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(71) Applicant (for all designated States except US): **SINGAPORE HEALTH SERVICES PTE. LTD.** [SG/SG]; 31 Third Hospital Avenue, #03-03 Bowyer Block C, Singapore 168753 (SG).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **YU, Wing Kwong Sidney** [SG/SG]; Department of Nuclear Medicine, Singapore General Hospital, Outram Road, Singapore 169608 (SG).

(74) Agent: **VIERING, JENTSCHURA & PARTNER LLP**; P.O. Box 1088, Rochor Post Office, Rochor Road, Singapore 911833 (SG).

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(54) Title: TUMOUR RADIOLABELLING IMAGING AGENTS COMPRISING ORNITHINE AND DERIVATIVES THERE-OF

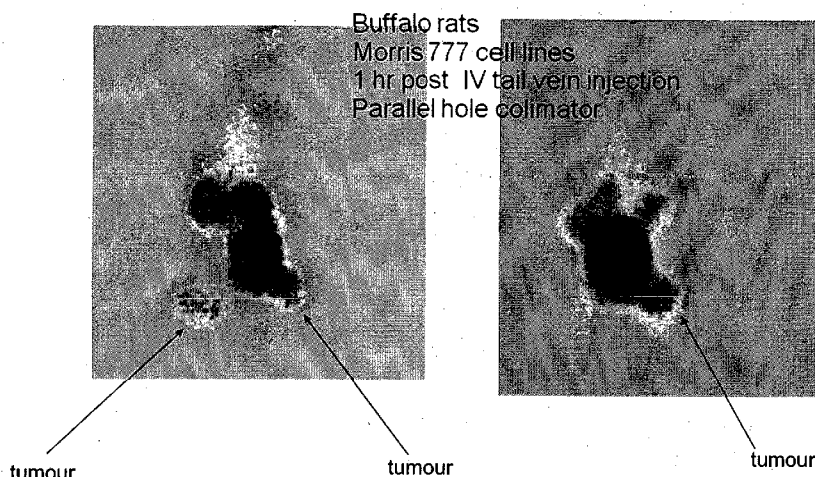


Figure 5

(57) Abstract: The present invention relates to a radiolabelling imaging agent comprising ornithine and derivatives thereof modified to incorporate a Technetium (^{99m}Tc) radiolabel. The radiolabelled imaging agents are substrates for Ornithine Decarboxylase (ODC) and can be used for imaging of cancers in which ODC expression is upregulated.

Tumour radiolabelling imaging agents comprising ornithine and derivatives thereof**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims the benefit of priority of US provisional application No. 61/326,676, filed April 22, 2010, the contents of it being hereby incorporated by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[0002] The present invention relates to novel radiolabeling imaging agents, methods of their production as well as methods of diagnosing cancer using these radiolabeling imaging agents.

BACKGROUND OF THE INVENTION

[0003] Cancer is a form of malignant disease that affects people of all ages and is the major cause of mortality in most developed and developing countries. For example, hepatocellular carcinoma (HCC) has been reported to be the fifth most prevalent malignant tumour worldwide and the third most prevalent in Singapore males. In addition, the mortality rate of HCC within 12 month of diagnosis has been reported to be up to 90% (Wai CT, Lee TM, Wang SC et al, "Liver transplantation for hepatocellular carcinoma in Singapore" *Singapore Med J* 2006; 47(7):584-587). The only cure for HCC is surgical resection. However, only 8% to 10% of patients are suitable for surgical resection because most of the HCC cases are diagnosed late, despite advances in diagnostic imaging modalities such as computerized tomography (CT) and magnetic resonance imaging (MRI). Therefore, early diagnosis is critical and the key to treatment of most cancers including HCC.

[0004] The role of nuclear medicine in oncology is particularly important in the diagnosis and management of malignant diseases. Nuclear Medicine Imaging (NMI) differs from other imaging modalities such as CT and MRI in that it detects biochemical changes rather than anatomical abnormalities. In most diseases, biochemical changes take place before anatomical abnormalities. Hence, Nuclear Medicine Imaging (NMI) can provide a diagnosis earlier than CT and MRI. In the field of nuclear medicine, certain pathological conditions are localized, or their extent is assessed, by detecting the distribution of small quantities of internally administered radioactively labeled compounds or otherwise known as radiopharmaceuticals.

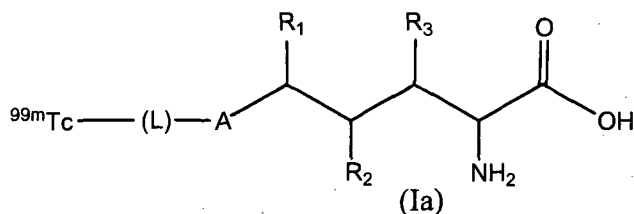
By tracing radiation emitted by the radiopharmaceuticals, it is therefore possible to locate the disease area.

[0005] A radioactive drug or radiopharmaceutical typically consists of a radioisotope attached to a ligand either directly or indirectly through a bi-functional chelate. The ligand is responsible for the bio-distribution of the drug, while the radioisotope enables the drug to be traced non-invasively. Technetium-99m (^{99m}Tc) is by far the most commonly used radioisotope in nuclear medicine because of its convenient half-life (6 hours), optimal energy (140 KeV) and wide availability. It has been used in over 20 million diagnostic medical procedures and approximately 85% of diagnostic imaging procedures in nuclear medicine use this radioisotope. The technetium radioisotope can be labeled to various ligands for imaging different organs of the body. For example, derivatives of aminoacetic acids can form negatively charged complexes with technetium for kidney and liver imaging. These aminoacetic acids include diethylenetriamine pentaacetic acid (DTPA), ethylenediaminetetraacetic acid (EDTA) and (N-(2,6-diethylacetanilido) iminodiacetic acid) (EHIDA). Another technetium complex for the imaging of renal excretion, is ^{99m}Tc -mercaptoacetyltriglycine (MAG3) complex. Other ligands that can be labeled to ^{99m}Tc include methylene diphosphonate (MDP) for use in bone imaging and methoxyisobutylisocyanide (MIBI) for use in heart imaging (Ulrich Abram et al, *Journal of the Brazilian Chemical Society*, 2006, vol. 17, pp. 1486-1500; Fritzberg, A. R. et al, *J. Nucl. Med.*, 1987, 28, 1180). Although various technetium radiopharmaceuticals exist for imaging almost all main organs and organ systems, there is still a need for new approaches and new labeling procedures.

[0006] This need is solved by the radiolabeling imaging agent and the respective compounds of the present invention including their diagnostic and pharmaceutical uses.

SUMMARY OF THE INVENTION

[0007] In a first aspect the invention provides a radiolabeling imaging agent of formula (I) consisting of technetium-99m (^{99m}Tc), a moiety of formula (Ia) covalently bound to a chelator (L)



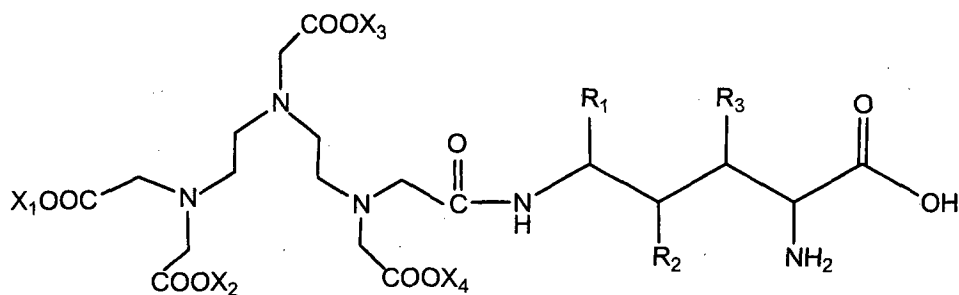
(I)

wherein

A is independently selected from the group consisting of an amino group, a mono-substituted amino group, a di-substituted amino group, and $-N=C(NH_2)_2$;

R_1 to R_3 are each independently selected from the group consisting of hydrogen, halogen, hydroxyl, oxo, optionally substituted C_1 - C_6 alkyl, optionally substituted C_2 - C_6 alkenyl, optionally substituted C_2 - C_6 alkynyl, optionally substituted C_1 - C_6 acyl, optionally substituted C_{3-8} cycloalkyl, and optionally substituted C_{3-8} cycloalkenyl.

[0008] In a second aspect the invention provides a compound of formula (II)



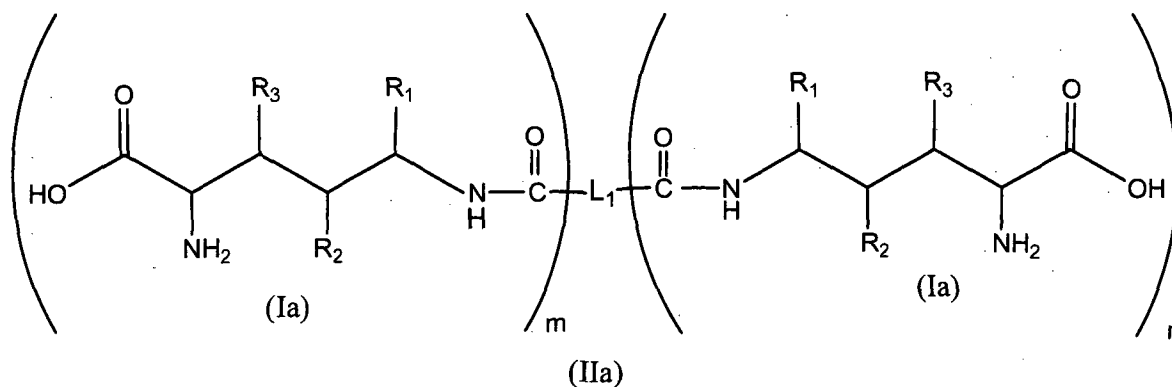
(II)

wherein

R_1 to R_3 are each independently selected from the group consisting of hydrogen, halogen, hydroxyl, oxo, optionally substituted C_1 - C_6 alkyl, optionally substituted C_2 - C_6 alkenyl, optionally substituted C_2 - C_6 alkynyl, optionally substituted C_1 - C_6 acyl, optionally substituted C_{3-8} cycloalkyl, and optionally substituted C_{3-8} cycloalkenyl;

X_1 to X_4 are each independently selected from the group consisting of hydrogen, optionally substituted C_1 - C_6 alkyl and optionally substituted C_2 - C_6 alkenyl.

[0009] In a third aspect the invention provides a compound of formula (IIa)



wherein

L_1 is a multidentate moiety comprising at least 2 reactive groups that react with the moiety of formula (Ia);

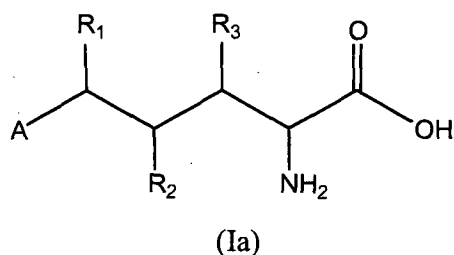
m is an integer 1 or 2; and

n is an integer 1 or 2.

[0010] In a fourth aspect the invention provides a method of identifying a tumour in a mammal. The method includes administering to the mammal the radiolabeling imaging agent as defined above; subjecting the mammal to nuclear medicine imaging to detect the radiation emitted by the radiolabeling imaging agent, wherein the presence of radioactivity indicates the presence of the tumour.

