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(54) Title: RADIOLABELLED PEPTIDE COMPOUNDS

(57) Abstract

The present invention relates to novel ligands and labelling techniques. The present invention further relates to a diagnostic composition suitable for administration to a warm-blooded animal comprising somatostatin or hirudin or a molecule capable of interacting with the somatostatin receptor or with the hirudin receptor labeled with a radionuclide by means of a chelate ligand capable of administration to an animal to produce reliable visual imaging of tumors or therapeutic effects on tumors and blood clots.

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RADIOLABELLED PEPTIDE COMPOUNDS

FIELD OF THE INVENTION

This invention relates generally to novel ligands and compounds for use in diagnostic tissue imaging and more particularly, to site specific radiolabelled peptides, to novel ligands for preparing radiolabelled compositions, to methods of preparing such site specific radiolabelled peptides, and to pharmaceutical compositions comprising these site specific radiolabelled peptides for diagnostic imaging or therapeutic use.

BACKGROUND OF THE INVENTION

Scintigraphic imaging and similar radiographic techniques for visualizing tissues in vivo are finding ever-increasing application in biological and medical research and in diagnostic and therapeutic procedures. Generally, scintigraphic procedures involve the preparation of radioactive agents which, upon introduction to a biological subject, become localized in the specific organ, tissue or skeletal structure of choice. When so localized, traces, plots or scintiphotos depicting the in vivo distribution of radiographic material can be made by various radiation detectors, e.g., traversing scanners and scintillation cameras. The distribution and corresponding relative intensity of the detected radioactive material not only indicates the space occupied by the targeted tissue, but also indicates a presence of receptors, antigens, aberrations, pathological conditions, and the like.

In general, depending on the type of radionuclide and the target organ or tissue of interest, the compositions comprise a radionuclide, a carrier agent designed to target the specific organ or tissue site, various auxiliary agents which affix the radionuclide to the carrier, water or other

delivery vehicles suitable for injection into, or aspiration by, the patient, such as physiological buffers, salts, and the like. The carrier agent, i.e. ligand, attaches or complexes the radionuclide to the peptide carrier agent, which results in localizing the radionuclide being deposited in the location where the carrier agent concentrates in the biological subject.

Technetium-99m(^{99m}Tc) is a radionuclide which is widely known for its uses in tissue imaging agents. Due to its safety and ideal imaging properties, this radionuclide is conveniently available commercially i the oxidized pertechnetate form ($^{99m}TcO_4^-$) hereinafter pertechnetate-Tc99m". However, pertechnetate will not plex with the most commonly used biological carriers radionuclide tissue imaging. Thus, technetium-labelled imaging agents are generally prepared by admixing a pertechnetate-Tc99m isotonic saline solution, a technetium reductant (reducing agent) such as stannous chloride or sodium dithionite, and a chelate conjugated to the desired peptide carrier agent for targeting the organ of interest. Alternatively, a transfer ligand may be added to the reduced pertechnetate prior to addition to the chelate-biological molecule to maintain the oxidation state within a desired level. Examples of such include 99m Tc-tartrate or 99m Tc-gluconate.

Another problem is that technetium-containing scintigraphic imaging agents are known to be unstable in the presence of oxygen, primarily since oxidation of the reductant and/or the technetium -99m destroys the reduced technetium -99m/targeting carrier complex. Accordingly, such imaging agents are generally made oxygen-free by saturating the compositions with oxygen-free nitrogen gas or by preparing the agents in an oxygen-free atmosphere.

Stabilization of imaging agents can also be achieved through chemical means. U.S. Patent Number 4,232,000, Fawzi, issued November 4, 1980, discloses the use of gentisyl alcohol as a stabilizer for technetium imaging agents. Similarly, U.S. Patent Number 4,233,284, Fawzi, issued November 11, 1980 discloses the use of gentisic acid as a stabilizer.

SUMMARY OF THE INVENTION

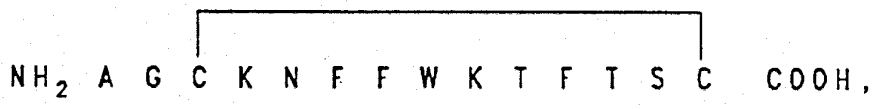
The present invention discloses novel ligands, particularly aminothiol ligands which are useful in preparing radiolabelled compositions. The ligands have been found to be particularly useful in labelling under a wide variety of acidic to basic labelling conditions.

The present invention further discloses novel radiolabelled peptide compounds, methods of preparing these compounds, pharmaceutical compositions comprising these compounds and the use of these compounds in kits for the therapeutic treatment of tumors and for diagnostic imaging of tumors. Certain tumors of endocrine-origin contain large numbers of receptors having a high affinity for somatostatin. Krenning, et al. (Lancet 8632, 242-244 (1989)). Examples of such tumors, having large numbers of high-affinity somatostatin receptors, are pituitary tumors, central nervous system tumors, breast tumors, gastro-entero-pancreatic tumors, small cell carcinoma of the lung, lymphomas, as well as their metastases.

In diagnostic tumor localization, a radiolabelled compound must be easily detectable and highly selective. High selectivity, which is essential in these compounds means that the diagnostic compound, after having been introduced into the body, accumulates to a greater degree

in the target tissue or tissues, i.e. a malignant tumor, than in surrounding tissues. In using somatostatin or other such peptides as carrier agents in radiolabelled compounds, the specific high selectivity of the particular peptide used provides for the strong accumulation of the diagnostic/therapeutic compound in the target tissue or tissues, such as in tumors in the case of somatostatin, compared with the concentration thereof in non-target tissues. Additionally, therapeutic treatment of malignant tumors is achieved when radiolabelled peptide compounds are constructed using high energy Beta or Alpha emitting isotopes rather than the pure gamma emitters customarily used for diagnostic purposes.

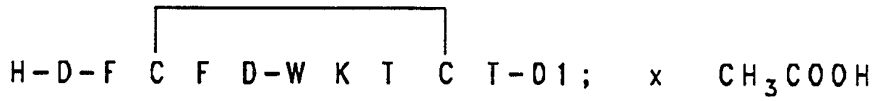
The radiolabelled peptide compounds of the present invention employ the somatostatin peptide:



wherein A represents Alanine, G represents Glycine, C represents Cysteine, K represents Lysine, N represents asparagine, F represents phenylalanine, W represents tryptophan, T represents threonine, and S represents serine, or a suitable derivative thereof.

In targeting particular receptors with radiolabelled somatostatin, it is not necessary that the complete fourteen (14) residue sequence of somatostatin be present. Binding is thought to reside primarily in the central core portion of the molecule, primarily, the phenylalanine-tryptophan-lysine-threonine or F W K T sequence. Through substitution in the somatostatin sequence, including some limited substitutions in the central core portion and perhaps incorporating (d) amino acid enantiomorphs,

additional useful peptides are developed without affecting the binding specificity and affinity desired. Likewise peptidomimetic molecules may be prepared to duplicate this specific binding function. An example of such a useful peptide is SANDOSTATIN™ manufactured by SANDOZ Pharmaceuticals, Ltd., Basel Switzerland. The sequence of the Sandostatin™ peptide is:



wherein x equals 1.4 to 2.5

In the present invention, the somatostatin peptide itself, or a molecule having somatostatin receptor specificity, may be radiolabelled using more than one method. The reaction generally takes place between the amino groups in the peptide and the carbonyl group in the active ester to form an amide bond. In particular, the peptides can be radiolabelled using either a conventional method referred to as "post-formed chelate approach" or by a recent method referred to as "pre-formed chelate approach" developed by Fritzberg et al., U.S. Patents Numbers 4,965,392 and 5,037,630 incorporated herein by reference. In the "pre-formed approach," the desired ligand is complexed with the radionuclide and then conjugated to somatostatin or a molecule having somatostatin receptor specificity. In the "post-formed approach," the desired ligand is first conjugated to the peptide and the resulting conjugate is incubated with 99mTc sodium pertechnetate solution obtained from $^{99}Mo/^{99m}Tc$ generator along with a reducing agent. In the present invention, the latter approach has the additional advantage of allowing preparation of the complex in kit form. Users merely add $Na^{99m}TcO_4$ to the ligand-somatostatin conjugate or

a derivative thereof for labelling to occur.

It is important to note a unique aspect of the present invention whereby the desired conjugation reaction should be directeed to occur only at the alpha amino group of the somatostatin. The critical binding region for the peptide includes the amino group at the lysine position, or K. Conjugation at the alpha amino group is important so as to avoid interference with the binding specificity of the somatostatin peptide. It is therfore necessary to take steps to assure that the conjugation reaction takes place at the alpha amino group. The desired conjugation reaction will take place most readily when the alpha amino group is in the "free base" form, i.e., deprotonated to the NH_2 form, while the epsilon amino group is protonated, i.e., in the NH_3^+ form. Therefore, according to the present invention, it is important to perform the conjugation reaction at neutral pH or within the range of pH 7.0 to pH 9.5. Performing the conjugation reaction at such a pH deactives the lysine position relative to the alpha amino group, and protonates the more basic epsilon amino group, thus making the epsilon amino group less reactive. In other words, by carrying out the conjugation reaction at the pH noted above, deprotonation of the epsilon amino group is prevented and conjugation occurs at the desired site of the alpha amino group.

Using either method of labelling the somatostatin peptide, any suitable ligand can be used to incorporate the preferred radionuclide metal ion such as technetium, rhenium, indium, gallium, samarium, holmium, yttrium, copper, or cobalt. The choice of the ligand entirely depends on the type of metal ion desired for diagnostic or therapeutic purposes. For example, if the radionuclide is a transition element such as technetium or rhenium, then

ligands containing amine, amide, and thiols are preferred to form a stable complex whereas if the radionuclide is a lanthanide element, then polyaminocarboxyates or phenolate type ligands are preferable.

The above-described unique characteristics of the present invention make radiolabelled somatostatin and its derivatives very attractive for diagnostic purposes as well as for radiotherapy. The compounds of the present invention may be labelled with any radionuclide favorable for these purposes. Such suitable radionuclides for radiotherapy include but are not limited to Re-186, copper-67, Re-188 and cobalt-60. For diagnostic purposes the most suitable radionuclides include but are not limited to the transition metals as exemplified by technetium-99m and copper-62.

Due to the unique mechanism employed in the present invention to label the alpha amino group of somatostatin and avoid the epsilon amino group(s) (which would inhibit the ability of somatostatin peptides to bind to its receptor) a significantly advantageous radiolabelled peptide compound for radiotherapy and diagnostic imaging of tumors is achieved.

It is therefore an object of the present invention to provide a selective agent, both for the diagnostic imaging and for the therapeutic treatment of tumors containing high-affinity somatostatin receptors having a significantly high target to background ratio.

The present invention also discloses novel radiolabelled peptide compounds, methods of preparing these compounds, pharmaceutical compositions comprising these compounds and the use of these compounds in kits for the

diagnostic imaging of thrombotic diseases. Thrombus hirudin contain large numbers of receptors having a high affinity for hirudin and derivatives thereof.

In diagnostic thrombus imaging, a radiolabelled compound must be easily detectable and highly selective and have low blood binding. High selectivity, which is essential in these compounds means that the diagnostic compound, after having been introduced into the body, accumulates to a greater degree in the target tissue or tissues, i.e. a thrombi, than in surrounding tissues. In using hirudin or derivatives thereof as carrier agents in radiolabelled compounds, the specific high selectivity of the particular peptide used provides for the strong accumulation of the diagnostic compound in the target tissue or tissues, such as in thrombus in the case of hirudin, compared with the concentration thereof in non-target tissues.

