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(54) **HIGH PURITY LIPOPEPTIDES**

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(58) **Field of Classification Search** 514/9, 11,
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See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,331,594 A 5/1982 Hamill et al.
4,482,487 A 11/1984 Abbott et al.
4,524,135 A 6/1985 Abbott et al.
4,537,717 A 8/1985 Abbott et al.
RE32,310 E 12/1986 Debono
RE32,311 E 12/1986 Debono
RE32,333 E 1/1987 Hamill et al.
RE32,455 E 7/1987 Hamill et al.
4,800,157 A 1/1989 Eaton et al.
4,874,843 A 10/1989 Baker
4,882,164 A 11/1989 Ferro et al.
4,885,243 A 12/1989 Huber et al.
5,271,935 A 12/1993 Franco et al.
5,387,670 A 2/1995 Roy et al.
5,573,936 A 11/1996 Kreuzman et al.
5,629,288 A 5/1997 Lattrell et al.
5,912,226 A 6/1999 Baker et al.
5,955,509 A 9/1999 Webber et al.

FOREIGN PATENT DOCUMENTS

EP 0095295 A1 11/1983
EP 0178152 A2 4/1986
EP 0294990 A2 12/1988
EP 0337731 61 10/1989
EP 0386951 A2 9/1990
WO WO 99/27954 6/1999
WO WO 99/27957 6/1999
WO WO 99/43700 9/1999
WO WO 00/18419 4/2000
WO WO 01/44271 6/2001
WO WO 01/44272 6/2001
WO WO 01/44274 6/2001

OTHER PUBLICATIONS

DeBono, M. et al.; "Enzymatic and Chemical Modifications of
Lipopeptide Antibiotic A21978C: The Synthesis and Evaluation of
Daptomycin (LY146032)," J. Antibiotics; 41; 1988; pp. 1093-1105.
Desai, J. D., et al.; "Microbial Production of Surfactants and Their
Commercial Potential," Microbiology and Molecular Biology
Review, vol. 61; No. 1; 1997; pp. 47-64; American Society for
Microbiology.
Fostel, Jennifer M., et al.; "Emerging Novel Antifungal Agents,"
DDT; vol. 5; No. 1; Jan. 2000; pp. 25-32; Elsevier Science Ltd.
Horowitz, Sarah, et al.; "Isolation and Characterization of a Surfactant
Produced by *Bacillus licheniformis* 86," J. Industrial Microbiol.; 6;
1990; pp. 243-248; Society for Industrial Microbiology.
Kirsch, Lee E., et al.; "Kinetics of the Aspartyl Transporation of
Daptomycin, a Novel Lipopeptide Antibiotic," Pharmaceutical
Research; vol. 6; No. 5; 1989; pp. 387-393; Plenum Publishing
Corporation.
Lasic, Dan D., et al.; "Novel Applications of Liposomes," Trends
Biotechnology; vol. 16; Jul. 1998; pp. 307-321; Elsevier Science Ltd.
Lasic, Danilo D., et al.; "Mixed Micelles in Drug Delivery," Nature;
vol. 355; Issue No. 6357; Jan. 16, 1992; pp. 279-280.
Lin, S.-C. et al., "General Approach for the Development of High-
Performance Liquid Chromatography Methods for Biosurfactant
Analysis and Purification," J. Chromatography; 825; 1998; pp. 149-
159.
Lin, S.-C. et al.; "Recovery and Purification of the Lipopeptide
Biosurfactant of *Bacillus subtilis* by Ultrafiltration," Biotechnology
Techniques; vol. 11; No. 6; Jun. 1997; pp. 413-416; Chapman Hall.

(Continued)

Primary Examiner — Chih-Min Kam

(57) **ABSTRACT**

The invention discloses highly purified daptomycin and to
pharmaceutical compositions comprising this compound.
The invention discloses a method of purifying daptomycin
comprising the sequential steps of anion exchange chroma-
tography, hydrophobic interaction chromatography and
anion exchange chromatography. The invention also dis-
closes a method of purifying daptomycin by modified buffer
enhanced anion exchange chromatography. The invention
also discloses an improved method for producing daptomycin
by fermentation of *Streptomyces roseosporus*. The invention
also discloses high pressure liquid chromatography methods
for analysis of daptomycin purity. The invention also dis-
closes lipopeptide micelles and methods of making the
micelles. The invention also discloses methods of using
lipopeptide micelles for purifying lipopeptide antibiotics,
such as daptomycin. The invention also discloses using
lipopeptide micelles therapeutically.