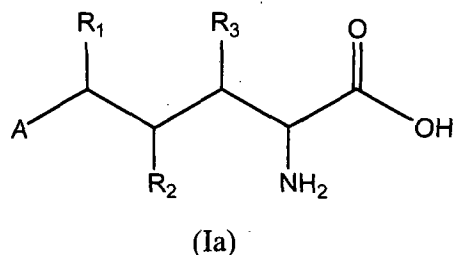
[0011] In a fifth aspect the invention provides a method of determining the likelihood of developing cancer in a non-cancerous cell. The method includes administering to the cell the radiolabeling imaging agent as defined above and measuring the radioactivity in the cell.

[0012] In a sixth aspect the invention provides a method of preparing the radiolabeling imaging agent as defined above. The method includes reacting the moiety of formula (Ia)

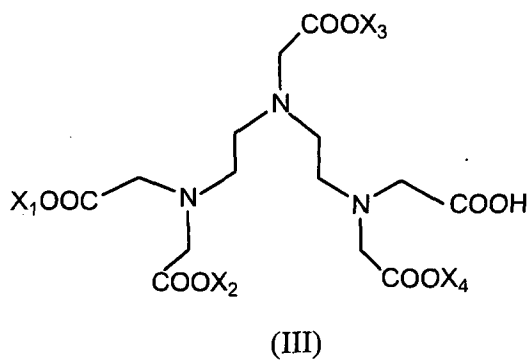


or an isomer thereof with a chelator (L). The method further includes adding a solution of $^{99m}\text{TcO}_4^-$ into the solution containing the moiety of formula (Ia) bound to the chelator (L) in the presence of a reducing agent, under conditions to form the radiolabeling imaging agent.

[0013] In a seventh aspect the invention provides a method of preparing the compound of formula (II) as defined above. The method includes reacting a compound of moiety (Ia)



with a compound of formula III



under conditions to form the compound of formula (II).

[0014] In an eighth aspect the invention provides a radiolabeling imaging agent as defined above for use in diagnosing cancer.

DETAILED DESCRIPTION OF THE INVENTION

[0015] The present invention is based on the surprising finding that a significant uptake of the radiolabeling imaging agent of the invention is achieved in cancer or tumour cells, as compared to known radiopharmaceuticals (Figure 4). Without wishing to be bound by any theory, the radiolabeling imaging agent according to the present invention that is taken up by cancer or tumour cells can react with the increased levels of ornithine decarboxylase (ODC) in cancer/tumour cells. Therefore, an increased radioactivity can be detected in tumour or cancer cells that have been administered with the radiolabeling imaging agent of the invention (Figure 2). Since ODC activity in normal cells is not increased, there will be minimum uptake of the radiolabeling imaging agent of the invention in normal cells. Thus, the inventors have

surprisingly found that the radiolabeling imaging agent according to the present invention is able to image the action of ODC on ornithine which is the rate limiting step in the polyamine pathway. Since the radiolabeling imaging agent of the present invention can specifically image the activity of ODC, the radiolabeling imaging agent can be used to detect cancer or tumour cells, thereby providing an earlier diagnosis of cancer.

[0016] The polyamine pathway has been discovered since the early 1970's and is one of the earliest cellular biochemical pathways leading to cell division (Figure 1). Ornithine is an amino acid in the polyamine pathway which is taken into the cell and converted by the enzyme ornithine decarboxylase (ODC) to putrescine. Putrescine is converted to spermidine, which is then converted to spermine. Spermine is a highly positively charged molecule which stimulates DNA replication and tumour growth (Thomas T, Thomas TJ, "Polyamines in cell growth and cell death: molecular mechanism and therapeutic applications" *CMLS* 2001; 58:244-258). Throughout the entire polyamine pathway, the step during which ornithine is converted to putrescine by ODC is the rate limiting step. It has been found that the ODC activity is increased in many cancers such as colon cancer and prostate cancer. Ornithine analogues such as difluoromethylornithine have been developed as drugs or chemotherapy agents in order to inhibit the action of ODC (Tamori KS, Nishiguchi S, Omura T, et al, "Relationship of polyamine metabolism to degree of malignancy of human hepatocellular carcinoma", *Oncol Rep* 1998; 5(6):1385-1388). There have been studies showing that ornithine uptake into HCC cells is increased according to increase in ODC (Tamori KS, Nishiguchi S, Omura T, et al; and Kubo S, Tamori A, Omura T, et al, "Ornithine decarboxylase activity in the non-cancerous hepatic tissue of patients with hepatocellular carcinoma" *Hepatogastroenterology* 2000; 47(33):920-823) and that ornithine analogue can be used to monitor cellular metabolic flux (Kramer D, Stanek J, Diegelman P, et al, "use of 4-fluoro-L-ornithine to monitor metabolic flux through the polyamine biosynthetic pathway" *Biochem Pharmacol* 1995; 50(9): 1433-1443).

[0017] It was also previously mentioned in Wan W et al, *Drug Development Research*, 2009, vol. 69, pp. 520-525 that ODC is the key enzyme in polyamine biosynthesis and represents a biochemical parameter for the assessment of tumour malignancy. However, it was noted by Wan et al that the clinical values of the measurements of ODC activity are restricted to quantitative biochemistry and are limited by cost and sampling errors caused by

tumour heterogeneity. Thus, Wan et al aims to develop agents suitable for imaging the polyamine metabolism, rather than imaging the action of ODC on ornithine. It should also be noted in this regard that the uptake of the polyamine analogs in tumour cells in Wan et al is lower than the uptake of the radiolabeling imaging agent of the present invention in tumour cells. As an illustrative context, the inventors found that the uptake of the radiolabeling imaging agent of the invention in liver cancer cells is 2.4% injected dose (ID)/mg of cell content at 1 hr post incubation (Figure 4), as compared to the uptake of ^{99m}Tc -DETA in Hep A tumour-bearing mice (0.65% injected dose/g at 4 hr post incubation).

[0018] The definitions of formulae (I), (Ia), (II) and (IIa) of the present invention are inclusive of all possible stereo-isomers of the respective compounds, including geometric isomers, e.g. Z and E isomers (*cis* and *trans* isomers), and optical isomers, e.g. diastereomers and enantiomers. Furthermore, the invention includes in its scope both the individual isomers and any mixtures thereof, e.g. racemic mixtures. In this context, the term "isomer" is meant to encompass all optical isomers of the compounds of the invention. It will be appreciated by those skilled in the art that the radiolabeling imaging agent and the compounds of the present invention contain at least one chiral center. Accordingly, the compounds of the invention may exist in optically active or racemic forms. It is to be understood that the compounds according to the present invention encompass any racemic or optically active form, or mixtures thereof. In one embodiment, the compounds of the invention can be pure (R)-isomers. In another embodiment, the compounds of the invention can be pure (S)-isomers. In another embodiment, the compounds of the invention can be a mixture of the (R) and the (S) isomers. In a further embodiment, the compounds of the invention can be a racemic mixture comprising an equal amount of the (R) and the (S) isomers. The individual isomers may be obtained using the corresponding isomeric forms of the starting material or they may be separated after the preparation of the end compound according to conventional separation methods. For the separation of optical isomers, e.g. enantiomers, from the mixture thereof, the conventional resolution methods for example fractional crystallization may be used.

[0019] In the radiolabeling imaging agent of the present invention, the moiety of formula (Ia) or the isomer thereof is bound to ^{99m}Tc via a chelator (L). The chelator (L) used in the present invention refers to any organic compound for example, a ligand that binds to a central metal atom such as ^{99m}Tc , to form a metal complex. The chelator (L) can for example, have

two, three, four or more donor atoms to form the respective two, three, four or more separate bonds with ^{99m}Tc . The bond(s) between ^{99m}Tc and the chelator (L) can include covalent bonds, ionic bonds, and/or coordinate covalent bonds. In some embodiments, the bond between the chelator (L) and ^{99m}Tc involves a donation of at least one pair of electrons from the donor atom of the chelator (L) to the central metal atom, ^{99m}Tc .

[0020] The term "ligand" as used herein refers to any molecular group that is associated with ^{99m}Tc . The ligand used herein can be a monodentate, bidentate (or didentate), a tridentate, a tetradentate, or a multidentate ligand. The terms "bidentate" (or didentate), "tridentate", "tetradentate", "multidentate" are used to indicate the number of potential binding sites of the ligand. For example, a carboxylic acid can be a bidentate or other multidentate ligand because it has at least two binding sites, the carboxyl oxygen and hydroxyl oxygen. In like manner, an amide has at least two binding sites, the carboxyl oxygen and the nitrogen atom. An amino sugar can have at least two binding sites and many amino sugars will have multiple binding sites including the amino nitrogen, a hydroxyl oxygen, an ethereal oxygen, an aldehyde carbonyl, and/or a ketonecarbonyl.

[0021] In some embodiments, the chelator (L) can be referred to as a bifunctional chelating agent having a i) chelating moiety capable of binding (chelating) ^{99m}Tc and ii) a moiety for example a multidentate moiety, which binds to the moiety of formula (Ia). In this context, the multidentate moiety can comprise one, two, three or more reactive groups that can react with the moiety of formula (Ia), thereby forming one, two, three or more covalent bonds with the moiety of formula (Ia). Examples of chelators (L) used in the present invention can include but are not limited to diethylenetriamine pentaacetic acid (DTPA) (CAS Number 67-43-6); ethylenediaminetetraacetic acid (EDTA) (CAS Number 60-00-4); (N-(2,6-diethylacetanilido) iminodiacetic acid) (EHIDA); triethylenetriaminehexaacetic acid (TTHA) (CAS Number 869-52-3); mercaptoacetyltriglycine (MAG3), dimercaptosuccinic acid (DMSA) (CAS Number 304-55-2); and 1,4,8,11-Tetraazacyclotetradecane (Cyclam) (CAS Number 295-37-4).

[0022] In some embodiments, the chelator (L) in the radiolabeling imaging agent of the invention binds covalently to the A substituent of the moiety of formula (Ia). In the moiety of formula (Ia), A is independently selected from the group consisting of an amino group, a mono-substituted amino group, a di-substituted amino group, and $-\text{N}=\text{C}(\text{NH}_2)_2$. Exemplary

mono-substituted amino groups can include but are not limited to a N-(C₁-C₆alkyl)amino group such as monoethylamino or monomethylamino; or an acetylamino group; or a tosylamino group. Exemplary di-substituted amino groups can include but are not limited to a N,N-(C₁-C₆alkyl)amino group such as diethylamino or dimethylamino; or a N,N-diacetylamino group; or a morpholino group, or a piperidino group, or an N-methylpiperazino group.

[0023] In formulae (I), (II) and (IIa), R₁ to R₃ are each independently selected from the group consisting of hydrogen, halogen, hydroxyl, oxo, optionally substituted C₁-C₆ alkyl, optionally substituted C₂-C₆ alkenyl, optionally substituted C₂-C₆ alkynyl, optionally substituted C₁-C₆ acyl, optionally substituted C₃₋₈ cycloalkyl, and optionally substituted C₃₋₈ cycloalkenyl.

[0024] In this context, the term “alkyl”, alone or in combination, refers to a fully saturated aliphatic hydrocarbon such as a straight or branched chain hydrocarbon group. The alkyl can for example be optionally substituted. In certain embodiments, an alkyl can comprise 1 to 6 carbon atoms, 1 to 5 carbon atoms, 1 to 4 carbon atoms, or 1 to 3 carbon atoms, wherein (whenever it appears herein in any of the definitions given below) a numerical range, such as “1 to 6” or “C₁-C₆”, refers to each integer in the given range, e.g. “C₁-C₆ alkyl” means that an alkyl group comprising only 1 carbon atom, 2 carbon atoms, 3 carbon atoms, etc., up to and including 6 carbon atoms. Examples of alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, tert-amyl, pentyl, hexyl, and the like.

