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				First Named Inventor	Bruce Scharschmidt
(use as many sheets as necessary)				**Art Unit**	1629
				Examiner Name	Sara Elizabeth Townsley
Sheet	5	of	10	**Attorney Docket Number**	HOR0027-201-US

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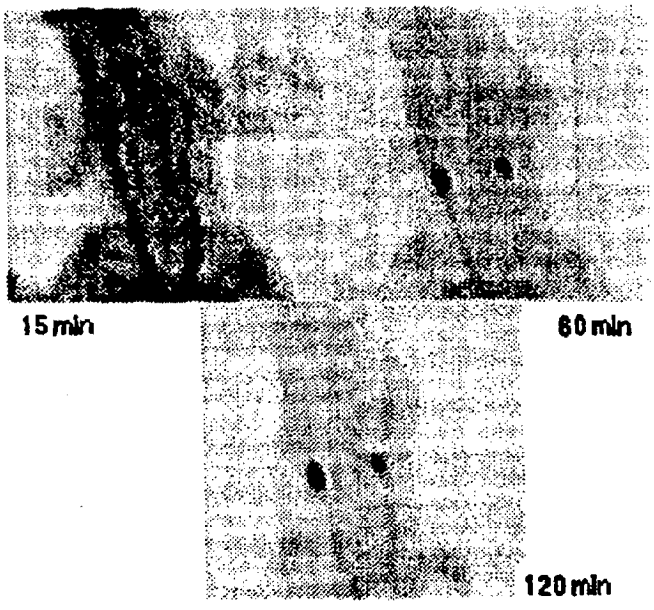
(54) Title: RADIOLABELED PLATELET GPIIb/IIIa RECEPTOR ANTAGONISTS AS IMAGING AGENTS FOR THE DIAGNOSIS OF THROMBOEMBOLIC DISORDERS

(57) Abstract

This invention provides novel radiopharmaceuticals that are radiolabeled cyclic compounds containing carbocyclic or heterocyclic ring systems which act as antagonists of the platelet glycoprotein IIb/IIIa complex; to methods of using said radiopharmaceuticals as imaging agents for the diagnosis of arterial and venous thrombi; to novel reagents for the preparation of said radiopharmaceuticals; and to kits comprising said reagents.



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TITLE

Radiolabeled Platelet GPIIb/IIIa Receptor Antagonists As Imaging Agents For The Diagnosis Of Thromboembolic Disorders

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application is a continuation-in-part of our copending application U.S.S.N. 08/040,336 filed March 30, 1993, the disclosure of which is hereby incorporated herein by reference.

FIELD OF THE INVENTION

This invention relates to novel radiopharmaceuticals that are radiolabeled cyclic compounds containing carbocyclic or heterocyclic ring systems; to methods of using said radiopharmaceuticals as imaging agents for the diagnosis of arterial and venous thrombi; to novel reagents for the preparation of said radiopharmaceuticals; and to kits comprising said reagents.

BACKGROUND OF THE INVENTION

The clinical recognition of venous and arterial thromboembolic disorders is unreliable, lacking in both sensitivity and specificity. In light of the potentially life threatening situation, the need to rapidly diagnose thromboembolic disorders using a non invasive method is an unmet clinical need. Platelet activation and resulting aggregation has been shown to be associated with various pathophysiological conditions including cardiovascular and cerebrovascular thromboembolic disorders such as unstable angina, myocardial infarction, transient ischemic attack, stroke, atherosclerosis and diabetes. The contribution

of platelets to these disease processes stems from their ability to form aggregates, or platelet thrombi, especially in the arterial wall following injury. See generally, Fuster et al., JACC, Vol. 5, No. 6, pp. 175B-183B (1985); Rubenstein et al., Am. Heart J., Vol. 102, pp. 363-367 (1981); Hamm et al., J. Am. Coll. Cardiol., Vol. 10, pp. 998-1006 (1987); and Davies et al., Circulation, Vol. 73, pp. 418-427 (1986). Recently, the platelet glycoprotein IIb/IIIa complex (GPIIb/IIIa), has been identified as the membrane protein which mediates platelet aggregation by providing a common pathway for the known platelet agonists. See Philips et al., Cell, Vol. 65, pp. 359-362 (1991).

Platelet activation and aggregation is also thought to play a significant role in venous thromboembolic disorders such as venous thrombophlebitis and subsequent pulmonary emboli. It is also known that patients whose blood flows over artificial surfaces, such as prosthetic synthetic cardiac valves, are at risk for the development of platelet plugs, thrombi and emboli. See generally Fuster et al., JACC, Vol. 5, No. 6, pp. 175B-183B (1985); Rubenstein et al., Am. Heart J., Vol. 102, pp. 363-367 (1981); Hamm et al., J. Am. Coll. Cardiol., Vol. 10, pp. 998-1006 (1987); and Davies et al., Circulation, Vol. 73, pp. 418-427 (1986).

A suitable means for the non-invasive diagnosis and monitoring of patients with such potential thromboembolic disorders would be highly useful, and several attempts have been made to develop radiolabeled agents targeted to platelets for non-invasive radionuclide imaging. For example, experimental studies have been carried out with 99mTc monoclonal antifibrin antibody for diagnostic imaging of arterial thrombus. See Cerqueira et al., Circulation, Vol., 85, pp. 298-304

(1992). The authors report the potential utility of such agents in the imaging of freshly formed arterial thrombus. Monoclonal antibodies labeled with 131I and specific for activated human platelets have also been reported to have potential application in the diagnosis of arterial and venous thrombi. However, a reasonable ratio of thrombus to blood (target/background) was only attainable at 4 hours after the administration of the radiolabeled antibody. See Wu et al., Clin. Med. J., Vol. 105, pp. 533-559 (1992). The use of 125I, 131I, 99mTc, and 111In radiolabeled 7E3 monoclonal antiplatelet antibody in imaging thrombi has also been recently discussed. Coller et al., PCT Application Publication No. WO 89/11538 (1989). The radiolabeled 7E3 antibody has the disadvantage, however, of being a very large molecular weight molecule. Other researchers have employed enzymatically inactivated t-PA radioiodinated with 123I, 125I and 131I for the detection and the localization of thrombi. See Ordm et al., Circulation, Vol. 85, pp. 288-297 (1992). Still other approaches in the radiologic detection of thromoboembolisms are described, for example, in Koblik et al., Semin. Nucl. Med., Vol. 19, pp. 221-237 (1989).

Arterial and venous thrombus detection and localization is of critical importance in accurately diagnosing thromboembolic disorders and determining proper therapy. New and better radiolabeled agents for non-invasive radionuclide imaging to detect thrombi are needed. The present invention is directed to this important end.

SUMMARY OF THE INVENTION

This invention provides novel radiopharmaceuticals that are radiolabeled cyclic compounds containing carbocyclic or heterocyclic ring systems which act as

antagonists of the platelet glycoprotein IIb/IIIa complex. It also provides methods of using said radiopharmaceuticals as imaging agents for the diagnosis of arterial and venous thrombi. It further provides novel reagents for the preparation of said radiopharmaceuticals. It further provides kits comprising said reagents.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1a. Illustrated are typical images of the radiopharmaceutical compound of Example 12 administered at 1 mCi/Kg,i.v. in a canine deep venous thrombosis model. In this model thrombi were formed in the jugular veins during a period of stasis which was followed by reflow. The compounds were administered beginning at reflow. Depicted is the uptake in a rapidly growing venous thrombus at 15, 60 and 120 min post-administration.

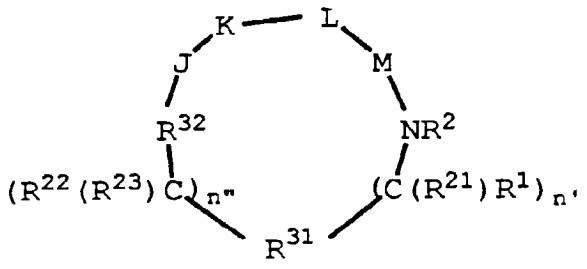
Figure 1b. Illustrated are typical images of the radiopharmaceutical compound of Example 19 administered at 1 mCi/Kg,i.v. in a canine deep venous thrombosis model. In this model thrombi were formed in the jugular veins during a period of stasis which was followed by reflow. The compounds were administered beginning at reflow. Depicted is the uptake in a rapidly growing venous thrombus at 15, 60 and 120 min post-administration.

DETAILED DESCRIPTION OF THE INVENTION

[1] The present invention is directed to novel reagents for preparing a radiopharmaceutical of formulae:

$(QL_n)_dC_h$; $(Q)_{d'}L_n-C_h$,

wherein, d is 1-3, d' is 2-20, L_n is a linking group, C_h is a metal chelator, and Q is a compound of formula (I):



(I)

or a pharmaceutically acceptable salt or prodrug form thereof, wherein:

R^{31} is a C_6-C_{14} saturated, partially saturated, or aromatic carbocyclic ring system, substituted with 0-4 R^{10} or R^{10a}, and optionally bearing a bond to L_n; a heterocyclic ring system, optionally substituted with 0-4 R^{10} or R^{10a}, and optionally bearing a bond to L_n;

R^{32} is selected from:

$-C(=O)-$;

$-C(=S)-$

$-S(=O)_2-$;

$-S(=O)-$;

$-P(=Z)(ZR^{13})-$;

Z is S or O;

n" and n' are independently 0-2;

R^1 and R^{22} are independently selected from the following groups:

hydrogen,
C_1-C_8 alkyl substituted with 0-2 R^{11};
C_2-C_8 alkenyl substituted with 0-2 R^{11};
C_2-C_8 alkynyl substituted with 0-2 R^{11};
C_3-C_{10} cycloalkyl substituted with 0-2 R^{11};

a bond to L_n;

aryl substituted with 0-2 R^{12};

a 5-10-membered heterocyclic ring system containing 1-4 heteroatoms independently selected from N, S, and O, said heterocyclic ring being substituted with 0-2 R^{12};

=O, F, Cl, Br, I, $-CF_3$, -CN, $-CO_2R^{13}$, $-C(=O)R^{13}$, $-C(=O)N(R^{13})_2$, -CHO, $-CH_2OR^{13}$, $-OC(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{14}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{14}SO_2N(R^{13})_2$, $-NR^{14}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-SR^{13}$, $-S(=O)R^{13a}$, $-SO_2N(R^{13})_2$, $-N(R^{13})_2$, $-NHC(=NH)NHR^{13}$, $-C(=NH)NHR^{13}$, $=NOR^{13}$, NO_2, $-C(=O)NHOR^{13}$, $-C(=O)NHNR^{13}R^{13a}$, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy;

R^1 and R^{21} can alternatively join to form a 3-7 membered carbocyclic ring substituted with 0-2 R^{12};

when n' is 2, R^1 or R^{21} can alternatively be taken together with R^1 or R^{21} on an adjacent carbon atom to form a direct bond, thereby to form a double or triple bond between said carbon atoms;

R^{21} and R^{23} are independently selected from:

hydrogen;
C_1-C_4 alkyl, optionally substituted with 1-6 halogen;
benzyl;

R^{22} and R^{23} can alternatively join to form a 3-7 membered carbocyclic ring substituted with 0-2 R^{12};

when n" is 2, R^{22} or R^{23} can alternatively be taken together with R^{22} or R^{23} on an adjacent carbon atom to form a direct bond, thereby to form a double or triple bond between the adjacent carbon atoms;

R^1 and R^2, where R^{21} is H, can alternatively join to form a 5-8 membered carbocyclic ring substituted with 0-2 R^{12};

R^{11} is selected from one or more of the following:

=O, F, Cl, Br, I, $-CF_3$, $-CN$, $-CO_2R^{13}$, $-C(=O)R^{13}$, $-C(=O)N(R^{13})_2$, $-CHO$, $-CH_2OR^{13}$, $-OC(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{14}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{14}SO_2N(R^{13})_2$, $-NR^{14}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-SR^{13}$, $-S(=O)R^{13a}$, $-SO_2N(R^{13})_2$, $-N(R^{13})_2$, $-NHC(=NH)NHR^{13}$, $-C(=NH)NHR^{13}$, $=NOR^{13}$, NO_2, $-C(=O)NHOR^{13}$, $-C(=O)NHNR^{13}R^{13a}$, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy,

C_1-C_5 alkyl, C_2-C_4 alkenyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_2-C_6 alkoxyalkyl, C_3-C_6 cycloalkoxy, C_1-C_4 alkyl (alkyl being substituted with 1-5 groups selected independently from: $-NR^{13}R^{14}$, $-CF_3$, NO_2, $-SO_2R^{13a}$, or $-S(=O)R^{13a}$),

aryl substituted with 0-2 R^{12},

a 5-10-membered heterocyclic ring system containing 1-4 heteroatoms independently selected from N, S, and O, said heterocyclic ring being substituted with 0-2 R^{12};

R^{12} is selected from one or more of the following:

phenyl, benzyl, phenethyl, phenoxy, benzyloxy, halogen, hydroxy, nitro, cyano, C_1-C_5 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_7-C_{10} arylalkyl, C_1-C_5 alkoxy, $-CO_2R^{13}$, $-C(=O)NHOR^{13a}$, $-C(=O)NHN(R^{13})_2$, $=NOR^{13}$, $-B(R^{34})(R^{35})$, C_3-C_6 cycloalkoxy, $-OC(=O)R^{13}$, $-C(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-(C_1$-C_4 alkyl$)-OR^{13}$, $-N(R^{13})_2$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{13}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{13}SO_2N(R^{13})_2$, $-NR^{13}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-S(=O)R^{13a}$, $-SR^{13}$, $-SO_2N(R^{13})_2$, C_2-C_6 alkoxyalkyl, methylenedioxy, ethylenedioxy, C_1-C_4 haloalkyl, C_1-C_4 haloalkoxy, C_1-C_4 alkylcarbonyloxy, C_1-C_4 alkylcarbonyl, C_1-C_4 alkylcarbonylamino, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy, C_1-C_4 alkyl (alkyl being substituted with $-N(R^{13})_2$, $-CF_3$, NO_2, or $-S(=O)R^{13a}$);

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, $-(C_1$-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, $-(C_1$-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may alternatively be taken together to form $-(CH_2)_{2-5}-$ or $-(CH_2)O(CH_2)-$;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

R^2 is H or C_1-C_8 alkyl;

R^{10} and R^{10a} are selected independently from one or more of the following:

phenyl, benzyl, phenethyl, phenoxy, benzyloxy, halogen, hydroxy, nitro, cyano, C_1-C_5 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_7-C_{10} arylalkyl, C_1-C_5 alkoxy, $-CO_2R^{13}$, $-C(=O)N(R^{13})_2$, $-C(=O)NHOR^{13a}$, $-C(=O)NHN(R^{13})_2$, $=NOR^{13}$, $-B(R^{34})(R^{35})$, C_3-C_6 cycloalkoxy, $-OC(=O)R^{13}$, $-C(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-(C_1$-C_4 alkyl$)-OR^{13}$, $-N(R^{13})_2$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{13}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{13}SO_2N(R^{13})_2$, $-NR^{13}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-S(=O)R^{13a}$, $-SR^{13}$, $-SO_2N(R^{13})_2$, C_2-C_6 alkoxyalkyl, methylenedioxy, ethylenedioxy, C_1-C_4 haloalkyl (including $-C_vF_w$ where v = 1 to 3 and w = 1 to (2v+1)), C_1-C_4 haloalkoxy, C_1-C_4 alkylcarbonyloxy, C_1-C_4 alkylcarbonyl, C_1-C_4 alkylcarbonylamino, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy, C_1-C_4 alkyl (alkyl being substituted with $-N(R^{13})_2$, $-CF_3$, NO_2, or $-S(=O)R^{13a}$);

J is β-Ala or an L-isomer or D-isomer amino acid of structure $-N(R^3)C(R^4)(R^5)C(=O)-$, wherein:

R^3 is H or C_1-C_8 alkyl;

R^4 is H or C_1-C_3 alkyl;

R^5 is selected from:

hydrogen;

C_1-C_8 alkyl substituted with 0-2 R^{11};

C_2-C_8 alkenyl substituted with 0-2 R^{11};

C_2-C_8 alkynyl substituted with 0-2 R^{11};

C_3-C_{10} cycloalkyl substituted with 0-2 R^{11};

a bond to L_n;

aryl substituted with 0-2 R^{12};

a 5-10-membered heterocyclic ring system containing 1-4 heteroatoms independently selected from N, S, or O, said heterocyclic ring being substituted with 0-2 R^{12};

=O, F, Cl, Br, I, $-CF_3$, -CN, $-CO_2R^{13}$, $-C(=O)R^{13}$, $-C(=O)N(R^{13})_2$, -CHO, $-CH_2OR^{13}$, $-OC(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{14}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{14}SO_2N(R^{13})_2$, $-NR^{14}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-SR^{13}$, $-S(=O)R^{13a}$, $-SO_2N(R^{13})_2$, $-N(R^{13})_2$, $-NHC(=NH)NHR^{13}$, $-C(=NH)NHR^{13}$, $=NOR^{13}$, NO_2, $-C(=O)NHOR^{13}$, $-C(=O)NHNR^{13}R^{13a}$, $=NOR^{13}$, $-B(R^{34})(R^{35})$, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy,

$-SC(=NH)NHR^{13}$, N_3, $-Si(CH_3)_3$, (C_1-C_5 alkyl)NHR^{16};

$-(C_0$-C_6 alkyl)X;

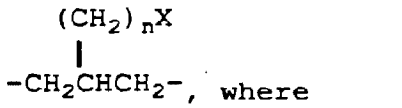

, where q is independently 0,1;

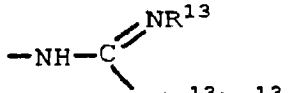

;

$-(CH_2)_mS(O)_{p'}(CH_2)_2X$, where m = 1,2 and p' = 0-2;

wherein X is defined below; and

R^3 and R^4 may also be taken together to form


, where

n = 0,1 and X is 
;

R^3 and R^5 can alternatively be taken together to form $-(CH_2)_t-$ or $-CH_2S(O)_{p'}C(CH_3)_2-$, where t = 2-4 and p' = 0-2; or

R^4 and R^5 can alternatively be taken together to form $-(CH_2)_u-$, where u = 2-5;

R^{16} is selected from:

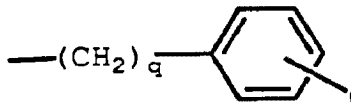
an amine protecting group;
1-2 amino acids;
1-2 amino acids substituted with an amine protecting group;

K is a D-isomer or L-isomer amino acid of structure
$-N(R^6)CH(R^7)C(=O)-$, wherein:

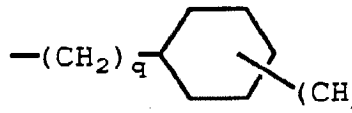
R^6 is H or C_1-C_8 alkyl;

R^7 is selected from:

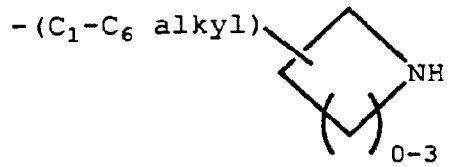
$-(C_1$-C_7 alkyl)X;



wherein each q is independently 0-2 and substitution on the phenyl is at the 3 or 4 position;



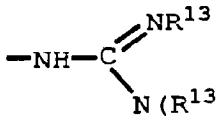
wherein each q is independently 0-2 and substitution on the cyclohexyl is at the 3 or 4 position;



$-(CH_2)_mO-(C_1-C_4$ alkyl)$-X$, where m = 1 or 2;

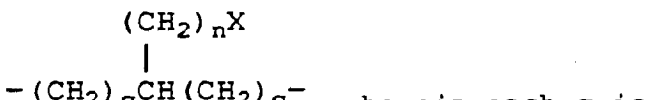
$-(CH_2)_mS(O)_{p'}-(C_1-C_4$ alkyl)$-X$, where m = 1 or 2 and p' = 0-2; and

X is selected from:



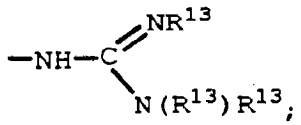
$N(R^{13})R^{13}$; $-N(R^{13})R^{13}$; $-C(=NH)(NH_2)$; $-SC(=NH)-NH_2$; $-NH-C(=NH)(NHCN)$; $-NH-C(=NCN)(NH_2)$; $-NH-C(=N-OR^{13})(NH_2)$;

R^6 and R^7 can alternatively be taken together to form



wherein each q is independently 1 or 2 and wherein

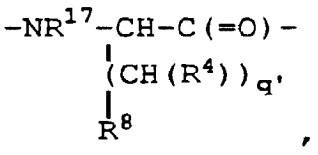
n = 0 or 1 and X is $-NH_2$ or



L is $-Y(CH_2)_vC(=O)-$, wherein:

Y is NH, N(C_1-C_3 alkyl), O, or S; and v = 1 or 2;

M is a D-isomer or L-isomer amino acid of structure



wherein:

q' is 0-2;

R^{17} is H, C_1-C_3 alkyl;

R^8 is selected from:
$-CO_2R^{13}$, $-SO_3R^{13}$, $-SO_2NHR^{14}$, $-B(R^{34})(R^{35})$, $-NHSO_2CF_3$, $-CONHNHSO_2CF_3$, $-PO(OR^{13})_2$, $-PO(OR^{13})R^{13}$, $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O) , $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O), $-SO_2NHCOR^{13}$, $-CONHSO_2R^{13a}$, $-CH_2CONHSO_2R^{13a}$, $-NHSO_2NHCOR^{13a}$, $-NHCONHSO_2R^{13a}$, $-SO_2NHCONHR^{13}$;

R^{34} and R^{35} are independently selected from:
-OH,
-F,
$-N(R^{13})_2$, or

C_1-C_8-alkoxy;

R^{34} and R^{35} can alternatively be taken together form:

a cyclic boron ester where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-4 heteroatoms independently selected from N, S, or O;

a divalent cyclic boron amide where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-4 heteroatoms independently selected from N, S, or O;

a cyclic boron amide-ester where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-4 heteroatoms independently selected from N, S, or O.

[2] Included in the present invention are those reagents in [1] above, wherein:

R^{31} is bonded to $(C(R^{23})R^{22})_{n"}$ and $(C(R^{21})R^{1})_{n'}$ at 2 different atoms on said carbocyclic ring.

[3] Included in the present invention are those reagents in [1] above, wherein:

n" is 0 and n' is 0;
n" is 0 and n' is 1;
n" is 0 and n' is 2;
n" is 1 and n' is 0;
n" is 1 and n' is 1;

n" is 1 and n' is 2;
n" is 2 and n' is 0;
n" is 2 and n' is 1; or
n" is 2 and n' is 2.

[4] Included in the present invention are those reagents in [1] above, wherein:
wherein R^6 is methyl, ethyl, or propyl.

[5] Included in the present invention are those reagents in [1] above, wherein:

R^{32} is selected from:
$-C(=O)-$;
$-C(=S)-$
$-S(=O)_2-$;

R^1 and R^{22} are independently selected from the following groups:

hydrogen,
C_1-C_8 alkyl substituted with 0-2 R^{11},
C_2-C_8 alkenyl substituted with 0-2 R^{11},
C_2-C_8 alkynyl substituted with 0-2 R^{11},
C_3-C_8 cycloalkyl substituted with 0-2 R^{11},
C_6-C_{10} bicycloalkyl substituted with 0-2 R^{11};

a bond to L_n;

aryl substituted with 0-2 R^{12};

a 5-10-membered heterocyclic ring system containing 1-4 heteroatoms independently selected from N, S, or O, said heterocyclic ring being substituted with 0-2 R^{12};

=O, F, Cl, Br, I, $-CF_3$, -CN, $-CO_2R^{13}$, $-C(=O)R^{13}$, $-C(=O)N(R^{13})_2$, -CHO, $-CH_2OR^{13}$, $-OC(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{14}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{14}SO_2N(R^{13})_2$, $-NR^{14}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-SR^{13}$, $-S(=O)R^{13a}$, $-SO_2N(R^{13})_2$, $-CH_2N(R^{13})_2$, $-N(R^{13})_2$, $-NHC(=NH)NHR^{13}$, $-C(=NH)NHR^{13}$, NO_2;

R^1 and R^{21} can alternatively join to form a 5-7 membered carbocyclic ring substituted with 0-2 R^{12};

when n' is 2, R^1 or R^{21} can alternatively be taken together with R^1 or R^{21} on an adjacent carbon atom to form a direct bond, thereby to form a double or triple bond between said carbon atoms;

R^{22} and R^{23} can alternatively join to form a 3-7 membered carbocyclic ring substituted with 0-2 R^{12};

when n" is 2, R^{22} or R^{23} can alternatively be taken together with R^{22} or R^{23} on an adjacent carbon atom to form

a direct bond, thereby to form a double or triple bond between said carbon atoms;

R^1 and R^2, where R^{21} is H, can alternatively join to form a 5-8 membered carbocyclic ring substituted with 0-2 R^{12};

R^{11} is selected from one or more of the following:

=O, F, Cl, Br, I, $-CF_3$, -CN, $-CO_2R^{13}$, $-C(=O)R^{13}$, $-C(=O)N(R^{13})_2$, -CHO, $-CH_2OR^{13}$, $-OC(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{14}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{14}SO_2N(R^{13})_2$, $-NR^{14}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-SR^{13}$, $-S(=O)R^{13a}$, $-SO_2N(R^{13})_2$, $-CH_2N(R^{13})_2$, $-N(R^{13})_2$, $-NHC(=NH)NHR^{13}$, $-C(=NH)NHR^{13}$, $=NOR^{13}$, NO_2;

C_1-C_5 alkyl, C_2-C_4 alkenyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_2-C_6 alkoxyalkyl, C_1-C_4 alkyl (substituted with $-NR^{13}R^{14}$, $-CF_3$, NO_2, $-SO_2R^{13}$, or $-S(=O)R^{13a}$)

aryl substituted with 0-2 R^{12},

a 5-10-membered heterocyclic ring system containing 1-4 heteroatoms independently selected from N, S, or O, said heterocyclic ring being substituted with 0-2 R^{12};

R^3 is H or CH_3;

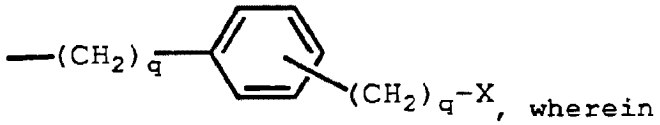
R^5 is H, C_1-C_8 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_1-C_6 cycloalkylethyl, phenyl, phenylmethyl, CH_2OH, CH_2SH, CH_2OCH_3, CH_2SCH_3, $CH_2CH_2SCH_3$, $(CH_2)_sNH_2$, $(CH_2)_sNHC(=NH)(NH_2)$, $(CH_2)_sNHR^{16}$, where s = 3-5;

a bond to L_n;

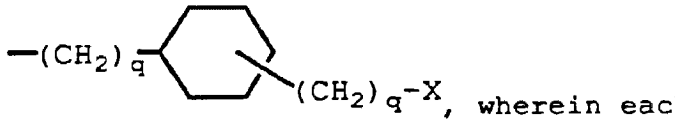
R^3 and R^5 can alternatively be taken together to form $-(CH_2)_t-$ (t = 2-4) or $-CH_2SC(CH_3)_2-$; or

R^7 is selected from:

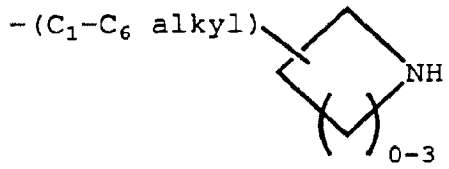
$-(C_1-C_7$ alkyl)X;



wherein each q is independently 0-2 and substitution on the phenyl is at the 3 or 4 position;



wherein each q is independently 0-2 and substitution on the cyclohexyl is at the 3 or 4 position;

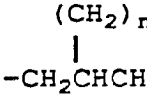


-$(CH_2)_m$O-(C_1-C_4 alkyl)-X, where m = 1 or 2;

-$(CH_2)_m$S-(C_1-C_4 alkyl)-X, where m = 1 or 2; and

X is selected from:
-NH-C(=NH)(NH_2), -NHR^{13}, -C(=NH)(NH_2), -SC(NH)-NH_2;

R^6 and R^7 can alternatively be taken together to form

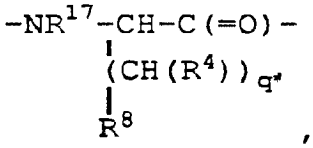


, where
n = 0 or 1 and X is -NH_2 or -NH-C(=NH)(NH_2);

L is -Y$(CH_2)_v$C(=O)-, wherein:

Y is NH, N(C_1-C_3 alkyl), O, or S; and v = 1 or 2;

M is a D-isomer or L-isomer amino acid of structure



wherein:

q' is 0-2;

R^{17} is H, C_1-C_3 alkyl;

R^8 is selected from:

$-CO_2R^{13}$, $-SO_3R^{13}$, $-SO_2NHR^{14}$, $-B(R^{34})(R^{35})$, $-NHSO_2CF_3$, $-CONHNHSO_2CF_3$, $-PO(OR^{13})_2$, $-PO(OR^{13})R^{13}$, $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O) , $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O), $-SO_2NHCOR^{13}$, $-CONHSO_2R^{13a}$, $-CH_2CONHSO_2R^{13a}$, $-NHSO_2NHCOR^{13a}$, $-NHCONHSO_2R^{13a}$, $-SO_2NHCONHR^{13}$;

R^{34} and R^{35} are independently selected from:
-OH,
-F,
$-NR^{13}R^{14}$, or
C_1-C_8-alkoxy;

R^{34} and R^{35} can alternatively be taken together form:
a cyclic boron ester where said chain or ring contains from 2 to 20 carbon atoms

and, optionally, 1-4 heteroatoms independently selected from N, S, or O;
a divalent cyclic boron amide where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-4 heteroatoms independently selected from N, S, or O;
a cyclic boron amide-ester where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-4 heteroatoms independently selected from N, S, or O.

[6] Included in the present invention are those reagents in [1] above, wherein:

R^{31} is selected from the group consisting of:

(a) a 6 membered saturated, partially saturated or aromatic carbocyclic ring substituted with 0-3 R^{10} or R^{10a}, and optionally bearing a bond to Ln;

(b) a 8-11 membered saturated, partially saturated, or aromatic fused bicyclic carbocyclic ring substituted with 0-3 R^{10} or R^{10a}, and optionally bearing a bond to Ln; or

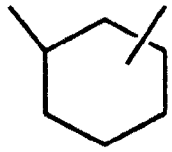
(c) a 14 membered saturated, partially saturated, or aromatic fused tricyclic carbocyclic ring substituted with 0-3 R^{10}

or R^{10a}, and optionally bearing a bond to Ln.

[7] Included in the present invention are those reagents in [1] above, wherein:

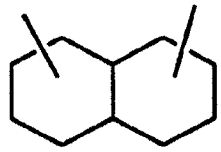
R^{31} is selected from the group consisting of:

(a) a 6 membered saturated, partially saturated, or aromatic carbocyclic ring of formulae:



wherein any of the bonds forming the carbocyclic ring may be a single or double bond, and wherein said carbocyclic ring is substituted with 0-3 R^{10}, and optionally bears a bond to Ln;

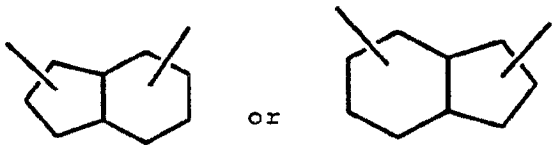
(b) a 10 membered saturated, partially saturated, or aromatic bicyclic carbocyclic ring of formula:



wherein any of the bonds forming the carbocyclic ring may be a single or double bond, wherein said carbocyclic

ring is substituted independently with 0-4 R^{10}, and optionally bears a bond to L_n;

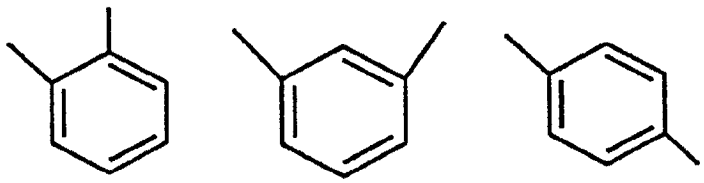
(c) a 9 membered saturated, partially saturated, or aromatic bicyclic carbocyclic ring of formula:



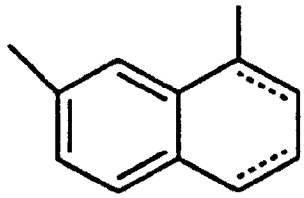
wherein any of the bonds forming the carbocyclic ring may be a single or double bond, wherein said carbocyclic ring is substituted independently with 0-4 R^{10}, and optionally bears a bond to L_n.

[8] Included in the present invention are those reagents in [1] above, wherein:

R^{31} is selected from (the dashed bond may be a single or double bond):



; or



wherein R^{31} may be independently substituted with 0-3 R^{10} or R^{10a}, and optionally bears a bond to L_n;

n" is 0 or 1; and

n' is 0-2.

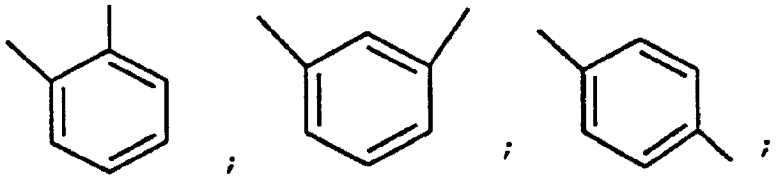
[9] Included in the present invention are those reagents in [1] above, wherein:

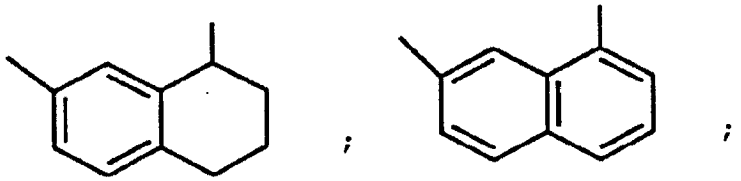
R^1 and R^{22} are independently selected from: phenyl, benzyl, phenethyl, phenoxy, benzyloxy, halogen, hydroxy, nitro, cyano, C_1-C_5 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_7-C_{10} arylalkyl, C_1-C_5 alkoxy, $-CO_2R^{13}$, $-C(=O)NHOR^{13a}$, $-C(=O)NHN(R^{13})_2$, $=NOR^{13}$, $-B(R^{34})(R^{35})$, C_3-C_6 cycloalkoxy, $-OC(=O)R^{13}$, $-C(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-(C_1$-C_4 alkyl$)-OR^{13}$, $-N(R^{13})_2$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{13}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{13}SO_2N(R^{13})_2$, $-NR^{13}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-S(=O)R^{13a}$, $-SR^{13}$, $-SO_2N(R^{13})_2$, C_2-C_6 alkoxyalkyl, methylenedioxy, ethylenedioxy, C_1-C_4 haloalkyl, C_1-C_4 haloalkoxy, C_1-C_4 alkylcarbonyloxy, C_1-C_4

alkylcarbonyl, C_1-C_4 alkylcarbonylamino, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy, C_1-C_4 alkyl (alkyl being substituted with $-N(R^{13})_2$, $-CF_3$, NO_2, or $-S(=O)R^{13a}$).

[10] Included in the present invention are those reagents in [1] above, wherein:

R^{31} is selected from:





wherein R^{31} may be independently substituted with 0-3 R^{10} or R^{10a}, and may optionally bear a bond to L_n;

R^{32} is $-C(=O)-$;

n" is 0 or 1;

n' is 0-2;

R^1 and R^{22} are independently selected from H, C_1-C_4 alkyl, phenyl, benzyl, phenyl-(C_2-C_4)alkyl, C_1-C_4 alkoxy; and a bond to L_n;

R^{21} and R^{23} are independently H or C_1-C_4 alkyl;

R^2 is H or C_1-C_8 alkyl;

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may alternatively be taken together to form $-(CH_2)_{2-5}-$ or $-(CH_2)O(CH_2)-$;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

R^{10} and R^{10a} are selected independently from: H, C_1-C_8 alkyl, phenyl, halogen, or C_1-C_4 alkoxy;

J is β-Ala or an L-isomer or D-isomer amino acid of structure $-N(R^3)C(R^4)(R^5)C(=O)-$, wherein:

R^3 is H or CH_3;

R^4 is H or C_1-C_3 alkyl;

R^5 is H, C_1-C_8 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_1-C_6 cycloalkylethyl, phenyl, phenylmethyl, CH_2OH, CH_2SH, CH_2OCH_3, CH_2SCH_3, $CH_2CH_2SCH_3$, $(CH_2)_sNH_2$, $-(CH_2)_sNHC(=NH)(NH_2)$, $-(CH_2)_sNHR^{16}$, where s = 3-5; and a bond to L_n; or

R^3 and R^5 can alternatively be taken together to form $-(CH_2)_t-$ (t = 2-4) or $-CH_2SC(CH_3)_2-$; or

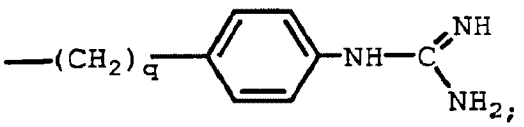
R^4 and R^5 can alternatively be taken together to form $-(CH_2)_u-$, where u = 2-5;

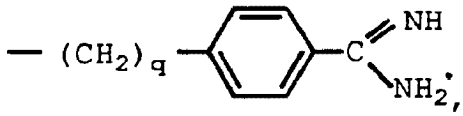
R^{16} is selected from:
an amine protecting group;
1-2 amino acids; or
1-2 amino acids substituted with an amine protecting group;

K is an L-isomer amino acid of structure $-N(R^6)CH(R^7)C(=O)-$, wherein:

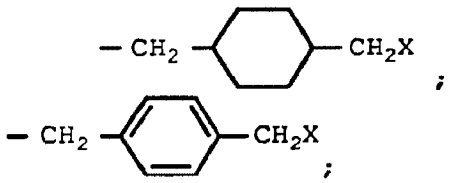
R^6 is H or C_1-C_8 alkyl;

R^7 is



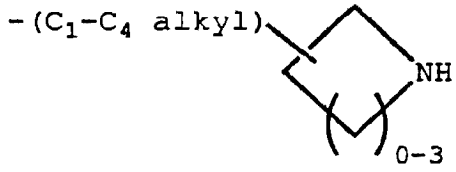

, where q = 0 or 1;

$-(CH_2)_rX$, where r = 3-6;


;

$-(CH_2)_mS(CH_2)_2X$, where m = 1 or 2;

$-(C_3-C_7$ alkyl)$-NH-(C_1-C_6$ alkyl);

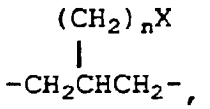


$-(CH_2)_m-O-(C_1-C_4$ alkyl)$-NH-(C_1-C_6$ alkyl), where m = 1 or 2;

$-(CH_2)_m-S-(C_1-C_4$ alkyl)$-NH-(C_1-C_6$ alkyl), where m = 1 or 2; and

X is $-NH_2$ or $-NHC(=NH)(NH_2)$; or

R^6 and R^7 can alternatively be taken together to form

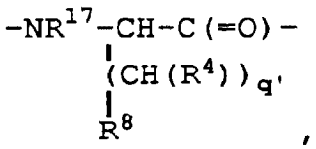

, where n = 0 or 1

and X is $-NH_2$ or $-NHC(=NH)(NH_2)$;

L is $-Y(CH_2)_vC(=O)-$, wherein:

Y is NH, O, or S; and v = 1 or 2;

M is a D-isomer or L-isomer amino acid of structure



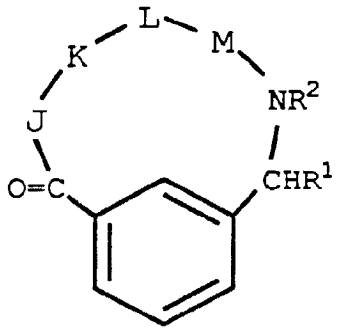
wherein:

q' is 0-2;

R^{17} is H, C_1-C_3 alkyl;

R^8 is selected from:
$-CO_2R^{13}$, $-SO_3R^{13}$, $-SO_2NHR^{14}$, $-B(R^{34})(R^{35})$, $-NHSO_2CF_3$, $-CONHNHSO_2CF_3$, $-PO(OR^{13})_2$, $-PO(OR^{13})R^{13}$, $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O) , $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O), $-SO_2NHCOR^{13}$, $-CONHSO_2R^{13a}$, $-CH_2CONHSO_2R^{13a}$, $-NHSO_2NHCOR^{13a}$, $-NHCONHSO_2R^{13a}$, $-SO_2NHCONHR^{13}$.

[11] Included in the present invention are those reagents in [1] above, wherein Q is a 1,3-disubstituted phenyl compound of the formula (II):



(II)

wherein:

the shown phenyl ring in formula (II) may be substituted with 0-3 R^{10}, and may optionally bear a bond to L_n;

R^{10} is selected independently from: H, C_1-C_8 alkyl, phenyl, halogen, or C_1-C_4 alkoxy;

R^1 is H, C_1-C_4 alkyl, phenyl, benzyl, phenyl-(C_1-C_4)alkyl, or a bond to L_n;

R^2 is H or methyl;

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may alternatively be taken together to form $-(CH_2)_{2-5}-$ or $-(CH_2)O(CH_2)-$;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

J is β-Ala or an L-isomer or D-isomer amino acid of structure $-N(R^3)C(R^4)(R^5)C(=O)-$, wherein:

R^3 is H or CH_3;

R^4 is H or C_1-C_3 alkyl;

R^5 is H, C_1-C_8 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_1-C_6 cycloalkylethyl, phenyl, phenylmethyl, CH_2OH, CH_2SH, CH_2OCH_3, CH_2SCH_3, $CH_2CH_2SCH_3$, $(CH_2)_sNH_2$, $-(CH_2)_sNHC(=NH)(NH_2)$, $-(CH_2)_sNHR^{16}$, where s = 3-5, or a bond to L_n;

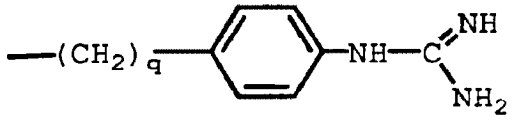
R^3 and R^5 can alternatively be taken together to form $-CH_2CH_2CH_2-$; or
R^4 and R^5 can alternatively be taken together to form $-(CH_2)_u-$, where u = 2-5;

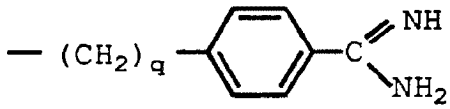
R^{16} is selected from:
an amine protecting group;
1-2 amino acids; or
1-2 amino acids substituted with an amine protecting group;

K is an L-isomer amino acid of structure $-N(R^6)CH(R^7)C(=O)-$, wherein:

R^6 is H or C_1-C_8 alkyl;

R^7 is:



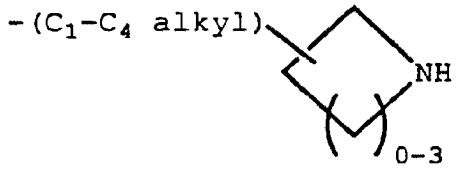


where q = 0 or 1;

$-(CH_2)_rX$, where r = 3-6;

$-(CH_2)_mS(CH_2)_2X$, where m = 1 or 2;

$-(C_3$-C_7 alkyl)-NH-(C_1-C_6 alkyl)



$-(CH_2)_m$-O-(C_1-C_4 alkyl)-NH-(C_1-C_6 alkyl), where m = 1 or 2;

$-(CH_2)_m-S-(C_1-C_4$ alkyl$)-NH-(C_1-C_6$ alkyl$)$,
where m = 1 or 2; and

X is $-NH_2$ or $-NHC(=NH)(NH_2)$, provided that X is not $-NH_2$ when r = 4; or

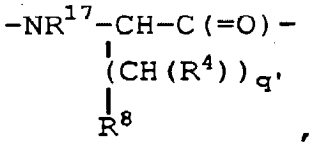
R^6 and R7 are alternatively be taken together to form

where n = 0,1 and X is $-NH_2$ or $-NHC(=NH)(NH_2)$;

L is $-Y(CH_2)_vC(=O)-$, wherein:

Y is NH, O, or S; and v = 1,2;

M is a D-isomer or L-isomer amino acid of structure



wherein:

q' is 0-2;

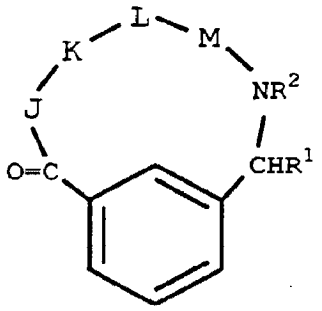
R^{17} is H, C_1-C_3 alkyl;

R^8 is selected from:

$-CO_2R^{13}$, $-SO_3R^{13}$, $-SO_2NHR^{14}$, $-B(R^{34})(R^{35})$, $-NHSO_2CF_3$, $-CONHNHSO_2CF_3$, $-PO(OR^{13})_2$, $-PO(OR^{13})R^{13}$, $-SO_2NH$-heteroaryl (said

heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O) , $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O), $-SO_2NHCOR^{13}$, $-CONHSO_2R^{13a}$, $-CH_2CONHSO_2R^{13a}$, $-NHSO_2NHCOR^{13a}$, $-NHCONHSO_2R^{13a}$, $-SO_2NHCONHR^{13}$.

[12] Included in the present invention are those reagents in [1] above, wherein Q is 1,3-disubstituted phenyl compound of the formula (II):



(II)

wherein:

the phenyl ring in formula (II) may be substituted with 0-3 R^{10} or R^{10a};

R^{10} or R^{10a} are selected independently from: H, C_1-C_8 alkyl, phenyl, halogen, or C_1-C_4 alkoxy;

R^1 is H, C_1-C_4 alkyl, phenyl, benzyl, or phenyl-(C_2- C_4)alkyl;

R^2 is H or methyl;

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may alternatively be taken together to form $-(CH_2)_{2-5}-$ or $-(CH_2)O(CH_2)-$;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

J is β-Ala or an L-isomer or D-isomer amino acid of structure $-N(R^3)C(R^4)(R^5)C(=O)-$, wherein:

R^3 is H or CH_3;

R^4 is H;

R^5 is H, C_1-C_8 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_1-C_6 cycloalkylethyl, phenyl, phenylmethyl, CH_2OH, CH_2SH, CH_2OCH_3, CH_2SCH_3, $CH_2CH_2SCH_3$, $(CH_2)_sNH_2$, $(CH_2)_sNHC(=NH)(NH_2)$, $(CH_2)_sR^{16}$, where s = 3-5; or a bond to L_n;

R^3 and R^5 can alternatively be taken together to form $-CH_2CH_2CH_2-$;

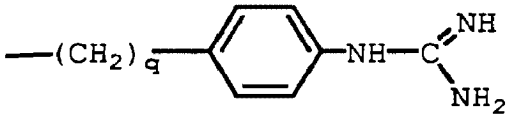
R^{16} is selected from:

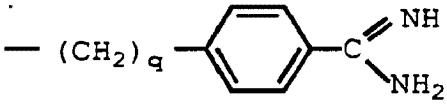
an amine protecting group;
1-2 amino acids;
1-2 amino acids substituted with an amine protecting group;

K is an L-isomer amino acid of structure $-N(R^6)CH(R^7)C(=O)-$, wherein:

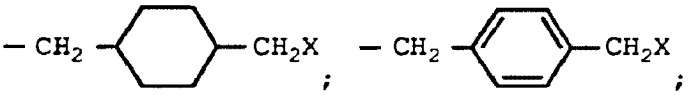
R^6 is H or C_3-C_8 alkyl;

R^7 is



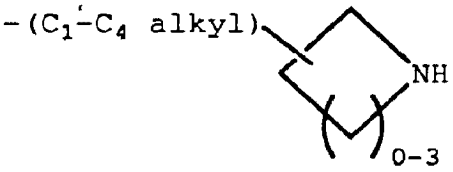

, where q = 0 or 1;

$-(CH_2)_rX$, where r = 3-6;


;

$-(CH_2)_mS(CH_2)_2X$, where m = 1 or 2;

$-(C_4$-C_7 alkyl$)$-NH-$(C_1$-C_6 alkyl$)$



$-(CH_2)_m-O-(C_1-C_4$ alkyl$)-NH-(C_1-C_6$ alkyl), where m = 1 or 2;

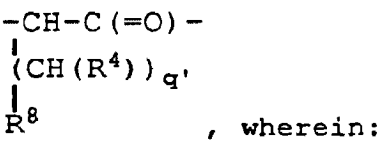
$-(CH_2)_m-S-(C_1-C_4$ alkyl$)-NH-(C_1-C_6$ alkyl), where m = 1 or 2; and

X is $-NH_2$ or $-NHC(=NH)(NH_2)$, provided that X is not $-NH_2$ when r = 4; or

L is $-YCH_2C(=O)-$, wherein:

Y is NH or O;

M is a D-isomer or L-isomer amino acid of structure



, wherein:

q' is 1;

R^{17} is H, C_1-C_3 alkyl;

R^8 is selected from:

$-CO_2H$ or $-SO_3R^{13}$.

[13] Included in the present invention are those reagents in [1] above, wherein:

the phenyl ring in formula (II) bears a bond to L_n, and may be further substituted with 0-2 R^{10} or R^{10a};

R^{10} or R^{10a} are selected independently from: H, C_1-C_8 alkyl, phenyl, halogen, or C_1-C_4 alkoxy;

R^1 is H;

R^2 is H;

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may alternatively be taken together to form $-(CH_2)_{2-5}-$ or $-(CH_2)O(CH_2)-$;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

J is β-Ala or an L-isomer or D-isomer amino acid of formula $-N(R^3)CH(R^5)C(=O)-$, wherein:

R^3 is H and R^5 is H, CH_3, CH_2CH_3, $CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$, $CH_2CH_2CH_3$, $CH_2CH_2CH_2CH_3$, $CH_2CH_2SCH_3$, $CH_2CH(CH_3)_2$, $(CH_2)_4NH_2$, (C_3-C_5 alkyl)NHR^{16};
or

R^3 is CH_3 and R^5 is H; or

R^3 and R^5 can alternatively be taken together to form $-CH_2CH_2CH_2-$;

R^{16} is selected from:

an amine protecting group;

1-2 amino acids;

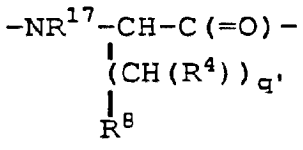
1-2 amino acids substituted with an amine protecting group;

K is an L-isomer amino acid of formula $-N(CH_3)CH(R^7)C(=O)-$, wherein:

R^7 is $-(CH_2)_3NHC(=NH)(NH_2)$;

L is $-NHCH_2C(=O)-$; and

M is a D-isomer or L-isomer amino acid of structure



, wherein:

q' is 1;

R^4 is H or CH_3;

R^{17} is H;

R^8 is

$-CO_2H$;

$-SO_3H$.

[14] Included in the present invention are those reagents in [1] above, wherein:

the phenyl ring in formula (II) bears a bond to L_n;

R^1 and R^2 are independently selected from H, methyl;

J is selected from D-Val, D-2-aminobutyric acid, D-Leu, D-Ala, Gly, D-Pro, D-Ser, D-Lys, β-Ala, Pro, Phe, NMeGly, D-Nle, D-Phg, D-Ile, D-Phe, D-Tyr, Ala, N^{ε}-p-azidobenzoyl-D-Lys, N^{ε}-p-benzoylbenzoyl-D-Lys, N^{ε}-tryptophanyl-D-Lys, N^{ε}-o-benzylbenzoyl-D-Lys, N^{ε}-p-acetylbenzoyl-D-Lys, N^{ε}-dansyl-D-Lys, N^{ε}-glycyl-D-Lys, N^{ε}-glycyl-p-benzoylbenzoyl-D-Lys, N^{ε}-p-phenylbenzoyl-D-Lys, N^{ε}-m-benzoylbenzoyl-D-Lys, N^{ε}-o-benzoylbenzoyl-D-Lys;

K is selected from NMeArg, Arg;

L is selected from Gly, β-Ala, Ala;

M is selected from Asp; αMeAsp; βMeAsp; NMeAsp; D-Asp.

[15] Included in the present invention are those reagents in [1] above, wherein:

R^{31} is a phenyl ring and bears a bond to L_n;

R^1 and R^2 are independently selected from H, methyl;

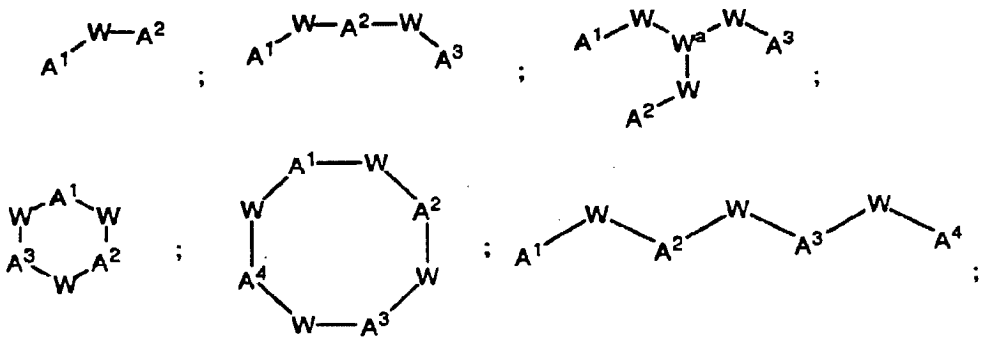
J is selected from: D-Val, D-2-aminobutyric acid, D-Leu, D-Ala, Gly, D-Pro, D-Ser, D-Lys, β-Ala, Pro, Phe, NMeGly, D-Nle, D-Phg, D-Ile, D-Phe, D-Tyr, Ala;

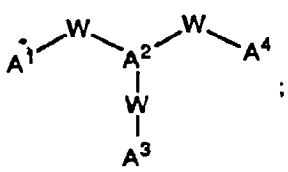
K is selected from NMeArg;

L is Gly;

M is selected from Asp; αMeAsp; βMeAsp; NMeAsp; D-Asp.

[16] Included in the present invention are those reagents in [1]-[15] above, wherein C_h is selected from the group:

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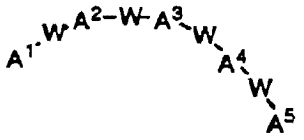
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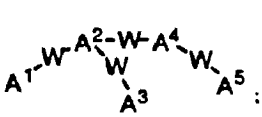
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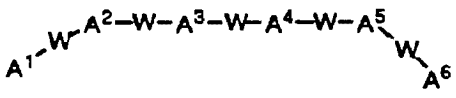
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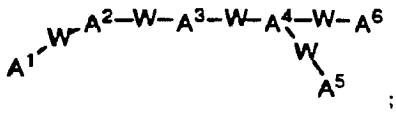
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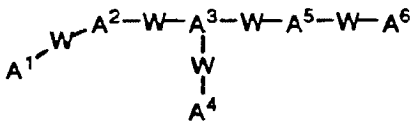
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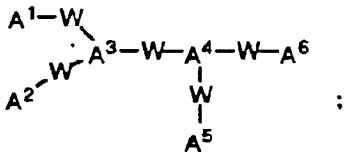
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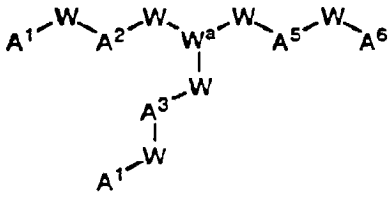
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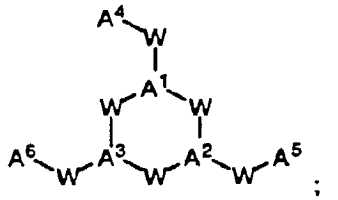
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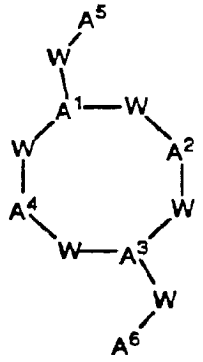
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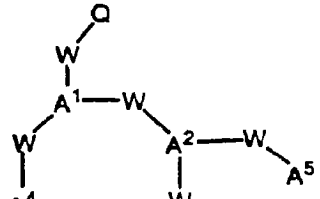
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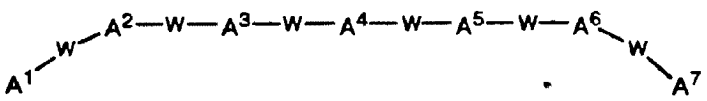

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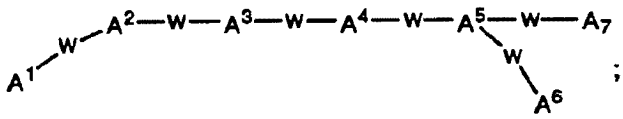

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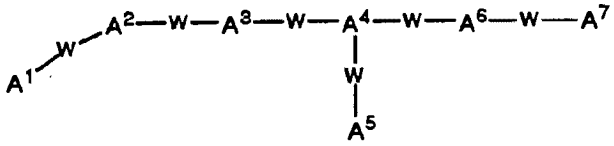

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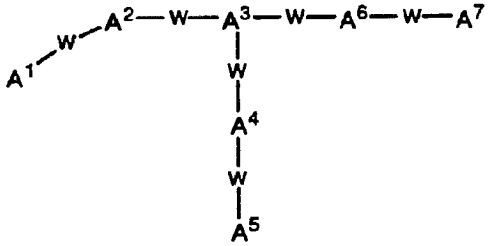

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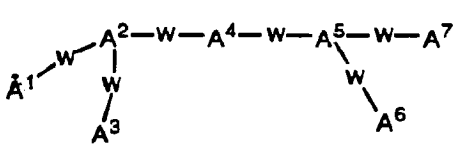
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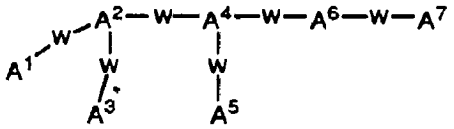
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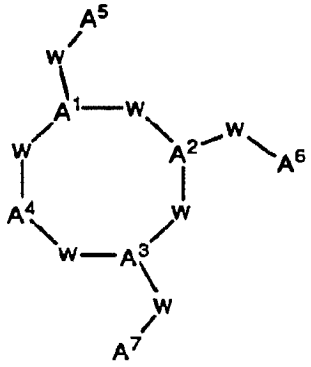
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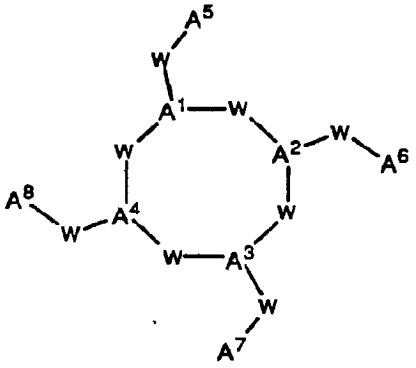
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wherein:

A^1, A^2, A^3, A^4, A^5, A^6, and A^7 are independently selected at each occurrence from the group: $NR^{40}R^{41}$, S, SH, S(Pg), O, OH, $PR^{42}R^{43}$, $P(O)R^{42}R^{43}$, $P(S)R^{42}R^{43}$, $P(NR^{44})R^{42}R^{43}$;

W is a bond, CH, or a spacer group selected from the group: C_1-C_{10} alkyl substituted with 0-3 R^{52}, aryl substituted with 0-3 R^{52}, cycloaklyl substituted with 0-3 R^{52}, heterocycloalkyl substituted with 0-3 R^{52}, aralkyl substituted with 0-3 R^{52} and alkaryl substituted with 0-3 R^{52};

W^a is a C_1-C_{10} alkyl group or a C_3-C_{14} carbocycle;

R^{40}, R^{41}, R^{42}, R^{43}, and R^{44} are each independently selected from the group: a bond to L_n, hydrogen, C_1-C_{10} alkyl substituted with 0-3 R^{52}, aryl substituted with 0-3 R^{52}, cycloaklyl substituted with 0-3 R^{52}, heterocycloalkyl substituted with 0-3 R^{52}, aralkyl substituted with 0-3 R^{52}, alkaryl substituted with 0-3 R^{52}substituted with 0-3 R^{52} and an electron, provided that when one of R^{40} or R^{41} is an electron, then the other is also an electron, and provided that when one of R^{42} or R^{43} is an electron, then the other is also an electron;

additionally, R^{40} and R^{41} may combine to form $=C(C_1\text{-}C_3$ alkyl)$(C_1\text{-}C_3$ alkyl);

R^{52} is independently selected at each occurrence from the group: a bond to L_n, =O, F, Cl, Br, I, $-CF_3$, $-CN$, $-CO_2R^{53}$, $-C(=O)R^{53}$, $-C(=O)N(R^{53})_2$, $-CHO$, $-CH_2OR^{53}$, $-OC(=O)R^{53}$, $-OC(=O)OR^{53a}$, $-OR^{53}$, $-OC(=O)N(R^{53})_2$, $-NR^{53}C(=O)R^{53}$, $-NR^{54}C(=O)OR^{53a}$, $-NR^{53}C(=O)N(R^{53})_2$, $-NR^{54}SO_2N(R^{53})_2$, $-NR^{54}SO_2R^{53a}$, $-SO_3H$, $-SO_2R^{53a}$, $-SR^{53}$, $-S(=O)R^{53a}$, $-SO_2N(R^{53})_2$, $-N(R^{53})_2$, $-NHC(=NH)NHR^{53}$, $-C(=NH)NHR^{53}$, $=NOR^{53}$, NO_2, $-C(=O)NHOR^{53}$,

$-C(=O)NHNR^{53}R^{53a}$, $-OCH_2CO_2H$,
2-(1-morpholino)ethoxy,

C_1-C_5 alkyl, C_2-C_4 alkenyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_2-C_6 alkoxyalkyl,

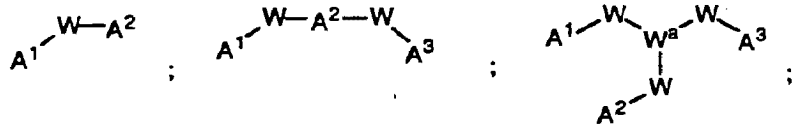
aryl substituted with 0-2 R^{53},

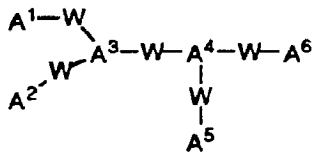
a 5-10-membered heterocyclic ring system containing 1-4 heteroatoms independently selected from N, S, and O;

R^{53}, R^{53a}, and R^{54} are independently selected at each occurrence from the group: a bond to L_n, C_1-C_6 alkyl, phenyl, benzyl, C_1-C_6 alkoxy, halide, nitro, cyano, and trifluoromethyl; and

Pg is a thiol protecting group capable of being displaced upon reaction with a radionuclide.

[17] Included in the present invention are those reagents in [1]-[15] above, wherein C_h is selected from the group:





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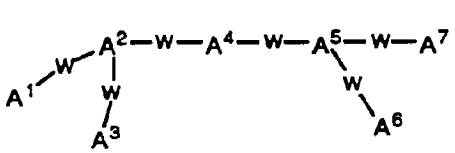

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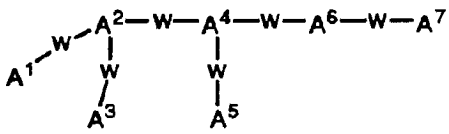

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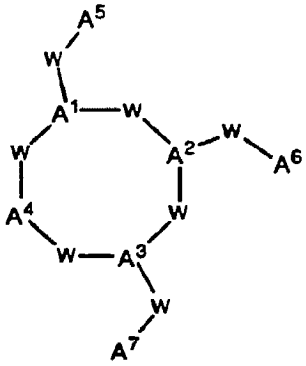

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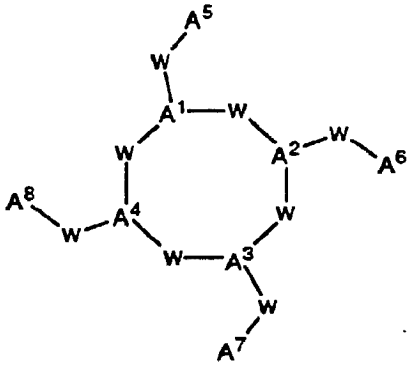


;

wherein:

A^1, A^2, A^3, A^4, A^5, A^6, and A^7 are independently selected at each occurrence from the group: $NR^{40}R^{41}$, S, SH, S(Pg), OH;

W is a bond, CH, or a spacer group selected from the group: C_1-C_3 alkyl substituted with 0-3 R^{52};

W^a is a methylene group or a C_3-C_6 carbocycle;

R^{40}, R^{41}, R^{42}, R^{43}, and R^{44} are each independently selected from the group: a bond to L_n, hydrogen, C_1-C_{10} alkyl substituted with 0-3 R^{52}, and an electron, provided that when one of R^{40} or R^{41} is an electron, then the other is also an electron, and provided that when one of R^{42} or R^{43} is an electron, then the other is also an electron;

additionally, R^{40} and R^{41} may combine to form, =C(C_1-C_3 alkyl)(C_1-C_3 alkyl);

R^{52} is independently selected at each occurrence from the group: a bond to L_n, =O, F, Cl, Br, I, $-CF_3$, -CN, $-CO_2R^{53}$, $-C(=O)R^{53}$, $-C(=O)N(R^{53})_2$, -CHO, $-CH_2OR^{53}$, $-OC(=O)R^{53}$, $-OC(=O)OR^{53a}$, $-OR^{53}$, $-OC(=O)N(R^{53})_2$, $-NR^{53}C(=O)R^{53}$, $-NR^{54}C(=O)OR^{53a}$, $-NR^{53}C(=O)N(R^{53})_2$, $-NR^{54}SO_2N(R^{53})_2$, $-NR^{54}SO_2R^{53a}$, $-SO_3H$, $-SO_2R^{53a}$, $-SR^{53}$, $-S(=O)R^{53a}$, $-SO_2N(R^{53})_2$, $-N(R^{53})_2$, $-NHC(=NH)NHR^{53}$, $-C(=NH)NHR^{53}$, $=NOR^{53}$, NO_2, $-C(=O)NHOR^{53}$, $-C(=O)NHNR^{53}R^{53a}$, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy,

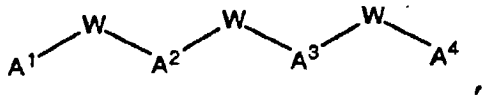
R^{53}, R^{53a}, and R^{54} are independently selected at each occurrence from the group: a bond to L_n, C_1-C_6 alkyl.

[18] Included in the present invention are those reagents in [1]-[15] above, of formula:

$$(QL_n)_dC_h,$$

wherein d is 1; and

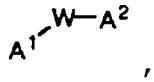
C_h is selected from:



wherein:

A^1 and A^4 are SH or SPg;
A^2 and A^3 are NR^{41};
W is independently selected from the group:
CHR^{52}, CH_2CHR^{52}, $CH_2CH_2CHR^{52}$ and $CHR^{52}C{=}O$; and
R^{41} and R^{52} are independently selected from hydrogen and a bond to L_n,

and,



wherein:

A^1 is NH_2 or $N{=}C(C_1\text{-}C_3$ alkyl$)(C_1\text{-}C_3$ alkyl);
W is a bond;

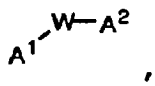
A^2 is NHR^{40}, wherein R^{40} is heterocycle substituted with R^{52}, wherein the heterocycle is selected from the group: pyridine, pyrazine, proline, furan, thiofuran, thiazole, and diazine, and R^{52} is a bond to L_n.

[19] Included in the present invention are those reagents in [1]-[15] above, of formula:

$$(QL_n)_dC_h,$$

wherein d is 1; and

wherein C_h is:



wherein:

A^1 is NH_2 or $N=C(C_1-C_3$ alkyl$)(C_1-C_3$ alkyl$)$;
W is a bond;
A^2 is NHR^{40}, wherein R^{40} is heterocycle substituted with R^{52}, wherein the heterocycle is selected from pyridine and thiazole, and R^{52} is a bond to L_n.

[20] Included in the present invention are those reagents in [1]-[15] above, wherein L_n is:

a bond between Q and C_h; or,
a compound of formula:

$$M^1-[Y^1(CR^{55}R^{56})_h(Z^1)_{h''}Y^2]_{h'}-M^2$$

wherein:

M^1 is $-[(CH_2)_gZ^1]_{g'}-(CR^{55}R^{56})_{g''}-$;
M^2 is $-(CR^{55}R^{56})_{g''}-[Z^1(CH_2)_g]_{g'}-$;
g is independently 0-10;
g' is independently 0-1;
g" is 0-10;
h is 0-10;
h' is 0-10;
h" is 0-1
Y^1 and Y^2, at each occurrence, are independently selected from:

a bond, O, NR^{56}, C=O, C(=O)O, OC(=O)O, C(=O)NH-, $C=NR^{56}$, S, SO, SO_2, SO_3, NHC(=O), $(NH)_2C(=O)$, $(NH)_2C=S$;

Z^1 is independently selected at each occurrence from a C_6-C_{14} saturated, partially saturated, or aromatic carbocyclic ring system, substituted with 0-4 R^{57}; a heterocyclic ring system, optionally substituted with 0-4 R^{57};

R^{55} and R^{56} are independently selected at each occurrence from:

hydrogen;
C_1-C_{10} alkyl substituted with 0-5 R^{57};

(C_1-C_{10} alkyl)aryl wherein the aryl is substituted with 0-5 R^{57};

R^{57} is independently selected at each occurrence from the group: hydrogen, OH, NHR^{58}, $C(=O)R^{58}$, $OC(=O)R^{58}$, $OC(=O)OR^{58}$, $C(=O)OR^{58}$, $C(=O)NR^{58}$-, C≡N, SR^{58}, SOR^{58}, SO_2R^{58}, $NHC(=O)R^{58}$, $NHC(=O)NHR^{58}$, $NHC(=S)NHR^{58}$; or, alternatively, when attached to an additional molecule Q, R^{57} is independently selected at each occurrence from the group: O, NR^{58}, C=O, C(=O)O, OC(=O)O, C(=O)N-, $C=NR^{58}$, S, SO, SO_2, SO_3, NHC(=O), $(NH)_2C(=O)$, $(NH)_2C=S$; and,

R^{58} is independently selected at each occurrence from the group:hydrogen; C_1-C_6 alkyl; benzyl, and phenyl.

[21] Included in the present invention are those reagents in [1]-[15] above, wherein Ln is:

a compound of formula:

$$M^1-[Y^1(CR^{55}R^{56})_h(Z^1)_{h''}Y^2]_{h'}-M^2$$

wherein:

M^1 is $-[(CH_2)_gZ^1]_{g'}-(CR^{55}R^{56})_{g''}-$;
M^2 is $-(CR^{55}R^{56})_{g''}-[Z^1(CH_2)_g]_{g'}-$;
g is independently 0-10;

g' is independently 0-1;
g" is 0-10;
h is 0-10;
h' is 0-10;
h" is 0-1
Y^1 and Y^2, at each occurrence, are independently selected from:

a bond, O, NR^{56}, C=O, C(=O)O, OC(=O)O, C(=O)NH-, $C=NR^{56}$, S, SO, SO_2, SO_3, NHC(=O), $(NH)_2C(=O)$, $(NH)_2C=S$;

Z^1 is independently selected at each occurrence from a C_6-C_{14} saturated, partially saturated, or aromatic carbocyclic ring system, substituted with 0-4 R^{57}; a heterocyclic ring system, optionally substituted with 0-4 R^{57};

R^{55} and R^{56} are independently selected at each occurrence from:

hydrogen;
C_1-C_{10} alkyl substituted with 0-5 R^{57};
(C_1-C_{10} alkyl)aryl wherein the aryl is substituted with 0-5 R^{57};

R^{57} is independently selected at each occurrence from the group: hydrogen, OH, NHR^{58}, $C(=O)R^{58}$, $OC(=O)R^{58}$, $OC(=O)OR^{58}$, $C(=O)OR^{58}$, $C(=O)NR^{58}$-,

C≡N, SR^{58}, SOR^{58}, SO_2R^{58}, $NHC(=O)R^{58}$, $NHC(=O)NHR^{58}$, $NHC(=S)NHR^{58}$; or, alternatively, when attached to an additional molecule Q, R57 is independently selected at each occurrence from the group: O, NR^{58}, C=O, C(=O)O, OC(=O)O, C(=O)N-, $C=NR^{58}$, S, SO, SO_2, SO_3, NHC(=O), $(NH)_2C(=O)$, $(NH)_2C=S$, and R^{57} is attached to an additional molecule Q; and,

R^{58} is independently selected at each occurrence from the group:hydrogen; C_1-C_6 alkyl; benzyl, and phenyl.

[22] Included in the present invention are those reagents in [1]-[15] above, wherein Ln is:

$$-(CR^{55}R^{56})_{g''}-[Y^1(CR^{55}R^{56})_hY^2]_{h'}-(CR^{55}R^{56})_{g''}-,$$

wherein:

g" is 1-10;
h is 0-10;
h' is 1-10;
Y^1 and Y^2, at each occurrence, are independently selected from:

a bond, O, NR^{56}, C=O, C(=O)O, OC(=O)O, C(=O)NH-, $C=NR^{56}$, S, SO, SO_2, SO_3, NHC(=O), $(NH)_2C(=O)$, $(NH)_2C=S$;

R^{55} and R^{56} are independently selected at each occurrence from:

hydrogen;
C_1-C_{10} alkyl substituted with 0-5 R^{57};
(C_1-C_{10} alkyl)aryl wherein the aryl is substituted with 0-5 R^{57};

R^{57} is independently selected at each occurrence from the group: hydrogen, OH, NHR^{58}, $C(=O)R^{58}$, $OC(=O)R^{58}$, $OC(=O)OR^{58}$, $C(=O)OR^{58}$, $C(=O)NR^{58}$-, C≡N, SR^{58}, SOR^{58}, SO_2R^{58}, $NHC(=O)R^{58}$, $NHC(=O)NHR^{58}$, $NHC(=S)NHR^{58}$; or, alternatively, when attached to an additional molecule Q, R57 is independently selected at each occurrence from the group: O, NR^{58}, C=O, C(=O)O, OC(=O)O, C(=O)N-, $C=NR^{58}$, S, SO, SO_2, SO_3, NHC(=O), $(NH)_2C(=O)$, $(NH)_2C=S$, and R^{57} is attached to an additional molecule Q; and,

R^{58} is independently selected at each occurrence from the group:hydrogen; C_1-C_6 alkyl; benzyl, and phenyl.

[23] Included in the present invention are those reagents in [1]-[15] above, wherein Ln is:

$-(CR^{55}R^{56})_{g''}-[Y^1(CR^{55}R^{56})_hY^2]_{h'}-(CR^{55}R^{56})_{g''}-$,

wherein:

g" is 1-5;
h is 0-5;
h' is 1-5;
Y^1 and Y^2, at each occurrence, are independently selected from:

O, NR^{56}, C=O, C(=O)O, OC(=O)O, C(=O)NH-, $C=NR^{56}$, S, SO, SO_2, SO_3, NHC(=O), $(NH)_2C(=O)$, $(NH)_2C=S$;

R^{55} and R^{56} are independently selected at each occurrence from:

hydrogen;
C_1-C_{10} alkyl;
(C_1-C_{10} alkyl)aryl.

[24] Included in the present invention are those reagents in [1]-[15] above, wherein Ln is:

$-(CR^{55}R^{56})_{g"}-[Y^1(CR^{55}R^{56})_hY^2]_{h'}-(CR^{55}R^{56})_{g"}-$,

wherein:

g" is 1-5;
h is 0-5;
h' is 1-5;
Y^1 and Y^2, at each occurrence, are independently selected from:

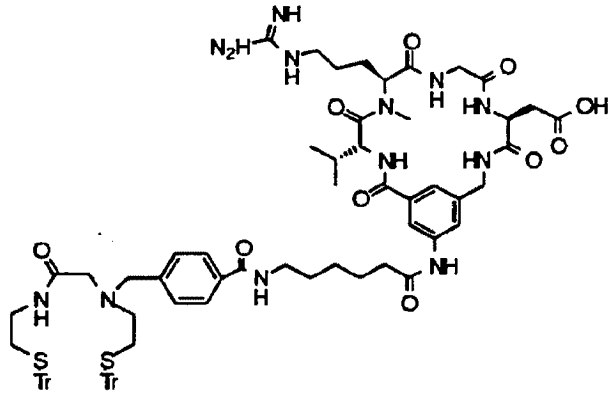
O, NR^{56}, C=O, C(=O)O, OC(=O)O,

C(=O)NH-, C=NR^{56}, S,
NHC(=O), (NH)$_2$C(=O), (NH)$_2$C=S;

R^{55} and R^{56} are independently selected at each occurrence from:

hydrogen.

[25] Included in the present invention are those reagents in [1] above, which are:



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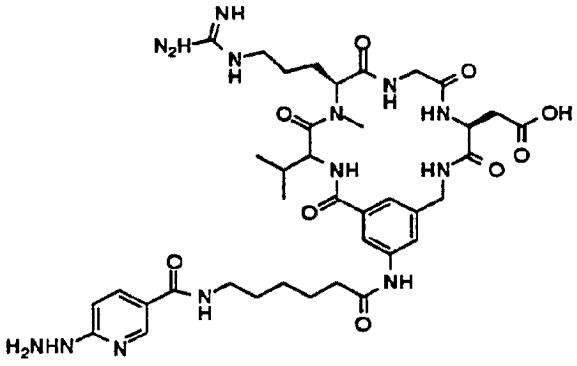

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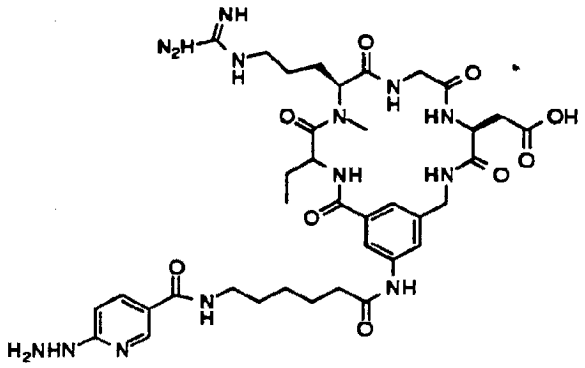
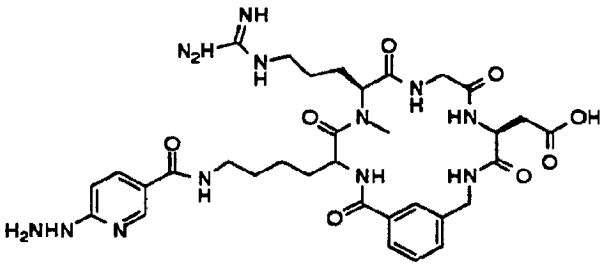
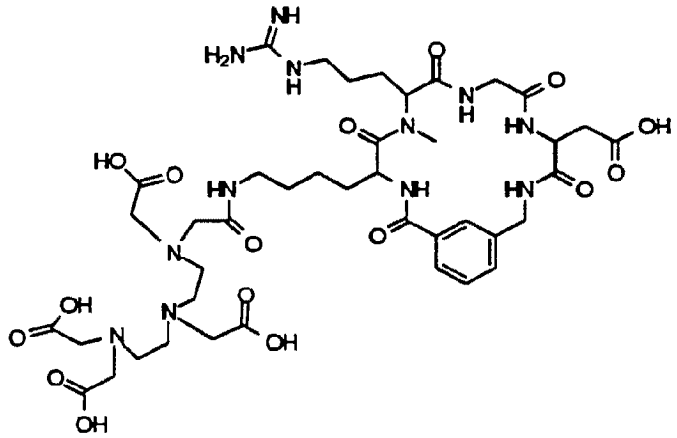
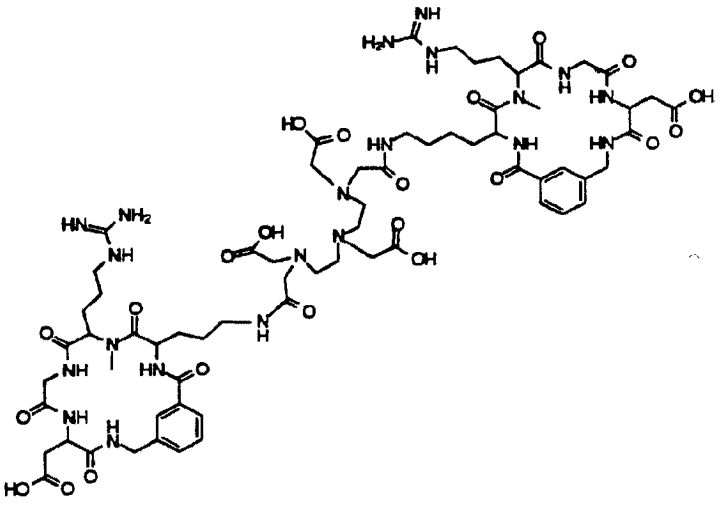








[26] Also included in the present invention is a kit for preparing a radiopharmaceutical comprising a predetermined quantity of a sterile, pharmaceutically acceptable reagent of [23].

[27] Also included in the present invention is a kit for preparing a radiopharmaceutical comprising a predetermined quantity of a sterile, pharmaceutically acceptable reagent of [24].

[28] Also included in the present invention is a kit for preparing a radiopharmaceutical comprising a predetermined quantity of a sterile, pharmaceutically acceptable reagent of [25].

[29] Also included in the present invention is a radiopharmaceutical comprising a complex of a reagent of [1]-[15] and a radionuclide selected

from the group ^{99m}Tc, ^{94m}Tc, ^{95}Tc, ^{111}In, ^{62}Cu, ^{43}Sc, ^{45}Ti, ^{67}Ga, ^{68}Ga, ^{97}Ru, ^{72}As, ^{82}Rb, and ^{201}Tl.

[30] Also included in the present invention is a radiopharmaceutical comprising a complex of a reagent of [16] and a radionuclide selected from the group ^{99m}Tc, ^{94m}Tc, ^{95}Tc, ^{111}In, ^{62}Cu, ^{43}Sc, ^{45}Ti, ^{67}Ga, ^{68}Ga, ^{97}Ru, ^{72}As, ^{82}Rb, and ^{201}Tl.

[31] Also included in the present invention is a radiopharmaceutical comprising a complex of a reagent of [17] and a radionuclide selected from the group ^{99m}Tc, ^{94m}Tc, ^{95}Tc, ^{111}In, ^{62}Cu, ^{43}Sc, ^{45}Ti, ^{67}Ga, ^{68}Ga, ^{97}Ru, ^{72}As, ^{82}Rb, and ^{201}Tl.

[32] Also included in the present invention is a radiopharmaceutical comprising a complex of a reagent of [18] and a radionuclide selected from the group ^{99m}Tc, ^{94m}Tc, ^{95}Tc, ^{111}In, ^{62}Cu, ^{43}Sc, ^{45}Ti, ^{67}Ga, ^{68}Ga, ^{97}Ru, ^{72}As, ^{82}Rb, and ^{201}Tl.

[33] Also included in the present invention is a radiopharmaceutical comprising a complex of a reagent of [19] and a radionuclide selected from the group ^{99m}Tc, ^{94m}Tc, ^{95}Tc, ^{111}In, ^{62}Cu, ^{43}Sc, ^{45}Ti, ^{67}Ga, ^{68}Ga, ^{97}Ru, ^{72}As, ^{82}Rb, and ^{201}Tl.

[34] Also included in the present invention is a radiopharmaceutical comprising a complex of a reagent of [20] and a radionuclide selected from the group ^{99m}Tc, ^{94m}Tc, ^{95}Tc, ^{111}In, ^{62}Cu, ^{43}Sc, ^{45}Ti, ^{67}Ga, ^{68}Ga, ^{97}Ru, ^{72}As, ^{82}Rb, and ^{201}Tl.

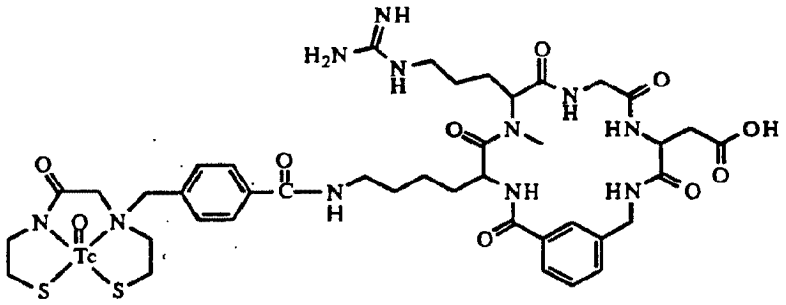
[35] Also included in the present invention is a radiopharmaceutical comprising a complex of a reagent of [21] and a radionuclide selected from the group ^{99m}Tc, ^{111}In, and ^{62}Cu.

[36] Also included in the present invention is a radiopharmaceutical comprising a complex of a reagent of [22] and a radionuclide selected from the group ^{99m}Tc, ^{111}In, and ^{62}Cu.

[37] Also included in the present invention is a radiopharmaceutical comprising a complex of a reagent of [23] and a radionuclide selected from the group ^{99m}Tc, ^{111}In, and ^{62}Cu.

[38] Also included in the present invention is a radiopharmaceutical comprising a complex of a reagent of [24] and a radionuclide selected from the group ^{99m}Tc, and ^{111}In.

[39] Also included in the present invention are the radiopharmaceuticals of [29] which are:



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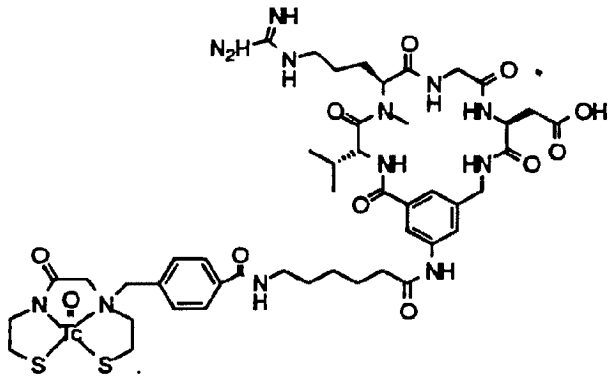

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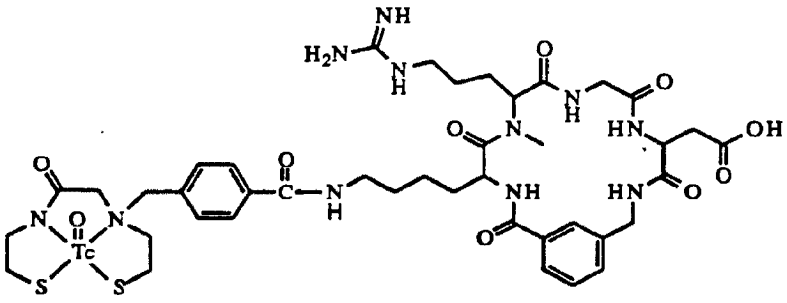

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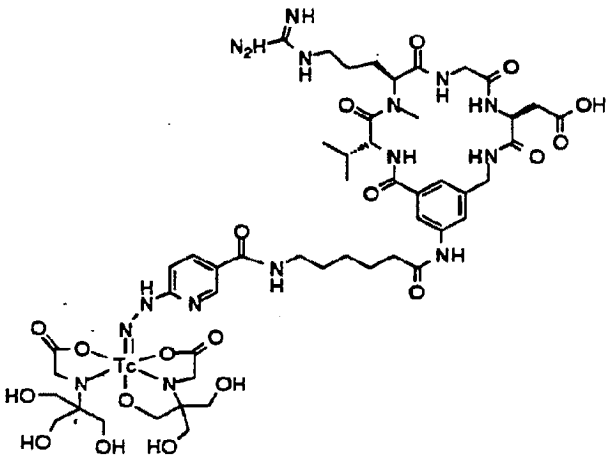

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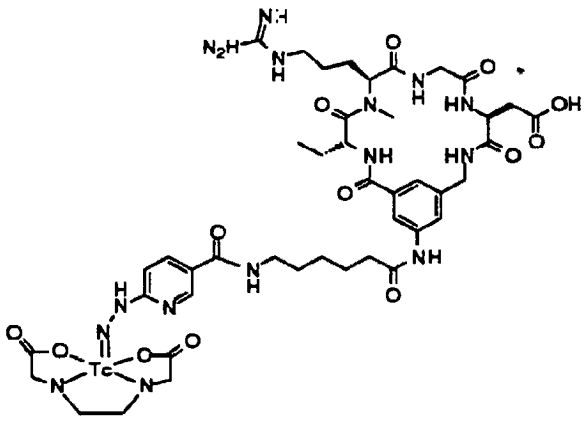

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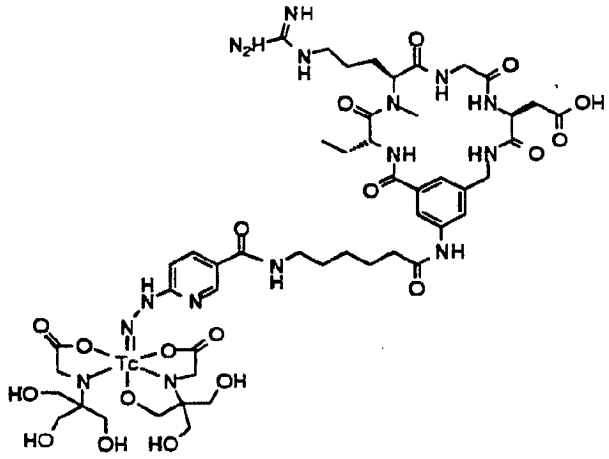

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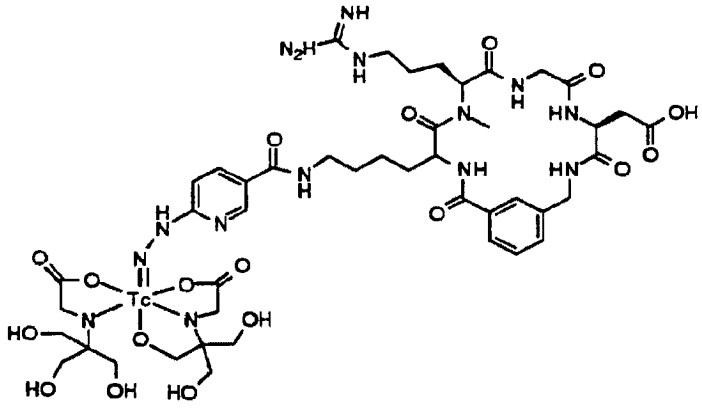

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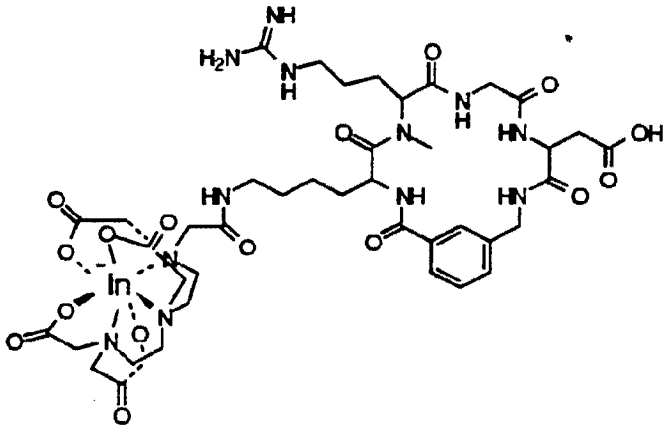

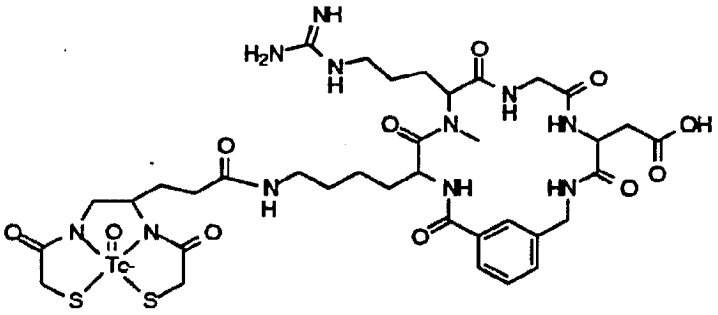

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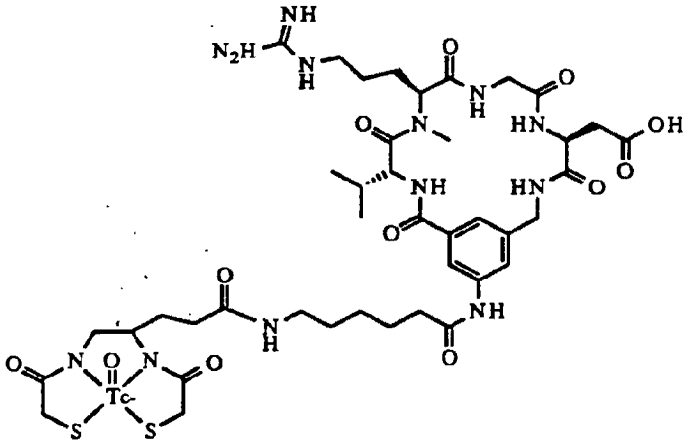

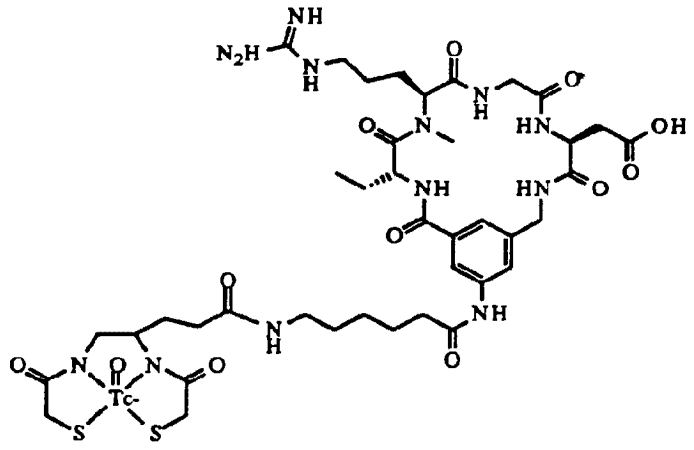

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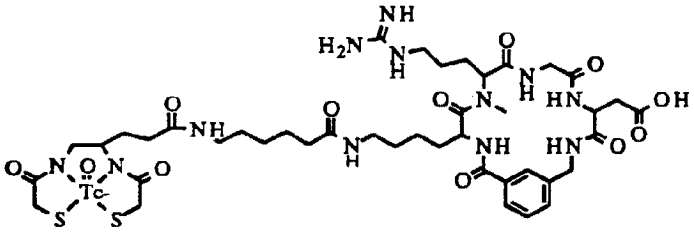

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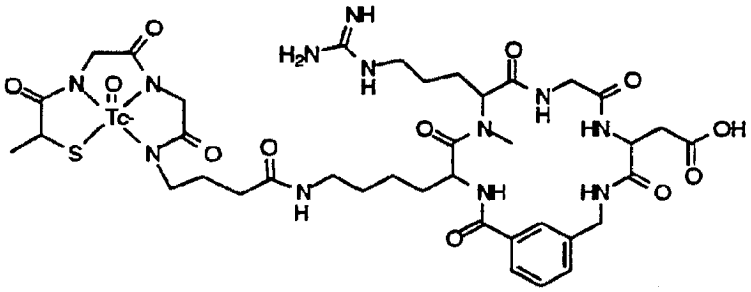

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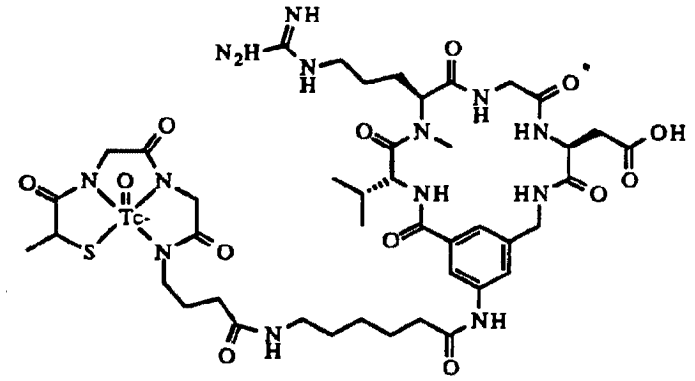

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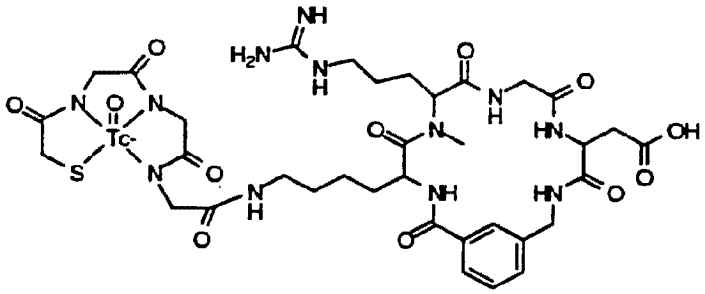

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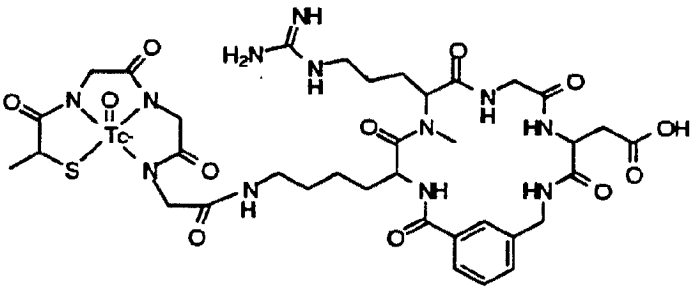

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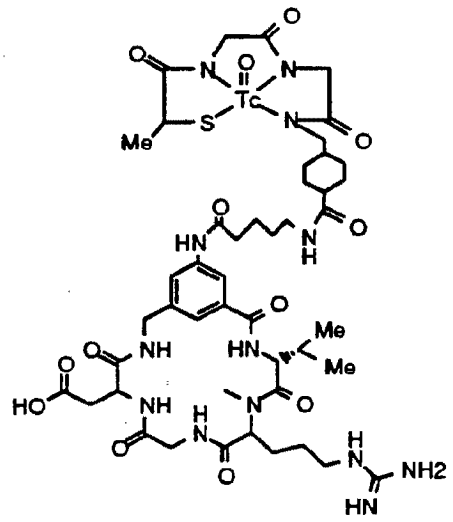

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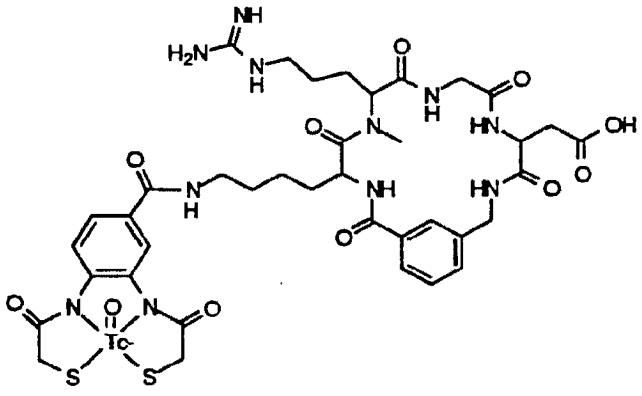

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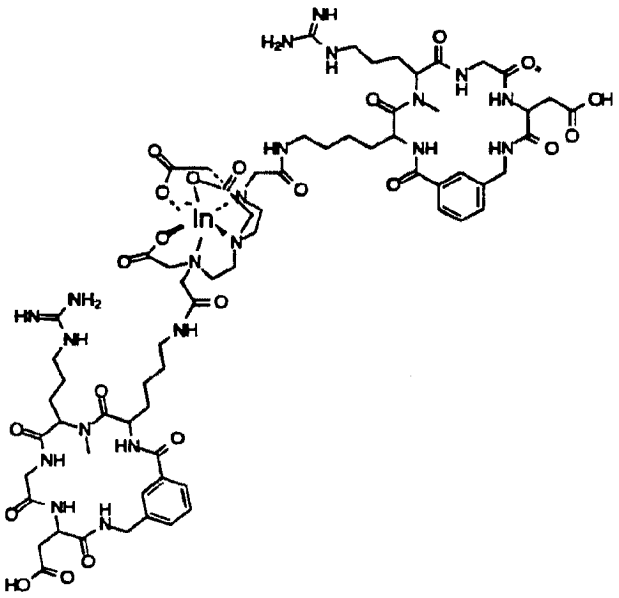


[40] Also included in the present invention is a method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of [29], and (ii) scanning the mammal using a radioimaging devise.

[41] Also included in the present invention is a method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of [30], and (ii) scanning the mammal using a radioimaging devise.

[42] Also included in the present invention is a method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of

a radiopharmaceutical of [31], and (ii) scanning the mammal using a radioimaging devise.

[43] Also included in the present invention is a method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of [32], and (ii) scanning the mammal using a radioimaging devise.

[44] A method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of [33], and (ii) scanning the mammal using a radioimaging devise.

[45] A method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of [34], and (ii) scanning the mammal using a radioimaging devise.

[46] A method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of [35], and (ii) scanning the mammal using a radioimaging devise.

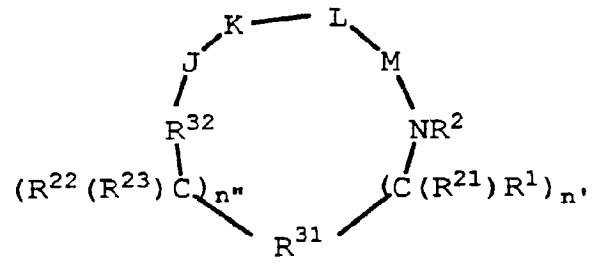
[47] A method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of [36], and (ii) scanning the mammal using a radioimaging devise.

[48] A method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of [37], and (ii) scanning the mammal using a radioimaging devise.

[49] A method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of [38], and (ii) scanning the mammal using a radioimaging devise.

[50] A method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of Claim 39, and (ii) scanning the mammal using a radioimaging devise.

[51] The present invention is also directed to direct radiolabeled compounds of formula (I):



(I)

or a pharmaceutically acceptable salt or prodrug form thereof wherein:

R^{31} is a C_6-C_{14} saturated, partially saturated, or aromatic carbocyclic ring system substituted with 0-4 R^{10} or R^{10a};

R^{32} is selected from:

-C(=O)-;

-C(=S)-

$-S(=O)_2-$;

-S(=O)-;

$-P(=Z)(ZR^{13})-$;

Z is S or O;

n" and n' are independently 0-2;

R^1 and R^{22} are independently selected from the following groups:

hydrogen,

C_1-C_8 alkyl substituted with 0-2 R^{11};

C_2-C_8 alkenyl substituted with 0-2 R^{11};

C_2-C_8 alkynyl substituted with 0-2 R^{11};

C_3-C_{10} cycloalkyl substituted with 0-2 R^{11};

aryl substituted with 0-2 R^{12};

a 5-10-membered heterocyclic ring system containing 1-4 heteroatoms independently selected from N, S, and O, said heterocyclic ring being substituted with 0-2 R^{12};

=O, F, Cl, Br, I, $-CF_3$, $-CN$, $-CO_2R^{13}$, $-C(=O)R^{13}$, $-C(=O)N(R^{13})_2$, $-CHO$, $-CH_2OR^{13}$, $-OC(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{14}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{14}SO_2N(R^{13})_2$, $-NR^{14}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-SR^{13}$, $-S(=O)R^{13a}$, $-SO_2N(R^{13})_2$, $-N(R^{13})_2$, $-NHC(=NH)NHR^{13}$, $-C(=NH)NHR^{13}$, $=NOR^{13}$, NO_2, $-C(=O)NHOR^{13}$, $-C(=O)NHNR^{13}R^{13a}$, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy;

R^1 and R^{21} can alternatively join to form a 3-7 membered carbocyclic ring substituted with 0-2 R^{12};

when n' is 2, R^1 or R^{21} can alternatively be taken together with R^1 or R^{21} on an adjacent carbon atom to form a direct bond, thereby to form a double or triple bond between said carbon atoms;

R^{22} and R^{23} can alternatively join to form a 3-7 membered carbocyclic ring substituted with 0-2 R^{12};

when n" is 2, R^{22} or R^{23} can alternatively be taken together with R^{22} or R^{23} on an adjacent carbon atom to form a direct bond, thereby to form a double or triple bond between the adjacent carbon atoms;

R^1 and R^2, where R^{21} is H, can alternatively join to form a 5-8 membered carbocyclic ring substituted with 0-2 R^{12};

R^{11} is selected from one or more of the following:

=O, F, Cl, Br, I, $-CF_3$, $-CN$, $-CO_2R^{13}$, $-C(=O)R^{13}$, $-C(=O)N(R^{13})_2$, $-CHO$, $-CH_2OR^{13}$, $-OC(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{14}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{14}SO_2N(R^{13})_2$, $-NR^{14}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-SR^{13}$, $-S(=O)R^{13a}$, $-SO_2N(R^{13})_2$, $-N(R^{13})_2$, $-NHC(=NH)NHR^{13}$, $-C(=NH)NHR^{13}$, $=NOR^{13}$, NO_2, $-C(=O)NHOR^{13}$, $-C(=O)NHNR^{13}R^{13a}$, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy,

C_1-C_5 alkyl, C_2-C_4 alkenyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_2-C_6 alkoxyalkyl, C_3-C_6 cycloalkoxy, C_1-C_4 alkyl (alkyl being substituted with 1-5 groups selected independently from: $-NR^{13}R^{14}$, $-CF_3$, NO_2, $-SO_2R^{13a}$, or $-S(=O)R^{13a}$),

aryl substituted with 0-2 R^{12},

a 5-10-membered heterocyclic ring system containing 1-4 heteroatoms independently selected from N, S, and O, said

heterocyclic ring being substituted with 0-2 R^{12};

R^{12} is selected from one or more of the following:

phenyl, benzyl, phenethyl, phenoxy, benzyloxy, halogen, hydroxy, nitro, cyano, C_1-C_5 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_7-C_{10} arylalkyl, C_1-C_5 alkoxy, $-CO_2R^{13}$, $-C(=O)NHOR^{13a}$, $-C(=O)NHN(R^{13})_2$, $=NOR^{13}$, $-B(R^{34})(R^{35})$, C_3-C_6 cycloalkoxy, $-OC(=O)R^{13}$, $-C(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-(C_1$-C_4 alkyl$)-OR^{13}$, $-N(R^{13})_2$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{13}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{13}SO_2N(R^{13})_2$, $-NR^{13}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-S(=O)R^{13a}$, $-SR^{13}$, $-SO_2N(R^{13})_2$, C_2-C_6 alkoxyalkyl, methylenedioxy, ethylenedioxy, C_1-C_4 haloalkyl, C_1-C_4 haloalkoxy, C_1-C_4 alkylcarbonyloxy, C_1-C_4 alkylcarbonyl, C_1-C_4 alkylcarbonylamino, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy, C_1-C_4 alkyl (alkyl being substituted with $-N(R^{13})_2$, $-CF_3$, NO_2, or $-S(=O)R^{13a}$);

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, $-(C_1$-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, $-(C_1$-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may alternatively be taken together to form $-(CH_2)_{2-5}-$ or $-(CH_2)O(CH_2)-$;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

R^{21} and R^{23} are independently selected from:

hydrogen;
C_1-C_4 alkyl, optionally substituted with 1-6 halogen;
benzyl;

R^2 is H or C_1-C_8 alkyl;

R^{10} and R^{10a} are selected independently from one or more of the following:

phenyl, benzyl, phenethyl, phenoxy, benzyloxy, halogen, hydroxy, nitro, cyano, C_1-C_5 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_7-C_{10} arylalkyl, C_1-C_5 alkoxy, $-CO_2R^{13}$, $-C(=O)N(R^{13})_2$, $-C(=O)NHOR^{13a}$, $-C(=O)NHN(R^{13})_2$, $=NOR^{13}$, $-B(R^{34})(R^{35})$, C_3-C_6 cycloalkoxy, $-OC(=O)R^{13}$, $-C(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-(C_1$-C_4 alkyl$)-OR^{13}$, $-N(R^{13})_2$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{13}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{13}SO_2N(R^{13})_2$, $-NR^{13}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-S(=O)R^{13a}$, $-SR^{13}$, $-SO_2N(R^{13})_2$, C_2-C_6 alkoxyalkyl, methylenedioxy,

ethylenedioxy, C_1-C_4 haloalkyl (including $-C_vF_w$ where v = 1 to 3 and w = 1 to (2v+1)), C_1-C_4 haloalkoxy, C_1-C_4 alkylcarbonyloxy, C_1-C_4 alkylcarbonyl, C_1-C_4 alkylcarbonylamino, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy, C_1-C_4 alkyl (alkyl being substituted with $-N(R^{13})_2$, $-CF_3$, NO_2, or $-S(=O)R^{13a}$);

J is β-Ala or an L-isomer or D-isomer amino acid of structure $-N(R^3)C(R^4)(R^5)C(=O)-$, wherein:

R^3 is H or C_1-C_8 alkyl;

R^4 is H or C_1-C_3 alkyl;

R^5 is selected from:

hydrogen;

C_1-C_8 alkyl substituted with 0-2 R^{11};

C_2-C_8 alkenyl substituted with 0-2 R^{11};

C_2-C_8 alkynyl substituted with 0-2 R^{11};

C_3-C_{10} cycloalkyl substituted with 0-2 R^{11};

aryl substituted with 0-2 R^{12};

a 5-10-membered heterocyclic ring system containing 1-4 heteroatoms independently selected from N, S, or O, said heterocyclic ring being substituted with 0-2 R^{12};

=O, F, Cl, Br, I, $-CF_3$, $-CN$, $-CO_2R^{13}$, $-C(=O)R^{13}$, $-C(=O)N(R^{13})_2$, $-CHO$, $-CH_2OR^{13}$, $-OC(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{14}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{14}SO_2N(R^{13})_2$, $-NR^{14}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-SR^{13}$, $-S(=O)R^{13a}$, $-SO_2N(R^{13})_2$, $-N(R^{13})_2$, $-NHC(=NH)NHR^{13}$, $-C(=NH)NHR^{13}$, $=NOR^{13}$, NO_2, $-C(=O)NHOR^{13}$, $-C(=O)NHNR^{13}R^{13a}$, $=NOR^{13}$, $-B(R^{34})(R^{35})$, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy, $-SC(=NH)NHR^{13}$, N_3, $-Si(CH_3)_3$, $(C_1-C_5$ alkyl)NHR^{16};

$-(C_0-C_6$ alkyl)X;

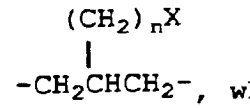
, where q is independently 0,1;

;

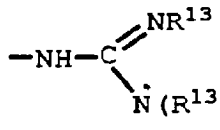
$-(CH_2)_mS(O)_{p'}(CH_2)_2X$, where m = 1,2 and p' = 0-2;

wherein X is defined below; and

R^3 and R^4 may also be taken together to form



, where

n = 0,1 and X is  ;

R^3 and R^5 can alternatively be taken together to form $-(CH_2)_t-$ or $-CH_2S(O)_{p'}C(CH_3)_2-$, where t = 2-4 and p' = 0-2; or

R^4 and R^5 can alternatively be taken together to form $-(CH_2)_u-$, where u = 2-5;

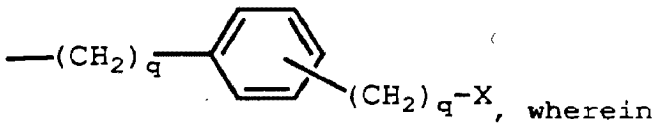
R^{16} is selected from:
an amine protecting group;
1-2 amino acids;
1-2 amino acids substituted with an amine protecting group;

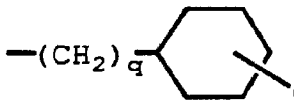
K is a D-isomer or L-isomer amino acid of structure
$-N(R^6)CH(R^7)C(=O)-$, wherein:

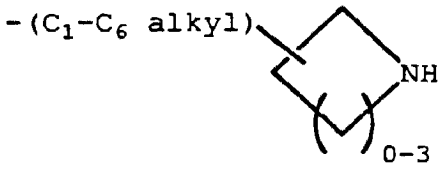
R^6 is H or C_1-C_8 alkyl;

R^7 is selected from:

$-(C_1-C_7$ alkyl)X;

 , wherein
each q is independently 0-2 and substitution on the phenyl is at the 3 or 4 position;

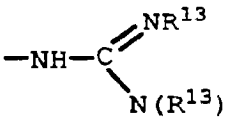

, wherein each q is independently 0-2 and substitution on the cyclohexyl is at the 3 or 4 position;



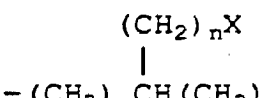
$-(CH_2)_mO-(C_1-C_4$ alkyl$)-X$, where m = 1 or 2;

$-(CH_2)_mS(O)_{p'}-(C_1-C_4$ alkyl$)-X$, where m = 1 or 2 and p' = 0-2; and

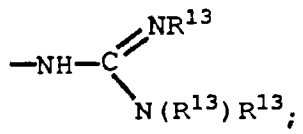
X is selected from:


; $-N(R^{13})R^{13}$; $-C(=NH)(NH_2)$; $-SC(=NH)-NH_2$; $-NH-C(=NH)(NHCN)$; $-NH-C(=NCN)(NH_2)$; $-NH-C(=N-OR^{13})(NH_2)$;

R^6 and R^7 can alternatively be taken together to form


, wherein each q is independently 1 or 2 and wherein

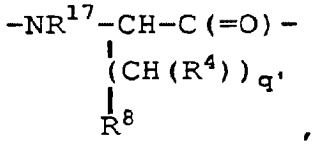
n = 0 or 1 and X is $-NH_2$ or



L is $-Y(CH_2)_vC(=O)-$, wherein:

Y is NH, $N(C_1-C_3$ alkyl), O, or S; and v = 1 or 2;

M is a D-isomer or L-isomer amino acid of structure



wherein:

q' is 0-2;

R^{17} is H, C_1-C_3 alkyl;

R^8 is selected from:
$-CO_2R^{13}$, $-SO_3R^{13}$, $-SO_2NHR^{14}$, $-B(R^{34})(R^{35})$, $-NHSO_2CF_3$, $-CONHNHSO_2CF_3$, $-PO(OR^{13})_2$, $-PO(OR^{13})R^{13}$, $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O) , $-SO_2NH$-heteroaryl

(said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O), $-SO_2NHCOR^{13}$, $-CONHSO_2R^{13a}$, $-CH_2CONHSO_2R^{13a}$, $-NHSO_2NHCOR^{13a}$, $-NHCONHSO_2R^{13a}$, $-SO_2NHCONHR^{13}$;

R^{34} and R^{35} are independently selected from:
-OH,
-F,
$-N(R^{13})_2$, or
C_1-C_8-alkoxy;

R^{34} and R^{35} can alternatively be taken together form:
a cyclic boron ester where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-4 heteroatoms independently selected from N, S, or O;
a divalent cyclic boron amide where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-4 heteroatoms independently selected from N, S, or O;
a cyclic boron amide-ester where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-4 heteroatoms independently selected from N, S, or O; and

wherein the radiolabel is selected from the group: ^{123}I, ^{125}I, ^{131}I, ^{18}F, ^{11}C, ^{13}N, ^{15}O, ^{75}Br.

[52] Included in the present invention are those direct radiolabeled compounds in [51] above, wherein:

R^{31} is bonded to $(C(R^{23})R^{22})_{n"}$ and $(C(R^{21})R^{1})_{n'}$ at 2 different atoms on said carbocyclic ring.

[53] Included in the present invention are those direct radiolabeled compounds in [51] above, wherein:

n" is 0 and n' is 0;
n" is 0 and n' is 1;
n" is 0 and n' is 2;
n" is 1 and n' is 0;
n" is 1 and n' is 1;
n" is 1 and n' is 2;
n" is 2 and n' is 0;
n" is 2 and n' is 1; or
n" is 2 and n' is 2.

[54] Included in the present invention are those direct radiolabeled compounds in [51] above, wherein R^6 is methyl, ethyl, or propyl.

[55] Included in the present invention are those direct radiolabeled compounds in [51] above, wherein:

R^{31} is selected from the group consisting of:

(a) a 6 membered saturated, partially saturated or aromatic carbocyclic ring substituted with 0-3 R^{10} or R^{10a};

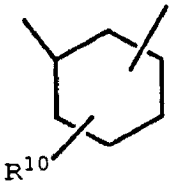
(b) a 8-11 membered saturated, partially saturated, or aromatic fused bicyclic carbocyclic ring substituted with 0-4 R^{10} or R^{10a}; or

(c) a 14 membered saturated, partially saturated, or aromatic fused tricyclic carbocyclic ring substituted with 0-4 R^{10} or R^{10a}.

[56] Included in the present invention are those direct radiolabeled compounds in [51] above, wherein:

R^{31} is selected from the group consisting of:

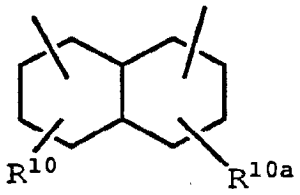
(a) a 6 membered saturated, partially saturated, or aromatic carbocyclic ring of formula:



wherein any of the bonds forming the carbocyclic ring may be a single or double bond,

and wherein said carbocyclic ring is substituted independently with 0-4 R^{10};

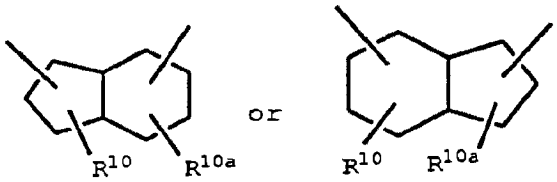
(b) a 10 membered saturated, partially saturated, or aromatic bicyclic carbocyclic ring of formula:



, wherein any of the bonds forming the carbocyclic ring may be a single or double bond,

and wherein said carbocyclic ring is substituted independently with 0-4 R^{10} or R^{10a};

(c) a 9 membered saturated, partially saturated, or aromatic bicyclic carbocyclic ring of formula:



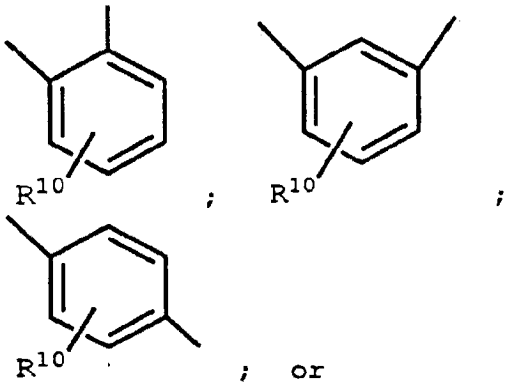
,

wherein any of the bonds forming the carbocyclic ring may be a single or double bond,

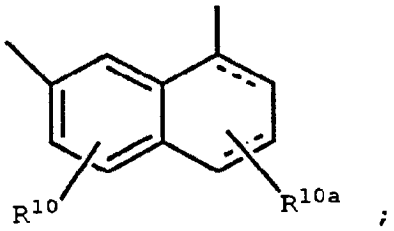
and wherein said carbocyclic ring is substituted independently with 0-4 R^{10} or R^{10a}.

[57] Included in the present invention are those direct radiolabeled compounds in [51] above, wherein:

R^{31} is selected from (the dashed bond may be a single or double bond):



; ; ; or



;

n" is 0 or 1; and

n' is 0-2.

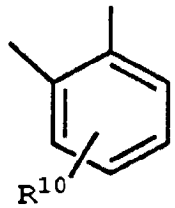
[58] Included in the present invention are those direct radiolabeled compounds in [51] above, wherein:

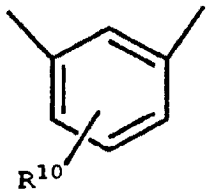
R^1 and R^{22} are independently selected from:

phenyl, benzyl, phenethyl, phenoxy, benzyloxy, halogen, hydroxy, nitro, cyano, C_1-C_5 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_7-C_{10} arylalkyl, C_1-C_5 alkoxy, $-CO_2R^{13}$, $-C(=O)NHOR^{13a}$, $-C(=O)NHN(R^{13})_2$, $=NOR^{13}$, $-B(R^{34})(R^{35})$, C_3-C_6 cycloalkoxy, $-OC(=O)R^{13}$, $-C(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-(C_1$-C_4 alkyl$)-OR^{13}$, $-N(R^{13})_2$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{13}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{13}SO_2N(R^{13})_2$, $-NR^{13}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-S(=O)R^{13a}$, $-SR^{13}$, $-SO_2N(R^{13})_2$, C_2-C_6 alkoxyalkyl, methylenedioxy, ethylenedioxy, C_1-C_4 haloalkyl, C_1-C_4 haloalkoxy, C_1-C_4 alkylcarbonyloxy, C_1-C_4 alkylcarbonyl, C_1-C_4 alkylcarbonylamino, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy, C_1-C_4 alkyl (alkyl being substituted with $-N(R^{13})_2$, $-CF_3$, NO_2, or $-S(=O)R^{13a}$).

[59] Included in the present invention are those direct radiolabeled compounds in [51] above, wherein:

R^{31} is selected from:



; 

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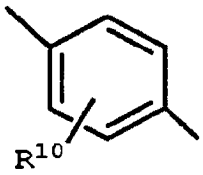
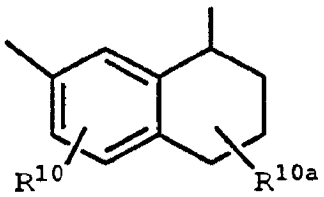
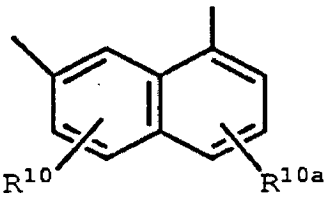


;



; or



wherein R^{31} may be substituted independently with 0-3 R^{10} or R^{10a};

R^{32} is -C(=O)-;

n" is 0 or 1;

n' is 0-2;

R^1 and R^{22} are independently selected from H, C_1-C_4 alkyl, phenyl, benzyl, phenyl-(C_2-C_4)alkyl, C_1-C_4 alkoxy;

R^{21} and R^{23} are independently H or C_1-C_4 alkyl;

R^2 is H or C_1-C_8 alkyl;

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may alternatively be taken together to form $-(CH_2)_{2-5}-$ or $-(CH_2)O(CH_2)-$;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

R^{10} and R^{10a} are selected independently from: H, C_1-C_8 alkyl, phenyl, halogen, or C_1-C_4 alkoxy;

J is β-Ala or an L-isomer or D-isomer amino acid of structure $-N(R^3)C(R^4)(R^5)C(=O)-$, wherein:

R^3 is H or CH_3;

R^4 is H or C_1-C_3 alkyl;

R^5 is H, C_1-C_8 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_1-C_6 cycloalkylethyl, phenyl, phenylmethyl, CH_2OH, CH_2SH, CH_2OCH_3, CH_2SCH_3,

$CH_2CH_2SCH_3$, $(CH_2)_sNH_2$,
$-(CH_2)_sNHC(=NH)(NH_2)$, $-(CH_2)_sNHR^{16}$, where
s = 3-5; or

R^{16} is selected from:
an amine protecting group;
1-2 amino acids; or
1-2 amino acids substituted with an amine protecting group;

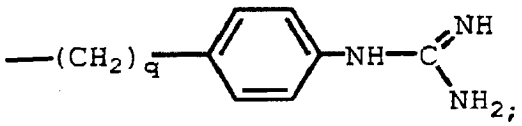
R^3 and R^5 can alternatively be taken together to form $-(CH_2)_t-$ (t = 2-4) or $-CH_2SC(CH_3)_2-$; or

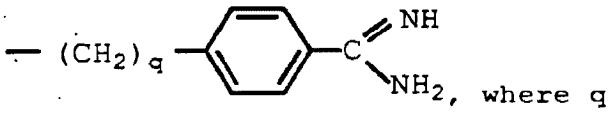
R^4 and R^5 can alternatively be taken together to form $-(CH_2)_u-$, where u = 2-5;

K is an L-isomer amino acid of structure $-N(R^6)CH(R^7)C(=O)-$, wherein:

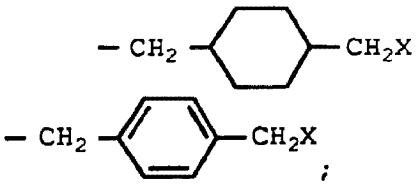
R^6 is H or C_1-C_8 alkyl;

R^7 is



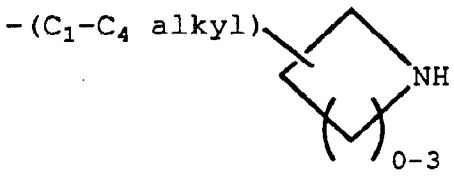


where q = 0 or 1;
$-(CH_2)_rX$, where r = 3-6;



$-(CH_2)_mS(CH_2)_2X$, where m = 1 or 2;

$-(C_3-C_7$ alkyl)$-NH-(C_1-C_6$ alkyl)

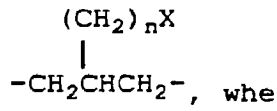


$-(CH_2)_m-O-(C_1-C_4$ alkyl)$-NH-(C_1-C_6$ alkyl), where m = 1 or 2;

$-(CH_2)_m-S-(C_1-C_4$ alkyl)$-NH-(C_1-C_6$ alkyl), where m = 1 or 2; and

X is $-NH_2$ or $-NHC(=NH)(NH_2)$; or

R^6 and R^7 can alternatively be taken together to form



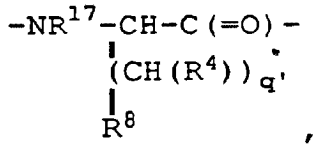
$-CH_2CHCH_2-$, where n = 0 or 1

and X is $-NH_2$ or $-NHC(=NH)(NH_2)$;

L is $-Y(CH_2)_vC(=O)-$, wherein:

Y is NH, O, or S; and v = 1 or 2;

M is a D-isomer or L-isomer amino acid of structure



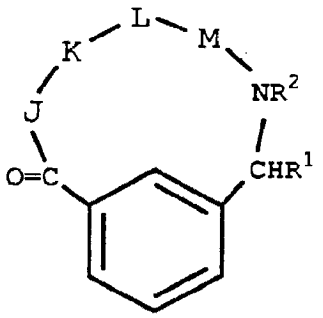
wherein:

q' is 0-2;

R^{17} is H, C_1-C_3 alkyl;

R^8 is selected from:
$-CO_2R^{13}$, $-SO_3R^{13}$, $-SO_2NHR^{14}$, $-B(R^{34})(R^{35})$, $-NHSO_2CF_3$, $-CONHNHSO_2CF_3$, $-PO(OR^{13})_2$, $-PO(OR^{13})R^{13}$, $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O) , $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O), $-SO_2NHCOR^{13}$, $-CONHSO_2R^{13a}$, $-CH_2CONHSO_2R^{13a}$, $-NHSO_2NHCOR^{13a}$, $-NHCONHSO_2R^{13a}$, $-SO_2NHCONHR^{13}$.

[60] Included in the present invention are those direct radiolabeled compounds in [51] above, that are radiolabeled 1,3-disubstituted phenyl compounds of the formula (II):



(II)

wherein:

the shown phenyl ring in formula (II) may be further substituted with 0-3 R^{10};

R^{10} is selected independently from: H, C_1-C_8 alkyl, phenyl, halogen, or C_1-C_4 alkoxy;

R^1 is H, C_1-C_4 alkyl, phenyl, benzyl, or phenyl-(C_1-C_4)alkyl;

R^2 is H or methyl;

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may alternatively be taken together to form -$(CH_2)_{2-5}$- or -$(CH_2)O(CH_2)$-;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

J is β-Ala or an L-isomer or D-isomer amino acid of structure $-N(R^3)C(R^4)(R^5)C(=O)-$, wherein:

R^3 is H or CH_3;

R^4 is H or C_1-C_3 alkyl;

R^5 is H, C_1-C_8 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_1-C_6 cycloalkylethyl, phenyl, phenylmethyl, CH_2OH, CH_2SH, CH_2OCH_3, CH_2SCH_3, $CH_2CH_2SCH_3$, $(CH_2)_sNH_2$, $-(CH_2)_sNHC(=NH)(NH_2)$, $-(CH_2)_sNHR^{16}$, where s = 3-5; or

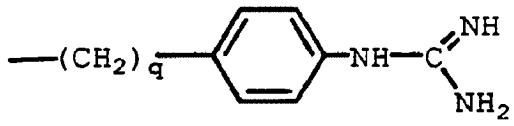
R^{16} is selected from:
an amine protecting group;
1-2 amino acids; or
1-2 amino acids substituted with an amine protecting group;

R^3 and R^5 can alternatively be taken together to form $-CH_2CH_2CH_2-$; or
R^4 and R^5 can alternatively be taken together to form $-(CH_2)_u-$, where u = 2-5;

K is an L-isomer amino acid of structure $-N(R^6)CH(R^7)C(=O)-$, wherein:

R^6 is H or C_1-C_8 alkyl;

R^7 is:

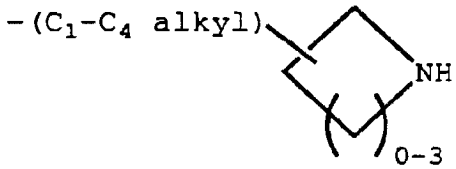


where q = 0 or 1;

$-(CH_2)_rX$, where r = 3-6;

$-(CH_2)_mS(CH_2)_2X$, where m = 1 or 2;

$-(C_3-C_7$ alkyl)$-NH-(C_1-C_6$ alkyl)



$-(CH_2)_m-O-(C_1-C_4$ alkyl)$-NH-(C_1-C_6$ alkyl), where m = 1 or 2;

$-(CH_2)_m-S-(C_1-C_4$ alkyl)$-NH-(C_1-C_6$ alkyl), where m = 1 or 2; and

X is $-NH_2$ or $-NHC(=NH)(NH_2)$, provided that X is not $-NH_2$ when r = 4; or

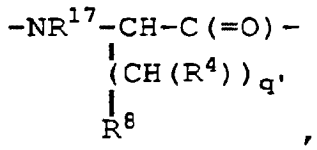
R^6 and R7 are alternatively be taken together to form

where n = 0,1 and X is $-NH_2$ or $-NHC(=NH)(NH_2)$;

L is $-Y(CH_2)_vC(=O)-$, wherein:

Y is NH, O, or S; and v = 1,2;

M is a D-isomer or L-isomer amino acid of structure



wherein:

q' is 0-2;

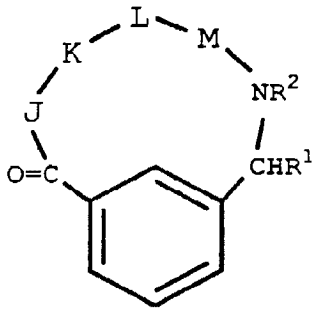
R^{17} is H, C_1-C_3 alkyl;

R^8 is selected from:

$-CO_2R^{13}$, $-SO_3R^{13}$, $-SO_2NHR^{14}$, $-B(R^{34})(R^{35})$, $-NHSO_2CF_3$, $-CONHNHSO_2CF_3$, $-PO(OR^{13})_2$, $-PO(OR^{13})R^{13}$, $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O) , $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected

independently from N, S, or O),
$-SO_2NHCOR^{13}$, $-CONHSO_2R^{13a}$,
$-CH_2CONHSO_2R^{13a}$, $-NHSO_2NHCOR^{13a}$,
$-NHCONHSO_2R^{13a}$, $-SO_2NHCONHR^{13}$.