OTHER PUBLICATIONS

- Mulligan, Catherine N., et al., "Recovery of Biosurfactants by Ultrafiltration," J. Chem. Tech. Biotechnology.; 47; 1990; pp. 23-29; Society of Chemical Industry; Printed in Great Britain.
- Schott, H.; "Colloidal Dispersions," Remington: The Science and Practice of Pharmacy; vol. 1; 19th Edition; 1995; pp. 252-277; Mack Publishing Company; Easton, Pennsylvania USA.
- Shaw, Duncan J.; "Liquid-Gas and Liquid-Liquid Interfaces," Introduction to Colloid and Surface Chemistry; 1989; pp. 64-114; 4th Edition; Butterworth-Heinemann Ltd. Great Britain.
- Sterling, John; "Membrane-Based System Combines Selective Separation with High-Volume Throughput," Genetic Engineering News; vol. 19; No. 20; Nov. 15, 1999; pp. 1, 34.
- Supersaxo, Andreas et al.; "Mixed Micelles as Proliposomal, Lymphotropic Drug Carrier," Pharmaceutical Research; vol. 8; No. 10; 1991; pp. 1286-1291; Plenum Publishing Corporation.
- Sweadner, Kathleen J. et al., "Filter Removal of Endotoxin (Pyrogens) In Solution in Different States of Aggregation," Applied and Environmental Microbiology; vol. 34; No. 4; 1977; pp. 382-385; American Society for Microbiology; Printed in the USA.
- Tally, F.P., et al.; "Daptomycin: A Novel Agent for Gram Positive Infections," Exp. Opin. Invest. Drugs; 8; 1999; 1223-1238.
- Thimon, L et al., "Surface-Active Properties of Antifungal Lipopeptides Produced by *Bacillus subtilis*," J. Am. Oil Chem. Soc.; 69; 1992; pp. 92-93.
- Yakimov, Michell M. et al.; "Characterization of a New Lipopeptide Surfactant Produced by Thermotolerant and Halotolerant Subsurface *Bacillus licheniformis* BAS50," Applied and Environmental Microbiology; vol. 61; No. 5; 1995; pp. 1706-1713; American Society for Microbiology.
- U.S. Appl. No. 07/060,148, filed Jun. 10, 1987, Baker et al.
- Agreement between Cubist Pharmaceuticals, Inc. and Eli Lilly and Company dated Nov. 7, 1997. (Redacted form from SEC Edgar).
- Agreement between Cubist Pharmaceuticals, Inc. and Eli Lilly and Company dated Oct. 6, 2000. (Redacted form from SEC Edgar).
- Assignment of US Re 39,071 from Eli Lilly and Company to Cubist Pharmaceuticals, Inc. recorded on Apr. 23, 2007. Reel/Frame: 019181/0916.
- Maio, et al., "Daptomycin biosynthesis in *Streptomyces roseosporus*: cloning and analysis of the gene cluster and revision of peptide stereochemistry," Microbiology, (vol. 151), (p. 1507-1523), (2005).
- Molloy, M. et al., Abstract, "Structure & Anhydro-Daptomycin and Iso-Daptomycin," ACS 200th Meeting, 1990.
- Molloy, M. et al., Poster, "Structure & Anhydro-Daptomycin and Iso-Daptomycin," ACS 200th Meeting, 1990.