[0025] The term “alkenyl” as used herein refers to an alkyl group that contains in the straight or branched hydrocarbon chain one or more double bonds. In certain embodiments, an alkenyl comprises 2 to 6 carbon atoms, for example 2 to 5 carbon atoms or 2 to 4 carbon atoms, wherein a numerical range, such as “2 to 6” or “C₂-C₆”, refers to each integer in the given range, e.g. “C₂-C₆ alkenyl” means that an alkenyl group comprising 2 carbon atoms, 3 carbon atoms, etc., up to and including 6 carbon atoms. An alkenyl used in this invention can for example be optionally substituted. Examples of alkenyl groups include, but are not limited to, ethenyl, propenyl, butenyl, 1,4-butadienyl, pentenyl, 4-methylhex-1-enyl, 4-ethyl-2-methylhex-1-enyl and the like.

[0026] The term “alkynyl” as used herein refers to an alkyl group that contains in the straight or branched hydrocarbon chain one or more triple bonds. In certain embodiments, an alkynyl comprises 2 to 6 carbon atoms, for example 2 to 6 carbon atoms, 2 to 5 carbon atoms, or 2 to 4 carbon atoms, wherein a numerical range, such as “2 to 6” or “C₂-C₆”, refers to each integer in the given range, e.g. “C₂-C₆ alkynyl” means that an alkynyl group comprising 2 carbon atoms, 3 carbon atoms, etc., up to and including 6 carbon atoms. An alkynyl group of this invention may be optionally substituted. Examples of alkyne groups include, but are not limited to, ethynyl, propynyl, butynyl, and the like.

[0027] The term “acyl” as used herein refers to the a group having the formula -RC(=O), an acyl group used in the present invention can for example be optionally substituted. In certain embodiments, an acyl group comprises 1 to 6 carbon atoms, for example 1 to 5 carbon atoms, 1 to 4 carbon atoms, or 1 to 3 carbon atoms, wherein a numerical range, such as “1 to 20” or “C₁-C₂₀”, refers to each integer in the given range, e.g. “C₁-C₆ acyl” means that an acyl group comprising 2 carbon atoms, 3 carbon atoms, etc., up to and including 6 carbon atoms. Examples of acyl groups include, but are not limited to formyl, acetyl, propanoyl, 2-methylpropanoyl, butanoyl and the like.

[0028] As used herein, the term “cycloalkyl” refers to a completely saturated hydrocarbon ring. The cycloalkyl group used in this invention may range from C₃ to C₈. A cycloalkyl group of this invention can for example be optionally substituted. Examples of cycloalkyl groups include, but are not limited to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and the like.

[0029] The term “cycloalkenyl” as used herein refers to a cycloalkyl group that contains one or more double bonds in the ring although, if there is more than one, they cannot form a fully delocalized pi-electron system in the ring (otherwise the group would be “aryl” as defined herein). Cycloalkyl groups of this invention may range from C₃ to C₈. A cycloalkenyl group use in this invention may for example be optionally substituted. Examples of cycloalkenyl groups include, but are not limited to cyclohexenyl, cyclohepta-1,3-dienyl, cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclohexenyl, cycloheptenyl and the like.

[0030] The term “optionally substituted” refers to a group in which none, one, or more than one of the hydrogen atoms has been replaced with one or more group(s) are independently selected from: alkyl, heteroalkyl, haloalkyl, heterohaloalkyl, cycloalkyl, aryl, arylalkyl, heteroaryl, non-aromatic heterocycle, hydroxy, alkoxy, aryloxy, mercapto,

alkylthio, arylthio, cyano, halo, carbonyl, thiocarbonyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, S-sulfonamido, N-sulfonamido, C-carboxy, O-carboxy, isocyanato, thiocyanato, isothiocyanato, nitro, silyl, trihalomethanesulfonyl, and amino, including mono- and di-substituted amino groups.

[0031] The term "aryl" refers to an aromatic ring wherein each of the atoms forming the ring is a carbon atom. Aryl rings may be formed by five, six, seven, eight, nine, or more than nine carbon atoms. Aryl groups may be optionally substituted.

[0032] The term "aromatic" refers to a group comprising a covalently closed planar ring having a delocalized [pi]-electron system comprising $4n+2$ [pi] electrons, where n is an integer. Aromatic rings may be formed by five, six, seven, eight, nine, or more than nine atoms. Aromatics may be optionally substituted. Examples of aromatic groups include, but are not limited to phenyl, naphthalenyl, phenanthrenyl, anthracenyl, tetralinyl, fluorenyl, indenyl, and indanyl. The term aromatic includes, for example, benzenoid groups, connected via one of the ring-forming carbon, atoms, and optionally carrying one or more substituents selected from an aryl, a heteroaryl, a cycloalkyl, a non-aromatic heterocycle, a halo, a hydroxy, an amino, a cyano, a nitro, an alkylamido, an acyl, a C_1 - C_6 alkoxy, a C_1 - C_6 alkyl, a C_1 - C_6 aminoalkyl, alkylamino, an alkylsulfenyl, an alkylsulfinyl, an alkylsulfonyl, an sulfamoyl, or a trifluoromethyl. In certain embodiments, an aromatic group is substituted at one or more of the para, meta, and/or ortho positions. Examples of aromatic groups comprising substitutions include, but are not limited to, phenyl, 3-halophenyl, 4-halophenyl, 3-hydroxyphenyl, 4-hydroxyphenyl, 3-aminophenyl, 4-aminophenyl, 3-methylphenyl, 4-methylphenyl, 3-methoxyphenyl, 4-methoxyphenyl, 4-trifluoromethoxyphenyl, 3-cyanophenyl, 4-cyanophenyl, dimethylphenyl, naphthyl, hydroxynaphthyl, hydroxymethylphenyl, (trifluoromethyl)phenyl, alkoxyphenyl, 4-morpholin-4-ylphenyl, 4-pyrrolidin-1-ylphenyl, 4-pyrazolylphenyl, 4-triazolylphenyl, and 4-(2-oxopyrrolidin-1-yl)phenyl.

[0033] The term "alkoxy", alone or in combination, refers to an aliphatic hydrocarbon having an alkyl-O-moiety. In certain embodiments, alkoxy groups are optionally substituted. Examples of alkoxy groups include, but are not limited to, methoxy, ethoxy, propoxy, butoxy and the like.

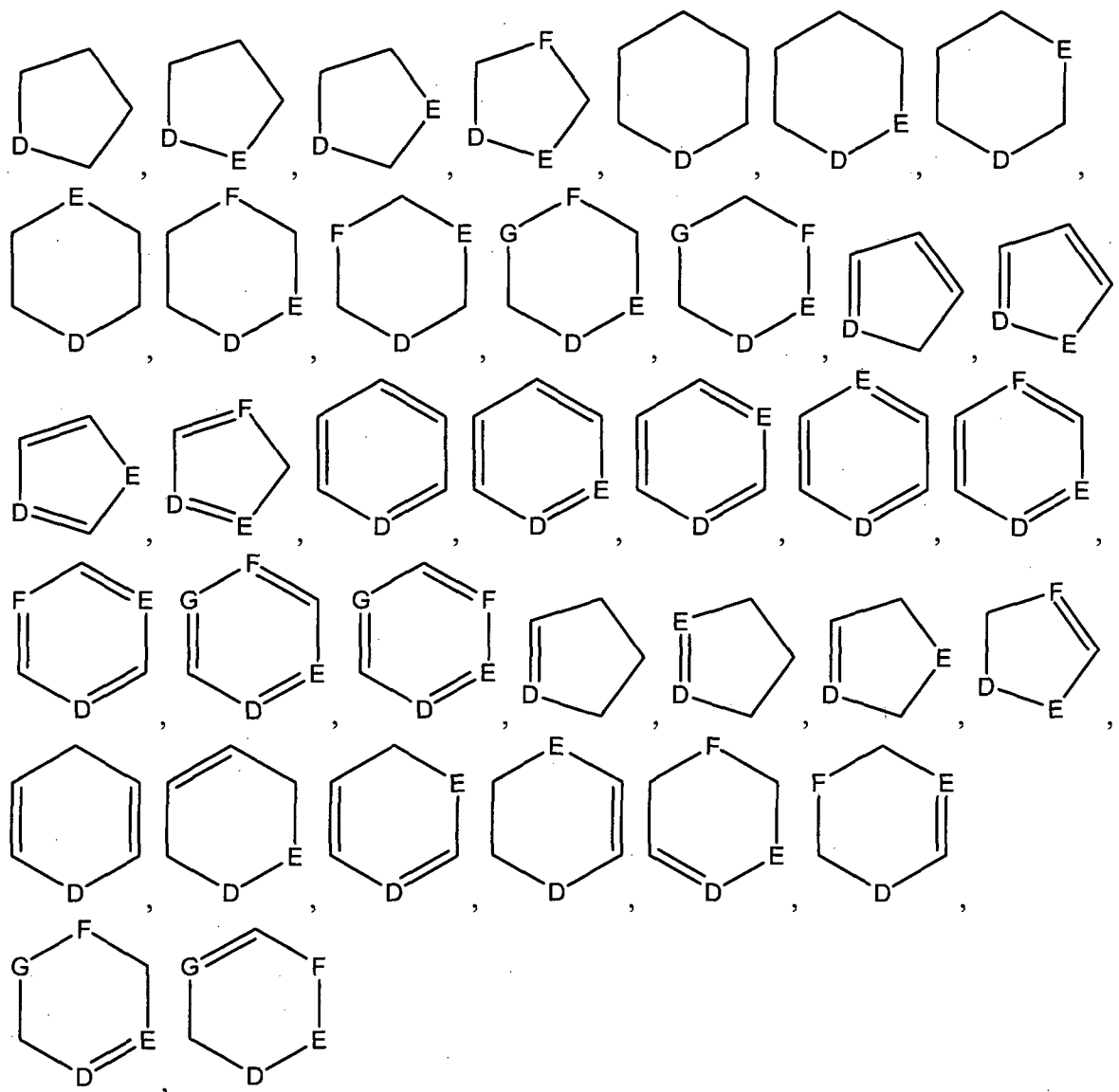
[0034] The term "arylalkyl" refers to a group comprising an aryl group bound to an alkyl group.

[0035] The term “heteroaryl” refers to an aromatic heterocycle. Heteroaryl rings may be formed by three, four, five, six, seven, eight, nine, or more than nine atoms. Heteroaryls may be optionally substituted. Examples of heteroaryl groups include, but are not limited to, aromatic C₃₋₈ heterocyclic groups comprising one oxygen or sulfur atom or up to four nitrogen atoms, or a combination of one oxygen or sulfur atom and up to two nitrogen atoms, and their substituted as well as benzo- and pyrido-fused derivatives, for example, connected via one of the ring-forming carbon atoms.