[61] Included in the present invention are those direct radiolabeled compounds in [51] above, that are radiolabeled 1,3-disubstituted phenyl compounds of the formula (II):



(II)

wherein:

the phenyl ring in formula (II) may be further substituted with 0-3 R^{10} or R^{10a};

R^{10} or R^{10a} are selected independently from: H, C_1-C_8 alkyl, phenyl, halogen, or C_1-C_4 alkoxy;

R^1 is H, C_1-C_4 alkyl, phenyl, benzyl, or phenyl-(C_2- C_4)alkyl;

R^2 is H or methyl;

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12}

alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may alternatively be taken together to form $-(CH_2)_{2-5}-$ or $-(CH_2)O(CH_2)-$;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

J is β-Ala or an L-isomer or D-isomer amino acid of structure $-N(R^3)C(R^4)(R^5)C(=O)-$, wherein:

R^3 is H or CH_3;

R^4 is H;

R^5 is H, C_1-C_8 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_1-C_6 cycloalkylethyl, phenyl, phenylmethyl, CH_2OH, CH_2SH, CH_2OCH_3, CH_2SCH_3, $CH_2CH_2SCH_3$, $(CH_2)_sNH_2$, $(CH_2)_sNHC(=NH)(NH_2)$, $(CH_2)_sR^{16}$, where s = 3-5;

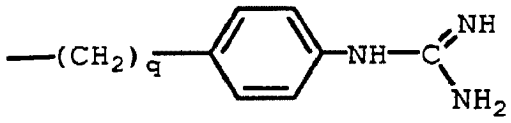
R^3 and R^5 can alternatively be taken together to form $-CH_2CH_2CH_2-$;

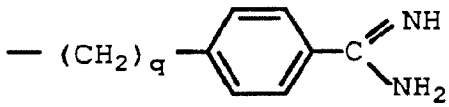
R^{16} is selected from:
an amine protecting group;
1-2 amino acids;
1-2 amino acids substituted with an amine protecting group;

K is an L-isomer amino acid of structure $-N(R^6)CH(R^7)C(=O)-$, wherein:

R^6 is H or C_3-C_8 alkyl;

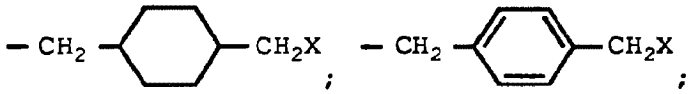
R^7 is





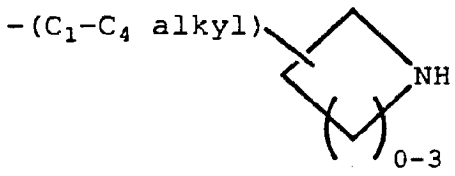
where q = 0 or 1;

$-(CH_2)_rX$, where r = 3-6;



$-(CH_2)_mS(CH_2)_2X$, where m = 1 or 2;

$-(C_4$-C_7 alkyl)-NH-(C_1-C_6 alkyl)



$-(CH_2)_m$-O-(C_1-C_4 alkyl)-NH-(C_1-C_6 alkyl), where m = 1 or 2;

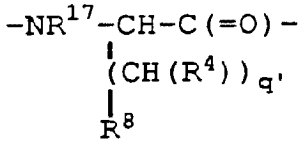
$-(CH_2)_m-S-(C_1-C_4$ alkyl)$-NH-(C_1-C_6$ alkyl), where m = 1 or 2; and

X is $-NH_2$ or $-NHC(=NH)(NH_2)$, provided that X is not $-NH_2$ when r = 4; or

L is $-YCH_2C(=O)-$, wherein:

Y is NH or O;

M is a D-isomer or L-isomer amino acid of structure



, wherein:

q' is 1;

R^{17} is H, C_1-C_3 alkyl;

R^8 is selected from:

$-CO_2H$ or $-SO_3R^{13}$.

[62] Included in the present invention are those direct radiolabeled compounds in of formula (II) above, wherein:

the phenyl ring in formula (II) may be further substituted with 0-2 R^{10} or R^{10a};

R^{10} or R^{10a} are selected independently from: H, C_1-C_8 alkyl, phenyl, halogen, or C_1-C_4 alkoxy;

R^1 is H;

R^2 is H;

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may alternatively be taken together to form $-(CH_2)_{2-5}-$ or $-(CH_2)O(CH_2)-$;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

J is β-Ala or an L-isomer or D-isomer amino acid of formula $-N(R^3)CH(R^5)C(=O)-$, wherein:

R^3 is H and R^5 is H, CH_3, CH_2CH_3, $CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$, $CH_2CH_2CH_3$, $CH_2CH_2CH_2CH_3$, $CH_2CH_2SCH_3$, $CH_2CH(CH_3)_2$, $(CH_2)_4NH_2$, (C_3-C_5 alkyl)NHR^{16};
or

R^3 is CH_3 and R^5 is H; or

R^3 and R^5 can alternatively be taken together to form $-CH_2CH_2CH_2-$;

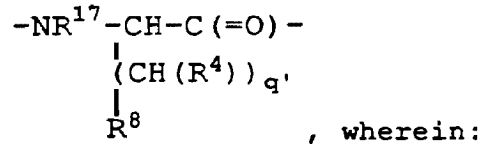
R^{16} is selected from:
an amine protecting group;
1-2 amino acids;
1-2 amino acids substituted with an amine protecting group;

K is an L-isomer amino acid of formula $-N(CH_3)CH(R^7)C(=O)-$, wherein:

R^7 is $-(CH_2)_3NHC(=NH)(NH_2)$;

L is $-NHCH_2C(=O)-$; and

M is a D-isomer or L-isomer amino acid of structure



, wherein:

q' is 1;

R^4 is H or CH_3;

R^{17} is H;

R^8 is
$-CO_2H$;
$-SO_3H$.

[63] Included in the present invention are those direct radiolabeled compounds in of formula (II) above, wherein:

R^1 and R^2 are independently selected from H, methyl;

J is selected from D-Val, D-2-aminobutyric acid, D-Leu, D-Ala, Gly, D-Pro, D-Ser, D-Lys, β-Ala, Pro, Phe, NMeGly, D-Nle, D-Phg, D-Ile, D-Phe, D-Tyr, Ala, N^{ε}-p-azidobenzoyl-D-Lys, N^{ε}-p-benzoylbenzoyl-D-Lys, N^{ε}-tryptophanyl-D-Lys, N^{ε}-o-benzylbenzoyl-D-Lys, N^{ε}-p-acetylbenzoyl-D-Lys, N^{ε}-dansyl-D-Lys, N^{ε}-glycyl-D-Lys, N^{ε}-glycyl-p-benzoylbenzoyl-D-Lys, N^{ε}-p-phenylbenzoyl-D-Lys, N^{ε}-m-benzoylbenzoyl-D-Lys, N^{ε}-o-benzoylbenzoyl-D-Lys;

K is selected from NMeArg, Arg;

L is selected from Gly, β-Ala, Ala;

M is selected from Asp; αMeAsp; βMeAsp; NMeAsp; D-Asp.

[64] Included in the present invention are those direct radiolabeled compounds in of formula (II) above, wherein:

R^1 and R^2 are independently selected from H, methyl;

J is selected from: D-Val, D-2-aminobutyric acid, D-Leu, D-Ala, Gly, D-Pro, D-Ser, D-Lys, β-Ala, Pro, Phe, NMeGly, D-Nle, D-Phg, D-Ile, D-Phe, D-Tyr, Ala;

K is selected from NMeArg;

L is Gly;

M is selected from Asp; αMeAsp; βMeAsp; NMeAsp; D-Asp.

[65] Included in the present invention are those direct radiolabeled compounds of [51] that are:

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-2-aminobutyric acid; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Leu; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Ala; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is Gly; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Pro; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Lys; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is β-Ala; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is NMeGly; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 is methyl (isomer 1); R^2 are H; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 is methyl (isomer 2); R^2 are H; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 is phenyl (isomer 1); R^2 are H; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein J = D-Met, K = NMeArg, L = Gly, M = Asp, R^1 = H, R^2 = H;

the radiolabeled compound of formula (II) wherein J = D-Abu, K = diNMe-guanidinyl-Orn , L = Gly, M = Asp, R^1 = H, R^2 = H;

the radiolabeled compound of formula (II) wherein J = D-Abu, K = diNMe-Lys, L = Gly, M = Asp, R^1 = H, R^2 = H;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-p-azidobenzoyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-p-benzoylbenzoyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-tryptophanyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-o-benzylbenzoyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp.

The radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-p-acetylbenzoyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-dansyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-glycyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

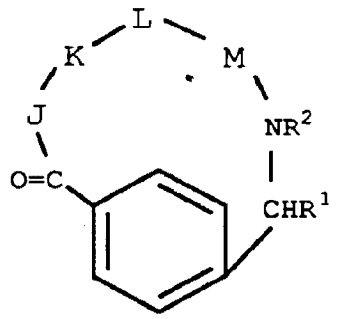
the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-glycyl-p-benzoylbenzoyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-p-phenylbenzoyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-m-benzoylbenzoyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-o-benzoylbenzoyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (III) wherein R^1 and R^2 are H; J is D-Val; K is NMeArg; L is Gly; and M is Asp;



(III);

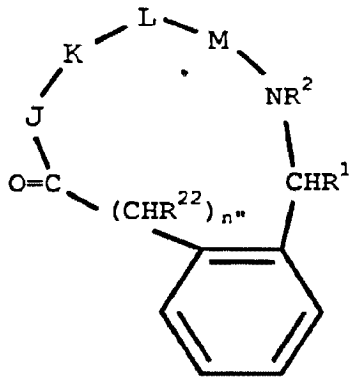
the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Val; K is D-NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Nle; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Phg; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Phe; K is NMeArg; L is Gly; and M is Asp;

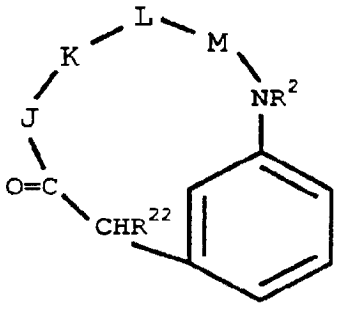
the radiolabeled compound of formula (V) wherein R^1 and R^2 are H; J is D-Ile; K is NMeArg; L is Gly; and M is Asp;



(V);

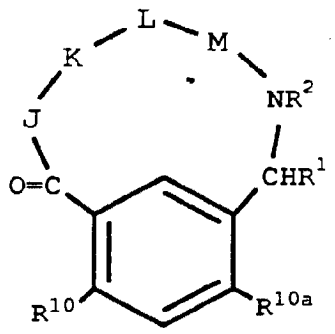
the radiolabeled compound of formula (V) wherein n"=1; R^1, R^2, and R^{22} are H; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (V) wherein n"=0; R^1 and R^2 are H; J is D-Val; K is NMeArg; L is Gly; and M is Asp;



(VI)

the radiolabeled compound of formula (VI) wherein R^2 and R^{22} are H; J is D-Val; K is NMeArg; L is Gly; and M is Asp;



(VII)

the radiolabeled compound of formula (VII) wherein R^1,R^2, and R^{10} are H; R^{10a} is Cl; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (VII) wherein R^1,R^2, and R^{10} are H; R^{10a} is I; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (VII) wherein R^1,R^2, and R^{10} are H; R^{10a} is I; J is D-Abu; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (VII) wherein R^1,R^2, and R^{10} are H; R^{10a} is Me; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (VII) wherein R^1,R^2, and R^{10a} are H; R^{10} is Cl; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (VII) wherein R^1,R^2, and R^{10a} are H; R^{10} is MeO; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (VII) wherein R^1,R^2, and R^{10a} are H; R^{10} is Me; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (VII) wherein R^1,R^2, and R^{10} are H; R^{10a} is Cl; J is D-Abu; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (VII) wherein R^1, R^2, and R^{10} are H; R^{10a} is I; J is D-Abu; K is NMeArg; L is Gly; and M is Asp.

The radiolabeled compound of formula (VII) wherein R^1, R^2, and R^{10} are H; R^{10a} is Me; J is D-Abu; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Tyr; K is NMeArg; L is Gly; and M is Asp;

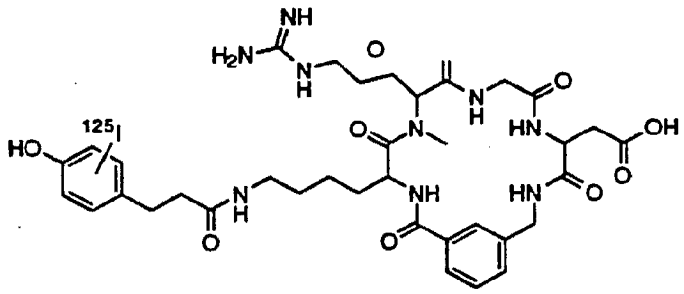
the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Val; K is NMeAmf; L is Gly; and M is Asp;

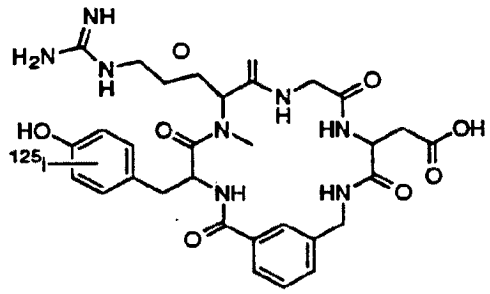
the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Val; K is NMeArg; L is Gly; and M is βMeAsp;

the radiolabeled compound of formula (II) wherein R^1 is H; R^2 is CH_3; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (III) wherein R^1 and R^2 are H; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (VIII) wherein J is D-Val; K is NMeArg; L is Gly; and M is Asp;





[66] Included in the present invention are those radiolabeled compound as in one of [51]-[65] wherein the radiolabel is selected from the group: ^{18}F, ^{11}C, ^{123}I, and ^{125}I.

[67] Included in the present invention are those radiolabeled compounds of [66] wherein the radiolabel is ^{123}I.

[68] Included in the present invention is a radiopharmaceutical composition comprising a radiopharmaceutically acceptable carrier and a radiolabeled compound of any of [51]-[67].

[69] Included in the present invention is a method of determining platelet deposition in a mammal comprising administering to said mammal a radiopharmaceutical composition comprising a compound of any of [51]-[67], and imaging said mammal.

[70] Included in the present invention is a method of diagnosing a disorder associated with platelet deposition in a mammal comprising administering to said mammal a radiopharmaceutical composition comprising a compound of any of [51]-[67], and imaging said mammal.

As noted above, the cyclic compounds of the present invention are radiolabeled. By "radiolabeled", it is meant that the subject cyclic platelet glycoprotein IIb/IIIa compounds contain a radioisotope which is suitable for administration to a mammalian patient. Suitable radioisotopes are known to those skilled in the art and include, for example, isotopes of halogens (such as chlorine, fluorine, bromine and iodine), and metals including technetium and indium. Preferred radioisotopes include 11C, 18F, ^{123}I, ^{125}I, ^{131}I, ^{99m}Tc, ^{94m}Tc, ^{95}Tc, ^{111}In, ^{62}Cu, ^{43}Sc, ^{45}Ti, ^{67}Ga, ^{68}Ga, ^{97}Ru, ^{72}As, ^{82}Rb, and ^{201}Tl. Most preferred are the isoptopes ^{123}I, ^{111}In, and ^{99m}Tc. Radiolabeled compounds of the invention may be prepared using standard radiolabeling procedures well known to those skilled in the art.

Suitable synthesis methodology is described in detail below. As discussed below, the cyclic platelet glycoprotein IIb/IIIa compounds of the invention may be radiolabeled either directly (that is, by incorporating the radiolabel directly into the compounds) or indirectly (that is, by incorporating the radiolabel into the compounds through a chelating agent, where the chelating agent has been incorporated into the compounds). Also, the radiolabeling may be isotopic or nonisotopic. With isotopic radiolabeling, one group already present in the cyclic compounds described above is substituted with (exchanged for) the radioisotope. With nonisotopic radiolabeling, the radioisotope is added to the cyclic compounds without substituting with (exchanging for) an already existing group. Direct and indirect radiolabeled compounds, as well as isotopic and nonisotopic radiolabeled compounds are included within the phrase "radiolabeled compounds" as used in connection with the present invention. Such radiolabeling should also be reasonably stable, both chemically and metabolically, applying recognized standards in the art. Also, although the compounds of the invention may be labeled in a variety of fashions with a variety of different radioisotopes, as those skilled in the art will recognize, such radiolabeling should be carried out in a manner such that the high binding affinity and specificity of the unlabeled cyclic platelet GPIIb/IIIa compounds of the invention to the GPIIb/IIIa receptor is not significantly affected. By not significantly affected, it is meant that the binding affinity and specificity is not affected more than about 3 log units, preferably not more than about 2 log units, more preferably not more than about 1 log unit, even more preferably not more than about 500%, and still even

more preferably not more than about 250%, and most preferably the binding affinity and specificity is not affected at all.

For radiolabeled compounds, the label may appear at any position on Q. Preferred radiolabeled compounds of the invention are radiolabeled compounds wherein the radiolabel is located on the carbocyclic ring system of R^{31}, the R^5 substituent on J, and at R^1 or R^{22}. Even more preferred radiolabeled compounds of the invention are those of formula (II), wherein the radiolabel is located on the carbocyclic ring system of R^{31}, or the R^5 substituent on J. With regard to the preferred and more preferred direct radiolabeled compounds, the preferred radiolabel is a halogen label, especially an iodine radiolabel. For indirect radiolabeled compounds, the preferred metal nuclides are ^{99m}Tc and ^{111}In. Preferred linking groups, Ln, and metal chelators, C_h, are described below.

It has been discovered that the radiolabeled compounds of the invention are useful as radiopharmaceuticals for non-invasive imaging to diagnose present or potential thromboembolic disorders, such as arterial or venous thrombosis, including, for example, unstable angina, myocardial infarction, transient ischemic attack, stroke, atherosclerosis, diabetes, thrombophlebitis, pulmonary emboli, or platelet plugs, thrombi or emboli caused by prosthetic cardiac devices such as heart valves. The radiolabeled compounds of the invention are useful with both newly formed and older thrombi. The radiolabeled compounds of the invention may also be used to diagnose other present or potential conditions where there is overexpression of the GPIIb/IIIa receptors, such as with metastatic cancer cells. The subject compounds may be effectively

employed in low doses, thereby minimizing any risk of toxicity. Also, the subject compounds are of a much smaller size than, for example, the radiolabeled 7E3 antibodies known in the art, allowing easier attainment of suitable target/background (T/B) ratio for detecting thrombi. The use of the radiolabeled compounds of the invention is further described in the utility section below.

In the present invention it has also been discovered that the radiolabeled compounds above are useful as inhibitors of glycoprotein IIb/IIIa (GPIIb/IIIa), and thus the radiolabeled compounds of the invention may also be employed for therapeutic purposes, in addition to the diagnostic usage described above. As discussed above, GPIIb/IIIa mediates the process of platelet activation and aggregation. The radiolabeled compounds of the present invention inhibit the activation and aggregation of platelets induced by all known endogenous platelet agonists.

The compounds herein described may have asymmetric centers. Unless otherwise indicated, all chiral, diastereomeric and racemic forms are included in the present invention. Many geometric isomers of olefins, C=N double bonds, and the like can also be present in the compounds described herein, and all such stable isomers are contemplated in the present invention. It will be appreciated that compounds of the present invention contain asymmetrically substituted carbon atoms, and may be isolated in optically active or racemic forms. It is well known in the art how to prepare optically active forms, such as by resolution of racemic forms or by synthesis, from optically active starting materials. Two distinct isomers (cis and trans) of the peptide bond are known to occur; both can

also be present in the compounds described herein, and all such stable isomers are contemplated in the present invention. Unless otherwise specifically noted, the L-isomer of the amino acid is used at positions J, K, L, and M of the compounds of the present invention. Except as provided in the preceding sentence, all chiral, diastereomeric, racemic forms and all geometric isomeric forms of a structure are intended, unless the specific stereochemistry or isomer form is specifically indicated. The D and L-isomers of a particular amino acid are designated herein using the conventional 3-letter abbreviation of the amino acid, as indicated by the following examples: D-Leu, D-Leu, L-Leu, or L-Leu.

When any variable (for example, R^1 through R^8, m, n, p, X, Y, etc.) occurs more than one time in any constituent or in any formula, its definition on each occurrence is independent of its definition at every other occurrence. Thus, for example, if a group is shown to be substituted with 0-2 R^{11}, then said group may optionally be substituted with up to two R^{11} and R^{11} at each occurrence is selected independently from the defined list of possible R^{11}. Also, by way of example, for the group $-N(R^{13})_2$, each of the two R^{13} substituents on N is independently selected from the defined list of possible R^{13}.

When a bond to a substituent is shown to cross the bond connecting two atoms in a ring, then such substituent may be bonded to any atom on the ring.

Combinations of substituents and/or variables are permissible only if such combinations result in stable compounds.

By "stable compound" or "stable structure" is meant herein a compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction

mixture, and formulation into an efficacious therapeutic agent.

The term "substituted", as used herein, means that an one or more hydrogen on the designated atom is replaced with a selection from the indicated group, provided that the designated atom's normal valency is not exceeded, and that the substitution results in a stable compound. When a substitent is keto (i.e., =O), then 2 hydrogens on the atom are replaced.

As used herein, "alkyl" is intended to include both branched and straight-chain saturated aliphatic hydrocarbon groups having the specified number of carbon atoms; "haloalkyl" is intended to include both branched and straight-chain saturated aliphatic hydrocarbon groups having the specified number of carbon atoms, substituted with 1 or more halogen (for example $-C_vF_w$ where v = 1 to 3 and w = 1 to (2v+1)); "alkoxy" represents an alkyl group of indicated number of carbon atoms attached through an oxygen bridge; "cycloalkyl" is intended to include saturated ring groups, including mono-,bi- or poly-cyclic ring systems, such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl and adamantyl; and "biycloalkyl" is intended to include saturated bicyclic ring groups such as [3.3.0]bicyclooctane, [4.3.0]bicyclononane, [4.4.0]bicyclodecane (decalin), [2.2.2]bicyclooctane, and so forth. "Alkenyl" is intended to include hydrocarbon chains of either a straight or branched configuration and one or more unsaturated carbon-carbon bonds which may occur in any stable point along the chain, such as ethenyl, propenyl and the like; and "alkynyl" is intended to include hydrocarbon chains of either a straight or branched configuration and one or

more triple carbon-carbon bonds which may occur in any stable point along the chain, such as ethynyl, propynyl and the like.

The phrase "boronic acid" as used herein means a group of the formula $-B(R^{34})(R^{35})$, wherein R^{34} and R^{35} are independently selected from: -OH; -F; $-NR^{13}R^{14}$; or C_1-C_8-alkoxy; or R^{34} and R^{35} can alternatively be taken together to form: a cyclic boron ester where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-4 heteroatoms independently selected from N, S, or O; a divalent cyclic boron amide where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-4 heteroatoms independently selected from N, S, or O; a cyclic boron amide-ester where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-4 heteroatoms independently selected from N, S, or O. Such cyclic boron esters, boron amides, or boron amide-esters may also be optionally substituted with 1-5 groups independently selected from R^{11}.

Boron esters include boronic acid protecting groups, including moieties derived from diols, for example pinanediol and pinacol to form pinanediol boronic acid ester and the pinacol boronic acid, respectively. Other illustrations of diols useful for deriving boronic acid esters are perfluoropinacol, ethylene glycol, diethylene glycol, 1,2-ethanediol, 1,3-propanediol, 1,2-propanediol, 1,2-butanediol, 1,4-butanediol, 2,3-butanediol, 2,3-hexanediol, 1,2-hexanediol, catechol, 1,2-diisopropylethanediol, 5,6-decanediol, 1,2-dicyclohexylethanediol.

"Halo" or "halogen" as used herein refers to fluoro, chloro, bromo and iodo; and "counterion" is used

to represent a small, negatively charged species such as chloride, bromide, hydroxide, acetate, sulfate and the like.

As used herein, "aryl" or "aromatic residue" is intended to mean phenyl or naphthyl. As used herein, "carbocycle" or "carbocyclic residue" is intended to mean any stable 3- to 7- membered monocyclic or bicyclic or 7- to 14-membered bicyclic or tricyclic or an up to 26-membered polycyclic carbon ring, any of which may be saturated, partially unsaturated, or aromatic. Examples of such carbocyles include, but are not limited to, cyclopropyl, cyclopentyl, cyclohexyl, phenyl, biphenyl, naphthyl, indanyl, adamantyl, or tetrahydronaphthyl (tetralin).

As used herein, the term "heterocycle" or "heterocyclic ring system" is intended to mean a stable 5- to 7- membered monocyclic or bicyclic or 7- to 10-membered bicyclic heterocyclic ring which may be saturated, partially unsaturated, or aromatic, and which consists of carbon atoms and from 1 to 4 heteroatoms selected independently from the group consisting of N, O and S and wherein the nitrogen and sulfur heteroatoms may optionally be oxidized, and the nitrogen may optionally be quaternized, and including any bicyclic group in which any of the above-defined heterocyclic rings is fused to a benzene ring. The heterocyclic ring may be attached to its pendant group at any heteroatom or carbon atom which results in a stable structure. The heterocyclic rings described herein may be substituted on carbon or on a nitrogen atom if the resulting compound is stable. Examples of such heterocycles include, but are not limited to, benzopyranyl, thiadiazine, tetrazolyl, benzofuranyl, benzothiophenyl, indolene, quinoline, isoquinolinyl or benzimidazolyl,

piperidinyl, 4-piperidone, 2-pyrrolidone, tetrahydrofuran, tetrahydroquinoline, tetrahydroisoquinoline, decahydroquinoline, octahydroisoquinoline, azocine, triazine (including 1,2,3-, 1,2,4-, and 1,3,5-triazine), 6*H*-1,2,5-thiadiazine, 2*H*,6*H*-1,5,2-dithiazine, thiophene, tetrahydrothiophene, thianthrene, furan, pyran, isobenzofuran, chromene, xanthene, phenoxathiin, 2*H*-pyrrole, pyrrole, imidazole, pyrazole, thiazole, isothiazole, oxazole (including 1,2,4- and 1,3,4-oxazole), isoxazole, triazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, 3*H*-indole, indole, 1*H*-indazole, purine, 4*H*-quinolizine, isoquinoline, quinoline, phthalazine, naphthyridine, quinoxaline, quinazoline, cinnoline, pteridine, 4a*H*-carbazole, carbazole, ß-carboline, phenanthridine, acridine, perimidine, phenanthroline, phenazine, phenarsazine, phenothiazine, furazan, phenoxazine, isochroman, chroman, pyrrolidine, pyrroline, imidazolidine, imidazoline, pyrazolidine, pyrazoline, piperazine, indoline, isoindoline, quinuclidine, or morpholine. Also included are fused ring and spiro compounds containing, for example, the above heterocycles.

As used herein, the term "any group that, when administered to a mammalian subject, cleaves to form a free hydroxyl, amino or sulfhydryl" means any group bonded to an O, N, or S atom, respectively, which is cleaved from the O, N, or S atom when the compound is administered to a mammalian subject to provide a compound having a remaining free hydroxyl, amino, or sulfhydryl group, respectively. Examples of groups that, when administered to a mammalian subject, are

cleaved to form a free hydroxyl, amino or sulfhydryl, include but are not limited to, C_1-C_6 alkyl substituted with 0-3 R^{11}, C_3-C_6 alkoxyalkyl substituted with 0-3 R^{11}, C_1-C_6 alkylcarbonyl substituted with 0-3 R^{11}, C_1-C_6 alkoxycarbonyl substituted with 0-3 R^{11}, C_1-C_6 alkylaminocarbonyl substituted with 0-3 R^{11}, benzoyl substituted with 0-3 R^{12}, phenoxycarbonyl substituted with 0-3 R^{12}, phenylaminocarbonyl substituted with 0-3 R^{12}. Examples of groups that, when administered to a mammalian subject, are cleaved to form a free hydroxyl, amino or sulfhydryl, include hydroxy, amine or sulfhydryl protecting groups, respectively.

As used herein, the term "amine protecting group" means any group known in the art of organic synthesis for the protection of amine groups. Such amine protecting groups include those listed in Greene, "Protective Groups in Organic Synthesis" John Wiley & Sons, New York (1981) and "The Peptides: Analysis, Sythesis, Biology, Vol. 3, Academic Press, New York (1981), the disclosure of which is hereby incorporated by reference. Any amine protecting group known in the art can be used. Examples of amine protecting groups include, but are not limited to, the following: 1) acyl types such as formyl, trifluoroacetyl, phthalyl, and p-toluenesulfonyl; 2) aromatic carbamate types such as benzyloxycarbonyl (Cbz or Z) and substituted benzyloxycarbonyls, 1-(p-biphenyl)-1-methylethoxycarbonyl, and 9-fluorenylmethyloxycarbonyl (Fmoc); 3) aliphatic carbamate types such as tert-butyloxycarbonyl (Boc), ethoxycarbonyl, diisopropylmethoxycarbonyl, and allyloxycarbonyl; 4) cyclic alkyl carbamate types such as

cyclopentyloxycarbonyl and adamantyloxycarbonyl; 5) alkyl types such as triphenylmethyl and benzyl; 6) trialkylsilane such as trimethylsilane; and 7) thiol containing types such as phenylthiocarbonyl and dithiasuccinoyl. Also included in the term "amine protecting group" are acyl groups such as azidobenzoyl, p-benzoylbenzoyl, o-benzylbenzoyl, p-acetylbenzoyl, dansyl, glycyl-p-benzoylbenzoyl, phenylbenzoyl, m-benzoylbenzoyl, benzoylbenzoyl.

As used herein, "pharmaceutically acceptable salts" refer to derivatives of the disclosed compounds wherein the parent compound of formula (I) is modified by making acid or base salts of the compound of formula (I). Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like.

Pharmaceutically acceptable salts of the compounds of the invention can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts are found in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, PA, 1985, p. 1418, the disclosure of which is hereby incorporated by reference.

The term "amino acid" as used herein means an organic compound containing both a basic amino group and an acidic carboxyl group. Included within this term are

modified and unusual amino acids, such as those disclosed in, for example, Roberts and Vellaccio (1983) The Peptides, 5: 342-429, the teaching of which is hereby incorporated by reference. Modified or unusual amino acids which can be used to practice the invention include, but are not limited to, D-amino acids, hydroxylysine, 4-hydroxyproline, ornithine, 2,4-diaminobutyric acid, homoarginine, norleucine, N-methylaminobutyric acid, naphthylalanine, phenylglycine, ß-phenylproline, tert-leucine, 4-aminocyclohexylalanine, N-methyl-norleucine, 3,4-dehydroproline, 4-aminopiperidine-4-carboxylic acid, 6-aminocaproic acid, trans-4-(aminomethyl)-cyclohexanecarboxylic acid, 2-, 3-, and 4-(aminomethyl)-benzoic acid, 1-aminocyclopentanecarboxylic acid, 1-aminocyclopropanecarboxylic acid, and 2-benzyl-5-aminopentanoic acid.

The term "amino acid residue" as used herein means that portion of an amino acid (as defined herein) that is present in a peptide.

The term "peptide" as used herein means a linear compound that consists of two or more amino acids (as defined herein) that are linked by means of a peptide bond. The term "peptide" also includes compounds containing both peptide and non-peptide components, such as pseudopeptide or peptide mimetic residues or other non-amino acid components. Such a compound containing both peptide and non-peptide components may also be referred to as a "peptide analog".

A "pseudopeptide" or "peptide mimetic" is a compound which mimics the structure of an amino acid residue or a peptide, for example, by using linking groups other than amide linkages between the peptide mimetic and an amino acid residue (pseudopeptide bonds)

and/or by using non-amino acid substituents and/or a modified amino acid residue.

A "pseudopeptide residue" means that portion of an pseudopeptide or peptide mimetic (as defined herein) that is present in a peptide.

The term "peptide bond" means a covalent amide linkage formed by loss of a molecule of water between the carboxyl group of one amino acid and the amino group of a second amino acid.

The term "pseudopeptide bonds" includes peptide bond isosteres which may be used in place of or as substitutes for the normal amide linkage. These substitute or amide "equivalent" linkages are formed from combinations of atoms not normally found in peptides or proteins which mimic the spatial requirements of the amide bond and which should stabilize the molecule to enzymatic degradation.

The terms "L_n", "linking group" and "linker", used interchangeably throughout, designate the group of atoms separating Q from the metal chelator, C_h.

The terms "activated L_n group", "activated L_n", "activated linking group" and "activated linker", used interchangeably throughout, refer to a linking group that bears one or more reactive group capable of reacting with, and forming a bond with, a chelator or a Q.

The terms "C_h", "metal chelator", and "chelator" are used interchangeably throughout to designate a chemical moiety capable of binding to or complexing with a metal nuclide.

The term "cyclizing moiety" means the intermediate compound that serves as the precursor to the R^{31} group of Q.

The term "ring substituted cyclizing moiety" is a cyclizing moiety bearin a substituent group one or more of its carbocyclic or heterocyclic rings.

The term "linker modified cyclizing moiety" refers to a cyclizing moiety that bears an activated L_n group.

The term "cyclic compound intermediate" means the intermediate compound that serves as the precursor to the Q group in the claimed compounds.

The term "linker modified cyclic compound intermediate" means a cyclic compound intermediate that bears an activated L_n group.

The compounds of the present invention can be prepared in a number of ways well known to one skilled in the art of organic synthesis. Preferred methods include but are not limited to those methods described below.

The following abbreviations are used herein:

Acm	acetamidomethyl
D-Abu	D-2-aminobutyric acid
5-Aca	5-aminocaproamide(5-aminohexanamide)
b-Ala, b-Ala or bAla	3-aminopropionic acid
Boc	t-butyloxycarbonyl
Boc-iodo-Mamb	t-butyloxycarbonyl-3-aminomethyl-4-iodo-benzoic acid
Boc-Mamb acid	t-butyloxycarbonyl-3-aminomethylbenzoic
Boc-ON	[2-(tert-butyloxycarbonyloxylimino)-2-phenylacetonitrile
Cl_2Bzl	dichlorobenzyl
CBZ, Cbz or Z	Carbobenzyloxy
DCC	dicyclohexylcarbodiimide

DIEA	diisopropylethylamine
di-NMeOrn	N-aMe-N-gMe-ornithine
DMAP	4-dimethylaminopyridine
HBTU	2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
NMeArg or MeArg	a-N-methyl arginine
NMeAmf	N-Methylaminomethylphenylalanine
NMeAsp	a-N-methyl aspartic acid
NMeGly or MeGly	N-methyl glycine
NMe-Mamb	N-methyl-3-aminomethylbenzoic acid
NMM	N-methylmorpholine
OcHex	O-cyclohexyl
OBzl	O-benzyl
oSu	O-succinimidyl
pNP	p-nitrophenyl
TBTU	2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
Teoc	2-(Trimethylsilyl)ethyloxycarbonyl
Tos	tosyl
Tr	trityl

The following conventional three-letter amino acid abbreviations are used herein; the conventional one-letter amino acid abbreviations are <u>not</u> used herein:

Ala	=	alanine
Arg	=	arginine
Asn	=	asparagine
Asp	=	aspartic acid
Cys	=	cysteine
Gln	=	glutamine

	Glu	=	glutamic acid
	Gly	=	glycine
	His	=	histidine
	Ile	=	isoleucine
	Leu	=	leucine
	Lys	=	lysine
	Met	=	methionine
	Nle	=	norleucine
	Phe	=	phenylalanine
	Phg	=	phenylglycine
	Pro	=	proline
	Ser	=	serine
	Thr	=	threonine
	Trp	=	tryptophan
	Tyr	=	tyrosine
	Val	=	valine

The compounds of the present invention can be synthesized using standard synthetic methods known to those skilled in the art. Preferred methods include but are not limited to those methods described below.

Generally, peptides are elongated by deprotecting the a-amine of the C-terminal residue and coupling the next suitably protected amino acid through a peptide linkage using the methods described. This deprotection and coupling procedure is repeated until the desired sequence is obtained. This coupling can be performed with the constituent amino acids in a stepwise fashion, or condensation of fragments (two to several amino acids), or combination of both processes, or by solid phase peptide synthesis according to the method originally described by Merrifield, J. Am. Chem. Soc.,

85, 2149-2154 (1963), the disclosure of which is hereby incorporated by reference.

The compounds of the invention may also be synthesized using automated peptide synthesizing equipment. In addition to the foregoing, procedures for peptide synthesis are described in Stewart and Young, "Solid Phase Peptide Synthesis", 2nd ed, Pierce Chemical Co., Rockford, IL (1984); Gross, Meienhofer, Udenfriend, Eds., "The Peptides: Analysis, Synthesis, Biology, Vol. 1, 2, 3, 5, and 9, Academic Press, New York, (1980-1987); Bodanszky, "Peptide Chemistry: A Practical Textbook", Springer-Verlag, New York (1988); and Bodanszky et al. "The Practice of Peptide Sythesis" Springer-Verlag, New York (1984), the disclosures of which are hereby incorporated by reference.

The coupling between two amino acid derivatives, an amino acid and a peptide, two peptide fragments, or the cyclization of a peptide can be carried out using standard coupling procedures such as the azide method, mixed carbonic acid anhydride (isobutyl chloroformate) method, carbodiimide (dicyclohexylcarbodiimide, diisopropylcarbodiimide, or water-soluble carbodiimides) method, active ester (p-nitrophenyl ester, N-hydroxysuccinic imido ester) method, Woodward reagent K method, carbonyldiimidazole method, phosphorus reagents such as BOP-Cl, or oxidation-reduction method. Some of these methods (especially the carbodiimide) can be enhanced by the addition of 1-hydroxybenzotriazole. These coupling reactions may be performed in either solution (liquid phase) or solid phase.

The functional groups of the constituent amino acids must be protected during the coupling reactions to avoid undesired bonds being formed. The protecting groups that can be used are listed in Greene,

"Protective Groups in Organic Synthesis" John Wiley & Sons, New York (1981) and "The Peptides: Analysis, Sythesis, Biology, Vol. 3, Academic Press, New York (1981), the disclosure of which is hereby incorporated by reference.

The a-carboxyl group of the C-terminal residue is usually protected by an ester that can be cleaved to give the carboxylic acid. These protecting groups include: 1) alkyl esters such as methyl and t-butyl, 2) aryl esters such as benzyl and substituted benzyl, or 3) esters which can be cleaved by mild base treatment or mild reductive means such as trichloroethyl and phenacyl esters. In the solid phase case, the C-terminal amino acid is attached to an insoluble carrier (usually polystyrene). These insoluble carriers contain a group which will react with the carboxyl group to form a bond which is stable to the elongation conditions but readily cleaved later. Examples of which are: oxime resin (DeGrado and Kaiser (1980) *J. Org. Chem.* **45**, 1295-1300) chloro or bromomethyl resin, hydroxymethyl resin, and aminomethyl resin. Many of these resins are commercially available with the desired C-terminal amino acid already incorporated.

The a-amino group of each amino acid must be protected. Any protecting group known in the art can be used. Examples of these are: 1) acyl types such as formyl, trifluoroacetyl, phthalyl, and p-toluenesulfonyl; 2) aromatic carbamate types such as benzyloxycarbonyl (Cbz) and substituted benzyloxycarbonyls, 1-(p-biphenyl)-1-methylethoxycarbonyl, and 9-fluorenylmethyloxycarbonyl (Fmoc); 3) aliphatic carbamate types such as tert-butyloxycarbonyl (Boc), ethoxycarbonyl, diisopropylmethoxycarbonyl, and allyloxycarbonyl; 4)

cyclic alkyl carbamate types such as cyclopentyloxycarbonyl and adamantyloxycarbonyl; 5) alkyl types such as triphenylmethyl and benzyl; 6) trialkylsilane such as trimethylsilane; and 7) thiol containing types such as phenylthiocarbonyl and dithiasuccinoyl. The preferred a-amino protecting group is either Boc or Fmoc. Many amino acid derivatives suitably protected for peptide synthesis are commercially available.

The a-amino protecting group is cleaved prior to the coupling of the next amino acid. When the Boc group is used, the methods of choice are trifluoroacetic acid, neat or in dichloromethane, or HCl in dioxane. The resulting ammonium salt is then neutralized either prior to the coupling or in situ with basic solutions such as aqueous buffers, or tertiary amines in dichloromethane or dimethylformamide. When the Fmoc group is used, the reagents of choice are piperidine or substituted piperidines in dimethylformamide, but any secondary amine or aqueous basic solutions can be used. The deprotection is carried out at a temperature between 0 °C and room temperature.

Any of the amino acids bearing side chain functionalities must be protected during the preparation of the peptide using any of the above-identified groups. Those skilled in the art will appreciate that the selection and use of appropriate protecting groups for these side chain functionalities will depend upon the amino acid and presence of other protecting groups in the peptide. The selection of such a protecting group is important in that it must not be removed during the deprotection and coupling of the a-amino group.

For example, when Boc is chosen for the a-amine protection the following protecting groups are

acceptable: *p*-toluenesulfonyl (tosyl) moieties and nitro for arginine; benzyloxycarbonyl, substituted benzyloxycarbonyls, tosyl or trifluoroacetyl for lysine; benzyl or alkyl esters such as cyclopentyl for glutamic and aspartic acids; benzyl ethers for serine and threonine; benzyl ethers, substituted benzyl ethers or 2-bromobenzyloxycarbonyl for tyrosine; *p*-methylbenzyl, *p*-methoxybenzyl, acetamidomethyl, benzyl, or t-butylsulfonyl for cysteine; and the indole of tryptophan can either be left unprotected or protected with a formyl group.

When Fmoc is chosen for the a-amine protection usually tert-butyl based protecting groups are acceptable. For instance, Boc can be used for lysine, tert-butyl ether for serine, threonine and tyrosine, and tert-butyl ester for glutamic and aspartic acids.

Once the elongation and cyclization of the peptide is completed all of the protecting groups are removed. For the liquid phase synthesis the protecting groups are removed in whatever manner as dictated by the choice of protecting groups. These procedures are well known to those skilled in the art.

When a solid phase synthesis is used, the peptide should be removed from the resin without simultaneously removing protecting groups from functional groups that might interfere with the cyclization process. Thus, if the peptide is to be cyclized in solution, the cleavage conditions need to be chosen such that a free a-carboxylate and a free a-amino group are generated without simultaneously removing other protecting groups. Alternatively, the peptide may be removed from the resin by hydrazinolysis, and then coupled by the azide method. Another very convenient method involves the synthesis of peptides on an oxime resin, followed by intramolecular

nucleophilic displacement from the resin, which generates a cyclic peptide (Osapay, Profit, and Taylor (1990) *Tetrahedron Letters* **43**, 6121-6124). When the oxime resin is employed, the Boc protection scheme is generally chosen. Then, the preferred method for removing side chain protecting groups generally involves treatment with anhydrous HF containing additives such as dimethyl sulfide, anisole, thioanisole, or p-cresol at 0 °C. The cleavage of the peptide can also be accomplished by other acid reagents such as trifluoromethanesulfonic acid/trifluoroacetic acid mixtures.

Unusual amino acids used in this invention can be synthesized by standard methods familiar to those skilled in the art ("The Peptides: Analysis, Sythesis, Biology, Vol. 5, pp. 342-449, Academic Press, New York (1981)). N-Alkyl amino acids can be prepared using procedures described in previously (Cheung et al., (1977) *Can. J. Chem.* **55**, 906; Freidinger et al., (1982) *J. Org. Chem.* **48**, 77 (1982)), which are incorporated here by reference.

The compounds of the present invention may be prepared using the procedures further detailed below.

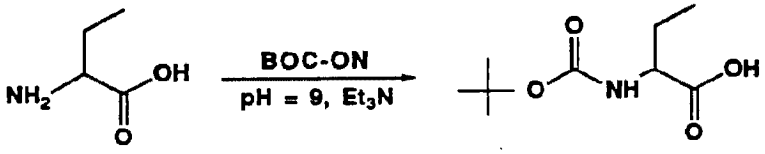
Representative materials and methods that may be used in preparing the compounds of the invention are described further below.

Manual solid phase peptide synthesis was performed in 25 mL polypropylene filtration tubes purchased from BioRad Inc., or in 60 mL hour-glass reaction vessels purchased from Peptides International. Oxime resin (substitution level = 0.96 mmol/g) was prepared according to published procedures (DeGrado and Kaiser (1980) *J. Org. Chem.* **45**, 1295), or was purchased from

Novabiochem (substitution level = 0.62 mmol/g). All chemicals and solvents (reagent grade) were used as supplied from the vendors cited without further purification. t-Butyloxycarbonyl (Boc) amino acids and other starting amino acids may be obtained commercially from Bachem Inc., Bachem Biosciences Inc. (Philadelphia, PA), Advanced ChemTech (Louisville, KY), Peninsula Laboratories (Belmont, CA), or Sigma (St. Louis, MO). 2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and TBTU were purchased from Advanced ChemTech. N-methylmorpholine (NMM), *m*-cresol, D-2-aminobutyric acid (Abu), trimethylacetylchloride, diisopropylethylamine (DIEA), 3-cyanobenzoic acid and [2-(tert-butyloxycarbonyloxylimino)-phenylacetonitrile] (Boc-ON) were purchased from Aldrich Chemical Company. Dimethylformamide (DMF), ethyl acetate, chloroform ($CHCl_3$), methanol (MeOH), pyridine and hydrochloric acid (HCl) were obtained from Baker. Acetonitrile, dichloromethane (DCM), acetic acid (HOAc), trifluoroacetic acid (TFA), ethyl ether, triethylamine, acetone, and magnesium sulfate were purchased from EM Science. Palladium on carbon catalyst (10% Pd) was purchased from Fluka Chemical Company. Absolute ethanol was obtained from Quantum Chemical Corporation. Thin layer chromatography (TLC) was performed on Silica Gel 60 F_{254} TLC plates (layer thickness 0.2 mm) which were purchased from EM Separations. TLC visualization was accomplished using UV light, iodine, ninhydrin spray and/or Sakaguchi spray. Melting points were determined using a Thomas Hoover or Electrothermal 9200 melting point apparatus and are uncorrected. HPLC analyses were performed on either a Hewlett Packard 1090, Waters Delta Prep 3000, Rainin, or DuPont 8800 system. NMR spectra were recorded on a 300 MHz General Electric QE-300,

Varian 300, or Varian 400 spectrometer. Fast atom bombardment mass spectrometry (FAB-MS) was performed on a VG Zab-E double-focusing mass spectrometer using a Xenon FAB gun as the ion source or a Finnigan MAT 8230.

Boc-D-2-aminobutyric acid (Boc-D-Abu) was prepared by a modification of procedures previously reported in the literature (Itoh, Hagiwara, and Kamiya (1975) *Tett. Lett.*, 4393), as shown in the scheme below.



D-2-aminobutyric acid

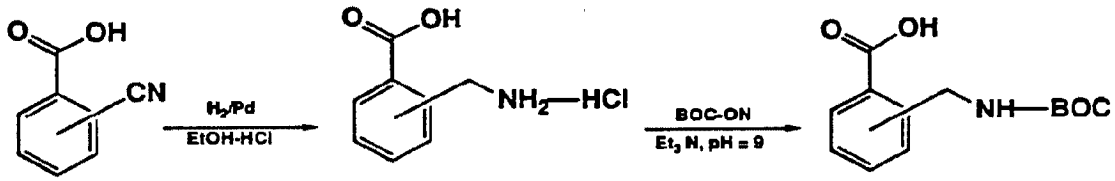
D-2-aminobutyric acid (1.0 g, 9.70 mmol) was dissolved in 20 ml H_2O and a solution of Boc-ON (2.62 g, 10.6 mmol) in 20 ml acetone was added. A white precipitate formed which dissolved upon addition of triethylamine (3.37 ml, 24.2 mmol) to give a pale yellow solution (pH = 9, wet pH paper). The solution was stirred at room temperature overnight at which time the acetone was removed under reduced pressure. The remaining aqueous layer was extracted with ether three times, acidified to pH 2 with concentrated HCl, and then extracted with ethyl acetate three times. The combined organic layers were dried over anhydrous magnesium sulfate and evaporated under reduced pressure to give t-butyloxycarbonyl-D-2-aminobutyric acid as an oil (2.05 g, greater than quantitative yield, contains solvent), which was used without further purification. ^1H NMR ($CDCl_3$) 0.98 (t, 3H), 1.45 (s, 9H), 1.73 (m, 1H), 1.90 (m, 1H), 4.29 (m, 1H), 5.05 (m, 1H).

Synthesis of R^{31} Cyclizing Moieties

This section teaches the synthesis of certain cyclizing moieties that serve as intermediates to the R^{31} groups in Q. Later sections teach the synthesis of other cyclizing moieties.

Synthesis of Boc-aminomethylbenzoic Acid, Boc-aminophenylacetic Acid and Boc-aminomethylphenylacetic Acid Derivatives

Boc-aminomethylbenzoic acid derivatives useful as cyclizing moieties in the synthesis of the compounds of the invention are prepared using standard procedures, for example, as described in *Tett. Lett.*, 4393 (1975); *Modern Synthetic Reactions*, H.O. House (1972); or Harting et al. *J. Am. Chem. Soc.*, 50: 3370 (1928), and as shown schematically below.



3-Aminomethylbenzoic acid•HCl

3-Cyanobenzoic acid (10.0 g, 68 mmol) was dissolved in 200 ml ethanol by heating in a 35-50°C water bath. Concentrated HCl (6.12 ml, 73 mmol) was added and the solution was transferred to a 500 ml nitrogen-flushed round bottom flask containing palladium on carbon catalyst (1.05 g, 10% Pd/C). The suspension was stirred under an atmosphere of hydrogen for 38 hours, filtered

through a scintered glass funnel, and washed thoroughly with H_2O. The ethanol was removed under reduced pressure and the remaining aqueous layer, which contained a white solid, was diluted to 250 ml with additional H_2O. Ethyl ether (250 ml) was added and the suspension was transferred to a separatory funnel. Upon vigorous shaking, all solids dissolved and the aqueous layer was then washed two times with ether, evaporated under reduced pressure to a volume of 150 ml, and lyophilized to give the title compound (3-aminomethylbenzoic acid·HCl) (8.10 g, 64%) as a beige solid. ^1H NMR (D_2O) 4.27 (s, 2H), 7.60 (t, 1H), 7.72 (d,1H), 8.06 (d, 2H).

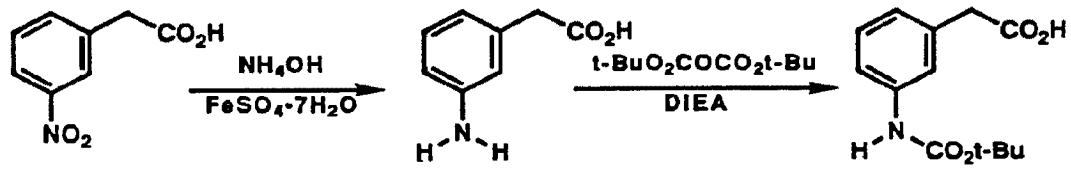
t-Butyloxycarbonyl-3-aminomethylbenzoic Acid (Boc-Mamb)

The title compound was prepared according to a modification of standard procedures previously reported in the literature (Itoh, Hagiwara, and Kamiya (1975) *Tett. Lett.*, 4393). 3-Aminomethylbenzoic acid (hydrochloride salt) (3.0 g, 16.0 mmol) was dissolved in 60 ml H_2O. To this was added a solution of Boc-ON (4.33 g, 17.6 mmol) in 60 ml acetone followed by triethylamine (5.56 ml, 39.9 mmol). The solution turned yellow and the pH was adjusted to 9 (wet pH paper) by adding an additional 1.0 ml (7.2 mmol) triethylamine. The solution was stirred overnight at room temperature at which time the acetone was removed under reduced pressure and the remaining aqueous layer was washed three times with ether. The aqueous layer was then acidified to pH 2 with 2N HCl and then extracted three times with ethyl acetate. The combined organic layers were washed three times with H_2O, dried over anhydrous magnesium sulfate, and evaporated to

dryness under reduced pressure. The material was recrystallized from ethyl acetate/ hexane, to give two crops of the title compound (2.58 g, 64%) as an off-white solid. mp 123-125°C ; ^{1}H NMR ($CDCl_3$) 1.47 (s, 9 H), 4.38 (br s, 2 H), 4.95 (br s, 1H), 7.45 (t, 1H), 7.55 (d, 1H), 8.02 (d, 2H).

Synthesis of t-Butyloxycarbonyl-3-aminophenylacetic Acid

t-Butyloxycarbonyl-3-aminophenylacetic acids useful as intermediates in the synthesis of the compounds of the invention are prepared using standard procedures, for example, as described in Collman and Groh (1982) *J. Am. Chem. Soc.*, 104: 1391, and as shown schematically below.



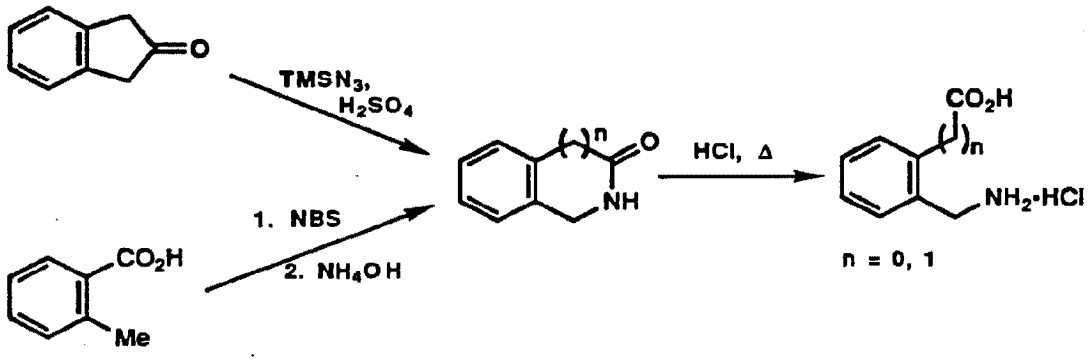
t-Butyloxycarbonyl-3-aminophenylacetic Acid

A solution of 3-aminophenylacetic acid (Aldrich, 10 g, 66 mmol), di-*tert*-butyl dicarbonate (15.8 g, 72 mmol), and DIEA (8.6 g, 66 mmol) in 50 ml of dichloromethane was stirred overnight at room temperature. The reaction mixture was concentrated, partitioned between dichloromethane-H_2O, the water layer was separated, acidified to pH 3 with 1N HCl, and extracted with dichloromethane. The extracts were washed with H_2O, brine, dried over anhydrous sodium sulfate,

and evaporated to dryness under reduced pressure. This material was purified by recrystallization from heptane to provide the title compound (3.7 g, 22%) as a white solid. mp 105°C; ^{1}H NMR ($CDCl_3$) 7.35 (s, 1H), 7.25 (m, 3H), 6.95 (m, 1H), 6.60 (br s, 1H), 3.65 (s, 2H), 1.50 (s, 9H).

Synthesis of 2-Aminomethylbenzoic Acid·HCl and 2-Aminomethylphenylacetic Acid·HCl

2-Aminomethylbenzoic acid·HCl and 2-aminomethylphenylacetic acid·HCl useful as intermediates in the synthesis of the compounds of the invention are prepared using standard procedures, for example, as described in Naito et al *J. Antibiotics*, 30: 698 (1977); or Young and Sweet *J. Am. Chem. Soc.*, 80: 800 (1958), and as shown schematically below.



2-Aminomethylphenylacetic Acid d-Lactam

The title compound was prepared by modification of procedures previously reported in the literature (Naito et al. (1977) *J. Antibiotics*, 30: 698). To an ice-cooled suspension of 2-indanone (10.8 g, 82 mmol) and azidotrimethylsilane (9.4 g, 82 mmol) in 115 ml of

chloroform was added 25 ml of concentrated sulfuric acid at a rate to maintain the temperature between 30-40°C. After an additional 3 hours, the reaction mixture was poured onto ice, and the water layer was made basic with concentrated ammonium hydroxide. The chloroform layer was separated, washed with H_2O, brine, dried over anhydrous magnesium sulfate, and evaporated to dryness under reduced pressure. This material was purified by sublimination (145°C, <1 mm), followed by recrystallization from benzene to give the title compound (5.4 g, 45%) as pale yellow crystals. mp 149-150°C; ^1H NMR ($CDCl_3$) 7.20 (m, 5H), 4.50 (s, 2H), 3.60 (s, 2H).

2-Aminomethylphenylacetic Acid•HCl

The title compound was prepared by modification of procedures previously reported in the literature (Naito et al. (1977) *J. Antibiotics*, 30: 698). A mixture of 2-aminomethylphenylacetic acid d-lactam (6.4 g, 44 mmol) and 21 ml of 6N HCl was heated to reflux for 4 hours. The reaction mixture was treated with activated carbon (Norit A), filtered, evaporated to dryness, and the residual oil triturated with acetone. Filtration provided the title compound (5.5 g, 62%) as colorless crystals. mp 168°C (dec); ^1H NMR (D_6-DMSO) 12.65 (br s, 1H), 8.35 (br s, 3H), 7.50 (m, 1H), 7.35 (m, 3H), 4.05 (ABq, 2H), 3.80 (s, 2H).

2-Aminomethylbenzoic Acid g-Lactam

The title compound was prepared by modification of procedures previously reported in the literature (Danishefsky et al. (1975) *J. Org. Chem.*, 40: 796). A mixture of methyl *o*-toluate (45 g, 33 mol), N-bromosuccinimide (57 g, 32 mol), and dibenzoyl peroxide

(0.64 g) in 175 ml of carbon tetrachloride was heated to reflux for 4 hours. The cooled reaction mixture was filtered, evaporated to dryness under reduced pressure, dissolved in 250 ml of methanol, and concentrated ammonium hydroxide (75 ml, 1.11 mol) was added. The reaction mixture was heated to reflux for 5 hours, concentrated, filtered, and the solid washed with H_2O followed by ether. This material was purified by recrystallization from H_2O to give the title compound (11.0 g, 26%) as a white solid. mp 150°C; ^1H NMR ($CDCl_3$) 7.90 (d, 1H), 7.60 (t, 1H), 7.50 (t, 2H), 7.00 (br s, 1H), 4.50 (s, 2H).

2-Aminomethylbenzoic Acid•HCl

The title compound was prepared using the general procedure described above for 2-aminomethylphenylacetic acid•HCl. The lactam (3.5 g, 26 mmol) was converted to the title compound (2.4 g, 50%) as colorless crystals. mp 233°C (dec); ^1H NMR (D_6-DMSO) 13.40 (br s, 1H), 8.35 (br s, 3H), 8.05 (d, 1H), 7.60 (m, 3H), 4.35 (br s, 2H).

Synthesis of Cyclic Compound Intermediates

This section teaches the synthesis of certain cyclic compound intermediates. These are the intermediate compounds that serve as the precursor to the Q group in the claimed compounds, $(QL_n)_dC_h$; $(Q)_{d'}L_n$-C_h. These compounds may be directly labeled with radioisotopes, or may be modified by attaching linker group(s) and chelator(s).

t-Butyloxycarbonyl-3-aminomethylbenzoic acid (Boc-Mamb) is coupled to oxime resin by a modification of the method described by DeGrado and Kaiser (1980) *J. Org.*

Chem. **45**, 1295 using 1 equivalent of the 3-aminomethylbenzoic acid (with respect to the substitution level of the resin), 1 equivalent of HBTU, and 3 equivalent of NMM. Alternatively, Boc-Mamb (1 equivalent) may be coupled to the oxime resin using 1 equivalent each of DCC and DMAP in methylene chloride. Coupling times range from 15 to 96 hours. The substitution level is then determined using either the picric acid test (Sarin, Kent, Tam, and Merrifield, (1981) *Anal. Biochem.* **117**, 145-157) or the quantitative ninhydrin assay (Gisin (1972) *Anal. Chim. Acta* **58**, 248-249). Unreacted oxime groups are blocked using 0.5 M trimethylacetylchloride / 0.5 M diisopropylethylamine in DMF for 2 hours. Deprotection of the Boc protecting group is accomplished using 25% TFA in DCM for 30 minutes. The remaining amino acids or amino acid derivatives are coupled using between a two and ten fold excess (based on the loading of the first amino acid or amino acid derivative) of the appropriate amino acid or amino acid derivatives and HBTU in approximately 8 ml of DMF. The resin is then neutralized in situ using 3 eq. of NMM (based on the amount of amino acid used) and the coupling times range from 1 hour to several days. The completeness of coupling is monitored by qualitative ninhydrin assay, or picric acid assay in cases where the amino acid was coupled to a secondary amine. Amino acids are recoupled if necessary based on these results.

After the linear peptide had been assembled, the N-terminal Boc group is removed by treatment with 25% TFA in DCM for 30 minutes. The resin is then neutralized by treatment with 10% DIEA in DCM. Cyclization with concomitant cleavage of the peptide is accomplished using the method of Osapay and Taylor ((1990) *J. Am. Chem. Soc.*, **112**, 6046) by suspending the resin in

approximately 10 ml/g of DMF, adding one equivalent of HOAc (based on the loading of the first amino acid), and stirring at 50-60°C for 60 to 72 hours. Following filtration through a scintered glass funnel, the DMF filtrate is evaporated, redissolved in HOAc or 1:1 acetonitrile: H_2O, and lyophilized to obtain protected, cyclized material. Alternatively, the material may be dissolved in methanol and precipitated with ether to obtain the protected, cyclized material. This is then treated using standard procedures with anhydrous hydrogen fluoride (Stewart and Young (1984) "Solid Phase Peptide Synthesis", 2nd. edition, Pierce Chemical Co., 85) containing 1 ml/g *m*-cresol or anisole as scavenger at 0°C for 20 to 60 minutes to remove side chain protecting groups. The crude product may be purified by reversed-phase HPLC using a 2.5 cm preparative Vydac C18 column with a linear acetonitrile gradient containing 0.1% TFA to produce pure cyclized material. The following N-a-Boc-protected amino acids may be used for the syntheses: Boc-Arg(Tos), Boc-N-a-MeArg(Tos), Boc-Gly, Boc-Asp(OcHex), Boc-3-aminomethyl-4-iodo-benzoic acid, Boc-D-Ile, Boc-NMeAsp(OcHex), Boc-NMe-Mamb, Boc-D-Phg, Boc-D-Asp(OBzl), Boc-L-Asp(OcHex), Boc-aMe-Asp(OcHex), Boc-bMe-Asp(OcHex), Boc-L-Ala, Boc-L-Pro, Boc-D-Nle, Boc-D-Leu, Boc-D-Val, Boc-D-2-aminobutyric acid (Boc-D-Abu), Boc-Phe, Boc-D-Ser(Bzl), Boc-D-Ala, Boc-3-aminomethylbenzoic acid (Boc-Mamb), Boc-D-Lys(2-ClZ), Boc-b-Ala, Boc-D-Pro, Boc-D-Phe, Boc-D-Tyr(Cl_2Bzl), Boc-NMe-Amf(CBZ), Boc-aminotetralin-carboxylic acid, Boc-aminomethylnaphthoic acid, Boc-4-aminomethylbenzoic acid, or Boc-NMeGly.

Preferable N-a-Boc-protected amino acids useful in these syntheses are Boc-Arg(Tos), Boc-N-a-MeArg(Tos), Boc-Gly, Boc-Asp(OcHex), Boc-D-Leu, Boc-D-Val, Boc-D-2-

aminobutyric acid (Boc-D-Abu), Boc-Phe, Boc-D-Ser(Bzl), Boc-D-Ala, Boc-3-aminomethylbenzoic acid (Boc-Mamb), Boc-D-Lys(2-ClZ), Boc-Ala,Boc-D-Pro, or Boc-NMeGly.

The synthesis of the compounds of the invention is further exemplified below. The Tables below set forth representative compounds of the present invention.

Cyclic Compound Intermediate 1

cyclo-(Gly-NMeArg-Gly-Asp-Mamb); the compound of formula (II) wherein J = Gly, K = NMeArg, L = Gly, M = Asp, R^1 = R^2 = H

The title compound was prepared using the general procedure described below for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb). The peptide was prepared on a 0.336 mmol scale to give the protected cyclic peptide (218 mg, 84%). The peptide (200 mg) and 200 mL of *m*-cresol were treated with anhydrous hydrogen fluoride at 0°C for 1 hour. The crude material was precipitated with ether, redissolved in aqueous HOAc, and lyophilized to generate the title compound as a pale yellow solid (158 mg, greater than quantitative yield; calculated as the acetate salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/ min. gradient of 2 to 11% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (21% recovery, overall yield 16.3%). Mass spectrum: M+H = 533.26.

Cyclic Compound Intermediate 2

cyclo-(D-Ala-NMeArg-Gly-Asp-Mamb); the compound of formula (II) wherein J = D-Ala, K = NMeArg, L = Gly, M = Asp, R^1 = R^2 = H

The title compound was prepared using the general procedure described below for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb). Recoupling of the Boc-N-MeArg(Tos) residue was found to be necessary. The peptide was prepared on a 0.244 mmol scale to give the protected cyclic peptide (117 mg, 61%). The peptide (110 mg) and 110 mL of *m*-cresol were treated with anhydrous hydrogen fluoride at 0°C for 1 hour. The crude material was precipitated with ether, redissolved in aqueous HOAc, and lyophilized to generate the title compound as a pale yellow solid. Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.25%/min. gradient of 2 to 11% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid.
Mass spectrum: M+H = 547.23.

Cyclic Compound Intermediate 3

cyclo-(D-Abu-NMeArg-Gly-Asp-Mamb); the compound of formula (II) wherein J = D-Abu, K = NMeArg, L = Gly, M = Asp, R^1 = R^2 = H

The title compound was prepared using the general procedure described below for Cyclic Compound Intermediate 4. The peptide was prepared on a 0.101 mmol scale to give the protected cyclic peptide (51 mg, 63%). The peptide (43 mg) and 50 µL of *m*-cresol were treated with anhydrous hydrogen fluoride at 0°C for 30

minutes The crude material was precipitated with ether, redissolved in aqueous HOAc, and lyophilized to generate the title compound as a pale yellow solid (23 mg, 68.7%; calculated as the acetate salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/min. gradient of 7 to 14% acetonitrile containing 0.1% trifluoroacetic acid and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (31% recovery; overall yield 12.4%).
Mass spectrum: M+H = 561.46.

Cyclic Compound Intermediate 3a

cyclo-(Abu-NMeArg-Gly-Asp-Mamb); the compound of formula (II) wherein J = Abu, K = NMeArg, L = Gly, M = Asp, R^1 = H, R^2 = H

The title compound was prepared using the general procedure described for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb) (Cyclic Compound Intermediate 4). The DCC/DMAP method was used for attachment of Boc-Mamb to the oxime resin. TBTU was used as the coupling reagent. The peptide was prepared on a 0.596 mmol scale to give the protected cyclic peptide (182 mg,38.4%). The peptide (176 mg) and 0.176 mL of anisole were treated with anhydrous hydrogen fluoride at 0°C for 20 minutes. The crude material was precipitated with ether, redissolved in aqueous acetonitrile, and lyophilized to generate the title compound (116 mg; 90.4%; calculated as the fluoride salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.45%/ min. gradient of 9 to 27% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy

white solid (1.92% recovery, overall yield 0.574%); FAB-MS: [M+H] = 561.39.

Cyclic Compound Intermediate 4

cyclo-(D-Val-NMeArg-Gly-Asp-Mamb); the compound of formula (II) wherein J = D-Val, K = NMeArg, L = Gly, M = Asp, $R^1 = R^2 = H$

To a 25 ml polypropylene tube fitted with a frit was added Boc-Mamb (0.126 g, 0.5 mmol) and 6 ml of DMF. To this was added HBTU (0.194 g, 0.5 mmol), oxime resin (0.52 g, substitution level = 0.96 mmol/g), and N-methylmorpholine (0.165 ml, 1.50 mmol). The suspension was mixed at room temperature for 24 hours. The resin was then washed thoroughly (10-12 ml volumes) with DMF (3x), MeOH (1x), DCM (3x), MeOH (2x) and DCM (3x). The substitution level was determined to be 0.389 mmol/g by quantitative ninhydrin assay. Unreacted oxime groups were blocked by treatment with 0.5 M trimethylacetylchloride/ 0.5M DIEA in DMF for 2 hours.

The following steps were then performed: (Step 1) The resin was washed with DMF(3x), MeOH (1x), DCM (3x), MeOH (2x), and DCM (3x). (Step 2) The t-Boc group was deprotected using 25% TFA in DCM for 30 minutes. (Step 3) The resin was washed with DCM (3x), MeOH (1x), DCM (2x), MeOH (3x) and DMF(3x) (Step 4) Boc-Asp(OcHex) (0.613 g, 1.94 mmol), HBTU (0.753 g, 1.99 mmol), 8 ml of DMF, and N-methylmorpholine (0.642 ml, 5.84 mmol) were added to the resin and the reaction allowed to proceed for 2.5 hours. (Step 5) The coupling reaction was found to be complete as assessed by the qualitative ninhydrin assay. Steps 1-5 were repeated until the desired sequence had been attained. The coupling of

Boc-D-Val to NMeArg was monitored by the picric acid test.

After the linear peptide was assembled, the N-terminal t-Boc group was removed by treatment with 25% TFA in DCM (30 min.) The resin was washed thoroughly with DCM (3x), MeOH (2x) and DCM (3x), and then neutralized with 10% DIEA in DCM (2 x 1 min.) The resin was washed thoroughly with DCM (3x) and MeOH (3x) and then dried. Half of the resin (0.101 mmol) was cyclized by treating with 6 ml of DMF containing HOAc (5.8 mL, 0.101 mmol) and heating at 50°C for 72 hours. The resin was then filtered through a scintered glass funnel and washed thoroughly with DMF. The DMF filtrate was evaporated to an oil, redissolved in 1:1 acetonitrile: H_2O, and lyophilized to give the protected cyclic peptide (49 mg, 60%). The peptide (42 mg) was treated with anhydrous hydrogen fluoride at 0°C, in the presence of 50 mL of *m*-cresol as scavenger, for 30 minutes to remove side chain protecting groups . The crude material was precipitated with ether, redissolved in aqueous HOAc, and lyophilized to generate the title compound as a pale yellow solid (23 mg, 70%; calculated as the acetate salt). Purification was accomplished using reversed-phase HPLC with a preparative Vydac C18 column (2.5 cm) and a 0.23%/ minute gradient of 7 to 18% acetonitrile containing 0.1% trifluoroacetic acid to give the TFA salt of the title compound as a fluffy white solid (24% recovery; overall yield 9.4%); FAB-MS: [M+H] = 575.45.

Solution Phase Synthesis of Cyclic Compound Intermediate 4

The following abbreviations are used below for TLC solvent systems: chloroform/methanol 95:5 = CM; chloroform/acetic acid 95:5 = CA; chloroform/methanol/acetic acid 95:5 = CMA

BocNMeArg(Tos)-Gly-OBzl -- 25 mmol BocNMeArg(Tos) (11.07 g, Bachem), 30 mmol Gly-OBzl tosylate (10.10 g, Bachem), 25 mmol HBTU (O-Benzotriazole-N,N,N',N',-tetramethyl-uronium-hexafluorophosphate; 9.48 g; Advanced Chemtech), and 75 mmol DIEA (diisopropylethylamine; Aldrich) were dissolved in 25 ml CH_2Cl_2. The reaction was allowed to proceed 1 hr, the solvent was evaporated under reduced pressure at 50° to a syrup, wich was dissolved in 400 ml ethyl acetate. This solution was extracted with (150 ml each) 2 x 5% citric acid, 1 x water, 2 x sat. $NaHCO_3$, 1 x sat. NaCl. The organic layer was dried over $MgSO_4$, and the solvent evaporated under reduced pressure. The resulting oil was triturated with petroleum ether and dried under high vacuum for a minimum of 1 hr. yield 14.7 g (99.5%); TLC $R_{f(CM)}$ = 0.18 $R_{f(CA)}$ = 0.10; NMR is consistent with structure; FABMS $M+H^+$ = 590.43 (expected 590.26).

NMeArg(Tos)-Gly-OBzl -- 14.5 g (BocNMeArg(Tos)-Gly-OBzl (24.5 mmol) was dissolved in 30 ml TFA, allowed to react for 5 min., and the solvent evaporated at 1 mm mecury pressure at r.t. The resulting syrup was dissolved in 400 ml ice cold ethyl acetate, and extracted with 100 ml ice cold sat. NaHCO3, the aqueous phase was extracted twice with 200 ml ethyl acetate, and the combined organic phases were extracted once with 25 ml sat. NaCl. The solvent was evaporated under reduced pressure giving a viscous oil that was triturated with 300 ml ether. The resulting solid was filtered and washed with ether,

giving a hydroscopic compound that was dried in a vacuum desiccator: yield 10.33 g (86.2%); TLC $R_{f(CM)}$ = 0.03; $R_{f(CMA)}$ = 0.20; NMR is consistent with structure; FABMS $M+H^+$ = 490.21 (expected 490.20).

Boc-D-Val-NMeArg(Tos)-Gly-OBzl -- 9.80 mmol NMeArg(Tos)-Gly-OBzl (4.80 g), 9.82 mmol Boc-D-Val (2.13 g, Bachem), and 10.0 mmol HBTU (3.79 g) were dissolved in 10 ml methylene chloride. The flask was placed on an ice bath, and 20 mmol DIEA (3.48 ml) was added. The reaction was allowed to proceed at 0° for 15 min and 2 days at r.t. The reaction mixture was diluted with 400 ml ethyl acetate, extracted (200 ml each) 2 x 5% citric acid, 1 x sat. NaCl, dried over $MgSO_4$ and evaporated under reduced pressure. The resulting oil was triturated with 50, then 30 ml ether for 30 min with efficient mixing: yield 4.58 g (69%); TLC $R_{f(CM)}$ = 0.27 (also contains a spot near the origin, which is an aromatic impurity that is removed during trituration of the product in the next step); NMR is consistent with structure; FABMS $M+H^+$ = 689.59 (expected 689.43).

Boc-D-Val-NMeArg(Tos)-Gly -- 4.50 g Boc-D-Val-NMeArg(Tos)-Gly-OBzl (4.44 mmol) dissolved in 80 ml methanol was purged with N_2 for 10 min. 1.30 g Pd/C catalyst (10% Fluka lot #273890) was then added, and then H_2 was passed directly over the surface of the reaction. TLC showed the reaction to be complete within approximately 0.5 hr. After 1 hr. the catalyst was removed by filtering through a bed of Celite, and the solvent removed at 40° under reduced pressure. The resulting solid was triturated well with 50 ml refluxing ether, filtered, and washed with petroleum ether: yield 3.05 g (78%); TLC $R_{f(CM)}$ = 0.03; $R_{f(CMA)}$ = 0.37; NMR is

consistent with structure; FABMS $M+H^+$ = 599.45 (expected 599.29).

4-Nitrobenzophenone Oxime (Ox) -- 50 g 4-nitrobenzophenone (220 mmol, Aldrich) and 30.6 g hydroxylamine hydrochloride (Aldrich, 440 mmol) were heated at reflux in 0.5 L methanol/pyridine (9:1) for 1 hr. The reaction mixture was evaporated under reduced pressure, dissolved in 500 ml ether, and extracted with 200 ml each of 5% citric acid (2 times) and sat. NaCl (1 time), dried over $MgSO_4$, evaporated under reduced pressure and triturated with ether giving 44.35 g (83%) of the oxime as a mixture of the cis and trans isomers: TLC $R_{f(CM)}$ = 0.50; $R_{f(CMA)}$ = 0.82; NMR is consistent with structure; FABMS $M+H^+$ = 242.07 (expected 242.07).

BocMamb-Ox -- 22 mmol BocMamb (5.522 g), 20 mmol nitrobenzophenone oxime (4.84 g), and 20 mmol DMAP (4-dimethylaminopyridine; Aldrich) were dissolved in 40 ml CH_2Cl_2. The flask was placed on an ice bath, and 21 mmol DCC (Dicyclohexylcarbodiimide; 4.33 g) was added. The reaction was allowed to proceed on ice for 30 min and at r.t. over night. The dicyclohexylurea formed was filtered, and washed with 40 ml methylene chloride. The filtrate was evaporated under reduced pressure at r.t. to a syrup, and dissolved in 400 ml ethyl acetate. This solution was extracted with (150 ml each) 2 x 5% citric acid, 1 x water, 2 x sat. $NaHCO_3$, 1 x sat. NaCl. The organic layer was dried over $MgSO_4$, and the solvent evaporated under reduced pressure. The resulting oil was triturated with petroleum ether and dried under high vacuum for a minimum of 1 hr.: yield 7.51 g (79%); TLC $R_{f(CM)}$ = 0.41; $R_{f(CMA)}$ = 0.66; NMR is consistent with structure; FABMS $M+H^+$ = 476.30 (expected 476.18).

TFA·MAMB-Ox -- BocMamb-Ox , 7.4 g (15.5 mmol) was dissolved in 30 ml methylene chloride plus 10 ml TFA (25% TFA). The reaction was allowed to proceed at r.t. for 1 hr, and the solvent evaporated under reduced pressure at r.t. for 10 min, then at 40° for 15 min. The resulting syrup was triturated with ether (200 ml) at -5°, giving. The resulting crystals were filtered after 1 hr and washed well with ether: yield 7.22 g (95%); $R_{f(CMA)}$ = 0.25; NMR is consistent with structure; FABMS $M+H^+$ = 376.22 (expected 376.12).

Boc-Asp(OcHex)-Mamb-Ox -- 20 mmol Boc-Asp(OcHex) (6.308 g, Bachem) and 44 mmol DIEA (7.66 ml) were dissolved in 20 ml DMF. 20 mmol HBTU (7.58 g, Advanced Chemtech) was added, and the reaction allowed to proceed for 2 minutes with vigorous stirring. TFA·Mamb-Ox (7.13 g, 15 mmol) was added, and the reaction allowed to proceed o.n. at r.t. The solvent was removed under reduced pressure giving an oil, which was dissolved in 500 ml ethyl acetate, and this solution was extracted with (150 ml each) 2 x 5% citric acid, 1 x water, 2 x sat. $NaHCO_3$, 1 x sat. NaCl. The organic layer was dried over $MgSO_4$, and the solvent evaporated under reduced pressure. The resulting oil was triturated with petroleum ether and dried under high vacuum: yield 9.76 g (97%); TLC $R_{f(CM)}$ = 0.55; NMR is consistent with structure; FABMS $M+H^+$ = 673.45 (expected 673.23).

TFA Asp(OcHex)-MAMB-Ox -- 15 mmol Boc-Asp(OcHex)-MAMB-Ox was dissolved in 50 ml 35% TFA in CH_2Cl_2, and allowed to react 90 min. The solvent was evaporated under reduced pressure at r.t. for 10 min, then at 40° for 15 min. To remove traces of TFA, 25 ml DMF was

added and the solvent evaporated at 50°. The resulting syrup was triturated with ether (200 ml), then dried under high vacuum: yield 9.61 g (93%); $R_{f(CMA)}$ = 0.45; NMR is consistent with structure; FABMS $M+H^+$ = 573.56 (expected 573.23).

Boc-D-Val-NMeArg(Tos)-Gly-Asp(OcHex)-MAMB-Ox 10.0 mmol each TFA Asp(OcHex)-MAMB-Ox, Boc-D-Val-NMeArg(Tos)-Gly, and HBTU, plus 30 mmol DIEA were dissolved in 20 ml DMF. After 4 hr., the solvent was removed under reduced pressure, and the residue taken up in 600 ml ethyl acetate, which was extracted with 300 ml each of 5% citric acid, water and sat. NaCl. The organic layer was dried over $MgSO_4$, evaporated under reduced pressure, triturated with ether and dried in vacuo: yield 9.90 g (86%); $R_{f(CM)}$ = 0.10; NMR is consistent with structure; FABMS $M+H^+$ = 1153.22 (expected 1153.47).

TFA·D-Val-NMeArg(Tos)-Gly-Asp(OcHex)-MAMB-Ox This compound was prepared from Boc-D-Val-NMeArg(Tos)-Gly-Asp(OcHex)-MAMB-Ox (9.8 g, 8.5 mmol) by treatment with TFA/CH_2Cl_2 (1:1) for 45 min. The solvent was evaporated and the product triturated with ether: yield 9.73 g (98%); $R_{f(CM)}$ = 0.10; NMR is consistent with structure; FABMS $M+H^+$ = 1053.22 (expected 1053.4).

cyclo(·D-Val-NMeArg(Tos)-Gly-Asp(OcHex)-MAMB) TFA·D-Val-NMeArg(Tos)-Gly-Asp(OcHex)-MAMB-Ox (1.80 g, 1.54 mmol), and 2 mmol each of DIEA and acetic acid were dissolved in 200 ml DMF. The mixture was heated to 50° for 2 days, then evaporated under reduced pressure. The syrup was dissolved in 400 ml ethyl acetate/n-butanol (1:1), and extracted with 200 ml each of 5% citric acid (3x) and sat. NaCl (1x). The organic layer was dried

over $MgSO_4$ and triturated twice with 200 ml ether: yield 1.07 g (86%); $R_{f(CM)}$ = 0.10; NMR is consistent with structure; FABMS $M+H^+$ = 811.25 (expected 811.38).

cyclo(·D-Val-NMeArg-Gly-Asp-MAMB) 0.50 g cyclo(D-Val-NMeArg(Tos)-Gly-Asp(OcHex)-MAMB) was treated with 5 ml HF at 0°C, in the presence of 0.5 ml of anisole for 30 min. The HF was removed under reduced pressure and the crude peptide triturated with ether, ethyl acetate and ether. The resulting solid was dissolved in 10% acetic acid and lyophilized: yield 0.321 g (82% calculated as the acetate salt). The product was purified with a recovery of approximately 40% using the same method as described for the material synthesized by the solid phase procedure.

Crystallization Cyclic Compound Intermediate 4

Preparation of Salt Forms of the Compound of Cyclic Compound Intermediate 4

It has been discovered that the compounds of the present invention may be isolated by crystallization of the compound from organic and aqueous solvents.

The zwitterion of Cyclic Compound Intermediate 4 was converted to the mesyl (methanesulfonate) salt of Cyclic Compound Intermediate 4 (Cyclic Compound Intermediate 4 (methane-sulfonate)) by refluxing the zwitterion with stirring in isopropanol at 25 mg/ml and slowly adding a solution of 1.0 molar equivalent methanesulfonic acid (correcting for the water content of the zwitterion) dissolved in isopropanol. The heat was turned off and the solution cooled to 5°C in an ice bath. After stirring 1 hour, the solution was filtered

and the solid rinsed three times with cold isopropanol and dried under vacuum to constant weight.

The following salts of the compound of Cyclic Compound Intermediate 4 were prepared using the same procedure, by adding 1.0 equivalent of the appropriate acid:

Cyclic Compound Intermediate 4 (biphenylsulfonate): zwitterion + 1.0 equivalent biphenylsulfonic acid.

Cyclic Compound Intermediate 4 (a-naphthalenesulfonate): zwitterion + 1.0 equiv. a-naphthalenesulfonic acid.

Cyclic Compound Intermediate 4 (b-naphthalenesulfonate): zwitterion + 1.0 equiv. b-naphthalenesulfonic acid.

Cyclic Compound Intermediate 4 (benzenesulfonate): zwitterion + 1.0 equiv. benezene-sulfonic acid.

Cyclic Compound Intermediate 4 (*p*-toluenesulfonate): zwitterion + 1.0 equiv. *p*-toluene-sulfonic acid.

The following salts of the compound of Cyclic Compound Intermediate 4 were prepared by crystallization of the compound from aqueous systems.

Cyclic Compound Intermediate 4 (sulfate): 10 mg amorphous Cyclic Compound Intermediate 4 (made by lyophilizing the zwitterion from a solution of 2 molar equivalents of acetic acid in water) dissolved per ml 1 N H_2SO_4, pH adjusted to 2.5. On standing at room

temperature, a precipitate formed. This was filtered through a sintered glass funnel and dried under vacuum to constant weight.

Cyclic Compound Intermediate 4 (methanesulfonate (mesyl)):
100 mg amorphous DMP728 dissolved per ml water + 1.2 molar equiv. methanesulfonic acid (this was obtained as a 4M aqueous solution). On standing at room temperature, a large flat crystal was formed.

Cyclic Compound Intermediate 4 (benzenesulfonate):
100 mg zwitterion dissolved per ml water + 1.2 equiv. benzenesulfonic acid added. On standing at room temeprature, a precipitate formed. This was filtered through a sintered glass funnel, rinsed with a small volume of isopropanol, and dried under vacuum to constant weight.

Cyclic Compound Intermediate 4 (*p*-toluenesulfonate):
100 mg zwitterion dissolved per ml water + 1.2 molar equiv. toluenesulfonic acid added. On standing at room temperature, a precipitate formed. This was filtered through a sintered glass funnel and dried under vacuum to constant weight.

Cyclic Compound Intermediate 4b

cyclo-(D-Val-D-NMeArg-Gly-Asp-Mamb); J = D-Val, K = D-NMeArg, L = Gly, M = Asp, R^1 = H, R^2 = H

The title compound was prepared using the general procedure described for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb) (Cyclic Compound Intermediate 4). The DCC/DMAP

method was used for attachment of Boc-Mamb to the oxime resin.. The peptide was prepared on a 0.596 mmol scale to give the protected cyclic peptide (186 mg, 38.6%). The peptide (183 mg) and 0.183 mL of anisole were treated with anhydrous hydrogen fluoride at 0°C for 30 minutes. The crude material was precipitated with ether, redissolved in aqueous acetonitrile, and lyophilized to generate the title compound (145 mg, greater than quantitative yield; calculated as the fluoride salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/ min. gradient of 9 to 22.5% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (14.8% recovery, overall yield 5.3%); FAB-MS: [M+H] = 575.31.

Cyclic Compound Intermediate 5

cyclo-(D-Leu-NMeArg-Gly-Asp-Mamb); the compound of formula (II) wherein J = D-Leu, K = NMeArg, L = Gly, M = Asp, R^1 = R^2 = H

The title compound was prepared using the general procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb). The peptide was prepared on a 0.115 mmol scale to give the protected cyclic peptide (92.4 mg, 98%). The peptide (92.4 mg) and 93 mL of *m*-cresol were treated with anhydrous hydrogen fluoride at 0°C for 20 minutes. The crude material was precipitated with ether, redissolved in aqueous HOAc, and lyophilized to generate the title compound as a pale yellow solid (45.7 mg, 63%; calculated as the acetate salt). Purification was accomplished by reversed-phase HPLC on a preparative

Vydac C18 column (2.5 cm) using a 0.23%/ min. gradient of 7 to 21% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (29% recovery, overall yield 16.5%);FAB-MS: [M+H] = = 589.48.

Cyclic Compound Intermediate 7

cyclo-(D-Nle-NMeArg-Gly-Asp-Mamb); the compound of formula (II) wherein J = D-Nle, K = NMeArg, L = Gly, M = Asp, R^1 = H, R^2 = H

The title compound was prepared using the general procedure described for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb) (Cyclic Compound Intermediate 4). The DCC/DMAP method was used for attachment of Boc-Mamb to the oxime resin. The peptide was prepared on a 0.586 mmol scale to give the protected cyclic peptide (305 mg, 63.3%). The peptide (295 mg) and 0.295 mL of anisole were treated with anhydrous hydrogen fluoride at 0°C for 30 minutes. The crude material was precipitated with ether, redissolved in aqueous acetonitrile, and lyophilized to generate the title compound (207 mg, 95.4%; calculated as the fluoride salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/ min. gradient of 5.4 to 18% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (44% recovery, overall yield 22.9%); FAB-MS: [M+H] = 589.26.

Cyclic Compound Intermediate 11

cyclo-(D-Phg-NMeArg-Gly-Asp-Mamb); the compound of formula (II) wherein J = D-Phg, K = NMeArg, L = Gly, M = Asp, R^1 = H, R^2 = H

The title compound was prepared using the general procedure described for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb) (Cyclic Compound Intermediate 4). The DCC/DMAP method was used for attachment of Boc-Mamb to the oxime resin. The peptide was prepared on a 0.611 mmol scale to give the protected cyclic peptide (296 mg, 57.4%). The peptide (286 mg) and 0.286 mL of anisole were treated with anhydrous hydrogen fluoride at 0°C for 30 minutes. The crude material was precipitated with ether, redissolved in aqueous acetonitrile, and lyophilized to generate the title compound (210 mg, 98.9%; calculated as the fluoride salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/ min. gradient of 5.4 to 18% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (24.2% recovery, overall yield 11.9%); FAB-MS: [M+H] = 609.27.

Cyclic Compound Intermediate 12

cyclo-(D-Phe-NMeArg-Gly-Asp-Mamb); the compound of formula (II) wherein J = D-Phe, K = NMeArg, L = Gly, M = Asp, R^1 = H, R^2 = H

The title compound was prepared using the general procedure described for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb) (Cyclic Compound Intermediate 4). The DCC/DMAP method was used for attachment of Boc-Mamb to the oxime resin. The peptide was prepared on a 0.611 mmol scale to give the protected cyclic peptide (140 mg, 26.7%).

The peptide (135 mg) and 0.135 mL of anisole were treated with anhydrous hydrogen fluoride at 0°C for 30 minutes. The crude material was precipitated with ether, redissolved in aqueous acetonitrile, and lyophilized to generate the title compound (108 mg, greater than quantitative yield; calculated as the fluoride salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/ min. gradient of 7.2 to 22.5% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (35% recovery, overall yield 8.7%); FAB-MS: [M+H] = 623.28.

Solid Phase Synthesis of Cyclic Compound Intermediate 13f

cyclo-(D-Lys-NMeArg-Gly-Asp-Mamb); the compound of formula (II) wherein J = D-Lys, K = NMeArg, L = Gly, M = Asp, $R^1 = R^2 = H$

The title compound was prepared using the general procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb). The DCC/DMAP method was used for attachment of Boc-Mamb to the oxime resin. The peptide was prepared on a 0.586 mmol scale to give the protected cyclic peptide (349 mg, 58.9%). The peptide (334 mg) and 334 mL of anisole were treated with anhydrous hydrogen fluoride at 0°C for 30 minutes. The crude material was precipitated with ether, redissolved in aqueous acetonitrile, and lyophilized to generate the title compound as a pale yellow solid (168 mg, 79.1%; calculated as the difluoride salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/ min. gradient

of 5.4 to 14.4% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (33.6% recovery, overall yield 12.1%); FAB-MS: [M+H] = 604.32

Solution Phase Synthesis of Cyclic Compound Intermediate 13f

A Scheme depicting the synthesis described below appears immediately after the description.

Cyclo-(D-Lys-NMeArg-Gly-Asp-Mamb); the compound of formula (yy) wherein

Part A - Boc-Asp(OBzl)

To a solution of Boc-Asp(OBzl) (45.80 g, 140 mmol) and HOSu (N-hydroxysuccinimide; 16.10 g, 140 mmol) in 300 ml p-dioxane at 5-10°C was added DCC (30.20 g, 140 mmol). The solution was stirred for 30 minutes at 5-10°C then the solids were filtered and washed with dioxane (3 X 50 ml). The combined organics were concentrated under reduced pressure to give a clear oil which crystallized to a colorless solid (42.98 g, 73%) when triturated with ethyl ether (3 x 100 ml). NMR is consistent with structure; MP = 98-99°C; DCI-MS: [M+NH_4] = 438.

Part B - Boc-Asp(OBzl)-Mamb

3-Aminomethylbenzoic acid·HCl (Mamb; 13.08 g, 70.0 mmol) was dissolved in 120 ml DMF and DIEA (24.32 ml, 140 mmol) was added, changing the pH from 4 to 7.5. The white suspension was stirred for 30 min at room temperature before a solution of Boc-Asp(OBzl)-OSu (29.40 g, 70.0 mmol) in DMF (50 ml) was added. The

mixture was allowed to stir 24 hr, during which time it turned to a gold solution. The solution was added to 5% citric acid (2000 ml) and cooled to 5°C for 3 hr. The solids were then collected by filtration, washed with ice cold water (200 ml) and ice cold ethyl ether (100 ml), and dried under reduced pressure to give the title compound as a colorless solid (29.62 g, 92%); MP = 149-151°C; DCI-MS: $[M+NH_4]$ = 474.

Part C - HCl·H-Asp(OBzl)-Mamb

Boc-Asp(OBzl)-Mamb (7.92 g, 17.4 mmol) was dissolved in 4N HCl in dioxane (50 ml), stirred for 2 hr, and the solution concentrated under reduced pressure to give the title compound as a colorless solid (6.80 g, 99%). DCI-MS: $[M+NH_4]$ = 374.

Part D - Boc-D-Lys(Tfa)-NMeArg(Tos)-Gly-OBzl

NMeArg(Tos)-Gly-OBzl (14.40 g, 29.4 mmol), Boc-D-Lys(Tfa) (10.00 g, 29.4 mmol), and HBTU (11.37 g, 62.0 mmol) were dissolved in methylene chloride (40 ml). After cooling to 0°C, DIEA (10.44 g, 62.0 mmol) was added and the reaction was allowed to proceed 20 minutes at 0°C and 2 days at room temperature. The reaction mixture was diluted with ethyl acetate (800 ml), extracted with 200 ml portions of 0.2 N HCl (1X), sat. $NaHCO_3$ (1X), and saturated NaCl (2X), dried (MgSO4), and evaporated under reduced pressure to a yellow solid. Purification by flash chromatography (silica gel; 5:1 EtOAc:acetonitrile) gave the title compound as a colorless solid (20.34 g, 85%). MP 78-85°C; DCI-MS: $[M+NH_4]$ = 831.

Part E - Boc-D-Lys(Tfa)-NMeArg(Tos)-Gly

A solution of Boc-D-Lys(Tfa)-NMeArg(Tos)-Gly-OBzl (11.00 g, 13.5 mmol) in methanol (200 ml), was placed in a Parr shaker bottle, purged with N_2 for 10 minutes, and treated with 10% palladium on carbon catalyst (10% Pd/C, 3.6 g). The shaker bottle was further purged with 7 pressurization-evacuation cycles, repressurized, and allowed to shake 90 minutes, during which time the calculated amount of hydrogen was consumed. The catalyst was removed by filtration through a bed of Celite and the filtrate was concentrated under reduced pressure yielding a solid. Trituration with refluxing ethyl ether (75 ml) gave pure product (9.18 g, 94%) as a colorless solid. DCI-MS: [M+H] = 724.

Part F - Boc-D-Lys(Tfa)-NMeArg(Tos)-Gly-OSu

Boc-D-Lys(Tfa)-NMeArg(Tos)-Gly (8.00 g, 11.0 mmol), HOSu (1.25 g, 10.8 mmol) and DCC (2.22 g, 10.8 mmol) were dissolved in DMF (75 ml) and stirred at room temperature for 2 days. The solids were removed by filtration and washed with DMF (2 x 15 ml). The filtrate was concentrated under reduced pressure and the resulting syrup dried under reduced pressure at 40°C to give a tan solid (6.50 g, 72%). MP = 66-69°C; FAB-MS: [M+H] = 821.

Part G - Boc-D-Lys(Tfa)-N-MeArg(Tos)-Gly-Asp(OBzl)-Mamb

A suspension of Boc-D-Lys(Tfa)-N-MeArg(Tos)-Gly-OSu (8.85 g, 10.8 mmol) and HCl•Asp(OBzl)-Mamb (4,24 g, 10.8 mmol) in 4:1 dioxane:DMF (100 ml) was treated with DIEA (1.39 g, 10.8 mmol) over 10 minutes. The resulting mixture was stirred 2 days at room temperature and concentrated under reduced pressure to a syrup. This syrup was dissolved in ethyl acetate (300 ml) and washed with 75 ml portions of 0.2N HCl (3X), sat. $NaHCO_3$ (2X),

H_2O (1X), and saturated NaCl (1X). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure at 40°C to a sticky amber solid (9.13 g, 78%). MP = 90-93°C; FAB-MS: [M+H] = 1062.

Part H - HCl•D-Lys(Tfa)-N-MeArg(Tos)-Gly-Asp(OBzl)-Mamb

Boc-D-Lys(Tfa)-N-MeArg(Tos)-Gly-Asp(OBzl)-Mamb (8.30 g, 7.8 mmol) was partially dissolved in 4N HCl in dioxane (50 ml), stirred at room temperature for 30 min, and concentrated under reduced pressure to give a yellow solid. Trituration with warm EtOAc (60 ml) afforded the product (7.65 g, 98%) as a yellow solid. FAB-MS: [M+H] = 962.

Part I - Cyclo-(D-Lys(Tfa)-N-MeArg(Tos)-Gly-Asp(OBzl)-Mamb)

HCl•D-Lys(Tfa)-N-MeArg(Tos)-Gly-Asp(OBzl)-Mamb (3.00 g, 3.0 mmol), DIEA (0.77 g, 6.0 mmol), and TBTU (0.98 g, 3.0 mmol) were dissolved in DMF (100 ml). The reaction was stirred at room temperature for 22 hours, and the pH was maintained at 7-8 by the addition of DIEA as necessary. The reaction was concentrated under reduced pressure and the resulting oil dissolved in 3.75:1 ethyl acetate:1-butanol (110 ml). The organic solution was washed with 50 ml portions of 0.2 N HCl (2X), saturated $NaHCO_3$ (1X), H_2O (1X), and saturated NaCl (1X), dried ($MgSO_4$), concentrated to a brown oil. Triturated with ethyl ether (100 ml) gave a brown solid which was purified by flash chromatography (silica gel; 5:1 EtOAc:EtOH) to give the title compound (1.62 g, 57%) as a colorless solid. MP = 128-130°C; FAB-MS: [M+H] = 944.

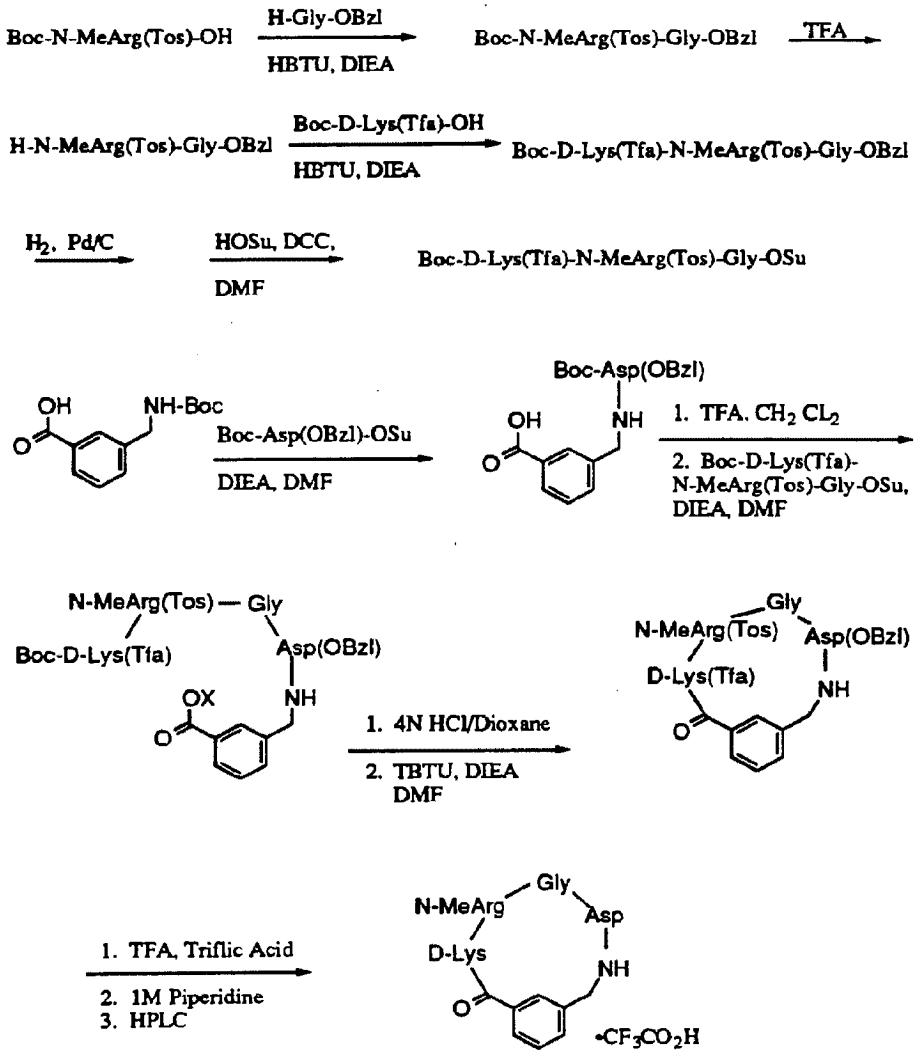
Part J - Cyclo-(D-Lys(Tfa)-N-MeArg-Gly-Asp-Mamb)

Cyclo-(D-Lys(Tfa)-N-MeArg(Tos)-Gly-Asp(OBzl)-Mamb) (0.85 g, 0.9 mmol) was dissolved in TFA (10 ml) and cooled to -10°C. Triflic acid (trifluoromethanesulfonic acid; 10 ml) was slowly added to the stirred reaction while maintaining the temperature at -5°C. Anisole (2 ml) was added and stirring was continued for 3 hours at -5°C. The temperature of the reaction was decreased to -78°C, ethyl ether (200 ml) was added, and the reaction was stirred for 1 hour. The white sticky solids were removed by filtration and washed with ice cold ether (50 ml). The solids were dissolved in 1:1 acetone:H_2O (10 ml) and lyophilized to give the product (0.63 g, 100%) as a fluffy colorless solid. FAB-MS: [M+H] = 700.

Part K - Cyclo-(D-Lys-N-MeArg-Gly-Asp-Mamb)

Cyclo-(D-Lys(Tfa)-N-MeArg-Gly-Asp-Mamb) (0.63 g, 0.9 mmol) was dissolved in 1.0 M aqueous piperdine (10 ml) at 0°C and the reaction was allowed to slowly warm to room temperature over 3 hours. The solution was lyophilized to give a yellow solid. Purification was accomplished by preparative HPLC with a Vydac protein-peptide C-18 column (2.1 cm) using a 0.36%/min. gradient of 9 to 18% acetonitrile containing 0.1% TFA, and then lyophilized to give the title compound (0.20 g, 90%) as a colorless fluffy solid. MP = 138-142°C; FAB-MS: [M+H] = 604.

Solution Phase Synthesis of 13f



Cyclic Compound Intermediate 13r

cyclo-(D-Ile-NMeArg-Gly-Asp-Mamb); the compound of formula (II) wherein J = D-Ile, K = NMeArg, L = Gly, M = Asp, R^1 = H, R^2 = H

The title compound was prepared using the general procedure described for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb) (Cyclic Compound Intermediate 4). The DCC/DMAP method was used for attachment of Boc-Mamb to the oxime resin. The peptide was prepared on a 0.611 mmol scale to give the protected cyclic peptide (349 mg, 69.2%). The peptide (342 mg) and 0.342 mL of anisole were treated with anhydrous hydrogen fluoride at 0°C for 30 minutes. The crude material was precipitated with ether, redissolved in aqueous acetonitrile, and lyophilized to generate the title compound (227 mg, 90%; calculated as the fluoride salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/ min. gradient of 10.8 to 19.8% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (22.5% recovery, overall yield 12.1%); FAB-MS: [M+H] = 589.34.

Cyclic Compound Intermediate 17

cyclo-(D-Met-NMeArg-Gly-Asp-Mamb); the compound of formula (II) wherein J = D-Met, K = NMeArg, L = Gly, M = Asp, R^1 = H, R^2 = H

The title compound was prepared using the general procedure described for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb) (Cyclic Compound Intermediate 4). The DCC/DMAP method was used for the attachment of Boc-Mamb to the resin. The peptide was prepared on a 0.179 mmol scale to give the protected cyclic peptide (105 mg, 69.7%). The peptide (105 mg) and 0.105 mL of anisole were treated with anhydrous hydrogen fluoride at 0°C for 20 minutes. The crude material was precipitated with ether, redissolved in aqueous acetonitrile, and

lyophilized to generate the title compound (72 mg; 92.3% yield; calculated as the fluoride salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/ min. gradient of 14.4 to 23.4% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (13.2% recovery, overall yield 7.4%); FAB-MS: [M+H] = 607.3.

Cyclic Compound Intermediate 18

cyclo-(NMeGly-NMeArg-Gly-Asp-Mamb); the compound of formula (II) wherein J = NMeGly, K = NMeArg, L = Gly, M = Asp, $R^1 = R^2 = H$

The title compound was prepared using the general procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb). The DCC/DMAP method was used for attachment of Boc-Mamb to the oxime resin. The peptide was prepared on a 0.43 mmol scale to give the protected cyclic peptide (205 mg, 60%). The peptide (200 mg) and 200 mL of *m*-cresol were treated with anhydrous hydrogen fluoride at 0°C for 30 minutes. The crude material was precipitated with ether, redissolved in aqueous HOAc, and lyophilized to generate (18) as a pale yellow solid (148 mg, 97%; calculated as the acetate salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/ min. gradient of 7 to 22% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of (18) as a fluffy white solid (14.7% recovery, overall yield 7.9%); FAB-MS: [M+H] = 547.34.

Cyclic Compound Intermediate 24

cyclo-(Pro-NMeArg-Gly-Asp-Mamb); the compound of formula (II) wherein J = Pro, K = NMeArg, L = Gly, M = Asp, $R^1 = R^2 = H$

The title compound was prepared using the general procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb). The DCC/DMAP method was used for attachment of Boc-Mamb to the oxime resin. The peptide was prepared on a 0.43 mmol scale to give the protected cyclic peptide (170 mg, 48.8%). The peptide (164 mg) and 164 mL of *m*-cresol were treated with anhydrous hydrogen fluoride at 0°C for 30 minutes. The crude material was precipitated with ether, redissolved in aqueous HOAc, and lyophilized to generate (24) as a pale yellow solid (101 mg, 79% ; calculated as the acetate salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/ min. gradient of 7 to 22% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of (24) as a fluffy white solid (5.8% recovery, overall yield 2.1%);FAB-MS: [M+H] = 573.46.

Cyclic Compound Intermediate 25

cyclo-(D-Pro-NMeArg-Gly-Asp-Mamb); the compound of formula (II) wherein J = D-Pro, K = NMeArg, L = Gly, M = Asp, $R^1 = R^2 = H$

The title compound was prepared using the general procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb). The DCC/DMAP method was used for attachment of Boc-Mamb to the oxime resin. The peptide was prepared on a 0.43 mmol scale to give the protected cyclic peptide (211mg, 60.8%). The peptide (200 mg) and 200 mL of *m*-cresol were treated with anhydrous hydrogen

fluoride at 0°C for 30 minutes. The crude material was precipitated with ether, redissolved in aqueous HOAc, and lyophilized to generate (25) as a pale yellow solid (145 mg, 93.3%; calculated as the acetate salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/ min. gradient of 7 to 22% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of (25) as a fluffy white solid (6.4% recovery, overall yield 3.3%); FAB-MS: [M+H] = = 573.35.

Cyclic Compound Intermediate 28c

cyclo-(b-Ala-NMeArg-Gly-Asp-Mamb); the compound of formula (II) wherein J = b-Ala, K = NMeArg, L = Gly, M = Asp, $R^1 = R^2 = H$

The title compound was prepared using the general procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb). The DCC/DMAP method was used for attachment of Boc-Mamb to the oxime resin. The peptide was prepared on a 0.586 mmol scale to give the protected cyclic peptide (264 mg, 57.5%). The peptide (258 mg) and 258 mL of anisole were treated with anhydrous hydrogen fluoride at 0°C for 30 minutes. The crude material was precipitated with ether, redissolved in aqueous acetonitrile, and lyophilized to generate the title compound as a pale yellow solid (231 mg, greater than quantitative yield; calculated as the fluoride salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/ min. gradient of 5.4 to 14.4% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid

(53.2% recovery, overall yield 32.5%); FAB-MS: [M+H] = 547.28;

Cyclic Compound Intermediate 28f

cyclo-(D-Tyr-NMeArg-Gly-Asp-Mamb); the compound of formula (II) wherein J = D-Tyr, K = NMeArg, L = Gly, M = Asp, R^1 = H, R^2 = H

The title compound was prepared using the general procedure described for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb) (Cyclic Compound Intermediate 4). The DCC/DMAP method was used for attachment of Boc-Mamb to the oxime resin. The peptide was prepared on a 0.313 mmol scale to give the protected cyclic peptide (342 mg, greater than quantitative yield). The peptide (331 mg) and 0.330 mL of anisole were treated with anhydrous hydrogen fluoride at 0°C for 30 minutes. The crude material was precipitated with ether, redissolved in aqueous acetonitrile, and lyophilized to generate the title compound (218 mg, greater than quantitative yield; calculated as the fluoride salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/ min. gradient of 9 to 18% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (11.3% recovery, overall yield 10.8%); FAB-MS: [M+H] = 639.54.

Cyclic Compound Intermediate 29

cyclo-(Gly-Arg-Gly-Asp-Mamb); the compound of formula (II) wherein J = Gly, K = Arg, L = Gly, M = Asp, R^1 = R^2 = H

The title compound was prepared using the general procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb). The peptide was prepared on a 0.283 mmol scale and half was cyclized to give the protected cyclic peptide (62 mg, 58%). The peptide (60 mg) and 60 mL of *m*-cresol were treated with anhydrous hydrogen fluoride at 0°C for 1 hour. The crude material was precipitated with ether, redissolved in aqueous HOAc, and lyophilized to generate the title compound as a pale yellow solid (48 mg, > quantitative yield; calculated as the acetate salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.30%/ min. gradient of 0 to 9% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (36% recovery, overall yield 19.9%); FAB-MS: [M+H] = 519.26.

Cyclic Compound Intermediate 30

cyclo-(D-Ala-Arg-Gly-Asp-Mamb); the compound of formula (II) wherein J = D-Ala, K = Arg, L = Gly, M = Asp, $R^1 = R^2 = H$

The title compound was prepared using the general procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb). The peptide was prepared on a 0.189 mmol scale to give the protected cyclic peptide (211 mg, >quantitative yield). The peptide (195 mg) and 195 mL of *m*-cresol were treated with anhydrous hydrogen fluoride at 0°C for 1 hour. The crude material was precipitated with ether, redissolved in aqueous HOAc, and lyophilized to generate the title compound as a pale yellow solid (125 mg, 83%; calculated as the acetate salt). Purification was accomplished by reversed-phase

HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/ min. gradient of 2 to 11% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (12.5% recovery, overall yield 13.8%); FAB-MS: [M+H] = 533.26.

Cyclic Compound Intermediate 31

cyclo-(Ala-Arg-Gly-Asp-Mamb); the compound of formula (II) wherein J = Ala, K = Arg, L = Gly, M = Asp, $R^1 = R^2 = H$

The title compound was prepared using the general procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb). The peptide was prepared on a 0.324 mmol scale to give the protected cyclic peptide (191 mg, 76.4%). The peptide (100 mg) and 100 mL of *m*-cresol were treated with anhydrous hydrogen fluoride at 0°C for 1 hour. The crude material was precipitated with ether, redissolved in aqueous HOAc, and lyophilized to generate the title compound as a pale yellow solid (75 mg, 97.4%; calculated as the acetate salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/ min. gradient of 2 to 11% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (15.5% recovery, overall yield 10.5%); FAB-MS: [M+H] = 533.25.

Cyclic Compound Intermediate 32

cyclo-(D-Val-Arg-Gly-Asp-Mamb); the compound of formula (II) wherein J = D-Val, K = Arg, L = Gly, M = Asp, R^1 = R^2 = H

The title compound was prepared using the general procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb). The peptide was prepared on a 0.193 mmol scale to give the protected cyclic peptide (199 mg, > quantitative yield). The peptide (193 mg) and 193 mL of *m*-cresol were treated with anhydrous hydrogen fluoride at 0°C for 1 hour. The crude material was precipitated with ether, redissolved in aqueous HOAc, and lyophilized to generate the title compound as a pale yellow solid (130 mg, 86%; calculated as the acetate salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/min. gradient of 2 to 13% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (57% recovery, overall yield 58.1%); FAB-MS: [M+H] = 561.22.

Cyclic Compound Intermediate 33

cyclo-(D-Leu-Arg-Gly-Asp-Mamb); the compound of formula (II) wherein J = D-Leu, K = Arg, L = Gly, M = Asp, R^1 = R^2 = H

The title compound was prepared using the general procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb). The peptide was prepared on a 0.202 mmol scale to give the protected cyclic peptide (152 mg, 93%). The peptide (150 mg) and 150 mL of *m*-cresol were treated with anhydrous hydrogen fluoride at 0°C for 1

hour. The crude material was precipitated with ether, redissolved in aqueous HOAc, and lyophilized to generate the title compound as a pale yellow solid (78 mg, 66%; calculated as the acetate salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/ min. gradient of 5 to 18% acetonitrile containing 0.1% trifluoroacetic acid and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (26% recovery, overall yield 14.8%); FAB-MS: [M+H] = 575.45.

Cyclic Compound Intermediate 34

cyclo-(D-Abu-Arg-Gly-Asp-Mamb); the compound of formula (II) wherein J = D-Abu, K = Arg, L = Gly, M = Asp, R^1 = R^2 = H

The title compound was prepared using the general procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb). The peptide was prepared on a 0.193 mmol scale to give the protected cyclic peptide (210 mg, > quantitative yield). The peptide (206 mg) and 206 mL of *m*-cresol were treated with anhydrous hydrogen fluoride at 0°C for 1 hour. The crude material was precipitated with ether, redissolved in aqueous HOAc, and lyophilized to generate the title compound as a pale yellow solid (158 mg, 99%; calculated as the acetate salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/ min. gradient of 2 to 11% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (57% recovery, overall yield 72.2%); FAB-MS: [M+H] = 547.21.

Cyclic Compound Intermediate 35

cyclo-(D-Ser-Arg-Gly-Asp-Mamb); the compound of formula (II) wherein J = D-Ser, K = Arg, L = Gly, M = Asp, R^1 = R^2 = H

The title compound was prepared using the general procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb). The peptide was prepared on a 0.193 mmol scale to give the protected cyclic peptide (224 mg, > quantitative yield). The peptide (210 mg) and 210 ml of *m*-cresol were treated with anhydrous hydrogen fluoride at 0°C for 1 hour. The crude material was precipitated with ether, redissolved in aqueous HOAc, and lyophilized to generate the title compound as a pale yellow solid (145 mg, 89%; calculated as the acetate salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/min. gradient of 2 to 13% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (22% recovery, overall yield 27%); FAB-MS: [M+H] = 549.31.

Cyclic Compound Intermediate 36

cyclo-(D-Phe-Arg-Gly-Asp-Mamb); the compound of formula (II) wherein J = D-Phe, K = Arg, L = Gly, M = Asp, R^1 = R^2 = H

The title compound was prepared using the general procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb). The peptide was prepared on a 0.266 mmol scale to give the protected cyclic peptide (202 mg, 90%). The peptide (157 mg) and 157 mL of *m*-cresol were treated with anhydrous hydrogen fluoride at 0°C for 1 hour. The crude material was precipitated with ether, redissolved in aqueous HOAc, and lyophilized to generate

the title compound as a pale yellow solid (125 mg, > quantitative yield; calculated as the acetate salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/min. gradient of 7 to 23% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (35% recovery, overall yield 29.3%); FAB-MS: [M+H] = 609.25

Cyclic Compound Intermediate 37

cyclo-(Phe-Arg-Gly-Asp-Mamb); the compound of formula (II) wherein J = Phe, K = Arg, L = Gly, M = Asp, $R^1 = R^2 = H$

The title compound was prepared using the general procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb). The peptide was prepared on a 0.335 mmol scale to give the protected cyclic peptide (306 mg, > quantitative yield). The peptide (275 mg) and 275 mL of *m*-cresol were treated with anhydrous hydrogen fluoride at 0°C for 1 hour. The crude material was precipitated with ether, redissolved in aqueous HOAc, and lyophilized to generate the title compound as a pale yellow solid (214 mg, 98%; calculated as the acetate salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/min. gradient of 9 to 23% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (32% recovery, overall yield 31.5%); FAB-MS: [M+H] = 609.26

Cyclic Compound Intermediate 40

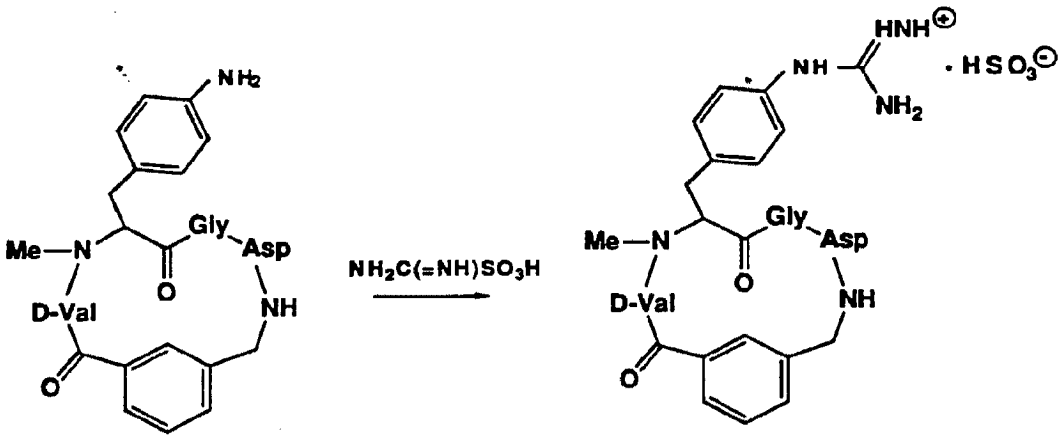
cyclo-(D-Val-NMeAmf-Gly-Asp-Mamb); the compound of formula (II) wherein J = D-Val,

K = NMeAmf, L = Gly, M = Asp, R^1 = R^2 = H

The title compound was prepared using the general procedure described for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb) (Cyclic Compound Intermediate 4). The DCC/DMAP method was used for attachment of Boc-Mamb to the oxime resin. The peptide was prepared on a 0.586 mmol scale to give the protected cyclic peptide (189 mg, 39.9%). The peptide (189 mg) and 0.189 mL of anisole were treated with anhydrous hydrogen fluoride at 0°C for 30 minutes. The crude material was precipitated with ether, redissolved in aqueous acetonitrile, and lyophilized to generate the title compound (212 mg, greater than quantitative yield; calculated as the fluoride salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/ min. gradient of 10.8 to 22.5% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (8.1% recovery, overall yield 4.1%); FAB-MS: [M+H] = 595.23.

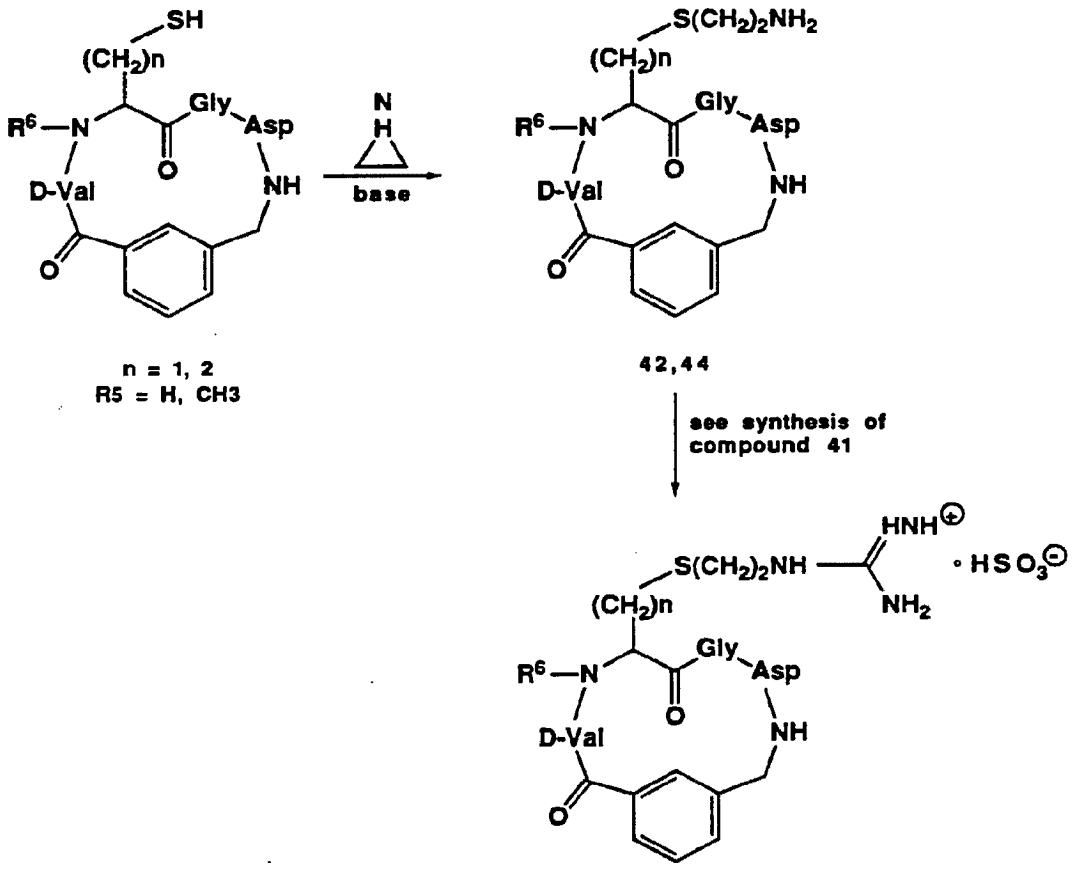
Cyclic Compound Intermediate 48a

The title compound may be synthesized using procedures described in Mosher et al. Tett. Lett. 29: 3183-3186, and as shown schematically below. This same procedure is a generally useful method for converting a primary amine into a guanidine functionality.



Cyclic Compound Intermediates 42-45

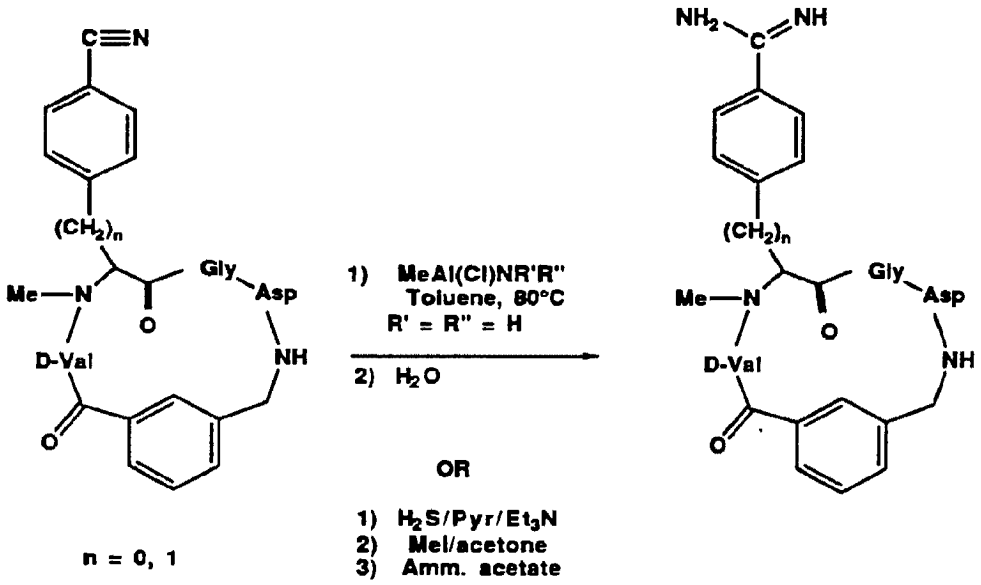
The synthesis of Cyclic Compound Intermediates 42-45 is shown schematically below.



Cyclic Compound Intermediate 46 and 47

Cyclic Compound Intermediates 46 and 47 are prepared according to standard procedures, for example, as described in Garigipati, *Tett. Lett.* (1990) 31: 1969-1972 and in Canadian Patent 2008311, as is shown

schematically below. The aspartic acid group may be protected (e.g., with a phenacyl protection group) to avoid side reactions.



Cyclic Compound Intermediate 54

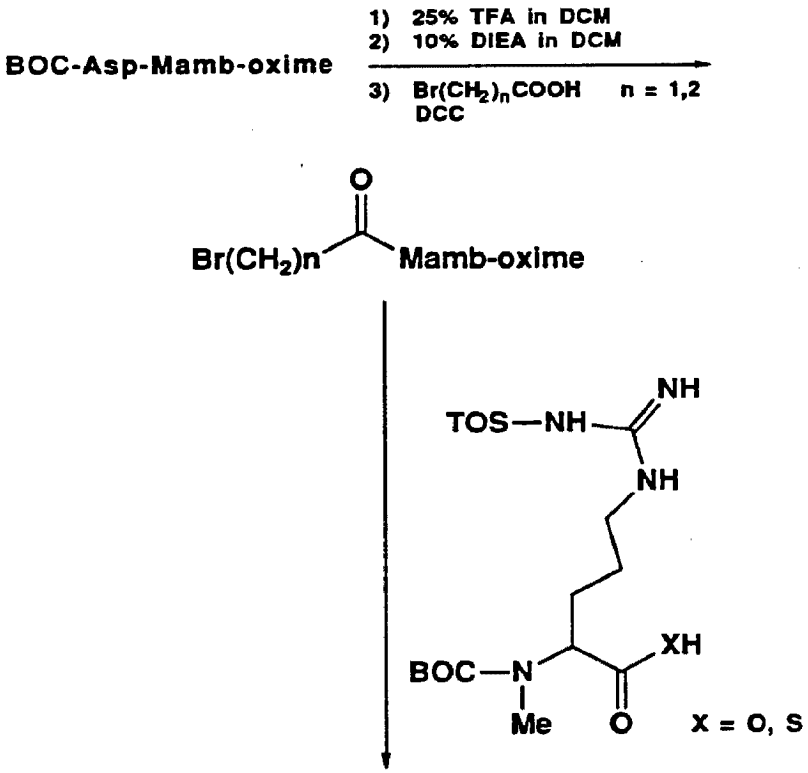
cyclo-(D-Val-NMeArg-b-Ala-Asp-Mamb); J = D-Val, K = NMeArg,
L = b-Ala, M = Asp, R^1 = R^2 = H

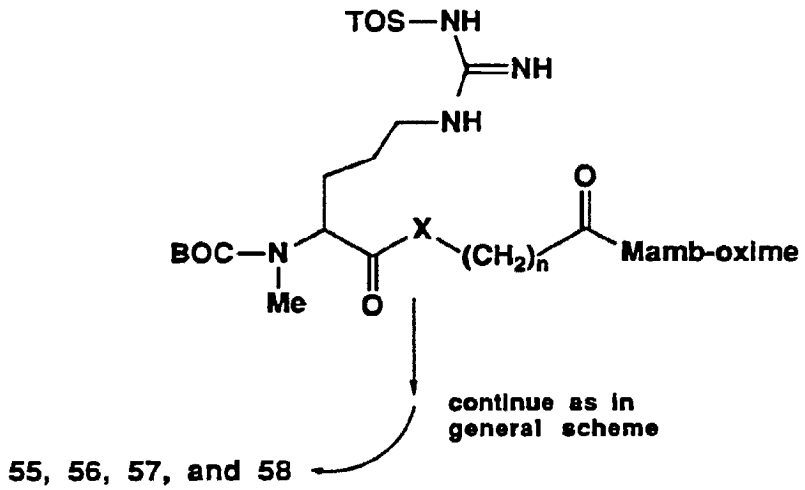
The title compound was prepared using the general procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb). The DCC/DMAP method was used for attachment of Boc-Mamb to the oxime resin. The peptide was prepared on a 0.586 mmol scale to give the protected cyclic peptide (227 mg, 46.9%). The peptide (219 mg) and 219 mL of anisole were treated with anhydrous hydrogen fluoride at 0°C for 30 minutes. The crude material was precipitated with ether, redissolved in aqueous acetonitrile, and lyophilized to generate (54) as a pale yellow solid (150 mg, 93.2%; calculated as the

fluoride salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/ min. gradient of 7.2 to 16.2% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of (54) as a fluffy white solid (43.6% recovery, overall yield 16.5%); FAB-MS: [M+H] = 589.32.

Cyclic Compound Intermediate 55-58

The synthesis of Cyclic Compound Intermediates 55-58 is shown schematically below.





Cyclic Compound Intermediate 58c

cyclo-(D-Val-NMeArg-L-Ala-Asp-Mamb); the compound of formula (II) wherein J = D-Val, K = NMeArg, L = L-Ala, M = Asp, R^1 = H, R^2 = H

The title compound was prepared using the general procedure described for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb) (Cyclic Compound Intermediate 4). The DCC/DMAP method was used for attachment of Boc-Mamb to the oxime resin. The peptide was prepared on a 0.611 mmol scale to give the protected cyclic peptide (375 mg, 74.6%). The peptide (360 mg) and 0.360 mL of anisole were treated with anhydrous hydrogen fluoride at 0°C for 30 minutes. The crude material was precipitated with ether, redissolved in aqueous acetonitrile, and lyophilized to generate the title compound (220 mg, 83%; calculated as the fluoride salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/ min. gradient of 9 to 18% acetonitrile containing 0.1% TFA and then

lyophilized to give the TFA salt of the title compound as a fluffy white solid (19.9% recovery, overall yield 10.6%); FAB-MS: [M+H] = 589.31.

Cyclic Compound Intermediate 63 and 63a

cyclo-(D-Val-NMeArg-Gly-a-MeAsp-Mamb); the compounds of formula (II) wherein J is D-Val; K is NMeArg; L is Gly; M is a-MeAsp; R^1 = R^2 = H

The title compound was prepared using the general procedure described for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb). The DCC/DMAP method was used for attachment of Boc-Mamb to the oxime resin. The peptide was prepared on a 0.794 mmol scale to give the protected cyclic peptide (237 mg, 36.1%). The peptide (237 mg) and 0.237 mL of anisole were treated with anhydrous hydrogen fluoride at 0°C for 30 minutes. The crude material was precipitated with ether, redissolved in aqueous acetonitrile, and lyophilized to generate the title compound (165 mg, 94.3%; calculated as the fluoride salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/ min. gradient of 9 to 18% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid; isomer #1 (8.36% recovery, overall yield 2.5%); FAB-MS: [M+H] = 589.29; isomer #2 (9.16% recovery, overall yield 2.7%); FAB-MS: [M+H] = 589.27.

