g. pages 755-757. Pavia, INTRODUCTION TO ORGANIC LABORATORY TECHNIQUES, 648 (1998). E.g. page 648. Priscinzano, Piperidine Analogues of 1-[2-[Bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine (GBR 12909): High Affinity Ligands for the Dopamine Transporter, J. Med. Chem., 45, 4371-4374 (2002). E.g. pages 4371-4374. Ohno, Development of Dual-Acting Benzofurans for Thromboxane A2 Receptor Antagonist and Prostacyclin Receptor

	Agonist: Synthesis, Structure-Activity Relationship, and Evaluation of Benzofuran Derivatives, J. Med. Chem., 48, 5279-5294 (2005). E.g. pages 5279-5294, compound 7. Burk, An Enantioselective Synthesis of (S)-(+)-3-Aminomethyl-5-methylhexanoic Acid via Asymmetric Hydrogenation, J. Org. Chem., 68, 5731-5734 (2003). E.g. pages 5731-34. The 2005 Physicians' Desk Reference for Bicillin® L-A (penicillin G benzathine suspension)
(b) hydrolyzing the product of formula III of step (a) with a base,	'117 Patent, 20:10-21:12. U.S. Patent Publication No. 2005/0085540 (April 2005), figures 15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024] Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil), J. Org. Chemistry., 69(6), 1890-1902 (2004). E.g. abstact, page 1892 (compound 7), page 1902. Lin et al., Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Org. Chemistry, 52,5594-5601 (1987). E.g. page 5595 Aristoff et al., Total Synthesis of a Novel Antiulcer Agent via a Modification of the Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Am. Chem., 107, 7967-7974 (1985). E.g. page 7971

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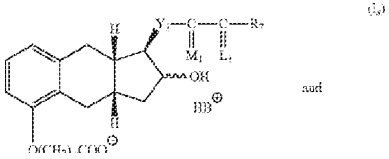
	Med. Chem., 45, 4371-4374 (2002). E.g. pages 4371-4374. Ohno, Development of Dual-Acting Benzofurans for Thromboxane A2 Receptor Antagonist and Prostacyclin Receptor Agonist: Synthesis, Structure-Activity Relationship, and Evaluation of Benzofuran Derivatives, J. Med. Chem., 48, 5279-5294 (2005). E.g. pages 5279-5294, compound 7. Burk, An Enantioselective Synthesis of (S)-(+)-3-Aminomethyl-5-methylhexanoic Acid via Asymmetric Hydrogenation, J. Org. Chem., 68, 5731-5734 (2003). E.g. pages 5731-34. The 2005 Physicians' Desk Reference for Bicillin® L-A (penicillin G benzathine suspension)
(c) contacting the product of step (h) with a base B to form a salt of formula I_S. 	'117 Patent, 20:10-21:12. U.S. Patent Publication No. 2005/0085540 (April 2005), figures 15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024] Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil), J. Org. Chemistry., 69(6), 1890-1902 (2004). E.g. abstact, page 1892 (compound 7), page 1902. Lin et al., Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Org. Chemistry, 52,5594-5601 (1987). E.g. page 5595

	Aristoff et al., Total Synthesis of a Novel Antiulcer Agent via a Modification of the Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Am. Chem., 107, 7967-7974 (1985). E.g. page 7971 McManus et al., Tetrazole Analogs of Plant Auxins, J. Org. Chemistry, 24, 1464-1467 (1959). E.g. pages 1465-1467. Arumugan et al., A New Purification Process for Pharmaceutical and Chemical Industries, Organic Process Research & Development 2005, 9, 319-320 (2005). E.g. page 319. Yu et al., Novel Synthetic Route of a Pivotal Intermediate for the Synthesis of 1â-Methyl Carbapenem Antibiotics, Organic Process Research & Development, 10, 829-832 (2006). E.g. page 832. Monson, ADVANCED ORGANIC SYNTHESIS, METHODS AND TECHNIQUES, 178-188 (1971). E.g. pages 181-183, 185 Harwood, EXPERIMENTAL ORGANIC CHEMISTRY: PRINCIPLES AND PRACTICE, 127-134 (1989). E.g. pages 127-134. Eliel, STEREOCHEMISTRY OF ORGANIC COMPOUNDS, 322-325 (1994). E.g. page 322. Jones, ORGANIC CHEMISTRY, 153-155 (2nd ed. 2000). E.g. pages 153-155. Sorrell, ORGANIC CHEMISTRY, 755-758 (1999). E.g. pages 755-757. Pavia, INTRODUCTION TO ORGANIC LABORATORY TECHNIQUES, 648 (1998). E.g. page 648.

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(d) optionally reacting the salt formed in step (c) with an acid to form the compound of formula I.	'117 Patent, 20:10-21:12. U.S. Patent Publication No. 2005/0085540 (April 2005), figures 15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024] Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil), J. Org. Chemistry., 69(6), 1890-1902 (2004). E.g. abstact, page 1892 (compound 7), page 1902.

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2. The product of claim 1, wherein the purity of compound of formula I in said product is at least 99.5%.	'117 Patent, 20:10-21:12. U.S. Patent Publication No. 2005/0085540 (April 2005), figures 15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024] Moriarty et al., The Intramolecular Asymmetric Pauson-Khan

	Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil), J. Org. Chemistry., 69(6), 1890-1902 (2004). E.g. abstact, page 1892 (compound 7), page 1902. Lin et al., Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Org. Chemistry, 52,5594-5601 (1987). E.g. page 5595 Aristoff et al., Total Synthesis of a Novel Antiulcer Agent via a Modification of the Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Am. Chem., 107, 7967-7974 (1985). E.g. page 7971 McManus et al., Tetrazole Analogs of Plant Auxins, J. Org. Chemistry, 24, 1464-1467 (1959). E.g. pages 1465-1467. Arumugan et al., A New Purification Process for Pharmaceutical and Chemical Industries, Organic Process Research & Development 2005, 9, 319-320 (2005). E.g. page 319. Yu et al., Novel Synthetic Route of a Pivotal Intermediate for the Synthesis of 1â-Methyl Carbapenem Antibiotics, Organic Process Research & Development, 10, 829-832 (2006). E.g. page 832. Monson, ADVANCED ORGANIC SYNTHESIS, METHODS AND TECHNIQUES, 178-188 (1971). E.g. pages 181-183, 185 Harwood, EXPERIMENTAL ORGANIC CHEMISTRY: PRINCIPLES AND PRACTICE, 127-134 (1989). E.g. pages 127-134. Eliel, STEREOCHEMISTRY OF ORGANIC COMPOUNDS, 322-325 (1994). E.g. page 322.

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3. The product of claim 1, wherein the alkylating agent is $Cl(CH_2)_wCN$, $Br(CH_2)_wCN$, or $I(CH_2)_wCN$.	'117 Patent, 20:10-21:12. U.S. Patent Publication No. 2005/0085540 (April 2005), figures

	15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024] Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil), J. Org. Chemistry., 69(6), 1890-1902 (2004). E.g. abstact, page 1892 (compound 7), page 1902. Lin et al., Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Org. Chemistry, 52,5594-5601 (1987). E.g. page 5595 Aristoff et al., Total Synthesis of a Novel Antiulcer Agent via a Modification of the Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Am. Chem., 107, 7967-7974 (1985). E.g. page 7971 McManus et al., Tetrazole Analogs of Plant Auxins, J. Org. Chemistry, 24, 1464-1467 (1959). E.g. pages 1465-1467. Arumugan et al., A New Purification Process for Pharmaceutical and Chemical Industries, Organic Process Research & Development 2005, 9, 319-320 (2005). E.g. page 319. Yu et al., Novel Synthetic Route of a Pivotal Intermediate for the Synthesis of 1â-Methyl Carbapenem Antibiotics, Organic Process Research & Development, 10, 829-832 (2006). E.g. page 832. Monson, ADVANCED ORGANIC SYNTHESIS, METHODS AND TECHNIQUES, 178-188 (1971). E.g. pages 181-183, 185

	Harwood, EXPERIMENTAL ORGANIC CHEMISTRY: PRINCIPLES AND PRACTICE, 127-134 (1989). E.g. pages 127-134. Eliel, STEREOCHEMISTRY OF ORGANIC COMPOUNDS, 322-325 (1994). E.g. page 322. Jones, ORGANIC CHEMISTRY, 153-155 (2nd ed. 2000). E.g. pages 153-155. Sorrell, ORGANIC CHEMISTRY, 755-758 (1999). E.g. pages 755-757. Pavia, INTRODUCTION TO ORGANIC LABORATORY TECHNIQUES, 648 (1998). E.g. page 648. Priscinzano, Piperidine Analogues of 1-[2-[Bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine (GBR 12909): High Affinity Ligands for the Dopamine Transporter, J. Med. Chem., 45, 4371-4374 (2002). E.g. pages 4371-4374. Ohno, Development of Dual-Acting Benzofurans for Thromboxane A2 Receptor Antagonist and Prostacyclin Receptor Agonist: Synthesis, Structure-Activity Relationship, and Evaluation of Benzofuran Derivatives, J. Med. Chem., 48, 5279-5294 (2005). E.g. pages 5279-5294, compound 7. Burk, An Enantioselective Synthesis of (S)-(+)-3-Aminomethyl-5-methylhexanoic Acid via Asymmetric Hydrogenation, J. Org. Chem., 68, 5731-5734 (2003). E.g. pages 5731-34. The 2005 Physicians' Desk Reference for Bicillin® L-A (penicillin G benzathine suspension)

4. The product of claim 1, wherein the base in step (b) is KOH or NaOH.	'117 Patent, 20:10-21:12. U.S. Patent Publication No. 2005/0085540 (April 2005), figures 15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024] Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil), J. Org. Chemistry., 69(6), 1890-1902 (2004). E.g. abstact, page 1892 (compound 7), page 1902. Lin et al., Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Org. Chemistry, 52,5594-5601 (1987). E.g. page 5595 Aristoff et al., Total Synthesis of a Novel Antiulcer Agent via a Modification of the Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Am. Chem., 107, 7967-7974 (1985). E.g. page 7971 McManus et al., Tetrazole Analogs of Plant Auxins, J. Org. Chemistry, 24, 1464-1467 (1959). E.g. pages 1465-1467. Arumugan et al., A New Purification Process for Pharmaceutical and Chemical Industries, Organic Process Research & Development 2005, 9, 319-320 (2005). E.g. page 319. Yu et al., Novel Synthetic Route of a Pivotal Intermediate for the Synthesis of 1â-Methyl Carbapenem Antibiotics, Organic Process

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	Chem., 68, 5731-5734 (2003). E.g. pages 5731-34. The 2005 Physicians' Desk Reference for Bicillin® L-A (penicillin G benzathine suspension)
5. The product of claim 1, wherein the base B in step (c) is selected from the group consisting of ammonia, N-methylglucamine, procaine, tromethamine, magnesium, L-lysine, L-arginine, triethanolamine, and diethanolamine.	'117 Patent, 20:10-21:12. U.S. Patent Publication No. 2005/0085540 (April 2005), figures 15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024] Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil), J. Org. Chemistry., 69(6), 1890-1902 (2004). E.g. abstact, page 1892 (compound 7), page 1902. Lin et al., Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Org. Chemistry, 52,5594-5601 (1987). E.g. page 5595 Aristoff et al., Total Synthesis of a Novel Antiulcer Agent via a Modification of the Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Am. Chem., 107, 7967-7974 (1985). E.g. page 7971 McManus et al., Tetrazole Analogs of Plant Auxins, J. Org. Chemistry, 24, 1464-1467 (1959). E.g. pages 1465-1467. Arumugan et al., A New Purification Process for Pharmaceutical

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6. The product of claim 1, wherein the acid in step (d) is HCl or H_2SO_4.	'117 Patent, 20:10-21:12. U.S. Patent Publication No. 2005/0085540 (April 2005), figures 15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024] Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil), J. Org. Chemistry., 69(6), 1890-1902 (2004). E.g. abstact, page 1892 (compound 7), page 1902. Lin et al., Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Org. Chemistry, 52,5594-5601 (1987). E.g. page 5595 Aristoff et al., Total Synthesis of a Novel Antiulcer Agent via a Modification of the Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Am. Chem., 107, 7967-7974 (1985). E.g. page 7971

	McManus et al., Tetrazole Analogs of Plant Auxins, J. Org. Chemistry, 24, 1464-1467 (1959). E.g. pages 1465-1467. Arumugan et al., A New Purification Process for Pharmaceutical and Chemical Industries, Organic Process Research & Development 2005, 9, 319-320 (2005). E.g. page 319. Yu et al., Novel Synthetic Route of a Pivotal Intermediate for the Synthesis of 1â-Methyl Carbapenem Antibiotics, Organic Process Research & Development, 10, 829-832 (2006). E.g. page 832. Monson, ADVANCED ORGANIC SYNTHESIS, METHODS AND TECHNIQUES, 178-188 (1971). E.g. pages 181-183, 185 Harwood, EXPERIMENTAL ORGANIC CHEMISTRY: PRINCIPLES AND PRACTICE, 127-134 (1989). E.g. pages 127-134. Eliel, STEREOCHEMISTRY OF ORGANIC COMPOUNDS, 322-325 (1994). E.g. page 322. Jones, ORGANIC CHEMISTRY, 153-155 (2nd ed. 2000). E.g. pages 153-155. Sorrell, ORGANIC CHEMISTRY, 755-758 (1999). E.g. pages 755-757. Pavia, INTRODUCTION TO ORGANIC LABORATORY TECHNIQUES, 648 (1998). E.g. page 648. Priscinzano, Piperidine Analogues of 1-[2-[Bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine (GBR 12909): High Affinity Ligands for the Dopamine Transporter, J.

	Med. Chem., 45, 4371-4374 (2002). E.g. pages 4371-4374. Ohno, Development of Dual-Acting Benzofurans for Thromboxane A2 Receptor Antagonist and Prostacyclin Receptor Agonist: Synthesis, Structure-Activity Relationship, and Evaluation of Benzofuran Derivatives, J. Med. Chem., 48, 5279-5294 (2005). E.g. pages 5279-5294, compound 7. Burk, An Enantioselective Synthesis of (S)-(+)-3-Aminomethyl-5-methylhexanoic Acid via Asymmetric Hydrogenation, J. Org. Chem., 68, 5731-5734 (2003). E.g. pages 5731-34. The 2005 Physicians' Desk Reference for Bicillin® L-A (penicillin G benzathine suspension)
7. The product of claim 1, wherein Y_1 is —CH_2CH_2—; M_1 is α-OH:β-H or α-H:β-OH; —$C(L_1)$-R_7 taken together is —$(CH_2)_4CH_3$; and w is 1.	'117 Patent, 20:10-21:12. U.S. Patent Publication No. 2005/0085540 (April 2005), figures 15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024] Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil), J. Org. Chemistry., 69(6), 1890-1902 (2004). E.g. abstact, page 1892 (compound 7), page 1902. Lin et al., Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Org.

	Chemistry, 52,5594-5601 (1987). E.g. page 5595 Aristoff et al., Total Synthesis of a Novel Antiulcer Agent via a Modification of the Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Am. Chem., 107, 7967-7974 (1985). E.g. page 7971 McManus et al., Tetrazole Analogs of Plant Auxins, J. Org. Chemistry, 24, 1464-1467 (1959). E.g. pages 1465-1467. Arumugan et al., A New Purification Process for Pharmaceutical and Chemical Industries, Organic Process Research & Development 2005, 9, 319-320 (2005). E.g. page 319. Yu et al., Novel Synthetic Route of a Pivotal Intermediate for the Synthesis of 1â-Methyl Carbapenem Antibiotics, Organic Process Research & Development, 10, 829-832 (2006). E.g. page 832. Monson, ADVANCED ORGANIC SYNTHESIS, METHODS AND TECHNIQUES, 178-188 (1971). E.g. pages 181-183, 185 Harwood, EXPERIMENTAL ORGANIC CHEMISTRY: PRINCIPLES AND PRACTICE, 127-134 (1989). E.g. pages 127-134. Eliel, STEREOCHEMISTRY OF ORGANIC COMPOUNDS, 322-325 (1994). E.g. page 322. Jones, ORGANIC CHEMISTRY, 153-155 (2nd ed. 2000). E.g. pages 153-155. Sorrell, ORGANIC CHEMISTRY, 755-758 (1999). E.g. pages 755-757. Pavia, INTRODUCTION TO ORGANIC LABORATORY TECHNIQUES,

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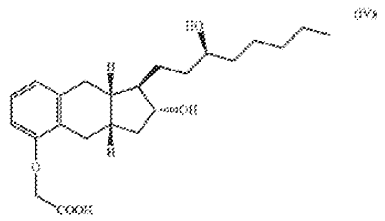
8. The product of claim 1, wherein the process does not include purifying the compound of formula (III) produced in step (a).	'117 Patent, 20:10-21:12. U.S. Patent Publication No. 2005/0085540 (April 2005), figures 15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024] Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil), J. Org. Chemistry., 69(6), 1890-1902 (2004). E.g. abstact, page

	1892 (compound 7), page 1902. Lin et al., Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Org. Chemistry, 52,5594-5601 (1987). E.g. page 5595 Aristoff et al., Total Synthesis of a Novel Antiulcer Agent via a Modification of the Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Am. Chem., 107, 7967-7974 (1985). E.g. page 7971 McManus et al., Tetrazole Analogs of Plant Auxins, J. Org. Chemistry, 24, 1464-1467 (1959). E.g. pages 1465-1467. Arumugan et al., A New Purification Process for Pharmaceutical and Chemical Industries, Organic Process Research & Development 2005, 9, 319-320 (2005). E.g. page 319. Yu et al., Novel Synthetic Route of a Pivotal Intermediate for the Synthesis of 1â-Methyl Carbapenem Antibiotics, Organic Process Research & Development, 10, 829-832 (2006). E.g. page 832. Monson, ADVANCED ORGANIC SYNTHESIS, METHODS AND TECHNIQUES, 178-188 (1971). E.g. pages 181-183, 185 Harwood, EXPERIMENTAL ORGANIC CHEMISTRY: PRINCIPLES AND PRACTICE, 127-134 (1989). E.g. pages 127-134. Eliel, STEREOCHEMISTRY OF ORGANIC COMPOUNDS, 322-325 (1994). E.g. page 322. Jones, ORGANIC CHEMISTRY, 153-155 (2nd ed. 2000). E.g. pages 153-155.

	Sorrell, ORGANIC CHEMISTRY, 755-758 (1999). E.g. pages 755-757. Pavia, INTRODUCTION TO ORGANIC LABORATORY TECHNIQUES, 648 (1998). E.g. page 648. Priscinzano, Piperidine Analogues of 1-[2-[Bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine (GBR 12909): High Affinity Ligands for the Dopamine Transporter, J. Med. Chem., 45, 4371-4374 (2002). E.g. pages 4371-4374. Ohno, Development of Dual-Acting Benzofurans for Thromboxane A2 Receptor Antagonist and Prostacyclin Receptor Agonist: Synthesis, Structure-Activity Relationship, and Evaluation of Benzofuran Derivatives, J. Med. Chem., 48, 5279-5294 (2005). E.g. pages 5279-5294, compound 7. Burk, An Enantioselective Synthesis of (S)-(+)-3-Aminomethyl-5-methylhexanoic Acid via Asymmetric Hydrogenation, J. Org. Chem., 68, 5731-5734 (2003). E.g. pages 5731-34. The 2005 Physicians' Desk Reference for Bicillin® L-A (penicillin G benzathine suspension)
9. A product comprising a compound having formula IV	'117 Patent, 20:10-21:12. U.S. Patent Publication No. 2005/0085540 (April 2005), figures 15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024]

 or a pharmaceutically acceptable salt thereof, wherein the product is prepared by the process comprising	Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil), J. Org. Chemistry., 69(6), 1890-1902 (2004). E.g. abstact, page 1892 (compound 7), page 1902. Lin et al., Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Org. Chemistry, 52,5594-5601 (1987). E.g. page 5595 Aristoff et al., Total Synthesis of a Novel Antiulcer Agent via a Modification of the Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Am. Chem., 107, 7967-7974 (1985). E.g. page 7971 McManus et al., Tetrazole Analogs of Plant Auxins, J. Org. Chemistry, 24, 1464-1467 (1959). E.g. pages 1465-1467. Arumugan et al., A New Purification Process for Pharmaceutical and Chemical Industries, Organic Process Research & Development 2005, 9, 319-320 (2005). E.g. page 319. Yu et al., Novel Synthetic Route of a Pivotal Intermediate for the Synthesis of 1â-Methyl Carbapenem Antibiotics, Organic Process Research & Development, 10, 829-832 (2006). E.g. page 832. Monson, ADVANCED ORGANIC SYNTHESIS, METHODS AND TECHNIQUES, 178-188 (1971). E.g. pages 181-183, 185 Harwood, EXPERIMENTAL ORGANIC CHEMISTRY: PRINCIPLES AND PRACTICE, 127-134 (1989). E.g. pages 127-134.

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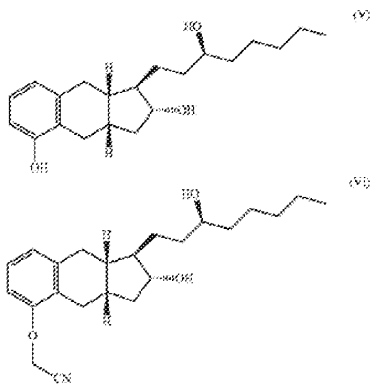
(a) alkylating a compound of formula V with an alkylating agent to produce a compound of formula VI,	'117 Patent, 20:10-21:12.

	U.S. Patent Publication No. 2005/0085540 (April 2005), figures 15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024] Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil), J. Org. Chemistry., 69(6), 1890-1902 (2004). E.g. abstact, page 1892 (compound 7), page 1902. Lin et al., Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Org. Chemistry, 52,5594-5601 (1987). E.g. page 5595 Aristoff et al., Total Synthesis of a Novel Antiulcer Agent via a Modification of the Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Am. Chem., 107, 7967-7974 (1985). E.g. page 7971 McManus et al., Tetrazole Analogs of Plant Auxins, J. Org. Chemistry, 24, 1464-1467 (1959). E.g. pages 1465-1467. Arumugan et al., A New Purification Process for Pharmaceutical and Chemical Industries, Organic Process Research & Development 2005, 9, 319-320 (2005). E.g. page 319. Yu et al., Novel Synthetic Route of a Pivotal Intermediate for the Synthesis of 1â-Methyl Carbapenem Antibiotics, Organic Process Research & Development, 10, 829-832 (2006). E.g. page 832. Monson, ADVANCED ORGANIC SYNTHESIS, METHODS AND TECHNIQUES, 178-188 (1971). E.g. pages 181-183, 185

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(b) hydrolyzing the product of formula VI of step (a) with a base,	'117 Patent, 20:10-21:12. U.S. Patent Publication No. 2005/0085540 (April 2005), figures 15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024] Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil), J. Org. Chemistry., 69(6), 1890-1902 (2004). E.g. abstact, page 1892 (compound 7), page 1902. Lin et al., Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Org. Chemistry, 52,5594-5601 (1987). E.g. page 5595 Aristoff et al., Total Synthesis of a Novel Antiulcer Agent via a Modification of the Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Am. Chem., 107, 7967-7974 (1985). E.g. page 7971 McManus et al., Tetrazole Analogs of Plant Auxins, J. Org. Chemistry, 24, 1464-1467 (1959). E.g. pages 1465-1467. Arumugan et al., A New Purification Process for Pharmaceutical and Chemical Industries, Organic Process Research & Development 2005, 9, 319-320 (2005). E.g. page 319. Yu et al., Novel Synthetic Route of a Pivotal Intermediate for the Synthesis of 1â-Methyl Carbapenem Antibiotics, Organic Process

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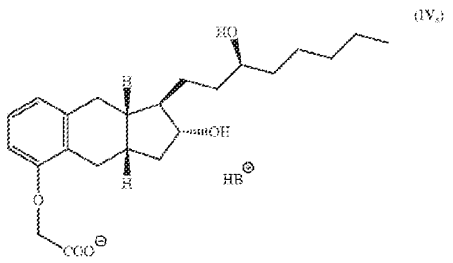
(c) contacting the product of step (h) with a base B to form a salt of formula IV_s, and 	'117 Patent, 20:10-21:12. U.S. Patent Publication No. 2005/0085540 (April 2005), figures 15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024] Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil), J. Org. Chemistry., 69(6), 1890-1902 (2004). E.g. abstact, page 1892 (compound 7), page 1902. Lin et al., Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Org. Chemistry, 52,5594-5601 (1987). E.g. page 5595 Aristoff et al., Total Synthesis of a Novel Antiulcer Agent via a Modification of the Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Am. Chem., 107, 7967-7974 (1985). E.g. page 7971 McManus et al., Tetrazole Analogs of Plant Auxins, J. Org. Chemistry, 24, 1464-1467 (1959). E.g. pages 1465-1467. Arumugan et al., A New Purification Process for Pharmaceutical and Chemical Industries, Organic Process Research &

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(d) optionally reacting the salt formed in step (c) with an acid to form the compound of formula IV.	'117 Patent, 20:10-21:12. U.S. Patent Publication No. 2005/0085540 (April 2005), figures 15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024] Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil), J. Org. Chemistry., 69(6), 1890-1902 (2004). E.g. abstact, page 1892 (compound 7), page 1902. Lin et al., Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Org. Chemistry, 52,5594-5601 (1987). E.g. page 5595 Aristoff et al., Total Synthesis of a Novel Antiulcer Agent via a Modification of the Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Am. Chem., 107, 7967-7974 (1985). E.g. page 7971 McManus et al., Tetrazole Analogs of Plant Auxins, J. Org.

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10. The product of claim 9, wherein the purity of product of step (d) is at least 99.5%.	'117 Patent, 20:10-21:12. U.S. Patent Publication No. 2005/0085540 (April 2005), figures 15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024] Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil), J. Org. Chemistry., 69(6), 1890-1902 (2004). E.g. abstact, page 1892 (compound 7), page 1902. Lin et al., Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Org. Chemistry, 52,5594-5601 (1987). E.g. page 5595

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11. The product of claim 9, wherein the alkylating agent is $ClCH_2CN$.	'117 Patent, 20:10-21:12. U.S. Patent Publication No. 2005/0085540 (April 2005), figures 15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024] Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil), J. Org. Chemistry., 69(6), 1890-1902 (2004). E.g. abstact, page 1892 (compound 7), page 1902.

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12. The product of claim 9, wherein the base in step (b) is KOH.	'117 Patent, 20:10-21:12. U.S. Patent Publication No. 2005/0085540 (April 2005), figures 15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024] Moriarty et al., The Intramolecular Asymmetric Pauson-Khan

	Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil), J. Org. Chemistry., 69(6), 1890-1902 (2004). E.g. abstact, page 1892 (compound 7), page 1902. Lin et al., Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Org. Chemistry, 52,5594-5601 (1987). E.g. page 5595 Aristoff et al., Total Synthesis of a Novel Antiulcer Agent via a Modification of the Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Am. Chem., 107, 7967-7974 (1985). E.g. page 7971 McManus et al., Tetrazole Analogs of Plant Auxins, J. Org. Chemistry, 24, 1464-1467 (1959). E.g. pages 1465-1467. Arumugan et al., A New Purification Process for Pharmaceutical and Chemical Industries, Organic Process Research & Development 2005, 9, 319-320 (2005). E.g. page 319. Yu et al., Novel Synthetic Route of a Pivotal Intermediate for the Synthesis of 1â-Methyl Carbapenem Antibiotics, Organic Process Research & Development, 10, 829-832 (2006). E.g. page 832. Monson, ADVANCED ORGANIC SYNTHESIS, METHODS AND TECHNIQUES, 178-188 (1971). E.g. pages 181-183, 185 Harwood, EXPERIMENTAL ORGANIC CHEMISTRY: PRINCIPLES AND PRACTICE, 127-134 (1989). E.g. pages 127-134. Eliel, STEREOCHEMISTRY OF ORGANIC COMPOUNDS, 322-325 (1994). E.g. page 322.

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13. The product of claim 9, wherein the base B in step (c) is selected from a group consisting of ammonia, N-methylglucamine, procaine, tromethamine, magnesium, L-lysine,	'117 Patent, 20:10-21:12. U.S. Patent Publication No. 2005/0085540 (April 2005), figures

L-arginine, triethanolamine, and diethanolamine.	15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024] Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil), J. Org. Chemistry., 69(6), 1890-1902 (2004). E.g. abstact, page 1892 (compound 7), page 1902. Lin et al., Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Org. Chemistry, 52,5594-5601 (1987). E.g. page 5595 Aristoff et al., Total Synthesis of a Novel Antiulcer Agent via a Modification of the Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Am. Chem., 107, 7967-7974 (1985). E.g. page 7971 McManus et al., Tetrazole Analogs of Plant Auxins, J. Org. Chemistry, 24, 1464-1467 (1959). E.g. pages 1465-1467. Arumugan et al., A New Purification Process for Pharmaceutical and Chemical Industries, Organic Process Research & Development 2005, 9, 319-320 (2005). E.g. page 319. Yu et al., Novel Synthetic Route of a Pivotal Intermediate for the Synthesis of 1â-Methyl Carbapenem Antibiotics, Organic Process Research & Development, 10, 829-832 (2006). E.g. page 832. Monson, ADVANCED ORGANIC SYNTHESIS, METHODS AND TECHNIQUES, 178-188 (1971). E.g. pages 181-183, 185

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14. The product of claim 9, wherein the base B is diethanolamine.	'117 Patent, 20:10-21:12. U.S. Patent Publication No. 2005/0085540 (April 2005), figures 15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024] Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil), J. Org. Chemistry., 69(6), 1890-1902 (2004). E.g. abstact, page 1892 (compound 7), page 1902. Lin et al., Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Org. Chemistry, 52,5594-5601 (1987). E.g. page 5595 Aristoff et al., Total Synthesis of a Novel Antiulcer Agent via a Modification of the Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Am. Chem., 107, 7967-7974 (1985). E.g. page 7971 McManus et al., Tetrazole Analogs of Plant Auxins, J. Org. Chemistry, 24, 1464-1467 (1959). E.g. pages 1465-1467. Arumugan et al., A New Purification Process for Pharmaceutical and Chemical Industries, Organic Process Research & Development 2005, 9, 319-320 (2005). E.g. page 319. Yu et al., Novel Synthetic Route of a Pivotal Intermediate for the Synthesis of 1â-Methyl Carbapenem Antibiotics, Organic Process

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15. The product of claim 9, wherein the acid in step (d) is HCl.	'117 Patent, 20:10-21:12. U.S. Patent Publication No. 2005/0085540 (April 2005), figures 15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024] Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil), J. Org. Chemistry., 69(6), 1890-1902 (2004). E.g. abstact, page 1892 (compound 7), page 1902. Lin et al., Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Org. Chemistry, 52,5594-5601 (1987). E.g. page 5595 Aristoff et al., Total Synthesis of a Novel Antiulcer Agent via a Modification of the Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Am. Chem., 107, 7967-7974 (1985). E.g. page 7971 McManus et al., Tetrazole Analogs of Plant Auxins, J. Org. Chemistry, 24, 1464-1467 (1959). E.g. pages 1465-1467. Arumugan et al., A New Purification Process for Pharmaceutical

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16. The product of claim 9, wherein the process does not include purifying the compound of formula (VI) produced in step (a).	'117 Patent, 20:10-21:12. U.S. Patent Publication No. 2005/0085540 (April 2005), figures 15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024] Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil), J. Org. Chemistry., 69(6), 1890-1902 (2004). E.g. abstact, page 1892 (compound 7), page 1902. Lin et al., Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Org. Chemistry, 52,5594-5601 (1987). E.g. page 5595 Aristoff et al., Total Synthesis of a Novel Antiulcer Agent via a Modification of the Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Am. Chem., 107, 7967-7974 (1985). E.g. page 7971

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17. The product of claim 16, wherein the base B in step (c) is selected from a group consisting of ammonia, N-methylglucamine, procaine, tromethamine, magnesium, L-lysinc, L-arginine, tricthanolamine, and diethanolamine.	'117 Patent, 20:10-21:12. U.S. Patent Publication No. 2005/0085540 (April 2005), figures 15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024] Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil), J. Org. Chemistry., 69(6), 1890-1902 (2004). E.g. abstact, page 1892 (compound 7), page 1902. Lin et al., Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Org.

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18. The product of claim 17, wherein the base B is diethanolamine.	'117 Patent, 20:10-21:12. U.S. Patent Publication No. 2005/0085540 (April 2005), figures 15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024] Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil), J. Org. Chemistry., 69(6), 1890-1902 (2004). E.g. abstact, page

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19. The product of claim 1, wherein the base in step (b) is KOH or NaOH and wherein the base 13 in step (c) is selected from the group consisting of ammonia. N-methyl glucamine, procaine, tromethamine, magnesium, L-lysine, L-arginine, triethanolamine, and diethanolamine.	'117 Patent, 20:10-21:12. U.S. Patent Publication No. 2005/0085540 (April 2005), figures 15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024]

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20. The product of claim 9, wherein the base in step (b) is KOH	'117 Patent, 20:10-21:12.

or NaOH and wherein the base B in step (c) is selected from the group consisting of ammonia, N-methylglucamine, procaine, tromethamine, magnesium, L-lysine, L-arginine, triethanolamine, and diethanolamine.	U.S. Patent Publication No. 2005/0085540 (April 2005), figures 15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024] Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil), J. Org. Chemistry., 69(6), 1890-1902 (2004). E.g. abstact, page 1892 (compound 7), page 1902. Lin et al., Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Org. Chemistry, 52,5594-5601 (1987). E.g. page 5595 Aristoff et al., Total Synthesis of a Novel Antiulcer Agent via a Modification of the Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Am. Chem., 107, 7967-7974 (1985). E.g. page 7971 McManus et al., Tetrazole Analogs of Plant Auxins, J. Org. Chemistry, 24, 1464-1467 (1959). E.g. pages 1465-1467. Arumugan et al., A New Purification Process for Pharmaceutical and Chemical Industries, Organic Process Research & Development 2005, 9, 319-320 (2005). E.g. page 319. Yu et al., Novel Synthetic Route of a Pivotal Intermediate for the Synthesis of 1â-Methyl Carbapenem Antibiotics, Organic Process Research & Development, 10, 829-832 (2006). E.g. page 832. Monson, ADVANCED ORGANIC SYNTHESIS, METHODS AND

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21. The product of claim 1, wherein step (d) is performed.	'117 Patent, 20:10-21:12. U.S. Patent Publication No. 2005/0085540 (April 2005), figures 15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024] Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil), J. Org. Chemistry., 69(6), 1890-1902 (2004). E.g. abstact, page 1892 (compound 7), page 1902. Lin et al., Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Org. Chemistry, 52,5594-5601 (1987). E.g. page 5595 Aristoff et al., Total Synthesis of a Novel Antiulcer Agent via a Modification of the Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Am. Chem., 107, 7967-7974 (1985). E.g. page 7971 McManus et al., Tetrazole Analogs of Plant Auxins, J. Org. Chemistry, 24, 1464-1467 (1959). E.g. pages 1465-1467. Arumugan et al., A New Purification Process for Pharmaceutical and Chemical Industries, Organic Process Research & Development 2005, 9, 319-320 (2005). E.g. page 319.

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22. The product of claim 21, wherein the product comprises a pharmaceutically acceptable salt formed from the product of step (d).	'117 Patent, 20:10-21:12. U.S. Patent Publication No. 2005/0085540 (April 2005), figures 15-22, [0051], [0105]. U.S. Patent Publication No. 2005/0165110 (July 2005), [0024] Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil), J. Org. Chemistry., 69(6), 1890-1902 (2004). E.g. abstact, page 1892 (compound 7), page 1902. Lin et al., Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Org. Chemistry, 52,5594-5601 (1987). E.g. page 5595 Aristoff et al., Total Synthesis of a Novel Antiulcer Agent via a Modification of the Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Am. Chem., 107, 7967-7974 (1985). E.g. page 7971 McManus et al., Tetrazole Analogs of Plant Auxins, J. Org. Chemistry, 24, 1464-1467 (1959). E.g. pages 1465-1467.

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IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF NEW JERSEY

UNITED THERAPEUTICS CORPORATION Plaintiff, v. TEVA PHARMACEUTICALS USA, INC., Defendant.	C.A. No.: 3:14-cv-05498 (PGS) (LHG) Hon. Peter G. Sheridan, U.S.D.J. Hon. Lois H. Goodman, U.S.M.J. **HIGHLY CONFIDENTIAL**

DEFENDANT TEVA PHARMACEUTICALS USA, INC.'S
AMENDED NON-INFRINGEMENT AND INVALIDITY CONTENTIONS

Pursuant to the Local Patent Rules 3.2A, 3.3 and 3.6, this Court's November 25, 2014 Scheduling Order, and this Court's April 15, 2015 Order, Teva submits the following Amended Non-Infringement and Invalidity Contentions for the asserted claims of United States Patent Nos. 6,765,117 ("the '117 patent"); 8,497,393 ("the '393 patent"); 7,999,007 ("the '007 patent"); 8,653,137 ("the '137 patent"); and 8,658,694 ("the '694 patent").

Teva has prepared these contentions in good faith based on information and discovery currently available to them. Fact discovery is in its beginning stages, claim construction has not

yet occurred, and expert discovery has not yet begun in this case. Teva has not had an opportunity to depose any individuals and Teva's investigation into the non-infringement and invalidity of the patents-in-suit continues. Therefore, Teva reserves the right to amend, alter, or supplement these contentions based on further investigation and discovery as the case progresses, any claim construction from the Court, Court decisions in any related cases (including the *United Therapeutics Corp. v. Sandoz, Inc.* case (case nos. 3:12-cv-01617 and 3:13-cv-00316) ("*UTC v. Sandoz* matter"), the contentions of any parties in litigations involving any of the patents-in-suit, or as a result of Plaintiff's asserted claims, contentions, and infringement positions. Teva reserves the right to serve additional, supplemental, and/or revised invalidity contentions as necessary or appropriate under the Local Rules and the Court's Order.

These contentions are made pursuant to Federal Rule of Evidence 502. To the extent these contentions contain any information that may be protected from discovery under the attorney-client privilege, the attorney work-product immunity, the common interest privilege, or any other applicable privilege or immunity, such disclosure is inadvertent and does not constitute a waiver of any such privilege or immunity. The information set forth in these contentions is provided without waiving: (1) the right to object to the use of any statement for any purpose, in this action or any other, on the grounds of privilege, relevance, materiality, or any other appropriate grounds; (2) the right to object to any request involving or relating to the subject matter of the statements herein; or (3) the right to revise, correct, supplement, or clarify any of the statements provided below at any time.

These contentions should not be taken as an indication of Teva's position with regard to the proper construction of any claim term. Rather, Teva has made reasonable assumptions, to the extent necessary and appropriate, as to the meaning of claim terms for the purpose of these

26	The method of claim 25, wherein the injection is intravenous injection.	*See* claim 11.

II. INVALIDITY CONTENTIONS

Pursuant to the Local Patent Rules, Teva hereby provides its invalidity contentions. Its contentions include (i) the identity of each item of prior art that allegedly anticipates each asserted claim or renders it obvious; (ii) if the prior art is a patent, its number, country of origin, and date of issue, and (iii) if the prior art is not a patent, its title, date of publication, and where feasible, author and publisher. Prior art under 35 U.S.C. § 102(b) is hereby identified by specifying the item offered for sale or publicly used or known, the date the offer or use took place or the information became known, and the identity of the person or entity which made the use or which made and received the offer, or the person or entity which made the information known or to whom it was made known. Prior art under 35 U.S.C. § 102(f) is identified by providing the name of the person(s) from whom and the circumstances under which the invention or any part of it was derived. Prior art under 35 U.S.C. § 102(g) is identified by providing the identities of the person(s) or entities involved in and the circumstances surrounding the making of the invention before the patent applicant(s).

Teva's contentions further disclose whether each item of prior art anticipates each asserted claim or renders it obvious. If obviousness is alleged, Teva's contentions include an explanation of why the prior art renders the asserted claim obvious, including an identification of any combination of prior art showing obviousness. In addition, Teva's contentions include a chart identifying where specifically in each alleged item of prior art each limitation of each asserted claim is found, including for each limitation that Teva contends is governed by 35 U.S.C. § 112(f), the identity of the structure(s), act(s), or material(s) in each item of prior art that performs the claimed function. Lastly, Teva's contentions also include, where appropriate, any

grounds of invalidity based on 35 U.S.C. § 101, indefiniteness under 35 U.S.C. § 112(b) or enablement or written description under 35 U.S.C. § 112(a) of any of the asserted claims.

The following table summarizes UTC's asserted claims in the case and Teva's invalidity contentions:

Patent No.	Patent Grant Date	Asserted Priority	Claims (Indep)	Asserted Claims	Invalidity Contentions
6,765,117	7/20/2004	10/24/1997	4 (3)	1-4	Pages 56-74
8,497,393	7/30/2013	12/17/2007	22 (2)	1-22	Pages 74-106
7,999,007	8/16/2011	9/7/2007	26 (3)	1-5, 7-17, 19-26	Pages 106-22
8,653,137	2/18/2014	9/7/2007	13 (1)	1-13	Pages 122-31
8,658,694	2/25/2014	9/7/2007	26 (2)	1-26	Pages 131-44

A. Invalidity of U.S. Patent No. 6,765,117

U.S. Patent No. 6,765,117 was issued on July 20, 2004 and it claims priority to October 24, 1997. The '117 patent issued from U.S. Patent Application No. 10/184,907, which is a divisional of U.S. Patent Application No. 09/541,521, filed April 3, 2000, now U.S. Pat. No. 6,441,245, which is a continuation-in-part of U.S. Patent Application No. 09/481,390, filed January 12, 2000, now abandoned, which is a continuation of U.S. Patent Application No. 08/957,736, filed October 24, 1997, now abandoned.

As further explained below, claims 1-4 of the '117 patent are invalid as anticipated or obvious in view of at least the following prior art references, which are exemplary of the state of the art at the time of the filing of the '117 patent:

- U.S. Patent No. 4,668,814 ("the '814 patent") (TEVA_TRE_0004219-49)
- Monson, Advanced Organic Synthesis, Methods and Techniques, *Introduction to the Techniques of Synthesis, Part III. Purification of Product,* 178-188 (1971) ("Monson") (TEVA_TRE_0004108-120)

- Harwood, *Experimental organic chemistry: Principles and Practice*, 127-134 (1989) ("Harwood") (TEVA_TRE_0004307-317)
- The references cited or disclosed during prosecution of the '117 patent

Teva expressly reserves the right to modify and/or supplement the above list at any time as necessary and/or as discovery progresses.[2]

As shown below, the asserted claims of the '117 patent are anticipated or obvious. Claims 1-4 are product-by-process claims directed to isomeric compounds including treprostinil. If the claim is a product-by-process claim, the focus of the invalidity analysis is the product produced by the claimed process. *Amgen Inc. v. F. Hoffmann-La Roche, Ltd.*, 580 F.3d 1340, 1369-70 (Fed. Cir. 2009). The prior art does not need to teach the process limitations so long as the prior art product is the same as the claimed product. *Id.* The prior art disclosed the same product, treprostinil and the pharmacologically acceptable salt form of treprostinil, as the claimed product and thus invalidates the claims. The '814 Patent col. 29, line 12-col. 33, line 4. Additionally, even if not anticipated, the claimed treprostinil would have been obvious to a person of ordinary skill in the art, because the POSA could have applied a standard purification technique such as recrystallization to arrive at the claimed treprostinil product.

1. Claims 1-4 of the '117 Patent Are Anticipated by U.S. Patent No. 4,668,814

Claims 1-4 of the '117 patent are anticipated by United States Patent No. 4,668,814 (TEVA_TRE_0004219-49). The '814 patent, entitled "Interphenylene carbacyclin derivatives," was issued on May 26, 1987 in the United States and is, thus, a 102(b) prior art to the '117 patent.

[2] Teva also asserts that if the Federal Circuit in the related *UTC v. Sandoz* case invalidates the '117 patent, UTC cannot assert and is estopped from asserting the '117 patent against Teva.

The '117 patent's named inventor is Paul A. Aristoff and its original assignee is the Upjohn Company. The '117 patent discloses treprostinil and an improved process for making treprostinil. Claim 1 is directed to a genus of compounds that includes treprostinil. '117 patent at col. 21:23-22:37. Claim 2 is dependent on claim 1 and adds the limitation that the claimed final isomeric compound is treprostinil. *Id.* at col. 22:38-41. Claim 3 is directed to the specific treprostinil compound. *Id.* at col. 22:42-23:52. Claim 4 of the '117 patent is directed to the treprostinil compound in "pharmacologically acceptable salt form."

The active pharmaceutical ingredient ("API") of Remodulin is treprostinil sodium. Treprostinil is an old compound, first synthesized more than 35 years ago by Dr. Paul Aristoff at The Upjohn Company ("Upjohn"). The treprostinil compound was disclosed and claimed in U.S. Patent No. 4,306,075, which issued on Dec. 15, 1981. Dr. Aristoff subsequently developed an improved process for making treprostinil, which was disclosed in the '814 patent. Thereafter, a different group of scientists led by Dr. Robert Moriarty developed a further improved process for making treprostinil, which is disclosed and claimed in the '117 patent.

As Dr. Aristoff testified in the *UTC v. Sandoz* trial, treprostinil was invented more than 35 years ago. These facts were confirmed at trial by Dr. Aristoff, who testified as an expert for UTC:

Q. You invented the treprostinil compound 35 years ago; right?
A. Roughly, yes.
Q. You patented the treprostinil compound in the '075 patent; right?
A. Yes.
Q. The '075 patent issued in 1981; correct?
A. Yes.
Q. The '075 patent sets out a process for making treprostinil; correct?
A. Yes.
Q. And you later developed an improved process for making treprostinil; correct?
A. That's correct.
Q. Now, that process was disclosed in the '814 patent; right?

A. Correct.
Q. And the process that was disclosed in the '814 patent was an improvement over your earlier process for making treprostinil; right?
A. That's correct.
Q. And after that Dr. Moriarty and his team developed another improved process for making treprostinil; correct?
A. Yes.
Q. And that's the process that's disclosed and claimed in the '117 patent; correct?
A. Yes.

UTC v. Sandoz, Nos. 2014-1821, -1849 (Fed. Cir. 2015) at Appendix A02061-62 at 1850:11-1851:12.

Example 3 of the '814 patent discloses the treprostinil compound, referred to as "9-Deoxy-13,14-dihydro-2',9α-methano-3-oxa-4,5,6-trinor-3,7-(1',3- interphenylene)-PGF1." The treprostinil compound disclosed in Example 3 is exactly the same treprostinil compound claimed in claims 1-3 of the '117 patent. Example 3 of the '814 patent provides a detailed description of an improved process for making treprostinil. A14533-35 (col. 29:11-33:4). The final steps in the process are disclosed as follows:

> The resulting pink to red solid was chromatographed on 400g of CC-4 acid washed silica gel eluting with 2L of 50% ethyl acetate in hexane followed by 3 L of 70% ethyl acetate in hexane to give 5.10g of solid which was crystallized from hot tetrahydrofuran and hexane to give 1.20g of 9-deoxy-13,14-dihydro-2',9α-methano-3-oxa-4,5,6-trinor- 3,7-(1',3-interphenylene)-PGF1 (m.p. 122° – 124° C).

'814 patent at col. 32:53-61. The intermediate "5.10 g of solid" disclosed in Example 3 was a 1:1 mixture of diastereomers. After a final purification step (crystallization from hot tetrahydrofuran and hexane), the resulting 1.20 g of treprostinil was approximately 95% pure.

The '117 patent claims are product-by-process claims. *See Bonito Boats, Inc. v. Thunder Craft Boats, Inc.*, 489 U.S. 141, 159 (1989) (A "product-by- process" claim is "one in which the product is defined at least in part in terms of the method or process by which it is made").

Product-by-process claims are anticipated by the disclosure of the same product in the prior art. *Amgen Inc. v. Hoffmann-La Roche, Ltd.*, 580 F.3d 1340, 1366 (Fed. Cir. 2009); *SmithKline Beecham Corp. v. Apotex Corp.*, 439 F.3d 1312, 1317 (Fed. Cir. 2006) ("It has long been established that one cannot avoid anticipation by an earlier product disclosure by claiming the same product more narrowly, that is, by claiming the product as produced by a particular process"); *Gen. Elec. Co. v. Wabash Appliance Corp.*, 304 U.S. 364, 373 (1938) ("a patentee who does not distinguish his product from what is old except by reference, express or constructive, to the process by which he produced it, cannot secure a monopoly on the product by whatever means produced"); *Cochrane v. Badische Anilin & Soda Farbrik*, 111 U.S. 293, 311 (1884) ("While a new process for producing [a chemical compound] was patentable, the product itself could not be patented, even though it was a product made [by a new process] for the first time" because the product was disclosed in the prior art).

"In determining validity of a product-by-process claim, the focus is on the product and not on the process of making it." *Amgen*, 580 F.3d at 1369-70; *In re Thorpe*, 777 F.2d 695, 697 (Fed. Cir. 1985) ("[E]ven though product-by-process claims are limited by and defined by the process, determination of patentability is based on the product itself. The patentability of a product does not depend on its method of production.") (internal citations omitted); SmithKline, 439 F.3d at 1317-19.

The product of the '117 patent claims is the treprostinil compound. The treprostinil compound depicted by the chemical formula set out in the '117 patent claims was disclosed in the '814 patent (as well as in the earlier '075 patent). Moreover, the treprostinil compound made by the '814 patent's processes is the exactly the same treprostinil compound made by the '117 patent process. Because the compound made by the '814 patent process and the compound made

by the '117 patent process are identical, there are no structural or functional differences between the product disclosed in the prior art and the product claimed in the '117 patent. *In re Papesch*, 315 F.2d 381, 391 (CCPA 1963) ("From the standpoint of patent law, a compound and all of its properties are inseparable").

The claims are still anticipated if the product of the '117 patent claims is not treprostinil compound but a mixture that includes treprostinil and various impurities. There is no threshold impurity profile required for a batch of treprostinil to fall within the scope of the claims. '117 patent, claims 1-4. The '814 patent discloses a final batch of 1.20 grams of treprostinil with a purity level of ~95%. As shown by a table in UTC's New Drug Application for Remodulin, this purity level is comparable to the purity level of a number of the batches made using the '117 patent process. *UTC v. Sandoz*, Nos. 2014-1821, -1849 (Fed. Cir. 2015), Br. of Defendant-Appellant at 14. Thus, even if the product of the '117 patent is a mixture with a certain level of purity, the 1.20 g batch of treprostinil disclosed in the '814 patent Example 3 is an embodiment that falls within the scope of the '117 patent claims. The '814 patent thus anticipates each of the claims of the '117 patent. *See, e.g., Titanium Metals Corp. of Am. v. Banner*, 778 F.2d 775, 782 (Fed. Cir. 1985) ("It is also an elementary principle of patent law that when, as by a recitation of ranges or otherwise, a claim covers several compositions, the claim is 'anticipated' if *one* of them is in the prior art") (emphasis in the original).[3]

[3] UTC also bears the burden of showing that the purity limitation, even if present in the '117 patent, was not present in the prior art product. *In re Marosi*, 710 F.2d 798, 802 (Fed. Cir. 1983) (The claims were directed to a zeolite manufactured by mixing together various inorganic materials in solution and heating the resultant gel to form a crystalline metal silicate essentially free of alkali metal. The prior art described a process of making a zeolite which, after ion exchange to remove alkali metal, appeared to be "essentially free of alkali metal." The court upheld the rejection because the applicant had not come forward with any evidence that the prior art was not "essentially free of alkali metal" and therefore a different and unobvious product.); *Ex parte Gray*, 10 U.S.P.Q.2d 1922 (Bd. Pat. App. & Inter. 1989) (The prior art disclosed human nerve growth factor (b-NGF) isolated from human placental tissue. The claim was directed to b-NGF produced through genetic engineering techniques. The factor produced seemed to be substantially the same whether isolated from tissue or produced through genetic engineering. While the applicant questioned the purity of the prior art factor, no concrete evidence of an unobvious difference was presented. The

2. Claims 1-4 of the '117 Patent Are Obvious Over the '814 Patent in view of Monson (1971), Harwood (1989) and Knowledge of One of Ordinary Skill in the Art.

If claims 1-4 of the '117 patent are not anticipated, claims 1-4 of the '117 patent are obvious over United States Patent No. 4,668,814 (TEVA_TRE_0004219-49) in view of Monson, Advanced Organic Synthesis, Methods and Techniques, Introduction to the Techniques of Synthesis, Part III. Purification of Product, 178-188 (1971) ("Monson") (TEVA_TRE_0004108-120), Harwood, Experimental organic chemistry: Principles and Practice, 127-134 (1989) ("Harwood") (TEVA_TRE_0004307-317) and knowledge of one of ordinary skill in the art. The '814 patent, Monson (1971), and Harwood (1989) are 102(b) prior arts to the '117 patent.

As explained in more detail above, claim 1 of the '117 patent is a product by process claims directed to isomeric compounds, including treprostinil. If the claim is a product-by-process claim, the focus of the anticipation analysis is the product produced by the claimed process. *Amgen Inc. v. F. Hoffmann-La Roche, Ltd.*, 580 F.3d 1340, 1369-70 (Fed. Cir. 2009). The prior art does not need to teach the process limitations so long as "the product in a product-by-process claim is the same as or obvious from a product of the prior art." *Id.* at 1366. Even if the Court rules that the claims of the '117 patent are not anticipated, the prior art disclosed obvious variations of the same product, treprostinil (or a mixture containing treprostinil) and the pharmacologically acceptable salt form of treprostinil, as the claimed product. The '814 patent col. 29, line 12-col. 33, line 4.

It would have been obvious to a person of ordinary skill in the art, for example, to apply standard and conventional purification techniques known in the art, such as distillation,

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Board stated that the dispositive issue is whether the claimed factor exhibits any unexpected properties compared with the factor disclosed by the prior art. The Board further stated that the applicant should have made some comparison between the two factors to establish unexpected properties since the materials appeared to be identical or only slightly different.).

recrystallization, drying of solids, sublimation, and chromatography, to arrive at the claimed treprostinil product or the treprostinil mixture with certain impurity levels. *See, e.g.*, '814 patent in view of Monson and Harwood. For instance, Professor Monson, in his book Advanced Organic Synthesis: Methods and Techniques (1971) describes in pages 178-188 these conventional purification methods known at the time. Drs. Harwood and Moody, in their book Experimental Organic Chemistry (1989), describe in pages 127-134 various ways for the purification of organic compounds, including at page 127 that "simplest and most effective" way is crystallization of solid organic compounds to obtain more pure compounds.

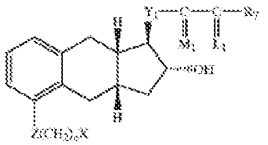
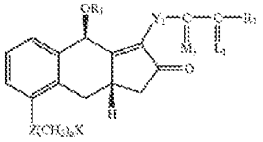
Dependent claim 2 claims the stereoselectively produced isomeric compound of claim 1, wherein Z is O, n is 1, X is COOH, Y_1 is $-CH_2CH_2-$ M_1 is α-OH:β-R_5, wherein R_5 is hydrogen, L_1 is α-R_3:β-R_4, wherein R_3 and R_4 are hydrogen and R_7 is butyl. The claim is anticipated or obvious for the same reasons as claim 1 above. Claim 3 is a product-by-process claims directed to treprostinil. If the claim is a product-by-process claim, the focus of the anticipation analysis is the product produced by the claimed process. *Amgen Inc. v. F. Hoffmann-La Roche, Ltd.*, 580 F.3d 1340, 1369-70 (Fed. Cir. 2009). The prior art does not need to teach the process limitations so long as the prior art product is the same as the claimed product. *Id.* The prior art disclosed the same product, treprostinil and the pharmacologically acceptable salt form of treprostinil, as the claimed product and thus anticipates the claim. The '814 Patent col. 29, line 12-col. 33, line 4. Additionally, it would be obvious to a person of ordinary skill in the art to apply a standard purification technique such as recrystallization to arrive at the claimed treprostinil product. *See e.g.* Monson; Harwood.

Claim 4 is a product by process claims directed to treprostinil. If the claim is a product-by-process claim, the focus of the anticipation analysis is the product produced by the claimed

process. *Amgen Inc. v. F. Hoffmann-La Roche, Ltd.*, 580 F.3d 1340, 1369-70 (Fed. Cir. 2009). The prior art does not need to teach the process limitations so long as the prior art product is the same as the claimed product. *Id.* The prior art disclosed the same product, treprostinil and the pharmacologically acceptable salt form of treprostinil, as the claimed product and thus anticipates the claim. The '814 Patent col. 29, line 12-col. 33, line 4. Additionally, it would be obvious to a person of ordinary skill in the art to apply a standard purification technique such as recrystallization to arrive at the claimed treprostinil product. *See e.g.* Monson; Harwood.

Plaintiff has not set forth its contentions concerning secondary considerations in this case. If Plaintiff relies on any secondary considerations of non-obviousness, Teva reserves the right to supplement its contentions.

The following chart incorporates the analysis set forth above and identifies where specifically in each alleged item of prior art each limitation of each asserted claim is found:

	'117 Patent Claim Language	**Invalidity Contentions**
1	A stereoselectively produced isomeric compound according to the following formula:  that is produced by a process for making 9-deoxy-PGF_1 -type compounds, the process comprising  by intramolecular cyclization of the enyne, wherein Z is O, S, CH_2, or NR_8 in which R_8 is H, alkyl or aryl; X is H, CN, OR_9, or $COOR_9$ in which R_9 is H, alkyl, a pharmacologically acceptable	**Anticipation:** Claim 1 is anticipated by the '814 patent, 29:12-33:4. If claim 1 is now anticipated, it is obvious over '814 patent in view of Monson, Advanced Organic Synthesis, Methods and Techniques, INTRODUCTION TO THE TECHNIQUES OF SYNTHESIS, PART III. PURIFICATION OF PRODUCT, 178-188 (1971) and Harwood, EXPERIMENTAL ORGANIC CHEMISTRY: PRINCIPLES AND PRACTICE, 127-132 (1989). E.g. pages 127-321. Claim 1 of the '117 patent is anticipated by United States Patent No. 4,668,814 (TEVA_TRE_0004219-49). The '814 patent, entitled "Interphenylene carbacyclin derivatives," was issued on May 26, 1987 in the United States and is, thus, a 102(b) prior art to the '117 patent. The '117 patent's named inventor is Paul A. Aristoff and its original assignee is the Upjohn Company. The '117 patent discloses treprostinil

	'117 Patent Claim Language	Invalidity Contentions
	cation, THP or TBDMS; wherein n is 0, 1, 2, or 3; wherein Y_1 is trans-CH=CH—, cis-CH=CH—, $CH_2(CH_2)_m$ -, or —C≡C—; m is 1,2, or 3; wherein R_1 is an alcohol protecting group; wherein R_7 is (1)—C_2 H_{2p}—CH_3, wherein p is an integer from one to 5, inclusive, (2) phenoxy optionally substituted by one, two or three chloro, fluoro, trifluoromethyl, (C_1-C_3)alkyl, or (C_1-C_3)alkoxy, with the proviso that not more than two substituents are other than alkyl, with the proviso that R_7 is phenoxy or substituted phenoxy, only when R_3 and R_4 are hydrogen or methyl, being the same or different, (3) phenyl, benzyl, phenylethyl, or phenylpropyl optionally substituted on the aromatic ring by one, two or three chloro, fluoro, trifluoromethyl, (C_1-C_3)alkyl, or (C_1-C_3)alkoxy, with the proviso that not more than two substituents are other than alkyl, (4) cis-CH=CH—CH_2—CH_3, (5) —$(CH_2)_2$—CH(OH) —CH_3, or (6) —$(CH_2)_3$—CH=$C(CH_3)_2$; wherein —$C(L_1)$—R_7 taken together is (1) (C_4-C_7)cycloalkyl optionally substituted by one to 3 (C_1-C_5) alkyl; (2) 2-(2-furyl)ethyl, (3) 2-(3-thienyl)ethoxy, or (4) 3-thienyloxymethyl; wherein M_1 is .α-OH:β-R_5 or α-R_5:β-OH or α-OR_1:β-R_5 or α-R_5:β-OR_1, wherein R_5 is hydrogen or methyl and R_1 is an alcohol protecting group; and wherein L_1 is α-R_3:β-R_4, α-R_4:β-R_3, or a mixture of .α-R_3:β-R_4 and α-R_4:β-R_3, wherein R_3 and R_4 are hydrogen, methyl, or fluoro, being the same or different, with the proviso that one of R_3 and R_4 is fluoro only when the	and an improved process for making treprostinil. Claim 1 is directed to a genus of compounds that includes treprostinil. '117 patent at col. 21:23-22:37. Claim 2 is dependent on claim 1 and adds the limitation that the claimed final isomeric compound is treprostinil. *Id.* at col. 22:38-41. Claim 3 is directed to the specific treprostinil compound. *Id.* at col. 22:42-23:52. Claim 4 of the '117 patent is directed to the treprostinil compound in "pharmacologically acceptable salt form." The active pharmaceutical ingredient ("API") of Remodulin is treprostinil sodium. Treprostinil is an old compound, first synthesized more than 35 years ago by Dr. Paul Aristoff at The Upjohn Company ("Upjohn"). The treprostinil compound was disclosed and claimed in U.S. Patent No. 4,306,075, which issued on Dec. 15, 1981. Dr. Aristoff subsequently developed an improved process for making treprostinil, which was disclosed in the '814 patent. Thereafter, a different group of scientists led by Dr. Robert Moriarty developed a further improved process for making treprostinil, which is disclosed and claimed in the '117 patent. As Dr. Aristoff testified in the *UTC v. Sandoz* trial, treprostinil was invented more than 35 years ago. These facts were confirmed at trial in the *UTC v. Sandoz* case by Dr. Aristoff, who testified as an expert for UTC: Q. You invented the treprostinil compound 35 years ago; right? A. Roughly, yes. Q. You patented the treprostinil compound in the '075 patent; right? A. Yes. Q. The '075 patent issued in 1981; correct? A. Yes. Q. The '075 patent sets out a

	'117 Patent Claim Language	Invalidity Contentions
	other is hydrogen or fluoro.	process for making treprostinil; correct? A. Yes. Q. And you later developed an improved process for making treprostinil; correct? A. That's correct. Q. Now, that process was disclosed in the '814 patent; right? A. Correct. Q. And the process that was disclosed in the '814 patent was an improvement over your earlier process for making treprostinil; right? A. That's correct. Q. And after that Dr. Moriarty and his team developed another improved process for making treprostinil; correct? A. Yes. Q. And that's the process that's disclosed and claimed in the '117 patent; correct? A. Yes. *UTC v. Sandoz*, Nos. 2014-1821, -1849 (Fed. Cr. 2015) at Appendix A02061-62 at 1850:11-1851:12. Example 3 of the '814 patent discloses the treprostinil compound, referred to as "9-Deoxy-13,14-dihydro-2',9α-methano-3-oxa-4,5,6-trinor-3,7-(1',3- interphenylene)-PGF1." The treprostinil compound disclosed in Example 3 is exactly the same treprostinil compound claimed in claims 1-3 of the '117 patent. Example 3 of the '814 patent provides a detailed description of an improved process for making treprostinil. A14533-35 (col. 29:11-33:4). The final steps in the process are disclosed as follows:

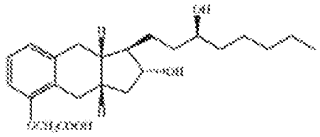
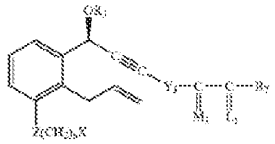
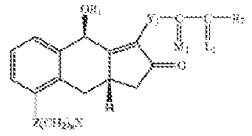
	'117 Patent Claim Language	Invalidity Contentions
		The resulting pink to red solid was chromatographed on 400g of CC-4 acid washed silica gel eluting with 2L of 50% ethyl acetate in hexane followed by 3 L of 70% ethyl acetate in hexane to give 5.10g of solid which was crystallized from hot tetrahydrofuran and hexane to give 1.20g of 9-deoxy-13,14-dihydro-2',9α-methano-3-oxa-4,5,6-trinor- 3,7-(1',3-interphenylene)-PGF1 (m.p. 122° – 124° C). '814 patent at col. 32:53-61. The intermediate "5.10 g of solid" disclosed in Example 3 was a 1:1 mixture of diastereomers. After a final purification step (crystallization from hot tetrahydrofuran and hexane), the resulting 1.20 g of treprostinil was approximately 95% pure. Claim 1 is a product-by-process claim. *See Bonito Boats, Inc. v. Thunder Craft Boats, Inc.*, 489 U.S. 141, 159 (1989) (A "product-by-process" claim is "one in which the product is defined at least in part in terms of the method or process by which it is made"). Product-by-process claims are anticipated by the disclosure of the same product in the prior art. *Amgen Inc. v. Hoffmann-La Roche, Ltd.*, 580 F.3d 1340, 1366 (Fed. Cir. 2009); *SmithKline Beecham Corp. v. Apotex Corp.*, 439 F.3d 1312, 1317 (Fed. Cir. 2006) ("It has long been established that one cannot avoid anticipation by an earlier product disclosure by claiming the same product more narrowly, that is, by claiming the product as produced by a particular process"); *Gen. Elec. Co. v. Wabash Appliance Corp.*, 304 U.S. 364, 373 (1938) ("a patentee who does not distinguish his product from what is old except by reference, express or constructive, to the process by which he produced it, cannot secure a monopoly on the product by whatever means produced"); *Cochrane v. Badische Anilin & Soda Farbrik*, 111

	'117 Patent Claim Language	Invalidity Contentions
		U.S. 293, 311 (1884) ("While a new process for producing [a chemical compound] was patentable, the product itself could not be patented, even though it was a product made [by a new process] for the first time" because the product was disclosed in the prior art). "In determining validity of a product-by-process claim, the focus is on the product and not on the process of making it." *Amgen*, 580 F.3d at 1369-70; *In re Thorpe*, 777 F.2d 695, 697 (Fed. Cir. 1985) ("[E]ven though product-by-process claims are limited by and defined by the process, determination of patentability is based on the product itself. The patentability of a product does not depend on its method of production.") (internal citations omitted); SmithKline, 439 F.3d at 1317-19. The product of the '117 patent claims is the treprostinil compound. The treprostinil compound depicted by the chemical formula set out in the '117 patent claims was disclosed in the '814 patent (as well as in the earlier '075 patent). Moreover, the treprostinil compound made by the '814 patent's processes is the exactly the same treprostinil compound made by the '117 patent process. Because the compound made by the '814 patent process and the compound made by the '117 patent process are identical, there are no structural or functional differences between the product disclosed in the prior art and the product claimed in the '117 patent. *In re Papesch*, 315 F.2d 381, 391 (CCPA 1963) ("From the standpoint of patent law, a compound and all of its properties are inseparable"). The claims are still anticipated if the product of the '117 patent claims is not treprostinil compound but a mixture that includes treprostinil and various impurities. There is no threshold impurity profile required for a batch of treprostinil to fall within the scope of the claims. '117 patent, claims 1-4. The '814 patent discloses a final batch of 1.20 grams of treprostinil with a

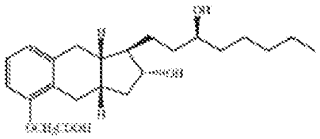
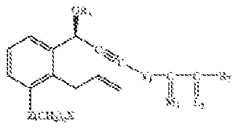
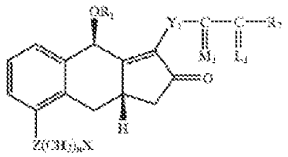
	'117 Patent Claim Language	Invalidity Contentions
		purity level of ~95%. As shown by a table in UTC's New Drug Application for Remodulin, this purity level is comparable to the purity level of a number of the batches made using the '117 patent process. Thus, even if the product of the '117 patent is a mixture with a certain level of purity, the 1.20 g batch of treprostinil disclosed in the '814 patent Example 3 is an embodiment that falls within the scope of the '117 patent claims. The '814 patent thus anticipates each of the claims of the '117 patent. *See, e.g.*, *Titanium Metals Corp. of Am. v. Banner*, 778 F.2d 775, 782 (Fed. Cir. 1985) ("It is also an elementary principle of patent law that when, as by a recitation of ranges or otherwise, a claim covers several compositions, the claim is 'anticipated' if *one* of them is in the prior art") (emphasis in the original). **Obviousness:** If claim 1 of the '117 patent is not anticipated, claim 1 of the '117 patent is obvious over United States Patent No. 4,668,814 (TEVA_TRE_0004219-49) in view of Monson, Advanced Organic Synthesis, Methods and Techniques, Introduction to the Techniques of Synthesis, Part III. Purification of Product, 178-188 (1971) ("Monson") (TEVA_TRE_0004108-120), Harwood, Experimental organic chemistry: Principles and Practice, 127-134 (1989) ("Harwood") (TEVA_TRE_0004307-317) and knowledge of one of ordinary skill in the art. The '814 patent, Monson (1971), and Harwood (1989) are 102(b) prior arts to the '117 patent.[4] As explained in more detail above, claim 1 of the '117 patent is a product-by-process claim directed to isomeric compounds, including treprostinil. If the claim is a product-by-process claim, the focus of the anticipation analysis is the product produced by the claimed process. *Amgen Inc. v. F. Hoffmann-La Roche, Ltd.*, 580 F.3d 1340,

[4] "[W]hen the prior art discloses a product which reasonably appears to be either identical with or only slightly different than a product claimed in a product-by-process claim, a rejection based alternatively on either section 102 or section 103 of the statute is eminently fair and acceptable." *In re Brown*, 459 F.2d 531, 535 (C.C.P.A. 1972).

	'117 Patent Claim Language	Invalidity Contentions
		1369-70 (Fed. Cir. 2009). The prior art does not need to teach the process limitations so long as "the product in a product-by-process claim is the same as or obvious from a product of the prior art." *Id.* at 1366. Even if the Court rules that the claims of the '117 patent are not anticipated, the prior art disclosed obvious variations of the same product, treprostinil (or a mixture containing treprostinil) and the pharmacologically acceptable salt form of treprostinil, as the claimed product. The '814 patent col. 29, line 12-col. 33, line 4. It would have been obvious to a person of ordinary skill in the art, for example, to apply standard and conventional purification techniques known in the art, such as distillation, recrystallization, drying of solids, sublimation, and chromatography, to arrive at the claimed treprostinil product or the treprostinil mixture with certain impurity levels. *See e.g.* '814 patent in view of Monson and Harwood. For instance, Professor Monson, in his book Advanced Organic Synthesis: Methods and Techniques (1971) describes in pages 178-188 these conventional purification methods known at the time. Drs. Harwood and Moody, in their book Experimental Organic Chemistry (1989), describe in pages 127-134 various ways for the purification of organic compounds various ways for the purification of organic compounds, including at page 127 that "simplest and most effective" way is crystallization of solid organic compounds to obtain more pure compounds. Plaintiff has not set forth its contentions concerning secondary considerations in this case. If Plaintiff relies on any secondary considerations of non-obviousness, Teva reserves the right to supplement its contentions or to rebut those arguments.
2	The stereoselectively produced isomeric compound of claim 1, wherein Z is O, n is 1, X is COOH, Y_1 is $-CH_2CH_2-$ M_1 is α-OH:β-R_5, wherein R_5 is hydrogen, L_1 is α-R_3:β-	Claim 2 incorporates the compound of claim 1 and is therefore invalid for the same reasons stated in claim 1. Claim 2 further specifies that the compound of claim 2 is directed to stereoselectively produced isomeric compound of

	'117 Patent Claim Language	Invalidity Contentions
	R_4, wherein R_3 and R_4 are hydrogen and R_7 is butyl.	claim 1, wherein Z is O, n is 1, X is COOH, Y_1 is $-CH_2CH_2-$ M_1 is α-OH:β-R_5, wherein R_5 is hydrogen, L_1 is α-R_3:β-R_4, wherein R_3 and R_4 are hydrogen and R_7 is butyl. As disclosed in the context of claim 1, treprostinil incorporates these limitations and this claim is anticipated or obvious for the same reasons as claim 1 above.
3	A steroselectively produced isomeric compound according to the following formula:  that is produced by a process for making 9-deoxy-PFG_1 -type compounds, the process comprising cyclizing a starting compound of the formula:  into a compound of the following formula:  by intramolecular cyclization of the enyne, wherein Z is O, S, CH_2, or NR_8 in which R_8 is H, alkyl or aryl; X is H, CN, OR_9, or $COOR_9$ in which R_9 is H; wherein n is 0, 1, 2, or 3; wherein Y_1 is trans-CH=CH-, cis-CH=CH-, $-CH_2(CH_2)_m-$, or -C≡C-; m is 1,2, or 3; wherein R_1 is an alcohol protecting group; wherein R_7 is	Claim 3 is a product-by-process claim that is directed to treprostinil. If the claim is a product-by-process claim, the focus of the anticipation analysis is the product produced by the claimed process. *Amgen Inc. v. F. Hoffmann-La Roche, Ltd.*, 580 F.3d 1340, 1369-70 (Fed. Cir. 2009). The prior art does not need to teach the process limitations so long as the prior art product is the same as the claimed product. *Id.* The '814 patent disclosed the same product, treprostinil, and the pharmacologically acceptable salt form of treprostinil, as the claimed product and thus anticipates the claim. The '814 Patent col. 29, line 12-col. 33, line 4. Even if not anticipated, it would have been obvious to a person of ordinary skill in the art to apply a standard purification technique such as recrystallization to arrive at the claimed treprostinil product. *See e.g.* Monson at 178-188 (disclosing standard purification methods, as discussed in more detail above) and Harwood at 127-134 (disclosing purifications methods known at the time, as discussed in more detail above).

	'117 Patent Claim Language	Invalidity Contentions
	(5) $-C_pH_{2p}-CH_3$, wherein p is an integer from one to 5, inclusive, (6) phenoxy optionally substituted by one, two or three chloro, fluoro, trifluoromethyl, (C_1 -C_3)alkyl, or (C_1 -C_3)alkoxy, with the proviso that not more than two substituents are other than alkyl, with the proviso that R_7 is phenoxy or substituted phenoxy, only when R_3 and R_4 are lydrogen[sic] or methyl, being the same or different, (7) phenyl, bnezyl[sic], phenylethyl, or phenylpropyl optionally substituted on the aromatic ring by one, two or three chloro, fluoro, trifluoromethyl, (C_1-C_3)alkyl, or (C_1-C_3)alkoyx, with the proviso that not more than two substituents are other than alkyl, (8) cis-$CH=CH-CH_2-CH_3$, (9) $-(CH_2)_2-CH(OH)-CH_3$, or (10) $-(CH_2)_3-CH=C(CH_3)_2$; wherein $-C(L_1)-R_7$ taken together is (11) (C_6-C_7)cycloalkyl optionally substituted by one to 3 (C_1-C_5) alkyl; (12) 2-(2-furyl)ethyl, (13) 2-(3-thienyl)ethoxy, or (14) 3-thienyloxymethyl; wherein M_1 is α-OH:β-R_5 or α-R_5:β-OH or α-OR_1:β-R_5 or α-R_5:β-OR_1, wherein R_5 is hydrogen or methyl and R_1 is an alcohol protecting group; and wherein L_1 is α-R_3:β-R_4, α-R_4:β-R_3, or a mixture of α-R_3:β-R_4 and α-R_4:β-R_3, wherein R_3 and R_4 are hydrogen, methyl, or fluoro, being the same or different, with the proviso that one of R_3 and R_4 is fluoro only when the other is hydrogen or fluoro.	
4	A steroselectively produced isomeric compound in pharmacologically acceptable salt form according to the following formula:	Claim 4 is a product-by-process claim directed specifically to treprostinil. If the claim is a product-by-process claim, the focus of the anticipation analysis is the product produced by

	'117 Patent Claim Language	Invalidity Contentions
	 that is produced by process for making 9-deoxy-PGF_1 -type compounds, the process comprising cyclizing a starting compound of the formula:  into a compound of the following formula:  by intramolecular cyclization of the enyne, wherein Z is O, S, CH_2, or NR_8 in which R_8 is H, alkyl or aryl; X is H, CN, OR_9, or $COOR_9$ in which R_9 is a pharmacologically acceptable cation; wherein n is 0, 1, 2, or 3; wherein Y_1 is trans-CH=CH-, cis-CH=CH-, $-CH_2(CH_2)_m-$, or -C=C-; m is 1,2, or 3; wherein R_1 is an alcohol protecting group; wherein R_7 is (1) $-C_p H_{2p}-CH_3$, wherein p is an integer from one to 5, inclusive, (2) phenoxy optionally substituted by one, two or three chloro, fluoro, trifluoromethyl, (C_1 - C_3)alkyl, or (C_1-C_3)alkoxy, with the proviso that not more than two substituents are other than alkyl, with the proviso that R_7 is phenoxy or substituted phenoxy, only when R_3 and R_4 are hydrogen or methyl, being	the claimed process. *Amgen Inc. v. F. Hoffmann-La Roche, Ltd.*, 580 F.3d 1340, 1369-70 (Fed. Cir. 2009). The prior art does not need to teach the process limitations so long as the prior art product is the same as the claimed product. *Id.* The prior art disclosed the same product, treprostinil, and the pharmacologically acceptable salt form of treprostinil, as the claimed product and thus anticipates the claim. The '814 Patent col. 29, line 12-col. 33, line 4. Even if not anticipated, it would be obvious to a person of ordinary skill in the art to apply a standard purification technique such as recrystallization to arrive at the claimed treprostinil product. *See e.g.* Monson at 178-188 (disclosing standard purification methods, as discussed in more detail above) and Harwood at 127-134 (disclosing purifications methods known at the time, as discussed in more detail above).

	'117 Patent Claim Language	Invalidity Contentions
	the same or different, (3) phenyl ,benzyl, phenylethyl, or phenylpropyl optionally substituted on the aromatic ring by one, tow or three chloro, fluoro, tribluoromethyl[sic], (C_1-C_3)alkyl, or (C_1 -C_3)alkoyx, with the proviso that not more than two substituents are other than alkyl, (4) cis-CH=CH-CH_2-CH_3, (5) -$(CH_2)_2$-CH(OH)-CH_3, or (6) -$(CH_2)_3$-CH=C$(CH_3)_2$; wherein -C(L_1)-R_7 taken together is (1) (C_4-C_7)cycloalkyl optionally substituted by one to 3 (C_1-C_5) alkyl; (2) 2-(2-furyl)ethyl, (3) 2-(3-thienyl)ethoxy, or (4) 3-thienyloxymethyl; wherein M_1 is α-OH:β-R_4 or α-R_5:β-OH or α-OR_1:β-R_5 or α-R_5:β-OR_1, wherein R_5 is hydrogen or methyl and R_1 is an alcohol protecting group; and wherein L_1 is α-R_3:β-R_4, α-R_4:β-R_3, or a mixture of α-R_3:β-R_4 and α-R_4:β-R_3, wherein R_3 and R_4 are kydrogen[sic], methyl, or fluoro, being the same or different, with the proviso that one of R_3 and R_4 is fluoro only when the other is hydrogen or fluoro.	

B. Invalidity of United States Patent No. 8,497,393

United States Patent No. 8,497,393, entitled "Process to prepare treprostinil, the active ingredient in Remodulin®," was issued on Jul 30, 2013 with 22 claims. UTC asserts that Teva infringes claims 1-22 of the '393 patent. The '393 patent contains product-by-process claims that cover making treprostinil. "In determining infringement of a product-by-process claim, . . . the focus is on the process of making the product as much as it is on the product itself." *Amgen Inc. v. F. Hoffmann-La Roche, Ltd.*, 580 F.3d 1340, 1369-70 (Fed. Cir. 2009). As explained

below, Teva hereby contends that all claims (claims 1-22) of the '393 patent are invalid as anticipated or obvious.

1. Claims 1-22 Of The '393 Patent Are Anticipated by U.S. Patent No. 6,765,117, Moriarty 2004, or Remodulin®

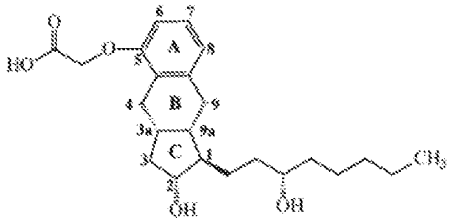
Claims 1–22 of the '393 patent are invalid as anticipated by U.S. Patent No. 6,765,117, Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil) J. Org. Chemistry. 2004, 69(6), 1890-1902 ("Moriarty 2004"), or Plaintiff's own Remodulin® (first approved in 2002).

Claims 1-22 are product-by-process claims directed to treprostinil or its pharmaceutically acceptable salt. The claimed process contains an alkylation of triol compound to a benzindene nitrile compound, hydrolysis of the nitrile compound, formation of a salt using "a base B," and optionally reacting the salt with an acid to form treprostinil. If the claim is a product-by-process claim, the focus of the anticipation analysis is the product produced by the claimed process. *Amgen Inc. v. F. Hoffmann-La Roche, Ltd.*, 580 F.3d 1340, 1369-70 (Fed. Cir. 2009). The prior art does not need to teach the process limitations so long as the prior art product is the same as the claimed product. *Id.* As explained in further detail below, the prior art discloses the same product, treprostinil, or its pharmaceutically acceptable salt, as the claimed product and thus anticipates the claims.

U.S. Patent No. 6,765,117, Moriarty 2004, and Remodulin® are 102(b) references to the '393 patent. As described in more detail above, the '117 patent is listed in the Orange Book as covering Remodulin® (treprostinil) and claims the same compound and its salt form as the '393

patent. Col. 20, line 10-col. 21, line 12.[5] As the applicants concede, treprostinil, the claimed product and active ingredient in Remodulin®, was well known and "was first described in U.S. Pat. No. 4,306,075." '393 patent, col. 1, lines 22-28. Indeed, "[t]reprostinil, and other prostacyclin derivatives have been prepared as described in Moriarty, et al in J. Org. Chem. 2004, 69, 1890-1902 …, 6,765,117 and 6,809,223." *Id.*

Similar to the disclosures of the '117 patent, Moriarty 2004 discloses compound 7 (page 1892), the same compound that falls within the claimed compound for all of the claims.



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Moriarty 2004 discloses an improved "route for synthesis and subsequent manufacture of a complex drug substance on a multikilogram scale." Moriarty 2004 at Abstract. Other than claims 2 and 10, there are no purity requirements in the asserted claims, and thus cannot be used to distinguish the prior art. *See Cubist Pharm., Inc. v. Hospira, Inc.*, No. CV 12-367-GMS, 2014 WL 6968046, at *19-20 (D. Del. Dec. 8, 2014). Claims 2 and 10 require a purity of the product of at least 99.5%. Moriarty 2004 discloses that the compound is produced with 99.7% purity (page 1902) and thus anticipates the claims.

Treprostinil that was used in UTC's commercial embodiment Remodulin®, first approved and marketed in 2002, with all its attributes and inherent qualities, also anticipates the '393 patent. Remodulin® was approved in 2002 and was publicly available at least 1 year prior

[5] *See also* Phares 2005 reference, where Phares discloses the claimed compound in at least two salt forms and further discloses that the sodium salt of the compound is sold as Remodulin® which is an FDA approved treatment. Paragraph [0051].

to the application of the '393 patent. *See, e.g.*, U.S. Patent Publication No. 2005/0085540 by Phares et al. ("Phares 2005") (TEVA_TRE_0004143-206) (disclosing the availability of treprostinil sodium (Remodulin®). [0004]); *see also* U.S. Patent Publication No. 2005/0165110 (July 2005), [0021, 0024] (disclosing treprostinil used in Remodulin® and its salt forms). Therefore, Remodulin® anticipated the '393 patent.

Claim 22 recited the limitation "[t]he product of claim 21 wherein the product comprises a pharmaceutically acceptable salt formed from the product of step (d). Page 1902 of Moriarty 2004 discloses that, "[c]ompound 7 was identical in all respects to an authentic sample of UT-15" and as disclosed on page 1890, UT-15 is Remodulin (Treprostinil Sodium). The '117 patent discloses the claimed compound in salt form. Col. 20, line 10-col. 21, line 12. Phares 2005 discloses the claimed compound in at least two salt forms and further discloses that the sodium salt of the compound is sold as Remodulin® which is an FDA-approved treatment. Paragraph [0051]. Thus, each of these prior art references anticipated claim 22.

2. Claims 1-22 Of The '393 Patent Are Obvious In View Of Remodulin®, '117 patent, And/Or Moriarty 2004 Over Monson (1971), Eliel (1994), Jones (1971 or 2000) and/or Wade 2005 In View Of The Knowledge Of One Of Ordinary Skill In The Art.

If the Court concludes that claims 1-22 of the '393 patent are not anticipated, claims 1-22 of the '393 patent are invalid as obvious to a person of ordinary skill in the art in view of the prior art— Remodulin®, '117 patent, and/or Moriarty 2004 over Monson (1971), Eliel (1994), Jones (1971 or 2000) and/or Wade 2005 in view of the knowledge of one of ordinary skill in the art. Claims 1-22 are product-by-process claims directed to treprostinil or its pharmaceutically acceptable salt. The claimed process contains an alkylation of triol compound to a benzindene nitrile compound, hydrolysis of the nitrile compound, formation of a salt using "a base B," and optionally reacting the salt with an acid to form treprostinil. If the claim is a product-by-process

claim, the focus of the invalidity analysis is the product produced by the claimed process. *Amgen Inc. v. F. Hoffmann-La Roche, Ltd.*, 580 F.3d 1340, 1369-70 (Fed. Cir. 2009). The prior art does not need to teach the process limitations so long as "the product in a product-by-process claim is the same as or obvious from a product of the prior art." *Id.* at 1366. Even if the Court rules that the claims of the '393 patent are not anticipated, the prior art disclosed obvious variations of the same product, treprostinil and the pharmacologically acceptable salt form of treprostinil, as the claimed product.

As disclosed in the anticipation section above, treprostinil and its pharmaceutically acceptable salts as claimed in the '393 patent were well-known in the art at the time. *See* Remodulin® product, the '117 patent, col. 20, line 10-col. 21, line 12; Moriarty 2004 page 1892 compound 7, page 1902; Phares 2005 Paragraph [0051]. In fact, the '393 patent incorporates Moriarity 2004 and the '117 patent, among other prior art, that describe purified treprostinil. Col. 1, lines 20-28. The prior art shows that it was well known to synthesize treprostinil via alkylation of benzindene triol and the hydrolysis of benzindene nitrile. *See* the '117 patent col. 20, line 10-col. 21, line 12; Moriarty 2004 page 1892 compound 7, page 1902. Such alkylation reactions adding $ClCH_2CN$ and then subsequent hydrolysis to the carboxylic acid were well-known in the art. *See, e.g.*, Lin at page 5595; Aristoff at page 7971; McManus at pages 1465-1467.

The prior art disclosed that synthesis of treprostinil utilizes purification by column chromatography. *See* the '117 Patent Col. 20, line 10-col. 21, Line 12; Moriarty 2004 page 1892 compound 7, page 1902. The prior art further taught that purification by chromatography is not favored for large-scale industrial production. *See* Monson page 185; Arumugam page 319; Yu page 832. The use of crystallization and recrystallization as a purification technique was well-

known. *See e.g.* Monson pages 181-183; Harwood pages 127-134; Pavia, Introduction To Organic Laboratory Techniques, at page 648 (1998). In fact, it has been known since at least 1853 from the work of Louis Pasteur that carboxylate ammonium salts are formed from adding a carboxylic acid with an amine and that those salts can be purified by recrystallization. *See* Eliel page 322; *see also* Jones pages 153-155; Sorrell, Organic Chemistry, at pages 755-758 (1999). Additionally, carboxylate ammonium salts are very common and well known for use in drugs and drug targets, including diethanolamine salts. *See e.g.*, Priscinzano, Piperidine Analogues of 1-[2-[Bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine (GBR 12909): High Affinity Ligands for the Dopamine Transporter, J. Med. Chem., 45: 4371-4374, at pages 4371-4374 (2002); Ohno, Development of Dual-Acting Benzofurans for Thromboxane A2 Receptor Antagonist and Prostacyclin Receptor Agonist: Synthesis, Structure-Activity Relationship, and Evaluation of Benzofuran Derivatives, J. Med. Chem., 48:5279-5294, at pages pages 5279-5294, compound 7 (2005); Burk, An Enantioselective Synthesis of (S)-(+)-3-Aminomethyl-5-methylhexanoic Acid via Asymmetric Hydrogenation, J. Org. Chem., 68:5731-5734, at pages 5731-5734 (2003); The 2005 Physicians' Desk Reference for Bicillin® L-A (penicillin G benzathine suspension).

The prior art also disclosed that treprostinil can be crystallized and the diethanolamine salt of treprostinil is particularly preferred. *See* Phares 2005 paragraph [00051], figures 15-22; Moriarty 2004 page 1892 compound 7, page 1902. The prior art also disclosed that other physiologically acceptable salts of treprostinil include salts derived from bases, such as ammonia, N-methyl-D-glucamine, magnesium, arginine and lysine. *See* Wade 2005 paragraph [0024]. It was also known in the art that salts of treprostinil could be reacted with diluted HCl to form treprostinil. *See* the '117 Patent col. 20, line 10-col. 21, line 12; Moriarty 2004 page 1892

compound 7, page 1902. In view of the known fact that purification by chromatography is not favored for large-scale industrial production, a POSA would have been motivated to address the problem by applying an obvious form of purification, salt crystallization, to form known salt forms of treprostinil.

As discussed below in detail, each step of independent claims 1 and 9 were known and disclosed in the prior art, and it would have been obvious to a person of ordinary skill in the art to combine known and standard steps disclosed in the prior art.

Step (a) – Alkylation: The prior art discloses alkylation of benzindene triol with an alkylating agent to produce benzedine nitrile. *See* the '117 Patent col. 20, line 10-col. 21, line 12; Moriarty 2004 page 1892 compound 7, page 1902. Such alkylation reactions adding $ClCH_2CN$ for subsequent hydrolysis to the carboxylic acid were well-known in the art. *See e.g.* Lin page 5595; Aristoff page 7971; McManus pages 1465-1467.

Step (b) – Hydrolyzation: The prior art discloses the hydrolysis of benzindene nitrile. *See* the '117 patent col. 20, line 10-col. 21, line 12; Moriarty 2004 page 1892 compound 7, page 1902. Such alkylation reactions adding $ClCH_2CN$ and then subsequent hydrolysis to the carboxylic acid compound were well-known in the art. *See e.g.* Lin page 5595; Aristoff page 7971; McManus pages 1465-1467.

Step (c) – formation of salt with base B: the prior art discloses that synthesis of treprostinil. The prior art further describes the well-known technique of purification by crystallization or recrystallization. *See, e.g.*, Monson pages 181-183; Harwood pages 127-134; Pavia reference page 648. In fact, it has been known since at least 1853 from the work of Louis Pasteur that carboxylate ammonium salts are formed from adding a carboxylic acid with an amine and that those salts can be purified by recrystallization. *See* Eliel page 322; *see also* Jones

pages 153-155; Sorrell pages 755-757. Additionally, carboxylate ammonium salts are very common and well known for use in drugs and drug targets, including diethanolamine salts. *See, e.g.*, Priscinzano pages 4371-4374; Ohno pages 5279-5294, compound 7; Burk pages 5731-34; PDR 2005 Bicillin® L-A. Moreover, the prior art disclosed that treprostinil can be crystallized and the diethanolamine salt of treprostinil is particularly preferred. *See* Phares 2005 paragraph [00051], figures 15-22; Moriarty 2004 page 1892 compound 7, page 1902. The prior art also disclosed that other physiologically acceptable salts of treprostinil include salts derived from bases, such as ammonia, N-methyl-D-glucamine, magnesium, arginine and lysine. *See* Wade 2005 paragraph [0024]. A POSA would have also have known that purification by column chromatography is disfavored for large-scale industrial production. *See* Monson page 185; Arumugam page 319; Yu page 832. Consequently, a person of ordinary skill in the art would have been motivated to apply an obvious and well-known procedure to purify a known compound synthesized by a known procedure.

Step (d) – optional reaction of the salt with acid to form the neutral compound: step d is optional, but the prior teaches that it was also known that salts of treprostinil could be reacted with diluted HCl acid to form treprostinil. *See* the '117 patent col. 20, line 10-col. 21, line 12; Moriarty 2004 page 1892 compound 7, page 1902. Therefore, it would have been obvious to react the salt formed during the crystallization step with an acid to get treprostinil.

Dependent claims 2 and 10 claim the product of claims 1 and 9, respectively, wherein the purity of compound is at least 99.5%. This claim are rendered obvious for the same reasons as above. Additionally, Moriarty 2004 discloses 99.7% purity for treprostinil. Page 1902.

Dependent claim 3 claims the product of claim 1, wherein the alkylating agent is $Cl(CH_2)_wCN$, $Br(CH_2)_wCN$, or $I(CH_2)_wCN$. This claim is rendered obvious for the same reasons

as above. Additionally, the prior art discloses that the alkylating agent is $Cl(CH_2)CN$. The '117 patent col. 20, line 10-col. 21, line 12; Moriarty 2004 page 1892 compound 7, page 1902.

Dependent claim 4 claims the product of claim 1, wherein the base in step (b) is KOH or NaOH. This claim is rendered obvious for the same reasons as above. Additionally, the prior art discloses that base in step (b) is KOH. The '117 patent col. 20, line 10-col. 21, line 12; Moriarty 2004 page 1892 compound 7, page 1902.

Dependent claim 5 claims the product of claim 1, wherein the base B in step (c) is selected from the group consisting of ammonia, N-methylglucamine, procaine, tromethanine, magnesium, L-lysine, L-arginine, triethanolamine, and diethanolamine. This claim is rendered obvious for the same reasons as above. Also, the claim includes common bases, and it would have been a matter of routine experimentation for a POSA to choose the bases included, and in particular the prior art specifically discloses that physiologically acceptable salts of treprostinil include salts derived from these bases. *See* Wade 2005 paragraph [0024]. Furthermore, the prior art also disclosed that treprostinil can be crystallized and the diethanolamine salt of treprostinil is particularly preferred. *See* Phares 2005 paragraph [0051]. Thus, it would have been obvious for a POSA to choose a base that was already known to form a salt with treprostinil.

Dependent claim 6 claims the product of claim 1, wherein the acid in step (d) is HCl or H_2SO_4. This claim is rendered obvious for the same reasons as above. Additionally, the prior art discloses salts of treprostinil could be reacted with diluted HCl to form treprostinil. *See* the '117 patent col. 20, line 10-col. 21, line 12; Moriarty 2004 page 1892 compound 7, page 1902. Therefore, it would have been obvious to react the salt formed during the crystallization step with diluted HCl to get treprostinil.

Dependent claim 7 claims the product of claim 1, wherein Y_1 is —CH_2CH_2—; M_1 is α-OH:β-H or α-H:β-OH; —$C(L_1)$-R_7 taken together is —$(CH_2)_4CH_3$; and w is 1. This claim is rendered obvious for the same reasons as above.

Dependent claim 8 claims the product of claim 1, wherein the process does not include purifying the compound of formula (III) produced in step (a). This claim is rendered obvious for the same reasons as above.

Dependent claim 11 claims the product of claim 9, wherein the alkylating agent is $ClCH_2CN$. This claim is rendered obvious for the same reasons as above. Additionally, the prior art discloses that the alkylating agent is $ClCH_2CN$. The '117 patent col. 20, line 10-col. 21, line 12; Moriarty 2004 page 1892 compound 7, page 1902.

Dependent claim 12 claims the product of claim 9, wherein the base in step (b) is KOH. This claim is rendered obvious for the same reasons as above. Additionally, the prior art discloses that base in step (b) is KOH. The '117 patent col. 20, line 10-col. 21, line 12; Moriarty 2004 page 1892 compound 7, page 1902.

Dependent claim 13 claims the product of claim 9, wherein the base B in step (c) is selected from a group consisting of ammonia, N-methylglucamine, procaine, tromethanine, magnesium, L-lysine, L-arginine, triethanolamine, and diethanolamine. This claim is rendered obvious for the same reasons as above. Also, the claim includes common bases, and it would have been a matter of routine experimentation for a POSA to choose the bases included, and in particular the prior art specifically discloses that physiologically acceptable salts of treprostinil include salts derived from these bases. *See* Wade 2005 paragraph [0024]. Furthermore, the prior art also disclosed that treprostinil can be crystallized and the diethanolamine salt of treprostinil is

particularly preferred. *See* Phares 2005 paragraph [00051]. Thus, it would have been obvious for a POSA to choose a base that was already known to form a salt with treprostinil.

Claim 14 claims the product of claim 9, wherein the base B is diethanolamine. This claim is rendered obvious for the same reasons as above. The prior art also disclosed that treprostinil can be crystallized and the diethanolamine salt of treprostinil is particularly preferred. *See* Phares 2005 paragraph [00051]. Thus, it would have been obvious for a POSA to choose a base that was already known to form a salt with treprostinil.

Claim 15 claims the product of claim 9, wherein the acid in step (d) is HCl. This claim is rendered obvious for the same reasons as above. Additionally the prior art discloses salts of treprostinil could be reacted with diluted HCl to form treprostinil. The '117 patent col. 20, line 10-col. 21, line 12; Moriarty 2004 page 1892 compound 7, page 1902. Therefore, it would have been obvious to react the salt formed during the crystallization step with diluted HCl to get treprostinil.

Dependent claim 16 claims the product of claim 9, wherein the process does not include purifying the compound of formula (VI) produced in step (a). This claim is rendered obvious for the same reasons as above.

Dependent claim 17 claims the product of claim 16, wherein the base B in step (c) is selected from a group consisting of ammonia, N-methylglucamine, procaine, tromethanine, magnesium, L-lysine, L-arginine, triethanolamine, and diethanolamine. This claim is rendered obvious for the same reasons as above. Also, the claim includes common bases, and it would have been a matter of routine experimentation for a POSA to choose the bases included and in particular the prior art specifically discloses that physiologically acceptable salts of treprostinil include salts derived from these bases. *See* Wade 2005 paragraph [0024]. Furthermore, the prior

art also disclosed that treprostinil can be crystallized and the diethanolamine salt of treprostinil is particularly preferred. *See* Phares 2005 paragraph [00051]. Thus, it would have been obvious for a POSA to choose a base that was already known to form a salt with treprostinil.

Dependent claim 18 claims the product of claim 17, wherein the base B is diethanolamine. This claim is rendered obvious for the same reasons as above. The prior art also disclosed that treprostinil can be crystallized and the diethanolamine salt of treprostinil is particularly preferred. *See* Phares 2005 paragraph [00051]. Thus, it would have been obvious for a POSA to choose a base that was already known to form a salt with treprostinil.

Dependent claim 19 claims the product of claim 1, wherein the base in step (b) is KOH or NaOH and wherein the base 13 in step (c) is selected from the group consisting of ammonia. N-methyl glucamine, procaine, tromethanine, magnesium, L-lysine, L-arginine, triethanolamine, and diethanolamine. This claim is rendered obvious for the same reasons as above.

Dependent claim 20 claims the product of claim 9, wherein the base in step (b) is KOH or NaOH and wherein the base B in step (c) is selected from the group consisting of ammonia, N-methylglucamine, procaine, tromethanine, magnesium, L-lysine, L-arginine, triethanolamine, and diethanolamine. This claim is rendered obvious for the same reasons as above.

Dependent claim 21 claims the product of claim 1, wherein step (d) is performed. This claim is rendered obvious for the same reasons as above.

Dependent claims 22 claims the product of claim 21, wherein the product comprises a pharmaceutically acceptable salt formed from the product of step (d). This claim is rendered obvious for the same reasons as above. Additionally, the Moriarty 2004, on page 1902 discloses that, "[c]ompound 7 was identical in all respects to an authentic sample of UT-15" and as disclosed on page 1890, UT-15 is Remodulin (Treprostinil Sodium). The '117 patent discloses

the claimed compound in salt form. Col. 20, line 10-col. 21, line 12. The Phares 2005 discloses the claimed compound in at least two salt forms and further discloses that the sodium salt of the compound is sold as Remodulin® which is an FDA approved treatment. Paragraph [0051].

No evidence of secondary considerations of non-obviousness were presented during the prosecution of the '393 patent, and Teva is not aware of any such secondary considerations that, when considered with the evidence of obviousness, would warrant a finding of non-obviousness of the claims of the '393 patent. If UTC relies on any secondary considerations of non-obviousness, Teva reserves the right to supplement its contentions.

As explained above, the claims would have been obvious in view of a host of prior art references because they are examples that show the steps in the claims are well-known procedures that would have been obvious to apply. Consequently, there are numerous different combinations of these prior art references as there are many exemplary references for each standard step. By way of example, Moriarty 2004 in view of Monson, Eliel, and Phares 2005.

By way of another example, '117 Patent in view of Monson, Jones, and Wade 2005. These are only two examples that support these Teva's invalidity defense, and Teva reserves the right to set forth examples such as discovery continues.

3. The '393 Patent Is Invalid For Obviousness Type Double Patenting Over the '117 Patent

The '393 patent is invalid for obviousness-type double patenting over the '117 patent. The doctrine of obviousness-type double patenting forbids obtaining more than one patent on the same invention, and is grounded in Section 101 of the Patent Act. 35 U.S.C. § 101 ("Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, . . . may obtain a patent therefor."); *see also In re Longi*, 759 F.2d 887, 892 (Fed. Cir. 1985); *Boehringer Ingelheim Int'l. GmbH v. Barr Labs., Inc.*, 592 F.3d 1340, 1346 (Fed. Cir.

2010); *Eli Lilly & Co. v. Barr Labs., Inc.*, 251 F.3d 955, 967 (Fed. Cir. 2001). Through judicial interpretation, "this prohibition has been extended to preclude a second patent on an invention which 'would have been obvious from the subject matter of the claims in the first patent, in light of the prior art.'" *Ortho Pharm. Corp. v. Smith*, 959 F.2d 936, 940 (Fed. Cir. 1992) (quoting In re Longi, 759 F.2d at 893). Accordingly, a claim in an issued patent that is not "patentably distinct" from an earlier issued claim in a separate patent is invalid for non-statutory double patenting, so long as the patents have at least one common inventor. *E.g., Eli Lilly*, 251 F.3d at 970-71; *Geneva Pharms. Inc. v. GlaxoSmithKline PLC*, 349 F.3d 1373, 1377-78 (Fed. Cir. 2003); *see also In re Hubbell*, 709 F.3d 1140, 1145-46 (Fed. Cir. 2013) (requiring only an "overlap in the inventors," not "identity of inventors"); *In re Longi*, 759 F.2d at 892.

An obviousness-type double patenting analysis begins by comparing the invention defined by the properly construed claims of the earlier-expiring patent (the "reference claims") with the claims of the later-expiring patent in a manner analogous to an anticipation analysis under 35 U.S.C. § 102 or an obviousness analysis under 35 U.S.C. § 103, except that the reference claims rather than the patent disclosure are the subject of the comparison. *See In re Braithwaite*, 379 F.2d 594, 597 n.4 (C.C.P.A. 1967). A later-expiring claim is invalid where the alleged invention "would have been obvious to those of ordinary skill in the art at the time the invention was made, taking into account the skill of the art and prior art other than the invention claimed in the [reference] patent." *In re Longi*, 759 F.2d at 892 (quoting In re Zickendraht, 319 F.2d 225, 232 (C.C.P.A. 1963) (Rich, J., concurring)). The supporting patent disclosures may be relevant for interpreting the scope and meaning of the reference and rejected claims. *In re Vogel*, 422 F.2d 438, 441-42; see also ("[T]he patent disclosure may . . . be used as a dictionary to learn the meaning of terms in a claim"); *see also Eli Lilly & Co. v. Teva Parenteral Medicines, Inc.*,

689 F.3d 1368, 1378-79 (Fed. Cir. 2012); *In re Avery*, 518 F.2d 1228, 1232 (C.C.P.A. 1975); *In re Zickendraht*, 319 F.2d at 228.

Here, the '117 patent and '393 patent share at least one common inventor (Raju Penmasta) and the same owner (United Therapeutics Corporation). The claims of the '117 patent are directed to the same subject matter, treprostinil and its pharmacologically acceptable salt form. *See* '117 patent, claims 1-4. There should be no dispute that the claims of the '393 patent, like the claims of the '117 patent, also are directed to the product treprostinil and its pharmacologically acceptable salt form. *See* '393 patent, claims 1-22. Any limitations not expressly claimed in the '117 patent would have been either inherent in the claims of the '117 patent or obvious to those of ordinary skill in the art at the time the invention was made, taking into account the skill of the person of ordinary skill in the art and prior art. Therefore, for the reasons explained in more detail above in the anticipation and obviousness analysis, the '393 patent is invalid for obviousness type double patenting over the '117 patent.

4. Claims 1-13 and 15-22 Of The '393 Patent Are Not Enabled Or Fail To Meet The Written Description Requirement

As discussed in the previous sections, it would have been obvious for a person of ordinary skill in the art to practice the claimed invention by applying known procedures described in the prior art. But if Plaintiff contends that it would have required undue experimentation for a person of ordinary skill to apply these prior art procedures to obtain the claimed methods (for example it would have required undue experimentation to find particular bases or a particular alkylating agent), the claims are not enabled. Such a contention by Plaintiff would not be supported by the specification or the prosecution history, and to the extent that Plaintiff contends that certain bases or reaction conditions, for example, are unique and that undue experimentation would have been required to practice the claimed method, then the claims

of the '393 patent are not enabled or fail to meet the written description requirement. Moreover, to the extent that Plaintiff takes a broad claim construction position and asserts infringement of certain processes and resulting intermediates—such as the use of intermediates or processes that are not sufficiently disclosed, taught or claimed in the '393 patent, including the intermediates and processes that are used to make Teva's treprostinil, the claims of the '393 patent are not enabled and/or lack written description.

As the following table shows, claims 1–22 of the '393 patent are invalid as anticipated or obvious in view of at least the following prior art references, which are exemplary of the state of the art at the time of the filing of the '393 patent.

- Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil) J. Org. Chemistry. 2004, 69(6), 1890-1902 ("Moriarty 2004") (TEVA_TRE_0004121-34)
- Lin et al., Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Org. Chemistry, 1987,52,5594-5601 ("Lin") (TEVA_TRE_0004096-103)
- Aristoff et al., Total Synthesis of a Novel Antiulcer Agent via a Modification of the Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Am. Chem. SOC. 1985, 107, 7967-7974 ("Aristoff 1985") (TEVA_TRE_003975-3982)
- McManus et al., Tetrazole Analogs of Plant Auxins, J. Org. Chemistry. 1959, 24, 1464-1467 ("McManus") (TEVA_TRE_0004104-7)
- U.S. Patent Publication No. 2005/0085540 April 2005, Phares et al. ("Phares 2005") (TEVA_TRE_0004143-206)
- U.S. Patent Publication No. 2005/0165110 July 2005, Wade et al. ("Wade 2005") (TEVA_TRE_0004213-218)
- U.S. Patent No. 6,765,117, July 2004, Moriarty et al. ("the '117 Patent") (TEVA_TRE_0004250-62)
- Arumugan et al., A New Purification Process for Pharmaceutical and Chemical Industries, Organic Process Research & Development 2005, 9, 319-320 ("Arumugan") (TEVA_TRE_0003983-4)

- Yu et al., Novel Synthetic Route of a Pivotal Intermediate for the Synthesis of 1â-Methyl Carbapenem Antibiotics, Organic Process Research & Development 2006, 10, 829-832 ("Yu") (TEVA_TRE_0004263-6)

- Monson, ADVANCED ORGANIC SYNTHESIS, METHODS AND TECHNIQUES, 178-188 (1971) ("Monson") (TEVA_TRE_0004108-120)

- Harwood, *Experimental organic chemistry: Principles and Practice,* 127-134 (1989) ("Harwood") (TEVA_TRE_0004307-317)

- Eliel, STEREOCHEMISTRY OF ORGANIC COMPOUNDS, 322-325 (1994) ("Eliel") (TEVA_TRE_0003985-90)

- Jones, ORGANIC CHEMISTRY, 153-155 (2nd ed. 2000) ("Jones") (TEVA_TRE_0004091-95)

- Sorrell, ORGANIC CHEMISTRY, 755-758 (1999) ("Sorrell") (TEVA_TRE_0004207-212)

- Pavia, INTRODUCTION TO ORGANIC LABORATORY TECHNIQUES, 648 (1998) ("Pavia") (TEVA_TRE_0004135-37)

- Priscinzano, Piperidine Analogues of 1-[2-[Bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine (GBR 12909): High Affinity Ligands for the Dopamine Transporter, J. Med. Chem. 2002, 45, 4371-4374 ("Priscinzano") (TEVA_TRE_0004067-70)

- Ohno, Development of Dual-Acting Benzofurans for Thromboxane A2 Receptor Antagonist and Prostacyclin Receptor Agonist: Synthesis, Structure-Activity Relationship, and Evaluation of Benzofuran Derivatives, J. Med. Chem. 2005, 48, 5279-5294 ("Ohno") (TEVA_TRE_0004071-86)

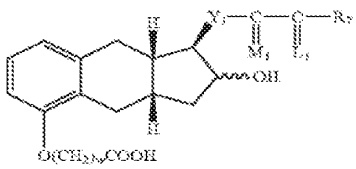
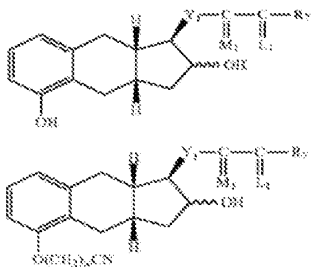
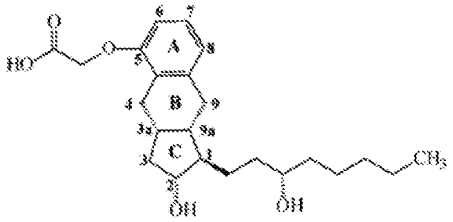
- Burk, An Enantioselective Synthesis of (S)-(+)-3-Aminomethyl-5-methylhexanoic Acid via Asymmetric Hydrogenation, J. Org. Chem. 2003, 68, 5731-5734 ("Burk") (TEVA_TRE_0004087-90)

- The 2005 Physicians' Desk Reference for Bicillin® L-A (penicillin G benzathine suspension) ("PDR 2005 Bicillin® L-A") (TEVA_TRE_0004138-42)

- The references cited or disclosed during prosecution of the '393 patent

Teva expressly reserves the right to modify and/or supplement the above list at any time as necessary and/or as discovery progresses. The following chart incorporates the analysis set

forth above and identifies where specifically in each alleged item of prior art each limitation of each asserted claim is found:

	'393 Patent Claim Language	Invalidity Contentions
1	A product comprising a compound of formula I  or a pharmaceutically acceptable salt thereof, wherein said product is prepared by a process comprising (a) alkylating a compound of structure II with an alkylating agent to produce a compound of formula III,  wherein w=1, 2, or 3; Y_1 is trans-CH=CH—, cis-CH=CH—, —$CH_2(CH_2)_m$—, or —C≡C—; m is 1, 2, or 3; R_7 is (1) —C_pH_{2p}—CH_3, wherein p is an integer from 1 to 5,	**Anticipation:** Claim 1 of the '393 patent is directed to a genus of compounds that include treprostinil. Claim 1 is invalid as anticipated by U.S. Patent No. 6,765,117, Moriarty et al., The Intramolecular Asymmetric Pauson-Khan Cyclization as a Novel and General Steroselective Route to Benzidindene Protacyclins: Synthesis of UT-15 (Treprostinil) J. Org. Chemistry. 2004, 69(6), 1890-1902 ("Moriarty 2004"), or UTC's commercially available drug Remodulin® (treprostinil). U.S. Patent No. 6,765,117, Moriarty 2004, and Remodulin® are 102(b) references to the '393 patent. The '117 patent is listed in the Orange Book as covering Remodulin® and claims treprostinil and its salt form. Col. 20, line 10-col. 21, line 12.[6] As the applicants concede, treprostinil, the claimed product and active ingredient in Remodulin®, was a known compound that "was first described in U.S. Pat. No. 4,306,075." '393 patent, col. 1, lines 22-28. Indeed, "[t]reprostinil, and other prostacyclin derivatives have been prepared as described in Moriarty, et al in J. Org. Chem. 2004, 69, 1890-1902 …, 6,765,117 and 6,809,223." *Id.* Moriarty 2004 also discloses treprostinil (compound 7 at page 1892), the same compound that falls within the claimed compound for all of the claims of the '393 patent.  7 Moriarty 2004 discloses an improved "route for synthesis and subsequent manufacture of a complex drug substance

[6] *See also* Phares 2005 reference, where Phares discloses the claimed compound in at least two salt forms and further discloses that the sodium salt of the compound is sold as Remodulin® which is an FDA approved treatment. Paragraph [0051].

	'393 Patent Claim Language	Invalidity Contentions
	inclusive, (2) phenoxy optionally substituted by one, two or three chloro, fluoro, trifluoromethyl, (C_1-C_3) alkyl, or (C_1-C_3)alkoxy, with the proviso that not more than two substituents are other than alkyl, with the proviso that R_7 is phenoxy or substituted phenoxy, only when R_3 and R_4 are hydrogen or methyl, being the same or different, (3) phenyl, benzyl, phenylethyl, or phenylpropyl optionally substituted on the aromatic ring by one, two or three chloro, fluoro, trifluoromethyl, (C_1-C_3)alkyl, or (C_1-C_3)alkoxy, with the proviso that not more than two substituents are other than alkyl, (4) cis-CH=CH—CH_2—CH_3, (5) —$(CH_2)_2$—$CH(OH)$—CH_3, or (6) —$(CH_2)_3$—CH=$C(CH_3)_2$; —$C(L_1)$—R_7 taken together is (1) (C_4-C_7)cycloalkyl optionally substituted by 1 to 3 (C_1-C_5)alkyl; (2) 2-(2-furyl)ethyl, (3) 2-(3-thienyl)ethoxy, or (4) 3-thienyloxymethyl; M_1 is α-OH:β-R_5 or α-R_5β-OH or α-OR_1:β-R_5 or α-R_5:β-OR_2, wherein R_5 is hydrogen or methyl, R_2 is an alcohol protecting group, and L_1 is α-	on a multikilogram scale." Moriarty 2004 at Abstract. There are no purity requirements in claim 1, and thus cannot be used to distinguish the prior art. *See Cubist Pharm., Inc. v. Hospira, Inc.*, No. CV 12-367-GMS, 2014 WL 6968046, at *19-20 (D. Del. Dec. 8, 2014). To the extent that a purity limitation is incorporated into claim 1, Moriarty 2004 discloses that the compound is produced with 99.7% purity (page 1902). Treprostinil that was used in UTC's commercial embodiment Remodulin®, with all its attributes and inherent qualities, also anticipates the '393 patent.[7] Remodulin® was approved in 2002 and was publicly available prior to the application of the '393 patent. *See, e.g.*, U.S. Patent Publication No. 2005/0085540 by Phares et al. ("Phares 2005") (TEVA_TRE_0004143-206) (disclosing the availability of treprostinil sodium (Remodulin®). [0004]); *see also* U.S. Patent Publication No. 2005/0165110 (July 2005), [0021, 0024] (disclosing treprostinil used in Remodulin® and its salt forms). As the Abstract of '393 notes, "[t]his present invention relates to an *improved process* to prepare prostacyclin derivatives," including "treprostinil," which were already known and disclosed in the art. (Emphasis added). Although the '393 patent vaguely asserts that it produces a "better quality" product, there is no evidence that the treprostinil product of the '393 patent is any different than the product that was known, disclosed, and used in the prior art.[8] **Obviousness:** If the Court concludes that claim 1 of the '393 patent is not anticipated, claim 1 is invalid as obvious to a person of ordinary skill in the art in view of the prior art— Remodulin®, '117 patent, and/or Moriarty

[7] *See, e.g., In re Thorpe*, 777 F.2d 695, 698 (Fed. Cir. 1985) ("[E]ven though product-by-process claims are limited by and defined by the process, determination of patentability is based on the product itself. The patentability of a product does not depend on its method of production. If the product in the product-by-process claim is the same as or obvious from a product of the prior art, the claim is unpatentable even though the prior product was made by a different process.").

[8] "Because validity is determined based on the requirements of patentability, a patent is invalid if a product made by the process recited in a product-by-process claim is anticipated by or obvious from prior art products, even if those prior art products are made by different processes." *Amgen Inc. v. F. Hoffman-La Roche Ltd.*, 580 F.3d 1340, 1370 n 14 (Fed. Cir. 2009).

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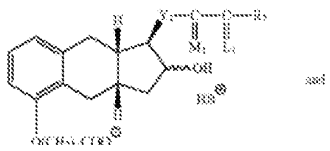
	R_3:β-R_4, α-R_4:β-R_3, or a mixture of α-R_3:β-R_4 and α-R_4:β-R_3, wherein R_3 and R_4 are hydrogen, methyl, or fluoro, being the same or different, with the proviso that one of R_3 and R_4 is fluoro only when the other is hydrogen or fluoro, (b) hydrolyzing the product of formula III of step (a) with a base, (c) contacting the product of step (h) with a base B to form a salt of formula I_s.  (d) optionally reacting the salt formed in step (c) with an acid to form the compound of formula I.	2004 over Monson, ADVANCED ORGANIC SYNTHESIS, METHODS AND TECHNIQUES, 178-188, at pages 181-183, 185 (1971), Eliel, STEREOCHEMISTRY OF ORGANIC COMPOUNDS, 322-325, at page 322 (1994), and U.S. Patent Publication No. 2005/0085540 (April 2005) (Phares 2005), Jones, ORGANIC CHEMISTRY, 153-155 (2nd ed. 2000), Jones, ADVANCED ORGANIC SYNTHESIS, METHODS AND TECHNIQUES, 178-188, at pages 181-183, 185 (1971) and/or U.S. Patent Publication No. 2005/0165110 July 2005, Wade et al. ("Wade 2005") in view of the knowledge of one of ordinary skill in the art. As explained more fully in the paragraphs above, the claims would have been obvious in view of a number of prior art references, because they are examples that show the knowledge of one of ordinary skill in the art at the time, which disclose that treprostinil product that was produced and the steps in the claims were well-known procedures that would have been obvious to apply. Claim 1 is a product by process claim directed to a genus of compounds that include treprostinil or its pharmaceutically acceptable salt. As discussed above in the anticipation section, treprostinil was known and disclosed in the prior art, including in UTC's commercial product Remodulin® (available as of 2002 and was described in numerous publications, including Phares 2005 Paragraph [0051]), the '117 patent at col. 20, line 10-col. 21, line 12 (which claims treprostinil, is listed in the Orange Book for treprostinil and is a patent in suit), and/or Moriarty 2004 page 1892 compound 7, page 1902 (which discloses treprostinil and improved processes for making same). In fact, the '393 patent incorporates Moriarity 2004 and the '117 patent, among other prior art, that describe purified treprostinil. Col. 1, lines 20-28. To the extent that these references do not disclose the compound of the '393 patent, it would have taken only routine experimentation of a person of ordinary skill in the art—in their natural desire to obtain improved versions of treprostinil—to make the product of claim 1. Moreover, the process claimed in claim 1 would have been obvious to a person of ordinary skill in the art. The claimed process contains an alkylation of triol compound to a benzindene nitrile compound, hydrolysis of the nitrile compound, formation of a salt using "a base B,"

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		and optionally reacting the salt with an acid to form treprostinil. The prior art shows that it was well known to synthesize treprostinil via alkylation of a known compound, benzindene triol, and the hydrolysis of the intermediate compound, benzindene nitrile. *See* the '117 patent col. 20, line 10-col. 21, line 12; Moriarty 2004 page 1892 compound 7, page 1902. The process of alkylation reactions adding $ClCH_2CN$ and then subsequent hydrolysis to make a carboxylic acid were well-known in the art. Lin et al., Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Org. Chemistry, 1987,52:5594-5601, at 5594 ("Lin") (TEVA_TRE_0004096-103) (disclosing improved methods of synthesizing benzindene prostaglandins); Aristoff et al., Total Synthesis of a Novel Antiulcer Agent via a Modification of the Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Am. Chem. SOC. 1985, 107:7967-7974, at 7971 ("Aristoff 1985") (TEVA_TRE_003975-3982) (disclosing improved process of making benzindene prostaglandins); and McManus et al., Tetrazole Analogs of Plant Auxins, J. Org. Chemistry. 1959, 24:1464-1467, at 1465-1467 ("McManus") (TEVA_TRE_0004104-7) (disclosing improved synthesis using nitriles). The prior art disclosed that synthesis of treprostinil utilizes purification by column chromatography. *See* the '117 Patent at col. 20, line 10-col. 21, Line 12; Moriarty 2004 at page 1892 compound 7, page 1902. The prior art further taught, however, that purification by chromatography is not favored for large-scale industrial production, and, thus, would have provided those of skill in the art strong motivation to improve upon its process through conventional, known, and routine optimization processes. *See* Monson, ADVANCED ORGANIC SYNTHESIS, METHODS AND TECHNIQUES, 178-188, at pages 181-183, 185 (1971); Arumugan et al., A New Purification Process for Pharmaceutical and Chemical Industries, Organic Process Research & Development 2005, 9, 319-320, at page 319 (2005) ("The separation and purification of organic compounds are very important to chemical and pharmaceutical industries. It is a challenging task to separate a required product from a

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		mixture of components during industrial production. Even though different distillation and recrystallization techniques are widely employed in industries, the application of the above methods are limited and time-consuming, leading to cost escalation. The column chromatographic method, used in some industries, is a process that is too complicated, particularly for large-scale production."); Yu et al., Novel Synthetic Route of a Pivotal Intermediate for the Synthesis of 1â-Methyl Carbapenem Antibiotics, Organic Process Research & Development, 10, 829-832, at page 832 (2006) (disclosing "novel synthetic method" which "requires no chromatographic purifications"). In view of the known fact that treprostinil was already an important commercialized product and purification by chromatography was not favored for large-scale industrial production, a POSA would have been motivated to address the problem by applying an obvious form of purification, salt crystallization, to form known salt forms of treprostinil. The '393 patent acknowledges this: "Because Treprostinil, and other prostacyclin derivatives are of great importance from a medicinal point of view, a need exists for an efficient process to synthesize these compounds on a large scale suitable for commercial production." '393 patent, at col. 1, line 58-61. The use of crystallization and recrystallization as a purification technique was well-known. *See e.g.* Monson at pages 181-183; Harwood at pages 127-134; Pavia, INTRODUCTION TO ORGANIC LABORATORY TECHNIQUES, at 648 (1998) ("Pavia") (TEVA_TRE_0004135-37) (explaining the conventional technique of "crystallization: purification of solids). In fact, it has been known since at least 1853 from the work of Louis Pasteur that carboxylate ammonium salts are formed from adding a carboxylic acid with an amine and that those salts can be purified by recrystallization. *See* Eliel, STEREOCHEMISTRY OF ORGANIC COMPOUNDS, 322-325, at page 322 (1994); *see also* Jones pages 153-155; Sorrell pages 755-757. Additionally, as the following references show, carboxylate ammonium salts are very common and well known for use in drugs and drug targets, including diethanolamine salts. Priscinzano, Piperidine Analogues

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		of 1-[2-[Bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine (GBR 12909): High Affinity Ligands for the Dopamine Transporter, J. Med. Chem. 2002, 45: 4371-4374, at 4371-74 ("Priscinzano") (TEVA_TRE_0004067-70) (; Ohno, Development of Dual-Acting Benzofurans for Thromboxane A2 Receptor Antagonist and Prostacyclin Receptor Agonist: Synthesis, Structure-Activity Relationship, and Evaluation of Benzofuran Derivatives, J. Med. Chem. 2005, 48, 5279-5294 ("Ohno") (TEVA_TRE_0004071-86); Burk, An Enantioselective Synthesis of (S)-(+)-3-Aminomethyl-5-methylhexanoic Acid via Asymmetric Hydrogenation, J. Org. Chem. 2003, 68, 5731-5734 ("Burk") (TEVA_TRE_0004087-90); ; The 2005 Physicians' Desk Reference for Bicillin® L-A (penicillin G benzathine suspension) ("PDR 2005 Bicillin® L-A") (TEVA_TRE_0004138-42). The prior art also disclosed that treprostinil can be crystallized and the diethanolamine salt of treprostinil is particularly preferred. *See* Phares 2005 paragraph [00051], figures 15-22; Moriarty 2004 page 1892 compound 7, at page 1902. The prior art also disclosed that other physiologically acceptable salts of treprostinil include salts derived from bases, such as ammonia, N-methyl-D-glucamine, magnesium, arginine and lysine. *See* Wade 2005 paragraph [0024]. It was also known in the art that salts of treprostinil could be reacted with diluted HCl to form treprostinil. *See* the '117 Patent col. 20, line 10-col. 21, line 12; Moriarty 2004 page 1892 compound 7, page 1902. Moreover, as discussed below in detail, each step of independent claim 1 was known and disclosed in the prior art, and it would have been obvious to a person of ordinary skill in the art to combine known and standard steps disclosed in the prior art. *Step (a) – Alkylation*: The prior art discloses alkylation of benzindene triol with an alkylating agent to produce benzedine nitrile. *See* the '117 Patent col. 20, line 10-col. 21, line 12; Moriarty 2004 page 1892 compound 7, page 1902. Such alkylation reactions adding $ClCH_2CN$ for subsequent hydrolysis to the carboxylic acid were well-known in the art. *See e.g.* Lin et al., Benzindene

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		Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Org. Chemistry, 52:5594-5601, at page 5595 (1987); Aristoff et al., Total Synthesis of a Novel Antiulcer Agent via a Modification of the Intramolecular Wadsworth-Emmons-Wittig Reaction, J. Am. Chem., 107:7967-7974, at page 7971 (1985); McManus et al., Tetrazole Analogs of Plant Auxins, J. Org. Chemistry, 24:1464-1467, at pages 1465-1467 (1959) *Step (b) – Hydrolyzation*: The prior art discloses the hydrolysis of benzindene nitrile. *See* the '117 patent col. 20, line 10-col. 21, line 12; Moriarty 2004 page 1892 compound 7, page 1902. Such alkylation reactions adding $ClCH_2CN$ and then subsequent hydrolysis to the carboxylic acid compound were well-known in the art. *See e.g.* Lin page 5595; Aristoff page 7971; McManus pages 1465-1467. *Step (c) – formation of salt with base B*: the prior art discloses that synthesis of treprostinil. The prior art further describes the well-known technique of purification by crystallization or recrystallization. *See e.g.* Monson pages 181-183; Harwood pages 127-134; Pavia reference page 648. In fact, it has been known since at least 1853 from the work of Louis Pasteur that carboxylate ammonium salts are formed from adding a carboxylic acid with an amine and that those salts can be purified by recrystallization. *See* Eliel at page 322; Jones at pages 153-155; Sorrell at pages 755-757. Additionally, carboxylate ammonium salts are very common and well known for use in drugs and drug targets, including diethanolamine salts. *See, e.g.*, Priscinzano at pages 4371-4374; Ohno at pages 5279-5294, compound 7; Burk at pages 5731-34; PDR 2005 Bicillin® L-A. Moreover, as discussed earlier, the prior art disclosed that treprostinil can be crystallized and the diethanolamine salt of treprostinil is particularly preferred. *See* Phares 2005 paragraph [00051], figures 15-22; Moriarty 2004 page 1892 compound 7, page 1902. The prior art also disclosed that other physiologically acceptable salts of treprostinil include salts derived from bases, such as ammonia, N-methyl-D-glucamine, magnesium, arginine and lysine. *See* Wade 2005

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		paragraph [0024]. A POSA would have also have known that purification by column chromatography is disfavored for large-scale industrial production. *See* Monson page 185; Arumugam page 319; Yu page 832. Therefore, a person of ordinary skill in the art would have been motivated to apply an obvious and well-known procedure to purify a known compound synthesized by a known procedure. *Step (d) – optional reaction of the salt with acid to form the neutral compound*: step d is optional, but the prior art teaches that it was also known that salts of treprostinil could be reacted with diluted HCl acid to form treprostinil. *See* the '117 patent col. 20, line 10-col. 21, line 12; Moriarty 2004 page 1892 compound 7, page 1902. Therefore, it would have been obvious to react the salt formed during the crystallization step with an acid to get treprostinil. No evidence of secondary considerations of non-obviousness were presented during the prosecution of the '393 patent, and Teva is not aware of any such secondary considerations that, when considered with the evidence of obviousness, would warrant a finding of non-obviousness of the claims of the '393 patent. If UTC relies on any secondary considerations of non-obviousness, Teva reserves the right to supplement its contentions. **Obviousness-Type Double Patenting:** The '393 patent also is invalid for obviousness type double patenting over the '117 patent. The doctrine of obviousness-type double patenting forbids obtaining more than one patent on the same invention, and is grounded in Section 101 of the Patent Act. 35 U.S.C. § 101 ("Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, . . . may obtain a patent therefor."); *see also In re Longi*, 759 F.2d 887, 892 (Fed. Cir. 1985); *Boehringer Ingelheim Int'l. GmbH v. Barr Labs., Inc.*, 592 F.3d 1340, 1346 (Fed. Cir. 2010); *Eli Lilly & Co. v. Barr Labs., Inc.*, 251 F.3d 955, 967 (Fed. Cir. 2001). Through judicial interpretation, "this prohibition has been extended to preclude a second patent on an invention which 'would have been obvious from the subject matter of the claims in the first patent, in light of the prior art.'" *Ortho Pharm. Corp. v. Smith*, 959 F.2d

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		936, 940 (Fed. Cir. 1992) (quoting In re Longi, 759 F.2d at 893). Accordingly, a claim in an issued patent that is not "patentably distinct" from an earlier issued claim in a separate patent is invalid for non-statutory double patenting, so long as the patents have at least one common inventor. *E.g.*, *Eli Lilly*, 251 F.3d at 970-71; *Geneva Pharms. Inc. v. GlaxoSmithKline PLC*, 349 F.3d 1373, 1377-78 (Fed. Cir. 2003); *see also In re Hubbell*, 709 F.3d 1140, 1145-46 (Fed. Cir. 2013) (requiring only an "overlap in the inventors," not "identity of inventors"); *In re Longi*, 759 F.2d at 892. An obviousness-type double patenting analysis begins by comparing the invention defined by the properly construed claims of the earlier-expiring patent (the "reference claims") with the claims of the later-expiring patent in a manner analogous to an anticipation analysis under 35 U.S.C. § 102 or an obviousness analysis under 35 U.S.C. § 103, except that the reference claims rather than the patent disclosure are the subject of the comparison. *See In re Braithwaite*, 379 F.2d 594, 597 n.4 (C.C.P.A. 1967). A later-expiring claim is invalid where the alleged invention "would have been obvious to those of ordinary skill in the art at the time the invention was made, taking into account the skill of the art and prior art other than the invention claimed in the [reference] patent." *In re Longi*, 759 F.2d at 892 (quoting In re Zickendraht, 319 F.2d 225, 232 (C.C.P.A. 1963) (Rich, J., concurring)). The supporting patent disclosures may be relevant for interpreting the scope and meaning of the reference and rejected claims. *In re Vogel*, 422 F.2d 438, 441-42; see also ("[T]he patent disclosure may . . . be used as a dictionary to learn the meaning of terms in a claim"); *see also Eli Lilly & Co. v. Teva Parenteral Medicines, Inc.*, 689 F.3d 1368, 1378-79 (Fed. Cir. 2012); *In re Avery*, 518 F.2d 1228, 1232 (C.C.P.A. 1975); *In re Zickendraht*, 319 F.2d at 228. Here, the '117 patent and '393 patent share at least one common inventor (Raju Penmasta) and the same owner (United Therapeutics Corporation). The claims of the '117 patent are directed to treprostinil and its pharmacologically acceptable salt form. *See* '117 patent, claims 1-4. The claims of the '393 patent also are directed to treprostinil and its pharmacologically

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		acceptable salt form. *See* '393 patent, claims 1-22. Any limitations not expressly claimed in the '117 patent (e.g., purity) would have been obvious to those of ordinary skill in the art at the time the invention was made, taking into account the skill of the art and prior art other than the invention claimed in '117 patent. Therefore, for the reasons explained in more detail above in the anticipation and obviousness analysis, the '393 patent is invalid for obviousness type double patenting over the '117 patent. **Section 112:** As discussed in the previous sections, it would have been obvious for a person of ordinary skill in the art to practice the claimed invention by applying known procedures described in the prior art. But if Plaintiff contends that it would have required undue experimentation for a person of ordinary skill to apply these prior art procedures to obtain the claimed methods (for example it would have required undue experimentation to find particular bases or a particular alkylating agent), the claims are not enabled. Such a contention by Plaintiff would not be supported by the specification or the prosecution history, and to the extent that Plaintiff contends that certain bases or reaction conditions, for example, are unique and that undue experimentation would have been required to practice the claimed method, then the claims of the '393 patent are not enabled or fail to meet the written description requirement. Moreover, to the extent that Plaintiff takes a broad claim construction position and asserts infringement of certain processes and resulting intermediates—such as the use of intermediates or processes that are not sufficiently disclosed, taught or claimed in the '393 patent, including the intermediates and processes that are used to make Teva's treprostinil, the claims of the '393 patent are not enabled and/or lack written description.
2	The product of claim 1, wherein the purity of compound of formula I in said product is at least 99.5%.	*See* analysis of claim 1. Claim 2 adds the additional limitation that the purity of compound of formula I in said product is at least 99.5%, but this limitation is an inherent property of the treprostinil of the prior art and is disclosed specifically in Moriarty 2004. Moriarty 2004 discloses that its compound is produced with 99.7% purity (page 1902).
3	The product of claim 1, wherein the alkylating agent is	Dependent claim 3 claims the product of claim 1, wherein the alkylating agent is $Cl(CH_2)_wCN$,

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	$Cl(CH_2)_wCN$, $Br(CH_2)_wCN$, or $I(CH_2)_wCN$.	$Br(CH_2)_wCN$, or $I(CH_2)_wCN$. This claim is rendered obvious for the same reasons as claim 1. Additionally, the prior art discloses that the alkylating agent is $Cl(CH_2)CN$. The '117 patent col. 20, line 10-col. 21, line 12; Moriarty 2004 page 1892 compound 7, page 1902.
4	The product of claim 1, wherein the base in step (b) is KOH or NaOH.	Dependent claim 4 claims the product of claim 1, wherein the base in step (b) is KOH or NaOH. This claim is rendered obvious for the same reasons as above. Additionally, the prior art discloses that base in step (b) is KOH. The '117 patent col. 20, line 10-col. 21, line 12; Moriarty 2004 page 1892 compound 7, page 1902.
5	The product of claim 1, wherein the base B in step (c) is selected from the group consisting of ammonia, N-methylglucamine, procaine, tromethamine, magnesium, L-lysine, L-arginine, triethanolamine, and diethanolamine.	Dependent claim 5 claims the product of claim 1, wherein the base B in step (c) is selected from the group consisting of ammonia, N-methylglucamine, procaine, tromethanine, magnesium, L-lysine, L-arginine, triethanolamine, and diethanolamine. This claim is rendered obvious for the same reasons as above. Also, the claim includes common bases, and it would have been a matter of routine experimentation for a POSA to choose the bases included, and in particular the prior art specifically discloses that physiologically acceptable salts of treprostinil include salts derived from these bases. *See* Wade 2005 paragraph [0024]. Furthermore, the prior art also disclosed that treprostinil can be crystallized and the diethanolamine salt of treprostinil is particularly preferred. *See* Phares 2005 paragraph [0051]. Thus, it would have been obvious for a POSA to choose a base that was already known to form a salt with treprostinil.
6	The product of claim 1, wherein the acid in step (d) is HCl or H_2SO_4.	Dependent claim 6 claims the product of claim 1, wherein the acid in step (d) is HCl or H_2SO_4. This claim is rendered obvious for the same reasons as above. Additionally, the prior art discloses salts of treprostinil could be reacted with diluted HCl to form treprostinil. *See* the '117 patent col. 20, line 10-col. 21, line 12; Moriarty 2004 page 1892 compound 7, page 1902. Therefore, it would have been obvious to react the salt formed during the crystallization step with diluted HCl to get treprostinil.
7	The product of claim 1, wherein Y_1 is $-CH_2CH_2-$; M_1 is α-OH:β-H or α-H:β-OH; $-C(L_1)-R_7$ taken together is $-(CH_2)_4CH_3$; and w is 1.	Dependent claim 7 claims the product of claim 1, wherein Y_1 is $—CH_2CH_2—$; M_1 is α-OH:β-H or α-H:β-OH; $—C(L_1)-R_7$ taken together is $—(CH_2)_4CH_3$; and w is 1. This claim is rendered obvious for the same reasons as above.
8	The product of claim 1, wherein the process does not include	Dependent claim 8 claims the product of claim 1, wherein the process does not include purifying the

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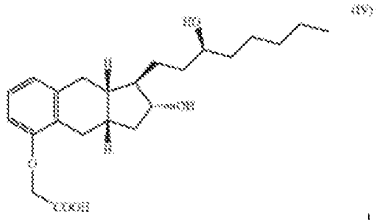
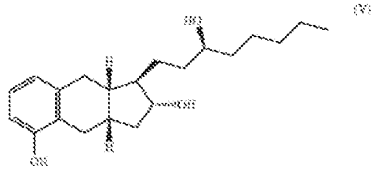
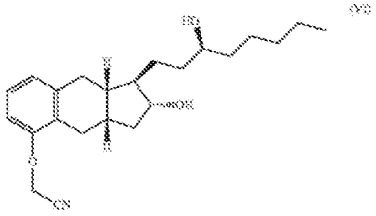
	purifying the compound of formula (III) produced in step (a).	compound of formula (III) produced in step (a). This claim is rendered obvious for the same reasons as above.
9	A product comprising a compound having formula IV  or a pharmaceutically acceptable salt thereof, wherein the product is prepared by the process comprising (a) alkylating a compound of formula V with an alkylating agent to produce a compound of formula VI,   (b) hydrolyzing the product of formula VI of step (a) with a base, (c) contacting the product of step (h) with a base B to form a salt of formula IV_s, and	Claim 9 is directed to a species of the genus of compounds in claim 1 and is directed more specifically to treprostinil. Therefore, for all of the reasons cited in claim 1's contentions, claim 9 is also invalid.

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	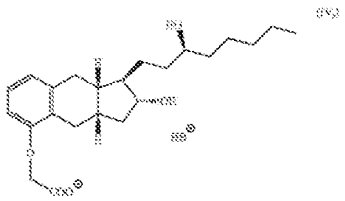 (d) optionally reacting the salt formed in step (c) with an acid to form the compound of formula IV.	
10	The product of claim 9, wherein the purity of product of step (d) is at least 99.5%.	Claim 10 depends on claim 9, so all of the contentions of claim 9 are incorporated herein. Claim 10 adds the additional limitation that the purity of compound of formula I in said product is at least 99.5%, but this limitation is an inherent property of the treprostinil of the prior art and is disclosed specifically in Moriarty 2004. Moriarty 2004 discloses that its compound is produced with 99.7% purity (page 1902).
11	The product of claim 9, wherein the alkylating agent is $ClCH_2CN$.	Dependent claim 11 claims the product of claim 9, wherein the alkylating agent is $ClCH_2CN$. This claim is rendered obvious for the same reasons as above. Additionally, the prior art discloses that the alkylating agent is $ClCH_2CN$. The '117 patent col. 20, line 10-col. 21, line 12; Moriarty 2004 page 1892 compound 7, page 1902.
12	The product of claim 9, wherein the base in step (b) is KOH.	Dependent claim 12 claims the product of claim 9, wherein the base in step (b) is KOH. This claim is rendered obvious for the same reasons as above. Additionally, the prior art discloses that base in step (b) is KOH. The '117 patent col. 20, line 10-col. 21, line 12; Moriarty 2004 page 1892 compound 7, page 1902.
13	The product of claim 9, wherein the base B in step (c) is selected from a group consisting of ammonia, N-methylglucamine, procaine, tromethamine, magnesium, L-lysine, L-arginine, triethanolamine, and diethanolamine.	Dependent claim 13 claims the product of claim 9, wherein the base B in step (c) is selected from a group consisting of ammonia, N-methylglucamine, procaine, tromethanine, magnesium, L-lysine, L-arginine, triethanolamine, and diethanolamine. This claim is rendered obvious for the same reasons as above. Also, the claim includes common bases, and it would have been a matter of routine experimentation for a POSA to choose the bases included, and in particular the prior art specifically discloses that physiologically acceptable salts of treprostinil include salts derived from these bases. *See*

	'393 Patent Claim Language	Invalidity Contentions
		Wade 2005 paragraph [0024]. Furthermore, the prior art also disclosed that treprostinil can be crystallized and the diethanolamine salt of treprostinil is particularly preferred. *See* Phares 2005 paragraph [00051]. Thus, it would have been obvious for a POSA to choose a base that was already known to form a salt with treprostinil.
14	The product of claim 9, wherein the base B is diethanolamine.	Claim 14 claims the product of claim 9, wherein the base B is diethanolamine. This claim is rendered obvious for the same reasons as above. The prior art also disclosed that treprostinil can be crystallized and the diethanolamine salt of treprostinil is particularly preferred. *See* Phares 2005 paragraph [00051]. Thus, it would have been obvious for a POSA to choose a base that was already known to form a salt with treprostinil.
15	The product of claim 9, wherein the acid in step (d) is HCl.	Claim 15 claims the product of claim 9, wherein the acid in step (d) is HCl. This claim is rendered obvious for the same reasons as above. Additionally the prior art discloses salts of treprostinil could be reacted with diluted HCl to form treprostinil. The '117 patent col. 20, line 10-col. 21, line 12; Moriarty 2004 page 1892 compound 7, page 1902. Therefore, it would have been obvious to react the salt formed during the crystallization step with diluted HCl to get treprostinil.
16	The product of claim 9, wherein the process does not include purifying the compound of formula (VI) produced in step (a).	Dependent claim 16 claims the product of claim 9, wherein the process does not include purifying the compound of formula (VI) produced in step (a). This claim is rendered obvious for the same reasons as above.
17	The product of claim 16, wherein the base B in step (c) is selected from a group consisting of ammonia, N-methylglucamine, procaine, tromethamine, magnesium, L-lysine, L-arginine, triethanolamine, and diethanolamine.	Dependent claim 17 claims the product of claim 16, wherein the base B in step (c) is selected from a group consisting of ammonia, N-methylglucamine, procaine, tromethanine, magnesium, L-lysine, L-arginine, triethanolamine, and diethanolamine. This claim is rendered obvious for the same reasons as above. Also, the claim includes common bases, and it would have been a matter of routine experimentation for a POSA to choose the bases included and in particular the prior art specifically discloses that physiologically acceptable salts of treprostinil include salts derived from these bases. *See* Wade 2005 paragraph [0024]. Furthermore, the prior art also disclosed that treprostinil can be crystallized and the diethanolamine salt of treprostinil is particularly preferred. *See* Phares 2005 paragraph [00051]. Thus, it would have been obvious for a POSA to choose a base

	'393 Patent Claim Language	Invalidity Contentions
		that was already known to form a salt with treprostinil.
18	The product of claim 17, wherein the base B is diethanolamine.	Dependent claim 18 claims the product of claim 17, wherein the base B is diethanolamine. This claim is rendered obvious for the same reasons as above. The prior art also disclosed that treprostinil can be crystallized and the diethanolamine salt of treprostinil is particularly preferred. *See* Phares 2005 paragraph [00051]. Thus, it would have been obvious for a POSA to choose a base that was already known to form a salt with treprostinil.
19	The product of claim 1, wherein the base in step (b) is KOH or NaOH and wherein the base 13 in step (c) is selected from the group consisting of ammonia. N-methyl glucamine, procaine, tromethamine, magnesium, L-lysine, L-arginine, triethanolamine, and diethanolamine.	Dependent claim 19 claims the product of claim 1, wherein the base in step (b) is KOH or NaOH and wherein the base 13 in step (c) is selected from the group consisting of ammonia. N-methyl glucamine, procaine, tromethanine, magnesium, L-lysine, L-arginine, triethanolamine, and diethanolamine. This claim is rendered obvious for the same reasons as above.
20	The product of claim 9, wherein the base in step (b) is KOH or NaOH and wherein the base B in step (c) is selected from the group consisting of ammonia, N-methylglucamine, procaine, tromethamine, magnesium, L-lysine, L-arginine, triethanolamine, and diethanolamine.	Dependent claim 20 claims the product of claim 9, wherein the base in step (b) is KOH or NaOH and wherein the base B in step (c) is selected from the group consisting of ammonia, N-methylglucamine, procaine, tromethanine, magnesium, L-lysine, L-arginine, triethanolamine, and diethanolamine. This claim is rendered obvious for the same reasons as above.
21	The product of claim 1, wherein step (d) is performed.	Dependent claim 21 claims the product of claim 1, wherein step (d) is performed. This claim is rendered obvious for the same reasons as above.
22	The product of claim 21, wherein the product comprises a pharmaceutically acceptable salt formed from the product of step (d).	Dependent claims 22 claims the product of claim 21, wherein the product comprises a pharmaceutically acceptable salt formed from the product of step (d). This claim is rendered obvious for the same reasons as above. Additionally, the Moriarty 2004, on page 1902 discloses that, "[c]ompound 7 was identical in all respects to an authentic sample of UT-15" and as disclosed on page 1890, UT-15 is Remodulin (Treprostinil Sodium). The '117 patent discloses the claimed compound in salt form. Col. 20, line 10-col. 21, line 12. The Phares 2005

	'393 Patent Claim Language	Invalidity Contentions
		discloses the claimed compound in at least two salt forms and further discloses that the sodium salt of the compound is sold as Remodulin® which is an FDA approved treatment. Paragraph [0051].

C. Invalidity of United States Patent No. 7,999,007

United States Patent No. 7,999,007 is entitled "Buffer solutions having selective bactericidal activity against gram negative bacteria and methods of using same." The '007 patent issued on August 16, 2011 and claims the priority date of September 7, 2007. The central feature of each of the asserted claims is the combination of treprostinil and glycine buffer with a pH of greater than 10.

In November 2004, Remodulin (treprostinil) was approved for intravenous use for the treatment of pulmonary hypertension. When administered intravenously, Remodulin must be diluted prior to injection. At the time, the approved diluents were Sterile Water for Injection or 0.9% Sodium Chloride for Injection. Around September 2006, Dr. Robyn Barst, a pulmonary hypertension specialist, contacted UTC and the CDC to inform them she was observing that patients receiving intravenous Remodulin were experiencing higher rates of blood stream infections than patients receiving intravenous administration of another pulmonary hypertension medication called Flolan®. Unlike Remodulin, which was diluted with water or saline, Flolan® was diluted with Sterile Diluent for Flolan®. Sterile Diluent for Flolan® is a glycine buffer with a pH of 10.5. In response, the CDC conducted an investigation and published its results in March, 2007, confirming that the incidence of blood stream infections was greater in patients receiving intravenous Remodulin® than in patients receiving intravenous Flolan®. On September 7, 2007, six months after the CDC report was published and approximately a year

after Dr. Barst first raised the infection issue, UTC filed the application that later matured into the '007 patent.

The asserted claims of the '007 patent generally are directed to methods of (i) selectively killing gram negative bacteria and inhibiting the growth of gram positive bacteria in a pharmaceutical preparation comprising supplying an active ingredient with "a buffer comprising glycine having a pH of greater than 10" (claims 1-10); (ii) methods of reducing the "occurrence of blood stream infections" in a mammal comprising "administering to a mammal the active agent with a buffer comprising glycine and having a pH of greater than 10" (claims 11-21); and (iii) pharmaceutical compositions in a solution "comprising glycine and with glycine and having a pH greater than 10" (claims 22-26).

1. Claims 1-5, 7-17 And 19-26 Of The '007 Patent Are Anticipated by EP 0347243A1 Or Obvious Over EP 0347243A1 In View Of Sterile Diluent for Flolan And Knowledge Of One Of Ordinary Skill In The Art.

The asserted patent claims 1-5, 7-17, and 19-26 of the '007 patent are invalid, because they are anticipated by European Patent Application EP 0347243A1 ("EP '243") or obvious over EP '243 in view of Sterile Diluent For Flolan and/or knowledge of one of ordinary skill in the prior art.

EP '243 issued on December 20, 1989, and is, thus, 102(b) prior art. EP '243 patent disclosed and claimed medicaments for the treatment of pulmonary hypertension that could be used subcutaneously or intravenously. EP '243, ¶¶ 22, 25. Example 1 discloses the combination of treprostinil and a "glycine buffer" with a pH of 10.5. EP '243 further describes the use of buffer solutions with treprostinil to treat pulmonary hypertension. EP '243 concludes that treprostinil used with a glycine buffer solution of greater than pH 10 "was found to reduce hypoxia-induced increase in pulmonary arterial pressure and pulmonary vascular resistance in a

dose-related manner without appreciably affecting cardiac output or heart rate." *Id.* at ¶¶ 32-34. EP '243 discloses that "sterile" acqueous solutions are preferred and that "[s]uch preparations may conveniently be prepared by admixing the compound with water or a glycine buffer and rendering the resulting solution sterile and isotonic with the blood." *Id.* at ¶ 25. Therefore, EP '243 discloses "a pharmaceutical preparation" or "pharmaceutical composition" comprising "treprostinil" and "a buffer comprising glycine and having a pH of greater than 10." Having a low buffer capcity and the intended purpose—killing bacteria and reducing infections—are inherent properties of a sterile solutions that are sterile and isotonic with the blood. *Id.*

If EP '243 does not anticipate the '007 patent, '007 patent is invalid as obvious over EP '243 patent in view of the commercial embodiment Sterile Diluent for Flolan®. Flolan® is a third party competitive product, containing epoprostenol, which was approved in 1995 for treating pulmonary hypertension. Flolan® is a powder that must be reconstituted with "Sterile Diluent for Flolan" ("SDF"). SDF is a solution containing the amino acid glycine and having a pH greater than 10 that physicians or patients may use to dilute Flolan prior to intravenous infusion. The use of SDF (or a buffer such as SDF) was described, for example, in 1999 Flolan® label and U.S. Patent No. 4,335,139[9]. SDF was available more than 1 year prior to the earliest priority date of the '007 patent and is 102(b) prior art to the '007 patent.

A person of ordinary would have found it obvious to combine Remodulin in combination with SDF, based on EP '243, SDF and knowledge of one of ordinary skill in the art, with a

[9] U.S. Patent No. 4,335,139 was cited by the Examiner during the prosecution of U.S. Patent No. 8,658,694 and appears on the face of 2000 Flolan® label. The '139 patent discloses the use of a prostacyclin with "a pharmaceutically acceptable buffer having a pH value of at least 9 and based on an amino acid as the principal buffering acid in the buffer." '139 patent, col. 1, lines 38-45. "Such a solution and all solutions hereinafter referred to are, for medicinal purposes, to be understood to be sterile solutions." *Id.* at col. 2, lines 4-6. "Glycine" is specifically disclosed as an amino acid of the buffer. *Id.* at Example 1. Example 7 specifically discloses a sterile diluent for injection of a prostacyclin, which contains glycine and has a pH of 10.5. The 2000 Flolan label incorporated by reference the '139 patent as covering Flolan® and SDF.

reasonable expectation of success. *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 421 (2007). As of the priority date of the '007 patent, Remodulin (treprostinil) was the commercially-available treprostinil product, and SDF was the only commercially-available glycine buffer with a pH of 10.5, already in use with another pulmonary hypertension medication. And as the district court in the related case, *UTC v. Sandoz*, 12-CV-01617, 13-CV-316 (D.N.J. 2014), expressly found, this combination would meet all of the asserted claims of the '007 patent. (Decision at 73.) UTC's expert in the related *Sandoz* matter, Dr. Michael Miller, admitted at trial that a person of ordinary skill in the art, seeking to practice the invention disclosed and claimed in EP '243, could easily have done so by combining Remodulin and Sterile Diluent for Flolan, both of which were commercially available products as of the priority date for the '007 patent.

A person of ordinary skill in the art also would have been motivated to use treprostinil with a high pH buffer comprising glycine with a reasonable expectation of success in inhibiting bacterial growth or reducing the occurrence of blood stream infections. The use of SDF resulted in a high pH glycine buffer solution that was sterile, antibacterial and anti-infective. Moreover, it was well-known in the prior art that glycine is an amino acid that has antibacterial properties. *See e.g.* Foegeding P.M. et al, "Chemical Food Preservatives," Ch. 47 at 825 in Disinfection, Sterilization and Preservation 4th Ed. 1991; Hammes et al., "Mode of Action of Glycine on the Biosynthesis of Peptidoglycoan," J. Bacteriology, Vol. 116, No. 2 pp. 1029-1053 (1973); Strominger et al., "Nucleotide Accumulation Induced in Staphylococcus aureus by Glycine," J. Bacteriology, Vol. 89, No. 4 pp. 1124-1127 (1965). Therefore, as of the priority date, it was also known that a solution in an alkali environment (high pH solutions) with glycine will have bactericidal antiinfective effects. *See, e.g.*, Mendonca, et al, "Destruction of Gram-Negative Food-Borne Pathogens by High pH Involves Disruption of the Cytoplasmic Membrane,: Applied

and Environmental Microbiology, vol. 60, No. 11, p. 4009-4014 (1994). ("Mendonca") (disclosed during the prosecution of the '007 patent); Crowther et al., "Growth of Microorganisms in Propofol, Thiopental, and a 1:1 Mixture Of Propofol and Thiopental," Anesth. Analg., 82: 475-478 (1996) ("Crowther") (TEVA_TRE_0004034-7); and Siqueira et al., Mechanisms of antimicrobial activity of calcium hydroxide: a critical review," Intern. Endodontic J., 32, pp. 361-369, (1999) ("Siqueira) (TEVA_TRE_0004298-306).

Consistent with the fact that glycine and high pH solutions have known antibacterial properties, the prior art describes glycine buffer solutions that have high pH used in pharmaceutical formulations. U.S. Appln. No. 10/137,331; 1999 Flolan Package Insert; EP '243; Wade 2005 [0030]; 2005 PDR. Indeed, the prior art specifically describes with treprostinil with high pH glycine buffer solutions for use in pharmaceutical compositions. EP '243; Wade 2005. Moreover, a person of ordinary skill would have been motivated to address possible complications from bacterial infections when the drug is administered intravenously.

A solution having "a low buffer capacity" also would have been known to a person of ordinary skill in the art. Claims 1-5, 7-10, 16-17, and 21 also require that the glycine buffer used in the claimed methods have a low buffer capacity. The '007 patent states that "the buffer capacity should be low to avoid pH changes in the blood upon infusion." Col. 2, lines 34-35. SDF inherently possesses this limitation. Moreover, it would have been obvious to a person of ordinary skill in the art that it is important to maintain the proper pH of blood to avoid possible severe complications. *See e.g.* Petrucci, R. and Harwood, W., General Chemistry Principles and Modern Applications, 6th Ed., 1993, pp. 656-57 (explaining that the normal pH of blood is 7.4 and increased pH of blood can lead to severe vomiting and hyperventilation). Consequently, it would have been obvious to a person of ordinary skill to formulate the high pH buffer solution

with a low buffer capacity, so that it would be safe and avoid any complications based on changes of blood pH when the treprostinil solution is administered. *See also* EP '243 at 5.

The dependent claims the depend from claim 1 are obvious for the same reasons as stated above. Furthermore, dependent claim 2 requires that the active agent is treprostinil sodium. The prior art specifically describes the use of glycine buffered solutions with treprostinil. EP '243; Wade 2005 [0030]. Also, the 2006 Remodulin Package Insert describes the use of treprostinil as the active ingredient. Dependent claim 3 requires the buffer to contain sodium hydroxide. SDF contained sodium hydroxide. Moreover, the 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem describes sodium hydroxide used in the glycine buffer solution. Dependent claims 4 and 5 require the buffer solution to have a pH between about 10 to about 12 or 10.2 to 10.8, respectively. The 1999 Flolan Package Insert and the 2005 PDR, and Calbiochem describe the pH of the glycine buffer from 10.2 to 10.8, and EP '243 describes such a formulation at Example 1. Dependent claim 7 requires the active agent to be at a concentration between about 0.001 mg/mL to about 1 mg/mL, and dependent claim 8 requires treprostinil sodium to be at a concentration between about 0.004 mg/mL to about 0.13 mg/mL. EP '243 and the 2006 Remodulin Package Insert disclose concentrations that cover this ranges. EP '243 at 5. Dependent claims 9 and 10 require that pharmaceutical preparation is injected, for claim 10 injected intravenously, into a mammal in need thereof. EP '243, 1999 Flolan Package Insert, the 2005 PDR, the 2006 Remodulin Package Insert, and Wade 2005 all describe the injection of the pharmaceutical preparation into mammals for treatment.

The dependent claims the depend from claim 11 are obvious for the same reasons as stated above. Furthermore, dependent claim 12 requires that a human subject undergoing the method has pulmonary arterial hypertension. EP '243, 1999 Flolan Package Insert, the 2005

PDR, the 2006 Remodulin Package Insert, and Wade 2005 all describe the use of the active ingredient to treat pulmonary arterial hypertension. Also, the dependent claims the depend from claim 22 are obvious for the same reasons as stated above.

Plaintiff has not set forth its contentions concerning secondary considerations in this case. If Plaintiff relies on any secondary considerations of non-obviousness, Teva reserves the right to supplement its contentions.

2. Claims 1-5, 7-17, And 19-26 Of The '007 Patent Are Not Enabled And/Or Lack A Written Description

As explained above, a person of ordinary skill in the art would have found the asserted claims anticipated or obvious in view of the prior art. A person of ordinary skill would have been able to take the information in the prior art and apply routine experimentation to arrive at the methods and compositions of the asserted claims. But if Plaintiff contends that that the asserted claims are not invalid because a person of ordinary skill would need to practice undue experimentation from the disclosures in the prior art, then the asserted claims are invalid because they are not enabled and the patent does not contain a sufficient written description.

The '007 patent generally describes solutions with an active ingredient, glycine, a high pH, and a low buffer capacity. Further, it gives general and broad ranges for these ingredients and requirements and specifically does not restrict them to indicate that only a narrow range for those ingredients and requirements will work. For example, it describes glycine concentrations "of about 30% to about 80%" and an active ingredient concentration of preferably 0.004 mg/mL to about 0.13 mg/mL. If Plaintiff contends that the prior art is not enabled or a person of ordinary skill would need to conduct undue experimentation based on the disclosures in the prior art, then the asserted claims of the '007 patent would lack enablement and fail to meet the written description requirement because a person of ordinary skill in the art would need to conduct

undue experimentation to enable the full scope of the claims, and the patent lacks any written description as to all of the alleged proper parameters for the claimed methods and compositions.

The following prior art shows all of the limitations of the '007 patent, including the use of glycine buffers to inhibit bacterial growth or reduce bloodbourne infections, prior to September 7, 2007, the priority date of the '007 patent:

- EP '243 (TEVA_TRE_0004270-80)
- U.S. Patent Publication No. 2005/0165110 July 2005, Wade et al. ("Wade 2005") (TEVA_TRE_0004213-218)
- Crowther et al., "Growth of Microorganisms in Propofol, Thiopental, and a 1:1 Mixture Of Propofol and Thiopental," *Anesth. Analg., 82:* 475-478 (1996) ("Crowther") (TEVA_TRE_0004034-7)
- Siqueira et al., Mechanisms of antimicrobial activity of calcium hydroxide: a critical review," Intern. Endodontic J., 32, pp. 361-369, (1999) ("Siqueira) (TEVA_TRE_0004298-306)
- Foegeding P.M. et al, "Chemical Food Preservatives," Ch. 47 in Disinfection, Sterilization and Preservation 4th Ed. 1991. (TEVA_TRE_0004267-9)
- Hammes et al., "Mode of Action of Glycine on the Biosynthesis of Peptidoglycoan," J. Bacteriology, Vol. 116, No. 2 pp. 1029-1053 (1973) (TEVA_TRE_0004042-66)
- Strominger et al., "Nucleotide Accumulation Induced in Staphylococcus aureus by Glycine," J. Bacteriology, Vol. 89, No. 4 pp. 1124-1127 (1965) (TEVA_TRE_0004038-41)
- "Buffers: A guide for the preparation and use of buffers in biological systems" by Calbiochem ("Calbiochem") (TEVA_TRE_0003997-4033)
- 2006 Remodulin Package Insert (TEVA_TRE_0004285-97)
- 1999 Flolan Package Insert (TEVA_TRE_0004281-84)
- The 2005 Physicians' Desk Reference for Flolan® (epoprostenol sodium for injection) ("2005 PDR") (TEVA_TRE_0003991-6)
- Petrucci, R. and Harwood, W., General Chemistry Principles and Modern Applications, 6th Ed., pp. 656-57 (1993) (Teva_TRE_0003971-4)

- Prior art disclosed or cited during prosecution of the '007, '137, and '694 patents.

Teva expressly reserves the right to modify and/or supplement the above list at any time as necessary and/or as discovery progresses.

The following chart incorporates the analysis set forth above and identifies where specifically in each alleged item of prior art each limitation of each asserted claim is found:

	'007 Patent Claim Language	Invalidity Contentions
1	A method of selectively killing gram negative bacteria and inhibiting the growth of gram positive bacteria in a pharmaceutical preparation comprising an active agent selected from the group consisting of treprostinil and treprostinil sodium, the method comprising supplying the active agent with a buffer comprising glycine and having a pH of greater than 10 with low buffer capacity.	**Anticipation:** Claim 1 of the '007 patent is invalid, because it is anticipated by European Patent Application EP 0347243A1 ("EP '243") or obvious over EP '243 in view of Sterile Diluent For Flolan and/or knowledge of one of ordinary skill in the prior art. EP '243 issued on December 20, 1989, and is, thus, 102(b) prior art. EP '243 patent disclosed and claimed medicaments for the treatment of pulmonary hypertension that could be used subcutaneously or intravenously. EP '243, ¶¶ 22, 25. Example 1 discloses the combination of treprostinil and a "glycine buffer" with a pH of 10.5. EP '243 further describes the use of buffer solutions with treprostinil to treat pulmonary hypertension. EP '243 concludes that treprostinil used with a glycine buffer solution of greater than pH 10 "was found to reduce hypoxia-induced increase in pulmonary arterial pressure and pulmonary vascular resistance in a dose-related manner without appreciably affecting cardiac output or heart rate." *Id.* at ¶¶ 32-34. EP '243 discloses that "sterile" acqueous solutions are preferred and that "[s]uch preparations may conveniently be prepared by admixing the compound with water or a glycine buffer and rendering the resulting solution sterile and isotonic with the blood." *Id.* at ¶ 25. Therefore, the pharmaceutical preparation of EP '243 inherently inhibits growth of gram positive bacteria and, if given to a person, will inherently kill gram negative bacteria. Therefore, EP '243 discloses "a pharmaceutical preparation" or "pharmaceutical composition" comprising "treprostinil" and "a buffer comprising glycine and having a pH of greater than 10" with the

	'007 Patent Claim Language	Invalidity Contentions
		inherent qualities described in the claims. Moreover, having a low buffer capcity is an inherent property of sterile solutions with a high pH that are isotonic with the blood and are intended to be given to humans, as the pH of the pharmaceutical preparation should quickly adjust to the pH of blood, which is substantially lower than pH of 10. *Id.* **Obviousness:** If EP '243 does not anticipate the '007 patent, '007 patent is invalid as obvious over EP '243 patent in view of the commercial embodiment Sterile Diluent for Flolan®. Flolan® is a third-party competitive product, containing epoprostenol, which was approved in 1995 for treating pulmonary hypertension. Flolan® is a powder that must be reconstituted with "Sterile Diluent for Flolan" ("SDF"). SDF is a solution containing the amino acid glycine and having a pH greater than 10 that physicians or patients may use to dilute Flolan prior to intravenous infusion. The use of SDF (or a buffer such as SDF) was described, for example, in 1999 Flolan® label and U.S. Patent No. 4,335,139[10]. SDF was available more than 1 year prior to the earliest priority date of the '007 patent and is 102(b) prior art to the '007 patent. A person of ordinary skill in the art would have found it obvious to combine Remodulin in combination with SDF, based on the teachings of EP '243, the existing knowledge and use of SDF, and knowledge of one of ordinary skill in the art, with a reasonable expectation of success at arriving at the claimed invention. *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 421 (2007). As of the priority date of the '007 patent, Remodulin (treprostinil) was the commercially-available treprostinil product, and SDF was the only commercially-available glycine buffer with a pH of 10.5, already in use with another pulmonary hypertension medication. And as the district court in the

[10] U.S. Patent No. 4,335,139 was cited by the Examiner during the prosecution of U.S. Patent No. 8,658,694 and appears on the face of 2000 Flolan® label. The '139 patent discloses the use of a prostacyclin with "a pharmaceutically acceptable buffer having a pH value of at least 9 and based on an amino acid as the principal buffering acid in the buffer." '139 patent, col. 1, lines 38-45. "Such a solution and all solutions hereinafter referred to are, for medicinal purposes, to be understood to be sterile solutions." *Id.* at col. 2, lines 4-6. "Glycine" is specifically disclosed as an amino acid of the buffer. *Id.* at Example 1. Example 7 specifically discloses a sterile diluent for injection of a prostacyclin, which contains glycine and has a pH of 10.5. The 2000 Flolan label incorporated by reference the '139 patent as covering Flolan® and SDF.

	'007 Patent Claim Language	Invalidity Contentions
		related case, *UTC v. Sandoz*, 12-CV-01617, 13-CV-316 (D.N.J. 2014), expressly found, this combination would meet all of the asserted claims of the '007 patent. (Decision at 73.) UTC's expert in the related *Sandoz* matter, Dr. Michael Miller, admitted at trial that a person of ordinary skill in the art, seeking to practice the invention disclosed and claimed in EP '243, could easily have done so by combining Remodulin and Sterile Diluent for Flolan, both of which were commercially available products as of the priority date for the '007 patent. A person of ordinary skill in the art also would have been motivated to use treprostinil with a high pH buffer comprising glycine with a reasonable expectation of success in inhibiting bacterial growth or reducing the occurrence of blood stream infections. The use of SDF resulted in a high pH glycine buffer solution that was sterile, antibacterial and anti-infective. Moreover, it was well-known in the prior art that glycine is an amino acid that has antibacterial properties. *See e.g.* Foegeding P.M. et al, "Chemical Food Preservatives," Ch. 47 at 825 in Disinfection, Sterilization and Preservation 4th Ed. 1991; Hammes et al., "Mode of Action of Glycine on the Biosynthesis of Peptidoglycoan," J. Bacteriology, Vol. 116, No. 2 pp. 1029-1053 (1973); Strominger et al., "Nucleotide Accumulation Induced in Staphylococcus aureus by Glycine," J. Bacteriology, Vol. 89, No. 4 pp. 1124-1127 (1965). Therefore, as the following prior art explains, as of the priority date, it was also known that a solution in an alkali environment (high pH solutions) with glycine will have bactericidal antiinfective effects. *See, e.g.*, Mendonca, et al, "Destruction of Gram-Negative Food-Borne Pathogens by High pH Involves Disruption of the Cytoplasmic Membrane,: Applied and Environmental Microbiology, vol. 60, No. 11, p. 4009-4014 (1994). ("Mendonca") (disclosed during the prosecution of the '007 patent); Crowther et al., "Growth of Microorganisms in Propofol, Thiopental, and a 1:1 Mixture Of Propofol and Thiopental," Anesth. Analg., 82: 475-478 (1996) ("Crowther") (TEVA_TRE_0004034-7); and Siqueira et al., Mechanisms of antimicrobial activity of calcium

	'007 Patent Claim Language	Invalidity Contentions
		hydroxide: a critical review," Intern. Endodontic J., 32, pp. 361-369, (1999) ("Siqueira) (TEVA_TRE_0004298-306). Consistent with the fact that glycine and high pH solutions have known antibacterial properties, the prior art describes glycine buffer solutions that have high pH for use in pharmaceutical formulations. *See* U.S. Appln. No. 10/137,331 (disclosed during prosecution of the '007 patent); 1999 Flolan Package Insert (TEVA_TRE_0004281-84) (disclosing the use of SDF and SDF's qualities); EP '243 (TEVA_TRE_0004270-80) (discussed in more detail above); U.S. Patent Publication No. 2005/0165110 (July 2005) by Wade et al. at [0030] ("Wade 2005") (TEVA_TRE_0004213-218); and The 2005 Physicians' Desk Reference for Flolan® (epoprostenol sodium for injection) ("2005 PDR") (TEVA_TRE_0003991-6) (disclosing SDF, its use, and qualities). Indeed, as discussed earlier, the prior art specifically describes, suggests, and combines treprostinil with a high pH glycine buffer solutions for use in pharmaceutical compositions for humans. See EP '243; Wade 2005. As is evident from thee disclosures, a person of ordinary skill would have been motivated to address possible complications from bacterial infections when treprostinil is administered intravenously, as suggested by the use of SDF and the disclosures of EP '243. A solution having "a low buffer capacity," if not inherent, also would have been known to a person of ordinary skill in the art. (Claims 1-5, 7-10, 16-17, and 21 also require that the glycine buffer used in the claimed methods have a low buffer capacity, so this analysis applies to the other claims with equal force.) The '007 patent states that "the buffer capacity should be low to avoid pH changes in the blood upon infusion." Col. 2, lines 34-35. SDF inherently possesses this limitation. Moreover, it would have been obvious to a person of ordinary skill in the art that it is important to maintain the proper pH of blood to avoid possible severe complications. *See e.g.* Petrucci, R. and Harwood, W., General Chemistry Principles and Modern Applications, 6th Ed., 1993, pp. 656-57 (explaining that the normal pH of blood is 7.4 and increased pH of blood can lead to

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		severe vomiting and hyperventilation). Consequently, it would have been obvious to a person of ordinary skill to formulate the high pH buffer solution with a low buffer capacity, so that it would be safe and avoid any complications based on changes of blood pH when the treprostinil solution is administered. *See also* EP '243 at 5. Plaintiff has not set forth its contentions concerning secondary considerations in this case. If Plaintiff relies on any secondary considerations of non-obviousness, Teva reserves the right to supplement its contentions.
2	The method of claim 1, wherein the active agent is treprostinil sodium.	Dependent claim 2 incorporates the method of claim 1; therefore, the contentions incorporate the analysis from claim 1 in its entirety. Claim 2 further specifies that the active agent is treprostinil sodium. The prior art specifically describes the use of glycine buffered solutions with treprostinil. EP '243; Wade 2005 [0030]. Also, the 2006 Remodulin Package Insert describes the use of treprostinil as the active ingredient.
3	The method of claim 1, wherein the buffer further comprises sodium hydroxide.	Dependent claim 3 incorporates the method of claim 1, therefore the contentions incorporate the analysis from claim 1 in its entirety. Dependent claim 3 further specifies that the buffer to contain sodium hydroxide. SDF contained sodium hydroxide. Moreover, the 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem describes sodium hydroxide as a basic agent that can be used to adjust the pH of a solution and can be used in the glycine buffer solution.
4	The method of claim 1, wherein the buffer has a pH between about 10 to about 12 with low buffer capacity.	Dependent claim 4 incorporates the method of claim 1, therefore the contentions incorporate the analysis from claim 1 in its entirety. Dependent claim 4 further requires the buffer solution to have a pH between about 10 to about 12. The 1999 Flolan Package Insert and the 2005 PDR, and Calbiochem describe the pH of the glycine buffer from 10.2 to 10.8, and EP '243 describes such a formulation at Example 1. Therefore, the pH range claimed herein is disclosed in the prior art.
5	The method of claim 4, wherein the buffer has a pH between about 10.2 to about 10.8 with low buffer capacity.	Dependent claim 5 incorporates the method of claim 1; therefore, the contentions incorporate the analysis from claim 1 in its entirety. Dependent claims 5 further requires the buffer solution to have a pH between about 10.2 to 10.8. The 1999 Flolan Package Insert and the 2005 PDR, and Calbiochem describe the pH of the glycine buffer from 10.2 to 10.8, and EP '243 describes

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		such a formulation at Example 1. Therefore, the pH range claimed herein is disclosed in the prior art.
7	The method of claim 1, wherein the active agent is supplied at a concentration between about 0.001 mg/mL to about 1 mg/mL.	Dependent claim 7 incorporates the method of claim 1; therefore, the contentions incorporate the analysis from claim 1 in its entirety. Dependent claim 7 further specifies the active agent to be at a concentration between about 0.001 mg/mL to about 1 mg/mL. EP '243 and the 2006 Remodulin Package Insert at dosing instruction disclose concentrations that specifically cover this ranges. *E.g.*, EP '243 at 5.
8	The method of claim 2, wherein the treprostinil sodium is supplied at a concentration between about 0.004 mg/mL to about 0.13 mg/mL.	Dependent claim 8 incorporates the method of claim 1; therefore, the contentions incorporate the analysis from claim 1 in its entirety. Dependent claim 8 further specifies the active agent to be at a concentration between about 0.004 mg/mL to about 0.13 mg/mL. EP '243 and the 2006 Remodulin Package Insert at dosing instruction disclose concentrations that specifically cover this ranges. *E.g.*, EP '243 at 5.
9	The method of claim 1 further comprising injecting the pharmaceutical preparation into a mammal in need thereof.	Dependent claim 9 incorporates the method of claim 1; therefore, the contentions incorporate the analysis from claim 1 in its entirety. Dependent claim 9 requires that pharmaceutical preparation is injected into a mammal in need thereof. This is specifically disclosed, as described in more detail above, in EP '243, 1999 Flolan Package Insert, the 2005 PDR, the 2006 Remodulin Package Insert, and Wade 2005. The prior art describes the injection of the pharmaceutical preparation into mammals for treatment.
10	The method of claim 4, wherein the pharmaceutical preparation is injected intravenously into a mammal in need thereof.	Dependent claim 9 incorporates the method of claim 1; therefore, the contentions incorporate the analysis from claim 1 in its entirety. Dependent claim 10 requires that pharmaceutical preparation is injected intravenously into a mammal in need thereof. This is specifically disclosed, as described in more detail above, in EP '243, 1999 Flolan Package Insert, the 2005 PDR, the 2006 Remodulin Package Insert, and Wade 2005. The prior art describes the injection of the pharmaceutical preparation into mammals for treatment.
11	A method of reducing the occurrence of blood stream infections in a mammal being treated with an active agent comprising administering to the mammal the active agent with a buffer comprising glycine and	Claim 11 is substantially the same as claim 1, except that it is directed to "reducing the occurrence of blood stream infections in a mammal being treated with an active agent." Teva hereby incprorates all analysis from claim 1 into this contention. The purpose of the active agent is an inherent quality of an agent that is injected into a mammal. Moreover, reducing the occurrence of blood

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	having a pH of greater than 10, wherein the active agent is selected from the group consisting of treprostinil and treprostinil sodium, and wherein the administration reduces the gram negative bacteria and inhibits the growth of gram positive bacteria.	stream infections would have been an important quality of any injectable.
12	The method of claim 11, wherein the human subject has pulmonary arterial hypertension.	Dependent claim 12 incorporates the method of claim 11; therefore, the contentions incorporate the analysis from claim 11 in its entirety. Dependent claim 12 further specifies that a human subject undergoing the method has pulmonary arterial hypertension. The prior art specifically discloses this additional limitation, as discussed more fully above, including at EP '243, 1999 Flolan Package Insert, the 2005 PDR, the 2006 Remodulin Package Insert, and Wade 2005. Therefore, the prior art describes the use of the active ingredient to treat pulmonary arterial hypertension. Moreover, this additional limitation would have required only routine optimization of the prior art combinations.
13	The method of claim 11, where in the active agent is administered intravenously.	Dependent claim 13 incorporates the method of claim 11; therefore, the contentions incorporate the analysis from claim 11 in its entirety (which in turn incorporates the analysis of claim 1). The prior art disclosed with respect to the analysis in claims 1 and 11 teaches the active agent that is administered intravenously. Moreover, this additional limitation would have required only routine optimization of the prior art combinations.
14	The method of claim 11, wherein the active agent is treprostinil sodium.	Dependent claim 14 incorporates the method of claim 11; therefore, the contentions incorporate the analysis from claim 11 in its entirety (which in turn incorporates the analysis of claim 1). The prior art disclosed with respect to the analysis in claims 1 and 11 teaches that the active agent is treprostinil sodium. Moreover, this additional limitation would have required only routine optimization of the prior art combinations.
15	The method of claim 11, wherein the buffer further comprises sodium hydroxide and has a pH between about 10.2 to about 10.8.	Dependent claim 15 incorporates the method of claim 11; therefore, the contentions incorporate the analysis from claim 11 in its entirety (which in turn incorporates the analysis of claim 1). The prior art disclosed with respect to the analysis in claims 1 and 11 teach that the buffer further comprises sodium hydroxide and has a pH between about 10.2 to about 10.8. Moreover, this

	'007 Patent Claim Language	Invalidity Contentions
		additional limitation would have required only routine optimization of the prior art combinations.
16	The method of claim 11, wherein the buffer has a pH between about 10 to about 12 with low buffer capacity.	Dependent claim 16 incorporates the method of claim 11; therefore, the contentions incorporate the analysis from claim 11 in its entirety (which in turn incorporates the analysis of claim 1). The prior art disclosed with respect to the analysis in claims 1 and 11 teaches that the buffer has a pH between about 10 to about 12 with low buffer capacity. Moreover, this additional limitation would have required only routine optimization of the prior art combinations.
17	The method of claim 16, wherein the buffer has a pH between about 10.2 to about 10.8 with low buffer capacity.	Dependent claim 17 incorporates the method of claim 11; therefore, the contentions incorporate the analysis from claim 11 in its entirety (which in turn incorporates the analysis of claim 1). The prior art disclosed with respect to the analysis in claims 1 and 11 teaches that the buffer has a pH between about 10.2 to about 10.8 with low buffer capacity. Moreover, this additional limitation would have required only routine optimization of the prior art combinations.
19	The method of claim 11, wherein the active agent is supplied at a concentration between about 0.004 mg/mL to about 0.13 mg/ml.	Dependent claim 19 incorporates the method of claim 11; therefore, the contentions incorporate the analysis from claim 11 in its entirety (which in turn incorporates the analysis of claim 1). The prior art disclosed with respect to the analysis in claims 1 and 11 teaches that the active agent is supplied at a concentration between about 0.004 mg/mL to about 0.13 mg/ml. Moreover, this additional limitation would have required only routine optimization of the prior art combinations.
20	The method of claim 14, wherein the treprostinil sodium is supplied at a concentration between about 0.004 mg/mL to about 0.13 mg/mL.	Dependent claim 20 incorporates the method of claim 14; therefore, the contentions incorporate the analysis from claim 14 in its entirety (which in turn incorporates the analysis of claims 1 and 11). The prior art disclosed with respect to the analysis in claims 1 and 11 teaches that treprostinil sodium is supplied at a concentration between about 0.004 mg/mL to about 0.13 mg/mL Moreover, this additional limitation would have required only routine optimization of the prior art combinations.
21	The method of claim 1 wherein the administering is injecting the pharmaceutical preparation into a mammal in need thereof.	Dependent claim 16 incorporates the method of claim 1; therefore, the contentions incorporate the analysis from claim 1 in its entirety. The prior art disclosed with respect to the analysis in claim 1 teaches injecting the pharmaceutical preparation into a mammal in need thereof. Moreover, this additional limitation would have required only routine optimization of the prior art

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		combinations.
22	A pharmaceutical composition comprising an active agent selected from the group consisting of treprostinil and treprostinil sodium in a solution comprising glycine and having a pH greater than 10.	Claim 2 is directed to a pharmaceutical composition comprising treprostinil in a solution comprising glycine having a pH greater than 10. All of the prior art and analysis cited in claim 1 are directly applicable to claim 22, as claim 22 is substantially same as the "pharmaceutical preparation" that is administered in claim 1.
23	The composition of claim 22, wherein the solution further comprises sodium hydroxide.	Dependent claim 23 incorporates the method of composition of 22; therefore, the contentions incorporate the analysis from claim 22 in its entirety (which in turn incorporates the analysis of claim 1). The prior art further discloses the solution has sodium hydroxide.
24	The composition of claim 22, wherein the solution has a pH between about 10 to about 12.	Dependent claim 24 incorporates the method of composition of 22, therefore the contentions incorporate the analysis from claim 22 in its entirety (which in turn incorporates the analysis of claim 1). The prior art further discloses the solution has pH between about 10-12. Moreover, this additional limitations would have required only routine optimization of the prior art combinations.
25	The composition of claim 24, wherein the solution has a pH between about 10.2 to about 10.8.	Dependent claim 25 incorporates the method of composition of 22, therefore the contentions incorporate the analysis from claim 22 in its entirety (which in turn incorporates the analysis of claim 1). The prior art further discloses the solution has pH between about 10.2-10.8. Moreover, this additional limitation would have required only routine optimization of the prior art combinations.
26	The composition of claim 22, wherein the active agent is treprostinil sodium.	Dependent claim 26 incorporates the method of composition of 22; therefore, the contentions incorporate the analysis from claim 22 in its entirety (which in turn incorporates the analysis of claim 1). The prior art further discloses the active agent is treprostinil sodium. Moreover, this additional limitation would have required only routine optimization of the prior art combinations.

D. Invalidity of United States Patent No. 8,653,137

United States Patent No. 8,653,137, entitled "Buffer solutions having selective bactericidal activity against gram negative bacteria and methods of using same," was issued on February 18, 2014 with 13 claims. Claims 1-13 of the '137 patent are directed to methods of

reducing the occurrence of a bacterial infection "comprising diluting a starting solution of an active pharmaceutical ingredient other than epoprostenol with a buffer comprising glycine and having a pH of greater than 10" and administering the buffered solution "to the human subject in need thereof."

UTC asserts that Teva infringes claims 1-13 of the '137 patent. Claim 1 is the only independent claim of the '137 patent:

> 1. A method of reducing occurrence of a bacterial infection in a human suffering from pulmonary arterial hypertension, who is undergoing treatment for said pulmonary hypertension, associated with occurrence of a bacterial infection comprising diluting a starting solution of an active pharmaceutical ingredient other than epoprostenol with a buffer comprising glycine and having a pH of greater than 10 to provide a final solution with a pH of greater than 10 and an amount of the active pharmaceutical ingredient other than epoprostenol effective for treating pulmonary arterial hypertension, and administering said final solution to the human subject in need thereof.

The '137 patent shares the same priority date as the '007 patent, substantially the same specification, and is in the same family of patent as the '007 patent. The scope of the claims, accordingly, are substantially similar and comprise generally the same subject matter (e.g., reducing infection with treprostinil plus buffer solution having glycine and having pH greater than 10). Therefore, Teva asserts that the claims of the '137 patent are invalid for substantially the same reasons as the '007 patent.

Teva is not aware of any such secondary considerations that, when considered with the evidence of obviousness, would warrant a finding of non-obviousness of the claims of the '137 patent. During the prosecution of the '137 patent, applicants contended, without any declaration, that the claimed methods showed an unexpected antibacterial effect. But as shown above in the contentions for the '007 patent, the prior art clearly demonstrates that the antibacterial effect of

the claimed buffer was well-known and expected. If UTC relies on any secondary considerations of non-obviousness, Teva reserves the right to supplement its contentions.

The following prior art shows all of the limitations of the '137 patent, including the use of glycine buffers to inhibit bacterial growth, prior to September 7, 2007, the priority date of the '137 patent:

- EP '243 (TEVA_TRE_0004270-80)
- U.S. Patent Publication No. 2005/0165110 July 2005, Wade et al. ("Wade 2005") (TEVA_TRE_0004213-218)
- Crowther et al., "Growth of Microorganisms in Propofol, Thiopental, and a 1:1 Mixture Of Propofol and Thiopental," *Anesth. Analg., 82:* 475-478 (1996) ("Crowther") (TEVA_TRE_0004034-7)
- Siqueira et al., Mechanisms of antimicrobial activity of calcium hydroxide: a critical review," Intern. Endodontic J., 32, pp. 361-369, (1999) ("Siqueira) (TEVA_TRE_0004298-306)
- Foegeding P.M. et al, "Chemical Food Preservatives," Ch. 47 in Disinfection, Sterilization and Preservation 4th Ed. 1991. (TEVA_TRE_0004267-9)
- Hammes et al., "Mode of Action of Glycine on the Biosynthesis of Peptidoglycoan," J. Bacteriology, Vol. 116, No. 2 pp. 1029-1053 (1973) (TEVA_TRE_0004042-66)
- Strominger et al., "Nucleotide Accumulation Induced in Staphylococcus aureus by Glycine," J. Bacteriology, Vol. 89, No. 4 pp. 1124-1127 (1965) (TEVA_TRE_0004038-41)
- "Buffers: A guide for the preparation and use of buffers in biological systems" by Calbiochem ("Calbiochem") (TEVA_TRE_0003997-4033)
- 2006 Remodulin Package Insert (TEVA_TRE_0004285-97)
- 1999 Flolan Package Insert (TEVA_TRE_0004281-84)
- The 2005 Physicians' Desk Reference for Flolan® (epoprostenol sodium for injection) ("2005 PDR") (TEVA_TRE_0003991-6)
- Petrucci, R. and Harwood, W., General Chemistry Principles and Modern Applications, 6th Ed., pp. 656-57 (1993) (Teva_TRE_0003971-4)

- Prior art disclosed or cited during prosecution of the '007, '137, and '694 patents.

Teva expressly reserves the right to modify and/or supplement the above list at any time as necessary and/or as discovery progresses.

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1	A method of reducing occurrence of a bacterial infection in a human suffering from pulmonary arterial hypertension, who is undergoing treatment for said pulmonary hypertension, associated with occurrence of a bacterial infection comprising diluting a starting solution of an active pharmaceutical ingredient other than epoprostenol with a buffer comprising glycine and having a pH of greater than 10 to provide a final solution with a pH of greater than 10 and an amount of the active pharmaceutical ingredient other than epoprostenol effective for treating pulmonary arterial hypertension, and administering said final solution to the human subject in need thereof.	Claim 1 of the '137 patent is invalid for the same reasons as the '007 patent and Teva hereby incorporates by reference the analysis set forth for claim 1 of the '007 patent. The '137 patent shares the same priority date as the '007 patent, substantially the same specification, and is in the same family of patent as the '007 patent. The scope of the claims, accordingly, are substantially similar and comprise generally the same subject matter (e.g., reducing infection with treprostinil plus buffer solution having glycine and having pH greater than 10). Claim 1 of the '137 patent specifies that "a human is suffering from pulmonary arterial hypertension, who is undergoing treatment for said pulmonary hypertension, associated with occurrence of a bacterial infection" and that the product is an "active pharmaceutical ingredient other than epoprostenol." The prior art treprostinil formulation is an active pharmaceutical ingredient other than epoprostenol and, as shown below, the teachings of the prior art sufficiently disclose and teach that the person to whom the pharmaceutical composition is administered suffers from PAH and may suffer a bacterial infection (due to having a non-sterile solution). Therefore, the same analysis and prior art would apply to the invalidity contention for the '137 patent, and if the '007 patent is invalid, the '137 patent would be invalid as well. **Anticipation:** Claim 1 of the '137 patent is invalid, because it is anticipated by European Patent Application EP 0347243A1 ("EP '243") or obvious over EP '243 in view of Sterile Diluent For Flolan and/or knowledge of one of ordinary skill in the prior art. EP '243 issued on December 20, 1989, and is, thus, 102(b) prior art. EP '243 patent disclosed and claimed medicaments for the treatment of persons suffering from pulmonary hypertension that could be used subcutaneously or intravenously. EP '243, ¶¶ 22, 25.

