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Pandey et al.

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(45) **Date of Patent: Aug. 29, 2006**

(54) **CHLORIN AND BACTERIOCHLORIN-BASED DIFUNCTIONAL AMINOPHENYL DTPA AND N_2S_2 CONJUGATES FOR MR CONTRAST MEDIA AND RADIOPHARMACEUTICALS**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 227 days.

(21) Appl. No.: **10/177,129**

(22) Filed: **Jun. 21, 2002**

(65) **Prior Publication Data**

US 2003/0053949 A1 Mar. 20, 2003

Related U.S. Application Data

(63) Continuation-in-part of application No. 09/739,155, filed on Dec. 18, 2000, now Pat. No. 6,534,040.

(60) Provisional application No. 60/171,961, filed on Dec. 23, 1999.

(51) **Int. Cl.**
A61B 5/055 (2006.01)

(52) **U.S. Cl.** **424/9.362**; 424/1.11; 424/9.1; 424/1.65; 424/9.3; 424/9.361; 534/14; 540/145

(58) **Field of Classification Search** 424/1.11, 424/1.65, 9.1, 9.3, 9.4, 9.36, 9.361, 9.362; 534/7, 10–14; 540/9.362, 145
See application file for complete search history.

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US 6,159,469, 12/2000, Choi et al. (withdrawn)

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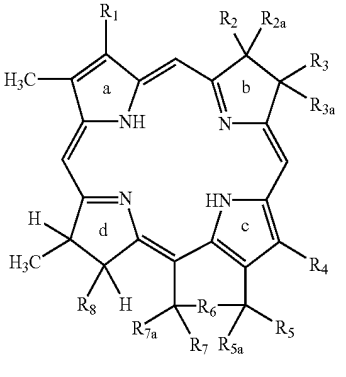
Primary Examiner—Dameron Jones
(74) *Attorney, Agent, or Firm*—Michael L. Dunn

(57) **ABSTRACT**

A compound comprising a chemical combination of a photodynamic tetra-pyrrolic compound with a plurality of radionuclide element atoms such that the compound may be used to enhance MR imaging and also be used as a photodynamic compound for use in photodynamic therapy to treat hyperproliferative tissue. The preferred compounds have the structural formula:



where R_1, R_2, R_{2a} R_3, R_{3a} R_4, R_5, R_{5a} R_6, R_7, R_{7a}, and R_8 cumulatively contain at least two functional groups that will complex or combine with an MR imaging enhancing element or ion. The compound is intended to include such complexes and combinations and includes the use of such compounds for MR imaging and photodynamic therapy treatment of tumors and other hyperproliferative tissue.

48 Claims, 17 Drawing Sheets
(1 of 17 Drawing Sheet(s) Filed in Color)

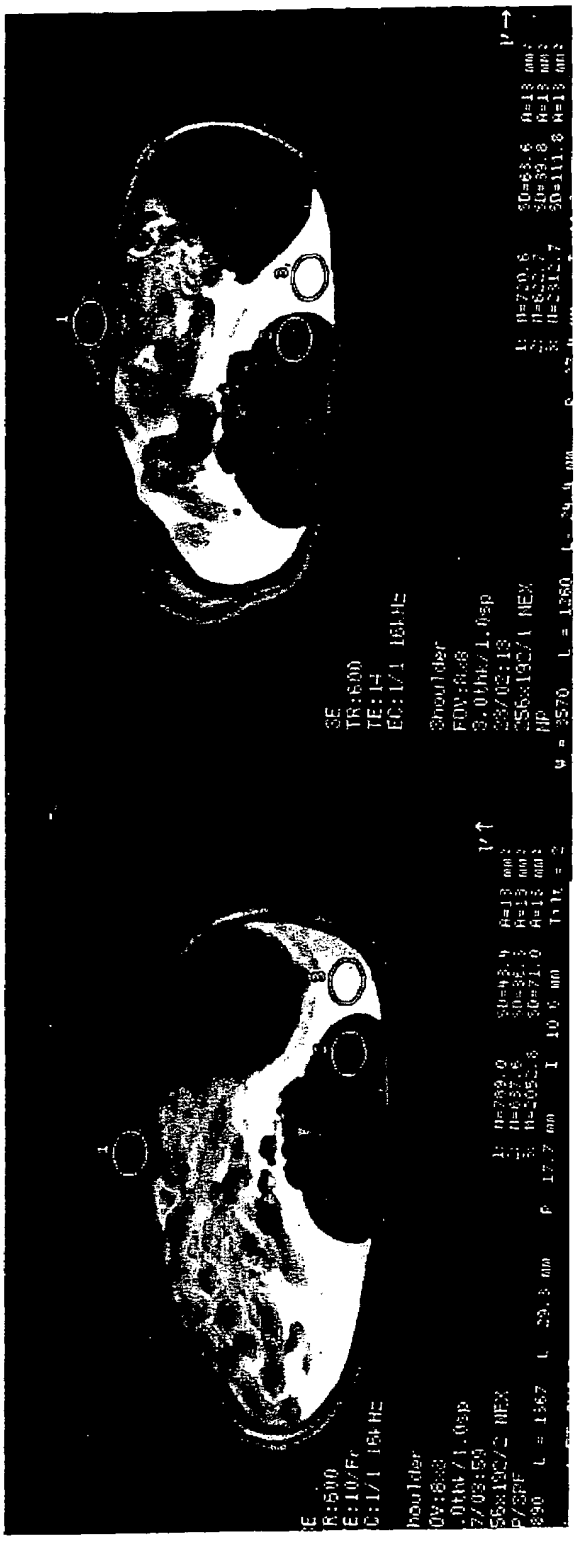


FIG. 1

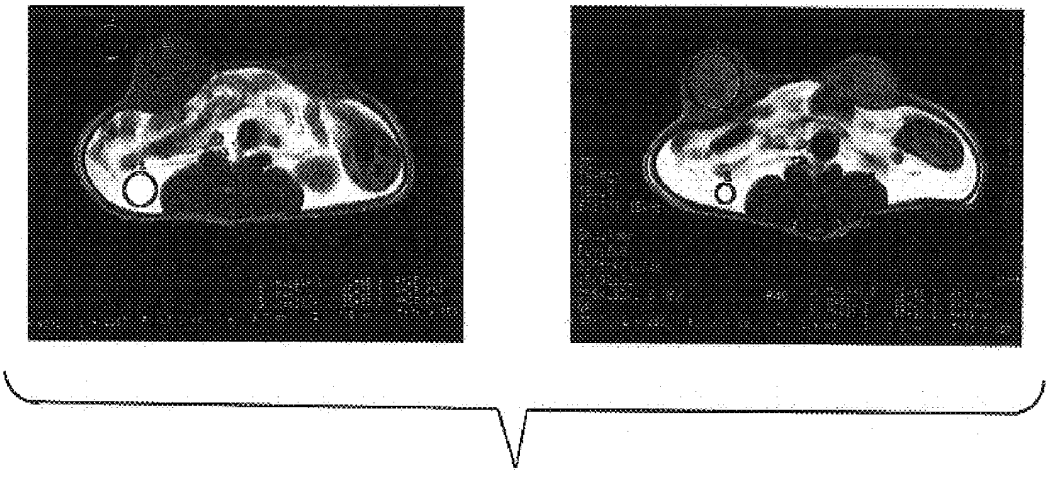


FIG. 2

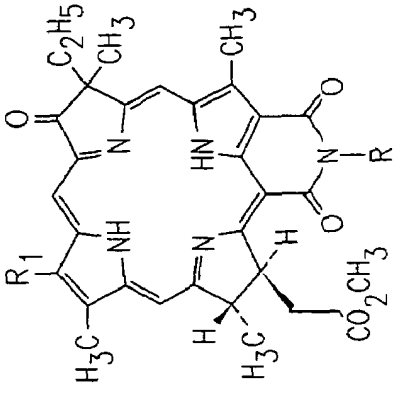


FIG. 3a

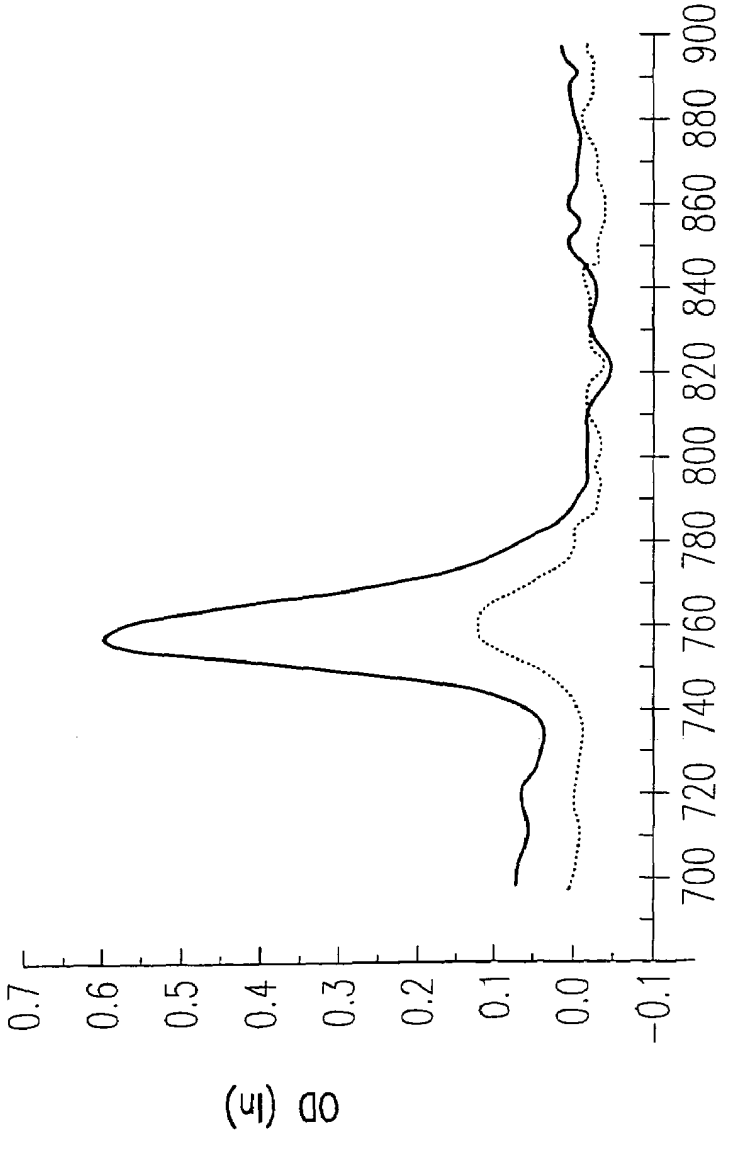

OD (ln)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
−0.1
700 720 740 760 780 800 820 840 860 880 900
nm

