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I, Colin Lewis Bodkin, of 31 Market Street, Sydney, New South Wales, 2000, Australia, Patent Attorney for the Applicant/Nominated Person in respect of Application No. 27783/95, state the following:-

The Applicant/Nominated Person has entitlement from the actual inventors as follows:

The Applicant/Nominated Person is the assignee of the actual inventors.

The Applicant/Nominated Person is entitled to rely on the application listed in the Declaration under Article 8 of the PCT as follows:

The Applicant/Nominated Person is the assignee of the basic applicants.

The basic application listed on the Declaration under Article 8 of the PCT is the first application made in a Convention country in respect of the invention.

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TECHNETIUM-99M LABELED PEPTIDES FOR IMAGING

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(57) Claim

1. A reagent comprising a specific binding peptide comprising 3 to 100 amino acid residues, a sidechain of one residue being covalently linked to a technetium-99m-binding moiety selected from the group consisting of:

I.

Cp(aa)Cp

wherein Cp is a protected cysteine and (aa) is any primary α- or β-amino acid not containing a thiol group;

and

II.

a technetium-99m complexing moiety comprising a single thiol-containing moiety having a formula:

$$A\text{-}CZ(B)\text{-}\{C(R^1R^2)\}_n\text{-}X$$

wherein A is H, HOOC, H_2NOC, (amino acid or peptide)-NHOC, (amino acid or peptide)-OOC or R^4;

B is H, SH, $-NHR^3$, $-N(R^3)$-(amino acid or peptide), or R^4;

X is H, SH, $-NHR^3$, $-N(R^3)$-(amino acid or peptide) or R^4;

Z is H or R^4;

R^1, R^2, R^3 and R^4 are independently H or lower straight or branched chain or cyclic alkyl;

n is 0, 1 or 2;

(peptide) is a peptide of 2 to about 10 amino acids;

.../2

and

where B is -NHR^3 or -$N(R^3)$-(amino acid or peptide), X is SH, and n is 1 or 2;
where X is -NHR^3 or -$N(R^3)$-(amino acid or peptide), B is SH, and n is 1 or 2;
where B is H or R^4, A is HOOC, H_2NOC, (amino acid or peptide)-NHOC, (amino acid or peptide)-OOC, X is SH, and n is 0 or 1;
where A is H or R^4, then where B is SH, X is -NHR^3 or -$N(R^3)$-(amino acid or peptide) and where X is SH, B is -NHR^3 or -$N(R^3)$-(amino acid or peptide);
where X is H or R^4, A is HOOC, H_2NOC, (amino acid or peptide)-NHOC, (amino acid or peptide OOC and B is SH;
where Z is methyl, X is methyl, A is HOOC, H_2NOC, (amino acid or peptide)-NHOC, (amino acid or peptide)-OOC, B is SH and n is 0;
and wherein the thiol moiety is in the reduced form and (amino acid) is any primary α- or β-amino acid not containing a thiol group.

2. The reagent of claim 1, wherein the peptide and the technetium-99m-binding moiety are covalently linked through a lysine residue or a homocysteine residue.

3. The reagent of claim 1, wherein the technetium-99m complexing moiety is

$$Cp(aa)Cp$$

and Cp has a formula

$$-CH_2-NH-CO-R$$

wherein R is a lower alkyl having 1 to 6 carbon atoms, 2-pyridyl, 3-pyridyl, 4-pyridyl, phenyl, or phenyl substituted with lower alkyl, hydroxy, lower alkoxy, carboxy, or lower alkoxycarbonyl.

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(54) Title: TECHNETIUM-99m LABELED PEPTIDES FOR IMAGING

(57) Abstract

This invention relates to radiolabeled peptides and methods for producing such peptides. Specifically, the invention relates to peptides, methods and kits for making such peptides, and methods for using such peptides to image sites in a mammalian body labeled with technetium-99m (Tc-99m) *via* a radiolabel-binding moiety covalently attached to a specific binding peptide *via* an amino acid side-chain of the peptide.

TECHNETIUM-99m LABELED PEPTIDES FOR IMAGING

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to radiodiagnostic reagents and peptides, and methods for producing labeled radiodiagnostic agents. Specifically, the invention relates to peptides, methods and kits for making such peptides, and methods for using such peptides to image sites in a mammalian body labeled with technetium-99m (Tc-99m) *via* a radiolabel-binding moiety which forms a neutral complex with Tc-99m.

2. Description of the Prior Art

In the field of nuclear medicine, certain pathological conditions are localized, or their extent is assessed, by detecting the distribution of small quantities of internally-administered radioactively labeled tracer compounds (called radiotracers or radiopharmaceuticals). Methods for detecting these radiopharmaceuticals are known generally as imaging or radioimaging methods.

In radioimaging, the radiolabel is a gamma-radiation emitting radionuclide and the radiotracer is located using a gamma-radiation detecting camera (this process is often referred to as gamma scintigraphy). The imaged site is detectable because the radiotracer is chosen either to localize at a pathological site (termed positive contrast) or, alternatively, the radiotracer is chosen specifically not to localize at such pathological sites (termed negative contrast).

A number of factors must be considered for optimal radioimaging in humans. To maximize the efficiency of detection, a radionuclide that emits gamma energy in the 100 to 200 keV range is preferred. To minimize the absorbed radiation dose to the patient, the physical half-life of the radionuclide should be as short as the imaging procedure will allow. To allow for examinations to be performed on any day and at any time of the day, it is advantageous to have a source of the radionuclide always available at the clinical site.

A variety of radionuclides are known to be useful for radioimaging, including ^{67}Ga, ^{99m}Tc (Tc-99m), ^{111}In, ^{123}I, ^{125}I, and ^{169}Yb. Tc-99m is a preferred radionuclide because it emits gamma radiation at 140 keV, it has a physical half-life of 6 hours, and it is readily available on-site using a molybdenum-99/technetium-99m generator.

The sensitivity of imaging methods using radioactively-labeled peptides is much higher than other radiopharmaceuticals known in the art, since the specific binding of the radioactive peptide concentrates the radioactive signal over the area of interest. Small synthetic peptides that bind specifically to targets of interest may be advantageously used as the basis for radiotracers. This is because: 1. they may be synthesized chemically (as opposed to requiring their production in a biological system such as bacteria or mammalian cells, or their isolation from a biologically-derived substance such as a fragment of a protein); 2. they are small, hence non-target bound radiotracer is rapidly eliminated from the body, thereby reducing background (non-target) radioactivity and allowing good definition of the target; and 3. small peptides may be readily manipulated chemically to optimize their affinity for a particular binding site.

Small readily synthesized labeled peptide molecules are preferred as routinely-used radiopharmaceuticals. There is clearly a need for small synthetic labeled peptides that can be directly injected into a patient and will image pathological sites by localizing at such sites. Tc-99m labeled small synthetic peptides offer clear advantages as radiotracers for gamma scintigraphy, due to the properties of Tc-99m as a radionuclide for imaging and the utility of specific-binding small synthetic peptides as radiotracer molecules.

Radiolabeled peptides have been reported in the prior art.

Ege *et al.*, U.S. Patent No. 4,832,940 teach radiolabeled peptides for imaging localized T-lymphocytes.

Olexa *et al.*, 1982, European Patent Application No. 823017009 disclose a pharmaceutically acceptable radiolabeled peptide selected from Fragment E_1 isolated from cross-linked fibrin, Fragment E_2 isolated from cross-linked fibrin, and peptides having an amino acid sequence intermediate between Fragments E_1 and E_2.

Ranby *et al.*, 1988, PCT/US88/02276 disclose a method for detecting fibrin deposits in an animal comprising covalently binding a radiolabeled compound to fibrin.

Hadley *et al.*, 1988, PCT/US88/03318 disclose a method for detecting a fibrin-platelet clot *in vivo* comprising the steps of (a) administering to a patient a labeled attenuated thrombolytic protein, wherein the label is selectively attached to a portion of the thrombolytic protein other than the fibrin binding domain; and (b) detecting the pattern of distribution of the labeled thrombolytic protein in the patient.

Lees *et al.*, 1989, PCT/US89/01854 teach radiolabeled peptides for arterial imaging.

Sobel, 1989, PCT/US89/02656 discloses a method to locate the position of one or more thrombi in an animal using radiolabeled, enzymatically inactive tissue plasminogen activator.

Stuttle, 1990, PCT/GB90/00933 discloses radioactively labeled peptides containing from 3 to 10 amino acids comprising the sequence arginine-glycine-aspartic acid (RGD), capable of binding to an RGD binding site *in vivo*.

Maraganore *et al.*, 1991, PCT/US90/04642 disclose a radiolabeled thrombus inhibitor comprising (a) a inhibitor moiety; (b) a linker moiety; and (c) and anion binding site moiety.

Rodwell *et al.*, 1991, PCT/US91/03116 disclose conjugates of "molecular recognition units" with "effector domains".

Tubis *et al.*, 1968, Int. J. Appl. Rad. Isot. 19: 835-840 describe labeling a peptide with technetium-99m.

Sundrehagen, 1983, Int. J. Appl. Rad. Isot. 34: 1003 describes labeling polypeptides with technetium-99m.

The use of chelating agents for radiolabeling polypeptides, and methods for labeling peptides and polypeptides with Tc-99m are known in the prior art and are disclosed in co-pending U.S. Patent Applications Serial Nos. 07/653,012 and 07/807,062, which are hereby incorporated by reference.

Although optimal for radioimaging, the chemistry of Tc-99m has not been as thoroughly studied as the chemistry of other elements and for this reason methods of radiolabeling with technetium are not abundant. Tc-99m is normally obtained as Tc-99m pertechnetate (TcO_4^-; technetium in the +7 oxidation state), usually from a molybdenum-99/technetium-99m generator. However, pertechnetate does not bind well to other compounds. Therefore, in order to radiolabel a peptide, Tc-99m pertechnetate must be converted to another form. Since technetium does not form a stable ion in aqueous solution, it must be held in such solutions in the form of a coordination complex that has sufficient kinetic and thermodynamic stability to prevent decomposition and resulting conversion of Tc-99m either to insoluble technetium dioxide or back to pertechnetate.

