(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 September 2005 (22.09.2005)

PCT

(10) International Publication Number
WO 2005/087275 A2

(51) International Patent Classification[7]: A61K 51/04 // 103:00, 123:00

(21) International Application Number: PCT/US2005/007686

(22) International Filing Date: 9 March 2005 (09.03.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/552,244	11 March 2004 (11.03.2004)	US
60/552,661	11 March 2004 (11.03.2004)	US

(71) Applicant *(for all designated States except US)*: **BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM** [US/US]; 201 W. 7th Street, Austin, TX 78701 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **CHAO, K.S. Clifford** [US/US]; 2705 Amherst, Houston, TX 77005 (US). **YANG, David J.** [US/US]; 1123 Spinnaker Way, Sugar Land, TX 77478 (US).

(74) Agent: **LECOINTE, Michelle M.**; Baker Botts L.L.P., 98 San Jacinto Blvd., 1500 San Jacinto Center, Austin, TX 78701-4039 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:

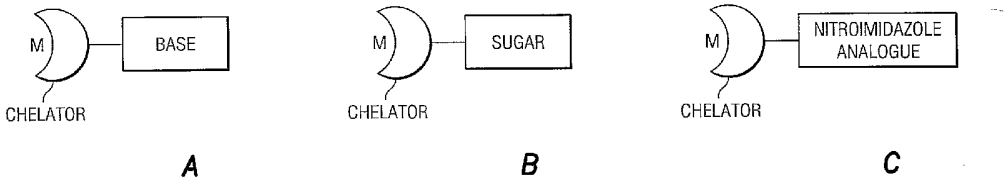
— *as to the identity of the inventor (Rule 4.17(i)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)*

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METAL RADIOLABELED PET IMAGING AGENTS



(57) Abstract: The invention relates to radiolabeled PET agents including a radioisotope, a chelator and a specific ligand. The radioisotope may include 60Cu, 62Cu, 61Cu, 64Cu and 68Ga. Generator-produced 68Ga radioisotopes may be used. Specific chelators include N2S2 moieties and N3S moieties. Specific ligands may include purine and pyrimidine bases, particularly adenine and guanine, a sugar-containing base, particularly glucosamine, galactosamine, or mannosamine, or a nitroimidazole analogue, such as a 2-nitroimidazole or a 5-nitroimidazole, particularly metronidazole. Radiolabeled agents may be formed by first forming a chelator-ligand complex, which may be stored until it is needed, at which time the radioisotope may be added. Radiolabeled agents may be used in traditional PET imaging or in dynamic PET imaging.

WO 2005/087275 A2

METAL RADIOLABELED PET IMAGING AGENTS

FIELD OF THE INVENTION

The present invention relates to radiolabeled agents containing a metal radioisotope suitable for PET imaging, a chelator, and a specific ligand. In particular embodiments, it relates to agents containing a generator-produced ^{68}Ga radioisotope or a purine or pyrimidine base ligand, a sugar-containing ligand, or a nitroimidazole analog ligand.

BACKGROUND

Radiolabeled agents are useful in a variety of imaging methods, including many *in vivo* imaging techniques. However, the usefulness of these techniques is often limited by the available methods of producing radioisotopes. Radioisotopes suitable for *in vivo* imaging frequently decay quickly and are difficult to produce. These and other problems limit the types of diagnostic applications for which *in vivo* imaging is suitable.

Positron emission tomography (PET) is a form of *in vivo* imaging that uses gamma-emitting radioisotopes to locate diseases or pathological processes in the body. Improvements in PET have resulted largely from improvements in the radioisotopes available for use. These radioisotopes now include ^{18}F, ^{60}Cu, ^{62}Cu, ^{61}Cu, ^{64}Cu, and ^{68}Ga. In order to obtain meaningful results, these radioisotopes are normally targeted to specific tissues. These tissues are often defined by physiological characteristics. For example, PET often uses an ^{18}F-fluorodeoxyglucose (FDG) radiolabeled agent. FDG is an

analog of glucose and is therefore taken up in larger quantities by cells with faster metabolisms. Because tumor cells tend to have relatively fast metabolisms, PET imaging using FDG may be used to detect many tumors.

However, FDG and other radiolabeled agents currently available for PET are not suitable to take full advantage of the diagnostic range PET offers. For example, hypoxia may indicate the stage of a tumor, as may the presence of cell surface markers. Such markers may also indicate the type and origin of a tumor or be useful in detecting small metastases. Changes in various other indictors of tumor metabolism may provide clues regarding whether a treatment is effective. Therefore, the development of additional radiolabeled agents suitable to detect various physiological states using PET is useful in advancing the diagnostic capabilities of PET.

Cyclotrons are extremely expensive to build and operate. Further, they require lengthy preparation and cool-down times. These and other factors dictate that a cyclotron be used approximately once a day to generate radioisotopes for PET. Most radioisotopes suitable for PET decay within a few hours. As a result, PET has been largely confined to diagnostic procedures that are scheduled in advance so that appropriate amounts of radioisotopes may be generated each day by the cyclotron. Although some PET techniques do allow "dynamic" imaging, i.e. imaging of conditions that change during the time span over which imaging occurs, most focus on a physiological state that is relatively static for the duration of the imaging process. The lack of availability of easily generated radioisotopes is at

least partially responsible for the lack of dynamic PET agents.

On possibility for improving the dynamic imaging capacity and general availability of PET lies in the production of radioisotopes without a cyclotron. For example, radioisotopes may be produced from a generator. The generator process uses a parent-daughter nuclidic pair where the parent isotope decays to a short-lived daughter isotope, which is used for imaging. One such generator has been developed for the production of ^{62}Cu from ^{62}Zn. However, ^{62}Cu has a half-life of only 10 minutes, therefore making it difficult to harvest the radioisotope, incorporate it in an imaging agent, administer it to the patient, and conduct a PET scan before the radioisotope has decayed to beyond its useable levels.

Because of the problems with ^{62}Cu, another generator and related process has been developed to produce ^{68}Ga. Because it is generator-produced, ^{68}Ga avoids the expense, scheduling and other problems of a cyclotron. In addition, because ^{68}Ga has a half-life of approximately 68 minutes, it is far easier to use in a clinical setting than ^{62}Cu. A ^{68}Ga generator has been previously described in a U.S. Provisional Patent Application filed on January 20, 2004, titled "System and Method for an Automated Synthesis of Gallium-68 Generator-Based Radiopharmaceutical Agents".

SUMMARY

The present invention relates to radiolabeled agents for PET imaging that include a metal radioisotope, a chelator and a specific ligand. In specific embodiments of the invention, the ligand may be purine or pyrimidine base ligand, a sugar-containing ligand or a nitroimidazole analogue. Although the ligand may be any compound sufficient to target the agent to a selected biochemical marker, in a specific embodiment it is selected to allow dynamic imaging. The radioisotope in certain embodiments may include ^{60}Cu, ^{62}Cu, ^{61}Cu, ^{64}Cu and ^{68}Ga, including a generator-produced ^{68}Ga radioisotope. The chelator in specific embodiments may contain oxygen, nitrogen, or sulfur. More specifically, it may contain an O_2S_2, NS_3, N_2S_2, or N_3S moiety for chelating the metal radioisotope.

Radiolabeled agents may be generally formed by first combining the specific ligand and chelator to create a ligand-chelator complex. The metal radioisotope may be added to this complex, for example, shortly before use of the radiolabelled agent. In specific embodiments, the ligand and chelator may be covalently bonded to form a single compound. In other specific embodiments, the radioisotope may be produced by a cyclotron or by a generator. The radioisotope source may be chosen based on the ultimate use of the radiolabeled agent.

In embodiments using a generator-produced ^{68}Ga radioisotope, shortly before imaging, ^{68}Ga may be produced using a ^{68}Ga generator and removed from the HCl or other acid in which it is initially present. This purified ^{68}Ga

is then provided to the chelator-ligand complex to form the completed radiolabeled agent.

Radiolabeled agents of the present invention may be used to detect a variety of physiological states, including DNA synthesis, glycolysis, or hypoxia. These states may be due to the presence of a tumor, a neurological disorder, a cardiovascular disorder, or because a subject has suffered a stroke, *inter alia*.

The invention may be better understood by reference to the following drawings in combination with the description presented herein.

BRIEF DESCRIPTION OF THE DRAWINGS

The following figures form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the description of embodiments presented herein.