FIG. 3

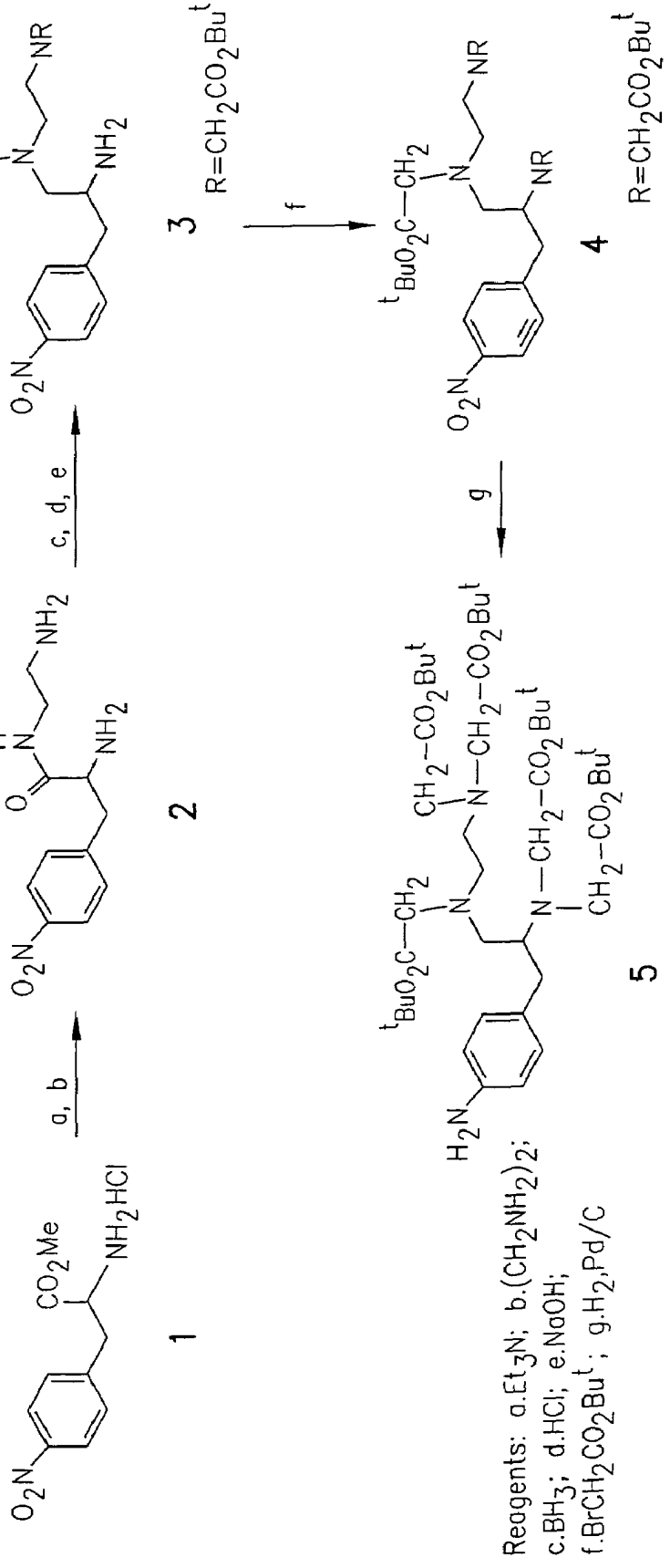

Reagents: a.Et3N; b.(CH2NH2)2;
c.BH3; d.HCl; e.NaOH;
f.BrCH2CO2But; g.H2,Pd/C

FIG. 4

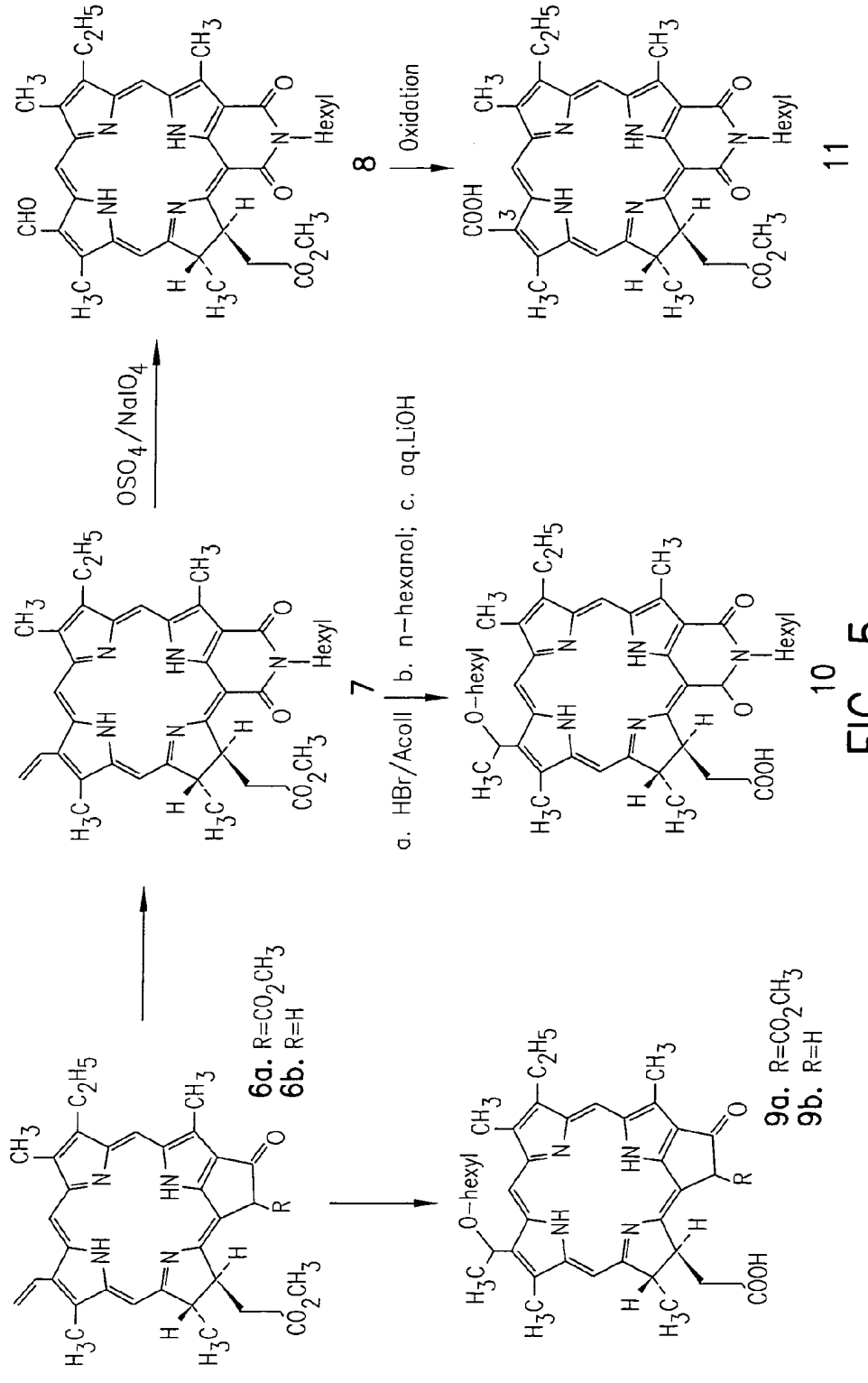

6a. R=CO2CH3
6b. R=H
OSO4/NaIO4
8
Oxidation
11
7
a. HBr/AcoII
b. n-hexanol; c. aq.LiOH
10
9a. R=CO2CH3
9b. R=H

FIG. 5

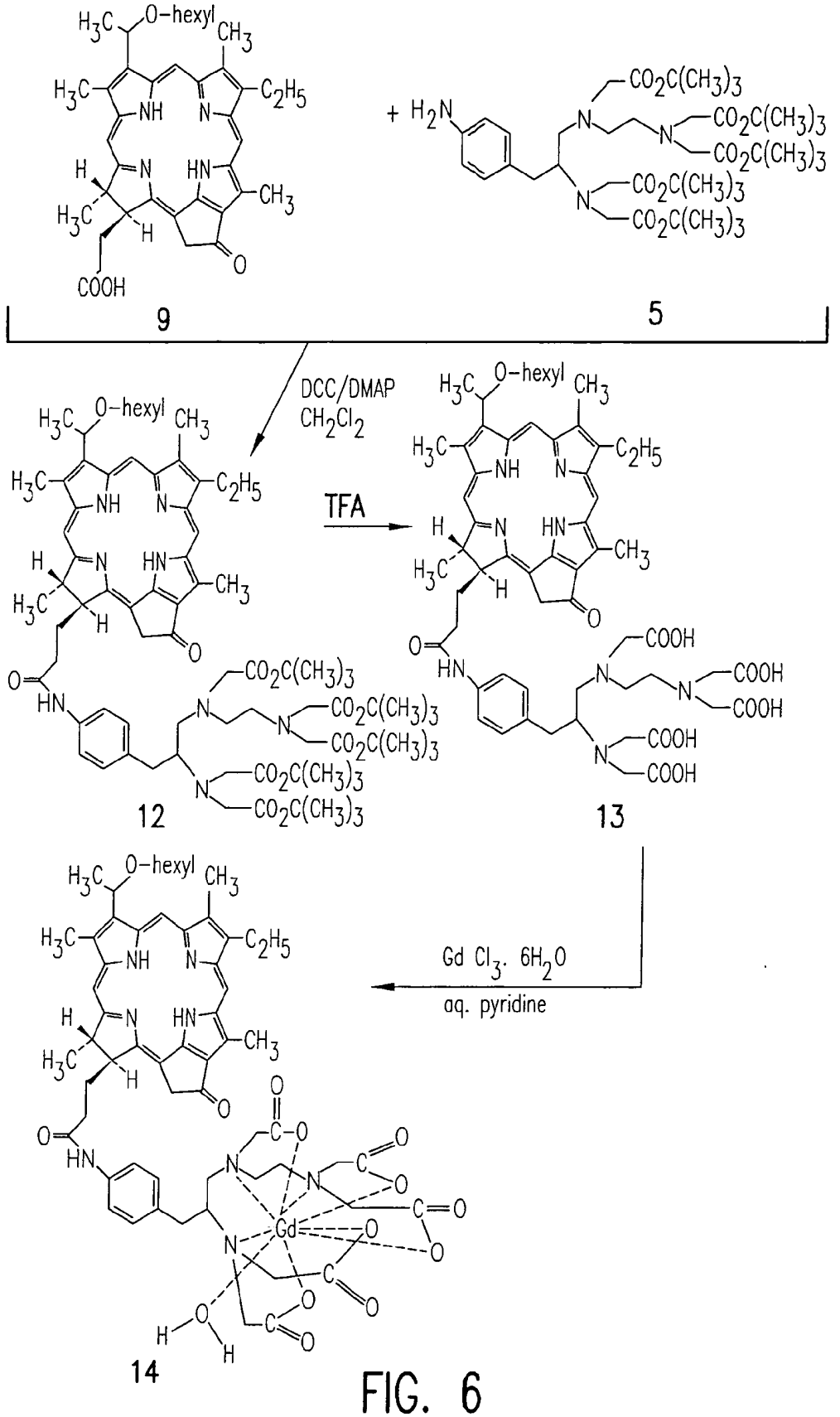


FIG. 6

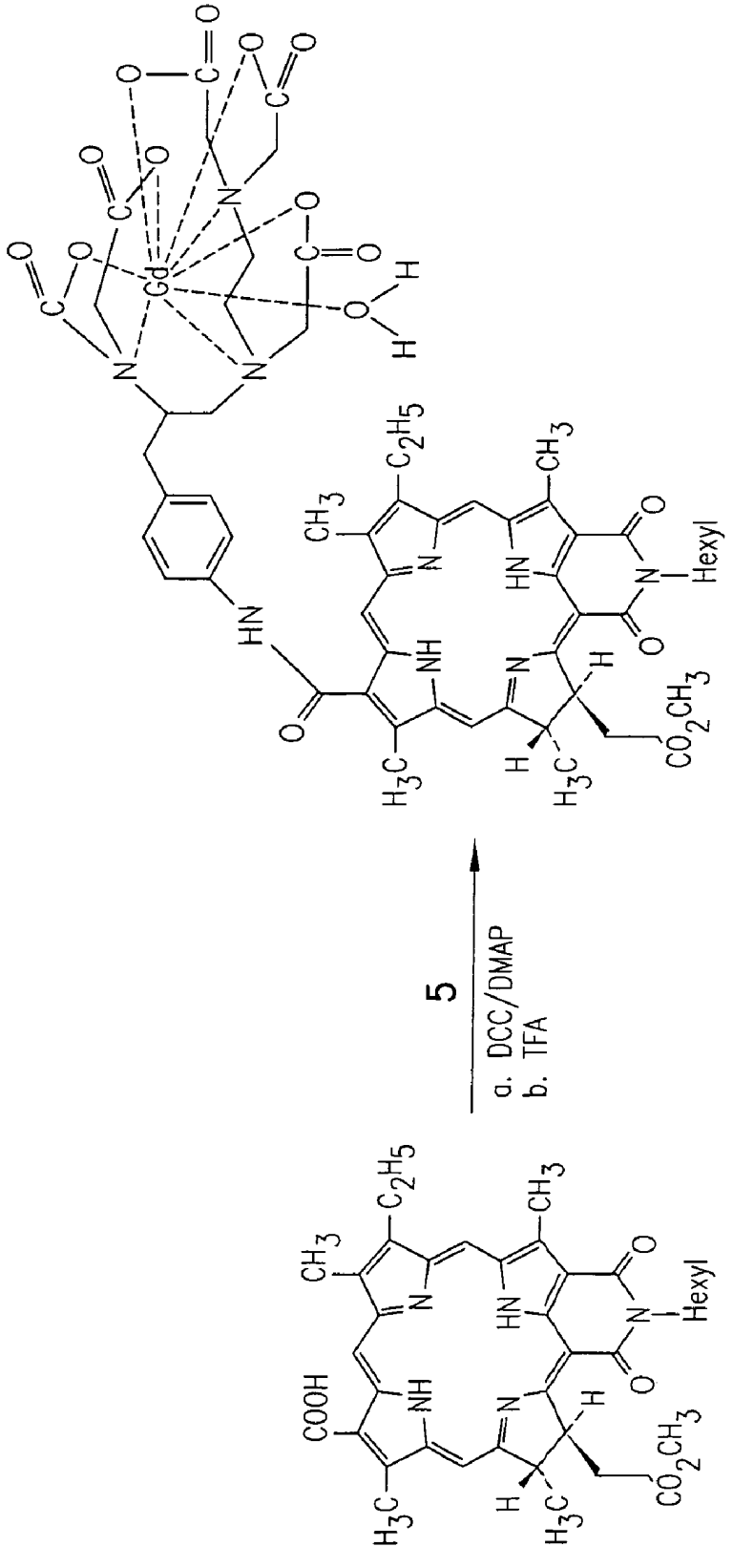


FIG. 7

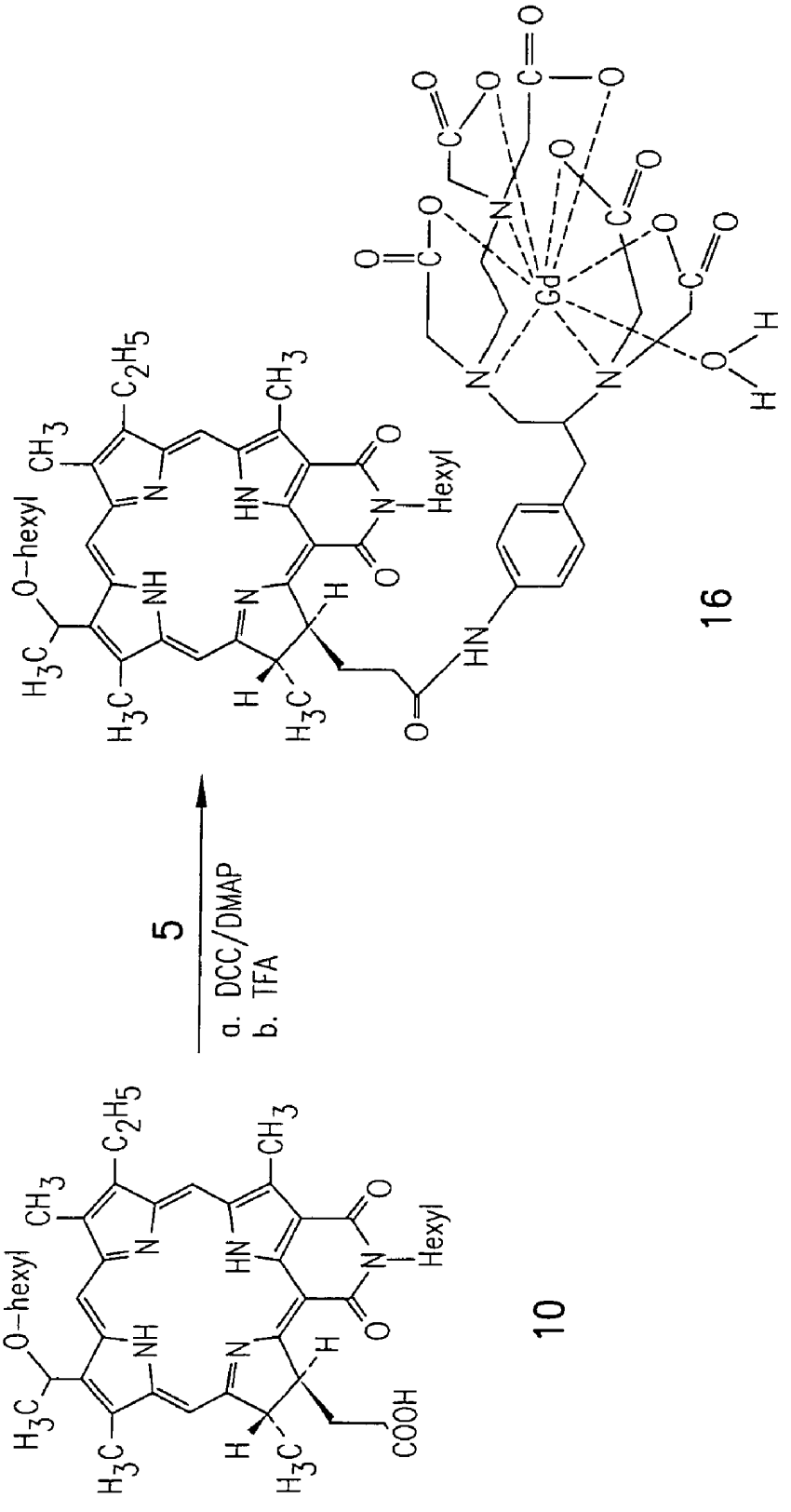


FIG. 8

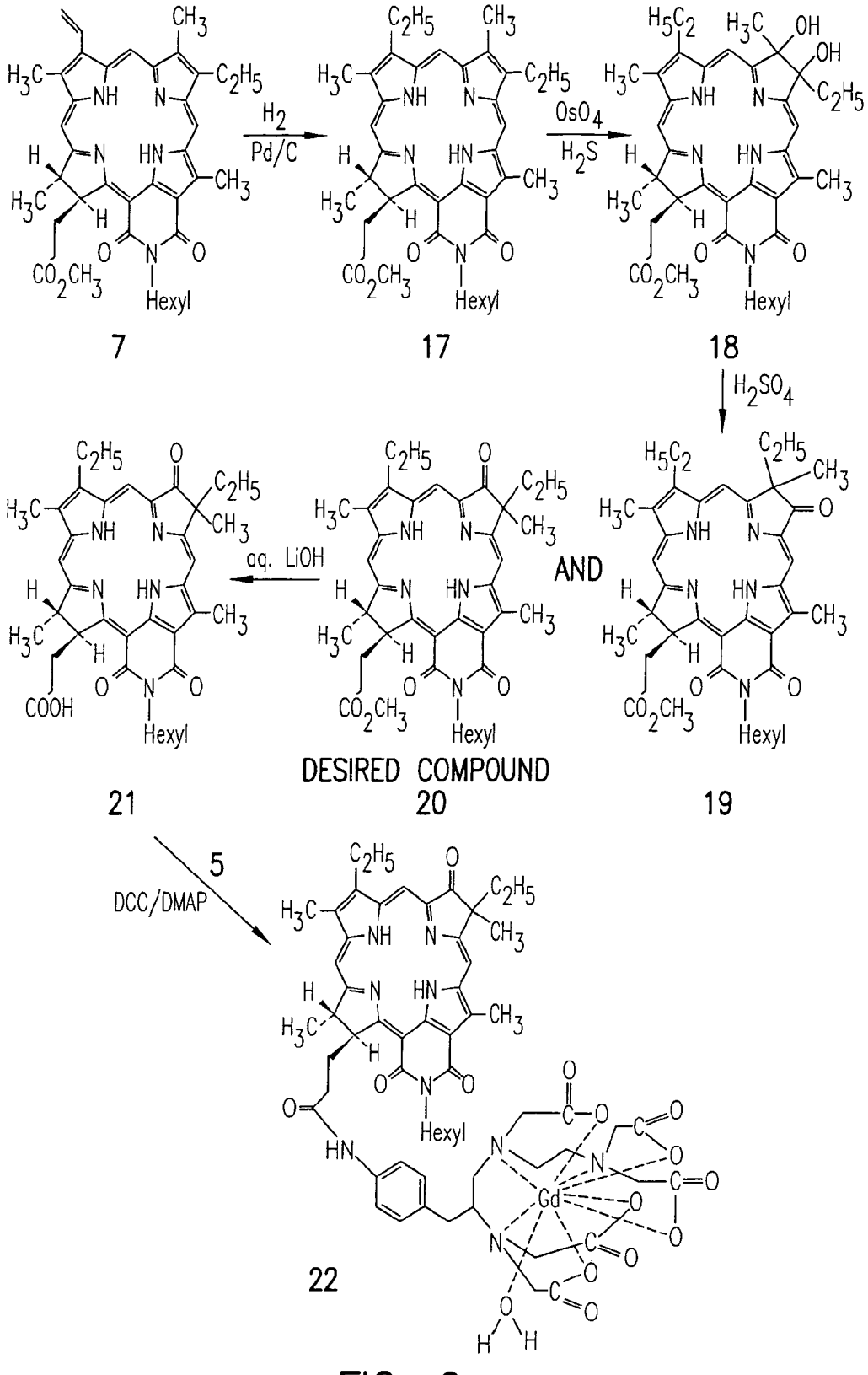

H2
Pd/C
OsO4
H2S
H2SO4
aq. LiOH
AND
DCC/DMAP
5
Hexyl
Gd
7
17
18
21
DESIRED COMPOUND
20
19
22