Such coordination complexes of Tc-99m (in the +1 to +6 oxidation states) are known. However, many of these complexes are inappropriate for radiolabeling due to the molecular geometry of the coordination complex. For the purpose of radiolabeling, it is particularly advantageous for the coordination complex to be formed as a chelate in which all of the donor groups surrounding the technetium ion are provided by a single chelating ligand. This allows the chelated Tc-99m to be covalently bound to a peptide through a single linker between the chelator and the peptide.

These ligands are sometimes referred to as bifunctional chelating agents having a chelating portion and a linking portion. Such compounds are known in the prior art.

Byrne *et al.*, U.S. Patent No. 4,434,151 describe homocysteine thiolactone-derived bifunctional chelating agents that can couple radionuclides to terminal amino-containing compounds that are capable of localizing in an organ or tissue to be imaged.

Fritzberg, U.S. Patent No. 4,444,690 describes a series of technetium-chelating agents based on 2,3-bis(mercaptoacetamido) propanoate.

Byrne *et al.*, U.S. Patent Nos. 4,571,430 describe novel homocysteine thiolactone bifunctional chelating agents for chelating radionuclides that can couple radionuclides to terminal amino-containing compounds that are capable of localizing in an organ or tissue to be imaged.

Byrne *et al.*, U.S. Patent Nos. 4,575,556 describe novel homocysteine thiolactone bifunctional chelating agents for chelating radionuclides that can couple radionuclides to terminal amino-containing compounds that are capable of localizing in an organ or tissue to be imaged.

Davison *et al.*, U.S. Patent No. 4,673,562 describe technetium chelating complexes of bisamido-bisthio-ligands and salts thereof, used primarily as renal function monitoring agents.

Nicolotti *et al.*, U.S. Patent No. 4,861,869 describe bifunctional coupling agents useful in forming conjugates with biological molecules such as antibodies.

Fritzberg *et al.*, U.S. Patent 4,965,392 describe various S-protected mercaptoacetylglycylglycine-based chelators for labeling proteins.

Fritzberg *et al.*, European Patent Application No. 86100360.6 describe dithiol, diamino, or diamidocarboxylic acid or amine complexes useful for making technetium-labeled imaging agents.

Dean *et al.*, 1989, PCT/US89/02634 describe bifunctional coupling agents for radiolabeling proteins and peptides.

Flanagan *et al.*, European Patent Application No. 90306428.5 disclose Tc-99m labeling of synthetic peptide fragments *via* a set of organic chelating molecules.

Albert *et al.*, European Patent Application No. WO 91/01144 disclose radioimaging using radiolabeled peptides related to growth factors, hormones, interferons and cytokines and comprised of a specific recognition peptide covalently linked to a radionuclide chelating group.

Dean, co-pending U.S. Patent Application Serial No. 07/653,012 teaches reagents and methods for preparing peptides comprising a Tc-99m chelating group covalently linked to a specific binding peptide for radioimaging *in vivo*, and is hereby incorporated by reference.

Baidoo & Lever, 1990, Bioconjugate Chem. 1: 132-137 describe a method for labeling biomolecules using a bisamine bisthiol group that gives a cationic technetium complex.

It is possible to radiolabel a peptide by simply adding a thiol-containing moiety such as cysteine or mercaptoacetic acid. Such procedures have been described in the prior art.

Schochat *et al.*, U.S. Patent No. 5,061,641 disclose direct radiolabeling of proteins comprised of at least one "pendent" sulfhydryl group.

Dean *et al.*, co-pending U.S. Patent Application 07/807,062 teach radiolabeling peptides *via* attached groups containing free thiols, and is incorporated herein by reference.

Goedemans *et al.*, PCT Application No. WO 89/07456 describe radiolabeling proteins using cyclic thiol compounds, particularly 2-iminothiolane and derivatives.

Thornback *et al.*, EPC Application No. 90402206.8 describe preparation and use of radiolabeled proteins or peptides using thiol-containing compounds, particularly 2-iminothiolane.

Stuttle, PCT Application No. WO 90/15818 describes Tc-99m labeling of RGD-containing oligopeptides.

Burns *et al.*, 1985, European Patent Application 85104959.3 describe bisamine bisthiol compounds for making small neutral Tc-99m brain imaging agents.

Kung *et al.*, 1986, European Patent Application 86105920.2 describe bisamine bisthiol compounds for making small neutral Tc-99m imaging agents.

Bergstein *et al.*, 1988, European Patent Application 88102252.9 describe bisamine bisthiol compounds for making small neutral Tc-99m brain imaging agents.

Bryson *et al.*, 1988, Inorg. Chem. 27: 2154-2161 describe neutral complexes of technetium-99 which are unstable to excess ligand.

Misra *et al.*, 1989, Tet. Let. 30: 1885-1888 describe bisamine bisthiol compounds for radiolabeling purposes.

Bryson *et al.*, 1990, Inorg. Chem. 29: 2948-2951 describe chelators containing two amide groups, a thiol group and a substituted pyridine that may form neutral Tc-99 complexes.

Taylor *et al.*, 1990, J. Nucl. Med. 31: 885 (Abst) describe a neutral Tc-99m complex for brain imaging.

The use of chelating agents for radiolabeling peptides, and methods for labeling peptides with Tc-99m are known in the prior art and are disclosed in co-pending U.S. Patent Applications Serial Nos. 07/653,012, 07/807,062, 07/871,282, 07/886,752, 07/893,981, 07/955,466, 08/019,864, 08/073,577, 08/210,822, 08/236,402 and 08/241,625, and radiolabeled peptides for use as scintigraphic imaging agents for imaging thrombi are known in the prior art and are disclosed in co-pending U.S. Patent Applications Serial Nos. 07/886,752, 07/893,981 and 08/044,825 and International Patent Applications Serial Nos. PCT/US92/00757,

PCT/US92/10716, PCT/US93/02320, PCT/US93/03687, PCT/US93/04794, PCT/US93/05372, PCT/US93/06029, PCT/US93/09387, PCT/US94/01894, PCT/US94/03878, and PCT/US94/05895, each of which are hereby incorporated by reference in its entirety.

SUMMARY OF THE INVENTION

The present invention provides scintigraphic imaging agents that are radioactively-labeled peptides. The radiolabeled peptides of the invention are comprised of peptides that specifically bind to a target *in vivo* and are covalently linked to a radiolabel-binding moiety wherein the moiety binds a radioisotope. It is a particular advantage in the present invention that the radiolabel-binding moiety is covalently linked to a side-chain of an amino acid residue comprising the peptide.

This mode of covalent linkage is advantageous for a number of reasons. First, covalent linkage to a side-chain of an amino acid constituent of the peptide avoids interference of the covalently linked radiolabel binding moiety with the specific binding properties of the specific binding peptide. Second, this arrangement permits cyclic peptides, which by definition not comprised of free amino or carboxy termini, to be used in conjunction with radiolabel binding moieties to be useful as scintigraphic imaging agents as disclosed here. Third, covalent linkage to the sidechain of a constituent amino acid permits each of the scintigraphic imaging agents of the invention to be more flexibly designed to achieve optimal increases efficacy and reductions in antigenicity, etc. Conjugation to an amino acid side-chain also allows the radiolabel binding moiety to be added to the peptide during synthesis as an amino acid conjugate, or after synthesis of the completed peptide has been achieved. Lastly, cyclic peptides are known to be resistant to exoprotease digestion, thereby incorporating the improved stability of such peptides *in vivo* into the scintigraphic imaging agents of the invention.

In a first aspect of the present invention, radiolabeled peptides are provided capable of imaging sites within a mammalian body. The peptides are comprised of a specific binding peptide having an amino acid sequence and a radiolabel-binding moiety covalently linked to the peptide. Further, the radiolabel-binding moiety is covalently linked to a side-chain of an amino acid comprising the peptide. In a preferred embodiment, the radiolabel-binding moiety is covalently linked to the side-chain of an amino acid having a side-chian comprising an amine or a thiol, the amino acid being most preferably lysine or homocysteine. In another preferred embodiment, the radiolabel is technetium-99m.

One aspect of the invention provides a reagent for preparing a a scintigraphic imaging agent for imaging sites within a mammalian body, comprising a specific binding peptide wherein a radiolabel binding moiety is covalently linked to the peptide *via* an amino acid side-chain of an amino acid of the peptide, the radiolabel binding moiety having the formula:

I.

$$C(pgp)^S\text{-}(aa)\text{-}C(pgp)^S$$

wherein $C(pgp)^S$ is a protected cysteine and (aa) is any primary α- or β-amino acid not containing a thiol group. In a preferred embodiment, the amino acid is glycine.