[0036] The term “non-aromatic heterocycle” refers to a group comprising a non- aromatic ring wherein one or more atoms forming the ring is a heteroatom. Non-aromatic heterocyclic rings may be formed by three, four, five, six, seven, eight, nine, or more than nine atoms. Non-aromatic heterocycles may be optionally substituted. In certain embodiments, non-aromatic heterocycles comprise one or more carbonyl or thiocarbonyl groups such as, for example, oxo- and thio-containing groups. Examples of non-aromatic heterocycles include, but are not limited to, lactams, lactones, cyclic imides, cyclic thioimides, cyclic carbamates, tetrahydrothiopyran, 4H-pyran, tetrahydropyran, 1,3-dioxane, 1,4-dioxin, 1,4-dioxane, piperazine, 1,3-oxathiane, 1,4-oxathiane, tetrahydro-1,4-thiazine, 2H- 1,2- oxazine, maleimide, succinimide, barbituric acid, thiobarbituric acid, dioxopiperazine, hydantoin, dihydrouacil, morphinone, trioxane, hexahydro-1,3,5-triazine, tetrahydrothiophene, tetrahydrofuran, pyrroline, pyrrolidine, pyridone, pyrroldione, pyrazone, pyrazolidine, imidazoline, imidazolidine, 1,3-dioxole, 1,3-dioxolane, 1,3-dithiole, 1,3-dithiolane, isoxazoline, isoxazolidine, oxazoline, oxazolidine, oxazolidone, thiazoline, thiazolidine, and 1,3-oxathiolane.

[0037] The term “heterocycle” refers to a group comprising a covalently closed ring wherein at least one atom forming the ring is a carbon atom and at least one atom forming the ring is a heteroatom. Heterocyclic rings may be formed by three, four, five, six, seven, eight, nine, or more than nine atoms. Any number of those atoms may be heteroatoms (i.e., a heterocyclic ring may comprise one, two, three, four, five, six, seven, eight, nine, or more than nine heteroatoms). Herein, whenever the number of carbon atoms in a heterocycle is indicated (e.g., C₁-C₆ heterocycle), at least one other atom (the heteroatom) must be present in the ring. Designations such as “C₁-C₆ heterocycle” refer only to the number of carbon atoms in the ring and do not refer to the total number of atoms in the ring. It is understood that the

heterocyclic ring will have additional heteroatoms in the ring. In heterocycles comprising two or more heteroatoms, those two or more heteroatoms may be the same or different from one another. Heterocycles may be optionally substituted. Binding to a heterocycle can be at a heteroatom or via a carbon atom. Examples of heterocycles include, but are not limited to the following:



wherein D, E, F, and G independently represent a heteroatom. Each of D, E, F, and G may be the same or different from one another.

[0038] The term "heteroatom" refers to an atom other than carbon or hydrogen. Heteroatoms are typically independently selected from oxygen, sulphur, nitrogen, and phosphorus, but are not limited to those atoms. In embodiments in which two or more heteroatoms are present, the two or more heteroatoms may all be the same as one another, or some or all of the two or more heteroatoms may each be different from the others.

[0039] The term "ring" as described herein refers to any covalently closed structure. Rings may include, for example, heterocycles (e.g. heteroaryls and non-aromatic heterocycles), aromatics (e.g. aryls and heteroaryls), and non-aromatics (e.g. non-aromatic heterocycles). Rings may be optionally substituted or rings may be fused to at least one ring to form part of a ring system. The term "ring system" refers to two or more rings, wherein two or more of the rings are fused. The term "fused" refers to structures which two or more rings share one or more bonds.

[0040] The term "O-carboxy" refers to a group of formula $\text{RC}(=\text{O})\text{O}$.

[0041] The term "C-carboxy" refers to a group of formula $-\text{C}(=\text{O})\text{OR}$.

[0042] The term "acetyl" refers to a group of formula $-\text{C}(=\text{O})\text{CH}_3$.

[0043] The term "trihalomethanesulfonyl" refers to a group of formula $\text{X}_3\text{CS}(=\text{O})_2$ - where X is a halogen.

[0044] The term "cyano" refers to a group of formula $-\text{CN}$.

[0045] The term "isocyanato" refers to a group of formula $-\text{NCO}$.

[0046] The term "thiocyanato" refers to a group of formula $-\text{CNS}$.

[0047] The term "isothiocyanato" refers to a group of formula $-\text{NCS}$.

[0048] The term "S-sulfonamido" refers to a group of formula $-\text{S}(=\text{O})_2\text{NR}$.

[0049] The term "N-sulfonamido" refers to a group of formula $\text{RS}(=\text{O})_2\text{NH}-$.

[0050] The term "O-carbamyl" refers to a group of formula $-\text{OC}(=\text{O})-\text{NR}$.

[0051] The term "N-carbamyl" refers to a group of formula $\text{ROC}(=\text{O})\text{NH}-$.

[0052] The term "O-thiocarbamyl" refers to a group of formula $-\text{OC}(=\text{S})-\text{NR}$.

[0053] The term "N-thiocarbamyl" refers to a group of formula $\text{ROC}(=\text{S})\text{NH}-$.

[0054] The term "C-amido" refers to a group of formula $-\text{C}(=\text{O})-\text{NR}_2$.

[0055] The term "N-amido" refers to a group of formula $\text{RC}(=\text{O})\text{NH}-$.

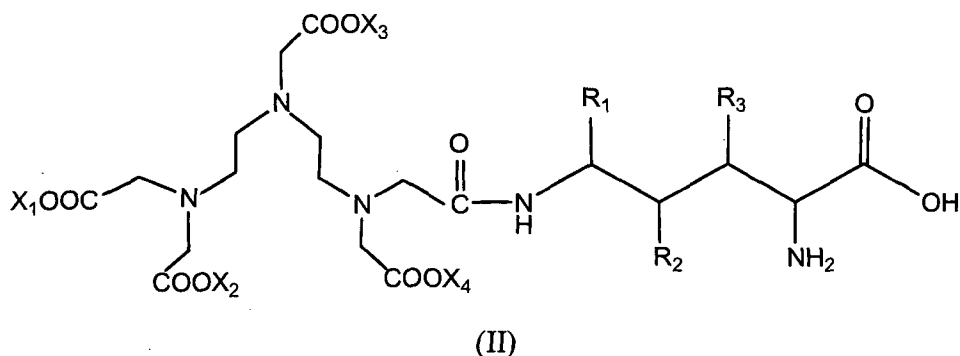
[0056] The substituent "R" appearing by itself and without a number designation refers to a substituent selected from alkyl, cycloalkyl, aryl, heteroaryl (bonded through a ring carbon) and non-aromatic heterocycle (bonded through a ring carbon).

[0057] The terms "amine," "hydroxy," and "carboxyl" include such groups that have been esterified or amidified. Procedures and specific groups used to achieve esterification and amidification are known to those of skill in the art.

[0058] The halogen used in the definitions of formulae (Ia), (II) and (IIa) can for example include fluorine, chlorine, iodine or bromine. In some embodiments, the R₂ substituent of the moiety of formula (Ia) of the present invention is fluorine.

[0059] In some embodiments, the moiety of formula (Ia) can be any derivatives of ornithine and can include, but are not limited to ornithine (CAS Number 128551-39-3); (DL)-ornithine, (L)-ornithine, (S)-ornithine, arginine (CAS number 74-79-3), (L)-arginine, 4-fluoro-L-ornithine (Kramer D et al, *Biochem Pharmacol* 1995; 50(9): 1433-1443); glutamic acid, (L)-glutamic acid or (D)-glutamic acid.

[0060] The present invention also provides a compound of formula (II)

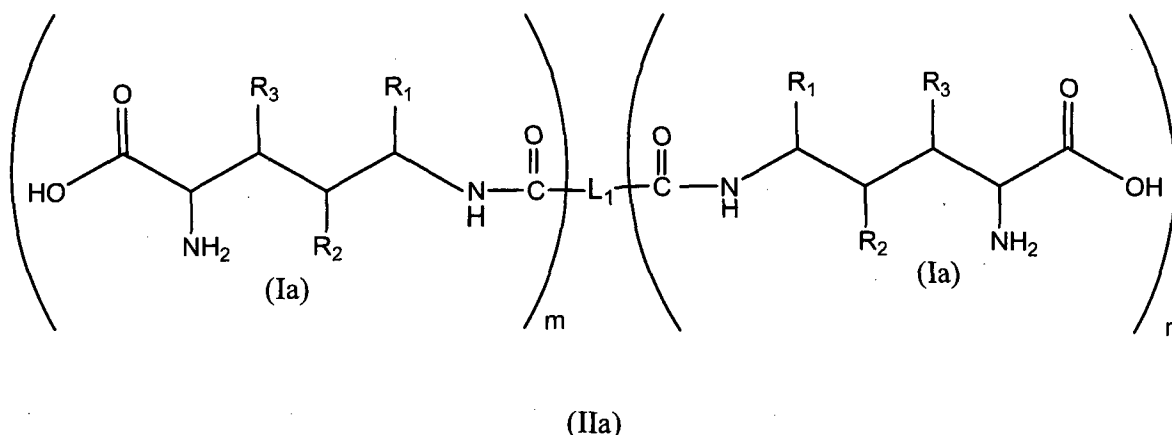


[0061] In the compound of formula (II), R₁ to R₃ are each independently selected from the group consisting of hydrogen, halogen, hydroxyl, oxo, optionally substituted C₁-C₆ alkyl, optionally substituted C₂-C₆ alkenyl, optionally substituted C₂-C₆ alkynyl, optionally substituted C₁-C₆ acyl, optionally substituted C₃₋₈ cycloalkyl, and optionally substituted C₃₋₈ cycloalkenyl. X₁ to X₄ are each independently selected from the group consisting of hydrogen, optionally substituted C₁-C₆ alkyl and optionally substituted C₂-C₆ alkenyl.

[0062] In some embodiments, the compound of formula (II) can further include ^{99m}Tc bound thereto, thereby forming the radiolabeling imaging agent of the present invention. The compound of formula (II) can for example, be covalently bound to ^{99m}Tc. In particular, the

compound of formula (II) can bind to ^{99m}Tc via the DTPA derivative moiety of the compound of formula (II). The DTPA derivative moiety of the compound of formula (II) can for example, form a chelating complex with ^{99m}Tc as described herein. In this context, the compound of formula (II) can react with a solution of $^{99m}\text{TcO}_4^-$ in the presence of a reducing agent such as stannous chloride solution (SnCl_2) or formamidine sulfinic acid (FSA) thereby forming a complex with ^{99m}Tc . Methods of complexing ^{99m}Tc with the DTPA derivative moiety of the compound of formula (II) are within the knowledge of a person of average skill in the art. Such complexing methods can for example, be described in Fritzberg A. R. et al, *J. Nuc. Med.*, 1977, vol. 18(6), pp. 553-557. In this context, the inventors found that the radiolabeling agent of the invention prepared according to the invention is stable for up to 6 hours after preparation (Example 3, Table 1). In addition, the radiochemical purity of the radiolabeling imaging agent of the invention is 97% at 6 hours after its preparation. In this context, methods for determining the radiochemical purity of the radiolabeling imaging agent of the invention can include high-performance liquid chromatograph (HPLC) or thin-layer chromatograph (TLC) such as instant thin-layer silica gel strips (ITLC-SG) as described in Chen F. et al, *Eur. J. Nuc. Med.*, 1993, vol. 20(3), pp. 334-338.