FIGURE 1 illustrates three radiolabeled agents, according to embodiments of the present invention.

FIGURE 2 illustrates several chelators, according to selected embodiments of the present invention.

FIGURE 3 illustrates a method of synthesizing a ^{68}Ga -chelator, according to an embodiment of the present invention.

FIGURE 4 illustrates another method of synthesizing a ^{68}Ga - chelator, according to an embodiment of the present invention.

FIGURE 5 illustrates yet another method of synthesizing a ^{68}Ga - chelator, according to an embodiment of the present invention.

FIGURE 6 illustrates a radiolabeled agent having a guanine ligand, according to an embodiment of the present invention.

FIGURE 7 presents a graph comparing cellular uptake of ^{64}Cu-N_2S_2 and ^{64}Cu-N_2S_2-Guan by cell phase cycle.

FIGURE 8 presents a graph comparing the cellular uptake by a rat breast adenocarcinomas (13762) or human glioma cells (U87) of a radiolabeled agent lacking a ligand with that of radiolabeled agents having a guanine, glucosamine or MN ligand, according to embodiments of the present invention. 50,000 cancer (U87 or 13762) were plated in each well. Cells were incubated with 2-4 μCi of the appropriate radiolabeled agent for 0.5 or 2 hours.

FIGURE 9 presents a brain autoradiogram of U87 tumor bearing mice injected with ^{64}Cu-N_2S_2.

FIGURE 10 presents a presents a brain autoradiogram of U87 tumor bearing mice injected with ^{64}Cu-N_2S_2-Guan.

FIGURE 11 presents a graph comparing the cellular uptake by a human lung cancer cell line of a radiolabeled agent lacking a ligand with that of a radiolabeled agent having a guanine ligand, according to an embodiment of the present invention. 50,000 human lung cancer cells (A549) were plated in each well. Cells were incubated with 4 μCi of ^{68}Ga-N_2S_2-Guanine (Guan) at 30 and 90 min.

FIGURE 12 presents a PET image of a tumor in a rat after injection of a radiolabeled agent having a guanine

ligand, according to an embodiment of the present invention.

FIGURE 13 presents a graph comparing the cellular uptake by a human lung cancer cell line of a radiolabeled agent lacking a ligand with that of a radiolabeled agent having a glucosamine ligand, according to an embodiment of the present invention. 50,000 human lung cancer cells (A549) were plated in each well. Cells were incubated with 4 μCi of ^{68}Ga-N_2S_2-Glucosamine at 30 and 90 min.

FIGURE 14A presents a PET image of a tumor in a rat after injection of a radiolabeled agent having a glucosamine ligand, according to an embodiment of the present invention. Muscle tissue is also indicated.

FIGURE 14B presents a graph illustrating the uptake of radiolabeled agent having a glucosamine ligand, according to an embodiment of the present invention, by a tumor as compared to by muscle tissue.

FIGURE 15A presents a PET image of a tumor in a rat after injection with either a ^{68}Ga radiolabeled agent having a glucosamine ligand, according to an embodiment of the present invention, or an ^{18}F-fluorodeoxyglucose (FDG) agent. FIGURE 15A presents the results obtained using a radiolabeled agent having a glucosamine ligand. FIGURE 15B presents the results obtained using ^{18}F-FDG.

FIGURE 15B presents a graph illustrating the uptake of radiolabeled agent having a glucosamine ligand, according to an embodiment of the present invention, by a tumor as compared to by muscle tissue.

FIGURE 16 presents a graph comparing the cellular uptake by a human lung cancer cell line of a radiolabeled

agent lacking a ligand with that of a radiolabeled agent having a metronidazole (MN) ligand, according to an embodiment of the present invention. 50,000 human lung cancer cells (A549) were plated in each well. Cells were incubated with 4 μCi of ^{68}Ga-N_2S_2-MN at 30 and 90 min.

FIGURE 17 presents a PET image of a tumor in a rat after injection of a radiolabeled agent having an MN ligand, according to an embodiment of the present invention.

DETAILED DESCRIPTION

The present invention includes radiolabeled agents for PET imaging that contain a metal radioisotope, a chelator and a specific ligand. In specific embodiments of the invention, the ligand may be purine or pyrimidine base ligand, a sugar-containing ligand or a nitroimidazole ligand. (See FIGURE 1.)

A variety of chelators exist that are suitable for use in the present invention. In a specific embodiment, such chelators include N, O, or S. More specifically, such chelators may include an O_2S_2, NS_3, N_2S_2, or N_3S moiety. Examples of suitable chelators are presented in FIGURE 2.

The metal radioisotope used in the present invention may include any suitable for PET imaging. In certain embodiments, the radioisotope may include ^{60}Cu, ^{62}Cu, ^{61}Cu, ^{64}Cu and ^{68}Ga. In more specific embodiments, the metal radioisotope may be a generator-produced radioisotope, such as ^{62}Cu or ^{68}Ga, particularly ^{68}Ga. The radioisotope may be specifically selected because its method of generation is best compatible with the ultimate use of the radiolabeled agent.

Generator-produced ^{68}Ga may be produced by the decay of ^{68}Ge. ^{68}Ga produced in this manner is normally initially present in HCl or another acid. The ^{68}Ga may be substantially purified from the HCl or other acid in order to make it more suitable for use with many chelators and ligands. The ^{68}Ga generator and method of purifying ^{68}Ga are described in further detail in a U.S. Provisional Patent Application filed on January 20, 2004, titled "System and Method for an Automated Synthesis of Gallium-68 Generator-Based Radiopharmaceutical Agents". The ^{68}Ga used in specific embodiment of the present invention includes such purified ^{68}Ga.

Generator-produced ^{68}Ga may also be from other generator sources in selected embodiments. Such sources may include a Ge/Ga generator, such as the type produced by Isotope Products Laboratories (Valencia, CA) or Oak Ridge National Laboratories (Oak Ridge, TN).

The ligand component of radiolabeled agents of the present invention may be selected based on the tissue and physiological state to be imaged. Some ligands may be suitable to detect relatively static conditions. Others may allow dynamic imaging of conditions that change during the course of the PET imaging procedure.

The ligand component of radiolabeled agents of the present invention may include a wide variety of compounds suitable to target the agent to a selected biochemical marker. In specific embodiments, ligands may include small molecules, peptides, proteins, nucleic acids, purine or pyrimidine bases, sugar-containing molecules, nitroimidazole analogues, or other metabolically significant compounds. Such ligands may

take advantage of the relatively quick time frame in which ^{68}Ga may be prepared using a generator.

More specifically, ligands may include therapeutic drugs, receptor agonists and antagonists, molecules capable of binding to adhesion molecules, antibodies to cell surface proteins or fragments of such antibodies, a purine or pyrimidine base, and sugars or sugar-containing molecules.

Ligands may target a variety of physiological states and activities including: glycolysis (e.g. using a sugar-containing ligand, such as glucosamine, mannosamine, or galactosamine), hypoxia (e.g. using a nitroimidazole ligand, such as a 2-nitroimidazole or a 5-nitroimidazole), and DNA content (e.g. using a purine or pyrimidine base ligand, such as guanine or adenine).

Radiolabeled PET agents of the present invention may also include a solubility enhancer, which in some embodiments may be attached between the ligand and the chelator. In other embodiments, it may simply be attached to the ligand or chelator. The solubility enhancer functions to increase the solubility of the radiolabeled agent, normally in water or an aqueous solution. In specific embodiments, solubility enhancers may include ethylene diamine, hydrazine or a poly(amino acid), such as poly(aspartic acid), poly(glutamic acid), or poly (lysine).

Radiolabeled PET agents of the present invention may be produced generally by first creating the ligand and the chelator and combining the two. The metal radioisotope may then be added to the ligand-chelator complex soon before use of the agent. If a solubility

enhancer is present, it may be added before, during, or after formation of the ligand-chelator complex, but normally before addition of the metal radioisotope.

Chelators may be formed in a variety of ways depending upon the chemical makeup of the chelator itself and also the need for functional groups or sites to which the ligand or solubility enhancer may attach. Examples of chelator synthesis reactions are provided in FIGURES 3-5. Some chelators may be available commercially.