In another embodiment, the invention provides a reagent for preparing a scintigraphic imaging agent for imaging sites within a mammalian body, comprising a specific binding peptide wherein a radiolabel binding moiety is covalently linked to the peptide via an amino acid side-chain of an amino acid of the peptide, the radiolabel binding moiety having the formula:

II.

$$A\text{-}CZ(B)\text{-}\{(C(R^1R^2)\}_n\text{-}X$$

wherein A is H, HOOC, H_2NOC, (amino acid or peptide)-NHOC, (amino acid or peptide)-OOC or R^4; B is H, SH or $-NHR^3$, $-N(R^3)$-(amino acid or peptide) or R^4; Z is H or R^4; X is SH or $-NHR^3$, $-N(R^3)$-(amino acid or peptide) or R^4; R^1, R^2, R^3 and R^4 are independently H or straight or branched chain or cyclic lower alkyl; n is 0, 1 or 2; wherein (peptide) is a peptide of 2 to about 10 amino acids; and: (1) where B is $-NHR^3$ or $-N(R^3)$-(amino acid or peptide), X is SH and n is 1 or 2; (2) where X is $-NHR^3$ or $-N(R^3)$-(amino acid or peptide), B is SH and n is 1 or 2; (3) where B is H or R^4, A is HOOC, H_2NOC, (amino acid or peptide)-NHOC, (amino acid or peptide)-OOC, X is SH and n is 0 or 1; (4) where A is H or R^4, then where B is SH, X is $-NHR^3$ or $-N(R^3)$-(amino acid or peptide) and where X is SH, B is -

NHR^3 or $-N(R^3)$-(amino acid or peptide); (5) where X is H or R^4, A is HOOC, H_2NOC, (amino acid or peptide)-NHOC, (amino acid or peptide)-OOC and B is SH; (6) where Z is methyl, X is methyl, A is HOOC, H_2NOC, (amino acid or peptide)-NHOC, (amino acid or peptide)-OOC and B is SH and n is 0; and (7) where B is SH and X is SH, n is not 0; and wherein the thiol moiety is in the reduced form and wherein (amino acid) is any primary α- or β-amino acid not containing a thiol group.

In particular embodiments of this aspect of the invention, the radiolabel-binding moiety has a formula that is:

IIa. -(amino acid)^1-(amino acid)^2-$\{A\text{-}CZ(B)\text{-}\{C(R^1R^2)\}_n\text{-}X\}$,

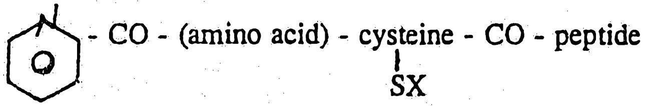
IIb. -$\{A\text{-}CZ(B)\text{-}\{C(R^1R^2)\}_n\text{-}X\}$-(amino acid)^1-(amino acid)^2,

IIc. -(a primary α,ω- or β,ω-diamino acid)-(amino acid)^1-$\{A\text{-}CZ(B)\text{-}\{C(R^1R^2)\}_n\text{-}X\}$, or

IId. -$\{A\text{-}CZ(B)\text{-}\{C(R^1R^2)\}_n\text{-}X\}$-(amino acid)^1-(a primary α,β- or β,γ-diamino acid)

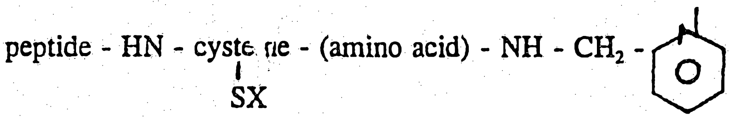
wherein (amino acid)^1 and (amino acid)^2 are each independently any naturally-ocurring, modified, substituted or altered α- or β-amino acid not containing a thiol group; A is H, HOOC, H_2NOC, (amino acid or peptide)-NHOC, (amino acid or peptide)-OOC or R^4; B is H, SH or $-NHR^3$, $-N(R^3)$-(amino acid or peptide) or R^4; Z is H or R^4; X is SH or $-NHR^3$, $-N(R^3)$-(amino acid or peptide) or R^4; R^1, R^2, R^3 and R^4 are independently H or straight or branched chain or cyclic lower alkyl; n is an integer that is either 0, 1 or 2; (peptide) is a peptide of 2 to about 10 amino acids; and: (1) where B is $-NHR^3$ or $-N(R^3)$-(amino acid or peptide), X is SH and n is 1 or 2; (2) where X is $-NHR^3$ or $-N(R^3)$-(amino acid or peptide), B is SH and n is 1 or 2; (3) where B is H or R^4, A is HOOC, H_2NOC, (amino acid or peptide)-NHOC, (amino acid or peptide)-OOC, X is SH and n is 0 or 1; (4) where A is H or R^4, then where B is SH, X is $-NHR^3$ or $-N(R^3)$-(amino acid or peptide) and where X is SH, B is $-NHR^3$ or $-N(R^3)$-(amino acid or peptide); (5) where X is H or R^4, A is HOOC, H_2NOC, (amino acid or peptide)-NHOC, (amino acid or peptide)-OOC and B is SH; (6) where Z is methyl, X is methyl, A is HOOC, H_2NOC, (amino acid or peptide)-NHOC, (amino acid or peptide)-OOC and B is SH and n is 0; and (7) where B is SH and X is SH, n is not 0; and wherein the thiol group is in the reduced form.

In another embodiment, the invention provides a radiolabeled peptide for imaging sites within a mammalian body, comprising a specific binding peptide peptide wherein a radiolabel binding moiety is covalently linked to the peptide via an amino acid side-chain of an amino acid of the peptide, the radiolabel binding moiety having the formula:



{for purposes of this invention, radiolabel-binding moieties having this structure will be referred to as picolinic acid (Pic)-based moieties}

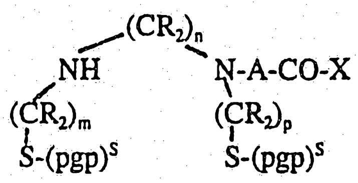
or



{for purposes of this invention, radiolabel-binding moieties having this structure will be referred to as picolylamine (Pica)-based moieties} wherein X is H or a protecting group; (amino acid) is any amino acid; the radiolabel-binding moiety is covalently linked to the peptide and the complex of the radiolabel-binding moiety and the radiolabel is electrically neutral. In a preferred embodiment, the amino acid is glycine and X is an acetamidomethyl protecting group. In additional preferred embodiments, the peptide is covalently linked to the radiolabel-binding moiety *via* an amino acid, most preferably glycine, and the radiolabel is technetium-99m.

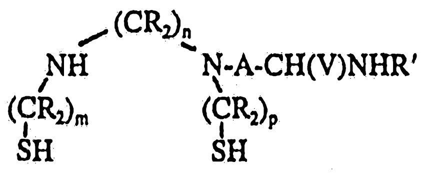
In yet another embodiment of the invention, a radiolabeled peptide is provided for imaging sites within a mammalian body, comprising a specific binding peptide and a bisamino bisthiol radiolabel-binding moiety covalently linked to the peptide *via* an amino acid side-chain of the peptide. The bisamino bisthiol radiolabel-binding moiety in this embodiment of the invention has a formula selected from the group consisting of:

I.



wherein each R can be independently H, CH_3 or C_2H_5; each $(pgp)^S$ can be independently a thiol protecting group or H; m, n and p are independently 2 or 3; A is linear or cyclic lower alkyl, aryl, heterocyclyl, combinations or substituted derivatives thereof; and X is peptide; and

II.



wherein each R is independently H, CH_3 or C_2H_5; m, n and p are independently 2 or 3; A is linear or cyclic lower alkyl, aryl, heterocyclyl, combinations or substituted derivatives thereof; V is H or CO-peptide; R' is H or peptide; provided that when V is H, R' is peptide and when R' is H, V is peptide. {For purposes of this invention, radiolabel-binding moieties having these structures will be referred to as "BAT" moieties}. In a preferred embodiment, the peptide is covalently linked to the radiolabel-binding moiety *via* an amino acid, most preferably glycine, and the radiolabel is technetium-99m.

In preferred embodiments of the aforementioned aspects of this invention, the specific binding compound is a peptide is comprised of between 3 and 100 amino acids. The most preferred embodiment of the radiolabel is technetium-99m.

Specific-binding peptides provided by the invention include but are not limited to peptides having the following sequences:

formyl-MLF
(VGVAPG)$_3$amide
(VPGVG)$_4$amide
RALVDTLKFVTQAEGAKamide
RALVDTEFKVKQEAGAKamide
PLARITLPDFRLPEIAIPamide
GQQHHLGGAKAGDV
PLYKKIIKKLLES
LRALVDTLKamide
GGGLRALVDTLKamide
GGGLRALVDTLKFVTQAEGAKamide
GGGRALVDTLKALVDTLamide
GHRPLDKKREEAPSLRPAPPPISGGGYR
PSPSPIHPAHHKRDRRQamide
GGGF$_D$.Cpa.YW$_D$KTFTamide

GGCNP.Apc.GDC
S-----S
{SYNRGDSTC}$_3$-TSEA
GGGLRALVDTLKamide
GCGGGLRALVDTLKamide
GCYRALVDTLKFVTQAEGAKamide
GC(VGVAPG)$_3$amide

The reagents of the invention may be formed wherein the specific binding compounds or the radiolabel-binding moieties are covalently linked to a polyvalent linking moiety. Polyvalent linking moieties of the invention are comprised of at least 2 identical linker functional groups capable of covalently bonding to specific binding compounds or radiolabel-binding moieties. Preferred linker functional groups are primary or secondary amines, hydroxyl groups, carboxylic acid groups or thiol-reactive groups. In preferred embodiments, the polyvalent linking moieties are comprised of *bis*-succinimdylmethylether (BSME), 4-(2,2-dimethylacetyl)benzoic acid (DMAB), *tris*(succinimidylethyl)amine (TSEA), *N*{2-(*N'*,*N'*-*bis*(2-succinimidoethyl) aminoethyl)}-N^6,N^9-*bis*(2-methyl-2-mercaptopropyl)-6,9-diazanonanamide (BAT-BS), *bis*-(acetamidoethyl)ether, *tris*(acetamidoethyl)amine, *bis*-(acetamidoethyl)ether, *bis*-(acetamidomethyl)ether, α,ε-*bis*acetyllysine, lysine and 1,8-*bis*-acetamido-3,6-dioxa-octane.

The invention also comprises complexes of the peptides of the invention with Tc-99m and methods for radiolabeling the peptides of the invention with Tc-99m. Radiolabeled complexes provided by the invention are formed by reacting the peptides of the invention with Tc-99m in the presence of a reducing agent. Preferred reducing agents include but are not limited to dithionite ion, stannous ion, and ferrous ion. Complexes of the invention are also formed by labeling the peptides of the invention with Tc-99m by ligand exchange of a prereduced Tc-99m complex as provided herein.