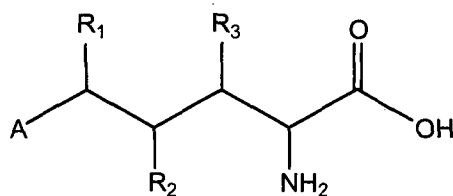
[0063] The present invention further provides a compound of formula (IIa)



[0064] In the compound of formula (IIa), the substituents R_1 to R_3 are each defined as above, m is an integer of 1 or 2, and n is an integer of 1 or 2. The multidentate moiety L_1 can be a chelator (L) as described above and can for example comprise at least two or three, or four or more reactive groups that react with the moiety of formula (Ia). In this context, the reactive group of the multidentate moiety L_1 can for example, be an acyl group or a carboxyl group. In some embodiments, the reactive group reacts with the amino group ($-\text{NH}_2$) of the

moiety of formula (Ia), thereby forming a covalent bond with the moiety of formula (Ia). Examples of multidentate moiety can include but are not limited to diethylenetriamine pentaacetic acid (DTPA) (CAS Number 67-43-6); ethylenediaminetetraacetic acid (EDTA) (CAS Number 60-00-4); (N-(2,6-diethylacetanilido) iminodiacetic acid) (EHIDA); triethylenetriaminehexaacetic acid (TTHA) (CAS Number 869-52-3); mercaptoacetyltriglycine (MAG3), dimercaptosuccinic acid (DMSA) (CAS Number 304-55-2); and 1,4,8,11-Tetraazacyclotetradecane (Cyclam) (CAS Number 295-37-4).

[0065] In some embodiments, the compound of formula (IIa) can further include ^{99m}Tc bound thereto, thereby forming the radiolabeling imaging agent of the present invention. When preparing the radiolabeling imaging agent of the invention, the moiety of formula (Ia) can undergo a modification process for example, by reacting the moiety of formula (Ia)



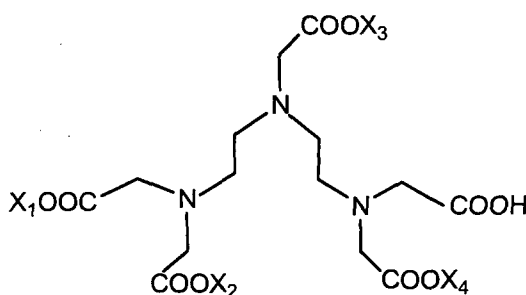
(Ia)

with any of the chelator (L) described herein; and adding of a solution of $^{99m}\text{TcO}_4^-$ into the solution containing the moiety of formula (Ia) bound to the chelator (L) in the presence of a reducing agent, for example, stannous chloride solution (SnCl_2) or formamidine sulfinic acid (FSA). Methods of complexing ^{99m}Tc with the moiety of formula (Ia) are within the knowledge of a person of average skill in the art, and also in line with the methods for complexing ^{99m}Tc with the compound of formula (II) as described above. When required, ^{99m}Tc can form an intermediate complex with a ligand such as glucoheptonate, prior to complexing ^{99m}Tc with the compounds of present invention.

[0066] In some embodiments, the moiety of formula (Ia) can be protected with at least one protecting group, such as an amine protecting group. Examples of such amine protecting groups can include but are not limited to di-tert-butyl dicarbonate (boc) or 9-fluorenylmethyloxycarbonyl (fmoc). It is to be understood that where the moiety of formula (Ia) contains a boc or fmoc functionality, boc groups may be replaced by fmoc groups and vice versa. In addition, the boc or fmoc groups may also be removed in their entirety, such as

to leave a free amine group. As an illustrative example, N-Boc-N'-Fmoc-L-ornithine (CAS Number 150828-96-9) or N-boc-L-ornithine (CAS Number 21887-64-9) can be used to react with the chelator (L).

[0067] Generally, about 2 moles of the moiety of formula (Ia) are used for each mole of the chelator (L). In this context, the chelator (L) can for example have an acyl group that reacts with the moiety of formula (Ia) by replacing a hydrogen atom of the A substituent with the acyl function. Therefore, in some embodiments, the method of preparing a compound of formula (II) includes reacting a moiety of formula (Ia) with a chelator (L), for example, a compound of formula (III)



(III)

under conditions to form the compound of formula (II). In this context, the compound of formula (III) may be in an anhydride form prior to reacting with the moiety of formula (Ia). In this context, the inventors have found that the compound of formula (II) prepared according to the invention gave a yield of 35% (Example 1).

[0068] The present invention also provides a method of identifying a tumour in a mammal. The method includes administering to the mammal the radiolabeling imaging agent as described herein. The method further includes subjecting the mammal to nuclear medicine imaging to detect the radiation emitted by the radiolabeling imaging agent, wherein the presence of radioactivity indicates the presence of the tumour.

[0069] Any mammal may be used in the present method of the invention. Examples of mammals include, but are not limited to, a rat, a cow, a goat, a sheep, a pig, a dog, a mouflon, a guinea pig, a hamster, a chimpanzee, a rhesus monkey and a human.

[0070] Any tumour or cancer may be used in the invention including for example, a benign tumour and a metastatic malignant tumour. Examples of tumours include, but are not limited to, haematological malignancies and solid tumours. Solid tumours include for instance

a sarcoma, arising from connective or supporting tissues, a carcinoma, arising from the body's glandular cells and epithelial cells or a lymphoma, a cancer of lymphatic tissue, such as the lymph nodes, spleen, and thymus. Examples of a solid tumour include, but are not limited to, breast tumour, lung tumour, brain tumour, a neuroblastoma, colon tumour, stomach tumour, rectal tumour, bladder cancer, a liver tumour, a pancreatic tumour, ovarian tumour, prostate tumour and a melanoma. In some embodiments, the tumour may be derived from a cancer including cancers of the organs mentioned above. In other embodiments, the tumour may be derived from a virus, for example, a cancer-causing or carcinogenic virus. Examples of such viruses can include but are not limited to Hepatitis B virus (HBV), Hepatitis C virus (HCV), human papilloma virus (HPV) including HPV strains 16 and 18. In further embodiments, the tumour may be derived from exposure to carcinogens including industrial chemicals such as dyes, naphthalene, lead, cobalt sulphate, diazoaminobenzene, and the like.

[0071] The radiolabeling imaging agent of the present invention that is administered to the mammal can be used to visualize specific sites, for example the liver, lung, or stomach in the mammalian body, in order to detect the presence of a tumour in the respective site. Therefore, in some embodiments, the radiolabeling imaging agent is used in diagnosing cancer. The radiolabeling imaging agent of the invention can be administered in any mode as long as the radiolabeling imaging agent is delivered to the target sites to diagnostically image the respective tumour(s). Exemplary modes of administration of the radiolabeling imaging agent include parenteral delivery, such as intramuscular, subcutaneous, intravenous, intramedullary injections. One may also administer the radiolabeling imaging agent in a local rather than systemic manner, for example, via injection of the compound directly into a solid tumour, such as in a depot or sustained release formulation. Furthermore, a respective radiolabeling imaging agent may be used in a targeted drug delivery system, for example, in a liposome coated with a tumour-specific antibody. Such liposomes may for example be targeted to and taken up selectively by a tumour.

[0072] The radiolabeling imaging agent of the invention can be administered in a single unit injectable dose. Any of the common carriers known to those with skill in the art, such as sterile saline solution or plasma, can be utilized for preparing the injectable solution to diagnostically image various organs, tumours and the like in accordance with the present invention. Generally, the unit dose to be administered has a radioactivity of about 0.01 mCi to about 100 mCi; about 0.5 mCi to about 80 mCi; about 1 mCi to about 50 mCi; or about 1 mCi to about 20 mCi. The solution to be injected at unit dosage is from about 0.01 ml to about 10

ml. Once the radiolabeling imaging agent has been administered into the mammal, imaging of the organ or tumour in vivo can take place in a few minutes, for example in about 15 to about 20 minutes. However, if desired, imaging can take place in hours for example about 2 to about 4 hours, or even longer. Generally, a sufficient amount of the administered dose will accumulate in the area to be imaged within about 5 to about 10 minutes to permit the taking of images. Any conventional imaging methods for diagnostic purposes can be utilized in accordance with the present invention. Examples of such methods include positron emission tomography (PET) and single photon emission computed tomography (SPECT).

[0073] The invention also provides a method of determining the likelihood of developing cancer in a non-cancerous cell. The method includes administering to the cell the radiolabeling imaging agent of the invention and measuring the radioactivity in the cell.

[0074] Any cell may be used in the present method of the invention, including cancerous cell or non-cancerous cell. In some embodiments, the cell used according to the invention may be comprised in a mammal. In other embodiments, the cell as used in the invention may be cultured. The cultured cell may also be obtained from a mammal. The cell may be infected with a cancer-causing virus or carcinogenic virus. The cell may in some embodiments be exposed to carcinogens as mentioned above.

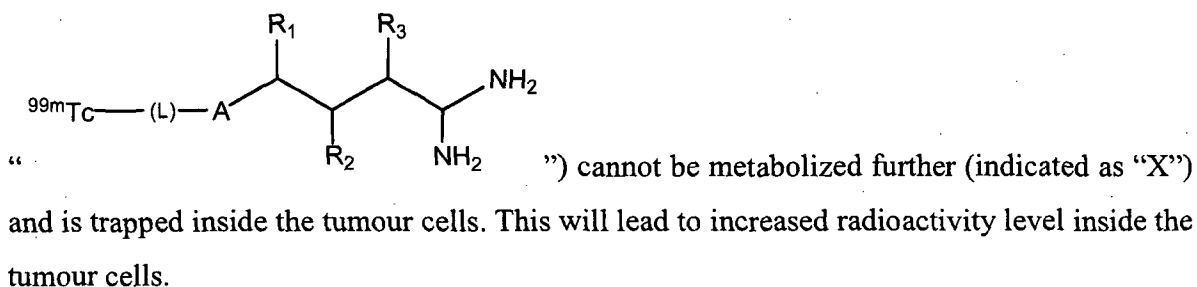
[0075] These aspects of the present invention and the advantages will be more fully understood in view of the following description of the drawings and the non-limiting examples.

BRIEF DESCRIPTION OF THE DRAWINGS

[0076] In order to better understand the present invention and to demonstrate how it may be carried out in practice, preferred embodiments will now be described by way of non-limiting examples only, with reference to the accompanying drawings, in which:

[0077] **Figure 1** depicts a schematic representation of the polyamine pathway. As shown in Figure 1, the step during which putrescine is formed by the decarboxylation of ornithine by ornithine decarboxylase (ODC) is the rate limiting step.

[0078] **Figure 2** depicts a schematic representation of the polyamine pathway in which the radiolabeling imaging agent of the present invention is taken up by the tumour cells and decarboxylated by ODC. The resulting product (indicated as



[0079] Figure 3A shows the percentage of free $^{99m}\text{TcO}_4^-$ (1.3%) present in the radiolabeling imaging agent according to the present invention. The percentage of free TcO_4^- is determined by ITLC-SG as described in Example 3.

[0080] Figure 3B shows the percentage of reduced hydrolyzed $^{99m}\text{TcO}_2$ (1.7%) present in the radiolabeling imaging agent according to the present invention. The percentage of reduced hydrolyzed $^{99m}\text{TcO}_2$ is determined by ITLC-SG as described in Example 3.