The radiolabelled peptide compounds of the present invention employ the hirudin peptide
NH_2-Ile-Thr-Tyr-Thr-Asp-Cys-Thr-Glu-Ser-Gly-Gln-Asn-Leu-Cys-Leu-Cys-Glu-Gly-Ser-Asn-Val-Cys-Gly-Lys-Gly-Asn-Lys-Cys-Ile-Leu-Gly-Ser-Asn-Gly-Lys-Gly-Asn-Gln-Cys-Val-Thr-Gly-Gly-Gly-Thr-Pro-Lys-Pro-Glu-Ser-His-Asn-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-Gln-COOH;

hirulog-1 peptide
NH_2-D-Phe-Pro-Arg-Pro-$(Gly)_4$-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-COOH;

hirulog-64 peptide
NH_2-D-Phe-Pro-Arg-Pro-$(Gly)_4$-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-Gly-Gly-Lys-COOH:

hirulog-133 peptide
NH_2-D-Phe-Pro-Arg-Pro-$(Gly)_4$-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-Gly-Gly-Cys-COOH; and like derivatives.

In targeting particular receptors with radiolabelled hirudin or its derivatives, it is not necessary that the complete sixty-five (65) residue sequence of hirudin be present. Binding is thought to reside primarily in the anion binding exosite. Through substitution in the hirudin sequence, including some limited substitutions in the anion binding exosite and perhaps incorporating D-amino acid enantiomorphs, additional useful peptides are developed without affecting the binding specificity and affinity desired. Likewise peptidomimetic molecules may be prepared to duplicate this specific binding function.

In the present invention, the hirudin peptide itself, or a molecule having hirudin receptor specificity, such as hirulog-1, hirulog-64 and hirulog-133 may be radiolabelled using more than one method. The reaction generally takes place between the amino groups in the peptide and the carbonyl group in the active ester to form an amide bond. In particular, the peptides can be radiolabelled using either a conventional method referred to as "post-formed chelate approach" or by a recent method referred to as "pre-formed chelate approach" developed by Fritzberg et al., U.S. Patents Numbers 4,965,392 and 5,037,630 incorporated herein by reference. In the "pre-formed approach," the desired ligand is complexed with the radionuclide and then conjugated to hirudin or a molecule having hirudin receptor specificity. In the "post-formed approach," the desired ligand is first conjugated to the peptide and the resulting conjugate is incubated with 99mTc sodium pertechnetate solution obtained from $^{99}Mo/^{99m}Tc$ generator along with a reducing agent. In the present

invention, the latter approach has the additional advantage of allowing preparation of the complex in kit form. Users merely add $Na^{99m}TcO_4$ to the ligand-hirudin conjugate or a derivative thereof for labelling to occur.

It is important to note that when forming the ligand-hirudin conjugate, it is important to direct the conjugation away from the alpha amino group. This is just the opposite of the case for somatostatin noted above. In other words, to avoid interference with the binding specificity of the hirudin peptide, it is desirable to have conjugation take place at the epsilon amino group. If the alpha-amino group is affected, such as by deprotonation, then the specificity and affinity of the peptide is altered. Therefore, for hirudin, it is important to perform the conjugation while protecting the alpha-amino group, such as through the use of blocking agents. For example, in the conjugation of hirulog-133, D-phenylalanine must be protected to ensure specificity. Further, in the case of labelling hirulog-1, hirulog-64 or hirulog-133 the epsilon amino group or the sulfhydryl groups are the groups preferably targeted for labelling.

Using either method of labelling the hirudin peptide or its derivatives, any suitable ligand can be used to incorporate the preferred radionuclide such as technetium, iodine, rhenium, indium, gallium, samarium, holmium, yttrium, copper, or cobalt. The choice of the radionuclide carrier entirely depends on the type of element desired for diagnostic purposes. For example, if the radionuclide is a transition element such as technetium or rhenium, then ligands containing amine, amide, and thiols are preferred to form a stable complex whereas if the radionuclide is a lanthanide element, then polyaminocarboxyates or phenolate type ligands are preferable. If the choice is iodine, then

a covalently bonded aromatic carrier would be selected.

The above-described unique characteristics of the present invention make radiolabelled hirudin and its derivatives very attractive for diagnostic purposes. The compounds of the present invention may be labelled with any radionuclide favorable for these purposes. For diagnostic purposes the most suitable radionuclides include but are not limited to the halogens or transition metals as exemplified by technetium-99m, copper-62 and iodine-123.

Due to the unique mechanism employed in the present invention to label by means of a chelate ligand the epsilon amino group of hirudin and avoid the alpha amino group(s) (which would inhibit the ability of hirudin or derivative peptides to bind to its receptor) a significantly advantageous radiolabelled peptide compound for diagnostic imaging of thrombus and thrombotic diseases is achieved.

It is therefore an object of the present invention to provide a selective agent, both for the diagnostic imaging and for the therapeutic treatment of thrombotic diseases containing high-affinity hirudin receptors having a significantly high target to background ratio.

DETAILED DESCRIPTION OF THE INVENTION

The novel aminothiol ligands may be defined according to the general formula:

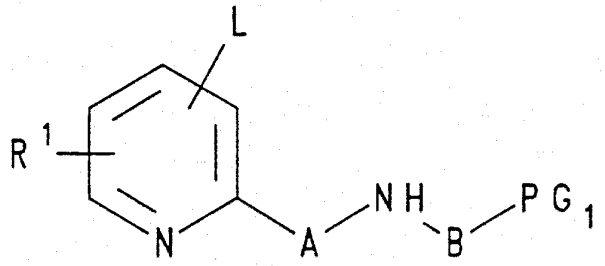


Figure 1

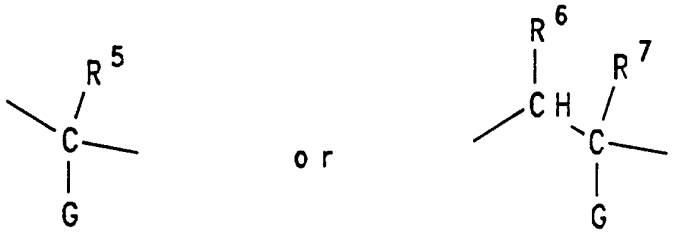
wherein R^1 is selected from the group consisting of hydrogen, alkyl, hydroxyl, alkoxyl, hydroxyalkyl, alkoxyalkyl, alkoxycarbonyl, or carbamoyl wherein the carbon containing portion of such groups contains 1 to 10 carbon atoms; PG_1 is a suitable sulfur protecting group selected from the group consisting of acetyl, methoxyacetyl, 1-3-dioxacyclohexyl, 1,3-dioxacyclopentyl, dialkoxyalkyl, tetrahydrofuranyl, benzhydryl, C_{1-20} S-acyl such as alkanoyl, benzoyl and substituted benzoyl, C_{1-20} S-acyl groups such as benzyl, t-butyl, trityl, 4-methoxybenzyl and 2,4-dimethoxybenzyl, C_{1-10} alkoxyalkyl such as methoxymethyl, ethoxyethyl and tetrahydropyranyl, carbamoyl, C_{1-10} alkoxy carbonyl such as t-butoxycarbonyl and methoxycarbonyl, and the like; L is selected from the group consisting of

$$-H \quad , \quad -(CH_2)_m-X \quad , \quad -(CH_2)_l-E-X \quad , \text{ or}$$

$$-(CH_2)_j-\underset{\displaystyle R^4}{\overset{\displaystyle |}{(CH_2)}}-E-\overset{\displaystyle O}{\overset{\displaystyle ||}{C}}-(CH_2)_k-X$$

wherein j, k, l and m are 0 to 10, preferably 1 to 6; E is -O-, -S-, or $-NR^3$, wherein R^3 and R^4 are defined in the same manner as R^1 above, wherein X is a suitable coupling moiety

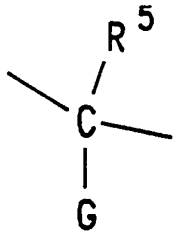
selected from the group consisting of hydrogen, formyl, carboxyl, hydroxyl, amino, t-butoxycarbonylamino, chlorocarbonyl, N-alkoxycarbamoyl, haloacetyl, imidate, succinimidoloxycarbonyl, maleimide, isocyanate, isothiocyanate, tetrafluorophenoxy, chlorosulfonyl, C_{1-10} N-alkoxycarbamoyl, and the like; A is selected from the group consisting of



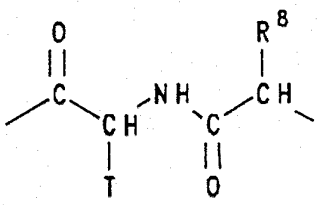
wherein R^5 to R^7 are defined in the same manner as R^1 above, and wherein G is defined in the same manner as L above; and B is selected from the group consisting of

wherein R^8 and R^9 are defined in the same manner as R^1 above, and wherein T is defined in the same manner as L above.

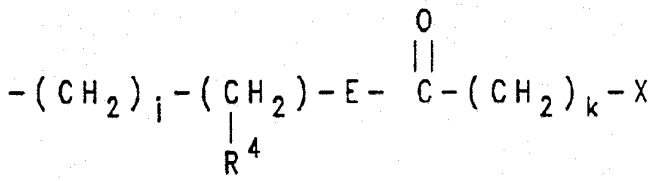
In a preferred embodiment, ligands according to the present invention have the general Formula 8 above, wherein A is



wherein R^5 and G are hydrogens; B is

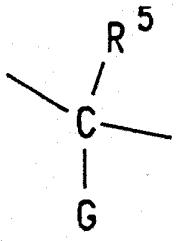


wherein R^8 is hydrogen and T is

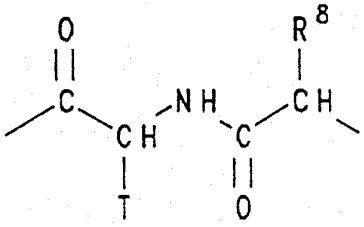


wherein R^4 is hydrogen, E is an -NH- group, k is 3, j is 2, and X is carboxyl; PG_1 is a benzoyl or a tetrahydropyranyl group; and L is hydrogen.

In another preferred embodiment, ligands according to the present invention have the general Formula 8 wherein A is



wherein R^5 and G are hydrogens; B is



wherein R^8 is hydrogen and T is $-(CH_2)_m-X$ wherein m is 2 or

4, and X is either an amino, a carboxyl or a hydroxyl; PG_1 is a benzoyl or a tetrahydropyranyl group; and L is hydrogen.

The novel ligands described above, may be incorporated into radionuclide complexes used as radiographic imaging agents. Further, these ligands or complexes can be covalently or non-covalently attached to biologically active carrier molecules, such as, antibodies, enzymes, peptides, peptidomimetics, hormones, and the like. The complexes of the present invention are prepared by reacting one of the aforementioned ligands with a radionuclide containing solution under radionuclide complex forming reaction conditions. In particular, if a technetium agent is desired, the reaction is carried out with a pertechnetate solution under technetium 99m complex forming reaction conditions. The solvent, if other than water or saline, may then be removed by any appropriate means, such as evaporation. The complexes are then prepared for administration to the patient by dissolution or suspension in a pharmaceutically acceptable vehicle.

One peptide employed in the present invention is a somatostatin peptide or derivatives thereof as described in U.S. Patent Number 4,395,403 incorporated herein by reference.

Another peptide employed in the present invention is a hirudin peptide as described in German Patents Numbered 136,103 (1902) and 150,805 (1903) incorporated herein by reference or derivatives thereof.

Both the somatostatin peptide and the hirudin peptide may be radiolabelled using a pre-formed or post-formed methodology. In a preferred embodiment according to the

present invention, the somatostatin or a molecule having somatostatin receptor specificity, or hirudin or a molecule having hirudin receptor specificity, is first bonded to an N_3S aminothiol ligand which is illustrated in Figure 2.