The invention also provides kits for preparing the peptides of the invention radiolabeled with Tc-99m. Kits for labeling the peptide of the invention with Tc-99m are comprised of a sealed vial containing a predetermined quantity of a peptide of the invention and a sufficient amount of reducing agent to label the peptide with Tc-99m.

This invention provides methods for preparing peptides of the invention by chemical synethesis *in vitro*. In a preferred embodiment, peptides are synthesized by solid phase peptide synthesis.

This invention provides methods for using Tc-99m labeled peptides for imaging site within a mammalian body by obtaining *in vivo* gamma scintigraphic images. These methods comprise administering an effective diagnostic amount of a Tc-99m radiolabeled peptide of the invention and detecting the gamma radiation emitted by the Tc-99m localized at the site within the mammalian body.

According to a first embodiment of the invention, there is provided a reagent comprising a specific binding peptide comprising 3 to 100 amino acid residues, a sidechain of one residue being covalently linked to a technetium-99m-binding moiety selected from the group consisting of:

I.

Cp(aa)Cp

wherein Cp is a protected cysteine and (aa) is any primary α- or β-amino acid not containing a thiol group;

and

II.

a technetium-99m complexing moiety comprising a single thiol-containing moiety having a formula:

$$\text{A-CZ(B)-}\{\text{C}(\text{R}^1\text{R}^2)\}_n\text{-X}$$

wherein A is H, HOOC, H_2NOC, (amino acid or peptide)-NHOC, (amino acid or peptide)-OOC or R^4;

B is H, SH, $-NHR^3$, $-N(R^3)$-(amino acid or peptide), or R^4;

X is H, SH, $-NHR^3$, $-N(R^3)$-(amino acid or peptide) or R^4;

Z is H or R^4;

R^1, R^2, R^3 and R^4 are independently H or lower straight or branched chain or cyclic alkyl;

n is 0, 1 or 2;

(peptide) is a peptide of 2 to about 10 amino acids;

and

where B is $-NHR^3$ or $-N(R^3)$-(amino acid or peptide), X is SH, and n is 1 or 2;

where X is $-NHR^3$ or $-N(R^3)$-(amino acid or peptide), B is SH, and n is 1 or 2;

where B is H or R^4, A is HOOC, H_2NOC, (amino acid or peptide)-NHOC, (amino acid or peptide)-OOC, X is SH, and n is 0 or 1;

where A is H or R^4, then where B is SH, X is $-NHR^3$ or $-N(R^3)$-(amino acid or peptide) and where X is SH, B is $-NHR^3$ or $-N(R^3)$-(amino acid or peptide);

where X is H or R^4, A is HOOC, H_2NOC, (amino acid or peptide)-NHOC, (amino acid or peptide OOC and B is SH;

where Z is methyl, X is methyl, A is HOOC, H_2NOC, (amino acid or peptide)-NHOC, (amino acid or peptide)-OOC, B is SH and n is 0;

and wherein the thiol moiety is in the reduced form and (amino acid) is any primary α- or β-amino acid not containing a thiol group.

According to a second embodiment of the invention, there is provided a scintigraphic imaging agent comprising the reagent according to the first embodiment of the invention and technetium-99m.

According to a third embodiment of the invention, there is provided a kit for preparing a radiopharmaceutical preparation, said kit comprising sealed vial containing a predetermined quantity of the reagent according to the first embodiment of the invention and a sufficient amount of a reducing agent to label the reagent with technetium-99m.

According to a fourth embodiment of the invention, there is provided a method of imaging a site of within a mammalian body, comprising the steps of administering an effective diagnostic amount of the agent according to the second embodiment of the invention.

Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

Brief Description of the Drawings

Figure 1 shows an image of ^{99m}Tc-P587 in a tumor-bearing rat.

Detailed Description of the Invention

The present invention provides Tc-99m labeled peptides for imaging target sites within a mammalian body comprising an amino acid sequence covalently linked through an amino acid side-chain to a radiolabel-binding moiety wherein the radiolabel-binding moiety binds a radioisotope.

Labeling with Tc-99m is an advantage of the present invention because the nuclear and radioactive properties of this isotope make it an ideal scintigraphic imaging agent. This isotope properties of this isotope make it an ideal scintigraphic imaging agent. This isotope has a single photon energy of 140 keV and a radioactive half-life of about 6 hours, and is readily available from a ^{99}Mo-^{99m}Tc generator. Other radionuclides known in the prior art have effective half-lives which are much longer (for example, ^{111}In, which has a half-life of 67.4 h) or are toxic (for example, ^{125}I).

In the radiolabel binding moieties and peptides covalently linked to such moieties that contain a thiol covalently linked to a thiol protecting groups [(pgp)^s] provided by the invention, the thiol-protecting groups may be the same or different and may be but are not limited to:

-CH_2-aryl (aryl is phenyl or alkyl or alkyloxy substituted phenyl);
-CH-$(aryl)_2$, (aryl is phenyl or alkyl or alkyloxy substituted phenyl);
-C-$(aryl)_3$, (aryl is phenyl or alkyl or alkyloxy substituted phenyl);
-CH_2-(4-methoxyphenyl);
-CH-(4-pyridyl)$(phenyl)_2$;
-$C(CH_3)_3$
-9-phenylfluorenyl;
-CH_2NHCOR (R is unsubstituted or substituted alkyl or aryl);
-CH_2-NHCOOR (R is unsubstituted or substituted alkyl or aryl);
-CONHR (R is unsubstituted or substituted alkyl or aryl);
-CH_2-S-CH_2-phenyl

Preferred protecting groups have the formula -CH_2-NHCOR wherein R is a lower alkyl having 1 and 8 carbon atoms, phenyl or phenyl-substituted with lower alkyl, hydroxyl, lower alkoxy, carboxy, or lower alkoxycarbonyl. The most preferred protecting group is an acetamidomethyl group.

Each specific-binding peptide-containing embodiment of the invention is comprised of a sequence of amino acids. The term amino acid as used in this invention is intended to include all L- and D- amino acids, naturally occurring and otherwise.

Peptides of the present invention can be chemically synthesized *in vitro*. Peptides of the present invention can generally advantageously be prepared on an amino acid synthesizer. The peptides of this invention can be synthesized wherein the radiolabel-binding moiety is covalently linked to the peptide during chemical synthesis *in vitro*, using techniques well known to those with skill in the art. Such peptides covalently-linked to the radiolabel-binding moiety during synthesis are advantageous because specific sites of covalent linkage can be determined.

It is a particular advantage of the present invention that a radiolabel-binding moiety is covalently linked to the peptide *via* an amino acid side-chain of the target specific binding peptide. This may be accomplished by either coupling the radiolabel binding moiety to the peptide by formation of a covalent bond with a particular amino acid side-chain or through incorporation of an amino acid conjugated to a radiolabel-binding moiety during peptide synthesis.

In the former case, for example, the radiolabel-binding moiety ClCH$_2$CO.Gly-Gly-Cys-Lys.amide (protected during synthesis, *inter alia*, by tritylation of the thiol group of the cysteine residue) is coupled at pH 8-10 to the somatostatin receptor binding peptide *cyclo*.(*N*-CH$_3$).Phe-Tyr-(D-Trp)-Lys-Val-Hcy to form the peptide *cyclo*.(*N*-CH$_3$).Phe-Tyr-(D-Trp)-Lys-Val-Hcy.(CH$_2$CO).Gly-Gly-Cys-Lys.amide (following de-protection). In this formula, it will be understood that the radiolabel-binding moiety is covalently linked to the sidechain sulfur atom of homocysteine.

Alternatively, the radiolabel-binding moiety BAT (N^6,N^9-*bis*(2-mercapto-2-methylpropyl)-6,9-diazanonanoic acid) is incorporated into the leukocyte binding peptide *formyl*.Met-Leu-Phe-Lys.amide by using the prepared lysine derivative, $N\alpha$(Fmoc)-$N\epsilon$(N^9-(*t*-butoxycarbonyl)-N^6, N^9-*bis*(2-methyl-2-triphenylmethylthiopropyl)-6,9-diazanonanoyl)lysine during peptide synthesis.

Other radiolabel binding moieties of the invention may be introduced into the target specific peptide during peptide synthesis. The picolinic acid-containing radiolabel-binding moiety may be covalently linked to the ϵ-amino group of lysine to give, for example, αN(Fmoc)-Lys-εN[Pic-Gly-Cys(protecting group)], which may be incorporated at any position in the peptide chain. This sequence is particularly advantageous as it affords an easy mode of incorporation into the target binding peptide.

In forming a complex of radioactive technetium with the peptides of this invention, the technetium complex, preferably a salt of Tc-99m pertechnetate, is reacted with the peptides of this invention in the presence of a reducing agent. Preferred reducing agents are dithionite, stannous and ferrous ions; the most preferred reducing agent is stannous chloride. In an additional preferred embodiment, the reducing agent is a solid-phase reducing agent. Complexes and means for preparing such complexes are conveniently provided in a kit form comprising a sealed vial containing a predetermined quantity of a peptide of the invention to be labeled and a sufficient amount of reducing agent to label the peptide with Tc-99m. Alternatively, the complex may be formed by reacting a peptide of this invention with a pre-formed labile complex of technetium and another compound known as a transfer ligand. This process is known as ligand exchange and is well known to those skilled in the art. The labile complex may be formed using such transfer ligands as tartrate, citrate, gluconate or mannitol, for example. Among the Tc-99m pertechnetate salts useful with the present invention are included the alkali metal salts such as the sodium salt, or ammonium salts or lower alkyl ammonium salts.