[0081] Figure 4 shows the uptake of the radiolabeling imaging agent according to the invention in HepG2 cells (1×10^6). HepG2 cells were incubated in RPMI cell culture media with 0.1 mCi (3.7 mBq) of the radiolabeling imaging agent of the present invention at 37°C for 1 hr. The method for determining the radioactivity inside the cells is described in Example 4. As shown in Figure 4, the *in vitro* uptake of the radiolabeling imaging agent of the invention (indicated as "HepG2+ORN") was 2.4% injected dose/mg of cell content, as compared to the control HepG2 cells (indicated as "HepG2+Tc99m") incubated with ^{99m}Tc only (0.17% injected dose/mg of cell content).

[0082] Figure 5 shows the images of 2 adult male buffalo rats injected subcutaneously with Morris 777 hepatoma cells in the inner side of the rats' femur. Three weeks later, the rats were injected with 1 mCi (37 mBq) of the radiolabeling imaging agent of the present invention and the images were taken at 1 hr after injection. The presence of tumours is indicated by the arrows.

EXAMPLES

Example 1

[0083] This example illustrates a method for modifying ornithine according to the present invention.

[0084] 1gm of $\text{N}_\alpha\text{Boc-N}_\delta$ Fmoc-Ornithine (Boc-Orn(Fmoc)-OH, obtained from Sigma Catalogue No.15539) was dissolved in 20% of piperidine in N,N-Dimethylformamide (DMF,

obtained from Sigma, Catalogue No.80645) at 37°C for 2 hour. N_αBoc-Ornithine was formed as white precipitate. The white precipitate was (238 mg) collected by filtration and washed with 20 ml acetonitrile (obtained from Sigma Cat # 271004), and dried in air overnight. The N_αBoc-Ornithine was dissolved in 5 ml of deionized water. 358 mg of DTPA anhydride (obtained from Sigma, Catalogue No. D6148) was added to the solution and stirred for 2 to 3 hours. Therefore, about 2 moles of N_αBoc-Ornithine was used for each mole of DTPA anhydride for preparing DTPA-ornithine. The solution was evaporated to dryness and then mixed with 3 ml of trifluoroacetic acid (obtained from Sigma T62200). The resultant DTPA-ornithine was then purified by gel filtration chromatography as described in Alper O Karacalioglu et al, Radiolabeled L-Lysine for Tumor Imaging, Acad Radiology 2006; 1327-1331. The yield of DTPA-ornithine prepared according to the present invention is 35%.

Example 2

[0085] This example illustrates a method for labeling ^{99m}Tc with DTPA-ornithine obtained from Example 1, thereby resulting in a radiolabeling imaging agent according to the present invention.

[0086] 100 mg of stannous chloride (SnCl₂) di-hydrate (obtained from Sigma, Catalogue No.452335) was dissolved in 10 ml 0.1 N Hydrochloric acid (obtained from Sigma, Catalogue No. 2104) to form a SnCl₂ stock solution. An aliquot (0.01 ml) of the SnCl₂ stock solution (0.1mg SnCl₂ di-hydrate) was added to 4-8 mg of the DTPA-ornithine. To the solution mixture, 50 mCi (1850 MBq) of ^{99m}TcO₄⁻ in 2 ml of normal saline was then added. The mixture was then left to stand at room temperature for 15 min, thereby obtaining a radiolabeling imaging agent of the present invention, ^{99m}Tc-DTPA-ornithine.

Example 3

[0087] This example illustrates a method for determining the radiochemical purity of the radiolabeling imaging agent obtained from Example 2.

[0088] The confirmation on the labeling of ^{99m}Tc and the radiochemical purity of the radiolabeling imaging agent (^{99m}Tc-DTPA-ornithine) was determined by instant thin layer chromatography silica gel strips (ITLC-SG) as described in for example, Chen F. et al, *Eur. J. Nuc. Med.*, 1993, vol. 20(3), pp. 334-338, with the exception that 0.9 % sodium chloride and acetone were used as the solvent systems.

[0089] The percentage of unlabeled ^{99m}Tc or free $^{99m}\text{TcO}_4^-$ was determined by ITLC-SG (1 x 10 cm) in acetone ($R_f = 1$) (Figure 3A). The percentage of reduced hydrolyzed Tc-99m was determined by ITLC-SG in normal saline ($R_f = 0$) (Figure 3B). The radiochemical purity of the radiolabeling imaging agent (the percentage of ^{99m}Tc -DTPA-ornithine) is calculated as follows:

$$100\% - \% \text{ unlabeled } ^{99m}\text{Tc} - \% \text{ reduced hydrolyzed } ^{99m}\text{Tc}$$

[0090] Referring to Figures 3A and 3B, the radiochemical purity of ^{99m}Tc -DTPA-ornithine according to an embodiment of the present invention is:

$$100\% - 1.3\% (\% \text{ free } ^{99m}\text{TcO}_4^-) - 1.7\% (\% ^{99m}\text{TcO}_2) = 97\%$$

[0091] The stability of the radiolabeling imaging agent according to the present invention (i.e. Tc-99m-DTPA-ornithine) was determined by first incubating Tc-99m-DTPA-ornithine in 10 ml of human serum at 37°C. The radiochemical purity was determined at 1 hour, 3 hr and 6 hr after its preparation. The Tc-99m ornithine was stable up to 6 hours after preparation (radiochemical purity of 97.1%). The results are shown in the Table 1 below.

Time after labeling	Radiochemical purity
15 min	98.1 %
1 hr	97.3%
3 hr	99.3%
6 hr	97.1%

N=3

Table 1

Example 4

[0092] This example illustrates the *in vitro* uptake of the radiolabeling imaging agent according to the invention in HepG2 cells (liver cancer cells). 1×10^6 HepG2 cells (liver cancer cells) obtained from ATCC were incubated in RPMI cell culture media (obtained from

Sigma, Catalogue No.51501C) with 0.1 mCi (3.7 mBq) of Tc-99m-DTPA-ornithine prepared from Example 2, at 37°C for 1 hour. The cells were then isolated by centrifugation and the radioactivity inside the cells was measured. As a control, HepG2 cells were incubated only with Tc-99m under the same conditions as above. As shown in Figure 4, there was a significant uptake of the radiolabeling imaging agent of the invention (indicated as "HepG2+ORN") (2.4% injected dose/mg of cell content), as compared to the control HepG2 cells (indicated as "HepG2+Tc99m") (0.17% injected dose/mg of cell content).

Example 5

[0093] This example illustrates the Nuclear Medicine imaging of liver cancer in buffalo rats. 2 adult male buffalo rats (250 grams by weight) which were bred in-house by the inventors, were injected subcutaneously with Morris 777 hepatoma cells in the inner side of femur. Three weeks later, the rats were injected with 1 mCi (37 mBq) of Tc-99m-DTPA-ornithine and images were taken using Phillips ADAC Genesis Dual Head Gamma Camera containing a parallel hole collimator at 1 hour after injection. The images are shown in Figure 5.

[0094] The listing or discussion of a previously published document in this specification should not necessarily be taken as an acknowledgement that the document is part of the state of the art or is common general knowledge. All documents listed are hereby incorporated herein by reference in their entirety.

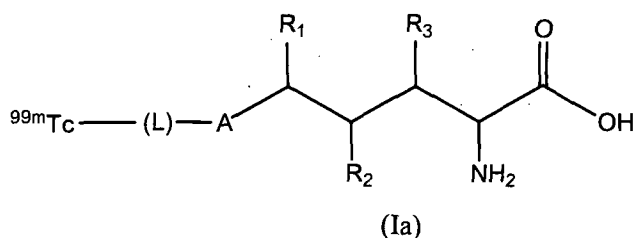
[0095] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[0096] Other embodiments are within the following claims. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognise that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

CLAIMS

What is claimed is:

1. A radiolabeling imaging agent of formula (I) consisting of technetium-99m (^{99m}Tc), and a moiety of formula (Ia) covalently bound to a chelator (L)



(I)

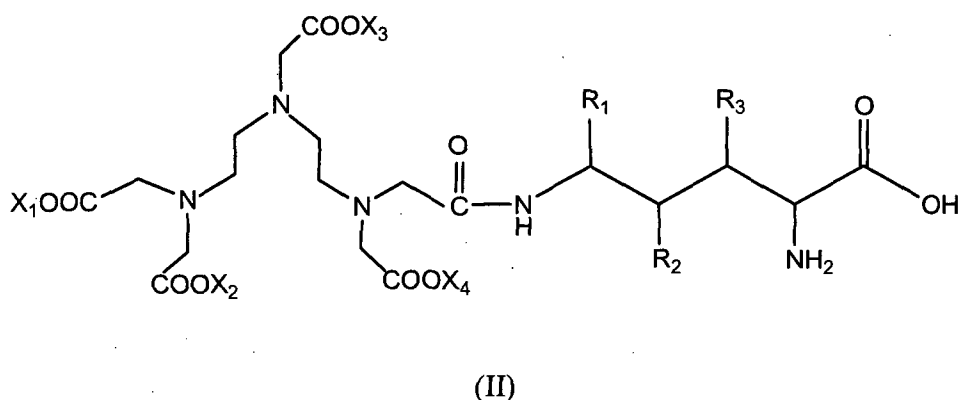
wherein

A is independently selected from the group consisting of an amino group, a mono-substituted amino group, a di-substituted amino group, and $-\text{N}=\text{C}(\text{NH}_2)_2$;

R_1 to R_3 are each independently selected from the group consisting of hydrogen, halogen, hydroxyl, oxo, optionally substituted $\text{C}_1\text{-C}_6$ alkyl, optionally substituted $\text{C}_2\text{-C}_6$ alkenyl, optionally substituted $\text{C}_2\text{-C}_6$ alkynyl, optionally substituted $\text{C}_1\text{-C}_6$ acyl, optionally substituted C_{3-8} cycloalkyl, and optionally substituted C_{3-8} cycloalkenyl.

2. The radiolabeling imaging agent according to claim 1, wherein the mono-substituted amino group is $\text{N-C}_1\text{-C}_6$ alkyl or acetylamino group.
3. The radiolabeling imaging agent according to claim 1 or 2, wherein the di-substituted amino group is $\text{N,N-C}_1\text{-C}_6$ alkyl or N,N -diacetylamino group.
4. The radiolabeling imaging agent according to any one of claims 1 to 3, wherein the halogen is selected from the group consisting of fluorine, chlorine, iodine and bromine.