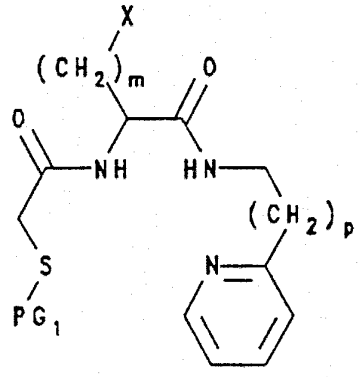


Figure 2

wherein m is a whole number less than eleven and preferably 4; p is either 0 or 1; PG_1 is a suitable sulfur protecting group selected from the group consisting of acetyl, methoxyacetyl, 1-3-dioxacyclohexyl, 1-3-dioxacyclopentyl, dialkoxyalkyl, tetrahydrofuranyl, benzhydryl, trityl, C_{1-20} S-acyl such as alkanoyl, benzoyl and substituted benzoyl -whereby alkanoyl is preferable, C_{1-20} S-acyl groups such as benzyl, t-butyl, trityl, 4-methoxybenzyl and 2,4-dimethoxybenzyl -whereby 2,4-dimethoxybenzyl is preferable, C_{1-10} alkoxyalkyl such as methoxymethyl, ethoxyethyl and tetrahydropyranyl -whereby tetrahydropyranyl is preferable, carbamoyl, and C_{1-10} alkoxy carbonyl such as t-butoxycarbonyl and methoxycarbonyl -whereby t-butoxycarbonyl is preferable; and X is a coupling moiety selected from the group consisting of hydrogen, formyl, carboxyl, hydroxyl, amino, t-butoxycarbonylamino, chlorocarbonyl, N-alkoxycarbamoyl, haloacetyl, imidate, succinimidoloxycarbonyl, maleimide, isocyanate, isothiocyanate, tetrafluorophenoxy, chlorosulfonyl, C_{1-10} N-alkoxycarbamoyl, and the like; -whereby N-methoxycarbamoyl

or succinimidoloxycarbonyl is preferable.

In another preferred embodiment according to the present invention, somatostatin or a molecule having somatostatin receptor specificity, or hirudin or a molecule having hirudin receptor specificity, is bonded to an N_2S_2 aminothiol ligand which is illustrated in Figure 3.

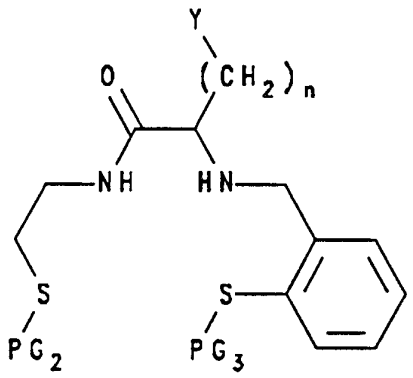


Figure 3

wherein n is a whole number less than eleven and preferably 3; PG_2 and PG_3 may be the same or different sulfur protecting groups selected from the group consisting of C_{1-20} S-acyl such as alkanoyl, benzoyl and substituted benzoyl -whereby alkanoyl is preferable, C_{1-20} alkyl groups such as benzyl, t-butyl, 4-methoxybenzyl, trityl and 2,4-dimethoxybenzyl -whereby 2,4-dimethoxybenzyl is preferable, C_{1-10} alkoxyalkyl such as for example methoxymethyl, ethoxyethyl, and tetrahydropyranyl -whereby tetrahydropyranyl is preferable, carbamoyl and C_{1-10} alkoxycarbonyl such as methoxycarbonyl, ethoxycarbonyl and t-butoxycarbonyl -whereby t-butoxycarbonyl is preferable; and Y is a coupling moiety selected from the group consisting of hydrogen, carboxyl, amino, isocyanate, isothiocyanate, imidate, maleimide, chlorocarbonyl, chlorosulfonyl, succinimidoloxycarbonyl, haloacetyl, and C_{1-10} N-alkoxycarbamoyl -whereby N-methoxycarbamoyl is

preferable.

In another preferred embodiment of the present invention, somatostatin or a molecule having somatostatin receptor specificity, or hirudin or a molecule having hirudin receptor specificity, is conjugated with the ligand illustrated in Figure 4.

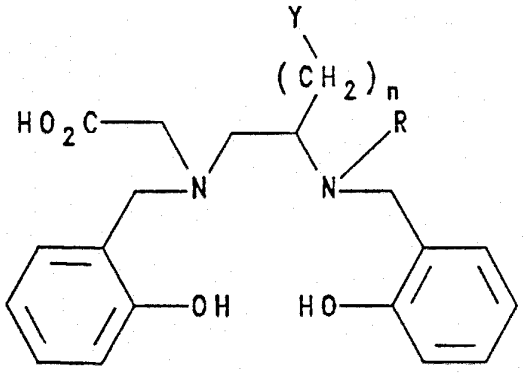


Figure 4

wherein n varies from 1 to 10, and Y is a coupling moiety ected rom the group consisting of carboxyl, amino, cyana , isothiocyanate, imidate, maleimide, chlorocarbonyl, chlorosulfonyl, succinimidoxycarbonyl, haloacetyl, and C_{1-10} N-alkoxycarbamoyl such as N-methoxycarbamoyl and t-butoxycarbamoyl -whereby N-methoxycarbamoyl is preferable; and R is selected from the group consisting of hydrogen and C_{1-} alkyl such as methyl and t-butyl -whereby methyl is preferable.

In another preferred embodiment, the somatostatin or a molecule having somatostatin receptor specificity, or hirudin or a molecule having hirudin receptor specificity, can be conjugated with the metal complex illustrated in

Figure 5.

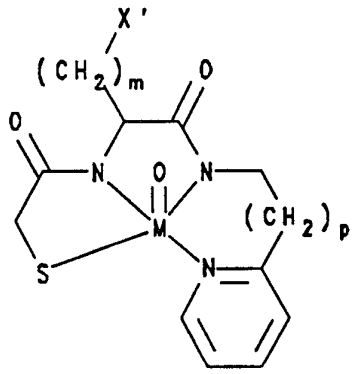


Figure 5

wherein m is a whole number less than eleven and more preferably 4; p is either 0 or 1; X' is a coupling moiety selected from the group consisting of carboxyl, amino, isocyanate, isothiocyanate, imidate, maleimide, chlorocarbonyl, chlorosulfonyl, succininimidyloxycarbonyl, haloacetyl and C_{1-10} N-alkoxycarbamoyl such as N-methoxycarbamoyl and t-butoxycarbamoyl -whereby N-methoxycarbamoyl is preferable and M is a radionuclide suitable for diagnostic imaging or therapeutic use such as technetium, rhenium, copper, cobalt, indium, gallium, samarium, yttrium and holmium.

In another preferred embodiment, the somatostatin or a molecule having somatostatin receptor specificity, or hirudin or a molecule having hirudin receptor specificity, can be conjugated with a metal complex as illustrated in Figure 6 wherein Y' and n are defined the same respectively as Y and n in Figure 4 and M is defined the same as M in Figure 5.

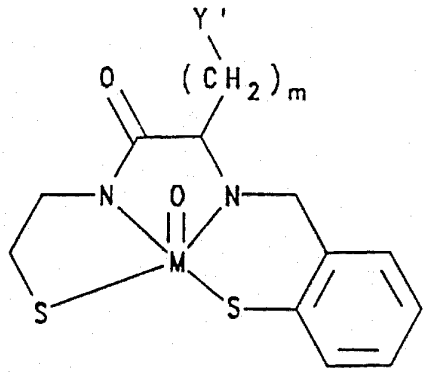


Figure 6

In another preferred embodiment, the somatostatin or a molecule having somatostatin receptor specificity, or hirudin or a molecule having hirudin receptor specificity, can be conjugated with a metal complex as shown in Figure 7.

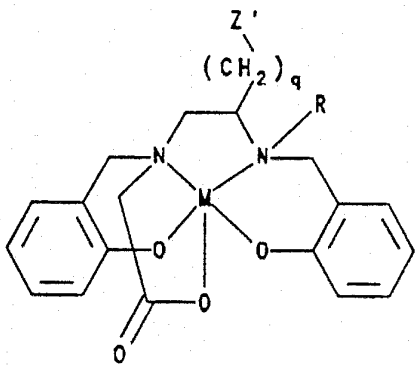


Figure 7

wherein Z', q and R are defined the same respectively as Y, n and R of Figure 4 and M is defined the same as M in Figure 5.

In another preferred embodiment, the somatostatin or a molecule having somatostatin receptor specificity, or hirudin or a molecule having hirudin receptor specificity,

can be conjugated with a metal complex as shown in Figure 8.

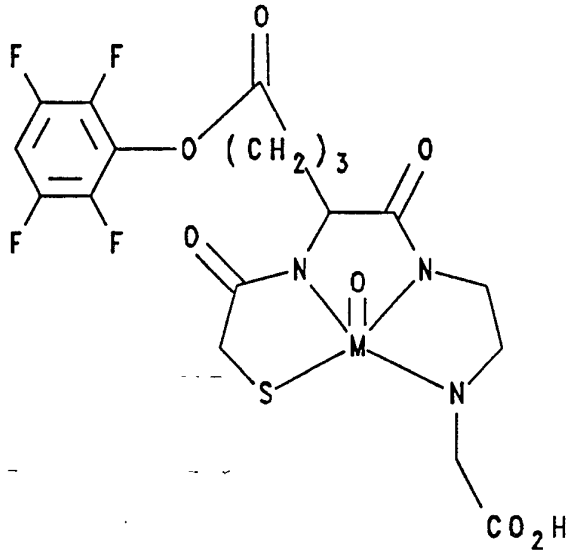


Figure 8

wherein M is defined the same as M in Figure 5.

Common esters which have been found useful in this labelling technique are o- and p- nitrophenyl, 2- chloro-4-nitrophenyl, cyanomethyl, 2-mercaptopyridyl, hydroxybenztriazole, N-hydroxysuccinimide, trichlorophenyl, tetrafluorophenyl, thiophenyl, tetrafluorothiophenyl, o-nitro-p-sulfophenyl, N-hydroxyphthalimide and the like. For the most part, the esters will be formed from the reaction of the carboxylate with an activated phenol, particularly, nitro-activated phenols, or a cyclic compound based on hydroxylamine.

The choice or an appropriate sulfur protecting group is essential to achieving the maximum utility form the invention. The protecting groups are displaced from the

compound during the labelling in what is believed to be a metal-assisted cleavage: i.e., the protective groups are displaced in the presence of a radionuclide during the labelling process. The radiolabelling procedure thus is simplified, which is a significant advantage when the chelating compounds are to be radiolabelled in a hospital laboratory shortly before use. Additionally, another advantage of the present invention is that extreme basic pH conditions and harsh conditions associated with certain known radiolabeling procedures or procedures for removal of other sulfur protected groups are avoided. Thus, both base-sensitive and acid-sensitive groups on the chelating compounds survive the radio-labelling step intact. Suitable sulfur-protecting groups, when taken together with the sulfur atom to be protected, include hemithioacetal groups such as ethoxyethyl, tetrahydrofuranyl, methoxymethyl, and tetrahydropyranyl. Other suitable sulfur protecting groups are C_{1-20} acyl groups, preferably alkanoyl, benzoyl, and C_{1-20} alkoxy-carbonyl groups, preferably N-methoxycarbonyl and t-butoxycarbonyl. Other possible formulas for the chelating compounds are described in the European Patent Application assigned publication number 0 284 071 incorporated herein by reference.

Synthesis of the Tc-99m bifunctional chelate and subsequent conjugation to a somatostatin peptide, or a derivative thereof, can be performed as described in the European Patent Application assigned publication number 0 284 071 and U.S. Patent Number 4,965,392 incorporated herein by reference and related technologies as covered by U.S. patent numbers 4,837,003, 4,732,974 and 4,659,839, each incorporated herein by reference.

After purification, technetium-99m labelled somatostatin peptide, or derivatives thereof, may be

injected into a patient for diagnostic imaging or therapeutic use. The technetium-99m somatostatin compound is capable of reliably visualizing tumors within minutes of post-injection. The somatostatin peptide when radiolabelled with the technetium-99m triamide thiolate bifunctional chelate is efficacious as an in vivo diagnostic agent for the imaging of tumors of the type described above.

The ligands of the present invention may be prepared from commercially available starting materials such as 2-(2-aminoethyl)pyridine, 2-aminomethyl pyridine, lysine, glutamic acid, aminoadipic acid, mercaptoacetic acid, etc. by standard synthetic methods as described in the following Examples.

Example 1

Preparation of 2-aza-4-[N-(S-benzoyl)mercaptoacetyl-8-[N-(t-butoxy)carbonyl]amino-3-oxo-1-(2-pyridyl)octane.