In a preferred embodiment of the invention, a kit for preparing technetium-labeled peptides is provided. The peptides of the invention can be chemically synthesized using methods and means well-known to those with skill in the art and described hereinbelow. Peptides thus prepared are comprised of between 3 and 100 amino acid residues, and are covalently linked to a radiolabel-binding moiety wherein the radiolabel-binding moiety binds a radioisotope. An appropriate amount of the peptide is introduced into a vial containing a reducing agent, such as stannous chloride or a solid-phase reducing agent, in an amount sufficient to label the peptide with Tc-99m. An appropriate amount of a transfer ligand as described (such as tartrate, citrate, gluconate or mannitol, for example) can also be included. Technetium-labeled peptides according to the present invention can be prepared by the addition of an appropriate amount of Tc-99m or Tc-99m complex into the vials and reaction under conditions described in Example 3 hereinbelow.

Radioactively labeled peptides provided by the present invention are provided having a suitable amount of radioactivity. In forming Tc-99m radioactive complexes, it is generally preferred to form radioactive complexes in solutions containing radioactivity at concentrations of from about 0.01 millicurie (mCi) to 100 mCi per mL.

Technetium-labeled peptides provided by the present invention can be used for visualizing sites in a mammalian body. In accordance with this invention, the technetium-labeled peptides are administered in a single unit injectable dose. Any of the common carriers known to those with skill in the art, such as sterile saline solution or plasma, can be utilized after radiolabeling for preparing the injectable solution to diagnostically image various organs, tumors and the like in accordance with this invention. Generally, the unit dose to be administered has a radioactivity of about 0.01 mCi to about 100 mCi, preferably 1 mCi to 20 mCi. The solution to be injected at unit dosage is from about 0.01 mL to about 10 mL. After intravenous administration, imaging of the organ or tumor *in vivo* can take place in a matter of a few minutes. However, imaging can take place, if desired, in hours or even longer, after the radiolabeled peptide is injected into a patient. In most instances, a sufficient amount of the administered dose will accumulate in the area to be imaged within about 0.1 of an hour to permit the taking of scintiphotos. Any conventional method of scintigraphic imaging for diagnostic purposes can be utilized in accordance with this invention.

The technetium-labeled peptides and complexes provided by the invention may be administered intravenously in any conventional medium for intravenous injection such as an aqueous saline medium, or in blood plasma medium. Such medium may also contain conventional pharmaceutical adjunct materials such as, for example, pharmaceutically acceptable salts to adjust the osmotic pressure, buffers, preservatives and the like. Among the preferred media are normal saline and plasma.

The methods for making and labeling these compounds are more fully illustrated in the following Examples. These Examples illustrate certain aspects of the above-described method and advantageous results. These Examples are shown by way of illustration and not by way of limitation.

EXAMPLE 1

Solid Phase Peptide Synthesis

Solid phase peptide synthesis (SPPS) was carried out on a 0.25 millimole (mmole) scale using an Applied Biosystems Model 431A Peptide Synthesizer and using 9-fluorenylmethyloxycarbonyl (Fmoc) amino-terminus protection, coupling with dicyclohexylcarbodiimide/hydroxybenzotriazole or 2-(1H-benzo-triazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate/ hydroxybenzotriazole (HBTU/HOBT), and using *p*-hydroxymethylphenoxy-methylpolystyrene (HMP) resin for carboxyl-terminus acids or Rink amide resin for carboxyl-terminus amides. Resin-bound products were routinely cleaved using a solution comprised of trifluoroacetic acid, water, thioanisole, ethanedithiol, and triethylsilane, prepared in ratios of 100 : 5 : 5 : 2.5 : 2 for 1.5 - 3 h at room temperature.

Where appropriate αN-formyl groups were introduced by treating the cleaved, deprotected peptide with excess acetic anhydride in 98% formic acid and stirring for about 18 hours followed by HPLC purification. Where appropriate N-terminal acetyl groups were introduced by treating the free N-terminal amino peptide bound to the resin with 20% v/v acetic anhydride in NMP (N-methylpyrrolidinone) for 30 min. Where appropriate, 2-chloroacetyl and 2-bromoacetyl groups were introduced either by using the appropriate 2-halo-acetic acid as the last residue to be coupled during SPPS or by treating the N-terminus free amino peptide bound to the resin with either the 2-halo-acetic acid/ diisopropylcarbodiimide/ N-hydroxysuccinimide in NMP of the 2-halo-acetic anhydride/ diisopropylethylamine in NMP. Where appropriate, HPLC-purified 2-haloacetylated peptides were cyclized by stirring an 0.1 - 1.0 mg/mL solution in bicarbonate or ammonia buffer (pH 8) with or without 0.5 - 1.0 mM EDTA for 1 - 48 hours, followed by acidification with acetic acid, lyophilization and HPLC purification. Where appropriate, Cys-Cys disulfide bond cyclizations were performed by treating the precursor cysteine-free thiol peptides at 0.1mg/mL in pH 7 buffer with aliquots of 0.006 M $K_3Fe(CN)_6$ until a stable yellow color persisted. The excess oxidant was reduced with excess cysteine, the mixture was lyophilized and then purified by HPLC.

Where appropriate the "Pica"' group was introduced by conjugating picolylamine to a precursor peptide using diisopropylcarbodiimide and N-hydroxysuccinimide. Where appropriate BAT ligands were introduced either by using the appropriate BAT acid as the last residue to be coupled during SPPS or by treating the N-terminus free amino peptide bound to the resin with BAT acid/ diisopropylcarbodiimide/ N-hydroxysuccinimide in NMP. Where appropriate, [BAM] was conjugated to the peptide by first activating the peptide carboxylate with a mixture of diisopropylcarbodiimide/N-hydroxysuccinimide or HBTU/HOBt in DMF, NMP or CH_2Cl_2, followed by coupling in the presence of diisopropylethylamine; after coupling, the conjugate was deprotected as described above.

Where appropriate, BSME adducts were prepared by reacting single thiol-containing peptides (5 to 50 mg/mL in 50 mM sodium phosphate buffer, pH 8) with 0.5 molar equivalents of BMME (*bis*-maleimidomethylether) pre-dissolved in acetonitrile at room temperature for approximately 1-18 hours. The solution was concentrated and the product was purified by HPLC.

Where appropriate, TSEA adducts were prepared by reacting single thiol-containing peptide (at concentrations of 10 to 100 mg/mL peptide in DMF, or 5 to 50 mg/mL peptide in 50mM sodium phosphate (pH 8)/ acetonitrile or THF) with 0.33 molar equivalents of TMEA (*tris*(2-maleimidoethyl)amine; as disclosed in U.S. Serial No. 08/044,825, incorporated by reference) pre-dissolved in acetonitrile or DMF, with or without 1 molar equivalent of triethanolamine, at room temperature for approximately 1-18h. Such reaction mixtures containing adducts were concentrated and the adducts were then purified using HPLC.

Where appropriate, BAT-BS adducts were prepared by reacting single thiol-containing peptide (at concentrations of 2 to 50 mg/mL peptide in 50mM sodium phosphate (pH 8)/ acetonitrile or THF) with 0.5 molar equivalents of BAT-BM (*N*-[2-(*N',N'*-*bis*(2-maleimidoethyl)aminoethyl)]-N^9-(*t*-butoxycarbonyl)-N^6,N^9,-*bis*(2-methyl-2-triphenylmethylthiopropyl)-6,9-diazanonanamide; as disclosed in U.S. Serial No. 08/044,825, incorporated by reference) pre-dissolved in acetonitrile or THF, at room temperature for approximately 1-18h. The solution was then evaporated to dryness and [BAT-BS]-peptide conjugates deprotected by treatment with 10mL TFA and 0.2mL triethylsilane for 1h. The solution was concentrated, the product adducts precipitated with ether, and then purified by HPLC.

Crude peptides were purified by preparative high pressure liquid chromatography (HPLC) using a Waters Delta Pak C18 column and gradient elution using 0.1% trifluoroacetic acid (TFA) in water modified with acetonitrile. Acetonitrile was evaporated from the eluted fractions which were then lyophilized. The identity of each product was confirmed by fast atom bombardment mass spectroscopy (FABMS).

EXAMPLE 2

A General Method for Radiolabeling with Tc-99m

0.1 mg of a peptide prepared as in Example 2 was dissolved in 0.1 mL of water or 50 mM potassium phosphate buffer (pH = 5, 6 or 7.4). Tc-99m gluceptate was prepared by reconstituting a Glucoscan vial (E.I. DuPont de Nemours, Inc.) with 1.0 mL of Tc-99m sodium pertechnetate containing up to 200 mCi and allowed to stand for 15 minutes at room temperature. 25 μl of Tc-99m gluceptate was then added to the peptide and the reaction allowed to proceed at room temperature or at 100°C for 15-30 min and then filtered through a 0.2 μm filter.

The Tc-99m labeled peptide purity was determined by HPLC using the following conditions: a Waters DeltaPure RP-18, 5μ, 150mm x 3.9mm analytical column was loaded with each radiolabeled peptide and the peptides eluted at a solvent flow rate equal to 1 mL/min. Gradient elution was performed beginning with 10% solvent A (0.1% CF3COOH/H_2O) to 40% solvent B_{90} (0.1% CF_3COOH/90% CH_3CN/H_2O) over the course of 20 min.

Radioactive components were detected by an in-line radiometric detector linked to an integrating recorder. Tc-99m gluceptate and Tc-99m sodium pertechnetate elute between 1 and 4 minutes under these conditions, whereas the Tc-99m labeled peptide eluted after a much greater amount of time.

The following Tables illustrates successful Tc-99m labeling of peptides prepared according to Example 2 using the method described herein.