5. The radiolabeling imaging agent according to any one of claims 1 to 4, wherein R_2 is fluorine.
6. The radiolabeling imaging agent according to any one of claims 1 to 5, wherein the moiety of formula (Ia) is selected from the group consisting of ornithine, (L)-ornithine, (S)-ornithine, arginine, (L)-arginine, 4-fluoro-L-ornithine, glutamic acid, (L)-glutamic acid and (D)-glutamic acid.
7. The radiolabeling imaging agent according to any one of claims 1 to 6, wherein the chelator (L) is selected from the group consisting of diethylenetriamine pentaacetic acid (DTPA), ethylenediaminetetraacetic acid (EDTA), (N-(2,6-diethylacetanilido) iminodiacetic acid) (EHIDA), triethylenetriaminehexaacetic acid (TTHA), mercaptoacetyltriglycine (MAG3), dimercaptosuccinic acid (DMSA), and Cyclam.
8. A compound of formula (II)

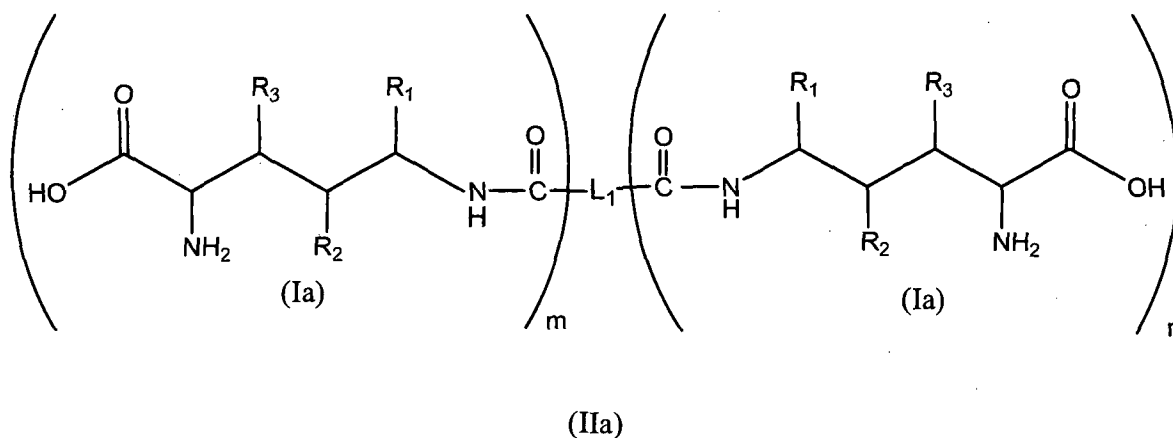


wherein

R_1 to R_3 are each independently selected from the group consisting of hydrogen, halogen, hydroxyl, oxo, optionally substituted C_1 - C_6 alkyl, optionally substituted C_2 - C_6 alkenyl, optionally substituted C_2 - C_6 alkynyl, optionally substituted C_1 - C_6 acyl, optionally substituted C_{3-8} cycloalkyl, and optionally substituted C_{3-8} cycloalkenyl; and

X_1 to X_4 are each independently selected from the group consisting of hydrogen, optionally substituted C_1 - C_6 alkyl and optionally substituted C_2 - C_6 alkenyl.

9. The compound according to claim 8, further comprising technetium-99m (^{99m}Tc) bound to the compound of formula (II).
10. The compound according to claim 8 or 9, wherein the halogen is selected from the group consisting of fluorine, chlorine, iodine and bromine.
11. The compound according to any one of claims 8 to 10, wherein R_2 is fluorine.
12. A compound of formula (IIa)



wherein

L_1 is a multidentate moiety comprising at least 2 reactive groups that react with the moiety of formula (Ia);

m is an integer 1 or 2; and

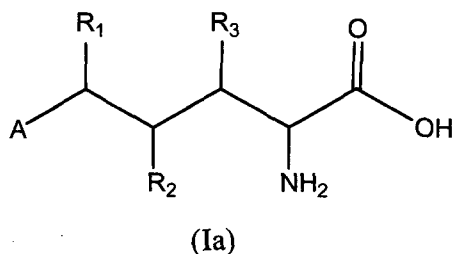
n is an integer 1 or 2.

13. The compound according to claim 12, wherein the reactive group is an acyl group or a carboxyl group.
14. The compound according to claim 12 or 13, wherein the multidentate moiety L_1 is selected from the group consisting of diethylenetriamine pentaacetic acid (DTPA), ethylenediaminetetraacetic acid (EDTA), (N-(2,6-diethylacetanilido) iminodiacetic acid) (EHIDA), triethylenetriaminehexaacetic acid (TTHA) and mercaptoacetyltriglycine (MAG3) and cyclam.

15. The compound according to any one of claims 12 to 14, further comprising technetium-99m (^{99m}Tc) bound to the multidentate moiety L_1 .
16. A method of identifying a tumour in a mammal comprising administering to the mammal the radiolabeling imaging agent according to any one of claims 1 to 7; subjecting the mammal to nuclear medicine imaging to detect the radiation emitted by the radiolabeling imaging agent, wherein the presence of radioactivity indicates the presence of the tumour.
17. The method according to claim 16, wherein the mammal is selected from the group consisting of a rat, a cow, a goat, a sheep, a pig, a dog, a mouflon, a guinea pig, a hamster, a chimpanzee, a rhesus monkey and a human.
18. The method according to claim 16 or 17, wherein the tumour is derived from a cancer selected from the group consisting of breast cancer, lung cancer, brain cancer, neuroblastoma, colon cancer, stomach cancer, rectal cancer, bladder cancer, liver cancer, pancreatic cancer, ovarian cancer, prostate cancer and melanoma.
19. A method of determining the likelihood of developing cancer in a non-cancerous cell comprising administering to the cell the radiolabeling imaging agent according to any one of claims 1 to 7 and measuring the radioactivity in the cell.
20. The method according to claim 19, wherein the cell is infected with a cancer-causing virus/carcinogenic virus.
21. The method according to claim 19 or 20, further comprising comparing the results obtained from claim 19 with that obtained from a control cell.
22. The method according to any one of claims 19 to 21, wherein the cell is comprised in a mammal.

23. The method according to claim 22, wherein the mammal is selected from the group consisting of a rat, a cow, a goat, a sheep, a pig, a dog, a mouflon, a guinea pig, a hamster, a chimpanzee, a rhesus monkey and a human.

24. A method of preparing the radiolabeling imaging agent according to any one of claims 1 to 7 comprising reacting the moiety of formula (Ia)

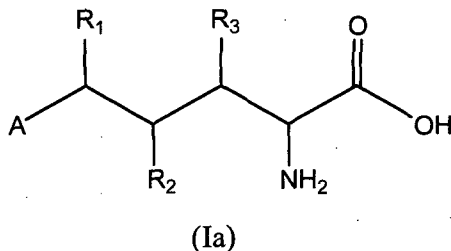


or an isomer thereof with a chelator (L) and adding a solution of $^{99m}\text{TcO}_4^-$ into the solution containing the moiety of formula (Ia) bound to the chelator (L) in the presence of a reducing agent, under conditions to form the radiolabeling imaging agent.

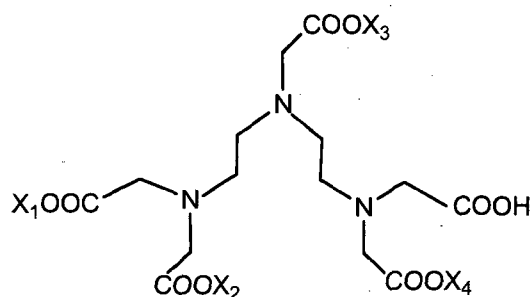
25. The method according to claim 24, wherein the moiety of formula (Ia) is protected with at least one amine protecting group.

26. The method according to claim 24, wherein the chelator L is selected from the group consisting of diethylenetriamine pentaacetic acid (DTPA), ethylenediaminetetraacetic acid (EDTA), (N-(2,6-diethylacetanilido) iminodiacetic acid) (EHIDA), triethylenetriaminehexaacetic acid (TTHA), mercaptoacetyltriglycine (MAG3), dimercaptosuccinic acid (DMSA) and Cyclam.

27. A method of preparing the compound of formula (II) according to any one of claims 8 to 11 comprising reacting a moiety of formula (Ia)



with a compound of formula III



(III)

under conditions to form the compound of formula (II).