FIG. 9

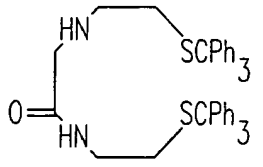


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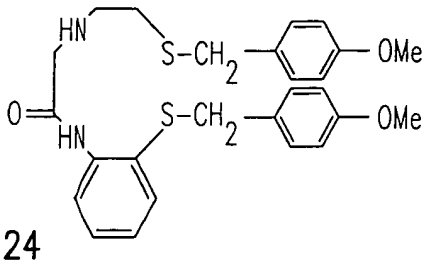
FIG. 10



24

FIG. 11

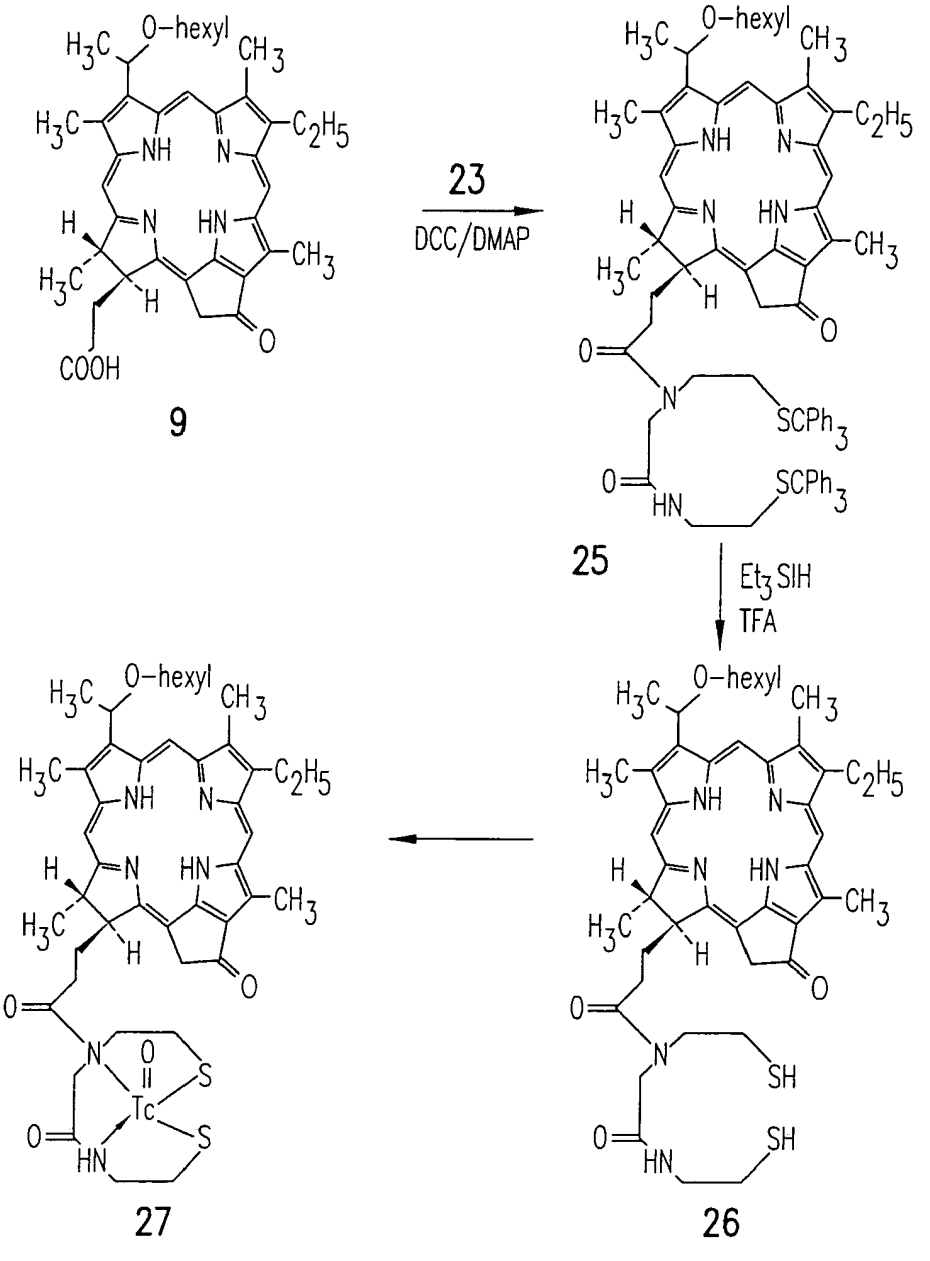


FIG. 12

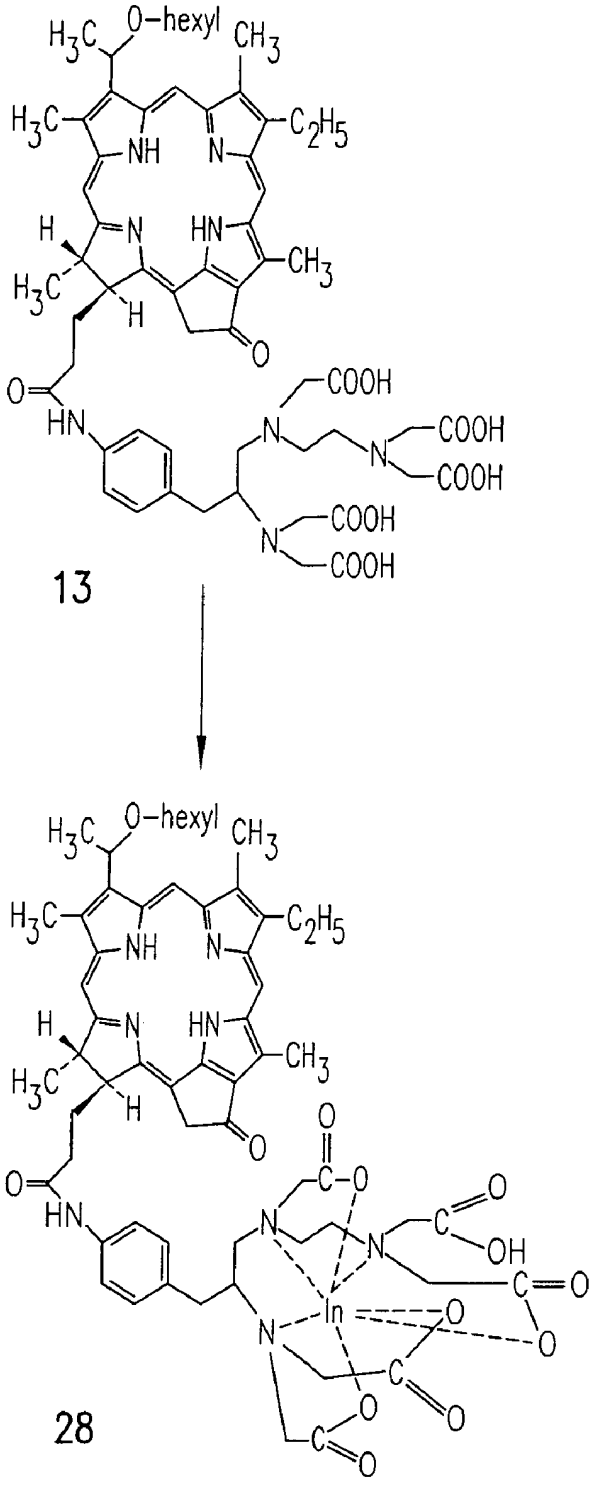
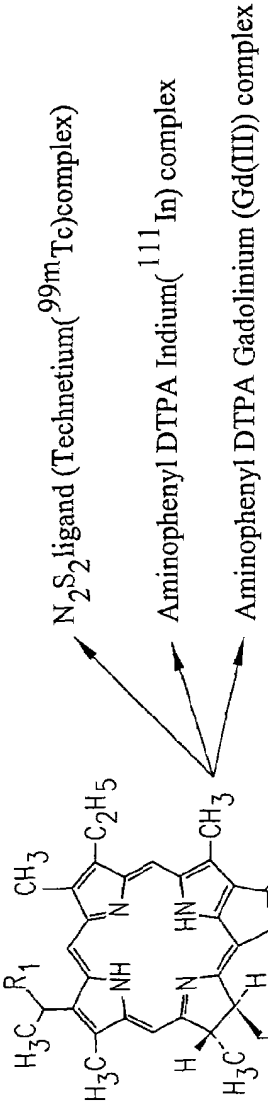
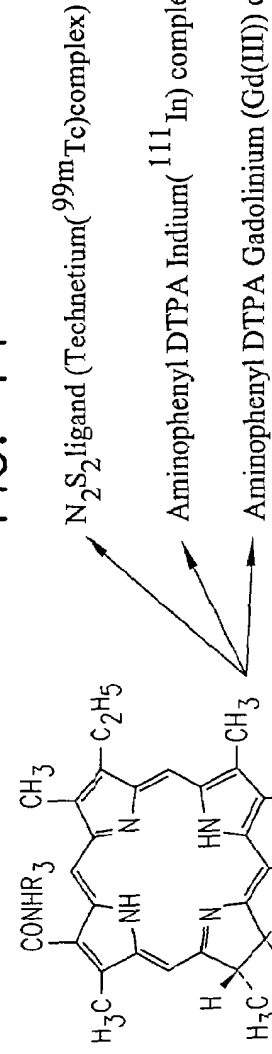