Cyclic Compound Intermediates 64 and 64a

cyclo-(D-Val-NMeArg-Gly-*B*-MeAsp-Mamb); the compounds of formula (II) wherein J = D-Val, K = NMeArg, L = Gly, M = *B*-MeAsp, R^1 = H, R^2 = H

The title compound was prepared using the general procedure described for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb) (Cyclic Compound Intermediate 4). The DCC/DMAP method was used for attachment of Boc-Mamb to the oxime resin. The peptide was prepared on a 0.611 mmol scale to give the protected cyclic peptide (201 mg, 40.0%). The peptide (200 mg) and 0.200 mL of anisole were treated with anhydrous hydrogen fluoride at 0°C for 30 minutes. The crude material was precipitated with ether, redissolved in aqueous acetonitrile, and lyophilized to generate the title compound (162 mg, greater than quantitative yield; calculated as the fluoride salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/ min. gradient of 9 to 18% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid; isomer #1 (12.7% recovery, overall yield 4.8%); FAB-MS: [M+H] = 589.43; isomer #2 (13.9% recovery, overall yield 5.3%); FAB-MS: [M+H] = 589.45.

Cyclic Compound Intermediate 64b

cyclo-(D-Val-NMeArg-Gly-NMeAsp-Mamb); the compound of formula (II) wherein J = D-Val, K = NMeArg, L = Gly, M = NMeAsp, R^1 = H, R^2 = H

The title compound was prepared using the general procedure described for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb) (Cyclic Compound Intermediate 4). The DCC/DMAP method was used for attachment of Boc-Mamb to the oxime resin. The peptide was prepared on a 0.611 mmol scale to give the

protected cyclic peptide (232 mg, 46.1%). The peptide (225 mg) and 0.225 mL of anisole were treated with anhydrous hydrogen fluoride at 0°C for 30 minutes. The crude material was precipitated with ether, redissolved in aqueous acetonitrile, and lyophilized to generate the title compound (160 mg, 96.4%; calculated as the fluoride salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/ min. gradient of 9 to 18% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (28.2% recovery, overall yield 10.9%); FAB-MS: [M+H] = 589.42.

Cyclic Compound Intermediate 64c

cyclo-(D-Val-NMeArg-Gly-D-Asp-Mamb); the compound of formula (II) wherein J = D-Val, K = NMeArg, L = Gly, M = D-Asp, R^1 = H, R^2 = H

The title compound was prepared using the general procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb). The DCC/DMAP method was used for attachment of Boc-Mamb to the oxime resin. The peptide was prepared on a 0.611 mmol scale to give the protected cyclic peptide (257 mg, 51.9%). The peptide (250 mg) and 0.250 mL of anisole were treated with anhydrous hydrogen fluoride at 0°C for 30 minutes. The crude material was precipitated with ether, redissolved in aqueous acetonitrile, and lyophilized to generate the title compound (192 mg, greater than quantitative yield; calculated as the fluoride salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18

column (2.5 cm) using a 0.23%/ min. gradient of 9 to 18% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (44.4% recovery, overall yield 20.7%); FAB-MS: [M+H] = 575.42.

Cyclic Compound Intermediate 89e

cyclo-(D-Abu-di-NMeOrn-Gly-Asp-Mamb); the compound of formula (II) wherein J = D-Abu, K = di-NMeOrn, L = Gly, M = Asp, $R^1 = R^2 = H$

The title compound was prepared using the general procedure described for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb) (Cyclic Compound Intermediate 4). The DCC/DMAP method was used for attachment of Boc-Mamb to the oxime resin. The peptide was prepared on a 0.498 mmol scale to give the protected cyclic peptide (150 mg, 39.3%). The peptide (150 mg) and 0.150 mL of anisole were treated with anhydrous hydrogen fluoride at 0°C for 30 minutes. The crude material was precipitated with ether, redissolved in aqueous acetonitrile, and lyophilized to generate the title compound (93 mg, 86%; calculated as the fluoride salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.45%/ min. gradient of 3.6 to 18% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title

compound as a fluffy white solid (49.3% recovery, overall yield 14.2%); FAB-MS: [M+H] = 533.34.

Cyclic Compound Intermediate 89f

cyclo-(D-Abu-NMeArg-Gly-D-Asp-Mamb); compound of formula (II) wherein J = D-Abu, K = NMeArg, L = Gly, M = D-Asp, R^1 = H, R^2 = H

The title compound was prepared using the general procedure described for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb) (Cyclic Compound Intermediate 4). The DCC/DMAP method was used for attachment of Boc-Mamb to the oxime resin. TBTU was used as the coupling reagent. The peptide was prepared on a 0.596 mmol scale to give the protected cyclic peptide (273 mg, 57.6%). The peptide (263 mg) and 0.263 mL of anisole were treated with anhydrous hydrogen fluoride at 0°C for 20 minutes. The crude material was precipitated with ether, redissolved in aqueous acetonitrile, and lyophilized to generate the title compound (218 mg; greater than quantitative yield; calculated as the fluoride salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/ min. gradient of 10.8 to 19.8% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (40.4% recovery, overall yield 21.9%); FAB-MS: [M+H] = 561.37.

Cyclic Compound Intermediate 89g

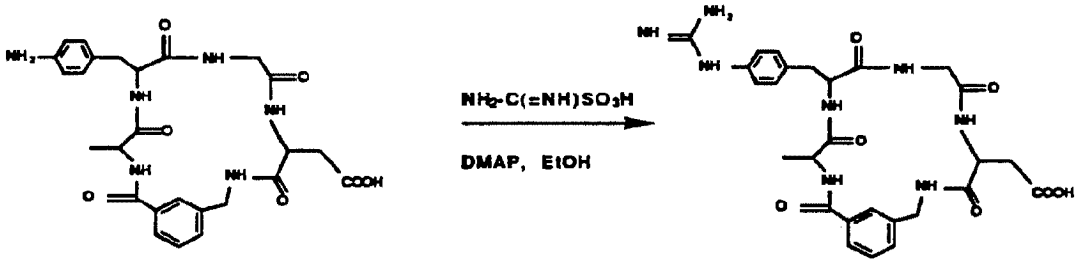
cyclo-(D-Abu-D-NMeArg-Gly-Asp-Mamb); the compound of formula (II) J = D-Abu, K = D-NMeArg, L = Gly, M = Asp, R^1 = H, R^2 = H

The title compound was prepared using the general procedure described for cyclo-(D-Val-NMeArg-Gly-Asp-

Mamb) (Cyclic Compound Intermediate 4). The DCC/DMAP method was used for attachment of Boc-Mamb to the oxime resin. TBTU was used as the coupling reagent. The peptide was prepared on a 0.596 mmol scale to give the protected cyclic peptide (241 mg, 50.8%). The peptide (235 mg) and 0.235 mL of anisole were treated with anhydrous hydrogen fluoride at 0°C for 20 minutes. The crude material was precipitated with ether, redissolved in aqueous acetonitrile, and lyophilized to generate the title compound (168 mg; 98.3%; calculated as the fluoride salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/ min. gradient of 12.6 to 21.6% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (2.3% recovery, overall yield 0.99%); FAB-MS: [M+H] = 561.36.

Cyclic Compound Intermediate 89h

Cyclo-(D-Ala-*p*-guanidinyl-Phe-Gly-Asp-Mamb); the compound of formula (II) wherein J = D-Ala, K = p-guanidinyl-Phe, L = Gly, M = Asp R^1 = H, R^2 = H

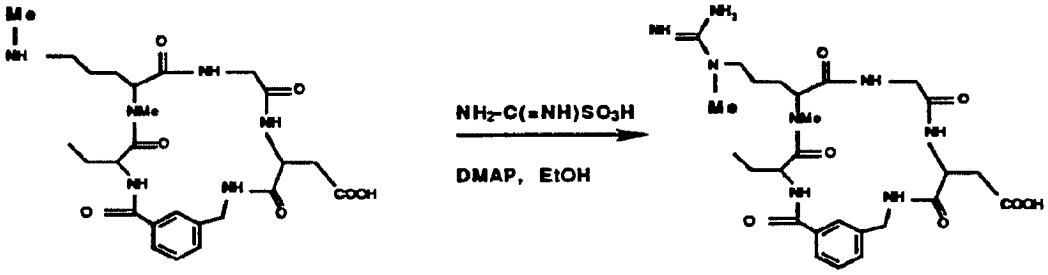


Dissolved 25 mg (38.3 mmoles) of cyclo-(D-Ala-*p*-amino-Phe-Gly-Asp-Mamb) (TFA salt), 14.3 mg (114.9 umoles) formamidine sulfonic acid, and 18.7 mg (153.2 umoles) of 4-dimethyl-aminopyridine in 5 ml of ethanol

in a 10 ml round bottom flask. Refluxed the mixture for 3 hours, then added an additional 14.3 mg of formamidine sulfonic acid and 18.7 mg of 4-dimethyl-aminopyridine. After refluxing for an additional 3 hours, the reaction was found to be ~75% complete by reversed-phase HPLC. The ethanol was evaporated under reduced pressure, and the residue was purified on a preparative Vydac C18 column (2.5 cm) using a 0.45%/min. gradient of 0 to 18% acetonitrile containing 0.1% TFA. Lyophilization afforded the TFA salt of the title compound as a white solid (28% recovery), overall yield 26.4%); FAB-MS: [M+H] = 581.30.

Cyclic Compound Intermediate 89i

cyclo-(D-Abu-(DiNMe,guanidinyl-Orn)-Gly-Asp-Mamb); the compound of formula (II) wherein J = D-Abu, K = diNMe,guanidinyl-Orn , L = Gly, D = Asp, R^1 = H, R^2 = H



Dissolved 10.53 mg (16.3 mmoles) of cyclo-(D-Abu-diNMeOrn-Gly-Asp-Mamb) (TFA salt), 6.08 mg (48.99 umoles) formamidine sulfonic acid, and 8.00 mg (65.57 umoles) of 4-dimethyl-aminopyridine in 2.5 ml of ethanol in a 10 ml round bottom flask. Refluxed the mixture for 2 hours and then stirred at room temperature overnight. Refluxed for one hour, added an additional 6.08 mg of formamidine sulfonic acid and 8.00 mg of 4-

dimethylaminopyridine and then refluxed for an additional 2 hours. Evaporated the ethanol under reduced pressure and purified the residue on a preparative Vydac C18 column (2.5 cm) using a 0.45%/min. gradient of 3.6 to 18% acetonitrile containing 0.1% TFA. Lyophilization afforded the TFA salt of the title compound as a white solid (57.2% recovery), overall yield 53.5%); FAB-MS: [M+H] = 575.34.

Cyclic Compound Intermediates 89j

cyclo-(D-Abu-Di-NMeLys-Gly-Asp-Mamb); the compound of formula (II) wherein J = D-Abu, K = Di-NMeLys, L = Gly, M = Asp, R^1 = H, R^2 = H

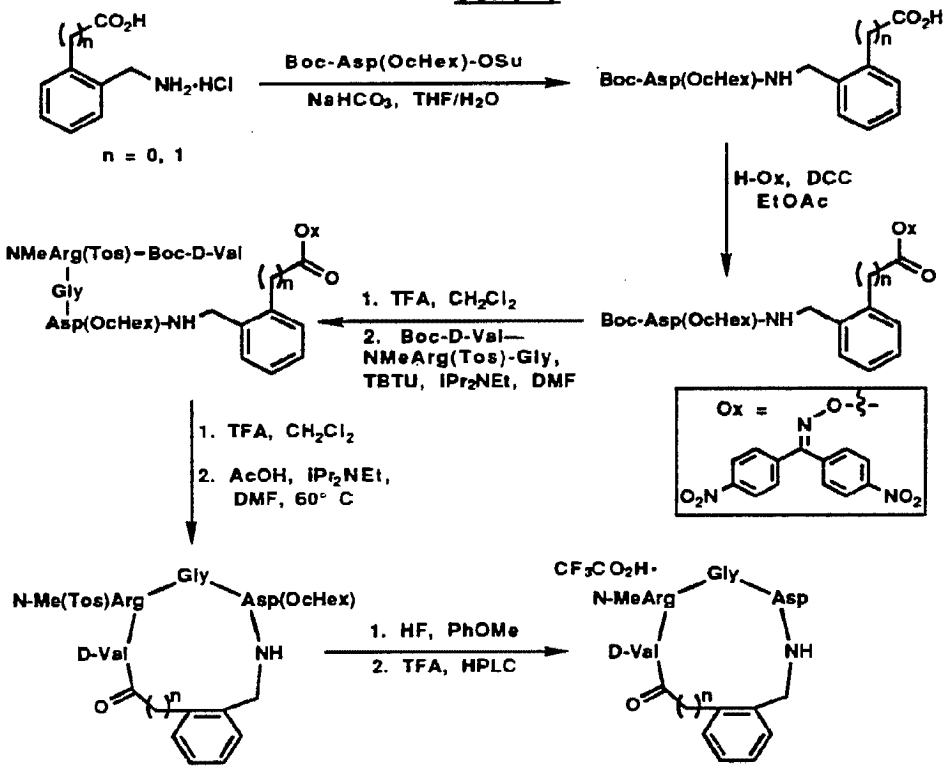
cyclo-(D-Abu-NMeLys-Gly-Asp-Mamb); the compound of formula (II) wherein J = D-Abu, K = NMeLys, L = Gly, M = Asp, R^1 = H, R^2 = H

Di-N-methyl amino acid derivatives may be prepared using methods which have been described previously (Olsen, *J. Org. Chem.* (1970) 35: 1912) or, alternatively, through the use of NaH/CH_3I. The mono-NMe-Lysine amino acid was obtained as a side product during the synthesis of the corresponding di-NMe-lysine derivative. The title compounds were prepared using conventional solution phase peptide chemistry techniques described previously. Cyclo-(D-Abu-diNMeLys-Gly-Asp-Mamb) was obtained in 0.31% overall yield, FAB-MS: [M+H] = 547.3. Cyclo-(D-Abu-NMeLys-Gly-Asp-Mamb) was obtained in 0.25% overall yield, FAB-MS: [M+H] = 533.3.

Cyclic Compound Intermediate 90
cyclo-(D-Val-NMeArg-Gly-Asp-2-aminomethylphenylacetic acid)

The title compound was prepared by a modification of the general solution-phase chemistry route. This approach employed an amino acid succinimide ester coupling to the aromatic cyclizing moiety, and the dinitrobenzophenone oxime as shown schematically below in the Scheme below (n = 1).

Scheme



Boc-Asp(OcHex)-2-aminomethylphenylacetic Acid

To a suspension of 2-aminomethylphenylacetic acid•HCl (4.0 g, 20 mmol) in H_2O (20 ml) was added

$NaHCO_3$ (5.0 g, 60 mmol), followed by a solution of Boc-Asp(OcHex)-OSu (7.5 g, 18 mmol) in THF (20 ml). The reaction mixture was stirred at room temperature for 3 hours, filtered, diluted with H_2O, acidified with 1N HCl, and extracted with ethyl acetate. The extracts were washed with H_2O, brine, dried over anhydrous magnesium sulfate, and evaporated to dryness under reduced pressure. This material was triturated with ether to provide the title compound (7.0 g, 83%) as a white powder. ^1H NMR (D_6-DMSO) 12.40 (br s, 1H), 8.30 (br t, 1H), 7.20 (m, 5H), 4.65 (m, 1H), 4.35 (q, 1H), 4.25 (m, 2H), 3.65 (s, 2H), 2.70 (dd, 1H), 2.55 (dd, 1H), 1.70 (m, 4H), 1.40 (s, 9H), 1.35 (m, 6H).

4,4'-Dinitrobenzophenone Oxime

The title compound was prepared by modification of procedures previously reported in the literature (Chapman and Fidler (1936) *J. Chem. Soc*, 448; Kulin and Leffek (1973) *Can. J. Chem.*, 51: 687). A solution of chromic anhydride (20 g, 200 mmol) in 125 ml of H_2O was added dropwise over 4 hours, to a suspension of bis(4-nitrophenyl)methane (25 g, 97 mmol) in 300 ml of acetic acid heated to reflux. The reaction mixture was heated at reflux for 1 hour, cooled to room temperature, and poured into water. The solid was collected by filtration, washed with H_2O, 5% sodium bicarbonate, H_2O, and air-dryed to provide a 1:1 mixture of bis(4-nitrophenyl)methane/4,4'-dinitrobenzophenone via ^1H NMR. This material was oxidized with a second portion of chromic anhydride (20 g, 200 mmol), followed by an identical work-up procedure to provide the crude product. Trituration with 200 ml of benzene heated to reflux for 16 hours provided 4,4'-dinitrobenzophenone (20.8 g, 79%) as a yellow powder.

A solution of hydroxylamine hydrochloride (10.2 g, 147 mmol) was added to a suspension of 4,4'-dinitrobenzophenone (19 g, 70 mmol) in 100 ml of ethanol. The reaction mixture was heated to reflux for 2 hours, cooled to room temperature, and the solid collected by filtration. Recrystallization from ethanol provided the title compound (14.0 g, 70%) as pale yellow crystals. mp 194°C; ^{1}H NMR (D_6-DMSO) 12.25 (s, 1H), 8.35 (d, 2H), 8.20 (d, 2H), 7.60 (d, 4H).

4,4'-Dinitrobenzophenone Oxime Boc-Asp(OcHex)-2-aminomethylphenylacetate

To an ice-cooled solution of Boc-Asp(OcHex)-2-aminomethylphenylacetic acid (3.5 g, 7.6 mmol) and 4,4'-dinitrobenzophenone oxime (2.2 g, 7.5 mmol) in 50 ml of ethyl acetate and 5 ml of DMF was added DCC (1.6 g, 7.8 mmol). The reaction mixture was stirred at room temperature for 8 hours, filtered, diluted with ethyl acetate, washed with saturated sodium bicarbonate solution, H_2O, brine, dried over anhydrous magnesium sulfate, and evaporated to dryness under reduced pressure. This material was purified by column chromatography on silica gel (EM Science, 230-400 mesh) using 10:1 dichloromethane/ethyl acetate to give the title compound (4.3 g, 78%) as pale yellow crystals. ^{1}H NMR (D_6-DMSO) 8.30 (dd, 5H), 7.80 (d, 2H), 7.65 (d, 2H), 7.15 (m, 5H), 4.65 (m, 1H), 4.35 (q, 1H), 4.15 (m, 2H), 3.90 (s, 2H), 2.70 (dd, 1H), 2.50 (dd, 1H), 1.70 (m, 4H), 1.40 (s, 9H), 1.35 (m, 6H).

4,4'-Dinitrobenzophenone Oxime Boc-D-Val-NMeArg(Tos)-Gly-Asp(OcHex)-2-aminomethylphenylacetate

To a solution of 4,4'-dinitrobenzophenone oxime Boc-Asp(OcHex)-2-aminomethylphenylacetate (1.5 g, 2 mmol) in 4 ml of dichloromethane was added 2 ml of trifluoroacetic acid. The reaction mixture was stirred at room temperature for 1 hour, diluted with dichloromethane, and evaporated to dryness under reduced pressure. The oily residue was concentrated under high vacuum to remove traces of excess trifluoroacetic acid.

To a sclution of the crude TFA salt and Boc-D-Val-NMeArg(Tos)-Gly (1.2 g, 2 mmol) in 5 ml of DMF was added TBTU (640 mg, 2 mmol) and DIEA (780 mg, 6 mmol). The reaction mixture was stirred at room temperature for 16 hours, concentrated under high vacuum, diluted with ethyl acetate, washed with 5% citric acid, H_2O, brine, dried over anhydrous magnesium sulfate, and evaporated to dryness under reduced pressure. This material was triturated with ether to provide the title compound (2.3 g, 95%) as a yellow powder. This material was used without further purification.

<u>cyclo-(D-Val-NMeArg(Tos)-Gly-Asp(OcHex)-2-aminomethylphenylacetic acid)</u>

To a solution of 4,4'-dinitrobenzophenone oxime Boc-D-Val-NMeArg(Tos)-Gly-Asp(OcHex)-2-aminomethylphenylacetate (1.2 g, 1 mmol) in 4 ml of dichloromethane was added 2 ml of trifluoroacetic acid. The reaction mixture was stirred at room temperature for 3 hours, diluted with dichloromethane, and evaporated to dryness under reduced pressure. The oily residue was concentrated under high vacuum to remove traces of excess trifluoroacetic acid.

To a solution of the crude TFA salt in 100 ml of DMF was added acetic acid (0.50 ml, 8.7 mmol) and DIEA (1.52 ml, 8.7 mmol). The reaction mixture was stirred at 60°C for 3 days, concentrated under high vacuum, diluted with ethyl acetate, and the solution allowed to crystallize overnight. Filtration provided the title compound (563 mg, 68%) as a yellow powder. ^{1}H NMR (D_6-DMSO) 8.70 (d, 1H), 8.40 (br s, 1H), 8.30 (br s, 1H), 8.05 (t, 1H), 7.65 (d, 2H), 7.25 (d, 2H), 7.20 (m, 4H), 7.10 (br d, 1H), 6.80 (br s, 1H), 6.60 (br s, 1H), 5.10 (dd, 1H), 4.65 (m, 1H), 4.55 (m, 1H), 4.40 (m, 2H), 3.85 (m, 2H), 3.65 (d, 1H), 3.45 (m, 2H), 3.05 (m, 2H), 2.80 (s, 3H), 2.80 (m, 1H), 2.60 (dd, 1H), 2.30 (s, 3H), 1.70 (m, 6H), 1.30 (m, 9H), 0.95 (d, 3H), 0.80 (d, 3H); DCI(NH_3)-MS: [M+H] = 825.

<u>cyclo-(D-Val-NMeArg-Gly-Asp-2-aminomethylphenylacetic acid)</u>

A mixture of 352 mg (0.43 mmol) of cyclo-(D-Val-NMeArg(Tos)-Gly-Asp(OcHex)-2-aminomethylphenylacetic acid) and 352 µl of anisole was treated at 0°C with 5 ml of HF for 20 minutes. The excess HF was removed under reduced pressure, the residue triturated with ether, dissolved in 50% acetonitrile/H_2O, and lyophilized to provide the crude cyclic peptide•HF salt as an off-white powder. Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.8% / minute gradient of 10 to 38% acetonitrile containing 0.1% trifluoroacetic acid to give the TFA salt of the title compound (225 mg, 75%) as a fluffy white solid; ^{1}H NMR (D_6-DMSO) 8.70 (d, 1H), 8.35 (d, 1H), 8.20 (t, 1H), 8.00 (t, 1H), 7.45 (t, 1H), 7.20 (m, 3H), 7.10 (m, 1H), 7.00 (br s, 4H), 5.10 (dd, 1H), 4.50 (dt, 1H), 4.40 (m, 2H), 3.85 (dt, 2H), 3.65 (d, 1H),

3.50 (dd, 1H), 3.45 (d, 1H), 3.10 (m, 2H), 2.90 (s, 3H), 2.75 (dd, 1H), 2.55 (dd, 1H), 2.00 (m, 1H), 1.85 (m, 1H), 1.65 (m, 1H), 1.30 (m, 2H), 0.95 (d, 3H), 0.85 (d, 3H); FAB-MS: [M+H] = 589.

Cyclic Compound Intermediate 91

cyclo-(D-Val-NMeArg-Gly-Asp-2-aminomethylbenzoic acid)

The title compound was prepared by the general solution-phase procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-2-aminomethylphenylacetic acid), and as shown schematically above in the Cyclic Compound Intermediate 90 Scheme (n = 0). The cyclic peptide (192 mg, 0.24 mmol) was deprotected with excess HF in the presence of anisole as scavenger. Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.8% / minute gradient of 10 to 38% acetonitrile containing 0.1% trifluoroacetic acid to give the TFA salt of the title compound (20 mg, 12%) as a fluffy white solid; ^{1}H NMR (D_6-DMSO) 8.75 (d, 1H), 8.50 (d, 1H), 7.65 (t, 1H), 7.60 (t, 1H), 7.50 (m, 2H), 7.40 (m, 3H), 7.00 (br s, 4H), 5.05 (dd, 1H), 4.50 (t, 1H), 4.30 (m, 2H), 4.10 (dd, 1H), 3.70 (m, 2H), 3.15 (q, 2H), 3.05 (s, 3H), 2.80 (dd, 1H), 2.55 (dd, 1H), 2.10 (m, 1H), 1.95 (m, 1H), 1.60 (m, 1H), 1.40 (m, 2H), 1.05 (d, 3H), 0.95 (d, 3H); FAB-MS: [M+H] = 575.

Cyclic Compound Intermediate 92

cyclo-(D-Val-NMeArg-Gly-Asp-3-aminophenylacetic acid)

The title compound was prepared by the general solution-phase procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb), and as shown schematically in

the Scheme below. The cyclic peptide (360 mg, 0.44 mmol) was deprotected with excess HF in the presence of anisole as scavenger. Purification was accomplished by reversed-phase HPLC on a preparative LiChrospher RP-18 column (5 cm) using a 2.3% / minute gradient of 22 to 90% acetonitrile containing 0.1% trifluoroacetic acid to give the TFA salt of the title compound (150 mg, 50%) as a fluffy white solid; ^{1}H NMR (D_6-DMSO) 12.40 (br s, 1H), 8.95 (s, 1H), 8.55 (m, 2H), 8.45 (t, 1H), 7.90 (d, 1H), 7.50 (m, 1H), 7.20 (t, 1H), 7.00 (br s, 4H), 6.90 (m, 2H), 5.15 (dd, 1H), 4.65 (q, 1H), 4.55 (t, 1H), 3.65 (m, 2H), 3.60 (dd, 1H), 3.10 (m, 2H), 2.85 (s, 3H), 2.85 (d, 1H), 2.70 (dd, 2H), 2.00 (m, 2H), 1.75 (m, 1H), 1.35 (m, 2H), 0.90 (d, 3H), 0.85 (d, 3H); FAB-MS: [M+H] = 575.

Cyclic Compound Intermediate 87, 88

cyclo-(D-Val-NMeArg-Gly-Asp-4-aminomethylbenzoic acid); the compound of formula (III) wherein J = D-Val, K = NMeArg, L = Gly, M = Asp, R^1 = H, R^2 = H

The title compound was prepared using the general procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb). The DCC/DMAP method was used for attachment of Boc-4-aminomethylbenzoic acid to the oxime resin. The peptide was prepared on a 0.43 mmol scale to give the protected cyclic peptide (212mg, 60.8%). The peptide (200 mg) and 200 mL of *m*-cresol were treated with anhydrous hydrogen fluoride at 0°C for 30 minutes. The crude material was precipitated with ether, redissolved in aqueous HOAc, and lyophilized to generate the crude peptide as a pale yellow solid (152 mg, 97% ; calculated as the acetate salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/ min. gradient

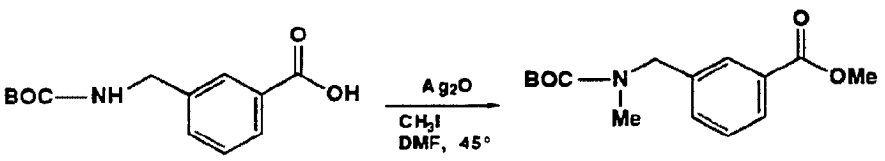
of 7 to 22% acetonitrile containing 0.1% TFA. Two peaks were isolated to give isomer #1 (87) (17.1% recovery, overall yield 9.3%) and isomer #2 (88) (13.4% recovery, overall yield 7.3%); FAB-MS: [M+H] = 575.41 (isomer #1; 87); 575.44 (isomer #2; 88).

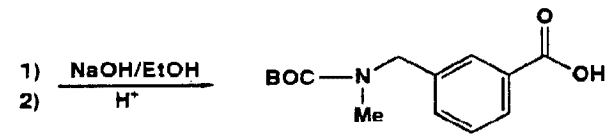
R^1 or R^2 Substituted Intermediates

Cyclic compound intermediates which incorporate substituents at R^1 or R^2 are synthesized from the corresponding substituted cyclizing moieties. The following Schemes, discussions, and examples teach the preparation of this class of cyclizing moiety and the corresponding cyclic compound intermediates.

t-Butyloxycarbonyl-N-methyl-3-aminomethylbenzoic Acid (Boc-NMeMamb)

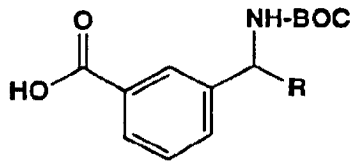
The title compound can be prepared according to standard procedures, for examples, as disclosed in Olsen, *J. Org. Chem.* (1970) 35: 1912), and as shown schematically below.





Synthesis of Aminomethylbenzoic Acid Analogs

Cyclizing moieties of the formula below may be prepared using standard synthetic procedures, for example, as shown in the indicated reaction schemes shown below.

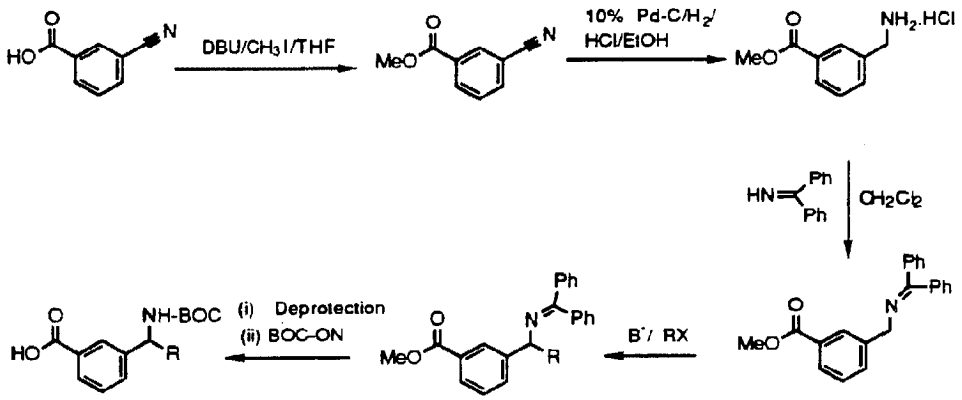


For R = CH_3, CH_2CH_3, $CH_2CH_2CH_3$, $CH_2CH_2CH_2CH_3$, $CH(CH_3)_2$, $C(CH_3)_3$, $CH(CH_3)CH_2CH_3$, benzyl, cyclopentyl, cyclohexyl; see Scheme 1.

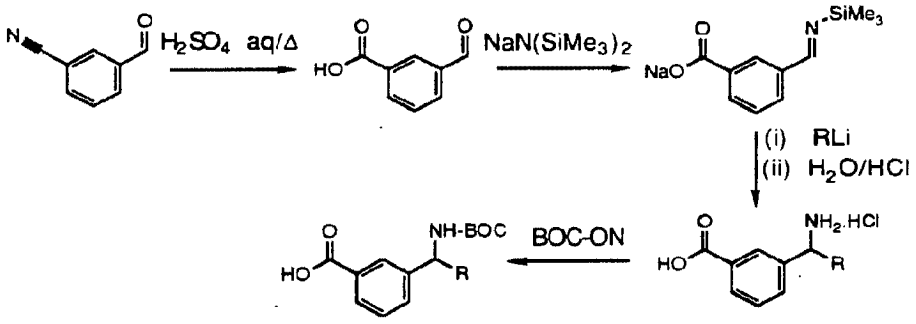
For R = CH_3, $CH_2CH_2CH_2CH_3$, phenyl; see Scheme 2.

For R = CH_3, phenyl; see Scheme 3 and 4.

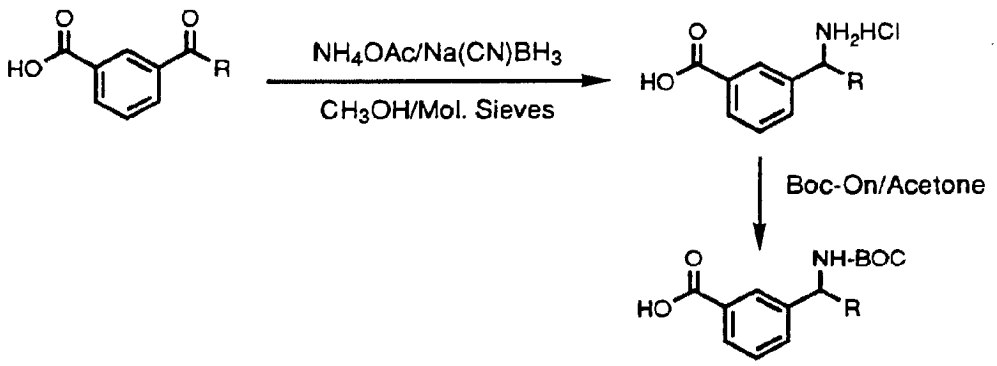
Scheme 1:



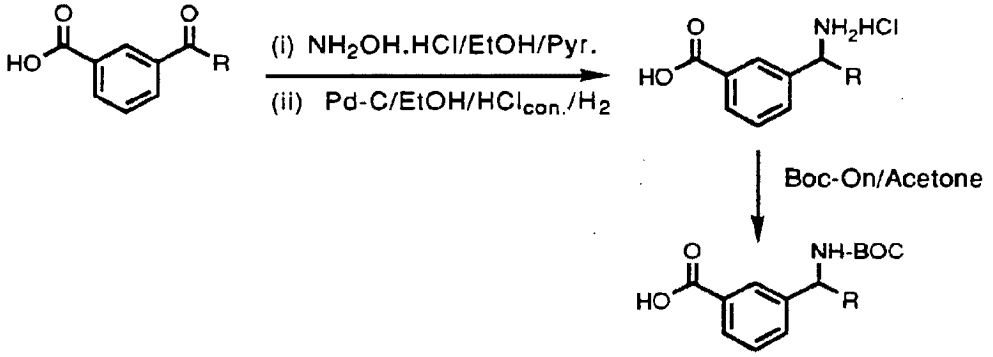
Scheme 2:



Scheme 3:



Scheme 4:



3-[1'-(t-butyloxycarbonyl)aminolethylbenzoic acid (BOC-MeMAMB)

The title compound for the purpose of this invention was prepared according to the Scheme 4 (above).

3-Acetylbenzoic acid (0.50 g, 3 mmol), hydroxylamine hydrochloride (0.70 g, 10 mmol) and pyridine (0.70 ml, 9 mmol) were refluxed in 10 ml ethanol, for 2 h. Reaction mixture was concentrated, residue triturated with water, filtered and dried. Oxime

was isolated as a white solid (0.51 g ; 94.4% yield). ^{1}HNMR (CD_3OD) 7.45-8.30(m, 4H), 2.30(s, 3H). MS (CH_4-CI) [M+H-O] = 164.

A solution of the oxime (0.51 g, 3 mmol) in ethanol, containing 10% Pd on carbon (1.5 g) and conc. HCl (0.25 ml, 3 mmol) was hydrogenated at 30 psi H_2 pressure in a Parr hydrogenator for 5 h. Catalyst was filtered and the filtrate concentrated. Residue was triturated with ether. Amine hydrochloride was isolated as a white solid (0.48 g ; 85.7% yield). ^{1}HNMR (CD_3OD) 7.6-8.15(m, 4H), 4.55(q, 1H), 1.70(s, 3H). MS [M+H] = 166.

Amine hydrochloride (0.40 g, 2 mmol) was dissolved in 15 ml water. A solution of BOC-ON (0.52 g, 2.1 mmol) in 15 ml acetone was added, followed by the addition of triethylamine (0.8 ml, 6 mmol). Reaction was allowed to proceed for 20 h. Reaction mixture was concentrated, partitioned between ethyl acetate and water. Aqueous layer was acidified to pH 2 using 10% HCl solution. Product was extracted in ethyl acetate, which after the usual work up and recrystallization from ethyl acetate/hexane, gave the title compound as a white solid (0.30 g ; 57% yield). m.p. 116-118° C. ^{1}HNMR ($CDCl_3$) 7.35-8.2(m, 4H), 4.6(bs, 1.5H), 1.50(d, 3H), 1.40(s, 9H). MS (NH_3-CI) [M+NH_4] = 283.

3-[1'-(t-butyloxycarbonyl)aminolbenzylbenzoic acid (BOC-PhMAMB)

The title compound for the purpose of this invention was prepared according to the Scheme 4 (above), by the procedure similar to that for the methyl derivative.

A solution of 3-benzoylbenzoic acid (2.00 g, 9 mmol), hydroxylamine hydrochloride (2.00 g, 29 mmol) and pyridine (2.00 ml, 25 mmol) in ethanol was refluxed for 12 h. After the usual extractive work up, white solid was obtained (2.41 g). The product still contained traces of pyridine, but was used in the next step without further purification.

The crude product (2.00 g, ~8 mmol) was dissolved in 200 ml ethanol. 10% Pd-C (2.00 g) and con. HCl (1.3 ml, 16 mmol) were added. Reaction mixture was hydrogenated at 30 psi for 1 h. The catalyst was filtered and the reaction mixture concentrated. Upon trituration of the residue with ether and drying under vacuum, amine hydrochloride was obtained as a white solid (2.12 g ; 97% yield). ^{1}HNMR (CD_3OD) 7.4-8.15(m, 10H), 5.75(s, 1H). MS (CH_4-CI) [M+H-OH] = 211.

Amine hydrochloride (1.00 g, 4 mmol) was converted to its BOC-derivative by a procedure similar to the methyl case. 0.60 g (48% yield) of the recrystallized (from ethanol/hexane) title compound was obtained as a white solid. m.p. 190-192° C. ^{1}HNMR (CD_3OD) 7.2-8.0(m, 10H), 5.90 (2s, 1H, 2 isomers), 1.40(s, 9H). MS (NH_3-CI) [M+NH_4-C_4H_8] = 289

Cyclic Compound Intermediates 68 and 68a

cyclo-(D-Val-NMeArg-Gly-Asp-MeMamb); the compound of formula (II) wherein J = D-Val, K = NMeArg, L = Gly, M = Asp, R^1 = CH_3, R^2 = H

MeMAMB cyclizing moiety was prepared via Scheme 4 (described earlier). The title compound was made by following the solution phase synthetic route to attach MeMAMB to the tripeptide. Cyclization gave the protected cyclic peptide. Deprotection was achieved by treatment

of the peptide (390 mg) and anisol (0.390 ml) with anhydrous HF at 0°C for 30 minutes. The crude material was precipitated with ether, redissolved in 10% aqueous acetic acid, and lyophilized to give a mixture of the two isomers (330 mg; greater than quantitative yield; calculated as the acetate salt). Purification and the separation of the isomers was accomplished by Reverse-Phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.48%/min gradient of 7 to 23% acetonitrile containing 0.1% TFA. Fractions collected at Rf 24.1 min and 26.8 min were lyophilized to give the TFA salts of the isomers 1 and 2 respectively. FAB-MS (Isomer 1): [M+H] = 589.31; FAB-MS (isomer 2): [M+H] = 589.31.

Cyclic Compound Intermediates 76 and 76a

cyclo-(D-Val-NMeArg-Gly-Asp-PhMamb); the compound of formula (II) wherein J = D-Val, K = NMeArg, L = Gly, M = Asp, R^1 = Ph, R^2 = H

PhMAMB cyclizing moiety was prepared via Scheme 4 (described earlier). The title compound was made by following the solution phase synthetic route to attach PhMAMB to the tripeptide. Cyclization gave the protected cyclic peptide. Deprotection was achieved by treatment of the peptide (470 mg) and anisol (0.470 ml) with anhydrous HF at 0°C for 30 minutes. The crude material was precipitated with ether, redissolved in 10% aqueous acetic acid, and lyophilized to give a mixture of the two isomers (310 mg; 82.4% overall recovery). Purification and the separation of the isomers was accomplished by Reverse-Phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.55%/min gradient of 18 to 36% acetonitrile containing 0.1% TFA. Fractions collected at Rf 22 min and 24.6 min were lyophilized to

give the TFA salts of the isomers 1 and 2 respectively. FAB-MS (Isomer 1): [M+H] = 651.33; FAB-MS (isomer 2): [M+H] = 651.33.

Cyclic Compound Intermediate 79

cyclo-(D-Val-NMeArg-Gly-Asp-NMeMamb); the compound of formula (II) wherein J = D-Val, K = NMeArg, L = Gly, M = Asp, R^1 = H, R^2 = CH_3

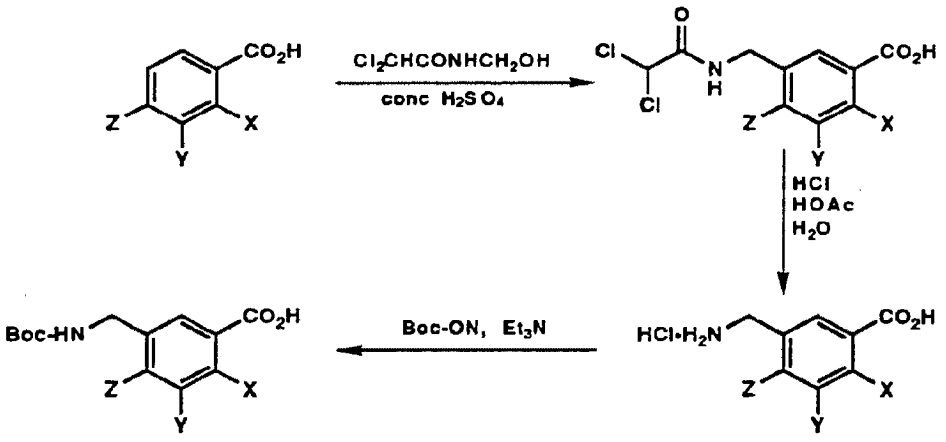
The title compound was prepared using the general procedure described for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb) (Cyclic Compound Intermediate 4). The DCC/DMAP method was used for attachment of Boc-NMeMamb to the oxime resin. The peptide was prepared on a 0.456 mmol scale to give the protected cyclic peptide (406 mg, greater than quantitative yield). The peptide (364 mg) and 0.364 mL of anisole were treated with anhydrous hydrogen fluoride at 0°C for 30 minutes. The crude material was precipitated with ether, redissolved in aqueous acetonitrile, and lyophilized to generate the title compound (251 mg, 93.5%; calculated as the fluoride salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/ min. gradient of 9 to 18% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (34.2% recovery, overall yield 29.9%); FAB-MS: [M+H] = 589.33.

Ring-Substituted R^{31} Cyclizing Moieties

Cyclizing moieties possessing an aromatic ring that bears a substituent group may be prepared using the methods taught in the following examples and Schemes.

Synthesis of 4, 5, and 6-Substituted 3-Aminomethylbenzoic Acid•HCl, and 4, 5, and 6-Substituted t-Butyloxycarbonyl-3-aminomethylbenzoic Acid Derivatives

4, 5, and 6-Substituted 3-aminomethylbenzoic acid•HCl, and 4, 5, and 6-substituted t-butyloxycarbonyl-3-aminomethylbenzoic acid derivatives useful as intermediates in the synthesis of the compounds of the invention are prepared using standard procedures, for example, as described in Felder et al *Helv. Chim. Acta*, 48: 259 (1965); de Diesbach *Helv. Chim. Acta*, 23: 1232 (1949); Truitt and Creagn *J. Org. Chem.*, 27: 1066 (1962); or Sekiya et al *Chem. Pharm. Bull.*, 11: 551 (1963), and as shown schematically below.



Synthesis of 4-Chloro-3-aminomethylbenzoic Acid•HCl

The title compound was prepared by modification of procedures previously reported in the literature (Felder

et al (1965) *Helv. Chim. Acta*, 48: 259). To a solution of 4-chlorobenzoic acid (15.7 g, 100 mmol) in 150 ml of concentrated sulfuric acid was added N-hydroxymethyl dichloroacetamide (23.7 g, 150 mmol) in portions. The reaction mixture was stirred at room temperature for 2 days, poured onto 375 g of ice, stirred for 1 hour, the solid was collected by filtration, and washed with H_2O. The moist solid was dissolved in 5% sodium bicarbonate solution, filtered, and acidified to pH 1 with concentrated HCl. The solid was collected by filtration, washed with H_2O, and air-dryed overnight to give 4-chloro-3-dichloroacetylaminomethylbenzoic acid (26.2 g, 89%) as a white powder.

A suspension of 4-chloro-3-dichloroacetylaminomethylbenzoic acid (26.2 g, 88 mmol) in 45 ml of acetic acid, 150 ml of concentrated HCl, and 150 ml of H_2O was heated to reflux for 3 hours, filtered while hot, and allowed to cool to room temperature. The solid was collected by filtration, washed with ether, washed with acetone-ether, and air-dryed overnight to give the title compound (7.6 g, 39%) as off-white crystals. mp 278-9°C; ^1H NMR (D_6-DMSO) 13.40 (br s, 1H), 8.75 (br s, 3H), 8.20 (s, 1H), 7.95 (dd, 1H), 7.70 (d, 1H), 4.20 (br s, 2H).

t-Butyloxycarbonyl-4-chloro-3-aminomethylbenzoic Acid

A suspension of 4-chloro-3-aminomethylbenzoic acid•HCl (6.7 g, 30 mmol) and triethylamine (9.3 g, 92 mmol) in 50 ml of H_2O, was added to a solution of Boc-ON (9.2 g, 38 mmol) in 50 ml of tetrahydrofuran cooled to 0°C. The reaction mixture was stirred at room temperature overnight, and the volatile compounds were

removed by concentration under reduced pressure. The residue was diluted with H_2O, washed with ether, acidified to pH 3 with 1N HCl, and extracted with ethyl acetate. The extracts were washed with H_2O, brine, dried over anhydrous magnesium sulfate, and evaporated to dryness under reduced pressure. This material was triturated with ether-hexane to provide the title compound (7.4 g, 87%) as a white powder. mp 159°C (dec); ^1H NMR (D_6-DMSO) 13.20 (br s, 1H), 7.90 (s, 1H), 7.80 (dd, 1H), 7.60 (br s, 1H), 7.55 (d, 1H), 4.20 (br d, 2H), 1.40 (s, 9H).

Synthesis of 3-Aminomethyl-6-iodobenzoic Acid•HCl

The title compound was prepared by modification of procedures previously reported in the literature (Felder et al. (1965) Helv. Chim. Acta, 48: 259). To a solution of 6-iodobenzoic acid (24.8 g, 100 mmol) in 150 ml of concentrated sulfuric acid was added N-hydroxymethyl dichloroacetamide (23.7 g, 150 mmol) in portions. The reaction mixture was stirred at room temperature for 7 days, poured onto 375 g of ice, and stirred for 1 hour. The solid was then collected by filtration, and washed with H_2O. The moist solid was dissolved in 5% sodium bicarbonate solution, filtered, and acidified to pH 1 with concentrated HCl. The solid was collected by filtration, washed with H_2O, and air-dried overnight to give 3-dichloroacetyl-aminomethyl-6-iodobenzoic acid (32.0 g, 82%) as a white powder.

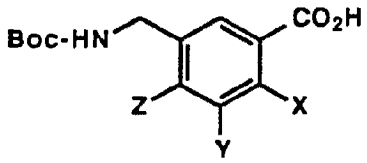
A suspension of 3-dichloroacetylaminomethyl-6-iodobenzoic acid (32.0 g, 82 mmol) in 51 ml of acetic acid, 170 ml of concentrated HCl, and 125 ml of$H_2O$was heated to reflux for 3 hours, and filtered while hot, and allowed to cool to room temperature. The solid was collected by filtration, washed with ether, washed with

acetone-ether, and air-dried overnight to give the title compound (13.2 g, 51%) as a beige powder; 1H NMR (D6-DMSO) 13.50 (br s, 1H), 8.50 (br s, 3H), 8.05 (d, 1H), 7.85 (s, 1H), 7.40 (d, 1H), 4.05 (br s, 2H).

t-Butyloxycarbonyl-3-Aminomethyl-6-Iodobenzoic Acid

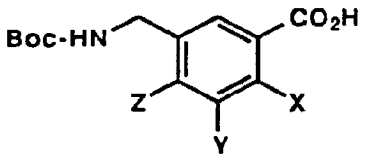
A suspension of 3-aminomethyl-6-iodobenzoic acid•HCl (8.0 g, 26 mmol) and triethylamine (8.7 g, 86 mmol) in 32 ml of H_2O, was added to a solution of Boc-ON (8.0 g, 32 mmol) in 23 ml of tetrahydrofuran cooled to 0°C. The reaction mixture was stirred at room temperature for overnight, and the volatile compounds were removed by concentration under reduced pressure. The residue was diluted with H2O, washed with ether, acidified to pH 3 with 1N HCl, and extracted with ethyl acetate. The extracts were washed with H2O, brine, dried over anhydrous magnesium sulfate, and evaporated to dryness under reduced pressure. This material was triturated from ether to provide the title compound (5.7 g, 59%) as a white powder; mp 182°C (dec); 1H NMR (D6-DMSO) 13.35 (br s, 1H), 7.95 (d, 1H), 7.60 (s, 1H), 7.50 (br t, 1H), 7.10 (d, 1H), 4.10 (d, 2H), 1.40 (s, 9H).

Other examples of ring-substituted R^{31} cyclizing moieties prepared using the general procedure described above for t-butyloxycarbonyl-3-aminomethyl-6-iodobenzoic acid are tabulated below.



X	Y	Z	mp.°C
H	H	Cl	159
H	H	I	168
H	H	Me	155
H	H	MeO	171
Cl	H	H	150
I	H	H	182
Me	H	H	166
MeO	H	H	79

4-Bromo and 6-Bromo derivatives useful as intermediates in the synthesis of the compounds of the invention may be prepared as described above for t-butyloxycarbonyl-3-aminomethyl-6-iodobenzoic acid. 4-Hydroxy and 6-Hydroxy derivatives useful as intermediates in the synthesis of the compounds of the invention may be prepared as described in Sekiya et al *Chem. Pharm. Bull.*, 11: 551 (1963). 5-Nitro and 5-Amino derivatives useful as intermediates in the synthesis of the compounds of the invention may be prepared as described in Felder et al *Helv. Chim. Acta*, 48: 259 (1965). The 5-amino derivative may be converted to the 5-iodo, 5-bromo, 5-chloro, or 5-fluoro derivatives via the diazonium salt as described in *Org. Syn. Coll. Vol.*, 2: 130 (1943); 2: 299 (1943); 2: 351 (1943); and 3: 185 (1955).



X	Y	Z
H	H	Br
Br	H	H
H	H	HO
HO	H	H
H	NO_2	H
H	NH_2	H
H	I	H
H	Br	H
H	Cl	H
H	F	H

Synthesis of Cyclic Compound Intermediates Using Ring Substituted R^{31} Cyclizing Moieties.

Cyclic compound intermediates in which the cyclizing moiety contains an aromatic ring bearing a substituent group may be prepared as taught in the following examples.

Cyclic Compound Intermediate 93
cyclo-(D-Val-NMeArg-Gly-Asp-3-aminomethyl-4-chlorobenzoic acid)

The title compound was prepared by the general solution-phase procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb). The cyclic peptide (240 mg, 0.28 mmol) was deprotected with excess HF in the presence of anisole as scavenger. Purification was accomplished by reversed-phase HPLC on a preparative LiChrospher RP-18 column (5 cm) using a 1.4% / minute gradient of 22 to 90% acetonitrile containing 0.1% trifluoroacetic acid to give the TFA salt of the title compound (80 mg, 39%) as a fluffy white solid; ^{1}H NMR (D_6-DMSO) 9.00 (d, 1H), 8.50 (d, 1H), 8.45 (t, 1H), 7.60 (d, 2H), 7.45 (s, 1H), 7.45 (d, 2H), 7.00 (br s, 4H), 5.15 (dd, 1H), 4.45 (m, 2H), 4.20 (m, 2H), 4.10 (d, 1H), 3.55 (d, 1H), 3.10 (m, 2H), 2.90 (s, 3H), 2.65 (dd, 1H), 2.50 (m, 1H), 2.05 (m, 2H), 1.50 (m, 1H), 1.30 (m, 2H), 1.05 (d, 3H), 0.85 (d, 3H); FAB-MS: [M+H] = 609.

Cyclic Compound Intermediate 94

cyclo-(D-Val-NMeArg-Gly-Asp-iodo-Mamb);
the compound of formula (VII) wherein J = D-Val, K = NMeArg, L = Gly, M = Asp, R^1 = R^2 = H, R^{10} = H, R^{10a} = I

The title compound was prepared using the general procedure described for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb) (Cyclic Compound Intermediate 4). The DCC/DMAP method was used for attachment of Boc-iodo-Mamb to the oxime resin. The peptide was prepared on a 1.05 mmol scale to give the protected cyclic peptide (460 mg, 46.8%). The peptide (438 mg) and 0.5 mL of anisole were treated with anhydrous hydrogen fluoride at 0°C for 30 minutes. The crude material was precipitated with ether, redissolved in aqueous acetic acid, and lyophilized to generate the title compound (340 mg, 95.6%; calculated as the acetate salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23%/ min. gradient of 12.6 to 22.5% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (39.7% recovery, overall yield 16.6%); 1H NMR (D6-DMSO) ∂ 9.05 (d, 1H), 8.55 (d, 1H), 8.55 (t, 1H), 7.90 (d, 1H), 7.65 (d, 1H), 7.55 (t, 1H), 7.20 (d, 1H), 7.15 (s, 1H),7.00 (br s, 4H), 5.15 (dd, 1H), 4.50 (g, 1H), 4.30 (m, 3H), 3.95 (dd, 1H), 3.60 (d, 1H), 3.10 (m, 2H), 3.00 (s, 3H), 2.75 (dd, 1H), 2.55 (dd, 1H), 2.10 (m, 2H), 1.60 (m, 1H), 1.35 (m, 2H), 1.10 (d, 3H), 0.90 (d, 3H); FAB-MS: [M+H] = 701.37.

Cyclic Compound Intermediate 95
cyclo-(D-Val-NMeArg-Gly-Asp-3-aminomethyl-4-methoxybenzoic acid)

The title compound was prepared by the general solution-phase procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb). The cyclic peptide (600 mg, 0.71 mmol) was deprotected with excess HF in the presence of anisole as scavenger. Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.33% / minute gradient of 7 to 18% acetonitrile containing 0.1% trifluoroacetic acid to give the TFA salt of the title compound (104 mg, 32%) as a fluffy white solid; ^{1}H NMR (D_6-DMSO) 12.40 (br s, 1H), 8.25 (d, 1H), 8.20 (br s, 1H), 8.00 (br s, 2H), 7.85 (d, 1H), 7.75 (s, 1H), 7.65 (br s, 1H), 7.05 (d, 1H), 7.05 (br s, 4H), 5.00 (dd, 1H), 4.60 (q, 1H), 4.30 (d, 1H), 4.25 (d, 2H), 3.85 (s, 3H), 3.85 (dd, 1H), 3.70 (dd, 1H), 3.10 (q, 2H), 3.00 (s, 3H), 2.70 (m, 1H), 2.50 (m, 1H), 2.10 (m, 1H), 1.90 (m, 1H), 1.65 (m, 1H), 1.35 (m, 2H), 1.00 (d, 3H), 0.90 (d, 3H); FAB-MS: $[M+H_2O+H]$ = 623.

Cyclic Compound Intermediate 96
cyclo-(D-Val-NMeArg-Gly-Asp-3-aminomethyl-4-methylbenzoic acid)

The title compound was prepared by the general solution-phase procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb). The cyclic peptide (210 mg, 0.25 mmol) was deprotected with excess HF in the presence of anisole as scavenger. Purification was accomplished by reversed-phase HPLC on a preparative LiChrospher RP-18 column (5 cm) using a 2.3% / minute

gradient of 22 to 90% acetonitrile containing 0.1% trifluoroacetic acid to give the TFA salt of the title compound (75 mg, 42%) as a fluffy white solid; ^{1}H NMR (D_6-DMSO) 12.30 (br s, 1H), 8.85 (d, 1H), 8.55 (d, 1H), 8.30 (t, 1H), 7.75 (d, 1H), 7.55 (m, 2H), 7.40 (s, 1H), 7.20 (s, 1H), 7.00 (br s, 4H), 5.20 (dd, 1H), 4.55 (q, 1H), 4.45 (dd, 1H), 4.30 (m, 2H), 4.05 (dd, 1H), 3.60 (d, 1H), 3.10 (q, 2H), 3.00 (s, 3H), 2.70 (dd, 1H), 2.50 (m, 1H), 2.25 (s, 3H), 2.10 (m, 2H), 1.60 (m, 1H), 1.35 (m, 2H), 1.10 (d, 3H), 0.90 (d, 3H); FAB-MS: [M+H] = 589.

Cyclic Compound Intermediate 97
cyclo-(D-Val-NMeArg-Gly-Asp-3-aminomethyl-6-chlorobenzoic acid)

The title compound was prepared by the general solution-phase procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb), except that 4,4'-dinitrobenzophenone oxime was employed. The cyclic peptide (550 mg, 0.65 mmol) was deprotected with excess HF in the presence of anisole as scavenger. Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.8% / minute gradient of 10 to 38% acetonitrile containing 0.1% trifluoroacetic acid to give the TFA salt of the title compound (254 mg, 54%) as a fluffy white solid; ^{1}H NMR (D_6-DMSO) 12.30 (br s, 1H), 9.05 (d, 1H), 8.45 (m, 2H), 7.50 (t, 1H), 7.35 (d, 1H), 7.30 (m, 2H), 7.10 (s, 1H), 7.05 (br s, 4H), 5.15 (dd, 1H), 4.45 (dd, 1H), 4.40 (q, 2H), 4.05 (dt, 2H), 3.55 (dd, 1H), 3.15 (q, 2H), 3.10 (s, 3H), 2.70 (dd, 1H), 2.50 (m, 1H), 2.05 (m, 2H), 1.65 (m, 1H), 1.35 (m, 2H), 1.10 (d, 3H), 0.90 (d, 3H); FAB-MS: [M+H] = 609.

Cyclic Compound Intermediate 99
cyclo-(D-Val-NMeArg-Gly-Asp-3-aminomethyl-6-methoxybenzoic acid)

The title compound was prepared by the general solution-phase procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb), except that 4,4'-dinitrobenzophenone oxime was employed. The cyclic peptide (256 mg, 0.30 mmol) was deprotected with excess HF in the presence of anisole as scavenger. Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.8% / minute gradient of 10 to 38% acetonitrile containing 0.1% trifluoroacetic acid to give the TFA salt of the title compound (137 mg, 63%) as a fluffy white solid; ^{1}H NMR (D_6-DMSO) 8.45 (d, 1H), 8.40 (d, 1H), 8.30 (t, 1H), 7.65 (d, 1H), 7.50 (t, 1H), 7.40 (s, 1H), 7.35 (d, 1H), 7.05 (d, 1H), 7.00 (br s, 4H), 5.20 (dd, 1H), 4.55 (dd, 1H), 4.50 (q, 1H), 4.35 (dd, 1H), 4.25 (dd, 1H), 3.95 (dd, 1H), 3.90 (s, 3H), 3.55 (d, 1H), 3.10 (q, 2H), 3.00 (s, 3H), 2.70 (dd, 1H), 2.50 (m, 1H), 2.05 (m, 2H), 1.60 (m, 1H), 1.35 (m, 2H), 1.10 (d, 3H), 0.95 (d, 3H); FAB-MS: [M+H] = 605.

Cyclic Compound Intermediate 100
cyclo-(D-Val-NMeArg-Gly-Asp-3-aminomethyl-6-methylbenzoic acid)

The title compound was prepared by the general solution-phase procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb), except that 4,4'-dinitrobenzophenone oxime was employed. The cyclic peptide (230 mg, 0.28 mmol) was deprotected with excess HF in the presence of anisole as scavenger. Purification

was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.8% / minute gradient of 10 to 38% acetonitrile containing 0.1% trifluoroacetic acid to give the TFA salt of the title compound (54 mg, 27%) as a fluffy white solid; ^{1}H NMR (D_6-DMSO) 12.30 (br s, 1H), 8.80 (d, 1H), 8.40 (d, 1H), 8.30 (t, 1H), 7.45 (m, 2H), 7.15 (q, 2H), 7.00 (s, 1H), 7.00 (br s, 4H), 5.15 (dd, 1H), 4.45 (m, 3H), 4.05 (m, 2H), 3.55 (dd, 1H), 3.10 (q, 2H), 3.05 (s, 3H), 2.70 (dd, 1H), 2.50 (m, 1H), 2.30 (s, 3H), 2.05 (m, 2H), 1.60 (m, 1H), 1.35 (m, 2H), 1.05 (d, 3H), 0.90 (d, 3H); FAB-MS: [M+H] = 589.

Cyclic Compound Intermediate 100a

cyclo-(D-Abu-NMeArg-Gly-Asp-3-aminomethyl-6-chlorobenzoic acid)

The title compound was prepared by the general solution-phase procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb), except that 4,4'-dinitrobenzophenone oxime was employed. The cyclic peptide (330 mg, 0.40 mmol) was deprotected with excess HF in the presence of anisole as scavenger. Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 1.0% / minute gradient of 10 to 38% acetonitrile containing 0.1% trifluoroacetic acid to give the TFA salt of the title compound (114 mg, 41%) as a fluffy white solid; ^{1}H NMR (D_6-DMSO) 9.00 (d, 1H), 8.40 (m, 2H), 7.50 (m, 1H), 7.40 (d, 1H), 7.30 (m, 2H), 7.15 (s, 1H), 7.00 (br s, 4H), 5.15 (dd, 1H), 4.65 (q, 1H), 4.50 (dd, 1H), 4.40 (q, 1H), 4.05 (dd, 1H), 3.95 (dd, 1H), 3.65 (dd, 1H), 3.10 (q, 2H), 3.05 (s, 3H), 2.75 (dd, 1H), 2.50 (m, 1H), 1.95

(m, 1H), 1.75 (m, 2H), 1.60 (m, 1H), 1.35 (m, 2H), 0.95 (t, 3H); FAB-MS: [M+H] = 595.4.

Cyclic Compound Intermediate 89d

cyclo-(D-Abu-NMeArg-Gly-Asp-iodo-Mamb); the compound of formula (VII) wherein J = D-Abu, K = NMeArg, L = Gly, M = Asp, R^1 = R^2 = H, R^{10} = H, R^{10a} = I

The title compound was prepared using the general procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb) (Cyclic Compound Intermediate 4). The DCC/DMAP method was used for attachment of Boc-iodo-Mamb to the oxime resin. The peptide was prepared on a 3.53 mmol scale to give the protected cyclic peptide (4.07 g, greater than quantitative yield). The peptide (4.07 g) and 4.0 mL of anisole were treated with anhydrous hydrogen fluoride at 0°C for 30 minutes. The crude material was precipitated with ether, redissolved in aqueous acetic acid, and lyophilized to generate the title compound (2.97 g, greater than quantitative yield; calculated as the acetate salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.16%/ min. gradient of 16.2 to 22.5% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (28.7% recovery, overall yield 30.2%); FAB-MS: [M+H] = 687.33.

Cyclic Compound Intermediate 100b

cyclo-(D-Abu-NMeArg-Gly-Asp-3-aminomethyl-6-iodobenzoic acid)

The title compound was prepared by the general solution-phase procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb), except that 4,4'-dinitrobenzophenone oxime was employed. The cyclic peptide (350 mg, 0.38 mmol) was deprotected with excess HF in the presence of anisole as scavenger. Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 1.0% / minute gradient of 10 to 38% acetonitrile containing 0.1% trifluoroacetic acid to give the TFA salt of the title compound (150 mg, 49%) as a fluffy white solid; ^{1}H NMR (D_6-DMSO) 8.90 (d, 1H), 8.40 (m, 2H), 7.70 (d, 1H), 7.50 (m, 1H), 7.30 (m, 1H), 7.05 (s, 1H), 7.00 (d, 1H), 7.00 (br s, 4H), 5.15 (dd, 1H), 4.65 (q, 1H), 4.45 (dd, 1H), 4.40 (q, 1H), 4.00 (q, 1H), 3.90 (q, 1H), 3.65 (dd, 1H), 3.10 (q, 2H), 3.05 (s, 3H), 2.70 (dd, 1H), 2.50 (m, 1H), 1.95 (m, 1H), 1.75 (m, 2H), 1.60 (m, 1H), 1.40 (m, 2H), 0.95 (t, 3H); FAB-MS: [M+H] = 687.3.

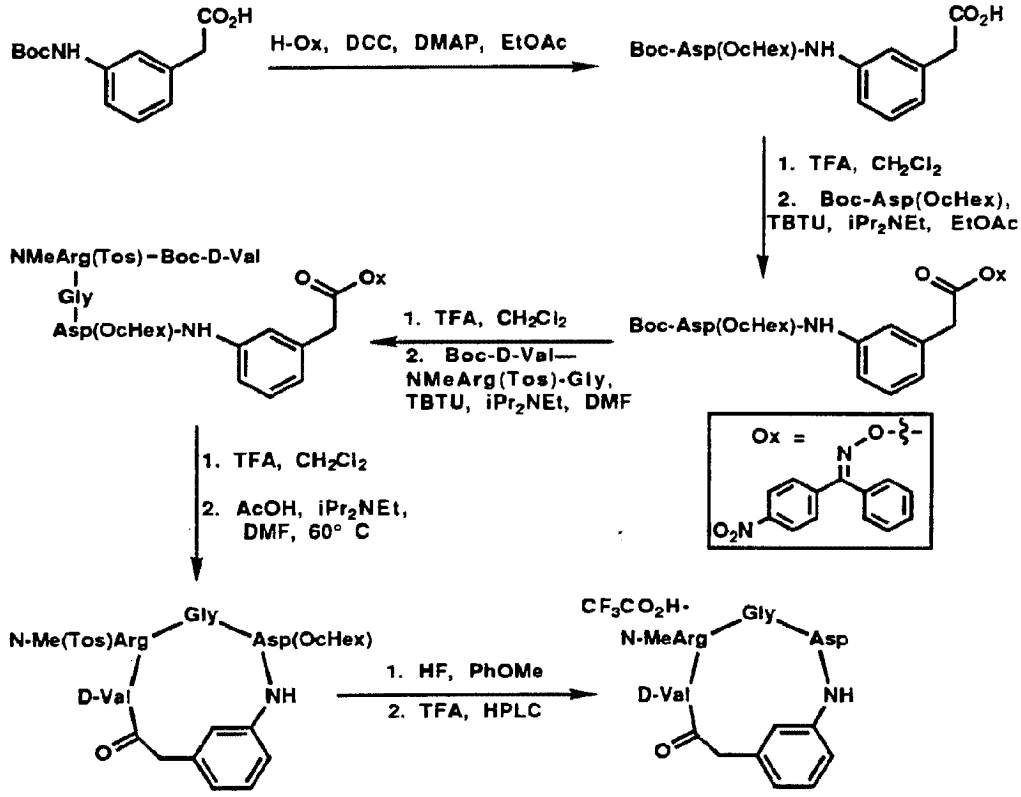
Cyclic Compound Intermediate 100c

cyclo-(D-Abu-NMeArg-Gly-Asp-3-aminomethyl-6-methylbenzoic acid)

(the compound of formula (VII) wherein J = D-Abu, K = NMeArg, L = Gly, M = Asp, R^{10} = Me)

The title compound was prepared by the general solution-phase procedure described above for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb), except that 4,4'-dinitrobenzophenone oxime was employed. The cyclic peptide (130 mg, 0.16 mmol) was deprotected with excess HF in the presence of anisole as scavenger. Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 1.0% / minute gradient of 10 to 38% acetonitrile containing 0.1%

trifluoroacetic acid to give the TFA salt of the title compound (31 mg, 28%) as a fluffy white solid; ^{1}H NMR (D_6-DMSO) 8.70 (d, 1H), 8.40 (d, 1H), 8.30 (t, 1H), 7.50 (m, 1H), 7.45 (m, 1H), 7.15 (q, 2H), 7.05 (s, 1H), 7.00 (br s, 4H), 5.15 (dd, 1H), 4.65 (q, 1H), 4.45 (m, 2H), 4.00 (m, 2H), 3.65 (dd, 1H), 3.10 (q, 2H), 3.05 (s, 3H), 2.75 (dd, 1H), 2.50 (m, 1H), 2.30 (s, 3H), 2.00 (m, 1H), 1.75 (m, 2H), 1.60 (m, 1H), 1.35 (m, 2H), 0.95 (t, 3H); FAB-MS: [M+H] = 575.4.



Scheme 5: procedure for synthesis of cyclic compound intermediate.

Solid-Phase Synthesis of Cyclic Compound Intermediate 101

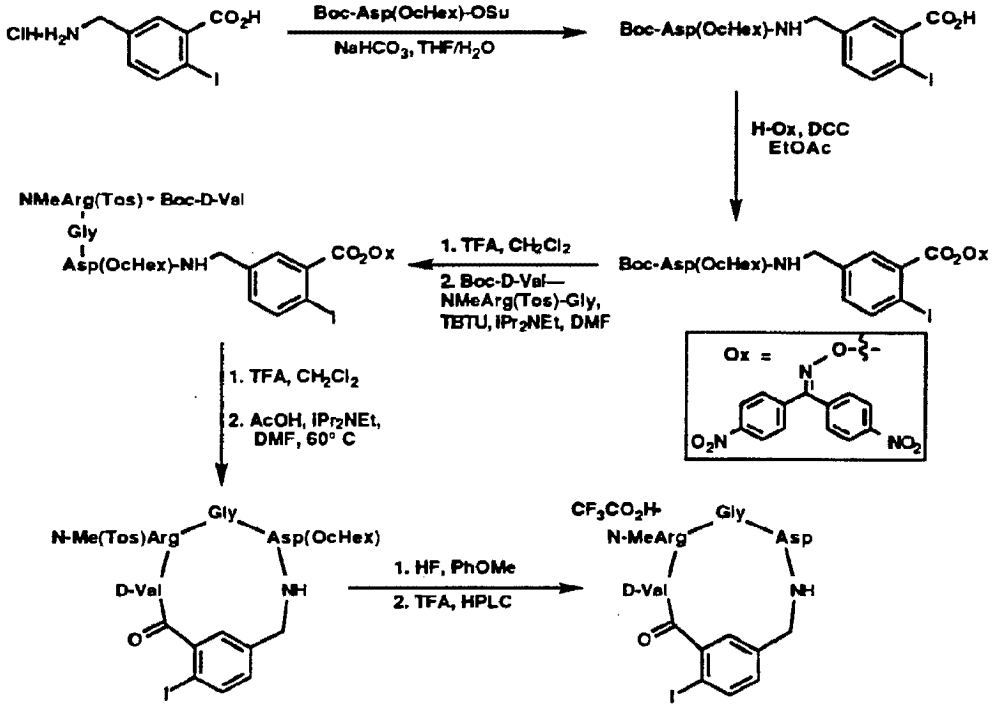
cyclo-(D-Val-NMeArg-Gly-Asp-3-aminomethyl-4-iodobenzoic Acid)

The title compound was prepared using the general procedure previously described for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb). The DCC/DMAP method was used for attachment of Boc-iodo-Mamb to the oxime resin. The peptide was prepared on a 1.05 mmol scale to give the protected cyclic peptide (460 mg, 46.8%). The peptide (438 mg) and 0.5 mL of anisole were treated with anhydrous hydrogen fluoride at 0°C for 30 minutes. The crude material was precipitated with ether, redissolved in aqueous acetic acid, and lyophilized to generate the title compound (340 mg, 95.6%; calculated as the acetate salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.23% / minute gradient of 12.6 to 22.5% acetonitrile containing 0.1% trifluoroacetic acid and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (39.7% recovery, overall yield 16.6%; ^{1}H NMR (D_6-DMSO) d 9.05 (d, 1H), 8.55 (d, 1H), 8.55 (t, 1H), 7.90 (d, 1H), 7.65 (d, 1H), 7.55 (t, 1H), 7.20 (d, 1H), 7.15 (s, 1H), 7.00 (br s, 4H), 5.15 (dd, 1H), 4.50 (q, 1H), 4.30 (m, 3H), 3.95 (dd, 1H), 3.60 (d, 1H), 3.10 (m, 2H), 3.00 (s, 3H), 2.75 (dd, 1H), 2.55 (dd, 1H), 2.10 (m, 2H), 1.60 (m, 1H), 1.35 (m, 2H), 1.10 (d, 3H), 0.90 (d, 3H); FAB-MS: [M+H] = 701.37.

Solution-Phase Synthesis of Cyclic Compound Intermediate 102

cyclo-(D-Val-NMeArg-Gly-Asp-3-aminomethyl-6-iodobenzoic Acid)

The title compound was prepared according to the method of Scheme 6, shown below.



Scheme 6

1. Boc-Asp(OcHex)-3-aminomethyl-6-iodobenzoic Acid

To a suspension of 3-aminomethyl-6-iodobenzoic acid•HCl (4.9 g, 16 mmol) in H_2O (16 ml) was added $NaHCO_3$ (3.9 g, 47 mmol), followed by a solution of Boc-Asp(OcHex)-OSu (5.9 g, 14 mmol) in THF (16 ml). The reaction mixture was stirred at room temperature overnight, filtered, diluted with H_2O, acidified with 1N HCl, and extracted with ethyl acetate. The extracts were washed with H_2O, brine, dried over anhydrous magnesium sulfate, and evaporated to dryness under reduced pressure. This material was triturated with ether to

provide the title compound (6.7 g, 82%) as a white powder. ^{1}H NMR d (D_6-DMSO) 8.45 (br t, 1H), 7.90 (d, 1H), 7.60 (s, 1H), 7.15 (m, 2H), 4.65 (m, 1H), 4.35 (m, 1H), 4.25 (d, 2H), 2.70 (m, 1H), 2.55 (m, 1H), 1.70 (m, 4H), 1.40 (s, 9H), 1.35 (m, 6H).

2. 4,4'-Dinitrobenzophenone Oxime

The title compound was prepared by modification of procedures previously reported in the literature (Chapman and Fidler (1936) *J. Chem. Soc*, 448; Kulin and Leffek (1973) *Can. J. Chem.*, 51: 687). A solution of chromic anhydride (20 g, 200 mmol) in 125 ml of H_2O was added dropwise over 4 hours, to a suspension of bis(4-nitrophenyl)methane (25 g, 97 mmol) in 300 ml of acetic acid heated to reflux. The reaction mixture was heated at reflux for 1 hour, cooled to room temperature, and poured into water. The solid was collected by filtration, washed with H_2O, 5% sodium bicarbonate, H_2O, and air-dryed to provide a 1:1 mixture of bis(4-nitrophenyl)methane/4,4'-dinitrobenzophenone via ^{1}H NMR. This material was oxidized with a second portion of chromic anhydride (20 g, 200 mmol), followed by an identical work-up procedure to provide the crude product. Trituration with 200 ml of benzene heated to reflux for 16 hours provided 4,4'-dinitrobenzophenone (20.8 g, 79%) as a yellow powder.

A solution of hydroxylamine hydrochloride (10.2 g, 147 mmol) was added to a suspension of 4,4'-dinitrobenzophenone (19 g, 70 mmol) in 100 ml of ethanol. The reaction mixture was heated to reflux for 2 hours, cooled to room temperature, and the solid collected by filtration. Recrystallization from ethanol provided the title compound (14.0 g, 70%) as pale yellow crystals. mp 194°C; ^{1}H NMR (D_6-DMSO) d 12.25 (s, 1H), 8.35 (d, 2H), 8.20 (d, 2H), 7.60 (d, 4H).

3. 4,4'-Dinitrobenzophenone Oxime Boc-Asp(OcHex)-3-aminomethyl-6-iodobenzoate

To an ice-cooled solution of Boc-Asp(OcHex)-3-aminomethyl-6-iodobenzoic acid (3.3 g, 5.7 mmol) and 4,4'-dinitrobenzophenone oxime (1.7 g, 5.9 mmol) in 32 ml of ethyl acetate was added DCC (1.2 g, 5.8 mmol). The reaction mixture was stirred at room temperature for 3 hours, filtered, diluted with ethyl acetate, washed with saturated sodium bicarbonate solution, H_2O, brine, dried over anhydrous magnesium sulfate, and evaporated to dryness under reduced pressure. This material was purified by column chromatography on silica gel (EM Science, 230-400 mesh) using 10:1 dichloromethane/ethyl acetate to give the title compound (1.8 g, 36%) as pale yellow crystals. ^1H NMR (D_6-DMSO) d 8.40 (dd, 5H), 7.90 (m, 5H), 7.45 (s,. 1H), 7.20 (m, 2H), 4.65 (m, 1H), 4.35 (m, 1H), 4.20 (m, 2H), 2.75 (dd, 1H), 2.50 (dd, 1H), 1.70 (m, 4H), 1.40 (s, 9H), 1.35 (m, 6H).

4. Boc-D-Val-NMeArg(Tos)-Gly

To a mixture of Boc-NMeArg(Tos) (11.07 g, 25 mmol), and Gly-OBzl tosylate (10.10 g, 30 mmol) in 25 ml of dichloromethane was added HBTU (9.48 g, 25 mmol) and DIEA (9.69 g, 75 mmol). The reaction mixture was stirred at room temperature for 1 hour, concentrated under high vacuum, diluted with ethyl acetate, washed with 5% citric acid, H_2O, saturated sodium bicarbonate solution, brine, dried over anhydrous magnesium sulfate, and evaporated to dryness under reduced pressure. The resulting oil was triturated with petroleum ether to provide Boc-NMeArg(Tos)-Gly-OBzl (14.7 g, 100%); FAB-MS: [M+H] = 590.43. This material was used without further purification.

A solution of Boc-NMeArg(Tos)-Gly-OBzl (14.5 g, 24.6 mmol) in 30 ml of trifluoroacetic acid was stirred

at room temperature for 5 minutes, and evaporated to dryness under reduced pressure. The oily residue was diluted with cold ethyl acetate, washed with cold saturated sodium bicarbonate solution, the aqueous phase was extracted with ethyl acetate. The combined organics were washed with brine, evaporated to dryness under reduced pressure, and the resulting oil triturated with ether. The resulting solid was filtered, washed with ether, and dried in a vacuum desiccator to provide NMeArg(Tos)-Gly-OBzl (10.3 g, 86%); FAB-MS: [M+H] = 490.21. This material was used without further purification.

To a solution of NMeArg(Tos)-Gly-OBzl (4.80 g, 9.8 mmol), and Boc-D-Val (2.13 g, 9.8 mmol) in 10 ml of dichloromethane, cooled in an ice-bath, was added HBTU (3.79 g, 10.0 mmol) and DIEA (2.58 g, 20.0 mmol). The reaction mixture was stirred at room temperature for 48 hours, diluted with ethyl acetate, washed with 5% citric acid, brine, dried over anhydrous magnesium sulfate, and evaporated to dryness under reduced pressure. The resulting oil was triturated with ether to provide Boc-D-Val-NMeArg(Tos)-Gly-OBzl (4.58 g, 68%); FAB-MS: [M+H] = 689.59. This material was used without further purification.

A solution of Boc-D-Val-NMeArg(Tos)-Gly-OBzl (4.50 g, 6.53 mmol) in 80 ml of methanol was purged with nitrogen gas, 1.30 g of 10% Pd/C was added, and hydrogen gas was passed over the reaction. After 1 hour the catalyst was removed by filtration through a bed of celite, and the solvent removed under reduced pressure. The resulting solid was triturated with ether, filtered, and washed with petroleum ether to provide Boc-D-Val-NMeArg(Tos)-Gly (3.05 g, 78%); ^{1}H NMR (D_6-DMSO) d 7.90 (br t, 1H), 7.65 (d, 2H), 7.30 (d, 2H), 7.00 (d, 1H),

6.85 (br d, 1H), 6.60 (br s, 1H), 5.00 (dd, 1H), 4.15 (t, 1H), 3.70 (m, 2H), 3.05 (m, 2H), 2.90 (s, 3H), 2.35 (s, 3H), 1.90 (m, 2H), 1.55 (m, 1H), 1.35 (s, 9H), 1.25 (m, 2H), 0.80 (br t, 6H); FAB-MS: [M+H] = 599.45.

5. 4,4'-Dinitrobenzophenone Oxime Boc-D-Val-NMeArg(Tos)-Gly-Asp(OcHex)-3-aminomethyl-6-iodobenzoate

To a solution of 4,4'-dinitrobenzophenone oxime Boc-Asp(OcHex)-3-aminomethyl-6-iodobenzoate (0.5 g, 0.59 mmol) in 1 ml of dichloromethane was added 0.5 ml of trifluoroacetic acid. The reaction mixture was stirred at room temperature for 90 minutes, diluted with dichloromethane, and evaporated to dryness under reduced pressure. The oily residue was concentrated under high vacuum to remove traces of excess trifluoroacetic acid.

To a solution of the crude TFA salt and Boc-D-Val-NMeArg(Tos)-Gly (0.52 g, 0.87 mmol) in 3.8 ml of DMF was added TBTU (0.28 g, 0.87 mmol) and DIEA (0.33 g, 2.58 mmol). The reaction mixture was stirred at room temperature overnight, concentrated under high vacuum, diluted with ethyl acetate, washed with 5% citric acid, H_2O, brine, dried over anhydrous magnesium sulfate, and evaporated to dryness under reduced pressure. This material was triturated with ether to provide the title compound (0.48 g, 61%) as a powder. This material was used without further purification.

6. cyclo-(D-Val-NMeArg(Tos)-Gly-Asp(OcHex)-3-aminomethyl-6-iodobenzoic Acid)

To a solution of 4,4'-dinitrobenzophenone oxime Boc-D-Val-NMeArg(Tos)-Gly-Asp(OcHex)-3-aminomethyl-6-iodobenzoate (0.48 g, 0.36 mmol) in 1 ml of dichloromethane was added 0.5 ml of trifluoroacetic acid. The reaction mixture was stirred at room temperature for 45 minutes, diluted with dichloromethane, and evaporated to dryness under reduced

pressure. The oily residue was concentrated under high vacuum to remove traces of excess trifluoroacetic acid.

To a solution of the crude TFA salt in 38 ml of DMF was added acetic acid (0.09 ml, 1.57 mmol) and DIEA (0.26 ml, 1.49 mmol). The reaction mixture was stirred at 60°C for 3 days, concentrated under high vacuum, diluted with ethyl acetate, washed with 5% citric acid, brine, dried over anhydrous magnesium sulfate, and evaporated to dryness under reduced pressure. This material was purified by column chromatography on silica gel (EM Science, 230-400 mesh) using 10:1 chloroform/isopropanol to give the title compound (0.13 g, 38%) as a powder; ^{1}H NMR (D_6-DMSO) d 8.95 (d, 1H), 8.50 (t, 1H), 8.45 (d, 1H), 7.70 (d, 1H), 7.60 (d, 2H), 7.30 (d, 3H), 7.05 (d, 1H), 7.00 (s, 1H), 6.80 (br s, 1H), 6.60 (br s, 1H), 5.10 (dd, 1H), 4.65 (m, 1H), 4.45 (m, 1H), 4.35 (m, 1H), 4.00 (m, 1H), 3.55 (dd, 1H), 3.05 (m, 2H), 3.00 (s, 3H), 2.70 (dd, 1H), 2.55 (dd, 1H), 2.35 (s, 3H), 2.05 (m, 1H), 1.90 (m, 1H), 1.75 (m, 1H), 1.65 (m, 1H), 1.35 (m, 13H), 1.15 (d, 3H), 0.85 (d, 3H); FAB(GLYC)-MS: [M+H] = 937.

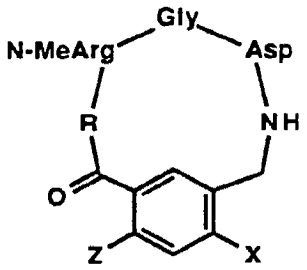
7. cyclo-(D-Val-NMeArg-Gly-Asp-3-aminomethyl-6-iodobenzoic Acid)

The cyclic peptide (490 mg, 0.52 mmol) was deprotected with excess HF in the presence of anisole as scavenger. Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.8% / minute gradient of 10 to 38% acetonitrile containing 0.1% trifluoroacetic acid to give the TFA salt of the title compound (194 mg, 46%) as a fluffy white solid; ^{1}H NMR (D_6-DMSO) d 12.30 (br s, 1H), 9.00 (d, 1H), 8.40 (m, 2H), 7.70 (d, 1H), 7.50 (m, 1H), 7.30 (m, 1H), 7.05 (d, 1H), 7.00 (s, 1H), 7.00 (br s, 4H), 5.15 (dd, 1H), 4.40 (d, 1H), 4.40 (q, 2H), 4.0 (m, 2H),

3.55 (dd, 1H), 3.15 (q, 2H), 3.10 (s, 3H), 2.70 (dd, 1H), 2.50 (m, 1H), 2.05 (m, 2H), 1.65 (m, 1H), 1.35 (m, 2H), 1.15 (d, 3H), 0.90 (d, 3H); FAB-MS: [M+H] = 701.

Table A shows the FAB-MS obtained for certain cyclic compound intermediates.

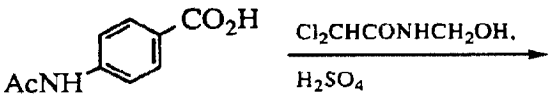
TABLE A

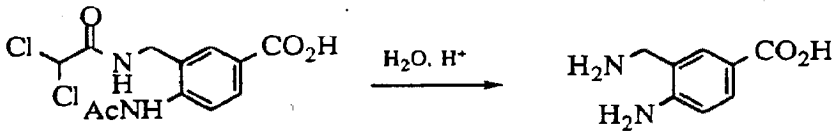


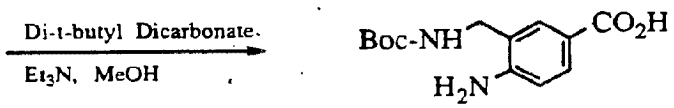
Cyclic Compound Intermediate Number	R	X	Z	FAB-MS (M+H)
101	D-Val	I	H	701.37
98, 102	D-Val	H	I	701
103	D-Abu	I	H	687.33
104	D-Abu	H	I	687.3
105	D-Val	Cl	H	609
106	D-Val	H	Cl	609
107	D-Abu	H	Cl	595.4
108	D-Val	Me	H	589
109	D-Val	H	Me	589
110	D-Abu	H	Me	575.4
111	D-Val	MeO	H	623 ($+H_2O$)

112	D-Val	H	MeO	605

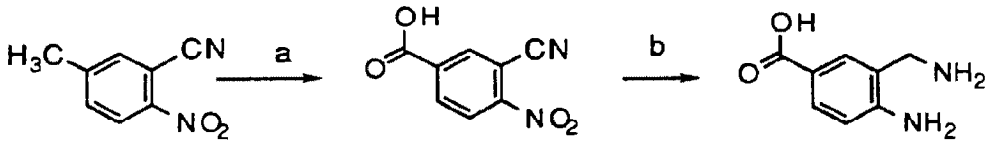
Other ring substituted cyclizing moieties can be synthesized as taught in the following schemes and discussion. The moiety of the formula above where Z = NH_2 can be synthesized by at least two different routes. For example, starting with 4-acetamidobenzoic acid (Aldrich Chemical Co.), a Friedel-Crafts alkylation with N-hydroxymethyldichloroacetamide would give the dichloroacetyl derivative of 3-aminomethyl-4-acetamidobenzoic acid (Felder, Pitre, and Fumagalli (1964), *Helv. Chim. Acta*, **48**, 259-274). Hydrolysis of the two amides would give 3-aminomethyl-4-aminobenzoic acid.





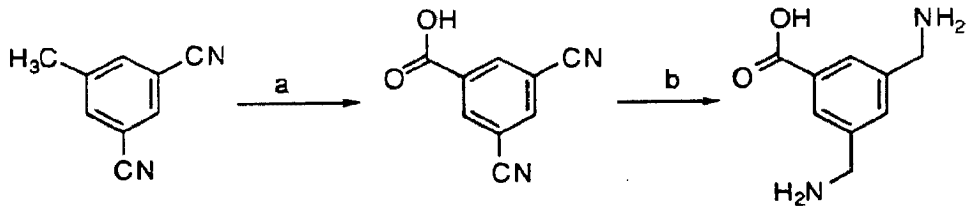


Alternatively, starting with 3-cyano-4-nitrotoluene, oxidation with chromium trioxide followed by reduction will give 3-aminomethyl-4-aminobenzoic acid.



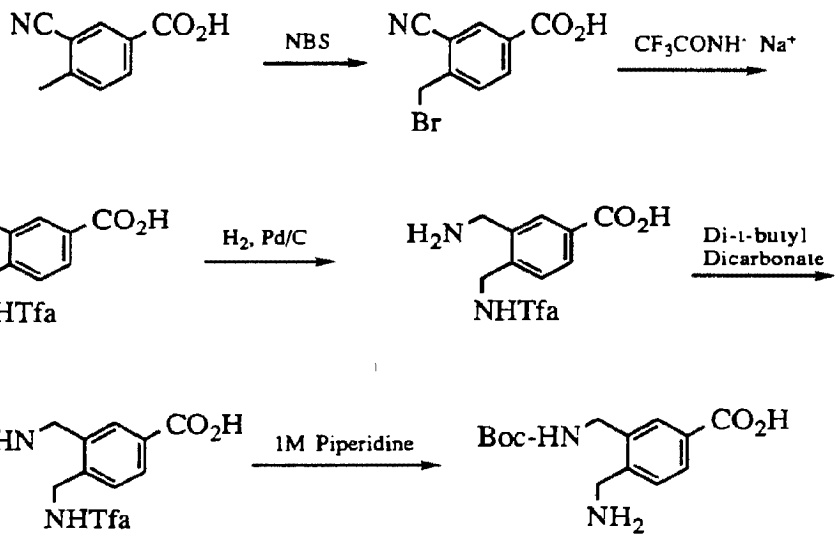
a] CrO_3 b] H_2-catalyst

The moiety of the formula above where Y = CH_2NH_2 can be synthesized from 3,5-dicyanotoluene by oxidation of the methyl group with chromium trioxide followed by reduction.

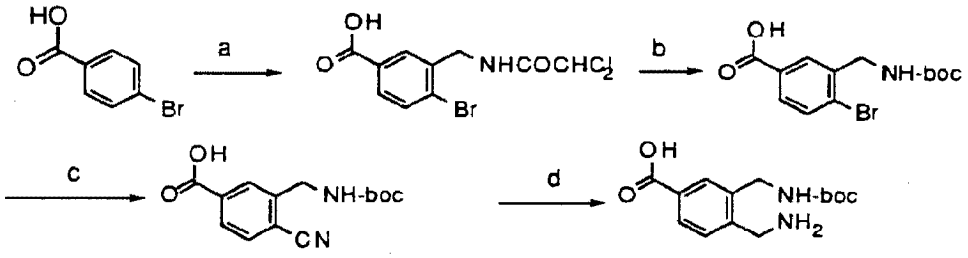


a] CrO_3 b] H_2-catalyst

The moiety of the formula above where Z = CH_2NH_2 can be synthesized from 3-cyano-4-methylbenzoic acid (K & K Rare and Fine Chemicals). Bromination using N-bromosuccinimide would give 4-bromomethyl-3-cyanobenzoic acid. A nucleophilic substitution reaction at the bromomethyl position using an amide anion would produce the protected amine. Amide anions which could be used in this reaction include potassium phthalimide (Gabriel synthesis), and the anion of trifluoroacetamide (Usui (1991), *Nippon Kagaku Kaishi*, 206-212) used in this example. Reduction of the nitrile would produce the second aminomethyl group, which would be protected by reaction with di-t-butyl dicarbonate. Removal of the trifluoroacetamide protecting group using aqueous piperidine would give the moiety.



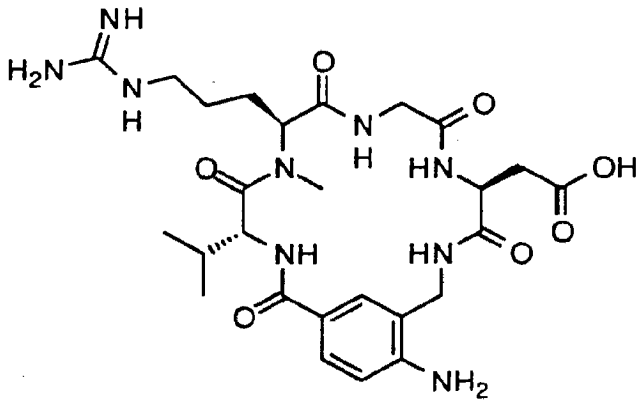
Alternatively, the moiety can be prepared from 4-bromobenzoic acid as shown in the scheme.



a] H_2SO_4, $HOCH_2NHCOCHCl_2$ b] H^+, boc-ON
c] CuCN, DMF d] H_2-catalyst

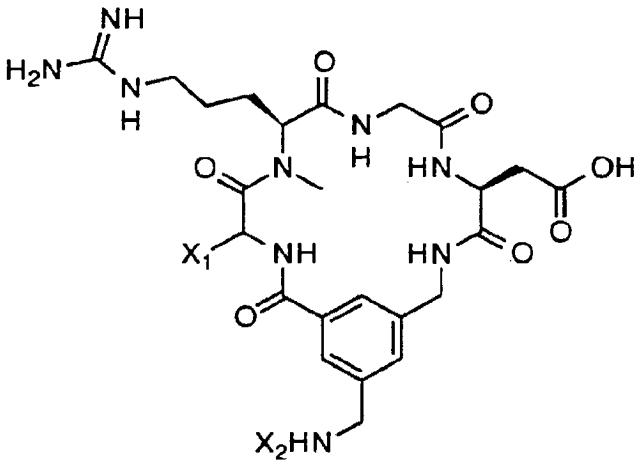
These ring substituted cyclizing moieties can be used to synthesize cyclic compound intermediates.

Cyclic Compound Intermediate 113
Cyclo(D-Val-NMeArg-Gly-Asp-Mamb(4-NH_2))



This compound can be prepared using the procedure described above for Cyclo(D-Val-NMeArg-Gly-Asp-Mamb substituting the ring substituted cyclizing moiety where Z = NH_2.

Cyclic Compound Intermediates 114, 115 and 116

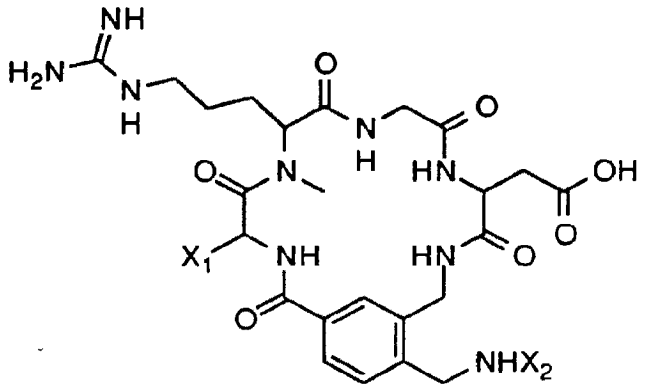


X_1 = 2-propyl, ethyl, or p-hydroxyphenylmethyl
X_2 = H.

Compounds cyclo(D-Val-NMeArg-Gly-Asp-Mamb(5-CH_2NHX_2), cyclo(D-Abu-NMeArg-Gly-Asp-Mamb(5-CH_2NHX_2), and cyclo(D-

Tyr-NMeArg-Gly-Asp-Mamb(5-CH_2NHX_2) can be prepared via the methods described above using the ring substituted cyclizing moiety where Y = CH_2NH_2.

Cyclic Compound Intermediates 117, 118 and 119.



X_1 = 2-propyl, ethyl, or p-hydroxyphenylmethyl
X_2 = H

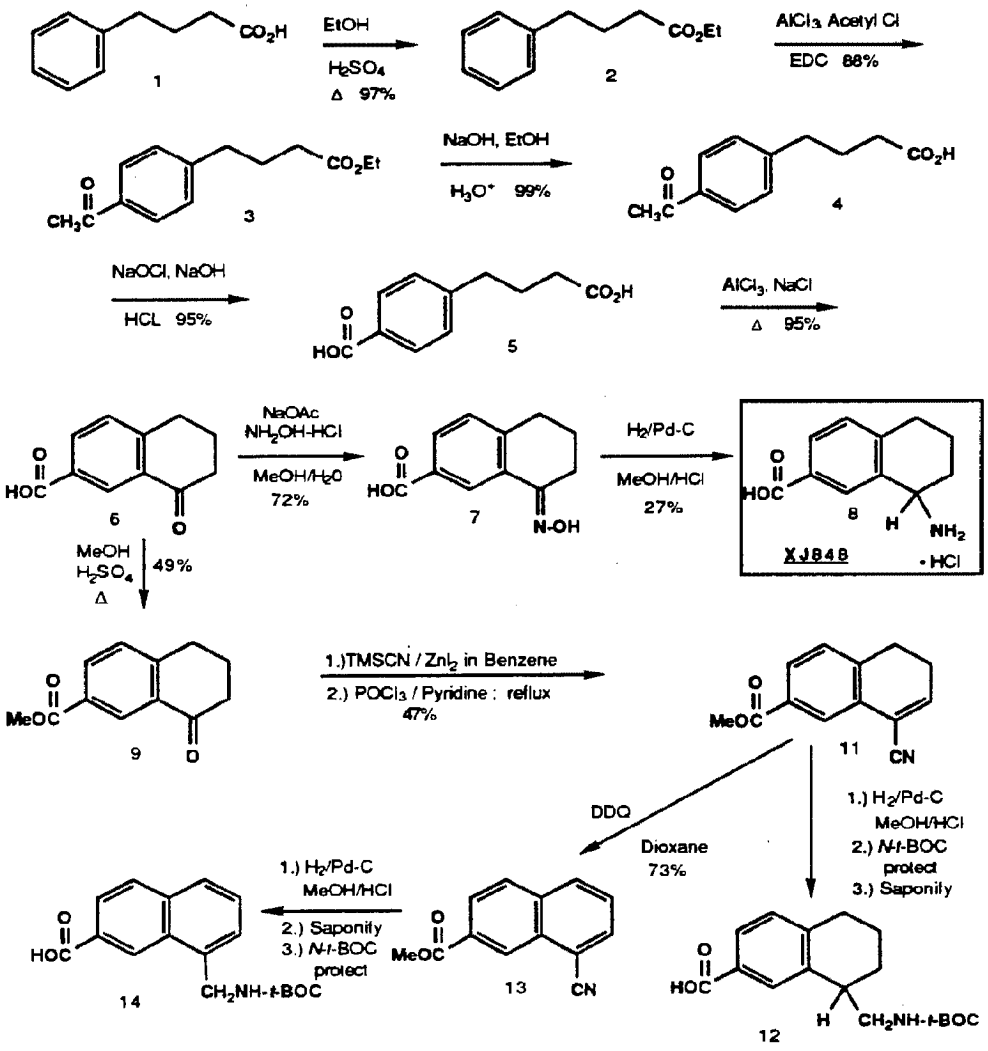
Compounds cyclo(D-Val-NMeArg-Gly-Asp-Mamb(4-CH_2NHX_2), cyclo(D-Abu-NMeArg-Gly-Asp-Mamb(4-CH_2NHX_2), and cyclo(D-Tyr-NMeArg-Gly-Asp-Mamb(4-CH_2NHX_2) can be prepared via the procedures described above using the ring substituted cyclizing moiety where Z = CH_2NH_2.

Other R^{31} Cyclizing Moieties

Alternatives to Mamb useful as cyclizing moieties R^{31} in the cyclic peptides of the invention include aminoalkyl-naphthoic acid and aminoalkyl-tetrahydronaphthoic acid residues. Representative aminoalkyl-naphthoic acid and aminoalkyl-tetrahydronaphthoic acid intermediates useful in the synthesis of cyclic peptides of the present invention

are described below. The synthesis of these intermediates is outlined below in Scheme 7.

Scheme 7



8-Amino-5,6,7,8-tetrahydro-2-naphthoic Acid Hydrochloride (8)

The title compound was prepared according to a modification of standard procedures previously reported in the literature (Earnest, I., Kalvoda, J., Rihs, G., and Mutter, M., Tett. Lett., Vol. 31, No. 28, pp 4011-4014, 1990).

As shown above in Scheme 7, 4-phenylbutyric acid (1) was converted to the ethyl ester (2) which was acylated via aluminum chloride and acetylchloride to give 4-acetylphenylbutyric acid ethyl ester (3). This ester was subjected to saponification to give 4-acetylphenylbutyric acid (4). Subsequently, the acetyl group was oxidized to give 4-carboxyphenylbutyric acid (5) which was converted to the 1-tetralin-7-carboxylic acid (6) using aluminum chloride in a Friedel-Crafts cyclization with resonably high yield. At that point, the tetralone was split into two portions and some was converted to the oxime (7) using sodium acetate and hydroxylamine hydrochloride.The oxime was subjected to hydrogenolysis to give the racemic mixture of 8-amino-5,6,7,8-tetrahydro-2-naphthoic acid as the hydrochloride (8) for use as an intermediate for incorporation into the cyclic peptide.

Part A - A solution of 4-phenylbutyric acid (50.0 g, 0.3 mol) in ethanol (140 mL) with concentrated sulfuric acid (0.53 mL) was stirred at reflux over 5 hours. The cooled solution was poured into ice water and extracted with ethyl acetate. The combined organic layers were backwashed with brine, dried over anhydrous magnesium sulfate and evaporated to dryness under reduced pressure to give 4-phenylbutyric acid ethyl ester (56.07 g, 0.29

mol, 97%) as a yellow liquid. ^{1}H NMR ($CDCl_3$) d 7.3-7.1 (m, 5H), 4.1 (q, 2H, J=7.1 Hz), 2.7 (t, 2H, J=7.7 Hz), 2.3 (t, 2H, J=7.5 Hz), 1.95 (quintet, 2H, J=7.5 Hz), 1.25 (t, 3H, J=7.1 Hz).

Part B - To a solution of aluminum chloride (153 g, 1.15 mol), and acetyl chloride (38.5 mL, 42.5 g, 0.54 mol) in dichloromethane (1500 mL) was added, dropwise, a solution of 4-phenylbutyric acid ethyl ester (50.0 g, 0.26 mol) in dichloromethane (500 mL). All was stirred at ambient temperature for 15 minutes. The solution was poured into cold concentrated hydrochloric acid (2000 mL) and then extracted with dichloromethane. The combined organic layers were backwashed with brine, dried over anhydrous magnesium sulfate and evaporated to dryness under reduced pressure to give 4-acetylphenylbutyric acid ethyl ester (53.23 g, 0.23 mol, 88%) as a dark yellow liquid. ^{1}H NMR ($CDCl_3$) d 7.9 (d, 2H, J=8.1 Hz), 7.25 (d, 2H, J=8.4 Hz), 4.1 (q, 2H, J=7.1 Hz), 2.75 (t, 2H, J=7.6 Hz), 2.6 (s, 3H), 2.35 (t, 2H, J=7.6 Hz), 2.0 (quintet, 2H, J=7.5 Hz), 1.25 (t, 3H, J=7.1 Hz).

Part C -To a solution of 4-acetylphenylbutyric acid ethyl ester (50.0 g, 0.21 mol) in ethanol (1250 mL) was added, dropwise, a solution of sodium hydroxide (50.0 g) in water (1250 mL). All was stirred at reflux over 4 hours. The solution was concentrated to half volume and then acidified to a pH equal to 1.0 using hydrochloric acid (1N). The resulting precipitate was collected and washed with water to give 4-acetylphenylbutyric acid (53.76 g, 0.26 mol, 99%) as a white solid. mp = 50-52°C; ^{1}H NMR ($CDCl_3$) d 7.9 (d, 2H, J=8.1 Hz), 7.25 (d, 2H,

J=9.1 Hz), 2.75 (t, 2H, J=7.7 Hz), 2.6 (s, 3H), 2.4 (t, 2H, J=7.3 Hz), 2.0 (quintet, 2H, J=7.4 Hz).

Part D -To a solution of sodium hypochlorite (330 mL, 17.32 g, 0.234 mol) in a solution of sodium hydroxide (50%, 172 mL), warmed to 55°C, was added, portionwise as a solid, 4-acetylphenylbutyric acid (16.0 g, 0.078 mol) while keeping the temperature between 60-70°C. All was stirred at 55°C over 20 hours. The cooled solution was quenched by the dropwise addition of a solution of sodium bisulfite (25%, 330 mL). The mixture was then transferred to a beaker and acidified by the careful addition of concentrated hydrochloric acid. The resulting solid was collected, washed with water and dried, then triturated sequentially with chlorobutane and hexane to give 4-carboxyphenylbutyric acid (15.31 g, 0.074 mol, 95%) as a white solid. mp = 190-195°C; ^{1}H NMR (DMSO) d 12.55 (bs, 1H), 8.1 (s, 1H), 7.85 (d, 2H, J=8.1 Hz), 7.3 (d, 2H, J=8.1 Hz), 2.7 (t, 2H, J=7.5 Hz), 2.2 (t, 2H, J=7.4 Hz), 1.8 (quintet, 2H, J=7.5 Hz).

Part E - A mixture of 4-carboxyphenylbutyric acid (10.40 g, 0.05 mol), aluminum chloride (33.34 g, 0.25 mol) and sodium chloride (2.90 g, 0.05 mol) was heated with continual stirring to 190°C over 30 minutes. As the mixture cooled to 60°C, cold hydrochloric acid (1N, 250 mL) was carefully added. The mixture was extracted with dichloromethane. The combined organic layers were backwashed with dilute hydrochloric acid and water, dried over anhydrous magnesium sulfate and evaporated to dryness under reduced pressure. The resulting solid was triturated with chlorobutane to give 1-tetralon-7-carboxylic acid (9.59 g, 0.05 mol, 100%) as a brown solid. mp = 210-215°C; ^{1}H NMR (DMSO) d 8.4 (s, 1H), 8.1

(d, 2H, J=8.0 Hz), 7.5 (d, 1H, J=7.9 Hz), 3.0 (t, 2H, J=6.0 Hz), 2.65 (t, 2H, J=6.6 Hz), 2.1 (quintet, 2H, J=6.3 Hz).

Part F - A solution of 1-tetralon-7-carboxylic acid (1.0 g, 0.0053 mol) and sodium acetate (1.93 g, 0.024 mol) and hydroxylamine hydrochloride (1.11 g, 0.016 mol) in a mixture of methanol and water (1:1, 15 mL) was stirred at reflux over 4 hours. The mixture was cooled and then added was more water (50 mL). The solid was collected, washed with water and dried, then triturated with hexane to give 1-tetralonoxime-7-carboxylic acid (0.78 g, 0.0038 mol, 72%) as a white solid. mp = 205-215°C; ^{1}H NMR (DMSO) d 11.3 (s, 2H), 8.4 (s, 1H), 7.8 (d, 1H, J=7.7 Hz), 7.3 (d, 1H, J=7.7 Hz), 2.8 (t, 2H, J=5.9 Hz), 2.7 (d, 2H, J=6.6 Hz), 1.9-1.7 (m, 2H).

Part G - A mixture of 1-tetralonoxime-7-carboxylic acid (0.75 g, 0.0037 mol) in methanol (25 mL) with concentrated hydrochloric acid (0.54 mL, 0.20 g, 0.0056 mol) and palladium on carbon catalyst (0.10 g, 5% Pd/C) was shaken for 20 hours at ambient temperature under an atmosphere of hydrogen (60 psi). The reaction mixture was filtered over Celite® and washed with methanol. The filtrate was evaporated to dryness under reduced pressure and the residue was purified by flash chromatography using hexane:ethyl acetate::1:1 to give the racemic mixture of 8-amino-5,6,7,8-tetrahydro-2-naphthoic acid hydrochloride (0.225 g, 0.001 mol. 27%) as a white solid. mp = 289-291°C; ^{1}H NMR (DMSO) d 8.55 (bs, 3H), 8.2-8.1 (m, 1H), 7.85-7.8 (m, 1H), 7.35-7.25 (m, 1H), 4.5 (m, 1H), 2.9-2.8 (m, 2H), 2.1-1.9 (m, 3H), 1.85-1.7 (m, 1H).

N-(BOC)-8-Aminomethyl-5,6,7,8-tetrahydro-2-naphthoic Acid (12)

As shown above in Scheme 7, the remaining tetralone was then converted to the methyl ester (9). Using a procedure from Gregory, G.B. and Johnson, A.L, JOC, 1990, 55, 1479, the tetralone methyl ester (9) was converted, first, to the cyanohydrin by treatment with trimethylsilylcyanide and zinc iodide and then, via the *in situ* dehydration with phosphorous oxychloride in pyridine, to the methyl 8-cyano-5,6-dihydro-2-naphthoate (11). This naphthoate was divided into two portions and some was subjected to hydrogenolysis, N-BOC-protection and saponification to give N-(BOC)-8-aminomethyl-5,6,7,8-tetrahydro-2-naphthoic acid (12) as an intermediate for incorporation into the cyclic peptide.

Part A - A mixture of 1-tetralon-7-carboxylic acid (7.0 g, 0.037 mol) in methanol (13.6 mL, 10.8 g, 0.30 mol) with a catalytic amount of hydrochloriic acid (0.07 mL, 0.12 g, 0.0012 mol) was stirred at reflux over 5 hours. The cooled reaction mixture was poured into ice water and extracted with ethyl acetate. The combined organic layers were backwashed with water and brine, dried over anhydrous magnesium sulfate and evaporated to dryness under reduced pressure. The resulting solid was purified by flash chromatography using hexane:ethyl acetate::75:25. The resulting solid was triturated with hexane to give 1-tetralon-7-carboxylic acid methyl ester (3.61 g, 0.018 mol, 49%) as a yellow solid. mp = 170-172°C; ^{1}H NMR ($CDCl_3$) d 8.7 (s, 1H), 8.15 (d, 1H, J=8.1 Hz), 7.35 (d, 1H, J=8.1 Hz), 3.95 (s, 3H), 3.05 (d, 2H,

J=6.1 Hz), 2.7 (t, 2H, J=6.4 Hz), 2.15 (quintet, 2H, J=6.2 Hz).

Part B - A solution of 1-tetralon-7-carboxylic acid methyl ester (3.50 g, 0.017 mol), trimethylsilylcyanide (1.98 g, 0.02 mol) and zinc iodide (0.10 g) in benzene (20 mL) was stirred at ambient temperature over 15 hours. Then added, sequentially and dropwise, was pyridine (20 mL) and phosphorous oxychloride (4.0 mL, 6.55 g, 0.0425 mol). The reaction mixture was stirred at reflux over 1 hour then evaporated to dryness under reduced pressure. The residue was taken up in chloroform, backwashed with water, dried over anhydrous magnesium sulfate and evaporated to dryness under reduced pressure to give methyl 8-cyano-5,6-dihydro-2-naphthoate (1.70 g, 0.008 mol, 47%) as a yellow solid. mp = 73-75°C; ^{1}H NMR ($CDCl_3$) d 8.0-7.9 (m, 1H), 7.3-7.2 (m, 1H), 6.95 (t, 1H, J=4.8 Hz), 3.95 (s, 3H), 2.9 (t, 2H, J=8.3 Hz), 2.6-2.4 (m, 3H)

Part C - A mixture of methyl 8-cyano-5,6-dihydro-2-naphthoate (0.80 g, 0.0038 mol) in methanol (25 mL) with concentrated hydrochloric acid (0.56 mL) and palladium on carbon catalyst (0.40 g, 5% Pd/C) was shaken for 20 hours at ambient temperature under an atmosphere of hydrogen (50 psi). The reaction mixture was filtered over Celite and washed with methanol. The filtrate was evaporated to dryness under reduced pressure and the residue was triturated with hexane to give the racemic mixture of methyl 8-aminomethyl-5,6,7,8-tetrahydro-2-naphthoate (0.80 g, 0.0037 mol, 97%) as a white solid. mp = 172-179°C; ^{1}H NMR (DMSO) d 8.2-8.0 (m, 4H), 7.9-7.7 (m, 6H), 7.5-7.2 (m, 4H), 3.9-3.8 (m, 7H), 3.3-2.7(m, 10H), 2.0-1.6 (m, 8H).

Part D - A solution of methyl 8-aminomethyl-5,6,7,8-tetrahydro-2-naphthoate (0.78 g, 0.0036 mol) and triethylamine (0.55 mL, 0.40 g, 0.004 mol) in aqueous tetrahydrofuran (50%, 75 mL) was added, portionwise as a solid, 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (0.99 g, 0.004 mol). All was stirred at ambient temperature over 3 hours. The solution was concentrated to half volume and extracted with diethylether. The aqueous layer was then acidified to a pH of 1.0 using hydrochloric acid (1N) and then extraced with ethyl acetate. The combined organic layers were dried over anhydrous magnesium sulfate and evaporated to dryness under reduced pressure. The residue was purified by flash chromatography using hexane:ethyl acetate::8:2 to give methyl N-(BOC)-8-aminomethyl-5,6,7,8-tetrahydro-2-naphthoate (0.54 g, 0.0017 mol, 47%) as a white solid. mp = 72-80°C; ^{1}H NMR (DMSO) d 13.8 (s, 1H), 7.8-7.65 (m, 3H), 7.6-7.5 (m, 3H), 7.25-7.20 (m, 1H), 7.15-7.05 (m, 1H), 3.9-3.8 (m, 1H), 3.2-2.8 (m, 4H), 1.8-1.6 (m, 3H), 1.4 (s, 6H).

Part E - To a solution of methyl N-(BOC)-8-aminomethyl-5,6,7,8-tetrahydro-2-naphthoate (0.50 g, 0.0016 mol) in ethanol (12.5 mL) was added, dropwise, a solution of sodium hydroxide (0.50 g) in water (12.5 mL). All was stirred a reflux over 4 hours. The reaction mixture was concentrated to half volume and then acidified to a pH equal to 1.0 using hydrochloric acid (1N). The residue was puified by flash chromatography using a gradient of hexane:ethyl acetate::1:1 to ethyl acetate to ethyl acetate: methanol::9:1 to give the racemic mixture of the title compound, N-(BOC)-2-aminomethyl-5,6,7,8-tetrahydro-2-naphthoic acid (0.19 g, 0.00062 mol, 39%)

as a white solid. mp = 172-176°C; ^{1}H NMR (DMSO) d 7.8 (s, 1H), 7.65 (d, 1H, J=8.1 Hz), 7.15 (d, 1H, J=8.1 Hz), 7.1-7.0 (m, 1H), 3.2-3.1 (m, 2H), 3.0-2.7 (m, 4H), 1.8-1.6 (m, 4H), 1.4 (s, 9H).

N-(BOC)-8-aminomethyl-2-naphthoic acid (14)

The remaining naphthoate (11) was treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in dioxane to aromatize the adjacent ring to give the methyl 8-cyano-2-naphthoate (13). Then, the nitrile was reduced via hydrogentation and the methyl ester saponified to the carboxylic acid. This acid was then N-BOC-protected to give N-(BOC)-8-aminomethyl-2-naphthoic acid (14) as an intermediate for incorporation into the cyclic peptide.

Part A - A solution of methyl 8-cyano-5,6-dihydro-2-naphthoate (1.0 g, 0.0047 mol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (1.07 g, 0.0047 mol) in dioxane (50 mL) was stirred at 120°C over 16 hours. The reaction mixture was poured into ice water and extracted with ethyl acetate. The combined organic layers were dried over anhydrous magnesium sulfate and evaporated to dryness under reduced pressure. The residue was purified by flash chromatography using ethyl acetate to give methyl 8-cyano-2-naphthoate (0.72 g, 0.0034 mol, 73%) as a tan solid. mp = 178-182°C; ^{1}H NMR ($CDCl_3$) d 8.95 (s, 1H), 8.3-8.2 (m, 1H), 8.15-8.10 (m, 1H), 8.0-7.95 (m, 2H), 7.7-7.6 (m, 1H), 4.05 (s, 1H).

Part B - A mixture of methyl 8-cyano-2-naphthoate (1.0 g, 0.0047 mol) in methanol (35 mL) with concentrated hydrochloric acid (0.69 mL) andpalladium on carbon catalyst (0.20 g, 5% Pd/C) was shaken for 6 hours at ambient temperature under anatmosphere of hydrogen (50 psi). The reaction mixture was filtered over Celite® and washed with methanol. The filtrate was evaporated to dryness under reduced pressure and the residue was triturated with hexane to give methyl 8-aminomethyl-2-naphthoate (0.76 g, 0.0035 mol, 75%) as an oil. ^{1}H NMR (DMSO) d 8.75 (s, 1H), 8.5 (bs, 2H), 8.2-8.05 (m, 3H), 7.75-7.70 (m, 2H), 4.6 (s, 2H), 3.95 (m, 3H).

Part C - To a solution of methyl 8-aminomethyl-2-naphthoate (0.75 g, 0.0035 mol) in dry tetrahydrofuran (50 mL), cooled to 0°C, was added a solution of lithium hydroxide (0.5 M, 5.83 mL). All was stirred at ambient temperature over 20 hours. Another aliquot of lithium hydroxide was added and all was stirred for an additional 20 hours. The solid was collected and the filtrate was evaporated to dryness under reduced pressure. The solids were triturated with diethyl ether to give 8-aminomethyl-2-naphthoic acid (0.67 g, 0.0033 mol, 95%) as a white solid. mp = 223-225°C; ^{1}H NMR (DMSO) d 8.6 (s, 1H), 8.1-7.9 (m, 1H), 7.8-7.7 (m, 4H), 7.55-7.5 (m, 1H), 7,45-7.35 (m, 2H), 4.2 (s, 2H).

Part D - A solution of 8-aminomethyl-2-naphthoic acid (0.50 g, 0.00025 mol) and triethylamine (0.038 mL, 0.028 g, 0.000275 mol) in aqueous tetrahydrofuran (50%, 5 mL) was added, portionwise as a solid, 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (0.068 g, 0.000275 mol). All was stirred at ambient temperature over 5 hours. The solution was concentrated to half

volume and extracted with diethylether. The aqueous layer was then acidified to a pH of 1.0 using hydrochloric acid (1N) and then extraced with ethyl acetate. The combined organic layers were dried over anhydrous magnesium sulfate and evaporated to dryness under reduced pressure to give the title compound, N-(BOC)-8-aminomethyl-2-naphthoic acid (0.050 g, 0.00017 mol) as a white solid. mp = 190-191°C; ^{1}H NMR (DMSO) d 13.1 (bs, 1H), 8.8 (s, 1H), 8.0 (q, 2H, J=7.9 Hz), 7.9 (d, 1H, J=8.1 Hz), 7.6 (t, 1H, J=7.5 Hz), 7.65-7.55 (m, 2H), 4.6 (d, 2H, J=5.5 Hz), 1.4 (s, 9H).

Cyclic Compound Intermediates 89a and 89b

cyclo-(D-Val-NMeArg-Gly-Asp-aminotetralincarboxylic acid); the compound of formula (VIII) wherein J = D-Val, K = NMeArg, L = Gly, M = Asp, $R^1 = R^2 = H$

The title compound was prepared using the general procedure described for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb) (Cyclic Compound Intermediate 4). The DCC/DMAP method was used for attachment of Boc-aminotetralin-carboxylic acid to the oxime resin. The peptide was prepared on a 0.164 mmol scale to give the protected cyclic peptide (69 mg, 49.3%). The peptide (69 mg) and 0.069 mL of anisole were treated with anhydrous hydrogen fluoride at 0°C for 30 minutes. The crude material was precipitated with ether, redissolved in aqueous acetonitrile, and lyophilized to generate the title compound (59.7 mg, greater than quantitative yield; calculated as the fluoride salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a

0.23%/ min. gradient of 16.2 to 27% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid. Two isomers were obtained; isomer #1 (12.5% recovery, overall yield 6.2%, FAB-MS: [M+H] = 615.34; isomer #2 (18.6% recovery, overall yield 9.3%, FAB-MS: [M+H] = 615.35.

Cyclic Compound Intermediate 89c

cyclo-(D-Val-NMeArg-Gly-Asp-aminomethylnaphthoic acid); the compound of formula (IX) wherein J = D-Val, K = NMeArg, L = Gly, M = Asp, R^1 = H, R^2 = H

The title compound was prepared using the general procedure described for cyclo-(D-Val-NMeArg-Gly-Asp-Mamb) (Cyclic Compound Intermediate 4). The DCC/DMAP method was used for attachment of Boc-aminomethyl-naphthoic acid to the oxime resin. The peptide was prepared on a 0.737 mmol scale to give the protected cyclic peptide (463 mg, 73.1%). The peptide (463 mg) and 0.463 mL of anisole were treated with anhydrous hydrogen fluoride at 0°C for 20 minutes. The crude material was precipitated with ether, redissolved in aqueous acetonitrile, and lyophilized to generate the title compound (349 mg, greater than quantitative yield; calculated as the fluoride salt). Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.5 cm) using a 0.45%/ min. gradient of 4.5 to 22.5% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy white solid (12.1% recovery, overall yield 7.8%); FAB-MS: [M+H] = 625.32.

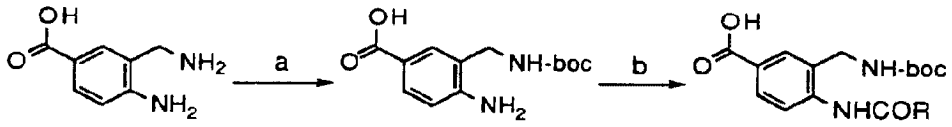
Synthesis of Linker Modified Cyclic Compound Intermediates

Linker modified cyclic compound intermediates can be synthesized either by incorporating an appropriately protected linker into a cyclizing moiety and then synthesizing the linker modified cyclic compound intermediate or by attaching the linker to a cyclic compound intermediate.

Linker Modified Cyclizing Moieties

Linker modified cyclizing moieties can be synthesized either by attaching the linker to a ring substituted cyclizing moiety synthesized as described above or by incorporating an appropriately protected linker into the synthesis of the cyclizing moiety.

For example, the ring substituted cyclizing moiety described above where X = NH_2 can be reacted with the succinimidyl linker, RCOOSu (R = $-(CH_2)_5-NH_2$ or $CH_2-C_6H_5-p-NH_2$), to give a linker attached at position X via an amide group.

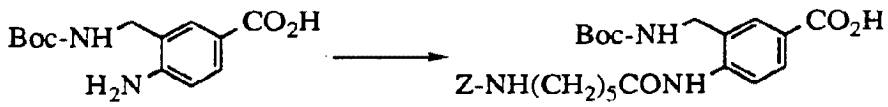


a] Boc-ON b] RCOOSu

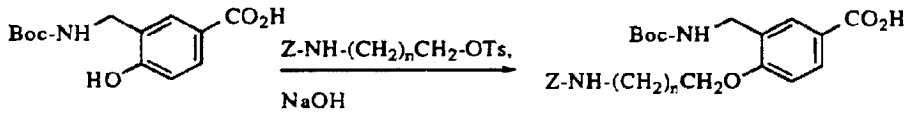
The ring substituted cyclizing moiety with X = OH can be reacted with a linker derived from tetraethylene glycol. This linker consists of four ethylene units separated by ether groups, and bearing a Z-protected

amine group at one end of the tether, and a leaving group such as tosylate at the other end of the tether. This will give a linker attached at position X via an ether group.

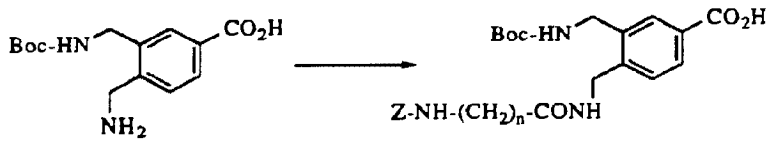
The ring substituted cyclizing moiety with Z = NH_2 can be reacted with $(Z\text{-}NH(CH_2)_5CO)_2O$ to give a linker attached at position Z via an amide group.



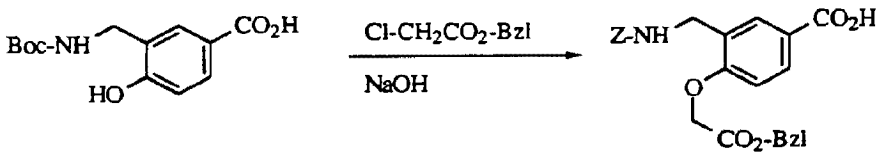
Linkers can be attached to the ring substituted cyclizing moiety with Z = OH. Attachment of the linkers to the ring will require the linker having a leaving group suitable for reaction with a phenolate ion. Such leaving groups include halides, aryl sulfonates (e.g., tosylate) and alkyl tosylates (e.g., mesylate). For example, an alkyl chain bearing a tosyl group at one end of the chain and a protected amine at the other end is used. The literature provides several examples of alkylation at a phenolic group in the presence of a carboxylic acid group (See, for example Brockmann, Kluge, and Muxfeldt (1957), *Ber. Deutsch. Chem. Ges.*, **90**, 2302.



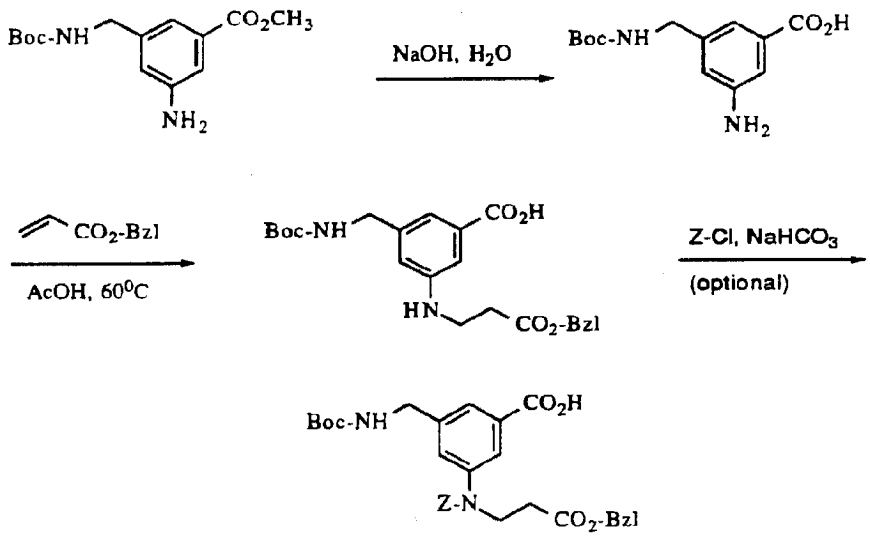
The ring substituted cyclizing moiety with Z = CH_2NH_2 can be reacted with $Z\text{-}NH(CH_2)_n\text{-}COOSu$ to give linkers attached at position Z via an amidomethyl group.



The previous examples have demonstrated the use of linkers which terminate in a protected amine. Linkers that terminate in a carboxylic acid or ester groups may also be desirable. Several such linkers can be attached to the cyclizing moieties described above. For example, in the following scheme, t-Boc protected 3-aminomethyl-4-hydroxybenzoic acid is treated with benzyl chloroacetate and base to introduce a short linker terminating in an ester.



A linker can be attached to the ring substituted cyclizing moiety where Y = NH_2. As shown in Scheme 8, hydrolysis of the methyl ester of t-Boc protected methyl 3-aminomethyl-5-aminobenzoate under mild base conditions, followed by treatment with benzyl acrylate (Lancaster Synthesis, Inc.) and acetic acid catalyst would produce the Michael addition product. Even though this linker modifed cyclizing moiety contains an unprotected secondary amine, it could be used directly in a solid phase synthesis. However, amine protection, if desired, could be accomplished by treatment with benzyl chloroformate and a mild base.



Scheme 8

The linker can also be incorporated into the synthesis of the cyclizing moieties. One example is the synthesis of linker modified cyclizing moiety 5-Aca-Mamb.

Synthesis of Boc-Mamb(Z-5-Aca)

This synthesis is depicted in Scheme 9, below.

Part A - Methyl 3-Nitro-5-hydroxymethylbenzoate

To a solution of monomethyl 3-nitroisophthalate (396.0 g, 1.76 mol) in anhydrous THF (1000 ml) was added 2.0 M BMS (borane methylsulfide complex) in THF (880 ml, 1.76 mol) dropwise over 1 hour. The resulting solution was heated to reflux for 12 hours, and MeOH (750 ml) was slowly added to quench the reaction. The solution was concentrated to give a yellow solid which was recrystalized from toluene (297.5 g, 80%). ^{1}H NMR

($CDCl_3$): 8.71-8.70 (m, 1H), 8.41-8.40 (m, 1H), 8.31-8.30 (m, 1H), 4.86 (s, 2H), 3.96 (s, 3H), 2.47 (s, 1H); MP = 76.5-77.5°C; DCI-MS: [M+H] = 212.

Part B - 3-Carbomethoxy-5-nitrobenzyl Methanesulfonate

Methyl 3-nitro-5-hydroxymethylbenzoate (296.0 g, 1.40 mol) and proton sponge (360.8 g, 1.68 mol) were dissolved in ethylene dichloride (150 ml). Triflic anhydride (292.3 g, 1.68 mol) dissolved in ethylene dichloride (800 ml) was added dropwise to the suspension over 90 minutes and the mixture allowed to stir 18 hour under nitrogen. The reaction was quenched with H_2O (2000 ml), the two layers were separated, and the organic layer was washed with 1000 ml portions of 1 N HCl, H_2O, saturated $NaHCO_3$, H_2O, and saturated NaCl. The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The resulting yellow solid was recrystalized from toluene to give the title compound as a tan solid (366.8 g, 91%). ^1H NMR ($CDCl_3$): 8.84-8.85 (m, 1H), 8.45-8.46 (m, 1H), 8.40-8.39 (m, 1H), 5.35 (s, 2H), 3.98 (s, 3H), 3.10 (s, 3H); MP = 96-97°C; DCI-MS: [M+NH_4] = 307.

Part C - Methyl 3-Azidomethyl-5-nitrobenzoate

3-Carbomethoxy-5-nitrobenzyl methanesulfonate (300.0 g, 1.04 mol) and sodium azide (81.0 g, 1.25 mol) were suspended in DMF (1700 ml) and stirred at room temperature for 5 hours. The reaction was diluted with ethyl acetate (2000 ml), washed with 1000 ml portions of H_2O (2X) and saturated NaCl (1X), dried ($MgSO_4$), and concentrated under reduced pressure. The resulting amber syrup was dried under vacuum at 40°C to yield the title compound as a tan solid (226.5 g, 92%). ^1H NMR

($CDCl_3$): 8.60 (s, 1H), 8.26 (s, 1H), 8.20 (s, 1H), 4.52 (s, 2H), 3.88 (s, 3H); MP = 44-46°C.

Part D - Methyl 3-Amino-5-aminomethylbenzoate

A solution of Methyl 3-Azidomethyl-5-nitrobenzoate (15.50 g, 65.7 mmol) and benzene sulfonic acid (22.14 g, 140 mmol) in warm methanol (320 ml) was placed in a Parr shaker bottle and purged with nitrogen for 15 minutes. Palladium on carbon catalyst (10% Pd/C, 4.0 g) was added and the shaker bottle was further purged with 7 pressurization-evacuation cycles, repressurized, and allowed to shake 18 hours, during which time the required amount of hydrogen was consumed. The catalyst was removed by filtration through a bed of Celite and the filtrate was concentrated under reduced pressure yielding a tan oil. Trituration with refluxing EtOAc (2 X 150 ml) followed by cooling 12 hours at -5°C gave a tan solid which was collected by filtration, washed with EtOAc (2 X 50 ml) and dried under vacuum (25.82 g, 80%). ^1H NMR (CD_3OD): 8.25-8.23 (m, 1H), 8.07-8.06 (m, 1H), 7.86-7.80 (m, 5H), 7.49-7.42 (m, 6H), 4.29 (s, 2H), 3.97 (s, 3H).