TABLE I

Peptides	FABMS MH$^+$	Radiochemical Yield(%)*	HPLC R$_T$(min)
cyclo(*N*-methyl)FYW$_D$KV.Hcy.(CH$_2$CO.GGC.amide)	1129	98^2	15.1, 17.2
cyclo(*N*-methyl)FYW$_D$KV.Hcy.(CH$_2$CO.GGCK.amide)	1258	99^2	15.0
cyclo(*N*-methyl)FYW$_D$KV.Hcy.(CH$_2$CO.GGCR.amide)	1285	99^1	15.1
cyclo(*N*-methyl)FYW$_D$KV.Hcy.(CH$_2$CO.GGCKK.amide)	1386	N.D.	N.D.
cyclo(*N*-methyl)FYW$_D$KV.Hcy.(CH$_2$CO.GGC.Orn.amide)	1244	98^3	7.0

* Superscripts refer to the following labeling conditions:

^{1} = in 10% HPCD at room temperature
^{2} = in 50/50 ethanol/water at room temperature
^{3} = in 0.9% NaCl at 100°C

HPLC methods (indicated by superscript after R$_T$):

Waters-1 column, 100% Solution A → 100% Solution B in 10 min

TABLE II

Peptides	FABMS MH^+	Radiochemical Yield(%)*	HPLC R_T(min)
formyl-MLFK.(BAT).amide	884	99	12.6
formyl-MLFK.(BAT)	884	96	11.9, 12.8
formyl-MLFK.(BAT).KKKKK.amide	1524	96	11.7, 12.2
formyl-MLFK.(BAT).GSGSGS.amide	1315	97	11.9, 12.8
formyl-MLFK.(BAT).E	1013	99	12.3
formyl-MLFK.(BAT).EGE	1200	98	13.7
(*formyl*-MLFK.(BAT.)GGC_{Acm}GC_{Acm}GGC.amide)$_2$-BSME	3477	99	11.9, 12.4
YRALVDTLKFVTQAEGAK.(BAT).amide	2329	98	11.4
K.(BAT).D.Nal.C_{Me}YW_DKVC_{Me}T.amide	1573	97	12.0, 12.5
(DTPA).{D-Nal.SYW_DKVTK.(BAT)}$_2$.amide	3210	97	12.1, 12.5
(DTPA).{D-Nal.SYW_DKVTK.(BAT)}.amide	1801	96	11.8, 12.0
(DTPA)K.(BAT).D-Nal.C_{Me}YW_DKVC_{Me}T.amide	1949	96	11.8, 12.0
*p*Glu.GVNDNEEGFFSARK.(BAT).amide	1997	N.D.	N.D.

* The following labeling conditions were used with the appropriate peptides:

1. The peptide is dissolved in 50 mM potassium phosphate buffer (pH 7.4) and labeled at room temperature.
2. The peptide is dissolved in 50 mM potassium phosphate buffer (pH 7.4) and labeled at 100°C.
3. The peptide is dissolved in water and labeled at room temperature.
4. The peptide is dissolved in water and labeled at 100°C.
5. The peptide is dissolved in 50 mM potassium phosphate buffer (pH 6.0) and labeled at 100°C.
6. The peptide is dissolved in 50 mM potassium phosphate buffer (pH 5.0) and labeled at room temperature.

** HPLC methods:

general:
solvent A = 0.1% CF3COOH/H_2O
solvent B_{70} = 0.1% CF_3COOH/70% CH_3CN/H_2O
solvent B_{90} = 0.1% CF_3COOH/90% CH_3CN/H_2O
solvent flow rate = 1 mL/min

Vydak column = Vydak 218TP54 RP-18, 5μ x 220mm x 4.6mm analytical column with guard column
Brownlee column = Brownlee Spheri-5 RP-18, 5μ x 220mm x 4.6mm column
Waters column = Waters Delta-Pak C18, 5μm, 39 X 150mm

Method 1:	Brownlee column	100% A to 100% B_{70} in 10 min
Method 2:	Vydak column	100% A to 100% B_{90} in 10 min
Method 3:	Vydak column	100% A to 100% B_{70} in 10 min
Method 4:	Brownlee column	100% A to 100% B_{90} in 10 min
Method 5:	Waters column	100% A to 100% B_{90} in 10 min

Single-letter abbreviations for amino acids can be found in G. Zubay, *Biochemistry* (2d. ed.), 1988 (MacMillen Publishing: New York) p.33; Ac = acetyl; Pic = picolinoyl (pyridine-2-carbonyl) = 6-aminocaproic acid; Hly = homolysine; Acm = acetamidomethyl; *p*Glu = *pyro*-glutamic acid; Mob = 4-methoxybenzyl; Pica = picolylamine (2-(aminomethyl)pyridine); Apc = L-[S-(3-aminopropyl)cysteine; F_D = D-phenylalanine; W_D= D-tryptophan; Y_D = D-tyrosine; Cpa = L-(4-chlorophenyl)alanine; Thp = 4-amino-tetrahydrothiopyran-4-carboxylic acid; ma = mercaptoacetic acid; D-Nal = D-2-naphthylalanine; Dpg = dipropylglycine; Nle = norleucine; BAT = N^6,N^9-*bis*(2-mercapto-2-methylpropyl)-6,9-diazanonanoic acid; BAT acid (protected) = N^9-(*t*-butoxycarbonyl)-N^6,N^9-*bis*(2-methyl-2-triphenylmethyl thiopropyl)-6,9-diazanonanoic acid; BAM = N^1,N^4-*bis*(2-mercapto-2-methylpropyl)-1,4,10-triazadecane; BAM (protected) = N^1-(*t*-butoxycarbonyl)-N^1,N^4-*bis*(2-methyl-2-triphenylmethylthiopropyl)-1,4,10-triazadecane; [BAT-BM] = *N*-[2-(*N'*,*N'*-*bis*(2-maleimidoethyl)aminoethyl]-N^9-(*t*-butoxycarbonyl)-N^6,N^9-*bis*(2-methyl-2-triphenylmethylthiopropyl)-6,9-diazanonanamide; [BAT-BS] = *N*-[2-(*N'*,*N'*-*bis*(2-

succinimidoethyl)aminoethyl]-N^6,N^9-*bis*(2-mercapto-2-methylpropyl)-6,9-diazanonanamide; [BMME] = *bis*-maleimidomethylether; [BSME] = *bis*-succinimidomethylether; [DTPA] = diethylenetriaminepentaacetic acid; Amp = 4-amidinophenylalanine.

EXAMPLE 3

Localization and *In Vivo* Imaging of Atherosclerotic Plaque using Tc-99m Labeled Compound P215 in the Hypercholesterol Rabbit Model

Twenty-two New Zealand White (NZW) rabbits of both sexes and weighing 2-3kg are divided into two groups. The control group of rabbits are housed and fed commercial rabbit chow (Purina). The HC group are fed a standardized, cholesterol-rich diet (rabbit chow mixed to a 1% w/w concentration of cholesterol) from seven weeks until 28 weeks of age. All animals are given water *ad libitum*.

Tc-99m labeled P215 ({BAT}.RALVDTLKFVTQAEGAK.amide) is prepared as described above in Example 1. Approximately 250-400μg of peptide is labeled with 140-160mCi of Tc-99m and prepared in unit doses of 7-8mCi (12.5-20.0 μg/rabbit; 6-7μg/kg) in 0.2mL volume doses. Adult rabbits are dosed with Tc-99m labeled peptide intravenously in a lateral ear vein by slow bolus infusion (approximately 0.1mL/min). A gamma camera fitted with a pin-hole collimator (5mm aperture) and energy window set for Tc-99m and programmed to accumulate 500,000 counts or scan for a desired time is used to acquire images. Shortly before imaging, animals are anesthetized with a mixture of ketamine and xylazine (5:1, 1mL/kg intramuscularly).

Gamma camera images are collected at 40°-45° just above the heart (left anterior oblique [LAO] view) to delineate the aortic arch and view the descending aorta. Images are acquired at 1 and 2h and occasionally at 3 and 5h after injection. Supplementary anesthesia is injected as needed prior to each image collection.

At 2.5 h (after a 2h scan), animals are sacrificed with an intravenous dose of sodium pentobarbital. Upon necropsy, the aorta is removed and branching vessels dissected free from the aortic valve to the mid-abdominal region. Using a parallel hole collimator, the aorta is also imaged *ex corpora*. Next, the aortae are opened longitudinally and stained with Sudan IV, thereby turning atherosclerotic plaque a deep red brick color. Lipid-free and uninjured aortic endothelium retains its normal, glistening white-pink appearance under these conditions.

Positive correlations among the *in vivo* and *ex corpora* Tc-99m P215 images and the deposition patterns of Sudan IV in the HC-treated rabbit aortae indicate that this scintigraphic imaging agent of the invention is capable of imaging atherosclerotic plaque.

EXAMPLE 4

In Vivo Imaging using Tc-99m Labeled Compound P357 of Deep Vein Thrombosis in a Canine Model

Mongrel dogs (25-35lb., fasted overnight) are sedated with a combination of ketamine and aceprozamine intramuscularly and then anesthetized with sodium pentobarbital intravenously. An 18-gauge angiocath is inserted in the distal half of the right femoral vein and an 8mm Dacron®-entwined stainless steel embolization coil (Cook Co., Bloomington IN) is placed in the femoral vein at approximately mid-femur in each animal. The catheter is removed, the wound sutured and the placement of the coil documented by X-ray. The animals are then allowed to recover overnight.

One day following coil placement, each animal is re-anesthetized, intravenous saline drips placed in each foreleg and a urinary bladder catheter inserted to collect urine. The animal is placed supine under a gamma camera which is equipped with a low-energy, all purpose collimator and photopeaked for Tc-99m. Images are acquired on a NucLear Mac computer system.