FIG. 13



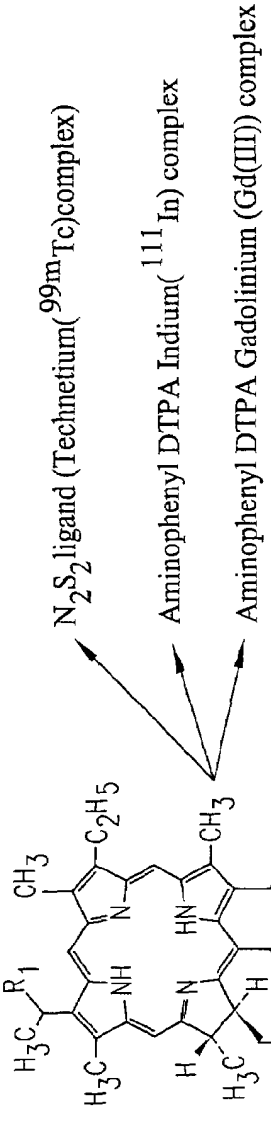
SERIES 1

FIG. 14



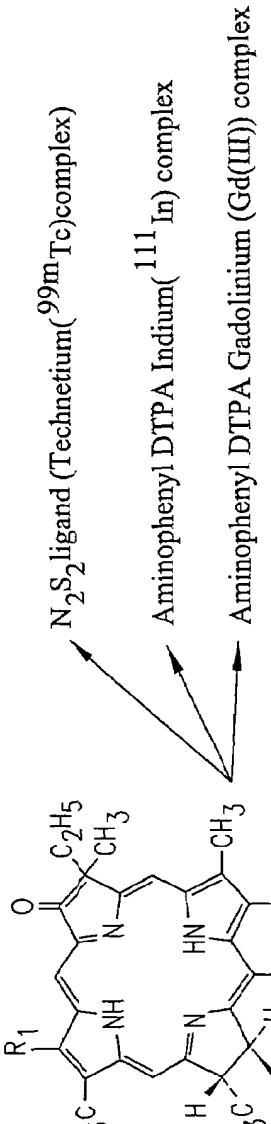
SERIES 2

FIG. 15



SERIES 3

FIG. 16



SERIES 4

FIG. 17

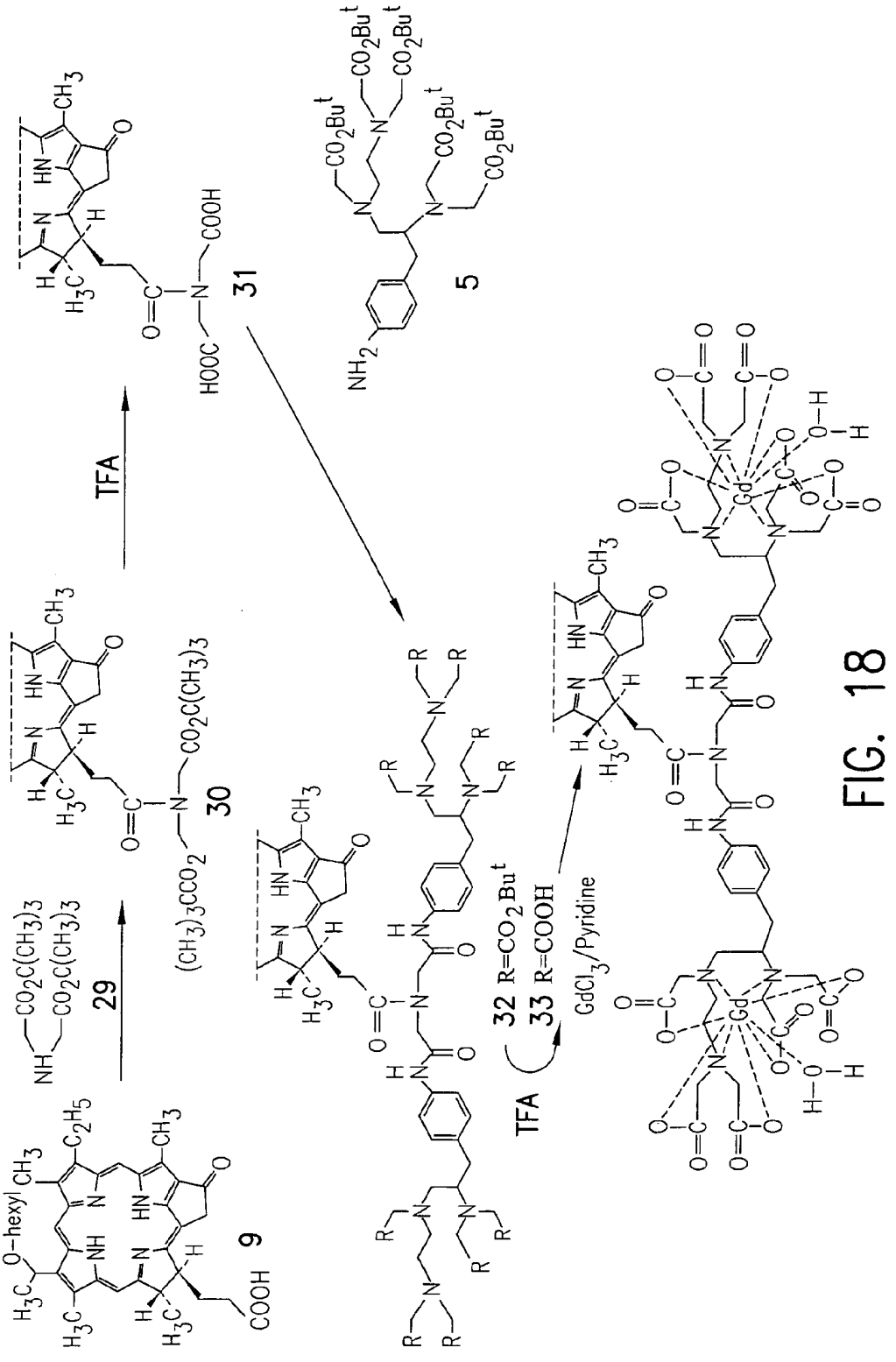


FIG. 18

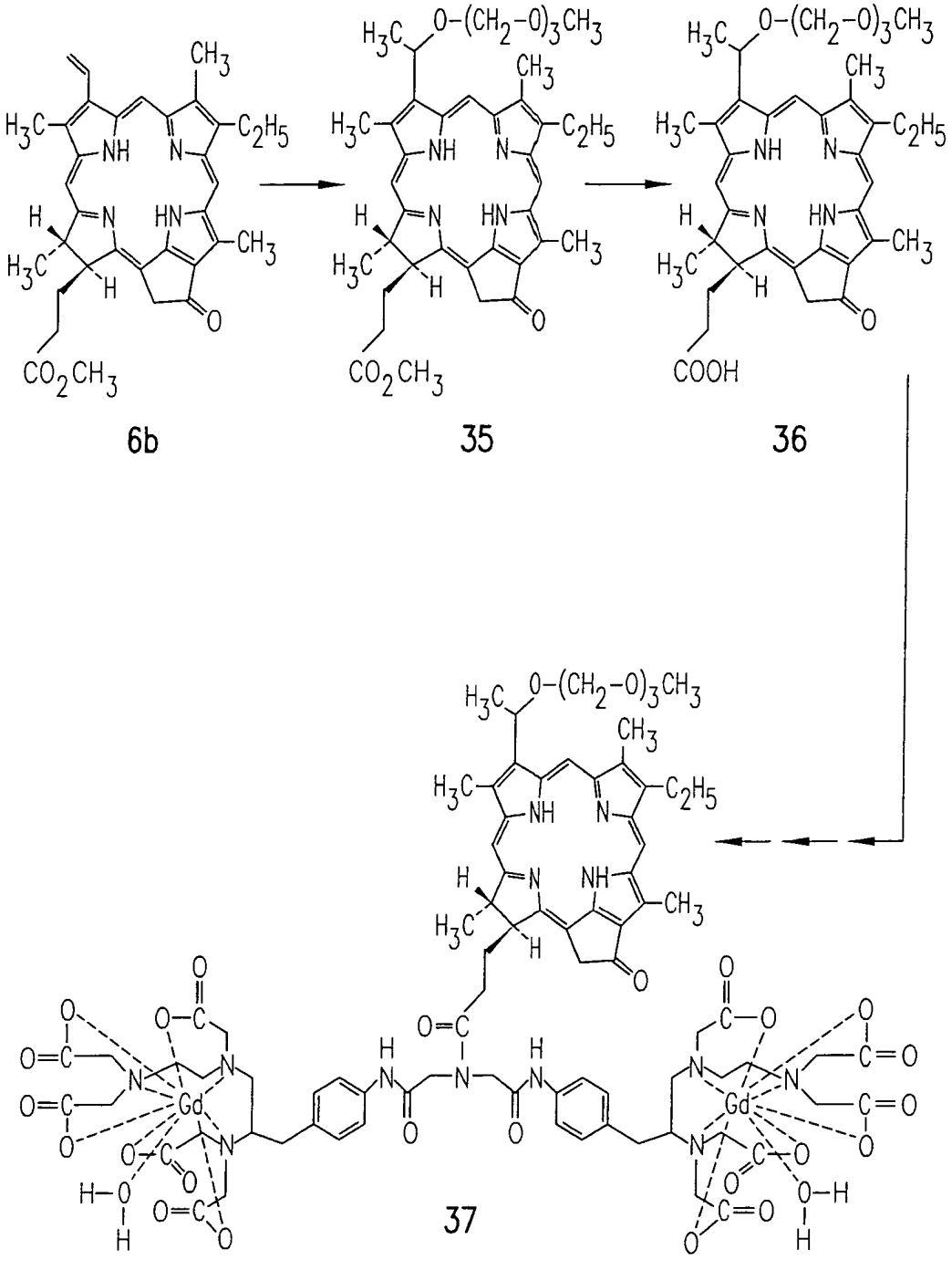


FIG. 19

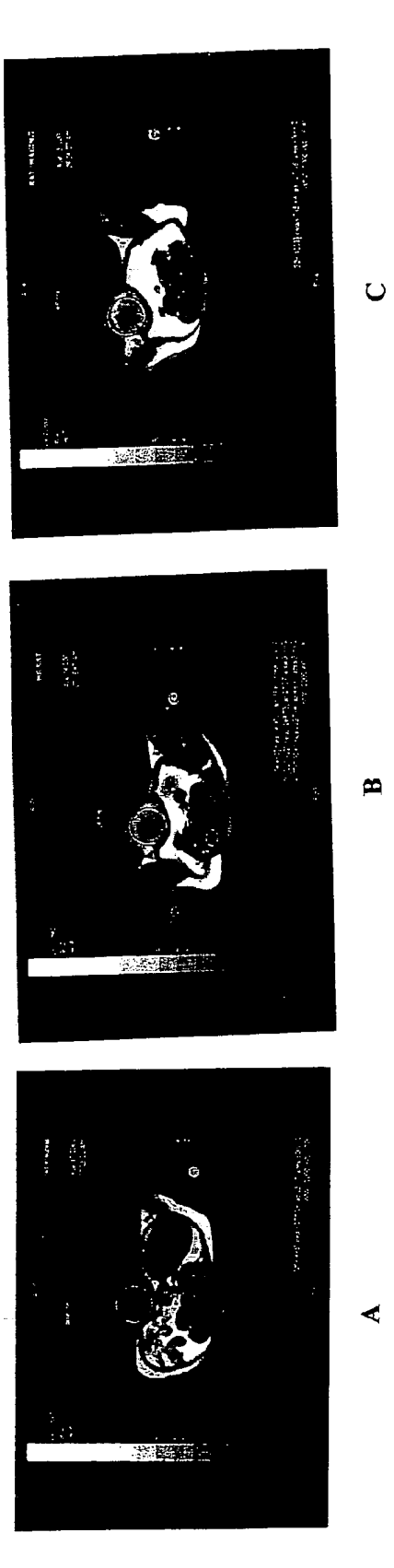


FIG. 20

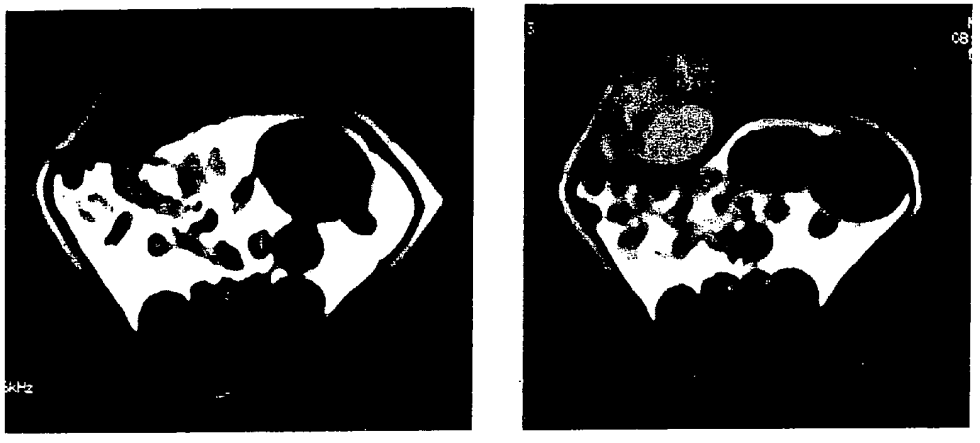


FIG. 21

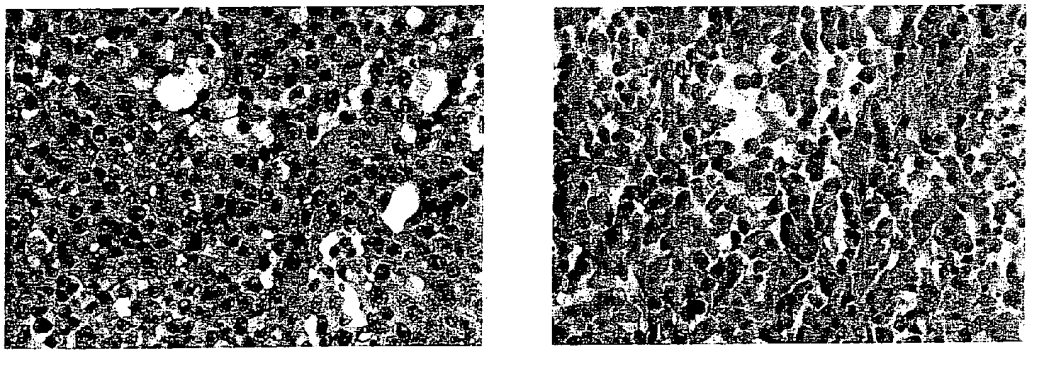


FIG. 22

CHLORIN AND BACTERIOCHLORIN-BASED DIFUNCTIONAL AMINOPHENYL DTPA AND N_2S_2 CONJUGATES FOR MR CONTRAST MEDIA AND RADIOPHARMACEUTICALS

This application is a continuation-in-part of U.S. patent application Ser. No. 09/739,155 filed Dec. 18, 2000, now U.S. Pat. No. 6,534,040 which claims priority from Provisional Patent Application No. 60/171,961 filed Dec. 23, 1999.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

This invention was made with funding from the National Institute of Health Grant Number NIH CA55792. The United States Government may have certain rights in this invention.

BACKGROUND OF THE INVENTION

Cancer is the second most common cause of death in the United States, accounting for 20% of all deaths. Until now, medicine has tried to overwhelm the cancer cell with brute force, slicing it out with surgery, zapping it with radiation, or poisoning it with chemotherapy. All too often, however, a few cells survive the onslaught and germinate, sometimes years later, into tumors that are impervious to treatment. If tumors can be diagnosed at early stages, it will certainly increase the survival rate of the cancer patients. Therefore, efforts are currently underway in our and various other laboratories to develop efficient tumor diagnostic imaging agents.

For many years, in vivo imaging of human anatomy was dependent upon the intravenous administration of radioactive atoms (nuclear medicine) or non-radioactive iodinated contrast media (various x-ray tests and computed tomography). However, over the last decade magnetic resonance imaging (MRI) has assumed a critical role in imaging, and, unlike x-rays or computed tomography, MR uses contrast media that contain paramagnetic ions, particularly Gadolinium (Gd(III)). Paramagnetic ions are not themselves "seen" by the MR scanner. Rather, they affect the water in body tissue so as to increase the "signal" emitted by tissue when it is placed in a magnetic field.

By and large, MR contrast media have been neither disease-specific nor organ-specific. Injected intravenously, most are rapidly excreted by the kidneys by glomerular filtration. Although several liver-specific contrast media have been created, other organs have not been successfully targeted, and no tumor-avid MR contrast agents are available to date.

Because of the importance of detection of unknown primary tumor and metastatic disease in diagnostic oncology imaging, a tumor-avid MR contrast medium would have high implications for prognosis, therapy selection, and patient outcomes. The entire issue of cure versus palliation would be impacted.

In recent years several reports focused on certain Gd-based macrocycles as potential magnetic resonance imaging agents (e.g. Z. D. Grossman and S. F. Rosebrough, Clinical Radioimmunoimaging, Grune & Stratton Inc., 1988, incorporated herein by reference as background art) and ^{99m}Tc or ^{111}In chelated compounds as radiopharmaceuticals (e.g. H. D. Burns, R. F. Gibson, R. F. Dannals and P. K. S. Siegel (Eds.); Nuclear imaging in Drug Discovery, Development and Approval, *Birkhauser,* 1993, and G. B. Saha, Fundamentals of Nuclear Pharmacy, Springer-Verlag, 1992, incorporated herein by reference as background art).

Since the approval of $(Gd(DTPA)(H_2O))^{2-}$ in 1988, more than 30 metric tons of Gadolinium have been administered to millions of patients worldwide. Approximately 30% of MRI exams include contrast agents, and this percentage is projected to increase as new agents and applications appear. Gadolinium is also finding a place in medical research. Over 600 references to Gadolinium appear each year in the basic science literature. While other types of MRI contrast agents, namely an iron-particle-based agent and a manganese (II) chelate have been approved, Gd(III) remains the dominant material. The reasons for this include the direction of MRI development and the nature of Gd chelates. The signal intensity in MRI stems largely from the local value of the longitudinal relaxation rate of water protons, $1/T_1$, and the transverse rate $1/T_2$. Signal tends to increase with increasing $1/T_1$ and decrease with increasing $1/T_2$. Pulse sequences that emphasize changes in $1/T_1$ are referred to as $1/T_1$-weighed, and the opposite is true for T_2-weighed scans. Contrast agents increase both $1/T_1$ and $1/T_2$ to varying degrees, depending on their nature as well as the applied magnetic field. Agents such as Gadolinium (III) that increases $1/T_1$ and $1/T_2$ by roughly similar amounts are best visualized using T_1-weighted images, because the percentage change in $1/T_1$ in tissue is much greater than that in $1/T_2$. The longitudinal and transverse relaxivity values r_1 and r_2 refer to the increase in $1/T_1$ and $1/T_2$, respectively, per millimole of agent. T_1 agents usually have r_2/r_1 ratios of 1–2, whereas that value for T_2 agents, such as iron oxide particles, is as high as 10 or more. Advances in MRI have strongly favored T_1 agents and thus Gadolinium (III). Faster scans with higher resolution require more rapid radio frequency pulsing and are thus generally T_1-weighed, since the MR signal in each voxel becomes saturated. T_1 agents relieve this saturation by restoring a good part of the longitudinal magnetization between pulses. At the same time a good T_1 agent would not significantly affect the bulk magnetic susceptibility of the tissue compartment in which it is localized, thus minimizing any inhomogeneities which can lead to image artifacts and/or decreased signal intensity.

The other important and interesting characteristic of Gadolinium (III) chelates is their stability. They remain chelated in the body and are excreted intact. For example, the off-the shelf ligands like DTPA form complexes so stable that while the agent is in vivo, there is no detectable dissociation. Owing to their large size, lanthanides tend to favor high coordination number in aqueous media. Currently, all Gd(III)-based chelates approved for use in MRI are nine-coordinate complexes in which the ligand occupies eight binding sites at the metal center and the ninth coordinate site is occupied by a solvent water molecule.