28. The radiolabeling imaging agent according to any one of claims 1 to 7 for use in diagnosing cancer.

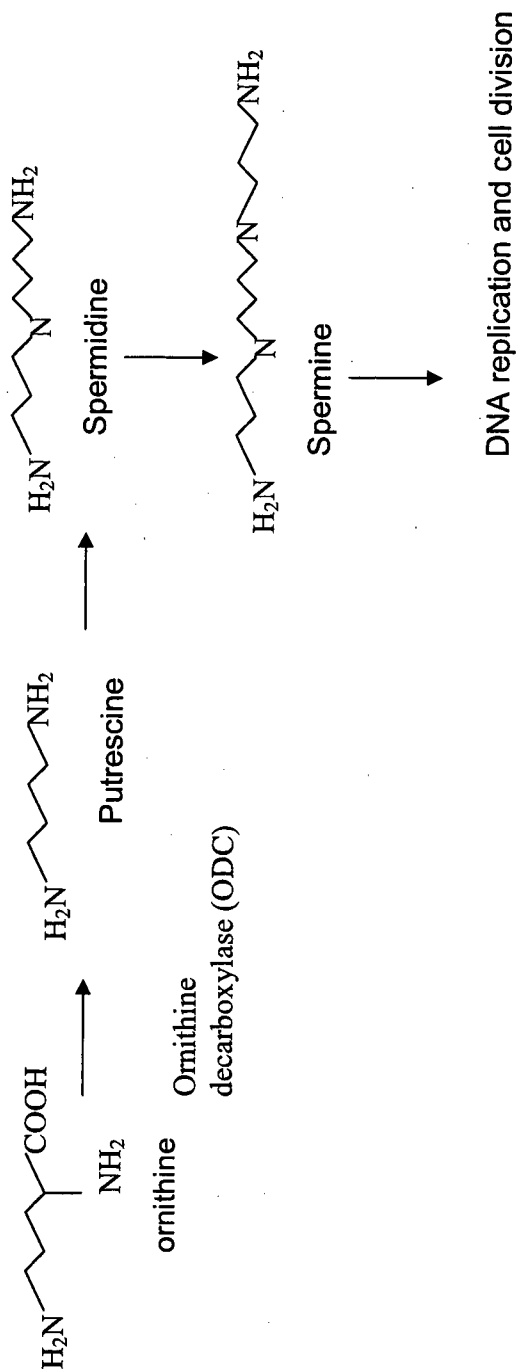


Figure 1

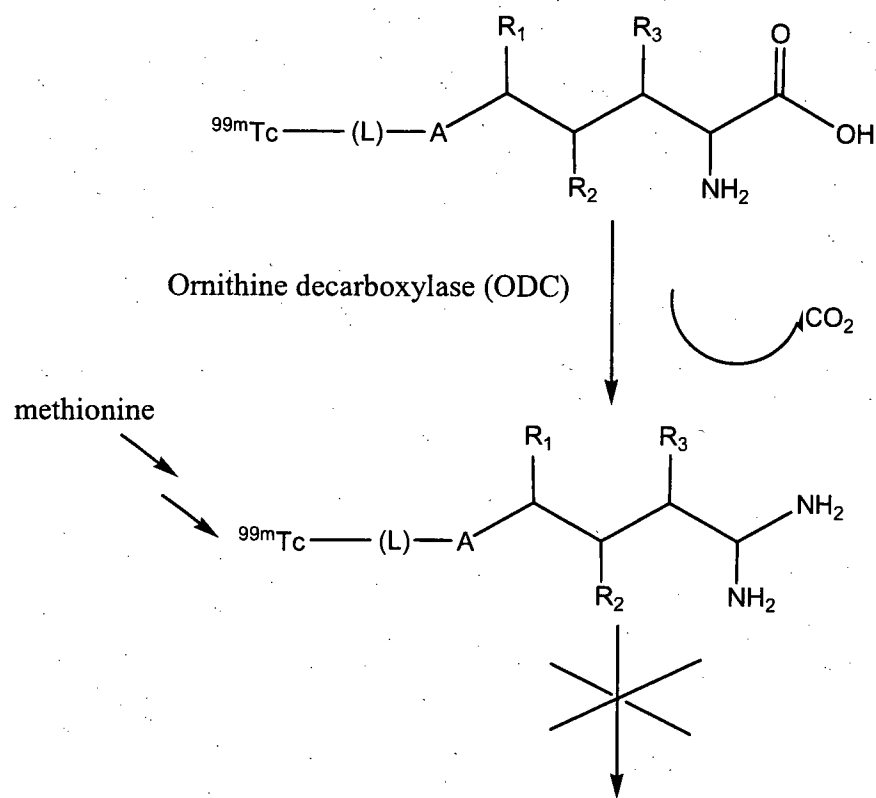


Figure 2

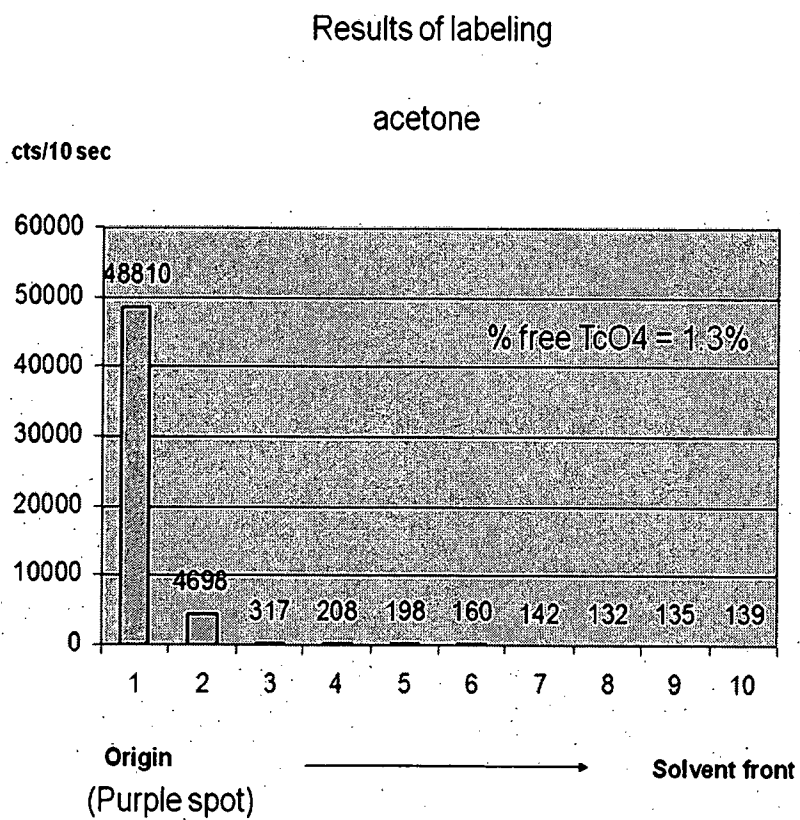


Figure 3A

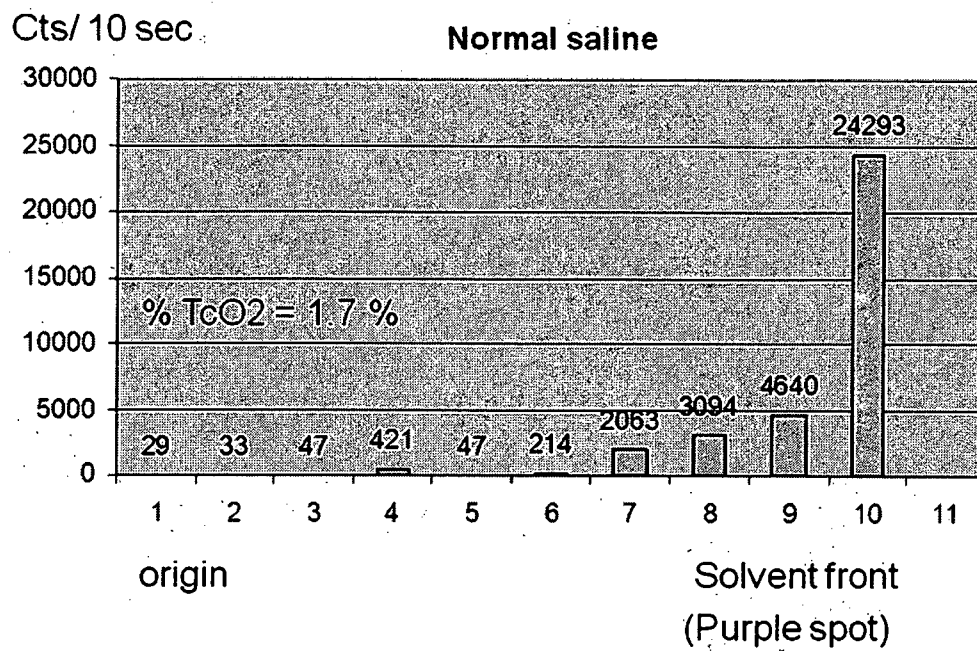


Figure 3B

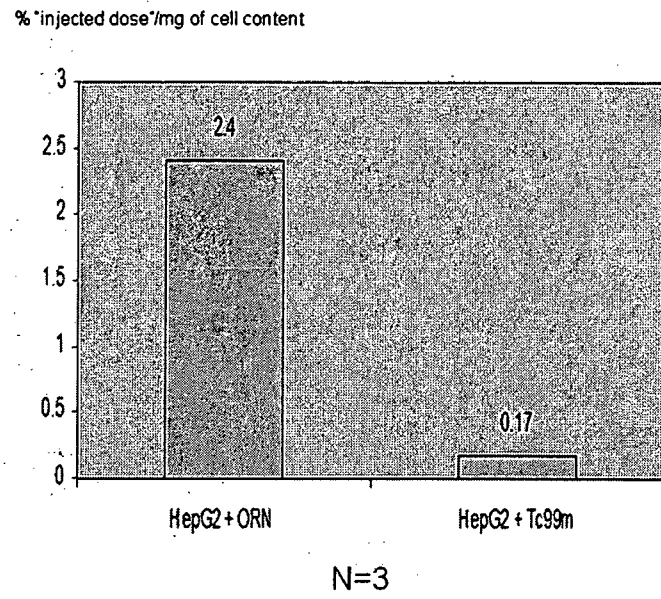


Figure 4

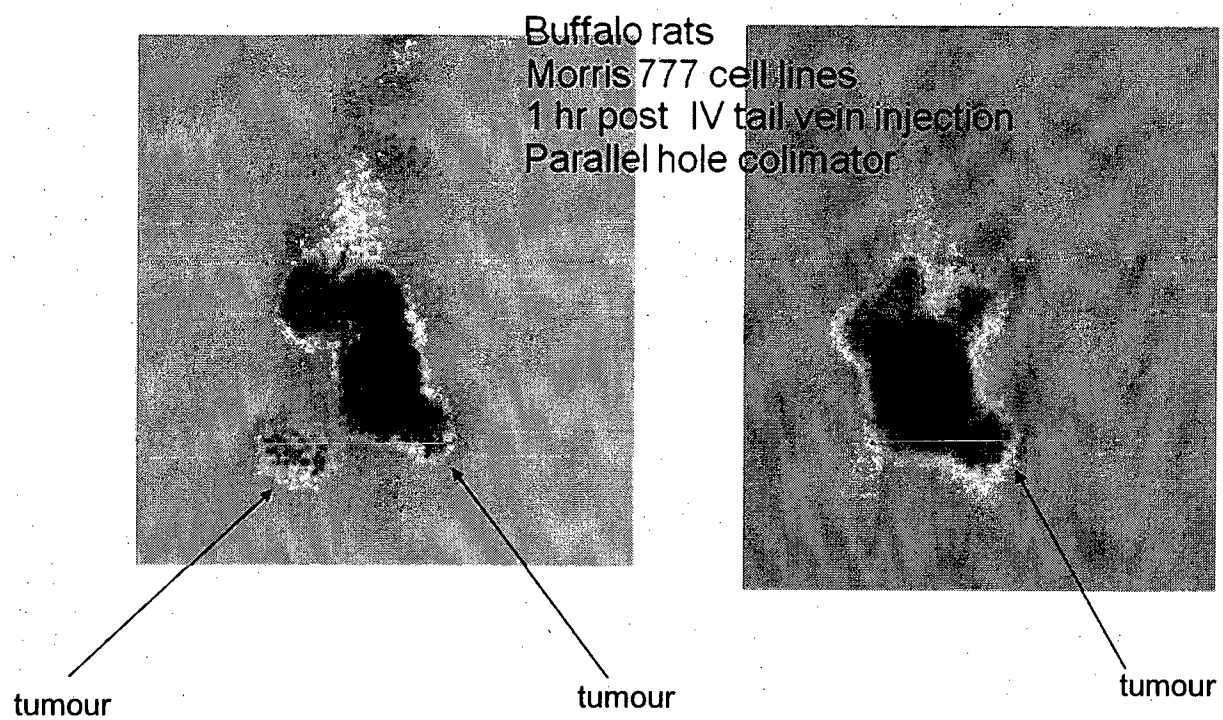


Figure 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2011/000160

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

C07F 13/00 (2006.01)**C07C 229/26** (2006.01)**C07C 229/76** (2006.01)**A61K 51/04** (2006.01)**A61K 103/10** (2006.01)

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)

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)

CA: STN Structure search based on structures of claims 1, 8, 12.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/118862 A1 (CARDAX PHARMACEUTICALS, INC.) 2 October 2008 Compound on bottom line of page 49.	12-13
X	Chen, J. <i>et al.</i> , "Darstellung von Isophthaloyl- und Terephthaloyl-di-[(β,γ,δ)-amino- α -aminocarboxylat]-verbrückten zweikernigen Ruthenium-, Rhodium und Iridium-Halbsandwich-Komplexen", <i>Journal fuer Praktische Chemie</i> , 1999, 341 (8), 792-796.	12-13
X	Two compounds of scheme 2, page 793. Anzai, K., "Bromodenitrogenation of <i>N</i> ² -Benzoylarginine Ethyl Ester with <i>N</i> -Bromosuccinimide", <i>Bulletin of the Chemical Society of Japan</i> , 1970, 43, 2539-2543.	12-13
X	Compound V page 2540.	12

☒ Further documents are listed in the continuation of Box C☒ See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"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
"E" earlier application or patent but published on or after the international filing date	"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
"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)	"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
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"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
06 June 2011Date of mailing of the international search report
14 JUN 2011Name and mailing address of the ISA/AU
AUSTRALIAN PATENT OFFICE
PO BOX 200, WODEN ACT 2606, AUSTRALIA
E-mail address: pct@ipaaustralia.gov.au
Facsimile No. +61 2 6283 7999Authorized officer
PAUL GARDNER
AUSTRALIAN PATENT OFFICE
(ISO 9001 Quality Certified Service)
Telephone No : +61 2 6283 2145

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2011/000160

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Wan, W. <i>et al.</i> , "[^{99m} Tc]Polyamine Analogs as Potential Tumor Imaging Agent", <i>Drug Development Research</i> , 2008, 69, 520-525. Abstract, methods page 521 right column, discussion pages 523-524.	1-28
Y	WO 2003/103722 A1 (BRACCO IMAGING S.P.A.) 18 December 2003. Example 2, example 13.	1-28
Y	Lattuada, L. <i>et al.</i> , "Magnetic resonance imaging of tumor cells by targeting the amino acid transport system" <i>Bioorganic & Medicinal Chemistry Letters</i> , 2006, 16, 4111-4114. Schemes 1 and 2, figure 2.	1-28

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/SG2011/000160

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member			
WO	2008118862	US	2009124574		
WO	03103722	AU	2003238192	EP	1369134
		US	2005175543	EP	1509254
				US	2010311962
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.					
END OF ANNEX					