	'137 Patent Claim Language	Invalidity Contentions
		Example 1 discloses the combination of treprostinil and a "glycine buffer" with a pH of 10.5. EP '243 further describes the use of buffer solutions with treprostinil to treat pulmonary hypertension. EP '243 concludes that treprostinil used with a glycine buffer solution of greater than pH 10 "was found to reduce hypoxia-induced increase in pulmonary arterial pressure and pulmonary vascular resistance in a dose-related manner without appreciably affecting cardiac output or heart rate." *Id.* at ¶¶ 32-34. EP '243 discloses that "sterile" acqueous solutions are preferred and that "[s]uch preparations may conveniently be prepared by admixing the compound with water or a glycine buffer and rendering the resulting solution sterile and isotonic with the blood." *Id.* at ¶ 25. Therefore, the pharmaceutical preparation of EP '243 inherently inhibits growth of gram positive bacteria associated with occurrence of a bacterial infection and, if given to a person, will inherently kill gram negative bacteria. Therefore, EP '243 discloses "a pharmaceutical preparation" or "pharmaceutical composition" comprising "treprostinil" (active ingredient other than epoprostenol") and "a buffer comprising glycine and having a pH of greater than 10" with the inherent qualities described in the claims. Moreover, having a low buffer capcity is an inherent property of a sterile solutions with a high pH that are isotonic with the blood and are intended to be given to humans, as the pH of the pharmaceutical preparation should quickly adjust to the pH of blood, which is substantially lower than pH of 10. *Id.* **Obviousness:** If EP '243 does not anticipate the '137 patent, the '137 patent is invalid as obvious over EP '243 patent in view of the commercial embodiment Sterile Diluent for Flolan®. Flolan® is a third party competitive product, containing epoprostenol, which was approved in 1995 for treating pulmonary hypertension. Flolan® is a powder that must be reconstituted with "Sterile Diluent for Flolan" ("SDF"). SDF is a solution containing the amino acid glycine and having a pH greater than 10 that physicians or patients may use to dilute Flolan prior to intravenous infusion. The use of SDF (or a buffer such

	'137 Patent Claim Language	Invalidity Contentions
		as SDF) was described, for example, in 1999 Flolan® label and U.S. Patent No. 4,335,139. SDF was available more than 1 year prior to the earliest priority date of the '137 patent and is 102(b) prior art to the '137 patent. A person of ordinary would have found it obvious to combine Remodulin in combination with SDF, based on the teachings of EP '243, the existing knowledge and use of SDF, and knowledge of one of ordinary skill in the art, with a reasonable expectation of success at arriving at the claimed invention. *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 421 (2007). As of the priority date of the '137 patent, Remodulin (treprostinil) was the commercially-available treprostinil product, and SDF was the only commercially-available glycine buffer with a pH of 10.5, already in use with another pulmonary hypertension medication. And as the district court in the related case, *UTC v. Sandoz*, 12-CV-01617, 13-CV-316 (D.N.J. 2014), expressly found, this combination would meet all of the asserted claims of the '007 patent (and therefore meet the limitations of the '137 patent). (Decision at 73.) UTC's expert in the related *Sandoz* matter, Dr. Michael Miller, admitted at trial that a person of ordinary skill in the art, seeking to practice the invention disclosed and claimed in EP '243, could easily have done so by combining Remodulin and Sterile Diluent for Flolan, both of which were commercially available products as of the priority date for the '137 patent. A person of ordinary skill in the art also would have been motivated to use treprostinil with a high pH buffer comprising glycine with a reasonable expectation of success in inhibiting bacterial growth or reducing the occurrence of blood stream infections. The use of SDF resulted in a high pH glycine buffer solution that was sterile, antibacterial and anti-infective. Moreover, it was well-known in the prior art that glycine is an amino acid that has antibacterial properties. *See e.g.* Foegeding P.M. et al, "Chemical Food Preservatives," Ch. 47 at 825 in Disinfection, Sterilization and Preservation 4th Ed. 1991; Hammes et al., "Mode of Action of Glycine on the Biosynthesis of Peptidoglycoan," J. Bacteriology, Vol. 116, No. 2 pp. 1029-1053 (1973); Strominger et al., "Nucleotide

	'137 Patent Claim Language	Invalidity Contentions
		Accumulation Induced in Staphylococcus aureus by Glycine," J. Bacteriology, Vol. 89, No. 4 pp. 1124-1127 (1965). Therefore, as the following prior art explains, as of the priority date, it was also known that a solution in an alkali environment (high pH solutions) with glycine will have bactericidal antiinfective effects. *See, e.g.*, Mendonca, et al, "Destruction of Gram-Negative Food-Borne Pathogens by High pH Involves Disruption of the Cytoplasmic Membrane,: Applied and Environmental Microbiology, vol. 60, No. 11, p. 4009-4014 (1994). ("Mendonca") (disclosed during the prosecution of the '007 patent); Crowther et al., "Growth of Microorganisms in Propofol, Thiopental, and a 1:1 Mixture Of Propofol and Thiopental," Anesth. Analg., 82: 475-478 (1996) ("Crowther") (TEVA_TRE_0004034-7); and Siqueira et al., Mechanisms of antimicrobial activity of calcium hydroxide: a critical review," Intern. Endodontic J., 32, pp. 361-369, (1999) ("Siqueira) (TEVA_TRE_0004298-306). Consistent with the fact that glycine and high pH solutions have known antibacterial properties, the prior art describes glycine buffer solutions that have high pH for use in pharmaceutical formulations. *See* U.S. Appln. No. 10/137,331 (disclosed during prosecution of the '007 patent); 1999 Flolan Package Insert (TEVA_TRE_0004281-84) (disclosing the use of SDF and SDF's qualities); EP '243 (TEVA_TRE_0004270-80) (discussed in more detail above); U.S. Patent Publication No. 2005/0165110 (July 2005) by Wade et al. at [0030] ("Wade 2005") (TEVA_TRE_0004213-218); and The 2005 Physicians' Desk Reference for Flolan® (epoprostenol sodium for injection) ("2005 PDR") (TEVA_TRE_0003991-6) (disclosing SDF, its use, and qualities). Indeed, as discussed earlier, the prior art specifically describes, suggests, and combines treprostinil with a high pH glycine buffer solutions for use in pharmaceutical compositions for humans. See EP '243; Wade 2005. As is evident from thee disclosures, a person of ordinary skill would have been motivated to address possible complications from bacterial infections when treprostinil is administered intravenously, as suggested by the use of SDF and the disclosures of EP '243.

	'137 Patent Claim Language	Invalidity Contentions
		A solution having "a low buffer capacity," if not inherent, also would have been known to a person of ordinary skill in the art. (Claims 1-5, 7-10, 16-17, and 21 also require that the glycine buffer used in the claimed methods have a low buffer capacity, so this analysis applies to the other claims with equal force.) The '137 patent states that "the buffer capacity should be low to avoid pH changes in the blood upon infusion." Col. 2, lines 34-35. SDF inherently possesses this limitation. Moreover, it would have been obvious to a person of ordinary skill in the art that it is important to maintain the proper pH of blood to avoid possible severe complications. *See, e.g.*, Petrucci, R. and Harwood, W., General Chemistry Principles and Modern Applications, 6th Ed., 1993, pp. 656-57 (explaining that the normal pH of blood is 7.4 and increased pH of blood can lead to severe vomiting and hyperventilation). Consequently, it would have been obvious to a person of ordinary skill to formulate the high pH buffer solution with a low buffer capacity, so that it would be safe and avoid any complications based on changes of blood pH when the treprostinil solution is administered. *See also* EP '243 at 5. Plaintiff has not set forth its contentions concerning secondary considerations in this case. If Plaintiff relies on any secondary considerations of non-obviousness, Teva reserves the right to supplement its contentions.
2	The method of claim 1, wherein the buffer further comprises sodium hydroxide.	Dependent claim 2 incorporates the method of claim 1, so the contentions incorporate herein by reference the analysis and prior art of claim 1. The additional limitation of sodium hydroxide is disclosed in the prior art cited therein and would have required only routine optimization. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
3	The method of claim 1, wherein the buffer has a pH between 10 and 12.	Dependent claim 3 incorporates the method of claim 1, so the contentions incorporate herein by reference the analysis and prior art of claim 1. The additional pH limitation is disclosed in the prior art cited therein (e.g., EP '243 discloses pH above 10) and would have required only routine optimization. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
4	The method of claim 3, wherein the buffer has a pH between	Dependent claim 4 incorporates the method of claim 1, so the contentions incorporate herein by reference the

	'137 Patent Claim Language	Invalidity Contentions
	10.2 and 10.8.	analysis and prior art of claim 1. The additional pH limitation is disclosed in the prior art cited therein (e.g., EP '243 discloses pH above 10) and would have required only routine optimization. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
5	The method of claim 1, wherein the final solution is administered at a concentration between about 0.001 mg/mL to about 1 mg/mL.	Dependent claim 5 incorporates the method of claim 1, so the contentions incorporate herein by reference the analysis and prior art of claim 1. The additional concentration limitation is disclosed in the prior art cited therein (e.g., 2005 PDR discloses the concentrations of Remodulin) and would have required only routine optimization. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
6	The method of claim 1, wherein the final solution is administered at a concentration between about 0.004 mg/mL to about 0.13 mg/mL.	Dependent claim 6 incorporates the method of claim 1, so the contentions incorporate herein by reference the analysis and prior art of claim 1. The additional concentration limitation is disclosed in the prior art cited therein (e.g., EP '243 discloses pH above 10) and would have required only routine optimization. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
7	The method of claim 1, wherein the administering is by injection.	Dependent claim 7 incorporates the method of claim 1, so the contentions incorporate herein by reference the analysis and prior art of claim 1. The additional "injection" limitation is disclosed in the prior art cited therein (e.g., EP '243 discloses injections and Flolan was an injection) and would have required only routine optimization. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
8	The method of claim 7, wherein the injection is intravenous injection.	Dependent claim 8 incorporates the method of claim 7, so the contentions incorporate herein by reference the analysis and prior art of claim 7. The additional intravenous injection limitation is disclosed in the prior art cited therein (e.g., EP '243 discloses intravenous injection and flolan was an intravenous injection) and would have required only routine optimization. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
9	The method according to claim 1, wherein the administration reduces the growth of gram negative bacteria.	Dependent claim 9 incorporates the method of claim 1, so the contentions incorporate herein by reference the analysis and prior art of claim 1. The additional bacterial limitation is disclosed in the prior art cited therein (e.g., EP '243 discloses a sterile solution that would have this inherent quality) and would have required only routine optimization. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.

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10	The method of claim 4, wherein the final solution is administered intravenously.	Dependent claim 10 incorporates the method of claim 4, so the contentions incorporate herein by reference the analysis and prior art of claim 4. The additional intravenous injection limitation is disclosed in the prior art cited therein (e.g., EP '243 discloses intravenous administration and Flolan was an intravenous administration) and would have required only routine optimization. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
11	The method of claim 1, wherein the buffer is a 50 mL solution of 94 mg of glycine, 73.3 mg of sodium chloride, and sodium hydroxide.	Dependent claim 11 incorporates the method of claim 1, so the contentions incorporate herein by reference the analysis and prior art of claim 1. The additional limitation specifying amount of ingredients is disclosed in the prior art cited therein (e.g., SDF) and would have required only routine optimization to arrive at the amounts to achieve the necessary pH and sterile qualities. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
12	The method of claim 11, wherein the administering is by injection.	Dependent claim 12 incorporates the method of claim 11, so the contentions incorporate herein by reference the analysis and prior art of claim 11. The additional injection limitation is disclosed in the prior art cited therein (e.g., EP '243 discloses injection and flolan was an injection) and would have required only routine optimization. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
13	The method of claim 12, wherein the injection is intravenous injection.	Dependent claim 13 incorporates the method of claim 12, so the contentions incorporate herein by reference the analysis and prior art of claim 12. The additional intravenous injection limitation is disclosed in the prior art cited therein (e.g., EP '243 discloses intravenous injection and flolan was an intravenous injection) and would have required only routine optimization. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.

E. Invalidity of United States Patent No. 8,658,694

United States Patent No. 8,658,694, entitled "Buffer solutions having selective bactericidal activity against gram negative bacteria and methods of using same," was issued on February 25, 2014 with 26 claims. UTC asserts that Teva infringes claims 1-26 of the '694 patent. Claims 1 and 11 are the only independent claims of the '694 patent:

1. A method of treating pulmonary arterial hypertension comprising diluting a starting solution of treprostinil or treprostinil sodium with a buffer comprising glycine and having a pH of greater than 10 to provide a final solution with a pH of greater than 10 and an effective amount of treprostinol or treprostinil sodium for treating pulmonary arterial hypertension, and administering said final solution to a human subject in need thereof.

11. A method of reducing occurrence of a bacterial infection in a human suffering from pulmonary arterial hypertension, who is undergoing treatment for said pulmonary hypertension, comprising diluting a starting solution of treprostinil or treprostinil sodium with a buffer comprising glycine and having a pH of greater than 10 to provide a final solution with a pH of greater than 10 and an amount of treprostinil or treprostinil sodium effective for treating pulmonary arterial hypertension, and administering said final solution to the human subject in need thereof.

The '694 patent shares the same priority date as the '007 patent, substantially the same specification, and is in the same family of patent as the '007 patent. The scope of the claims, accordingly, is substantially similar and comprises generally the same subject matter (e.g., reducing infection or treating PAH with treprostinil plus buffer solution having glycine and having pH greater than 10). Therefore, Teva asserts that the claims of the '694 patent are invalid for substantially the same reasons as the '007 patent.

No evidence of secondary considerations of non-obviousness were presented during the prosecution of the '694 patent, and Teva is not aware of any such secondary considerations that, when considered with the evidence of obviousness, would warrant a finding of non-obviousness of the claims of the '694 patent. To the extent Plaintiff is relying on their contentions during the prosecution history of the '694 patent, then the prior art rebuts this contention of unexpected results. If UTC relies on any secondary considerations of non-obviousness, Teva reserves the right to supplement its contentions.

The following prior art shows all of the limitations of the '694 patent, including the use of glycine buffers to inhibit bacterial growth, prior to September 7, 2007, the priority date of the '694 patent:

- EP '243 (TEVA_TRE_0004270-80)
- U.S. Patent Publication No. 2005/0165110 July 2005, Wade et al. ("Wade 2005") (TEVA_TRE_0004213-218)
- Crowther et al., "Growth of Microorganisms in Propofol, Thiopental, and a 1:1 Mixture Of Propofol and Thiopental," *Anesth. Analg., 82:* 475-478 (1996) ("Crowther") (TEVA_TRE_0004034-7)
- Siqueira et al., Mechanisms of antimicrobial activity of calcium hydroxide: a critical review," Intern. Endodontic J., 32, pp. 361-369, (1999) ("Siqueira) (TEVA_TRE_0004298-306)
- Foegeding P.M. et al, "Chemical Food Preservatives," Ch. 47 in Disinfection, Sterilization and Preservation 4th Ed. 1991. (TEVA_TRE_0004267-9)
- Hammes et al., "Mode of Action of Glycine on the Biosynthesis of Peptidoglycoan," J. Bacteriology, Vol. 116, No. 2 pp. 1029-1053 (1973) (TEVA_TRE_0004042-66)
- Strominger et al., "Nucleotide Accumulation Induced in Staphylococcus aureus by Glycine," J. Bacteriology, Vol. 89, No. 4 pp. 1124-1127 (1965) (TEVA_TRE_0004038-41)
- "Buffers: A guide for the preparation and use of buffers in biological systems" by Calbiochem ("Calbiochem") (TEVA_TRE_0003997-4033)
- 2006 Remodulin Package Insert (TEVA_TRE_0004285-97)
- 1999 Flolan Package Insert (TEVA_TRE_0004281-84)
- The 2005 Physicians' Desk Reference for Flolan® (epoprostenol sodium for injection) ("2005 PDR") (TEVA_TRE_0003991-6)
- Petrucci, R. and Harwood, W., General Chemistry Principles and Modern Applications, 6th Ed., pp. 656-57 (1993) (Teva_TRE_0003971-4)
- Prior art disclosed or cited during prosecution of the '007, '137, and '694 patents.

Teva expressly reserves the right to modify and/or supplement the above list at any time as necessary and/or as discovery progresses.

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1	1. A method of treating pulmonary arterial hypertension comprising diluting a starting solution of treprostinil or treprostinil sodium with a buffer comprising glycine and having a pH of greater than 10 to provide a final solution with a pH of greater than 10 and an effective amount of treprostinol[sic] or treprostinil sodium for treating pulmonary arterial hypertension, and administering said final solution to a human subject in need thereof.	Claim 1 of the '694 patent is invalid for the same reasons as the '007 patent and Teva hereby incorporates by reference the analysis set forth for claim 1 of the '007 patent. The '694 patent shares the same priority date as the '007 patent, substantially the same specification, and is in the same family of patent as the '007 patent. The scope of the claims, accordingly, are substantially similar and comprise generally the same subject matter (e.g., treating pulmonary arterial hypertension with treprostinil plus buffer solution having glycine and having pH greater than 10). Claim 1 of the '694 patent specifies treating a person with pulmonary arterial hypertension and administering the solution with trepsotinil and glycine having pH above 10 to such a person. There is no meaningful patentable difference between claims of the '694 patent and the '007 patent. The prior art teaches that the person to whom the pharmaceutical composition is administered suffers from PAH. Therefore, the same analysis and prior art would apply to the invalidity contention for the '694 patent and if the '007 patent is invalid, so would be the '694 patent. **Anticipation:** Claim 1 of the '694 patent is invalid, because it is anticipated by European Patent Application EP 0347243A1 ("EP '243") or obvious over EP '243 in view of Sterile Diluent For Flolan and/or knowledge of one of ordinary skill in the prior art. EP '243 issued on December 20, 1989, and is, thus, 102(b) prior art. EP '243 patent disclosed and claimed medicaments for the treatment of persons suffering from pulmonary hypertension that could be used subcutaneously or intravenously. EP '243, ¶¶ 22, 25. Example 1 discloses the combination of treprostinil and a "glycine buffer" with a pH of 10.5. EP '243 further describes the use of buffer solutions with treprostinil to treat pulmonary hypertension. EP '243 concludes that treprostinil used with a glycine buffer solution of greater than pH 10 "was found to reduce

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		hypoxia-induced increase in pulmonary arterial pressure and pulmonary vascular resistance in a dose-related manner without appreciably affecting cardiac output or heart rate." *Id.* at ¶¶ 32-34. EP '243 discloses that "sterile" acqueous solutions are preferred and that "[s]uch preparations may conveniently be prepared by admixing the compound with water or a glycine buffer and rendering the resulting solution sterile and isotonic with the blood." *Id.* at ¶ 25. Therefore, the pharmaceutical preparation of EP '243 inherently inhibits growth of gram positive bacteria associated with occurrence of a bacterial infection and, if given to a person, will inherently kill gram negative bacteria. Therefore, EP '243 discloses "a pharmaceutical preparation" or "pharmaceutical composition" comprising "treprostinil" and "a buffer comprising glycine and having a pH of greater than 10" with the inherent qualities described in the claims. Moreover, having a low buffer capcity is an inherent property of a sterile solutions with a high pH that are isotonic with the blood and are intended to be given to humans, as the pH of the pharmaceutical preparation should quickly adjust to the pH of blood, which is substantially lower than pH of 10. *Id.* **Obviousness:** If EP '243 does not anticipate the '694 patent, '694 patent is invalid as obvious over EP '243 patent in view of the commercial embodiment Sterile Diluent for Flolan®. Flolan® is a third-party competitive product, containing epoprostenol, which was approved in 1995 for treating pulmonary hypertension. Flolan® is a powder that must be reconstituted with "Sterile Diluent for Flolan" ("SDF"). SDF is a solution containing the amino acid glycine and having a pH greater than 10 that physicians or patients may use to dilute Flolan prior to intravenous infusion. The use of SDF (or a buffer such as SDF) was described, for example, in 1999 Flolan® label and U.S. Patent No. 4,335,139. SDF was available more than 1 year prior to the earliest priority date of the '694 patent and is 102(b) prior art to the '694 patent. A person of ordinary would have found it obvious to combine Remodulin in combination with SDF, based on the teachings of EP '243, the existing knowledge and use

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		of SDF, and knowledge of one of ordinary skill in the art, with a reasonable expectation of success at arriving at the claimed invention. *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 421 (2007). As of the priority date of the '694 patent, Remodulin (treprostinil) was the commercially-available treprostinil product, and SDF was the only commercially-available glycine buffer with a pH of 10.5, already in use with another pulmonary hypertension medication. And as the district court in the related case, *UTC v. Sandoz*, 12-CV-01617, 13-CV-316 (D.N.J. 2014), expressly found, this combination would meet all of the asserted claims of the '007 patent (and therefore would meet the limitations of the '694 patent). (Decision at 73.) UTC's expert in the related *Sandoz* matter, Dr. Michael Miller, admitted at trial that a person of ordinary skill in the art, seeking to practice the invention disclosed and claimed in EP '243, could easily have done so by combining Remodulin and Sterile Diluent for Flolan, both of which were commercially available products as of the priority date for the '694 patent. A person of ordinary skill in the art also would have been motivated to use treprostinil with a high pH buffer comprising glycine with a reasonable expectation of success in inhibiting bacterial growth or reducing the occurrence of blood stream infections. The use of SDF resulted in a high pH glycine buffer solution that was sterile, antibacterial and anti-infective. Moreover, it was well-known in the prior art that glycine is an amino acid that has antibacterial properties. *See e.g.* Foegeding P.M. et al, "Chemical Food Preservatives," Ch. 47 at 825 in Disinfection, Sterilization and Preservation 4th Ed. 1991; Hammes et al., "Mode of Action of Glycine on the Biosynthesis of Peptidoglycoan," J. Bacteriology, Vol. 116, No. 2 pp. 1029-1053 (1973); Strominger et al., "Nucleotide Accumulation Induced in Staphylococcus aureus by Glycine," J. Bacteriology, Vol. 89, No. 4 pp. 1124-1127 (1965). Therefore, as the following prior art explains, as of the priority date, it was also known that a solution in an alkali environment (high pH solutions) with glycine will have bactericidal antiinfective effects. *See, e.g.*, Mendonca, et al, "Destruction of Gram-Negative Food-Borne Pathogens by High pH Involves Disruption of the

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		Cytoplasmic Membrane,: Applied and Environmental Microbiology, vol. 60, No. 11, p. 4009-4014 (1994). ("Mendonca") (disclosed during the prosecution of the '007 patent); Crowther et al., "Growth of Microorganisms in Propofol, Thiopental, and a 1:1 Mixture Of Propofol and Thiopental," Anesth. Analg., 82: 475-478 (1996) ("Crowther") (TEVA_TRE_0004034-7); and Siqueira et al., Mechanisms of antimicrobial activity of calcium hydroxide: a critical review," Intern. Endodontic J., 32, pp. 361-369, (1999) ("Siqueira) (TEVA_TRE_0004298-306). Consistent with the fact that glycine and high pH solutions have known antibacterial properties, the prior art describes glycine buffer solutions that have high pH for use in pharmaceutical formulations. *See* U.S. Appln. No. 10/137,331 (disclosed during prosecution of the '007 patent); 1999 Flolan Package Insert (TEVA_TRE_0004281-84) (disclosing the use of SDF and SDF's qualities); EP '243 (TEVA_TRE_0004270-80) (discussed in more detail above); U.S. Patent Publication No. 2005/0165110 (July 2005) by Wade et al. at [0030] ("Wade 2005") (TEVA_TRE_0004213-218); and The 2005 Physicians' Desk Reference for Flolan® (epoprostenol sodium for injection) ("2005 PDR") (TEVA_TRE_0003991-6) (disclosing SDF, its use, and qualities). Indeed, as discussed earlier, the prior art specifically describes, suggests, and combines treprostinil with a high pH glycine buffer solutions for use in pharmaceutical compositions for humans. See EP '243; Wade 2005. As is evident from thee disclosures, a person of ordinary skill would have been motivated to address possible complications from bacterial infections when treprostinil is administered intravenously, as suggested by the use of SDF and the disclosures of EP '243. A solution having "a low buffer capacity," if not inherent, also would have been known to a person of ordinary skill in the art. (Claims 1-5, 7-10, 16-17, and 21 also require that the glycine buffer used in the claimed methods have a low buffer capacity, so this analysis applies to the other claims with equal force.) The '137 patent states that "the buffer capacity should be low to

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		avoid pH changes in the blood upon infusion." Col. 2, lines 34-35. SDF inherently possesses this limitation. Moreover, it would have been obvious to a person of ordinary skill in the art that it is important to maintain the proper pH of blood to avoid possible severe complications. *See e.g.* Petrucci, R. and Harwood, W., General Chemistry Principles and Modern Applications, 6th Ed., 1993, pp. 656-57 (explaining that the normal pH of blood is 7.4 and increased pH of blood can lead to severe vomiting and hyperventilation). Consequently, it would have been obvious to a person of ordinary skill to formulate the high pH buffer solution with a low buffer capacity, so that it would be safe and avoid any complications based on changes of blood pH when the treprostinil solution is administered. *See also* EP '243 at 5. Plaintiff has not set forth its contentions concerning secondary considerations in this case. If Plaintiff relies on any secondary considerations of non-obviousness, Teva reserves the right to supplement its contentions.
2	The method of claim 1, wherein the buffer further comprises sodium hydroxide.	This dependent claim incorporates the method of claim 1, so the contentions incorporate herein by reference the analysis and prior art of claim 1. Moreover, the additional limitation of this claim is substantially identical to the dependent limitations of the '007 and '137 patent and has been dicussed in full detail with respect to those patents and the dependent claims therein. The additional limitation is disclosed in the prior art cited in those discusions. *See, e.g.*, 1999 Flolan Package Insert (disclosing a buffer with sodium hydroxide), the 2005 PDR, and Calbiochem.
3	The method of claim 1, wherein the buffer has a pH between 10 and 12.	This dependent claim incorporates the method of claim 1, so the contentions incorporate herein by reference the analysis and prior art of claim 1. Moreover, the additional limitation of this claim is substantially identical to the dependent limitations of the '007 and '137 patent and has been dicussed in full detail with respect to those patents and the dependent claims therein. The additional limitation is disclosed in the prior art cited in those discusions. *See, e.g.*, 1999 Flolan Package Insert (disclosing a solution with pH of over 10), the 2005 PDR, and Calbiochem.
4	The method of claim 3, wherein the buffer has a pH	This dependent claim incorporates the method of claim 1, so the contentions incorporate herein by reference the

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	between 10.2 and 10.8.	analysis and prior art of claim 1. Moreover, the additional limitation of this claim is substantially identical to the dependent limitations of the '007 and '137 patent and has been dicussed in full detail with respect to those patents and the dependent claims therein. The additional limitation is disclosed in the prior art cited in those discusions. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
5	The method of claim 1, wherein the final solution is administered at a concentration between about 0.004 mg/mL to about 0.13 mg/mL.	This dependent claim incorporates the method of claim 1, so the contentions incorporate herein by reference the analysis and prior art of claim 1. Moreover, the additional limitation of this claim is substantially identical to the dependent limitations of the '007 and '137 patent and has been dicussed in full detail with respect to those patents and the dependent claims therein. The additional limitation is disclosed in the prior art cited in those discusions. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
6	The method of claim 1, wherein the administering is by injection.	This dependent claim incorporates the method of claim 1, so the contentions incorporate herein by reference the analysis and prior art of claim 1. Moreover, the additional limitation of this claim is substantially identical to the dependent limitations of the '007 and '137 patent and has been dicussed in full detail with respect to those patents and the dependent claims therein. The additional limitation is disclosed in the prior art cited in those discusions. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
7	The method of claim 6, wherein the injection is intravenous injection.	This dependent claim incorporates the method of claim 6, so the contentions incorporate herein by reference the analysis and prior art of claim 6. Moreover, the additional limitation of this claim is substantially identical to the dependent limitations of the '007 and '137 patent and has been dicussed in full detail with respect to those patents and the dependent claims therein. The additional limitation is disclosed in the prior art cited in those discusions. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
8	The method of claim 6, wherein the active pharmaceutical ingredient is treprostinil sodium.	This dependent claim incorporates the method of claim 6, so the contentions incorporate herein by reference the analysis and prior art of claim 6. Moreover, the additional limitation of this claim is substantially identical to the dependent limitations of the '007 and '137 patent and has been dicussed in full detail with respect to those patents and the dependent claims therein.

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		The additional limitation is disclosed in the prior art cited in those discusions. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
9	The method of claim 4, wherein the final solution is administered intravenously.	This dependent claim incorporates the method of claim 4, so the contentions incorporate herein by reference the analysis and prior art of claim 4. Moreover, the additional limitation of this claim is substantially identical to the dependent limitations of the '007 and '137 patent and has been dicussed in full detail with respect to those patents and the dependent claims therein. The additional limitation is disclosed in the prior art cited in those discusions. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
10	The method of claim 1, wherein the buffer is a 50 mL solution of 94 mg of glycine, 73.3 mg of sodium chloride, and sodium hydroxide.	This dependent claim incorporates the method of claim 1, so the contentions incorporate herein by reference the analysis and prior art of claim 1. Moreover, the additional limitation of this claim is substantially identical to the dependent limitations of the '007 and '137 patent and has been dicussed in full detail with respect to those patents and the dependent claims therein. The additional limitation is disclosed in the prior art cited in those discusions. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
11	A method of reducing occurrence of a bacterial infection in a human suffering from pulmonary arterial hypertension, who is undergoing treatment for said pulmonary hypertension, comprising diluting a starting solution of treprostinil or treprostinil sodium with a buffer comprising glycine and having a pH of greater than 10 to provide a final solution with a pH of greater than 10 and an amount of treprostinil or treprostinil sodium effective for treating pulmonary arterial hypertension, and administering said final solution to the human subject in need thereof.	This independent claim is substantially similar to claim 1. Therefore, Teva hereby incorporates herein the analysis set forth in claim 1. The minor differences in claim language, such as "reducing occurrence of a bacterial infection in a human suffering from pulmonary arterial hypertension, who is undergoing treatment for said pulmonary hypertension" has been addressed in the analysis for claim 1, and, therefore, does not require any further explanation here. To the extent that claim 1 is invalid, claim 11 should also be invalid.
12	The method of claim 11,	This dependent claim incorporates the method of claim

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	wherein the buffer further comprises sodium hydroxide.	11, so the contentions incorporate herein by reference the analysis and prior art of claim 11. Moreover, the additional limitation of this claim is substantially identical to the dependent limitations of the '007 and '137 patent and has been dicussed in full detail with respect to those patents and the dependent claims therein. The additional limitation is disclosed in the prior art cited in those discusions. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
13	The method of claim 11, wherein the buffer has a pH between 10 and 12.	This dependent claim incorporates the method of claim 11, so the contentions incorporate herein by reference the analysis and prior art of claim 11. Moreover, the additional limitation of this claim is substantially identical to the dependent limitations of the '007 and '137 patent and has been dicussed in full detail with respect to those patents and the dependent claims therein. The additional limitation is disclosed in the prior art cited in those discusions. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
14	The method of claim 13, wherein the buffer has a pH between 10.2 and 10.8.	This dependent claim incorporates the method of claim 13, so the contentions incorporate herein by reference the analysis and prior art of claim 13. Moreover, the additional limitation of this claim is substantially identical to the dependent limitations of the '007 and '137 patent and has been dicussed in full detail with respect to those patents and the dependent claims therein. The additional limitation is disclosed in the prior art cited in those discusions. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
15	The method of claim 11, wherein the final solution is administered at a concentration between about 0.001 mg/mL to about 1 mg/mL.	This dependent claim incorporates the method of claim 11, so the contentions incorporate herein by reference the analysis and prior art of claim 11. Moreover, the additional limitation of this claim is substantially identical to the dependent limitations of the '007 and '137 patent and has been dicussed in full detail with respect to those patents and the dependent claims therein. The additional limitation is disclosed in the prior art cited in those discusions. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
16	The method of claim 11, wherein the final solution is administered at a concentration between about 0.004 mg/mL to about 0.13 mg/mL.	This dependent claim incorporates the method of claim 11, so the contentions incorporate herein by reference the analysis and prior art of claim 11. Moreover, the additional limitation of this claim is substantially identical to the dependent limitations of the '007 and '137 patent and has been dicussed in full detail with

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		respect to those patents and the dependent claims therein. The additional limitation is disclosed in the prior art cited in those discusions. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
17	The method of claim 11, wherein the administering is by injection.	This dependent claim incorporates the method of claim 11, so the contentions incorporate herein by reference the analysis and prior art of claim 11. Moreover, the additional limitation of this claim is substantially identical to the dependent limitations of the '007 and '137 patent and has been dicussed in full detail with respect to those patents and the dependent claims therein. The additional limitation is disclosed in the prior art cited in those discusions. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
18	The method of claim 17, wherein the injection is intravenous injection.	This dependent claim incorporates the method of claim 11, so the contentions incorporate herein by reference the analysis and prior art of claim 11. Moreover, the additional limitation of this claim is substantially identical to the dependent limitations of the '007 and '137 patent and has been dicussed in full detail with respect to those patents and the dependent claims therein. The additional limitation is disclosed in the prior art cited in those discusions. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
19	The method of claim 17, wherein the active pharmaceutical ingredient is treprostinil sodium.	This dependent claim incorporates the method of claim 11, so the contentions incorporate herein by reference the analysis and prior art of claim 11. Moreover, the additional limitation of this claim is substantially identical to the dependent limitations of the '007 and '137 patent and has been dicussed in full detail with respect to those patents and the dependent claims therein. The additional limitation is disclosed in the prior art cited in those discusions. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
20	The method according to claim 11, wherein the administration reduces the growth of gram negative bacteria.	This dependent claim incorporates the method of claim 11, so the contentions incorporate herein by reference the analysis and prior art of claim 11. Moreover, the additional limitation of this claim is substantially identical to the dependent limitations of the '007 and '137 patent and has been dicussed in full detail with respect to those patents and the dependent claims therein. The additional limitation is disclosed in the prior art cited in those discusions. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
21	The method of claim 14,	This dependent claim incorporates the method of claim

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	wherein the final solution is administered intravenously.	14, so the contentions incorporate herein by reference the analysis and prior art of claim 14. Moreover, the additional limitation of this claim is substantially identical to the dependent limitations of the '007 and '137 patent and has been dicussed in full detail with respect to those patents and the dependent claims therein. The additional limitation is disclosed in the prior art cited in those discusions. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
22	The method of claim 11, wherein the buffer is a 50 mL solution of 94 mg of glycine, 73.3 mg of sodium chloride, and sodium hydroxide.	This dependent claim incorporates the method of claim 11, so the contentions incorporate herein by reference the analysis and prior art of claim 11. Moreover, the additional limitation of this claim is substantially identical to the dependent limitations of the '007 and '137 patent and has been dicussed in full detail with respect to those patents and the dependent claims therein. The additional limitation is disclosed in the prior art cited in those discusions. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
23	The method of claim 22, wherein the administering is by injection.	This dependent claim incorporates the method of claim 22, so the contentions incorporate herein by reference the analysis and prior art of claim 22. Moreover, the additional limitation of this claim is substantially identical to the dependent limitations of the '007 and '137 patent and has been dicussed in full detail with respect to those patents and the dependent claims therein. The additional limitation is disclosed in the prior art cited in those discusions. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
24	The method of claim 23, wherein the injection is intravenous injection.	This dependent claim incorporates the method of claim 23, so the contentions incorporate herein by reference the analysis and prior art of claim 23. Moreover, the additional limitation of this claim is substantially identical to the dependent limitations of the '007 and '137 patent and has been dicussed in full detail with respect to those patents and the dependent claims therein. The additional limitation is disclosed in the prior art cited in those discusions. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
25	The method of claim 10, wherein the administering is by injection.	This dependent claim incorporates the method of claim 10, so the contentions incorporate herein by reference the analysis and prior art of claim 10. Moreover, the additional limitation of this claim is substantially identical to the dependent limitations of the '007 and '137 patent and has been dicussed in full detail with

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		respect to those patents and the dependent claims therein. The additional limitation is disclosed in the prior art cited in those discusions. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.
26	The method of claim 25, wherein the injection is intravenous injection.	This dependent claim incorporates the method of claim 25, so the contentions incorporate herein by reference the analysis and prior art of claim 25. Moreover, the additional limitation of this claim is substantially identical to the dependent limitations of the '007 and '137 patent and has been dicussed in full detail with respect to those patents and the dependent claims therein. The additional limitation is disclosed in the prior art cited in those discusions. *See, e.g.*, 1999 Flolan Package Insert, the 2005 PDR, and Calbiochem.

Dated: April 24, 2015

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Organic Process Research & Development 2005, 9, 319–320

A New Purification Process for Pharmaceutical and Chemical Industries

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Abstract:

A novel separation and purification process suitable for pharmaceutical and chemical industries has been developed. The process is based on the difference in adsorption and solubility of organic compounds. The process was carried out under mechanical stirring, and individual components were isolated in short time with excellent purity. The process can be suitably adopted for the purification of organic compounds in large scale.

The separation and purification of organic compounds are very important to chemical and pharmaceutical industries. It is a challenging task to separate a required product from a mixture of components during industrial production. Even though different distillation[1] and recrystallization[2] techniques are widely employed in industries, the application of the above methods are limited and time-consuming, leading to cost escalation. The column chromatographic method,[3−5] used in some industries, is a process that is too complicated, particularly for large-scale production (Table 1).

To overcome the above barriers, herein we bring a preliminary communication of our new invention for the separation of organic compounds, which can be applied in kilogram reactors to purify drugs and chemicals. The process is very simple and does not require any special kind of glassware. The process is carried out under mechanical stirring in a round-bottom flask.

Thus, the crude reaction mixture to be purified was dissolved in a minimum amount of a suitable solvent, selected preferably from low-boiling solvents such as hexane, dichloromethane, chloroform, ethanol, etc. To this solution 3−4-fold (if the spots are close as in aniline and 4-nitroaniline 5−6-fold) of a selected adsorbent was added and mixed well. Then the solvent was removed completely under vacuum. To the above solvent-free slurry, a selected solvent or mixture of solvent was added and stirred mechanically; the solution was decanted, and the solvent was evaporated. When the quantity of solvent and length of stirring time were increased, comparatively more quantity of a particular compound was isolated. When the polarity of the solvent was slowly increased, successive components were isolated.

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10.1021/op048790d CCC: $30.25
Published on Web 04/27/2005

The success of the process is evident by the fact that it is able to separate a mixture of very close-moving (chromatographically) aniline and 4-nitroaniline. Aniline and 4-nitroaniline are moving in 5% ethyl acetate:petroleum ether, and the R_f difference between aniline and 4-nitroaniline is just 0.09. A variety of organic compounds that were mixed and isolated successfully are summarized in Table 2.

To demonstrate suitability the method for separation of the required component from a chemical reaction mixture, the technique was applied to Biginelli condensation, and pure 6-methyl-4-phenyl-1,2,3,4-tetrahydropyrimidin-5-carboxylic acid ethyl ester was isolated from a mixture of benzaldehyde, ethyl acetoacetate, and 6-methyl-4-phenyl-1,2,3,4-tetrahydropyrimidin-5-carboxylic acid ethyl ester. This reaction was carried out in 1-mole scale, and by employing our technique the quantitative separation of 6-methyl-4-phenyl-1,2,3,4-tetrahydropyrimidin-5-carboxylic acid ethyl ester was achieved with very high purity (99%).

Explanation of the Process with an Example. *p*-Methyl acetophenone (500 g) and resorcinol (500 g) were dissolved in 2 L of ethanol. To this solution 3 kg of neutral alumina activity I−II was added and mixed well; the solvent was removed completely under vacuum. To the above solvent-free slurry was added 2% ethyl acetate:petroleum ether 40−60 °C (7 L); the solution was stirred for 20 min and decanted, and the solvent was evaporated. The residue weighed 120 g of *p*-methyl acetophenone. Thus, five elutions (each elution was carried out with 7 L of solvent and 20 min stirring) in 2% ethyl acetate:petroleum ether at 40−60 °C separated 455 g of *p*-methyl acetophenone with 100% purity (based on gas chromatography). Then the polarity of solvent was increased to 5% ethyl acetate:petroleum ether 40−60 °C. In 5% ethyl acetate:petroleum ether 40−60 °C (7 L) mixture were isolated *p*-methyl acetophenone and resorcinol. The elution in 5% ethyl acetate:petroleum ether 40−60 °C was continued until the isolated resorcinol was single on TLC. Then 7 L of ethyl acetate was added and stirred for 20 min and then decanted; the solvent was evaporated. The elution in ethyl acetate was repeated for three times to complete the isolation of resorcinol. Thus 400 g of pure (100%, based on gas chromatography) resorcinol was isolated. The mixture of *p*-methyl acetophenone and resorcinol isolated in 5% ethyl acetate:petroleum ether 40−60 °C were combined, solvent was removed, and the process was repeated. Thus, each component was isolated in almost pure state in a short time.

The results of our study indicate the following salient features: (1) Direct separation based on solubility differences gave a mixture of two products, whereas adsorption on silica gel followed by elution with same solvent gave separation

Table 1. **Comparison between chromatographic method and our new method**

s. no.		purification based on column chromatographic method	purification based on our new method
1	ratio of adsorbent (employed in the purification process) to compound	25−50:1[6]	4−6:1
2	quantity of solvent required	large excess of solvent is required for continuous elution	minimal solvent consumption; 2−3 times the slurry weight is sufficient
3	time (for 1-kg batch)	several hours	10 h
4	apparatus	very large size colum is required	does not require special equipment; performed in reactor vessel.

Table 2. **Separation of some mixed compounds based on our new technique**

				yield[a] %		purity[b]	
s. no.	cmpd 1	cmpd 2	adsorbent	cmpd 1	cmpd 2	cmpd 1	cmpd 2
1	benzophenone	dimedone	silica gel 60−120 mesh	98	97	100	98
2	aniline	4-nitroaniline	neutral alumina activity I−II	98	98	99	98
3	*p*-chlorobenzaldehyde	acetoacetanilide	neutral alumina activity I−II	99	97	100	99
4	*p*-methylacetophenone	resorcinol	neutral alumina activity I−II	97	96	100	100[c]

[a] After purification. [b] Based on gas chromatography. [c] Carried out in 40-g as well as in 1-kg scale.

of the chemical mixture. Example: dimedone and benzophenone.

(2) The nature of the adsorbent plays a vital role in the above separation process.[7] Low-grade adsorbent is preferable when the compounds are not close moving on TLC. When ethyl acetoacetate, benzaldehyde, and 6-methyl-4-phenyl-1,2,3,4-tetrahydropyrimidin-5-carboxylic acid ethyl ester were adsorbed on silica gel, the isolation of product was very easy compared to the adsorption on neutral alumina activity I−II.

(3) High-grade adsorbent is preferable when the compounds are close moving on TLC. For example, aniline and 4-nitroaniline adsorption on silica gel gave a mixture of two compounds, whereas adsorption on neutral alumina activity I−II resulted in the separation of individual compounds.

(4) The eluent for the separation of a particular compound from a mixture was chosen on the basis of a trial and error method. The prepared slurry was collected in different vials, solvent systems of increasing polarity were added, and the eluates were analyzed by TLC to scout for the best solvent system. It is recommended to use a solvent of slightly reduced polarity and then choose one from TLC analysis to perform large-scale elution.

We strongly believe that further intensive research in this technique will enhance its application to the separation of all kinds of organic compounds of industrial importance.

Acknowledgment

P.A. dedicates this article to Mr. P. G. Jayapalan, M.A. B.Ed., and Mrs. P. G. J. Vijayalakshmi, Kanchipuram. We thank the Council of Scientific and Industrial Research, India, for financial support.

Received for review November 24, 2004.

OP049790D

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JOC Note

An Enantioselective Synthesis of (*S*)-(+)-3-Aminomethyl-5-methylhexanoic Acid via Asymmetric Hydrogenation

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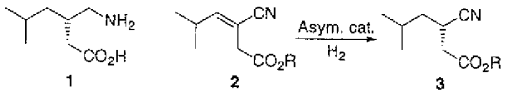
Received March 27, 2003

Abstract: A concise enantioselective synthesis of (*S*)-(+)-3-aminomethyl-5-methylhexanoic acid (**1**, Pregabalin) has been developed. The key step is the asymmetric hydrogenation of a 3-cyano-5-methylhex-3-enoic acid salt **2** with a rhodium Me-DuPHOS catalyst, providing the desired (*S*)-3-cyano-5-methylhexanoate **3** in very high ee. Subsequent hydrogenation of the nitrile **3** with a heterogeneous nickel catalyst provides Pregabalin **1** in excellent overall yield and purity.

(*S*)-(+)-3-Aminomethyl-5-methylhexanoic acid (**1**, Pregabalin) is a potent anticonvulsant related to the inhibitory neurotransmitter γ-aminobutyric acid.[2] Since the biological activity resides in the (*S*)-enantiomer, an enantioselective synthesis is required. During the initial development of Pregabalin **1** several routes were examined in considerable detail.[3] The preferred process to emerge from these studies starts with the condensation of diethyl malonate and isobutyraldehyde. After a further 4 steps, resolution with (*S*)-(+)-mandelic acid provides (*S*)-Pregabalin **1** in 25−29% overall yield. Although this route is cost-effective, the use of a late-stage resolution without the opportunity to efficiently recycle the off-isomer is inefficient and there was clearly scope for developing a more economical process. Asymmetric catalytic hydrogenation of a suitable prochiral precursor such as **2** was identified as a potential route to **1** via intermediate **3**.



1 **2** **3**

Considerable precedent exists for the asymmetric hydrogenation of β-substituted itaconic acid derivatives. Rhodium−phosphine complexes generally provide the desired 2-substituted succinates with high enantioselectivity.[4] In particular, hydrogenation of itaconate salts with Rh-DuPHOS catalysts provides significant rate enhancement, increases the selectivity, and allows mixtures of geometrical isomers to be hydrogenated to a single product. This is in sharp contrast to previous catalyst systems for which considerable differences were noted for the different geometrical isomers. Chiral ruthenium complexes have also found some application, but in general these are less effective than rhodium complexes, and require higher catalyst loading, higher temperatures, and longer reaction times.[5] There are surprisingly few reports on the asymmetric hydrogenation of acrylonitrile derivatives. In one example, (*Z*)-*N*-(1-cyano-2-phenylvinyl)benzamide was hydrogenated in the presence of [(*R*,*R*)-(DIPAMP)Rh(COD)]BF_4, giving the desired product in 89% ee. However, the reaction was less selective and considerably slower than the hydrogenation of the corresponding acrylic acid.[6]

Thus, while there was no direct precedent for the hydrogenation of this class of compounds we had considerable confidence that a suitable catalyst could be identified for this reaction. Herein we report a succinct synthesis of Pregabalin **1**, utilizing a rhodium-catalyzed asymmetric hydrogenation to furnish the key intermediate **3** in high yield and excellent enantiomeric excess.[7]

The required precursor for the hydrogenation reaction was readily prepared following a literature procedure for similar compounds, summarized in Scheme 1.[8] Baylis−Hillman reaction between isobutyraldehyde and acrylonitrile furnished hydroxy nitrile **4**.[9] This was then converted to the ethyl carbonate **5** (the reaction also works with the corresponding acetate), which was used directly in a palladium-catalyzed carbonylation to give 3-cyano-5-methylhex-3-enoic acid ethyl ester (**2a**) as a 3.5:1 (*Z*/*E*) mixture of isomers (83%, Scheme 1). Initial attempts at using the crude product from this reaction in the hydrogenation step failed, presumably due to residual impurities. The ester was further purified by vacuum distillation. The other hydrogenation substrates, *tert*-butylammonium and potassium salts **2b** and **2c**,

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10.1021/jo034397b CCC: $25.00
Published on Web 06/06/2003

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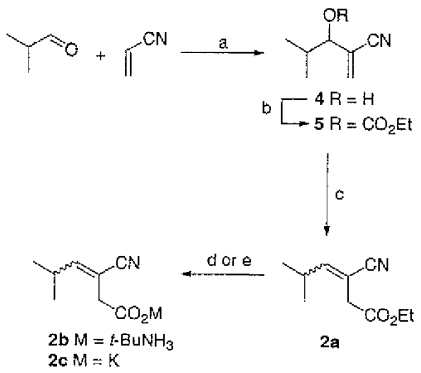
SCHEME 1[a]



[a] Reagents and conditions: (a) DABCO, H_2O, 2,6-di-*tert*-butyl-4-methylphenol, 50 °C, 97%; (b) $ClCO_2Et$, pyridine, CH_2Cl_2, rt, 95%; (c) $Pd(OAc)_2$, PPh_3, EtOH, CO (300 psi), 50 °C, 83%; (d) **2b** (i) LiOH, H_2O, THF, rt; (ii) HCl; (iii) *tert*-$BuNH_2$, EtOAc, 89%; (e) **2c** KOH, MeOH, 45 °C, 88%.

TABLE 1. Asymmetric Hydrogenation of 3-Cyano-5-methylhex-3-enoic Acid Ethyl Ester 2a

entry[a]	precatalyst	temp (°C)	conv (%)[b]	ee (%)[b,c]
1	[(*R,R*)-(Me-DuPHOS)Rh(COD)]BF_4	rt	10	8 (*S*)
2	[(*R,R*)-(Me-BPE)Rh(COD)]OTf	rt	54	10 (*R*)
3	[(*R,R*)-(Me-DuPHOS)Rh(COD)]BF_4	55	100	19 (*R*)
4	[(*R,R*)-(Et-DuPHOS)Rh(COD)]BF_4	55	100	42 (*R*)
5	[(*R,R*)-(*i*Pr-DuPHOS)Rh(COD)]BF_4	55	79	44 (*S*)
6	[(*R,R*)-(Me-BPE)Rh(COD)]OTf	55	100	13 (*R*)
7	[(*R,R*)-(Et-BPE)Rh(COD)]BF_4	55	100	13 (*R*)
8	[(*S,S*)-(*i*Pr-BPE)Rh(COD)]BF_4	55	67	<2
9	[(*R,R*)-(Me-FerroTANE)Rh(COD)]BF_4	rt	51	37 (*S*)
10	[(*R,R*)-(Et-FerroTANE)Rh(COD)]BF_4	rt	41	7 (*S*)

[a] 1 mmol of substrate in 5 mL of methanol was hydrogenated with 10 μmol of precatalyst in a glass lined stainless steel pressure vessel with hydrogen at 90 psi. [b] Conversion and enantiomeric excess were determined by GC (see Experimental Section). [c] The absolute stereochemistry was established by conversion to Pregabalin.

were readily prepared from the ester by standard methods. In both cases, mixtures of geometrical isomers were obtained in approximately the same ratio as observed for ester **2a**. The *tert*-butylammonium salt **2b** could also be prepared directly from the crude ester **2a** (62%), removing the need for vacuum distillation.

Efforts were initially focused on the asymmetric hydrogenation of the ethyl ester **2a**. A range of chiral rhodium complexes were examined under typical hydrogenation conditions (Table 1). Although the hydrogenation reactions proceeded slowly at room temperature, upon heating to 55 °C complete conversion was achieved for the majority of catalyst systems examined. Unfortunately, the enantiomeric excess obtained for this substrate was disappointingly low.

In contrast, however, the hydrogenation of *tert*-butylammonium salt **2b** not only proceeded rapidly at room temperature (reaction complete in under 15 min with some catalysts) but also gave the product with excellent enantioselectivity (Table 2).

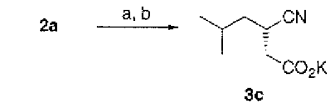
From this initial screen, three precatalysts were clearly outstanding in terms of both high reactivity and selectivity, namely [(Me-DuPHOS)Rh(COD)]BF_4, [(Et-DuPHOS)-Rh(COD)]BF_4, and [(*R,R*)-(Me-FerroTANE)Rh(COD)]BF_4 (entries 1, 2, and 7). In all cases the (*R,R*)-enantiomer of the catalyst provided the desired (*S*)-enantiomer of the product. A similar screen of catalysts was also conducted for the potassium salt **2c**, with comparable results being obtained in terms of rate and selectivity. These screening reactions were conducted at a molar substrate-to-catalyst ratio (S/C) of 100:1. For this to be an economically viable route, comparable rates and selectivity would need to be achieved at much lower S/C ratios. After some scale-up work, [(*R,R*)-(Me-DuPHOS)Rh(COD)]BF_4 was selected as the best catalyst for further development due to a combination of rate and selectivity at reduced catalyst loading. Under slightly modified reaction conditions the hydrogenation of **2b** with [(*R,R*)-(Me-DuPHOS)Rh(COD)]-BF_4 was demonstrated at a molar S/C of 2700/1, which corresponds to a substrate to catalyst w/w ratio of 1000/1. The reaction was complete in 4 h and the crude product was obtained in 97.7% e.e.

TABLE 2. Asymmetric Hydrogenation of *tert*-Butylammonium 3-Cyano-5-methylhex-3-enoate 2b

entry[a]	precatalyst	react. time	conv (%)[b]	ee (%)[b]
1	[(*R,R*)-(Me-DuPHOS)Rh(COD)]BF_4	15 min[c]	100	95.0 (*S*)
2	[(*R,R*)-(Et-DuPHOS)Rh(COD)]BF_4	15 min[c]	100	97.4 (*S*)
3	[(*R,R*)-(*i*Pr-DuPHOS)Rh(COD)]BF_4	6 h	72	24.0 (*R*)
4	[(*R,R*)-(Me-BPE)Rh(COD)]OTf	45 min[c]	100	83.3 (*S*)
5	[(*R,R*)-(Et-BPE)Rh(COD)]BF_4	45 min[c]	100	81.0 (*S*)
6	[(*S,S*)-(*i*Pr-BPE)Rh(COD)]OTf	6 h	59	8.0 (*S*)
7	[(*R,R*)-(Me-FerroTANE)Rh(COD)]BF_4	20 min[c]	100	95.4 (*S*)
8	[(*R,R*)-(Et-FerroTANE)Rh(COD)]BF_4	20 min[c]	100	84.6 (*S*)

[a] 1 mmol of substrate in 5 mL of methanol was hydrogenated with 10 μmol of precatalyst in a glass-lined stainless steel pressure vessel with hydrogen at 90 psi at room temperature. [b] Conversion and enantiomeric excess were determined by GC (see Experimental Section). [c] Time within which hydrogen uptake had ceased.

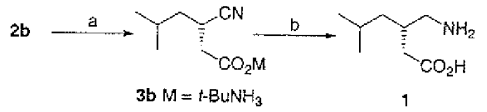
SCHEME 2[a]



[a] Reagents and conditions: (a) KOH, H_2O, MeOH; (b) [(*R,R*)-(Me-DuPHOS)Rh(COD)]BF_4, H_2 (45 psi), 55 °C, 99% conversion, 96.6% ee.

To circumvent the need to isolate 3-cyano-5-methylhex-3-enoic acid or a salt, ethyl ester **2a** was hydrolyzed with potassium hydroxide in a mixture of methanol and water to give a solution of potassium salt **2c**. Addition of a solution of the precatalyst, [(*R,R*)-(Me-DuPHOS)Rh(COD)]-BF_4 (S/C 2000/1), followed by hydrogenation gave potassium (*S*)-3-cyano-5-methylhexanoate (**3c**) in 96.6% ee (Scheme 2). An important point to note is that this reaction is conducted in a mixed methanol−water solvent system (presumably the water assists in the hydrolysis), demonstrating the utility of the Rh-DuPHOS catalyst under partially aqueous conditions. While this is a more direct approach, the drawback of this procedure is that any residual ethyl ester **2a** that may be present will be hydrogenated to the *opposite* enantiomer of the product **3**, thus reducing the enantiomeric excess. The rate of reaction was also somewhat slower under these reaction

SCHEME 3[a]



[a] Reagents and conditions: (a) [(*R,R*)-(Me-DuPHOS)Rh(COD)]-BF_4, H_2 (45 psi), MeOH, 55 °C, 100% conversion, 97.7% ee; (b) (i) Sponge Ni, KOH, H_2 (50 psi), H_2O, EtOH; (ii) AcOH, 61% (two steps), 99.8% ee.

conditions. Thus, the favored process is to prepare the *tert*-butylammonium salt **2b** from the purified ethyl ester **2a**, followed by asymmetric hydrogenation to *tert*-butylammonium salt **3b** (Scheme 3). This process has been scaled up to multi-kilogram quantities without significant difficulties (see Supporting Information).

The final step in the synthesis of (*S*)-(+)-3-aminomethyl-5-methylhexanoic acid (**1**), the reduction of the nitrile group, was accomplished via a heterogeneous hydrogenation of the *tert*-butylammonium salt **3b** over sponge nickel. The crude product was crystallized from a mixture of ethanol, water, and acetic acid to give Pregabalin **1** in 61% yield and 99.8% ee (Scheme 3).

The much higher reactivity and selectivity observed for the asymmetric hydrogenation of salts **2b** and **2c** compared to the ethyl ester **2a** is due to enhanced coordination between the substrate and the catalyst. It is well established that the bisphosphine rhodium catalysts of this type are most effective when the substrate is able to behave as a bidentate ligand **7** (Scheme 4).[10] In the presence of the strongly coordinating nitrile ligand this chelating binding mode is disrupted. The $^{31}P\{^1H\}$ NMR spectrum of the complex formed between the catalytic intermediate [(*R,R*)-(Me-DuPHOS)Rh$(CD_3OD)_2$]-BF_4 (**6**) and *tert*-butylammonium salt **2b** shows a dynamic mixture of species, characterized by complex and broadened signals. Within this is a pair of double doublets at δ 79.3 and 88.6 ppm (J_{PP} = 34 Hz, J_{PRh} = 149 Hz) which can be assigned to the rhodium chelate **7**. The spectroscopic data for this complex are similar to those previously reported for an analogous vinyl acetate complex.[10] Upon treatment with hydrogen, the olefin is reduced and the $^{31}P\{^1H\}$ NMR spectrum collapses to a doublet at δ 95.4 ppm (J_{PRh} = 171 Hz), assigned to the bis-(*S*)-3-cyano-5-methylhexanoate complex **8** as the $^{31}P\{^1H\}$ NMR spectrum is almost identical with that of [(*R,R*)-(Me-DuPHOS)Rh$(NCCH_3)_2$]BF_4 [δ 95.3 (d, J_{PRh} = 175 Hz)]. It is proposed that the small standing concentration of **7** in the reaction mixture (ca. 10%) serves as a conduit through which all the hydrogenation substrate is converted to product. The relatively low reactivity observed in this reaction compared to, for example, itaconic salts[4c] or amido itaconates[11] is attributed to the low levels of the reactive intermediate **7** in the reaction mixture. Similar observations, where a minor component of a mixture gives rise to the major product, are well established in the asymmetric hydrogenation of prochiral olefins by chiral bisphosphine rhodium complexes.[12]

(10) Burk, M. J.; Bienwald, F.; Challenger, S.; Derrick, A.; Ramsden. J. A. *J. Org. Chem.* **1999**, *64*, 3290.

(11) Berens, U.; Burk, M. J.; Gerlach, A.; Hems, W. *Angew. Chem., Int. Ed.* **2000**, *39*, 1981.

(12) Landis, C. R.; Halpern, J. *J. Am. Chem. Soc.* **1987**, *109*, 1746.

In conclusion we have demonstrated a six-step synthesis of (*S*)-(+)-3-aminomethyl-5-methylhexanoic acid (**1**) that delivers the product in high yield and excellent enantiopurity. The synthetic sequence described is as short as the previously preferred route,[3] but potentially provides significant improvements in cost of goods, waste reduction, and throughput.

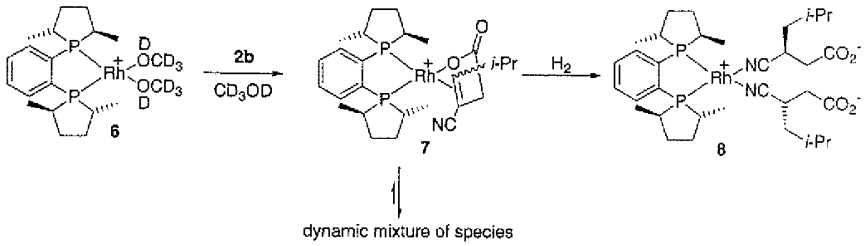
Experimental Section

***tert*-Butylammonium 3-Cyano-5-methyl-hex-3-enoate (2b).** Ethyl ester **2a** (20.0 g, 110 mmol, see Supporting Information) and lithium hydroxide hydrate (13.0 g, 310 mmol) were suspended in a mixture of tetrahydrofuran (75 mL) and water (25 mL). The slurry was vigorously stirred for 4 h at room temperature. The mixture was acidified to pH 2 (HCl, 3 N) and extracted into ethyl acetate (3 × 150 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to give crude 3-cyano-5-methylhex-3-enoic acid: IR (film) ν_{max} 2222, 1714 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ (major isomer) 1.09 (3H, d, J = 7.0 Hz), 2.91 (1H, dheptet, J = 10.0, 7.0 Hz), 3.25 (2H, br), 6.16 (1H, d, J = 10.0 Hz), (minor isomer) 1.05 (3H, d, J = 6.7 Hz), 2.63 (1H, dheptet, J = 10.0, 6.7 Hz), 3.31 (2H, s), 6.40 (1H, d, J = 10.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (major isomer) 22.1, 31.9, 39.0, 104.6, 116.9, 159.7, 175.4 (minor isomer) 21.9, 28.9, 34.2, 104.8, 119.5, 159.1, 175.0. *m/z* 152 (M − H), 305 (2M − H). The acid was dissolved in ethyl acetate (400 mL) and a solution of *tert*-butylamine in ethyl acetate (20 mL) was added. The temperature of the solution rose by approximately 10 °C as the salt **2b** precipitated as a white crystalline solid. The product was collected by filtration and dried in vacuo (22.15 g, 89%): mp 161 °C; IR (KBr) ν_{max} 2216, 1557 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ (major isomer) 1.09 (6H, d, J = 6.5 Hz), 1.37 (9H, s), 2.81 (1H, dheptet, J = 10.0, 6.5 Hz), 3.04 (2H, d, J = 1 Hz), 6.13 (1H, d, J = 10.0 Hz), (minor isomer) 1.05 (6H, d, J = 6.5 Hz), 1.37 (9H, s), 2.74 (1H, dheptet, J = 10.1, 6.5 Hz), 3.11 (2H, s), 6.25 (1H, d, J = 10.1 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ (major isomer) 22.7, 28.3, 33.0, 44.1, 52.9, 110.3, 119.2, 157.3, 177.1, (minor isomer) 22.1, 28.3, 29.7, 38.8, 52.9, 110.8, 122.1, 157.0, 176.5; *m/z* 74 ($tBuNH_3^+$), 305 (2M + H).

Representative Procedure for Hydrogenation Screening Reactions. A solution of ethyl ester **2a** (0.19 mL, 1.0 mmol) in methanol (4 mL) was placed in a glass-lined 50-mL PARR microreactor modified with an injection septum and valve. The vessel was heated to an internal temperature of 55 °C. A hydrogen atmosphere was established and a solution of [(*R,R*)-(^{i}Pr-DuPHOS)Rh(COD)]BF_4 (7.2 mg, 10 μmol) in methanol (1 mL) was added via syringe. The vessel was pressurized with hydrogen to 100 psi and stirred overnight. The pressure was then released and the solvent was removed in vacuo. ^1H NMR analysis showed approximately 80% conversion to **3a**. GC analysis showed 86.4% conversion, 43.8% ee (*S*): IR (film) ν_{max} 2242, 1738 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.96 (3H, d, J = 6.8 Hz), 0.98 (3H, d, J = 6.5 Hz), 1.29 (3H, t, J = 7.1 Hz), 1.34 (1H, ddd, J = 13.4, 9.4, 5.0 Hz), 1.64 (1H, ddd, J = 13.8, 10.9, 4.7 Hz), 1.87 (1H, m), 2.53 (1H, dd, J = 16.6, 6.9 Hz), 2.69 (1H, dd, J = 16.3, 7.3 Hz), 3.06 (1H, m), 4.20 (2H, q, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.5, 21.6, 23.2, 25.7, 26.2, 26.5, 37.5, 41.1, 61.6, 121.5, 170.1. Screening reactions at room temperature were carried out via a modified procedure. The liner was charged with a stir bar, the substrate, and catalyst. The vessel was assembled and a hydrogen atmosphere established as described above. Methanol was added via the septum, the vessel was again purged before pressurizing to the reaction pressure and stirring was then initiated.

***tert*-Butylammonium (*S*)-3-Cyano-5-methylhexanoate (3b).** A pressure reactor was charged with a solution of *tert*-butylammonium salt **2b** (125.8 g, 0.56 mol) in methanol (1 L). A hydrogen atmosphere was established and the vessel was heated to 45 °C. A solution of [(*R,R*)-(Me-DuPHOS)Rh(COD)]-BF_4 (0.125 g, 0.206 mmol) in methanol (15 mL) was added via syringe. The vessel was charged with hydrogen to 65 psi and the reaction was stirred at 45 °C until hydrogen uptake ceased

SCHEME 4. Proposed Mechanism for the Hydrogenation of *tert*-Butylammonium Salt 2b



(4 h). The solvent was removed in vacuo to give the product as a white crystalline solid (125 g, 99%), GC analysis showed >99% conversion, 97.7% ee: mp 148 °C dec; $[\alpha]^{25}_D$ −16.8° (*c* 1.2, MeOH); IR (KBr) ν_{max} 2238, 1557 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 0.98 (3H, d, J = 5.8 Hz), 1.00 (3H, d, J = 6.1 Hz), 1.37 (9H, s), 1.41 (1H, ddd, J = 13.4, 9.4, 5.0 Hz), 1.59 (1H, ddd, J = 13.8, 10.9, 5.1 Hz), 1.83 (1H, m), 2.39 (1H, dd, J = 15.2, 6.9 Hz), 2.49 (1H, dd, J = 15.5, 7.9 Hz), 3.10 (1H, m); ^{13}C NMR (CD_3OD, 100 MHz) 22.2, 23.9, 27.9, 28.3, 28.7, 42.2, 42.6, 52.9, 124.2, 177.7; *m/z* 74 (tBuNH$_3^+$), 309 (2M + H).

Potassium (*S*)-3-Cyano-5-methylhexanoate (3c) (in situ generation of salt). A pressure reactor was charged with a solution of ethyl ester **2a** (10.8 g, 59.7 mmol) in methanol (100 mL) and water (18 mL). A solution of potassium hydroxide in methanol (5 M, 11.7 mL, 58.4 mol) was added, a nitrogen atmosphere was established, and the vessel was heated to 55 °C and held at this temperature for 2 h. A hydrogen atmosphere was established and a solution of [(*R*,*R*)-(Me-DuPHOS)Rh(COD)]-BF_4 (0.018 g, 0.030 mmol) in methanol (20 mL) was added via syringe. The vessel was charged with hydrogen to 60 psi and the reaction was stirred at 55 °C until hydrogen uptake ceased (5 h). The solvent was removed in vacuo to give the product as a white crystalline solid (11.2 g, 99%), GC analysis showed >99% conversion, 97.7% ee: mp 102 °C dec; $[\alpha]^{25}_D$ −20.6° (*c* 1.1, MeOH); IR (KBr) ν_{max} 2240, 1580 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 0.98 (3H, d, J = 6.6 Hz), 1.00 (3H, d, J = 6.6 Hz), 1.41 (1H, ddd, J = 13.5, 9.7, 5.2 Hz), 1.58 (1H, ddd, J = 15.2, 10.7, 4.8 Hz), 1.84 (1H, m), 2.39 (1H, dd, J = 15.2, 6.9 Hz), 2.49 (1H, dd, J = 15.2, 7.6 Hz), 3.11 (1H, m); ^{13}C NMR (CD_3OD, 100 MHz) 22.2, 23.8, 27.9, 28.7, 42.2, 42.5, 124.6, 178.0; *m/z* 154 (M), 309 (2M + H).

(*S*)-3-Aminomethyl-5-methylhexanoic Acid (1). A solution of *tert*-butylammonium salt **3b** (8.0 g, 35.0 mmol) in water (15 mL) and ethanol (11 mL) was added to nickel sponge (A-7000, 5 g, water wet), followed by potassium hydroxide (91% flake, 2.2 g, 35.6 mmol), and the resulting slurry was shaken under 50 psi of hydrogen overnight. The mixture was filtered (Supercel) and the cake was rinsed with water (20 mL) and ethanol (7 mL). Acetic acid (4.1 mL, 71.6 mmol) was added to the combined filtrates which were then heated to 70 °C, then cooled slowly to room temperature over several hours, followed by aging for 6 h at 0−5 °C. The product was collected by filtration, rinsed with propan-2-ol (50 mL), and dried under vacuum to give 1 as a white crystalline solid (3.4 g, 61%, 99.8% ee), identical with that prepared previously.[3] Anal. Calcd for $C_8H_{17}NO_2$: C, 60.35; H, 10.76; N, 8.80. Found: C, 60.59; H, 10.78; N, 8.80.

Acknowledgment. The authors thank Cara Dykstra (Pfizer) and Will Spearing and Jonathan Hill (Chirotech) for analytical support.

Supporting Information Available: Experimental details for the synthesis of **4**, **5**, **2a**, **2c**, and **3c** and for the multikillogram conversion of **2b** to **1**, copies of the ^{31}P NMR spectra of **6**−**8**, and ^{1}H and ^{13}C NMR spectra of **2a**−**c**, **3a**−**c**, 3-cyano-5-methylhex-3-enoic acid, and 3-cyano-5-methyl-hexanoic acid. This material is available free of charge via the Internet at http://pubs.acs.org.

JO034397B

Stereochemistry of Organic Compounds

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A Wiley-Interscience Publication
JOHN WILEY & SONS, INC.
New York • Chichester • Brisbane • Toronto • Singapore

This text is printed on acid-free paper.

Library of Congress Cataloging in Publication Data:

Eliel, Ernest Ludwig, 1921-
Stereochemistry of organic compounds / Ernest L. Eliel, Samuel H. Wilen, Lewis N. Mander.
p. cm.
"A Wiley-Interscience publication."
Includes index.
ISBN 0-471-01670-5
1. Stereochemistry. 2. Organic compounds. I. Wilen, Samuel H. II. Mander, Lewis N. III. Title.
QD481.E52115 1993
547.1'223--dc20 93-12476

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

this type of enantiomerization has much in common with asymmetric transformations of diastereomers. The latter are described in Section 7-3.c.

7-3. CHEMICAL SEPARATION OF ENANTIOMERS VIA DIASTEREOMERS

a. Formation and Separation of Diastereomers. Resolving Agents

The largest number of recorded resolutions has been effected by conversion of a racemate to a mixture of diastereomers. In this type of reaction, the substrate to be resolved is treated with one enantiomer of a chiral substance (the resolving agent). The first such resolution, described by Pasteur in 1853, is outlined in Figure 7.17 (Jacques et al., 1981a, pp. 253, 257). Diastereomer pairs prepared in connection with resolutions may be ionic (diastereomeric salts), covalent, charge-transfer complexes, or inclusion compounds. The latter two types of diastereomers are discussed in Section 7-3.c.

The vast majority of resolutions mediated by diastereomers (diastereomeric salt mixtures, in particular) have been based on solubility differences of solids; however, in the contemporary literature, covalent diastereomer separations based on chromatography in all of its variants are used with great frequency. Chromatography has freed resolutions from the constraint of dependency on crystallization as the technique on which diastereomer separation has traditionally depended. As a result, resolutions in general are much more successful at present than they were in the past.

Not infrequently oily covalent diastereomer mixtures eventually crystallize and their resolution may then be performed in the more traditional way by taking advantage of solubility differences. Separation of diastereomeric salt mixtures by chromatography is also now possible (Section 7-3.d). Because of this interplay between ionic and covalent structure and the several ways of separating diastereomer mixtures, we have chosen in this section not to treat resolving agents separately according to whether they form covalent or ionic diastereomer mixtures.

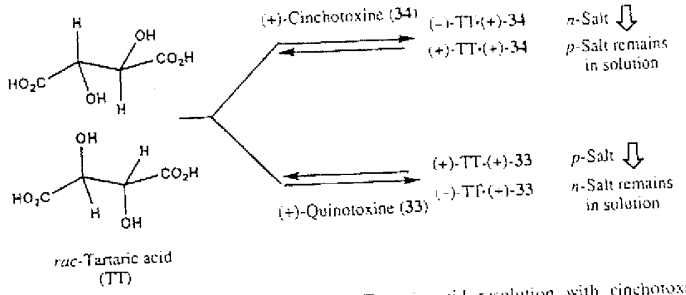


Figure 7.17. First resolution via diastereomers. Tartaric acid resolution with cinchotoxine and quinotoxine (Fig. 7.19) as resolving agents (Pasteur, 1853). The *n* and *p* symbols are defined on p. 326.

Details of chromatographic resolutions are examined in Section 7-3.d. The differential stability of diastereomers is another basis for their separation. Transient diastereomeric species are formed in chromatographic columns as flowing racemate samples interact with chiral stationary phases or with achiral stationary phases in the presence of nonracemic chiral mobile phases. This type of resolution is also dealt with in Section 7-3.d. Section 7-3 concludes with an examination of the asymmetric transformation of diastereomers (Section 7-3.e).

In this section, our analysis focuses on resolving agents with emphasis on the recent literature and with examples of their use. The desirable characteristics of a good resolving agent are (Wilen, 1971):

(a) Ready availability
(b) Stability of supply
(c) Stability in use and in storage
(d) Low price or ease of preparation
(e) Ease of recovery and reuse
(f) Low molecular weight
(g) Availability in high enantiomeric purity
(h) Availability of both enantiomers
(i) Low toxicity
(j) Reasonable solubility

α-Methyl-β-phenylethylamine, $C_6H_5CH_2CH(NH_2)CH_3$, illustrates the application of feature (a). This amine is a potentially useful resolving agent (for a recent application to the resolution of gossypol, see Kai, Liang, et al., 1985). However, the amine (amphetamine) is a central nervous system (CNS) active compound, and accordingly it is a controlled substance. Like all such substances (e.g., deoxyephedrine and morphine) it is difficult to obtain. The acquisition of controlled substances for use as resolving agents is so complicated and time consuming (at least in the United States) that their use for this purpose is essentially precluded.

The supply of resolving agents that are derived from natural sources, such as brucine and 10-camphorsulfonic acid, may be shut off by economic or political problems that impede access to the sources (feature b).

Some resolving agents are awkward to use and to store without precaution. Liquid primary amines, such as α-methylbenzylamine and dehydroabietylamine (Fig. 7.19), readily form solid carbamates on exposure to air (Rosan, 1989). It may consequently be desirable to store these amines as salts [feature (c)]; if so, one may profitably choose salts that are conglomerates, since enantiomer purification of such salts would be concomitant with chemical purification (e.g., during recovery). α-Methylbenzylamine hydrogen sulfate (Fig. 7.1) and α-(1-naphthyl)-ethylamine phenylacetate are examples of salts that are conglomerates (Jacques et al., 1981a). All other things being equal, high expense is a negative feature in the choice of a resolving agent, although this feature (d) may be mitigated by the possibility of recovery and reuse (feature e). When preparation of a resolving

agent is required, the yield and complexity of the synthesis is likely to be a consideration.

Since resolving agents are purchased by weight but are used on a molar basis, low molecular weight (feature f) is an advantage. This is a significant consideration especially in resolutions carried out on an industrial scale. Unfortunately, many naturally occurring resolving agents, notably alkaloids, have high molecular weights (e.g., brucine, MW 394.4); this is less likely to be the case for synthetic ones (for lists of resolving agents giving molecular weights, see Jacques et al., 1981a, pp. 255–256). Moreover, synthetic resolving agents are usually obtainable in both enantiomeric forms and this feature (h) is advantageous, since it permits the preparation of both enantiomers of a compound by means of mirror-image resolutions (*Marckwald principle*, Marckwald, 1896; type a in Table 7.3). A fair number of such pairs of enantiomers are available commercially (e.g., α-methylbenzylamine, ephedrine, tartaric acid, or 10-camphorsulfonic acid). Some synthetic resolving agents have been designed that explicitly incorporate many of the features listed above (e.g., ten Hoeve and Wynberg, 1985).

Use of synthetic resolving agents requires their prior resolution. This requirement leads us to discuss the possibility of effecting *reciprocal resolutions*: If *rac*-*N*-benzyloxycarbonylalanine [(±)-Z-Ala] is resolvable with (−)-ephedrine [(−)-Eph], then, as is often (but not invariably) the case, the resolving agent (±)-Eph will be resolvable with either (+)- or (−)-Z-Ala (type b in Table 7.3; Overby and Ingersoll, 1960; Jacques et al., 1981a, p. 306).

TABLE 7.3 Types of Diastereomer-mediated Resolutions[a]

Type of Resolution[b]	Resolution Substrate	Resolving Agent	Diastereomeric Products: Less Soluble	Diastereomeric Products: More Soluble
a. Normal	(±)-**Z-Ala**	+ (−)-Eph ⟶	(−)-**Z-Ala**·(−)-Eph	+ (+)-Z-Ala·(−)-Eph
Marckwald	(±)-**Z-Ala**	+ (+)-Eph ⟶	(+)-**Z-Ala**·(+)-Eph	+ (−)-Z-Ala·(+)-Eph
b. Normal	(±)-**Z-Ala**	+ (−)-Eph ⟶	(−)-**Z-Ala**·(−)-Eph	+ (+)-Z-Ala·(−)-Eph
Reciprocal	(±)-**Eph**	+ (+)-Z-Ala ⟶	(+)-Z-Ala·(+)-**Eph**	+ (±)-Z-Ala·(−)-Eph
c. Mutual	(±)-**Z-Ala**[c]	+ (±)-Eph ⟶	(+)-**Z-Ala**·(±)-**Eph**	+ (±)-Z-Ala·(−)-Eph
d. Mutual	(±)-**Z-Ala**	+ (±)-**Eph** ⟶	{(+)-**Z-Ala**·(±)-**Eph**[d] + (−)-Z-Ala·(−)-Eph[e]}	

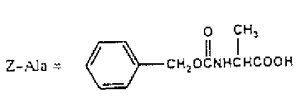
[a] Types of resolutions: (a) Normal and Marckwald resolutions. (b) Normal and reciprocal resolutions (Overby and Ingersoll, 1960); (c) Mutual resolution; see text (Ingersoll, 1925); (d) Mutual resolutions (Wong and Wang, 1978). Resolution substrates and products are in boldface.

[b] This resolution was carried out on partially resolved Ala enriched in (+)-Z-Ala.

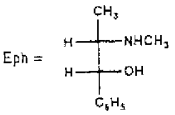
[c] On seeding with (+, +) salt.

[d] On seeding with (−, −) salt.

[e] The (+)-Z-Ala·(−)-Eph and (−)-Z-Ala·(+)-Eph diastereomeric salts did not crystallize.



N-Benzyloxycarbonylalanine



(1*R*,2*S*)-(−)-Ephedrine

The reciprocal resolutions shown in Table 7.3 (type b) lead to diastereomeric combinations of salt pairs (the two sets of resolution products). It follows that separability of the diastereomeric salts in one case does not guarantee such separability in the other (Mislow, 1962). However, it does not preclude it either; reciprocal resolutions very often are successful. Moreover, the likelihood of success in reciprocal resolutions has served as a guide in the design of new resolvings agents (including the design of new enantioselective stationary phases for chromatography; Section 7-3.c). Factors leading to the prediction of success in reciprocal resolutions have been evaluated (Fogassy et al., 1981).

Another potentially useful approach to the preparation of synthetic resolving agents is the application of *mutual resolution*. The idea of effecting the mutual resolution of a racemic acid and of a racemic base was first advanced by Ingersoll (1925) in connection with the resolution of phenylglycine with (+)-10-camphorsulfonic acid. The compound (−)-phenylglycine was recovered from the less soluble diastereomeric salt and (+)-phenylglycine (ee = 63%) was recovered from the mother liquor. Reaction of the latter with *rac*-10-camphorsulfonic acid led to formation of a precipitate from which pure (+)-phenylglycine *and* (−)-10-camphorsulfonic were recovered. This process is schematically illustrated in Table 7.3 (type c) for partially resolved (+)-Z-Ala (admixed with *rac*-Z-Ala) recovered from the more soluble product (+)-Z-Ala·(−)-Eph (method a, top line). The less soluble product isolated from reaction with (±)-Eph contains both pure (+)-Z-Ala and resolved "resolving agent" (+)-Eph. Although the method is attractive for the isolation of the substrate enantiomer incorporated in the more soluble diastereomeric product (Table 7.3), we are unaware of any application of this process other than the cases described by Ingersoll (1925).

Although it is not implicit in Table 7.3 (type a), only *one* enantiomer of the substrate is readily obtained in conventional (hence, also in reciprocal) diastereomer-mediated resolutions, for example, (−)-Z-Ala in the resolution of (±)-Z-Ala with (−)-Eph, and (+)-Eph in the reciprocal resolution of (±)-Eph with (+)-Z-Ala (a and b in Table 7.3). In either case, it is only the enantiomer incorporated in the less soluble product that is readily obtained. A change in resolving agent or use of the enantiomeric resolving agent [(+)-Eph as in Table 7.3 (type a), second line (Marckwald principle)] is usually required to obtain the other enantiomer of the substrate to be resolved (see, e.g., Saigo et al., 1986b). On the other hand, crystallization of solutions containing equivalent amounts of *racemic* substrate and *racemic* "resolving agent" may permit the isolation of either enantiomer of the material to be resolved and simultaneously either enantiomer of the "resolving agent" provided that the racemic salt is a conglomerate. Wong and Wang (1978) demonstrated the possibility of effecting such mutual resolutions by alternately seeding racemic solutions containing the four possible salts with crystals of one of the less soluble salts and then with crystals of the enantiomeric salt. In each case, the salt that precipitated had the same composition as that of the seeds; the enantiomers of only one of two possible diastereomeric salts crystallized (d in Table 7.3). As expected, on admixture, the two precipitated enantiomers formed a conglomerate. Hence, mutual resolution, though performed on diastereomeric salts, has the attributes of preferential crystallization. The mutual resolution of (±)-malic acid and of (±)-α-methylbenzylamine has

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23 Ainslie Place, Edinburgh EH3 6AJ
3 Cambridge Center, Suite 208,
Cambridge, Massachusetts 02142, USA
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Victoria 3053, Australia

First published 1989

Set by Times Graphics, Singapore
and printed and bound in Great Britain by
Butler & Tanner Ltd
Frome, Somerset and London

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(*Orders*: Tel: (03) 347 0300)

British Library
Cataloguing in Publication Data

Harwood, Laurence
Experimental organic chemistry.
1. Organic chemistry. Experiments
I. Title II. Moody, Christopher
547'.00724

ISBN 0-632-02016-4
ISBN 0-632-02017-2 pbk

Library of Congress
Cataloging-in-Publication Data

Harwood, Laurence
Experimental organic chemistry.
Includes index.
1. Chemistry, Organic--Laboratory manuals. I. Moody, Christopher. II. Title.
QD261.H265 1989 547'.0078 88-7680
ISBN 0-632-02016-4
ISBN 0-632-02017-2

Contents

The most important factor in drying organic solutions is the choice of drying agent. Ideally the solid drying agent should be totally insoluble in organic solvents, inert to a wide range of organic compounds (including solvents) and able to take up water quickly and efficiently to give a hydrated form which is an easily filterable solid. The most commonly used drying agents are listed in Table 3.5, which gives information on their *capacity* (how much water they can take up), *speed* (rate of water uptake), *efficiency* (how dry they leave the solution) and *applicability* (suitability for different classes of compound). Clearly the choice will depend on a number of factors, the most crucial of which is the nature of the organic compound that is dissolved in the solvent, and that is ultimately to be isolated. As a good general purpose drying agent, magnesium sulfate finds the widest use.

It is important to note that drying agents that are suitable for drying *organic solutions* are not usually appropriate for drying *organic solvents* for use with moisture-sensitive compounds (see pp. 77–87). The drying of organic solvents is an entirely separate problem that is referred to on p. 79, and dealt with specifically in Appendix 2.

Crystallization

The simplest and most effective technique for the purification of solid organic compounds is crystallization. Crystalline compounds are easy to handle, their purity is readily assessed (Chapter 4) and they are often easier to identify than liquids or oils. Crystals can be obtained in one of three ways: from the melted solid on cooling, by sublimation (pp. 154–155) or from a supersaturated solution. The last method is by far the most common in the organic laboratory.

Crystallization of Organic Compounds

A general plan for the purification of an organic compound by *crystallization* is shown in Figure 3.37. The process involves five stages: dissolution, filtration, crystallization, collection of the crystals and drying the crystals. The purity of the crystals can then be determined (Chapters 4 and 5), and if necessary further purification by *recrystallization* can be carried out. Before discussing each of the stages in the process in detail, we should briefly consider how crystallization succeeds in purifying compounds at all.

How crystallization works

The technique involves dissolving the impure solid in the minimum volume of a hot solvent and filtering to remove insoluble impurities. The resulting hot saturated solution of the compound, together with any soluble impurities, is set aside to cool slowly, whereupon crystals of pure compound will separate from solution. The solution remaining after crystallization is usually known as the *mother liquor*. Why are the crystals

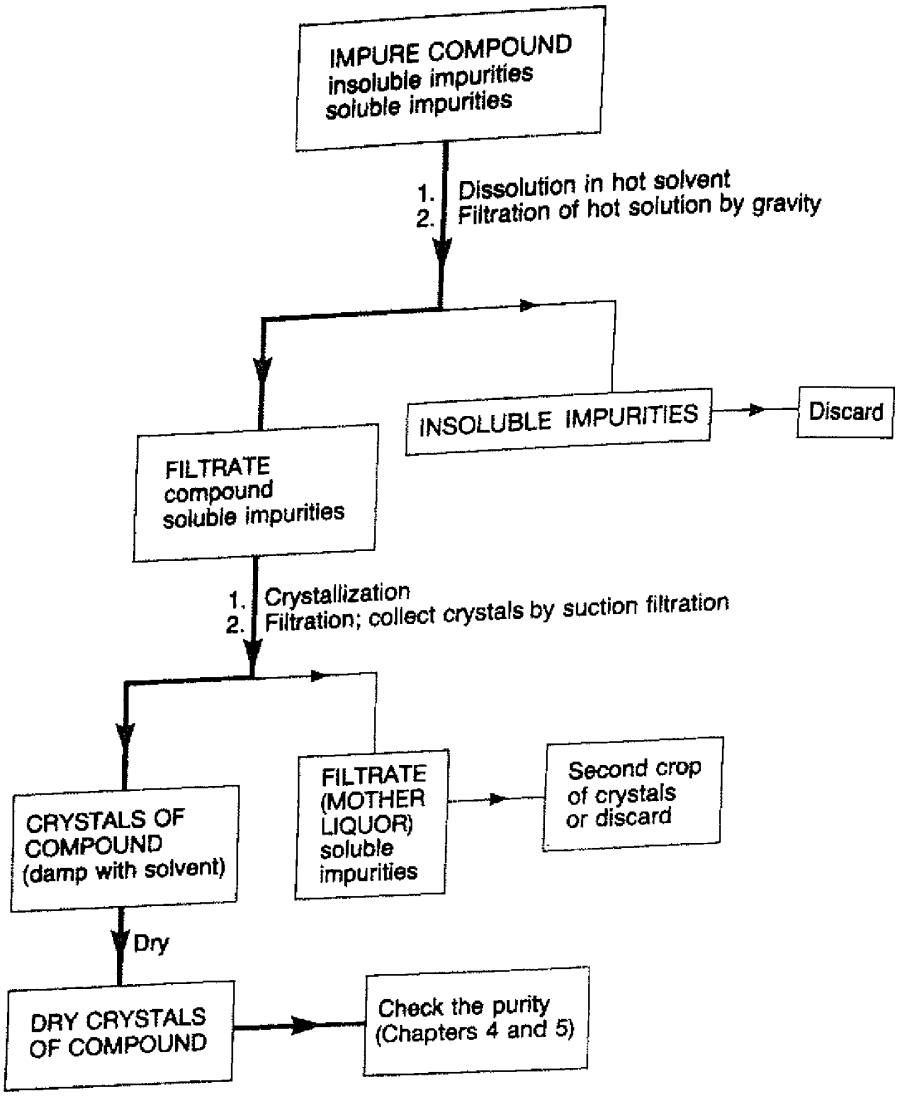


Figure 3.37. Plan for purification of an organic compound by crystallization.

pure? The process of crystallization is an equilibrium: molecules in solution are in equilibrium with those in the crystal lattice. Since a crystal lattice is highly ordered, other different molecules, such as impurities, will be excluded from the lattice and will return to the solution. Therefore only molecules of the required compound are retained in the crystal lattice and the impurities will remain in solution. For a crystallization to be successful, *the solution must be allowed to cool slowly*, so that the crystals are formed slowly, and the equilibrium process which excludes the impurities is allowed to operate. If a solution is cooled rapidly, impurity molecules will be trapped or included in the rapidly growing crystal lattice. This rapid

ooling

formation of solid material from solution is *precipitation*, and is not the same as crystallization.

At this stage it should be pointed out that crystallization does not always work. Substances which are grossly impure will often refuse to crystallize, and in these cases some preliminary purification by another technique, such as extraction (pp. 114–125) or chromatography (pp. 155–205), may be necessary.

Dissolution

The first problem is to dissolve the impure substance in a suitable solvent. The ideal solvent for a crystallization should not react with the compound, should be fairly volatile so that it is easy to remove from the crystals, should have a boiling point that is lower than the melting point of the compound to be crystallized, should be nontoxic and nonflammable, but most important of all, the compound should be very soluble in hot solvent and insoluble in cold solvent. In many cases, particularly when crystallizing known compounds, you will know what solvent to use because the literature or your laboratory text will tell you. In other cases, you will have to decide what solvent to use. Choosing a solvent for crystallization is not always easy, but organic chemists tend to follow the rule that 'like dissolves like'. So, for the crystallization of nonpolar substances such as hydrocarbons, use a nonpolar solvent such as hexane or light petroleum. Compounds containing polar groups such as OH are best crystallized from polar OH containing solvents such as ethanol. Indeed polar solvents are often preferred for other compounds because they tend to give better crystals. Some suggestions for crystallization solvents for the most common classes of organic compound, arranged in order of increasing polarity, are given in Table 3.6.

Like dissolves like

If the crystallization solvent is not known for certain, do not commit all your solid and attempt to dissolve it up. Rather, carry out some preliminary solubility tests. To do this, place a small quantity of the solid

Test the solubility

Table 3.6. Suggested solvents for crystallization.

Class of compound	Suggested solvents
Hydrocarbons	Light petroleum, hexane, cyclohexane, toluene
Ethers	Ether, dichloromethane
Halides	Dichloromethane, chloroform
Carbonyl compounds	Ethyl acetate, acetone
Alcohols, acids	Ethanol
Salts	Water

Use a hood if the solvent is toxic

Use a steam bath for heating flammable solvents

(*ca.* 20 mg or the amount that fits on the tip of a micro-spatula) in a small test tube — an ignition tube or a 10 × 75 mm test tube is ideal — and add a few drops of solvent to the tube. If the substance dissolves easily in cold solvent, try again with a different solvent. If the substance is insoluble in cold solvent, warm the tube on a steam or water bath, and if the substance remains insoluble, add more solvent with continued heating. If the compound still refuses to dissolve, try again with a different solvent. Once you have found a solvent that dissolves the compound when hot, you need to check that the solid will separate again on cooling. Place the tube in a beaker of ice-water, and leave it to stand for a minute or two. If a solid forms on cooling, the solvent is probably suitable for crystallization of the bulk material. With experience, these preliminary solubility tests can be carried out quickly, and provide a satisfactory guide to the choice of crystallization solvent.

Always keep a seed crystal

Once you have found a suitable solvent, you are ready to dissolve up the solid for crystallization. Before doing so, it is a good idea to weigh the solid, if you have not already done so, so that the recovery of material from the crystallization process can be determined. If the substance is already crystalline, do not dissolve all of it. Always retain a few crystals in case they are needed for seeding purposes (see p. 132). Large crystals are often difficult to dissolve, and should be ground up before adding the crystallization solvent.

Mixed solvents for crystallization

If a suitable crystallization solvent cannot be found, then you may have to use a *mixed solvent system*. A mixed solvent system is a pair of miscible solvents, chosen so that one of them (the good solvent) dissolves the compound readily, and the other (the poor solvent) does not. For example, many moderately polar organic compounds are soluble in ether, but not in light petroleum, and therefore a mixture of the two solvents may be suitable for crystallization. There are two schools of thought on how to carry out a crystallization using mixed solvents. One method is to dissolve the solid in the minimum volume of hot good solvent, add the poor solvent dropwise until the solution starts to become slightly turbid or cloudy, and then set the solution aside to crystallize. The second method is to suspend the solid in hot poor solvent, and then add the good solvent dropwise with continued heating until the solid *just* dissolves; then set the solution aside as before. Typical mixed solvent systems that often work quite well include ether–light petroleum, dichloromethane–light petroleum, ether–acetone and ethanol–water. If possible choose a system in which the good solvent is the lower boiling solvent. One final word of warning: the use of mixed solvents often encourages *oiling out* (see p. 132), and therefore crystallization from a single solvent is preferred.

Filtration

Once your compound is in solution in a hot solvent, the solution should be filtered to remove any insoluble material. This material may be an

insoluble impurity or by-product or may simply be pieces of extraneous material such as dust, glass or paper. The solution should be filtered under gravity through a fluted filter paper into an Erlenmeyer flask using the technique described in pp. 74–75.

Use hand protection for hot filtration

In some cases the solution of your organic compound will be strongly colored by impurities. This is not a problem provided that the colored impurities remain in solution. However, occasionally they are adsorbed by the crystals as they form, to give an impure, colored product. Luckily the fact that such impurity molecules are easily adsorbed can be used to remove them from solution. This process is usually known as *decolorization*, and involves treating the hot solution with activated charcoal, often known as decolorizing carbon or under the tradename Norit®. To decolorize a solution add a small quantity of activated charcoal, usually about 2% by weight of the sample, to the hot, but not boiling, solution. If the solution is at or close to its boiling point, the addition of the finely divided charcoal will cause it to boil over. Continue to heat the solution containing the charcoal for about 5–10 min with occasional swirling or stirring. By this time the impurity molecules responsible for the color should have been adsorbed by the charcoal, and filtration of the mixture should give a decolorized solution of the organic compound. The filtration can be carried out under gravity through a fluted filter paper, although a second filtration may be necessary to remove all the fine particles of charcoal.

Decolorization

Use a steam bath for flammable solvents

Crystallization and What To Do if No Crystals are Formed

Having filtered your hot solution into an Erlenmeyer flask, cover the flask with a watch glass to prevent contamination by atmospheric dust, and then set it aside so that the solution can cool slowly. The rate of cooling determines the size of the crystals, rapid cooling favoring the formation of a lot of small crystals, and slow cooling encouraging the growth of fewer, but much larger, crystals. A convenient compromise between speed of crystallization and crystal quality is to allow the hot solution to cool to room temperature by placing the flask on a surface such as glass or cork that does not conduct the heat away too quickly. The *rate* of crystallization is usually greatest at about 50 °C below the melting point of the substance, and maximum formation of crystals occurs at about 100 °C below the melting point. Once the crystals have formed, it is usually a good idea to cool the solution from room temperature to about 0 °C by placing the Erlenmeyer in an ice bath. This will ensure that the maximum amount of crystals are obtained. It is not usually good practice to cool the solution below 0 °C, unless there are special problems in getting crystals to form in the first place (see p. 132), because this results in condensation of water vapor into the solution unless special precautions are taken.

What do you do when no crystallization occurs after cooling the solution to room temperature? You should attempt to induce crystalliza-

Seeding

Scratching

Cooling

tion by one of the following methods. Add a seed crystal which was saved from the original material before dissolution. This will provide a nucleus on which other crystals can grow. If this fails, try scratching the side of the flask with a glass rod. This is thought to produce micro-fragments of glass which then serve as nuclei to induce crystallization. If this fails, try cooling the flask in an acetone–solid CO_2 bath (see p. 104), and then scratch the side of the flask as the solution warms up to room temperature. If the substance still refuses to crystallize, it probably means that you have too much solvent; the excess solvent should be boiled off (**hood — check for flames in the vicinity**), and the reduced volume of solution should be set aside again until crystallization occurs.

Oiling out

The final problem that may be encountered in crystallization is the separation of the substance as an oil rather than as crystals. This is known as *oiling out*, and usually occurs when the compound is very impure or when it has a melting point that is lower than the boiling point of the solvent. Even if the oil eventually solidifies, the compound will not be pure, and the material should be redissolved by heating the solution. You may need to add a little more solvent at this stage, or more good solvent if mixed solvents are being used. Indeed, crystallization from a slightly more dilute solution may prevent oiling out. Slower cooling also favors the formation of crystals rather than oils. If the compound completely refuses to crystallize, the chances are that it is too impure, and it should be purified by some other means such as chromatography.

Collecting the Crystals

After crystallization the crystals are separated from the *mother liquor* by suction filtration, a technique which has already been discussed in detail on pp. 75–77. After filtration, the crystals should be washed with a little fresh solvent. Remember that if the crystallization has been performed using mixed solvents, the wash solvent should be the same mixture.

Second crop will be less pure

The mother liquor from the crystallization (which is now the filtrate) may still contain a significant quantity of your organic product. In this case a second batch of crystals, known as the *second crop*, can often be obtained by concentrating the mother liquor by boiling off some of the solvent (**hood — check for flames in the vicinity**) and then allowing the solution to cool and crystallize as before. However, be warned, the second crop is usually less pure than the first simply because the impurities were concentrated in the mother liquor during the first crystallization. Do not combine the two crops of crystals until you have checked the purity of each batch.

Drying the Crystals

After filtration and washing, the crystals should be dried to constant weight. Techniques for drying solids are discussed in pp. 136–138.

Special Crystallization Techniques

Crystallization of Very Small Quantities

When the amount of material to be crystallized is less than about 100 mg, the normal techniques of crystallization are inappropriate because of the losses of material that would occur, particularly during filtration. To crystallize small quantities (10–100 mg) of organic compounds, place the solid in a *very small* test tube, and dissolve it up in the minimum volume of hot solvent in the usual way. It is impossible to filter very small volumes of solution using the normal technique, so another method is needed. One way is to put a small plug of cotton wool in the tip of a Pasteur pipet and then slowly draw the hot solution through the cotton wool into the pipet (Figure 3.38(a)). The cotton wool will retain all but the finest of insoluble impurities. Quickly remove the wool from the end of the pipet using a pair of tweezers, and then release the hot solution from the pipet into the *pre-weighed* vessel where it will be allowed to crystallize. To avoid spills, it is safer to hold the Pasteur pipet over the crystallization vessel whilst removing the cotton wool. The ideal vessels for the crystallization of small quantities of material are small conical-bottomed centrifuge tubes or tubes specially designed for the purpose known as *Craig tubes*. The idea is to minimize the number of transfers and to avoid having to collect the crystals by filtration. If the crystallization is allowed to take place in a

Craig tubes

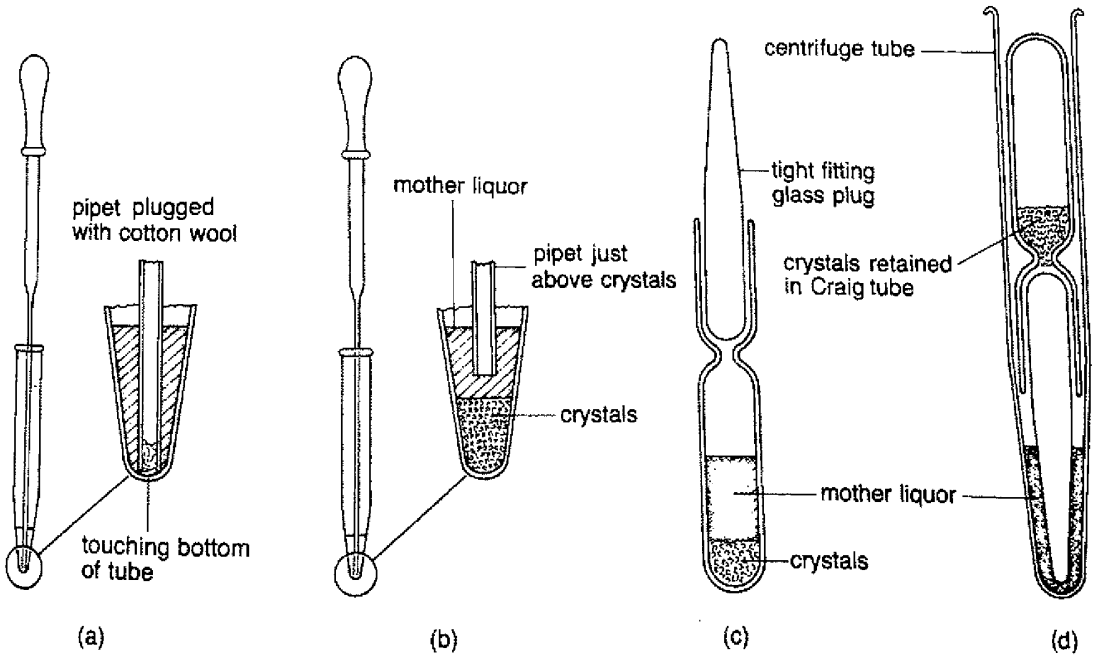


Figure 3.38. (a) Using a Pasteur pipet and cotton wool for filtration; (b) removing the mother liquor with a Pasteur pipet; (c) Craig tube before centrifugation; (d) Craig tube after centrifugation.

small centrifuge tube, the mother liquor should be removed using a Pasteur pipet, taking care not to suck up any crystals (Figure 3.38(b)). A small amount of wash solvent can be added, and can then be removed by pipet. The damp crystals should be dried in the same tube by placing it in a suitable drying apparatus (Figure 3.40).

Craig tubes (Figure 3.38(c, d)) are designed so that the mother liquor from the crystallization can be removed by *centrifugation*. The hot filtrate is transferred to the Craig tube as described above, and the crystallization is allowed to proceed. When crystallization is complete, insert the well-fitting glass 'plug' of the Craig tube, place an empty inverted centrifuge tube over the Craig tube, and invert the whole, making sure that the two parts of the Craig tube do not separate. Place the tube in the centrifuge, make sure the centrifuge is balanced, and turn it on for 20–30 s. The centrifugation will force the mother liquor past the glass plug, but the crystals will be retained by the plug (Figure 3.38(d)). The Craig tube plus crystals is then placed in a suitable drying apparatus to dry the crystals.

Always balance the centrifuge rotor arm

When the crystals are dry, the crystallization tube can be weighed, and provided that the empty weight was recorded, the weight of crystals can be determined. The crystals can be removed from the tube by inverting it over a piece of filter or weighing paper, and gently tapping it.

Fractional Crystallization

Fractional crystallization is a rather special technique for separating two compounds by repeated crystallization. Although chromatography has largely supplanted fractional crystallization as a separation method, the technique still has its uses, particularly in the resolution of racemic acids or bases by separation of their crystalline diastereomeric salts formed by reaction with optically active bases or acids respectively. A schematic plan for a fractional crystallization is shown in Figure 3.39. The first crystallization gives crystals (C_1) and mother liquor (ML_1). These are separated in the normal way, and the crystals are recrystallized to give crystals C_2 and mother liquor ML_2'. The first mother liquor is evaporated to dryness, and the residue is redissolved and crystallized to give crystals C_2' and mother liquor ML_2. The crystals C_2' are combined with ML_2'; the solvent is evaporated, and the residue is crystallized further. As the scheme unfolds, pure crystals of the less soluble component are obtained and the mother liquor becomes enriched in the more soluble component. In practice it is fairly easy to obtain a pure, less soluble component after two or three crystallizations, but the more soluble component may require further purification by some other technique.

Crystals for X-Ray Crystallography

Good crystals are an essential requirement if the material is to be submitted for X-ray structure analysis. Therefore the growth of X-ray

Organic Chemistry

Second Edition

MAITLAND JONES, Jr. Princeton University

W · W · NORTON & COMPANY NEW YORK · LONDON

Printed in the United States of America

Editor: Joseph Wisnovsky
Manuscript editor: Jeannette Stiefel
Project editor: Katherine Barry
Editorial assistants: Paul Fyfe, Amy Sievers
Manufacturing director: Roy Tedoff
Illustrations: J/B Woolsey Associates, TSI Graphics
Composition: TSI Graphics
Manufacturing: R.R. Donnelly & Sons Company
Text design: Jack Meserole
Cover design: Maitland Jones, III
Cover blackboard photographs: Nat Clymer
Cover molecular image: Greg Hart

The text of this book is composed in Trump Medieval with the display set in Helvetica

Library of Congress Cataloging-in-Publication Data
Jones, Maitland, Jr., 1937–
Organic chemistry / Maitland Jones, Jr.—2nd ed.
p. cm.
Includes bibliographical references and index.
ISBN 0-393-97405-7
1. Chemistry, Organic. I. Title.
QD253.J65 2000 99-052222
547—dc21

W. W. Norton & Company, Inc., 500 Fifth Avenue, New York, N.Y. 10110
www.wwnorton.com

W. W. Norton & Company Ltd., 10 Coptic Street, London, WC1A 1PU

1 2 3 4 5 6 7 8 9 0

4.8 PHYSICAL PROPERTIES OF DIASTEREOMERS: OPTICAL RESOLUTION

The formation of diastereomers allows the separation of enantiomers. Separation of enantiomers, called **resolution**, is a serious experimental difficulty. So far we have ignored it. Enantiomers have identical physical properties (except for the ability to rotate the plane of plane-polarized light), and one might legitimately wonder how in the world we are ever going to get them apart. At several points we used a single enantiomer without giving any hint of how a pair of enantiomers might be separated. The key to this puzzle is that diastereomers, unlike enantiomers, have different physical properties—melting point, boiling point, and so on.

One general procedure for separating enantiomers is to allow them to react with a naturally occurring chiral molecule to form a pair of diastereomers.* These can then be separated by taking advantage of one of their different physical properties. One typically can separate such a pair by crystallization, because the members of the diastereomeric pair will have different solubilities. Then, if the original chemical reaction can be reversed, we have the pair of enantiomers separated. Figure 4.42 outlines the general scheme and begins with a schematic recapitulation of Figure 4.33, which first described the reaction of a single enantiomer with a racemic mixture to give a pair of diastereomers. Be sure to compare the two figures.

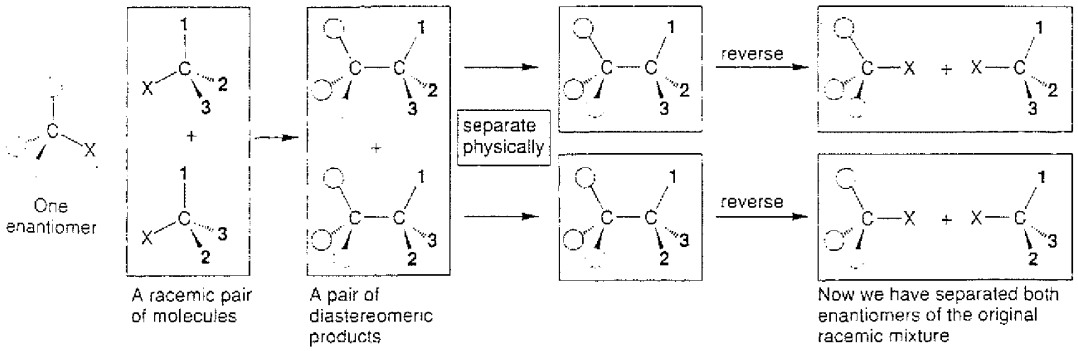


FIGURE 4.42 Resolution is a general method for separating the constituent enantiomers of a racemic mixture. A single enantiomer of a chiral molecule is used to form a pair of diastereomers, which can be separated physically. If the original chemical reaction can be reversed, the enantiomers can be isolated.

It is not even necessary to form covalent bonds. For example, in the traditional method for separating enantiomers of organic acids, optically active nitrogen-containing molecules, called alkaloids, are used to form a pair of diastereomeric salts, which can then be separated by crystallization. These alkaloids have wondrously complex structures (for more on these fascinating molecules, see Chapter 20, pp. 1047–51). Two examples, brucine and the notorious strychnine, are shown in Figure 4.43 along with the general procedure for this kind of resolution.

*Of course, there is no magic in using a molecule derived from natural sources. One made in the laboratory will do as well. However, many molecules found in Nature are easily isolable as pure enantiomers, and it is often convenient to use them.

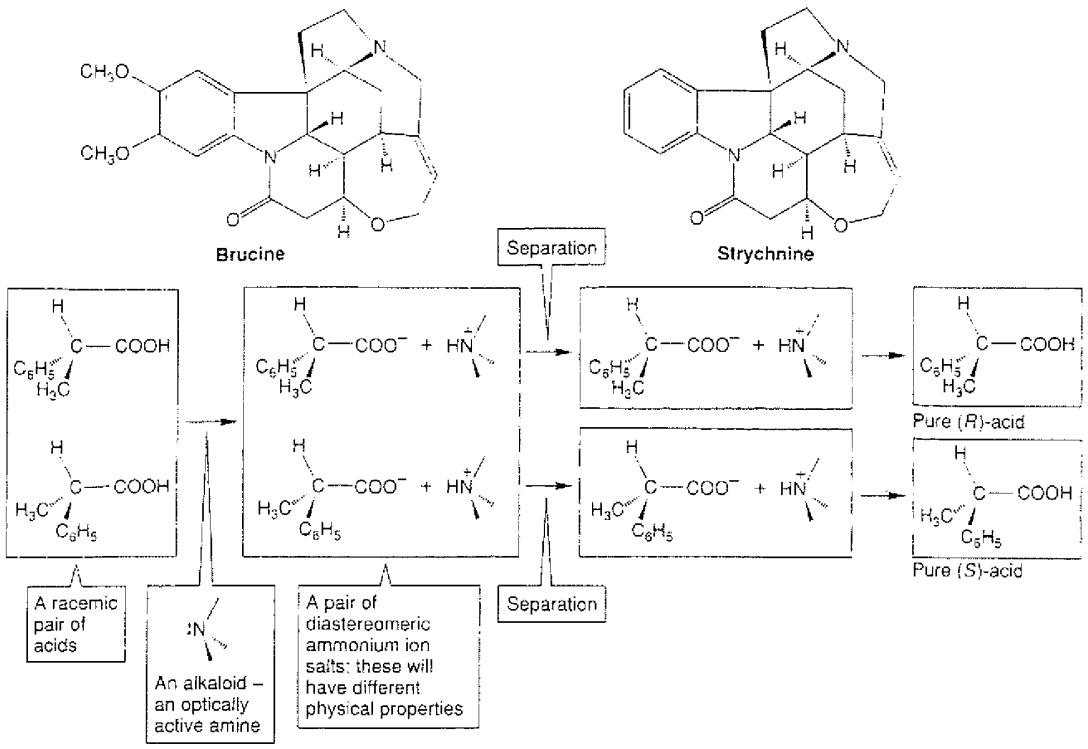
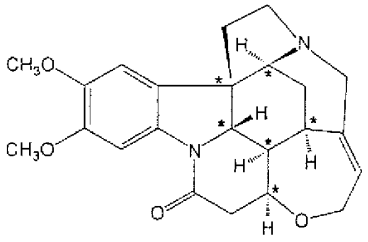


FIGURE 4.43 Two alkaloids, brucine and strychnine, are commonly used to separate the enantiomers of chiral organic acids. Diastereomeric salts are first formed, then separated by crystallization, and the individual enantiomeric acids are regenerated.

PROBLEM 4.20 Identify with an asterisk (*) all the stereogenic carbons in brucine.

ANSWER



PROBLEM 4.21 Identify each stereogenic carbon in brucine as (*R*) or (*S*).

These days, this general procedure has been extended so that all manner of enantiomeric pairs can be separated by chromatography. In such a technique, covalent chemical bonds are not formed, as they are not in the salt formation shown in Figure 4.43. Rather, advantage is taken of the formation of partial bonds—complexes—as the pair of enantiomers passes over an optically active substrate. The complexes are diastereomeric and

thus one will be more stable (contain a stronger partial bond) than the other. One enantiomer will be held more tightly than the other and will pass through the chromatography apparatus more slowly (Fig. 4.44).

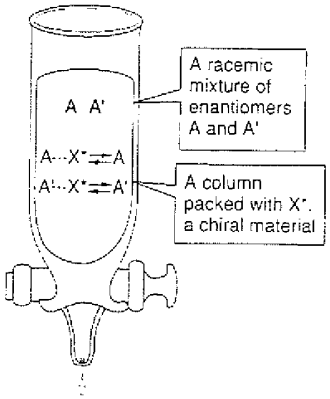


FIGURE 4.44 A chromatography column for separating enantiomers. The column is packed with an optically active substrate (X*) that forms a complex with the enantiomers A and A'. These complexes are diastereomers and have different physical properties, including bond strengths of A···X and A'···X. Both AX and A'X are in equilibrium with the free enantiomers, and these equilibria will be different for the two diastereomeric complexes. Therefore, A and A' will move through the column at different rates and emerge at different times.

4.9 DETERMINATION OF ABSOLUTE CONFIGURATION (*R* OR *S*)

Now that we have achieved the separation of our racemic mixture of enantiomers into a pair of optically active stereoisomers, we face the difficult task of finding out which enantiomer is (*R*) and which is (*S*). This problem is not trivial! Indeed, in Chapter 23, when we deal with sugars, we'll find that until rather recently, there was *no* way to be certain, and one just had to guess (correctly in the case of the sugars, it turns out). One would like to peer directly at the structures, of course, and under some circumstances this is possible.

X-ray crystallography can determine the relative positions of atoms in a crystal, and a special kind of X-ray diffraction called "anomalous dispersion" can tell the absolute configuration of the molecule. But this is not a generally applicable technique—one needs a crystalline compound, for example. It does serve to give us some benchmarks, though. If we know the absolute configuration (*R* or *S*) of some compounds, we may be able to determine the absolute configurations of other molecules by relating them to the few compounds of known absolute configuration. We must be very careful, however. The chemical reactions that interconvert the molecules of known and unknown absolute configurations must not alter the stereochemical arrangement at the stereogenic atoms, or if they do, it must be in a known fashion. How do we know whether a given chemical reaction will or will not change the stereochemistry? We need to know the reaction mechanism—to know how the chemical changes occur—in order to answer this question. This reason is just one of many for the study of reac-

Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction

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Received June 11, 1987

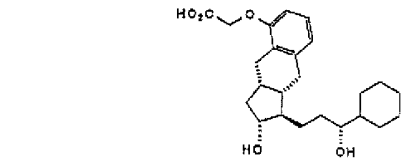
The optically pure [[1(*R*)-(3-cyclohexylpropyl)-2,3,3a,4,9,9a-hexahydro-2(*R*)-hydroxy-(3a*S*,9a*S*)-1*H*-benz[*f*]-inden-5-yl]oxy]acetic acid (**2a**) and its 1,2,3a,9a-tetraepi isomer (**2b**), 15-deoxy analogues (prostaglandin numbering) of the potent antiulcer agent **1** (U-68,215), have been synthesized. The resolution of the optical isomers was accomplished by LC (liquid chromatography) separation of the 2(*RS*)-(*S*)-α-methylbenzyl carbamates **8a,b**, which were obtained from the racemic alcohols **7a,b**. The racemic alcohols **7a,b** were initially synthesized via condensation of the enol lactone **4** with the phosphonate **3c** followed by the catalytic hydrogenation and sodium borohydride reduction of the adduct **5**. A second and improved route involved the coupling reaction of the anion of the phosphonate **3c** and the enolate of the γ-keto ester **12a**, both generated in situ in the presence of the excess lithium diisopropylamide, to give cyclopentenone **5** directly in 75% yield. The absolute configuration of the resolved isomers **7a** and **7b** was confirmed by comparison with authentic samples which were synthesized independently via selective deoxygenation of the side chain hydroxyl group from **16a** and **16b**, prepared with known absolute configuration. The racemic alcohols **7a,b** and the resolved optically pure isomers **7a** and **7b** were then converted separately in three steps to the racemic analogue **2a,b** and the optically pure isomers **2a** and **2b**, respectively. The absolute configuration of the optically pure isomers **2a** and **2b** was further established by comparing with the authentic samples synthesized independently from the authentic alcohols **7a** and **7b**, prepared from **16a** and **16b**, respectively. Interestingly, most of the biological activity seemed to reside with **2b**, the "unnatural" isomer in terms of prostaglandin analogues.

Introduction

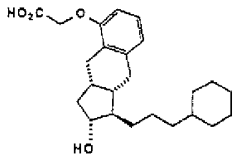
A benzindene prostaglandin analogue with a cyclohexyl side chain (**1**, U-68,215) was recently shown by us to be a potent antiulcer agent.[1,2] Moreover, unlike most prostaglandin analogues of interest in the gastrointestinal area, this compound is considerably more stable and exhibits no enteropooling, uterotonic, or gastrointestinal mucosa cellular proliferative activity.[1] However, it does have significant cardiovascular effects which could limit its therapeutic utility in antiulcer therapy. We were therefore interested in synthesizing some selected analogues of **1** in hopes of completely separating out the cardiovascular activity while maintaining the potent antiulcer behavior. The side chain deoxy analogue of **1** was chosen as one of the analogues to examine this possibility and was initially prepared in racemic form for the initial biological screening. Encouragingly, the racemate **2a,b** was found to demonstrate significant cytoprotective and gastric antisecretory activity while having only minor effects on blood pressure. However, unlike the corresponding dihydroxy analogue **1**, the racemic analogue **2a,b** exhibited enteropooling (the accumulation of fluid in the small intestine, an index of the diarrheogenic activity of prostaglandin) activity. We therefore set out to synthesize the optically pure isomer **2a** with natural configuration and its enantiomer (**2b**). The biological activity of each isomer, then, would enable us to determine which component of the racemate was responsible for the desirable cytoprotective/antisecretory activity and which one contributed to the undesirable enteropooling activity.

Results and Discussion

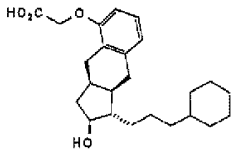
The synthesis of the racemic side chain deoxy analogue **2a,b** was accomplished in a straightforward manner as shown in Scheme I. Bromination of alcohol **3a** followed



1 (U-68,215)



2a



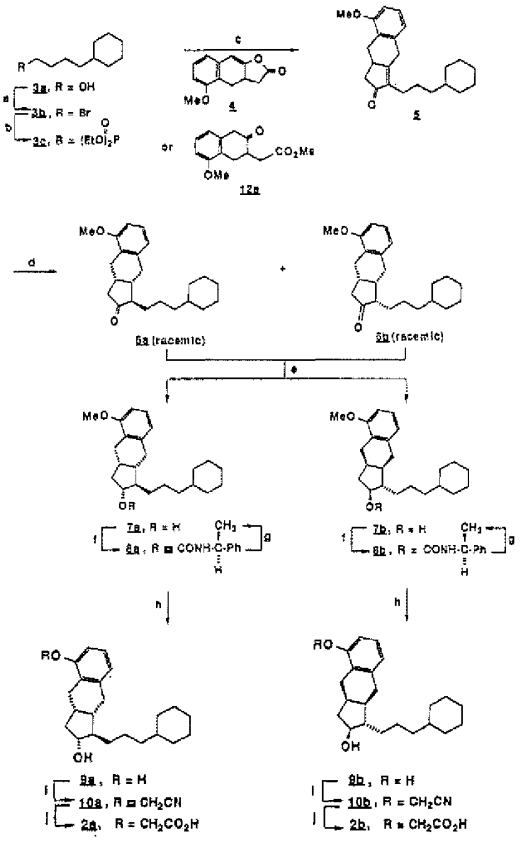
2b

by displacement of the bromide **3b** with the anion of diethyl phosphite afforded the phosphonate reagent **3c** in 78% overall yield. The anion of this phosphonate, **3d**, generated in situ by reacting **3c** with 1 equiv of *n*-butyllithium at −78 °C, was coupled with 0.5 equiv of enol lactone **4**.[2] When the reaction mixture was warmed to room temperature, protonated with 0.5 equiv of acetic acid, and heated, the coupled product undergoes the intramolecular Wadsworth-Emmons-Wittig reaction to give cyclopentenone derivative **5** in 68% yield. A similar transformation has already been described in detail in the synthesis of the dihydroxy analogue **1**.[2] As shown in Scheme II, the mechanism of the coupling reaction of the enol lactone **4** with 2 equiv of the phosphonate anion **3d** proceeds via the initial adduct **13a**, a hemiketal intermediate which collapses to intermediate **13b**, which rapidly reacts with excess phosphonate anion **3d** to generate the dianion **13d**. Addition of 1 equiv of acetic acid (against 2 equiv of base used) as an external proton source upon warming to room temperature generates the monoanion **13e**. This monoanion undergoes an intramolecular Wadsworth-Emmons-Wittig reaction upon heating at 65

(1) Robert, A.; Aristoff, P. A.; Wendling, M. G.; Kimball, F. A.; Miller, W. L.; Gorman, R. R. *Prostaglandins* **1985**, *30*, 619.
(2) Aristoff, P. A.; Johnson, P. D.; Harrison, A. W. *J. Am. Chem. Soc.* **1985**, *107*, 7967.

0022-3263/87/1952-5594$01.50/0

Scheme I[a]

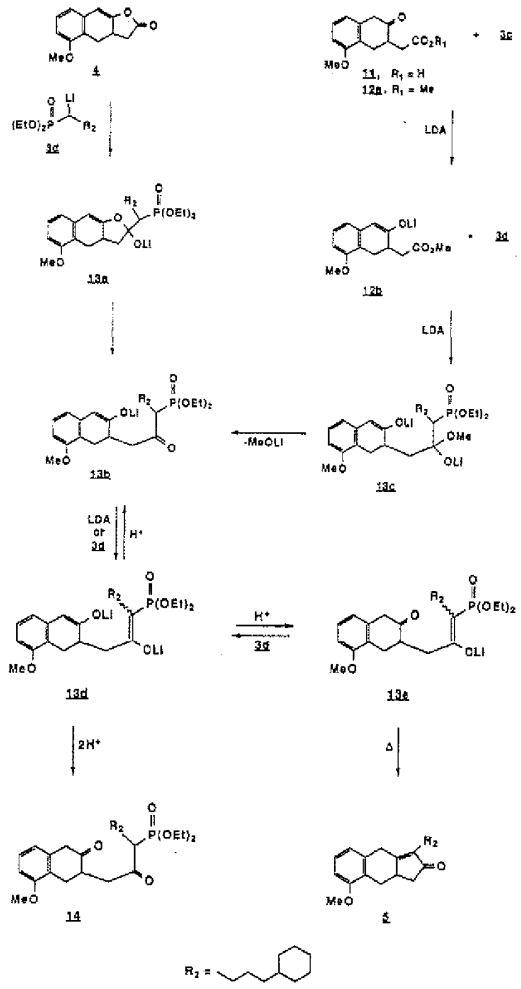


[a] (a) PBr_3, Δ; (b) $(EtO)_2P(O)Li$, THF, Δ; (c) 4 or 12a (see Scheme II), base, THF; HOAc, Δ; (d) Pd/C, H_2, K_2CO_3, EtOH; (e) $NaOH/H_2O/EtOH/NaBH_4$, −10 °C; (f) phosgene, triethylamine, (S)-(−)-α-methylbenzylamine, toluene; (g) $LiAlH_4$/THF, Δ; (h) Ph_2PLi, THF, Δ; (i) K_2CO_3, $ClCH_2CN$, acetone, Δ; (j) KOH, EtOH, H_2O, Δ.

°C to give the desired cyclopentenone derivative **5**.

More recently, on the basis of this postulated mechanism, we have developed an improved process (Scheme II) which involves the direct coupling of the anion **3d** of the phosphonate **3c** and the lithium enolate **12b** of the γ-keto ester **12a**.[3] We rationalized that the enolate ester **12b** can also undergo a similar coupling reaction with the phosphonate anion **3d**. If the resulting coupled hemiketal intermediate, **13c**, suffers loss of lithium methoxide, intermediate **13b** would be obtained, which is then converted to **5** as described previously. This mechanistic consideration prompted us to try an in situ generation of both anions **3d** and **12b**. Since the enolate formation of the ketone is much faster than of the ester with a base such as lithium diisopropylamide, we decided to try this possibility. Thus, when 3 equiv of lithium diisopropylamide in tetrahydrofuran were added to a tetrahydrofuran solu-

Scheme II



tion containing 1 equiv of keto ester **12a**[3] and 1.5 equiv of phosphonate **3c** at −78 °C, followed by slow warming to room temperature, addition of 1.5–1.8 equiv of acetic acid, and heating at 65 °C, the desired cyclopentenone derivative **5** was isolated in 75% yield after chromatography. This result indicates that the deprotonation of **3c** and **12a** to form the phosphonate anion **3d** and the enolate anion **12b**, respectively, is much faster than the deprotonation to form the enolate of the ester in **12a**. Actually, when we monitored the reaction by quenching an aliquot with saturated aqueous ammonium chloride, 5–10 min after the addition of lithium diisopropylamide at −78 °C, the keto phosphonate **14** was isolated, indicating that the coupling reaction of **3d** and **12b** occurs rather rapidly. When the reaction was interrupted by quenching with excess acid before undergoing intramolecular Wadsworth–Emmons–Wittig reaction, i.e., before protonation with acetic acid and heating, the coupled product **14** was isolated in 75% yield after chromatography. This result shows that the intramolecular Wadsworth–Emmons–Wittig reaction (**13e** to **5**) is a high-yield process. Although the overall yield of this process, from the keto acid **11** to the enone **5** via the γ-keto ester **12a** (49%), is no better than the original

(3) The γ-keto ester **12a** used in the modified process was easily obtained in 66% yield from the keto acid **11**, which is the precursor to enol lactone **4** and an intermediate in the synthesis of 15-hydroxy analogue 1[2] (see Experimental Section). This successful conversion, in conjunction with the modified phosphonate chemistry described in this report, has provided the more viable and improved synthesis of the benzindene analogs.

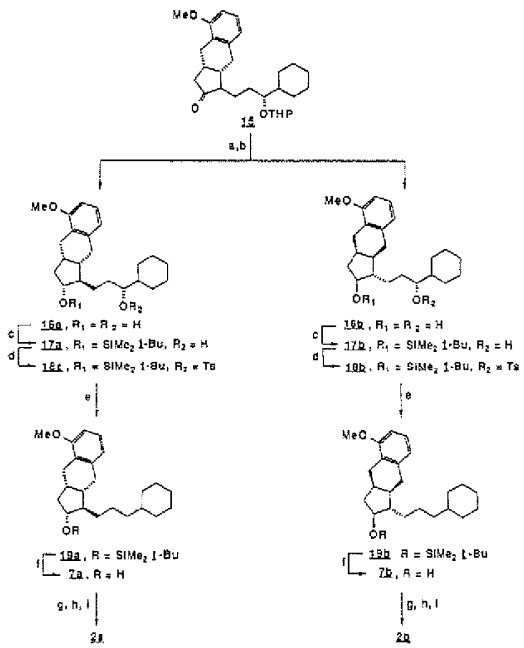
process via enol lactone (57%), in the large-scale preparation (>300 g), the process via the γ-keto ester has been found to have the following advantages: (1) the reproducibility of the conversion of the γ-keto ester **12a** to enone **5** was far better than the enol lactone **11**; (2) the conversion of the keto acid **11** to the γ-keto ester **12a** was much easier to accomplish on large scale than the conversion to the somewhat labile enol lactone, which required removal of large quantities of acetic anhydride in the workup; (3) when the resolved phosphonate is used for the condensation–cyclization, as in the case of synthesizing 15-hydroxy analogue **1**, there is a distinctive advantage in using less phosphonate for the reaction. Overall, this remarkable result indicates that this reaction is a potentially useful method for achieving the direct synthesis of cyclopentenone or cyclohexenone derivatives from γ- or δ-keto esters, respectively. We are currently examining the scope and limitations of this transformation.[4]

Hydrogenation of the enone **5** (Scheme I) using 10% palladium on carbon in ethanol with a catalytic amount of potassium carbonate present gave after 2 days at room temperature a difficultly separated mixture of the initial product **6b** (racemic) and its equilibrated isomer **6a** (racemic) in 80% yield (3:1 ratio of **6a** to **6b**). Apparently the long reaction time allowed significant equilibration of the initially formed product **6b** to **6a** in the presence of potassium carbonate. When the same hydrogenation was carried out with a different lot of 10% palladium on carbon, the hydrogenation was completed in 7 h, and the product ratio was reversed to 1:3 for **6a** to **6b**. Thus, the variations in activity of the catalyst play an important role in this hydrogenation reaction. The more active catalyst, however, also lowered the yield of **6a,b** to 73% and caused the formation of the side products such as overreduction to the alcohol (9%) and the deoxy compound (17%).

The mixture of racemic ketones **6a** and **6b** was converted directly in a one-pot operation to the racemic alcohol mixture **7a,b** in 84% yield by treatment with aqueous sodium hydroxide and sodium borohydride in ethanol. This reaction succeeds because whereas equilibration of **6b** to **6a** is fast, hydride reduction of **6b** is slow and that of **6a** is relatively fast.[2] The net effect is therefore that all of **6b** is converted to the racemic mixture of alcohols **7a,b**. Without resolution of the optical isomers **7a** and **7b**, initially the alcohol mixture **7a,b** was converted in three steps (90% overall yield) to the racemic 15-deoxy benzindene analogue **2a,b**, by using the same chemistry developed to prepare the parent 15-hydroxy compound **1**.[2] Thus, the alcohols **7a,b** were first demethylated with lithium diphenylphosphide in tetrahydrofuran (70 °C, 7 h) to give the diols **9a,b** in 95% yield.[5] The racemic diol mixture **9a,b** was then selectively alkylated with chloroacetonitrile in the presence of potassium carbonate in acetone (65 °C, 24 h) to afford the cyanomethyl ethers **10a,b** (99%). Finally, the hydrolysis of the cyano group was smoothly accomplished by heating **10a,b** in 25% aqueous potassium hydroxide in methanol or ethanol (90 °C, 5 h) in 97% yield. Thus, keto ester **12a** was converted in six steps and 46% overall yield to the racemic benzindene analogue **2a,b** (13 steps and 25% overall yield from 5-methoxy-2-tetralone).

For the synthesis of the optically active isomer **2a** with natural configuration and its enantiomer (**2b**), the resolution of the racemic mixtures at some stage of the synthesis was necessary. We decided to pursue the resolution of the racemic alcohols **7a,b** by reacting the alcohols with a chiral reagent and separating the diastereomers chromatographically. The absolute configuration of these two diastereomers was then determined by matching the HPLC peaks with the authentic diastereomer synthesized independently from the established route (vide infra). On an analytical scale, the carbamate formation from the racemic **7a,b**, and authentic **7a** and **7b** (vide infra), was initially carried out by using (*S*)-(−)-α-methylbenzyl isocyanate[6,7] in toluene at room temperature with dibutyltin acetate as the catalyst.[8] Apparently the catalyst was very effective for this transformation, and the reaction could be completed in 3–4 h at room temperature. Without this catalyst, however, refluxing in toluene for an extended period of time was required. From this analytical scale carbamate formation the absolute configuration of each carbamate was assigned by matching the HPLC peaks of the carbamates **8a** and **8b**, obtained from the authentic alcohols **7a** and **7b**, prepared from compounds of known configuration as shown in Scheme III. The less polar carbamate corresponded to the diastereomer **8a** and the more polar carbamate to the diastereomer **8b**. When the reaction was scaled-up for the synthesis, however, the reaction conditions to form carbamates **8a** and **8b** were altered to use the less expensive (*S*)-(−)-α-methylbenzylamine. Thus, the racemic alcohol mixture **7a,b** was reacted

Scheme III[a]



[a] (a) NaOH, H_2O, EtOH, $NaBH_4$, −10 °C; (b) HOAc–THF–H_2O (3:1.5:1), 45 °C, 3 h; (c) *t*-BDMSiCl, imidazole, THF; (d) TsCl, pyridine; (e) $LiAlH_4$, Et_2O; (f) HCl, 2-PrOH, H_2O; (g) Ph_2PLi, THF, Δ; (h) K_2CO_3, $ClCH_2CN$, acetone, Δ; (i) KOH, EtOH, H_2O, Δ.

(4) The direct conversion of a γ-keto ester with a hindered carbonyl group to a cyclopentenone derivative has also been reported, see: Haltman, R. L.; Vollhardt, K. P. C. *Tetrahedron Lett.* **1986**, 1461.

(5) Ireland, R. E.; Walba, D. M. *Tetrahedron Lett.* 1976, 1071. See, for a useful review: Bhatt, M. V.; Kulkami, S. U. *Synthesis* 1983, 249.

(6) Pirkle, W. H.; Hoekstra, M. S. *J. Org. Chem.* 1974, 39, 3904 and references cited therein.

(7) Morrison, J. D., Ed. In *Asymmetric Synthesis*; Academic: Orlando, 1983; Vol. 1, Chapter 6.

(8) Thomas, F.; Thorne, M. P. *Can. J. Chem.* 1976, *54*, 24.

in toluene with phosgene, triethylamine, and the optically active amine, to form the mixture of carbamates **8a** and **8b**.[6] Preparative LC separation (liquid chromatography) afforded >99% pure **8a** and **8b** (see Experimental Section). Each of these carbamates **8a** and **8b**, was then converted back to the optically pure alcohols **7a** and **7b**, respectively, in 100% yield by treating the carbamates in tetrahydrofuran with lithium aluminum hydride. Each of these alcohols **7a** and **7b** was then converted in three steps, as described earlier for the synthesis of the racemic **2a,b**, to the optically pure **2a** and **2b**, respectively. These optically pure **2a** and **2b** showed physical properties identical with those of the racemic **2a,b** except for optical rotation and melting point (**2a,b**, mp 158–160 °C; **2a**, mp 133.5–135.5 °C; **2b**, mp 133.5–135.5 °C). The specific rotations of **2a** ($[\alpha]_D$ + 30.69°) and **2b** ($[\alpha]_D$ −28.16°) also showed **2a** and **2b** are enantiomers. The HPLC analyses also confirmed that **2a** and **2b** exhibited retention times identical with those of the authentic samples synthesized from the material with known absolute configuration.

The confirmation of the absolute configuration of **2a** and **2b** synthesized from the resolved alcohols **7a** and **7b** via chiral carbamates shown in Scheme I required the synthesis of authentic acids **2a** and **2b** for comparison. As shown in Scheme III, the 15-hydroxy intermediates **16a** and **16b** were synthesized from optically pure 15[2] and separated during the course of the synthesis of 1.[2] The diols 16a (98.5% pure) and **16b** (>99% pure) were independently converted to alcohols **7a** and **7b**, respectively, via selective removal of the side chain hydroxyl group. These alcohols were then used as the reference for matching the peaks with the resolved alcohols **7a** and **7b**, thereby establishing the absolute configuration of each isomer. This selective removal was made possible based on the observation that the ring hydroxyl group was preferentially silylated over the side chain hydroxyl group. Thus, as shown in Scheme III, the diols **16a** and **16b** were therefore separately silylated using *tert*-butyldimethylsilyl chloride and imidazole in tetrahydrofuran.[9] The major products obtained were assigned as **17a** and **17b**, respectively. Each of these isomers was then subjected to tosylation (*p*-toluenesulfonyl chloride/pyridine) to give the tosylates **18a** and **18b** from **17a** and **17b**, respectively. The tosylates 18a and 18b were then treated with lithium aluminum hydride in ether to give the silyloxy products **19a** and **19b**, respectively. The removal of the silyl group from **19a** and **19b** in aqueous hydrochloric acid/2-propanol resulted in the formation of the authentic alcohols **7a** and **7b**, respectively. Alcohols **7a** and **7b** exhibited the opposite specific rotations but otherwise were spectroscopically and chromatographically identical. Finally, the alcohols **7a** and **7b** prepared from 16a and **16b**, respectively, were converted to the authentic acids **2a** and **2b**, respectively, as previously described. The spectroscopic data, specific rotations, and retention times by HPLC of **2a** and **2b** obtained by the resolution process (Scheme I) matched the authentic acids 2a and **2b** formed via the route shown in Scheme III.

The biological activities of the racemate **2a,b**, the "natural" isomer **2a**, and the "unnatural isomer" **2b** were tested orally in rats. The racemate **2a,b** was found to be cytoprotective (against ethanol-induced lesions, ED_{50} = 40 μg/kg), antiulcer (against aspirin-induced ulcers, ED_{50} = 35 μg/kg) and antisecretory (ED_{50} = 500 μg/kg). This racemate **2a,b** also did not lower blood pressure when given orally to anesthesized rats at 20 times the antisecretory ED_{50}. However, orally in rats, it was enteropooling (ED_{50} = 50 μg/kg) and diarrheogenic (ED_{50} < 150 μg/kg). When given subcutaneously, **2a,b** was not cytoprotective, enteropooling, nor antisecretory. Its two optical isomers, **2a** and **2b**, were also cytoprotective orally (ED_{50}'s = 65 and 15 μg/kg, respectively). Paradoxically, the isomer with an unnatural configuration, **2b**, was much more active than the isomer with a natural configuration, **2a** (antisecretory ED_{50}'s = 150 and 3000 μg/kg, respectively, for **2b** and **2a**). The unnatural isomer **2b** was also more enteropooling than the natural isomer **2a** when administered orally (ED_{50}'s = 50 and 2000 μg/kg, respectively). However, both were inactive at 5000 μg/kg when administered subcutaneously.

In conclusion, the synthesis of the racemic (**2a,b**) as well as the optically pure side chain deoxy analogue (**2a**) of a potent antiulcer agent 1 and its enantiomer (**2b**) has been achieved via the modification of the synthesis of 1 reported previously. The benzindene nucleus has been formed via a convergent cyclopentane annulation route, the key step either involving formation of **5** from enol lactone **4** or, via an improved process, from the keto ester **12a**. The synthesis of the optically pure isomers **2a** and **2b** has been achieved by resolving the intermediates **7a,b** via the preparative liquid chromatographic separation of their carbamates, **8a** and **8b**. The absolute configuration of each optically pure isomer has been established by comparing the physical and HPLC properties with the authentic samples, synthesized independently via deoxygenation from the side chain of the intermediates **16a** and **16b** with known configuration. It has also been found, for the first time, that the isomer with the "unnatural" configuration among the benzindene prostaglandin analogues has shown more potent biological activity than the isomer with the "natural" configuration.

Experimental Section[10]

1-Bromo-4-cyclohexylbutane (3b). To 8.1 mL (47 mmol) of 4-cyclohexyl-1-butanol (3a) was added dropwise 2.2 mL (23 mmol) of phosphorus tribromide. The mixture was stirred at 0 °C for 15 min, at room temperature for 2 h, and then at 100 °C for 1.5 h, cooled to 0 °C, quenched with 50 g of ice, diluted with 100 mL of brine, and extracted with ether. The organic layer was washed with brine, dried (Na_2SO_4), filtered, concentrated, and bulb-to-bulb distilled at 100 °C at 2 mmHg, to give 9.84 g (96%)

(9) Corey, E. J.; Venkateswarlu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190. In this particular case, however, we found tetrahydrofuran to be a superior solvent to dimethylformamide for the selective silylation.

(10) All melting points were obtained with a Thomas-Hoover melting point apparatus and are uncorrected. The ^1H NMR spectra of chloroform-*d* solutions were obtained on a Varian EM-390 spectrometer operating at 90 MHz. Chemical shifts are reported in δ (parts per million) relative to internal tetramethylsilane. Combustion analyses, mass spectra (including high resolution), and infrared spectra (IR) were obtained by the Physical and Analytical Chemistry Unit of The Upjohn Company with IR spectra being obtained either on neat samples (oils) or on Nujol mulls (solids). GC/MS were obtained with a Hewlett-Packard 5992A GC/MS system. HPLC analyses were obtained with a Varian 5500 or 5560 HPLC chromatograph with appropriate columns and solvent systems. Optical rotations were measured by a Perkin-Elmer 241 polarimeter. Thin-layer chromatography (TLC) was conducted with silica gel GF plates (Analtech Uniplates). The TLC plates were visualized first by UV light (Mineralight UVS-11) and then sprayed with either 50% aqueous or methanolic sulfuric acid followed by heating. For the phosphonates, 0.5% zinc chloride/0.5% diphenylamine in acetone was used as the spraying agent. For flash chromatography and preparative liquid chromatography (LC), silica gel 60 (40–63 μm, E. Merck) was used. The liquid chromatography (LC) was performed either by various sizes of Michel-Miller columns (Ace Glass, Inc.) dry-packed with silica gel or by prepacked columns (E. Merck). The solvents were delivered by Milton-Roy pumps. For gravity column chromatography, silica gel 60 (63–200 μm, E. Merck) was used. The analyses of fractions were performed by TLC using an appropriate solvent or a mixture of solvents. All solvents were reagent grade or reagent grade distilled from glass (Burdick and Jackson). The dry solvents used in reactions, such as THF, DMF, and DMSO, were Burdick and Jackson brand high-purity solvents dried over 4-Å molecular sieves. All reactions were degassed and were conducted under an inert atmosphere.

of **3b** as a colorless liquid: ^{1}H NMR ($CDCl_3$, TMS) δ 3.43 (t, J = 7 Hz, 2 H), 2.3–0.6 (m, 17 H); IR (film) ν_{max} 732,648 cm^{-1}; high-resolution MS, m/z calcd for $C_{10}H_{19}{}^{79}Br$ (M^+) 218.0671, found 218.0664; R_f 0.54 (hexane).

Diethyl (4-Cyclohexylbutyl)phosphonate (3c). A solution of 6.7 mL (52 mmol) of diethyl phosphite in 400 mL of THF was cooled to −78 °C, and 36.4 mL (57 mmol) of n-butyllithium in hexane (1.57 M) was added dropwise. The resulting mixture was stirred at −78 °C for 30 min and at 0 °C for 30 min and then treated with 9.4 g (43 mmol) of 1-bromo-4-cyclohexylbutane (**3b**) in 50 mL of THF dropwise over 10 min. The resulting solution was stirred at room temperature for 1 h and at 60 °C for 4 h and then quenched with 500 mL of brine containing 40 mL of 1 N aqueous hydrochloric acid. The layers were separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried (Na_2SO_4), filtered, and concentrated. The crude product was flash chromatographed on 200 g of silica gel 60, eluting with 1 L of 50%, 1 L of 60%, and 2 L of 70% ethyl acetate in hexane to give 9.6 g (81%) of **3c** as a colorless oil: ^{1}H NMR ($CDCl_3$, TMS) δ 4.13 (2 overlapping q, J = 7 Hz, J_{P-H} = 7 Hz, 4 H), 1.9–0.5 (m, including t at 1.33, 25 H); IR (film) ν_{max} 1392, 1244, 1229, 1164, 1098, 1059, 1032 cm^{-1}; high-resolution MS, m/z calcd for $C_{14}H_{29}O_3P$ (M^+) 276.1854, found 276.1852; R_f 0.14 (50% ethyl acetate/hexane). Anal. Calcd for $C_{14}H_{29}O_3P$: C, 60.84; H, 10.58. Found: C, 61.01; H, 10.96.

Methyl 5-Methoxy-2-oxo-1,2,3,4-tetrahydro-3-naphthaleneacetate (12a). The keto acid 11[2] (33.1 mmol) dissolved in 27 mL of acetonitrile was treated with a solution of anhydrous hydrochloric acid in methanol, prepared by adding 3.7 mL of acetyl chloride to 23 mL of methanol at 0 °C.[11] The mixture was stirred at room temperature for 3 h and then treated with 5.7 mL of water. The resulting mixture was stirred at room temperature overnight, quenched with saturated aqueous sodium bicarbonate to pH 5, and concentrated in vacuo. The residue was extracted with ethyl acetate, and the organic layer was washed with brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue (7.4 g) was purified by column chromatography on 200 g silica gel (63–200 μm), eluting with 15% ethyl acetate/hexane, and then recrystallized from *tert*-butyl methyl ether/hexane, to give in the first crop 4.93 g and in the second crop 0.5 g, with a total of 5.43 g (66%), of pure keto ester **12a** as a pale yellow solid: mp 71–72 °C; ^{1}H NMR ($CDCl_3$) δ 7.40–6.68 (m, 3 H), 3.84 (s, 3 H), 3.72 (s, 3 H), 3.72–2.32 (m, 7 H); IR (mull) 1731, 1709, 1588 cm^{-1}; high-resolution MS, m/z calcd for $C_{14}H_{16}O_4$ (M^+) 248.1049, found 248.1046, other ions at 217, 216, 188, 175, 174, 160, 146, 131, 115, 104, 91; R_f 0.35 (25% ethyl acetate/hexane). Anal. Calcd for $C_{14}H_{16}O_4$: C, 67.73; H, 6.50. Found: C, 67.50; H, 6.60.

(3a*RS*)-1-(3-Cyclohexylpropyl)-3,3a,4,9-tetrahydro-5-methoxy-2*H*-benz[*f*]inden-2-one (5). Method A. A solution of 496.5 mg (2.0 mmol) of keto ester **12a**, 829.1 mg (3.0 mmol) of phosphonate **3c**, and 10 mL of THF at −78 °C was treated dropwise over 1–2 h with 6.0 mmol of lithium diisopropylamide in 6 mL of 2:1 hexane/THF. The resulting light pink solution was stirred at −78 °C for 3 h and then at room temperature overnight, treated with 0.21 mL (3.6 mmol) of glacial acetic acid, and then heated to reflux (bath temperature, 70 °C). TLC analysis showed R_f 0.71, 0.66, and 0.21 for **12a**, **5**, and **14**, respectively, in 50% ethyl acetate/hexane. The phosphonate **3c** stayed at the origin in the same solvent system. After 7 h the mixture was cooled to 0–5 °C and quenched with 10% aqueous sodium bisulfate to pH 7–8. The THF was removed in vacuo, and the residue was extracted with ethyl acetate. The organic extract was washed with water, brine, and saturated aqueous sodium bicarbonate, dried (Na_2SO_4), filtered, and concentrated to give the crude product as a yellow oil. This oil was purified by liquid chromatography (LC), eluting with 2.8 L of 10% ethyl acetate/hexane and 3 L of ethyl acetate, to give 504.5 mg (74.5%) of pure cyclopentenone **5** as a near colorless oil and 220 mg (26.5%) of the recovered phosphonate **3c** as a light brown oil.

Method B. A solution of 2.17 g (7.85 mmol) of phosphonate **3c** and 150 mL of THF at −78 °C was treated with 5 mL (8.0 mmol) of 1.6 M n-butyllithium, in hexane, stirred at −78 °C for 1 h, and then treated dropwise with a solution of 0.80 g (3.7 mmol) of enol lactone **4**[2] in 20 mL of THF. The resulting mixture was stirred for 1 h at −78 °C and 2 h while warming to 0 °C, then treated with 0.21 mL (3.6 mmol) of glacial acetic acid, stirred at 0 °C for 15 min and at 55–60 °C for 6 h, cooled to 0 °C, and quenched with 250 mL of brine containing 6 mL of 1 N aqueous hydrochloric acid. The layers were separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine/saturated aqueous sodium bicarbonate (3:1) and brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. The crude product was flash chromatographed on 200 g of silica gel 60, eluting with 1 L 10%, 1 L 15%, 1 L 20%, 2 L 50%, and 2 L 75% ethyl acetate in hexane to give 0.855 g (68%) of pure **5** as a near colorless oil.

The physical properties of compound **5** were as follows: ^{1}H NMR ($CDCl_3$, TMS) δ 7.30–6.60 (m, 3 H), 3.87 (s, 3 H), 4.10–3.40 (m, 3 H), 3.10–0.60 (m, 21 H); IR (film) ν_{max} 1700, 1655, 1595 cm^{-1}; high-resolution MS, m/z calcd for $C_{23}H_{30}O_2$ (M^+) 338.2246, found 338.2252; R_f 0.50 and 0.66 (25% and 50% ethyl acetate/hexane, respectively). Anal. Calcd for $C_{23}H_{30}O_2$: C, 81.61; H, 8.93. Found: C, 81.66; H, 9.42.

Diethyl [1-(3-Cyclohexylpropyl)-2-oxo-3-(2-oxo-5-methoxy-1,2,3,4-tetrahydro-3-naphthyl)propyl]phosphonate (14). The reaction was run in an identical manner as in the previous experiment (method A) for the preparation of cyclopentenone **5**. However, the reaction was interrupted by quenching with 10% aqueous sodium bisulfate (to pH 7) after the reaction mixture was stirred overnight at room temperature rather than treating with acetic acid and heating. The reaction mixture was then extracted with ethyl acetate, and the organic extract was washed with saturated sodium bicarbonate and brine, dried (Na_2SO_4), filtered, and concentrated in vacuo to give a brown oil. The oil was purified by LC on 200 g silica gel 60, eluting with 50% ethyl acetate/hexane to give 740.2 mg (75%) of **14** as a near colorless oil: ^{1}H NMR ($CDCl_3$, TMS) δ 7.34–6.65 (m, 3 H), 4.16 (quintet, J = 7 Hz, 4 H), 3.80 (s, 3 H), 3.78–0.78 (m, 25 H), 1.40 (t, J = 7 Hz, 6 H); IR (film) ν_{max} 1713, 1589, 1265, 1250 cm^{-1}; high-resolution MS, m/z calcd for $C_{27}H_{41}O_6P$ (M^+) 492.2641, found 492.2639; R_f 0.44 in 66% ethyl acetate/hexane. This material appeared to be unstable. The color of this oil turned to deep brown even though it was stored in the refrigerator at 0–5 °C.

1(*R*)-(3-Cyclohexylpropyl)-1,3,3a,4,9,9a-hexahydro-5-methoxy-(3a*S*,9a*S*)-2*H*-benz[*f*]inden-2-one and Its 1,3a,9a-Triepi Isomer (6a) and 1(*S*)-(3-Cyclohexylpropyl)-1,3,3a,4,9,9a-hexahydro-5-methoxy-(3a*S*,9a*S*)-2*H*-benz[*f*]inden-2-one and Its 1,3a,9a-Triepi Isomer (6b). A mixture of 1.37 g (4.05 mmol) of enone **5**, 490 mg of 10% palladium on carbon, and 50 mg (0.36 mmol) of anhydrous potassium carbonate in 75 mL of absolute ethanol was hydrogenated at 50 psi. After 46 h at room temperature, the mixture was filtered through a Celite pad, the pad being washed with ethyl acetate. The filtrate was concentrated in vacuo and then chromatographed on 100 g of silica gel 60, eluting with 10% ethyl acetate in hexane to give 1.10 g (80%) of 3:1 mixture of **6a** and **6b** as a colorless oil: ^{1}H NMR ($CDCl_3$, TMS) δ 7.30–6.60 (m, 3 H), 3.83 (s, 3 H), 3.30–0.60 (m, 26 H); IR (film) 1740, 1595 cm^{-1}; high-resolution MS, m/z calcd for $C_{23}H_{32}O_2$ (M^+) 340.2402, found 340.2427; R_f 0.55 (20% ethyl acetate/hexane). HPLC analyses were carried out by using a Varian 5560 with HP 3390A integrator, with Waters Resolve 3.9 × 150 mm column (5-μm SiO_2), eluting with 1% THF/hexane with 2 mL/min flow rate and detecting at 278 nm (0.05 AU/mV). Two peaks with retention times 5.80 and 6.60 min in a 3:1 ratio were assigned as **6a** and **6b**, respectively. Anal. Calcd for $C_{23}H_{34}O_2$: C, 81.15; H, 9.47; Found: C, 80.76; H, 9.55. When the hydrogenation was carried out by using a different lot of palladium on carbon, presumably more active, the reaction was completed in 7 h. The ratio was reversed to 1:3 for **6a** to **6b**, indicating the shorter reaction time gave nonequilibrated mixture in favor of the unnatural isomer **6b**.

1(*R*)-(3-Cyclohexylpropyl)-2,3,3a,4,9,9a-hexahydro-5-methoxy-(3a*S*,9a*S*)-1*H*-benz[*f*]inden-2(*R*)-ol and Its 1,2,3a,9a-Tetraepi Isomer (7a,b). A solution of 400 mg (1.17 mmol) of the mixture of **6a** and **6b**, 40 mL of absolute ethanol, and 4 mL of methylene chloride was cooled to −10 °C, treated with 8 mL of 10% aqueous sodium hydroxide, and stirred for 15 min, and 60 mg (1.59 mmol) of sodium borohydride was added.

(11) Fieser, L. F.; Fieser, M. In *Reagents for Organic Synthesis*; Vol. 1, p 11.

At 1, 3, and 6 h, an additional 60 mg (1.59 mmol) of sodium borohydride was added to the reaction mixture at −10 °C. After being stirred for an additional 2 h at −10 °C, the mixture was quenched with 2.9 mL of glacial acetic acid, diluted with 250 mL of brine, and extracted with ethyl acetate. The organic layers were combined, washed with saturated sodium bicarbonate and brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. The crude product was purified by flash chromatography using 250 g of silica gel 60, eluting with 10% ethyl acetate/hexane, to give 373 mg (93%) of **7a,b** as a white oily solid: ^1H NMR ($CDCl_3$, TMS) δ 7.30–6.60 (m, 3 H), 3.83 (s, 3 H), 3.96–3.34 (m, 1 H), 2.90–0.60 (m, 27 H); IR (film) 3350, 1595 cm^{-1}; high-resolution MS, m/z calcd for $C_{23}H_{34}O_2$ (M^+) 342.2599, found 342.2547; R_f (20% ethyl acetate/hexane). Anal. Calcd for $C_{23}H_{34}O_2$: C, 80.65; H, 10.01; Found: C, 80.70; H, 10.23.

1(*R*)-(3-Cyclohexylpropyl)-2,3,3a,4,9,9a-hexahydro-(3a*S*,9a*S*)-1*H*-benz[*f*]indene-2(*R*),5-diol and Its 1,2,3a,9a-Tetraepi Isomer (9ab). A solution of 0.9 mL of diphenylphosphine and 30 mL of THF at 0 °C was treated with 3.2 mL (5.1 mmol) of *n*-butyllithium (1.6 M in hexane). After the mixture was stirred for 30 min, 557 mg (1.6 mmol) of **7a,b** in 10 mL of THF was added, and the resulting mixture was heated at 70 °C for 7 h, then cooled to 0 °C, treated with an additional 0.9 mL (5 mmol) of diphenylphosphine and 3.2 mL (5.2 mmol) of *n*-butyllithium in hexane, and stirred at room temperature for 30 min and then at 70 °C for 18 h. The mixture was then cooled to 0 °C, diluted with 100 mL of brine containing 10 mL of 1 N aqueous hydrochloric acid, and extracted with ethyl acetate. The combined organic layers were washed with brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. The crude product was purified by flash chromatography on 200 g of silica gel 60, eluting with 5% acetone/methylene chloride to give 509 mg (95%) of **9a,b** as a white foam: ^1H NMR ($CDCl_3$, TMS) δ 7.20–6.50 (m, 3 H), 3.90–3.40 (m, 1 H), 2.90–0.50 (m, 28 H); IR (mull) 3430, 3160, 1600 cm^{-1}; high-resolution MS, m/z calcd for $C_{22}H_{32}O_2$ (M^+) 328.2402, found 328.2409; R_f 0.24 (5% acetone/methylene chloride). Anal. Calcd for $C_{22}H_{32}O_2$: C, 80.44; H, 9.82; Found: C, 80.09; H, 10.14.

[[1(*R*)-(3-Cyclohexylpropyl)-2,3,3a,4,9,9a-hexahydro-2-(*R*)-hydroxy-(3a*S*,9a*S*)-1*H*-benz[*f*]inden-5-yl]oxy]acetonitrile and Its 1,2,3a,9a-Tetraepi Isomer (10a,b). A mixture of 450 mg (1.37 mmol) of **9a,b**, 1.8 mL (28 mmol) of chloroacetonitrile, 2.2 g (16 mmol) of anhydrous potassium carbonate, and 20 mL of acetone was heated at 65 °C for 24 h, then cooled, diluted with brine, and extracted with ethyl acetate. The combined organic layers were washed with brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. The crude product was purified by flash chromatography on 200 g of silica gel 60, eluting with 25% ethyl acetate/hexane, to give 498 mg (99%) of **10a,b** as a colorless oil, which solidified in the refrigerator: ^1H NMR ($CDCl_3$, TMS) δ 7.30–6.70 (m, 3 H), 4.74 (s, 2 H), 3.90–3.50 (m, 1 H), 3.0–0.6 (m, 27 H); IR (film) 3400, 1595 cm^{-1}; high-resolution MS, m/z calcd for $C_{24}H_{33}NO_2$ 367.2511, found 367.2500; R_f 0.33 (30% ethyl acetate/hexane). Anal. Calcd for $C_{24}H_{33}O_2N$: C, 78.43; H, 9.06; N, 3.81. Found: C, 78.18; H, 9.08; N, 3.97.

[[1(*R*)-(3-Cyclohexylpropyl)-2,3,3a,4,9,9a-hexahydro-2-(*R*)-hydroxy-(3a*S*,9a*S*)-1*H*-benz[*f*]inden-5-yl]oxy]acetic Acid and Its 1,2,3a,9a-Tetraepi Isomer (2a,b). A solution of 450 mg (1.22 mmol) of **10a,b**, 30 mL of methanol, and 10 mL of 25% aqueous potassium hydroxide was heated at 90 °C for 5 h, then cooled to 0 °C, acidified to pH 5–6 with 1 N aqueous hydrochloric acid, diluted with brine, and extracted with ethyl acetate. The combined organic extracts were washed with brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. The crude product was chromatographed on 90 g of CC-4 acid-washed silica gel, eluting with 250 mL of 20%, 30%, 40%, and 50% ethyl acetate/hexane, to give 461 mg (97%) of **2a,b**, which was crystallized from hot THF/hexane (1:2, 3 mL/100 mg) to give 305 mg of **2a,b** as a white solid (mp 158–160 °C), and 156 mg of a white solid (mp 150–155 °C) from the mother liquor: ^1H NMR ($CDCl_3$, TMS) δ 7.30–6.70 (m, 3 H), 4.73 (s, 2 H), 4.60–3.40 (m, 3 H), 3.0–0.6 (m, 26 H); IR (film) 3420, 1735, 1590 cm^{-1}; high-resolution MS, m/z calcd for $C_{30}H_{50}O_4Si_2$ (as $(TMS)_2$ derivative) 530.3247, found 530.3227; R_f 0.57 (ethyl acetate/acetic acid/cyclohexane/water (9:2:5:10)). HPLC analyses were carried out by using a Varian 5560 with an HP 3390A integrator equipped with an Altex Ultrasphere ODS C18 10 × 250 mm column, eluting with water/acetonitrile/methanol/85% phosphoric acid (500:350:150:1) with a 3-mL/min flow rate and detection at 217 nm (0.02 AU/mV). A single peak at retention time of 48.89 min was observed. Anal. Calcd for $C_{24}H_{34}O_4$: C, 74.57; H, 8.87. Found: C, 74.57; H, 9.12.

[[1(*R*)-(Cyclohexylpropyl)-2,3,3a,4,9,9a-hexahydro-5-methoxy-1*H*-(3a*S*,9a*S*)-benz[*f*]inden-2(*R*)-yl]oxy]-(*S*)-α-methylbenzyl Carbamate (8a) and Its 1,2,3a,9a-Tetraepi Isomer (8b). A solution of 572.4 mg (1.67 mmol) of **7a,b** and 75 mL of toluene was treated with 1.66 mL of 12.5% solution of phosgene in toluene and 0.59 mL of triethylamine. After 1.25 h, the resulting mixture was treated with 1.3 mL of 98% (*S*)-(−)-α-methylbenzylamine. The reaction was exothermic and the mixture became gelatinous within 1 min after the addition. After 25 min the mixture was diluted with 100 mL of Skellysolve B and filtered through a medium frittered disk Buchner funnel, washing with Skellysolve B. The filtrate was concentrated in vacuo at 35 °C to give 1.11 g of a yellow oil. This oil was chromatographed on 100 g of silica gel 60, which was slurry packed, and eluted with 5% acetone/Skellysolve B to give 785.1 mg of the mixture of **8a** and **8b**. This mixture was chromatographed on two Merck columns (size B) connected in series, conditioned, and eluted with 15% *tert*-butyl methyl ether/hexane. The mixture was applied to the column in toluene, eluting at 7 mL/min to give 328 mg (40%) of compound **8a** as a white solid (mp 108 °C, R_f 0.31 (15% *tert*-butyl methyl ether/Skellysolve B)), 117.3 mg of the mixture of **8a** and **8b**, and 273.8 mg (33.5%) of compound **8b** as a white solid (mp 115 °C, R_f 0.28 (the same solvent system as **8a**)). HPLC analyses were carried out by using a Varian 5560 with a Varian 4270 integrator equipped with an Altex Ultrasphere ODS C18, 10 × 250 mm column, eluting with acetonitrile/methanol/water (45:40:15), with a 3 mL/min flow rate and detection at 217 nm (0.05 AU/mV). The compound **8a** showed a single peak at 34.12 min whereas the compound **8b** showed a peak at 35.36 min with a minor contaminant at 34.08. The assignment of carbamates **8a** and **8b** was based on the comparison of the HPLC peaks with authentic carbamates **8a** and **8b**, prepared independently from authentic alcohols **7a** and **7b**, respectively. The carbamates were prepared in analytical scale by dissolving ca. 1 mg of these alcohols in 0.5 mL of toluene and adding 1 μL of dibutyltin diacetate and 5 μL of (*S*)-(−)-α-methylbenzyl isocyanate. After the mixture was stirred at room temperature for 4 h, the reaction was quenched with brine and extracted with ethyl acetate. The organic extract was dried (Na_2SO_4), filtered, and concentrated in vacuo.

1(*R*)-(3-Cyclohexylpropyl)-2,3,3a,4,9,9a-hexahydro-5-methoxy-(3a*S*,9a*S*)-1*H*-benz[*f*]inden-2(*R*)-ol (7a). A solution of 328 mg (0.67 mmol) of **8a** and 25 mL of THF was treated with lithium aluminum hydride (102.6 mg, 2.7 mmol), and the resulting mixture was heated at 60–65 °C for 4 h. The mixture was then cooled to room temperature and carefully quenched with 1 mL of water. Addition of brine was followed by aqueous sodium bisulfate until pH was 4. The mixture was then extracted three times with ethyl acetate, and the combined organic extracts were filtered through anhydrous sodium sulfate powder. The filtrate was concentrated in vacuo to give 285.9 mg of a yellow oil, which was chromatographed on 64 g of silica gel 60, eluting with 12% ethyl acetate/hexane to give 229.5 mg (100%) of pure **7a** as a colorless glass: ^1H NMR and IR spectra were identical with those of **7a,b**; $[\alpha]_D$ 30.6° (c 1.215, 95% ethanol); R_f 0.36 (15% ethyl acetate/Skellysolve B) and 0.20 (15% *tert*-butyl methyl ether/Skellysolve B); HPLC analysis (same condition as in the analyses of **8a** and **8b**) as in previous experiment, but eluting with acetonitrile/water/methanol (60:20:20), gave a single peak at 41.06 min.

1(*S*)-(3-Cyclohexylpropyl)-2,3,3a,4,9,9a-hexahydro-5-methoxy-(3a*R*,9a*R*)-1*H*-benz[*f*]inden-2(*S*)-ol (7b). A 273.8 mg (0.56 mmol) sample of **8b** was reacted and worked up in an identical manner as the reaction of **8a** to **7a** to obtain 267.2 mg of crude product as a yellow glass. This material was chromatographed on 65 g of silica gel 60, which was eluted with 10% ethyl acetate/hexane to give 191.8 mg (100%) of pure **7b** as a colorless glass: $[\alpha]_D$ −27.7° (c 1.139, 95% ethanol); HPLC analysis gave a single peak at 41.38 min.

1(*R*)-(3-Cyclohexylpropyl)-2,3,3a,4,9,9a-hexahydro-(3a*S*,9a*S*)-1*H*-benz[*f*]indene-2(*R*),5-diol (9a). A 233.9 mg (0.68 mmol) sample of **7a** was converted to **9a** in an identical manner as in the preparation of **9a,b** from **7a,b** to give 212.0 mg

(94.5%) of **9a** as a white foam: ^{1}H NMR and IR spectra were identical with those of **9a,b**; $[\alpha]_D$ 30.6° (*c* 1.09, 95% ethanol); R_f 0.25 (25% ethyl acetate/hexane).

1(*S*)-(3-Cyclohexylpropyl)-2,3,3a,4,9,9a-hexahydro-(3a*R*,9a*R*)-1*H*-benz[*f*]indene-2(*S*),5-diol (9b). A 194.6 mg (0.57 mmol) sample of **7b** was converted to **9b** in an identical manner as in the preparation of **9a,b** from **7a,b** to give 167.6 mg (90%) of **9b** as a white foam: ^{1}H NMR and IR spectra were identical with those of **9a,b**; $[\alpha]_D$ −29.2° (*c* 1.17, 95% ethanol); R_f 0.25 (25% ethyl acetate/hexane).

[[1(*R*)-(3-Cyclohexylpropyl)-2,3,3a,4,9,9a-hexahydro-2-(*R*)-hydroxy-(3a*S*,9a*S*)-1*H*-benz[*f*]inden-5-yl]oxy]acetonitrile (10a). A 212.0 mg (0.65 mmol) sample of **9a** was converted to **10a** in a similar manner as in the alkylation of **9a,b** to **10a,b** to give 211.6 mg (89%) of **10a**: mp 99–99.5 °C; ^{1}H NMR spectrum was identical with that of **10a,b**; $[\alpha]_D$ 30.5° (*c* 1.032, 95% ethanol); R_f 0.34 (25% ethyl acetate/Skellysolve B).

[[1(*S*)-(3-Cyclohexylpropyl)-2,3,3a,4,9,9a-hexahydro-2-(*S*)-hydroxy-(3a*R*,9a*R*)-1*H*-benz[*f*]inden-5-yl]oxy]acetonitrile (10b). A 167.6 mg (0.51 mmol) sample of **9b** was converted to **10b** in a similar manner as to conversion of **9a,b** to **10a,b** to give 164.9 mg (88%) of **10b**: mp 99.5–100 °C; ^{1}H NMR spectrum was identical with that of **10a,b**; $[\alpha]_D$ −28.4° (*c* 1.017, 95% ethanol); R_f 0.34 (25% ethyl acetate/Skellysolve B).

[[1(*R*)-(3-Cyclohexylpropyl)-2,3,3a,4,9,9a-hexahydro-2-(*R*)-hydroxy-(3a*S*,9a*S*)-1*H*-benz[*f*]inden-5-yl]oxy]acetic Acid (2a). A 211.6 mg (0.58 mmol) sample of **10a** was converted to **2a** in an identical manner as the conversion of **10a,b** to **2a,b** to give 203.3 mg of a pale yellow solid, which was recrystallized from ethyl acetate/hexane to give 156.1 mg (70%) of pure **2a** as a white solid: mp 133.5–135.5 °C, mixed mp with **2a** obtained from **16a** (Scheme III) was unchanged; ^{1}H NMR spectrum was identical with that of **2a,b**; MS, *m/z* calcd for $C_{24}H_{34}O_4$ (M^+) 386; other ions 368, 309, 243, 203, 157; $[\alpha]_D$ 30.69° (*c* 1.167, 95% ethanol), R_f 0.19 (acetone/methylene chloride/acetic acid (4:95:1)), 0.27 (the organic phase of ethyl acetate/acetic acid/2,2,4-trimethylpentane/water (9:2:5:10)), and 0.35 (ethyl acetate/Skellysolve B/acetic acid (35:64:1)).

[[1(*S*)-(3-Cyclohexylpropyl)-2,3,3a,4,9,9a-hexahydro-2-(*S*)-hydroxy-(3a*R*,9a*R*)-1*H*-benz[*f*]inden-5-yl]oxy]acetic Acid (2b). A 164.9 mg (0.45 mmol) sample of **10b** was converted to **2b** in an identical manner as the synthesis of **2a** from **10a** to give 139.7 mg (80%) of pure **2b** as a white solid: mp 133.5–135.5 °C, mixed mp with **2b** obtained from **16b** (Scheme III) was unchanged; ^{1}H NMR and MS spectra and R_f's in three-solvent system as described in the previous experiment were identical with those of **2a**; $[\alpha]_D$ −28.16 (*c* 1.079, 95% ethanol).

1(*R*)-(3-Cyclohexyl-3(*R*)-hydroxypropyl)-2,3,3a,4,9,9a-hexahydro-2(*R*)-[(*tert*-butyldimethylsilyl)oxy]-5-methoxy-(3a*S*,9a*S*)-1*H*-benz[*f*]indene (17a). A solution of 229.5 mg (0.64 mmol) of **16a** (98.5% pure, contaminated with 1.5% **16b**),[2] 87.1 mg (1.28 mmol) of imidazole, and 6.4 mL of THF at 0–5 °C was treated with 192.9 mg (1.28 mmol) of *tert*-butyldimethylsilyl chloride dissolved in 3.2 mL of THF. After the mixture was stirred 30 h at room temperature another portion of imidazole (17.4 mg, 0.256 mmol) and *tert*-butyldimethylsilyl chloride (38.6 mg, 0.256 mmol) was added, and the mixture was stirred for an additional 18 h, quenched at 0–5 °C with 5 mL of water, stirred for 30 min, and extracted with ethyl acetate. The organic extract was washed with water and brine, dried ($MgSO_4$), filtered, and concentrated in vacuo to give a near colorless oil. The oil was purified by LC on 215 g of silica gel 60, eluting with 2.4 L of 9% ethyl acetate/hexane and 600 mL ethyl acetate to give 21.2 mg (6.4%) of bis-silylated product, 197.9 mg (65.4%) of **17a**, and 46.2 mg (20%) of the recovered starting material **16a**. This material was resilylated by reacting with 122.5 mg (1.8 mmol) of imidazole and 271.3 mg (1.8 mmol) of *tert*-butyldimethylsilyl chloride in 5 mL of THF at room temperature for 48 h. Workup and LC purification using 52.5 g silica gel 60, as described above, gave an additional 56.5 mg of **17a**. The combined products yielded 254.2 mg (84%) of the desired monosilylated product **17a** as a colorless oil: ^{1}H NMR ($CDCl_3$, TMS) δ 7.30–6.68 (m, 3 H), 3.80 (s, 3 H), 3.86–3.12 (m, 2 H), 3.08–0.95 (m, 24 H), 0.88 (s, 9 H), 0.04 (s, 6 H); IR (film) 3430, 1580, 870, 840, 775, 730 cm^{-1}; R_f 0.48 (17% ethyl acetate/hexane).

1(*S*)-(3-Cyclohexyl-3(*R*)-hydroxypropyl)-2,3,3a,4,9,9a-hexahydro-2(*S*)-[(*tert*-butyldimethylsilyl)oxy]-5-methoxy-(3a*R*,9a*R*)-1*H*-benz[*f*]indene (17b). A 358.5 mg (1.0 mmol) sample of **16b** (>99% pure)[2] was converted to **17b** in an identical manner as the silylation of **16a** to **17a** to give 29.7 mg (5.1%) of bis-silylated product, 385.8 mg (81.6%) of **17b** as a colorless oil, and 40.7 mg (11.4%) of the recovered starting material **16b**. Spectral properties of **17b** were identical with those of **17a**.

1(*R*)-(3-Cyclohexylpropyl)-2,3,3a,4,9,9a-hexahydro-2-(*R*)-[(*tert*-butyldimethylsilyl)oxy]-5-methoxy-(3a*S*,9a*S*)-1*H*-benz[*f*]indene (19a). A solution of 245.9 mg (0.52 mmol) of **17a** and 5.2 mL of pyridine at 0–5 °C was treated with 594.8 mg (3.12 mmol) of *p*-toluenesulfonyl chloride. The resulting pink-colored solution was stirred at room temperature for 24 h, cooled to 0–5 °C again, treated with 0.52 mL of water, stirred for 30 min at room temperature, and extracted with ethyl acetate. The combined organic extracts were washed with water, 10% aqueous sodium bisulfate, saturated aqueous sodium bicarbonate, and brine, dried ($MgSO_4$), filtered, and concentrated in vacuo to give 0.33 g of tosylate **18a** as a light brown oil: ^{1}H NMR ($CDCl_3$, TMS) δ 7.94–6.68 (m, 7 H), 4.62–4.32 (m, 1 H), 3.80 (s, 3 H), 3.90–3.40 (m, 1 H), 3.04–0.95 (m, 24 H), 2.38 (s, 3 H), 0.88 (s, 9 H), 0.04 (s, 6 H); R_f 0.56 (17% ethyl acetate/hexane). Without further purification of the tosylate **18a**, this material was dissolved in 26.0 mL of anhydrous ether, cooled to 0–5 °C with an ice–water bath, and treated over 5 min with 197.3 mg (5.2 mmol) of lithium aluminum hydride. The cooling bath was then removed, and the gray suspension was stirred at room temperature for 24 h, cooled again to 0–5 °C, and treated carefully with 1 N hydrochloric acid until the pH of the mixture was about 7. The resulting mixture was extracted with ethyl acetate and the combined organic extracts were washed with water and brine, dried ($MgSO_4$), filtered, and concentrated in vacuo to give an oil. This oil was purified on 215 g of LC grade silica gel 60, eluting with 1.5 L of 5% and 1 L of 33% ethyl acetate/hexane to give 187.4 mg (78.9%) of pure **19a** as a colorless oil: ^{1}H NMR ($CDCl_3$, TMS) δ 7.24–6.66 (m, 3 H), 3.82 (s, 3 H), 3.92–3.50 (m, 1 H), 3.10–0.95 (m, 26 H), 0.88 (s, 9 H), 0.02 (s, 6 H); IR (film) 1600, 1580, 870, 835, 770 cm^{-1}; R_f 0.89 (17% ethyl acetate/hexane).

1(*S*)-(3-Cyclohexylpropyl)-2,3,3a,4,9,9a-hexahydro-2-(*S*)-[(*tert*-butyldimethylsilyl)oxy]-5-methoxy-(3a*R*,9a*R*)-1*H*-benz[*f*]indene (19b). A 378.2 mg (0.8 mmol) sample of **17b** was converted to **19b** in an identical manner as the conversion of **17a** to **19a**. However, the lithium aluminum hydride reduction was run in tetrahydrofuran instead of diethyl ether. This change of solvent resulted in isolation of 214.0 mg (58.6%) of the desired **19b** as a colorless oil: ^{1}H NMR and IR spectra and R_f were identical with those of **19a**.

1(*R*)-(3-Cyclohexylpropyl)-2,3,3a,4,9,9a-hexahydro-5-methoxy-(3a*S*,9a*S*)-1*H*-benz[*f*]inden-2(*R*)-ol (7a). A solution of 182.7 mg (0.40 mmol) of **19a**, 3.0 mL of 2-propanol, 2 mL of THF, and 1 mL of 3 N hydrochloric acid was stirred at room temperature for 24 h, treated with saturated aqueous sodium bicarbonate to pH 7–8, and extracted with ethyl acetate. The organic extract was washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo to give a colorless oil. This oil was chromatographed on 215 g of LC grade silica gel 60, eluting with 20% ethyl acetate/hexane to give 123.8 mg (90.4%) of **7a** as a colorless oil: ^{1}H NMR and IR spectra and R_f were identical with those of **7a,b**; $[\alpha]_D$ 31.8° (*c* 1.22, 95% ethanol). The carbamate **8a** of compound **7a** had an identical HPLC retention time as the carbamate **8a** resolved by liquid chromatography from the mixture **8a,b**.

1(*S*)-(3-Cyclohexylpropyl)-2,3,3a,4,9,9a-hexahydro-5-methoxy-(3a*R*,9a*R*)-1*H*-benz[*f*]inden-2(*S*)-ol (7b). A 205.6 mg (0.45 mmol) sample of **19b** was converted to **7b** in an identical manner as in the conversion of **19a** to **7a** to give 125.6 mg (81.5%) of **7b** as a colorless oil: ^{1}H NMR and IR spectra and R_f were identical with those of **7a,b**; $[\alpha]_D$ −31.5° (*c* 1.24, 95% ethanol). The carbamate **8b** of compound **7b** had an identical HPLC retention time as the carbamate **8b**, resolved by liquid chromatography from the mixture **8a,b**.

[[1(*R*)-(3-Cyclohexylpropyl)-2,3,3a,4,9,9a-hexahydro-2-(*R*)-hydroxy-(3a*S*,9a*S*)-1*H*-benz[*f*]inden-5-yl]oxy]acetic Acid (2a). Compound **7a** (obtained from **16a**) was converted to **2a** in an identical manner as previously described for the conversion of **7a** (obtained via resolution of **7a,b**) to **2a**. The specific

rotations of each intermediate were recorded: **9a**, $[\alpha]_D$ 31.4° (c 0.873, 95% ethanol); **10a**, $[\alpha]_D$ 29.5° (c 0.818, 95% ethanol). The HPLC analysis of the final product **2a** showed an identical retention time as that of **2a** obtained from the resolution of intermediate **7a,b** as shown on Scheme I. The spectral properties were also identical: $[\alpha]_D$ 31.3° (c 0.713, 95% ethanol); high-resolution MS (as TMS derivative), m/z calcd for $C_{36}H_{50}O_4Si_2$ 530.3247, found 530.3248.

[[1(*S*)-(3-Cyclohexylpropyl)-2,3,3a,4,9,9a-hexahydro-2-(*S*)-hydroxy-(3a*R*,9a*R*)-1*H*-benz[*f*]inden-5-yl]oxy]acetic Acid (2b). Compound **7b** (obtained from **16b**) was converted to **2b** in an identical manner as previously described for the conversion of **7b** (obtained via resolution of **7a,b**) to **2b**. The specific rotations of each intermediate were recorded: **9b**, $[\alpha]_D$ -31.8° (c 0.916, 95% ethanol); **10b**, $[\alpha]_D$ -30.0° (c 0.83, 95% ethanol). The HPLC analysis of the final product **2b** showed an identical retention time as that of **2b** obtained from the resolution of intermediate **7a,b** as shown in Scheme I. The spectral properties were also identical: $[\alpha]_D$ -31.4° (c 0.72, 95% ethanol); high-resolution MS (as TMS derivative), m/z calcd for $C_{30}H_{50}O_4Si_2$ 530.3247, found 530.3237.

Acknowledgment. Helpful discussions with Dr. D. R. Morton are acknowledged. The supply of the intermediates as well as the technical assistance of D. L. Alexander, A. W. Harrison, P. G. Brewer, and D. P. Carvell are gratefully acknowledged. We also thank C. Lancaster, A. S. Olafsson, and K. P. Kolbasa for their assistance in providing the biological data.

Novel Mitomycin C Amidines:[1] Synthesis and Their Reactions with Amines

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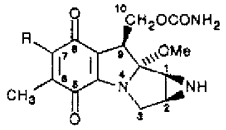
Received April 10, 1987

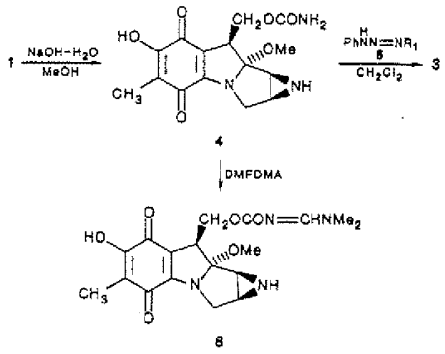
Reactions of formamide acetals (e.g., DMFDMA, **10**, **12**) with mitomycin C (**1**) has afforded novel amidine derivatives (e.g., 7–9, 11, 13). Investigation of reactions of amines with bisamidine **8** in both polar and nonpolar solvents (e.g., MeOH vs $CHCl_3$) has led to the discovery that **8**, in its reactions with primary amines in methanol, behaves as a mitomycin A (**2**) equivalent to afford 7-*N*-substituted mitosanes (e.g., **16**–**19**). In contrast, bisamidine **8** undergoes a selective deamidination reaction with primary amines in chloroform to afford monoamidine **14**.

Fermentation-derived[3] antineoplastic antibiotics, mitomycin C (**1**) and mitomycin A (**2**),[4] are of great significance in cancer chemotherapy. While **1** is currently[5] in clincal use for the management of a variety of neoplasms, mitomycin A (**2**) is continuing to play a pivotal role in analogue research[6] which is directed toward discovery of new clinical agents endowed with less myelosuppressive properties and a broader spectrum of antitumor activity.



Recently,[7] we reported a practical approach to the synthesis of **2** and its analogues, namely 7-alkoxymitosanes **3**[8] from mitomycin C. The key reaction of this process

Scheme I



(Scheme I) involves O-alkylation of 7-hydroxymitosane (**4**) with an appropriate triazene (**5**) in a nonpolar solvent. During a similar attempt to methylate **4** with another well-established methylating agent, namely *N*,*N*-dimethylformamide dimethyl acetal (DMFDMA),[9] an amidine derivative, **6**, was obtained as the sole product in place of the desired product **2**. This finding was not surprising in light of the fact that DMFDMA is known to react with amines, amides, and urethanes to yield corresponding amidines. However, the observed functionalization[10] of the carbamoyl moiety of **4** is unprecedented. This encouraged us to investigate the reactions of formamide acetals with mitomycin C, which bears potentially three reactive amino

(1) Presented in part at the 187th National Meeting of the American Chemical Society, St. Louis, MO, April 8–13, 1984; Abstracts, MEDI 30.
(2) Present address: Hoechst-Roussel Pharmaceuticals, Inc. Somerville, NJ 08876.
(3) Remers, W. A. In *Chemistry of Antitumor Antibiotics*; Wiley: New York, 1979; Vol 1, pp 221–276 and references therein.
(4) According to the trivial system of nomenclature, which has found wide use in mitomycin literature, mitomycin C (**1**) is named as 7-amino-9a-methoxymitosane and mitomycin A (**2**) as 7,9a-dimethoxymitosane.
(5) Carter, S. K.; Crooke, S. T. *Mitomycin C, Current Status and New Developments*; Academic: New York, 1979; Chapter 15.
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(9) For a review on the chemistry of formamide acetals, see: Abdulla, R. F.; Brinkmeyer, R. S. *Tetrahedron* **1979**, *35*, 1675.
(10) Under reductive conditions, thionucleophiles are known to displace the carbamoyl moiety. See: Bean, M.; Kohn, H. *J. Org. Chem.* **1985**, *50*, 293.

0022-3263/87/1952-5601$01.50/0

was obtained as a colorless, crystalline solid from water, yield 33.4 g. (91%), m.p. 211–211.5° with decomposition.[15]

Anal. Calcd. for $C_6H_6N_6$: C, 49.0; H, 3.4; N, 47.6. Found: C, 49.2; H, 3.7; N, 47.8.

5-(3'-Pyridyl)tetrazole was prepared from 3-cyanopyridine. Using the same quantities of reagents as in the foregoing example, the product was obtained as a colorless, crystalline solid from water, yield 33.3 g. (91%), m.p. 234–235° with decomposition.[15]

Anal. Calcd. for $C_6H_6N_6$: C, 49.0; H, 3.4; N, 47.6. Found: C, 49.2; H, 3.4; N, 47.7.

5-(4'-Pyridyl)tetrazole was prepared from 4-cyanopyridine in the same way with the same quantities of reagents. It crystallized from water as a colorless solid, yield 34.3 g. (93%), m.p. 253–254° with decomposition.[15]

Anal. Calcd. for $C_6H_6N_6$: C, 49.0; H, 3.4; N, 47.6. Found: C, 49.2; H, 3.6; N, 47.3.

2,6-Di(5'-tetrazolyl)pyridine. A solution of 27.5 g. (0.21 mole) of 2,6-dicyanopyridine in 100 ml. of *n*-butyl alcohol was refluxed for 2 days with 38.2 g. (0.59 mole) of sodium azide and 38 ml. of glacial acetic acid.[14] At this point another 10 g. of sodium azide and 20 ml. of glacial acetic acid were added. Refluxing continued for 2 days. The crude product, 45.6 g. (99%), was obtained by diluting the reaction mixture with water, distilling and acidifying as in the foregoing examples. The product was purified by dissolving it in aqueous sodium hydroxide and reprecipitating from the hot, colorless solution with acid. The analytical sample was recrystallized from hot water in which the product was only sparingly soluble, m.p. 290° with decomposition.

Anal. Calcd. for $C_7H_5N_9$: C, 39.1; H, 2.3; N, 58.6. Found: C, 39.2; H, 2.6; N, 58.6.

5-(2'-Piperidyl)tetrazole. A suspension of 11 g. of 5-(2'-pyridyl)tetrazole in 150 ml. of glacial acetic acid was shaken with 250 mg. of platinum oxide and hydrogen at an initial pressure of 50 p.s.i. Hydrogenation was complete in 24 hr. After removal of the catalyst by filtration the solution was evaporated to a small volume and diluted with ether to precipitate the product. Purification was effected by dissolving the colorless solid in the minimum amount of warm water, treating with Norit and reprecipitating with acetone, yield 10.5 g. (92%), m.p. 287° with decomposition.

Anal. Calcd. for $C_6H_{11}N_5$: C, 47.1; H, 7.2; N, 45.7. Found: C, 47.0; H, 7.1; N, 46.0.

The *acetyl* derivative was prepared by refluxing for 2 hrs. in glacial acetic acid with an equimolar amount of acetic anhydride. After removal of the solvent under reduced pressure, the residue of acetyl derivative was obtained as a colorless, crystalline solid from water, m.p. 135.5–136.5°.

Anal. Calcd. for $C_8H_{13}N_5O$: C, 49.2; H, 6.7; N, 35.9. Found: C, 49.1; H, 6.6; N, 35.6.

For preparative purposes it was advantageous to form the acetyl derivative directly by hydrogenation of the pyridyltetrazole as just described; after removal of the catalyst, acetic anhydride was added to the glacial acetic acid solution and acetylation was completed as just described. The over-all yield from the pyridyltetrazole was 84%.

5-(3'-Piperidyl)tetrazole was obtained in almost quantitative yield as a colorless, crystalline solid by hydrogenation of the pyridyltetrazole in a completely analogous manner, m.p. 296–297° with decomposition. The analytical sample was recrystallized from the minimum amount of water; the remainder of the product was precipitated from water with acetone.

Anal. Calcd. for $C_6H_{11}N_5$: C, 47.1; H, 7.2; N, 45.7. Found: C, 47.1; H, 7.3; N, 45.7.

The *acetyl* derivative, prepared as described for the isomer, separated from isopropyl alcohol as a colorless, crystalline solid, m.p. 170–171°.

Anal. Calcd. for $C_8H_{13}N_5O$: C, 49.2; H, 6.7; N, 35.9. Found: C, 49.5; H, 6.7; N, 36.1.

5-(4'-Piperidyl)tetrazole was obtained in 86% yield by hydrogenation of the pyridyltetrazole in a completely analogous manner. The product crystallized from water as dense colorless prisms; it did not decompose below 370° but showed some shrinking and browning at 237°.

Anal. Calcd. for $C_6H_{11}N_5$: C, 47.1; H, 7.2; N, 45.7. Found: C, 47.0; H, 7.2; N, 46.0.

The *acetyl* derivative, obtained as described for the isomers, separated from isopropyl alcohol as a colorless, crystalline solid, m.p. 156.5–157.5°.

Anal. Calcd. for $C_8H_{13}N_5O$: C, 49.2; H, 6.7; N, 35.9. Found: C, 49.3; H, 6.8; N, 35.8.

EAST LANSING, MICHIGAN

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MICHIGAN STATE UNIVERSITY]

Tetrazole Analogs of Plant Auxins[1]

J. M. McMANUS[2,3] AND ROBERT M. HERBST

Received March 30, 1959

A group of chlorinated 5-phenoxymethyltetrazoles has been prepared as analogs of the corresponding substituted phenoxyacetic acids. Two methods of synthesis were used to corroborate the structure of the products. The tetrazole analog of the natural plant auxin, 3-indolylacetic acid, in which the carboxyl group is replaced by the acidic tetrazole moiety, has been prepared from the corresponding nitrile. An improved method for the synthesis of phenoxyacetonitriles is described.

The isolation and identification of 3-indolylacetic acid as a natural growth hormone in plants[4] initiated a search for other substances which could elicit this type of activity. Among those synthetic materials shown to stimulate growth was a group of chlorinated compounds derived from phenoxyacetic acid. Varying degrees of activity were demonstrated depending on the number and position of the chlorine atoms in the benzenoid portion of the structure; the most active are 2,4-dichloro-

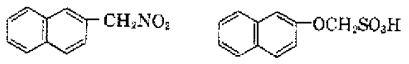
(1) Based on a doctoral thesis submitted to Michigan State University in 1958 by James M. McManus.

(2) White Laboratories Fellow, 1956–1958.

(3) Present address: Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

(4) F. Kögl, A. J. Haagen-Smit and H. Erxleben, *Z. physiol. Chem.*, 228, 90 (1934).

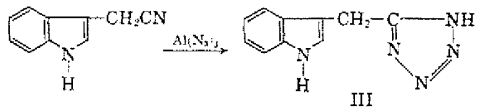
phenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T).[5] The requirement that there be a carboxyl group on the side chain[6] finds exception in that the corresponding aldehydes, nitriles, esters and amides also show, to a certain extent, hormonal activity. Exceptions to the carboxylic acid rule have been shown by active compounds in which the carboxyl group is replaced by a nitro group (I) or a sulfonic acid moiety (II).[7]



I II

Because of the acidic nature of 5-mono substituted tetrazoles,[8,9,10,11] it appeared of interest to incorporate a tetrazole nucleus into the chemical structure of an active plant auxin in place of the carboxyl group. In this study the tetrazole analogs of 3-indolylacetic acid and various chlorophenoxyacetic acids were synthesized.