Radiopharmaceuticals are drugs containing a radionuclide and are used routinely in nuclear medicine department for the diagnosis or therapy. Radiopharmaceuticals can be divided into two primary classes: Those whose biodistribution is determined exclusively by their chemical and physical properties (like iodine-131) and those whose ultimate distribution is determined by their biological interactions (like a radiolabeled antibody). The latter class includes more target-specific radiopharmaceuticals. A target-specific radiopharmaceutical consists of four parts: a targeting molecule, a linker, a chelating ligand and a radionuclide. The targeting molecule serves as the vehicle, which carries the radionuclide to the target site in diseased tissue. The radionuclide is the radiation source.

Metallic radionuclides offer many opportunities for designing new radiopharmaceuticals by modifying the coordination environment around the metal with a variety of chelators. Most of the radiopharmaceuticals used in conventional nuclear medicine are ^{99m}Tc labeled, because of its short half-life (6 hours) and ideal gamma emission (140 KeV). Millicurie quantities can be delivered without excessive radiation to the patient. The monoenergetic 140-KeV photons are readily collimated, producing images of superior spatial resolution. Furthermore, ^{99m}TC is readily available in a sterile, pyogen-free, and carrier-free state from ^{99}MO-^{99m}TC generators. Its 6h half-life is sufficiently long to synthesize the labeled radiopharmaceuticals, assay for purity, inject the patient, image, and yet short enough to minimize radiation dose. Another radionuclide successfully used is ^{111}In. The success of the pharmaceutical IN-DTPA-Octreotide (OCTREOSCAN), used for diagnosis of somatostatin receptor-positive tumors, has intensified the search for new target-specific radiopharmaceuticals. Compared to ^{99m}Tc, the half-life of ^{111}In is much longer (72 hours).

Certain porphyrins and related tetrapyrrolic compounds tend to localize in malignant tumors and other hyperproliferative tissue, such as hyperproliferative blood vessels, at much higher concentrations than in normal tissues, so they are useful as a tool for the treatment of various type of cancers and other hyperproliferative tissue by photodynamic therapy (PDT) (T. J. Dougherty, C. J. Gomer, B. W. Henderson, G. Jori, D. Kessel, M. Kprbelik, J. Moan, Q. Peng, *J. Natl. Cancer Inst.,* 1998, 90, 889 incorporated here by reference as background art). However, most of the porphyrin-based photosensitizers including PHOTOFRIN® (approved worldwide for the treatment of tumors) clear slowly from normal tissue, so patients must avoid exposure to sunlight for a significant time after treatment. In recent years, a number of chlorophyll analogs have been synthesized and evaluated for their use as photosensitizers for PDT (e.g. R. K. Pandey, D. Herman, *Chemistry & Industry,* 1998, 739 incorporated herein by reference as background art). Among these photosensitizers, the hexyl ether derivative of pyropheophorbide-a 9 (HPPH) (e.g. R. K. Pandey, A. B. Sumlin, S. Constantine, M. Aoudia, W. R. Potter, D. A. Bellnier, B. W. Henderson, M. A. Rodgers, K. M. Smith and T. J. Dougherty, *Photochem. Photobiol.,* 1996, 64, 194; B. W. Henderson, D. A. Bellnier, W. R. Graco, A. Sharma, R. K. Pandey, L. A. Vaughan, W. R. Weishaupt and T. J. Dougherty, *Cancer Res.,* 1997, 57, 4000; and R. K. Pandey, T. J. Dougherty, U.S. patent, 1993, U.S. Pat. No. 5,198,460; U.S. patent, 1994, U.S. Pat. No. 5,314,905 and U.S. patent, 1995, U.S. Pat. No. 5,459,159, incorporated herein by reference as background art) and the hexyl-ether derivative of purpurin-18-N-hexylimide 10 (e.g. R. K. Pandey, W. R. Potter and T. J. Dougherty, U.S. patent, 1999, U.S. Pat. No. 5,952,366, incorporated herein by reference as background art) have shown high tumor uptake and minimal skin phototoxicity compared with PHOTOFRIN®. HPPH is currently in phase I/II clinical trials for treatment of various types of cancer by photodynamic therapy at the Roswell Park Cancer Institute, Buffalo, N.Y. and the results are promising.

Sessler et al. have recently discovered a new class of expanded porphyrins known as "texaphyrins" ("Texaphyrins: Synthesis and Applications", Acc. Chem. Res., 27, 43, 1994). Compared with natural porphyrins, texaphyrins possess a larger core size and are capable of forming complexes with certain lanthanides such as gadolinium (III). Gd (III) texaphyrin is being tested as a tumor-avid MRI agent. Such texaphyrin compounds are able to form complexes within the ring structure due to an expanded ring size, i.e. more than four fused pyrol rings. As a result, the texaphyrins have different characteristics than true porphyrins with respect to solubility, tumor avidity and photodynamic properties.

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

FIG. **1** shows an MR image control using a commercially available contrast agent vs. no use of contrast enhancement agent. The tumor area of the images shows little or no enhancement using the commercially available contrast agent. Baseline (left) and 24 hour post-injection images (right) of a tumor bearing rat are shown. Contrast medium used was Magnavist, the standard commercially available agent, tumor area of interest "1" revealed no signal enhancement, visually or quantitatively.

FIG. **2** shows the MR image using a Gd-HPPH contrast agent of the invention vs. no contrast agent. The image formed using the contrast agent of the invention shows dramatic image enhancement of the tumor area. The images show a baseline (left) and 24 hours post-injection (right) of a tumor bearing rat. Contrast medium was Gd-HPPH. The area of interest "3" increases markedly from 623 to 881. The effect is striking both visually as well as quantitatively. Signal enhancement appears largely restricted to tumor. Fat is unchanged (1998 goes to 1939, and muscle enhancement is minimal.

FIG. **3** is a graph of in vivo measurement of tumor vs. muscle uptake by reflection spectroscopy of the compound shown in FIG. **3***a*.

FIG. **3***a* shows the schematic structure of the compound used in the reflection spectroscopy represented by the graph in FIG. **3**.

FIG. **4** is a schematic diagram showing chemical synthesis of 4-aminophenyl DTPA penta-tert-butyl esters.

FIG. **5** is a schematic diagram showing chemical synthesis of carboxy 3-(hexyloxy)ethyl pyropheophorbide-a from methylpheophorbide-a.

FIG. **6** is a schematic diagram showing chemical synthesis of HPPH-aminophenyl DTPA from carboxy 3-(hexyloxy)ethyl pyropheophorbide-a and 4-aminophenyl DTPA penta-tert-butyl ester followed by reaction with Gadolinium (III) trichloride to form HPPH-aminophenyl DTPA.

FIG. **7** is a schematic diagram showing chemical synthesis of purpurin-18-imide-Gd(III) aminophenyl DTPA (16).

FIG. **8** is a schematic diagram showing preparation of Gd(III) aminophenyl DTPA complex from purpurin 7.

FIG. **9** is schematic diagram showing preparation of bacteriochlorin based Gd(III) aminophenyl DTPA.

FIG. **10** is a schematic formula for bisaminoethanethiol compound 23.

FIG. **11** is a schematic formula for bisaminoethanethiol compound 24.

FIG. **12** is a schematic diagram showing preparation of HPPH based bisaminoethanethiol conjugate 27.

FIG. **13** is a schematic diagram showing preparation of HPPH based In Aminophenyl DTPA conjugate 28.

FIG. **14** is a schematic diagram showing preparation of N_2S_2 ligand ^{99m}Tc complex, Aminophenyl DTPA ^{111}In complex and Aminophenyl DTPA Gd(III) complex, e.g. 3-devinyl-3-(1'-alkoxy ethyl)-17-(3'-(4"-amidobenzyl gadolinium (III)DTPA))ethyl pyropheophorbide-a, from a DTPA or N_2S_2 dihydro tetrapyrrole compound of the invention.

FIG. **15** is a schematic diagram showing N_2S_2 ligand ^{99m}Tc complex, Aminophenyl DTPA "In complex, and Aminophenyl DTPA ^{111}In Complex, and Aminophenyl DTPA Gd(III) complex, e.g. purpurin-18-(30devinyl-3-(4"-amidobenzyl gadoliniumDTPA))-N-substituted imide, from a DTPA or N_2S_2 dihydro tetrapyrrole compound of the invention.

FIG. **16** is a schematic diagram showing N_2S_2 ligand ^{99m}Tc complex, Aminophenyl DTPA "In complex, and Aminophenyl DTPA ^{111}In Complex, and Aminophenyl DTPA Gd(III) complex, e.g. purpurin-18-(3-devinyl-3-(1'alkoxy ethyl)-17-(3'-(4"-amidobenzyl gadolinium(III)DTPA))ethyl pyropheophorbide-a, from a DTPA or N_2S_2 dihydro tetrapyrrole compound of the invention.

FIG. **17** is a schematic diagram showing N_2S_2 ligand ^{99m}Tc complex, Aminophenyl DTPA "In complex, and Aminophenyl DTPA ^{111}In Complex, and Aminophenyl DTPA Gd(III) complex, e.g. bacteriopurpurin 18-3-(alkyl or alkoxyalkyl)-7-keto-17-(3'-(4"-amidobenzyl gadolinium (III)DTPA))-N-substituted imide, from a DTPA or N_2S_2 tetrahydro tetrapyrrole compound of the invention.

FIG. **18** is a schematic diagram showing synthesis of HPPH Di-Gd(III)DTPA conjugate 34.

FIG. **19** is a schematic diagram showing synthesis of polyethylene glycol ether analog of pyropheophorbide-a 35 and the related Di-Gd(III) DTPA conjugate 37

FIG. **20** shows comparative magnetic resonance tumor images pre-injection (A), 24 hours post injection (B) and 48 hours post injection (C) with HPPH-diGD(III)conjugate 34 at a drug dose of 10.0 μmol/Kg

FIG. **21** shows comparative magnetic resonance tumor images pre-injection (A), and 24 hours post injection (B) with PEG-pyro-di-Gd(III) conjugate 37 at a drug dose of 10.0 μmol/Kg. A remarkable tumor enhancement can be seen.

FIG. **22** shows a comparison of histopathology sections of Word Colon Carcinoma tumors implanted in rat, treated with light after injection with HPPH-diGD(III)conjugate 34. (A) shows a section resulting from injection of HPPH-diGD(III) conjugate 34 at a dose of 10.0 μmol/Kg and treated with light at 200J/cm^2 at 400 mw for 8 minutes at fours hours post imaging (28 hours post injection). No tumor damage is observed. (B) shows a similar section treated with light at 24 hours post imaging (48 hours post injection). Complete tumor necrosis is observed.

BRIEF DESCRIPTION OF THE INVENTION

The invention includes compositions that are chemical combination of porphyrins and chlorins and related tetrapyrrolic compounds with radioactive elements such as Technetium^{99}, Gadolinium, Indium^{111} and radioactive iodine. When the element can form cations, the compound is usually a chelate with the porphyrin or chlorin structure. When the element forms anions, the compound is usually a direct chemical combination of the radioactive element into the porphyrin or chlorin structure.

Examples of porphyrin and chlorin structures that can form compounds with radioactive elements, when modified in accordance with the present invention, are for example described in U.S. Pat. Nos. 5,756,541; 5,028,621; 4,866, 168; 4,649,151; 5,438,071; 5,198,460; 5,002,962; 5,093, 349; 5,171,741; 5,173,504; 4,968,715; 5,314,905; 5,459, 159; 5,770,730; 5,864,035; 5,190,966; and 5,952,366 all of which are incorporated by reference as background art.

The invention further includes the method of using the compounds of the invention for diagnostic imaging of hyperproliferative tissue such as tumors and new blood vessel growth as is associated with the wet form of age related macular degeneration.

Unexpectedly, porphyrins and chlorins, as above described, upon injection, carry the element to cells of hyperproliferative tissue and dramatically enhance the signal produced by tumor tissue in MR imaging.

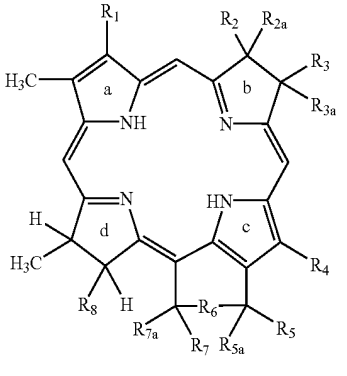
It has further been discovered that porphyrin based photosensitizers, such as HPPH, when conjugated with an MR image enhancing compound as previously described, can simultaneously operate as both tumor-avid magnetic resonance imaging (MRI) agents and as photosensitizers for photodynamic therapy thus permitting a tumor to precisely located and then treated by photodynamic therapy using the same compound.

It is to be understood that porphyrin and chlorin compounds (including bacteriochlorins) may be chemically altered to other forms by substitutions and modifications; provided that, the base tetrapyrrolic structure that allows selective entry and retention in hyperproliferative tissue cells (e.g. tumors) is retained.

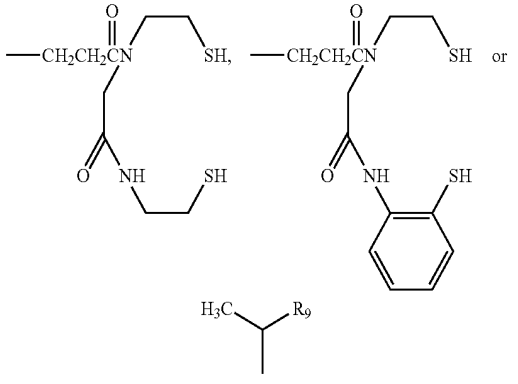
It has now been discovered that even better MR results can be obtained when the tetrapyrolic ring is directly or indirectly complexed with more than one, e.g. di-complexed, element, that enhances MR image quality, e.g. Technetium^{99}, Indium^{111}, lanthanide metals such as Gadolinium, and heavy anionic elements such as radioactive iodine.

It has further been discovered that such di-complexed compounds improve relaxivity per conjugated porphyrin molecule by increasing the "payload" of imaging enhancing agent, e.g. gadolinium, to the tumor per porphyrin molecule and that such enhanced imaging agents still retain the properties required to act as photodynamic treatment agents.

Compounds of the invention usually have the formula:



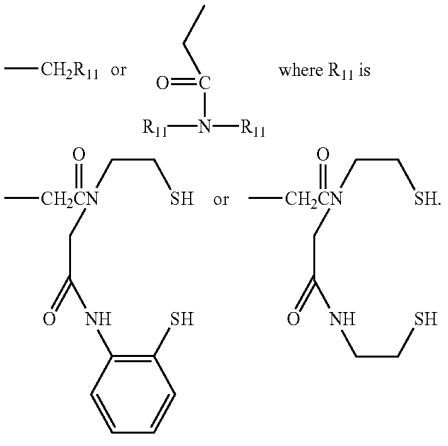
where R_1, R_2, R_{2a} R_3, R_{3a} R_4, R_5, R_{5a} R_6, R_7, R_{7a}, and R_8 cumulatively contain at least two functional groups that will complex or combine with an MR imaging enhancing element or ion as above described. R_1 is usually —CH═CH$_2$, —CHO, COOH,



where R_9=—OR_{10} where R_{10} is lower alkyl of 1 through 6 carbon atoms, —$((CH^2)_2O)_nCH_3$ where n is 0 through 3, or —$(CH_2)_2CONHphenyleneCH_2DTPA$.