A mixture of 4-amino-2-aza-8-[N-(t-butoxy)carbonyl]-amino-3-oxo-1-(2-pyridyl)octane (1.70 g, 5 mmol) and N-[(S-benzoyl)mercapto]acetoxy-succinimide (1.53 g, 5.5 mmol) in acetonitrile (15 mL) was stirred at ambient temperature for 4 hours. The reaction mixture was poured onto water (100 mL) and kept at 4 to 8 °C (refrigerator) for about 16 hours. The precipitate was collected by filtration, washed well with water, dried, and recrystallized from acetonitrile to give 1.2 g of colorless solid, mp 133-135 °C. Anal. Calcd. for $C_{25}H_{34}N_4O_5S$: C, 60.70; H, 6.61; N, 10.89; S, 6.26. Found: C, 60.79; H, 6.65; N, 10.91; S, 6.30.

Example 2

Preparation of 2-aza-4-[N-(S-tetrahydropyranyl)-mercapto] acetyl-8-N-(t-butoxy)carbonyl]amino-3-oxo-1-(2-pyridyl)-octane.

A mixture of 4-amino-2-aza-8-[N-(t-butoxy)carbonyl)]-amino-3-oxo-1-(2-pyridyl)octane (3.36 g, 10 mmol) and N-[(S-tetrahydropyranyl)mercapto-acetoxy]-succinimide (2.40 g, 10 mmol) in acetonitrile (25 mL) was stirred at ambient temperature for 4 hours. The reaction mixture was poured onto water (100 mL) and extracted with methylene chloride (3 x 25 mL). The combined organic extracts were washed with water, dried ($MgSO_4$), filtered, and the filtrate taken to dryness under reduced pressure. The gummy residue was chromatographed over silica gel (200 g) using chloroform/methanol (95:5) as eluent to give 3.2 g of off-white solid, mp 87-90 °C. 13C-NMR ($CDCl_3$) δ 171.8, 171.7, 170.0, 156.9, 156.7, 156.3, 149.3, 137.0, 122.5, 121.9, 84.0, 83.6, 79.0, 66.2, 65.7, 53.2, 44.4, 44.3, 40.0, 35.0, 34.6, 31.7, 31.0, 29.4, 28.2, 25.0, 24.9, 22.4, 21.9, 21.6.

Example 3

Preparation of 6-aza-4-[N-(S-benzoyl)mercapto]acetyl-5-oxo-7-(2-pyridyl)-heptanoic acid.

A mixture of t-butyl 6-aza-4-[N-(S-benzoyl)-mercapto]-acetyl-5-oxo-7-(2-pyridyl)heptanoate (2.35 g, 5 mmol) and trifluoroacetic acid (5 mL) was kept at ambient temperature for 1 hour. The solution was then poured onto ether (100 mL). The precipitate was then collected by filtration, washed well with ether, and dried to yield 1.5 g of off white solid. ^{1}H-NMR (DMSO-d_6) δ 8.49-8.71 (m, 3H), 7.85-8.00 (m, 3H), 7.60-7.70 (m, 1H), 7.40-7.60 (m, 4H), 4.45 (d, 2H), 4.31 (m, 1H), 3.87 (dd, 2H), 2.27 (m, 2H), 1.95 (m, 1H), 1.80 (m, 1H). ^{13}C-NMR (DMSO-d_6) δ 191.1,

174.4, 172.0, 167.7, 157.5, 146.7, 140.3, 136.3, 134.5, 129.5, 127.2, 123.5, 122.5, 52.7, 42.9, 32.6, 30.1, 27.0. FAB mass spectrum, m/Z 416 (M + 1).

Example 4

Preparation of 7-aza-5-N-[(5-benzoyl)mercapto]acetyl-1-N-(t-butoxy-carbonyl)amino-6-oxo-9-(2-pyridyl)nonane.

A mixture of N-t-BOC-lysine-2-(2-pyridyl)ethylamide (1.75 g, 5 mmol) and N-[(5-benzoyl)mercapto]acetoxy-succinimide (1.53 g, 5.5 mmol) in acetonitrile (15 mL) was stired at ambient temperature for four hours. The reaction mixture was poured onto water (100 ml) and cooled in ice-salt bath for two hours. The precipitate was collected by filtration, washed with water, dried, and recrystalized from acetronitrile to give 2.3 g (88 %) of colorless solid. m.p. 138-140 C. Anal. Calcd. for $C_{26}H_{36}N_4O_5S$: C, 61.36; H, 7.27; N, 10.67; S, 6.10. Found: C, 61.39; H, 7.18; N, 10.62; S, 6.01.

Example 5

Preparation of 10-[(S-tetrahydropyranyl)mercapto]acetamido-5,12-diaza-4,11-dioxo-13(2-pyridyl)tridecanoic acid.

A mixture of 4-(4-amino)butyl-3,6-diaza-2,5-dioxo-1-(S-tetrahydropyranyl)mercapto-7-(2-pyridyl)heptane (790 mg, 2.0 mmol) and S-tetrahydropyranylmercaptoacetic acid (220 mg, 2.2 mmol) in acetonitrile (10 mL) was heated under reflux for four hours and stired at ambient temperature for sixteen hours. The solvent was removed under reduced pressure and the residue was purified by flash chromatography over reverse phase (25 g) eluted with water followed by methanol/water (1:1). Evaporation of the solvent afforded the desired ligand (510 mg) as colorless, amorphous solid. Anal. Calcd. for $C_{23}H_{34}N_4O_6S \times 0.33\ H_2O$: C,

55.20; H, 6.93; N, 11.20; S, 6.40: H_2O, 1.20. Found: C, 54.81; H, 6.99; N, 11.18; S, 6.39: H_2O, 1.19. Mass spectrum (thermospray) M/Z 495 (M + 1).

As noted above, the choice of protecting groups for the ligands according to the present invention has been found to be important. In particular, finding the proper protecting group for protection of the sulfur moiety has created difficulty in past ligand technology. It has been discovered that the use of hemithioacetal protecting groups such as tetrahydropyrannyl (THP) are especially useful during the labelling procedures.

Labelling of pyridine ligands as described above having a hemithioacetal protecting group has been carried out as shown in the following examples.

Example 6

Preparations were made as follows:

To 0.1 mL stannous gluconate (from a lyophilized kit containing 50 mg sodium gluconate and 1.2 mg stannous chloride, and reconstituted with 1.0 mL of degassed water) was added 1.0 mL pertechnetate, Tc-99m (about 3 mCi). The above is allowed to stand for 5 min at room temperature, before it is adjusted for pH with either HCl or NaOH (target Ph were 5, 6, 7 and 8). 0.12 mL of a pyridine ligand (SN_2Py) (0.88 mg/mL, 33% IPA/water) was then added. The preparations were incubated in a boiling water bath for 5 minutes.

An aliquot of the preparation was injected on an HPLC (C18 reverse phase), and the results of the radioactive profiles were integrated. Radiolabelling yields (RCY) are expressed as a percent of the peak of interest (Tc-99m

SN_2Py). Recovery studies were performed by measuring the amount of activity injected on the system vs recovered. The pH of the preparations were also measured with a pH electrode.

Example 6: Results

Target pH	RCY	Recovery (%)	Measured pH
5	43.1	90	5.1
6	53.6	ND	6.0
7	89.9	84	7.6
8	86.7	91	8.8

Example 7

Three preparations were done following the same protocol set forth in Example 6, except that dilute Tc-99 pertechnetate was added to the Tc-99m in order to carry more Tc mass.

One prepartion was a control (prep pH 7) and the two other prepartions contained an additional 5 nanomoles of Tc-99 (since 1 mL TcO_4^- is used, the prepartion woudl be made with 5 μM Tc, the highest usually eluted from a Mo-99/Tc-99m generator). Amoung these preparations, one was done at 50°C for 30 min instead of the 100°C (boiling water bath) for 5 min.

Example 7: Results

Preparation	RCY	Recovery (%)	Measured pH
control	89.9	89	7.3
100°C, 5 min	70.2	83	7.6
50°C, 30 min	25.4	79	ND

The results above clearly indicate that a pyridine ligands having a THP protecting group can be labelled in a wide rage of pH conditions ranging from acidic to basic. The preparations made with additional Tc-99 showed somewhat reduced kinetics but still provided good yield of product. This precludes the possibility that the results could be explained by radiolabelling of an impurity of the ligand. Radiolabelling was shown to occur even at reduced temperature.

Based of the above results, it is believed that the pyridine ligand plays a major role in the radiolabelling properties. In addition, it is believed that the THP protecting group, previously thought to be an acid cleavable protector can be used to protect the ligand and allow excellent radiolabelling of the product, even under neutral and basic conditions.

The radiolabelled somatostatin compound of the present invention are described in still greater detail in the illustrative examples which follow.

Example 8

A solution of somatostatin, or derivatives thereof, (0.01 mmol) in 2 mL of carbonate/bicarbonate buffer at pH 8.5 ± 0.5 is treated with a solution of 0.1 mmol of the ligand in Figure 2 (wherein m=2, p=1, PG_1 is benzoyl, and X is succinimidoloxycarbonyl) in dimethylformamide (0.5 mL)

and the entire mixture is kept at room temperature for 2 hours. The mixture is then diluted with water (2.5 mL) and dialyzed extensively against water. After dialysis, the solution is lyophilized to give the desired somatostatin conjugate.

Example 9

A solution of somatostatin, or derivatives thereof, (0.01 mmol) in 2 mL of carbonate/bicarbonate buffer at pH 8.5 ± 0.5 is treated with a solution of 0.1 mmol of the ligand in Figure 3 (wherein n=2, PG_2 and PG_3 are benzoyl, and Y is succinimidoloxycarbonyl) in dimethylformamide (0.5 mL) and the entire mixture is kept at room temperature for 2 hours. The mixture is then diluted with water (2.5 mL) and dialyzed extensively against water. After dialysis, the solution is lyophilized to give the desired somatostatin conjugate.

Example 10

A solution of somatostatin, or derivatives thereof, (0.01 mmol) in 2 mL of carbonate/bicarbonate buffer at pH 8.5 ± 0.5 is treated with a solution of 0.1 mmol of the ligand in Figure 4 (wherein q=4, and Z is succinimidoloxycarbonyl) in dimethylformamide (0.5 mL) and the entire mixture is kept at room temperature for 2 hours. The mixture is then diluted with water (2.5 mL) and dialyzed extensively against water. After dialysis, the solution is lyophilized to give the desired somatostatin conjugate.

Example 11

To 100 uL of a solution containing 5 mg of sodium gluconate and 0.1 mg of stannous chloride in water, 500 ul of 99m-Tc04 (pertechnetate) is added. After incubation at room temperature for about 10 minutes at room temperature,

a solution of 500 uL of the somatostatin, or derivatives thereof, conjugates (1 mg/mL in 0.1 M carbonate/bicarbonate buffer, pH 9.5) in Examples 8 or 9 is then added and the entire mixture is incubated at 37°C for about 1 hour. The desired labelled peptide is separated from unreacted 99mTc-gluconate and other small molecular weight impurities by gel filtration chromatography (Sephadex G-50) using phosphine buffered physiological saline, (hereinafter PBS), 0.15M NaCl, pH 7.4 as eluent.

Example 12

A mixture of gentisic acid (25 mg), inositol (10 mg), and the somatostatin, or derivatives thereof, conjugate (500 uL, 1 mg/mL in water) was treated with In-111 indium chloride in 0.05 M HCl. The solution was allowed to incubate at room temperature for about 30 minutes. The desired labelled peptide is separated from unreacted In-111 indium salts and other small molecular weight impurities by gel filtration chromatography (Sephadex G-50) using phosphine buffered physiological saline, (PBS), 0.15M NaCl as eluent.

Synthesis of the Tc-99m bifunctional chelate and subsequent conjugation to a hirudin peptide, or a derivative thereof, can be performed as described in the European Patent Application assigned publication number 0 284 071 and U.S. Patent Number 4,965,392 incorporated herein by reference and related technologies as covered by U.S. patent numbers 4,837,003, 4,732,974 and 4,659,839, each incorporated herein by reference.