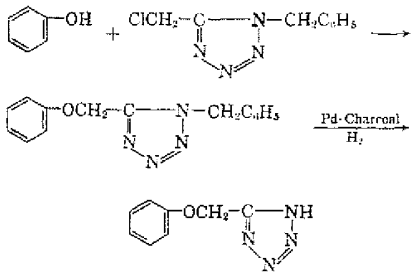
Behringer and Kohl[12] have shown that certain nitriles will react with aluminum azide in tetrahydrofuran to form 5-substituted tetrazoles. The preparation of 5-(3′-indolylmethyl)tetrazole (III) was accomplished by application of this general procedure to 3-indolylacetonitrile. It was found advantageous to modify the isolation technique recommended by these authors. Better results were obtained when the tetrahydrofuran was displaced from the reaction mixture by distillation while constant volume was maintained by simultaneous addition of water. The insoluble aluminum salt of the tetrazole which remained after all the tetrahydrofuran had been removed was decomposed with dilute hydrochloric acid, leaving an aqueous suspension of the tetrazole.



III

The substituted 5-phenoxymethyltetrazoles were synthesized by application of two general procedures: The first involved interaction of nitriles with sodium azide and acetic acid in *n*-butyl alcohol[10]; the second, interaction of nitriles with aluminum azide in tetrahydrofuran.[12] The first procedure was used successfully for the synthesis of 5-phenoxymethyltetrazole and the corresponding 2,4-dichloro- and 2,4,5-trichlorophenoxymethyl analogs from the appropriate nitriles. Attempts to prepare 5-(2′,4′,6′-trichlorophenoxymethyl)tetrazole in this way were not successful; the reaction mixture became very dark because of extensive decomposition, and no definite product was isolated. The interaction of 2-chloro-, 4-chloro-, and 2,4,6-trichlorophenoxyacetonitrile with aluminum azide in refluxing tetrahydrofuran resulted in good yields of the corresponding tetrazoles. After completion of this work an improved technique involving interaction of nitriles with lithium or an ammonium azide in dimethylformamide appeared.[13]

An alternate method used for the preparation of some of the phenoxymethyltetrazoles involved interaction of the appropriately substituted phenol with 1-benzyl-5-chloromethyltetrazole in an alkaline medium, followed by hydrogenolytic removal of the benzyl group with palladium on charcoal and hydrogen. In several instances, namely 5-(2,′4′-dichloro- and 2′,4′,6′-trichlorophenoxymethyl)-1-benzyltetrazole, debenzylation was accompanied by partial dehalogenation and possibly reduction. Isolation of pure compounds of unequivocal structure for comparison with the compounds prepared by other routes was not feasible in these two cases. In other instances compounds identical with those formed from the nitriles were obtained by this method.



The tetrazole analogs are similar to the phenoxyacetic acids in physical properties. All are solids with melting points in the same range as and similar solubilities to the corresponding carboxylic acids. No regular differences in melting points are noted, some are slightly higher some lower than those of the corresponding phenoxyacetic acids.

The nitriles used as intermediates for the phenoxymethyltetrazole syntheses were prepared from the phenol, chloroacetonitrile and potassium carbonate in refluxing acetone. This method of preparation offered a distinct advantage over methods which involved synthesis of the nitrile either from

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TABLE I

PHENOXYACETONITRILES ARYL-OCH_2CN

Aryl	M.P.	Yield, %	Formula	Analyses: Calcd. Cl	Calcd. N	Found Cl	Found N
C_6H_5	[a]	82					
2-ClC_6H_4	[b]	44	C_8H_6ClNO	21.2	8.4	21.1	8.1
4-ClC_6H_4	46.5–47.5	93	C_8H_6ClNO	21.2	8.4	21.2	8.2
2,4-$Cl_2C_6H_3$	48.5–49[c]	85	$C_8H_5Cl_2NO$	35.1	6.9	35.2	6.8
2,4,5-$Cl_3C_6H_2$	91.5–92.5	88	$C_8H_4Cl_3NO$	45.0	5.9	44.8	5.8
2,4,6-$Cl_3C_6H_2$	102–103[d]	98	$C_8H_4Cl_3NO$	45.0	5.9	44.9	5.7

[a] B.p. 73–76° at 1 mm., Powell and Adams[18] reported b.p. 132° at 30 mm. [b] B.p. 109° at 1 mm. [c] M.p. 44–46° previously reported.[14] [d] M.p. 103° previously reported.[19]

the acid by way of the acid chloride and amide or from phenoxymethyl chloride and sodium cyanide[14] as these latter methods involved a series of steps. The structure of the phenoxyacetonitriles was established by comparison of physical constants with those recorded in the literature, elemental analysis and, in several cases, by hydrolysis to the known phenoxyacetic acids.

5-(3′-Indolylmethyl)tetrazole appears to stimulate cell elongation in the *Avena* test at concentrations about 200 times as great as those of 3-indolylacetic acid required to produce the same effect. 5(2′,4′-Dichlorophenoxymethyl)tetrazole is inactive but appears to be a competitive antagonist for 2,4-dichlorophenoxyacetic acid in the *Avena* test. Details of these studies are to be published elsewhere.[15]

The preparation of both 5-(3′-indolylmethyl)- and 5-(2′,4′-dichlorophenoxymethyl)tetrazole by somewhat different techniques has just been reported by van de Westeringh and Veldstra.[16]

EXPERIMENTAL[17]

5-(3′-Indolylmethyl)tetrazole. Seven and eight-tenths g. (0.12 mole) of sodium azide and 5.3 g. (0.04 mole) of anhydrous aluminum chloride were refluxed together in 120 ml. of dry tetrahydrofuran for 1 hr. 5.8 g. (0.04 mole) of 3-indolylacetonitrile was added to the mixture and refluxing with stirring continued for 24 hrs. The tetrahydrofuran was then distilled from the reaction mixture while water was added slowly at such a rate that the volume remained constant. After the organic solvent had been removed, the suspended solid was filtered off, resuspended in 250 ml. of water, and treated with sufficient hydrochloric acid to bring the suspension to *p*H 2. After 10 min. stirring, the solid was filtered off and washed with water. Drying gave 6.5 g. of crude product which was recrystallized first from ethylene chloride and then from water, yield 4.5 g. (61%), m.p. 179–180° with decomposition.

Anal. Calcd. for $C_{10}H_9N_5$: C, 60.3; H, 4.6; N, 35.2. Found: C, 60.3; H, 4.8; N, 35.0.

The *monopicrate* crystallized from water, m.p. 131–132°.

Anal. Calcd. for $C_{16}H_{12}N_8O_7$: C, 44.9; H, 2.8; N, 26.2. Found: C, 45.5; H, 3.2; N, 25.8.

Phenoxyacetonitriles. The preparation of phenoxyacetonitrile will serve as a typical example. A mixture of 23.5 g. of phenol, 18.7 g. of chloroacetonitrile and 34.5 g. of anhydrous potassium carbonate in 75 ml. of dry acetone was heated under reflux for 8 hr. The mixture was then poured into 200 ml. of water containing 10 g. of sodium hydroxide and extracted with ether. The ether layer was separated and dried over sodium sulfate, and the ether was removed by distillation. Fractionation of the residual reddish oil gave the product as a colorless, oily liquid, yield 27.2 g. Physical properties, yields, and analytical data for the phenoxyacetonitriles prepared in this way are given in Table I. Except for 2,4,6-trichlorophenoxyacetonitrile, which was recrystallized from absolute ethanol, the solid chlorophenoxyacetonitriles were recrystallized from petroleum ether.

Phenoxyacetic acid. Phenoxyacetonitrile (5.3 g.) was refluxed in 100 ml. of 25% sodium hydroxide solution for 12 hr. The resulting solution was filtered and the filtrate was cooled and acidified with 6*N* hydrochloric acid. The yield of product after recrystallization from water was 4.9 g. (81%), m.p. 98–99°. Sabanejeff and Dworkowitsch[20] report m.p. 97°.

2,4-Dichlorophenoxyacetic acid, m.p. 138.5–139° was obtained from the nitrile in similar manner; previously reported[21] m.p. 138°.

2,4,5-Trichlorophenoxyacetic acid was obtained from the nitrile in similar manner and recrystallized from benzene, m.p. 150.5–152°. Porkorny[21] reported m.p. 153°.

Preparation of Phenoxymethyltetrazoles. 5-Phenoxymethyltetrazole. Procedure Ia. A mixture of 16.3 g. (0.125 mole) of phenoxyacetonitrile, 11 g. (0.165 mole) of sodium azide and 10 g. (0.165 mole) of glacial acetic acid in 60 ml. of *n*-butyl alcohol was heated under reflux for 4 days. Heating was continued for 2 days after addition of 2.5 g. of sodium azide and 5 g. of glacial acetic acid. The reaction mixture was diluted with 200 ml. of water, and the mixture was distilled until the alcohol was removed. Acidification of the residual aqueous solution with dilute sulfuric acid gave the product as a colorless solid, yield 22 g. Recrystallization from water gave the pure product, m.p. 127.5–129°.

(14) H. Barber, R. Fuller, M. Green and H. Zwartouw *J. Appl. Chem. (London)*, 3, 266 (1953).

(15) We are indebted to Mr. R. H. Hamilton, Dr. A. Kivilaan and Dr. R. S. Bandurski of the Department of Botany at Michigan State University for their enthusiastic cooperation in these studies. Their results will be published separately in *Plant Physiology*.

(16) C. van de Westeringh and H. Veldstra, *Rec. trav. chim.*, 77, 1107 (1958).

(17) Microanalyses were done on all compounds by Micro-Tech Laboratories, Skokie, Ill. Melting points were taken in open capillaries and are not corrected.

(18) S. Powell and R. Adams, *J. Am. Chem. Soc.*, 42, 646 (1920).

(19) D. Drain, D. Peak, and F. Whitmont, *J. Chem. Soc.*, 2680 (1949).

(20) A. Sabanejeff and P. Dworkowitsch, *Ann.*, 216, 284 (1883).

(21) R. Porkorny, *J. Am. Chem. Soc.*, 63, 1768 (1941).

Anal. Calcd. for $C_9H_8N_4O$: C, 54.5; H, 4.6; N, 31.8. Found: C, 54.5; H, 4.7; N, 31.9.

5-(2'-Chlorophenoxymethyl)tetrazole. Procedure Ib. To a suspension of 16.7 g. (0.1 mole) of 2-chlorophenoxyacetonitrile and 19.5 g. (0.3 mole) of sodium azide in 50 ml. of dry tetrahydrofuran was added a solution of 13.3 g. (0.1 mole) of anhydrous aluminum chloride in 160 ml. of the same solvent. The mixture was refluxed with continuous stirring for 24 hr. The tetrahydrofuran was then distilled from the reaction mixture while water was added slowly at such a rate that the volume of the mixture remained constant. The solid which had separated was filtered off, resuspended in 250 ml. of water and treated with 30 ml. of concentrated hydrochloric acid. After being stirred for 1 hr. the solid was filtered off and dried, yield 18.8 g. of crude product which was recrystallized from toluene, m.p. 134.5–135.5°.

Anal. Calcd. for $C_9H_7ClN_4O$: C, 45.6; H, 3.4; Cl, 16.8; N, 26.6. Found: C, 45.9; H, 3.6; Cl, 16.9; N, 26.6.

5-(4'-Chlorophenoxymethyl)tetrazole. Following *Procedure Ib* a mixture of 16.7 g. (0.1 mole) of 4-chlorophenoxyacetonitrile, 19.5 g. (0.3 mole) of sodium azide, and 13.3 g. (0.1 mole) of anhydrous aluminum chloride in 210 ml. of dry tetrahydrofuran gave 20.6 g. of crude product. Recrystallization from aqueous ethanol gave 13.9 g. (66%) of pure product, m.p. 165–166°.

Anal. Calcd. for $C_9H_7ClN_4O$: C, 45.6; H, 3.4; Cl, 16.8; N, 26.6. Found: C, 45.7; H, 3.6; Cl, 16.8; N, 26.5.

5-(2' 4'-Dichlorophenoxymethyl)tetrazole. Using *Procedure Ia* a mixture of 25.2 g. (0.125 mole) of 2,4-dichlorophenoxyacetonitrile, 11 g. (0.165 mole) of sodium azide, and 10 g. of glacial acetic acid in 60 ml. of *n*-butyl alcohol gave 25.6 g. of crude product which was purified by recrystallization from toluene, m.p. 124.5–125.5°.

Anal. Calcd. for $C_9H_6Cl_2N_4O$: C, 39.2; H, 2.5; Cl, 28.9; N, 22.9. Found: C, 39.4; H, 2.6; Cl, 29.0; N, 23.0.

5-(2',4',5'-Trichlorophenoxymethyl)tetrazole. Following *Procedure Ia* a mixture of 29.6 g. (0.125 mole) of 2,4,5-trichlorophenoxyacetonitrile, 11 g. (0.165 mole) of sodium azide, and 10 g. of glacial acetic acid in 60 ml. of *n*-butyl alcohol gave 25.4 g. of crude product that was purified by recrystallization from toluene, m.p. 163.5–165°.

Anal. Calcd. for $C_9H_5Cl_3N_4O$: C, 34.4; H, 1.8; Cl, 38.1; N, 20.1. Found: C, 34.7; H, 1.8; Cl, 38.3; N, 20.1.

5-(2',4',6'-Trichlorophenoxymethyl)tetrazole. Using *Procedure Ib* 5.8 g. (0.025 mole) of 2,4,6-trichlorophenoxyacetonitrile, 4.8 g. (0.074 mole) of sodium azide, and 2.98 g. (0.025 mole) of anhydrous aluminum chloride in 90 ml. of dry tetrahydrofuran gave 6.6 g. of crude product which was recrystallized first from toluene and then from ethanol, m.p. 164–165°.

Anal. Calcd.: for $C_9H_5Cl_3N_4O$: C, 34.4; H, 1.8; Cl, 38.1; N, 20.1. Found: C, 34.6; H, 2.1; Cl, 37.9; N, 20.0.

Several attempts to prepare this compound using *Procedure Ia* were accompanied by extensive decomposition; no definite product was isolated from the reaction mixtures.

1-Benzyl-5-phenoxymethyltetrazole. A mixture of 8.3 g. (0.04 mole) of 1-benzyl-5-chloromethyltetrazole,[22] 4.7 g. (0.05 mole) of phenol, and 2.7 g. (0.05 mole) of sodium methoxide in 75 ml. of absolute methanol was heated under reflux with stirring for 10 hr. The contents of the flask were then poured into 150 ml. of water, the precipitate was filtered off and recrystallized from aqueous methanol to give 3.8 g. (36%) of the desired product, m.p. 66.5–67°.

Anal. Calcd. for $C_{15}H_{14}N_4O$: C, 67.7; H, 5.3; N, 21.0. Found: C, 67.4; H, 5.4; N, 21.1.

1-Benzyl-5-(2',4'-dichlorophenoxymethyl)tetrazole. Under similar conditions 8.3 g. of 1-benzyl-5-chloromethyltetrazole, 8.15 g. of 2,4-dichlorophenol, and 2.7 g. of sodium methoxide in 75 ml. of absolute methanol gave 12.4 g. of crude product from which, after recrystallization from methanol, 8.6 g. of pure product, m.p. 107.5–108°, was obtained.

Anal. Calcd. for $C_{15}H_{12}Cl_2N_4O$: C, 53.8; H, 3.6; Cl, 21.2; N, 16.7. Found: C, 53.8; H, 3.9; Cl, 21.0; N, 16.8.

1-Benzyl-5-(2',4',5'-trichlorophenoxymethyl)tetrazole. In similar manner 6.9 g. of 1-benzyl-5-chloromethyltetrazole, 8.2 g. of 2,4,5-trichlorophenol, and 2.2 g. of sodium methoxide in 75 ml. of absolute methanol gave 10.6 g. of crude product which on recrystallization from methanol gave 6.4 g. of pure product, m.p. 113.5–114.5°.

Anal. Calcd. for $C_{15}H_{11}Cl_3N_4O$: C, 48.7; H, 3.0; Cl, 28.8; N, 15.2. Found: C, 48.6; H, 3.1; Cl, 28.7; N, 15.3.

1-Benzyl-5-(2',4',6'-trichlorophenoxymethyl)tetrazole. Similarly 6.9 g. of 1-benzyl-5-chloromethyltetrazole, 8.15 g. of 2,4,6-trichlorophenol, and 2.2 g. of sodium methoxide in 75 ml. of absolute methanol gave 12.3 g. of crude product and after recrystallization from methanol, 9.1 g. of pure material, m.p. 112–113°.

Anal. Calcd. for $C_{15}H_{11}Cl_3N_4O$: C, 48.7; H, 3.0; Cl, 28.8; N, 15.2. Found: C, 48.8; H, 3.0; Cl, 28.9; N, 15.0.

Debenzylation of 1-Benzyl-5-phenoxymethyltetrazole. A solution of 2.7 g. (0.01 mole) of 1-benzyl-5-phenoxymethyltetrazole in 100 ml. of absolute ethanol was shaken for 12 hr. with 1 g. of 5% palladium on charcoal at an initial hydrogen pressure of 50 p.s.i. The catalyst was filtered off and the solvent was removed from the filtrate in a vacuum. The residue was treated with dilute sodium hydroxide and filtered. From the alkali insoluble solid, 1.3 g. (49%) of the starting material was recovered. Acidification of the alkaline solution with dilute hydrochloric acid gave a precipitate of 5-phenoxymethyltetrazole, 400 mg. (43%), which was recrystallized from water, m.p. and mixture m.p. 127.5–128.5°.

Debenzylation of 1-benzyl-5-(2',4',5'-trichlorophenoxymethyl)tetrazole. A mixture of 1.8 g. of 1-benzyl-5-(2',4',5'-trichlorophenoxymethyl)tetrazole and 1 g. of palladium on charcoal in 75 ml. of absolute ethanol was shaken for 12 hr. at an initial hydrogen pressure of 50 p.s.i. The catalyst was filtered and washed with warm ethanol. Removal of the solvent from the combined filtrate and washings in a vacuum left a residue which after repeated crystallization from toluene gave 5-(2',4',5'-trichlorophenoxymethyl)tetrazole, m.p. and mixture m.p. 160–162°.

Both 1-benzyl-5-(2',4'-dichloro- and 2',4',6'-trichlorophenoxymethyl)tetrazole were debenzylated in a similar manner, but in neither case was a pure product isolated from the resulting mixture of products. Apparently debenzylation was accompanied by dehalogenation and possibly reduction in varying degrees which would have vitiated this approach as an unequivocal synthesis.

EAST LANSING, MICH.

(22) E. K. Harvill, R. M. Herbst, and E. C. Schreiner, *J. Org. Chem.*, 17, 1597 (1952).

Advanced Organic Synthesis

METHODS AND TECHNIQUES

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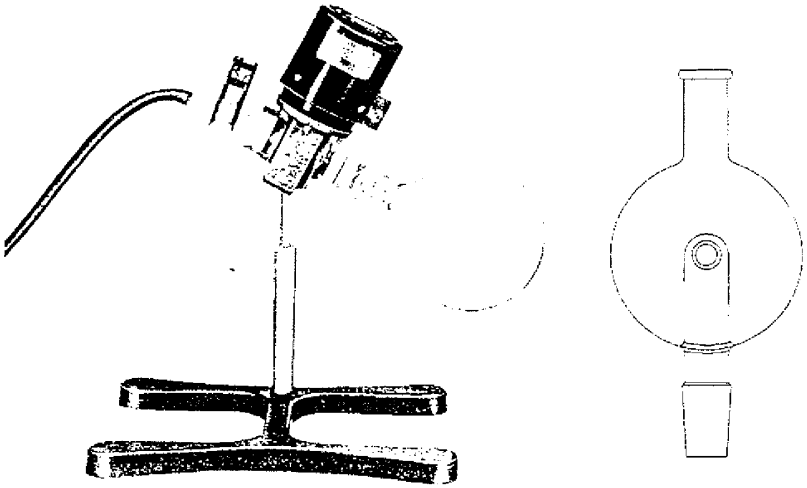
ACADEMIC PRESS
New York and London

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
Berkeley Square House, London W1X 6BA

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 75-165531

PRINTED IN THE UNITED STATES OF AMERICA



(a) (b)

FIG. A3.12. (a) Rotary evaporator (Buchler Instruments) (b) Trap used with rotary evaporator.

walls of the rotating flask provides a large surface area for rapid evaporation, while the rotation action mixes the solution and inhibits actual boiling. Figure A3.12b illustrates a trap which may be used with the rotary evaporator to prevent loss of the solution in case of bumping. The trap may also be cooled, if desired, for recovery of the solvent.

III. Purification of the Product

A. DISTILLATION (2)

Setups for simple and fractional distillation at atmospheric pressure are shown (Fig. A3.13). A 30-cm Vigreux column (Fig. A3.13b) is convenient if the components boil at least 50° apart at atmospheric pressure. For better separation, a column packed with glass helices is suitable. All columns employed in fractional distillation should be wrapped or jacketed to minimize heat loss.

Heat sources for distillation must be closely controlled to prevent overheating or too rapid distillation. The best heat sources are electrically heated liquid baths. Mineral oil or wax is a satisfactory medium for heat exchange up to about 240°. The medium may be

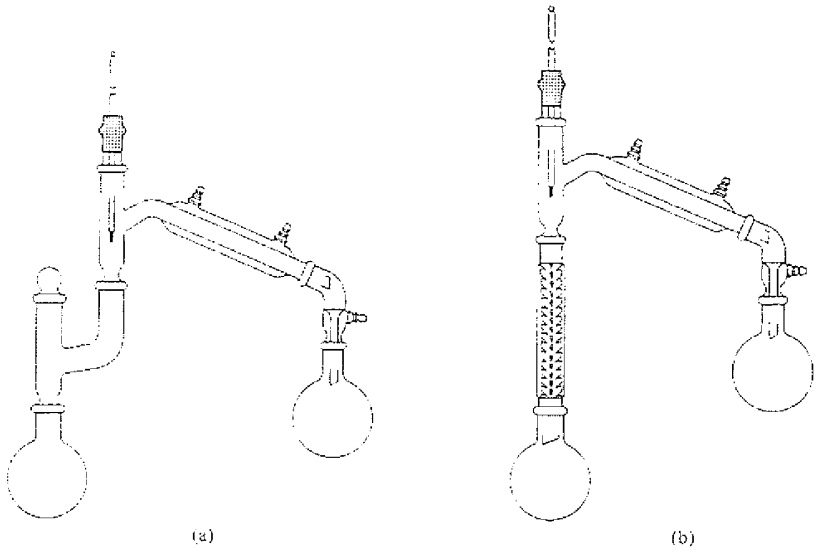


FIG. A3.13. Setups for atmospheric pressure distillation (a) for simple distillation (b) Vigreux column for fractional distillation.

contained in a stainless steel beaker or sponge dish and heated by an electric hot plate or immersion coil. The bath temperature (20–80° above the boiling point) is easily monitored by an immersed thermometer.

Distillation at reduced pressure is advisable with the majority of organic compounds boiling above 150° at 1 atmosphere. Aspirator pressure (20–30 mm depending on water temperature and system leaks) is sufficient for many reduced pressure distillations. A liquid boiling at 200°/1 atm, for example, will have a boiling point of approximately 100° at 30 mm. (Estimates of observed boiling points at reduced pressure can be made by use of the pressure-temperature alignment chart shown in Fig. A3.14). The aspirator pump is simple and is not affected by organic or acid vapors. The pressure in such a system is best monitored by a manometer.

A vacuum system employing an oil pump is shown schematically in Fig. A3.15. Protection of the pump requires that the system be well trapped between the pump and the distillation setup. The pressure can be regulated by introducing an air leak through a needle valve (a bunsen burner needle valve is satisfactory). The pressure is monitored by use of a tipping McLeod gauge (Fig. A3.16) which gives intermittant (as opposed to continuous) reading of pressure down to about 0.05 mm, of sufficient precision for the purpose.

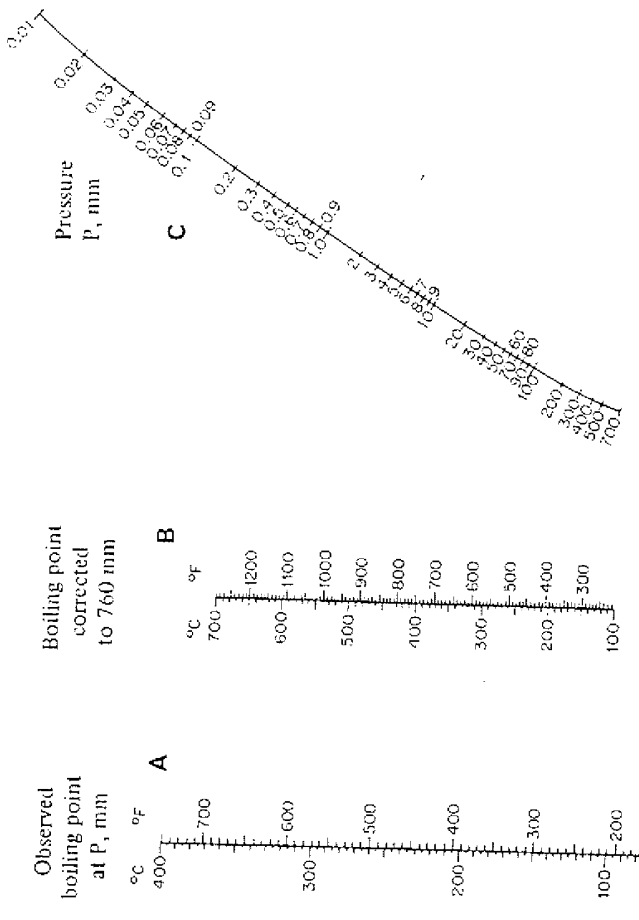


FIG. A3.14. Pressure–temperature alignment chart (reprinted by permission from MCR Manufacturing Chemists, Norwood, Ohio).

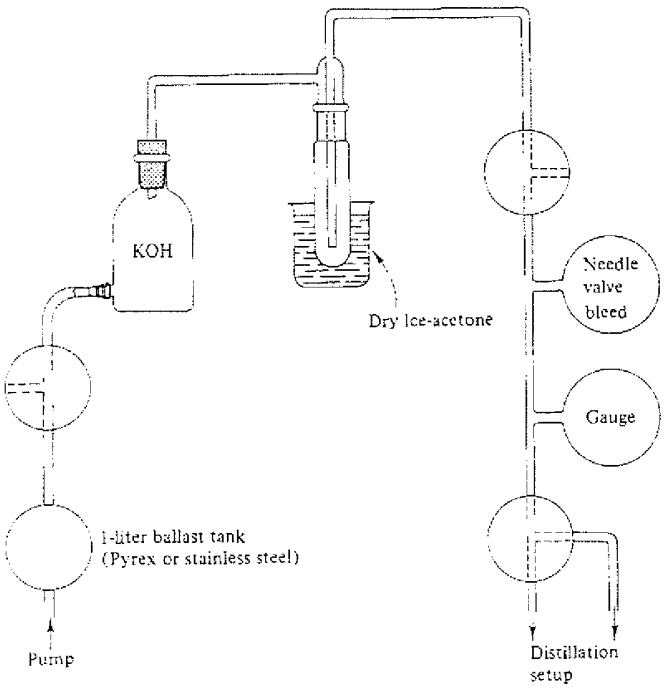


FIG. A3.15. Schematic diagram of a vacuum system for distillation.

The prevention of bumping in reduced pressure distillations requires special precautions. Boiling chips rarely function well over the course of a long distillation under vacuum, and one of several alternative techniques should be employed. The following methods are listed in decreasing order of effectiveness: (1) Introduction of a fine stream of air or nitrogen through a capillary bleed tube (Fig. A3.17); (2) the use of 12–15 microporous boiling chips (Todd Scientific Co.); (3) covering the boiling liquid with a mesh of Pyrex wool; (4) the use of boiling sticks.

B. CRYSTALLIZATION AND RECRYSTALLIZATION

Several techniques are usually employed to induce crystallization from saturated solutions of organic solids. The introduction of seed crystals will invariably work, although with new compounds such crystals are not available. Seeding with crystals of a

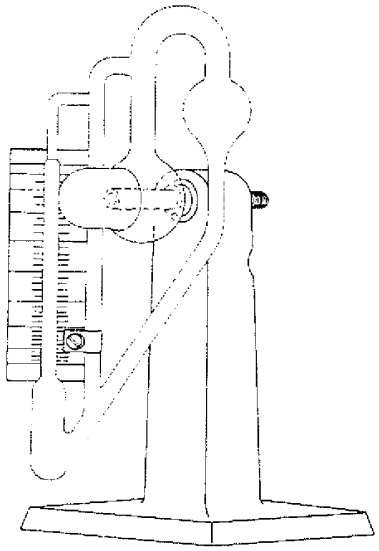


FIG. A3.16. Tipping McLeod guage.

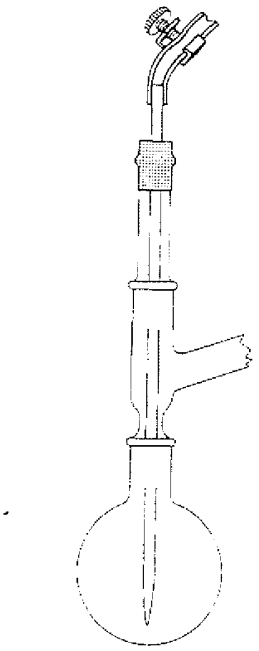


FIG. A3.17. Capillary bleed tube for reduced pressure distillation.

compound with related molecular or crystal structure is frequently successful. Alternatively, cooling the solution and scratching the interior of the vessel with a glass rod is successful in a surprising number of cases.

The technique of trituration is frequently useful. The organic product is stripped of solvent and the oily residue is placed in a mortar and covered with a layer of a solvent in which it is only slightly soluble. The mass is ground with a pestle mixing in the solvent as thoroughly as possible. In favorable cases, the solvent removes traces of impurities that may be inhibiting crystallization, and grinding action induces crystallization.

Successful recrystallization of an impure solid is usually a function of solvent selection. The ideal solvent, of course, dissolves a large amount of the compound at the boiling point but very little at a lower temperature. Such a solvent or solvent mixture must exist (one feels) for the compound at hand, but its identification may necessitate a laborious trial and error search. Solvent polarity and boiling point are probably the most important factors in selection. Benzhydrol, for example, is only slightly soluble in 30–60° petroleum ether at the boiling point but readily dissolves in 60–90° petroleum ether at the boiling point.

Until one develops a "feel" for recrystallization, the best procedure for known compounds is to duplicate a selection in the literature. For new compounds, a literature citation of a solvent for an analogous structure is often a good beginning point. To assist in the search, Table A3.4 lists several of the common recrystallizing solvents with useful data. The dielectric constant can be taken to be a rough measure of solvent polarity.

TABLE A3.4
RECRYSTALLIZING SOLVENTS

Solvent	B.P. (°C)	Dielectric constant	Water solubility (g 100 g)
Acetic acid	188	6.2	Misc.
Acetone	56.5	21	Misc.
Acetonitrile	82	38	Misc.
Benzene	80	2.3	0.07
t-Butyl alcohol	82	17	Misc.
Carbon tetrachloride	77	2.2	0.08
Chloroform	61	4.8	1.0
Cyclohexane	81	2.0	Sl. sol.
DMF	154	38	Misc.
Dioxane	101	2.2	Misc.
DMSO	189	45	Misc.
Ethanol	78	25	Misc.
Ethyl acetate	77	6.0	9
Ethyl ether	35	4.3	7.5
Ethylene chloride	83	10	0.83
Heptane	98	2.0	Insol.
Hexane	69	1.9	Insol.
Isopropyl alcohol	82	18	Misc.
Methanol	65	34	Misc.
Methylene chloride	40	9.1	2.0
Nitromethane	101	38	10
Pentane	36	2.0	0.03
Pyridine	115	12	Misc.
Water	100	80	—

C. DRYING OF SOLIDS

A solid insensitive to air is easily dried by spreading the material over a large piece of filter paper and allowing moisture or solvent to evaporate. However, many organic solids are sensitive to air or moisture and must be dried under reduced pressure in a vacuum desiccator or vacuum oven. Moreover, complete drying of a sample to be analyzed by combustion analysis necessitates vacuum drying. For vacuum drying of small samples, an Abderhalden (drying pistol) is a convenient arrangement (Fig. A3.18).

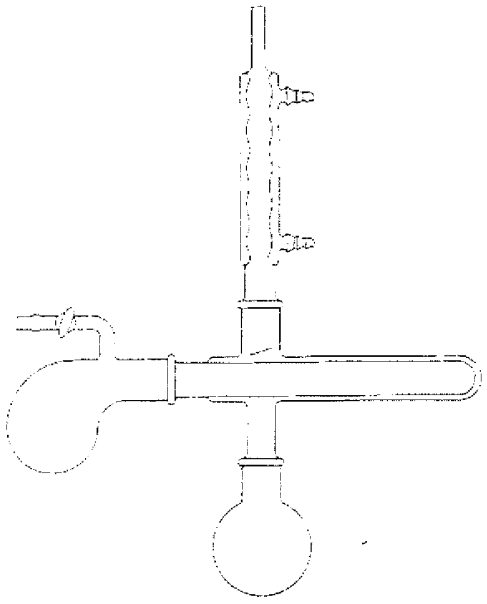


FIG. A3.18. Abderhalden (drying pistol).

The sample is placed in the barrel of the pistol and a drying agent (usually P_2O_5) placed in the "handle." The evacuated system is heated by refluxing a liquid of the desired boiling point over the sample.

D. SUBLIMATION

When a solid compound possesses a relatively high vapor pressure below its melting point, it may be possible to purify it by sublimation. Selenium dioxide, for example, is easily purified prior to use by sublimation at atmospheric pressure (Chapter 1, Section XI). More commonly, the method of choice is sublimation at reduced pressure, which allows more ready evaporation of solids with limited volatility. A convenient vacuum sublimation apparatus is shown in Fig. A3.19. The impure sample is placed in the lower cup, which is attached to the condenser by an O-ring seal and spring. Water is run through the condenser and the system is evacuated. The cup is heated gradually with an oil bath, and sublimation follows. The sublimate is recovered by scraping it off the walls of the condenser with a spatula.

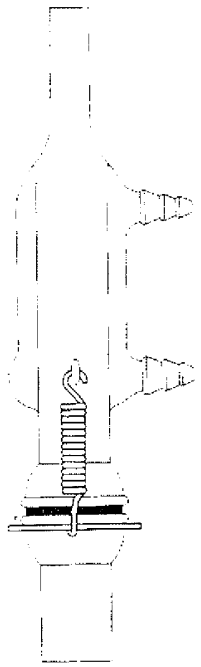


FIG. A3.19. Apparatus for vacuum sublimation.

E. CHROMATOGRAPHY

1. *Column Chromatography:* Column Chromatography is a useful separation technique for mixtures resulting from intermediate to small scale synthetic processes. For example, nitroferrocene is conveniently isolated from a mixture of the product, ferrocene, and 1,1'-dinitroferrocene by chromatography on Activity I basic alumina at about the 100-g scale (Chapter 7, Section XI).

The column (20–30 cm by 1–2 cm diameter or larger in the proportion 10:1) is prepared by filling it with a dry solvent of low polarity (e.g., pentane), pushing a plug of cotton to the bottom, covering the cotton with a layer of sand, and dusting in the adsorbant. About 25 g of adsorbant per gram of mixture is a good approximation for a first trial. The adsorbant is covered with a layer of sand, excess solvent is drained, and the sample, dissolved in a minimum amount of a suitable solvent, is introduced with a dropper.

Alumina is the most frequently employed adsorbant. Its activity (i.e., the extent to which it adsorbs polar compounds) is largely a function of the amount of water present. Alumina of Activity I is prepared by heating the material in an oven to 200–230° and allowing it to cool in a desiccator. Addition of water to the extent of 3%, 6%, 10%, or 15% to the dry material gives alumina of Activity II, III, IV, and V, respectively.

The column is eluted with dry solvents of gradually increasing eluting power. The order of eluting power of the common dry solvents is shown in Table A3.5. The compounds are eluted from the column in order of their increasing polarity. The usual order of elution of organic compounds is shown in Table A3.6. The progress of the

TABLE A3.5
ORDER OF ELUTING POWER OF COMMON DRY SOLVENTS

1. 30–60° Petroleum ether	8. Chloroform
2. 60–90° Petroleum ether	9. Ethyl acetate
3. Carbon tetrachloride	10. Ethylene chloride
4. Cyclohexane	11. Ethanol
5. Benzene	12. Methanol
6. Ether	13. Water
7. Acetone	14. Acetic acid

TABLE A3.6
ORDER OF ELUTION OF ADSORBED COMPOUNDS

1. Aliphatic hydrocarbons	7. Ketones
2. Alkyl halides	8. Aldehydes
3. Olefins	9. Thiols
4. Aromatic hydrocarbons	10. Amines
5. Ethers	11. Alcohols
6. Esters	12. Carboxylic acids

elution is followed by collecting small samples of the eluant and evaporating the solvent. The melting points and spectra of the residual materials serve as a guide to the development of the column. When one compound has been completely eluted, changing to a solvent or a solvent mixture of higher eluting power will hasten the recovery of subsequent fractions.

2. *Thin-Layer Chromatography (TLC)*: The function of TLC in organic synthesis is primarily one of allowing the experimenter to follow the progress of the reaction without actually interrupting the reaction. Since successful TLC can be carried out on a minute scale, only a very small fraction of the reaction mixture need be withdrawn and subjected to analysis. The following example of the TLC analysis of the chromic acid oxidation of borneol, described by Davis (3), is a useful model.

(a) *Preparation of the plates* (4): Microscope slides are washed thoroughly with soap, rinsed with distilled water followed by methanol, and allowed to dry on edge. A suspension of 35 g of Silica Gel G in 100 ml of chloroform is placed in a wide-mouth bottle. Two slides, held face to face with forceps, are immersed in the suspension which is briefly stirred. The slides are withdrawn evenly, resulting in a smooth deposit of the adsorbant. The rate of withdrawal of the slides controls the thickness of the silica gel layer. The slides are separated and allowed to dry. Each slide is then held in a slow stream of steam for 5 seconds to allow the binder to set. Prior to use, the slides are activated by heating for 45 minutes in an oven at 125° or by placing them on a hot plate over a wire gauze for the same length of time.

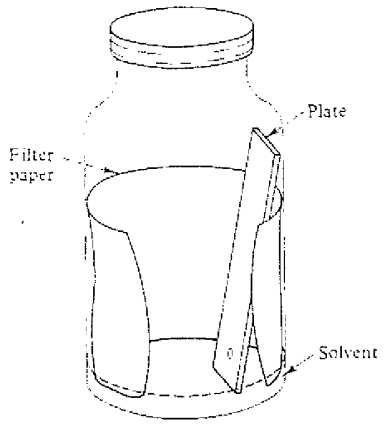


FIG. A3.20. Apparatus for the development of TLC plates.

(b) *Chromic acid oxidation of borneol:* The following solutions are prepared: 2% borneol in ether; 10% chromic anhydride and 5%, sulfuric acid in water; 2% camphor in ether.

One milliliter each of the borneol solution and the oxidizing solution are mixed in a test tube and briefly shaken. A TLC slide is spotted with the borneol solution, the camphor solution, and the ether layer of the reaction mixture. Spotting is done by means of a capillary melting point tube used as a dropper and filled with a 5 mm sample. The slide is developed in a wide-mouth jar containing a filter paper liner and a few milliliters of chloroform (Fig. A3.20). After development (the solvent front rises to within 1 cm of the top), the slide is removed, the solvent is allowed to evaporate, and the slide is placed in a covered wide-mouth jar containing a few crystals of iodine. The spots readily become visible and the progress of the reaction can easily be followed. With periodic shaking, the oxidation is complete in about 30 minutes.

A variety of reaction mixtures can be analyzed by this simple technique, although a suitable solvent or solvent mixture for the development of the slide must be determined for the particular compounds involved.

3. *Gas–Liquid Phase Chromatography (glpc):* glpc is certainly a technique of high utility to the synthetic chemist, both for analysis of reaction mixtures and for their separation on a synthetic scale. However, a detailed treatment of the techniques of glpc would be beyond the intention of the present book, since, by and large, such matters as sampling techniques, flow rates, column temperature and packing, as well as other variables, can usually be determined only in connection with the problem at hand. Instead, the student is advised to consult the instruction manuals of individual commercial instruments for operating details. An excellent discussion of the practical aspects of glpc by Ettre and Zlatkis is also available (5). Finally, a useful summary of column packing materials with many references is published periodically by Analabs, Inc. (6).

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Development of Dual-Acting Benzofurans for Thromboxane A_2 Receptor Antagonist and Prostacyclin Receptor Agonist: Synthesis, Structure−Activity Relationship, and Evaluation of Benzofuran Derivatives

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Received March 3, 2005

Prostacyclin (PGI_2) is an unstable, powerful endogenous inhibitor of platelet aggregation, and thromboxane A_2 (TXA_2) is an unstable endogenous arachidonic acid metabolite that plays a pivotal role in platelet aggregation and vasoconstriction. The balance between TXA_2 and PGI_2 greatly affects maintenance of the homeostasis of the circulatory system. A novel series of benzofuran-7-yloxyacetic acid derivatives was discovered as potent dual-acting agents to block the thromboxane A_2 receptor and to activate the prostacyclin receptor. Synthesis, structure−activity relationship, and in vitro and ex vivo pharmacology of this series of compounds are described. The most potent in the series was {3-[2-(1,1-diphenylethylsulfanyl)ethyl]-2-hydroxymethylbenzofuran-7-yloxy}acetic acid diethanolamine salt (**7**) with K_i of 4.5 nM for thromboxane receptor antagonism and K_i of 530 nM for prostacyclin receptor agonism. Remarkably, compound **7** is a promising candidate for novel treatment as an antithrombotic agent with other cardiovascular actions to avoid hypotensive side effects.

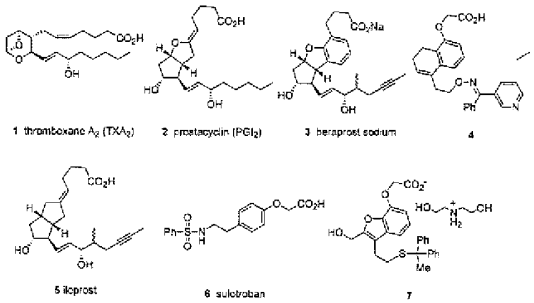
Introduction

Thromboxane A_2 (TXA_2) (**1**), discovered by Samuelsson, is an unstable endogenous arachidonic acid metabolite that plays a pivotal role in platelet aggregation and vasoconstriction[1] and has been implicated as a contributor to cardiovascular, renal, and pulmonary diseases.[2,3] Because of the lack of clinical efficacy with these agents,[4] a combined therapy using thromboxane receptor antagonists (TRAs) and thromboxane synthase inhibitors (TSIs) has been developed. This therapy has the advantage that its TSI activity would prevent the biosynthesis of TXA_2 while the accumulated PGH_2 would be redirected to produce beneficial prostaglandin metabolites such as prostacyclin (PGI_2), PGD_2, and PGE_2. However, this conventional TRA/TSI therapy exhibits unsatisfactory clinical effects.[5]

Prostacyclin (**2**), discovered by Vane, is a powerful endogenous inhibitor of platelet aggregation and also plays an important role in biological homeostasis as an endogenous autacoid distributed widely in various tissues.[6] Although these actions attract notice in the cardiovascular field, the therapeutic application of PGI_2 itself is limited by both chemical and metabolic instability because of its labile enol−ether moiety. Thus, the extensive efforts that have been focused on the synthesis of PGI_2 mimics were directed toward the stabilization of the enol−ether moiety (i.e., **3**).[7,8] Recently, nonprostanoids PGI_2 mimetics with chemical and metabolic instability have been reported (i.e., **4**)[9−14] (Chart 1).

TXA_2 and PGI_2, both of which are synthesized from arachidonic acid, have opposite effects on platelet aggregation. Also, the balance between TXA_2 and PGI_2 greatly affects maintenance of the homeostasis of the circulatory system. In the case of ischemic disorders, the TXA_2/PGI_2 balance is shifted to the TXA_2 side, and phenomena such as platelet activation, subsequent thrombogenesis, and vascular contraction appear. Thus, it is clinically important to achieve the proper TXA_2/PGI_2 balance. A combination of an agent for inhibiting TXA_2 activity and an agent acting as a PGI_2 receptor agonist is thought to be effective. Moreover, researchers at Schering AG reported that the PGI_2 mimetic **5** (iloprost) showed strong antithrombotic action when it was combined with the TXA_2/PGH_2 receptor antagonist **6** (sulotroban).[15,16] Therefore, we are interested in developing agents that combine the TXA_2 receptor (TP) antagonist activity with prostacyclin receptor (IP) agonist activity within a single molecule. Such agents would not only maximize the beneficial effects of each agent but also address the potential clinical problem of using two drugs with different pharmacokinetics. Moreover, one could expect a synergistic effect from combining two therapeutic actions in a single chemical entity to avoid the hypotensive effect of PGI_2.

Chart 1. Chemical Structures of Thromboxane A_2, Prostacyclin, Prostacyclin Mimetics, and Thromboxane A_2 Antagonists



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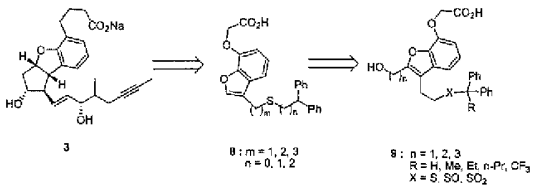
10.1021/jm050194z CCC: $30.25
Published on Web 07/15/2005

Chart 2. Molecular Design of Benzofuran Derivatives from **3**



In this paper, we report the first dual-acting benzofuran **7** that possesses TXA_2 antagonism and PGI_2 agonism within a single molecule. We describe the design, synthesis, and the biological evaluation of benzofuran derivatives.

Chemistry

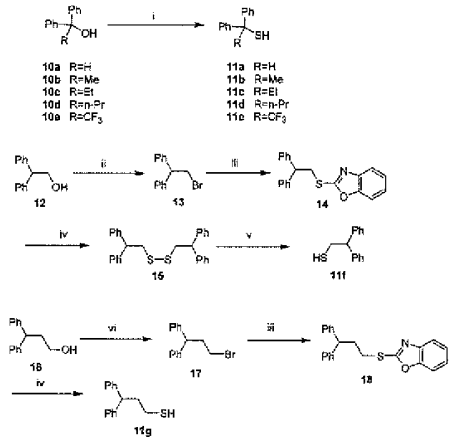
We started to design our new compounds from **3**. To avoid enantiomeric problems, we chose benzofuran, which is regarded as a characteristic structure of **3**, for our scaffold. We chose an oxyacetic acid group for the α-chain and attached it at the 7-position of benzofuran for the following reasons: (1) This derivatization at the 7-position is known to maintain the PGI_2 agonistic properties. (2) This derivatization can avoid ω-oxidation as a route of metabolic degradation of the α-chain. (3) It enables us to shorten synthetic steps. For comparison, we also attached the ω-side chain at the 3-position of benzofuran. A wide range of ω-side chains was screened from a series of functional groups, which we examined in the course of research on **3**. We began with the synthesis and evaluation of compound **8**, which have a sulfide ω-side chain, because some thromboxane antagonists have sulfide groups or sulfonamide groups (i.e., **6**) in their ω-chains.

In the following optimization, we introduced a hydroxyl group at the 2-position of compound **8** through the carbon chain and designed compound **9** to enhance the TXA_2 antagonism and/or the PGI_2 agonism. The hydroxyl group at the 2-position of benzofuran corresponds to that of the 11-position of PGI_2. The product in which the sulfide in compound **9** was oxidized was also screened in the optimization (Chart 2).

Compounds in Tables 1 and 2 were prepared as described in Schemes 1−8. The exploration of conventional methods for thiol synthesis was the first key objective of this project. First, we tried to synthesize thiols **11a−e** by alkali hydrolysis of the 2-alkylated isothioureas but only succeeded in the case of **11a**. Isothioureas for **11b−e** have undergone elimination reactions to produce styrenes under hydrolytic conditions. Primary thiols (**11f** and **11g**) were synthesized by hydrolysis of 2-mercaptobenzoxazole derivatives. Compounds **11b−d** were also obtained in poor yield by this method.

Nishio reported the single-step conversion from secondary and tertiary alcohols to the corresponding thiols by treatment with Lawesson's reagent.[17,18] The original procedure, reported by Nishio, worked well for **11a** but gave low yields for **11b−d**. We isolated styrene-type byproducts in the reaction mixtures of **11b−d**, which suggests that the thiols produced had undergone elimination reactions to produce styrenes. Moreover, the reaction rate under the original conditions (using DME)

Scheme 1[a]



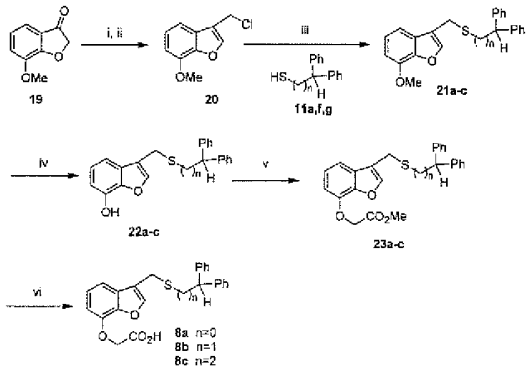
[a] Reagents: (i) Lawesson's reagent, toluene−H_2O; (ii) Ph_3P, CBr_4; (iii) 2-mercaptobenzoxazole, K_2CO_3; (iv) NaOH; (v) Zn, AcOH; (vi) Ph_3P, NBS.

was fast, and the thiol conversion reaction at room temperature was complete within 15 min, which made the control of the reaction difficult. We later found that the addition of a small amount of the water would slow both the thiol conversion and the elimination reactions. By heating the corresponding alcohols with Lawesson's reagent in toluene with water (1 equiv for Lawesson's reagent), we succeeded in obtaining **11b−e** in good yield, and our condition also enabled the large-scale preparation of **11b**.

The second key objective of this project was the synthesis of 3-substituted 7-oxybenzofurans. The preparation of compounds **8a−c** is described in Scheme 2. We began the synthesis from 7-methoxy-2*H*-benzofuran-3-one (**19**), which was easily prepared by the procedure of Bryant.[19] Thus, compound **19** was treated with lithium chloromethylene to obtain compound **20** in 10% yield. The low yield occurred because the carbonyl group of **19** was easily enolized upon treatment with base, and compound **19** was subject to intermolecular aldol condensation. Compound **20** was coupled with thiols, and the methyl protection of the phenol group at the 7-position of **21a−c** was removed using *n*-PrSK. Compounds **22a−c** were treated with methyl bromoacetate to introduce the oxyacetic α-chain moiety. Methyl esters of **23a−c** were hydrolyzed to give **8a−c**.

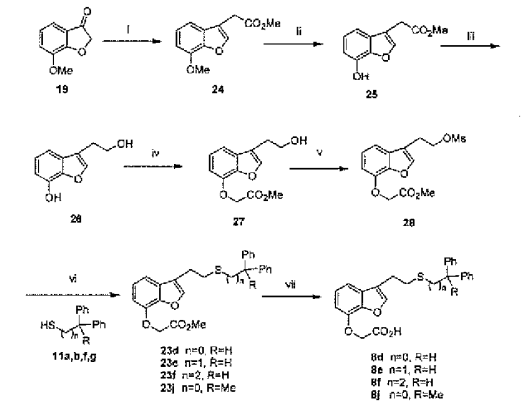
The preparation of compounds **8d−f** and **8j** is described in Scheme 3. To avoid the aldol side reaction described in the synthesis of **8a−c**, we used the stable Wittig ylide for the preparation of compound **24**. Since this reagent was isolated as a neutral salt-free form, the reaction did not require any base, resulting in the isolation of compound **24** in 46% yield. By use of BBr_3, the methyl protection of phenol group at the 7-position of **24** was selectively removed. The methyl ester of **25** was reduced to the alcohol using $LiAlH_4$, and the resulting compound **26** was treated with methyl bromoacetate to selectively introduce the oxyacetic α-chain moiety at the 7-position of **26**. The alcohol **27** was treated with mesyl chloride, and the resulting mesylate **28** was coupled with thiols. Compounds **8d−f** and **8j**

Scheme 2[a]



[a] Reagents: (i) *n*-BuLi, CH_2BrCl; (ii) *p*-toluenesulfonic acid, toluene; (iii) *t*-BuOK, thiols, DMF; (iv) *t*-BuOK, *n*-PrSH, DMF; (v) $BrCH_2CO_2Me$, K_2CO_3, DMF; (vi) NaOH.

Scheme 3[a]



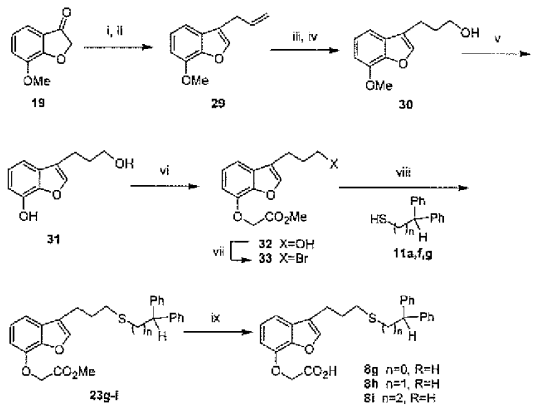
[a] Reagents: (i) Ph_3PCHCO_2Me, xylene; (ii) BBr_3, CH_2Cl_2; (iii) $LiAlH_4$, THF; (iv) $BrCH_2CO_2Me$, K_2CO_3, DMF; (v) methanesulfonyl chloride, Et_3N; (vi) *t*-BuOK, thiols, DMF; (vii) NaOH.

were obtained upon hydrolysis of the methyl ester groups of **23d–f** and **23j**.

The preparation of compounds **8g–i** is described in Scheme 4. To avoid aldol side reactions described in the synthesis of **8a–c**, we used an organocerium reagent, which was prepared in situ from $CeCl_3$ and allylmagnesium bromide.[20,21] Since the basicity of the allylcerium reagent was lower than that in Grignard reagent, compound **29** was obtained in 72% yield, including the dehydration step. The alcohol group was introduced using a hydroboration procedure on **29**. After cleaving the methyl protection of the phenol group at the 7-position of **30** using BBr_3, the oxyacetic α-chain moiety was introduced selectively at the 7-position of **31** by treating with methyl bromoacetate. The alcohol group of **32** was converted to bromine using *N*-bromosuccinimde-Ph_3P, and the resulting compound **33** was coupled with thiols. Compounds **8g–i** were obtained by hydrolysis of the methyl ester groups of **23g–i**.

The preparation of compounds **9a–e** is described in Scheme 5. The 2-substituted benzofuran scaffold was synthesized from **34** using a Dieckmann condensation. The methyl ester of **35** was selectively reduced to the

Scheme 4[a]



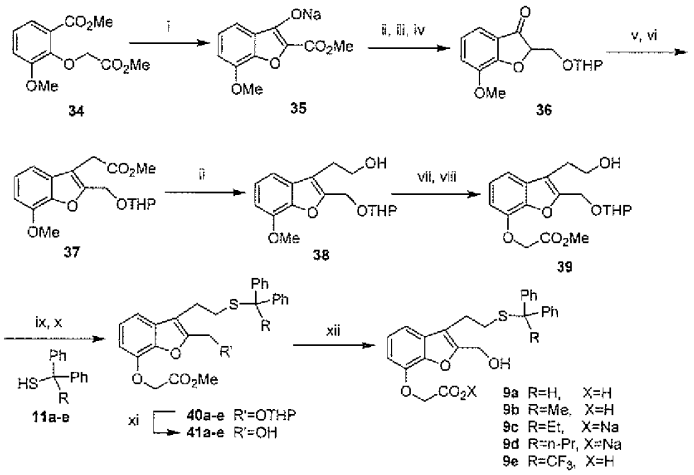
[a] Reagents: (i) allylmagnesium bromide, $CeCl_3$, THF; (ii) *p*-toluenesulfonic acid, benzene; (iii) BH_3-Me_2S, THF; (iv) H_2O_2, NaOH; (v) BBr_3, CH_2Cl_2; (vi) $BrCH_2CO_2Me$, K_2CO_3, DMF; (vii) Ph_3P, *N*-bromosuccinimde; (viii) *t*-BuOK, thiols, DMF; (ix) NaOH.

alcohol using $LiAlH_4$ because the carbonyl group of the 3-position was protected as sodium enolate. During the acidic workup, the carbonyl group of the 3-position was restored. After the protection of the primary alcohol at the 2-position of benzofuran by THP, compound **36** was obtained in 75% yield as a 1:1 mixture of diastereomers. In the synthesis of this series, we also tried a Wittig reaction using the stable ylides to avoid the aldol side reaction as described in the synthesis of **8d**, but the stable Wittig ylide did not react with **36** because of the steric interference by 2-position substitution. Then we performed Reformatski reaction. The reactivity of the Reformatski reagent with each diastereomer was similar, so we used the 1:1 diastereomers mixture of **36** for the scale-up synthesis. The dehydration of the Reformatski product was achieved by using Tf_2O–pyridine in toluene, and compound **37** was obtained in 78% yield in two steps. The methyl ester of compound **37** was reduced to the alcohol using $LiAlH_4$. After cleaving the methyl protection of the phenol group at the 7-position of **38** was cleaved using *n*-PrSK, the oxyacetic α-chain moiety was introduced selectively at this position by treating with methyl bromoacetate. The alcohol **39** was coupled with thiols **11a–e** via the mesylate. The THP group of **40a–e** was removed under mild acidic conditions, and compounds **9a–e** were obtained by hydrolysis of methyl ester groups of **41a–e**.

The preparation of compounds **7** and **9h–i** is described in Scheme 6. Compound **7** was obtained in 71% yield by treating **9b** with diethanolamine and crystallizing from ethanol. The sulfoxide analogue **9h** was synthesized by direct oxidation of **9b** with H_2O_2. The sulfone analogue **9i** was synthesized by oxidation of **41b** with *m*-CPBA followed by hydrolysis of the methyl ester.

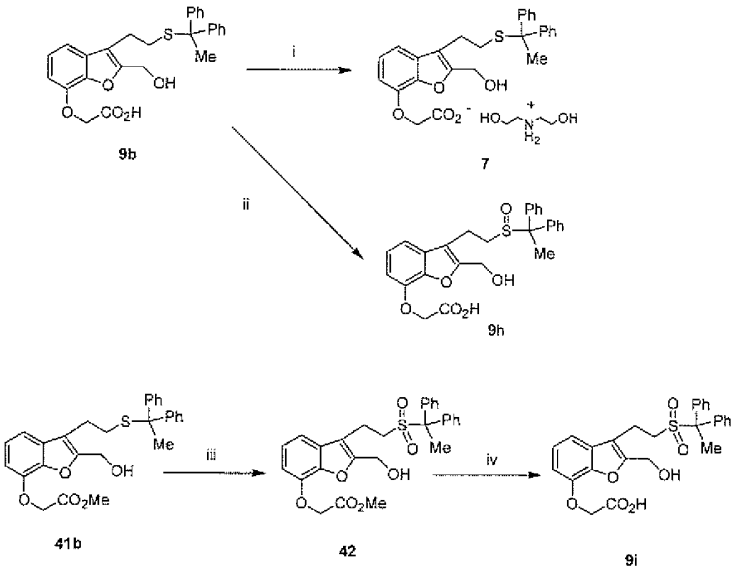
The preparation of **9f** is described in Scheme 7. The 2-hydroxyethylbenzofuran scaffold was also synthesized from **35**, and the side chain at the 2-position of benzofuran was introduced using a Claisen rearrangement. First, the hydroxyl group of enolate **35** was allylated by treating with allyl bromide, and the allylic group then migrated to the 2-position upon heating to give **43**. The ester group of compound **43** underwent hydrolysis

Scheme 5[a]



[a] Reagents: (i) NaH, toluene; (ii) $LiAlH_4$, THF; (iii) HCl (aq); (iv) 3,4-dihydro-2*H*-pyran, pyridinium *p*-toluenesulfonate; (v) Zn, $BrCH_2CO_2Me$; (vi) Tf_2O, pyridine; (vii) *t*-BuOK, *n*-PrSH, DMF; (viii) $BrCH_2CO_2Me$, K_2CO_3, DMF; (ix) methanesulfonyl chloride, Et_3N; (x) *t*-BuOK, thiols, DMF; (xi) pyridinium *p*-toluenesulfonate, MeOH; (xii) NaOH.

Scheme 6[a]

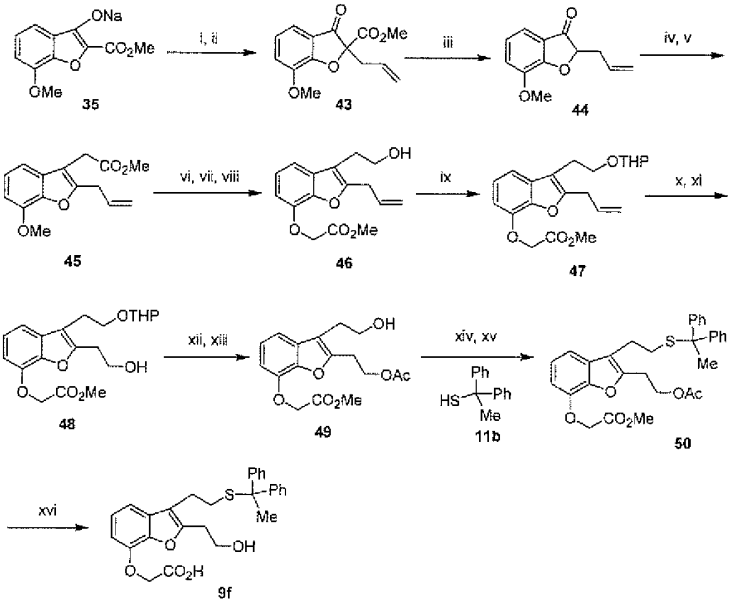


[a] Reagents: (i) diethanol amine, EtOH; (ii) H_2O_2, MeOH; (iii) *m*-CPBA; (iv) NaOH.

and decarboxylation under acidic condition. The side chain at the 3-position of benzofuran was introduced using Reformatski reaction, and the dehydration of the Reformatski product was performed using *p*-toluenesulfonic acid. After cleaving the methyl protection of the phenol group at the 7-position of **45** using BBr_3, the methyl ester was reduced to the alcohol by $LiAlH_4$. Then, the oxyacetic α-chain moiety was introduced selectively at the 7-position by treating with methyl bromoacetate. The primary alcohol of **46** was protected with THP, followed by cleavage of the olefin at the 2-position of **47** using OsO_4–$NaIO_4$. The alcohol of the 2-position side chain of compound **48** was protected with acetyl group, and the THP group was removed. After the coupling with thiol **11b** via the mesylate of **49**, compound **9f** was obtained by hydrolysis of the methyl ester and the acetyl group.

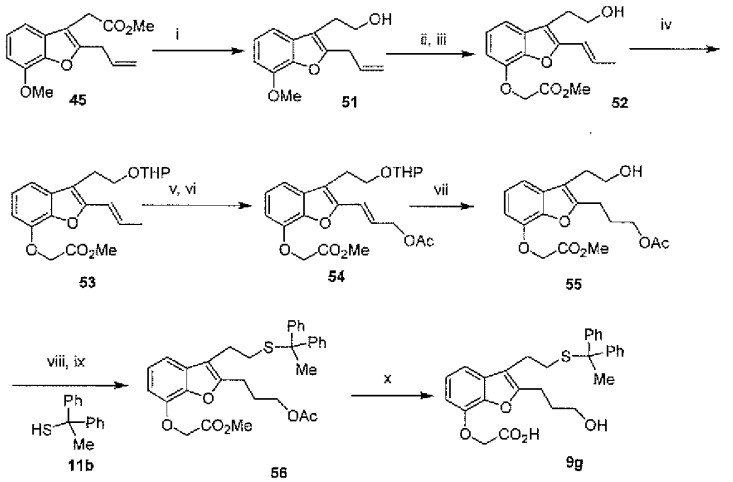
The preparation of **9g** is described in Scheme 8. We planned to introduce a hydroxyl group by using hydroboration of the olefin. After the reduction of the methyl ester on the side chain of compound **45** to alcohol **51** by $LiAlH_4$, the methyl protection of the phenol group at 7-position was removed using *n*-PrSK instead of BBr_3, which was accompanied by double bond isomerization of the olefin at the 2-position. Then, we changed the original plan by introducing the hydroxyl group via bromination at the allylic position. We isolated **52** after the introduction of the oxyacetic α-chain moiety at the

Scheme 7[a]



[a] Reagents: (i) allyl bromide; (ii) toluene, reflux; (iii) H_2SO_4, *t*-BuOH; (iv) Zn, $BrCH_2CO_2Me$; (v) *p*-toluenesulfonic acid; (vi) BBr_3, CH_2Cl_2; (vii) $LiAlH_4$, THF; (viii) $BrCH_2CO_2Me$, K_2CO_3, DMF; (ix) 3,4-dihydro-2*H*-pyran, *p*-toluenesulfonic acid; (x) OsO_4, $NaIO_4$; (xi) $NaBH_4$, THF; (xii) Ac_2O, pyridine; (xiii) HCl, MeOH; (xiv) methanesulfonyl chloride, Et_3N; (xv) *t*-BuOK, **11b**, DMF; (xvi) NaOH.

Scheme 8[a]



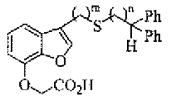
[a] Reagents: (i) $LiAlH_4$, THF; (ii) *t*-BuOK, *n*-PrSH, DMF; (iii) $BrCH_2CO_2Me$, K_2CO_3, DMF; (iv) 3,4-dihydro-2*H*-pyran, pyridinium *p*-toluenesulfonate; (v) *N*-bromosuccinimide, AIBN; (vi) AcOK, DMF; (vii) H_2, 10% Pd/C, MeOH; (viii) methanesulfonyl chloride, Et_3N; (ix) NaH, **11b**, DMF; (x) NaOH.

7-position by treating with methyl bromoacetate. After THP protection of the primary alcohol on the side chain, the allylic position on the side chain of **53** was brominated by *N*-bromosuccinimide and compound **54** was obtained by treatment with potassium acetate. The double bond on the side chain was reduced by catalytic hydrogenation, which was accompanied by removal of the THP group. After coupling with thiol **11b** via mesylate of **55**, compound **9g** was obtained by hydrolysis of the methyl ester and the acetyl group.

Pharmacology

All compounds synthesized were evaluated as the sodium salt, diethanolamine salt, or free acid. Compounds synthesized were evaluated in terms of inhibition of aggregation in human platelet rich plasma (PRP) induced by the P2Y receptor agonist adenosine diphosphate (ADP) or by a stable TXA_2 agonist (U46619). To confirm the mechanistic profile of these compounds, we also performed receptor binding assays in the

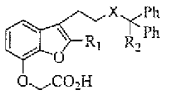
Table 1. In Vitro Activities of Benzofuran Sulfides



compd	m	n	antiaggregatory activity IC_{50} (μM)[a] ADP[b]	antiaggregatory activity IC_{50} (μM)[a] U46619[c]	receptor affinty K_i (μM) IP	receptor affinty K_i (μM) TP
8a	1	0	>100	>100	4.7 ± 1.4	5.1 ± 0.4
8b	1	1	19.9 ± 6.4	17.3 ± 0.6	0.68 ± 0.07	2.5 ± 0.1
8c	1	2	12.8 ± 3.2	10.0 ± 3.6	0.58 ± 0.02	3.1 ± 0.2
8d	2	0	5.9 ± 1.8	0.56 ± 0.02	0.41 ± 0.05	0.31 ± 0.02
8e	2	1	0.73 ± 0.24	0.51 ± 0.01	0.08 ± 0.02	3.1 ± 0.2
8f	2	2	5.2 ± 1.4	5.4 ± 0.14	0.56 ± 0.09	>10
8g	3	0	1.9 ± 0.2	1.6 ± 0.1	0.27 ± 0.03	3.1 ± 0.3
8h	3	1	1.7 ± 0.3	1.4 ± 0.2	0.40 ± 0.07	>15
8i	3	2	>100	>100	2.9 ± 0.5	>15

[a] IC_{50} represents the concentration that inhibits induced aggregation by 50%. [b] Inhibition of platelet aggregation induced by ADP (5 μM) in human platelet rich plasma. [c] Inhibition of platelet aggregation induced by U46619 (2μM) in human platelet rich plasma.

Table 2. In Vitro Activities of 2-Substituted Benzofuran Sulfides



compd	R_1	R_2	X	antiaggregatory activity IC_{50} (μM)[a] ADP[b]	antiaggregatory activity IC_{50} (μM)[a] U46619[c]	receptor affinty K_i (μM) IP	receptor affinty K_i (μM) TP
8d	H	H	S	5.9 ± 1.8	0.56 ± 0.02	0.41 ± 0.05	0.31 ± 0.02
8j	H	Me	S	8.1 ± 1.1	0.58 ± 0.03	0.57 ± 0.07	0.026 ± 0.001
9a	CH_2OH	H	S	3.3 ± 0.6	0.46 ± 0.08	0.75 ± 0.07	0.088 ± 0.012
9b	CH_2OH	Me	S	2.2 ± 0.4	0.17 ± 0.01	0.53 ± 0.07	0.0045 ± 0.0002
9c[d]	CH_2OH	Et	S	56 ± 8	3.8 ± 0.8	3.40 ± 0.15	0.180 ± 0.01
9d[d]	CH_2OH	*n*-Pr	S	>100	56 ± 2	>2.3	>5.9
9e	CH_2OH	CF_3	S	1.5 ± 0.2	0.52 ± 0.01	0.76 ± 0.19	0.150 ± 0.04
9f	$(CH_2)_2OH$	Me	S	0.71 ± 0.07	0.54 ± 0.01	0.21 ± 0.01	0.078 ± 0.008
9g	$(CH_2)_3OH$	Me	S	9.0 ± 0.7	2.9 ± 0.2	5.80 ± 0.09	0.072 ± 0.003
9h	CH_2OH	Me	SO	7.9 ± 1.7	1.7 ± 0.1	>10	0.051 ± 0.012
9i	CH_2OH	Me	SO_2	3.0 ± 0.8	0.31 ± 0.09	1.90 ± 0.22	0.0043 ± 0.0004

[a] IC_{50} represents the concentration that inhibits induced aggregation by 50%. [b] Inhibition of platelet aggregation induced by ADP (5 μM) in human platelet rich plasma. [c] Inhibition of platelet aggregation induced by U46619 (2 μM) in human platelet rich plasma. [d] This compound was provided as sodium salt.

human platelet membrane fraction. These receptor binding assays were carried out by using [^{3}H]-SQ-29548 (a selective TXA_2 receptor (TP) antagonist) and [^{3}H]-APS314d sodium salt (a selective PGI_2 receptor (IP) agonist), which is one of the component of **3**. Scatchard analysis of binding of [^{3}H]-SQ-29548 revealed a single binding site (K_d = 10.2 ± 0.51 nM, B_{max} = 5.89 ± 0.62 nM/mg protein). [^{3}H]-APS314d sodium salt also had one binding site (K_d = 14.3 ± 0.51 nM, B_{max} = 6.08 ± 0.60 nM/mg protein).

Results and Discussion

We screened a wide range of ω-side chain functionality based on our work with **3**. We identified lead compound **8e**, which contains sulfide in its ω-side chain. The sulfide side chain in conjunction with the benzofuran scaffold results in a PGI_2 receptor agonist. To probe the width and depth of the ω-side chain binding pocket, various lengths of carbon chains were tested (Table 1).

In this series, compound **8e** possesses the lowest inhibitory potency of ADP-induced platelet aggregation, which was derived from its agonism at the PGI_2 receptor. Compound **8d** possesses the second lowest inhibitory potency of U46619-induced platelet aggregation, which was derived from its antagonism at the TXA_2 receptor and its agonism at the PGI_2 receptor. Agonism at PGI_2 receptor proved to be tolerated on the length of the side chain, and compounds **8b**−**h** showed inhibitory potency (induced by ADP). On the other hand, TXA_2 receptor antagonism is very sensitive to the length of the side chain. Only compound **8d** shows significant TXA_2 antagonistic property (K_i = 0.31 μM) (Table 1).

Compound **8j**, which has a diphenylethyl sulfide group at the end of its side chain, and compound **9a**, which has a hydroxymethyl group at the 2-position of benzofuran, also display TXA_2 receptor antagonistic and PGI_2 receptor agonistic properties (Table 2). This is evidence of the utility of terminal sulfide group on the ω-side chain in the search for dual prostanoids.

We investigated the influence of alkyl substitution groups at the end of the side chain on compound **9a**. The methyl analogue **9b**, ethyl analogue **9c**, *n*-propyl analogue **9d**, and trifluoromethyl analogue **9e** were synthesized. Compound **9b** shows excellent potency as both a PGI_2 receptor agonist and a TXA_2 receptor

Table 3. Solubility of Compound **9b** with Diethanolamine Salt and Sodium Salt

salt form of **9b**	solubility (mg/mL) in distilled H_2O	saline	5% xylitol
diethanolamine salt (**7**)	>30	<0.5[a]	10
sodium salt	10	<0.5	1

[a] The compound was precipitated as a sodium salt.

antagonist. Other compounds were not as potent as **9b** at these receptors. Compound **9b** is the best dual prostanoid in this series.

Next we checked the influence of carbon chain length of the hydroxymethyl group attached to the 2-position on the benzofuran ring, which was designed to correspond to the 11-position hydroxyl group of PGI_2. Compound **8j**, which lacks the hydroxymethyl group, has almost the same agonist potency as compound **9b** at the PGI_2 receptor, but it is less potent as a TXA_2 receptor antagonist. In contrast, compound **9f**, which bears a hydroxyethyl group instead of hydroxymethyl, is more potent than compound **9b** as a PGI_2 receptor agonist but is also less potent as a TXA_2 receptor antagonist. The hydroxypropyl-bearing compound **9g** is less potent in both properties.

We also tested oxidized forms of the sulfide in compound **9b**. The sulfoxide analogue **9h** and the sulfone analogue **9i** were synthesized. Compound **9h** completely loses efficacy at the PGI_2 receptor and is a pure TXA_2 receptor antagonist. Compound **9i** has almost the same potency as compound **9b** as a TXA_2 receptor antagonist, but its potency as a PGI_2 receptor agonist is less than that of compound **9b**.

In the next study, we examined the pharmacological profile of compound **9b** in terms of its antiplatelet effects. Compound **9b** is a novel compound having potent TXA_2 receptor antagonistic activity together with a moderate PGI_2 receptor agonist activity. In fact, compound **9b** shows 117-fold higher affinity compared to TP receptor than to IP receptor, as evidenced by the K_d values determined in binding assays using human platelet membrane.

To eliminate the effect of DMSO in pharmacological experiments, we made the sodium salt and the diethanolamine salt and compared the solubility of these salts. (Table 3). Both salts dissolve well in distilled water. Compound **9b** having two aromatic rings at the end of the side chain, however, is highly lipophilic, so sodium salt does not dissolve well in saline and 5% xylitol. Otherwise, the diethanolamine salt of compound **9b** (**7**), which could be easily crystallized from ethanol, showed excellent solubility in the 5% xylitol. In saline, neither salt dissolved more than 0.5 mg/mL, since the diethanolamine salt turned into the sodium salt. From these results, we found out that compound **7** with 5% xylitol is the practical formula for pharmacological experiments.

The TXA_2 receptor antagonistic and PGI_2 receptor agonistic activities of compound **7** were examined in in vitro platelet aggregation (Table 4). Compound **7** exhibited inhibitory effects on the ADP and U46619-induced aggregation. The IC_{50} value of inhibitory effects on the ADP-induced aggregation was about 18-fold less potent than that on the U46619-induced aggregation. A similar tendency was observed with the TXA_2 receptor

Table 4. Effects of Compounds **7**, **3**, **4**, and SQ-29548 on in Vitro Platelet Aggregation in Human PRP[a]

aggregating agent	IC_{50} (nM) **7**	**3**	**4**	SQ-29548
U46619	120 ± 30	7.6 ± 0.7	170 ± 11	21 ± 3
ADP	2200 ± 320	5.7 ± 1.0	170 ± 3	>10000

[a] The platelet aggregation was induced by U46619 (4 μM) or by ADP (5 μM). Values are the mean ± SE of three to four determinations.

antagonist SQ-29548. On the other hand, the IC_{50} value of inhibitory effects by the selective PGI_2 receptor agonists **3** and compound **4** were almost the same on both ADP and U46619 induced platelet aggregation. These results are consistent with the fact that compound **7** has PGI_2 receptor agonistic activity in addition to the TXA_2 receptor antagonistic activity. This is also supported by the evidence that these results, together with the results of binding assay, indicate that the PGI_2 receptor agonistic activity of compound **7** is relatively weaker than its TXA_2 receptor antagonistic activity.

To confirm the antithrombotic character of compound **7**, we tried ex vivo platelet aggregation experiment by monitoring blood pressure and heart rate. The inhibitory effects observed with cynomolgus monkey PRP were IC_{50} = 3.7 ± 1.5 μM (induced by 5 μM of ADP) and IC_{50} = 0.14 ± 0.20 μM (induced by 600 μM of arachidonic acid). Since these data were quite similar to those observed with human PRP, the ex vivo experiment was carried out in monkeys (Figure 1). In the ex vivo experiment, the arachidonic acid induced aggregation was completely inhibited by the infusion of compound **7** even at the lowest dose examined (3 $\mu g\ kg^{-1}\ min^{-1}$). Infusion of **7** also caused dose-dependent inhibitions of the ADP-induced platelet aggregation, which was completely inhibited at the highest dose examined (30 $\mu g\ kg^{-1}\ min^{-1}$). In the similar manner, the IP receptor agonist **4** showed dose-dependent inhibition of ADP-induced platelet aggregation but did not show clear inhibition of arachidonic acid induced aggregation. Furthermore, compound **4** showed a dose-dependent decrease in blood pressure in the examined dose range, and the decrease was accompanied by an increase in heart rate. The antiplatelet activity of compound **4** is linked to its potent vasodilation. On the other hand, compound **7** does not show any significant change in blood pressure and heart rate even at the highest dose examined (30 $\mu g\ kg^{-1}\ min^{-1}$). These results suggest that the antiplatelet activity of compound **7** is not related to vasodilation.

In conclusion, a variety of benzofuran-7-oxyacetic acid analogues with many kinds of 2- and 3-position side chains were prepared by versatile synthetic routes, which allow large-scale preparation. Among the benzofuran analogues synthesized, we found the first dual-acting benzofuran **7** possessing a potent TXA_2 antagonism and a moderate PGI_2 agonism. The TP receptor antagonistic and IP receptor agonistic activities of compound **7** are also demonstrated in in vitro platelet aggregation induced by various platelet stimulating agents. The ex vivo experiment of compound **7** illustrated the beneficial properties of PGI_2 stable mimetics in terms of avoiding hypotensive side effects. Remarkably, compound **7** was found to be a promising

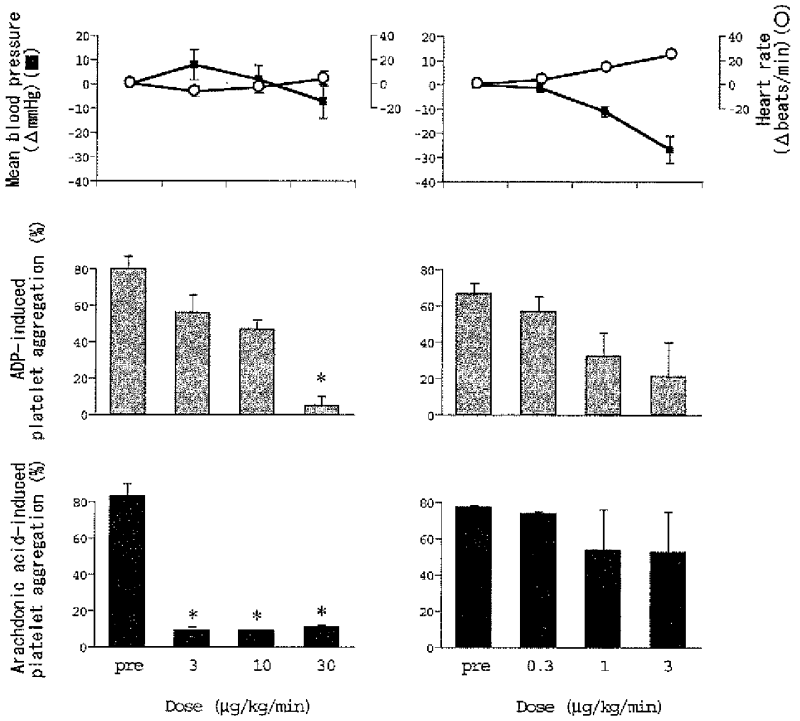


Figure 1. Effects of compound **7** (left) and compound **4** (right) on blood pressure, heart rate, and ex vivo platelet aggregation in monkey. Drugs were infused for 30 min at each of the doses in a dose-escalation manner. Platelet aggregation was induced by ADP (10 μM) or by arachidonic acid (600 μM). Data are expressed as the mean ± SE of three to four determinations: (*) significantly different from the vehicle group ($p < 0.01$).

candidate as novel medicine in antithrombotic and cardiovascular fields. Further experimental evaluations are now in progress on pharmacological properties.

Experimental Section

All of the reagents and solvents used were reagent grade or were purified by standard methods before use. All melting points were obtained with Yanagimoto melting point apparatus and are uncorrected. Infrared (IR) spectra were measured on a JASCO FT/IR-410 infrared spectrophotometer. ^{1}H NMR spectra were recorded with Varian Gemini-2000 spectrometer (300 MHz) with tetramethylsilane as an internal standard. Low mass spectra (MS) or high-resolution mass spectra (HR-MS) were obtained with a JEOL JMS-DX303 mass spectrometer. The fast atom bombardment mass spectra (FAB-MS) were obtained by using glycerol as the matrix. Optical rotations were determined at the sodium D line using a HORIBA high-sensitivity polarimeter. Elemental analysis was performed by Toray Research Center. Thin-layer chromatography was performed on precoated TLC plates (silica gel 60 F-254, layer thickness of 0.25 mm, or DIOL F-254s) manufactured by E. Merck. Silica gel column chromatography was performed on silica gel 60 (0.063−0.200 mm) manufactured by E. Merck. Synthetic reagents were purchased from Aldrich (Milwaukee, WI), Kanto Kagaku Co. (Tokyo, Japan), TCI (Tokyo, Japan), and Sigma Chemical Co. (St. Louis, MO). Anhydrous tetrahydrofuran, methanol, dichloromethane, dimethylformamide, and pyridine were purchased from Kanto Kagaku Co. (Tokyo, Japan). The active isomer of beraprost, [^{3}H]APS-314d sodium, and [^{3}H]SQ-29548 were synthesized at Daiichi Pure Chemicals (Tokyo, Japan). SQ-29548 and U46619 were purchased from Cayman Chemical (MI), ADP from Sigma (MO), 3.8% sodium citrate was purchased from Yamanouchi Pharmaceutical (Tokyo, Japan), and a low molecular weight heparin sodium dalteparin was purchased from Kissei Pharmaceutical (Nagano, Japan).

In general, reactions were carried out in dry solvents under an argon atmosphere unless otherwise mentioned. All reactions that required anhydrous conditions were performed under argon or nitrogen, and all glassware was either oven-dried or flame-dried before use.

[3-[2-(1,1-Diphenylethylsulfanyl)ethyl]-2-hydroxymethylbenzofuran-7-yloxy]acetic Acid Diethanolamine Salt (7). To a stirred solution of **9b** (886 mg, 1.92 mmol) in EtOH (10 mL) was added diethanolamine (230 mg, 2.19 mmol) in EtOH (3 mL), which was stood at room temperature. The resulting crystals were collected and were washed with small amount of cold EtOH to afford **7** (776 mg, 71%). Colorless plates, mp 181.5 °C; ^{1}H NMR (D_2O) δ 1.95 (3H, s), 2.61 (4H, m), 3.36 (4H, bs), 4.01 (4H, bs), 4.61 (2H, s), 4.71 (2H, s), 6.79 (2H, m), 7.04 (1H, m), 7.18 (6H, bs), 7.36 (4H, bs). Anal. ($C_{31}H_{37}NO_7S$) C, H, N, S.

General Procedure for Hydrolysis of Methyl Ester. [3-Benzhydrylsulfanylmethylbenzofuran-7-yloxy]acetic Acid (8a). To a stirred solution of **23a** (73 mg, 0.17 mmol) in MeOH (3.0 mL) was added 1.0 N NaOH (aq) (0.010 mL, 0.49 mmol) and stirred at room temperature for 1 h. The reaction mixture was poured into 1 N HCl (aq) and was extracted with AcOEt. The organic layer was sequentially washed with water and brine and dried over Na_2SO_4. Removal of the solvent gave an oily residue, which was recrystallized from AcOEt/*n*-hexane to afford **8a** (70 mg, 99%). White powder, mp 135.5−137 °C; ^{1}H NMR ($CDCl_3$) δ 3.64 (2H, d, J = 1.0 Hz), 4.92 (2H, s), 5.00 (1H, s), 6.83 (1H, d, J = 6.0 Hz), 7.14−7.39 (13H, m); IR (KBr) 1742 cm^{-1} (COOH); LR-MS (EI) 404 (M^+). Anal. ($C_{24}H_{20}O_4S$) C, H, N, S.

[3-(2,2-Diphenylethylsulfanylmethyl)benzofuran-7-yloxy]acetic Acid (8b). Compound **8b** (70%) was prepared from **23b**. White powder, mp 91.0−93.5 °C; ^{1}H NMR ($CDCl_3$) δ 3.13 (2H, d, J = 8.0 Hz), 3.65 (2H, s), 4.11 (1H, t, J = 8.0 Hz), 4.92 (2H, s), 6.83 (1H, d, J = 8.0 Hz), 7.13−7.31 (13H, m); IR (KBr) 1738 cm^{-1} (COOH); LR-MS (EI) 418 (M^+). Anal. ($C_{25}H_{22}O_4S$) C, H, N, S.

[3-(3,3-Diphenylpropylsulfanylmethyl)benzofuran-7-yloxy]acetic Acid (8c). Compound **8c** (96%) was prepared from **23c**. White powder, mp 154.5−155.5 °C; ^{1}H NMR (DMSO-d_6) δ 2.15−2.35 (4H, m), 3.82 (2H, s), 4.02 (1H, t, J = 6.0 Hz), 4.85 (2H, s), 6.84 (1H, d, J = 8.0 Hz), 7.10−7.30 (13H, m), 7.70 (1H, s); IR (KBr) 1748 cm^{-1} (COOH); LR-MS (EI) 432 (M^+). Anal. ($C_{26}H_{24}O_4S$) C, H, N, S.

[3-(2-Benzhydrylsulfanylethyl)benzofuran-7-yloxy]acetic Acid (8d). Compound **8d** (97%) was prepared from **23d**. Colorless prisms, mp 139−141 °C; ^{1}H NMR ($CDCl_3$) δ 2.57 (2H, t, J = 8.0 Hz), 2.88 (2H, t, J = 8.0 Hz), 4.90 (2H, s), 5.17 (1H, s), 6.78 (1H, dd, J = 1.0, 8.0 Hz), 6.97 (1H, dd, J = 1.0, 8.0 Hz), 7.09 (1H, t, J = 8.0 Hz), 7.21−7.41 (11H, m); IR (KBr) 1738 cm^{-1} (COOH); LR-MS (EI) 418 (M^+). Anal. ($C_{25}H_{22}O_4S$) C, H, N, S.

{3-[2-(2,2-Diphenylethylsulfanyl)ethyl]benzofuran-7-yloxy}acetic Acid (8e). Compound **8e** (78%) was prepared from **23e**. Colorless prisms, mp 116−118 °C; ^{1}H NMR ($CDCl_3$) δ 2.74−2.79 (2H, m), 2.87−2.91 (2H, m), 3.24 (2H, d, J = 8.0 Hz), 4.16 (1H, t, J = 8.0 Hz), 4.91 (2H, s), 6.82 (1H, dd, J = 2.0, 7.0 Hz), 7.12−7.32 (12H, m), 7.40 (1H, m); IR (KBr) 1744 cm^{-1} (COOH); LR-MS (EI) 432 (M^+). Anal. ($C_{26}H_{24}O_4S$) C, H, N, S.

{3-[2-(3,3-Diphenylpropylsulfanylmethyl)ethyl]benzofuran-7-yloxy}acetic Acid (8f). Compound **8f** (97%) was prepared from **23f**. White powder, mp 61−62 °C; ^{1}H NMR ($CDCl_3$) δ 2.29−2.36 (2H, m), 2.49 (2H, dd, J = 7.0, 9.0 Hz), 2.78−2.89 (4H, m), 4.07 (1H, t, J = 8.0 Hz), 4.91 (2H, s), 6.82 (1H, dd, J = 2.0, 7.0 Hz), 7.11−7.30 (12H, m), 7.44 (1H, m); IR (KBr) 1734 cm^{-1} (COOH); LR-MS (EI) 446 (M^+). Anal. ($C_{27}H_{26}O_4S$) C, H, N, S.

[3-(3-Benzhydrylsulfanylpropyl)benzofuran-7-yloxy]acetic Acid (8g). Compound **8g** (85%) was prepared from **23g**. Colorless needles, mp 116−118 °C; ^{1}H NMR ($CDCl_3$) δ 1.93 (2H, sept, J = 7.0 Hz), 2.46 (2H, t, J = 7.0 Hz), 2.72 (2H, t, J = 7.0 Hz), 4.91 (2H, s), 5.14 (1H, s), 6.81 (1H, d, J = 7.0 Hz), 7.10−7.42 (13H, m); IR (KBr) 1738 cm^{-1} (COOH); LR-MS (EI) 432 (M^+). Anal. ($C_{26}H_{24}O_4S$) C, H, N, S.

{3-[3-(2,2-Diphenylethylsulfanyl)propyl]benzofuran-7-yloxy}acetic Acid (8h). Compound **8h** (84%) was prepared from **23h**. Colorless needles, mp 94 °C; ^{1}H NMR ($CDCl_3$) δ 1.94 (2H, quint, J = 7.0 Hz), 2.51 (2H, t, J = 7.0 Hz), 2.72 (2H, t, J = 7.0 Hz), 3.21 (2H, d, J = 8.0 Hz), 4.17 (1H, t, J = 8.0 Hz), 4.92 (2H, s), 6.81 (1H, d, J = 7.0 Hz), 7.10−7.40 (13H, m); IR (KBr) 1740 cm^{-1} (COOH); LR-MS (EI) 446 (M^+). Anal. ($C_{27}H_{26}O_4S$) C, H, N, S.

{3-[3-(3,3-Diphenylpropylsulfanyl)propyl]benzofuran-7-yloxy}acetic Acid (8i). Compound **8i** (85%) was prepared from **23i**. Colorless prisms, mp 94 °C; ^{1}H NMR ($CDCl_3$) δ 1.92 (2H, quint, J = 7.0 Hz), 2.32 (2H, q, J = 7.0 Hz), 2.45 (2H, t, J = 7.0 Hz), 2.54 (2H, t, J = 7.0 Hz), 2.74 (2H, t, J = 7.0 Hz), 4.08 (1H, t, J = 8.0 Hz), 4.91 (2H, s), 6.81 (1H, d, J = 7.0 Hz), 7.10−7.40 (13H, m); IR (KBr) 1738 cm^{-1} (COOH); LR-MS (EI) 460 (M^+). Anal. ($C_{28}H_{28}O_4S$) C, H, N, S.

{3-[2-(1,1-Diphenylethylsulfanyl)ethyl]benzofuran-7-yloxy}acetic Acid (8j). Compound **8j** (70%) was prepared from **23j**. Colorless prisms, mp 117 °C; ^{1}H NMR ($CDCl_3$) δ 2.11 (3H, s), 2.60 (2H, m), 2.70 (2H, m), 4.89 (2H, s), 6.79 (1H, dd, J = 1.0, 7.5 Hz), 6.99 (1H, dd, J = 1.0, 7.5 Hz), 7.10 (1H, t, J = 7.5 Hz), 7.18−7.33 (6H, m), 7.38−7.43 (5H, m); IR (KBr) 1740 cm^{-1} (COOH); LR-MS (EI) 432 (M^+). Anal. ($C_{26}H_{24}O_4S$) C, H, N, S.

[3-(2-Benzhydrylsulfanylethyl)-2-hydroxymethylbenzofuran-7-yloxy]acetic Acid (9a). Compound **9a** (80%) was prepared from **41a**. Colorless plates, mp 144 °C; ^{1}H NMR ($CDCl_3$) δ 2.66 (2H, t, J = 7.0 Hz), 2.88 (2H, t, J = 7.0 Hz), 4.65 (2H, s), 4.85 (2H, s), 5.04 (1H, s), 6.77 (1H, d, J = 8.0 Hz), 6.92 (1H, d, J = 8.0 Hz), 7.07 (1H, t, J = 8.0 Hz), 7.19−7.36 (10H, m); IR (KBr) 1736 cm^{-1} (COOH); LR-MS (EI) 448 (M^+). Anal. ($C_{26}H_{24}O_5S$) C, H, N, S.

[3-[2-(1,1-Diphenylethylsulfanyl)ethyl]-2-hydroxymethylbenzofuran-7-yloxy]acetic Acid (9b). Compound **9b** (89%) was prepared from **41b**. Colorless plates, mp 140.5 °C; ^{1}H NMR ($CDCl_3$) δ 2.00 (3H, s), 2.59 (2H, m), 2.67 (2H, m), 4.62 (2H, s), 4.85 (2H, s), 6.76 (1H, d, J = 7.0 Hz), 6.92 (1H, d, J = 7.0 Hz), 7.06 (1H, t, J = 7.0 Hz), 7.16−7.28 (6H, m), 7.33 (4H, m); IR (KBr) 1742 cm^{-1} (COOH); LR-MS (FAB, negative) 461 (M^+ − H). Anal. ($C_{27}H_{26}O_5S$) C, H, N, S.

[3-[2-(1,1-Diphenylpropylsulfanyl)ethyl]-2-hydroxymethylbenzofuran-7-yloxy]acetic Acid Sodium Salt (9c). Compound **9c** (83%) was prepared from **41c**. White powder, mp 193 °C; ^{1}H NMR (D_2O) δ 0.52 (3H, bs), 2.07 (2H, bs), 2.20 (2H, m), 2.35 (2H, m), 4.34 (2H, s), 4.46 (2H, s), 6.50 (1H, m), 6.58 (1H, m), 6.80 (1H, m), 6.97 (6H, bs), 7.10 (4H, bs); LR-MS (FAB, negative) 475 (M^+ − Na). Anal. ($C_{28}H_{27}O_5SNa$) C, H, N, S.

[3-[2-(1,1-Diphenylbutylsulfanyl)ethyl]-2-hydroxymethylbenzofuran-7-yloxy]acetic Acid Sodium Salt (9d). Compound **9d** (76%) was prepared from **41d**. Colorless needles, mp 178 °C; ^{1}H NMR (D_2O) δ 0.48 (3H, m), 0.93 (2H, m), 2.02 (2H, m), 2.22 (4H, m), 4.21 (2H, bs), 4.36 (2H, s), 6.35 (1H, s), 6.48 (1H, m), 6.67 (1H, m), 6.90 (6H, m), 7.09 (4H, m); LR-MS (FAB, positive) 513 (M^+ + H). Anal. ($C_{29}H_{29}O_5SNa$) C, H, N, S.

{2-Hydroxymethyl-3-[2-(2,2,2-trifluoro-1,1-diphenylethylsulfanyl)ethyl]benzofuran-7-yloxy}acetic Acid (9e). Compound **9e** (93%) was prepared from **41e**. Colorless prisms, mp 129−131 °C; ^{1}H NMR ($CDCl_3$) δ 2.61 (2H, m), 2.74 (2H, m), 4.64 (2H, s), 4.89 (2H, s), 6.77 (1H, dd, J = 0.8, 8.0 Hz), 6.83 (1H, dd, J = 0.8, 8.0 Hz), 7.06 (1H, t, J = 8.0 Hz), 7.24−7.29 (6H, m), 7.32−7.39 (4H, m); IR (KBr) 1738 cm^{-1} (COOH); LR-MS (EI) 516 (M^+). Anal. ($C_{27}H_{23}F_3O_5S$) C, H, N, S.

[3-[2-(1,1-Diphenylethylsulfanyl)-ethyl]-2-(2-hydroxyethyl)benzofuran-7-yloxy]acetic Acid (9f). Compound **9f** was prepared from **50** (87%). Colorless prisms, mp 129−131 ° C; ^{1}H NMR (CD_3OD) δ 2.01 (3H, s), 2.56 (2H, m), 2.69 (2H, m), 2.89 (2H, t, J = 6.9 Hz), 3.83 (2H, t, J = 6.9 Hz), 4.83 (2H, s), 6.74 (1H, dd, J = 7.8, 1.0 Hz), 6.81 (1H, dd, J = 7.8, 1.0 Hz), 7.01 (1H, t, J = 7.8 Hz), 7.15−7.40 (10H, m); IR (KBr) 1742 cm^{-1} (COOH); LR-MS (EI) 476 (M^+). Anal. ($C_{28}H_{23}O_5S$) C, H, N, S.

[3-[2-(1,1-Diphenylethylsulfanyl)ethyl]-2-(3-hydroxypropyl)benzofuran-7-yloxy]acetic Acid (9g). Compound **9g** was prepared from **55** (84%). Colorless prisms, mp 152−153 ° C; ^{1}H NMR (CD_3OD) δ 1.90 (2H, m), 2.00 (3H, s), 2.54 (2H, m), 2.66 (2H, m), 2.74 (2H, t, J = 7.5 Hz), 3.57 (2H, t, J = 6.4 Hz), 4.84 (2H, s), 6.73 (1H, dd, J = 7.8, 1.0 Hz), 6.81 (1H, dd, J = 7.8, 1.0 Hz), 7.01 (1H, t, J = 7.8 Hz), 7.15−7.39 (10H, m); IR (KBr) 1748 cm^{-1} (COOH); LR-MS (EI) 490 (M^+). Anal. ($C_{29}H_{30}O_5S$) C, H, N, S.

{3-[2-(Diphenylethanesulfonyl)ethyl]-2-hydroxymethylbenzofuran-7-yloxy}acetic Acid (9i). Compound **9i** (84%) was prepared from **42**. Colorless prisms, mp 156−158 °C; ^{1}H NMR (CD_3OD) δ 2.20 (3H, s), 2.96−3.10 (4H, m), 4.60 (2H, s), 4.83 (2H, s), 6.80 (1H, dd, J = 0.8, 8.0 Hz), 6.86 (1H, dd, J = 0.8, 8.0 Hz), 7.07 (1H, t, J = 8.0 Hz), 7.33−7.42 (6H, m), 7.55−7.64 (4H, m); IR (KBr) 1740 cm^{-1} (COOH); LR-MS (EI) 494 (M^+). Anal. ($C_{27}H_{26}O_7S$) C, H, N, S.

{3-[2-(Diphenylethanesulfinyl)ethyl]-2-hydroxymethylbenzofuran-7-yloxy}acetic Acid (9h). To a stirred solution of **9b** (197 mg, 0.43 mmol) in MeOH (3 mL) was added 30% H_2O_2 (0.5 mL), and the reaction mixture was stirred at room temperature for 4.5 h. The reaction mixture was poured into 1 N HCl (aq) and was extracted with AcOEt. The organic layer was sequentially washed with water and brine and dried over $MgSO_4$. Removal of the solvent gave an oily residue, which was recrystallized from AcOEt/*n*-hexane to afford **9h** (164 mg, 81%). Colorless prisms, mp 131−132 °C; ^{1}H NMR (CD_3OD) δ 1.94 (3H, s), 2.51 (2H, t, J = 7.4 Hz), 3.00−3.10 (2H, m), 4.62 (2H, s), 4.86 (2H, s), 6.82 (1H, dd, J = 0.8, 7.9 Hz), 6.83 (1H, dd, J = 0.8, 7.9 Hz), 7.04 (1H, t, J = 7.9 Hz), 7.20−7.42

(10H, m); IR (KBr) 1745 cm^{-1} (COOH); LR-MS (EI) 478 (M^+). Anal. ($C_{27}H_{26}O_6S$) C, H, N, S.

1,1-Diphenylpropane-1-ol (10c). To a stirred solution of benzophenone (3.50 g, 19.2 mmol) in THF (30 mL) was added 1.0 M EtMgBr in THF (24.5 mL, 24.5 mmol), and the mixture was stirred at 0 °C for 5.0 h. The reaction mixture was poured into 5% citric acid (aq) and was extracted with ethyl acetate. The combined organic layer was sequentially washed with water and brine and was dried over $MgSO_4$. Removal of the solvent afforded an oily residue, which was purified by silica gel chromatography (AcOEt/*n*-hexane = 1/4) to afford **10c** (635 mg, 16%). Colorless oil; ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, J = 7.5 Hz), 2.08 (1H, s), 2.32 (2H, q, J = 7.5 Hz), 7.19−7.34 (6H, m), 7.39−7.44 (4H, m); LR-MS (EI) 212 (M^+).

1,1-Diphenylbutane-1-ol (10d). By the procedure used in **10c**, compound **10d** (80%) was prepared from benzophenone and *n*-PrMgBr. Colorless oil; ^1H NMR ($CDCl_3$) δ 0.93 (3H, t, J = 7.0 Hz), 1.30 (2H, m), 2.09 (1H, s), 2.26 (2H, m), 7.19−7.33 (6H, m), 7.39−7.43 (4H, m); LR-MS (EI) 226 (M^+).

2,2,2-Trifluoro-1,1-diphenylethanol (10e). By the procedure used in **10c**, compound **10e** (96%) was prepared from trifluoroacetophenone and PhMgBr. Colorless oil; ^1H NMR ($CDCl_3$) δ 2.87 (1H, s), 7.33−7.39 (6H, m), 7.46−7.53 (4H, m); LR-MS (EI) 252 (M^+).

General Procedure for Preparation of Thiols. 1,1-Diphenylethanthiol (11b). To solution of 1,1-diphenylethane-1-ol (50 g) and Lawesson's reagent (50 g) in toluene (1300 mL) was added water (6.5 mL), and the reaction mixture was stirred at 50 °C. Water (200 mL) was added, and the resulting mixture was cooled to room temperature. The organic layer was separated and sequentially washed with saturated $NaHCO_3$ (200 mL) and brine (200 mL). The organic layer was dried over Na_2SO_4 and evaporated. The resulting oil was purified by silica gel chromatography (eluent: *n*-hexane), which afforded **11b** (25.0 g, 46%). Colorless solid; ^1H NMR ($CDCl_3$) δ 2.16 (3H, s), 2.49 (1H, s), 7.20−7.34 (6H, m), 7.41−7.45 (4H, m); LR-MS (CI) 213 ($M^+ - H$).

Diphenylmethanethiol (11a). Compound **11a** (94%) was prepared from diphenylmethanol. Colorless oil; ^1H NMR ($CDCl_3$) δ 2.27 (1H, d, J = 5.0 Hz), 5.44 (1H, d, J = 5.0 Hz), 7.20−7.45 (10H, m); LR-MS (FAB) 200 (M^+), 199 ($M^+ - H$).

1,1-Diphenylpropane-1-thiol (11c). Compound **11c** (49%) was prepared from **10c**. Colorless oil; ^1H NMR ($CDCl_3$) δ 0.86 (3H, t, J = 7.0 Hz), 2.25 (1H, s), 2.51 (2H, q, J = 7.0 Hz), 7.19−7.40 (10H, m); LR-MS (EI) 228 (M^+).

1,1-Diphenylbutane-1-thiol (11d). Compound **11d** (44%) was prepared from **10d**. Colorless oil; ^1H NMR ($CDCl_3$) δ 0.91 (3H, t, J = 7.0 Hz), 1.24 (2H, m), 1.55 (1H, s), 2.42 (2H, m), 7.16−7.40 (10H, m); LR-MS (EI) 242 (M^+).

2,2,2-Trifluoro-1,1-diphenylethanol (11e). Compound **11e** (20%) was prepared from **10e**. Colorless oil; ^1H NMR ($CDCl_3$) δ 2.86 (1H, s), 7.30−7.39 (6H, m), 7.40−7.49 (4H, m); LR-MS (EI) 268 (M^+).

2,2-Diphenylethanethiol (11f). To a stirred solution of **15** (30 mg, 0.07 mmol) in AcOH (5 mL) was added zinc powder (5 mg, 0.08 mmol), and the reaction mixture was stirred at 90 °C for 1 h. The reaction mixture was filtered, and the solvent was removed under reduced pressure. The resulting oily residue was purified by silica gel chromatography (AcOEt/*n*-hexane = 1/5) to afford **11f** (27 mg, 90%). Colorless oil; ^1H NMR ($CDCl_3$) δ 1.35 (1H, t, J = 8.0 Hz), 3.18 (2H, q, J = 8.0 Hz), 4.13 (1H, t, J = 8.0 Hz), 7.10−7.42 (10H, m); LR-MS (EI) 214 (M^+).

3,3-Diphenylpropane-1-thiol (11g). To a stirred solution of **18** (5.16 g, 15 mmol) in EtOH (50 mL) and H_2O (20 mL) was added NaOH (950 mg, 24 mmol), and the reaction mixture was refluxed for 5.5 h. The solvent was removed, and the residue was purified by silica gel chromatography (AcOEt/*n*-hexane = 1/12) to afford **11g** (2.94 g, 86%). Colorless oil; ^1H NMR ($CDCl_3$) δ 2.28−2.51 (5H, m), 4.09 (1H, t, J = 8.0 Hz), 7.15−7.32 (10H, m); LR-MS (EI) 228 (M^-).

1-Bromo-2,2-diphenylethane (13). To a stirred solution of 2,2-diphenylethanol (**12**) (10.0 g, 50 mmol) in dichloromethane (200 mL) was added Ph_3P (16.0 g, 61 mmol) and CBr_4 (25 g, 75.6 mmol). After being stirred at room temperature for 4 h, the reaction mixture was sequentially washed with saturated $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and evaporated. The resulting oil was distilled under reduced pressure to afford **13** (10.9 g, 83%). Colorless oil, bp 170−171 °C at 0.40 mmHg; ^1H NMR ($CDCl_3$) δ 3.87−4.00 (2H, m), 4.29−4.40 (1H, m), 7.00−7.50 (10H, m); LR-MS (EI) 260, 262 (M^+) (relative peak height ratio is 1:1).

2-(2,2-Diphenylethylsulfanyl)benzoxazole (14). To a stirred solution of **13** (2.03 g, 7.77 mmol) in DMF (15 mL) was added 2-mercaptobenzoxazole (1.31 g, 8.66 mmol) and K_2CO_3 (1.47 g, 10.6 mmol), and the reaction mixture was stirred at room temperature for 4 h. Saturated aqueous NH_4Cl (5 mL) was added to the reaction mixture and was extracted with AcOEt. The combined organic layer was sequentially washed with water and brine and dried over Na_2SO_4. Removal of the solvent afforded an oily residue that was purified by silica gel chromatography (AcOEt/*n*-hexane = 1/50 to 1/20) to afford **14** (694 mg, 27%). Colorless prisms, mp 89.0−90.5 °C; ^1H NMR ($CDCl_3$) δ 3.95−4.05 (2H, m), 4.42−4.52 (1H, m), 7.00−7.70 (14H, m); LR-MS (EI) 331(M^+).

Di-(2,2-diphenylethyl) Disulfide (15). To a stirred solution of **14** (110 mg, 0.33 mmol) in EtOH (5 mL) and THF (1 mL) was added 1 N NaOH (1.0 mL), and the reaction mixture was stirred at 40 °C for 4 h. Saturated aqueous NH_4Cl (5 mL) was added to the reaction mixture and was extracted with AcOEt. The combined organic layer was sequentially washed with water and brine and dried over Na_2SO_4. Removal of the solvent afforded an oily residue that was purified by silica gel chromatography (AcOEt/*n*-hexane = 1/25) to afford **15** (60 mg, 85%). Colorless oil; ^1H NMR ($CDCl_3$) δ 3.31 (4H, d, J = 8.0 Hz), 4.28 (2H, t, J = 8.0 Hz), 7.16−7.30 (20H, m); LR-MS (EI) 426 (M^+).

1-Bromo-3,3-diphenylpropane (17). To a stirred solution of 3,3-diphenylpropan-1-ol (**16**) (10.8 g, 48 mmol) and Ph_3P (15.2 g, 58 mmol) in THF (120 mL) was added *N*-bromosuccinimide (10.1 g, 57 mmol). After being stirred at 0 °C for 2 h, the reaction mixture was filtered and the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (dichloromethane) to afford **17** (13.4 g, 96%). Colorless plates, mp 35−37 °C; ^1H NMR ($CDCl_3$) δ 2.58 (2H, q, J = 7.0 Hz), 3.32 (2H, t, J = 7.0 Hz), 4.20 (1H, t, J = 7.0 Hz), 7.15−7.34 (10H, m); LR-MS (EI) 274, 276 (M^+) (relative peak height ratio is 1:1).

2-(3,3-Diphenylpropylsulfanyl)benzoxazole (18). By the procedure used in **14**, compound **18** (90%) was prepared from **17**. Colorless plates, mp 91 °C; ^1H NMR ($CDCl_3$) δ 2.61 (2H, q, J = 7.0 Hz), 3.23 (2H, t, J = 7.0 Hz), 4.20 (1H, t, J = 7.0 Hz), 7.15−7.60 (14H, m); LR-MS (EI) 345 (M^+).

3-Chloromethyl-7-methoxybenzofuran (20). To a solution of 7-methoxy-2*H*-benzofuran-3-one (**19**) (7.13 g, 43.4 mmol) and bromochloromethane (11.3 mL) in THF (200 mL) was added *n*-BuLi (1.6 M in *n*-hexane) (80 mL, 128 mmol) at −78 °C, and the reaction mixture was stirred at −78 °C for 2 h. AcOH (7.3 mL) was added to the reaction mixture, and the solvent was removed. To the resulting oil was added toluene (100 mL) and *p*-toluenesulfonic acid monohydrate (20 mg), and the mixture was stirred at 50 °C for 2 h. The reaction mixture was sequentially washed with saturated $NaHCO_3$ and brine and was dried over Na_2SO_4 and evaporated. The resulting oil was purified by silica gel chromatography (AcOEt/*n*-hexane = 1/8 then 1/4) to afford **20** (0.88 g, 10%). Colorless prisms, mp 43−44 °C; ^1H NMR ($CDCl_3$) δ 4.00 (3H, s), 4.72 (2H, d, J = 3.0 Hz), 6.80 (1H, d, J = 3.0 Hz), 6.87 (1H, d, J = 3.0 Hz), 7.11−7.27 (1H, m), 7.65 (1H, s); LR-MS (EI) 196 (M^+).

General Procedure for Coupling with Thiols. 3-Benzhydrylsulfanylmethyl-7-methoxybenzofuran (21a). To a solution of diphenylmethanethiol (**11a**) (121 mg, 0.604 mmol) in DMF (2.0 mL) was added *t*-BuOK (81 mg, 0.722 mmol) and **20** (118 mg, 0.600 mmol), and the reaction mixture was stirred at room temperature for 1 h. Saturated aqueous NH_4Cl was added to the reaction mixture and was extracted with AcOEt. The combined organic layer was sequentially washed with

water and brine and dried over Na_2SO_4. Removal of the solvent afforded an oily residue that was purified by silica gel chromatography (AcOEt/*n*-hexane = 1/20) to afford **21a** (183 mg, 85%). Colorless oil; ^{1}H NMR ($CDCl_3$) δ 3.63 (2H, s), 4.02 (3H, s), 5.00 (1H, s), 6.83 (1H, dd, J = 1.0, 8.0 Hz), 7.15−7.39 (13H, m); LR-MS (EI) 360 (M^+).

3-(2,2-Diphenylethylsulfanylmethyl)-7-methoxybenzofuran (21b). Compound **21b** (99%) was prepared from **20**. Colorless oil; ^{1}H NMR ($CDCl_3$) δ 3.12 (2H, d, J = 8.0 Hz), 3.65 (2H, s), 4.00 (3H, s), 4.11 (1H, t, J = 8.0 Hz), 6.82 (1H, d, J = 8.0 Hz), 7.13−7.29 (12H, m), 7.46 (1H, s); LR-MS (EI) 374 (M^+).

3-(3,3-Diphenylpropylsulfanylmethyl)-7-methoxybenzofuran (21c). Compound **21c** (62%) was prepared from **20**. Colorless oil; ^{1}H NMR ($CDCl_3$) δ 2.20−2.60 (4H, m), 3.74 (2H, s), 4.00 (3H, s), 3.90−4.20 (1H, m), 6.81 (1H, dd, J = 2.0, 8.0 Hz), 7.10−7.50 (12H, m), 7.47 (1H, s); LR-MS (EI) 388 (M^+).

General Procedure for Deprotection of Methyl on Phenolic Hydroxyl Group Using *n*-PrSH. 3-Benzhydrylsulfanylmethylbenzofuran-7-ol (22a). To a solution of **21a** (45 mg, 0.125 mmol) in DMF (3.0 mL) was added *t*-BuOK (47 mg, 0.42 mmol) and *n*-PrSH (0.20 mL), and the reaction mixture was stirred at 100 °C for 4 h. Saturated aqueous NH_4Cl was added to the reaction mixture and was extracted with AcOEt. The combined organic layer was sequentially washed with water and brine and dried over Na_2SO_4. Removal of the solvent afforded an oily residue that was purified by silica gel chromatography (AcOEt/*n*-hexane = 1/5) to afford **22a** (26 mg, 60%). Colorless oil; ^{1}H NMR ($CDCl_3$) δ 3.63 (2H, s), 5.02 (1H, s), 5.47 (1H, s), 6.85 (1H, dd, J = 1.0, 8.0 Hz), 7.15−7.39 (13H, m); LR-MS (EI) 346 (M^+).

3-(2,2-Diphenylethylsulfanylmethyl)benzofuran-7-ol (22b). Compound **22b** (98%) was prepared from **21b**. Colorless oil; ^{1}H NMR ($CDCl_3$) δ 3.13 (2H, d, J = 7.5 Hz), 3.65 (2H, d, J = 1.0 Hz), 4.12 (1H, t, J = 7.5 Hz), 5.26 (1H, s), 6.83−6.86 (1H, m), 7.11−7.30 (12H, m), 7.45 (1H, s); LR-MS (EI) 360 (M^+).

3-(3,3-Diphenylpropylsulfanylmethyl)benzofuran-7-ol (22c). Compound **22c** (89%) was prepared from **21c**. Colorless oil; ^{1}H NMR ($CDCl_3$) δ 2.10−2.60 (4H, m), 3.74 (2H, d, J = 1.0 Hz), 3.90−4.20 (1H, m), 5.60−6.20 (1H, s), 6.70−6.90 (1H, m), 6.90−7.40 (13H, m); LR-MS (EI) 374 (M^+).

General Procedure for Reaction with Methyl Bromoacetate. [3-Benzhydrylsulfanylmethylbenzofuran-7-yloxy]acetic Acid Methyl Ester (23a). To a solution of **22a** (67 mg, 0.19 mmol) in DMF (2.0 mL) was added methyl bromoacetate (0.18 mL) and K_2CO_3 (145 mg, 1.05 mmol), and the reaction mixture was stirred at room temperature for 1.5 h. Saturated aqueous NH_4Cl was added to the reaction mixture and was extracted with AcOEt. The combined organic layer was sequentially washed with water and brine and dried over Na_2SO_4. Removal of the solvent afforded an oily residue that was purified by silica gel chromatography (AcOEt/*n*-hexane = 1/5) to afford **23a** (73 mg, 90%). Colorless oil; ^{1}H NMR ($CDCl_3$) δ 3.63 (2H, d, J = 0.5 Hz), 3.82 (3H, s), 4.88 (2H, s), 5.00 (1H, s), 7.12−7.40 (14H, m); IR (neat) 1748 cm^{-1} (COOMe); LR-MS (EI) 418 (M^+).

[3-(2,2-Diphenylethylsulfanylmethyl)benzofuran-7-yloxy]acetic Acid Methyl Ester (23b). Compound **23b** (93%) was prepared from **22b**. Colorless oil; ^{1}H NMR ($CDCl_3$) δ 3.12 (2H, d, J = 7.5 Hz), 3.64 (2H, d, J = 1.0 Hz), 3.81 (3H, s), 4.11 (1H, t, J = 7.5 Hz), 4.88 (2H, s), 6.78 (1H, d, J = 7.0 Hz), 7.11−7.30 (12H, m), 7.47 (1H, s); IR (neat) 1760 cm^{-1} (COOMe); LR-MS (EI) 432 (M^+).

[3-(3,3-Diphenylpropylsulfanylmethyl)benzofuran-7-yloxy]acetic Acid Methyl Ester (23c). Compound **23c** (70%) was prepared from **22c**. Colorless oil; ^{1}H NMR ($CDCl_3$) δ 2.20−2.60 (4H, m), 3.78 (2H, d, J = 2.0 Hz), 3.80 (3H, s), 3.90−4.20 (1H, m), 4.86 (2H, s), 6.77 (1H, dd, J = 1.0, 7.5 Hz), 7.00−7.50 (13H, m); IR (neat) 1763 cm^{-1} (COOMe); LR-MS (EI) 446 (M^+).

[3-(2-Benzhydrylsulfanylethyl)benzofuran-7-yloxy]acetic Acid Methyl Ester (23d). By the procedure used in **21a**, compound **23d** (80%) was prepared from **28**. Colorless prisms, mp 94−95 °C; ^{1}H NMR ($CDCl_3$) δ 2.59−3.02 (4H, m), 3.79 (3H, s), 4.87 (2H, s), 5.17 (1H, s), 6.69−7.45 (14H, m); IR (KBr) 1763 cm^{-1} (COOMe); LR-MS (EI) 432 (M^+).

{3-[2-(2,2-Diphenylethylsulfanyl)ethyl]benzofuran-7-yloxy}acetic Acid Methyl Ester (23e). By the procedure used in **21a**, compound **23e** (80%) was prepared from **28**. Colorless oil; ^{1}H NMR ($CDCl_3$) δ 2.73−2.80 (2H, m), 2.85−2.92 (2H, m), 3.24 (2H, d, J = 8.0 Hz), 3.80 (3H, s), 4.17 (1H, t, J = 8.0 Hz), 4.88 (2H, s), 6.76−6.79 (1H, m), 7.12−7.33 (12H, m), 7.40 (1H, m); IR (neat) 1736 cm^{-1} (COOMe); LR-MS (EI) 446 (M^+).

{3-[2-(3,3-Diphenylpropylsulfanylmethyl)ethyl]benzofuran-7-yloxy}acetic Acid Methyl Ester (23f). By the procedure used in **21a**, compound **23f** (29%) was prepared from **28**. Colorless oil; ^{1}H NMR ($CDCl_3$) δ 2.30−2.36 (2H, m), 2.46−2.52 (2H, m), 2.76−2.89 (4H, m), 3.80 (3H, s), 4.08 (1H, t, J = 8.0 Hz), 4.87 (2H, s), 6.77 (1H, dd, J = 2.0, 7.0 Hz), 7.11−7.31 (12H, m), 7.44 (1H, m); IR (neat) 1742 cm^{-1} (COOMe); LR-MS (EI) 460 (M^+).

[3-(3-Benzhydrylsulfanylpropyl)benzofuran-7-yloxy]acetic Acid Methyl Ester (23g). By the procedure used in **21a**, compound **23g** (89%) was prepared from **33**. Colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.93 (2H, sept, J = 7.0 Hz), 2.46 (2H, t, J = 7.0 Hz), 2.72 (2H, t, J = 7.0 Hz), 3.82 (3H, s), 4.88 (2H, s), 5.13 (1H, s), 6.77 (1H, dd, J = 2.0, 6.0 Hz), 7.00−7.50 (13H, m); IR (neat) 1763 cm^{-1} (COOMe); LR-MS (EI) 446 (M^+).

{3-[3-(2,2-Diphenylethylsulfanyl)propyl]benzofuran-7-yloxy}acetic Acid Methyl Ester (23h). By the procedure used in **21a**, compound **23h** (67%) was prepared from **33**. Colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.94 (2H, quint, J = 7.0 Hz), 2.50 (2H, t, J = 7.0 Hz), 2.71 (2H, t, J = 7.0 Hz), 3.20 (2H, d, J = 8.0 Hz), 3.81 (3H, s), 4.15 (1H, t, J = 8.0 Hz), 4.89 (2H, s), 6.77 (1H, dd, J = 1.0, 7.0 Hz), 7.09−7.34 (13H, m); IR (neat) 1765 cm^{-1} (COOMe); LR-MS (EI) 460 (M^+).

{3-[3-(3,3-Diphenylpropylsulfanyl)propyl]benzofuran-7-yloxy}acetic Acid Methyl Ester (23i). By the procedure used in **21a**, compound **23i** (53%) was prepared from **33**. Colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.92 (2H, quint, J = 7.0 Hz), 2.31 (2H, q, J = 7.0 Hz), 2.45 (2H, t, J = 7.0 Hz), 2.54 (2H, t, J = 7.0 Hz), 2.74 (2H, t, J = 7.0 Hz), 3.81 (3H, s), 4.08 (1H, t, J = 8.0 Hz), 4.88 (2H, s), 6.77 (1H, d, J = 7.0 Hz), 7.05−7.35 (12H, m), 7.39 (1H, s); IR (neat) 174 cm^{-1} (COOMe); LR-MS (EI) 474 (M^+)

{3-[2-(1,1-Diphenylethylsulfanyl)ethyl]benzofuran-7-yloxy}acetic Acid Methyl Ester (23j). By the procedure used in **21a**, compound **23j** (74%) was prepared from **28**. Colorless oil; ^{1}H NMR ($CDCl_3$) δ 2.07 (3H, s), 2.61 (2H, m), 2.70 (2H, m), 3.80 (3H, s), 4.87 (2H, s), 6.75 (1H, dd, J = 1.0, 8.0 Hz), 6.97 (1H, dd, J = 1.0, 8.0 Hz), 7.08 (1H, t, J = 8.0 Hz), 7.19−7.34 (6H, m), 7.38−7.43 (5H, m); IR (neat) 1765 cm^{-1} (COOMe); LR-MS (EI) 446 (M^+).

(7-Methoxybenzofuran-3-yl)acetic Acid Methyl Ester (24). To a solution of 7-mehtoxy-3(2*H*)-benzofuranone (**19**) (1.80 g, 11.0 mmol) in xylene (40 mL) was added $Ph_3PCHCOOMe$ (4.10 g, 12.3 mmol), and the mixture was stirred at 140 °C for 18 h. The reaction mixture was cooled to room temperature, and the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (AcOEt/*n*-hexane = 1/3) to afford **24** (1.11 g, 46%). Pale-yellow oil; ^{1}H NMR ($CDCl_3$) δ 3.70 (2H, d, J = 1.0 Hz), 3.73 (3H, s), 4.01 (3H, s), 6.82 (1H, dd, J = 2.0, 7.0 Hz), 7.15 (1H, dd, J = 2.0, 7.0 Hz), 7.19 (1H, t, J = 7.0 Hz), 7.64 (1H, s); IR (KBr) 1742 cm^{-1} (COOMe); LR-MS (EI) 220 (M^+).

(7-Hydroxybenzofuran-3-yl)acetic Acid Methyl Ester (25). To a solution of **24** (5.35 g, 24.3 mmol) in dichloromethane (100 mL) was added 1.0 M BBr_3 in dichloromethane (55 mL, 55 mmol) at −78 °C, and the mixture was stirred at 0 °C for 90 min. The reaction mixture was poured into water and was extracted with dichloromethane. The combined organic layer was sequentially washed with water and brine and dried over $MgSO_4$. Removal of the solvent afforded **25** (5.00 g, 99%). Pale-brown prisms, mp 48−50 °C; ^{1}H NMR ($CDCl_3$) δ 3.71 (2H, d, J = 1.0 Hz), 3.74 (3H, s), 5.30 (1H, bs), 6.82−6.88 (1H, m), 7.10−7.17 (2H, m), 7.64 (1H, t, J = 1.0 Hz); IR (KBr) 1696 cm^{-1} (COOMe); LR-MS (EI) 206 (M^+).

3-(2-Hydroxyethyl)benzofuran-7-ol (26). To a solution of **25** (8.11 g, 39 mmol) in THF (600 mL) was added $LiAlH_4$ (1.53 g, 40 mmol) at 0 °C and was stirred at 0 °C for 6 h. The reaction mixture was poured into 1.0 N HCl and was extracted with AcOEt. The combined organic layer was sequentially washed with water and brine and dried over Na_2SO_4. The solvent was removed, and the residue was recrystallized from AcOEt to afford **26** (5.37 g, 77%). Colorless prisms, mp 113.0 °C; ^{1}H NMR ($CDCl_3$) δ 2.94 (2H, dt, J = 1.0, 6.0 Hz), 3.94 (2H, t, J = 6.0 Hz), 5.26 (1H, bs), 6.85 (1H, m), 7.12–7.14 (2H, m), 7.52 (1H, d, J = 1.0 Hz); LR-MS (EI) 178 (M^+).

[3-(2-Hydroxyethyl)benzofuran-7-yloxy]acetic Acid Methyl Ester (27). By the procedure used in **23a**, compound **27** (80%) was prepared from **26**. Pale-yellow oil; ^{1}H NMR ($CDCl_3$) δ 2.94 (2H, dt, J = 1.0, 6.0 Hz), 3.81 (3H, s), 3.93 (2H, t, J = 6.0 Hz), 4.89 (2H, s), 6.79 (1H, dd, J = 1.0, 8.0 Hz), 7.15 (1H, t, J = 8.0 Hz), 7.22 (1H, dd, J = 1.0, 8.0 Hz), 7.54 (1H, d, J = 1.0 Hz); IR (KBr) 1746 (COOMe); LR-MS (EI) 250 (M^+).

[3-(2-Methanesulfonyloxyethyl)benzofuran-7-yloxy]-acetic Acid Methyl Ester (28). To a stirred solution of **27** (4.12 g, 16.5 mmol) in CH_2Cl_2 (120 mL) was added Et_3N (3.0 mL, 21.6 mmol) and methanesulfonyl chloride (1.35 mL, 17.4 mmol) at 0 °C, and the reaction mixture was stirred at 0 °C for 3.5 h. The solvent was removed under reduced pressure, and the residue was poured into 1 N HCl and was extracted with ethyl acetate. The combined organic layer was sequentially washed with water, saturated $NaHCO_3$, water, and brine and dried over Na_2SO_4. The solvent was removed, and the residue was recrystallized from AcOEt/*n*-hexane to afford **28** (5.25 g, 97%). Colorless prisms, mp 102.0 °C; ^{1}H NMR ($CDCl_3$) δ 3.15 (2H, dt, J = 1.0, 7.0 Hz), 3.81 (3H, s), 3.92 (3H, s), 4.48 (2H, t, J = 7.0 Hz), 4.89 (2H, s), 6.79 (1H, dd, J = 1.5, 7.5 Hz), 7.17 (1H, t, J = 7.5 Hz), 7.21 (1H, dd, J = 1.5, 7.5 Hz), 7.56 (1H, s); IR (KBr) 1763 (COOMe); LR-MS (EI) 328 (M^+).

3-Allyl-7-methoxybenzofuran (29). $CeCl_3$ (5.63 g, 22.8 mmol) was dried with stirring at 150 °C for 4 h under reduced pressure. Anhydrous THF (30 mL) was added to this flask and was stirred at room temperature overnight. Allylmagnesium bromide (0.79 M in diethyl ether) (28.9 mL, 22.8 mmol) was added to this suspension at 0 °C dropwise, which afforded an orange suspension. To this suspension was added **19** (2.5 g, 22.8 mmol) at 0 °C, and the reaction mixture was stirred at 0 °C for 1.5 h. The reaction mixture was poured into water (200 mL) and AcOH (3.0 mL) and was extracted with ethyl acetate. The combined organic layer was sequentially washed with saturated $NaHCO_3$ and brine and dried over Na_2SO_4. The solvent was removed, and to the residue was added benzene (20 mL) and *p*-TsOH (50 mg). The mixture was stirred at 60 °C for 0.5 h. The reaction mixture was sequentially washed with saturated $NaHCO_3$ and brine and was dried over Na_2SO_4 and evaporated. The residue was purified by silica gel chromatography (AcOEt/*n*-hexane = 1/20) to afford **29** (2.05 g, 72%). Pale-yellow oil; ^{1}H NMR ($CDCl_3$) δ 3.40–3.44 (2H, m), 4.10 (3H, s), 5.09–5.23 (2H, m), 5.95–6.10 (1H, m), 6.80 (1H, dd, J = 3.0, 6.0 Hz), 7.13–7.16 (2H, m), 7.42 (1H, s); LR-MS (EI) 188 (M^-).

3-(7-Methoxybenzofuran-3-yl)propan-1-ol (30). To a stirred solution of **29** (2.19 g, 11.65 mmol) in THF (25 mL) was added BH_3-Me_2S (2.0 M in THF) (6.1 mL, 12.2 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 2 h. To the reaction mixture was added EtOH (20 mL), 3 N NaOH (5 mL), and 30% H_2O_2 (1.5 mL), and the resulting solution was stirred at room temperature for 30 min. The reaction mixture was poured into saturated NH_4Cl and was extracted with ethyl acetate. The combined organic layer was sequentially washed with water, saturated $NaHCO_3$, water, and brine and dried over Na_2SO_4. The solvent was removed and the residue was purified by silica gel chromatography (AcOEt/cyclohexane = 1/3) to afford **30** (1.42 g, 59%). Colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.49 (1H, bs), 1.98 (2H, m), 2.77 (2H, dt, J = 1.0, 8.0 Hz), 3.74 (2H, t, J = 6.0 Hz), 4.01 (3H, s), 6.81 (1H, m), 7.16 (2H, m), 7.44 (1H, s); LR-MS (EI) 206 (M^+).

3-(3-Hydroxypropyl)benzofuran-7-ol (31). By the procedure used in **25**, compound **31** (90%) was prepared from **30**. Colorless prisms, mp 101.0–101.5 °C; ^{1}H NMR ($CDCl_3$) δ 1.90 (2H, quint, J = 7.0 Hz), 2.72 (2H, t, J = 7.0 Hz), 3.62 (2H, t, J = 7.0 Hz), 6.70 (1H, dd, J = 2.0, 7.0 Hz), 7.03 (2H, m), 7.49 (1H, s); LR-MS (EI) 192 (M^+).

[3-(3-Hydroxypropyl)benzofuran-7-yloxy]acetic Acid Methyl Ester (32). By the procedure used in **23a**, compound **32** (83%) was prepared from **31**. Colorless powder, mp 72–73 °C; ^{1}H NMR ($CDCl_3$) δ 1.98 (2H, quint, J = 6.0 Hz), 2.78 (2H, t, J = 6.0 Hz), 3.74 (2H, t, J = 6.0 Hz), 3.82 (3H, s), 4.89 (2H, s), 6.78 (1H, d, J = 7.0 Hz), 7.14 (1H, m), 7.22 (1H, m), 7.45 (1H, s); IR (KBr) 1715 cm^{-1} (COOMe); LR-MS (EI) 264 (M^+).

[3-(3-Bromopropyl)benzofuran-7-yloxy]acetic Acid Methyl Ester (33). By the procedure used in **17**, compound **33** (95%) was prepared from **32**. Colorless oil; ^{1}H NMR ($CDCl_3$) δ 2.24 (2H, quint, J = 6.0 Hz), 2.86 (2H, t, J = 6.0 Hz), 3.45 (2H, t, J = 6.0 Hz), 3.82 (3H, s), 4.89 (2H, s), 6.78 (1H, dd, J = 1.0, 8.0 Hz), 7.15 (1H, t, J = 8.0 Hz), 7.21 (1H, dd, J = 1.0, 8.0 Hz), 7.48 (1H, s); IR (neat) 1769 cm^{-1} (COOMe); LR-MS (EI) 326, 328 (M^+) (relative peak hight ratio is 1:1).

7-Methoxy-2-(tetrahydropyran-2-yloxymethyl)benzofuran-3-one (36). Sodium hydride (60% in mineral oil, 5.60 g, 0.140 mmol), which was washed with *n*-hexane before use, was suspended in toluene (100 mL). To this suspension was added a solution of 3-methoxy-2-methoxycarbonylmethoxybenzoic acid methyl ester (**34**) (35.6 g, 0.140 mol) in toluene (400 mL), and the reaction mixture was refluxed for 22 h. After the mixture was cooled to room temperature, the precipitate was collected and washed with a small amount of toluene to give **35** (34.18 g, 100%) as pale-red powder.

To a stirred solution of **35** (11.3 g, 46.4 mmol) in THF (1100 mL) was added $LiAlH_4$ (1.83 g, 49 mmol) in four portions at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, and 1 N HCl (200 mL) and brine (200 mL) were added. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layer was washed with brine and dried over $MgSO_4$. Removal of the solvent afforded an oily residue, which was dissolved in CH_2Cl_2 (180 mL). To this solution was added 3,4-dihydro-2*H*-pyran (7.26 g, 86 mmol) and pyridinium *p*-toluenesulfonate (2.50 g, 110 mmol), and the reaction mixture was stirred at room temperature for 5 h. The reaction mixture was diluted with CH_2Cl_2 (180 mL) and was sequentially washed with water and brine and dried over $MgSO_4$. Removal of the solvent afforded an oily residue, which was purified by silica gel chromatography (AcOEt/*n*-hexane = 1/3) to afford **36** (9.71 g, 75%) as a 1:1 mixture of two diastereomers.

Polar Isomer of 36. Pale-yellow solid; ^{1}H NMR ($CDCl_3$) δ 1.35–1.65 (6H, m), 3.50 (1H, m), 3.78 (1H, m), 3.97 (3H, s), 3.99 (1H, dd, J = 2.7, 11.5 Hz), 4.21 (1H, dd, J = 4.1, 11.5 Hz), 4.67 (1H, m), 4.76 (1H, dd, J = 2.7, 4.1 Hz), 7.03 (1H, t, J = 7.8 Hz), 7.13 (1H, dd, J = 1.2, 7.8 Hz), 7.27 (1H, dd, J = 1.2, 7.8 Hz); LR-MS (EI) 278 (M^+).

Less Polar Isomer of 36. Pale-yellow liquid; ^{1}H NMR ($CDCl_3$) δ 1.42–1.65 (6H, m), 3.52 (1H, m), 3.84 (1H, dd, J = 5.8, 11.5 Hz), 3.85 (1H, m), 3.97 (3H, s), 4.29 (1H, dd, J = 2.5, 11.5 Hz), 4.69 (1H, m), 4.82 (1H, dd, J = 2.5, 5.8 Hz), 7.02 (1H, t, J = 7.7 Hz), 7.12 (1H, dd, J = 1.4, 7.7 Hz), 7.25 (1H, dd, J = 1.4, 7.7 Hz); LR-MS (EI) 278 (M^+).

[7-Methoxy-2-(tetrahydropyran-2-yloxymethyl)benzofuran-3-yl]acetic Acid Methyl Ester (37). To a stirred suspension of zinc powder (7.81 g, 120 mmol) and catalytic amount of iodine in THF (10 mL) was added a solution of **36** (16.1 g, 58 mmol) and methyl bromoacetate (11.0 mL, 116 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 60 min and at 50 °C for 30 min. The reaction was quenched by addition of acetic acid (5.5 mL), and the mixture was filtered. The filtrate was concentrated under reduced pressure, and the residue was dissolved in toluene (140 mL) and pyridine (140 mL). The solution was cooled to 0 °C, and Tf_2O (14.5 mL, 88 mmol) was added. The reaction mixture was stirred at 0 °C for 90 min. The reaction was

quenched by addition of brine (400 mL), and the mixture was extracted with ethyl acetate. The combined organic layer was sequentially washed with water, 5% citric acid, water, and brine and dried over $MgSO_4$. Removal of the solvent afforded an oily residue, which was purified by silica gel chromatography (AcOEt/*n*-hexane = 1/2) to afford **37** (15.0 g, 78%). Colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.45−1.90 (6H, m), 3.56 (1H, m), 3.69 (3H, s), 3.76 (2H, s), 3.90 (1H, m), 4.01 (3H, s), 4.70 (1H, d, J = 13.0 Hz), 4.72 (1H, t, J = 3.0 Hz), 4.85 (1H, d, J = 13.0 Hz), 6.81 (1H, dd, J = 1.0, 8.0 Hz), 7.11−7.20 (2H, m); IR (neat) 1742 cm^{-1} (COOMe); LR-MS (EI) 334 (M^+).

2-[7-Methoxy-2-(tetrahydropyran-2-yloxymethyl)benzofuran-3-yl]ethanol (38). By the procedure used in **26**, compound **38** (51%) was prepared from **37**. Pale-yellow oil; ^{1}H NMR ($CDCl_3$) δ 1.49−1.87 (6H, m), 2.98 (2H, t, J = 6.0 Hz), 3.57 (1H, m), 3.87 (2H, t, J = 6.0 Hz), 3.92 (1H, m), 4.01 (3H, s), 4.64 (1H, d, J = 13.0 Hz), 4.81 (1H, t, J = 3.0 Hz), 4.86 (1H, d, J = 13.0 Hz), 6.83 (1H, dd, J = 1.0, 8.0 Hz), 7.12 (1H, dd, J = 1.0, 8.0 Hz), 7.17 (1H, t, J = 8.0 Hz); IR (neat) 1734 cm^{-1} (COOMe); LR-MS (EI) 306 (M^+).

[3-(2-Hydroxyethyl)-2-(tetrahydropyran-2-yloxymethyl)benzofuran-7-yloxy]acetic Acid Methyl Ester (39). To a stirred mixture of *t*-BuOK (9.71 g, 87 mmol) and **38** (7.67 g, 25 mmol) in DMF (150 mL) was added *n*-PrSH (8.50 mL, 94 mmol), and the reaction mixture was stirred at 140 °C for 1 h. The solvent was removed under reduced pressure, and the residue was poured into 5% citric acid and extracted with ethyl acetate. The combined organic layer was sequentially washed with water and brine and dried over $MgSO_4$. Removal of the solvent afforded an oily residue, which was dissolved in DMF (100 mL). To this solution was added K_2CO_3 (10.23 g, 74 mmol) and methyl bromoacetate (5.0 mL, 54 mmol), and the reaction mixture was stirred at room temperature for 15 h. The solvent was removed under reduced pressure, and the residue was poured into 5% citric acid and was extracted with ethyl acetate. The combined organic layer was sequentially washed with water and brine and dried over $MgSO_4$. Removal of the solvent afforded an oily residue, which was purified by silica gel chromatography (AcOEt/*n*-hexane = 1/1, then 2/1) to afford **39** (7.54 g, 84%). Pale-yellow oil; ^{1}H NMR ($CDCl_3$) δ 1.47−1.88 (6H, m), 2.31 (1H, t, J = 3.0 Hz), 2.98 (2H, t, J = 6.0 Hz), 3.81 (3H, s), 3.83−3.96 (3H, m), 4.65 (1H, d, J = 13.0 Hz), 4.81 (1H, t, J = 3.0 Hz), 4.85 (1H, d, J = 13.0 Hz), 4.90 (2H, s), 6.78 (1H, dd, J = 1.5, 7.5 Hz), 7.14 (1H, t, J = 7.5 Hz), 7.18 (1H, dd, J = 1.5, 7.5 Hz); IR (neat) 1763 cm^{-1} (COOMe); LR-MS (EI) 364 (M^+).

[3-[2-(1,1-Diphenylethylsulfanyl)ethyl]-2-(tetrahydropyran-2-yloxymethyl)benzofuran-7-yloxy]acetic Acid Methyl Ester (40b). Compound **39** was mesylated by the procedure used in **28**. And by the procedure used in **23d**, compound **40b** (80% in two steps) was synthesized from **39**. Colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.47−1.82 (6H, m), 2.04 (3H, s), 2.57 (2H, t, J = 7.0 Hz), 2.77 (2H, t, J = 7.0 Hz), 3.54 (1H, m), 3.80 (3H, s), 3.89 (1H, m), 4.55 (1H, d, J = 13.0 Hz), 4.68 (1H, t, J = 3.0 Hz), 4.73 (1H, d, J = 13.0 Hz), 4.87 (2H, s), 6.74 (1H, dd, J = 1.0, 8.0 Hz), 6.89 (1H, dd, J = 1.0, 8.0 Hz), 7.06 (1H, t, J = 8.0 Hz), 7.18−7.30 (6H, m), 7.39 (4H, m); LR-MS (FAB, positive) 583 (M^+ + Na).

[3-(2-Benzhydrylsulfanylethyl)-2-(tetrahydropyran-2-yloxymethyl)benzofuran-7-yloxy]acetic Acid Methyl Ester (40a). By the procedure used in **40b**, compound **40a** (73%) was synthesized from **39**. Colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.47−1.82 (6H, m), 2.66 (2H, t, J = 7.0 Hz), 2.95 (2H, t, J = 7.0 Hz), 3.54 (1H, m), 3.80 (3H, s), 3.89 (1H, m), 4.54 (1H, d, J = 13.0 Hz), 4.68 (1H, t, J = 3.0 Hz), 4.72 (1H, d, J = 13.0 Hz), 4.88 (2H, s), 5.15 (1H, s), 6.78 (1H, dd, J = 1.0, 8.0 Hz), 6.83 (1H, dd, J = 1.0, 8.0 Hz), 7.08 (1H, t, J = 8.0 Hz), 7.19−7.33 (6H, m), 7.37−7.41 (4H, m); LR-MS (FAB, positive) 569 (M^+ + Na).

[3-[2-(1,1-Diphenylpropylsulfanyl)ethyl]-2-(tetrahydropyran-2-yloxymethyl)benzofuran-7-yloxy]acetic Acid Methyl Ester (40c). By the procedure used in **40b**, compound **40c** (40%) was synthesized from **39**. Colorless oil; ^{1}H NMR ($CDCl_3$) δ 0.77 (3H, t, J = 7.0 Hz), 1.45−1.82 (6H, m), 2.33 (2H, q, J = 7.0 Hz), 2.40 (2H, t, J = 7.0 Hz), 2.69 (2H, t, J = 7.0 Hz), 3.54 (1H, m), 3.80 (3H, s), 3.88 (1H, m), 4.52 (1H, d, J = 13.0 Hz), 4.68 (1H, t, J = 3.0 Hz), 4.71 (1H, d, J = 13.0 Hz), 4.87 (2H, s), 6.73 (1H, dd, J = 1.0, 8.0 Hz), 6.84 (1H, dd, J = 1.0, 8.0 Hz), 7.05 (1H, t, J = 8.0 Hz), 7.16−7.29 (m, 6H), 7.33 (m, 4H); LR-MS (FAB, positive) 597 (M^+ + Na).

[3-[2-(1,1-Diphenylbutylsulfanyl)ethyl]-2-(tetrahydropyran-2-yloxymethyl)benzofuran-7-yloxy]acetic Acid Methyl Ester (40d). By the procedure used in **40b**, compound **40d** (86%) was synthesized from **39**. Colorless oil; ^{1}H NMR ($CDCl_3$) δ 0.82 (3H, t, J = 7.0 Hz), 1.14 (2H, m), 1.45−1.88 (6H, m), 2.25 (2H, m), 2.41 (2H, t, J = 7.0 Hz), 2.68 (2H, t, J = 7.0 Hz), 3.53 (1H, m), 3.80 (3H, s), 3.88 (1H, m), 4.52 (1H, d, J = 13.0 Hz), 4.68 (1H, t, J = 3.0 Hz), 4.70 (1H, d, J = 13.0 Hz), 4.87 (2H, s), 6.73 (1H, dd, J = 1.0, 8.0 Hz), 6.84 (1H, dd, J = 1.0, 8.0 Hz), 7.45 (1H, t, J = 8.0 Hz), 7.16−7.28 (6H, m), 7.34 (4H, m); LR-MS (FAB, positive) 611 (M^+ + Na).

{2-(Tetrahydropyran-2-yloxymethyl)-3-[2-(2,2,2-trifluoro-1,1-diphenylethylsulfanyl)ethyl]benzofuran-7-yloxy}-acetic Acid Methyl Ester (40e). By the procedure used in **40b**, compound **40e** (56%) was synthesized from **39**. Colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.42−1.95 (6H, m), 2.59 (2H, m), 2.78 (2H, m), 3.56 (1H, m), 3.80 (3H, s), 3.89 (1H, m), 4.53 (1H, d, J = 13.0 Hz), 4.68 (1H, t, J = 3.3 Hz), 4.72 (1H, d, J = 13.0 Hz), 4.87 (2H, s), 6.73 (1H, dd, J = 0.8, 7.9 Hz), 6.78 (1H, dd, J = 0.8, 7.9 Hz), 7.03 (1H, t, J = 8.0 Hz), 7.25−7.30 (6H, m), 7.36−7.42 (4H, m); LR-MS (EI) 614 (M^+).

General Procedure for Deprotection of the THP Group. [3-[2-(1,1-Diphenylethylsulfanyl)ethyl]-2-hydroxymethylbenzofuran-7-yloxy]acetic Acid Methyl Ester (41b). To a stirred solutoin of **40b** (550 mg, 0.98 mmol) in THF (10 mL) and MeOH (10 mL) was added pyridinium *p*-toluenesulfonate (103 mg, 0.41 mmol), and the reaction mixture was stirred at 80 °C for 6.5 h. The solvent was removed under reduced pressure, and the residue was poured into 5% citric acid and was extracted with ethyl acetate. The combined organic layer was sequentially washed with water and brine and was dried over $MgSO_4$. Removal of the solvent afforded an oily residue, which was purified by silica gel chromatography (AcOEt/*n*-hexane = 1/1) to afford **41b** (387 mg, 83%). Colorless oil; ^{1}H NMR ($CDCl_3$) δ 2.00 (3H, s), 2.26 (1H, t, J = 6.5 Hz), 2.60 (2H, m), 2.69 (2H, m), 3.81 (3H, s), 4.63 (2H, d, J = 6.5 Hz), 4.88 (2H, s), 6.74 (1H, dd, J = 1.0, 7.0 Hz), 6.92 (1H, dd, J = 1.0, 7.0 Hz), 7.07 (1H, t, J = 7.0 Hz), 7.16−7.29 (6H, m), 7.34 (m, 4H); LR-MS (FAB, positive) 477 (M^+ + H).

[3-(2-Benzhydrylsulfanylethyl)-2-hydroxymethylbenzofuran-7-yloxy]acetic Acid Methyl Ester (41a). Compound **41a** (73%) was prepared from **40a**. Colorless oil; ^{1}H NMR ($CDCl_3$) δ 2.15 (1H, t, J = 7.0 Hz), 2.67 (2H, t, J = 7.0 Hz), 2.92 (2H, t, J = 7.0 Hz), 3.80 (3H, s), 4.68 (2H, d, J = 7.0 Hz), 4.88 (2H, s), 5.04 (1H, s), 6.81 (1H, dd, J = 1.0, 8.0 Hz), 6.89 (1H, dd, J = 1.0, 8.0 Hz), 7.11 (1H, t, J = 8.0 Hz), 7.18−7.36 (10H, m); LR-MS (FAB, positive) 463 (M^+ + H).

[3-[2-(1,1-Diphenylpropylsulfanyl)ethyl]-2-hydroxymethylbenzofuran-7-yloxy]acetic Acid Methyl Ester (41c). Compound **41c** (78%) was prepared from **40c**. Colorless oil; ^{1}H NMR ($CDCl_3$) δ 0.73 (3H, t, J = 7.0 Hz), 2.27 (1H, t, J = 6.0 Hz), 2.31 (2H, q, J = 7.0 Hz), 2.47 (2H, m), 2.55 (2H, m), 3.81 (3H, s), 4.59 (2H, d, J = 6.0 Hz), 4.88 (2H, s), 6.74 (1H, dd, J = 1.0, 8.0 Hz), 6.88 (1H, dd, J = 1.0, 8.0 Hz), 7.06 (1H, t, J = 8.0 Hz), 7.14−7.32 (10H, m); LR-MS (FAB, positive) 491 (M^+ + H).

[3-[2-(1,1-Diphenylbutylsulfanyl)ethyl]-2-hydroxymethylbenzofuran-7-yloxy]acetic Acid Methyl Ester (41d). Compound **41d** (91%) was prepared from **40d**. Colorless oil; ^{1}H NMR ($CDCl_3$) δ 0.80 (3H, t, J = 7.0 Hz), 1.11 (2H, m), 2.23 (2H, m), 2.26 (1H, t, J = 6.5 Hz), 2.48 (2H, m), 2.55 (2H, m), 3.81 (3H,s), 4.59 (2H, d, J = 6.5 Hz), 4.88 (2H, s), 6.74 (1H, dd, J = 1.0, 8.0 Hz), 6.88 (1H, dd, J = 1.0, 8.0 Hz), 7.06 (1H, t, J = 8.0 Hz), 7.15−7.32 (10H, m); LR-MS (FAB, positive) 505 (M^+ + H).

{2-Hydroxymethyl-3-[2-(2,2,2-trifluoro-1,1-diphenylethylsulfanyl)ethyl]benzofuran-7-yloxy}acetic Acid Methyl Ester (41e). Compound **41e** (84%) was prepared from **40e**. Colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.98 (1H, t, J = 6.3 Hz), 2.57−2.65 (2H, m), 2.70−2.78 (2H, m), 3.81 (3H, s), 4.64 (2H, d, J = 6.3 Hz), 4.87 (2H, s), 6.73 (1H, dd, J = 0.8, 8.0 Hz), 6.81 (1H, dd, J = 0.8, 8.0 Hz), 7.05 (1H, t, J = 8.0 Hz), 7.24−7.29 (6H, m), 7.35−7.40 (4H, m); LR-MS (EI) 530 (M^+).

2-(3-(2-(1,1-Diphenylethylsulfonyl)ethyl)-2-(hydroxymethyl)benzofuran-7-yloxy)acetic Acid Methyl Ester (42). To a stirred solution of **41b** (216 mg, 0.45 mmol) in dichloromethane (3 mL) was added *m*-CPBA (196 mg) at 0 °C. The reaction mixture was stirred at this temperature for 3.5 h and was poured into water. The organic layer was separated, and the water layer was extracted twice with dichloromethane. The combined organic layer was sequentially washed with water and brine and dried over $MgSO_4$. Removal of the solvent gave an oily residue, which was purified by silica gel chromatography (AcOEt/*n*-hexane = 2/3) to afford **42** (183 mg, 79%). Colorless prisms, mp 53−54 °C; ^{1}H NMR ($CDCl_3$) δ 2.17 (3H, s), 2.49 (1H, t, J = 6.3 Hz), 2.97−3.12 (4H, m), 3.80 (3H, s), 4.69 (2H, d, J = 6.3 Hz), 4.87 (2H, s), 6.78 (1H, dd, J = 0.8, 8.0 Hz), 6.88 (1H, dd, J = 0.8, 8.0 Hz), 7.08 (1H, t, J = 8.0 Hz), 7.32−7.40 (6H, m), 7.52−7.60 (4H, m); LR-MS (EI) 508 (M^+).

2-Allyl-7-methoxy-3-oxo-2,3-dihydrobenzofuran-2-carboxylic Acid Methyl Ester (43). Compound **35** (18.6 g, 76.4 mmol) was dissolved in DMF (150 mL). To this solution was added allyl bromide (8.6 mL, 99 mmol), and the reaction mixture was stirred at room temperature for 15.5 h. Acetic acid (2.0 mL) was added to the reaction mixture, and the solvent was removed under reduced pressure. The residue was dissolved in toluene (200 mL) and was refluxed for 1 h. The reaction mixture was cooled to room temperature and poured into water (200 mL). The organic layer was separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layer was sequentially washed with saturated $NaHCO_3$, water, and brine and dried over Na_2SO_4. Removal of the solvent afforded an oily residue, which was purified by silica gel column chromatography (AcOEt/*n*-hexane = 1/3) to afford **43** (18.4 g, 92%). Colorless oil; ^{1}H NMR ($CDCl_3$) δ 2.91 (1H, ddt, J = 14.5, 7.0, 1.0 Hz), 3.08 (1H, ddt, J = 14.5, 7.0, 1.0 Hz), 3.75 (3H, s), 3.99 (3H, s), 5.11−5.07 (1H, m), 5.27−5.20 (1H, m), 5.67 (1H, ddt, J = 17.0, 10.0, 7.0 Hz), 7.06 (1H, t, J = 8.0 Hz), 7.15 (1H, dd, J = 8.0, 1.5 Hz), 7.24 (1H, dd, J = 8.0, 1.5 Hz); LR-MS (EI) 262 (M^+).

2-Allyl-7-methoxybenzofuran-3-one (44). To a solution of **43** (18.42 g, 70 mmol) in *t*-BuOH (150 mL) was added concentrated H_2SO_4 (2 mL), and the mixture was refluxed for 22.5 h. The reaction mixture was cooled to room temperature and was poured into saturated aqueous $NaHCO_3$. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layer was sequentially washed with saturated aqueous $NaHCO_3$, water, and brine and dried over Na_2SO_4. Removal of the solvent afforded an oily residue, which was purified by silica gel column chromatography (AcOEt/*n*-hexane = 1/4) to afford **44** (11.39 g, 80%). Colorless oil; ^{1}H NMR ($CDCl_3$) δ 2.55−2.66 (1H, m), 2.78−2.89 (1H, m), 3.96 (3H, s), 4.68 (1H, dd, J = 7.0, 5.0 Hz), 5.09−5.14 (1H, m), 5.24 (1H, ddd, J = 7.0, 2.0, 1.5 Hz), 5.82 (1H, ddt, J = 17.0, 10.0, 7.0 Hz), 7.02 (1H, t, J = 8.0 Hz), 7.15 (1H, dd, J = 8.0, 1.0 Hz), 7.24 (1H, dd, J = 8.0, 1.0 Hz); LR-MS (EI) 204 (M^+).

(2-Allyl-7-methoxybenzofuran-3-yl)acetic Acid Methyl Ester (45). By the procedure used in **37**, the Reformatski reaction of compound **44** was performed. The intermediate was dissolved in toluene (100 mL), and *p*-toluenesulfonic acid monohydrate (536 mg, 2.8 mmol) was added. The reaction mixture was stirred at room temperature for 2 h and was poured into water and extracted with ethyl acetate. The combined organic layer was washed with brine and dried over Na_2SO_4. Removal of the solvent afforded an oily residue, which was purified by silica gel chromatography (AcOEt/*n*-hexane = 1/3) and was recrystallized from AcOEt/*n*-hexane to afford **45** (5.23 g, 67%). Colorless prisms, mp 65−66 °C; ^{1}H NMR ($CDCl_3$) δ 3.57 (2H, dt, J = 6.0, 1.5 Hz), 3.63 (2H, s), 3.68 (3H, s), 3.99 (3H, s), 5.15 (1H, dq, J = 25.0, 1.5 Hz), 5.24 (1H, m), 5.82 (1H, ddt, J = 17.0, 10.0, 6.0 Hz), 6.77 (1H, dd, J = 7.7, 1.4 Hz), 7.09 (1H, dd, J = 7.7, 1.4 Hz), 7.15 (1H, t, J = 7.7 Hz); LR-MS (EI) 260 (M^+).

[2-Allyl-3-(2-hydroxyethyl)benzofuran-7-yloxy]acetic Acid Methyl Ester (46). To a solution of **45** (464 mg, 1.78 mmol) in dichloromethane (4 mL) was added 1.0 M BBr_3 dichloromethane solution (3.9 mL, 3.9 mmol) at −78 °C, and the mixture was stirred at 0 °C for 2 h. The reaction mixture was poured into saturated $NaHCO_3$ and was extracted with ethyl acetate. The combined organic layer was sequentially washed with water and brine and dried over Na_2SO_4. Removal of the solvent afforded an oily residue, which was dissolved in THF (15 mL). To this solution was added $LiAlH_4$ (91 mg, 2.40 mmol), and the mixture was stirred at 0 °C for 30 min and at room temperature for 1.5 h. The reaction mixture was poured into saturated $NaHCO_3$ and extracted with ethyl acetate. The combined organic layer was sequentially washed with water and brine and dried over Na_2SO_4. Removal of the solvent afforded an oily residue, which was dissolved in DMF (5 mL). To this solution was added methyl bromoacetate (0.5 mL, 5.28 mmol) and K_2CO_3 (606 mg, 4.38 mmol), and the mixture was stirred at room temperature for 17 h. The reaction mixture was poured into water and extracted with ethyl acetate. The combined organic layer was sequentially washed with water and brine and dried over Na_2SO_4. Removal of the solvent afforded an oily residue, which was purified by silica gel column chromatography (AcOEt/*n*-hexane = 1/1) to afford **46** (450 mg, 87%). Colorless oil; ^{1}H NMR ($CDCl_3$) δ 2.89 (2H, t, J = 6.3 Hz), 3.56 (2H, dt, J = 6.0, 1.5 Hz), 3.81 (3H, s), 3.85 (2H, t, J = 6.3 Hz), 4.89 (2H, s), 5.10−5.19 (2H, m), 5.99 (1H, ddt, J = 17.0, 10.0, 6.0 Hz), 6.73 (1H, dd, J = 7.0, 1.5 Hz), 7.08−7.16 (2H, m); LR-MS (EI) 290 (M^+).

{2-Allyl-3-[2-(tetrahydropyran-2-yloxy)ethyl]benzofuran-7-yloxy}acetic Acid Methyl Ester (47). To a solution of **46** (450 mg, 1.55 mmol) in THF (2 mL) were added 3,4-dihydro-2*H*-pyran (0.21 mL, 2.30 mmol) and *p*-toluenesulfonic acid monohydrate (15 mg, 0.08 mmol), and the mixture was stirred at room temperature for 1.5 h. The reaction mixture was poured into water and was extracted with ethyl acetate. The combined organic layer was sequentially washed with water and brine and dried over Na_2SO_4. Removal of the solvent afforded an oily residue, which was purified by silica gel chromatography (AcOEt/*n*-hexane = 1/3) to afford **47** (544 mg, 94%). Colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.45−1.84 (6H, m), 2.92 (2H, t, J = 7.0 Hz,), 3.41−3.49 (1H, m), 3.53−3.61 (3H, m), 3.72−3.80 (1H, m), 3.81 (3H, s), 3.94 (1H, dt, J = 9.5, 7.0 Hz), 4.57−4.59 (1H, m), 4.88 (2H, s), 5.09−5.20 (2H, m), 5.98 (1H, ddt, J = 17.0, 10.2, 6.3 Hz), 6.70 (1H, dd, J = 8.0, 1.0 Hz), 7.09 (1H, t, J = 8.0 Hz), 7.16 (1H, dd, J = 8.0, 1.0 Hz); LR-MS (EI) 374 (M^+).

{2-(2-Hydroxyethyl)-3-[2-(tetrahydropyran-2-yloxy)ethyl]benzofuran-7-yloxy}acetic Acid Methyl Ester (48). To a solution of **47** (0.97 g, 2.59 mmol) in dioxane (15 mL) and water (5 mL) was added 0.07 M OsO_4 in *t*-BuOH (0.37 mL, 26 umol) at 0 °C. $NaIO_4$ (1 38 g 6 45 mmol) was added to this solution in several portions, and the mixture was stirred at 0 °C for 30 min and at room temperature for 30 min. The reaction mixture was filtered, and the filtrate was diluted with THF (12 mL). To this solution was added $NaBH_4$ (98 mg, 2.59 mmol), and the mixture was stirred at room temperature for 40 min. The reaction mixture was poured into water and was extracted with ethyl acetate. The combined organic layer was sequentially washed with water and brine and dried over Na_2SO_4. Removal of the solvent afforded an oily residue, which was purified by silica gel column chromatography (AcOEt/*n*-hexane = 1/1) to afford **48** (412 mg, 42%). Colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.42−1.76 (6H, m), 2.96 (2H, t, J = 6.0 Hz), 3.05 (2H, t, J = 5.8 Hz), 3.35−3.43 (1H, m), 3.58−3.71 (2H, m), 3.81 (3H, s), 3.93 (2H, t, J = 5.8 Hz), 4.04−4.11 (1H, m), 4.52−4.54 (1H, m), 4.87 (2H, s), 6.70 (1H, dd, J =

8.0, 1.5 Hz), 7.09 (1H, t, J = 8.0 Hz), 7.14 (1H, dd, J = 8.0, 1.5 Hz); LR-MS (EI) 378 (M^+).

[2-(2-Acetoxyethyl)-3-(2-hydroxyethyl)benzofuran-7-yloxy]acetic Acid Methyl Ester (49). To a solution of **48** (403 mg, 1.06 mmol) in THF (5 mL) were added pyridine (0.13 mL, 1.61 mmol) and acetic anhydride (0.3 mL, 3.18 mmol). The reaction mixture was stirred at room temperature for 16 h and was poured into water and extracted with ethyl acetate. The combined organic layer was sequentially washed with water and brine and dried over Na_2SO_4. Removal of the solvent afforded an oily residue, which was dissolved in MeOH (4 mL). To this solution was added 1 N HCl (1 mL), and the reaction mixture was stirred at room temperature for 2 h and was poured into water and extracted with ethyl acetate. The combined organic layer was sequentially washed with water and brine and dried over Na_2SO_4. Removal of the solvent afforded an oily residue, which was purified by silica gel chromatography (AcOEt/*n*-hexane = 2/1) and recrystallized from AcOEt/*n*-hexane to afford **49** (283 mg, 79%). Colorless prisms, mp 80−81 °C; ^{1}H NMR ($CDCl_3$) δ 2.03 (3H, s), 2.90 (2H, t, J = 6.3 Hz), 3.13 (2H, t, J = 6.6 Hz), 3.81 (3H, s), 3.87 (2H, t, J = 6.3 Hz), 4.43 (2H, t, J = 6.6 Hz), 4.88 (2H, s), 6.74 (1H, dd, J = 7.5, 2.5 Hz), 7.09−7.14 (2H, m); LR-MS (EI) 336 (M^+).

{2-(2-Acetoxyethyl)-3-[2-(1,1-diphenylethylsulfanyl)-ethyl]benzofuran-7-yloxy}acetic Acid Methyl Ester (50). By the procedure used in the preparation of **40b**, compound **50** (87%) was prepared from **49** and **11b**. Colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.99 (3H, s), 2.04 (3H, s), 2.50−2.59 (2H, m), 2.62−2.70 (2H, m), 2.98 (2H, t, J = 6.6 Hz), 3.81 (3H, s), 4.32 (2H, t, J = 6.6 Hz), 4.87 (2H, s), 6.69 (1H, dd, J = 8.0, 1.0 Hz), 6.85 (1H, dd, J = 8.0, 1.0 Hz), 7.04 (1H, t, J = 8.0 Hz), 7.18−7.32 (6H, m), 7.36−7.42 (4H, m); LR-MS (EI) 532 (M^+).

2-(2-Allyl-7-methoxybenzofuran-3-yl)ethanol (51). By the procedure used in **26**, compound **51** (86%) was prepared from **45**. Colorless oil; ^{1}H NMR ($CDCl_3$) δ 2.90 (2H, t, J = 6.4 Hz), 3.56 (2H, dt, J = 4.6, 1.6 Hz), 3.86 (2H, t, J = 6.4 Hz), 4.00 (3H, s), 5.09−5.15 (1H, m), 5.18 (1H, q, J = 1.6 Hz), 5.99 (1H, ddt, J = 17.0, 9.9, 6.3 Hz), 6.77 (1H, dd, J = 7.4, 1.4 Hz), 7.08−7.17 (2H, m); LR-MS (EI) 232 (M^+).

[3-(2-Hydroxyethyl)-2-propenylbenzofuran-7-yloxy]-acetic Acid Methyl Ester (52). By the procedure used in **39**, compound **52** (35%) was prepared from **51**. Colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.94 (3H, dd, J = 6.6, 1.4 Hz), 2.93 (2H, t, J = 6.5 Hz), 3.82 (3H, s), 3.85 (2H, t, J = 6.5 Hz), 4.92 (2H, s), 6.38−6.61 (2H, m), 6.76 (1H, dd, J = 7.0, 1.5 Hz), 7.08−7.14 (2H, m); LR-MS (EI) 290 (M^+).

{2-Propenyl-3-[2-(tetrahydropyran-2-yloxy)ethyl]benzofuran-7-yloxy}acetic Acid Methyl Ester (53). By the procedure used in **47**, compound **53** (92%) was prepared from **52**. Colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.44−1.86 (6H, m), 1.93 (3H, d, J = 5.2 Hz), 2.95 (2H, t, J = 7.0 Hz), 3.40−3.48 (1H, m), 3.58 (1H, dt, J = 9.5, 7.0 Hz), 3.72−3.82 (1H, m), 3.81 (3H, s), 3.93 (1H, dt, J = 9.5, 7.0 Hz), 4.56−4.59 (1H, m), 4.91 (2H, s), 6.35−6.56 (2H, m), 6.73 (1H, dd, J = 8.0, 1.0 Hz), 7.07 (1H, t, J = 8.0 Hz), 7.15 (1H, dd, J = 8.0, 1.0 Hz); LR-MS (EI) 374 (M^+).

{2-(3-Acetoxypropenyl)-3-[2-(tetrahydropyran-2-yloxy)ethyl]benzofuran-7-yloxy}acetic Acid Methyl Ester (54). To a solution of **53** (523 mg, 1.40 mmol) in benzene (5 mL) were added *N*-bromosuccimide (299 mg, 1.68 mmol) and AIBN (23 mg, 0.14 mmol). The reaction mixture was stirred at room temperature for 4 h and was poured into water and extracted with ethyl acetate. The combined organic layer was sequentially washed with water and brine and dried over Na_2SO_4. Removal of the solvent afforded an oily residue, which was dissolved in DMF (4 mL). To this solution was added KOAc (205 mg, 2.09 mmol), and the mixture was stirred at room temperature for 50 min. The reaction mixture was poured into water and was extracted with ethyl acetate. The combined organic layer was sequentially washed with water and brine and dried over Na_2SO_4. Removal of the solvent afforded an oily residue, which was purified by silica gel chromatography (AcOEt/*n*-hexane = 1/2) to afford **54** (217 mg, 36%). Colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.44−1.84 (6H, m), 2.12 (3H, s), 2.98 (2H, t, J = 7.0 Hz), 3.39−3.47 (1H, m), 3.59 (1H, dt, J = 9.5, 7.0 Hz), 3.69−3.77 (1H, m), 3.82 (3H, s), 3.95 (1H, dt, J = 9.5, 7.0 Hz), 4.57 (1H, m), 4.77 (2H, dd, J = 6.0, 1.5 Hz), 4.90 (2H, s), 6.52 (1H, dt, J = 16.0, 6.0 Hz), 6.69 (1H, dt, J = 16.0, 1.5 Hz), 6.77 (1H, dd, J = 8.0, 1.0 Hz), 7.10 (1H, t, J = 8.0 Hz), 7.18 (1H, dd, J = 8.0, 1.0 Hz); LR-MS (EI) 432 (M^+).

[2-(3-Acetoxypropyl)-3-(2-hydroxyethyl)benzofuran-7-yloxy]acetic Acid Methyl Ester (55). To a solution of **54** (199 mg, 0.460 mmol) in MeOH (4 mL) was added 5% Pd/C (28 mg), and the reaction mixture was stirred at room temperature for 50 min under hydrogen atmosphere. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel chromatography (AcOEt/*n*-hexane = 1/1) to afford **55** (80 mg, 50%). Colorless oil; ^{1}H NMR ($CDCl_3$) δ 2.03 (3H, s), 2.09 (2H, quint, J = 7.0 Hz), 2.87 (4H, m), 3.81 (3H, s), 3.86 (2H, t, J = 7.0 Hz), 4.12 (2H, t, J = 7.0 Hz), 4.88 (2H, s), 6.71 (1H, dd, J = 7.0, 2.0 Hz), 7.07−7.14 (2H, m); LR-MS (EI) 350 (M^+).

{2-(3-Acetoxypropyl)-3-[2-(1,1-diphenylethylsulfanyl)-ethyl]benzofuran-7-yloxy}acetic Acid Methyl Ester (56). By the procedure used in the preparation of **40b**, compound **56** (78%) was prepared from **55**. Colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.92 (2H, m), 2.01 (3H, s), 2.05 (3H, s), 2.50−2.57 (2H, m), 2.65−2.70 (2H, m), 2.76 (2H, t, J = 7.3 Hz), 3.80 (s, 3H), 4.05 (2H, t, J = 6.0 Hz), 4.86 (2H, s), 6.68 (1H, dd, J = 8.0, 1.0 Hz), 6.86 (1H, dd, J = 8.0, 1.0 Hz), 7.04 (1H, t, J = 8.0 Hz), 7.18−7.31 (6H, m), 7.36−7.41 (4H, m); LR-MS (EI) 546 (M^+).

Blood Samples. Blood samples were collected from healthy male human volunteers under the approval by the Institutional Ethics Committee of the Pharmaceutical Research Laboratories, Toray Industries, Inc. Written informed consent was obtained from each of the volunteers. The volunteers did not take any drugs at least within 2 weeks before their participation in this study. Blood samples were also collected from male cynomolgus monkeys (Japan SLC, Shizuoka, Japan) in accordance with the guidelines for the animal care and use established at the Pharmaceutical Research Laboratories, Toray Industries, Inc.

Binding Assay for TP and IP Receptors in Human Platelet Membrane. Blood was collected from human volunteers by venous puncture. An amount of nine volumes of the collected blood was mixed with one volume of a solution containing 85 mM sodium citrate, 65 mM citric acid, 2% glucose, and 0.1 mM indomethacin. Platelet-rich plasma (PRP) was prepared by centrifugation at 120*g* for 10 min at 4 °C. The platelets were washed twice in washing buffer, pH 6.5, containing 115 mM NaCl, 4.3 mM K_2HPO_4, 24.4 mM Na_2HPO_4, 5 mM glucose, 1 mM EDTA-2Na, and 0.01 mM indomethacin, and resuspended in 10 mM Tris buffer, pH 7.4, containing 5 mM $MgCl_2$, and 2 mM EDTA-2Na. The platelets were alternately frozen and thawed three times and then centrifuged at 40000*g* for 20 min at 4 °C. The membrane preparation was resuspended at 4 °C in assay buffer, pH 7.4, containing 50 mM Tris and 5 mM $MgCl_2$, and stored at −80 °C until use. For TP receptor binding assay, human platelet membrane (10 μg of protein) was incubated in assay buffer in the presence of the selective TP receptor antagonist, [^{3}H]SQ-29548, and **7** for 30 min at 25 °C. For IP receptor binding assay, human platelet membrane (10 μg of protein) was incubated in assay buffer in the presence of the selective IP receptor agonist, [^{3}H]APS-314d sodium, and **7** for 60 min at 4 °C. The reaction mixture was separated into bound and free radiolabeled ligand by rapid filtration through GF/C filters presoaked in 10 mM Tris-HCl buffer. Filters were washed, and the residual [^{3}H]SQ-29548 or [^{3}H]APS-314d sodium bound to the filter was determined by liquid scintillation counting. Specific binding was defined as the difference between total binding and nonspecific binding, which was determined in the presence of 10 μM SQ-29548 or 10 μM APS-314d sodium. K_i was calculated using the equation $K_i = IC_{50}/(1 + L/K_d)$, where L is the concentration of ligand.

Platelet Aggregation in PRP. Nine volumes of blood collected from human volunteers were mixed with one volume of 3.8% sodium citrate in a tube. The citrated blood samples were immediately centrifuged at 90−140*g* for 10 min at room temperature. The resulting supernatant was used as the PRP fraction. The remaining blood was further centrifuged at 1400*g* for 10 min. The resulting supernatant was used as the platelet-poor plasma fraction. Human PRP were pretreated with compound at various concentrations for 1 min before the addition of U46619 (2 μM), arachidonic acid (600 μM), collagen (1 μg/mL), or ADP (5 μM). The platelet stimulation with ADP was carried out in the presence or absence of SQ-29548 (10 μM).

Platelet aggregation was monitored by recording transmittance on a four-channel light transmission aggregometer (NBS Hematracer 601, MC Medical, Japan) for 5 min after the addition of a platelet-stimulating agent. For evaluating the effect of the test drugs, the percent inhibition values of platelet aggregation were calculated from the increases in transmittance observed with the test drugs ($N = 3$), on the assumption that no inhibition was observed in the control incubation of PRP with vehicle alone. And the optical density of platelet-poor plasma was taken to represent 100% aggregation.

Blood Pressure, Heart Rate, and ex Vivo Platelet Aggregation in Monkeys. Cynomolgus monkeys were anesthetized with sodium pentobarbital (35 mg/kg iv) and given compound **7** at doses of 3, 10, and 30 μg kg^{-1} min^{-1} or compound **4** at doses of 0.3, 1, and 3 μg kg^{-1} min^{-1} both in a manner of dose escalation by infusion for 30 min for each dose via the catheter inserted into the forearm or saphenous vein. Arterial blood pressure and heart rate were monitored with a polygraph system through a femoral catheter and during the infusion period, and the blood pressure and heart rate were recorded at baseline and at the end of the infusion at each dose. Arterial blood was drawn to examine ex vivo platelet aggregation at baseline and at the end of the infusion at each dose. The collected blood samples were processed to prepare PRP for determining by the light transmission method, as described above.

Statistics. The data are shown as the mean ± standard error. Statistical comparisons between mean values were performed by one-way ANOVA and Dunnett's test at a significance level of $p < 0.05$.

Acknowledgment. We thank Dr. Katsuhiko Iseki (Toray, Kamakura) and Dr. Kiyotaka Ohno (Toray, Kamakura) for helpful discussions. We thank Dr. Masafumi Isogaya and Mika Nukaya for providing us with the biological data. We thank Dr. Kenneth A. Jacobson and Heng T. Duong (NIDDK, Bethesda, MD) for helpful discussions and proofreading of the manuscript.

Supporting Information Available: Results from elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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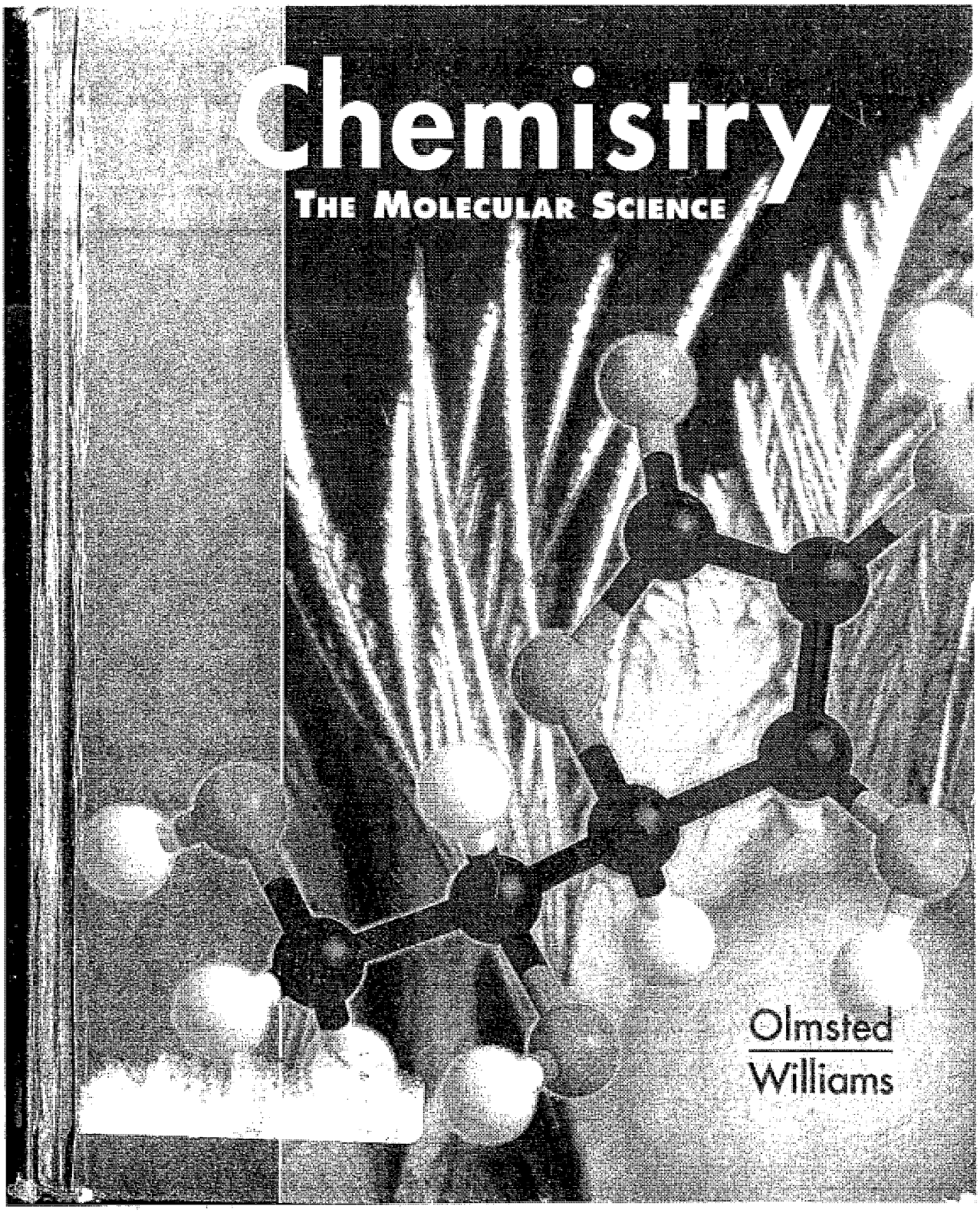
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Printed in the United States of America
Composition by Graphic World, Inc.
Printing/binding by Von Hoffmann Press

Mosby–Year Book, Inc.
11830 Westline Industrial Drive
St. Louis, Missouri 63146

Library of Congress Cataloging in Publication Data

Olmsted, John III
Chemistry, the molecular science / John Olmsted III, Gregory M. Williams
p. cm.
Includes index.
ISBN 0-8016-7485-9
1. Chemistry. I. Williams, Gregory M. II. Title.
QD33.046 1994
540—dc20 93-37165
CIP

94 95 96 97 98 / 9 8 7 6 5 4 3 2

CHAPTER 10 EFFECTS OF INTERMOLECULAR FORCES

The properties of ice crystals, icebergs, and liquid water are consequences of intermolecular forces.

As we have developed ideas about chemistry, we have emphasized the forces that bind atoms together into molecules. In Chapters 8 and 9, for example, we described the bonding forces that exist *within molecules*. These are called **intramolecular forces.** In Chapter 5, on the other hand, we described the properties of a gas using the ideal gas model, which assumes that the forces acting *between molecules* of a gas are negligible. In reality, there are indeed forces between molecules. These forces are called **intermolecular forces.** They affect the properties of gases and explain the existence and properties of liquids and solids.

Intermolecular forces are considerably weaker than intramolecular forces. In a liquid, for example, intermolecular forces are weak enough to allow individual molecules to move about relatively freely. On the other hand, intramolecular forces are strong enough to prevent atoms from breaking away from the molecules to which they are bonded.

Our discussion of bonding ignored relatively weak intermolecular interactions, but to understand the properties of liquids and solids, we must take these interactions into account. Intermolecular forces "lock" molecules into the fixed positions that characterize a solid and prevent vaporization of molecules in the liquid phase. This chapter is devoted to describing intermolecular forces and their role in the world of chemistry.

10.1 THE NATURE OF INTERMOLECULAR FORCES

We can begin an exploration of intermolecular forces by considering the properties of the elements. At room temperature and pressure, all but 13 of the elements are solids. Two others, mercury and bromine, are liquids, leaving only 11 elements that are gases. Only for these 11 gases are intermolecular forces small enough to neglect at room temperature. More commonly, intermolecular forces are strong enough to lock molecules in place in the solid state.

THE HALOGENS

The halogens, the elements from column VII of the periodic table, provide a good introduction to intermolecular forces. The halogens are most stable as diatomic molecules: F_2, Cl_2, Br_2, I_2, and At_2. At room temperature and pressure, fluorine and chlorine are gases, bromine is a liquid, and iodine is a solid. Figure 10-1 shows the strikingly different physical appearances of these elements.

The bonding patterns of the four halogens are identical. Each molecule contains two atoms held together by a single covalent bond that can be described by the overlap of valence *p* orbitals. In contrast to this common bonding pattern, bromine and iodine differ from chlorine and fluorine in their macroscopic physical appearance and in their molecular behavior, as Figure 10-2 illustrates.

Fluorine and chlorine molecules move freely throughout their gaseous volume, traveling many molecular diameters before colliding with one another or with the

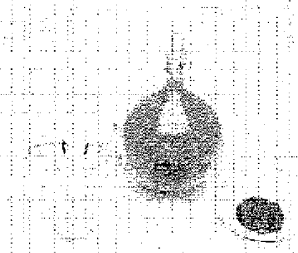


FIGURE 10-1
Under ambient conditions, chlorine is a pale yellow-green gas, bromine is a dark red liquid, and iodine is a purple crystalline solid.

The heaviest halogen, astatine, is a highly unstable radioactive element. Astatine is extremely rare and has no chemical applications.

A bonding description of F_2 is given in Chapters 8 and 9. The other diatomic molecules form bonds in an analogous manner, using valence p orbitals.

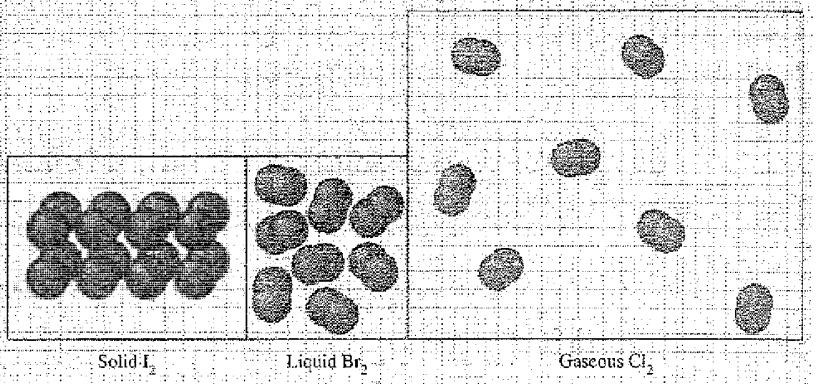


FIGURE 10-2
Molecular representations of solid I_2, liquid Br_2, and gaseous Cl_2 demonstrate why gases, liquids, and solids behave differently. A gas is mostly empty space, so the molecules are free to move about the entire volume of their container. Molecules in a liquid, on the other hand, are packed closely together but can still move past one another. A crystalline solid contains a regular array of molecules that vibrate about favored positions but cannot move freely by one another.

walls of their container. Because much of the volume of a gas is empty space, samples of gaseous F_2 and Cl_2 readily expand or contract in response to changes in pressure. This freedom of motion indicates that the intermolecular forces between these molecules are quite small.

Molecules of liquid bromine also move about relatively freely, but there is not much empty space between molecules. A liquid cannot be compressed significantly by increasing the pressure because molecules are already in close contact with one another. Also, a liquid does not expand significantly if the pressure above it is reduced. This is because intermolecular forces in a liquid are large enough to prevent the molecules from breaking away from one another.

Solid iodine has even less empty space between molecules than liquid bromine. Furthermore, the molecules in this solid do not move freely past one another. A sample of solid iodine contains highly regular crystals in which I_2 molecules are arranged in ordered arrays. Each molecule vibrates back and forth about a single lowest-energy position, but it cannot slide easily past its neighbors. Like liquids, solids do not expand or contract significantly when pressure decreases or increases.

Bromine does not exist as a gas at room temperature and iodine molecules cannot move freely because intermolecular forces between these molecules are relatively strong. Attractive intermolecular forces pull molecules toward one another, and energy is released as they get closer together. Molecules in a gas remain separated from one another because they have sufficient kinetic energy to overcome these attractive forces. Molecules in a liquid or solid remain close to one another because they lack sufficient kinetic energy to overcome these attractive forces. Hence whether a substance is a gas, liquid, or solid depends on the balance between the energy of motion of its molecules and the stabilization energy generated by its intermolecular forces.

The graph in Figure 10-3 shows that the intermolecular stabilization energy is substantially greater for Br_2 than for F_2. At room temperature, fluorine molecules have more kinetic energy of motion than the stabilization energy of F_2-F_2 interactions, whereas bromine molecules have enough kinetic energy to move freely about but insufficient energy of motion to overcome the intermolecular forces that hold them together in the liquid phase. At room temperature, iodine molecules are locked in position in the solid state because the stabilization energy between I_2 molecules is even larger than that between Br_2 molecules. To summarize, whether a substance is a gas, a liquid, or a solid depends on the balance between its intermolecular stabilization energy and its average molecular energy of motion.

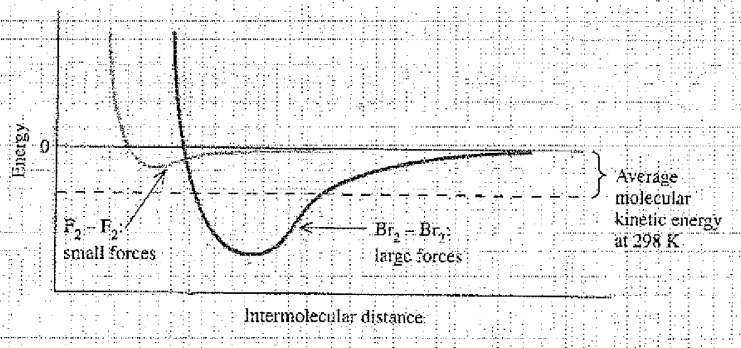


FIGURE 10-3
Intermolecular attractive forces stabilize molecules that are close to one another. The plot shows that there are larger attractive energies between bromine molecules than between fluorine molecules. This is the reason that at room temperature, fluorine is more stable as a gas, but bromine is more stable as a liquid.

Because the energy of motion depends on temperature, changing the temperature changes the balance between interaction energy and energy of motion and eventually changes the stable form of matter. For example, liquid bromine boils when it is heated to 59 °C at atmospheric pressure, forming gaseous bromine. Similarly, gaseous chlorine condenses when it is cooled to −34 °C at atmospheric pressure, forming liquid chlorine. At −101 °C, moreover, liquid chlorine becomes a solid. Fluorine liquefies at −188 °C and solidifies at −220 °C.

The link between kinetic energy and temperature is described in Chapter 5.

REAL GASES

Fluorine and chlorine are gases under ambient conditions. Yet both gases can be liquefied by lowering the temperature sufficiently. This shows the existence of attractive forces sufficient to hold molecules in the confined volume of the liquid phase at low temperature. Therefore the assumption of the ideal gas model—that intermolecular forces in a gas can be neglected—cannot be correct under conditions that cause a gas to liquefy. In other words, neither Cl_2 nor F_2 behaves ideally under all conditions.

The ideal gas model also assumes that molecular sizes can be neglected, yet no substance can be compressed indefinitely. When the distance between molecules gets small enough, repulsive forces among their electron clouds strongly resist further reduction of the volume. This is shown by the steeply rising plots of Figure 10-3. Thus finite molecular sizes also lead to deviations from ideal gas behavior.

What effect do intermolecular forces and molecular volumes have on real gases? In other words, how close do real gases come to ideal behavior? To see how far real gases stray from the ideal gas model, we can compare experimental values of real gas properties with those computed from the ideal gas equation. A convenient way to make these comparisons is to examine the experimental ratio, PV/nRT. For an ideal gas, this ratio, which is called the *compressibility*, must equal 1.