The method of creating a ligand depends largely on the nature of the ligand. Many ligands may be created by routine chemical synthesis. Others, such as short polypeptides, may require more complicated peptide synthesis or may be produced in a cellular system. Still others, such as antibodies or fragments thereof, may be best produced in a cellular system or an animal then purified and modified as needed. Methods of producing various suitable ligands are well known to the art. Many ligands, including guanine, adenine, glucosamine, and MN are available commercially.

In general, after their synthesis both the chelator and ligand are substantially purified before they are combined with one another. Addition of one or more protective groups, particularly to the ligand, may also be advisable prior to combination. Such protective groups may be removed after the chelator and ligand are combined. In a specific embodiment, the chelator and ligand covalently bond to one another to form a single compound. In another specific embodiment, the solubility enhancer is covalently bonded to the ligand-chelator complex to form a single compound.

After the ligand-chelator complex is formed, it may be further treated to enhance its preservation or ease of storage. For example, the complex may be lyophilized. Prior to addition of the metal radioisotope, the complex should be prepared such that it is capable of chelating the radioisotope. For example, a lyophilized complex may be reconstituted in an aqueous solution. Adjustments to pH and other properties useful to achieve chelation may also be made. If the solution will not be further purified prior to administration to a patient, it may also be adjusted to meet physiological requirements either before or after addition of the radioisotope. In certain embodiments, the solution may be heated to allow more thorough complexation. However, the length of time available after generation of the radioisotope should be taken into consideration when determining appropriate steps for adding the radioisotope to the ligand-chelator complex or any further processing.

In some embodiments of the invention, for example those in which the ligand includes a nitroimidazole analogue, it may be appropriate to also add a carrier. Addition of this carrier increases the concentration of metal overall, thereby improving chelation and actually increasing radiochemical yield in the completed solution of radiolabeled agent. For example, $GaCl_3$ may be added to improve radiochemical yield when a ^{68}Ga radioisotope is used.

The invention additionally includes methods of using radiolabeled PET agents containing specific ligands. Such uses include traditional PET techniques as well as dynamic PET. PET imaging may be used for initial diagnosis to detect the presence of a physiological state

or its severity. It may also be used to monitor the effects of treatment.

In specific embodiments, the radiolabeled agents may be used for PET imaging of tumors. Tumor imaging may be used to determine the pathology of the tumor or the progress of treatment. Specifically, tumors and their physiological states may be identified, *inter alia*, by glycolysis, incorporation of nucleic acid components, presence of selected receptor, apoptosis, and hypoxia.

In another specific embodiment, the radiolabeled agents may be used to detect hypoxia in patients thought to be suffering from stroke. Such detection may allow a definitive diagnosis of stroke. It may also provide information about areas deprived of oxygen as a result of the stroke and the duration of oxygen depravation, which may be useful in directing the appropriate response or in providing more useful rehabilitation to the patient afterwards, if necessary.

In other embodiments, radiolabeled agents of the present invention may also be used in detection of physiological characteristics related to neurological, cardiovascular and other diseases.

In still other embodiments, the radiolabeled agents may be used to detect the presence of a drug in the body, which may be useful for diagnosis or the evaluation of treatment effectiveness.

The following examples are included to demonstrate specific embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent techniques discovered by the inventors to

function well in the practice of the invention. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLES

Example 1:Synthesis of 9-[4-amino-3-(hydroxymethyl)butyl]guanine

N^2-(p-anisyldiphenylmethyl)-9-[(4-tosyl)-3-p-anisyldiphenylmethoxy-methylbutyl]guanine (Compound 1) was synthesized according to previously reported methods. (See Alauddin, M.M. & Conti, P.S., *Nucl. Med. Biol.* *25*:175-180 (1998).) Compound 1 (500mg, 0.52mmol) was placed in dry round bottom flask and dissolved in DMF. Sodium azide (160mg, 2.5mmol) was added to the reaction mixture and heated at 100°C for overnight with stirring. After reaction the mixture was cooled to room temperature and extracted with ethyl acetate twice. The organic extracts were washed with distilled water and evaporated *in vacuo* to give crude yield (100%) azido compound 2.

Compound 2 (453mg) was placed in a dry round bottom flask and dissolved in dry THF. A solution of triphenylphosphine (655mg, 2.5mmol) was add to stirred solution of azido compound 2 and stirred at 20 °C overnight. Concentrated HCl (5N, 1ml) was added to the solution and heated under reflux for 5 hours before being evaporated completely. The residue was mixed with water and washed with Ethyl acetate twice and pH was adjusted to 7-8. The water solution was lyophilized (Labconco, Kansas City, MO) to give de-protected trityl and free

amino compound 3 (100mg, 80% yield). Ninhydrin (2% in methanol) spray indicated the positivity of the amino group.

Compounds 1, 2 and 3 are further characterized as follows:

Compound 1; ^{1}H NMR ($CDCl_3$): δ 8.62 (s, 1H), 7.74 (d, 2H, aromatic, j=9.0Hz), 7.14-7.33 (m, 22H), 6.80 (d, 4H, J=9.0Hz), 6.74 (d, 4H, d=9.0Hz), 3.77 (s, 3H), 3.71 (s, 3H), 3.47-3.52 (m, 4H), 2.85-2.95 (m, 2H), 2.44 (s, 3H), 1.45-1.53 (m, 1H), 1.25-1.35 (m, 2H). FAB MS: 952 $(M+H)^+$. Exact mass calculated for $C_{57}H_{54}N_5O_5S$ $(M+H)^+$ 952.37 found 952.37.

Compound 2; ^{1}H NMR ($CDCl_3$): δ 8.04 (s, 1H), 7.22-7.45 (m, 20H), 6.86 (d, 4H, J=9.0Hz), 6.78 (d, 4H, J=9.0Hz), 3.79 (s, 3H), 3.76 (s, 3H), 3.55-3.65 (m, 2H), 3.25-3.35 (m, 2H), 1.63-1.73 (m, 2H), 1.32-1.42 (m, 1H), 1.16 (d, 2H, J=3.6Hz). FAB MS: 952 $(M+H)^+$. Exact mass calculated for $C_{50}H_{46}N_8O_4$ $(M+H)^+$ 823.36 found 823.4.

Compound 3; ^{1}H NMR ($CDCl_3$): δ 7.60 (s, 1H), 3.98 (t, 2H, J=7.2Hz), 3.46-3.61 (m, 2H), 2.90 (d, 2H, J=6.0Hz), 1.72 (m, 2H), 1.43 (m, 1H).

Example 2: Synthesis of N_2S_2-9-[4-amino-3-(hydroxymethyl)butyl]guanine conjugate (N_2S_2-Guan)

To dissolve N_2S_2, Sodium hydroxide (1N, 0.2ml) was added to a stirred solution of N_2S_2 (50mg, 0.19mmol) in water (5ml). To this colorless solution, sulfo-NHS (95.5mg, 0.44 mmol), EDAC (84.5mg, 0.44mmol) and finally amino compound 3 (50mg, 0.20mmol) were added. The mixture was stirred at room temperature for overnight. The mixture was dialyzed for 24 hours using Spectra/POR dialysis membrane with molecule cut-off at 500 (Spectrum

Medical Industries Inc., Houston TX). After dialysis, the products were lyophilized (Labconco, Kansas City, MO) to give final compound (N_2S_2-Guan) (50mg, 0.08mmol, yield 40%). N_2S_2-Guan; ^1H NMR ($CDCl_3$): δ 8.04 (s, 1H), 7.22-7.45 (m, 20H), 6.86 (d, 4H, J=9.0Hz), 6.78 (d, 4H, J=9.0Hz), 3.79 (s, 3H), 3.76 (s, 3H), 3.55-3.65 (m, 2H), 3.25-3.35 (m, 2H), 1.63-1.73 (m, 2H), 1.32-1.42 (m, 1H), 1.16 (d, 2H, J=3.6Hz). FAB MS: 569 $(M+H)^+$. Exact mass calculated for $C_{18}H_{27}N_8Na_3O_5S_2$ $(M+H)^+$ 568.56 found 568.93. The structure of N_2S_2-Guanine is shown in FIGURE 6.

Example 3: Radiolabelling of N_2S_2-Guan with ^{64}Cu

N_2S_2-Guanine (5mg) was dissolved in 0.2 ml water. ^{64}Cu-acetate solution (0.1 ml, 0.5 mCi) was added and well mixed with N_2S_2-Guan. The product was warmed up at 50 °C for 15 min. Finally water was added up to 1 ml with this solution. Radiochemical purity was determined by TLC (ITLC SG, Gelman Sciences, Ann Arbor, MI) eluted with Dichloromethane: Methanol : Ammonium acetate (85 : 14 : 1). From radio-TLC (Bioscan, Washington, DC) analysis, the radiochemical purity was more than 95%.