Part E - Methyl 3-Amino-5-(t-butoxycarbonylamino)-methylbenzoate

A solution of methyl 3-amino-5-aminomethylbenzoate (19.32 g, 39.0 mmol), TEA (7.89 g, 78.0 mmol), and di-t-butyl dicarbonate (8.51 g, 39.0 mmol) in MeOH (350 ml) was allowed to react 24 hours at room temperature and concentrated to yield a colorless solid. Purification by flash chromatography (silica gel; 1:1 hexane:EtOAc) gave the product (9.21 g, 84%) as a colorless solid. ^1H NMR (CD_3OD): 7.26-7.25 (m, 2H), 6.86-6.85 (m, 1H), 4.16

(s, 2H), 3.88 (s, 3H), 1.48 (s, 9H); MP = 57-65°C. ESI-MS: [M+H] = 281.

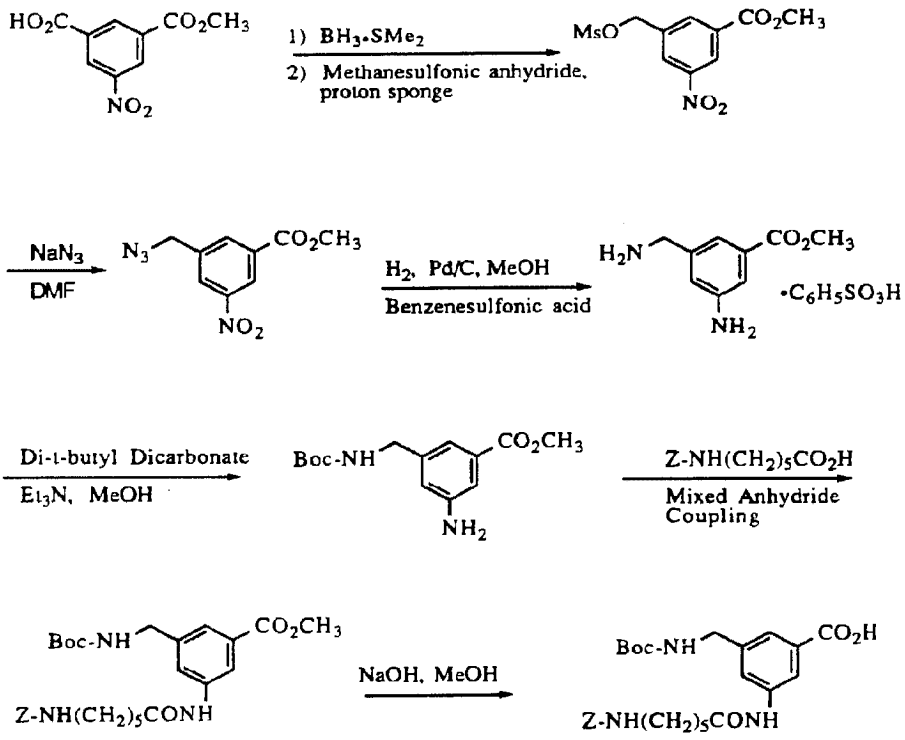
Part F - Boc-Mamb(Z-5-Aca)-OMe

N-CBZ-e-aminocaproic acid (7.77 g, 29.3 mmol) and TEA (2.97 g, 29.3 mmol) were dissolved in anhydrous THF (250 ml) and cooled to -20°C. Isobutylchloroformate (4.00 g, 29.3 mmol) was added dropwise and the mixture allowed to react for 5 minutes at -20°C. Methyl 3-Amino-5-(t-butoxycarbonylamino)methylbenzoate (8.20 g, 29.3 mmol) dissolved in anhydrous THF (50 ml) was cooled to -20°C and added to the reaction. The reaction mixture was allowed to slowly warm to room temperatures and was stirred for an additional 2 days. The solids were removed by filtration and the filtrate was concentrated under reduced pressure. The resulting residue was dissolved in EtOAc (125 ml) and washed with two 50 ml portions each of 0.2 N HCl, saturated $NaHCO_3$, and saturated NaCl. The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by flash chromatography (silica gel; 1:2 hexane:EtOAc), and recrystallization from CCl_4 to give the title compound (10.09 g, 65%) as a colorless solid. ^1H NMR ($CDCl_3$): 8.03-7.63 (m, 3H), 7.32-7.28 (m, 5H), 5.12-4.92 (m, 4H), 4.27-4.25 (m, 2H), 3.85 (s, 3H), 3.17-3.12 (m, 2H), 2.34-2.28 (m, 2H), 1.72-1.66 (m, 2H), 1.48-1.53 (m, 2H), 1.43 (s, 9H), 1.36-1.34 (m, 2H); MP = 52-54°C. ESI-MS: [M+H] = 528.

Part G - Boc-Mamb(Z-5-Aca)

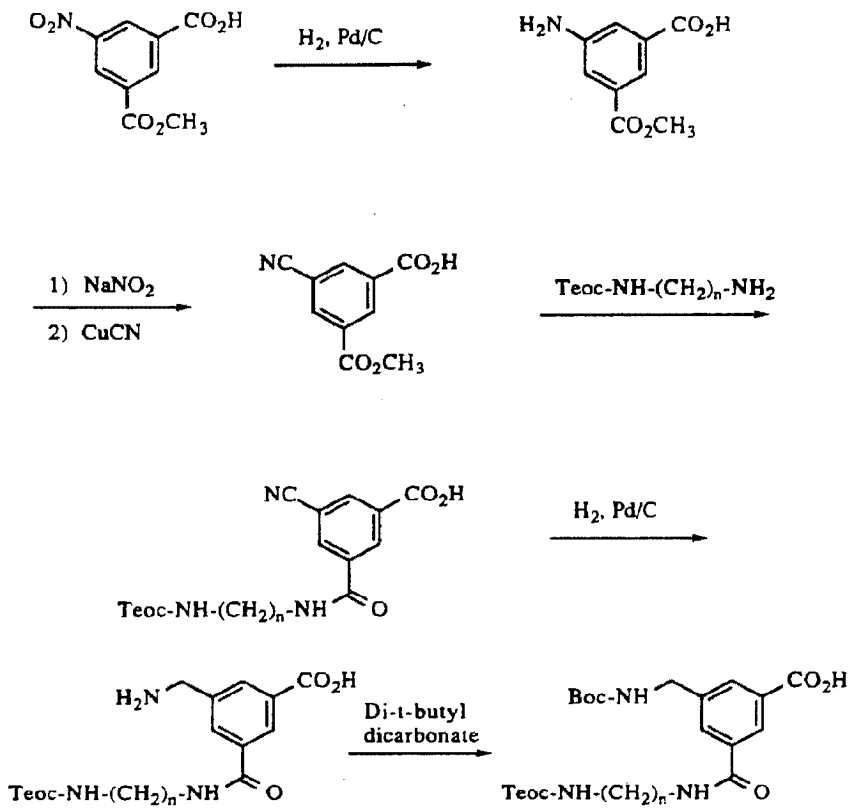
Boc-Mamb(Z-5-Aca)-OMe (22.58 g, 43.0 mmol) was dissolved in 1:1 1 N NaOH:MeOH (500 ml) and allowed to stir 18 hours at room temperature. The reaction was partitioned between EtOAc (300 ml) and H_2O (200 ml) and

the two layers were separated. The pH of the aqueous layer was lowered to 4.5, and the resulting oily precipitate was extracted into EtOAc (2 X 300 ml). The organic extract was dried ($MgSO_4$) and concentrated to a yellow solid. The solid was triturated with refluxing CCl_4 (3 X 100 ml) to give the product (14.17 g, 64%) as a colorless solid. ^1H NMR (CD_3OD): 8.04 (s, 1H), 7.71-7.66 (m, 2H), 7.30-7.23 (m, 5H), 5.02 (s, 2H), 4.24 (s, 2H), 3.32 (s, 3H), 3.11 (t, J = 6.8 Hz, 2H), 2.34 (t, J = 6.8 Hz, 2H), 1.74-1.35 (m, 15H); MP = 168-169°C. DCI-MS: $[M+NH_4]$ = 531.



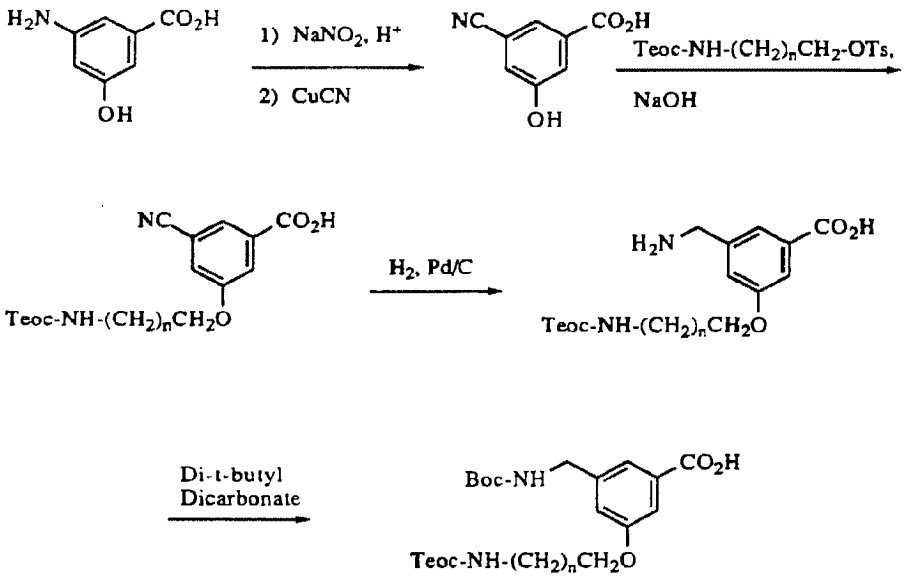
Scheme 9

Scheme 10 teaches how a linker attached to the cyclizing moiety via a reverse amide functional group can also be synthesized. Reduction of the nitro group of monomethyl 3-nitroisophthalate (Fluka) using palladium on carbon would give monomethyl 3-aminoisophthalate, which can be converted to the corresponding nitrile by the Sandmeyer procedure. Treatment of this ester with a mono-protected diamine would yield the corresponding amide. The protecting group on the diamine must be stable to hydrogenation conditions. The Scheme demonstrates the used of the Teoc (2-trimethylsilylethyloxycarbonyl) group, but others familiar to those skilled in the art can also be used. Reduction of the nitrile using palladium on carbon would give the linker modified cyclizing moiety.



Scheme 10

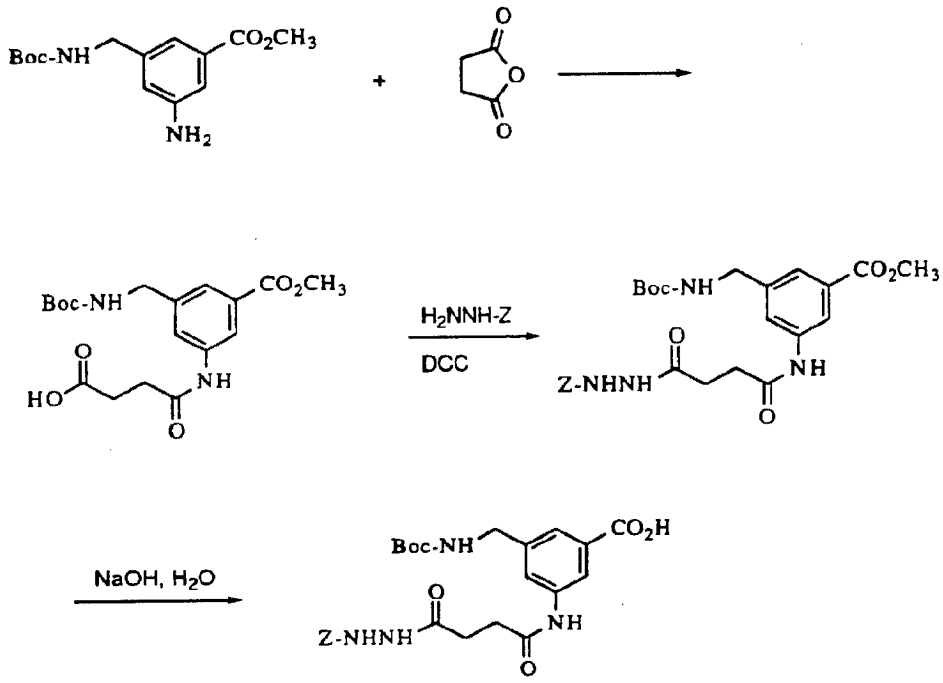
Linkers attached at position Y of the ring substituted cyclizing moieties via an ether linkage can be synthesized, starting from 3-hydroxy-5-aminobenzoic acid. A Sandmeyer reaction can be used to convert the amine to a 3-hydroxy-5-cyanobenzoic acid. Alkyklation as above introduces the linker. Reduction of the nitrile using palladium on carbon catalyst would provide the aminomethyl group. Protection of the amine with the t-Boc group using di-t-butyl dicarbonate would provide linker modified cyclizing moieties ready for use in a solid phase synthesis. This is shown in Scheme 11.



Scheme 11

Linkers terminating in a carboxylic acid group can be synthesized using cyclic anhydrides. Scheme 12 illustrates such a synthesis using succinic anhydride. Reaction of t-Boc protected methyl 3-aminomethyl-5-aminobenzoate with succinic anhydride would give the carboxylic acid linker. Activation of the carboxylic acid and condensation with benzyl carbazate (Lancaster Synthesis, Inc.) would give the protected hydrazide. This hydrazide serves to protect the carboxylic acid during the remainder of the synthesis. Hydrolysis of the methyl ester provides the linker modified cyclizing moiety in a form ready to be used in the solid phase synthesis. After synthesis is complete, removal of the Cbz protecting group from the hydrazide opens the way for the preparation of an azide and azide coupling to the chelator (Hofmann, Magee, and Lindenmann (1950) *J.*

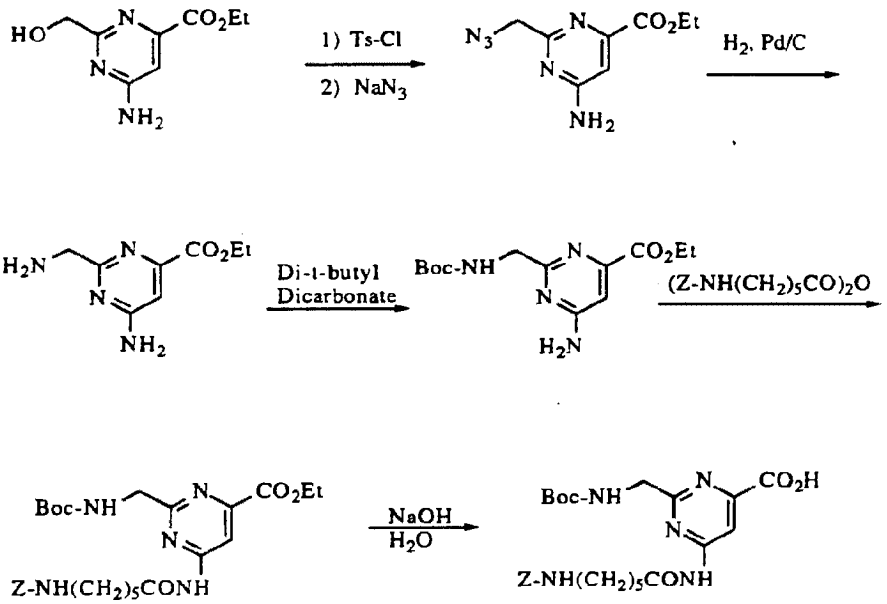
Amer. Chem. Soc., **72**, 2814). This is shown in Scheme 12.



Scheme 12

Linkers can also be incorporated into the syntheses of alternate cyclizing moieties. For example, a linker modified heterocyclic cyclizing moiety can be synthesized from 4-amino-6-carbethoxy-1-hydroxymethylpyrimidine (Boger (1994), *J. Amer. Chem. Soc.*, **116**, 82-92). The alcohol would be converted to the amine in three steps. First, treatment with toluenesulfonyl chloride and base would give the tosylate, which on treatment with sodium azide would give the azide. Reduction of the azide over palladium on carbon catalyst would yield the diamine. The large

difference in nucleophilicity of the two amines will allow the selective protection of the aminomethyl group using di-t-butyl dicarbonate. Attachment of a protected linker, such as Z-5-Aca, to the remaining amine would be accomplished using mixed anhydride or symmetrical anhydride chemistry. Finally, hydrolysis of the ethyl ester would give the linker modified heterocyclic cyclizing moiety ready to be coupled to solid phase synthesis resin. This is shown in Scheme 13.

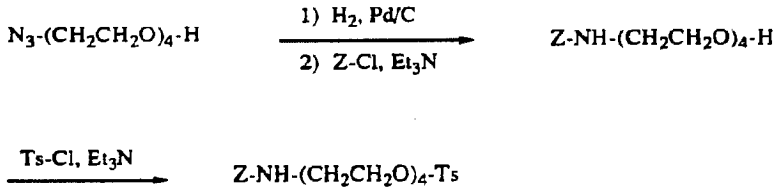


Scheme 13

Linkers

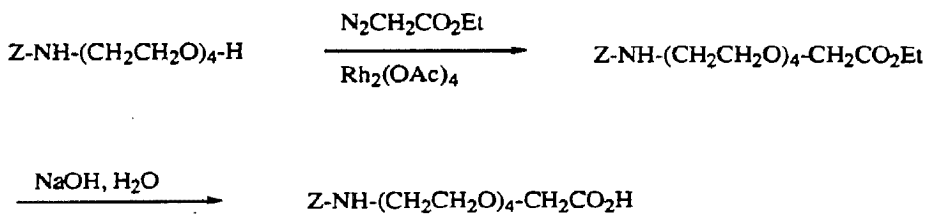
The preparation of the tetraethylene glycol tether discussed above is shown in Scheme 14. The synthesis begins with 1-amino-11-azido-3,6,9-trioxaundecane (Bertozzi and Bednarski (1990), *J. Org. Chem.*, **56**, 4326-

4329). Reduction of the azide with palladium on carbon catalyst gives the amine, which is protected with the Cbz group (designated as "Z" in Scheme 14, and thereafter). The alcohol is now converted to the tosylate using toluenesulsonyl chloride and base.



Scheme 14

A second type of linker composed of ethylene glycol units is shown in the next Scheme. This linker bears a carboxylic acid group on one end, allowing it to be attached to cyclizing moieties containing amine functional groups. The synthesis begins with the Cbz-protected amino alcohol described above. Treatment of the alcohol with ethyl diazoacetate and rhodium(II) acetate dimer would give the e glycolic acid ester having the tetraethylene glycol tail. Hydrolysis of the ethyl ester would provide the linker ready to be coupled to the cyclizing moiety. This is shown in Scheme 15.



Scheme 15

As taught below, these linker modified cyclizing moieties can be used to synthesize linker modified cyclic compound intermediates.

Linker Modified Cyclic Compound 1
Cyclo-(D-Val-NMeArg-Gly-Asp-Mamb(5-Aca))

The synthesis of the title compound is depicted in Scheme 16, shown below.

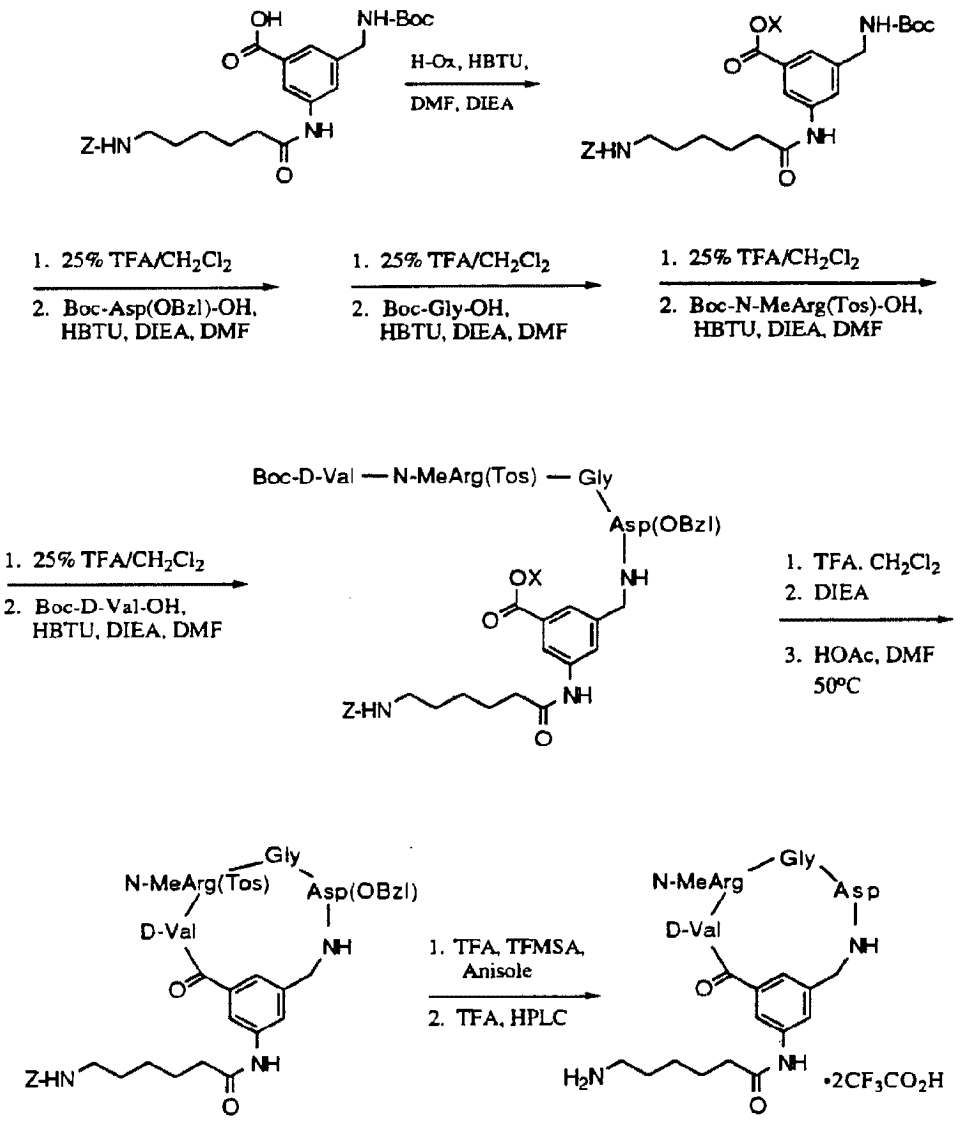
To a 60 ml peptide reaction vessel was added oxime resin (1.61 g, substitution level = 0.62 mmol/g). The resin was swelled by washing once with DMF (30 ml). To the reaction vessel was added Boc-Mamb(Z-5-Aca) (513 mg, 1.0 mmol), HBTU (379 mg, 1.0 mmol), and DIEA (0.52 ml, 3 mmol). The suspension was mixed at room temperature for 96 hr. The resin was washed thoroughly with 30 ml portions of DMF (3X), MeOH (1X), DCM (3X), MeOH (2X), and DCM (3X). The substitution level was determined to be 0.381 mmol/g by the picric acid test. Unreacted oxime groups were blocked by treatment with 30 ml of 0:5 M trimethylacetylchloride/0.5 M DIEA in DMF for 2 hours.

The following steps were then performed: (Step 1) The resin was washed with 30 ml portions of DMF (3X), MeOH (1X), DCM (3X), MeOH (2X), and DCM (3X). (Step 2) The resin was washed with 30 ml of 50% TFA in DCM, and the t-Boc group was deprotected using 30 ml of 50% TFA in DCM for 30 minutes. (Step 3) The resin was washed thoroughly with DCM (3X), MeOH (1X), DCM (2X), MeOH (3X), and DMF (3X). (Step 4) Boc-Asp(OBzl) (0.982 g, 3.04 mmol), HBTU (1.153 g, 3.04 mmol), DIEA (1.59 ml, 9.14 mmol), and DMF (14 ml) were added to the resin and the reaction was allowed to proceed for 22 hours. (Step 5) The completeness of the coupling reaction was

monitored by the picric acid test. Steps 1-5 were repeated until the desired sequence had been attained.

After the linear peptide was assembled, the N-terminal t-Boc group was removed first washing with 50% TFA in DCM, followed by treatment with 30 ml of 50% TFA in DCM for 30 minutes. The resin was washed thoroughly with DCM (3X), MeOH (2X), DCM (3X), and then neutralized with 30 ml portions of 10 DIEA in DCM (2 X 1 min.) The resin was washed with DCM (3X) and MeOH (3X), and dried under vacuum to give 1.965 g of brown resin. The resin was cyclized by suspending in DMF (20 ml) containing HOAc (35 µl, 0.609 mmol) and heating at 50°C for 72 hours. The resin was filtered in a scintered glass funnel and washed thoroughly with 10 ml of DMF (3X). The DMF filtrate was evaporated, and the resulting oil was redissolved in 1:1 acetonitrile:H_2O (20 ml), and lyophilized to give the protected cyclic peptide (342 mg). Purification was accomplished using reversed-phase HPLC with a preparative Vydac C18 column (2.1 cm) and an isocratic mobile phase of 1:1 acetonitrile:H_2O containing 0.1% TFA. Lyophilization of the product fraction gave purified protected peptide (127 mg).

The peptide (120 mg, 0.11 mmol) was deprotected by treating with TFA (1 ml) and triflic acid (1 ml) containing anisole (0.2 ml) for three hours at -10°C. The peptide was precipitated by the addition of ether and cooling to -35°C for 1.5 hours. The peptide was collected by filtration, washed with ether, and dried. The resulting solid was dissolved in 1:1 acetone:H_2O (12 ml) and the pH is adjusted to 4-6 by treatment with Bio-Rad AG1-8X acetate ion exchange resin. The resin was filtered and washed with water. The filtrate was lyophilized to give HPLC pure peptide (75 mg, overall yield 13.5%); FAB-MS: [M+H] = 703.3951.



Scheme 16

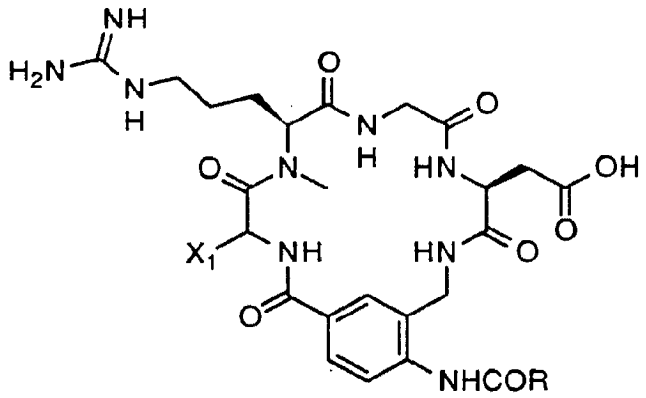
Linker Modified Cyclic Compound 2

Cyclo-(D-Abu-NMeArg-Gly-Asp-Mamb(5-Aca))

The title compound was prepared using the general procedure described for cyclo-(D-Val-NMeArg-Gly-Asp-

Mamb(5-Aca)). The peptide was prepared on a 1.35 mmol scale to give the crude cyclic protected peptide (1.05 g, 73%). The peptide (500 mg) was deprotected by treating with TFA (4 ml) and triflic acid (4 ml) containing anisole (0.8 ml) for three hours at -10°C. The peptide was precipitated by the addition of ether and cooling to -35°C for 1.5 hours. The peptide was collected by filtration, washed with ether, and dried. The resulting solid was dissolved in 1:1 acetone:H_2O (50 ml) and lyophilized. Purification was accomplished by reversed-phase HPLC on a preparative Vydac C18 column (2.1 cm) using a 0.36%/min. gradient of 9 to 18% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy colorless solid (218 mg, 69% recovery, overall yield 37%); FAB-MS: [M+H] = 689.3735.

Linker Modified Cyclic Compounds 3-8



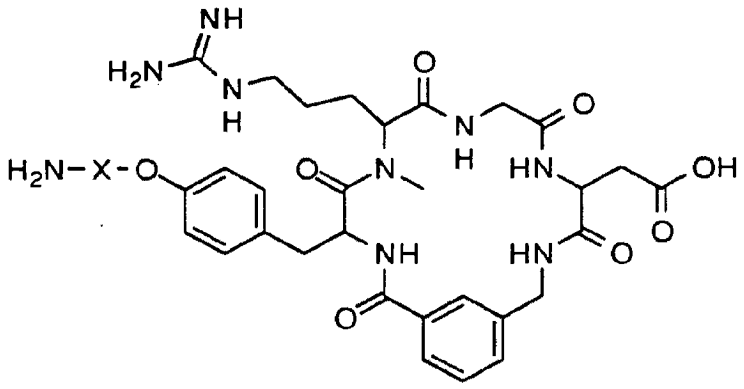
R = $-(CH_2)_5-NH_2$ or $CH_2-C_6H_5-p-NH_2$
X_1 = 2-propyl, ethyl, or p-hydroxyphenylmethyl

Compounds cyclo(D-Val-NMeArg-Gly-Asp-Mamb(4-NHCOR), cyclo(D-Abu-NMeArg-Gly-Asp-Mamb(4-NHCOR), and cyclo(D-

Tyr-NMeArg-Gly-Asp-Mamb(4-NHCOR) can be prepared via the procedure described above.

Linkers can be incorporated into the synthesis of cyclic compound intermediates.

Linker Modified Cyclic Compounds 9,10 and 11



X = CH_2CH_2, $CH_2CH_2CH_2$, $CH_2CH_2CH_2CH_2$

Cyclo(O-2-aminoethyl-D-Tyr)-NMeArg-Gly-Asp-Mamb),
Cyclo(O-3-aminopropyl-D-Tyr)-NMeArg-Gly-Asp-Mamb),
Cyclo(O-4-amino-butyl-D-Tyr)-NMeArg-Gly-Asp-Mamb):

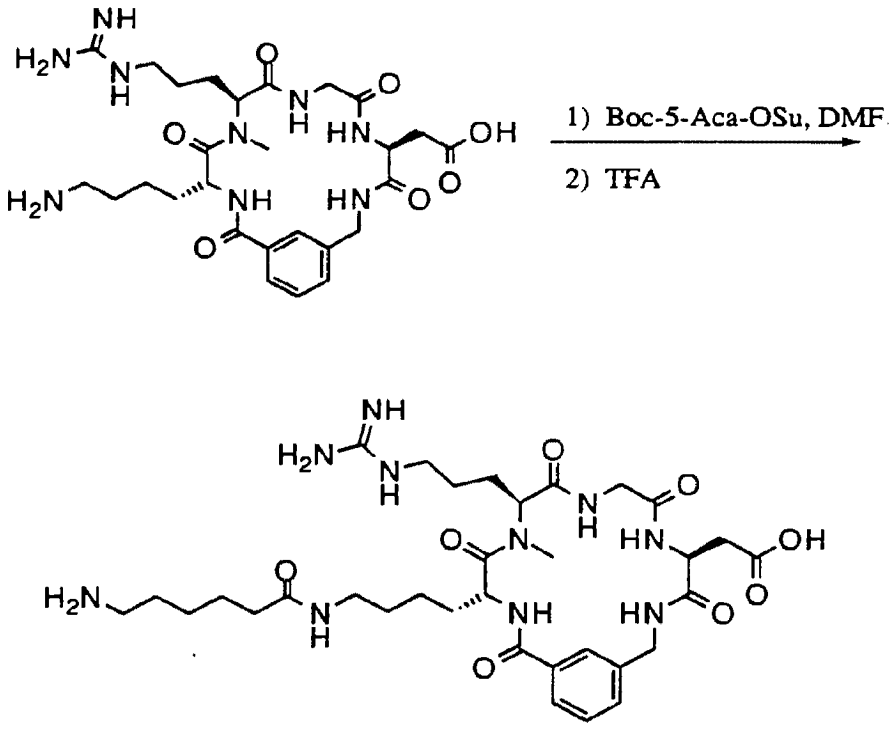
These compounds can be prepared using the procedure described above for Cyclo(D-Tyr-NMeArg-Gly-Asp-Mamb) using linker modified D-Tyr. The O-derivatized D-Tyr can be prepared via the alkylation of boc-D-Tyr with the aminoprotected 2-bromoethylamine (or 3-bromopropylamine, 4-bromobutylamine) in the presence of a base.

Linkers can also be attached to cyclic compound intermediates.

Linker Modified Cyclic Compound 12
Cyclo-(D-Lys(5-Aca)-NMeArg-Gly-Asp-Mamb)

The preparation of the title compound is depicted in Scheme 17, shown below.

A solution of cyclo-(D-Lys-NMeArg-Gly-Asp-Mamb) (100 mg, 0.12 mmol), Boc-5-aminocaproic acid hydroxysuccinimide ester (47 mg, 0.144 mmol), and Et_3N (50 µl, 0.36 mmol) in DMF (1.50 ml) was allowed to react at room temperature for 60 minutes. The progress of the reaction was monitored by normal phase TLC (90:8:2 $CHCl_3$:MeOH:HOAc) using the ninhydrin and Sakaguchi tests. The DMF was removed under reduced pressure. The crude conjugate was treated with TFA (3 ml) at room temperature for 45 minutes to remove the t-Boc protecting group. The TFA was removed under reduced pressure and the conjugate was purified using reversed-phase HPLC with a preparative Vydac C18 column (2.1 cm) using 6% acetonitrile containing 0.1% TFA for 20 minutes, followed by a 3.0%/min. gradient of 6 to 36% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy colorless solid (80 mg, 70%); FAB-MS: [M+H] =



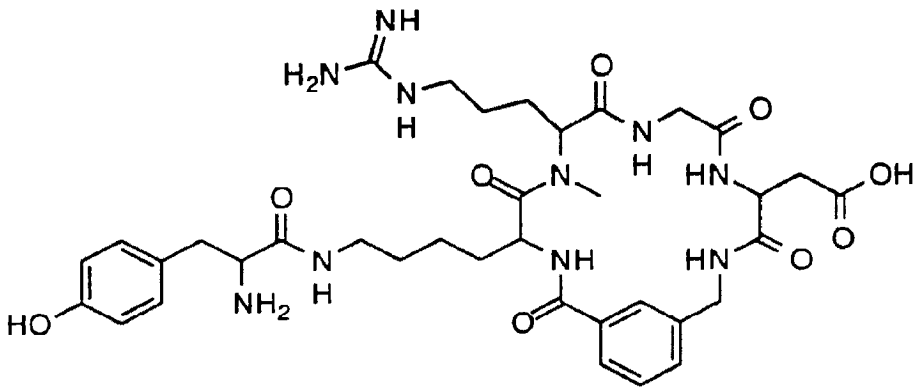
Scheme 17

Linker Modified Cyclic Compound 13
Cyclo-([3-(4-hydroxyphenyl)propyl-D-Lys]-NMeArg-Gly-Asp-Mamb)

A solution of N-succinimidyl-3-(4-hydroxyphenyl)-propionate (Bolton-Hunter reagent; 0.022 g, 0.08 mmol) and DIEA (0.02 ml, 0.10 mmol) in dioxane (5 ml) was added to a solution of cyclo[D-Lys-N-MeArg-Gly-Asp-MAMB] (0.026 g, 0.04 mmol) in pH 9 phosphate buffer (5 ml) and the reaction was allowed to stir for 2 days at room temperature. The solution was lyophilized and the resulting white solid was purified by reversed-phase preparative HPLC on a Vydac C-18 column (2.1 cm) using a 0.36%/min. gradient of 9 to 18% acetonitrile containing

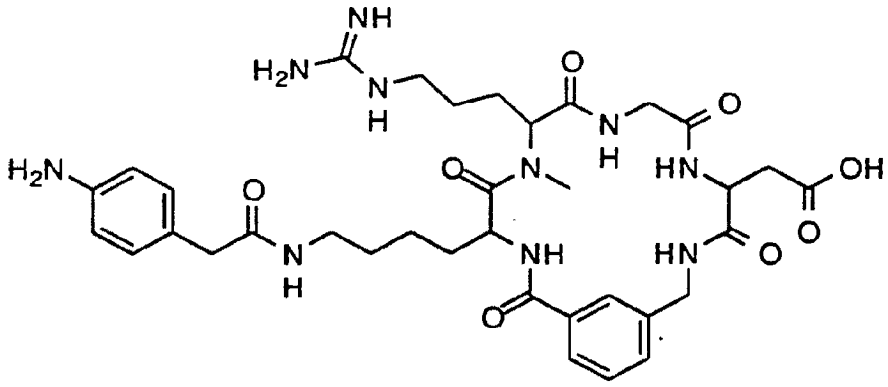
0.1% TFA to give the product (0.018 g, 60%) as a colorless solid. MP = 146-155°C; ESI-MS: [M] = 751.

Linker Modified Cyclic Compound 14
Cyclo((N-E-Tyr-D-Lys)-NMeArg-Gly-Asp-Mamb)



The desired compound can be prepared from the reaction of Cyclo(D-Lys-NMeArg-Gly-Asp-Mamb) with boc-Tyr-OSu in a solvent such as DMF in the presence of a base such as triethylamine, followed by deprotection.

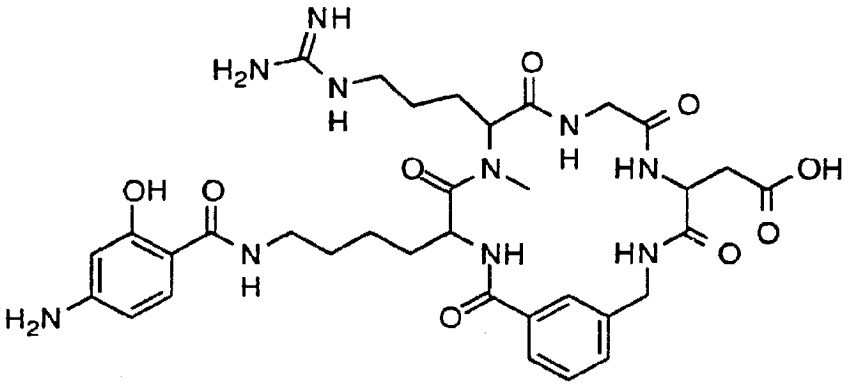
Linker Modified Cyclic Compound 15
Cyclo((N-E-(4-aminophenylacetyl)-D-Lys)-NMeArg-Gly-Asp-Mamb)



The desired compound can be prepared from the reaction of Cyclo(D-Lys-NMeArg-Gly-Asp-Mamb) with succinimidyl fmoc-4-aminophenylacetate in a solvent such as DMF in the presence of a base such as triethylamine, followed by deprotection.

Linker Modified Cyclic Compound 16

Cyclo((N-E-(4-amino-2-hydroxybenzoyl)-D-Lys)-NMeArg-Gly-Asp-Mamb)



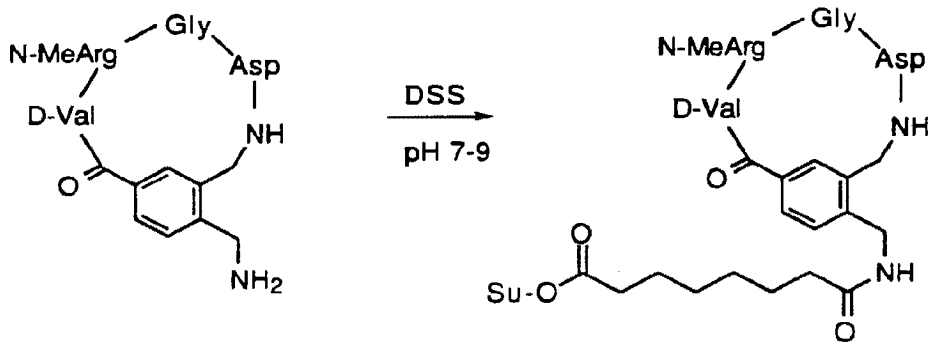
The desired compound can be prepared from the reaction of Cyclo(D-Lys-NMeArg-Gly-Asp-Mamb) with succimidyl 4-amino-2-hydroxybenzoate in a solvent such

as DMF or THF in the presence of a base such as triethylamine.

A variety of linker modifed cyclic compounds can be synthesized using bifunctional cross-linking reagents developed for the derivatization of proteins. These reagents consist of two electrophilic groups, such as active esters or isocyanates, separated by a spacer. The reagents can be homobifunctional, meaning that the two reactive groups are identical, or heterobifunctional. The spacer can be aliphatic or aromatic and may contain additional functionality to modify the lipophilicity of the conjugates, or to allow cleavage of the chain. The following examples will illustrate the use of several commercially available cross-linking reagents using as a starting point a cyclic compound intermediate synthesized with the 4-aminomethyl Mamb unit.

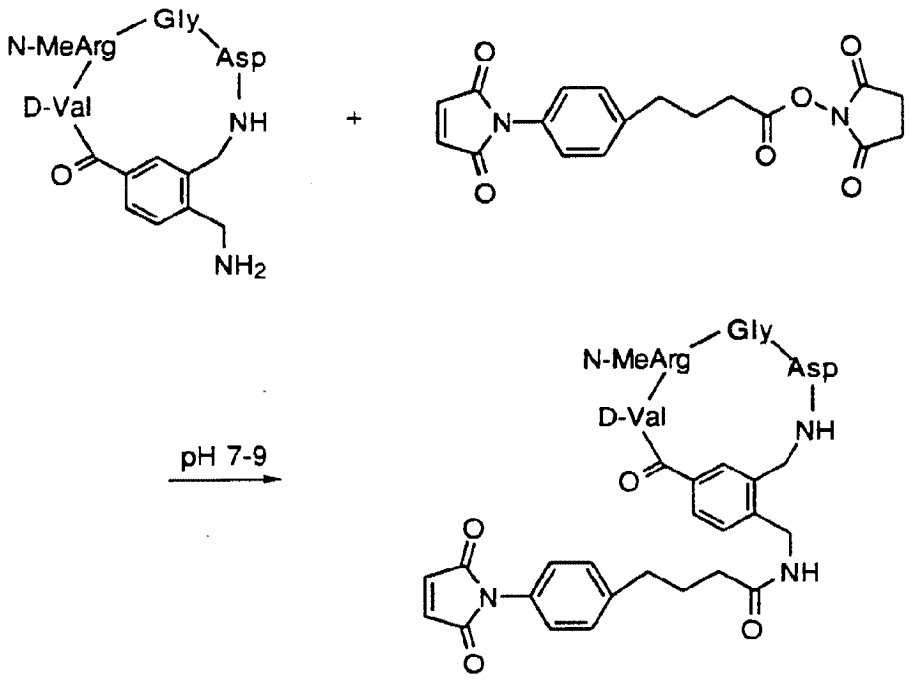
In the first example, the cyclic compound is treated with an excess of DSS (disuccinimidyl suberate, Pierce Chemical Co.) in either aqueous or organic solvent at a pH of between 7 and 9. These are typical reaction conditions for these cross-linking reagents. The excess of cross-linker minimizes the amount of dimeric species formed. The pH of 7-9 allows the amine to react at a reasonable rate but does not produce any appreciable hydrolysis of the second reactive group and prevents reaction with the guanidino group on arginine. The active ester at the end of the linker is stable enough to allow purification by HPLC or flash chromatography. Once purified, the linker modified cyclic compound can be conjugated to a chelator

containing a nucleophilic group, such as an amine or thiol. This is depicted in Scheme 18.



Scheme 18

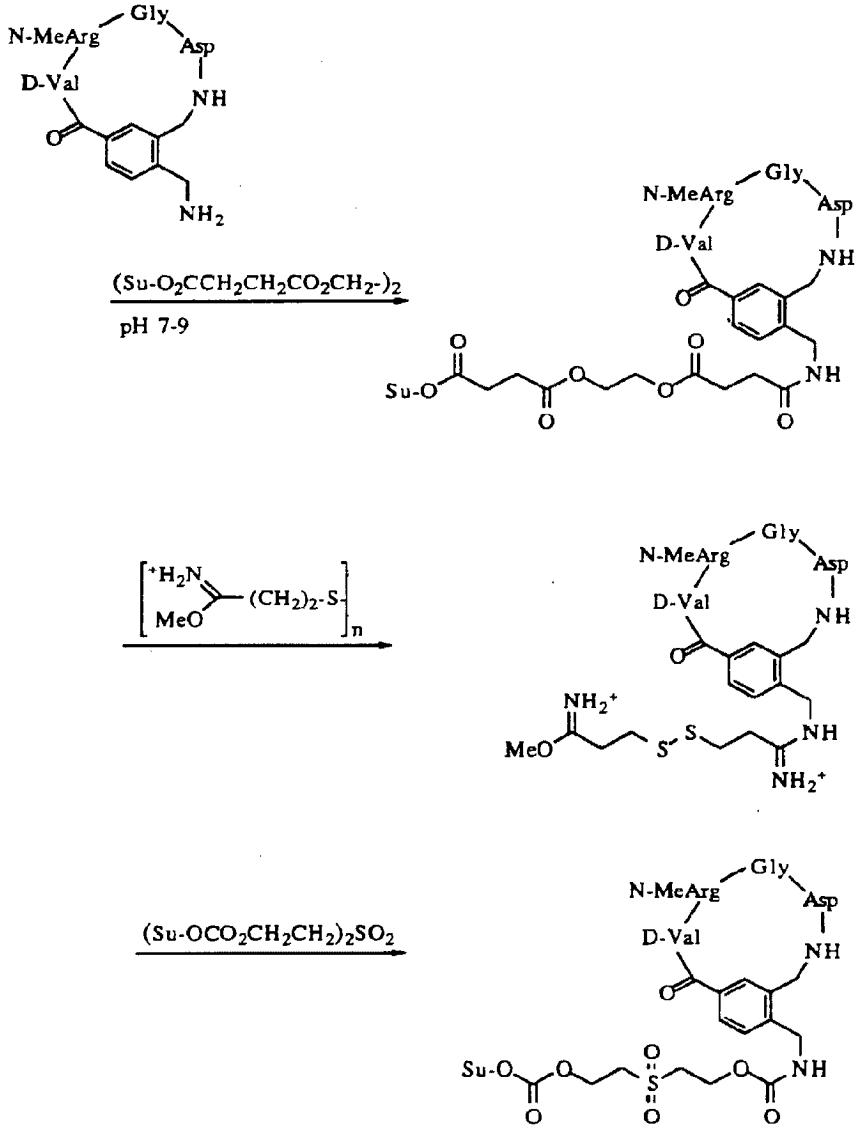
Heterobifunctional reagents are typically used to achieve very selective activatation of peptides and proteins. In the following example SMPB (succinimidyl 4-(p-maleimidophenyl)butyrate, Pierce Chemical Co.) is used to modify an amine-containing cyclic compound and prepare it for coupling to a thiol-containing chelator. Treatment of the cyclic compound with SMPB under slightly basic conditions gives the linker modified cyclic compound in which the linker terminates in a maleimido group. Selectivity is achieved because the maleimido group shows low reactivity towards amine groups, and dimerization is minimized. After purification, the maleimido group can be coupled to a thiol-containing chelator. This is depicted in Scheme 19.



Scheme 19

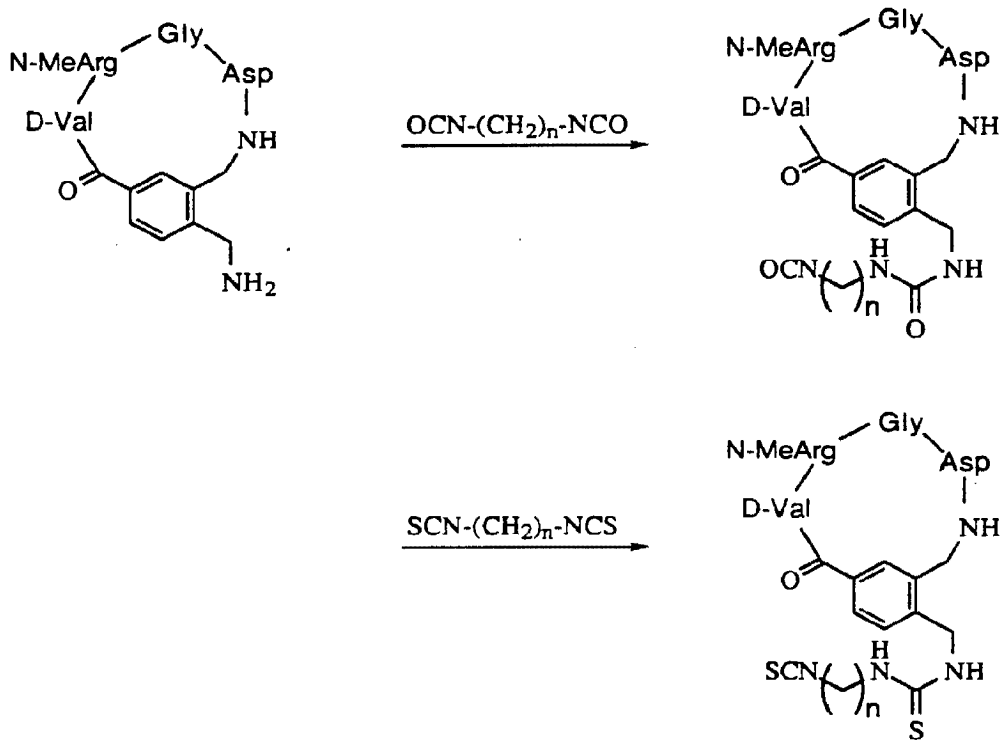
Linkers containing interior functional groups can be prepared with the reagents shown in Scheme 20. EGS (ethylene glycolbis(succinimidylsuccinimidate), Sigma Chemical Co.) is a bis-succinimidyl ester which reacts preferentially with amines. Dimethyl 3,3'-dithiobispropionimidate (DTBP, also called the Wang and Richards reagent, Pierce Chemical Co.) also reacts preferentially with amines. The disulfide is cleaved by thiols. Meares and coworkers have shown (*Int. J. Cancer*: Supplement 2, **1988**, 99-102) that ^{111}In labeled antibody-chelate conjugates joined by a disulfide-containing linker show more rapid clearance of radioactivity from mice than conjugates which did not contain a cleavable linker. The third example of Scheme

20 demonstrates the use of BSOCOES (bis[2-(succinimidooxycarbonyloxy)ethyl]sulfone, Pierce Chemical Co.), a homobifunctional cross-linker which contains an interior sulfone group. This reagent produces a carbamate group on conjugation with an amine.



Scheme 20

Scheme 21 illustrates the use of bisisocyanates and bisisothiocyanates in the preparation of linker modified cyclic compounds. These reagents react with amines to for urea and thiourea groups, respectively. The reagents would be used in excess to minimize the formation of dimers. The isocyanate and isothiocyanate groups at the end of the linkers are sufficiently stable to allow purification of the products.



Scheme 21

Chelators

The present invention also provides novel reagents useful for the preparation of radiopharmaceuticals.

These reagents consist of a chelator, C_h, attached via a linking group, L_n, to a cyclic compound intermediate, Q. These reagents can be synthesized in several ways, either by attaching a chelator to a linker modified cyclic compound intermediate or by attaching a chelator bearing a linking group to the cyclic compound intermediate. Preferably, the chelator is attached to linker modified cyclic compound intermediate.

Any chelator can be used in this invention provided it forms a stable complex to a radioactive isotope. Typically the radioactive isotope is a metal or transition metal and the complex with the chelator is a metal chelate complex. Examples of metal chelate complexes can be found in a recent review (S. Jurisson et. al., Chem Rev., 1993, 93, 1137-1156) herein incorporated by reference.

The chelators can be attached to the linkers by a variety of means known to those skilled in the art. In general, a reactive group on the linker can react with the chelator or alternatively a reactive group on the chelator can react with the linker. Suitable reactive groups include active esters, isothiocyanates, alkyl and aryl halides, amines, thiols, hydrazines, maleimides, and the like. Several linker modified cyclic compounds bearing reactive groups are described in the examples below.

Representative chelators include: diethylenetriamine- pentaacetic acid (DTPA), ethylenediamine-tetraacetic acid (EDTA), 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA), 1,4,7,10-tetraaza-cyclododecane-N,N',N''-

triacetic acid, hydroxybenzyl-ethylene-diamine diacetic acid, N,N'-bis(pyridoxyl- 5-phosphate)ethylene diamine, N,N'-diacetate, 3,6,9-triaza-12- oxa-3,6,9-tricarboxymethylene-10-carboxy-13-phenyl-tridecanoic acid, 1,4,7-triazacyclononane-N,N',N''-triacetic acid, 1,4,8,11- tetraazacyclo-tetradecane-N,N'N'',N'''-tetraacetic acid, 2,3-bis(S- benzoyl)mercaptoacetamido-propanoic acid and the chelators described below. Other chelators may include metal binding regions derived from metal binding proteins such as, for example, metallothionines which are sulfhydryl-rich cytoplasmic proteins present in vertebrates, invertebrates and fungi.

Synthesis of Chelators

Synthesis of 4,5 bis((S-benzoyl)mercaptoacetamido)pentanoic acid (mapt).

The chelator was synthesized as described in Fritzberg et. al., Appl. Radiat. Isot. 1991, 42, 525-530.

Synthesis of (S-benzoyl)mercaptoacetylglycylglycylglycine (MAG_3)

The chelator was synthesized as described in Brandau, W. et al., *Appl. Radiat. Isot.* **1988**, *39*, 121-129.

Synthesis of Succinimidyl 6-Boc-hydrazinopyridine-3-carboxylate (SHNH)

The chelator was synthesized as described in Schwartz et. al., 1990, European Patent Application 90301949.5.

Synthesis of N-[4-(Carboxy)benzyl]-N,N'-bis[(2-triphenylmethylthio)ethyl]glycinamide N-hydroxysuccinimide ester

The synthesis of the title compound is depicted below in Scheme 22.

Part A - S-Triphenylmethyl-2-aminoethanethiol

A solution of cysteamine hydrochloride (79.5 g, 0.7 mol) in TFA (500 ml) was treated with triphenylmethanol (182 g, 0.7 mol), and stirred at room temperature for one hour. TFA was removed under reduced pressure at a temperature of 45°C and the resulting dark orange oil was dissolved in EtOAc (700 ml). The EtOAc solution was washed with cold 2N NaOH (3 X 350 ml), H_2O (2 X 350 ml), saturated $NaHCO_3$ (350 ml), and saturated NaCl (350 ml). The combined aqueous washings were back extracted with EtOAc (350 ml). The combined organic layers were dried ($MgSO_4$) and concentrated to a yellow solid. Trituration with ether (500 ml) gave product (97.2 g, 43%) as a colorless solid, MP 90-92°C (D. Brenner et al., J. Inorg. Chem. 1984, 23, 3793-3797, MP 93-94°C). Concentration of the ether triturant to a volume of 100 ml and cooling produced an additional 40.9 g of product, MP 89-91°C, for a combined yield of 62%.

Part B - N-2-Bromoacetyl-S-triphenylmethyl-2-aminoethanethiol

A solution S-triphenylmethyl-2-aminoethanethiol (48 g, 0.15 mol) and Et_3N (20.9 ml, 0.15 mol) in DCM (180

ml) was slowly added to a stirred solution of bromoacetyl bromide (13.9 ml, 0.15 mol) in DCM (100 ml) at a temperature of -20°C. The reaction was allowed to warm to room temperature over a one hour period. The reaction was washed with 500 ml portions of H_2O, 0.2 N HCl, saturated $NaHCO_3$, and saturated NaCl. The organic solution was dried ($MgSO_4$) and concentrated to an oil. This oil was crystallized from DCM-hexane to give product (54.9 g, 83%) as a colorless solid, MP 137-139.5°C (J.A. Wolff, Ph.D. Thesis, Massachusetts Institute of Technology, February 1992, MP 130-135°C.

Part C - N,N'-Bis[(2-triphenylmethylthio)ethyl]glycinamide

A solution of N-2-Bromoacetyl-S-triphenylmethyl-2-aminoethanethiol (35.2 g, 0.08 mol), S-triphenylmethyl-2-aminoethanethiol (25.5 g, 0.08 mol), and Et_3N (16.7 ml, 0.12 mol) in DCM (375 ml) was kept at room temperature for 24 hours. The solution was washed with 200 ml portions of H_2O (1X), saturated $NaHCO_3$ (2X), H_2O (1X), and saturated NaCl (1X), dried ($MgSO_4$), and concentrated to give a viscous oil. The oil was dissolved in 70:30 DCM:EtOAc (150 ml) and cooled in an ice bath. The solid which formed was removed by filtration. The filtrate was concentrated to a viscous oil. This oil was purified by flash chromatography over 200-400 mesh, 60Å silica gel using 70:30 DCM:EtOAc mobile phase to give product (34.4 g, 63%) as a colorless, amorphous foamy solid. ^1H NMR ($CDCl_3$) 7.42-7.18 (m, 30H), 3.12-3.01 (m, 4H), 2.48-2.27 (m, 6H).

Part D - Methyl 4-(Methanesulfonylmethyl)benzoate

A solution of methyl 4-(hydroxymethyl)benzoate (10.8 g, 0.065 mol) and proton sponge (19.5 g, 0.091

mol) in DCM (200 ml) was treated with methanesulfonic anhydride (13.94 g, 0.08 mol) and stirred at room temperature for 20 hours. The reaction mixture was washed with 100 ml portions of H_2O (1X), 1N HCl (2X), H_2O (1X), saturated $NaHCO_3$ (1X), and H_2O (1X). The organic phase was dried ($MgSO_4$) and concentrated to give 15.5 g of pale yellow solid. Recrystallization from CCl_4 (150 ml) using decolorizing carbon gave product (14.2 g, 90%) as colorless needles, MP 91-94°C.

Part E - N-[4-(Carbomethoxy)benzyl]-N,N'-bis[(2-triphenylmethylthio)ethyl]glycinamide

A solution of N,N'-Bis[(2-triphenylmethylthio)ethyl]glycinamide (16.27 g, 0.024 mol) and methyl 4-(methanesulfonylmethyl)benzoate (4.88 g, 0.02 mol) in ethylene dichloride (200 ml) was heated to reflux for 28 hours. The reaction was washed with 200 ml portions of saturated $NaHCO_3$ and H_2O, dried ($MgSO_4$), and concentrated to a light brown oil (30 g). This oil was purified by flash chromatography over 200-400 mesh, 60Å silica gel using DCM:EtOAc mobile phase to give product (9.9 g, 60%) as a colorless, amorphous foamy solid. ^1H NMR ($CDCl_3$) 7.90 (d, 2H, J = 6.5 Hz), 7.49-7.18 (m, 32H), 3.91 (s, 3H), 3.47 (s, 2H), 3.01 (q, 2H, J = 6.2 Hz), 2.88 (s, 2H), 2.43 (t, 2H, J = 6.2 Hz), 2.39-2.27 (m, 4H).

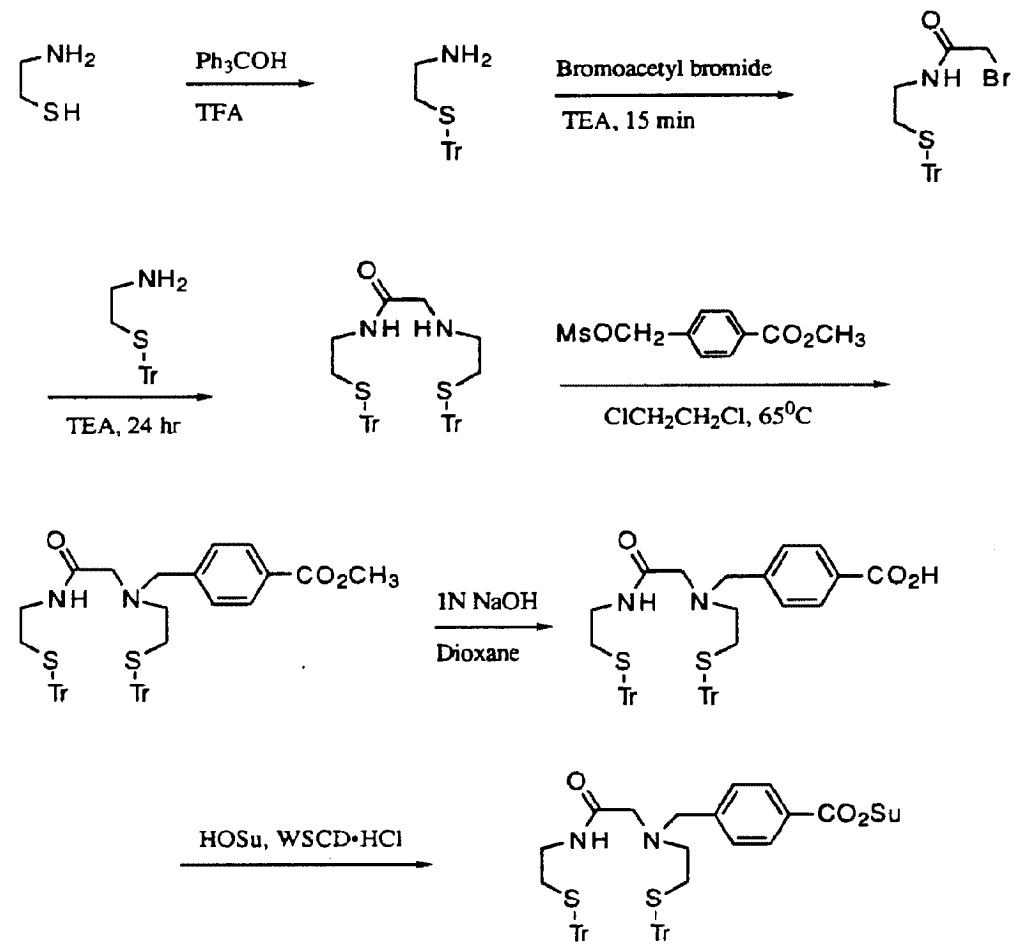
Part F - N-[4-(Carboxy)benzyl]-N,N'-bis[(2-triphenylmethylthio)ethyl]glycinamide

A mixture of N-[4-(carbomethoxy)benzyl]-N,N'-bis[(2-triphenylmethylthio)ethyl]glycinamide (6.00 g, 7.26 mmol) in dioxane (65 ml) and 1N NaOH (65 ml) was stirred at room temperature for 24 hours. The mixture was acidified with 2.5 M citric acid (100 ml) and the

gummy precipitate which formed was extracted into EtOAc (400 ml). The EtOAc solution was washed with H_2O (3 X 200 ml) and saturated NaCl (100 ml), dried ($MgSO_4$), and concentrated to give product (5.90 g, 100%) as a colorless, amorphous foamy solid. ^1H NMR ($CDCl_3$) 7.96 (d, 2H, J = 8.1 Hz), 7.40-7.16 (m, 32H), 3.71 (s, 3H), 3.49 (s, 2H), 3.00 (q, 2H, J = 5.4 Hz), 2.91 (s, 2H), 2.44 (t, 2H, J = 5.4 Hz), 2.38-2.30 (m, 4H).

Part G - N-[4-(Carboxy)benzyl]-N,N'-bis[(2-triphenylmethylthio)ethyl]glycinamide N-hydroxysuccinimide ester

A solution of N-[4-(carboxy)benzyl]-N,N'-bis[(2-triphenylmethylthio)ethyl]glycinamide (450 mg, 0.55 mmol) and N-hydroxysuccinimide (76 mg, 0.66 mmol) in DCM (10 ml) was treated with a solution of WSCD•HCl (122 mg, 0.66 mmol) in DCM (7 ml) and stirred at room temperature for 22 hours. The reaction mixture was concentrated and the solids redissolved in EtOAc (60 ml). The EtOAc solution was washed with H_2O (2 X 25 ml), 0.1 N NaOH (35 ml), H_2O (2 X 25 ml), and saturated NaCl (35 ml), dried (Na_2SO_4), and concentrated to give product (469 mg, 93%) as a colorless solid.



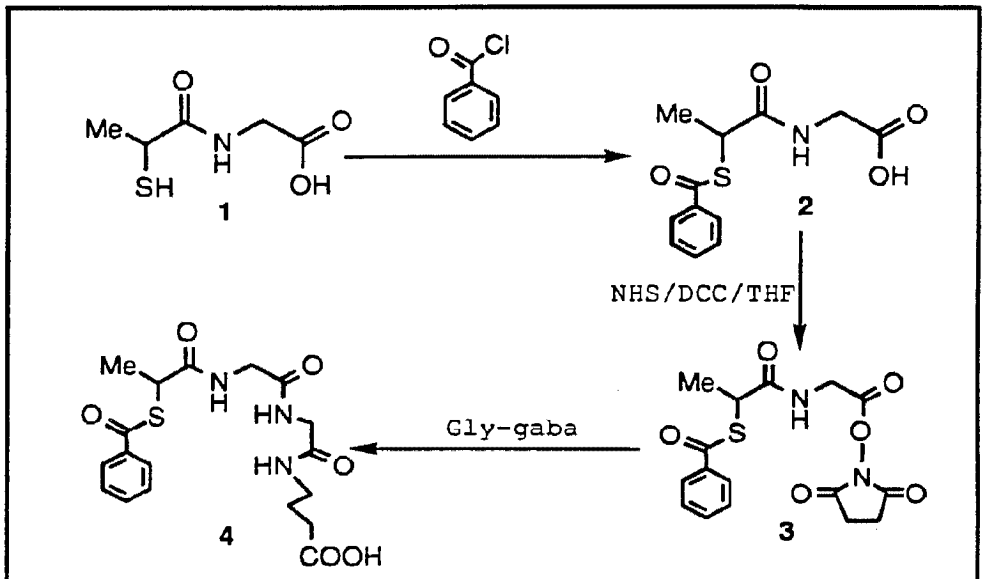
Scheme 22

5

Synthesis of N-[2-(Benzoylthio)propionyl]glycylglycyl-g-Amino-butyric Acid(Bz-Me-MAG2-gaba).

10 The title compound was prepared according to Scheme 23 from N-(2-mercaptopropionyl)-glycine (**1**), which is commercially available from Aldrich. The protection of

the thiol group in compound **1** is achieved by reacting with benzoyl chloride under basic conditions to give compund **2**. The carboxylic group can be activated by forming its succinimide ester (**3**), which reacts with glycyl-g-aminobutyric acid in 90% methanol solution to give the benzoyl-protected Me-MAG_2-gaba (**4**). The spectral (IR, ^1H NMR and FAB-MS) data are completely consistent with the proposed formulation.



Scheme 23. Synthesis of Benzoyl-Protected Me-MAG_2-gaba.

Step 1: N-[2-(benzoylthiol)propionyl]glycine (2). Sodium hydroxide (4.5 g, 0.109 mol) and N-(2-mercaptopropionyl)glycine (8.20 g, 0.05 mol) were dissolved in a mixture of water (40 mL) and toluene (30 mL). The temperature was lowered to 5-15 °C using an ice bath. Benzoyl chloride (4.6 mL, 0.051 mol) in toluene (10 mL) was added dropwise with vigorously stirring. After addition, the mixture was stirred at 5-

15 °C for another 30 min., and then at room temperature for 2 hr. The organic layer was separated, washed with H_2O (2x20 mL), and discarded. Aqueous fractions were combined and acidified to pH ~ 1.5 using concentrated HCl while white solid formed. The precipitate was collected by filtration, washed with H_2O and small amount of ethanol, and dried under vacuum. The yield was 13.0 g (97%). Anal. Calcd (found) for $C_{12}H_{13}NO_4S$: C, 53.90 (53.89); H, 4.90 (4.81); N, 5.24 (5.22). IR (KBr disk, in cm^{-1}): 3375 (s, n_{N-H}) 3200-2500 (br, n_{O-H}); 1745 (vs, thioester $n_{C=O}$); 1663, 1625 (vs, amide and carboxylic $n_{C=O}$). ^1H NMR (DMSO-d_6, d in ppm): 1.47 (d, 3H, CH_3, J = 7.0 Hz); 3.79 (d, 2H, CH_2, J = 5.9 Hz); 4.40 (q, 1H, CH, J = 7.0 Hz); 7.53 (m, 2H, =CH); 7.69 (m, 1H, =CH); 7.90 (dd, 2H, =CH, J = 7.0 Hz); 8.59 (t, 1H, NH, J = 5.8 Hz); 12.6 (bs, 1H, COOH). DCI-MS: *m/z* = 268 ($[M+H]^+$).

Step 2: N-[2-(Benzoylthio)propionyl]glycine Succinimide Ester (3). To a suspension of N-hydroxysuccinimide (5.80 g, 0.05 mol) and N-[2-(benzoylthiol)propionyl]glycine (13.35 g, 0.05 mol) in dry THF (400 mL) was added DCC (12.0 g, 0.052 mol) in the same solvent (100 mL THF) at 5-10 °C. The mixture was stirred at 5 - 10 °C for 2hr, and then at room temperature for 2 days. To the reaction mixture was added 2-3 mL of acetic acid and then stirred for another 2 hr. The solid was filtered off, washed with 2x150 mL of THF. The organic fractions were combined and the solvent was removed under reduced pressure to give a white solid, which was collected, washed with diethyl ether, and dried in air. The yield was 14.5 g (80%). Anal. Calcd (found) for $C_{16}H_{16}N_2O_6S$: C, 52.72 (52.70); H 4.43 (4.21); N, 7.69 (7.69). IR (KBr disk, in cm^{-1}):

3290 (s, n_{N-H}); 1820 (m, succinimide $n_{C=O}$); 1785 (m, ester $n_{C=O}$); 1735 (vs, thioester $n_{C=O}$); 1600 (vs, amide $n_{C=O}$). ^1H NMR ($CDCl_3$, d in ppm): 1.57 (d, 3H, CH_3, J = 7.0 Hz); 2.79 (s, 4H, CH_2); 4.33 (q, 1H, CH, J = 7.0 Hz); 4.39 (m, 2H, CH_2); 7.00 (t, 1H, NH, J = 5.8 Hz); 7.44 (m, 2H, =CH); 7.59 (m, 1H, =CH); 7.93 (dd, 2H, =CH, J = 7.0 Hz). DCI-MS: *m/z* = 365 ($[M+H]^+$).

Step 3: N-[2-(Benzoylthio)propionyl]glycylglycyl-g-Amino-butyric Acid (Bz-Me-MAG$_2$-gaba, 4). N-[2-(Benzoylthio)-propionyl]glycine succinimide ester (1.82 g, 5 mmol) and glycyl-g-aminobutyric acid (0.80 g, 5 mmol) were suspended in a mixture of methanol (150 mL) and water (30 mL). The mixture was heated to reflux for 5 hr, during which time the cloudy mixture became a clear solution. The solution was then cooled to room temperature and was kept stirring overnight. Evaporation of solvents under reduced pressure give a white solid, which was purified by washing with water, and dried under vacuum. The yield was 1.85 g (93%). Anal. Calcd (found) for $C_{18}H_{23}N_3O_6S$: C, 52.78 (52.69); H, 5.66 (5.70); N, 10.27 (10.17). IR (KBr disk, in cm^{-1}): 3380, 3320 (s, n_{N-H}); 3100-2500 (br, n_{O-H}); 1725 (vs, thioester $n_{C=O}$); 1680, 1640, 1624 (vs, amide $n_{C=O}$). ^1H NMR (DMSO-d_6, d in ppm): 1.49 (d, 3H, CH_3, J = 7.0 Hz); 1.62 (qin, 2H, CH_2, J = 7.1 Hz); 2.21 (t, 2H, CH_2COOH, J = 7.5 Hz); 3.05 (qart, 2H, $NH-CH_2$, J = 7.0 Hz); 3.67 (d, 2H, $NH-CH_2$, J = 5.7 Hz); 3.75 (d, 2H, $NH-CH_2$, J = 7.0 Hz); 4.42 (q, 1H, CH, J = 7.0 Hz); 7.57 (m, 2H, =CH); 7.70 (m, 1H, =CH); 7..80 (t, 1H, NH, J = 3.0 Hz); 7.90 (dd, 2H, =CH, J = 7.0 Hz); 8.14 (t, 1H, NH, J = 5.70 Hz); 8.57 (t, 1H, NH, J =

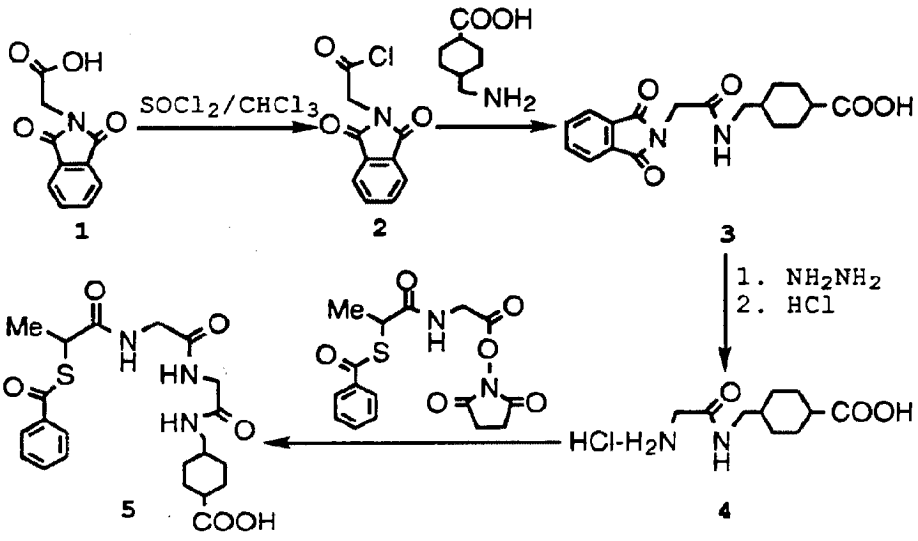
5.90 Hz), 12.0 (bs, 1H, COOH). DCI-MS: *m/z* = 410 ([M+H]$^+$).

Synthesis of N-[2-(Benzoylthio)propionyl]glycylglycylglycine (Bz-Me-MAG$_3$)

The title compound was synthesized as described for Bz-Me-MAG$_2$-gaba by substituting glycylglycine for glycyl-g-aminobutyric acid. The yield was 83%. Anal. Calcd (found) for $C_{16}H_{19}N_3O_6S$: C, 50.39 (50.59); H, 5.02(5.78); N, 11.02 (10.70). IR (KBr disk, in cm^{-1}): 3380, 3300 (s, n_{N-H}); 3100-2500 (br, n_{O-H}); 1738 (vs, thioester $n_{C=O}$); 1680, 1660 (vs, amide $n_{C=O}$). ^{1}H NMR (DMSO-d_6, d in ppm): 1.48 (d, 3H, CH_3, J = 7.05 Hz); 3.78(m, 4H, CH_2); 3.85 (d, 2H, CH_2, J = 6.00 Hz); 4.41 (m, 1H, CH); 7.52 (m, 2H, =CH); 7.70 (m, 1H, =CH), 7.90 (m, 2H, =CH); 8.15 (t, 1H, NH, J = 3.00 Hz); 8.51 (t, 1H, NH, J = 3.00 Hz); 8.80 (t, 1H, NH, J = 3.00 Hz). FAB-MS: *m/z* = 382 ([M+H]$^+$). ESI-MS: *m/z* = 381.9 ([M+H]$^+$).

Synthesis of N-[2-(Benzoylthio)propionylglycylglycyl-4-Amino-methylcyclohexane Carboxylic Acid (Bz-Me-MAG$_2$-ACA).

Synthesis of Bz-Me-MAG$_2$-ACA involves several steps (Scheme 24). Compound 1 could be easily converted to its chloride 2, which reacted with 4-*trans*-amino-methylcyclohexane carboxylic acid to give compound 3. Deprotection of 3 using hydrazine in ethanol, followed by addition of HCl produces 4. Reaction of 4 with Bz-Me-MAG-Succ in methanol in presence of Et_3N afforded Bz-Me-MAG$_2$-ACA 5.



Scheme 24. Synthesis of Bz-Me-MAG$_2$-ACA.

Step 1: Phthaloylglycyl Chloride. Phthaloylglycine (40 g) was suspended in chloroform (400 mL), followed by addition of thionyl chloride (60 mL). The mixture was heated to reflux for 2 hr, during which time the mixture became a homogeneous clear solution. The solvent and excess of thionyl chloride was removed under reduced pressure to give an off-white solid, which was dried under vacuum and used without further purification. ^{1}H NMR was consistent with the proposed structure.

Step 2: 4-*trans*-[(Phthaloylglycyl)aminomethyl]cyclohexane Carboxylic Acid. Suspended were 4-*trans*-aminomethylcyclohexane carboxylic acid (7.85 g, 50 mmol) and K_2CO_3 (5 g, 50 mmol) in DMF (150 mL). To the suspension was added phthaloylglycyl chloride (11.85 g, 50 mmol) in acetonitrile (150 mL). The reaction mixture was

refluxed for 3 hr and then filtered while hot. Solvents were removed under reduced pressure to give an oil. Upon addition of diethyl ether (50 mL), a white solide formed. The solid was collected by filtration, washed with diethyl ether, and dried in air. The yield was 10.32 g (60%). ^{1}H NMR (in DMSO-d_6, d in ppm relative to TMS): 0.87-2.00 (m, 9H, CH_2 and CH from cyclohexane ring); 2.10 (m, 1H, CHCOOH); 2.92 (t, 2H, CH_2, J = 4.6 Hz); 4.19 (s, 2H, CH_2); 7.85 (m, 4H, -CH=); 8.21 (t, 1H, NH, J = 4.1 Hz).

Step 3: Glycyl-4-*trans*-(Aminomethyl)cyclohexane Carboxylic Acid Hydrochloride (Gly-ACA·HCl). To a suspension of 4-*trans*-[(Phthaloylglycyl)aminomethyl]cyclohexane carboxylic acid (10.32 g, 30 mmol) in ethanol (300 mL) was added 85% hydrazine hydrate (100 mL). The mixture was heated to reflux for 12 hr, during which time a white precipitate formed. After solvent was removed, 2 N HCl (200 mL) was added to the residue. The mixture was warmed up to 60-70 °C for 20 min and the solid was filtered off and discarded. The filtrate was concentrated to 1/3 of its original volume. The mixture was cooled in an ice bath for 2 hr. The precipitate was collected by filtration, washed with a small amount of water and ethanol, and dried under vacuum. The yield was 3.45 g (45%). ^{1}H NMR (in D_2O, d in ppm relative to TMS): 1.04 (m, 2H, CH_2); 1.45 (m, 2H, CH_2); 1.57 (m, 1H, CH), 1.81-2.05 (m, 4H, CH_2); 2.35 (m, 1H, CHCOOH); 3.15 (d, 2H, CH_2, J = 4.9 Hz); 3.84 (s, 2H, CH_2).

Step 4: N-[2-(Benzoylthio)propiony]glycylglycyl-4-Amino-methylcyclohexane Carboxylic Acid (Bz-Me-MAG_2-

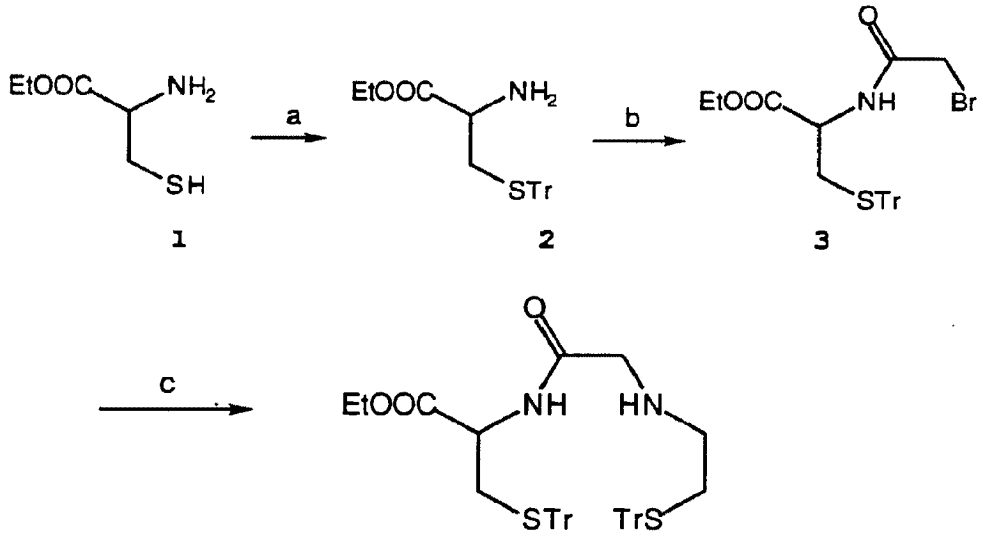
ACA). Gly-ACA·HCl (1.25 g, 5 mmol), Et_3N (1.0 g, 10 mmol) and Bz-Me-MAG-Succ (1.82 g, 5 mmol) were suspended in a mixture of methanol (200 mL) and acetonitrile (100 mL). The mixture was refluxed overnight. Solvents were removed under reduced pressure to give a white solid residue, to which was added 6 N HCl (10 mL). The solid was separated by filtration, washed with water and small amount of ethanol, and dried under vacuum. The yield was 1.35 g (58%). Anal. Calcd (found) for $C_{22}H_{29}N_3O_6S$: C, 57.00 (58.41); H, 6.31 (6.70); N, 9.06 (9.72). IR (KBr disk, in cm^{-1}): 3600-2000 (br, OH---N); 3270 (s, n_{N-H}); 1720, 1655, 1625, and 1565 (vs, $n_{C=O}$). FAB-MS: m/z = 464 (M+1). ^1H NMR (in DMSO-d_6, d in ppm relative to TMS): 0.81-1.90 (m, 9H, CH_2 and CH from cyclohexane ring); 1.48 (d, 3H, CH_3, J = 5.2 Hz); 2.10 (t, 1H, CHCOOH, J = 9.0 Hz); 2.91 (t, 2H, CH_2, J = 4.6 Hz); 3.68 (d, 2H, CH_2, 4.2 Hz); 3.75 (d, 2H, CH_2, J = 4.1 Hz); 4.42 (q, 1H, CH, J = 5.2 Hz); 7.50 (t, 2H, -CH=, J = 5.8 Hz); 7.71 (t, 2H, -CH=, J = 5.4 Hz); 7.91 (d, 1H, -CH=, J = 6.4 Hz); 8.14 (t, 1H, NH, J = 4.2 Hz); 8.60 (t, 1H, NH, J = 4.1 Hz), 12.00 (bs, 1H, COOH).

Synthesis of 3,4-Bis[3-(Benzoylthioacetyl)amido]benzoic Acid (Bz-MABA).

To a solution of S-benzoylthioacetyl chloride (8.69g, 40 mmol), freshly prepared from the reaction of S-benzoylthioacetic acid with excess of thionyl chloride in chloroform, in dry THF (300 mL) was added 3,4-diaminobenzoic acid (3.04 g, 20 mmol) while the solution became brown. The solution was refluxed over night, during which time a precipitate formed. The mixture was cooled, and the solid was separated by filtration,

washed with THF, ethanol and diethyl ether, and dried under vacuum to give a pale gray solid. The yield was 5.8 g (54%). Anal. Calcd (found) for $C_{25}H_{20}N_2O_6S_2$: C, 59.04 (58.82); H, 3.96 (4.04); N, 5.51 (5.46). IR (KBr disk, in cm^{-1}): 3600-2000 (br, OH---N); 3340 (s, n_{N-H}); 1690, 1670, 1655, 1610 and 1595 (s or m, $n_{C=O}$). FAB-MS: *m/z* = 509 (M+1). ^1H NMR (in $CDCl_3$, d in ppm relative to TMS): 4.12 and 4.14 (s, 4H, CH_2); 7.50-8.30 (m, 13H, aromatic H's); 9.85 and 9.89 (s, 2H, NH); 12.99 (bs, 1H, COOH).

Synthesis of 2-(S-Triphenylmethylmercapto)ethylaminoacetyl-S-triphenylmethyl-L-cysteine ethyl ester(Tr_2-MA-MAMA).



1 2 3 4

a: Triphenylmethanol, TFA; b: bromoacetyl bromide, TEA, THF; c: S-triphenylmethyl-2-aminoethanethiol, TEA, methylene chloride

Scheme 25

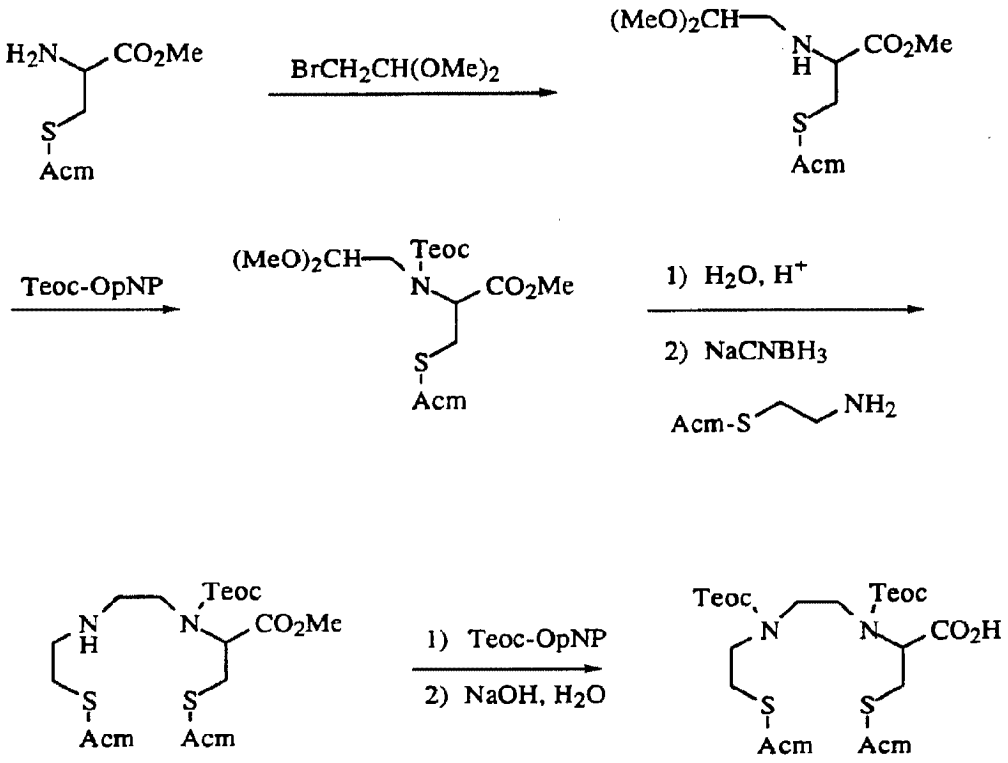
S-Triphenylmethyl-L-cysteine ethyl ester (**2**): To a solution of L-cysteine ethyl ester hydrochloride (18.6 g, 0.1 mole) in 200 mL TFA was added triphenylmethanol (52 g, 0.2 mole). The resulting dark brown solution was allowed to stir for 2 h at room temperature under nitrogen. The solvent was removed in vacuo and ethanol (100 mL) added to the residue. A 1 M solution of sodium ethoxide (50 mL) was added to the ethanolic solution and stirred for 90 min. during which time the solution turned cloudy. The mixture was filtered, the filtrated was concentrated in vacuo to give an oily residue. Flash column chromatography using ethyl acetate:hexane (1:3) and ethyl acetate gave the desired product (containing some ethyl acetate which is difficult to remove) which was stored under vacuum.

N-Bromoacetyl-S-triphenylmethyl-L-cysteine ethyl ester (**3**): A solution of S-triphenylmethyl-L-cysteine ethyl ester (18 g, 46 mmol.) and triethylamine (6.4 mL, 46 mmol.) in dry THF (250 mL) under nitrogen was cooled to 0 °C. A solution of bromoacetyl bromide (9.28 g, 46 mmol.) in dry THF (60 mL) was added dropwise during which time the solution tirned cloudy. The reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 1 h. The reaction mixture was filtered and the filtrate was concentrated in vacuo to give an oil. The oil was partitioned between methylene chloride and water (60 mL each), the organic layer washed with 5% HCl, $NaHCO_3$, dried (magnesium sulfate), filtered, and the volatiles removed to give the desired product (69%).

2-(S-Triphenylmethylmercapto)ethylaminoacetyl-S-triphenylmethyl-L-cysteine ethyl ester (**4**): To a solution of N-bromoacetyl-S-Triphenylmethyl-L-cysteine

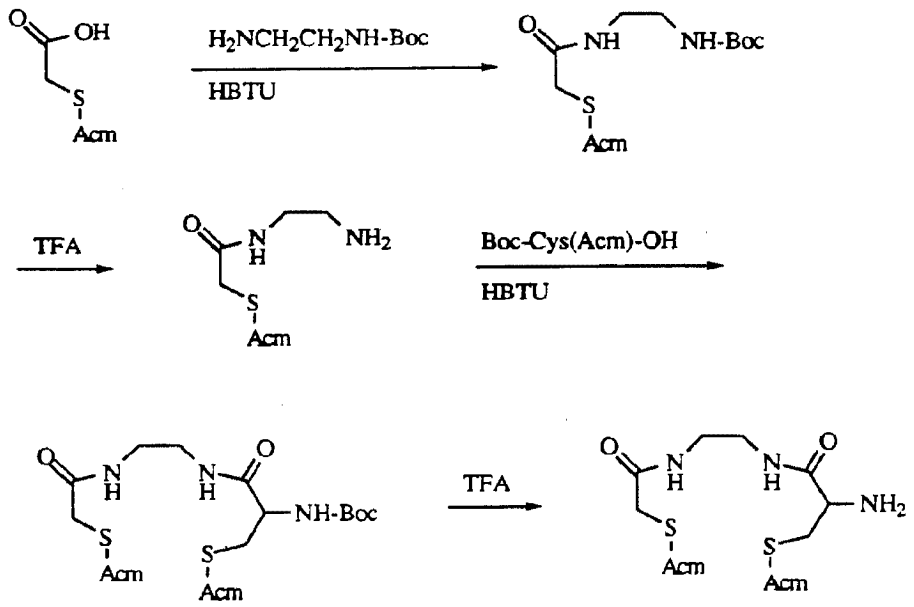
ethyl ester (1.0 g, 1.98 mmol.) and triethylamine (0.4 mL, 2.9 mmol.) in methylene chloride (10 mL) was added S-triphenylmethyl-2-aminoethanethiol (0.64 g, 2.0 mmol.). The reaction mixture allowed to stir at room temperature for seven days. Water (10 mL) was added. The organic layer was washed with NaHCO3 (2x10 mL), water (2x10 mL), and brine (10 mL), dried (magnesium sulfate), and concentrated in vacuo to give a foamy product. Flash chromatography using ethyl acetate:hexane (3:1) gave the product in 22% yield. MS (M+H) = 751, calculated 751.3

The synthesis of a chelator having a single carboxylic acid group availible for attaching the linker is shown in Scheme 26. The synthesis begins with the N-alkylation of Cys(Acm)OMe with bromoacetaldehyde dimethylacetal. The secondary amine of the alkylation product is now protected from further reaction with the Teoc group. Other protecting groups which are stable to both mild acid and mild base, and can be removed in the presence of sulfur may also be used. The Teoc group is introduced by the use of 2-(trimethylsilyl)ethyl p-nitrophenyl carbonate. The acetal is now hydrolyzed with mild aqueous acid and the aldehyde is reductively aminated with S-triphenylmethyl-2-aminoethanethiol. The one free amine of the chelator is protected with the Teoc group and the methyl ester is hydrolyzed with aqueous base to give the carboxylic acid ready for reaction with the reactive group of a linker modified cyclic compound.



Scheme 26

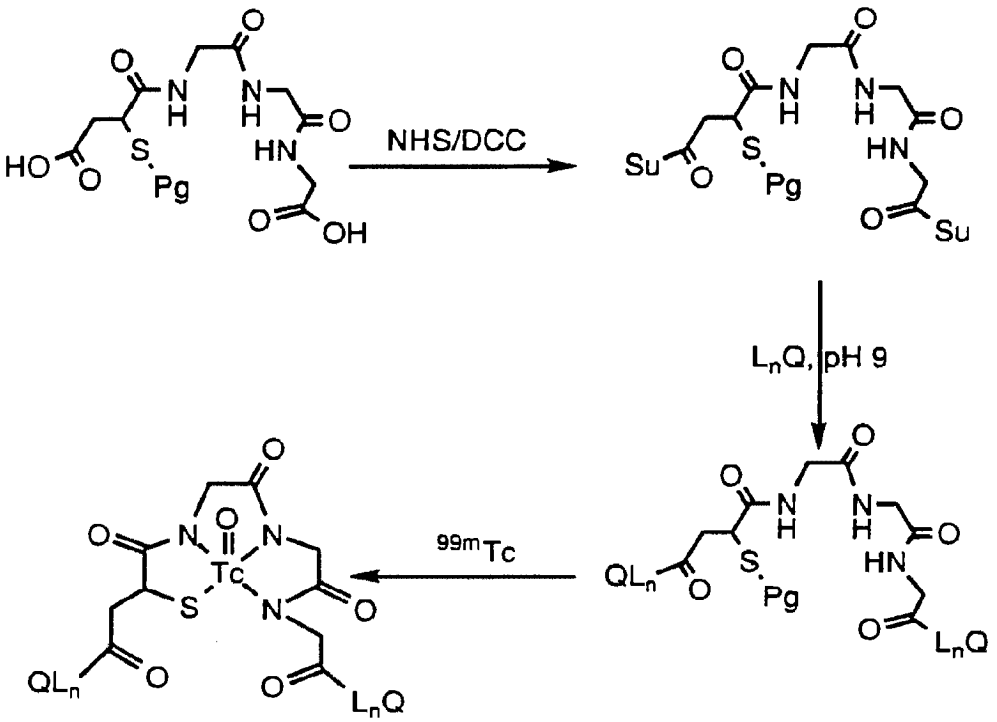
A chelator having one additional amine available for conjugation to the linker modified cyclic compound can be synthesized according to the procedure of Scheme 27. Acm protected thioglycolic acid would be coupled to N-t-butoxycarbonylethylenediamine using any of the standard coupling methods of peptide synthesis. The Boc protecting group would be removed by the use of TFA, and the resulting amine would be coupled to Boc-Cys(Acm)-OH. Removal of the Boc protecting group provides the S-protected chelator in a form appropriate for reaction with the reactive group of a linker modified cyclic compound.



Scheme 27

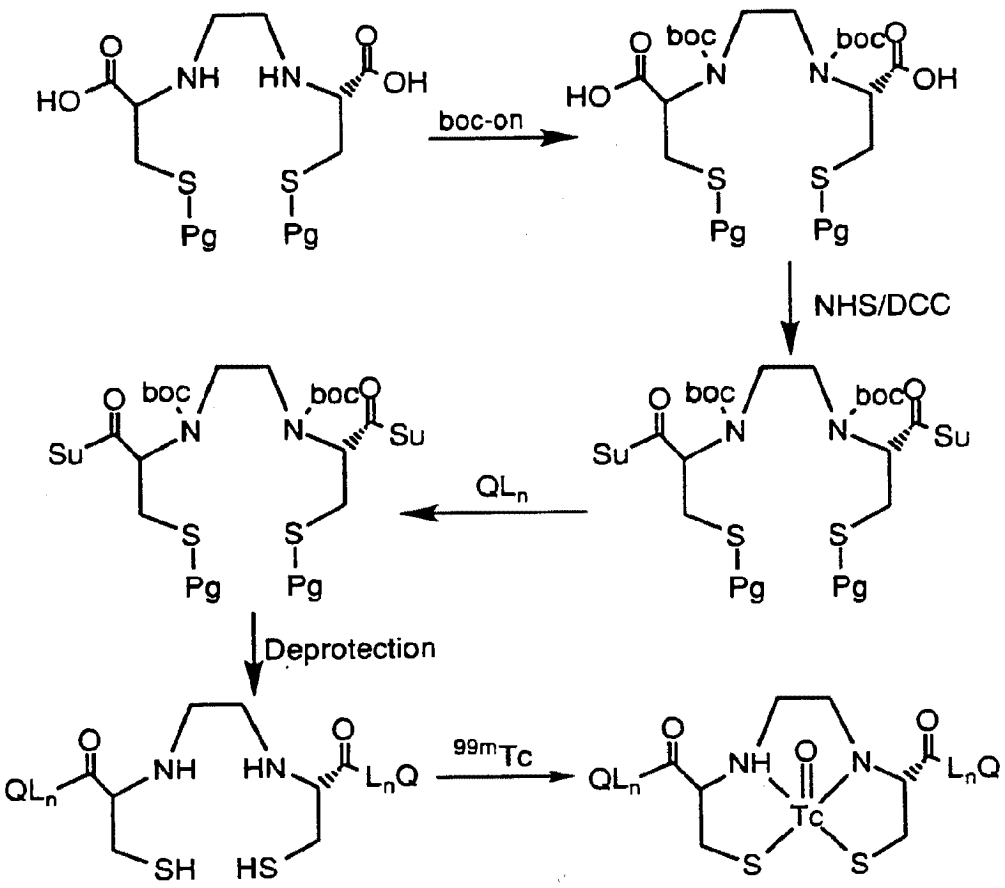
Also subject to this invention are reagents of the formula $(QL_n)_dC_h$ for radiolabeling which comprise more than one linker modified cyclic compound intermediate attached to a chelator as well as reagents of the formula $(Q)_{d'}L_n-C_h$, having two or more cyclic compound intermediates attached to a common linker that also bears a chelator.

An example of a reagent comprising two linker modified cyclic compound intermediates attached to a chelator is shown below (Schemes 28 and 29). Other representative examples are shown in the following schemes. In this scheme, amine groups on two linker intermediate compounds react with the shown two activated ester groups to afford a compound of this invention of formula $(QL_n)_2C_h$.



Scheme 28

The sulfur protecting group, Pg, shown above, as well as all Pg groups claimed herein, may be any sulfur protecting group capable of being displaced upon reaction with the metal nuclide. Such protecting groups are well known by those skilled in the art. Examples of suitable protecting are taught in U.S. Patents Nos. 4,897,255, 4,965,392, and 4,980,147, each of which is herebu incorporated herein by reference.

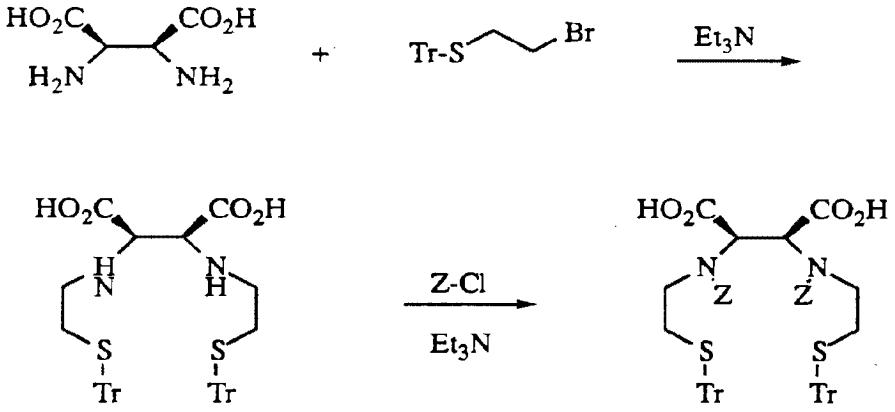


Scheme 29

Chelators useful in the synthesis of these reagents are described in Chervu et. al., U.S. Patent 4,883,862 and Bergstein et. al., U.S. Patent 5, 279,811. The synthesis of other useful chelators is described in the following schemes.

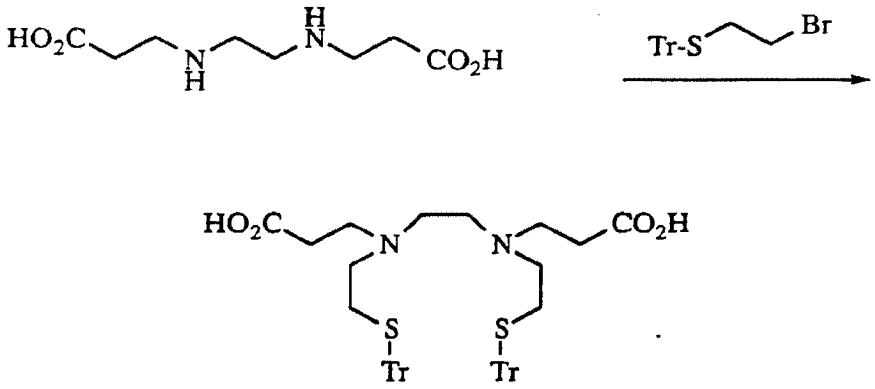
The following examples illustrate how three such chelators could be prepared. Scheme 30 outlines the synthesis of a N_2S_2 ligand having two carboxylic acid group to which the targeting cyclic compound can be

conjugated. The synthesis begins with an alkylation reaction on the two amines of DL-2,3-diaminosuccinic acid (Sigma Chemical Co.), using S-triphenylmethyl-2-bromoethanethiol. The secondary amines must now be protected to avoid self-condensation when the carboxylic acids are activated. This can be accomplished with any of the standard amine protecting groups. The Z group would be a good choice because it can be removed under acidic conditions (HBr/HOAc or TFA/trifluoromethanesulfonic acid) at the same time as the trityl protection on sulfur.



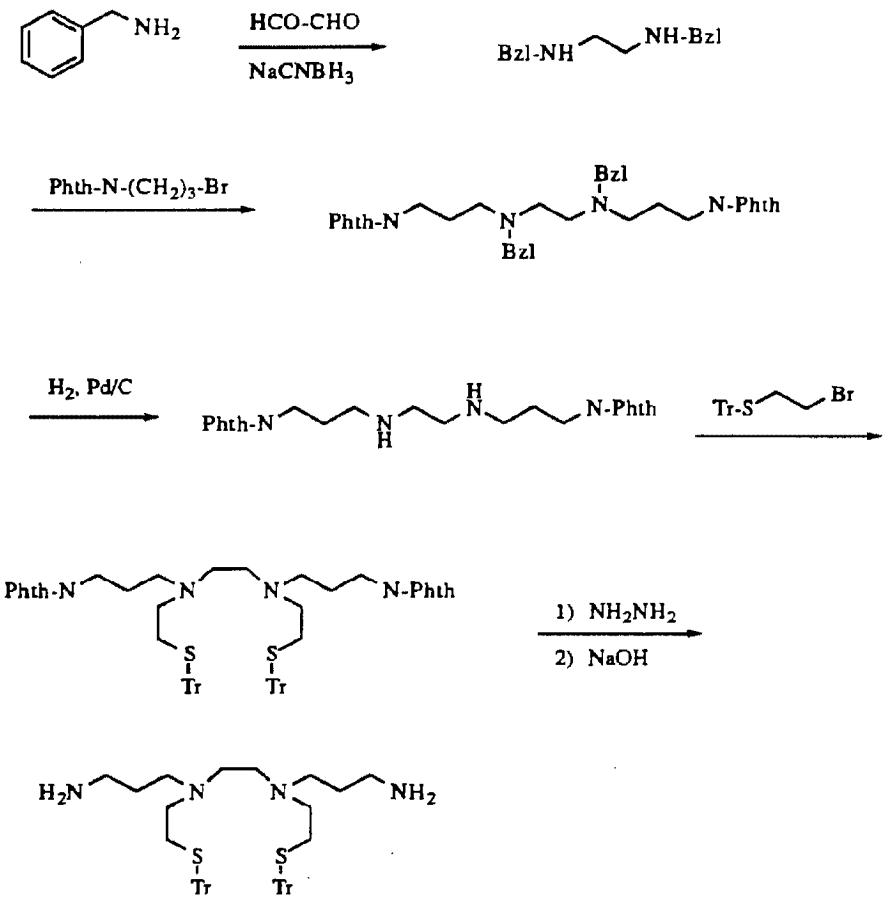
Scheme 30

The synthesis of a second N_2S_2 having two carboxylic acid groups is shown in Scheme 31. Alkylation of ethylenediamine-N,N'-dipropionic acid (American Tokyo Kasei) with S-triphenylmethyl-2-bromoethanethiol would give the N_2S_2 ready for conjugation. The amines are tertiary and no additional protection is required.



Scheme 31

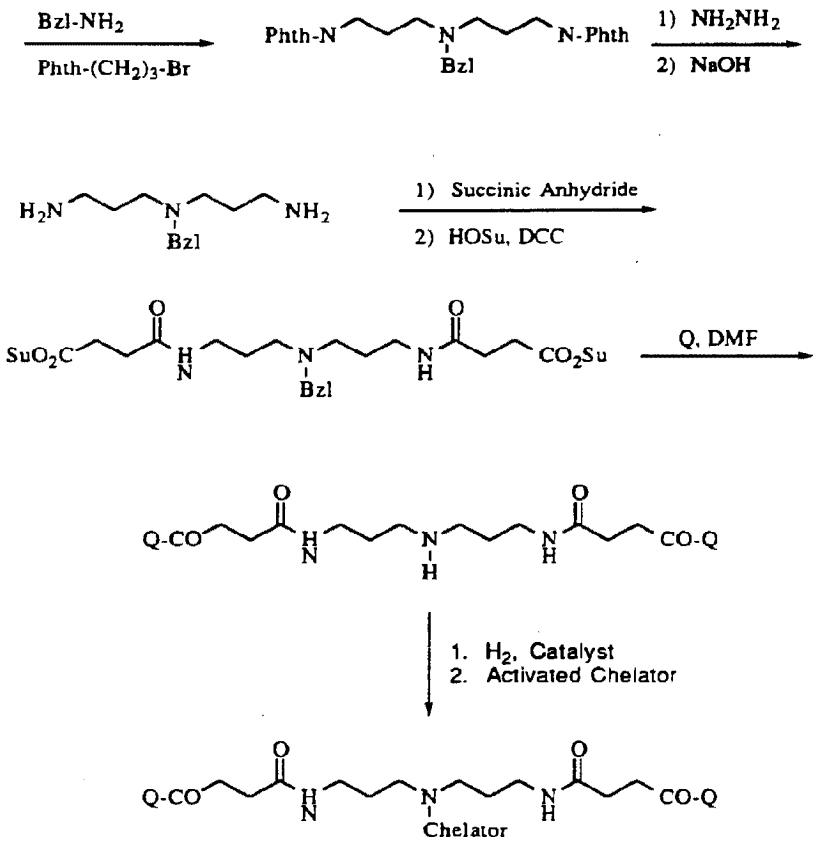
Scheme 32 outlines the synthesis of an N_2S_2 ligand having two additional amine groups for conjugation to targeting cyclic compounds bearing reactive electrophilic groups (e.g., active esters). A reductive amination reaction between benzyl amine and glyoxal would give N,N'-dibenzylethylenediamine. Alkylation of the two amines with N-(3-bromopropyl)phthalimide would give the fully protected tetraamine. The benzyl protection on the two secondary amines would be removed by catalytic reduction, and the free amines would then be alkylated with S-triphenylmethyl-2-bromoethanethiol to give the fully protected ligand. Selective deprotection of the primary amines would be accomplished with hydrazine.



Scheme 32

Reagents having two targeting groups and one chelator bound to a common linker can be synthesized according to the route shown in Scheme 33. Reaction of benzylamine with N-(3-bromopropyl)phthalimide will yield N,N-bis(3-phthalimidopropyl)benzylamine (Niitsu and Samejima (1986), *Chem. Pharm. Bul.*, **34**, 1032-1038). Treatment with hydrazine will remove the phthalimido protecting groups. N,N-Bis(3-aminopropyl)benzylamine would then be reacted with succinic anhydride to give the diacid, which would be converted to the bis active

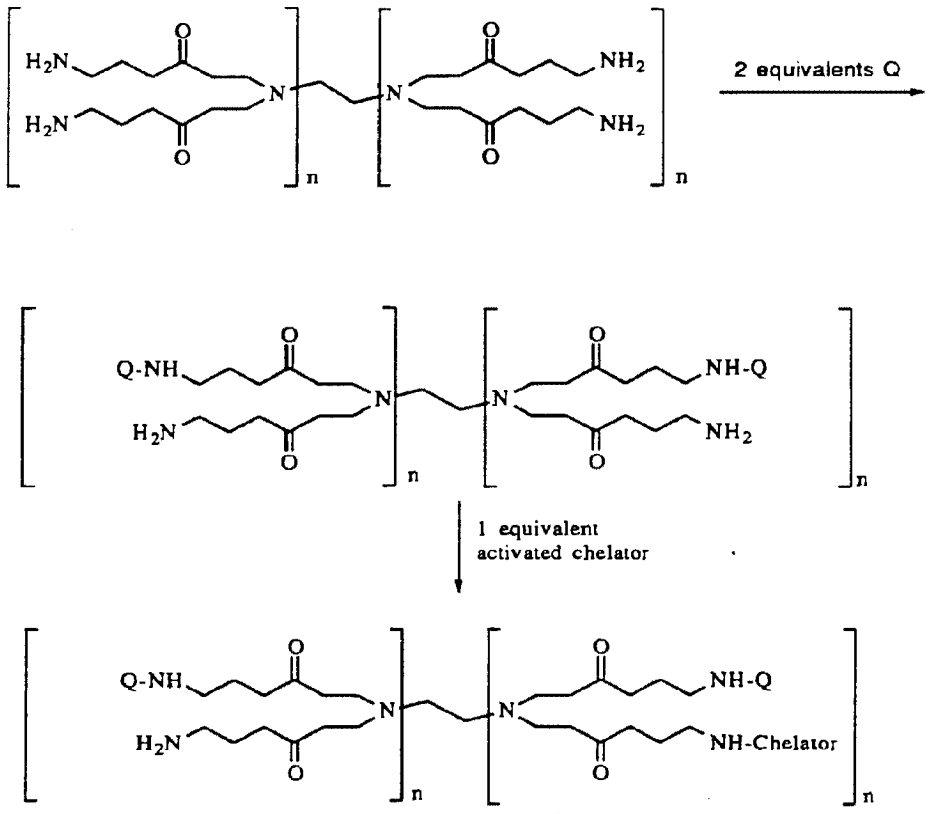
ester with DCC and N-hydroxysuccinimide. This bis active ester would then be conjugated to a linker modified cyclic compound. Hydrogenation to remove the benzyl protecting group and conjugation with an activated chelator would yield the final product.



Scheme 33

More than two compounds Q and more than one chelator can be joined together by using starburst or cascade dendrimers as linkers. Dendrimers are constructed by adding branched segments onto a

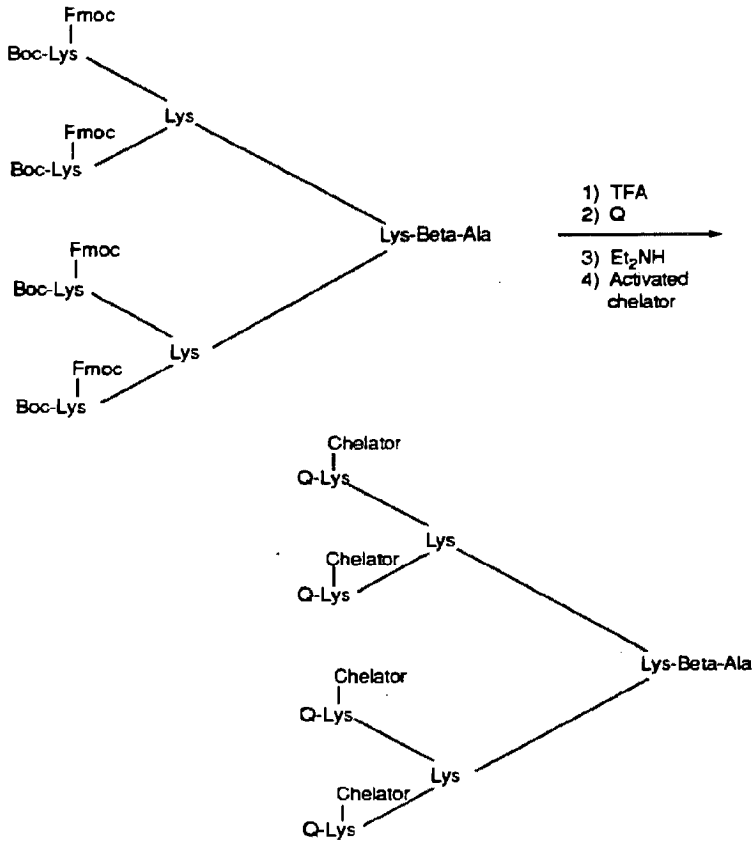
functionalized core, producing a product having twice the number of functional groups as the original core. This addition of branched units can be carried through several generations to product large polyfunctional molecules. One example is the PAMAM (polyamidoamine) dendrimers (Aldrich Chemical Co.), which use ethylenediamine as the initiator core. Scheme 34 shows the generalized preparation of a radiopharmaceutical based on PAMAM dendrimer containing targeting cyclic compounds and chelators in a 2:1 ratio. For this structure a generation = 0 (n = 1) dendrimer would have two targeting cyclic compounds and one chelator. A generation = 1 (n = 2) dendrimer would have four targeting cyclic compounds and two dendrimers. The ratio and absolute number of targeting cyclic compounds and chelators would be controlled by the stoichiometry of the conjugation reactions.



Scheme 34

A similar system, called the multiple antigen peptide (MAP) system was developed by Posnett, McGrath, and Tam (*J. Biol. Chem.*, **263**, (1988), 1719) to facilitate the generation of antibodies. This system constructs a branching network on a solid support using the two amino groups of lysine. Because the two different amino groups on lysine can be orthogonally protected, this system allows a higher level of control of the conjugation reactions. In Scheme 35 a MAP system terminating in four lysine groups is conjugated first to four targeting cyclic compounds at the alpha amino

groups, and them to four chelators at the epsilon amino groups.



Scheme 35

Synthesis of Radiolabeled Compounds

The radiolabeled cyclic platelet glycoprotein IIb/IIIa compounds of the present invention can be synthesized using standard synthetic methods known to those skilled in the art, using radioisotopes of halogens (such as chlorine, fluorine, bromine and

iodine), technetium and indium, as well as others. Preferable radioisotopes include ^{123}I, ^{125}I, ^{131}I, ^{99m}Tc, and ^{111}In.

The cyclic platelet glycoprotein IIb/IIIa compounds of the invention may be labeled either directly (that is, by incorporating the radiolabel directly into the compounds) or indirectly (that is, by incorporating the radiolabel into the compounds through a chelator which has been incorporated into the compounds. For direct labeling, as those skilled in the art will recognize, the labeling may be isotopic or nonisotopic. With isotopic labeling, one group already present in the cyclic compound is substituted with (exchanged for) the radioisotope. With nonisotopic labeling, the radioisotope is added to the cyclic compounds without substituting with (exchanging for) an already existing group.

Generally, labeled compounds are prepared by procedures which introduce the labeled atom at a late stage of the synthesis. This allows for maximum radiochemical yields, and reduces the handling time of radioactive materials. When dealing with short half-life isotopes, a major consideration is the time required to conduct synthetic procedures, and purification methods. Protocols for the synthesis of radiopharmaceuticals are described in Tubis and Wolf, Eds., "Radiopharmacy", Wiley- Interscience, New York (1976); Wolf, Christman, Fowler, Lambrecht, "Synthesis of Radiopharmaceuticals and Labeled Compounds Using Short-Lived Isotopes", in Radiopharmaceuticals and Labeled Compounds, Vol 1, p. 345-381 (1973), the disclosures of each of which are hereby incorporated herein by reference, in their entirety.

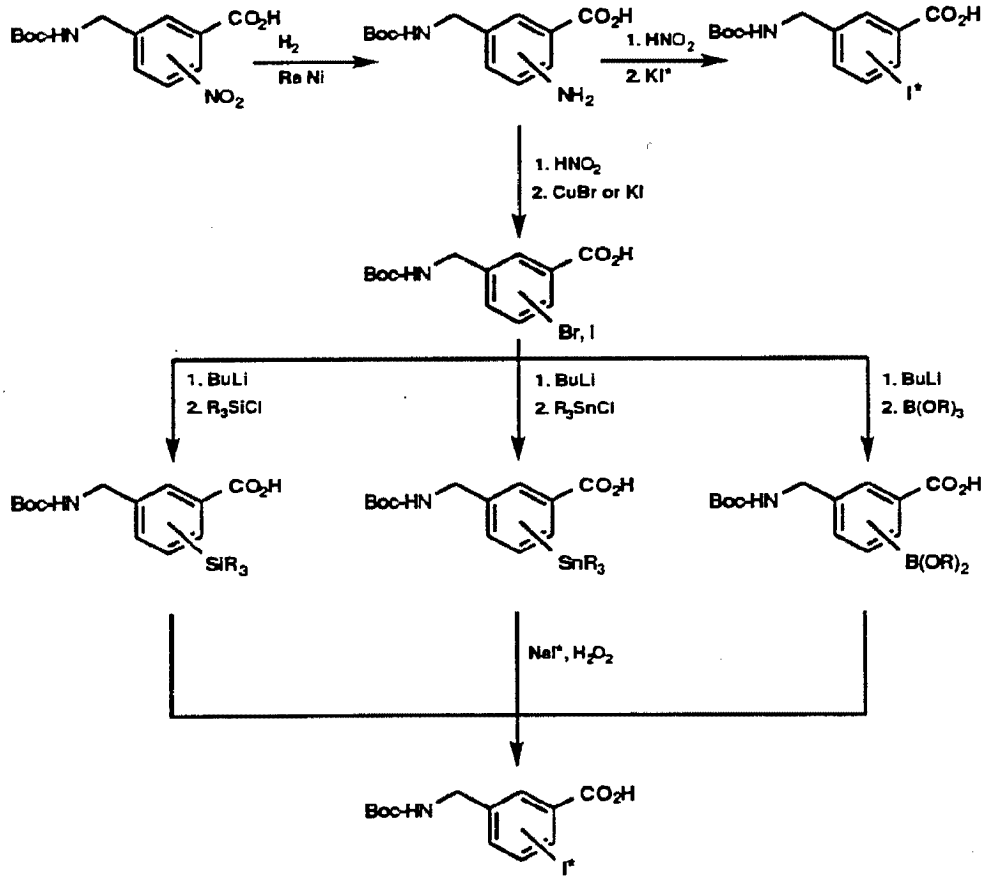
Various procedures may be employed in preparing the radiolabeled compounds of the invention where the radiolabel is a halogen. Some common synthetic methodologies for isotopic halogen labeling of aromatic compounds such as the type present here are iododediazonization, iododeborobation, iododestannylation, iododesilation, iododethallation, and halogen exchange reactions. The most common synthetic methodology for nonisotopic halogen labeling of aromatic compounds such as the type present here is iododeprotonation or electrophilic aromatic substitution reactions. These methods and additional procedures are described in Merkushev, Synthesis, 923 (1988), and Seevers et al, Chem. Rev., 82: 575 (1982), the disclosures of each of which are hereby incorporated herein by reference, in their entirety.

By way of example, isotopically radiolabeled 4, 5 and 6-halo t-butyloxycarbonyl-3-aminomethylbenzoic acid derivatives may be prepared using the general procedures described above for the synthesis of the unlabeled compounds. In carrying out such radiolabeling, it is important that the half-life of the isotope chosen be much longer than the handling time of the reaction sequences. Known starting materials include the 2, 3, and 4-iodo (123I, 125I, and 131I) benzoic acids.

The iodo-radiolabeled Mamb derivatives may also be isotopically prepared from the anilines by the Sandmeyer reaction as described in Ellis et at Aust. J. Chem., 26: 907 (1973).

Alternatively, such compounds may prepared by way of isotopic labeling from the unlabeled bromo or iodo derivatives by various two step reaction sequences, such as through the use of trialkylsilyl synthons as described in Wilson et at J. Org. Chem., 51: 483 (1986)

and Wilbur et al J. Label. Compound. Radiopharm., 19: 1171 (1982), the use of trialkylsilyl synthons as described in Chumpradit et al J. Med. Chem., 34: 877 (1991) and Chumpradit et al J. Med. Chem., 32: 1431 (1989), and the use of boronic acid synthons as described in Kabalka et al J. Label. Compound. Radiopharm., 19: 795 (1982) and Koch et al Chem. Ber., 124:2091 (1991). These synthetic transformations are outlined in the Scheme 36 below.

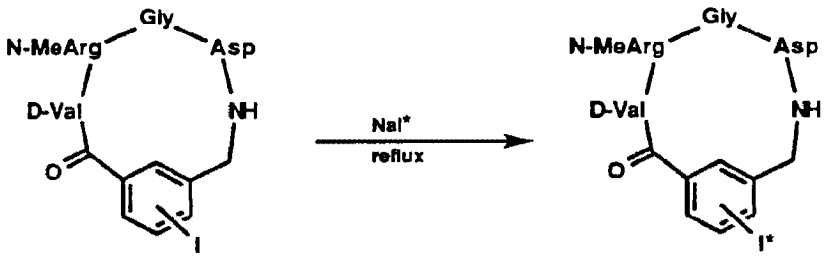


Scheme 36

Although the foregoing protocol may be employed in preparing radiolabeled compounds of the present invention, to maximize radiochemical yields, to reduce the handling time of radioactive materials, and to prepare short half-life halogen labeled compounds, it is preferable to perform the isotopic halogen labeling as one of the final steps in the cyclic compound synthesis. The following provides exemplary proceudres for such late stage labeling.

The unlabeled iodo compounds are versatile precursors which can be converted to the labeled derivatives by any of the two step reaction sequences described above. Useful functionality to incorporate into the Mamb portion of the cyclic compound includes the bromo, the nitro, the trialkylsilyl, the trialkyltin, and the boronic acid groups. The synthesis and application of each of these precursors is described above.

The least complex means of radioiodination of the cyclic compounds of the present invention via isotopic labeling during the final stages of their preparation is the substitution of radioactive iodide for a stable iodine atom already present in the molecule. This can often be done by heating the compound with radioactive iodide in an appropriate solvent as described in Ellis et al., Aust. J. Chem., 26: 907 (1973). When applied to aromatic iodides, the extremely small quantities and low concentration of radioactive iodide employed leads to the incorporation of only modest specific activity. This reaction sequence is outlined in the Scheme 37.



Scheme 37

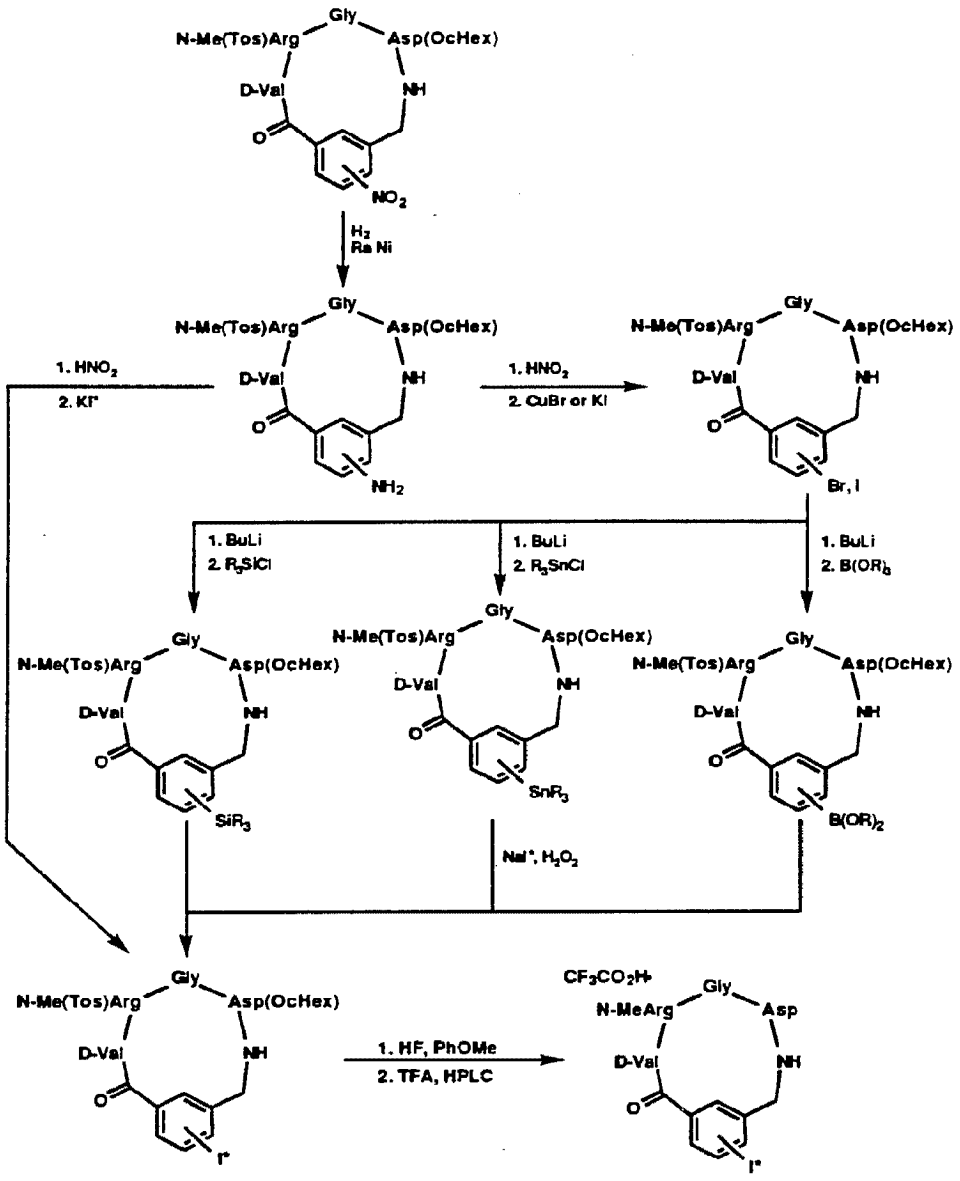
The cyclic compounds may also be isotopically iodo-labeled during the final stages of their preparation from the anilines by the Sandmeyer reaction as described in Ellis et al., Aust. J. Chem., 26: 907 (1973). This approach leads to a labeled cyclic compound with high specific activity. To avoid complications in the synthesis of the cyclic compound, the nitro group provides an ideal synthon for the aniline.

Alternatively, the cyclic compounds may be isotopically labeled late in the reaction scheme from the unlabeled bromo or iodo derivatives by various two step reaction sequences, as described above, such as through the use of trialkylsilyl synthons as described in Wilson et al., J. Org. Chem., 51: 4833 (1986) and Wilbur et al., J. Label. Compound. Radiopharm., 19: 1171 (1982), through the use of trialkylsilyl synthons as described in Chumpradit et al., J. Med. Chem., 34: 877 (1991) and Chumpradit et al., J. Med. Chem., 32: 1431 (1989), and through the use of boronic acid synthons as described in Kabalka et al., J. Label. Compound. Radiopharm., 19: 795 (1982) and Koch et al., Chem. Ber., 124:2091 (1991).

A related approach where the isotopic halogen radiolabeling may be carried out late in the synthesis scheme involves converting the substituted Mamb

derivatives to cyclic compounds that already incorporate the trialkylsilyl, trialkyltin, or boronic acid groups. The synthesis of each Mamb derivative has been described in an earlier section.

The forgoing synthetic transformations on the cyclic compounds are outlined in the Scheme 38.

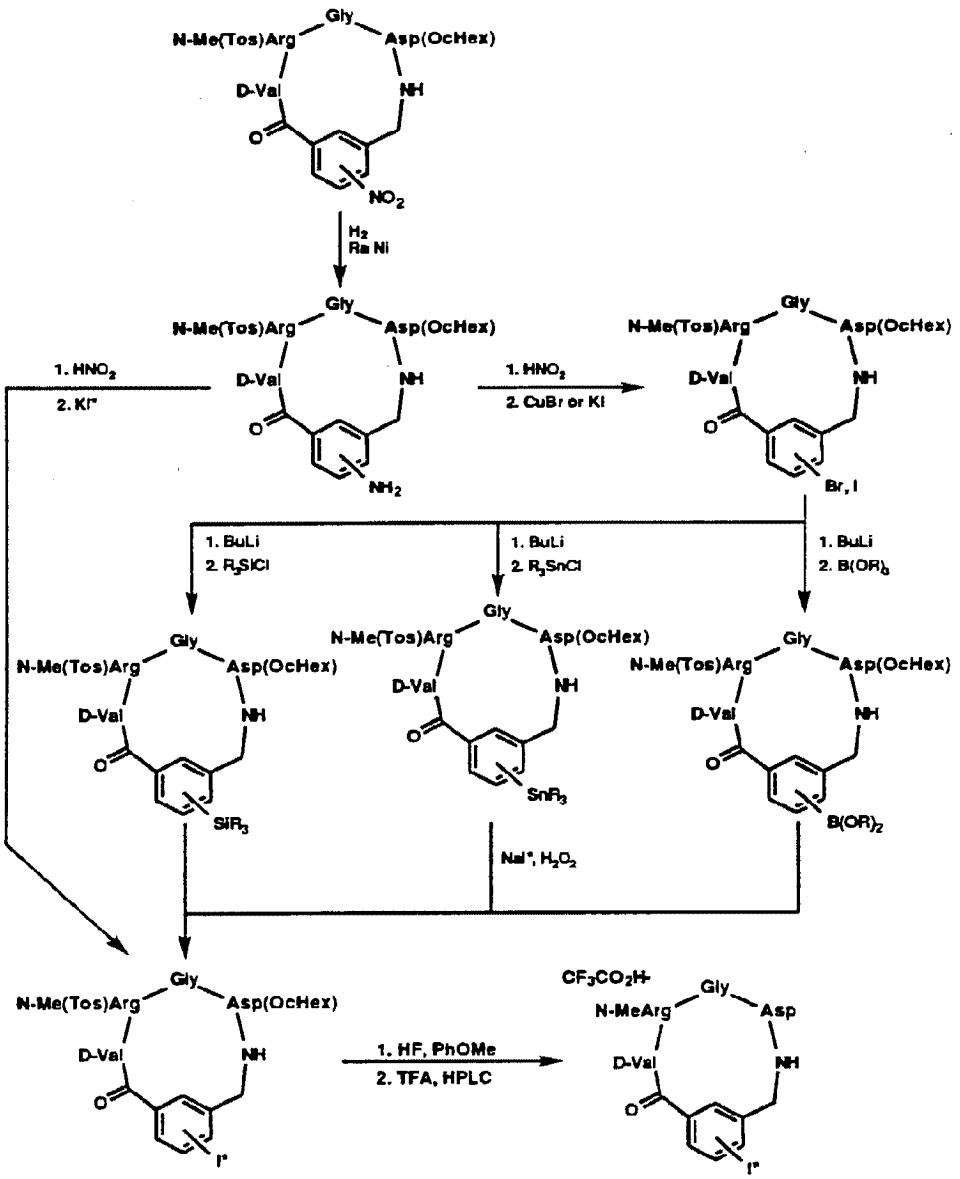


Scheme 38

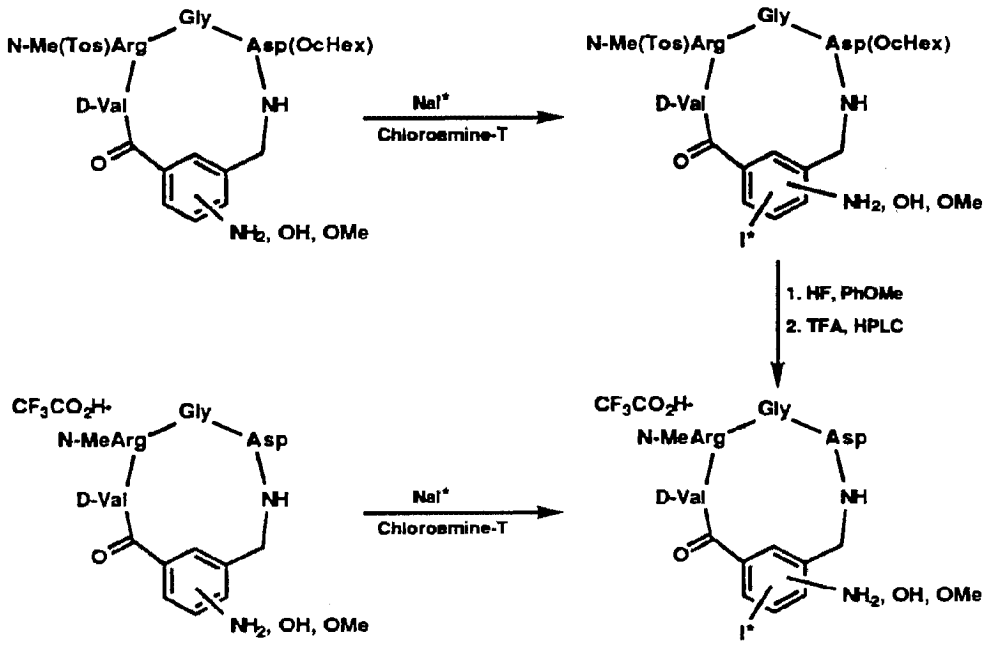
Labeled iodo derivatives may also be readily prepared nonisotopically from the amino, hydroxy, or methoxy substituted cyclic compounds as described in

Arora et al J. Med. Chem., 30:918 (1987). Electrophilic aromatic substitution reactions are enhanced by the presence of such electron-donating substituents. This synthetic sequence is outlined in Schemes 39 and 40.