After purification, technetium-99m labelled hirudin peptide, or derivatives thereof, may be injected into a patient for diagnostic imaging. The technetium-99m hirudin compound is capable of reliably visualizing thrombus within

minutes of post-injection. The hirudin peptide when radiolabelled with the technetium-99m triamide thiolate bifunctional chelate is efficacious as an in vivo diagnostic agent for the imaging of thrombus of the type described above. The radiolabelled hirudin compound of the present invention are described in still greater detail in the illustrative examples which follow.

Example 13

A solution of hirudin, or derivatives thereof, (0.01 mmol) in 2 mL of carbonate/bicarbonate buffer at pH 8.5 ± 0.5 is treated with a solution of 0.1 mmol of the ligand in Figure 2 (wherein m=2, p=1, PG_1 is benzoyl, and X is succinimidoloxycarbonyl) in dimethylformamide (0.5 mL) and the entire mixture is kept at room temperature for 2 hours. The mixture is then diluted with water (2.5 mL) and dialyzed extensively against water. After dialysis, the solution is lyophilized to give the desired hirudin conjugate.

Example 14

A solution of hirudin, or derivatives thereof, (0.01 mmol) in 2 mL of carbonate/bicarbonate buffer at pH 8.5 ± 0.5 is treated with a solution of 0.1 mmol of the ligand in Figure 3 (wherein n=2, PG_2 and PG_3 are benzoyl, and Y is succinimidoloxycarbonyl) in dimethylformamide (0.5 mL) and the entire mixture is kept at room temperature for 2 hours. The mixture is then diluted with water (2.5 mL) and dialyzed extensively against water. After dialysis, the solution is lyophilized to give the desired hirudin conjugate.

Example 15

A solution of hirudin, or derivatives thereof, (0.01 mmol) in 2 mL of carbonate/bicarbonate buffer at pH 8.5 ±

0.5 is treated with a solution of 0.1 mmol of the ligand in Figure 4 (wherein q=4, and Z is succinimidoloxycarbonyl) in dimethylformamide (0.5 mL) and the entire mixture is kept at room temperature for 2 hours. The mixture is then diluted with water (2.5 mL) and dialyzed extensively against water. After dialysis, the solution is lyophilized to give the desired hirudin conjugate.

Example 16

To 100 uL of a solution containing 5 mg of sodium gluconate and 0.1 mg of stannous chloride in water, 500 ul of 99m-Tc04 (pertechnetate) is added. After incubation at room temperature for about 10 minutes at room temperature, a solution of 500 uL of the hirudin, or derivatives thereof, conjugates (1 mg/mL in 0.1 M carbonate/bicarbonate buffer, pH 9.5) in Examples 13 or 14 is then added and the entire mixture is incubated at 37°C for about 1 hour. The desired labelled peptide is separated from unreacted 99mTc-gluconate and other small molecular weight impurities by gel filtration chromatography (Sephadex G-50) using phosphine buffered physiological saline, (hereinafter PBS), 0.15M NaCl, pH 7.4 as eluent.

Example 17

A mixture of gentisic acid (25 mg), inositol (10 mg), and the hirudin, or derivatives thereof, conjugate (500 uL, 1 mg/mL in water) was treated with In-111 indium chloride in 0.05 M HCl. The solution was allowed to incubate at room temperature for about 30 minutes. The desired labelled peptide is separated from unreacted In-111 indium salts and other small molecular weight impurities by gel filtration chromatography (Sephadex G-50) using phosphine buffered physiological saline, (PBS), 0.15M NaCl as eluent.

After the somatostatin or a derivative thereof or

hirudin or a derivative thereof is prepared and labelled according to a procedure described above, the compound is used with a pharmaceutically acceptable carrier in a method of performing a diagnostic imaging procedure using a gamma camera or like device. This procedure involves injecting or administering, for example in the form of an injectable liquid, to a warm-blooded animal an effective amount of the present invention and then exposing the warm-blooded animal to an imaging procedure using a suitable detector, e.g. a gamma camera. Images are obtained by recording emitted radiation of tissue or the pathological process in which the radioactive peptide has been incorporated, which in the present case are thrombus, thereby imaging thrombus in the body of the warm-blooded animal. Pharmaceutically acceptable carriers for either diagnostic or therapeutic use include those that are suitable for injection or administration such as aqueous buffer solutions, e.g. tris (hydroxymethyl)aminomethane (and its salts), phosphate, citrate, bicarbonate, etc., sterile water for injection, physiological saline, and balanced ionic solutions containing chloride and/or bicarbonate salts of normal blood plasma cations such as Ca^{2+}, Na^{+}, K^{+} and Mg^{2+}. Other buffer solutions are described in Remington's Practice of Pharmacy, 11th edition, for example on page 170. The carriers may contain a chelating agent, e.g. a small amount of ethylenediaminetetraacidic acid, calcium disodium salt, or other pharmaceutically acceptable chelating agents.

The concentration of labeled peptide and the pharmaceutically acceptable carrier, for example in an aqueous medium, varies with the particular field of use. A sufficient amount is present in the pharmaceutically acceptable carrier in the present invention when satisfactory visualization of the tumor is achievable or therapeutic results are achievable.

The composition is administered to the warm-blooded animals so that the composition remains in the living animal for about six to seven hours, although shorter and longer residence periods are normally acceptable.

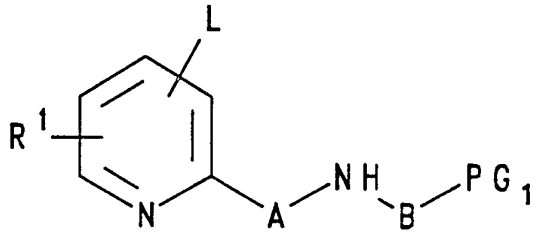
The radiolabelled somatostatin compounds of the present invention or somatostatin derivatives thereof, prepared as described herein, provide means of in vivo diagnostic imaging of tumors or therapeutic treatment of tumors which provides many advantages over prior known procedures for targeting the particular tumors of choice.

The radiolabelled hirudin compounds of the present invention or hirudin derivatives thereof, prepared as described herein, provide means of in vivo diagnostic imaging or therapeutic treatment of thrombus which provides many advantages over prior known procedures for diagnosis and treatment of thrombotic disease.

After consideration of the above specification, it will be appreciated that many improvements and modifications in the details may be made without departing from the spirit and scope of the invention. It is to be understood, therefore, that the invention is in no way limited, except as defined by the appended claims.

We claim:

1. A ligand useful in forming radionuclide complexes, said ligand having the general formula:

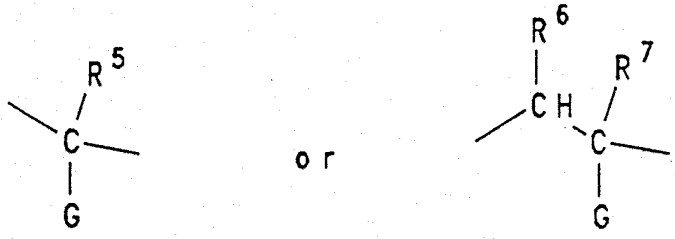


wherein R^1 is selected from the group consisting of hydrogen, alkyl, hydroxyl, alkoxyl, hydroxyalkyl, alkoxyalkyl, alkoxycarbonyl, or carbamoyl wherein the carbon containing portion of such group contains 1 to 10 carbon atoms; PG_1 is a suitable sulfur protecting group selected from the group consisting of acetyl, methoxyacetyl, 1-3-dioxacyclohexyl, 1,3-dioxacyclopentyl, dialkoxyalkyl, tetrahydrofuranyl, benzhydryl, C_{1-20} S-acyl such as alkanoyl, benzoyl and substituted benzoyl, C_{1-20} S-acyl groups such as benzyl, t-butyl, trityl, 4-methoxybenzyl and 2,4-dimethoxybenzyl, C_{1-10} alkoxyalkyl such as methoxymethyl, ethoxyethyl and tetrahydropyranyl, carbamoyl, C_{1-10} alkoxy carbonyl such as t-butoxycarbonyl and methoxycarbonyl, and the like; L is selected from the group consisting of

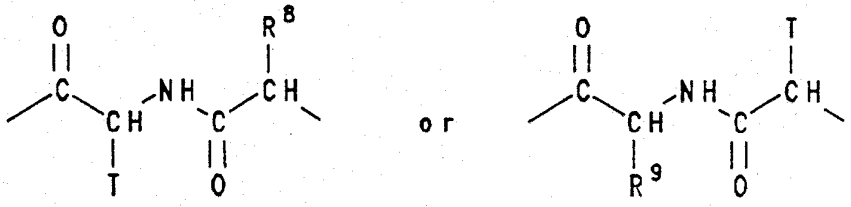
$$-H \ , \ -(CH_2)_m-X \ , \ -(CH_2)_i-E-X \ , \text{ or}$$

$$-(CH_2)_i-\underset{\underset{R^4}{|}}{(CH_2)}-E-\overset{\overset{O}{||}}{C}-(CH_2)_k-X$$

wherein j, k, l and m are 0 to 10; E is -O-, -S-, or $-NR^3$, wherein R^3 and R^4 are defined in the same manner as R^1 above, wherein X is a suitable coupling moiety selected from the group consisting of hydrogen, formyl, carboxyl, hydroxyl, amino, t-butoxycarbonylamino, chlorocarbonyl, N-alkoxycarbamoyl, haloacetyl, imidate, maleimide, succinimidoloxycarbonyl, isocyanate, isothiocyanate, tetrafluorophenoxy, chlorosulfonyl, C_{1-10} N-alkoxycarbamoyl, and the like; A is selected from the group consisting of

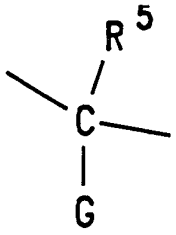


wherein R^5 to R^7 are defined in the same manner as R^1 above, and wherein G is defined in the same manner as L above; and B is selected from the group consisting of

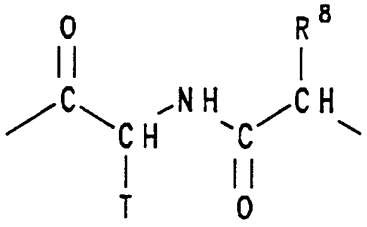


wherein R^8 and R^9 are defined in the same manner as R^1 above, and wherein T is defined in the same manner as L above.

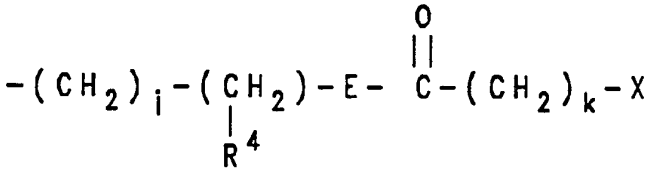
2. A ligand according to claim 1, wherein A is



wherein R^5 and G are hydrogens; B is

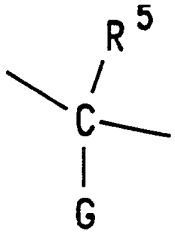


wherein R^8 is hydrogen and T is

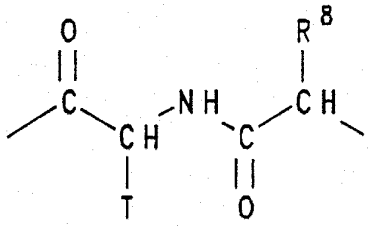


wherein R^4 is hydrogen, E is an -NH- group, k is 2, j is 3, and X is carboxyl; PG_1 is a benzoyl or a tetrahydropyranyl group; and L is hydrogen.

3. A ligand according to claim 1, wherein A is



wherein R^5 and G are hydrogens; B is



wherein R^8 is hydrogen and T is $-(CH_2)_m-X$ wherein m is 2 or 4, and X is either an amino, a carboxyl or a hydroxyl; PG_1 is a benzoyl or a tetrahydropyranyl group; and L is hydrogen.