Example 4: Cell Cycle Uptake Assays

Cell cycles of human lung tumor cells (A549, 2M) were sorted using FACS. The cells at different cycles were plated to 12 wells tissue culture plate that contained 50,000 cells per each well. 2-4 μCi (0.074-0.148 MBq) of ^{64}Cu-N_2S_2-Guan or ^{64}Cu-N_2S_2 was added to each well. Cells were incubated with radiotracer at 37°C for 0.5, 2 or 4 hours. After incubation, cells were washed with ice-cold phosphate-buffered saline (PBS) twice and trypsinized with 0.5 ml of trypsin solution. Then cells were collected test tubes and counted for radioactivity

(Packard Instrument, Downers Grove, IL). Data are expressed in mean±SD percent uptake ratio of triplicate. The data showed that ^{64}Cu-N_2S_2-Guan had high uptake in cell cycle S-phase compared to control groups (FIGURE 7).

Example 5: In Vitro Cellular Uptake Studies of ^{64}Cu-N_2S_2-Guan and ^{64}Cu-N_2S_2

Two different cell lines, rat breast adenocarcinoma and U87 human glioma cells, were used for in vitro cellular uptake assays. The cells were plated to 12 well tissue culture plates that contained 50,000 cells per each well. 2-4 μCi (0.074-0.148 MBq) of ^{64}Cu-N_2S_2-Guan or ^{64}Cu-N_2S_2 was added to each well. Cells were incubated with radiotracer at 37°C for 0.5, 2 or 4 hours. After incubation, cells were washed with ice-cold phosphate-buffered saline (PBS) twice and trypsinized with 0.5 ml of trypsin solution. Then cells were collected in test tubes and counted for radioactivity (Packard Instrument, Downers Grove, IL). Data are expressed in mean±SD percent uptake ratio of triplicates. The data showed that ^{64}Cu-N_2S_2-Guan had high uptake compared to control groups (FIGURE 8).

Example 6: Autoradiographic Studies

Brain autoradiograms were performed using U87 tumor-bearing nude mouse. The mice were sacrificed at 0.8 and 2 hours after ^{64}Cu- N_2S_2-Guan or ^{64}Cu- N_2S_2 injection, and immediately the brain was fixed in carboxymethyl cellulose (4%) and put on dry ice for 10 minutes until totally frozen. The frozen brain was mounted onto a cryostat (LKB 2250 cryomicrotome) and cut into 30 μm coronal sections. Each section was placed in contact with a multipurpose phosphor imaging screen (MP, 7001480)

and exposed for 15 hours. The screen was read by quantitative image analyzer (Cyclone Storage Phosphor System, Packard, Meridien, CT). At 0.8 h, tumors could be visualized by both agents, suggesting early blood flow images. However, at 2h the tumor could be visualized only by ^{64}Cu- N_2S_2-Guan (FIGURES 9 and 10). The findings indicate that ^{64}Cu-N_2S_2-Guan is a tumor-seeking agent.

Example 7: Radiolabelling of N_2S_2-Guan with ^{68}Ga

For ^{68}Ga-labeling, ^{68}Ga was eluted from a Ge/^{68}Ga generator (Isotope Products Laboratories, Valencia, CA) using 1N HCl. The acidic solution was evaporated to dryness with either $GaCl_3$ carrier added (10 μg) or no carrier added. The solution was reconstituted in water. N_2S_2-*Guan* (5mg) dissolved in 0.2 ml water was then added to the radioactive solution. The complexation process was completed by heating at 65-70°C for 30 min. From radio-TLC analysis (Bioscan, Washington, DC), the radiochemical purity was more than 90%.

Example 8: *In vitro* Cellular Uptake Studies of ^{68}Ga-N_2S_2-Guan and ^{68}Ga-N_2S_2

Two different cancer cell lines (human lung NSCLC A549, breast 13762) were used for cellular uptake assays. The cell lines were obtained from American Type Culture Collection (Rockville, MD). The cells were plated on 12 well tissue culture plate that contained 50,000 cells per each well. 4 μCi (0.148 MBq) of ^{68}Ga- N_2S_2-Guan or ^{68}Ga-N2S2 (0.1 mg/well) was added to each well. Cells were incubated with the radiotracers at 37°C for different time intervals. After incubation, cells were washed with ice-cold phosphate-buffered saline (PBS) twice and trypsinized with 0.5 ml of trypsin solution. Then cells

were collected and the radioactivity was measured by gamma counter. Data are expressed in mean±SD percent uptake ratio of three measurements. There was an increased uptake of ^{68}Ga- N_2S_2-Guan as a function of incubation time in the lung cancer cell line (FIGURE 11). Uptake of ^{68}Ga- N_2S_2 as the control group was less that 0.2% at any time point. (FIGURE 11).

Example 9: *In vivo* Animal microPET imaging

Female Fischer 344 rats (150±25 g) (Harlan Sprague-Dawley, Indianapolis, IN) were inoculated subcutaneously with 0.1 ml of mammary tumor cells from the 13762 tumor cell line suspension (10^6 cells/rat, a tumor cell line specific to Fischer rats) into the hind legs. Imaging studies were performed 12 to 15 days after inoculation. Tumor sizes of approximately 1-1.5 cm were measured. MicroPET was used for PET imaging studies. Each rat was administered the radiotracer (0.5 mCi/rat) and the images were acquired immediately and up to 2 hrs after administration. A selected coronal image of one rat is shown in FIGURE 12.

Example 10: Synthesis of ^{68}Ga-N_2S_2-Glucosamine

Sodium hydroxide (1N, 1ml) was added to a stirred solution of N_2S_2 (110 mg, 0.41 mmol) in water (5 ml). To this colorless solution, sulfo-NHS (241.6 mg, 1.12 mmol) and EDC (218.8 mg, 1.15 mmol) were added. D-Glucosamine hydrochloride salt (356.8 mg, 1.65 mmol) was then added. The mixture was stirred at room temperature for 24 hours and pH was adjusted to 6.4-7.0. The mixture was dialyzed for 48 hours using Spectra/POR molecular porous membrane with cut-off at 500 (Spectrum Medical Industries Inc., Houston, TX). After dialysis, the product was frozen

dried using a lyophilizer (Labconco, Kansas City, MO). The product weighed 291 mg (yield 60%). ^{1}H-NMR (D_2O) δ 2.60-2.90 (m, 4H and -$\underline{CH_2}$-SH of EC), 2.95 (t, 2H, glucosamine 5-$\underline{CH}$-CH_2OH) 3.20 (d, 4H, glucosamine 6-$\underline{CH_2}$OH), 3.30-3.95(m, 6H glucosamine1,3,4-$\underline{CH}$ and 4H $\underline{CH_2}$-SH of EC) 3.30-3.66 (m, 4H, CH_2-$\underline{CH_2}$- of EC), 4.15-4.30 (t, 2H, NH-$\underline{CH}$-CO of EC), 4.60 (d, 2H, glucosamine 2-$\underline{CH}$ -NH_2). FAB MS m/z 591 (M^+, 20). ^{68}Ga-labeling was similar to that described above for ^{68}Ga- N_2S_2-Guan (Example 7). N_2S_2-*glucosamine* (10mg) dissolved in 0.2 ml water was added to the radioactive solution. The complexation process was completed by heating at 65-70°C for 30 min.

Example 11: In vitro cell culture studies

The same conditions were used as described in Example 4. High uptake of ^{68}Ga-N_2S_2-Glucosamine was observed as compared with a ^{68}Ga- N_2S_2 control (FIGURE 13).

Example 12: *In vivo* Animal microPET imaging

Female Fischer 344 rats (150±25 g) (Harlan Sprague-Dawley, Indianapolis, IN) were inoculated subcutaneously with 0.1 ml of mammary tumor cells from the 13762 tumor cell line suspension (10^6 cells/rat, a tumor cell line specific to Fischer rats) into the hind legs. Imaging studies were performed 12 to 15 days after inoculation. Tumor sizes of approximately 1-1.5 cm were measured. MicroPET was used for PET imaging studies. Each rat was administered the radiotracer (0.5 mCi/rat) and the images were acquired immediately and up to 2 hrs. FIGURES 14A and 14B depict the results of dynamic tumor uptake of ^{68}Ga-N_2S_2-Glucosamine in a microPET imaging study. Further comparison of the imaging quality of ^{68}Ga-N_2S_2-Glucosamine and ^{18}FDG is shown in FIGURES 15A and 15B.