5

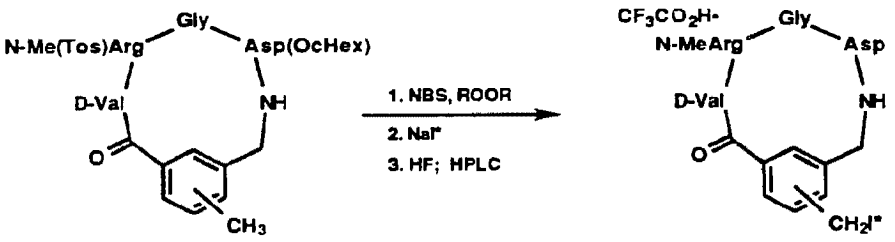


Scheme 39



Scheme 40

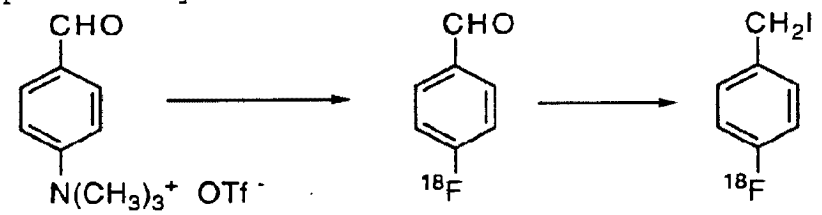
As an alternate approach to the incorporation of a radiolabeled halogen, the methyl substituted cyclic compounds may be converted to the a-halotoluene derivative with NBS or NCS under free-radical halogenation conditions. The benzylic halides may be smoothly replaced by radiolabeled iodide through a nucleophilic substitution reaction. This synthetic sequence is outlined in Scheme 41.



Scheme 41

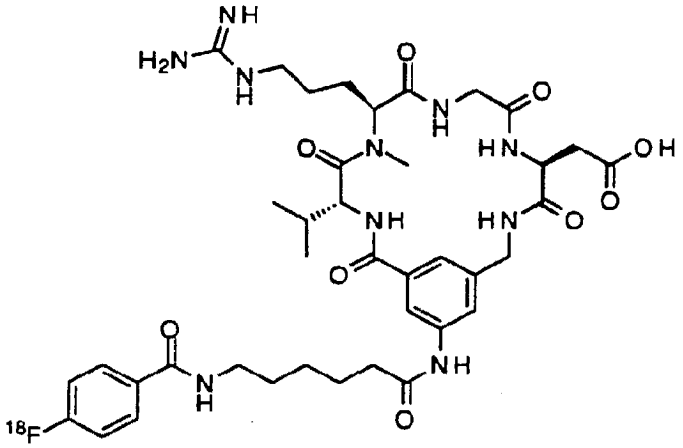
Although primarily illustrated for the radiolabeled iodo compounds, the above described process chemistry can be used to prepare any radioactive halogen isotope.

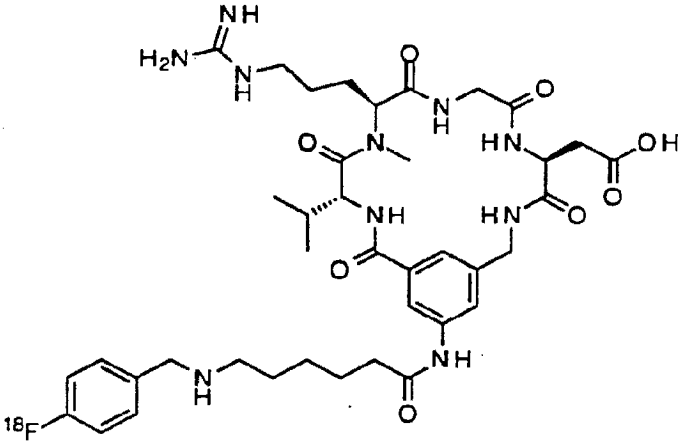
^{18}F derivatives of these cyclic compounds can be prepared by conjugation of ^{18}F functionalized phenyl intermediates. ^{18}F-functionalized cyclic compounds can be prepared as shown in Scheme 42 (R.H. Mach et al., J. Med. Chem., 1993, 36,3707-3720). Reaction of p-trimethylammonium-benzaldehyde with [^{18}F]CsF/aqueous DMF at 120 °C for 10 min.(aqueous [^{18}F]KF/kryptofix/ACN can also be used to generate the ^{18}F-phenyl compounds from the corresponding trimethylammonium or nitro groups), followed by LAH/THF/pentane and 57% aqueous HI gives the p-^{18}F-benzyl iodide.



Scheme 42

Reaction with the amine funtionality of the cyclic compound intermediate cyclo(D-Lys-NMeArg-Gly-Asp-Mamb) or the linker modifed cyclic compound Cyclo(D-Val-NMeArg-Gly-Asp-Mamb(5-Aca)) can give the ^{18}F labeled products suitable for use in positron emission tomography(PET):

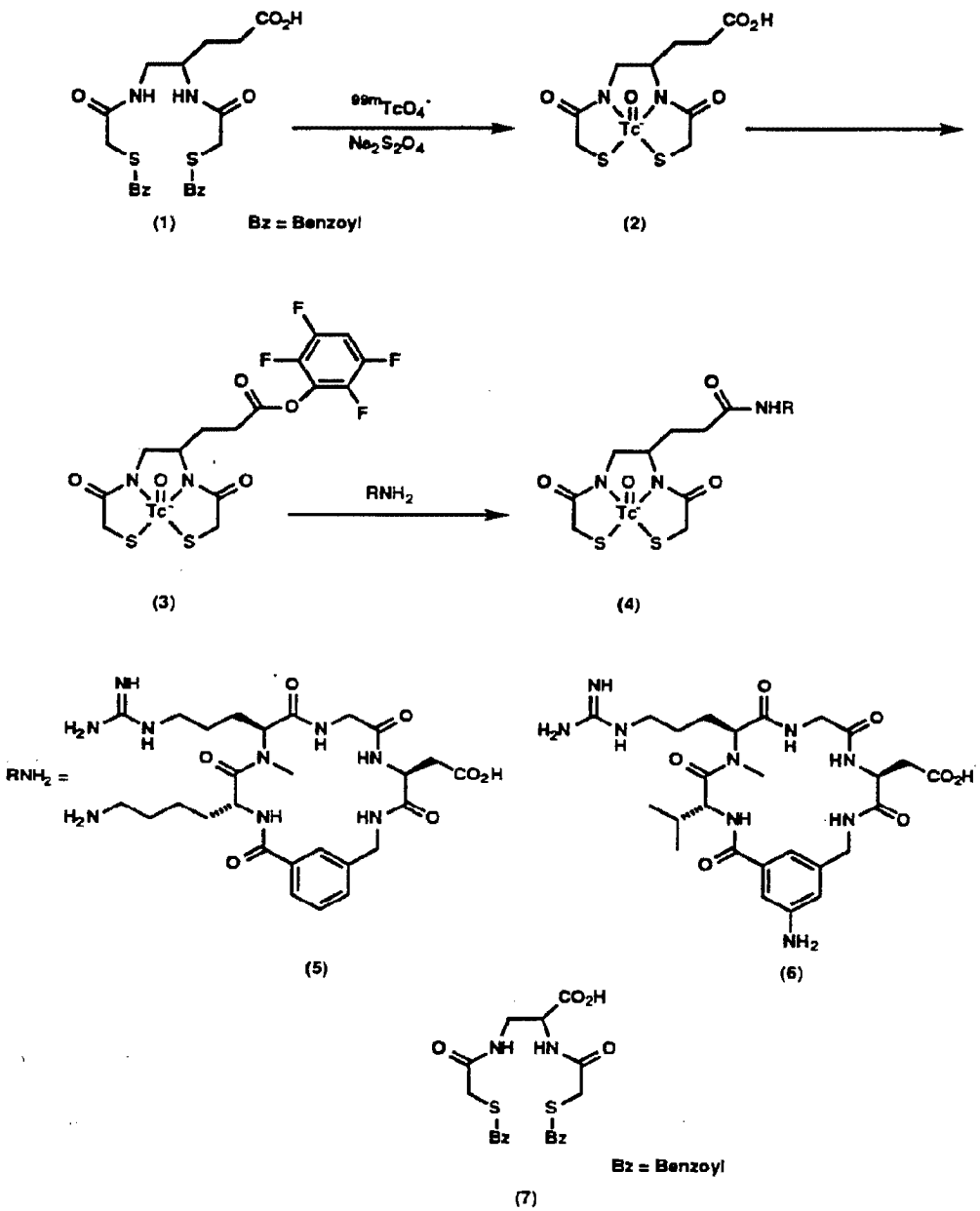




Various procedures may also be employed in preparing the radiolabeled compounds of the invention where the radiolabel is a metal, such as where the radiolabel is technetium or indium. These procedures are utilized for labeling compounds of this invention of formulae: $(QL_n)_dCh$ and $(Q)_{d'}L_n$-Ch. Exemplary procedures for such technetium or indium labeling are disclosed, for example, in Cerqueira et al., Circulation, Vol. 85, No. 1, pp. 298-304 (1992), Pak et al., J. Nucl. Med., Vol.

30, No. 5, p. 793, 36th Ann. Meet. Soc. Nucl. Med. (1989), Epps et al., J. Nucl. Med., Vol. 30, No. 5, p. 794, 36th Ann. Meet. Soc. Nucl. Med. (1989), Pak et al., J. Nucl. Med., Vol. 30, No. 5, p. 794, 36th Ann. Meet. Soc. Nucl. Med. (1989), and Dean et al., J. Nucl. Med., Vol. 30, No. 5, p. 794, 36th Ann. Meet. Soc. Nucl. Med. (1989), the disclosures of each of which are hereby incorporated herein by reference, in their entirety. In additon, specific procedures are provided in the examples below.

Another useful method for labeling the cyclic compounds of the present invention involves preparing a ^{99m}Tc chelator (at the tracer level) and conjugating it to either a cyclic compound intermediate or a linker modified cyclic compound. This method is termed the prechelate approach. As shown, for example, in the scheme below, 4,5-bis(S-benzoyl)mercaptoacetamidopentanoic acid (1) is complexed with $^{99m}TcO4$ under reducing conditions to form (2). Then (2) is converted to the active ester (3) containing the tetrafluorophenyl group. Complex (3) then may be reacted with an appropriate cyclic compound intermediate such as (5) or (6), to yield radiolabeled compounds (4). Another appropriate technetium chelator is 2,3-bis(S-benzoyl)mercaptoacetamido-propanoic acid (7). HPLC purification of the ^{99m}Tc complex may be performed at each step. This approach is depicted in Scheme 43.



Scheme 43

Examples

Section A. Reagents for Radiolabeling

Example 1

Cyclo-(D-Val-NMeArg-Gly-Asp-Mamb(5-Aca)) - N-[4-(carboxy)benzyl]-N,N'-bis[(2-triphenylmethylthio)ethyl]-glycinamide Conjugate

A solution of N-[4-(carboxy)benzyl]-N,N'-bis[(2-triphenylmethylthio)ethyl]glycinamide N-hydroxysuccinimide ester (0.017 mmol), cyclo-(D-Val-NMeArg-Gly-Asp-Mamb(5-Aca)) (13.9 mg, 0.015 mmol), and Et_3N (6.25 µl, 0.045 mmol) in DMF (350 µl) was allowed to stir at room temperature for 14 hours. The progress of the reaction was monitored by normal phase TLC (90:8:2 $CHCl_3$:MeOH:HOAc) using the ninhydrin and Sakaguchi tests. The DMF was removed under reduced pressure. The conjugate was purified using reversed-phase HPLC with a preparative Vydac C18 column (2.1 cm) using a 1.0%/min. gradient of 18 to 36% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy colorless solid (11 mg, 53%); FAB-MS: [M+H] =

Example 2

Cyclo-(D-Lys-NMeArg-Gly-Asp-Mamb) - N-[4-(carboxy)benzyl]-N,N'-bis[(2-triphenylmethylthio)ethyl]-glycinamide Conjugate

A solution of N-[4-(carboxy)benzyl]-N,N'-bis[(2-triphenylmethylthio)ethyl]glycinamide N-

hydroxysuccinimide ester (30 mg, 0.033 mmol), cyclo-(D-Lys-NMeArg-Gly-Asp-Mamb) (23.8 mg, 0.029 mmol), and Et_3N (12 µl, 0.087 mmol) in DMF (0.60 ml) was allowed to stir at room temperature for 63 hours. The progress of the reaction was monitored by normal phase TLC (90:8:2 $CHCl_3$:MeOH:HOAc) using the ninhydrin and Sakaguchi tests. The DMF was removed under reduced pressure. The conjugate was purified using reversed-phase HPLC with a preparative Vydac C18 column (2.1 cm) using a 0.9%/min. gradient of 18 to 36% acetonitrile containing 0.1% TFA and then lyophilized to give the TFA salt of the title compound as a fluffy colorless solid (24 mg, 60%); ESI-MS: [M] = 1397.3.

Example 3

Cyclo(D-Val-NMeArg-Gly-Asp-Mamb(N-hydrazino-nicotinyl-5-Aca)) TFA salt

Part A. Synthesis of Cyclo(D-Val-NMeArg-Gly-Asp-Mamb(N-boc-hydrazino-nicotinyl-5-Aca)) TFA salt

To a solution of cyclo(D-Val-NMeArg-Gly-Asp-Mamb(5-Aca) (10 mg, 0.011 mmol), succinimidyl boc-hydrazinonicotinate (4.6 mg, 0.0132 mmol) in DMF (0.3 mL) was added triethylamine (0.0061 mL, 0.044 mmol) and the reaction stirred at room temperature under nitrogen for 24 hours. The solvent was removed in vacuo and the residue dissolved in a solution of acetonitrile-water and lyophilized overnight to give an off-white solid. Purification of part of the product was accomplished by reversed-phase HPLC on a preparative Vydac C-18 column using a 2.0%/min. gradient of 6.3-72% aqueous acetonitrile containing 0.1% TFA and lyophilized to give

the TFA salt of the title compound as a fluffy solid. MS (M+H = 938.4849, calc. 938.4848).

Part B. Deprotection to Cyclo(D-Val-NMeArg-Gly-Asp-Mamb(N-hydrazinonicotinyl-5-Aca)) TFA salt

Cyclo(D-Val-NMeArg-Gly-Asp-Mamb(N-boc-hydrazinonicotinyl-5-Aca) TFA salt was dissolved in a mixture of 98:2 TFA:anisole (2 mL) and the reaction mixture stirred for 15 min. The solvent was removed in vacuo and the residue disolved in a solution of acetonitrile-water and lyophilized to give a white solid. Purification was accomplished by reversed-phase HPLC on a preparative Vydac C-18 column using a 2.0%/min. gradient of 6.3-72% aqueous acetonitrile containing 0.1% TFA and lyophilized to give the TFA salt of the title compound as a fluffy solid. MS (M+H = 838.4324, calc. 838.4324).

Example 4

Cyclo(D-Abu-NMeArg-Gly-Asp-Mamb(N-hydrazino-nicotinyl-5-Aca)) TFA salt

Part A. Synthesis of Cyclo(D-Abu-NMeArg-Gly-Asp-Mamb(N-boc-hydrazino-nicotinyl-5-Aca)) TFA salt

To a solution of cyclo(D-Abu-NMeArg-Gly-Asp-Mamb(5-Aca) TFA salt (10 mg, 0.0109 mmol), succinimidyl boc-hydrazinonicotinate (4.55 mg, 0.0131 mmol) in DMF (0.4 mL) was added triethylamine (0.0061 mL, 0.044 mmol) and the reaction stirred at room temperature under nitrogen for 24 hours. The solvent was removed in vacuo and the

residue dissolved in a solution of acetonitrile-water and lyophilized overnight to give an off-white solid. Purification of part of the product was accomplished by reversed-phase HPLC on a preparative Vydac C-18 column using a 2.0%/min. gradient of 6.3-72% aqueous acetonitrile containing 0.1% TFA and lyophilized to give the TFA salt of the title compound as a fluffy solid. MS (M+H = 924.4699, calc. 924.4692).

Part B. Deprotection to Cyclo(D-Abu-NMeArg-Gly-Asp-Mamb(N-hydrazino-nicotinyl-5-Aca)) TFA salt

Cyclo(D-Abu-NMeArg-Gly-Asp-Mamb(N-hydrazinonicotinyl-5-Aca)) TFA salt: Cyclo(D-Abu-NMeArg-Gly-Asp-Mamb(N-boc-hydrazinonicotinyl-5-Aca)) TFA salt was dissolved in a mixture of 98:2 TFA:anisole (2 mL) and the reaction mixture stirred for 15 min. The solvent was removed in vacuo and the residue disolved in a solution of acetonitrile-water and lyophilized to give a white solid. Purification was accomplished by reversed-phase HPLC on a preparative Vydac C-18 column using a 2.07%/min. gradient of 6.3-85.5% aqueous acetonitrile containing 0.1% TFA and lyophilized to give the TFA salt of the title compound as a fluffy solid. MS (M+H = xx, calc. xx).

Example 5

Cyclo((N-E-hydrazinonicotinyl-D-Lys)-NMeArg-Gly-Asp-Mamb) TFA salt

Part A. Synthesis of Cyclo((N-E-boc-hydrazinonicotinyl-D-Lys)-NMeArg-Gly-Asp-Mamb) TFA salt

To a solution of cyclo(D-Lys-NMeArg-Gly-Asp-Mamb).2TFA (4.2 mg, 0.005 mmol), succinimidyl boc-hydrazinonicotinate (2.1 mg, 0.006 mmol) in DMF (0.15 mL) was added triethylamine (0.003 mL, 0.02 mmol) and the reaction stirred at room temperature under nitrogen for 48 hours. The solvent was removed in vacuo and the residue dissolved in a solution of acetonitrile-water and lyophilized overnight to give an off-white solid. Purification was accomplished by reversed-phase HPLC on a preparative Vydac C-18 column using a 1.7%/min. gradient of 6.3-85.5% aqueous acetonitrile containing 0.1% TFA and lyophilized to give the TFA salt of the title compound as a fluffy solid. MS (M+H = 839.4157, calc. 839.4164).

Part B. Deprotection to Cyclo((N-E-hydrazinonicotinyl-D-Lys)-NMeArg-Gly-Asp-Mamb) TFA salt

Cyclo((N-E-hydrazinonicotinyl-D-Lys)-NMeArg-Gly-Asp-Mamb) TFA salt: Cyclo((N-E-boc-hydrazinonicotinyl-D-Lys)-NMeArg-Gly-Asp-Mamb) TFA salt (3 mg) was dissolved in a mixture of 98:2 TFA:anisole (2 mL) and the reaction mixture stirred for 15 min. The solvent was removed in vacuo and the residue disolved in a solution of acetonitrile-water and lyophilized to give a white solid. Purification was accomplished by reversed-phase HPLC on a preparative Vydac C-18 column using a 2.0%/min. gradient of 6.3-72% aqueous acetonitrile containing 0.1% TFA and lyophilized to give the TFA salt of the title compound as a fluffy solid. MS (M+H = 739.3629, calc. 739.3640).

Example 6.

Cyclo-([DTPA-D-Lys]-NMeArg-Gly-Asp-Mamb) Conjugate

To a solution of 250 mg (2 mmol.) of cyclo(D-Lys-NMeArg-Gly-Asp-Mamb) in 208 mL of 0.1 M Borate (pH 9.88) at room temperature was added DTPA anhydride (743 mg, 10 mmol.) with constant stirring. The reaction was allowed to stir for 2 h. The crude mixture of products obtained after removal of the solvent was purified by preparative HPLC (Vydac C_{18} column, gradient of 0-50% ACN containing 0.1% TFA over 60 min., flow rate 20 mL/min). Two major components were isolated. Component A is Cyclo-([DTPA-D-Lys]-NMeArg-Gly-Asp-Mamb). MS: 979.1 ($M+H^+$)

Example 7.

$[Cyclo-(D-Lys-NMeArg-Gly-Asp-Mamb)]_2$ - DTPA Conjugate

Component B from the synthesis described in Example 6 is $[Cyclo-(D-Lys-NMeArg-Gly-Asp-Mamb)]_2$ - DTPA. MS: 1565.4 (M^+)

Section B. Radiolabeled Compounds

Direct Labeling

Example 8.

Cyclo-((^{125}I)D-Tyr-NMeArg-Gly-Asp-Mamb)

To a 5 mL vial was added 22 mCi (45 µL) aqueous $Na^{125}I$, 100 µL 0.5 M phosphate buffer pH 7.5, 4.5 µL 1 N HCl, 75 µg of the cyclic compound intermediate Cyclo-(D-

Tyr-NMeArg-Gly-Asp-Mamb) dissolved in 75 µL 0.1% aqueous TFA, and 50 µg Chloramine-T dissolved in 50 µL H_2O. The reaction was allowed to proceed for 1 minute then 50 µg of sodium metabisulfite dissolved in H_2O was added. The product was purified by preparative HPLC. (Zorbax-Rx C_{18} column, flow = 1 mL/min, gradient from 100% A to 100% B over 30 minutes; Solvent A = 0.1% TFA in H_2O, Solvent B = 40% ethanol in A. The product had a retention time of 30 min.

Example 9.

[(^{125}I)N-3-(4-hydroxyphenyl)propionyl]-Cyclo-(D-Lys-NMeArg-Gly-Asp-Mamb)

To a 5 mL vial was added 11.4 mCi (25 µL) aqueous $Na^{125}I$, 100 µL 0.5 M phosphate buffer pH 7.5, 4.5 µL 1 N HCl, 50 µg of the linker modified cyclic compound [N-3-(4-hydroxyphenyl)propionyl]-Cyclo-(D-Tyr-NMeArg-Gly-Asp-Mamb) dissolved in 50 µL 0.1% aqueous TFA, and 50 µg Chloramine-T dissolved in 50 µL H_2O. The reaction was allowed to proceed for 1 minute then 50 µg of sodium metabisulfite dissolved in H_2O was added. The product was purified by preparative HPLC, using the condition described in Example 10. The product had a retention time of 32 min.

Indirect Labeling

Example 10.

^{99m}TcO(MAMA)-Cyclo-(D-Val-NMeArg-Gly-Asp-Mamb(5-Aca))

Part A. Deprotection

The trityl protecting groups on the reagent described in Example 1 are removed: To a separate, clean 10 cc vial was added the reagent and 0.1 mL trifluoroacetic acid (TFA). The solid dissolved to give a yellow solution.

Part B. Synthesis of ^{99m}Tc-glucoheptonate

A Glucoscan® vial was reconstituted with 1.0 mL Milli-Q H_2O. 0.2 mL of the solution was removed and added to a clean 10 cc vial followed by ~200 mCi $^{99m}TcO_4^-$. The reaction proceeded at room temperature for 20 minutes.

Part C. Synthesis of ^{99m}TcO(MAMA)-Cyclo-(D-Val-NMeArg-Gly-Asp-Mamb(5-Aca))

To the deprotected reagent solution from Part A was added 0.2 mL 5 N NaOH, and 0.4 mL 0.2 M phosphate buffer pH 6. The pH was measured and adjusted as needed to 6. This solution was immediately added to the ^{99m}Tc-glucoheptonate solution vial, crimped and heated at 100 °C for 15 minutes. After cooling ~2 minutes, 20 µL of the solution was analyzed by HPLC using Method 1.(See Table 1)

Example 11.

^{99m}TcO(MAMA)-Cyclo-(D-Lys-NMeArg-Gly-Asp-Mamb)

Part A. Deprotection

The trityl protecting groups on the reagent described in Example 2 are removed: To a separate, clean 10 cc vial was added the reagent and 0.1 mL trifluoroacetic acid (TFA). The solid dissolved to give a yellow solution.

Part B. Synthesis of ^{99m}TcO(MAMA)-Cyclo-(D-Lys-NMeArg-Gly-Asp-Mamb)

To the deprotected reagent solution from Part A was added 0.2 mL 5 N NaOH, and 0.4 mL 0.2 M phosphate buffer pH 6. The pH was measured and adjusted as needed to 6. This solution was immediately added to the ^{99m}Tc-glucoheptonate solution vial, generated as described in Example 11, Part B, crimped and heated at 100 °C for 15 minutes. After cooling ~2 minutes, 20 µL of the solution was analyzed by HPLC using Method 1.(See Table 1)

Example 12.

^{99m}Tc(tricine)$_2$-Cyclo(D-Val-NMeArg-Gly-Asp-Mamb(hydrazino-nicotinyl-5-Aca))

To a solution of 70 mg tricine in 1.0 mL of water was added 0.05 mL 1.0 N NaOH to raise the pH to 7. 0.1 - 1.0 mL of $^{99m}TcO_4^-$ in saline (10 - 100 mCi) was added followed by 10 µg of the reagent described in Example 3 dissolved in 100 µL of 0.1 N HCl and 100 µg of $SnCl_2 \cdot 2H_2O$ dissolved in 0.1 N HCl. The reaction proceeded at room temperature for 45 minutes. The product was analyzed by HPLC using the method 1 and by TLC using method 2.(see Table 1)

Example 13.

^{99m}Tc(EDDA)-Cyclo(D-Val-NMeArg-Gly-Asp-Mamb(hydrazino-nicotinyl-5-Aca))

To a solution of 10 mg ethylenediamine-N,N'-diacetic acid (EDDA) in 1.0 mL of water was added 0.05 mL 1.0 N NaOH to raise the pH to 7. 0.1 - 1.0 mL of $^{99m}TcO_4^-$ in saline (10 - 100 mCi) was added followed by 50 µg of the reagent described in Example 3 dissolved in 100 µL of 0.1 N HCl and 100 µg of $SnCl_2 \cdot 2H_2O$ dissolved in 0.1 N HCl. The reaction proceeded at room temperature for 45 minutes. The product was analyzed by HPLC using the method 1 and by TLC using method 2.(see Table 1)

Example 14.

^{99m}Tc(tricine)$_2$-Cyclo(D-Abu-NMeArg-Gly-Asp-Mamb(hydrazino-nicotinyl-5-Aca))

To a solution of 70 mg tricine in 1.0 mL of water was added 0.05 mL 1.0 N NaOH to raise the pH to 7. 0.1 - 1.0 mL of $^{99m}TcO_4^-$ in saline (10 - 100 mCi) was added followed by 10 µg of the reagent described in Example 4 dissolved in 100 µL of 0.1 N HCl and 100 µg of $SnCl_2 \cdot 2H_2O$ dissolved in 0.1 N HCl. The reaction proceeded at room temperature for 45 minutes. The product was analyzed by HPLC using the method 1 and by TLC using method 2.(see Table 1)

Example 15.

^{99m}Tc(tricine)$_2$-Cyclo(D-Lys-NMeArg-Gly-Asp-Mamb(hydrazino-nicotinyl-5-Aca))

To a solution of 70 mg tricine in 1.0 mL of water was added 0.05 mL 1.0 N NaOH to raise the pH to 7. 0.1 - 1.0 mL of $^{99m}TcO_4^-$ in saline (10 - 100 mCi) was added followed by 10 µg of the reagent described in Example 5 dissolved in 100 µL of 0.1 N HCl and 100 µg of $SnCl_2 \cdot 2H_2O$ dissolved in 0.1 N HCl. The reaction proceeded at room temperature for 45 minutes. The product was analyzed by HPLC using the method 1 and by TLC using method 2.(see Table 1)

Table 1. Analytical and Yield Data for ^{99m}Tc Labeled Reagents

	HPLC Retention Time(min)	% Yield
Example 10	20.4	66
Example 11	19.6	95
Example 12	13.4	95
Example 13	11.5	60
Example 14	11.5	97
Example 15	8.8	90

Example 16.

Cyclo-([^{111}In-DTPA-D-Lys]-NMeArg-Gly-Asp-Mamb)

50 µL of $^{111}InCl_3$ (~100 mCi/mL in 0.05 M HCl) obtained from DuPont-NEN Products, Billerica, MA, was combined with an equal volume of freshly prepared 1.0 M ammonium acetate. After about five minutes, 0.1 - 1 mg of the reagent described in Example 6 dissolved in 0.25 mL water was added. The reaction proceeded at room temperature for 30 minutes. The product was analyzed by HPLC using method 3.

Example 17.

^{111}In-DTPA-[Cyclo-(D-Lys-NMeArg-Gly-Asp-Mamb)]$_2$

To 0.5 mL of a solution of the reagent described in Example 7 in water (0.9 mg/1 mL) was added $^{111}InCl_3$ (~3 mCi) in 0.5 mL of 1 N NH_4OAc solution. The mixture was allowed to stand at room temperature for 30 minutes then analyzed by HPLC using method 3. (See Table 2)

Table 2. Analytical and Yield Data for ^{111}In-labeked Reagents

	HPLC Retention Time (min)	% Yield
Example 16	13.3	97
Example 17	14.5	98

Section C. ^{99m}Tc Labeled Reagents Via the Prechelate Approach.

The ^{99m}Tc-labeled reagents described in these examples were synthesized using the prechelate approach. The prechelate approach involves the steps: (1) chelation of ^{99m}Tc by the chelator; (2) activation of a non-coordinated carboxylic group on the resulting complex by forming its tetrafluorophenyl (TFP) ester; and (3) conjugation of the TFP-ester complex by forming an amide bond with a cyclic compound intermediate or linker modified cyclic compound.

Example 18.

Cyclo-([[^{99m}TcO(mapt)]$^-$-D-Lys]-NMeArg-Gly-Asp-Mamb)

Part A. Chelation of ^{99m}Tc

To a clean 10 cc vial was added 0.35 mL Bz-mapt (3.0 mg/mL in 1 N NaOH), 0.10 mL $SnCl_2 \cdot 2H_2O$ (10 mg/mL in 1 N HCl), and 200 mCi $^{99m}TcO_4^-$ in saline. The vial was crimped and placed in a 100 °C water bath for 25 minutes. After cooling ~2 minutes, 10 µL of the solution was analyzed by HPLC using Method 1.

Part B. Activation

To the solution from Part A was added 0.3 mL 0.5 M sodium phosphate pH 6, 0.3 mL 2,3,5,6-tetrafluorophenol (100 mg/mL in 90% acetonitrile), 0.3 mL 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide (100 mg/mL in 90% acetonitrile), and ~0.1 mL 1 N HCl. The pH was adjusted as needed to pH 6. The vial was crimped and heated at 40 °C for 25 minutes. After cooling ~ 2 minutes, 20 µL of the solution was analyzed by HPLC using Method 1.

Part C. Conjugation

1.0 - 2.5 mg of the cyclic compound intermediate Cyclo-(D-Lys-NMeArg-Gly-Asp-Mamb) was dissolved in 0.3 mL 0.5 M pH 9 phosphate buffer and added to the solution from Part B. Using 1 N NaOH, the pH was adjusted to 9. The reaction was heated at 40 °C for 30 minutes. After cooling ~2 minutes, 25 µL of the solution was analyzed by HPLC using Method 1. (See Table 3)

Example 19.

Cyclo-(D-Val-NMeArg-Gly-Asp-Mamb([^{99m}TcO(mapt)]$^-$-5-Aca))

1.0 - 2.5 mg of the linker modified cyclic compound Cyclo-(D-Val-NMeArg-Gly-Asp-Mamb(5-Aca)) was dissolved in 0.3 mL 0.5 M pH 9 phosphate buffer and added to the solution from Example 18, Part B. Using 1 N NaOH, the pH was adjusted to 9. The reaction was heated at 40 °C for 30 minutes. After cooling ~2 minutes, 25 µL of the solution was analyzed by HPLC using Method 1. (See Table 3)

Example 20.

Cyclo-(D-Abu-NMeArg-Gly-Asp-Mamb([^{99m}TcO(mapt)]$^{-}$-5-Aca))

1.0 - 2.5 mg of the linker modified cyclic compound Cyclo-(D-Abu-NMeArg-Gly-Asp-Mamb(5-Aca)) was dissolved in 0.3 mL 0.5 M pH 9 phosphate buffer and added to the solution from Example 18, Part B. Using 1 N NaOH, the pH was adjusted to 9. The reaction was heated at 40 °C for 30 minutes. After cooling ~2 minutes, 25 µL of the solution was analyzed by HPLC using Method 1. (See Table 3)

Example 21.

Cyclo-([([^{99m}TcO(mapt)]$^{-}$-5-Aca)D-Lys]-NMeArg-Gly-Asp-Mamb)

1.0 - 2.5 mg of the linker modified cyclic compound Cyclo-((5-Aca)D-Lys-NMeArg-Gly-Asp-Mamb) was dissolved in 0.3 mL 0.5 M pH 9 phosphate buffer and added to the solution from Example 18, Part B. Using 1 N NaOH, the pH was adjusted to 9. The reaction was heated at 40 °C for 30 minutes. After cooling ~2

minutes, 25 µL of the solution was analyzed by HPLC using Method 1. (See Table 3)

Example 22.

Cyclo-([[^{99m}TcO(MeMAG$_2$gaba)]$^-$-D-Lys]-NMeArg-Gly-Asp-Mamb)

Part A. Chelation

To a 10 mL vial was added 100-250 mCi $^{99m}TcO_4^-$ in 1.0 mL of saline, 1.0 mL of Bz-MeMAG$_2$gaba solution (1 mg/1 mL in 0.5M pH 12 phosphate buffer), followed by of 0.15-0.20 mL of $SnCl_2 \cdot 2H_2O$ solution (15 mg/3 mL in 1N HCl). The pH was adjusted to ~11 and the mixture was heated for 30 min at 100 °C. The solution was analyzed by HPLC using Method 1.

Part B. Activation

To the solution from Part A was added 0.2 mL of 1N HCl, 0.5 mL of tetrafluorophenol solution (100 mg/mL in 90% CH_3CN), and 0.5 mL of (1-[3-(dimehtylamino)propyl]-3-ethylcarbodiimide chloride) solution (100 mg/mL in 90% CH_3CN). The pH was adjusted to 6.0 and the mixture was heated at 50 °C for 30 min.

Part C. Conjugation

1.0 - 2.5 mg of the cyclic compound intermediate Cyclo-(D-Lys-NMeArg-Gly-Asp-Mamb) dissolved in 0.3 mL 0.5 M pH 9 phosphate buffer and added to the solution from Part B. Using 1 N NaOH, the pH was adjusted to 9. The reaction was heated at 40 °C for 30 minutes. After cooling ~2 minutes, 25 µL of the solution was analyzed by HPLC using Method 1.(See Table 3)

Example 23.

Cyclo-(D-Val-NMeArg-Gly-Asp-Mamb([^{99m}TcO(MeMAG$_2$gaba)]$^-$-5-Aca))

1.0 - 2.5 mg of the linker modified cyclic compound Cyclo-(D-Val-NMeArg-Gly-Asp-Mamb(5-Aca)) was dissolved in 0.3 mL 0.5 M pH 9 phosphate buffer and added to the solution from Example 22, Part B. Using 1 N NaOH, the pH was adjusted to 9. The reaction was heated at 40 °C for 30 minutes. After cooling ~2 minutes, 25 µL of the solution was analyzed by HPLC using Method 1. (See Table 3)

Example 24.

Cyclo-(D-Abu-NMeArg-Gly-Asp-Mamb([^{99m}TcO(MeMAG$_2$gaba)]$^-$-5-Aca))

1.0 - 2.5 mg of the linker modified cyclic compound Cyclo-(D-Abu-NMeArg-Gly-Asp-Mamb(5-Aca)) was dissolved in 0.3 mL 0.5 M pH 9 phosphate buffer and added to the solution from Example 22, Part B. Using 1 N NaOH, the pH was adjusted to 9. The reaction was heated at 40 °C for 30 minutes. After cooling ~2 minutes, 25 µL of the solution was analyzed by HPLC using Method 1. (See Table 3)

Example 25.

Cyclo-([[^{99m}TcO(MAG$_3$)]$^-$-D-Lys]-NMeArg-Gly-Asp-Mamb)

This example was synthesized following the procedure described in Example 22, substituting Bz-MAG$_3$ as the chelator. (See Table 3)

Example 26.

Cyclo-([[^{99m}TcO(Me-MAG$_3$)]$^-$-D-Lys]-NMeArg-Gly-Asp-Mamb)

This example was synthesized following the procedure described in Example 22, substituting Bz-Me-MAG$_3$ as the chelator. (See Table 3)

Example 27.

Cyclo-(D-Val-NMeArg-Gly-Asp-Mamb([^{99m}TcO(MeMAG$_2$ACA)]$^-$-5-Aca))

The title compund was prepared according to the procedure procedure described in Example 22, substituting Bz-Me-MAG$_2$-ACA as the chelator in Part A and using Cyclo-(D-Val-NMeArg-Gly-Asp-Mamb(5-Aca)) as the linker modifed cyclic compound in Part C. (See Table 3)

Example 28.

Cyclo-([[^{99m}TcO(MABA)]$^-$-D-Lys]-NMeArg-Gly-Asp-Mamb)

Part A. Chelation

To a 10 mL vial was added 50-300 mCi $^{99m}TcO_4^-$ in 0.5 mL of saline, followed by 0.5 mL of Bz-MABA solution (1 mg/1 mL in 0.5 M pH 12 phosphate buffer) and 0.15 mL of $Na_2S_2O_4$ solution (5mg/mL in 0.5 M in pH 11.5 phosphate buffer) The pH was adjusted to 10-12 using 1 N NaOH and the mixture was heated for 30 min. at 100 °C then analyzed by HPLC using method 1.

Part B. Activation

To the solution from Part A was added 0.2 mL of 1 N HCl, 0.5 mL of TFP solution (50 mg/0.5 mL in 90% CH_3CN),

and 0.5 mL of DCI solution (50 mg in 0.5 mL in 90% CH_3CN). The pH was adjusted to 6 if necessary and the mixture was heated at 45-50 °C for 30 min then analyzed by HPLC using method 1.

Part C. Conjugation

To the solution from Part B was added 2-3 mg of the cyclic compound intermediate Cyclo-(D-Lys-NMeArg-Gly-Asp-Mamb) dissolved in 0.5 mL 0.5 M phosphate buffer pH 9 and pH was then adjusted to 9.5-10. The solution was heated at 50 °C for 30 min,then analyzed by HPLC using method 1. (See Table 3)

Example 29.

Cyclo-(D-Val-NMeArg-Gly-Asp-Mamb([^{99m}TcO(MABA)]$^-$-5-Aca))

The title compound was synthesized following the procedure described in Example 28, substituting the linker modified cyclic compound Cyclo-(D-Val-NMeArg-Gly-Asp-Mamb(5-Aca)) for the cyclic compound intermediate Cyclo-(D-Lys-NMeArg-Gly-Asp-Mamb) in Part C.

Example 30.

Cyclo-(D-Abu-NMeArg-Gly-Asp-Mamb([^{99m}TcO(MABA)]$^-$-5-Aca))

The title compound was synthesized following the procedure described in Example 28, substituting the linker modified cyclic compound Cyclo-(D-Abu-NMeArg-Gly-Asp-Mamb(5-Aca)) for the cyclic compound intermediate Cyclo-(D-Lys-NMeArg-Gly-Asp-Mamb) in Part C.

Example 31.

Cyclo-([[^{99m}TcO(MA-MAMA)]-D-Lys]-NMeArg-Gly-Asp-Mamb)

Part A. Deprotection.

The trityl groups on the chelator MA-MAMA were removed by dissolving 6 mg in 1 mL of anhydrous trifluoroacetic acid (TFA). The resulting yellow solution was allowed to stand at room temperature for 5 minutes. Triethylsilane (0.5 mL) was added to the yellow solution to give a clear two-layered mixture. Volatiles were removed under reduced pressure to give a white residue.

Part B. Hydrolysis of the Ethyl Ester.

To the white residue from Part A was added 0.5 mL of 5 N NaOH and 1 mL of THF. The mixture was heated in a water bath (100 °C) for 5 minutes, by which time most of THF was evaporated. To the reaction mixture was added 3 mL of 0.5 M phosphate buffer pH 11.5. The pH was adjusted to 10-12 and sodium dithionite (15-30 mg) was added. The mixture was filtered and the total volume was adjusted to 6 mL using 0.5 M pH 11.5 phosphate buffer.

Part C. Chelation.

To a 10 mL vial was added 50-150 mCi $^{99m}TcO_4^-$ in 0.5 mL of saline, followed by 0.5 mL of ligand solution from Part B. The pH was adjusted to 10-12 using 1 N NaOH and the mixture was heated for 30 min at 100 °C then analyzed by HPLC using method 1.

Part D. Activation.

To the solution from Part C was added 0.2 mL of 1 N HCl, 0.5 mL of TFP solution (50 mg/0.5 mL 90% CH_3CN), and 0.5 mL of DCI solution (50 mg in 0.5 mL 90% CH_3CN). The pH was adjusted to 6 if necessary and the mixture was heated at 45-50 °C for 30 min.then analyzed by HPLC using method 1.

Part E. Conjugation.

To the solution from Part D was added 2.5 mg of the cyclic compound intermediate Cyclo-(D-Lys-NMeArg-Gly-Asp-Mamb) dissolved in 0.5 mL 0.5 M phosphate buffer pH 9 and the pH was then adjusted to 9.5-10. After heating at 50 °C for 30 min, the solution was analyzed by HPLC using method 1.

Example 32.

Cyclo-(D-Val-NMeArg-Gly-Asp-Mamb([^{99m}TcO(MA-MAMA)]-5-Aca))

The title compound was synthesized following the procedure described in Example 31, substituting the linker modified cyclic compound Cyclo-(D-Val-NMeArg-Gly-Asp-Mamb(5-Aca)) for the cyclic compound intermediate Cyclo-(D-Lys-NMeArg-Gly-Asp-Mamb) in Part E.

Table 3. Analytical and Yield Data for ^{99m}Tc-labeled Reagents

	HPLC Retention Time (min)	% Yield
Example 18	15.0	60
Example 19	16.2	45
Example 20	15.3	35
Example 21	15.5	55
Example 22	14.3	44
Example 23	15.5	34
Example 24	14.5	70
Example 25	13.2	50
Example 26	13.0	55
Example 27	14.3	40
Example 28	18.2	10
Example 29	19.1	22

Example 30	19.3	22
Example 31	14.8	23
Example 32	16.2	34

Analytical Methods

HPLC Method 1
Column: Vydac C_{18}, 250 mm x 4.6 mm, 300 Å pore size
Solvent A: 10 mM sodium phosphate, pH 6.0
Solvent B: 100% acetonitrile
Gradient:

0%B	30%B	75%B
0'	15'	25'

Flow rate: 1.0 mL/min
Detection by NaI probe

TLC Method 2
ITLC-SG strip, 1 cm x 7.5 cm, developed in 1:1 acetone:water.

HPLC Method 3
Column: Vydac C_{18}, 250 mm x 4.6 mm, 300 Å pore size
Solvent A: 10 mM sodium phosphate, pH 6.0
Solvent B: 75% acetonitrile in Solvent A
Gradient:

5%B	5%B	100%B
0'	5'	40'

Flow rate: 1.0 mL/min
Detection by NaI probe

Utility

The radiolabeled compounds of the invention are useful as radiopharmaceuticals for imaging a thrombus

such as may be present in a patient with unstable angina, myocardial infarction, transient ischemic attack, stroke, atherosclerosis, diabetes, thrombophlebitis, pulmonary emboli, or prosthetic cardiac devices such as heart valves, and thus may be used to diagnose such present or potential disorders. The patient may be any type of a mammal, but is preferably a human. The radiolabeled compounds may be used alone, or may be employed as a composition with a radiopharmaceutically acceptable carrier, and/or in combination with other diagnostic or therapeutic agents. Suitable radiopharmaceuticals carriers and suitable amounts thereof are well known in the art, and can be found in, for example, Remington's Pharmaceutical Sciences, Gennaro, A.R., ed., Mack Publishing Company, Easton, PA (1985), and The United States Pharmacopia - The National Formulary, 22nd Revision, Mack Printing Company, Easton, PA (1990), standard reference texts in the pharmaceutical field. Other materials may be added, as convenient, to stabilize the composition, as those skilled in the art will recognize, including antioxidizing agents such as sodium bisulfite, sodium sulfite, ascorbic acid, gentisic acid or citric acid (or their salts) or sodium ethylenediamine tetraacetic acid (sodium EDTA), as is well known in the art. Such other materials, as well as suitable amounts thereof, are also described in Remington's Pharmaceutical Sciences and The United States Pharmacopia - The National Formulary, cited above.

The present invention also includes radiopharmaceutical kits containing the labeled compounds of the invention. Such kits may contain the labeled compounds in sterile lyophilized form, and may include a sterile container of a radiopharma-ceutically

acceptable reconstitution liquid. Suitable reconstitution liquids are disclosed in Remington's Pharmaceutical Sciences and The United States Pharmacopia - The National Formulary, cited above. Such kits may alternatively contain a sterile container of a composition of the radiolabeled compounds of the invention. Such kits may also include, if desired, other conventional kit components, such as, for example, one or more carriers, one or more additional vials for mixing. Instructions, either as inserts or labels, indicating quantities of the labeled compounds of the invention and carrier, guidelines for mixing these components, and protocols for administration may also be included in the kit. Sterilization of the containers and any materials included in the kit and lyophilization (also referred to as freeze-drying) of the labeled compounds of the invention may be carried out using conventional sterilization and lyophilization methodologies known to those skilled in the art.

To carry out the method of the invention, the radiolabeled compounds are generally administered intravenously, by bolus injection, although they may be administered by any means that produces contact of the compounds with platelets. Suitable amounts for administration will be readily ascertainable to those skilled in the art, once armed with the present disclosure. The dosage administered will, of course, vary depending up such known factors as the particular compound administered, the age, health and weight or the nature and extent of any symptoms experienced by the patient, the amount of radiolabeling, the particular radionuclide used as the label, the rate of clearance of the radiolabeled compounds from the blood.

Acceptable ranges for administration of radiolabeled materials are tabulated, for example, in the Physicians Desk Reference (PDR) for Nuclear Medicine, published by Medical Exonomics Company, a well-known reference text. A discussion of some of the aforementioned considerations is provided in Eckelman et al., J. Nucl. Med., Vol. 209, pp. 350-357 (1979). By way of general guidance, a dosage range of the radiolabeled compounds of the invention may be between about 1 and about 40 mCi.

Once the radiolabeled compounds of the invention are administered, the presence of thrombi may be visualized using a standard radioscintographic imaging system, such as, for example, a gamma camera or a computed tomographic device, and thromboembolic disorders detected. Such imaging systems are well known in the art, and are discussed, for example, in Macovski, A., Medical Imaging Systems, Information and Systems Science Series, Kailath, T., ed., Prentice-Hall, Inc., Englewood Cliffs, NJ (1983). Particularly preferred are single-photon emission computed tomography (SPECT) and positron emission tomography (PET). Specifically, imaging is carried out by scanning the entire patient, or a particular region of the patient suspected of having a thrombus formation, using the radioscintographic system, and detecting the radioisotope signal. The detected signal is then converted into an image of the thrombus by the system. The resultant images should be read by an experienced observer, such as, for example, a nuclear medicine physician. The foregoing process is referred to herein as "imaging" the patient. Generally, imaging is carried out about 1 minute to about 48 hours following

administration of the radiolabeled compound of the invention. The precise timing of the imaging will be dependant upon such factors as the half-life of the radioisotope employed, and the clearance rate of the compound administered, as will be readily apparent to those skilled in the art. Preferably, imaging is carried out between about 1 minute and about 4 hours following administration.

The advantage of employing the radiolabeled compounds of the invention, which have the ability to localize specifically and with high affinity in thrombi, to detect the presence of thrombi and/or to diagnose thromboembolic disorders in a patient, will be readily apparent to those skilled in the art, once armed with the present disclosure.

Arteriovenous Shunt Model: Adult mongrel dogs of either sex (9-13kg) were anesthetized with pentobarbital sodium (35 mg/kg,i.v.) and ventilated with room air via an endotracheal tube (12 strokes/min,25 ml/kg). For arterial pressure determination, the left carotid artery was cannulated with a saline-filled polyethylene catheter (PE-240) and connected to a Statham pressure transducer (P23ID; Oxnard,CA). Mean arterial blood pressure was determined via damping the pulsatile pressure signal. Heart rate was monitored using a cardiotachometer (Biotach, Grass Quincy, MA) triggered from a lead II electrocardiogram generated by limb leads. A jugular vein was cannulated (PE-240) for drug administration. The both femoral arteries and femoral veins were cannulated with silicon treated (Sigmacote, Sigma Chemical Co. St Louis, MO), saline filled polyethylene tubing (PE-200) and connected with a 5 cm section of silicon treated tubing (PE-240) to form

an extracorporeal arterio-venous shunts (A-V). Shunt patency was monitored using a doppler flow system (model VF-1, Crystal Biotech Inc, Hopkinton, MA) and flow probe (2-2.3 mm, Titronics Med. Inst., Iowa City, IA) placed proximal to the locus of the shunt. All parameters were monitored continuously on a polygraph recorder (model 7D Grass) at a paper speed of 10 mm/min or 25 mm/sec.

On completion of a 15 min post surgical stabilization period, an occlusive thrombus was formed by the introduction of a thrombogenic surface (4-0 braided silk thread, 5 cm in length, Ethicon Inc., Somerville, NJ) into the shunt one shunt with the other serving as a control. Two consecutive 1hr shunt periods were employed with the test agent administered as an infusion over 5 min beginning 5 min before insertion of the thrombogenic surface. At the end of each 1 hr shunt period the silk was carefully removed and weighed and the % incorporation determined via well counting. Thrombus weight was calculated by subtracting the weight of the silk prior to placement from the total weight of the silk on removal from the shunt. The results are shown in Table 4. Arterial blood was withdrawn prior to the first shunt and every 30 min thereafter for determination of blood clearance, whole blood collagen-induced platelet aggregation, thrombin-induced platelet degranulation (platelet ATP release), prothrombin time and platelet count. Template bleeding time was also performed at 30 min intervals.

Canine Deep Vein Thrombosis Model: This model incorporates the triad of events (hypercoagulatible state, period of stasis, low shear environment) essential for the formation of a venous fibrin-rich

actively growing thrombus. The procedure was as follows: Adult mongrel dogs of either sex (9-13 kg) were anesthetized with pentobarbital sodium (35 mg/kg,i.v.) and ventilated with room air via an endotracheal tube (12 strokes/min, 25 ml/kg). For arterial pressure determination, the right femoral artery was cannulated with a saline-filled polyethylene catheter (PE-240) and connected to a Statham pressure transducer (P23ID; Oxnard,CA). Mean arterial blood pressure was determined via damping the pulsatile pressure signal. Heart rate was monitored using a cardiotachometer (Biotach, Grass Quincy, MA) triggered from a lead II electrocardiogram generated by limb leads. The right femoral vein was cannulated (PE-240) for drug administration. A 5 cm segment of both jugular veins was isolated, freed from fascia and circumscribed with silk suture. A microthermister probe was placed on the vessel which serves as an indirect measure of venous flow. A balloon embolectomy catheter was utilized to induce the 15 min period of stasis during which time a hypercoagulatible state was then induced using 5 U thrombin (American Diagnosticia, Greenwich CT) administered into the occluded segment. Fifteen minutes later, flow was reestablished by deflating the balloon. The agent was infused during the first 5 min of reflow and the rate of incorporation monitored using gamma scintigraphy. The results for Examples 12 and 19 are shown in Figure 1.

Example 33

Table 4. Experimental Data from the Arteriovenous Shunt Model

(mean± SEM, T/B = thrombus/background)

Ex. #	Venous Conditions		Arterial Conditions	
	Uptake (%id/g)	T/B ratio	Uptake (%id/g)	T/B ratio
8	0.25±0.15	19±9	1.81±0.18	173±22
9	0.45±0.11	8±3	2.60±.005	44±4
10	0.16±0.02	7±0.6	5.00±0.51	221±16
12	0.46±0.19	7.0±2	6.15±0.66	111±6
13	1.64±1.32	33±27	8.50±0.20	163±14
16	0.08	14	0.95±0.29	128±24
18	0.04±.01	13±3	0.47±0.12	147±44
19	0.58±0.22	13±4	5.75±1.28	142±24
21	0.06±0.03	4.0±2	1.6±0.12	113±1
22	0.045±0.02	7±4	1.28±0.44	158±5
23	0.21±0.05	7±0.4	5.41±0.70	195±39
32	0	0	7.4	102

Platelet Aggregation Assay: Canine blood was collected into 10 ml citrated Vacutainer tubes. The blood was centrifuged for 15 minutes at 150 x g at room temperature, and platelet-rich plasma (PRP) was removed. The remaining blood was centrifuged for 15 minutes at 1500 x g at room temperature, and platelet-poor plasma (PPP) was removed. Samples were assayed on a aggregometer (PAP-4 Platelet Aggregation Profiler), using PPP as the blank (100% transmittance). 200 µl of PRP was added to each micro test tube, and transmittance was set to 0%. 20 µl of various agonists (ADP, collagen, arachidonate, epinephrine, thrombin) were added to each tube, and the aggregation profiles were plotted (% transmittance versus time). The results were expressed as % inhibition of agonist-induced platelet aggregation. For the IC_{50} evaluation, the test

compounds were added at various concentrations prior to the activation of the platelets.

Platelet-Fibrinogen Binding Assay: Binding of ^{125}I-fibrinogen to platelets was performed as described by Bennett et al. (1983) Proc. Natl. Acad. Sci. USA 80: 2417-2422, with some modifications as described below. Human PRP (h-PRP) was applied to a Sepharose column for the purification of platelet fractions. Aliquots of platelets (5 X 10^8 cells) along with 1 mM calcium chloride were added to removable 96 well plates prior to the activation of the human gel purified platelets (h-GPP). Activation of the human gel purified platelets was achieved using ADP, collagen, arachidonate, epinephrine, and/or thrombin in the presence of the ligand, ^{125}I-fibrinogen. The ^{125}I-fibrinogen bound to the activated, platelets was separated from the free form by centrifugation and then counted on a gamma counter. For an IC_{50} evaluation, the test compounds were added at various concentrations prior to the activation of the platelets.

The novel cyclic glycoprotein IIb/IIIa compounds of the invention may also possess thrombolytic efficacy, that is, they are capable of lysing (breaking up) already formed platelet-rich fibrin blood clots, and thus may useful in treating a thrombus formation, as evidenced by their activity in the tests described below. Preferred cyclic compounds of the present invention for use in thrombolysis would include those compounds having an IC_{50} value (that is, the molar concentration of the cyclic compound capable of achieving 50% clot lysis) of less than about 1 mM, more preferably an IC_{50} value of less than about 0.1 mM, even

more preferably an IC_{50} value of less than about 0.01 mM, still more preferably an IC_{50} value of less than about 0.001 mM, and most preferably an IC_{50} value of about 0.0005 mM.

IC_{50} determinations may be made using a standard thrombolysis assay, as described below. Another class of preferred thrombolytic compounds of the invention would include those compounds which have a Kd of < 100 nM, preferably < 10 nM, most preferably 0.1 to 1.0 nM.

Thrombolytic Assay: Venous blood was obtained from the arm of a healthy human donor who was drug-free and aspirin free for at least two weeks prior to blood collection, and placed into 10 ml citrated Vacutainer tubes. The blood was centrifuged for 15 minutes at 1500 x g at room temperature, and platelet rich plasma (PRP) was removed. To the PRP was then added 1 x 10^{-3} M of the agonist ADP, epinephrine, collagen, arachidonate, serotonin or thrombin, or a mixture thereof, and the PRP incubated for 30 minutes. The PRP was centrifuged for 12 minutes at 2500 x g at room temperature. The supernatant was then poured off, and the platelets remaining in the test tube were resuspended in platelet poor plasma (PPP), which served as a plasminogen source. The suspension was then assayed on a Coulter Counter (Coulter Electronics, Inc., Hialeah, FL), to determine the platelet count at the zero time point. After obtaining the zero time point, test compounds were added at various concentrations. Test samples were taken at various time points and the platelets were counted using the Coulter Counter. To determine the percent of lysis, the platelet count at a time point subsequent to the addition of the test compound was subtracted from the platelet count at the zero time point, and the resulting

number divided by the platelet count at the zero time point. Multiplying this result by 100 yielded the percentage of clot lysis achieved by the test compound. For the IC_{50} evaluation, the test compounds were added at various concentrations, and the percentage of lysis caused by the test compounds was calculated.

The disclosures of each patent and publication cited in this document are hereby incorporated herein by reference, in their entirety.

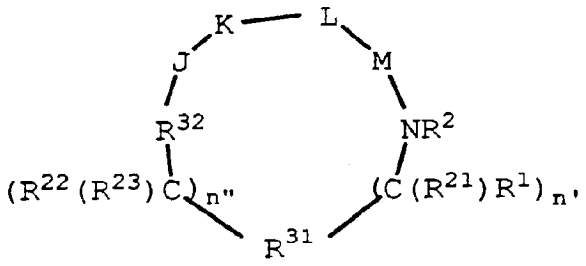
Various modifications in the invention, in addition to those shown and described herein will be readily apparent to those skilled in the art from the foregoing description. Such modifications are intended to be within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A reagent for preparing a radiopharmaceutical of formulae:

$$(QL_n)_dC_h \; ; \; (Q)_{d'}L_n\text{-}C_h,$$

wherein, d is 1-3, d' is 2-20, L_n is a linking group, C_h is a metal chelator, and Q is a compound of formula (I):



(I)

or a pharmaceutically acceptable salt or prodrug form thereof, wherein:

R^{31} is a C_6-C_{14} saturated, partially saturated, or aromatic carbocyclic ring system, substituted with 0-4 R^{10} or R^{10a}, and optionally bearing a bond to L_n; a heterocyclic ring system, optionally substituted with 0-4 R^{10} or R^{10a}, and optionally bearing a bond to L_n;

R^{32} is selected from:
-C(=O)-;

-C(=S)-
-S(=O)$_2$-;
-S(=O)-;
-P(=Z)(ZR^{13})-;

Z is S or O;

n" and n' are independently 0-2;

R^1 and R^{22} are independently selected from the following groups:

hydrogen,
C_1-C_8 alkyl substituted with 0-2 R^{11};
C_2-C_8 alkenyl substituted with 0-2 R^{11};
C_2-C_8 alkynyl substituted with 0-2 R^{11};
C_3-C_{10} cycloalkyl substituted with 0-2 R^{11};

a bond to L_n;

aryl substituted with 0-2 R^{12};

a 5-10-membered heterocyclic ring system containing 1-4 heteroatoms independently selected from N, S, and O, said heterocyclic ring being substituted with 0-2 R^{12};

=O, F, Cl, Br, I, -CF$_3$, -CN, -CO$_2$R^{13}, -C(=O)R^{13}, -C(=O)N(R^{13})$_2$, -CHO, -CH$_2$OR^{13}, -OC(=O)R^{13}, -OC(=O)OR^{13a}, -OR^{13}, -OC(=O)N(R^{13})$_2$, -NR^{13}C(=O)R^{13}, -NR^{14}C(=O)OR^{13a}, -NR^{13}C(=O)N(R^{13})$_2$,

$-NR^{14}SO_2N(R^{13})_2$, $-NR^{14}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-SR^{13}$, $-S(=O)R^{13a}$, $-SO_2N(R^{13})_2$, $-N(R^{13})_2$, $-NHC(=NH)NHR^{13}$, $-C(=NH)NHR^{13}$, $=NOR^{13}$, NO_2, $-C(=O)NHOR^{13}$, $-C(=O)NHNR^{13}R^{13a}$, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy;

R^1 and R^{21} can alternatively join to form a 3-7 membered carbocyclic ring substituted with 0-2 R^{12};

when n' is 2, R^1 or R^{21} can alternatively be taken together with R^1 or R^{21} on an adjacent carbon atom to form a direct bond, thereby to form a double or triple bond between said carbon atoms;

R^{21} and R^{23} are independently selected from:

hydrogen;
C_1-C_4 alkyl, optionally substituted with 1-6 halogen;
benzyl;

R^{22} and R^{23} can alternatively join to form a 3-7 membered carbocyclic ring substituted with 0-2 R^{12};

when n" is 2, R^{22} or R^{23} can alternatively be taken together with R^{22} or R^{23} on an adjacent carbon atom to form a direct bond, thereby to form a double or triple bond between the adjacent carbon atoms;

R^1 and R^2, where R^{21} is H, can alternatively join to form a 5-8 membered carbocyclic ring substituted with 0-2 R^{12};

R^{11} is selected from one or more of the following:

=O, F, Cl, Br, I, $-CF_3$, $-CN$, $-CO_2R^{13}$, $-C(=O)R^{13}$, $-C(=O)N(R^{13})_2$, $-CHO$, $-CH_2OR^{13}$, $-OC(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{14}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{14}SO_2N(R^{13})_2$, $-NR^{14}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-SR^{13}$, $-S(=O)R^{13a}$, $-SO_2N(R^{13})_2$, $-N(R^{13})_2$, $-NHC(=NH)NHR^{13}$, $-C(=NH)NHR^{13}$, $=NOR^{13}$, NO_2, $-C(=O)NHOR^{13}$, $-C(=O)NHNR^{13}R^{13a}$, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy,

C_1-C_5 alkyl, C_2-C_4 alkenyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_2-C_6 alkoxyalkyl, C_3-C_6 cycloalkoxy, C_1-C_4 alkyl (alkyl being substituted with 1-5 groups selected independently from: $-NR^{13}R^{14}$, $-CF_3$, NO_2, $-SO_2R^{13a}$, or $-S(=O)R^{13a}$),

aryl substituted with 0-2 R^{12},

a 5-10-membered heterocyclic ring system containing 1-4 heteroatoms independently selected from N, S, and O, said

heterocyclic ring being substituted with 0-2 R^{12};

R^{12} is selected from one or more of the following:

phenyl, benzyl, phenethyl, phenoxy, benzyloxy, halogen, hydroxy, nitro, cyano, C_1-C_5 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_7-C_{10} arylalkyl, C_1-C_5 alkoxy, $-CO_2R^{13}$, $-C(=O)NHOR^{13a}$, $-C(=O)NHN(R^{13})_2$, $=NOR^{13}$, $-B(R^{34})(R^{35})$, C_3-C_6 cycloalkoxy, $-OC(=O)R^{13}$, $-C(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-(C_1$-C_4 alkyl)$-OR^{13}$, $-N(R^{13})_2$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{13}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{13}SO_2N(R^{13})_2$, $-NR^{13}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-S(=O)R^{13a}$, $-SR^{13}$, $-SO_2N(R^{13})_2$, C_2-C_6 alkoxyalkyl, methylenedioxy, ethylenedioxy, C_1-C_4 haloalkyl, C_1-C_4 haloalkoxy, C_1-C_4 alkylcarbonyloxy, C_1-C_4 alkylcarbonyl, C_1-C_4 alkylcarbonylamino, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy, C_1-C_4 alkyl (alkyl being substituted with $-N(R^{13})_2$, $-CF_3$, NO_2, or $-S(=O)R^{13a}$);

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, $-(C_1$-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, $-(C_1$-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may alternatively be taken together to form $-(CH_2)_{2-5}-$ or $-(CH_2)O(CH_2)-$;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

R^2 is H or C_1-C_8 alkyl;

R^{10} and R^{10a} are selected independently from one or more of the following:

phenyl, benzyl, phenethyl, phenoxy, benzyloxy, halogen, hydroxy, nitro, cyano, C_1-C_5 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_7-C_{10} arylalkyl, C_1-C_5 alkoxy, $-CO_2R^{13}$, $-C(=O)N(R^{13})_2$, $-C(=O)NHOR^{13a}$, $-C(=O)NHN(R^{13})_2$, $=NOR^{13}$, $-B(R^{34})(R^{35})$, C_3-C_6 cycloalkoxy, $-OC(=O)R^{13}$, $-C(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-(C_1$-C_4 alkyl$)-OR^{13}$, $-N(R^{13})_2$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{13}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{13}SO_2N(R^{13})_2$, $-NR^{13}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-S(=O)R^{13a}$, $-SR^{13}$, $-SO_2N(R^{13})_2$, C_2-C_6 alkoxyalkyl, methylenedioxy, ethylenedioxy, C_1-C_4 haloalkyl (including $-C_vF_w$ where v = 1 to 3 and w = 1 to (2v+1)), C_1-C_4 haloalkoxy, C_1-C_4 alkylcarbonyloxy, C_1-C_4 alkylcarbonyl, C_1-C_4 alkylcarbonylamino, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy, C_1-C_4 alkyl

(alkyl being substituted with $-N(R^{13})_2$, $-CF_3$, NO_2, or $-S(=O)R^{13a}$);

J is β-Ala or an L-isomer or D-isomer amino acid of structure $-N(R^3)C(R^4)(R^5)C(=O)-$, wherein:

R^3 is H or C_1-C_8 alkyl;

R^4 is H or C_1-C_3 alkyl;

R^5 is selected from:

hydrogen;

C_1-C_8 alkyl substituted with 0-2 R^{11};

C_2-C_8 alkenyl substituted with 0-2 R^{11};

C_2-C_8 alkynyl substituted with 0-2 R^{11};

C_3-C_{10} cycloalkyl substituted with 0-2 R^{11};

a bond to L_n;

aryl substituted with 0-2 R^{12};

a 5-10-membered heterocyclic ring system containing 1-4 heteroatoms independently selected from N, S, or O, said heterocyclic ring being substituted with 0-2 R^{12};

=O, F, Cl, Br, I, $-CF_3$, $-CN$, $-CO_2R^{13}$, $-C(=O)R^{13}$, $-C(=O)N(R^{13})_2$, $-CHO$, $-CH_2OR^{13}$, $-OC(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{14}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$,

$-NR^{14}SO_2N(R^{13})_2$, $-NR^{14}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-SR^{13}$, $-S(=O)R^{13a}$, $-SO_2N(R^{13})_2$, $-N(R^{13})_2$, $-NHC(=NH)NHR^{13}$, $-C(=NH)NHR^{13}$, $=NOR^{13}$, NO_2, $-C(=O)NHOR^{13}$, $-C(=O)NHNR^{13}R^{13a}$, $=NOR^{13}$, $-B(R^{34})(R^{35})$, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy, $-SC(=NH)NHR^{13}$, N_3, $-Si(CH_3)_3$, (C_1-C_5 alkyl)NHR^{16};

$-(C_0$-C_6 alkyl)X;

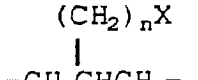
, where q is independently 0,1;

;

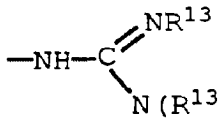
$-(CH_2)_mS(O)_{p'}(CH_2)_2X$, where m = 1,2 and p' = 0-2;

wherein X is defined below; and

R^3 and R^4 may also be taken together to form



, where

n = 0,1 and X is  ;

R^3 and R^5 can alternatively be taken together to form $-(CH_2)_t-$ or $-CH_2S(O)_{p'}C(CH_3)_2-$, where t = 2-4 and p' = 0-2; or

R^4 and R^5 can alternatively be taken together to form $-(CH_2)_u-$, where u = 2-5;

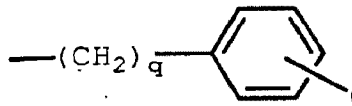
R^{16} is selected from:
an amine protecting group;
1-2 amino acids;
1-2 amino acids substituted with an amine protecting group;

K is a D-isomer or L-isomer amino acid of structure
$-N(R^6)CH(R^7)C(=O)-$, wherein:

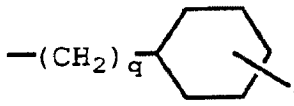
R^6 is H or C_1-C_8 alkyl;

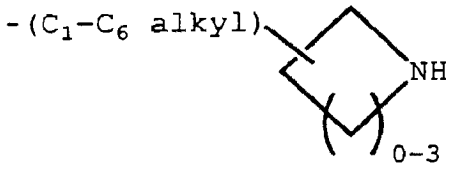
R^7 is selected from:

$-(C_1-C_7$ alkyl$)X$;



, wherein each q is independently 0-2 and substitution on the phenyl is at the 3 or 4 position;

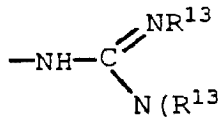

, wherein each q is independently 0-2 and substitution on the cyclohexyl is at the 3 or 4 position;



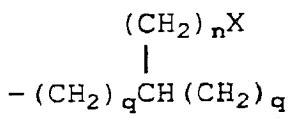
$-(CH_2)_mO-(C_1-C_4$ alkyl$)-X$, where m = 1 or 2;

$-(CH_2)_mS(O)_{p'}-(C_1-C_4$ alkyl$)-X$, where m = 1 or 2 and p' = 0-2; and

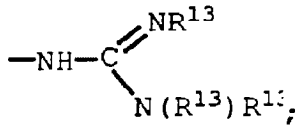
X is selected from:


; $-N(R^{13})R^{13}$; $-C(=NH)(NH_2)$; $-SC(=NH)-NH_2$; $-NH-C(=NH)(NHCN)$; $-NH-C(=NCN)(NH_2)$; $-NH-C(=N-OR^{13})(NH_2)$;

R^6 and R^7 can alternatively be taken together to form


, wherein each q is independently 1 or 2 and wherein

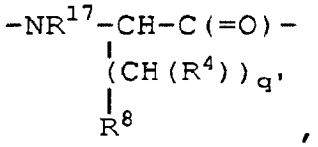
n = 0 or 1 and X is $-NH_2$ or



L is $-Y(CH_2)_vC(=O)-$, wherein:

Y is NH, $N(C_1-C_3$ alkyl), O, or S; and v = 1 or 2;

M is a D-isomer or L-isomer amino acid of structure



wherein:

q' is 0-2;

R^{17} is H, C_1-C_3 alkyl;

R^8 is selected from:
$-CO_2R^{13}$, $-SO_3R^{13}$, $-SO_2NHR^{14}$, $-B(R^{34})(R^{35})$, $-NHSO_2CF_3$, $-CONHNHSO_2CF_3$, $-PO(OR^{13})_2$, $-PO(OR^{13})R^{13}$, $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O) , $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and

having 1-4 heteroatoms selected independently from N, S, or O), $-SO_2NHCOR^{13}$, $-CONHSO_2R^{13a}$, $-CH_2CONHSO_2R^{13a}$, $-NHSO_2NHCOR^{13a}$, $-NHCONHSO_2R^{13a}$, $-SO_2NHCONHR^{13}$;

R^{34} and R^{35} are independently selected from:
-OH,
-F,
$-N(R^{13})_2$, or
C_1-C_8-alkoxy;

R^{34} and R^{35} can alternatively be taken together form:
a cyclic boron ester where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-4 heteroatoms independently selected from N, S, or O;
a divalent cyclic boron amide where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-4 heteroatoms independently selected from N, S, or O;
a cyclic boron amide-ester where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-4 heteroatoms independently selected from N, S, or O.

2. A reagent of Claim 1, wherein:

R^{31} is bonded to $(C(R^{23})R^{22})_{n''}$ and $(C(R^{21})R^{1})_{n'}$ at 2 different atoms on said carbocyclic ring.

3. A reagent of Claim 1, wherein:

n" is 0 and n' is 0;
n" is 0 and n' is 1;
n" is 0 and n' is 2;
n" is 1 and n' is 0;
n" is 1 and n' is 1;
n" is 1 and n' is 2;
n" is 2 and n' is 0;
n" is 2 and n' is 1; or
n" is 2 and n' is 2.

4. A reagent of Claim 1 wherein R^6 is methyl, ethyl, or propyl.

5. A reagent of Claim 1 wherein:

R^{32} is selected from:
$-C(=O)-$;
$-C(=S)-$
$-S(=O)_2-$;

R^1 and R^{22} are independently selected from the following groups:

hydrogen,
C_1-C_8 alkyl substituted with 0-2 R^{11},
C_2-C_8 alkenyl substituted with 0-2 R^{11},
C_2-C_8 alkynyl substituted with 0-2 R^{11},
C_3-C_8 cycloalkyl substituted with 0-2 R^{11},

C_6-C_{10} bicycloalkyl substituted with 0-2 R^{11};

a bond to L_n;

aryl substituted with 0-2 R^{12};

a 5-10-membered heterocyclic ring system containing 1-4 heteroatoms independently selected from N, S, or O, said heterocyclic ring being substituted with 0-2 R^{12};

=O, F, Cl, Br, I, $-CF_3$, -CN, $-CO_2R^{13}$, $-C(=O)R^{13}$, $-C(=O)N(R^{13})_2$, -CHO, $-CH_2OR^{13}$, $-OC(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{14}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{14}SO_2N(R^{13})_2$, $-NR^{14}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-SR^{13}$, $-S(=O)R^{13a}$, $-SO_2N(R^{13})_2$, $-CH_2N(R^{13})_2$, $-N(R^{13})_2$, $-NHC(=NH)NHR^{13}$, $-C(=NH)NHR^{13}$, NO_2;

R^1 and R^{21} can alternatively join to form a 5-7 membered carbocyclic ring substituted with 0-2 R^{12};

when n' is 2, R^1 or R^{21} can alternatively be taken together with R^1 or R^{21} on an adjacent carbon atom to form a direct bond, thereby to form a double or triple bond between said carbon atoms;

R^{22} and R^{23} can alternatively join to form a 3-7 membered carbocyclic ring substituted with 0-2 R^{12};

when n" is 2, R^{22} or R^{23} can alternatively be taken together with R^{22} or R^{23} on an adjacent carbon atom to form a direct bond, thereby to form a double or triple bond between said carbon atoms;

R^1 and R^2, where R^{21} is H, can alternatively join to form a 5-8 membered carbocyclic ring substituted with 0-2 R^{12};

R^{11} is selected from one or more of the following:

=O, F, Cl, Br, I, $-CF_3$, -CN, $-CO_2R^{13}$, $-C(=O)R^{13}$, $-C(=O)N(R^{13})_2$, -CHO, $-CH_2OR^{13}$, $-OC(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{14}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{14}SO_2N(R^{13})_2$, $-NR^{14}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-SR^{13}$, $-S(=O)R^{13a}$, $-SO_2N(R^{13})_2$, $-CH_2N(R^{13})_2$, $-N(R^{13})_2$, $-NHC(=NH)NHR^{13}$, $-C(=NH)NHR^{13}$, $=NOR^{13}$, NO_2;

C_1-C_5 alkyl, C_2-C_4 alkenyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_2-C_6 alkoxyalkyl, C_1-C_4 alkyl (substituted with $-NR^{13}R^{14}$, $-CF_3$, NO_2, $-SO_2R^{13}$, or $-S(=O)R^{13a}$)

aryl substituted with 0-2 R^{12},

a 5-10-membered heterocyclic ring system containing 1-4 heteroatoms independently selected from N, S, or O, said heterocyclic ring being substituted with 0-2 R^{12};

R^3 is H or CH_3;

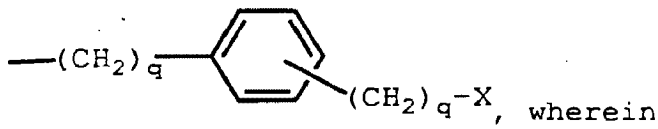
R^5 is H, C_1-C_8 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_1-C_6 cycloalkylethyl, phenyl, phenylmethyl, CH_2OH, CH_2SH, CH_2OCH_3, CH_2SCH_3, $CH_2CH_2SCH_3$, $(CH_2)_sNH_2$, $(CH_2)_sNHC(=NH)(NH_2)$, $(CH_2)_sNHR^{16}$, where s = 3-5;

a bond to L_n;

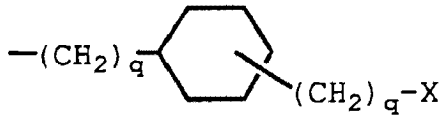
R^3 and R^5 can alternatively be taken together to form $-(CH_2)_t-$ (t = 2-4) or $-CH_2SC(CH_3)_2-$; or

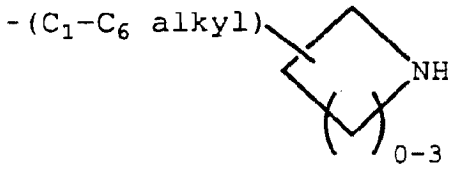
R^7 is selected from:

$-(C_1-C_7$ alkyl$)X$;



, wherein each q is independently 0-2 and substitution on the phenyl is at the 3 or 4 position;


, wherein each q is independently 0-2 and substitution on the cyclohexyl is at the 3 or 4 position;

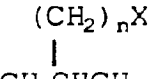


$-(CH_2)_mO-(C_1-C_4$ alkyl)-X, where m = 1 or 2;

$-(CH_2)_mS-(C_1-C_4$ alkyl)-X, where m = 1 or 2; and

X is selected from:
$-NH-C(=NH)(NH_2)$, $-NHR^{13}$, $-C(=NH)(NH_2)$, $-SC(NH)-NH_2$;

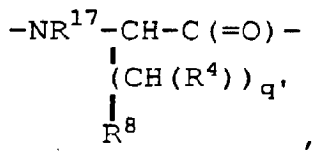
R^6 and R^7 can alternatively be taken together to form


, where n = 0 or 1 and X is $-NH_2$ or $-NH-C(=NH)(NH_2)$;

L is $-Y(CH_2)_vC(=O)-$, wherein:

Y is NH, N(C_1-C_3 alkyl), O, or S; and v = 1 or 2;

M is a D-isomer or L-isomer amino acid of structure



wherein:

q' is 0-2;

R^{17} is H, C_1-C_3 alkyl;

R^8 is selected from:

$-CO_2R^{13}$, $-SO_3R^{13}$, $-SO_2NHR^{14}$, $-B(R^{34})(R^{35})$, $-NHSO_2CF_3$, $-CONHNHSO_2CF_3$, $-PO(OR^{13})_2$, $-PO(OR^{13})R^{13}$, $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O) , $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O), $-SO_2NHCOR^{13}$, $-CONHSO_2R^{13a}$, $-CH_2CONHSO_2R^{13a}$, $-NHSO_2NHCOR^{13a}$, $-NHCONHSO_2R^{13a}$, $-SO_2NHCONHR^{13}$;

R^{34} and R^{35} are independently selected from:

-OH,

-F,

$-NR^{13}R^{14}$, or

C_1-C_8-alkoxy;

R^{34} and R^{35} can alternatively be taken together form:
a cyclic boron ester where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-4 heteroatoms independently selected from N, S, or O;
a divalent cyclic boron amide where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-4 heteroatoms independently selected from N, S, or O;
a cyclic boron amide-ester where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-4 heteroatoms independently selected from N, S, or O.

6. A reagent of Claim 1, wherein:

R^{31} is selected from the group consisting of:

(a) a 6 membered saturated, partially saturated or aromatic carbocyclic ring substituted with 0-3 R^{10} or R^{10a}, and optionally bearing a bond to Ln;

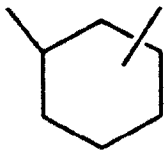
(b) a 8-11 membered saturated, partially saturated, or aromatic fused bicyclic carbocyclic ring substituted with 0-3 R^{10} or R^{10a}, and optionally bearing a bond to Ln; or

(c) a 14 membered saturated, partially saturated, or aromatic fused tricyclic carbocyclic ring substituted with 0-3 R^{10} or R^{10a}, and optionally bearing a bond to Ln.

7. A reagent of Claim 1, wherein:

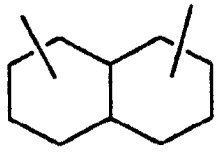
R^{31} is selected from the group consisting of:

(a) a 6 membered saturated, partially saturated, or aromatic carbocyclic ring of formulae:



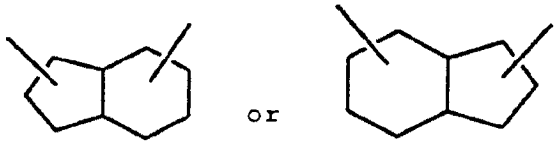
wherein any of the bonds forming the carbocyclic ring may be a single or double bond, and wherein said carbocyclic ring is substituted with 0-3 R^{10}, and optionally bears a bond to Ln;

(b) a 10 membered saturated, partially saturated, or aromatic bicyclic carbocyclic ring of formula:



wherein any of the bonds forming the carbocyclic ring may be a single or double bond, wherein said carbocyclic ring is substituted independently with 0-4 R^{10}, and optionally bears a bond to L_n;

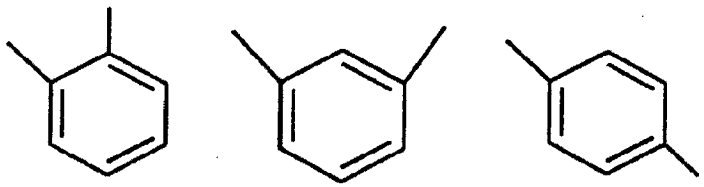
(c) a 9 membered saturated, partially saturated, or aromatic bicyclic carbocyclic ring of formula:

 or ,

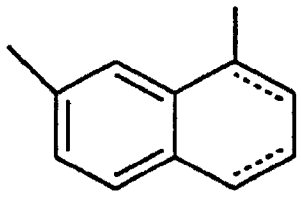
wherein any of the bonds forming the carbocyclic ring may be a single or double bond, wherein said carbocyclic ring is substituted independently with 0-4 R^{10}, and optionally bears a bond to L_n.

8. A reagent of Claim 1, wherein:

R^{31} is selected from (the dashed bond may be a single or double bond):



; or



;

wherein R^{31} may be independently substituted with 0-3 R^{10} or R^{10a}, and optionally bears a bond to L_n;

n" is 0 or 1; and

n' is 0-2.

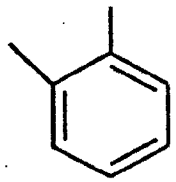
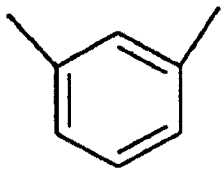
9. A reagent of Claim 1, wherein:

R^1 and R^{22} are independently selected from: phenyl, benzyl, phenethyl, phenoxy, benzyloxy, halogen, hydroxy, nitro, cyano, C_1-C_5 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_7-C_{10} arylalkyl, C_1-C_5 alkoxy, $-CO_2R^{13}$, $-C(=O)NHOR^{13a}$, $-C(=O)NHN(R^{13})_2$, $=NOR^{13}$, $-B(R^{34})(R^{35})$, C_3-C_6 cycloalkoxy, $-OC(=O)R^{13}$, $-C(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-(C_1$-C_4 alkyl$)-OR^{13}$, $-N(R^{13})_2$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{13}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{13}SO_2N(R^{13})_2$, $-NR^{13}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-S(=O)R^{13a}$, $-SR^{13}$, $-SO_2N(R^{13})_2$, C_2-C_6 alkoxyalkyl, methylenedioxy, ethylenedioxy, C_1-C_4 haloalkyl, C_1-C_4 haloalkoxy, C_1-C_4 alkylcarbonyloxy, C_1-C_4 alkylcarbonyl, C_1-C_4 alkylcarbonylamino,

$-OCH_2CO_2H$, 2-(1-morpholino)ethoxy, C_1-C_4 alkyl (alkyl being substituted with $-N(R^{13})_2$, $-CF_3$, NO_2, or $-S(=O)R^{13a}$).

10. A reagent of Claim 1, wherein:

R^{31} is selected from:


;

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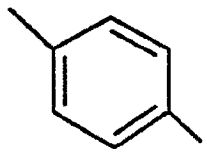
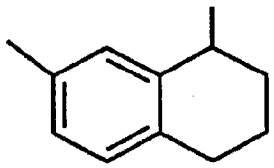

;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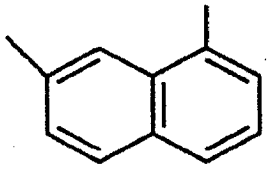

;

wherein R^{31} may be independently substituted with 0-3 R^{10} or R^{10a}, and may optionally bear a bond to L_n;

R^{32} is $-C(=O)-$;

n" is 0 or 1;

n' is 0-2;

R^1 and R^{22} are independently selected from H, C_1-C_4 alkyl, phenyl, benzyl, phenyl-(C_2-C_4)alkyl, C_1-C_4 alkoxy; and a bond to L_n;

R^{21} and R^{23} are independently H or C_1-C_4 alkyl;

R^2 is H or C_1-C_8 alkyl;

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may alternatively be taken together to form $-(CH_2)_{2-5}-$ or $-(CH_2)O(CH_2)-$;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

R^{10} and R^{10a} are selected independently from: H, C_1-C_8 alkyl, phenyl, halogen, or C_1-C_4 alkoxy;

J is β-Ala or an L-isomer or D-isomer amino acid of structure $-N(R^3)C(R^4)(R^5)C(=O)-$, wherein:

R^3 is H or CH_3;

R^4 is H or C_1-C_3 alkyl;

R^5 is H, C_1-C_8 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_1-C_6 cycloalkylethyl, phenyl, phenylmethyl, CH_2OH, CH_2SH, CH_2OCH_3, CH_2SCH_3, $CH_2CH_2SCH_3$, $(CH_2)_sNH_2$, $-(CH_2)_sNHC(=NH)(NH_2)$, $-(CH_2)_sNHR^{16}$, where s = 3-5; and a bond to L_n; or

R^3 and R^5 can alternatively be taken together to form $-(CH_2)_t-$ (t = 2-4) or $-CH_2SC(CH_3)_2-$; or

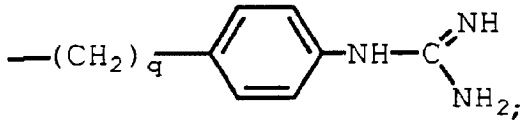
R^4 and R^5 can alternatively be taken together to form $-(CH_2)_u-$, where u = 2-5;

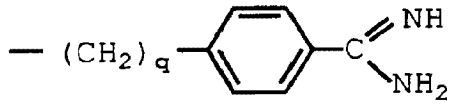
R^{16} is selected from:
an amine protecting group;
1-2 amino acids; or
1-2 amino acids substituted with an amine protecting group;

K is an L-isomer amino acid of structure $-N(R^6)CH(R^7)C(=O)-$, wherein:

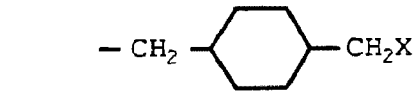
R^6 is H or C_1-C_8 alkyl;

R^7 is




, where q = 0 or 1;

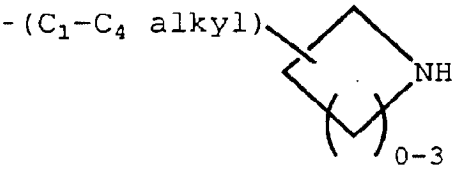
$-(CH_2)_rX$, where r = 3-6;


;


;

$-(CH_2)_mS(CH_2)_2X$, where m = 1 or 2;

$-(C_3-C_7$ alkyl)$-NH-(C_1-C_6$ alkyl);

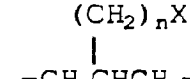


$-(CH_2)_m-O-(C_1-C_4$ alkyl)$-NH-(C_1-C_6$ alkyl), where m = 1 or 2;

$-(CH_2)_m-S-(C_1-C_4$ alkyl)$-NH-(C_1-C_6$ alkyl), where m = 1 or 2; and

X is $-NH_2$ or $-NHC(=NH)(NH_2)$; or

R^6 and R^7 can alternatively be taken together to form

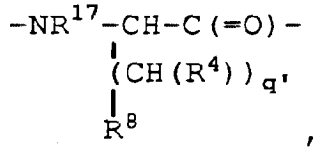

, where n = 0 or 1

and X is $-NH_2$ or $-NHC(=NH)(NH_2)$;

L is $-Y(CH_2)_vC(=O)-$, wherein:

Y is NH, O, or S; and v = 1 or 2;

M is a D-isomer or L-isomer amino acid of structure



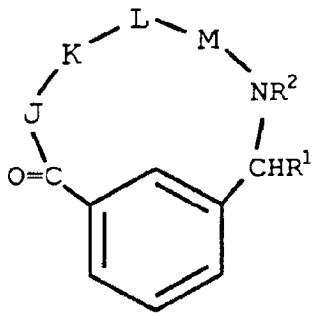
wherein:

q' is 0-2;

R^{17} is H, C_1-C_3 alkyl;

R^8 is selected from:
$-CO_2R^{13}$, $-SO_3R^{13}$, $-SO_2NHR^{14}$, $-B(R^{34})(R^{35})$, $-NHSO_2CF_3$, $-CONHNHSO_2CF_3$, $-PO(OR^{13})_2$, $-PO(OR^{13})R^{13}$, $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O) , $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O), $-SO_2NHCOR^{13}$, $-CONHSO_2R^{13a}$, $-CH_2CONHSO_2R^{13a}$, $-NHSO_2NHCOR^{13a}$, $-NHCONHSO_2R^{13a}$, $-SO_2NHCONHR^{13}$.

11. The reagent of Claim 1 that is a 1,3-disubstituted phenyl compound of the formula (II):



(II)

wherein:

the shown phenyl ring in formula (II) may be substituted with 0-3 R^{10}, and may optionally bear a bond to L_n;

R^{10} is selected independently from: H, C_1-C_8 alkyl, phenyl, halogen, or C_1-C_4 alkoxy;

R^1 is H, C_1-C_4 alkyl, phenyl, benzyl, phenyl-(C_1-C_4)alkyl, or a bond to L_n;

R^2 is H or methyl;

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may

alternatively be taken together to form $-(CH_2)_{2-5}-$ or $-(CH_2)O(CH_2)-$;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

J is β-Ala or an L-isomer or D-isomer amino acid of structure $-N(R^3)C(R^4)(R^5)C(=O)-$, wherein:

R^3 is H or CH_3;

R^4 is H or C_1-C_3 alkyl;

R^5 is H, C_1-C_8 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_1-C_6 cycloalkylethyl, phenyl, phenylmethyl, CH_2OH, CH_2SH, CH_2OCH_3, CH_2SCH_3, $CH_2CH_2SCH_3$, $(CH_2)_sNH_2$, $-(CH_2)_sNHC(=NH)(NH_2)$, $-(CH_2)_sNHR^{16}$, where s = 3-5, or a bond to L_n;

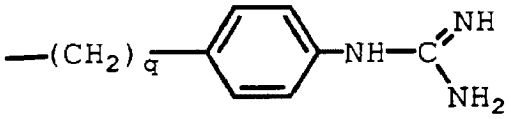
R^3 and R^5 can alternatively be taken together to form $-CH_2CH_2CH_2-$; or
R^4 and R^5 can alternatively be taken together to form $-(CH_2)_u-$, where u = 2-5;

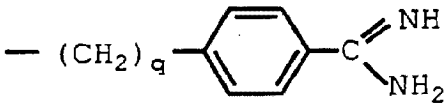
R^{16} is selected from:
an amine protecting group;
1-2 amino acids; or
1-2 amino acids substituted with an amine protecting group;

K is an L-isomer amino acid of structure $-N(R^6)CH(R^7)C(=O)-$, wherein:

R^6 is H or C_1-C_8 alkyl;

R^7 is:





, where q = 0 or 1;

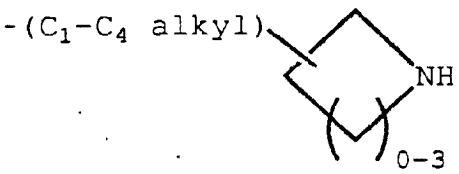
$-(CH_2)_rX$, where r = 3-6;

;

;

$-(CH_2)_mS(CH_2)_2X$, where m = 1 or 2;

$-(C_3$-C_7 alkyl)-NH-(C_1-C_6 alkyl)



$-(CH_2)_m$-O-(C_1-C_4 alkyl)-NH-(C_1-C_6 alkyl), where m = 1 or 2;

$-(CH_2)_m$-S-(C_1-C_4 alkyl)-NH-(C_1-C_6 alkyl), where m = 1 or 2; and

X is $-NH_2$ or $-NHC(=NH)(NH_2)$, provided that X is not $-NH_2$ when r = 4; or

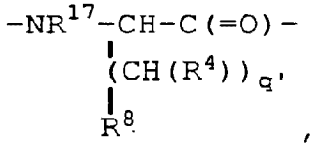
R^6 and R7 are alternatively be taken together to form

where n = 0,1 and X is $-NH_2$ or $-NHC(=NH)(NH_2)$;

L is $-Y(CH_2)_vC(=O)-$, wherein:

Y is NH, O, or S; and v = 1,2;

M is a D-isomer or L-isomer amino acid of structure



wherein:

q' is 0-2;

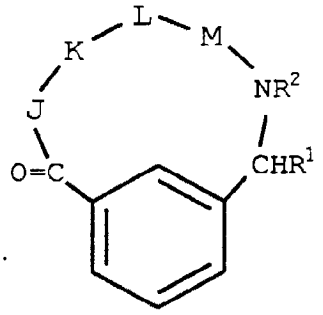
R^{17} is H, C_1-C_3 alkyl;

R^8 is selected from:

$-CO_2R^{13}$, $-SO_3R^{13}$, $-SO_2NHR^{14}$, $-B(R^{34})(R^{35})$, $-NHSO_2CF_3$, $-CONHNHSO_2CF_3$, $-PO(OR^{13})_2$, $-PO(OR^{13})R^{13}$, $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O) , $-SO_2NH$-heteroaryl

(said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O), $-SO_2NHCOR^{13}$, $-CONHSO_2R^{13a}$, $-CH_2CONHSO_2R^{13a}$, $-NHSO_2NHCOR^{13a}$, $-NHCONHSO_2R^{13a}$, $-SO_2NHCONHR^{13}$.

12. The reagent of Claim 1 that is a 1,3-disubstituted phenyl compound of the formula (II):



(II)

wherein:

the phenyl ring in formula (II) may be substituted with 0-3 R^{10} or R^{10a};

R^{10} or R^{10a} are selected independently from: H, C_1-C_8 alkyl, phenyl, halogen, or C_1-C_4 alkoxy;

R^1 is H, C_1-C_4 alkyl, phenyl, benzyl, or phenyl-(C_2- C_4)alkyl;

R^2 is H or methyl;

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12}

alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may alternatively be taken together to form $-(CH_2)_{2-5}-$ or $-(CH_2)O(CH_2)-$;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

J is β-Ala or an L-isomer or D-isomer amino acid of structure $-N(R^3)C(R^4)(R^5)C(=O)-$, wherein:

R^3 is H or CH_3;

R^4 is H;

R^5 is H, C_1-C_8 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_1-C_6 cycloalkylethyl, phenyl, phenylmethyl, CH_2OH, CH_2SH, CH_2OCH_3, CH_2SCH_3, $CH_2CH_2SCH_3$, $(CH_2)_sNH_2$, $(CH_2)_sNHC(=NH)(NH_2)$, $(CH_2)_sR^{16}$, where s = 3-5; or a bond to L_n;

R^3 and R^5 can alternatively be taken together to form $-CH_2CH_2CH_2-$;

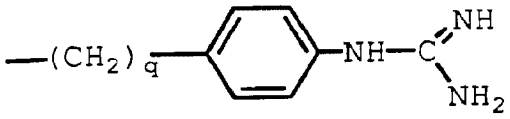
R^{16} is selected from:
an amine protecting group;
1-2 amino acids;

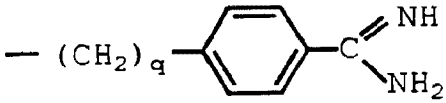
1-2 amino acids substituted with an amine protecting group;

K is an L-isomer amino acid of structure $-N(R^6)CH(R^7)C(=O)-$, wherein:

R^6 is H or C_3-C_8 alkyl;

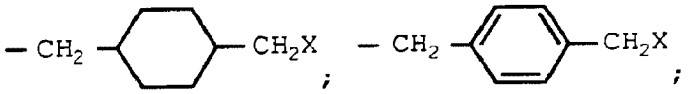
R^7 is





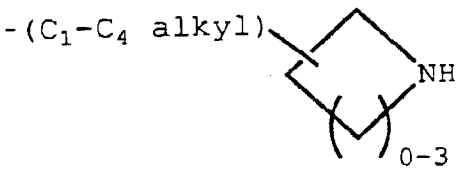
where q = 0 or 1;

$-(CH_2)_rX$, where r = 3-6;



$-(CH_2)_mS(CH_2)_2X$, where m = 1 or 2;

$-(C_4$-C_7 alkyl$)$-NH-$(C_1$-C_6 alkyl$)$



$-(CH_2)_m$-O-$(C_1$-C_4 alkyl$)$-NH-$(C_1$-C_6 alkyl), where m = 1 or 2;

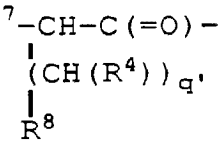
$-(CH_2)_m-S-(C_1-C_4$ alkyl$)-NH-(C_1-C_6$ alkyl), where m = 1 or 2; and

X is $-NH_2$ or $-NHC(=NH)(NH_2)$, provided that X is not $-NH_2$ when r = 4; or

L is $-YCH_2C(=O)-$, wherein:

Y is NH or O;

M is a D-isomer or L-isomer amino acid of structure



, wherein:

q' is 1;

R^{17} is H, C_1-C_3 alkyl;

R^8 is selected from:
$-CO_2H$ or $-SO_3R^{13}$.

13. The reagent of Claim 1 that that is a compound of formula (II) above, wherein:

the phenyl ring in formula (II) bears a bond to L_n, and may be further substituted with 0-2 R^{10} or R^{10a};

R^{10} or R^{10a} are selected independently from: H, C_1-C_8 alkyl, phenyl, halogen, or C_1-C_4 alkoxy;

R^1 is H;

R^2 is H;

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may alternatively be taken together to form $-(CH_2)_{2-5}-$ or $-(CH_2)O(CH_2)-$;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

J is β-Ala or an L-isomer or D-isomer amino acid of formula $-N(R^3)CH(R^5)C(=O)-$, wherein:

R^3 is H and R^5 is H, CH_3, CH_2CH_3, $CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$, $CH_2CH_2CH_3$, $CH_2CH_2CH_2CH_3$, $CH_2CH_2SCH_3$, $CH_2CH(CH_3)_2$, $(CH_2)_4NH_2$, (C_3-C_5 alkyl)NHR^{16};
or

R^3 is CH_3 and R^5 is H; or

R^3 and R^5 can alternatively be taken together to form $-CH_2CH_2CH_2-$;

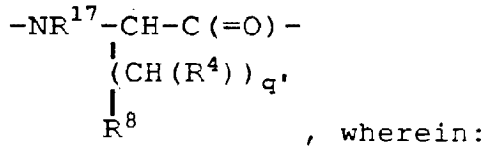
R^{16} is selected from:
an amine protecting group;
1-2 amino acids;
1-2 amino acids substituted with an amine protecting group;

K is an L-isomer amino acid of formula $-N(CH_3)CH(R^7)C(=O)-$, wherein:

R^7 is $-(CH_2)_3NHC(=NH)(NH_2)$;

L is $-NHCH_2C(=O)-$; and

M is a D-isomer or L-isomer amino acid of structure



, wherein:

q' is 1;

R^4 is H or CH_3;

R^{17} is H;

R^8 is
$-CO_2H$;
$-SO_3H$.

14. The reagent of Claim 1 that that is a compound of formula (II) above, wherein:

the phenyl ring in formula (II) bears a bond to L_n;

R^1 and R^2 are independently selected from H, methyl;

J is selected from D-Val, D-2-aminobutyric acid, D-Leu, D-Ala, Gly, D-Pro, D-Ser, D-Lys, βAla, Pro, Phe, NMeGly, D-Nle, D-Phg, D-Ile, D-Phe, D-Tyr, Ala, N^{ε}-p-azidobenzoyl-D-Lys, N^{ε}-p-benzoylbenzoyl-D-Lys, N^{ε}-tryptophanyl-D-Lys, N^{ε}-o-benzylbenzoyl-D-Lys, N^{ε}-p-acetylbenzoyl-D-Lys, N^{ε}-dansyl-D-Lys, N^{ε}-glycyl-D-Lys, N^{ε}-glycyl-p-benzoylbenzoyl-D-Lys, N^{ε}-p-phenylbenzoyl-D-Lys, N^{ε}-m-benzoylbenzoyl-D-Lys, N^{ε}-o-benzoylbenzoyl-D-Lys;

K is selected from NMeArg, Arg;

L is selected from Gly, βAla, Ala;

M is selected from Asp; αMeAsp; βMeAsp; NMeAsp; D-Asp.

15. The reagent of Claim 1, wherein:

R^{31} bears a bond to L_n;

R^1 and R^2 are independently selected from H, methyl;

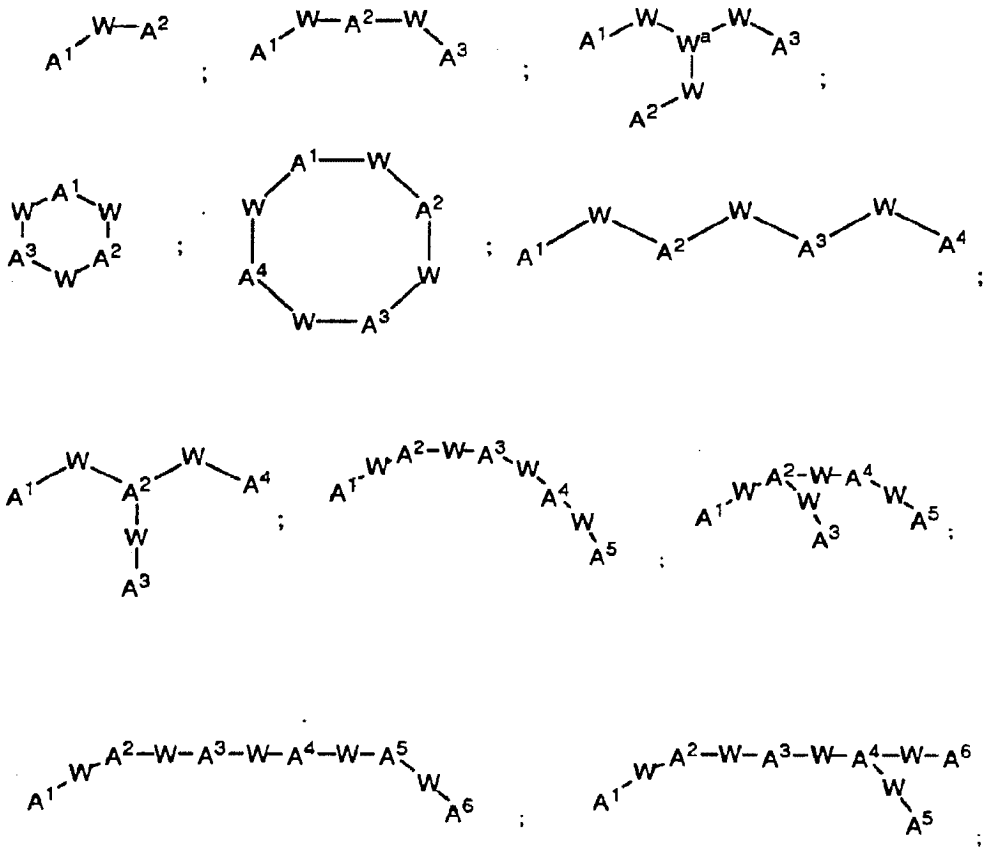
J is selected from: D-Val, D-2-aminobutyric acid, D-Leu, D-Ala, Gly, D-Pro, D-Ser, D-Lys, βAla, Pro, Phe, NMeGly, D-Nle, D-Phg, D-Ile, D-Phe, D-Tyr, Ala;

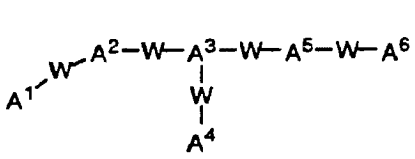
K is selected from NMeArg;

L is Gly;

M is selected from Asp; αMeAsp; βMeAsp; NMeAsp; D-Asp.

16. A reagent as in one of claims 1-15, wherein C_h is selected from the group:

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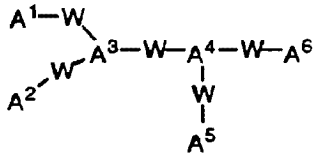
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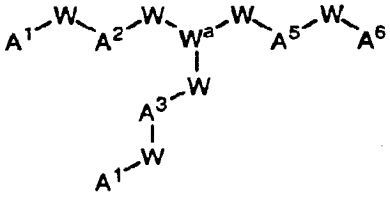
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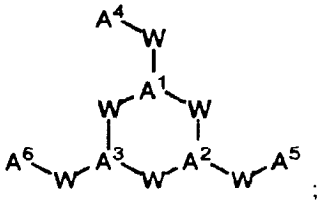
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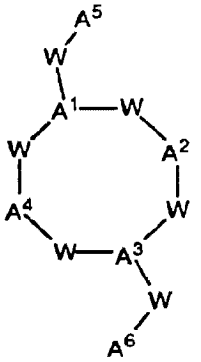
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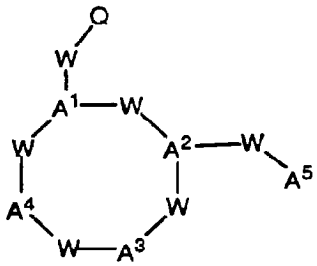
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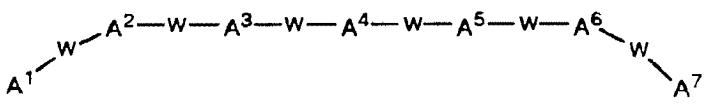
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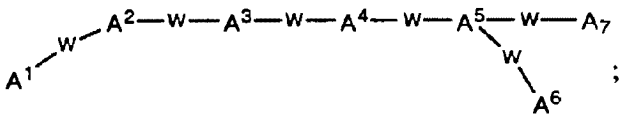
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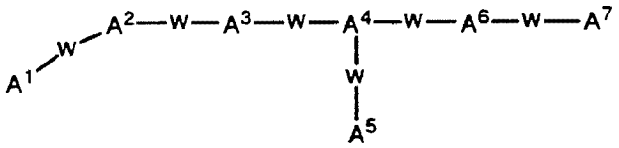
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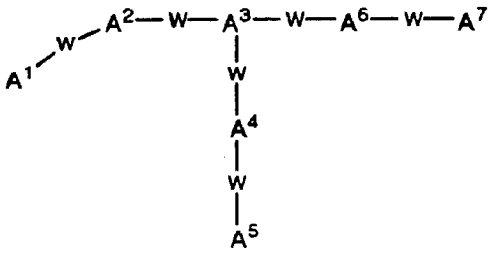
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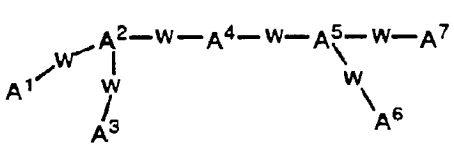
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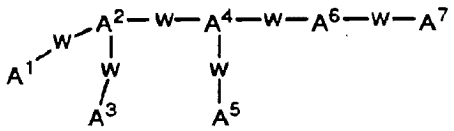
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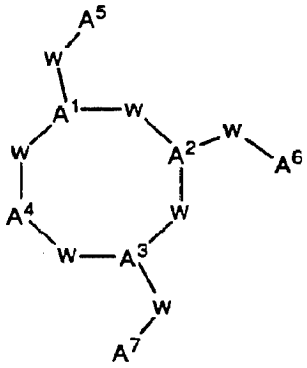
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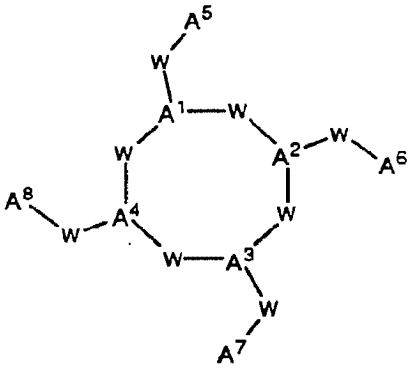
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wherein:

A^1, A^2, A^3, A^4, A^5, A^6, and A^7 are independently selected at each occurrence from the group: $NR^{40}R^{41}$, S, SH, S(Pg), O, OH, $PR^{42}R^{43}$, $P(O)R^{42}R^{43}$, $P(S)R^{42}R^{43}$, $P(NR^{44})R^{42}R^{43}$;

W is a bond, CH, or a spacer group selected from the group: C_1-C_{10} alkyl substituted with 0-3 R^{52}, aryl substituted with 0-3 R^{52}, cycloaklyl substituted with 0-3 R^{52}, heterocycloalkyl substituted with 0-3 R^{52}, aralkyl substituted with 0-3 R^{52} and alkaryl substituted with 0-3 R^{52};

W^a is a C_1-C_{10} alkyl group or a C_3-C_{14} carbocycle;

R^{40}, R^{41}, R^{42}, R^{43}, and R^{44} are each independently selected from the group: a bond to L_n, hydrogen, C_1-C_{10} alkyl substituted with 0-3 R^{52}, aryl substituted with 0-3 R^{52}, cycloaklyl substituted with 0-3 R^{52}, heterocycloalkyl substituted with 0-3 R^{52}, aralkyl substituted with 0-3 R^{52}, alkaryl substituted with 0-3 R^{52}substituted with 0-3 R^{52} and an electron, provided that when one of R^{40} or R^{41} is an electron, then the other is also an electron, and provided that when one of R^{42} or R^{43} is an electron, then the other is also an electron;

additionally, R^{40} and R^{41} may combine to form =C(C_1-C_3 alkyl)(C_1-C_3 alkyl);

R^{52} is independently selected at each occurrence from the group: a bond to L_n, =O, F, Cl, Br, I, $-CF_3$, -CN, $-CO_2R^{53}$, $-C(=O)R^{53}$, $-C(=O)N(R^{53})_2$, -CHO, $-CH_2OR^{53}$, $-OC(=O)R^{53}$, $-OC(=O)OR^{53a}$, $-OR^{53}$, $-OC(=O)N(R^{53})_2$, $-NR^{53}C(=O)R^{53}$, $-NR^{54}C(=O)OR^{53a}$, $-NR^{53}C(=O)N(R^{53})_2$, $-NR^{54}SO_2N(R^{53})_2$, $-NR^{54}SO_2R^{53a}$, $-SO_3H$, $-SO_2R^{53a}$, $-SR^{53}$, $-S(=O)R^{53a}$, $-SO_2N(R^{53})_2$, $-N(R^{53})_2$, $-NHC(=NH)NHR^{53}$, $-C(=NH)NHR^{53}$, $=NOR^{53}$, NO_2, $-C(=O)NHOR^{53}$, $-C(=O)NHNR^{53}R^{53a}$, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy,

C_1-C_5 alkyl, C_2-C_4 alkenyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_2-C_6 alkoxyalkyl,

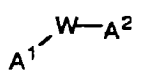
aryl substituted with 0-2 R^{53},

a 5-10-membered heterocyclic ring system containing 1-4 heteroatoms independently selected from N, S, and O;

R^{53}, R^{53a}, and R^{54} are independently selected at each occurrence from the group: a bond to L_n, C_1-C_6 alkyl, phenyl, benzyl, C_1-C_6 alkoxy, halide, nitro, cyano, and trifluoromethyl; and

Pg is a thiol protecting group capable of being displaced upon reaction with a radionuclide.

17. A reagent as in one of Claims 1-15, wherein C_h is selected from the group: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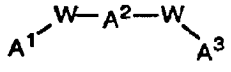
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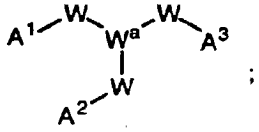
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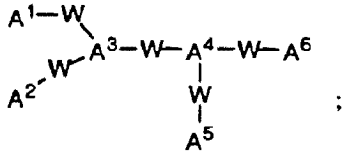
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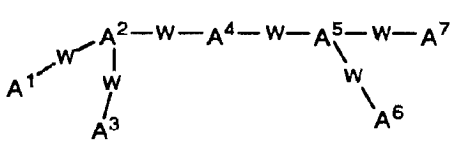
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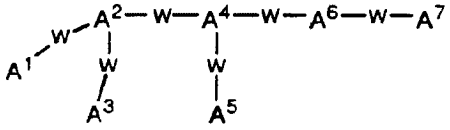
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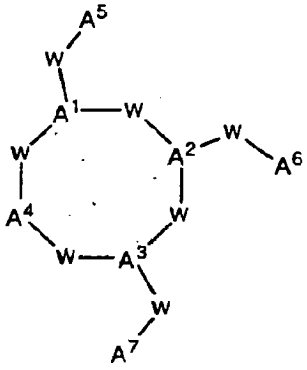
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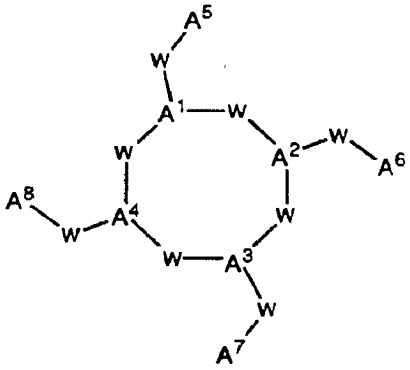

;

wherein:

A^1, A^2, A^3, A^4, A^5, A^6, and A^7 are independently selected at each occurrence from the group: $NR^{40}R^{41}$, S, SH, S(Pg), OH;

W is a bond, CH, or a spacer group selected from the group: C_1-C_3 alkyl substituted with 0-3 R^{52};

W^a is a methylene group or a C_3-C_6 carbocycle;

R^{40}, R^{41}, R^{42}, R^{43}, and R^{44} are each independently selected from the group: a bond to L_n, hydrogen, C_1-C_{10} alkyl substituted with 0-3 R^{52}, and an electron, provided that when one of R^{40} or R^{41} is an electron, then the other is also an electron, and provided that when one of R^{42} or R^{43} is an electron, then the other is also an electron;

additionally, R^{40} and R^{41} may combine to form, =C(C_1-C_3 alkyl)(C_1-C_3 alkyl);

R^{52} is independently selected at each occurrence from the group: a bond to L_n, =O, F, Cl, Br, I, $-CF_3$, -CN, $-CO_2R^{53}$, $-C(=O)R^{53}$, $-C(=O)N(R^{53})_2$, -CHO, $-CH_2OR^{53}$, $-OC(=O)R^{53}$, $-OC(=O)OR^{53a}$, $-OR^{53}$, $-OC(=O)N(R^{53})_2$, $-NR^{53}C(=O)R^{53}$, $-NR^{54}C(=O)OR^{53a}$, $-NR^{53}C(=O)N(R^{53})_2$, $-NR^{54}SO_2N(R^{53})_2$, $-NR^{54}SO_2R^{53a}$, $-SO_3H$,

$-SO_2R^{53a}$, $-SR^{53}$, $-S(=O)R^{53a}$, $-SO_2N(R^{53})_2$, $-N(R^{53})_2$, $-NHC(=NH)NHR^{53}$, $-C(=NH)NHR^{53}$, $=NOR^{53}$, NO_2, $-C(=O)NHOR^{53}$, $-C(=O)NHNR^{53}R^{53a}$, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy; and

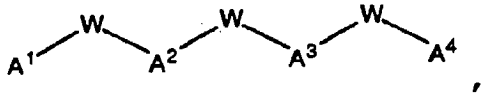
R^{53}, R^{53a}, and R^{54} are independently selected at each occurrence from the group: a bond to L_n, C_1-C_6 alkyl.

18. A reagent as in one of Claims 1-15, of formula:

$$(QL_n)_dC_h,$$

wherein d is 1; and

C_h is selected from:



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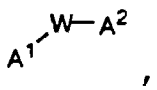
wherein:

A^1 and A^4 are SH or SPg;
A^2 and A^3 are NR^{41};
W is independently selected from the group:
CHR^{52}, CH_2CHR^{52}, $CH_2CH_2CHR^{52}$ and $CHR^{52}C=O$; and
R^{41} and R^{52} are independently selected from hydrogen and a bond to L_n,

and,



wherein:

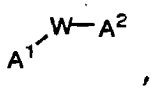
A^1 is NH_2 or $N=C(C_1-C_3$ alkyl$)(C_1-C_3$ alkyl);
W is a bond;
A^2 is NHR^{40}, wherein R^{40} is heterocycle substituted with R^{52}, wherein the heterocycle is selected from the group: pyridine, pyrazine, proline, furan, thiofuran, thiazole, and diazine, and R^{52} is a bond to L_n.

19. A reagent as in one of Claims 1-15, of formula:

$$(QL_n)_dC_h,$$

wherein d is 1; and

wherein C_h is:



wherein:

A^1 is NH_2 or $N=C(C_1-C_3$ alkyl$)(C_1-C_3$ alkyl);
W is a bond;
A^2 is NHR^{40}, wherein R^{40} is heterocycle substituted with R^{52}, wherein the

heterocycle is selected from pyridine and thiazole, and R^{52} is a bond to L_n.

20. A reagent as in one of Claims 1-15, wherein Ln is:

a bond between Q and C_h; or,
a compound of formula:

$$M^1-[Y^1(CR^{55}R^{56})_h(Z^1)_{h"}Y^2]_{h'}-M^2$$

wherein:

M^1 is $-[(CH_2)_gZ^1]_{g'}-(CR^{55}R^{56})_{g"}-$;
M^2 is $-(CR^{55}R^{56})_{g"}-[Z^1(CH_2)_g]_{g'}-$;
g is independently 0-10;
g' is independently 0-1;
g" is 0-10;
h is 0-10;
h' is 0-10;
h" is 0-1
Y^1 and Y^2, at each occurrence, are independently selected from:

a bond, O, NR^{56}, C=O, C(=O)O, OC(=O)O, C(=O)NH-, $C=NR^{56}$, S, SO, SO_2, SO_3, NHC(=O), $(NH)_2C(=O)$, $(NH)_2C=S$;

Z^1 is independently selected at each occurrence from a C_6-C_{14} saturated, partially saturated, or aromatic carbocyclic ring system, substituted with 0-4 R^{57}; a heterocyclic ring

system, optionally substituted with 0-4 R^{57};

R^{55} and R^{56} are independently selected at each occurrence from:

hydrogen;
C_1-C_{10} alkyl substituted with 0-5 R^{57};
(C_1-C_{10} alkyl)aryl wherein the aryl is substituted with 0-5 R^{57};

R^{57} is independently selected at each occurrence from the group: hydrogen, OH, NHR^{58}, $C(=O)R^{58}$, $OC(=O)R^{58}$, $OC(=O)OR^{58}$, $C(=O)OR^{58}$, $C(=O)NR^{58}$-, C≡N, SR^{58}, SOR^{58}, SO_2R^{58}, $NHC(=O)R^{58}$, $NHC(=O)NHR^{58}$, $NHC(=S)NHR^{58}$; or, alternatively, when attached to an additional molecule Q, R^{57} is independently selected at each occurrence from the group: O, NR^{58}, C=O, C(=O)O, OC(=O)O, C(=O)N-, $C=NR^{58}$, S, SO, SO_2, SO_3, NHC(=O), $(NH)_2C(=O)$, $(NH)_2C=S$; and,

R^{58} is independently selected at each occurrence from the group:hydrogen; C_1-C_6 alkyl; benzyl, and phenyl.

21. A reagent as in Claim 16, wherein L_n is:

a compound of formula:

$$M^1-[Y^1(CR^{55}R^{56})_h(Z^1)_{h"}Y^2]_{h'}-M^2$$

wherein:

M^1 is $-[(CH_2)_gZ^1]_{g'}-(CR^{55}R^{56})_{g"}-$;
M^2 is $-(CR^{55}R^{56})_{g"}-[Z^1(CH_2)_g]_{g'}-$;
g is independently 0-10;
g' is independently 0-1;
g" is 0-10;
h is 0-10;
h' is 0-10;
h" is 0-1
Y^1 and Y^2, at each occurrence, are independently selected from:

a bond, O, NR^{56}, C=O, C(=O)O, OC(=O)O, C(=O)NH-, $C=NR^{56}$, S, SO, SO_2, SO_3, NHC(=O), $(NH)_2C(=O)$, $(NH)_2C=S$;

Z^1 is independently selected at each occurrence from a C_6-C_{14} saturated, partially saturated, or aromatic carbocyclic ring system, substituted with 0-4 R^{57}; a heterocyclic ring system, optionally substituted with 0-4 R^{57};

R^{55} and R^{56} are independently selected at each occurrence from:

hydrogen;
C_1-C_{10} alkyl substituted with 0-5 R^{57};

(C_1-C_{10} alkyl)aryl wherein the aryl is substituted with 0-5 R^{57};

R^{57} is independently selected at each occurrence from the group: hydrogen, OH, NHR^{58}, $C(=O)R^{58}$, $OC(=O)R^{58}$, $OC(=O)OR^{58}$, $C(=O)OR^{58}$, $C(=O)NR^{58}$-, C≡N, SR^{58}, SOR^{58}, SO_2R^{58}, $NHC(=O)R^{58}$, $NHC(=O)NHR^{58}$, $NHC(=S)NHR^{58}$; or, alternatively, when attached to an additional molecule Q, R57 is independently selected at each occurrence from the group: O, NR^{58}, C=O, C(=O)O, OC(=O)O, C(=O)N-, $C=NR^{58}$, S, SO, SO_2, SO_3, NHC(=O), $(NH)_2C(=O)$, $(NH)_2C=S$, and R^{57} is attached to an additional molecule Q; and,

R^{58} is independently selected at each occurrence from the group:hydrogen; C_1-C_6 alkyl; benzyl, and phenyl.

22. A reagent as in Claim 17, wherein L_n is:

$$-(CR^{55}R^{56})_{g"}-[Y^1(CR^{55}R^{56})_hY^2]_{h'}-(CR^{55}R^{56})_{g"}-,$$

wherein:

g" is 1-10;
h is 0-10;
h' is 1-10;
Y^1 and Y^2, at each occurrence, are independently selected from:

a bond, O, NR^{56}, C=O, C(=O)O,
OC(=O)O,
C(=O)NH-, $C=NR^{56}$, S, SO, SO_2, SO_3,
NHC(=O), $(NH)_2C(=O)$, $(NH)_2C=S$;

R^{55} and R^{56} are independently selected at each occurrence from:

hydrogen;
C_1-C_{10} alkyl substituted with 0-5 R^{57};
(C_1-C_{10} alkyl)aryl wherein the aryl is substituted with 0-5 R^{57};

R^{57} is independently selected at each occurrence from the group: hydrogen, OH, NHR^{58}, $C(=O)R^{58}$, $OC(=O)R^{58}$, $OC(=O)OR^{58}$, $C(=O)OR^{58}$, $C(=O)NR^{58}$-, C≡N, SR^{58}, SOR^{58}, SO_2R^{58}, $NHC(=O)R^{58}$, $NHC(=O)NHR^{58}$, $NHC(=S)NHR^{58}$; or, alternatively, when attached to an additional molecule Q, R57 is independently selected at each occurrence from the group: O, NR^{58}, C=O, C(=O)O, OC(=O)O, C(=O)N-, $C=NR^{58}$, S, SO, SO_2, SO_3, NHC(=O), $(NH)_2C(=O)$, $(NH)_2C=S$, and R^{57} is attached to an additional molecule Q; and,

R^{58} is independently selected at each occurrence from the group:hydrogen; C_1-C_6 alkyl; benzyl, and phenyl.

23. A reagent as in Claim 18, wherein L_n is:

$$-(CR^{55}R^{56})_{g"}-[Y^1(CR^{55}R^{56})_hY^2]_{h'}-(CR^{55}R^{56})_{g"}-,$$

wherein:

g" is 1-5;
h is 0-5;
h' is 1-5;
Y^1 and Y^2, at each occurrence, are independently selected from:

O, NR^{56}, C=O, C(=O)O, OC(=O)O, C(=O)NH-, $C=NR^{56}$, S, SO, SO_2, SO_3, NHC(=O), $(NH)_2C(=O)$, $(NH)_2C=S$;

R^{55} and R^{56} are independently selected at each occurrence from:

hydrogen;
C_1-C_{10} alkyl;
(C_1-C_{10} alkyl)aryl.

24. A reagent as in Claim 19, wherein L_n is:

$$-(CR^{55}R^{56})_{g"}-[Y^1(CR^{55}R^{56})_hY^2]_{h'}-(CR^{55}R^{56})_{g"}-,$$

wherein:

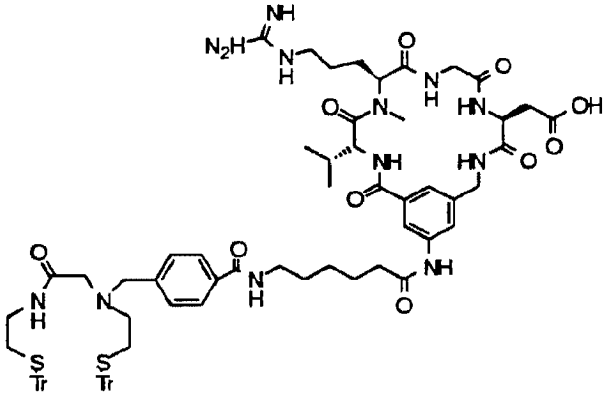
g" is 1-5;
h is 0-5;
h' is 1-5;
Y^1 and Y^2, at each occurrence, are independently selected from:

O, NR^{56}, C=O, C(=O)O, OC(=O)O,
C(=O)NH-, $C=NR^{56}$, S,
NHC(=O), $(NH)_2C(=O)$, $(NH)_2C=S$;

R^{55} and R^{56} are independently selected at each occurrence from:

hydrogen.

25. The reagents of Claim 1, which are:



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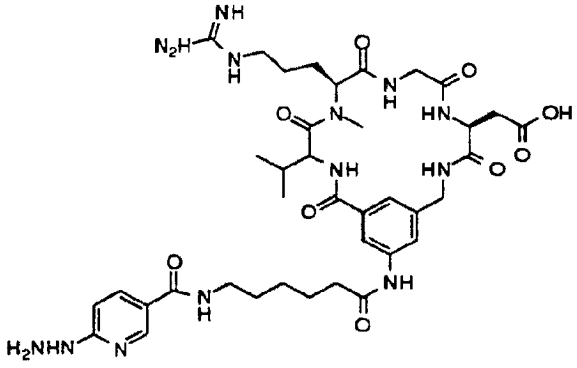

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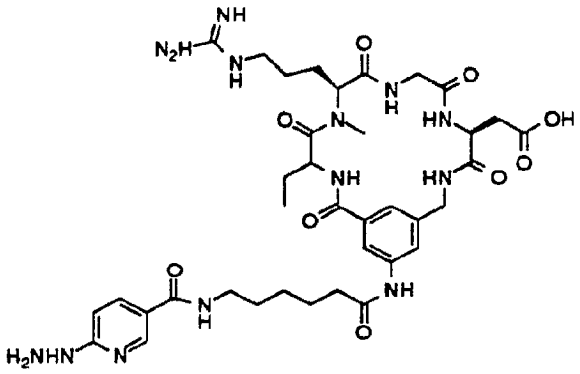

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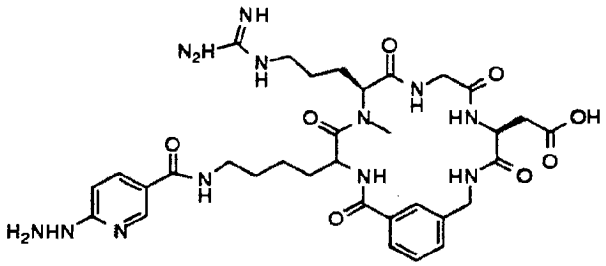
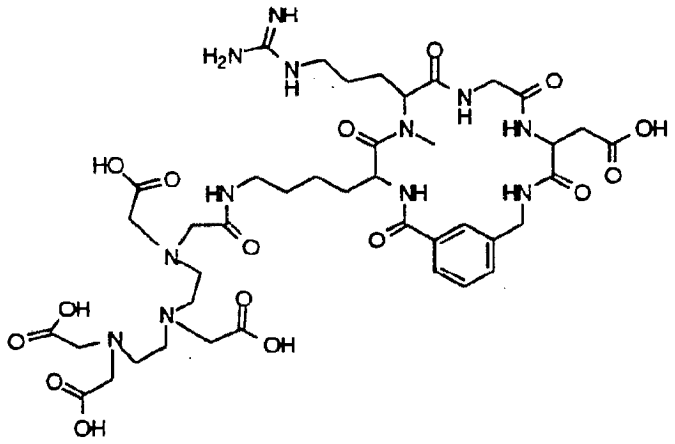


; and



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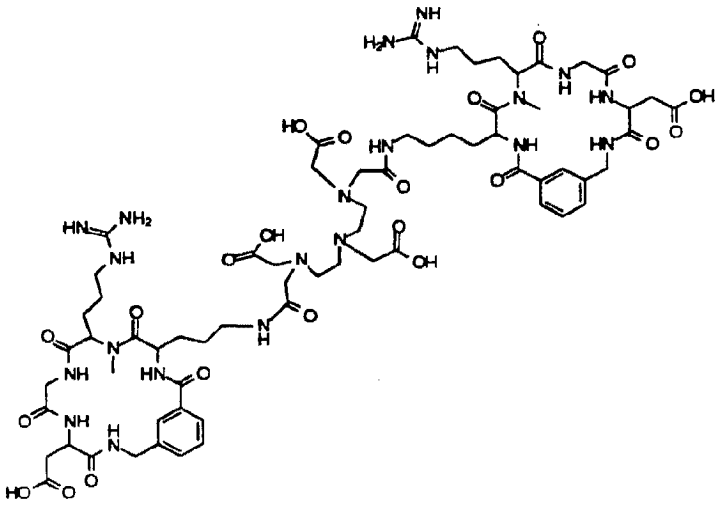


26. A kit for preparing a radiopharmaceutical comprising a predetermined quantity of a sterile, pharmaceutically acceptable reagent of Claim 23.