Figure 10-4 shows how compressibility varies with pressure for chlorine gas at room temperature. If chlorine were ideal, the compressibility would always be 1, as shown by the red line on the graph. Notice in the inset of Figure 10-4 that chlorine behaves very nearly ideally at pressures around 1 atmosphere (atm). In fact, its compressibility deviates from 1.0 by less than 4% at pressures below 4 atm. As the pressure increases, however, the deviations become increasingly significant. At 100 atm, chlorine is far from ideal because chlorine molecules are close enough together for attractive forces to play a significant role. Figure 10-4 also indicates that up to about 375 atm pressure, the compressibility of Cl_2 is *smaller* than 1, which means that intermolecular attractions hold chlorine molecules somewhat closer

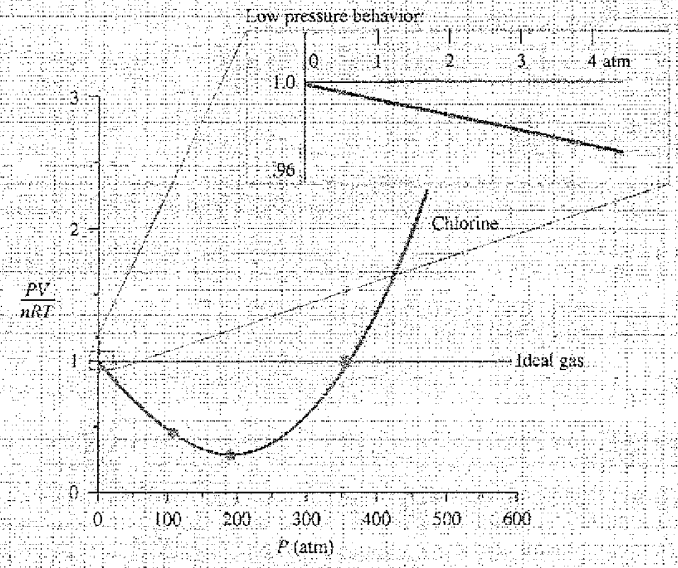


FIGURE 10-4
Variation in PV/nRT with pressure for chlorine gas at room temperature. The inset at the upper right shows the low-pressure region on an expanded scale.

together, on average, than would be the case for an ideal gas. At pressures greater than 375 atm, the compressibility becomes *larger* than 1. This is the effect of finite molecular size. At high enough pressure, molecules are so close together that repulsive interactions outweigh attractive ones.

Every gas deviates from ideal behavior. Given this fact, does it make sense to use the ideal gas model to discuss the properties of real gases? The answer is "yes," as long as conditions do not become too extreme. The gases that chemists usually work with, such as chlorine, helium, and nitrogen, are nearly ideal at and above room temperature at pressures below about 10 atm.

At high pressure, every gas shows deviations from ideal behavior. Figure 10-5 shows compressibilities of He, F_2, CH_4, and N_2, which are gases at room temperature. Notice that the compressibility of helium increases steadily as pressure increases. Interatomic forces are too small to reduce the compressibility below 1, but the finite size of helium atoms generates deviations from ideality that become significant at pressures above 100 atm.

Deviations from ideal behavior always decrease as temperature increases. Figure 10-6 shows compressibility plots for fluorine at several temperatures. Notice that

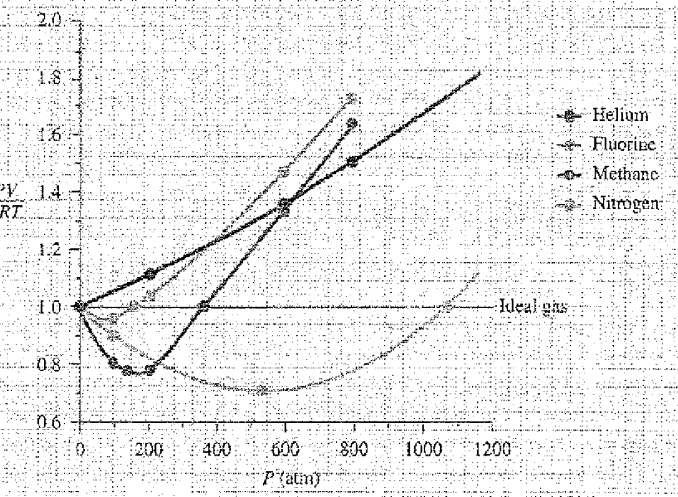


FIGURE 10-5
Compressibilities of He, F_2, CH_4, and N_2 at 300 K. Even substances that we normally think of as gases are not completely ideal.

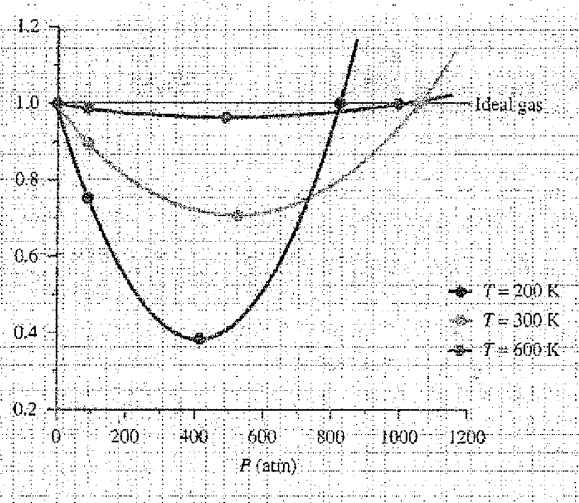


FIGURE 10-6
Variation in PV/nRT with pressure for fluorine gas at several temperatures. As temperature increases, the behavior of all gases becomes more nearly ideal.

fluorine deviates considerably from ideal behavior at 200 K and high pressure but that it is nearly ideal at 600 K, even at 1000 atm. High temperature means high average kinetic energy, and molecules with high energy have more than enough energy to overcome intermolecular forces of attraction.

DISPERSION FORCES

The strength of intermolecular interactions in a liquid determines its normal boiling point, which is the temperature at which liquid converts to vapor at a pressure of 1.00 atm. A liquid boils when the average kinetic energy of its molecules becomes larger than the stabilization energy between molecules. Thus a low boiling point signifies small intermolecular forces, whereas a high boiling point signifies large intermolecular forces. Among the halogens, fluorine boils at −188 °C, chlorine at −34 °C, bromine at 59 °C, and iodine at 185 °C. These boiling points indicate that intermolecular forces between halogen molecules increase with atomic number.

To explain this trend in forces, we can examine what happens when two halogen molecules approach each other. Each molecule contains positive nuclei surrounded by a cloud of negative electrons. As two molecules approach each other, the nucleus of one molecule attracts the electron cloud of the other. At the same time the two electron clouds repel each other. Because electrons are highly mobile, however, their orbitals can change shape to minimize electron-electron repulsion, as shown in Figure 10-7. This distortion of the electron cloud creates a temporary charge imbalance, giving the molecule a slight positive charge at one end and a slight negative charge at the other. The net attractive forces generated by all these temporary charge imbalances are called **dispersion forces.**

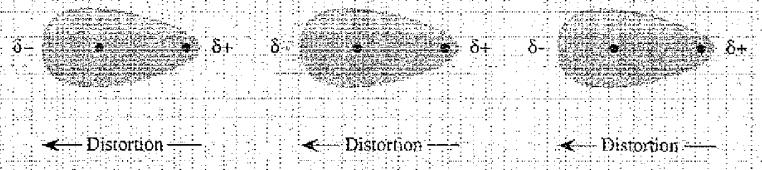


FIGURE 10-7
Schematic view of how dispersion forces arise. As the molecule in the center approaches the one on the left, its electron cloud distorts slightly in response to coulombic attraction to the nuclei of the other molecule. This creates a small, temporary positive charge at the right end of the center molecule, which in turn distorts the electron cloud of the molecule on the right.

FIGURE 10-8
Iodine's electron cloud is much larger and much more polarizable than fluorine's. Iodine therefore has stronger dispersion forces than fluorine, which is why F_2 is a gas and I_2 is a solid at room temperature.

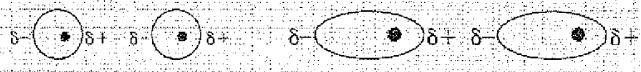
The magnitude of dispersion forces depends on how easy it is to distort the electron cloud of the molecule. The ease of distortion is called molecular **polarizability** because distortion of an electron cloud generates a temporary polarity within the molecule. As Figure 10-8 illustrates, the large electron cloud of an I_2 molecule distorts more readily than the small electron cloud of F_2. Both F_2 and I_2 contain 14 valence electrons, but those of F_2 are in relatively compact $n = 2$ orbitals, whereas those of I_2 occupy highly diffuse $n = 5$ orbitals. As a result, the valence orbitals of I_2 distort much more readily than those of F_2, generating large dispersion forces that make I_2 a solid at room temperature. This reasoning is extended to include the elemental rare gases in Sample Problem 10-1.

SAMPLE PROBLEM 10-1 BOILING POINT TRENDS

At room temperature, neon and xenon are gases, but both become liquids if the temperature is low enough. Draw a molecular picture showing the relative sizes and polarizabilities of atoms of neon and xenon, and use the picture to determine which substance has a lower boiling point.

METHOD: The boiling point of a substance depends on the magnitude of its intermolecular forces, which in turn depend on the polarizability of its electron cloud. Monatomic gases contain atoms rather than molecules, so we must assess *interatomic* forces for these substances.

The only force acting between atoms of rare gases is due to the polarizability of their electron clouds. The valence electrons of neon are in small, $n = 2$ atomic orbitals that have low polarizability, whereas those of xenon are in relatively large, polarizable $n = 5$ orbitals. The smaller electron cloud of neon distorts less than the larger electron cloud of xenon when two atoms approach each other, as a molecular picture illustrates.



Neon ($n = 2$) Xenon ($n = 5$)

Less polarizability means smaller partial charges and weaker intermolecular forces. Thus neon has the lower boiling point.

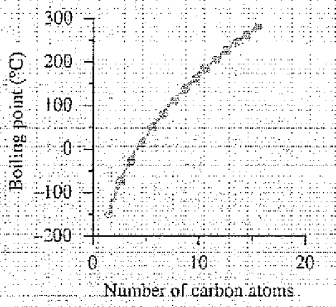


FIGURE 10-9
The boiling points of alkanes increase with the length of their carbon chains.

It is easier to distort the electron cloud of a large molecule than of a small molecule. Thus size also affects polarizability. Figure 10-9 shows how the boiling points of alkanes change as the carbon chain gets longer. As alkanes get longer, their electron clouds become larger and more polarizable, making dispersion forces larger and raising the boiling point. For example, at room temperature methane (CH_4) is a gas, pentane (C_5H_{12}) is a liquid, and eicosane ($C_{20}H_{42}$) is a waxy solid. Figure 10-10 compares the polarizabilities of pentane and decane.

Among otherwise similar substances, more extended molecules have higher polarizabilities than more compact molecules. This trend is illustrated by the boiling points of the three isomers with the formula C_5H_{12}. Figure 10-11 shows that the

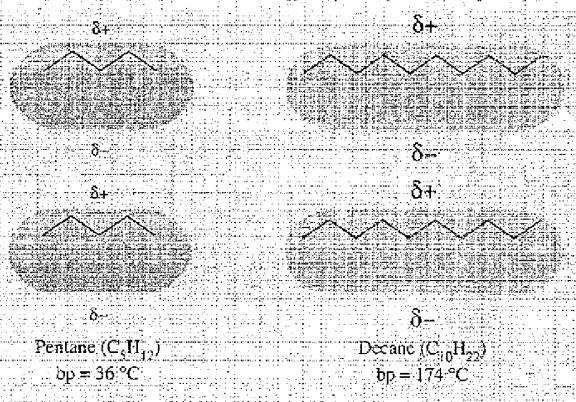


FIGURE 10-10
Large alkanes have higher boiling points than small alkanes. Their dispersion forces are larger because of the increased polarizability of their larger electron clouds.

highest-boiling isomer, pentane, is most extended and the lowest-boiling isomer, 2,2-dimethylpropane, is most compact, with 2-methylbutane in between in compactness and boiling point.

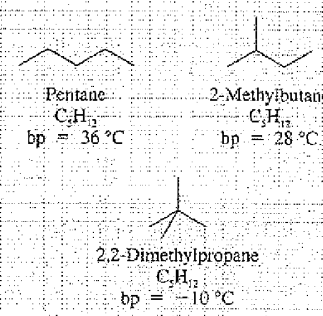


FIGURE 10-11
The three isomers with chemical formula C_5H_{12} have somewhat different boiling points because polarizability increases as molecules become more extended.

DIPOLAR FORCES

Dispersion forces exist between all molecules, but some substances remain liquid at much higher temperatures than can be accounted for by dispersion forces alone. As examples, consider 2-methylpropane and acetone, whose structures are shown in Figure 10-12. These two molecules have the same molar mass, similar shape, and nearly the same number of valence electrons (34 vs. 32). They are so similar that we might expect the two compounds to have similar boiling points, but acetone is a liquid at room temperature, whereas 2-methylpropane is a gas. Acetone boils at 56 °C, whereas 2-methylpropane boils at −12 °C. Why does acetone remain a liquid at temperatures well above the boiling point of 2-methylpropane? The cause is charge asymmetry in the molecular structure of acetone.

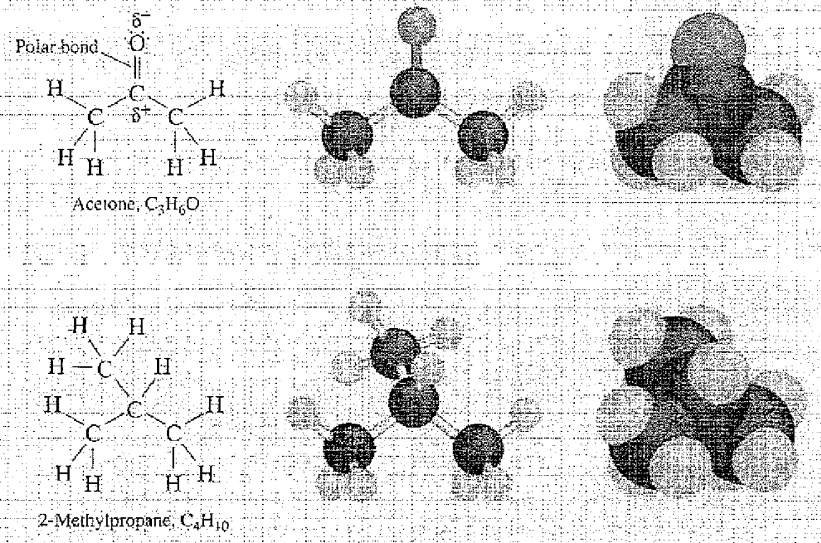


FIGURE 10-12
Models and Lewis structures of 2-methylpropane and acetone show that they have similar molecular shapes. The important difference between them is the polar bond in acetone.

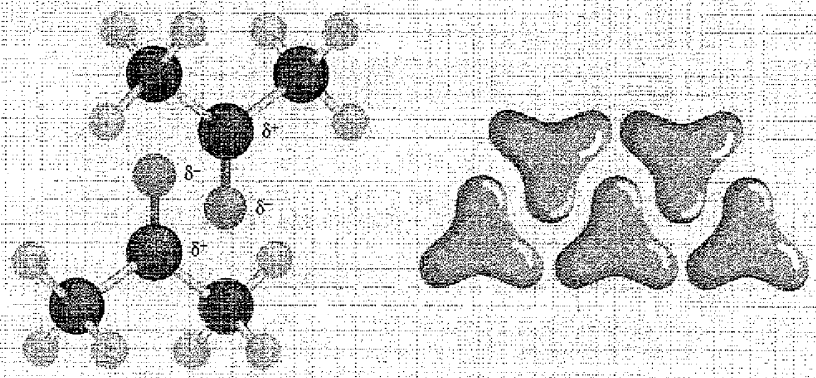


FIGURE 10-13
In liquid acetone the permanent dipoles tend to align with positive ends nearer negative ones and negative ends nearer positive ones.

Electronegativity and polarized bonds were introduced in Section 8.2.

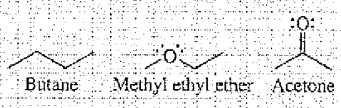
Remember from the bonding picture presented in Chapter 8 that chemical bonds are polarized toward the more electronegative atom. Whereas carbon ($\chi = 2.5$) and hydrogen ($\chi = 2.2$) have nearly equal electronegativity, the electronegativity of oxygen is considerably larger ($\chi = 3.4$). Thus a C—O bond is highly polarized, with a partial negative charge on the oxygen atom and a partial positive charge on the carbon atom.

When two polar acetone molecules approach each other, they align with the positive end of one molecule close to the negative end of the other. In a liquid array, this repeating pattern of head-to-tail alignment gives rise to significant net attractive **dipolar forces** among the molecules. Figure 10-13 illustrates this schematically.

The dispersion forces in acetone are about the same as those in 2-methylpropane, but the addition of dipolar forces makes the total amount of intermolecular attraction between acetone molecules substantially greater than that between molecules of 2-methylpropane. Consequently, acetone boils at a considerably higher temperature than 2-methylpropane. Sample Problem 10-2 provides some additional comparisons of dispersion forces and dipole forces.

SAMPLE PROBLEM 10-2 BOILING POINTS AND STRUCTURE

The line structures of butane, methyl ethyl ether, and acetone are as follows. Explain the trend in boiling points: butane (0 °C), methyl ethyl ether (8 °C), and acetone (56 °C).



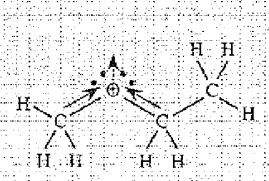
METHOD: We can explain these boiling points in terms of dispersion forces and dipolar forces. First, assess the magnitudes of dispersion forces, which are present in all substances, and then look for molecular polarity.

Dispersion forces depend primarily on the size of the electron cloud and secondarily on the shape of the molecule. A table helps organize the available information.

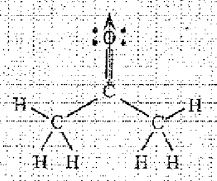
Substance	Boiling point	Electrons	Shape
Butane	0 °C	34	Elongated
Methyl ethyl ether	8 °C	34	Elongated
Acetone	56 °C	32	Compact

The table shows that dispersion forces alone cannot account for the range in boiling temperatures. Methyl ethyl ether and butane have the same number of electrons and similar shapes; yet their boiling points are different. Acetone, which has fewer electrons and a more compact shape than the other compounds, has smaller dispersion forces; yet it boils at a higher temperature. The order of boiling points indicates that acetone is a more polar molecule than methyl ethyl ether, which in turn is more polar than butane.

We expect butane to have a low polarity because of the small electronegativity difference between carbon and hydrogen. Acetone and methyl ethyl ether, on the other hand, contain polar C—O bonds. The molecular geometry about the polar C—O bonds reveals why acetone is more polar than methyl ethyl ether. The full Lewis structures of these molecules show that the oxygen atom in the ether has a steric number of four and bent geometry. Arrows show the charge displacement for each polar bond.



Methyl ethyl ether



Acetone

Notice that the two C—O bond dipoles in methyl ethyl ether partially cancel each other, leaving a relatively small polarity, whereas the polar C—O bond in acetone is unopposed. Thus acetone is more polar than methyl ethyl ether.

SECTION EXERCISES

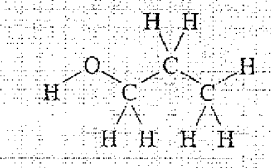
10.1.1 On the basis of the behavior of the other elements of Group VII, predict whether At$_2$ will be a gas, liquid, or solid at room temperature. Sketch its intermolecular stabilization energy curve relative to that of F_2.

10.1.2 From the compressibility curves shown in Figure 10-5, determine which of the four gases has the largest intermolecular forces and which has the smallest. State your reasoning.

10.1.3 Explain the following differences in normal boiling points:
(a) Kr boils at −152 °C, and propane boils at −42 °C.
(b) $C(CH_3)_4$ boils at 10 °C, and CCl_4 boils at 77 °C.
(c) N_2 boils at −196 °C, and CO boils at −91.5 °C.

10.2 HYDROGEN BONDING

Methyl ethyl ether is a gas at room temperature (boiling point, or bp, = 8 °C), whereas 1-propanol, whose structure is shown in Figure 10-14, is a liquid (bp = 97 °C). Both compounds have the same molecular formula, C_3H_8O, and both have chains of four atoms, C—O—C—C and O—C—C—C. Consequently, the electron clouds of these two molecules are about the same size, and their dispersion forces are comparable. Each molecule has an sp^3-hybridized oxygen atom with two polar single bonds, so their dipolar forces should be similar. The very different boiling points of 1-propanol and methyl ethyl ether make it clear that dispersion and dipolar forces do not reveal the entire story of intermolecular attractions.



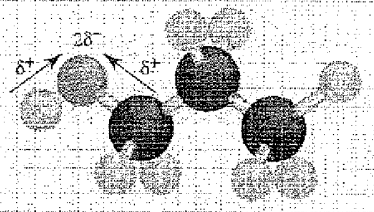


FIGURE 10-14
The Lewis structure and ball-and-stick model of 1-propanol. Polar bonds to the oxygen atom have been highlighted in the ball-and-stick model.

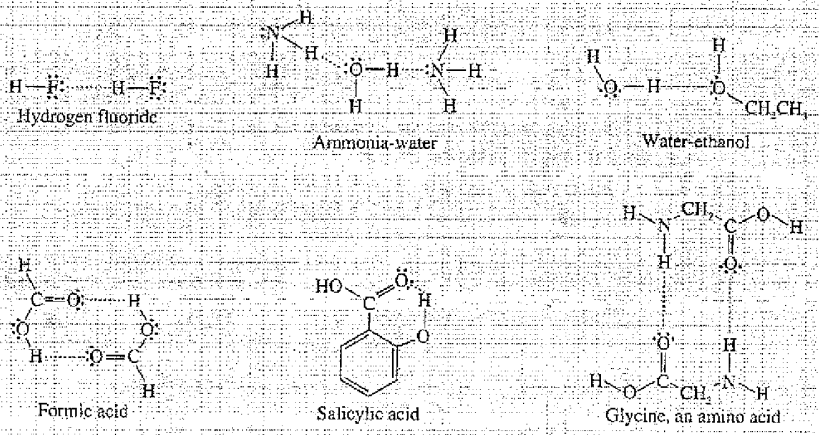


FIGURE 10-15
Examples of hydrogen bonding among some small molecules.

The forces of attraction between 1-propanol molecules are stronger than those between methyl ethyl ether molecules because of a special intermolecular interaction called a **hydrogen bond.** A hydrogen bond occurs when electrons from a highly electronegative atom are partially shared with a positively polarized hydrogen atom. Hydrogen bonds are only 5% to 10% as strong as covalent bonds, but they are comparable to and sometimes stronger than dipolar and dispersion interactions.

There are two requirements for hydrogen bond formation. The covalent bond to hydrogen must be highly polar, and there must be nonbonding electrons on a highly electronegative atom. These requirements restrict hydrogen bond formation to molecules that have hydrogen atoms bonded to fluorine, oxygen, and nitrogen. The presence of any of these elements signals that hydrogen bonding may occur. Figure 10-15 shows representative examples of hydrogen bonding. Dashed lines designate hydrogen bonds to indicate the partially bonding nature of these interactions.

Chlorine and sulfur atoms are also sufficiently electronegative to participate in hydrogen bonding, and there is some evidence for such bonding in HCl. However, the nonbonding electrons on these atoms are in diffuse 3p orbitals that do not interact as strongly with a hydrogen atom as electrons in more compact 2p orbitals.

Notice from the examples shown in Figure 10-15 that hydrogen bonds can form between *different* molecules (for example NH_3···H_2O) and *identical* molecules (for example, HF···HF). Also notice that molecules can form more than one hydrogen bond (for example, glycine) and that hydrogen bonds can form within a molecule (for example, salicylic acid) and between molecules. Sample Problem 10-3 explores the possibilities for hydrogen bond formation.

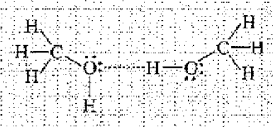
SAMPLE PROBLEM 10-3 FORMATION OF HYDROGEN BONDS

In which of the following systems will hydrogen bonding play an important role: CH_3F, $(CH_3)_2CO$ (acetone), CH_3OH, and NH_3 dissolved in $(CH_3)_2CO$?

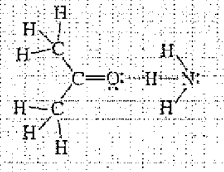
METHOD: Hydrogen bonds occur when both polar H—X bonds and electronegative atoms with nonbonding pairs of electrons are present. Lewis structures provide the best starting point in determining whether these requirements are met:

Acetone and CH_3F each has an electronegative atom with nonbonding pairs, but neither has highly polar H—X bonds. Thus there is no hydrogen bonding between molecules of these substances.

Its O—H bond gives CH_3OH an electronegative atom with nonbonding pairs and a polar O—H bond. Hydrogen bonding occurs between the O—H hydrogen atom on one molecule and the oxygen atom of a neighboring molecule:



For a solution of ammonia in acetone, we must examine both components. Acetone has an electronegative oxygen atom with nonbonding pairs, whereas NH_3 has a polar N—H bond. Consequently, a mixture of these two compounds will display hydrogen bonding between ammonia's hydrogen atoms and acetone's oxygen atoms:



For extra practice, draw a similar picture that shows the hydrogen bonding in a solution of acetone in water.

BINARY HYDROGEN COMPOUNDS

The graph in Figure 10-16 shows that there are regular periodic trends in the boiling points of the binary hydrogen compounds. For each column of the periodic table the boiling points of the binary hydrogen compounds increase from top to bottom of the column. This trend can be attributed to increasing dispersion forces: The more electrons the molecule has, the stronger the dispersion forces and the higher the boiling

Many elements in the *p* block of the periodic table have electronegativities close to that of hydrogen. This means that the H-X bonds have low bond polarity, so dispersion forces dominate the intermolecular interactions.

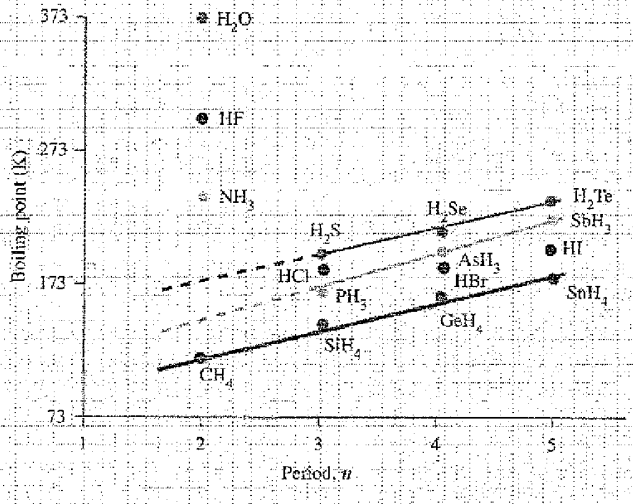


FIGURE 10-16
Periodic trends in the boiling points of binary hydrogen compounds. Notice that H_2O, HF, and NH_3 are exceptions to the trends.

point. For example, H_2S (18 electrons) boils at −60 °C, whereas H_2Se (36 electrons) and H_2Te (54 electrons) boil at −41 °C and −4 °C, respectively.

Ammonia, water, and hydrogen fluoride depart dramatically from the periodic behavior illustrated in Figure 10-16. This is because their molecules experience particularly large intermolecular forces resulting from hydrogen bonding. In hydrogen fluoride, for instance, partial donation of an electron pair from the highly electronegative fluorine atom of one HF molecule to the electron-deficient hydrogen atom of another HF molecule creates a hydrogen bond. Similar interactions among many HF molecules result in a network of hydrogen bonds that gives HF a boiling point much higher than those of HCl, HBr, and HI.

Water has a substantially higher boiling point than hydrogen fluoride, which indicates that the overall strength of hydrogen bonding in H_2O is greater than that in HF. Fluorine has the highest electronegativity, however, so the strongest *individual* hydrogen bonds are those in HF. The higher boiling point of water reflects the fact that it forms more hydrogen bonds *per molecule* than hydrogen fluoride.

Every hydrogen atom in liquid HF is involved in a hydrogen bond, but there is only one polar hydrogen atom per molecule. Thus each HF molecule participates in two hydrogen bonds with two other HF partners. There is one hydrogen bond involving the partially positive hydrogen atom and a second involving the partially negative fluorine atom. In contrast, a water molecule has *two* hydrogen atoms that can form hydrogen bonds and *two* nonbonding electron pairs on each oxygen atom. This permits every water molecule to form *four* hydrogen bonds.

Hydrogen bonding in liquid water and, even more strikingly, in solid ice creates a three-dimensional network that puts each oxygen atom at the center of a distorted tetrahedron. Figure 10-17 shows that two arms of the tetrahedron are regular covalent O—H bonds, whereas the other two arms of the tetrahedron are hydrogen bonds to two different water molecules.

HYDROGEN BONDING IN BIOMOLECULES

Hydrogen bonding is particularly important in biochemical systems because biomolecules contain many oxygen and nitrogen atoms that participate in hydrogen bonding. For example, the amino acids from which proteins are made contain NH_2

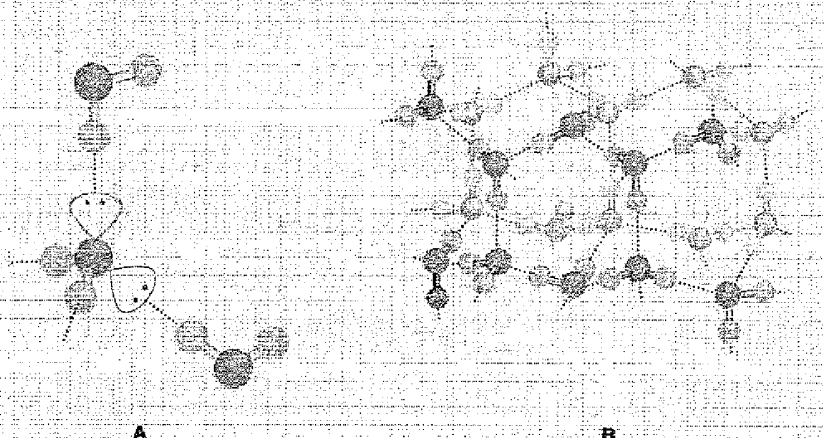
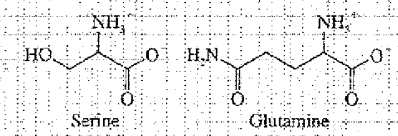


FIGURE 10-17
The structure of ice. **A,** Each oxygen atom is at the center of a distorted tetrahedron of hydrogen atoms. The tetrahedron is composed of two short covalent O····H bonds and two long H—O hydrogen bonds. **B,** Water molecules are held in a network of these tetrahedra.

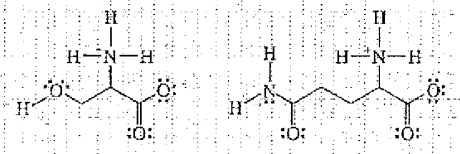
(amino) and CO_2H (carboxylic acid) groups. Four different types of hydrogen bonds exist in these systems: O—H···N, N—H···O, O—H···O and N—H···N. Hydrogen bonding between glycine molecules is shown in Figure 10-15, and Sample Problem 10-4 provides further illustrations. We examine more details of hydrogen bonding in biomolecules in Chapter 11.

SAMPLE PROBLEM 10-4 HYDROGEN BONDING IN AMINO ACIDS

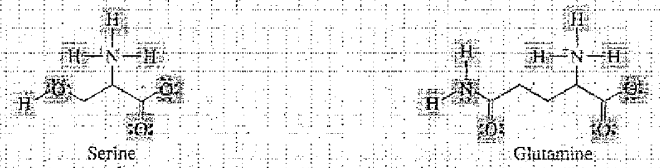
In aqueous solution, amino acids undergo intramolecular proton transfer to form ions. The line structures of two amino acid ions, serine and glutamine, follow. For each molecule, identify the hydrogen atoms that can form hydrogen bonds and the electronegative atoms to which hydrogen bonds can form.



METHOD: Hydrogen atoms in polar H—X bonds can form hydrogen bonds with lone pairs on other nitrogen, oxygen, or fluorine atoms. To identify hydrogen bonding possibilities, we need Lewis structures to determine the locations of nonbonding pairs of electrons. Carbon atoms are never involved in hydrogen bonds, so we can ignore the carbon framework of the molecules. The following partial Lewis structures show the nonbonding pairs of electrons and the polar H—X bonds:



Any N—H or O—H hydrogen atom in these molecules can participate in hydrogen bonding. These are highlighted in blue in the following structures. The N atoms and O atoms with lone pairs of electrons can also participate in hydrogen bonding. These atoms are highlighted in yellow.



Dispersion forces, dipole interactions, and hydrogen bonds are all much weaker than covalent intramolecular bonds. For example, the average C—C bond energy is 345 kJ/mol, whereas dispersion forces are just 0.1 to 5 kJ/mol for small alkanes such as propane. Moreover, dipolar interactions between polar molecules such as acetone range from 5 to 20 kJ/mol, and hydrogen bonds vary from 5 to 50 kJ/mol.

Recall from Chapter 9 that bond energy is the amount of energy required to break 1 mol of a particular bond. Table 9-2 lists bond energies.

SECTION EXERCISES

10.2.1 Which has a higher boiling point, CH_3F or CH_3OH? Why? Illustrate your answer with a molecular picture.

10.2.2 Acetone and methanol have nearly equal boiling points. What types of intermolecular forces does each exhibit? What does the similarity in boiling points tell you about the relative magnitudes of each type of force in these two compounds?

10.2.3 There are nine important hydrogen bonding interactions. One of them is O—H···O. Draw the other eight. For each of the nine, draw a Lewis structure of a specific example using real molecules.

10.3 PROPERTIES OF LIQUIDS

Liquids are intermediate in behavior between gases and solids. Whereas intermolecular forces among gas molecules are weak enough to allow molecules of a gas to move freely throughout their container, intermolecular forces in a solid are strong enough to hold the molecules fixed in place. In a liquid, intermolecular forces confine the molecules to a specific volume, but they are insufficient to keep the molecules from moving from place to place within the body of the liquid. Table 10-1 summarizes the physical properties of the three states of matter. Notice how the properties of liquids fall in between those of gases and solids.

SURFACE TENSION

Water drips from a faucet in nearly spherical liquid droplets rather than in a film. Drops are more stable than films because of intermolecular attractions. The physical property describing this increased stability is **surface tension,** which is the resistance of a liquid to an increase in its surface area.

Figure 10-18 illustrates at the molecular level why liquids exhibit surface tension. A molecule in the *interior* of a liquid is completely surrounded by other molecules, each of which exerts attractive forces as described in the previous sections. A molecule at a liquid *surface*, on the other hand, has other molecules beside it and beneath it but *none above it*. As a result, the net intermolecular force on molecules at the surface pulls them toward the interior of the liquid.

Molecules at the surface of a liquid are less stable than those within it, so a liquid is most stable when the fewest molecules are at its surface. This occurs when the liquid has minimum surface area. Spheres have less area per unit volume than any

It is not absolutely correct to say that there are *no* molecules above a surface, for there are always molecules in the gas above the liquid. However, the concentration of molecules in the gas phase is so low that they can be ignored.

TABLE 10-1 PHYSICAL PROPERTIES OF THE STATES OF MATTER

PROPERTY	GAS	LIQUID	SOLID
Volume	Variable	Fixed	Fixed
Shape	Variable	Variable	Fixed
Compressibility	Large	Almost zero	Almost zero
Fluidity	Very high	High	Very low
Diffusion rate	High	Moderate	Very low

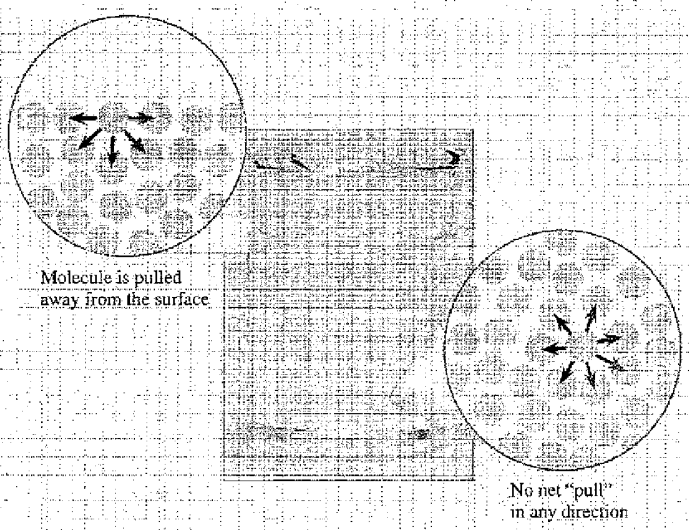
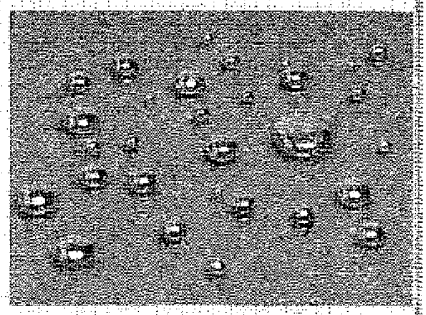


FIGURE 10-18
In the interior of a liquid *(right)*, each molecule experiences equal forces in all directions. A molecule at the surface of a liquid *(left)* is pulled back into the liquid by intermolecular forces.

other shape, so small drops of a liquid tend to be spheres. Large drops are affected by gravitational forces, which distort them from spheres.



A small drop of mercury adopts a spherical shape, which minimizes the number of atoms at the surface. Gravitational force "flattens out" larger, more massive drops.

ADHESIVE FORCES

Molecules in liquids in contact with the solid surfaces of their containers experience two different sets of forces. First, they are attracted to the other molecules in the liquid, as just described. The intermolecular forces between liquid molecules are called **cohesive forces.** Second, they are attracted to molecules in the container walls. The intermolecular forces between molecules in the wall of a container and the molecules of a liquid are called **adhesive forces.**

One manifestation of adhesive forces is the curved surface of an aqueous solution contained in a narrow glass tube. Glass surfaces are mostly silicates with many exposed oxygen atoms and O—H groups. These form strong hydrogen bonds with water molecules. As a result, water adheres well to glass. The liquid maximizes its contact with the walls by forming a concave surface.

Figure 10-19 shows that the curvature of the liquid surface increases as the diameter of the tubing becomes smaller. When the tube diameter is smaller than a few millimeters, water actually "climbs the walls," pulled upward by forces of adhesion. The upward movement of water against the force of gravity is called **capillary action.** Capillary action can also operate in reverse. For example, water molecules do not adhere well to a surface coated with a film of wax, so immersing a wax-coated capillary tube in water will cause the water level to fall rather than rise. Sample Problem 10-5 treats another example of reverse capillary action.

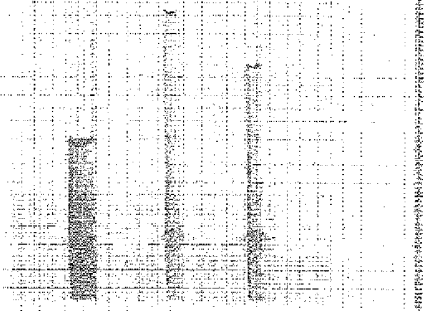


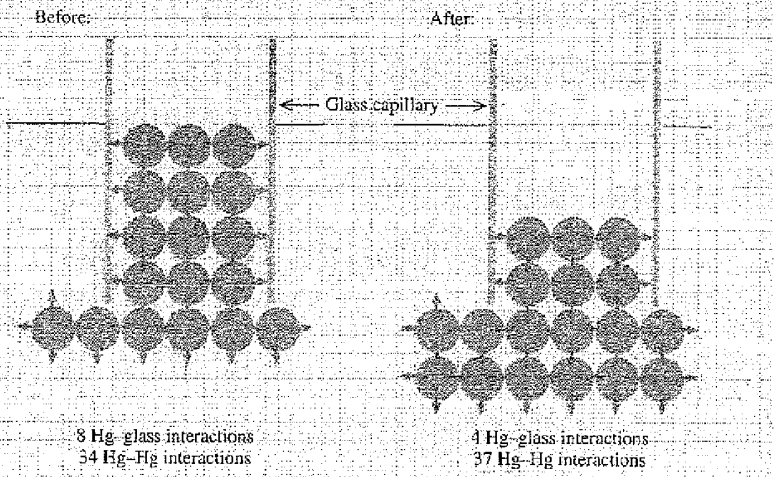
FIGURE 10-19
When adhesive forces exceed cohesive forces, a liquid takes on a shape that maximizes its contact with the walls of the container. Water forms a concave surface in cylindrical glass containers and rises inside narrow-diameter tubing.

SAMPLE PROBLEM 10-5 CAPILLARY ACTION IN MERCURY

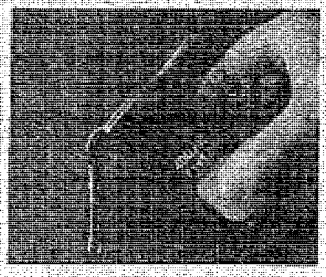
When a narrow tube is inserted into liquid mercury, the liquid level inside the tube *drops*. Explain this observation in terms of intermolecular forces, and illustrate it with a drawing.

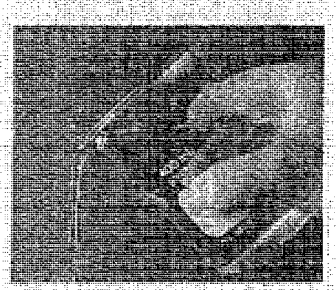
METHOD: The balance between cohesive intermolecular forces (within the liquid) and adhesive intermolecular forces (between liquid and solid molecules) determines how a liquid behaves in contact with a solid surface. We must interpret the observation in terms of the contacts between liquid and solid molecules.

The observation is that the liquid level *drops* when a tube is inserted into liquid mercury. A picture helps show what takes place.



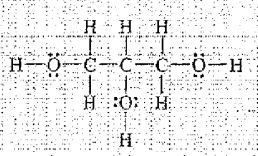
When the liquid inside the tube falls to a lower height, the number of *adhesive* interactions decreases, because fewer liquid atoms are in contact with the solid glass surface. Conversely, the number of *cohesive* interactions increases because the mercury now has less surface area. We conclude that cohesive forces in liquid mercury are *greater* than adhesive forces between mercury atoms and glass.





Honey is far more viscous than water.

Glycerol is highly viscous because its molecules are large enough for some tangling to occur and because it forms extensive hydrogen bonding networks via its three polar O—H bonds:



VISCOSITY

Water can be poured very quickly from one container to another, salad oil pours more slowly, and honey on a cold day seems to take forever. A liquid's resistance to flow is called its **viscosity**; the larger its viscosity, the more slowly the liquid pours. Viscosity can be determined by measuring the time it takes for a specific amount of liquid to flow through a tube of known diameter under the force of gravity.

Viscosity measures how easily molecules slide by one another, and this depends on molecular shapes and intermolecular forces. Molecular shape strongly influences viscosity. Liquids such as water, acetone, and benzene, whose molecules are small and compact, have low viscosity. In contrast, large molecules such as the hydrocarbons found in oils tend to get tangled up with each other. Tangling inhibits the flow of molecules and leads to high viscosity. In addition, strong cohesive forces make it harder for molecules to move about. As a result, substances whose molecules form hydrogen bonds have higher viscosity than those whose molecules do not. Hydrogen bonding makes water more viscous than acetone (C_3H_6O). Glycerol, whose molecules combine a hydrocarbon's tendency to tangle with the "stickiness" of hydrogen bonding, has a very high viscosity.

Molecules move faster as temperature increases, and this allows them to slide by one another more easily. Consequently, viscosity decreases as temperature increases. This dependence is quite noticeable for highly viscous substances such as honey and syrup, which are much easier to pour when hot than when cold.

SECTION EXERCISES

10.3.1 Water forms a film on the surface of a "clean" buret and drains without forming droplets. Water forms beads on the surface of a "dirty" buret. What can you conclude about the nature of the forces between clean glass and water molecules and those between dirty glass and water molecules?

10.3.2 Aqueous solutions do not adhere well to polyethylene because polyethylene contains very long chains of nonpolar CH_2 groups: $CH_3—(CH_2)_n—CH_3$, where n is larger than 200. Does the water rise or fall when a polyethylene straw is dipped into a glass of water? Draw a picture that shows what happens to the water molecules inside the straw. You need not show the details of structure of the surface or water molecules, but your drawing should indicate the adhesive and cohesive interactions.

10.3.3 In what way is a beaker of octadecane like a plate of spaghetti? Octadecane is the C_{18} linear alkane.

10.4 PROPERTIES OF SOLIDS

One of the most active areas of research in modern chemistry, physics, and engineering is the development of new solid materials. Solids play an ever-expanding role in modern society, from complex materials that act as high-temperature superconductors, to heat-resistant tiles for the outer "skin" of the space shuttle, to new tissue-compatible solids used for surgical implants.

BONDING IN SOLIDS

In preceding chapters, we introduced ionic, metallic, and covalent solids; each is held together by a different type of interaction. As described in Chapter 7, ionic solids contain cations and anions strongly attracted to each other through coulombic interactions. These forces are *interionic* rather than *intermolecular*. As we described in Chapter 9, the solid structure of metals comes from electrons in highly delocalized valence orbitals. Each metal atom can be viewed as a cation embedded in a "sea" of mobile valence electrons. Semimetals such as silicon and germanium also contain delocalized orbitals extending throughout their entire structures. Covalent solids such as quartz, graphite, and diamonds, described in Chapters 8 and 9, contain infinite arrays of atoms, all linked by covalent bonds in a single huge three-dimensional network.

A fourth type of solid contains individual molecules that are held in place by combinations of dispersion and dipolar forces, with hydrogen bonding playing an important role when it is present. Examples of such molecular solids include iodine, ice, table sugar, and wax. Molecular solids tend to have relatively low melting points as a consequence of the relatively small forces that hold their molecules in place.

Solids are classified as **crystalline** or **amorphous**. Crystalline solids have a highly regular appearance because they contain ordered arrays of atoms, molecules, or ions at the microscopic level. Diamonds, sugar crystals, quartz, and table salt are examples of crystalline solids. Amorphous solids, on the other hand, show much less regularity in appearance because their molecules are distributed irregularly throughout the solid. Cotton candy, glass, and wax are examples of amorphous solids.

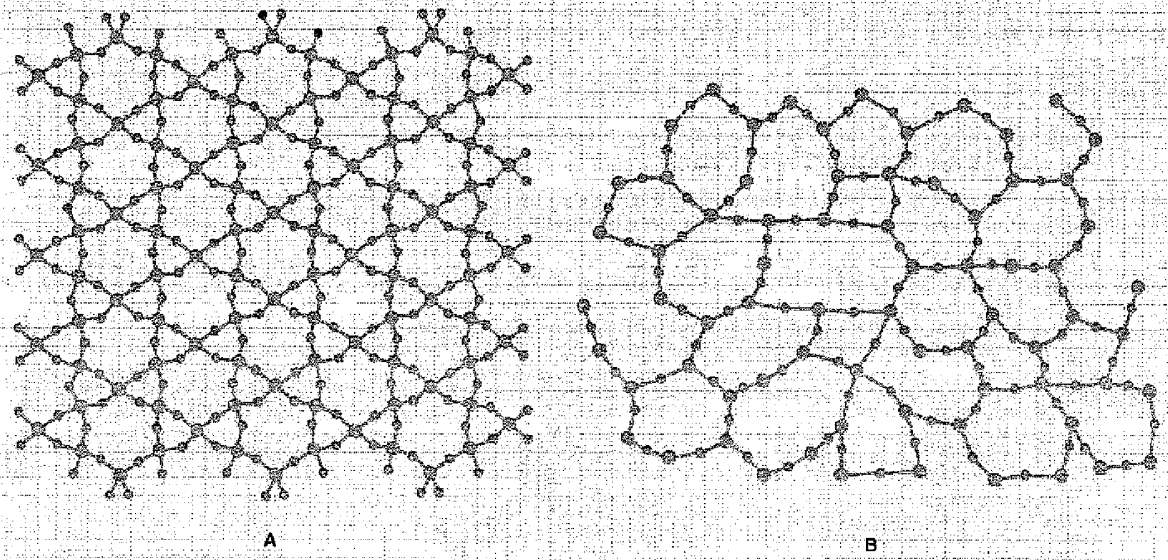
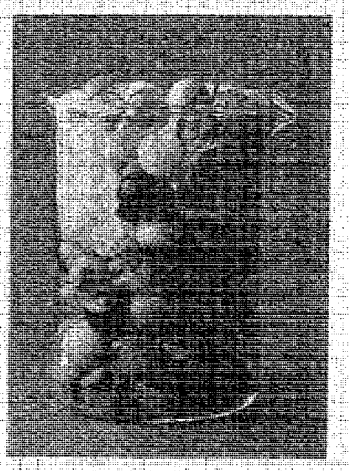


FIGURE 10-20
A, Quartz is a crystalline form of silicon dioxide containing a three-dimensional array of SiO_2 units linked by highly regular covalent bonding. **B,** Glass also has a three-dimensional array of SiO_2 units, but in this case bond arrangement is irregular, and the solid is amorphous. Both **A** and **B** are two-dimensional representations. The actual structures are three-dimensional.

Silicon dioxide forms crystalline and amorphous solids. Quartz is a crystalline form of silicon dioxide found in minerals all over the world. Each tetrahedral silicon atom is bonded to a total of four oxygens in a highly symmetrical three-dimensional network. This strong bonding network gives silicon dioxide its high melting point of 1710 °C. Slow cooling of molten SiO_2 gives crystalline quartz, but rapid cooling gives an amorphous material called *glass*. Figure 10-20 shows representations of quartz and glass. Both structures are three-dimensional networks, but the two-dimensional view given in the figure is sufficient to show the differences between crystalline and amorphous solids.



A close-packed arrangement of marbles packs the maximum number into the minimum volume.

CRYSTALLINE SOLIDS

Crystals (Figure 10-21) often have a high degree of symmetry. Some of the most valued materials of society are precious gemstones, which are crystals of rare and richly colored minerals. The following general features characterize crystalline solids:

- Crystals are uniform in structure. Crystals of a particular substance have common geometric features regardless of how they are formed.
- The shape of a crystal is characterized by its parallel faces and edges. The edges of a crystal usually intersect at fixed angles.
- When a crystal breaks into smaller pieces, fragmentation occurs along crystal edges. The smaller pieces have the same characteristic angles as the original crystal.
- Crystals have a high degree of symmetry.

CLOSE-PACKED CRYSTALS

A solid is most stable when each atom or molecule is close to as many others as possible. An arrangement that accomplishes this is described as a **close-packed**

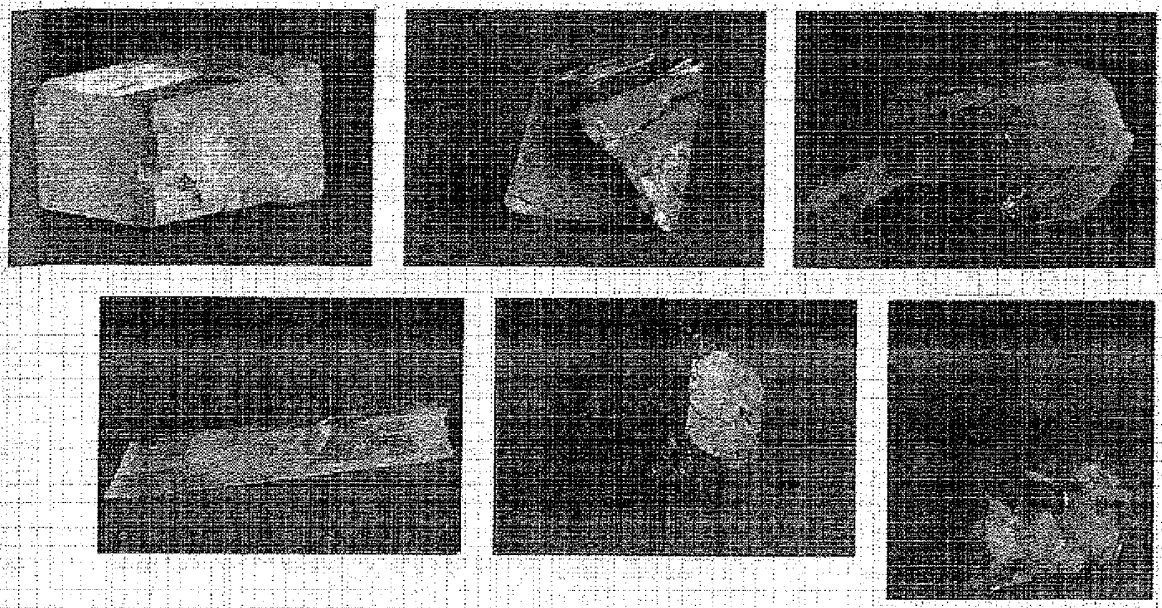


FIGURE 10-21
Crystalline materials can have many shapes. Often, they are highly regular and strikingly beautiful.

structure. Close-packed structures have atoms or molecules arranged so that the empty space around them is minimized.

To visualize a close-packed atomic solid, think of the atoms as spheres that are packed as compactly as possible. Begin by assembling a single planar layer as shown in Figure 10-22, *A*. Notice that the most compact planar arrangement places each sphere within a regular hexagon formed by six others. Now add a second layer of spheres. To achieve a most compact arrangement, each sphere will nestle in one of the "dimples" between a trio of spheres in the first layer, as shown in Figure 10-22, *B*. As additional spheres are added, the second layer eventually looks identical to the lower layer, except that it is offset slightly to allow the spheres to nestle in the dimples formed by the layer below.

Now consider adding a third layer of spheres. This new layer will look just like the other two, but it can nest in two different ways because there are two sets of dimples in the second layer. As Figure 10-22, *C* shows, one set is located directly above the spheres in the first layer. If spheres in the third layer lie in these dimples, the third layer is directly above the first, and the resulting three-dimensional structure is a **hexagonal close-packed structure.** If spheres in the third layer lie in the other set of dimples, the third layer is offset from both of the lower layers. This arrangement is a **cubic close-packed structure.**

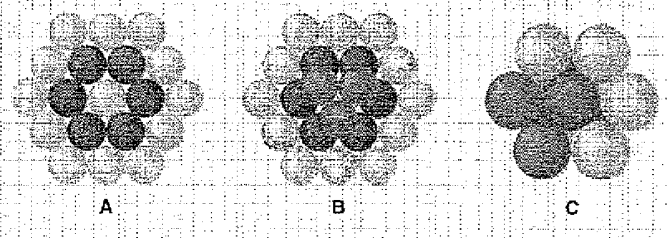
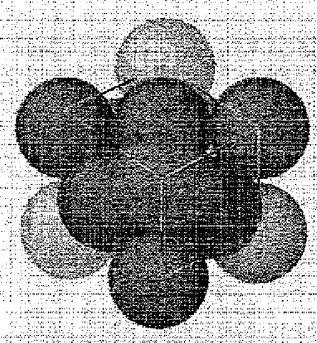
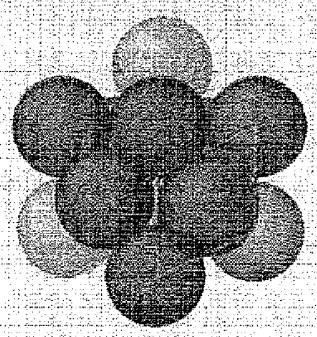


FIGURE 10-22
A, Spheres close-packed in a layer generate a hexagonal pattern. **B,** When a second layer is packed on top of the first, each sphere in the second layer nestles in the dimple created by three adjacent spheres in the lower layer. **C,** The second layer has two different sets of dimples, one directly above the spheres in the first layer (shaded in blue) and the other offset from the spheres in the first layer (shaded in orange).



A



B

FIGURE 10-23
A cubic close-packed array viewed at an angle to reveal its cubic structure. **A,** Two faces of the cube have been outlined. A sphere sits in the center of each face. **B,** One corner sphere has been removed to show more clearly the underlying hexagonal plane of spheres.

To see the cubes in a cubic close-packed structure, we need to rotate the array so that the hexagonal planes tilt upward at a 45-degree angle, as shown in Figure 10-23, *A*. Notice that we have rotated the entire array but have not changed the relative positions of the spheres. From this perspective, three spheres from one hexagonal plane lie along a diagonal of a square, with one sphere from each adjacent plane forming the other two corners of the square. At right angles to this first square are other sets of squares that form cubes. Figure 10-23, *B* shows one such cube with one corner sphere removed to reveal the original hexagonal planar array.

The exploded views in Figure 10-24 show yet another way of looking at the hexagonal close-packed and cubic close-packed crystal types. In the hexagonal close-packed structure, notice that the *third* layer lies directly above the first, the fourth above the second, and so on. Thus we can label the layers ABAB, and so on. In the cubic close-packed structure, the third layer is offset from the other two, but the *fourth* layer is directly above the first. Thus this arrangement can be labelled ABCABC, and so on.

Atoms and molecules with spherical symmetry often form crystals with hexagonal close-packed or cubic close-packed geometry. For instance, magnesium and zinc crystallize with their atoms in a hexagonal close-packed array. Silver, aluminum, and gold, on the other hand, crystallize in the cubic close-packed arrangement. Argon solidifies at low temperature as a cubic close-packed crystal, and neon can solidify in either form.

The packing in ionic crystals requires that ions of opposite charges alternate with one another to maximize interionic attraction. For many 1:1 ionic crystals such as NaCl, the most stable arrangement is two interlocking face-centered cubic arrays, as is illustrated in Figure 10-25.

Another type of arrangement, which is shown in Figure 10-26, *A*, is a **body-centered cubic structure.** A body-centered cube can be constructed by assembling a set of spheres in a *square* planar array, as shown in Figure 10-26, *B*, and then nesting a second set of spheres in the dimples of the first set, as shown in Figure

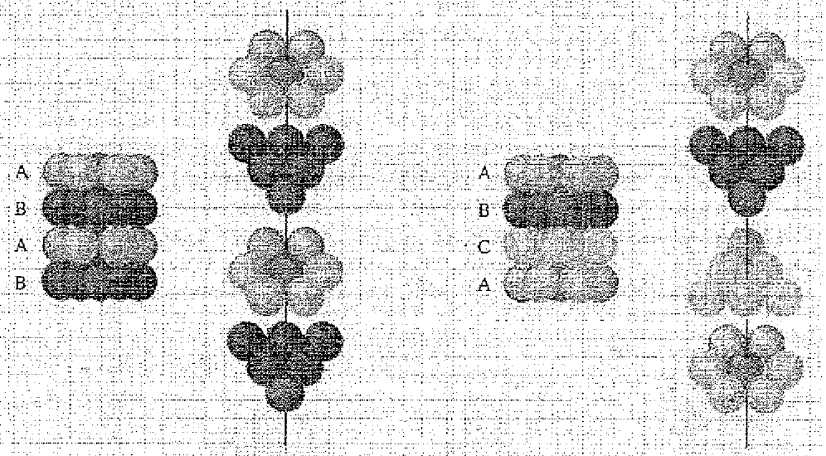


FIGURE 10-24
Side and expanded views of the hexagonal close-packed and cubic close-packed crystal types. In the hexagonal close-packed structure, spheres on both sides of any plane are in the same positions, and the third layer is directly above the first. In the cubic close-packed structure, layers take up three different positions, and the fourth layer is directly above the first.

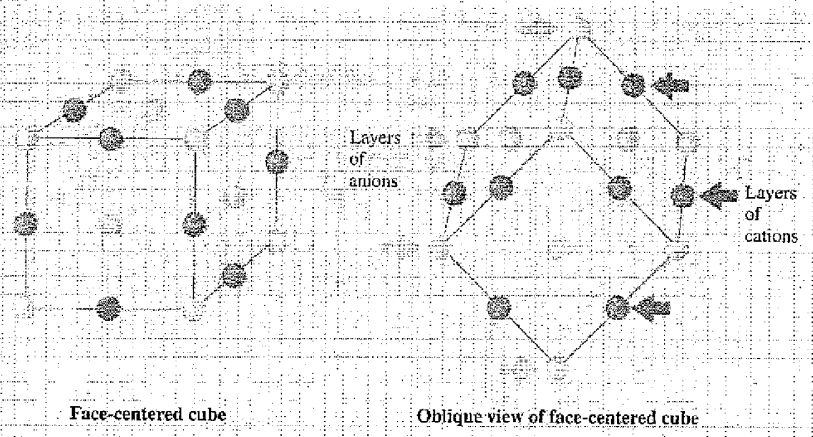


FIGURE 10-25
Ionic crystals such as NaCl contain face-centered cubic arrangements of each ion. In this view a cube is drawn with the cations, shown as yellow spheres, at its corners and in the centers of the faces. The anions, shown as blue spheres, occupy positions at the center of each edge of the cube. A view from an oblique angle reveals that this structure contains alternating hexagonal planar arrays of cations and anions.

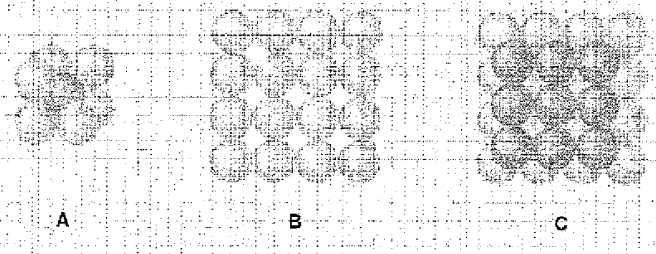


FIGURE 10-26
A body-centered cubic array is made up of layers of spheres arranged in a square pattern. The basic pattern **(A)** has one corner sphere removed to reveal the sphere nested in the center of the cube. The array can be constructed by laying down a square layer of spheres **(B)**, and then placing a second square layer in the dimples between the spheres **(C)**. A third layer directly above the first completes the cubes.

10-26, *C*. This arrangement gives a second square array, on which yet another set can be nested.

UNIT CELLS

Any crystal is a near-infinite array of atoms, molecules, or ions arranged in some regular repeating pattern. Because of this repeating pattern, every crystal has one smallest unit from which the entire pattern can be assembled. This minimum unit is called a **unit cell**. The idea of the unit cell is illustrated in two dimensions by the art of M.C. Escher, as shown in Figure 10-27. Escher often used symmetrical patterns aligned together to create an overall design. The repeating units can be visualized as "tiles" placed edge to edge. A unit cell in a crystal is a three-dimensional fragment stacked together like a set of blocks.

The body-centered cubic crystal provides a good illustration of three-dimensional unit cells. A drawing of the unit cell of this crystal is shown in Figure 10-28, *A*. Notice that it is a cube defined by the centers of eight iron atoms that surround a central iron atom. This cube contains a central Fe atom and portions of additional Fe atoms. The body-centered cubic crystal is built up by stacking together many unit cells, as shown in Figure 10-28, *B*. It takes eight unit cells stacked together to complete one of the corner atoms, so each unit cell contains one eighth of an atom at each of its corners. The cell has eight such corners, so each unit cell contains two complete iron atoms.

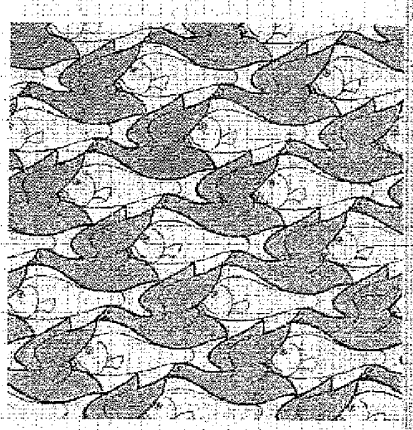


FIGURE 10-27
M.C. Escher used repeating patterns to create works of art with exquisite symmetry. The repeating patterns in Escher's work are two-dimensional analogs of the unit cells that define the symmetry of a crystalline solid.

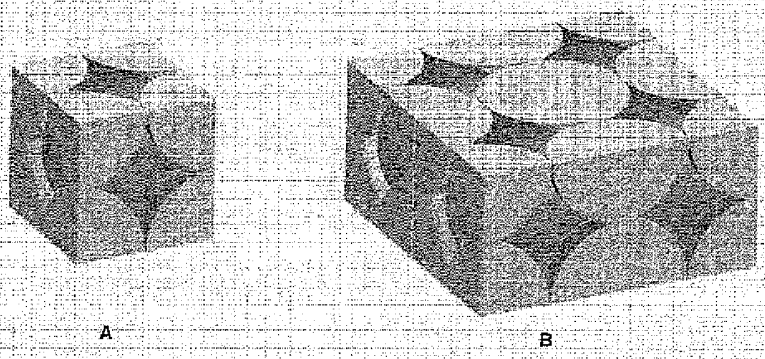


FIGURE 10-28
A, The unit cell of iron, which forms body-centered cubic crystals. There is an iron atom at the center of the unit cell, and each of the eight corners contains one eighth of an iron atom, giving a total of one complete atom. **B,** Four unit cells stacked together. The four unit cells touch at the center, forming half of an iron atom. When a second set of four unit cells is placed on top of the first, the iron atom becomes complete and is surrounded by eight other iron atoms.

MOLECULAR SOLIDS

Up to now, we have described the crystalline arrays favored by spherical objects such as atoms, but most molecules are far from spherical. The photo of stacks of produce in Figure 10-29 illustrates that nonspherical objects require more elaborate arrays to achieve maximum stability. Compare the stack of bananas in the figure with the stack of oranges. Just as the stacking pattern for bananas is less symmetrical than that for oranges, the structural patterns of most molecular crystals are less symmetrical than crystals of spherical atoms, reflecting the lower symmetry of the molecules that make up the crystal.

Dispersion forces, dipolar forces, and hydrogen bonds hold the molecules of molecular crystals in place. Two examples of molecular crystals are naphthalene and benzoic acid. Naphthalene, which is sold as moth balls, forms white crystals in which the planar naphthalene molecules are held in place only by dispersion forces. The lattice structure of this crystal is shown in Figure 10-30. Benzoic acid forms white crystals, too, but its molecules are held in place by a combination of dispersion and hydrogen bonding forces, as illustrated in Figure 10-31.

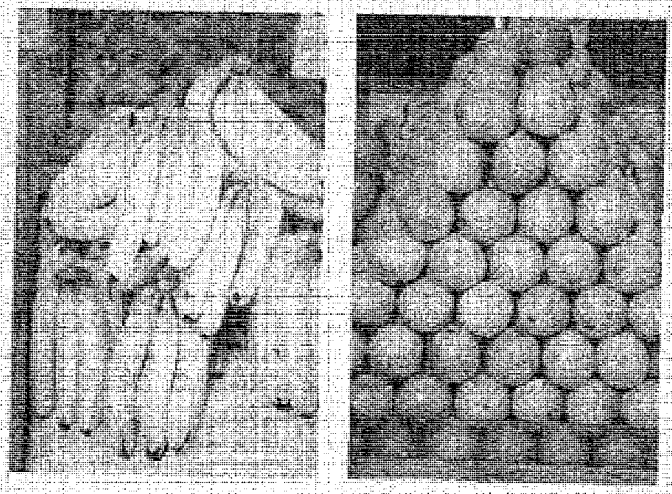
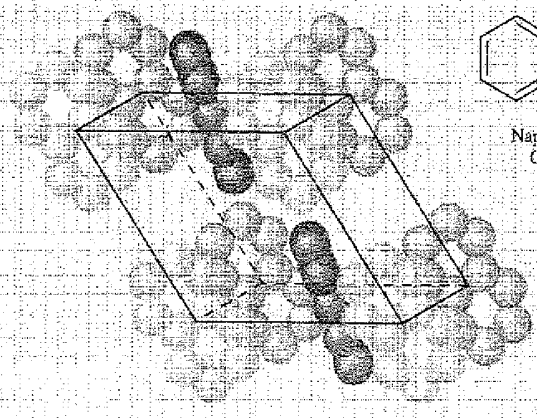


FIGURE 10-29
Nonspherical objects such as bananas require more elaborate packing schemes than spherical objects such as oranges.



Naphthalene
$C_{10}H_8$

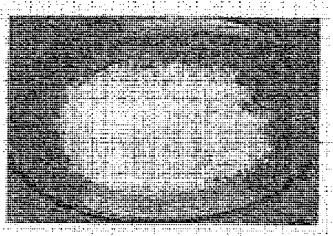
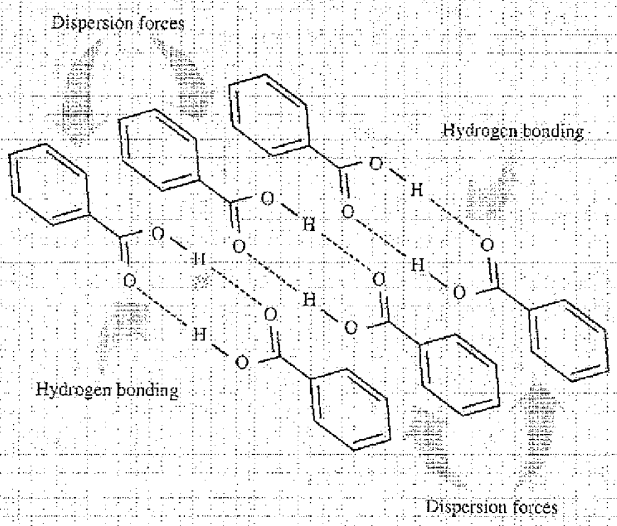


FIGURE 10-30
Naphthalene is a planar molecule. It forms crystals whose unit cell has a molecule at each corner of a prism and two molecules tilted at an angle inside the prism. The crystal is held together by dispersion forces generated primarily by the electrons of the delocalized π system.



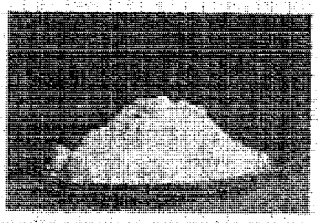


FIGURE 10-31
Crystals of benzoic acid contain pairs of molecules held together head-to-head by hydrogen bonds. These pairs then form stacks, which are held together by dispersion forces.

COVALENT SOLIDS

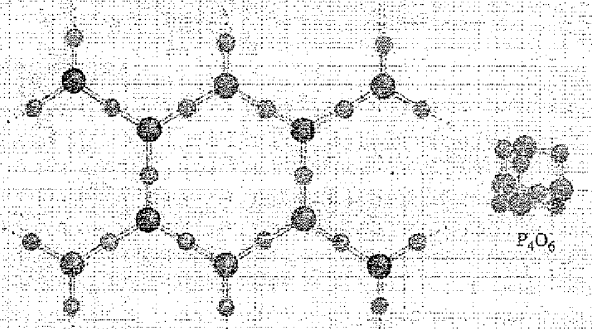
We described the covalent network structure of silicon dioxide in Chapter 8 and at the beginning of this section. Two other covalent solids, diamond and graphite, were introduced in Chapter 9. These two forms of carbon have very different structures (see Box 9-3), but both contain near-infinite arrays of atoms bonded covalently to one another.

Covalent bonds make covalent solids extremely durable. Examples include the "everlasting sands" and the longevity of granite formations such as the Rock of Gibraltar. Diamonds and other valuable gemstones are covalent solids, too. Rubies and sapphires are covalent crystals of aluminum oxide with small amounts of colored transition metal ion impurities. Carborundum is a 1:1 covalent solid of silicon and

carbon that has the same lattice structure as diamonds. Carborundum is considerably less expensive than diamond but almost as strong and wear-resistant, so it is used for the edges of cutting tools. Sample Problem 10-6 compares the structures and properties of a covalent solid and a molecular solid.

SAMPLE PROBLEM 10-6 COVALENT AND MOLECULAR SOLIDS

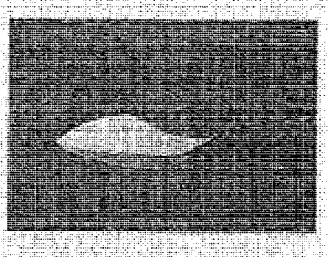
Whereas SiO_2 melts at 1710 °C, other nonmetal oxides melt at much lower temperatures. For example, As_2O_3 sublimes directly to the gas phase at 315 °C, and P_4O_6 melts at 25 °C. Referring to the following bonding pictures and to the bonding pattern of silica in Figure 10-20, describe the forces that hold these solids together.



P_4O_6

As_2O_3

METHOD: Solids may be covalent, ionic, metallic, or molecular, with different forces accounting for the stability of each type of solid. Because these are nonmetal oxides, they cannot be described as metallic. None of these oxides contains ions, so they must be covalent or molecular. The bonding patterns provide the information we need to categorize them and explain their melting temperatures.



SiO_2: The bonding pattern in silica is a three-dimensional array of strong covalent bonds. Many of these bonds must be broken before silica melts. Silica melts at 1710 °C because its three-dimensional covalent bonding network is highly stable.

As_2O_3: The bonding picture shows a two-dimensional network of covalent bonds, with no bonding between molecular planes. In this respect, arsenic trioxide is similar to graphite. It sublimes at a relatively low temperature, indicating that a small amount of energy is required to disrupt this planar bonding network. This is a covalent solid with weak covalent bonds. Actually, when As_2O_3 sublimes, it forms As_4O_6 molecules whose bonding pattern resembles that of P_4O_6. Thus much of the energy required to break the planar bonding network is recovered through the formation of new As—O bonds.

P_4O_6: The molecular structure shows that P_4O_6 is composed of discrete molecular units rather than arrays of covalent bonds. Strong covalent bonding holds the atoms in each molecule together, but each molecular unit is attracted to others only by dispersion forces. This is a molecular solid, so very little energy is required to overcome dispersion forces and allow P_4O_6 molecules to move around in the liquid state.

AMORPHOUS SOLIDS

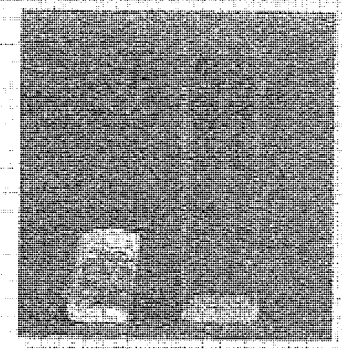
Solid materials are most stable in crystalline form, so when a liquid is cooled slowly, it generally solidifies as crystals. When solids form rapidly, on the other hand, their atoms or molecules may become locked into positions other than those of a regular crystal, giving amorphous materials. Ordinary cane sugar is crystalline as it comes

from the package, but it forms an amorphous solid when it is carefully heated until it melts and then is rapidly cooled. Cotton candy contains long threads of amorphous sugar.

Amorphous comes from Greek, *a* meaning "without," and *morph*, meaning "form."

What we call "glass" turns out to be an entire family of amorphous solids based on silica (SiO_2). Pure silica is usually found as crystals containing the regular array of covalent bonds shown in Figure 10-20. Quartz contains crystals of pure silica, whereas sand is generally crystals of silica and other minerals. When quartz is melted and then quickly cooled, however, it forms fused silica, an amorphous solid glass. Silica glass resists corrosion, transmits light well, and withstands wide variations in temperature, but it is very difficult to work with because its melting point of 1710 °C is so high. Despite its advantageous properties, therefore, pure silica glass is used only for special applications.

Sodium oxide (Na_2O) is mixed with silica to make glass that can be shaped at a lower temperature. Sodium oxide is ionic, and it breaks the Si—O—Si chain of covalent bonds. This weakens the lattice strength of the glass, lowers its melting point, and reduces the viscosity of the resulting liquid. Unfortunately, it also reduces the resistance to chemical attack so much that a stoichiometric mixture of sodium oxide and silica, Na_2SiO_3, dissolves in water and is called *water glass*.



An important scientific application of silica glass is in high-precision spectrophotometer cells for measuring ultraviolet absorption spectra.

The most desirable glass melts at a reasonable temperature and is easy to work with, yet is chemically inert. Such a glass can be prepared by adding a third component that has bonding characteristics intermediate between those of purely ionic sodium oxide and those of purely covalent silicon dioxide. Several different components are used, depending on the properties desired in the glass.

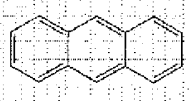
The glass used for windowpanes and bottles is soda-lime-silica glass, a composite of sodium oxide, calcium oxide, and silicon dioxide. The addition of CaO strengthens the lattice enough to make it chemically inert to most common substances. (Strong bases and HF, however, attack this glass.) Pyrex, the glass used in coffeepots and laboratory glassware, can withstand rapid temperature changes that would crack soda-lime-silica glass. Pyrex is a composite of B_2O_3, CaO, and SiO_2. Lenses and other optical components are made from glass that contains PbO. Light rays are strongly bent as they pass through lenses made of this glass. Colored glasses are obtained by adding small amounts of colored metal oxides such as Cr_2O_3 (amber), NiO (green), or CoO (brown).

Many materials that we use in this age of plastics are amorphous solids composed of extremely large molecules called *polymers*. Polymeric solids are intermediate between molecular and covalent solids. They have discrete but extremely large molecular units held together by dispersion forces. Because the molecules are so large, their covalent bonding plays an important role in determining the properties of the solid. A "plastic" can be shaped and molded because of the weak dispersion forces between polymer molecules, but it has relatively high strength because its long-chain molecules are held together by strong covalent bonds.

We discuss giant molecules in Chapter 11.

SECTION EXERCISES

10.4.1 Describe the forces that exist in (a) solid CO_2 (dry ice); (b) crystalline yellow elemental sulfur, S_8; (c) triacontane, the linear C_{30} hydrocarbon, whose melting point is 65 °C; and (d) anthracene ($C_{14}H_{10}$), whose line structure follows:



10.4.2 Predict the angles found in the crystalline fragments broken from a hexagonal close-packed crystal. What additional angles would be found in fragments from cubic close-packed crystals?

10.4.3 The unit cell of cubic close-packed crystals is a cube defined by the centers of spheres at its eight corners; there is an additional sphere embedded in the center of each face (see Figure 10-22 on p. 447). Draw this unit cell. Determine what fraction of each type of atom (corner and center of face) is within the unit cell. (HINT: How many unit cells must be stacked together to give one complete face atom?)

10.5 THE NATURE OF SOLUTIONS

Our discussion so far has focused primarily on pure substances, but much of the chemistry that occurs in the world around us involves mixtures of substances. Recall from Chapter 1 that a homogeneous mixture of chemical substances is commonly called a **solution.** The component that determines the state of the solution is the **solvent.** Normally, this component is present in greatest quantity. All other substances in the solution are called **solutes.** Solutions have special properties that we describe in the next several sections.

Most of us think of solutions as liquid mixtures. The oceans, for example, are liquid solutions in which water is the solvent and dissolved ions, gases, and molecules are the solutes. Vinegar is a liquid solution of acetic acid in water, and gasoline is a liquid solution that contains many different hydrocarbons. Gases can be solutions, as well. Two examples are the Earth's atmosphere, a solution of nitrogen, oxygen, and small amounts of several other gases, as well as natural gas, a solution of methane, ethane, and minor amounts of other hydrocarbons. Even solids can be solutions. For instance, brass is a solution of copper and zinc, and steel is a solution of a small amount of carbon dissolved in iron.

A solution is characterized by its concentration, which can vary. For example, if we add more sugar to a cup of coffee, the solution becomes more concentrated in sugar. The concentration of pollutants in the air is higher in urban areas than in rural ones. Steel can be made harder and stronger by controlling the concentration of carbon in iron. Concentration is usually expressed as molarity or as mole fraction of the solute.

Molarity (M) was first described in Section 3.7, and mole fraction (X) was defined in Section 5.5.

Except for gaseous solutions, there is usually an upper limit to the amount of solute that will dissolve in a given amount of solvent. When that limit has been reached, the solution can hold no more solute. It is then said to be a **saturated solution.** The concentration of a saturated solution is called the **solubility** of the substance in that particular solvent.

Solubilities vary widely because they depend on the intermolecular forces in the solute and solvent. For example, the solubility of NaCl in water is about 6 mol/L (M), the solubility of AgCl in water is only 10^{-5} M, and the solubility of NaCl in gasoline is virtually zero. Some substances form solutions in all proportions and are said to be completely **miscible.** Acetone and water, for example, can be mixed in any proportion from pure water to pure acetone. A few salts, such as $FeCl_3 \cdot 6H_2O$, mix with hot water in any proportion. The energetic interactions involved in the solution process underlie such variations in solubility.

"LIKE DISSOLVES LIKE"

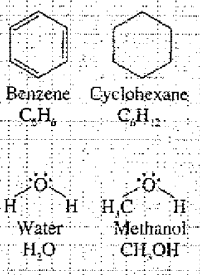
Whether or not a given substance dissolves in a liquid depends primarily on intermolecular coulombic forces of attraction. These interactions are of three types: those between ions or molecules of the pure solute, those between solvent molecules, and those between solvent and solute in the solution.

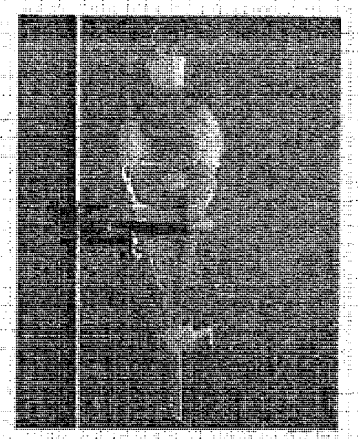
A substance dissolves if the forces of attraction between the solvent molecules and the solute molecules are comparable to or greater than the solute-solute and solvent-solvent interactions. Substances that dissolve in each other usually have similar types of intermolecular interactions, a generalization that can be summarized by the expression *like dissolves like*.

When two liquids are mixed, all three sets of intermolecular interactions are important. Consider water, methanol, benzene, and cyclohexane, which are all liquids at room temperature. Water and methanol are miscible because molecules in the pure liquids and their mixtures form many hydrogen bonds. Benzene and cyclohexane are also completely miscible because molecules in the pure liquids and their mixtures interact through the dispersion forces caused by their polarizable electron clouds. In contrast, water and benzene are nearly insoluble in each other. When mixed, benzene and water partition into distinct layers, one of nearly pure water and the other of nearly pure benzene. Water and cyclohexane are also insoluble in each other and form two layers when mixed.

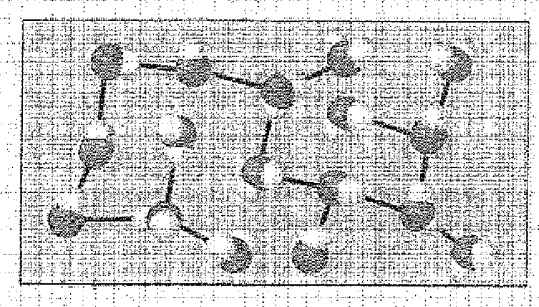
The solubilities of water, methanol, benzene, and cyclohexane in one another are examples of like dissolving like. Water and methanol form hydrogen bonds. When these liquids are mixed, the degree of hydrogen bonding in the solution is about the same as that in the pure liquids. Likewise, dispersion forces in solutions of benzene and cyclohexane are about the same as those in the pure liquids. As Figure 10-32 shows, however, benzene molecules cannot dissolve in water unless they disrupt part of water's hydrogen bonding network. Because benzene does not form hydrogen bonds, the only forces of attraction between water molecules and benzene molecules are dispersion forces, and for water, hydrogen bonds are much stronger than dispersion forces. The cost of disrupting water's hydrogen bonding network is far greater than the stability gained from benzene-water dispersion forces.

Some liquids can interact with other substances in multiple ways. Acetone, for instance, has a polar C═O double bond and a three-carbon bonding framework. The bonding framework is similar to a hydrocarbon, so acetone will mix with cyclohexane. The polar C═O group makes acetone compatible with other polar molecules such as acetonitrile (CH_3CN). Finally, the polar oxygen atom in acetone has two lone pairs of electrons that can form hydrogen bonds with hydrogen atoms from ammonia or water. Because of its versatility as a solvent, acetone is widely used to clean and rinse laboratory glassware. Sample Problem 10-7 treats several alcohols, which also display multiple types of interactions.





Water and benzene, which are insoluble in each other, form two layers when mixed.



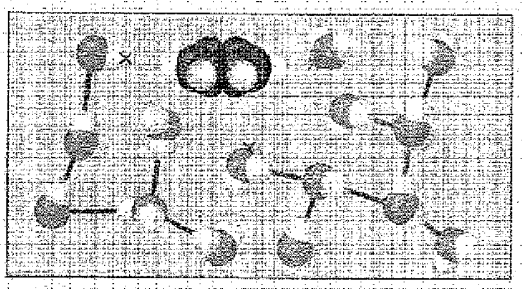


FIGURE 10-32
A collection of water molecules *(left)* has an intricate hydrogen bonding network *(blue lines)*. The presence of the benzene molecule *(right)* disrupts all the hydrogen bonds marked with *x*'s.

SAMPLE PROBLEM 10-7 SOLUBILITY TRENDS

Give a molecular explanation for the following trend in alcohol solubilities in water:

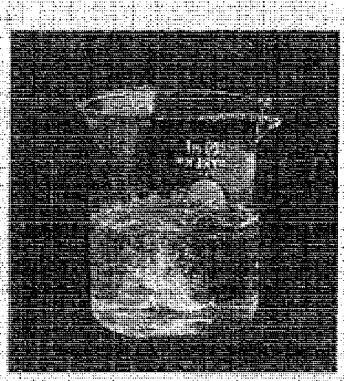
Propanol	$CH_3CH_2CH_2OH$	Completely miscible
Butanol	$CH_3CH_2CH_2CH_2OH$	1.1 M
Pentanol	$CH_3CH_2CH_2CH_2CH_2OH$	0.30 M
Hexanol	$CH_3CH_2CH_2CH_2CH_2CH_2OH$	0.056 M

METHOD: Solubility limits depend on the relative magnitude of the destabilization that occurs when solvent bonding networks and solute bonding networks are disrupted, compared with the stabilization generated by solute-solvent interactions. This problem refers to a series of aqueous solutions, which are dominated by hydrogen bonding interactions.

When any alcohol dissolves in water, the hydrogen-bonding network of water is disrupted by the nonpolar hydrocarbon part of the alcohol. Counterbalancing these disruptions, hydrogen-bonding interactions are generated in the solution between the OH groups of the alcohol and water molecules.

As the nonpolar region of an alcohol grows longer, more and more hydrogen bonds are disrupted by each solute molecule. At the same time, each alcohol listed has only one OH group, so the amount of compensating solute-solvent hydrogen bonding is the same for all the alcohols.

This explains why longer-chain alcohols are progressively less soluble in water. As the hydrocarbon chain gets longer, more destabilization is involved in inserting the alcohol into the water matrix, so the alcohol gets increasingly less soluble as the chain grows.



Zinc methyl reacts with aqueous acids.

The network bonding of metals was discussed in Section 9.6.

Recall from Section 4.6 that strong acids generate hydronium ions in aqueous solution.

SOLUBILITY OF SOLIDS

Like dissolves like also describes the solubility properties of solids. There are four different kinds of solids: covalent, ionic, molecular, and metallic. Each is held together by a different kind of interaction, and each has its own solubility characteristics.

Covalent solids such as diamond, graphite, or silica cannot dissolve without breaking covalent chemical bonds. Because intermolecular forces of attraction are always much weaker than covalent bonds, solvent-solute interactions are never strong enough to offset the energy cost of breaking bonds. Covalent solids are insoluble in all solvents, but they may be chemically attacked by some liquids or vapors.

Metals are the next most difficult solids to dissolve because they contain extensive delocalized bonding networks that must be disrupted before the metal can dissolve.

When an alkali metal contacts water or when other metals such as Ca, Zn, or Fe are treated with aqueous acid, the metal *reacts* with the solution, producing hydrogen gas and a solution of the metal cation (for example, Na^+ and Ca^{2+}). A chemical reaction has occurred, so the aqueous medium has *not* dissolved the metal. Zinc metal, for example, reacts with hydrochloric acid to generate H_2 gas and displace Zn^{2+} cations in solution:

$$Zn_{(s)} + 2\,H_3O^+_{(aq)} \rightarrow Zn^{2+}_{(aq)} + H_{2(g)} + 2\,H_2O_{(l)}$$

The solution produced when zinc reacts with hydrochloric acid is an aqueous solution of zinc ions from the chemical reaction and chloride ions from HCl, not a solution of zinc metal in water. If this solution is boiled to dryness, the remaining solid is $ZnCl_2$, not zinc metal.

A few metals *react* with water, and several *react* with aqueous acids, but no metals will simply *dissolve* in water. Likewise, metals do not dissolve in nonpolar liquid solvents.

Metals are insoluble in common liquid solvents but can dissolve in each other (like dissolves like). A mixture of substances with metallic properties is called an **alloy.** Some alloys are solutions, and others are heterogeneous mixtures. Brass, for instance, is a homogeneous solution of copper (20% to 97%) and zinc (80% to 3%), but common plumber's solder is a heterogeneous alloy of lead (67%) and tin (33%). When solder is examined under a microscope, separate regions of solid lead and solid tin can be seen. When brass is examined, no such regions can be seen.

Mercury, the only metal that is a liquid at room temperature, dissolves a number of metals to give liquid solutions. Any solution of another metal in mercury is called an **amalgam.** Metals close to mercury in the periodic table, such as silver, gold, zinc, and tin, are particularly soluble in mercury. An amalgam of silver, tin, and mercury has been widely used to make dental fillings. When the intermetallic compound Ag_3Sn is ground with mercury, it forms a semisolid amalgam that can be shaped to fill a cavity. On standing, mercury reacts with the other metals to form a hard solid mixture of Ag_2Hg_8 and Sn_7Hg_8. The mixture expands slightly during reaction, forming a tight fit within the cavity.

The mercury atoms in dental fillings are chemically bound and do not dissolve, so they are safe for the wearer, despite the fact that mercury is highly toxic. Dentists who mix the amalgams, however, may be at risk of mercury poisoning.

As described in Section 7.7, *ionic solids* contain cations and anions held in a three-dimensional ionic lattice by strong coulombic attractions. Thus ionic solids do not dissolve unless considerable solvent-ion interactions exist to counterbalance the energy cost of breaking the ions free from the lattice. There are no ionic liquids at room temperature, so at first we might think there are no solvents suitable for ionic solids. Some ionic solids dissolve in water, however, because water is a *highly polar* liquid in which strong ion-dipole interactions exist between water molecules and ions in aqueous solution. Figure 10-33 illustrates the solvation of Na^+ and Cl^- ions as NaCl dissolves in water.

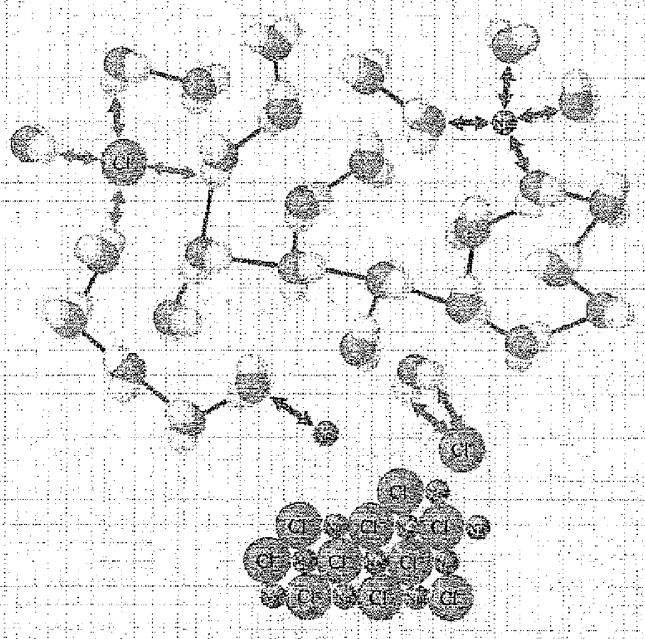


FIGURE 10-33
A molecular picture showing the ion-dipole interactions that help a solid ionic crystal dissolve in water. Arrows indicate ion-dipole interactions.

The solubility guidelines presented in Chapter 4 categorize ionic solids as soluble or insoluble. For soluble salts the stabilization due to ion-dipole interactions compares favorably with the coulombic forces of the ionic solid. For insoluble salts, ion-dipole interactions provide too little stabilization to overcome the forces that hold the ions in the solid lattice.

Molecular solids are held together by dispersion forces, dipole forces, and sometimes hydrogen bonds. Such solids dissolve readily in solvents with similar types of intermolecular forces. Nonpolar iodine, for instance, dissolves readily in a nonpolar liquid such as carbon tetrachloride (CCl_4). Many organic compounds are molecular solids that dissolve in organic liquids such as benzene or cyclohexane.

Hydrogen bonding in the aqueous environment allows water to dissolve materials that can form hydrogen bonds. For example, we have seen that acetone is fully miscible with water because of significant hydrogen bonding between the polar C=O oxygen atoms of acetone and the hydrogen atoms of water molecules. Hydrogen bonding also makes sugars such as sucrose and glucose very soluble in water. Glucose, whose structure is shown in Figure 10-34, is an organic molecule with five polar O—H groups, each of which forms hydrogen bonds with water molecules. Thus glucose ($C_6H_{12}O_6$) is quite soluble in water, whereas a hydrocarbon such as benzene (C_6H_6), which cannot form hydrogen bonds, is nearly insoluble in water.

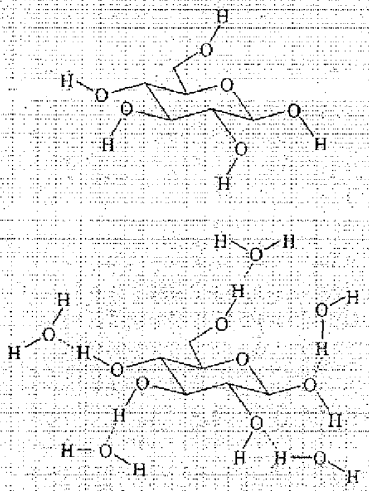


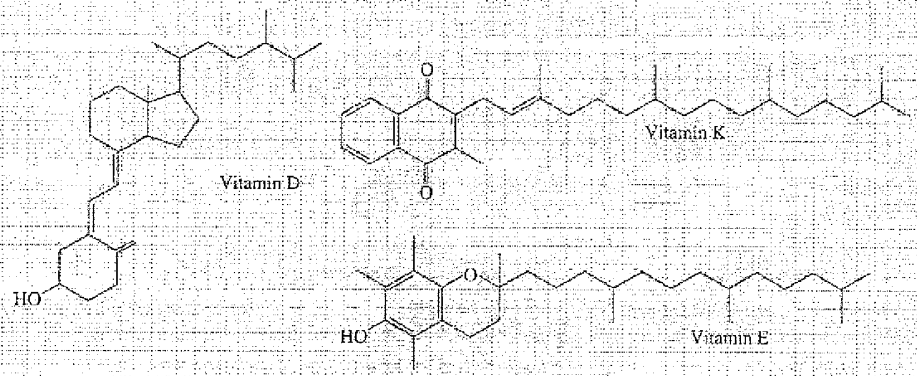
FIGURE 10-34
The line structure of glucose (top). All five polar O—H groups can form hydrogen bonds with water molecules (dotted lines, bottom).

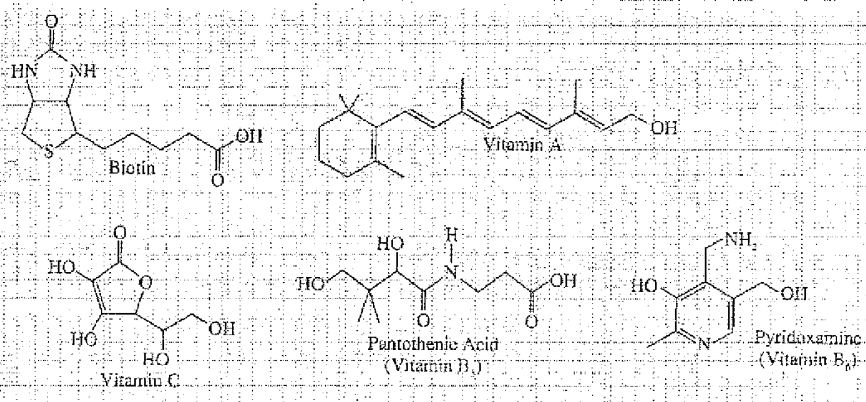
Glucose and other sugars are discussed in more detail in Section 11.7.

The best solvent for a molecular solid is one whose intermolecular forces match the forces holding the molecules in the crystal. For a solid held together by dispersion forces, good solvents are nonpolar liquids such as CCl_4 and C_6H_6. For polar solids a polar solvent such as acetone works well. The best solvent for ionic salts is water. This does not mean, however, that *every* polar solid dissolves in acetone or that *every* ionic salt dissolves in water. A substance dissolves when there is a favorable balance of coulombic forces. We can predict that the balance will be *unfavorable* for a salt in a nonpolar solvent or a nonpolar organic molecule in water, but there is no foolproof method for predicting a *favorable* balance for potentially favorable cases. Solubility guidelines are only guidelines, and they are highly empirical. Sample Problem 10-8 provides some practice in recognizing solubility types.

SAMPLE PROBLEM 10-8 SOLUBILITIES OF VITAMINS

Vitamins, organic molecules required by the body for proper function but not synthesized by the body, must be present in the foods we eat. Vitamins can be grouped into two categories: fat-soluble, which dissolve in fatty hydrocarbon-like tissues, and water-soluble. The structures of several vitamins are shown below. Assign each one to the appropriate category.





METHOD: At first glance, it may seem that the like-dissolves-like guideline does not apply here. Certainly, none of these complex molecules look like water, and the resemblance to simple hydrocarbons such as cyclohexane is also remote. Keep in mind, however, that the basis for the like-dissolves-like principle is that similar compounds dissolve in each other because they have *common patterns* of intermolecular interactions. We have seen, for instance, that alcohols with large nonpolar segments do not dissolve well in water. We can categorize vitamins similarly by the amount of the structure that can be stabilized by hydrogen bonding to water molecules.

A hydrogen bond donor must have a hydrogen atom bonded to F, O, or N, and a hydrogen bond acceptor is an electronegative atom with a lone pair of electrons. By these criteria, all the vitamins shown above are capable of some hydrogen bonding. However, vitamins A, D, E, and K have large regions where there are only nonpolar C—C and C—H bonds. Like the longer alcohols, these molecules have too few hydrogen bonding sites for them to be soluble in water. As a result, these are fat-soluble vitamins.

The four remaining molecules, vitamin C, biotin, pantothenic acid, and pyridoxamine, have a comparatively large number of O—H and N—H groups. These groups make these vitamins strong hydrogen bonders, so they are all water soluble. (In fact, all the B vitamins are soluble in water.)

The different solubilities of these two kinds of vitamins have important metabolic consequences. Fat-soluble vitamins can be stored in fatty body tissue for a long time because they do not dissolve in aqueous body fluids. As a result, too much of a fat-soluble vitamin can overload the storage capabilities and lead to a toxic reaction. Water-soluble vitamins, on the other hand, cannot be stored, and the body will excrete anything more than the amount it can use immediately. We must therefore have a regular supply of water-soluble vitamins in our diets to remain healthy.

SOLUBILITY OF GASES IN LIQUIDS

Substances are gases when their intermolecular forces are negligible. Molecular oxygen is a typical example. Oxygen molecules are nonpolar, so they do not form dipole-dipole or hydrogen-bonding interactions. The valence electrons in O_2 molecules have $n = 2$, which means that they are in compact orbitals that generate small dispersion interactions. Intermolecular interactions between oxygen molecules and molecules of a solvent such as water are minimal, so oxygen is not very soluble in water. Water in contact with the Earth's atmosphere contains O_2 at a concentration of only about 3×10^{-4} M.

A few gases interact strongly with water to form concentrated aqueous solutions. For example, a 12 M solution can be prepared by bubbling HCl gas through water.

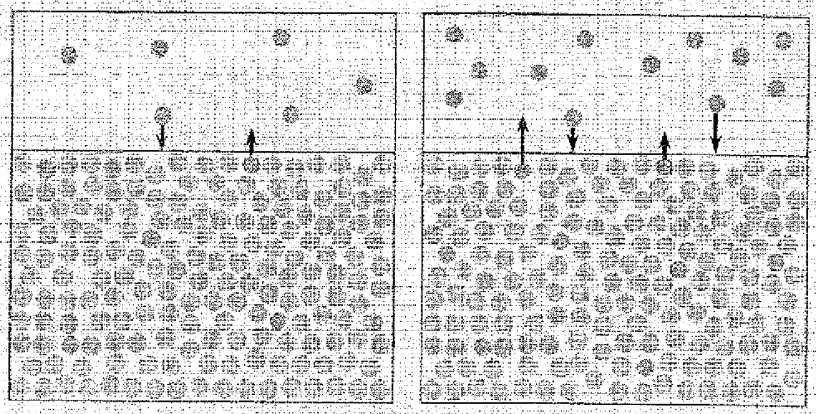


FIGURE 10-35
When the partial pressure of a gas above a solution increases *(right)*, the capture rate goes up, so the concentration of gas in the solution increases.

This is because when HCl gas dissolves in water, proton transfer occurs to generate H_3O^+ ions:

$$HCl_{(g)} + H_2O_{(l)} \rightarrow Cl^-_{(aq)} + H_3O^+_{(aq)}$$

Although this is a chemical reaction, we say that HCl dissolves in water rather than reacts with water because boiling the solution regenerates HCl and H_2O vapors.

Ammonia is another gas that is very soluble in water, giving solutions as concentrated as 14.8 M. Ammonia dissolves because it forms hydrogen bonds with water molecules. When an ammonia molecule displaces a water molecule, one hydrogen-bonding interaction is exchanged for another.

Gas solubility increases with the partial pressure of the gas in contact with the solution. The molecular view of a solution in Figure 10-35 shows the reasons. Gas molecules that collide with a liquid surface can be captured into solution, and as the partial pressure increases, the number of collisions between gas molecules and the solution surface also increases. This, in turn, causes more gas molecules to be captured by the solution and increases the concentration of dissolved gas.

At the same time that gas molecules are being captured at the liquid surface, dissolved gas molecules escape from the liquid if their motion brings them to the surface. As more and more gas molecules enter the solution, the escape rate of molecules returning to the gas phase increases accordingly. At any given partial pressure, the concentration of gas dissolved in the liquid changes until the number of gas molecules that leave the solution matches the number of molecules that enter the solution. The gas-liquid system is then in a condition of dynamic equilibrium, in which molecules are continually transferred between phases without any net change in concentrations.

Dynamic equilibrium was introduced in Section 2.1 and is treated in detail in Chapter 15.

The rate of capture from the gas phase is directly proportional to pressure, and the rate of escape from the solution is directly proportional to the concentration of dissolved gas molecules. The solubility of a gas is the concentration at which these two rates exactly balance. Thus gas solubility is directly proportional to partial pressure. **Henry's law** expresses this quantitatively:

$$C_i = K_H p_i \qquad (10\text{-}1)$$

TABLE 10-2 HENRY'S LAW CONSTANTS

GAS	K_H (10^{-3} M/atm) 0 °C	25 °C	30 °C
N_2	1.1	0.67	0.40
O_2	2.5	1.3	0.89
CO	1.6	0.96	0.44
Ar	2.5	1.5	1.0
He	0.41	0.38	0.40
CO_2	78	34	16

Aqueous solutions.

We usually express dissolved gas concentration in molarity and gas pressure in atmospheres, so K_H has units of molarity/atmosphere.

Here, C_i is the concentration of gas in the solution, and p_i is the partial pressure of the same gas in the vapor phase above the solution. These two variables are linked by the Henry's law constant (K_H). The value of K_H depends on the identity of the gas, the solvent, and on the temperature of the system. Table 10-2 lists values for the Henry's law constants for several gases dissolving in water. Sample Problem 10-9 makes use of Henry's law to determine the concentrations of atmospheric gases that dissolve in water, and Box 10-1 discusses how Henry's law applies to deep-sea diving.

SAMPLE PROBLEM 10-9 SOLUBILITIES OF ATMOSPHERIC GASES

The Earth's atmosphere is 78% N_2, 21% O_2, and minor amounts of other gases, including CO_2 (0.31%). Find the concentration of N_2, O_2, and CO_2 in water at equilibrium with the Earth's atmosphere at 25 °C.

METHOD: Each gas establishes its own dynamic equilibrium with water. The concentration depends on the partial pressure of the gas in the atmosphere and on the value of the Henry's law constant at 25 °C.

Recall from Chapter 5 that the partial pressure of any gas in a mixture is given by the mole fraction (X_i) multiplied by total pressure. Using 1.0 atmosphere (atm) for the total pressure:

$$p_{O_2} = X_{O_2} P = \left(\frac{21\%\ O_2}{100\%}\right) \times (1.0\ \text{atm}) = 0.21\ \text{atm}\ O_2$$

Likewise, the partial pressure of N_2 is 0.78 atm and that of CO_2 is 3.1×10^{-3} atm.

Now we can use Henry's law to calculate the concentrations of dissolved gas:

$$C_{N_2} = \left(6.7 \times 10^{-4} \frac{\text{M}}{\text{atm}}\right)(0.78\ \text{atm}) = 5.2 \times 10^{-4}\ \text{M}\ N_2$$

$$C_{O_2} = \left(1.3 \times 10^{-3} \frac{\text{M}}{\text{atm}}\right)(0.21\ \text{atm}) = 2.7 \times 10^{-4}\ \text{M}\ O_2$$

$$C_{CO_2} = \left(3.4 \times 10^{-2} \frac{\text{M}}{\text{atm}}\right)(3.1 \times 10^{-3}\ \text{atm}) = 1.1 \times 10^{-4}\ \text{M}\ CO_2$$

BOX 10-1

HENRY'S LAW AND THE BENDS

According to Henry's law, gases become more soluble as pressure increases. Normally, this variation has few consequences because atmospheric pressure varies slowly with changing altitude or weather. However, very large pressure changes are routine in deep-sea diving. As a result, divers returning from the depths to the surface must take special precautions to allow their bodies to adjust to changes in the solubility of the gases in their blood.

Carbonated beverages illustrate what happens when a gas dissolved in a liquid experiences a rapid drop in pressure. Soft drinks, soda water, champagne, and beer are all bottled under several atmospheres' pressure of carbon dioxide. When a bottle is uncapped, the total pressure quickly falls to 1 atm, and the partial pressure of CO_2 drops to 0.003 atm. At this lower pressure, the concentration of CO_2 in the solution is much higher than its solubility, so the excess CO_2 forms gas bubbles and escapes from the liquid.

Deep-sea divers experience pressure changes similar to those of bottled drinks. For every 30 feet a diver descends, the pressure increases by 1 atmosphere. As a result, the amount of nitrogen gas dissolved in the diver's blood increases significantly as the diver descends. If a diver returns to the surface too quickly after a deep dive, gas dissolved in the blood may form bubbles in the same way as the CO_2 in a freshly opened carbonated drink. These bubbles interfere with the transmission of nerve impulses and restrict the flow of blood. This condition, known as *the bends*, is extremely painful and can cause paralysis or death.

Divers avoid the bends by returning to the surface slowly, taking short "decompression stops" at intermediate depths to allow excess gas to escape from the blood without forming bubbles. Another way of preventing the bends is by using helium-oxygen gas mixtures instead of air in divers' breathing apparatus. Helium is only half as soluble as nitrogen in blood, so less extra gas dissolves in blood.

SECTION EXERCISES

10.5.1 List the types of intermolecular interactions that stabilize a solution of acetone in methanol, and draw molecular pictures that illustrate any dipole-dipole and hydrogen-bonding interactions that exist between molecules of these substances.

10.5.2 Gases can be collected by bubbling them through water into an evacuated container. If 0.18 mol of CO_2 at $P = 0.98$ atm is bubbled through 450 mL of water into an empty glass vessel at 298 K, what fraction of the gas dissolves in the water?

10.5.3 On the basis of their molecular structures, predict which of the following silicon-containing materials are water soluble: elemental Si, SiO_2, Na_2SiO_3, and $SiCl_4$.

10.6 DUAL-NATURE MOLECULES: SURFACTANTS AND BIOLOGICAL MEMBRANES

Now that we have described intermolecular forces, solutions, and solubility properties, we can apply these concepts to examples of solute-solvent interactions of key importance in the chemical industry and in biology.

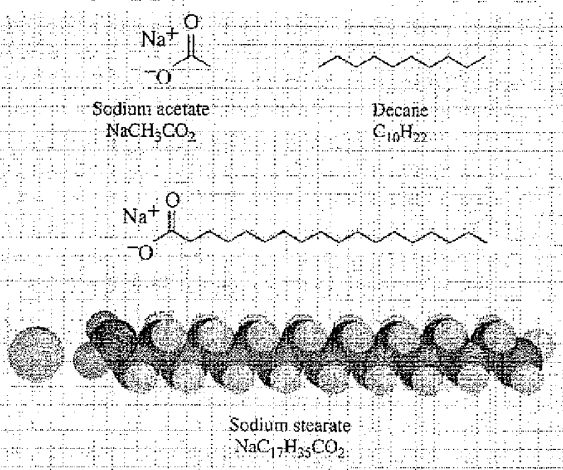


FIGURE 10-36
Sodium stearate is a typical dual-nature molecule. It has a hydrophilic polar head that resembles sodium acetate and a hydrophobic nonpolar tail that is a hydrocarbon similar to decane.

Substances that do not dissolve in water, such as organic fats and oils, are called **hydrophobic.** Substances that are miscible with water, such as the organic but hydrogen-bonding molecules methanol and acetone, are called **hydrophilic.** Some molecules contain both hydrophilic and hydrophobic regions. Such dual-nature molecules may have a polar or ionic "head" that is compatible with water and a long hydrocarbon "tail" that is incompatible with water. Sodium stearate, whose structure is shown in Figure 10-36, is a dual-nature molecule. The head of the stearate anion resembles the water soluble acetate anion, and the tail is a hydrocarbon chain containing 17 carbon atoms.

Figure 10-37 shows three different structures that dual-nature molecules such as sodium stearate can form when they are placed in water. They may form a molecular **monolayer** on the surface, in which the polar head groups are immersed in the water while the nonpolar tails are aggregated together on the surface. Agitating the solution may cause the molecules to arrange into spherical aggregates called **micelles,** in which the hydrophobic tails point inward and the polar heads lie on the outside of the structure, where they interact with the aqueous solvent. Dual-nature molecules may also form enclosed **bilayers,** called **vesicles,** which have two parallel rows of molecules oriented so that their hydrocarbon tails are clustered together.

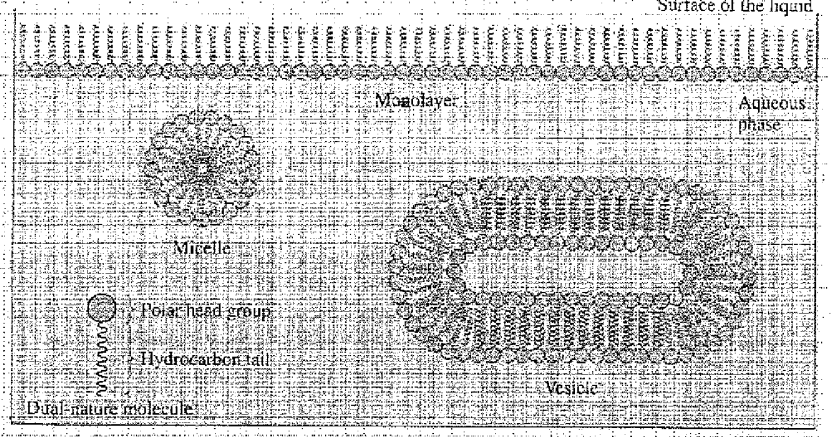


FIGURE 10-37
Cross-sectional molecular views of the structures that can form when dual-nature molecules are placed in water. The molecules may form a monolayer at the surface, spherical clusters called *micelles*, or bilayer structures called *vesicles*. In all three structures the hydrocarbon tails cluster together to minimize their interactions with water molecules, and the polar head groups are positioned to maximize their interactions with water molecules.

All these arrangements obey the principle of like dissolving like. The hydrocarbon tails aggregate through dispersion forces because they are incompatible with the aqueous medium. The hydrogen-bonding network of the solvent would be disrupted by incorporating these tails into the solution. The polar heads, on the other hand, interact strongly with water to maximize hydrogen bonding and ion-dipole interactions.

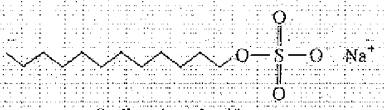
SURFACTANTS

Dual-nature molecules are widely used in industrial chemistry to modify the behavior of aqueous solutions. In this context they are called **surfactants.** Common surfactant head groups include carboxylate ($-CO_2^-$), sulfonate ($-SO_3^-$), sulfate ($-OSO_3^-$), and ammonium ($-NH_3^+$). Sodium is the most common counter-ion for anionic surfactants and chloride for cationic surfactants because these ions are nontoxic and their salts are highly soluble.

Surfactants are widely used as soaps and detergents. Clothing becomes soiled by a wide variety of substances; some are water soluble, and others are not. Surfactants remove water-insoluble grease (for example, butter, fat, and oil) from solid surfaces. Dispersion forces stabilize grease particles in the hydrocarbon tails of surfactant aggregates. Agitation removes these aggregates from the fabric, suspending them in solution as a large number of tiny micelles with grease particles trapped inside. The micelles do not redeposit on the fabric because their hydrophilic heads hold them in solution. When water is drained from the washing machine, the grease-containing micelles are swept away, leaving clean clothes behind.

Soap made by boiling animal fat in an alkaline solution obtained from ashes has been known since the time of the ancient Sumerians, 2500 BC.

Soaps are carboxylate surfactants derived from natural sources such as animal fats that contain stearic acid and other long-chain organic acids. These carboxylate surfactants form insoluble salts with Ca^{2+} and Mg^{2+}. In regions where water is "hard," these soaps precipitate calcium and magnesium stearate as a "scum" that inhibits cleansing action and is responsible for bathtub rings. Detergents, on the other hand, contain sulfonate and sulfate surfactants that are "synthetic" compounds, originally prepared in the laboratory. Detergents such as sodium lauryl sulfate do not form precipitates with divalent cations, but they have a tendency to lather and foam. Foaming is a disadvantage in washing machines but is considered to be an advantage in hair shampoos. The cleaning action of soaps and detergents is similar, but detergents have largely replaced soaps because of their superior behavior in hard water.



Sodium lauryl sulfate
(common ingredient in shampoo)

Surfactants are used in such a wide variety of ways that billions of dollars are spent on them every year. They appear in many household products, including cleansing agents and shampoos. Some surfactants are used as emulsifiers in processed foods such as bottled salad dressing. An emulsifier causes normally incompatible liquids such as the oil and water in salad dressing to disperse in each other. Surfactants emulsify by forming molecular connections between the liquids. Their hydrophobic tails interact with oil molecules, whereas their hydrophilic heads interact with water molecules.

Gasoline contains surfactants designed to prevent the accumulation of high-boiling compounds on the surfaces of fuel injectors and carburetors. These deposits interfere with the flow of air and cause rough idling and poor gas mileage. In this case the hydrophilic polar ends interact strongly with the solid surface, and the hydrophobic ends are compatible with liquid gasoline. The polar ends adhere to the metal walls of the injection system, whereas their tails extend into the fuel mixture. This creates a thin nonpolar film that protects the surface from gummy deposits. These same films help prevent the formation of rust by screening the metal surface from water molecules.

Figure 10-38 illustrates that surfactants also decrease the surface tension of water. In the figure, the drop of water that contains a surfactant is flattened and deformed, giving it a larger surface area than the drop of water that contains no surfactant. Surfactant molecules reduce surface tension by forming a monolayer on the aqueous surface. Unlike water molecules at the surface, this monolayer does not experience an attractive force drawing molecules back into the bulk of the liquid.

A surfactant also causes water to form a film coating on any surface it contacts. In this sense, surfactants make water "wetter." Because of its improved ability to coat surfaces, surfactant-treated water is used occasionally to fight fires.

Chemists and engineers in the petroleum industry are studying ways to use surfactants to increase the amount of oil that can be recovered from wells. The goal is to develop inexpensive, environmentally safe surfactants that can be mixed with water and injected into existing oil wells. The surfactant will promote formation of an oil-water emulsion that has a lower viscosity than oil and should be easier to extract from the well.

Approximately half of the surfactants produced in the United States are used in household and industrial cleaning products, but the remaining half are used in a wide range of industries. In agriculture, surfactants are used as wetting agents that assist in the uniform application of sprayed pesticides. They also are used to prevent caking of fertilizers. Surfactants used in agricultural products must not interfere with the active agents and must be biodegradable and environmentally benign. In the food industry, different surfactants are used as emulsifiers, cleaners, foaming agents, and antifoaming agents. Paints are dispersions of dyes, binding agents, and fillers. Most paints contain surfactants that convey improved flow and mixing properties. Surfactants are used widely in the plastics industry as foaming agents to assist in the production of plastic foams and to improve moldability and extrudability of specially shaped products. In the manufacture of textiles, surfactants are used to clean natural fibers, as lubricants that reduce friction during the spinning and weaving processes, emulsifiers that improve the application of dyes and finishes, and antistatic agents.

This is just a sampling of the industrial applications of these versatile materials. A host of other industries also uses surfactants in significant amounts. Examples include pharmaceuticals, paper, mining, petroleum, tanning, photography, electroplating, and adhesives.

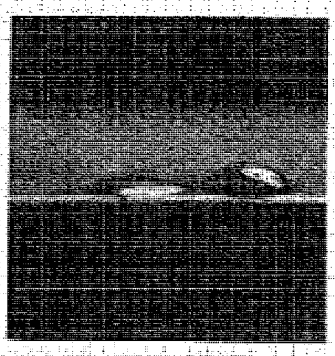


FIGURE 10-38
Surfactants reduce surface tension by forming a monolayer at the water-air interface. The water droplet on the left contains a surfactant, making its surface tension lower and causing it to flatten and spread out.

CELL MEMBRANES

It may seem like a huge conceptual leap from industrial surfactants to biological cell membranes, but the same principles apply to both sets of substances.

Every biological cell is surrounded by a thin membrane only a few molecules thick. Among the major components of membranes are molecules called *phospholipids*, which are dual-nature molecules. Although their chemical structures are much more complex than simple surfactants such as sodium stearate, phospholipids nevertheless have hydrophilic heads and hydrophobic tails. Figure 10-39 shows the structure of lecithin, which is a common membrane phospholipid. The hydrophilic end of lecithin has a cationic $N(CH_3)_3^+$ group and eight oxygen atoms with nonbonding pairs of electrons, all of which form hydrogen bonds with water molecules. The hydrophobic portion of lecithin consists of two hydrocarbon tails.

Phospholipids form bilayers in aqueous media. The molecules form two approximately parallel rows with tails aligned and heads in contact with the solution. This arrangement, shown in Figure 10-40, is analogous to the vesicles in Figure 10-37. The bilayer forms a closed sac that contains the aqueous cytoplasm and all the cellular components. Thus a cell can be viewed as a large and complex vesicle.

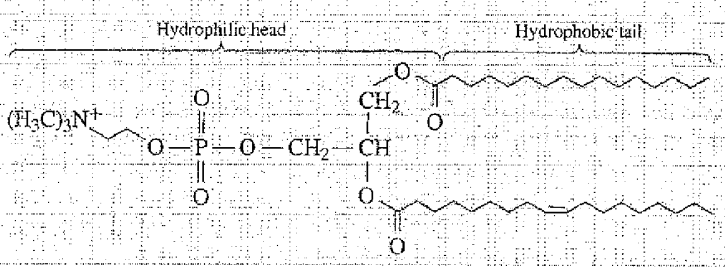


FIGURE 10-39
The chemical structure of lecithin. Lecithin is one of the most common phospholipids used for the construction of cell membranes. It is also used as a "natural" emulsifier in beauty products.

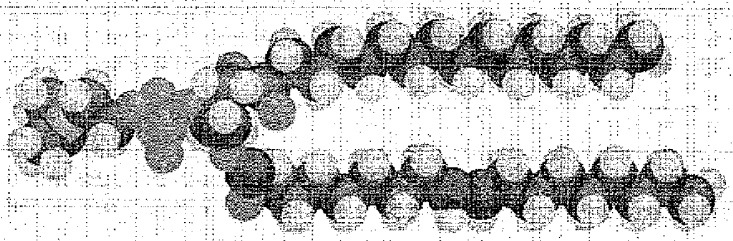
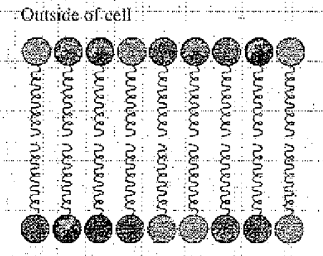
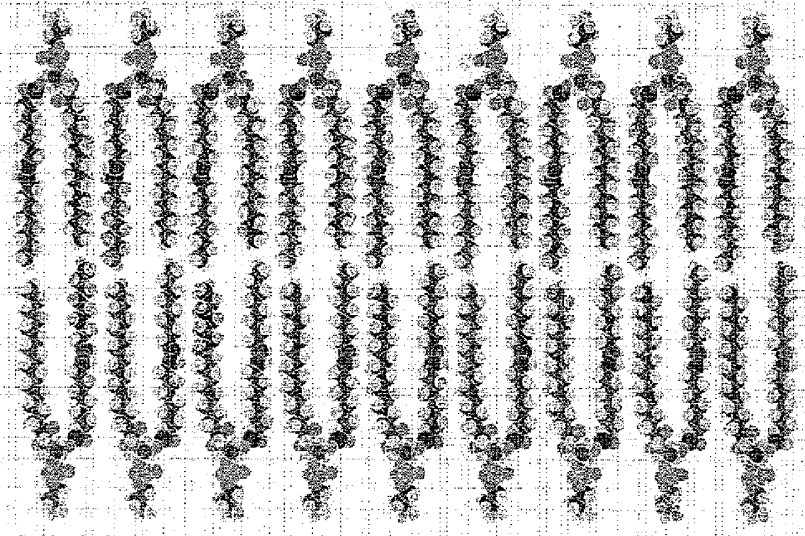


FIGURE 10-40
A lipid bilayer contains two layers of dual-nature molecules arranged tail to tail. The polar head groups face outward and are stabilized by dipolar and hydrogen-bonding interactions with water molecules.



A Schematic of a lipid bilayer



B Space-filling model of a lipid bilayer

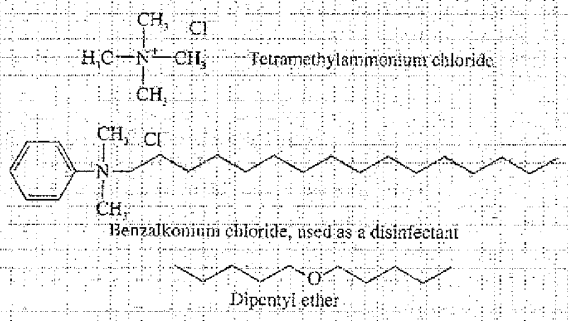
One purpose of a cellular lipid bilayer is to control which molecules pass into and out of the cell. Uncharged small molecules such as water, ammonia, and oxygen can diffuse through the membrane. Hydrophobic molecules such as hydrocarbons can also pass through because they are soluble in the overlapping tails that make up the interior of the bilayer. Ions and water-soluble polar molecules such as glucose and urea, on the other hand, cannot get through the membrane.

For cells to carry out their functions, glucose and other nutrients must be brought in, and urea and other waste products must be expelled. This would be an impossible task if cell membranes were composed only of phospholipids. Specific large biomolecules act as molecular "gates" through the membranes. These proteins are embedded in the bilayers but protrude into the surrounding water and/or into the cell interiors.

The structures of proteins are described in Chapter 11.

SECTION EXERCISES

10.6.1 Line drawings of some molecules follow. Identify the hydrophilic and hydrophobic regions of each, and determine which are surfactants.



Tetramethylammonium chloride

Benzalkonium chloride, used as a disinfectant

Dipentyl ether

10.6.2 Explain why glucose and other large, water-soluble molecules cannot pass through a lipid bilayer. (The structure of glucose is shown in Figure 10-34.)

10.7 PROPERTIES OF AQUEOUS SOLUTIONS

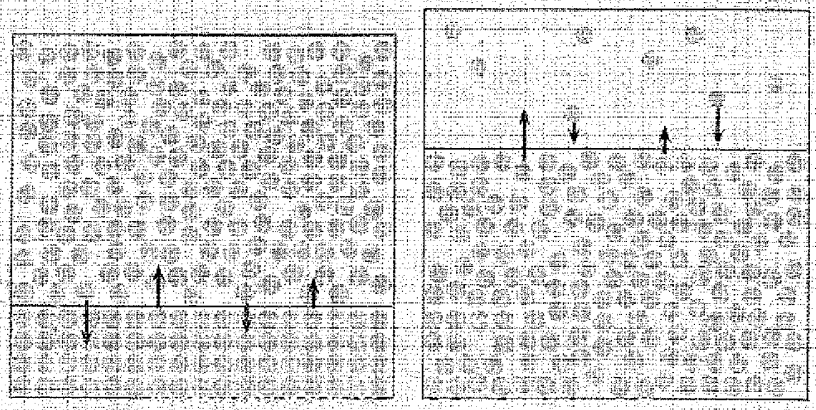
Solute molecules alter many properties of a liquid. For instance, adding salt to water gives a solution that boils at a higher temperature than pure water, and adding ethylene glycol to the water in an automobile radiator gives a solution that protects against freezing because the solution freezes at a lower temperature than pure water. Changes such as these in the behavior of liquids can be understood from a molecular perspective if we first describe phase changes from a molecular viewpoint and then examine the effect of added solute molecules. We consider aqueous solutions specifically because they are by far the most important in general chemistry, biology, and geology.

PHASE EQUILIBRIA

If pure water at 0 °C is cooled, it freezes, and if ice at 0 °C is warmed, it melts. The temperature at which this transformation between the liquid and solid forms of H_2O occurs is the freezing point of water. At exactly 0 °C, solid ice and liquid water are equally stable, so in a thoroughly insulated container, ice and water could coexist at 0 °C indefinitely.

The molecular view shown in Figure 10-41, *A*, reveals that two processes occur in a mixture of ice and water at 0 °C. First, water molecules in the liquid that collide

The temperature 0 °C can also be characterized as the melting point of ice.



A Solid–pure liquid equilibrium **B** Pure liquid–gas equilibrium

FIGURE 10-41
Molecular views of the dynamic equilibria between pure phases. **A,** The equilibrium between liquid and solid. **B,** The equilibrium between liquid and gas. At the equilibrium temperature, exactly the same number of molecules escape from the liquid phase as are captured by the liquid phase. Remember that all molecules are constantly in motion; for clarity, however, the motions of molecules confined within a phase have not been shown.

Dynamic equilibrium is consistent with the kinetic theory of molecular motion. One proof of molecular transfer between phases comes from radioactivity studies. If radioactive ice is placed in nonradioactive water at 0 °C, the water slowly becomes radioactive because of molecular transfer of radioactive water molecules between phases.

When water boils in an open container, the steam diffuses into the surrounding atmosphere, leading to a continual escape of molecules. Consequently, the liquid-vapor equilibrium can be observed only when the gas is confined to a closed space.

Four common properties of solutions are modified by the presence of solute molecules. These properties are freezing point, boiling point, vapor pressure, and osmotic pressure. They are called the *colligative properties*.

with the crystals are sometimes captured and added to the solid phase. Second, molecules on the surface of the ice crystals sometimes become detached and enter the surrounding liquid. The mixture reaches a state of dynamic equilibrium when equal numbers of molecules move in each direction in any given time. When the pressure exerted on the mixture is 1 atm, this ice-water equilibrium exists only at 0 °C because any change of temperature throws the rates out of balance. Lowering the temperature decreases the rate at which molecules escape from the surface of the ice, whereas raising the temperature increases the rate of escape.

A dynamic equilibrium also exists between liquid water and steam when the pressure exerted on the liquid is 1 atm and the temperature is 100 °C (Figure 10-41, *B*). Some molecules at the liquid surface have sufficient energy to escape into the gas phase, and some molecules in the gas phase are captured when they strike the liquid surface. Under conditions of dynamic equilibrium, equal numbers of molecules move in each direction in any given time. At a pressure of 1 atm, this equilibrium exists only at 100 °C because lowering the temperature reduces the rate at which molecules escape from the liquid phase and condensation occurs. Raising the temperature, on the other hand, increases the rate of escape from the liquid phase, and the liquid boils.

These two equilibria provide the basis for precise definitions of the normal freezing point and the normal boiling point. The **normal freezing point** *(fp)* of a substance is the temperature at which solid and liquid coexist at equilibrium under a pressure of 1 atm. The **normal boiling point** *(bp)* of a liquid is the temperature at which liquid and vapor coexist at equilibrium under a pressure of 1 atm.

EFFECT OF SOLUTES

The molecular view of freezing and boiling provides a basis for determining the influence of dissolved substances on melting and boiling points. In a solution, solute molecules displace some of the solvent molecules, so a given volume of a solution contains a smaller number of solvent molecules than the same volume of pure solvent. Consequently, the presence of solute molecules reduces the rate at which solvent molecules leave the liquid phase. Figure 10-42 shows that changing one rate without changing the other rate throws the dynamic equilibrium out of balance.

The addition of solutes *decreases* the freezing point of a solution because collisions between solvent molecules and crystals of solid solvent occur less frequently

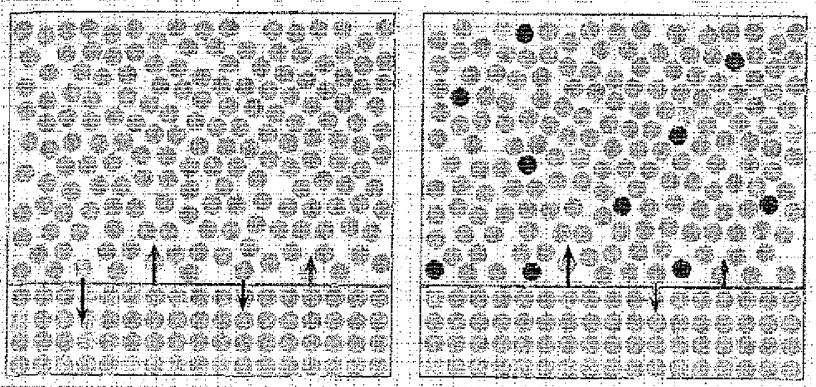


FIGURE 10-42
Molecular views of the rates of solid-liquid phase transfer of a pure liquid and a solution at the normal freezing point. The addition of solute does not change the rate of escape from the solid, but it decreases the rate at which the solid captures solvent molecules from the solution. This disrupts the dynamic equilibrium between escape and capture.

than in the pure solvent. Consequently, fewer molecules are captured by the solid phase than escape from the solid to the liquid. Cooling the solution restores dynamic equilibrium because it simultaneously reduces the number of molecules that have sufficient energy to break away from the surface of the solid and increases the number of molecules in the liquid with low enough kinetic energy to be captured by the solid.

Experiments show that at low solute concentration the change in freezing point of a solution, ΔT_f, obeys a simple equation:

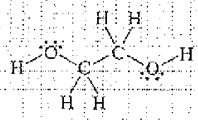
$$\Delta T_f = K_f X_{\text{solute}} \qquad (10\text{-}2)$$

where X_{solute} is the total mole fraction of solutes and K_f is a constant, called the **freezing point depression constant.** The constant is different for different *solvents* but does not depend on the identity of the *solutes.* For water, K_f is 105.0 °C. Sample Problem 10-10 illustrates the use of Equation 10-2.

Equation 10-2 can be derived from our simple molecular picture and principles of kinetic molecular theory. The derivation is independent of the nature of solute and solvent, so Equation 10-2 is valid for other solvents besides water, except that K_f has a different value for each solvent.

SAMPLE PROBLEM 10-10 FREEZING POINT DEPRESSION

Ethylene glycol (1,2-ethanediol) is added to automobile radiators to prevent cooling water from freezing. What is the freezing point of radiator coolant that contains 2.00 kg of ethylene glycol and 5.00 L of water?


Ethylene glycol

METHOD: The question asks for the freezing point of a solution. The phrase *to prevent the water from freezing* reveals that we are dealing with the depression of the freezing point of water. Equation 10-2 describes this process quantitatively: $\Delta T_f = K_f X_{\text{solute}}$.

The freezing point depression constant for water is known from experiments and can be found in tables: $K_f = 105.0$ °C. To calculate the freezing point, we must first determine the mole fraction of the solute in this solution.

The mole fraction of solute is defined to be moles of solute divided by total moles of solution. The number of moles of solute is found from the mole-mass relationship, and the number of moles of solvent can be found from the density of water and the mole-mass relationship. The molar mass (MM) of ethylene glycol is obtained from its chemical formula, $C_2H_6O_2$: $MM = 62.07$ g/mol:

$$n(\text{ethylene glycol}) = \frac{(2.00\text{ kg})(10^3\text{ g/kg})}{62.07\text{ g/mol}} = 32.22\text{ mol}$$

We find the mass of water from its density, 1.00 g/mL, and then convert to moles using the molar mass of water, $MM = 18.02$ g/mol:

$$m(\text{water}) = dV = (1.00\text{ g/mL})(5.00\text{ L})(10^3\text{ mL/L}) = 5.000 \times 10^3\text{ g}$$

$$n(\text{water}) = \frac{5.000 \times 10^3\text{ g}}{18.02\text{ g/mol}} = 277.5\text{ mol}$$

Now calculate the mole fraction of ethylene glycol:

$$X_{\text{solute}} = \frac{n_{\text{solute}}}{n_{\text{solute}} + n_{\text{solvent}}} = \frac{32.22\text{ mol}}{32.22\text{ mol} + 277.5} = 0.1040$$

Substitute this value for the mole fraction into Equation 10-2 to find the difference between the freezing point of the solution and that of pure water:

$$\Delta T_f = K_f X_{\text{solute}} = (105.0\text{ °C})(0.1040) = 10.9\text{ °C}$$

The result is rounded to three significant figures to be consistent with the data given in the problem. This is the amount by which the freezing point of the solution *differs* from that of pure water. Because the freezing point of water is 0 °C and freezing points are depressed by adding solutes, the new freezing point is below 0 °C: $T_f = -10.9$ °C.

A nonvolatile solute is one that has a negligible vapor pressure at the boiling point of the solution.

The effect of a solute on the boiling point of a solution is opposite to its effect on the freezing point. A boiling point is *increased* by adding a nonvolatile solute. This is because the solute reduces the rate of escape of solvent molecules into the gas phase. To get back to dynamic equilibrium, the solution must be heated so that more molecules acquire sufficient energy to escape from the liquid phase.

Molecular analysis and experimental studies show that the change in the boiling point of a solution obeys the same type of equation as the change in the freezing point:

$$\Delta T_b = K_b X_{\text{solute}} \qquad (10\text{-}3)$$

In Equation 10-3, ΔT_b is the elevation of the boiling point, and K_b is a constant called the **boiling point elevation constant.** The constant depends on the identity of the *solvent* but not on the identities of the nonvolatile *solutes*. Thus there is a different boiling point elevation constant for every solvent; for water, $K_b = 28.9$ °C. As Sample Problem 10-11 illustrates, Equation 10-3 is used in the same way as Equation 10-2.

SAMPLE PROBLEM 10-11 BOILING POINT ELEVATION

Determine the mole fraction of ethylene glycol required to raise the boiling point of radiator coolant to 110 °C, and calculate the mass of ethylene glycol that must be mixed with 5.00 L of water to give a solution with this mole fraction.

METHOD: The problem asks for the amount of solute required to raise the boiling point of a solution. Equation 10-3 applies, but it must be rearranged to solve for mole fraction:

$$\Delta T_b = K_b X_{solute} \qquad \textit{from which} \qquad X_{solute} = \frac{\Delta T_b}{K_b}$$

The tabulated value of K_b is 28.9 °C. We find ΔT_b from the normal boiling point of water and the desired boiling point of the solution:

$$\Delta T_b = (110\ °C - 100\ °C) = 10\ °C \qquad X_{solute} = \frac{10\ °C}{28.9\ °C} = 0.346$$

To apply this to an actual solution of radiator coolant containing 5.00 L of water, we must convert from mole fraction to mass using the definition of mole fraction. First, we calculate the number of moles of ethylene glycol required to give a solution whose mole fraction is 0.346. We determined in Sample Problem 10-10 that 5.00 L of water contains 277.5 moles:

$$0.346 = \frac{n_{solute}}{n_{solute} + n_{solvent}} = \frac{n_{solute}}{n_{solute} + 277.5\ mol}$$

Solving for n_{solute} requires some algebra:

$$n_{solute} = (0.346)(n_{solute} + 277.5\ mol) = (0.346)(n_{solute}) + 96.02\ mol$$

$$96.02\ mol = n_{solute} - (0.346)(n_{solute}) = (0.654)(n_{solute})$$

$$n_{solute} = 149\ mol$$

Finally, we convert from moles to mass using the molar mass of ethylene glycol:

$$m = n(MM) = (149\ mol)(62.07\ g/mol) = 9.2 \times 10^3\ g$$

The result is rounded to two significant figures to be consistent with ΔT_b.

Traditionally, K_b and K_f values have been expressed by using a different concentration unit called *molality* (c_m). *Molality* is moles of solute divided by kilograms of solvent. Then, $\Delta T_f = K_f c_m$ and $\Delta T_b = K_b c_m$. For water, $K_b = 0.512\ °C/c_m$ and $K_f = 1.858\ °C/c_m$. We prefer to use *mole fraction*, however, because it is a concentration measure that is already familiar to you. Furthermore, the mole fraction emphasizes the molecular nature of these effects. Keep in mind, however, that tabulations in reference sources are likely to be in molality units.

IONIC SOLUTIONS

Equations 10-2 and 10-3 describe how the freezing and boiling points of a solution depend on the mole fraction of solute. These changes occur because each solute species reduces the concentration of solvent molecules, thereby reducing the rate of escape of solvent molecules from the solution phase. This effect is cumulative, meaning that if two types of solute species are present, each reduces the rate of escape of solvent molecules. The change in freezing and boiling points of a solution is therefore determined by the *total* mole fraction of *all* solute species present.

This cumulative effect is particularly important for solutions of ionic substances because these solutions always contain cations and anions. As a result, the total mole fraction of solutes in an aqueous salt solution is always greater than the mole fraction of the salt itself. For example, when sodium chloride dissolves in water, each mole of the salt yields 1 mol of Na^+ ions and 1 mol of Cl^- ions, making the mole fraction of all solutes twice the mole fraction of the salt. This is taken into account by including an additional term in Equations 10-2 and 10-3:

$$\Delta T_f = i K_f X_{solute} \qquad \Delta T_b = i K_b X_{solute}$$

The factor i is a dimensionless number that gives the number of ions generated in solution by one formula unit of solute. For NaCl, $i = 2$ because each NaCl unit generates two ions in solution. For a salt such as $MgCl_2$, $i = 3$, reflecting the fact that each $MgCl_2$ unit yields one Mg^{2+} cation and two Cl^- anions.

OSMOSIS

Water molecules can pass through cell membranes, but most solutes cannot. This is a **semipermeable membrane,** and the movement of water through it is **osmosis.**

If a semipermeable membrane separates two identical solutions, solvent molecules move in both directions at the same rate, and there is no net osmosis. The two sides of the membrane are at dynamic equilibrium. The situation changes when the solution on the two sides of the membrane are different. Consider the membrane in Figure 10-43, which has pure water on one side and a solution of sugar in water on the other. The sugar molecules in the solution reduce the concentration of solvent molecules in the solution. Consequently, more solvent molecules pass through the membrane from the solvent side to the solution side than from the solution side to the solvent side. Now water flows from the solvent side to the solution side, and there is a net rate of osmosis.

What can be done to increase the rate of solvent flow from the solution side of the membrane? An increase in pressure on the solution side accomplishes this, because as pressure increases, the flow rate of any liquid also increases. An increase in pressure on the solution side of the membrane increases the rate of transfer of water molecules from the solution side to the solvent side.

Figure 10-44 shows that when the pressure is increased until the rate of solvent transfer is equal in both directions, dynamic equilibrium has been reestablished and net osmosis falls to zero. The pressure increase needed to equalize the transfer rates is called the **osmotic pressure** (Π). Osmotic pressure is a pressure *difference.* Both sides of a semipermeable membrane have some pressure exerted on them, and Π is the *extra pressure* that must be exerted on the solution to maintain dynamic equilibrium.

Like freezing point depression and boiling point elevation, osmotic pressure is proportional to the concentration of solute molecules. Osmotic pressure does not involve a temperature change, however, so there is no disadvantage in using the usual measure of solution concentration, molarity. Experiments also show that osmotic pressure increases as temperature increases:

$$\Pi = MRT \qquad (10\text{-}4)$$

The osmotic pressure equation seems very simple, but its derivation requires the molecular model, differential calculus, and detailed principles of physical chemistry that are beyond the scope of this book.

In Equation 10-4, M is the total molarity of all solutes, T is the temperature in kelvins, and R is the gas constant. If osmotic pressure is expressed in atmospheres, the fact that molarity is in moles per liter requires us to use $R = 0.08206$ L atm/mol K.

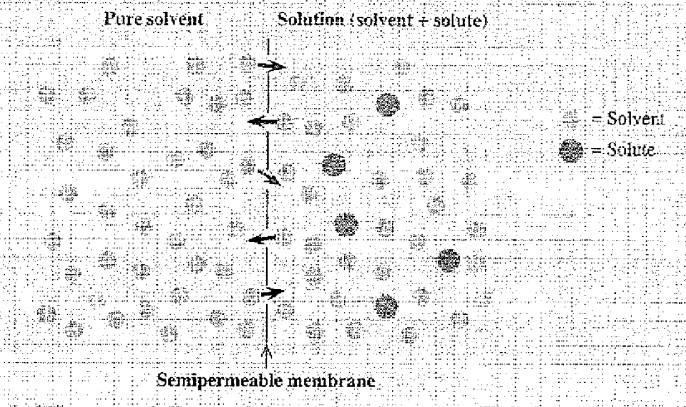


FIGURE 10-43
Small solvent molecules can pass back and forth freely through the pores of a semipermeable membrane, but solute molecules cannot. The presence of solute molecules in a solution reduces the concentration of solvent molecules, and this in turn reduces the rate at which solvent molecules pass out of the solution. There is an imbalance in transfer rates, which leads to osmosis.

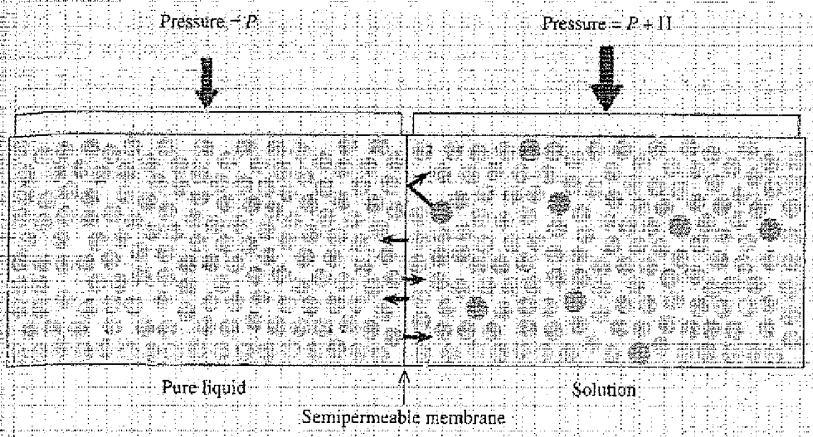


FIGURE 10-44
To equalize the rates of transfer of solvent molecules from a solution and from pure solvent, an additional pressure Π (osmotic pressure) must be exerted on the solution.

Osmotic pressure effects can be substantial. For example, the waters of the oceans contain dissolved salts at a total ionic molarity of about 1.13 M. We can calculate the osmotic pressure of ocean water:

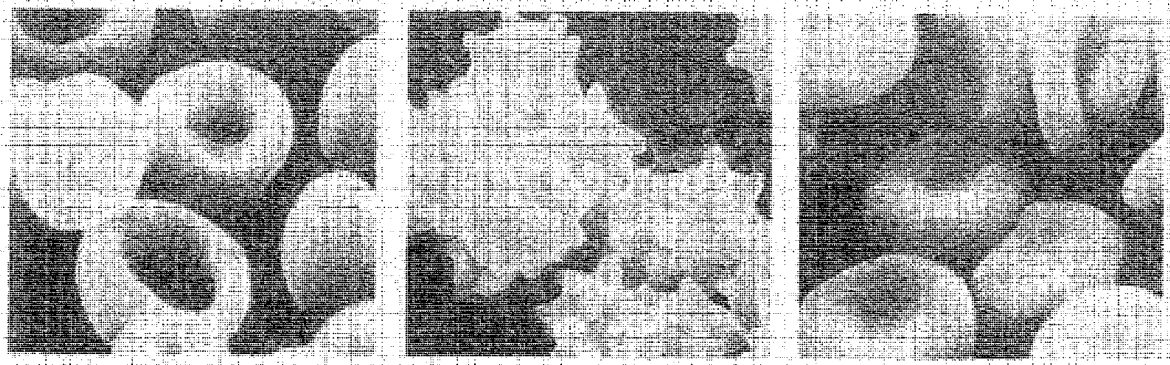
$$\Pi = MRT = (1.13 \text{ mol/L})(0.08206 \text{ L atm/mol K})(298 \text{ K}) = 27.6 \text{ atm}$$

Thus the osmotic pressure of ocean water is *more than 25 times atmospheric pressure*. By comparison the freezing point of ocean water is depressed by only about 1% from the freezing point of pure water, from 273 K to about 271 K (−2 °C).

Osmotic pressure plays a key role in biological chemistry because the cells of the human body are encased in semipermeable membranes and bathed in body fluids. Under normal physiological conditions the body fluid outside the cells has the same total solute molarity as the fluid inside the cells, and there is no *net* osmosis across cell membranes. Solutions with the same solute molarity are isotonic solutions.

The situation changes if a molarity imbalance is created. Figure 10-45 shows red blood cells immersed in solutions of different molarities. When the fluid outside the cell is at *higher* solute molarity, transport of water across the membrane into the cell slows. The result is that water leaves the cell, causing it to shrink. When the fluid

FIGURE 10-45
When bathed in isotonic solution *(left)*, red blood cells retain their normal shape because there is no net osmosis across their membranes. In a solution of higher concentration *(center)*, the net osmotic flow removes water from the cell interior, causing cells to shrink and wrinkle. In a solution of lower concentration *(right)*, the net osmotic flow pumps water into cells, expanding them until they may rupture.



outside the cell is at *lower* molarity, movement of water into the cell increases. The extra water in the cell causes an increase in internal pressure. Eventually, the internal pressure of the cell matches the osmotic pressure, and water transport reaches dynamic equilibrium. Unfortunately, osmotic pressures are so large that cells can burst under the increased pressure before they reach equilibrium.

Red blood cells are particularly susceptible to these potentially damaging concentration changes because they are suspended in the aqueous medium of the blood. Consequently, solutions used for intravenous feeding must be isotonic. Sample Problem 10-12 deals with isotonic solutions.

SAMPLE PROBLEM 10-12 ISOTONIC SOLUTIONS

Isotonic intravenous solutions contain 49 g/L of glucose ($C_6H_{12}O_6$). What is the osmotic pressure of blood?

METHOD: Isotonic solutions, by definition, exert equal osmotic pressure. Therefore Π for blood is the same as Π for the glucose solution. We can calculate Π from Equation 10-4 after converting the concentration into moles per liter:

$$\Pi = MRT \qquad M = \frac{\text{mol}}{\text{L}} = \frac{m}{(MM)(\text{L})}$$

According to the formula of glucose, MM is 180 g/mol. Substituting, we find the molarity of the glucose solution:

$$\frac{49\ \text{g}}{(180\ \text{g/mol})(1\ \text{L})} = 0.272\text{M}$$

Because we are working with blood in the human body, T is human body temperature, which is 37 °C.

$$T = 37\ ^\circ\text{C} = 37 + 273 = 310\ \text{K} \qquad R = 0.08206\ \text{L atm/mol K}$$

$$P = (0.272\ \text{mol/L})(0.08206\ \text{L atm/mol K})(310\ \text{K}) = 6.9\ \text{atm}$$

The result is rounded to two significant figures to match the initial data (49 g/L).

An additional pressure of 6.9 atm is more than enough to destroy the cell membrane, so it is hardly surprising that red blood cells burst when immersed in dilute solutions.

DETERMINATION OF MOLAR MASS

The magnitude of osmotic pressure is large enough that measurements of Π provide a convenient way to determine the molar mass of a compound. We can solve the osmotic pressure equation (Equation 10-4) for molar mass after expressing molarity in terms of mass and molar mass:

$$\Pi = MRT \qquad \Pi = \frac{mRT}{(MM)(V)}$$

A simple rearrangement gives an equation for calculating molar mass:

$$MM = \frac{mRT}{\Pi V} \tag{10-5}$$

To determine the molar mass of an unknown compound, a measured mass of material is dissolved to give a measured volume of solution. The system is held at constant temperature, and the osmotic pressure is determined by using an apparatus such as the one shown in Figure 10-46. Osmotic pressure measurements are particularly useful for determining the molar mass of large molecules such as polymers and biological materials, as Sample Problem 10-13 illustrates.

SAMPLE PROBLEM 10-13 DETERMINING MOLAR MASSES

A 25.00 mL aqueous solution containing 0.420 g of hemoglobin has an osmotic pressure of 4.6 torr at 27 °C. What is the molar mass of hemoglobin?

METHOD: Equation 10-5, which is used to calculate the molar mass by osmometry, is derived from the osmotic pressure expression, Equation 10-4.

All of the necessary data are given in the problem:

$$m_{solute} = 0.420 \text{ g} \qquad R = 0.08206 \text{ L atm/mol K} \qquad T = 27 \text{ °C} + 273 = 300 \text{ K}$$

$$\Pi = (4.6 \text{ torr})(1 \text{ atm}/760 \text{ torr}) = 0.00605 \text{ atm}$$

$$V_{solution} = (25.00 \text{ mL})(1 \text{ L}/1000 \text{ mL}) = 0.02500 \text{ L}$$

$$MM = \frac{mRT}{\Pi V} = \frac{(0.420 \text{ g})(0.08206 \text{ L atm/mol K})(300 \text{ K})}{(0.00605 \text{ atm})(0.02500 \text{ L})}$$

$$MM = 6.8 \times 10^4 \text{ g/mol}$$

As in any calculation, be careful to express all data in appropriate units. The osmotic pressure was measured to two significant figures, so the result has two significant figures.

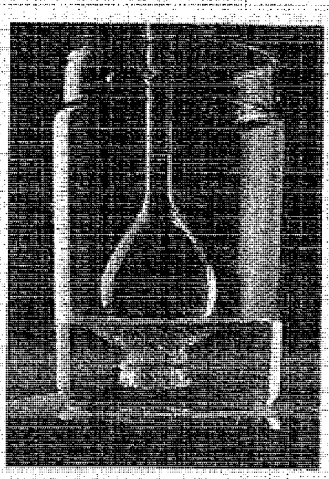


FIGURE 10-16
In a standard thistle-tube osmometer the liquid level of the solution at equilibrium is higher than that of the exterior solvent, generating an additional pressure equal to the osmotic pressure.

SECTION EXERCISES

10.7.1 A water-soluble protein molecule has a molar mass of 985 g/mol. Calculate the freezing point depression, boiling point elevation, and osmotic pressure at 300 K of an aqueous solution containing 0.750 g/L of this protein. (Assume that the solution has a density of 1.000 g/mL.)

10.7.2 The process called **reverse osmosis** occurs when a solution in contact with pure solvent across a semipermeable membrane is subjected to an external pressure that is *greater* than its osmotic pressure. Reverse osmosis can be used to desalinate seawater. Redraw Figure 10-44 using arrows to represent water movement across the membrane during reverse osmosis.

10.7.3 In your own words, write a detailed, molecular-level description of how reverse osmosis can be used to desalinate seawater.

10.8 SEPARATION PROCESSES

Water leaving a community is contaminated with a variety of impurities that must be removed before the water is pure enough to be used again. When a synthetic chemist makes a new compound, it is likely to be contaminated with by-products and unreacted starting materials, which must be removed before the new compound can be identified and studied. An oil refinery is a huge chemical plant that separates the components of petroleum and converts them into useful fuels. A biochemist who wants to study the properties of a particular enzyme must first isolate the molecule from its natural source. These are but four scenarios out of many in which separation and purification are essential parts of the chemical operations.

Phase behavior is at the heart of most purification techniques. When a solution goes through a phase change, its different components are likely to move between the phases at different rates. Chemists take advantage of these differences to purify chemical compounds. In this section, we survey purification techniques.

RECRYSTALLIZATION

Most laboratory syntheses are carried out in liquid solution. If the product is a solid, it may spontaneously precipitate from the reaction solvent, or it may be isolated by boiling off the solvent. In either case the solid product almost always contains impurities. Recrystallization is the classic way of removing impurities from a crude solid.

Recrystallization takes advantage of the way in which the solubilities of solids vary with temperature. Most solid solutes are more soluble in hot than in cold solvent because fast-moving, high-energy molecules are less likely to be captured by the solid phase than slow-moving ones, and solute molecules move faster in hot than in cold solutions.

If a solid substance is dissolved in a minimum volume of hot solvent that is then allowed to cool, the solubility of the solid is exceeded, and it crystallizes from the solution. In favorable cases the impurities remain dissolved in the cold solvent, and the solid has been purified. Purification by recrystallization works best when the crude solid contains a low percentage of impurities. If a large amount of an impurity is present, the impurity is likely to crystallize with the desired substance. The example in Sample Problem 10-14 illustrates this feature.

SAMPLE PROBLEM 10-14 PURIFICATION BY RECRYSTALLIZATION

A chemist has synthesized 10.0 g of crude organic solid that contains an estimated 10% impurities. The desired product is less soluble in cold ethanol (5.0 g/100 mL) than in hot ethanol (15 g/100 mL). The chemist estimates that the impurity is similar to the product and therefore has the same solubility properties. Can the compound be purified by recrystallization from ethanol?

METHOD: If the sample is dissolved in the minimum amount of hot ethanol, chilling the solution will cause the solid to precipitate. This will purify the compound if none of the impurity precipitates at the same time. We need to determine the minimum volume of hot solvent needed to dissolve the entire sample and then find out whether the impurity precipitates when that volume of solvent is chilled.

Because 10% of the crude sample is impurity, the 10.0-g sample contains 9.00 g of the desired compound. From the solubility of 15 g/100 mL in hot ethanol, we can calculate the minimum volume of solvent that will dissolve the entire sample:

$$(9.0\text{ g})\left(\frac{100\text{ mL}}{15\text{ g}}\right) = 60\text{ mL}$$

There is an estimated 1.0 g of impurities in the sample. If the impurities have solubility properties similar to those of the desired product, 60 mL of hot ethanol will dissolve 9.0 g of impurities, too, so both the desired product and the impurities will dissolve completely in 60 mL of hot ethanol.

When the ethanol is cooled, the mass of solid that it can hold can be calculated from the solubility in cold ethanol:

$$(60\text{ mL})\left(\frac{5\text{ g}}{100\text{ mL}}\right) = 3.0\text{ g}$$

The cold solution can contain 3.0 g each of the desired solid and its impurity.

Of the 9.0 g of the desired substance, 6.0 g will recrystallize on cooling. All of the 1.0 g of impurity will remain dissolved. A single recrystallization of the contaminated sample will give 6.0 g of pure compound.

Chemists frequently recover a "second crop" of substance by boiling off some of the solvent and then rechilling the solution. You should be able to determine how much additional pure substance could be recovered from this solution before the impurity begins to precipitate.

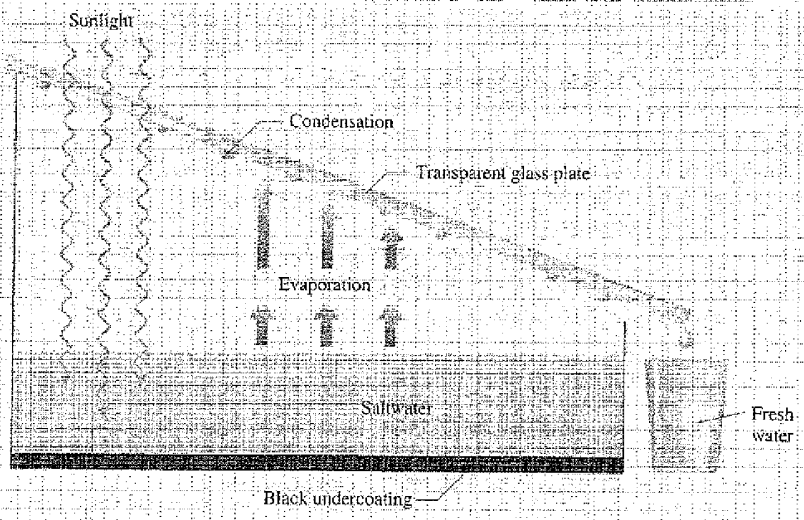


FIGURE 10-47
A simple solar saltwater still. Stills designed along these lines find commercial use in areas where sunlight and saltwater are plentiful and natural supplies of fresh water are scarce.

As Sample Problem 10-14 shows, some product is always lost during recrystallization. In the single recrystallization described, 3.0 g of the original material remains in solution. Thus the yield of the one-step process is 67%. By taking a second crop, an additional 2.0 g can be recovered, increasing the yield to 89%, but the remaining 1.0 g remains mixed with 1.0 g of impurity and cannot be recovered by further recrystallization. Chemical syntheses seldom give 100% yields, in part because the process of purifying the product always results in some losses.

DISTILLATION

The most common method for purifying liquids is **distillation,** which is based on a liquid-vapor phase change. A liquid solution is heated until it boils, and if the solutes remain nonvolatile, pure solvent boils off. This pure solvent vapor is captured by condensing it on a chilled surface.

Fresh water can be obtained by distilling saltwater. Figure 10-47 shows a simple solar still, in which the energy needed to vaporize the water comes from sunlight absorbed by the black coating on the bottom of the still. As the solution is heated, water evaporates, leaving the nonvolatile salt behind. The water vapor comes into contact with the underside of the glass plate, which is cooled by natural air flow. Fresh water condenses on the cool plate and trickles down to a collection vessel at the bottom of the still.

Obtaining high-purity liquids in the laboratory often requires a more elaborate procedure because many liquids decompose or react with oxygen at high temperature. For this reason, high-boiling liquids are often distilled under reduced pressure to lower the boiling temperature. Figure 10-48 shows a common laboratory apparatus used for reduced-pressure distillation.

When a liquid is contaminated with volatile impurities, simple boiling gives a mixture of compounds rather than a pure solvent. In these cases, chemical treatment of the solution can be used to convert the volatile impurity into a nonvolatile solid. For example, small amounts of water are removed from organic solvents such as cyclohexane and diethyl ether by placing a piece of sodium metal in the distilling flask. Water in the solution reacts with sodium to give sodium hydroxide, which is nonvolatile, and hydrogen gas, which does not recondense. Air is excluded from the still, moreover, to prevent immediate contamination of the distilled solvent with

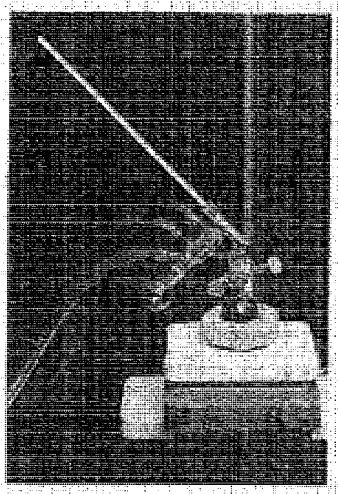


FIGURE 10-48
Liquids susceptible to oxidation or thermal decomposition can be purified by distilling them under reduced pressure.

The effect of pressure on boiling points is considered in Chapter 13. Qualitatively, reducing the pressure reduces the rate of capture of molecules from the gas phase, and this lowers the temperature at which liquid-vapor equilibrium exists.

$2\ Na + 2\ H_2O \rightarrow 2\ NaOH + H_2$. Dry solvents are needed for reactions that give undesirable side products when water is present.

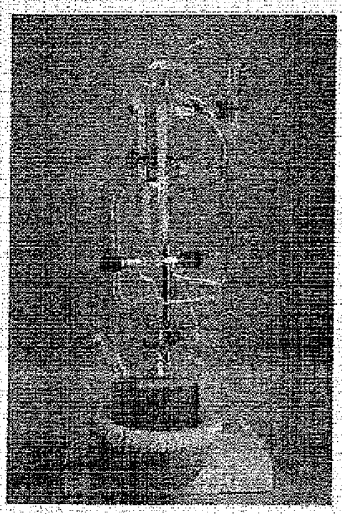


FIGURE 10-49
To produce scrupulously dry solvents, the solvent must be treated with a chemical purifying agent and then distilled under an atmosphere of a dry inert gas. The blue color in the distillation flask is due to the drying agent. Diethyl ether, which is colorless, is collected by condensing the solvent into a bulb above the boiling solvent.

water vapor from the atmosphere. Figure 10-49 shows a laboratory still for producing scrupulously dry diethyl ether.

Distillations in the chemical industry are performed on an enormous scale. Distillation is an essential step in the refining of petroleum, for example. Crude oil is a complex mixture of hydrocarbons without a single well-defined boiling point. Instead, crude oil boils over a broad range of temperatures as the lighter, more volatile hydrocarbons boil off first. As the temperature increases gradually, heavier and heavier components of the oil distill out of the mixture. The end product is asphalt, a gooey black tar. In the first step of petroleum refining, crude oil is separated into several fractions according to specific ranges of boiling point. Figure 10-50 shows the huge distillation towers used for these kinds of separations.

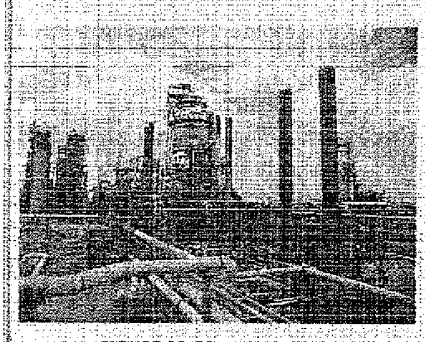


FIGURE 10-50
Oil refineries use immense distillation towers to separate crude oil into its various useful fractions.

The different fractions of hydrocarbons that can be obtained from petroleum are listed in Table 8-2.

CHROMATOGRAPHY

There are many types of **chromatography,** but all are based on the same essential principles. A *mobile phase* carries the compounds to be separated, and a *stationary phase* binds these compounds through intermolecular forces.

Figure 10-51 shows how chromatography separates compounds. The mobile phase dissolves the compounds of interest and carries them over the stationary phase. The rate of movement of compounds depends on how strongly they interact with the stationary phase. Because solutes move only when in the mobile phase, molecules that have a very low affinity for the stationary phase move quickly, whereas those that bind tightly to the stationary phase lag behind. After the materials have traveled a sufficient distance, they become separated into distinct "bands"; each band may contain one pure material. As the mobile phase comes off the lower end of the column, it can be collected in small volumes called *fractions* or *cuts*. When the separations are complete, the various components of the original mixture are found in different fractions.

Chromatography is extremely versatile because the stationary phase and the mobile phase can be varied to match the types of compounds that need to be separated. For example, some stationary phases separate solutes according to their polarity. Polar groups on the stationary phase bind solutes through dipole-dipole or hydrogen-bonding interactions. The binding is reversible, and eventually the solvent washes the solutes off the stationary phase. The more polar the solute, the tighter it binds to the stationary phase. Thus the faster the solutes move through the column, the lower their polarity.

In other cases the stationary phase binds solutes according to their size. Here, the stationary phase is made up of particles that are perforated with holes or channels, much like a sponge or a Wiffle ball (Figure 10-52). Small molecules can pass through the holes into the interior of the particle. Eventually these molecules make their way back out of the stationary phase. The smallest molecules move into and out of particles many times as they travel along the column. Larger molecules enter fewer times because they do not fit inside the pores as easily. The more particles a solute molecule enters, the more time it spends bound to the stationary phase, and the more slowly it moves along the column. Thus the largest solute molecules emerge from the chromatography column first, and the smallest molecules emerge last. Pore size in these stationary phases can be controlled to accommodate an enormous range of molecular sizes, from mixtures of small gas molecules to huge biomolecules with molar masses in excess of 100,000 g/mol.

METHODS OF CHROMATOGRAPHY

Chromatographic techniques are classified according to the nature of their mobile and stationary phases. Gas chromatography (GC) is used to separate mixtures of

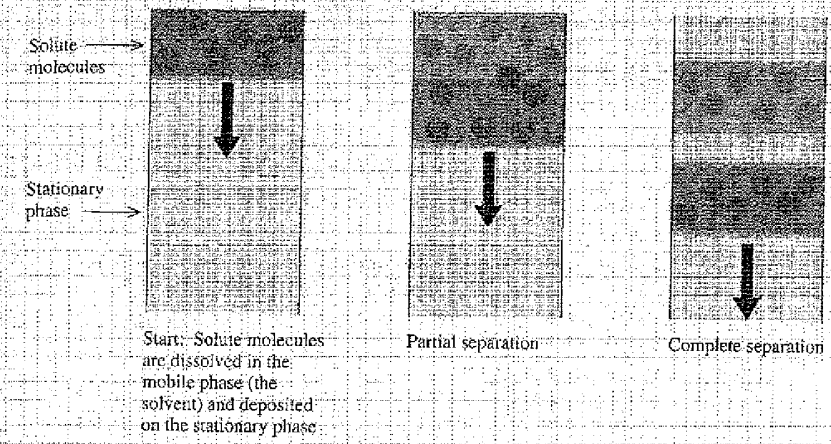


FIGURE 10-51
Diagrammatic view of how column chromatography works. Solute molecules that bind strongly to the stationary phase (*red circles*) move down the column more slowly than those that bind only weakly (*blue circles*). For clarity, solvent molecules and the detailed structure of the stationary phase are not shown.

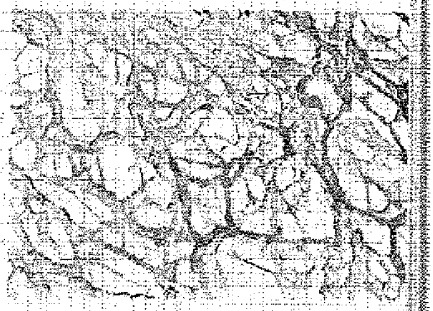
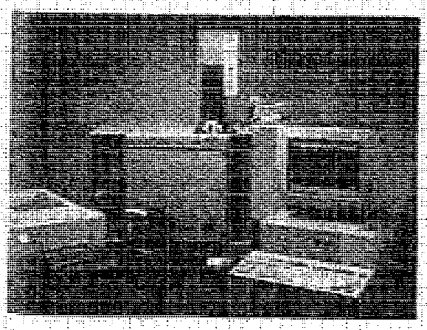


FIGURE 10-52
Chromatographic columns that separate substances according to molecular sizes have stationary phases made up of many tiny porous beads. This image was magnified 50,000 times.

gases or volatile liquids. The mixture to be separated is vaporized in an oven, and the gaseous mobile phase passes through a long, narrow column packed with a finely divided solid that may be impregnated with a nonvolatile liquid (stationary phase). As the components of the mixture emerge from the column, their presence is sensed by a detector and displayed as a graph on a computer screen. Figure 10-53 shows a photograph of a gas chromatograph. The most widespread use of GC is in identifying trace components of a mixture. Among other things, GC is used to test urine for the presence of illegal drugs, identify pollutants and measure their concentrations in groundwater, assay the purity of a volatile compound isolated in the laboratory, and follow the progress of a chemical reaction by monitoring the disappearance of starting materials or the appearance of products.

Liquid chromatography (LC) uses a liquid mobile phase that passes down the stationary phase of a finely divided solid. An LC apparatus is shown in Figure 10-54. This technique is widely used to purify chemical substances on a multigram scale.

In thin-layer chromatography (TLC) a minute amount of a mixture is placed as a small spot at the bottom of a plate coated with a thin layer of a solid stationary phase, usually SiO_2 or Al_2O_3. The plate is placed "spotted" end down in a chamber containing a small amount of a suitable liquid solvent that acts as the mobile phase. Capillary action carries the solvent and the mixture up the plate, and the dissolved



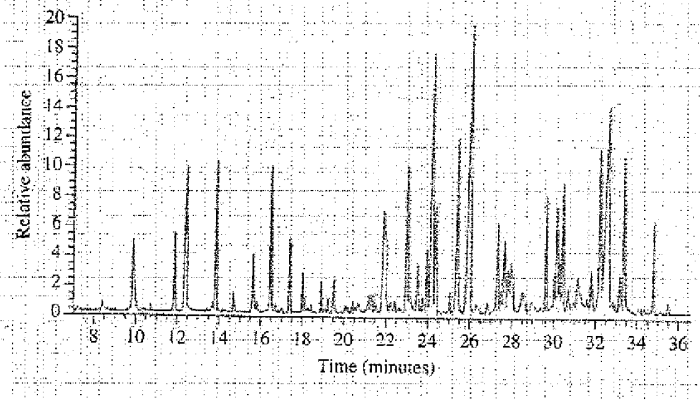


FIGURE 10-53
A gas chromatograph separates a small sample of a mixture into its individual components. The printout shown here highlights the large number of compounds used to make perfume.

BOX 19-2

ION-EXCHANGE CHROMATOGRAPHY

The cations or anions in solutions of ionic compounds can be exchanged for other cations or anions by using the technique of ion-exchange chromatography. The stationary phases used in ion-exchange columns are large polymer molecules with charged functional groups. In an anion-exchange column the polymer is linked covalently to a positively charged group. Negative counter-ions are loosely associated with the polymer through ion-ion attractions. In a cation-exchange column the polymer contains covalently bound substituents with a negative charge, and the positive counter-ions are loosely associated through ion-ion attractions.

Before an ion exchange column can be used, the stationary phase must first be *charged*. In the charging process a highly concentrated solution of a specific cation or anion is passed through the column. All the mobile ions associated with the resin are replaced by the specific cation or anion. For example, to charge a cation-exchange resin with sodium ions, the column is treated with concentrated aqueous sodium chloride. After the column is charged, an aqueous solution containing other cations (for example, calcium cations) can be passed through the column, and the column will attract these cations, releasing sodium ions to enter the solution. Cations have exchanged places between solution and polymer, hence the term **ion exchange.**

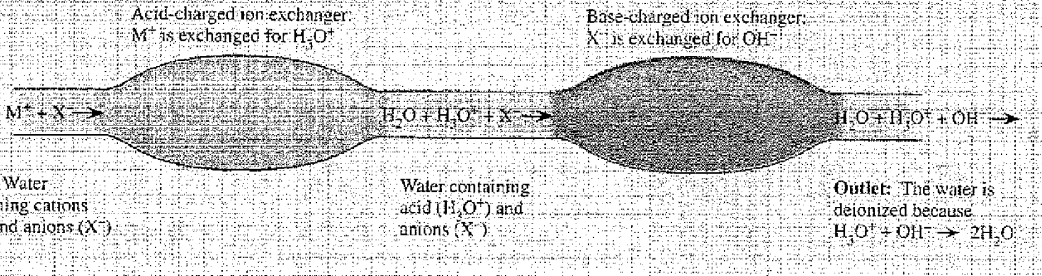
As this description suggests, ion-exchange chromatography does not *remove* ions from a solution. Instead, it *replaces* them with other ions. Nonetheless, this method is used widely in the water treatment industry to soften and deionize water.

"Hard" water has high concentrations of divalent Mg^{2+} and Ca^{2+} cations. We explained earlier that the large molecules that make up soaps contain negatively charged groups that form precipitates with these divalent metal cations. The sodium salts of soaps, on the other hand, do not precipitate from solution. The function of a water softener is to exchange the "hard" Ca^{2+} and Mg^{2+} cations for the "soft" Na^+ cation. Thus even though soft water is no more pure than hard water, it dissolves soaps better. This makes soft water a better medium than hard water for household and industrial cleaning.

Deionization, shown below, removes ions from solution. An aqueous salt solution passes in sequence through a cation-exchange column charged with hydronium ions and an anion-exchange column charged with hydroxide ions. In the first column H_3O^+ replaces metal cations in the solution. In the second column OH^- replaces the anions present in the original salt solution. Hydroxide ions and hydronium ions immediately combine to give water:

$$H_3O^+_{(aq)} + OH^-_{(aq)} \rightarrow 2\,H_2O_{(l)}$$

Although ion exchange is a cost-effective way to produce ion-free water for laboratory and home use, it cannot be applied economically to the desalinization of seawater. After a short period of use, the columns become depleted of H_3O^+ and OH^- ions and must be recharged by passing aqueous HCl through the cation exchanger and aqueous NaOH through the anion exchanger. Because seawater is much higher in total ion content than fresh water, the cost of the chemicals for recharging quickly becomes prohibitive.



solutes spread out according to their polarity. The plate is removed from the chamber when the solvent nears the top. The plate dries as the solvent evaporates, leaving the nonvolatile components of the mixture as spots located at different positions on the plate. The TLC in Figure 10-55 shows that common blue ink is a mixture of several different colored compounds. TLC is often used to monitor the progress of chemical reactions. It is also used to determine the optimum separation conditions for larger-scale chromatographic techniques such as LC. Box 10-2 explains another chromatographic technique, ion-exchange chromatography.

FIGURE 10-54
A liquid chromatograph involves the same principles as GC but on a larger scale.

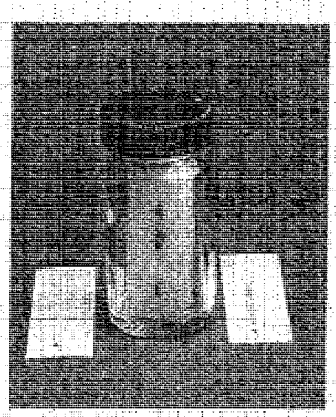


FIGURE 10-55
In thin-layer chromatography a solvent moves along a plate by capillary action, carrying different components with it at different rates. The photograph shows the separation of a blue ink into its component pigments.

SECTION EXERCISES

Explain in molecular terms the following features of purification techniques.

10.8.1 When a precipitate forms too quickly, it is likely to be less pure than if it is allowed to crystallize slowly from the same solution.

10.8.2 Distillation of an organic liquid that contains a volatile impurity always gives a distilled liquid that still contains some of the impurity.

10.8.3 If your home water softener runs out of salt, your water soon feels hard again.

CHAPTER SUMMARY

1. Attractive forces between molecules cause most substances to be liquids or solids under normal conditions, as well as leading to nonideal behavior of gases at high pressure and low temperature. These forces include dispersion forces, dipole-dipole interactions, and hydrogen bonding.
2. Hydrogen bonds, which are particularly important in aqueous environments, involve partial sharing of electrons between a fluorine, oxygen, or nitrogen atom and a hydrogen atom in a highly polar bond.
3. The molecules in liquids cohere but move freely. Liquid properties include surface tension, capillary action, and viscosity. Solids, on the other hand, are held in fixed structures by ionic, metallic, covalent, or intermolecular interactions.
4. Amorphous solids lack a regular structure, but any crystalline solid is composed of a repeating pattern whose smallest complete part is a unit cell. The simplest of these repeat patterns, adopted by many atomic and metallic solids, are hexagonal close-packed, face-centered cubic, and body-centered cubic structures.
5. A solution is a homogeneous mixture of varying amounts of solutes contained in a solvent. Substances that are subject to similar intermolecular forces tend to dissolve in each other, leading to the generalization, like dissolves like.
6. Gaseous solutions have unrestricted composition ranges, but most liquid solutions have an upper limit on the amount of solute they can hold. The solubility of a gas in a liquid depends not only on the natures of solvent and solute, but also on the partial pressure of solute in the gas phase.
7. Surfactants, which are molecules that contain water-compatible and water-incompatible structures, form monolayers, micelles, and vesicles in aqueous media.
8. Solutes depress the freezing point, raise the boiling point, and generate an osmotic pressure of a solution. The magnitudes of these colligative properties are concentration-dependent.
9. Transfers between phases form the basis for separation and purification techniques, including recrystallization, distillation, and chromatography.

KEY TERMS

adhesive forces
cohesive forces
dipolar forces
dispersion forces
hydrogen bond
intermolecular forces
intramolecular forces
polarizability

capillary action
surface tension
viscosity

amorphous
body-centered cubic structure
close-packed structure
crystalline
cubic close-packed structure
hexagonal close-packed structure
unit cell

alloy
amalgam
Henry's law
miscible
saturated solution
solubility
solute
solution
solvent

bilayer
hydrophilic
hydrophobic
micelle
monolayer
surfactant
vesicle

boiling point elevation constant
freezing point depression constant
normal boiling point (bp)
normal freezing point (fp)
osmosis
osmotic pressure (Π)
semipermeable membrane

chromatography
distillation
ion exchange

SKILLS TO MASTER

- Explaining variations in boiling points
- Identifying hydrogen bonds
- Describing surface tension, capillary action, and viscosity
- Recognizing types of solids
- Depicting simple crystal types
- Predicting solubility patterns
- Calculating gas solubilities
- Describing surfactant properties
- Calculating colligative properties
- Drawing molecular pictures of solutions
- Describing separation techniques

LEARNING EXERCISES

10.1 Write a chapter summary of two pages or less that summarizes the important ideas and concepts presented in this chapter.

10.2 List all the types of interactions that can act to hold a solid together. Organize the list from strongest to weakest.

10.3 Draw molecular pictures that show every type of hydrogen bond that exists in a solution containing methanol, water, and ammonia.

10.4 Write a paragraph that describes the factors that make glycerol highly viscous and explains why its viscosity falls as temperature rises.

10.5 Define and give an example of each of the following: (a) close-packed structure; (b) unit cell; (c) molecular solid; (d) covalent solid; (e) amorphous solid; and (f) surfactant.

10.6 Update your list of memory-bank equations. Be sure to mention how the equations in this chapter are used.

10.7 Write a paragraph that describes the types of substances that form monolayers, micelles, and vesicles in water. Explain the differences among these structures.

10.8 Describe how each of the following separation processes works: recrystallization, distillation, and chromatography.

10.9 Prepare a list of the terms in Chapter 10 that are new to you. Write a one-sentence definition for each, using your own words. If you need help, consult the glossary.

PROBLEMS

THE NATURE OF INTERMOLECULAR FORCES

10.1 Methane condenses at 121 K, but carbon tetrachloride boils at 350 K. Sketch an energy-distance plot similar to that of Figure 10-3 that shows the behavior of both of these substances.

10.2 Draw pictures showing the atomic arrangements in samples of $Ag_{(s)}$, $Ar_{(g)}$, and $Hg_{(l)}$.

10.3 Predict whether intermolecular attractions become more or less significant when the following changes are imposed:
(a) A gas is expanded to a larger volume at constant temperature.
(b) More gas is forced into the same volume at constant temperature.
(c) The temperature of the gas is lowered at constant volume.

10.4 Predict whether molecular volume becomes more or less significant when each of the changes in Problem 10.3 is imposed.

10.5 From the following experimental data, calculate the percent deviation from ideal behavior for each gas:
(a) 1.00 mol CO_2 in a 1.20-L container at 40.0 °C exerts 19.7 atm pressure.
(b) 3.00 g H_2 at 0.00 °C and 200 atm occupies a volume of 189.18 cm^3.

10.6 Arrange the following in order of increasing boiling point: Ar, He, Ne, and Xe. Explain your ranking.

10.7 Arrange the following in order of ease of liquefaction: CCl_4, CH_4, and CF_4. Explain your ranking.

10.8 Benzene (C_6H_6), naphthalene ($C_{10}H_8$), and anthracene ($C_{14}H_{10}$) are three ring compounds with similar molecular structures. One is a liquid, another is a relatively volatile solid, and the third is a less volatile solid. Which is which? Explain your assignments.

Benzene C_6H_6 — Napthalene $C_{10}H_8$ — Anthracene $C_{14}H_{10}$

10.9 Which of the following ions have the stronger interaction with water molecules in an aqueous solution? Explain your choices. (a) Na^+ or Mg^{2+}; (b) Na^+ or K^+; and (c) SO_4^{2-} or SO_3^{2-}.

HYDROGEN BONDING

10.10 Draw Lewis structures that show the hydrogen bonding interactions for each of the following: (a) two NH_3 molecules; (b) two CH_3OH molecules; and (c) an HF molecule and an acetone molecule [$(CH_3)_2C{=}O$].

10.11 List ethanol (C_2H_5OH), propane (C_3H_8), and *n*-pentane (C_5H_{12}) in order of increasing boiling point, and explain what features determine this order.

10.12 How many hydrogen bonds can be formed by one glycerol molecule ($HOCH_2CHOHCH_2OH$)? Draw Lewis structures that show the hydrogen bonding of a glycerol molecule dissolved in water.

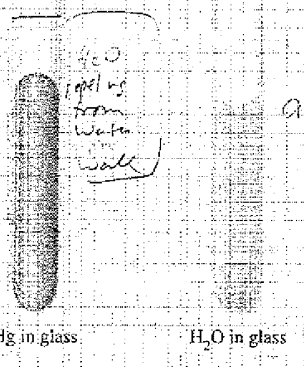
10.13 Which of the following will hydrogen bond? (a) CH_2Cl_2; (b) H_2SO_4; (c) H_3COCH_3; and (d) $H_2NCH_2CO_2H$.

PROPERTIES OF LIQUIDS

10.14 Given that a lubricant must flow easily to perform its function, which grade of motor oil is preferred for winter use: high or low viscosity? Why?

10.15 Pentane is a C_5 hydrocarbon, gasoline contains mostly C_8 hydrocarbons, and fuel oil contains hydrocarbons in the C_{12} range. List these three hydrocarbons in order of increasing viscosity, and explain what molecular feature accounts for the variation.

10.16 Water in a glass tube takes on a concave shape, whereas mercury in a glass tube takes on a convex shape. Explain why the two liquids display different shapes.



Hg in glass — H_2O in glass

10.17 To make a good solder joint, the liquid metal solder must adhere well to the metal surfaces being joined. "Flux" is used to clean the metal surfaces. What types of substances must flux remove?

10.18 A pipet is considered to be "dirty" when water forms beads on its walls rather than forming a thin film that drains well. Which of the following on the surface of a pipet wall will make it dirty? In each case, explain the intermolecular forces underlying your classification. (a) grease; (b) Mg^{2+} ions; (c) acetone; and (d) SiO_2.

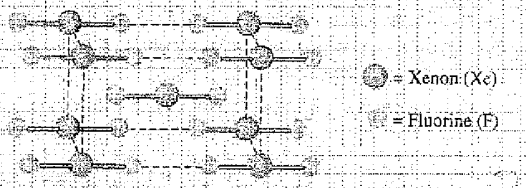
PROPERTIES OF SOLIDS

10.19 Classify each of the following as ionic, covalent, molecular, or metallic solids: Sn, S_8, Se, SiO_2, and Na_2SO_4.

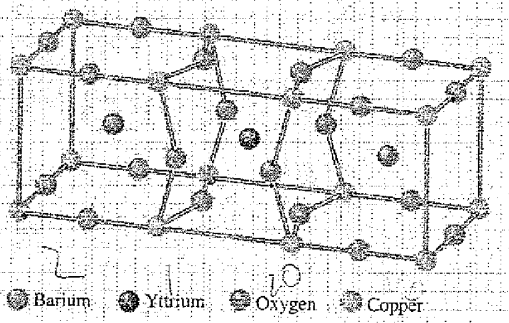
10.20 Amorphous silica has a density of around 2.3 g/cm^3, whereas crystalline quartz has a density of 2.65 g/cm^3. Why do these two forms of the same substance have different densities?

10.21 Construct part of the Lewis structure of carborundum, the diamondlike compound of empirical formula SiC.

10.22 The unit cell of a compound of xenon and fluorine follows. What is the formula of the compound?



10.23 Recently, a new group of solids was prepared that can act as superconductors at temperatures near the boiling point of liquid nitrogen. (A superconductor is a material whose electrical resistance is zero.) The unit cell of one of these new superconductors is shown here. Identify the formula of the compound.



10.24 Draw the unit cell of the NaCl crystal and determine the number of nearest neighbors of opposite charge for each ion in these unit cells.

THE NATURE OF SOLUTIONS

10.25 Do you expect gasoline to dissolve in water? Knowing that gasoline is less dense than water, would you use water to fight a gasoline fire? Explain.

10.26 Acetone, $(CH_3)_2CO$, is miscible with both water and cyclohexane (C_6H_{12}), but water and cyclohexane are nearly insoluble in each other. Explain.

10.27 Ammonia can be condensed to a liquid at low temperature. What kinds of solids would you expect to be soluble in liquid ammonia?

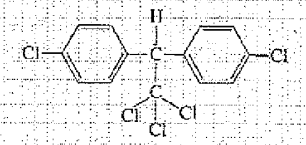
10.28 One of the detrimental effects of the "thermal pollution" of water supplies is that a rise in temperature reduces the amount of dissolved oxygen available for fish. Using the information in Table 10-2, calculate the number of liters of water a fish requires at 30 °C to obtain the same amount of oxygen that it could obtain from 1 L of water at 25 °C.

10.29 Using the information in Table 10-2, calculate the number of grams of CO_2 that can be dissolved in 250 mL of a carbonated beverage at 1.10 atm pressure and 25 °C.

10.30 If a bottle of the carbonated beverage in Problem 10.29 is stored in an ice chest at 0 °C, what is the partial pressure of CO_2 in the gas space above the liquid?

DUAL-NATURE MOLECULES: SURFACTANTS AND BIOLOGICAL MEMBRANES

10.31 Dichlorodiphenyltrichloroethane (DDT) has the following structure:



Is this compound hydrophilic or hydrophobic? Is it readily excreted by animals, or will it concentrate in fatty tissues? Does your answer explain why DDT has been banned as a pesticide?

10.32 Of the following compounds, which will be the best and which will be the worst surfactant? Support your choices with molecular pictures. (a) propionic acid, $H_3CCH_2CO_2H$; (b) lauryl alcohol, $H_3C(CH_2)_{11}OH$; and (c) sodium lauryl sulfate $H_3C(CH_2)_{11}OSO_3^-Na^+$.

10.33 Some surfactants form membranes that span small holes between two aqueous solutions. These membranes are liquid bilayers two molecules thick. Draw a molecular picture of one of these membranes.

10.34 Stearic acid forms a monolayer on the surface of gasoline. Draw a molecular picture that shows how stearic acid molecules are arranged in this monolayer.

PROPERTIES OF AQUEOUS SOLUTIONS

10.35 Compute the freezing point of a wine that is 12% ethanol by mass. (Ignore all other solutes.)

10.36 Do you have enough information to calculate the boiling point of the wine in Problem 10.35? If so, calculate it. If not, explain what feature of wine prevents you from doing this calculation.

10.37 An aqueous solution contains 1.00 g/L of a derivative of the detergent lauryl alcohol. The osmotic pressure of this solution at 25 °C is 17.8 torr.
(a) What is the molar mass of the detergent?
(b) The hydrocarbon portion of the molecule is an 11-carbon chain. What is the molar mass of the polar portion?

10.38 Calculate the boiling point of a solution that contains 2.50 g NaCl in 155 mL of water.

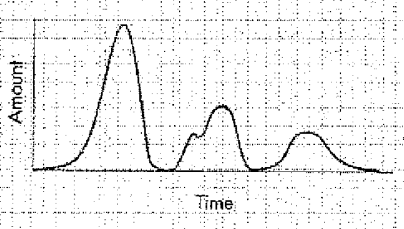
SEPARATION PROCESSES

10.39 Describe how you would purify diethyl ether, $(C_2H_5)_2O$, which is contaminated with a small amount of water.

10.40 You have prepared a new, highly colored solid compound and want to determine whether your product is pure or contains several components. What technique would provide this information most conveniently? Describe how the technique works.

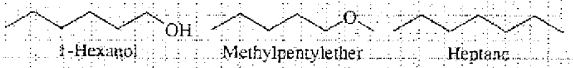
10.41 The solubility of $HgCl_2$ in water is 380 g/L at 100 °C and 30 g/L at 0 °C. What is the minimum volume of water needed to recrystallize a crude sample of this compound whose mass is 250 g? What fraction of the crude sample will be recovered? (For calculation purposes, assume that the crude sample is 95% $HgCl_2$ and that the impurity is more soluble than $HgCl_2$.)

10.42 You have prepared a sample of polymer and have performed liquid chromatography using molecular sieves to determine its molecular size. The chromatogram follows:



(a) How many components does your sample contain?
(b) Is there a larger amount of long-, medium-, or short-chain polymer molecules in the sample? Explain.

10.43 A sample for gas chromatography contains the following compounds:



If the GC column separates molecules according to their polarity, in what order will the compounds come off the column? Explain.

ADDITIONAL PROBLEMS

10.44 Will water, ethanol, or acetone rise the highest in a glass capillary tube? Which will rise the least? Explain why in terms of intermolecular forces.

10.45 An aqueous solution containing 1.00 g of a sugar in 100 mL of solution has an osmotic pressure of 1.36 atm at 25 °C. What is the molar mass of this sugar?

10.46 Classify each of the following solids as covalent, metallic, ionic, or molecular: (a) a solid that conducts electricity; (b) a solid that does not conduct electricity but dissolves in water to give a conducting solution; and (c) a solid that does not conduct electricity and melts below 100 °C to give a nonconducting liquid.