Example 13: Synthesis of N_2S_2-Metronidazole (N_2S_2-MN)

Sodium hydroxide (2N, 0.2 ml) was added to a stirred solution of EC (134 mg, 0.50 mmol) in water (5 ml). To this colorless solution, sulfo-NHS (217 mg, 1.0 mmol) and EDC (192 mg, 1.0 mmol) were added. MN-NH_2 dihydrochloride salt (340 mg, 2.0 mmol) was then added. The mixture was stirred at room temperature for 24 hours. The mixture was dialyzed for 48 hrs using Spectra/POR molecular porous membrane with cut-off at 500 (Spectrum Medical Industries Inc., Houston, TX). After dialysis, the product was freeze dried using a lyophilizer (Labconco, Kansas City, MO). The product weighed 315 mg (yield 55%). ^{1}H-NMR (D_2O) δ 2.93 (s, 6H, nitroimidazole-$\underline{CH_3}$), 2.60-2.95 (m, 4H and -$\underline{CH_2}$-SH of EC), 3.30-3.66 (m, 8H, ethylenediamine of EC and nitromidazole-CH_2-$\underline{CH_2}$-NH_2), 3.70-3.99 (t, 2H, NH-$\underline{CH}$-CO of EC), 5.05 (t, 4H, metronidazole-$\underline{CH_2}$-CH_2-NH_2) (s, 2H, nitroimidazole C=$\underline{CH}$). FAB MS m/z 572 (M^+, 20). ^{68}Ga-labeling was conducted in a manner similar to that for ^{68}Ga- N_2S_2-Guan (Example 7). *N_2S_2-MN* (10mg) dissolved in 0.2 ml water was added to the radioactive solution. The complexation process was completed by heating at 65-70°C for 30 min.

Example 14: *In vitro* cell culture studies

The same conditions were used as described in Example 4. High uptake of ^{68}Ga- N_2S_2-MN was observed (FIGURE 16).

Example 15: *In vivo* Animal microPET imaging

Female Fischer 344 rats (150±25 g) (Harlan Sprague-Dawley, Indianapolis, IN) were inoculated subcutaneously

with 0.1 ml of mammary tumor cells from the 13762 tumor cell line suspension (10^6 cells/rat, a tumor cell line specific to Fischer rats) into the hind legs. Imaging studies were performed 12 to 15 days after inoculation. Tumor sizes of approximately 1-1.5 cm were measured. microPET was used for PET imaging studies. Each rat was administered the radiotracer (0.5 mCi/rat) and the images were acquired immediately and up to 2 hrs after administration. A selected coronal image of one rat is shown in FIGURE 17.

Although only exemplary embodiments of the invention are specifically described above, it will be appreciated that modifications and variations of these examples are possible without departing from the spirit and intended scope of the invention.

CLAIMS

1. A radiolabeled PET agent comprising:

a metal radioisotope;

a chelator; and

a pyrimidine or purine base ligand.

2. The radiolabeled agent of Claim 1, further comprising the metal radioisotope selected from the group consisting of: ^{60}Cu, ^{62}Cu, ^{61}Cu, ^{64}Cu, ^{68}Ga, and any combination thereof.

3. The radiolabeled agent of Claim 1, further comprising generator-produced ^{68}Ga substantially free of acid.

4. The radiolabeled agent of Claim 1, wherein the chelator further comprises nitrogen, oxygen, or sulfur.

5. The radiolabeled agent of Claim 1, wherein the chelator comprises an O_2S_2, NS_3, N_2S_2 or NS_3 moiety.

6. The radiolabeled agent of Claim 1, further comprising the ligand operable to detect DNA content.

7. The radiolabeled agent of Claim 1, wherein the ligand comprises adenine or guanine.

8. The radiolabeled agent of Claim 1, further comprising the agent operable in dynamic PET imaging.

9. The radiolabeled agent of Claim 1, further comprising a solubility enhancer.

10. The radiolabeled agent of Claim 9, further comprising the solubility enhancer selected from the group consisting of: ethylene diamine, hydrazine, poly(aspartic acid), poly(glutamic acid), or poly(lysine), other poly(amino acid), and any combinations thereof.

11. A radiolabeled PET agent comprising:

a metal radioisotope;

a chelator; and

a sugar-containing ligand.

12. The radiolabeled agent of Claim 11, further comprising the radioisotope selected from the group consisting of: ^{60}Cu, ^{62}Cu, ^{61}Cu, ^{64}Cu, ^{68}Ga, and any combination thereof.

13. The radiolabeled agent of Claim 11, further comprising generator-produced ^{68}Ga substantially free of acid.

14. The radiolabeled agent of Claim 11, wherein the chelator further comprises nitrogen, oxygen, or sulfur.

15. The radiolabeled agent of Claim 11, wherein the chelator comprises an O_2S_2, NS_3, N_2S_2 or NS_3 moiety.

16. The radiolabeled agent of Claim 11, further comprising the ligand operable to detect glycolysis.

17. The radiolabeled agent of Claim 11, further comprising the sugar-containing molecule selected from the group consisting of: glucosamine, galactosamine, mannosamine, and any combination thereof.

18. The radiolabeled agent of Claim 11, further comprising a solubility enhancer.

19. The radiolabeled agent of Claim 18, further comprising the solubility enhancer selected from the group consisting of: ethylene diamine, hydrazine, poly(aspartic acid), poly(glutamic acid), or poly(lysine), other poly(amino acid), and any combinations thereof.

20. A radiolabeled PET agent comprising:

a metal radioisotope;

a chelator; and

a nitroimidazole analogue ligand.

21. The radiolabeled agent of Claim 20, further comprising the radioisotope selected from the group consisting of: ^{60}Cu, ^{62}Cu, ^{61}Cu, ^{64}Cu, ^{68}Ga, and any combination thereof.

22. The radiolabeled agent of Claim 20, further comprising generator-produced ^{68}Ga substantially free of acid.

23. The radiolabeled agent of Claim 20, wherein the chelator further comprises nitrogen, oxygen, or sulfur.

24. The radiolabeled agent of Claim 20, wherein the chelator comprises an O_2S_2, NS_3, N_2S_2 or NS_3 moiety.

25. The radiolabeled agent of Claim 20, further comprising the ligand operable to detect hypoxia.

26. The radiolabeled agent of Claim 20, further comprising the nitroimidazole analogue ligand selected from the group consisting of: a 2-nitroimidazole, a 5-nitroimidazole, metronidazole, and any combination thereof.

27. The radiolabeled agent of Claim 20, further comprising a solubility enhancer.

28. The radiolabeled agent of Claim 27, further comprising the solubility enhancer selected from the group consisting of: ethylene diamine, hydrazine, poly(aspartic acid), poly(glutamic acid), or poly(lysine), other poly(amino acid), and any combinations thereof.

29. A radiolabeled PET agent comprising:

generator-produced ^{68}Ga;

a chelator; and

a ligand.

30. The radiolabeled agent of Claim 29, further comprising the generator-produced ^{68}Ga substantially free of acid.

31. The radiolabeled agent of Claim 29, wherein the chelator further comprises nitrogen, oxygen, or sulfur.

32. The radiolabeled agent of Claim 29, wherein the chelator comprises an O_2S_2, NS_3, N_2S_2 or NS_3 moiety.

33. The radiolabeled agent of Claim 29, further comprising a solubility enhancer.

34. The radiolabeled agent of Claim 33, further comprising the solubility enhancer selected from the group consisting of: ethylene diamine, hydrazine,

poly(aspartic acid), poly(glutamic acid), or poly(lysine), other poly(amino acid), and any combinations thereof.

35. The radiolabeled agent of Claim 29, further comprising the ligand operable to target the agent to a selected biochemical marker or drug.

36. The radiolabeled agent of Claim 29, further comprising the ligand selected from the group consisting of: small molecules, peptides, proteins, nucleic acids, purine or pyrimidine bases, metabolically significant compounds, therapeutic drugs, receptor agonists, receptor antagonists, molecules capable of binding to adhesion molecules, molecules capable of binding to cell-surface receptors, antibodies and fragments thereof, sugars, sugar-containing compounds, nitroimidazole analogues, and any combinations thereof.