27. A kit for preparing a radiopharmaceutical comprising a predetermined quantity of a sterile, pharmaceutically acceptable reagent of Claim 24.

28. A kit for preparing a radiopharmaceutical comprising a predetermined quantity of a sterile, pharmaceutically acceptable reagent of Claim 25.

29. A radiopharmaceutical comprising a complex of a reagent of Claims 1-15 and a radionuclide selected from the group ^{99m}Tc, ^{94m}Tc, ^{95}Tc, ^{111}In, ^{62}Cu, ^{43}Sc, ^{45}Ti, ^{67}Ga, ^{68}Ga, ^{97}Ru, ^{72}As, ^{82}Rb, and ^{201}Tl.

30. A radiopharmaceutical comprising a complex of a reagent of Claim 16 and a radionuclide selected from the group ^{99m}Tc, ^{94m}Tc, ^{95}Tc, ^{111}In, ^{62}Cu, ^{43}Sc, ^{45}Ti, ^{67}Ga, ^{68}Ga, ^{97}Ru, ^{72}As, ^{82}Rb, and ^{201}Tl.

31. A radiopharmaceutical comprising a complex of a reagent of Claim 17 and a radionuclide selected from the group ^{99m}Tc, ^{94m}Tc, ^{95}Tc, ^{111}In, ^{62}Cu, ^{43}Sc, ^{45}Ti, ^{67}Ga, ^{68}Ga, ^{97}Ru, ^{72}As, ^{82}Rb, and ^{201}Tl.

32. A radiopharmaceutical comprising a complex of a reagent of Claim 18 and a radionuclide selected from the group ^{99m}Tc, ^{94m}Tc, ^{95}Tc, ^{111}In, ^{62}Cu, ^{43}Sc, ^{45}Ti, ^{67}Ga, ^{68}Ga, ^{97}Ru, ^{72}As, ^{82}Rb, and ^{201}Tl.

33. A radiopharmaceutical comprising a complex of a reagent of Claim 19 and a radionuclide selected from the group ^{99m}Tc, ^{94m}Tc, ^{95}Tc, ^{111}In, ^{62}Cu, ^{43}Sc, ^{45}Ti, ^{67}Ga, ^{68}Ga, ^{97}Ru, ^{72}As, ^{82}Rb, and ^{201}Tl.

34. A radiopharmaceutical comprising a complex of a reagent of Claim 20 and a radionuclide selected from the group ^{99m}Tc, ^{94m}Tc, ^{95}Tc, ^{111}In, ^{62}Cu, ^{43}Sc, ^{45}Ti, ^{67}Ga, ^{68}Ga, ^{97}Ru, ^{72}As, ^{82}Rb, and ^{201}Tl.

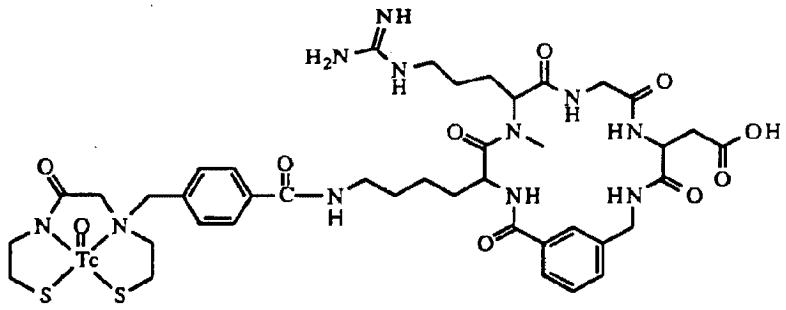
35. A radiopharmaceutical comprising a complex of a reagent of Claim 21 and a radionuclide selected from the group ^{99m}Tc, ^{111}In, and ^{62}Cu.

36. A radiopharmaceutical comprising a complex of a reagent of Claim 22 and a radionuclide selected from the group ^{99m}Tc, ^{111}In, and ^{62}Cu.

37. A radiopharmaceutical comprising a complex of a reagent of Claim 23 and a radionuclide selected from the group ^{99m}Tc, ^{111}In, and ^{62}Cu.

38. A radiopharmaceutical comprising a complex of a reagent of Claim 24 and a radionuclide selected from the group ^{99m}Tc, and ^{111}In.

39. The radiopharmaceuticals of Claim 29, which are:



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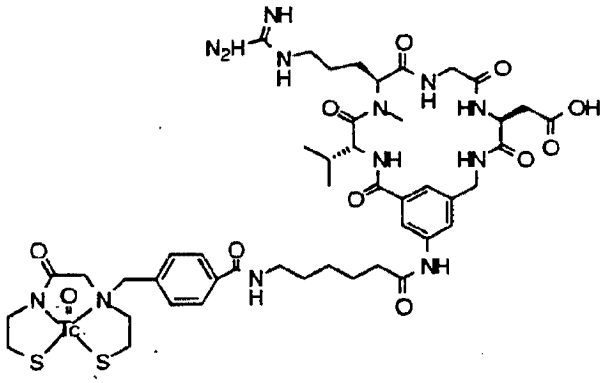

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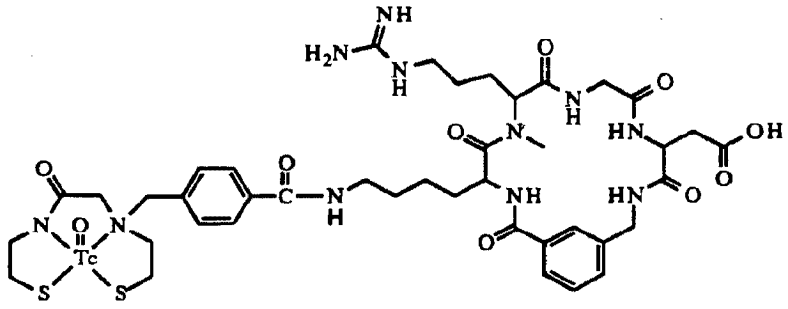

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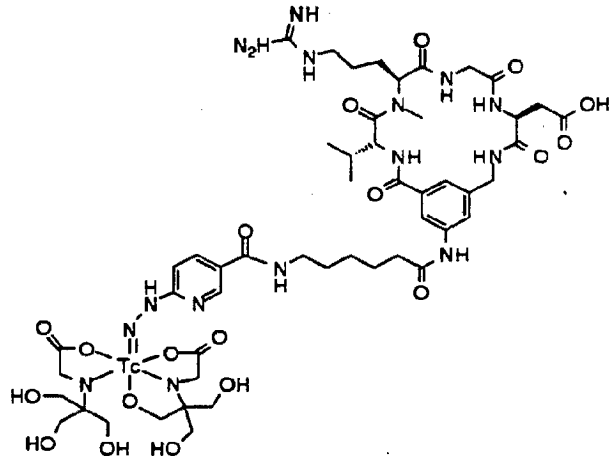

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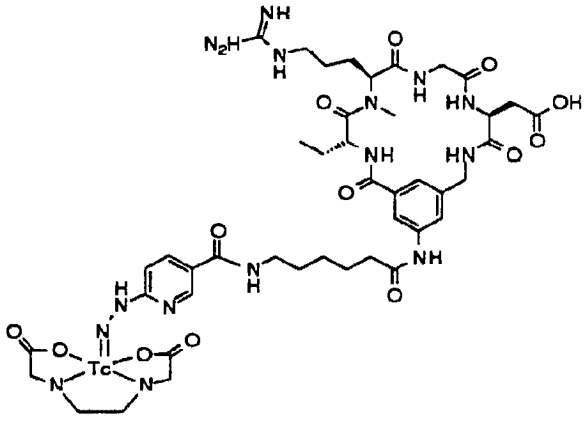

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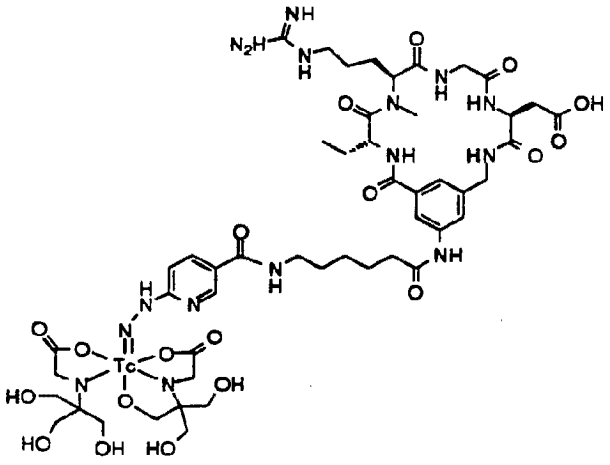

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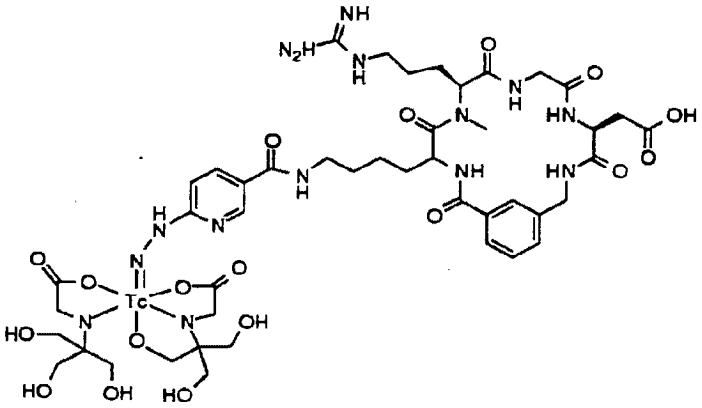

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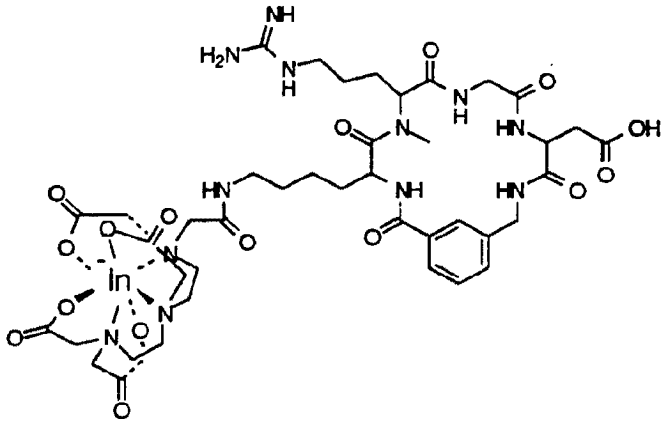

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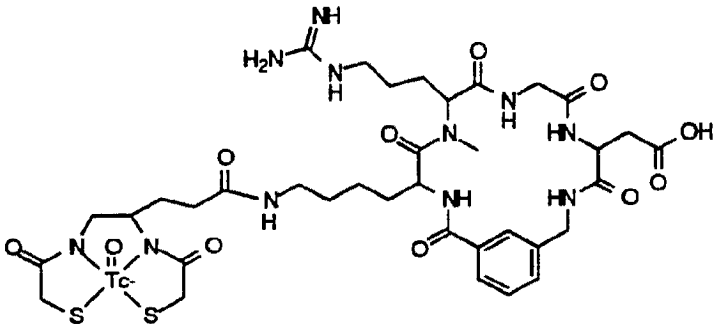

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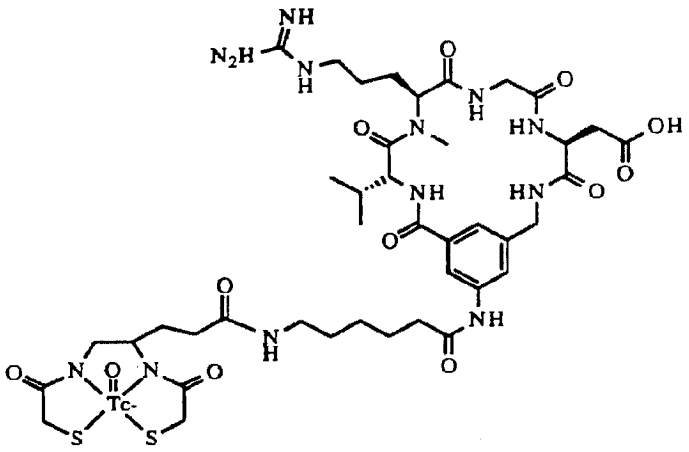

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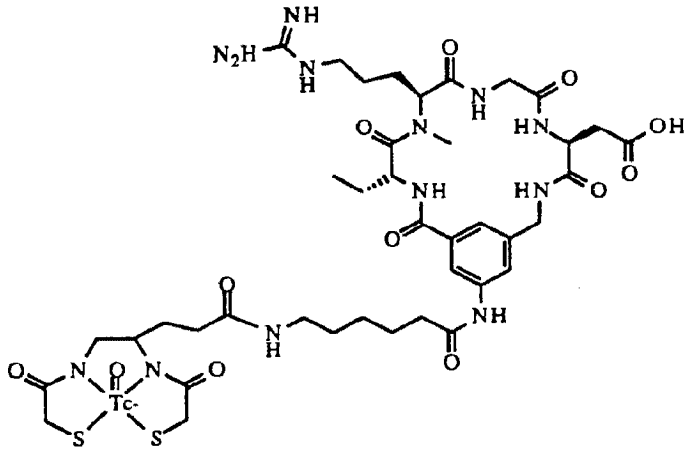

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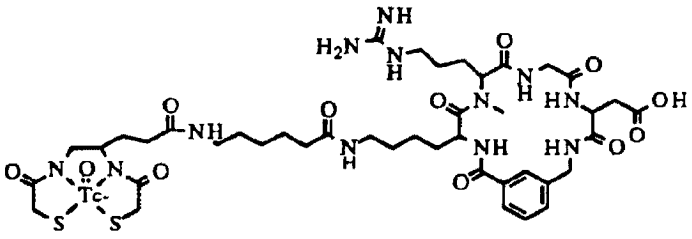

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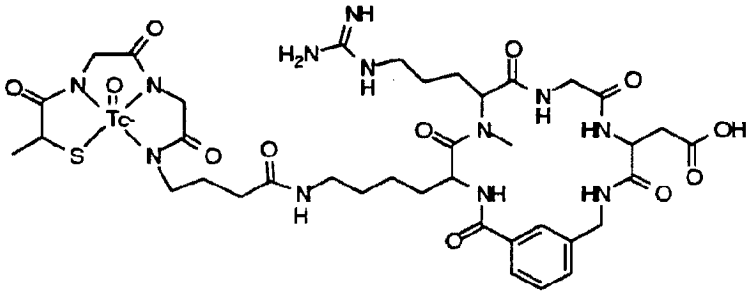

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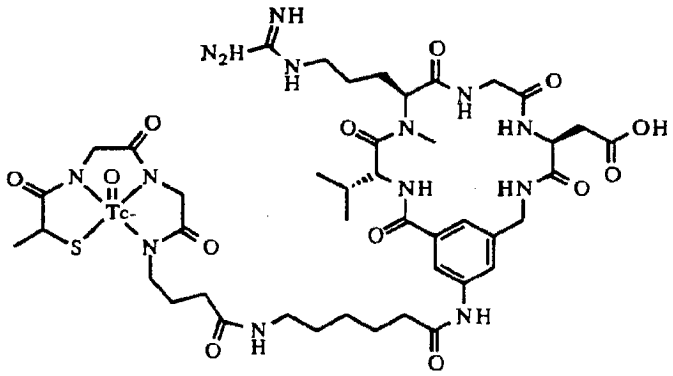

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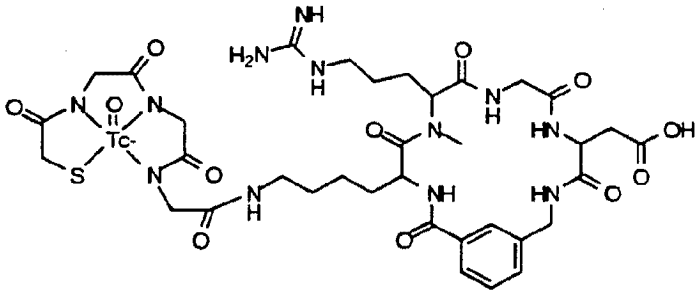

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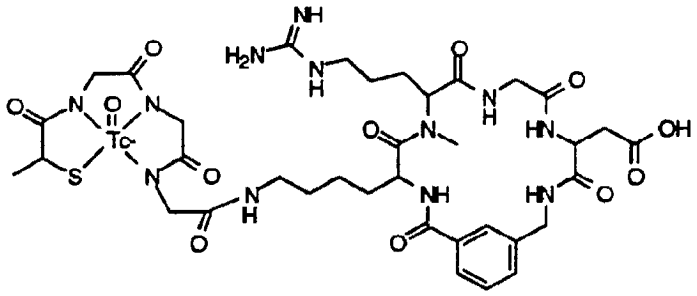

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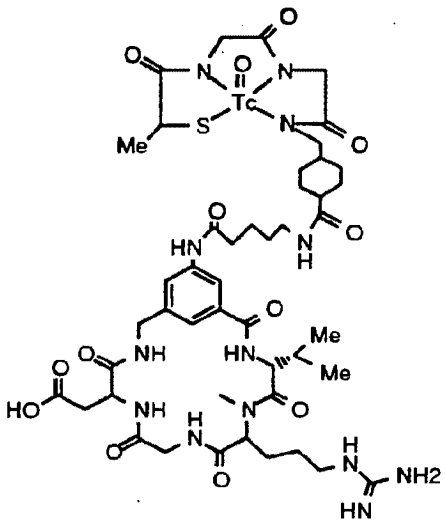

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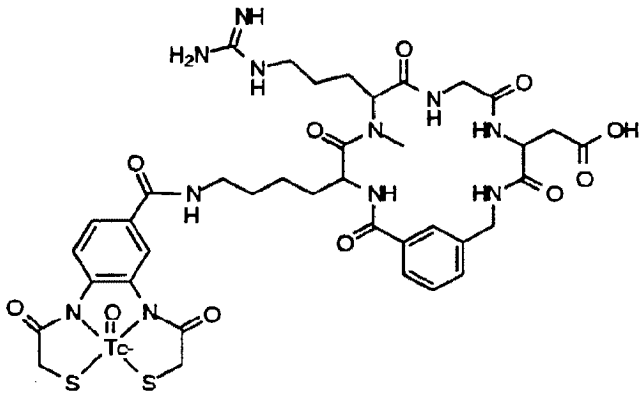

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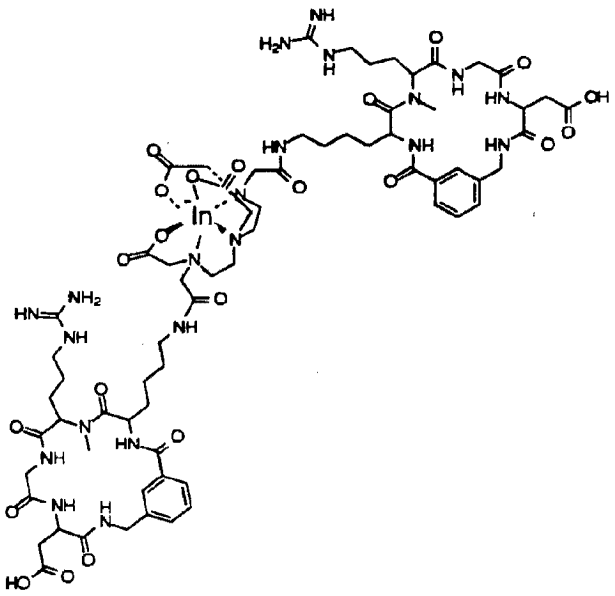


40. A method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of Claim 29, and (ii) scanning the mammal using a radioimaging devise.

41. A method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of Claim 30, and (ii) scanning the mammal using a radioimaging devise.

42. A method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of Claim 31, and (ii) scanning the mammal using a radioimaging devise.

43. A method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of Claim 32, and (ii) scanning the mammal using a radioimaging devise.

44. A method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of Claim 33, and (ii) scanning the mammal using a radioimaging devise.

45. A method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of Claim 34, and (ii) scanning the mammal using a radioimaging devise.

46. A method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of Claim 35, and (ii) scanning the mammal using a radioimaging devise.

47. A method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of Claim 36, and (ii) scanning the mammal using a radioimaging devise.

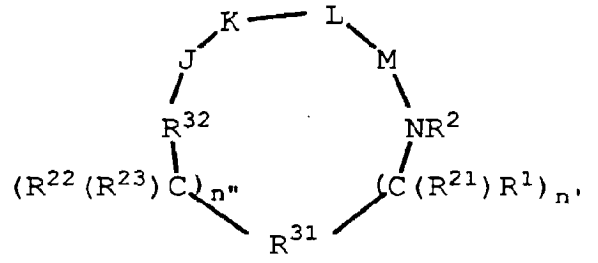
48. A method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount

of a radiopharmaceutical of Claim 37, and (ii) scanning the mammal using a radioimaging devise.

49. A method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of Claim 38, and (ii) scanning the mammal using a radioimaging devise.

50. A method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of Claim 39, and (ii) scanning the mammal using a radioimaging devise.

51. A direct radiolabeled compound of formula (I):



(I)

or a pharmaceutically acceptable salt or prodrug form thereof wherein:

R^{31} is a C_6-C_{14} saturated, partially saturated, or aromatic carbocyclic ring system substituted with 0-4 R^{10} or R^{10a};

R^{32} is selected from:

$-C(=O)-$;
$-C(=S)-$
$-S(=O)_2-$;
$-S(=O)-$;
$-P(=Z)(ZR^{13})-$;

Z is S or O;

n" and n' are independently 0-2;

R^1 and R^{22} are independently selected from the following groups:

hydrogen,
C_1-C_8 alkyl substituted with 0-2 R^{11};
C_2-C_8 alkenyl substituted with 0-2 R^{11};
C_2-C_8 alkynyl substituted with 0-2 R^{11};
C_3-C_{10} cycloalkyl substituted with 0-2 R^{11};

aryl substituted with 0-2 R^{12};

a 5-10-membered heterocyclic ring system containing 1-4 heteroatoms independently selected from N, S, and O, said heterocyclic ring being substituted with 0-2 R^{12};

=O, F, Cl, Br, I, $-CF_3$, $-CN$, $-CO_2R^{13}$, $-C(=O)R^{13}$, $-C(=O)N(R^{13})_2$, $-CHO$, $-CH_2OR^{13}$, $-OC(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{14}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{14}SO_2N(R^{13})_2$, $-NR^{14}SO_2R^{13a}$, $-SO_3H$,

$-SO_2R^{13a}$, $-SR^{13}$, $-S(=O)R^{13a}$, $-SO_2N(R^{13})_2$, $-N(R^{13})_2$, $-NHC(=NH)NHR^{13}$, $-C(=NH)NHR^{13}$, $=NOR^{13}$, NO_2, $-C(=O)NHOR^{13}$, $-C(=O)NHNR^{13}R^{13a}$, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy;

R^1 and R^{21} can alternatively join to form a 3-7 membered carbocyclic ring substituted with 0-2 R^{12};

when n' is 2, R^1 or R^{21} can alternatively be taken together with R^1 or R^{21} on an adjacent carbon atom to form a direct bond, thereby to form a double or triple bond between said carbon atoms;

R^{22} and R^{23} can alternatively join to form a 3-7 membered carbocyclic ring substituted with 0-2 R^{12};

when n" is 2, R^{22} or R^{23} can alternatively be taken together with R^{22} or R^{23} on an adjacent carbon atom to form a direct bond, thereby to form a double or triple bond between the adjacent carbon atoms;

R^1 and R^2, where R^{21} is H, can alternatively join to form a 5-8 membered carbocyclic ring substituted with 0-2 R^{12};

R^{11} is selected from one or more of the following:

=O, F, Cl, Br, I, $-CF_3$, -CN, $-CO_2R^{13}$, $-C(=O)R^{13}$, $-C(=O)N(R^{13})_2$, -CHO, $-CH_2OR^{13}$, $-OC(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{14}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{14}SO_2N(R^{13})_2$, $-NR^{14}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-SR^{13}$, $-S(=O)R^{13a}$, $-SO_2N(R^{13})_2$, $-N(R^{13})_2$, $-NHC(=NH)NHR^{13}$, $-C(=NH)NHR^{13}$, $=NOR^{13}$, NO_2, $-C(=O)NHOR^{13}$, $-C(=O)NHNR^{13}R^{13a}$, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy,

C_1-C_5 alkyl, C_2-C_4 alkenyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_2-C_6 alkoxyalkyl, C_3-C_6 cycloalkoxy, C_1-C_4 alkyl (alkyl being substituted with 1-5 groups selected independently from: $-NR^{13}R^{14}$, $-CF_3$, NO_2, $-SO_2R^{13a}$, or $-S(=O)R^{13a}$),

aryl substituted with 0-2 R^{12},

a 5-10-membered heterocyclic ring system containing 1-4 heteroatoms independently selected from N, S, and O, said heterocyclic ring being substituted with 0-2 R^{12};

R^{12} is selected from one or more of the following:

phenyl, benzyl, phenethyl, phenoxy, benzyloxy, halogen, hydroxy, nitro,

cyano, C_1-C_5 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_7-C_{10} arylalkyl, C_1-C_5 alkoxy, $-CO_2R^{13}$, $-C(=O)NHOR^{13a}$, $-C(=O)NHN(R^{13})_2$, $=NOR^{13}$, $-B(R^{34})(R^{35})$, C_3-C_6 cycloalkoxy, $-OC(=O)R^{13}$, $-C(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-(C_1$-C_4 alkyl$)-OR^{13}$, $-N(R^{13})_2$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{13}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{13}SO_2N(R^{13})_2$, $-NR^{13}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-S(=O)R^{13a}$, $-SR^{13}$, $-SO_2N(R^{13})_2$, C_2-C_6 alkoxyalkyl, methylenedioxy, ethylenedioxy, C_1-C_4 haloalkyl, C_1-C_4 haloalkoxy, C_1-C_4 alkylcarbonyloxy, C_1-C_4 alkylcarbonyl, C_1-C_4 alkylcarbonylamino, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy, C_1-C_4 alkyl (alkyl being substituted with $-N(R^{13})_2$, $-CF_3$, NO_2, or $-S(=O)R^{13a}$);

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, $-(C_1$-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, $-(C_1$-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may alternatively be taken together to form $-(CH_2)_{2-5}-$ or $-(CH_2)O(CH_2)-$;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

R^{21} and R^{23} are independently selected from:

hydrogen;
C_1-C_4 alkyl, optionally substituted with 1-6 halogen;
benzyl;

R^2 is H or C_1-C_8 alkyl;

R^{10} and R^{10a} are selected independently from one or more of the following:

phenyl, benzyl, phenethyl, phenoxy, benzyloxy, halogen, hydroxy, nitro, cyano, C_1-C_5 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_7-C_{10} arylalkyl, C_1-C_5 alkoxy, $-CO_2R^{13}$, $-C(=O)N(R^{13})_2$, $-C(=O)NHOR^{13a}$, $-C(=O)NHN(R^{13})_2$, $=NOR^{13}$, $-B(R^{34})(R^{35})$, C_3-C_6 cycloalkoxy, $-OC(=O)R^{13}$, $-C(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-(C_1$-C_4 alkyl$)-OR^{13}$, $-N(R^{13})_2$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{13}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{13}SO_2N(R^{13})_2$, $-NR^{13}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-S(=O)R^{13a}$, $-SR^{13}$, $-SO_2N(R^{13})_2$, C_2-C_6 alkoxyalkyl, methylenedioxy, ethylenedioxy, C_1-C_4 haloalkyl (including $-C_vF_w$ where v = 1 to 3 and w = 1 to (2v+1)), C_1-C_4 haloalkoxy, C_1-C_4 alkylcarbonyloxy, C_1-C_4 alkylcarbonyl, C_1-C_4 alkylcarbonylamino, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy, C_1-C_4 alkyl (alkyl being substituted with $-N(R^{13})_2$, $-CF_3$, NO_2, or $-S(=O)R^{13a}$);

J is β-Ala or an L-isomer or D-isomer amino acid of structure
$-N(R^3)C(R^4)(R^5)C(=O)-$, wherein:

R^3 is H or C_1-C_8 alkyl;

R^4 is H or C_1-C_3 alkyl;

R^5 is selected from:
hydrogen;
C_1-C_8 alkyl substituted with 0-2 R^{11};
C_2-C_8 alkenyl substituted with 0-2 R^{11};
C_2-C_8 alkynyl substituted with 0-2 R^{11};
C_3-C_{10} cycloalkyl substituted with 0-2 R^{11};

aryl substituted with 0-2 R^{12};

a 5-10-membered heterocyclic ring system containing 1-4 heteroatoms independently selected from N, S, or O, said heterocyclic ring being substituted with 0-2 R^{12};

=O, F, Cl, Br, I, $-CF_3$, $-CN$, $-CO_2R^{13}$, $-C(=O)R^{13}$, $-C(=O)N(R^{13})_2$, $-CHO$, $-CH_2OR^{13}$, $-OC(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{14}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{14}SO_2N(R^{13})_2$, $-NR^{14}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-SR^{13}$, $-S(=O)R^{13a}$, $-SO_2N(R^{13})_2$, $-N(R^{13})_2$, $-NHC(=NH)NHR^{13}$, $-C(=NH)NHR^{13}$, $=NOR^{13}$, NO_2, $-C(=O)NHOR^{13}$,

$-C(=O)NHNR^{13}R^{13a}$, $=NOR^{13}$, $-B(R^{34})(R^{35})$, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy, $-SC(=NH)NHR^{13}$, N_3, $-Si(CH_3)_3$, (C_1-C_5 alkyl)NHR^{16};

$-(C_0$-C_6 alkyl)X;

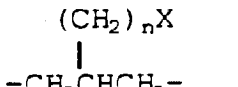
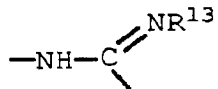

, where q is independently 0,1;


;

$-(CH_2)_mS(O)_{p'}(CH_2)_2X$, where m = 1,2 and p' = 0-2;

wherein X is defined below; and

R^3 and R^4 may also be taken together to form


, where n = 0,1 and X is  ;

R^3 and R^5 can alternatively be taken together to form $-(CH_2)_t-$ or $-CH_2S(O)_{p'}C(CH_3)_2-$, where t = 2-4 and p' = 0-2; or

R^4 and R^5 can alternatively be taken together to form $-(CH_2)_u-$, where u = 2-5;

R^{16} is selected from:

an amine protecting group;

1-2 amino acids;

1-2 amino acids substituted with an amine protecting group;

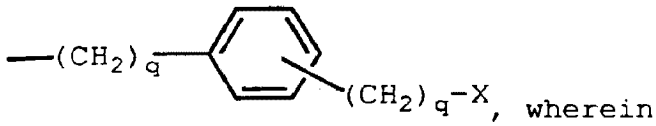
K is a D-isomer or L-isomer amino acid of structure

$-N(R^6)CH(R^7)C(=O)-$, wherein:

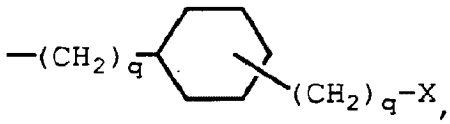
R^6 is H or C_1-C_8 alkyl;

R^7 is selected from:

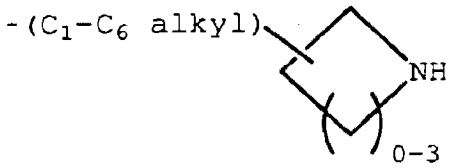
$-(C_1-C_7$ alkyl$)X$;



each q is independently 0-2 and substitution on the phenyl is at the 3 or 4 position;



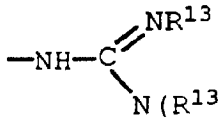
is independently 0-2 and substitution on the cyclohexyl is at the 3 or 4 position;



$-(CH_2)_mO-(C_1-C_4$ alkyl)$-X$, where m = 1 or 2;

$-(CH_2)_mS(O)_{p'}-(C_1-C_4$ alkyl)$-X$, where m = 1 or 2 and p' = 0-2; and

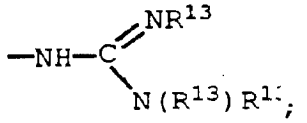
X is selected from:


$N(R^{13})R^{13}$; $-N(R^{13})R^{13}$; $-C(=NH)(NH_2)$; $-SC(=NH)-NH_2$; $-NH-C(=NH)(NHCN)$; $-NH-C(=NCN)(NH_2)$; $-NH-C(=N-OR^{13})(NH_2)$;

R^6 and R^7 can alternatively be taken together to form


, wherein each q is independently 1 or 2 and wherein

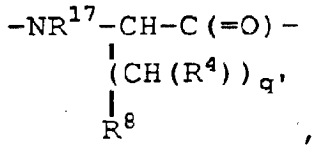
n = 0 or 1 and X is $-NH_2$ or



L is $-Y(CH_2)_vC(=O)-$, wherein:

Y is NH, N(C_1-C_3 alkyl), O, or S; and v = 1 or 2;

M is a D-isomer or L-isomer amino acid of structure



wherein:

q' is 0-2;

R^{17} is H, C_1-C_3 alkyl;

R^8 is selected from:
$-CO_2R^{13}$, $-SO_3R^{13}$, $-SO_2NHR^{14}$, $-B(R^{34})(R^{35})$, $-NHSO_2CF_3$, $-CONHNHSO_2CF_3$, $-PO(OR^{13})_2$, $-PO(OR^{13})R^{13}$, $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O) , $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O), $-SO_2NHCOR^{13}$, $-CONHSO_2R^{13a}$, $-CH_2CONHSO_2R^{13a}$, $-NHSO_2NHCOR^{13a}$, $-NHCONHSO_2R^{13a}$, $-SO_2NHCONHR^{13}$;

R^{34} and R^{35} are independently selected from:
-OH,
-F,
$-N(R^{13})_2$, or

C_1-C_8-alkoxy;

R^{34} and R^{35} can alternatively be taken together form:
a cyclic boron ester where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-4 heteroatoms independently selected from N, S, or O;
a divalent cyclic boron amide where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-4 heteroatoms independently selected from N, S, or O;
a cyclic boron amide-ester where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-4 heteroatoms independently selected from N, S, or O; and

wherein the radiolabel is selected from the group: ^{123}I, ^{125}I, ^{131}I, ^{18}F, ^{11}C, ^{13}N, ^{15}O, ^{75}Br.

52. A radiolabeled compound of Claim 51, wherein:

R^{31} is bonded to $(C(R^{23})R^{22})_{n"}$ and $(C(R^{21})R^{1})_{n'}$ at 2 different atoms on said carbocyclic ring.

53. A radiolabeled compound of Claim 51, wherein:

n" is 0 and n' is 0;
n" is 0 and n' is 1;

n" is 0 and n' is 2;
n" is 1 and n' is 0;
n" is 1 and n' is 1;
n" is 1 and n' is 2;
n" is 2 and n' is 0;
n" is 2 and n' is 1; or
n" is 2 and n' is 2.

54. A radiolabeled compound of Claim 51 wherein R^6 is methyl, ethyl, or propyl.

55. A radiolabeled compound of Claim 51, wherein:

R^{31} is selected from the group consisting of:

(a) a 6 membered saturated, partially saturated or aromatic carbocyclic ring substituted with 0-3 R^{10} or R^{10a};

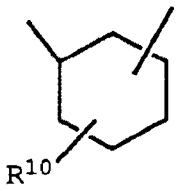
(b) a 8-11 membered saturated, partially saturated, or aromatic fused bicyclic carbocyclic ring substituted with 0-4 R^{10} or R^{10a}; or

(c) a 14 membered saturated, partially saturated, or aromatic fused tricyclic carbocyclic ring substituted with 0-4 R^{10} or R^{10a}.

56. A radiolabeled compound of Claim 51, wherein:

R^{31} is selected from the group consisting of:

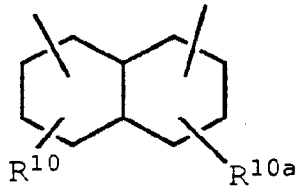
(a) a 6 membered saturated, partially saturated, or aromatic carbocyclic ring of formula:



wherein any of the bonds forming the carbocyclic ring may be a single or double bond,

and wherein said carbocyclic ring is substituted independently with 0-4 R^{10};

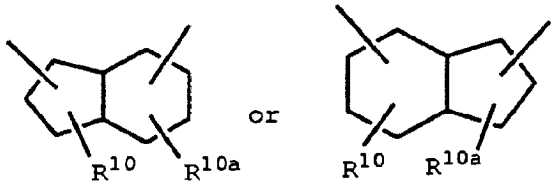
(b) a 10 membered saturated, partially saturated, or aromatic bicyclic carbocyclic ring of formula:



, wherein any of the bonds forming the carbocyclic ring may be a single or double bond,

and wherein said carbocyclic ring is substituted independently with 0-4 R^{10} or R^{10a};

(c) a 9 membered saturated, partially saturated, or aromatic bicyclic carbocyclic ring of formula:

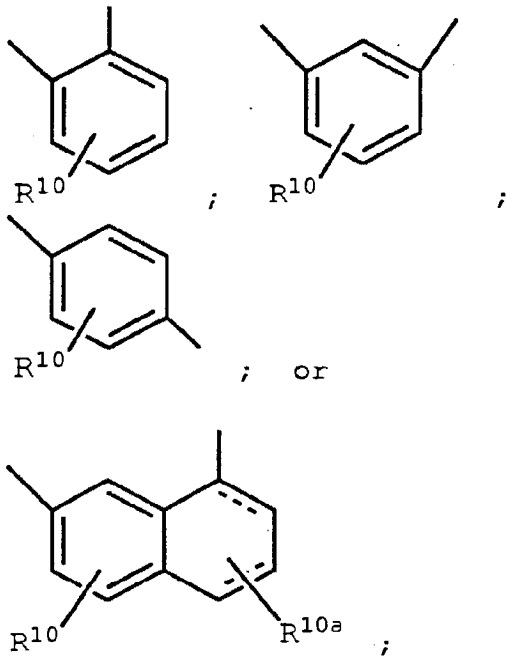


wherein any of the bonds forming the carbocyclic ring may be a single or double bond,

and wherein said carbocyclic ring is substituted independently with 0-4 R^{10} or R^{10a}.

57. A radiolabeled compound of Claim 51, wherein:

R^{31} is selected from (the dashed bond may be a single or double bond):



n" is 0 or 1; and

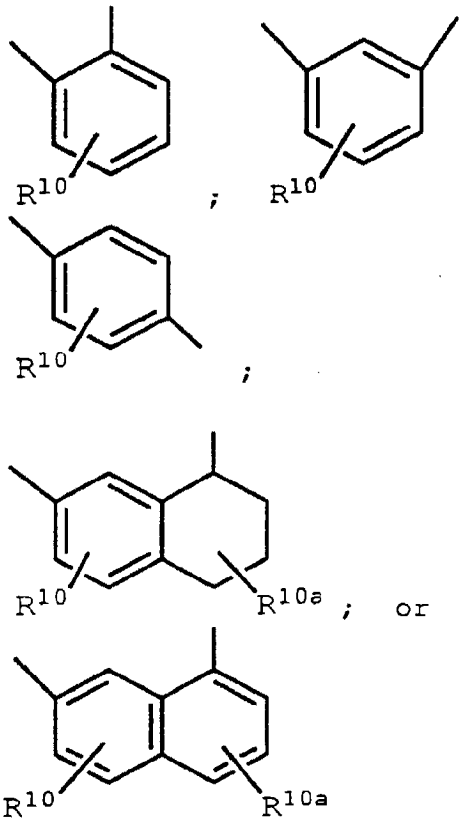
n' is 0-2.

58. A radiolabeled compound of Claim 51, wherein:

R^1 and R^{22} are independently selected from: phenyl, benzyl, phenethyl, phenoxy, benzyloxy, halogen, hydroxy, nitro, cyano, C_1-C_5 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_7-C_{10} arylalkyl, C_1-C_5 alkoxy, $-CO_2R^{13}$, $-C(=O)NHOR^{13a}$, $-C(=O)NHN(R^{13})_2$, $=NOR^{13}$, $-B(R^{34})(R^{35})$, C_3-C_6 cycloalkoxy, $-OC(=O)R^{13}$, $-C(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-(C_1$-C_4 alkyl$)-OR^{13}$, $-N(R^{13})_2$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{13}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{13}SO_2N(R^{13})_2$, $-NR^{13}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-S(=O)R^{13a}$, $-SR^{13}$, $-SO_2N(R^{13})_2$, C_2-C_6 alkoxyalkyl, methylenedioxy, ethylenedioxy, C_1-C_4 haloalkyl, C_1-C_4 haloalkoxy, C_1-C_4 alkylcarbonyloxy, C_1-C_4 alkylcarbonyl, C_1-C_4 alkylcarbonylamino, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy, C_1-C_4 alkyl (alkyl being substituted with $-N(R^{13})_2$, $-CF_3$, NO_2, or $-S(=O)R^{13a}$).

59. A radiolabeled compound of Claim 51, wherein:

R^{31} is selected from:



wherein R^{31} may be substituted independently with 0-3 R^{10} or R^{10a};

R^{32} is -C(=O)-;

n" is 0 or 1;

n' is 0-2;

R^1 and R^{22} are independently selected from H, C_1-C_4 alkyl, phenyl, benzyl, phenyl-(C_2-C_4)alkyl, C_1-C_4 alkoxy;

R^{21} and R^{23} are independently H or C_1-C_4 alkyl;

R^2 is H or C_1-C_8 alkyl;

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may alternatively be taken together to form $-(CH_2)_{2-5}-$ or $-(CH_2)O(CH_2)-$;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

R^{10} and R^{10a} are selected independently from: H, C_1-C_8 alkyl, phenyl, halogen, or C_1-C_4 alkoxy;

J is β-Ala or an L-isomer or D-isomer amino acid of structure $-N(R^3)C(R^4)(R^5)C(=O)-$, wherein:

R^3 is H or CH_3;

R^4 is H or C_1-C_3 alkyl;

R^5 is H, C_1-C_8 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_1-C_6 cycloalkylethyl, phenyl, phenylmethyl, CH_2OH, CH_2SH, CH_2OCH_3, CH_2SCH_3,

$CH_2CH_2SCH_3$, $(CH_2)_sNH_2$,
$-(CH_2)_sNHC(=NH)(NH_2)$, $-(CH_2)_sNHR^{16}$, where
s = 3-5; or

R^{16} is selected from:
an amine protecting group;
1-2 amino acids; or
1-2 amino acids substituted with an amine protecting group;

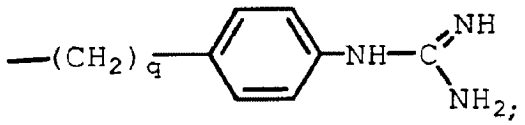
R^3 and R^5 can alternatively be taken together to form $-(CH_2)_t-$ (t = 2-4) or $-CH_2SC(CH_3)_2-$; or

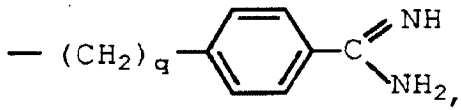
R^4 and R^5 can alternatively be taken together to form $-(CH_2)_u-$, where u = 2-5;

K is an L-isomer amino acid of structure $-N(R^6)CH(R^7)C(=O)-$, wherein:

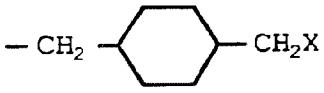
R^6 is H or C_1-C_8 alkyl;

R^7 is





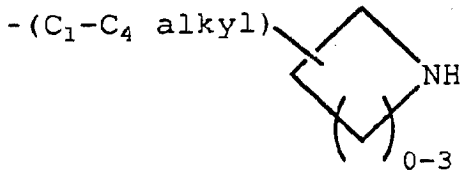
where q = 0 or 1;
$-(CH_2)_rX$, where r = 3-6;


;


;

$-(CH_2)_mS(CH_2)_2X$, where m = 1 or 2;

$-(C_3-C_7$ alkyl)$-NH-(C_1-C_6$ alkyl)

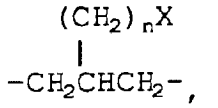


$-(CH_2)_m-O-(C_1-C_4$ alkyl)$-NH-(C_1-C_6$ alkyl), where m = 1 or 2;

$-(CH_2)_m-S-(C_1-C_4$ alkyl)$-NH-(C_1-C_6$ alkyl), where m = 1 or 2; and

X is $-NH_2$ or $-NHC(=NH)(NH_2)$; or

R^6 and R^7 can alternatively be taken together to form

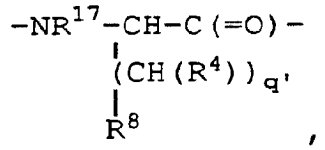

, where n = 0 or 1

and X is $-NH_2$ or $-NHC(=NH)(NH_2)$;

L is $-Y(CH_2)_vC(=O)-$, wherein:

Y is NH, O, or S; and v = 1 or 2;

M is a D-isomer or L-isomer amino acid of structure



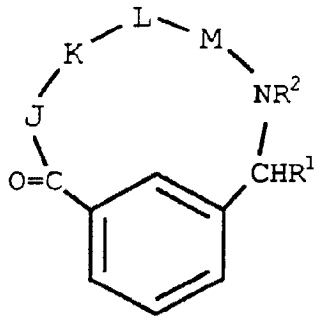
wherein:

q' is 0-2;

R^{17} is H, C_1-C_3 alkyl;

R^8 is selected from:
$-CO_2R^{13}$, $-SO_3R^{13}$, $-SO_2NHR^{14}$, $-B(R^{34})(R^{35})$, $-NHSO_2CF_3$, $-CONHNHSO_2CF_3$, $-PO(OR^{13})_2$, $-PO(OR^{13})R^{13}$, $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O) , $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O), $-SO_2NHCOR^{13}$, $-CONHSO_2R^{13a}$, $-CH_2CONHSO_2R^{13a}$, $-NHSO_2NHCOR^{13a}$, $-NHCONHSO_2R^{13a}$, $-SO_2NHCONHR^{13}$.

60. A radiolabeled compound of Claim 51 that is a radiolabeled 1,3-disubstituted phenyl the formula (II):



(II)

wherein:

the shown phenyl ring in formula (II) may be further substituted with 0-3 R^{10};

R^{10} is selected independently from: H, C_1-C_8 alkyl, phenyl, halogen, or C_1-C_4 alkoxy;

R^1 is H, C_1-C_4 alkyl, phenyl, benzyl, or phenyl-(C_1-C_4)alkyl;

R^2 is H or methyl;

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may alternatively be taken together to form -$(CH_2)_{2-5}$- or -$(CH_2)O(CH_2)$-;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

J is β-Ala or an L-isomer or D-isomer amino acid of structure $-N(R^3)C(R^4)(R^5)C(=O)-$, wherein:

R^3 is H or CH_3;

R^4 is H or C_1-C_3 alkyl;

R^5 is H, C_1-C_8 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_1-C_6 cycloalkylethyl, phenyl, phenylmethyl, CH_2OH, CH_2SH, CH_2OCH_3, CH_2SCH_3, $CH_2CH_2SCH_3$, $(CH_2)_sNH_2$, $-(CH_2)_sNHC(=NH)(NH_2)$, $-(CH_2)_sNHR^{16}$, where s = 3-5; or

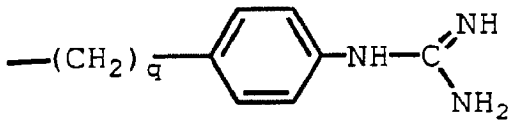
R^{16} is selected from:
an amine protecting group;
1-2 amino acids; or
1-2 amino acids substituted with an amine protecting group;

R^3 and R^5 can alternatively be taken together to form $-CH_2CH_2CH_2-$; or
R^4 and R^5 can alternatively be taken together to form $-(CH_2)_u-$, where u = 2-5;

K is an L-isomer amino acid of structure $-N(R^6)CH(R^7)C(=O)-$, wherein:

R^6 is H or C_1-C_8 alkyl;

R^7 is:



, where q = 0 or 1;

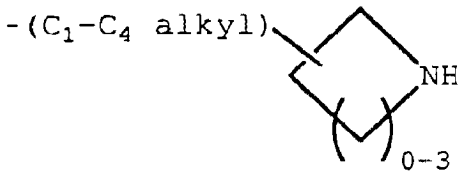
$-(CH_2)_rX$, where r = 3-6;

;

;

$-(CH_2)_mS(CH_2)_2X$, where m = 1 or 2;

$-(C_3-C_7$ alkyl)$-NH-(C_1-C_6$ alkyl)



$-(CH_2)_m-O-(C_1-C_4$ alkyl)$-NH-(C_1-C_6$ alkyl), where m = 1 or 2;

$-(CH_2)_m-S-(C_1-C_4$ alkyl)$-NH-(C_1-C_6$ alkyl), where m = 1 or 2; and

X is $-NH_2$ or $-NHC(=NH)(NH_2)$, provided that X is not $-NH_2$ when r = 4; or

R^6 and R7 are alternatively be taken together to form

$$-CH_2CH(CH_2)_nXCH_2-,$$

where n = 0,1 and X is $-NH_2$ or $-NHC(=NH)(NH_2)$;

L is $-Y(CH_2)_vC(=O)-$, wherein:

Y is NH, O, or S; and v = 1,2;

M is a D-isomer or L-isomer amino acid of structure

$$-NR^{17}-CH(CH(R^4))_{q'}-C(=O)-$$
$$\qquad\qquad\quad R^8$$

wherein:

q' is 0-2;

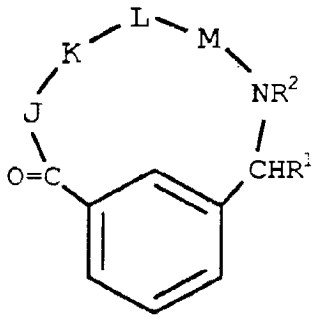
R^{17} is H, C_1-C_3 alkyl;

R^8 is selected from:

$-CO_2R^{13}$, $-SO_3R^{13}$, $-SO_2NHR^{14}$, $-B(R^{34})(R^{35})$, $-NHSO_2CF_3$, $-CONHNHSO_2CF_3$, $-PO(OR^{13})_2$, $-PO(OR^{13})R^{13}$, $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O) , $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O),

$-SO_2NHCOR^{13}$, $-CONHSO_2R^{13a}$,
$-CH_2CONHSO_2R^{13a}$, $-NHSO_2NHCOR^{13a}$,
$-NHCONHSO_2R^{13a}$, $-SO_2NHCONHR^{13}$.

61. A radiolabeled compound of Claim 51 that is a radiolabeled 1,3-disubstituted phenyl of the formula (II):



(II)

wherein:

the phenyl ring in formula (II) may be further substituted with 0-3 R^{10} or R^{10a};

R^{10} or R^{10a} are selected independently from: H, C_1-C_8 alkyl, phenyl, halogen, or C_1-C_4 alkoxy;

R^1 is H, C_1-C_4 alkyl, phenyl, benzyl, or phenyl-(C_2- C_4)alkyl;

R^2 is H or methyl;

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may alternatively be taken together to form $-(CH_2)_{2-5}-$ or $-(CH_2)O(CH_2)-$;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

J is β-Ala or an L-isomer or D-isomer amino acid of structure $-N(R^3)C(R^4)(R^5)C(=O)-$, wherein:

R^3 is H or CH_3;

R^4 is H;

R^5 is H, C_1-C_8 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_1-C_6 cycloalkylethyl, phenyl, phenylmethyl, CH_2OH, CH_2SH, CH_2OCH_3, CH_2SCH_3, $CH_2CH_2SCH_3$, $(CH_2)_sNH_2$, $(CH_2)_sNHC(=NH)(NH_2)$, $(CH_2)_sR^{16}$, where s = 3-5;

R^3 and R^5 can alternatively be taken together to form $-CH_2CH_2CH_2-$;

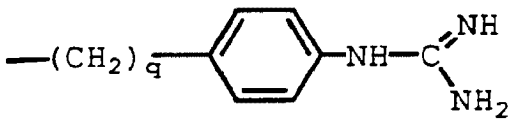
R^{16} is selected from:
an amine protecting group;
1-2 amino acids;
1-2 amino acids substituted with an amine protecting group;

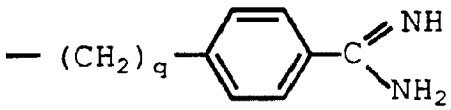
K is an L-isomer amino acid of structure

$-N(R^6)CH(R^7)C(=O)-$, wherein:

R^6 is H or C_3-C_8 alkyl;

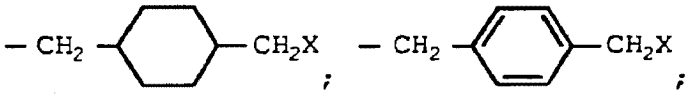
R^7 is





where q = 0 or 1;

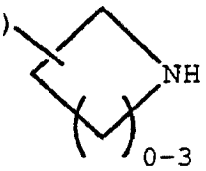
$-(CH_2)_rX$, where r = 3-6;



$-(CH_2)_mS(CH_2)_2X$, where m = 1 or 2;

$-(C_4$-C_7 alkyl)$-NH-(C_1$-C_6 alkyl)

$-(C_1$-C_4 alkyl)



$-(CH_2)_m-O-(C_1$-C_4 alkyl)$-NH-(C_1$-C_6 alkyl), where m = 1 or 2;

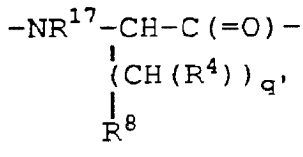
$-(CH_2)_m-S-(C_1$-C_4 alkyl)$-NH-(C_1$-C_6 alkyl), where m = 1 or 2; and

X is $-NH_2$ or $-NHC(=NH)(NH_2)$, provided that X is not $-NH_2$ when r = 4; or

L is $-YCH_2C(=O)-$, wherein:

Y is NH or O;

M is a D-isomer or L-isomer amino acid of structure



, wherein:

q' is 1;

R^{17} is H, C_1-C_3 alkyl;

R^8 is selected from:
$-CO_2H$ or $-SO_3R^{13}$.

62. A radiolabeled compound of Claim 51 that is a radiolabeled compound of formula (II) above, wherein:

the phenyl ring in formula (II) may be further substituted with 0-2 R^{10} or R^{10a};

R^{10} or R^{10a} are selected independently from: H, C_1-C_8 alkyl, phenyl, halogen, or C_1-C_4 alkoxy;

R^1 is H;

R^2 is H;

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may alternatively be taken together to form $-(CH_2)_{2-5}-$ or $-(CH_2)O(CH_2)-$;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

J is β-Ala or an L-isomer or D-isomer amino acid of formula $-N(R^3)CH(R^5)C(=O)-$, wherein:

R^3 is H and R^5 is H, CH_3, CH_2CH_3, $CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$, $CH_2CH_2CH_3$, $CH_2CH_2CH_2CH_3$, $CH_2CH_2SCH_3$, $CH_2CH(CH_3)_2$, $(CH_2)_4NH_2$, (C_3-C_5 alkyl)NHR^{16};
or

R^3 is CH_3 and R^5 is H; or

R^3 and R^5 can alternatively be taken together to form $-CH_2CH_2CH_2-$;

R^{16} is selected from:
an amine protecting group;
1-2 amino acids;

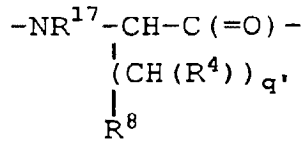
1-2 amino acids substituted with an amine protecting group;

K is an L-isomer amino acid of formula $-N(CH_3)CH(R^7)C(=O)-$, wherein:

R^7 is $-(CH_2)_3NHC(=NH)(NH_2)$;

L is $-NHCH_2C(=O)-$; and

M is a D-isomer or L-isomer amino acid of structure



, wherein:

q' is 1;

R^4 is H or CH_3;

R^{17} is H;

R^8 is
$-CO_2H$;
$-SO_3H$.

63. A radiolabeled compound of Claim 51 that is a radiolabeled compound of formula (II), or a pharmaceutically acceptable salt thereof, wherein:

R^1 and R^2 are independently selected from H, methyl;

J is selected from D-Val, D-2-aminobutyric acid, D-Leu, D-Ala, Gly, D-Pro, D-Ser, D-Lys, βAla, Pro, Phe, NMeGly, D-Nle, D-Phg, D-Ile, D-Phe, D-Tyr, Ala, N^{ε}-p-azidobenzoyl-D-Lys, N^{ε}-p-benzoylbenzoyl-D-Lys, N^{ε}-tryptophanyl-D-Lys, N^{ε}-o-benzylbenzoyl-D-Lys, N^{ε}-p-acetylbenzoyl-D-Lys, N^{ε}-dansyl-D-Lys, N^{ε}-glycyl-D-Lys, N^{ε}-glycyl-p-benzoylbenzoyl-D-Lys, N^{ε}-p-phenylbenzoyl-D-Lys, N^{ε}-m-benzoylbenzoyl-D-Lys, N^{ε}-o-benzoylbenzoyl-D-Lys;

K is selected from NMeArg, Arg;

L is selected from Gly, βAla, Ala;

M is selected from Asp; αMeAsp; βMeAsp; NMeAsp; D-Asp.

64. A radiolabeled compound of Claim 51 that is a radiolabeled compound of formula (II), or a pharmaceutically acceptable salt thereof, wherein:

R^1 and R^2 are independently selected from H, methyl;

J is selected from: D-Val, D-2-aminobutyric acid, D-Leu, D-Ala, Gly, D-Pro, D-Ser, D-Lys, βAla, Pro, Phe, NMeGly, D-Nle, D-Phg, D-Ile, D-Phe, D-Tyr, Ala;

K is selected from NMeArg;

L is Gly;

M is selected from Asp; αMeAsp; βMeAsp; NMeAsp; D-Asp.

65. The radiolabeled compounds of Claim 51 that are:

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-2-aminobutyric acid; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Leu; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Ala; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is Gly; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Pro; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Lys; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is β-Ala; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is NMeGly; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 is methyl (isomer 1); R^2 are H; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 is methyl (isomer 2); R^2 are H; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 is phenyl (isomer 1); R^2 are H; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein J = D-Met, K = NMeArg, L = Gly, M = Asp, R^1 = H, R^2 = H;

the radiolabeled compound of formula (II) wherein J = D-Abu, K = diNMe-guanidinyl-Orn , L = Gly, M = Asp, R^1 = H, R^2 = H;

the radiolabeled compound of formula (II) wherein J = D-Abu, K = diNMe-Lys, L = Gly, M = Asp, R^1 = H, R^2 = H;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-p-azidobenzoyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-p-benzoylbenzoyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-tryptophanyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-o-benzylbenzoyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp.

The radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-p-acetylbenzoyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-dansyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-glycyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

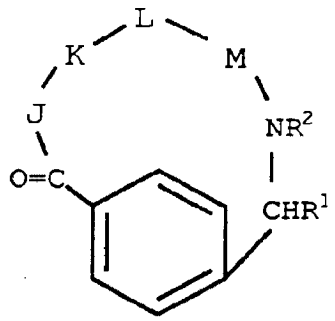
the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-glycyl-p-benzoylbenzoyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-p-phenylbenzoyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-m-benzoylbenzoyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-o-benzoylbenzoyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (III) wherein R^1 and R^2 are H; J is D-Val; K is NMeArg; L is Gly; and M is Asp;



(III);

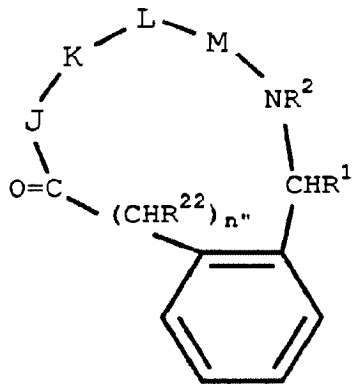
the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Val; K is D-NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Nle; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Phg; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Phe; K is NMeArg; L is Gly; and M is Asp;

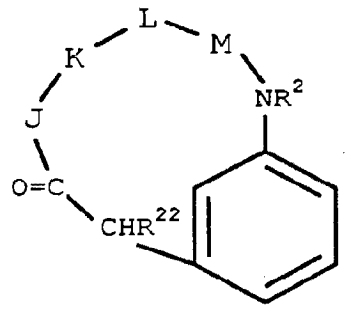
the radiolabeled compound of formula (V) wherein R^1 and R^2 are H; J is D-Ile; K is NMeArg; L is Gly; and M is Asp;



(V);

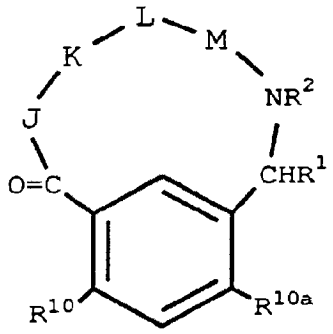
the radiolabeled compound of formula (V) wherein n"=1; R^1, R^2, and R^{22} are H; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (V) wherein n"=0; R^1 and R^2 are H; J is D-Val; K is NMeArg; L is Gly; and M is Asp;



(VI)

the radiolabeled compound of formula (VI) wherein R^2 and R^{22} are H; J is D-Val; K is NMeArg; L is Gly; and M is Asp;



(VII)

the radiolabeled compound of formula (VII) wherein R^1,R^2, and R^{10} are H; R^{10a} is Cl; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (VII) wherein R^1,R^2, and R^{10} are H; R^{10a} is I; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (VII) wherein R^1,R^2, and R^{10} are H; R^{10a} is I; J is D-Abu; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (VII) wherein R^1,R^2, and R^{10} are H; R^{10a} is Me; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (VII) wherein R^1,R^2, and R^{10a} are H; R^{10} is Cl; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (VII) wherein R^1,R^2, and R^{10a} are H; R^{10} is MeO; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (VII) wherein R^1,R^2, and R^{10a} are H; R^{10} is Me; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (VII) wherein R^1,R^2, and R^{10} are H; R^{10a} is Cl; J is D-Abu; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (VII) wherein R^1, R^2, and R^{10} are H; R^{10a} is I; J is D-Abu; K is NMeArg; L is Gly; and M is Asp.

The radiolabeled compound of formula (VII) wherein R^1, R^2, and R^{10} are H; R^{10a} is Me; J is D-Abu; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Tyr; K is NMeArg; L is Gly; and M is Asp;

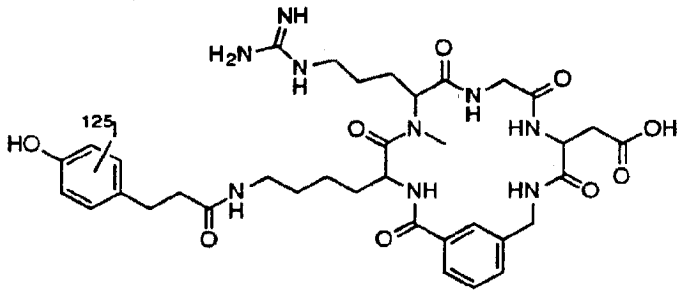
the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Val; K is NMeAmf; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Val; K is NMeArg; L is Gly; and M is βMeAsp;

the radiolabeled compound of formula (II) wherein R^1 is H; R^2 is CH_3; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (III) wherein R^1 and R^2 are H; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (VIII) wherein J is D-Val; K is NMeArg; L is Gly; and M is Asp;



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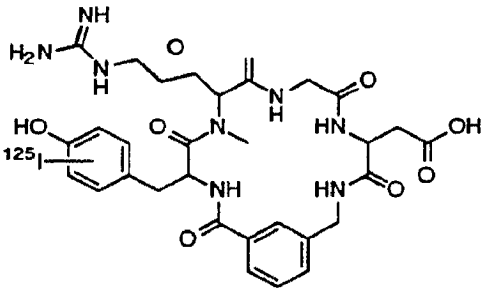


66. A radiolabeled compound as in one of Claims 51-65 wherein the radiolabel is selected from the group: ^{18}F, ^{11}C, ^{123}I, and ^{125}I.

67. A radiolabeled compound of Claim 66 wherein the radiolabel is ^{123}I.

68. A radiopharmaceutical composition comprising a radiopharmaceutically acceptable carrier and a radiolabeled compound of any of Claims 51-67.

69. A method of determining platelet deposition in a mammal comprising administering to said mammal a radiopharmaceutical composition comprising a compound of any of Claims 51-67, and imaging said mammal.

70. A method of diagnosing a disorder associated with platelet deposition in a mammal comprising administering to said mammal a radiopharmaceutical composition comprising a compound of any of Claims 51-67, and imaging said mammal.

Fig. 1a

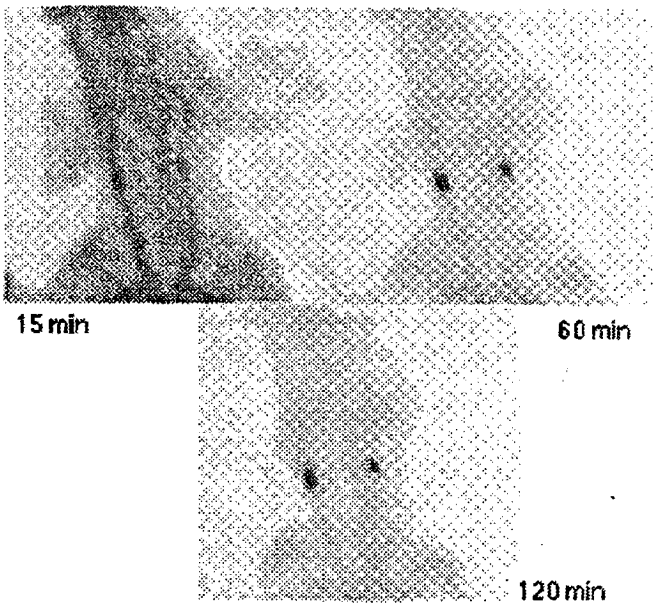
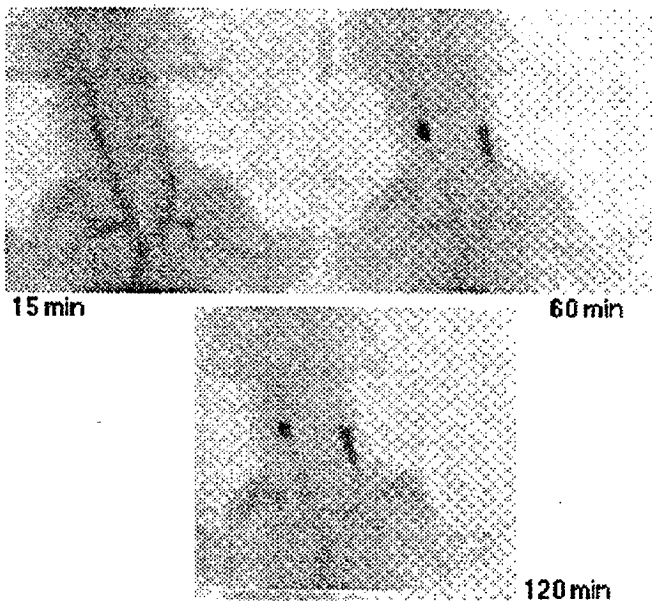


Fig. 1b



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/03256

A. CLASSIFICATION OF SUBJECT MATTER
IPC(5) :A61K 49/02
US CL :424/1.69; 530/317
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/1.69,1.45,1.65; 530/317; 930/270; 514/9, 11, 2, D1G 802

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
A61K 49/02 Digest (1988-date)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	WO,A, 93/07170 (DUPONT MERCK) 15 APRIL 1993. See entire document.	1-70
A	EP, A, 341915 (SMITHKLINE BECKMAN) 15 NOVEMBER 1989.	
Y	EP, A, 425212 (SMITHLKINE BEECHAM) 02 MAY 1991. See pages 1-2, and page 7, line 41.	1-70
Y	WO, A, 91/02750 (BIOGEN, INC.) 07 MARCH 1991. See page 47.	51-70
Y,P	US, A, 5,279,812 (KRSTENANSKY ET AL.) 18 JANUARY 1994. See column 2, lines 5-43, and columns 9 and 10.	51-70
A,P	US, A, 5,236,898 (KRSTENANSKY ET AL.) 17 AUGUST 1993. See columns 1 and 2.	

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer - JOHN M. COVERT
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0444

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/03256

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	WO, A, 93/15770 (MALLINCKRODT MEDICAL, INC.) 19 AUGUST 1993. See pages 8-11.	1-50
A	US, A, 5,041,380 (RUOSLAHTI ET AL.) 20 AUGUST 1991. See column 2.	
A	US, A, 5,192,380 (RUOSLAHTI ET AL.) 20 AUGUST 1991. See column 2.	
A	US, A, 5,192,746 (LOBL ET AL.) 09 MARCH 1993.	
A	US, A, 5, 192,745 (KRSTENANSKY ET AL.) 09 MARCH 1993.	
A	US, A, 5,023,233 (NUTT ET AL.) 11 JUNE 1991.	

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

STN-file CA

L1	39073	S	THOMB? /AB, BI
L2	1133	S	"IIB/IIIA" /AB, BI
L3	1234	S	CYCLIC(W) PEPTID?/AB,BI
L4	2	S	L1 AND L2 AND L3
L5	112986	S	ANTAGON? /AB, BI
L6	27	S	L1 AND L2 AND L5
		E	MOUSA, S/AU
		E	MOUSA, SHA? / AU
L7	0	S	MOUSA SH/AU
		E	MOUSA S/AU
L8	18	S	E7 - E9
L9	3	S	L2 AND L8
L10	15	S	L8 NOT L9

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(74) Agent: **MORRIS, Patrick D.**; Perkins Coie LLP, P.O. Box 1208, Seattle, Washington 98111-1208 (US).

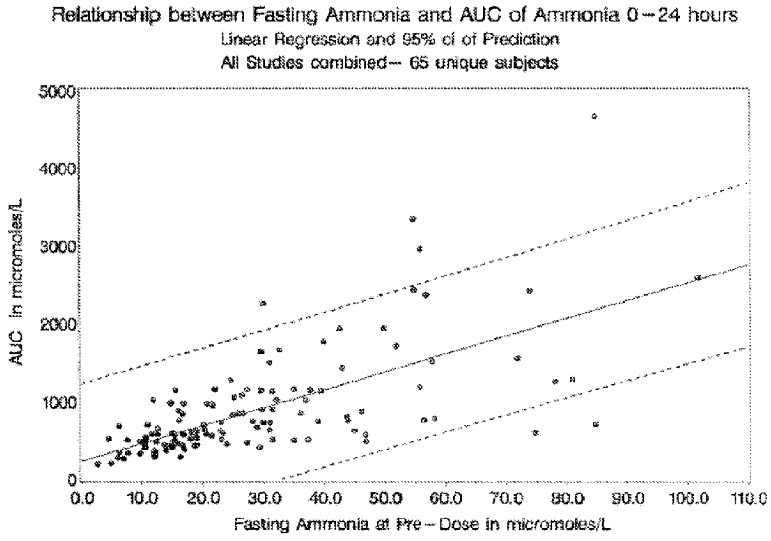
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU,

[Continued on next page]

(54) Title: METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS

Figure 2



(57) Abstract: The present disclosure provides methods for evaluating daily ammonia exposure based on a single fasting ammonia blood level measurement, as well as methods that utilize this technique to adjust the dosage of a nitrogen scavenging drug, determine whether to administer a nitrogen scavenging drug, and treat nitrogen retention disorders.

WO 2013/048558 A2

LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS

RELATED APPLICATIONS

[0001] The present application claims the benefit of U.S. Provisional Application No. 61/564,668, filed November 29, 2011, and U.S. Provisional Application No. 61/542,100, filed September 30, 2011, the disclosures of which are incorporated by reference herein in their entirety, including drawings.

BACKGROUND

[0002] Nitrogen retention disorders associated with elevated ammonia levels include urea cycle disorders (UCDs) and hepatic encephalopathy (HE).

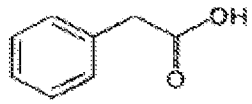
[0003] UCDs include several inherited deficiencies of enzymes or transporters necessary for the synthesis of urea from ammonia, including enzymes involved in the urea cycle. The urea cycle is depicted in Figure 1, which also illustrates how certain ammonia-scavenging drugs act to assist in elimination of excessive ammonia. With reference to Figure 1, N-acetyl glutamine synthetase (NAGS)-derived *N*-acetylglutamate binds to carbamyl phosphate synthetase (CPS), which activates CPS and results in the conversion of ammonia and bicarbonate to carbamyl phosphate. In turn, carbamyl phosphate reacts with ornithine to produce citrulline in a reaction mediated by ornithine transcarbamylase (OTC). A second molecule of waste nitrogen is incorporated into the urea cycle in the next reaction, mediated by arginosuccinate synthetase (ASS), in which citrulline is condensed with aspartic acid to form argininosuccinic acid. Argininosuccinic acid is cleaved by argininosuccinic lyase (ASL) to produce arginine and fumarate. In the final reaction of the urea cycle, arginase (ARG) cleaves arginine to produce ornithine and urea. Of the two atoms of nitrogen incorporated into urea, one originates from free ammonia (NH_4^+) and the other from aspartate. UCD individuals born with no meaningful residual urea synthetic capacity typically present in the first few days of life (neonatal presentation). Individuals with residual function typically present later in childhood or even in adulthood, and symptoms may be precipitated by increased dietary protein or physiological stress (e.g., intercurrent illness).

[0004] Hepatic encephalopathy (HE) refers to a spectrum of neurologic signs and symptoms believed to result from hyperammonemia, which frequently occur in subjects with cirrhosis or certain other types of liver disease. Subjects with HE typically show altered mental status ranging from subtle changes to coma, features similar to subjects with UCDs.

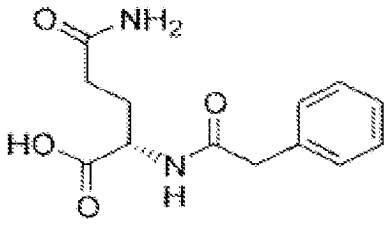
[0005] Subjects with nitrogen retention disorders whose ammonia levels and/or symptoms are not adequately controlled by dietary restriction of protein and/or dietary supplements are generally treated with nitrogen scavenging agents such as sodium phenylbutyrate (NaPBA, approved in the United States as BUPHENYL® and in Europe as AMMONAPS®) or sodium benzoate. These are often referred to as alternate pathway drugs because they provide the body with an alternate pathway to urea for excretion of waste nitrogen (Brusilow 1980; Brusilow 1991). NaPBA is a phenylacetic acid (PAA) prodrug. Another nitrogen scavenging drug currently in development for the treatment of nitrogen retention disorders is glyceryl tri-[4-phenylbutyrate](HPN-100), which is described in U.S. Patent No. 5,968,979. HPN-100, which is commonly referred to as GT4P or glycerol PBA, is a prodrug of PBA and a pre-prodrug of PAA.

[0006] HPN-100 and NaPBA share the same general mechanism of action: PBA is converted to PAA via beta oxidation, and PAA is conjugated enzymatically with glutamine to form phenylacetylglutamine (PAGN), which is excreted in the urine. The structures of PBA, PAA, and PAGN are set forth below.

phenylbutyrate



Phenylacetic acid



Phenylacetylglutamine

[0007] The clinical benefit of NaPBA and HPN-100 with regard to nitrogen retention disorders derives from the ability of PAGN to effectively replace urea as a vehicle for waste nitrogen excretion and/or to reduce the need for urea synthesis (Brusilow 1991; Brusilow 1993). Because each glutamine contains two molecules of nitrogen, the body rids itself of two waste nitrogen atoms for every molecule of PAGN excreted in the urine. Therefore, two equivalents of nitrogen are removed for each mole of PAA converted to PAGN. PAGN

represents the predominant terminal metabolite, and one that is stoichiometrically related to waste nitrogen removal, a measure of efficacy in the case of nitrogen retention states. The difference between HPN-100 and NaPBA with respect to metabolism is that HPN-100 is a triglyceride and requires digestion, presumably by pancreatic lipases, to release PBA (McGuire 2010).

[0008] In contrast to NaPBA or HPN-100, sodium benzoate acts when benzoic acid is combined enzymatically with glycine to form hippuric acid. For each molecule of hippuric acid excreted in the urine, the body rids itself of one waste nitrogen atom.

[0009] Methods of determining an effective dosage of PAA prodrugs such as NaPBA or HPN-100 for a subject in need of treatment for a nitrogen retention disorder are described in WO09/1134460 and WO10/025303. Daily ammonia levels, however, may vary greatly in a subject. This can lead to overestimation by the physician of the average daily ammonia levels, which may result in overtreatment. Thus, there is a need in the art for improved methods for PAA prodrug dose determination and adjustment based on ammonia levels in subjects with nitrogen retention disorders such as UCDs or HE.

SUMMARY

[0010] Provided herein in certain embodiments are methods for determining whether to increase a dosage of a nitrogen scavenging drug in a subject with a nitrogen retention disorder by measuring a fasting blood ammonia level and comparing the fasting blood ammonia level to the upper limit of normal (ULN) for blood ammonia, where a fasting blood ammonia level that is greater than half the ULN for blood ammonia indicates that the dosage needs to be increased. In certain embodiments, the nitrogen retention disorder is a UCD or HE. In certain embodiments, the nitrogen scavenging drug is HPN-100, PBA, NaPBA, sodium benzoate, or any combination thereof (i.e., any combination of two or more of HPN-100, PBA, NaPBA). In certain embodiments, the ULN is around 35 μmol/L or 59 μg/mL. In certain embodiments, the methods include an additional step of administering an increased dosage of the nitrogen scavenging drug if the need exists, and in certain of these embodiments administration of the nitrogen scavenging drug produces a normal average daily ammonia level in the subject. In certain embodiments wherein a determination is made to administer an increased dosage of nitrogen scavenging drug and wherein the nitrogen scavenging drug is a PAA prodrug, the methods include an additional step of measuring urinary PAGN excretion and determining an effective dosage of the PAA prodrug based on a mean conversion of PAA prodrug to urinary PAGN of 60-75%.

[0011] Provided herein in certain embodiments are methods for determining whether to administer a nitrogen scavenging drug to a subject with a nitrogen retention disorder by measuring a fasting blood ammonia level and comparing the fasting blood ammonia level to the ULN for blood ammonia, where a fasting blood ammonia level that is greater than half the ULN for blood ammonia indicates that the nitrogen scavenging drug needs to be administered. In certain embodiments, the nitrogen retention disorder is a UCD or HE. In certain embodiments, the nitrogen scavenging drug is HPN-100, PBA, NaPBA, sodium benzoate, or any combination thereof (i.e., any combination of two or more of HPN-100, PBA, NaPBA). In certain embodiments, the ULN is around 35 μmol/L or 59 μg/mL. In certain embodiments, the methods include an additional step of administering a nitrogen scavenging drug if the need exists, and in certain of these embodiments administration of the nitrogen scavenging drug produces a normal average daily ammonia level in the subject. In certain embodiments wherein a determination is made to administer a nitrogen scavenging drug and wherein the nitrogen scavenging drug is a PAA prodrug, the methods further include a step of determining an effective initial dosage of the PAA prodrug by determining a target urinary PAGN output based on a target nitrogen output and calculating an effective initial dosage that results in the target urinary PAGN output based on a mean conversion of PAA prodrug to urinary PAGN of 60-75%. In certain embodiments, the methods include a step of administering the calculated effective initial dosage.

[0012] Provided herein in certain embodiments are methods for treating a nitrogen retention disorder in a subject who has previously been administered a nitrogen scavenging drug by measuring a fasting blood ammonia level, comparing the fasting blood ammonia level to the ULN for blood ammonia, and administering an increased dosage of the nitrogen scavenging drug if the fasting ammonia level is greater than half the ULN for blood ammonia. In certain embodiments, administration of an increased dosage of the nitrogen scavenging drug produces a normal average daily ammonia level in the subject. In certain embodiments, the nitrogen retention disorder is a UCD or HE. In certain embodiments, the nitrogen scavenging drug is HPN-100, PBA, NaPBA, sodium benzoate, or any combination thereof (i.e., any combination of two or more of HPN-100, PBA, NaPBA). In certain embodiments, the ULN is around 35 μmol/L or 59 μg/mL. In certain embodiments wherein the nitrogen scavenging drug is a PAA prodrug, the methods include an additional step of measuring urinary PAGN excretion and determining an effective dosage of the PAA prodrug based on a mean conversion of PAA prodrug to urinary PAGN of 60-75%. In certain embodiments, the methods include a step of administering the calculated effective dosage.

BRIEF DESCRIPTION OF DRAWINGS

[0013] Figure 1: The urea cycle and how certain nitrogen-scavenging drugs may assist in elimination of excessive ammonia.

[0014] Figure 2: Relationship between fasting ammonia and average ammonia UCD patients.

[0015] Figure 3: Venous blood ammonia values over 24 hours in (A) adult and (B) pediatric UCD patients.

DETAILED DESCRIPTION

[0016] The following description of the invention is merely intended to illustrate various embodiments of the invention. As such, the specific modifications discussed are not to be construed as limitations on the scope of the invention. It will be apparent to one skilled in the art that various equivalents, changes, and modifications may be made without departing from the scope of the invention, and it is understood that such equivalent embodiments are to be included herein.

[0017] In subjects with a nitrogen retention disorder, the desired effect of treatment with a nitrogen scavenging drug is control of blood ammonia level. Control of blood ammonia level generally refers to ammonia values within the normal range and avoidance of hyperammonemic crises, which are often defined in the art as transient ammonia values exceeding 100 µmol/L or 178 µg/mL accompanied by clinical signs and symptoms of hyperammonemia. Dosing of nitrogen scavenging drugs is usually based upon clinical assessment and measurement of ammonia. However, assessment of treatment effect and interpretation of ammonia levels is confounded by the fact that individual ammonia values vary several-fold over the course of a day and are impacted by timing of the blood draw in relation to the last meal and dose of drug (see, e.g., Lee 2010; Lichter-Konecki 2011; Diaz 2011).

[0018] A random ammonia value obtained during an outpatient visit may fail to provide a reliable measure of a subject's status and the drug effect. For example, basing treatment on a blood sample taken after eating a meal might overestimate average daily ammonia level and result in overtreatment. Conversely, basing treatment on a blood sample taken after drug administration might underestimate average daily ammonia level and result in undertreatment. A fasting ammonia level at or near the ULN might be taken as an indication of satisfactory control without appreciating the fact that the ammonia burden during the day (average and/or highest possible value) might be significantly higher. Thus, a fasting level at or near the ULN may actually reflect undertreatment in a subject already a receiving nitrogen

scavenging drug or the need for treatment in a subject not currently prescribed a nitrogen scavenging drug. A more accurate view of daily ammonia level could be obtained by multiple blood draws in a controlled setting over an extended period of time. Although this is currently done in clinical trials, it is clinically impractical.

[0019] As set forth below, the relationship between fasting ammonia levels and daily ammonia exposure was evaluated in subjects with nitrogen retention disorders. It was found that fasting ammonia correlates strongly with daily ammonia exposure, assessed as a 24 hour area under the curve for ammonia, daily average, or maximal daily concentration, and that a target fasting value which does not exceed half of the ULN is a clinically useful and practical predictor of ammonia values over 24 hours. As such, provided herein are clinically practical methods of evaluating ammonia exposure in subjects with nitrogen retention disorders based on fasting ammonia levels, as well as methods of using the resultant information to adjust the dosage of a nitrogen scavenging drug, determine whether to administer a nitrogen scavenging drug, treat a nitrogen retention disorder, and predict daily ammonia burden. The use of fasting ammonia levels to predict ammonia exposure provides a significant advantage over previously developed methods by reducing the number of required blood draws and eliminating the confusion associated with conflicting ammonia levels over the course of the day.

[0020] As further disclosed herein, the relationship between ammonia control and neurocognitive outcome was evaluated in UCD patients. Previous research has demonstrated that UCD patients often exhibit lower IQ overall and deficient executive function manifested by difficulty in goal setting, planning, monitoring progress and purposeful problem solving. As set forth herein, it was found that ammonia control with GPB resulted in a significant improvement in executive functions in pediatric patients. Based on these results, methods are provided herein for improving executive function in a pediatric subject with a UCD by administering one or more nitrogen scavenging drugs.

[0021] As further disclosed herein, the relationship between elevated PAA levels and neurological adverse events (AEs) was analyzed. Many of the over 30 reports of administration of NaPBA and/or sodium PAA to humans describe AEs, particularly when administered intravenously. IV administration of PAA to cancer patients was shown previously to result in AEs that included fatigue, dizziness, dysgeusia, headache, somnolence, lightheadedness, pedal edema, nausea, vomiting, and rash (Thibault 1994; Thibault 1995). These AEs correlated with PAA levels from 499 to 1285 μg/mL. Although NaPBA has been used in UCD treatment for over two decades and AEs reportedly associated with PAA are

similar to those associated with hyperammonemia, little was known previously about the relationship between PAA levels and neurological AEs in UCD patients. As shown herein, increased PAA levels did not correlate with increased neurological AEs in subjects with UCD. However, PAA levels were associated with an increase in neurological AEs in healthy subjects. Based on these results, methods are provided herein for predicting or diagnosing AEs in a subject by measuring PAA levels. Further provided herein are methods of treating and/or preventing AEs in a subject with elevated PAA levels by administering one or more nitrogen scavenging drugs.

[0022] Provided herein are specific target values for blood ammonia upon which an effective dosage of a nitrogen scavenging drug can be based. In certain embodiments, an effective dosage of a nitrogen scavenging drug may be an initial dosage, subsequent/maintenance dosage, improved dosage, or a dosage determined in combination with other factors. In certain embodiments, the effective dosage may be the same as or different than the initial dosage. In other embodiments, the effective dosage may be higher or lower than the initial dosage. In certain embodiments, methods are provided for adjusting the dose or regimen of a nitrogen scavenging drug to achieve a target ammonia level that is predictive of the average daily ammonia level and/or the highest ammonia value that the subject is likely to experience during the day.

[0023] Using the methods herein, a subject's fasting blood ammonia level may be used as a predictor of daily ammonia burden, average daily ammonia level, and/or highest daily ammonia value. Whether a subject with a nitrogen retention disorder is receiving an optimum dosage of nitrogen scavenging drug may be determined based on predicted daily ammonia exposure. By optimizing the therapeutic efficacy of a nitrogen scavenging drug, the therapeutic dosage of the nitrogen scavenging drug is adjusted so that the subject experiences the desired nitrogen scavenging effect. In particular, the dose is adjusted so that the subject may experience a normal average daily ammonia level. In certain embodiments, the effective dosage of nitrogen scavenging drug is determined by adjusting (e.g., increasing) a dosage to achieve a fasting blood ammonia level for a subject that is less than or equal to half the ULN for blood ammonia.

[0024] Provided herein in certain embodiments are methods of determining whether the dosage of a nitrogen scavenging drug needs to be increased in a subject with a nitrogen retention disorder comprising comparing a fasting blood ammonia level for the subject to a ULN for blood ammonia. If the fasting blood ammonia level has a value that greater than half the ULN, the dosage of the nitrogen scavenging drug needs to be increased. In certain

embodiments, the methods further comprise increasing the dosage of the nitrogen scavenging drug if the need exists, and in certain of these embodiments the methods further comprise administering the increased dosage. In certain of these embodiments, administration of the increased dosage results in a normal average daily ammonia level in the subject.

[0025] Provided herein in certain embodiments are methods of determining whether the dosage of a nitrogen scavenging drug needs to be increased in a subject with a nitrogen retention disorder comprising measuring a fasting blood ammonia level for the subject and comparing the fasting blood ammonia level to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, the dosage of the nitrogen scavenging drug needs to be increased. In certain embodiments, the methods further comprise increasing the dosage of the nitrogen scavenging drug if the need exists, and in certain of these embodiments the methods further comprise administering the increased dosage. In certain of these embodiments, administration of the increased dosage results in a normal average daily ammonia level in the subject.

[0026] Provided herein in certain embodiments are methods of adjusting the dosage of a nitrogen scavenging drug in a subject with a nitrogen retention disorder comprising comparing a fasting blood ammonia level for the subject to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, the dosage of the nitrogen scavenging drug is increased, and if the dosage is less than or equal to half the ULN the dosage of the nitrogen scavenging drug is not increased. In certain embodiments, the methods further comprise administering the increased dosage. In certain of these embodiments, administration of the increased dosage results in a normal average daily ammonia level in the subject.

[0027] Provided herein in certain embodiments are methods of adjusting the dosage of a nitrogen scavenging drug in a subject with a nitrogen retention disorder comprising measuring a fasting blood ammonia level for the subject and comparing the fasting blood ammonia level to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, the dosage of the nitrogen scavenging drug is increased, and if the dosage is less than or equal to half the ULN the dosage of the nitrogen scavenging drug is not increased. In certain embodiments, the methods further comprise administering the increased dosage. In certain of these embodiments, administration of the increased dosage results in a normal average daily ammonia level in the subject.

[0028] Provided herein in certain embodiments are methods of adjusting the dosage of a nitrogen scavenging drug in a subject with a nitrogen retention disorder comprising

measuring a fasting blood ammonia level for the subject and comparing the fasting blood ammonia level to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, the dosage of the nitrogen scavenging drug is increased, and if the dosage is significantly less than half the ULN, the dosage of the nitrogen scavenging drug may be decreased. In certain embodiments, the methods further comprise administering the adjusted dosage. In certain of these embodiments, administration of the adjusted dosage results in a normal average daily ammonia level in the subject.

[0029] Provided herein in certain embodiments are methods of adjusting the dosage of a nitrogen scavenging drug in a subject with a nitrogen retention disorder comprising administering an initial dosage of the nitrogen scavenging drug, measuring fasting blood ammonia level, and comparing the fasting blood ammonia level to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, subsequent maintenance dosages of the nitrogen scavenging drug are adjusted to be greater than the initial dosage. In certain embodiments, the methods further comprise administering the increased maintenance dosage, and in certain of these embodiments, administration of the increased maintenance dosage results in a normal average daily ammonia level in the subject.

[0030] Provided herein in certain embodiments are methods of adjusting the dosage of a nitrogen scavenging drug in a subject with a nitrogen retention disorder to achieve a fasting blood ammonia level that is less than or equal to half the ULN for blood ammonia comprising measuring a fasting blood ammonia level for the subject and comparing the fasting blood ammonia level to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, the subject is administered an increased dosage of the nitrogen scavenging drug. After a time period sufficient for the drug to reach steady state (e.g., 48 hours, 48 to 72 hours, 72 hours to 1 week, 1 week to 2 weeks, greater than 2 weeks), fasting blood ammonia level is measured again and compared to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, the dosage of the nitrogen scavenging drug is increased. This process is repeated until a fasting blood ammonia level of less than or equal to half the ULN is obtained.

[0031] Provided herein in certain embodiments are methods for assessing whether a subject with a nitrogen retention disorder is more or less likely to need a dosage adjustment of a nitrogen scavenging drug comprising measuring a fasting blood ammonia level for the subject and comparing the fasting blood ammonia level to a ULN for blood ammonia, wherein a fasting blood ammonia level that is greater than half the value of ULN indicates that the subject is more likely to need a dosage adjustment and a fasting blood ammonia level

less than or equal to half the value of ULN indicates that the subject is less likely to need a dosage adjustment.

[0032] Provided herein in certain embodiments are methods of determining whether to administer a nitrogen scavenging drug to a subject with nitrogen retention disorder comprising comparing a fasting blood ammonia level for the subject to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, a nitrogen scavenging drug needs to be administered to the subject. In certain embodiments, these methods further comprise administering the nitrogen scavenging drug. In certain embodiments, the subject may not have been administered any nitrogen scavenging drugs prior to the determination. In other embodiments, the subject may have previously been administered a nitrogen scavenging drug other than the one being evaluated. In these embodiments, the methods provided herein can be used to determine whether to administer a new nitrogen scavenging drug to a subject.

[0033] Provided herein in certain embodiments are methods of determining whether to administer a nitrogen scavenging drug to a subject with nitrogen retention disorder comprising measuring a fasting blood ammonia level for the subject and comparing the fasting blood ammonia level to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, a nitrogen scavenging drug needs to be administered to the subject. In certain embodiments, these methods further comprise administering the nitrogen scavenging drug. In certain embodiments, the subject may not have been administered any nitrogen scavenging drugs prior to the determination. In other embodiments, the subject may have previously been administered a nitrogen scavenging drug other than the one being evaluated. In these embodiments, the methods provided herein can be used to determine whether to administer a new nitrogen scavenging drug to a subject.

[0034] Provided herein in certain embodiments are methods for selecting a dosage of a nitrogen scavenging drug for treating a nitrogen retention disorder in a subject based on blood ammonia levels comprising selecting a dosage that results in a fasting blood ammonia level that is less than or equal to half the ULN for blood ammonia. In certain embodiments, selecting the effective dosage is further based on diet, endogenous waste nitrogen excretion capacity, or any combination thereof. In certain embodiments, the methods further comprise administering the selected dosage.

[0035] Provided herein in certain embodiments are methods of treating a subject with a nitrogen retention disorder who has previously been administered a nitrogen scavenging drug comprising measuring a fasting blood ammonia level for the subject and comparing the

fasting blood ammonia level to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, the subject is administered an increased dosage of the nitrogen scavenging drug. If the fasting blood ammonia level has a value that is less than or equal to half the ULN, the subject is administered the same dosage or a decreased dosage of the nitrogen scavenging drug. In certain embodiments, administration of an increased dosage results in a normal average daily ammonia level in the subject.

[0036] Provided herein in certain embodiments are methods of treating a subject with a nitrogen retention disorder who has previously been administered an initial dosage of a nitrogen scavenging drug comprising measuring a fasting blood ammonia level for the subject and comparing the fasting blood ammonia level to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, the subject is administered a maintenance dosage that is greater than the initial dosage of the nitrogen scavenging drug. If the fasting blood ammonia level has a value that is less than or equal to half the ULN, the subject is administered the initial dosage or a lower dosage. In certain embodiments, administration of an increased maintenance dosage results in a normal average daily ammonia level in the subject.

[0037] Provided herein in certain embodiments are methods of treating a subject with a nitrogen retention disorder comprising administering a nitrogen scavenging drug, then measuring a fasting blood ammonia level for the subject at some point after drug administration and comparing the fasting blood ammonia level to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, the subject is administered an increased dosage of the nitrogen scavenging drug. If the fasting blood ammonia level has a value that is less than or equal to half the ULN, the subject is administered the original or a lower dosage of the drug.

[0038] Provided herein in certain embodiments are methods of treating a subject with a nitrogen retention disorder comprising administering a first dosage of a nitrogen scavenging drug, measuring a fasting blood ammonia level for the subject, and comparing the fasting blood ammonia level to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, a second dosage of a nitrogen scavenging drug that is greater than the first dosage is administered to the subject. A fasting ammonia blood level is measured again in the subject and compared to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, a third dosage of a nitrogen scavenging drug that is greater than the second dosage is administered to the subject.

This process is repeated until the subject exhibits a fasting blood ammonia level with a value less than or equal to half the ULN.

[0039] Provided herein in certain embodiments are methods of monitoring the efficacy of nitrogen scavenging drug administration in a subject with a nitrogen retention disorder who has previously been administered a nitrogen scavenging drug comprising measuring a fasting blood ammonia level for the subject and comparing the fasting blood ammonia level to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, the previously administered dosage of the nitrogen scavenging drug is considered inadequate to treat the nitrogen retention disorder. If the fasting blood ammonia level has a value that is less than or equal to half the ULN, the previously administered dosage is considered adequate to treat the nitrogen retention disorder. In certain embodiments where the previously administered dosage is considered inadequate to treat the nitrogen retention disorder, the methods provided herein further comprise administering an increased dosage of the nitrogen scavenging drug.

[0040] Provided herein in certain embodiments are methods for monitoring therapy with a nitrogen scavenging drug in a subject having a nitrogen retention disorder comprising measuring a fasting blood ammonia level from the subject and comparing the fasting blood ammonia level to a ULN for blood ammonia, wherein a fasting blood ammonia level that is greater than half the ULN indicates that the subject is more likely to need a dosage adjustment of the nitrogen scavenging drug, and wherein a fasting blood ammonia level less than or equal to half the ULN indicates that the subject is less likely to need a dosage adjustment.

[0041] A nitrogen retention disorder as used herein refers to any condition associated with elevated blood nitrogen/ammonia levels. In certain embodiments, a nitrogen retention disorder may be a UCD. In other embodiments, a nitrogen retention disorder may be HE.

[0042] A nitrogen scavenging drug as used herein refers to any drug that decreases blood nitrogen and/or ammonia levels. In certain embodiments, a nitrogen scavenging drug may remove nitrogen in the form of PAGN, and in certain of these embodiments the nitrogen scavenging drug may be an orally administrable drug that contains or is metabolized to PAA. For example, a nitrogen scavenging drug may be a PAA prodrug such as PBA or HPN-100, a pharmaceutically acceptable salt of PBA such as NaPBA, or a pharmaceutically acceptable ester, acid, or derivative of a PAA prodrug. In other embodiments, a nitrogen scavenging drug may remove nitrogen via hippuric acid. In certain of these embodiments, a nitrogen scavenging drug may be benzoic acid, a pharmaceutically acceptable salt of benzoic acid

such as sodium benzoate, or a pharmaceutically acceptable ester, acid, or derivative of benzoic acid.

[0043] Increasing the dosage of a nitrogen scavenging drug may refer to increasing the amount of drug per administration (e.g., an increase from a 3 mL dosage to a 6 mL dosage), increasing the number of administrations of the drug (e.g., an increase from once-a-day dosing to twice- or three-times-a-day), or any combination thereof.

[0044] A subject that has previously been administered a nitrogen scavenging drug may have been administered the drug for any duration of time sufficient to reach steady state. For example, the subject may have been administered the drug over a period of 2 to 7 days, 1 week to 2 weeks, 2 weeks to 4 weeks, 4 weeks to 8 weeks, 8 weeks to 16 weeks, or longer than 16 weeks.

[0045] In certain embodiments of the methods disclosed herein, the fasting period for obtaining a fasting blood ammonia level is overnight. In certain embodiments, the fasting period is 4 hours or more, 5 hours or more, 6 hours or more, 7 hours or more, 8 hours or more, 9 hours or more, 10 hours or more, 11 hours or more, or 12 hours or more, and in certain embodiments the fasting period is 4-8 hours, 6-8 hours, or 8-12 hours. During the fasting period, the subject preferably does not ingest any food. In certain embodiments, the subject may also refrain from ingesting certain non-food substances during the fasting period. For example, in certain embodiments the subject does not ingest any supplements and/or nitrogen scavenging drugs during the fasting period. In certain of these embodiments, the subject may nonetheless ingest one or more drugs other than nitrogen scavenging drugs during the fasting period. In certain embodiments, the subject does not ingest any high calorie liquids during the fasting period. In certain of these embodiments, the subject does not ingest any liquids other than water during the fasting period. In other embodiments, the subject may ingest small amounts of low calorie beverages, such as tea, coffee, or diluted juices.

[0046] In certain embodiments of the methods disclosed herein, blood samples used for measuring fasting blood ammonia levels and/or ULN blood ammonias are venous blood samples. In certain embodiments, a blood sample is a plasma blood sample. Any methods known in the art may be used to obtain a plasma blood sample. For example, blood from a subject may be drawn into a tube containing heparin or ethylenediaminetetraacetic acid (EDTA). In certain embodiments, the sample can be placed on ice and centrifuged to obtain plasma within 15 minutes of collection, stored at 2-8°C (36-46°F) and analyzed within 3 hours of collection. In other embodiments, the blood plasma sample is snap frozen, stored at

≤-18°C (≤0°F) and analyzed at a later time. For example, the sample may be analyzed at 0-12 hours, 12-24 hours, 24-48, 48-96 hours after freezing, or within any other timeframe over which the sample has demonstrated stability. In certain embodiments, blood samples are taken in a laboratory or hospital setting. In certain embodiments, a single fasting blood sample is used to measure fasting blood ammonia level. However, in other embodiments, multiple fasting blood samples may be obtained. In certain embodiments, a subject's blood ammonia level may be monitored throughout the day. Further, in certain embodiments, the methods disclosed herein comprise an additional step of obtaining one or more blood samples from a subject prior to or after measuring fasting blood ammonia level.

[0047] In certain embodiments, a blood sample is analyzed immediately after collection. In other embodiments, the blood sample is stored for some period between collection and analysis. In these embodiments, the sample may be stored for less than 1 hour, 1 hour to 6 hours, 1 hour to 12 hours, 1 hour to 24 hours, or 1 hour to 48 hours. In certain of these embodiments, the blood sample is stored at a temperature between 0-15°C, such as 2-8°C. In other embodiments, the blood sample is stored below 0°C or below -18°C.

[0048] Measurement of ammonia levels in a fasting blood sample is carried out using techniques known in the art. For example, ammonia levels may be measured using a colorimetric reaction or an enzymatic reaction. In certain embodiments, a colorimetric reaction may involve the use of bromophenol blue as an ammonia indicator. In these embodiments, ammonia may react with bromophenol blue to yield a blue dye. In certain embodiments, an enzymatic reaction may involve glutamate dehydrogenase catalyzing the reductive amination of 2-oxoglutarate with NH^{4+} and NADPH to form glutamate and $NADP^{+}$. The formation of $NADP^{+}$ formed is directly proportional to the amount of ammonia present in the blood sample. Therefore, the concentration of ammonia is measured based on a decrease in absorbance.

[0049] In certain embodiments of the methods disclosed herein, a subject exhibiting a fasting blood ammonia level less than or equal to half the ULN for blood ammonia has an average likelihood within a confidence interval that their average daily ammonia level will remain within a normal average daily ammonia level. In certain embodiments, the average likelihood of having a normal daily ammonia value is 80% to 90%. In certain embodiments, one may predict with 95% confidence that a blood ammonia level will fall within a certain range. In certain embodiments, one can predict with 95% confidence that a true probability of predicting normal values based on fasting blood ammonia is between 65% and 93%. In other embodiments, one can predict with 80% confidence that a true probability of predicting

normal values based on fasting blood ammonia is at least 70%. In certain embodiments, the average likelihood of predicting normal ammonia value based on fasting blood ammonia is about 84% with 95% confidence that the true probability is between 65% and 93%.

[0050] In certain embodiments of the methods disclosed herein, a subject exhibiting a fasting blood ammonia level less than or equal to half the ULN for blood ammonia has an average likelihood within a confidence interval that their maximum daily blood ammonia level will not exceed 1.5 times the ULN for blood ammonia. In certain of these embodiments, the average likelihood is about 70% to 80%. In certain embodiments, the confidence interval is a 95% confidence interval. In certain embodiments, the average likelihood is about 75% with 95% confidence that the true probability is between 58% and 86%.

[0051] In certain embodiments of the methods disclosed herein, a subject exhibiting a fasting blood ammonia level less than or equal to half the ULN for blood ammonia has an average likelihood within a confidence interval that their maximum daily blood ammonia level will be less than 100 μmol/L. In certain of these embodiments, the average likelihood is 90% to 98%. In certain embodiments, the confidence interval is 95%. In certain embodiments, the average likelihood is about 93% with 95% confidence that the true probability is between 77% and 100%.

[0052] The maximal ammonia value refers to the maximum amount of ammonia that may be detected in a subject following consumption of meals, if repeated measurement of blood ammonia can be instituted to detect such maximum value over an extended period of time. Based on well-controlled clinical trials with repeated blood sampling over 24 hours, the maximum blood ammonia has been observed to occur following the third major meal of the day in the early to mid evening hours (4-8PM, assuming that breakfast is approximately 8AM; see, e.g., Lee 2010; Lichter-Konecki 2011).

[0053] The ULN for blood ammonia typically represents the highest level in the range of normal values, which may be influenced by a variety of factors such as the assay method, types of regents, standard reference samples used, and specifications and calibration of equipment used to perform the measurement. In certain embodiments of the methods disclosed herein, the ULN for blood ammonia is determined for a subject individually. In other embodiments, the ULN for blood ammonia may be based on measurements obtained across a range of subjects (i.e., subjects with UCD or with a particular subtype of UCD, subjects with HE, healthy subjects, etc.). In certain embodiments, the ULN for blood ammonia may represent a standard reference value disclosed in the art, such as a mean ULN

developed across a particular subset of subjects. In other embodiments, the ULN for blood ammonia may represent a standard measurement that has been developed by a particular entity that performs blood draws and/or blood evaluations, such as a particular clinical laboratory. In certain embodiments, the ULN is a standard reference value utilized by the same entity that measures the fasting blood ammonia level. In these embodiments, one skilled in the art will appreciate that interpretation of average daily ammonia in subject with a nitrogen retention disorder must be made relative to the reference range of normal values at the laboratory in which the ammonia was measured. Furthermore, the units of ammonia measurement may also vary from lab to lab (e.g., μg/mL or μmol/L), emphasizing the importance of interpreting the subject's ammonia levels relative to the ULN at the laboratory in which the measurement was performed. In certain embodiments, the ULN for blood ammonia may be in the range of 26-64 μmol/L. In certain of these embodiments, the ULN for blood ammonia may be in the range of 32-38 μmol/L or 34-36 μmol/L, and in certain of these embodiments the ULN for blood ammonia is 35 μmol/L. In certain embodiments, the ULN for blood ammonia may be in the range of 50-65 μg/mL. In certain of these embodiments, the ULN for blood ammonia may be in the range of 55-63 μg/mL or 57-61 μg/mL, and in certain of these embodiments the ULN for blood ammonia is 59 μg/mL.

[0054] In certain embodiments, the average daily ammonia is the average amount of ammonia an individual may experience during the day, if serial blood sampling were performed for ammonia measurements. In well-controlled clinical studies, it has been established that ammonia fluctuates several fold during the day, depending on the timing of blood draw relative to food and drug intake. Due to these fluctuations, the timing of individual or serial blood sampling should be controlled relative to the timing of food and drug intake. Even serial sampling may not be enough to capture the peaks and troughs of the fluctuating ammonia values, unless samples are taken frequently enough. Therefore, obtaining a simple average of several measurements may provide inadequate or misleading information regarding the total ammonia burden a subject may experience during the day.

[0055] Provided herein are methods to better estimate a subject's average daily ammonia assessed as the area under the curve for 24-hr ammonia (ammonia $AUC_{0\text{-}24hr}$) obtained from adequate and well-spaced samples over 24 hours. This ammonia $AUC_{0\text{-}24hr}$ can be further normalized for the entire actual period of sampling, i.e., ammonia $AUC_{0\text{-}24hr}$ is divided by the sampling period (e.g., 24 hours). For example, if an AUC of 1440 μmol*hr/L is calculated using the trapezoidal rule based on 8-11 ammonia values obtained over 24 hours, then the average daily ammonia value or time-normalized $AUC_{0\text{-}24hr}$ would be equal to 1440

μmol*hr/ml divided by the sampling time of 24 hr, or 60 μmol/L. If the normal reference range at the laboratory which performed the ammonia analysis was 10-35 μmol/L, then the average daily ammonia value for this subject would be approximately 1.71 times the ULN of 35 μmol/L. Similarly, if the ammonia $AUC_{0\text{-}24hr}$ was determined to be equal to 840 μmol*hr/L based on multiple, well-spaced samples over 24 hours and analyzed at the same laboratory, and the sampling period was 24 hours, then the time-normalized $AUC_{0\text{-}24hr}$ would be 35 μmol/L. This corresponds to an average ammonia or daily ammonia burden within the ULN. Finally, subjects with nitrogen retention disorders such as UCDs may experience a hyperammonemic crisis, which is often defined clinically as a blood level exceeding 100 μmol/L and clinical manifestations of hyperammonemia, which may require intervention to prevent irreversible hard and enable recovery.

[0056] Provided herein are methods of adjusting nitrogen scavenging drug dosage by measuring fasting blood ammonia to minimize the likelihood a subject may experience an ammonia value (Cmax) over 24 hours that exceeds 100 μmol/L. It has been found that 100 μmol/L corresponds to approximately 2-3 times the ULN in most laboratories. Previously, if a subject with a nitrogen retention disorder such as UCD had a blood ammonia level within or slightly above the normal reference range for the laboratory which performed the analysis, the subject was considered to be in good clinical control regardless of the timing of the blood draw in relation to meals and last administration of drug dose. However, it has been shown that a subject with a UCD who has a fasting blood ammonia level between the ULN and 1.5 times the ULN (e.g., 35 to 52 μmol/L) has an average likelihood of only 45% (with a 95% confidence interval of 21% to 70%) that his or her average daily ammonia is within the normal range; an average likelihood of only 35% (with a 95% confidence interval of 13% to 60%) that his or her maximal level of ammonia during the day is less than 1.5 times the ULN (e.g., 52 μmol/L); and an average likelihood of 25% that his or her maximal daily ammonia level exceeds 100 μmol/L during the day. Thus, after measuring a UCD subject's fasting blood ammonia, the dosage of a nitrogen scavenging drug may be progressively increased and/or his or her protein intake progressively decreased until the fasting ammonia value is less than or equal to half of the ULN for the local laboratory in which the ammonia analysis was performed.

[0057] In certain embodiments of the methods disclosed herein, one or more factors other than ammonia level may be taken into consideration when evaluating nitrogen scavenging drug dosage. For example, blood ammonia measurements may be combined with urinary PAGN measurements in determining whether to administer a nitrogen scavenging drug,

adjusting the dosage of a nitrogen scavenging drug, or treating a nitrogen retention disorder. US Patent Publication No. 2010/0008859 discloses that urinary PAGN levels correlate more closely to PBA prodrug dosage than plasma PAA, PBA, or PAGN levels, and further discloses that PBA prodrugs are converted to urinary PAGN with a mean efficiency of 60-75%. Therefore, certain embodiments of the methods disclosed herein comprise an additional step wherein urinary PAGN levels are measured. In certain of these embodiments, calculation of an effective dosage of nitrogen scavenging drug is based in part on a mean 60-75% conversion of PAA prodrug to urinary PAGN. For example, in certain embodiments the methods disclosed herein for determining whether to administer a nitrogen scavenging drug to a subject comprise an additional step of measuring urinary PAGN and calculating an effective initial dosage based on a mean conversion of PAA prodrug to urinary PAGN of 60-75%. Similarly, in certain embodiments the methods disclosed herein for adjusting the dosage of a nitrogen scavenging drug comprise an additional step of measuring urinary PAGN and calculating an effective dosage based on a mean conversion of PAA prodrug to urinary PAGN of 60-75%. In certain of these embodiments, the effective dosage is calculated based on a target nitrogen output. In certain embodiments, urinary PAGN may be determined as a ratio of the concentration of urinary PAGN to urinary creatinine. In certain embodiments, urinary PAGN is a factor that is taken into consideration when determining whether to administer or increase the dosage of a nitrogen scavenging drug, i.e., urinary PAGN is evaluated in combination with ammonia level to determine whether to administer or increase the dosage of the drug. In other embodiments, ammonia level alone is used to determine whether to administer or increase the dosage of a nitrogen scavenging drug, and urinary PAGN is simply used to calculate the initial or adjusted dosage.

[0058] One skilled in the art will recognize that a variety of other factors may be taken into consideration when determining the effective dosage of a nitrogen scavenging drug. For example, factors such as diet (e.g., protein intake) and endogenous waste nitrogen capacity (e.g., urea synthesis capacity) may be considered.

[0059] Provided herein in certain embodiments are kits for carrying out the methods disclosed herein. In certain embodiments, kits are provided for determining whether to administer or adjust the dosage of a nitrogen scavenging drug for a subject with a nitrogen retention disorder. The kits disclosed herein may include one or more nitrogen scavenging drugs and/or one or more reagents (e.g., bromophenol blue) or enzymes (e.g., glutamate dehydrogenase) to measure blood ammonia levels in a sample. The kit may additionally include other pigments, binders, surfactants, buffers, stabilizers, and/or chemicals necessary

to obtain a blood sample and to measure the ammonia level in the sample. In certain embodiments, the kits provided herein comprise instructions in a tangible medium.

[0060] One of ordinary skill in the art will recognize that the various embodiments described herein can be combined.

[0061] The following examples are provided to better illustrate the claimed invention and are not to be interpreted as limiting the scope of the invention. To the extent that specific materials are mentioned, it is merely for purposes of illustration and is not intended to limit the invention. One skilled in the art may develop equivalent means or reactants without the exercise of inventive capacity and without departing from the scope of the invention. It will be understood that many variations can be made in the procedures herein described while still remaining within the bounds of the present invention. It is the intention of the inventors that such variations are included within the scope of the invention.

Examples

Example 1: Analysis of predictability of pharmacodynamic ammonia values from fasting ammonia in UCD patients:

[0062] This example demonstrates the relationship between fasting ammonia and the pharmacodynamic (PD) profile of daily ammonia in patients receiving PAA prodrugs for UCDs. Ammonia values vary many-fold over the course of 24 hours in UCD patients. As depicted in Figures 3a and 3b, venous ammonia was measured for 24 hours following one week of dosing with either NaPBA or glycerol phenylbutyrate (GPB). The graphs display ammonia values as mean ±SD over 24 hours, where time zero corresponds to just prior to dosing and breakfast (i.e., fasting state). In view of this variability in daily ammonia levels, a single measurement may not be very informative in determining whether a UCD patient is optimally dosed. The ability to predict the highest potential ammonia a UCD patient may experience during the day and the average 24-hour ammonia from a single measurement such as fasting levels has important practical implications for nitrogen scavenging drug dosing guidelines and patient management.

[0063] Data from two Phase 2 studies and one Phase 3 study comparing ammonia control assessed by 24-hour sampling during steady state treatment with HPN-100 versus NaPBA in 65 UCD patients were used for the analysis. The two Phase 2 studies include protocols UP 1204-003 and HPN-100-005 (Lee 2010; Lichter-Konecki 2011). The Phase 3 study includes protocols from HPN-100-006 (Diaz 2011).

[0064] Ammonia values obtained from different hospital laboratories with different normal ranges were normalized to a standard laboratory range of 9-35 μmol/L. The patient

population included a broad range of ages, UCD subtypes, and doses of drug, and is summarized in Table 1 below.

Table 1: UCD demographics in studies UP 1204-003, HPN-100-005, and HPN-100-006:

Gender n (%)	Male	18 (27.7)
	Female	47 (72.3)
Age at screening (years)	N	65
	Mean (SD)	29.46 (15.764)
	Median	24.00
	Range	6.0-75.0
UCD diagnosis n (%)	OTC deficiency	57 (87.7)
	CPS1 deficiency	1 (1.5)
	ASS deficiency	5 (7.7)
	ASL deficiency	1 (1.5)
	Missing	1 (1.5)
Duration of NaPBA treatment (months)	N	63
	Mean (SD)	114.14 (90.147)
	Median	101.00
	Range	0.2-300.0
Daily dose NaPBA	N	64
	Mean (SD)	14.10 (6.255)
	Median	13.50
	Range	1.5-36.0

[0065] Exploratory analysis:

[0066] Several PD parameters for steady-state ammonia were explored: $AUC_{0\text{-}24hr}$, time-normalized AUC, log AUC, maximal ammonia value over 24 hours (Cmax), and average ammonia. Data from 65 subjects from all three studies with steady-state ammonia and fasting ammonia were used. Missing data were imputed per procedures specified in the protocol and statistical analysis plan, except that no imputations were made for subjects who had no PK sampling conducted while on a given study drug.

[0067] Sample collection times of 0-hr (before first daily dose) and 24-hours post-dose (before first daily dose of the following day) were both evaluated as representative of fasting ammonia. No noticeable difference in the shape or quality of the relationship due to the choice of time point was observed.

[0068] The relationship between fasting ammonia and pharmacokinetic profile was evaluated separately for HPN-100 and NaPBA, with no apparent difference in the strength or magnitude of the relationship. Therefore, all data from both HPN-100 and NaPBA treatments were used and conclusions regarding fasting ammonia pertain to both HPN-100 and NaPBA.

[0069] The relationships between (1) fasting ammonia and $AUC_{0\text{-}24hr}$ and (2) fasting ammonia and maximum observed ammonia (Cmax) were visually explored for the whole population. The effects of the following covariates were also observed: age, weight, gender, and dietary protein intake. A positive and strong relationship was observed between fasting ammonia and $AUC_{0\text{-}24hr}$, with increasing fasting ammonia being associated with higher $AUC_{0\text{-}24hr}$ and maximum observed ammonia (Figure 2).

[0070] Prediction of $AUC_{0\text{-}24hr}$ through GEE Modeling:

[0071] The aim of this modeling was to predict average daily or highest achieved ammonia based on the subject's fasting ammonia. In order to take into account the differences in normal ranges at different laboratories, all ammonia values were normalized to a reference range of 9-35 μmol/L, and the predictions were referenced to the ULN rather than a fixed value.

[0072] Generalized Estimating Equations (GEE) were used to model the predictive ability of fasting ammonia against various ammonia PD properties. GEE methodology can be used to analyze repeated measures of categorical data, in which the repeated measures are assumed to be correlated (Liang 1986). The model allows for the specification of the assumed correlation structure without the knowledge of the magnitude of the correlation.

[0073] The 24-hour ammonia profile was divided into ordered categories using a variety of endpoints and cutpoints as follows:

1) AUC [0-1.0*ULN, >1.0*ULN];

2) AUC [0-1.5*ULN, >1.5*ULN];

3) Cmax [0-1.0*ULN, >1.0*ULN];

4) Cmax [0-1.5*ULN, >1.5*ULN]; and

5) Cmax [0-100] μmol/L.

[0074] Three levels of fasting ammonia were considered in separate models as input:

1) [0-0.5*ULN];

2) [>0.5*ULN-<1.0 ULN]; and

3) [>1.0*ULN-1.5*ULN].

[0075] Using Statistical Analysis Software (SAS) Proc Genmod, generalized linear models were fit with a logit link function. Pre-dose fasting ammonia was the only predictor variable in the model. The repeated nature of the data (two study periods per subject) was modeled using GEE with exchangeable correlation matrix. ULN for fasting ammonia was set at 35 μmol/L. ULN for AUC over 24 hours was taken as 840 (35 μmol/L * 24 hours); i.e., the AUC which corresponds to an average daily ammonia less than or equal to 35 μmol/L, which

was the normalized ULN among the participating study sites and is derived by dividing the 24-hour area under the curve by the sampling time of 24 hours. The GEE model was bootstrap-resampled 1,000 times according to the method outlined in Davison, A.C. & Hinkley, D.V., Bootstrap Methods and their Application, Cambridge University Press, London (1997), pp.358-362. The results of these models are shown in Table 2 below.

Table 2: Summary of results from GEE model to predict ability of fasting ammonia against various ammonia PD properties:

Model #	Fasting ammonia level	Ammonia PK outcome	Probability of outcome in category	Bootstrap 95% c.i.	Bootstrap 80% c.i.	Bootstrap pred. error rate* (%)
1	[0-0.5 ULN]	AUC in 24 hours [0-1.0 ULN]	0.84	0.67, 0.93	0.71, 0.89	11.5
2		AUC in 24 hours [0-1.5 ULN]	Did not converge			
3		Cmax observed [0-1.0 ULN]	0.53	0.38, 0.65	0.42, 0.61	45.8
4		Cmax observed [0-1.5 ULN]	0.76	0.61, 0.86	0.66, 0.82	23.3
5		Cmax observed [0-100]	0.93	0.78, 1.00	0.85, 0.97	5.7
6	[0-<1.0 ULN]	AUC in 24 hours [0-1.0 ULN]	0.58	0.42, 0.73	0.48, 0.68	42.8
7		AUC in 24 hours [0-1.5 ULN]	0.88	0.78, 0.97	0.82, 0.94	11.1
8		AUC in 24 hours [0-2 ULN]	0.97	0.90, 1.00	0.93, 1.00	2.2
9		Cmax observed [0-1.0 ULN]	0.21	0.11, 0.38	0.14, 0.33	20.0
10		Cmax observed [0-1.5 ULN]	0.52	0.35, 0.66	0.42, 0.61	46.0
11		Cmax observed [0-2.0 ULN]	0.74	0.62, 0.85	0.91, 1.00	27.2
12		Cmax observed [0-	0.95	0.88, 1.00	0.66, 0.81	4.3

		100]				
13	[>1.0-1.5 ULN]	AUC in 24 hours [0-1.0 ULN]	0.45	0.24, 0.71	0.30, 0.63	43
14		AUC in 24 hours [0-1.5 ULN]	Did not converge			
15		AUC in 24 hours [0-2 ULN]	0.80	0.49, 0.99	0.63, 0.92	27
16		Cmax observed [0-1.0 ULN]	Did not converge			
17		Cmax observed [0-1.5 ULN]	0.35	0.16, 0.58	0.23, 0.51	33
18		Cmax observed [0-2.0 ULN]	Did not converge			
19		Cmax observed [0-100]	Did not converge			

[0076] From Table 2 above, we can conclude that in the population of UCD patients described in Table 1, we can be 95% confident that, given a fasting ammonia less than or equal to half the ULN, the true probability of having an AUC in the range [0-840] is on average 84%, at least 67%, and as high as 93%.

[0077] Row 1 of Table 2 above suggests that a UCD patient with a fasting ammonia of 17 μmol/L as determined by a laboratory with a normal reference range of 9-35 μmol/L (i.e., a fasting ammonia in the range [0-0.5 ULN]) has an 84% chance (with a 95% confidence interval of 67% to 93%) of having a time normalized $AUC_{0\text{-}24hr}$ in the normal range [$AUC_{0\text{-}24hr}$ of 0-840 or an average daily ammonia of 35 μmol/L], a 76% chance (with a 95% confidence interval of 61% to 86%) of having a Cmax of less than 1.5 ULN, and a 93% chance (with a 95% confidence interval of 78% to 100%) of never having an ammonia of more than 100 μmol/L. Therefore, this patient would be optimally controlled and unlikely to suffer from high ammonia during the day.

[0078] This Example shows that fasting ammonia correlates strongly with daily ammonia exposure, assessed as a daily average or as maximal daily concentration, and that a target fasting value which does not exceed half of the upper level of normal for the local lab appears to be a clinically useful as well as practical predictor of ammonia values over 24 hours as well. Furthermore, this Example shows that a subject with a fasting ammonia in the range 0-

0.5 ULN has an 84% chance of having an AUC_{0-24hr} in the normal range (0-840 or an average daily ammonia of 35 μmol/L).

Example 2: Selecting and adjusting HPN-100 dosage based on fasting blood ammonia levels in a patient with UCD:

[0079] Patient A is an adult with UCD being managed with amino acid supplements and dietary protein restriction only. Patient A consumes neither his supplements nor food for approximately 8 hours prior to a fasting morning blood draw. A venous blood draw is performed, and fasting blood ammonia level is determined to be 52 μmol/L. This fasting blood ammonia level is compared to the ULN for blood ammonia in the laboratory performing the blood draw, which is 35 μmol/L. Based on the correlation of fasting ammonia level to average ammonia level, it is determined that Patient A's fasting blood ammonia level of approximately 1.5 times the ULN represents only a 45% chance on average of having an average ammonia during the day within the normal range. Thus, the ratio of fasting blood ammonia level to ULN for blood ammonia indicates that Patient A will benefit from treatment with a nitrogen scavenging drug.

[0080] The physician elects to treat Patient A with HPN-100. Initial dosage is determined based on body surface area or as otherwise instructed according to HPN-100 drug labeling. Patient A's body surface area is 1.4 m^2, and therefore the initial dosage is determined to be 9 mL per day or 3 mL TID, which is approximately 60% of the maximum allowed dosage per HPN-100 label. Patient A is treated with 9mL/day of HPN-100 for at least 7 days, and returns for an additional blood draw. The fasting blood ammonia level at this time is 33 μmol/L, which is slightly below the ULN and falls into the range of 0.5 to 1.0 times normal. Patient A's blood ammonia level is monitored throughout the day after administration of a 3 mL dose of HPN-100 with each meal. It is observed that Patient A's maximum ammonia reaches 95 μmol/L after dinner with an average daily ammonia of 66 μmol/L, which is almost two times the upper normal range. Therefore, Patient A's dosage of HPN-100 is increased by approximately one-third to 12 mL total or 4 mL TID. Patient A returns after at least 7 days of treatment with HPN-100. Patient A's fasting ammonia level is 15 μmol/L, which is less than half of the ULN range. It is determined that Patient A has reached satisfactory ammonia control.

[0081] It is expected that if Patient A adheres to his prescribed diet, his maximal daily ammonia is not expected to exceed approximately 52 μmol/L, i.e., approximately 1.5 times the ULN, with an average likelihood of 75% with 95% confidence. The average ammonia level during the day is expected to remain within normal range with greater than 84%

likelihood and 95% confidence. Moreover, Patient A's maximal daily ammonia is highly unlikely to reach 100 μmol/L during the day.

Example 3: Adjusting HPN-100 dosage based on fasting blood ammonia levels in a patient with UCD:

[0082] Patient B is an 11-year UCD patient receiving 24 pills of BUPHENYL® per day, amino acid supplements, and restricted dietary protein intake. Patient B does not consume BUPHENYL®, supplements, or food for approximately 6 hours prior to a fasting morning blood draw. A venous blood draw is performed, and fasting blood ammonia level is determined to be 40 μmol/L. This fasting blood ammonia level is compared to the ULN for blood ammonia for the laboratory performing the blood draw, which is 35 μmol/L. Based on the correlation of fasting ammonia level to average ammonia level, it is determined that Patient B's fasting blood ammonia level falling between 1 and 1.5 times the ULN represents a 55% chance of having an average ammonia during the day that is greater than the normal range, and as high as a 65% chance that her ammonia will go above 52 μmol/L or 1.5 times ULN during the day.

[0083] Based on discussion with the patient and her mother, the physician suspects that Patient B is noncompliant with her medication, and decides to change her to HPN-100. The initial dosage is determined based on the amount of BUPHENYL® Patient B was receiving, and it is determined that Patient B needs to take 10.5 mL of HPN-100 per day. Patient B is treated with 3.5mL of HPN-100 3 times a day for at least 7 days, and returns for additional blood draws. Her fasting blood ammonia level at this time is 17 μmol/L, which is below the ULN and falls into the range of 0 to 0.5 times normal. It is determined that Patient B has reached satisfactory ammonia control.

[0084] It is expected that if Patient B adheres to her prescribed diet, her maximal daily ammonia will not go above approximately 50 μmol/L, which is less than 1.5 times the ULN. Her average ammonia level during the day is expected with greater than 84% average likelihood to remain within normal range. Moreover, there is only a small chance (7%) that Patient B's maximal daily ammonia will exceed 100 μmol/L during the day.

Example 4: Selecting and adjusting sodium benzoate dosage based on fasting blood ammonia levels in a patient with UCD:

[0085] Patient C is an adult UCD patient who is allergic to PBA and is therefore being managed with amino acid supplements and dietary protein restriction only. Patient C complains of chronic headache and frequent nausea. Patient C consumes neither his supplements nor food for approximately 8 hours prior to a fasting morning blood draw. A

venous blood draw is performed, and fasting blood ammonia level is determined to be 77 μmol/L. This fasting blood ammonia level is compared to the ULN for blood ammonia for the laboratory performing the blood draw, which is 35 μmol/L. Based on the correlation of fasting ammonia level to average ammonia level, it is determined that Patient C's fasting blood ammonia level of approximately 2 times the ULN represents a high likelihood of ammonia levels going over 100 μmol/L during the day. Thus, the ratio of fasting blood ammonia level to ULN for blood ammonia indicates that Patient C will benefit from treatment with a nitrogen scavenging drug.

[0086] The physician decides to treat Patient C with 15 g of sodium benzoate per day since the patient is allergic to PBA. Patient C is treated with 15 g/day of sodium benzoate for at least 7 days, and returns for additional blood draws. Fasting blood ammonia level at this time is 35 μmol/L, which is equal to the ULN. Patient C's dosage of sodium benzoate is increased by approximately 30% to 18 grams per day. After at least 7 days of treatment, Patient C's fasting ammonia level is 15 μmol/L, which is less than half of the ULN. It is determined that Patient C has reached satisfactory ammonia control.

[0087] It is expected that if Patient C adheres to his prescribed diet and medication, his maximal daily ammonia will not exceed approximately 52 μmol/L, which is approximately 1.5 times the ULN. His average ammonia level during the day is expected with greater than 80% likelihood to remain within normal range. Moreover, Patient C's maximal daily ammonia is highly unlikely to reach 100 μmol/L during the day.

Example 5: Evaluation of the effect of ammonia control on neurocognitive outcome:

[0088] It has been shown that UCD patients are likely to suffer from diminished intelligence and impaired neurocognitive functions (Kirvitsky 2009). These neuropsychological impairments have been attributed to repeated episodes of acute hyperammonemia interspersed on chronically elevated ammonia. Abnormalities in neuropsychological function and/or brain imaging have been detected even in UCD patients with mild disorders who exhibit normal IQ and/or appear clinical normal (Gropman 2008a; Gropman 2008b). Therefore, it was hypothesized that maintaining average daily ammonia within normal limits and thereby reducing the long term ammonia burden could result in improved cognition.

[0089] The relationship between reducing ammonia burden by maintaining fasting ammonia at or close to half ULN and neuropsychological outcomes in pediatric UCD patients was explored in clinical trials. Eleven pediatric patients ages 6-17 were enrolled in short term switch over comparison of NaPBA and HPN-100 in controlling ammonia. These patients

underwent 24-hr serial sample collection in a confined setting where the last sample at 24 hr was considered fasting and under supervision of the study personnel. At the end of treatment with HPN-100 the average fasting ammonia at 24-hr time point was 15.5 μmol/L or less than half ULN, indicating good clinical control. These 11 patients along with another 15 pediatric patients were enrolled in two long term studies and received HPN-100 for 12 months, during which monthly fasting ammonia were collected. At the time of enrollment and at the end of the study, all patients underwent assessment for neuropsychological outcomes including the following: BRIEF (Behavior Rating Inventory of Executive Function) to assess day-to-day executive functioning, CBCL (Child Behavior Checklist) to evaluate internalizing (e.g., mood/anxiety) and externalizing behaviors, and WASI (Wechsler Abbreviated Scale of Intelligence) to estimate of intellectual ability.

[0090] During the 12 month treatment with HPN-100, pediatric UCD patients experienced fewer episodes of acute hyperammonemia than in the 12 months preceding enrollment (5 episodes during the study versus 9 before enrollment), with peak ammonia dropping from a mean of 233 μmol/L before enrollment to 166 μmol/L during the study. Fasting ammonia remained controlled and monthly averages were at or close to half ULN, ranging from 17 to 22 μmol/L. Although patients had been instructed to remain fasting before monthly study visits, some ammonia samples were taken in a non-fasted state, resulting in average monthly ammonia of slightly above half ULN.