10.47 Rank the following substances in order of increasing solubility in water, and state the reasons for your rankings: C_6H_6 (benzene), $HOCH_2CH(OH)CH(OH)CH_2OH$ (erythritol), and $C_5H_{11}OH$ (pentanol).

10.48 What mole fraction of ethanol is required to protect the water in an automobile cooling system from freezing at −20 °C?

10.49 Aqueous solutions of 0.5 M acetic acid and 0.5 M $MgSO_4$ each have freezing points higher than the freezing point of 1 M glucose but lower than the freezing point of 0.5 M glucose. Explain these observations.

10.50 The osmotic pressure of a 0.10 M solution of H_3PO_4 at 300 K is 3.03 atm. What is the *total* molarity of the solutes under these conditions? On the basis of this result, would you call H_3PO_4 a strong acid?

10.51 The solubility of NaCl is 26 g/100 mL at 0 °C and 28 g/100 mL at 100 °C. Is it practical to purify NaCl by recrystallization from water? Explain your answer.

10.52 Why is the boiling point of H_2S lower than the boiling point of H_2O? Why is it also lower than the boiling point of H_2Te?

10.53 List the following liquids in order of increasing viscosity at room temperature, and explain the order of your list: (a) butanol, C_4H_9OH; (b) pentane, C_5H_{12}; and (c) propane-1,3-diol, $HOCH_2CH_2CH_2OH$.

10.54 Rank the following substances in order of increasing solubility in cyclohexane (C_6H_{12}) and explain the order of your list: KCl, C_4H_9OH, and C_4H_8.

10.55 Approximately what value of *total* solute molarity would you expect to find for 0.1 M aqueous solutions of each of the following: (a) citric acid (a weak organic acid); (b) $FeCl_3$; (c) NaOH; and (d) $(NH_4)_2CO_3$.

10.56 Brackish water, with a salt content around 0.5% by mass, is found in semiarid regions such as the American Southwest. Assuming that brackish water contains only sodium chloride, estimate the osmotic pressure of brackish water.

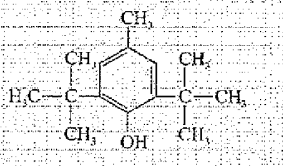
10.57 The freezing point of 0.050 M $KHSO_3$ is −0.19 °C. Which of the following equations best represents what happens when this compound dissolves in water? Explain your choice.

(a) $KHSO_{3\,(s)} \rightarrow KHSO_{3\,(aq)}$

(b) $KHSO_{3\,(s)} \rightarrow K^+_{(aq)} + HSO_{3\,(aq)}^-$

(c) $KHSO_{3\,(s)} + H_2O \rightarrow K^+_{(aq)} + H_2O^+_{(aq)} + SO_3^{2-}{}_{(aq)}$

10.58 Butylated hydroxytoluene (BHT) is used as a food preservative. It has the following molecular structure:



Would you expect to find this compound in urine or stored in body fat? BHT is nontoxic to humans.

10.59 Water and carbon tetrachloride are not miscible. When mixed, they form two layers, like water and oil. If an aqueous solution of I_2 is shaken with CCl_4, the iodine is "extracted" into the CCl_4 layer. Explain this behavior on the basis of your knowledge of intermolecular forces.

10.60 Some chemists interpret the boiling point of HCl as evidence for hydrogen bonding in this compound. How does the location of HCl on the graph in Figure 10-16 suggest that it may form hydrogen bonds? Draw a molecular picture that shows the possible hydrogen bonds between HCl molecules.

10.61 List the following aqueous solutions in order of increasing osmotic pressure, and explain your rankings: (a) 3.0×10^{-3} M KBr; (b) 3.0×10^{-3} M glucose; and (c) 4.0×10^{-3} M glucose.

10.62 Identify two elements that form molecular crystals, two that form metallic crystals, and two that form covalent crystals. Identify regions of the periodic table where elements of these three kinds are located.

10.63 One of the earliest methods of preserving fish was by salting. Explain what happens when fish is placed in a strong salt solution.

10.64 Would water dissolve salts as well as it does if it had a linear structure (such as CO_2) instead of a bent one? Explain.

10.65 List all the intermolecular forces that stabilize the liquid phase of each of the following compounds: (a) NH_3; (b) Xe; (c) SF_4; (d) CF_4; and (e) CH_3CO_2H (acetic acid).

10.66 Fish have blood that is isotonic with seawater, which freezes at −2.30 °C. What is the osmotic pressure of fish blood at 15 °C?

10.67 Homemade ice cream is frozen by churning it in a bucket suspended in an ice-water-salt mixture. A typical mix calls for 1.1 kg of rock salt (NaCl) and 7.25 kg of ice. Compute the mole fraction of NaCl in this mixture after all the ice melts, and estimate its freezing point.

10.68 Compute the molar mass of vitamin C if a solution containing 22.0 g in 100 g of water freezes at −2.33 °C.

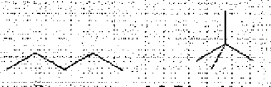
10.69 For each of the following pairs, identify which has the higher boiling point, and identify the type of force that is responsible: (a) CH_3OCH_3 and CH_3OH; (b) SO_2 and SiO_2; (c) HF and HCl; and (d) Br_2 and I_2.

10.70 When an aqueous solution is cooled to a low temperature, part of the water freezes as pure ice. What happens to the freezing point of the remaining solution when this occurs? A glass of wine placed in a freezer at −10 °C for a very long time forms some ice crystals but does not completely freeze. Compute the mole fraction of ethanol in the remaining liquid phase.

10.71 Molecular hydrogen and atomic helium have two electrons, but He boils at 4.2 K, whereas H_2 boils at 20 K. Neon boils at 27.1 K, whereas methane, which has the same number of electrons, boils at 114 K. Explain why molecular substances boil at a higher temperature than atomic substances with the same number of electrons.

10.72 Arrange the following liquids in order of increasing viscosity, and state the factors that determine the ranking: 1-butanol, $CH_3CH_2CH_2CH_2OH$; *n*-pentane, $CH_3CH_2CH_2CH_2CH_3$; 2,2-dimethylpropane, $(CH_3)_4C$; and propane-1,3-diol, $HOCH_2CH_2CH_2OH$.

10.73 The structures and boiling points of *n*-pentane and 2,2-dimethylpropane follow:



Pentane	2,2-Dimethylpropane
C_5H_{12}	C_5H_{12}
bp = 36 °C	bp = 10 °C

Use the boiling point data and molecular drawings to explain how shape affects the magnitude of dispersion forces. (See Figures 10-10 and 10-11.)

INTRODUCTION TO

Organic Laboratory Techniques

SMALL-SCALE APPROACH

FIRST EDITION

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Saunders Golden Sunburst Series
SAUNDERS COLLEGE PUBLISHING
Harcourt Brace College Publishers

Fort Worth Philadelphia San Diego New York Orlando Austin
San Antonio Toronto Montreal London Sydney Tokyo

Publisher: John Vondeling
Developmental Editor: Sandra Kiselica
Cover Designer: Kathleen Flanagan
Cover Photo Credit: Reginald Wickham Photography
Production Services: York Production Services
Product Manager: Angus McDonald

Printed in the United States of America
Introduction to Organic Laboratory Techniques: Small Scale Approach
ISBN: 0-03-024519-2
Library of Congress Catalog Card Number: 94-066238
890123 039 109876543

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4.8 CENTRIFUGATION

Sometimes centrifugation is more effective in removing solid impurities than are conventional filtration techniques. Centrifugation is particularly effective in removing suspended particles which are so small that the particles would pass through most filtering devices. Another situation in which centrifugation may be useful is when the mixture must be kept hot to prevent premature crystallization while the solid impurities are removed.

Centrifugation is performed by placing the mixture in one or two centrifuge tubes (be sure to balance the centrifuge) and centrifuging for several minutes. The supernatant liquid is then decanted (poured off) or removed with a Pasteur pipet.

PROBLEMS

1. In each of the following situations, what type of filtration device would you use?
   (a) Remove powdered decolorizing charcoal from 20 mL of solution.
   (b) Collect crystals obtained from crystallizing a substance from about 1 mL of solution.
   (c) Remove a very small amount of dirt from 1 mL of liquid.
   (d) Isolate 2.0 g of crystals from about 50 mL of solution after performing a crystallization.
   (e) Remove dissolved colored impurities from about 3 mL of solution.
   (f) Remove solid impurities from 5 mL of liquid at room temperature.

TECHNIQUE 5

Crystallization: Purification of Solids

Organic compounds that are solid at room temperature are usually purified by crystallization. The general technique involves dissolving the material to be crystallized in a *hot* solvent (or solvent mixture) and cooling the solution slowly. The dissolved material has a decreased solubility at lower temperatures and will separate from the solution as it is cooled. This phenomenon is called either **crystallization** if the crystal growth is relatively slow and selective or **precipitation** if the process is rapid and nonselective. Crystallization is an equilibrium process and produces very pure material. A small seed crystal is formed initially, and it then grows layer by layer in a reversible manner. In a sense, the crystal "selects" the correct molecules from the solution. In precipitation, the crystal lattice is formed so rapidly that impurities are trapped within the lattice. Therefore, any attempt at purification with too rapid a process should be avoided.

The method of crystallization described in detail in this chapter is called **standard-scale crystallization.** This technique, which is carried out with an Erlenmeyer flask to dissolve the material and a Büchner funnel to filter the crystals, is normally used when the weight of solid to be crystallized is more than 0.1 g. Another method, which is performed with a Craig tube, is used with smaller amounts of solid. Referred to as **microscale crystallization,** this technique is discussed briefly in Section 5.4.

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ISBN: 1-56363-497-X

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Aplisol—Cont.

A separate, sterile, single-use disposable syringe and needle should be used for each individual patient to prevent possible transmission of serum hepatitis virus and other infectious agents from one person to another.

Special care should be taken to ensure that the product is injected intradermally and not into a blood vessel.

Before administration of Aplisol, a review of the patient's history with respect to possible immediate-type hypersensitivity to the product, determination of previous use of Aplisol and the presence of any contraindication to the test should be made (see CONTRAINDICATIONS).

As with any biological product, epinephrine should be immediately available in case an anaphylactoid or acute hypersensitivity reaction occurs.

Failure to store and handle Aplisol as recommended may result in a loss of potency and inaccurate test results.[2,3]

Reactivity to the test may be depressed or suppressed for as long as 5–6 weeks in individuals following immunization with certain live viral vaccines, viral infections or discontinuation of corticosteroids or immunosuppressive agents.[5,6]

Information to Patients

Patients should be instructed to report adverse events such as vesiculation, ulceration or necrosis which may occur at the test site in highly sensitive individuals. Patients should be informed that pain, pruritus and discomfort may occur at injection site.

Patient should be informed of the need to return to their physician or health care provider for the reading of the test and of the need to keep and maintain a personal immunization record.

Drug Interactions

In patients who are receiving corticosteroids or immunosuppressive agents, reactivity to the test may be depressed or suppressed. This reduced reactivity may be present for as long as 5–6 weeks after discontinuation of therapy (see PRECAUTIONS—General).[2]

The reactivity to PPD may be temporarily depressed by certain live virus vaccines. Therefore, if a tuberculin test is to be performed, it should be administered either before or simultaneously with the use of oral polio and/or injection of measles, mumps and rubella vaccines in combined form or as separate antigens, or testing should be postponed for 4–6 weeks.[10]

Carcinogenesis, Mutagenesis, Impairment of Fertility

No long term studies have been conducted in animals or in humans to evaluate carcinogenic or mutagenic potential or effects on fertility with Aplisol.

Pregnancy

Teratogenic effects: Pregnancy Category C. Animal reproduction studies have not been conducted with Aplisol. It is also not known whether Aplisol can cause fetal harm when administered to a pregnant woman or can affect the reproduction capacity. Aplisol should be given to a pregnant woman only if clearly needed.

However, the risk of unrecognized tuberculosis and the postpartum contact between a mother with active disease and an infant leaves the infant in grave danger of tuberculosis and complications such as tuberculous meningitis. Although there have not been any reported adverse effects upon the fetus recognized as being due to tuberculosis skin testing, the prescribing physician will want to consider if the potential benefits outweigh the possible risks for performing the tuberculin test on a pregnant woman or a woman of childbearing age, particularly in certain high risk populations.

Tuberculin skin testing is considered valid and safe throughout pregnancy.[8]

ADVERSE REACTIONS

In highly sensitive individuals, strongly positive reactions including vesiculation, ulceration or necrosis may occur at the test site; however, there were no reports of these reactions for the period 1995 through 1998. Cold packs or topical steroid preparations may be employed for symptomatic relief of the associated pain, pruritus and discomfort.

Strongly positive test reactions may result in scarring at the test site.

Immediate erythematous or other reactions may occur at the injection site.

DOSAGE AND ADMINISTRATION

Aplisol vials should be inspected visually for both particulate matter and discoloration prior to administration and discarded if either is seen. Vials in use for more than 30 days should be discarded.

Standard Method (Mantoux Test)

The Mantoux test is performed by intradermally injecting with a syringe and needle exactly 0.1mL of Aplisol. The result is read 48 to 72 hours later and induration only is considered in interpreting the test. Induration is a hard, raised area with clearly defined margins at and around the injection site. Erythema may develop at the injection site but has no diagnostic value. The standard test is performed as follows:

1. The site of the test is usually the flexor or dorsal surface of the forearm about 4" below the elbow. Other skin sites may be used, but the flexor surface of the forearm is preferred. The use of a skin area free of lesions and away from any veins is recommended.[7]
2. The skin at the injection site is cleansed with 70% alcohol and allowed to dry.
3. The test material is administered with a tuberculin syringe (0.5 or 1.0mL) fitted with a short (1/2") 26 or 27 gauge needle.
4. A separate, sterile, single-use disposable syringe and needle should be used for each individual patient.
5. The diaphragm of the vial-stopper should be wiped with 70% alcohol.
6. The needle is inserted through the stopper diaphragm of the inverted vial. Exactly 0.1mL is filled into the syringe with care being taken to exclude air bubbles and to maintain the lumen of the needle filled.
7. The point of the needle is inserted into the most superficial layers of the skin with the needle bevel pointed upward. As the Tuberculin solution is injected, a pale bleb 6 to 10mm in size (1/3") will rise over the point of the needle. This is quickly absorbed and no dressing is required.

In the event the injection is delivered subcutaneously (i.e., no bleb will form), or if a significant part of the dose leaks from the injection site, the test should be repeated immediately at another site at least 5 cm (2") removed.

The Mantoux test is the standard of comparison for all other tuberculin tests.

Interpretation of Tuberculin Reaction

Readings of Mantoux reactions should be made during the period from 48 to 72 hours after the injection. Induration only should be considered in interpreting the test. The diameter of induration should be measured transversely to the long axis of the forearm and recorded in millimeters. Erythema has no diagnostic value and should be disregarded. The presence and size of necrosis and edema if present should be recorded although not used in the interpretation of the test. In the absence of induration, an area of erythema greater than 10 mm is diameter may indicate the injection was made too deeply and retesting is indicated.

Reactions should be interpreted as follows:

Positive—A positive reaction to the tuberculin skin test may not be seen until 2–10 weeks after the infection.[9] Based in current guidelines,[1,12] interpretation of positive reactions (depending on the age, immune status or risk factors of the persons tested) is:

1. An induration of >5 mm is classified as positive in the following:
   - Persons who have had recent close contact with persons who have active TB;
   - Persons who have human immunodeficiency virus (HIV) infection or risk factors for HIV infection but unknown HIV status;
   - Persons who have fibrotic chest radiographs consistent with healed TB.
2. An induration of >10 mm is classified as positive in all persons who do not meet any of the above criteria, but who belong to one or more of the following groups at high risk for TB:
   - Injecting-drug users known to be HIV seronegative;
   - Persons who have other medical conditions that have been reported to increase the risk for progressing from latent TB infection to active TB. These medical conditions include diabetes mellitus, conditions requiring prolonged high-dose corticosteroid therapy and other immunosuppressive therapy (including bone marrow and organ transplantation), chronic renal failure, some hematologic disorders (e.g., leukemias and lymphomas), other specific malignancies (e.g., carcinoma of the head or neck), weight loss of >10% below ideal body weight, silicosis, gastrectomy, jejunoileal bypass;
   - Residents and employees of high-risk congregate settings: prisons and jails, nursing homes and other long-term facilities for the elderly, health-care facilities (including some residential mental health facilities), and homeless shelters;
   - Foreign-born persons recently arrived (i.e., within the last 5 years) from countries having a high prevalence or incidence of TB;
   - Some medically underserved, low-income populations, including migrant farm workers and homeless persons;
   - High-risk racial or ethnic minority populations, as defined locally;
   - Children <4 years of age or infants, children and adolescents exposed to adults in high-risk categories.
3. An induration of > 15mm is classified as positive in persons who do not meet any of the above criteria.

Negative—Induration of less than 5 mm. This indicates a lack of hypersensitivity to tuberculoprotein and tuberculous infection is highly unlikely.

Booster Effect—Infection of an individual with tubercle bacilli or other mycobacteria or BCG vaccination results in a delayed hypersensitivity response to tuberculin which is demonstrated by the skin test. The delayed hypersensitivity response may gradually wane over a period of years. If a person receives a tuberculin test at this time, a significant reaction may not be detected. However, the stimulus of the test may boost or increase the size of the reaction to a second test, sometimes causing an apparent conversion or development of sensitivity. This booster effect can be seen on a second test done one week after the initial stimulating test and can persist for a year, and perhaps longer. When routine periodic tuberculin testing of adults is done, initially two-stage testing should be considered to minimize the likelihood of interpreting a boosted reaction as a conversion.[13,14] It should be noted that reactivity to tuberculin may be depressed or suppressed for as long as 5–6 weeks by viral infections, live virus vaccines (i.e., measles, smallpox, polio, rubella and mumps), or after discontinuation of therapy with corticosteroids or immunosuppressive agents. Malnutrition may also have a similar effect. When of diagnostic importance, a negative test should be accepted as proof that hypersensitivity is absent only after normal reactivity to non-specific irritants has been demonstrated. A primary injection of tuberculin may possibly have a boosting effect on subsequent tuberculin reactions. A pediatric patient who is known to have been exposed to a person with tuberculosis must not be adjudged free of infection until that patient has a negative tuberculin reaction at least ten weeks after contact with tuberculous person has ceased.[15] Annual testing is generally recommended for pediatric patients in high risk populations, such as persons from countries with a high prevalence of tuberculosis and low-income groups.[16,17] A positive tuberculin reaction does not necessarily signify the presence of active disease. Further diagnostic procedures (e.g., chest radiograph, sputum smear and/or culture examination) should be carried out before a diagnosis of tuberculosis is made. A small percentage of responders may not have been infected with *M. tuberculosis* but by some other mycobacterium. The negative tuberculin skin test should never be used to exclude the possibility of active tuberculosis among persons for whom the diagnosis is being considered (symptoms compatible with tuberculosis).

HOW SUPPLIED

Tuberculin PPD-Aplisol bioequivalent to 5US units (TU) PPD-S per test dose (0.1mL) is available in the following presentations:

NDC 64029-4525-1 (Bio. 1525)
1 mL (10 tests) – rubber-diaphragm-capped vial

NDC 64029-4525-2 (Bio. 1607)
5 mL (50 tests) – rubber-diaphragm-capped vial

This product is ready for use without further dilution.

Storage

DO NOT FREEZE

This product should be stored at 2°–8°C (36°–46°F) and protected from light.

Vials in use more than 30 days should be discarded due to possible oxidation and degradation which may affect potency.

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Rx only.

Prescribing Information as of May 2002

PARKEDALE PHARMACEUTICALS

Manufactured by:
Parkedale Pharmaceuticals, Inc.
Rochester, MI 48307

Shown in Product Identification Guide, page 379

BICILLIN® L-A
[bi-sil' in]
(penicillin G benzathine suspension)
INJECTION
FOR DEEP IM INJECTION ONLY

DESCRIPTION

Bicillin L-A (penicillin G benzathine suspension) is prepared by the reaction of dibenzylethylene diamine with two molecules of penicillin G. It is chemically designated as 6*R*, 6*R*) 3,3-Dimethyl-7-oxo -6- (2-phenylacetamido)-1-azabicyclo[3.2.0]heptane-2-carboxylic acid compound with *N,N'*-dibenzylethylenediamine (2:1), tetrahydrate. It is available for deep intramuscular injection. It contains penicillin G benzathine in aqueous suspension with sodium

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drate buffer and, as w/v, approximately 0.5% lecithin, 0.6% carboxymethylcellulose, 0.6% povidone, 0.1% methylparaben; and 0.01% propylparaben. It occurs as a white, crystalline powder and is very slightly soluble in water and sparingly soluble in alcohol.

Bicillin L-A suspension in the multiple-dose vial formulation, disposable syringe formulation and TUBEX formulation is viscous and opaque. The multiple-dose vial formulation contains the equivalent of 300,000 units per mL of penicillin G as the benzathine salt. The disposable syringe formulation is available in a 4 mL size containing the equivalent of 2,400,000 units of penicillin G as the benzathine salt. The TUBEX formulation is available in 1 mL and 2 mL TUBEX Sterile Cartridge-Needle Units containing the equivalent of 600,000 units and 1,200,000 units respectively of penicillin G as the benzathine salt. Read CONTRAINDICATIONS, WARNINGS, PRECAUTIONS, and DOSAGE AND ADMINISTRATION sections prior to use.

CLINICAL PHARMACOLOGY

General

Penicillin G benzathine has an extremely low solubility and, thus, the drug is slowly released from intramuscular injection sites. The drug is hydrolyzed to penicillin G. This combination of hydrolysis and slow absorption results in blood serum levels much lower but much more prolonged than other parenteral penicillins.

Intramuscular administration of 300,000 units of penicillin G benzathine in adults results in blood levels of 0.03 to 0.05 units per mL, which are maintained for 4 to 5 days. Similar blood levels may persist for 10 days following administration of 600,000 units and for 14 days following administration of 1,200,000 units. Blood concentrations of 0.003 units per mL may still be detectable 4 weeks following administration of 1,200,000 units.

Approximately 60% of penicillin G is bound to serum protein. The drug is distributed throughout the body tissues in widely varying amounts. Highest levels are found in the kidneys with lesser amounts in the liver, skin, and intestines. Penicillin G penetrates into all other tissues and the spinal fluid to a lesser degree. With normal kidney function, the drug is excreted rapidly by tubular excretion. In neonates and young infants and in individuals with impaired kidney function, excretion is considerably delayed.

Microbiology

Penicillin G exerts a bactericidal action against penicillin-susceptible microorganisms during the stage of active multiplication. It acts through the inhibition of biosynthesis of cell-wall mucopeptide. It is not active against the penicillinase-producing bacteria, which include many strains of staphylococci.

The following *in vitro* data are available, but their clinical significance is unknown. Penicillin G exerts high *in vitro* activity against staphylococci (except penicillinase-producing strains), streptococci (Groups A, C, G, H, L, and M), and pneumococci. Other organisms susceptible to penicillin G are *Neisseria gonorrhoeae, Corynebacterium diphtheriae, Bacillus anthracis,* Clostridia species, *Actinomyces bovis, Streptobacillus moniliformis, Listeria monocytogenes,* and *Leptospira* species. *Treponema pallidum* is extremely susceptible to the bactericidal action of penicillin G.

Susceptibility Test: If the Kirby-Bauer method of disc susceptibility is used, a 20-unit penicillin disc should give a zone greater than 28 mm when tested against a penicillin-susceptible bacterial strain.

INDICATIONS AND USAGE

Intramuscular penicillin G benzathine is indicated in the treatment of infections due to penicillin-G-sensitive microorganisms that are susceptible to the low and very prolonged serum levels common to this particular dosage form. Therapy should be guided by bacteriological studies (including sensitivity tests) and by clinical response.

The following infections will usually respond to adequate dosage of intramuscular penicillin G benzathine:

Mild-to-moderate infections of the upper respiratory tract due to susceptible streptococci.

Venereal infections—Syphilis, yaws, bejel, and pinta.

Medical Conditions in which Penicillin G Benzathine Therapy is Indicated as Prophylaxis:

Rheumatic fever and/or chorea—Prophylaxis with penicillin G benzathine has proven effective in preventing recurrence of these conditions. It has also been used as follow-up prophylactic therapy for rheumatic heart disease and acute glomerulonephritis.

CONTRAINDICATIONS

A history of a previous hypersensitivity reaction to any of the penicillins is a contraindication.

Do not inject into or near an artery or nerve.

WARNINGS

Penicillin G benzathine should only be prescribed for the indications listed in this insert.

SERIOUS AND OCCASIONALLY FATAL HYPERSENSITIVITY (ANAPHYLACTIC) REACTIONS HAVE BEEN REPORTED IN PATIENTS ON PENICILLIN THERAPY. THESE REACTIONS ARE MORE LIKELY TO OCCUR IN INDIVIDUALS WITH A HISTORY OF PENICILLIN HYPERSENSITIVITY AND/OR A HISTORY OF SENSITIVITY TO MULTIPLE ALLERGENS. THERE HAVE BEEN REPORTS OF INDIVIDUALS WITH A HISTORY OF PENICILLIN HYPERSENSITIVITY WHO HAVE EXPERIENCED SEVERE REACTIONS WHEN TREATED WITH CEPHALOSPORINS. BEFORE INITIATING THERAPY WITH BICILLIN L-A, CAREFUL INQUIRY SHOULD BE MADE CONCERNING PREVIOUS HYPERSENSITIVITY REACTIONS TO PENICILLINS, CEPHALOSPORINS AND OTHER ALLERGENS. IF AN ALLERGIC REACTION OCCURS, BICILLIN L-A SHOULD BE DISCONTINUED AND APPROPRIATE THERAPY INSTITUTED. SERIOUS ANAPHYLACTIC REACTIONS REQUIRE IMMEDIATE EMERGENCY TREATMENT WITH EPINEPHRINE, OXYGEN, INTRAVENOUS STEROIDS AND AIRWAY MANAGEMENT, INCLUDING INTUBATION, SHOULD ALSO BE ADMINISTERED AS INDICATED.

Pseudomembranous colitis has been reported with nearly all antibacterial agents, including penicillin, and may range in severity from mild to life-threatening. Therefore, it is important to consider this diagnosis in patients who present with diarrhea subsequent to the administration of any antibacterial agent.

Treatment with antibacterial agents alter the normal flora of the colon and may permit overgrowth of clostridia. Studies indicate that a toxin produced by *Clostridium difficile* is one primary cause of "antibiotic-associated colitis".

After the diagnosis of pseudomembranous colitis has been established, appropriate therapeutic measures should be initiated. Mild cases of pseudomembranous colitis usually respond to drug discontinuation alone. In moderate to severe cases, consideration should be given to management with fluids and electrolytes, protein supplementation, and treatment with an antibacterial drug clinically effective against *C. difficile* colitis.

Inadvertent intravascular administration, including inadvertent direct intra-arterial injection or injection immediately adjacent to arteries, of Bicillin L-A and other penicillin preparations has resulted in severe neurovascular damage, including transverse myelitis with permanent paralysis, gangrene requiring amputation of digits and more proximal portions of extremities, and necrosis and sloughing at and surrounding the injection site. Such severe effects have been reported following injections into the buttock, thigh, and deltoid areas. Other serious complications of suspected intravascular administration which have been reported include immediate pallor, mottling, or cyanosis of the extremity both distal and proximal to the injection site, followed by thick formation; severe edema requiring anterior and/or posterior compartment fasciotomy in the lower extremity. The above-described severe effects and complications have most often occurred in infants and small children. Prompt consultation with an appropriate specialist is indicated if any evidence of compromise of the blood supply occurs at, proximal to, or distal to the site of injection.[1-5] See CONTRAINDICATIONS, PRECAUTIONS, and DOSAGE AND ADMINISTRATION sections.

Quadriceps femoris fibrosis and atrophy have been reported following repeated intramuscular injections of penicillin preparations into the anterolateral thigh.

Injection into or near a nerve may result in permanent neurological damage.

PRECAUTIONS

General

Penicillin should be used with caution in individuals with histories of significant allergies and/or asthma.

Care should be taken to avoid intravenous or intra-arterial administration, or injection into or near major peripheral nerves or blood vessels, since such injection may produce neurovascular damage. See CONTRAINDICATIONS, WARNINGS, and DOSAGE AND ADMINISTRATION sections.

Prolonged use of antibiotics may promote the overgrowth of nonsusceptible organisms, including fungi. Should superinfection occur, appropriate measures should be taken.

Laboratory Tests

In streptococcal infections, therapy must be sufficient to eliminate the organism; otherwise, the sequelae of streptococcal disease may occur. Cultures should be taken following completion of treatment to determine whether streptococci have been eradicated.

Drug Interactions

Tetracycline, a bacteriostatic antibiotic, may antagonize the bactericidal effect of penicillin, and concurrent use of these drugs should be avoided.

Concurrent administration of penicillin and probenecid increases and prolongs serum penicillin levels by decreasing the apparent volume of distribution and slowing the rate of excretion by competitively inhibiting renal tubular secretion of penicillin.

Pregnancy Category B

Reproduction studies performed in the mouse, rat, and rabbit have revealed no evidence of impaired fertility or harm to the fetus due to penicillin G. Human experience with the penicillins during pregnancy has not shown any positive evidence of adverse effects on the fetus. There are, however, no adequate and well-controlled studies in pregnant women showing conclusively that harmful effects of these drugs on the fetus can be excluded. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Nursing Mothers

Soluble penicillin G is excreted in breast milk. Caution should be exercised when penicillin G benzathine is administered to a nursing woman.

Carcinogenesis, Mutagenesis, Impairment Of Fertility

No long-term animal studies have been conducted with this drug.

Pediatric Use

See INDICATIONS AND USAGE and DOSAGE AND ADMINISTRATION.

ADVERSE REACTIONS

As with other penicillins, untoward reactions of the sensitivity phenomena are likely to occur, particularly in individuals who have previously demonstrated hypersensitivity to penicillins or in those with a history of allergy, asthma, hay fever, or urticaria.

As with other treatments for syphilis, the Jarisch-Herxheimer reaction has been reported.

The following have been reported with parenteral penicillin G:

General: Hypersensitivity reactions including the following: skin eruptions (maculopapular to exfoliative dermatitis), urticaria, laryngeal edema, fever, eosinophilia; other serum sickness-like reactions (including chills, fever, edema, arthralgia, and prostration); and anaphylaxis including shock and death. Note: Urticaria, other skin rashes, and serum sickness-like reactions may be controlled with antihistamines and, if necessary, systemic corticosteroids. Whenever such reactions occur, penicillin G should be discontinued unless, in the opinion of the physician, the condition being treated is life-threatening and amenable only to therapy with penicillin G. Serious anaphylactic reactions require immediate emergency treatment with epinephrine. Oxygen, intravenous steroids, and airway management, including intubation, should also be administered as indicated.

Gastrointestinal: Pseudomembranous colitis. Onset of pseudomembranous colitis symptoms may occur during or after antibacterial treatment. See WARNINGS.

Hematologic: Hemolytic anemia, leukopenia, thrombocytopenia.

Neurologic: Neuropathy.

Urogenital: Nephropathy.

The following adverse events have been temporally associated with parenteral administration of penicillin G benzathine:

Body as a Whole: Hypersensitivity reactions including allergic vasculitis, pruritus, fatigue, asthenia, and pain; aggravation of existing disorder; headache.

Cardiovascular: Cardiac arrest; hypotension; tachycardia; palpitations; pulmonary hypertension; pulmonary embolism; vasodilatation; vasovagal reaction; cerebrovascular accident; syncope.

Gastrointestinal: Nausea, vomiting; blood in stool; intestinal necrosis.

Hemic and Lymphatic: Lymphadenopathy.

Injection Site: Injection site reactions including pain, inflammation, lump, abscess, necrosis, edema, hemorrhage, cellulitis, hypersensitivity, atrophy, ecchymosis, and skin ulcer. Neurovascular reactions including warmth, vasospasm, pallor, mottling, gangrene, numbness of the extremities, and neurovascular damage.

Metabolic: Elevated BUN, creatinine, and SGOT.

Musculoskeletal: Joint disorder; periostitis; exacerbation of arthritis; myoglobinuria; rhabdomyolysis.

Nervous System: Nervousness; tremors; dizziness; somnolence; confusion; anxiety; euphoria; transverse myelitis; seizures; coma. A syndrome manifested by a variety of CNS symptoms such as severe agitation with confusion, visual and auditory hallucinations, and a fear of impending death (Hoigne's syndrome), has been reported after administration of penicillin G procaine and, less commonly, after injection of the combination of penicillin G benzathine and penicillin G procaine. Other symptoms associated with this syndrome, such as psychosis, seizures, dizziness, tinnitus, cyanosis, palpitations, tachycardia, and/or abnormal perception in taste, also may occur.

Respiratory: Hypoxia; apnea; dyspnea.

Skin: Diaphoresis.

Special Senses: Blurred vision; blindness.

Urogenital: Neurogenic bladder; hematuria; proteinuria; renal failure; impotence; priapism.

OVERDOSAGE

Penicillin in overdosage has the potential to cause neuromuscular hyperirritability or convulsive seizures.

DOSAGE AND ADMINISTRATION

Due to the viscous nature of this medication, a 23 gauge or larger bore needle should be used to withdraw medication from the vial and for patient administration. A smaller bore needle, such as a 24 or 25 gauge, is not recommended.

Streptococcal (Group A) Upper-respiratory infections (for example, pharyngitis)

Adults—a single injection of 1,200,000 units; older pediatric patients—a single injection of 900,000 units; infants and pediatric patients under 60 lbs.—300,000 to 600,000 units.

Syphilis

Primary, secondary, and latent—2,400,000 units (1 dose).

Late (tertiary and neurosyphilis)—2,400,000 units at 7-day intervals for three doses.

Congenital—under 2 years of age: 50,000 units/kg/body weight; ages 2 to 12 years: adjust dosage based on adult dosage schedule.

Yaws, Bejel, and Pinta—1,200,000 units (1 injection).

Prophylaxis—for rheumatic fever and glomerulonephritis. Following an acute attack, penicillin G benzathine (parenteral) may be given in doses of 1,200,000 units once a month or 600,000 units every 2 weeks.

Continued on next page

Bicillin L-A—Cont.

Administer by DEEP INTRAMUSCULAR INJECTION in the upper, outer quadrant of the buttock. In neonates, infants and small children, the midlateral aspect of the thigh may be preferable. When doses are repeated, vary the injection site.

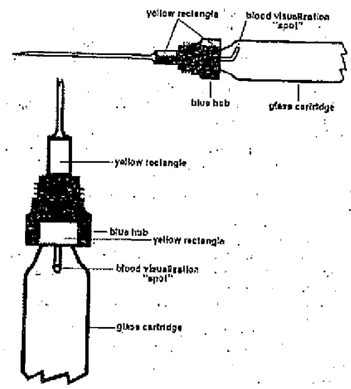
When using the multiple-dose vial:

After selection of the proper site and insertion of the needle into the selected muscle, aspirate by pulling back on the plunger. While maintaining negative pressure for 2 to 3 seconds, carefully observe the barrel of the syringe immediately proximal to the needle hub for appearance of blood or any discoloration. Blood or "typical blood color" may *not* be seen if a blood vessel has been entered—only a mixture of blood and Bicillin L-A. The appearance of any discoloration is reason to withdraw the needle and discard the syringe. If it is elected to inject at another site, a new syringe and needle should be used. If no blood or discoloration appears, inject the contents of the syringe slowly. Discontinue delivery of the dose if the subject complains of severe immediate pain at the injection site or if, especially in neonates, infants and young children symptoms or signs occur suggesting onset of severe pain.

Because of the high concentration of suspended material in this product, the needle may be blocked if the injection is not made at a slow, steady rate.

When using the TUBEX cartridge:

The Wyeth-Ayerst TUBEX® cartridge for this product incorporates several features that are designed to facilitate the visualization of blood on aspiration if a blood vessel is inadvertently entered.



The design of this cartridge is such that blood which enters its needle will be quickly visualized as a red or dark-colored "spot." This "spot" will appear on the barrel of the glass cartridge immediately proximal to the blue hub. The TUBEX is designed with two orientation marks, in order to determine where the "spot" can be seen. First insert and secure the cartridge in the TUBEX injector in the usual fashion. Locate the yellow rectangle at the base of the blue hub. This yellow rectangle is aligned with the blood visualization "spot." An imaginary straight line, drawn from this yellow rectangle to the shoulder of the glass cartridge, will point to the area on the cartridge where the "spot" can be visualized. When the needle cover is removed, a second yellow rectangle will be visible. The second yellow rectangle is also aligned with the blood visualization "spot" to assist the operator in locating this "spot." If the 2 mL metal or plastic syringe is used, the glass cartridge should be rotated by turning the plunger of the syringe clockwise until the yellow rectangle is visualized. If the 1 mL metal syringe is used, it will not be possible to continue to rotate the glass cartridge clockwise once it is properly engaged and fully threaded; it can, however, then be rotated counterclockwise as far as necessary to properly orient the yellow rectangles and locate the observation area. (In this same area in some cartridges, a dark spot may sometimes be visualized prior to injection. This is the proximal end of the needle and does not represent a foreign body in, or other abnormality of, the suspension.)

Thus, before the needle is inserted into the selected muscle, it is important for the operator to orient the yellow rectangle so that any blood which may enter after needle insertion and during aspiration can be visualized in the area on the cartridge where it will appear and not be obscured by any obstructions.

After selection of the proper site and insertion of the needle into the selected muscle, aspirate by pulling back on the plunger. While maintaining negative pressure for 2 to 3 seconds, carefully observe the barrel of the cartridge in the area previously identified (see above) for the appearance of a red or dark-colored "spot."

Blood or "typical blood color" may not be seen if a blood vessel has been entered—only a mixture of blood and Bicillin L-A. The appearance of any discoloration is reason to withdraw the needle and discard the glass TUBEX cartridge. If it is elected to inject at another site, a new cartridge should be used. If no blood or discoloration appears, inject the contents of the cartridge slowly. Discontinue delivery of the dose if the subject complains of severe immediate pain at the injection site or if, especially in infants and young children, symptoms or signs occur suggesting onset of severe pain.

Some TUBEX cartridges may contain a small air bubble which may be disregarded, since it does not affect administration of the product.

DO NOT clear any air bubbles from the cartridge or needle as this may interfere with the visualization of any blood or discoloration during aspiration.

Because of the high concentration of suspended material in this product, the needle may be blocked if the injection is not made at a slow, steady rate.

When using the disposable syringe:

The Wyeth-Ayerst disposable syringe for this product incorporates several features that are designed to facilitate its use.

A single, small indentation, or "dot," has been punched into the metal ring that surrounds the neck of the syringe near the base of the needle. It is important that this "dot" be placed in a position so that it can be easily visualized by the operator following the intramuscular insertion of the syringe needle.

After selection of the proper site and insertion of the needle into the selected muscle, aspirate by pulling back on the plunger. While maintaining negative pressure for 2 to 3 seconds, carefully observe the barrel of the syringe immediately proximal to the location of the "dot" for appearance of blood or any discoloration. Blood or "typical blood color" may *not* be seen if a blood vessel has been entered—only a mixture of blood and Bicillin L-A. The appearance of any discoloration is reason to withdraw the needle and discard the syringe. If it is elected to inject at another site, a new syringe should be used. If no blood or discoloration appears, inject the contents of the syringe slowly. Discontinue delivery of the dose if the subject complains of severe immediate pain at the injection site or if, especially in neonates, infants and young children, symptoms or signs occur suggesting onset of severe pain.

Some disposable syringes may contain a small air bubble which may be disregarded, since it does not affect administration of the product. DO NOT clear any air bubbles from the disposable syringe or needle as this may interfere with the visualization of any blood or discoloration during aspiration.

Because of the high concentration of suspended material in this product, the needle may be blocked if the injection is not made at a slow, steady rate.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit.

HOW SUPPLIED

Bicillin® L-A (penicillin G benzathine suspension) is supplied in packages of 10 TUBEX® Sterile Cartridge-Needle Units as follows:

1 mL size, containing 600,000 units per TUBEX® (21 gauge, thin-wall 1 inch needle for pediatric use), NDC 61570-146-10.

2 mL size, containing 1,200,000 units per TUBEX® (21 gauge, thin-wall 1–1/4 inch needle), NDC 61570-147-10.

Store in a refrigerator.

Keep from freezing.

ALSO AVAILABLE

Bicillin L-A (penicillin G benzathine suspension) is also available in packages of 10 disposable syringes as follows:

4 mL size, containing 2,400,000 units per syringe (18 gauge × 2 inch needle), NDC 61570-148-10

Store in a refrigerator.

Keep from freezing.

Shake multiple-dose vials well before using.

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1. SHAW, E.: Transverse myelitis from injection of penicillin. *Am. J. Dis. Child., 111:* 548, 1966.
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Refer to the Tubex® Closed Injection System instructions in the Wyeth-Ayerst section of the 2002 PDR®.

Revised March 14, 2001 CI 4656-

BREVITAL® SODIUM

[*brĕ-vĭ-tŏl*]

METHOHEXITAL SODIUM FOR INJECTION, USP

For Intravenous Use in Adults

For Rectal and Intramuscular Use Only in Pediatric Patients

Prescribing information as of November 2003. 3000370 242

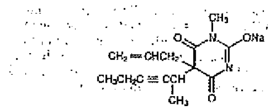
WARNING

Brevital should be used only in hospital or ambulatory care settings that provide for continuous monitoring of respiratory (e.g. pulse oximetry) and cardiac function. Immediate availability of resuscitative drugs and age- and size-appropriate equipment for bag/valve/mask ventilation and intubation and personnel trained in their use and skilled in airway management should be assured. For deeply sedated patients, a designated individual other than the practitioner performing the procedure should be present to continuously monitor the patient. (See WARNINGS)

DESCRIPTION

Brevital® Sodium (Methohexital Sodium for Injection, USP) is 2,4,6 (1*H*, 3*H*, 5*H*)-Pyrimidinetrione, 1-methyl-5-(1-methyl-2-pentynyl)-5-(2-propenyl)-, (±)-, monosodium salt, and has the empirical formula $C_{14}H_{17}N_2NaO_3$. Its molecular weight is 284.29.

The structural formula is as follows:



Methohexital sodium is a rapid, ultrashort-acting barbiturate anesthetic. Methohexital sodium for injection is a freeze-dried, sterile, nonpyrogenic mixture of methohexital sodium with 6% anhydrous sodium carbonate added as a buffer. It contains not less than 90% and not more than 110% of the labeled amount of methohexital sodium. It occurs as a white, freeze-dried plug that is freely soluble in water.

This product is oxygen sensitive. The pH of the 1% solution is between 10 and 11; the pH of the 0.2% solution in 5% dextrose is between 9.5 and 10.5.

Methohexital sodium may be administered by direct intravenous injection or continuous intravenous drip, intramuscular or rectal routes (see PRECAUTIONS—*Pediatric Use*). Reconstituting instructions vary depending on the route of administration (see DOSAGE AND ADMINISTRATION).

CLINICAL PHARMACOLOGY

Compared with thiamylal and thiopental, methohexital is at least twice as potent on a weight basis, and its duration of action is only about half as long. Although the metabolic fate of methohexital in the body is not clear, the drug does not appear to concentrate in fat depots to the extent that other barbiturate anesthetics do. Thus, cumulative effects are fewer and recovery is more rapid with methohexital than with thiobarbiturates. In experimental animals, the drug cannot be detected in the blood 24 hours after administration.

Methohexital differs chemically from the established barbiturate anesthetics in that it contains no sulfur. Little analgesia is conferred by barbiturates; their use in the presence of pain may result in excitation.

Intravenous administration of methohexital results in rapid uptake by the brain (within 30 seconds) and rapid induction of sleep.

Following intramuscular administration to pediatric patients, the onset of sleep occurs in 2 to 10 minutes. A plasma concentration of 3 μg/mL was achieved in pediatric patients 15 minutes after an intramuscular dose (10 mg/kg) of a 5% solution. Following rectal administration to pediatric patients, the onset of sleep occurs in 5 to 15 minutes. Plasma methohexital concentrations achieved following rectal administration tend to increase both with dose and with the use of more dilute solution concentrations when using the same dose. A 25 mg/kg dose of a 1% methohexital solution yielded plasma concentrations of 6.9 to 7.9 μg/mL 15 minutes after dosing. The absolute bioavailability of rectal methohexital sodium is 17%.

With single doses, the rate of redistribution determines duration of pharmacologic effect. Metabolism occurs in the liver through demethylation and oxidation. Side-chain oxidation is the most important biotransformation involved in termination of biologic activity. Excretion occurs via the kidneys through glomerular filtration.

INDICATIONS AND USAGE

Brevital Sodium can be used in adults as follows:

1. For *intravenous* induction of anesthesia prior to the use of other general anesthetic agents.

PRODUCT INFO

2. For *intravenous* in... junct to subpotent ... as nitrous oxide in ... Brevital Sodium m... injection.
3. For use along with ... cotic analgesics, t... anesthetic agents ... longer surgical pro...
4. As *intravenous* an... or therapeutic pro... ful stimuli (see W...
5. As an agent for in...

Brevital Sodium can ... than 1 month as foll...

1. For *rectal* or *intra*... to the use of other ...
2. For *rectal* or *intra*... as an adjunct to su... for short surgical ...
3. As *rectal* or *intra*... diagnostic, or the... minimal painful s...

CONTRAINDICAT...

Brevital Sodium is ... general anesthesia i... of manifest porphyr... sensitivity to barbit...

WARNINGS

See boxed Warning. As with all potent a... should be used only ... that provide for con... pulse oximetry) and ... ity of resuscitative ... equipment for bag/ ... and personnel trai...

Brief Articles

Piperidine Analogues of 1-[2-[Bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine (GBR 12909): High Affinity Ligands for the Dopamine Transporter

Thomas Prisinzano,† Elisabeth Greiner,† Edward M. Johnson II,† Christina M. Dersch,‡ Jamila Marcus,‡ John S. Partilla,‡ Richard B. Rothman,‡ Arthur E. Jacobson,† and Kenner C. Rice*,†

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Received June 21, 2002

A series of 4-[2-[bis(4-fluorophenyl)methoxy]ethylpiperidines were examined for their ability to bind to the dopamine transporter (DAT), the norepinephrine transporter, and the serotonin transporter (SERT). In particular, the role of the *N*-substituent on affinity and selectivity for the DAT was probed. 4-[2-[Bis(4-fluorophenyl)methoxy]ethyl-1-(2-naphthylmethyl)piperidine was found to possess subnanomolar affinity (K_i = 0.7 nM) and good selectivity for the DAT (SERT/DAT = 323).

Introduction

Cocaine is a widely abused drug, and its abuse has had great effects on public health, through the spread of human immunodeficiency virus (HIV), hepatitis, and tuberculosis.[1−9] Unfortunately, there are no U.S. Food and Drug Administration (FDA)-approved therapeutic agents available for the treatment of cocaine abuse or for the prevention of relapse.[10] Among the various agents tested clinically, the best results appear to have been achieved with dextroamphetamine,[11] supporting the hypothesis that agonist substitution therapy is a reasonable approach to developing pharmacotherapies for cocaine dependence.

On a molecular level, cocaine inhibits the reuptake of dopamine (DA), serotonin (5-HT), and norepinephrine (NE). Evidence suggests, however, that its binding to the dopamine transporter (DAT) and subsequent inhibition of DA reuptake may be responsible for its reinforcing properties and a good target for the design of an agonist substitution type medication for cocaine abuse.[12−16]

Our approach to developing this type of potential therapeutic for cocaine abuse is to find a competitive inhibitor of the DAT that dissociates very slowly.[17] 1-[2-[Bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)-piperazine (GBR 12909) (**1**, Figure 1) was among the first agents to be characterized as a high-affinity and selective inhibitor of DA reuptake.[18,19] Studies with rhesus monkeys have shown that in cocaine and food self-administration studies, **1** decreases cocaine-maintained responding without affecting food-maintained responding.[20,21] Given the promising properties of **1** and its analogues, these compounds have been identified as novel agents for the potential pharmacotherapy of cocaine abuse in humans.

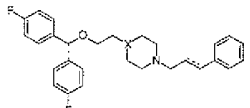

Figure 1. Structure of 1-{2-[bis-(4-fluoro-phenyl)methoxy]-ethyl}-4-(3-phenylpropyl)piperazine analogues.

The binding of these agents at the transporter for norepinephrine (NET) is a possible source of sympathomimetic side effects. In our continuing efforts to develop new agents that might reduce cocaine self-administration, we are attempting to find more selective and high-affinity DAT inhibitors.[20−26] Our efforts were focused on analogues, where a piperidine ring was substituted for the piperazine ring in **1**. Several piperidine analogues have been prepared.[27] Compound **2** was reported to have good affinity for the DAT but was not very selective.[27] Previous reports have shown that modification of this compound may lead to analogues with enhanced selectivity for the DAT over the serotonin transporter (SERT).[28,29]

Chemistry

The *N*-substituted piperidines were synthesized from the commercially available 4-piperidineethanol (Scheme 1). The reaction of 4-piperidineethanol with hydrocinnamoyl chloride, followed by reduction of the corresponding amide with LAH, and ether formation using 4,4′-difluorobenzhydrol in toluene under azeotropic distillation conditions gave **2** in good yield.[27] Alternately, a three step sequence of *N*-protection, ether formation, and *N*-deprotection afforded **5**.[27,30] The coupling of piperidine **5** with *trans*-cinnamic acid using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI), followed by reduction of the corresponding amide with AlH_3, afforded alkene **4**.[31,32]

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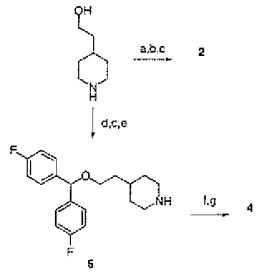
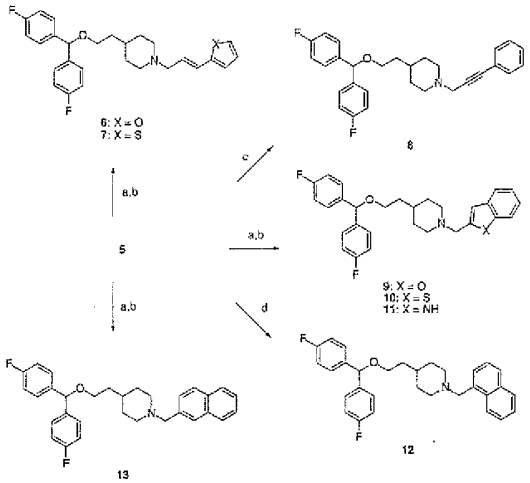
10.1021/jm020264r
Published on Web 08/17/2002

Scheme 1[a]



[a] Reagents: (a) Hydrocinnamoyl chloride, NEt_3, CH_2Cl_2. (b) LAH, THF. (c) 4,4-Difluorobenzhydrol, *p*-TsOH·H_2O, toluene. (d) Benzoyl chloride, NEt_3, CH_2Cl_2. (e) NaOH, EtOH. (f) *trans*-Cinnamic acid, EDCI, CH_2Cl_2. (g) LAH, H_2SO_4, THF.

Scheme 2[a]



[a] Reagents: (a) Appropriate acid, EDCI, CH_2Cl_2. (b) LAH, H_2SO_4, THF. (c) Phenylacetylene, $(CH_2O)_n$, $CuSO_4$, THF. (d) 1-Chloromethylnaphthalene, K_2CO_3, DMF.

Targets **6**, **7**, **9**−**11**, and **13** were prepared from **5** using a procedure similar to the preparation of **4** (Scheme 2). The coupling of **5** with the appropriate acid using EDCI followed by reduction of the corresponding amide with AlH_3 gave **6**, **7**, **9**−**11**, and **13**.[33,34] A modified Mannich reaction using **5**, phenylacetylene, and paraformaldehyde gave **8**. The treatment of **5** with 1-chloromethylnaphthalene under basic conditions afforded **12**.

Results and Discussion

We resynthesized **2** to compare (Table 1) its binding affinities with those of our new analogues. It was hoped that the introduction of different functional groups into the *N*-alkyl group of **2** would give us a compound with high affinity and better selectivity than **1**. These analogues might represent a second generation cocaine abuse treatment agent.

We found that the affinity of **2** in our binding assay (Table 1) was higher than previously reported.[27] It had higher affinity (K_i = 1.1 nM) for the DAT and greater selectivity over the SERT (68-fold) than **1**. This might be due to the use of [^{125}I]RTI-55 to label a site on the DAT rather than the formerly used[27] [^{3}H]WIN 35,428.

Table 1. Binding Affinities at the DAT and SERT Labeled with [^{125}I]RTI-55 of **2**, **4**, and **6**−**13** ($K_i \pm$ SD, nM)

compds[a]	DAT[b]	SERT[b]	SERT/DAT
1	3.7 ± 0.4	126 ± 27	34
2	1.1 ± 0.1	68 ± 8	65
4	0.45 ± 0.03	47 ± 2	104
6	0.99 ± 0.07	41 ± 6	41
7	1.3 ± 0.1	45 ± 5	35
8	5.3 ± 0.4	164 ± 26	31
9	1.01 ± 0.18	85 ± 10	84
10	1.61 ± 0.15	246 ± 33	153
11	0.73 ± 0.07	88 ± 10	121
12	16 ± 1	370 ± 27	23
13	0.71 ± 0.06	229 ± 21	323

[a] Prepared and tested as oxalate salt. [b] Values determined as in ref 23 using [^{125}I]RTI-55 as radioligand for the DAT and SERT.

On the basis of this observation and our previous discovery that 1-{2-[bis-(4-fluorophenyl)methoxy]ethyl}-4-(3-phenylallyl)piperazine (GBR 13069, **3**) also had higher affinity for the DAT than **1**,[23] we thought it might be of great interest to prepare **4**. Although it was prepared previously, we thought it possible that **4** might have greater affinity for the DAT in our assay. We found that **4** had higher affinity for the DAT (K_i = 0.45 nM) than **2** (K_i = 1.1 nM). This result, however, was also in contrast to previous findings where the affinity of **4** was reported to be 41.4 nM.[32] In an attempt to better understand these results, we evaluated several additional analogues with *N*-substituents that had been previously investigated in the piperazine series.[22] In particular, we chose to examine *N*-substituents in the piperidine series that displayed high affinity ($K_i \leq$ 10 nM) in the piperazine series, such as a 3-furan-2-ylallyl and a 3-thiophen-2-ylallyl substituent, as well as several others.[22] It was hoped that these specific alterations might provide an explanation for the mode of binding of the two series relative to one another.

The binding results showed that the replacement of the phenyl ring in **4** with a 2-furyl, **6**, or 2-thienyl, **7**, group resulted in a decrease in affinity for the DAT as compared to **4**. These compounds were similar to **4** in their affinity for the SERT.

The role of the trans-alkene in **4** was then tested. The introduction of an alkyne, i.e., **8**, decreased affinity for the DAT (10-fold) as compared to **4**. This seemed to indicate that a trans-alkene was favored over a linear conformation, i.e., **8**. Furthermore, the trans-alkene in **4** was then incorporated into several heterocycles, **9**−**11**. These modifications decreased affinity for the DAT and the SERT as compared to **4**. We noted that the introduction of an indole moiety, **11**, was optimal for affinity (K_i = 0.73 nM) among the heterocycles, **9**−**11**. However, the benzothiophene analogue, **10**, had the best selectivity over the SERT (153-fold).

In an attempt to further investigate the binding region of the *N*-substituent, we synthesized two naphthalene isomers. 1-Naphthylmethyl analogue **12** had the least affinity for the DAT (K_i = 16 nM) and the SERT (K_i = 370 nM) of this series of ligands. Remarkably, 2-naphthylmethyl analogue, **13**, was found to have subnanomolar affinity (K_i = 0.71 nM) for the DAT and the best selectivity over the SERT (323-fold) of the compounds examined.

The functional binding assays (Table 2) were carried out for two of the most interesting ligands, **10** and **13**.

Table 2. Reuptake Inhibition Studies of **1**, **10**, and **13** ($IC_{50} \pm$ SD, nM)

compds[a]	DA[b]	5-HT[b]	NE[b]	5-HT/DA	NE/DA
1	4.3 ± 0.3	73 ± 2	79 ± 5	17	18
10	11.9 ± 1.0	1037 ± 92	260 ± 21	87	22
13	7.2 ± 0.4	277 ± 15	93 ± 8	38	13

[a] Prepared and tested as oxalate salt. [b] Values determined as in ref 23b.

These compounds were chosen because they had the best selectivity over the SERT in this series. The uptake assay showed that both **10** and **13** had slightly lower potency than **1** in inhibiting DA uptake. However, both had increased selectivity over inhibiting 5-HT uptake as compared to **1**, and **10** showed the desired increase in selectivity over NE.

In comparison with the piperazine series, the piperidine analogues generally had higher affinity for both the DAT and the SERT.[22] In agreement with the piperazine series,[22] 2-furyl analogue **6** (K_i = 0.99 nM) had slightly higher affinity for the DAT than the 2-thienyl analogue **7** (K_i = 1.3 nM). Compounds **9** and **10** had higher affinity and better selectivity than the corresponding piperazine analogues. Compound **11** was nearly identical to its piperazine counterpart. The only difference was a slightly higher affinity for the SERT that was responsible for a lower selectivity. Compounds **12** and **13** showed very different results. In the piperazine series, the 2-naphthylmethyl derivative had lower affinity for the DAT than the 1-naphthylmethyl derivative. It was thought that if the piperidine series was binding in an identical manner to the piperazine series, then compound **12** would have higher affinity than **13** for the DAT. Interestingly, **13** had subnanomolar affinity for the DAT and the best selectivity over the SERT in this series of ligands. Apparently, an extended conformation is preferred in the piperidine series. It also appears that previous piperazine structure–activity relationships (SAR) may not be applicable to the piperidine series, suggesting that these two series are not binding in an identical manner at the DAT.

Conclusions

A series of 4-[2-[bis(4-fluorophenyl)methoxy]ethylpiperidines were synthesized and evaluated. Several ligands were identified with subnanomolar affinity to DAT. The 2-naphthyl derivative (**13**) was more than 300-fold selective for DAT over the SERT, and in reuptake inhibition studies, the benzothiophene compound **10** was found to have somewhat better selectivity for DAT over the NET than **1**. This study indicates that previous SAR seen in the GBR-12909 piperazine series do not hold for the corresponding piperidine series. Further exploration of this is currently underway.

Experimental Section

Unless otherwise indicated, all reagents were purchased from commercial suppliers and were used without further purification. The instrumentation used has been previously noted.[26]

4-{2-[Bis-(4-fluorophenyl)methoxy]ethyl}piperidine Oxalate (5). A solution of benzoyl chloride (16.3 g, 116.1 mmol) in dry CH_2Cl_2 (200 mL) was added in a dropwise manner to a solution of 4-piperidineethanol (15.0 g, 116.1 mmol) and triethylamine (11.7 g, 116.1 mmol) in dry CH_2Cl_2 (400 mL) at 0 °C. The mixture was stirred at room temperature overnight, washed successively with H_2O (3 × 100 mL), 2 N HCl (3 × 100 mL), and saturated NaCl (2 × 200 mL), and dried (Na_2SO_4). Removal of the solvent under reduced pressure afforded a crude oil that was used without further purification. A mixture of crude oil, *p*-toluenesulfonic acid monohydrate (13.8 g, 72.6 mmol), and 4,4-difluorobenzhydrol (26.8 g, 121.9 mmol) in dry toluene (800 mL) was heated at reflux under azeotropic distillation conditions overnight. The solvent was removed under reduced pressure, and EtOAc (600 mL) was added. The EtOAc portion was washed with H_2O (3 × 100 mL) and saturated NaCl (2 × 100 mL) and dried (Na_2SO_4). Removal of the solvent under reduced pressure afforded a crude oil that solidified upon addition of hexane. A solution of the crude solid, NaOH (21.2 g, 529 mmol), H_2O (50 mL), and absolute EtOH (500 mL) was heated at reflux for 36 h. The solvent was removed under reduced pressure, and H_2O (300 mL) was added to the residue. The mixture was extracted with CH_2Cl_2 (3 × 100 mL). The combined CH_2Cl_2 portion was washed with saturated NaCl (2 × 100 mL) and dried (Na_2SO_4). Removal of the solvent under reduced pressure afforded a crude oil that was dissolved in dry acetone. Oxalic acid (1.1 equiv) was added, and precipitate was collected and dried to afford 36.1 g (74%) of **5** as a white solid; mp 146–148 °C. ^{1}H NMR (DMSO-d_6): δ 7.1–7.4 (m, 8H, aromatic); 5.5 (s, 1H, CH–O); 3.9 (bs, NH); 3.1–3.4 (m, 4H); 2.5–2.9 (m, 5H); 1.1–2.0 (m, 5H).

4-{2-[Bis-(4-fluorophenyl)methoxy]ethyl}-1-(3-phenylallyl)piperidine Oxalate (4). A solution of the free base of **5** (1.5 g, 4.5 mmol), *trans*-cinnamic acid (0.7 g, 4.5 mmol), and EDCI (0.9 g, 5.0 mmol) in dry CH_2Cl_2 (25 mL) was stirred at room temperature overnight. The solvent was removed under reduced pressure, and the ethyl acetate (125 mL) was added to the residue. The ethyl acetate solution was washed successively with 1 N HCl (2 × 50 mL), 10% K_2CO_3 (2 × 50 mL), and saturated NaCl (50 mL) and dried (Na_2SO_4). The solvent was removed under reduced pressure to afford 2.0 g (96%) of the corresponding amide as an oil that was used without further characterization. A 100% amount of H_2SO_4 (d = 1.84) (1.1 g, 11.0 mmol) was added cautiously to a suspension of LAH (0.8 g, 22 mmol) in dry tetrahydrofuran (THF, 100 mmol) at 0 °C. After the addition was complete, the mixture was stirred at room temperature for 1 h. A solution of the crude amide in dry THF (50 mL) was added in a dropwise manner. The resulting mixture was stirred for 2 h, and 10% NaOH (150 mL) was added cautiously. The layers separated, and the aqueous layer was extracted with ethyl acetate (2 × 100 mL). The combined organic portion was washed with H_2O (100 mL) and saturated NaCl (2 × 100 mL) and dried (Na_2SO_4). Removal of the solvent under reduced pressure afforded a crude oil that was dissolved in dry acetone. Oxalic acid (1.1 equiv) was added, and the solvent was removed under reduced pressure. Anhydrous Et_2O was added, and the precipitate was collected and dried to afford 1.6 g (67%) of **4** as a white solid; mp 158–160 °C (literature[2] 162.7–163.5 °C). Anal. ($C_{29}H_{31}F_2NO \cdot C_2H_2O_4 \cdot 0.5H_2O$): C, H, N.

4-{2-[Bis-(4-fluorophenyl)methoxy]ethyl}-1-(3-phenylprop-2-ynyl)piperidine Oxalate (8). A mixture of the free base of **5** (1.5 g, 4.7 mmol), phenylacetylene (0.7 g, 7.1 mmol), $CuSO_4$ (0.8 g, 4.7 mmol), and paraformaldehyde (0.4 g, 14.1 mmol) in dry THF (45 mL) was heated at reflux for 2 h. Et_2O (100 mL) was added, and the mixture was filtered through a pad of Celite. The filtrate was evaporated to dryness under reduced pressure, and the residue was dissolved in CH_2Cl_2 (100 mL). The CH_2Cl_2 portion was washed with H_2O (5 × 50 mL) and saturated NaCl (2 × 50 mL) and dried (Na_2SO_4). The solvent was removed under reduced pressure affording a crude oil that was dissolved in anhydrous Et_2O. Oxalic acid (1.1 equiv) was added, and the precipitate was collected, washed with cold absolute EtOH (20 mL), and dried to afford 2.0 g (80%) of **8** as a white solid; mp 134–136 °C. ^{1}H NMR (DMSO-d_6): δ 7.1–7.6 (m, 13H, aromatic); 5.5 (s, 1H, CH–O); 4.0 (s, 2H, NCH_2); 3.1–3.4 (m, 4H); 2.5–2.9 (m, 5H); 1.0–2.0 (m, 5H). Anal. ($C_{29}H_{29}F_2NO \cdot C_2H_2O_4$): C, H, N.

4-{2-[Bis-(4-fluorophenyl)methoxy]ethyl}-1-naphthalen-1-ylmethylpiperidine Oxalate (12). A suspension of **5** (1.0 g, 2.4 mmol), K_2CO_3 (1.0 g, 7.2 mmol), a catalytic amount of NaI, and 1-chloromethylnaphthalene (0.5 g, 2.6 mmol) in dimethylformamide (DMF, 30 mL) was stirred at 100 °C overnight. The mixture was poured into H_2O (200 mL) and extracted with ethyl acetate (3 × 60 mL). The combined ethyl acetate portion was washed with H_2O (2 × 75 mL) and saturated NaCl (2 × 75 mL) and dried (Na_2SO_4). Removal of the solvent under reduced pressure afforded a residue that was dissolved in anhydrous Et_2O. Oxalic acid (1.1 equiv) was added, and the precipitate was collected, recrystallized from absolute EtOH, and dried to afford 0.9 g (67%) of **12** as a white solid; mp 176−178 °C. ^1H NMR (DMSO-d_6): δ 7.1−8.4 (m, 15H, aromatic); 5.5 (s, 1H, CH−O); 4.5 (s, 2H, $ArCH_2$-); 3.1−3.4 (m, 4H); 2.5−2.9 (m, 5H); 1.1−2.0 (m, 5H). Anal. ($C_{31}H_{31}F_2NO\cdot C_2H_2O_4$): C, H, N.

Acknowledgment. We (LMC, NIDDK) thank the National Institute on Drug Abuse, NIH, for partial support of this work.

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(33) All of the new compounds were found to be within ±0.4 of the theoretical values for C, H, and N. ^1H NMR (300 MHz, DMSO-d_6) was in agreement with assigned structures.

(34) Uncorrected melting points of oxalate salts: **4**, mp 158−160 °C; **5**, mp 146−148 °C; **6**, mp 148−149 °C; **7**, mp 150−152 °C; **8**, mp 134−136 °C; **9**, mp 156−159 °C; **10**, mp 155−159 °C; **11**, mp 180−183 °C; **12**, mp 176−178 °C; **13**, mp 161−162 °C.

JM020264R

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use REMODULIN safely and effectively. See full prescribing information for REMODULIN.

REMODULIN® (treprostinil) Injection, for subcutaneous or intravenous use
Initial U.S. Approval: May 2002

----------RECENT MAJOR CHANGES----------

Dosage and Administration (2.1, 2.5) 12/2014

----------INDICATIONS AND USAGE----------

Remodulin is a prostacyclin vasodilator indicated for:

- Treatment of pulmonary arterial hypertension (PAH) (WHO Group 1) to diminish symptoms associated with exercise. Studies establishing effectiveness included patients with NYHA Functional Class II-IV symptoms and etiologies of idiopathic or heritable PAH (58%), PAH associated with congenital systemic-to-pulmonary shunts (23%), or PAH associated with connective tissue diseases (19%) (1.1)
- Patients who require transition from Flolan®, to reduce the rate of clinical deterioration. The risks and benefits of each drug should be carefully considered prior to transition. (1.2)

----------DOSAGE AND ADMINISTRATION----------

PAH in patients with NYHA Class II-IV symptoms:

- Initial dose for patients new to prostacyclin infusion therapy: 1.25 ng/kg/min, increase based on clinical response (increments of 1.25 ng/kg/min per week for the first 4 weeks of treatment, later 2.5 ng/kg/min per week). Avoid abrupt cessation. (2.2, 2.3)
- Mild to moderate hepatic insufficiency: Decrease initial dose to 0.625 ng/kg/min.
- Severe hepatic insufficiency: No studies performed. (2.4)

Transition from Flolan:

Increase the Remodulin dose gradually as the Flolan dose is decreased, based on constant observation of response. (2.6)

Administration:

Continuous subcutaneous infusion (undiluted) is the preferred mode. Use intravenous (IV) infusion (dilution required) if subcutaneous infusion is not tolerated. (2.1, 2.5)

----------DOSAGE FORMS AND STRENGTHS----------

- Remodulin is supplied in 20 mL vials containing 20, 50, 100, or 200 mg of treprostinil (1, 2.5, 5 or 10 mg/mL). (3)

----------CONTRAINDICATIONS----------

None

----------WARNINGS AND PRECAUTIONS----------

- For intravenous infusion use an indwelling central venous catheter. This route is associated with the risk of blood stream infections (BSIs) and sepsis, which may be fatal. (5.1)
- Do not abruptly lower the dose or withdraw dosing. (5.2)

----------ADVERSE REACTIONS----------

Most common adverse reactions (incidence >3%) reported in clinical studies with Remodulin: subcutaneous infusion site pain and reaction, headache, diarrhea, nausea, jaw pain, vasodilatation, edema, and hypotension. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact United Therapeutics Corp. at 1-866-458-6479 or contact FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

----------DRUG INTERACTIONS----------

- Blood pressure lowering drugs (e.g., diuretics, antihypertensive agents, or vasodilators): Risk of increased reduction in blood pressure (7.1)
- Remodulin inhibits platelet aggregation. Potential for increased risk of bleeding, particularly among patients on anticoagulants. (7.2)
- Remodulin dosage adjustment may be necessary if inhibitors or inducers of CYP2C8 are added or withdrawn. (7.6)

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 12/2014

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1. INDICATIONS AND USAGE

1.1 Pulmonary Arterial Hypertension

Remodulin is indicated for the treatment of pulmonary arterial hypertension (PAH) (WHO Group 1) to diminish symptoms associated with exercise. Studies establishing effectiveness included patients with NYHA Functional Class II-IV symptoms and etiologies of idiopathic or heritable PAH (58%), PAH associated with congenital systemic-to-pulmonary shunts (23%), or PAH associated with connective tissue diseases (19%) *[see Clinical Studies (14.1)]*.

It may be administered as a continuous subcutaneous infusion or continuous intravenous (IV) infusion; however, because of the risks associated with chronic indwelling central venous catheters, including serious blood stream infections (BSIs), reserve continuous intravenous infusion for patients who are intolerant of the subcutaneous route, or in whom these risks are considered warranted *[see Warnings and Precautions 5.1]*.

1.2 Pulmonary Arterial Hypertension in Patients Requiring Transition from Flolan®

In patients with pulmonary arterial hypertension requiring transition from Flolan (epoprostenol sodium), Remodulin is indicated to diminish the rate of clinical deterioration. Consider the risks and benefits of each drug prior to transition.

2 DOSAGE AND ADMINISTRATION

2.1 General

Remodulin can be administered without further dilution for subcutaneous administration, or diluted for intravenous infusion with Sterile Diluent for Remodulin or similar approved high-pH glycine diluent (e.g. Sterile Diluent for Flolan or Sterile Diluent for Epoprostenol Sodium), Sterile Water for Injection, or 0.9% Sodium Chloride Injection prior to administration. See Table 1 below for storage and administration time limits for the different diluents.

Table 1. Selection of Diluent

Route	Diluent	Storage limits	Administration limits
SC	None	See section 16	72 hours at 37°C
IV	Sterile Diluent for Remodulin Sterile Diluent for Flolan Sterile Diluent for Epoprostenol Sodium	14 days at room temperature	48 hours at 40 °C
	Sterile water for injection 0.9% Sodium Chloride for injection	4 hours at room temperature or 24 hours refrigerated	48 hours at 40°C

2.2 Initial Dose for Patients New to Prostacyclin Infusion Therapy

Remodulin is indicated for subcutaneous (SC) or intravenous (IV) use only as a continuous infusion. Remodulin is preferably infused subcutaneously, but can be administered by a central intravenous line if the subcutaneous route is not tolerated, because of severe site pain or reaction. The infusion rate is initiated at 1.25 ng/kg/min. If this initial dose cannot be tolerated because of systemic effects, reduce the infusion rate to 0.625 ng/kg/min.

2.3 Dosage Adjustments

The goal of chronic dosage adjustments is to establish a dose at which PAH symptoms are improved, while minimizing excessive pharmacologic effects of Remodulin (headache, nausea, emesis, restlessness, anxiety and infusion site pain or reaction).

The infusion rate should be increased in increments of 1.25 ng/kg/min per week for the first four weeks of treatment and then 2.5 ng/kg/min per week for the remaining duration of infusion, depending on clinical response. Dosage adjustments may be undertaken more often if tolerated. Avoid abrupt cessation of infusion *[see Warnings and Precautions (5.4)]*. Restarting a Remodulin infusion within a few hours after an interruption can be done using the same dose rate. Interruptions for longer periods may require the dose of Remodulin to be re-titrated.

2.4 Patients with Hepatic Insufficiency

In patients with mild or moderate hepatic insufficiency, decrease the initial dose of Remodulin to 0.625 ng/kg/min ideal body weight. Remodulin has not been studied in patients with severe hepatic insufficiency *[see Warnings and Precautions (5.3), Use In Specific Populations (8.6)* and *Clinical Pharmacology (12.3)]*.

2.5 Administration

Inspect parenteral drug products for particulate matter and discoloration prior to administration whenever solution and container permit. If either particulate matter or discoloration is noted, do not use.

Subcutaneous Infusion

Remodulin is administered subcutaneously by continuous infusion without further dilution, via a subcutaneous catheter, using an infusion pump designed for subcutaneous drug delivery. To avoid potential interruptions in drug delivery, the patient must have immediate access to a backup infusion pump and subcutaneous infusion sets. The ambulatory infusion pump used to administer Remodulin should: (1) be small and lightweight, (2) be adjustable to approximately 0.002 mL/hr, (3) have occlusion/no delivery, low battery, programming error and motor malfunction alarms, (4) have delivery accuracy of ±6% or better and (5) be positive pressure driven. The reservoir should be made of polyvinyl chloride, polypropylene or glass.

Remodulin is administered subcutaneously by continuous infusion at a calculated subcutaneous infusion rate (mL/hr) based on a patient's dose (ng/kg/min), weight (kg), and the vial strength (mg/mL) of Remodulin being used. During use, a single reservoir (syringe) of undiluted Remodulin can be administered up to 72 hours at 37°C. The subcutaneous infusion rate is calculated using the following formula:

$$\textbf{Subcutaneous Infusion Rate (mL/hr)} = \frac{\textbf{Dose} \text{ (ng/kg/min)} \times \textbf{Weight} \text{ (kg)} \times \textbf{0.00006*}}{\textbf{Remodulin Vial Strength} \text{ (mg/mL)}}$$

**Conversion factor of 0.00006 = 60 min/hour x 0.000001 mg/ng*

Example calculations for ***Subcutaneous Infusion*** are as follows:

Example 1:

For a 60 kg person at the recommended initial dose of 1.25 ng/kg/min using the 1 mg/mL Remodulin, the infusion rate would be calculated as follows:

$$\text{Subcutaneous Infusion Rate (mL/hr)} = \frac{1.25 \text{ ng/kg/min} \times 60 \text{ kg} \times 0.00006}{1 \text{ mg/mL}} = 0.005 \text{ mL/hr}$$

Example 2:

For a 65 kg person at a dose of 40 ng/kg/min using the 5 mg/mL Remodulin, the infusion rate would be calculated as follows:

$$\text{Subcutaneous Infusion Rate (mL/hr)} = \frac{40 \text{ ng/kg/min} \times 65 \text{ kg} \times 0.00006}{5 \text{ mg/mL}} = 0.031 \text{ mL/hr}$$

Intravenous Infusion

Diluted Remodulin is administered intravenously by continuous infusion via a surgically placed indwelling central venous catheter using an infusion pump designed for intravenous drug delivery. If clinically necessary, a temporary peripheral intravenous cannula, preferably placed in a large vein, may be used for short term administration of Remodulin. Use of a peripheral intravenous infusion for more than a few hours may be associated with an increased risk of thrombophlebitis. To avoid potential interruptions in drug delivery, the patient must have immediate access to a backup infusion pump and infusion sets. The ambulatory infusion pump used to administer Remodulin should: (1) be small and lightweight, (2) have occlusion/no delivery, low battery, programming error and motor malfunction alarms, (3) have delivery accuracy of ±6% or better of the hourly dose, and (4) be positive pressure driven. The reservoir should be made of polyvinyl chloride, polypropylene or glass.

Infusion sets with an in-line 0.22 or 0.2 micron pore size filter should be used.

Diluted Remodulin has been shown to be stable at ambient temperature when stored for up to 14 days using high-pH glycine diluent at concentrations as low as 0.004 mg/mL (4,000 ng/mL).

Select the intravenous infusion rate to allow for a desired infusion period length of up to 48 hours between system changeovers. Typical intravenous infusion system reservoirs have volumes of 50 or 100 mL. With this selected intravenous infusion rate (mL/hr) and the patient's dose (ng/kg/min) and weight (kg), the diluted intravenous Remodulin concentration (mg/mL) can be calculated using the following formula:

Step 1

$$\text{Diluted Intravenous Remodulin Concentration (mg/mL)} = \frac{\text{Dose (ng/kg/min)} \times \text{Weight (kg)} \times 0.00006}{\text{Intravenous Infusion Rate (mL/hr)}}$$

The volume of Remodulin Injection needed to make the required diluted intravenous Remodulin concentration for the given reservoir size can then be calculated using the following formula:

Step 2

$$\text{Volume of Remodulin Injection (mL)} = \frac{\text{Diluted Intravenous Remodulin Concentration (mg/mL)}}{\text{Remodulin Vial Strength (mg/mL)}} \times \text{Total Volume of Diluted Remodulin Solution in Reservoir (mL)}$$

The calculated volume of Remodulin Injection is then added to the reservoir along with the sufficient volume of diluent to achieve the desired total volume in the reservoir.

Example calculations for ***Intravenous Infusion*** are as follows:

Example 3:

For a 60 kg person at a dose of 5 ng/kg/min, with a predetermined intravenous infusion rate of 1 mL/hr and a reservoir of 50 mL, the diluted intravenous Remodulin concentration would be calculated as follows:

Step 1

$$\text{Diluted Intravenous Remodulin Concentration (mg/mL)} = \frac{5 \text{ ng/kg/min} \times 60 \text{ kg} \times 0.00006}{1 \text{ mL/hr}} = 0.018 \text{ mg/mL (18,000 ng/mL)}$$

The volume of Remodulin Injection (using 1 mg/mL Vial Strength) needed for a total diluted Remodulin concentration of 0.018 mg/mL and a total volume of 50 mL would be calculated as follows:

Step 2

$$\text{Volume of Remodulin Injection (mL)} = \frac{0.018 \text{ mg/mL}}{1 \text{ mg/mL}} \times 50 \text{ mL} = 0.9 \text{ mL}$$

The diluted intravenous Remodulin concentration for the person in Example 3 would thus be prepared by adding 0.9 mL of 1 mg/mL Remodulin Injection to a suitable reservoir along with a sufficient volume of diluent to achieve a total volume of 50 mL in the reservoir. The pump flow rate for this example would be set at 1 mL/hr.

Example 4:

For a 75 kg person at a dose of 30 ng/kg/min, with a predetermined intravenous infusion rate of 2 mL/hr, and a reservoir of 100 mL, the diluted intravenous Remodulin concentration would be calculated as follows:

Step 1

$$\text{Diluted Intravenous} = \frac{30 \text{ ng/kg/min} \times 75 \text{ kg} \times 0.00006}{} = 0.0675 \text{ mg/mL (67,500 ng/mL)}$$

$$\text{Remodulin Concentration (mg/mL)} = \frac{\text{Remodulin Concentration (mg/mL)}}{2 \text{ mL/hr}}$$

The volume of Remodulin Injection (using 2.5 mg/mL Vial Strength) needed for a total diluted Remodulin concentration of 0.0675 mg/mL and a total volume of 100 mL would be calculated as follows:

Step 2

$$\text{Volume of Remodulin Injection (mL)} = \frac{0.0675 \text{ mg/mL}}{2.5 \text{ mg/mL}} \times 100 \text{ mL} = 2.7 \text{ mL}$$

The diluted intravenous Remodulin concentration for the person in Example 4 would thus be prepared by adding 2.7 mL of 2.5 mg/mL Remodulin Injection to a suitable reservoir along with a sufficient volume of diluent to achieve a total volume of 100 mL in the reservoir. The pump flow rate for this example would be set at 2 mL/hr.

2.6 Patients Requiring Transition from Flolan

Transition from Flolan to Remodulin is accomplished by initiating the infusion of Remodulin and increasing it, while simultaneously reducing the dose of intravenous Flolan. The transition to Remodulin should take place in a hospital with constant observation of response (e.g., walk distance and signs and symptoms of disease progression). Initiate Remodulin at a recommended dose of 10% of the current Flolan dose, and then escalate as the Flolan dose is decreased (see Table 2 for recommended dose titrations).

Patients are individually titrated to a dose that allows transition from Flolan therapy to Remodulin while balancing prostacyclin-limiting adverse events. Increases in the patient's symptoms of PAH should be first treated with increases in the dose of Remodulin. Side effects normally associated with prostacyclin and prostacyclin analogs are to be first treated by decreasing the dose of Flolan.

Table 2: Recommended Transition Dose Changes

Step	Flolan Dose	Remodulin Dose
1	Unchanged	10% Starting Flolan Dose
2	80% Starting Flolan Dose	30% Starting Flolan Dose
3	60% Starting Flolan Dose	50% Starting Flolan Dose
4	40% Starting Flolan Dose	70% Starting Flolan Dose
5	20% Starting Flolan Dose	90% Starting Flolan Dose
6	5% Starting Flolan Dose	110% Starting Flolan Dose
7	0	110% Starting Flolan Dose + additional 5-10% increments as needed

3 DOSAGE FORMS AND STRENGTHS

20-mL vial containing 20 mg treprostinil (1 mg per mL).

20-mL vial containing 50 mg treprostinil (2.5 mg per mL).
20-mL vial containing 100 mg treprostinil (5 mg per mL).
20-mL vial containing 200 mg treprostinil (10 mg per mL).

4 CONTRAINDICATIONS

None

5 WARNINGS AND PRECAUTIONS

5.1 Risk of Catheter-Related Bloodstream Infection

Chronic intravenous infusions of Remodulin are delivered using an indwelling central venous catheter. This route is associated with the risk of blood stream infections (BSIs) and sepsis, which may be fatal. Therefore, continuous subcutaneous infusion (undiluted) is the preferred mode of administration.

In an open-label study of IV treprostinil (n=47), there were seven catheter-related line infections during approximately 35 patient years, or about 1 BSI event per 5 years of use. A CDC survey of seven sites that used IV treprostinil for the treatment of PAH found approximately 1 BSI (defined as any positive blood culture) event per 3 years of use. Administration of IV Remodulin with a high pH glycine diluent has been associated with a lower incidence of BSIs when compared to neutral diluents (sterile water, 0.9% sodium chloride) when used along with catheter care guidelines.

5.2 Worsening PAH upon Abrupt Withdrawal or Sudden Large Dose Reduction

Avoid abrupt withdrawal or sudden large reductions in dosage of Remodulin, which may result in worsening of PAH symptoms.

5.3 Patients with Hepatic or Renal Insufficiency

Titrate slowly in patients with hepatic or renal insufficiency, because such patients will likely be exposed to greater systemic concentrations relative to patients with normal hepatic or renal function *[see Dosage and Administration (2.4, 2.5), Use In Specific Populations (8.6, 8.7), and Clinical Pharmacology (12.3)]*.

5.4 Effect of Other Drugs on Treprostinil

Co-administration of a cytochrome P450 (CYP) 2C8 enzyme inhibitor (e.g., gemfibrozil) increases exposure (both C_{max} and AUC) to treprostinil. Co-administration of a CYP2C8 enzyme inducer (e.g., rifampin) decreases exposure to treprostinil *[see Drug Interactions (7.5)* and *Clinical Pharmacology (12.3)]*.

6 ADVERSE REACTIONS

The following adverse reactions are discussed elsewhere in labeling: Infections associated with intravenous administration *[see Warnings and Precautions (5.1)]*.

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

Adverse Events with Subcutaneously Administered Remodulin

Patients receiving Remodulin as a subcutaneous infusion reported a wide range of adverse events, many potentially related to the underlying disease (dyspnea, fatigue, chest pain, right ventricular heart failure, and pallor). During clinical trials with subcutaneous infusion of Remodulin, infusion site pain and reaction were the most common adverse events among those treated with Remodulin. Infusion site reaction was defined as any local adverse event other than pain or bleeding/bruising at the infusion site and included symptoms such as erythema, induration or rash. Infusion site reactions were sometimes severe and could lead to discontinuation of treatment.

Table 3: Percentages of subjects reporting subcutaneous infusion site adverse events

	Reaction		Pain	
	Placebo	Remodulin	Placebo	Remodulin
Severe	1	38	2	39
Requiring narcotics*	NA[†]	NA[†]	1	32
Leading to discontinuation	0	3	0	7

* based on prescriptions for narcotics, not actual use

† medications used to treat infusion site pain were not distinguished from those used to treat site reactions

Other adverse events included diarrhea, jaw pain, edema, vasodilatation and nausea, and these are generally considered to be related to the pharmacologic effects of Remodulin, whether administered subcutaneously or intravenously.

Adverse Reactions during Chronic Dosing

Table 4 lists adverse reactions defined by a rate of at least 3% more frequent in patients treated with subcutaneous Remodulin than with placebo in controlled trials in PAH.

Table 4: Adverse Reactions in Controlled 12-Week Studies of Subcutaneous Remodulin and at least 3% more frequent than on Placebo.

Adverse Reaction	Remodulin (N=236) Percent of Patients	Placebo (N=233) Percent of Patients
Infusion Site Pain	85	27
Infusion Site Reaction	83	27
Headache	27	23
Diarrhea	25	16
Nausea	22	18
Rash	14	11
Jaw Pain	13	5
Vasodilatation	11	5
Edema	9	3

Reported adverse reactions (at least 3% more frequent on drug than on placebo) are included except those too general to be informative, and those not plausibly attributable to the use of the drug, because they were associated with the condition being treated or are very common in the treated population.

While hypotension occurred in both groups, the event was experienced twice as frequently in the Remodulin group as compared to the placebo group (4% in Remodulin treatment group verses 2% in placebo-controlled group). As a potent vasodilator, hypotension is possible with the administration of Remodulin.

The safety of Remodulin was also studied in a long-term, open-label extension study in which 860 patients were dosed for a mean duration of 1.6 years, with a maximum exposure of 4.6 years. Twenty-nine (29%) percent achieved a dose of at least 40 ng/kg/min (max: 290 ng/kg/min). The safety profile during this chronic dosing study was similar to that observed in the 12-week placebo controlled study except for the following suspected adverse drug reactions (occurring in at least 3% of patients): anorexia, vomiting, infusion site infection, asthenia, and abdominal pain.

Adverse Events Attributable to the Drug Delivery System

In controlled studies of Remodulin administered subcutaneously, there were no reports of infection related to the drug delivery system. There were 187 infusion system complications reported in 28% of patients (23% Remodulin, 33% placebo); 173 (93%) were pump related and 14 (7%) related to the infusion set. Eight of these patients (4 Remodulin, 4 Placebo) reported non-serious adverse events resulting from infusion system complications. Adverse events resulting from problems with the delivery systems were typically related to either symptoms of excess Remodulin (e.g., nausea) or return of PAH symptoms (e.g., dyspnea). These events were generally resolved by correcting the delivery system pump or infusion set problem such as replacing the syringe or battery, reprogramming the pump, or straightening a crimped infusion line. Adverse events resulting from problems with the delivery system did not lead to clinical instability or rapid deterioration. In addition to these adverse events due to the drug delivery system during subcutaneous administration, the following adverse events may be attributable to the IV mode of infusion including arm swelling, paresthesias, hematoma and pain *[see Warnings and Precautions (5.1)]*.

6.2 Post-Marketing Experience

In addition to adverse reactions reported from clinical trials, the following events have been identified during post-approval use of Remodulin. Because they are reported voluntarily from a population of unknown size, estimates of frequency cannot be made. The following events have been chosen for inclusion because of a combination of their seriousness, frequency of reporting, and potential connection to Remodulin. These events are thrombophlebitis associated with peripheral intravenous infusion, thrombocytopenia bone pain, pruritus and dizziness. In addition, generalized rashes, sometimes macular or papular in nature, and cellulitis have been infrequently reported.

7 DRUG INTERACTIONS

Pharmacokinetic/pharmacodynamic interaction studies have been conducted with treprostinil administered subcutaneously (Remodulin) and orally (treprostinil diethanolamine).

Pharmacodynamics

7.1 Antihypertensive Agents or Other Vasodilators

Concomitant administration of Remodulin with diuretics, antihypertensive agents or other vasodilators may increase the risk of symptomatic hypotension.

7.2 Anticoagulants

Since treprostinil inhibits platelet aggregation, there may be an increased risk of bleeding, particularly among patients receiving anticoagulants.

Pharmacokinetics

7.3 Bosentan

In a human pharmacokinetic study conducted with bosentan (250 mg/day) and an oral formulation of treprostinil (treprostinil diethanolamine), no pharmacokinetic interactions between treprostinil and bosentan were observed.

7.4 Sildenafil

In a human pharmacokinetic study conducted with sildenafil (60 mg/day) and an oral formulation of treprostinil (treprostinil diethanolamine), no pharmacokinetic interactions between treprostinil and sildenafil were observed.

7.5 Effect of Treprostinil on Cytochrome P450 Enzymes

In vitro studies of human hepatic microsomes showed that treprostinil does not inhibit cytochrome P450 (CYP) isoenzymes CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A. Additionally, treprostinil does not induce cytochrome P450 isoenzymes CYP1A2, CYP2B6, CYP2C9, CYP2C19, and CYP3A. Thus Remodulin is not expected to alter the pharmacokinetics of compounds metabolized by CYP enzymes.

7.6 Effect of Cytochrome P450 Inhibitors and Inducers on Treprostinil

Human pharmacokinetic studies with an oral formulation of treprostinil (treprostinil diethanolamine) indicated that co-administration of the cytochrome P450 (CYP) 2C8 enzyme inhibitor gemfibrozil increases exposure (both C_{max} and AUC) to treprostinil. Co-administration of the CYP2C8 enzyme inducer rifampin decreases exposure to treprostinil. It has not been determined if the safety and efficacy of treprostinil by the parenteral (subcutaneously or intravenously) route are altered by inhibitors or inducers of CYP2C8 *[see Warnings and Precautions (5.4)]*.

Remodulin has not been studied in conjunction with Flolan or Tracleer® (bosentan).

7.7 Effect of Other Drugs on Treprostinil

Drug interaction studies have been carried out with treprostinil (oral or subcutaneous) co-administered with acetaminophen (4 g/day), warfarin (25 mg/day), and fluconazole (200 mg/day), respectively in healthy volunteers. These studies did not show a clinically significant effect on the pharmacokinetics of treprostinil. Treprostinil does not affect the pharmacokinetics or pharmacodynamics of warfarin. The pharmacokinetics of R- and S- warfarin and the INR in healthy subjects given a single 25 mg dose of warfarin were unaffected by continuous subcutaneous infusion of treprostinil at an infusion rate of 10 ng/kg/min.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category B - In pregnant rats, continuous subcutaneous infusions of treprostinil during organogenesis and late gestational development, at rates as high as 900 ng treprostinil/kg/min (about 117 times the starting human rate of infusion, on a ng/m^2 basis and about 16 times the average rate achieved in clinical trials), resulted in no evidence of harm to the fetus. In pregnant rabbits, effects of continuous subcutaneous infusions of treprostinil during organogenesis were limited to an increased incidence of fetal skeletal variations (bilateral full rib or right rudimentary rib on lumbar 1) associated with maternal toxicity (reduction in body weight and food consumption) at an infusion rate of 150 ng treprostinil/kg/min (about 41 times the starting human rate of infusion, on a ng/m^2 basis, and 5 times the average rate used in clinical trials). In rats, continuous subcutaneous infusion of treprostinil from implantation to the end of lactation, at rates of up to 450 ng treprostinil/kg/min, did not affect the growth and development of offspring. Animal reproduction studies are not always predictive of human response.

8.2 Labor and Delivery

No treprostinil treatment-related effects on labor and delivery were seen in animal studies. The effect of treprostinil sodium on labor and delivery in humans is unknown.

8.3 Nursing Mothers

It is not known whether treprostinil is excreted in human milk or absorbed systemically after ingestion. Many drugs are excreted in human milk.

8.4 Pediatric Use

Safety and effectiveness in pediatric patients have not been established. Clinical studies of Remodulin did not include sufficient numbers of patients aged ≤16 years to determine whether they respond differently from older patients.

8.5 Geriatric Use

Clinical studies of Remodulin did not include sufficient numbers of patients aged 65 and over to determine whether they respond differently from younger patients. In general, dose selection for an elderly patient should be cautious, reflecting the greater frequency of decreased hepatic, renal, or cardiac function, and of concomitant disease or other drug therapy.

8.6 Patients with Hepatic Insufficiency

Remodulin clearance is reduced in patients with hepatic insufficiency. In patients with mild or moderate hepatic insufficiency, decrease the initial dose of Remodulin to 0.625 ng/kg/min ideal body weight, and monitor closely. Remodulin has not been studied in patients with severe hepatic insufficiency *[see Dosage and Administration (2.4), Warnings and Precautions (5.3) and Clinical Pharmacology (12.3)]*.

8.7 Patients with Renal Insufficiency

No studies have been performed in patients with renal insufficiency. No specific advice about dosing in patients with renal impairment can be given *[see Clinical Pharmacology (12.3)]*.

10 OVERDOSAGE

Signs and symptoms of overdose with Remodulin during clinical trials are extensions of its dose-limiting pharmacologic effects and include flushing, headache, hypotension, nausea, vomiting, and diarrhea. Most events were self-limiting and resolved with reduction or withholding of Remodulin.

In controlled clinical trials, seven patients received some level of overdose and in open-label follow-on treatment seven additional patients received an overdose; these occurrences resulted from accidental bolus administration of Remodulin, errors in pump programmed rate of administration, and prescription of an incorrect dose. In only two cases did excess delivery of Remodulin produce an event of substantial hemodynamic concern (hypotension, near-syncope).

One pediatric patient was accidentally administered 7.5 mg of Remodulin via a central venous catheter. Symptoms included flushing, headache, nausea, vomiting, hypotension and seizure-like activity with loss of consciousness lasting several minutes. The patient subsequently recovered.

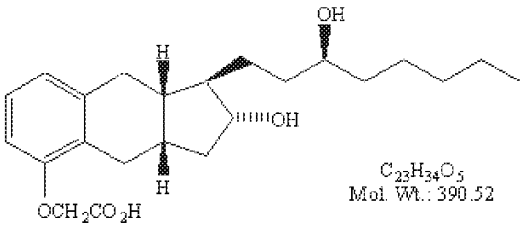
11 DESCRIPTION

Remodulin (treprostinil) Injection is a sterile solution of treprostinil formulated for subcutaneous or intravenous administration. Remodulin is supplied in 20 mL multidose vials in four strengths, containing 20 mg, 50 mg, 100 mg, or 200 mg (1 mg/mL, 2.5 mg/mL, 5 mg/mL or 10 mg/mL) of treprostinil. Each mL also contains 5.3 mg sodium chloride (except for the 10 mg/mL strength which contains 4.0 mg sodium chloride), 3 mg metacresol, 6.3 mg sodium citrate, and water for injection. Sodium hydroxide and hydrochloric acid may be added to adjust pH between 6.0 and 7.2.

Treprostinil is chemically stable at room temperature and neutral pH.

Treprostinil is (1R,2R,3aS,9aS)-[[2,3,3a,4,9,9a-Hexahydro-2-hydroxy-1-[(3S)-3-hydroxyoctyl]-1H-benz[f]inden-5-yl]oxy]acetic acid. Treprostinil has a molecular weight of 390.52 and a molecular formula of $C_{23}H_{34}O_5$.

The structural formula of treprostinil is:



Sterile Diluent for Remodulin is a high-pH (pH~10.4) glycine diluent supplied in a 50 mL vial containing 50 mL of Sterile Diluent for Remodulin. Each vial contains 94 mg glycine, 73.3 mg sodium chloride, sodium hydroxide (to adjust pH), and water for injection.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

The major pharmacologic actions of treprostinil are direct vasodilation of pulmonary and systemic arterial vascular beds, and inhibition of platelet aggregation.

12.2 Pharmacodynamics

In animals, the vasodilatory effects reduce right and left ventricular afterload and increase cardiac output and stroke volume. Other studies have shown that treprostinil causes a dose-related negative inotropic and lusitropic effect. No major effects on cardiac conduction have been observed.

Treprostinil produces vasodilation and tachycardia. Single doses of treprostinil up to 84 mcg by inhalation produce modest and short-lasting effects on QTc, but this is apt to be an artifact of the rapidly changing heart rate. Treprostinil administered by the subcutaneous or intravenous routes has the potential to generate concentrations many-fold greater than those generated via the inhaled route; the effect on the QTc interval when treprostinil is administered parenterally has not been established.

12.3 Pharmacokinetics

The pharmacokinetics of continuous subcutaneous Remodulin are linear over the dose range of 1.25 to 125 ng/kg/min (corresponding to plasma concentrations of about 15 pg/mL to 18,250 pg/mL) and can be described by a two-compartment model. Dose proportionality at infusion rates greater than 125 ng/kg/min has not been studied.

Subcutaneous and intravenous administration of Remodulin demonstrated bioequivalence at steady state at a dose of 10 ng/kg/min.

Absorption

Remodulin is relatively rapidly and completely absorbed after subcutaneous infusion, with an absolute bioavailability approximating 100%. Steady-state concentrations occurred in approximately 10 hours. Concentrations in patients treated with an average dose of 9.3 ng/kg/min were approximately 2,000 pg/mL.

Distribution

The volume of distribution of the drug in the central compartment is approximately 14L/70 kg ideal body weight. Remodulin at *in vitro* concentrations ranging from 330-10,000 mcg/L was 91% bound to human plasma protein.

Metabolism and Excretion

Treprostinil is substantially metabolized by the liver, primarily by CYP2C8. In a study conducted in healthy volunteers using [^{14}C] treprostinil, 78.6% and 13.4% of the subcutaneous dose was recovered in the urine and feces, respectively, over 10 days. Only 4% was excreted as unchanged treprostinil in the urine. Five metabolites were detected in the urine, ranging from 10.2% to 15.5% and representing 64.4% of the dose administered. Four of the metabolites are products of oxidation of the 3-hydroxyloctyl side chain and one is a glucuroconjugated derivative (treprostinil glucuronide). The identified metabolites do not appear to have activity.

The elimination of treprostinil (following subcutaneous administration) is biphasic, with a terminal elimination half-life of approximately 4 hours using a two compartment model. Systemic clearance is approximately 30 L/hr for a 70 kg person.

Based on in vitro studies treprostinil does not inhibit or induce major CYP enzymes *[see Drug Interactions (7.5)]*.

Special Populations

Hepatic Insufficiency

In patients with portopulmonary hypertension and mild (n=4) or moderate (n=5) hepatic insufficiency, Remodulin at a subcutaneous dose of 10 ng/kg/min for 150 minutes had a C_{max} that was 2-fold and 4-fold, respectively, and an AUC_{0-} that was 3-fold and 5-fold, respectively, values observed in healthy subjects. Clearance in patients with hepatic insufficiency was reduced by up to 80% compared to healthy adults.

Renal Insufficiency

No studies have been performed in patients with renal insufficiency, so no specific advice about dosing in such patients can be given. Although only 4% of the administered dose is excreted unchanged in the urine, the five identified metabolites are all excreted in the urine.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term studies have not been performed to evaluate the carcinogenic potential of treprostinil. *In vitro* and *in vivo* genetic toxicology studies did not demonstrate any mutagenic or clastogenic effects of treprostinil. Treprostinil did not affect fertility or mating performance of male or female rats given continuous subcutaneous infusions at rates of up to 450 ng treprostinil/kg/min [about 59 times the recommended starting human rate of infusion (1.25 ng/kg/min) and about 8 times the average rate (9.3 ng/kg/min) achieved in clinical trials, on a ng/m^2 basis]. In this study, males were dosed from 10 weeks prior to mating and through the 2-week mating period. Females were dosed from 2 weeks prior to mating until gestational day 6.

14 CLINICAL STUDIES

14.1 Clinical Trials in Pulmonary Arterial Hypertension (PAH)

Two 12-week, multicenter, randomized, double-blind studies compared continuous subcutaneous infusion of Remodulin to placebo in a total of 470 patients with NYHA Class II (11%), III (81%), or IV (7%) pulmonary arterial hypertension (PAH). PAH was idiopathic/heritable in 58% of patients, associated with connective tissue diseases in 19%, and the result of congenital systemic-to-pulmonary shunts in 23%. The mean age was 45 (range 9 to 75 years). About 81% were female and 84% were Caucasian. Pulmonary hypertension had been diagnosed for a mean of 3.8 years. The primary endpoint of the studies was change in 6-minute walking distance, a standard measure of exercise capacity. There were many assessments of symptoms related to heart failure, but local discomfort and pain associated with Remodulin may have substantially unblinded those assessments. The 6-minute walking distance and an associated subjective measurement of shortness of breath during the walk (Borg dyspnea score) were administered by a person not participating in other aspects of the study. Remodulin was administered as a subcutaneous infusion, described in Section 2, DOSAGE AND ADMINISTRATION, and the dose averaged 9.3 ng/kg/min at Week 12. Few subjects received doses > 40 ng/kg/min. Background therapy,

determined by the investigators, could include anticoagulants, oral vasodilators, diuretics, digoxin, and oxygen but not an endothelin receptor antagonist or epoprostenol. The two studies were identical in design and conducted simultaneously, and the results were analyzed both pooled and individually.

Hemodynamic Effects

As shown in Table 5, chronic therapy with Remodulin resulted in small hemodynamic changes consistent with pulmonary and systemic vasodilation.

Table 5: Hemodynamics during Chronic Administration of Remodulin in Patients with PAH in 12-Week Studies

Hemodynamic Parameter	Baseline		Mean change from baseline at Week 12	
	Remodulin (N=204-231)	Placebo (N=215-235)	Remodulin (N=163-199)	Placebo (N=182-215)
CI ($L/min/m^2$)	2.4 ± 0.88	2.2 ± 0.74	+0.12 ± 0.58*	-0.06 ± 0.55
PAPm (mmHg)	62 ± 17.6	60 ± 14.8	-2.3 ± 7.3*	+0.7 ± 8.5
RAPm (mmHg)	10 ± 5.7	10 ± 5.9	-0.5 ± 5.0*	+1.4 ± 4.8
PVRI ($mmHg/L/min/m^2$)	26 ± 13	25 ± 13	-3.5 ± 8.2*	+1.2 ± 7.9
SVRI ($mmHg/L/min/m^2$)	38 ± 15	39 ± 15	-3.5 ± 12*	-0.80 ± 12
SvO_2 (%)	62 ± 100	60 ± 11	+2.0 ± 10*	-1.4 ± 8.8
SAPm (mmHg)	90 ± 14	91 ± 14	-1.7 ± 12	-1.0 ± 13
HR (bpm)	82 ± 13	82 ± 15	-0.5 ± 11	-0.8 ± 11

*Denotes statistically significant difference between Remodulin and placebo, $p<0.05$.
CI = cardiac index; PAPm = mean pulmonary arterial pressure; PVRI = pulmonary vascular resistance indexed; RAPm = mean right atrial pressure; SAPm = mean systemic arterial pressure; SVRI = systemic vascular resistance indexed; SvO2 = mixed venous oxygen saturation; HR = heart rate.

Clinical Effects

The effect of Remodulin on 6-minute walk, the primary end point of the 12-week studies, was small and did not achieve conventional levels of statistical significance. For the combined populations, the median change from baseline on Remodulin was 10 meters and the median change from baseline on placebo was 0 meters from a baseline of approximately 345 meters. Although it was not the primary endpoint of the study, the Borg dyspnea score was significantly improved by Remodulin during the 6-minute walk, and Remodulin also had a significant effect, compared with placebo, on an assessment that combined walking distance with the Borg dyspnea score. Remodulin also consistently improved indices of dyspnea, fatigue and signs and symptoms of pulmonary hypertension, but these indices were difficult to interpret in the context of incomplete blinding to treatment assignment resulting from infusion site symptoms.

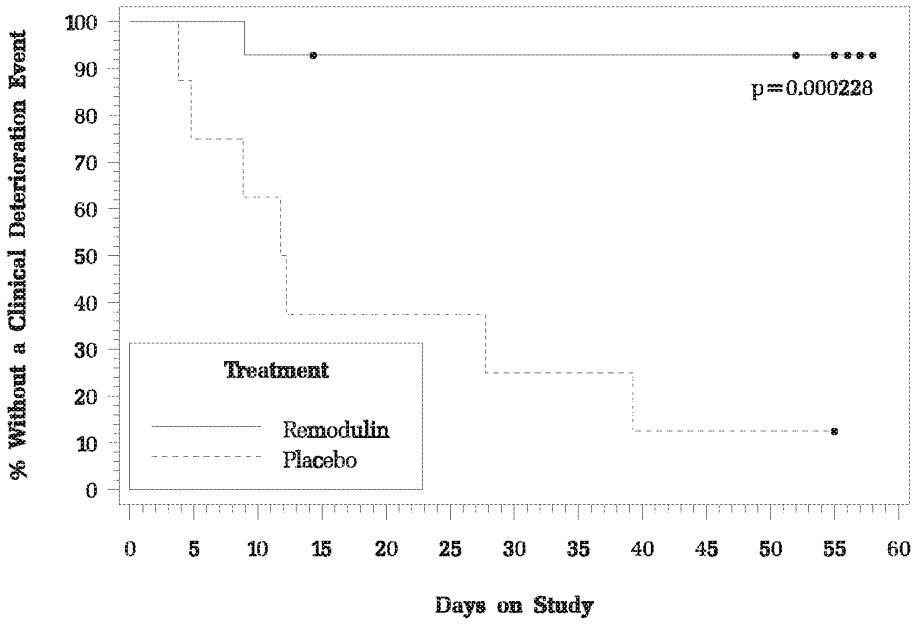
14.2 Flolan-To-Remodulin Transition Study

In an 8-week, multicenter, randomized, double-blind, placebo-controlled study, patients on stable doses of Flolan were randomly withdrawn from Flolan to placebo or Remodulin. Fourteen

Remodulin and 8 placebo patients completed the study. The primary endpoint of the study was the time to clinical deterioration, defined as either an increase in Flolan dose, hospitalization due to PAH, or death. No patients died during the study.

During the study period, Remodulin effectively prevented clinical deterioration in patients transitioning from Flolan therapy compared to placebo (Figure 1). Thirteen of 14 patients in the Remodulin arm were able to transition from Flolan successfully, compared to only 1 of 8 patients in the placebo arm (p=0.0002).

Figure 1: Time to Clinical Deterioration for PAH Patients Transitioned from Flolan to Remodulin or Placebo in an 8-Week Study



16 HOW SUPPLIED / STORAGE AND HANDLING

Remodulin is supplied in 20-mL multidose vials as sterile solutions in water for injection, individually packaged in cartons. Unopened vials of Remodulin are stable until the date indicated when stored at 25°C (77°F), with excursions permitted to 15-30°C (59-86°F) [see USP Controlled Room Temperature]. A single vial of Remodulin should be used for no more than 30 days after the initial introduction into the vial.

Remodulin Injection is supplied as:

Remodulin	Concentration	NDC 66302-xxx-xx
20 mg / 20 mL	1 mg/ mL	101-01
50 mg / 20 mL	2.5 mg/ mL	102-01
100 mg / 20 mL	5 mg/ mL	105-01
200 mg / 20 mL	10 mg/ mL	110-01

Sterile Diluent for Remodulin is supplied separately as:
50 mL vial, carton of 1 (NDC 66302-150-50).

17 PATIENT COUNSELING INFORMATION

Patients receiving Remodulin should be given the following information: Remodulin is infused continuously through a subcutaneous or surgically placed indwelling central venous catheter, via an infusion pump. Patients receiving intravenous infusion should use an infusion set with an in-line filter. Therapy with Remodulin will be needed for prolonged periods, possibly years, and the patient's ability to accept and care for a catheter and to use an infusion pump should be carefully considered. In order to reduce the risk of infection, aseptic technique must be used in the preparation and administration of Remodulin. Additionally, patients should be aware that subsequent disease management may require the initiation of an alternative intravenous prostacyclin therapy, Flolan (epoprostenol sodium).

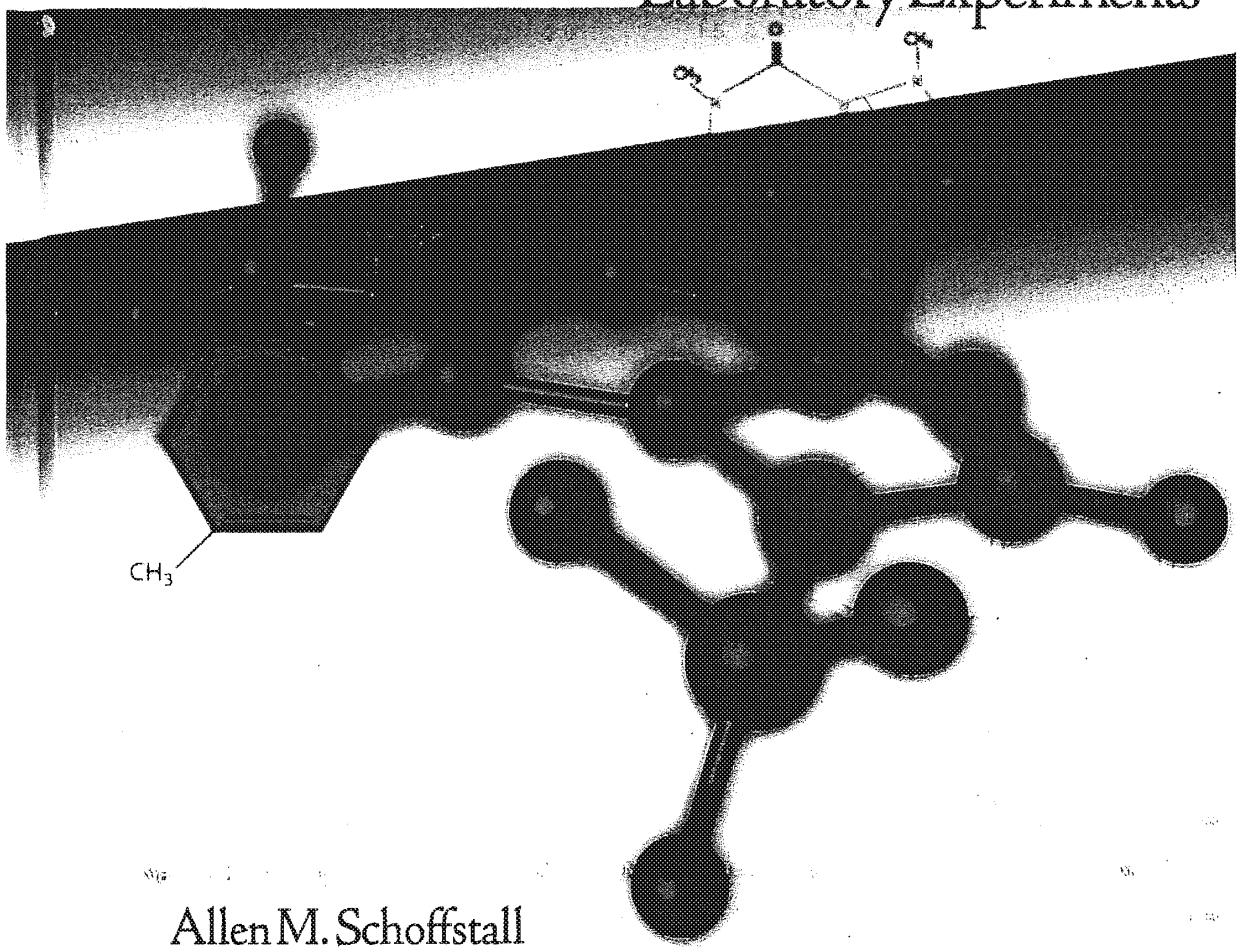
REMODULIN manufactured for:

United Therapeutics Corp.
Research Triangle Park, NC 27709

Microscale and Miniscale

ORGANIC CHEMISTRY

Laboratory Experiments



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Melvin L. Druelinger

Second Edition

Results and Conclusions for Part B

1. Calculate the percent recovery for the recrystallization process. Explain why it is not 100%.
2. Explain and evaluate the effectiveness of the recrystallization solvent in terms of percent recovery and purity of the recrystallized solid.
3. Suggest other solvents or solvent pairs that might have been used for this recrystallization.

Cleanup & Disposal

Place the solvents used for recrystallization in a container labeled "nonhalogenated organic solvent waste." Aqueous solutions can be washed down the drain with water.

Critical Thinking Questions *(The harder one is marked with a ❖.)*

1. List the main criteria for selecting a recrystallization solvent.
2. When is it necessary to use a solvent-pair recrystallization?
3. Why should the recrystallization solvent have a fairly low boiling point?
4. ❖ Will the following pairs of solvents be suitable for doing a solvent-pair recrystallization? Explain.
   a. ethanol (bp 78.5°C) and water
   b. methylene chloride (bp 40°C) and water
   c. dimethylformamide (bp 153°C) and diethyl ether (bp 37°C)
5. If a solute is soluble in cold solvent, is it necessary to test the solubility of the solute in the same solvent when hot? Explain.
6. Arrange the following solvents in order of increasing polarity: ethanol, ethyl acetate, petroleum ether, toluene, and acetone.
7. Methylene chloride (CH_2Cl_2) is polar, whereas carbon tetrachloride (CCl_4) is nonpolar. Explain.
8. Carbon disulfide (CS_2) is sometimes used as a recrystallization solvent. Will this solvent dissolve polar or nonpolar compounds? Explain.

Experiment 3.5: Separations Based upon Acidity and Basicity

Extraction is a technique in which a solute is transferred from one solvent to another. In this experiment, you will investigate acid-base extraction. You will:

- determine the solubilities of an organic acid, an organic base, and a neutral organic compound.
- design a flow scheme to separate an organic acid, an organic base, and a neutral compound.
- use microscale extraction techniques to separate and isolate each component of a mixture of naphthalene, benzoic acid, and ethyl 4-aminobenzoate.
- use miniscale extraction techniques to separate and isolate a mixture of benzoic acid and ethyl 4-aminobenzoate.

Techniques

Technique C	Melting point
Technique F	Vacuum filtration
Technique I	Drying and extraction

Background

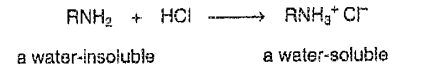
A water-insoluble, acidic organic compound such as a carboxylic acid or phenol can be easily separated from neutral and basic organic compounds by conversion to a water-soluble salt.

a water-insoluble carboxylic acid — a water-soluble carboxylate salt

Neutral and basic organic compounds remain in the organic layer. The two layers can then be separated. Addition of HCl to the aqueous layer regenerates the water-insoluble carboxylic acid, which can then be filtered or extracted into an organic solvent:

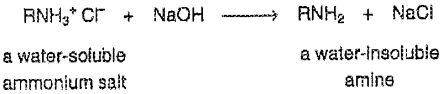
a water-soluble carboxylate salt — a water-insoluble carboxylic acid

A similar scheme can be used to separate a basic compound, such as a water-insoluble amine, from neutral or acidic organic compounds by conversion of the amine to a water-soluble salt:



a water-insoluble amine — a water-soluble ammonium salt

Neutral compounds and acidic organic compounds remain in the organic solvent, where they can be removed. Addition of sodium hydroxide to the aqueous layer regenerates the amine, which is now insoluble in the aqueous solution. The amine can be filtered or extracted into an organic solvent.



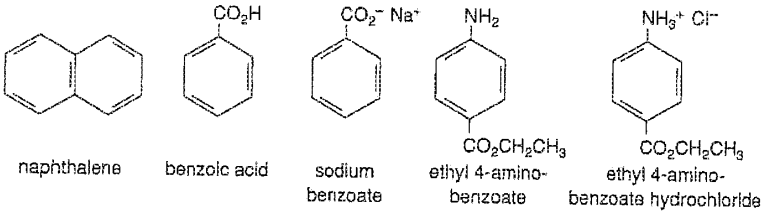
a water-soluble ammonium salt — a water-insoluble amine

The neutral compound remains in the organic solvent, where it can be recovered by drying the solution to remove traces of water, filtering off the drying agent, and evaporating the solvent.

In this exercise, the solubilities of an organic acid (benzoic acid), an organic base (ethyl 4-aminobenzoate), a neutral compound (naphthalene), and the organic salts (ethyl 4-aminobenzoate hydrochloride and sodium benzoate) will be tested in methylene chloride and water.

From the solubilities, you will construct a flow scheme outlining the separation of naphthalene, benzoic acid, and ethyl 4-aminobenzoate. In Part B, you will use the flow

scheme to separate a mixture of naphthalene, benzoic acid, and ethyl 4-aminobenzoate in microscale. In Part C, you will use the flow scheme to separate a mixture of benzoic acid and ethyl 4-aminobenzoate in miniscale.



naphthalene | benzoic acid | sodium benzoate | ethyl 4-amino-benzoate | ethyl 4-amino-benzoate hydrochloride

The instructor may substitute other compounds for those shown here.

Prelab Assignment

1. Read Technique I on the theory and technique of extraction and do all assigned problems.
2. Construct a solubility table similar to Table 3.5-1 in the experimental section.
3. Identify the conjugate acid/conjugate base pairs for the structures above.
4. Write the reaction (if any) and give the products for the reaction of each pair of reagents below. If no reaction occurs, write NR. Indicate whether the product will be water-soluble or water-insoluble.
   a. benzoic acid with NaOH.
   b. sodium benzoate with HCl.
   c. ethyl 4-aminobenzoate with HCl.
   d. ethyl 4-aminobenzoate hydrochloride with NaOH.
   e. naphthalene and NaOH.
   f. ethyl 4-aminobenzoate with NaOH.
5. Determine whether each of the five compounds is predominantly ionically or covalently bonded. Based upon this answer, indicate whether the compound would be expected to be more soluble in water or more soluble in methylene chloride.

Experimental Procedure

Safety First!

Always wear eye protection in the laboratory.

1. Wear eye protection at all times in the laboratory.
2. Wear gloves when handling reagents in this experiment.
3. Methylene chloride is a toxic irritant and a suspected carcinogen. Do not breathe the vapors. Work under the hood or in a well-ventilated area.
4. NaOH and HCl are corrosive and toxic and can cause burns.

Part A: Determination of Solubilities

Obtain 20 small, dry test tubes or a spot plate. Place approximately 10–20 mg of benzoic acid into four of the test tubes or wells; place 10–20-mg of sodium benzoate into four other test tubes or wells. Repeat, using 10–20-mg samples of the other solutes. It is

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SECOND EDITION

This book is printed on acid-free paper.

1 2 3 4 5 6 7 8 9 0 VNH/VNH 0 9 8 7 6 5 4 3

ISBN 0–07–242456–7

Publisher: *Kent A. Peterson*
Sponsoring editor: *Thomas D. Timp*
Senior developmental editor: *Shirley R. Oberbroeckling*
Senior marketing manager: *Tamara L. Good-Hodge*
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Cover/interior designer: *Rokusek Design*
Cover image: *Rokusek Design*
Senior photo research coordinator: *Lori Hancock*
Compositor: *Precision Graphics*
Typeface: *10/12 Times Roman*
Printer: *Von Hoffmann Corporation*

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Library of Congress Cataloging-in-Publication Data

Schoffstall, Allen M.
Microscale and miniscale organic chemistry laboratory experiments / Allen M. Schoffstall, Barbara A. Gaddis, Melvin L. Druelinger.—2nd ed.
p. cm.
Includes bibliographical references and index.
ISBN 0–07–242456–7 (acid-free paper)
1. Chemistry, Organic—Laboratory manuals. I. Gaddis, Barbara A. II. Druelinger, Melvin L. III. Title.

QD261 .S34 2004
547.0078—dc21 2003008663
CIP

www.mhhe.com

ORGANIC CHEMISTRY

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THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

University Science Books
Sausalito, California

University Science Books
55D Gate Five Road
Sausalito, CA 94965
Fax: (415) 332-3393
www.uscibooks.com

Production manager: *Susanna Tadlock*
Manuscript editor: *Ann McGuire*
Designer: *Robert Ishi*
Illustrators: *John and Judy Waller*
Compositor: *Compset Inc.*
Printer & binder: *Edwards Brothers, Inc.*

This book is printed on acid-free paper.

Library of Congress Cataloging-in-Publication Data

Sorrell, Thomas N.
Organic chemistry / Thomas N. Sorrell.
p. cm.
Includes bibliographical references (p. –) and index.
ISBN 0-935702-47-4 (hardcover)
1. Chemistry, Organic. I. Title.
QD251.2.S67 1997
547—dc21

98-47383
CIP

Printed in the United States of America
10 9 8 7 6 5 4 3 2 1

Closely related to economic concerns are environmental issues. Chapter 13 introduced the notion of the **atom economy** of a reaction (page 620). If a chemist makes a racemic mixture of products and has to carry out a resolution (discussed below), a large amount of byproduct may be generated that has to be disposed of or recycled.

THERE ARE TWO COMMON METHODS FOR OBTAINING OPTICALLY ACTIVE COMPOUNDS, RESOLUTION AND ASYMMETRIC SYNTHESIS

Faced with the need to obtain a chiral substance, a chemist has two choices that have general applicability. A third method is physical separation of enantiomeric forms, a method successfully employed by Louis Pasteur to obtain enantiomeric crystals of tartaric acid salts (page 12 and 176). This method is limited to only a handful of cases, however, so we will not consider it further.

The first general method is by **resolution** of enantiomers. As noted in the introduction to this chapter, enantiomers have *identical* chemical and physical properties but diastereomers have *different* properties. Converting a pair of enantiomers to diastereomers often makes it possible to separate them. Subsequently, the diastereomeric derivatives can be converted back to the desired enantiomerically pure substances.

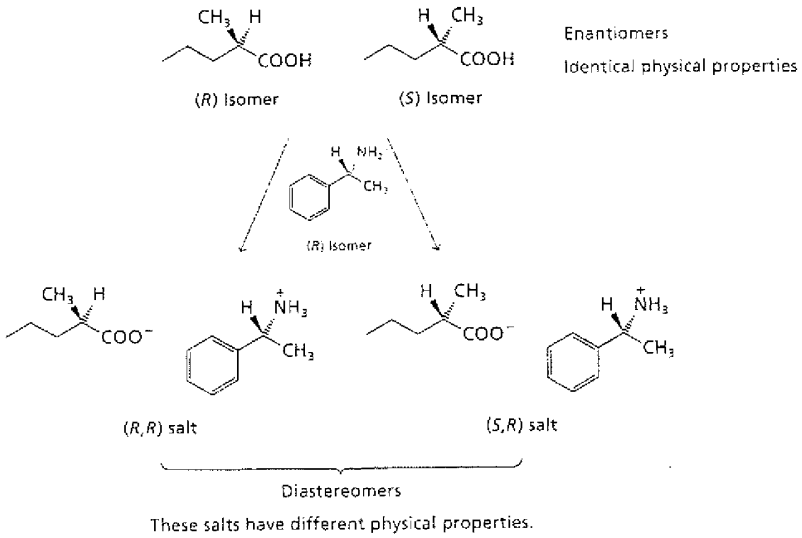
The other route for making an optically active product is called a **kinetically controlled asymmetric transformation**. These processes have attracted the most interest in recent years because they allow chemists to create enantiomerically enriched products from achiral starting materials by employing a chiral catalyst or reagent. We will look at examples of both catalytic and stoichiometric processes.

RESOLUTION OF A RACEMATE MAKES USE OF DIASTEREOMERIC FORMS

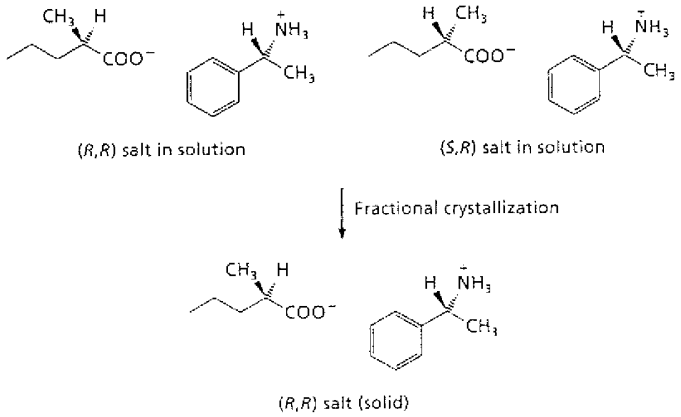
The most general method for obtaining optically pure compounds from a racemic mixture is based on exploiting differences in chemical and physical properties of diastereomeric derivatives of the mirror-image isomers. In the process that constitutes a classical **resolution**, the racemate is first treated with an optically active reagent, producing a pair of diastereoisomers. These are then separated by crystallization, chromatography, or any other means that makes use of the different physical properties of the derivatives. Once the diastereomeric pair is separated, the pure isomer is treated with a second reagent to regenerate the resolving agent and the original substrate, in its enantiomerically pure form.

The procedure will be illustrated for the resolution of a carboxylic acid via formation of a salt, a process frequently employed to resolve amino acids. In this example, the resolving agent is α-phenethylamine (1-amino-1-phenylethane), an amine that has a single asymmetric carbon atom.

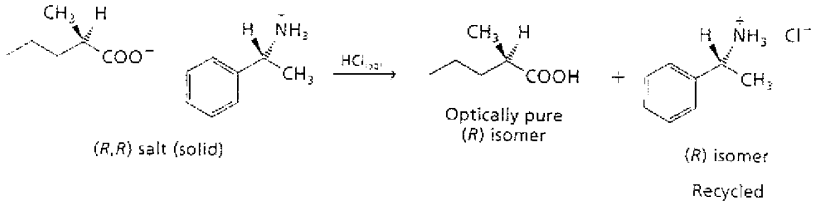
The carboxylic acid and amine undergo an acid–base reaction, producing the ammonium carboxylate salt. Because only one enantiomer of the amine is added to the racemic mixture of carboxylic acid, *the resulting salt, which comprises two asymmetric centers, is a diastereomeric mixture.*



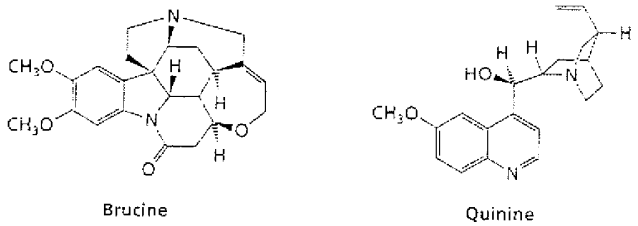
Diastereomeric salts can often be separated by fractional crystallization, taking advantage of their different solubility characteristics.



The pure salt is subsequently treated with hydrochloric acid, which regenerates the optically active carboxylic acid and liberates the chloride salt of the amine. The latter can be separated by extraction and recovered by neutralization.



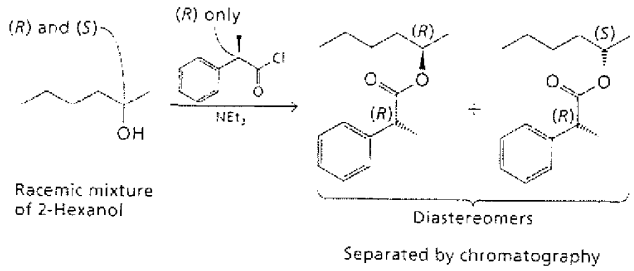
The resolution process illustrated here employs an optically active amine that has a single stereogenic center. In practice, the optically active amine is normally a substance that can be isolated as a pure compound from nature. Many amines used for resolutions are alkaloids such as brucine and quinine, which have multiple stereogenic centers. The salts formed when they are treated with a racemic carboxylic acid are still diastereomers, so they can be separated by fractional crystallization.



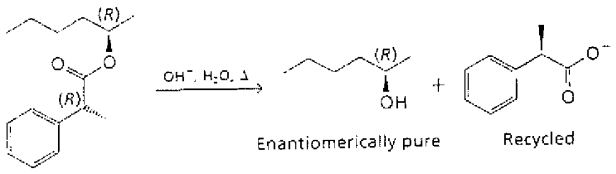
EXERCISE 16.1

How many stereogenic centers are there in brucine and quinine? Recall that some nitrogen atoms can be chiral (review Chapter 5, page 194).

Another way to carry out the resolution of a racemic compound is to make a *covalent* derivative of the racemate. For example, enantiomeric alcohols can be treated with an optically active acid chloride to produce esters, a reaction that will be described in Chapter 19. These esters are diastereomers and can be separated



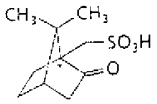
by chromatography, a technique amenable for use with neutral substances. Hydrolysis of the ester after chromatography regenerates the optically active carboxylic acid as its salt and yields each of the optically active alcohols [only recovery of the (*R*)-2-hexanol is illustrated below].



This reaction is discussed in Chapter 19.

EXERCISE 16.2

Amines and sulfonic acids form salts by an acid–base reaction. Show how racemic α-phenethylamine can be resolved using optically active 10-camphorsulfonic acid (shown below).

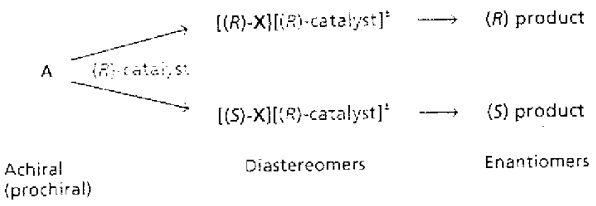


What is the configuration of the carbon atom marked with an asterisk?

A KINETICALLY CONTROLLED ASYMMETRIC TRANSFORMATION RELIES ON DIFFERENCES IN THE TRANSITION-STATE ENERGIES

Apart from a resolution, the only other general method for making an optically active substance is that by which an achiral starting material is transformed into a chiral product. Reactions that accomplish this goal define an **asymmetric synthesis**. Such transformations exploit the use of a chiral species that generates transition states with unequal energies as a result of the spatial interaction of the reactant with the reagent or catalyst.

In the abstract, we can represent an asymmetric reaction by the following scheme in which an achiral substance reacts with a chiral catalyst, generating diastereomeric transition states but enantiomeric products.



Laboratory Technique in Organic Chemistry

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McGRAW-HILL BOOK COMPANY, INC.

1960 New York Toronto London

LABORATORY TECHNIQUE IN ORGANIC CHEMISTRY

 Library of Congress Catalog Card Number 59-11950

IV

70095

THE MAPLE PRESS COMPANY, YORK, PA.

PREFACE

Although there are a number of monographs available which deal with an aspect of the techniques required in dealing with organic compounds, there has for some time been no book which gives a brief description of most of the important techniques. This book is written in an effort to fill this need and is directed mainly to the advanced undergraduate or beginning graduate student who is about to undertake a program of research work.

Each of the three types of matter, liquids, solids and gases, is considered with respect to both its properties and the methods of purification. It is felt that an understanding of the properties of the substances adds materially to the appreciation of the methods of purification. Methods which involve distribution between two phases are then considered. Finally, the reaction itself is examined in relation to the apparatus and techniques involved.

In organic chemical laboratory technique, the use of the proper apparatus is important. A drawing of a commonly used piece of equipment has generally been provided to accompany the description of each method. These drawings are for the most part derived from the working drawings used in the shops at the University of Washington, and in most cases all important dimensions are given in millimeters.

In writing a book of this type, it is very difficult to give credit to

a specific designer for a piece of equipment or to the originator of a technique. The art of laboratory work in organic chemistry has evolved from the experiments and modifications of many technicians, and only rarely can the contribution of an individual be specifically recognized.

Kenneth B. Wiberg

CONTENTS

ing homogeneity, particularly of natural products. If the material is fractionally crystallized, giving perhaps 8 to 10 fractions from the head fraction to the tail fraction (8 to 10 layers), and if these fractions are compared and found to be identical, it is reasonable to assume that the material is homogeneous.

The alembic shown in Fig. 2-21 is particularly useful in fractional crystallization, since it permits convenient adjustment of the amount of solvent and prevents loss of solvent during the prolonged refluxing sometimes required to bring the material into solution.

Precipitation

In some cases, the most convenient method for the purification of a solid consists in precipitating it from a solution in which it is contained as a derivative. A typical example is the purification of a water-insoluble solid carboxylic acid by dissolving it in sodium hydroxide solution, filtering, and precipitating the compound by the addition of acid. A similar procedure may be used with amines: dissolve the compound in acid and precipitate it with a base. These procedures usually work quite well in that they utilize a chemical reaction to aid in separation from nonacidic or nonbasic impurities.

Another method of precipitation involves precipitating the compound as a derivative and then converting the derivative back to the original compound. An example of this is to dissolve an amine in ether, precipitate it as the hydrochloride by passing in hydrogen chloride, and convert the hydrochloride back to the amine with sodium hydroxide solution. Again, this method is useful because it involves separation through the use of a reaction.

One method of precipitation which is usually relatively unsuccessful involves dissolving the compound in one solvent and precipitating by the addition of another solvent in which it is insoluble. This procedure usually leads to coprecipitation and relatively little purification. If two solvents are to be used, the compound should be recrystallized from a mixture of the two solvents as described in the preceding section.

Distillation

If the compound is relatively impure, crystallization usually entails considerable loss of material, and several recrystallizations are required to effect complete purification. The procedure often may be

Organic Process Research & Development 2006, 10, 829–832

Technical Notes

Novel Synthetic Route of a Pivotal Intermediate for the Synthesis of 1β-Methyl Carbapenem Antibiotics

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Abstract:

A novel synthetic method using an original and practical procedure for the preparation of the *N*-PNZ protected 2-aminomethylpyrrolidin-4-ylthio-containing side chain of doripenem hydrate (S-4661), a new parenteral 1β-methylcarbapenem antibiotic, is described. *trans*-4-Hydroxy-L-proline was converted through an efficient process to (2*S*,4*S*)-4-acetylthio-2-(*N*-sulfamoyl-4-nitro-benzyloxycarbonyl-aminomethyl)-1-(4-nitrobenzyloxycarbonyl) pyrrolidine with 60−70% overall yield via a two-step sequence. This procedure requires no chromatographic purifications, no cryogenic temperatures, no haloalkane solvent, and shorter operating times and avoids the side reaction brought by acid hydrolysis. Furthermore, the product was obtained as a crystal rather than an oil, which made it to be an advantage for quantization in the pilot-scale manufacture. Several kilograms of the side chain were prepared by using this method.

1. Introduction

Members of the carbapenem family are important among the β-lactam antibiotics for their broad and potent antibacterial activity and their relatively high resistance to most clinically encountered β-lactamases.[1] So far many products, such as imipenem,[2] panipenem,[3] meropenem,[4] biapenem,[5] and ertapenem,[6] have been put into market. The introduction of a 1β-methyl group to the carbapenem skeleton in meropenem, biapenem, and ertapenem enhances metabolic stability to renal dehydropeptidase-1 (DHP-1) and leads to high antibacterial potency.[7] Doripenem hydrate (S-4661, **1**, Shionogi Research Laboratories, Shionogi & Co., Ltd., Osaka, Japan) is a novel parenteral 1β-methylcarbapenem antibiotic.[8] Compound **1** is superior to meropenem against Gram positive bacteria and, meanwhile, is superior to imipenem against Gram negative bacteria. Furthermore, **1** has an antibacterial potency against *Pseudomonas aeruginosa* which is up to twice as strong as that of imipenem or meropenem. With its potent, broad, and well-balanced antibacterial activity against a wide range of both Gram-positive and Gram-negative bacteria, doripenem is now under phase 3 clinical trials for the treatment of serious infections such as pneumonia, pyelonephritis, and respiratory tract infections.

According to the conventional retrosynthetic analysis of a carbapenem, doripenem can be assembled from 4-nitrobenzyl-protected 1β-methylcarbapenem enolphosphate **2**[7,10] and 2-aminomethyl-pyrrolidin-4-ylthio-containing side chain **3** (Scheme 1). SAR studies revealed that the acylation and sulfamoylamination of the side chain pyrrolidine would benefit the enhancement of the antibacterial activity.

Several papers have been published regarding the synthesis of the side chain aminomethylpyrrolidine derivatives,[8,9,11a,11b] among which two are important. In 1996, Iso and co-workers reported the synthesis of *N*-*p*-methoxybenzyl (PMZ)-protected aminomethylpyrrolidine **3a** (R^2 = PMZ) or *N*-BOC-protected aminomethylpyrrolidine **3b** (R^2 =

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‡ Chengdu Di-Ao Pharmaceuticals Company.
§ Chengdu Institute of Biology, The Chinese Academy of Science.
∥ State Key Laboratory of Biotherapy, Sichuan University.

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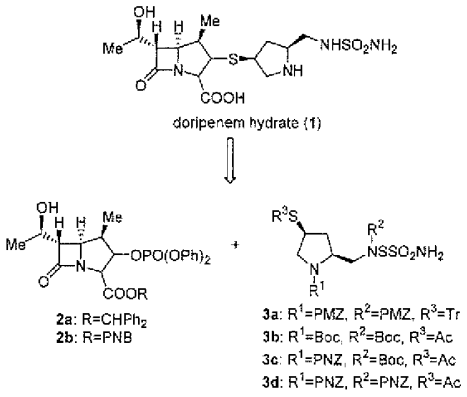
10.1021/op0600714 CCC: $33.50
Published on Web 08/29/2006

Scheme 1. **Retrosynthetic analysis of a carbapenem**



BOC),[8] after coupling with the diphenylmethyl-protected enolphosphate **2a**, compound **1** was prepared by deprotection with $AlCl_3$-anisole.

Although this route facilitated the SAR studies and led to rapid optimization of the lead derivatives, it had several drawbacks for multikilogram-scale preparation of compound **1**. The two most serious problems resided in the isolation and deprotection steps. Compound **1** was isolated as a foam, which needed further purification on a Diaion HP-20. Later, a modified process was developed, and compound **1** was obtained as a crystalline monohydrate.[9] However, the process still required chromatographic purification, and the yield of compound **1** through the deprotection, purification, and crystallization steps on a pilot scale (49%) was lower than that through the deprotection and purification steps on a bench scale (72%). During the column chromatography and concentration of the eluents, decomposition of the target compound **1** was observed, resulting in a 16% yield decrease due to longer operating times on scale-up. Furthermore, this process included several severe conditions such as cryogenic reaction temperatures (three reactions required −45 ℃), long operation times, and the use of haloalkane solvent (CH_2Cl_2). To reduce the cost of processing time and to make the process more environmentally suitable, Nishino and co-workers[11b] reported *N*-PNZ-protected aminomethylpyrrolidine (R^2 = Boc) **3c**, which was prepared from *trans*-4-hydroxy-L-proline. But the deprotection of the *tert*-butylcarbonyl group by 98% H_2SO_4 results in an oily product at room temperature, and the carbonium ion brought many side reactions, while scavenger usage will increase the producing cost.

To increase the yield and to avoid the chromatographic purification, we developed an improved process. Compound **1** was synthesized from PNB-protected enolphophate **2b** and *N*-[*p*-nitrobenzyloxycarbonyl] (PNZ)-protected aminomethylpyrrolidine **3d**. In this contribution, we describe the preparation of the new pyrrolidine derivative **3d** and its coupling with enolphosphate **2b** (Scheme 2). This process avoided the side reaction brought by deprotection of the *tert*-butoxycarbonyl group and made it easier for purification. Furthermore, the product was obtained as a crystal rather than an oil, which made it an advantage for quantization in the pilot-scale manufacture.

(11) (a) A portion of this study was patented. Nishino, Y.; Yuasa, T.; Komurasaki, T.; Kakinuma, M.; Masui, T.; Kobayashi, M. Patent Application No. JP 2001-140782. (b) Nishino, Y.; Komurasaki, T.; Yuasa, T.; Kakinuma, M.; Izumi, K.; Kobayashi, M.; Fujiie, S.; Gotoh, T.; Masui, Y.; Hajima, M.; Takahira, M.; Okuyama, A.; Kataoka, T. *Org. Process Res. Dev.* **2003**, *7*, 649−654.

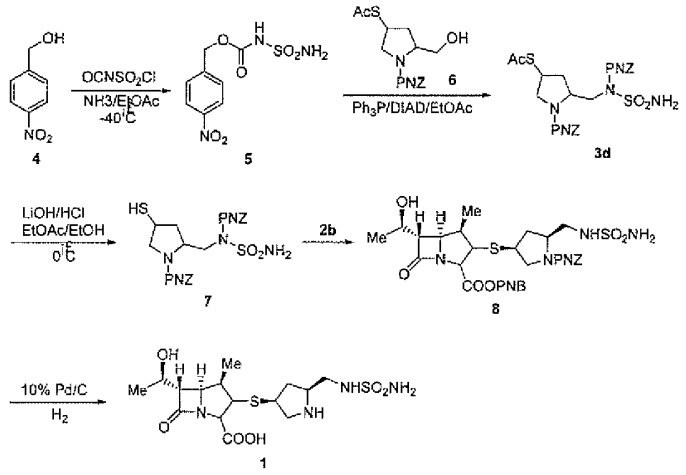
2. Experimental Section

2.1. Materials and Instrumentations. ESI-MS spectral data were measured on a Finnigan LCQ^{DECA} mass spectrometer. ^{1}H NMR and ^{13}C NMR experiments were measured on a Bruker Avance 600 spectrometer. Chemical shifts are reported in ppm (δ scale) using tetramethylsilane as an internal standard. Melting points were determined with a micromelting point apparatus and are uncorrected. All commercially available materials and solvents were used as received without any further purification. 4-Nitrobenzyl (4*R*,5*S*,6*S*)-3-[(diphenylphosphono)oxy]-6-[(1*R*)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3, 2, 0]hept-2-ene-2-carboxylate (**2b**) is commercially available.

2.2. Preparation of the Compounds. 2.2.1. Preparation of the *N*-PNZ-Protected Pyrrolidine Derivative 3d. 2.2.1.a. Preparation of *N*-4-Nitrobenzyloxycarbonyl-sulfonamide (5). To a solution of 4-nitrobenzyl alcohol (38.25 g, 250 mmol) in THF, chlorosulfonyl isocyanate (21.75 mL, 250 mmol) was added dropwise at −40 ℃, and the mixture was stirred at −40 ℃ for 30 min. After cooling the mixture to −60 ℃, gaseous amine was bubbled into the reaction with stirring. After bubbling, the mixture was stirred for 30 min at 15 ℃ and then acidified with 1 N HCl until no more precipitation was generated. The precipitate was collected by filtration and dissolved in EtOAc, washed with water and brine, dried over anhydrous Na_2SO_4, and evaporated in a vacuum. The residue was crystallized from EtOAc−hexane to give 55 g of *N*-4-nitrobenzyl sulfonamide as a colorless crystal (80%). Mp: 160−162 ℃ (dec). FT-IR (KBr, cm^{-1}): 3348, 3258, 3223, 1721, 1610, 1473, 1454, 1348, 1260, 1156, 847. ^{1}H NMR (600 MHz, DMSO-d_6) δ 5.29 (2H, s, −O−CH_2−Ar), 7.54 (br, 2H, $-SO_2NH_2$), 7.64 (A_2B_2, 2H, J = 8.7 Hz, −ArH), 8.26 (A_2B_2, 2H, J = 8.7 Hz, −ArH), 11.38 (br, 1H, −CO−NH−SO_2−). ^{13}C NMR (600 MHz, DMSO-d_6) δ 65.6 (−O−CH_2−Ar), 124.1 (o, −Ar−NO_2), 128.8 (m, −Ar−NO_2), 144.3 (p, −Ar−NO_2), 147.6 (−Ar−NO_2 in situ), 152.0 (C=O, PNZ−). ESI-MS: 298 [M + Na]$^-$, 314 [M + K]$^+$. FTICR/MS: Calculated for [$C_8H_9N_2Na_1O_6S_1$], 298.0110; found, 298.0104.

2.2.1.b. Preparation of Acetylthiol-pyrrolidine Derivative (3d). A solution of diisopropyl azodicarboxylate (DIAD, 22 mL, 130 mmol) in EtOAc was added dropwise to a mixture of (2*S*,4*S*)-4-acetylthio-2-hydroxymethyl-1-(4-nitrobenzyl-oxycarbonyl)pyrrolidine (**6**, 35.5 g, 100 mmol), *N*-4-nitrobenzylsulfamide (**5**, 41.25 g, 150 mmol), triphenyl phospine (34.125 g, 130 mmol), and THF (1000 mL) at 0 ℃. The reaction mixture was stirred at 20 ℃ for 2 h. After the reaction was completed, the reaction mixture was concentrated to 200 mL, and 500 mL of anhydrate alcohol were then added. The solution was stored in a refrigerator over-

Scheme 2. **Improved method for the preparation of 1**



night and resulted in 54 g of amorphous yellowish powder (88%). FT-IR (KBr, cm^{-1}): 3383, 3082, 2960, 1695, 1607, 1522, 1430, 1395, 1296, 1267, 1181, 1154, 1114, 850. ^1H NMR (600 MHz, $CDCl_3$) δ 1.60 (1H, m, pyrrolidine, H-3β), 2.33 (3H, s, AcS—), 2.59 (dt, 1H, J = 14.0, 8.7 Hz, pyrrolidine, H-3α), 3.17 (dd, 1H, J = 11.9, 6.24 Hz, pyrrolidine H-5β), 3.71 (dd, 1H, J = 14.9 Hz, —CH_2N(PNZ)SO_2—), 3.92 (1H, m, pyrrolidine H-4), 4.10 (dd, 1H, J = 15.3,10.2 Hz, —CH_2N(PNZ)SO_2—), 4.20 (dd, 1H, J = 11.7, 7.5 Hz, pyrrolidine H-5α), 4.52 (1H, m, pyrrolidine H-2α), 5.16 (AB_q, 2H, J = 13.4 Hz, —O—CH_2—Ar), 5.25 (br, 2H, —O—CH_2—Ar), 5.86 (br, 2H, —SO_2NH_2), 7.47 (A_2B_2, m, 2H, J = 8.46 Hz, —$ArNO_2$), 7.51 (A_2B_2, m, 2H, J = 8.46 Hz, —$ArNO_2$), 8.21 (A_2B_2, 2H, o, J = 8.52 Hz, —$ArNO_2$), 8.23 (A_2B_2, 2H, o, J = 8.28 Hz, —$ArNO_2$). ^{13}C NMR (600 MHz, $CDCl_3$) δ 30.5 (Me—, AcS—), 34.7 (pyrrolidine C-3), 39.1 (pyrrolidine C-4), 50.6 (—CH_2NSO_2—), 52.2 (pyrrolidine C-5), 56.7 (pyrrolidine C-2), 66.1 (—OCH_2—Ar), 74.0 (—OCH_2—Ar), 123.9 (o, —$ArNO_2$), 124.0 (o, —$ArNO_2$), 128.0 (m, —$ArNO_2$), 128.5 (m, —$ArNO_2$), 141.7 (p, —$ArNO_2$), 143.1 (p, —$ArNO_2$), 147.8 (—$ArNO_2$, in situ), 148.0 (—$ArNO_2$, in situ), 152.7 (C=O, PNZ—), 155.4 (C=O, PNZ—), 194.6-(C=O, AcS—). ESI-MS: 610.2 $[M - H]^-$. FTICR/MS: Calculated for $[C_{23}H_{25}N_5O_{11}S_2Na]$, 634.0890; found, 634.0884.

2.2.2. Preparation of Doripenem (S-4661). 2.2.2.a. Preparation of Thiol-pyrrolidine Derivative (2*S*,4*S*)-1-*tert*-Nitrobenzyl-oxycarbonyl-2—4*S*)-1-*tert*-nitrobenzyloxycarbonyl-2-(*N*-4-nitrobenzyloxycarbonylbenzyl-*N*-sulfamoulaminomethyl)-4-mercaptopyrrolidine (7). To a solution of 50 g (81.8 mmol) of (2*S*,4*S*)-1-*tert*-nitrobenzyl-2-(*N*-*tert*-nitrobenzylcarbonyl-*N*-aminosulfamide)methyl-4-acetylthiopyrrolidine (**3d**) in 200 mL of THF, 6 g of lithium hydroxide in 20 mL of water were added with an ice bath. After stirring for 2 h, the mixture was acidified with 6 N HCl and gave a sticky solid. The solid was collected by filtration, was dissolved with EtOAc and alcohol, and then stored in a refrigerator overnight. The product was precipitated as a yellowish amorphous powder (32 g, 68.8%). FT-IR (KBr, cm^{-1}): 3381, 2959, 1717, 1607, 1521, 1432, 1393, 1347, 1268, 1181, 1154, 1112, 850. ^1H NMR (600 MHz, $CDCl_3$) δ 1.52 (m, 1H, H-3β of pyrrolidine), 1.83 (d, 1H, J = 6.12 Hz, H of HS—), 2.62 (m, 1H, H-3α of pyrrolidine), 3.13 (dd, 1H, J = 11.64 and 7.68 Hz, H-5β of pyrrolidine), 3.39 (m, 1H, H-4 of pyrrolidine), 3.75 (d, 1H, J = 15.3 Hz, one of —CH_2N(PNZ)SO_2—), 4.10 (dd, 1H, J = 11.64 and 7.26 Hz, H-5α of pyrrolidine), 4.27 (dd, 1H, J = 15.3 and 10.32 Hz, one of —CH_2N(PNZ)SO_2—), 4.48 (m, 1H, H-2α of pyrrolidine), 5.15 (AB_q, 2H, J = 13.74 Hz, —OCH_2—Ar), 5.26 (AB_q, 2H, J = 13.74 Hz, —OCH_2—Ar), 5.84 (br, 2H, —SO_2NH_2), 7.45 (A_2B_2, 2H, J = 8.22 Hz, meta-H of nitrophenyl), 7.51 (A_2B_2, 2H, J = 8.40 Hz, *meta*-H of nitrophenyl), 8.21 (m, 4H, *ortho*-H of nitrophenyl). ^{13}C NMR (600 MHz, $CDCl_3$) δ: 34.5 (pyrrolidine C-3), 39.4 (pyrrolidine C-4), 50.9 (—CH_2N(PNZ)SO_2—), 55.3 (pyrrolidine C-5), 57.2 (pyrrolidine C-2), 66.1 (—OCH_2—Ar), 67.3 (—OCH_2—Ar), 123.9 and 124.0 (nitrophenyl *ortho*-C), 128.0 and 128.5 (nitrophenyl *meta*-C), 141.9 and 143.2 (nitrophenyl *para*-C), 147.8 and 148.0 (nitrophenyl ipso-C), 152.8 and 155.3 (C=O of PNZ—). ESI-MS: 592.1 $[M + Na]^+$, 608.1 $[M + K]^+$. FTICR/MS: Calculated for $[C_{21}H_{23}N_5Na_1O_{10}S_2]$, 592.0784; found, 592.0779.

2.2.2.b. Preparations of 8. To a DMF solution (250 mL) of (1*R*,5*S*,6*S*)-2-diphenoxy-phosphonyloxy-6-[(1*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylic acid-4-nitrobenzyl ester (**7**, 17.82 g, 30 mmol) and the corresponding mercaptopyrrolidine (**2b**, 22 g, 38.66 mmol), diisopropylethylamine (7.23 mL, 42.5 mmol) was added with an ice bath. After stirring for 2 h, the mixture was diluted with 500 mL of EtOAc and washed with 1 N HCl, saturated Na_2CO_3, and saturated brine, dried over anhydrous Na_2SO_4, and evaporated in a vacuum. Toluene was added to deposit the product. After filtration, the product was obtained as a yellowish amorphous powder (27 g, 98.5%). FT-IR (KBr cm^{-1}): 3405, 2969, 1771, 1716, 1607, 1522,1433, 1392, 1347, 1276, 1181, 1141, 1111, 850.^1H NMR (600 MHz, $CDCl_3$) δ 1.26 (d, 3H, J = 7.14 Hz, CH_3— on 4-position), 1.37 (d, 3H, J = 6.18 Hz, CH_3CHOH—), 1.64 (m, 1H, H-3β of pyrrolidine), 2.62 (m, 1H, H-3α of pyrrolidine), 3.27 (m,

1H, H-4α), 3.27 (m, 1H, H-6), 3.27 (m, 1H, H-5β of pyrrolidine), 3.73 (m, 1H, one of $-CH_2N(PNZ)SO_2-$), 3.73 (m, 1H, H-4 of pyrrolidine), 4.10 (m, 1H, H-5α of pyrrolidine), 4.25 (m, 1H, H-5), 4.25 (m, 1H, $-CH(OH)\ CH_3$), 4.25 (m, 1H, one of $-CH_2N(PNZ)SO_2-$), 4.54 (m, 1H, H-2α of pyrrolidine), 5.12−5.48 (m, 6H, $-OCH_2-Ar$), 5.85 (br, 2H, $-SO_2NH_2$), 7.47 (m, 4H, *meta*-H of nitrophenyl), 7.63 (A_2B_2, 2H, $J = 8.70$ Hz, *meta*-H of nitrophenyl), 8.16 (A_2B_2, 2H, $J = 8.28$ Hz, *ortho*-H of nitrophenyl), 8.20 (m, 4H, *ortho*-H of nitrophenyl).^{13}C NMR (600 MHz, $CDCl_3$) δ 16.9 (Me— of 4-position), 22.0 (Me— of CH_3CHOH-), 34.7 (pyrrolidine C-3), 40.5 (pyrrolidine C-4), 44.0 (C-4), 50.7 (C of $-CH_2N(PNZ)SO_2-$), 54.0 (pyrrolidine C-5), 56.2 (C-5), 56.8 (pyrrolidine C-2), 59.8 (C-6), 65.5 (—CH— of CH_3CHOH-), 66.2 and 67.4 and 68.4 ($-OCH_2-Ar$), 123.8 and 123.9 and 124.0 (*ortho*-C of nitrophenyl), 125.8 (C-2), 128.3 and 128.4 (*meta*-C of nitrophenyl), 141.8 and 142.6 and 143.0 (*para*-C of nitrophenyl), 147.7 and 147.8 and 148.0 (ipso-C of nitrophenyl), 148.2 (C-3), 152.7 and 155.0 (C=O of PNZ—), 160.0 (C=O of PNB—), 172.4(C=O of C-7). FTICR/MS: Calculated for $[C_{38}H_{39}N_7Na_1O_{16}S_2]$, 936.1792; found, 936.1787.

2.2.2.c. Deprotection and Preparation of Doripenem (S-4661, 1). To a solution (180 mL) of (1*R*,5*S*,6*S*)-2-[(3*S*,5*S*)-1-*p*-nitrobenzyloxycarbonyl-5-(*N*-*p*-nitrobenzyloxycarbonyl-*N*-aminosulfonyl-amide)methylpyrrolidine]-sulfur-6-[(1*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylic acid-4-nitrobenzyl ester (**8**, 10 g, 10.95 mmol), 120 mL of water and 10 g of 10% Pd/C (contents 54% H_2O) were added, and the reaction mixture stirred under 0.5 mpa H_2 pressure for 4 h and then was filtered to remove the catalyst. Then 4 g of $MgCl_2 \cdot H_2O$ were added followed by partitioning with 300 mL of THF. 2-Propanol was added to the separated aqueous layer, which was then stored in a refrigerator overnight. The product S-4661 was precipitated and collected by filtration and dried in a vacuum as a white powder (2.269 g, 49%). FT-IR(KBr cm^{-1}): 3532, 3391, 3261, 3080, 2949, 2922, 2853, 1713, 1630, 1567, 1455, 1378, 1350, 1321, 1278, 1264, 1162, 1092, 1071, 930, 764. ^{1}H NMR (600 MHz, D_2O) δ 1.11 (d, $J = 7.26$ Hz, 3H), 1.18 (d, $J = 6.48$ Hz, 3H), 1.62−1.67 (m, 1H), 2.60−2.65 (m, 1H), 3.25−3.35 (m, 3H), 3.36 (dd, $J = 2.58$, 6 Hz, 1H), 3.43 (dd, $J = 4.77$, 10.11 Hz, 1H), 3.60 (dd, $J = 6.96$, 12.48 Hz, 1H), 3.8−3.84 (m, 1H), 3.92−3.96 (m, 1H), 4.12−4.16 (m, 2H). ^{13}C NMR (600 MHz, D_2O) δ 15.76, 20.05, 32.67, 39.22, 42.42, 43.06, 52.15, 55.88, 58.08, 59.57, 65.03, 133.69, 138.11, 167.63, 176.60. ESI-MS: 421.1 $[M + H]^+$. FTICR/MS: Calculated for $[C_{15}H_{24}N_4Na_1O_6S_2]$, 443.4940; found, 443.1029. The data are coincident with literature.

3. Conclusions

We developed and described a practical multikilogram scale synthesis of doripenem hydrate (**1**) by deprotection of compound **8**, which was prepared from enolphosphate **2b** and *N*-PNZ protected aminomethylpyrrolidine **3d**. We found effective extraction conditions to remove *p*-toluidine and most other organic impurities using THF/water and $MgCl_2$. The reported process requires no chromatographic purification and affords compound **1** as a sterile crystalline monohydrate in satisfactory yield. This process is practical and efficient. In fact, this process is now under pilot-scaled study to make compound **1** for clinical studies.

Acknowledgment

We acknowledge the useful help from Di-Ao Pharmaceutical Company for its financial support. This work was also financially supported by the National Natural Science Foundation of China (Nos.: 20372051, 20471038), Program for New Century Excellent Talents in University, Specialized Research Fund for the Doctoral Program of Higher Education, and Scientific Fund of Sichuan Province for Outstanding Young Scientist.

Received for review March 31, 2006.

OP0600714

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Inventor Name:	Hitesh BATRA
Title:	AN IMPROVED PROCESS TO PREPARE TREPROSTINIL, THE ACTIVE INGREDIENT IN REMODULIN®
Application No.:	14/849,981
Filing Date:	9/10/2015
Examiner:	Yevgeny Valenrod
Art Unit:	1672
Confirmation No.:	6653

INFORMATION DISCLOSURE STATEMENT UNDER 37 CFR §1.56

Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

Applicant submits herewith documents for the Examiner's consideration in accordance with 37 CFR §§1.56, 1.97 and 1.98.

Applicant respectfully requests that each listed document be considered by the Examiner and be made of record in the present application and that an initialed copy of Form PTO/SB/08 be returned in accordance with MPEP §609.

The submission of any document herewith is not an admission that such document constitutes prior art against the claims of the present application or that such document is considered material to patentability as defined in 37 CFR §1.56(b). Applicants do not waive any rights to take any action which would be appropriate to antedate or otherwise remove as a

competent reference any document submitted herewith. However, in accordance with MPEP § 609.04(a)(I), Applicant hereby states that for items for which the date of publication supplied does not include the month of publication, the year of publication is sufficiently earlier than the effective U.S. filing date and any foreign priority date so that the particular month of publication is not in issue.

CONCISE EXPLANATION OF RELEVANCE

Invalidity contentions filed against parent U.S. Patent 8,497,393 ("the '393 parent patent") and prior art mentioned therein are being filed in this submission. With respect to certain invalidity contentions that contain "confidential" designations, those documents were previously designated confidential at one time in the litigation, but they are no longer subject to confidentiality, except where certain information has been redacted.

Recent Patent Owner documents are also being cited herein from the related proceeding IPR2016-00006, *Steadymed Ltd. (Petitioner), v. United Therapeutics Corporation (Patent Owner)*, Case IPR2016-00006, US Patent 8,497,393, which involves the same '393 parent patent of the above-captioned patent application. Although these documents were previously submitted, the versions filed with this Statement are new versions of certain documents filed recently in the IPR that have some information unredacted that was previously redacted in prior versions.

TIMING OF THE DISCLOSURE

The listed documents are being submitted in compliance with 37 CFR §1.97(b), before the mailing of a first Office action after the filing of a RCE.

STATEMENT UNDER 37 CFR §1.97(e)

The undersigned hereby states in accordance with 37 CFR §1.97(e)(2) that no item of information contained in this information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application and, to the knowledge of the undersigned, after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR §1.56(c) more than three months prior to the filing of the information disclosure statement.

FEE

Fees in the amount of $180.00 to cover the fee associated with an information disclosure statement are being paid by credit card via EFS-Web.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this submission under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account Number 19-0741.

Respectfully submitted,

Date DEC 29 2016

By _____

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Substitute for form 1449/PTO				Complete if Known	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT				**Application Number**	14/849,981
Date Submitted: ~~DEC 29 2016~~				**Filing Date**	9/10/2015
(use as many sheets as necessary)				**First Named Inventor**	Hitesh BATRA
Sheet	1	of	3	Art Unit	1672
				Examiner Name	Yevgeny Valenrod
				Attorney Docket Number	080618-1581

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number / Number-Kind Code[2] (*if known*)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] (*if known*)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Documents	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.) date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[6]
	D1	Redacted Petitioner's Reply to Patent Owner's Response to Petition filed on September 27, 2016 in *Steadymed Ltd. (Petitioner), v. United Therapeutics Corporation (Patent Owner)*, Case IPR2016-00006, US Patent 8,497,393, with Exhibits 1022-1028.	
	D2	Petitioner's Demonstratives filed November 28, 2016, in *Steadymed Ltd. (Petitioner), v. United Therapeutics Corporation (Patent Owner)*, Case IPR2016-00006, US Patent 8,497,393	
	D3	Patent Owner Response to Petition filed November 23, 2016, in *Steadymed Ltd. (Petitioner), v. United Therapeutics Corporation (Patent Owner)*, Case IPR2016-00006, US Patent 8,497,393, with Redacted Exhibits 2006, 2020, 2022, 2058 and 2059 filed November 23, 2016, 1151 pages.	
	D4	Patent Owner Demonstratives filed November 23, 2016, in *Steadymed Ltd. (Petitioner), v. United Therapeutics Corporation (Patent Owner)*, Case IPR2016-00006, US Patent 8,497,393, 62 pages.	
	D5	Decision Redacted Institute of *Inter Partes* Review dated November 23, 2016, in *Steadymed Ltd. (Petitioner), v. United Therapeutics Corporation (Patent Owner)*, Case IPR2016-00006, US Patent 8,497,393, 53 pages.	
	D6	Service copy of Third Party Submission dated October 16, 2016, filed but not entered in US 14/849,981 on October 16, 2016, with 6 indicated attachments, 822 pages.	
	D7	Redacted Defendant Sandoz Inc.'s Invalidity Contentions dated February 5, 2015, *United Therapeutics Corporation (Plaintiff) v. Sandoz Inc. (Defendant),* In The United States District Court for the District of New Jersey, Civil Action No. 3:14-cv-5499(PGH)(LHG), 90 pages.	
	D8	Defendant Sandoz Inc.'s Invalidity Contention Chartss dated February 5, 2015, *United Therapeutics Corporation (Plaintiff) v. Sandoz Inc. (Defendant),* In The United States District Court for the District of New Jersey, Civil Action No. 3:14-cv-5499(PGH)(LHG), 189 pages.	

Examiner Signature		Date Considered	

Substitute for form 1449/PTO				Complete if Known	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT				**Application Number**	14/849,981
Date Submitted: DEC 29 2016				**Filing Date**	9/10/2015
				First Named Inventor	Hitesh BATRA
(use as many sheets as necessary)				Art Unit	1672
				Examiner Name	Yevgeny Valenrod
Sheet	2	of	3	Attorney Docket Number	080618-1581

NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.) date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[6]
	D9	Defendant Actavis Laboratories FL, Inc. Preliminary Invalidity Contentions, dated August 30, 2016, *United Therapeutics Corporation, and Supernus Pharmaceuticals, Inc., (Plaintiff) v. Actavis Laboratories FL, Inc., (Defendant)*, In The United States District Court for the Distritc of New Jersey, Civil Action No. 3:16-cv-01816-PGS-LHG, Civil Action No. 3:16-cv-03642-PGS-LHG, 330 pages, (see particularly pages 18-20, 42-62 and 269-280).	
	D10	Exhibit G, Invalidity Claim Chart for the '393 patent, January 12, 2015, 66 pages.	
	D11	Defendant Teva Pharmaceuticals USA, Inc.'s Amended Non-Infringement and Invalidity Contentions, dated April 24, 2015, *United Therapeutics Corporation (Plaintiff) v. Teva Pharmaceuticals USA, Inc. (Defendant)*, In The United States District Court for the District of New Jersey, Civil Action No. 3:14-cv-05498(PGS)(LHG), 94 pages, (see particularly pages 22-54).	
	D12	Arumugan et al., "A New Purification Process for Pharmaceutical and Chemical Industries," Organic Process Research & Development, 2005, 9:319-320.	
	D13	Burk et al., "An Enantioselective Synthesis of (S)-(+)-3-Aminomethyl-5-methylhexanoic Acid via Asymmetric Hydrogenation," J. Org. Chem., 2003, 68:5731-5734.	
	D14	Eliel et al., Stereochemistry of Organic Compounds, 1994, 322-325.	
	D15	Harwood et al., Experimental organic chemistry: Principles and Practice, 1989, 127-134.	
	D16	Jones, Maitland Jr., Organic Chemistry, 2nd Ed., 2000, 153-155.	
	D17	Lin et al., "Benzindene Prostaglandins. Synthesis of Optically Pure 15-Deoxy-U-68,215 and Its Enantiomer via a Modified Intramolecular Wadsworth-Emmons-Wittig Reaction," J. Org. Chem., 1987, 52:5594-5601.	
	D18	McManus et al., "Tetrazole Analogs of Plant Auxins," J. Org. Chem., 1959, 24:1464-1467.	
	D19	Monson, Richard S., Advanced Organic Synthesis, Methods and Techniques, 1971, 178-188.	
	D20	Ohno et al., "Development of Dual-Acting Benzofurans for Thromboxane A2 Receptor Antagonist and Prostacyclin Receptor Agonist: Synthesis, Structure-Activity Relationship, and Evaluation of Benzofuran Derivatives," J. Med. Chem., 2005, 48:5279-5294.	
	D21	Olmsted III et al., Chemistry, The Molecular Science, Mosby-Year Book, Inc., Chapter 10 "Effects of Intermolecular Forces," 1994, 428-486.	
	D22	Pavia et al., Introduction to Organic Laboratory Techniques, First Edition, 1998, 648.	
	D23	Physicians' Desk Reference, 59 Edition, 2005, for Bicillin® L-A (penicillin G benzathine suspension), 5 pages.	
	D24	Priscinzano et al., "Piperidine Analogues of 1-[2-[Bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine (GBR 12909): High Affinity Ligands for the Dopamine Transporter," J. Med. Chem., 2002, 45:4371-4374.	

Examiner Signature		Date Considered	

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** Date Submitted: DEC 29 2016 *(use as many sheets as necessary)* Sheet 3 of 3	*Complete if Known*	
	Application Number	14/849,981
	Filing Date	9/10/2015
	First Named Inventor	Hitesh BATRA
	Art Unit	1672
	Examiner Name	Yevgeny Valenrod
	Attorney Docket Number	080618-1581

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.) date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[6]
	D25	REMODULIN® label, 2014, 17 pages.	
	D26	Schoffstall, et al., Microscale and Miniscale Organic Chemistry Laboratory Experiments, 2004, 2nd Ed., 200-202.	
	D27	Sorrell, Thomas N., Organic Chemistry, 1999, 755-758.	
	D28	Wiberg, Laboratory Technique in Organic Chemistry, 1960, 112.	
	D29	Yu et al., "Novel Synthetic Route of a Pivotal Intermediate for the Synthesis of 1β-Methyl Carbapenem Antibiotics," Organic Process Research & Development, 2006,10:829-832.	

Examiner Signature		Date Considered	

Electronic Patent Application Fee Transmittal	
Application Number:	14849981
Filing Date:	10-Sep-2015
Title of Invention:	PROCESS TO PREPARE TREPROSTINIL, THE ACTIVE INGREDIENT IN REMODULIN®
First Named Inventor/Applicant Name:	Hitesh BATRA
Filer:	Stephen Bradford Maebius/Karen Strawderman
Attorney Docket Number:	080618-1581
Filed as Large Entity	
Filing Fees for Utility under 35 USC 111(a)	

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
RCE- 1st Request	1801	1	1200	1200
STATUTORY OR TERMINAL DISCLAIMER	1814	1	160	160
		Total in USD ($)		**1360**

Electronic Acknowledgement Receipt	
EFS ID:	27925600
Application Number:	14849981
International Application Number:	
Confirmation Number:	6653
Title of Invention:	PROCESS TO PREPARE TREPROSTINIL, THE ACTIVE INGREDIENT IN REMODULIN®
First Named Inventor/Applicant Name:	Hitesh BATRA
Customer Number:	22428
Filer:	Stephen Bradford Maebius/Karen Strawderman
Filer Authorized By:	Stephen Bradford Maebius
Attorney Docket Number:	080618-1581
Receipt Date:	29-DEC-2016
Filing Date:	10-SEP-2015
Time Stamp:	12:04:25
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$1360
RAM confirmation Number	122916INTEFSW12062000
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for Continued Examination (RCE)	RCE.pdf	105762 671af64f4ef01d46fba1738a9d1c8dc37ce51d97	no	4
Warnings:					
This is not a USPTO supplied RCE SB30 form.					
Information:					
2		Amendment.pdf	132577 7f16764273168 58d65dec86e8382a072a43ecad8	yes	5

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Amendment Submitted/Entered with Filing of CPA/RCE	1	1
	Claims	2	3
	Applicant Arguments/Remarks Made in an Amendment	4	5

Warnings:					
Information:					
3	Terminal Disclaimer Filed	TerminalDisclaimer.pdf	90939 b369ca48846a5fe8221f0b7c319d6d014d07925d	no	1
Warnings:					
Information:					
4	Other Reference-Patent/App/Search documents	9-27-2016PetitionerReplytoPatentOwnerResponse.pdf	13659957 897263441a6cca3c6e002b07d5bb97ded57975da	no	322
Warnings:					
Information:					

5	Other Reference-Patent/App/Search documents	11-28-2016PetitionerDemonstratives.pdf	12565962 7142b69357b8c2f269caedc971e12d5cef5f9321	no	82
Warnings:					
The page size in the PDF is too large. The pages should be 8.5 x 11 or A4. If this PDF is submitted, the pages will be resized upon entry into the Image File Wrapper and may affect subsequent processing					
Information:					
6	Other Reference-Patent/App/Search documents	11-23-2016PatentOwnerResponseandExhibits.pdf	5048622 85f049c2e11a99e2bc14f25f70b687446d908145	no	1151
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Information:					
7	Other Reference-Patent/App/Search documents	11-23-2016PatentOwnerDemonstratives.pdf	11944838 839a943270b3278a2096b8e53ab34487aac3be0e	no	62
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Information:					
8	Other Reference-Patent/App/Search documents	11-23-2016RedactedDecisiontoInstitute.pdf	2124535 d1f286e1b0f08b79783924384114df8d52f823ed	no	53
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9	Other Reference-Patent/App/Search documents	ThirdPtySubmPARTONE.pdf	12222250 d40157d94a5db5e8a25674bab24daf46cccf44d1	no	400
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Information:					
10	Other Reference-Patent/App/Search documents	ThirdPtySubmPARTTWO.pdf	12521559 79faefa4d23284fca37c8c72e55d40db25e88d7f	no	422
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Information:					
11	Other Reference-Patent/App/Search documents	SandozInvCont2-5-2015Redacted.pdf	603522 e6a3a54cee4303307b4a54ccb540ddb9c308eb80	no	90
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Information:					

12	Other Reference-Patent/App/Search documents	SandozInvContCharts2-5-2015.pdf	698449 b63f37bd11c3839e30470758af75d70041231fd9	no	189
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13	Other Reference-Patent/App/Search documents	ActavisPrelInvCont8-30-2016.pdf	1806962 c4469aba665c0ada06bd1cb9c46c2f66d783d254	no	330
Warnings:					
Information:					
14	Other Reference-Patent/App/Search documents	Treprostinil_-_Ex__G_invalidity_chart_for_the__393_patent_1-12-2015.pdf	304304 6bc080014897a484b8d06175e4d7985d11b1ec92	no	66
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Information:					
15	Other Reference-Patent/App/Search documents	TevaInvCont4-24-2015.pdf	567620 856442f52f1d8f3a02c1a3892acfcb7b123cb0c4	no	94
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Information:					
16	Non Patent Literature	Arumugam.pdf	510030 437a3975900966a808334a109ba44ad67432f70b	no	2
Warnings:					
Information:					
17	Non Patent Literature	Burk.pdf	704672 18b841deda8a39082b82cf4da79b3d470fa0af71	no	4
Warnings:					
Information:					
18	Non Patent Literature	Eliel.pdf	643300 aab21634720bd173fac8b09074dc0e550aba6b99	no	6
Warnings:					
Information:					

19	Non Patent Literature	Harwood.pdf	904588 9ebe079787102934b9284aa2a9f29b4b0240fac7	no	11
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Information:					
20	Non Patent Literature	Jones.pdf	544757 115e7f7ef706251e05ce52a08662401312d12185	no	5
Warnings:					
Information:					
21	Non Patent Literature	Lin.pdf	1300793 77655d6bb757ad96b8b08737c99e80e57bc60ff4	no	8
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Information:					
22	Non Patent Literature	McManus.pdf	745927 43532b7572f451474c2b10cd7cd91bd74c8eb9bc	no	4
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23	Non Patent Literature	Monson.pdf	787507 c11a6382504698cca5cdcbbceef885dc8d3b7a98	no	13
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Information:					
24	Non Patent Literature	Ohno.pdf	2055739 01c03319fb6a8140a0c95478a9c1586decff546a	no	16
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Information:					
25	Non Patent Literature	Olmsted.pdf	14163672 c4e45e76ce502d6f097a9ca771ca1911d1bcd175	no	62
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Information:					

26	Non Patent Literature	Pavia.pdf	479524 74f5a68bdb2973f0492dde185e6d40d6ebc0985f	no	3
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27	Non Patent Literature	PDR2005.pdf	1176914 83b70e21d08abeaefcfee3c4b70abd631686f6e5	no	5
Warnings:					
Information:					
28	Non Patent Literature	Prisinzano.pdf	754866 aa23db551f329816a2997cfbff0f6bd9e23bc772	no	4
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Information:					
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Information:					
30	Non Patent Literature	Schoffstall.pdf	7105291 e97a314c08d1ec7a795ed31c69ee90fb172b9d76	no	5
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31	Non Patent Literature	Sorrell.pdf	560691 9a6f445c1399544cb4cba882cb28bcfc927d498b	no	6
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32	Non Patent Literature	Wiberg.pdf	5760043 414bd0a91ea52145029ba4b60477a777fc400b3c	no	6
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Information:					

33	Non Patent Literature	Yu.pdf	689895 9e21b8aced370b80d93c796cfd434a72a159d29f	no	4
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Information:					
34		IDS.pdf	375470 d23f8be59968d5cf7063f26ae51796f596829059	yes	6
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Transmittal Letter		1	3	
	Information Disclosure Statement (IDS) Form (SB08)		4	6	
Warnings:					
Information:					
35	Fee Worksheet (SB06)	fee-info.pdf	32547 8c0d9552dc342026bf0c28b2a041b827740652f46	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	113982196		

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Atty. Dkt. No. 080618-1581

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Inventor Name:	Hitesh BATRA
Title:	AN IMPROVED PROCESS TO PREPARE TREPROSTINIL, THE ACTIVE INGREDIENT IN REMODULIN®
Appl. No.:	14/849,981
Filing Date:	9/10/2015
Examiner:	Yevgeny Valenrod
Art Unit:	1672
Confirmation Number:	6653

STATEMENT RE IDS CERTIFICATION

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

In the Information Disclosure Statement Transmittal filed in the above application today, on page 3, a Statement Under 37 CFR 1.97(e)(2) is erroneously included. The documents cited in the IDS were not all recently discovered. However, in view of the RCE which accompanied the IDS, no certification or IDS fee needed to be included and the submission was properly filed in the absence of such certification and fee.

Respectfully submitted,

Date Dec. 29, 2016

By /Stephen B. Maebius/

FOLEY & LARDNER LLP
Customer Number: 22428
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Stephen B. Maebius
Attorney for Applicant
Registration No. 35,264

4844-0586-7583.1

Electronic Acknowledgement Receipt	
EFS ID:	27929099
Application Number:	14849981
International Application Number:	
Confirmation Number:	6653
Title of Invention:	PROCESS TO PREPARE TREPROSTINIL, THE ACTIVE INGREDIENT IN REMODULIN®
First Named Inventor/Applicant Name:	Hitesh BATRA
Customer Number:	22428
Filer:	Stephen Bradford Maebius/Karen Strawderman
Filer Authorized By:	Stephen Bradford Maebius
Attorney Docket Number:	080618-1581
Receipt Date:	29-DEC-2016
Filing Date:	10-SEP-2015
Time Stamp:	14:32:17
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	StatementreIDSCert.pdf	93590 9c97bae0af669f9c28c9229ddc64a900c4643b57	no	1
Warnings:					

Information:	
Total Files Size (in bytes):	93590

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 14/849,981	Filing Date 09/10/2015	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT	12/29/2016	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 11	Minus	** 20	= 0	X $80 =	0
	Independent (37 CFR 1.16(h))	* 2	Minus	***3	= 0	X $420 =	0
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	**0**

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

SLIE
HELENA PAYTON

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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UNITED STATES DEPARTMENT OF COMMERCE
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/849,981	09/10/2015	Hitesh BATRA	080618-1581	6653

22428 7590 11/30/2016
Foley & Lardner LLP
3000 K STREET N.W.
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WASHINGTON, DC 20007-5109

EXAMINER	
VALENROD, YEVGENY	

ART UNIT	PAPER NUMBER
1672	

NOTIFICATION DATE	DELIVERY MODE
11/30/2016	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

ipdocketing@foley.com

Office Action Summary	Application No. 14/849,981	Applicant(s) BATRA ET AL.	
	Examiner YEVGENY VALENROD	Art Unit 1672	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *8/24/16*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1,2 and 4-11* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1,2 and 4-11* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date *2/29/16*.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

The present application is being examined under the pre-AIA first to invent provisions.

DETAILED ACTION

Withdrawn rejections / reasons for allowability

Rejection of claims 1-2, 4-10 under 35USC 103(a) as being unpatentable over Moriarty in view of Phares is withdrawn in view of applicants' arguments. Moriarty fails to teach formation of the salt prior to purification of treprostinil in the process described on page 1902. Moriarty also discloses that the sample of treprostinil obtained is in all respects identical to the authentic sample. In this respects the final product of Moriarty does not have the impurities from alkylation and hydrolysis because its a pure sample.

With respect to the sample prior to chromatography, Moriarty obtains crystals from the free acid of treprostinil. The crystals are also described as pure treprostinil and there is no basis to presume that the described impurities are present in the crystalized sample.

Furthermore, the declarations by Dr Williams and Dr. Ruffolo describe the product of Moriarty to have a different impurity profile from the product the instant claims where salt formation step is present.

Maintained Double Patenting Rejection

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted

by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the claims at issue are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); In re Vogel, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the reference application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(l)(1) - 706.02(l)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/forms/. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out

completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to http://www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

Claims 1-2 and 4-11 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 24 and 26 of U.S. Patent No. 8,242,305 (‘305). Although the claims at issue are not identical, they are not patentably distinct from each other because:

Claim 24 of ‘305 is directed to a process for the preparation of compound IV (treprostinil). Said method comprises alkylation od benzindene triol to prepare compound (VI) followed by hydrolyzing compound (VI) and contacting the hydrolysis product with a base. In claim 26 the contacting base is diethanolamine.

Claims 1-2 and 4-11 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-3, 8-14, of copending Application No. 14/754,932 (reference application). Although the claims at issue are not identical, they are not patentably distinct from each other because both the instant claims and claims of '932 are directed to a pharmaceutical product comprising treprostinil diethanolamine and a method of preparing said product via alkylation of benzindene triol, hydrolysis, contacting with a base to form a salt and isolation of the salt.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Conclusion

Claims 1-2 and 4-11 are pending

Claims 1-2 and 4-11 are rejected

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to YEVGENY VALENROD whose telephone number is (571)272-9049. The examiner can normally be reached on mon-fri 8-4:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Fereydoun G. Sajjadi can be reached on 571-572-3311. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/YEVGENY VALENROD/
Primary Examiner, Art Unit 1672

PTO/SB/08 (modified)

Substitute for form 1449/PTO				Complete if Known	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT				**Application Number**	14/849,981
Date Submitted: FEB 29 2016				**Filing Date**	9/10/2015
(use as many sheets as necessary)				**First Named Inventor**	Hitesh BATRA
Sheet	1	of	1	Art Unit	1672
				Examiner Name	Yevgeny Valenrod
				Attorney Docket Number	080618-1581

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	C1	2001/0038855 A1	11/08/2001	Desjardin et al.	
	C2	2001/0056095 A1	12/27/2001	Mylari	
	C3	4,434,164 A	02/28/1984	Lombardino	
	C4	5,466,713 A	11/14/1995	Blitstein-Willinger et al.	
	C5	5,506,265 A	04/09/1996	Blitstein-Willinger	
	C6	6,706,283 B1	03/16/2004	Appel et al.	

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Documents	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]
	C7	WO 98/18452 A1	05/07/1998	Shire Laboratories, Inc.		

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.) date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[6]
	C8	BIGHLEY et al., "Salt Forms of Drugs and Absorption," Encyclopedia of Pharmaceutical Technology, Swarbrick et al., Eds., 1995, 13:453-499.	
	C9	SIMONNEAU et al., "Continuous Subcutaneous Infusion of Treprostinil, a Prostacyclin Analogue, in Patients with Pulmonary Arterial Hypertension," Am. J. Respir. Crit. Care Med., 2002, 165:800-804.	

Examiner Signature	/YEVGENY VALENROD/	Date Considered	11/23/2016

4829-6306-5134.1

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /Y.V/

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	1	("8497393").PN.	US-PGPUB; USPAT; USOCR	OR	OFF	2016/11/23 18:45
L2	1	("8242305").PN.	US-PGPUB; USPAT; USOCR	OR	OFF	2016/11/23 18:45
L3	1	("4683330").PN.	US-PGPUB; USPAT; USOCR	OR	OFF	2016/11/23 18:45
L4	1	("4306075").PN.	US-PGPUB; USPAT; USOCR	OR	OFF	2016/11/23 18:45
L5	32	((Hitesh) near2 (Batra)).INV.	US-PGPUB; USPAT; USOCR	OR	ON	2016/11/23 18:45
L6	24	((Sudersan) near2 (Tuladhar)).INV.	US-PGPUB; USPAT; USOCR	OR	ON	2016/11/23 18:45
L7	30	((Raju) near2 (Penmasta)).INV.	US-PGPUB; USPAT; USOCR	OR	ON	2016/11/23 18:45
L8	245	((David) near2 (Walsh)).INV.	US-PGPUB; USPAT; USOCR	OR	ON	2016/11/23 18:45
L9	273	L5 or L6 or L7 or L8	US-PGPUB; USPAT; USOCR	OR	ON	2016/11/23 18:45
L10	24	L9 and treprostinil	US-PGPUB; USPAT; USOCR	OR	ON	2016/11/23 18:45
L11	534	c07c59/72.cpc.	US-PGPUB; USPAT; USOCR	OR	ON	2016/11/23 18:45
L12	870	(562/466).CCLS.	US-PGPUB; USPAT; USOCR	OR	OFF	2016/11/23 18:45
L13	1277	L11 or L12	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2016/11/23 18:45
L14	44	L13 and treprostinil	US-PGPUB; USPAT; USOCR	OR	ON	2016/11/23 18:45

EAST Search History (Prior Art)

L15	40	L14 and purity	US-PGPUB; USPAT; USOCR	OR	ON	2016/11/23 18:45
L16	37	L15 and HPLC	US-PGPUB; USPAT; USOCR	OR	ON	2016/11/23 18:45
L17	1	("6765117").PN.	US-PGPUB; USPAT; USOCR	OR	OFF	2016/11/23 18:45
L18	2	wo "2005007081"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2016/11/23 18:45
L19	2	"9242350"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2016/11/23 18:45
L20	1	("8242305").PN.	US-PGPUB; USPAT; USOCR	OR	OFF	2016/11/23 18:45
L21	1	("9156786").PN.	US-PGPUB; USPAT; USOCR	OR	OFF	2016/11/23 18:45

EAST Search History (Interference)

		< This search history is empty>				

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14849981	BATRA ET AL.
	Examiner	**Art Unit**
	YEVEGENY VALENROD	1672

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	02/22/2016	11/23/2016							
	1	✓	✓							
	2	✓	✓							
	3	✓	-							
	4	✓	✓							
	5	✓	✓							
	6	✓	✓							
	7	✓	✓							
	8	✓	✓							
	9	✓	✓							
	10	✓	✓							
	11		✓							

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14849981	BATRA ET AL.
	Examiner	Art Unit
	YEVEGENY VALENROD	1672

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
EAST	11/23/2016	YV
Inventor	11/23/2016	YV

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

	/YEVEGENY VALENROD/ Primary Examiner.Art Unit 1672

To: ipdocketing@foley.com,,
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 22428

Nov 30, 2016 03:36:57 AM

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UNITED STATES

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Application	Document	Mailroom Date	Attorney Docket No.
14849981	CTFR	11/30/2016	080618-1581
	1449	11/30/2016	080618-1581

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Thank you for prompt attention to this notice,

UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Inventor Name:	Hitesh BATRA
Title:	AN IMPROVED PROCESS TO PREPARE TREPROSTINIL, THE ACTIVE INGREDIENT IN REMODULIN®
Appl. No.:	14/849,981
Filing Date:	9/10/2015
Examiner:	Yevgeny Valenrod
Art Unit:	1672
Confirmation Number:	6653

AMENDMENT AND REQUEST FOR RECONSIDERATION

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

This amendment is submitted in response to the outstanding, non-final Office Action mailed on February 25, 2016.

Amendments to the Claims are reflected in the listing of claims that begins on page 2 of this document.

Remarks begin on page 4 of this document.

Amendments to the Claims:

This listing of claims will replace all prior versions and listings of claims in the application:

Listing of Claims:

1. (Previously Presented) A pharmaceutical composition comprising treprostinil or a pharmaceutically acceptable salt thereof, said composition prepared by a process comprising providing a starting batch of treprostinil having one or more impurities resulting from prior alkylation and hydrolysis steps, forming a salt of treprostinil by combining the starting batch and a base, isolating the treprostinil salt, and preparing a pharmaceutical composition comprising treprostinil or a pharmaceutically acceptable salt thereof from the isolated treprostinil salt, whereby a level of one or more impurities found in the starting batch of treprostinil is lower in the pharmaceutical composition, and wherein said alkylation is alkylation of benzindene triol.

2. (Previously Presented) The pharmaceutical composition of claim 1, wherein the salt is isolated in crystalline form.

3. (Canceled).

4. (Previously Presented) The pharmaceutical composition of claim 1, wherein the base is selected from the group consisting of sodium, ammonia, potassium, calcium, ethanolamine, diethanolamine, N-methylglucamine, and choline.

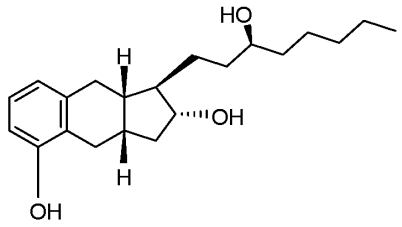
5. (Previously Presented) The pharmaceutical composition of claim 4, wherein the base is diethanolamine.

6. (Previously Presented) The pharmaceutical composition of claim 1, wherein the base is combined with treprostinil that has not been previously isolated.

7. (Previously Presented) The pharmaceutical composition of claim 1, wherein the isolated salt is stored at ambient temperature.

8. (Previously Presented) The pharmaceutical composition of claim 1, which is a pharmaceutical solution.

9. (Previously Presented) A process of preparing a pharmaceutical product comprising treprostinil or a pharmaceutically acceptable salt thereof, comprising alkylating a triol intermediate of the formula:



hydrolyzing the resulting compound to form treprostinil, forming a salt of treprostinil stable at ambient temperature, storing the treprostinil salt at ambient temperature, and preparing a pharmaceutical product from the treprostinil salt after storage, wherein the pharmaceutical product comprises treprostinil or a pharmaceutically acceptable salt thereof.

10. (Previously Presented) A pharmaceutical product prepared by the process of claim 9.

11. (New) The process as claimed in claim 9, wherein forming the salt of treprostinil stable at ambient temperature is performed by adding diethanolamine to treprostinil.

REMARKS

Applicants respectfully request reconsideration and allowance of the present application.

Status of Claims

Applicants have canceled claim 3 and added claim 11 to depend from claim 9. Support for claim 11 can be found in examples 4-6. No new matter has been added.