37. The radiolabeled agent of Claim 29, wherein the ligand comprises annexin V and the radiolabeled agent is operable to detect apoptosis.

38. The radiolabeled agent of Claim 29, wherein the ligand comprises a receptor ligand and the radiolabeled agent is operable to detect the presence of a receptor.

39. The radiolabeled agent of Claim 29, wherein the ligand comprises TRAIL and the radiolabeled agent is operable to detect the presence of a TRAIL receptor.

40. The radiolabeled agent of Claim 29, wherein the ligand comprises EGF and the radiolabeled agent is operable to detect the presence of an EGF receptor.

41. The radiolabeled agent of Claim 29, wherein the ligand comprises folate and the radiolabeled agent is operable to detect the presence of a folate receptor.

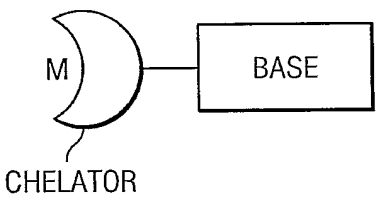


FIG. 1A

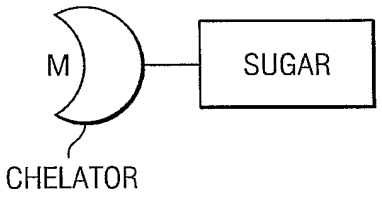


FIG. 1B

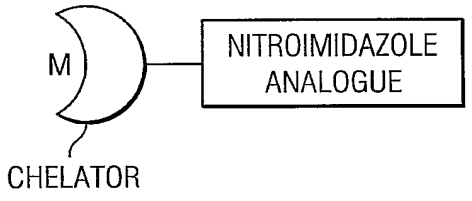


FIG. 1C

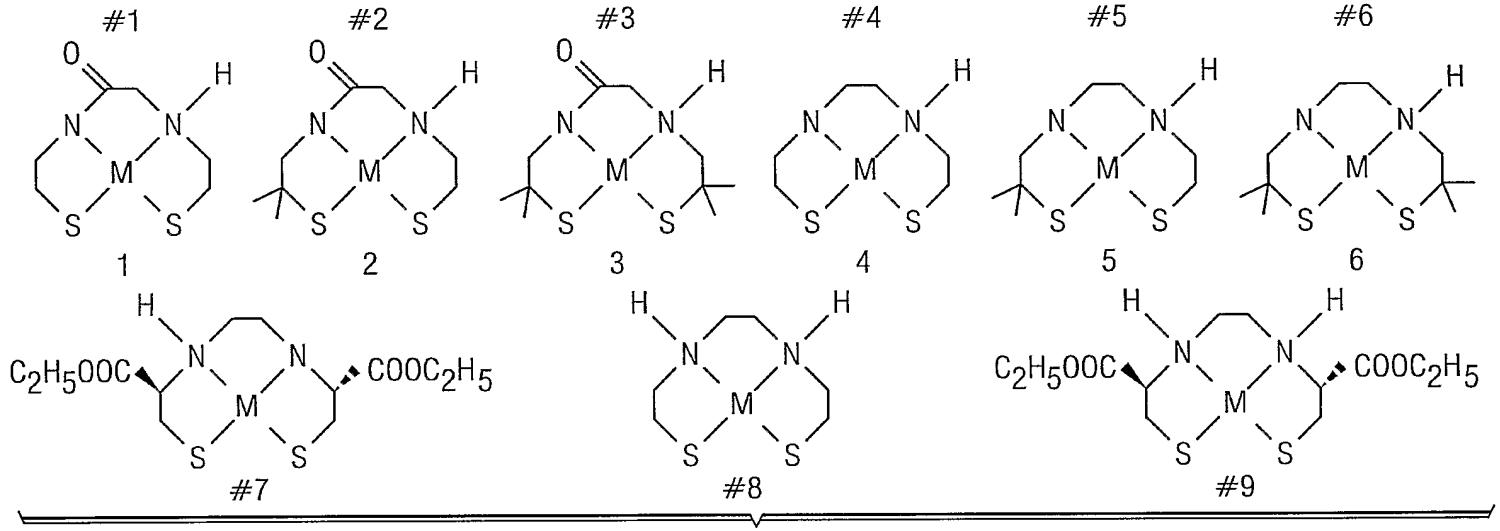


FIG.2

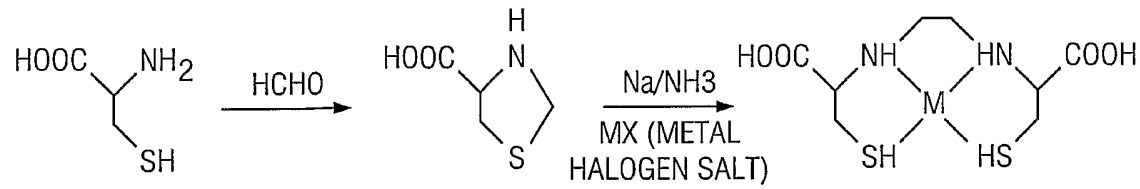

HOOC
NH2
SH
HCHO
H
HOOC
N
S
Na/NH3
MX (METAL
HALOGEN SALT)
HOOC
NH
HN
COOH
M
SH
HS

FIG.3

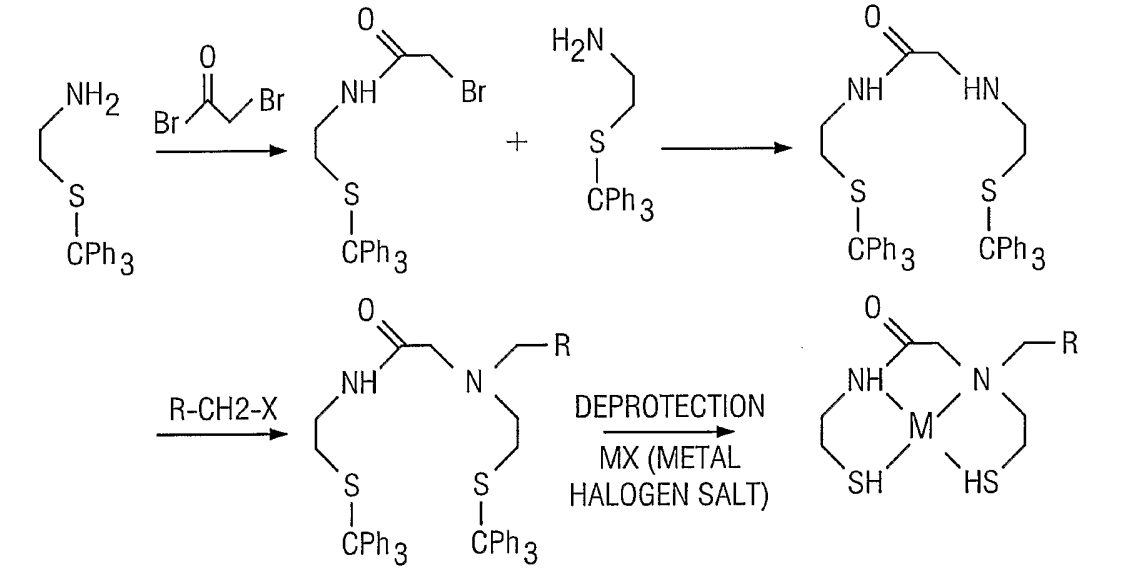

NH2
S
CPh3
O
Br
Br
NH
Br
S
CPh3
+
H2N
S
CPh3
O
NH
HN
S
CPh3
S
CPh3
R-CH2-X
O
NH
N
R
S
CPh3
S
CPh3
DEPROTECTION
MX (METAL
HALOGEN SALT)
O
NH
N
R
M
SH
HS