4. A ligand according to claim 1, wherein j, k, l and m are 1 to 6.

5. A method of labelling pyridine containing ligands with a radionuclide, wherein said method includes the step of providing a pyridine ligand having a hemithioacetal protecting group.

6. A method according to claim 5, wherein said radionuclide is technetium.

7. A method according to claim 5, wherein said ligand is an pyridine based aminothiol ligand.

8. A method according to claim 5, wherein said hemithioacetal protecting group is tetrahydropyranyl.

9. A method according to claim 5, wherein said labelling is carried out under acidic conditions.

10. A method according to claim 5, wherein said labelling is carried out under neutral conditions.

11. A method according to claim 5, wherein said labelling is carried out under basic conditions.

12. A diagnostic composition suitable for administration to a warm-blooded animal comprising a somatostatin peptide labeled with Tc-99m by means of a triamide thiolate (N_3S) chelate capable of administration to an animal to produce reliable diagnostic imaging of tumors.

13. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an imaging-effective amount of a somatostatin peptide labeled with Tc-99m by means of a triamide thiolate (N_3S) chelate to allow for diagnostic imaging of tumors.

14. A somatostatin peptide labeled with Tc-99m by means of a triamide thiolate (N_3S) chelate.

15. The somatostatin peptide of claims 12, 13, or 14 wherein said somatostatin peptide labeled with Tc-99m by means of a triamide thiolate (N_3S) chelate is capable of administration to a warm-blooded animal to produce reliable diagnostic imaging of tumors within two and one half hours post-injection.

16. A therapeutic composition suitable for administration to a warm-blooded animal comprising a somatostatin peptide labeled with Re-186 or Re-188 by means of a triamide thiolate (N_3S) chelate capable of administration to an animal to produce therapeutic effects on tumors.

17. A method of performing a therapeutic procedure, which comprises administering to a warm-blooded animal an therapeutically-effective amount of a somatostatin peptide labeled with Re-186 or Re-188 by means of a triamide

thiolate (N_3S) chelate to allow for therapeutic effects on tumors.

18. A somatostatin peptide labeled with Re-186 or Re-188 by means of a triamide thiolate (N_3S) chelate.

19. The somatostatin peptide of claims 16, 17, or 18 wherein said somatostatin peptide labeled with Re-186 or Re-188 Re by means of a triamide thiolate (N_3S) chelate is capable of administration to a warm-blooded animal to produce therapeutic effects on tumors post-injection.

20. A diagnostic composition suitable for administration to a warm-blooded animal comprising a somatostatin peptide labeled with Tc-99m by means of a triamide thiolate (N_3S) or a diamide dithiolate (N_2S_2) chelate capable of administration to an animal to produce reliable diagnostic imaging of tumors.

21. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an imaging-effective amount of a somatostatin peptide labeled with Tc-99m by means of a triamide thiolate (N_3S) or diamide dithiolate (N_2S_2) chelate to allow for diagnostic imaging of tumors.

22. A somatostatin peptide labeled with Tc-99m by means of a triamide thiolate (N_3S) or a diamide dithiolate (N_2S_2) chelate.

23. The somatostatin peptide of claims 20, 21, or 22 wherein said somatostatin peptide labeled with Tc-99m by means of a triamide thiolate (N_3S) or a diamide dithiolate (N_2S_2) chelate is capable of administration to a warm-blooded animal to produce reliable diagnostic imaging of tumors within two and one half hours post-injection.

24. A therapeutic composition suitable for administration to a warm-blooded animal comprising a somatostatin peptide labelled with a triamide thiolate (N_3S) or a diamide dithiolate (N_2S_2) chelate bound to a radioactive isotope of copper or cobalt capable of administration to an animal to produce therapeutic effects on tumors.

25. A method of performing a therapeutic procedure, which comprises administering to a warm-blooded animal a therapeutically-effective amount of a somatostatin peptide labeled with a triamide thiolate (N_3S) or a diamide dithiolate (N_2S_2) chelate bound to a radioactive isotope of copper or cobalt to produce therapeutic effects on tumors.

26. A somatostatin peptide labeled with a triamide thiolate (N_3S) or a diamide dithiolate (N_2S_2) chelate bound to a radioactive isotope of copper or cobalt.

27. A diagnostic composition suitable for administration to a warm-blooded animal comprising a peptide with somatostatin receptor specificity labeled with a radionuclide by means of a triamide thiolate (N_3S) or a diamide dithiolate (N_2S_2) chelate capable of administration to an animal to produce reliable diagnostic imaging of tumors.

28. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an imaging-effective amount of a peptide with somatostatin receptor specificity labeled with a radionuclide by means of a triamide thiolate (N_3S) or a diamide dithiolate (N_2S_2) chelate to allow for diagnostic imaging of tumors.

29. A peptide with somatostatin receptor specificity labeled with a radionuclide by means of a triamide thiolate (N_3S) or a radionuclide diamide dithiolate (N_2S_2) chelate.

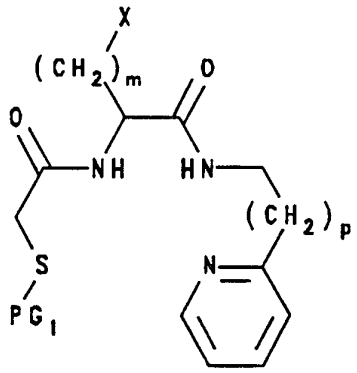
30. The peptide with somatostatin receptor specificity of claims 27, 28, or 29 wherein said peptide labeled with a radionuclide by means of a triamide thiolate (N_3S) or a diamide dithiolate (N_2S_2) chelate is capable of administration to a warm-blooded animal to produce reliable diagnostic imaging of tumors within two and one half hours post-injection.

31. A therapeutic composition suitable for administration to a warm-blooded animal comprising a peptide with somatostatin receptor specificity labeled with a radionuclide by means of a triamide thiolate (N_3S) or a diamide dithiolate (N_2S_2) chelate capable of administration to an animal to provide therapeutic effects on tumors.

32. A method of performing a therapeutic procedure, which comprises administering to a warm-blooded animal a therapeutically-effective amount of a peptide with somatostatin receptor specificity labeled with a radionuclide by means of a triamide thiolate (N_3S) or a diamide dithiolate (N_2S_2) chelate to provide therapeutic effects on tumors.

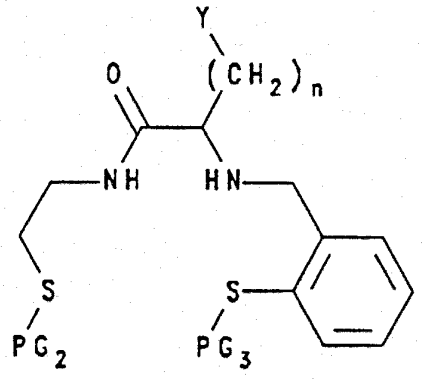
33. A peptide with somatostatin receptor specificity labeled with a radionuclide by means of a triamide thiolate (N_3S) or a diamide dithiolate (N_2S_2) chelate.

34. A composition comprising somatostatin or a peptide which retains somatostatin receptor specificity conjugated with a N_3S ligand having the general structure



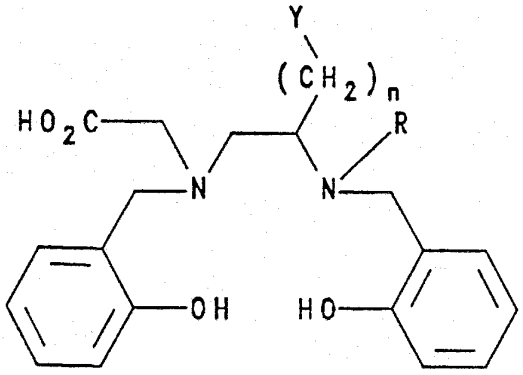
wherein m is a whole number less than eleven; p is either 0 or 1; PG_1 is a sulfur protecting group selected from the group consisting of acetyl, methoxyacetyl, 1-3-dioxacyclohexyl, 1,3-dioxacyclopentyl, dialkoxyalkyl, tetrahydrofuranyl, benzhydryl, C_{1-20} S-acyl such as alkanoyl, benzoyl and substituted benzoyl, C_{1-20} S-acyl groups such as benzyl, t-butyl, trityl, 4-methoxybenzyl and 2,4-dimethoxybenzyl, C_{1-10} alkoxyalkyl such as methoxymethyl, ethoxyethyl and tetrahydropyranyl, carbamoyl, C_{1-10} alkoxy carbonyl such as t-butoxycarbonyl and methoxycarbonyl, and the like; and X is a coupling moiety selected from the group consisting of hydrogen, formyl, carboxyl, hydroxyl, amino, t-butoxycarbonylamino, chlorocarbonyl, N-alkoxycarbamoyl, haloacetyl, imidate, succinimidoloxycarbonyl, maleimide, isocyanate, isothiocyanate, tetrafluorophenoxy, chlorosulfonyl, C_{1-10} N-alkoxycarbamoyl, and the like.

35. A composition comprising somatostatin or a molecule having somatostatin receptor specificity conjugated with a N_2S_2 ligand having the general structure



wherein n is a whole number less than eleven; PG_2 and PG_3 may be the same or different sulfur protecting groups selected from the group consisting of C_{1-20} S-acyl, C_{1-20} alkyl, C_{1-10} alkoxyalkyl, carbamoyl and C_{1-10} alkoxycarbonyl and Y is a coupling moiety selected from the group consisting of carboxyl, amino, isocyanate, isothiocyanate, imidate, malaeimide, chlorocarbonyl, chlorosulfonyl, succinimidoloxycarbonyl, haloacetyl and C_{1-10} N-alkoxycarbamoyl.

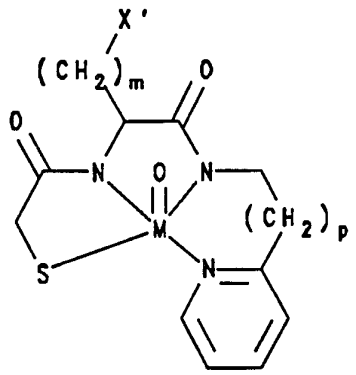
36. A composition comprising somatostatin or a molecule having somatostatin receptor specificity conjugated with a phenolic ligand having the general structure



wherein n is a whole number less than eleven; Y is a

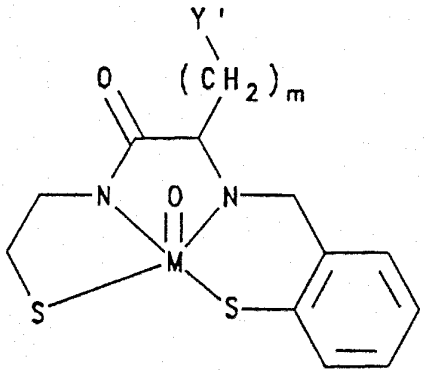
coupling moiety selected from the group consisting of carboxyl, amino, isocyanate, isothiocyanate, imidate, malaeimide, chlorocarbonyl, chlorosulfonyl, succinimidoloxycarbonyl, haloacetyl and C_{1-10} N-alkoxycarbamoyl; and R is hydrogen or a C_{1-10} alkyl.

37. A composition comprising somatostatin or a molecule having somatostatin receptor specificity conjugated with a metal complex having the general structure



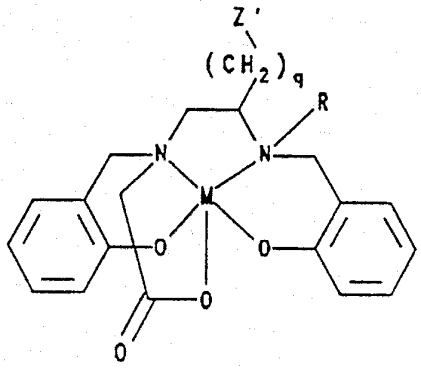
wherein m is a whole number less than eleven; p is either 0 or 1; X' is a coupling moiety selected from the group consisting of carboxyl, amino, isocyanate, isothiocyanate, imidate, malaeimide, chlorocarbonyl, chlorosulfonyl, succinimidoloxycarbonyl, haloacetyl and C_{1-10} N-alkoxycarbamoyl; and M is technetium, rhenium, indium, yttrium, gallium, samarium, holmium, copper or cobalt.