Tc-99m labeled P357 {(CH_2CO-Y_D.Apc.GDCGGC$_{Acm}$GC$_{Acm}$GGC.amide$)_2$-[BAT-BS]} {185-370 mBq (5-10 mCi) Tc-99m and 0.2-0.4mg P357} is injected into one foreleg intravenous line at its point of insertion. The second line is maintained for blood collection. Anterior images over the legs are acquired for 500,000 counts or 20 min (whichever was shorter), at approximately 10-20 min, and at approximately 1, 2, 3 and 4h post-injection. Following the collection of the final image, each animal is deeply anesthetized with pentobarbital. Two blood samples are collected on a cardiac puncture using a heparinized syringe followed by a euthanasing dose of saturated potassium chloride solution administered by intercardiac or bolus intravenous injection. The femoral vein containing the thrombus and samples of thigh muscle are then carefully dissected out. The thrombus is then dissected free of the vessel and placed in a pre-weighed test tube. The thrombus samples are then weighed and counted in a gamma well counter in the Tc-99m channel. Known fractions of the injected doses are counted as well.

Fresh thrombus weight, percent injected dose (%ID)/g in the thrombus and blood obtained just prior to euthanasia and thrombus/blood and thrombus/muscle ratios are determined. Thrombus/background ratios are determined by analysis of the counts/pixel measured in regions-of-interest (ROI) drawn over the thrombus and adjacent muscle from computer-stored images.

These results are used to demonstrate that deep vein thrombi can be rapidly and efficiently located *in vivo*.

EXAMPLE 5

Scintigraphic Imaging and Biodistribution of Tc-99m Labeled Peptides

In order to demonstrate the effectiveness of Tc-99m labeled peptide reagents as provided above, New Zealand white rabbits were innoculated intramuscularly in the left calf with a potent stain of *E. coli*. After 24 h, the animals were sedated by i.m. injection of ketamine and xylazine, and then injected i.v. with Tc-99m labeled peptide ($\leq$ 150μg, 2-10 mCi). The animals were positioned supine in the field of view of a gamma camera (LEAP collimator/ photopeaked for Tc-99m) and imaged over the first hour post-injection, and then at approximately 1h intervals over the next three hours post injection. Animals were allowed to recover between image acquisitions and re-anesthetized as needed.

Upon completion of the final imaging, each animal was sacrificed by overdose of phenobarbital i.v. and dissected to obtain samples of blood and of infected and control muscle tissue. The tissue samples were weighed, and along with a standard amount of the injected dose, were counted using a gamma counter, and the percent injected dose (per gram of tissue) remaining in the tissues was determined. Ratios of percent of injected dose per gram of infected *versus* non-infected muscle tissue, and of infected muscle tissue *versus* blood, were calculated for each peptide. These results are presented in the following Table for the Tc-99m labeled reagent of the invention, having the formula

*formyl*MLFK(BAT).amide.

TABLE III

Peptide	A	B	C	D	E
*formyl*MLFK(BAT).amide	0.0215	0.0028	7.68	0.006	3.58

A = %ID/gram infected muscle
B = %ID/gram control muscle
C = Ratio infected muscle:control muscle
D = %ID/gram blood
E = Ratio infected muscle:blood

EXAMPLE 6

Localization and *In Vivo* Imaging of Somatostatin Receptor (SSTR)-Expressing Tumors in Rats

In vivo imaging of somatostatin receptors expressed by rat tumor cells was performed essentially as described by Bakker *et al.* (1991, *Life Sciences* 49: 1593-1601).

CA20948 rat pancreatic tumor cells, thawed from frozen harvested tumor brei, were implanted intramuscularly in a suspension of 0.05 to 0.1 mL/animal, into the right hind thigh of 6 week old Lewis rats. The tumors were allowed to grow to approximately 0.5 to 2g, harvested, and tumor brei was used to implant a second, naive set of Lewis rats. Passaging in this fashion was repeated to generate successive generations of tumor-bearing animals. The tumor-bearing animals used for the *in vivo* studies were usually from the third to fifth passage and carried 0.2 to 2g tumors.

For studies of the specificity of radiotracer localization in the tumors, selected animals were given an subcutaneous SSTR-blocking dose (4 mg/kg) of octreotide 30 minutes prior to injection of the radiotracer. (This protocol has been shown by Bakker *et al.* to result in a lowering of ^{111}In-[DTPA]octreotide tumor uptake by

40%.)

Third- to fifth-passage CA20948 tumor-bearing Lewis rats were restrained and injected intravenously *via* the dorsal tail vein with a dose of 0.15-0.20 mCi ^{99m}Tc-labeled peptide corresponding to 3 to 8 μg peptide in 0.2 to 0.4 mL.

At selected times, the animals were sacrificed by cervical dislocation and selected necropsy was performed. Harvested tissue samples were weighed and counted along with an aliquot of the injected dose in a gamma well-counter.

The 90-minute biodistribution results of selected radiolabeled peptides are presented in Table I. Notably, ^{99m}Tc-P587, ^{99m}Tc-P617, ^{99m}Tc-P726, and ^{99m}Tc-P736 showed very high tumor uptake and tumor/blood ratios demonstrating their high specific uptake in target (tumor) tissue.

Figure 1 shows an image of ^{99m}Tc-P587 in a tumor-bearing rat. The high uptake in the tumor in the lower leg (arrow) is clearly visible.

^{99m}Tc-P587 uptake in tumors in rats was compared with and without pre-injection treatment with octreotide, a somatostatin analogue known to bind to the somatostatin receptor *in vivo*. In these experiments, receptor-blocking by administration of octreotide prior to administration of ^{99m}Tc-P587 reduced specific tumor uptake of the radiolabeled peptide by 76%. These results confirmed that binding of ^{99m}Tc-P587 *in vivo* was SSTR-specific.

TABLE III

		%ID/g		
No.	Peptides	Tumor	Blood	Tumor/Blood
P736	*cyclo*(*N*-methyl)FYW$_D$KV.Hcy.(CH$_2$CO.GGCRK.amide)	2.1	0.24	9
P587	*cyclo*(*N*-methyl)FYW$_D$KV.Hcy.(CH$_2$CO.GGCK.amide)	3.4	0.61	6
P617	*cyclo*(*N*-methyl)FYW$_D$KV.Hcy.(CH$_2$CO.GGCR.amide)	6.7	0.73	9
P726	*cyclo*(*N*-methyl)FYW$_D$KV.Hcy.(CH$_2$CO.KKC.amide)	2.5	0.30	8

It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.

The claims defining the invention are as follows:

1. A reagent comprising a specific binding peptide comprising 3 to 100 amino acid residues, a sidechain of one residue being covalently linked to a technetium-99m-binding moiety selected from the group consisting of:

I.

Cp(aa)Cp

wherein Cp is a protected cysteine and (aa) is any primary α- or β-amino acid not containing a thiol group;

and

II.

a technetium-99m complexing moiety comprising a single thiol-containing moiety having a formula:

$$A\text{-}CZ(B)\text{-}\{C(R^1R^2)\}_n\text{-}X$$

wherein A is H, HOOC, H_2NOC, (amino acid or peptide)-NHOC, (amino acid or peptide)-OOC or R^4;

B is H, SH, $-NHR^3$, $-N(R^3)$-(amino acid or peptide), or R^4;

X is H, SH, $-NHR^3$, $-N(R^3)$-(amino acid or peptide) or R^4;

Z is H or R^4;

R^1, R^2, R^3 and R^4 are independently H or lower straight or branched chain or cyclic alkyl;

n is 0, 1 or 2;

(peptide) is a peptide of 2 to about 10 amino acids;

and

where B is $-NHR^3$ or $-N(R^3)$-(amino acid or peptide), X is SH, and n is 1 or 2;

where X is $-NHR^3$ or $-N(R^3)$-(amino acid or peptide), B is SH, and n is 1 or 2;

where B is H or R^4, A is HOOC, H_2NOC, (amino acid or peptide)-NHOC, (amino acid or peptide)-OOC, X is SH, and n is 0 or 1;

where A is H or R^4, then where B is SH, X is $-NHR^3$ or $-N(R^3)$-(amino acid or peptide) and where X is SH, B is $-NHR^3$ or $-N(R^3)$-(amino acid or peptide);

where X is H or R^4, A is HOOC, H_2NOC, (amino acid or peptide)-NHOC, (amino acid or peptide OOC and B is SH;

where Z is methyl, X is methyl, A is HOOC, H_2NOC, (amino acid or peptide)-NHOC, (amino acid or peptide)-OOC, B is SH and n is 0;

and wherein the thiol moiety is in the reduced form and (amino acid) is any primary α- or β-amino acid not containing a thiol group.

2. The reagent of claim 1, wherein the peptide and the technetium-99m-binding moiety are covalently linked through a lysine residue or a homocysteine residue.

3. The reagent of claim 1, wherein the technetium-99m complexing moiety is

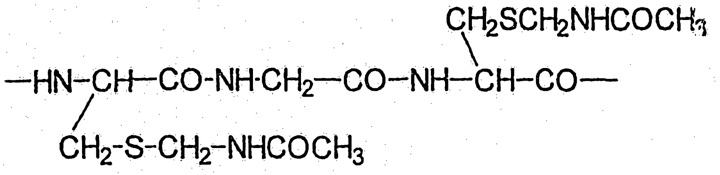
$$Cp(aa)Cp$$

and Cp has a formula

$$-CH_2-NH-CO-R$$

wherein R is a lower alkyl having 1 to 6 carbon atoms, 2-pyridyl, 3-pyridyl, 4-pyridyl, phenyl, or phenyl substituted with lower alkyl, hydroxy, lower alkoxy, carboxy, or lower alkoxycarbonyl.