FIG.4

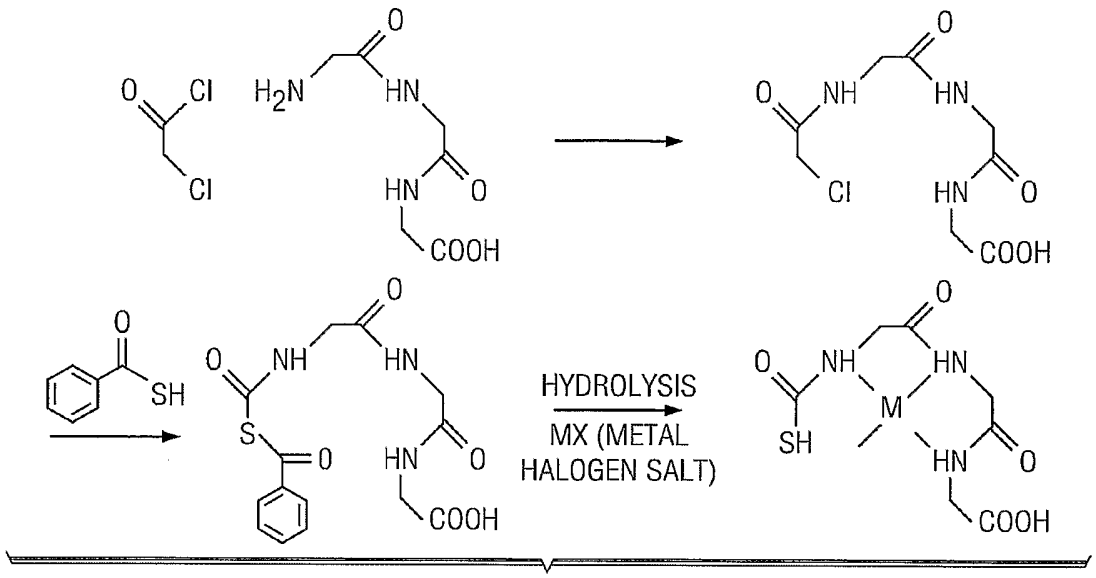


FIG.5

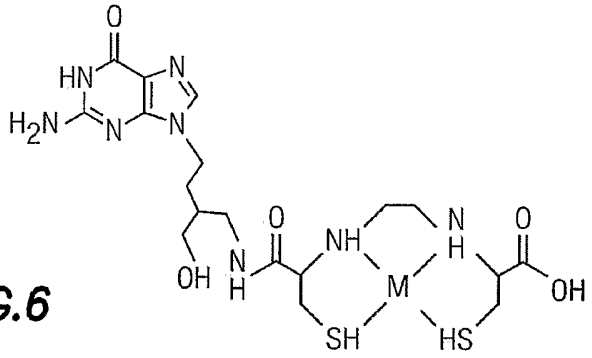


FIG.6

FIG.12

FIG.7

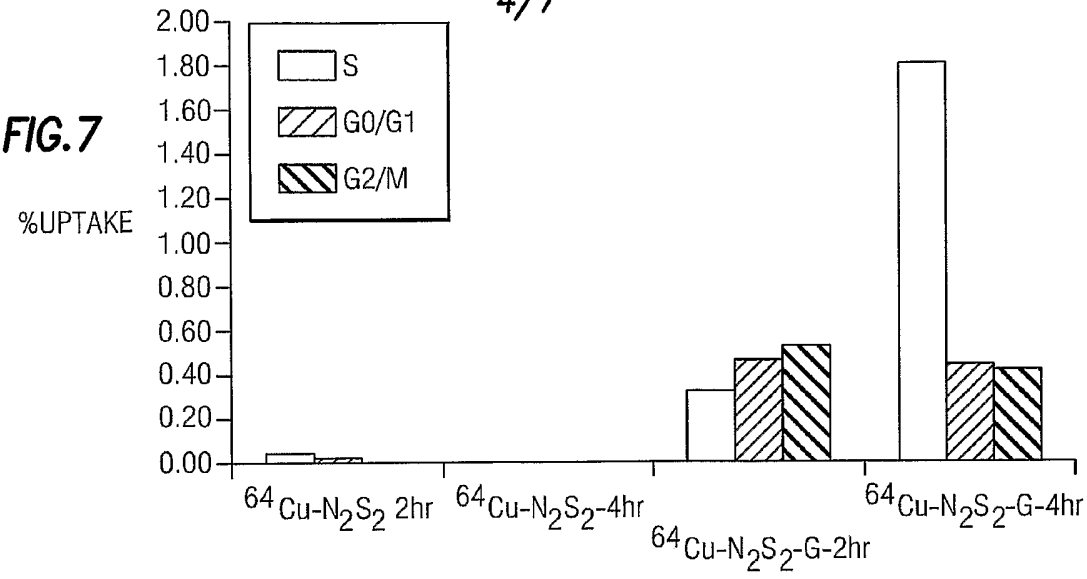


FIG.8

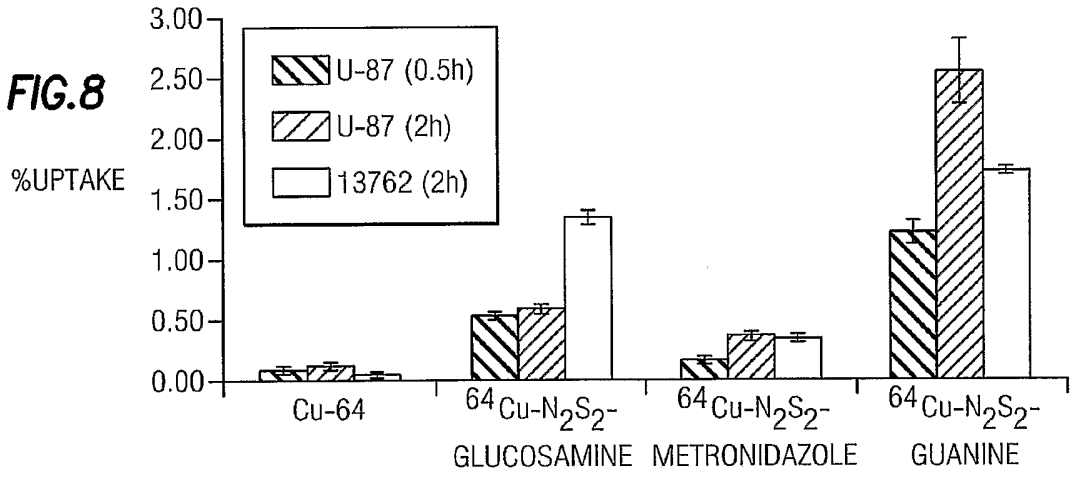
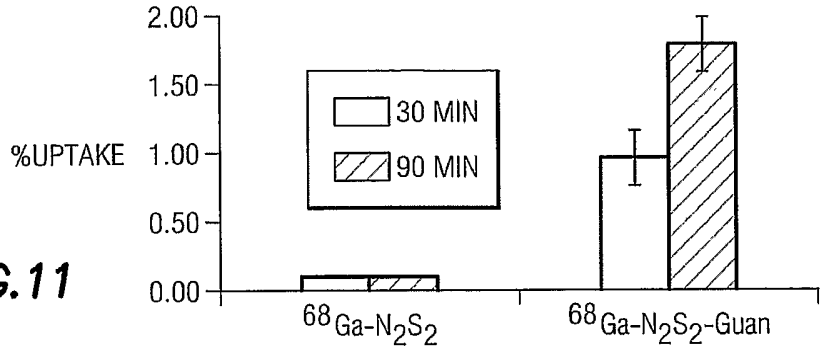