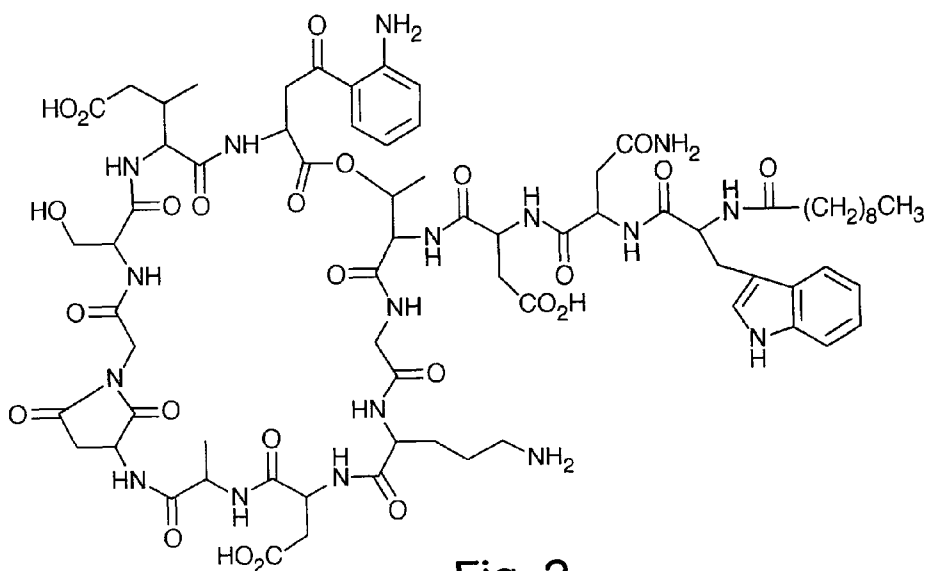


Fig. 3

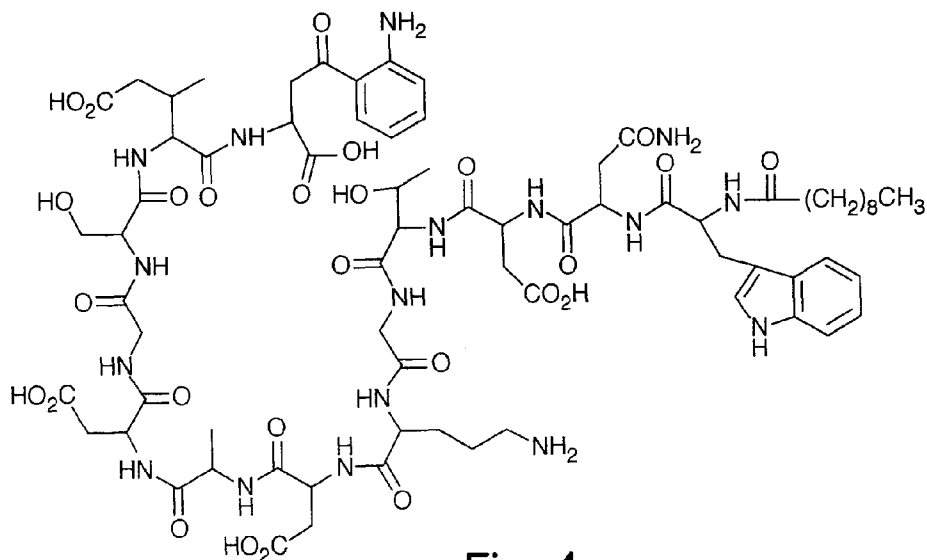


Fig. 4

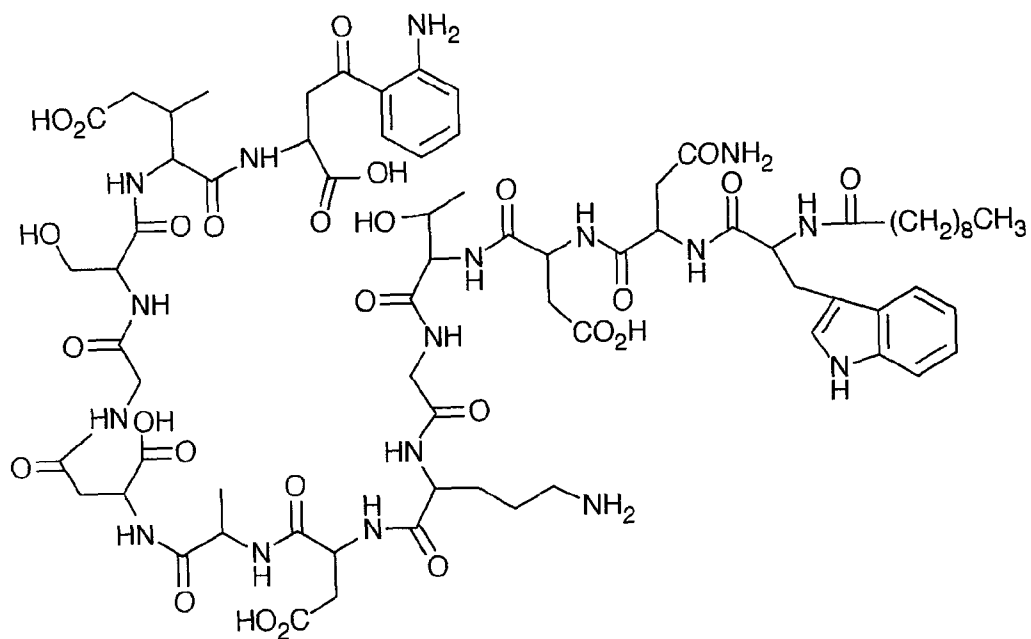


Fig. 5

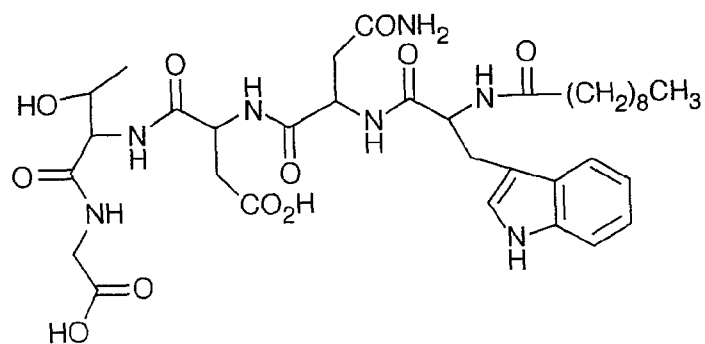


Fig. 6

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