After the amendment, claims 1, 2, and 4-11 are pending.

35 U.S.C. § 103

Claims 1-3, 6, 8, and 9 stand rejected under 35 U.S.C. § 103(a) as allegedly obvious over Moriarty (2004) in view of Phares (WO 2005/007081 A2). Applicants respectfully request reconsideration of the rejection.

Applicants filed a notification of related proceedings to bring to the Examiner's attention documents from IPR2016-00006, which involves parent U.S. Patent No. 8,497,393. Certain information is redacted in those documents due to confidentiality. Documents provided in that notification include the Patent Owner's Response and expert declarations from Dr. Williams and Ruffolo. These documents address the subject matter of the '393 patent claims although certain information is relevant to the present claims as explained herein.

Claim 1 recites steps, including "forming a salt", which read on a commercial process used by the assignee of the present application. Prior to the current commercial process, the assignee used a process based on Moriarty 2004. Because the assignee used both processes, the assignee had the opportunity to analyze the resulting pharmaceutical products as reflected in certificates of analysis. In the IPR, Dr. Williams and Dr. Ruffolo used these certificates of analysis to explain that a pharmaceutical batch produced according to a salt formation process as covered by claim 1 is different from the product produced by the process described in Moriarty 2004. Williams Dec. at ¶¶94-99; Ruffolo Dec. at ¶¶66-72. Specifically, the processes result in products having different impurity profiles, and in fact, the pharmaceutical composition of claim 1 has higher average purity. Patent Owner's Response at Section III.C.

The differences are not merely academic, but critical to the successful manufacture of a clinical product. FDA uses both overall purity and levels of individual impurities ("purity specification") as a basis to regulate the manufacturing of pharmaceuticals. Batches that fall outside of the purity specification cannot be sold or used to treat patients. As noted in the Patent Owner's IPR Response, the differences between claim 1's pharmaceutical composition and a product produced according to the process of Moriarty were significant enough to result in FDA's acceptance of a new purity specification for the commercial product, thus proving that the products are not the same in the eyes of the FDA. Patent Owner's Response at Section III.C. Furthermore, this change constitutes a "major" change according to the classification system for manufacturing changes used by FDA. Ruffolo Dec. at ¶¶70- 72.

The rejection further cites Phares for showing that it would have been obvious to form a diethanolamine salt using Moriarty's treprostinil. However, the differences in the resulting products, as explained above, would not have been expected based on the prior art. In particular, it would not have been obvious to use the salt formation step of Phares to decrease amounts of stereoisomer impurities of treprostinil, which are acidic rather than neutral or basic. Williams Dec. at ¶102. When subject to salt-forming conditions, one of ordinary skill in the art would expect that any undesired stereoisomer of treprostinil would be included in the final salt product because the stereoisomer would also be converted to the corresponding salt under such salt-forming conditions. One of ordinary skill in the art would have had no reasonable expectation of success in removing any undesired treprostinil stereoisomer impurities by salt formation.

In addition, FDA's decision to adopt a new purity specification for the resulting product further establishes unobviousness of the presently claimed invention. Indeed, as noted above, the specification change is classified as a "major" change according to the FDA's classification system for manufacturing changes. *See Knoll Pharm. Co., Inc. v. Teva. Pharm. USA, Inc.*, 367 F.3d 1381, 1385 (Fed. Cir. 2004) (explaining that while FDA approval is not determinative of nonobviousness, it can be relevant in evaluating the objective indicia of nonobviousness). As noted in Dr. Ruffolo's Declaration, even small changes in impurity are important to FDA: "Regulatory agencies have also sought to increase levels of purity, and consequently decrease levels of impurities, in order to provide to the maximum extent possible, the highest level of

safety to patients." Ruffolo Dec. at ¶36. This is due to the fact that even trace amounts of impurities can sometime pose serious health concerns.

Accordingly, withdrawal of the rejection under 35 U.S.C. § 103(a) is requested.

Double Patenting

Claims 1-10 have been rejected for alleged non-statutory double patenting as unpatentable over claims 24 and 26 of US Patent No. 8,242,305. Applicants will address the rejection by filing a terminal disclaimer or other action if still necessary after the PTO's consideration of the above arguments and confirmation that the present claims are otherwise in condition for allowance.

Concluding Remarks

Applicants believe that the application is in condition for allowance. Favorable reconsideration is respectfully requested. The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance prosecution.

The Commissioner is hereby authorized to charge any additional fees that may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing or a credit card payment form being unsigned, providing incorrect information resulting in a rejected credit card transaction, or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicants hereby petition for such extension under 37 C.F.R. § 1.136 and authorize payment of any such extension fees to Deposit Account No. 19-0741.

Respectfully submitted,

Date Aug. 24, 2016

By /Stephen B. Maebius/

FOLEY & LARDNER LLP
Customer Number: 22428
Telephone: (202) 672-5569
Facsimile: (202) 672-5399

Stephen B. Maebius
Attorney for Applicant
Registration No. 35,264

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Inventor Name:	Hitesh BATRA
Title:	AN IMPROVED PROCESS TO PREPARE TREPROSTINIL, THE ACTIVE INGREDIENT IN REMODULIN®
Appl. No.:	14/849,981
Filing Date:	9/10/2015
Examiner:	Yevgeny Valenrod
Art Unit:	1672
Confirmation Number:	6653

PETITION FOR EXTENSION OF TIME

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

Applicant hereby petitions the Commissioner under 37 C.F.R. §1.136(a) for a three-month extension of time for response in the above-identified application for the period required to make the attached response timely.

The extension fee for response within the third month is $1,400.00.

The above-identified fees of $1,400.00 are being paid by credit card via EFS-Web.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by the credit card payment instructions in EFS-Web being incorrect or absent, resulting in a rejected or incorrect credit card transaction, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741.

Respectfully submitted,

Date Aug. 24, 2016

By /Stephen B. Maebius/

FOLEY & LARDNER LLP
Customer Number: 22428
Telephone: (202) 672-5569
Facsimile: (202) 672-5399

Stephen B. Maebius
Attorney for Applicant
Registration No. 35,264

Electronic Patent Application Fee Transmittal	
Application Number:	14849981
Filing Date:	10-Sep-2015
Title of Invention:	PROCESS TO PREPARE TREPROSTINIL, THE ACTIVE INGREDIENT IN REMODULIN®
First Named Inventor/Applicant Name:	Hitesh BATRA
Filer:	Stephen Bradford Maebius/Mary Jo Boyce
Attorney Docket Number:	080618-1581
Filed as Large Entity	
Filing Fees for Utility under 35 USC 111(a)	

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 3 months with $0 paid	1253	1	1400	1400
Miscellaneous:				
		Total in USD ($)		**1400**

Electronic Acknowledgement Receipt	
EFS ID:	26733306
Application Number:	14849981
International Application Number:	
Confirmation Number:	6653
Title of Invention:	PROCESS TO PREPARE TREPROSTINIL, THE ACTIVE INGREDIENT IN REMODULIN®
First Named Inventor/Applicant Name:	Hitesh BATRA
Customer Number:	22428
Filer:	Stephen Bradford Maebius/Mary Jo Boyce
Filer Authorized By:	Stephen Bradford Maebius
Attorney Docket Number:	080618-1581
Receipt Date:	24-AUG-2016
Filing Date:	10-SEP-2015
Time Stamp:	13:14:09
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$1400
RAM confirmation Number	9977
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		Amendment.pdf	134812 343357de95eb8800052830f12cdc8dd26e7b677b	yes	7
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Amendment/Req. Reconsideration-After Non-Final Reject		1	1	
	Claims		2	3	
	Applicant Arguments/Remarks Made in an Amendment		4	7	
Warnings:					
Information:					
2	Extension of Time	3EOT.pdf	97084 bbfc21febfc8066cf79a696684a9bc6dd1c5374c	no	2
Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	31318 e4abe29bfcd0ff5a1498e888d49cbccc04490776	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	263214		

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Inventor Name:	Hitesh BATRA
Title:	AN IMPROVED PROCESS TO PREPARE TREPROSTINIL, THE ACTIVE INGREDIENT IN REMODULIN®
Appl. No.:	14/849,981
Filing Date:	9/10/2015
Examiner:	Yevgeny Valenrod
Art Unit:	1672
Confirmation Number:	6653

NOTIFICATION OF RELATED PROCEEDINGS

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

Applicant hereby updates the Office concerning the status of a related proceeding styled *Steadymed Ltd. (Petitioner), v. United Therapeutics Corporation (Patent Owner)*, Case IPR2016-00006, US Patent 8,497,393, which involves the issued parent of the above-captioned patent application. Other documents from the above-identified Inter Partes Review (IPR) were submitted in the present application with an Information Disclosure Statement filed on December 8, 2015, and a Notification of Related Proceedings filed on February 29, 2016, for the Examiner's consideration. The purpose of this notice is to provide a copy of the public Patent Owner's Response to Petition and public exhibits filed on July 6 and 13, 2016, and the public

Decision Instituting the IPR from the IPR proceeding. Certain information is redacted and certain exhibits are not provided due to their filing under seal in the IPR proceeding.

Respectfully submitted,

Date JUL 19 2016

By

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Paper No. 28
Entered: May 12, 2016

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

STEADYMED LTD.,
Petitioner,

v.

UNITED THERAPEUTICS CORPORATION,
Patent Owner.

Case IPR2016-00006
Patent 8,497,393 B2

Before LORA M. GREEN, JONI Y. CHANG, and JACQUELINE T. HARLOW, *Administrative Patent Judges.*

HARLOW, *Administrative Patent Judge.*

DECISION
Redacted Institution of *Inter Partes* Review
37 C.F.R. § 42.108

I. INTRODUCTION

Petitioner, SteadyMed LTD ("SteadyMed"), filed a Petition requesting an *inter partes* review of claims 1–22 of U.S. Patent No. 8,497,393 B2 (Ex. 1001, "the '393 patent"). Paper 1 ("Pet."). Patent Owner, United Therapeutics Corporation ("UTC"), filed a Preliminary Response on January 14, 2016. Paper 10[1] ("Prelim. Resp."). We have jurisdiction under 35 U.S.C. § 314, which provides that an *inter partes* review may not be instituted unless the information presented in the petition "shows that there is a reasonable likelihood that the petitioner would prevail with respect to at least 1 of the claims challenged in the petition."

For the reasons set forth below, we institute an *inter partes* review of claims 1–22 of the '393 patent.

A. Related Matters

The '393 patent is asserted in: *United Therapeutics Corp. v. Sandoz, Inc.*, No. 14-cv-05499 (D.N.J.); *United Therapeutics Corp. v. Teva Pharmaceuticals U.S.A., Inc.*, No. 14-cv-05498 (D.N.J.); and *United Therapeutics Corp. v. Watson Laboratories, Inc.*, No. 15-cv-05723 (D.N.J). Pet. 1. SteadyMed is not party to the above identified litigations. *Id.*

[1] Paper 10 is the Unredacted Preliminary Response. Paper 8, filed concurrently with Paper 10, is a redacted version of the Preliminary Response.

B. The '393 Patent

The '393 patent, titled "Process to Prepare Treprostinil, the Active Ingredient in Remodulin®," issued July 30, 2013, from U.S. Patent Application No. 13/548,446 ("the '446 application") (Ex. 1002), filed July 13, 2012. Ex. 1001, [54], [45], [21], [22]. The '446 application is a continuation of U.S. Patent Application No. 12/334,731 ("the '731 application") (Ex. 1002), filed on December 15, 2008, now issued as U.S. Patent No. 8,242,305 ("the '305 patent"). Ex. 1001, [63]. The '393 patent claims priority to U.S. Provisional Patent Application No. 61/014,232 (Ex. 2008), filed December 17, 2007. Ex. 1001, [60].

The '393 patent recites 22 product-by-process claims for prostacyclin derivatives, including treprostinil.[2] *Id.* at 17:51–21:16; Pet. 5; Prelim. Resp. 3. The process disclosed by the '393 patent takes advantage of carbon treatment and salt formation steps to remove impurities, eliminating the need for purification by column chromatography. *Id.* at 17:29–32; *see also id.* at 5:41–45 ("purification by column chromatography is eliminated . . . . [T]he salt formation is a much easier operation than column chromatography.").

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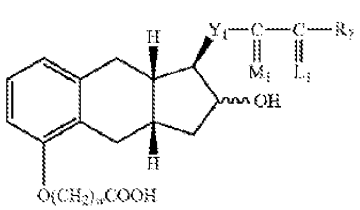
[2] The '305 patent, which issued from the parent to the application for the '393 patent, recites claims to a process for the preparation of prostacyclin derivatives comprising steps similar to those set forth in the product-by-process claims of the '393 patent. *Compare* Ex. 1001, 17:51–21:16, *with* Ex. 2007, 17:39–24:3.

The process for forming prostacyclin derivatives described in the '393 patent includes four steps: (a) alkylating a prostacyclin derivative to form an alkylated prostacyclin derivative; (b) hydrolyzing the alkylated prostacyclin derivative with a base to form a prostacyclin acid; (c) contacting the prostacyclin acid with a base to form a prostacyclin carboxylate salt; and (d) optionally reacting the prostacyclin carboxylate salt formed in (c) with an acid to form the desired compound, or pharmaceutically acceptable salt thereof. *Id.* at 1:65–3:19.

C. Illustrative Claim

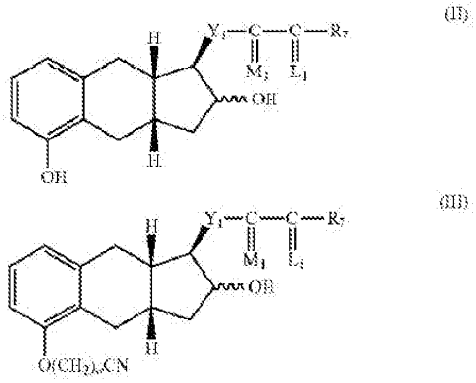
Each of the challenged claims is a product-by-process claim. Of the challenged claims, claims 1 and 9 are independent. Claim 1, reproduced below, is illustrative of the claimed subject matter.

1. A product comprising a compound of formula I



or a pharmaceutically acceptable salt thereof, wherein said product is prepared by a process comprising

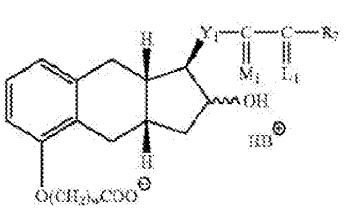
a) alkylating a compound of structure II with an alkylating agent to produce a compound of formula III,



wherein [recitation of Markush groups for the specified structures],

b) hydrolyzing the product of formula III of step (a) with a base,

c) contacting the product of step (h)[3] with a base B to form a salt of formula I_s.



and

d) optionally reacting the salt formed in step (c) with an acid to form the compound of formula I.

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[3] We note that the reference to "step (h)," rather than "step (b)," in claim 1 is an apparent typographical error. *See* Ex. 1001, 3:66–67 ("(c) contacting the product of step (b) with a base B to for a salt of formula IV_s"); *see also* Pet. 25; Ex. 1009 ¶ 51.

Ex. 1001, 17:51–19:29. Claim 9 is drawn to a product comprising a specific treprostinil compound within the genus set forth in claim 1, and made by the process recited in claim 1. *Id.* at 19:48–20:46.

D. Prior Art Relied Upon

SteadyMed relies upon the following prior art references (Pet. 4–6):

Phares	WO 2005/007081 A2	Jan. 27, 2005	(Ex. 1005)
Kawakami	JP 56-122328A	Sept. 25, 1981	(Ex. 1006[4])

Moriarty et al., *The Intramolecular Asymmetric Pauson-Khand Cyclization as a Novel and General Stereoselective Route to Benzindene Prostacyclins: Synthesis of UT-15 (Treprostinil)*, 69 J. Org. Chem. 1890–1902 (2004) ("Moriarty") (Ex. 1004); and

Seyhan N. Eğe, ORGANIC CHEMISTRY 543–547 (2d ed. 1989) ("Eğe") (Ex. 1008).

E. Asserted Grounds of Unpatentability

SteadyMed asserts the following grounds of unpatentability (Pet. 3–4):

Claims	Basis	Reference(s)
1–5, 7–9, 11–14, and 16–20	§ 102(b)	Phares
1–5, 7–9, 11–14, and 16–20	§ 103(a)	Moriarty and Phares or Kawakami
6, 10, 15, 21, and 22	§ 103(a)	Moriarty, Phares, Kawakami, and Eğe

[4] SteadyMed submitted a certified English translation of Kawakami as Ex. 1007. As discussed in Part II.F below, UTC argues the admissibility of this translation.

II. ANALYSIS

A. 35 U.S.C. § 325(d)

UTC urges the exercise of our discretion under 35 U.S.C. § 325(d) to deny some or all of the grounds of unpatentability presented by SteadyMed because the same, or substantially similar issues were addressed during prosecution. Prelim. Resp. 25–26. UTC states that the Patent Office considered Moriarty alone, and in combination with Phares, during prosecution of the '393 patent. *Id.* at 8–10, 26. UTC also reports that Phares was considered alone, and in combination with Moriarty, during prosecution of U.S. Patent Application No. 13/910,583 ("the '583 application") (Ex. 2010) filed June 5, 2013, which is a continuation of the '446 application. *Id.* at 11–14.

Regarding the patentability of claims 6, 15, 21, and 22, in particular, UTC asserts that Eğe "is nothing more than a first-year organic chemistry textbook," and that SteadyMed "relies on nothing more than conclusory statements in three paragraphs of the [Declaration of Jeffery D. Winkler]" to support its unpatentability arguments. *Id.* at 26. UTC therefore contends that SteadyMed "has provided no evidence of probative value that is any different than what was already before the Patent Office during prosecution." *Id.* at 26–27.

Although it is within our discretion to "reject the petition or request because, the same or substantially the same prior art or arguments previously were presented to the Office" pursuant to 35 U.S.C. § 325(d), we decline to do so here.

We note that during prosecution of the '446 application, which issued as the '393 patent, the Examiner rejected the claims as anticipated by Moriarty, but subsequently withdrew that rejection, without elaboration, in response to a declaration filed by David A. Walsh ("Walsh Declaration") (Ex. 1002, 346–350), one of the named inventors of the '393 patent, and the Executive Vice President of Chemical Research and Development at UTC. Ex. 1002, 344, 346–360. Although Phares is listed as a cited reference on the face of the '393 patent (Ex. 1001, [56]), we observe that the Examiner neither relied on, nor otherwise discussed Phares during prosecution of the '446 application (Ex. 1002, 295–296, 327–330, 359). In addition, neither Eğe nor Kawakami was considered during prosecution of the '446 application. *Id.* at 235–359. The grounds of unpatentability asserted in the instant Petition likewise differ from the rejections entered by the Examiner during prosecution of the '731 application, the parent to the '446 application. *See* Ex. 1002, 122–124.

Moreover, as discussed in detail in Part II.B below, the Declaration of Jeffrey D. Winkler ("Winkler Declaration") (Ex. 1009), submitted in support of SteadyMed's Petition, calls into question Dr. Walsh's conclusion that treprostinil prepared according to the process claimed in the '393 patent is "physically different from treprostinil prepared according to the process of 'Moriarty'" (Ex. 1002, 347 (¶ 6)). Ex. 1009 ¶¶ 63–71. In addition, as set forth in Part II.F, we disagree with UTC's characterization of Dr. Winkler's testimony as conclusory. *See, e.g.,* Ex. 1009 ¶¶ 80–90.

We, therefore, decline to exercise our discretion to deny the Petition pursuant to 35 U.S.C. § 325(d). *See Nestle USA, Inc. v. Steuben Foods, Inc.*, Case IPR2014-01235, slip op. at 7 (PTAB Dec. 22, 2014) (Paper 12) ("[W]e conclude that Petitioner's arguments regarding the unpatentability of claims 18–20, which include arguments relating to Biewendt and a combination of references previously not considered and supported by a declaration previously not considered, are persuasive. . ."); *Merial Ltd., v. Virbac*, Case IPR2014-01279, slip op. at 9 (PTAB Jan. 22, 2015) (Paper 13) (noting the different burdens of proof and evidentiary standards applicable to *ex parte* examination and *inter partes review* proceedings).

B. Claim Construction

In an *inter partes* review, claim terms in an unexpired patent are given their broadest reasonable interpretation in light of the specification of the patent in which they appear. 37 C.F.R. § 42.100(b); *see also In re Cuozzo Speed Techs., LLC*, 793 F.3d 1268, 1278–79 (Fed. Cir. 2015) ("Congress implicitly approved the broadest reasonable interpretation standard in enacting the AIA," and "the standard was properly adopted by PTO regulation."), *cert. granted sub nom. Cuozzo Speed Techs., LLC v. Lee*, 136 S. Ct. 890 (2016) (mem.). Under this standard, we may take into account definitions or other explanations provided in the written description of the specification. *In re Morris*, 127 F.3d 1048, 1054 (Fed. Cir. 1997). Any special definition for a claim term must be set forth in the specification with reasonable clarity, deliberateness, and precision. *In re Paulsen*, 30 F.3d

1475, 1480 (Fed. Cir. 1994). Only those terms that are in controversy need be construed, and only to the extent necessary to resolve the controversy. *Vivid Techs., Inc. v. Am. Sci. & Eng'g, Inc.*, 200 F.3d 795, 803 (Fed. Cir. 1999).

"Product" / "A product comprising a compound [of/having] formula [I/IV] . . . or a pharmaceutically acceptable salt thereof"

Independent claims 1 and 9 recite the phrase "[a] product comprising a compound [of/having] formula [I/IV] . . . or a pharmaceutically acceptable salt thereof . . . ." Ex. 1001, 19:48–20:46. In addition, each challenged dependent claim recites the term "product." *Id.* at 17:51–21:16. Because the parties advance similar arguments pertaining to the construction of these terms, we address these terms together.

SteadyMed asserts that the phrase "[a] product comprising a compound [of/having] formula [I/IV] . . . or a pharmaceutically acceptable salt thereof" should be interpreted to mean "a chemical composition that includes, but is not limited to, a compound of Formula I, or a pharmaceutically acceptable salt thereof, and that may also include other non-mentioned substances (including impurities), additives, or carriers, without limitation as to the types or relative amounts thereof." Pet. 11. SteadyMed contends that because independent claims 1 and 9 recite "[a] product comprising," the claim term "product" should be construed to include "the treprostinil compound along with other substances (including impurities)," i.e., a "chemical composition." *Id.* at 11.

UTC counters that "[a] product comprising a compound [of/having] formula [I/IV] . . . or a pharmaceutically acceptable salt thereof" should be interpreted as "a substance resulting from a chemical reaction constituted primarily of formula I/IV or a pharmaceutically acceptable salt thereof." Prelim. Resp. 21. As an initial matter, UTC notes that SteadyMed's proposed construction refers only to Formula I, and asserts that SteadyMed "inexplicably read[s] Formula IV out of the term entirely." *Id.* at 22.

UTC further argues that the claims and Specification of the '393 patent use "product" to refer to a substance resulting from a chemical reaction. *Id.* at 17. UTC also contends that the prosecution history for the '393 patent supports its proposed construction because "during prosecution, the Patent Owner and Examiner explicitly discussed the 'product' of the claims as a real world substance that results from employing a specific chemical process, as differentiated from the substance obtained from employing a different chemical process." *Id.* at 18–19. UTC points to chemistry textbooks as buttressing its position that a skilled artisan would understand the claim term "product" as referring to "a substance resulting from a chemical reaction." *Id.* at 19. UTC further reasons that "the 'product' claimed in a product-by-process claim is necessarily a substance that results from the process specified in that claim" (*id.*), and that SteadyMed's proposed construction "contradicts this inherent limitation of the claims" (*id.* at 22).

On this record, and for purposes of this decision, we interpret the phrase "[a] product comprising a compound [of/having] formula [I/IV] or a

pharmaceutically acceptable salt thereof," to mean "a product including, but not limited to, a compound [of/having] formula [I/IV] or a pharmaceutically acceptable salt thereof."

The claim term "product," as it is used in the '393 patent, does not require construction because the claimed "product" is defined by the limitations recited in the challenged claims. This is evidenced by independent claims 1 and 9, which recite "[a] product comprising . . . ," and go on to define the essential elements of the claimed product. The transitional term "'comprising' is a term of art used in claim language which means that the named elements are essential, but other elements may be added and still form a construct within the scope of the claim." *Genentech, Inc. v. Chiron Corp.*, 112 F.3d 495, 501 (Fed. Cir. 1997); *see also* Ex. 1001, 4:23–25 (defining "comprising" as "including, but not limited to"). Thus, the open-ended structure of the challenged claims forecloses limitation of the term "product" beyond that achieved by the recited claim elements.

Indeed, neither UTC nor SteadyMed identifies any disclosure in the '393 patent or its prosecution history that necessitates a contrary understanding of the term "product." For example, the portions of the Specification to which UTC points comport with an understanding of "product" as being defined only by the recited claim elements. *See* Ex. 1001, 5:45–46, 7:16–20, 17:37–40. Furthermore, far from disavowing or otherwise limiting claim scope, the portions of the prosecution history identified by UTC are consistent with an understanding that the claimed "product" is defined solely by the recited claim elements. *See* Ex. 1002,

315, 328–329, 346–350. We similarly are unpersuaded that the chemistry textbook glossaries to which UTC points (Exs. 2011, 2012, 2014) provide a basis for narrowly interpreting "product" to require that the product result from a chemical reaction.

Regarding the larger claim phrase "[a] product comprising a compound [of/having] formula [I/IV] . . . or a pharmaceutically acceptable salt thereof," as explained above, we determine that the embedded claim term "comprising" means "including, but not limited to." *See Genentech*, 112 F.3d at 501; *see also* Ex. 1001, 4:23–25. Accordingly, we reject UTC's proposal that claims 1 and 9 be read to require a product "*constituted primarily of* formula I/IV or a pharmaceutically acceptable salt thereof." Prelim. Resp. 21 (emphasis added).

"[A/the] process comprising"

SteadyMed argues that the claim phrase "[a/the] process comprising," which appears in independent claims 1 and 9, should be interpreted as "a process that includes, but is not limited to, the recited process steps, and may include, without limitation, any other non-recited steps." Pet. 12. UTC counters that this claim phrase should be construed to mean "a/the process including but not limited to." Prelim. Resp. 23–24. For the reasons set forth above, we agree with UTC that these claim phases should be interpreted to mean "a/the process including, but not limited to."

Product-by-Process Claims

Each of the challenged claims is a product-by-process claim. Ex. 1001, 17:51–21:16; Pet. 5; Prelim. Resp. 3. The general rule when determining patentability of a product-by-process claim is to "focus . . . on the product and not on the process of making it." *Amgen, Inc. v. Hoffman-La Roche Ltd.*, 580 F.3d 1340, 1369 (Fed. Cir. 2009). This general rule embodies the long-standing principle that "an old product is not patentable even if it is made by a new process." *Id.* at 1370. An exception applies when process steps recited in the claim impart "structural and functional differences" to the claimed product. *Greenliant Sys., Inc. v. Xicor LLC*, 692 F.3d 1261, 1267–1268 (Fed. Cir. 2012). If the exception applies, the structural and functional differences conveyed by the recited process steps "'are relevant as evidence of no anticipation' although they 'are not explicitly part of the claim.'" *Id.* at 1268 (citing *Amgen*, 580 F.3d at 1370).

SteadyMed contends that the challenged claims do not yield a treprostinil product having structural or functional differences as compared to treprostinil products produced by prior art methods. Pet. 19–22. Specifically, SteadyMed asserts that the Walsh Declaration, relied on by UTC during prosecution as evidencing differences in the treprostinil products of the '393 patent and Moriarty, fails to demonstrate any functional or structural differences between the instantly claimed and prior art treprostinil products. *Id.* SteadyMed relies on the Winkler Declaration (Ex. 1009) to support its position. *Id.*

UTC acknowledges that "at the time of the '393 patent, there existed at least three prior art methods" for making treprostinil. Prelim. Resp. 33. Relying on the Walsh Declaration, UTC asserts that the process steps recited in independent claims 1 and 9 are entitled to patentable weight because they yield a "physically different and improved final product with significantly reduced overall impurities and a distinct and unexpected impurity profile" as compared to treprostinil produced using prior art methods. *Id.* at 3.

The Walsh Declaration compares the impurity profile of treprostinil free acid "prepared according to the process of 'Moriarty'" to the impurity profiles of treprostinil free acid and treprostinil diethanolamine "prepared according to the process specified in claim 1 or [9]" of the '393 patent.[5] Ex. 1002, 347–348 (¶ 6). Dr. Walsh concludes that the treprostinil free acid and treprostinil diethanolamine prepared according to the process of claims 1 and 9 is physically different from the treprostinil diethanolamine prepared according to the process of Moriarty "at least because neither of [the '393 patent products] contains a detectable amount of any of benzindene triol, treprostinil methyl ester, 1AU90 treprostinil stereoisomer and 2AU90 treprostinil stereoisomer, each of which were present in detectable amounts in treprostinil produced according to the process of 'Moriarty'." *Id.* at 349 (¶ 8). In addition, Dr. Walsh provides "data obtained from representative Certificates of Analysis" indicating that treprostinil free acid "prepared

[5] Issued claim 9 of the '393 patent is identified as claim 10 in the Walsh Declaration, and other documents in the prosecution history in the '393 patent.

according to 'Moriarty'" is 99.4% pure, while the treprostinil free acid and treprostinil diethanolamine "prepared according to the process specified in claim 1 or [9]" are 99.8% pure and 99.9% pure, respectively. *Id.* at 347–348 (¶ 6).

SteadyMed disputes Dr. Walsh's contention that there are physical differences between the treprostinil products of the '393 patent and prior art. Pet. 19–22; *see also* Ex. 1009 ¶¶ 63–71. As an initial matter, SteadyMed points out that the 99.7% treprostinil purity reported by Moriarty (Ex. 1004, 13) is higher than the 99.5% purity recited in claims 2 and 10 of the '393 patent, the only challenged claims that recite a purity level. Pet. 20; *see also* Ex. 1009 ¶ 65. In addition, Dr. Winkler testifies that the limited sample set, consisting of "*only two specific batches* of treprostinil" (Ex. 1009 ¶ 66), and absence of any disclosure concerning the reaction conditions, reagents, and solvents used in carrying out the process of claims 1 and 9 of the '393 patent (*id.* ¶ 67), undermine the veracity of Dr. Walsh's conclusion regarding the purity of these products. *Id.* ¶¶ 66–67. SteadyMed also observes that the statement in the Specification of the '393 patent that in one embodiment the purity of treprostinil is "at least 90.0%, 95.0%, 99.0%, 99.5%" (Ex. 1001, 8:66–67), supports the conclusion that the 99.8% purity purportedly achieved by Dr. Walsh "is based on a particular set of process steps that are not claimed and which must have been found after the filing date." Pet. 20.

Dr. Winkler additionally testifies that the alleged differences in purity between the treprostinil batches described by Dr. Walsh are attributable to

experimental error. *Id.* ¶¶ 68–70. Dr. Winkler testifies that "the literature on [High Performance Liquid Chromatography's ("HPLC's")] precision indicates that the 'RSD' or 'relative standard deviation' for a typical instrument is about 1%. (Ex. 1017.)." *Id.* ¶ 70. Dr. Winkler further observes that "[i]n the present case, we can estimate the precision of the equipment the inventors actually used, since the inventors found that Example 4's Batch 1 had an HPLC Assay of 100.4%, which is obviously greater than the 100% value theoretically achievable. (Ex. 1001, col. 13, lines 50-65)." *Id.* Dr. Winkler, thus, concludes that "[t]his deviation between experimental and theoretical shows that the instrument can have variations of at least 0.4%, which is greater than the differences in purity that the inventors offered to support their contention regarding greater purity over the prior art." *Id.* On this record, we credit Dr. Winkler's testimony, as it is consistent with the disclosures of the prior art and the disclosure of the '393 patent itself.

UTC does not challenge SteadyMed's arguments concerning the shortcomings of the Walsh Declaration. Rather, UTC points to correspondence with, and reports submitted to, the Food and Drug Administration ("FDA") relating to the acceptance of a supplemental new drug application for treprostinil. Prelim. Resp. 36–38. UTC contends that these reports show that "the purity of the treprostinil improved close to 100%" for treprostinil prepared as described in claims 1 and 9 of the '393 patent as opposed to the prior process implemented by UTC. Prelim. Resp. 38; *see also* Ex. 2006, 3–4.

On the record before us, and for purposes of this decision, we conclude that the process steps recited in the challenged claims do not impart structural or functional differences to the claimed product.

As an initial matter, we observe that the challenged product-by-process claims are drawn to "[a] product comprising a compound" of either formula I or formula IV, or a pharmaceutically acceptable salt of the recited formula. Ex. 1001, 17:51–19:29, 19:48–20:46). "'Comprising' is a term of art used in claim language which means that the named elements are essential, but other elements may be added and still form a construct within the scope of the claim." *Genentech*, 112 F.3d at 501. Thus, a product comprising a particular compound must contain that compound, but may additionally include other substances, such as impurities. On this record, therefore, it is unclear how claims 1, 3–9, and 11–22, which claim a product comprising a particular compound, but do not recite limitations concerning the purity profile of that product, could be restricted to a product including the claimed compound, but also having a particular purity profile. In addition, although claims 2 and 10 require a purity of at least 99.5% (Ex. 1001, 19:29–30, 20:47–48), these claims similarly are drawn to a product comprising a compound, and do not specify the type of impurities that may be present in the compound or restrict the amount of any particular impurity that may be present, so long as the product remains at least 99.5% pure.

Furthermore, the evidence presently before us, including UTC's own testing results, suggests that inter-batch variability in impurity profiles,

experimental error in impurity measuring equipment, and variations in reagents, solvents, and reaction conditions, rather than the instantly recited process steps, account for any purported improvements in purity reported by UTC. We observe that UTC offers no explanation for the variation between the 99.7% purity reported by Moriarty, and the 99.4% purity Dr. Walsh obtained for treprostinil purportedly prepared according to the process described by Moriarty. Neither does UTC offer reasoning for crediting Dr. Walsh's results over those reported by Moriarty himself. Similarly, UTC neglects Dr. Winker's assessment of the experimental error present, but unaccounted for, in the impurity measurements reported in the Walsh Declaration, and fails to account for the absence of any disclosure regarding the experimental protocols followed by Dr. Walsh, such as the reaction conditions, or the solvents or reagents used, in synthesizing treprostinil according to Moriarty or the '393 patent.

Moreover, the Process Optimization Report (Ex. 2005) proffered by UTC supports the conclusion that the process steps recited in the '393 patent do not produce a treprostinil product that differs, either structurally or functionally, from that produced using prior art methods.

The Process Optimization Report discloses the impurity analyses for five batches of treprostinil identified by UTC as having been prepared using the process recited in the '393 patent. Ex. 2005, 4–6; *see also* Prelim. Resp. 36 ("Ex. 2005 is a Process Optimization Report that provides results

for batches resulting from step (d) of claims 1 and 10 in the '393 patent,[6] which was performed on specific batches of the diethanolamine salt intermediate produced by steps (a)-(c) [redacted]."). The Process Optimization Report states that the purity of these batches, as determined by HPLC analysis, ranged from [redacted] to [redacted].[7] Ex. 2005, 6. Additionally, the Process Optimization Report indicates that each of the following impurities were detected by HPLC analysis in one or more of the above referenced treprostinil batches: [redacted]. *Id.*

We also observe that although UTC sought, and obtained from the FDA, modification of the specification for the HPLC assay for treprostinil to require a purity range of [redacted], rather than [redacted] Ex. 2006, 3–4, 6; Ex. 2003. Notably, UTC's specification for treprostinil produced according to the '393 patent permits

[6] We note that UTC likely intended to reference independent claim 9 of the '393 patent, rather than dependent claim 10; however our analysis is equally applicable to claim 9 or claim 10.

[7] The reported batch purity values were [redacted], for an average purity of [redacted]. Ex. 2005, 6.

each of the following impurities: [redacted]. Ex. 2006, 6. The analysis of treprostinil purportedly prepared according to the process of Moriarty, set forth in the Walsh Declaration, reveals that each of the impurities detected in Moriarty treprostinil was present in an amount [redacted]. *Compare* Ex.1002, 347, *with* Ex. 2006, 6.

Accordingly, on the record before us, and for purposes of this decision, we conclude that the process steps recited in the challenged claims of '393 patent do not impart structural or functional differences to the claimed product as compared to prior art processes, and therefore, that these process steps do not patentably limit the claimed product. We note, however, that the factual dispute between the parties concerning the existence of any structural or functional differences between treprostinil products produced according to the process recited in the '393 patent and prior art processes, as well as arguments addressing our concerns regarding the relevance of the impurity profile of a product obtained by the recited process to the patentability of claims drawn to a product *comprising* a compound, are appropriate for further development at trial.

C. Principles of Law

To establish anticipation, each and every element in a claim, arranged as recited in the claim, must be found in a single prior art reference. *Net MoneyIN, Inc. v. VeriSign, Inc.*, 545 F.3d 1359, 1369 (Fed. Cir. 2008). "A reference anticipates a claim if it discloses the claimed invention 'such that a skilled artisan could take its teachings in combination with his own knowledge of the particular art and be in possession of the invention.'" *In re Graves*, 69 F.3d 1147, 1152 (Fed. Cir. 1995) (emphasis omitted) (quoting *In re LeGrice*, 301 F.2d 929, 936 (CCPA 1962)).

A patent claim is unpatentable under 35 U.S.C. § 103(a) if the differences between the claimed subject matter and the prior art are such that the subject matter, as a whole, would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 406 (2007). The question of obviousness is resolved on the basis of underlying factual determinations including: (1) the scope and content of the prior art; (2) any differences between the claimed subject matter and the prior art; (3) the level of ordinary skill in the art; and (4) objective evidence of nonobviousness. *Graham v. John Deere Co.*, 383 U.S. 1, 17–18 (1966).

> When a work is available in one field of endeavor, design incentives and other market forces can prompt variations of it, either in the same field or a different one. If a person of ordinary skill can implement a predictable variation, § 103 likely bars its patentability. For the same reason, if a technique has been used to improve one device, and a person of ordinary skill in the art would recognize that it would improve similar devices in the

> same way, using the technique is obvious unless its actual application is beyond his or her skill. *Sakraida* [*v. Ag Pro, Inc.*, 425 U.S. 273 (1976)] and *Anderson's–Black Rock* [*v. Pavement Salvage Co.*, 396 U.S. 57 (1969)] are illustrative—a court must ask whether the improvement is more than the predictable use of prior art elements according to their established functions.

KSR, 550 U.S. at 417.

The level of ordinary skill in the art is reflected by the prior art of record. *See Okajima v. Bourdeau*, 261 F.3d 1350, 1355 (Fed. Cir. 2001); *In re GPAC Inc.*, 57 F.3d 1573, 1579 (Fed. Cir. 1995); *In re Oelrich*, 579 F.2d 86, 91 (CCPA 1978).

D. Anticipation Grounds of Unpatentability Based on Phares

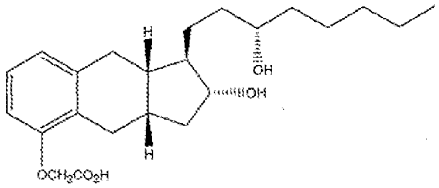
SteadyMed asserts that claims 1–5, 7–9, 11–14, and 16–20 are unpatentable under § 102(b) as anticipated by Phares. Pet. 22–37. Claims 2–5, 7, 8, and 19 depend directly from claim 1, and claims 11–14, 16–18, and 20 depend, directly or indirectly, from claim 9. In support of its assertion, SteadyMed provides detailed explanations as to how Phares discloses each claim limitation (*id.*), and relies upon the Winkler Declaration (Ex. 1009) to support its positions.

UTC counters that the treprostinil product of Phares is physically different from that produced by the process disclosed in the '393 patent, and, therefore, that the process steps disclosed in the claims of the '393 patent are limiting for purposes of the patentability determination. Prelim. Resp. 33–36. UTC also argues that SteadyMed improperly engages in picking and choosing among distinct embodiments in Phares to piece together an

anticipation argument as to the recited process steps. *Id.* at 29–31. UTC further asserts that explicit disclosure of certain claimed process steps is absent from SteadyMed's anticipation analysis, and that SteadyMed fails to show that those limitations are inherently disclosed by Phares. *Id.* at 31–36.

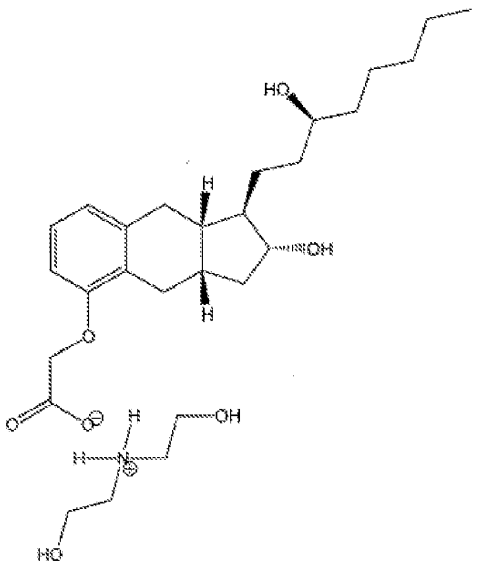
Phares

Phares describes "compounds and methods for inducing prostacyclin-like effects in a subject or patient," including treprostinil and derivatives thereof. Ex. 1005, 10. The chemical structure of treprostinil disclosed by Phares, on page 10 of Exhibit 1005, is reproduced below:



Id. Phares explains that "[t]reprostinil is a chemically stable analog of prostacyclin, and as such is a potent vasodilator and inhibitor of platelet aggregation." *Id.*

Phares further discloses that "[a] preferred embodiment of the present invention is the diethanolamine salt of treprostinil. . . . A particularly preferred embodiment of the present invention is form B of treprostinil diethanolamine." *Id.* at 11. The structure of the diethanolamine salt of treprostinil described by Phares, on page 99 of Exhibit 1005, is reproduced below:

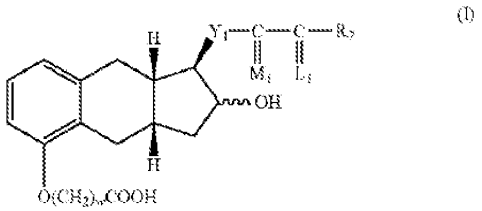


Id. at 99 (claim 49). Phares reports that form B of the diethanolamine salt of treprostinil "appears to be a crystalline material which melts at 107°C." *Id.* at 91.

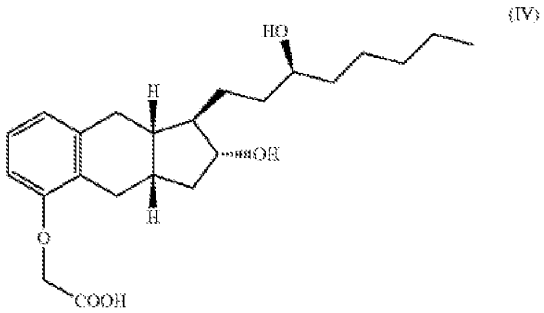
Phares describes the synthesis of (-)-treprostinil, the enantiomer of treprostinil. Ex. 1005, 41–42. Phares explains that "[e]nantiomers of these compounds . . . can be synthesized using reagents and synthons of enantiomeric chirality of the above reagents." *Id.* at 41. In particular, Phares teaches that "the enantiomer of the commercial drug (+)-Treprostinil was synthesized using the stereoselective intramolecular Pauson Khand reaction as a key step and Mitsunobu inversion of the side-chain hydroxyl group." *Id.* at 42. Phares discloses the following reaction procedure: "i. $ClCH_2CN$, K_2CO_3. ii, KOH, CH_3OH, reflux. 83 % (2 steps)." *Id.*

A product comprising a compound [of/having] formula [I/IV] . . . or a pharmaceutically acceptable salt thereof

Claim 1 of the '393 patent recites "[a] product comprising a compound of formula I



or a pharmaceutically acceptable salt thereof," and sets forth a series of process steps for obtaining the claimed product. Claim 9 recites "[a] product comprising a compound having formula IV

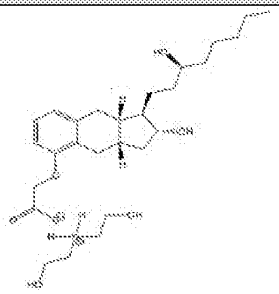
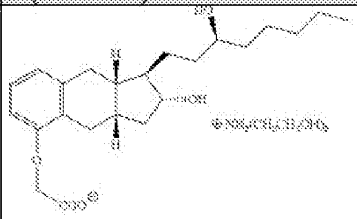


or a pharmaceutically acceptable salt thereof," and includes the same process steps for obtaining the claimed product as recited in claim 1. Claim 9 is identical to claim 1, except that it is drawn to a product comprising the specific treprostinil compound, a species of the genus of claim 1. Accordingly, we address these claims together.

SteadyMed contends that "Phares discloses in its Claim 49 the identical, pharmaceutically acceptable treprostinil diethanolamine salt" claimed in the '393 patent. Pet. 26; *see also* Ex. 1005, 24, 85–93, 99

(claim 49); Ex. 1009 ¶¶ 50–53. In support of SteadyMed's position, Dr. Winkler testifies that "[o]ther than a change in formatting, the two structures [for treprostinil diethanolamine salt] from Phares and the '393 Patent are identical." Ex. 1009 ¶ 53.

Paragraph 52 of the Winkler Declaration depicts a side-by-side comparison of the chemical structures disclosed in claim 49 of Phares, and column 8, lines 50–63 of the '393 patent, reproduced below:

Phares (Ex. 1005), Claim 49	'393 Patent (Ex. 1001), Claims 1 & 9 (column 8)
	

Id. ¶ 52. As shown in the figure from paragraph 52 of the Winkler Declaration, the treprostinil diethanolamine salt disclosed by Phares is structurally identical to that disclosed in the '393 patent.

As set forth in Part II.B above, SteadyMed, relying on the Winkler Declaration, further asserts that the process disclosed in claims 1 and 9 of the '393 patent does not result in a treprostinil product that is physically different or unique from treprostinil produced by prior art methods. Pet. 19–22; *see also* Ex. 1009 ¶¶ 63–71. In support of this position, Dr. Winkler testifies that "[i]n both the '393 Patent and Phares (Ex. 1005), treprostinil diethanolamine salt Form B is made . . . . Phares further discloses a melting point of 107° C (Ex. 1005, p. 91 & Fig. 21) for the Form B salt."

Ex. 1009 ¶ 59; *see also* Ex. 1005, 90–93; Pet. 27. Dr. Winkler also testifies that Phares discloses the same procedure as is claimed in the '393 patent, but describes this procedure in reference to the synthesis of the enantiomer of treprostinil. Ex. 1009 ¶¶ 55–57; Ex. 1005, 41–42; Pet. 25–26. Dr. Winkler thus concludes that in "making the most stable crystal form (Form B) and preparing a product that melts at a higher temperature higher than that described in the '393 Patent, Phares necessarily discloses a salt of at least equal purity to the salt in the '393 Patent." Ex. 1009 ¶ 62; *see also id.* ¶ 60 (citing Ex. 1018, 6); Pet. 27–28.

SteadyMed also contends that Phares anticipates the process steps recited in claim 1. Pet. 24–28; Ex. 1005, 24, 41–42, 85–93, 99 (claim 49); Ex. 1009 ¶¶ 44–71.

UTC does not dispute Phares' disclosure of a treprostinil product; rather, as previewed in relation to its claim construction arguments above, UTC contends that the treprostinil product of Phares is "physically different" from that claimed in the '393 patent, and, therefore, not anticipatory. Prelim. Resp. 33–36. UTC argues that as Phares does not disclose which treprostinil starting material is used, it "cannot inherently anticipate the final treprostinil product of the '393 patent because each method would result in a distinct impurity profile." Prelim. Resp. 34. Referring to the Walsh Declaration, UTC further asserts that "even if the Moriarty treprostinil was used for Phares, Petitioner has failed to provide any evidence that the final Phares treprostinil product would necessarily be the same as the products claimed in the '393 patent." *Id.* UTC also asserts that SteadyMed's reliance

on the melting point of the treprostinil product of Phares as a proxy for purity is misplaced because "melting point does not disclose any specific impurity level and instead may demonstrate a different form, or polymorph, of treprostinil diethanolamine altogether." *Id.* at 35.

UTC additionally argues that Phares does not disclose the same process for generating treprostinil as recited in claims 1 and 9, and that SteadyMed improperly "cobbles together disclosure from four disparate portions of Phares covering multiple distinct embodiments" to arrive at the claimed invention. Prelim. Resp. 27. Further, UTC asserts that even if SteadyMed were permitted to pick and choose steps from various embodiments of Phares, SteadyMed nevertheless must rely on inherency to prove anticipation because "Phares lacks express disclosure of certain claim elements." *Id.* at 28.

The present record supports SteadyMed's contention that the treprostinil diethanolamine salt taught by Phares is identical in structure to the pharmaceutically acceptable treprostinil diethanolamine salt recited in claims 1 and 9. Pet. 24; *see also* Ex. 1005, 24, 99 (claim 49); Ex. 1009 ¶¶ 52–53. Dr. Winkler testifies that the process for producing treprostinil disclosed by Phares yields the same form (Form B) of treprostinil diethanolamine salt as the process of the '393 patent, and that the treprostinil diethanolamine salt of Phares is at least equal in purity to the treprostinil product of the '393 patent. Ex. 1009 ¶¶ 59–62. Dr. Winkler further testifies that Phares discloses the same process for synthesizing treprostinil as the

'393 patent. Ex. 1009 ¶¶ 55–57, 62; Ex. 1005, 41–42; Pet. 25–26. On this record, we credit Dr. Winkler's testimony.

We are not persuaded by UTC's arguments concerning the possibility that treprostinil produced according to Phares might have a different impurity profile than that produced according to the process disclosed in the '393 patent. First, for the reasons set forth in Part II.B above, it is unclear on this record how the use of the transitional phrase "comprising" excludes any impurities that may possibly be produced by the process of Phares. In addition, the present record supports a finding that the impurity profiles for treprostinil diethanolamine salt prepared as described by Phares and that prepared according to the '393 patent are the same. As explained above, Dr. Winkler's testimony regarding the form and melting point of Phares' treprostinil product, is consistent with the conclusion that the products of Phares and the '393 patent are the same.

Furthermore, we note that, as explained in Parts II.A and II.B above, the inter-batch variability in treprostinil impurity profiles, experimental error inherent in impurity measurements, and the variety and extent of impurities permitted in UTC's specification for the manufacture of treprostinil according to the process of the '393 patent, which remained unchanged when UTC migrated from a prior art process to the process of the '393 patent, support the conclusion that the process steps recited in claims 1 and 9 of the '393 patent do not impart any structural or functional differences over prior art treprostinil products.

Accordingly, given the evidence before us in this record, we conclude that SteadyMed has established adequately for purposes of this decision that Phares teaches the treprostinil diethanolamine salt product recited in claims 1 and 9. Because we determine, on the record before us, and for purposes of this decision, that the process steps recited in claims 1 and 9 do not impart structural or functional differences to the claimed treprostinil product and are therefore not limiting, we do not address the parties' contentions concerning Phares' anticipation of the recited process steps.

Conclusion

UTC has not raised any additional arguments with regard to the dependent claims other than those addressed above. We have reviewed SteadyMed's evidence, arguments, and claim charts, and conclude that SteadyMed has sufficiently demonstrated that the dependent claims are also anticipated by Phares. Thus, for the foregoing reasons, we conclude that SteadyMed has shown a reasonable likelihood of prevailing on its assertions that claims 1–5, 7–9, 11–14, and 16–20 are anticipated by Phares.

E. Obviousness Grounds of Unpatentability Based on Moriarty and Phares

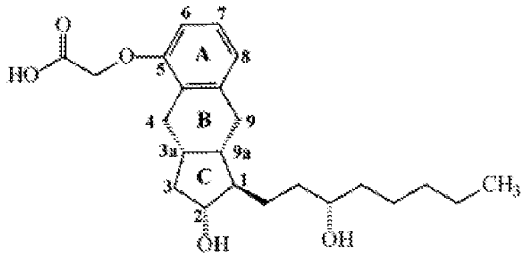
SteadyMed asserts that claims 1–5, 7–9, 11–14, and 16–20 are unpatentable under § 103(a) as obvious in view of Moriarty and Phares. Pet. 37–52. Claims 2–5, 7, 8, and 19 depend directly from claim 1, and claims 11–14, 16–18, and 20 depend, directly or indirectly, from claim 9. In support of its assertion, SteadyMed provides detailed explanations as to how

the combination of Moriarty and Phares discloses each claim limitation (*id.*), and relies upon the Winkler Declaration (Ex. 1009) to support its positions.

UTC counters that "Phares fails to disclose the synthetic route or purity of the claimed treprostinil product. Moriarty adds nothing to cure these deficiencies." Prelim. Resp. 43. UTC asserts that the process described in the '393 patent "unexpectedly reduced the impurity level in the claimed treprostinil product even more" than Moriarty, and reiterates its position that treprostinil produced according to the process of the '393 patent has "a superior purity profile compared to the prior art." *Id.* at 44.

Moriarty

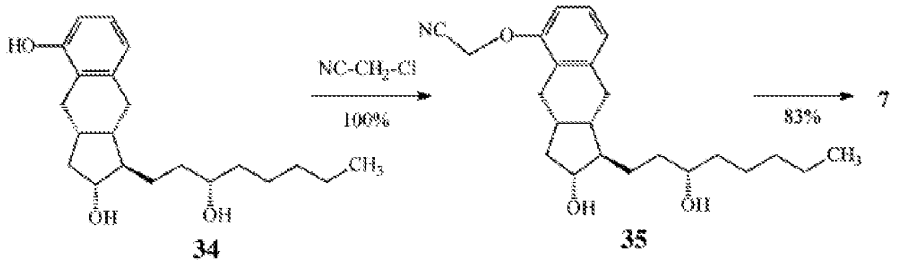
Moriarty describes the synthesis of treprostinil "via the stereoselective intramolecular Pauson-Khand cyclization." Ex. 1004, 1. Formula 7 of Moriarty is reproduced below:



7

Id. at 3. Formula 7 of Moriarty depicts the chemical structure of treprostinil. *Id.*

An excerpt of Scheme 4 of Moriarty is reproduced below:



Id. at 6. The excerpted portion of Scheme 4 of Moriarty illustrates the alkylation Formula 34 to yield Formula 35, and subsequent hydrolysis of Formula 35 with a base (followed by acidification) to yield Formula 7, treprostinil. Ex. 1004, 6, 13.

A product comprising a compound [of/having] formula [I/IV] . . . or a pharmaceutically acceptable salt thereof

SteadyMed contends that Moriarty and Phares respectively disclose treprostinil acid and treprostinil diethanolamine salt, as recited in claims 1 and 9 of the '393 patent. Pet. 22–23, 24, 33, 39, 48; *see also* Ex. 1004, 6, 13; Ex. 1005, 24, 99 (claim 49); Ex. 1009 ¶¶ 74, 76. Furthermore, Dr. Winkler testifies that the combination of Moriarty and Phares "discloses the same process steps and same product of the '393 Patent. For the same reasons discussed above regarding Phares, the purity of the combinations would be of at least equal purity to that claimed in the '393 Patent." Ex. 1009 ¶ 76.

SteadyMed asserts that Moriarty discloses steps (a) and (b) of claims 1 and 9, and that Phares discloses step (c) of these claims. Pet. 43; *see also* Ex. 1004, 6, 13; Ex. 1005, 24; Ex. 1009 ¶ 74. Dr. Winkler testifies

that a relevant skilled artisan would have recognized that the treprostinil acid produced in Moriarty could be purified by contacting it with a base as described by Phares. Ex. 1009 ¶ 74. In addition, as discussed in Part II.D above, Dr. Winkler testifies that Phares "details the same Claim 1 and 9 steps (a) or (b) as were used to make treprostinil in the '117 Patent and Moriarty reference, but applies them to make (-)-treprostinil, the enantiomer of (+)- treprostinil (Ex. 1005, p. 42)." *Id.* ¶55. Dr. Winkler further testifies that a relevant skilled artisan would have had "more than a reasonable expectation of success that the reaction of treprostinil with diethanolamine would be successful" because "Phares (Ex. 1005, p. 24, p. 99, Claim 49) performed the same reaction and it was successful." Ex. 1009 ¶ 80.

UTC reasserts the arguments described above concerning the purity of treprostinil produced according to the process disclosed in the '393 patent. UTC acknowledges that Moriarty itself was an improvement over the prior art, but contends that "the '393 patent unexpectedly reduced the impurity level in the claimed treprostinil product even more." Prelim. Resp. 44. Specifically, UTC contends that "performing step (c) on a product that resulted from steps (a) and (b) provided a product with reduced impurities." *Id.* UTC also reiterates its arguments concerning the Walsh Declaration, and highlights the purported differences in the impurity profile of treprostinil produced according to Moriarty compared to that produced according to the '393 patent.

The present record supports SteadyMed's contention that the treprostinil diethanolamine salt disclosed by the combination of Moriarty

and Phares is identical in structure to the pharmaceutically acceptable treprostinil diethanolamine salt recited in claims 1 and 9. Pet. 41–42; *see also* Ex. 1004, 6, 13; Ex. 1005, 24, 99 (claim 49); Ex. 1009 ¶ 76.

First, as explained in Part II.B above, the present record does not support the conclusion that claims drawn to "[a] product comprising a compound . . ." can be distinguished from prior art products on the basis of differences in the impurity profiles of those products.

Moreover, as explained in detail in Parts II.A, II.B, and II.D above, we determine that the present record supports the contention that the treprostinil product of Moriarty and Phares is the same as that produced according to the steps recited in claims 1 and 9 of '393 patent.

As discussed in Part II.B, the Walsh Declaration fails to disclose the protocols followed in producing the Moriarty and '393 patent treprostinil samples analyzed, and fails to account for the experimental error in Dr. Walsh's impurity measurements. In addition, the inter-batch variability in the types and amounts of impurities observed in treprostinil prepared according to the '393 patent, and the fact that the treprostinil Dr. Walsh prepared according to Moriarty satisfies the FDA purity specification for treprostinil prepared per the '393 patent, lends further support to the conclusion that no structural or functional differences exist between treprostinil produced according to Moriarty, and that produced according to the'393 patent.

Similarly, as discussed in Part II.D, the present record supports a finding that the impurity profile of treprostinil diethanolamine salt prepared

as described by Moriarty in combination with Phares is the same as that prepared according to the '393 patent. Dr. Winkler's testimony regarding the form and melting point of Phares' treprostinil product (Ex. 1009 ¶¶ 59–60, 62), as well as his testimony regarding the disclosure by Phares of the same synthesis process as described by Moriarty (Ex. 1009 ¶¶ 55–57), is consistent with the conclusion that treprostinil diethanolamine generated by reacting Formula 7 of Moriarty with a base, as disclosed by Phares, to form a salt of Formula 7 would result in a treprostinil diethanolamine salt of at least equal purity to that disclosed in the '393 patent.

Accordingly, given the evidence before us in this record, we conclude that SteadyMed has established adequately for purposes of this decision that the combination of Moriarty and Phares renders obvious the treprostinil diethanolamine salt product recited in claims 1 and 9. Because we determine, on the record before us, and for purposes of institution, that the process steps recited in claims 1 and 9 do not impart structural or functional differences to the claimed treprostinil product and are therefore not limiting, we need not address the parties' contentions concerning the obviousness of the recited process steps.

Conclusion

UTC has not raised any additional arguments with regard to the dependent claims other than those addressed above. We have reviewed SteadyMed's evidence, arguments, and claim charts, and conclude that SteadyMed has sufficiently demonstrated that the dependent claims are also rendered obvious by the combination of Moriarty and Phares. Thus, for the

foregoing reasons, we conclude that SteadyMed has shown a reasonable likelihood of prevailing on its assertions that claims 1–5, 7–9, 11–14, and 16–20 are obvious in view of Moriarty and Phares.

F. Obviousness Grounds of Unpatentability Based on Moriarty, Phares, Kawakami, and Eğe

SteadyMed asserts that claims 6, 10, 15, 21, and 22 are unpatentable under § 103(a) as obvious in view of Moriarty, Phares or Kawakami, and Eğe. Pet. 37–52. Although SteadyMed nominally identifies this ground of unpatentability as being over "Moriarty (Ex. 1004) with Phares (Ex. 1005) or Kawakami (Exs. 1006 & 1007) and in further combination with Ege (Ex. 1008)" (Pet. 53 (emphasis omitted), as discussed below, SteadyMed explicitly relies on Kawakami in arguing unpatentability in view of Moriarty, Phares, and Eğe. Accordingly, we understand SteadyMed's stated ground of unpatentability as relying on the combination of Moriarty, Phares, Kawakami, and Eğe. Claims 6, 21, and 22 depend, directly or indirectly, from claim 1, and claims 10 and 15 depend directly from claim 9. In support of its assertion, SteadyMed provides detailed explanations as to how the combination of Moriarty, Eğe, Phares, and Kawakami discloses each claim limitation (*id.*), and relies upon the Winkler Declaration (Ex. 1009) to support its positions.

UTC contends that Kawakami should not be considered as evidence of unpatentability because the declaration certifying the accuracy of the translation is deficient. Prelim. Resp. 38–39. UTC also asserts that Eğe is merely a generic introductory chemistry text, and irrelevant to the

'393 patent. *Id.* at 47. UTC further argues that SteadyMed has not identified a rationale for, or expectation of success in, combining either Moriarty, Phares, and Eğe, or Moriarty, Kawakami, and Eğe. *Id.* In addition, UTC contends that SteadyMed improperly asserts that the cited combination would inherently result in the claimed product. *Id.* at 54.

Kawakami

Kawakami describes "a crystalline dicyclohexylamine salt of a methanoprostacyclin derivative, a manufacturing method thereof, and a purifying method thereof." Ex. 1007, 3. Kawakami discloses obtaining a dicyclohexylamine salt by "mixing a methanoprostacyclin derivative [I] . . . with dicyclohexylamine in an appropriate solvent." Ex. 1007, 5–6. Kawakami explains that "[t]he dicyclohexylamine salt of the methanoprostacyclin derivative [I] thus obtained generally has fairly high purity, and the purity can be further improved by recrystallization as needed with the use of an appropriate solvent." *Id.* at 6.

Kawakami further teaches that "[t]he dicyclohexylamine salt obtained by the present invention can be easily reverted to a free methanoprostacyclin derivative [I] by conventional methods, and the resulting methanoprostacyclin derivative exhibits excellent crystallinity compared with substances not purified according to the present invention." *Id.*

Eğe

Eğe is an organic chemistry textbook. Ex. 1008, 1. Eğe discloses:

> Carboxylic acids that have low solubility in water, such as benzoic acid, are converted to water-soluble salts by reaction

> with aqueous base. Protonation of the carboxylate anion by a strong acid regenerates the water-insoluble acid. These properties of carboxylic acids are useful in separating them from reaction mixtures containing neutral and basic compounds.

Id. at 8 (reference omitted).

Compliance with 37 C.F.R. § 42.63(b)

Kawakami is a Japanese patent application. Ex. 1006. SteadyMed submitted an English translation of Kawakami (Ex. 1007), as well as an affidavit certifying that translation (Ex. 1011) with its Petition.

UTC nevertheless contends that Kawakami should not be considered as evidence of unpatentability because the President of the translation service, rather than the individual who prepared the translation, executed the certification affidavit. Prelim. Resp. 38–39. UTC asserts that certification affidavit is objectionable because the affiant lacks personal knowledge of the relevant facts, the accuracy of the translation cannot be determined, and the translator is shielded from cross-examination. *Id.* at 39.

In view of the record before us, and for purposes of this decision, we decline UTC's invitation to disregard Kawakami. No credible prejudice to UTC has been called to our attention, and none is apparent. An English translation of Kawakami was available to UTC in time to prepare its Preliminary Response.[8] Furthermore, UTC has not identified any error in

[8] It does not appear that UTC has served objections on SteadyMed concerning the adequacy of the English translation of Kawakami or the certifying affidavit.

the translation that would call into question its authenticity. Regarding UTC's contention that the accuracy of the translation cannot be determined absent a certification affidavit from the translator himself, we note that the commission of an independent translation would confirm the veracity of the translation submitted by SteadyMed. We also observe that even if the individual personally responsible for generating the English translation of Kawakami had submitted a certification affidavit, UTC would not have had the opportunity to cross-examine him prior to the submission of its Preliminary Response.

Accordingly, on the record before us, and for purposes of this decision, we decline UTC's request that we disregard Kawakami. We observe, however, that the adequacy of the Kawakami translation and certification affidavit may be subject to further challenge during trial.[9]

Rationale to Combine Prior Art Teachings

Building on the rationale for combining Moriarty and Phares discussed in Part II.E above, SteadyMed contends that a relevant skilled

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[9] Pursuant to 37 C.F.R. § 42.64(b)(1), "[a]ny objection to evidence submitted during a preliminary proceeding must be served within ten business days of the institution of the trial. . . . The objection must identify the grounds for the objection with sufficient particularity to allow correction in the form of supplemental evidence." "The party relying on evidence to which an objection is timely served may respond to the objection by serving supplemental evidence within ten business days of service of the objection." 37 C.F.R. § 42.64(b)(2). Furthermore, "[a] motion to exclude evidence must be filed to preserve any objection. . . . The motion may be filed without prior authorization from the Board." 37 C.F.R. § 42.64(c)

artisan would add further purification steps from Kawakami and Eğe because Kawakami "discloses that the dicyclohexylamine salt of a methanoprostacyclin derivative 'can be easily reverted to the free methanoprostacyclin derivative by *conventional methods*,'" and that the "fairly high purity" of the salt obtained "can be further improved by recrystallization as needed with the use of an appropriate solvent." Pet. 53; *see also* Ex. 1007, 6; Ex. 1009 ¶ 83. Dr. Winkler testifies that, as evidenced by Eğe, a relevant skilled artisan "would understand that one such conventional method for converting the dicyclohexylamine salt of a methanoprostacyclin derivative to the free methanoprostacyclin derivative, or converting the treprostinil diethanolamine salt to treprostinil (*i.e.*, the free acid) is by treating the salt with a strong acid such as HCl or H_2SO_4." Ex. 1009 ¶ 84; *see also* Pet. 53–54.

Dr. Winkler elaborates on this rationale for combining the cited references, testifying that a relevant skilled artisan

> would want to form the treprostinil diethanolamine salt, purify it, and then convert it back to its free form (*i.e.*, treprostinil) in order to obtain excellent crystallinity and increased purity. And Ege (Ex. 1008, p. 8) teaches that one such method for obtaining the free form of treprostinil or any carboxylic acid would be by treatment of the carboxylate salt with a strong acid.

Ex. 1009 ¶ 88; *see also* Ex. 1008, 8; Pet. 54.

UTC does not address the combination of Moriarty, Eğe, Phares, and Kawakami. Instead, UTC addresses Moriarty, Eğe, and Phares as one combination, and Moriarty, Eğe, and Kawakami as an alternative combination. Prelim. Resp. 46–47.

As an initial matter, UTC asserts that Eğe is irrelevant to the '393 patent because it does not discuss prostacyclin derivatives or pharmaceutical synthesis. *Id.* at 47. UTC argues that Eğe in fact "would teach away or discourage the use of salt formation for purifying a mixture of compounds that includes other carboxylic-acid containing compounds as impurities." *Id.* at 48.

Regarding the combination of Moriarty, Eğe, and Phares, UTC contends that "even though Phares discloses forming a salt from treprostinil free acid, and Ege generally discusses that carboxylate salt formation was known in the art, there would have been no motivation or expectation of success in using these teachings on the already-formed free acid disclosed in Moriarty." Prelim. Resp. 50. Pertaining to the combination of Moriarty, Eğe, and Kawakami, UTC asserts that SteadyMed "fails to establish that a [relevant skilled artisan] would reasonably expect the teachings of Kawakami to extend to the products in Moriarty." *Id.* at 52.

UTC also argues that Dr. Winkler's testimony regarding the reasons a relevant skilled artisan would want to form treprostinil diethanolamine salt, and treat it with a strong acid to convert it back to its free form (treprostinil) is improperly conclusory. *Id.* at 50, 52.

On the record before us, and for purposes of this decision, we agree that SteadyMed has sufficiently demonstrated that a relevant skilled artisan would have had reason to include the carboxylate salt formation and regeneration of the neutral carboxylic acid with the syntheses of Moriarty and Phares based on the teachings of Kawakami and Eğe.

We recognize, but do not find persuasive, UTC's position that Eğe is irrelevant to the synthesis of prostacyclin derivatives, and that it teaches away from the use of salt formation for purifying a mixture of compounds that includes other carboxylic-acid containing compounds as impurities. First, we observe that SteadyMed relies on Eğe not for any teachings specific to prostacyclin derivative synthesis, but rather, to support the contention that the addition of a strong acid to a carboxylate salt to regenerate the neutral carboxylic acid is a conventional purification technique in organic chemistry. Pet. 53–55; Ex. 1009 ¶¶ 86, 88. In particular, Dr. Winkler testifies that the "addition of a strong acid to a carboxylate salt to regenerate the neutral carboxylic acid is a common reaction in organic chemistry and this process is well within the skill of one of ordinary skill in the art (indeed, a process that I teach to my organic chemistry students)" (Ex. 1009 ¶ 85), and that Eğe, an introductory organic chemistry text, "discloses that sodium benzoate (i.e., a carboxylate salt) can be converted back to benzoic acid (i.e., a carboxylic acid) by treatment with the acid HCl" (*id.* ¶ 86). On this record, we credit Dr. Winkler's testimony, as it is consistent with the prior art.

Second, we note that even crediting UTC's position that the use of salt formation would not be effective for purifying treprostinil from its stereoisomers (Prelim. Resp. 47–48), the present record suggests that it would be effective for removing other impurities (Pet. 53–55; Ex. 1009 ¶¶ 86, 88). Moreover, as explained below, the present record, including Kawakami, indicates that treprostinil diethanolamine salt formation followed

by regeneration of treprostinil using a strong acid is an effective purification step. Pet. 53–55; *see also* Ex. 1007, 6; Ex. 1008, 8; Ex. 1009 ¶¶ 82–90.

Additionally, we agree with SteadyMed that a relevant skilled artisan would have had reason to combine Moriarty, Phares, Kawakami, and Eğe. Pet. 53–55; Ex. 1009 ¶¶ 82–90. For example, Dr. Winkler testifies that a relevant skilled artisan would want to include a carboxylate salt formation and regeneration of the neutral carboxylic acid as described by Eğe with the syntheses of Moriarty and Phares because Kawakami teaches that "'the dicyclohexylamine salt obtained by the present invention can be easily reverted to a free methanoprostacyclin derivative [I] by conventional methods, and the resulting methanoprostacyclin derivative exhibits excellent crystallinity compared with substances not purified according to the present invention.'" Ex. 1009 ¶ 86; *see also* Ex. 1007, 6; Pet. 53–55. Dr. Winkler additionally testifies that a skilled artisan would be motivated to form treprostinil diethanolamine salt, and treat it with a strong acid to "obtain excellent crystallinity and increased purity" of the final treprostinil product (Ex. 1009 ¶ 88), and that a skilled artisan would have a reasonable expectation of success in performing such reaction because it is "a common reaction in organic chemistry and this process is well within the skill of one of ordinary skill in the art" (*id.* ¶ 90).

On this record, we credit Dr. Winkler's testimony, as it is consistent with the prior art. Moreover, we disagree with UTC that Dr. Winkler's testimony is improperly conclusory. Rather, as illustrated by the excerpts of his testimony referenced above, Dr. Winkler supports his opinions with

reference to the cited art, as well as his experience as a chemist and chemistry professor.

Accordingly, on the record before us, we agree that SteadyMed has sufficiently demonstrated that one of ordinary skill in the art would have included the carboxylate salt formation and regeneration of the neutral carboxylic acid of Eğe with the syntheses of Moriarty and Phares based on Kawakami's disclosure that the conversion of salts of prostacyclin derivatives to their free forms by conventional methods increases purity of the final product. *See KSR*, 550 U.S. at 417 ("[I]f a technique has been used to improve one device, and a person of ordinary skill in the art would recognize that it would improve similar devices in the same way, using the technique is obvious unless its actual application is beyond his or her skill.").

Claims 6, 15, and 21

Claims 6, 15, and 21 each recite the product of either claim 1 or claim 9, subject to additional process steps. For example, claim 6 recites "[t]he product of claim 1, wherein the acid in step (d) is HCl or H_2SO_4." Ex. 1001, 19:39–40. Claim 15 similarly recites "[t]he product of claim 9, wherein the acid in step (d) is HCl." *Id.* at 20:59–60. Claim 21 simply recites "[t]he product of claim 1, wherein step (d) is performed." *Id.* at 21:13.

The present record supports SteadyMed's contention that claims 6, 15, and 21 would have been obvious in view of Moriarty, Eğe, Phares, and

Kawakami. Pet. 53–56; Ex. 1009 ¶¶ 82–90. For example, Dr. Winkler testifies that

> the combination of Moriarty (Ex. 1004) and Phares (Ex. 1005) (or Kawakami, Exs. 1006 & 1007) and Ege (Ex. 1008) would disclose . . . treprostinil of at least equal purity to that claimed in the '393 Patent, since the combination of these references discloses the same product and same process of Claims 1 and 9.

Ex. 1009 ¶ 89; *see also* Pet. 54. In addition, as explained above, Dr. Winkler testifies that a skilled artisan would have made the cited combination, with an expectation of success, in order to obtain a treprostinil product of improved purity. Ex. 1009 ¶¶ 88–90; Pet. 54–55. On this record, we credit Dr. Winkler's testimony.

UTC does not offer evidence or argument to suggest that the additional process steps recited in claims 6, 15, and 21 impart structural or functional differences to the claimed product beyond that discussed above in Parts II.B, II.D, and II.E. Rather, UTC contends that SteadyMed has not asserted that the products of claims 6, 15, and 21 would have been obvious in view of the cited art. Prelim. Resp. 54. UTC frames SteadyMed's position as an argument that the recited process steps would have been obvious, and would have inherently resulted in the claimed product. *Id.*

We do not find UTC's contentions persuasive. We observe that claims 6, 15, and 21 differ from their respective independent claims only in that they require the performance of optional step (d) from claims 1 and 9, and in the case of claims 6 and 15, specify the acid to be used in carrying out that process step. Ex. 1001, 19:39–40, 20:59–60. As set forth in detail in Parts II.A, II.B, II.D, and II.E, on the record before us, and for purposes of

this decision, we conclude that the process steps recited in the challenged claims, including step (d), do not impart structural or functional differences over prior art treprostinil products.

Furthermore, we disagree with UTC's characterization of SteadyMed's obviousness argument. We note, for example, that under the general rule for the interpretation of product-by-process claims, which we determine applies here, the products of claims 1, 6, and 21 are interpreted to be the same, namely, the product of claim 1. Likewise, the same analysis applies for the products of claims 9 and 15.

Accordingly, given the evidence before us in this record, we conclude that SteadyMed has established adequately for purposes of this decision that the combination of Eğe, Phares, and Kawakami renders obvious the treprostinil products of claims 6, 15, and 21. Because we determine, on the record before us, and for purposes of institution, that the process steps recited in claims 6, 15, and 21 do not impart structural or functional differences to the claimed treprostinil product, we do not address the parties' contentions concerning the obviousness of the recited process steps.

Claim 10

Claim 10 recites "[t]he product of claim 9, wherein the purity of product of step (d) is at least 99.5%." Ex. 1001, 20:47–48. The present record supports SteadyMed's contention that claim 10 is obvious in view of Moriarty, Eğe, Phares, and Kawakami. Pet. 55–56; *see also* Ex. 1009 ¶¶ 82–90. As detailed in Parts II.B, II.D, and II.E, the present record supports SteadyMed's position that Moriarty discloses treprostinil free acid having a

purity of 99.7% (Pet. 20; *see also* Ex. 1004, 13; Ex. 1009 ¶ 65), and Phares discloses treprostinil diethanolamine salt of the same form and at least the same purity as that claimed in the '393 patent (Pet. 27–28; Ex. 1005, 88–93; Ex. 1009 ¶¶ 59–62). The present record further supports SteadyMed's contention that even if Dr. Walsh's impurity measurements are credited, the 0.1% difference between the purity of the sample prepared according to Moriarty, and claim 10 is within the expected level experimental error for impurity measurements, and the degree of inter-batch variability in impurity content is such that Dr. Walsh's results are insufficient to support a conclusion of nonobviousness. Pet. 19–22; *see also* Ex. 1009 ¶¶ 63–71.

UTC does not offer evidence or argument to suggest that the additional process step recited in claim 10 imparts structural or functional differences to the claimed product beyond that discussed above in Parts II.A, II.B, II.D, and II.E. Neither does UTC present any additional argument regarding the recited purity requirement beyond those already addressed above. UTC does reassert its position, discussed with regard to claims 6, 15, and 21, that SteadyMed has not asserted that the product of claim 10 would have been obvious in view of the cited art. Prelim. Resp. 54. For the reasons set forth above, however, we do not find this contention persuasive.

Accordingly, given the evidence before us in this record, we conclude that SteadyMed has established adequately for purposes of this decision that the combination of Eğe, Phares, and Kawakami renders obvious the treprostinil product of claim 10. Because we determine, on the record before us, and for purposes of institution, that the process steps recited in claim 10

do not impart structural or functional differences to the claimed treprostinil product, we do not address the parties' contentions concerning the obviousness of the recited process steps at this time.

Claim 22

Claim 22 recites "[t]he product of claim 21, wherein the product comprises a pharmaceutically acceptable salt formed from the product of step (d)." Ex. 1001, 21:14–16. The present record supports SteadyMed's contention that claim 22 is obvious in view of Moriarty, Eğe, Phares, and Kawakami. Pet. 56–57; *see also* Ex. 1009 ¶¶ 82–90. As discussed above in Parts II.D and II.E, the present record supports SteadyMed's position that the cited combination renders obvious a pharmaceutically acceptable treprostinil salt.

UTC does not offer evidence or argument to suggest that the additional process step recited in claim 22 imparts structural or functional differences to the claimed product beyond that discussed above in Parts II.A, II.B, II.D, and II.E. Neither does UTC present any additional argument regarding the recited purity requirement beyond those already addressed above. UTC does reassert its position, discussed with regard to claims 6, 15, and 21, that SteadyMed has not asserted that the product of claim 22 would have been obvious in view of the cited art. Prelim. Resp. 54. For the reasons set forth above, however, we do not find this contention persuasive

Accordingly, given the evidence before us in this record, we conclude that SteadyMed has established adequately for purposes of this decision that the combination of Eğe, Phares, and Kawakami renders obvious the

treprostinil products of claim 22. Because we determine, on the record before us, and for purposes of institution, that the process steps recited in claims 22 do not impart structural or functional differences to the claimed treprostinil product, we do not address the parties' contentions concerning the obviousness of the recited process steps at this time.

Conclusion

For the foregoing reasons, we conclude that SteadyMed has shown a reasonable likelihood of prevailing on its assertions that claims 6, 10, 15, 21, and 22 are obvious in view of Moriarty, Eğe, Phares, and Kawakami.

G. Secondary Considerations of Non-Obviousness

UTC contends that objective indicia of non-obviousness, such as purported evidence of long-felt but unmet need, unexpected results, commercial success, and copying support the patentability of the challenged claims of the '393 patent. Prelim. Resp. 55–58.

We conclude that the evidence of secondary considerations currently of record is not sufficient, at this point in the proceeding, to support UTC's contention. As an initial matter, we observe that "secondary considerations are better considered in the context of a trial when the ultimate determination of obviousness is made." *Crocs, Inc. v. Polliwalks, Inc.*, Case IPR2014-00424, slip op. 16 (PTAB Aug. 20, 2014) (Paper 8). In addition, we note that UTC's contentions regarding long-felt need and unexpected results are predicated on UTC's claim that treprostinil made according to the process described in the '393 patent has fewer impurities than treprostinil produced by other methods. However, as explained in Parts II.B, II.D, and

II.E above, the present record does not support that contention. We also observe that UTC does not offer evidence of a nexus between the claimed invention and its commercial success. For example, UTC does not offer evidence concerning its relative share of the market for treprostinil products, or demonstrating that its revenues or market share increased after it began manufacturing treprostinil according to the process described in the '393 patent. Finally, we note that the mere existence of litigation concerning the '393 patent alone is insufficient to establish copying. *See Iron Grip Barbell Co. v. USA Sports, Inc.*, 392 F.3d 1317, 1325 (Fed. Cir. 2004) ("Not every competing product that arguably fails within the scope of a patent is evidence of copying. Otherwise every infringement suit would automatically confirm the nonobviousness of the patent.").

H. Other Asserted Grounds of Unpatentability

SteadyMed also asserts the following ground of unpatentability:

Claims	Basis	Reference(s)
1–5, 7–9, 11–14, and 16–20	§ 103(a)	Moriarty and Kawakami

In light of the grounds specifically discussed above, on the basis of which we institute review, we exercise our discretion and decline to consider these other grounds asserted in the Petition. *See* 37 C.F.R. § 42.108(a). We observe that SteadyMed presents the above ground of unpatentability and the obviousness of claims 1–5, 7–9, 11–14, and 16–20 in view of Moriarty and Phares, a ground on which we institute review, in the alternative.

III. CONCLUSION

For the foregoing reasons, we determine that the information presented in the Petition establishes that there is a reasonable likelihood that SteadyMed would prevail in challenging claims 1–22 of the '393 patent. At this juncture, we have not made a final determination with respect to the patentability of the challenged claims, nor with respect to claim construction.

IV. ORDER

For the foregoing reasons, it is

ORDERED that pursuant to 35 U.S.C. § 314(a), an *inter partes* review is hereby instituted for the following grounds of unpatentability:

Claims	Basis	Reference(s)
1–5, 7–9, 11–14, and 16–20	§ 102(b)	Phares
1–5, 7–9, 11–14, and 16–20	§ 103(a)	Moriarty and Phares
6, 10, 15, 21, and 22	§ 103(a)	Moriarty, Phares, Kawakami, and Eğe

FURTHER ORDERED that no other ground of unpatentability asserted in the Petition is authorized for this *inter partes* review; and

FURTHER ORDERED that pursuant to 35 U.S.C. § 314(c) and 37 C.F.R. § 42.4, notice is hereby given of the institution of a trial; the trial will commence on the entry date of this decision.

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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

STEADYMED LTD.,

Petitioner,

v.

UNITED THERAPEUTICS CORPORATION,

Patent Owner.

Case IPR2016-00006
Patent 8,497,393

Patent Owner Response to Petition

4814-0612-4340.3

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I. INTRODUCTION

United Therapeutics Corporation ("UTC") submits this Response in accordance with 35 U.S.C. § 316(a)(8) and 37 C.F.R. § 42.120, responding to the instituted grounds of the Petition for *Inter Partes* Review filed by SteadyMed Ltd. ("SteadyMed") challenging claims 1-22 of U.S. Patent No. 8,497,393 ("the '393 patent"). The Declaration of Dr. Williams ("Ex. 2020") and of Dr. Ruffolo ("Ex. 2022") are filed herewith in support of the Response (Ex. 2020 and Ex. 2022, respectively). The Board should conclude that SteadyMed has failed to prove by a preponderance of the evidence that the instituted claims are unpatentable, as required under 35 U.S.C. § 316(e).

II. SUMMARY OF THE ARGUMENT

SteadyMed's anticipation and obviousness arguments are flawed for two fundamental reasons. First, SteadyMed's arguments rely on Moriarty (Moriarty *et al.*, J. Org. Chem. 2004, 1890-1902; Ex. 1004) and Phares (International Publication No. WO 2005/007081; Ex. 1005), but neither reference discloses the same highly pure treprostinil or treprostinil diethanolamine product claimed by the '393 patent when properly construed, let alone the same synthesis recited in the instituted claims. In fact, the Office considered both references during prosecution of the '393 patent, and the Office construed the claims of the '393 patent in a way that distinguished the product of the '393 patent specifically from the Moriarty

product. Moreover, a person of ordinary skill in the art ("POSA") would not look to either Eğe (Seyhan N. Eğe, Organic Chemistry 543-547 (2d ed. 1989) (Ex. 1008) or Kawakami (JP 56-122328A) (Ex. 1007) as neither reference is relevant to further purification of the complex treprostinil carboxylic acid structure that is at issue in the '393 patent, and a POSA would have no reasonable expectation of success in combining these references with either Moriarty or Phares.

Second, SteadyMed's anticipation and obviousness arguments are flawed because they misunderstand, both the error associated with such measurements and the difference between "assay purity" against a standard and measurements of purity that directly measure the level of impurities. As explained in the Williams and Ruffolo Declarations, this misunderstanding resulted in Petitioner's incorrect assertion that there are inconsistencies between the purity values recited in the '393 specification, the Walsh Declaration, and the Moriarty prior art. Ex. 2020 at ¶¶88-89; Ex. 2022 at ¶¶73-74. Dr. Williams notes that the '393 patent itself expressly refers to assay purity values as "HPLC (assay)" values whenever it uses such measurements, as opposed to other purity values based on measuring amount of impurities. Ex. 2020 at ¶89. Dr. Ruffolo further explains that FDA drug approval system rests on precise measurements of individual impurities that make up a purity "specification" for a drug, which can be reliably determined within the detection limits of HPLC measurements. Ex. 2022 at ¶¶32-35 and 44-50. Dr.

Ruffolo also specifically notes that it is routine to have assay purity values above 100% because it is a relative value measurement. Ex. 2022 at ¶53.

SteadyMed's purported expert, Dr. Winkler, confirmed this misunderstanding. Dr. Winkler acknowledged at his deposition that FDA's purity specification of less than [redacted]% for the impurity [redacted] indicates that precise measurements of impurities are possible: "I would think that the error in the measurement for [redacted] would be, should be less than [redacted] percent." Ex. 2051 at 64:7-9. Dr. Winkler further acknowledged that he did not know how the treprostinil purity specification adopted by FDA could change from [redacted]% to [redacted]% and stated that he viewed purity levels above 100% as errors: "I think the thing that I am able to conclude from the data that is on page 6 of this, of this letter [Ex. 2006] is that the error in the HPLC assay could be as high as [redacted] percent in the first column and by my analysis could be as high as [redacted] percent in the second column." Ex. 2051 at 86:15-21; 24-25; 87:2-9. As Dr. Williams explained, Dr. Winkler's conclusions on this point appear "to arise from Dr. Winkler's fundamental misunderstanding of how assay purity values are calculated." Ex. 2020 at ¶¶90-92; *see also* Ex. 2022 at ¶¶74. Moreover, Dr. Winkler admitted he did not know what the actual error was associated with the measurements submitted in the Walsh declaration. Ex. 2051 at 62:16-25; 63:2-14. Because Dr. Winkler does not understand the basic differences in types of purity measurements and their related

errors that are used in the '393 patent, discussed in the Walsh Declaration, and which form the basis for FDA's regulation of drug product manufacturing, his declaration should not be credited.

Moreover, the Williams Declaration establishes that there are measurable structural differences between the average impurity profiles of the Moriarty product and the claimed product based on data obtained from 175 batches. Ex. 2020 ¶¶94-99, Appendices A-B; see also Ex. 2005, Ex. 2036, Ex. 2037, Ex. 2052, Ex. 2053. The average impurity profiles show that Moriarty process and the '393 process produce two physically distinct products that contain different total and specific impurities. *Id.* Specifically, the claimed product essentially lacks certain impurities found in the Moriarty product, such as [redacted], and [redacted]. Ex.2020 at ¶¶96-97. The claimed product also contains much smaller amounts of other impurities that are found in the Moriarty product, such as [redacted], [redacted]. *Id.* at ¶96.

Furthermore, based on the same 175 batches, the average purity of the '393 product is [redacted] greater than the average purity of the Moriarty product, thereby corroborating that the Moriarty process and the '393 process produces two physically distinct products that contain measurable and significant structural differences. *Id.* at ¶98.

Finally, the initial claim construction of the preamble "a product… comprising" urged by SteadyMed and adopted by the Board would violate the canon that patent claims may not be construed to encompass material that was clearly disavowed in order to obtain allowance of claims. Even under the broadest reasonable interpretation standard, the Board has found in its own cases that the prosecution history may limit the plain meaning of a limitation in a claim, which otherwise is presumed to apply. The '393 claims were allowed after submission of the Walsh Declaration, which established the differences between the '393 products and the Moriarty product. This disavowal of the Moriarty subject matter is further reinforced by additional intrinsic evidence. The '393 patent includes a side-by-side comparison in Example 6 to show the difference between the Moriarty product and the '393 product and repeatedly references higher purity and different impurity profile compared to Moriarty. In the face of this disavowal, it is improper to construe "a product …comprising" to allow the impurities "without limitation," as such a construction would encompass the impurity profile of Moriarty.

In addition, the Williams Declaration explains why Phares cannot anticipate the claimed products because of the particular conditions used to prepare the Phares product for polymorph screening and because of the uncertain provenance of starting treprostinil used to make the diethanolamine salt.

As to instituted grounds 2 and 3, Dr. Williams also explains why the references in the instituted obviousness grounds would not have been combined in the asserted manner due to lack of motivation and the failure of the references to provide an expectation of success for achieving the purity level and impurity profile of the '393 patent in the specific case of treprostinil. Kawakami teaches away from the selection of diethanolamine, the salt specifically claimed in claims 14 and 18. Lastly, secondary considerations of long-felt need and unexpected results would rebut any case of obviousness as to grounds 2 and 3.

In view of the foregoing, SteadyMed has not met its burden of proving the unpatentability of claims 1-22 by a preponderance of the evidence, as required under 35 U.S.C. § 316(e).

III. STRUCTURAL/FUNCTIONAL DIFFERENCES OF THE CLAIMED PRODUCTS OVER THE CITED ART

The combined Declarations of Dr. Williams and Dr. Ruffolo establish that the '393 product has a different impurity profile than the Moriarty product, and in fact, that the '393 product has higher average purity. These differences matter. FDA uses both overall purity and levels of individual impurities ("purity specification") as a basis to regulate the manufacturing of pharmaceuticals. Batches that fall outside of the purity specification cannot be sold or used to treat

patients. Thus, differences in purity and impurity profile are not merely academic, but critical to the successful manufacture of a clinical product.

A. The Importance of Purity in Pharmaceuticals

As noted by the '393 patent itself, "because Treprostinil, and other prostacyclin derivatives are of great importance from a medicinal point of view, a need exists for an efficient process to synthesize these compounds on a large scale suitable for commercial production." Ex. 1001, col. 1:57-61. The invention therefore "provides for a process that is more economical, safer, faster, greener, easier to operate, and provides higher purity." *Id.*, col. 5:47-50. As the treprostinil product is a drug product subject to the rules of FDA, the reduction of impurities is of great importance in the drug. Drug purity is defined by FDA as "relative freedom from extraneous matter in the finished product, whether or not harmful to the recipient or deleterious to the product." See, Ex. 2022 at ¶33; see also 21 C.F.R. §600.3 (r) (2015). The purity of a drug is of such importance to FDA that the purity level of a drug substance must appear in the drug product specification, which is a collection of data about the drug required by FDA. *See*, Ex. 2022 at ¶¶32-34. "Regulatory agencies have also sought to increase levels of purity, and consequently decrease levels of impurities, in order to provide to the maximum extent possible, the highest level of safety to patients." *Id.* at ¶36. This is due to

the fact that even trace amounts of impurities can sometime pose serious health concerns.

For example, the drug penicillin is one of the best known and extensively studied examples of trace impurities that can cause serious, life-threatening adverse events. *Id.* at ¶62. While penicillin is safe and effective for most people, it can cause serious allergic reactions resulting in anaphylaxis and death. *Id.* Because the amount of trace impurity of penicillin needed to cause an allergic reaction is so low, FDA has mandated the production of penicillin active pharmaceutical ingredient (API) and finished product to be made in buildings entirely separate from buildings that manufacture other APIs or finished drug product. *Id.*, *see also* FDA Guidance for Industry, Non-Penicillin Beta-Lactam Drugs: A CGMP Framework for Preventing Cross-Contamination, (2013) (Ex. 2047) at 1-6. The same is true for the drug cephalosporin. Ex. 2022 at ¶63; *see also* Ex. 2047 at 1-6.

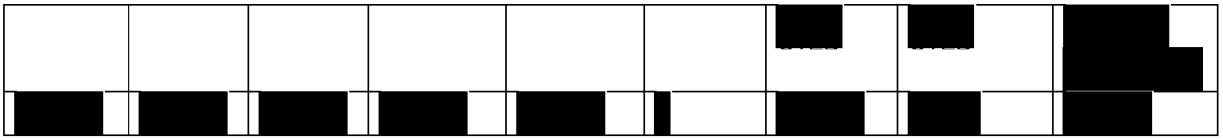
Additionally, human insulin is another example. For many years, human insulin was derived from pig pancreases, but then it became possible to produce human insulin in the bacteria *E. coli* using large bioreactors. Ex. 2022 at ¶64. Even though the human insulin derived from *E. coli* was highly pure, it contained very small trace amounts of *E. coli*, a very dangerous bacteria causing reactions (directly from the trace amounts of bacteria, and not due to infection) in some people even in trace amounts. *Id.* As a result, the product needed to be even more

highly purified to further minimize or eliminate the trace bacterial contaminants. *Id.* These examples highlight the importance of drug purity in pharmaceutical formulations and the potential risks to patients between two products that differ in their impurity profile and purity. By having a different impurity profile and overall purity, two products are structurally and functionally different.

B. The '393 Product Has A Different Impurity Profile and a Higher Purity Than Moriarty

As detailed in Dr. Williams' Declaration and supporting exhibits, comparing the average impurity profiles for the '393 product and the Moriarty product using data obtained from over 175 batches reveals measurable structural differences, as the two processes produce physically different products which contain different total and specific amounts of impurities. Ex. 2020 ¶¶94-99 and Appendices A-B; *see also* Ex. 2005, Ex. 2036, Ex. 2037, Ex. 2052, Ex. 2053. The batch reports show that the Moriarty product and the claimed product exhibit different impurity profiles and that the claimed product has a higher average purity than Moriarty's product. *Id.*

Moriarty Process Impurities (Average Percent Detected)								
1AU90	**2AU90**	**3AU90**	**750W93**	**751W93**	**97W86**	**ethyl ester**	**methyl ester**	**Total Related Substance**
0.0473	0.0407	0.2545	0.1646	0.1025	0.0405	0.0889	0.1028	**0.9545**
'393 patent Process Impurities (Average Percent Detected)								
[redacted]								



In total, the '393 product has [redacted] times fewer impurities than the Moriarty product.[1] Ex. 2020 ¶¶94-95. Additionally, certain specific impurities found in the prior art Moriarty product are essentially eliminated in the '393 product, as the '393 product does not contain detectable amounts of the impurity [redacted], and none of the commercial batches of the '393 product contain detectable amounts of [redacted] or [redacted]. Ex. 2020 ¶¶94, 96-97. Other impurities, including [redacted], [redacted], [redacted], and [redacted], are also greatly reduced in the '393 product as compared to the Moriarty product, while the level of the [redacted] impurity is slightly increased in the '393 product. Ex. 2020 ¶96. These substantial differences between the impurity profiles of the '393 product and the Moriarty product constitute structural differences between the claimed product and the prior art.

Furthermore, the average purity based on data from over 175 batches is higher for the '393 product than that of Moriarty. As shown above, the average purity of a Moriarty batch was 99.05% while the average purity of a '393 batch

[1] Moriarty Total Related Substances: 0.9545; '393 patent Process Total Related Substances: [redacted]

was [redacted]. Ex. 2020 ¶¶94-99. This is a marked improvement in overall purity. Moreover, the purity analyzed in these batches – the total related substances – is exactly the same type of analysis Dr. Walsh referred to in his declaration when referring to purity of the '393 patent process versus that of the Moriarty process. Thus, this analysis is consistent with how the inventor interpreted the purity of the '393 patent. And this analysis also persuaded the Office to allow the claims.

The Institution Decision cited to the Walsh Declaration for revealing "that each of the impurities detected in [the tested batch of] Moriarty treprostinil was present in an amount below that identified as acceptable in UTC's own specification for treprostinil produced according to the process disclosed in the '393 patent." Paper 12 at 20-21. First, the above data shows that the average amount of each impurity and the average purity is different between Moriarty treprostinil and the '393 product. Second, whether an isolated batch of Moriarty treprostinil does or does not satisfy the new FDA purity specification is not relevant to patentability. The question for patentability is whether or not a given batch of *starting* Moriarty treprostinil (steps a and b of the '393 independent claims) will be physically changed when step (c) is performed *on that batch*. The above averages show that it does change, as do the large scale synthesis examples 4-6 in the '393 patent. While Moriarty treprostinil may show inter-batch variation in overall purity and impurity profiles, the data of record establishes that

performing step (c) *on a given starting batch* of Moriarty treprostinil will lead to a higher purity and a different impurity profile in the end product. Petitioner has not established that any specific batch of Moriarty treprostinil is not physically changed by performing step (c), and all the evidence suggests that it is.

C. The Differences In Impurity Profile And Average Purity Between The '393 Product And Moriarty Are Functionally Important

The higher purity of the claimed product resulted in FDA approving a new assay purity for the treprostinil drug as noted in the January 2009 letter submitted to FDA by UTC. Ex. 2006 at 4-6; Ex. 2022 at ¶¶66-68; Ex. 2020 at ¶91. Furthermore, this change constitutes a "major" change according to the classification system for manufacturing changes used by FDA. Ex. 2022 at ¶¶70-72. FDA requires continuous testing of pharmaceutical batches to ensure that they fall within the established purity specification. Ex. 2022 at ¶¶32-40. If a given batch falls outside the established purity specification, then it will be rejected by FDA and cannot be sold for patient use. *Id.* at ¶32. FDA is so concerned about purity of pharmaceuticals that it requires companies to test for very tiny amounts of individual known impurities carried over into the final product based on the manufacturing process. *Id.* at ¶¶32-40. Thus, the change in the '393 product is commercially important and has real-world value.

IV. CLAIM CONSTRUCTION

In the Decision on Institution (Paper 28), the preliminary claim construction construes "[a] product comprising a compound [of/having] formula [I/IV] or a pharmaceutically acceptable salt thereof" and "product" in an unreasonably broad manner. The Board is not bound by that preliminary construction based on an incomplete record. *See e.g., The Scotts Co., LLC v. Encap, LLC*, IPR2013-00110, Paper 79 (PTAB June 24, 2014) (overturning preliminary claim construction in final written opinion) (Ex. 2024). On the fuller record now available to it, the Board should adopt UTC's construction of the disputed terms.

A. Intrinsic Evidence Can Override The Presumption That "Comprising" Creates An "Open" Claim Construction

The claims at issue in an IPR must be given their broadest reasonable interpretation (BRI) in light of the specification, but the Board must still interpret claim terms according to established principles. The transition phrase "comprising" is only *presumed* to be an "open" phrase. *Crystal Semiconductor Corp. v. TriTech Microelectronics Int'l, Inc.*, 246 F.3d 1336, 1348 (Fed. Cir. 2001) ("In the parlance of patent law, the transition 'comprising' creates a presumption that the recited elements are only a part of the device, that the claim does not exclude additional, unrecited elements."). "While it is true that, as a general rule, the words of a patent claim are to be given their plain, ordinary and accustomed

meaning to one of ordinary skill in the relevant art, *Toro Co. v. White Consol. Indus., Inc.*, 199 F.3d 1295, 1299 (Fed. Cir. 1999), a court must nevertheless examine the remaining intrinsic evidence to determine whether the patentee has set forth an explicit definition of a term contrary to its ordinary meaning, has disclaimed subject matter, or has otherwise limited the scope of the claims." *Day Intern., Inc. v. Reeves Brothers, Inc.*, 260 F.3d 1343, 1349 (Fed. Cir. 2001).

The intrinsic record, both the specification and the prosecution history, must be reviewed to determine if there are limits to terms in the claims that would otherwise be given their presumptive plain meanings. Prosecution history "limits the interpretation of claims so as to exclude any interpretation that may have been disclaimed or disavowed during prosecution in order to obtain claim allowance." *Standard Oil Co. v. American Cyanamid Co.*, 774 F.2d 448, 452 (Fed. Cir. 1985). Similarly, the specification may contain repeated statements distinguishing the prior art that limit the claims. *SafeTCare Mfg., Inc. v.Tele-Made, Inc.*, 497 F.3d 1262, 1269-70 (Fed. Cir. 2007) (finding disclaimer where the specification repeatedly indicated that the invention operated by "pushing (as opposed to pulling) forces," and then characterized the "pushing forces" as "an important feature of the present invention").

Under the BRI standard, the Board should take into account both the specification and the prosecution history because the patent examiner and the

applicant have already worked together to determine the scope of the claimed invention. *See In re Buszard*, 504 F.3d 1364, 1366-67 (Fed. Cir. 2007) ("The patent examiner and the applicant, in the give and take of rejection and response, work toward defining the metes and bounds of the invention to be patented."); *In re Zletz*, 893 F.2d 319, 321 (Fed. Cir. 1989) ("When the applicant states the meaning that the claim terms are intended to have, the claims are examined with that meaning, in order to achieve a complete exploration of the applicant's invention and its relation to the prior art.").

The Board has followed these principles of claim construction in other IPR proceedings. *See, e.g.*, *The Scotts Co., LLC v. Encap, LLC*, IPR2013-00110, Ex. 2024 at 14-16. In *Scotts*, the Board changed its preliminary claim construction of "being in a solid state at time of coating" because the Board found that the patent owner had disavowed claim scope during prosecution in order to overcome a specific prior art reference. Ex. 2024 at 15. The Board relied on statements made in Examiner Interview Summaries which confirmed that claim amendments and arguments presented overcame the prior art. *Id.*; *see also* Prosecution History of U.S. Patent No. 6,209,259 (Ex. 2025). As another example, the Board recently construed a phrase to exclude trace amounts of a substance based on statements made during prosecution distinguishing prior art containing trace amounts of the substance. *Daicel Corp. v. Celanese Int'l Corp.*, IPR2015-00171, Paper 86 at 41

(PTAB June 23, 2016). Thus, the BRI cannot be divorced from the intrinsic evidence, including the prosecution history. Such a construction is not reasonable.

B. The Distinct Impurity Profile And Higher Purity Of the '393 Patent Product Were Clearly Considered Part of the Claimed Product During Prosecution

As explained during prosecution,"[e]ach of treprostinil as the free acid and treprostinil diethanolamine prepared according to the process specified in claim 1 or 10 . . . is physically different from treprostinil prepared according to the process of 'Moriarty' due to differences in their impurity profiles." Ex. 1002 at 344. In fact, the Examiner required UTC to provide evidence in declaration form showing that the product of claims 1 and 10 was different than Moriarty's product. *Id.* at 328. In response, UTC filed the Walsh Declaration, which demonstrated that the claimed product had a different impurity profile and higher purity than Moriarty's product. *Id.* at 347-349. It was upon these statements and evidence that Moriarty was overcome, and shortly thereafter the Examiner issued a Notice of Allowance. *Id.* at 354-360.

In addition, the '393 specification repeatedly refers to the differences of the '393 product compared to Moriarty. The entirety of Example 6 in the '393 specification is a large scale, side-by-side comparison between Moriarty and the '393 product, which shows a purity of 99.0% for Moriarty and 99.9% for the '393 product. Ex. 1001, 17:step 53. At the end of this example, the '393 specification

further states that "impurities carried over from intermediate steps (i.e., alkylation of triol and hydrolysis of benzindene nitrile) are removed during the carbon treatment and salt formation step" (Ex. 1001, 17:29-32), which are the same differences (higher purity and different impurity profile) that UTC relied upon in the Walsh Declaration during prosecution as noted above.

These statements by UTC demonstrate that the claimed "product" must have an impurity profile conferred by its process steps. *See Purdue Pharma L.P. v. Endo Pharms. Ins.*, 438 F.3d 1123, 1136 (Fed. Cir. 2006); *see also Atofina v. Great Lakes Chem. Corp.*, 441 F.3d 991, 997 (Fed. Cir. 2006) (statements made during prosecution history that distinguished the claimed invention from the prior art constituted a prosecution disclaimer); *see also United Therapeutics Corp. v. Sandoz, Inc.*, 2014 WL 4259153, *54-56 (D.N.J. Aug 29, 2014) (finding compounds made by different processes resulted in different impurity profiles meaning they were structurally different).

D. The Plain Meaning Of "Product" In The Context Of The '393 Product-By-Process Claims Requires The Characteristics Conferred By The Process Steps Be Present

The term "product" in the context of the '393 patent should be construed as "a substance resulting from a chemical reaction." This is consistent with the '393 patent itself (Ex. 1001 at col. 3, lines 3, 4, 65, and 66; col. 5, line 45; col. 6, lines 65 and 66; and col. 7, line 17), as well as the understanding of a POSA and the

generally accepted definition in chemistry. Ex. 2020 at ¶¶60-62. Additionally, Dr. Williams and Dr. Winkler both use the term product to refer to the result of a chemical reaction in their own work. Id. at ¶¶63-65; *see also* Ex. 2031 at 155:2-11 ("the product of a chemical reaction would be essentially all of the substances that result from the treatment of a particular reactant with a particular set of reagents."). To construe the term "product" as "a chemical composition" is too broad and improperly disregards a significant portion of the intrinsic record. As described above, a product is the result of a chemical reaction and has its own impurity profile depending upon how it is made. "A chemical composition" could be anything and is in no way limiting to what the term "product" actually means. Ex. 2020 at ¶¶66-68.

V. GROUND 1: PHARES FAILS TO EXPLICITLY OR INHERENTLY DISCLOSE EACH AND EVERY LIMITATION OF CLAIMS 1-5, 7-9, 11-14 OR 16-20

The Board instituted Ground 1 based on the conclusion that Phares teaches the treprostinil diethanolamine salt product recited in claims 1 and 9, and that the recited process steps of the claims do not impart structural or functional differences over Phares' treprostinil diethanolamine salt. As discussed below, SteadyMed has failed to establish anticipation based on Phares.

A. SteadyMed Cannot Pick and Choose From Unrelated Portions of Phares to Establish Anticipation

In attempting to show anticipation, SteadyMed cites four different portions of Phares, Ex. 1005, as teaching the combined elements of claims 1 and 9. However, SteadyMed selectively ignores other portions in the Phares disclosure that suggest the four disparate portions of Phares should not be cobbled together to a single allegedly anticipatory embodiment. Petition at 22-24 and 33-34.

The portions of Phares cited by SteadyMed each relate to distinct subject matter, and Phares provides no description that would lead to the combination of these separate disclosures. Ex. 2020 at ¶¶79-84. Phares' only disclosure of steps (a) and (b) is directed to the enantiomer (-)-treprostinil, which are not the same as the synthesis for treprostinil. Ex. 2020 at ¶¶79-81. In fact, the intermediate products disclosed in the enantiomer synthesis as well as several reagents are different than the synthesis of treprostinil. *Id.* at ¶81. In contrast, Phares' separate alleged disclosure of step (c) is silent as to how the starting treprostinil acid was prepared. Ex. 1005 at 85. Thus, there is no reason set forth in Phares to combine the single teaching of steps (a) and (b) directed to one enantiomer with the other teachings of step (c), which are all directed to the other enantiomer. Ex. 2020 at ¶¶79-81.

Despite the alleged disclosure in Phares' that enantiomers of the disclosed compounds can be prepared using the proper chiral reagents, Phares itself teaches that treprostinil can be prepared in other ways that do not include steps (a) and (b), including the processes disclosed in US Patent Nos. 4,306,075 (Ex. 2032) and 5,153,222 (Ex. 2033). Ex. 1005 at 11; Ex. 2020 at ¶78. Thus, a POSA would reasonably conclude that the diethanolamine salts of Phares were prepared based on other disclosed methods that do not require steps (a) and (b). Ex. 2020 at ¶78. If the diethanolamine salts of Phares were prepared differently than the recited process steps, nothing in Phares establishes that the diethanolamine salts are necessarily the claimed product.

B. The Proper Construction of a "product comprising a compound [of/having] formula [I/IV] or a pharmaceutically acceptable salt thereof" Precludes A Finding That Phares Anticipates the Present Claims

The Board's institution of Ground 1 was partly based on its preliminary finding that "comprising" does not exclude impurities that may possibly be produced by the process of Phares and that the impurity profile of Phares' diethanolamine salt is identical to that of the claimed product. *See* Paper 12 at 30. However, such a finding does not take into consideration the reasonable construction of "product comprising a compound [of/having] formula [I/IV] or a

pharmaceutically acceptable salt thereof," which is set forth in this Response and supported by the record now before the Board.

As discussed above in Section IV, both the specification and the prosecution history of the '393 patent distinguish the claimed product from prior art treprostinil products based on its higher purity and different impurity profile, which is achieved through the recited process steps. Thus, to prevail on Ground 1, SteadyMed must show that the Phares' diethanolamine salt necessarily possesses an impurity profile that is distinct from that of the Moriarty product and with higher purity.

Steadymed simply assumes that the diethanolamine salt discussed by Dr. Winkler is prepared from Moriarty treprostinil and does not acknowledge that the source of treprostinil would impact both the overall purity and impurity profile of the resulting salt. As exemplified in the '393 patent, the claimed process provides an improved treprostinil product due to its superior purity. As evidenced by the Williams Declaration and the batch record data, the claimed product has an average purity of [redacted] and a distinct impurity profile from Moriarty's product. Ex. 2020 at ¶¶94-99. Importantly, SteadyMed has failed to show that, at a minimum, the Phares' diethanolamine salt possesses an impurity profile that is distinct from that of the Moriarty product and contains fewer overall impurities than the Moriarty product. Nor has SteadyMed shown that the Phares'

diethanolamine salt has a higher purity than the Moriarty product. Indeed, SteadyMed's only argument regarding the purity of Phares' diethanolamine salt is based on the theory that the higher melting point of Phares' diethanolamine salt necessarily means that it must be at least equal in purity to that of the exemplified batches in the '393 patent. *See* Petition at 27-28. However, for the reasons noted below, that is an incorrect conclusion based on the evidence now in the record.

C. The Higher Melting Point of Phares' Diethanolamine Salt Does Not Necessarily Mean That it is of Higher Purity Than the Diethanolamine Salts of the '393 Patent

The Board relied on incorrect statements in the Winkler Declaration alleging that Phares' diethanolamine salt must be more or at least equally pure as the claimed product solely because the former has a higher melting point. Paper 12 at 28-29. However, melting point is just one factor in assessing a compound's purity and is not necessarily a reliable metric of purity. This is especially applicable to Phares because only one melting point value was obtained in a sample for a polymorph screen. A POSA would not rely upon a single melting point value, absent any other impurity information, to determine the purity of a substance made under unspecified conditions. Ex. 2020 ¶76. Indeed, the "higher" melting point of Phares' diethanolamine salt could be indicative of the inclusion of impurities or the result of the use of different solvent systems for the crystal forms. *Id.* Accordingly,

the purity of a compound cannot be assessed based solely on its melting point value.

Moreover, even if the melting point could be relied upon, the data cited by Dr. Winkler does not indicate a product of high purity. To the contrary, Fig. 21 of Phares "shows a broad melting peak with a range of close to 10 degrees which is indicative of a lower purity substance." Ex. 2020 ¶76; *see also*, Marti, E., *Purity determination by differential scanning calorimetry*, Thermochimica Acta, 5(1972) 173-220 at 214 ("The melting of diphenyl is extremely sharp because of the purity level; on the other hand, the melting region of phenacetin-benzamide is rather broad.") (Ex. 2031).

Additionally, Phares discloses several different conditions for preparing Polymorph A of the diethanolamine salt and that Polymorph A is required to make Polymorph B. Ex. 2020 at ¶73. The '393 patent does not indicate that making Polymorph A first is required. *Id.* Phares also indicates many conditions used to make Polymorph A and Polymorph B, but it is not clear what conditions were specifically used for the sample analyzed in Figure 21 that Dr. Winkler relies upon. *Id.* at ¶¶73-74. It is well known that the use of different solvent systems in forming different crystal forms can have a significant effect on the melting point of a substance, as well as other characteristics, including purity, and a higher melting point does not always mean a higher purity. *Id.* at ¶¶75-76; *see also* R. Adhiyaman,

et.al., *Crystal modification of dipyridamole using different solvents and crystallization conditions*, Int'l J. Pharm.321 (2006) 27-34 at 33 ("Adhiyaman") ("In conclusion, it can be said that the crystallization conditions and medium used have major effect on dipyridamole crystals habit modification under ambient conditions. The crystals showed significant changes in the shape, size, melting points, dissolution rate, XRD patterns and DSC curves.") (Ex. 2030).

Dr. Williams, therefore, has concluded that "[i]t is known in the art that sample size, rate of heating, the recrystallization solvent(s) used, and the conditions under which the crystalline sample was obtained can significantly affect the DSC data. Dr. Winkler's conclusion based on this single vague and incompletely described DSC data is not scientifically sound." *Id.* at ¶76.

Thus, nothing in Phares establishes that the disclosed diethanolamine salt is at least of equal purity to the diethanolamine salts of the '393 patent. With respect to claim 2 of the '393 patent specifically, nothing in Phares discloses a purity of at least 99.5%. Ex. 2020 at ¶82. For this additional reason, Phares cannot anticipate claim 2.

D. Phares Fails To Disclose the Claimed Process for Making Treprostinil or Any Purity or Impurity Profile for Treprostinil Diethanolamine

SteadyMed has failed to establish that Phares' diethanolamine salt (Form B) is the claimed product.

First, as Dr. Williams notes, the samples of treprostinil diethanolamine disclosed in Phares were "made for a polymorph screen, not large scale batches." Ex. 2020 ¶73. Accordingly, "the samples of polymorph B described in Phares are prepared in a completely different way under different conditions than those described in the '393 patent." Ex. 2020 ¶75. Specifically, Phares discloses first preparing polymorph A by any one of a variety of methods and then preparing polymorph B from some sample of polymorph A. In contrast, the '393 patent makes no mention of first forming polymorph A. Ex. 2020 ¶¶73-74. Additionally, Phares describes reaction conditions for making the polymorph samples that are not described anywhere in the '393 patent. *Id.* In particular, the reaction conditions disclosed for the sample of polymorph B characterized by Phares, heated slurries of form A in 1,4-dioxane and toluene, are not described anywhere in the '393 patent. *Id.* It is well-known that the use of different reaction conditions, including different solvents, can significantly affect the characteristics of a given crystal form. Ex. 2020 ¶75. As a result, the diethanolamine salt disclosed in Phares cannot be directly compared to the diethanolamine salt disclosed in the '393 patent.

Second, the Williams Declaration clearly establishes that the claimed product has an average purity of [redacted], thus giving it a superior purity and distinct impurity profile over that of the prior art treprostinil products. Ex. 2020 ¶¶94-99. The purity of the claimed product provides a structural difference from the prior art

treprostinil, as evidenced by the differences in the average impurity profiles for the Moriarty product and the '393 product. *Id.*, Ex. 2036, Ex. 2037. Indeed, the higher purity of the claimed product resulted in FDA approving a new purity specification for the treprostinil drug as noted in the January 2009 letter submitted to FDA by UTC. Ex. 2006 at 4-6; Ex. 2022 at ¶¶70-72; Ex. 2020 at ¶91.

The impurity profile of the *starting* treprostinil acid used to prepare the Phares diethanolamine salt is crucial to assess whether the diethanolamine salt is the same as the claimed product, *i.e.*, whether the impurity profile of the diethanolamine salt in Phares is identical to that of the claimed product. Ex. 2020 ¶¶76-78. However, nowhere does Phares disclose the process of preparing the treprostinil acid used to prepare the diethanolamine salt. As acknowledged in both Phares and the '393 patent, several different processes can produce treprostinil acid. *See, e.g.*, Ex. 1005 at 11; *see also,* Ex. 2020 ¶78. Each known process can produce a treprostinil acid with a unique impurity profile. Ex. 2020 ¶78. Because Phares does not disclose the process of preparing the starting treprostinil acid for the diethanolamine salt, the impurity profile of the diethanolamine salt cannot be established. Without knowing the impurity profile and level of purity of Phares' diethanolamine salt, SteadyMed cannot show that it is necessarily identical to the claimed product or has equal purity to the claimed product.

Consequently, SteadyMed has not carried its burden on Ground 1.

VI. GROUND 2: MORIARTY AND PHARES FAIL TO RENDER OBVIOUS CLAIMS 1-5, 7-9, 11-14, OR 16-20

Moriarty does not teach salt formation and regeneration of the free acid. SteadyMed attempts to cure this deficiency in Moriarty by citing Phares for allegedly teaching step (c). However, Moriarty teaches three distinct methods of preparing the treprostinil free acid. Nothing in Moriarty directs a POSA to select one specific process over the three disclosed for purposes of further modification by adding a salt formation step. Furthermore, SteadyMed fails to recognize that the performance of step (c) after steps (a) and (b) unexpectedly results in a product with an improved average purity over that of the prior art. Indeed, the Williams Declaration demonstrates that, out of 122 samples, the claimed product has an average purity of greater than [redacted]. Ex. 2020 at ¶¶94-95 and Appendices A-B.

As discussed above, the claimed product is structurally different from Moriarty's product because the claimed product has a distinct impurity profile, including a marked reduction in several specific impurities, and a higher average purity relative to Moriarty's product. Ex. 2020 at ¶¶94-99 and Appendices A-B. This evidence shows that, in the recited combination, performing step (c) in conjunction with steps (a) and (b) of the present claims produces a treprostinil product that is significantly improved over that of the prior art. Ex. 2020 at ¶¶48-49, 70.

Moreover, Moriarty's product cannot render obvious the claimed product because during prosecution of the '393 patent, UTC overcame a rejection based upon Moriarty by providing evidence of representative sample impurity profiles, showing the physical difference between the product of the '393 patent and the Moriarty product. Ex. 1002 at p. 347. Phares does not cure this deficiency because, as noted above, nothing in Phares establishes that the diethanolamine salt either 1) has an impurity profile similar to the claimed product or 2) has an overall purity at least equal to the claimed product.

In particular, it would not have been obvious to use the salt formation step of Phares to decrease amounts of at least [redacted] and [redacted], which are stereoisomers of treprostinil, and accordingly, are acidic rather than neutral or basic. Ex. 2020 at ¶102. Thus, when subject to salt-forming conditions, a POSA would expect that any undesired stereoisomer of treprostinil would be included in the final salt product because the stereoisomer would also be converted to the corresponding salt under such salt-forming conditions. A POSA has no reasonable expectation of success in removing any undesired treprostinil stereoisomer impurities by salt formation and subsequent regeneration of the free acid. *Id.* Instead, a POSA would expect the salt formation and subsequent regeneration to produce a final product with the same initial amount of stereoisomer impurities before the salt formation step. *Id.* Yet these impurities are each detected in only a single optimization batch

of the '393 product, and in none of the commercial batches. Even taking these optimization batches into consideration, this represents a greater than 100-fold reduction as compared to the Moriarty product. *Id.* at ¶¶94-96.

Additionally, as described above, there is no basis for comparing the "purity" in Moriarty with the purity described in the Walsh Declaration. *Id.* at ¶88. Walsh's Declaration makes clear that purity in terms of the '393 patent is assessed by looking to the total related substances of a batch. *Id.* at ¶¶88-89. The Moriarty reference, while not specifying a reference standard, does refer to a comparison to an authentic sample. *Id.* As a result, it is not clear what method was used to determine the purity in Moriarty and therefore a direct comparison of the value reported in Moriarty cannot be made to the '393 patent.

Moreover, Dr. Winkler fundamentally misunderstands the error associated with various purity measurements used in the Walsh Declaration, the '393 patent, the prior art, and FDA. Dr. Winkler states in his declaration that:

> even a difference of 0.4% as discussed below, between the claimed processes of the '393 Patent and the prior art, such as Moriarty (Ex. 1004), would be attributable to experimental error, and thus the claimed degree of purity under the claimed processes of the '393 Patent presents no distinction from the prior art.

Ex. 1009 at ¶69.

He goes on to state that "HPLC's precision indicates that the 'RSD' or 'relative standard deviation' for a typical instrument is about 1%." *Id.* at ¶70.

This is wrong for several reasons. First, during his deposition, Dr. Winkler admitted he did not know what the actual error in the measurement was for the data submitted in the Walsh Declaration during prosecution of the '393 patent. Ex. 2051 at 62:16-25; 63:2-14.[2] While he did not know the error associated with the measurements made in the data submitted with the Walsh Declaration, he did admit that "the error in the measurement for the [redacted] [treprostinil impurity] would be, should be less than .1 percent," and in general, "[t]he error should be less than the maximum number reported, that's correct, for the measurement of the materials described here." Ex. 2051 at 63:25-64:4; 64:7-16. By his own admission, the error associated with the measurement of impurities in treprostinil batch records such as those submitted in Walsh's Declaration are therefore far less than the alleged error of 1% or 0.4% he stated in his declaration.

[2] Indeed, Dr Winkler admitted he was not familiar with FDA guidelines regarding impurity profiles for a drug, did not know what is required in order to change a drug specification, and was not familiar with published guidances from FDA regarding changes to new drug applications or abbreviated new drug applications. Ex. 2051 at 19:3-24.

In contrast, FDA requires that impurity determinations must be measured at or below 0.05% for drugs such as treprostinil. *See*, Ex. 2022 at ¶47; Ex. 2020 at ¶92. As Dr. Ruffolo explains, impurities in drug substances such as treprostinil that are administered in dosages less than 2 grams per day require that impurities be reported if they are present at a level less than or equal to 0.05%. *See, e.g.*, Ex. 2022 at ¶¶44-47; *see also* ICH Impurities in New Drug Substances Q3A(R2) monograph at 5-11 (Ex. 2038). "As a result of these thresholds, by definition, the limit of detection for impurities (and therefore total related substances) must be at least as low as 0.05%." Ex. 2022 at ¶50.

Furthermore, the '393 patent is directed to an improved and more pure treprostinil product. *See, e.g.*, Ex. 1001, 17:27-40. Given that Moriarty discloses the use of column chromatography for purification, a POSA would not be motivated to create the salt form in Phares, as Phares does not disclose any benefit or increased purity as a result of using the diethanolamine salt. Ex. 2020 at ¶101. "In fact, Phares does not allege that the diethanolamine salt is superior in any way to the treprostinil product of Moriarty and instead identifies other earlier treprostinil disclosures as a means to create the treprostinil used to form the diethanolamine salt." *Id.* A POSA would not have a reasonable expectation of success by using salt formation as a purification step separate from or in addition to the column chromatography of Moriarty, as Phares does not disclose any alleged

benefit to forming the salt and a POSA would have no expectation that only certain acidic and neutral impurities would be reduced or completely eliminated while others remained. *Id.* at ¶102. Thus, the combination of Moriarty and Phares cannot render obvious claims 1-5, 7-9, 11-14, or 16-20.

Similarly, as described above, there is no basis to compare the purity disclosed in Moriarty to the measurements obtained in the '393 patent or those obtained by Dr. Walsh in his declaration, and therefore, claim 2 would also not be rendered obvious by the combination of Phares and Moriarty for this additional reason. *Id.* at ¶103.

Claims 8 and 16 also require the additional limitation that the formula (VI) compound of step (a) is not purified. In fact, the '393 patent specifically distinguishes this limitation over the prior art. Ex. 1001, Example 6. Moriarty expressly discloses that the compound of formula (VI) from step (a) is purified. Ex. 2020 at ¶104. Phares does not disclose any synthesis for treprostinil and, even in the abbreviated synthesis of the enantiomer, no details of purification are disclosed. *Id.* Thus, claims 8 and 16 are not rendered obvious by the combination of Phares and Moriarty for this additional reason. Process advantages should be considered as secondary considerations to rebut obviousness, even if the process steps or advantages are not considered in the initial determination of whether there is *prima*

facie obviousness (where the products are compared regardless of how they are made).

Consequently, SteadyMed has not carried its burden on Ground 2.

VII. GROUND 3: MORIARTY, PHARES, KAWAKAMI, AND EĞE FAIL TO RENDER OBVIOUS CLAIMS 6, 10, 15, 21, AND 22

A. The Product of Claims 6, 15, and 21 Are Different Than the Prior Art Treprostinil Products

The Board concluded that the process steps of claims 6, 15, and 21, including step (d), do not impart structural or functional differences over prior art treprostinil products. Paper 12 at 46-47.

Based on the evidentiary record now before the Board, and in view of the reasons set forth in Section III, above, the free acid substance formed by step (d) of claims 6, 10, 15, 21 and 22 is structurally different from the prior art treprostinil products in Phares and Moriarty. The evidentiary record shows that the free acid substance of claims 6, 10, 15, 21 and 22 contains a distinct impurity profile and a higher average purity over the treprostinil free acid of Moriarty, and thus is structurally different. Further, Phares' diethanolamine salt of treprostinil is structurally and functionally distinct from the free acid substance formed by step (d) of claims 6, 15 and 21.

1. The '393 Patent Product is Structurally and Functionally Distinct from Moriarty's Product

As explained in the Williams Declaration and discussed above, the free acid substances of claims 6, 10, 15, 21 and 22 are structurally distinct from Moriarty's product because the formation of the salt in step (c) leads to a product that has a distinct and improved impurity profile. *See* Sections III, VI, *supra*. Additionally, the average purity of the product of claim 21 is about [redacted] greater than that of Moriarty. Ex. 2020 ¶¶94-99 and Appendices A-B. Indeed, as evidenced by Dr. Ruffolo's Declaration, a [redacted] difference in average purity for a highly potent drug, such as treprostinil is a very significant difference. *See, e.g.*, Ex. 2022 at ¶70.

B. There Is No Motivation For A POSA To Combine Moriarty and Phares with Eğe and Kawakami

In the Institution Decision, the Board determined "on the record before us, and for purposes of institution, that the process steps recited in claims 6, 15, and 21 do not impart structural or functional differences to the claimed treprostinil product, we do not address the parties' contentions concerning the obviousness of the recited process steps." Paper 12 at 47. However, the fuller record now indicates that the claimed treprostinil product is structurally and/or functionally different from Moriarty's treprostinil free acid and Phares' treprostinil diethanolamine salt. Thus, the recited process steps must now be considered.

Similarly, the board credited Dr. Winkler's opinion regarding the combination of Kawakami and Eğe with Moriarty and Phares. Paper at 42. Dr. Winkler, however, too easily dismisses the complexity and difficulty associated with further purifying a drug substance as complex as treprostinil. Dr. Winkler attempts to portray the chemistry involved in the '393 patent as "nothing more than basic organic chemistry techniques – in my view 'organic chemistry 101'" in an effort to minimize the significant invention of the '393 patent. Ex.1009 at ¶3. Yet, Dr. Winkler contradicts himself by defining a POSA as having "a master's degree or Ph.D. in medicinal or organic chemistry, or a closely related field. Alternatively a person of ordinary skill would include a bachelor's degree and at least five years of practical experience in medicinal or organic chemistry." *Id.* at ¶14. Indeed, Dr. Winkler goes on to testify that to understand the science and chemistry of the patent, you would need that level of skill in the art. Ex. 2051 at 29:12-16. As a result, a POSA would not look to an undergraduate textbook like Eğe, for example, to figure out improved purification techniques for a complex drug substance such as treprostinil.

1. There Is No Motivation to Follow the Carboxylate Salt Formation With Regeneration of the Carboxylic Acid

The Board credited Dr. Winkler's opinion regarding the combination of Kawakami and Eğe with Moriarty and Phares. Paper 12 at 42. Dr. Winkler,

however, too easily dismisses the complexity and difficulty associated with further purifying a drug substance as complex as treprostinil. After first referencing "organic chemistry 101" to minimize the significance of the '393 patent (Ex. 1009 at ¶3), Dr. Winkler contradicts himself by defining a POSA as having "a master's degree or Ph.D. in medicinal or organic chemistry, or a closely related field. Alternatively a person of ordinary skill would include a bachelor's degree and at least five years of practical experience in medicinal or organic chemistry." *Id.* at ¶14. At his deposition, Dr. Winkler conceded that, to understand the science and chemistry of the '393 patent, you would need this higher level of skill in the art. Ex. 2051 at 29:12-16. As a result, a POSA would not look to an undergraduate textbook like Eğe, for example, to figure out improved purification techniques for a complex drug substance such as treprostinil.

As explained previously, the claimed free-acid compounds, including treprostinil, produced by the processes of claims 6, 10, 15, and 21 provide a new product that induced FDA to adopt a new purity standard for treprostinil free acid due to the excellent purity of the final product. Furthermore, UTC demonstrated that treprostinil free acid made by the claimed methods provides a compound that lacks or reduces the levels of the impurities found in the free acid treprostinil of the Moriarty process.

Neither Phares nor Eğe provide a reason that a POSA would include a "carboxylate salt formation and regeneration of the neutral carboxylic acid" step. *See* Petition, p. 54. Phares merely discloses forming a salt from treprostinil free acid of undisclosed origin. *See* Section V.E., *supra*. There is no suggestion that this salt should then be converted *back* to the free acid (*e.g.*, there is no suggestion of using the salt formation as a purification method). "Given that the purification techniques disclosed in Moriarty include chromatography and recrystallization after many years of research to optimize the process of making treprostinil, a POSA would not have been motivated to use a salt purification technique disclosed in an undergraduate chemistry textbook. More importantly, a POSA would not have had a reasonable expectation of success in further purifying the treprostinil product of Moriarty by using such a technique. To the extent a POSA was motivated to further purify treprostinil, a POSA would have focused on the known impurities and investigated methods of removing those." Ex. 2020 at ¶106. Indeed, stereoisomers were known impurities in treprostinil. *Id.* Eğe, however, simply discloses that "carboxylic acids that have low solubility in water, such as benzoic acid, are converted to water-soluble salts by reaction with aqueous base. Protonation of the carboxylate anion by a strong acid regenerates the water-insoluble acid. These properties of carboxylic acids are useful in separating them from reaction mixtures containing neutral and basic compounds." *Id.* at ¶107.

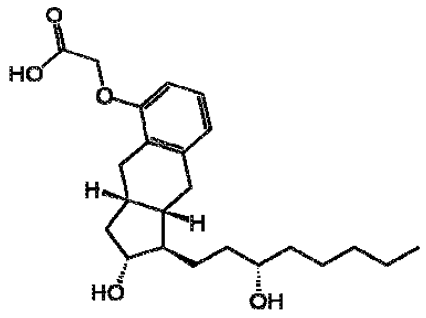
Indeed, the only example given in Eğe is of benzoic acid – a very simple aromatic acid that is quite different from the structure of treprostinil, as it has no chiral centers and therefore no stereoisomeric impurities. *Id.* at ¶108. Given that Eğe only predicts the removal of neutral and basic compounds by a salt purification step followed by acidification and only describes a simple non-chiral carboxylic acid, a POSA would have no motivation to look to Eğe for purification and no reasonable expectation of success given that many of the impurities in treprostinil are acidic stereoisomers. *Id.* at ¶¶108-109.

As discussed above, the average impurities found in samples of the Moriarty product include three different stereoisomers of treprostinil free acid. Eğe suggests that a "carboxylate salt formation and regeneration of the neutral carboxylic acid" step would not remove these compounds from the product. Thus, a POSA would have understood Moriarty, Phares, and Eğe to suggest simply making the treprostinil free acid product of Moriarty, and not undergoing the additional time and expense of a "carboxylate salt formation and regeneration of the neutral carboxylic acid" step because Eğe actually teaches away from the usefulness of this step when impurities include acidic stereoisomers are present because a POSA would have to ignore Eğe's teaching that these types of impurities could not be removed by carboxylate salt formation. *See* Ex. 2020 ¶¶107-109; *see also United States v. Adams*, 383 U.S. 39, 42-43 (1966).

The Institution Decision cites *KSR* for the proposition that "a technique has been used to improve one device, and a POSA would recognize that it would improve similar devices in the same way, using the technique is obvious unless its actual application is beyond his or her skill." Paper 12 at 45. However, the simple application of this proposition regarding devices (a predictable art) should not be applied to an unpredictable field, such as the chemical arts, without truly examining whether the technique would improve *similar compounds* in the *same way*. *See, e.g., In re Fisher*, 427 F.2d 833, 839 (C.C.P.A., 1970)(contrasting "predictable factors, such as mechanical or electrical elements" from "unpredictable factors, such as most chemical reactions"); *see also, Ortho-McNeil Pharm., Inc. v. Mylan Labs., Inc.*, 520 F.3d 1358, 1364 (Fed. Cir. 2008).

For example, Kawakami teaches purification of a methanoprostacyclin derivative by forming the dicyclohexyl amine salt and then regenerating the free acid to achieve a "fairly high" purity. Analogizing to the language cited from *KSR*, a POSA must have recognized that the "technique" of salt formation followed by regeneration of the free acid would improve *similar compounds* in the *same way*.

However, as can be seen by the below comparison, the structures of treprostinil and the methanoprostacyclin derivative of Kawakami are structurally very different – they are not *similar compounds/devices*.



Treprostinil

methanoprostacyclin compound in Kawakami

First, the methanoprostocyclin compound in Kawakami is a-two fused-ring structure, while treprostinil is a three-fused-ring structure. Ex. 2020 at ¶112. Second, Kawakami does not actually disclose a purification method for separating diastereomers, but instead one for separating E and Z isomers. Ex. 2020 ¶¶112-113.

Indeed, Kawakami teaches that the starting material does not vary at each chiral center other than the alkene double bond. *Id.* In other words, Kawakami discloses a mixture of two compounds: (1) the E-isomer of a stereoisomerically pure compound and (2) the Z-isomer of a stereoisomerically pure compound. *Id.* at ¶113. Treprostinil contains no mixture of E and Z isomers because it does not contain a carbon-carbon double bond that is capable of forming E and Z isomers. Indeed, the use of a specific salt to isolate a specific E/Z isomer does not reasonably suggest that salt formation of a much more complex compound with

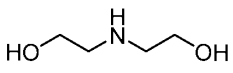
multiple chiral centers such as treprostinil could be isolated from entirely different impurities and then converted back to the free acid form. *Id.*

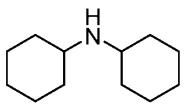
Thus, the purification of treprostinil from its stereoisomers and related impurities is quite different from the purification of the methanoprostacyclin derivative from its structural isomer – the compositions are not improved in the *same way*.

As a result of these differences, "a POSA would not have looked to Kawakami (or Eğe) if they were looking for additional purification techniques for treprostinil because neither reference discloses how to remove stereoisomeric impurities." *Id* at ¶112.

2. Kawakami Would Have Motivated One of Ordinary Skill In The Art To Select A Dicyclohexyl Amine Salt, Teaching Away From The Diethanolamine Salt of Claims 14 and 18

Not only are there structural differences between treprostinil and the "methanoprostacyclin compound" in Kawakami, but the counter-ion used to prepare the salt is structurally different. *Id.* at ¶114. Specifically, Kawakami teaches preparing the dicyclohexyl amine salt, whereas particular claims of the '393 patent require use of the diethanolamine salt.





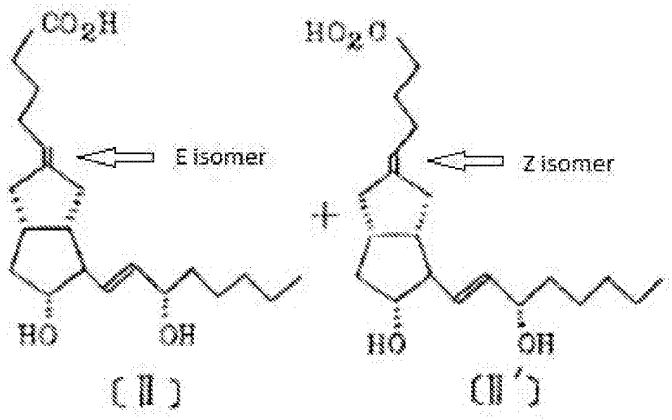
Diethanolamine **dicyclohexyl amine**

Because Kawakami uses a different salt to remove a different sort of impurity from a different structure, a POSA would have no reason to combine the teachings of Kawakami with Moriarty and Phares in the particular manner of the asserted grounds in the Petition, or a reasonable expectation of success of achieving a more pure treprostinil product by such a combination. Ex. 2020 ¶114. For this reason, claims 14 and 18 are separately patentable.

3. Kawakami Does Not Provide A Reasonable Expectation Of Success That Treprostinil Products Could Be Further Purified Because Different Impurities Are Targeted

The purification of treprostinil from its stereoisomers and related impurities is quite different from the purification of the methanoprostacyclin derivative from its structural isomer, and thus, Kawakami provides no reasonable expectation of success. Ex. 2020 ¶¶112-114

To illustrate this point further, Kawakami is directed to purifying E- and Z-isomers of methanoprostacyclin compound from one another. In order for the E- and Z-isomers to exist, the "prostacyclin compound" must have an alkene. For example, Kawakami discusses separating a mixture of the following compounds:



Treprostinil, on the other hand, contains no mixture of E/Z isomers. In fact, it cannot because it does not contain an alkene capable of E/Z isomerization. SteadyMed has failed to provide a factual basis as to how or why the separation of E/Z isomers of an alkene would provide a motivation to combine or reasonable expectation of success in a compound not containing an alkene capable of E/Z isomerization, such as treprostinil. As explained in the Williams Declaration, the use of a specific salt to isolate a specific E/Z isomer does not reasonably suggest that salt formation of an entirely different compound, such as treprostinil, could be isolated from entirely different impurities, such as stereoisomers and related impurities. Ex. 2020 ¶¶112-114.

Furthermore, the Kawakami reference would have provided no motivation or rationale to attempt to remove the trace impurities of the Moriarty treprostinil free acid through the process of salt formation followed by conversion back to the

free acid. Indeed, Kawakami was concerned with isolating a particular isomer from a 7:2 E/Z isomeric mixture. Ex. 1007 at 4. In other words, the composition in Kawakami contained, at most, a purity of 77.8% prior to the salt formation step. Kawakami provides a crude purification of the desired E-isomer through a particular salt formation, and suggests that not all impurities were removed by formation of a salt and conversion back to the free acid. *Id.* at 5 ("purity can be further improved by recrystallization"). Nothing in the reference suggests that a substance as pure as the Moriarty treprostinil free acid (a substance with about 99.4% assay purity) – a substance that had already been "further improved" by recrystallization (*see* Ex. 1004 at 13, right column) – would be improved by formation of a salt and conversion back to the free acid. Ex. 2020 ¶¶113-114.

Thus, even if formation of a salt and conversion back to the free acid was known in the art, it would not have rendered the present claims obvious without some motivation and expectation of success in its use on the Moriarty treprostinil free acid. To put it another way, there would have been no reason to incur additional time and expense to form a salt of the valuable, relatively pure Moriarty treprostinil free acid only to then convert it back to the free acid, even though the addition would have been technologically possible. *In re Omeprazole Patent Litigation,* 536 F.3d 1361 (Fed. Cir. 2008).

4. Any "Close" Structural Similarity of the Moriarty Free Acid Does Not Render the Claims Obvious

As explained above, the claimed substance is structurally different from Moriarty's treprostinil free acid because the claimed substance has an improved and different impurity profile. Even if the Board views an improvement in impurity profile of, e.g., [redacted], as a close relationship between the substances of the present claims and of Moriarty, there is no obviousness because there was not a known or obvious process for making the claimed free acid substance. *See In re Hoeksema*, 399 F.2d 269, 274 (C.C.P.A. 1968)("the absence of a known or obvious process for making the claimed compounds overcomes any presumption that the compounds are obvious based on close relationships between their structures and those of prior art compounds"). For the reasons set forth in the previous sections, conducting a salt-formation purification step on the known treprostinil free acid of Moriarty would not have been obvious, so the mere existence of a "close relationship" in the products cannot be used to deny patentability.

5. Additional Claim Limitations Are Not Disclosed by the Cited Prior Art

In addition to the reasons above, certain dependent claims would also not have been obvious in light of the combination of Phares, Moriarty, Eğe, and Kawakami. Claim 6 requires the acid in step (d) to be either HCl or H_2SO_4 and

claim 15 requires the acid to be HCl. Similarly, claim 21 requires step (d) is performed. Phares, Moriarty, and Kawakami all do not disclose the use of either HCl or H_2SO_4 and do not disclose converting a carboxylic acid salt back to its salt form using an acid. Ex. 2020 at ¶115. "Eğe cites HCl as an example in the conversion of benzoic acid, but as described above, a POSA would not have looked to Eğe to further purify a complex carboxylic acid such as treprostinil from its stereoisomers and other impurities and would have no reasonable expectation of success by using HCl based on this disclosure." *Id.* In addition to the reasons above, claims 6, 15, and 21 would not be obvious in light of any combination of the cited prior art.

Like claim 2, claim 10 requires that the product be 99.5% pure and that step (d) be performed. The only purity limitation disclosed in any cited prior art reference is in Moriarty and, as explained above, that purity cannot be directly compared to the purity recited by the claims. Similarly, Moriarty does not perform steps (c) or (d). *Id.* at ¶116. A POSA would have no motivation to look to Phares, Kawakami or Eğe to improve the purity to at least 99.5% and, given that none of these references disclose a purity amount, would have no reasonable expectation of success in achieving that purity. *Id.* Finally, claim 22 requires an extra step of forming a pharmaceutically acceptable salt from the product of step (d). SteadyMed and Dr. Winkler cite no evidence whatsoever for this additional step.

"In fact, none of the references cited even suggest converting a carboxylic acid to a salt form, then regenerating the carboxylic acid, then forming a pharmaceutically acceptable salt from that." *Id.* at ¶117. For this additional reason, claim 22 is not obvious in light of the combination of Phares, Moriarty, Kawakami, or Eğe.

Consequently, SteadyMed has not carried its burden on Ground 3.

VIII. SECONDARY CONSIDERATIONS REBUT ANY POSSIBLE CASE OF OBVIOUSNESS

SteadyMed has not established a *prima facie* case of obviousness. Thus, UTC is not obligated to provide evidence of objective indicia of non-obviousness. Nonetheless, objective indicia of non-obviousness confirm that the claims of the '393 patent would not have been obvious and, in fact, represent a surprising solution to the problem of minimizing impurities and providing a safer and purer treprostinil product.

A. Long-Felt Unmet Need

At the time of the invention, there was a long-felt need to have a more efficient synthesis to produce treprostinil in a more pure form and in a cost-effective manner. *See generally*, Ex. 2022 at ¶¶31, 65. Treprostinil has five chiral centers resulting in 32 possible diastereomers, so the potential for diastereomeric impurities is high; only the treprostinil stereoisomer has the desired pharmaceutical effect. Ex. 2013, at pp. 11, ll. 18-25, pp. 15, ll. 1-pp. 16, ll. 8, pp. 19, ll. 14-25.

Treprostinil is also a very potent drug so any diastereomeric impurities would also potentially be potent. *Id.*; Ex. 2022 at ¶54. Specifically, the FDA as a matter of course seeks to minimize all impurities in drug substances and particularly in highly potent drug substances such as treprostinil. Ex. 2022 at ¶¶ 31, 54. The reduction and removal of several types of impurities met the long-felt need expressed by the FDA to minimize impurities as much as possible. *Id.* at ¶¶ 31, 75. Additionally, because the '393 patent product was so successful, it resulted in a change in the drug specification submitted to FDA. *Id.* at ¶¶66-67. The change indicated that the assay purity of the new drug substance made by the '393 patent process increased in purity from an assay range of [redacted] to [redacted] - a full [redacted] increase in assay purity. *Id.* at ¶ 70. The range of assay values of [redacted] as well as the amount above 100% does not indicate an error associated with the measurement, but just the acceptable value of this measurement approved by the FDA. *Id.* at ¶¶ 69-70. The fact that UTC submitted a [redacted] increase in assay purity to FDA is considered a "major" change by FDA. *Id.* at ¶ 72. *See Knoll Pharm. Co., Inc. v. Teva. Pharm. USA, Inc.*, 367 F.3d 1381, 1385 (Fed.Cir. 2004) (while FDA approval is not determinative of nonobviousness, it can be relevant in evaluating the objective indicia of nonobviousness). In fact, even a change as small as 0.1% of impurities can have an impact on a drug substance. *See, e.g., id.* at ¶¶ 32, 45. Given that FDA consistently wants drug substances to have fewer

impurities and in less amounts, the '393 patent invention met that need by further reducing and removing certain specific impurities and by increasing the overall assay purity of the drug substance.

B. Unexpected Results

The results of the claimed inventions in the '393 were unexpected. The use of a salt form of treprostinil to further purify the treprostinil acid in a cheaper and better way than the previously used methods of purification was an unexpected result. Moreover, it was unexpected that the salt purification step reduced not only diastereomeric impurities, but also certain non-acidic impurities as well. *See, supra*, Section XI.B.1; Ex. 2020 ¶¶94-97, 102, 108-109. Indeed, Eğe itself predicted that a salt formation followed by regeneration using an acid would remove only basic and neutral impurities. *Id.* at ¶107. The unpredictability of this result is supported by the fact that the salt purification step did not reduce all non-acidic impurities; in fact, the '393 product has slightly increased levels of one such impurity, [redacted]. Ex. 2020 ¶96. Thus, a person of skill in the art would not have expected the results of the '393 patent to be so successful at reducing the levels of so many impurities.

IX. Conclusion

For the foregoing reasons, the Board should hold that SteadyMed has failed to carry its burden attacking the patentability of the instituted claims because none

of the prior art cited by SteadyMed anticipates or renders obvious any claim of the '393 patent.

Respectfully submitted,

Date: July 6, 2016

/Stephen B. Maebius/
Stephen B. Maebius
Reg. No. 35,264

CERTIFICATE OF COMPLIANCE

This Paper contains 11,230 words according to the word processing program in which it was created, excluding the portions exempted by 37 C.F.R. ¶42.24(a)(1). Accordingly, this Paper complies with the requirements of 37 C.F.R. § 42.24(b)(1).

Date: July 6, 2016

Signature: /Stephen B. Maebius/
Stephen B. Maebius

CERTIFICATE OF SERVICE

The undersigned hereby certifies that a copy of the foregoing Patent Owner Response and accompanying exhibits was served on counsel of record for Petitioner on July 6, 2016 by filing through the Board's PRPS system and by delivering a copy via email to Stuart Pollack and Lisa Haile (the counsel of record for the Petitioner) at the following address:

Steadymed-IPR@dlapiper.com

Date: July 6, 2016 Signature: /Stephen B. Maebius/
Stephen B. Maebius

4814-0612-4340.3

Paper _____

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

STEADYMED LTD.,

Petitioner,

v.

UNITED THERAPEUTICS CORPORATION,

Patent Owner.

Case IPR2016-00006
Patent 8,497,393

DECLARATION OF ROBERT M. WILLIAMS, Ph.D., IN SUPPORT OF PATENT OWNER RESPONSE TO PETITION

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I have been retained by the law firm of Wilson Sonsini Goodrich & Rosati ("WSGR") as an expert consultant to United Therapeutics Corporation ("UTC") in connection with the above-identified matter to provide expert testimony concerning U.S. Patent No. 8,497,393 ("the '393 Patent", Ex. 1001) by Batra *et al.*, entitled "Process to prepare Treprostinil, the active ingredient in Remodulin," issued on July 30, 2013. At the request of Counsel for UTC, I hereby submit this expert declaration.

I. Qualifications and Background

A. Education and Experience

1. I am a tenured University Distinguished Professor of Chemistry at Colorado State University (CSU). I currently serve as the Director for the Colorado Center for Drug Discovery. I also served as co-Director (Experimental Therapeutics) for the Infectious Diseases Supercluster Initiative and also served as co-Director for the Cancer Supercluster Initiative at CSU. My *curriculum vitae* is attached hereto as Exhibit A (Ex. 2021).

2. I received a B.A. in Chemistry from Syracuse University in 1975, and did laboratory research in the field of synthetic organic chemistry under the guidance of the recent Nobel Laureate Professor Ei-ichi Negishi. In 1979, I received both a Master's degree and Ph.D. degree in Organic Chemistry from the Massachusetts Institute of Technology (MIT) under the direction of Professor William H. Rastetter. Upon graduating from MIT, I spent one year (1979-80) as a postdoctoral fellow at Harvard University in the laboratories of the Nobel Laureate, the late Professor Robert B. Woodward, whose laboratory was subsequently managed by Professor Yoshito Kishi.

3. Subsequent to my fellowship at Harvard, I served as an Assistant Professor at Colorado State University from 1980–84. I was tenured and promoted early, to the rank of

Associate Professor in 1985, and in 1988, I was promoted to the rank of Full Professor. In 2002, I was named a University Distinguished Professor, which is my current position. University Distinguished Professor is the highest academic rank at Colorado State University, and there are a maximum of twelve University Distinguished Professors at any given time out of a faculty of 1,200. This is a lifetime appointment until retirement, whereupon Emeritus status is granted. In addition to my positions at Colorado State University, I was a Visiting Professor of Chemistry at Harvard University from 1994–95, at which time I was sponsored by Professor Stuart L. Schreiber and taught a sophomore organic chemistry course for pre-medical students, Chem 17. I was also a Visiting Professor of Chemistry at the University of California at Berkeley in 1990 and worked in the laboratory of Professor Peter G. Schultz.

4. I have extensive experience in the field of synthetic organic chemistry and medicinal chemistry with an emphasis on biologically active compounds including anti-tumor agents, heterocycles, antibiotics, anti-fungal agents, anti-viral agents, immunomodulators, amino acids, peptides and alkaloids, among many other classes of biologically active organic substances. My organic chemistry research interests include the total synthesis of novel natural and synthetic products, heterocyclic chemistry, asymmetric synthesis, synthetic methodology, process chemistry, and reaction mechanisms. I have extensive experience in the synthesis, chemistry, conformational analysis, biochemical activity, and biological activity of a range of organic compounds.

5. My research laboratory at Colorado State University has worked extensively on the chemistry and biology of numerous drugs over my career, including Quinocarcin (Quinocarmycin citrate), Tetrazomine, Bioxalomycin, Ecteinascidin 743 (Yondelis® or trabectidin), Renieramycin, Cribrostatin-4, Jorumycin, the Mitomycins, FR900482, FK973,

FK317, FK228 (Romidepsin), Largazole, Stephacidins A and B, Avrainvillamide, Spirotryprostatins, TMC-95A/B, Rottlerin, and Antimycin, amongst many others.

6. I have been the Principal Investigator on numerous research grants from Federal agencies, such as the National Institutes of Health (NIH) and the National Science Foundation (NSF) as well as from various Foundations, and corporations to synthesize biologically active compounds on both small laboratory scale as well as larger industrial scales.

7. I held a funded research collaboration with the Infectious Diseases Research Institute (IDRI), in Seattle, Washington, to develop several novel adjuvants for the treatment and prevention of autoimmune diseases, infectious diseases and cancer (2010).

8. From 1991-1993, I held a research grant from Symphony Pharmaceuticals, located in Philadelphia, Pennsylvania, to prepare anti-HIV drugs based on inhibition of the HIV protease. I supervised a graduate student who prepared several very potent peptide isosteres that exhibited in vitro activity against HIV.

9. I have taught undergraduate and graduate courses in organic chemistry, organic synthesis, biosynthesis, biological chemistry, drug design, and the synthesis of natural products. I have also lectured at numerous professional conferences, universities, and in corporate R&D laboratories in those areas.

10. I am a Scientific Founder, Acting President, and Chair of the Scientific Advisory Board of Cetya Therapeutics, a company that is developing several drugs, including drugs for the treatment of various cancers, multiple myeloma, autoimmune diseases, and hemoglobinopathies. I also direct all of the process scale synthesis optimization and drug formulation studies being conducted on Cetya's HDAC inhibitors. This includes injectable formulations as well as oral formulations. Specifically, I directed and supervised post-doctoral researchers in my laboratory

(on behalf of Cetya Therapeutics) to formulate the poorly water-soluble drug Largazole, including a myriad of synthetic analogs of Largazole prepared in my laboratory, as a polysorbate-80/ethanol co-solvent excipient system. This formulation has been used in animal studies for obtaining critical dose-escalation and pharmacokinetic data. I have also specifically directed and supervised the formulation of Largazole and related analogs in various PEG-based (polyethylene glycol) excipient systems. This work is currently being conducted in collaboration with oncologist Dr. Douglas Thamm of the Colorado State University Animal Cancer Center, pharmacologist Dr. Dan Gustafson of the Colorado State University Animal Cancer Center, Dr. Kimberly Stegmaier of the Dana-Farber Cancer Institute/Harvard Medical School and Dr. James E. Bradner of the Dana-Farber Cancer Institute/Harvard Medical School. The animal studies commenced in 2010, and the drug formulation studies are being conducted in my laboratory at Colorado State University under my direction.

11. I was a Scientific Founder, Member of the Scientific Advisory Board, and Member of the Corporate Board of Directors for Xcyte Therapies, a company devoted to developing *ex vivo* T-cell therapies for treating cancer, autoimmune, and infectious diseases, including HIV. As a Scientific Founder and Member of the Board of Directors of Xcyte Therapies, I was deeply involved in writing the patents and developing formulation strategies for both topical and injectable drugs based on FK228 (Romidepsin).

12. As a Scientific Founder and Acting Vice-President of Discovery Chemistry of HemaQuest Pharmaceuticals (Seattle, Washington), I have directed the pre-clinical and clinical synthesis, scale-up and formulation studies of several of the companies' drugs. These include both water-soluble drugs and hydrophobic, poorly water-soluble drugs for therapeutic applications in both cancer and hemoglobinopathies. I directed both the medicinal chemistry

efforts as well as the pre-process optimization work for potential industrial-scale syntheses of our lead drug candidates.

13. In addition, I am a Scientific Founder and member of the Scientific Advisory Board of Sapientia Therapeutics, located in Philadelphia, Pennsylvania. I am the acting Director of the Medicinal Chemistry, Process Chemistry and Drug Formulation efforts of this company to develop novel small-molecule inhibitors of protein kinase C-delta for autoimmune diseases, cancer and scleroderma. My laboratory has synthesized the first lead compounds, which are protein kinase C-delta (PKC-Δ) inhibitors and are water-insoluble substances. Under my direction we have engaged in early scale-up and route optimization for our leading drug candidates.

14. As a chemist with expertise in structure-activity studies and synthesis of biologically active agents, I have been retained to consult for a number of pharmaceutical and biopharmaceutical companies for both drug discovery and process research applications over the past thirty years. I consulted for Ajinomoto Co., Japan from 2002-2014 in the general area of process chemistry in the manufacture of amino acids, their derivatives, pharmaceutical intermediates and peptide synthesis. I served as a consultant for Cubist Pharmaceutical Company (2000–03) in the general field of antibacterial agents. I consulted for NewBiotics, Inc. (2001–02) in the general fields of anti-infective agents and anti-cancer agents. I consulted for Hoffman-La Roche, Inc. (1989–92) in the field of cephalosporin-fluoroquinolone dual-action antibacterial agents, as well as on a project concerned with inhibitors of diaminopimelic acid (DAP) biosynthesis as potential antibacterial agents. I consulted for W.R. Grace (1985–90) in the area of specialty chemicals and pharmaceutical intermediates process manufacturing and process development. I was a Scientific Founder, Member of the Scientific Advisory Board,

Consultant and sub-contractor for Microcide Pharmaceutical Co. (Microcide) in their drug discovery and early process research efforts. Microcide was a biopharmaceutical company devoted to developing antibacterial agents against a range of drug-resistant bacterial and fungal infectious diseases. In addition, I have consulted for EPIX Medical, G. D. Searle, Nutrasweet, and Boehringer-Ingelheim, among others. The consulting work I performed for Nutrasweet (1990-1991), was concerned with large-scale manufacturing process chemistry for Aspartame.

15. I was a co-organizer of a special Symposium on process chemistry at The International Chemical Congress of Pacific Basin Societies, PacifiChem 2015 (December 15-18. Honolulu, Hawaii) entitled: *"New Horizon of Process Chemistry by Scalable Reactions and Technology."*

16. I have directed the research activities of more than sixty PhD students and eighty post-doctoral fellows; most of my former co-workers have gone on to successful careers in the pharmaceutical industry in both process research and medicinal chemistry.

17. I have delivered numerous named and plenary lectures at Universities, corporations, and scientific societies on the synthesis, chemistry, biology, and mechanism of action of numerous classes of therapeutic agents, as detailed in my *curriculum vitae* attached hereto as Exhibit A.

18. I have published more than 315 scientific research articles, authored numerous chapters in books, and have written a well-known textbook on the synthesis of optically active amino acids. I have particular expertise in the large-scale industrial synthesis of amino acids and their derivatives. I am also a named inventor on seventeen issued U.S. patents and published patent applications. My publications and patents are listed on my CV, provided in Exhibit 2021.

19. I currently serve on the Editorial board for *Chemistry & Biology*. I have served as Editor for the *Organic Chemistry Series* published by Pergamon Press and Elsevier (1997-2012), and *Mini Reviews in Organic Chemistry* (Bentham Science). I have also served as an editor for several other journals in the past, including *Tetrahedron: Asymmetry*, *Tetrahedron Publications*, *Amino Acids*, and the *Journal of the American Chemical Society*.

20. I am a member of the American Chemical Society, the Japan Antibiotics Research Association, the International Society of Heterocyclic Chemistry, and the American Association for the Advancement of Science. I am a Member of the University of Colorado Cancer Center, located in Aurora, Colorado. I have served as organizer or co-organizer of numerous scientific meetings and symposia, and served as the Vice President of the International Society of Heterocyclic Chemistry, Chairing the 2003 International Congress of Heterocyclic Chemistry.

21. I serve on the Scientific Advisory Board of Arch Therapeutics, located in Boston, Massachusetts, that is developing self-assembling peptides for wound healing and surgical closure.

22. I have also served on the Scientific Advisory Boards for a number of other companies. I currently serve on the External Advisory Committee for the Puerto Rico Alliance for the Advancement of Biomedical Research Excellence. I was a Scientific Founder, Director of Chemistry, and member of the Scientific Advisory Board for HemaQuest Pharmaceuticals. I was a Founding Scientist and Member of the Scientific Advisory Board of Microcide Pharmaceuticals from 1993 to 1998.

23. I have expertise in drug formulation for injectable, topical and oral medications. I have directed research programs, both through my academic laboratory at Colorado State University as well as through my various consulting engagements and as a research director

and/or consultant for companies developing medicines for numerous therapeutic indications. I have consulted on many aspects of pharmaceutical drug discovery, development, formulation, and manufacturing. This includes basic discovery and optimization, early process research, large-scale manufacturing, and drug formulation.

24. I have served as a consultant for a number of companies for both drug discovery and process research applications, including, for example, W.R. Grace Company (1985-1990, fine chemicals synthesis); Symphony Pharmaceuticals (1991-1993, anti-HIV drugs); G.D. Searle Co. (1988-1990, memory and learning enhancement agents based on NMDA receptor antagonists); Nutrasweet Co. (1990-1991, artificial sweeteners); EPIX Medical (1993-1997, MRI imaging and contrast agents); Hoffman-La Roche, Inc. (1989-1992, cephalosporin-fluoroquinolone dual-action antibacterial agents); Boehringer-Ingelheim Pharmaceuticals (1992-1993, antiviral agents); Cubist Pharmaceutical Company (2000-2003, macrocyclic peptide antibacterial agents); NewBiotics, Inc. (2001-2002, anti-infective agents and anti-cancer agents); Microcide Pharmaceutical Co. (1993-1998, analogs of macrocyclic anti-fungal agents related to echinocandin, cephalosporins, and quinolones); Xcyte Therapies (1996-2006, T-cell activation); Ajinomoto Co, Japan (2002-2014, amino acids, peptides, and other specialty chemicals); HemaQuest Pharmaceuticals (2006-2014, short chain fatty acids for treating hemoglobinopathies); Sapientia Therapeutics (2012-present, small-molecule inhibitors of protein kinase C-delta); Arch Therapeutics (2010-present, self-assembling peptides for wound healing); and most recently, Cetya Therapeutics (2012-present, histone deacetylase inhibitors as therapeutic agents for treating cancers, multiple myeloma, autoimmune diseases, and hemoglobinopathies).

25. Under my direction, my laboratory developed the technology for the asymmetric synthesis of amino acids in 1985 that was commercialized by Aldrich Chemical Co. in 1988. My laboratory devised several large-scale (multi-kilogram) process routes for the manufacture of the so-called "Williams Lactone" that has been sold by Sigma-Aldrich Chemical Company since 1988. Early manufacturing was conducted in China by several of my former co-workers at the Chengdu Institute of Organic Chemistry.

26. I have been awarded numerous prizes and awards including the NIH Research Career Development Award (1984-89), the Eli Lilly Young Investigator Award (1986), the Merck, Sharp & Dohme Academic Development Award (1991), an award from the Japanese Society for the Promotion of Science Fellowship (1999), the Arthur C. Cope Scholar Award sponsored by The American Chemical Society (2002), the Multiple Myeloma Research Foundation Senior Award (2010), the ACS Ernest Guenther Award in the Chemistry of Natural Products sponsored by Givoudan and The American Chemical Society (2011), an award from the Japanese Society for the Promotion of Science Long-term Fellowship (2012-2013), and the Organic Synthesis Award from the local Rocky Mountain section of the American Chemical Society (2012).

27. I have testified numerous times as an expert witness in process chemistry patent litigation in the following matters: Great Lakes Chemical *versus* Archimica SPA. Civil Action No. 99–728-JJF; Ranbaxy Laboratories *versus* Abbott Laboratories. Case No. 04 C 8078; Lundbeck *versus* Infosint. 06 Civ. 2869 (LAK); United Therapeutics Corp. *versus* Sandoz, Inc. C.A. Nos.: 12-1617 (PGS)(LHG) and 13-316 (PGS) (LHG); Gilead Sciences, Inc. and Emory University *versus* Cipla, Limited. Civil Action No.: 1:12-cv-06350-RJS; United Therapeutics

Corp. *versus* Teva Pharmaceuticals, USA, Inc. C.A. No.: 3:14-cv-05498 (PGS)(LHG); United Therapeutics Corp. *versus* Sandoz, Inc. C.A. No.: 3:14-cv-05499 (PGS)(LHG).

B. Materials Considered

28. In forming my opinions in this report, I have relied upon my professional experience and personal knowledge. I have also reviewed a number of documents in this case including all documents cited by the SteadyMed and UTC as well as the materials I have cited in this declaration. In this report, I have provided representative citations to exemplary documents that I have relied upon in reaching my opinions. If I am provided additional information or documents in this proceeding, I may offer further opinions regarding the additional information.

II. Legal Standards Provided By Counsel

29. I have been informed by Counsel that, during an *inter partes* review (IPR), a petitioner must prove invalidity by a preponderance of the evidence. Accordingly, I understand that the burden is on a petitioner to prove invalidity, rather than a patent owner to prove validity. I have been informed by Counsel that because each claim defines a separate invention, the validity of each claim in a patent is addressed independently of the validity of the other claims in that patent.

30. I have also been informed by Counsel that the claims of the '393 patent are "product-by-process" claims. I have also been informed by Counsel that when evaluating the validity of a patent claim, the "product" of product-by-process claims must include structural and/or functional differences over the prior art, even if they are not explicitly claimed.

A. The Person of Ordinary Skill in the Art

31. I have been informed by Counsel that a patent is to be interpreted from the perspective of a hypothetical person referred to as the person of ordinary skill in the art ("POSA")

to which the patent pertains. I am further informed that a determination of the level of ordinary skill is based on, among other things, the type of problems encountered in the art, prior art solutions to those problems, rapidity with which innovations are made, sophistication of the art, and the educational level of active workers in the field. I have been informed that in any particular case, every factor may not be present, and one or more factors may predominate. I understand the person of ordinary skill in the art is presumed to know all prior art that is reasonably relevant to the subject matter of the claimed invention.

32. I understand from Counsel that the validity of a patent claim must be assessed from the perspective of a POSA at the time of the invention.

33. Given the complexity of the chemistry involved in the '393 patent, it is my opinion that a POSA with respect to the patent-in-suit would have had, at the time of the claimed invention, a doctorate degree in chemistry, pharmaceutics, pharmaceutical sciences, medicine, or a related discipline. Alternatively, the POSA may have had a lesser degree in one of those fields, with correspondingly more experience. To the extent necessary, a POSA may have collaborated with others of skill in the art, such that the individual and/or team collectively would have had experience in synthesizing and analyzing complex organic compounds. It is my understanding that a patent is to be interpreted from the perspective of a person of ordinary skill in the art at the time of the patent's priority date.

34. I understand that SteadyMed's expert Dr. Winkler has opined that a POSA would have "a master's degree or a Ph.D. in medicinal or organic chemistry, or a closely related field. Alternatively, a person of ordinary skill would include an individual with a bachelor's degree and at least five years of practical experience in medicinal or organic chemistry." Ex. 1009 at ¶14.

35. All of my opinions regarding validity contained in this report are expressed from the view of a POSA at the time of the priority date of the '393 patent. These opinions apply equally whether my definition of a POSA or Dr. Winkler's is applied.

B. Anticipation

36. I understand from Counsel that anticipation requires that each and every element of a claim is set forth in a single prior art reference, and that these elements are arranged or combined in that reference in the same way as recited by the claim. I further understand from Counsel that if there is any difference between the prior art reference and the claimed invention, there is no anticipation by that reference. Further, I understand that there is no anticipation if the elements disclosed in a prior art reference must be combined with the knowledge of one skilled in the art to achieve the subject matter of the claim. I understand that for a prior art reference to be anticipatory, it must enable a POSA to make or practice the invention without undue experimentation.

37. I also understand from Counsel that if the single prior art reference is missing a claimed feature, the reference may inherently anticipate if that missing feature is necessarily present in the single prior art reference.

38. I also understand from Counsel that if there are structural or functional differences in the product of the product by process claims of the invention from the product of the prior art that arise from the process in which it was made, those differences may be evidence of no anticipation even if those differences are not explicitly claimed.

C. Obviousness

39. I understand from Counsel that obviousness requires that a POSA would have been able to arrive at the claimed invention by modifying a single prior art reference or by

combining two or more prior art references. I also understand from Counsel that obviousness analysis must be conducted from the point of view of a POSA at the time of the invention, and that it is improper to employ hindsight or consider the inventors' own path to the invention as proof of obviousness.

40. Counsel has also informed me that obviousness requires that a POSA would have had a reasonable expectation of success in achieving the claimed invention.

41. I understand from Counsel that four factual issues are relevant to obviousness analysis: the scope and content of the prior art; the level of ordinary skill in the field of the art at the time of the invention; the differences between the claimed invention and the prior art; and various objective indicia of non-obviousness.

42. I understand from Counsel that, in addition to considering the prior art, certain objective indicia may also provide evidence that a claimed invention is not obvious. I am informed by Counsel that these objective indicia, which are also referred to as secondary considerations, may include factors such as commercial success, unexpected results, the resolution of long-felt but previously unmet needs, skepticism by others prior to achieving the invention, failure of others to achieve the invention, praise from others for the invention, and copying by others.

43. I understand from Counsel that, like anticipation, if there are structural or functional differences in the product of the product by process claims of the invention from the product of the prior art that arise from the process in which it was made, those differences may be evidence of non-obviousness even if those differences are not explicitly claimed.

III. Summary of Opinions

44. It is my opinion that the term "product" as it is used in the claims of the '393 patent should be construed using UTC's construction: "a substance resulting from a chemical reaction."

45. It is my opinion that the term "[a] product comprising a compound of formula I/IV or a pharmaceutically acceptable salt thereof" as it is used in the claims of the '393 patent should be construed using UTC's construction: "a substance resulting from a chemical reaction constituted primarily of formula I/IV or a pharmaceutically acceptable salt thereof."

46. It is also my opinion that none of the claims of the '393 patent are anticipated by or rendered obvious by the prior art.

47. My opinions and the bases for them are based on information that I know, that I have reviewed, and that I am currently aware exists. I reserve the right to supplement or amend my opinions in light of any additional evidence, testimony, or other information that may be provided to me after the date of this declaration. Additionally, I may use the cited materials to assist me in preparing demonstratives such as graphics and animations if I am asked to testify.

IV. The '393 Patent

48. The '393 patent is directed to an improved treprostinil product and improved process for making the product. I understand from Counsel that the priority date for the '393 patent is December 17, 2007.

49. The synthesis of treprostinil is complex as several improvements resulting in improved products are disclosed in the '393 patent itself. The structure of treprostinil has five chiral centers (stereogenic centers) resulting in 32 possible stereoisomers of treprostinil.

50. The '393 patent has two independent claims: Claims 1 and 9. Claim 1 requires "a product comprising a compound of formula I…or a pharmaceutically acceptable salt thereof," in which formula I can be several structures including treprostinil. Claim 9 requires "[a] product comprising a compound having formula IV…or a pharmaceutically acceptable salt thereof," in which is the structure of treprostinil. Both Claims 1 and 9 then specify that the product is prepared by a process comprising (a) alkylating a compound of Formula II or V [a benzindene triol structure] with an alkylating agent to produce a compound of Formula III or VI [a benzindene nitrile intermediate], (b) hydrolyzing the product of formula III or VI of step (a) with a base, (c) contacting the product of step (b) with a base B to form a salt of Formula Is or IVs [indicating a salt form of treprostinil with an HB+ counterion], and (d) optionally reacting the salt formed in step (c) with an acid to form the compound of formula I or IV. Dependent Claim 7 further identifies the specific structure of Formula I of the product of Claim 1 as treprostinil. Because the other possible structures of Claim 1 are not at issue here, I will consider these Claims 1, 7, and 9 together in my analysis. Likewise, I will consider the following dependent claims together that have similar claim limitations.

51. Dependent Claims 2 and 10 provide a further purity limitation. Claim 2 further requires "[t]he product of claim 1 wherein the purity of compound of formula I in said product is at least 99.5%." Similarly, Claim 10 requires "[t]he product of claim 9, wherein the purity of product of step (d) is at least 99.5%." Thus, step (d) must be performed in claim 10, but both of these claims require a purity of at least 99.5%.

52. Dependent Claims 3 and 11 provide a further limitation on what alkylating agent may be used. Claim 3 requires the alkylating agent be $Cl(CH_2)_wCN$, $Br(CH_2)_wCN$, or $I(CH_2)_wCN$. Claim 11 requires the alkylating agent be $Cl(CH_2)_wCN$.

53. Dependent Claims 4 and 12 specify what base may be used in step (b). Claim 4 requires the base in step (b) to be KOH or NaOH and Claim 12 requires the base to be KOH.

54. Dependent Claims 5, 13, 14, 17 and 18 specify what the base B in step (c) may be selected from certain specific bases. Claims 5, 13, and 17 limit base B to the group consisting of ammonia, N-methylglucamine, procaine, tromethamine, magnesium, L-lysine, L-arginine, triethanolamine, and diethanolamine. Claims 14 and 18 specify that the base B is diethanolamine.

55. Dependent Claims 6 and 15 specify what acid is used in step (d). Claim 6 specifies the acid is HCl or H_2SO_4. Claim 15 specifies the acid is HCl.

56. Dependent Claims 8 and 16 specify that the process does not include purifying the compound of formula III or VI produced in step (a).

57. Dependent Claims 19 and 20 depend on Claims 1 and 9, respectively. Each dependent claim further specifies the base in step (b) is KOH or NaOH and the base in step (c) is selected from the same group specified in Claims 5, 13, and 17.

58. Claim 21 depends on Claim 1 and requires that step (d) is performed. Claim 22 depends on Claim 21 and further requires that the product comprises a pharmaceutically acceptable salt formed from the product of step (d).

V. Claim Construction

59. I understand from Counsel that different claim constructions for certain terms used in the claims of the '393 patent have been proposed by SteadyMed and UTC, and that the U.S. Patent and Trademark Office ("PTO") has entered a preliminary claim construction for certain terms.

60. I agree with UTC's construction of the term "product" as "a substance resulting from a chemical reaction" which is consistent with the plain and ordinary meaning of the term.

61. In the chemical context, "product" generally refers to the real world outcome or result of a reaction:

Generalized Chemical Reaction

Reactants → Products

I agree with UTC that the '393 patent itself distinguishes "product" to identify it as what comes at the end of a chemical process or chemical reaction. Prelim. Resp. at pp.17-18.

62. I also agree with the consistent definitions given by the several textbooks cited by UTC all referring to "product" as the result of a chemical reaction. *Id.* at 19.

63. In fact, I have used the term "product" consistently in my own publications to refer to the real world result of a chemical reaction. *See, e.g.*, Williams, et.al., *Asymmetric, Stereocontrolled Total Synthesis of Paraherquamide A*, J. Am. Chem. Soc. 2003, 125, 12172-178. ("However, the reaction was very slow and gave the desired cyclization product 64 in only 25% yield, accompanied by products from competing pathways.") (Ex. 2026); Williams, et.al., *Stereocontrolled Total Synthesis of (+)-Paraherquamide B*, J. Am. Chem. Soc. 1996, 118, 557-579 ("Compound 66 was refluxed in benzene with 20 equiv of sodium hydride, resulting in a very clean and high yielding cyclization reaction furnishing the desired product 68 in 93% yield.") (Ex. 2027); Williams, et.al., *Synthetic Studies on Et-743. Assembly of the Pentacyclic Core and a Formal Total Synthesis*, J. Org. Chem. 73.24 (2008): 9594-9600. ("The scarcity of the natural product from marine sources renders Et-743 an important target for synthesis.") (Ex. 2028).

64. Dr. Winkler also uses the term "product" as the result of a chemical reaction in his own publications and confirmed that understanding during his deposition. *See, e.g.*, Winkler, J., et.al., *A Pauson-Khand Approach to the Synthesis of Ingenol*, Org. Lett., 2005, 8, 1489-1491 at Abstact ("Pauson-Khand cyclization of dioxanone photoadduct 21 leads to the formation of a single product in good yield.") (Ex. 2029); *see also* Ex. 2051 at 155:12-157:3.

65. Specifically, Dr. Winkler confirmed that "the product of a chemical reaction would be essentially all of the substances that result from the treatment of a particular reactant with a particular set of reagents." Ex. 2051 at 155:2-11. This is consistent with UTC's definition as well as how Dr. Walsh interpreted the product in his Declaration submitted during prosecution of the '393 Patent. Ex. 1002 at 346-347 (showing the products containing certain other substances as impurities).

66. I disagree with the PTO's preliminary construction and SteadyMed's construction of "product" as "a chemical composition." I believe that this proposed definition is too broad and does not accurately describe the term as it is customarily used in the art and in the context of how it is defined in the '393 patent. In the chemical context, there can be no "product" if there is no corresponding reaction, process, or synthesis that it refers to. A "chemical composition" could be used to describe the starting materials, solvents, reagents, catalysts, and even the glassware used during a chemical reaction as there is no limitation on SteadyMed's construction of the term "product" on how it relates to the chemical reaction at issue.

67. In the '393 patent and each of the references I describe above, the word "product" is exclusively used to describe a substance resulting from a chemical reaction, and it is not used to describe any and all "chemical compositions."

68. SteadyMed's construction is therefore inconsistent with the understanding of a POSA and inconsistent with the '393 patent specification regarding the term "product" because "a chemical composition" is not an accurate and specific definition of the term.

69. For the reasons I previously described regarding the term "product", a POSA would understand the plain and ordinary meaning of the claim term "A product comprising a compound of formula I/IV or a pharmaceutically acceptable salt thereof," as UTC's construction: "a substance resulting from a chemical reaction constituted primarily of formula I/IV or a pharmaceutically acceptable salt thereof." This definition is consistent with how a POSA would understand the term and is consistent with its plain and ordinary meaning.

70. I disagree with the PTO's preliminary construction and SteadyMed's construction of "[a] product comprising a compound of formula I/IV or a pharmaceutically acceptable salt thereof" as "a chemical composition that includes, but is not limited to, a compound of Formula I, or a pharmaceutically acceptable salt thereof, and that may also include other non-mentioned substances (including impurities), additives, or carriers, without limitation as to the types of or relative amounts thereof." I believe that this proposed definition is too broad and does not accurately describe the term. The entirety of the '393 patent is directed to an improved product with lower amounts of impurities and therefore the product includes its own impurity profile which provides a high level of purity and does not indiscriminately include other substances and impurities "without limitation as to the types of or relative amounts thereof."

VI. Phares Does Not Anticipate Claims 1-5, 7-9, 11-14, or 16-20 of the '393 Patent

71. I have reviewed Dr. Winkler's opinions alleging that Phares (Ex. 1005) inherently anticipates Claims, 1-5, 7-9, 11-14, and 16-20. I have also reviewed the Institution Decision in which the Board credited Dr. Winkler's opinion regarding this lack of physical differences

between the treprostinil products of the '393 patent and Phares. Paper 12 at 23-31. I disagree. Additionally, the Board credited Dr. Winkler's opinion that Phares discloses the same process for synthesizing treprostinil as the '393 patent. Paper 12 at 29-30. This is not true. Because no synthesis of treprostinil is disclosed in Phares, the diethanolamine salt described would have an unknown impurity profile and therefore cannot anticipate any claim of the '393 patent.

A. The Product Disclosed in Phares is Physically Different Than the Products Disclosed in the '393 Patent Claims

72. In order for Phares to anticipate any claim of the '393 patent, Phares must disclose every claim limitation of the product. Phares does not disclose the same product as claimed in the '393 patent.

73. Contrary to Dr. Winkler's opinion, the polymorph form and purity of the treprostinil diethanolamine salt is not the same as that claimed in the '393 patent. Specifically, Phares discloses samples made for a polymorph screen, not large scale batches. *See, e.g.*, Ex. 1005 at 85-86. In fact Phares notes several different conditions to form polymorph A including preparation using fast evaporation, slow evaporation, freeze drying, heating, and slow cooling in a variety of solvent systems including water and ethanol; water, toluene, and tetrahydrofuran. *Id.* Once polymorph A is prepared, Phares then further states that polymorph form B must be made from polymorph A, listing several conditions under which polymorph B is prepared. *Id.* Phares further notes that the polymorph B sample that was used for characterization was made from heated slurries of form A in 1,4-dioxane and toluene. *Id.* at 87. In fact, it is not clear which sample of polymorph form A was further used to create the characterized sample of polymorph B that Dr. Winkler discusses. Ex. 1009 at ¶¶58-61.

74. The '393 patent does not discuss that polymorph A must be formed first. *See, e.g.*, Ex. 1001 at col. 12-13 and 15. The '393 patent also does not describe the use of 1,4 dioxane or toluene and only describes forming the diethanolamine salt followed by cooling and filtering the salt with ethyl acetate and ethanol, and then drying. *Id.* Thus, the treprostinil diethanolamine salt formed in Phares required an extra step to first form polymorph A, under different reaction conditions with different solvents.

75. It is well-known that the use of different solvent systems in forming different crystal forms can have a significant effect on the melting point of a substance as well as other characteristics including purity. *See, e.g.*, R. Adhiyaman, et.al., *Crystal modification of dipyridamole using different solvents and crystallization conditions*, Int'l J. Pharm.321, 2006, 27-34 at 33 ("Adhiyaman") ("In conclusion, it can be said that the crystallization conditions and medium used have major effect on dipyridamole crystals habit modification under ambient conditions. The crystals showed significant changes in the shape, size, melting points, dissolution rate, XRD patterns and DSC curves.") (Ex. 2030). Given that the samples of polymorph B described in Phares are prepared in a completely different way under different conditions than those described in the '393 patent, their melting points and other analytical data cannot be directly compared.

76. Furthermore, the only data that Dr. Winkler relies upon to conclude that the polymorph B sample of treprostinil diethanolamine salt in Phares has a "higher purity than the '393 product" is that the recorded melting point was higher in one sample than the melting point of the diethanolamine salt sample of the '393 patent. Ex. 1009 at ¶¶ 59-60. This is incorrect for several reasons. First, as mentioned above, the different solvents and conditions used to form the salt can greatly affect the melting point – which is the only purported evidence

that Dr. Winkler cites for purity. Second, there is absolutely no actual purity data disclosed in Phares for the diethanolamine salt or treprostinil free acid and a POSA would not have concluded based on a single melting point example of polymorph B prepared under unknown conditions (e.g., recrystallization solvent and recrystallization conditions are not identified) would be of a higher purity than the known purity of the '393 patent. Third, even if the diethanolamine salt samples were prepared under the same work-up and purification conditions, a higher melting point does not mean that the substance must be of a higher purity. *See*, Ex. 2030 at Fig. 5 showing modified crystals in several different solvents had a higher melting point than the pure dipyridamole). Fourth, the DSC curve cited by Dr. Winkler in Fig. 21 of Phares (Ex. 1009 at ¶59) shows a broad melting peak with a range of close to 10 degrees which is indicative of a lower purity substance. *See*, Marti, E., *Purity determination by differential scanning calorimetry*, Thermochimica Acta, 5(1972) 173-220 at 214 ("The melting of diphenyl is extremely sharp because of the purity level; on the other hand, the melting region of phenacetin-benzamide is rather broad.") (Ex. 2031). Additionally, the DSC data provided does not describe the sample size, the rate of temperature increase as a function of time and does not compare this with an authentic standard of known purity melted under identical conditions. It is known in the art that sample size, rate of heating, the recrystallization solvent(s) used, and the conditions under which the crystalline sample was obtained can significantly affect the DSC data. Dr. Winkler's conclusion based on this single vague and incompletely described DSC data is not scientifically sound.

77. Dr. Winkler also points to the brief description of the formation of the treprostinil diethanolamine salt (Ex. 1009 at ¶¶50-54), but that description does not indicate what treprostinil free acid was used to make it. While the Board agreed with Dr. Winkler regarding the similarity

of the products of Phares and the '393 patent, the source of the treprostinil used to make treprostinil diethanolamine is very important and would greatly affect the impurity profile and other analytical characteristics, including DSC, of the sample.

78. In fact, Phares itself describes several references that could be used to make treprostinil, but does not identify which one, if any, was used to make the sample for the treprostinil diethanolamine salt. *See, e.g.*, Ex. 1005 at 9 ("Compounds of the present invention can also be provided by modifying the compounds found in U.S. Patent Nos. 4,306,075 ("the '075 patent", Ex. 2032) and 5,153,222 ("the '222 patent", Ex. 2033) in like manner."). The '075 patent, for example, discloses a very different and less pure treprostinil product than that of Moriarty (Ex. 1004). *See, e.g.*, Ex. 1004 at 1892-93. Thus, without knowing the source of the treprostinil used in Phares to make the treprostinil diethanolamine salt, the resulting product could have a very different purity and impurity profile and would necessarily have a distinct impurity profile if it were made by a different process than that disclosed in the '393 patent.

B. Phares Does Not Disclose Several Other Claim Limitations

79. Dr. Winkler alleges that Phares discloses the same synthesis to make treprostinil diethanolamine as the synthesis described in the '393 patent and the Board credited his opinion on this point. *See*, Ex. 1009 at ¶¶51-57; Paper 12 at 29-30. I disagree. First, there is no description whatsoever in Phares of how to make treprostinil free acid. Instead, Dr. Winkler points to the synthesis of the enantiomer of treprostinil ((-) treprostinil) which is a completely different synthesis for a different stereoisomer. Ex. 1009 at ¶57. Winkler alleges that because certain steps are used in forming the enantiomer, those steps are inherently disclosed for use with treprostinil. Ex. 1009 at ¶¶56-57.

80. I understand the Board decision did not address the additional limitations of independent Claims 1 and 9 nor the dependent claim limitations in its anticipation analysis because "the process steps recited in claims 1 and 9 do not impart structural or functional differences to the claimed treprostinil product." Paper 12 at 31. I disagree with this assertion. Even if Phares used the synthesis of Moriarty to make treprostinil, there are significant differences between the product of Moriarty and the product of the '393 patent. *See*, Section VII(A) below. Because the products are different, the process differences are relevant to the anticipation analysis.

81. The synthesis for the enantiomer of treprostinil disclosed in Phares, however, is different than the synthesis of treprostinil disclosed in the '393 patent. First, contrary to Dr. Winkler's claims, the earlier part of the synthesis used in Phares to make the enantiomer is not the same synthesis disclosed in Moriarty. Specifically, the Moriarty reference obviously does not describe the synthesis of the enantiomer of treprostinil, but also does not include the Mitsunobu inversion step described by Phares wherein the stereochemistry of the secondary alcohol moiety has to be chemically reversed. Ex. 1005 at 40. In fact, because (S)-2-methyl-CBS-oxazaborolidine is used on structure 5, the resulting structures 6-11 are diastereoisomers of the intermediates used in the synthesis of the '393 patent. As a result, intermediate products of formulas (II) and (III) of Claim 1 and intermediate products of formulas (V) and (VI) of Claim 9 of the '393 patent are not disclosed in Phares. Thus, because steps (a) – (c) of *every claim* of the patent requires these products, Phares cannot anticipate any claim of the '393 patent.

82. Second, Claim 2 requires a specific purity of 99.5%. As I discussed above, there are no specific purity measurements disclosed in Phares and a single broad melting point determination with a large melting point range does not provide evidence that the purity of the

treprostinil diethanolamine sample is at least 99.5%. *See*, Section VI(A) above. For this additional reason, Phares does not anticipate Claim 2. The purity of that sample was not calculated from the DSC data as no control to an authentic standard of known purity was performed or reported.

83. SteadyMed claims that because the synthesis of the enantiomer of treprostinil in Phares does not describe a purification step, that the claim limitation of Claims 8 and 16 that the process does not include purifying the compound of Formula III (or VI) produced in step (a) is satisfied. That is not correct. In fact, Phares does not disclose any specific details of those steps whatsoever. Indeed, if the same synthesis from Moriarty was used as Dr. Winkler suggests, purification at step (a) is specifically described in that reference. Ex. 1004 at 1901-1902. Regardless of what synthesis was used, however, the fact remains that compounds of Formula III and VI do not appear in Phares as described above.

84. Under my interpretation of the highly pure product described in each of the claims of the '393 patent, Phares does not anticipate Claims 1-5, 7-9, 11-14, or 16-20 because it does not disclose the highly-pure product of the '393 patent, the synthesis of treprostinil, nor compounds of structures (II) and (III) from independent Claim 1 or structures (V) and (VI) from independent Claim 9, which are required by all of the claims.

VII. None of the Claims of the '393 patent Are Rendered Obvious by the Prior Art

85. I understand that the Board cited additional grounds for unpatentability including obviousness based on the combination of Moriarty and Phares and obviousness based on the combination of Moriarty, Phares, Kawakami (Ex. 1007), and Eğe (Ex. 1008). I disagree that any claim of the '393 patent is rendered obvious by any combination of these references.

A. The Product of the '393 Patent Is Structurally Different Than the Product of the Prior Art

86. In his declaration, Dr. Winkler expresses his opinion that "the '393 patent processes do not result in a physically different or unique product than that disclosed in the prior art." Ex. 1009 at ¶71. I am aware that, in the Institution Decision, the Board credited Dr. Winkler's opinion regarding this lack of physical differences between the treprostinil products of the '393 patent and the prior art. Paper 12 at 16-17. I disagree with Dr. Winkler's opinion for at least the following reasons.

87. Dr. Winkler appears to base his opinion on a comparison between the '393 patent process batches identified in the declaration submitted by Dr. David Walsh, one of the inventors of the '393 patent, during prosecution (Walsh Declaration), and a single prior art process batch identified in a particular prior art publication by Moriarty . Ex. 1009 at ¶¶63-71. However, Dr. Winkler's comparison suffers from several critical flaws.

88. First, and most fundamentally, there is no basis for comparing the "purity" reported in Moriarty with the purity discussed in the Walsh Declaration. When purity is determined by comparison of a sample to a reference standard such as assay purity (*see, e.g.*, ICH Guidance For Industry: Q7A Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients (2001) ("Q7A") at 28-29 (Ex. 2034); see also Reviewer Guidance: Validation of Chromatographic Methods (1994) ("Reviewer Guidance") at 5-8) (Ex. 2035), one cannot directly compare the purity values of two samples in any meaningful way unless each value was achieved by comparison to the same reference standard. Neither the Walsh Declaration nor Moriarty identifies a specific reference standard. While Moriarty notes that the

treprostinil product obtained was compared to an authentic sample of UT-15, there is no mention of any such comparison in the Walsh Declaration.

89. Instead, with respect to the Walsh Declaration, purity must be understood not with respect to any reference standard, but with respect to the amount of total impurities reported as detected in each of the sample batches. The term "purity" must also be understood with respect to the amount of total impurities detected in the context of the '393 patent itself; wherever assay purity is referred to, the '393 patent specifies that the number indicated refers to "HPLC (Assay)." For each of the representative batches discussed in the Walsh Declaration, impurity data is presented in the same way, and thus the purity of these samples can properly be compared to each other; the same cannot necessarily be said of the sample data reported in Moriarty.