FIG.11



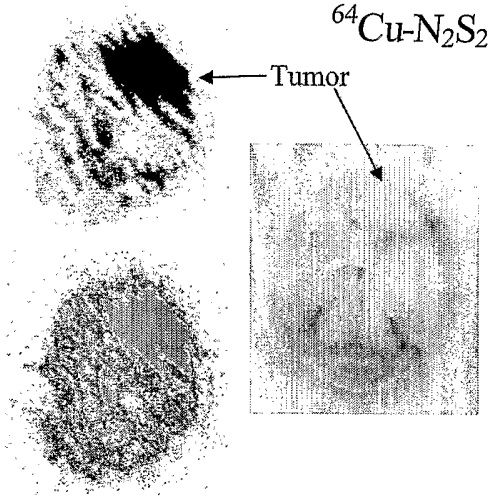


FIG.9

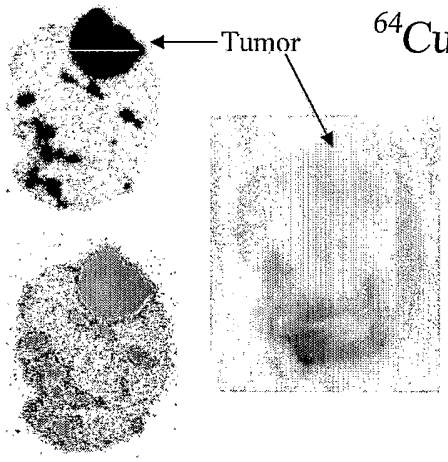


FIG.10

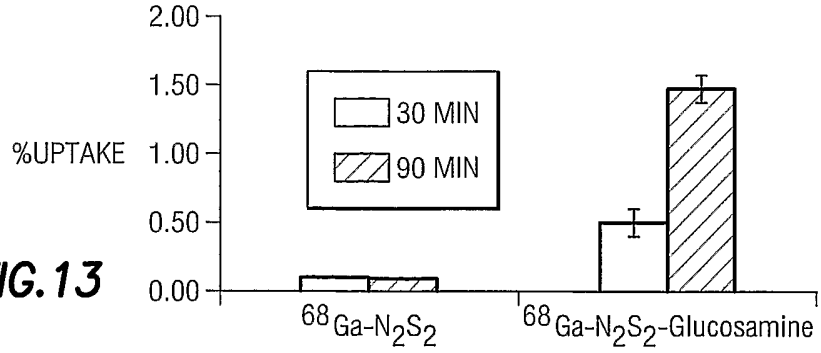


FIG.13

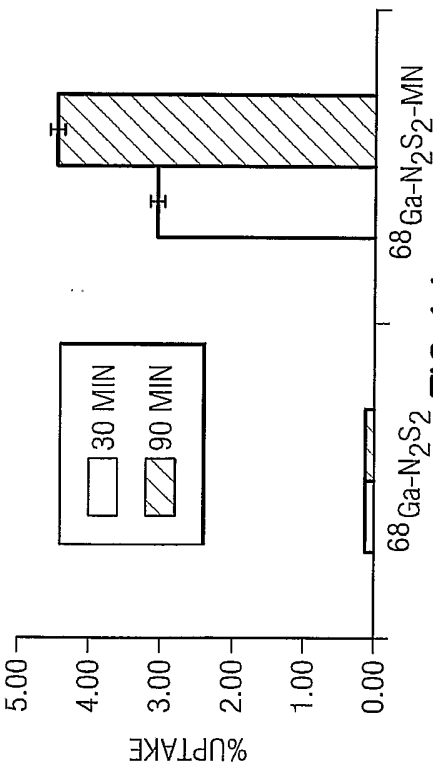


FIG. 14

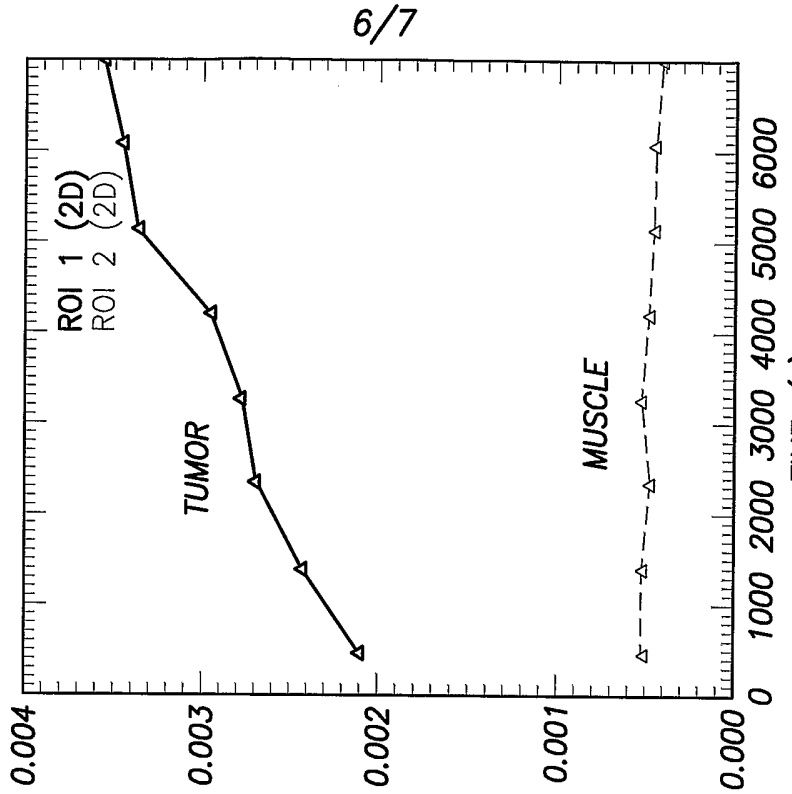


FIG. 15B

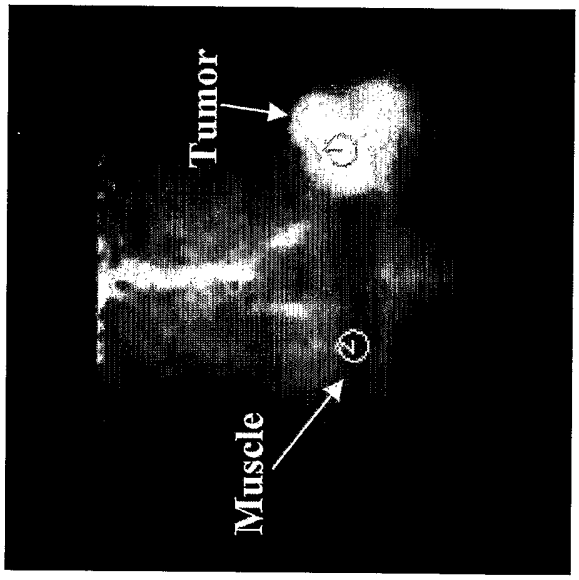
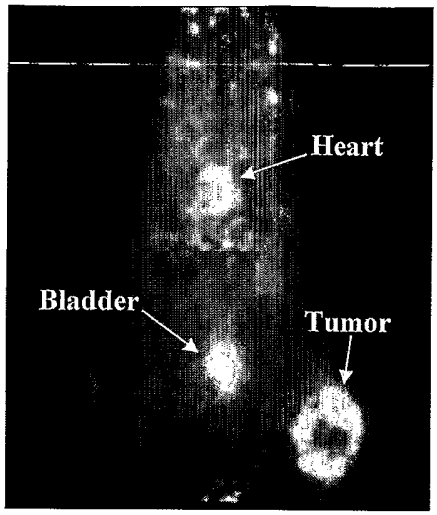
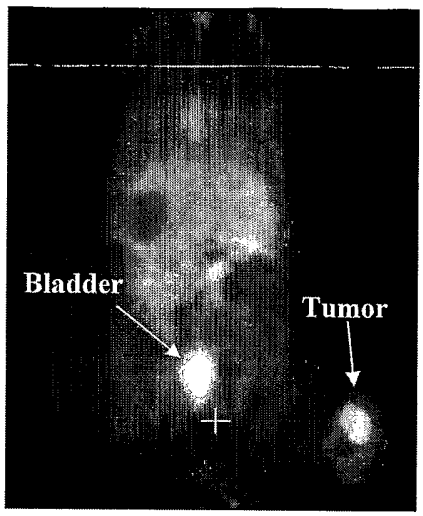


FIG. 15A



^{68}Ga-N_2S_2-Glucosamine

FIG. 16A



^{18}F-FDG

FIG. 16B

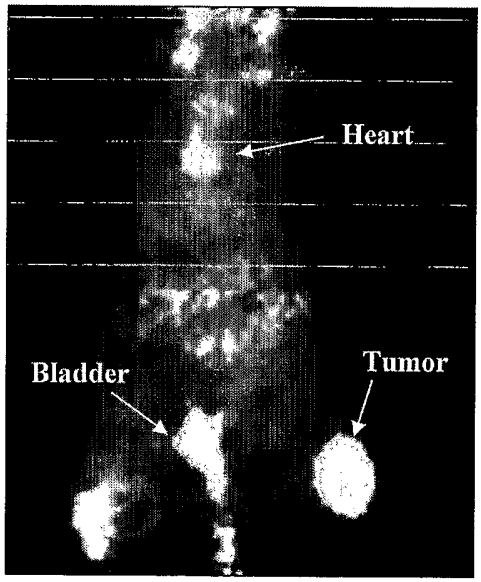


FIG. 17