[0091] In pediatric patients, WASI and CBCL scores were stable in comparison to baseline. The majority of the BRIEF subscales at baseline were at or close to 65, consistent with borderline and/or clinically significant dysfunction. Among 22 pediatric subjects who completed the neuropsychological testing at 12 months, all BRIEF domains were improved (lower T scores) with means (SD) at end of study compared to baseline for Behavioral Regulation Index 53.7 (9.79) vs. 60.4 (14.03) ($p<0.05$); Metacognition Index 57.5 (9.84) vs. 67.5 (13.72) ($p<0.001$), and Global Executive Scale 56.5 (9.71) vs. 66.2 (14.02) ($p<0.001$).

[0092] The significant improvement in executive functions in this group of pediatric UCD patients indicates the importance of long term ammonia control and achieving target levels of fasting ammonia.

Example 6: Correlation of elevated PAA levels to neurological AEs in UCD and healthy subjects:

[0093] Elevated plasma levels of PAA may cause symptoms that mimic those associated with hyperammonemia, including headache, nausea, somnolence, etc. Since such symptoms are common and nonspecific, an ammonia level below half the upper limit of normal in a

subject with a nitrogen retention disorder who exhibits such symptoms and is receiving a PAA prodrug would prompt a physician to check plasma PAA levels.

[0094] The relationship between elevated PAA levels and neurological AEs was evaluated in three populations: (1) 130 healthy adults dosed with 4 to 12 mL TID of GPB in a thorough QTc study, (2) 54 adult and 11 pediatric UCD patients (ages 6-17) enrolled in one of 3 protocols involving short term (2-4 week) switchover comparisons of NaPBA vs. GPB, and (3) 77 patients enrolled in two nearly identical 12-month GPB treatment protocols. In populations 1 and 2, maximal PAA (i.e., Cmax) levels were analyzed in relation to neurological AEs as defined by MEDDRA using an Exact non-parametric Mann-Whitney test and Generalized Estimating Equations (GEE) with a logit link function and effects for dose and PAA level. The relationship between PAA levels and the occurrence of the AEs reported by Thiebault was also explored in population 3.

[0095] No statistically significant relationship was observed between neurological AEs and PAA levels for either GPB or NaPBA. The odds ratio of a neurological AE occurring for each 20 μg/mL increase in PAA levels for the two drugs combined was 0.95, very close to 1. Thus, among UCD patients dosed with HPN-100 or NaPBA over the ranges used in these studies, increasing levels of PAA (ranging up to 244 μg/mL) were not associated with an increase in neurological AEs. Similarly, in population 3, PAA levels did not increase over time and exhibited no apparent relationship to neurological AEs, which also did not increase in frequency over time. The pediatric patient with the highest PAA level (410 μg/mL) did not report neurological AEs close to the timing of the blood draw.

[0096] Unlike UCD subjects, healthy adult volunteers who reported a nervous system AE had statistically significantly higher PAA C_{max} levels than those who did not. While this analysis in healthy adults is compromised by the fact that PAA levels were not always available at the time of occurrence of the AEs, as well as by the small sample size in the higher dose groups, the odds ratio of 1.75 (p=0.006) suggests that increasing levels of PAA are associated with increased probability of experiencing a nervous system AE among healthy adults. AEs reported by healthy adults generally began within 36 hours of dosing and, among those adults who remained on study, most resolved with continued dosing.

[0097] A significant relationship between PAA levels and occurrence of neurological AEs, which generally resolved with continued dosing, was detected in healthy volunteers. Unlike in healthy adults, PAA C_{max} did not correlate with nervous system AEs in UCD patients over a similar range of doses and PAA levels. These findings may reflect metabolic differences

among the populations (e.g., UCD patients exhibit high glutamine levels compared with healthy humans) and/or metabolic adaptation with continued dosing.

[0098] Population PK model building was performed on 65 UCD patients who participated in the short-term switchover Hyperion studies using NONMEM (version 7.2) based on 2981 ([PBA], [PAA], [PAGN], and urine PAGN [UPAGN])) data points from 53 adult and 11 pediatric UCD patients (ages 6-17) who participated in 3 switchover studies of NaPBA and GPB. The median GPB dose, expressed as grams of PBA per m2, was 8.85 and 7.01 for pediatric and adult subjects, respectively. Diagnostic plots and statistical comparisons were used to select among candidate models, and covariates were assessed by graphical analyses and covariate modeling. Using the final popPK model and parameter estimates, Monte Carlo simulations were performed in ~1000 virtual patients for a range of NaPBA and GPB doses to predict systemic metabolite exposure and UPAGN output.

[0099] The final model that best fit the data was characterized by (a) partial conversion of PBA to PAGN prior to reaching the systemic circulation, (b) saturable conversion of PAA to PAGN (Km ~161ug/ml), and (c) ~60% slower PBA absorption when delivered as GPB vs. NaPBA. Body surface area (BSA) was a significant covariate such that metabolite clearance was proportionally related to BSA. Fractional presystemic metabolism of PBA was higher for adults than for pediatric patients receiving GPB (43% vs. 14%), whereas the reverse was true for NaPBA (23% vs. 43%). Predicted median PAA exposure based on simulated GPB dosing at the PBA equivalent of 13g/m2 of NaPBA was ~13%-22% lower in adults than NaPBA (Cmax = 82 vs. 106 µg/mL; AUC_{0-24} = 649 vs. 829 µg.h/m) and ~13% higher in pediatric subjects ages 6-17 than NaPBA (Cmax = 154 vs. 138 µg/mL; AUC_{0-24} = 1286 vs. 1154 µg.h/ml); predicted upper 95th percentile PAA exposure was below 500 µg/mL and 25%-40% lower for adult subjects on GPB versus NaPBA and similar for pediatric subjects. Simulated dosing at the PBA equivalent of ~5g/m^2 of NaPBA yielded similar and less variable PAA exposure for both drugs and for pediatric and adult patients. Recovery of PBA as UPAGN was very similar whether delivered orally as GPB or NaPBA.

[00100] These findings based on PopPK modeling and dosing simulations suggest that while most patients treated with PAA prodrugs including NaPBA or HPN-100 will have PAA levels below those reportedly associated with toxicity and while no relationship between PAA levels and neurological AEs was found on a population basis, individual patients exhibiting symptoms such as headache or nausea might be suffering from either hyperammonemia or high PAA levels and that a fasting ammonia level equal to or below half the upper limit of normal would prompt the physician to check plasma PAA levels.

[00101] As stated above, the foregoing is merely intended to illustrate various embodiments of the present invention. The specific modifications discussed above are not to be construed as limitations on the scope of the invention. It will be apparent to one skilled in the art that various equivalents, changes, and modifications may be made without departing from the scope of the invention, and it is understood that such equivalent embodiments are to be included herein. All references cited herein are incorporated by reference as if fully set forth herein.

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What is claimed is:

1. A method for determining whether to increase a dosage of a nitrogen scavenging drug in a subject currently receiving the nitrogen scavenging drug, comprising:

a) measuring a fasting blood ammonia level for the subject; and

b) comparing the fasting blood ammonia level to the upper limit of normal for blood ammonia level to determine whether to increase the dosage of a nitrogen scavenging drug, wherein the dosage needs to be increased if the fasting blood ammonia level is greater than half the upper limit of normal for blood ammonia level.

2. A method for determining whether to administer a nitrogen scavenging drug to a subject having a nitrogen retention disorder comprising:

a) measuring a fasting blood ammonia level for the subject; and

b) comparing the fasting blood ammonia level to the upper limit of normal for blood ammonia level to determine whether to administer a nitrogen scavenging drug to the subject, wherein a nitrogen scavenging drug needs to be administered to the subject if the fasting blood ammonia level is greater than half the upper limit of normal for blood ammonia level.

3. A method of treating a subject with a nitrogen retention disorder who has previously been administered a nitrogen scavenging drug comprising:

a) measuring a fasting blood ammonia level for the subject; and

b) comparing the fasting blood ammonia level to the upper limit of normal for blood ammonia level and administering an increased dosage of the nitrogen scavenging drug if the fasting blood ammonia level is greater than half the upper limit of normal for blood ammonia level.

4. The method of claim 1, further comprising:

c) administering an increased dosage of the nitrogen scavenging drug if the need exists.

5. The method of any of claims 1-3, wherein the nitrogen retention disorder is selected from the group consisting of a urea cycle disorder and hepatic encephalopathy.

6. The method of any of claims 1-3, wherein the nitrogen scavenging drug is a PAA prodrug.

7. The method of claim 6, wherein the PAA prodrug is selected from the group consisting of glyceryl tri-[4-phenylbutyrate] (HPN-100), phenylbutyric acid (PBA), sodium PBA (NaPBA), and a combination of two or more of HPN-100, PBA, and NaPBA.

8. The method of any of claims 1-3, wherein the nitrogen scavenging drug is sodium benzoate.

9. The method of claim 3 or 4, wherein administering an increased dosage of the nitrogen scavenging drug produces a normal average daily ammonia level in the subject.

10. The method of any of claims 1-3, further comprising the step of determining an upper limit of normal for blood ammonia level for the subject prior to step (b).

11. The method of any of claims 1-3, wherein the upper limit of normal blood ammonia level is 35 μmol/L.

12. The method of claim 6, further comprising:

c) measuring urinary PAGN excretion; and

e) determining an effective dosage of the PAA prodrug based on a mean conversion of PAA prodrug to urinary PAGN of 60-75%.

Figure 1

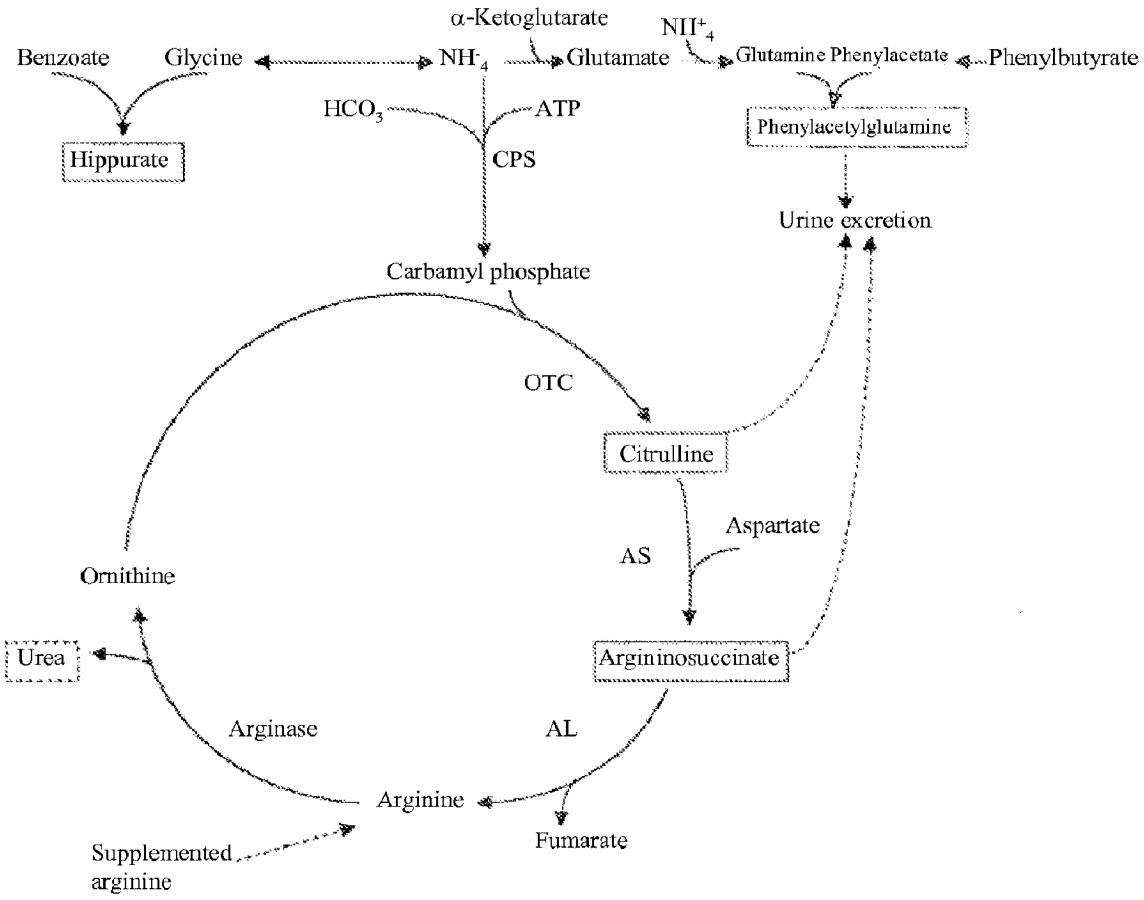


Figure 2

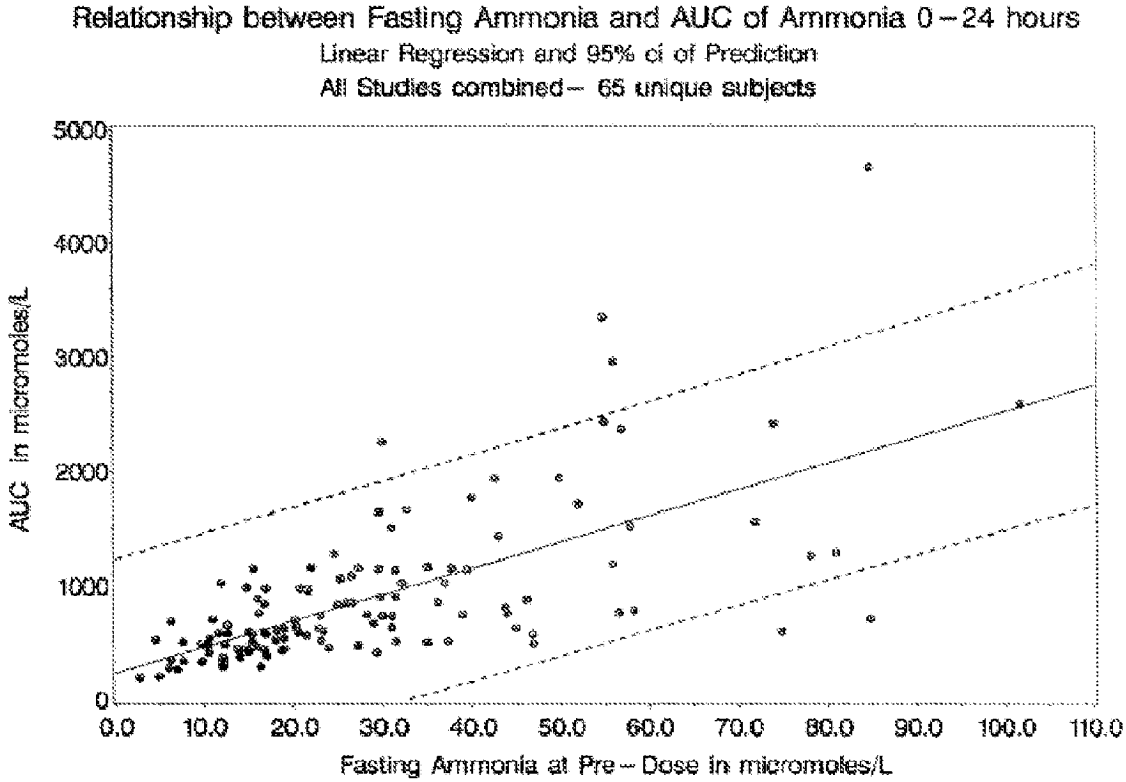
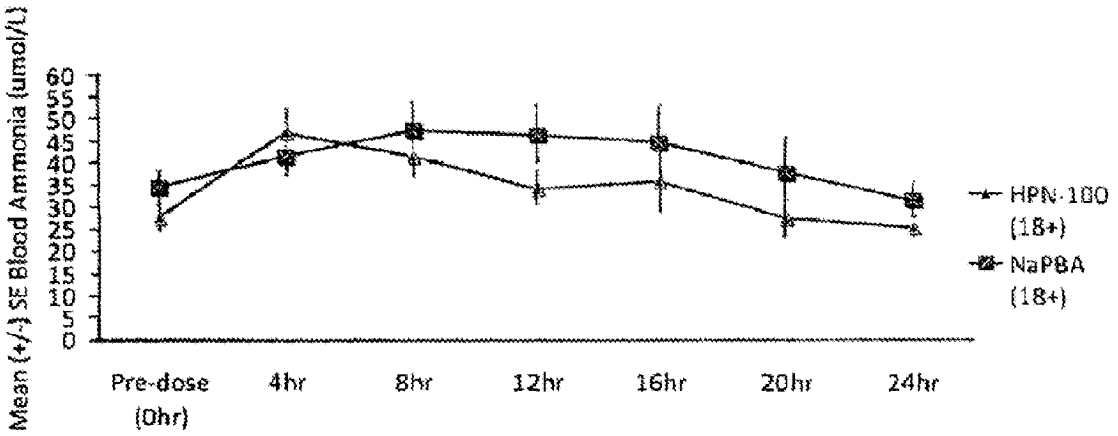
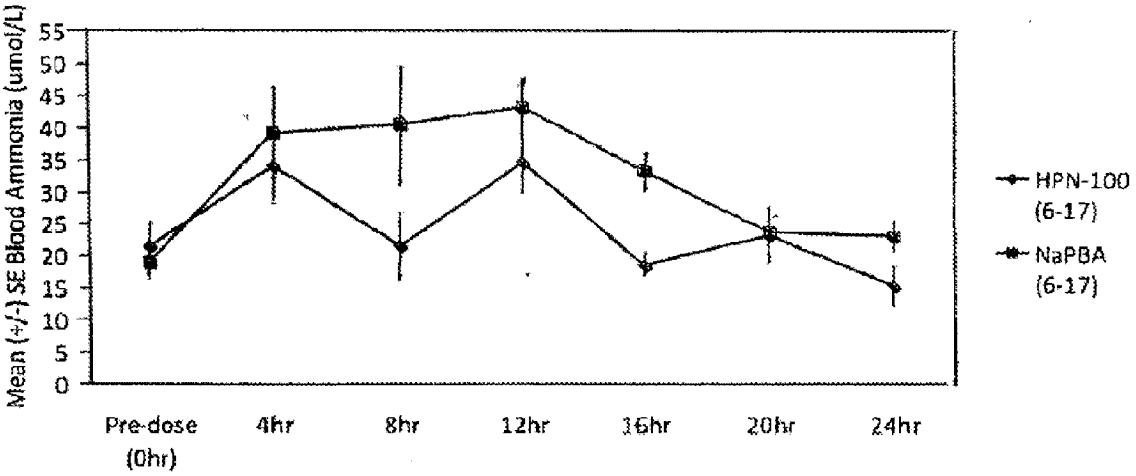


Figure 3

A.



B.



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(54) **Title**: METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS

(57) **Abstract**: The present disclosure provides methods for adjusting the dosage of PAA prodrugs (e.g., HPN-100, PBA) based on measurement of PAA and PAGN in plasma and calculating the PAA:PAGN ratio so as to determine whether PAA to PAGN conversion is saturated.

METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS

RELATED APPLICATIONS

[0001] The present application claims priority to U.S. Provisional Application No. 61/636,256, filed April 20, 2012, the disclosure of which is incorporated by reference herein in its entirety, including drawings.

BACKGROUND

[0002] Nitrogen retention disorders associated with elevated ammonia levels include urea cycle disorders (UCDs), hepatic encephalopathy (HE), and advanced kidney disease or kidney failure, often referred to as end-stage renal disease (ESRD).

[0003] UCDs include several inherited deficiencies of enzymes or transporters necessary for the synthesis of urea from ammonia, including enzymes involved in the urea cycle. The urea cycle is depicted in Figure 1, which also illustrates how certain ammonia-scavenging drugs act to assist in elimination of excessive ammonia. With reference to Figure 1, N-acetyl glutamine synthetase (NAGS)-derived *N*-acetylglutamate binds to carbamyl phosphate synthetase (CPS), which activates CPS and results in the conversion of ammonia and bicarbonate to carbamyl phosphate. In turn, carbamyl phosphate reacts with ornithine to produce citrulline in a reaction mediated by ornithine transcarbamylase (OTC). A second molecule of waste nitrogen is incorporated into the urea cycle in the next reaction, mediated by arginosuccinate synthetase (ASS), in which citrulline is condensed with aspartic acid to form argininosuccinic acid. Argininosuccinic acid is cleaved by argininosuccinic lyase (ASL) to produce arginine and fumarate. In the final reaction of the urea cycle, arginase (ARG) cleaves arginine to produce ornithine and urea. Of the two atoms of nitrogen incorporated into urea, one originates from free ammonia (NH_4^+) and the other from aspartate. UCD individuals born with no meaningful residual urea synthetic capacity typically present in the first few days of life (neonatal presentation). Individuals with residual function typically present later in childhood or even in adulthood, and symptoms may be precipitated by increased dietary protein or physiological stress (e.g., intercurrent illness). For UCD patients, lowering blood ammonia is the cornerstone of treatment.

[0004] HE refers to a spectrum of neurologic signs and symptoms believed to result from hyperammonemia, which frequently occur in subjects with cirrhosis or certain other types of liver disease. HE is a common manifestation of clinically decompensated liver disease and most

commonly results from liver cirrhosis with diverse etiologies that include excessive alcohol use, hepatitis B or C virus infection, autoimmune liver disease, or chronic cholestatic disorders such as primary biliary cirrhosis. Patients with HE typically show altered mental status ranging from subtle changes to coma, features similar to patients with UCDs. It is believed that an increase in blood ammonia due to dysfunctional liver in detoxifying dietary protein is the main pathophysiology associated with HE (Ong 2003).

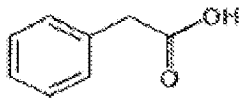
[0005] ESRD results from a variety of causes including diabetes, hypertension, and hereditary disorders. ESRD is manifested by accumulation in the bloodstream of substances normally excreted in the urine, including but not limited to urea and creatinine. This accumulation in the bloodstream of substances, including toxins, normally excreted in the urine is generally believed to result in the clinical manifestations of ESRD, sometimes referred to also as uremia or uremic syndrome. ESRD is ordinarily treated by dialysis or kidney transplantation. To the extent that urea, per se, contributes to these manifestations and that administration of a phenylacetic (PAA) prodrug may decrease synthesis of urea (see, e.g., Brusilow 1993) and hence lower blood urea concentration, PAA prodrug administration may be beneficial for patients with ESRD.

[0006] Subjects with nitrogen retention disorders whose ammonia levels and/or symptoms are not adequately controlled by dietary restriction of protein and/or dietary supplements are generally treated with nitrogen scavenging agents such as sodium phenylbutyrate (NaPBA, approved in the United States as BUPHENYL® and in Europe as AMMONAPS®), sodium benzoate, or a combination of sodium phenylacetate and sodium benzoate (AMMONUL®). These are often referred to as alternate pathway drugs because they provide the body with an alternate pathway to urea for excretion of waste nitrogen (Brusilow 1980; Brusilow 1991). NaPBA is a PAA prodrug. Another nitrogen scavenging drug currently in development for the treatment of nitrogen retention disorders is glyceryl tri-[4-phenylbutyrate] (HPN-100), which is described in U.S. Patent No. 5,968,979. HPN-100, which is commonly referred to as GT4P or glycerol PBA, is a prodrug of PBA and a pre-prodrug of PAA. The difference between HPN-100 and NaPBA with respect to metabolism is that HPN-100 is a triglyceride and requires digestion, presumably by pancreatic lipases, to release PBA (McGuire 2010), while NaPBA is a salt and is readily hydrolyzed after absorption to release PBA.

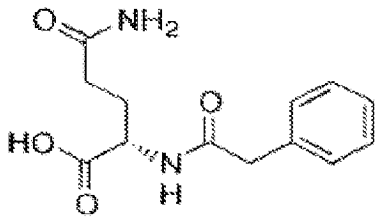
[0007] HPN-100 and NaPBA share the same general mechanism of action: PBA is converted to PAA via beta oxidation, and PAA is conjugated enzymatically with glutamine to

form phenylacetylglutamine (PAGN), which is excreted in the urine. The structures of PBA, PAA, and PAGN are set forth below:

phenylbutyrate



Phenylacetic acid



Phenylacetylglutamine

[0008] The clinical benefit of NaPBA and HPN-100 with regard to nitrogen retention disorders derives from the ability of PAGN to effectively replace urea as a vehicle for waste nitrogen excretion and/or to reduce the need for urea synthesis (Brusilow 1991; Brusilow 1993). Because each glutamine contains two molecules of nitrogen, the body rids itself of two waste nitrogen atoms for every molecule of PAGN excreted in the urine. Therefore, two equivalents of nitrogen are removed for each mole of PAA converted to PAGN. PAGN represents the predominant terminal metabolite, and one that is stoichiometrically related to waste nitrogen removal, a measure of efficacy in the case of nitrogen retention states.

[0009] In addition to nitrogen retention states, PAA prodrugs may be beneficial in a variety of other disorders for which PBA and/or PAA are believed to modify gene expression and/or exert post-translational effects on protein function. In the case of maple syrup urine disease (MSUD, also known as branched-chain ketoaciduria), for example, the apparently beneficial effect of NaPBA in lowering plasma levels of branched chain amino acids is reported to be mediated by PBA-induced inhibition of the kinase that regulates activity of branched chain alpha-keto acid dehydrogenase complex or BCKDC. BCKDC is the enzyme that normally breaks down branched-chain amino acids and is genetically defective in MSUD patients (Bruneti-Pieri 2011). Similarly, the putative beneficial effects of PAA prodrugs for the

treatment of cancer (Chung 2000), neurodegenerative diseases (Ryu 2005), and sickle cell disease (Perrine 2008) all involve alteration of gene expression and/or post-translational effects on protein function via PBA and/or PAA.

[0010] Numerous publications reports adverse events following administration of PBA and/or PAA (Mokhtarani 2012), and PAA is reported to cause reversible toxicity when present in high levels in circulation. While many of these publications have not recorded PAA blood levels and/or temporally correlated adverse events with PAA levels, toxicities such as nausea, headache, emesis, fatigue, weakness, lethargy, somnolence, dizziness, slurred speech, memory loss, confusion, and disorientation have been shown to be temporally associated with PAA levels ranging from 499–1285 μg/mL in cancer patients receiving PAA intravenously, and these toxicities have been shown to resolve with discontinuation of PAA administration (Thiebault 1994; Thiebault 1995). Therefore, when administering PAA prodrugs for treatment of nitrogen retention disorders and other conditions, it is important to optimize dosing so as to achieve the desired therapeutic effect while minimizing the risk of PAA associated toxicity.

SUMMARY

[0011] Provided herein is a clinically practical approach for utilizing and interpreting blood levels of PAA and PAGN to adjust the dose of a PAA prodrug in order to minimize the risk of toxicities and maximize drug effectiveness.

[0012] Provided herein in certain embodiments are methods of treating a nitrogen retention disorder or a condition for which PAA prodrug administration is expected to be beneficial in a subject comprising the steps of administering a first dosage of a PAA prodrug, measuring plasma PAA and PAGN levels, calculating a plasma PAA:PAGN ratio, and determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range. In certain embodiments, the target range is 1 to 2.5, 1 to 2, 1 to 1.5, 1.5 to 2, or 1.5 to 2.5. In certain embodiments, a PAA:PAGN ratio above the target range indicates that the dosage of the PAA prodrug needs to be decreased. In other embodiments, a PAA:PAGN ratio above the target range indicates that the dosage may need to be decreased, with the final determination of whether to decrease the dosage taking into account other characteristics of the subject such as biochemical profile or clinical characteristics such as target nitrogen excretion, actual nitrogen excretion, symptom severity, disorder duration, age, or overall health. In certain embodiments, a PAA:PAGN ratio below the target range indicates that the dosage of the PAA prodrug needs to be increased. In other embodiments, a PAA:PAGN

ratio below the target range indicates that the dosage may need to be increased, with the final determination of whether to increase the dosage taking into account other characteristics of the subject such as biochemical profile or clinical characteristics such as target nitrogen excretion, actual nitrogen excretion, symptom severity, disorder duration, age, or overall health. In certain embodiments, a PAA:PAGN ratio that is within the target range but within a particular subrange (e.g., 1 to 1.5 or 2 to 2.5 where the target range is 1 to 2.5) indicates that the dosage of the PAA prodrug does not need to be adjusted, but that the subject needs to be subjected to more frequent monitoring. In certain embodiments, the methods further comprise a step of administering an adjusted second dosage if such an adjustment is determined to be necessary based on the PAA:PAGN ratio and, optionally, other characteristics of the subject. In other embodiments, the methods further comprise a step of administering a second dosage that is the same as or nearly the same as the first dosage if no adjustment in dosage is deemed to be necessary. In certain embodiments, the nitrogen retention disorder is UCD, HE, or ESRD. In certain embodiments, the condition for which PAA prodrug administration is expected to be beneficial is cancer, a neurodegenerative diseases, a metabolic disorder, or sickle cell disease. In certain embodiments, the PAA prodrug is HPN-100 or NaPBA. In certain embodiments, measurement of plasma PAA and PAGN levels takes place after the first dosage of the PAA prodrug has had sufficient time to reach steady state, such as at 48 hours to 1 week after administration.

[0013] Provided herein in certain embodiments are methods of treating a nitrogen retention disorder or a condition for which PAA prodrug administration is expected to be beneficial in a subject who has previously received a first dosage of PAA prodrug comprising the steps of measuring plasma PAA and PAGN levels, calculating a plasma PAA:PAGN ratio, and determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range. In certain embodiments, the target range is 1 to 2.5, 1 to 2, 1 to 1.5, 1.5 to 2, or 1.5 to 2.5. In certain embodiments, a PAA:PAGN ratio above the target range indicates that the dosage of the PAA prodrug needs to be decreased. In other embodiments, a PAA:PAGN ratio above the target range indicates that the dosage may need to be decreased, with the final determination of whether to decrease the dosage taking into account other characteristics of the subject such as biochemical profile or clinical characteristics such as target nitrogen excretion, actual nitrogen excretion, symptom severity, disorder duration, age, or overall health. In certain embodiments, a PAA:PAGN ratio below the target range indicates that the dosage of the PAA prodrug needs to be increased. In other embodiments, a PAA:PAGN

ratio below the target range indicates that the dosage may need to be increased, with the final determination of whether to increase the dosage taking into account other characteristics of the subject such as biochemical profile or clinical characteristics such as target nitrogen excretion, actual nitrogen excretion, symptom severity, disorder duration, age, or overall health. In certain embodiments, a PAA:PAGN ratio that is within the target range but within a particular subrange (e.g., 1 to 1.5 or 2 to 2.5 where the target range is 1 to 2.5) indicates that the dosage of the PAA prodrug does not need to be adjusted, but that the subject needs to be subjected to more frequent monitoring. In certain embodiments, the methods further comprise a step of administering an adjusted second dosage if such an adjustment is determined to be necessary based on the PAA:PAGN ratio and, optionally, other characteristics of the subject. In other embodiments, the methods further comprise a step of administering a second dosage that is the same as or nearly the same as the first dosage if no adjustment in dosage is deemed to be necessary. In certain embodiments, the nitrogen retention disorder is UCD, HE, or ESRD. In certain embodiments, the condition for which PAA prodrug administration is expected to be beneficial is cancer, a neurodegenerative diseases, a metabolic disorder, or sickle cell disease. In certain embodiments, measurement of plasma PAA and PAGN levels takes place after the first dosage of the PAA prodrug has had sufficient time to reach steady state, such as at 48 hours to 1 week after administration.

[0014] Provided herein in certain embodiments are methods of adjusting the dosage of a PAA prodrug to be administered to a subject comprising the steps of administering a first dosage of a PAA prodrug, measuring plasma PAA and PAGN levels, calculating a plasma PAA:PAGN ratio, and determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range. In certain embodiments, the target range is 1 to 2.5, 1 to 2, 1 to 1.5, 1.5 to 2, or 1.5 to 2.5. In certain embodiments, a PAA:PAGN ratio above the target range indicates that the dosage of the PAA prodrug needs to be decreased. In other embodiments, a PAA:PAGN ratio above the target range indicates that the dosage may need to be decreased, with the final determination of whether to decrease the dosage taking into account other characteristics of the subject such as biochemical profile or clinical characteristics such as target nitrogen excretion, actual nitrogen excretion, symptom severity, disorder duration, age, or overall health. In certain embodiments, a PAA:PAGN ratio below the target range indicates that the dosage of the PAA prodrug needs to be increased. In other embodiments, a PAA:PAGN ratio below the target range indicates that the dosage may need to be increased, with the final

determination of whether to increase the dosage taking into account other characteristics of the subject such as biochemical profile or clinical characteristics such as target nitrogen excretion, actual nitrogen excretion, symptom severity, disorder duration, age, or overall health. In certain embodiments, a PAA:PAGN ratio that is within the target range but within a particular subrange (e.g., 1 to 1.5 or 2 to 2.5 where the target range is 1 to 2.5) indicates that the dosage of the PAA prodrug does not need to be adjusted, but that the subject needs to be subjected to more frequent monitoring. In certain embodiments, the methods further comprise a step of administering an adjusted second dosage if such an adjustment is determined to be necessary based on the PAA:PAGN ratio and, optionally, other characteristics of the subject. In other embodiments, the methods further comprise a step of administering a second dosage that is the same as or nearly the same as the first dosage if no adjustment in dosage is deemed to be necessary. In certain embodiments, measurement of plasma PAA and PAGN levels takes place after the first dosage of the PAA prodrug has had sufficient time to reach steady state, such as at 48 hours to 1 week after administration.

[0015] Provided herein in certain embodiments are methods of determining whether a first dosage of a PAA prodrug can be safely administered to a subject comprising the steps of administering the first dosage of a PAA prodrug, measuring plasma PAA and PAGN levels, calculating a plasma PAA:PAGN ratio, and determining whether the first dosage can be safely administered based on whether the PAA:PAGN ratio falls above a target range. In certain embodiments, the target range is 1 to 2.5, 1 to 2, 1 to 1.5, 1.5 to 2, or 1.5 to 2.5. In certain embodiments, a PAA:PAGN ratio above the target range indicates that the first dosage is unsafe and needs to be decreased. In other embodiments, a PAA:PAGN ratio above the target range indicates that the first dosage is potentially unsafe and may need to be decreased, with the final determination of whether to decrease the dosage taking into account other characteristics of the subject such as biochemical profile or clinical characteristics such as target nitrogen excretion, actual nitrogen excretion, symptom severity, disorder duration, age, or overall health. In certain embodiments, a PAA:PAGN ratio that is within the target range but within a particular subrange (e.g., 2 to 2.5 where the target range is 1 to 2.5) indicates that the first dosage is likely safe, but that the subject needs to be subjected to more frequent monitoring. In certain embodiments, the methods further comprise a step of administering an adjusted second dosage if such an adjustment is determined to be necessary based on the PAA:PAGN ratio and, optionally, other characteristics of the subject. In certain embodiments, measurement of plasma PAA and PAGN

levels takes place after the first dosage of the PAA prodrug has had sufficient time to reach steady state, such as at 48 hours to 1 week after administration.

[0016] Provided herein in certain embodiments are methods of determining whether a first dosage of a PAA prodrug is likely to be effective for treating a nitrogen retention disorder or another disorder for which PAA prodrug administration is expected to be beneficial comprising the steps of administering the first dosage of a PAA prodrug, measuring plasma PAA and PAGN levels, calculating a plasma PAA:PAGN ratio, and determining whether the first dosage is likely to be effective based on whether the PAA:PAGN ratio falls below a target range. In certain embodiments, the target range is 1 to 2.5, 1 to 2, 1 to 1.5, 1.5 to 2, or 1.5 to 2.5. In certain embodiments, a PAA:PAGN ratio below the target range indicates that the first dosage is unlikely to be effective needs to be increased. In other embodiments, a PAA:PAGN ratio below the target range indicates that the first dosage is potentially ineffective and may need to be increased, with the final determination of whether to increase the dosage taking into account other characteristics of the subject such as biochemical profile or clinical characteristics such as target nitrogen excretion, actual nitrogen excretion, symptom severity, disorder duration, age, or overall health. In certain embodiments, a PAA:PAGN ratio that is within the target range but within a particular subrange (e.g., 1 to 1.5 where the target range is 1 to 2.5) indicates that the first dosage is likely effective, but that the subject needs to be subjected to more frequent monitoring. In certain embodiments, the methods further comprise a step of administering an adjusted second dosage if such an adjustment is determined to be necessary based on the PAA:PAGN ratio and, optionally, other characteristics of the subject. In certain embodiments, measurement of plasma PAA and PAGN levels takes place after the first dosage of the PAA prodrug has had sufficient time to reach steady state, such as at 48 hours to 1 week after administration.

[0017] In certain embodiments, methods are provided for optimizing the therapeutic efficacy of a PAA prodrug in a subject who has previously been adminsitered a first dosage of PAA prodrug comprising the steps of measuring plasma PAA and PAGN levels, calculating a plasma PAA:PAGN ratio, and determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range. In certain embodiments, the target range is 1 to 2.5, 1 to 2, 1 to 1.5, 1.5 to 2, or 1.5 to 2.5. In certain embodiments, a PAA:PAGN ratio above the target range indicates that the dosage of the PAA prodrug needs to be decreased. In other embodiments, a PAA:PAGN ratio above the target range indicates that the dosage may

need to be decreased, with the final determination of whether to decrease the dosage taking into account other characteristics of the subject such as biochemical profile or clinical characteristics such as target nitrogen excretion, actual nitrogen excretion, symptom severity, disorder duration, age, or overall health. In certain embodiments, a PAA:PAGN ratio below the target range indicates that the dosage of the PAA prodrug needs to be increased. In other embodiments, a PAA:PAGN ratio below the target range indicates that the dosage may need to be increased, with the final determination of whether to increase the dosage taking into account other characteristics of the subject such as biochemical profile or clinical characteristics such as target nitrogen excretion, actual nitrogen excretion, symptom severity, disorder duration, age, or overall health. In certain embodiments, a PAA:PAGN ratio that is within the target range but within a particular subrange (e.g., 1 to 1.5 or 2 to 2.5 where the target range is 1 to 2.5) indicates that the dosage of the PAA prodrug does not need to be adjusted, but that the subject needs to be subjected to more frequent monitoring. In certain embodiments, the methods further comprise a step of administering an adjusted second dosage if such an adjustment is determined to be necessary based on the PAA:PAGN ratio and, optionally, other characteristics of the subject. In other embodiments, the methods further comprise a step of administering a second dosage that is the same as or nearly the same as the first dosage if no adjustment in dosage is deemed to be necessary. In certain embodiments, measurement of plasma PAA and PAGN levels takes place after the first dosage of the PAA prodrug has had sufficient time to reach steady state, such as at 48 hours to 1 week after administration.

[0018] In certain embodiments, methods are provided for obtaining a plasma PAA:PAGN ratio within a target range in a subject comprising the steps of administering a first dosage of a PAA prodrug, measuring plasma PAA and PAGN levels, calculating a plasma PAA:PAGN ratio, and determining whether the PAA:PAGN ratio falls within the target range. If the PAA:PAGN ratio does not fall within the target range, an adjusted second dosage is administered, and these steps are repeated until a plasma PAA:PAGN ratio falling within the target range is achieved. In certain embodiments, the target range is 1 to 2.5, 1 to 2, 1 to 1.5, 1.5 to 2, or 1.5 to 2.5. In certain embodiments, a PAA:PAGN ratio above the target range indicates that the dosage of the PAA prodrug needs to be decreased and a PAA:PAGN ratio below the target range indicates that the dosage of the PAA prodrug needs to be increased. In certain embodiments, measurement of plasma PAA and PAGN levels takes place after the first dosage

of the PAA prodrug has had sufficient time to reach steady state, such as at 48 hours to 1 week after administration.

BRIEF DESCRIPTION OF DRAWINGS

[0019] Figure 1: Urea cycle.

[0020] Figure 2: Plasma PAA levels versus plasma PAA:PAGN ratio in (A) all subjects combined (healthy adults, patients age 2 months and above with UCDs, and patients with cirrhosis), (B) patients age 2 months and above with UCDs, and (C) patients with cirrhosis.

[0021] Figure 3: Estimated probability (95% confidence interval (c.i.)) of correctly detecting elevated plasma PAA:PAGN ratio (≥2.0) with a single blood sample at a designated time.

[0022] Figure 4:Distribution of plasma PAA:PAGN ratio (log scale) by time since dosing (hours) and category of maximum PAA:PAGN ratio in all subjects combined.

[0023] Figure 5: Distribution of plasma PAA concentrations (μg/mL) by PAA:PAGN ratio for (A) all subjects and (B) UCD and HE subjects.

DETAILED DESCRIPTION

[0024] The following description of the invention is merely intended to illustrate various embodiments of the invention. As such, the specific modifications discussed are not to be construed as limitations on the scope of the invention. It will be apparent to one skilled in the art that various equivalents, changes, and modifications may be made without departing from the scope of the invention, and it is understood that such equivalent embodiments are to be included herein.

[0025] The enzymes responsible for beta oxidation of PBA to PAA are present in most cell types capable of utilizing fatty acids as energy substrates, and the widespread distribution of these enzymes presumably accounts for the rapid and essentially complete conversion of PBA to PAA. However, the enzymes that conjugate PAA with glutamine to form PAGN are found primarily in the liver and to a lesser extend in kidneys (Moldave 1957). Therefore, the conversion of PAA to PAGN may be affected under several circumstances, including the following: a) if conjugation capacity is saturated (e.g., by high doses of PAA prodrug); b) if conjugation capacity is compromised (e.g., by severe hepatic and/or renal dysfunction); c) if the substrate (glutamine) for PAA to PAGN conjugation is rate limiting; d) genetically determined variability (i.e., polymorphisms) in the enzymes responsible for PAA to PAGN conversion, or e) in young children, since the capacity to convert PAA to PAGN varies with body size measured

as body surface area (Monteleone 2012). The presence of any one of these conditions may lead to accumulation of PAA in the body, which causes reversible toxicity.

[0026] The goal of PAA prodrug administration in subjects with nitrogen retention disorders is to provide a sufficient dosage to obtain a desired level of nitrogen removal while avoiding excess build-up of PAA. The goal of PAA prodrug administration in patients without a nitrogen retention disorder (e.g., a neurodegenerative disease) is to achieve circulating metabolite levels necessary to produce a clinical benefit by alteration of gene expression and/or protein folding or function. However, there are several difficulties associated with determining the proper dosage in patients with nitrogen retention disorders.

[0027] Plasma PAA and PAGN levels are affected by various factors, including timing of the blood draw in relation to drug administration, hepatic function, availability of metabolizing enzymes, and availability of substrates required for metabolism. A random PAA level drawn during an outpatient visit to determine if levels are in the toxicity range without considering concomitant PAGN level is insufficient to inform dosing. First, PAA levels vary many-fold over the course of the day, fluctuating a great deal between peak and trough levels. For example, in the Hyperion pivotal study evaluating HPN-100 for use in treating adult UCD (Study ID HPN-100-006, Clinical Trials ID NCT00992459), serial blood samples were obtained for PK studies over a 24 hour period during which subjects were receiving HPN-100 or NaPBA. The fluctuation index for PAA over a 24 hour period, which represents the fluctuation between maximum concentration (typically observed after the last daily dose or at approximately 12 hours) and minimum concentration (typically observed in the morning after overnight fasting or at 0 hours), indicated a very high degree of variability (2150% for NaPBA and 1368% for HPN-100). Therefore, a single plasma PAA level may not be representative of the highest PAA level a patient may experience during the day. Second, a high plasma PAA level may only be indicative of the high doses a subject is receiving rather than a point of concern if the subject is effectively conjugating PAA with glutamine to form PAGN. Therefore, basing dose adjustment on only on a high PAA level without considering concomitant plasma PAGN level may result in unnecessary dose reduction and under-treatment of the patient. Conversely, a PAA level seemingly below the levels associated with toxicity might be taken as an indication of satisfactory dosing without appreciating the fact that the concomitant PAGN level may not be proportional to PAA, indicating that PAA is not being efficiently utilized and may be accumulating.

[0028] Previous studies have shown that conversion of PAA to PAGN is a saturable process that varies considerably among individuals (see, e.g., Monteleone 2012), and that patients with hepatic impairment have higher PAA levels than patients without hepatic impairment (Ghabril et al., "Glycerol phenylbutyrate (GPD) administration in patients with cirrhosis and episodic hepatic encephalopathy (HE)," submitted to Digestive Disease Week, 2012). If PAGN formation is affected by any of the above factors, PAA will be accumulated and waste nitrogen may not be removed from the body. Previous studies have also shown that a small proportion of individuals, including both healthy adults ad patients with UCDs or HE, have higher PAA levels than the remainder of the population, presumably due to individual differences in conjugating PAA to PAGN, and that PAA levels fluctuate many-fold during the day depending on the dose and the timing of blood sample relative to the last dose so that a single plasma level may not be informative (Lee 2010; Lichter 2011).

[0029] Although the goal of PAA prodrug therapy for nitrogen retention disorders is to achieve ammonia levels within a normal limit, there is no correlation between plasma PAA levels and blood ammonia. Nitrogen retention disorder subjects are normally "dosed to effect," meaning that subjects with absent or severely deficient urea synthetic capacity require higher doses of PAA prodrugs than do mildly deficient UCD patients. These higher dosages are generally associated with higher PAA levels, such that the conventional PK/PD response (higher active moiety, i.e., PAA, correlates with lower harmful substance, i.e., ammonia) does not apply. Therefore, there is no single target plasma PAA level that can be applied to patients with UCDs or other nitrogen retention disorders based on their blood ammonia.

[0030] Patients with severe hepatic impairment are at increased risk of PAA accumulation due to inadequate levels of PAA conjugating enzymes if treated with PAA-prodrugs. UCD patients without hepatic impairment whose PAA conjugating enzymes are readily saturated are also at increased risk of PAA accumulation if treated with PAA-producing compounds. Other patients without nitrogen retention are at increased risk of PAA accumulation due to limited availability of glutamine as the substrate to form PAGN if treated with PAA-producing compounds, which accumulates in patients with nitrogen retention states.

[0031] WO09/134460 and WO10/025303 disclose methods for determining an effective dosage of a PAA prodrug based on urinary PAGN levels, which was found to be a more reliable indictor of effective dosage than plasma levels of PAA or other metabolites. Although such

measurements are highly useful for evaluating waste nitrogen removal, they do not provide complete information regarding a subject's ability to utilize the prodrug.

[0032] Since PAA, PAGN, and ammonia levels do not provide the information necessary to determine whether a subject is effectively converting PBA to PAGN (i.e., effectively utilizing the PAA prodrug), there is a need for improved methods of adjusting PAA prodrug dosage and incorporating such adjustments into methods of treating nitrogen retention disorders.

[0033] As disclosed herein, plasma PAA:PAGN ratio has been found to provide an unexpectedly accurate measure of PAA prodrug metabolism in subjects with nitrogen retention disorders and/or hepatic impairment. It was found that subjects who can readily convert PAA to PAGN and have not reached the saturation point with respect to PAA to PAGN conversion will have a plasma PAA:PAGN ratio of 2.5 or below (when both are measured in μg/mL), and that subjects with PAA:PAGN ratios above 2.5 have a significantly higher chance of experience a PAA level above 400 μg/mL or 500 μg/mL over a 24 hour period. A PAA/PAGN ratio of less than 2.5 was associated primarily with healthy adult or adolescent subjects and normal liver function, with subjects having a ratio below 2.5 exhibiting a 1% probability of experiencing a PAA level greater than 400 μg/mL and almost no chance of exhibiting a PAA level greater than 500 μg/mL at any point during a 24 hour period. A ratio greater than 2.5, on the other hand, was generally seen in subjects with moderate hepatic impairment, a subset of healthy subjects or UCD patients with relatively lower saturation point and difficulty conjugating PAA to form PAGN, and patients with a low body surface area. Subjects with a ratio greater than 2.5, on the other hand, exhibited a 20-36% likelihood of experiencing a PAA level greater than 400 μg/mL during the day, and an approximately 10% likelihood of experiencing a PAA level of 500 μg/L or greater. In subjects with a ratio greater than 3, the likelihood of experiencing a PAA level higher than 500 μg/mL increased to as high as 25%. These results show that a plasma PAA:PAGN ratio exceeding 2.5 in a patient with unexplained neurological adverse events and normal ammonia indicates that dosage adjustment should be considered. Thus, plasma PAA:PAGN ratio provides a clinically useful surrogate for evaluating the efficiency of PAA to PAGN conversion.

[0034] Plasma PAA:PAGN ratio indicates whether a PAA prodrug is being effectively utilized and scavenging nitrogen, and therefore provides an indirect and simple measure of saturation of conjugating enzymes, availability of substrate, and possible effect of hepatic or renal impairment on this process. Calculating this ratio will allow effective treatment and dose

adjustment in subjects with known hepatic impairment, subjects presenting with signs and symptoms overlapping between hyperammonemia and PAA toxicities, and subjects who are not clinically controlled despite increasing the dosage of drugs.

[0035] One of ordinary skill in the art would generally not consider the ratio of an active metabolite such as PAA to a terminal metabolite such as PAGN when making therapeutic decisions because they would expect that higher levels of the active metabolite would result in a proportionately higher response (as measured by PAGN production) and increased efficacy (i.e., waste nitrogen removal). However, the results provided herein show that the use of plasma PAA:PAGN ratios to evaluate and adjust PAA prodrug dosage is unexpectedly superior to the use of PAA or PAGN levels alone. Once a subject exceeds a specific PAA:PAGN ratio, there is a high likelihood that they are not effectively utilizing the active moiety and that further increasing PAA prodrug dosage may not increase efficacy and may actually result in PAA accumulation and toxicity.

[0036] Based on these findings, methods are provided herein for treating nitrogen retention disorders and evaluating and adjusting the dosage of a PAA prodrug based on plasma PAA:PAGN ratio. Generally, these methods comprise steps of measuring plasma PAA and PAGN levels, calculating the PAA:PAGN ratio, and determining whether the ratio falls within a target range, with this determination being used at least in part to decide whether to adjust PAA prodrug dosage. In these methods, PAA:PAGN ratio can be used to ensure that urinary PAGN output, plasma ammonia concentration, and/or PAA levels fall within a predefined target range. Such methods represent an improvement over previously developed methods for evaluating PAA prodrug dosage and efficacy in that they allow for more accurate dosing, greater efficacy, and decreased risk of toxicity associated with PAA accumulation.

[0037] Disclosed herein are target ranges for the ratio of plasma PAA to PAGN in subjects who are receiving PAA prodrug therapy. In certain embodiments, a subject exhibiting a PAA:PAGN ratio falling within a target range is classified as properly dosed, meaning that they do not require a PAA prodrug dosage adjustment, while a subject exhibiting a PAA:PAGN ratio falling outside the target range is classified as improperly dosed, meaning that they require an adjustment in PAA prodrug dosage. In certain of these embodiments, a subject exhibiting a plasma PAA:PAGN ratio falling above a target range is classified as requiring a decreased dosage of PAA prodrug, while a subject exhibiting a plasma PAA:PAGN ratio falling below a target range is classified as requiring an increased dosage of PAA prodrug. In other

embodiments, a subject exhibiting a plasma PAA:PAGN ratio falling above a target range is classified as requiring a decreased dosage of PAA prodrug, while a subject exhibiting a plasma PAA:PAGN ratio falling below a target range is classified as potentially requiring an increase in PAA prodrug dosage. In still other embodiments, a subject exhibiting a plasma PAA:PAGN ratio falling above a target range is classified as potentially requiring a decreased dosage of PAA prodrug, while a subject exhibiting a plasma PAA:PAGN ratio falling below a target range is classified as potentially requiring an increase in PAA prodrug dosage. In those embodiments where a subject is classified as potentially requiring an increase or decrease in PAA prodrug dosage based on their PAA:PAGN ratio, a decision as to whether to increase or decrease dosage may be based on one or more additional characteristics of the subject such as biochemical profile or clinical characteristics such as target nitrogen excretion, actual nitrogen excretion, symptom severity, disorder duration, age, or overall health.

[0038] In certain embodiments, the target range for plasma PAA:PAGN ratio is 1 to 2.5, meaning that a subject exhibiting a PAA:PAGN falling within this range is classified as properly dosed. In other embodiments, the target range for plasma PAA:PAGN ratio is 1 to 2, 1 to 1.5, 1.5 to 2, or 1.5 to 2.5. In certain of those embodiments where the target range is 1 to 2.5, a subject with a PAA:PAGN ratio above 2.5 is classified as requiring a decrease in PAA prodrug dosage, while a subject with a PAA:PAGN ratio falling below 1 is classified as potentially requiring an increase in PAA prodrug dosage. In certain of these embodiments, a subject is necessarily classified as requiring an increase in PAA prodrug dosage if their ratio is below 1. In other embodiments, a subject with a PAA:PAGN ratio of less than 1 is only classified as requiring an increase in PAA prodrug dosage if one or more additional clinical or biochemical characteristics are satisfied (e.g., the subject is exhibiting severe symptoms of a nitrogen retention disorder).

[0039] In certain embodiments, the target range for plasma PAA:PAGN ratio may comprise one or more subranges, with subjects falling within different subranges being treated differently despite falling within the target range. For example, where a target range is 1 to 2.5, a subject exhibiting a PAA:PAGN ratio below 1 or above 2.5 may be classified as requiring an adjustment in PAA prodrug dosage. Within the target range, subjects with a PAA:PAGN ratio falling within a particular subrange may be treated as properly dosed, improperly dosed (i.e., requiring a dosage adjustment), or properly dosed but requiring more frequent monitoring. For example,

subjects having a PAA:PAGN ratio greater than 2 but not greater than 2.5 may be classified as properly dosed but requiring more frequent monitoring.

[0040] In certain embodiments, subrange boundaries or the treatment of subjects falling within a particular subrange will depend in part on a subject's specific characteristics, including for example biochemical profile or clinical characteristics such as target nitrogen excretion, actual nitrogen excretion, symptom severity, disorder duration, age, or overall health. For example, in certain embodiments a first subject with a PAA:PAGN ratio falling within the subrange of 2 to 2.5 may be classified as properly dosed but requiring frequent monitoring, while a second subject falling within the same subrange may be classified as requiring a decreased dosage of PAA prodrug. Similarly, a first subject with a PAA:PAGN ratio falling within the subrange of 1 to 1.5 may be classified as properly dosed but requiring frequent monitoring, while a second subject falling within the same subrange may be classified as requiring an increased dosage of PAA prodrug. For example, a subject who has recently exhibited particularly acute symptoms associated with a particular disorder may be classified as requiring an increased dosage of PAA prodrug when exhibiting a PAA:PAGN ratio of 1 to 1.5, while a subject who is clinically controlled may be classified as properly dosed despite a ratio falling within the same subrange.

[0041] In certain embodiments, methods are provided herein for treating a nitrogen retention disorder or a condition for which PAA prodrug administration is expected to be beneficial in a subject that has previously received a first dosage of a PAA prodrug. These methods comprise measuring plasma PAA and PAGN levels, calculating the plasma PAA:PAGN ratio, determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, and administering a second dosage of the PAA prodrug. In certain embodiments, the target range for PAA:PAGN ratio is 1 to 2.5 or 1 to 2. In certain of these embodiments, the second dosage is greater than the first dosage if the PAA:PAGN ratio is less than 1 (i.e., the dosage is increased) and less than the first dosage if the PAA:PAGN ratio is greater than 2.5 (i.e., the dosage is decreased). In other embodiments, the second dosage may or may not be greater than the first dosage if the PAA:PAGN ratio is less than 1, depending on one or more other characteristics of the subject. In certain embodiments, the second dosage is equal to the first dosage when the PAA:PAGN ratio is 1 to 2.5, i.e., falling within the target range. In certain embodiments, the target range is divided into one or more subranges. In certain of these embodiments, the second dosage may be equal to the first dosage if the PAA:PAGN ratio is 1 to

1.5 or 2 to 2.5, but the subject may be subjected to more frequent monitoring. In certain other embodiments, the second dosage may be greater than the first dosage if the PAA:PAGN ratio is 1 to 1.5 or 1 to 2 and the subject has recently exhibited particularly acute symptoms of a nitrogen retention disorder or another condition for which PAA prodrug administration is expected to be beneficial. Similarly, the second dosage may be less than the first dosage if the PAA:PAGN ratio is greater than 1.5 or 2 but not greater than 2.5, depending on the subject's specific characteristics. In certain embodiments, the increase or decrease in the second dosage versus the first dosage depends on the precise plasma PAA:PAGN ratio. For example, where the plasma PAA:PAGN ratio is only slightly less than 1, the dosage may be increased only slightly, but where the PAA:PAGN ratio is significantly less than 1, the dosage may be increased more. Similarly, the decrease in dosage for subjects exhibiting a ratio above 2.5 may vary depending on how far above 2.5 the ratio extends. In certain embodiments, measurement of plasma PAA and PAGN ratio takes place after the PAA prodrug has had sufficient time to reach steady state (e.g., 48 hours, 48 to 72 hours, 72 hours to 1 week, 1 week to 2 weeks, or greater than 2 weeks after PAA prodrug administration). In certain embodiments, the above steps may be repeated until a desired plasma PAA:PAGN ratio (e.g., 1 to 2.5 or 1 to 2) is achieved. For example, the methods may comprise measuring plasma PAA and PAGN levels after administration of the second dosage, calculating the plasma PAA:PAGN ratio, determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within the target range, and administering a third dosage of the PAA prodrug.

[0042] In certain embodiments, methods are provided for treating a nitrogen retention disorder or a condition for which PAA prodrug administration is expected to be beneficial in a subject that has not previously been administered a PAA prodrug. These methods comprise administering a first dosage of a PAA prodrug, measuring plasma PAA and PAGN levels, calculating the plasma PAA:PAGN ratio, determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, and administering a second dosage of the PAA prodrug. In certain embodiments, the target range for PAA:PAGN ratio is 1 to 2.5 or 1 to 2. In certain of these embodiments, the second dosage is greater than the first dosage if the PAA:PAGN ratio is less than 1 (i.e., the dosage is increased) and less than the first dosage if the PAA:PAGN ratio is greater than 2.5 (i.e., the dosage is decreased). In other embodiments, the second dosage may or may not be greater than the first dosage if the PAA:PAGN ratio is less than 1, depending on one or more additional characteristics of the

subject. In certain embodiments, the second dosage is equal to the first dosage when the PAA:PAGN ratio is 1 to 2.5, i.e., falling within the target range. In certain embodiments, the target range is divided into one or more subranges. In certain of these embodiments, the second dosage may be equal to the first dosage if the PAA:PAGN ratio is 1 to 1.5 or 2 to 2.5, but the subject may be subjected to more frequent monitoring. In certain other embodiments, the second dosage may be greater than the first dosage if the PAA:PAGN ratio is 1 to 1.5 or 1 to 2 and the subject has recently exhibited particularly acute symptoms of a nitrogen retention disorder or another condition for which PAA prodrug administration is expected to be beneficial. Similarly, the second dosage may be less than the first dosage if the PAA:PAGN ratio is greater than 1.5 or 2 but not greater than 2.5, depending on the subject's specific clinical or biochemical characteristics. In certain embodiments, the increase or decrease in the second dosage versus the first dosage depends on the precise plasma PAA:PAGN ratio. For example, where the plasma PAA:PAGN ratio is only slightly less than 1, the dosage may be increased only slightly, but where the PAA:PAGN ratio is significantly less than 1, the dosage may be increased more. Similarly, the decrease in dosage for subjects exhibiting a ratio above 2.5 may vary depending on how far above 2.5 the ratio extends. In certain embodiments, measurement of plasma PAA and PAGN ratio takes place after the PAA prodrug has had sufficient time to reach steady state (e.g., 48 hours, 48 to 72 hours, 72 hours to 1 week, 1 week to 2 weeks, or greater than 2 weeks after PAA prodrug administration). In certain embodiments, the above steps may be repeated until a desired plasma PAA:PAGN ratio (e.g., 1 to 2.5 or 1 to 2) is achieved. For example, the methods may comprise measuring plasma PAA and PAGN levels after administration of the second dosage, calculating the plasma PAA:PAGN ratio, determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within the target range, and administering a third dosage of the PAA prodrug.

[0043] A method of administering a PAA prodrug to a subject with a nitrogen retention disorder or another condition for which PAA prodrug administration is expected to be beneficial. These methods comprise administering a first dosage of the PAA prodrug, measuring plasma PAA and PAGN levels, calculating the plasma PAA:PAGN ratio, determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, and administering a second dosage of the PAA prodrug. In certain embodiments, the target range for PAA:PAGN ratio is 1 to 2.5 or 1 to 2. In certain of these embodiments, the second dosage is greater than the first dosage if the PAA:PAGN ratio is less than 1 (i.e., the

dosage is increased) and less than the first dosage if the PAA:PAGN ratio is greater than 2.5 (i.e., the dosage is decreased). In other embodiments, the second dosage may or may not be greater than the first dosage if the PAA:PAGN ratio is less than 1, depending on one or more additional characteristics of the subject. In certain embodiments, the second dosage is equal to the first dosage when the PAA:PAGN ratio is 1 to 2.5, i.e., falling within the target range. In certain embodiments, the target range is divided into one or more subranges. In certain of these embodiments, the second dosage may be equal to the first dosage if the PAA:PAGN ratio is 1 to 1.5 or 2 to 2.5, but the subject may be subjected to more frequent monitoring. In certain other embodiments, the second dosage may be greater than the first dosage if the PAA:PAGN ratio is 1 to 1.5 or 1 to 2 and the subject has recently exhibited particularly acute symptoms of a nitrogen retention disorder or another condition for which PAA prodrug administration is expected to be beneficial. Similarly, the second dosage may be less than the first dosage if the PAA:PAGN ratio is greater than 1.5 or 2 but not greater than 2.5, depending on the subject's specific biochemical or clinical characteristics. In certain embodiments, the increase or decrease in the second dosage versus the first dosage depends on the precise plasma PAA:PAGN ratio. For example, where the plasma PAA:PAGN ratio is only slightly less than 1, the dosage may be increased only slightly, but where the PAA:PAGN ratio is significantly less than 1, the dosage may be increased more. Similarly, the decrease in dosage for subjects exhibiting a ratio above 2.5 may vary depending on how far above 2.5 the ratio extends. In certain embodiments, measurement of plasma PAA and PAGN ratio takes place after the PAA prodrug has had sufficient time to reach steady state (e.g., 48 hours, 48 to 72 hours, 72 hours to 1 week, 1 week to 2 weeks, or greater than 2 weeks after PAA prodrug administration). In certain embodiments, the above steps may be repeated until a desired plasma PAA:PAGN ratio (e.g., 1 to 2.5 or 1 to 2) is achieved. For example, the methods may comprise measuring plasma PAA and PAGN levels after administration of the second dosage, calculating the plasma PAA:PAGN ratio, determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within the target range, and administering a third dosage of the PAA prodrug.

[0044] In certain embodiments, methods are provided herein for achieving a target plasma PAA:PAGN ratio in a subject with a nitrogen retention disorder or another condition for which PAA prodrug administration is expected to be beneficial. These methods comprise administering a first dosage of a PAA prodrug, measuring plasma PAA and PAGN levels, calculating the plasma PAA:PAGN ratio, determining whether the PAA prodrug dosage needs to

be adjusted based on whether the PAA:PAGN ratio falls within a target range, and administering a second dosage of the PAA prodrug based on the PAA:PAGN ratio. If the PAA:PAGN ratio is above the target range, the second dosage is less than the first dosage. If the PAA:PAGN ratio is below the target range, the second dosage is greater than the first dosage. These steps are repeated until a target plasma PAA:PAGN ratio is achieved. In certain embodiments, the target ratio falls within a target range of 1 to 2.5 or 1 to 2. In certain embodiments, the increase or decrease in the second dosage versus the first dosage depends on the precise plasma PAA:PAGN ratio. For example, where the plasma PAA:PAGN ratio is only slightly less than 1, the dosage may be increased only slightly, but where the PAA:PAGN ratio is significantly less than 1, the dosage may be increased more. Similarly, the decrease in dosage for subjects exhibiting a ratio above 2.5 may vary depending on how far above 2.5 the ratio extends. In certain embodiments, measurement of plasma PAA and PAGN ratio takes place after the PAA prodrug has had sufficient time to reach steady state (e.g., 48 hours, 48 to 72 hours, 72 hours to 1 week, 1 week to 2 weeks, or greater than 2 weeks after PAA prodrug administration).

[0045] In certain embodiments, methods are provided for evaluating the dosage of a PAA prodrug in a subject who has previously been administered a first dosage of a PAA prodrug. These methods comprise measuring plasma PAA and PAGN levels, calculating the plasma PAA:PAGN ratio, and determining whether the first dosage of the PAA prodrug is effective based on whether the PAA:PAGN ratio falls within a target range. In certain embodiments, the target range for PAA:PAGN ratio is 1 to 2.5 or 1 to 2. In certain of these embodiments, the first dosage is considered too low if the PAA:PAGN ratio is less than 1, and too high if the PAA:PAGN ratio is greater than 2.5. In other embodiments, the first dosage is considered potentially too low if PAA:PAGN ratio is less than 1, with a final decision depending on one or more additional characteristics of the subject. In certain embodiments, the target range is divided into one or more subranges. In certain of these embodiments, the first dosage is considered potentially effective if the PAA:PAGN ratio is 1 to 1.5 or 2 to 2.5, but the subject may be subjected to more frequent monitoring. In certain other embodiments, the first dosage may be considered too low if the PAA:PAGN ratio is 1 to 1.5 or 1 to 2 and the subject has recently exhibited particularly acute symptoms of a nitrogen retention disorder or another condition for which PAA prodrug administration is expected to be beneficial. Similarly, in certain embodiments the first dosage may be considered too high if the PAA:PAGN ratio is greater than 1.5 or 2 but not greater than 2.5, depending on the subject's specific biochemical or

clinical characteristics. In certain embodiments, measurement of plasma PAA and PAGN ratio takes place after the PAA prodrug has had sufficient time to reach steady state (e.g., 48 hours, 48 to 72 hours, 72 hours to 1 week, 1 week to 2 weeks, or greater than 2 weeks after PAA prodrug administration). In certain embodiments, the methods further comprise a step of administering a second dosage that differs from the first dosage, and in certain of these embodiments the above steps may be repeated until a desired plasma PAA:PAGN ratio (e.g., 1 to 2.5 or 1 to 2) is achieved. For example, the methods may comprise administering a second dosage that differs from the first dosage, measuring plasma PAA and PAGN levels after administration of the second dosage, calculating the plasma PAA:PAGN ratio, and determining whether the second dosage of the PAA prodrug is effective based on whether the PAA:PAGN ratio falls within a target range.

[0046] In certain embodiments, methods are provided for adjusting the dosage of a PAA prodrug in a subject who has previously been administered a first dosage of a PAA prodrug. These methods comprise measuring plasma PAA and PAGN levels, calculating the plasma PAA:PAGN ratio, and determining whether to adjust the dosage of the PAA prodrug based on whether the PAA:PAGN ratio falls within a target range. In certain embodiments, the target range for PAA:PAGN ratio is 1 to 2.5 or 1 to 2. In certain of these embodiments where the target range is 1 to 2.5, a PAA:PAGN ratio of less than 1 indicates the PAA prodrug dosage needs to be adjusted upwards, while a PAA:PAGN ratio above 2.5 indicates the PAA prodrug dosage needs to be adjusted downwards. In other embodiments, a PAA:PAGN ratio of less than 1 indicates that the PAA prodrug dosage potentially needs to be adjusted upwards, with a final decision depending on one or more additional characteristics of the subject. In certain embodiments, the target range is divided into one or more subranges. In certain of these embodiments, a PAA:PAGN ratio of 1 to 1.5 or 2 to 2.5 indicates that the dosage need not be adjusted, but that the subject should be subjected to more frequent monitoring. In certain other embodiments, a PAA:PAGN ratio of 1 to 1.5 or 1 to 2 indicates that the dosage needs to be increased when the subject has recently exhibited particularly acute symptoms of a nitrogen retention disorder or another condition for which PAA prodrug administration is expected to be beneficial. Similarly, in certain embodiments a PAA:PAGN ratio greater than 1.5 or 2 but not greater than 2.5 may indicate that the dosage needs to be decreased, depending on the subject's specific biochemical or clinical characteristics. In certain embodiments, measurement of plasma PAA and PAGN ratio takes place after the PAA prodrug has had sufficient time to reach steady

state (e.g., 48 hours, 48 to 72 hours, 72 hours to 1 week, 1 week to 2 weeks, or greater than 2 weeks after PAA prodrug administration). In certain embodiments where a determination is made that the dosage needs to be adjusted, the methods further comprise a step of administering a second dosage that differs from the first dosage, and in certain of these embodiments the above steps may be repeated until a desired plasma PAA:PAGN ratio (e.g., 1 to 2.5 or 1 to 2) is achieved. For example, the methods may comprise administering a second dosage that differs from the first dosage, measuring plasma PAA and PAGN levels after administration of the second dosage, calculating the plasma PAA:PAGN ratio, and determining whether the second dosage of the PAA prodrug needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range. In certain embodiments, the increase or decrease in the second dosage versus the first dosage depends on the precise plasma PAA:PAGN ratio. For example, where the plasma PAA:PAGN ratio is only slightly less than 1, the dosage may be increased only slightly, but where the PAA:PAGN ratio is significantly less than 1, the dosage may be increased more. Similarly, the decrease in dosage for subjects exhibiting a ratio above 2.5 may vary depending on how far above 2.5 the ratio extends.

[0047] In certain embodiments, methods are provided for optimizing the therapeutic efficacy of a PAA prodrug for use in treating a nitrogen retention disorder in a subject. These methods comprise measuring plasma PAA and PAGN levels in a subject who has previously been administered a PAA prodrug, calculating the plasma PAA:PAGN ratio, determining whether to adjust the dosage of the PAA prodrug based on whether the PAA:PAGN ratio falls within a target range, and administering an adjusted dosage of the PAA prodrug as necessary. These steps are repeated until the subject exhibits a plasma PAA:PAGN ratio falling within the target range (e.g., 1 to 2.5 or 1 to 2). In certain embodiments where the target range is 1 to 2.5, a plasma PAA:PAGN ratio of less than 1 indicates that the dosage needs to be adjusted upwards, while a ratio greater than 2.5 indicates that the dosage needs to be decreased. In certain embodiments, the target range is divided into one or more subranges. In certain of these embodiments, a PAA:PAGN ratio of 1 to 1.5 or 2 to 2.5 indicates that the dosage does not need to be adjusted, but that the subject should be subjected to more frequent monitoring. In certain other embodiments, a PAA:PAGN ratio of 1 to 1.5 or 1 to 2 indicates that the dosage needs to be increased when the subject has recently exhibited particularly acute symptoms of a nitrogen retention disorder or another condition for which PAA prodrug administration is expected to be beneficial. Similarly, in certain embodiments a PAA:PAGN ratio greater than 1.5 or 2 but not

greater than 2.5 may indicate that the dosage needs to be decreased, depending on the subject's specific biochemical or clinical characteristics. In certain embodiments, measurement of plasma PAA and PAGN ratio takes place after the PAA prodrug has had sufficient time to reach steady state (e.g., 48 hours, 48 to 72 hours, 72 hours to 1 week, 1 week to 2 weeks, or greater than 2 weeks after PAA prodrug administration). In certain embodiments, the magnitude of the increase or decrease in dosage may be based on the precise PAA:PAGN ratio. For example, a PAA:PAGN ratio that is slightly less than 1 may indicate that the dosage needs to be increased slightly, while a ratio significantly less than 1 may indicate the dosage needs to be increased to a greater degree. In certain embodiments, the above steps are repeated until the subject exhibits a PAA:PAGN ratio falling within the target range.

[0048] In certain embodiments, methods are provided for determining whether a prescribed first dosage of a PAA prodrug can be safely administered to a subject. These methods comprise administering the prescribed first dosage to the subject, measuring plasma PAA and PAGN levels, calculating the plasma PAA:PAGN ratio, and determining whether the prescribed first dosage is safe for the subject based on whether the PAA:PAGN ratio falls above a target range, wherein a PAA:PAGN ratio falling above the target range indicates that the first dosage cannot be or potentially cannot be safely administered to the subject. In certain embodiments, the target range for PAA:PAGN ratio is 1 to 2.5 or 1 to 2. In certain of these embodiments where the target range is 1 to 2.5, a PAA:PAGN ratio above 2.5 indicates the PAA prodrug dosage is unsafe and needs to be adjusted downwards. In certain embodiments, the target range is divided into one or more subranges. In certain of these embodiments, a PAA:PAGN ratio of 2 to 2.5 indicates that the first dosage is safe, but that the subject should be subjected to more frequent monitoring. In other embodiments, a PAA:PAGN ratio of 2 to 2.5 indicates that the first dosage is potentially unsafe, with a final determination of safety taking into account the subject's specific biochemical or clinical characteristics. In certain embodiments, measurement of plasma PAA and PAGN ratio takes place after the PAA prodrug has had sufficient time to reach steady state (e.g., 48 hours, 48 to 72 hours, 72 hours to 1 week, 1 week to 2 weeks, or greater than 2 weeks after PAA prodrug administration). In certain embodiments where a determination is made that the first dosage is unsafe and needs to be decreased, the methods further comprise a step of administering a second dosage that is lower than the first dosage, and in certain of these embodiments the above steps may be repeated until a desired plasma PAA:PAGN ratio (e.g., 1 to 2.5 or 1 to 2) is achieved. For example, the methods may comprise administering a second

dosage that is lower than the first dosage, measuring plasma PAA and PAGN levels after administration of the second dosage, calculating the plasma PAA:PAGN ratio, and determining whether the second dosage of the PAA prodrug can be safely administered to the subject based on whether the PAA:PAGN ratio falls above a target range.

[0049] In certain embodiments, methods are provided for determining whether a prescribed first dosage of a PAA prodrug will be effective for treating a nitrogen retention disorder or another disorder for which PAA prodrug administration is expected to be beneficial. These methods comprise administering the prescribed first dosage to the subject, measuring plasma PAA and PAGN levels, calculating the plasma PAA:PAGN ratio, and determining whether the prescribed first dosage will be effective for the subject based on whether the PAA:PAGN ratio falls below a target range, wherein a PAA:PAGN ratio falling below the target range indicates that the first dosage will not be or potentially will not be effective for treating a disorder. In certain embodiments, the target range for PAA:PAGN ratio is 1 to 2.5 or 1 to 2. In certain of these embodiments where the target range is 1 to 2.5, a PAA:PAGN ratio below 1 indicates the PAA prodrug dosage is unlikely to be effective and needs to be adjusted upwards. In other embodiments, a PAA:PAGN ratio below 1 indicates that the first dosage is potentially ineffective, with a final determination of whether the dosage is likely to be ineffective based on the subject's specific biochemical or clinical characteristics. In certain embodiments, the target range is divided into one or more subranges. In certain of these embodiments, a PAA:PAGN ratio of 1 to 1.5 indicates that the first dosage is likely to be effective, but that the subject should be subjected to more frequent monitoring. In other embodiments, a PAA:PAGN ratio of 1 to 1.5 indicates that the first dosage is potentially ineffective, with a final determination of whether the dosage is likely to be ineffective taking into account the subject's specific biochemical or clinical characteristics. In certain embodiments, measurement of plasma PAA and PAGN ratio takes place after the PAA prodrug has had sufficient time to reach steady state (e.g., 48 hours, 48 to 72 hours, 72 hours to 1 week, 1 week to 2 weeks, or greater than 2 weeks after PAA prodrug administration). In certain embodiments where a determination is made that the first dosage is likely to be ineffective and needs to be increased, the methods further comprise a step of administering a second dosage that is higher than the first dosage, and in certain of these embodiments the above steps may be repeated until a desired plasma PAA:PAGN ratio (e.g., 1 to 2.5 or 1 to 2) is achieved. For example, the methods may comprise administering a second dosage that is higher than the first dosage, measuring plasma PAA and PAGN levels after

administration of the second dosage, calculating the plasma PAA:PAGN ratio, and determining whether the second dosage of the PAA prodrug is likely to be ineffective for treating a disorder based on whether the PAA:PAGN ratio falls above a target range.

[0050] Provided herein in certain embodiments are methods for monitoring therapy with a PAA prodrug in patients with a nitrogen retention disorder. These methods comprise administering a PAA prodrug to the subject, measuring plasma PAA and PAGN levels, and calculating the plasma PAA:PAGN ratio. In these methods, a PAA:PAGN ratio falling within a target range (e.g., 1 to 2.5 or 1 to 2) indicates that the therapy is effective, while a ratio falling outside this range indicates that the therapy may need to be adjusted. In certain embodiments, the plasma PAA:PAGN ratio is compared to a previously obtained PAA:PAGN ratio from the same subject to evaluate the effectiveness of PAA prodrug administration.

[0051] In certain embodiments, the methods provided herein may be used in conjunction with the methods described in WO09/134460 and WO10/025303. In these embodiments, urinary PAGN levels may be determined in addition to plasma PAA:PAGN ratio, with both measurements being used to evaluate or adjust PAA prodrug dosage.

[0052] A "PAA prodrug" as used herein refers to any drug that contains or is converted to PAA following administration to a subject, or to any pharmaceutically acceptable salt, ester, acid, or derivative thereof. A PAA prodrug may be administered via any route, including oral or parenteral administration. A PAA prodrug may be converted directly to PAA (e.g., a salt or ester of PAA; PBA or a salt or ester thereof such as NaPBA), or it may be converted to PAA via an intermediate (e.g., a pre-prodrug such as HPN-100). Other examples of PAA prodrugs include butyroyloxymethyl-4-phenylbutyrate.

[0053] An adjustment to the dosage of a PAA prodrug as discussed herein may refer to a change in the amount of drug per administration (e.g., an increase from a first dosage of 3 mL to a second dosage of 6 mL), a change in the number of administration within a particular time period (e.g., an increase from once a day to twice a day), or any combination thereof.

[0054] A "subject in need thereof" as used herein refers to any individual having a condition or suspected of having a condition for which administration of a PAA prodrug is expected to be beneficial. For example, a subject may be an individual with a nitrogen retention disorder or suspected of having a nitrogen retention disorder, including for example UCD, HE, and/or kidney failure/ESRD (Lee 2010; McGuire 2010; Lichter 2011). Likewise, a subject may have or be suspected of having another condition for which PAA prodrug administration is expected to

be beneficial, including for example cancer (Thiebault 1994; Thiebault 1995), neurodegenerative disorders such as Huntington's Disease (Hogarth 2007), amyotrophic lateral sclerosis (ALS) (Cudkowicz 2009), and spinal muscular atrophy (SMA) (Mercuri 2004; Brahe 2005), metabolic disorders (e.g., maple syrup urine disease (MSUD) (Bruneti-Pieri 2011), or sickle cell disease (Hines 2008).

[0055] A subject that has previously been administered a PAA prodrug may have been administered the drug for any duration of time sufficient to reach steady state. For example, the subject may have been administered the drug over a period of 2 to 7 days, 1 week to 2 weeks, 2 weeks to 4 weeks, 4 weeks to 8 weeks, 8 weeks to 16 weeks, or longer than 16 weeks.

[0056] A "PAA prodrug" as used herein refers to any drug that contains or is converted to PAA following administration to a subject, or to any pharmaceutically acceptable salt, ester, acid, or derivative thereof. A PAA prodrug may be administered via any route, including oral or parenteral administration. A PAA prodrug may be converted directly to PAA (e.g., PBA or a salt thereof such as NaPBA), or it may be converted to PAA via an intermediate (e.g., a pre-prodrug such as HPN-100). Other examples of PAA prodrugs include butyroyloxymethyl-4-phenylbutyrate.

[0057] An adjustment to the dosage of a PAA prodrug as discussed herein may refer to a change in the amount of drug per administration (e.g., an increase from a first dosage of 3 mL to a second dosage of 6 mL), a change in the number of administration within a particular time period (e.g., an increase from once a day to twice a day), or any combination thereof.

[0058] The terms "treat," "treating," or "treatment" as used herein may refer to preventing a disorder, slowing the onset or rate of development of a disorder, reducing the risk of developing a disorder, preventing or delaying the development of symptoms associated with a disorder, reducing or ending symptoms associated with a disorder, generating a complete or partial regression of a disorder, or some combination thereof. For example, where the disorder being treated is a nitrogen retention disorder, "treating" may refer to lowering waste nitrogen levels below a threshold level, preventing waste nitrogen levels from reaching a threshold level, decreasing the likelihood of waste nitrogen levels exceeding a threshold level, reducing or ending symptoms associated with elevated waste nitrogen levels, or a combination thereof.

[0059] With regard to the methods of treatment disclosed herein, interpretation of the PAA:PAGN ratio must be performed in the context of the therapeutic objective. For example, in subjects being treated for a nitrogen retention disorder, the therapeutic objective is elimination of

waste nitrogen in the form of PAGN. In subjects being treated for other disorders for which PAA prodrug administration is expected to be beneficial (e.g., neurodegenerative disorders, MSUD), the therapeutic objective is safely achieving target plasma levels of PAA and/or PBA.

[0060] Any methods known in the art may be used to obtain a plasma blood sample. For example, blood from a subject may be drawn into a tube containing heparin or ethylenediaminetetraacetic acid (EDTA). In certain embodiments, the sample can be placed on ice and centrifuged to obtain plasma within 15 minutes of collection, stored at 2-8°C (36-46°F) and analyzed within 3 hours of collection. In other embodiments, the blood plasma sample is snap frozen, stored at ≤-18°C (≤0°F) and analyzed at a later time. For example, the sample may be analyzed at 0-12 hours, 12-24 hours, 24-48, 48-96 hours after freezing, or within any other timeframe over which the sample has demonstrated stability. In certain of these embodiments, the blood sample is stored at a temperature between 0-15°C, such as 2-8°C. In other embodiments, the blood sample is stored below 0°C or below -18°C.

[0061] Measurement of PAA and PAGN levels in a plasma sample is carried out using techniques known in the art. For example, PAA and PAGN levels may be measured using liquid chromatography/mass spec analyses.

[0062] Any combination of embodiments described herein can be envisioned. Although individual features may be included in different claims, these may be advantageously combined.

[0063] The following examples are provided to better illustrate the claimed invention and are not to be interpreted as limiting the scope of the invention. To the extent that specific materials are mentioned, it is merely for purposes of illustration and is not intended to limit the invention. One skilled in the art may develop equivalent means or reactants without the exercise of inventive capacity and without departing from the scope of the invention. It will be understood that many variations can be made in the procedures herein described while still remaining within the bounds of the present invention. It is the intention of the inventors that such variations are included within the scope of the invention.

EXAMPLES

Example 1: Analysis of PAA:PAGN ratio in UCD and HE subjects:

[0064] Plasma PAA and PAGN levels and PAA:PAGN ratio were analyzed in more than 4000 plasma samples obtained from various clinical trials of healthy adults, severely hepatic impaired adults with clinically decompensated Child-Pugh B or C cirrhosis, and UCD patients ages 29 days or older. Healthy and hepatically impaired adults received HPN-100, while UCD

subjects received both HPN-100 and NaPBA. Clinical trial populations are summarized in Tables 1 and 2.

Table 1: Clinical studies and analysis populations

Study Group	Description	Demographics	Protocols Included	Analysis Populations
1	Short-term (<= 2-4 weeks) exposure in UCD subjects	Adults and children ages 29 days or greater (N=81)	UP 1204-003 HPN-100-005SO HPN-100-006 HPN-100-012	A, B
2	Long-term exposure in UCD and HE subjects	Adults and children ages 6 years or greater (N=180)	HPN-100-005SE HPN-100-007 HPN-100-008 Part B	A
3	Short-term (<= 4 weeks) exposure in hepatic impaired subjects	Adults (N=15)	HPN-100-008 Part A	A, B
4	Short-term exposure (<= 4 weeks) in healthy subjects	Adults (N=98)	HPN-100-010	A, B

Table 2: Demographics and number of samples used

	Attribute	No. of subjects		No. of sample points (Population A)		No. of time-specific PK sample points (Population B)	
		Count	Percent	Count	Percent	Count	Percent
Population	Healthy	86	17.0	2126	34.4	2126	38.5
	Hepatic Encephalopathy (HE)	103	20.4	830	13.4	830	15.0
	UCD	158	31.3	1616	26.1	1281	23.2
	Total	**347**	**100.0**	**4572**	**100.0**	**4237**	**100.0**
Age	29 days -< 6 yrs	15	4.3	110	2.4	110	2.6
	6 -< 18 yrs	47	13.5	373	8.2	213	5.0
	18+ yrs	285	82.1	4089	89.4	3914	92.4
Sex	F	199	57.3	2394	52.4	2152	50.8
	M	148	42.7	2178	47.6	2085	49.2

[0065] Analysis Population A consisted of quantifiable levels of PAA and PAGN metabolites derived from all studies described above. All PAA and PAGN levels used for analysis came from blood samples drawn once dosing with NaPBA or HPN-100 had reached steady state. Analysis Population B consisted of quantifiable levels of PAA and PAGN metabolites during studies in which pharmacokinetics were analyzed and for which blood draws were performed over 12 or 24 hours at steady state and for which the timing of the blood sample in relation to dosing was known. Subjects in study groups 1, 3 and 4 above contributed to these

points. Analysis Population B was the source of analyses that examined how PAA levels changed with time relative to dosing, where dosing could have been with either NaPBA or HPN-100. To be eligible for Analysis Population B, the time of the blood draw relative to the time of initiation of dosing during the dosing period had to have been recorded.

[0066] Data on metabolite levels were pooled across a wide range of age levels- infants, toddlers, children, adolescents, and adults. All children, defined as ages under 18, were UCD patients. The majority of the blood sampling points came from adults (89.4%). Newborn infants (< 29 days old) were not studied in any of the clinical trials for the investigational agent HPN-100. The population of blood sampling points were roughly equally divided between female and male (57.3% female, 42.7% male).

[0067] To examine the predictive ability of PAA:PAGN ratios, a subject was considered to have achieved a high value of PAA if any PAA value up to 24 hours since initiation of dosing equaled or exceeded 400 μg/mL or equaled or exceeded 500 μg/mL. PAA:PAGN ratios were grouped into one of three categorization schemes: a.) [0-<= 2.0] , [> 2.0] , b.) [0- <= 2.5, > 2.5], c.) [0- <= 3.0, > 3.0]. The repeated measures categorical outcome was modeled using GEE with a logit link function, ratio category as the independent variable, and SUBJECTID as the repeated measures factor. Confidence intervals for the predicted probabilities were computed by bootstrap estimation of 1000 resamplings of the original data, as detailed in Davison & Hinkley, "Bootstrap Methods and Their Application," Cambridge Univ. Press (1997), pp. 358-362.

[0068] Results are summarized in Figures 2-5. A striking curvilinear relationship was observed between plasma PAA levels and PAA:PAGN ratio at any given timepoint. Figure 2A shows the relationship between the ratio of PAA:PAGN concentrations and absolute PAA levels in micrograms per milliliter among blood samples that had quantifiable values for both PAA and PAGN. The ratio axis (i.e. 'X' axis) is plotted on a logarithmic (base e) scale. For ratios less than 1.0, increases in ratio are not associated with correspondingly elevated or increased levels of PAA. Above ratios of 1.0, there is a gradual increase in PAA levels, and a noticeable upswing in PAA levels that begins in the vicinity of a ratio of 2.0. This finding suggests that when the ratio of PAA precursor to PAGN product approaches higher values, the values of PAA are also correspondingly high. This increase in the ratio of precursor (PAA) to product (PAGN) implies ineffective PAA to PAGN conversion, regardless of whether the PAA is derived from HPN-100 or NaPBA.

[0069] To determine whether excessive PAA build-up is a function of dosing, the plots mentioned above were repeated, but this time adjusting for assigned dose level of NaPBA or HPN-100 at the time of the blood draw. Since the UCD population consisted of a mixture of children and adults undergoing both short-term therapy and long-term therapy, total assigned daily dose for UCD patients was standardized to body surface area and reported in PBA-equivalent grams meter^2. Healthy and HE subjects were all adults and their assigned dose was not adjusted by body surface area. Dose levels for healthy and HE subjects were reported in HPN-100 equivalent mL. Dose levels for UCD subjects were reported in NaPBA-equivalent grams.

[0070] The excess of PAA over PAGN, indicated by larger ratios as PAA increases, was evident across all dosage groups, disease populations, and types of treatment in UCD patients (i.e., applies to both NaPBA and HPN-100). This finding suggests that analysis of the precursor (PAA) to product (PAGN) ratio may be predictive of the efficiency of conversion among patients with or without liver dysfunction (UCD patients have normal liver function apart from their urea cycle dysfunction) and independently of dose. As a corollary, the presence of liver dysfunction (e.g. cirrhosis) by itself, is not necessarily a reliable determinant of whether a particular patient is at risk for high PAA levels.

[0071] The ability of PAA:PAGN ratios to predict extremely high plasma PAA concentrations was determined by modeling the probability that a subject would exceed a PAA value of 400 or 500 μg/mL anytime during a 24 hour dosing period, based on the ratio of PAA to PAGN computed at pre-dose (presumably trough), 12 hours after dosing (presumably peak), and the maximum ratio encountered anytime between pre-dose and 12 hours post-dose. This interval of 0-12 hours was chosen for practical reasons, as it would encompass the entire interval corresponding to the usual outpatient visit.

[0072] Since subjects could have multiple dosing periods within a given clinical study, the probability was modeled using Generalized Estimating Equations. Three categorizations of ratios were modeled: a.) [0-<= 2.0] [> 2.0] , b.) [0- <= 2.5, > 2.5], c.) [0- <= 3.0, > 3.0]. The models were repeated with PAA values greater than or equal to 500 μg/mL considered extreme. Results are summarized in Table 3.

Table 3: Probabilities of extreme PAA values encountered during 24 hour PK sampling with PAA:PAGN ratios (all subjects combined)

PAA Value Considered High		Time of Blood Draw Used For Ratio Classification	Observed Ratio of PAA/PAGN	Probability that a Subject With This Ratio Will Exceed High Value* (%)	Bootstrapped 95% Confidence Interval**
[<=2.0, >2.0]	>=400 µg/mL	t=0 (fasting)	<= 2.0	0.005 (0.5%)	0.004, 0.020
			> 2.0	0.164 (16.4%)	0.041, 0.281
		t = 12 hours	<= 2.0	0.003 (0.3%)	0.004, 0.021
			> 2.0	0.227 (22.7%)	0.048, 0.412
		MAX(0-12)	<= 2.0	0.002 (0.2%)	0.004, 0.010
			> 2.0	0.143 (14.3%)	0.036, 0.263
	>=500 µg/mL	t=0 (fasting)	<= 2.0	did not converge	
			> 2.0		
		t = 12 hours	<= 2.0	did not converge	
			> 2.0		
		MAX(0-12)	<= 2.0	did not converge	
			> 2.0		
[<=2.5, >2.5]	>=400 µg/mL	t=0 (fasting)	<= 2.5	0.008 (0.8%)	0.004, 0.023
			> 2.5	0.191 (19.1%)	0.053, 0.366
		t = 12 hours	<= 2.5	0.007 (0.7%)	0.004, 0.016
			> 2.5	0.364 (36.4%)	0.125, 0.752
		MAX(0-12)	<= 2.5	0.003 (0.3%)	0.004, 0.013
			> 2.5	0.200 (20.0%)	0.050, 0.381
	>=500 µg/mL	t=0 (fasting)	<= 2.5	0.003 (0.3%)	0.004, 0.011
			> 2.5	0.084 (8.4%)	0.029, 0.214
		t = 12 hours	<= 2.5	did not converge	
			> 2.5		
		MAX(0-12)	<= 2.5	did not converge	
			> 2.5		
[<=3, >3]	>=400 µg/mL	t=0 (fasting)	<= 3.0	0.010 (1.0%)	0.004, 0.025
			> 3.0	0.205 (20.5%)	0.059, 0.398
		t = 12 hours	<= 3.0	0.013 (1.3%)	0.004, 0.028
			> 3.0	0.250 (25.0%)	0.113, 0.576
		MAX(0-12)	<= 3.0	0.003 (0.3%)	0.004, 0.014
			> 3.0	0.229 (22.9%)	0.059, 0.438
	>=500 µg/mL	t=0 (fasting)	<= 3.0	0.003 (0.3%)	0.004, 0.010
			> 3.0	0.102 (10.2%)	0.032, 0.255
		t = 12 hours	<= 3.0	did not converge	
			> 3.0		
		MAX(0-12)	<= 3.0	did not converge	
			> 3.0		

Analysis repeated for each ratio cut off category independently.
* Probability derived from Generalized Estimating Equations model with logit link function.
** Confidence interval derived from method disclosed in Davison & Hinkley, "Bootstrap Methods and Their Application," Cambridge Univ. Press (1997), pp. 358-362, using 1000 re-samplings of original data.

[0073] Because of the sparseness of samples in which PAA equaled or exceeded 500 µg/mL, 400 µg/mL proved to be a more stable and predictable target (i.e. high) value. Of the three categorizations of ratio considered, the cutpoint of 2.5 was the best discriminator and predictor

of the risk of experiencing an high value. For example, referring to Table 3, a subject with a PAA:PAGN ratio > 2.5 at t=12 hours after dosing has a 36.4% chance (95% c. i.= 0.125, 0.752) of exceeding 400 μg/mL in PAA sometime during the 24-hour PK sampling period.

[0074] Results were similar whether the ratio was computed from plasma drawn at pre-dose, 12 hours after initiation of dosing, or the maximum ratio encountered anytime between pre-dose and 12 hours after initiation of dosing.

[0075] Due to the very high intra-day variability of plasma PAA levels, a PAA:PAGN ratio observed as exceeding 2.0 at a certain time following dosing may not remain greater than 2.0 in subsequent times. To evaluate the optimal time for obtaining a PAA:PAGN ratio measurement (i.e., the time that gives the greatest probability of correctly detecting a subject whose PAA:PAGN ratio ever equals or exceeds 2.0 during the dosing period), ratios were evaluated at 0 (pre-dose) and 2, 4, 6, 8, 10, and 12 hours post-dosing and modeled using GEE methodology. Pairwise differences in sensitivity between time points were evaluated using LS means and confidence intervals were computed.

[0076] Figure 3 plots the estimated probabilities of correctly detecting a ratio profile that ever equals of exceeds 2.0. With the exception of time= 2 hours and time=10 hours, time points of 0, 4, 6, 8, and 12 hours post-dosing were equally effective in detecting subjects who equal or exceed a PAA:PAGN ratio of 2.0 at some point during the dosing period. Sensitivities were in the range of 75-90 percent. There were too few blood samples collected at t=10 hours to analyze inter-time differences. Differences in predictive value were observed. For example, blood samples collected at t= 2 hours post-dosing had a significantly lower probability of detecting subjects who equal or exceed a PAA:PAGN ratio of 2.0 than samples collected at t=0 (p = 0.036), 4 (p = 0.032), or 6 hours (p = 0.017) post-dosing (p values are comparisons of t=2 hour probability with other time points). Similarly, a sample collected at t=12 hours following initiation of dosing had the highest probability (87%) of detecting a subject whose ratio ever equals or exceeds 2.0. However, for practical clinical purposes, the differences in predictive value among time points was trivial relative to the dramatically greater variability in PAA values themselves, meaning that random blood draws can be used for measurement of PAA:PAGN ratio.

[0077] Further exploration of the fluctuation of PAA:PAGN ratios over time was conducted by dividing the subject population into cohorts according to the maximum PAA:PAGN ratio achieved during the 24-hour PK sampling time during the dosing period. Cohorts were divided

into "low" (maximum ratio <= 2.0), "medium" (maximum ratio: 2.01-2.50), and "high" (maximum ratio > 2.50). Each cohort was then followed over time during the dosing period at t= 0 hours(pre-dose), 4, 6, and 8 hours post-dosing and the distribution of PAA:PAGN ratios within the cohort summarized using a box-and-whisker plot at each time point. This analysis was conducted for the PK-timepoint-specific population as a whole (analysis population B) as well as for each disease subpopulation separately.

[0078] Figure 4 plots the progression of ratios for all subjects combined. Each "panel" of the plot that divides the graphing space into thirds represents one cohort. Subjects in the high cohort had high ratios throughout the day and not only at a particular time point. Therefore, subjects in this cohort (n=73 subject/dosing periods) started with high ratios (median ratio > 2.5) and remained high throughout the first 12 hours. This finding is consistent with the findings plotted in Figure 3 which revealed the consistency of sensitivity in ratios.

[0079] The relationship between PAA levels and PAA:PAGN ratios was further analyzed by categorizing ratios into "low" (maximum ratio <= 2.0), "medium" (maximum ratio: 2.01-2.50), and "high" (maximum ratio > 2.50). Unlike the previous analysis, this analysis did not associate subject/dosing periods with particular cohorts (i.e., all samples and all time points are combined with regard to the subject or dosing period).

[0080] Figure 5A shows the box-and-whisker plots of PAA levels grouped by the above categories of PAA:PAGN ratio for all subjects, while Figure 5B shows the same for UCD and HE subjects only. The results were very similar in both analysis sets. Following a statistically significant overall Kruskal-Wallis test ($p < 0.0001$), pairwise comparisons of PAA levels were conducted using Wilcoxon-Mann-Whitney with a Bonferroni alpha correction of (0.0167). In both analysis sets, ratios greater than 2.5 had significantly higher PAA levels ($p < 0.001$) than either ratios between 2.0 – 2.5 or ratios less than 2.0. Furthermore, ratios between 2.0 – 2.5 were associated with significantly higher PAA levels than ratios less than 2.0 ($p < 0.001$).

Example 2: Analysis of PAA:PAGN ratio as a guide to dose adjustment and monitoring in a UCD patient:

[0081] Patient 1 was a 15 year old partial OTC female receiving HPN-100 as maintenance therapy for her UCD at a dose of 9 mL/day. The patient's ammonia had been controlled since her last routine visit around 6 months ago, but she was complaining of headache and lack of appetite for the past 3 days. Ammonia and metabolite levels were tested after overnight fasting and showed the following results: ammonia 55 μmol/L, PAA and PAGN below levels of

quantification. The physician suspected non-compliance with drug and repeated the tests in midday several hours after lunch and found the following results: ammonia: 117 μmol/L; PAA 55 μg/L, PAGN 121 μg/L, and PAA:PAGN ratio approximately 0.5. The patient indicated that she had been fully compliant with her medication. Based on the PAA to PAGN ratio of 0.5 and ammonia of 117, the physician decided to increase the dosage of HPN-100 to 12 mL/day. After one week of treatment with the new dose of HPN-100, all symptoms resolved and the laboratory tests after overnight fasting showed the following: ammonia 9 μmol/L; PAA 12.9 μg/L, PAGN of 9 μg/L, and PAA:PAGN ratio of 1.3. Midday tests showed the following: ammonia 35 μmol/L, PAA 165 μg/L, PAGN 130 μg/L, and PAA:PAGN ratio of ~1.2. The patient was considered controlled and the dose remained at 12 mL/day.

Example 3: Analysis of PAA:PAGN ratio as a guide to dose adjustment in a UCD patient:

[0082] Patient 2 was a 1 year old male OTC receiving 600 mg/kg of NaPBA per day. The patient presented with poor feeding and somnolence. Laboratory tests showed ammonia levels of <9 μmol/L, PAA levels of 530 μg/L, PAGN levels of 178 μg/L, and a PAA:PAGN ratio of >2.5, suggesting that the dose of NaPBA was greater than the patient could effectively convert to PAGN. The treating physician decided to decrease the dose of NaPBA to 450 mg/Kg/day. After one week of treatment with the new dosage, the patient's mother reported that he was eating well and was no longer somnolent. Laboratory tests showed the following: ammonia 20 μmol/L, PAA 280 μg/L, and PAGN 150 μg/L.

Example 4: Analysis of PAA:PAGN ratio as a guide to assessment of importance of a high PAA level in a UCD patient:

[0083] Patient 3 is a 25 year old OTC female who is being treated with HPN-100. The physician had to increase the dose of HPN-100 several times in order to achieve clinical and blood ammonia within normal limits. Patient 3 was treated at a dose of 18 mL/day for her UCD for the past month. In her next office visit, she did not have any complaints and the following lab results were reported: ammonia 22 μmol/L, PAA 409 μg/L, PAGN 259 μg/L, and PAA:PAGN ratio of 1.5. Despite the patient's relatively high PAA levels, the PAA:PAGN ratio indicated that the subject was being adequately treated and that the patient was able to effectively metabolize the high dose of HPN-100 that she was receiving. The physician decided to continue the treatment as planned.

Example 5: Analysis of PAA:PAGN ratio as a guide to dose adjustment in a patient with spinal muscular atrophy and concomitant liver disease:

[0084] Patient 4 was a 2 year old female being treated with a liquid form of NaPBA for her type II SMA. The patient also suffered from chronic hepatitis C virus infection acquired perinatally from her infected mother. The patient had been having mild to moderate elevation of transaminases since birth, with episodes of icterus and a recent liver biopsy has confirmed presence of chronic hepatitis and cirrhosis. The patient was receiving 4 g of NaPBA per day, and the physician wanted to increase the dosage due to the patient's growth but was concerned about the effects of liver dysfunction on drug metabolism. The physician ordered plasma PAA and PAGN levels and the results were as follows: PAA 110 μg/L, PAGN 85 μg/L, PAA:PAGN ratio of 1.2. The physician decided to increase the dosage of NaPBA to 6 g/day, and repeated the plasma metabolite level measurements after one week of treatment with the new regimen. The results were as follows: PAA 155 μg/L, PAGN 110 μg/L, and PAA:PAGN ratio of 1.4. The physician decided to leave the patient on 6 g/day of NaPBA since his liver seems to have adequate capacity to metabolize 6 g of NaPBA.

Example 6: Analysis of PAA:PAGN ratio as a guide to dose adjustment in a patient with Huntington's Disease and concomitant liver disease:

[0085] Patient 5 was a 56 year old male diagnosed with Huntington's disease several years ago. He also had a history of alcohol abuse and was diagnosed with alcoholic cirrhosis last year. His wife enrolled him in clinical trials that involved an experimental drug delivering PBA at a slow rate, thereby enabling once-a-day dosing of the drug. The study had an option for dose escalation after 2 weeks of treatment if clinically safe. Although the protocol did not exclude patients with liver dysfunction, the investigator was concerned about PBA metabolism and possible accumulation of PAA in higher doses due to the patient's liver dysfunction. The investigator enrolled the patient in the low dose group and performed plasma PBA, PAA and PAGN measurements after 6 weeks of treatment with experimental drug. The patient reported improvement in his HD symptoms with no specific complains. Plasma metabolite levels after six weeks of treatment were as follows: PBA 45 μg/L; PAA 159 μg/L, and PAGN 134 μg/L. The dosage of the drug was increased by 50%. After four days of treatment at the new dosage, the patient started to complain about short episodes of somnolence. The investigator performed a blood test and observed the following: PBA 44 μg/L; PAA 550 μg/L, PAGN 180 μg/L, and PAA:PAGN ratio of >3. The PAA:PAGN ratio of greater than 2.5 indicated that the patient's

liver could not effectively metabolize the higher dose of the drug, and the investigator therefore decided to reduce the dosage of the experimental drug and not continue dose escalation.

Example 7: Analysis of PAA:PAGN ratio as a guide to dose adjustment in a patient with MSUD:

[0086] Patient 6 was a 4 year old female being treated with HPN-100 for MSUD. The patient was receiving 6 mL of HPN-100 once a day, and the physician wanted to increase the dosage due to the patient's growth. Midday plasma PAA and PAGN measurements after the dose of medication were as follows: PAA 550 μg/L, PAGN 180 μg/L, and PAA:PAGN ratio of >2.5. The physician believed a lower dosage of HPN-100 would not be as effective for the patient, and decided to change the dosing regimen to 3 mL BID instead of 6 mL QD based on the high PAA:PAGN ratio. The tests were repeated after one week of treatment with the new BID regimen, with the following results: PAA 350 μg/L, PAGN 190 μg/L, and PAA:PAGN ratio of 1.8. Based on the ratio of 1.8, the physician decided to leave the patient on 3 mL BID since she can efficiently use a total dose of 6 mL/day given in divided doses but not as a bolus.

Example 8: Analysis of PAA:PAGN ratio as a guide to monitor a patient with HE and hepatic impairment:

[0087] Patient 7 was a 55 year old Caucasian male diagnosed with alcoholic cirrhosis 3 years ago. His transaminase levels had been mildly elevated and he had recently experienced mild episodes of HE. In the last assessment at the time of hospital admission for a grade 2 HE episode, the patient had a blood ammonia of 85 μmol/L, ALT of 55 U/L, and AST of 47 U/L, and a calculated MELD score of 11. The physician decided to start an ammonia scavenging therapy for the patient and treated him with HPN-100 6 mL BID. The patient returned for a follow up visit after 3 months, during which time he had experienced no episodes of HE. His laboratory assessments showed the following: ammonia of 30 μmol/L, plasma PAA level of 285 μg/mL, PAGN level of 120 μg/L, ALT of 66 U/L, AST of 50 U/L, and calculated MELD score of 13. The physician suspected that the patient's hepatic function may be deteriorating and was concerned about possible accumulation of PAA. She calculated the ratio of PAA to PAGN as 2.4, and confirmed that the patient had not experienced any unusual symptoms such as dizziness, headache, or nausea. Considering patient's ammonia control, lack of specific side effects, and clinical remission, the physician decided not to change the dose and to see the patient in two weeks to repeat the laboratory tests. The physician also warned the patient to call her immediately if he experienced any of these symptoms. In two weeks, the patient's laboratory

assessments were essentially unchanged from the previous visit, with a PAA to PAGN ratio of 2.3, and the patient did not report any unusual symptoms. Based on the PAA:PAGN ratio of less than 2.5, the physician decided to continue dosing with 6 mL BID of HPN-100 until the next routine visit.

Example 9: Analysis of PAA:PAGN ratio as a guide to monitoring treatment in a patient with Parkinson's Disease:

[0088] HPN-100 treatment was initiated at a dose of 4mL twice a day in a patient with Parkinson's Disease to produce target circulating levels of PAA expected to produce clinical benefit. After one week of treatment, the patient's circulating PAA level of 50 µg/mL was below the target range, and the PAA:PAGN ratio was determined to be 0.9. The physician concluded that the HPN-100 dose could be safely adjusted upward, and the dose was increased by 50% to 6 mL BID. The PAA level and PAA/PAGN ratio one week later were found to be 75 µg/mL and 1.4, respectively. Since 75 µg/mL was still below the therapeutic PAA target level and the PAA:PAGN ratio of 1.4 indicated that conversion of PAA to PAGN had not been saturated, the patient's dosage was increased again by 50% to 9 mL BID. One week later, the patient's PAA and PAA:PAGN ratio were found to be 159 µg/mL and 2.6, respectively. Since the target PAA level was now approximately therapeutic but the PAA:PAGN ratio indicated that PAA to PAGN conversion was approaching saturation, HPN-100 dosage was decreased to 8 mL BID, at which time the patient's circulating PAA level was determined to be close to the target range and his PAA:PAGN ratio was determined to be 2. The patient's dose was not further adjusted and he continued to be monitored.

[0089] As stated above, the foregoing is merely intended to illustrate various embodiments of the present invention. The specific modifications discussed above are not to be construed as limitations on the scope of the invention. It will be apparent to one skilled in the art that various equivalents, changes, and modifications may be made without departing from the scope of the invention, and it is understood that such equivalent embodiments are to be included herein. All references cited herein are incorporated by reference as if fully set forth herein.

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What is claimed is:

1. A method of treating a nitrogen retention disorder in a subject comprising:

(a) administering a first dosage of a PAA prodrug,

(b) measuring plasma PAA and PAGN levels,

(c) calculating a plasma PAA:PAGN ratio,

(d) determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(e) administering a second dosage of the PAA prodrug based on the determination in (d).

2. A method of treating a nitrogen retention disorder in a subject who has previously been administered a first dosage of a PAA prodrug comprising:

(a) measuring plasma PAA and PAGN levels,

(b) calculating a plasma PAA:PAGN ratio,

(c) determining whether the first PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(d) administering a second dosage of the PAA prodrug based on the determination in (c).

3. A method of treating a condition for which PAA prodrug administration is expected to be beneficial in a subject comprising:

(a) administering a first dosage of a PAA prodrug,

(b) measuring plasma PAA and PAGN levels,

(c) calculating a plasma PAA:PAGN ratio,

(d) determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(e) administering a second dosage of the PAA prodrug based on the determination in (d).

4. A method of treating a condition for which PAA prodrug administration is expected to be beneficial in a subject who has previously been administered a first dosage of a PAA prodrug comprising:

(a) measuring plasma PAA and PAGN levels,

(b) calculating a plasma PAA:PAGN ratio,

(c) determining whether the first PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(d) administering a second dosage of the PAA prodrug based on the determination in (c).

5. A method of adjusting the dosage of a PAA prodrug comprising:

(a) administering a first dosage of a PAA prodrug,

(b) measuring plasma PAA and PAGN levels,

(c) calculating a plasma PAA:PAGN ratio,

(d) determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(e) administering a second dosage of the PAA prodrug based on the determination in (d).

6. A method of optimizing the therapeutic efficacy of a PAA prodrug in a subject who has previously been administered a first dosage of a PAA prodrug comprising:

(a) measuring plasma PAA and PAGN levels,

(b) calculating a plasma PAA:PAGN ratio,

(c) determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(e) administering a second dosage of the PAA prodrug as necessary based on the determination in (c).

7. The method of claim 1 or 2, wherein the nitrogen retention disorder is selected from the group consisting of UCD, HE, and ESRD.

8. The method of claim 3 or 4, wherein the disorder is selected from the group consisting of cancer, a neurodegenerative diseases, a metabolic disorder, and sickle cell disease.

9. The method of any of claims 1-6, wherein the target range is 1 to 2.5.

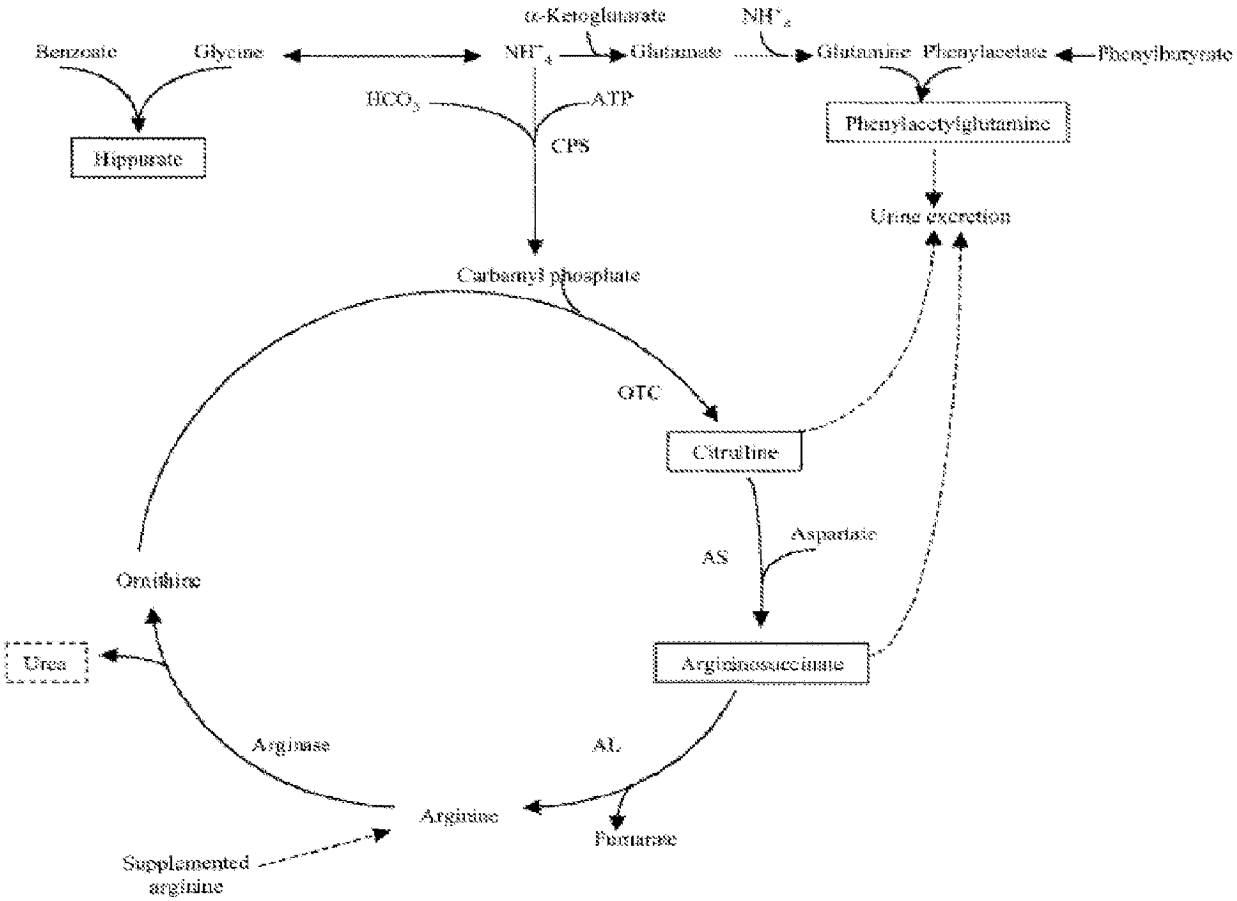
10. The method of any of claims 1-6, wherein the target range is 1 to 2.

11. The method of any of claims 1-6, wherein measurement of PAA and PAGN levels is carried out after the first dosage of PAA prodrug has had sufficient time to reach steady state.

12. The method of claim 11, wherein measurement of PAA and PAGN levels is carried out 48 hours to 1 week after the first dosage of PAA prodrug is administered.

13. The method of any of claims 1-6, wherein the PAA prodrug is selected from the group consisting of NaPBA and HPN-100.

Figure 1


α-Ketoglutarate
NH^+_2
Benzoate
Glycine
NH^+_4
Glutamate
Glutamine
Phenylacetate
Phenylbutyrate
HCO_3
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CPS
Hippurate
Phenylacetylglutamine
Urine excretion
Carbamyl phosphate
GTC
Citrulline
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Urea
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Arginase
AL
Arginine
Fumarate
Supplemented arginine

Figure 2A

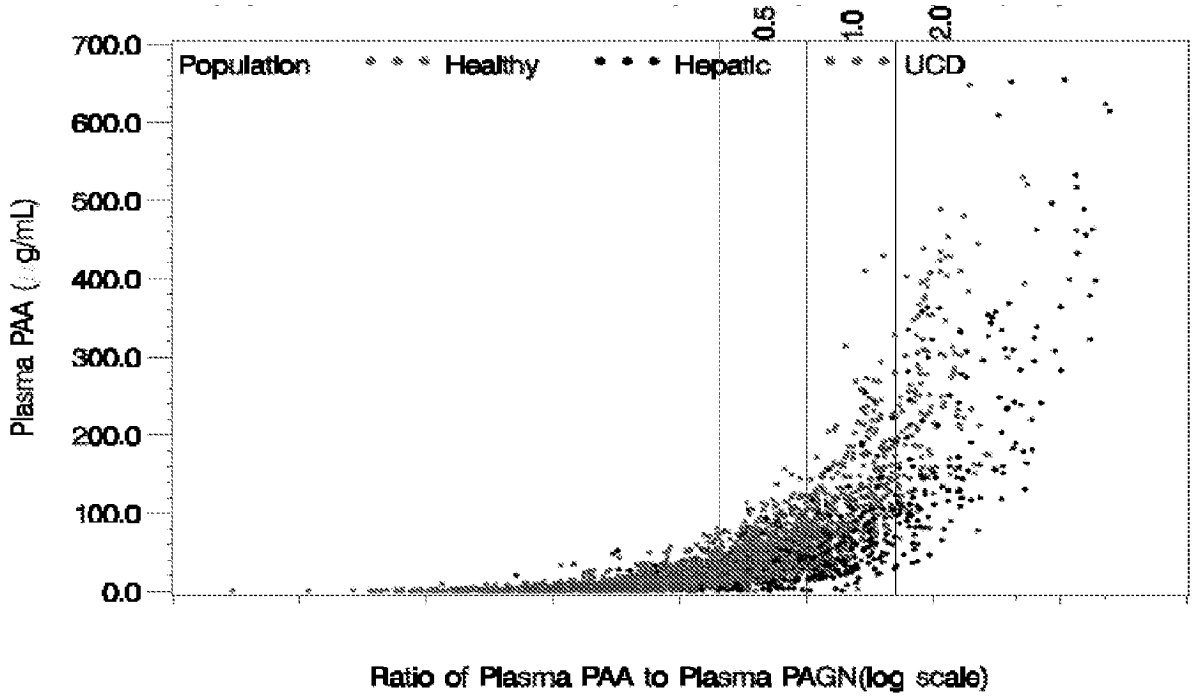

0.5
1.0
2.0
Population
Healthy
Hepatic
UCD
700.0
600.0
500.0
400.0
300.0
200.0
100.0
0.0
Plasma PAA (g/mL)
Ratio of Plasma PAA to Plasma PAGN(log scale)

Figure 2B

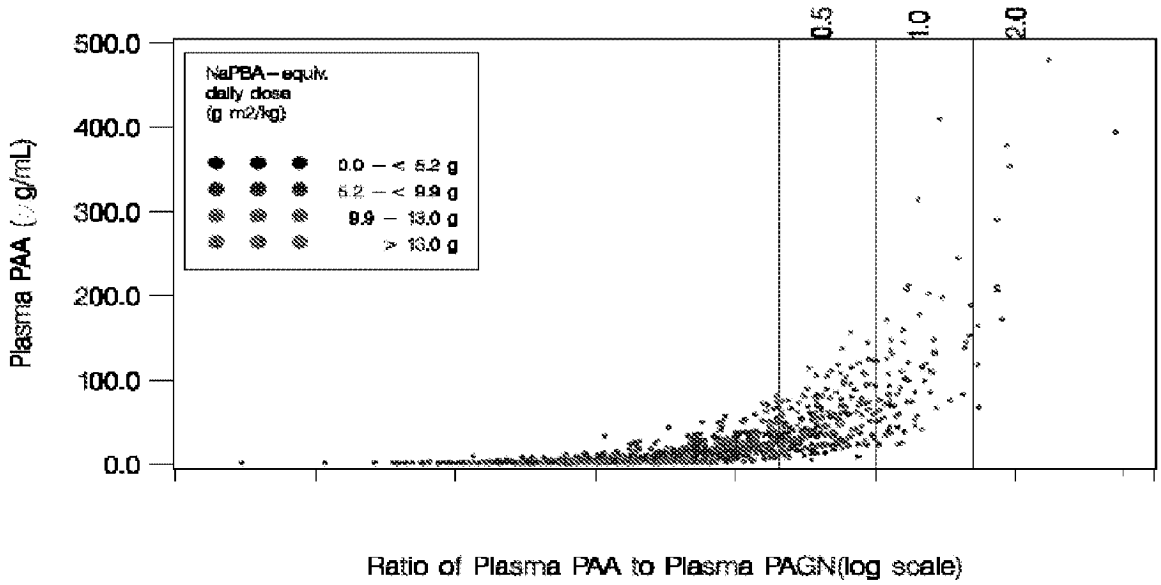

0.5
1.0
2.0
500.0
400.0
300.0
200.0
100.0
0.0
NaPBA–equiv.
daily dose
(g m2/kg)
0.0 – < 5.2 g
5.2 – < 9.9 g
9.9 – 13.0 g
> 13.0 g
Plasma PAA (µg/mL)
Ratio of Plasma PAA to Plasma PAGN(log scale)

Figure 2C

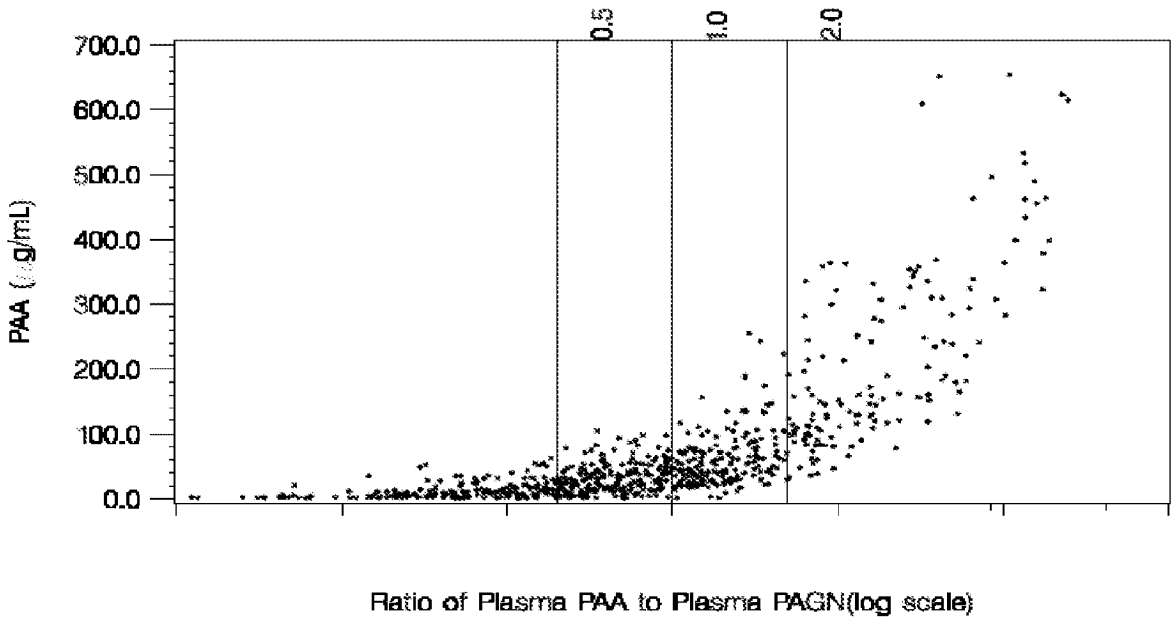
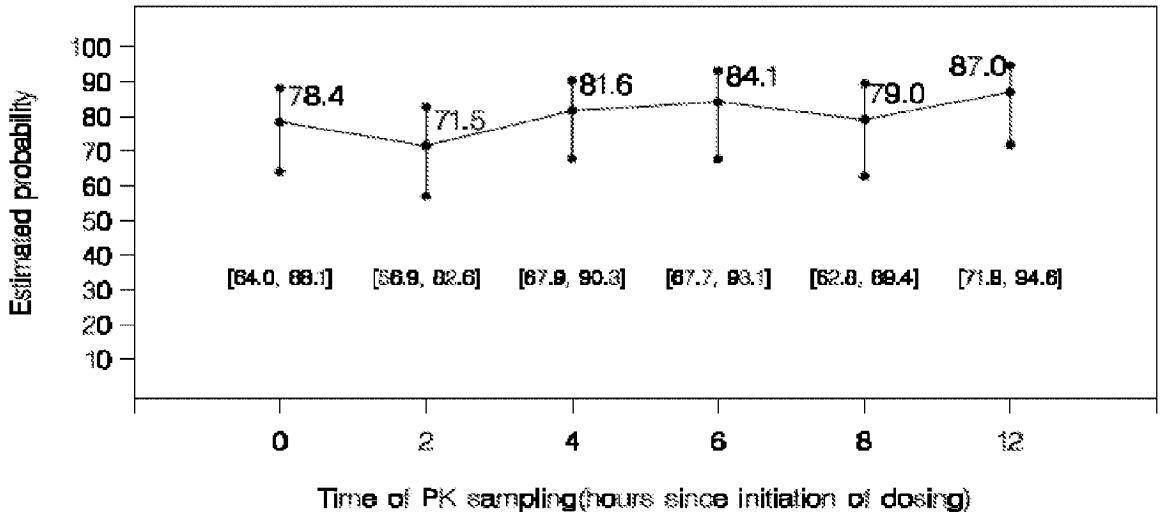

0.5
1.0
2.0
700.0
600.0
500.0
400.0
300.0
200.0
100.0
0.0
PAA (ng/mL)
Ratio of Plasma PAA to Plasma PAGN(log scale)

Figure 3



t=2 hrs signif. less than t=0(p=0.036), t=4(p=0.032), and t=8(p=0.017)
No other time differences statistically significant. Time=10 omitted due to too few observations

Figure 4

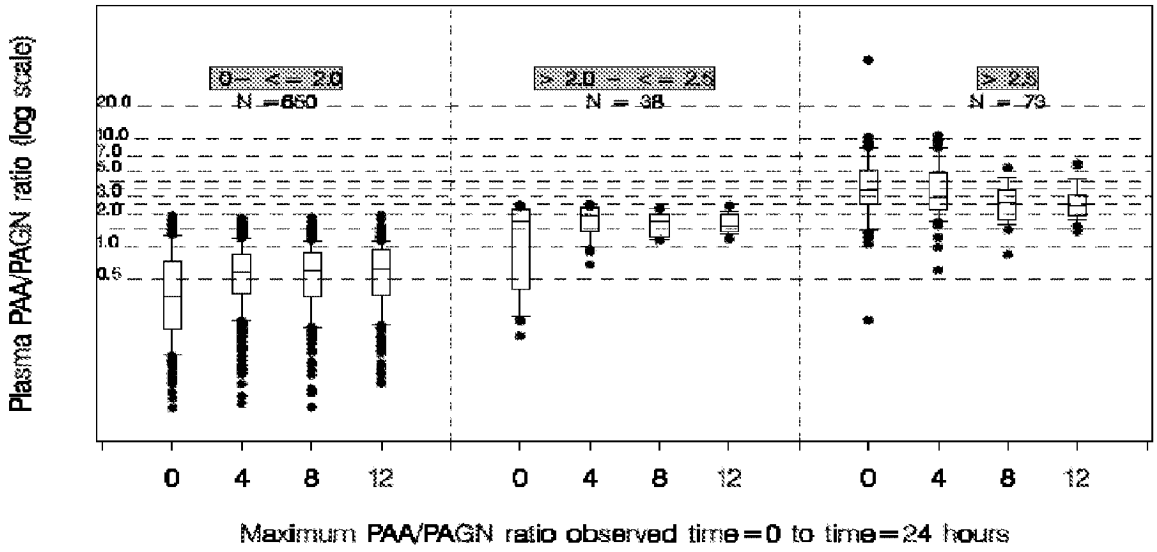

Plasma PAA/PAGN ratio (log scale)
0 - < = 2.0
N = 650
> 2.0 - < = 2.5
N = 38
> 2.5
N = 79
20.0
10.0
5.0
2.5
2.0
1.0
0.5
0
4
8
12
Maximum PAA/PAGN ratio observed time=0 to time=24 hours

Figure 5A

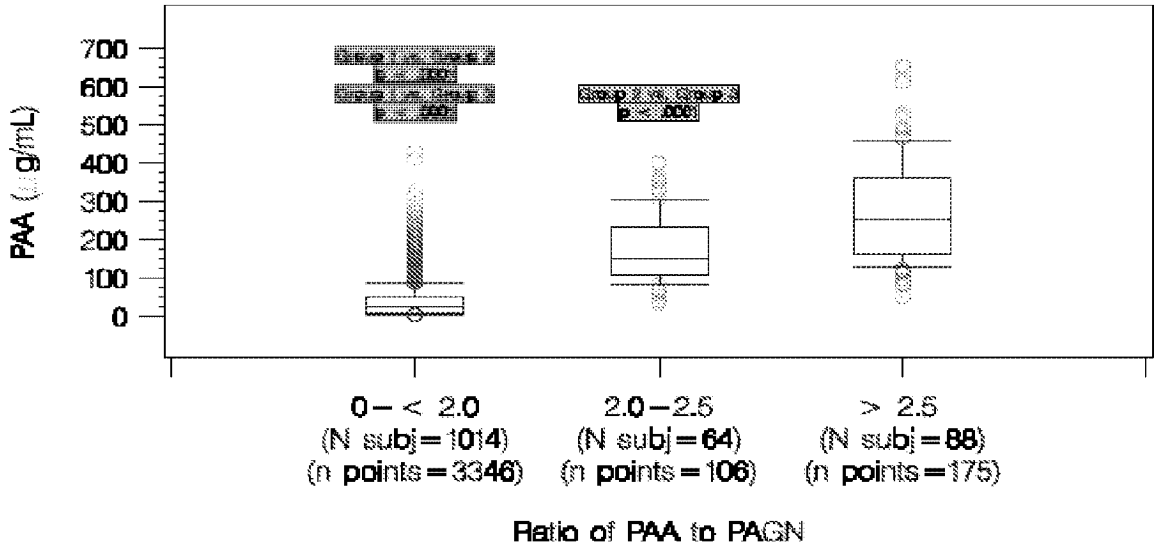
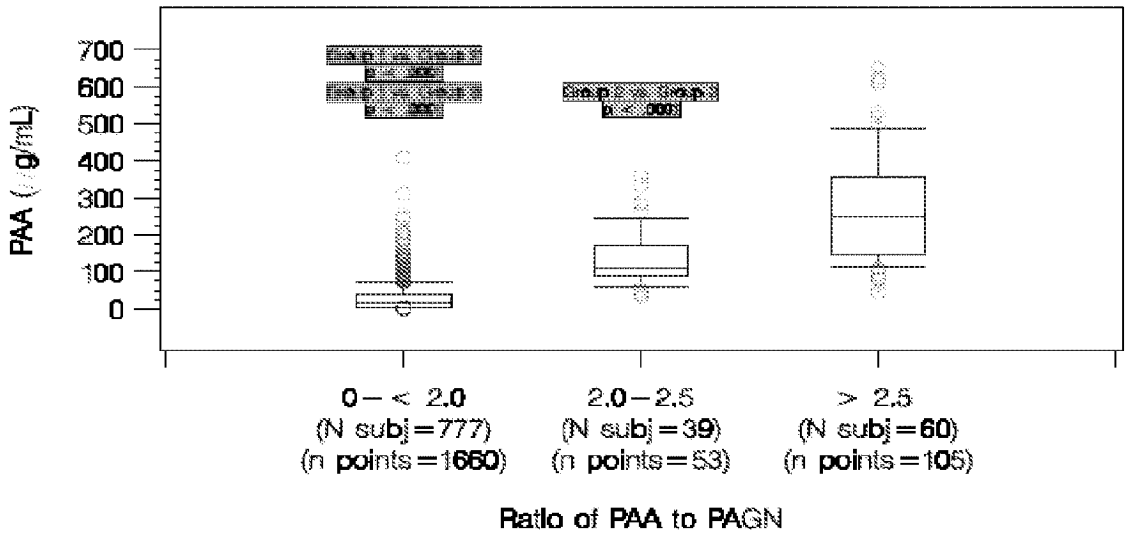


Figure 5B



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/54673

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/216; A61K 31/185
USPC - 514/533; 514/576; 514/532, 514/553; 554/220, 554/227
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A61K 31/216; A61K 31/185 (2012.01)
USPC: 514/533; 514/576

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 514/532, 514/553; 554/220, 554/227 (search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST, PatBase, Google Scholar: Plasma, PAA, PAGN, nitrogen retention, phenylacetic acid, phenylacetylglutamine, levels, NaPBA, NPH-100, nitrogen retention disorders, target range, dose

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2012/0022157 A1 (SCHARSCHMIDT) 26 January 2012 (26.01.2012); para [0021], [0089], [0097], [0106], [0116], [0118], [0160], [0173], [0174], [0181], [0297]	1-13
Y	MCGUIRE et al., Pharmacology and Safety of Glycerol Phenylbutyrate in Healthy Adults and Adults with Cirrhosis, HEPATOLOGY, June 2010, Vol. 51, pages 2077-2085; abstract; page 2079, col 2, para 3, page 2081, col 1, para 2;	1-13

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 October 2012 (24.10.2012)	20 NOV 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 09/30362

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A01N 37/10; A61K 31/19 (2009.01)
USPC - 514/570
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A01N 37/10; A61K 31/19 (2009.01)
USPC: 514/570

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC(8): A01N 37/10; A61K 31/19 (2009.01)
USPC: 514/570

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
US WEST(PGPB,USPT,EPAB,JPAB), Google Scholar, Dialog PRO (Engineering)
ammonia scavenging, accumulation, retention, hepatic encephalopathy, urea cycle disorder, phenylacetyl glutamine, PAGN, HPN-100, phenyl butyrate, glyceryl tri-(4-phenyl butyrate)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2004/0229948 A1 (SUMMAR, et al.) 18 November 2004 (18.11.2004), para [0022], [0029], [0035]	1-11, 19-22, 28, 29
Y	US 4,284,647 A (BRUSILOW, et al.) 18 August 1981 (18.08.1981) col 2, ln 26-32; Fig. 3; col 4, ln 35-46.	1-5, 9-18, 23-27, 29
Y	US 5,968,979 A (BRUSILOW) 19 October 1999 (19.10.1999), col 1, ln 27-34; col 1, ln 41-45; col 2, ln 25-34; col 3, ln 3-7; col 3, ln 42-59; col 4, ln 1-26; col 4, ln 54-58; col 5, ln 3-15; ln 29-35	6-29

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 February 2009 (24.02.2009)	02 MAR 2009
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (April 2007)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:
MICHAEL G. SMITH
MORRISON & FOERSTER LLP
12531 HIGH BLUFF DRIVE, SUITE 100
SAN DIEGO, CA 92130-2040

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	02 MAR 2009
Applicant's or agent's file reference 643982000140	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 09/30362	07 January 2009 (07.01.2009)	29 April 2008 (29.04.2008)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A01N 37/10; A61K 31/19 (2009.01)
USPC - 514/570

Applicant HYPERION THERAPEUTICS

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	24 February 2009 (24.02.2009)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (April 2007)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 09/30362

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of:

   a. type of material

      ☐ a sequence listing

      ☐ table(s) related to the sequence listing

   b. format of material

      ☐ on paper

      ☐ in electronic form

   c. time of filing/furnishing

      ☐ contained in the international application as filed

      ☐ filed together with the international application in electronic form

      ☐ furnished subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 09/30362

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-29	YES
	Claims	None	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-29	NO
Industrial applicability (IA)	Claims	1-29	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-5 lack an inventive step under PCT Article 33(3) as being obvious over US 2004/0229948 A1 to Summar, et al. (hereinafter "Summar") in view of US 4,284,647 A to Brusilow, et al. (hereinafter "Brusilow-647").