90. Second, Dr. Winkler concludes from Example 4 of the '393 patent that the instrumentation used to measure purity "can have variations of at least 0.4%," and thus any detected difference less than that can be attributed to experimental error. Ex. 1009 at ¶¶69-70. Dr. Winkler bases his estimate of experimental error on the statement "that Example 4's Batch 1 had an HPLC Assay of 100.4%, which is obviously greater than the 100% value theoretically achievable." Ex. 1009 at ¶70. This is unsupported and appears to arise from Dr. Winkler's fundamental misunderstanding of how assay purity values are calculated. HPLC assay values are calculated with respect to a reference standard; thus, any time that the sample you are measuring has a greater purity than the reference standard, the assay value will exceed 100%. As such, it is incorrect to conclude that an assay value of 100.4% must indicate an error of at least 0.4%. Dr. Winkler's conclusion on this point is therefore fundamentally flawed.

91. This explains why the assay value for drug specification submitted to the FDA changed from a range of [redacted] to [redacted]. *See*, Ex. 2003 at 6. This change was not due to

an increase in impurities, but because the purity of the product using the '393 patent process improved (as compared to the already-established reference standard) thus moving the acceptability range to a higher purity specification. *Id.* The letter notes that the scope of the range remained unchanged which simply indicates the acceptability criteria was increased, and does not index an error rate or limit of detection. Indeed, the change to the specification is further evidence that the product of the '393 patent is physically different than the product of Moriarty.

92. Indeed, Dr. Winkler's conclusion is contradicted by the impurity data actually measured for the treprostinil product made by both the '393 patent process and the prior art process according to Moriarty. For both processes, impurities are reported with specific numbers unless the amount detected fell below 0.05%; in cases where some amount of an impurity less than 0.05% was detected, it was reported as simply "less than 0.05%" or "< 0.05%." This means that the level of detection for measuring impurities in these treprostinil samples was somewhere between 0 and 0.05%, not something in excess of 0.4% as Dr. Winkler erroneously concludes.

93. Third, as Dr. Winkler himself points out, there is the possibility for "significant batch-to-batch variations in the impurity profile of each batch of treprostinil." Dr. Walsh stated that the data presented in his declaration came from representative samples of each synthetic process. Ex. 1002 at 346-347. However, there is no such indication that the purity data reported in Moriarty comes from a representative sample of the prior art process. Due to the possibility of batch-to-batch variations, if a small number of batches are to be used as the basis for comparison, it is critical that those batches be representative of their respective products and processes. Thus while one could reasonably rely on a comparison between the representative batches presented in

the Walsh Declaration, one could not reasonably add the batch discussed in Moriarty to that comparison. It is exactly this scientifically unsound comparison to Moriarty upon which Dr. Winkler bases his opinion.

94. Ideally, to avoid the risk of batch-to-batch variations unintentionally biasing the data, a comparison should be made between the average impurities detected in treprostinil products made by the '393 patent process and treprostinil products made by the prior art process. To this end, I have prepared a chart containing impurity data for 56 samples of treprostinil product as produced by the prior art process according to Moriarty through 2004 (the date of the publication), attached as Appendix A to this declaration[1], and another chart containing impurity data for 122 samples of treprostinil product as produced by the '393 patent processes, attached as Appendix B to this declaration. I have prepared these charts using impurity data from release testing of samples of the respective treprostinil products that were produced by or for UTC for the purposes of obtaining regulatory approval and/or commercial sale. *See* Appendix A, Appendix B; Ex. 2005; Ex. 2036; Ex. 2037; Ex. 2052; Ex. 2053. As the purpose of these charts is to calculate the average impurities – both specific and total – found in the treprostinil products of each process, I have necessarily assigned a value of zero where the level of impurities was

[1] I am aware that UTC's Process Optimization Report for treprostinil prepared according to the '393 process included Table 2, which provided average impurity data for 96 batches of treprostinil made according to the prior art process. UT Ex. 2005, at 7. However, Table 2 does not provide exact values for four of the eight impurities under consideration, [redacted] and does not identify the underlying batch data. *Id.* As such, I have prepared my own chart using data on 56 treprostinil samples made by the prior art method and have based my analysis, including my calculations of average for total and individual impurities, upon this chart. While I believe my chart allows for a more precise comparison between Moriarty treprostinil products and '393 treprostinil products, the averages presented in the Process Optimization Report still show significant differences between '393 treprostinil products and the Moriarty treprostinil products. Specifically, Table 2 of the Process Optimization Report shows that on average [redacted] was detectable in these 96 batches, and that these 96 batches contained higher average levels of [redacted], and total impurities as compared to the averages for the '393 treprostinil product. Ex. 2005 at 7; Appendix B.

reported as "ND" (Not Detected), and a value of 0.05 where the level of impurities was reported as being less than 0.05%. From these data, I have found the following average impurity levels:

Moriarty Process Impurities (Average Percent Detected)								
1AU90	**2AU90**	**3AU90**	**750W93**	**751W93**	**97W86**	**ethyl ester**	**methyl ester**	**Total Related Substance**
0.0473	0.0407	0.2545	0.1646	0.1025	0.0405	0.0889	0.1028	**0.9545**
'393 patent Process Impurities (Average Percent Detected)								
[redacted]								

95. These averages make clear that the '393 patent process does result in a treprostinil product that is physically different from the prior art treprostinil product. In terms of total volume of impurities, the Moriarty process resulted in [redacted] times the amount of impurities that is achieved with the '393 patent process.

96. The products from the two processes also differ significantly with respect to the individual impurities in each product's impurity profile. Notably, the '393 patent process produces a treprostinil product that does not contain any detectable amounts of [redacted]. Additionally, the '393 patent process produces a treprostinil product that, on average, contains only [redacted] each of [redacted] and [redacted] and only [redacted] of [redacted]; as compared to the Moriarty process, this represents greater than a [redacted] reduction in each of the [redacted] and [redacted] impurities and a [redacted] reduction in the [redacted] impurity. The '393 patent process also produces a treprostinil product that, on average, has significantly reduced amounts of several other identified impurities; as compared to the average of the Moriarty process, the '393 patent process produces a treprostinil product with less than [redacted] the amount of [redacted], approximately [redacted] the amount of [redacted], and approximately [redacted] the amount of

[redacted]. Conversely, the '393 patent process produces a treprostinil product which actually contains slightly more [redacted] impurity than was detected in the treprostinil product of the Moriarty process.

97. Looking past the average data, it is also worth noting that, out of all the batches of treprostinil product made by the '393 patent process which I reviewed, [redacted] was only detected in a single batch [redacted]) and [redacted] was also only detected in a single batch ([redacted]), and both impurities were only detected at a level of 0.05% or less. Furthermore, batches [redacted] and [redacted] were both identified as "optimization batches" (as distinguished from commercial batches) and thus are not properly representative of treprostinil products made by the '393 patent process.

98. From these data, it is clear that the treprostinil product produced by the '393 patent process has a markedly different impurity profile than the treprostinil product of the Moriarty prior art process, and as such is physically distinct from the prior art product. Moreover, it could not have been obvious that employing the process of the '393 patent would result in a reduction of impurities as compared to the Moriarty process. Indeed, the '393 patent process actually results in an [redacted] in one detected impurity, [redacted]. Furthermore, it is also clear that the treprostinil product produced by the '393 patent process has a higher average purity than the Moriarty product. The treprostinil product of the '393 patent has an average purity of [redacted] while the Moriarty product has an average purity of 99.05%. Thus, the treprostinil product of the '393 patent has an average purity that is [redacted] higher than that of Moriarty's.

99. Therefore, it is my opinion that the treprostinil product produced by the process used in the '393 patent Claims 1 and 9 is physically different than the treprostinil product produced by Moriarty.

B. Claims 1-5, 7-9, 11-14, and 16-20 Are Not Rendered Obvious by the Combination of Moriarty and Phares

100. As described above, the product of Moriarty is physically different than the product of the '393 patent process. Even if the Moriarty synthesis was used to make treprostinil, a POSA would not have been motivated to make the diethanolamine salt identified in Phares.

101. Specifically, the '393 patent notes that the salt formation step results in an improved and more pure treprostinil product. Given that Moriarty discloses the use of column chromatography for purification, a POSA would not have been motivated to create the salt form in Phares as Phares does not disclose any benefit or increased purity as a result of using the diethanolamine salt. In fact, Phares does not allege that the diethanolamine salt is superior in any way to the treprostinil product of Moriarty and instead identifies other earlier treprostinil disclosures as a means to create the treprostinil used to form the diethanolamine salt. *See*, Section VI(A) above.

102. Additionally, a POSA would not have had a reasonable expectation of success in making the higher purity treprostinil product claimed in the '393 patent by the use of a salt formation step. As identified above, the impurities of treprostinil include [redacted] ([redacted]), [redacted] ([redacted]), the [redacted] starting material ([redacted]), and the [redacted]. As described above, the '393 patent process essentially eliminated the [redacted] impurities [redacted], and [redacted] impurity [redacted], but did not eliminate another [redacted] which likely has the same [redacted] as the other

stereoisomers. Similarly, the [redacted] impurity increased while the [redacted] impurity decreased. A POSA would have expected that all of the stereoisomers would remain as salt impurities, but that is not the case. Instead, the impurity profile of the '393 patent process yields an unexpected result by removing [redacted] while [redacted] impurity and [redacted] another. A POSA could not have predicted this outcome based on the salt formation described in Phares.

103. Regarding Claim 2, neither Moriarty nor Phares discloses treprostinil or treprostinil diethanolamine at a purity of 99.5%. As described above, Phares does not disclose any purity measurement (see Section VI above) and the purity measurement identified in Moriarty does not identify how the measurement was taken (see Section VII(A) above). Regardless of the purity identified in Moriarty, a further analysis of all batches made by the Moriarty process up to the time of the reference itself reveals an average purity of 99.05% while the average purity of the '393 patent batches is [redacted]. Given that the error rate must be below 0.05% for these measurements (see Section VII(A) above), the '393 patent process batches are significantly better in terms of overall purity. For this additional reason, Claim 2 is not rendered obvious by the combination of Moriarty and Phares.

104. Regarding Claims 8 and 16, Phares does not disclose any synthesis for treprostinil and therefore cannot disclose whether purification was needed for step (a). (*See*, Section VI(B) above). As previously described, Moriarty specifically discloses that purification is performed at step (a). See Section VII(B) above). In fact and most significantly, the '393 patent itself identifies that as a distinguishing feature over the prior art. *See, e.g.*, Ex. 1001 at Example 6. For this additional reason, Claims 8 and 16 are not rendered obvious by the combination of Moriarty and Phares.

C. Claims 6, 10, 15, 21, and 22 Are Not Rendered Obvious by the Combination of Moriarty, Phares, Kawakami, and Ege

105. Each of Claims 6, 10, 14, 21, and 22 require the additional step (d) of independent Claims 1 and 9 which is to react the salt formed in step (c) with an acid to form the compound of formula I or IV (treprostinil). Claim 22 further requires a pharmaceutically acceptable salt formed from the product of step (d). Step (d) is not disclosed in any way in Moriarty, Phares, Kawakami, or Eğe. Additionally, it is my opinion that it would not have been obvious to combine these references to arrive at the claimed inventions of Claims 6, 10, 15, 21, or 22.

106. First, there is no teaching or suggestion to perform step (d) in either Moriarty or Phares and similarly no reference to reverting back to treprostinil free acid from any treprostinil salt. Given that the purification techniques disclosed in Moriarty include chromatography and recrystallization after many years of research to optimize the process of making treprostinil, a POSA would not have been motivated to use a salt purification technique disclosed in an undergraduate chemistry textbook. More importantly, a POSA would not have had a reasonable expectation of success in further purifying the treprostinil product of Moriarty by using such a technique. To the extent a POSA was motivated to further purify treprostinil, a POSA would have focused on the known impurities and investigated methods of removing those. At the time of the invention, it was known that the formation of diastereomers occurred in the formation of treprostinil. *See*, Ex. 1004 at 1897-99. Thus, a POSA would have focused on how to remove those types of impurities.

107. Eğe simply discloses that "carboxylic acids that have low solubility in water, such as benzoic acid, are converted to water-soluble salts by reaction with aqueous base. Protonation of the carboxylate anion by a strong acid regenerates the water-insoluble acid. These properties

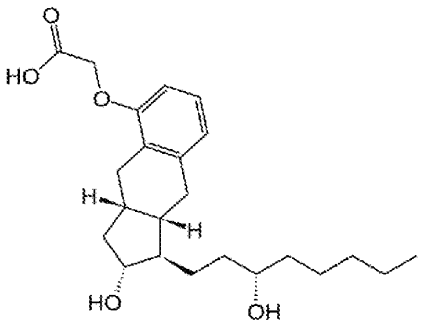
of carboxylic acids are useful in separating them from reaction mixtures containing neutral and basic compounds." Ex. 1008 at 8. This disclosure, however, would not have provided a POSA with a motivation to make the treprostinil free acid disclosed in Moriarty, convert that to the salt form of Phares, then convert the salt form back to the free acid.

108. First, Eğe does not provide any detail regarding how this reaction could be applied to more complex carboxylic acids or if it even could be applied. Specifically, the only carboxylic acid referenced in Eğe as an example is benzoic acid, a very simple aromatic acid, which is structurally very different from treprostinil acid. Indeed, benzoic acid has no chiral centers and therefore no stereoisomers and there is no suggestion in Eğe that this step could be used in purifying more complex carboxylic acids such as treprostinil which have stereoisomeric impurities. Second, Eğe specifically notes that "these properties of carboxylic acids are useful in separating them from reaction mixtures containing neutral and basic compounds," therefore Eğe would not apply to purifying carboxylic acids with stereoisomeric impurities because each stereoisomer would necessarily be an acidic impurity. As described above, the impurities that are removed from the '393 patent product include some, but not all acidic impurities and some but not all neutral impurities. *See*, Section VII(B) above. For these reasons a POSA would not have been motivated to combine Eğe with either Moriarty or Phares and would not have had a reasonable expectation of success in further purifying treprostinil using the acid reformation step described in Eğe.

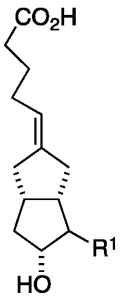
109. Indeed, given that Eğe predicts that only neutral and basic impurities would be removed, the actual average impurity profile for the '393 patent product is an unexpected result given that some but not all neutral impurities are removed as well as some but not all acidic impurities. *See*, Section VII(B) above.

110. Kawakami similarly does not provide any motivation for combining with either Phares or Moriarty and a POSA would not have had a reasonable expectation of success in preparing the products of Claims 6, 10, 15, 21, or 22 by combining these references.

111. Kawakami discloses the purification of a methanoprostacyclin derivative by forming the dicyclohexyl amine salt then regenerating the free acid to achieve a "fairly high" purity. Ex. 1007 at 6. Treprostinil and methanoprostacyclin, however, are very different structures:



Treprostinil



methanoprostacyclin compound in Kawakami

112. As shown here, the methanoprostacylin compound in Kawakami is a two-fused ring structure which is different than the three-fused ring structure of treprostinil that also includes an aromatic ring absent in the Kawakami methanoprostacyclin. These differences matter because a POSA would not have looked to Kawakami (or Eğe) if they were looking for additional purification techniques for treprostinil because neither reference discloses how to remove stereoisomeric impurities.

113. Instead, Kawakami provides a purification method for separating E and Z isomers of a starting material that is otherwise free of impurities, and not diastereomers that result from the various chiral centers that treprostinil was known to have as impurities. In fact, treprostinil

contains no mixture of E and Z isomers because it does not contain a carbon-carbon double bond that is capable of forming E and Z isomers. Indeed, the use of a specific salt to isolate a specific E/Z isomer does not reasonably suggest that salt formation of a much more complex compound with multiple chiral centers such as treprostinil could be isolated from entirely different impurities and then converted back to the free acid form. In fact, nothing in Kawakami suggests that this method could be used for a substance that was already fairly pure such as the treprostinil disclosed in Moriarty.

114. Similarly, Kawakami uses a dicyclohexylamine salt and does not use a diethanolamine salt, nor any salt counterion disclosed in the '393 patent. A POSA would have had no reason to combine the synthesis of Moriarty, use the salt only disclosed by Phares, and convert back to the free acid based on the teaching of Kawakami because Kawakami uses a different salt to separate a different structure from different types of impurities. Even if a POSA did combine these references in this way, a POSA would not have had a reasonable expectation of success in forming a more pure treprostinil product because Kawakami does not provide any information regarding the high level of purity required by the '393 patent and does not describe the separation of the types of stereoisomeric impurities known to be present in the treprostinil product. Dr. Winkler's obviousness analysis using these combinations is flawed and suffers from hindsight analysis.

115. Claim 6 requires the acid in step (d) be either HCl or H_2SO_4 and Claim 15 requires the acid to be HCl. Claim 21 requires that step (d) is performed. Phares, Moriarty, and Kawakami all do not disclose the use of either HCl or H_2SO_4 in converting a salt back to a carboxylic acid of any kind. Eğe cites HCl as an example in the conversion of benzoic acid, but as described above, a POSA would not have looked to Eğe to further purify a complex

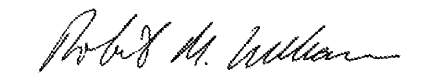
carboxylic acid such as treprostinil from its stereoisomers and other impurities and would have no reasonable expectation of success by using HCl based on this disclosure. For this additional reason, Claims 6 and 15 would not have been rendered obvious by any combination of Phares, Moriarty, Kawakami or Eğe. Similarly, given the deficiencies described above regarding Eğe and Kawakami, Claim 21 would not have been rendered obvious by any combination of Phares, Moriarty, Eğe, or Kawakami.

116. Claim 10 requires that step (d) is performed and further requires the product to be at least 99.5% pure. The only purity limitation disclosed in any of the cited prior art references is to Moriarty in which neither step (c) or (d) is performed. There is absolutely no other disclosure of a purity of at least 99.5% in any other cited prior art reference. A POSA looking to improve the purity of treprostinil above that level would have had no reason to look to Phares, Kawakami, or Eğe and based on their disclosures, would have had no reasonable expectation of success in making a treprostinil product with that level of purity as it simply is not present in the prior art allegedly disclosing step (d).

117. Claim 22 depends on Claim 21 and further requires a pharmaceutically acceptable salt be formed from the product of step (d). Dr. Winkler cites no evidence for this additional step in the prior art. In fact, none of the references cited even suggest converting a carboxylic acid to a salt form, then regenerating the carboxylic acid, then forming a pharmaceutically acceptable salt from that. It is my opinion that there is no evidence in the prior art supporting the additional claim limitation of Claim 22 and therefore no combination of Moriarty, Phares, Kawakami, or Eğe would render this claim obvious.

I declare under penalty of perjury that the foregoing is true and correct.

Date: July 6, 2016



Robert M. Williams, Ph.D.

APPENDIX A

Sample of product of Moriarty process	Impurities (Percent Detected)									Data Source
	1AU90	2AU90	3AU90	750W93	751W93	97W86	ethyl ester	methyl ester	Total Related Substances	
LRX-97J01	0.3	0.3	0.4	1.2	0.7	0.1	0	0.7	5.4	Ex. 2052, pp. 25-27
LRX-98A01	0.4	0.07	0.5	0.1	0.09	0.2	0	0.3	4.4	Ex. 2052, pp. 25-27
LRX-98B01	0.4	0.1	1	0.1	0.06	0.2	0	0.3	4.8	Ex. 2052, pp. 25-27
UT15-98H01	0.2	0.07	0.4	0.6	0.3	0	0	1.2	3.6	Ex. 2052, pp. 25-27
UT15-98I01	0.2	0.07	0.4	0.6	0.4	0.05	0	0.8	3.8	Ex. 2052, pp. 25-27
UT15-98I001	0.3	0.06	0.4	0.8	0.4	0	0	0.8	3.5	Ex. 2052, pp. 25-27
UT15RP-98K001	0.1	0.06	0.3	0.4	0.2	0	0	0.1	1.6	Ex. 2052, pp. 25-27
UT15-RP99D002	0.05	0.05	0	0.2	0.1	0.05	0.1	0.05	0.4	Ex. 2052, pp. 28-30
UT15-99E001	0.05	0.05	0.2	0.1	0.1	0	0	0.05	0.7	Ex. 2052, pp. 28-30
UT15MIX-99G001	0.05	0.05	1.1	0.3	0.2	0.6	0.6	0.05	2.8	Ex. 2052, pp. 28-30
UT15-99H001	0.05	0.05	0	0.5	0.3	0	0.1	0.06	1.0	Ex. 2052, pp. 28-30; Ex. 2036, pp. 2-3
UT15-000701	0	0.05	0.1	0.06	0.05	0	0	0.05	0.2	Ex. 2053, p. 19; Ex. 2036, pp. 88-89
UT15-000801	0	0.05	0.2	0.07	0.05	0	0	0.05	0.4	Ex. 2053, p. 19; Ex. 2036, pp. 91-92
UT15-000802	0	0.05	0.1	0.1	0.07	0	0	0.05	0.3	Ex. 2053, p. 19; Ex. 2036, pp. 94-95
UT15-000803	0	0.05	0.2	0.2	0.09	0	0	0.05	0.6	Ex. 2053, p. 19; Ex. 2036, pp. 100-101
UT15-000901	0	0.05	0.3	0.05	0.05	0	0.05	0.05	0.05	Ex. 2053, p. 19; Ex. 2036, pp. 33-34
UT15-000902	0	0.05	0.2	0.1	0.06	0	0.05	0.05	0.5	Ex. 2053, p. 19; Ex. 2036, pp. 97-98

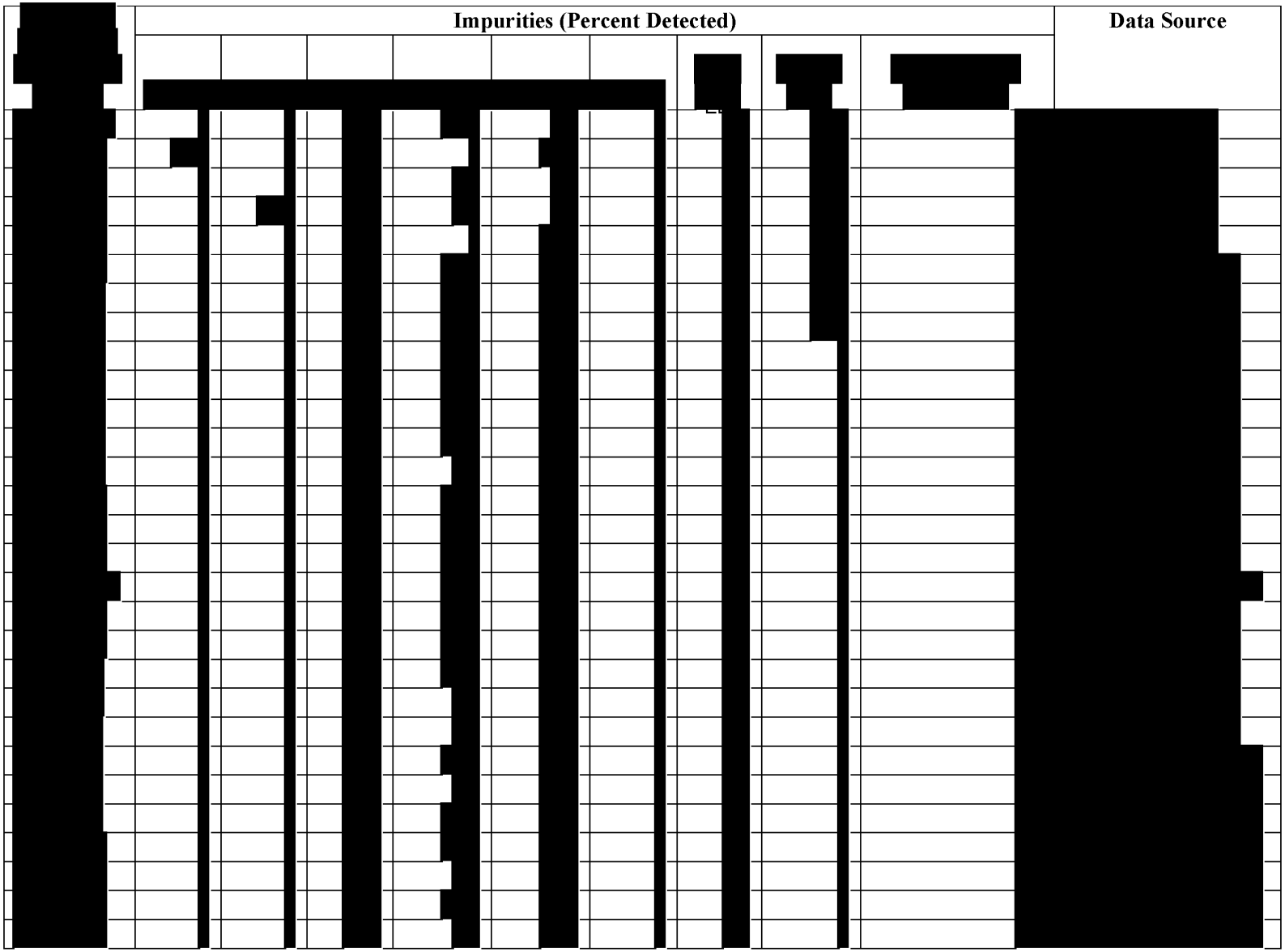
UT15-001001	0.05	0.05	0.2	0.09	0.06	0	0.05	0.05	0.4	Ex. 2053, p. 19; Ex. 2036, pp. 35-36
UT15-010201	0	0.05	0.2	0.09	0.05	0.05	0	0	0.4	Ex. 2053, p. 19; Ex. 2036, pp. 37-38
UT15-010202	0	0.05	0.2	0.09	0.05	0.05	0	0.05	0.4	Ex. 2053, p. 19; Ex. 2036, pp. 39-40
UT15-010203	0.2	0.05	0.3	0.4	0.2	0.08	0.05	0.05	1.5	Ex. 2053, p. 19; Ex. 2036, pp. 41-42
UT15-010301	0	0.05	0.3	0.09	0.05	0.05	0.05	0	0.5	Ex. 2053, p. 19; Ex. 2036, pp. 43-44
UT15-010302	0.05	0	0.2	0.05	0.05	0.05	0.08	0	0.3	Ex. 2053, p. 19; Ex. 2036, pp. 45-46
UT15-010303	0	0	0.2	0.1	0.05	0.05	0	0	0.3	Ex. 2053, p. 19; Ex. 2036, pp. 47-48
UT15-010801-RP	0	0.05	0.1	0.2	0.1	0.05	0.2	0	0.6	Ex. 2053, p. 20; Ex. 2036, pp. 60-61
UT15-010802	0.05	0.05	0.2	0.05	0.05	0	0.05	0.05	0.2	Ex. 2053, p. 20; Ex. 2036, pp. 50-52
UT15-010803	0.05	0.05	0.2	0.1	0.06	0	0.07	0.05	0.4	Ex. 2053, p. 20; Ex. 2036, pp. 52-53
UT15-010901	0	0.05	0.2	0.1	0.08	0.07	0.09	0	0.6	Ex. 2053, p. 20; Ex. 2036, pp. 54-55
UT15-010902	0	0.05	0.2	0.05	0.05	0	0.1	0	0.4	Ex. 2053, p. 20; Ex. 2036, pp. 56-57
UT15-011001	0	0.05	0.3	0.08	0.05	0.05	0.1	0	0.6	Ex. 2053, p. 20; Ex. 2036, pp. 58-59
UT15-020101	0	0.05	0.2	0.05	0.05	0	0.05	0	0.4	Ex. 2053, p. 20
UT15-020201	0	0.05	0.2	0.1	0.1	0	0.1	0	0.4	Ex. 2053, p. 20
UT15-020202	0	0.05	0.1	0.1	0.1	0.05	0.2	0	0.6	Ex. 2053, p. 20; Ex. 2036, pp. 62-63
UT15-020203	0	0	0.05	0.05	0.05	0	0.1	0.05	0.2	Ex. 2053, p. 20; Ex. 2036, pp. 64-65

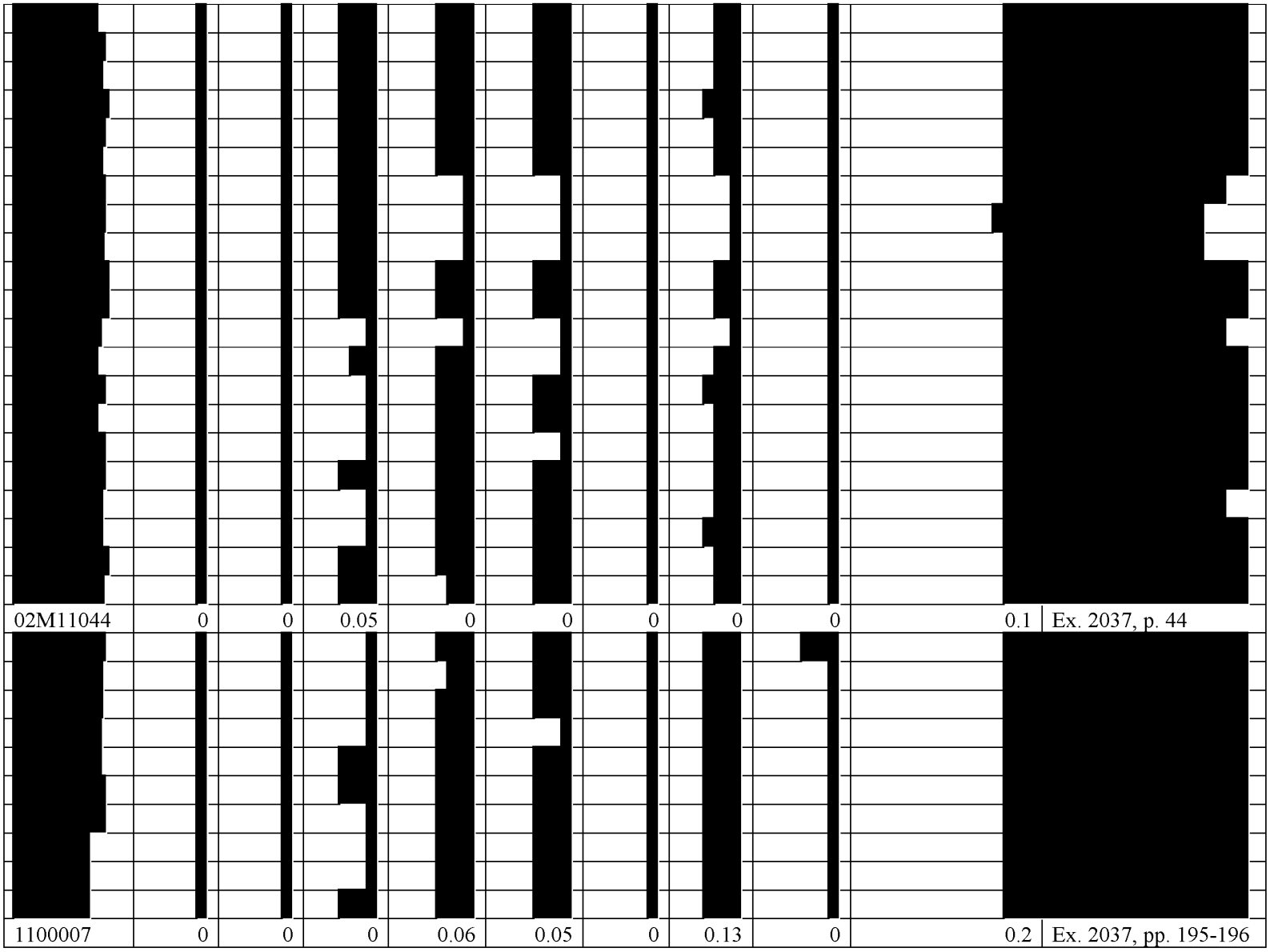
UT15-020301	0	0.05	0.2	0.05	0.05	0	0.1	0	0.3	Ex. 2053, p. 20; Ex. 2036, pp. 66-67
UT15-020302	0	0.05	0.2	0.06	0.05	0	0.1	0	0.4	Ex. 2053, p. 20; Ex. 2036, pp. 68-69
UT15-020303	0	0.05	0.2	0.05	0.05	0	0.1	0	0.3	Ex. 2053, p. 20; Ex. 2036, pp. 70-71
UT15-021001	0	0	0.4	0.1	0.08	0.05	0.1	0.05	0.8	Ex. 2053, p. 21; Ex. 2036, pp. 72-73
UT15-021002	0	0.05	0.3	0.06	0.05	0.05	0.2	0.05	0.6	Ex. 2053, p. 21; Ex. 2036, pp. 74-76
UT15-021003	0	0	0.4	0.05	0.05	0	0.1	0.05	0.6	Ex. 2053, p. 21; Ex. 2036, pp. 78-79
UT15-021101	0	0	0.2	0.09	0.06	0	0.1	0	0.5	Ex. 2053, p. 21; Ex. 2036, pp. 80-82
UT15-021102	0	0	0.1	0.2	0.1	0.07	0.1	0	0.6	Ex. 2053, p. 21; Ex. 2036, pp. 83-85
UT15-030401	0	0	0.3	0.06	0.05	0	0.2	0.05	0.5	Ex. 2053, p. 21; Ex. 2036, pp. 31-32
UT15-030501	0	0	0.3	0.1	0.07	0	0.1	0.05	0.6	Ex. 2036, pp. 29-30
UT15-030502	0	0	0.3	0.1	0.06	0	0.1	0.05	0.6	Ex. 2036, pp. 27-28
UT15-030503	0	0	0.3	0.2	0.1	0.05	0.2	0.05	0.9	Ex. 2036, pp. 25-26
UT15-030504	0.05	0.05	0.2	0.06	0.05	0.05	0.1	0.05	0.4	Ex. 2036, pp. 23-24
UT15-030601	0.05	0.05	0.2	0.05	0.05	0.05	0.09	0.05	0.3	Ex. 2036, pp. 21-22
UT15-030602	0.05	0.05	0.2	0.06	0.05	0.05	0.1	0.05	0.4	Ex. 2036, pp. 19-20
UT15-031001	0	0	0.2	0.2	0.08	0.05	0.1	0.05	0.6	Ex. 2036, pp. 17-18
UT15-031002	0	0	0.2	0.05	0.05	0	0.1	0	0.4	Ex. 2036, pp. 15-16
UT15-031003	0	0	0.2	0.1	0.06	0.05	0.2	0.05	0.6	Ex. 2036, pp. 13-14
UT15-031101	0	0	0.2	0.05	0.05	0	0.2	0	0.5	Ex. 2036, pp. 11-12
UT15-031102	0	0	0.1	0.1	0.06	0.05	0.1	0.05	0.4	Ex. 2036, pp. 8-10
UT15-031201	0	0	0.2	0.09	0.05	0	0.1	0.05	0.4	Ex. 2036, pp. 6-7

UT15-031202	0	0	0.2	0.07	0.05	0	0.2	0.05	0.5	Ex. 2036, pp. 4-5
Average	**0.0473**	**0.0407**	**0.2545**	**0.1646**	**0.1025**	**0.0405**	**0.0889**	**0.1028**	**0.9545**	
	1AU90	**2AU90**	**3AU90**	**750W93**	**751W93**	**97W86**	**ethyl ester**	**methyl ester**	**Total Related Substances**	

Note: For impurities reported as not detected ("ND") a value of 0 has been assigned; for impurities reported as <0.05, a value of 0.05 has been assigned.

APPENDIX B





02M11044	0	0	0.05	0	0	0	0	0	0.1	Ex. 2037, p. 44
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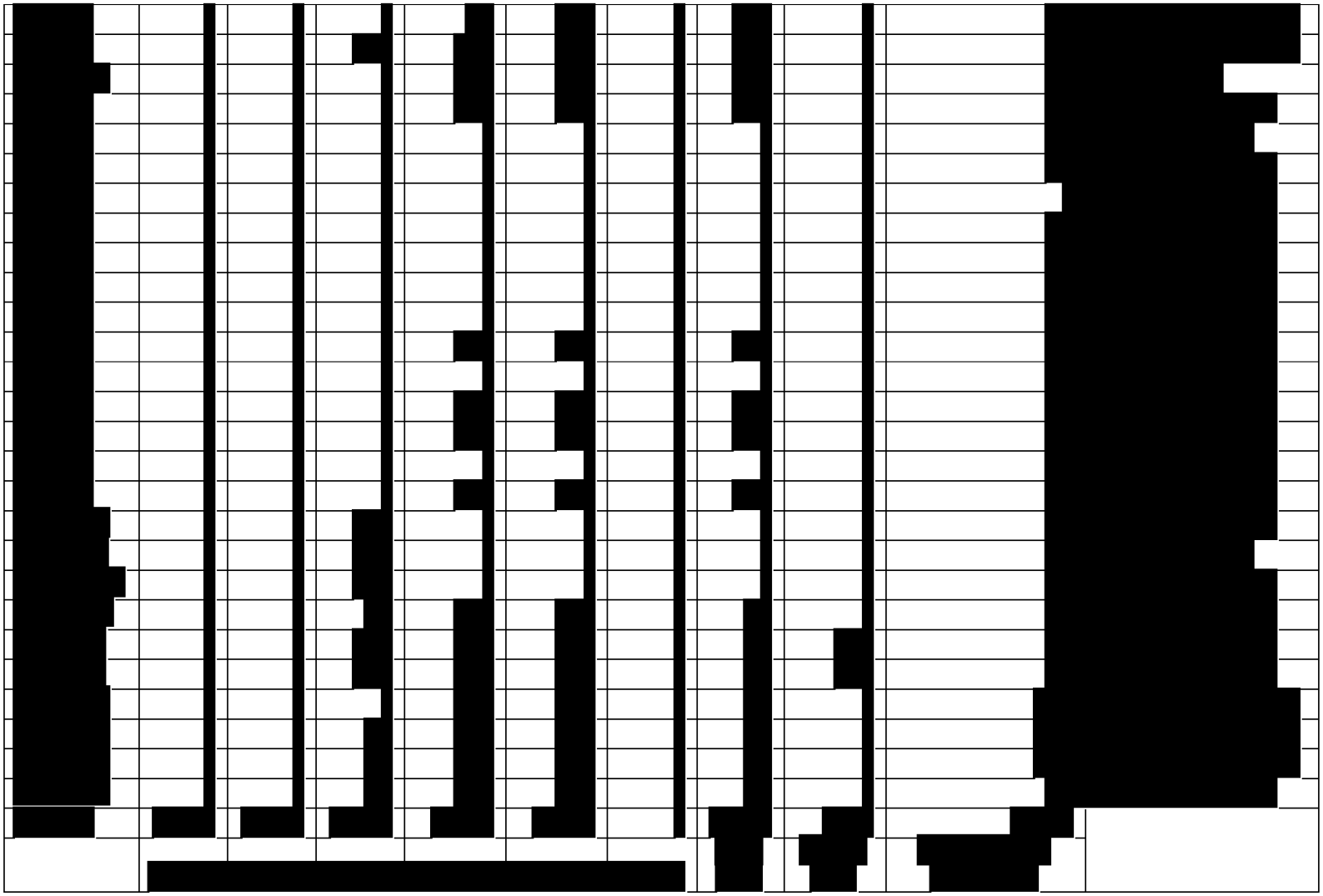
4851-2371-9220.1



Note: For impurities reported as not detected ("ND") a value of 0 has been assigned; for impurities reported as <0.05, a value of 0.05 has been assigned.

4851-2371-9220.1

CURRICULUM VITAE

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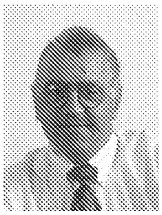

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Date of birth: February 8, 1953
Married: Jill Janssen Williams
Children: Ridge Janssen Williams (born February 23, 2001)
Rainier Valentine Williams (born August 20, 2005)

Education:
B.A., Chemistry (with highest distinction), May, 1975. Syracuse University, Syracuse, NY. Thesis under Professor Ei-ichi Negishi on *"A Stereoselective Synthesis of Partially Substituted 1,2,3-Butatriene Derivatives via Hydroboration".*

Ph.D., Organic Chemistry, June, 1979. Massachusetts Institute of Technology, Cambridge, MA. Thesis advisor, Dr. W. H. Rastetter. Thesis title: *"Epidithiapiperazinedione Syntheses".*

Postdoctoral Fellow, September 1979-September 1980, Harvard University, Cambridge, MA. The late Professor R. B. Woodward group (Y. Kishi, principal investigator). *Total synthesis of erythromycin A.*

Honors and Awards:
Organic Synthesis Award, Local Rocky Mountain ACS Section, Reaching New Heights (2012)
JSPS Invitation Fellowship Program for Research in Japan (Long-Term) 2012-2013
Ernest Guenther Award in the Chemistry of Natural Products, American Chemical Society (2011)
Multiple Myeloma Research Foundation Senior Award (2009-2011)
University Distinguished Professor, Colorado State University (2002)
Arthur C. Cope Scholar Award, American Chemical Society (2002)
Japanese Society for the Promotion of Science (JSPS) Fellowship (1999)
Merck & Co. Academic Development Award (1991-93)
Fellow of the Alfred P. Sloan Foundation (1986-88)
Eli Lilly Young Investigator Grantee (1986-88)
NIH Research Career Development Awardee (1984-89)
Phi Beta Kappa, Syracuse University (1975)
Kanner Prize for Chemistry and Physics, Syracuse University (1974)

Research and Professional Experience:
Director, Colorado Center for Drug Discovery (C2D2), April 2012 -present
Co-Director, Infectious Diseases Supercluster in Developmental Therapeutics at CSU
Co-Director, Cancer Supercluster in Developmental Therapeutics at CSU
Member, University of Colorado Cancer Center 2004-present
University Distinguished Professor, Colorado State University 2002-present
Professor, Colorado State University; Fort Collins, Colorado, January 1988-2002
Associate Professor, Colorado State University; Fort Collins, Colorado, July 1985-December 1987
Assistant Professor, Colorado State University; Fort Collins, Colorado, September 1980-June 1985
Visiting Professor, University of California, Berkeley, CA September 1990-December 1990
Visiting Professor of Chemistry, Harvard University, Cambridge, Mass. Sept. 15, 1994-Feb. 15, 1995

Professional Society Memberships:
American Association for the Advancement of Science
American Chemical Society
Japan Antibiotics Research Association
International Society of Heterocyclic Chemistry
Phi Beta Kappa

Editorial:

(1) Associate Editor, *Tetrahedron: Asymmetry* (2006-2008)

(2) Member, Editorial Board *"Chemistry and Biology"* Current Biology, Ltd. (1994-present).

(3) Member, Editorial Advisory Board for *Mini-Reviews in Organic Chemistry*, Bentham Science Pubs. (2004-2012).

(4) Co-Editor for the *"Organic Chemistry Series",* Elsevier (Sir J.E. Baldwin co-Ed.) 1997-2012.

(5) Editor-in-Chief for *"Amino Acids"*, Springer-Verlag (1991-1998).

(6) Acting Associate Editor for the *Journal of the American Chemical Society*; Sept. 1983-Jan. 1984.

Consulting Activities:

Cetya Therapeutics, Fort Collins, Colorado (2012-present)

Sapientia Therapeutics, Philadelphia, Pennsylvania (2012-present)

Arch Therapeutics, Cambridge, Massachusetts (2010-present)

HemaQuest Pharmaceuticals, Boston, Massachusetts (2006-2014)

Ajinomoto Co., Yokohama Japan (2002-present)

Xcyte Therapies, Inc., Seattle, Washington (1996-2006)

NewBiotics, Inc., SanDiego, California (2001-2002)

Cubist Pharmaceutical Co., Cambridge, Massachusetts (2000-2003)

Microcide Pharmaceuticals, Mountainview, California (1993-1998)

Boehringer Ingelheim Pharmaceuticals, Ridgefield, Connecticut (1992-1993)

Hoffman-LaRoche, Inc., Nutley, New Jersey (1989-1992)

EPIX Medical, Cambridge, Massachusetts (1993-1997)

W.R. Grace Company, Columbia, Maryland (1985-1990)

G.D. Searle Co., St. Louis, Missouri (1988-1990)

Nutrasweet Co., Skokie, Illinois (1990-1991)

Symphony Pharmaceutical Co., Philadelphia, Pennsylvania (1991-1993)

Venture Capital Corporate Boards and Scientific Advisory Boards:

(1) Founding Scientist, Acting President, Member of the Board of Directors and Member of the Scientific Advisory Board of Cetya Therapeutics, Fort Collins, Colorado 2012-present.

(2) Founding Scientist and Member of the Scientific Advisory Board for Sapientia Therapeutics (2012-present).

(3) Consultant and Member of the Scientific Advisory Board for Arch Therapeutics, Cambridge, Mass. 2010-present.

(4) Founding Scientist, Acting Vice-President of Discovery Chemistry, consultant and Member of the Scientific Advisory Board of HemaQuest, Seattle, Washington. 2006-present.

(5) Founding Scientist and Member of the Corporate Board of Directors for Xcyte Therapies, Seattle, Washington. 1996-2006.

(6) Scientific Advisory Board Member of Xcyte Therapies, Seattle, Washington. 1996-2006.

(7) Scientific Advisory Board Member of NewBiotics, Inc., San Diego, CA. 2001-2004.

(8) Founding Scientist and Scientific Advisory Board Member for Microcide Pharmaceuticals, Inc., Mountainview, California 1993-1998.

Advisory Boards:

Member of the Advisory Board of the Los Alamos Stable Isotope Resource (1999-2007)

Member of the Advisory Board for the *Puerto Rico Alliance for the Advancement of Biomedical Research Excellence* (PRAABRE) (2006-present)

Teaching

Graduate

C651 - "Molecular Basis for Drug Action and Design: Mechanism-Based Enzyme Inhibitors"
C651 - "Total Synthesis of Natural Products"
C651 - "Biosynthesis of Primary and Secondary Metabolites"
C541 - "Spectroscopic Methods"
C545 - "Advanced Organic Synthesis I"
C549 – "Advanced Organic Synthesis II"
C-547 – "Chemical Biology"

Undergraduate

C340H - "Honors Organic Chemistry I"
C341 - "Organic Chemistry I"
C343 - "Organic Chemistry II"
C245 - "Organic and Biological Chemistry"
C345 - "Organic Chemistry I"
C346 – "Organic Chemistry II"
Chem 17 (Harvard University, 1994 - 1995)

Ph.D. Theses Directed:

Robert W. Armstrong	(1984)	Chester Yuan	(1997)	Xiangna Jia	(2009)
Andrew O. Stewart	(1987)	Bradley Herberich	(1999)	Daniel A. Gubler	(2009)
Peter J. Sinclair	(1987)	Jeffrey Cao	(2000)	Tenaya Newkirk	(2009)
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Myeong-Nyeo Im	(1991)	Emily M. Stocking	(2001)	Jennifer M. Finefield	(2011)
James A. Hendrix	(1992)	Paul R. Sebahar	(2003)	Timmy McAfoos	(2011)
Sean C. Esslinger	(1993)	Ted C. Judd	(2003)	Ryan J. Rafferty	(2011)
Glenn J. Fegley	(1993)	Steven R. Lenger	(2003)	Guojun Pan	(2011)
Gregory F. Miknis	(1993)	Duane E. DeMong	(2003)	Paul T. Schuber	(2011)
Timothy D. Cushing	(1993)	Brian K. Albrecht	(2003)	Guojun Pan	(2011)
Mark E. Flanagan	(1995)	Ryan E. Looper	(2004)	Timothy R. Welch	(2011)
Steve Rubenstein	(1996)	Yuyin Chen	(2006)	Jennifer M. Bubb	(2012)
Jennifer Travers	(1995)	Alan W. Grubbs	(2006)	Aaron Pearson	(2013)
Samuel B. Rollins	(1997)	Siyuan Chen	(2007)	Alberto Jimenez	(2013)
Scott R. Rajski	(1997)	Chandele Gray	(2008)	Michelle Sanchez	(2014)

Masters Theses Directed:

Tracy N. Tippie	(1995)	Christie Kosogof	(2001)	Annie Youngman	(2009)
Paul B. Gansle	(1997)	Meriah W.N. Valente	(2006)	Andrea Geiser	(2009)
David M. Bender	(1998)	Nick Gearhart	(2008)	Tatyana Sabodash	(2011)
				Marie Trujillo	(2013)

Reviewer for Journals:

Journal of the American Chemical Society
Chemical Society Reviews
Journal of Organic Chemistry
Canadian Journal of Chemistry]
Archives of Biochemistry and Biophysics
Bioorganic and Medicinal Chemistry Letters
Bioorganic and Medicinal Chemistry
Amino Acids
Science
Tetrahedron
Tetrahedron Letters
Synthesis
Organic Letters
Chemistry & Biology
Accounts of Chemical Research
Chemical Communications
Angewandte Chemie
Chemical Reviews
Syn. Lett.
Nature Protocols
Nature Chemistry

Reviewer for Proposals:

National Institutes of Health Synthetic and Biological Chemistry B (SBCB) Study Section 2005
National Institutes of Health Medicinal Chemistry Study Section (MCHA) (2002-2005)
National Science Foundation
National Institutes of Health Medicinal Chemistry Study Section (Feb. 1987)
National Institutes of Health BNP Study Section (Feb. 1989)
Research Corporation
Petroleum Research Fund (ACS)
State Board of Education, Idaho
Ad-hoc reviewer for the Medicinal Chemistry Study Section (MCHA) February, 1987
Ad-hoc reviewer for the Bioorganic and Natural Products Chemistry Study Section (BNP) Feb., 1990
Member, NIH T-32 Chemistry / Biology Interface Training Grant Study Section, 1993
Ad-hoc reviewer for the Medicinal Chemistry Study Section (MCHA) October, 1996

Committee Memberships (Colorado State University)

Mis-conduct in Science Investigation Committee
Council of Research Associate Deans
University Distinguished Professors Selection Committee
Colorado State University Patent Committee
Graduate Operations Committee, Department of Chemistry
Graduate Admissions Committee, Department of Chemistry
Colorado State University Biosafety Committee
Promotion and Tenure Committee, Department of Chemistry
Executive Committee, Department of Chemistry
Industrial Liaison Committee, Department of Chemistry
Awards Committee, Department of Chemistry

Current Research Funding:

***Active Grants* (Robert M. Williams, PI: all projects)**

(1)	2RO1 CA70375-13 (NIH/NCI) Title: *"Total Synthesis and Biosynthesis of Bioactive Substances"*	08/01/14 – 07/31/19 $268,515 Annual Amount $1,292,938 Total Award
(2)	1 RO1 CA152314-01 (NIH/NCI) Title: *"Multiple Myeloma and Cancer Therapies via Largazole Analogs"*	09/01/10 – 12/30/15 $338,173 Annual Amount $1,599,830 Total Award

Chair, Organizer or Co-organizer of Scientific Meetings
Robert M. Williams, Professor of Chemistry

(1) The 1987 NSF Workshop on Environmental Chemistry, Stanford Sierra Camp, Lake Tahoe, California, Sept. 25-27, 1987.

(2) The 1989 Chemical Congress of Pacific Basin Societies, Honolulu, Hawaii, December 17-22, 1989. Co-organizer of a Symposium entitled: *"Recent Developments in the Chemistry of Amino Acids".*

(3) Special Bilateral U.S.-Britain Workshop entitled: *"Asymmetric Synthesis"* July 3-8, 1990. Pingree Park, Colorado. Co-organizer of this workshop.

(4) NSF-JSPS Bilateral Seminar entitled: *"Selectivity in Synthetic and Bio-Organic Chemistry",* Tokyo, Japan, June 3-7, 1991. Co-organizer of this bilateral seminar.

(5) The 3rd International Congress on Amino Acids, Vienna, Austria, August 23-27, 1993. Title: *"Asymmetric Synthesis of Non-proteinogenic Amino Acids via Chiral Glycinates".* Co-organizer of this meeting.

(6) The John K. & Dolores Stille Symposium on *"Biological Chemistry",* September 12, 1997. Organizer of this symposium.

(7) The 60th Birthday Celebration for Professor Yoshito Kishi, Harvard University. April 13, 1997, Harvard University. Co-organizer of this symposium and reception.

(8) The PacifiChem 2000 Conference, Honolulu, Hawaii, December 14-19, 2000. Organizer of a Symposium entitled: *"Frontiers in Antibiotics: Synthesis, Design and Mode of Action".*

(9) *The 19th International Congress of Heterocyclic Chemistry*, Fort Collins, Colorado. August, 2003. Vice-President, Chairman and Organizer of this meeting.

(10) The PacifiChem 2005 Congress, Honolulu, Hawaii, December 15-20, 2005. Co-Chair, Symposium entitled: *"Total Synthesis of Natural Products"*

(11) The 70th Birthday Celebration for Professor Yoshito Kishi, Harvard University. April 13, 2007, Harvard University. Co-organizer of this symposium and reception.

(12) *The Albert & Joan Meyers Symposium*. October 24, 2008, Colorado State University. Co-organizer of this symposium and reception.

(13) *The PacifiChem 2010 Conference*, Honolulu, Hawaii. Organizer of a Symposium entitled: *"Natural Products Synthesis"*

(14) *Robert Burns Woodward Memorial Symposium*. National ACS Meeting in Boston, Massachusetts, August 22-26, 2010. Principal Co-organizer.

Robert M. Williams
Brief Biographical Sketch

Robert M. Williams was born in New York in 1953 and was raised in Huntington, Long Island by Edith and Valentine Williams. He attended Syracuse University from 1971-1975 and received the B.A. degree in Chemistry in 1975. While at Syracuse, he did undergraduate research with the recent Nobel Laureate, Prof. Ei-ichi Negishi in the area of hydroboration methodology. He then moved to Cambridge, Massachusetts and entered the Ph.D. program at MIT and obtained his Ph.D. degree in 1979 under the supervision of Prof. William H. Rastetter. His doctoral studies were concerned with the total synthesis of two fungal metabolites, gliovictin and hyalodendrin. Following completion of his doctoral studies, he joined the laboratories of the late Prof. R.B. Woodward in 1979 whose postdoctoral group was subsequently managed by Professor Yoshito Kishi. His postdoctoral work was concerned with the completion of the total synthesis of erythromycin A. Upon completion of his postdoctoral tenure at Harvard, he joined the faculty at Colorado State University in 1980. He was promoted to Associate Professor with tenure in 1985, Full Professor in 1988, and University Distinguished Professor in 2002, his current position. Dr. Williams has received several Honors and Awards including the NIH Research Career Development Award (1984-1989); The Eli Lilly Young Investigator Award (1986); Fellow of the Alfred P. Sloan Foundation (1986); the Merck, Academic Development Award (1991), the Japanese Society for the Promotion of Science Fellowship (1999), the ACS Arthur C. Cope Scholars Award (2002), the ACS Ernest Guenther Award in the Chemistry of Natural Products (2011) and the Japanese Society for the Promotion of Science Long-term Fellowship (2012-2013). In 2002, he was named one of the twelve University Distinguished Professors, the highest honor and rank that Colorado State University bestows upon faculty. He spent a sabbatical at the University of California, Berkeley in 1990 in the laboratories of Professor Peter G. Schultz and was a visiting Professor at Harvard University in 1994 where he spent a sabbatical with Professor Stuart L. Schreiber and also taught Chem 17. He serves on the Editorial Board of the journal *Chemistry & Biology* and was an Editor for the journal *Amino Acids* (1991-1998). He has served as a Series co-Editor for *The Organic Chemistry Series*, published by Pergamon Press/Elsevier with Professor Sir Jack E. Baldwin of Oxford. Dr. Williams was a Scientific Founder and member of the Scientific Advisory Board of Microcide Pharmaceutical Co. from 1993-1998 located in Mountainview, California and was a Founding Scientist, Member of the Scientific Advisory Board and Member of the Board of Directors of Xcyte Therapies, located in Seattle, Washington from 1995-2006. Dr. Williams is currently a Scientific Founder, consultant, and member of the Scientific Advisory Board for HemaQuest Pharmaceuticals, located in Seattle, Washington from 2006-present and also serves as a consultant and Member of the Scientific Advisory Board for Arch Therapeutics, Cambridge, Mass. 2010-present. Dr. Williams is a Scientific Founder, Member of the Board of Directors, Chairman of the Scientific Advisory Board and acting President of Cetya Therapeutics, located in Fort Collins, Colorado. Dr. Williams is a Scientific Founder and Member of the Scientific Advisory Board of Sapientia Therapeutics, located in Philadelphia, Pennsylvania. Dr. Williams was named as Director for the Colorado Center for Drug Discovery (C2D2) in April, 2012.

Dr. Williams' research results from the interplay of synthetic organic chemistry, microbiology, biochemistry and molecular biology. Dr. Williams research interests have included the total synthesis of natural products, the asymmetric synthesis of amino acids and peptide isosteres, studies on anti-tumor drug-DNA interactions, design and synthesis of antibiotics and DNA-cleaving molecules, combinatorial phage libraries and biosynthetic pathways. He has utilized natural products synthesis to probe and explore biomechanistic and biosynthetic problems with a particular emphasis on antitumor and antimicrobial antibiotics. He has developed technology for the asymmetric synthesis of α-amino acids and peptide isosteres that has been commercialized by Aldrich Chemical Co. and has written a monograph on this subject.

Personal Interests: Downhill, cross-country, randonee and telemark skiing; rock electric & acoustic guitars; backpacking; mountain biking; running; bicycling; technical rock climbing; scuba diving; water skiing; surfing; sailing; photography; oil painting; oriental carpets; fine woodworking, furniture design and construction; golf; squash racquets; gardening; orchids; salt water aquarist.

PUBLICATIONS

Robert M. Williams, Colorado State University

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[ʃ] Syracuse University

[¶] MIT

[§] Harvard University

**Colorado State University*

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8. **Bicyclomycin Synthetic Studies: Utilization of Bridgehead Carbanions*, Williams, R. M., *Tetrahedron Lett.,* **1981**, *22*, 2341-2344.

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16. **Synthesis and Antimicrobial Evaluation of Bicyclomycin Analogs*. Williams, R.M.; Armstrong, R.W.; Dung, J-S., *J. Med. Chem.*, **1985**, *28*, 733-740.

17. **Unusual Bridgehead Hydroxylations via Selenoxides: Evidence for Bridgehead Carbocations*. Williams, R.M.; Dung, J-S., *Tetrahedron Lett.*, **1985**, *26*, 37-38.

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*The Third Biennial Lilly Grantee Symposium, Eli Lilly, Indianapolis, Indiana, March 8, 1988. Title: "*Approach to Understanding the Molecular Mechanism of Action of Bicyclomycin*" (Plenary Lecture)

The 9th Rocky Mountain Regional ACS Meeting, Symposium on Natural Products, Las Vegas, Nevada, March 29, 1988. Title: "*Stereofacial Selectivity of the Intramolecular S_N2' Cyclization: Brevianamide B*"

Ciba-Geigy Pharmaceuticals, Basel Switzerland; April 27, 1988. Title: "*Bicyclomycin: Mechanistic and Synthetic Investigations*"

University of Geneva, Geneva Switzerland; April 28, 1988. Title: "*Stereofacial Selectivity of the Intramolecular S_N2' Cyclization: Asymmetric Total Synthesis of Brevianamide B*"

*Burgenstock Conference on Stereochemistry; Burgenstock Switzerland; May 1-7, 1988. Title: "*Bicyclomycin: A Mechanistic, Biological and Synthetic Pandoras' Box*" (Plenary Lecture)

ETH (Eidgenössischen Technischen Hochschule), Zurich Switzerland; May 9, 1988. Title: "*Bicyclomycin: A Mechanistic, Biological and Synthetic Pandoras' Box*"

Chengdu Institute of Organic Chemistry, Chengdu, People's Republic of China; May 18-20 1988. Title: "*Recent Advances in Stereocontrolled Synthesis*"

IUPAC 88 Kyoto 16th International Symposium on the Chemistry of Natural Products, Kyoto Japan; May 31, 1988. Title: "*Stereofacial Selectivity of the Intramolecular S_N2' Cyclization: Asymmetric Total Synthesis of Brevianamide B*"

Fujisawa Pharmaceutical Co., Osaka Japan; June 3, 1988. Title: *"Asymmetric Synthesis of α-Amino Acids"*

Nagoya Post-Symposium "New Topics on Natural Products", Toba Japan; June 7, 1988. Title: *"Facial Selectivity of the Intramolecular SN2' Reaction: Asymmetric Total Synthesis of Brevianamide B"*

Takeda Pharmaceutical Co., Osaka Japan; June 5, 1988. Title: *"Asymmetric Synthesis of α-Amino Acids"*

Sumitomo Chemical Co., Osaka Japan; June 9, 1988. Title: *"Asymmetric Synthesis of α-Amino Acids"*

Sankyo Pharmaceutical Co., Tokyo Japan; June 10, 1988. Title: *"Asymmetric Synthesis of α-Amino Acids"*

Hoffmann-LaRoche, Nutley, New Jersey, June 24, 1988: Title: *"Asymmetric Synthesis of α-Amino Acids"*

The University of Gottingen, Gottingen West Germany, June 27, 1988. Title: *"Novel Syntheses of Chiral Amino Acids"*

Cambridge University, Cambridge, England, June 29, 1988. Title: "*Bicyclomycin: Synthetic, Mechanistic and Biological Studies*"

Oxford University, Oxford, England, June 30, 1988. Title: "*Practical Asymmetric Synthesis of α-Amino Acids"*

Imperial Chemical Industries, Alderly Edge, England, July 1, 1988. Title: *"Asymmetric Synthesis of α-Amino Acids"*

Churchill College, Cambridge, England, July 4, 1988. Title: "*Synthesis and Properties of 1,3-Bridged β-Lactams: Novel Anti-Bredt β-Lactams*"

Imperial College, London, England, July 7, 1988. Title: "*Bicyclomycin: Recent Mechanistic, Biological and Synthetic Investigations*"

Pfizer Pharmaceutical Co., Sandwich, England, July 8, 1988. Title: "*Stereofacial Selectivity of the Intramolecular S_N2' Cyclization: Asymmetric Total Synthesis of Brevianamide B*"

Texas A & M University, College Station, Texas October 13, 1988. Title: "*Studies on the Chemistry of Amino Acids and Selected Derivatives*"

The University of Texas, Austin, Texas, October 14, 1988. Title: "*Stereofacial Selectivity of the Intramolecular S_N2' Cyclization: Asymmetric Total Synthesis of Brevianamide B*"

Ciba-Geigy Pharmaceutical, Summit, New Jersey, October 19, 1988. Title: "*Asymmetric Synthesis of Biologically Important Non-Proteinogenic α-Amino Acids*"

Merck, Sharp & Dohme Research Laboratories, Rahway, New Jersey, October 20, 1988. Title: "*Asymmetric Synthesis of Biologically Important Non-Proteinogenic α-Amino Acids"*

1989

*****The Carl Marvel Symposium, Tucson, Arizona, March 7, 1989. Title: "*Total Synthesis and Biogenetic Intrigue of the Brevianamides*" (Plenary Lecture)

Abbott Laboratories, Abbott Park, Illinois, May 4, 1989. Title: *"Synthetic and Mechanistic Investigations on the Anti-Tumor Antibiotic Quinocarcin"*

Lederle Laboratories, Pearl River, New York, May 23, 1989. Title: *"Synthetic and Mechanistic Investigations on the Anti-Tumor Antibiotic Quinocarcin"*

The UpJohn Company, Kalamazoo, Michigan, June 5-7, 1989. 3-Day Short course on the "*Synthesis of Optically Active α-Amino Acids*"

Eli Lilly Company, Indianapolis, Indiana, June 23, 1989. Title: *"Probing the Mode of Action Bicyclomycin and Anti-tumor Action of Quinocarcin"*

W.R. Grace and Company, Columbia, Maryland, June 29, 1989. Title: "*Synthesis of Optically Active α-Amino Acids*"

Hoffman-LaRoche, Inc., Nutley, New Jersey, July 21, 1989. Title: "*Synthesis of Optically Active α-Amino Acids*"

Yale University, New Haven, Connecticut, November 15, 1989. Title: *"Oxazolidines, Carbinolamines and Amides: Intrigue at Nitrogen"*

1990

Nutrasweet Co., Mt. Prospect, Illinois, March 20, 1990. Title: *"Synthesis of Optically Active α-Amino Acids"*

Syntex Research, Palo Alto, California, May 11, 1990. Title: *"Studies on the Mechanism of DNA Cleavage by Quinocarmycin"*

*CU-SYNTEX Synthetic Chemistry Symposium, June 7, 1990. Title: *"Mechanistic and Synthetic Studies on the Mode of Cleavage of Superhelical DNA by Quinocarcin"*

Special Bilateral U.S.-Britain Workshop on "Asymmetric Synthesis," July 3-8, 1990, Pingree Park, Colorado (RMW Co-organizer)

Gordon Research Conference on "Organic Reactions and Processes, New Hampton, New Hampshire, July 15-20, 1990. Title: *"The Mechanism of Oxygen Reduction by Quinocarcin"*

Gordon Research Conference on "Natural Products," New Hampton, New Hampshire, July 22-27, 1990. Title: *"Bioorganic, Mechanistic and Synthetic Chemistry of Biologically Significant Nitrogenous Substances"*

Hoffman-LaRoche, Inc., Nutley, N.J., August 24, 1990. Title: *"Probing the Mechanism of DNA Cleavage by the Antitumor Drug Quinocarcin"*

Bio Mega, Inc., Montreal, Canada, September 18, 1990. Title: *"Asymmetric Synthesis of α-Amino Acids"*

The University of California, Santa Barbara, California, November 9, 1990. Title: *"In Search of the Biosynthetic Diels-Alder Construction: Total Synthesis as a Periscope"*

Stanford University, Palo Alto, California, November 28, 1990. Title: *"In Search of the Biosynthetic Diels-Alder Construction: Total Synthesis as a Periscope"*

1991

Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan, May 27, 1991. Title: *"Mechanistic and Synthetic Studies on Anti-Tumor Antibiotics"*

SunBor (Suntory Institute of Bio-Organic Research) Osaka, Japan, May 28, 1991. Title: *"In Search of the Biosynthetic Diels-Alder Construction: Total Synthesis as a Periscope"*

Sagami Research Institute, Kanagawa, Japan, May 29, 1991. Title: *"Mechanistic and Synthetic Studies on Quinocarcin: Superoxide-Mediated DNA Cleavage"*

Ajinomoto Co., Kawasaki, Japan, May 30, 1991. Title: *"Recent Studies on the Asymmetric Synthesis of α-Amino Acids"*

Shionogi Pharmaceutical Co., Tokyo, Japan, May 31, 1991. Title:*" Recent Studies on the Asymmetric Synthesis of α-Amino Acids"*

Tokyo Institute of Technology, Tokyo, Japan, June 1, 1991. Title: *"Facial Selectivity of the [2,3] Wittig Rearrangement and S_N2' Cyclization Reactions in Total Synthesis"*

NSF-JSPS Bilateral Seminar on "Selectivity in Synthetic and Bio-Organic Chemistry", Tokyo, Japan, June 3-7, 1991. Title: *"Unusual Facial Selectivity in the Biosynthesis and Synthesis of*

the Brevianamide / Paraherquamide Class of Mycotoxins: In Search of the Biosynthetic Diels-Alder Construction"

*Otsuka Pharmaceutical Co., Tokushima, Japan. June 11, 1991. Title: *"Asymmetric Synthesis of Non-Proteinogenic α-Amino Acids"*

*International Congress of New Drug Development, Seoul, Korea, August 21, 1991. Title: *"Synthetic and Mechanistic Studies on Anti-Tumor Antibiotics"*

*4th Chemical Congress of North America, Division of Organic Chemistry, New York, August 29, 1991. Title: *"Unusual Stereofacial Selectivity in the Biosynthesis and Synthesis of the Brevianamide / Paraherquamide Class of Mycotoxins: In Search of the Biosynthetic Diels-Alder Construction"*

DowElanco Co., Walnut Creek, California, November 8, 1991. Title: *"Design and Synthesis of Lysine Biosynthesis Inhibitors"*

The University of California, Santa Cruz, California, November 11, 1991. Title: *"Synthetic and Biosynthetic Studies on the Brevianamides and Paraherquamides"*

The University of California, Davis, California, November 12, 1991. Title: *"Cannizzarro-Based Oxygen-Dependent Cleavage of DNA by Quinocarcin and Tetrazomine"*

1992

Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, Conn., March 18, 1992. Title: *"Asymmetric Synthesis of α-Amino Acids and What's Next?"*

24th Central Regional ACS Meeting (CMACS-92), Cincinnati, Ohio, Symposium on Amino Acids and Peptides, May 28, 1992. Title: *"Asymmetric Synthesis of Non-Proteinogenic α-Amino Acids"*

Hoffman-La Roche, Inc., Workshop on Prospects for Antibacterial Agents Based on Diaminopimelic Acid, May 11, 1992. Title: *"Stereochemistry and DAP Inhibitors"*

Amgen Boulder, Colorado, August 14, 1992. Title: *"A Combinatorial Approach to Vancomycin Resistance"*

Parke-Davis Pharmaceutical Research Division, Ann Arbor, Michigan, September 25, 1992, Title: *" Chemical, Biological and Combinatorial Adventures with Amino Acids"*

*48th Southwest Regional ACS Meeting, Lubbock, Texas, October 21-23, 1992, A.I. Meyers Symposium. Title: *"Recent Studies in Asymmetric and Bioorganic Methods"*

Purdue University, November 10, 1992, West Lafayette, Indiana, Title: *"Chemical, Biological and Combinatorial Adventures with Amino Acids"*

Monsanto Corporate Research, St. Louis, Missouri, November 11, 1992, Title: *" Recent Adventures in the Synthesis of Amino Acids and Complex Natural Products"*

Pfizer Central Research, Groton, Conn., November 12, 1992, Title: *"A Combinatorial Approach to Multiple Drug-Resistant Bacterial Infections"*

R.W. Johnson Pharmaceutical Research Co., Raritan, New Jersey, November 13, 1992, Title: *"Chemical and Biological Adventures with Amino Acids"*

1993

* BioEast '93, Washington, D.C., January 27, 1993, Title: *"New Approaches to Antibiotic Design and Synthesis"*

Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, N.J., February 22, 1993, Title: *"Utility of Natural Products Synthesis in Studying Biosynthesis and Biomechanism"*

Bristol-Myers Squibb Pharmaceutical Research Institute, New Brunswick, N.J., February 23, 1993, Title: *"Utility of Natural Products Synthesis in Studying Biosynthesis and Biomechanism"*

Scripps Research Institute, Department of Chemistry, La Jolla, California, April 12, 1993. Title: *"Chemical, Biological and Combinatorial Adventures with Amino Acids"*

Microcide Pharmaceutical Co., Menlo Park, California, April 18, 1993. Title: *"Combinatorial Approaches to Treating Drug-Resistant Mycobacterial Infections"*

Hoffman-La Roche, Inc., Basel Switzerland, April 26, 1993. Title: *"Unnatural α-Amino Acids as a Vehicle for Biological, Mechanistic and Synthetic Inquiries"*

Sandoz Pharmaceutical Co., Inc., Basel Switzerland, April 27, 1993. Title: *"Recent Studies in Asymmetric and Bioorganic Methods"*

Pasteur Institute, Paris, France, April 29, 1993. Title: *"New Approaches to Antibiotic Design and Synthesis"*

3rd International Congress on Amino Acids, Vienna, Austria, August 23-27, 1993. Title: *"Asymmetric Synthesis of Non-proteinogenic α-Amino Acids Acids via Chiral Glycinates"*

Burroughs-Wellcome Pharmaceutical Co., Research Triangle, October 11, 1993. Title: *"Studies on the Total Synthesis and Biogenesis of the Paraherquamide / Brevianamide Class of Alkaloids"*

***** 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Symposium on "Glycopeptide Resistance and the Cell Wall", New Orleans, Louisiana, October 18, 1993. Title: *"Implications for Drug Discovery"*

1994

***** Sino-American Symposium on Asymmetric Synthesis, Taichung, Taiwan, April 8-9, 1994. Title:*"The Asymmetric Synthesis of α-Amino Acids"*

E.I. Dupont De Nemours & Co. / Dupont-Merck Pharmaceutical Co., Newark, Delaware, May 16, 1994. Title: *"The Use of Total Synthesis of Indole Alkaloids as a Biosynthetic Periscope"*

Hoechst-Roussel Pharmaceuticals, Inc., Sommerville, N.J., July 13, 1994. Title: *"Bioxalomycins, Tetrazomine and Quinocarcin: Synthetic, Mechanistic and Biological Studies"*

*****The 4th International Conference on Chemical Synthesis of Antibiotics and Related Microbial Products, Nashville, Indiana, September 11-16, 1994. Title: *" Studies on the Mechanism of Action of Bioactive Nitrogenous Substances"*

Brandeis University, Waltham, Mass. October 27, 1994. Title: *"Synthesis and Biosynthesis of the Paraherquamides and Brevianamides"*

Eisai Pharmaceutical Co., Andover, Mass., December 1, 1994. Title: *"Asymmetric Synthesis of α-Amino Acids, Peptidomimetics and Other Nitrogenous Substances"*

Harvard University, Cambridge, Mass., December 12, 1994. Title: *"Chemical Probes and Microbes"*

1995

Rice University, Houston, Texas, February 22, 1995. Title: *"Total Synthesis of Natural Products: A Useful Periscope for Exploring Biosynthesis and Biomechanism"*

Texas A & M University, College Station, Texas, February 23, 1995. Title: *"Synthetic and Biosynthetic Studies on the Paraherquamides and Brevianamides"*

The University of Texas at Austin, Austin, Texas, February 24, 1995. Title: *"O_2-Dependent Cleavage of DNA by Tetrazomine, Quinocarcin and Synthetic Analogs"*

Imperial College, London England, May 15, 1995. Title: *"Recent Adventures in the Total Synthesis of Biosynthetically and Biomechanistically Intriguing Natural Products"*

*The SCI Meeting on Amino Acids, London, England, May 16, 1995. Title: *"Overview of the Major Conceptual Approaches to the Construction of Amino Acids"*

Rhone Poulenc Rorer Pharmaceutical Co., England, May 17, 1995. Title: *"Studies on the Biosynthesis of Taxol and the Brevianamide / Paraherquamide Class of Alkaloids"*

Eli Lilly Pharmaceuticals, England, May 18, 1995. Title: *"The Asymmetric Synthesis of α-Amino Acids"*

Astra-Zeneca Agrochemicals, Jealott's Hill, Bracknell, England, May 19, 1995. Title: *"Studies on the Total Synthesis and Mechanism of Action of Anti-tumor Antibiotics FR-900482 and Quinocarcin"*

Darwin Molecular Corp., Bothell, Washington, October 16, 1995. Title: *"Organic Synthesis: An Important Vehicle to Probe Biomechanism and Biosynthesis"*

The University of Washington, Seattle, Washington, October 17, 1995. Title: *"Organic Synthesis: An Important Vehicle to Probe Biomechanism and Biosynthesis"*

Hokkaido University, Sapporo, Japan, November 20, 1995. Title: *"Biosynthetic Studies on the Brevianamides and Paraherquamides: A Quest for the Biosynthetic Diels-Alder Construction"*

Takeda Pharmaceutical Company, Osaka, Japan, November 21, 1995. Title: *"Studies on the Total Synthesis of TAN-1057 and Novel Approaches to MRSA"*

Osaka University, Osaka, Japan, November 22, 1995. Title: *"Organic Synthesis as a Vehicle for Probing Biomechanism and Biosynthesis"*

Kyowa Hakko Pharmaceutical Co., Tokyo, Japan, November 24, 1995. Title: *"Synthetic and Bio-Mechanistic Studies on Quinocarcin, Tetrazomine and the Bioxalomycins"*

Tokyo University, Tokyo, Japan, November 24, 1995. Title: *"Short Stories in Natural Products Chemistry"*

Tokyo Institute of Technology, Tokyo, Japan, November 25, 1995. Title: *"Natural Products Synthesis as a Vehicle for Probing Biosynthesis and Discovering New Reactions"*

1996

Case Western Reserve University, Cleveland, Ohio, February 15, 1996. Title: *"Probing Biosynthesis and Biomechanism through Natural Products Synthesis"*

1997

The Upjohn Company, Kalamazoo, Michigan. April 2, 1997. Title: *"Studies on the Asymmetric Total Synthesis and Biosynthesis of the Paraherquamide/Marcfortine/Brevianamide Class of Alkaloids"*

Michigan State University, April 3, 1997. Title: *"Recent Studies on the Synthesis of Antitumor Antibiotics: Exploiting Drug-DNA Interactions"*

Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, Connecticut. April 11, 1997. Title: *"Recent Studies on the Synthesis of Antitumor Antibiotics: Drug-DNA Interactions"*

*CU Hauser Symposium, May 29 & 30, 1997, Boulder, Colorado. Title: *"Organic Synthesis: An Important Vehicle to Probe Biosynthesis"*

*Hokkaido University, Sapporo, Japan, June 24 & 25, 1997. Title: *"In Search of the Enzyme-Catalyzed Diels-Alder Construction: The Paraherquamide/Brevianamide Paradox"*

Dai-ichi Pharmaceutical Co., Tokyo, Japan, June 27, 1997. Title: *"Recent Studies in Natural Products Synthesis and Biomechanism"*

The University of Alberta, Edmonton, Canada. October 1, 1997. Title: *"Synthetic and Biomechanistic Studies on the Antitumor Antibiotics Bioxalomycin, Quinocarcin and FR900482"*

NexStar Pharmaceutical Co., Boulder, Colorado, December 2, 1997. Title: *"New and Old Approaches to Scaling the Bacterial Cell Wall: Design and Synthesis of Antibiotics".*

Tularik Co., San Francisco, California, December 4, 1997. Title: *"Organic Synthesis: A Probe for Studying Biomechanism and Biosynthesis".*

1998

Ligand Pharmaceutical Co., LaJolla, California, February 19, 1998. Title: *"The Asymmetric Synthesis of Amino Acids and Peptide Isosteres".*

The Scripps Research Institute, La Jolla California, February 20, 1998. Title: *"Natural Products Synthesis: An Important Probe for Studying Biosynthesis and Biomechanism".*

The University of Arizona, Tucson, Arizona, Department of Pharmaceutical Sciences April 20, 1998. Title: *"The Oxazolidine Family of Antitumor Antibiotics: Deconvoluting Multiple Modes of Action".*

The University of Arizona, Tucson, Arizona, Department of Chemistry April 21, 1998. Title: *"The Stereochemical Paradox of the Paraherquamide and Brevianamide Biosyntheses".*

The University of Gottingen, Gottingen, Germany, June 19, 1998. Title: *"Total Synthesis Reveals the Stereochemical Paradox of the Paraherquamide and Brevianamide Biosyntheses".*

The University of Konstanz, Konstanz, Germany, June 23, 1998. Title: *"Total Synthesis Reveals the Stereochemical Paradox of the Paraherquamide and Brevianamide Biosyntheses".*

The University of Geneva, Geneva Switzerland, June 29, 1998. Title: *"Total synthesis of Natural Alkaloids of Biological and Biosynthetic Interest".*

*The Gordon Research Conference on Natural Products, July 5-9, 1998. Title: *"Natural Product Synthesis as a Probe for Studying Biosynthesis and Biomechanism".*

The University of Illinois at Urbana Champaign, October14, 1998. Title: *"Natural Product Synthesis as a Probe for Studying Biosynthesis and Biomechanism".*

1999

Array BioPharma, Boulder, Colorado, January 14, 1999. Title: *"Recent Studies in the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis".*

The University of Tokyo, Tokyo, Japan, May 11, 1999. Title: *"Adventures in the Total Synthesis of Natural Products: Discovery and Surprises".*

Riken Institute, Tokyo, Japan. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis".*

Waseda University, Tokyo, Japan May 13, 1999. Title: "*Total Synthesis Reveals the Stereochemical Paradox of the Paraherquamide and Brevianamide Biosyntheses".*

Tohoku University, Sendai, Japan. May 14, 1999. Title: "*Total Synthesis Reveals the Stereochemical Paradox of the Paraherquamide and Brevianamide Biosyntheses".*

Tokyo Institute of Technology, Tokyo, Japan. May 17, 1999. Title: *"Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis and Studies on the Biosynthesis of Taxol: Synthesis and Labeling of Biosynthetically Relevant Taxoids".*

Keio University, Tokyo, Japan. May 18, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis".*

Tokyo University School of Pharmacy and Life Science, Hachioji, Japan. May 19, 1999. Title: "*Total Synthesis Reveals the Stereochemical Paradox of the Paraherquamide and Brevianamide Biosyntheses".*

Teikyo University, Sagamiko, Kanagawa, Japan. May 20, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis".*

Ajinomoto Co., Tokyo, Japan. May 21, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis".*

Sankyo Co., Tokyo, Japan. May 24, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis".*

Yamanouchi Pharmaceutical Co., Tsukuba, Japan. June 3, 1999. Title: "*The Oxazolidine Family of Antitumor Antibiotics: Total Synthesis and Deconvoluting Multiple Modes of Nucleic Acid Damage"*

Eisai Pharmaceutical Co., Tsukuba, Japan. June 4, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis".*

Pfizer Pharmaceutical Co., Nagoya, Japan. June 7, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis".*

Nagoya University, Nagoya, Japan. June 8, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis".*

Nagoya City University, Nagoya, Japan. June 9, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of Natural Products Synthesis and Studies on the Biosynthesis of Taxol: Synthesis and Labeling of Biosynthetically Relevant Taxoids".*

Nara Institute of Science and Technology, Nara, Japan. June 10, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis".*

Osaka City University, Osaka, Japan. June 11, 1999. Title: "*The Oxazolidine Family of Antitumor Antibiotics: Total Synthesis and Deconvoluting Multiple Modes of Nucleic Acid Damage".*

Osaka University, Osaka, Japan. June 14, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis".*

Kyowa Hakko Kogyo Co., Osaka, Japan. June 15, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis and Total Synthesis Reveals the Stereochemical Paradox of the Paraherquamide and Brevianamide Biosyntheses".*

Fujisawa Pharmaceutical Co., Osaka, Japan. June 16, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis and Studies on the Total Synthesis and Mechanism of Action of FR900482".*

Kyoto University, Kyoto, Japan. June 17, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis".*

Mitsubishi Corporation, Tokyo, Japan. June 18, 1999. Title: "*Total Synthesis Reveals the Stereochemical Paradox of the Paraherquamide and Brevianamide Biosyntheses".*

Sagami Chemical Research Institute, Tokyo, Japan. June 21, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis".*

Meiji Pharmacy College, Tokyo, Japan. June 22, 1999. Title: "*Recent Studies on the Asymmetric Synthesis of α-Amino Acids and Natural Products Synthesis".*

Monsanto Company, St. Louis, Missouri, September 28, 1999. Title: *"Recent Studies on the Synthesis of Amino Acid Derivatives, Peptide Isosteres and Natural Products".*

The State University of New York at Buffalo, Buffalo, N.Y., Oct. 15, 1999. Title: *"Total Synthesis Reveals the Stereochemical Paradox of the Paraherquamide and Brevianamide Biosyntheses".*

2000

Cubist Pharmaceutical Co., Cambridge, Mass., January 18, 2000. Title: *"Total Synthesis of the Anti-MRSA Peptide Antibiotic TAN 1057 and Analogs".*

SmithKline Beecham Pharmaceutical Co., Philadelphia, Penn. April 17, 2000. Title: *"Studies on the Asymmetric Synthesis of Amino Acids and Natural Products: Tools for Exploring and Exploiting Biomechanisms".*

Schering-Plough Pharmaceutical Co., April 18, 2000. Title: *"Studies on the Asymmetric Synthesis of Amino Acids and Natural Products; Tools for Exploring and Exploiting Biomechanisms".*

The University of California at San Diego, May 22, 2000. Title: *"Asymmetric Stereocontrolled Total Synthesis of the Spirooxindole Alkaloids Spirotryprostatin B and Paraherquamide A".*

*The 83rd Canadian Society for Chemistry Conference, Calgary, Canada, May 28-31, 2000. Title: *"Asymmetric Total Synthesis of the Oxindole Alkaloids Paraherquamide A and Spirotryprostatin B".*

*Herbert C. Brown Distinguished Professorship Symposium, Purdue University, Sept. 28-29, 2000. Title: "*Total Synthesis of Natural Products: Tools for Probing and Exploiting Biomechanism and Biosynthesis".*

Gilead Sciences, San Francisco, California, October 27, 2000. Title: *"Studies on the Asymmetric Synthesis of Amino Acids and Natural Products; Tools for Exploring and Exploiting Biomechanisms".*

SmithKline Beecham Pharmaceutical Co., Philadelphia, Pennsylvania, November 1, 2000. Title: *"Studies on the Asymmetric Total Synthesis of FR900482, Tetrazomine and Spirooxindole Alkaloids".*

The PacifiChem 2000 Conference, Honolulu, Hawaii, December 14-19, 2000. Title: "*Synthesis and Antimicrobial Evaluation of TAN1057A/B Analogs".*

2001

*University of California Irvine, UCI Synthesis Symposium on *Biological Tools and Targets*. January 20, 2001. Title: "*DNA Crosslinking Agents: Synthesis, New Methods for Activation and the Elucidation of New Targets in the Nucleus in vivo".*

*Michigan State University, Pfizer Lecturer (May 7-9, 2001). Titles: (1) *"Extraordinarily Versatile Amino Acid Templates for the Total Synthesis of Natural Products, Peptide Isosteres and Amino Acids"*; (2) *"Antitumor Antibiotics: Mechanistic Discoveries, Synthesis and Exploitation"*; (3) *"Organic Synthesis: An Important Vehicle to Probe Biosynthesis".*

*The 2001 CU-Array Biopharma Symposium on Medicinal and Synthetic Organic Chemistry, June 6-8, 2001, Boulder, Colorado. Title: *"Extraordinarily Versatile Amino Acid Templates for the Total Synthesis of Natural Products, Peptide Isosteres and Amino Acids".*

The 18th International Congress of Heterocyclic Chemistry, Yokohama, Japan, July 29 - August 3, 2001. Title: *"Extraordinarily Versatile Amino Acid Templates for the Total Synthesis of Natural Products, Peptide Isosteres and Amino Acids".*

The Kyowa Hakko Kogyo Co., Osaka, Japan. August 1, 2001. Title: *"Studies on the Total Synthesis of Tetrazomine, Bioxalomycin and Et743".*

57th Southwest Regional ACS Meeting, Symposium Honoring Al Meyers Retirement. San Antonio, Texas. October 18-19, 2001. Title: *"Extraordinarily Versatile Amino Acid Templates for the Total Synthesis of Natural Products, Peptide Isosteres and Amino Acids".*

2002

Ohio State University, Columbus, Ohio, March 7, 2002 Title: *"Studies on the Asymmetric Synthesis of Amino Acids and Natural Products: Tools for Exploring and Exploiting Biomechanisms".*

Montana State University, Bozeman, Montana, March 22, 2002. Abbott Distinguished Lecturer. Title: *"Studies on the Asymmetric Synthesis of Amino Acids and Natural Products: Tools for Exploring and Exploiting Biomechanisms".*

Reaction Mechanisms Conference June 28 - July 1, 2002. Ohio State University. Title: *"Mechanistic Studies on Synthetic and Natural DNA-Reactive Alkaloids"*

*University of Wyoming Distinguished Summer Lecture Series. Laramie, Wyoming.

June 25 lecture #1: Title: *"Studies on the Asymmetric Synthesis of Amino Acids"*

June 26 lecture #2: Title: *"Elucidating the Biosynthesis of Taxol"*

July 17 lecture #3: Title: *"Total Synthesis and Biosynthesis of the Paraherquamides"*

July 18 lecture #4: Title: *"Synthesis and Mechanism of Action of FR900482"*

July 19 lecture #5: Title: *"Antitumor Agents Armed with an Oxazolidine: Synthetic and Biomechanistic Studies"*

*Belgian Organic Synthesis Symposium (BOSS IX), Namur, Belgium, July 8-12, 2002. Title: *"Extraordinarily Versatile Amino Acid Templates for the Total Synthesis of Natural Products, Peptide Isosteres and Amino Acids"*

Karolinska Institute, Dept. of Biosciences at Novum, Huddinge, Sweden. August 16, 2002. Title: *"Total Synthesis of Natural Products of Biological Intrigue"*

*American Chemical Society Meeting, Boston, Massachusetts, August 20, 2002, Arthur C. Cope Scholar Awardee lecture. Title: *"Total Synthesis of Natural Products: Tools for Probing and Exploiting Biomechanism and Biosynthesis"*

Western Washington University, Bellingham, Washington. October 15, 2002. Title: *"Total Synthesis of Natural Products of Biological Intrigue"*

*November 4, 2002, College Colloquium Series, Colorado State University. Title: *"Vitalism in Natural Products: Finessing Molecular Secrets from Nature"*

November 6, 2002, Emory University, Atlanta, Georgia. Title: *"Total Synthesis of Natural Products of Biological Intrigue"*

December 2, The University of Minnesota, Minneapolis, Minnesota. Title: *"Studies on the Synthesis and Biology of FR900482 and the Paraherquamides"*

2003

January 24, 2003, Brigham Young University, Provo, Utah. Title: *"Total Synthesis of Natural Products of Biological Intrigue"*

March 17, 2003, Amgen, Inc., Thousand Oaks, California. Title: *"Total Synthesis of Biologically Intriguing Alkaloids"*

April 17, 2003, The University of Florida, Gainseville, Florida. Title: *"Total Synthesis of Biologically Intriguing Alkaloids"*

April 28, 2003. Colorado State University Department of Biochemistry. Title: *"Utilizing Organic Synthesis to Penetrate Drug-Nucleic Acid Interactions"*

October 10, 2003. Pennsylvania State University, State College, Pennsylvania. Title: *"Total Synthesis of Natural Products as a Vehicle for Penetrating Secondary Metabolism"*

*October 22, 2003, The 8th Loughborough Synthesis Symposium Sponsored by Astra Zeneca, Loughborough, Leicestershire, UK. Title: *"Total Synthesis of Natural Products of Biological Intrigue"*

*November 4, 2003, The Barton Symposium. Heron Island, Australia. Title: *"Total Synthesis of Natural Products of Biological Intrigue"*

*The University of Colorado, Boulder, Colorado. December 2, 2003. Roche Distinguished Lecture. Title: *"Total Synthesis of Natural Products of Biological Intrigue"*

2004

Celera Co., South San Francisco, CA January 13, 2004. Title: *"Synthesis and Mechanistic Studies on Antitumor Antibiotics"*

*March 1,2, Eli Lilly Grantee Symposium, Indianapolis, Indiana. Title: *"From Bicyclomycin to Biosynthesis"*

March 17, 2004. Merck & Co. West Point, Pennsylvania. Title: *"Synthesis of Biologically Intriguing Alkaloids"*

March 18, 2004. Aventis Pharmaceutical Co., New Jersey. Title: *"Mechanistic Studies on Synthetic and Natural DNA-Reactive Alkaloids"*

*March 19, 2004. *Chemistry as a Life Science Symposium XII*. Rutgers University, New Jersey. Title: *"Total Synthesis of Natural Products of Biological Intrigue"*

*March 29, 30, 2004. ACS Meeting in Anaheim California. Tohru Fukuyama ACS Award Symposium. Title: *"Total Synthesis of Biologically Intriguing Natural Products"*

April 9, 2004. The University of Nebraska, Lincoln, Nebraska. Title: *"Total Synthesis of Natural Products of Biological Intrigue"*

April 27, 2004. The University of Iowa. Title: *"Total Synthesis of Natural Products of Biological Intrigue"*

April 28, 2004. Wayne State University. Title: *"Total Synthesis of Natural Products of Biological Intrigue"*

November 1, 2004. Colorado State University Veterinary Cancer Center. Title: "*Studies on Antitumor Antibiotics."*

*December 13, 2004. University of California, SanDiego *UCSD/Merck Symposium, Perspectives in Organic Synthesis*. Title: "*Studies on Biologically Intriguing Natural Products."*

2005

*January 28, 2005. Yale University, Connecticut Organic Chemistry Symposium, New Haven, Connecticut. Title: "*Exploiting Total Synthesis for Biological Intrigue."*

April 22, 2005. The University of Chicago. Title: "*Exploiting Total Synthesis for Biological Intrigue."*

April 25, 2005. Abbott Laboratories, Chicago, Illinois. Title: *"Total Synthesis of Biomedically Significant Alkaloids"*

April 26, 2005. The University of Illinois at Chicago. Title: "*Total Synthesis of Biologically Intriguing Natural Products."*

May 5, 2005. The University of Kansas. Title: *"Total Synthesis of Biologically Significant Alkaloids"*

*May 24, 2005. Imperial College, London, UK. Eli Lilly Lecture. Title: *"Vitalism in Natural Products: Finessing Molecular Secrets from Nature"*

May 25, 2005. Eli Lilly, England. Title: *"Total Synthesis of Biologically Significant Natural Alkaloids"*

*July 21, 2005 ***"****Synthesis in Organic Chemistry"* sponsored by the Perkin Division of the Royal Society of Chemistry, Oxford University, Oxford, England. Title: *"Total Synthesis of Biologically Significant Nitrogenous Substances"*

December 15-20, 2005. Pacifichem Congress Symposium on *"New Aspects of Heterocyclic Chemistry"* Title: *"Total Synthesis of Biologically Intriguing Natural Products"*

2006

*February 26 – March 2, 2006. 7th Winter Conference on Medicinal and Bioorganic Chemistry (WCMBC), Clearwater, Florida. Title: *Total Synthesis of Biomedically Significant Nitrogenous Substances*

*April 27, 2006. University of Oklahoma, *"Frontiers in Chemical Research"* Distinguished J. Clarence Karcher Lecturer. Title: *Total Synthesis of Biologically Intriguing Alkaloids*

May 1, 2006. Amgen, Inc., Cambridge, Mass. Title: "*New Tricks in Amino Acid Synthesis: Applications to Complex Natural Products"*

*May 2, 2006. Boston College, Boston, Mass. *Bristol-Myers Squibb Organic Chemistry Symposium*. Title: "*Total Synthesis as a Vehicle to Penetrate Biosynthesis and Biomechanism"*

May 17, 2006. Abbott Bioresearch Center, Worcester, Mass. Title: "*New Tricks in Amino Acid Synthesis: Applications to Complex Natural Products"*

*May 18, 2006. MIT, Organic Syntheses Lecture. Title: *"Penetrating Biomechanistic and Biosynthetic Puzzles; Challenges in Natural Products Chemistry"*

May 19, 2006. Eisai Research Institute, Andover, Mass. Title: *"Total Synthesis of Biologically Intriguing Alkaloids"*

*July 21-23, Tokushima 2006 Presymposium-Natural Product Chemistry, Tokushima, Japan. Title: *"Total Synthesis of Natural Products of Biological Intrigue"*

*July 23-28, 2006 Kyoto, Japan. ICOB-5 & ISCNP-25 IUPAC International Conference on Biodiversity and Natural Products Chemistry. Title: *Total Synthesis of Biologically and Structurally Intriguing Alkaloids*

*July 30,31, Post-Symposium at Hokkaido University, Sapporo, Japan. Title: *"Penetrating Biomechanistic and Biosynthetic Puzzles; Challenges in Natural Products Chemistry"*

*September 15-16, 2006. Inaugural Negishi-Brown Lectures. Purdue University, West Lafayette, Indiana. Title: "*Total Synthesis of Biologically and Structurally Intriguing Alkaloids"*

September 25, 2006. Mexican Chemical Society 50th Anniversary Symposium. Mexico City, Mexico. Title: *"Penetrating Biomechanistic and Biosynthetic Puzzles; Challenges in Natural Products Chemistry"*

2007

March 25-29, 2007. 233rd ACS National Meeting, Chicago, Illinois. Symposium on *"Biomimetic Natural and Unnatural Product Synthesis".* Title: *"Biomimetic Total Syntheses of Prenylated Indole Alkaloids"*

March 26, 2007. 233rd ACS National Meeting, Chicago, Illinois. Symposium on *"Asymmetric Synthesis of α-Amino Acids. Novel Developments and Future Directions".* Title: *"New Tricks in Amino Acid Synthesis: Applications to Complex Natural Products"*

April 27, 2007. University of Missouri-Columbia. Title*: "Harnessing Total Synthesis of Natural Products to Probe Their Biosynthesis"*

April 28, 2007. University of Missouri-Columbia. Organic Chemistry Day. Title: "New Tricks in Amino Acid Synthesis: Applications to Complex Natural Products"*

May 27 – June 2, 2007. Barton Symposium. *Organic Chemistry: Perspectives on the 21st Century IV.* Anse Chatanet Resort, St. Lucia, Caribbean Islands. Title: *"Total Synthesis of Biologically Intriguing Alkaloids"*

September 19, Pfizer La Jolla Labs, San Diego, CA. Title: *"The Grubbs Methodological Series and Profound Implications for the Future of Natural Product Synthesis"*

September 20, 2007. Johnson & Johnson Pharmaceutical Research & Development, LaJolla, CA. Title: *"Total Synthesis of Biologically Intriguing Alkaloids"*

*September 21, 2007. Scripps Research Institute. Roche Lecture. Title: *"Penetrating Biomechanistic and Biosynthetic Puzzles: Challenges in Natural Products Chemistry and the Exploitation of Synthesis"*

October 2, 2007. The University of Wisconsin, Madison, Wisconsin. Title: *"Harnessing Total Syntheses of Natural Products to Probe Their Biosynthesis"*

October 4, 2007. The University of Missouri, Kansas City, Missouri. Title: *"Total Synthesis of Biologically Intriguing Alkaloids"*

November 8, 2007.Queen's University, Kingston, Ontario. Title*: "Total Synthesis as a Vehicle for Penetrating Biomechanistic and Biosynthetic Puzzles; Challenges in Natural Products Chemistry"*

*November 9-11, 2007. *Quebec-Ontario Mini-symposium in Bio-Organic and Organic Chemistry* (QOMSBOC), University of Montreal, Quebec, Canada. Title: "*Harnessing Total Syntheses of Natural Products to Probe Their Biosynthesis"*

2008

*May 24-28, 2008. 91st Canadian Society for Chemistry Conference, Edmonton, Alberta, Canada. Title: "*Total Synthesis of Natural Products of Biological Intrigue"*

June 15-18, 2008. Regional ACS meeting, Park City, Utah. Title: "*Total Synthesis of Natural Products of Biological Intrigue"*

*July 22-25, 2008. The 9th Tetrahedron Symposium, Berkeley, California. Title: "*Total Synthesis of Natural Products of Biological Intrigue"*

July 24, 2008. Genentech. South SanFrancisco, CA. Title: *"Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles; Challenges in Natural Products Chemistry"*

October 13, 2008. Bristol-Myers Squibb Pharmaceutical Co., Princeton, N.J. Title: *"Total Synthesis and Biological Mode of Action of Macrocyclic Histone Deacetylase Inhibitors"*

October 14, 2008. Princeton University, Princeton, N.J. Title: *"Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

October 23, 2008. Symposium in Honor of Dr. Robin D.G. Cooper's 70th Birthday, Rigel Pharmaceutical Co., SanFrancisco, CA. Title: *"Total Synthesis and Biological Mode of Action of Macrocyclic Histone Deacetylase Inhibitors"*

2009

*January 25-29, 2009. Steamboat Winter Medicinal Chemistry Conference. Plenary Lecture Title: *"Quinine! A Story of Chemistry, History, Personalities and Ethics"*

May 11, 2009. Indiana University, Bloomington, Indiana. Title: "*Total Synthesis of Natural Products of Biological Intrigue"*

June 28-July 3, 2009. Gordon Research Conference on Heterocyclic Chemistry, Newport, Rhode Island. Title: *"Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Heterocyclic Natural Products Chemistry"*

*August 2-7, 2009. 22nd International Congress of Heterocyclic Chemistry, St. Johns, Newfoundland, Labrador, Canada. Title: *"Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Heterocyclic Natural Products Chemistry"*

September 11, 2009. University of Notre Dame, South Bend, Indiana. Title: "*Total Synthesis of Natural Products of Biological Intrigue"*

2010

*August 22-26, 2010. Boston, Massachusetts. Symposium in Honor of Robert Burns Woodward. Title: *"Quinine! A Story of Chemistry, History, Personalities and Ethics"*

Honolulu, Hawaii, December 2010. Pacifichem Symposium #148: *Design and Synthesis of Biologically Active Compounds for Elucidating Mode of-Action*. Title: *Total Synthesis and Biological Mode of Action of Macrocyclic Histone Deacetylase Inhibitors*

2011

*March 28, 2011. American Chemical Society 241st National Meeting, Anaheim, California. Ernest Guenther Award Symposium in Honor of Robert M. Williams, Award address. Title: *Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry*

*June 26-30. Conference on Advances in Organic Synthesis. Hradec, Kralov, Czech Republic. Title: *Total Synthesis as a Vehicle for Interrogating Biosynthetic and Biomechanistic Puzzles*

October 3, 2011. Givaudan Co., Cincinnati, Ohio. Title: *Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry*

October 28, 2011. University of Michigan, Ann Arbor, Michigan. Title: *Total Synthesis as a Vehicle for Interrogating Biosynthetic and Biomechanistic Puzzles*

2012

*February 21, 2012 Texas Tech University. Henry J. Shine Endowment Lectureship. Title: *Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry*

February 22, 2012 Texas Tech University. Public Lecture: Title: *"Quinine! A Story of Chemistry, History, Personalities and Ethics"*

May 9, 2012. Merck & Co., Rahway, New Jersey. Title: *Total Synthesis as a Vehicle for Interrogating Biosynthetic and Biomechanistic Puzzles*

*June 4, 2012. Oregon State University, Corvalis, Oregon. James D. White Honorary Lecture. Title: *"Quinine! A Story of Chemistry, History, Personalities and Ethics"*

*June 26-29, 2012. 13th Tetrahedron Symposium, Amsterdam, the Netherlands. Title: *Total Synthesis as a Vehicle for Interrogating Biosynthetic and Biomechanistic Puzzles*

July 22-27, 2012. Proctor Academy, Andover, New Hampshire. Natural Products Gordon Research Conference. Title: "*Enantiomeric Natural Products: Biosynthetic, Synthetic and Genetic Revelations"*

August 20, 2012. University of Alberta, Edmonton, Canada. Title: "*Enantiomeric Natural Products: Biosynthetic, Synthetic and Genetic Revelations"*

October 18, 2012. ACS Rocky Mountain Regional Meeting, Cope Scholars Symposium. Title: *"Total Synthesis and Biological Mode of Action of Macrocyclic Histone Deacetylase Inhibitors"*

*November, 27-30, 2012. 13th Tetrahedron Symposium, Taiwan. Title: *Total Synthesis as a Vehicle for Interrogating Biosynthetic and Biomechanistic Puzzles*

2013

January 11, 2013. University of Shizuoka, Shizuoka, Japan. Title: "*Total Synthesis as a Vehicle for Interrogating Biosynthetic and Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

January 15, 2013. Kyoto University, Kyoto, Japan. Title: "*Tetrahydroisoquinoline Antitumor Alkaloids: Total Synthesis, Mechanism of Action and Biosynthesis"*

January 16, 2013. Osaka City University, Osaka, Japan. Title: "*Asymmetric Synthesis of Amino Acids: Gateway to Alkaloids and Peptides"*

January 17, 2013. Osaka University, Osaka, Japan. Title: "*Total Synthesis as a Vehicle for Interrogating Biosynthetic and Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

January 18, 2013. Kwansei, Gakuin University, Osaka, Japan. Title: "*Total Synthesis as a Vehicle for Interrogating Biosynthetic and Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

January 21, 2013. Keio University, Tokyo, Japan. Title: "*Total Synthesis as a Vehicle for Interrogating Biosynthetic and Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

January 22, 2013. Tokyo Institute of Technology, Tokyo, Japan. Title: "*Total Synthesis as a Vehicle for Interrogating Biosynthetic and Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

January 23, 2013. Waseda University, Tokyo, Japan. Title: "*Total Synthesis and Biological Mode of Action of Macrocyclic Histone Deacetylase Inhibitors"*

January 31, 2013. Kumamoto University, Kumamoto, Japan. Title: *"Total Synthesis and Biosynthesis of Prenylated Indole Alkaloids of the Notoamide and Paraherquamide Families".*

February 5, 2013. Tokyo University of Science, Chiba, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

February 6, 2013. Tsukuba University, Tsukuba, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

February 6, 2013. Eisai Pharmaceutical Company, Tsukuba, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

February 7, 2013. Toray Company, Kamakura, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

February 12, 2013. Dainippon-Sumitomo Company, Osaka, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

February 13, 2013. Kaneka Corporation, Osaka, Japan. Title: "*Asymmetric Synthesis of Amino Acids: Gateway to Peptides and Alkaloids"*

February 14, 2013. Shionogi Pharmaceutical Company, Osaka, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

February 20, 2013. Kobe Pharmaceutical University, Kobe, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

February 21, 2013. Asubio Pharma, Co., Ltd., Kobe, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

February 22, 2013. Kyoto Pharmaceutical University, Kyoto, Japan. Title: *"Tetrahydroisoquinoline Antitumor Alkaloids: Total Synthesis, Mechanism of Action and Biosynthesis"*

February 26, 2013. Institute of Microbial Chemistry, Tokyo, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

February 28, 2013. Otsuka Pharmaceutical Co., Tokushima, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

March 1, 2013. Tokushima University, Tokushima, Japan. Title: "*Asymmetric Synthesis of Amino Acids: Gateway to Peptides and Alkaloids"*

March 6, 2013. Tokyo University of Science, Tokyo, Japan. Title: "*Total Synthesis and Biological Mode of Action of Macrocyclic Histone Deacetylase Inhibitors"*

March 12, 2013. Keio University, Tokyo, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

March 13, 2013. Chugai, Co., Tokyo, Japan. Title: "*Total Synthesis and Biological Mode of Action of Macrocyclic Histone Deacetylase Inhibitors and the Asymmetric Total Synthesis of Capreomycin"*

March 19, 2013. Mitsubishi Tanabe Pharma, Inc., Yokohama, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

March 21, 2013. Astellas Pharma, Tsukuba, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

April 2, 2013. Daiichi Fine Chemicals, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

April 3, 2013. Gifu University, Gifu, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

April 4, 2013. Meiji Pharmaceutical University, Tokyo, Japan. Title: *"Tetrahydroisoquinoline Antitumor Alkaloids: Total Synthesis, Mechanism of Action and Biosynthesis"*

April 9, 2013. ACS Meeting, New Orleans, Louisiana. Special Award Symposium in Honor of Professor Dale L. Boger, recipient of the 2013 Ralph Hirschmann Award, Title: "*Asymmetric Synthesis of Amino Acids: Gateway to Alkaloids and Peptides"*

April 15, 2013. Kyowa-Kirin Co., Shizuoka, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

April 17, 2013. Takasago International, Tokyo, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

April 18, 2013. Kyoto University, Faculty of Science, Kyoto, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

April 22, 2013. Tokyo University of Pharmacy and Life Sciences. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

April 23, 2013. Kitasato Institute, Tokyo, Japan. Title: "*Enantiomeric Natural Products: Biosynthetic, Synthetic and Genetic Revelations"*

April 24, 2013. Tokyo University, Tokyo, Japan. Title: "*Enantiomeric Natural Products: Biosynthetic, Synthetic and Genetic Revelations"*

May 20, 2013. Tohoku University, Faculty of Science, Sendai, Japan. Title: "*Enantiomeric Natural Products: Biosynthetic, Synthetic and Genetic Revelations"*

May 21, 2013. Tohoku University, Faculty of Pharmaceutical Sciences, Sendai, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

May 22 & 23, 2013. Tokyo University, Faculty of Pharmaceutical Sciences, Tokyo, Japan.

Lecture 1, Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

Lecture 2, Title: *"Quinine! A Story of Chemistry, History, Personalities and Ethics"*

Lecture 3, Title: "*Total Synthesis and Biological Mode of Action of Macrocyclic Histone Deacetylase Inhibitors"*

Lecture 4, Title: "*Enantiomeric Natural Products: Biosynthetic, Synthetic and Genetic Revelations"*

May 24, 2013. Hokkaido University, Sapporo, Japan. Title: "*Enantiomeric Natural Products: Biosynthetic, Synthetic and Genetic Revelations"*

May 27, 2013. Teijin Pharma, Tokyo, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

May 29, 2013. Nagoya City University, Nagoya, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

May 30, 2013. Nagoya University, Nagoya, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

May 31, 2013. Kinjo Gakuin University, Nagoya, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

June 6, 2013. Kyushu University, Kyushu, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

June 7, 2013. Nagasaki University, Nagasaki, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

June 10, 2013. Taisho Company, Tokyo, Japan. Title: "*Asymmetric Synthesis of Amino Acids: Gateway to Peptides and Alkaloids"*

July 12, 2013. Daiichi Sankyo Co., Tokyo, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

July 16, 2013. Astellas Pharma, Tokyo, Japan. Title: "*Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

July 17, 2013. Ajinomoto Company, Tokyo, Japan. Title: "*Total Synthesis and Biological Mode of Action of Macrocyclic Histone Deacetylase Inhibitors"*

July 18 & 19, 2013. Japanese Society for Process Chemistry, Tsukuba, Japan. Title: *"The Evolution of Synthetic Strategies: Lessons from Nature"*

July 22, 2013. Hamari Chemical Co., Osaka, Japan. Title: "*Asymmetric Synthesis of Amino Acids: Gateway to Peptides and Alkaloids"*

October 7, 2013. Richmond University, Richmond, Virginia. Title: "*Total Synthesis as a Vehicle for Interrogating Biosynthetic and Biomechanistic Puzzles: Challenges in Natural Products Chemistry"*

October 8, 2013. College of William and Mary, Williamsburg, Virginia. Title: "*Enantiomeric Natural Products: Biosynthetic, Synthetic and Genetic Revelations"*

October 21, 2013. Colorado State University, Department of Biochemistry & Molecular Biology. Title: "*Synthesis and Biological Mode of Action of Macrocyclic Histone Deacetylase Inhibitors"*

November 16, 2013. Charles R. Martin 60th Birthday Symposium, University of Florida, Gainseville, Florida. Title: *"Quinine! A Story of Chemistry, History, Personalities and Ethics"*

December 2, University of Pennsylvania, Philadelphia, Pennsylvania. Title: "*Total Synthesis and Biological Mode of Action of Macrocyclic Histone Deacetylase Inhibitors"*

2014

*June 30, 2014. The Barton Lecture, Imperial College, London. Title: "*Enantiomeric Natural Products: A Marine and Terrestrial Fungi Conundrum"*

July 3, 2014. University of St. Andrews, St. Andrews, Scotland. Title: "*Enantiomeric Natural Products: A Marine and Terrestrial Fungi Conundrum"*

July 4, 2014. Edinburgh University, Edinburgh, Scotland. Title: "*Enantiomeric Natural Products: A Marine and Terrestrial Fungi Conundrum"*

2015

June 1-5, 2015. TERPNET 2015, Vancouver, Canada. Title: *"Prenylated Indole Alkaloid Biosynthesis: Reverse and Normal Prenyl Transferases Direct Rich Molecular Diversity"*

June 21-26, 2015. Salve Regina University, Rhode Island. Gordon Research Conference on Heterocyclic Compounds. Title: *"Synthesis and Biosynthesis of Heterocyclic Ring Systems in Natural Products Derived from Marine and Terrestrial Fungi"*

*November 15-19, 2015, 16th Brazilian Meeting on Organic Synthesis, Rio de Janeiro, Brazil. Title: "*Enantiomeric Natural Products: Biosynthetic, Synthetic and Genetic Revelations"*

(*Plenary Lecture or Distinguished Named Lecture)

Conferences Attended

1. 183rd National ACS Meeting, Las Vegas, Nevada; March 1982 (Abstract #17), Division of Organic Chemistry.
2. Fourth International Conference on Organic Synthesis (IUPAC), Tokyo, Japan; August 1982 (Abstract #A-I-4301).
3. Post ICOS IV Symposium, "Highlights in Organic Synthesis", Hokkaido University, Sapporo, Japan; August 30-31, 1982. Title: *"A New and Efficient Cyclization Reaction to Construct the Bicyclomycin Ring System".*
4. 185th National ACS Meeting, Seattle, Washington; March 1983 (Abstract #10), Division of Organic Chemistry.
5. 187th National ACS Meeting, St. Louis, Missouri; April 1984 (Abstract #24), Division of Carbohydrate Chemistry.
6. 188th National ACS Meeting, Philadelphia, Pennsylvania; August 1984 (Abstract #92), Division of Organic Chemistry.
7. The 1984 International Chemical Congress of Pacific Basin Societies; December 1984 (Abstract #10E57), Division of Organic Chemistry.
8. The 1985 NSF Synthesis Workshop, Pingree Park, Colorado.
9. The 1985 Gordon Research Conference on "Organic Reactions and Processes", New Hampshire; July 17, 1985. Title: *"New Carbon-Carbon Bond-Forming Reactions via Electrophilic Glycine Derivatives".*
10. The 1985 Gordon Research Conference on "Natural Products", New Hampshire; July 24, 1985. Title: *"Progress Toward Understanding the Mechanism of Action of Bicyclomycin".*
11. The Third International Kyoto Conference on New Aspects of Organic Chemistry; November 1985.
12. The 1986 Gordon Research Conference on "Heterocycles"; July 9, 1986. Title: *"Electrophilic Glycinates: Versatile Templates for Amino Acid Synthesis".*
13. The 192nd National ACS Meeting, Anaheim, California; September 1986 (Abstract #152 and #272), Division of Organic Chemistry.
14. The 193rd National ACS Meeting, Denver, Colorado, April 1987 (Abstracts #22 and #81), Division of Organic Chemistry.
15. The 1987 Gordon Research Conference on "Heterocycles" July 6-10, 1987. Title: *"Electrophilic Glycinates: Versatile Templates for Amino Acid Synthesis".*
16. The 1987 NSF Workshop on Environmental Chemistry, Stanford Sierra Camp, Lake Tahoe, California, Sept. 25-27, 1987.
17. *Eli Lilly Grantee Symposium, Indianapolis, Indiana, March 7-8, 1988. Title: *"Approach to Understanding the Molecular Mechanism of Action of Bicyclomycin"*.
18. The 9th Rocky Mountain Regional ACS Meeting, Symposium on Natural Products, Las Vegas, Nevada, March 28-30, 1988. Title: *"Stereofacial Selectivity of the Intramolecular S_N2' Cyclization: Brevianamide B".*
19. *Burgenstock Stereochemistry Conference, Burgenstock, Switzerland; April 28, 1988. Title: *"Stereofacial Selectivity of the Intramolecular S_N2' Cyclization: Asymmetric Total Synthesis of Brevianamide B"*.
20. IUPAC 88 Kyoto 16th International Symposium on the Chemistry of Natural Products, Kyoto, Japan; May 31, 1988. Title: *"Stereofacial Selectivity of the Intramolecular S_N2' Cyclization: Asymmetric Total Synthesis of Brevianamide B"*.
21. Nagoya Post-Symposium "New Topics on Natural Products", Toba, Japan; June 1988. Title: *"Stereofacial Selectivity of the Intramolecular S_N2' Cyclization: Asymmetric Total Synthesis of Brevianamide B"*.

22. Fourth International Symposium on "Recent Advances in the Chemistry of β-Lactam Antibiotics", Churchill College, Cambridge, England, July 4, 1988. Title: *"Synthesis and Properties of 1,3-Bridged β-Lactams: Novel Anti-Bredt β-Lactams"*.
23. *Eighth Biennial Carl S. Marvel Symposium, Tucson, Arizona, March 7, 1989. Title: *"Total Synthesis and Biogenetic Intrigue of the Brevianamide"* (Plenary Lecture).
24. The 1989 Chemical Congress of Pacific Basin Societies, Honolulu, Hawaii, December 17-22, 1989 (co-organizer of a Symposium on Amino Acids).
25. *The 1990 CU-Syntex Symposium, Boulder, Colorado, June 5-8, 1990. Title: *"Mechanistic and Synthetic Studies on the Mode of Cleavage of Superhelical DNA by Quinocarcin"*. (Plenary Lecture).
26. Special Bilateral U.S.-Britain Workshop on "Asymmetric Synthesis." July 3-8, 1990. Pingree Park, Colorado (RMW Co-organizer).
27. The 1990 Gordon Research Conference on "Organic Reactions and Processes," July 15-20, New Hampton, New Hampshire. Title: *"The Mechanism of Oxygen Reduction by Quinocarcin"*.
28. The 1990 Gordon Research Conference on "National Products," July 22-27, New Hampton, New Hampshire. Title: *"Bioorganic, Mechanistic and Synthetic Chemistry of Biologically Significant Nitrogenous Substances"*.
29. The 200th National ACS Meeting, August 27-31, 1990, Washington, DC.
30. NSF-JSPS Bilateral Seminar on "Selectivity in Synthetic and Bio-Organic Chemistry", Tokyo, Japan, June 3-7, 1991. Title: *"Unusual Facial Selectivity in the Biosynthesis and Synthesis of the Brevianamide / Paraherquamide Class of Mycotoxins: In Search of the Biosynthetic Diels-Alder Construction"*.
31. *International Congress of New Drug Development, Seoul, Korea, August 21, 1991. Title: *"Synthetic and Mechanistic Studies on Anti-Tumor Antibiotics"*. (Plenary Lecture).
32. *4th Chemical Congress of North America, Division of Organic Chemistry, New York, August 29, 1991. Title: *"Unusual Stereofacial Selectivity in the Biosynthesis and Synthesis of the Brevianamide / Paraherquamide Class of Mycotoxins: In Search of the Biosynthetic Diels-Alder Construction"*.
33. 24th Central Regional ACS Meeting (CMACS-92), Cincinnati, Ohio, Symposium on Amino Acids and Peptides, May 28, 1992. Title: *"Asymmetric Synthesis of Non-Proteinogenic α-Amino Acids".*
34. Hoffman-La Roche, Inc., Workshop on Prospects for Antibacterial Agents Based on Diaminopimelic Acid, May 11, 1992. Title: *"Stereochemistry and DAP Inhibitors".*
35. *48th Southwest Regional ACS Meeting, Lubbock, Texas, October 21-23, 1992, A.I. Meyers Symposium. Title: *"Recent Studies in Asymmetric and Bioorganic Methods".* (Plenary Lecture).
36. * BioEast '93, Washington, D.C., January 27, 1993, Title: *"New Approaches to Antibiotic Design and Synthesis"*. (Plenary Lecture).
37. 3rd International Congress on Amino Acids, Vienna, Austria, August 23-27, 1993. Title: *"Asymmetric Synthesis of Non-proteinogenic α-Amino Acids via Chiral Glycinates".*
38. *33rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Symposium on *"Glycopeptide Resistance and the Cell Wall",* New Orleans, Louisiana, October 18, 1993. Title: *"Implications for Drug Discovery".* (Plenary Lecture).
39. *Sino-American Symposium on Asymmetric Synthesis, Taichung, Taiwan, April 8-9, 1994. Title: *"The Asymmetric Synthesis of α-Amino Acids".* (Plenary Lecture).
40. *The 4th International Conference on Chemical Synthesis of Antibiotics and Related Microbial Products, Nashville, Indiana, September 11-16, 1994. Title: *"Studies on the Mechanism of Action of Bioactive Nitrogenous Substances".* (Plenary Lecture).
41. *The SCI Meeting on Amino Acids, London, England, May 16, 1995. Title: *"Overview of the Major Conceptual Approaches to the Construction of Amino Acids"*. (Plenary Lecture).

42. *CU Hauser Symposium, May 29 & 30, 1997, Boulder, Colorado. Title: *"Organic Synthesis: An Important Vehicle to Probe Biosynthesis".*
43. *The Gordon Research Conference on Natural Products, July 5-9, 1998. Title: *"Natural Product Synthesis as a Probe for Studying Biosynthesis and Biomechanism".*
44. *The 83rd Canadian Society for Chemistry Conference, Calgary, Canada, May 28-31, 2000. Title: *"Asymmetric Total Synthesis of the Oxindole Alkaloids Paraherquamide A and Spirotryprostatin B".*
45. *Herbert C. Brown Distinguished Professorship Symposium, Purdue University, Sept. 28-29, 2000. Title: "*Total Synthesis of Natural Products: Tools for Probing and Exploiting Biomechanism and Biosynthesis".* (Plenary Lecture).
46. The PacifiChem 2000 Conference, Honolulu, Hawaii, December 14-19, 2000. Title: "*Synthesis and Antimicrobial Evaluation of TAN1057A/B Analogs".*
47. *University of California Irvine, UCI Synthesis Symposium on *Biological Tools and Targets*. January 20, 2001. Title: "*DNA Crosslinking Agents: Synthesis, New Methods for Activation and the Elucidation of New Targets in the Nucleus in vivo".* (Plenary Lecture).
48. *The 2001 CU-Array Biopharma Symposium on Medicinal and Synthetic Organic Chemistry, June 6-8, 2001, Boulder, Colorado. Title: *"Extraordinarily Versatile Amino Acid Templates for the Total Synthesis of Natural Products, Peptide Isosteres and Amino Acids".* (Plenary Lecture).
49. The 18th International Congress of Heterocyclic Chemistry, Yokohama, Japan, July 29 - August 3, 2001. Title: *"Extraordinarily Versatile Amino Acid Templates for the Total Synthesis of Natural Products, Peptide Isosteres and Amino Acids".*
50. 57th Southwest Regional ACS Meeting, Symposium Honoring Al Meyers Retirement. San Antonio, Texas. October 18-19, 2001. Title: *"Extraordinarily Versatile Amino Acid Templates for the Total Synthesis of Natural Products, Peptide Isosteres and Amino Acids".*
51. *Reaction Mechanisms Conference June 28 - July 1, 2002. Ohio State University. Title: *Mechanistic Studies on Synthetic and Natural DNA-Reactive Alkaloids.* (Plenary Lecture).
52. *Belgian Organic Synthesis Symposium (BOSS IX), Namur, Belgium, July 8-12, 2002. Title: *Extraordinarily Versatile Amino Acid Templates for the Total Synthesis of Natural Products, Peptide Isosteres and Amino Acids.* (Plenary Lecture).
53. *American Chemical Society Meeting, Boston, Massachusetts, August 20, 2002, Arthur C. Cope Scholar Awardee lecture. Title: *"Total Synthesis of Natural Products: Tools for Probing and Expoliting Biomechanism and Biosynthesis"*
54. *The 8th Loughborough Synthesis Symposium Sponsored by Astra Zeneca, Loughborough, Leicestershire, UK, October 22, 2003. Title: *Total Synthesis of Natural Products of Biological Intrigue.* (Plenary Lecture).
55. *The Barton Symposium. Heron Island, Australia, November 4, 2003. Title: *"Total Synthesis of Natural Products of Biological Intrigue".* (Plenary Lecture).
56. *March 1,2, 2004. Eli Lilly Grantee Symposium, Indianapolis, Indiana. Title: *"From Bicyclomycin to Biosynthesis"*
57. *March 19, 2004. *Chemistry as a Life Science Symposium XII*. Rutgers University, New Jersey. Title: *"Total Synthesis of Natural Products of Biological Intrigue"* (Plenary Lecture).
58. *March 29, 30, 2004. ACS Meeting in Anaheim California. Tohru Fukuyama ACS Award Symposium. Title: *"Total Synthesis of Biologically Intriguing Natural Products"*
59. *December 13, 2004. University of California, SanDiego *UCSD/Merck Symposium, Perspectives in Organic Synthesis*. Title: "*Studies on Biologically Intriguing Natural Products."*
60. *January 28, 2005. Yale University, Connecticut Organic Chemistry Symposium, New Haven, Connecticut. Title: "*Exploiting Total Synthesis for Biological Intrigue."*
61. *July 21, 2005 *"Synthesis in Organic Chemistry"* sponsored by the Perkin Division of the Royal Society of Chemistry, Oxford University, Oxford, England. Title: *"Total Synthesis of Biologically Significant Nitrogenous Substances."*

62. December 15-20, 2005. Pacifichem Congress Symposium on *"New Aspects of Heterocyclic Chemistry"* Title: *"Total Synthesis of Biologically Intriguing Natural Products."*

63. February 26 – March 2, 2006. 7th Winter Conference on Medicinal and Bioorganic Chemistry (WCMBC), Clearwater, Florida. Title: "*Total Synthesis of Biomedically Significant Nitrogenous Substances."*

64. July 21-23, Tokushima 2006 Presymposium-Natural Product Chemistry, Tokushima, Japan. Title: *"Total Synthesis of Natural Products of Biological Intrigue"*

65. July 23-28, 2006 Kyoto, Japan. ICOB-5 & ISCNP-25 IUPAC International Conference on Biodiversity and Natural Products Chemistry. Title: "*Total Synthesis of Biologically and Structurally Intriguing Alkaloids."*

66. July 30,31, Post-Symposium at Hokkaido University, Sapporo, Japan. Title: *"Penetrating Biomechanistic and Biosynthetic Puzzles; Challenges in Natural Products Chemistry"*

67. *September 15-16, 2006. Inaugural Negishi-Brown Lectures. Purdue University, West Lafayette, Indiana. Title: "*Total Synthesis of Biologically and Structurally Intriguing Alkaloids"* (Plenary Lecture).

68. September 25, 2006. Mexican Chemical Society 50th Anniversary Symposium. Mexico City, Mexico. Title: *"Penetrating Biomechanistic and Biosynthetic Puzzles; Challenges in Natural Products Chemistry"*

69. March 25-19, 2007. The 233rd American Chemical Society National Meeting, Chicago, Illinois. Symposium on *"Biomimetic Natural and Unnatural Product Synthesis".* Title: "*Biomimetic Total Syntheses of Prenylated Indole Alkaloids"*

70. March 26, 2007. 233rd ACS National Meeting, Chicago, Illinois. Symposium on *"Asymmetric Synthesis of α-Amino Acids. Novel Developments and Future Directions".* Title: *"New Tricks in Amino Acid Synthesis: Applications to Complex Natural Products"*

71. May 27 – June 2, 2007. Barton Symposium. *Organic Chemistry: Perspectives on the 21st Century IV.* Anse Chatanet Resort, St. Lucia, Caribbean Islands. Title: *"Total Synthesis of Biologically Intriguing Alkaloids"*

72. *November 9-11, 2007. *Quebec-Ontario Mini-symposium in Bio-Organic and Organic Chemistry* (QOMSBOC), University of Montreal, Quebec, Canada. Title: "*Harnessing Total Syntheses of Natural Products to Probe Their Biosynthesis".* (Plenary Lecture).

73. *May 24-28, 2008. 91st Canadian Society for Chemistry Conference, Edmonton, Alberta, Canada. Title: "*Total Synthesis of Natural Products of Biological Intrigue"*

74. June 15-18, 2008. Regional ACS meeting, Park City, Utah. Title: "*Total Synthesis of Natural Products of Biological Intrigue"*

75. *July 22-25, 2008. The 9th Tetrahedron Symposium, Berkeley, California. Title: "*Total Synthesis of Natural Products of Biological Intrigue".* (Plenary Lecture).

76. October 23, 2008. Symposium in Honor of Dr. Robin D.G. Cooper's 70th Birthday, Rigel Pharmaceutical Co., SanFrancisco, CA. Title: *"Total Synthesis and Biological Mode of Action of Macrocyclic Histone Deacetylase Inhibitors"*

77. October 25, 2008. The Albert I. and Joan Meyers Symposium, Fort Collins, Colorado. (Symposium Organizer)

78. *January 25-29. Steamboat Winter Medicinal Chemistry Conference. Plenary Lecture Title: *"Quinine! A Story of Chemistry, History, Personalities and Ethics"*

79. June 28-July 3, 2009. Gordon Research Conference on Heterocyclic Chemistry, Newport, Rhode Island. Title: *"Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Heterocyclic Natural Products Chemistry"*

80. *August 22-26, 2010. 240th National American Chemical Society Meeting, Boston, Massachusetts. Robert Burns Woodward Memorial Symposium. Title: *"Quinine! A Story of Chemistry, History, Personalities and Ethics"*

81. *March 28, 2011. American Chemical Society 241st National Meeting, Anaheim, California. Ernest Guenther Award Symposium in Honor of Robert M. Williams, Award address. Title: *Total Synthesis as a Vehicle for Penetrating Biomechanistic Puzzles: Challenges in Natural Products Chemistry*

82. *June 26-30. Conference on Advances in Organic Synthesis. Hradec, Kralov, Czech Republic. Title: *Total Synthesis as a Vehicle for Interrogating Biosynthetic and Biomechanistic Puzzles*

83. *June 26-29, 2012. 13th Tetrahedron Symposium, Amsterdam, the Netherlands. Title: "*Total Synthesis as a Vehicle for Interrogating Biosynthetic and Biomechanistic Puzzles"*

84. July 22-27, 2012. Natural Products Gordon Research Conference. Proctor Academy, Andover, New Hampshire. Title: "*Enantiomeric Natural Products: Biosynthetic, Synthetic and Genetic Revelations"*

85. *October 18, 2012. ACS Rocky Mountain Regional Meeting, Cope Scholars Symposium. Title: *"Total Synthesis and Biological Mode of Action of Macrocyclic Histone Deacetylase Inhibitors"*

86. *November, 27-30, 2012. 13th Tetrahedron Symposium, Taiwan. Title: *Total Synthesis as a Vehicle for Interrogating Biosynthetic and Biomechanistic Puzzles*

87. *July 18 & 19, 2013. Japanese Society for Process Chemistry, Tsukuba, Japan. Title: *"The Evolution of Synthetic Strategies: Lessons from Nature"*

88. July 28 – August 1, 2013. Natural Products Gordon Research Conference. Proctor Academy, Andover, New Hampshire.

89. November 16, 2013. Charles R. Martin 60th Birthday Symposium, University of Florida, Gainseville, Florida. Title: *"Quinine! A Story of Chemistry, History, Personalities and Ethics"*

90. June 1-5, 2015. TERPNET 2015, Vancouver, Canada. Title: *"Prenylated Indole Alkaloid Biosynthesis: Reverse and Normal Prenyl Transferases Direct Rich Molecular Diversity"*

91. June 21-26, 2015. Salve Regina University, Rhode Island. Gordon Research Conference on Heterocyclic Compounds. Title: *"Synthesis and Biosynthesis of Heterocyclic Ring Systems in Natural Products Derived from Marine and Terrestrial Fungi"*

92. November 15-19, 2015, 16th Brazilian Meeting on Organic Synthesis, Búzios, Brazil. Title: "*Enantiomeric Natural Products: Biosynthetic, Synthetic and Genetic Revelations"*

(*Plenary Lecture or Distinguished Named Lecture)

Former Co-workers of Robert M. Williams

Department of Chemistry, Colorado State University

Former Graduate Students:

1. Robert W. Armstrong (Ph.D., August 1984)
Thesis entitled: *Total Synthesis of (±)- and (+)-Bicyclomycin*
NIH Postdoctoral Fellow with Y.Kishi, Harvard University (1984-86).
Professor of Chemistry, UCLA, 405 Hilgard Ave., Los Angeles, CA. August 1986-present
Director of Drug Discovery, Amgen Co., Thousand Oaks, California (1995-1999); Vice President of Discovery Research, Eli Lilly Pharmaceutical Co., Indianapolis, IN. (1999-2011). **Current position:** Independent Venture Capitalist.

2. Andrew O. Stewart (Ph.D., December 1985)
Thesis entitled: *Carbohydrates as Chiral Templates*
Current position: Volweiler Research Scientist at Abbott Laboratories, Abbott Park, IL.

3. Peter J. Sinclair (Ph.D., April 1987)
Thesis entitled: *Asymmetric Synthesis of Amino Acids via Electrophilic Glycinates*
Current position: Research Scientist in Drug Discovery at Merck & Co. Research Laboratories, Rahway, N.J.

4. Lynn K. Maruyama (Ph.D., September 1987)
Thesis entitled: *Synthesis and Study of Bicyclomycin Analogs*
Current position: Professor at Southern Oregon University, Ashland, Oregon

5. Paul P. Ehrlich (Ph.D., Spring 1989)
Thesis entitled: *Synthetic and Pharmacophoric Studies of Quinocarcin"*
Current position: Research Scientist at Bayer, AG, Germany

6. Myeong-Nyeo Im (Ph.D. Spring 1991)
Thesis entitled: *The Asymmetric Synthesis of Amino Acids via Glycine Enolate Alkylation*
Current position: Instructor in Seoul, Korea.

7. James A. Hendrix (Ph.D., Spring 1992)
Thesis Entitled: *The Asymmetric Synthesis of Arylglycines*
Postdoctoral Fellow with Prof. A.G.M. Barrett, Colorado State University, (1991-1992)
Current position: Head, Medicinal Chemistry at Sanofi Aventis Pharma, Bridgewater, New Jersey

8. Christopher Sean Esslinger (Ph.D., Fall 1996)
Thesis Entitled: *Studies Toward the Total Synthesis of Fusarin C*
Postdoctoral Fellow with Prof. Richard A. Chamberlin, The University of California , Irvine, September (1992-1995).
Current position: Deceased; formerly Associate Professor at the University of Montana, Missoula, Montana

9. Glenn J. Fegley (Ph.D., Fall 1994)
Thesis Entitled: *Asymmetric Synthesis of 1-Aminocyclopropane Carboxylic Acids*
Postdoctoral Fellow with Prof. William R. Roush, Indiana University, September 1993- August 1995.
Current position: Research Scientist at Onconova Therapeutics, Wynnewood Pennsylvania

10. Timothy D. Cushing (Ph.D., Fall 1993)
Thesis Entitled: *Total Synthesis of (+)-Paraherquamide B*
NIH Postdoctoral Fellow with Prof. Gregory L. Verdine, Harvard University, September 1993- June 1995.
Current position: Chemistry Principal Investigator at Amgen Co., SanFrancisco, California

11. Gregory F. Miknis (Ph.D., Fall 1993)
Thesis Entitled: *The Total Synthesis of Aspirochlorine*
NIH Postdoctoral Fellow with Prof. Phil Magnus, The University of Texas at Austin, (1993- 1995)
Current position: Associate Director, Colorado Center for Drug Discovery (C2D2), Fort Collins, Colorado

12. Tracy N. Tippie (MS., Summer 1995)
Thesis entitled: *Preparation of Azidobrevianamide A and Synthetic and Biological Studies of Tetrazomine*
Current position: physician

13. Mark E. Flanagan (Ph.D., Spring 1995)
Thesis entitled: *Synthesis and Biomechanistic Studies of Quinocarcin and Structural Analogs*
NIH Postdoctoral Fellow with Prof. Peter G. Schultz, The University of California at Berkeley, (1995-1997).
Current position: Research Scientist in Drug Discovery at Pfizer, Inc., Groton, Connecticut

Former Graduate Students of Robert M. Williams (continued)

14. Steven M. Rubenstein (Ph.D. Spring 1996)
Thesis entitled: *Elucidating the Biosynthetic Pathway of Taxol*
Postdoctoral Fellow with Prof. David A. Evans, Harvard University (1996-1998).
Current position: Senior Chemistry Scientist in Drug Discovery at Albany Molecular, Albany, New York.

15. Jennifer Travers (Ph.D., Spring 1996)
Thesis entitled: *Panning Peptide Libraries on Filamentous Phage*
Current position: Instructor at Oregon State University

16. Chester C. Yuan (Ph.D. Spring 1997)
Thesis entitled: *Part I. Asymmetric Synthesis of 2,6-Diaminopimelic Acids (DAP) and g-D(L)-Glutamyl-L-meso-Diaminopimelic Acid Dipeptide. Part II. Total Synthesis of TAN-1057 and Analogues*
Current position: Research Scientist at Amgen, Inc., Thousand Oaks, California.

17.Scott R. Rajski (Ph.D., Spring 1997)
Thesis entitled: *Mechanism of Action Studies on the FR900482 Class of Antitumor Antibiotics*
ACS Postdoctoral Fellow with Prof. Jacqueline Barton, Cal.Tech., Pasadena, California (1997-2000)
Current position: Research Associate at the University of Wisconsin, Madison, Department of Pharmacy

18. Samuel B. Rollins (Ph.D., Fall 1997)
Thesis entitled: *Synthesis of a Photoactivated Analog of the Antitumor Antibiotic FR900482*
Current position: Patent Attorney for Kilpatrick Stockton LLP, Winston-Salem, North Carolina

19. Paul B. Gansle (M.S., Fall, 1997)
Thesis Entitled: *Synthesis of D-Alanyl-D-Alanyl Dipeptide Isosteres and Cephalosporin Prodrugs*
Current position: Local High School Science Teacher.

20. David M. Bender (M.S., Spring 1998)
Thesis entitled: *Design and Synthesis of Analogs of the Peptidylnucleoside Antibiotics the Mureidomycins*
Current position: Research Associate in Drug Discovery at Eli Lilly Co., Indianapolis, Indiana

21. Brad Herberich (Ph.D., Fall 1999)
Thesis entitled: *Synthetic and DNA Cross-Linking Studies of Bioxalomycin a2*
NIH Postdoctoral Fellow with Prof. Peter G. Scultz, at the Scripps Institute. (1999-2001)
Current position: Research Scientist at Amgen, Inc., Thousand Oaks, California

22. Jeffrey Cao (Ph.D., Spring 2000)
Thesis entitled: *The Total Synthesis of (-)-Paraherquamide A*
Current position: Research Scientist in Drug Discovery at Merck & Co., New Jersey

23. Kathleen M. Halligan (Ph.D. Fall 2000)
Thesis entitled: *Synthetic and Biosynthetic Studies of the Brevianamides*
Current position: Assistant Professor, York College, York, Pennsylvania

24. Jack D. Scott (Ph.D. 2001)
Thesis entitled: *Total Synthesis of (-)-Tetrazomine and Biochemical Studies*
Current position: Research Scientist in Drug Discovery at Merck & Co., Rahway, New Jersey

25. Emily M. Stocking (Ph.D. 2001)
Thesis entitled: *Studies on the Biosynthesis of Paraherquamide A and a Total Synthesis of (+)-VM55599*
Current position: Research Scientist at R.W. Johnson Pharmaceutical Co., San Diego, California.

26. Christi Kosogof (M.S. 2001)
Thesis entitled: *Synthesis and Biochemical Activity of Pyrrolizidine Alkaloid Derivatives*
Current position: Research Associate at Abbott Laboratories, Chicago, Illinois.

27. Paul R. Sebahar (Ph.D. 2002)
Thesis entitled: *Asymmetric, Stereocontrolled Total Synthesis of (+)- and (-)-Spirotryprostatin B.*
Current position: Research Scientist at University of Utah, Salt Lake City, Utah.

28. Ted C. Judd (Ph.D. 2003)
Thesis entitled: *Asymmetric Total Synthesis of FR900482*
NIH Postdoctoral Fellow with Professor Yoshito Kishi, Harvard University, Cambridge, Mass. 2003-2005
Current position: Research Scientist at Amgen, Inc., Thousand Oaks, California

Former Graduate Students of Robert M. Williams (continued)

29. Steve Lenger (Ph.D. 2003)
Thesis entitled: *Studies on the total synthesis of putative intermediates in the biosynthesis of Taxol*
Current position: Research Scientist in Process Research at Array BioPharma, Boulder, Colorado.

30. Duane E. DeMong (Ph.D. 2003)
Thesis entitled: *Asymmetric Total Synthesis of Capreomycidine and Capreomycin IB*
Current position: Research Scientist at Merck & Co., Rahway, New Jersey

31. Brian K. Albrecht (Ph.D. 2003)
Thesis Entitled: *A Concise Total Synthesis of the TMC-95A and TMC-95B Proteasome Inhibitors*
Current position: Consulting Scientist, Third Rock Ventures, Cambridge, Mass.

32. Ryan E. Looper (Ph.D. 2004)
Thesis Entitled: *Concise Asymmetric Synthesis of the Cylindrospermopsin Alkaloids*
Current position: Associate Professor at The University of Utah, Salt Lake City, Utah

33. Chandele Gray (Ph.D. 2006)
Thesis Entitled: *Asperparaline A: Biosynthetic Studies and Synthetic Efforts*
Current position: High School teacher, Sumner, Maine

34. Yuyin Chen (Ph.D. 2006)
Thesis Entitled: *Approaches to the Total Synthesis of Lemonomycin*
Current position: Project Manager at BioDuro, China

35. Meriah W.N. Valente (M.S. 2006)
Thesis Entitled: *Studies Towards the Biomimetic Synthesis of the Stephacidin Family of Natural Products and the Concise and Versatile Synthesis of D,L-Brevianamide B, C-12A-epi-Malbrancheamide and Structurally Related Analogs*
Current position: Associate Research Scientist at Bristol-Myers Squibb Pharmaceutical Co., New Jersey

36. Alan R. Grubbs (Ph.D. 2007)
Thesis entitled: *"Concise Synthesis of Notoamides B-E and Stephacidin A"*
Current position: Research Scientist at Ardea Biosciences, San Diego, California.

37. Siyuan Chen (Ph.D. 2007)
Thesis entitled: *Studies Toward the Total Synthesis of Spiroquinazoline*
Current position: Research Scientist at Anichem, Inc.

38. Nick Gearhart (M.S. 2008)
Thesis entitled: *Studies Towards the Synthesis of a Bicyclo[2.2.2]diazaoctane Ring System and Efforts Towards the Synthesis of SB-219383*
Current position: Research Associate Scientist at Eisai Co.

39. Andrea Geiser (M.S. 2009)
Thesis entitled: *Progress Towards Proposed Biosynthetic Intermediates of Stephacidin A*
Current position: Research Associate Scientist at Merck & Co.

40. Ann E. Troutman (M.S. 2009)
Thesis entitled: *Progress Towards the Improved Synthesis of FK228 and Analogs; and the Total Synthesis of Largazole-Azumamide Hybrid*
Current position: Research Associate Scientist at Merck & Co.

41. Daniel A. Gubler (Ph.D. 2009)
Thesis entitled: *Mitomycin Alkaloids: Synthetic Studies*
Current position: Director of Unicity International, Orem, Utah.

42. Cameron Burnett (Ph.D. 2009)
Thesis entitled: *Studies Towards the Total Synthesis of Microsclerodermin G*
Current position: Ensign and Instructor for the U.S. Navy, Goose Creek, South Carolina.

43. Xiangna Jia (Ph.D. 2009)
Thesis entitled: *Progress Toward an Asymmetric Total Synthesis of the Stemona Alkaloid Tuberostemoninol.*
Current position: unknown (in China)

44. Tenaya L. Newkirk (Ph.D. 2009)
Thesis entitled: *Towards the Total Synthesis of 14-Acetoxygelsenicine and Synthesis of Largazole Analogs*
Current position: Instructor at Colorado State University

Former Graduate Students of Robert M. Williams (continued)

45. Brandon English (Ph.D. 2010)
Thesis entitled: *A Divergent Synthesis of Secologanin Derived Natural Products*
Current position: Assistant Professor at Red Rocks Community College, Lakewood, Colorado.

46. Tatyana Sabodash (M.S. 2011)
Thesis entitled: *Studies Toward the Total Synthesis of Lydiamycin A*
Current position: Trainee European patent attorney, Lederer and Keller, Germany

47. Timmy McAfoos (Ph.D. 2011)
Thesis entitled: *Studies on the Biosynthesis of the Stephacidins and Notoamides. Total Synthesis of Notoamide S and Notoamide T and Progress Toward the Synthesis of Chrysogenamide A.*
Current position: Research Associate at the M.D. Anderson, Cancer Research Center, Texas

48. Jennifer Finefield (Ph.D. 2011)
Thesis entitled: *Studies on the Biosynthesis of Prenylated Indole Secondary Metabolites from Aspergillus versicolor and Aspergillus sp. and A Novel Approach to Tumor Specific Drug Delivery: Use of a Naphthyridine Drug Linker with a DNA Hairpin.*
Current position: Technology Transfer Manager, Indiana University, Bloomington, Indiana

49. Ryan Rafferty (Ph.D. 2011)
Thesis entitled: *Total Synthesis of Hapalindoles J and U, Formal Synthesis of Hapalindole O, Synthesis of of the Proposed Biosynthetic Precursor to Hapalindole K and Work Towards the Ambiguine Family of Alkaloids.*
Current position: Assistant Professor, Kansas State University, Manhattan, Kansas.

50. Paul Schuber (Ph.D. 2011)
Thesis entitled: *Studies on the Total Synthesis of MPC1001*
Current position: Research Associate at the M.D. Anderson, Cancer Research Center, Texas

51. Guojun Pan (Ph.D. 2011)
Thesis Entitled: *Total Syntheses of (+)-Fawcettimine, (+)-Fawcettidine, (+)-Lycoflexine and (+)-Lycoposerramine B.*
Current Position: post-doctoral research associate with Prof. Liming Zhang, University of California, Santa Barbara

52. Timothy R. Welch (Ph.D. 2012)
Thesis entitled: *Epidithiodioxopiperazines: Synthetic Studies of (+)-Chetomin and (-)-Sporidesmin A.*
Current Position: Consulting Scientist, Wilson, Sonsini, Goodrich & Rosati, San Francisco, California

53. Aaron Pearson (Ph.D. 2013)
Research Project: *Asymmetric Total Synthesis of Zetekitoxin and Saxitoxin*
Current Position: post-doctoral research associate with Prof. Peter G. Schultz, Scripps Research Institute, La Jolla, California

54. Marie Trujillo (M.S. 2013)
Research Project: *Synthesis & Biosynthesis of the Notoamides*
Current Position: QC Analyst at Leprino Foods, Greeley, Colorado

55. Alberto Jimenez (Ph.D. 2013)
Thesis Entitled: *Synthetic Studies on (-)-Lemonomycin: Construction of the Tetracyclic Core.*
Current Position: Industrial post-doctoral Research Associate at Emory Institute for Drug Development, Emory University.

56. Michelle Sanchez (Ph.D. 2014)
Thesis Entitled: *The Synthesis of the Pentacyclic Carbon Framework of the PF1270 Family of Natural Products.*
Current Position: Post-doctoral research associate at the University of Pennsylvania with Prof. Jeff Winkler, Philadelphia, Pennsylvania.

Former Postdoctoral Fellows of Robert M. Williams
Department of Chemistry, Colorado State University

1. Dr. Jen-Sen Dung (January 1982-October 1984)
Research Project: *Total Synthesis of Bicyclomycin and Bicyclomycin Analogs*
Current position: Research Scientist at Johnson Matthey Pharmaceuticals, West Deptford, N.J.

2. Dr. Kohtaro Tomizawa (December 1984 - December 1986)
Research Project: *Mechanism of Action of Bicyclomycin*
Current position: Associate Professor at Suzuka College of Tech. Shiroko-cho, Suzuka, Nie 510-02, Japan

3. Dr. Mark Kirms (January 1985 - April 1987)
Research Project: *Development of Synthetic Methodology for the Synthesis of Carbocycles*
Employed at Astronautics Laboratories, AL/LSX, Building 8451, Edwards, AFB, CA 93523
Current position: Associate Professor at Southern Oregon State College

4. Dr. Dongguan Zhai (April 1985 - November 1986)
Research Project: *Practical Synthesis of the Williams Amino Acid Templates and the Asymmetric Synthesis of α-Amino Acids*
Current position: Research Professor, at the Chinese Academy of Sciences, Chengdu Institute of Organic Chemistry, Chengdu, PRC

5. Dr. Tomasz Glinka (April 1985 - February 1987 and April 1989 - November 1990)
Research Project: *Asymmetric total synthesis of Brevianamide B*
Research Scientist at the Polish Academy of Sciences, Warsaw, Poland 1987-1989
Current position: Independent consultant

6. Dr. Eduard J. Brunner (April 1987 - November 1987)
Research Project: *Total Synthesis of Verruculotoxin*
Current position: Production Chemist at Novartis Widhagweg, Kaiseraugst, Switzerland

7. Dr. Byung H. Lee (September 1985 -January 1988)
Research Project: *Synthesis of the First [1,3]-Bridged β-Lactam*
Current position: Research Scientist in Drug Discovery at Pfizer, Kalamazoo, Michigan

8. Dr. Weixu Zhai (December 1985 -December 1987)
Research Project: *Methods Development for the Asymmetric Synthesis of α-Amino Acids and Peptide Isosteres.*
Associate Professor at the Lanzhou Institute of Chemical Physics, Academia Sinica, Chinese Academy of Sciences, Lanzhou, Gansu, PRC
Current position: Research Scientist at Bristol-Myers Squibb PRI, Wallingford, Connecticut.

9. Dr. Ewa Kwast (April 1987-October 1988)
Research Project: *Asymmetric total synthesis of Brevianamide B*
Current position: Translator for the Polish Government

10. Dr. Andrejz Kwast (April 1987-October 1988)
Research Project: *Development of Synthetic Methodology to Synthesize Bicyclomycin Analogs*
Current position: Research Scientist at the Polish Academy of Sciences, Institute of Organic Chemistry

11. Dr. Daimo Chen (April 1987-April 1989 and September 24, 1999 - December 8, 2000)
Research Project: *Practical Synthesis of the Williams Lactone and Synthesis of TAN-1057 Analogs*
Current position: Research Scientist at the Chinese Academy of Sciences, Chengdu Institute of Organic Chemistry)

12. Dr. Maria Wudlikow (April 1989 - November 1990)
Research Project: *Asymmetric Total Synthesis of Brevianamide B*
Current position: Research Scientist in Drug Discovery at Essential Therapeutics Co., Mountainview, CA

13. Dr. Mark Sabol (October 1987-August 1989)
Research Project: *Total Synthesis of Carbabicyclomycin*
Current position: retired

14. Dr. David J. Aldous (February 1988-October 1989)
Research Project: *Asymmetric Synthesis of α-Amino Acids via [1,3]-Dipolar Cycloadditions; Synthesis of Ethynylglycine*
Current position: Director of Drug Discovery at Sanofi-Aventis Pharma, Bridgewater, N.J.

Former Postdoctoral Fellows of Robert M. Williams (continued)

15. Suzanne C. Aldous (July 1989-October 1989)
Research Project: *Asymmetric Synthesis of α-Amino Acids via [1,3]-Dipolar Cycloadditions; Synthesis of Ethynylglycine*
Current position: Research Scientist in Drug Discovery at Sanofi-Aventis Pharmaceutical Co., Bridgewater, N.J.

16. Dr. Gyoosoon Park (October 1988-February 1, 1990)
Research Project: *Studies on the Total Synthesis of Quinocarcin and Quinocarcin Analogs*
Current position: Professor of Chemistry at Kookmin University, Seoul, Korea)

17. Dr. Hee-do Kim (November 1989-September 1990)
Research Project: *Total Synthesis of Carbabicyclomycin*
Current position: Assistant Professor at Soak Myoung Woman's University, School of Pharmacy, Seoul, Korea

***18. Dr. Nobuyoshi Yasuda** (July 1988 - August 1989)
Research Project: *Approach to the Total Synthesis of FR900482*
Current position: Senior Investigator, Process Research & Development at Merck & Co. Rahway, NJ

19. Dr. Norbert Richter (February 1991-August, 1991)
Research Project: *Development of Synthetic Methodology for the Asymmetric Synthesis of Amino Acids*
Current position: Research Scientist at Boehringer Mannheim, Germany

***20. Dr. Mary Dosch-Doubleday** (July 1991-December 3, 1992)
Research Project: *Display of Phage Combinatorial Libraries*
Merck Postdoctoral Fellow (1991-1993)
Current position: Research Scientist at Protarga Co., Exton, Pennsylvania

***21. Dr. Yusuke Amino** (July 1991-August, 1993)
Research Project: *Asymmetric Total Synthesis of Actinoidic Acid*
Current position: Research Scientist in Drug Discovery at Ajinomoto Co., Japan

22. Dr. Pierre-Jean Colson (March 1, 1994 - August 31, 1994)
Research Project: *Asymmetric Synthesis of Hydroxymethylene Peptide Isosteres and a Total Synthesis of Statine*
Current position: Research Scientist in Drug Discovery at G.D. Searle Co., Skokie, Illinois

***23. Dr. Matt A. Peterson** (December 1, 1992 - December 1, 1994)
Research Project: *Display of Phage Combinatorial Libraries*
**NIH Postdoctoral Fellow, Colorado State University, 1992-1994*
Current position: Associate Professor of Chemistry, Brigham Young University, Provo, Utah

***24. Dr.Monica Baloga** (June 1, 1994-May 31, 1995)
Merck Postdoctoral Fellow, Colorado State University 1994-1995
Research Project: *Display of Phage Combinatorial Libraries*
Current position: Assistant Professor at Florida Institute of Technology, Melbourne, Florida

25. Dr. Jiwen Liu (October 29, 1996 - October 15, 1997)
Research Project: *Asymmetric Synthesis of 2,7-Diaminosuberic Acid*
Current position: Research Scientist in Drug Discovery at Amgen, Inc., SanFrancisco, California)

26. Dr. Claude Quesnelle (September 23, 1996 -July 17, 1998)
Research Project: *Total Synthesis of Lightly Oxygenated Natural Taxoids*
Current position: Research Scientist at Bristol-Myers Squibb Pharmaceutical Co., New Jersey

27. Dr. Florenci V. Gonzalez Adelantado (July 8, 1998 - October 30, 1998, Visiting Professor from Spain)
Research Project: *Total Synthesis of Asperparaline A*
Current position: Assistant Professor at the University of Jaume, Campus de Borriol, Spain

28. Dr. David Hennings (December 1, 1997 - November 30, 1999)
Research Project: *Asymmetric Total Synthesis of Mureidomycin*
Current position: Research Scientist at Array Biopharma Co., Boulder, Colorado

29. Dr. Jetze Tepe (January 9, 1998 - July 12, 2000)
Research Project: *Synthesis of Photoactivated Progenitors of Dehydromonocrotaline*
Current position: Associate Professor at Michigan State University

30. Dr. Alfredo Vazquez (March 1, 1999 - August 15, 2000)
Total Synthesis of Lightly Oxygenated, Natural Taxoids, Asymmetric & Total Synthesis of Bioxalomycin α_2.
Current position: Assistant Professor at the University of Mexico

Former Postdoctoral Fellows of Robert M. Williams (continued)

***31. Dr. Hidekazu Tsujishima** (May 10, 1999 - March 31, 2000)
JSPS Fellowship at Colorado State University
Research Project: *Asymmetric Total Synthesis of Paraherquamide A*
Current position: Research Scientist at Tanabe Pharmaceutical Co., Japan

***32. Dr. Masahiko Kinugawa** (October 4, 1999 - September 30, 2000)
Research Project: *Asymmetric Total Synthesis* of *Bioxalomycin α2*
Current position: Research Scientist at Kyowa Hakko Kogyo Pharmaceutical Co., Japan

33. Professor Juan F. Sanz-Cervera (June 24, 1996 - August 31, 1996; July 9, 1997 - September 12, 1997; July 8, 1998 - September 11, 1998; June 10, 1999 - September 25, 1999; June 1, 2000 - September 25, 2000; February 1, 2001 - September 15, 2001 Associate Professsor from the University of Valencia)
Research Project: *Asymmetric Total Synthesis of (+)-Paraherquamide B, Biomimetic Total Synthesis of Brevianamide B, Biomimetic Total Synthesis of VM55599, Biosynthesis of Paraherquamide A, Brevianamide B, Austamide and VM55599.*
Current position: Professor at the University of Valencia, Spain.

34. Dr. Yutaka Aoyagi (February 18, 1997 - August 12, 1998)
Research Project: *Asymmetric Synthesis of Amino Acids and Peptide Isosteres*
Current position: Professor, College of Pharmacy, Kinjo Gakuin University, 2-1723 Omori, Moriyama-ku, Nagoya

35. Dr. Rajendra P. Jain (July 3, 2000 – June 28, 2002)
Research Project: *Asymmetric Synthesis of Amino Acids and Peptide Isosteres*
Current position: Associate Director, Medivation, Inc. India.

***36. Dr. Kosuke Namba** (April 26, 2001-May 1, 2003)
Research Project: *Asymmetric Total Synthesis of Palau'amine*
**JSPS Postdoctoral Fellow*
Current position: Professor at Tokushima University, Tokushima, Japan.

37. Dr. Sammy Metobo (Ph.D. from Rutgers University with Prof. Leslie S. Jimenez)
Research Project: *Asymmetric Total Synthesis of Bioxalomycin α2*
Current position: Research Scientist at Gilead Sciences, California

38. Dr. Rhona J. Cox (Ph.D. from Oxford University, UK, with Prof. Sir Jack E. Baldwin)
Research Project: *Biomimetic Total Synthesis of Paraherquamide A*
Current position: Research Scientist at Astra-Zeneca Pharmaceutical Co., Loughborough, UK

39. Dr. Mick Grady (Ph.D. from The University of Bristol with Prof. Kevin I. Booker-Milburn)
Research Project: *Asymmetric Total Synthesis of TMC-95A-D Analogs*
Current position: Research Scientist at Rhodia ChiRex, UK

***40. Dr. Makoto Mori** (Ph.D. from Gifu University)
Research Project: *Asymmetric, Stereocontrolled Total Synthesis of Quinine*
Current position: Research Scientist at Sankyo Pharmaceutical Co., Japan

41. Dr. Kim Dastlik (Ph.D. from Murdoch University, Australia)
Research Project*: Asymmetric Synthesis of Amino Acids*
Current position: unknown

***42. Dr. Tomoyuki Onishi** (PhD from Tokyo Institute of Technology)
Research Project: *Asymmetric Synthesis of Spirotryprostatin A and the Asymmetric Synthesis of Peptide Isosteres*
Current position: Research Scientist at Ajinomoto Co., Japan

43. Dr. Guiru Zhang (Ph.D. from Vanderbilt University with Prof. Ned Porter)
Research Project: *Asymmetric Total Synthesis of Mitomycin C*
Current position: Research Scientist at Procter & Gamble Co., Cincinnati, Ohio

44. Dr. Uta Sundermeier (Ph.D. from the Institut für organische Katalyseforschung an der Universität Rostock, Germany with Prof. Matthias Beller)
Research Project*: Asymmetric Synthesis of styloguanidine*
Current position: Research Scientist at Henkel KGAA, Duesseldorf, Germany

Former Postdoctoral Fellows of Robert M. Williams (continued)

45. Dr. Alan Stewart (Ph.D. from Imperial College with Prof. Donald Craig)
Research Project*: Asymmetric Synthesis of Stephacidins A,B, avrainvillamide and paraherquamide F*
Current position: Postdoctoral fellow in Finland

***46. Dr. Kateri Ahrendt** (PhD, University of California, Berkeley with Profs. Jon Ellman and Robert Bergman).
Research Project: *Asymmetric Synthesis of Nakadomarin.*
**NIH post-doctoral fellow*
Current Position: Associate Professor at Regis College, Denver, Colorado

47. Dr. Luke Adams (Ph.D. from the University of Bristol, England with Prof. Russell J. Cox)
Research Project: *Asymmetric Total Synthesis of Asperparaline A*
Current Position: Research Scientist at Cellaura Co., Nottingham, UK

48. Dr. Pascal Ducept (Ph.D. from Imperial College, London with Prof. Donald Craig)
Research Project: *Synthesis of Biological Probes Based on FR900482*
Current Position: unknown

***49. Dr. Tohru Horiguchi** (Ph.D. from Tohoku University, Japan with Prof. Kyouzou Suyama).
Research Project: *Synthesis of Taxol Biosynthetic Intermediates.*
**JSPS Postdoctoral Fellow*
Current Position: Research Associate at Nagase & Co., LTD, Japan

***50. Dr. Jonathan Lane** (Ph.D. from the University of Colorado, Boulder, Colorado with Prof. Randall Halcomb).
Research Projects: *Asymmetric Total Synthesis of Jorumycin, Renieramycin G, Saframycin A and Communesin.*
**NIH post-doctoral fellow*
Current Position: Research Scientist at Array BioPharma, Boulder, Colorado

***51. Dr. Yasuo Noguchi** (Ph.D. from Tokyo University with Prof. Susumu Kobayashi).
Research Project: *Total Synthesis of FK228.*
**Sponsored by Sankyo Co., Japan*
Current Position: Research Scientist at Sankyo Co., Japan

52. Dr. Hidenori Namiki (Ph.D. from Hoshi University with Prof. Toshio Honda).
Research Project: *Asymmetric Synthesis of Biosynthetic Intermediates for FR900482 and Mitomycin C*
Current Position: Research Scientist at Sankyo Co., Japan

***53. Dr. Konrad Sommer** (Ph.D. from the University of Göttingen, Germany with Prof. Lutz Tietze).
Research Project*: Asymmetric Synthesis of Paraherquamide F and Paraherquamide Biosynthetic Intermediates.*
Current Position: Research scientist at Carbogen Amcis, Switzerland.

***54. Dr. Gerald D. Artman III.** (Ph.D. from Pennsylvania State University with Prof. Steven M. Weinreb)
Research Project: Asymmetric Total Synthesis of Stephacidin A, Stephacidin B and Notoamide B.
**NIH post-doctoral fellow*
Current Position: Research Scientist at Kalexsyn, Inc., Kalamazoo, Michigan.

55. Dr. Esther González Cantalapiedra (Ph.D. from the Universidad Autónoma de Madrid with Prof. Antonio Echavarren)
Research Project: *Asymmetric Total Synthesis of Nakadomarin A*
Current Position: Research Scientist at the Medicinal Chemistry Department at the Spanish National Cancer Research Centre (Centro Nacional de Investigaciones Oncologicas-CNIO) in Madrid.

56. Dr. Guillaume Vincent (Ph.D. from Laboratoire de Synthese et Methodologie Organiques Universite Claude Bernard Lyon with Prof. Marco Ciufolini)
Research Project: *Asymmetric Total Synthesis of Cribrostatin-4*
Current position: *"Chargé de Recherche"* CNRS at the Université Paris-Sud XI at Orsay

***57. Dr. Deidre M. Johns** (Ph.D. from the University of Colorado, Boulder, Colorado with Prof. Tarek Sammakia).
Research Project: *Asymmetric Total Synthesis of Quinine and Antimycin A3.*
**American Cancer Society post-doctoral fellow*
Current Position: Research Assistant Professor at the Oregon State University

58. Dr. Dan Fishlock (Ph.D. from the University of Waterloo with Prof. Eric Fillion)
Research Project: *Asymmetric Total Synthesis of Ecteinascidin 743*
Current Position: Research Scientist at Hoffman-La Roche, Inc., Basel, Switzerland.

Former Postdoctoral Fellows of Robert M. Williams (continued)

59. Dr. Stephen Chamberland (Ph.D. from the University of California, Irvine with Prof. Keith Woerpel)
Research Project: *Synthesis of isotopically labeled mitomycin C and FR900482 biosynthetic intermediates*
Current Position: Assistant Professor of Chemistry, Central Washington University

60. Dr. Thomas J. Greshock (Ph.D. from Pennsylvania State University with Prof. Raymond L. Funk)
Research Project: *Total Synthesis of FK228;Total Synthesis of Stephacidins A&B; Total Synthesis of Palau'amine*
Current Position: Research Scientist at Merck & Co., West Point, Pennsylvania

61. Dr. Aaron Smith (Ph.D. from the University of North Carolina at Chapel Hill with Prof. Michael Crimmins)
Research Project: *Asymmetric Total Synthesis of Quinine and Oligonucleotide Silencing of Gene-Shuffling in Cancer Cells*
Current Position: Research Scientist at Pfizer, Inc., Groton, Connecticut

***62. Albert A. Bowers** (Ph.D. from the University of Illinois, Chicago, with Prof. David Crich)
Research Project: *Total Synthesis of Largazole and Related HDAC Inhibitors*
Current Position: Assistant Professor at University of North Carolina, Chapel Hill

63. Dr. Hui Li (Ph.D. from the University of Texas at Austin with Prof. Stephen H. Martin)
Research Project: *Asymmetric Total Synthesis of Palau'amine and Taxol Biosynthetic Intermediates*
Synthesis of Inducers of Fetal Hemoglobin (HemaQuest Pharmaceuticals)
Current Position: Research Scientist at Anichem

64. Dr. Carolyn Selenski (Ph.D. from the University of California, Santa Barbara with Prof. Thomas R.R. Pettus)
Research Project: *Asymmetric Total Synthesis of Bioxalomycin and Lemonomycin*
Current Position: Research Scientist at Glaxo SmithKline, Research Triangle Park, North Carolina

65. Dr. Kenny Miller (Ph.D. from the University of Texas at Austin with Prof. Stephen H. Martin)
Research Project: *Asymmetric synthesis of Versicolamide B and related prenylated indole alkaloids; synthesis of largazole analogs and other HDAC inhibitors.*
Current Position: Assistant Professor at Fort Lewis College, Durango, Colorado.

66. Dr. Takeshi Yamada (Ph.D. from Osaka City University, Japan with Prof. Yasufumi Ohfune)
Research Project: *Asymmetric Total Synthesis of Nakadomarin A and Et-743*
Current Position: Assistant Professor at the Kitasato Institute, Japan with Prof. Sunazuka

67. Dr. Cameron Burnett (Ph.D. from Colorado State University, with Prof. Robert M. Williams)
Research Project: *Synthesis of Adjuvants* (Infectious Diseases Research Institute)
Current Position: Instructor for the U.S. Naval Academy

***68. Dr. Makoto Inai** (Ph.D. from Shizouka University, Japan with Prof. Toshiyuki Kan)
Research Project: *Asymmetric Total Synthesis of Palau'amine and Et-743*
Current Position: Assistant Professor, Tokushima Bunri University, Tokushima, Japan
**Uehara Foundation Post-doctoral Fellow*

69. Dr. Ludwig Kaspar (Ph.D. at Ludwig-Maximilians-University, Munich with Prof. Dr. Lutz Ackermann)
Research Project: *Synthesis of Adjuvants*
Current Position: Research Scientist at HWR Chemie, Germany

70. Dr. Michael A. Christiansen (Ph.D. from Brigham Young University with Prof. Merit Andrus)
Research Project: Asymmetric Total *Synthesis of Et-743 and the Synthesis of Et-743 Biosynthetic Intermediates.*
Current Position: Assistant Professor at Utah State University

71. Dr. Sumit Dey (Ph.D. from Indian Institute of Chemical Biology, West Bengal, India, with Prof. P.Jaisankar)
Research Project: *Synthesis of Inducers of Fetal Hemoglobin* (HemaQuest Pharmaceuticals); *Synthesis of HDAC Inhibitors; Synthesis of Adjuvants.*
Current Position: currently unemployed

72. Dr. James E. Sunderhaus (Ph.D. from the University of Texas at Austin with Prof. Stephen H. Martin)
Research Project: Total Synthesis of Notoamides and Stephacidins.
Current Position: Generic Pharmaceutical

73. Dr. Jennifer M. Finefield (Ph.D. from Colorado State University, with Prof. Robert M. Williams)
Research project: Synthesis of new HDAC inhibitors.
Current Position: Indiana University, Technology Transfer Officer

Former Postdoctoral Fellows of Robert M. Williams (continued)

74. Dr. Santhosh Reddy Jangari (Ph.D. from the Indian Institute of Chemical Technology, with Prof. B. Venkateswara Rao)
Research Project: *Synthesis of Adjuvants, pkC-δ Inhibitors.*
Current position: unemployed

75. Dr. Masashi Yokoya (Ph.D. from Meiji Pharmaceutical University, Japan with Prof. Naoki Saito)
Research project: *Synthesis of Et743 and Aclindomycin*
Current position: Assistant Professor at Meiji Pharmaceutical University, Japan

76. Dr. Dane Clausen (Ph.D. from the University of Pittsburgh, with Prof. Paul E. Floreancig)
Research Project: Synthesis of Largazole Analogs
Current position: Research Scientist at Merck & Co., Rahway, New Jersey

77. Dr. Amber Somoza (Ph.D. from the University of Southern California, with Prof. Clay C.C. Wang)
Research project: *Synthesis and Biosynthesis of Prenylated Indole Alkaloids*
Current Position: Research Scientist at Gilead Pharmaceuticals, San Dimas, California

78. Dr. Jiyu Wang (Ph.D. from Chengdu Institute of Organic Chemistry)
Research Project: Synthesis of Largazole Analogs & PKC-δ Inhibitors
Current Position: Professor at the Chengdu Institute of Organic Chemistry, Chengdu, China

***79. Dr. Kazutada Ikeuchi** (Ph.D. from Shizuoka University with Prof. Toshiyuki Kan)
Research Project: *Total Synthesis of Citrinalin*
Current Position: Assistant Professor of Chemistry, Kwansei Gaikun University, Japan

**Post-docs with their own fellowship support or corporate sponsor.*

Current Research Group of Robert M. Williams
Department of Chemistry, Colorado State University

Current Graduate Students

1. Vy Le (Ph.D. expected 2015)
Research Project: *Synthesis of Et-743*

2. Nathan Bair (Ph.D. expected 2015)
Research Project: *Synthesis of Okaramine M*

3. Christine Dunne (Ph.D. expected 2019)
Research Project: (not yet assigned)

4. Jonathan Thielman (Ph.D. expected 2019)
Research Project: (not yet assigned)

5. Kimberly Klas (Ph.D. expected 2019)
Research Project: (not yet assigned)

Current Post-doctoral Associates

1. Dr. Le Zhao (Ph.D. from Tohoku University with Prof. Masahiro Hirama)
Research Project: Synthesis of Largazole Analogs

Former Co-workers of Robert M. Williams in Academia:

Robert W. Armstrong	University of California at Los Angeles, U.S.A. (adjunct)
Scott R. Rajski	University of Wisconsin, Madison, U.S.A.
Jetze Tepe	Michigan State University, U.S.A.
Matt A. Peterson	Brigham Young University, U.S.A.
Lynn Maruyama-Kirms	Southern Oregon State University, U.S.A.
Christopher Sean Esslinger	University of Montana, U.S.A. (deceased)
Jennifer Travers	Oregon State University, U.S.A.
Ryan E. Looper	University of Utah
Monica Baloga	Florida Institute of Technology, U.S.A.
Kohtaro Tomizawa	Suzuka College of Technology, Japan
Gyoosoon Park	Kookmin University, Korea
Dongguan Zhai	Chengdu Institute of Organic Chemistry, China
Daimo Chen	Chengdu Institute of Organic Chemistry, China
Hee-do Kim	Soak Myoung Woman's University, Korea
Florenci V. Gonzalez Adelantado	University of Jaume, Spain
Alfredo Vazquez	University of Mexico, Mexico
Juan F. Sanz-Cervera	University of Valencia, Spain
Yutaka Aoyagi	Kinjo Gaikuin University, Japan
Stephen Chamberland	Central Washington University
Kenny Miller	Fort Lewis College, Colorado
Brandon English	Red Rocks Community College
Tenaya Newkirk	Colorado State University
Takeshi Yamada	Kitasato Institute, Japan
Makoto Inai	University of Shizuoka, Japan
Kosuke Namba	Tokushima University, Japan
Michael Christiansen	Utah State University
Deidre M. Johns	Oregon State University
Ryan E. Looper	University of Utah
Daniel Gubler	Brigham Young University
Kazutada Ikeuchi	Kwansei Gaikun University, Japan
Masashi Yokoya	Meiji Pharmaceutical University, Japan

Former Co-workers of Robert M. Williams in Industry:

Robert W. Armstrong	Eli Lilly Pharmaceutical Co. (retired as Vice President)
Andrew O. Stewart	Abbott Laboratories (retired)
Peter J. Sinclair	Merck & Co. (retired)
Paul P. Ehrlich	Bayer, AG
James A. Hendrix	Aventis Pharma
Glenn J. Fegley	Onconova Therapeutics, Inc.
Timothy D. Cushing	Amgen (retired)
Gregory F. Miknis	Array Biopharma; Colorado State University C2D2 Program
Mark E. Flanagan	Pfizer, Inc.
Steven M. Rubenstein	Albany Molecular Co.
Chester C. Yuan	Amgen
David M. Bender	Eli Lilly Pharmaceutical Co.
Brad Herberich	Amgen, Inc.
Jeffrey Cao	Merck & Co.
Jack D. Scott	Merck & Co.
Emily M. Stocking	R.W. Johnson Pharmaceutical Research Institute
Christi Kosogof	Abbott Laboratories
Paul R. Sebahar	Myriad Pharmaceuticals
Steven Lenger	Array BioPharma
Duane E. DeMong	Merck & Co.
Jen-sen Dung	Johnson Matthey
Tomasz Glinka	Rempex Pharmaceuticals
Eduard J. Brunner	Novartis
Byung H. Lee	Pfizer, Inc.
Maria Wudlikow	Essential Therapeutics

Mark Sabol	Dow Chemical Co.
David J. Aldous	Aventis Pharma
Suzanne C. Aldous	Aventis Pharma
Nobuyoshi Yasuda	Merck & Co.
Norbert Richter	Boehringer Mannheim AG
Mary Dosch-Doubelday	Bristol-Myers Squibb Co.
Yusuke Amino	Ajinomoto Co.
Pierre-Jean Colson	Pfizer, Inc.
Jiwen Liu	Amgen, Inc.
Claude Quesnelle	Bristol-Myers Squibb Pharmaceutical Co.
David D. Hennings	Array Biopharma
Hidekazu Tsujishima	Tanabe Pharmaceutical Co.
Masahiko Kinugawa	Kyowwa-Kirin Co.
Rhona J. Cox	Astra-Zeneca Pharmaceutical Co.
Tomoyuki Onishi	Ajinomoto Co.
Guiru Zhang	Procter & Gamble Co.
Uta Sundermeier	Henkel KGAA (Duesseldorf, Germany)
Jonathan Lane	Array BioPharma
Hidenori Namiki	Daiichi Sankyo Co.
Yasuo Noguchi	Daiichi Sankyo Co.
Meriah W.N. Valente	Bristol-Myers Squibb Co.
Brian K. Albrecht	Constellation Pharmaceuticals
Alan R. Grubbs	Ardea Biosciences
Gerald D. Artman III	Kalexsyn, Inc.
Dan Fishlock	Hoffman-La Roche, Inc.
Deidre M. Johns	Eli Lilly
Aaron Smith	Pfizer, Inc.
Thomas J. Greshock	Merck & Co.
Carolyn Selenski	Glaxo SmithKline
Andrea Geiser	Merck & Co.
Ann E. Troutman	Merck & Co.
Ludwig Kaspar	HWR Chemie
Dane Clausen	Merck & Co.

Collaborations and Biological Support. We have ongoing formal collaborations with the following laboratories around the world that are relevant to numerous ongoing research efforts.

Collaborator	Institution	Role on Project/Expertise
Prof. James E. Bradner	Dana-Farber Cancer Research Inst.	Biology & biochemistry of HDAC inhibitors. NIH sub-contractor.
Prof. Kimberly Stegmaier	Dana-Farber Cancer Research Inst.	Biology & biochemistry of HDAC inhibitors. NIH sub-contractor.
Prof. Olaf Wiest	University of Notre Dame	Design & conformational properties, protein binding of HDAC inhibitors. NIH sub-contractor.
Prof. David Sherman	University of Michigan	Biosynthesis of secondary metabolites from microorganisms and plants; Meta-omics, biosynthetic gene identification, cloning and expression. NIH sub-contractor.
Prof. Sachiko Tsukamoto	Kumamoto University, Japan	Biosynthesis of stephacidins, notoamides
Prof. Douglas V. Faller	Boston University Medical Center	Inhibitors of Protein Kinase C-δ
Prof. Susan M. Perrine	Boston University Medical Center	Development of drugs for hemoglobinopathies
Dr. William V. Williams	Incyte & Sapientia Therapeutics	Inhibitors of Protein Kinase C-δ
Prof. Chris Walsh	Harvard Medical School	Biosynthesis of the kutznerides
Prof. Stuart L. Schreiber	Harvard University The Broad Institute of Harvard & MIT	Cytotoxicity & mode of action of HDAC inhibitors
Prof. Douglas Thamm	Colorado State University Animal Cancer Center	Cytotoxicity of HDAC inhibitors, anti-cancer agents
Prof. Dan Gustafson	Colorado State University Animal Cancer Center	Pharmacokinetics of HDAC inhibitors
Prof. Hiroaki Suga	University of Tokyo	Preparation of HDAC inhibitor libraries
Dr. James R. Berenson, M.D.	Institute for Myeloma and Bone Cancer Research, Los Angeles	HDAC inhibitors for treating multiple myeloma
Prof. George Stamatoyannopoulos	Markey Molecular Medicine Center University of Washington	HDAC inhibitors as erythropoiesis agents.
Dr. Jens C. Frisvad	Technical University of Denmark	Fungi that produce prenylated indole alkaloids.
Prof. Ian Orme	Colorado State University	Synthesis of drugs that inhibit the growth of drug-resistant *Mycobacteria tuberculosis*
Prof. Rod Croteau	Washington State University	Taxol biosynthesis: genetics, cloning, *in vivo* incorporation, metabolite identification, biotransformation, enzymology.
Prof. Juan F. Sanz-Cervera	University of Valencia (Spain)	Visiting scholar: structure determination, synthetic co-worker biosynthesis.
Prof. Hideo Hayashi	Osaka Prefecture University (Japan)	Asperparaline A biosynthesis in *Aspergillus japonicus* strains.
Professor Karolin Luger	Colorado State University	Interaction of FR900482 with nucleosome core particles.
Professor Raymond Reeves	Washington State University	High Mobility Group A1 (HMG A1) oncoprotein interactions with FR900482 and related antitumor agents.
Professor Yutaka Aoyagi	Kinjo Gakuin University	Asymmetric synthesis of amino acids.
Dr. Tomoyuki Onishi	Ajinomoto Co. (Japan)	Asymmetric synthesis of important chiral compounds.
Prof. James B. Gloer	University of Iowa	Biosynthesis of sclerotiamide, versicolamide
Dr. Hiroyuki Osada	RIKEN Institute (Japan)	Cytotoxicity and mammalian cell cycle inhibition assays; microtubule disruption.

Paper _____

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

STEADYMED LTD.,

Petitioner,

v.

UNITED THERAPEUTICS CORPORATION,

Patent Owner.

Case IPR2016-00006
U.S. Patent 8,497,393

DECLARATION OF ROBERT R. RUFFOLO, Jr., Ph.D. IN SUPPORT OF PATENT OWNER RESPONSE TO PETITION

TABLE OF CONTENTS

I have been retained by the law firm of Wilson Sonsini Goodrich & Rosati ("WSGR") as an expert consultant to United Therapeutics Corporation ("UTC") in connection with the above-identified matter to provide expert testimony concerning U.S. Patent No. 8,497,393 ("the '393 patent", Ex. 1001) by Batra *et al.*, entitled "Process to prepare treprostinil, the active ingredient in Remodulin®," issued on July 30, 2013. At the request of Counsel for UTC, I hereby submit this expert declaration.

I. Qualifications and Background

A. Education and Experience

1. I am the retired (as of 2008) President of Research and Development for Wyeth Pharmaceuticals (now Pfizer Inc.) and Corporate Senior Vice President of Wyeth (now Pfizer Inc.). I am currently Managing Director of Ruffolo Consulting, LLC, a consulting company serving the pharmaceutical and biotechnology industries.

2. I have studied, researched, taught (in medical and pharmacy schools), worked and managed all aspects of the pharmaceutical drug discovery and development fields for over 35 years. I received my Bachelor of Science (B.S.) degree in Pharmacy (*summa cum laude,* and *With Distinction*) in 1973 from The Ohio State University, and was licensed to practice Pharmacy in 1973. I received my Doctor of Philosophy (Ph.D.) degree in Pharmacology in the fields of autonomic and cardiovascular pharmacology in 1976 also from The Ohio State University. My doctoral research included the areas of drug-receptor interactions, autonomic pharmacology, cardiovascular pharmacology, adrenergic drugs, stereochemistry and the study of the stereochemical aspects of adrenergic drugs and their receptors. During the period of my undergraduate and graduate education, I authored or co-authored a number of peer-reviewed research articles describing that work.

3. Upon earning my Ph.D. degree, I remained at The Ohio State University as a Postdoctoral Fellow for six months, and extended my research on drug-receptor interactions and drug-receptor theory. From 1977-1978, I worked as a Staff Fellow and Postdoctoral Fellow [Pharmacology Research Associate Training (PRAT) Fellow] at the National Heart Lung and Blood Institute of the National Institutes of Health (NIH) in the laboratory of Dr. Marshall Nierenberg (Nobel Laureate for breaking the genetic code), where my research focused on neurobiology, and in particular on synapse formation in brain, spinal cord and skeletal muscle.

4. In 1978, I began my independent career in the pharmaceutical industry at Eli Lilly & Company as Senior Pharmacologist in the Department of Cell Biology. I subsequently became Senior Pharmacologist in the Department of Cardiovascular Pharmacology in 1981, and was promoted to Research Scientist in 1982. I then became Chairman of the Cardiovascular Research Committee in 1983, where I continued my research in cardiovascular pharmacology, adrenergic drugs, drug-receptor theory, stereochemistry and the stereochemical basis of drug action. My work also expanded into the area of structure-activity relationships and drug design. Shortly after joining Eli Lilly & Company, I was also assigned to supervise a medicinal chemistry laboratory that was dedicated to my work in stereochemistry and structure-activity relationships, and which I personally directed. While working at Eli Lilly & Company, I was credited with discovering the complex mechanism of action of the newly marketed drug for the treatment of acute congestive heart failure, dobutamine (Dobutrex®), which involved the complex interplay of the different pharmacological activities of both enantiomers of the drug, each acting on multiple adrenergic receptors and their subtypes..

5. In 1984, I joined SmithKline Beckman Pharmaceuticals (now GlaxoSmithKline PLC) as Director of Cardiovascular Pharmacology, where I continued my work in cardiovascular

pharmacology, adrenergic drugs, drug-receptor theory, stereochemistry, the stereochemical basis of drug action, structure-activity relationships and drug design. As Director of the Department of Cardiovascular Pharmacology, I supervised a staff of approximately 40 researchers and scientists in the field of cardiovascular drug discovery and development. Throughout my tenure at SmithKline Beckman Pharmaceuticals (and its subsequent corporate identities that changed through mergers and acquisitions), I also maintained my own laboratory and conducted studies on the pharmacology of cardiovascular drugs, drug-receptor interactions, adrenergic pharmacology, stereochemistry, the steric aspects of drug action, and structure-activity relationships related to new drug discovery.

6. I remained at SmithKline Beckman Pharmaceuticals (and its subsequent corporate identities) for approximately 17 years, over which time I rose to the position of Senior Vice President and Director of Biological Sciences Worldwide, where I was responsible for a staff of approximately 500 scientists. During my last year at the company, I became the Senior Vice President and Director of all Discovery Research for the Corporation Worldwide, which included all of the areas of Biological Sciences, Chemical Sciences, Medicinal Chemistry, Physical Chemistry, Process Chemistry, Molecular and Cellular Biology, and Genetics, with responsibility for a staff of approximately 1,700 scientists and an annual budget of approximately $1.2 billion.

7. It was during my tenure at SmithKline Beckman Pharmaceuticals (and its subsequent corporate identities) that I was personally responsible for the discovery and subsequent development of Coreg® (carvedilol) for the treatment of chronic congestive heart failure, for which I was awarded the *Discoverers Award* in 2008 by the Pharmaceutical Research and Manufacturers Association (PhRMA), which is the major trade association for the

pharmaceutical industry and is comprised by Industry CEOs and Senior Executives, as well as a group of my peers (i.e., Presidents of R&D). Coreg® revolutionized the treatment of chronic congestive heart failure by markedly reducing death, hospitalization and morbidity from this devastating disease. Coreg® is now the "standard of care" for the treatment of congestive heart failure. The FDA approved Coreg® in 1997, after more than 10 years of research and development work that I researched and personally led, and the drug is currently prescribed globally to treat congestive heart failure. The drug has saved tens of millions of lives throughout the world.

8. Also during my tenure at SmithKline Beckman Pharmaceuticals (and its subsequent corporate identities) beginning in 1984, I personally led and managed the discovery of ropinirole (Requip®) for the treatment of Parkinson's disease. Ropinirole is a highly selective dopamine DA2 receptor agonist. Ropinirole was approved by the FDA in 1997 for the treatment of the signs and symptoms of Parkinson's disease, both as monotherapy and as adjunctive treatment in combination with Levodopa.

9. Also during my tenure at SmithKline Beecham Pharmaceuticals (and its subsequent corporate identities), I personally initiated and led the Angiotensin II Receptor Antagonist Program, and I was personally involved in the discovery and development of the marketed angiotensin II receptor antagonist, eprosartan mesylate (Teveten®), which was approved by the FDA in 2001 for the treatment of hypertension.

10. As a result of my research at The Ohio State University, Eli Lilly & Company and SmithKline Beckman Pharmaceuticals (and its subsequent corporate identities), I gained considerable experience in all aspects of drug discovery and development. In addition, throughout this entire period, I maintained my own personal laboratories and conducted my own

independent research in cardiovascular pharmacology, drug-receptor theory, autonomic pharmacology, stereochemistry and the stereochemical requirements of drug action and structure-activity relationships. It was during this period that my laboratory was the first to discover that three subtypes existed for both alpha-1 and alpha-2 adrenoceptors, which was subsequently proven to be correct when the human genome was sequenced a decade later, confirming indeed that three subtypes existed for each of these two adrenoceptor subtypes. My personal laboratory also collaborated with many internationally recognized scientists and their laboratories throughout the world. In addition, I have been invited to lecture at international symposia and at leading research institutions and hospitals around the world on most areas of my research.

11. In 2000, I assumed the positions of Executive Vice President of Research and Development at Wyeth Pharmaceuticals as well as Corporate Vice President, and I was appointed to the Corporate Management Committee and the Board of Directors (as a non-voting member), both of which were chaired by the CEO. Eighteen months later, I was promoted to the positions of President of Research and Development, as well as Corporate Senior Vice President, and I was also appointed as Chair of the Science Subcommittee of the Board of Directors. I was responsible for a staff of approximately 7,000 employees globally, with an annual budget in excess of $3 billion. During this period, I was credited with changing the paradigm for drug discovery and development at Wyeth by markedly improving R&D productivity. This work has been highlighted in *BusinessWeek* magazine, and was the subject of a "Case Study" conducted by the Harvard Business School, which was published in the *Harvard Business Review* in 2007. The Harvard Business School "Case Study" has been covered extensively in business school textbooks, and is a commonly taught case study in many leading business schools throughout the

world, including the Harvard Business School, Wharton Business School, Columbia Business School, Duke University Business School and the London School of Economics. The re-engineering of Research and Development at Wyeth under my direction was also the subject of many articles appearing in major newspapers and trade journals globally. In my role as a scientist and senior pharmaceutical executive, I oversaw and managed each and every aspect of the pharmaceutical drug discovery and development processes. My areas of responsibility included Pharmacological Sciences, Biological Sciences, Biochemical Sciences, Medicinal Chemistry, Physical Chemistry, Molecular Modeling, Spectral Sciences, Pharmaceutics and Pharmaceutical Sciences, Drug Safety and Toxicology, Drug Metabolism, Clinical R&D (which included all clinical trials from Phase 1 through Phase 3), Regulatory Affairs [for FDA (U.S.), EMA (Europe), PMDA (Japan) and every regulatory agency in the world], Medical Affairs, Global Safety Surveillance and Epidemiology, Process Chemistry at the pilot plant and kilo plant levels, as well as the transfer of chemical processes to manufacturing scale, and Post-Marketing Research and Surveillance for all Wyeth drugs throughout their lifetimes on the market.

12. Following my retirement from Wyeth in 2008, I served for one year as a consultant to Wyeth Pharmaceuticals and Pfizer, Inc. Since then, I have been a consultant to most of the major large and mid-sized pharmaceutical companies and many biotechnology companies, as well as other industries outside of biomedical research, as Managing Director of Ruffolo Consulting, LLC. My consulting responsibilities include the areas of R&D Leadership, Leadership Development, Management of Scientific Innovators, Managing Innovation and Managing Organizational Change.

13. During my career as an executive in the pharmaceutical industry, both at SmithKline Beckman Pharmaceuticals (and its subsequent corporate identities) and Wyeth

Pharmaceuticals, I managed and oversaw the discovery and development of over two-dozen innovative new drugs that were approved by the FDA and other regulatory agencies around the world.

14. During my career, I have authored or co-authored nearly 500 full-length scientific publications, over 200 abstracts, and I have edited 17 books. I was founder and editor-in-chief of three international scientific journals, and have served on the editorial boards of 29 international scientific journals devoted to the fields of pharmacology, biochemistry, pharmaceutical sciences, medicinal chemistry, physical chemistry, analytical chemistry, stereochemistry and stereoselectivity of drugs. I have lectured extensively in scientific and industrial forums worldwide. I have also been invited to speak extensively on the topics of Pharmaceutical Research and Development Management, Research and Development Productivity, Organizational Change, Federal Regulation of Drug Approval and the Principles of Executive Leadership at national and international scientific and management meetings and symposia, and since my retirement, also as a consultant to most of the mid-sized and large pharmaceutical companies and many biotechnology companies..