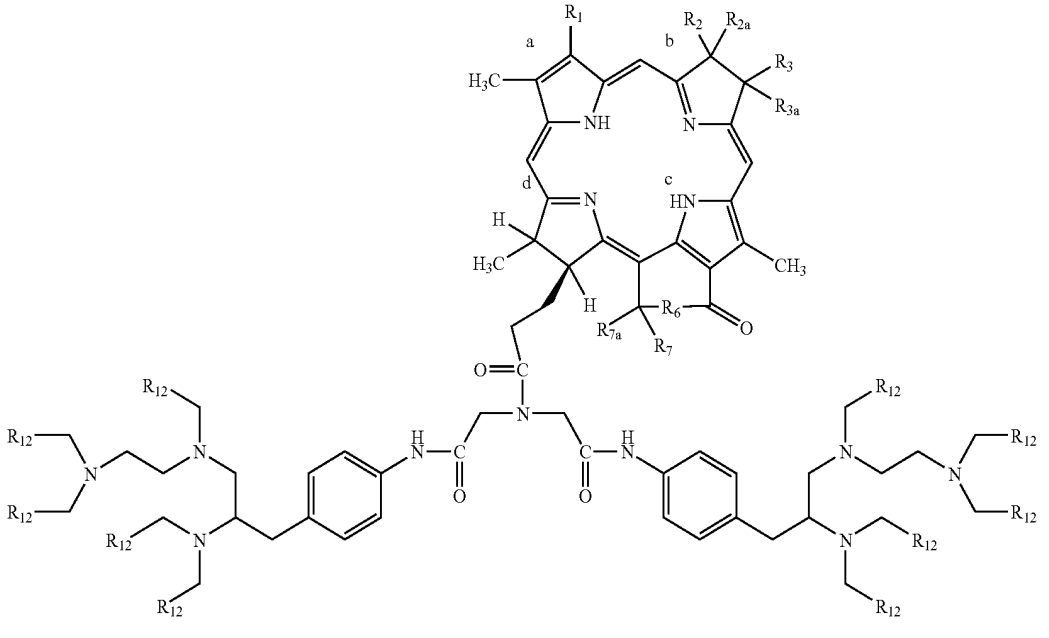
R_2, R_{2a}, R_3, R_{3a}, R_4, R_5, R_{5a}, R_6, R_7, and R_{7a} are independently hydrogen, lower alkyl or substituted lower alkyl or two R_2, R_{2a}, R_3, R_{3a}, R_5, R_{5a}, R_7, and R_{7a} groups on adjacent carbon atoms may be taken together to form a covalent bond or two R_2, R_{2a}, R_3, R_{3a}, R_5, R_{5a}, R_7, and R_{7a} groups on the same carbon atom may form a double bond to a divalent pendant group. R_2 and R_3 may together form a 5 or 6 membered oxygen, nitrogen or sulfur containing heterocyclic ring.

R_6 is —CH_2—, —NR_{11}— or a covalent bond; R_8 is —$(CH_2)_2CO_2CH_3$, —$(CH_2)_2CONHphenyleneCH_2DTPA$, —$CH_2CH_2CON(CONHphenyleneCH_2DTPA)_2$,



The above generic formula is intended to cover di-metallic complexes with elements that enhance MR imaging as previously described. Such elements that enhance MR imaging are referred to herein as "radionuclides" or "nuclides."

The compound of the invention has the preferred generic formula:



where R_1, R_2, R_{2a}, R_3, R_{3a}, R_6, R_7, and R_{7a} are as previously described. R_{12} is —COORa where Ra is hydrogen or lower alkyl of 1 through 8 carbon atoms. The above preferred generic formula is intended to include di-complexes with metals that enhance MR imaging, especially di-gadolinium complexes.

DETAILED DESCRIPTION OF THE INVENTION

An objective of the invention was to use these photosensitizers as a vehicle for delivering the desired conjugate (mono-chelated or poly-chelated with Gd or other radionuclides) to tumor which may optionally be followed with treatment of the tumor with light to obtain tumor necrosis. The di-chelates, in addition to containing at least two radionuclides, are "bifunctional" because they bind the nuclide, e.g. Gd, at one end and bind the target specific vehicle at the other. The chelate is a multidentate ligand, which has appropriate ligating groups for coordination to the metal. In a preferred embodiment, the invention includes:

Development of chlorin and bacteriochlorin-based di-Gd (III)aminophenyl DTPA conjugates with variable lipophilicity as tumor diagnostic agent by MRI.

Development of chlorin and bacteriochlorin-based di-^{111}In aminophenyl DTPA and di-^{99m}Tc N_2S_2 conjugates with variable lipophilicity as tumor diagnostic radiopharmaceuticals.

In accordance with the invention, nuclides, e.g. gadolinium, have been successfully bound to a tumor-avid porphyrin, originally designed for photodynamic therapy (PDT), by modification of the porphyrin substituents to permit both mono and poly-complexing (e.g. di-complexing) with the nuclides. The resulting compounds have shown striking tumor uptake at 24 hours to enhance the MRI "signal" produced by tumor, thus dramatically increasing its conspicuity on MR imaging. In addition related ^{99m}Tc and ^{111}In labeled radiopharmaceuticals in complexes with modified porphyrins of the invention form diagnostic agents for nuclear medicine.

This invention includes the synthesis and application of certain chlorin and bacteriochlorin-based bisaminoethanethiol (N_2S_2) and modified ditetratriethylamine penta carboxylic acid (DTPA) conjugates as MR contrast media and radiopharmaceuticals for diagnosis, and optionally treatment, of primary malignancy and metastatic disease.

The following examples describe examples for synthesis and use of magnetic resonance imaging agents.

Synthesis of HPPH-Gd(III)aminophenylDTPA 14: For the preparation of the title compound, pyropheophorbide-a 6b was obtained from methylpheophorbide-a 6a (which in turn was extracted from Spirulina Algae) by following the literature procedure. It was then converted into methyl 3-(hexyloxy)ethyl analog 9a by following a methodology developed in our laboratory. Hydrolysis of the methyl ester functionality with aqueous LiOH/methanol/THF produced the corresponding carboxylic acid 9b in quantitative yield. The reaction of 9b with 4-aminophenyl DTPA penta-tert-butyl esters prepared by following the methodology in FIG. **4** via the carbodiimide approach (R. K. Pandey, F.-Y. Shiau, A. B. Sumlin, T. J. Dougherty and K. M. Smith, *Bioorg. Med. Chem. Lett.,* 1994, 4, 1263, incorporated herein by reference as background art) produced the corresponding analog 12 in 57% yield (FIGS. **5** and **6**). The structure was confirmed by NMR and mass spectrometry analyses.

Before preparing the Gd(III) complex, the tert-butyl groups of the conjugate were converted into corresponding carboxylic acid by reacting with trifluoroacetic acid (yield 100%). For the preparation of Gd(III) complex 14, the conjugate was dissolved in pyridine and Gadolinium chloride hexahydrate dissolved in deionized water. The mixture was stirred at room temperature for 2h. After the completion of the reaction (monitored by TLC), pyridine was removed under high vacuum. The residue was washed with water to remove the excess of Gadolinium chloride, dried under vacuum and the title compound was isolated in 92% yield. The structure of the final product was confirmed by mass spectrometry. Synthesis of Purpurin-18-imide-Gd(III)aminophenylDTPA 16: Methylpheophorbide-a 7a was converted into the hexylether derivative of N-hexyl purpurinimide in 70% yield. The methyl ester group was then hydrolyzed to the corresponding carboxylic acid 10 by following the methodology as discussed for the preparation of 9b. Purpurin-imide 10 was then reacted with aminophenylDTPA penta tert-butyl ester 5 by following a reaction sequence depicted in FIG. **7** and the intermediate conjugate was isolated in 45% yield. Further reaction with trifluoroacetic acid and then with $GdCL_3.6H_2O$ produced the Gd(III) complex 16 in >90% yield. The structures of the conjugates were confirmed by NMR and mass spectrometry.

In our attempt to investigate the effect of the position of the Gd(III) conjugate in the macrocycle, purpurin-imide 7 was converted into the related carboxylic acid analog 11 by conventional procedures. Reaction of 10 with aminophenyl DTPA 5 will produce Gd(III) aminophenyl DTPA conjugate 15, purpurin 18-3-devinyl-3(4'-amidophenyl Gadolinium (III) DTPA)-N-hexylimide.

In this series of compounds, the overall lipophilicity of the molecule can be altered by varying the length of the carbon chain of either the alkyl ether substituents and/or N-substituted alkyl chain. Thus, these compounds provide a unique opportunity to investigate the correlation of tumor uptake and lipophilicity.

Synthesis of Bacteriochlorin basedGD(III)aminophenylDTPA

Bacteriochlorins are a class of tetrapyrroles in which the two pyrrole units diagonal to each other are reduced. Starting from N-hexyl-purpurin imide 7 we have prepared ketobacteriochlorin 20 by following a reaction sequence illustrated in FIG. **9**. In our approach purpurinimide 7 containing a vinyl group at position 3 was converted into the 3-devinyl-3-ethyl analog 17 (can also be named as meso-N-hexyl-purpurin-18-imide) by reacting with hydrogen using Pd/C as a catalyst. It was then reacted with osmium tetroxide/pyridine/H_2S (A. N. Kozyrev. T. J. Dougherty and R. K. Pandey, *Tetrahedron Lett.,* 1996, 37, 3781, incorporated herein by reference as background art) and the corresponding vic-dihydroxybacteriochlorin 18 was isolated in 75% yield as a mixture of diasteriomers (cis-hydroxy groups up or down relative to trans-reduced ring D). The dihydroxy analog as a diasteriomeric mixture was treated with sulfuric

acid under pinacol-pinacolone reaction conditions, (R. K. Pandey, T. Tsuchida, S. Constantine, G. Zheng, C. Medforth, A Kozyrev, A. Mohammad, M. A. J. Rodgers, K. M. Smith and T. J. Dougherty, *J. Med. Chem.,* 1997, 40, 2770, incorporated herein by reference as background art) and the ketobacteriochlorin, containing a keto-group either at 7—(compound 20) or 8-position (compound 19) respectively were isolated in 70% yield. Among these bacteriochlorins, the 7-keto analog 20 showed high tumor uptake as determined by in vivo reflectance spectroscopy in mice model transplanted with RIF tumor (see FIG. **3**). The structures of bacteriochlorins 19 and 20 were confirmed by NMR and mass spectrometry analyses.

Our next step was to hydrolyze the methyl ester group in purpurinimide 20 into carboxylic acid 21 before converting it into the corresponding 4-aminophenylDTPA conjugate 22 by following the methodology discussed previously for the preparation of related HPPH and purpurin-imide analogs.

Synthesis of HPPH-based Bisaminoethanethiol conjugates 27: For preparing the ^{99m}Tc labeled radiopharmaceuticals, two bisaminoethanethiols 23 and 24 were prepared by following the methodology developed in our laboratory (G. Li, Q. Ma, B. Ma, Z. D. Grossman and R. K. Pandey, (1999) *Heterocycles* 51(12):2849–2854; and G. Li, B. Ma, J. R. Missert, Z. D. Grossman and R. K. Pandey, (1999) *Heterocycles* 51(12):2855–2860; incorporated herein by reference as background art). For the synthesis of N_2S_2 conjugate 26, HPPH was reacted with N_2S_2 chelate 23 and the thioprotected HPPH conjugate 25 was isolated in 40% yield. Subsequent deprotection of the thiols with triethylsilane/TFA afforded the corresponding bis-aminoethanethiol 26 in quantitative yield. The structure of the newly synthesized compound was confirmed by NMR and mass spectrometry analyses.

The Tc-99m complex 27 was prepared by ligand-exchange reaction with ^{99m}Tc pertechnatate reduced by Sn(II) glucoheptonate by following the methodology of Kung and coworkers (S. K. Meegalla, K. Plossl, M-P. Kung, S. Chumpradit, D. A. Stevenson, S. A. Kushner, W. T. McElgin, P. D. Mozley and H. F. Kung. *J. Med. Chem.,* 1997, 40, 9, incorporated herein by reference as background art). The radiolabeling yield was >80%. The purity of the Tc-99m complex was >95%, by chromatography.

Syntheses of HPPH based ^{111}In AminophenylDTPA conjugate 28: For the preparation of the title compound, the HPPH-aminophenylDTPA 13 was reacted with ^{111}In(III) chloride, following the methodology reported by Low and coworkers (S. Wang J. Juo, D. A. Lantrip, D. A. Waters, C. J. Mathias, W. A. Green, P. L. Fuchs and P. S. Low, *Bioconjugate Chem.,* 1997, 8, 673, incorporated herein by reference as background art) for the preparation of ^{111}In-DTPA-Folate and the ^{111}In-labeled compound was obtained in 82% yield.

Body Tumor MR Imaging:

HPPH-Gd(III)AminophenylDTPA conjugate 14:

Following the synthesis of GD-labeled HPPH, a series of three rats were injected intravenously and studied immediately after injection, at 1 hour, and at 24 hours, to establish whether the Gd-HPPH remained in the circulation longer than the current standard contrast medium (Magnavist or Gd-DTPA).

Whereas Magnavist clears rapidly from the mammalian circulation by glomerular filtration, with a circulatory half-time of 16–20 minutes, the newly-synthesized contrast medium Gd-HPPH, was evident in the cerebral circulation at 1 hour. Subsequently, to establish whether the GD-HPPH is tumor-avid, a single rat with a subcutaneously-implanted Ward colon carcinoma was imaged, 24 hours after intravenous GD-HPPH. A second tumor-bearing rat was imaged 24 hours after injection of Magnavist (See FIGS. **1** and **2**). Clearly, the enhanced tumor signal after Gd-HPPH injection indicated that GD-HPPH 14 has potential as a contrast medium for MRI. HPPH (a chlorophyll-a derivative) represents the vehicle by which the Gd complex is carried into the tumor. Addition of the Gd chelate to HPPH does not hinder its ability to form singlet oxygen producing efficacy, so this contrast medium also has the potential for dual action: enhanced localization on MR imaging (diagnosis), followed by directed light exposure with tumor injury (treatment). Also, because of its excellent tumor selectivity and high fluorescence, the newly synthesized conjugate can be used for IR imaging.

In addition to the above monocomplexed nuclide-modified porphyrin compounds, di-complexed compounds have been prepared and tested.

C. Description and Operation:

Synthesis of HPPH Di-Gd(III)DTPA Conjugate 34:

For the preparation of the title compound HPPH 9 was reacted with di-tert-butyl imidodiacetate 29 by following the DCC approach very frequently used in peptide synthesis and compound 30 was obtained in 60% yield. Reaction of 30 with trifluoroacetic acid (TFA) produced the corresponding carboxylic acid in quantitative yield, which on reacting with DTPA containing aminophenyl group 5 afforded the conjugate 32 in 70% yield. The related carboxylic acid derivative 33 obtained after reacting 32 with TFA was converted into the corresponding Gd analog 34 by reacting with gadolinium chloride ($GdCl_3$) in quantitative yield (FIG. **18**).

Synthesis of polyethyleneglycol ether analog of pyropheophorbide-a 35 and the related Di-Gd(III) DTPA conjugate 37

Methyl pyropheophorbide-a 6b was reacted with 30% HBr/acetic acid for 2h at room temperature. The acetic acid was removed under high vacuum and the residue was reacted with polyethyleneglycol at room temperature for 45 min. It was then poured in water and extracted with dichloromethane. The dichloromethane layer was dried over anhydrous sodium sulfate. Evaporation of the solvent gave a residue, which was purified by column chromatography, eluting with methanol/dichloromethane. The appropriate eluates were collected, solvent was removed and the PEG analog 35 was isolated in 70% yield. The methyl ester functionality present in 35 was converted into the corresponding carboxylic acid 36 on reacting with aqueous LiOH/methanol-THF mixture in quantitative yield. Com-

pound 36 was then converted into the corresponding di-Gd (III)DTPA conjugate 37 by following the methodology described for the preparation of the related HPPH conjugate 34 (FIG. **19**).