Regarding claim 1, Summar teaches a method to determine an effective dosage of HPN-100 for a patient in need of treatment for a nitrogen retention disorder, which comprises monitoring the effect of an initial dosage of HPN-100 (para [0022], "glyceryl-tri(4-phenyl butyrate)"; para [0029], "hepatic encephalopathy"; para [0035]). Summar does not teach monitoring the patient's urinary phenylacetyl glutamine (PAGN) output. However, Brusilow-647 teaches a method of determining the patient's urinary PAGN output (col 2, ln 26-32; Fig. 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to use the method of determining the urinary PAGN output taught in Brusilow-647, in order to determine the effective dosage of HPN-100 for a patient and/or how to adjust the initial dosage of HPN-100 to produce a desired ammonia scavenging effect, as a correlation of phenylacetyl glutamine to phenylacetate administration is disclosed in Brusilow-647 (col 2, ln 26-32), a correlation similar to which would be likely between the administration of HPN-100 and urinary phenylacetyl glutamine output, phenyl acetate being a metabolite of HPN-100 (Summar, para [0005]).

Regarding claim 2, Brusilow-647 further teaches the method of claim 1, wherein urinary PAGN output is determined as a ratio of the concentration of urinary PAGN to urinary creatinine (Fig. 3; col 4, ln 35-46).

Regarding claim 3, Summar further teaches the method of claim 1, wherein the nitrogen retention disorder is chronic hepatic encephalopathy (para [0029]).

Regarding claim 4, Summar further teaches the method of claim 1, wherein administering the effective dosage of HPN-100 to the patient produces a change in plasma ammonia level in the patient (para [0035]). Summar does not explicitly teach achieving normal plasma ammonia levels. However, it would have been obvious to one of ordinary skill in the art to produce normal plasma ammonia levels by administration of HPN-100, as a reduction in plasma ammonium levels following administration of a metabolite of HPN-100, namely phenyl acetic acid, is taught in Brusilow-647 (col 4, ln 46-50; col 4, ln 64-68).

Regarding claim 5, Summar teaches a method to determine an effective dosage of HPN-100 for a patient in need of treatment for a nitrogen retention disorder, which comprises monitoring the effect of an initial dosage of HPN-100 (para [0022], "glyceryl-tri(4-phenyl butyrate)"; para [0029], "hepatic encephalopathy"; para [0035]). Summar does not teach monitoring the patient's urinary phenylacetyl glutamine (PAGN) output. However, Brusilow-647 teaches a method of determining the patient's urinary phenylacetyl glutamine output and total urinary nitrogen (col 2, ln 26-32; Fig. 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to use the method of determining the urinary phenylacetyl glutamine output taught in Brusilow-647, in order to determine the effective dosage of HPN-100 for a patient and/or how to adjust the initial dosage of HPN-100 to produce a desired ammonia scavenging effect, as a correlation of phenylacetyl glutamine to phenylacetate administration is disclosed in Brusilow-647 (col 2, ln 26-32), a correlation similar to which would be likely between the administration of HPN-100 and urinary phenylacetyl glutamine output, phenyl acetate being a metabolite of HPN-100 (Summar, para [0005]).

Claims 6-8, 19-22 and 28 lack an inventive step under PCT Article 33(3) as being obvious over Summar in view of US 5,968,979 A to Brusilow (hereinafter "Brusilow-979").

Regarding claim 6, Summar teaches a method to determine an effective dosage of HPN-100 for a patient in need of treatment for a nitrogen retention disorder (para [0022], "glyceryl-tri(4-phenyl butyrate)"; para [0029], "hepatic encephalopathy"; para [0035]). Summar does not teach HPN-100 conversion to PAGN. However, Brusilow-979 teaches HPN-100 conversion to PAGN (col 4, ln 1-26, "n = 2"; col 5, ln 3-15; col 5, ln 29-35). It would have been obvious to one of ordinary skill in the art to calculate the dosage of HPN-100 based on a utilization efficiency for HPN-100 conversion into PAGN of about 60% to about 75%, in order to achieve effective plasma concentrations of phenylacetate for acetylation of glutamine, by routine experimentation, as Brusilow-979 teaches the intermediate formation of phenylacetate that produces PAGN by acetylation of glutamine (col 3, ln 3-7).

==========Continued in Supplemental Box==========

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 09/30362

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Box V.2. Citations and Explanations:

Regarding claim 7, Summar (para [0022], [0029], [0035]) and Brusilow-979 (col 4, ln 1-26; col 5, ln 29-35) teach the method of claim 6. Neither Summar nor Brusilow teaches a method wherein the dosage of HPN-100 is calculated from the patient's dietary protein intake. However, it would have been obvious to one of ordinary skill in the art to determine the dosage of HPN-100, in order to effectively deplete accumulated nitrogen via acetylation of glutamine, as taught in Brusilow-979 (col 3, ln 3-7), as the plasma level of glutamine would be likely to depend on the protein intake of the patient, as taught in Brusilow-979 (col 1, ln 41-45).

Regarding claim 8, Summar (para [0022], [0029], [0035]) and Brusilow-979 (col 4, ln 1-26; col 5, ln 29-35) teach the method of claim 7. Neither Summar nor Brusilow-979 teaches a method wherein the dosage of HPN-100 is reduced to account for the patient's residual urea synthesis capacity. However, it would have been obvious to one of ordinary skill in the art to reduce the dosage to account for the patient's residual urea synthesis capacity, by routine experimentation, as urea synthesis would be likely to lesson the plasma nitrogen accumulation, as taught in Brusilow-979 (col 1, ln 27-34).

Regarding claim 19, Brusilow-979 teaches a method to treat a UCD patient with a PBA prodrug, wherein the prodrug produces equivalent or better ammonia level control compared to PBA (col 2, ln 25-34; col 3, ln 42-59, "triglycerides of phenyl alkanoic acid"; col 4, ln 1-26). Brusilow-979 does not teach determining the AUC and Cmax for PBA when the patient receives the PBA prodrug. However, Summar teaches determining the blood levels of phenyl butyrate in a patient (para [0035]). It would have been obvious to one of ordinary skill in the art to determine the effective dosage of the PBA prodrug, in order to treat UCD without the excessive sodium intake associated with administration of phenyl butyrate, as taught in Brusilow-979 (col 2, ln 15-24), by comparing the AUC and Cmax for the prodrug with those when the patient receives an equimolar amount of PBA, by routine experimentation, as the pharmacokinetic parameters would be a measure of the plasma-level of PBA in the patient, measurement of which for determining dosage has been disclosed in Summar (para [0035], "sodium phenyl butyrate and its metabolites").

Regarding claim 20, Brusilow-979 further teaches the method of claim 19, wherein the PBA prodrug is HPN-100 (col 4, ln 1-26, "n = 2").

Regarding claims 21 and 22, Brusilow-979 (col 2, ln 25-34; col 3, ln 42-59) and Summar (para [0035]) teach the method of claim 20. Neither Brusilow nor Summar teaches a method wherein the AUC for PBA exposure is lower with the prodrug than with PBA by at least about 20% or by at least 30%. However, it would have been obvious to one of ordinary skill in the art to expect AUC for PBA exposure to be lower by 20-30% for PBA prodrug than with PBA, in order to treat UCD with minimum exposure to PBA, as taught in Brusilow-979 (col 2, ln 15-24), as the triglyceride of PBA would be likely to produce a stable drug level by gradual beta-oxidation of the prodrug, as taught in Brusilow-979 (col 2, ln 25-34).

Regarding claim 28, Brusilow-979 teaches a method to treat a patient having a nitrogen retention disorder with the PBA prodrug HPN-100 (col 3, ln 42-59, "triglycerides of phenyl alkanoic acid"; col 4, ln 1-26). Brusilow-979 does not teach the AUC or Cmax of PBA. However, Summar teaches determining the blood levels of phenyl butyrate in a patient (para [0035]). It would have been obvious to one of ordinary skill in the art to determine the effective dosage of the PBA prodrug so that AUC for PBA is less than about 600 and the Cmax for PBA is less than about 100 when the PBA prodrug is administered, in order to treat UCD without the excessive sodium intake associated with administration of phenyl butyrate, as taught in Brusilow-979 (col 2, ln 15-24), through routine experimentation, as the pharmacokinetic parameters would be a measure of the plasma-level of PBA in the patient, measurement of which for determining dosage has been disclosed in Summar (para [0035], "sodium phenyl butyrate and its metabolites").

Claims 12-18 and 23-27 lack an inventive step under PCT Article 33(3) as being obvious over Brusilow-647 in view of Brusilow-979.

Regarding claim 12, Brusilow-979 teaches a method to treat a patient having an ammonia retention disorder with a suitable dosage of a PAA prodrug comprising administering to the patient the suitable dosage of the PAA prodrug (col 4, ln 1-26; col 3, ln 56-59). Brusilow-979 does not teach a method of determining the urinary PAGN output of the patient. However, Brusilow-647 teaches a method of determining the urinary PAGN output in a patient (col 2, ln 26-32; Fig 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to estimate the target urinary PAGN output based on 60-75% convertion of the pro-drug, taking into account the residual urea synthesis capacity and dietary protein intake of the patient, by the method taught in Brusilow-647, in order to determine the amount of the PAA prodrug needed to produce the target amount of urinary PAGN for a patient, as a correlation of urinary PAGN output to the residual urea synthesis capacity and dietary protein intake of the patient and to PAA prodrug administration is disclosed in Brusilow-979 (col 1, ln 27-34; ln 41-45; col 5, ln 3-15; ln 29-35).

Regarding claim 13, Brusilow-979 further teaches the method of claim 12, wherein the PAA prodrug is HPN-100 (col 4, ln 1-26, "n = 2").

Regarding claim 14, Brusilow-979 further teaches the method of claim 12, wherein the PAA prodrug is HPN-100, administered in fewer doses per day (col 3, ln 42-55; col 4, ln 1-26). Brusilow-979 does not teach administering two or three doses of HPN-100 per day. However, it would have been obvious to one of ordinary skill in the art to administer two or three doses of HPN-100 to the patient with clinically significant residual urea synthetic capacity, in order to reduce plasma ammonium to normal levels, as the urea synthetic capacity would be likely to aid in the depletion of nitrogen, as taught in Brusilow-979 (col 1, ln 27-34), thus reducing the number of doses per day of HPN-100 required to be administered to the patient.

==========Continued in Next Supplemental Box==========

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 09/30362

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Prior Supplemental Box:

Regarding claim 15, Brusilow-979 teaches a method of treatment to a patient comprising substituting HPN-100 for phenylacetate or phenylbutyrate (col 2, ln 25-34; col 3, ln 42-55). Brusilow-979 does not teach a method of determining the urinary PAGN output of the patient. However, Brusilow-647 teaches a method of determining the urinary PAGN output (col 2, ln 26-32; Fig. 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to transition a patient receiving treatment with an initial amount of phenylacetate or phenylbutyrate to a final amount of HPN-100, by monitoring the amount of urinary PAGN excreted by the patient, in order to assess the effectiveness of the replacement amount of the HPN-100 by the method taught in Brusilow-647, by routine experimentation, as the urinary PAGN output would be a measure of the effectiveness of the waste nitrogen depletion by the drug administered, as taught in Brusilow-647 (col 2, ln 26-32).

Regarding claim 16, Brusilow-979 teaches the method of claim 15 (col 2, ln 25-34; col 3, ln 42-55). Brusilow-979 does not teach determining the urinary PAGN. However, Brusilow-647 teaches a method of determining the urinary PAGN output (col 2, ln 26-32; Fig. 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to reduce the amount of HPN-100 based on the increase in the amount of urinary PAGN caused by the transition, in order to effectively treat nitrogen-retention disorders, by routine experimentation, as a correlation between urinary PAGN output and HPN-100 is taught in Brusilow-979 (col 5, ln 3-15; ln 29-35).

Regarding claim 17, Brusilow-979 teaches a method of treatment to a patient comprising substituting HPN-100 for phenylacetate or phenylbutyrate (col 2, ln 25-34; col 3, ln 42-55). Brusilow-979 does not teach a method of determining the urinary PAGN output of the patient. However, Brusilow-647 teaches a method of determining the urinary PAGN output (col 2, ln 26-32; Fig. 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to gradually transition a patient receiving treatment with an initial amount of phenylacetate or phenylbutyrate to a final amount of HPN-100 in small amounts, by monitoring the amount of urinary PAGN excreted by the patient, in order to assess the effectiveness of the replacement amount of the HPN-100 in depleting waste nitrogen as PAGN, by routine experimentation, as the urinary PAGN output would be a measure of the effectiveness of the waste nitrogen depletion by the drug administered, as taught in Brusilow-647 (col 2, ln 26-32).

Regarding claim 18, Brusilow-979 teaches a method of treatment with HPN-100 (col 3, ln 42-55). Brusilow-979 does not teach a method of determining the urinary PAGN output of the patient. However, Brusilow-647 teaches a method of determining the urinary PAGN output (col 2, ln 26-32; Fig. 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to initiate treatment with HPN-100 in a step-wise fashion and increase the amount of HPN-100 gradually, by monitoring the urinary PAGN based on 60-75% convertion by the method taught in Brusilow-647, taking into account the residual urea synthesis capacity and dietary protein intake of the patient, in order to determine the maintenance dose of HPN-100 effective for the treatment of nitrogen-retention disorders, as a correlation of urinary PAGN output to the residual urea synthesis capacity and dietary protein intake of the patient and HPN-100 administration is disclosed in Brusilow-979 (col 1, ln 27-34; ln 41-45; col 5, ln 3-15; ln 29-35).

Regarding claim 23, Brusilow-647 teaches a method to determine the nitrogen elimination capacity of a patient having a nitrogen retention disorder, being treated with a nitrogen scavenging drug (col 2, ln 26-32; Fig. 3; col 4, ln 35-46, "urinary phenylacetyl glutamine"). Brusilow-647 does not teach a method to determine a suitable dietary protein level for a patient. However, it would have been obvious to one of ordinary skill in the art to use the method taught in Brucilow-647 to determine the patient's endogenous nitrogen elimination capacity with and without the nitrogen scavenging drug, in order to determine the amount of dietary protein the patient can have while being treated with the selected dosage of the nitrogen scavenging drug, through routine experimentation, since the dietary protein intake would be likely to influence the nitrogen elimination capacity of the patient, as taught in Brucilow-979 (col 1, ln 27-34; ln 41-45; col 5, ln 3-15; ln 29-35).

Regarding claim 24, Brusilow-979 further teaches the method of claim 23, wherein the nitrogen scavenging drug is HPN-100 (col 4, ln 1-26, "n = 2").

Regarding claim 25, Brusilow-647 (col 2, ln 26-32; Fig. 3; col 4, ln 35-46) and Brusilow-979 (col 1, ln 27-34; col 1, ln 41-45; col 5, ln 3-15) teach the method of claim 24, wherein Brusilow-979 teaches the selected dosage of HPN-100 (col 4, ln 54-58). Neither Brusilow-647 nor Brusilow-979 teaches a dosage of HPN-100 of up to about 19 grams per day. However, it would have been obvious to one of ordinary skill in the art to determine the dosage of HPN-100 based on the dietary protein the patient intake of the patient, in order to provide effective elimination of waste nitrogen, as PAGN as taught in Brusilow-979 (col 5, ln 3-15), by routine experimentation, as the patient's inherent ability to process nitrogen and the dietary protein intake would be likely to influence the nitrogen elimination capability, measured by the method taught in Brucilow-647 (col 2, ln 26-32; Fig 3; col 4, ln 35-46, "urinary phenylacetyl glutamine").

Regarding claim 26, Brusilow-979 teaches a method to treat a patient with a PBA prodrug, comprising administering HPN-100 to a subject having HE or UCD (col 3, ln 42-59, "triglycerides of phenyl alkanoic acid"; col 4, ln 1-26; col 4, ln 54-58). Brusilow does not teach a daily dose in excess of 19 g per day of the prodrug. However, it would have been obvious to one of ordinary skill in the art to determine the dosage of HPN-100 based on the dietary protein the patient intake of the patient, in order to provide effective elimination of waste nitrogen as PAGN as taught in Brusilow-979 (col 5, ln 3-15), through routine experimentation, since the patient's inherent ability to process nitrogen and the dietary protein intake would likely influence the nitrogen elimination capability, measured by the method taught in Brucilow-647 (col 2, ln 26-32; Fig. 3; col 4, ln 35-46, "urinary phenylacetyl glutamine").

==========Continued in Next Supplemental Box==========

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 09/30362

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Prior Supplemental Box:

Regarding claim 27, Brusilow-647 (col 2, ln 26-32; Fig. 3; col 4, ln 35-46) and Brusilow-979 (col 1, ln 27-34; col 1, ln 41-45; col 5, ln 3-15) teach the method of claim 26. Neither Brusilow-647 nor Brusilow-979 teaches a daily dose of HPN-100 is between about 199 and about 57 g. However, it would have been obvious to one of ordinary skill in the art to determine the dosage of HPN-100 based on the dietary protein the patient intake of the patient, in order to provide effective elimination of waste nitrogen as PAGN, as taught in Brusilow-979 (col 5, ln 3-15), through routine experimentation, as the patients inherent ability to process nitrogen and the dietary protein intake would likely influence the nitrogen elimination capability, measured by the method taught in Brucilow-647 (col 2, ln 26-32; Fig. 3; col 4, ln 35-46, "urinary phenylacetyl glutamine").

Claims 9-11 and 29 lack an inventive step under PCT Article 33(3) as being obvious over Summar in view of Brusilow-647 and further in view of Brusilow-979.

Regarding claim 9, Summar teaches a method to determine a dosage of a PAA prodrug for a patient having an ammonia retention disorder (para [0022],"glyceryl-tri(4-phenyl butyrate)"; para [0029], "hepatic encephalopathy"; para [0035]). Summar does not explicitly teach determining the patient's residual urea synthesis capacity or dietary intake or estimating the urinary PAGN output. However, Brusilow-647 teaches a method of determining the urinary PAGN output (col 2, ln 26-32; Fig. 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to estimate the target urinary PAGN output for a patient based on 60-75% convertion of the pro-drug, by the method taught in Brusilow-647, by taking into account the residual urea synthesis capacity and dietary protein intake of the patient, in order to determine the amount of the PAA prodrug needed to produce the target amount of urinary PAGN, as a correlation of urinary PAGN output to the residual urea synthesis capacity and dietary protein intake of the patient and to PAA prodrug administration is disclosed in Brusilow-979 (col 1, ln 27-34; col 1, ln 41-45; col 5, ln 3-15; col 5, ln 29-35).

Regarding claim 10, Summar further teaches the method of claim 9, wherein the PAA prodrug is phenylbutyric acid (PBA) or a pharmaceutically acceptable salt thereof (para [0022]).

Regarding claim 11, Summar further teaches the method of claim 9, wherein the PAA prodrug is HPN-100 (para [0022], "glyceryl-tri(4-phenyl butyrate)").

Regarding claim 29, Brusilow-979 (col 3, ln 42-59, "triglycerides of phenyl alkanoic acid"; col 4, ln 1-26) and Summar (para [0035]) teach the method of claim 28, wherein Summar further teaches that administering the effective dosage of HPN-100 to the patient produces a change in plasma ammonia level in the patient (para [0035]). Neither Brusilow-979 nor Summar explicitly teaches achieving normal plasma ammonia levels. However, it would have been obvious to one of ordinary skill in the art to produce normal plasma ammonia levels by administration of HPN-100, as a reduction in plasma ammonium levels following administration of a metabolite of HPN-100, namely phenyl acetic acid, is taught in Brusilow-647 (col 4, ln 46-50; ln 64-68).

Claims 1-29 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

International application No
PCT/US2009/055256

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SIMELL O ET AL: "Waste nitrogen excretion via amino acid acylation: Benzoate and phenylacetate in lysinuric protein intolerance" PEDIATRIC RESEARCH, WILLIAMS AND WILKINS, BALTIMORE, MD, US, vol. 20, no. 11, 1 January 1986 (1986-01-01), pages 1117-1121, XP009127277 ISSN: 0031-3998	30-33
Y	the whole document	1-29
	-/--	

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 December 2009	30/12/2009

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Moreno de Vega, C

Form PCT/ISA/210 (second sheet) (April 2005)

International application No
PCT/US2009/055256

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MACARTHUR ROBERT B ET AL: "Pharmacokinetics of sodium phenylacetate and sodium benzoate following intravenous administration as both a bolus and continuous infusion to healthy adult volunteers" MOLECULAR GENETICS AND METABOLISM, ACADEMIC PRESS, SAN DIEGO, CA, US, vol. 81, no. Suppl.1, 1 April 2004 (2004-04-01), pages S67-S73, XP009127291 ISSN: 1096-7192 the whole document	1-33
Y	TANNER L M ET AL: "Nutrient intake in lysinuric protein intolerance" JOURNAL OF INHERITED METABOLIC DISEASE, KLUWER ACADEMIC PUBLISHERS, DO, vol. 30, no. 5, 21 June 2007 (2007-06-21), pages 716-721, XP019548954 ISSN: 1573-2665 page 716 - page 717	1-33
X	LEE B ET AL: "Preliminary data on adult patients with urea cycle disorders (UCD) in an open-label, switch-over, dose-escalation study comparing a new ammonia scavenger, glyceryl tri(4-phenylbutyrate) (HPN-100), to buphenyl (sodium phenylbutyrate (PBA))" JOURNAL OF INHERITED METABOLIC DISEASE, KLUWER, DORDRECHT, NL, vol. 31, no. suppl. 1, 1 August 2008 (2008-08-01), page 91, XP009127344 ISSN: 0141-8955	1-5, 15-17, 19-22, 30-33
Y	the whole document	1-33

Electronic Patent Application Fee Transmittal

Application Number:	13610580
Filing Date:	11-Sep-2012
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS
First Named Inventor/Applicant Name:	Bruce Scharschmidt
Filer:	Lauren Stevens
Attorney Docket Number:	HOR0027-201-US

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($)
Miscellaneous:				
Submission- Information Disclosure Stmt	1806	1	180	180
		Total in USD ($)		**180**

Electronic Acknowledgement Receipt

EFS ID:	23049641
Application Number:	13610580
International Application Number:	
Confirmation Number:	1957
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS
First Named Inventor/Applicant Name:	Bruce Scharschmidt
Customer Number:	101325
Filer:	Lauren Stevens
Filer Authorized By:	
Attorney Docket Number:	HOR0027-201-US
Receipt Date:	29-JUL-2015
Filing Date:	11-SEP-2012
Time Stamp:	11:16:02
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$180
RAM confirmation Number	11774
Deposit Account	504297
Authorized User	LECHNER, VALERIE

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

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Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Information Disclosure Statement (IDS) Form (SB08)	HOR0027-201-US_IDS.pdf	154251 569fc23a12b65bdb6c731ec217e44e7297be6b67	no	10
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Information:					
This is not an USPTO supplied IDS fillable form					
2	Foreign Reference	WO9422494A1.pdf	12620304 3c4bf1d6042a077773a3ce513df7225657faf744	no	460
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Information:					
3	Foreign Reference	WO2013048558A2.PDF	1888960 22400ae411d096f1cd99145aeff6b5f3c4c6489b	no	37
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4	Foreign Reference	WO2013158145A1.pdf	2539118 0d5a792e8d76a2e55c5cebc1dab781a5c54c6170	no	50
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5	Non Patent Literature	Amodio_JHepatol_2008.PDF	10178291 e023c89f552d47a9386de234659c25f7dd3cd110	no	8
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6	Non Patent Literature	ANDA_Hyperion.pdf	1828552 353709de30629160d699c3f58bb3c88d5f6cdafb	no	27
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8	Non Patent Literature	Barsotti_2001.pdf	6404000 18d4317a40b5b4c45db7054449ad11294a0e3a40	no	10
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12	Non Patent Literature	Blei_AmJGastroenterol_2001.PDF	227027 fc3b5ed203fad8fad2619130db53a0de1654fedf	no	9
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16	Non Patent Literature	CDER_Ammonaps_Med_Review_Part1.pdf	16640989 a22da4b3c827c27ef62f9b760a170732da23e467	no	27
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24	Non Patent Literature	CDER_Ammonaps_Label.pdf	12642794 c256d47cc011b7bb52bfcb4cc11837d0532b7f7d	no	20
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30	Non Patent Literature	EurMedAgencyAnnex.pdf	16323565 be6351760ffcdae2554c3cb87ea90463262b3a1c	no	33
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31	Non Patent Literature	EuroMedAgency_2009.pdf	157626 6ff01398745080dd7a0de135e6e23e61d7fd7617	no	2
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34	Non Patent Literature	Feillet_1998.pdf	7152935 e04ce74238c3b463debefe70fa195a1a467e0b3b	no	11
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35	Non Patent Literature	FDA_Carbaglu_Label_2010.pdf	2753804 dc59e8d75403150467c1cff9a44ab474868f1bd2	no	7
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36	Non Patent Literature	Feoli_Fonseca_1996.pdf	4802821 fdbcbca624b8860f3f8fd454e510b05a767f26f1	no	6
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37	Non Patent Literature	Ferenci_Hepatology_2002.pdf	116563 fc1bf6551ecb0dcc4f17fe4e118ea4d16a28122a	no	6
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38	Non Patent Literature	Fernandes_2000.pdf	3678938 c3a3c703d5877ee1da6b6b0f57deacf958090d07	no	8
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39	Non Patent Literature	Geraghty_2001.pdf	14402723 799396dfc117dcaaf43e2bcc05ada560f664280b	no	19
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40	Non Patent Literature	GhabrilM_ClinPharmainDrugDev_2013.pdf	290154 fcc71dd56d49e17102c155bce28ea98bd0d8d355	no	7
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41	Non Patent Literature	Gilbert_2001.pdf	8059098 5a769918beaae445c0e630488e9b9f0fbb20735c	no	10
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43	Non Patent Literature	Gropman_2007.pdf	17152815 1c0674d3fb3d4a5de4d79fb4a6a53f1c9da20859	no	26
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44	Non Patent Literature	Hassanein_AmJGastroenterol_2009.pdf	211886 0847c8721514dd408069540f64a3e34ebdb44834	no	9
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45	Non Patent Literature	Hassanein_DigDisSci_2008.pdf	22039102 207cede7e5fb9495559504be5345dc5c0b030290	no	10
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46	Non Patent Literature	Hassanein_Hepatology_2007.pdf	481035 b7d7c14d4993dff95b13bf0ec7fb011d9be9b81c	no	10
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48	Non Patent Literature	ISRandWOofISA_PCT_US2009_030362.pdf	469369 bcf873398e7d2e8c77b72a69df12b8a76e5e641e	no	7
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49	Non Patent Literature	ISRandWOofISA_PCT_US2009_055256.PDF	101384 01c5c816dea1bdd4c2422b284619853c4f3d3973	no	2
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50	Non Patent Literature	Kleppe_2003.pdf	7683625 768f79f170e1b8e94c6d7b1bf0115e0cffcb2d4a	no	11
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52	Non Patent Literature	Lee_2001.pdf	7977588 d4c375f0e78bf1e1a963e0a75be3378e46fe707b	no	10
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54	Non Patent Literature	IPR_US8404215_Petition.pdf	589626 e1427bb49e001ec971a224d722f15ad2be77f280	no	68
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55	Non Patent Literature	IPR_US8642012_Petition.pdf	546453 d2fb362659177cf0b127473ae7b496c2cbcc1db1	no	68
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56	Non Patent Literature	Lee_2013.pdf	76104 96ae17e263d71c8085e19d558cec699beaa893e7	no	2
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57	Non Patent Literature	Leonard_2002.pdf	6155259 fe3818002b4c3fcab9c54a4db3efb20cc5dc07dd	no	9
Warnings:					
Information:					
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Information:					
59	Non Patent Literature	MaestriNE_JPediatr_1991.pdf	3965613 b1f57ed403c0ae2135a0c0577b8d5a806baffa1f	no	6
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Information:					
60	Non Patent Literature	Maestri_1995.pdf	2431046 a79699bd040299f284cdaa7dd1dd2b8e7c261794	no	7
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Information:					
61	Fee Worksheet (SB06)	fee-info.pdf	30622 9c50880828a619078213eb2b3008c16c933a8e63	no	2
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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.

Doc Code: DIST.E.FILE Document Description: Electronic Terminal Disclaimer - Filed	PTO/SB/26 U.S. Patent and Trademark Office Department of Commerce

Electronic Petition Request	**TERMINAL DISCLAIMER TO OBVIATE A DOUBLE PATENTING REJECTION OVER A "PRIOR" PATENT**
Application Number	13610580
Filing Date	11-Sep-2012
First Named Inventor	Bruce Scharschmidt
Attorney Docket Number	HOR0027-201-US
Title of Invention	METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS

☒ Filing of terminal disclaimer does not obviate requirement for response under 37 CFR 1.111 to outstanding Office Action

☒ This electronic Terminal Disclaimer is not being used for a Joint Research Agreement.

Owner	Percent Interest
Horizon Therapeutics, Inc.	100%

The owner(s) with percent interest listed above in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of prior patent number(s)

8642012

as the term of said prior patent is presently shortened by any terminal disclaimer. The owner hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and the prior patent are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the owner does not disclaim the terminal part of the term of any patent granted on the instant application that would extend to the expiration date of the full statutory term of the prior patent, "as the term of said prior patent is presently shortened by any terminal disclaimer," in the event that said prior patent later:
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- is found invalid by a court of competent jurisdiction;
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- has all claims canceled by a reexamination certificate;
- is reissued; or
- is in any manner terminated prior to the expiration of its full statutory term as presently shortened by any terminal disclaimer.

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○	I certify, in accordance with 37 CFR 1.4(d)(4), that the terminal disclaimer fee under 37 CFR 1.20(d) required for this terminal disclaimer has already been paid in the above-identified application.

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○ Small Entity

○ Micro Entity

◉ Regular Undiscounted

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

THIS PORTION MUST BE COMPLETED BY THE SIGNATORY OR SIGNATORIES

I certify, in accordance with 37 CFR 1.4(d)(4) that I am:

◉ An attorney or agent registered to practice before the Patent and Trademark Office who is of record in this application

Registration Number 36691

○ A sole inventor

○ A joint inventor; I certify that I am authorized to sign this submission on behalf of all of the inventors as evidenced by the power of attorney in the application

○ A joint inventor; all of whom are signing this request

Signature	/Lauren Stevens/
Name	Lauren Stevens

*Statement under 37 CFR 3.73(b) is required if terminal disclaimer is signed by the assignee (owner).
Form PTO/SB/96 may be used for making this certification. See MPEP § 324.

Electronic Patent Application Fee Transmittal

Application Number:	13610580
Filing Date:	11-Sep-2012
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS
First Named Inventor/Applicant Name:	Bruce Scharschmidt
Filer:	Lauren Stevens/Valerie Lechner
Attorney Docket Number:	HOR0027-201-US

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Statutory or Terminal Disclaimer	1814	1	160	160
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**160**

Doc Code: DISQ.E.FILE
Document Description: Electronic Terminal Disclaimer – Approved

Application No.: 13610580

Filing Date: 11-Sep-2012

Applicant/Patent under Reexamination: Scharschmidt et al.

Electronic Terminal Disclaimer filed on July 29, 2015

☒ APPROVED

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EFS ID:	23054751
Application Number:	13610580
International Application Number:	
Confirmation Number:	1957
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS
First Named Inventor/Applicant Name:	Bruce Scharschmidt
Customer Number:	101325
Filer:	Lauren Stevens/Valerie Lechner
Filer Authorized By:	Lauren Stevens
Attorney Docket Number:	HOR0027-201-US
Receipt Date:	29-JUL-2015
Filing Date:	11-SEP-2012
Time Stamp:	11:44:21
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$160
RAM confirmation Number	12117
Deposit Account	504297
Authorized User	LECHNER, VALERIE

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

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Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Electronic Terminal Disclaimer-Filed	eTerminal-Disclaimer.pdf	33376 93148bd6869dee8bd568cf5cd86855537c875b4c	no	2
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30586 88349bfcf5da8bc623b10da2fc8cd06b1635ae7c	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	63962		

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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
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 13/610,580	Filing Date 09/11/2012	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

	(Column 1)	(Column 2)		
FOR	NUMBER FILED	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

		(Column 1)		(Column 2)	(Column 3)		
	07/29/2015	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
AMENDMENT	Total (37 CFR 1.16(i))	* 17	Minus	** 40	= 0	X $80 =	0
	Independent (37 CFR 1.16(h))	* 4	Minus	***6	= 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

		(Column 1)		(Column 2)	(Column 3)		
		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
AMENDMENT	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.

LIE
/EFREM WARREN/

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.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/610,580	09/11/2012	Bruce Scharschmidt	HOR0027-201-US	1957

101325 7590 05/19/2016

GLOBAL PATENT GROUP - HOR
1005 NORTH WARSON ROAD
SUITE 404
SAINT LOUIS, MO 63132

EXAMINER
TOWNSLEY, SARA ELIZABETH

ART UNIT	PAPER NUMBER
1629	

NOTIFICATION DATE	DELIVERY MODE
05/19/2016	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):

admin@globalpatentgroup.com
vtruman@globalpatentgroup.com
LStevens@horizonpharma.com

Office Action Summary	Application No. 13/610,580	Applicant(s) SCHARSCHMIDT ET AL.	
	Examiner SARA E. TOWNSLEY	Art Unit 1629	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 *7/29/2015*.

☐ 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) *1,2,5,6 and 9-12* 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) *1,2,5,6 and 9-12* 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 *7/29/2015*.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

FINAL REJECTION

Receipt is acknowledged of Applicants' Amendments and Remarks, filed Jul. 29, 2015.

Rejections and/or objections not reiterated from previous Office Actions are hereby withdrawn. The rejections and/or objections set forth below are either maintained or newly applied, and constitute the complete set presently applied to the instant claims.

STATUS OF THE CLAIMS

Claims 3, 4, 7, 8, and 13 have been cancelled.

Claims 1, 2, 5, 6, 11, and 12 have been amended and incorporate no new matter.

No new claims have been added.

Claims 1, 2, 5, 6, and 9-12 now represent all claims currently pending and under consideration.

INFORMATION DISCLOSURE STATEMENT

The information disclosure statement (IDS) submitted on Jul. 29, 2015 was filed after the mailing date of the non-final action on Feb. 27, 2015. The submission is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

TERMINAL DISCLAIMER

The terminal disclaimer filed on Jul. 29, 2015 disclaiming the terminal portion of any patent granted on this application which would extend beyond the expiration date of USPN 8,642,012 has been reviewed and is accepted. The terminal disclaimer has been recorded.

MAINTAINED REJECTIONS

The following rejection is maintained from the previous Office Action dated Feb. 27, 2015, on the ground that the references cited therein continue to read on the limitations of the amended claims.

Claim Rejections - 35 USC § 103

Claims 1, 2, 5, 6, and 9-12 stand rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Scharschmidt (US Pub. 2012/0022157) in view of McGuire et al. (*Hepatology* 51, 2077-2085 (2010)).

Independent claim 1 recites a method of treating urea cycle disorders in a subject; and **independent claim 5** recites a method of adjusting the dosage of glyceryl tri-[4-phenylbutyrate], a PAA prodrug, each comprising the steps of

(a) administering a first dosage of glyceryl tri-[4-phenylbutyrate],

(b) measuring plasma PAA and PAGN levels,

(c) calculating a plasma PAA:PAGN ratio,

(d) determining whether the glyceryl tri-[4-phenylbutyrate] dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range,
where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased, and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and
(e) administering a second dosage of the glyceryl tri-[4-phenylbutyrate] based on the determination in (d).

Scharschmidt discloses the treatment of nitrogen retention disorders, including UCDs (urea cycle disorders), by administering a PAA prodrug, e.g., HPN-100 (para. [0097]), a.k.a. glyceryl tri-[4-phenylbutyrate], as recited by the amended claims.

Scharschmidt discloses methods for determining and adjusting the schedule and dose of orally administered nitrogen scavenging drugs, including glyceryl tri-[4-phenyl-butyrate] (a.k.a. HPN-100 or GPB), based upon the urinary excretion of the drug metabolite phenylacetylglutamine (PAGN) and/or total urinary nitrogen (para. [0021]).

In particular, Scharschmidt discloses methods of (a) administering a first dosage of HPN-100 (glyceryl tri-[4-phenylbutyrate]) (para. [0173]) and (b) measuring urinary PAGN levels (para. [0174]). Scharschmidt further teaches the step of determining whether the PAA prodrug dosage needs to be adjusted based on whether the measured levels of PAGN falls within a target range (paras. [0106], [0174]). Scharschmidt further discloses measuring plasma PAA levels and plasma PAGN levels (Table 4).

Scharschmidt also discloses the step of (e) administering a second dosage of the PAA prodrug based on the determination in (d) (paras. [0106], [0174]).

However, Scharschmidt does not disclose calculating a plasma PAA:PAGN ratio, and comparing the PAA:PAGN ratio to a target range to determine whether the dosage needs to be increased or decreased.

McGuire discloses measuring metabolites in blood and urine after administration of the claimed PAA prodrug, GPB (a.k.a. glyceryl tri-[4-phenylbutyrate]) (abstract), wherein the metabolites include plasma PAA and PAGN (p. 2079, col 2, ¶ 3), which values can easily be compared as a ratio (p. 2081, col. 1, ¶ 2). McGuire further teaches that metabolites important in the monitoring of PAA prodrugs include both PAA and PAGN; and that urinary testing is not as complete and thorough as plasma testing (p. 2081, col. 2, ¶ 1).

Therefore, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Scharschmidt by measuring plasma levels of PAA and PAGN, instead of urinary PAA and PAGN levels, and comparing them as a ratio, in order to more accurately assess the patient's metabolism of PAA prodrugs, e.g., glyceryl tri-[4-phenylbutyrate], and evaluate any need to adjust the dosage, with a reasonable expectation of success, because McGuire teaches that urinary testing is not as complete and thorough as testing for plasma levels of PAA and PAGN.

Independent claim 2 recites a method of treating urea cycle disorders in a subject who has previously been administered a first dosage of a PAA prodrug; and **independent claim 6** recites a method of optimizing the therapeutic efficacy of a PAA

prodrug in a subject who has previously been administered a first dosage of a PAA prodrug, each comprising the steps of

(a) measuring plasma PAA and PAGN levels,

(b) calculating a plasma PAA:PAGN ratio,

(c) determining whether the first PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(d) administering a second dosage of the PAA prodrug based on the determination in (c).

Scharschmidt discloses methods of treating urea cycle disorders in a subject who has previously been administered a first dosage of a PAA prodrug (para [0106], [0173]) comprising measuring PAGN levels (para [0174]). Scharschmidt also teaches a method of optimizing the therapeutic efficacy of a PAA prodrug in a subject (para [0297],[0173]) who has previously been administered a first dosage of a PAA prodrug (para [0106]) comprising measuring PAGN levels (para [0174]).

Scharschmidt further teaches the step of determining whether the PAA prodrug dosage needs to be adjusted based on whether the measured levels of PAGN falls within a target range (paras. [0106], [0174]).

Scharschmidt also discloses the step of (d) administering a second dosage of the PAA prodrug based on the determination in (c) (paras. [0106], [0174]).

However, Scharschmidt does not disclose calculating a plasma PAA:PAGN ratio, and comparing the PAA:PAGN ratio to a target range to determine whether the dosage needs to be increased or decreased.

McGuire discloses measuring metabolites in blood and urine after administration of the claimed PAA prodrug, GPB (a.k.a. glyceryl tri-[4-phenylbutyrate]) (abstract), wherein the metabolites include plasma PAA and PAGN (p. 2079, col 2, ¶ 3), which values can easily be compared as a ratio (p. 2081, col. 1, ¶ 2). McGuire further teaches that metabolites important in the monitoring of PAA prodrugs include both PAA and PAGN; and that urinary testing is not as complete and thorough as plasma testing (p. 2081, col. 2, ¶ 1).

Therefore, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Scharschmidt by measuring plasma levels of PAA and PAGN, instead of urinary PAA and PAGN, and comparing them as a ratio, in order to more accurately assess the patient's metabolism of PAA prodrugs, and evaluate any need to adjust (optimize) the dosage, with a reasonable expectation of success, because McGuire teaches that urinary testing is not as complete and thorough as testing for plasma levels of PAA and PAGN.

While Scharschmidt does not disclose that the PAA:PAGN ratio falls within a target range of 1 to 2.5, as recited by claim 9, or within a target range of 1 to 2, as recited by claim 10, it would have been *prima facie* obvious to an ordinarily skilled clinician to determine the optimal target range for the plasma PAA:PAGN ratio for the subject being treated, by routine experimentation.

Scharschmidt further teaches that measuring PAGN levels is carried out after the first dosage of PAA prodrug has had sufficient time to reach steady state (para. [0160]), but does not disclose measurement of both PAA and PAGN levels after the first dosage of PAA prodrug has had sufficient time to reach steady state, as recited by claim 11. However, it would have been *prima facie* obvious to an ordinarily skilled clinician to further measure PAA as well as PAGN in order to maintain comparable results, by routine experimentation.

Scharschmidt further teaches measurement of PAGN levels 48 hours to 1 week after the first dosage of PAA prodrug is administered (para (0160), 3 days), but does not disclose measurement of both PAA and PAGN levels 48 hours to 1 week after the first dosage of PAA prodrug is administered, as recited by claim 12. However, it would have been *prima facie* obvious to an ordinarily skilled clinician to further measure PAA as well as PAGN in order to maintain comparable results, by routine experimentation.

The rationale to combine Scharschmidt and McGuire is premised on the findings that (1) the prior art includes each element claimed, with the only difference between the claimed invention and the prior art being the lack of actual combination of the elements in a single prior art reference; (2) one of ordinary skill in the art could have combined the elements as claimed by known methods, and that in combination, each element merely performs the same function as it does separately; and (3) one of ordinary skill in the art would have recognized that the results of the combination were predictable.

As recognized by MPEP §2143, combining prior art elements according to known methods to yield predictable results would motivate the skilled artisan to modify the references with a reasonable expectation of success. The rationale to support a conclusion of *prima facie* obviousness is that all the claimed elements were known in the prior art, and a skilled artisan could have combined the elements as claimed by known methods with no change in their respective functions, and the combination yielded nothing more than predictable results to one of ordinary skill in the art. See *KSR Int'l Co. v. Teleflex Inc.* (550 U.S. 398, 409).

RESPONSE TO ARGUMENTS

Applicant's arguments filed Jul. 29, 2015 have been fully considered but they are not persuasive.

With respect to the rejection under 35 U.S.C. § 103, Applicant contends that McGuire describes a statistical approach to assess bioequivalency of 2 different drugs: glycerol phenylbutyrate (GPB) and sodium phenylbutyrate (NaPBA), each of which are metabolized to phenylbutyric acid (PBA). Applicant contends that McGuire compares the ratio of PBA blood levels following administration of GPB with PBA blood levels following administration of NaPBA, wherein the systemic exposure is calculated based on PBA levels taken at multiple time points from multiple patients during dosing with each of the two different drugs. Thus, Applicant contends that McGuire simply utilizes conventional methodology for assessing bioequivalence of one drug to another; McGuire does not teach the novel and unexpected finding that the ratio of two different

metabolites, PAA and PAGN, taken at the same time from the same patient receiving GPB (glyceryl tri-[4-phenylbutyrate]) is of utility in assessing the effectiveness of PAA to PAGN conversion (Remarks, pp. 1-2).

Applicant further contends that nothing in McGuire teaches or suggests measuring two different metabolites from glyceryl tri-[4-phenylbutyrate] in the same patient, and using the ratio of the two metabolites from the same patient to adjust the glyceryl tri-[4-phenylbutyrate] dosage (Remarks, p. 3).

However, McGuire reports two studies. The comparison of the bioequivalence of GPB and NaPBA summarized by Applicant refers to study UP 1204-001; whereas the rejection references study UP 1204-002, in which GPB only was orally administered to 32 subjects (8 healthy and 24 with cirrhosis). The last dose of GPB was administered on day 15, followed by 48 hours of plasma PK sampling and urine collection, and measurement of PAA and PAGN levels, which values are easily compared as a ratio (p. 2079, para. bridging cols. 1-2; Table 2, lower half). McGuire reports that PAA and PAGN predose concentrations increased during the first 2 to 4 days of multiple dosing, but did not increase consistently thereafter, indicating that a steady state had been reached (p. 2082, col. 1; Fig. 3).

In other words, McGuire in fact exemplifies administration of the claimed PAA prodrug, GPB (a.k.a. glyceryl tri-[4-phenylbutyrate]), followed by measuring PAA and PAGN levels in both blood and urine; i.e., measuring two different plasma metabolites from glyceryl tri-[4-phenyl-butyrate] in the same patient.

While it is acknowledged that the cited references do not explicitly disclose that glyceryl tri-[4-phenylbutyrate] dosage can be optimized by comparing plasma metabolite ratios, various methods of optimizing drug dosage regimens are generally known and/or within the capability of those of ordinary skill in the art. In addition, the cited references disclose the active steps of administering glyceryl tri-[4-phenylbutyrate], followed by measuring plasma metabolite levels of PAA and PAGN. Manipulating those values, e.g., by making a comparison or calculation, constitutes a purely mental step, not an active step in carrying out a new method.

For the foregoing reasons, the rejection of claims 1, 2, 5, 6, and 9-12 under 35 U.S.C. § 103 over Scharschmidt and McGuire is maintained.

CONCLUSION

No claims are allowed.

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.

CORRESPONDENCE

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SARA E. TOWNSLEY whose telephone number is 571-270-7672. The examiner can normally be reached on Mon-Fri from 9:00 am to 5:00 pm (EST). If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jeff S. Lundgren, can be reached at 571-272-5541. 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://portal.uspto.gov/external/portal. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/SARA E. TOWNSLEY/
Examiner, Art Unit 1629

/JEFFREY S. LUNDGREN/
Supervisory Patent Examiner, Art Unit 1629

PTO/SB/08 (09-06)
Approved for use through 03/31/2007. OMB 0651-0031
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

Date Submitted: March 12, 2012

(use as many sheets as necessary)

Sheet 1 of 10

Complete if Known	
Application Number	13/610,580
Filing Date	September 11, 2012
First Named Inventor	Bruce Scharschmidt
Art Unit	1629
Examiner Name	Sara Elizabeth Townsley
Attorney Docket Number	HOR0027-201-US

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
	P1	4,457,942	07-03-1984	Brusilow, S.W.	
	P2	5,654,333	08-05-1997	The United States Of America As Represented By The Department Of Health And Human Services	
	P3	8,094,521	01-10-2012	Nightengale Products LLC	
	P4	8,404,215	03-26-2013	Hyperion Therapeutics, Inc.	
	P5	2003/0195255	10-16-2003	Marshall L. Summar	
	P6	2005/0273359	12-08-2005	Young, D.E.	
	P7	2010/0016207	01-21-2010	Wurtman, RJ et al	
	P8	2014/0142186	05-22-2014	Hyperion Therapeutics, Inc.	
	P9	8,642,012	02-04-2014	Hyperion Therapeutics, Inc.	

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]
	F1	WO1994/22494	10-13-1994	The DuPont Merck Pharmaceutical Company		
	F2	WO2013/048558	04-04-2013	Hyperion Therapeutics, Inc.		
	F3	WO2013/158145	10-24-2013	Hyperion Therapeutics, Inc.		

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.E.T./

Examiner Signature	/Sara E. Townsley/	Date Considered	05/16/2016

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. 1 Applicant's unique citation designation number (optional). 2 See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. 3 Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). 4 For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. 5 Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. 6 Applicant is to place a check mark here if English language Translation is attached.

PTO/SB/08 (09-06)
Approved for use through 03/31/2007. OMB 0651-0031
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Substitute for form 1449/PTO				***Complete if Known***	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT				**Application Number**	13/610,580
				Filing Date	September 11, 2012
Date Submitted: March 12, 2012				**First Named Inventor**	Bruce Scharschmidt
				Art Unit	1629
(use as many sheets as necessary)				**Examiner Name**	Sara Elizabeth Townsley
Sheet	2	of	10	**Attorney Docket Number**	HOR0027-201-US

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]
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				First Named Inventor	Bruce Scharschmidt
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	D89	UNITED STATES PATENT AND TRADEMARK OFFICE, International Search Report and Written Opinion for PCT/ US2014/060543 dated January 23, 2015.	
	D90	VILSTRUP, H., et al., "Hepatic Encephalopathy in Chronic Liver Disease: 2014 Practice Guideline by the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver," Hepatology 60 (2):715-735 (2014).	
	D91	Walsh et al., Chemical Abstract vol. 112, No. 231744	
	D92	Welbourne, T. et al., The Effect of Glutamine Administration on Urinary Ammonium Excretion in Normal Subjects and Patients with Renal Disease, 51 J. Clin. Investigation 1852 (1972).	
	D93	Wilcken, B., Problems in the Management of Urea Cycle Disorders, 81 Molecular Genetics and Metabolism 85 (2004).	
	D94	Wilson, C.J., et al., Plasma Glutamine and Ammonia Concentrations in Ornithine Carbamoyltransferase Deficiency and Citrullinaemia, 24 J. Inherited Metabolic Disease 691 (2001).	
	D95	Wright, G., et al., Management of Hepatic Encephalopathy, 2011 International Journal of Hepatology 1 (2011).	

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.E.T./

Examiner Signature	/Sara E. Townsley/	Date Considered	05/16/2016

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. 1 Applicant's unique citation designation number (optional). 2 See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. 3 Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). 4 For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. 5 Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. 6 Applicant is to place a check mark here if English language Translation is attached.

PTO/SB/08 (09-06)
Approved for use through 03/31/2007. OMB 0651-0031
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Substitute for form 1449/PTO				***Complete if Known***	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT				**Application Number**	13/610,580
Date Submitted: March 12, 2012				**Filing Date**	September 11, 2012
				First Named Inventor	Bruce Scharschmidt
(use as many sheets as necessary)				**Art Unit**	1629
				Examiner Name	Sara Elizabeth Townsley
Sheet	10	of	10	**Attorney Docket Number**	HOR0027-201-US

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]
	D96	Wright, P., Review: Nitrogen Excretion: Three End Products, Many Physiological Roles, 198 J. Experimental Biology 273 (1995).	
	D97	Yajima, et al. Diurnal Fluctuations of Blood Ammonia Levels in Adult-Type Citrullinemia, 137 Tokohu J. Ex/ Med, 213-220 (1982)	
	D98	Yu, Ryan and Potter, Murray, Diagnosis of Urea Cycle Disorders in Adulthood: Late- Onset Carbamyl Phosphate Synthetase 1 Deficiency, 7 MUMJ 30 (2010).	
	D99	Yudkoff, M. et al., In Vivo Nitrogen Metabolism in Ornithine Transcarbamylase Deficiency, 98 J. Clin. Invest. 2167 (1996).	
	D100	Zeitlin, P., Novel Pharmacologic Therapies for Cystic Fibrosis, 103 J. Clinical Investigation 447 (1999).	
	D101	AHRENS, M. et al. (January 2001). "Consensus Statement From a Conference for the Management of Patients With Urea Cycle Disorders." *Supp. Journal of Pediatrics* 138(1):S1-S5.	
	D102	LEE, B. et al. (August 2008). "Preliminary Data on Adult Patients with Urea Cycle Disorders (UCD) in An Open-Label, Swirch-Over, Dose Escalation Study Comparing a New Ammonia Scavenger, Glyceryl Tri (4-Phenylbutyrate) [HPN-100], to Buphenyl® (Sodium Phenylbutyrate [PBA])", *abstract presented at SSSIEM 2008*, Lisbon, Portugal, one page.	
	D103	LEE, B. et al. (August 2008). "Preliminary Data on Adult Patients with Urea Cycle Disorders (UCD) in An Open-Label, Swirch-Over, Dose Escalation Study Comparing a New Ammonia Scavenger, Glyceryl Tri (4-Phenylbutyrate) [HPN-100], to Buphenyl® (Sodium Phenylbutyrate [PBA])", *presented at SSSIEM 2008*, Lisbon, Portugal, Poster, one page.	

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.E.T./

Examiner Signature	/Sara E. Townsley/	Date Considered	05/16/2016

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. 1 Applicant's unique citation designation number (optional). 2 See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. 3 Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). 4 For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. 5 Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. 6 Applicant is to place a check mark here if English language Translation is attached.

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13610580	SCHARSCHMIDT ET AL.
	Examiner	Art Unit
	SARA E TOWNSLEY	1629

✓	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	10/05/2014	02/20/2015	05/16/2016						
	1	÷	✓	✓						
	2	÷	✓	✓						
	3	-	-	-						
	4	-	-	-						
	5	÷	✓	✓						
	6	÷	✓	✓						
	7	÷	✓	-						
	8	-	-	-						
	9	÷	✓	✓						
	10	÷	✓	✓						
	11	÷	✓	✓						
	12	÷	✓	✓						
	13	÷	✓	-						

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Scharschmidt et al.

Application No.: 13/610,580

Filing Date: September 11, 2012

For: METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS

Group Art Unit: 1629

Examiner: Sara Elizabeth Townsley

Docket No.: HOR0027-201-US

Confirmation No.: 1957

RESPONSE TO FINAL OFFICE ACTION UNDER 37 C.F.R. § 1.113

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

This document is timely filed in response to the Final Office Action mailed May 19, 2016. No additional fees are believed due in connection with this filing, however, should any such fees become due under 37 C.F.R. §§ 1.16 to 1.21 for any reason relating to the instant paper, the Commissioner is authorized to deduct said fees from Global Patent Group, LLC Deposit Account No. 50-4297.

Amendments to the Claims begin on page 2.

Remarks follow the Amendments to the Claims.

AMENDMENTS TO THE CLAIMS

Please amend the claims as follows:

1. (Currently Amended) A method of treating urea cycle disorders in a subject comprising:

(a) administering a first dosage of glyceryl tri-[4-phenylbutyrate],

(b) measuring plasma phenylacetic acid (PAA) and phenylacetyl glutamine (PAGN) levels,

(c) calculating a plasma PAA:PAGN ratio,

(d) determining whether the glyceryl tri-[4-phenylbutyrate] dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(e) administering a second dosage of the glyceryl tri-[4-phenylbutyrate] based on the determination in (d);

wherein the target range is 1 to 2:5.

2. (Currently Amended) A method of treating urea cycle disorders in a subject who has previously been administered a first dosage of glyceryl tri-[4-phenylbutyrate] comprising:

(a) measuring plasma phenylacetic acid (PAA) and phenylacetyl glutamine (PAGN) levels,

(b) calculating a plasma PAA:PAGN ratio,

(c) determining whether the first dosage of glyceryl tri-[4-phenylbutyrate] needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(d) administering a second dosage of the glyceryl tri-[4-phenylbutyrate] based on the determination in (c);

wherein the target range is 1 to 2:5.

3-4. (Canceled)

5. (Currently Amended) A method of adjusting the dosage of glyceryl tri-[4-phenylbutyrate] comprising:

(a) administering a first dosage of glyceryl tri-[4-phenylbutyrate],

(b) measuring plasma phenylacetic acid (PAA) and phenylacetyl glutamine (PAGN) levels,

(c) calculating a plasma PAA:PAGN ratio,

(d) determining whether the glyceryl tri-[4-phenylbutyrate] dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(e) administering a second dosage of the glyceryl tri-[4-phenylbutyrate] based on the determination in (d);

wherein the target range is 1 to 2:5.

6. (Currently Amended) A method of optimizing the therapeutic efficacy of glyceryl tri-[4-phenylbutyrate] in a subject who has previously been administered a first dosage of glyceryl tri-[4-phenylbutyrate] comprising:

(a) measuring plasma phenylacetic acid (PAA) and phenylacetyl glutamine (PAGN) levels,

(b) calculating a plasma PAA:PAGN ratio,

(c) determining whether the dosage of glyceryl tri-[4-phenylbutyrate] needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(e) administering a second dosage of the glyceryl tri-[4-phenylbutyrate] as necessary based on the determination in (c);

wherein the target range is 1 to 2:5.

7-9. (Canceled)

10. (Previously Presented) The method of any of claims 1, 2, 5, or 6, wherein the target range is 1 to 2.

11. (Previously Presented) The method of any of claims 1, 2, 5, or 6, wherein measurement of PAA and PAGN levels is carried out after the first dosage of glyceryl tri-[4-phenylbutyrate] has

had sufficient time to reach steady state.

12. (Previously Presented) The method of claim 11, wherein measurement of PAA and PAGN levels is carried out 48 hours to 1 week after the first dosage of glyceryl tri-[4-phenylbutyrate] is administered.

13. (Canceled)

REMARKS

Status of Claims

Claims 1, 2, 5, and 6 are amended herein. Claim 9 is canceled herein. No new matter has been added by these amendments. With the entry of this amendment, claims 1, 2, 5, 6, and 10-12 are pending.

Rejections Under 35 U.S.C. § 103(a) (pre-AIA)

The Action rejects claims 1, 2, 5, 6, and 9-12 under 35 U.S.C. § 103(a), as allegedly obvious over Scharschmidt et al. (US 2012/0022157; "Scharschmidt") in view of McGuire et al. (Hepatology 51:2077-85, 2010; "McGuire").

In rejecting independent claims 1 and 5 the Action asserts that "it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Scharschmidt by measuring plasma levels of PAA and PAGN, instead of urinary PAA and PAGN levels, and comparing them as a ratio, in order to more accurately assess the patient's metabolism of PAA prodrugs, e.g., glyceryl tri-[4-phenylbutyrate], and evaluate any need to adjust the dosage, with a reasonable expectation of success, because McGuire teaches that urinary testing is not as complete and thorough as testing for plasma levels of PAA and PAGN." Action, p. 5. Applicant respectfully disagrees.

The present claims are based on the unexpected observation that the plasma PAA:PAGN ratio provides an accurate measure of PAA prodrug metabolism. *See, e.g.,* Specification as filed, ¶ [0033]. The ratio of an active metabolite, such as PAA, to its terminal metabolite (here, PAGN), would not normally be taken into consideration by the person of ordinary skill in making therapeutic decisions regarding drug dosing. The skilled artisan would expect that higher levels of the active metabolite (PAA) would lead to a proportionately higher response (as measured by PAGN levels) and increased nitrogen waste removal. The results described in the present application demonstrate the surprising and unexpected result that the use of plasma PAA:PAGN ratios to evaluate and adjust PAA prodrug dosage is superior to the use of either PAA or PAGN levels alone.

For example, Figures 2-5 demonstrate the surprising non-linear relationship between

plasma PAA levels and PAA:PAGN ratios in patients at any given time point. When the PAA:PAGN ratio exceeds 1, there is an increase in plasma PAA levels, and at ratios above 2 there is a sharp upswing in plasma PAA levels, with levels of PAA hitting 400 μg/mL or higher. Figures 2A-C. As shown in Table 3, measuring PAA and PAGN and calculating the ratio was predictive of the probability that the patient would subsequently achieve a high level of plasma PAA. Thus, a patient whose PAA:PAGN ratio was greater than 2.5 at 12 hours post-dosing has a 36.4% chance of exceeding 400 mg/mL in plasma PAA sometime during the 24 hour period. Specification as filed, ¶ [0073]. As the specification explains, "basing dose adjustment [] only on a high PAA level without considering concomitant plasma PAGN level may result in unnecessary dose reduction and under-treatment of the patient. Conversely, a PAA level seemingly below the levels associated with toxicity might be taken as an indication of satisfactory dosing without appreciating the fact that the concomitant PAGN level may not be proportional to PAA, indicating that PAA is not being efficiently utilized and may be accumulating." *Id.* at ¶ [0027]. Therapeutically, this is an important discovery not taught or suggested by the prior art. Specifically, once a subject exceeds a specific PAA:PAGN ratio, there is an indication that the active moiety is not being effectively utilized, and increasing the prodrug dosage may actually be deleterious, resulting in accumulation of PAA and associated toxicity. *Id.* at ¶ [0035].

Scharschmidt notes the "evidence that that for certain prodrugs of phenylacetic acid (PAA), measuring the blood level of the prodrug (e.g. PBA [phenylbutyric acid]) or of PAA formed from it is unreliable in assessing drug effect; drug levels in the blood do not correlate with efficacy in this case." Scharschmidt, ¶ [0004]. In particular, Scharschmidt "is based in part on the discovery that bioavailability of these drugs as conventionally assessed based on systemic blood levels of the drugs themselves or of the active species produced in vivo from these drugs does not accurately predict removal of waste nitrogen or reduction of plasma ammonia in healthy human volunteers, adults with liver disease, or patients with UCDs receiving ammonia scavenging drugs." *Id.* at ¶ [0021]. Scharschmidt further explains that, "systemic levels of PAA or PBA are not reliably correlated with the efficacy of HPN-100 as an ammonia scavenger." *Id.* at ¶ [0027].

Scharschmidt observes that "data from three clinical test groups show the inconsistent relationship between plasma PAA and PBA levels among healthy volunteers, patients with cirrhosis and UCD patients, despite the fact that, as described in detail below, all groups exhibited

similar ammonia scavenging activity based on urinary excretion of PAGN." *Id.* at ¶ [0042]. Partly on the basis of those results, Scharschmidt discloses methods of utilizing urinary PAGN levels to determine doses and making dose adjustments of PBA prodrugs such as HPN-100. As such, Scharschmidt teaches away from the use of measured plasma levels of PBA prodrugs or their metabolites for determining dosages and dose adjustment.

The Action also asserts that the teachings in McGuire regarding measuring metabolites, including PAA and PAGN, of PAA prodrugs in plasma, together with the teachings of Scharschmidt, would lead the person of ordinary skill in the art at the time the present invention was made to measure plasma levels of PAA and PAGN in a patient taking a PAA prodrug, and use the PAA/PAGN ratio to adjust the dosage of the PAA prodrug. However, the teaching away of Scharschmidt is not altered by McGuire.

McGuire describes the results of two Phase 1 studies designed to assess safety, tolerability, pharmacokinetic equivalence, and bioequivalence of PBA and GPB (glyceryl phenylbutyrate, HPN-100). McGuire states that PAGN was detectable in the plasma at 24 hours, and therefore urine collection was not complete at 24 hours. On the basis of the pattern of plasma levels and urinary excretion, the urine collection (done for a total of 48 hours) was split into two groups, 0-24 hours and 24-48 hours. McGuire has nothing to say regarding the nature of the sampling, plasma versus urinary, and the correlation of the detected levels of prodrug or metabolite with efficacy of the prodrug as an ammonia scavenger. Rather, McGuire describes safety, tolerability, and bioequivalence.

Nothing in McGuire suggests utilizing PAA:PAGN ratios for therapeutic purposes. McGuire states that "[u]rinary PAGN excretion was significantly greater in all groups after multiple dosing … a result consistent with the larger daily GPB doses and higher plasma PAA and plasma PAGN observed." McGuire, p. 2081, col. 2. McGuire also discloses that, "[u]rinary PAGN is also of particular interest because it is stoichiometrically related to nitrogen scavenging." *Id.* at p. 2084, col. 2. These statements suggest that PAA or PAGN levels alone are sufficient for evaluating and monitoring PAA prodrug dosage, and do not suggest or provide a motivation for calculating PAA:PAGN ratios for these purposes. Therefore, in view of McGuire and the later published Scharschmidt, one of skill in the art would have had the view that urinary PAGN levels, not plasma levels, should be used to assess drug efficacy for purposes of guiding dosing.

Furthermore, the cited references, alone or in combination, fail to teach the target range for the PAA:PAGN ratio is 1 to 2.5 or 1 to 2. The Action acknowledges that "the cited references do not explicitly disclose that glyceryl tri-[4-phenylbutyrate] dosage can be optimized by comparing plasma metabolite ratios," but then vaguely, and generally, asserts that "various methods of optimizing drug dosage regimens are generally known and/or within the capability of those of ordinary skill in the art." Action, p. 11. However, the Action fails to provide any factual evidence in support of a suggestion or motivation in the cited references, alone or in combination, to calculate and utilize the PAA:PAGN ratios described in the present specification, for the purpose of adjusting drug dosage.

In view of the above, the Action has failed to establish a *prima facie* case of obviousness and withdrawal of the rejections is respectfully requested.

Conclusion

In light of the foregoing amendments and arguments, Applicant submits that the application is in condition for allowance and favorable consideration is requested. The Examiner is invited to contact the undersigned by telephone or email if it is felt that an interview would advance the prosecution of the present application.

Respectfully submitted,

/Chris Marion/

Chris L. Marion
Reg. No. L0931
Attorney for Applicant

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17014 New College Avenue, Suite 201
Grover, MO 63040
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Date: July 7, 2016

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Scharschmidt et al.

Application No.: 13/610,580

Filing Date: September 11, 2012

For: METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS

Group Art Unit: 1629

Examiner: Sara Elizabeth Townsley

Docket No.: HOR0027-201-US

Confirmation No.: 1957

NOTICE OF RELATED LITIGATION

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Further to the Notice of Related Litigation filed July 29, 2015, Applicant hereby notifies the U.S. Patent and Trademark Office ("USPTO") that the subject matter of the present application is involved in litigation in the United States.

Specifically, on September 4, 2015, Lupin, Ltd. sent Horizon Therapeutics, Inc. ("Horizon") a letter indicating that Lupin, Ltd. had filed an Abbreviated New Drug Application ("ANDA") with respect to RAVICTI® (Glycerol Phenylbutyrate) Oral Liquid, with a certification under 21 U.S.C. § 355(j)(2)(A)(vii)(IV) ("Paragraph IV") alleging that U.S. Patent Nos. 8,404,215 and 8,642,012 are invalid, unenforceable, and/or will not be infringed by the commercial manufacture, use or sale of the Lupin, Ltd. drug product. On November 6, 2015, Lupin, Ltd. sent Horizon a second ANDA notice letter indicating that Lupin, Ltd. had also filed a Paragraph IV certification with respect to U.S. Patent No. 9,095,559, issued August 4, 2015.

Under 21 U.S.C. § 355(j)(5)(B)(iii), Horizon had forty-five days from receipt of the first ANDA notice letter to file suit against Lupin, Ltd. for patent infringement. Accordingly, on October 19, 2015, Horizon brought suit on those patents against Lupin, Ltd. and Lupin Pharmaceuticals (collectively, "Lupin") in the United States District Court for the District of

New Jersey. The Complaint alleged that Lupin infringes U.S. Patent Nos. 8,404,215, 8,642,012, and 9,095,559. Horizon subsequently filed an Amended Complaint on April 6, 2016, alleging infringement of only U.S. Patent No. 9,095,559.

On February 9, and May 3, 2016, the USPTO issued U.S. Patent Nos. 9,254,278, and 9,326,966, respectively, which cover RAVICTI® (Glycerol Phenylbutyrate) Oral Liquid. Accordingly, on June 30, 2016, Horizon brought suit against Par Pharmaceutical, Inc. ("Par") in the United States District Court for the District of New Jersey. The Complaint alleged that Par infringes US Patent Nos. 9,095,559, 9,254,278, and 9,326,966.

Respectfully submitted,

/Chris Marion/

Chris L. Marion
Reg. No. L0931
Attorney for Applicant

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17014 New College Avenue, Suite 201
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(314) 812-8020

Date: July 7, 2016

Electronic Acknowledgement Receipt

EFS ID:	26286013
Application Number:	13610580
International Application Number:	
Confirmation Number:	1957
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS
First Named Inventor/Applicant Name:	Bruce Scharschmidt
Customer Number:	101325
Filer:	Christopher Lee Marion
Filer Authorized By:	
Attorney Docket Number:	HOR0027-201-US
Receipt Date:	07-JUL-2016
Filing Date:	11-SEP-2012
Time Stamp:	17:46:03
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		20160707_Response.pdf	112949 334467490daddf75ec2fbaa87f55bebe8e665dd9	yes	8

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Response After Final Action	1	1
	Claims	2	4
	Applicant Arguments/Remarks Made in an Amendment	5	8

Warnings:					
Information:					
2	Miscellaneous Incoming Letter	20160707_NRL.pdf	69502 3b27c8f2baa61867b4269ccfb12014e00a93c314	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	182451		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

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 13/610,580	Filing Date 09/11/2012	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

	(Column 1)	(Column 2)		
FOR	NUMBER FILED	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	07/07/2016	(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))	* 17	Minus	** 40	= 0	X $80 =	0
	Independent (37 CFR 1.16(h))	* 4	Minus	***6	= 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.

LIE
/DORIS BURNS/

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/610,580	09/11/2012	Bruce Scharschmidt	HOR0027-201-US	1957

101325 7590 07/20/2016

GLOBAL PATENT GROUP - HOR
17014 NEW COLLEGE AVENUE
SUITE 201
WILDWOOD, MO 63040

EXAMINER
TOWNSLEY, SARA ELIZABETH

ART UNIT	PAPER NUMBER
1629	

NOTIFICATION DATE	DELIVERY MODE
07/20/2016	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):

admin@globalpatentgroup.com
vtruman@globalpatentgroup.com
LStevens@horizonpharma.com

Advisory Action Before the Filing of an Appeal Brief	**Application No.** 13/610,580	**Applicant(s)** SCHARSCHMIDT ET AL.	
	Examiner SARA E. TOWNSLEY	**Art Unit** 1629	**AIA (First Inventor to File) Status** No

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

THE REPLY FILED 07 July 2016 FAILS TO PLACE THIS APPLICATION IN CONDITION FOR ALLOWANCE.

NO NOTICE OF APPEAL FILED

1. ☒ The reply was filed after a final rejection. No Notice of Appeal has been filed. To avoid abandonment of this application, applicant must timely file one of the following replies: (1) an amendment, affidavit, or other evidence, which places the application in condition for allowance;

(2) a Notice of Appeal (with appeal fee) in compliance with 37 CFR 41.31; or (3) a Request for Continued Examination (RCE) in compliance with 37 CFR 1.114 if this is a utility or plant application. Note that RCEs are not permitted in design applications. The reply must be filed within one of the following time periods:

a) ☐ The period for reply expires _____months from the mailing date of the final rejection.

b) ☒ The period for reply expires on: (1) the mailing date of this Advisory Action; or (2) the date set forth in the final rejection, whichever is later. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of the final rejection.

c) ☐ A prior Advisory Action was mailed more than 3 months after the mailing date of the final rejection in response to a first after-final reply filed within 2 months of the mailing date of the final rejection. The current period for reply expires months from the mailing date of *the prior Advisory Action* or SIX MONTHS from the mailing date of the final rejection, whichever is earlier.

Examiner Note: If box 1 is checked, check either box (a), (b) or (c). ONLY CHECK BOX (b) WHEN THIS ADVISORY ACTION IS THE FIRST RESPONSE TO APPLICANT'S FIRST AFTER-FINAL REPLY WHICH WAS FILED WITHIN TWO MONTHS OF THE FINAL REJECTION. ONLY CHECK BOX (c) IN THE LIMITED SITUATION SET FORTH UNDER BOX (c). See MPEP 706.07(f).

Extensions of time may be obtained under 37 CFR 1.136(a). The date on which the petition under 37 CFR 1.136(a) and the appropriate extension fee have been filed is the date for purposes of determining the period of extension and the corresponding amount of the fee. The appropriate extension fee under 37 CFR 1.17(a) is calculated from: (1) the expiration date of the shortened statutory period for reply originally set in the final Office action; or (2) as set forth in (b) or (c) above, if checked. Any reply received by the Office later than three months after the mailing date of the final rejection, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

NOTICE OF APPEAL

2. ☐ The Notice of Appeal was filed on _____. A brief in compliance with 37 CFR 41.37 must be filed within two months of the date of filing the Notice of Appeal (37 CFR 41.37(a)), or any extension thereof (37 CFR 41.37(e)), to avoid dismissal of the appeal. Since a Notice of Appeal has been filed, any reply must be filed within the time period set forth in 37 CFR 41.37(a).

AMENDMENTS

3. ☒ The proposed amendments filed after a final rejection, but prior to the date of filing a brief, will not be entered because

a) ☒ They raise new issues that would require further consideration and/or search (see NOTE below);

b) ☒ They raise the issue of new matter (see NOTE below);

c) ☐ They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal; and/or

d) ☐ They present additional claims without canceling a corresponding number of finally rejected claims.

NOTE: *See Continuation Sheet*. (See 37 CFR 1.116 and 41.33(a)).

4. ☐ The amendments are not in compliance with 37 CFR 1.121. See attached Notice of Non-Compliant Amendment (PTOL-324).

5. ☐ Applicant's reply has overcome the following rejection(s): _____.

6. ☐ Newly proposed or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment canceling the non-allowable claim(s).

7. ☒ For purposes of appeal, the proposed amendment(s): (a) ☒ will not be entered, or (b) ☐ will be entered, and an explanation of how the new or amended claims would be rejected is provided below or appended.

AFFIDAVIT OR OTHER EVIDENCE

8. ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

9. ☐ The affidavit or other evidence filed after final action, but before or on the date of filing a Notice of Appeal will not be entered because applicant failed to provide a showing of good and sufficient reasons why the affidavit or other evidence is necessary and was not earlier presented. See 37 CFR 1.116(e).

10. ☐ The affidavit or other evidence filed after the date of filing the Notice of Appeal, but prior to the date of filing a brief, will not be entered because the affidavit or other evidence failed to overcome all rejections under appeal and/or appellant fails to provide a showing of good and sufficient reasons why it is necessary and was not earlier presented. See 37 CFR 41.33(d)(1).

11. ☐ The affidavit or other evidence is entered. An explanation of the status of the claims after entry is below or attached.

REQUEST FOR RECONSIDERATION/OTHER

12. ☒ The request for reconsideration has been considered but does NOT place the application in condition for allowance because: See Continuation Sheet.

13. ☐ Note the attached Information *Disclosure Statement*(s). (PTO/SB/08) Paper No(s). _____

14. ☐ Other: _____.

STATUS OF CLAIMS

15. The status of the claim(s) is (or will be) as follows:

Claim(s) allowed: .
Claim(s) objected to: .
Claim(s) rejected: 1,2,5,6 and 9-12.
Claim(s) withdrawn from consideration: .

/Barbara Badio/ Primary Examiner, Art Unit 1628	/SARA E. TOWNSLEY/ Examiner, Art Unit 1629

Continuation of 3. NOTE: Applicant has proposed to amend claims 1, 2, 5, and 6 to recite the limitation "wherein the target range is 1 to 2:5." This limitation was not previously considered, and does not appear to be supported by the instant specification. Thus, further search and consideration would be required.

Continuation of 12. does NOT place the application in condition for allowance because: Applicant's arguments that the newly amended claims are patentable over the prior art references are moot at this time due to non-entry of the proposed amendment..

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Scharschmidt et al.

Application No.: 13/610,580

Filing Date: September 11, 2012

For: METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS

Group Art Unit: 1629

Examiner: Sara Elizabeth Townsley

Docket No.: HOR0027-201-US

Confirmation No.: 1957

RESPONSE TO FINAL OFFICE ACTION UNDER 37 C.F.R. § 1.113

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

This document is timely filed in response to the Final Office Action mailed May 19, 2016. No additional fees are believed due in connection with this filing, however, should any such fees become due under 37 C.F.R. §§ 1.16 to 1.21 for any reason relating to the instant paper, the Commissioner is authorized to deduct said fees from Global Patent Group, LLC Deposit Account No. 50-4297.

Amendments to the Claims begin on page 2.

Remarks follow the Amendments to the Claims.

Doc Code: A.NE.AFCP
Document Description: After Final Consideration Pilot Program Request

PTO/SB/434 (05-13)

CERTIFICATION AND REQUEST FOR CONSIDERATION UNDER THE AFTER FINAL CONSIDERATION PILOT PROGRAM 2.0

Practitioner Docket No.:	Application No.:	Filing Date:
HOR0027-201-US	13/610,580	September 11, 2012
First Named Inventor:	**Title:**	
Scharschmidt et al.	METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS	

APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS CONSIDERATION UNDER THE AFTER FINAL CONSIDERATION PILOT PROGRAM 2.0 (AFCP 2.0) OF THE ACCOMPANYING RESPONSE UNDER 37 CFR 1.116.

1. The above-identified application is (i) an original utility, plant, or design nonprovisional application filed under 35 U.S.C. 111(a) [a continuing application (*e.g.*, a continuation or divisional application) is filed under 35 U.S.C. 111(a) and is eligible under (i)], or (ii) an international application that has entered the national stage in compliance with 35 U.S.C. 371(c).
2. The above-identified application contains an outstanding final rejection.
3. Submitted herewith is a response under 37 CFR 1.116 to the outstanding final rejection. The response includes an amendment to at least one independent claim, and the amendment does not broaden the scope of the independent claim in any aspect.
4. This certification and request for consideration under AFCP 2.0 is the only AFCP 2.0 certification and request filed in response to the outstanding final rejection.
5. Applicant is willing and available to participate in any interview requested by the examiner concerning the present response.
6. This certification and request is being filed electronically using the Office's electronic filing system (EFS-Web).
7. Any fees that would be necessary consistent with current practice concerning responses after final rejection under 37 CFR 1.116, *e.g.*, extension of time fees, are being concurrently filed herewith. [There is no additional fee required to request consideration under AFCP 2.0.]
8. By filing this certification and request, applicant acknowledges the following:
   - Reissue applications and reexamination proceedings are not eligible to participate in AFCP 2.0.
   - The examiner will verify that the AFCP 2.0 submission is compliant, *i.e.*, that the requirements of the program have been met (see items 1 to 7 above). For compliant submissions:
     - The examiner will review the response under 37 CFR 1.116 to determine if additional search and/or consideration (i) is necessitated by the amendment and (ii) could be completed within the time allotted under AFCP 2.0. If additional search and/or consideration is required but cannot be completed within the allotted time, the examiner will process the submission consistent with current practice concerning responses after final rejection under 37 CFR 1.116, *e.g.*, by mailing an advisory action.
     - If the examiner determines that the amendment does not necessitate additional search and/or consideration, or if the examiner determines that additional search and/or consideration is required and could be completed within the allotted time, then the examiner will consider whether the amendment places the application in condition for allowance (after completing the additional search and/or consideration, if required). If the examiner determines that the amendment does not place the application in condition for allowance, then the examiner will contact the applicant and request an interview.
       - The interview will be conducted by the examiner, and if the examiner does not have negotiation authority, a primary examiner and/or supervisory patent examiner will also participate.
       - If the applicant declines the interview, or if the interview cannot be scheduled within ten (10) calendar days from the date that the examiner first contacts the applicant, then the examiner will proceed consistent with current practice concerning responses after final rejection under 37 CFR 1.116.

Signature	Date
/Chris Marion/	July 29, 2016
Name (Print/Typed) Chris L. Marion	Practitioner Registration No. L0931

***Note**: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.*

☑ * Total of 1 forms are submitted.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Scharschmidt et al.

Application No.: 13/610,580

Filing Date: September 11, 2012

For: METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS

Group Art Unit: 1629

Examiner: Sara Elizabeth Townsley

Docket No.: HOR0027-201-US

Confirmation No.: 1957

AMENDMENT, RESPONSE TO ADVISORY ACTION, AND AFCP 2.0

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

This document is timely filed in response to the Advisory Action mailed July 20, 2016. Also filed concurrently herewith is an After Final Consideration Pilot Program 2.0 Request. No additional fees are believed due in connection with this filing, however, should any such fees become due under 37 C.F.R. §§ 1.16 to 1.21 for any reason relating to the instant paper, the Commissioner is authorized to deduct said fees from Global Patent Group, LLC Deposit Account No. 50-4297.

Amendments to the Claims begin on page 2.

Remarks follow the Amendments to the Claims.

AMENDMENTS TO THE CLAIMS

Please amend the claims as follows:

1. (Currently Amended) A method of treating urea cycle disorders in a subject comprising:

(a) administering a first dosage of glyceryl tri-[4-phenylbutyrate],

(b) measuring plasma phenylacetic acid (PAA) and phenylacetyl glutamine (PAGN) levels,

(c) calculating a plasma PAA:PAGN ratio,

(d) determining whether the glyceryl tri-[4-phenylbutyrate] dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(e) administering a second dosage of the glyceryl tri-[4-phenylbutyrate] based on the determination in (d);

wherein the target range is 1 to 2.5.

2. (Currently Amended) A method of treating urea cycle disorders in a subject who has previously been administered a first dosage of glyceryl tri-[4-phenylbutyrate] comprising:

(a) measuring plasma phenylacetic acid (PAA) and phenylacetyl glutamine (PAGN) levels,

(b) calculating a plasma PAA:PAGN ratio,

(c) determining whether the first dosage of glyceryl tri-[4-phenylbutyrate] needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(d) administering a second dosage of the glyceryl tri-[4-phenylbutyrate] based on the determination in (c);

wherein the target range is 1 to 2.5.

3-4. (Canceled)

5. (Currently Amended) A method of adjusting the dosage of glyceryl tri-[4-phenylbutyrate] comprising:

(a) administering a first dosage of glyceryl tri-[4-phenylbutyrate],

(b) measuring plasma phenylacetic acid (PAA) and phenylacetyl glutamine (PAGN) levels,

(c) calculating a plasma PAA:PAGN ratio,

(d) determining whether the glyceryl tri-[4-phenylbutyrate] dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(e) administering a second dosage of the glyceryl tri-[4-phenylbutyrate] based on the determination in (d);

wherein the target range is 1 to 2.5.

6. (Currently Amended) A method of optimizing the therapeutic efficacy of glyceryl tri-[4-phenylbutyrate] in a subject who has previously been administered a first dosage of glyceryl tri-[4-phenylbutyrate] comprising:

(a) measuring plasma phenylacetic acid (PAA) and phenylacetyl glutamine (PAGN) levels,

(b) calculating a plasma PAA:PAGN ratio,

(c) determining whether the dosage of glyceryl tri-[4-phenylbutyrate] needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(e) administering a second dosage of the glyceryl tri-[4-phenylbutyrate] as necessary based on the determination in (c);

wherein the target range is 1 to 2.5.

7-9. (Canceled)

10. (Previously Presented) The method of any of claims 1, 2, 5, or 6, wherein the target range

is 1 to 2.

11. (Previously Presented) The method of any of claims 1, 2, 5, or 6, wherein measurement of PAA and PAGN levels is carried out after the first dosage of glyceryl tri-[4-phenylbutyrate] has had sufficient time to reach steady state.

12. (Previously Presented) The method of claim 11, wherein measurement of PAA and PAGN levels is carried out 48 hours to 1 week after the first dosage of glyceryl tri-[4-phenylbutyrate] is administered.

13. (Canceled)

REMARKS

Status of Claims

Entry into the record of the amendment to the claims presented herein, and the remarks previously presented in the Response to Final Office Action filed July 7, 2016, is respectfully requested. Claims 1, 2, 5, and 6 are amended herein. Claim 9 is canceled herein. No new matter has been added by these amendments. With the entry of this amendment, claims 1, 2, 5, 6, and 10-12 are pending.

Comments in Advisory Action

The Advisory Action asserts that amendment to claims 1, 2, 5, and 6, as presented in the Response to the Final Office Action filed July 7, 2016, recites a limitation not previously considered and not supported by the specification. Advisory Action, p. 2. Thus, the Advisory Action asserts that further search and consideration would be required. *Ibid.*

In response, Applicant notes that a typographical error in the previously presented amendment to the claims has been corrected herein. Specifically, recitation of the limitation from now canceled claim 9 was inadvertently presented in amended independent claims 1, 2, 5, and 6 as "wherein the target range is 1 to 2:5" (emphasis added) instead of "wherein the target range is 1 to 2.5" (emphasis added). The amendment to the claims presented herein corrects this typographical error and reference to the remarks related to the rejections under 35 U.S.C. § 103(a) presented in the Response to Final Office Action filed July 7, 2016, is respectfully requested.

Conclusion

In view of the above, entry into the record of the amendments presented herein, and the remarks previously presented in the Response to the Final Office Action filed July 7, 2016, Applicant respectfully submits that all outstanding rejections should be withdrawn and the application allowed. The Examiner is invited to contact the undersigned by telephone or email, if it is felt that an interview would advance the prosecution of the present application.

Respectfully submitted,

/Chris Marion/

Chris L. Marion
Reg. No. L0931
Attorney for Applicant

Global Patent Group, LLC
17014 New College Avenue, Suite 201
Grover, MO 63040
(314) 812-8020

Date: July 29, 2016

Electronic Acknowledgement Receipt

EFS ID:	26486845
Application Number:	13610580
International Application Number:	
Confirmation Number:	1957
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS
First Named Inventor/Applicant Name:	Bruce Scharschmidt
Customer Number:	101325
Filer:	Christopher Lee Marion/Vicki Truman
Filer Authorized By:	Christopher Lee Marion
Attorney Docket Number:	HOR0027-201-US
Receipt Date:	01-AUG-2016
Filing Date:	11-SEP-2012
Time Stamp:	13:03:11
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	After Final Consideration Program Request	20160729_Request_Pilot.pdf	226523 5731019194bba117127e725a528f4c74b0992cd0	no	2

Warnings:

Information:					
2		20160729_Response1.pdf	96967 15ae79d4ffddab90d593fd2211323590d7049586	yes	6

Multipart Description/PDF files in .zip description		
Document Description	**Start**	**End**
Response After Final Action	1	1
Claims	2	4
Applicant Arguments/Remarks Made in an Amendment	5	6

Warnings:	
Information:	
Total Files Size (in bytes):	323490

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

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 13/610,580	Filing Date 09/11/2012	☐ 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 **08/01/2016**

	(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))	* 16	Minus	** 40	= 0	X $80 =	0
Independent (37 CFR 1.16(h))	* 4	Minus	***6	= 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.

LIE
CAROLYN THOMAS

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/610,580	09/11/2012	Bruce Scharschmidt	HOR0027-201-US	1957

101325 7590 11/03/2016

GLOBAL PATENT GROUP - HOR
17014 NEW COLLEGE AVENUE
SUITE 201
WILDWOOD, MO 63040

EXAMINER	
TOWNSLEY, SARA ELIZABETH	

ART UNIT	PAPER NUMBER
1629	

NOTIFICATION DATE	DELIVERY MODE
11/03/2016	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):

admin@globalpatentgroup.com
vtruman@globalpatentgroup.com
LStevens@horizonpharma.com

Examiner-Initiated Interview Summary	Application No.	Applicant(s)	
	13/610,580	SCHARSCHMIDT ET AL.	
	Examiner	Art Unit	
	SARA E. TOWNSLEY	1629	

All participants (applicant, applicant's representative, PTO personnel):

(1) *SARA E. TOWNSLEY*. (3)_____.

(2) *LAUREN STEVENS (Applicant's representative)*. (4)_____.

Date of Interview: *24 August 2016*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☐ No.
If Yes, brief description: _____.

Issues Discussed ☒101 ☐112 ☐102 ☒103 ☒Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *All*.

Identification of prior art discussed: *All*.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

Agreed that the claimed steps of calculating a patient's plasma PAA:PAGN ratio, and adjusting the drug dosage if said ratio lies outside the target range of 1 to 2.5, are not specifically disclosed by the cited references. Discussed whether optimizing the dosage of a drug on the basis of metabolite ratios is routine, in particular with respect to the claimed patient population, which may be inherently limited to infants and children due to the nature of the disease.

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/SARA E. TOWNSLEY/ Examiner, Art Unit 1629	/JEFFREY S. LUNDGREN/ Supervisory Patent Examiner, Art Unit 1629

Advisory Action Before the Filing of an Appeal Brief	**Application No.** 13/610,580	**Applicant(s)** SCHARSCHMIDT ET AL.	
	Examiner SARA E. TOWNSLEY	**Art Unit** 1629	**AIA (First Inventor to File) Status** No

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

THE REPLY FILED 01 August 2016 FAILS TO PLACE THIS APPLICATION IN CONDITION FOR ALLOWANCE.

NO NOTICE OF APPEAL FILED

1. ☒ The reply was filed after a final rejection. No Notice of Appeal has been filed. To avoid abandonment of this application, applicant must timely file one of the following replies: (1) an amendment, affidavit, or other evidence, which places the application in condition for allowance;

(2) a Notice of Appeal (with appeal fee) in compliance with 37 CFR 41.31; or (3) a Request for Continued Examination (RCE) in compliance with 37 CFR 1.114 if this is a utility or plant application. Note that RCEs are not permitted in design applications. The reply must be filed within one of the following time periods:

a) ☒ The period for reply expires *3* months from the mailing date of the final rejection.

b) ☐ The period for reply expires on: (1) the mailing date of this Advisory Action; or (2) the date set forth in the final rejection, whichever is later. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of the final rejection.

c) ☐ A prior Advisory Action was mailed more than 3 months after the mailing date of the final rejection in response to a first after-final reply filed within 2 months of the mailing date of the final rejection. The current period for reply expires months from the mailing date of *the prior Advisory Action* or SIX MONTHS from the mailing date of the final rejection, whichever is earlier.

Examiner Note: If box 1 is checked, check either box (a), (b) or (c). ONLY CHECK BOX (b) WHEN THIS ADVISORY ACTION IS THE FIRST RESPONSE TO APPLICANT'S FIRST AFTER-FINAL REPLY WHICH WAS FILED WITHIN TWO MONTHS OF THE FINAL REJECTION. ONLY CHECK BOX (c) IN THE LIMITED SITUATION SET FORTH UNDER BOX (c). See MPEP 706.07(f).

Extensions of time may be obtained under 37 CFR 1.136(a). The date on which the petition under 37 CFR 1.136(a) and the appropriate extension fee have been filed is the date for purposes of determining the period of extension and the corresponding amount of the fee. The appropriate extension fee under 37 CFR 1.17(a) is calculated from: (1) the expiration date of the shortened statutory period for reply originally set in the final Office action; or (2) as set forth in (b) or (c) above, if checked. Any reply received by the Office later than three months after the mailing date of the final rejection, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

NOTICE OF APPEAL

2. ☐ The Notice of Appeal was filed on _____. A brief in compliance with 37 CFR 41.37 must be filed within two months of the date of filing the Notice of Appeal (37 CFR 41.37(a)), or any extension thereof (37 CFR 41.37(e)), to avoid dismissal of the appeal. Since a Notice of Appeal has been filed, any reply must be filed within the time period set forth in 37 CFR 41.37(a).

AMENDMENTS

3. ☐ The proposed amendments filed after a final rejection, but prior to the date of filing a brief, will not be entered because
   a) ☐ They raise new issues that would require further consideration and/or search (see NOTE below);
   b) ☐ They raise the issue of new matter (see NOTE below);
   c) ☐ They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal; and/or
   d) ☐ They present additional claims without canceling a corresponding number of finally rejected claims.

   NOTE: _____. (See 37 CFR 1.116 and 41.33(a)).

4. ☐ The amendments are not in compliance with 37 CFR 1.121. See attached Notice of Non-Compliant Amendment (PTOL-324).

5. ☐ Applicant's reply has overcome the following rejection(s): _____.

6. ☐ Newly proposed or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment canceling the non-allowable claim(s).

7. ☒ For purposes of appeal, the proposed amendment(s): (a) ☐ will not be entered, or (b) ☒ will be entered, and an explanation of how the new or amended claims would be rejected is provided below or appended.

AFFIDAVIT OR OTHER EVIDENCE

8. ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

9. ☐ The affidavit or other evidence filed after final action, but before or on the date of filing a Notice of Appeal will not be entered because applicant failed to provide a showing of good and sufficient reasons why the affidavit or other evidence is necessary and was not earlier presented. See 37 CFR 1.116(e).

10. ☐ The affidavit or other evidence filed after the date of filing the Notice of Appeal, but prior to the date of filing a brief, will not be entered because the affidavit or other evidence failed to overcome all rejections under appeal and/or appellant fails to provide a showing of good and sufficient reasons why it is necessary and was not earlier presented. See 37 CFR 41.33(d)(1).

11. ☐ The affidavit or other evidence is entered. An explanation of the status of the claims after entry is below or attached.

REQUEST FOR RECONSIDERATION/OTHER

12. ☒ The request for reconsideration has been considered but does NOT place the application in condition for allowance because: See Continuation Sheet.

13. ☐ Note the attached Information *Disclosure Statement*(s). (PTO/SB/08) Paper No(s). _____

14. ☒ Other: PTO-2323 and interview summary attached.

STATUS OF CLAIMS

15. The status of the claim(s) is (or will be) as follows:

Claim(s) allowed: .
Claim(s) objected to: .
Claim(s) rejected: 1,2,5,6 and 10-12.
Claim(s) withdrawn from consideration: .

/JEFFREY S. LUNDGREN/ Supervisory Patent Examiner, Art Unit 1629	/SARA E. TOWNSLEY/ Examiner, Art Unit 1629

Continuation of 12. does NOT place the application in condition for allowance because: Applicant's arguments filed Jul. 7, 2016 and Aug. 1, 2016 have been fully considered but they are not persuasive.

With respect to the rejection under 35 U.S.C. § 103(a), Applicant contends that a prima facie case of obviousness has not been established because the cited references fail to disclose, teach, or suggest methods of adjusting the dosage of glyceryl tri-[4-phenylbutyrate] ("GPB") by measuring the plasma levels of GPB's active metabolite, PAA, and its terminal metabolite, PAGN; calculating the plasma PAA:PAGN ratio; and determining whether said ratio falls within the target range of 1 to 2.5, as recited by independent claims 1, 2, 5, and 6, or 1 to 2, as recited by dependent claim 10. Applicant contends that the instant claims are based on the unexpected finding that the plasma PAA:PAGN ratio provides an accurate measure of GPB metabolism, which is superior to previously known methods of adjusting GPB dosage based on one of PAA or PAGN levels alone (Remarks, p. 5).

However, on the basis of Mayo Collaborative Services v. Prometheus Laboratories Inc., 132 S. Ct. 1289 (U.S. 2012), the claimed steps of "calculating" a plasma PAA:PAGN ratio, and "determining" whether the GPB dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within the target range of 1 to 2.5, are not given patentable weight, for the following reasons.

The claims at issue in Mayo are nearly identical to the instant claims. Prometheus was the sole and exclusive licensee of the two patents at issue, which concerned the use of thiopurine drugs to treat autoimmune diseases. When ingested, the body metabolizes the drugs, producing metabolites in the bloodstream. Because patients metabolize these drugs differently, doctors have found it difficult to determine whether a particular patient's dose is too high, risking harmful side effects, or too low, and so likely ineffective. Prometheus' claims set forth processes embodying researchers' findings that identified correlations between metabolite levels and likely harm or ineffectiveness with precision. Each claim recited (1) an "administering" step, instructing a doctor to administer the drug to a patient; (2) a "determining" step, telling the doctor to measure the resulting metabolite levels in the patient's blood; and (3) a "wherein" step, describing the metabolite concentrations above which there is a likelihood of harmful side-effects and below which it is likely that the drug dosage is ineffective, i.e., a target range.

The Court held that such claims are directed to laws of nature or natural phenomena and as such are not patent eligible. The relationships between concentrations of certain metabolites in the blood and the likelihood that a drug dosage will prove ineffective or cause harm are not themselves patentable. The three additional steps were not themselves natural laws, but were also insufficient to transform the nature of the claims, because they were conventional and well known.

The "determining" step tells a doctor to measure patients' metabolite levels, through whatever process the doctor wishes to use. Because methods for making such determinations were well known in the art, this step simply tells doctors to engage in well-understood, routine, conventional activity. Such activity is normally not sufficient to transform an unpatentable law of nature into a patent-eligible application of such a law. In telling a doctor to measure metabolite levels and to consider the resulting measurements in light of the correlations they describe, the claimed methods would tie up subsequent treatment decisions, and threaten to inhibit the development of more refined treatment recommendations that combine the claimed correlations with later discoveries.

Here, the cited references establish that the remaining steps recited by the instant claims - administering a first dosage of GPB to a patient with a urea cycle disorder, measuring the plasma levels of PAA and PAGN, and administering a second dosage of GPB - were routine, conventional steps which were known in the art.

For the foregoing reasons, the rejection under 35 U.S.C. § 103 is maintained.
.

Examiner-Initiated Interview Summary	Application No.	Applicant(s)	
	13/610,580	SCHARSCHMIDT ET AL.	
	Examiner	Art Unit	
	SARA E. TOWNSLEY	1629	

All participants (applicant, applicant's representative, PTO personnel):

(1) *SARA E. TOWNSLEY*. (3)_____.

(2) *LAUREN STEVENS (Applicant's representative)*. (4)_____.

Date of Interview: *24 August 2016*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☐ No.
If Yes, brief description: _____.

Issues Discussed ☒101 ☐112 ☐102 ☒103 ☒Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *All*.

Identification of prior art discussed: *All*.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

Agreed that the claimed steps of calculating a patient's plasma PAA:PAGN ratio, and adjusting the drug dosage if said ratio lies outside the target range of 1 to 2.5, are not specifically disclosed by the cited references. Discussed whether optimizing the dosage of a drug on the basis of metabolite ratios is routine, in particular with respect to the claimed patient population, which may be inherently limited to infants and children due to the nature of the disease.

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Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/SARA E. TOWNSLEY/ Examiner, Art Unit 1629	/JEFFREY S. LUNDGREN/ Supervisory Patent Examiner, Art Unit 1629

AFCP 2.0 Decision	Application No.	Applicant(s)
	13/610,580	SCHARSCHMIDT ET AL.
	Examiner	Art Unit
	SARA E. TOWNSLEY	1629

This is in response to the After Final Consideration Pilot request filed 01 August 2016.

1. **Improper Request** – The AFCP 2.0 request is improper for the following reason(s) and the after final amendment submitted with the request will be treated under pre-pilot procedure.

   ☐ An AFCP 2.0 request form PTO/SB/434 (or equivalent document) was not submitted.

   ☐ A non-broadening amendment to at least one independent claim was not submitted.

   ☐ A proper AFCP 2.0 request was submitted in response to the most recent final rejection.

   ☐ Other:

2. **Proper Request**

   A. After final amendment submitted with the request will not be treated under AFCP 2.0.
   The after final amendment cannot be reviewed and a search conducted within the guidelines of the pilot program.

   ☐ The after final amendment will be treated under pre-pilot procedure.

   B. Updated search and/or completed additional consideration.
   The examiner performed an updated search and/or completed additional consideration of the after final amendment within the time authorized for the pilot program. The result(s) of the updated search and/or completed additional consideration are:

   ☐ 1. All of the rejections in the most recent final Office action are overcome and a Notice of Allowance is issued herewith.

   ☒ 2. The after final amendment would not overcome all of the rejections in the most recent final Office action. See attached interview summary for further details.

   ☐ 3. The after final amendment was reviewed, and it raises a new issue(s). See attached interview summary for further details.

   ☐ 4. The after final amendment raises new issues, but would overcome all of the rejections in the most recent final Office action. A decision on determining allowability could not be made within the guidelines of the pilot. See attached interview summary for further details, including any newly discovered prior art.

   ☐ 5. Other:

   Examiner Note: Please attach an interview summary when necessary as described above.

OK TO ENTER: /S.E.T./ 08/24/2016

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Scharschmidt et al.

Application No.: 13/610,580

Filing Date: September 11, 2012

For: METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS

Group Art Unit: 1629

Examiner: Sara Elizabeth Townsley

Docket No.: HOR0027-201-US

Confirmation No.: 1957

AMENDMENT, RESPONSE TO ADVISORY ACTION, AND AFCP 2.0

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

This document is timely filed in response to the Advisory Action mailed July 20, 2016. Also filed concurrently herewith is an After Final Consideration Pilot Program 2.0 Request. No additional fees are believed due in connection with this filing, however, should any such fees become due under 37 C.F.R. §§ 1.16 to 1.21 for any reason relating to the instant paper, the Commissioner is authorized to deduct said fees from Global Patent Group, LLC Deposit Account No. 50-4297.

Amendments to the Claims begin on page 2.

Remarks follow the Amendments to the Claims.

PTO/SB/30EFS (07-14)
Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

REQUEST FOR CONTINUED EXAMINATION(RCE)TRANSMITTAL
(Submitted Only via EFS-Web)

Application Number	13610580	Filing Date	2012-09-11	Docket Number (if applicable)	HOR0027-201-US	Art Unit	1629
First Named Inventor	Scharschmidt, Bruce			Examiner Name	Townsley, Sara Elizabeth		

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, to any international application that does not comply with the requirements of 35 U.S.C. 371, or to any design application. The Instruction Sheet for this form is located at WWW.USPTO.GOV.

SUBMISSION REQUIRED UNDER 37 CFR 1.114

Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the order in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s) entered, applicant must request non-entry of such amendment(s).

☐ Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.

- ☐ Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____
- ☐ Other _____

☒ Enclosed

- ☒ Amendment/Reply
- ☐ Information Disclosure Statement (IDS)
- ☐ Affidavit(s)/ Declaration(s)
- ☐ Other _____

MISCELLANEOUS

☐ Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of months _____ (Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)

☐ Other _____

FEES

The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.

☒ The Director is hereby authorized to charge any underpayment of fees, or credit any overpayments, to Deposit Account No 504297

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

☒ Patent Practitioner Signature

Applicant Signature

PTO/SB/30EFS (07-14)
Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Signature of Registered U.S. Patent Practitioner			
Signature	/Chris Marion/	Date (YYYY-MM-DD)	2016-11-18
Name	Chris Marion	Registration Number	60931

This collection of information is required by 37 CFR 1.114. 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 1.11 and 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.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal

Application Number:	13610580
Filing Date:	11-Sep-2012
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS
First Named Inventor/Applicant Name:	Bruce Scharschmidt
Filer:	Christopher Lee Marion
Attorney Docket Number:	HOR0027-201-US

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
REQUEST FOR PRIORITIZED EXAMINATION	1817	1	4000	4000
Pages:				
Claims:				
Miscellaneous-Filing:				
PUBL. FEE- EARLY, VOLUNTARY, OR NORMAL	1504	1	0	0
PROCESSING FEE, EXCEPT PROV. APPLS.	1830	1	140	140
Petition:				
Patent-Appeals-and-Interference:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Extension - 3 months with $0 paid	1253	1	1400	1400
Miscellaneous:				
RCE- 1st Request	1801	1	1200	1200
		Total in USD ($)		**6740**

Electronic Acknowledgement Receipt

EFS ID:	27559525
Application Number:	13610580
International Application Number:	
Confirmation Number:	1957
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS
First Named Inventor/Applicant Name:	Bruce Scharschmidt
Customer Number:	101325
Filer:	Christopher Lee Marion
Filer Authorized By:	
Attorney Docket Number:	HOR0027-201-US
Receipt Date:	18-NOV-2016
Filing Date:	11-SEP-2012
Time Stamp:	16:19:15
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$6740
RAM confirmation Number	112116INTEFSW00003221504297
Deposit Account	504297
Authorized User	Valerie Lechner

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

37 CFR 1.16 (National application filing, search, and examination fees)

37 CFR 1.17 (Patent application and reexamination processing fees)

37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		20161118_Response.pdf	91419 7234c7f391015bb21cb7451f040dbcabafd27f20	yes	4
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Response After Final Action		1	1	
	Claims		2	3	
	Applicant Arguments/Remarks Made in an Amendment		4	4	
Warnings:					
Information:					
2	TrackOne Request	20161118_Track_1.pdf	124867 e239c1124c5d9b63fbd8865c900902f27a745904	no	2
Warnings:					
Information:					
3	Request for Continued Examination (RCE)	20161118_RCE.pdf	1349885 3fc1a47e15f5797694fc921e580e4b3ca5f0c947	no	3
Warnings:					
Information:					
4	Fee Worksheet (SB06)	fee-info.pdf	39332 fceecc16c18a4adafa45c03d29b24387fe2fd210	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	1605503		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

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.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Scharschmidt et al.

Application No.: 13/610,580

Filing Date: September 11, 2012

For: METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS

Group Art Unit: 1629

Examiner: Sara Elizabeth Townsley

Docket No.: HOR0027-201-US

Confirmation No.: 1957

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT, RESPONSE TO ADVISORY ACTION, AND REQUEST FOR CONTINUED EXAMINATION

Commissioner:

This document is timely filed in response to the Advisory Action mailed November 3, 2016, and the Final Office Action dated May 19, 2016. Also filed concurrently herewith is a Request for Continued Examination. No additional fees are believed due in connection with this filing, however, should any such fees become due under 37 C.F.R. §§ 1.16 to 1.21 for any reason relating to the instant paper, the Commissioner is authorized to deduct said fees from Global Patent Group, LLC Deposit Account No. 50-4297.

Amendment to the Claims begins on page 2.

Remarks follow the Amendments to the Claims.

AMENDMENT TO THE CLAIMS

Please amend the claims as follows:

1-13. (Canceled)

14. (New) A method of treating a urea cycle disorder in a subject in need thereof, the method comprising:

(a) administering a first dosage of glyceryl tri-[4-phenylbutyrate] to the subject, wherein the first dosage results in a ratio of plasma phenylacetic acid (PAA) to phenylacetylglutamine (PAGN) greater than 2 in the subject; and

(b) administering a second dosage of glyceryl tri-[4-phenylbutyrate] to the subject, wherein the second dosage is less than the first dosage.

15. (New) The method of claim 14, further comprising measuring the PAA level and the PAGN level in the subject after administering the first dosage and reaching a steady state of glyceryl tri-[4-phenylbutyrate] in the subject.

16. (New) The method of claim 14, further comprising measuring the PAA level and the PAGN level in the subject about 48 hours to about one week after the first dosage is administered to the subject.

17. (New) A method of treating a urea cycle disorder in a subject in need thereof, the method comprising:

(a) administering a first dosage of glyceryl tri-[4-phenylbutyrate] to the subject, wherein the first dosage results in a ratio of plasma phenylacetic acid (PAA) to phenylacetylglutamine (PAGN) greater than 2.5 in the subject; and

(b) administering a second dosage of glyceryl tri-[4-phenylbutyrate] to the subject, wherein the second dosage is less than the first dosage.

18. (New) The method of claim 17, further comprising measuring the PAA level and the PAGN level in the subject after administering the first dosage and reaching a steady state of glyceryl tri-[4-phenylbutyrate] in the subject.

19. (New) The method of claim 17, further comprising measuring the PAA level and the PAGN level in the subject about 48 hours to about one week after the first dosage is administered to the subject.

20. (New) A method of treating a urea cycle disorder in a subject in need thereof, the method comprising:

(a) administering a first dosage of glyceryl tri-[4-phenylbutyrate] to the subject, wherein the first dosage results in a ratio of plasma phenylacetic acid (PAA) to phenylacetylglutamine (PAGN) less than 1 in the subject; and

(b) administering a second dosage of glyceryl tri-[4-phenylbutyrate] to the subject, wherein the second dosage is greater than the first dosage.

21. (New) The method of claim 20, further comprising measuring the PAA level and the PAGN level in the subject after administering the first dosage and reaching a steady state of glyceryl tri-[4-phenylbutyrate] in the subject.

22. (New) The method of claim 20, further comprising measuring the PAA level and the PAGN level in the subject about 48 hours to about one week after the first dosage is administered to the subject.

REMARKS

Status of Claims

Claims 1-13 are canceled and claims 14-22 are added. Support for the amendment to the claims can be found in the specification. No new matter has been added by these amendments. With the entry of this amendment, claims 14-22 are pending.

Reference to the remarks related to the rejections under 35 U.S.C. § 103(a) presented in the Response to Final Office Action filed July 7, 2016, is respectfully requested.

Conclusion

In view of the above, entry into the record of the amendments presented herein, and the remarks previously presented in the Response to the Final Office Action filed July 7, 2016, Applicant respectfully submits that all outstanding rejections should be withdrawn and the application allowed. The Examiner is invited to contact the undersigned by telephone or email, if it is felt that an interview would advance the prosecution of the present application.

Respectfully submitted,

/Chris Marion/

Chris L. Marion
Reg. No. L0931
Attorney for Applicant

Global Patent Group, LLC
17014 New College Avenue, Suite 201
St. Louis, MO 63040
(314) 812-8020

Date: November 18, 2016

Doc Code: TRACK1.REQ
Document Description: TrackOne Request

PTO/AIA/424 (04-14)

CERTIFICATION AND REQUEST FOR PRIORITIZED EXAMINATION UNDER 37 CFR 1.102(e) (Page 1 of 1)

First Named Inventor:	Scharschmidt, Bruce	Nonprovisional Application Number (if known):	13/610,580
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS		

APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS PRIORITIZED EXAMINATION FOR THE ABOVE-IDENTIFIED APPLICATION.

1. The processing fee set forth in 37 CFR 1.17(i)(1) and the prioritized examination fee set forth in 37 CFR 1.17(c) have been filed with the request. The publication fee requirement is met because that fee, set forth in 37 CFR 1.18(d), is currently $0. The basic filing fee, search fee, and examination fee are filed with the request or have been already been paid. I understand that any required excess claims fees or application size fee must be paid for the application.

2. I understand that the application may not contain, or be amended to contain, more than four independent claims, more than thirty total claims, or any multiple dependent claims, and that any request for an extension of time will cause an outstanding Track I request to be dismissed.

3. The applicable box is checked below:

I. ☐ Original Application (Track One) - Prioritized Examination under § 1.102(e)(1)

i. (a) The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a). This certification and request is being filed with the utility application via EFS-Web.

---OR---

(b) The application is an original nonprovisional plant application filed under 35 U.S.C. 111(a). This certification and request is being filed with the plant application in paper.

ii. An executed inventor's oath or declaration under 37 CFR 1.63 or 37 CFR 1.64 for each inventor, **or** the application data sheet meeting the conditions specified in 37 CFR 1.53(f)(3)(i) is filed with the application.

II. ☑ Request for Continued Examination - Prioritized Examination under § 1.102(e)(2)

i. A request for continued examination has been filed with, or prior to, this form.
ii. If the application is a utility application, this certification and request is being filed via EFS-Web.
iii. The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a), or is a national stage entry under 35 U.S.C. 371.
iv. This certification and request is being filed prior to the mailing of a first Office action responsive to the request for continued examination.
v. No prior request for continued examination has been granted prioritized examination status under 37 CFR 1.102(e)(2).

Signature /Chris Marion/	Date 2016-11-18
Name (Print/Typed) Chris Marion	Practitioner Registration Number L0931

Note: *This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required.**

☑ *Total of 1 forms are submitted.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

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 13/610,580	Filing Date 09/11/2012	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

	(Column 1)	(Column 2)		
FOR	NUMBER FILED	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	11/18/2016	(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))	* 9	Minus	** 20	= 0	X $80 =	0
	Independent (37 CFR 1.16(h))	* 3	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.

LIE
GOIGA DUCKETT

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
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Alexandria, VA 22313-1450
www.uspto.gov

GLOBAL PATENT GROUP - HOR
17014 NEW COLLEGE AVENUE
SUITE 201
WILDWOOD MO 63040

Doc Code: TRACK1.GRANT

Decision Granting Request for Prioritized Examination (Track I or After RCE)	Application No.: 13/610,580

1. THE REQUEST FILED November 18, 2016 IS **GRANTED.**

The above-identified application has met the requirements for prioritized examination

A. ☐ for an original nonprovisional application (Track I).
B. ☒ for an application undergoing continued examination (RCE).

2. **The above-identified application will undergo prioritized examination.** The application will be accorded special status throughout its entire course of prosecution until one of the following occurs:

A. filing a **petition for extension of time** to extend the time period for filing a reply;

B. filing an **amendment to amend the application to contain more than four independent claims, more than thirty total claims**, or a multiple dependent claim;

C. filing a **request for continued examination**;

D. filing a notice of appeal;

E. filing a request for suspension of action;

F. mailing of a notice of allowance;

G. mailing of a final Office action;

H. completion of examination as defined in 37 CFR 41.102; or

I. abandonment of the application.

Telephone inquiries with regard to this decision should be directed to Brian W. Brown at 571-272-5338.

/Brian W. Brown/
[*Signature*]

Petitions Examiner, Office of Petitions
(Title)

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
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NOTICE OF ALLOWANCE AND FEE(S) DUE

101325 7590 12/16/2016

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17014 NEW COLLEGE AVENUE
SUITE 201
WILDWOOD, MO 63040

EXAMINER
TOWNSLEY, SARA ELIZABETH

ART UNIT	PAPER NUMBER
1629	

DATE MAILED: 12/16/2016

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/610,580	09/11/2012	Bruce Scharschmidt	HOR0027-201-US	1957

TITLE OF INVENTION: METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	03/16/2017

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

101325 7590 12/16/2016
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Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/610,580	09/11/2012	Bruce Scharschmidt	HOR0027-201-US	1957

TITLE OF INVENTION: METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	03/16/2017

EXAMINER	ART UNIT	CLASS-SUBCLASS
TOWNSLEY, SARA ELIZABETH	1629	514-533000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
- ❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.
1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. The following fee(s) are submitted:
- ❑ Issue Fee
- ❑ Publication Fee (No small entity discount permitted)
- ❑ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
- ❑ A check is enclosed.
- ❑ Payment by credit card. Form PTO-2038 is attached.
- ❑ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
- ❑ Applicant certifying micro entity status. See 37 CFR 1.29
  NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
- ❑ Applicant asserting small entity status. See 37 CFR 1.27
  NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
- ❑ Applicant changing to regular undiscounted fee status.
  NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

PTOL-85 Part B (10-13) Approved for use through 10/31/2013. OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/610,580	09/11/2012	Bruce Scharschmidt	HOR0027-201-US	1957

101325 7590 12/16/2016

GLOBAL PATENT GROUP - HOR
17014 NEW COLLEGE AVENUE
SUITE 201
WILDWOOD, MO 63040

EXAMINER	
TOWNSLEY, SARA ELIZABETH	
ART UNIT	PAPER NUMBER
1629	

DATE MAILED: 12/16/2016

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. 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, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. 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.

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2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 13/610,580	Applicant(s) SCHARSCHMIDT ET AL.	
	Examiner SARA E. TOWNSLEY	Art Unit 1629	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *Applicant's reply filed Nov. 18, 2016*.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on_____.
2. ☐ 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.
3. ☒ The allowed claim(s) is/are *14 and 17*. As a result of the allowed claim(s), 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.
4. ☐ 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)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
4. ☒ Interview Summary (PTO-413), Paper No./Mail Date *20161208* .
5. ☒ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/SARA E. TOWNSLEY/ Examiner, Art Unit 1629	/JEFFREY S. LUNDGREN/ Supervisory Patent Examiner, Art Unit 1629

EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in an interview with Applicant's representative, Lauren Stevens, on Dec. 8, 2016.

The application has been amended as follows:

Claims 15, 16, and 18-22 are canceled.

Claim 14 is amended in its entirety as follows:

A method of treating a urea cycle disorder in a subject comprising administering to a subject having a plasma PAA to PAGN ratio outside the target range of 1 to 2, a dosage of glyceryl tri-[4-phenylbutyrate] (HPN-100) effective to achieve a plasma PAA to PAGN ratio within the target range of 1 to 2.

Claim 17 is amended in its entirety as follows:

A method of treating a urea cycle disorder in a subject comprising administering to a subject having a plasma PAA to PAGN ratio outside the target range of 1 to 2.5, a dosage of glyceryl tri-[4-phenylbutyrate] (HPN-100) effective to achieve a plasma PAA to PAGN ratio within the target range of 1 to 2.5.

CORRESPONDENCE

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SARA E. TOWNSLEY whose telephone number is (571)270-7672. The examiner can normally be reached on Mon - Fri, 9:00 am - 5:00 pm (EST).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jeff S. Lundgren can be reached on 571-272-5541. 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.

/SARA E. TOWNSLEY/
Examiner, Art Unit 1629

/JEFFREY S. LUNDGREN/
Supervisory Patent Examiner, Art Unit 1629

Examiner-Initiated Interview Summary	Application No.	Applicant(s)	
	13/610,580	SCHARSCHMIDT ET AL.	
	Examiner	Art Unit	
	SARA E. TOWNSLEY	1629	

All participants (applicant, applicant's representative, PTO personnel):

(1) *SARA E. TOWNSLEY*. (3)_____.

(2) *LAUREN STEVENS (Applicant's representative)*. (4)_____.

Date of Interview: *08 December 2016*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☒ No.
If Yes, brief description: _____.

Issues Discussed ☐101 ☒112 ☐102 ☐103 ☐Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *All*.

Identification of prior art discussed: *N/A*.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

Agreed to amend independent claims 14 and 17 to overcome potential issues under 35 U.S.C. 112, and to cancel claims 15, 16, and 18-22.

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/SARA E. TOWNSLEY/ Examiner, Art Unit 1629	/JEFFREY S. LUNDGREN/ Supervisory Patent Examiner, Art Unit 1629

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13610580	SCHARSCHMIDT ET AL.
	Examiner	Art Unit
	SARA E TOWNSLEY	1629

CPC- SEARCHED

Symbol	Date	Examiner
A61K31/192	12/9/2016	set
A61K31/216	12/9/2016	set

CPC COMBINATION SETS - SEARCHED

Symbol	Date	Examiner

US CLASSIFICATION SEARCHED

Class	Subclass	Date	Examiner

SEARCH NOTES

Search Notes	Date	Examiner
61/636,256 considered	2/20/2015	set
Inventor name/assignee search (PALM, EAST)	2/20/2015	set
EAST keyword search (USPAT, PGPub, USOCR, EPO, JPO, Derwent)	2/20/2015	set
Patentability conference (Jeff Lundgren)	12/9/2016	set

INTERFERENCE SEARCH

US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
A61K	31/192	12/9/2016	set
A61K	31/216	12/9/2016	set

/SARA E TOWNSLEY/ Examiner, Art Unit 1629	

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13610580	SCHARSCHMIDT ET AL.
	Examiner	Art Unit
	SARA E TOWNSLEY	1629

✓	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	10/05/2014	02/20/2015	05/16/2016	12/08/2016					
	1	÷	✓	✓	-					
	2	÷	✓	✓	-					
	3	-	-	-	-					
	4	-	-	-	-					
	5	÷	✓	✓	-					
	6	÷	✓	✓	-					
	7	÷	✓	-	-					
	8	-	-	-	-					
	9	÷	✓	✓	-					
	10	÷	✓	✓	-					
	11	÷	✓	✓	-					
	12	÷	✓	✓	-					
	13	÷	✓	-	-					
1	14				=					
	15				-					
	16				-					
2	17				=					
	18				-					
	19				-					
	20				-					
	21				-					
	22				-					

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13610580	SCHARSCHMIDT ET AL.
	Examiner	**Art Unit**
	SARA E TOWNSLEY	1629

CPC						
Symbol					**Type**	**Version**
A61K		31	/	192	F	2013-01-01
A61K		31	/	216	I	2013-01-01
G01N		33	/	6812	I	2013-01-01
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CPC Combination Sets							
Symbol					**Type**	**Set**	**Ranking**	**Version**
			/					
			/					

/SARA E TOWNSLEY/ Examiner.Art Unit 1629	12/9/2016	**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	2	
/JEFFREY S LUNDGREN/ Supervisory Patent Examiner.Art Unit 1629	12/12/2016	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	NONE

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13610580	SCHARSCHMIDT ET AL.
	Examiner	**Art Unit**
	SARA E TOWNSLEY	1629

US ORIGINAL CLASSIFICATION		INTERNATIONAL CLASSIFICATION														
CLASS	SUBCLASS	CLAIMED									NON-CLAIMED					
514	533	A	6	1	K	31 / 192 (2006.01.01)										
CROSS REFERENCE(S)		A	6	1	K	31 / 216 (2006.01.01)										
		A	6	1	K	31 / 225 (2006.01.01)										
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)															
514	570															

/SARA E TOWNSLEY/ Examiner.Art Unit 1629	12/9/2016	**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	2	
/JEFFREY S LUNDGREN/ Supervisory Patent Examiner.Art Unit 1629	12/12/2016	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	NONE

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13610580	SCHARSCHMIDT ET AL.
	Examiner	**Art Unit**
	SARA E TOWNSLEY	1629

☒ **Claims renumbered in the same order as presented by applicant** ☐ **CPA** ☐ **T.D.** ☐ **R.1.47**

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	14														
2	17														

/SARA E TOWNSLEY/ Examiner.Art Unit 1629	12/9/2016	**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	2	
/JEFFREY S LUNDGREN/ Supervisory Patent Examiner.Art Unit 1629	12/12/2016	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	NONE

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

101325 7590 12/16/2016
GLOBAL PATENT GROUP - HOR
17014 NEW COLLEGE AVENUE
SUITE 201
WILDWOOD, MO 63040

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

VIA EFS-WEB	(Depositor's name)
	(Signature)
12/22/2016	(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/610,580	09/11/2012	Bruce Scharschmidt	HOR0027-201-US	1957

TITLE OF INVENTION: METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	03/16/2017

EXAMINER	ART UNIT	CLASS-SUBCLASS
TOWNSLEY, SARA ELIZABETH	1629	514-533000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
- ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.
1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE: Horizon Therapeutics, LLC

(B) RESIDENCE: (CITY and STATE OR COUNTRY): Lake Forest, IL

Please check the appropriate assignee category or categories (will not be printed on the patent) : ☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:
- ☒ Issue Fee
- ☐ Publication Fee (No small entity discount permitted)
- ☐ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
- ☐ A check is enclosed.
- ☐ Payment by credit card. Form PTO-2038 is attached.
- ☒ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number 50-4297 (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
- ☐ Applicant certifying micro entity status. See 37 CFR 1.29
  NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
- ☐ Applicant asserting small entity status. See 37 CFR 1.27
  NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
- ☐ Applicant changing to regular undiscounted fee status.
  NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature /Chris Marion/ Date December 22, 2016

Typed or printed name Chris Marion Registration No. L0931

PTOL-85 Part B (10-13) Approved for use through 10/31/2013. OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Electronic Patent Application Fee Transmittal

Application Number:	13610580
Filing Date:	11-Sep-2012
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS
First Named Inventor/Applicant Name:	Bruce Scharschmidt
Filer:	Christopher Lee Marion/Valerie Lechner
Attorney Docket Number:	HOR0027-201-US

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:				
UTILITY APPL ISSUE FEE	1501	1	960	960

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**960**

Electronic Acknowledgement Receipt

EFS ID:	27887112
Application Number:	13610580
International Application Number:	
Confirmation Number:	1957
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS
First Named Inventor/Applicant Name:	Bruce Scharschmidt
Customer Number:	101325
Filer:	Christopher Lee Marion/Valerie Lechner
Filer Authorized By:	Christopher Lee Marion
Attorney Docket Number:	HOR0027-201-US
Receipt Date:	22-DEC-2016
Filing Date:	11-SEP-2012
Time Stamp:	18:11:32
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$960
RAM confirmation Number	122316INTEFSW00006220504297
Deposit Account	504297
Authorized User	Valerie Lechner
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: 37 CFR 1.20 (Post Issuance fees) 37 CFR 1.21 (Miscellaneous fees and charges)	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Issue Fee Payment (PTO-85B)	HOR0027_IssueFeeTransmittal.pdf	92962 f689fee6c0632232c341be6d6c50f9902388b7dc	no	1
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30716 ea2208971312a1d1db213b290d181fd527993314	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	123678		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

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/08 (09-06)
Approved for use through 03/31/2007. OMB 0651-0031
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Substitute for form 1449/PTO				***Complete if Known***	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT				**Application Number**	13/610,580
				Filing Date	September 11, 2012
Date Submitted: March 12, 2012				**First Named Inventor**	Bruce Scharschmidt
				Art Unit	1629
(use as many sheets as necessary)				**Examiner Name**	Sara Elizabeth Townsley
Sheet	1	of	10	**Attorney Docket Number**	HOR0027-201-US

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
	P1	4,457,942	07-03-1984	Brusilow, S.W.	
	P2	5,654,333	08-05-1997	~~The United States Of America As Represented By The Department Of Health And Human Services~~	Samid
Change(s) applied to document, /K.S.S./ 12/21/2016	P3	8,094,521	01-10-2012	~~Nightengale Products LLC~~	Levy
	P4	8,404,215	03-26-2013	~~Hyperion Therapeutics, Inc.~~	Scharschmidt et al.
	P5	2003/0195255	10-16-2003	Marshall L. Summar	
	P6	2005/0273359	12-08-2005	Young, D.E.	
	P7	2010/0016207	01-21-2010	Wurtman, RJ et al	
	P8	2014/0142186	05-22-2014	~~Hyperion Therapeutics, Inc.~~	Scharschmidt et al.
	P9	8,642,012	02-04-2014	~~Hyperion Therapeutics, Inc.~~	Scharschmidt

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]
	F1	WO1994/22494	10-13-1994	The DuPont Merck Pharmaceutical Company		
	F2	WO2013/048558	04-04-2013	Hyperion Therapeutics, Inc.		
	F3	WO2013/158145	10-24-2013	Hyperion Therapeutics, Inc.		

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.E.T./

Examiner Signature	/Sara E. Townsley/	Date Considered	05/16/2016

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. 1 Applicant's unique citation designation number (optional). 2 See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. 3 Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). 4 For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. 5 Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. 6 Applicant is to place a check mark here if English language Translation is attached.

Receipt date: 11/19/2014

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

Form PTO-1449 (Modified)

(Use several sheets if necessary)

Sheet	1	of	11

COMPLETE IF KNOWN	
Application Number	13/610,580
Confirmation Number	1957
Filing Date	September 11, 2012
First Named Inventor	SCHARSCHMIDT, Bruce
Group Art Unit	1765
Examiner Name	
Attorney Docket No.	79532.8004.US01

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.	U.S. Patent or Application NUMBER	Kind Code (if known)	Name of Patentee or Inventor of Cited Document	Date of Publication or Filing Date of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
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	A2	5,968,979		BRUSILOW	10/19/1999	
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FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.	Foreign Patent or Application Office	NUMBER	Kind Code (if known)	Name of Patentee or Applicant of Cited Document	Date of Publication or Filing Date of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T
	B1	WO	2005/053607		Medicis Pharmaceuticla Corp.	6/16/2005		
	B2	WO	2006/056794		UCL Business PCL	6/01/2006		
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	B5	WO	2009/134460		Hyperion Therapeutics	11/05/2009		
	B6	WO	2010/025303		Hyperion Therapeutics	03/04/2010		
	B7	WO	2012/028620		INSERM	03/08/2012		

OTHER PRIOR ART-NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.	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

EXAMINER	/Sara E. Townsley/	DATE CONSIDERED	12/01/2014

*EXAMINER: Initial if reference considered, whether or not criteria is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to application(s).

79532-8004.US01/LEGAL124080222.1

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.E.T.

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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/610,580	02/07/2017	9561197	HOR0027-201-US	1957

101325 7590 01/18/2017

GLOBAL PATENT GROUP - HOR
17014 NEW COLLEGE AVENUE
SUITE 201
WILDWOOD, MO 63040

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 649 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

Bruce Scharschmidt, San Francisco, CA;
Masoud Mokhtarani, Walnut Creek, CA;

AO 120 (Rev. 08/10)

TO:	**Mail Stop 8** **Director of the U.S. Patent and Trademark Office** **P.O. Box 1450** **Alexandria, VA 22313–1450**	**REPORT ON THE FILING OR DETERMINATION OF AN ACTION REGARDING A PATENT OR TRADEMARK**

In Compliance with 35 U.S.C. § 290 and/or 15 U.S.C. § 1116 you are hereby advised that a court action has been filed in the **U.S. District Court for the District of New Jersey** on the following:
___ Trademarks or **X** Patents. (____ the patent action involves 35 U.S.C. § 292.)

DOCKET NO.	DATE FILED	U.S. DISTRICT COURT
2:17-cv-05901-KM-MAH	8/9/2017	NEWARK, NJ
PLAINTIFF HORIZON THERAPEUTICS, LLC		DEFENDANT PAR PHARMACEUTICAL, INC.

PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1 ~~PLEASE SEE ATTACHED COMPLAINT AND EXHIBIT A~~		
2 9561197		
3		
4		
5		

In the above—entitled case, the following patent(s)/ trademark(s) have been included:

DATE INCLUDED	INCLUDED BY ___ Amendment ___ Answer ___ Cross Bill ___ Other Pleading	
PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1		
2		
3		
4		
5		

In the above—entitled case, the following decision has been rendered or judgement issued:

DECISION/JUDGEMENT

CLERK	(BY) DEPUTY CLERK	DATE
William T. Walsh	s/ Donato Marucci	8/9/2017

Copy 1—Upon initiation of action, mail this copy to Director Copy 3—Upon termination of action, mail this copy to Director
Copy 2—Upon filing document adding patent(s), mail this copy to Director Copy 4—Case file copy