15. I am a member of several professional organizations including the American Society for Pharmacology and Experimental Therapeutics (ASPET), the British Pharmacological Society, the International Union of Pharmacology (IUPHAR), where I was also Chairman of the Committee on Drug Receptor Nomenclature which was responsible for the naming of all drug receptors and ion channels worldwide, and the professional organization comprised of the international Presidents of Research & Development from large Pharmaceutical Companies (a group called "Hever"). I have served as an elected officer of many of these organizations.

16. I have received a number of prestigious awards for accomplishments throughout my career, including two *Lifetime Achievement Awards* (one from the Scrip Awards and the other from The Ohio State University; one of only three ever to be awarded), two *Honorary Doctorates* (one from the University of Catania, Italy, and the other from West Virginia University), *Chief Scientific Officer of the Year* (for being the best leader of R&D in the pharmaceutical and biotechnology industries), the *John Jacob Able Award*, the *Lorenzini Gold Medal for Biomedical Research*, and the *Prix Galien Special Commendation for Excellence and Innovation in Research* to name but a few. I was also the winner of *"The Great Oxford Debate"* at the world-renowned Oxford Union of Oxford University, UK. Recently, the American Society for Pharmacology and Experimental Therapeutics (ASPET) has established an annual award in my name to honor the contributions that I have made to drug discovery and development; the Award is entitled the "*Robert R. Ruffolo Career Achievement in Pharmacology Medal,*" which is awarded annually to the most prestigious scientists in the world at the height of their careers. The American Society for Information Science & Technology has designated me as a *Highly Cited Scientist* for being among the top 100 most cited Pharmacologists in the world for over two decades.

17. My *curriculum vitae* is submitted herewith as Ex. 2023.

II. Legal Standards Provided By Counsel

18. I have been informed by Counsel that because each claim defines a separate invention, the validity of each claim in a patent is addressed independently of the validity of the other claims in that patent.

19. I have also been informed by Counsel that the claims of the '393 patent are "product-by-process" claims. I have also been informed by Counsel that the "product" of

product-by-process claims include structural and functional differences that are present even if they are not explicitly claimed.

20. I understand from Counsel that, in addition to considering the prior art, certain objective indicia may also provide evidence that a claimed invention is not obvious. I am informed by Counsel that these objective indicia, which are also referred to as secondary considerations, may include factors such as commercial success, unexpected results, the resolution of long-felt, but previously unmet needs, skepticism by others prior to achieving the invention, failure of others to achieve the invention, praise from others for the invention, and copying by others.

21. I have been informed by Counsel that a patent is to be interpreted from the perspective of a hypothetical person referred to as the person of ordinary skill in the art ("POSA") to which the patent pertains. I have also been informed by Counsel that a determination of the level of ordinary skill is based on, among other things, the type of problems encountered in the art, prior art solutions to those problems, rapidity with which innovations are made, sophistication of the art, and the educational level of active workers in the field. I have been informed that in any particular case, every factor may not be present, and one or more factors may predominate. I understand the POSA is presumed to know all prior art that is reasonably relevant to the subject matter of the claimed invention.

22. I understand from Counsel that the validity of a patent claim must be assessed from the perspective of a POSA at the time of the invention.

23. I have reviewed Dr. Williams' Declaration (Ex. 2020) and his definition of a POSA with respect to the patent-in-suit and I agree with his opinion that a POSA would have had, at the time of the claimed invention, a doctorate degree in chemistry, pharmaceutics,

pharmaceutical sciences, medicine, or a related discipline. Alternatively, the POSA may have had a lesser degree in one of those fields, with correspondingly more experience. To the extent necessary, a POSA may have collaborated with others of skill in the art, such that the individual and/or team collectively would have had experience in synthesizing and analyzing complex organic compounds.

24. I understand that SteadyMed's expert, Dr. Winkler, in his declaration has opined that a POSA would have "a master's degree or a Ph.D. in medicinal or organic chemistry, or a closely related field. Alternatively, a person of ordinary skill would include an individual with a bachelor's degree and at least five years of practical experience in medicinal or organic chemistry." Ex. 1009 at ¶14.

25. My opinions in this declaration are expressed from the view of a POSA at the time of the priority date of the '393 patent. These opinions apply equally whether Dr. Williams' definition of a POSA or Dr. Winkler's is applied.

III. The '393 Patent

26. This case relates to a process to prepare an improved treprostinil product, the active ingredient in Remodulin®, as described in the '393 patent. As described in the '393 patent, treprostinil is prepared as an improved drug substance and active pharmaceutical ingredient (API) in a more pure form. The new preparation of treprostinil described in the '393 patent also has lower levels of impurities.

IV. Summary of Opinions

27. This report contains a statement of my present opinions and includes the bases and reasons therefore, and the data and other information that I have considered in forming these opinions. In this report, I offer herein my opinions on the importance of drug purity and

impurities, and on the improvements made in these properties as a result of the new preparation of treprostinil as described in this patent.

28. In forming my opinions, I have reviewed several documents, such as the documents cited by SteadyMed and UTC in this case, the '393 patent and its file history, as well as references that I have found through my own research. I have also based my opinions on my own extensive general knowledge, comprising nearly 40 years of experience, of the areas of pharmaceutical drug synthesis, production of API, manufacturing, formulation and preparation of final drug product.

29. If called to testify, I will, as needed, explain the principles and terminology used in this report, as well as in the materials referenced herein. I may use demonstrative aids and exhibits to illustrate these principles and the opinions expressed. I have not yet prepared any such demonstrative aids.

30. I may also testify or provide an opinion in rebuttal to testimony or opinions offered by other witnesses in response to the opinions stated herein. I reserve the right to supplement or otherwise amend my opinions.

31. It is my opinion that the invention of the '393 patent satisfied a long-felt unmet need by providing a commercial scale synthesis of treprostinil that results in a treprostinil product with higher overall purity and lower levels of individual impurities. As with all drug substances such as treprostinil, the FDA seeks to list, quantitate, and minimize impurities, and maximize the overall purity, of such drug substances as much as possible for the benefit of patients. The claimed invention of the '393 patent invention meets this need.

V. Background

A. The Importance of Purity in Pharmaceutical Preparations

32. The purity of a pharmaceutical drug substance, both active pharmaceutical ingredient (API) and final or finished drug product, is of the utmost importance to regulatory agencies, and especially the FDA. Accordingly, the first sentence of the Code of Federal Regulations (C.F.R.) Title 21, Part 610, Subpart B, Section 610.13 is *"Products shall be free of extraneous material except that which is unavoidable in the manufacturing process described in the approved biologics license application."* 21 C.F.R. § 610.13(b) (2015). Although the FDA provides no absolute level of purity required for any given drug, based on my experience of approximately 40 years in the pharmaceutical industry interacting with the FDA on regulatory issues, it is commonly assumed that, with rare exception, licensed drugs will have purities in excess of 99%, and often significantly higher. ICH Impurities in New Drug Substances Q3A(R2) (2006) ("Q3A(R2)", Ex. 2038) at 12; ICH M7 Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk, 2015 ("ICH M7", Ex. 2039) at 24-25. There is so much concern with the purity of drug substance and drug product that the highest level of purity possible should be achieved, even if that means changing the synthetic method as has been done in the '393 patent. Olsen, Bernard A., *What's New with Impurities in Pharmaceuticals?*, Southern California Pharmaceutical Discussion Group, January 15, 2015 (Ex. 2040) at 14. Drug purity is of such importance to regulatory agencies that the purity level of a drug substance and API ***must*** appear in the drug product specification, which is the quality control document of the drug's Certificate of Analysis for each batch of drug substance to be released for subsequent formulation into the final drug product. 21 C.F.R. § 600.3 (kk). If a batch of drug substance falls short of its lowest purity limit listed in the

specification, that batch of the drug substance must be rejected, even if the deviation in purity is as low as 0.1%. For example, if the actual purity of an API is 99.4% and the lowest limit of purity in the Drug Specification of the Certificate of Analysis is 99.5%, the entire batch of API must be rejected. As the FDA clearly states, *"Each component* [of API] *shall be tested for conformity with all appropriate written specifications for purity, strength and quality." Id.* at § 211.84(d)(2).

33. The FDA defines purity as *"relative freedom from extraneous matter in the finished product, whether or not harmful to the recipient or deleterious to product." Id.* at § 600.3 (r). Any batches of drug substance that fail to meet the levels of purity indicated in the product specification must not only be rejected, but rejected batches must also be *"identified and controlled under a quarantine system designed to prevent their use in manufacturing or processing operations for which they are unsuitable." Id.* at § 211.110(a). The position of the FDA on the significance of drug purity is absolutely clear, and would be understood by a POSA.

34. The function of the FDA is to approve new drugs based on their safety and efficacy, as well as the balance between the benefits and risks of new drugs to patients. Biotech, Janet Woodcock, The Political Economy of FDA Drug Review: Processing, Politics, And Lessons For Policy, FDA) (Ex. 2041) at 1-2. The FDA's focus on purity relates specifically to their many analyses that are related to the overall assessment of drug safety, and relative risk, of the new product to be marketed, and is done in the interest of patient safety. Ex. 2039 at 5-9, and 20.

35. Guidelines and requirements for the levels of purity of new drug substances and new drug products have been increasing over the past few decades. The FDA's requirements for increases in drug purity are based on their prior experiences (both positive and negative) in

approving drugs with varying degrees of purity. Based on my experience, the FDA understands that the levels of purity that they require are dependent upon improvements in technologies available to purify drug substances, as well as improvements in the levels of detection of various drug components, including impurities, that are available to pharmaceutical companies to produce, and equally important, manufacture, highly pure compounds. Ex. 2038 at 6-9. The trends for improvements in these technologies have unmistakably improved over the decades, and accordingly, so have the FDA's requirements for drug purity. Accordingly, in my experience, the drug purity requirements of the FDA represent a constantly moving (and improving) target.

36. Regulatory agencies have also sought to increase levels of purity, and consequently decrease levels of impurities, in order to provide to the maximum extent possible, the highest level of safety to patients. Ex. 2038 at 13-15. As indicated above, impurities are extraneous substances that are present in the API and final dosage form which add no value to the new drug product or to the patient. 21 C.F.R. § 600.3(5)(r). Because impurities add no value or benefit to the new drug product, they are, at best, irrelevant, and at worst, sources of potential adverse toxicities to patients. Impurities, therefore, can only add to the risk assessments, which are often unknown, made by regulatory agencies in the evaluation of new drug products. Ex. 2040 at 3-4 and 5-8.

37. Impurities may be introduced into the API or final dosage form during any of the many steps involved in the synthesis, formulation and manufacturing of the drug product. ICH Q3D Elemental Impurities ("ICH Q3D", Ex. 2043) at 5; Ex. 2038 at 6-7. It has long been the desire of regulatory agencies, and especially the FDA, to require pharmaceutical companies to produce the highest levels of drug purity that are possible and practicable.

38. Regulatory agencies have observed toxicities, or adverse events, resulting from drugs in clinical development as well as approved drugs that were not related to the new drug product itself, but rather to the impurities present in these new drugs (see examples below at ¶58). Because impurities add nothing to the benefit of a new drug, by extension, it is the view of regulatory agencies that impurities represent only potential risk to patients. Ex. 2039 at 12-16. Accordingly, regulatory agencies encourage (and may mandate) pharmaceutical manufacturers to increase levels of purity of their new drug substance. Even for products already approved by the FDA and on the market, it has been my experience that the FDA often encourages manufactures to continue to develop new synthetic and/or manufacturing processes to improve purity, and decrease levels of impurities, even further. This desirable goal is one of the objects of the invention of the '393 patent with respect to the new preparation of treprostinil with a higher level of purity.

39. The opening sentences of ICH M7 begin as follows: "*The synthesis of drug substances involves the use of reactive chemicals, reagents, solvents, catalysts, and other processing aids. As a result of chemical synthesis or subsequent degradation, impurities reside in all drug substances and associated drug products.*" *Id.* at 5. These sentences alone address the importance that regulatory agencies, including the FDA, attach to drug impurities, and the significant risks that may be associated with them. These sentences are also among the fundamentals taught in all introductory courses in Pharmacology and Toxicology (as an adjunct faculty member at Baylor University Medical School, West Virginia University Medical School, and The Ohio State University Department of Pharmacology, I teach this very concept to medical and pharmacy students in their second year pharmacology courses).

40. All drug substances contain impurities, and these impurities can range from harmless to extremely toxic, and often regulatory agencies do not know the risks of all (and sometimes any) of the impurities present in new drug substances. Accordingly, regulatory agencies focus intensely on the numbers and levels of impurities that exist in a drug substance, such as API and the finished drug product. Ex. 2043 at 13-16. With the information on impurities provided by pharmaceutical companies, and analyzed by the regulatory agencies, risk assessments are made or estimated by regulators with respect to the relative hazard that impurities, including trace impurities, may have on patients. Ex. 2039 at 6-10. This task becomes more complex and uncertain when one considers that there will also likely exist impurities that are present but not detectable (based on limitations in levels of detection; see penicillin example below at ¶62), yet may still present risks to individual patients, and the population of patients at large, who take any given medication.

41. In their assessment, the FDA and other regulatory agencies attempt to determine whether the impurities that are known to be present in the new drug substance have the capacity to induce injury or adverse effects in patients, as well as the types of injuries or adverse effects that may occur at the anticipated exposure level to the patient. *Id.* In so doing, regulatory agencies rely on the concept of the "threshold" for producing an adverse health effect. With respect to threshold, the concept of Threshold of Toxicological Concern (TTC) is considered (as discussed below), which is most often associated with impurities that are known or suspected to be mutagenic compounds that have the capacity to damage DNA and therefore the possibility of causing cancer, or impurities known to be highly toxic to humans or animals. *Id.* at 7-8 and 12-13. Regulators will also consider the type of adverse health effect that each impurity may produce, such as direct cell, tissue or organ damage, carcinogenicity, teratogenicity, reproductive

injury, chemical injury, and other types of injuries that may occur from exposure to an impurity. These considerations by regulatory agencies will also be viewed within the overall context of the benefit/risk ratio of the drug substance itself, as well as the severity of the disease to be treated, the availability of other potentially safer therapeutic alternatives or options, the types of patients being treated (i.e., male, female, children, elderly) and the duration of treatment (acute vs. chronic). *See, e.g., id.* at 12-16.

42. Among the many considerations that the FDA will explore with respect to impurities in pharmaceutical preparations is the dose-response relationship, or more appropriately, the dose-toxicity relationship, of the impurity. Accordingly, regulatory agencies also rely heavily on the Permitted Daily Exposure (PDE) of the impurity in the individual patient and the population of patients taking the drug (i.e., the maximum allowable level of the impurity that the patient or patient population can be exposed to), the length or duration of exposure to the impurity (acute vs. chronic administration of the drug), and the overall risk assessment made for each impurity. *Id.* at 13-16; *see also*, Ex. 2038 at 14-15; Ex. 2043 at 5-15. This is to say that regulatory agencies are realistic that patients will undoubtedly be exposed to potentially toxic impurities, and as such, regulatory agencies work diligently to determine how many patients may be injured from the anticipated level of exposure to the impurity in the new drug product. *Id.* These assessments are not viewed in isolation, but rather within the context of the other considerations, such as the benefits and risks of the new drug substance, the severity of the disease to be treated and the duration of therapy, as well as the availability of other potentially safer therapeutic alternatives. *Id.* Regulatory agencies will (reluctantly) accept potential or possible risks from impurities when the new drug product is directed to a serious disease with high unmet medical need, and for which there are few or no other therapeutic options. Ex. 2038

at 14-15. But even in these situations, regulatory agencies still press for the highest levels of purity that are possible and practicable. Again, this desire of regulatory agencies for the highest levels of purities in pharmaceuticals is one of the subjects of the inventions in the '393 patent.

43. Based on my personal experience in the pharmaceutical industry, depending upon the nature and number of impurities, and their levels in the drug substance, the FDA may request that individual impurities be synthesized and tested ***directly*** in a variety of animal safety test systems. Alternately, it is possible to evaluate ***indirectly*** the drug substance itself containing the impurities at very high doses in animal safety test systems, such that the absolute level of the impurities are considered to be sufficiently high to provide a level of exposure to the impurity that would be considered to be appropriately high to detect, with a high level of confidence, potentially injurious events in the safety test systems. For practical considerations, the latter indirect course is the most commonly taken path. When one or the other of these approaches is taken to assess potential safety risks in animal safety studies, either directly or indirectly, and the results of those safety studies indicate that little or no risk is likely, the potential safety risks of one or more of the impurities in the new drug substance are considered to be "***qualified***", and generally considered by regulatory agencies to present little or no risk to patients. Ex. 2038 at 9-14. One significant limitation of the testing of impurities in the drug substance to qualify the impurities, as well as the safety testing done for the drug substance itself, is that these safety test systems rely primarily or exclusively on animal safety models and test systems, and these test systems do not always translate with fidelity to the human patient (*see* below at ¶55). Differences in safety assessment of drugs (and impurities) between animals and humans can result because of differences in biological and biochemical processes between animals and humans, differences in metabolism and elimination, or differences in the duration of exposure

(animal testing is relatively limited in duration for a new drug substance or impurity, compared to exposure in humans which may extend for decades). As a result, there is no way for regulatory agencies to be absolutely certain of the safety risks associated with impurities in new drug substances. To accommodate for this, the analyses used by regulatory agencies to assess the risks of impurities are highly conservative, yet they still cannot guarantee that patient safety will not be compromised by an impurity. Ex. 2039 at 13-16. As a result, regulatory agencies require that impurities be limited to the lowest levels that are possible and practicable. Again, lowering the levels of impurities in treprostinil is one of the major benefits of the '393 patent, and results in higher levels of purity for the treprostinil product.

44. Because impurities in a pharmaceutical preparation are so critically important, regulatory agencies have issued specific guidelines that are consistent with those guidelines outlined in the ICH Impurities in New Drug Substances Q3A(R2) monograph. Ex. 2038 at 11-12. Inasmuch as most drugs are administered in dosages of less than 2 grams per day, only the guidelines for these drugs will be discussed herein. And indeed, treprostinil and the '393 patent fall into this category.

45. Because of the critical significance attached by regulatory agencies to impurities in pharmaceutical preparations, the levels and types of impurities must be included in routine batch specifications for all new drug substances. Ex. 2038 at 7-10. When levels of individual or total impurities exceed those levels listed in the specifications of the batch records required for the release of the drug substance, the batch must be rejected. 21 C.F.R. §§ 211.1 and 211.22(c). For example, if an individual impurity, or the total of all impurities found in a given batch of drug substance, exceeds the limits set in the specifications, even by amounts as low as 0.1%, the entire batch must be rejected. Accordingly, even very small differences in the levels of purity, or

the levels of impurities, can mean the difference between acceptance or rejection of an entire batch of drug substance.

46. Regulatory agencies categorize the treatment of impurities based on the levels that exist in the drug substance, and they categorize them into three different ways: a) Reporting Threshold, b) Identification Threshold and c) Qualification Threshold. Ex. 2038 at 10-12. These threshold levels are extremely low (i.e., conservative) because of the importance assigned by regulators to impurities, and the potential for toxicities caused by them. The specific requirements, as set forth by the ICH, are described below:

47. Reporting Threshold: when a given impurity is present at a level that is less than or equal to 0.05%, the impurity must be ***reported*** in the specification of the drug substance for manufacturing. *Id.*

48. Identification Threshold: when the amount of a given impurity exists at a level that is equal to or greater than 0.1%, or when the total dose of impurity administered per day is equal to or greater than 1.0 mg per day (whichever is lower), the impurity must be ***identified*** chemically in the specification of the drug substance for manufacturing. *Id.*

49. Qualification Threshold: when a given impurity exists at a level that is equal to or greater than 0.15%, or when the total dose administered per day is equal to or greater than 1.0 mg per day (whichever is lower), the impurity must be ***qualified***, which means that the biological safety must be established in standard safety and toxicology studies as described above. *Id.; see also* 13-15. This may either be done indirectly by studying in animal safety models very high doses of the drug substance containing sufficiently high levels of the impurity to be ***qualified***, or directly by studying the isolated and purified impurity to be ***qualified*** by itself in the safety studies. *Id.* at 9-10. In practice, ***qualification*** of an impurity is most commonly done indirectly

by studying high doses of the drug substance containing the impurity in animal safety test systems.

50. As a result of these thresholds, by definition, the limit of detection for impurities (and therefore total related substances) *must be* at least as low as 0.05%.

51. Often the FDA also requires a measure of the Total Related Substances, which is a measurement of all of the identified impurities added together. *See, e.g.*, Ex. 2006 at 6. This number is essentially a measurement of the purity of the sample itself.

52. For each impurity measurement and for the total related substances measurement, the Drug Specification in the Certificate of Analysis also includes a limit for those impurities, reported as "Not More Than", or "NMT", a certain percentage. *Id.* This number is not a measurement of the error, but is an indication of what level in the API or final product the FDA has permitted for that impurity.

53. An assay is a measure of the purity of a sample of the drug substance (or drug product) often performed in comparison to a "reference standard". *See, e.g.*, Ex. 2006 at 6; *see also* ICH Guidance For Industry: Q7A Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients (2001) ("Q7A", Ex. 2044) at 34-35; *see also* Reviewer Guidance: Validation of Chromatographic Methods (1994) ("Reviewer Guidance", Ex. 2035) at 8-11. The "reference standard" is a high quality and relatively highly purified sample against which all future samples of the drug are compared and measured. Ex. 2035 at 8. It is important to note that these reference standards do not represent absolute purity (which is impossible), and they also do not represent the maximum level of purity that can be obtained for any given drug. Reference standards are simply deemed to be high quality standards that all future samples of the drug will be measured against. A reference standard should always be included in assays required for

determination of the acceptability or unacceptability of manufacturing batches of API or final product in accord with the Drug Specification of the Certificate of Analysis. Ex. 2035 at 9-10. Accordingly, it is possible, and quite common, for a pharmaceutical company to develop an even more highly purified version of a drug than the reference standard for that drug, and as a result, purity levels for the drug will be in excess of 100% (relative to the reference standard) because it is more pure than the reference standard. This is a highly desirable outcome inasmuch as it indicates that the drug is even more pure than the "accepted" highly purified reference standard. Importantly, purities of a drug in excess of 100% (relative to the reference standard) do ***not*** represent an error in the assay, but simply indicate that the preparation of the drug is ***more pure*** than the reference standard. It cannot be overstated that a reported purity of a drug of over 100% (relative to the reference standard) does not indicate an error in the measurement, but simply that the sample being analyzed has a higher purity than the reference standard. As is the case for most assays, reported assay values may span a range of acceptable limits that can include and go beyond 100%, indicating that certain samples may be even more pure than the reference standard they are compared to.

54. It is also important to note that because some impurities are extremely toxic at very low levels of exposure, Thresholds of Toxicological Concern can, and often are, lowered, beyond the guidelines described above, in the specifications for the synthesis and manufacturing of a drug substance in order to be conservative. Ex. 2039 at 12-15. Conservative specifications, which provide a higher level of certainty that the drug product is safe from the standpoint of impurities, can make it especially difficult for API production plants and manufacturing plants that produce final drug product to meet the specifications established for a new drug substance. This creates the situation where exceedingly small differences in the allowable levels of purity

and impurities make it difficult to meet synthesis and manufacturing specifications. It has been my experience that this is particularly important for drugs of extremely high potency, such as the prostaglandins, prostanoids and prostaglandin-like drugs in general, and treprostinil specifically, which may contain trace amounts of potent structural analogs as impurities. As such, establishing specifications for purity and levels of impurities for extremely potent drugs, such as treprostinil, and being able to meet those specifications for synthesis of API and manufacturing of final drug product, is especially difficult for such highly potent drugs. Accordingly, the potential risk from impurities of even very potent drugs is not minimized because of the lower amount of API used in the final drug product.

55. In addition to the discussion above of the regulatory approach to dealing with the critical issue of impurities in drug substances, there are a number of other issues involved in coping with impurities that regulatory agencies must consider. Among the most important of these other considerations is the fear is that toxic effects of impurities, and especially *qualified* impurities, in drug substances may not be detectable in animal safety and toxicology studies, but yet may still occur in humans, and especially for those drugs that are used on a more chronic basis where they may be administered to patients for decades (especially in view of the relatively short-term nature of animal safety and toxicology testing). Diethylstilbestrol is one such example. This drug was determined to be safe in animal studies, but was subsequently found to produce higher incidences of vaginal and cervical cancer in second-generation female offspring who were exposed to the drug *in utero* while their mothers were being treated. Schrager et al., Diethylstilbestrol Exposure, 2004 (Ex. 2045) at 2-3. These types of results represent false negatives, or Type I Error, in that the animal safety studies failed to predict human toxicities. Carpenter, The Political Economy of FDA Drug Review: Processing, Politics, and Lessons for

Policy (2013) (Ex. 2042) at 4. And the converse may be true as well, as in the case of saccharin, which was found to be carcinogenic in animals, but not in humans. NTP Report on Carcinogens Background Document for Saccharin, March 1999 (Ex. 2046) at 102. This situation represents a false positive, or a Type II error (Ex. 2042 at 4) which will often result in the termination of development of an otherwise safe and potentially highly beneficial and medically necessary new drug. This latter situation is most unfortunate when a medically necessary product never reaches the human patient.

56. For these reasons, and others, the types and levels of impurities in drug substances are of the utmost importance to the FDA, and as a result, the FDA insists on the highest level of purity that is possible in a drug substance, and alternatively, the lowest levels of impurities possible. Although the FDA and ICH make recommendations with respect to the levels of impurities that are present, for example a threshold of 0.1%, or a total dose of 1.0 mg per day, whichever is lower (Ex. 2038 at 12), it is important to note that these are simply guidelines, and previously unknown impurities, or known impurities of high risk, can be held to even lower thresholds at the discretion of the regulatory agencies, or potentially not permissible at all down to the level of detection. Ex. 2039 at 12-16. Based on the present FDA and ICH guidelines, a potentially toxic impurity that is not demonstrated to be a risk in *animals*, could be present in a drug substance at a level resulting in exposures of up to 1.0 mg per day that could, in fact, be toxic to *humans*, and yet may not need to be *identified* or *qualified* (i.e., they were shown or assumed to be safe based on animal studies). Given the number of compounds known to be highly toxic at doses of 1.0 mg or less per day, even these relatively stringent criteria for reporting impurities in specifications for the synthesis and manufacture of drug substance could still expose humans to significant risk, and regulators are acutely aware of this. Accordingly,

any procedure or method that provides for a higher level of drug purity and/or lower levels of impurities is of critical importance to regulatory agencies and to patients in general. Again, one of the major inventions in the '393 patent is a new synthesis of treprostinil that results in a higher level of purity.

57. The regulatory agencies have set specific limits on levels of impurities in drug substances as described above, and they use detailed and complex analyses to assess risk to patients from impurities in drug substances. Accordingly, regulatory agencies insist that drug substances must have the highest levels of purity as possible, and contain the lowest levels of impurities as possible. As a result, new synthetic processes for an existing drug that increase purity and reduce impurities, both in number and level, are considered to be critically important to regulators, patients and public health (Ex. 2040 at 14). Even small increases in purity and decreases in impurities can have significant impacts on patient safety, as well as for acceptance or rejection of batches of manufactured drug substance. My personal experience has been that when considering the safety and toxicology profile of impurities, it is often more efficient to reduce the levels of impurities in the drug substance by altering or changing the synthetic method (*id.*), as opposed to simply adding additional purification steps, to achieve the desired reduction or elimination of impurities, as UTC has done and demonstrated for treprostinil in the '393 patent.

B. Examples of Toxic Contaminants in Pharmaceutical Preparations

58. Toxic impurities in drug substances are not a hypothetical or theoretical discussion. Indeed, highly toxic impurities in drug substances, API and final formulated product are, indeed, a reality with sometimes tragic outcomes. Specific examples of marketed drugs with highly toxic impurities are presented in this section as well as several examples of the negative

impact that toxic impurities in pharmaceutical preparations have had on patients. These examples highlight the critical importance of producing drug product of the highest possible quality and purity and with the lowest possible level of impurities, with both objectives strongly supported and often required by regulatory agencies globally, as reflected in the current ICH Guidelines. Ex. 2039 at 12-16.

59. The existence of highly toxic impurities in commonly used pharmaceutical preparations is not new and is not uncommon. And despite the strict limitations placed on toxic impurities by regulatory agencies, following the recommendations of the ICH discussed above, even newly approved pharmaceuticals may contain highly toxic impurities. It is important to note that even with the precautions taken by regulatory agencies to restrict or limit the levels of toxic impurities present in pharmaceutical substances, and limitations in the maximum allowable level of exposure to these impurities, that have been discussed previously, there is no guarantee that these toxic impurities are harmless even at the low levels that are permitted to exist in drug products. And this is especially true when these assessments are made in experimental animal safety/toxicology studies, which as discussed above, do not necessarily translate to safety in humans. In fact, regulatory agencies acknowledge that even these low levels of impurities allowed by their guidelines can still result in adverse toxicities in patients, and they have even calculated the risks of adverse toxicities, including cancer, that may be associated with these low levels. Ex. 2039 at 13-16; *see also* Ex. 2043 at 15-16.

60. As a result, it is still considered absolutely essential for pharmaceutical manufacturers to produce pharmaceutical products with the highest levels of purity, and the lowest levels of impurities, and I have observed over the course of my career that regulatory agencies have at times recommended changing synthetic and manufacturing procedures to

develop improved procedures to enhance purity and decrease the number and levels of impurities, as has been done in the '393 patent for treprostinil. *Id.*

61. Despite these significant limitations, which are well-known to regulatory agencies, there do exist examples of trace impurities in pharmaceutical substances that have produced significant, and often tragic, toxic adverse effects in patients. Some representative examples of these toxic or potentially toxic effects of impurities in drug substances and drug products are presented below:

62. Penicillins: Penicillins represent a class of drugs that are among the best-known and most extensively studied examples of trace impurities that can cause serious and potentially life-threatening adverse events. Penicillins refer to a group of important antibiotics belonging to the class of beta-lactam antibiotics. FDA Guidance for Industry, Non-Penicillin Beta-Lactam Drugs: A CGMP Framework for Preventing Cross-Contamination, (2013) ("Penicillin Guidance", Ex. 2047) at 4-9. Despite the very high level of safety and efficacy associated with the penicillins (they may be administered at doses of millions of international units, even to newborn babies), they are, as a class, known to produce serious and life-threatening allergic reactions, which, at their most serious levels, can result in anaphylaxis and death. *Id.* Penicillins have been known to be trace impurities in other non-penicillin products that are manufactured by companies that also manufacture penicillins. Penicillin impurities at minute levels have been introduced from the environment (e.g., air in the manufacturing facility) into both the API and finished products manufactured in the same facilities as the penicillins. *Id.* This resulted in immeasurable amounts of penicillin impurities to occur in other unrelated products which, when consumed by patients, have resulted in allergic reactions ranging from minor itching (e.g., rashes) to death (e.g., from anaphylaxis). *Id.* Because penicillins are so highly sensitizing to the human

immune system, and the level of penicillin impurity can be exceedingly low and not quantifiable, the FDA (and now other regulatory agencies) has determined that a zero level of penicillin impurity in a drug substance must be guaranteed. Accordingly, the FDA has mandated that the production of penicillin API and finished product must be performed in entirely different buildings, or buildings that are completely physically isolated from all other buildings, that manufacture other API and finished products for all other drugs. *Id.*

63. Cephalosporins: Cephalosporins are another class of beta-lactam antibiotics that, although chemically different from the penicillins, are associated with the same problem of sensitization and allergic reaction when they exist as trace (and often immeasurable) impurities in the API and finished products of other pharmaceutical drug materials. *Id.* Accordingly, the entire discussion above applies to the cephalosporins as well, including the need for completely isolated synthetic and manufacturing facilities for cephalosporins that are physically (or practically) isolated from all other facilities involved in the production of API and the production of final drug product for all other drugs. *Id.*

64. Human Insulin: Human insulin is an important protein that regulates glucose levels in the blood. In patients with diabetes, there is a low level of insulin secreted from the pancreas, and as a result, blood glucose levels rise producing the signs and symptoms of diabetes. Heinemann and Richter, Clinical Pharmacology of Human Insulin, 1993 (Ex. 2048) at 3. For decades, patients with diabetes were treated with insulin isolated from the pancreases of pigs inasmuch as no human source of insulin was available. With the advent of recombinant DNA technology, it became possible to produce human insulin in the bacteria *E. coli* using large bioreactors. Ex. 2048 at 2. The *E. coli* containing human insulin is then recovered, and the human insulin is isolated free from the *E. coli* and purified for human use to treat diabetes (this

represents the first drug to be produced by recombinant DNA technology). This was, and is today, considered to be a major advance in human health. Initially, in the isolation of human insulin from *E. coli*, the bacteria in which it was produced, the drug product contained trace impurities from *E. coli*, despite the fact that the human insulin was considered to be highly pure. As a member of the Department of Cell Biology at Eli Lilly and Company that developed recombinant human insulin, I am personally aware that when human insulin was initially developed to treat diabetes, allergic reactions were experienced in some diabetic patients, and these reactions were ultimately attributed to the trace impurities of antigens (foreign proteins capable of producing an allergic reaction) derived from the *E. coli* that was used to produce the human insulin. Ex. 2048 at 2. Subsequently, the company needed to manufacture an even more highly purified formulation of human insulin to ensure that the bacterial contaminants were minimized or eliminated. This phenomenon is not limited to human insulin, but is a fairly common occurrence among human proteins produced as therapeutic drugs through recombinant DNA technologies where the human protein is produced in non-human, and therefore potentially antigenic, cells (many human therapeutic proteins are produced in *E. coli* or Chinese Hamster Ovary Cells, or CHO cells, and trace quantities of foreign *E. coli* or CHO proteins can exist in the final product and elicit allergic responses).

65. These representative examples described above, and many others, highlight the importance of drug purity in pharmaceutical preparations, and more importantly, the risks that impurities existing in pharmaceutical API or finished drug product can have on patients. It is for these reasons that regulatory agencies encourage the highest levels of purity possible, and the lowest levels of impurities possible, in pharmaceutical product synthesis and production of drug

product. Ex. 2038 at 5-9. These highly valued goals of regulatory agencies with respect to purity and impurities are embodied in the new preparation of treprostinil described in the '393 patent.

VI. The Invention of the '393 Patent Met a Long-Felt Unmet Need

66. I have reviewed the letter submitted by UTC to the FDA on January 2, 2009 including the revised Drug Substance Specification (Ex. 2006), as well as Dr. Williams' Declaration. It is my opinion that the reduction of individual impurities and the change to the specification of the drug substance of treprostinil indicates that the invention of the '393 patent met a long-felt unmet need by providing a higher purity drug substance. If there is any doubt as to the extreme concern that the FDA places on purity, one need only read the letter submitted by UTC to the FDA on January 2, 2009 where UTC responds to the concerns raised by the FDA with respect to the request to change the Drug Specification for treprostinil in the Certificate of Analysis. The FDA raised only three specific concerns with respect to the Supplemental New Drug Application for treprostinil, and two of these concerns were related to impurities. Ex. 2006 at 1-7. In FDA Comment 1, the FDA has expressed concern about impurities in the supplies of [redacted] that are sourced through outside vendors, and which could potentially carry through to the final product in the synthesis of treprostinil. *Id.* at 1-3. And in FDA Comment 3, the FDA raises a concern about the "residual [redacted] present in treprostinil", which is a potential impurity. *Id.* at 6-7.

67. I have also reviewed Table 1 from Dr. Williams' Declaration where I observe that the level of Total Related Substances (i.e., total impurities) has been reduced from a level of 0.9545% using the Moriarty Process to [redacted] using the '393 patent process, which I calculate to represent a reduction of approximately [redacted] in total impurities. In addition, a reduction was observed in 7 out of 8 impurities using the '393 patent process (compared to the Moriarty

Process), and three impurities were essentially eliminated. Ex. 2020 at ¶¶94-96. These results would most definitely be considered to be significant and clinically meaningful to the FDA, and is consistent with the fact that the FDA allowed the Drug Specification in the Certificate of Analysis to be changed accordingly.

68. Specifically, as UTC explained to the FDA, the '393 patent process was not previously used in their prior process, but the use of the diethanolamine salt intermediate (UT-15C) "affords an additional purification step and an improvement in the process to synthesize treprostinil API" which showed the reduction of all but one of the impurities. Ex. 2006, at 2-3. I understand that the prior synthesis used by UTC was the same as Moriartycited by SteadyMed. *See*, Ex. 1004, *see also*, Ex. 1001, Ex. 6. I have also reviewed the analysis of Dr. Williams and it is my opinion that the removal of [redacted] impurities ([redacted]) was a long-felt unmet need given the FDA's desire to minimize impurities. I note specifically that the FDA concern identified in FDA Comment 1 of the UTC letter to the FDA dated January 2, 2009 demonstrates FDA's worries over the levels of [redacted], which was one of the impurities that was significantly reduced through the '393 patent process. Ex. 2006 at 1-3. As a result, I do not believe that it can be reasonably argued that the improvement in purity, and the reduction in impurities, resulting from the '393 patent process can represent anything other than a long-felt unmet need.

69. Additionally, UTC noted that there was a [redacted] variability in the assay, which meant that the specification was allowable for [redacted] above and below the average purity for the prior synthesis. Ex. 2006 at 3. This variability does not indicate an error in the measurement, but rather a limit on the acceptable specification, so that subsequent batches can be reliably and consistently approved for use by the current manufacturer, and subsequently by generic

manufactures, as a practical and realistic matter, which is also of relevance to the FDA. I have repeatedly observed during the course of my career that the FDA balances their strong desire for the highest levels of purity against the practical need for a company to be able to manufacture the drug product reliably. There are numerous examples of failures to supply patients with much needed medications because Drug Specifications could not be met consistently, resulting in shortages in drug supply. FDA: Drug Shortages, FDA.gov (Ex. 2049) at 1-5. This has been the focus of much attention in recent years, so much so that the FDA has developed an App so that patients can monitor supplies, and observe shortages in drug supply when they occur *Id.* A specification that is too limited or restrictive could result in multiple failing batches, and ultimately the inability to provide a medically necessary drug to patients on a consistent and reliable basis. As a result, there is a [redacted] range in the specification, and this does not represent an error in the assay, but in contrast represents a level of purity that assures the FDA that batches of treprostinil can be consistently manufactured and supplied to patients that need the medication on a reliable basis, while at the same time maintaining levels of purity that are consistent with patient safety.

70. As a result of the change in the synthesis to use the '393 patent process and product, the assay value was changed from a range of [redacted] to [redacted], because the increased purity of the drug substance prepared by the '393 patent process could now be centered at an increased mean of [redacted], and no longer at the [redacted] level as it was for the Moriarty process. Ex. 2006 at 3-4 and 6. This is typical of how assays are reported. *See, e.g.*, Guidance for Industry: Changes to an Approved NDA or ANDA, (2004) (Ex. 2050) at 20. The change in the upper limit for the assay from [redacted] to [redacted] did ***not*** indicate an increase in error from [redacted] to [redacted], but simply indicated that the average purity of the samples ***increased***, from a

midpoint of [redacted] which as indicated above, is always the objective of the FDA. Simply put, the original range of purity ([redacted]) of [redacted] (i.e., +/- [redacted], the variability in the assay) from [redacted] was shifted upward to a range of [redacted] ([redacted]), which unmistakably can ***only*** represent an upward shift in overall purity of the product, which is clearly a primary objective of the FDA and a benefit to patients. Likewise, the increase in the lower limit of purity of [redacted] using the old Moriarty process to [redacted] with the '393 patent process unquestionably represents an ***increase*** of the minimum level of purity required for release of any API or final product batch of the drug. This cannot be argued to represent anything other than an ***absolute increase*** in the required level of purity in the Drug Specification for the treprostinil product. It is also not relevant whether a previous process (*i.e.*, Moriarty) produces batches that are above this new level of [redacted]. What is relevant is that any process that results in a lower routine level of purity, as is the case for the Moriarty process, will have a higher probability of failing to meet the new minimum release specification of [redacted] than a process that routinely produces a higher level of purity, such as the '393 patent process, simply because its lower level of purity is that much closer to the lower limit of [redacted] required for release. Additionally, as shown by the 175 batch records, the average purity of the treprostinil product prepared by the process of the '393 patent is [redacted] while the average purity of the Moriarty product is 99.05%. Ex. 2020 at ¶99 and Appendices A-B. Thus, the average purity of the treprostinil product prepared by the process of the '393 patent has a [redacted] higher average purity than the Moriarty product.

71. As I discussed above, the assay values are a relative measurement that provide a comparison to a reference standard. Therefore, this change in the assay value indicates that the new batches made by the '393 patent process resulted in several batches with purities above that

of the highly purified reference sample. No matter how an assay is performed, an increase in the minimum level of purity from [redacted] in the Drug Specification can ***only*** represent an increased level of required purity of the API and final drug product required for release of any given batch. This clearly represents an improvement in quality and purity, and a reduction in the level of impurities, which is a major goal of the FDA.

72. This change in the assay value of the Drug Specification for treprostinil represents a significant change for the FDA. Specifically, any change in the synthesis or manufacture of the drug substance that may affect its impurity profile and/or the physical, chemical, or biological properties of a drug is considered a major change. Ex. 2050 at 17. Because the FDA allowed the drug specification for purity to be changed to reflect the higher level of purity, from a lower limit of [redacted], around means of [redacted], respectfully, resulting from the '393 patent process, it is clear that the FDA considered this to represent a major/significant change.

73. I have also reviewed the declaration and deposition transcript of Dr. Jeffery D. Winkler. In his opinion, a difference of 0.4% in a purity measurement "would be attributable to experimental error, and thus the claimed degree of purity under the claimed processes of the '393 patent presents no distinction from the prior art." Ex. 1009 at ¶¶ 68-69. I understand that he also acknowledged that he did not know how a purity specification for an FDA-approved product could change from [redacted], and stated that he viewed purity levels above 100% as errors: "I think the thing that I am able to conclude from the data that is on page 6 of this, of this letter [Ex. 2006] is that the error in the HPLC assay could be as high as [redacted] percent in the first column and by my analysis could be as high as [redacted] percent in the second column." Ex. 2051 at 86:15-87:9.

74. I disagree with Dr. Winkler. As I explain above, assay measurements are comparisons to reference standards and therefore can easily be greater than 100%. This is

different than the purity measurement reporting Total Related Substances reported by Dr. Walsh and separate from the Total Related Substances in the drug specification. Dr. Winkler admits he does not know the difference. *Id.* at 89:2-6.

75. It is therefore my opinion that the invention of the '393 patent met a long-felt unmet need that led to an increase in the purity of the treprostinil drug substance by reducing and/or removing several individual impurities and improving the overall purity as analyzed by Dr. Williams. It is because of this major change that the FDA permitted the change in the Drug Specification assay measurement submitted by UTC to be included in the revised specifications.

IPR2016-00006
patent 8,497,393

I declare under penalty of perjury that the foregoing is true and correct.

Date: July 6, 2016

Robert R. Ruffolo, Jr., Ph.D.

CURRICULUM VITAE

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Personal

Born:	April 14, 1950, Yonkers, New York
Marital Status:	Married, Stephany Ruffolo
Children:	Michael Robert Ruffolo, Born October 4, 1983
	Brian John Ruffolo, Born February 7, 1989
	Jennifer Suzanne Ruffolo, Born June 18, 1991

Education

1968-1973	The Ohio State University	B.S., *summa cum laude* and with Distinction in Pharmacy.
1973-1976	The Ohio State University	Ph.D. in Pharmacology

Professional Experience

2008- Present	President, Ruffolo Consulting, LLC (Pharmaceutical Consulting)
2008-2009	Consultant, Wyeth Pharmaceuticals
2002-2008	President, Research and Development, Wyeth (Responsibility for approximately 9,000 staff, >$3 Billion Budget)
2002-2008	Senior Vice President, Wyeth (Corporation)
2007-2008	Director, Wyeth Pharmaceuticals Inc. (Domestic Entity)
2007-2008	Director, Genetics Institute Europe, Inc.
2000-2002	Executive Vice President, Research and Development, Wyeth Research (Responsibility for approximately 4500 staff, $1.6 Billion Budget)
1998-2000	Senior Vice President and Director, Research Worldwide, SmithKline Beecham Pharmaceuticals (acting, while Head of Research recovered

from automobile accident) (Responsible for 1700 staff, $180 Million Budget) [Received a Special Commendation for performance]

Professional Experience (Continued)

1998-2000	Senior Vice President and Director, Biological Sciences, Worldwide, SmithKline Beecham Pharmaceuticals (Responsibility for approximately 500 staff, $105 Million Budget)
1995-1998	Vice President and Director, Pharmacological Sciences, Worldwide, and Medicinal Chemistry, Europe, SmithKline Beecham Pharmaceuticals (Responsibility: >300 staff)
1992-1995	Vice President and Director, Pharmacological Sciences, U.S., U.K., Europe and Australia, SmithKline Beecham Pharmaceuticals (Responsibility: >300 staff)
1990-1992	Vice President and Director, Pharmacological Sciences, U.S., SmithKline Beecham Pharmaceuticals (Responsibility: 120 staff)
1989-1990	Vice President, Pharmacological Sciences, Smith Kline & French Laboratories (Responsibility: 120 staff)
1987-1989	Group Director, Department of Pharmacology and Department of Molecular Pharmacology, Smith Kline & French Laboratories (Responsibility: 140 staff)
1985-1987	Director, Cardiovascular and Renal Pharmacology, Smith Kline & French Laboratories (Responsibility: 41 staff)
1984-1985	Director, Cardiovascular Pharmacology, Smith Kline & French Laboratories (Responsibility: 24 staff)
1983-1984	Chairman, Cardiovascular Research Committee, Lilly Research Laboratories
1982-1984	Research Scientist, Lilly Research Laboratories, Department of Cardiovascular Pharmacology
1981-1982	Senior Pharmacologist, Lilly Research Laboratories, Department of Cardiovascular Pharmacology
1978-1981	Senior Pharmacologist, Lilly Research Laboratories, Department of Cell Biology
1977-1978	Staff Fellow, Postdoctoral Research Associate, National Heart, Lung and Blood Institute, The National Institutes of Health, Laboratory of Biochemical Genetics (Chief, Dr. Marshall Nirenberg, Nobel Laureate)
1976-1977	Postdoctoral Research Associate, The Ohio State University
1973-1976	Graduate Fellow, The Ohio State University (Thesis Advisor, Dr. Popat N. Patil)

Honors, Awards and Recognitions

2013	Resolution: Scroll of Appreciation, West Virginia University Foundation
2013	Chauncey D. Leake Award, The Ohio State University, Columbus, Ohio
2012	Robert R. Ruffolo Career Achievement in Pharmacology Award (Medal) established by the American Society for Pharmacology and Experimental Therapeutics (ASPET)
2011	Elected, Fellow of The College of Physicians of Philadelphia (FCPP)
2009	Winner, Great Oxford Debate, Oxford, England
2009	Lifetime Achievement Award, The Ohio State University, Columbus, Ohio
2009	Target Leadership Lectureship, College of Pharmacy, University of Florida
2008	Scrip Lifetime Achievement Award, Scrip Annual Awards, London, UK
2008	"Best Pharmaceutical Company, R. Ruffolo, Scrip Awards, London, UK
2008	Legislative Commemoration, the State of South Dakota. Awarded for Discovery and Development of Coreg
2008	Tribute, Office of the Governor, State of Delaware. Awarded for Discovery and Development of Coreg
2008	Citation, Commonwealth of Pennsylvania, House of Representatives. Awarded for Discovery and Development of Coreg
2008	Resolution of Congratulations, Senate of the State of Pennsylvania. Awarded for Discovery and Development of Coreg
2008	Maurice Seevers Lectureship, Department of Pharmacology, Univ. Michigan
2008	William E. Hassan Distinguished Rho Chi Memorial Lecture, Massachusetts College of Pharmacy and Health Sciences
2008	Discoverer's Award. Awarded for Coreg® (Carvedilol/Kredex) by PhRMA
2008	David Perlman Memorial Lectureship. American Chemical Society
2008	"Top Ten Pipeline: Strongest Women's Health Pipeline". Awarded to Robert R. Ruffolo by R&D Directions at the Drug Development Summit
2008	RADEX (R&D Executive Committee) Award for re-building Wyeth's R&D
2008	Visionary Leadership Award, Wyeth Pharmaceuticals
2008	Pharmaceutical R&D Achievement Award, Accenture

Honors, Awards and Recognitions (Continued)

2008	Sino-American Pharmaceutical Professionals Special Service Award
2007-2013	Professor of Physiology and Pharmacology. West Virginia University
2007	Honorary Doctorate of Science (D.Sc.). West Virginia University
2007	Honorary Doctorate in Engineering (D.Eng.). University of Catania, Italy
2007	Best Drug Development Pipeline. Awarded to R. Ruffolo for Wyeth Pipeline
2007	John S. O'Brien Memorial Lectureship. University of Pennsylvania
2007	138th Commencement Speaker, West Virginia University School of Medicine. West Virginia University, Morgantown, WV
2007	Profiled in The Star Ledger of New Jersey. Feature article entitled "Researcher Makes His Mark", April 25, 2007.
2007	Profiled in Harvard Business Review. Wyeth Pharmaceuticals: Spurring Scientific Creativity with Metrics, February 2, 2007.
2007	"Top Ten Pipeline: Strongest CNS Pipeline". Awarded to Robert R. Ruffolo by R&D Directions at the Drug Development Summit.
2006	Profiled in BusinessWeek Magazine. Feature article on re-engineering R&D, February 6, 2006.
2006	Centennial Award for Drug Discovery. Awarded for the discovery and development of Coreg® (Carvedilol) by Temple University.
2006	Listed as one of the "Most Notable Graduates" of The Ohio State University
2006	Research's Shining Star. Designated by Med Ad News for Leading and Re-engineering Wyeth's R&D
2006	Key To The City, Catania, Italy (awarded by Mayor)
2006	Designated as "Notable Alumni" by The Ohio State University.
2006	SAPA Special Award for Outstanding Contributions. Awarded by Sino-American Pharmaceutical Professionals Association (SAPA).
2006	Management Team of the Year. Awarded to R. Ruffolo by Scrip for his leadership of the Wyeth Research and Development Executive Committee
2006	Renowned Pharmaceutical Scientist. Named to list of top 200 Renowned Pharmaceutical Scientists by Pharmer.

Honors, Awards and Recognitions (Continued)

2006	"Top Ten Pipelines: The Strongest Primary Cure Pipeline". Awarded to Robert R. Ruffolo by R&D Directions and Drug Discovery Summit.
2005	Profiled in Philadelphia Inquirer. Feature article entitled "Wyeth's Front Man", June 5, 2005.
2005	Designated as One of the 100 Most Inspiring People in the Life-Sciences Industry. Awarded by PharmaVOICE
2005	George B. Koelle Award for Scientific Excellence. Awarded by the Mid-Atlantic Pharmacology Society.
2005	Executive of The Year Finalist. Scrip Awards.
2005	"Top Ten Pipelines: The Pipeline to Watch". Awarded to Robert R. Ruffolo by R&D Directions and Drug Discovery Summit.
2005	SAPA-GP Excellence Award. Awarded by Sino-American Pharmaceutical Professionals Association
2004	Chief Scientific Officer of the Year. Awarded at the Third Annual Pharmaceutical Achievement Awards for exemplary innovation, scientific competence, leadership and organizational creativity in managing R&D.
2004	Corporate Recognition Award for Innovation by American Chemical Society
2002-2008	Principal Corporate Officer, Senior Vice President, Wyeth (Corporation)
2002	Corporate Officer, Senior Vice President, Wyeth (Corporation)
2002	Designated ISI Highly Cited Researcher, in recognition of being in the top 100 cited Pharmacologists worldwide over the past 20 years (1981-2001)
2002-2008	Member, Pharmaceutical Research and Manufacturing Association (PhRMA) Foundation Board
2003-2008	Member, BIO Board of Directors
2002-2008	Member, Robert F. Furchgott Endowed Chair Committee
2001	Corporate Officer, American Home Products Corporation
2001-Present	Member, Pharmaceutical Research and Manufacturers Association (PhRMA), Science and Regulatory Executive Committee
2000	SmithKline Beecham "Battlefield Award" for exceptional performance in SmithKline Beecham in litigation on Phentermine. Cash Award.
1999-2000	Principle Actor; National television and print media campaign for the Pharmaceutical Research and Manufacturers of America. Selected for the discovery and development of Coreg (carvedilol) for congestive heart failure

Honors, Awards and Recognitions (Continued)

1999	Lorenzini Gold Medal for Biomedical Research, Lorenzini Medical Science Foundation
1998	Member (elected), Mark Nickerson Lecture Award Endowment Committee
1998	Chairman, International Union of Pharmacology (IUPHAR) Committee on Receptor Classification and Nomenclature
1998	John V. Croker Lecturer, The American Society for Pharmacology and Experimental Therapeutics
1998	Special Commendation awarded by SmithKline Beecham's Corporate Management Team for supervising Worldwide Discovery while head of Discovery recuperated from a serious automobile accident. Cash award.
1997	Visiting Lecturer of the Hungarian Academy of Sciences (elected by HAS), Budapest, Hungary
1997	Albert Szentgyörgy Medal, Szentgyörgy Medical School, Szeged, Hungary
1997	U.S. Pharmaceutical President's Award in Recognition of Outstanding Contribution for Research on Carvedilol (Coreg), U.S. Pharmaceuticals Division, SmithKline Beecham Pharmaceuticals
1997	R&D President's Gold Impact Award in Recognition of Outstanding Contribution to Carvedilol (Coreg) Approval for Congestive Heart Failure, Research and Development, SmithKline Beecham Pharmaceuticals.
1994-1997	Secretary/Treasurer, American Society for Pharmacology and Experimental Therapeutics (ASPET; Elected Position)
1996	Prix Galien Research Award Citation and Commendation for Innovation and Excellence in Research; Nonpeptide Endothelin Receptor Antagonists UK
1996	Prix Galien Innovation Research Award. United Kingdom
1995	Maloney-Booker Lecturer in Pharmacology. College of Medicine, Howard University, Washington, D.C.
1995	Distinguished Service Award, Publications Committee, Federation of American Societies for Experimental Biology
1995	Simply the Best Award, presented by CEO of SmithKline Beecham
1994	Distinguished Visiting Professor, Department of Pharmacological and Pharmaceutical Sciences, University of Houston, Houston, Texas
1991	Diploma - El Postgrado de Farmacologia, Universidad Central de Venezuela, Facultad de Farmacia, Caracas, Venezuela

Honors, Awards and Recognitions (Continued)

1989	Distinguished Alumni Award, The Ohio State University
1989	Certificate of Appreciation, Food and Drug Administration, Committee for Advanced Scientific Education
1988	John Jacob Abel Award in Pharmacology, American Society for Pharmacology and Experimental Therapeutics
1988	R&D President's Award, SmithKline Beckman Pharmaceuticals, for Exceptional Performance with CEO in presentation of R&D Pipeline to Analysts and Investors. Cash Award.
1984	Travel Award granted by the American Society for Pharmacology and Experimental Therapeutics to attend the Ninth IUPHAR International Congress for Pharmacology, London, England.
1982	Queen Beatrix Medal, Koningin Der Nederlanden, The Netherlands.
1981	Travel Award granted by the American Society for Pharmacology and Experimental Therapeutics to attend the Eighth IUPHAR International Congress for Pharmacology, Tokyo, Japan
1977-1978	PRAT Fellow: Fellow of the National Institutes of General Medical Sciences. Pharmacology Research Associate Training Fellowship
1976	Phi Kappa Phi Honor Society, The Ohio State University
1973-1976	Fellowship from the American Foundation for Pharmaceutical Education
1973	B.S. *Summa cum laude*, The Ohio State University
1973	Rho Pi Phi Scholastic Award, The Ohio State University
1973	George B. Kauffman Memorial Award for Scholarship, The Ohio State University
1972	Rho Chi Pharmaceutical Honor Society, The Ohio State University
1972	Rho Chi Scholarship Recognition Award, The Ohio State University
1972	Miami Valley Pharmaceutical Association Scholastic Award, The Ohio State University

Major Personal Role in the Discovery and Development of the Following Marketed Products

- Dobutrex (Dobutamine) for Congestive Heart Failure
- Requip (Ropinerole) for Parkinson's Disease
- Teveten (Eprosartan) for Hypertension
- Coreg/Kredex (Carvedilol) for Congestive Heart Failure and Acute Myocardial Infarction

The Following Products Were Approved During Tenure as President of Research & Development, Wyeth Pharmaceuticals

- Effexor for Depression and multiple indications
- Enbrel for Rheumatoid Arthritis and other Indications
- Pristiq (Desvenlefaxine) for Depression
- Relistor (Methylnaltrexone) for Opiate-Induced Consitpation
- InFuse (rhBMP-2) for Bone Healing
- Torisel (Temsirilomus) for Renal Cancer
- Lybrel (Levo Ethynylestradiol) for Continuous Contraception
- Xyntha (rhFactor VIII) for Hemophilia
- Tygacil (Tygacycline) Broad Spectrum Injectable Antibiotic
- Mylotarg (Gemtuzumab Ozogamicin) for Acute Myelogenous Leukemia
- Premarin (Conjugated Estrogens), Low Dose
- PremPro (Conjugated Estrogens/Progestin), Low Dose
- DuaVee (Conjugated Estrogens/Bazedoxifene) for Menopausal Symptoms
- Prevnar and Prevnar 13 vaccines for Pneumococcal Disease (children and adult)

Philanthropic Activities

- Established the *Ruffolo Charitable Fund* to support many charities, humanitarian projects and educational programs throughout the World

- With the American Society for Pharmacology and Experimental Therapeutics (ASPET), established the *Robert R. Ruffolo Career Achievement in Pharmacology Award (Medal) in Pharmacology*

- Underwrote the renovation of the *Robert & Stephany Ruffolo Lecture Hall* in the College of Pharmacy at The Ohio State University

- Established the *Robert & Stephany Ruffolo Endowed Scholarship* at The Ohio State University College of Pharmacy

- Established the *Popat N. Patil Endowed Scholarship* at The Ohio State University College of Pharmacy in honor of his former Professor and Advisor

- Established the *Robert & Stephany Ruffolo Endowed Scholarship* at The Ohio State University Fisher School of Business

- Established the *Robert & Stephany Ruffolo Endowed Scholarship* at West Virginia University School of Pharmacy

- Established the *Robert & Stephany Ruffolo Endowed Research Fellowship* at West Virginia University School of Pharmacy

- Funded over 20 Non-Endowed *Robert & Stephany Ruffolo Scholarships* at West Virginia University College of Pharmacy

Philanthropic Activities (Continued)

- Established the *Robert & Stephany Ruffolo Endowed Research Fellowship* at the University of Florida College of Medicine
- Major Donor to the *Mali Health Organization Project*
- Named *"American Patriot"* by Bill O'Reilly live "on air" on the Fox News Network and on the Bill O'Reilly website for his "*Generous Donation to the Fisher House Foundation*" which is dedicated to help fallen and disabled military and their families.

Ruffolo Consulting, LLC, Clients (Past and Present)

- Wyeth Pharmaceuticals
- Pfizer Pharmaceuticals
- Takeda Pharmaceuticals
- Merck, Sharp & Dohme
- EMD Serono Pharmaceuticals (Merck KGaA)
- Johnson & Johnson
- Novartis
- GlaxoSmithKline
- Teva Pharmaceuticals
- Shire Pharmaceuticals
- The Carlyle Group
- PPD (Pharmaceutical Product Development)
- Accenture
- McKinsey
- UCB
- Alcon
- Highfields Capital Management
- Tessella
- Trevena Pharmaceuticals
- Keddem Biotech
- Gillead
- HemoShear Technologies
- GLG Institute
- Gardner Roberts, LLP
- Goodwin-Proctor, LLP

Board Memberships and Consultancies (Past and Present)

- Trevena Pharmaceuticals (Board of Directors and Scientific Advisory Board)
- West Virginia University Foundation (Board of Directors)
- Gene Network Sciences (Scientific Advisory Board)
- HemoShear Pharmaceuticals (Scientific Advisory Board)
- Ore Pharmaceuticals (Scientific Advisory Board)
- West Virginia University School of Pharmacy Dean's Board of Directors
- University of Michigan Health Sciences Center
- The Ohio State University School of Business
- Mali Health Organization Project Board
- EMD Serono (International Advisory Board)
- DNDi (Drugs for Neglected Diseases Initiative) (Scientific Advisory Board)
- Sapience Pharmaceuticals (Board of Directors)
- Sigilon Pharmaceuticals (Board of Directors)
- Aridis Pharmaceuticals (Board of Directors)

Expert Witness in Lawsuits

- SmithKline Beecham Litigation on Diet Drug-induced Primary Pulmonary Hypertension and Valvular Heart Disease (1998-2000)
- Wyeth Pharmaceuticals Hormone Therapy Litigation following publication of the Women's Health Initiative (2007)
- Patent Litigation, Gardner Roberts LLP (2009-2010)
- Patent Litigation; Goodwin-Procter LLP (2010-2013)

Academic Appointments

2007-2014	Adjunct Professor of Physiology and Pharmacology, School of Medicine, West Virginia University
2004-2008	Corporate Advisory Board for the University of Michigan Medical School
2004-2008	Corporate Advisory Board, University of Pennsylvania School of Nursing
1989-Present	Adjunct Professor, Division of Pharmacology, College of Pharmacy, The Ohio State University, Columbus, Ohio
2004-2008	University of Pennsylvania School of Nursing Board of Overseers
2004-2009	Ohio State University College of Pharmacy Dean's Corporate Council
2000-2008	West Virginia University School of Pharmacy Dean's Advisory Board
1990-2001	Adjunct Professor, Department of Pharmacology, School of Medicine, Baylor University, Houston, Texas
1982-1989	Adjunct Professor, Department of Pharmacology, School of Medicine, McGill University, Montreal, Canada
1993-1996	Board of Visitors, School of Pharmacy, University of Wisconsin, Madison, Wisconsin

Professional Affiliations

Coalition Against Major Diseases, The Brookings Institute (Chaired by Dr. Mark McClellan, Former Commissioner of the FDA and CMS)

American Society for Pharmacology and Experimental Therapeutics (ASPET)

British Pharmacological Society (BPS)

International Union of Pharmacology (IUPHAR)

Federation of American Societies of Experimental Biology (FASEB)

Experimental Biology (EB)

Society of Critical Care Medicine (SCCM)

Mid-Atlantic Pharmacology Society (MAPS)

Professional Affiliations (Continued)

Screen Actors Guild (SAG); Principal Performer in Television Advertisement for Pharmaceutical Research and Manufacturers of America

SAPA Board of Advisors

Center for Biomedical Innovation; MIT

Drugs for Neglected Diseases (DNDi)

University of Florida College of Pharmacy Dean's National Advisory Board

Editorial Responsibilities

Editor-in-Chief, Current Opinions in Pharmacology (2000-2008)

Founding Editor, Current Opinions in Pharmacology (2000)

Editor-in-Chief, Pharmacology Reviews and Communications (1996-2000)

Founding Editor, Pharmacology Reviews and Communications (1996)

Editor, British Journal of Pharmacology (1998-2000).

Editor-in-Chief, Pharmacology Communications (1991-1996).

Founding Editor, Pharmacology Communications (1991)

Editor, Critical Reviews in Pharmacology (1993-1995)

Editor, The Journal of Pharmacology and Experimental Therapeutics; Autonomic Pharmacology (1985-1992).

Editor, Cardiovascular Drugs and Therapy; Adrenergic Modulation (1994-Present)

Editor, Cardiovascular and Renal Drugs, Current Opinion in Investigational Drugs (1992-present).

Co-Editor, Neuropharmacology, Textbook of Basic and Clinical Pharmacology (1990-1994)

Guest Editor, Autonomic Pharmacology, The Journal of Pharmacology and Experimental Therapeutics, 1982-1985.

Editorial Advisory Boards

British Journal of Pharmacology (1998 - 2000)

The Journal of Pharmacology and Experimental Therapeutics (1981 - 1986)

Journal of Cardiovascular Pharmacology (1991 - present)

Cardiovascular Drugs and Therapy (1993 - present)

Annual Reviews of Pharmacology (1991 - 1995)

Journal of Autonomic Pharmacology (1984 - present)

Fundamental and Clinical Pharmacology (1986 - 1992)

Journal of Medicinal Chemistry (1989 - 1995)

Trends in Pharmacological Sciences (1990 - present)

The Spilker Report (2004 - present)

Medicinal Research Reviews (1994 - 2003)

FASEB Journal Publications Committee (1989 - 1995)

Receptor (1989 - 1996)

Drug Development Research (1995 - Present)

Year Book of Pharmacology (1988 - 1992)

Journal of Chirality (1988 - 1996)

Drug News & Perspective (1989 - present)

Pharmacology Communications (1991 - 1996)

CRC Critical Reviews in Pharmacology (1992 - 1996)

Investigational Drugs Database (1993 - present)

Pharmaceutical News (1994 - present)

Research Biochemicals International (1993 - 1997)

Current Protocols in Pharmacology (1995 - 2001)

Receptors and Signal Transduction (1995 - 1996)

Pharmacology Reviews and Communications (1996 - Present)

Current Opinions in Pharmacology (2000 - Present)

Phacilitate: R&D Leaders Forum (2001 - Present)

Expert Review on Drug Metabolism and Toxicology (2004 – Present)

Expert Opinion on Drug Discovery (2005 – Present)

Reviewer for Research Grants

National Institutes of Health, National Institute on Drug Abuse, Study Section: Drug Abuse Biomedical Research Review Committee, 1984, Special Review Consultant

National Institutes of Health, Bio-organic and Natural Products Chemistry Study Section, 1984, Special Review Consultant

National Science Foundation, Molecular and Cellular Neurobiology Section 1984

Medical Research Council of Canada, Montreal

Tobacco and Health Institute, Lexington, Kentucky

National Institutes of Health, Study Section, Cardiovascular Pharmacology; Special Review Consultant, 1986

National Institutes of Health Study Section, Autonomic Pharmacology; Special Review Consultant, 1986

National Institutes of Health Study Section, Receptor Pharmacology; Special Review Consultant, 1986

National Institutes of Health, Study Section; Special Review Consultant, 1987

Member, Site Visit Committee, National Heart, Lung and Blood Institute, Stanford University, 1988

Member, Site Visit Committee, National Heart, Lung and Blood Institute, University of Chicago, 1988

American (ASPET) and International (IUPHAR) Pharmacology Society Roles

Council Member, Drug Discovery, Drug Development and Regulatory Affairs, Division of the American Society for Pharmacology and Experimental Therapeutics (1999-2008)

Officer, IUPHAR 2002 World Congress, 1996-2002.

Chairman, IUPHAR Committee for Receptor Nomenclature and Drug Classification (1998 - 2002)

ASPET Sollmann Award Selection Committee (1997)

Chairman, IUPHAR Finance Committee, International Union for Pharmacology (1995 - 2002)

IUPHAR Subcommittee for Receptor Nomenclature and Drug Classification: Endothelin Receptors (1995 - 2002), International Union for Pharmacology (IUPHAR)

ASPET Sollmann Award Selection Committee (1995)

IUPHAR Committee for Receptor Nomenclature and Drug Classification (1994-present), International Union for Pharmacology

Member, ASPET Council (1994-1997)

Member, ASPET Finance Committee (1994-1997)

Chairman, ASPET Finance Committee (1995-1996)

Member, ASPET Investment Subcommittee (1995-1996)

Secretary/Treasurer, American Society for Pharmacology and Experimental Therapeutics (1994-1997) (Elected Position)

Committee on Industrial - Academic Relations, American Society for Pharmacology and Experimental Therapeutics (1993 - 1994)

Nomination Committee, American Society For Pharmacology and Experimental Therapeutics (1992 - 1993) (Elected Position).

Subcommittee on Pharmacology in Industry, (1990-1992), American Society for Pharmacology and Experimental Therapeutics (ASPET)

IUPHAR Subcommittee for Receptor Nomenclature and Drug Classification: Adrenoceptors (1990-present), International Union for Pharmacology (IUPHAR).

Publications Committee, the FASEB Journal (1989-present), Federation of American Societies for Experimental Biology (FASEB)

American (ASPET) and International (IUPHAR) Pharmacology Society Roles (continued)

Selection Committee (1985), Pharmacology Research Associate Training (PRAT) Fellowships National Institute of General Medical Sciences, National Institutes of Health

Program Committee (1984-1989), American Society for Pharmacology and Experimental Therapeutics

Invited Lectures

University of Kansas, Department of Pharmacology, Lawrence, Kansas. Synapse turnover: A mechanism for acquiring synaptic specificity, 1978.

Mayo Clinic, Department of Pharmacology, Rochester, Minnesota. Alpha-Adrenergic Activity of Clonidine-like Imidazolines, 1979.

University of Connecticut, Department of Neurobiology, Storrs, Connecticut. Synapse Turnover: A Mechanism for Acquiring Synaptic Specificity, 1979.

Northeastern University, Section of Pharmacology, College of Pharmacy, Boston, Massachusetts. Alpha-Adrenergic Effects of Imidazolines and β-Phenethylamines, 1981.

University of Kentucky, Department of Pharmacology, College of Medicine, Lexington, Kentucky. Peripheral and Central Effects of Adrenergic Agonists, 1981.

Merck Institute for Therapeutic Research, Department of Cardiovascular Pharmacology, West Point, Pennsylvania. Evaluation of Clonidine-like Imidazolines from the Standpoint of Receptor Theory and Antihypertensive Activity, January 18, 1982.

McGill University, Department of Pharmacology, College of Medicine, Montreal, Canada. Quantitative Analysis of Drug-Receptor Interactions in Classical Pharmacological Studies, March 2, 1982.

McGill University, Department of Pharmacology, College of Medicine, Montreal, Canada. An Evaluation of the Antihypertensive Activity of Clonidine-Like Imidazolines From the Standpoint of Classical Receptor Theory, March 3, 1982.

Dow Chemical Company, Department of Cardiovascular Pharmacology, Zionsville, Indiana. Central Alpha-Adrenergic Mechanisms in the Regulation of Blood Pressure. Evaluation From the Standpoint of Receptor Theory, May 3, 1982.

Laboratoires D'Etudes et de Recherches SYNTHELABO, Department of Cardiovascular Pharmacology, Paris, France. Stereochemical Requirements of Alpha-2 Adrenergic Receptors, May 24, 1982.

University of Amsterdam, Department of Pharmacodynamics, Amsterdam, The 1982. Differences Between the Interactions of Imidazolines and Phenethylamines with Alpha-Adrenergic Receptors, October 25, 1982.

Emory University, Department of Pharmacology, Atlanta, Georgia. Peripheral and Central Effects of α-Adrenergic Agonists, November 5, 1982.

Emory University, Department of Pharmacology, Atlanta, Georgia. The Current Status of α-Adrenergic Receptors. November 5, 1982.

Invited Lectures (Continued)

Smith Kline & French Laboratories, Department of Pharmacology, Philadelphia, Pennsylvania. Stereochemical requirements of α_1 and α_2-adrenoceptors. July, 1982.

State University of New York at Buffalo, Department of Biochemical Pharmacology, Buffalo, New York. Role of Central and Peripheral α-Adrenergic Receptors in the Regulation of Blood Pressure. December 1, 1982.

The Ohio State University, Division of Pharmacology, College of Pharmacy, Columbus, Ohio. The Role of Central and Peripheral α-Adrenergic Receptors in the Regulation of Blood Pressure. February 9, 1983.

Tulane University, Department of Pharmacology, School of Medicine, New Orleans, Louisiana. The Role of Central and Peripheral α-Adrenoceptors in the Regulation of Blood Pressure. May 24, 1983.

University of Mainz, Institute of Pharmacology, School of Medicine, Mainz, West Germany. The Role of Central and Peripheral α-Adrenoceptors in the Regulation of Blood Pressure. June 21, 1983.

University of Wurzburg, Department of Pharmacology, School of Medicine, Wurzburg, West Germany. The Role of Central and Peripheral α-Adrenoceptors in the Regulation of Blood Pressure. June 22, 1983.

Doctor's Hospital, Section on Critical Care Medicine, Grand Rounds, Columbus, Ohio. Central and Peripheral Regulation of the Cardiovascular System. September 7, 1983.

Mayo Clinic, Department of Physiology and Biophysics, Rochester, Minnesota. The Role of Central and Peripheral α-Adrenoceptors in the Regulation of Blood Pressure. December 19, 1983.

Sloan-Kettering Memorial Hospital, Department of Critical Care Medicine, Grand Rounds, New York. Drug, Neurotransmitter and Hormone Receptors in the Regulation of the Cardiovascular System. December 16, 1983.

Cook County Hospital, Department of Critical Care Medicine, Grand Rounds, Chicago. Central and Peripheral Regulation of the Cardiovascular System. May 10, 1984.

University of Illinois, Department of Neural and Behavioral Biology, Urbana, Illinois. The Role of Central and Peripheral α-Adrenoceptors in the Regulation of Blood Pressure. November 22, 1983.

Indiana University, Department of Pharmacology, Indianapolis, Indiana. The Role of Central and Peripheral α-Adrenoceptors in the Regulation of Blood Pressure. December 2, 1983.

Invited Lectures (Continued)

Presbyterian University Hospital, Anesthesia Research Conference, Pittsburgh, Pennsylvania. New Insights into the Inotropic Activity of Dobutamine: Future Directions. February 9, 1984.

Mayo Clinic, Department of Physiology and Biophysics, Rochester, Minnesota. The Existence of Spare α_1-Adrenoceptors, but not α_2-Adrenoceptors in the Vasculature: Caution about the Calcium Dependence of α_2-Adrenoceptors. December 19, 1983.

Presbyterian University Hospital, Department of Critical Care Medicine, Pittsburgh, Pennsylvania. Central and Peripheral Regulation of the Cardiovascular System. February 8, 1984.

Presbyterian University Hospital, Anesthesia Grand Rounds, Pittsburgh, Pennsylvania. Sympathomimetic Amines in the Treatment of Heart Failure and Shock. February 9, 1984.

University of Brussels, Society of Emergency and Critical Care Medicine. New Insights Into the Inotropic Action of Dobutamine. March 30, 1984.

Laboratoires D'Etudes et de Rechereches SYNTHELABO, Department of Cardiovascular Pharmacology, Paris, France. Existence of Spare α_1-Adrenoceptors, but not α_2-Adrenoceptors, in the Vasculature of the Pithed Rat: Possible Relationship to Calcium Utilization. March 26, 1984.

Ohio State University, Department of Clinical Pharmacology, College of Medicine, Columbus, Ohio. Drug, Neurotransmitter and Hormone Receptors in the Regulation of the Cardiovascular System. March 1, 1984.

Ohio State University, Department of Pharmacology, College of Pharmacy, Columbus, Ohio. Possible Relationship Between Spare α_1- and α_2-Adrenoceptors and the Differential Utilization of Calcium for Vasoconstriction In Vivo. March 1, 1984.

Glaxo Group Research Ltd., Department of Cardiovascular Pharmacology, Ware, England. Existence of Spare α_1-Adrenoceptors, but not α_2-Adrenoceptors, in the Vasculature of the Pithed Rat: Possible Relationship to Calcium Utilization. July 26, 1984.

Wishard Hospital, 11th International Cardiovascular Opinion Leaders Meeting, Indianapolis, Indiana. New Insights into the Mechanism of the Inotropic Activity of Dobutamine. May 11, 1984.

Medical College of Wisconsin, Department of Pharmacology, School of Medicine, Milwaukee, Wisconsin. Peripheral Adrenoceptors and Dopamine Receptors in the Cardiovascular System. October 2, 1984.

Veterans Administration Hospital, Research Services, Wood (Milwaukee), Wisconsin. The Role of Central and Peripheral α-Adrenoceptors in the Regulation of Blood Pressure. October 3, 1984.

Invited Lectures (Continued)

Medical College of Pennsylvania, Department of Pharmacology, Philadelphia, Pennsylvania. The Role of Central and Peripheral α-Adrenoceptors in the Regulation of Blood Pressure. November 17, 1984.

Temple University School of Medicine, Department of Pharmacology, Philadelphia, Pennsylvania. The Role of Central and Peripheral α-Adrenoceptors in the Regulation of Blood Pressure. January 16, 1985.

Medical College of Pennsylvania, Department of Physiology, Philadelphia, Pennsylvania. Graduate Course in The Biology of the Arterial Wall. Vascular Adrenergic Receptors. May 1, 1985.

McGill University, Department of Medicine, College of Medicine, Montreal, Canada. Recent Advances in α-Adrenoceptor Research. September 17, 1985.

McGill University, Department of Pharmacology, College of Medicine, Montreal, Canada. Quantitative Analysis of Drug-Receptor Interactions. September 18, 1985.

Wayne State University, Department of Pharmacology, School of Medicine, Detroit, Michigan. Mechanism for the Positive Inotropic Effect of Dobutamine. November 1, 1985.

University of Utah, Department of Biochemical Pharmacology and Toxicology, Salt Lake City, Utah. The Role of Central and Peripheral α-Adrenoceptors in the Regulation of Blood Pressure. November, 1985.

Louisiana State University, Department of Pharmacology, School of Medicine, New Orleans, Louisiana. The Role of Central and Peripheral α-Adrenoceptors in the Regulation of Blood Pressure. April 29, 1986.

West Virginia University Medical Center, Department of Pharmacology and Toxicology, Morgantown, West Virginia. The Role of Central and Peripheral α-Adrenoceptors in the Regulation of Blood Pressure. September 29, 1986.

Warner-Lambert Company, Pharmaceutical Research Division, Ann Arbor, Michigan. Existence of Spare α_1-Adrenoceptors, but not α_2-Adrenoceptors, in the Vasculature: Possible Relationship to Calcium Utilization. May 14, 1986.

McGill University, Department of Medicine, College of Medicine, Montreal, Canada. Recent Developments in α-Adrenoceptor Research. September 22, 1986.

McGill University, Department of Pharmacology, College of Medicine, Montreal, Canada. Existence of Spare α_1-Adrenoceptors, but not α_2-Adrenoceptors, in the Vasculature: Relationship to Calcium Utilization. September 23, 1986.

Laboratoires d'Etudes et de Recherches, SYNTHELABO, Department of Biology, Paris, France. The Pharmacologic Differentiation of Pre- and Postjunctional α_2-Adrenoceptors. September 15, 1986.

Invited Lectures (Continued)

Allergan, Department of Pharmacology, Irvine, California. The Pharmacologic Differentiation of Pre- and Postjunctional α_2-Adrenoceptors. October 9, 1986.

Universidad Central de Venezuela, Division of Pharmacology, Facultad de Farmacia, Caracus, Venezuela. Short Course: The Pharmacologic Basis of Drug-Receptor Interactions. February 23, 1987.

Universidad Central de Venezuela, Division of Pharmacology, Facultad de Farmacia, Caracus, Venezuela. Short Course: Distribution and Function of α- and β-Adrenoceptors in the Cardiovascular System. February 24, 1987.

Universidad Central de Venezuela, Division of Pharmacology, Facultad de Farmacia, Caracus, Venezuela. Short Course: The Role of Central and Peripheral α-Adrenoceptors in the Regulation of Blood Pressure. February 25, 1987.

Universidad Central de Venezuela, Division of Pharmacology, Facultad de Farmacia, Caracus, Venezuela. Short Course: The Pharmacologic Differentiation of Pre- and Postjunctional α_2-Adrenoceptors. February 26, 1987.

Universidad Central de Venezuela, Division of Pharmacology, Facultad de Farmacia, Caracus, Venezuela. Short Course: Future Trends in Pharmacological Sciences. February 27, 1987.

Medical College of Pennsylvania, Department of Physiology Graduate Course entitled "Biology of the Arterial Wall". Lecture title: "Vascular Adrenergic Receptors". Philadelphia, Pennsylvania. May 6, 1987.

Analysts' and Investors' Meeting, SmithKline Beckman Corporation, Wyndham Franklin Plaza Hotel. The Future of Smith Kline & French Pharmaceutical R&D. Philadelphia, Pennsylvania. December 16, 1986.

McGill University, Department of Medicine, College of Medicine, Montreal, Canada. Recent Developments in α-adrenoceptor Research. September 21, 1987.

University of Vermont, Department of Pharmacology, College of Medicine, Burlington, Vermont. The Role of Central and Peripheral α-Adrenoceptors in the Control of Cardiovascular Function. October 8, 1987.

Blood Vessel Club, University of Vermont, Burlington, Vermont. The Pharmacologic Differentiation of Pre- and Postjunctional α_2-Adrenoceptors. October 8, 1987.

American Analysts and Investors' Colloquium, King of Prussia, Pennsylvania. Future Therapeutic Approaches to Congestive Heart Failure. December 2, 1987.

European Analysts and Investors' Colloquium, Welwyn Spring Garden, England. Future Therapeutic Approaches to Congestive Heart Failure. December 4, 1987.

Invited Lectures (Continued)

Food and Drug Administration, Department of Health, Education and Welfare, Bethesda, Maryland. The Pharmacologic Differentiation of Pre- and Postjunctional α_2-Adrenoceptors. February 4, 1988.

Dahlhouse University, Department of Pharmacology, Halifax, Nova Scotia. The Role of Central and Peripheral α-Adrenoceptors in the Control of Blood Pressure. April 12, 1988.

Wayne State University, Department of Pharmacology, School of Medicine, Detroit, Michigan. The Role of Central and Peripheral α-Adrenoceptors in the Control of Blood Pressure. April 1, 1988.

Ohio State University, Department of Veterinary Physiology and Pharmacology, School of Agriculture, Columbus, Ohio. The Role of Central and Peripheral α-Adrenoceptors in the Control of Blood Pressure. May 17, 1988.

Baylor University, School of Medicine, Houston, Texas. Congestive Heart Failure, March 28, 1988.

Baylor University, School of Medicine, Houston, Texas. The Pharmacology of Inotropic Agents. March 28, 1988.

Baylor University, School of Medicine, Houston, Texas. The Pharmacology of Vasodilators, March 28, 1988.

Baylor University, School of Medicine, Houston, Texas. Angina and Antianginal Drugs. March 29, 1988.

Baylor University, School of Medicine, Houston, Texas. Coronary Thrombosis and Thrombolytic Agents. March 29, 1988.

Eli Lilly & Company, Research and Development, Indianapolis, Indiana. 1988 John Jacob Abel Award. Lecture entitled: "The Role of Central and Peripheral α-Adrenoceptors in the Control of Blood Pressure". June 22, 1988.

Food and Drug Administration, Department of Health, Education and Welfare, Bethesda, Maryland. Future Therapeutic Uses of α-Adrenoceptor Agonists and Antagonists. January 11, 1989.

Medical College of Pennsylvania, Department of Pharmacology, Philadelphia, PA. The Role of Guanine Nucleotide Regulatory Proteins in α_1- and α_2-Adrenoceptor Mediated Vasoconstriction. May 8, 1989.

Baylor University, School of Medicine, Houston, Texas. Series of six lectures entitled: "Congestive Heart Failure; Inotropic Agents; Vasodilators; Angina and Antianginal Drugs; Thrombolytic Agents; Antiarrhythmic Drugs". April 3-6, 1989.

Invited Lectures (Continued)

National Institutes of Health, National Institute of Drug Abuse, Bethesda, Maryland. Lecture entitled: "The Role of Guanine Nucleotide Regulatory Proteins and Calcium Utilization in α_1- and α_2-Adrenoceptor Mediated Vasoconstriction". December 6, 1988.

Northeastern University School of Pharmacy, Departments of Pharmacology and Medicinal Chemistry, Boston, Massachusetts. Lecture entitled: "The Role of Guanine Nucleotide Regulatory Proteins in α_1- and α_2-Adrenoceptor Mediated Vasoconstriction". December 13, 1988.

Harvard University, The Brigham and Women's Hospital, Department of Medicine, Boston, Massachusetts. Lecture entitled: "Signal Transduction Processes Involved in α-Adrenoceptor-Mediated Vasoconstriction". December 13, 1988.

University of Houston, School of Pharmacy, Department of Pharmacology, Houston, Texas. Lecture entitled: "Signal Transduction Processes Involved in α-Adrenoceptor-Mediated Vasoconstriction". April 4, 1989.

Ohio State University, College of Pharmacy, Division of Pharmacology, Columbus, Ohio. Lecture entitled: "Signal Transduction Processes Involved in α-Adrenoceptor-Mediated Vasoconstriction". May 12, 1989.

Medical College of Wisconsin, Department of Pharmacology, School of Medicine, Milwaukee, Wisconsin. Lecture entitled: "The Physiology and Pharmacology of α_1- and α_2-Adrenoceptors". September 26, 1989.

Medical College of Wisconsin, Department of Pharmacology, School of Medicine, Milwaukee, Wisconsin. Lecture entitled: "Signal Transduction Processes Involved in α-Adrenoceptor Mediated Vasoconstriction". September 27, 1989.

Laboratoires d'Etudes et de Recherches, SYNTHELABO, Department of Pharmacology, Paris, France. Lecture entitled: "The Role of Guanine Nucleotide Regulatory Proteins and α_1- and α_2-Adrenoceptor Mediated Vasoconstriction". June 28, 1989.

Uniformed Services, Department of Pharmacology, School of Medicine, Bethesda, Maryland. Lecture entitled: "Signal Transduction Processes Involved α-Adrenoceptor-Mediated Vasoconstriction". December 12, 1989.

Laboratoires d'Etudes et de Recherches, SYNTHELABO, Department of Pharmacology, Paris, France. Lecture entitled: " Renal α_2-Adrenoceptors". October 16, 1989.

Department of the Navy, Naval Hospital, Naval Medical Research Institute, Bethesda, Maryland. Lecture entitled: "Signal Transduction Processes Involved in α-Adrenoceptor-Mediated Vasoconstriction". January 8, 1990.

Department of Medicine, Baylor University, Houston, Texas. Series of seven lectures entitled: "Congestive Heart Failure, Inotropic Agents, Vasodilators; Angina and Antianginal Drugs; Thrombolytic Agents; Antiarrhythmic Drugs; Diuretics". April 2-6, 1990.

Invited Lectures (Continued)

Department of Biology, Les Laboratoires Beecham, Rennes, France. Lecture entitled: "Signal Transduction Processes Involved in α-Adrenoceptor-Mediated Vasoconstriction". September 24, 1990.

Laboratoires d'Etudes et de Recherches, SYNTHELABO, Department of Biology, Paris, France. Lecture entitled: "Evidence that a Single α_1-Adrenoceptor is Coupled to Two Signal Transduction Processes in the Vasculature". October 15, 1990.

Department of Pharmacology, Philadelphia College of Pharmacy and Sciences, Philadelphia, Pennsylvania. Lecture entitled: "Signal Transduction Processes Involved in α-Adrenoceptor Mediated Vasoconstriction". December 20, 1990.

Department of Physiology and Biophysics, University of Tennessee, Memphis, Tennessee. Lecture entitled: "Signal Transduction Processes Involved in α-Adrenoceptor Mediated Vasoconstriction". April 19, 1991.

Department of Pharmacology, The Ohio State University, Columbus, Ohio. Lecture entitled: "Signal Transduction Processes Involved in α-Adrenoceptor Mediated Vasoconstriction". May 9, 1991.

Department of Pharmacology, University of Vermont, Burlington, Vermont. Lecture entitled: "A Single α_1-Adrenoceptor Subtype is Coupled to Two Signal Transduction Processes in Vascular Smooth Muscle". June 13, 1991.

Department of Medicine, Baylor University, Houston, Texas. Series of seven lectures entitled: "Congestive Heart Failure, Inotropic Agents, Vasodilators, Angina and Antianginal Drugs, Thrombolytic Agents, Antiarrhythmic Drugs, Diuretics". April 8-11, 1991.

Department of Pharmacology, The Ohio State University, Columbus, Ohio. Lecture entitled: "Molecular Structure and Function of G-Protein Coupled Receptors". May 6, 1991.

Department of Pharmacology, The Ohio State University, Columbus, Ohio. Lecture entitled: "Interactions of Receptors with G-Proteins". May 7, 1991.

Department of Pharmacology, The Ohio State University, Columbus, Ohio. Lecture entitled: "Signal Transduction Processes Activated by G-Protein Coupled Receptors". May 8, 1991.

Department of Pharmacology, The Ohio State University, Columbus, Ohio. Lecture entitled: "Receptor Regulation". May 9, 1991.

Meeting of Opinion Leaders in Hypertension, Troon, Scotland. Lecture entitled: "Cardioprotection by Carvedilol". August 24-25, 1991.

Meeting of Opinion Leaders in Hypertension, Troon, Scotland. Lecture entitled: "The Pharmacology of the Angiotensin II Receptor Antagonists, SK&F 108566 and SB 200220". August 25, 1991.

Invited Lectures (Continued)

Meeting of Opinion Leaders in Hypertension, Troon, Scotland. Lecture entitled: "Basic and Clinical Pharmacology of Carvedilol". August 25, 1991.

Department of Biology, Les Laboratoires Beecham, Rennes, France. Lecture entitled: "Cardioprotection by Carvedilol". September 20, 1991.

Society of Scandinavian Cardiologists, Monte Carlo, Monaco. Lecture entitled: "Cardioprotection by Carvedilol". January 23, 1992.

Department of Medicine, Baylor University, Houston, Texas. Series of seven lectures entitled: "Congestive Heart Failure; Inotropic Agents; Vasodilators; Angina and Antianginal Drugs; Thrombolytic Agents; Antiarrhythmic Drugs; Diuretics". April 27-30, 1992.

Division of Cardiology, Department of Medicine, University of Gothenburg, Gothenburg, Sweden. Lecture entitled: "Protection of Major Cardiovascular Organ Systems by Carvedilol". May 20, 1992.

Department of Medicine, Malmo University, Malmo, Sweden. Lecture entitled: "Protection of Major Cardiovascular Organ Systems; Not Only β-Blockade". May 21, 1992.

Department of Medicine, Karolinska Institute, Stockholm, Sweden. Lecture entitled: "Protection of Major Cardiovascular Organ Systems by Carvedilol". May 25, 1992.

Department of Cardiology, School of Medicine, Upsala, Sweden. Lecture entitled: "Protection of Major Cardiovascular Organ Systems by Carvedilol". May 27, 1992.

Department of Medicine, International Cardiological Institute for Therapeutic Research, Oslo, Norway. Lecture entitled: "Protection of Major Cardiovascular Organ Systems by Carvedilol; Not Only β-Blockade". May 26, 1992.

Department of Medicine, University Hospital, Tromso, Norway. Lecture entitled: "Protection of Major Cardiovascular Organ Systems by Carvedilol; Not Only β-Blockade". May 27, 1992.

Toronto Hypertension Society, Toronto, Canada. Lecture entitled: "α-Adrenoceptors in Hypertension". January 26, 1993.

Division of Cardiology, Department of Medicine, University of Toronto, Toronto, Canada. Lecture entitled: "Signal Transduction Processes Involved in α-Adrenoceptors Mediated Vasoconstriction". January 27, 1993.

Department of Medicine, University of Antwerp, Antwerp, Belgium. Lecture entitled: "Major Organ Protection by Carvedilol". October 27, 1992.

Division of Cardiology, Department of Medicine, University Hospital, Ghent, Belgium. Lecture entitled: "Major Organ Protection by Carvedilol". October 28, 1992.

Invited Lectures (Continued)

Department of Cardiology, University Hospital, Leuven, Belgium. Lecture entitled: "Major Organ Protection by Carvedilol". October 29, 1992.

Division of Cardiology, Department of Medicine, University of Brussels, Belgium. Lecture entitled: "Major Organ Protection by Carvedilol". October 29, 1992.

Department of Medicine, Baylor University, Houston, Texas. Series of seven lectures entitled: "Congestive Heart Failure; Inotropic Agents; Vasodilators; Angina and Antianginal Drugs; Thrombolytic Agents; Antiarrhythmic Drugs; Diuretics". April 12-15, 1993.

Department of Pharmacology, College of Medicine, University of Minnesota, Minneapolis, Minnesota. Lecture entitled: "Stereochemical Requirements of α_1- and α_2-Adrenoceptors". June 25, 1993.

Department of Molecular Biology, Human Genome Sciences, Gaithersburg, Maryland. Lecture entitled: "Drug Development Pipeline at SmithKline Beecham Pharmaceuticals". August 9, 1993.

Department of Medicine, University of Hawaii, Honolulu, Hawaii. Lecture entitled: "The Drug Discovery Process in the Pharmaceutical Industry: Myths and Realities". February 2, 1994.

Department of Medicine, Albert Einstein College of Medicine of Yeshiva University, Bronx, New York. Lecture entitled: "Drug Discovery and Development in the Pharmaceutical Industry". February 7, 1994.

Department of Medicine, Baylor University, Houston, Texas. Series of seven lectures entitled: "Congestive Heart Failure; Inotropic Agents. Vasodilators; Angina and Antianginal Agents; Thrombolytic Agents; Antiarrhythmic Agents; Diuretics". April 4-7, 1994.

Department of Pharmacology, University of Houston, Houston, Texas. Lecture entitled: "Effect of Point Mutations in Transmembrane Helices II and III on the Stereoselective Interaction of Catecholamines with α-Adrenoceptors". April 6, 1994.

University of Houston, Distinguished Visiting Professor Lecture and Award Ceremony, Houston, Texas. Lecture entitled: "Impact of Health Care Reform on the Pharmaceutical Industry". April 5, 1994.

Department of Pharmacology, University of Kansas, Kansas City, Kansas. Lecture entitled: "The Use of Site-Directed Mutagenesis in α_2-Adrenoceptors to Establish the Molecular Basis of Chirality". November 1, 1994.

Department of Medicine, Baylor University Medical School, Houston, Texas. Series of seven lectures entitled: "Congestive Heart Failure; Inotropic Agents, Vasodilators; Angina and Antianginal Agents; Thrombolytic Agents; Antiarrhythmic Agents; Diuretics". March 20-24, 1995.

Invited Lectures (Continued)

Department of Medicine, Albert Einstein College of Medicine of Yeshiva University, Bronx, New York. Lecture entitled: "Drug Discovery and Development in the Pharmaceutical Industry". February 6, 1995.

PhRMA Education and Research Institute Basic Pharmacology Training Course, Merck Corporate Conference Center, Woodridge, New Jersey. Lecture entitled: "Principles of Pharmacodynamics I". April 4, 1995.

PhRMA Education and Research Institute Basic Pharmacology Training Course, Merck Corporate Conference Center, Woodridge, New Jersey. Lecture entitled: "Principles of Pharmacodynamics I". April 4, 1995.

Department of Pharmacology, Stanford University, College of Medicine, Palo Alto, California. Lecture entitled: "Molecular Basis of Chirality for the Interaction of Catecholamines with the Adrenoceptors". July 5, 1995.

Department of Medicine, Stanford University, College of Medicine, Palo Alto, CA, California. Lecture entitled: "The Pharmacology of Carvedilol". July 6, 1995.

World Bank, Washington, D.C. Lecture entitled: "The Process of Drug Discovery and Development: Risks and Pitfalls". June 8, 1995.

National Hospital, Department of Medicine, Oslo, Norway. Lecture entitled: "The Pharmacology of Carvedilol; Rationale for Use in Congestive Heart Failure". June 19, 1995.

Department of Medicine, Bergen, Norway. Lecture entitled: "The Pharmacology of Carvedilol; Rationale for Use in Congestive Heart Failure". June 20, 1995.

Department of Pharmacology, Institute of Pharmacological Sciences, University of Milan, Milan, Italy. Lecture entitled: "Molecular Basis for Stereoselectivity in the Interaction of Catecholamines with α-adrenoceptors". October 25, 1995.

PhRMA Education and Research Institute Basic Pharmacology Training Course, Arlington, Virginia. Lecture entitled: "Principles of Pharmacodynamics I". October 17, 1995.

PhRMA Education and Research Institute Basic Pharmacology Training Course, Arlington, Virginia. Lecture entitled: "Principles of Pharmacodynamics II". October 17, 1995.

Department of Molecular and Medical Pharmacology, University of California, Los Angeles, California. Lecture entitled: "Molecular Basis of Chirality for the Interaction of Catecholamines with the Adrenoceptors". November 8, 1995.

Invited Lectures (Continued)

Congestive Heart Failure Advisory Board Meeting on Carvedilol, Philadelphia, Pennsylvania. Lecture entitled: "The Pharmacology of Carvedilol: Rationale for Use in Congestive Heart Failure". October 12, 1995.

Department of Pharmacology, College of Medicine, Howard University, Washington, D.C. Lecture entitled: "Molecular Basis for Stereoselectivity in the Interaction of Catecholamines with α-Adrenoceptors". November 29, 1995.

Department of Pharmacology, University of Vancouver, Vancouver, Canada. Lecture series entitled "Frontiers in Cardiovascular Research". Lecture entitled: "The Pharmacology of Carvedilol". May 17, 1996.

Laboratoires D'Etudes et de Recherches Synthelabo, Division of Biological Research, Paris, France. Lecture entitled: "Molecular Basis for Stereoselectivity in the Interaction of Catecholamines with α-Adrenoceptors". January 26, 1996.

Laboratoires D'Etudes et de Recherches Synthelabo, Division of Biological Research, Paris, France. Lecture entitled: "The Use of Genomics to Discover Novel Drug Targets". January 26, 1996.

Department of Medicine, Albert Einstein College of Medicine of Yeshiva University, Bronx, New York. Lecture entitled: "Drug Discovery and Development in the Pharmaceutical Industry". February 5, 1996.

Department of Physiology, University of Glasgow, Glasgow, Scotland. Lecture entitled: "Molecular Basis for Stereoselectivity in the Interaction of Catecholamines with α-Adrenoceptors". September 26, 1996.

Department of Medicine, Baylor University College of Medicine, Houston, Texas. Series of Lectures entitled: "Congestive Heart Failure; Inotropic Agents, Vasodilators; Angina and Antianginal Drugs". August 8-9, 1996.

Department of Cardiology, Hospital General Universitari Vall D'Hebron, Barcelona, Spain. Lecture entitled: "The Antioxidant Activity of Carvedilol: Clinical Implications". December 13, 1996.

Italian Ministry of Health, Instituto di Sanita, Rome, Italy. Lecture entitled: "Innovation in Research and Development of Drugs: A Global Approach". May 28, 1997.

Joint Research & Development, Boehringer Mannheim and Daiichi Pharmaceutical Companies, Tokyo, Japan. Lecture entitled: "The Antioxidant Activity of Carvedilol and its Relationship to Congestive Heart Failure". September, 1997.

Department of Medicine, Baylor University College of Medicine, Houston, Texas. Series of Lectures entitled: "Congestive Heart Failure; Inotropic Agents; Vasodilators; β-Blockers in Heart Failure; Angina and Antianginal Drugs". August 18-19, 1997.

Invited Lectures (Continued)

Grand Rounds; Department of Medicine, Louisiana State University, New Orleans, Louisiana. Lecture entitled: "Rationale for the Use of β-Blockers in Congestive Heart Failure: Experience with Carvedilol". October 31, 1997.

Yamanouchi Tsukuba Research Center, Tsukuba, Japan. Lecture entitled: "The Research and Development Pipeline at SmithKline Beecham". September 17, 1997.

Hungarian Academy of Sciences, Budapest, Hungary. Lecture entitled: "The Molecular Basis for the Stereoselective Interactions of Drugs with α-Adrenoceptors". October 14, 1997.

Albert Szentgyörgy Medical School, Department of Pharmacology, Szeged, Hungary. Lecture entitled: "The Pharmacology of Carvedilol: Use in Congestive Heart Failure". October 17, 1997.

Department of Pharmacology, University of West Virginia, Morgantown, Virginia. Lecture entitled: "The Molecular Basis for the Stereoselective Interactions of Drugs with α-Adrenoceptors". November 17, 1997.

Department of Medicine, Albert Einstein University, College of Medicine, Bronx, New York. Lecture entitled: "The Discovery and Development of Carvedilol: From the test-tube to the Patient". December 17, 1997.

John V. Croker Lecture, The American Society for Pharmacology and Experimental Therapeutics, San Francisco, California. Lecture entitled: "Pharmacology and the Pharmaceutical Industry: An assessment of the present and a prediction of the future". April 19, 1998.

Program Introduction Meeting, SB The Netherlands, New York, New York. Lecture entitled: "The Effect of Teveten on the Sympathetic Nervous System". April 26, 1998.

Department of Medicine, Baylor University College of Medicine, Houston, Texas. Series of lectures entitled: "Congestive Heart Failure, Angina and Antianginal Drugs". August 13, 1998.

Wharton Business School, Philadelphia, Pennsylvania: Lecture entitled: "Drug Discovery in the Pharmaceutical Industry". April 26, 1999.

Phentermine Legal Defense Team (approx. 120 Attorneys), Wyndham Franklin Plaza Hotel, Philadelphia, Pennsylvania: Lecture entitled: "The Pharmacology of Phentermine and Related Sympathomimetic Amines". January 14, 1999.

Department of Medicine, Tulane University and Louisiana State University, New Orleans, Louisiana. Lecture entitled: "New Frontiers in the Pharmacotherapy of Congestive Heart Failure: The Pre-Clinical and Clinical Pharmacology of Carvedilol". June 16, 1999.

Clinical Development Partners Annual Meeting, Phoenix, Arizona. Lecture entitled: "The Impact of Genomics on Drug Discovery". April 30, 1999.

Invited Lectures (Continued)

Meeting of the Key Opinion Leaders in Nephrology, New York, NY. Lecture entitled: "The Renal Protective Effects of Carvedilol (Coreg®) and Rosiglitazone (Avandia®)". April 19, 1999.

Department of Medicine, Baylor University College of Medicine, Houston, Texas. Series of lectures entitled: "Congestive Heart Failure, Angina and Antianginal Drugs". August 23, 1999.

Grand Rounds, Department of Medicine, Tallahassee, Florida. Lecture entitled: "New Frontiers in the Pharmacotherapy of Congestive Heart Failure: The Pre-Clinical and Clinical Pharmacology of Carvedilol". September 1, 1999.

Grand Rounds, Department of Medicine, Tampa, Florida. Lecture entitled: " New Frontiers in the Pharmacotherapy of Congestive Heart Failure: Recent Experience with β-Blockers". November 11, 1999.

Grand Rounds, Department of Medicine, Panama City, Florida. Lecture entitled: "New Frontiers in the Pharmacotherapy of Congestive Heart Failure: The Role of β-Blockers". November 17, 1999.

Division of Pharmacology, Johns Hopkins University, Baltimore, Maryland: Lecture entitled: "The Discovery and Development of Carvedilol: From the Test-Tube to the Patient". March 1, 2000.

Division of Pharmacology, Johns Hopkins University, Baltimore, Maryland: Lecture entitled: "The Molecular Basis of the Stereoselective Interactions of Catecholamines with the Adrenoceptors". March 1, 2000.

Grand Rounds, Tampa, Florida. Lecture entitled: "New Frontiers in the Pharmacotherapy of Congestive Heart Failure: The Pre-Clinical and Clinical Pharmacology of Carvedilol". March 7, 2000.

Wharton Business School, Philadelphia, Pennsylvania: Lecture entitled: "Drug Discovery in the Pharmaceutical Industry". May 1, 2000.

Grand Rounds, Department of Medicine, Panama City, Florida. Lecture entitled: "New Frontiers in the Pharmacotherapy of Congestive Heart Failure: The Role of β-Blockers". May 11, 2000.

Grand Rounds, Department of Medicine, Pensacola, Florida. Lecture entitled: "New Frontiers in the Pharmacotherapy of Congestive Heart Failure: The Role of β-Blockers". May 12, 2000.

Grand Rounds, Department of Medicine, Tampa, Florida. Lecture entitled: "New Frontiers in the Pharmacotherapy of Congestive Heart Failure: The Role of β-Blockers". November 2, 2000.

Invited Lectures (Continued)

Grand Rounds, Department of Medicine, Fort Walton Beach, Florida. Lecture entitled: "New Frontiers in the Pharmacotherapy of Congestive Heart Failure: The Role of β-Blockers". October 12, 2000.

Grand Rounds, Department of Medicine, Mount Sinai School of Medicine, New York. Lecture entitled: "New Frontiers in the Pharmacotherapy of Congestive Heart Failure: The Role of β-Blockers". October 19, 2000.

University of Minnesota, College of Medicine; Department of Pharmacology, Minneapolis, Minnesota. Lecture entitled: "Modern Drug Discovery and Development", 2002.

John Hopkins University, School of Medicine; Department of Pharmacology, Baltimore, Maryland. Lecture entitled: "Drug Discovery and Development; Experience with Carvedilol (Coreg)". March 4, 2002.

Accenture Discovery Advisory Board, Philadelphia, Pennsylvania. Lecture entitled: "Increasing Productivity in the R&D Process". June 3, 2002.

Heidrick and Struggles Global Healthcare Meeting, Princeton, New Jersey. Lecture entitled: "Filling the R&D Pipeline". April 29, 2002.

University of Minnesota, Department of Pharmacology, College of Medicine, Minneapolis, Minnesota. Lecture entitled: "Drug Discovery in the New Millennium". April 25, 2003.

Division of Pharmacology, School of Medicine, John Hopkins University, Baltimore, Maryland. Lecture entitled: "Drug Discovery and Development; Experience with Carvedilol (Coreg)". February 11, 2004.

College of Pharmacy, The Ohio State University, Columbus, OH. Lecture entitled: "The Discovery and Development of Carvedilol: From the Test Tube to the Patient". May 7, 2004.

FDA Grand Rounds, Rockville, Maryland. Lecture entitled: "Issues Affecting R&D Productivity: Obstacles and Solutions". September 2, 2004.

Grand Rounds, Department of Obstetrics and Gynecology, University of Florida, Gainesville, Florida. Lecture entitled: "The Future of Women's Health: A Pipeline Perspective". November 19, 2004.

Grand Rounds, Department of Cardiology, University of Florida, Gainesville, Florida. Lecture entitled: "The Discovery of Carvedilol (Coreg) for the Treatment of Congestive Heart Failure: From the Test Tube to the Patient". November 19, 2004.

Grand Rounds, Department of Obstetrics and Gynecology, Emory University, Atlanta, Georgia. Lecture entitled: "The Future of Women's Health: A Pipeline Perspective". March 30, 2005.

Invited Lectures (Continued)

John Hopkins University, School of Medicine; Department of Pharmacology, Baltimore, Maryland. Lecture entitled: "Drug Discovery and Development; Experience with Carvedilol (Coreg)". March 3, 2005.

Department of Pharmacology, Vanderbilt University, College of Medicine. Lecture entitled: "Opportunities in the Pharmaceutical Industry in the 21st Century". Nashville, Tennessee, July 26, 2005.

Grand Rounds, Health Sciences Grand Rounds, West Virginia University Medical School. Lecture entitled: "The Discovery and Development of Coreg (Carvedilol) for the Treatment of Congestive Heart Failure: The Saga of Carvedilol". Morgantown, West Virginia, August 31, 2006.

Grand Rounds, Department of Medicine, West Virginia University School of Medicine. Lecture entitled: "Drug Discovery and Development: Impact on the Cost of Health Care Delivery". Morgantown, West Virginia, September 1, 2006.

Distinguished Lecture Series, Temple University. Lecture entitled: "The Discovery and Development of Coreg (Carvedilol): A New Paradigm for the Treatment of Congestive Heart Failure". Philadelphia, Pennsylvania, November 30, 2006.

John Hopkins University, School of Medicine; Department of Pharmacology, Baltimore, Maryland. Lecture entitled: "Drug Discovery and Development; Experience with Carvedilol (Coreg)". March 10, 2006.

Health Sciences Center, West Virginia University Medical School. Lecture entitled: "Management of Change to Increase Productivity in a Scientific/Technical Environment". Morgantown, West Virginia, January 16, 2007.

John Hopkins University, School of Medicine; Department of Pharmacology, Baltimore, Maryland. Lecture entitled: "Drug Discovery and Development; Experience with Carvedilol (Coreg)". March 12, 2007.

Massachusetts College of Pharmacy and Health Sciences. Lecture entitled: "The Trials and Tribulations of a Medical Breakthrough: The Discovery and Development of Coreg (Carvedilol) for the Treatment of Congestive Heart Failure". Boston, Massachusetts, April 17, 2008.

Department of Pharmacology, University of Michigan School of Medicine. Lecture entitled: "The Trials and Tribulations Behind a Medical Breakthrough: The Discovery and Development of Coreg (Carvedilol) for the Treatment of Congestive Heart Failure". Ann Arbor, Michigan, October 8, 2008.

John Hopkins University, School of Medicine; Department of Pharmacology, Baltimore, Maryland. Lecture entitled: "Drug Discovery and Development; Experience with Carvedilol (Coreg)". March 4, 2008.

Columbia University School of Business, New York, New York. Lecture entitled: "Clinical Development and the Drug Approval Process-Interactions with the FDA". February 2, 2009.

Invited Lectures (Continued)

University of Delaware, Department of Psychiatry, Newark, Delaware. Lecture entitled: "The Drug Discovery and Development Process". April 1, 2009

Johns Hopkins University, School of Medicine, Department of Pharmacology, Baltimore, Maryland. Lecture entitled: "Drug Discovery and Development; Experience with Carvedilol (Coreg). April 1, 2009.

Columbia University, School of Business, New York, New York. Lecture entitled: "The Drug Discovery and Development Process". April 3, 2009.

Ohio State University, Division of Pharmacology, College of Pharmacy, Columbus, Ohio. Lecture entitled: "Overview of the Pharmaceutical Industry; The World's Most Unique Industry". May 6, 2009.

Ohio State University, Division of Pharmacology, College of Pharmacy, Columbus, Ohio. Lecture entitled: "The Research & Development Process in the Pharmaceutical Industry; The Source of Most New Medicines". May 6, 2009.

Ohio State University, Division of Pharmacology, College of Pharmacy, Columbus, Ohio. Lecture entitled: "The Interplay Between the Pharmaceutical Industry, Academia, Research Institutes and the Government in the Development of New Drugs". May 7, 2009.

Ohio State University, Division of Pharmacology, College of Pharmacy, Columbus, Ohio. Lecture entitled: "Training Needs for the Pharmaceutical Industry; Expectations of Graduate Education". May 7, 2009.

Ohio State University, Division of Pharmacology, College of Pharmacy, Columbus, Ohio. Lecture entitled: "My Journey from OSU to the Pharmaceutical Industry and Back Again to OSU". May 8, 2009.

The Great Oxford Debate, Oxford University, United Kindgom. The Role of the Pharmaceutical Industry and Academia in Conducting Clinical Trials. September 23, 2009.

Columbia University School of Business, New York, New York. Lecture entitled: "Clinical Development and the Drug Approval Process-Interactions with the FDA". August 18, 2009

Columbia University School of Business, New York, New York. Lecture entitled: "Clinical Development and the Drug Approval Process-Interactions with the FDA". January 22, 2009

Department of Pharmacology, University of Florida, College of Pharmacy. Lecture entitled: "The Trials and Tribulations Behind a Medical Breakthrough: The Discovery and Development of Coreg (Carvedilol) for the Treatment of Congestive Heart Failure". Gainesville, Florida, February 19, 2009.

Invited Lectures (Continued)

Columbia University School of Business, New York, New York. Lecture entitled: "Clinical Development and the Drug Approval Process-Interactions with the FDA". March 31, 2010

Columbia University School of Business, New York, New York. Lecture entitled: "Clinical Development and the Drug Approval Process-Interactions with the FDA". March 30, 2011

Columbia University School of Business, New York, New York. Lecture entitled: "Clinical Development and the Drug Approval Process-Interactions with the FDA". April 4, 2012

Columbia University School of Business, New York, New York. Lecture entitled: "Clinical Development and the Drug Approval Process-Interactions with the FDA". April 3, 2013

Columbia University School of Business, New York, New York. Lecture entitled: "Clinical Development and the Drug Approval Process-Interactions with the FDA". April 2, 2014

IMPACT Branding & Design, LLC, Wallingford, Connecticut: Lecture entitled: "The Qualities of Leadership". September 19, 2014.

Invited Lectures (Continued)

The Hartford, Hartford, Connecticut. Lecture Entitled: "Leadership Practices Relevant to the 21st Century Business Environment". June 18, 2010

Columbia University School of Business, New York, New York. Lecture entitled: "Clinical Development and the Drug Approval Process-Interactions with the FDA". April 3, 2009

XenoBiotic, Plainsboro, New Jersey. Lecture entitled: "The Trials and Tribulations Behind a Medical Breakthrough: The Discovery and Development of Coreg (Carvedilol) for the Treatment of Congestive Heart Failure". September 29, 2011.

Columbia University School of Business, New York, New York. Lecture entitled: "Clinical Development and the Drug Approval Process-Interactions with the FDA". March 29, 2010.

Chauncey D. Leake Award Lecture, Columbus, Ohio. Lecture entitled: "The Drug Discovery and Development Process: A Case Study with Carvedilol for the Treatment of Heart Failure". Spring, 2013

Columbia University School of Business, New York, New York. Lecture entitled: "Clinical Development and the Drug Approval Process-Interactions with the FDA". March 26, 2012.

University of Florida, College of Pharmacy, Orlando, Florida. Lecture entitled: "Drug Discovery and Development: Past Present and Future". November 8, 2012.

Columbia University School of Business, New York, New York. Lecture entitled: "Clinical Development and the Drug Approval Process-Interactions with the FDA". April 3, 2013.

Columbia University School of Business, New York, New York. Lecture entitled: "Clinical Development and the Drug Approval Process-Interactions with the FDA". April 3, 2014.

Columbia University School of Business, New York, New York. Lecture entitled: "Clinical Development and the Drug Approval Process-Interactions with the FDA". April 3, 2015.

Servier, Paris, France. Lecture entitled: "Qualities of Leadership Necessary to Change the Direction of a Large R&D Organization", September 7, 2015.

Invited Symposium Speaker

American Society for Pharmacology and Experimental Therapeutics, Graduate Student Convocation, Columbus, Ohio. Lecture Title: Pharmacological and Biochemical Characterization of Adrenoceptors. August, 1977.

International Symposium on Neuroreceptors. Lecture entitled: Alpha-Adrenoceptors. Terre Haute Center for Medical Education, Terre Haute, Indiana, September 25, 1982.

International Symposium on Neuroreceptors. Lecture entitled: The Use of Isolated Intact Tissues to Study Neurotransmitter Receptors. Terre Haute Center for Medical Education, Terre Haute, Indiana, September 25, 1982.

International Symposium, "Stereochemistry and Biological Activity of Drugs". Stereoselectivity in Adrenergic Agonists and Adrenergic Blocking Agents. Noordwijkerhout, Holland, October 21-22, 1982.

American Chemical Society Symposium: "Alpha$_2$-Adrenergic Receptors". Stereochemical Requirements of Alpha$_2$-Adrenergic Receptors. Seattle, Washington, March 21, 1983.

Society of Critical Care Medicine Symposium, Plenary Lecture: Drug, Neurotransmitter and Hormone Receptors in the Regulation of the Cardiovascular System. New Orleans, Louisiana, May 27, 1983.

Society of Critical Care Medicine Symposium, Pharmacological Vignettes: Dobutamine Enantiomers, Contribution of α- and β-Adrenoceptor Activity to Inotropic Selectivity. New Orleans, Louisiana, May 26, 1983.

American Society for Pharmacology and Experimental Therapeutics, Symposium on "Peripheral Alpha-Adrenergic Receptors". Interactions of Agonists with Peripheral Alpha-Adrenergic Receptors. Philadelphia, Pennsylvania, August 11, 1983.

Symposium on Centroperipheral Resetting Loops, organized by the Department of Medical Pharmacology, Pharmacology Institute of Milan, Subclassification of Adrenoceptors, Florence, Italy, November 13-17, 1983.

Fourth International Symposium of Intensive Care and Emergency Medicine, Plenary Lecture. The Role of Central and Peripheral Alpha- and Beta-Adrenoceptors in the Control of Cardiovascular Function. Brussels, Belgium, March 28-30, 1984.

Workshop on Alpha-Adrenoceptors, Session Title: How Many Types of α-Adrenoceptors are Currently Required. Lecture Title: Agonist Potency Series, Ross Priory on Loch Lomondside, Glasgow, Scotland, July 27-28, 1984.

Satellite Symposium of the 9th International Congress of Pharmacology, Pharmacology of Adrenoceptors: Selective α_1-Adrenoceptor Agonists and Antagonists. Manchester, England, August 6, 1984.

Invited Symposium Speaker (Continued)

American Society for Pharmacology and Experimental Therapeutics, Symposium entitled "α-Adrenoceptor Distribution and Function". Lecture Title: Distribution and Function of Peripheral α-Adrenoceptors. Indianapolis, Indiana, August, 1984.

Symposium on Contemporary Issues in the Management of Chronic Congestive Heart Failure. Lecture Title: Importance of Receptor Regulation in the Pathophysiology and Therapy of Congestive Heart Failure, Baltimore, Maryland, May 3, 1985.

Federation of American Societies for Experimental Biology (FASEB) Symposium Title: "Subtypes of Alpha-Adrenoreceptors in Systemic and Pulmonary Vascular Beds". Lecture Title: Spare Alpha-Adrenoceptors: Excitation-Contraction Coupling. Anaheim, California, April, 1985.

Joint Statistical Meetings of the American Statistical Association Symposium entitled "Statistical Aspects of Drug-Receptor Interactions". Lecture Title: Pharmacological Basis of Drug-Receptor Interaction. Las Vegas, Nevada, August 5-8, 1985.

International Symposium entitled "Brain Epinephrine Neuronal Functions". Lecture Title: Alpha-Adrenoceptor Coupling to Vasoconstriction in the Peripheral Circulation. Baltimore, Maryland, September 29 - October 2, 1985.

Symposium entitled "The Adrenergic Receptors". Lecture Title: Alpha-Adrenoceptor Location and Function in the Cardiovascular System. University of Michigan, Ann Arbor, Michigan, May 15, 1986.

Symposium entitled "The Adrenergic Receptors". Lecture Title: The Role of Central and Peripheral Alpha-Adrenoceptors in the Regulation of Blood Pressure. University of Michigan, Ann Arbor, Michigan, May 15, 1986.

Symposium Honoring The Centennial Celebration for The Ohio State University College of Pharmacy. Lecture Title: Visions of the Future in Pharmacology. Columbus, Ohio, September 13, 1985.

Future of Cardiovascular/Renal Therapeutics Symposium. Lecture Title: The Functional Role of α_2-Adrenoceptors in the Peripheral Arterial and Venous Circulation. Naples, Florida, February 22, 1986.

Smooth Muscle Function Symposium, Satellite Symposium of XXX International Congress of the Physiology. Lecture Title: Pharmacologic Basis of Drug-Receptor Interaction. Banff, Alberta, Canada, July 2, 1986.

International Committee on Medicinal Chemistry Symposium entitled "Recent Advances in Receptor Chemistry". Lecture Title: The Mode of Action and Structure - Activity Relationships Among Imidazoline-like Compounds Acting at the α-Adrenoceptor. Camerino, Italy, September 6-10, 1987.

Invited Symposium Speaker (Continued)

New York Academy of Sciences and the Giovanni Lorenzini Medical Foundation Symposium on "Calcium Antagonists: Pharmacology and Clinical Research". Lecture Title: Causes of Heterogeneity in the Importance of Calcium Entry in Vascular Smooth Muscle. New York, February 11-13, 1987.

Satellite Symposium to the 11th Scientific Meeting of the International Society of Hypertension entitled "Adrenergic Receptor Function and Cardiovascular Reactivity in Human Hypertension". Lecture Title: Arterial α_2-Adrenoceptor Blockade: A New Approach to Antihypertensive Therapy. Essen, Federal Republic of Germany, September 8, 1986.

Vascular Neuroeffector Mechanisms 6th International Symposium, session on "α-Adrenoceptors in the Vasculature". Lecture Title: The relationship between agonist efficacy and receptor reserve to the sensitivity of α-adrenoceptor-mediated vasoconstriction to inhibition by calcium entry blockers. Melbourne, Australia, August 30-September 2, 1987.

Federation of the American Societies for Experimental Biology (FASEB) Symposium entitled "Vasomotor Regulatory Mechanisms: Central and Peripheral Aspects". Lecture title: Adrenergic Receptor Distribution and Function. Washington, DC, April, 1987.

American Society for Pharmacology and Experimental Therapeutics (ASPET) Symposium entitled "Spare α-Adrenoceptors in the Vasculature". Lecture title: The relationship between α_1-adrenoceptor reserve and calcium utilization in the vasculature. Honolulu, Hawaii, August, 1987.

14th Annual Harvard Medical School Postgraduate Course entitled "Intensive Care Medicine-Mastering the New Skills". Lecture title: Clinical Implications of Adrenergic Physiology. Boston, Massachusetts, April 27-29, 1987.

Federation of the American Societies for Experimental Biology (FASEB). Catecholamine Club Annual Dinner. Lecture title: Pharmacologic Differentiation Between Pre- and Postjunctional α_2-Adrenoceptors. Washington, DC, March 31, 1987.

Future of Cardiovascular/Renal Therapeutics Symposium. Lecture title: The Pharmacologic Differentiation Between Pre- and Postjunctional α_2-Adrenoceptors: Relevance to Cardiovascular Disease. Tempe, Arizona, March 14, 1987.

Federation of the American Societies for Experimental Biology (FASEB). Symposium entitled "Renal α_2-Adrenoceptors". Lecture title: Pharmaceutical Aspects of α_2-Adrenoceptors. Las Vegas, Nevada, May, 1988.

Xth International Congress of Pharmacology, Symposium entitled "Drug Metabolism and its Pharmacokinetic and Pharmacodynamic Consequences". Lecture title: Enantioselectivity: Its Biological Basis and Pharmacological Consequences. Sydney, Australia, August 27, 1987.

Invited Symposium Speaker (Continued)

Future Therapeutic Approaches to Congestive Heart Failure. Lecture title: "α-Adrenoceptor Antagonists", London, England, November 7-8, 1987.

Future Therapeutic Approaches to Ischemic Heart Disease. Lecture title: "Acute Myocardial Infarction: The Role of Thromboxane A_2 Receptor Antagonists in Coronary Thrombosis and Thrombolysis", San Juan, Puerto Rico, February 12-14, 1988.

Mid-Atlantic Pharmacology Society Symposium entitled "Molecular Pharmacology: Future Drug Development". Lecture title: "Molecular Pharmacology of α-Adrenoceptors: Future Drug Development", Jefferson University, Philadelphia, Pennsylvania, April 8, 1988.

International Carvedilol Symposium. Lecture entitled: "Preclinical Pharmacology of Carvedilol". Nice, France, June 11, 1988.

Federation of the American Societies for Experimental Biology (FASEB). Overview entitled "Distribution Function, Isolation and Subclassification of α_2-Adrenoceptors. Las Vegas, Nevada, May 2, 1988.

16th Annual Harvard Medical Course in Intensive Care Medicine, Harvard Medical School. Lecture title: "The α-Adrenergic Receptor: New Insights into Lung and Cardiovascular Function", Boston, Massachusetts, April 27, 1989.

American Motility Society Symposium on "Cell Membrane Receptors". Lecture title: "Adrenergic Receptors", Monterey, California, October 3, 1988.

National Institutes of Health Symposium on "Animal Use in Research". Lecture title: "Use of Animal Model Systems in Drug Discovery". Washington, D.C. May 1-2, 1989.

Federation of American Societies for Experimental Biology (FASEB) symposium entitled "Subclassification of α-Adrenoceptors". Lecture entitled: Interaction of Vascular α_1-Adrenoceptors with Multiple Signal Transduction Pathways". Washington, DC, 1990.

13th Annual Conference on Shock, symposium on "Basic Pharmacologic Principles Applied to Shock Research" Lecture entitled: "Characterization of Receptors by Physiologic Assays". Durango, Colorado, June 11, 1990

XIth International Congress of Pharmacology, Satellite Symposium entitled "Pharmacology of Adrenoceptors." Lecture entitled: "Structure Activity Relationships". Manchester, England, June 27, 1990.

10th International Symposium on Intensive Care and Emergency Medicine. Lecture entitled: "The Role of α_1- and α_2-Adrenoceptors in the Regulation of the Cardiovascular System". Brussels, Belgium, March 28, 1990.

Invited Symposium Speaker (Continued)

Roussel Scientific Institute - Table Ronde on "Chirality and Drug Design". Lecture entitled: "Stereospecificity at Receptors". Oxford, England, July 12-13, 1990.

Federation of American Societies for Experimental Biology (FASEB) symposium entitled: "Impact of Federal Agencies on Drug Development and Utilization". Lecture entitled: "Identification of Novel α-Adrenoceptor Agonists and Antagonists for Drug Development". Washington, DC, 1990.

Second Congress on "Strategies and Prospects in Cardiovascular Research", Satellite symposium on Carvedilol. Lecture entitled: "The Pharmacology of Carvedilol". Antwerp, Belgium, February 20, 1990.

International Symposium on Presynaptic Receptors and Neuronal Transporters. Lecture entitled: "Possible Heterogeneity Between Prejunctional and Postjunctional $\alpha 2$-Adrenoceptors in the Cardiovascular System". Rouen, France, June 26-29, 1990.

Vascular Neuroeffector Mechanism 7th International Symposium. Lecture entitled: "Interaction of Vascular α_1-Adrenoceptors with Multiple Signal Transduction Pathways". Bonn, West Germany, July 8-11, 1990.

Thrombolytics Symposium. Lecture entitled: "The Future of Cardiovascular Therapeutics". Bermuda, March 30, 1990.

Intensive Care and Emergency Medicine Symposium; Tutorial entitled: Adrenergic Receptors, Brussels, Belgium, March 29, 1990.

Twentieth Annual Meeting of New England Pharmacologists, Wallingford, Connecticut. Lecture entitled: "Signal Transduction Processes Involved α-Adrenoceptor-Mediated Vasoconstriction". February 1, 1991.

International Symposium on "Carvedilol; Refining Antihypertensive Therapy". Lecture entitled "The Pharmacology of Carvedilol", Paris, France, October 19, 1990.

XIth International Congress of Pharmacology, Satellite Symposium entitled "Pharmacology of Adrenoceptors". Lecture entitled: Molecular Structure and Genetics of Adrenoceptors. Manchester, England, June 27, 1990.

International Symposium Entitled "Receptors: Actualization and Clinical Importance". Lecture entitled: "Adrenoceptors". Santiago, Chile, July 8-31, 1991.

International Symposium on "Carvedilol; Refining Antihypertensive Therapy". Lecture entitled "Antihypertensive Drugs and the Coronary Circulation", Paris, France, October 19, 1990.

First International Symposium on "Imidazoline Specific Receptors". Lecture entitled: "Evolution of the concept from $\alpha 2$-adrenoceptors to imidazoline specific receptors". Paris, March, 1992.

Invited Symposium Speaker (Continued)

Twentieth Annual Meeting of New England Pharmacologists, Wallingford, Connecticut. Lecture Panel on "Pharmacology in the Industrial Setting". February 1, 1991.

Symposium in Medicinal Chemistry on "Structure-Activity Relationships". Lecture entitled: "Structure-activity relationships of α-adrenoceptor agonists and antagonists". Minneapolis, Minnesota, June 28, 1991.

German-Austrian Society of Critical Care Medicine. Lecture entitled: "Preclinical Pharmacology of Fenoldopam". Hannover, Germany, October 25, 1991.

Symposium on "Carvedilol: The Promise of the Future". Lecture entitled: "The Pharmacology of Carvedilol", Handbury Manor, England, March 23, 1991.

Swedish Academy of Pharmaceutical Sciences Symposium on "Neuromedicinal Chemistry of G-Protein Coupled Receptors". Lecture entitled: Medicinal Chemistry of Adrenoceptor Agonists", Lund, Sweden, May 20-22, 1992.

Conference on Vascular Smooth Muscle. Lecture entitled: "Signal transduction processes involved in α-adrenoceptor mediated vasoconstriction", Burlington, Vermont, June 13, 1991.

Annual Conference of the Chairmen of Pharmacology in United States Medical Schools. Lecture entitled: "Training Needs of the Pharmaceutical Industry in Pharmacology", Cloisters, Georgia, February 15, 1992.

Annual Medical Sciences Symposium of the University of Calgary. Lecture entitled: "Signal transduction processes involved in α-adrenoceptor mediated vasoconstriction", The University of Calgary, Calgary, Alberta, Canada, March, 1992.

International Cardiological Institute for Therapeutic Research Symposium on "Carvedilol: Wider Therapeutic Potential in Cardiovascular Syndromes", Satellite Symposium of the 13th Congress of the European Society of Cardiology. Lecture entitled: Cardio-protective Potential of Carvedilol, Scheveningen, The Netherlands, August 23, 1991.

International Symposium Entitled "Hypertension and Concomitant Diseases in the Elderly: New Perspectives". Lecture entitled: "Carvedilol - Novel Pharmacology with Far Reaching Clinical Potential". Monte Carlo, Monaco, January 22-25, 1992.

International Symposium Entitled "From α_2-Adrenoceptors to the Imidazoline-Preferring Receptors". Satellite Symposium of the 7th International Catecholamine Symposium. Keynote lecture entitled "From α_2-Adrenoceptors to the Imidazoline-Preferring Receptors: An Historical Overview". Paris, France, June 29-30, 1992.

International Symposium entitled: "Peptide Therapies in Developmental Gastroenterology and Nutrition". Lecture entitled "The G-Protein Family of Peptide Receptors", Columbus, Ohio, October 8-10, 1992.

Invited Symposium Speaker (Continued)

International Symposium entitled: "Anti-atherosclerotic Drugs: Medicinal, Chemical and Biochemical Aspects". Sponsored by the Division of Medical Chemistry, American Chemical Society. Lecture entitled: "Adrenoceptors and Lipid Lipoprotein Metabolism", Cincinnati, Ohio, May 28, 1992.

International Symposium entitled: "Vasodilating β-Blockers: Hemodynamics, Clinical Implications and Promises for the Future". Continuing Medical Education Symposium. Lecture entitled: "Cardioprotective Potential of Carvedilol". Reims, France, April 28, 1992.

American Society for Pharmacology and Experimental Therapeutics (ASPET) Symposium entitled: "Graduate Pharmacology Instruction in the Age of Molecular Biology". Lecture entitled: "Traditional vs. Molecular/Cellular-Based Pharmacology: A View from Industry". Orlando, Florida, August 14-18, 1992.

Twenty Second Annual Meeting of the New England Pharmacologists. Lecture entitled: Training Needs in the Pharmaceutical Industry in Pharmacology. Lake Morey Resort, Sarlee, Vermont, February 2-6, 1993.

International Symposium on Carvedilol. Lecture entitled: "Major Organ Protection by Carvedilol". Brussels, Belgium, October 26-30, 1992.

First International Symposium on Imidazoline Preferring Receptors. Led and Chaired Discussion Group. Lecture entitled: "Future Directions", Paris, France, June 30, 1992.

International Symposium on Polychlorinated Biphenyls (Sponsored by the Environmental Protection Agency). Lecture entitled: "Receptor Theory: Agonists, Partial Agonists and Competitive Antagonists". Chicago, Illinois, August 17, 1992.

International Symposium on Carvedilol. Lecture entitled "Major Organ Protection by Carvedilol". London, England, October 21-22, 1992.

Australian Society of Pharmacology/Western Society of Pharmacology Joint Meeting. Symposium entitled "Novel Nonpeptide Angiotensin II Receptor Antagonists". Lecture entitled: "Cardiovascular Effects of the Nonpeptide Angiotensin II Receptor Antagonists". Lake Tahoe, Nevada, February 1-5, 1993.

International Symposium on Carvedilol. Lecture entitled: "The Pharmacology of Carvedilol: Major Organ Protection". Stockholm, Sweden, March 9, 1993.

Pharmaceutical Manufactures Education and Research Institute. Symposium on "Basic Pharmacology". Lecture entitled: "General Principles: Pharmacodynamics I". Philadelphia, Pennsylvania, February 23, 1993.

Pharmaceutical Manufactures Education and Research Institute. Symposium on "Basic Pharmacology". Lecture entitled: "General Principles: Pharmacodynamics II". Philadelphia, Pennsylvania, February 23, 1993.

Invited Symposium Speaker (Continued)

XIIIth International Symposium on Medicinal Chemistry. Symposium on "Low Urinary Tract Diseases". Lecture entitled: "Recent Progress in the Treatment of Low Urinary Tract Diseases", Paris, France, September 19-23, 1994

International Symposium on "Multiple Action Antihypertensives, Hemodynamics, Clinical Implications and Promises for the Future: An Update. Lecture entitled: "Free radicals in ischemic tissue damage; role for carvedilol. Luxemburg, May 8, 1993.

Satellite Symposium of the XVth Congress of the European Society of Cardiology Symposium entitled: "The Heart Failure Syndrome - From Prevention to Treatment". Lecture entitled: "A Role for the Antiproliferative and Antioxidative Effects of Carvedilol". Nice, France, August 29, 1993.

Symposium entitled "Ischemic Episodes: Strategies in Cardioprotection and the Role of Free Radicals". Lecture entitled: "The Promise of Protection: Carvedilol is a Potent Antioxidant. Oslo, Norway, March 8, 1993.

International Launch Symposium on Carvedilol. Lecture entitled: "The Pharmacology of Carvedilol: Antiproliferative and Antioxidant Activities. Oslo, Norway, August 27, 1993.

37th Annual Meeting of the Western Pharmacology Society. Symposium on "α_1-Adrenoceptors". Lecture entitled: "The Pharmacological Classification of α_1-Adrenoceptors". January 30 - February 4, 1994, Kona, Hawaii.

8th Annual Meeting on Adrenergic Mechanisms. Symposium on "Adrenoceptors and Second Messengers". Lecture entitled: "Signal Transduction Mechanisms Utilized by Vascular α-Adrenoceptors". September 19-22, 1993, Porto, Portugal.

Symposium entitled "The Pharmacology of Adrenoceptors". Satellite symposium to the XII IUPHAR Congress. Lecture entitled "Overview: α_1-Adrenoceptors". July 21, 1994, King of Prussia, Pennsylvania.

2nd International Meeting on Imidazoline Receptors. Satellite symposium to the XII IUPHAR Congress. Lecture entitled: "Relationships Between Imidazoline and α_2-Adrenergic Receptors". July 19, 1994, New York, New York.

Pharmaceutical Manufacturers Education and Research Institute. Symposium on "Basic Pharmacology". Lecture entitled: "General Principles: Pharmacodynamics I". Washington, D.C., September 27, 1993.

Pharmaceutical Manufacturers Education and Research Institute. Symposium on "Basic Pharmacology". Lecture entitled: General Principles: Pharmacodynamics II". Washington, D.C., September 27, 1993.

8th International Symposium on Vascular Neuroeffector Mechanisms. Lecture entitled "Receptor, Receptor Interactions and Vascular Smooth Muscle Function: Overview". August 3, 1994, Kananaskis, Alberta, Canada.

Invited Symposium Speaker (Continued)

The Irvington Institute for Medical Research Symposium on "New York Area Careers in Industry". Lecture entitled "Career Opportunities in the Pharmaceutical Industry". October 15, 1993, New York, New York.

Pharmacology-Medicinal Chemistry Annual Symposium, University of Minnesota. Lecture entitled: "Structure-Activity Relationships and Stereochemical Requirements for α_1- and α_2-Adrenoceptors". June 23, 1993. Minneapolis, Minnesota.

Oxford International Biomedical Centre symposium on "Biomedicine Today and Tomorrow". Lecture entitled: "Drug Discovery Into the Next Millennium: From the Gene to the Human". December 17, 1993, King of Prussia, Pennsylvania.

XIIth World Congress of Cardiology and XVth Congress of the European Society of Cardiology symposium entitled "Beta-Blockers in Heart Failure: Myths and Realities". Lecture entitled: Vasodilating beta-blockers in heart failure: their experimental potential. September 10-14, 1994, Berlin, Germany.

Symposium on Carvedilol. Lecture entitled: "The Pharmacology of Carvedilol: antiproliferative and antioxidant activities". Copenhagen, Denmark, February 22, 1994.

International Symposium on Carvedilol. Lecture entitled: Major organ protection with carvedilol, a potent antioxidant and antiproliferative agent. Budapest, Hungary, April 9, 1994.

International Symposium on Vasodilating β-Blocking Drugs: A New Generation of Antihypertensives. Lecture entitled: Protection of cardiovascular organs by carvedilol. Helsinki, Finland, April 18, 1994.

Maine Society of Hospital Pharmacists. Lecture entitled: Impact of Health Care Reform on the Pharmaceutical Industry". Portland, Maine, October 27, 1994.

Pharmacology of Adrenoceptors. Satellite Symposium to the 12th International Congress of Pharmacology. Lecture entitled: "The Adrenoceptors: Historical perspectives, current status and future directions". King of Prussia, Pennsylvania, July 21, 1994.

Western Pharmacology Society Annual Meeting. Lecture entitled: α_1-Adrenoceptors: Pharmacological Subclassification and Newer Therapeutic Applications." Maui, Hawaii, January 23, 1995.

Society of Neurosciences, Lecture to the Catecholamine Club. Lecture entitled: Molecular Basis of Chirality in the Interaction of Ligands with Adrenoceptors." San Diego, California, November 15, 1995.

Invited Symposium Speaker (Continued)

Joint Meeting of the Inter-American Congress of Cardiology and the International Society for Heart Research: Symposium entitled "Cardiovascular Pharmacotherapy: From Prevention to Treatment." Lecture entitled: A Role for Antiproliferative and Antioxidative Effects of Carvedilol in Coronary Artery Disease." Santiago de Chile, December 4, 1995.

European Congress of Pharmacology. Symposium entitled: "Adrenoceptor Pharmacology". Lecture entitled "New Concepts in Adrenoceptor Pharmacology". June 16-19, 1995, Milan, Italy.

Vascular Biology Symposium, University of Vermont. Lecture entitled "The Discovery and Development of Carvedilol for Congestive Heart Failure". May 19, 1995, Burlington, Vermont.

American College of Cardiology, Carvedilol Investigators Symposium entitled "Carvedilol in Congestive Heart Failure: Results of Phase III Clinical Trials". Lecture entitled "The Pharmacology of Carvedilol: Role of Antiischemic Activity to Congestive Heart Failure". March 19, 1995, New Orleans, Louisiana.

Experimental Biology Symposium entitled: Industrial-Academic Relations". Lecture entitled "Industrial Perspectives". April 12, 1995, Atlanta, Georgia.

Symposium/Panel Discussion on Barriers to Health Care. Lecture entitled: The Role of the Pharmaceutical Industry in Health Care Reform". Albert Einstein College of Medicine, May 17, 1995, Bronx, New York.

Symposium entitled “Alpha-1 Adrenoceptor Subtype Selectivity.” Lecture entitled “Current Status of α_1-Adrenoceptor Nomenclature.” November 6, 1995, London, England.

Association of American Medical Colleges Symposium on “Reassessing the Biomedical Ph.D.” Lecture entitled: Industrial Expectations for the New Generation of Biomedical Ph.D.s.” October 8, 1995, Ft. Lauderdale, Florida.

Scientific Therapeutics Information “Consensus Conference on Carvedilol.” Lecture entitled: “Overview of the Pharmacology of Carvedilol: Rationale for Use in Cardiovascular Disease.” October 11, 1995, Philadelphia, Pennsylvania.

International Symposium on “New Drugs for the Treatment of Congestive Heart Failure.” Lecture entitled: “The Pharmacology of Carvedilol: Rationale for Use in Congestive Heart Failure.” October 1, 1995, Oslo, Norway.

American Heart Association Symposium on Carvedilol. Lecture entitled: “The Pharmacology of Carvedilol: Rationale for Use in Congestive Heart Failure.” November 12, 1995, Anaheim, California.

Association of Black Cardiologists Symposium. Lecture entitled: “Protection of Major Organ Systems by Carvedilol.” November 11, 1995, Anaheim, California.

Invited Symposium Speaker (Continued)

Hypertension Investigators Meeting on Carvedilol. Lecture entitled: "The Use of Carvedilol in Hypertension: Prevention of Secondary Organ Damage." November 11, 1995, Anaheim, California.

Congestive Heart Failure Investigators Symposium. Lecture entitled: "Carvedilol Rationale for Use in Congestive Heart Failure." November 11, 1995, Anaheim, California.

International Symposium on "Cell Cycle Regulation". Lecture entitled: "Regulation of the Cell Cycle: Overview". November 5, 1995, King of Prussia, Pennsylvania.

EUROCONFERENCE on "Receptors in Cardiovascular Diseases as Drug Targets". Lecture entitled: "The Role of α- and β-Adrenoceptors in Cardiovascular Diseases: Recent Developments". October 3-4, 1996, Paris, France.

Winter Cardiology Meeting Symposium. Lecture entitled: "The Pharmacology of Carvedilol". February 26, 1996, Sugar Bush, Vermont.

European Society of Cardiology Symposium on "Advances in the Treatment of Congestive Heart Failure: Therapeutic Targets for the New Millennium". Lecture entitled: "The Prevention of Disease Progression: New Approaches". August 25, 1996, Birmingham, United Kingdom.

Symposium on Carvedilol in Congestive Heart Failure. Lecture entitled: Carvedilol: Basic Pharmacology and Ancillary Properties. February 23, 1996, Dallas, Texas.

Symposium on Basic Cardiovascular Research in Scottish Universities. Lecture entitled: Major Unmet Needs in Cardiovascular Diseases. September 27, 1996, Glasgow, Scotland.

American Society of Hypertension Symposium on Hypertension and the Heart: Left Ventricular Hypertrophy and Heart Failure. Lecture entitled: Recent Observations with ß-Blockade: Beneficial Effects in Hypertension and Heart Failure. May 18, 1996, New York, New York.

American College of Cardiology Symposium on Evolution of Heart Failure Management: Traditional Endpoints Versus New Outcomes. Lecture entitled: Pharmacology of ß-Blockade. March 23, 1996, Orlando, Florida.

Symposium on Carvedilol. Lecture entitled: Unique Activities of Carvedilol and their Relevance to Congestive Heart Failure. April 12, 1996, Budapest, Hungary.

2nd International Conference on Lipoprotein and Atherosclerosis: Biological and Clinical Aspects. Lecture entitled: Carvedilol, an α- and ß-Adrenoceptor Antagonist Inhibiting LDL Oxidation. September 14, 1996, Pavia, Italy.

Invited Symposium Speaker (Continued)

28th Annual Joint Symposium of the German and Austrian Societies of Intensive Care Medicine. Symposium entitled "Antioxidants and Limitation of Reperfusion Damage". Lecture entitled: "Carvedilol: A Potent Antioxidant that Limits Reperfusion Damage in the Heart." November 21-23, 1996, Vienna, Austria

Joint Meeting of the Finnish and Hungarian Cardiac Societies. Symposium on "Update in Heart Failure". Lecture entitled: "Cardiovascular Protective Properties of Carvedilol: Role of Antioxidant Activity". May 25, 1996, Stockholm, Sweden.

9th Meeting on Adrenergic Mechanisms. Lecture entitled: "Adrenoceptors." September 23, 1996, Porto, Portugal.

Scottish Biomedical Research Trust Symposium, Cardiovascular Symposium. Keynote Lecture "Major Unmet Needs in Cardiovascular Disease." September 27, 1996, Glasgow, Scotland.

Annual Meeting of the Canadian Cardiovascular Society. Symposium entitled "Evolution of Heart Failure Management: Emerging Role of Beta-Blockers." Lecture entitled: "Pharmacology of Beta-Blockade." October 29, 1996, Montreal, Canada.

American Society of Consultant Pharmacists (ASCP) Annual Meeting. Symposium entitled "Progress in Managing Congestive Heart Failure." Lecture entitled: "New Developments in Heart Failure Management: Role of β-Blockade." November 16, 1996, Nashville, Tennessee.

British Pharmacological Society Meeting. Symposium on "Genetics and the Therapy of Cardiovascular Disease." Lecture entitled: "What Does the Pharmaceutical Industry Expect from Genetic Analysis in Cardiovascular Diseases?" December 13, 1996, Brighton, United Kingdom.

Joint Meeting of the American Society of Pharmacology and Experimental Therapeutics and the British Pharmacological Society. Symposium on "G-Protein Coupled Receptors Regulation: Evolving Concepts and Clinical Implications. Lecture entitled "New Strategies in Drug Development for the Regulation of G-Protein Coupled Receptor Function." March 9, 1997, San Diego, California.

Finnish Society of Cardiology Meeting. Symposium on "The Effect of Medical Treatment on Symptoms and Prognosis of Congestive Heart Failure". Lecture entitled "New Approaches in Preventing the Progression of Heart Failure." October 10, 1996, Helsinki, Finland.

Austrian Cardiac Society Meeting. Symposium on "Congestive Heart Failure". Lecture entitled "The Role of Carvedilol in Preventing the Progression of Congestive Heart Failure." January 16, 1997, Bad Gastein, Austria.

Portuguese Congress of Cardiology Symposium on "Congestive Heart Failure". Lecture entitled "The Pharmacology of Carvedilol." April 21-23, 1997, Lisbon, Portugal.

Invited Symposium Speaker (Continued)

Keynote Speaker, 1997 Pharmacology Day, University of Toronto. Lecture entitled "The History of Adrenergic Pharmacology", May 23, 1997, Toronto, Canada.

Dutch Society of Cardiology Annual Symposium. Lecture entitled: "The Pharmacology of Carvedilol", May 31, 1997, Amsterdam, The Netherlands.

Symposium on Remodeling of Cardiovascular Organs. Lecture entitled: "Overview of Cardiac and Vascular Remodelling". February 17, 1997, King of Prussia, PA.

International Union of Pharmacology (IUPHAR) World Congress Meeting. Symposium on "Receptor Nomenclature: Principles and Applications". Lecture entitled: "The Resolution of the Problem of α-Adrenoceptor Classification". August, 1998, Munich, Germany.

United States Launch Symposium for Coreg (Carvedilol). Lecture entitled: "The Pharmacology of Coreg: Mechanisms for Inhibition of Progression of Congestive Heart Failure". April 28, 1997, Atlanta, GA.

Satellite Symposium to the 45th Annual Meeting of the Japanese College of Cardiology. Symposium entitled: "Cardiovascular Disease and β-Blockers". Lecture entitled: "Multiple Actions of β-Blockers for Cardiovascular Disease". September 25, 1997, Sapporo, Japan.

International Society for Heart Research, World Congress of Cardiology. Symposium entitled: "Adrenergic Receptor Modulation: A Molecular and Pharmacological Adventure in Heart Failure Territory". Lecture entitled: "Adrenergic Receptor Pharmacology". May 27-31, 1998, Rhodes, Greece.

Western Pharmacology Society Annual Meeting. Symposium entitled: "Alpha-1-Adrenoceptors". Lecture entitled: "The Molecular Basis for the Stereoselective Interactions of Agonists with α-Adrenoceptors". January 25-30, 1998, Mazatlan, Mexico.

Satellite Symposium to the 5th International Congress on Endothelin. Symposium entitled: "Endothelin in Disease". Lecture entitled: "The Effects of Carvedilol on Endothelin: Inhibition of Endothelin Synthesis". September 12, 1997, Kyoto, Japan.

American Heart Association Annual Symposium. Satellite Symposium on Teveten. Lecture entitled: "The Pharmacology of Teveten". November 8, 1997, Orlando, Florida.

9th International Symposium on Vascular Neuroeffector Mechanisms. Satellite Symposium to the IUPHAR Congress. Symposium entitled "Adrenoceptors". Lecture entitled: "New Perspective in the use of β-blockers in the treatment of congestive heart failure". August 2-5, 1998, Porto, Portugal.

Invited Symposium Speaker (Continued)

International Society of Hypertension. Symposium on "The Importance of Systolic Control: A New Paradigm for Effective Bleed Pressure Management". Lecture entitled: "Pharmacological Mechanisms of Angiotensin II Receptor Antagonists: Implication for the Treatment of Elevated Systolic Blood Pressure". June 7, 1998, Amsterdam, The Netherlands.

XIIIth World Congress of Pharmacology, Satellite Symposium on "α_1-Adrenoceptors as Targets for Therapeutic Agents in Urology". Lecture entitled "Adrenoceptor Pharmacology". July 23, 1998, Paris, France.

World Congress of Neurosciences. Lecture entitled: "Adrenoceptors". July 11-16, 1999, Jerusalem, Israel.

International Symposium on Medicinal Chemistry, European Federation of Medicinal Chemistry. Plenary Lecture "Pharmacotherapy of the Major Cardiovascular Diseases". Bologna, Italy, September 19-23, 2000.

XIV Lorenzini Annual Lecture. 5th International Symposium Multiple Risk Factors in Cardiovascular Disease: Global Assessment and Intervention". Lecture entitled: "New Frontiers in the Pharmacotherapy of Congestive Heart Failure: The Role of β-Blockers". Venice, Italy, October 28-31, 1999.

British Pharmacology Society Annual Meeting. Symposium on "Pharmacology for the New Millennium-Directions". Lecture entitled: "Orphan Receptors and the Discovery of Orexins". Cambridge, England, January 5-7, 2000.

Experimental Biology Meeting. ASPET Colloquium on "Functional Genomics and Proteonomics". Lecture entitled: "Functional Genomics: Overview". Boston, Massachusetts, June 4, 2000.

Annual Meeting of the Regional Medical Associates for SmithKline Beecham. Symposium on Coreg. Lecture entitled: "New Frontiers in the Pharmacotherapy of Congestive Heart Failure: The Role of β-Blockers". Atlanta, GA, November 3, 1999.

10th Meeting on Adrenergic Mechanisms. Session on Adrenoceptors I. Lecture entitled: "Current Status of Adrenoceptors: Concluding Remarks". Porto, Portugal, September 25, 2000.

American Diabetes Association Annual Meeting, Symposium on the Use of Carvedilol (Coreg) in Heart Failure Patients with Diabetes. Lecture entitled: "Carvedilol: Pharmacologic Profile". San Antonio, Texas, June 10, 2000.

Mid-Atlantic Pharmacology Society Annual Meeting. Plenary Lecture entitled: "The Role of β-Blockers in Congestive Heart Failure: Challenging Dogma". Collegeville, Pennsylvania, September 15, 2000.

Invited Symposium Speaker (Continued)

Rapamune Internal Launch Meeting. Session on Shaping the Art of Immunosuppression. Lecture entitled: "Wyeth - A Commitment to R&D". Marbella, Spain, March 4, 2001.

Joint Meeting of the Australasian, British Canadian and the Western Pharmacology Societies. Plenary lecture entitled: "Drug Discovery in the New Millennium". Vancouver, Canada, March 25, 2001.

Heart and Kidney Institute, University of Houston. Lecture entitled: "The Role of β-Blockers in the Management of Congestive Heart Failure ". Houston, Texas, June 14, 2002.

International Congress of Pharmacology. Lecture entitled: "Receptor Classification in the Post Genomic World ". San Francisco, California, July 9, 2002.

Drug Discovery Technology 2003 Symposium. Keynote Lecture entitled: "R&D Productivity in the Pharmaceutical Industry: Issues and Challenges". Boston, Massachusetts, August 12, 2003.

Biopharmaceutical Conference in Europe. Lecture entitled: "Pharmaceutical R&D: Former and Future Perspectives". Monaco, June 20, 2003.

R&D Leader's Forum. Lecture entitled: "R&D Productivity: Can it be Increased?". Coral Gables, Florida, March 1-3, 2004.

Annual Meeting of the Association of American Medical Colleges. Lecture entitled: "Measuring Productivity: Industry Models". Philadelphia, Pennsylvania, October 11, 2002.

Symposium of New Molecules in Cancer Therapeutics. Lecture entitled: "Overview of the Wyeth Oncology Pipeline". Athens, Greece, October 27, 2002.

Symposium on Futures in Biomedical Research: A Look Ahead VII. Lecture entitled: "Building Success in Tomorrow's Pharmaceutical Industry". University of Maryland Baltimore Campus, Baltimore, Maryland, November 12, 2003.

2003 Retail Advisory Board Meeting. Lecture entitled: "R&D Productivity in the Pharmaceutical Industry". Philadelphia, Pennsylvania, August 23, 2003

Research & Development Leaders Forum. Lecture entitled: "How can the Pharmaceutical Industry Counter the Growing Costs of Drug Development Amid Increasingly Stringent Regulatory Requirements?" Coral Gables, Florida, March 1-3, 2004.

Drug Discovery Technology World Congress Summit. Lecture entitled: "Is R&D Productivity Really Falling? Or is this Oft-Quoted "Fact" Based on Outdated Parameters?" Boston, Massachusetts, August 8, 2004.

The World Pharmaceutical Congress. Lecture entitled: "Optimizing R&D Productivity". Philadelphia, Pennsylvania, May 18, 2004.

Invited Symposium Speaker (Continued)

2004 BIO Annual Meeting. Lecture entitled: "Productivity of Drug Discovery". San Francisco, California, June 8, 2004.

Annual Congress of the Florida American College of Obstetrics and Gynecology. Lecture entitled: "The Future of Women's Health". Naples, Florida, July 24, 2004.

11th Annual Human Genome Discovery Symposium. Lecture entitled: "Redefining the Pre-Competitive Opportunity Within the Pharmaceutical Industry". San Francisco, California, March 26, 2004.

Experimental Biology 2004 Annual Meeting. Lecture entitled: "What the Pharmaceutical Industry of the 21st Century is Looking for in a Pharmacologist". Washington, D.C., April 18, 2004.

2004 Retail Advisory Board Meeting. Lecture entitled: "R&D Productivity in the Pharmaceutical Industry: Issues and Challenges". San Diego, California, August 28, 2004.

2004 Windhover AudioConference. Lecture entitled: "Future Direction of R&D at Wyeth". Philadelphia, Pennsylvania, September 17, 2004.

All Florida Obstetrics and Gynecology Symposium. Lecture entitled: "The Future of Women's Health: New Therapies in Development". Orlando, FL, November 20, 2004.

Drug Discovery Technology Europe 2005. Lecture entitled: "Re-Engineering Discovery and Development: Impact on the Pharmaceutical Industry of Tomorrow". London, England, March 14-17, 2005.

Mid-Winter Symposium of the University of South Florida. Lecture entitled: "The Future of Women's Health: New Therapies in Development". February 19, 2005.

Drug Development Summit. Lecture entitled: "Wyeth: The R&D Pipeline to Watch". Phoenix, Arizona, February 7, 2005.

Pharmaceutical Strategy Series: Executive Leadership Summit. Lecture entitled: "Strategies to Integrate Discovery, Development and Marketing". Orlando, Florida, February 10, 2005.

Sino-American Pharmaceutical Professionals Association-Greater Philadelphia Chapter (SAPA-GP). Annual Symposium on Globalization of Pharmaceutical/Biotech R&D. Lecture entitled: "Strategies to Integrate Discovery, Development and Marketing". Blue Bell, Pennsylvania, June 18, 2005.

PhRMA Science and Regulatory Annual Meeting. Town Hall Session. Lecture entitled: "From Basic Research to Marketed Products: What Value Does Each Stake Holder Add". Washington, DC, May 2, 2005.

Invited Symposium Speaker (Continued)

Drug Discovery Summit Genius Symposium. Lecture entitled: "What Role Does Genius Play in Drug Discovery and Development". Phoenix, Arizona, February 8, 2005.

Pennsylvania Biotech Annual Dinner. Lecture entitled: "The Challenges of Drug Discovery and Development". Philadelphia, Pennsylvania, January 26, 2005.

CMR International Workshop on a New Paradigm for Clinical Research. Lecture entitled: "A New Paradigm for Clinical Research – A Path to Improve Drug Development at Wyeth". Washington, D.C., October 4, 2005.

Economist Conference: The 12th Annual Pharmaceuticals Conference. Lecture entitled: "Collaborating for R&D Success". London, England, February 21, 2006.

Sino-American Pharmaceutical Professionals Association (SAPA) Annual Meeting. Lecture entitled: "Strategies to Integrate Discovery, Development and Global Marketing". Philadelphia, Pennsylvania, June 18, 2005.

Drug Development Summit. Lecture entitled: "Wyeth Pharmaceutical's Approach to Redesigning R&D to Increase Innovation, Output and to Expedite Discoveries into Therapeutic Products". Phoenix, Arizona, February 14, 2006

Scrip R&D Productivity Summit. Lecture entitled: "If You Can't Measure It, You Can't Manage It; Accurately Measuring and Comparing Productivity Improvements Across Multiple Deliverables". London, England, April 26, 2006.

Drug Discovery Technology and Development World Congress. Lecture entitled: "Growing Costs of Healthcare: Impact on Government, Regulatory Agencies, Industry and Patients". Boston, Massachusetts, August 8, 2005.

R&D Executive Leadership Summit. Lecture entitled: "R&D Productivity Improvements at Wyeth". Boston, Massachusetts, August 9, 2005.

IBC Conference on R&D Productivity. Lecture entitled: "Rethinking the Productivity Challenge". New York, New York, September 27, 2005.

PharmaDiscovery Conference. Lecture entitled: "Maximizing the Tools of Drug Discovery and Development to Improve R&D Productivity". Washington, DC, May 11, 2005.

Drug Discovery & Technology Leaders Summit. Lecture entitled: "Re-Engineering the Drug Discovery and Development Process and Overcoming Technological Challenges in the Drug Discovery Process". Orlando, Florida, February 24, 2005.

Mid-Winter Florida Obstetrics & Gynecology Conference. Lecture entitled: "Developing and Delivering Health Care: The Pharmaceutical Industry Perspective". St. Pete Beach, Florida, February 20, 2005.

2005 BIO Symposium on Intellectual Property. Lecture entitled: "Capturing the Value in Non-Progressed Assets". Philadelphia, Pennsylvania, June 22, 2005.

Invited Symposium Speaker (Continued)

Drug Discovery Technology Europe 2005. Lecture entitled: "Strategies for Re-Engineering Discovery and Development to Address R&D Productivity". London, England, March 14, 2005.

2005 BIO Annual Conference. Lecture entitled: "Maximizing the Tools of Drug Discovery and Development to Improve R&D Productivity". Philadelphia, Pennsylvania, June 21, 2005.

Cambridge Health Institute (CHI) Pharmaceutical Leadership Summit. Lecture entitled: "Strategies to Integrate Discovery, Development and Marketing". Lake Buena Vista, Florida, February 10, 2005.

12th Annual Pharmaceuticals Conference: Building a Strategy for Pharma's New Role. Lecture entitled: "Collaborating for R&D Success". London, England, February 21, 2006.

Annual Meeting of the Directors of Graduate Studies in Pharmacology. Lecture entitled: "Training Needs for the Pharmaceutical Industry". Salt Lake City, Utah, July 25-28, 2007.

2006 Adaptive Designs: Opportunities, Challenges and Scope in Drug Development. Keynote address entitled: "Improving the Efficiency of Drug Development". Bethesda, Maryland, November 13-14, 2006.

Sino-American Pharmaceutical Professionals Association (SAPA) Annual Meeting. Keynote lecture entitled: "21st Century Innovation and Globalization of Drug Development: Wyeth's New Clinical Development Paradigm". Philadelphia, Pennsylvania, June 17, 2006.

5th Annual Evolution Summit. Keynote lecture entitled: "21st Century Innovation in the Discovery and Development of New Medicines – Changing the Clinical Development Paradigm". Monte Carlo, Monaco, October 22, 2006.

Ohio State University College of Pharmacy Annual Symposium. Keynote lecture entitled: "The Discovery and Development of Coreg (Carvedilol) for the Treatment of Congestive Heart Failure". Columbus, Ohio, May 16, 2006.

Drug Development Summit. Keynote lecture entitled: "Development of the Wyeth Neurosciences Pipeline: Transformation in Discovery and Clinical Development". Phoenix, Arizona, January 22, 2006.

Enbrel Summit: Progress and Promise: A Decade of Scientific Innovation. Lecture entitled: "Inflammation Research at Wyeth: Bringing Novel Therapies to the Clinic". Munich, Germany, March 15, 2007.

Second Trial Design Innovation Conference. Keynote lecture entitled: "Bringing Statistical Methodology to the Board Room: How Adaptive Designs Influence Portfolio Management Decisions". Washington, D.C., July 16, 2007.

Invited Symposium Speaker (Continued)

40th Mid-Atlantic Graduate Student Symposium. Keynote lecture entitled: "New Drug Development: The Saga of Carvedilol". Morgantown, West Virginia, June 10, 2007.

Annual Meeting of the National Directors of Graduate Studies in Pharmacology. Keynote lecture entitled: "Future Training Needs in Pharmacology". Salt Lake City, Utah, July 25, 2007.

ASPET Centennial Meeting. Symposium entitled "Drug Discovery Paradigms: Past, Present, Future". Lecture entitled: "Drug Discovery of the Future". San Diego, California, April 5-9, 2008.

Asia-Pacific Enbrel Summit: Keynote lecture entitled: "Inflammation Research at Wyeth: Bringing Novel Therapies to the Clinic". Seoul, Korea, July 3, 2007.

John S. O'Brien Memorial Lectureship at the Annual University of Pennsylvania Graduate Students in Pharmacology Symposium. Lecture entitled: "New Drug Development: The Saga of Carvedilol". Philadelphia, Pennsylvania, November 1, 2007.

David Perlman Memorial Lectureship at the American Chemical Society Annual Meeting. Lecture entitled: "The Discovery and Development of Coreg (Carvedilol) for the Treatment of Congestive Heart Failure: A Paradigm Shift". Philadelphia, Pennsylvania, August 17, 2008.

WV-INBRE Summer Research Symposium. Keynote lecture entitled: "The Discovery and Development of Carvedilol (Coreg) for the Treatment of Congestive Heart Failure". Morgantown, West Virginia, July 31, 2008.

Induction Ceremony for the Rho Chi Society. Keynote lecture entitled: "The Discovery and Development of Coreg (Carvedilol) for the Treatment of Congestive Heart Failure: A Paradigm Change". Boston, Massachusetts, April 17, 2008.

Drug Development Summit. Keynote lecture entitled: "Integrated Small Molecule, Biotechnology and Vaccine Technologies in Pharmaceutical Research & Development". Amelia Island, Florida, February 12-15, 2008.

Drug Development Summit. Panel Discussion: "Critical Issues for R&D in 2008". Amelia Island, Florida, February 12-15, 2008.

16th Sino-American Pharmaceutical Professionals Association (SAPA) Annual Meeting. Keynote lecture entitled: "Future Trends for Integrated Drug Discovery and Development". Princeton, New Jersey, June 15, 2008.

Institute for Regulatory Science Symposium. Keynote Lecture entitled: "Predictable Outcomes: Why Do Potential Winners Fail". Washington, D.C., September 30-October 1, 2008.

Invited Symposium Speaker (Continued)

University of Florida, College of Pharmacy Annual Research Showcase. Keynote Lecture entitled: "The Trials and Tribulations of a Medical Breakthrough: The Discovery and Development of Coreg (Carvedilol) for the Treatment of Congestive Heart Failure". Gainesville, Florida, February 18, 2009.

University of Florida, College of Pharmacy. Target Leadership Speaker. Lecture entitled: "Leadership Lessons from the School of Life". Gainesville, Florida, February 18, 2009.

FDA/CMS Summit. Plenary Lecture entitled: "Interactions Between the Pharmaceutical Industry and Global Regulatory Agencies". Washington, D.C., December 3, 2009.

West Virginia University, College of Pharmacy, White Coat Induction Ceremony. Lecture Entitled: "The Role the Research Scholarships Played in My Career", Morgantown, West Virginia, December 14, 2009.

Great Oxford Debate, Oxford University. Lecture entitled: "There Exists No Conflict of Interest in the Pharmaceutical Industry Conducting Its Own Clinical Trials". Oxford, England, September 23, 2009.

50th Year Anniversary of the PRAT Program at the National Institute of General Medical Sciences, Lecture entitled: "The Discovery and Development of Carvedilol for the Treatment of Congestive Heart Failure: From the Laboratory Bench to the Patient", Bethesda, MD, November 6, 2015.

Chairman or Organizer of the Following Symposia

American Society for Pharmacology and Experimental Therapeutics, Symposium of Peripheral Alpha-Adrenergic Receptors, Philadelphia, Pennsylvania, August 11, 1983 (Chairman).

Satellite Symposium of the 9th International Congress of Pharmacology entitled Pharmacology of Adrenoceptors, Manchester, England, August 6, 1984 (Organizing Committee).

30th International Congress of Physiology, Satellite Symposium on Smooth Muscle Function. Ligand Binding and Approaches to Receptor Characterization, Banff, Alberta, Canada, July 2, 1986 (Chairman).

Federation of American Societies for Experimental Biology (FASEB), Symposium entitled: The Role of Peripheral Dopamine Receptors in Cardiovascular Function, Anaheim, California, April, 1985 (Organizer).

American Society for Pharmacology and Experimental Therapeutics, Symposium on the Pharmacology of the Prostate, Boston, Massachusetts, August, 1985 (Organizer).

Satellite Symposium to the 11th Scientific Meeting of the International Society of Hypertension. Adrenergic Receptor Function and Cardiovascular Reactivity in Human Hypertension. Session on Postsynaptic α_1- and α_2-Adrenoceptors in Hypertension, Essen, Federal Republic of Germany, September 7-8, 1986 (Chairman).

Symposium Entitled "Cardiac and Renal Failure", Phoenix, Arizona, March 13-15, 1987 (organizer).

Federation of the American Societies for Experimental Biology, Symposium entitled "Vasomotor Regulatory Mechanisms: Central and Peripheral Aspects", Washington, DC, April, 1987 (Chairman).

Vascular Neuroeffector Mechanisms, 6th International Symposium. Symposium entitled "α-Adrenoceptor II", Melbourne, Australia, August 30-September 2, 1987 (Chairman).

Smith Kline & French, Symposium entitled " Future Therapeutic Approaches to Ischemic Heart Disease", San Juan, Puerto Rico, February 12-14, 1988 (Organizer).

UCLA Symposia on Molecular and Cellular Biology, Symposium entitled "Molecular Biology of the Cardiovascular System", Keystone, Colorado, April, 1988 (Organizing Committee).

Federation of the American Societies for Experimental Biology (FASEB). Symposium entitled "Renal α_2-Adrenoceptors", Las Vegas, Nevada, May 3, 1988 (Co-Chairman).

Chairman or Organizer of the Following Symposia (Continued)

Federation of the American Societies for Experimental Biology (FASEB). Symposium entitled "Mechanisms of Signal Transduction: Receptors Related to Tyrosine Kinase", New Orleans, Louisiana, March 20 1989 (Co-Organized with Dr. A. Robinson).

Federation of the American Societies for Experimental Biology (FASEB). Symposium entitled "Mechanisms of Signal Transduction: Regulation of Adenylyl Cyclase", New Orleans, Louisiana, March 20 1989 (Co-Organized with Dr. A. Robinson).

Federation of the American Societies for Experimental Biology (FASEB). Symposium entitled "Mechanisms of Signal Transduction: Calcium, Phosphoinositides, and C Kinase", New Orleans, Louisiana, March 20, 1989 (Co-Organized with Dr. A. Robinson).

Federation of the American Societies for Experimental Biology (FASEB). Symposium entitled "Mechanisms of Signal Transduction: Receptors Related to Ion Pumps and Channels", New Orleans, Louisiana, March 22,1989 (Co-Organized with Dr. A. Robinson).

Federation of the American Societies for Experimental Biology (FASEB). Symposium entitled "Mechanisms of Signal Transduction: The Steroid Supra-Family of Receptors", New Orleans, Louisiana, March 22, 1989 (Co-Organized with Dr. A. Robinson).

Federation of the American Societies for Experimental Biology (FASEB). Symposium entitled "Mechanisms of Signal Transduction: Sensory Signal Transduction", New Orleans, Louisiana, March 22, 1989 (Co-Organized with Dr. A. Robinson).

Federation of the American Societies for Experimental Biology (FASEB). Symposium entitled "Mechanisms of Signal Transduction: Receptors Related to Guanylate Cyclase", New Orleans, Louisiana, March 23, 1989 (Co-Organized with Dr. A. Robinson).

Federation of the American Societies for Experimental Biology (FASEB). Symposium entitled: "Endothelin and Endothelial-Derived Contractile Substance", New Orleans, Louisiana, March, 1989 (Chairman).

Smith Kline & French, Symposium entitled "Future Therapeutic Approaches to Cerebrovascular Disease", Orlando, Florida, March 10-12, 1989 (Organizer).

Satellite Symposium of the 11th International Congress of Pharmacology entitled "Pharmacology of Adrenoceptors". Manchester, England, June 27-29, 1990 (Organizing Committee).

Satellite Symposium of the 11th International Congress of Pharmacology entitled "Presynaptic Receptors and Neuronal Transporters". Rouen, France, June 26-29, 1990 (Organizing Committee).

Chairman or Organizer of the Following Symposia (Continued)

American Society for Pharmacology and Experimental Therapeutics (ASPET).Short Course: Endothelial Biology. Milwaukee, Wisconsin, August, 1990 (Chairman and Organizer).

American Society for Pharmacology and Experimental Therapeutics (ASPET).Symposium entitled: "Endothelium-Derived Relaxing Factors", Milwaukee, Wisconsin, August, 1990 (Organizer).

American Society for Pharmacology and Experimental Therapeutics (ASPET).Symposium entitled: "Endothelium-Derived Contracting Factors", Milwaukee, Wisconsin, August, 1990 (Chairman and Organizer).

Cardio/Renal Conference entitled "Cell and Molecular Biology of Atherosclerosis", Phoenix, Arizona, February 23-25, 1990 (Organizer).

American Society for Pharmacology and Experimental Therapeutics (ASPET).Symposium entitled: "Cellular Interactions of Endothelium", Milwaukee, Wisconsin, August, 1990 (Organizer).

Satellite Symposium of the XIth International Congress of Pharmacology, Session entitled "Molecular Structure and Genetics of Adrenoceptors. Manchester, England, June 27, 1990 (Chairman).

Symposium Entitled" Molecular and Cellular Biology of Atherogenesis: Alteration in Progression/Regression". Phoenix, Arizona, April 5-7, 1991 (Chairman).

International Symposium Entitled "From α_2-Adrenoceptors to the Imidazoline-Preferring Receptors". Satellite symposium of the 7th International Catecholamine Symposium. Paris, France, June 29-30, 1992 (Scientific Committee).

Symposium Entitled "Vascular Remodeling: Thrombosis and Smooth Muscle Function". West Palm Beach, Florida, June 26-28, 1992 (Organizer).

International Symposium on Endothelin, Houston, TX, 1993 (Scientific Advisory Board).

XIIIth International Symposium on Medicinal Chemistry, organized by Societe de Chimie Therapeutique on behalf of the European Federation for Medicinal Chemistry. Paris, France, September 19-23, 1994 (International Advisory Board and Chairman).

International Symposium Entitled "Angiotensin II Receptor Antagonists". Organized by the International Academy of Biomedical and Drug Research, Monte Carlo, Monaco, March 20-22, 1993 (Organizing Committee).

8th Meeting on Adrenergic Mechanisms, Symposium on "Adrenoceptors and Second Messengers". Porto, Portugal, September 19-22, 1993 (Chairman).

Chairman or Organizer of the Following Symposia (Continued)

International Symposium entitled "The Pharmacology of Adrenoceptors". Satellite symposium to the 12th International Pharmacology (IUPHAR) Congress. King of Prussia, Pennsylvania, July 21-23, 1994 (Organizer).

Vascular α-Adrenoceptors: From the Gene to the Human. 8th International Symposium on Vascular Neuroeffector Mechanisms, Satellite Symposium to the 12th International Pharmacology Congress, Kananaskis, Alberta, Canada, August 1, 1994 (Chairman).

Fourth International Conference on Endothelin, London, England, April 23-26, 1995 (Scientific Advisory Board).

2nd International Meeting on Imidazoline Receptors. Satellite symposium to the 12th International Pharmacology (IUPHAR) Congress. New York, New York, July 19-20, 1994 (Scientific Advisory Board).

8th International Symposium on Vascular Neuroeffector Mechanisms. Satellite Symposium to the 12th International Pharmacology (IUPHAR) Congress. Kananaskis, Alberta, Canada. August 1-5, 1994 (Co-Organizer).

International Symposium on Cell Cycle Regulation, King of Prussia, Pennsylvania, November 5-7, 1995 (Co-Organizer).

9th International Symposium on Adrenergic Mechanisms, Porto, Portugal, September 23-25, 1996 (Scientific Advisory Board).

6th World Congress of the World Federation of Societies of Biological Psychiatry. Symposium entitled: The Genomic Alliance and Innovative Drug Discovery in the Neurosciences. Nice, France, June 23, 1997 (Co-Chairman).

Fifth International Conference on Endothelin. Kyoto, Japan, September 12-15, 1997 (Scientific Advisory Board and Co-Chairman).

International Society for Heart Research, World Congress of Cardiology Symposium on "Adrenergic Receptor Modulation: A Molecular and Pharmacological Adventure in Heart Failure Territory". Rhodes, Greece, May 27-31, 1998 (Organizer and Chairman).

6th World Congress of Biological Psychiatry. Symposium on "The Genomic Alliance and Innovative Drug Discovery in the Neurosciences". Nice, France, June 23, 1997 (Chairman).

9th International Symposium on Vascular Neuroeffector Mechanisms; Satellite Symposium to the IUPHAR Congress. Porto, Portugal, August 2-5, 1998 (Scientific Advisory Committee).

10th International Symposium on Vascular Neuroeffector Mechanisms; Satellite Symposium to the IUPHAR Congress. Porto, Portugal, September 24-27, 2000 (Scientific Advisory Committee).

Chairman or Organizer of the Following Symposia (Continued)

Fifth International Conference on Endothelin; Session entitled "Endothelin Receptors and Endothelin Receptor Antagonists". Kyoto, Japan, September 13, 1997 (Chairman).

9th International Symposium on Vascular Neuroeffector Mechanisms; Satellite Symposium to the IUPHAR Congress. Session entitled "Adrenoceptors". Porto, Portugal, August 2-5, 1998 (Chairman).

XIIIth World Congress of Pharmacology, Satellite Symposium on "α_1-Adrenoceptors as Targets for Therapeutic Agents in Urology". Paris, France, July 23-24, 1998 (Chairman).

XVI International Symposium on Medicinal Chemistry. Bologna, Italy, September 18-23, 2000 (Scientific Advisory Board).

ET-6 International Symposium, Montreal, Canada, October 10-14, 1999 (Scientific Advisory Board).

Experimental Biology Meeting, ASPET Colloquium on Functional Genomics, Symposium on "Functional Genomics and Proteonomics". Boston, Massachusetts, June 4, 2000 (Chairman).

First International Symposium on PPARs: From Basic Science to Clinical Applications. Florence, Italy, April 4-7, 2001 (International Advisory Board).

11th Meeting on Adrenergic Mechanisms. Porto, Portugal, September 25-27, 2003 (Scientific Advisor).

2nd International Symposium on PPARs: From Basic Science to Clinical Applications. Florence, Italy, March 19-22, 2003 (International Advisory Board).

International Congress of Pharmacology. Symposium on “Receptor Classification in the Post Genomic World”. San Francisco, California, July 9, 2002 (Organizer).

Sixth IUPHAR Satellite Symposium on Adrenoceptors. Rohnert Park, California, July 12-14, 2002 (Organizer).

Second International Symposium on “PPARs: From Basic Science to Clinical Applications”. Florence, Italy, March 19-22, 2003 (International Advisory Board).

World Drug Discovery Congress 2004. Copenhagen, Denmark, January 19-21, 2004 (Scientific Advisory Committee).

rEVOLUTION 2004 Summit for Chief Scientific Officers. Greensboro, Georgia, May 5-7, 2004 (Steering Committee)

Research & Development Leaders Forum. Coral Gables, Florida, May 1-3, 2004 (Scientific Advisory Committee).

Chairman or Organizer of the Following Symposia (Continued)

rEVOLUTION 2005 Summit for Chief Scientific Officers, September 29-30, 2005 (Steering Committee)

4th World Drug Discovery and Development Summit, Copenhagen, Denmark, January 25-26, 2005 (Advisory Board).

IBC Drug Discovery and Technology Conference. R&D Executive Summit. Boston, Massachusetts, August 9, 2005 (Chairman).

PhRMA Science and Regulatory Annual Meeting. Town Hall Session entitled "From Basic Research to Marketed Products". Washington, D.C., May 2, 2005 (Chairman).

Drug Development Summit 2007, Phoenix, Arizona, January 21-24, 2007 (Advisory Board).

ASPET Centennial Meeting. Symposium entitled: "Drug Discovery Paradigms: Past, Present and Future". San Diego, California, April 7, 2008 (Chairman).

Expert Opinion-Evolution Summit 2008. Monte Carlo, Monaco, October 22-24, 2008 (Scientific Advisory Board)..

7th Annual R&D Leaders Forum. San Diego, California, October 28-30, 2008 (Scientific Advisory Board).

rEvolution Summit 2009. Miami, Florida, March 25-27, 2009 (Scientific Advisory Board).

Publications

Books Edited

1. The Alpha-1 Adrenergic Receptors, edited by R.R. Ruffolo, Jr., Humana Press, Clifton, New Jersey, 1987 (543 pages).

2. Beta-Adrenoceptors: Molecular Biology, Biochemistry and Pharmacology, Progress in Basic and Clinical Pharmacology, Volume 7, edited by R.R. Ruffolo, Jr., S. Karger, A.G., Basel, 1991 (240 pages).

3. Alpha-Adrenoceptors: Molecular Biology, Biochemistry and Pharmacology, Progress in Basic and Clinical Pharmacology, Volume 8, edited by R.R. Ruffolo, Jr., S. Karger, A.G., Basel, 1991 (226 pages).

4. Angiotensin II Receptors, Volume 1: Molecular Biology, Biochemistry, Pharmacology and Clinical Perspective, edited by R.R. Ruffolo, Jr., CRC Press, Boca Raton, 1994 (170 pages).

5. Angiotensin II Receptors, Volume 2: Medicinal Chemistry, edited by R.R. Ruffolo, Jr., CRC Press, Boca Raton, 1994 (225 pages).

6. Endothelin Receptors: From the Gene to the Human, edited by R.R. Ruffolo, Jr., CRC Press, Boca Raton, 1995 (285 pages).

7. Adrenoceptors: Structure, Function and Pharmacology, edited by R.R. Ruffolo, Jr., Harwood Academic Publishers, London, 1995 (287 pages).

8. G-Protein Coupled Transmembrane Signaling Mechanisms, edited by R.R. Ruffolo, Jr. and M. Hollinger, CRC Press, Boca Raton, 1995 (204 pages).

9. Inflammation: Mediators and Pathways, edited by R.R. Ruffolo, Jr. and M. Hollinger, CRC Press, Boca Raton, 1995 (206 pages).

10. Pharmacology of Adrenoceptors, edited by R.R. Ruffolo, Jr., Harwood Academic Publishers, London, 1995 (279 pages).

11. Cell Cycle Regulation, edited by R.R. Ruffolo, Jr., B. Metcalf and G. Poste. Harwood Academic Publishers, London, 1997 (174 pages).

12. Carvedilol: A Multiple Action Neurohormonal Antagonists, edited by R.R. Ruffolo, Jr., G. Poste and C. Sohn, Harwood Academic Publishers, London, in preparation.

13. Inflammatory Cells and Mediators in CNS Diseases. Edited by R.R. Ruffolo, Jr., G. Feuerstein, J. Hunter, G. Poste and B. Metcalf. Harwood Academic Publishers, London, 1998 (518 pages).

14. The IUPHAR Compendium of Receptor Characterization and Classification. Edited by T. Godfraind, P.M. Vanhoutte, R.R. Ruffolo, Jr. and P. Humphrey. Published by IUPHAR Media, Burlington Press, Cambridge, 1998 (267 pages).

Books Edited (continued)

15. The Alpha-1 Adrenergic Receptors. Edited by J.P. Hieble, A. Leonardi and R.R. Ruffolo, Jr. Humana Press, Totowa, New Jersey, in preparation.

16. Apoptosis in Health and Disease. Edited by R.R. Ruffolo, Jr. and F. Walsh, Harwood Academic Publishers, London, 2000 (249 pages).

17. IUPHAR Compendium of Receptor Characterization and Classification, Volume II. Published by IUPHAR Media, Burlington Press, Cambridge, 398 pages, 2000.

Theses

The Mechanism of Action of Sympathomimetic Amines. Submitted in partial fulfillment for the degree, Bachelor of Science in Pharmacy with Distinction. The Ohio State University, Columbus, Ohio, 1973.

Biochemical and Pharmacological Characterization of the Alpha-Adrenoreceptor. Submitted in partial fulfillment for the degree, Doctor of Philosophy in Pharmacology. The Ohio State University, Columbus, Ohio, 1976.

Publications (Full Papers)

1. Krell, R. D., Ruffolo, R. R., Jr. and Patil, P. N.: Steric aspects of adrenergic drugs. XXI. Drug-induced release of (-)- and (+)- ^{14}C-norepinephrine from the isolated superfused rat vas deferens. Naunyn-Schmiedeberg's Arch. Pharmacol. 274: 394-403, 1972.

2. Witiak, D. T., Sinha, B. K., Ruffolo, R. R., Jr. and Patil, P. N.: cis- and trans-2-Mercaptocyclo-butylamines, their benzylmercapto analogs, and aminomethyl homologs. Influence on bradykinin-induced contraction of the guinea pig ileum. J. Med. Chem. 16: 232-235, 1973.

3. Ruffolo, R. R., Jr. and Patil, P. N.: Catecholamine content of pigmented and nonpigmented tissues of the rabbit. Eur. J. Pharmacol. 25: 255-258, 1974.

4. Bingham, W. G., Ruffolo, R. R., Jr. and Friedman, S. J.: Catecholamine levels in the injured spinal cord of monkeys. J. Neurosurg. 42: 174-178, 1975.

5. Bingham, W. G., Ruffolo, R. R., Jr., Goodman, J. H., Knofel, J. and Friedman, S. J.: Norepinephrine and dopamine levels in normal dog and monkey spinal cord. Life Sci. 16: 1521-1526, 1975.

6. Ruffolo, R. R., Jr., Miller, D. D. and Patil, P. N.: Biochemical correlates for the pharmacological effects of L(+)-isomers and beta-desoxy-sympathomimetic amines. Biochem. Pharmacol. 25: 399-404, 1976.

7. Ruffolo, R. R., Jr., McCreery, R. L. and Patil, P. N.: A kinetic analysis of a catechol-specific binding site in the microsomal fraction from the rabbit aorta. Eur. J. Pharmacol. 38: 221-232, 1976.

8. Ruffolo, R. R., Jr., Fowble, J. W., Miller, D. D. and Patil, P. N.: Binding of ^{3}H-dihydroazapetine to alpha-adrenoreceptor-related proteins from rat vas deferens. Proc. Natl. Acad. Sci. U.S.A. 73: 2730-2734, 1976.

9. Miller, D. D., Hsu, F. L., Ruffolo, R. R., Jr. and Patil, P. N.: Stereochemical studies of adrenergic drugs. Optically active derivatives of imidazolines. J. Med. Chem. 19:1382-1384, 1976.

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11. Ruffolo, R. R., Jr., Turowski, B. S. and Patil, P. N.: Lack of cross-desensitization between structurally dissimilar alpha-adrenoceptor agonists. J. Pharm. Pharmacol. 29: 378-380, 1977.

12. Ruffolo, R. R., Jr. and Patil, P. N.: Kinetics of blockade of different receptors by chlorpromazine in rabbit stomach strips. Eur. J. Pharmacol. 48: 151-157, 1978.

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13. Ruffolo, R. R., Jr., Turowski, B. S. and Patil, P. N.: Further biochemical characterization of ^{3}H-dihydroazapetine binding to alpha-adrenoreceptor-related proteins from the rat vas deferens. J. Pharm. Pharmacol. 30: 498-502, 1978.

14. Ruffolo, R. R., Jr., Eisenbarth, G. S., Thompson, J. M. and Nirenberg, M.: Synapse turnover: A mechanism for acquiring synaptic specificity. Proc. Natl. Acad. Sci. U.S.A. 75: 2281-2285, 1978.

15. Eisenbarth, G. S., Ruffolo, R. R., Walsh, F. W., and Nirenberg, M: Lactose sensitive lectin of chick retina and spinal cord. Biochem. Biophys. Res. Commun. 83: 1246-1252, 1978.

16. Ruffolo, R. R., Jr., Miller, D. D. and Patil, P. N.: Some thoughts on the chemical and pharmacological aspects of adrenoreceptors. In: Recent Advances in the Pharmacology of Adrenoceptors. ed. by E. Szabadi, C. M. Bradshaw and P. Bevan, pp. 45-50, Elsevier/North-Holland Biomedical Press, 1978.

17. Ruffolo, R. R., Jr. and Patil, P. N.: Kinetics of alpha-adrenoreceptor blockade by phentolamine in the normal and denervated rabbit aorta and rat vas deferens. Blood Vessels, 16: 135-143, 1979.

18. Ruffolo, R. R., Jr., Rosing, E. L. and Waddell, J. E.: Receptor interactions of imidazolines. I. Affinity and efficacy for alpha-adrenergic receptors in rat aorta. J. Pharmacol. Exp. Ther. 209: 429-436, 1979.

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22. Patil, P. N. and Ruffolo, R. R., Jr.: Evaluation of adrenergic alpha- and beta-receptor activators and adrenergic alpha- and beta-receptor blocking agents. In: The Handbook of Experimental Pharmacology. Vol. 54/1, ed. by L. Szekeres, Springer-Verlag (Berlin), pp. 89-134, 1980.

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23. Ruffolo, R. R., Jr., Yaden, E. L., Waddell, J. E. and Dillard, R. D.: Receptor interactions of imidazolines. V. Clonidine differentiates postsynaptic alpha-adrenergic receptor subtypes in tissues from the rat. J. Pharmacol. Exp. Ther. 213: 557-561, 1980.

24. Ruffolo, R. R., Jr., Yaden, E. L. and Waddell, J. E.: Receptor interactions of imidazolines. VI. Significance of carbon bridge separating phenyl and imidazoline rings of tolazoline-like alpha-adrenergic imidazolines. J. Pharmacol. Exp. Ther. 214: 535-540, 1980.

25. Zaborowsky, B. R., McMahan, W. C., Griffin, W. A., Norris, F. H. and Ruffolo, R. R., Jr.: Computerized graphic methods for determining dissociation constants of agonists, partial agonists and competitive antagonists in isolated smooth muscle preparations. J. Pharmacol. Methods 4: 165-178, 1980.

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28. Ruffolo, R. R., Jr., Waddell, J. E. and Yaden, E. L.: Postsynaptic alpha-adrenergic receptor subtypes differentiated by yohimbine in tissues from the rat. Existence of $alpha_2$-adrenergic receptors in rat aorta. J. Pharmacol. Exp. Ther. 217: 235-240, 1981.

29. Ruffolo, R. R., Jr., Yaden, E. L. and Waddell, J. E.: Receptor interactions of imidazolines. VII. Peripherally-mediated pressor and centrally-mediated depressor effects of clonidine and a series of mono- and dimethoxy-substituted tolazoline derivatives. J. Pharmacol. Exp. Ther. 218: 154-160, 1981.

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31. Hoffman, B. B., Lavin, T. N., Lefkowitz, R. J. and Ruffolo, R. R., Jr.: Alpha-adrenergic receptor subtypes in rabbit uterus: Mediation of myometrial contraction and regulation by estrogens. J. Pharmacol. Exp. Ther. 219: 290-295, 1981.

32. Ruffolo, R. R., Jr.: Structure-activity relationships of alpha adrenoceptor agonists. In: Adrenoceptors and Catecholamine Action, Part B, G. Kunos (editor), John Wiley and Sons, Inc., New York, pp. 1-50, 1983.

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33. Ruffolo, R. R., Jr., Anderson, K. and Miller, D. D.: Conformational requirements of alpha$_1$- and alpha$_2$-adrenergic receptors. Mol. Pharmacol. 21: 259-261, 1982.

34. Ruffolo, R. R., Jr., Yaden, E. L. and Ward, J. S.: Receptor interactions of imidazolines. VIII. Influence of ionization constant on the diffusion of clonidine and a series of structurally related imidazolidines into and out of the central nervous system. Eur. J. Pharmacol. 81: 367-375, 1982.

35. Ruffolo, R. R., Jr., Waddell, J. E. and Yaden, E. L.: Heterogeneity of postsynaptic alpha-adrenergic receptors in mammalian aortas. J. Pharmacol. Exp. Ther. 221: 309-314, 1982.

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