38. A composition comprising somatostatin or a molecule having somatostatin receptor specificity conjugated with a metal complex having the general structure



wherein Y' is a coupling moiety selected from the group consisting of carboxyl, amino, isocyanate, isothiocyanate, imidate, malaeimide, chlorocarbonyl, chlorosulfonyl, succinimidoloxycarbonyl, haloacetyl and C_{1-10} N-alkoxycarbamoyl; n is a whole number less than eleven; and M is technetium, rhenium, indium, yttrium, gallium, samarium, holmium, copper or cobalt.

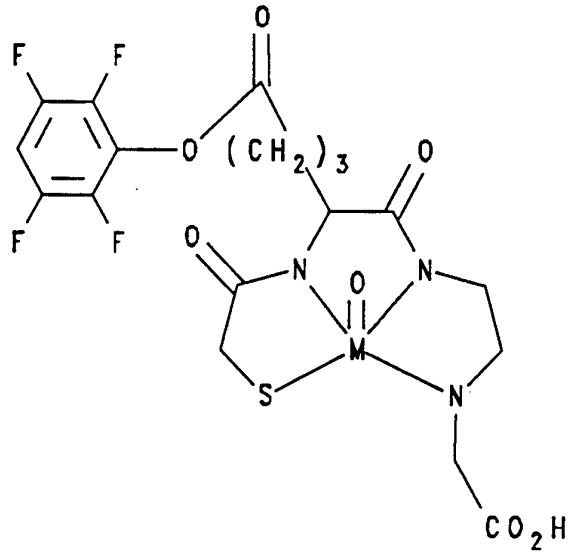
39. A composition comprising somatostatin or a molecule having somatostatin receptor specificity conjugated with a metal complex having the general structure



wherein q is a whole number less than eleven; wherein Z' is a coupling moiety selected from the group consisting of carboxyl, amino, isocyanate, isothiocyanate, imidate,

malaeimide, chlorocarbonyl, chlorosulfonyl, succinimidoloxycarbonyl, haloacetyl and C_{1-10} N-alkoxycarbamoyl; R is selected from the group consisting of hydrogen, and C_{1-10} alkyl; and M is technetium, rhenium, indium, yttrium, gallium, samarium, holmium, copper or cobalt.

40. A composition comprising somatostatin or a molecule having somatostatin receptor specificity conjugated with a metal complex having the general structure



wherein M is technetium, rhenium, indium, yttrium, gallium, samarium, holmium, copper or cobalt.

41. The composition of any one of claims 34 to 40 labelled in a ^{99m}Tc-pertechnetate solution containing a reducing agent, a buffering agent, and a transfer ligand such as sodium gluconate or tartarate.

42. The composition of any one of claims 34 to 40 labelled with ^{111}In-indium derivatives such as indium chloride, citrate or tartarate.

43. The composition of any one of claims 34 to 40 labelled in a 186/188 Re-perrheneate solution containing a reducing agent, a buffering agent, and a transfer ligand such as sodium gluconate or tartarate.

44. The composition of any one of claims 34 to 40 labelled with ^{90}Yt derivatives such as yttrium chloride, citrate or tartarate.

45. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 41 for diagnostic imaging of tumors.

46. A method of performing a therapeutic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 42 to destroy tumors.

47. The composition of any of claims 37 to 40, wherein M is ^{99m}technetium.

48. The composition of any of claims 37 to 40, wherein M is indium-111.

49. The composition of any of claims 37 to 40, wherein M is rhenium-186 or rhenium-188.

50. The composition of any of claims 37 to 40, wherein M is yttrium-90.

51. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 43 to image tumors.

52. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 44 to destroy tumor cells.

53. A diagnostic composition suitable for administration to a warm-blooded animal comprising a hirudin peptide labeled with Tc-99m by means of a triamide thiolate (N_3S) chelate capable of administration to an animal to produce reliable diagnostic imaging of thrombus.

54. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an imaging-effective amount of a hirudin peptide labeled with Tc-99m by means of a triamide thiolate (N_3S) chelate to allow for diagnostic imaging of thrombus.

55. A hirudin peptide labeled with Tc-99m by means of a triamide thiolate (N_3S) chelate.

56. The hirudin peptide of claims 53, 54, or 55 wherein said hirudin peptide labeled with Tc-99m by means of a triamide thiolate (N_3S) chelate is capable of administration to a warm-blooded animal to produce reliable diagnostic imaging of thrombus within two and one half hours.

57. A diagnostic composition suitable for administration to a warm-blooded animal comprising a hirudin peptide labeled with Iodine-123 to allow for diagnostic imaging of thrombus.

58. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an imaging-effective amount of a hirudin peptide labeled with Iodine-123 to allow for diagnostic imaging of thrombus.

59. A hirudin peptide labeled with Iodine-123.

60. The hirudin peptide of claims 57, 58, or 59 wherein said hirudin peptide labeled with Iodine-123 is capable of administration to a warm-blooded animal to produce reliable diagnostic imaging of thrombus within two and one half hours.

61. A diagnostic composition suitable for administration to a warm-blooded animal comprising a hirudin peptide labeled with Tc-99m by means of a triamide thiolate (N_3S) or a diamide dithiolate (N_2S_2) chelate capable of administration to an animal to produce reliable diagnostic imaging of thrombus.

62. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an imaging-effective amount of a hirudin peptide labeled with Tc-99m by means of a triamide thiolate (N_3S) or diamide dithiolate (N_2S_2) chelate to allow for diagnostic imaging of thrombus.

63. A hirudin peptide labeled with Tc-99m by means of a triamide thiolate (N_3S) or a diamide dithiolate (N_2S_2) chelate.

64. The hirudin peptide of claims 61, 62, or 63 wherein said hirudin peptide labeled with Tc-99m by means of a triamide thiolate (N_3S) or a diamide dithiolate (N_2S_2) chelate is capable of administration to a warm-blooded animal to produce reliable diagnostic imaging of thrombus within two and one half hours.

65. A diagnostic composition suitable for administration to a warm-blooded animal comprising a hirudin peptide labelled with a triamide thiolate (N_3S) or a diamide dithiolate (N_2S_2) chelate bound to a radioactive isotope of iodine capable of administration to an animal to allow for diagnostic imaging of thrombus.

66. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an imaging-effective amount of a hirudin peptide labeled with a triamide thiolate (N_3S) or a diamide dithiolate (N_2S_2) chelate bound to a radioactive isotope of iodine to allow for diagnostic imaging of thrombus.

67. A hirudin peptide labeled with a triamide thiolate (N_3S) or a diamide dithiolate (N_2S_2) chelate bound to a radioactive isotope of iodine.

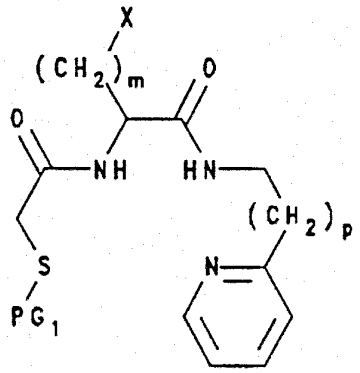
68. A diagnostic composition suitable for administration to a warm-blooded animal comprising a peptide with hirudin receptor specificity labeled with a radionuclide by means of a triamide thiolate (N_3S) or a diamide dithiolate (N_2S_2) chelate capable of administration to an animal to produce reliable diagnostic imaging of thrombus.

69. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an imaging-effective amount of a peptide with hirudin receptor specificity labeled with a radionuclide by means of a triamide thiolate (N_3S) or a diamide dithiolate (N_2S_2) chelate to allow for diagnostic imaging of thrombus.

70. A peptide with hirudin receptor specificity labeled with a radionuclide by means of a triamide thiolate (N_3S) or a radionuclide diamide dithiolate (N_2S_2) chelate.

71. The peptide with hirudin receptor specificity of claims 68, 69, or 70 wherein said peptide labeled with a radionuclide by means of a triamide thiolate (N_3S) or a diamide dithiolate (N_2S_2) chelate is capable of administration to a warm-blooded animal to produce reliable diagnostic imaging of thrombus within two and one half hours post-injection.

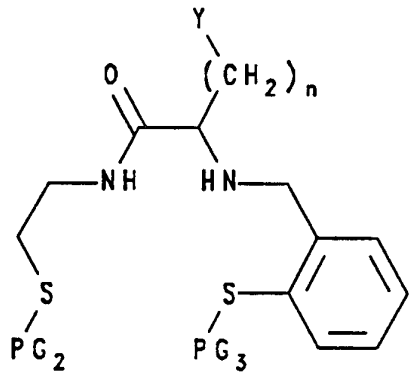
72. A composition comprising hirudin or a peptide which retains hirudin receptor specificity conjugated with a N_3S ligand having the general structure



wherein m is a whole number less than eleven; p is either 0 or 1; PG_1 is a sulfur protecting group selected from the group consisting of acetyl, methoxyacetyl, 1-3-dioxacyclohexyl, 1,3-dioxacyclopentyl, dialkoxyalkyl,

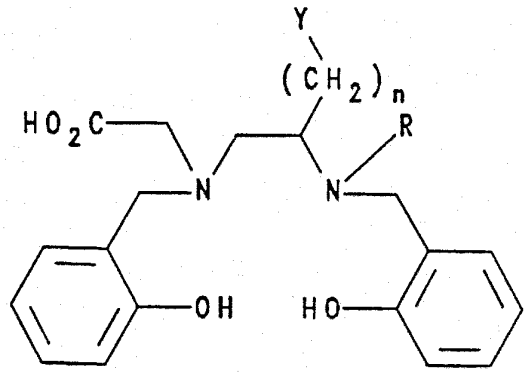
tetrahydrofuranyl, benzhydryl, C_{1-20} S-acyl such as alkanoyl, benzoyl and substituted benzoyl, C_{1-20} S-acyl groups such as benzyl, t-butyl, trityl, 4-methoxybenzyl and 2,4-dimethoxybenzyl, C_{1-10} alkoxyalkyl such as methoxymethyl, ethoxyethyl and tetrahydropyranyl, carbamoyl, C_{1-10} alkoxy carbonyl such as t-butoxycarbonyl and methoxycarbonyl, and the like; and X is a coupling moiety selected from the group consisting of hydrogen, formyl, carboxyl, hydroxyl, amino, t-butoxycarbonylamino, chlorocarbonyl, N-alkoxycarbamoyl, haloacetyl, imidate, succinimidoloxycarbonyl, maleimide, isocyanate, isothiocyanate, tetrafluorophenoxy, chlorosulfonyl, C_{1-10} N-alkoxycarbamoyl, and the like.

73. A composition comprising hirudin or a molecule having hirudin receptor specificity conjugated with a N_2S_2 ligand having the general structure



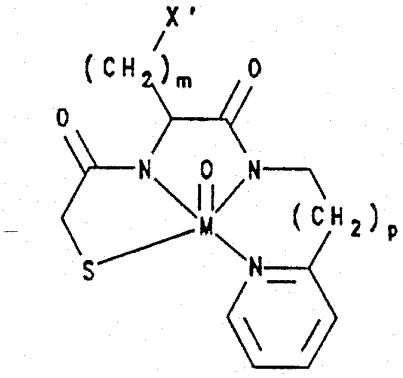
wherein n is a whole number less than eleven; PG_2 and PG_3 may be the same or different sulfur protecting groups selected from the group consisting of C_{1-20} S-acyl, C_{1-20} alkyl, C_{1-10} alkoxyalkyl, carbamoyl and C_{1-10} alkoxycarbonyl and Y is a coupling moiety selected from the group consisting of carboxyl, amino, isocyanate, isothiocyanate, imidate, malaeimide, chlorocarbonyl, chlorosulfonyl, succinimidoloxycarbonyl, haloacetyl and C_{1-10} N-alkoxycarbamoyl.