Formulation: (liposomal encapsulation)

Liposomal encapsulation of di-Gd-HPPH: To dissolve a sufficient quantity of drug for imaging and PDT, di-Gd-HPPH was liposome encapsulated. 120 mg of the egg-phosphatidylcholine (Sigma) and cholesterol (50 mg) were dissolved in dichloromethane (3 ml). Nitrogen was slowly bubbled through the solution and the product was dried under a vacuum. To this, HPPH-DTPA(GdIII) conjugate (50 mg) in 9.0 ml PBS (pH=7.4, 0.01M) were added and sonicated for 4 hours. The solution was filtered and the concentration of the conjugate in solution was measured spectrophotometrically (2.65 μM/ml).

Body tumor MR Imaging of conjugate 34

Four rats with a subcutaneously implanted Ward colon carcinoma were imaged using a 1.5 tesla magnetic resonance imaging system with a standard wrist coil (GE Horizon 5.8, GE Medical Systems, Milwakee, Wis.). Pre-injection imaging was T_1 weighed (TR=500 ms, TE=14 ms) in axial and coronal planes. Images thickness was 3 mm with a 1 mm inter-scan gap. Matrix size was 256×192 with 1.5 excitations and an 8×8 cm field-of-view. Imaging was repeated at 24 hour and 48 hours post-injection of HPPH-di-Gd(III) conjugate (10.0 μmol/Kg) with identical imaging parameters.

From the results shown in FIG. **20**, it can be seen that the signal enhancement is largely restricted to tumor, with intensity rising markedly from 370 (pre-injectin) to 582 (24 hr) to 715 (48 hr) post-injection. The effect is virtually striking.

Body tumor MR Imaging of conjugate 37

Under similar experimental conditions, conjugate 37 was evaluated for tumor imaging. The images are shown in FIG. **21**.

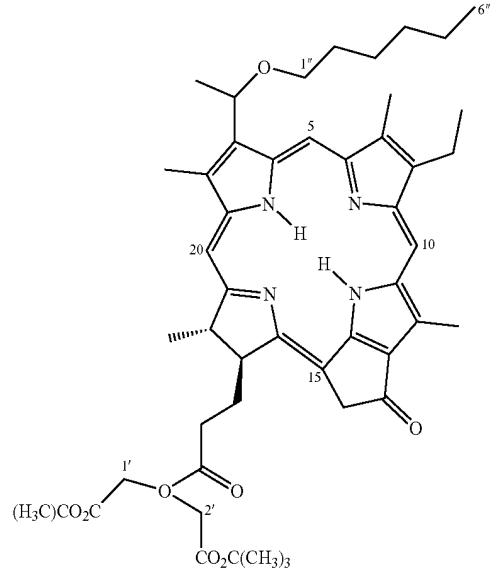
In vivo photosensitizing efficay of DiGd(III) at its imaging dose

Two 250 gram (approximately) female SD rats were anesthetized with xylazine/ketamine, the abdomen cleansed with alcohol, and a 2×2 mm fragment of Ward colon carcinoma was inserted under the skin through a small (2–3 mm) incision. The wound was closed with 5/0 silk suture. Ten days later the rats were injected intravenously with 10 μmole HPPH-2×GD, and 24 hr later a 1 mm cylindrical fiber was inserted centrally into the tumor (approximately 1×1 cm tumor size), and irradiated with 200 joules of 660 nm laser light (400 mw for 8 min). Four and twenty hours after irradiation the tumors were removed, fixed in 10% buffered formalin, sectioned (5 microns) and stained (routine hematoxylin and eosin). Four hours after irradiation the tumor appears healthy (see FIG. **22**A) with no evidence of tumor cell death. By twenty-four hours the tumor showed evidence of complete necrosis (with scattered apoptotic cells) by histopathologic exam (FIG. **3**B).

The structures of the intermediates and the final products were confirmed by UV-vis,

^{1}H NMR, mass spectrometry/elemental analyses.

Di-tert-buty iminodiacetate imide analog of HPPH



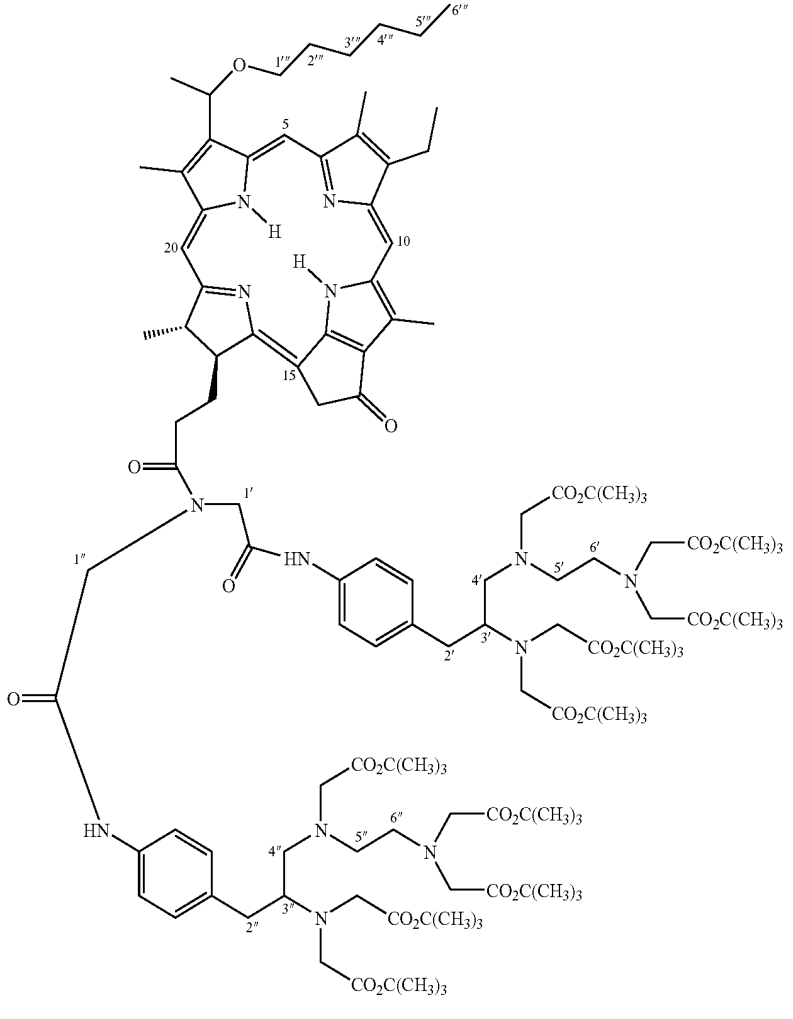
UV Vis in CH_2Cl_2 (λ_{max} (ε)): 318 (25650), 410 (128140), 505 (11750), 536 (11750), 605 (9720), 661 (60760). ^{1}H NMR ($CDCl_3$): δ 9.79 (1H, s, H-5), 9.53 (1H, s, H-10), 8.53 (1H, s, H-20), 5.92 (1H, q, J=6.6 Hz, CH_3(O-hexyl)CH-3), 5.22 (2H, dd, AB system, J=20.7 Hz, —$COCH_2$— 15), 4.51 (1H, q, J=6.9 Hz, H-18), 4.39 (1H, m, H-17), 4.03 (2H, splitting s, H-1' or 2'), 3.79–3.53 (9H, m, CH_3CH_2-8, 1×ring CH_3, H-1' or 2', H-1"), 3.39, 3.28 (each 3H, s, 2×ring CH_3), 2.75, 2.47 (each 1H, m, —$HNCOCH_2CH_2$-17), 2.42, 2.14 (each 1H, m, —$HNCOCH_2CH_2$-17), 2.12 (3H, two set of doublets, J=6.6, 7.3 Hz, CH_3(O-hexyl)CH-3), 1.81 (3H, d, J=7.6 Hz, CH_3-18), 1.75 (2H, m, H-2"), 1.72 (3H, t, J=7.6 Hz, CH_3CH_2-8), 1.50–1.40 (11H, m, H-3", 1×—OCOC$(CH_3)_3$), 1.23 (4H, m, H-4" and H-5"), 1.04, 1.03 (9H, two singlets, 1×—OCOC$(CH_3)_3$), 0.79 (3H, m, H-6"), 0.43 (1H, br, —NH), −1.72 (1H, s, —NH). Mass calculated for $C_{51}H_{69}N_5O_7$:863. Found (FAB): m/z 864.4 (MH^+, 100). HRMS (FAB): Calcd for $C_{51}H_{70}N_5O_7$ (MH^+) 864.5275. Found 864.5280

Bis-(N,N.N',N',N"-Pentakris(tert-butoxycarbonyl) methyl)-1-((4-amido-HPPH)methyl) diethylenetriamine

UV Vis in CH_2Cl_2 (λ_{max} (ε)): 319 (21870), 411 (109150), 506 (9960), 537 (10010), 604 (8280), 660 (50080). ^{1}H NMR ($CDCl_3$): δ 10.59 (1H, br, 1×—CONH-phenyl), 9.76 (1H, splitting s, H-5), 9.49 (1H, s, H-10), 8.46 (1H, s, H-20), 8.14 (1H, br, 1×—CONH-phenyl), 7.65 (2H, m, 2×phenyl H),

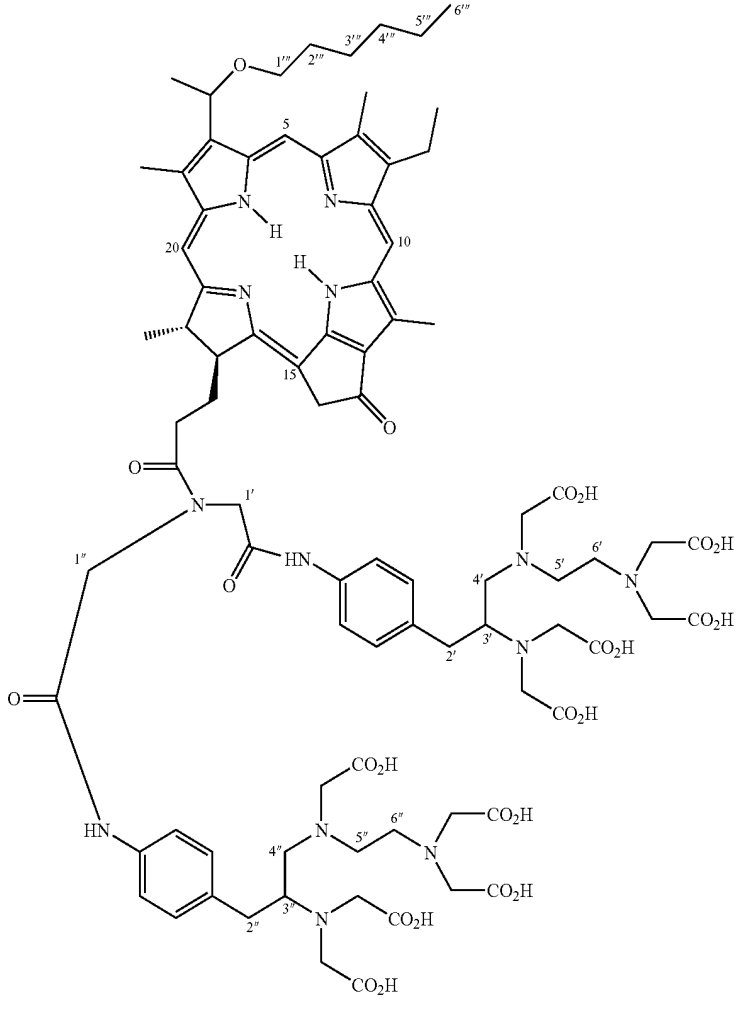
7.48 (2H, m, 2×phenyl H), 7.23 (4H, m, 4×phenyl H), 5.87 (1H, m, CH_3(O-hexyl)CH-3), 5.16 (2H, dd, AB system, J=20.0 Hz, —$COCH_2$-15), 4.40 (1H, m, H-18), 4.28 (1H, m, H-17), 4.07, 3.84 (4H, m, H-1' and H-1"), 3.76–3.53 (7H, m, H-1', CH_3-12, CH_3CH_2-8), 3.53–3.28 (23H, CH_3-2 and 10×—NCH_2CO_2C—), 3.25 (3H, s, CH_3-7), 3.10 (2H, m, H-3', and H-3"), 2.98–2.15 (20H, m, —$HNCOCH_2CH_2$-17, H-2', 2", 4', 4", 5', 5", 6', 6"), 2.07 (3H, m, CH_3(O-hexyl) CH-3), 1.69 (8H, m, H-2"', CH_3CH_2-8, CH_3-18), 1.61–1.28 (92H, m, H-3"', 10×—$OCOC(CH_3)_3$), 1.20 (4H, m, H-4"' and H-5"'), 0.76 (3H, t, J=6.8 Hz, H-6"), 0.43 (1H, br, —NH), −1.75 (1H, s, —NH).

$C_{125}H_{189}N_{13}O_{25}$, MS (FAB) m/z 2274.0 ($MH^+$, 100). HRMS (FAB): Calcd for $C_{125}H_{189}N_{13}O_{25}Na$ (MNa^+) 2295.3814; Found 2295.3820.

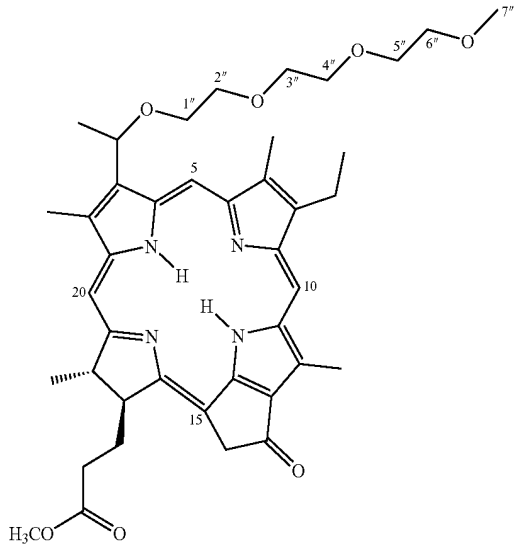


Bis-(N,N,N'N',N"-Pentakris(carboxymethyl)))-1-((4-amido-HPPH)methyl)diethylenetriamine

^{1}H NMR (60% $CDCl_3$, 40% CD_3OD and one drop of C_5D_5N, TMS as internal standard): δ9.83 (1H, splitting s, H-5), 9.57 (1H, s, H-10), 8.58 (1H, s, H-20), 7.68–7.37 (4H, m, 4×phenyl H), 7.22–6.94 (4H, m, 4×phenyl H), 5.92 (1H, m, CH_3(O-hexyl)CH-3), 5.13 (2H, dd, AB system, J=19.8 Hz, —$COCH_2$-15), 4.58–3.99 (6H, m, overlapped with water signal, H-18, H-17, H-1' and H-1"), 3.84–2.20 (55H, m, H-1'", CH_3-2, CH_3-7, CH_3-12, CH_3CH_2-8, 10×—NCH_2CO_2C—, —$HNCOCH_2CH_2$-17, H-2', 2", 3', 3", 4', 4', 5', 5", 6', 6"), 2.12 (3H, m, CH_3(O-hexyl)CH-3), 1.85–1.63 (8H, m, H-2'", CH_3CH_2-8, CH_3-18), 1.35 (2H, m, H-3'"), 1.22 (4H, m, H-4'" and H-5'""), 0.76 (3H, m, H-6").