4. The reagent of claim 1, wherein the technetium-99m complexing moiety has the formula:



$$-HN-\underset{\displaystyle CH_2-S-CH_2-NHCOCH_3}{CH}-CO-NH-CH_2-CO-NH-\overset{\displaystyle CH_2SCH_2NHCOCH_3}{CH}-CO-$$

5. The reagent of claim 1, wherein technetium-99m complexing moiety II has a formula selected from the group consisting of:

IIa. -(amino acid)^1-(amino acid)^2-{A-CZ(B)-{C(R^{1}R^2)}$_n$-X},

IIb. -{A-CZ(B)-{C(R^{1}R^2)}$_n$-X}-(amino acid)^1-(amino acid)^2,

IIc. -(a primary α,ω- or β,ω-diamino acid)-(amino acid)^1-{A-CZ(B)-{C(R^{1}R^2)}$_n$-X}, and

IId. -{A-CZ(B)-{C(R^{1}R^2)}$_n$-X}-(amino acid)^1-(a primary α,β- or β,γ-diamino acid)

wherein (amino acid)^1 and (amino acid)^2 are each independently any naturally-occurring, modified, substituted or altered α- or β-amino acid not containing a thiol group.

6. The reagent of claim 1, wherein the peptide is selected from the group consisting of:

formyl-MLF;
(VGVAPG)$_3$amide;
(VPGVG)$_4$amide;
RALVDTLKFVTQAEGAKamide;
RALVDTEFKVKQEAGAKamide;
PLARITLPDFRLPEIAIPamide;
GQQHHLGGAKAGDV;
PLYKKIIKKLLES;
LRALVDTLKamide;
GGGLRALVDTLKamide;
GGGLRALVDTLKFVTQAEGAKamide;

GGGRALVDTLKALVDTLamide;
GHRPLDKKREEAPSLRPAPPPISGGGYR;
PSPSPIHPAHHKRDRRQamide;
GGGF$_D$.Cpa.YW$_D$KTFTamide;
[SYNRGDSTC]$_3$-TSEA;
GGGLRALVDTLKamide;
GCGGGLRALVDTLKamide;
GCYRALVDTLKFVTQAEGAKamide; and
GC(VGVAPG)$_3$amide.

7. The reagent of claim 1, wherein the peptide is a cyclic peptide.

8. A multimer having a molecular weight of less than about 20,000 daltons, which comprises a polyvalent linker covalently linked to at least two reagents of claim 1.

9. The multimer of claim 8, wherein the linker is selected from the group consisting of *bis*-succinimidylmethylether, 4-(2,2-dimethylacetyl)benzoic acid, *N*-[2-(*N'*,*N'*-*bis*(2-succinimido-ethyl)aminoethyl)]-*N*^{6},*N*^{9}-*bis*(2-methyl-2-mercaptopropyl)-6,9-diazanonanamide, *tris*(succinimidylelthyl)amine; *tris*(acetamidoethyl)amine, *bis*-(acetamidoethyl)ether, *bis*-(acetamidomethyl)ether, α,ε-*bis*acetyllysine, lysine, 1,8-*bis*-acetamido-3,6-dioxa-octane, a derivative of *bis*-succinimidylmethylether, a derivative of 4-(2,2-dimethylacetyl)benzoic acid, a derivative of *N*-[2-(*N'*,*N'*-*bis*(2-succinimido-ethyl)aminoethyl)]-*N*^{6},*N*^{9}-*bis*(2-methyl-2-mercaptopropyl)-6,9-diazanonanamide, a derivative of *tris*(succinimidylethyl)amine, a derivative of *tris*(acetamidoethyl)amine, a derivative of *bis*-(acetamidoethyl)ether, a derivative of *bis*-(acetamidomethyl)ether, a derivative of α,ε-*bis*acetyllysine, a derivative of lysine, and a derivative of 1,8-*bis*-acetamido-3,6-dioxa-octane.

10. A scintigraphic imaging agent comprising the reagent according to any one of claims 1-7 and technetium-99m.

11. A scintigraphic imaging agent comprising the multimer according to claim 8 or 9 and technetium-99m.

12. A complex formed by reacting the reagent of any one of claims 1-7 with technetium-99m in the presence of a reducing agent.

13. A complex formed by reacting the multimer of claim 8 or 9 with technetium-99m in the presence of a reducing agent.

14. The complex of claim 12 or 13, wherein the reducing agent is selected from the group consisting of a dithionite ion, a stannous ion, and a ferrous ion.

15. A complex formed by labeling the reagent of any one of claims 1-7 with technetium-99m by ligand exchange of a prereduced technetium-99m complex.

16. A complex formed by labeling the multimer of claim 8 or 9 with technetium-99m by ligand exchange of a prereduced technetium-99m complex.

17. A kit for preparing a radiopharmaceutical preparation, said kit comprising a sealed vial containing a predetermined quantity of the reagent according to any one of

claims 1-7 and a sufficient amount of a reducing agent to label the reagent with technetium-99m.

18. A kit for preparing a radiopharmaceutical preparation, said kit comprising a sealed vial containing a predetermined quantity of the reagent of claim 8 or 9 and a sufficient amount of a reducing agent to label the reagent with technetium-99m.

19. A method of imaging a site within a mammalian body, comprising the steps of administering an effective diagnostic amount of the agent of claim 10 or 11 and detecting technetium-99m localised at the site.

20. The agent of claim 10 or 11 when used to image a site within a mammalian body.

21. Use of the agent of claim 10 or 11 in the preparation of medicament for imaging a site within a mammalian body.

22. A process of preparing the reagent according to any one of claims 1-7, wherein the peptide is chemically synthesized *in vitro*.

23. A reagent prepared by the process of claim 22.

24. A process of preparing the multimer according to claim 8 or 9, wherein the peptide is chemically synthesized *in vitro*.

25. A multimer prepared by the process of claim 24.

26. The process of claim 22 or 24, wherein the peptide is synthesized by solid phase peptide synthesis.

27. A reagent for preparing a scintigraphic imaging agent for imaging sites within a mammalian body, substantially as hereinbefore described with reference to any one of examples 2-6.

28. A scintigraphic imaging agent comprising the reagent according to claim 27 bound to technetium-99m.

29. Use of the reagent of claim 28 for imaging a site within a mammalian body.

30. A process for preparing a scintigraphic imaging agent for imaging sites within a mammalian body, substantially as hereinbefore described with reference to Examples 1 and 2.

31. A scintigraphic imaging agent prepared according to the process of claim 30.

Dated 11 August, 1998
Diatech, Inc.

Patent Attorneys for the Applicant/Nominated Person
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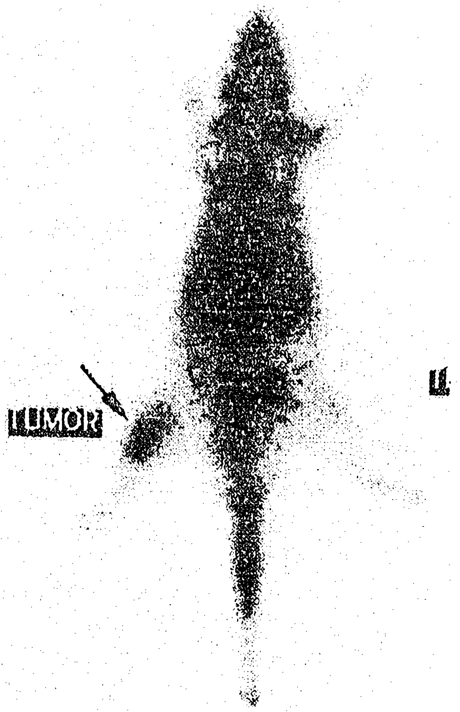

Figure 1

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/07017

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K51/08

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 6 A61K

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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO,A,95 00553 (DIATECH) 5 January 1995 see abstract see page 14, line 14 - page 21, line 16 see page 24, line 7 - page 29, line 28; claims; examples; tables ---	1-13, 15-24
P,X	WO,A,94 23758 (DIATECH) 27 October 1994 cited in the application see the whole document ---	1-13, 15-24
X	WO,A,93 25244 (DIATECH) 23 December 1993 cited in the application see abstract see page 7, line 1 - page 12, line 2 see page 16, line 1 - page 17, line 2; examples 1D,3,4; tables I,II see page 40, line 1 - line 15; claims --- -/--	1-13, 15-24

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in 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

1

Date of the actual completion of the international search
16 October 1995

Date of mailing of the international search report
27.10.95

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Authorized officer
Hoff, P

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/07017

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,93 21962 (DIATECH) 11 November 1993 cited in the application see abstract see page 8, line 1 - page 12, line 5 see page 13, line 10 - page 14, line 13; claims; examples; table ---	1-5,8,9, 14-24
X	WO,A,93 17719 (DIATECH) 16 September 1993 cited in the application see abstract see page 5, line 1 - page 9, line 5 see page 11, line 1 - page 14, line 18; claims; examples; tables ---	1-24
X	EP,A,0 398 143 (THE GENERAL HOSPITAL CORPORATION) 22 November 1990 see the whole document ---	1-4,14, 20-23
X	THE JOURNAL OF NUCLEAR MEDICINE, vol. 35, no. 5, May 1994 pages 257P-258P, J. LISTER-JAMES ET AL. 'A STRUCTURE-ACTIVITY-RELATIONSHIP (SAR) STUDY OF SOMATOSTATIN RECEPTOR-BINDING PEPTIDES RADIOLABELED WITH TC-99M' see the whole document ---	1-13, 15-24
A	WO,A,93 23085 (DIATECH) 25 November 1993 cited in the application see the whole document ---	1-24
A	WO,A,92 13572 (DIATECH) 20 August 1992 cited in the application see the whole document ---	1-24
A	WO,A,93 10747 (DIATECH) 10 June 1993 cited in the application see the whole document ---	1-24
A	WO,A,94 00489 (DIATECH) 6 January 1994 cited in the application see the whole document -----	1-24

International application No.
PCT/US95/07017

INTERNATIONAL SEARCH REPORT

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark:Although claim 21 is directed to a method of treatment of the human /animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Information on patent family members

PCT/US 95/07017

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9310747		EP-A- 0614379	14-09-94
		JP-T- 7506086	06-07-95
WO-A-9400489	06-01-94	AU-B- 4768893	24-01-94
		CA-A- 2138647	06-01-94
		EP-A- 0649434	26-04-95