74. A composition comprising hirudin or a molecule having hirudin receptor specificity conjugated with a phenolic ligand having the general structure



wherein n is a whole number less than eleven; Y is a coupling moiety selected from the group consisting of carboxyl, amino, isocyanate, isothiocyanate, imidate, malaeimide, chlorocarbonyl, chlorosulfonyl, succinimidoloxycarbonyl, haloacetyl and C_{1-10} N-alkoxycarbamoyl; and R is hydrogen or a C_{1-10} alkyl.

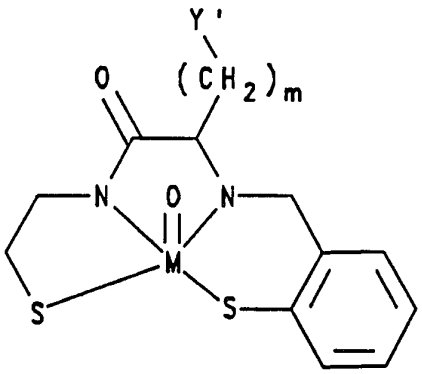
75. A composition comprising hirudin or a molecule having hirudin receptor specificity conjugated with a metal complex having the general structure



wherein m is a whole number less than eleven; p is either 0 or 1; X' is a coupling moiety selected from the group consisting of carboxyl, amino, isocyanate, isothiocyanate, imidate, malaeimide, chlorocarbonyl, chlorosulfonyl,

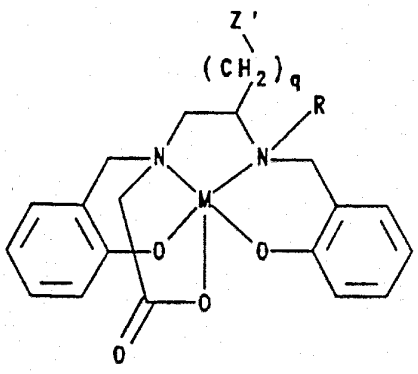
succinimidoloxycarbonyl, haloacetyl and C_{1-10} N-alkoxycarbamoyl; and M is technetium, rhenium, indium, yttrium, gallium, samarium, holmium, copper, iodine or cobalt.

76. A composition comprising hirudin or a molecule having hirudin receptor specificity conjugated with a metal complex having the general structure



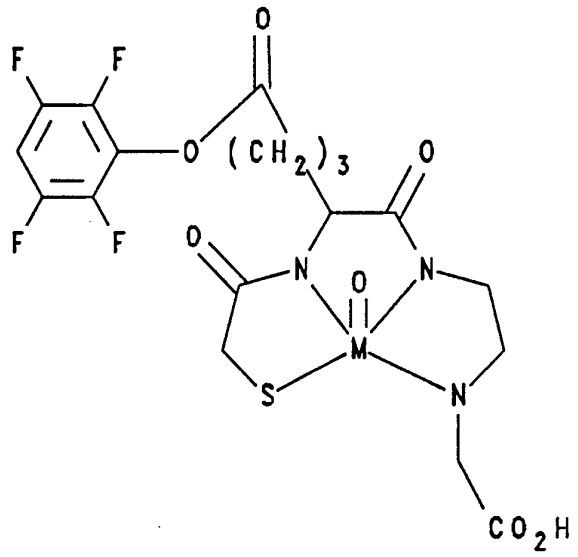
wherein Y' is a coupling moiety selected from the group consisting of carboxyl, amino, isocyanate, isothiocyanate, imidate, malaeimide, chlorocarbonyl, chlorosulfonyl, succinimidoloxycarbonyl, haloacetyl and C_{1-10} N-alkoxycarbamoyl; n is a whole number less than eleven; and M is technetium, rhenium, indium, yttrium, gallium, samarium, holmium, copper, iodine or cobalt.

77. A composition comprising hirudin or a molecule having hirudin receptor specificity conjugated with a metal complex having the general structure



wherein q is a whole number less than eleven; wherein Z' is a coupling moiety selected from the group consisting of carboxyl, amino, isocyanate, isothiocyanate, imidate, malaeimide, haloacetyl, chlorocarbonyl, chlorosulfonyl, succinimidoloxycarbonyl, and C_{1-10} N-alkoxycarbamoyl; R is selected from the group consisting of hydrogen, and C_{1-10} alkyl; and M is technetium, rhenium, indium, yttrium, gallium, samarium, holmium, copper, iodine or cobalt.

78. A composition comprising hirudin or a molecule having hirudin receptor specificity conjugated with a metal complex having the general structure



wherein M is technetium, rhenium, indium, yttrium, gallium, samarium, holmium, copper, iodine or cobalt.

79. The composition of any of claims 72 to 78 labelled in a ^{99m}Tc-pertechnetate solution containing a reducing agent, a buffering agent, and a transfer ligand such as sodium gluconate or tartarate.

80. The composition of any of claims 72 to 78 labelled with ^{111}In-indium derivatives such as indium chloride, citrate or tartarate.

81. The composition of any of claims 72 to 78 labelled in a 186/188 Re-perrheneate solution containing a reducing agent, a buffering agent, and a transfer ligand such as sodium gluconate or tartarate.

82. The composition of any of claims 72 to 78 labelled with ^{90}Yt derivatives such as yttrium chloride, citrate or tartarate.

83. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 79 for diagnostic imaging of thrombus.

84. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 80 to image thrombus.

85. The composition of any of claims 75 to 78, wherein M is ^{99m}technetium.

86. The composition of any of claims 75 to 78, wherein M is indium-111.

87. The composition of any of claims 75 to 78, wherein M is rhenium-186 or rhenium-188.

88. The composition of any of claims 75 to 78, wherein M is yttrium-90.

89. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 81 to image thrombus.

90. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an effective amount of the composition of claim 82 to destroy thrombi cells.

INTERNATIONAL SEARCH REPORT

PCT/US93/00939

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 49/02, 43/00 C07K 7/00, 5/08, 7/06
US CL :424/1.1
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. : 424/1.1 530/311,324,326

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 practicable, search terms used)

STN- file Reg structure search and name search- somatostatin", "hirudin"

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	EP,A, 0,333,356 (Biogen, Inc.) 20 September 1989 See page 5, page 9, lines 1-10, and page 21, lines 40-59.	57-60 53-90
Y	US,A, 5,037,630 (Fritzberg et al.) 06 August 1991 See columns 2,3, and 50.	5-11
Y	US,A, 4,837,003 (Nicolotti) 06 June 1989 See column 2, lines 25-60.	1-90
Y	US,A, 4,965,392 (Critzberg et al.) 23 October 1990 See figure 1; column 2, line 32 bridging column 4, line 18; column 7, lines 1-52 and column 18.	12-33,40 53-71,73 78 and 79-90

[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 18 JUNE 1993	Date of mailing of the international search report 27 JUL 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer JOHN M. COVERT Telephone No. (703) 308-0444

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/00939

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Inorganic Chemistry, Volume 29, No. 16., August 1990 (ACS, Easton, PA USA) Bryson et al., "Protecting Groups in the Preparation of Thiolate Complexes of Technetium", pp. 2948-2951, See especially page 2950.	1-11,34,37 72,75 and 79-90
A	EP,A, 0,421,366 (Merrell Dow Pharm.) 10 April 1991	
A	WO,A, 90/15818 (Antisoma Limited) 27 December 1990	
X Y	GB,A, 2,225,579 (Sandoz) 06 June 1990 See pages 11-15,19 and 20.	20-33 12-52
A	Journal of Nuclear Medicine, Volume 32, number 10, October 1991. Kwekkeboom et al., "Radioiodinated Somatostatin Analog Scintigraphy in Small-Cell Lung Cancer", pp. 1845-1848.	20-52
A	Journal of Nuclear Medicine, Volume 32, No. 6, June 1991. Larson, "Receptors on Tumors Studied with Radionuclide Scintigraphy," pages 1189-1191.	20-52
A	Seventh International Symposium on Radiopharmacology, (1991) Cox et al., "Technetium Labelled Somatostatin: A Potential Agent for In Vivo Tumor Localisation", page 16.	20-52
Y	Journal of Nuclear Medicine, Volume 32, No. 5, May 1991 Kwekkeboom et al., "[In-111-DTPA-D-Phe]- Octreotide Suntigraphy in Neuro- Endocrine Tumors", Abstract No. 305, page 981.	20-52
A,P	WO,A, 92/05154 (Mallinckrodt Medical) 02 April 1992 See pages 6 and 14.	1-11
A,P	WO,A, 92/21383 (Mallinckrodt Medical) 10 December 1992 See the Abstract.	
A	Tetrahedron Letters, Volume 30, No. 15 (1989) Misra et al., "Sythesis of a Novel Diaminodithiol Ligand for Labeling Proteins and Small Molecules with Technetium-99M", pages 1885-1888.	
A	US,A, 4,746,505 (Jones et al.) 24 May 1988 See columns 7 and 8.	
T	US,A, 5,187,264 (Verbruggen) 16 February 1993	
Y	US,A, 4,746,507 (Quag) 24 May 1988. See entire document	36.39.41-50,74,77,79-90

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/00939

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

This international 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:

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:
Please See Extra Sheet.

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 searched 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.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I. Claims 1-11,34,37,72 and 75 claims 35,38 and 40, and claims 41/34, 42/34, 43/34, 44/34, 45/34, 46/34, 47/37, 48/37, 49/37, 50/37, 51/34, 52/34, 79/72, 80/72, 81/72, 82/72, 83/72, 84/72, 89/72 and 90/72, drawn to compounds, radionuclide complexes and hirusin or somatostatin conjugates, all processing the special technical feature of a pyridine chelating ligand, classified in Class 544, subclass 1+, class 534, subclass 10+ and class 530, subclass 350+;

II. Claims 12-33, and claims 41/35, 42/35, 43/35, 44/35, 45/35, 46/35, 47/38, 48/38, 49/38, 50/38, 51/35, and 52/35, drawn to peptide/ligand conjugates peptide/ligand conjugates labeled with radionuclides diagnsotic and therapeutic compositions thereof and methods of in vivo therapy and in vivo diagnosis employing said compositions wherein each claim recites the special technical feature of a somatostain peptide conjugated to N2S2 ligands, classified in Class 530, subclass 350+ class 424, subclass 1.1.

III. Claims 36-39, and claims 41/36, 42/36, 43/36, 44/36, 45/36, 46/36, 47/39, 48/39, 49/39, 50/39, 51/36, and 52/36, drawn to peptide/ligand conjugates, peptide/ligand conjugates labeled with radionuclides diagnostic and therapeutic compositions thereof and methods of in vivo therapy and in vivo diagnosis employing said compositions wherein each claim recites the special technical feature of a somatostain peptide conjugated to a phenolic ligand, classified in Class 530, subclass 350+, class 424, subclass 1.1

IV. Claims 53-71, and claims 79/73, 80/73, 81/73, 82/73, 83/73, 84/73, 85/78, 86/78, 87/78, 88/78, 89/73 and 90/73 drawn to peptide/ligand conjugates peptide/ligand conjugates labeled with radionuclides diagnostic and therapeutic compositions thereof and methods of in vivo therapy and in vivo diagnosis employing said compositions wherein each claim recites the special technical feature of a hirudin peptide conjugated to a N2S2 ligand or an N3S ligand, classified in Class 530, subclass 350+, class 424, subclass 1.1.

V. Claims 74 and 77, and claims 79/74, 80/74, 81/74, 82/74, 83/74, 84/74, 85/77, 86/77, 87/77, 88/77, 89/74, and 90/74, drawn to peptide/ligand conjugates, peptide/ligand conjugates labeled with radionuclides diagnostic and therapeutic compositions thereof and methods of in vivo therapy and in vivo diagnosis employing said compositions wherein each claim recites the special technical feature of a hirudin peptide conjugated to a phenolic ligand, classified in Class 530, subclass 350+, class 424, subclass 1.1.

The inventions are distinct, each from the other because of the following reasons:

The Groups of claims are independent and distinct because each grouping employs a structurally different core ligand for labeling a particulary defined peptide, either hirudin or somatostain.

Group I defines a group of inventions which all employ a pyridine ligand which is distinct from the remaining ligands employed in the application.