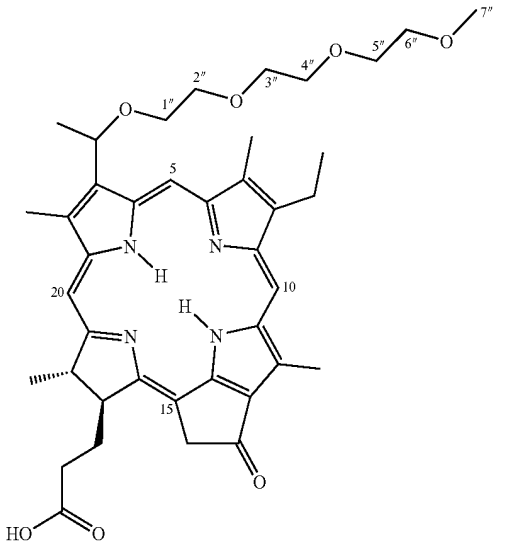
3-Devinyl-3-((1'-O-tri(ethylene glycol)monomethyl-ether)ethyl-pyropheophorbide-a methyl ester



^{1}H NMR ($CDCl_3$): δ 9.75 (1H, s, H-5), 9.50 (1H, s, H-10), 8.54 (1H, s, H-20), 6.02 (1H, q, J=6.7 Hz, CH_3(O-PEG) CH-3), 5.18 (2H, dd, AB system, J=19.8 Hz, —$COCH_2$-15), 4.49 (1H, m, H-18), 4.30 (1H, m, H-17), 3.66, 3.62, 3,40, 3.28 (15H, each s, 3×ring CH_3, —$COOCH_3$, H-7"), 3.95–3.48, 3.40 (14H, m, CH_3CH_2-8, H-1", 2", 3", 4", 5", 6"), 2.69, 2.55, 2.28 (1H, 1H, 2H, m, $CH_3OOCCH_2CH_2$-17), 2.15 (3H, two set of doublets, J=6.8, 6.6 Hz, CH_3(O-hexyl) CH-3), 1.82 (3H, d, J=7.8 Hz, CH_3-18), 1.72 (3H, t, J=7.6 Hz, CH_3CH_2-8), 0.43 (1H, br, —NH), −1.72 (1H, s, —NH).

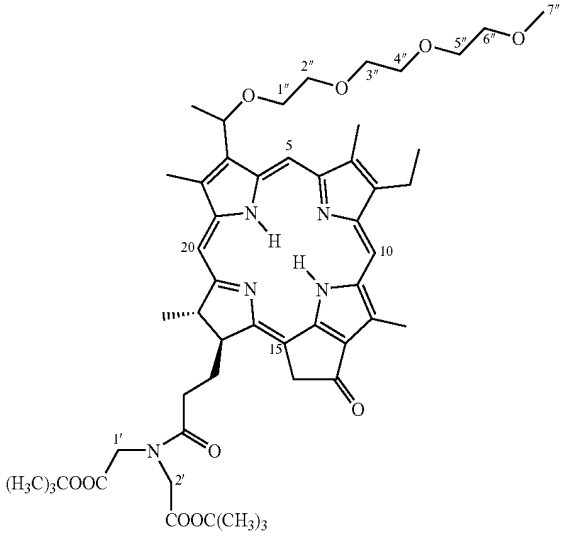
3-Devinyl-3-((1'-O-tri(ethylene glycol)monomethyl ether)ethyl-pyropheophorbide-a

^{1}H NMR ($CDCl_3$): δ 9.72 (1H, splitting s, H-5), 9.49 (1H, s, H-10), 8.51 (1H, s, H-20), 5.99 (1H, q, J=6.4 Hz, CH_3(O-PEG)CH-3), 5.18 (2H, dd, AB system, J=20.2 Hz, —$COCH_2$-15), 4.47 (1H, q, J=7.0 Hz, H-18), 4.31 (1H, m, H-17), 3.65, 3.37, 3,26, 3.25 (12H, each s, 3×ring CH_3, H-7"), 3.89–3.45, 3.39 (14H, m, CH_3CH_2-8, H-1", 2", 3", 4", 5", 6"), 2.69, 2.59, 2.29 (1H, 1H, 2H, m, $CH_3OOCCH_2CH_2$-17), 2.12 (3H, two set of doublets, J=6.6, 6.7 Hz, CH_3(O-hexyl)CH-3), 1.80 (3H, d, J=6.9 Hz, CH_3-18), 1.69 (3H, t, J=7.7 Hz, CH_3CH_2-8), -1.72 (1H, s, —NH). Mass calculated for $C_{40}H_{50}N_4O_7$: 698. Mass (FAB) found: m/z 699.2 (MH^+, 100). HRMS (FAB): Calcd for $C_{40}H_{51}N_4O_7$ (MH^+) 699.3757. Found 699.3730.



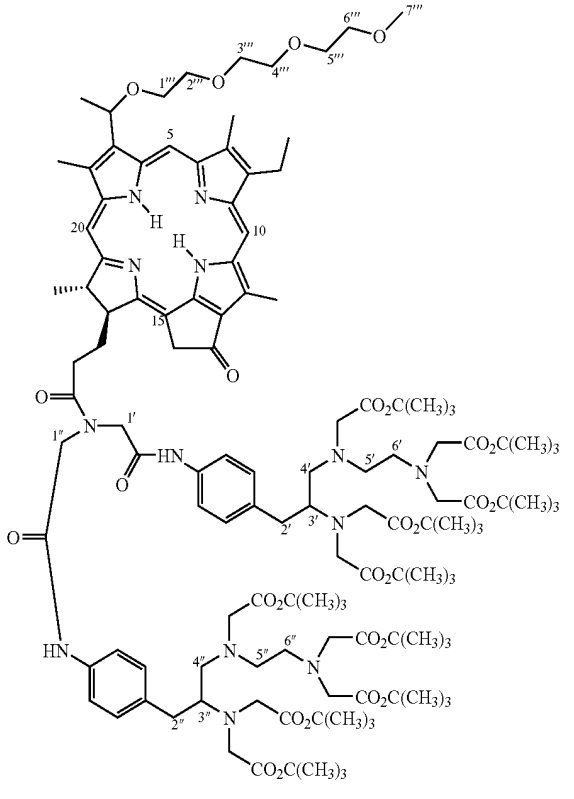
Di-tert-buty iminodiacetate imide analog of PEG-ether analog of pyropheophorbide-a

^{1}H NMR ($CDCl_3$): δ9.74 (1H, splitting s, H-5), 9.53 (1H, s, H-10), 8.53 (1H, s, H-20), 6.01 (1H, m, CH_3(O-hexyl) CH-3), 5.21 (2H, dd, AB system, J=19.8 Hz, —COCH2-15), 4.50 (1H, m, H-18), 4.38 (1H, m, H-17), 4.02 (2H, splitting s, H-1' or 2'), 3.68, 3.34, 3,29, 3.27 (12H, each s, 3×ring CH_3, H-7"), 3.91–3.50, 3.42 (14H, m, CH_3CH_2-8, H-1", 2", 3", 4", 5", 6"), 3.39 (2H, s, H-1' or 2'), 2.74, 2.43, 1.94 (1H, 2H, 1H, m, $CH_3OOCCH_2CH_2$-17), 2.13(3H, d, J=6.7 Hz, CH_3(O-hexyl)CH-3), 1.80 (3H, d, J=7.5 Hz, CH_3-18), 1.72 (3H, t, J=7.5 Hz, CH_3CH_2-8), 1.47, 1.43 1.07, 1.03 (6H, 6H, 3H, 3H, each s, 2×—$OCOC(CH_3)_3$), 0.79 (3H, m, H-6"), 0.39 (1H, br, —NH), −1.75 (1H, s, —NH). Mass calculated for $C_{52}H_{71}N_5O_{10}$: 925. Found MS (FAB): m/z 926.3 (MH^+, 100).HRMS (FAB): Calcd for $C_{52}H_{72}N_5O_{10}$ (MH^+) 926.5279. Found 926.5316.



Bis-(N,N.N',N',N''-Pentakris(tert-butoxycarbonyl) methyl)-1-((4-amido-PEG-ether-pyropheophorbide-a)methyl) diethylenetriamine

^{1}H NMR ($CDCl_3$): δ 10.79 (1H, br, 1×—CONH-phenyl), 9.72 (1H, splitting s, H-5), 9.45 (1H, splitting s, H-10), 8.76 (1H, br, 1×—CONH-phenyl), 8.47 (1H, s, H-20), 7.71 (2H, m, 2×phenyl H), 7.54 (2H, d, J=7.9 Hz, 2×phenyl H), 7.25 (2H, m, 2×phenyl H), 7.20 (2H, d, J=8.2 Hz, 2×phenyl H), 5.96 (1H, m, CH_3(O-hexyl)CH-3), 5.15 (2H, dd, AB system, J=20.4 Hz, —$COCH_2$-15), 4.41 (1H, m, H-18), 4.26 (1H, m, H-17), 3.94 (2H, m, H-1' or H-1''), 3.87–3.20 (48H, m, H-1' or H-1'', H-1''', 2''', 3''', 4''', 5''', 6''', 7''', 10×—NCH_2CO_2C—, CH_3-2, CH_3-7, CH_3-12, CH_3CH_2-8), 3.11 (2H, m, H-3' and H-3''), 2.98–2.30 (20H, m, —$HNCOCH_2CH_2$-17, H-2', 2'', 4', 4'', 5', 5'', 6', 6''), 2.10 (3H, m, CH_3(PEG)CH-3), 1.68 (6H, m, CH_3CH_2-8, CH_3-18), 1.55–1.30 (90H, m, 10×—$OCOC(CH_3)_3$), 0.38 (1H, s, —NH), −1.77 (1H, s, —NH). Mass calculated for $C_{126}H_{191}N_{13}O_{28}$; 2335. Mass (FAB) found: m/z 2336.1 (MH^+, 68), 2358.1 (MNa^+, 100). HRMS (FAB): Calcd for $C_{126}H_{19}N_{13}O_{28}Na$ (MNa^+) 2357.3818. Found 2357.3820.



HPPH-2 (Gd-DTPA) 34

The title compound was insoluble in all the organic solvents suitable for the NMR spectrometry analysis. Therefore, the structure was confirmed by elemental analyses Analysis calculated for $C_{85}H_{107}N_{13}O_{27}Gd_2$: C, 49.57; H, 5.24; N, 8.85. Found: C, 50.63; H, 5.10; N, 8.21.

PyroPEG-2 (Gd-DTPA) 37

The title compound was prepared by following the approach described for the synthesis of 8. This conjugate was also found to be insoluble in all the organic solvents suitable for the NMR spectrometry analysis. Therefore, the structure was confirmed by elemental analyses. Analysis calculated for $C_{86}H_{109}N_{13}O_{30}Gd_2$: C, 48.69; H, 5.18; N, 8.59.

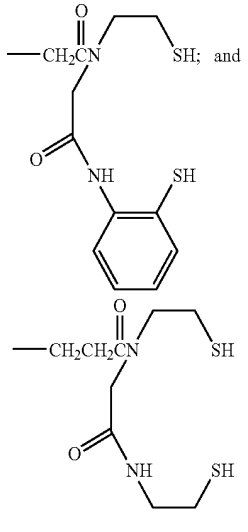
Found:

C, 47.91; H, 4.99; N, 7.85.

Also, Indium or other radionuclides like Tc-99m (the latter conjugated by an N_2S_2 ligand) bound to chlorins and bacteriochlorins synthesized and proposed in this invention have potential as imaging agents for nuclear medicine.

The invention claimed is:

1. A composition comprising a chemical combination of a photodynamic tetra-pyrrolic compound having a porphyrin or chlorin structure having four pendant groups attached to at least one pyrolle ring, said compound being provided with a plurality of radionuclide element atoms connected to a plurality of functional groups that will complex or combine with an MR imaging enhancing element or ion selected from the group consisting of —$(CH_2)_2$ CONHphenyleneCH$_2$DTPA; —CH_2CH_2CON(CONHphenylene-CH_2DTPA)$_2$



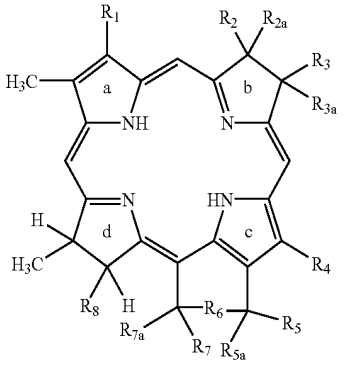
such that the compound may be used to enhance MR imaging wherein —CH_2CH_2CON(CONHphenylene-CH_2DTPA)$_2$ counts as two such functional groups.

2. The composition of claim **1** wherein the radionuclide element is selected from the group consisting of Technetium^{99}, Indium^{111}, radioactive iodine and a Lanthanum series element including Gadolinium.

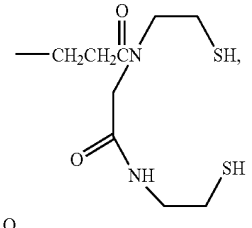
3. The composition of claim **1** wherein the radionuclide element forms cations and the compound is a chelate of the radionuclide element with the porphyrin or chlorin structure.

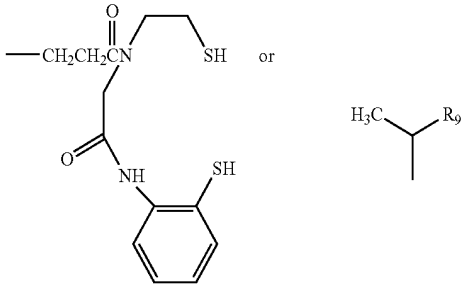
4. The composition of claim **1** wherein the radionuclide element forms anions and the compound is a direct chemical combination of the radionuclide element with a porphyrin or chlorin structure.

5. A compound having the structural formula:

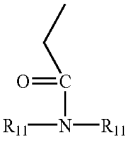


where R_1, R_2, R_{2a} R_3, R_{3a} R_4, R_5, R_{5a} R_6, R_7, R_{7a}, and R_8 cumulatively contain at least two functional groups that complex or combine with an MR imaging enhancing element or ion; R_1 is —CH═CH_2, —CHO, COOH,

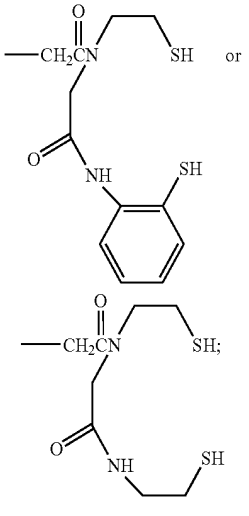




where R_9=—OR_{10} where R_{10} is lower alkyl of 1 through 8 carbon atoms, —$((CH_2)_2O)_nCH_3$ where n is 0 through 3, or —$(CH_2)_2$CONHphenyleneCH_2DTPA; R_2, R_{2a}, R_3, R_{3a}, R_4, R_5, R_{5a}, R_7, and R_{7a} are independently hydrogen, lower alkyl or substituted lower alkyl so as to contain a functional group that will combine with a radionuclide element selected from the group consisting of Technetium^{99}, Indium^{111}, radioactive iodine and a Lanthanum series element including Gadolinium or two R_2, R_{2a}, R_3, R_{3a}, R_5, R_{5a}, R_7, and R_{7a} groups on adjacent carbon atoms may be taken together to form a covalent bond or two R_2, R_{2a}, R_3, R_{3a}, R_5, R_{5a}, R_7, and R_{7a} groups on the same carbon atom may form a double bond to a divalent pendant group; R_2 and R_3 may together form a 5 or 6 membered heterocyclic ring containing oxygen, nitrogen or sulfur; R_6 is —CH_2—, —NR_{11}— or a covalent bond; R_8 is —$(CH_2)_2CO_2CH_3$, —$(CH_2)_2$CONHphenyleneCH_2DTPA, —CH_2CH_2CON(CONHphenyleneCH_2DTPA)$_2$, —CH_2R_{11} or



where R_{11} is



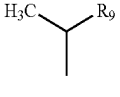
and polynuclide complexes thereof; wherein the functional groups that will complex or combine with an MR imaging enhancing element or ion are selected from the group consisting of —$(CH_2)_2$CONHphenyleneCH_2DTPA; —CH_2CH_2CON(CONHphenyleneCH_2DTPA)$_2$;

wherein —CH_2CH_2CON(CONHphenylene-CH_2DTPA)$_2$ counts as two such functional groups.

6. The compound of claim 5 wherein the compound is a dinuclide complex of gadolinium III.

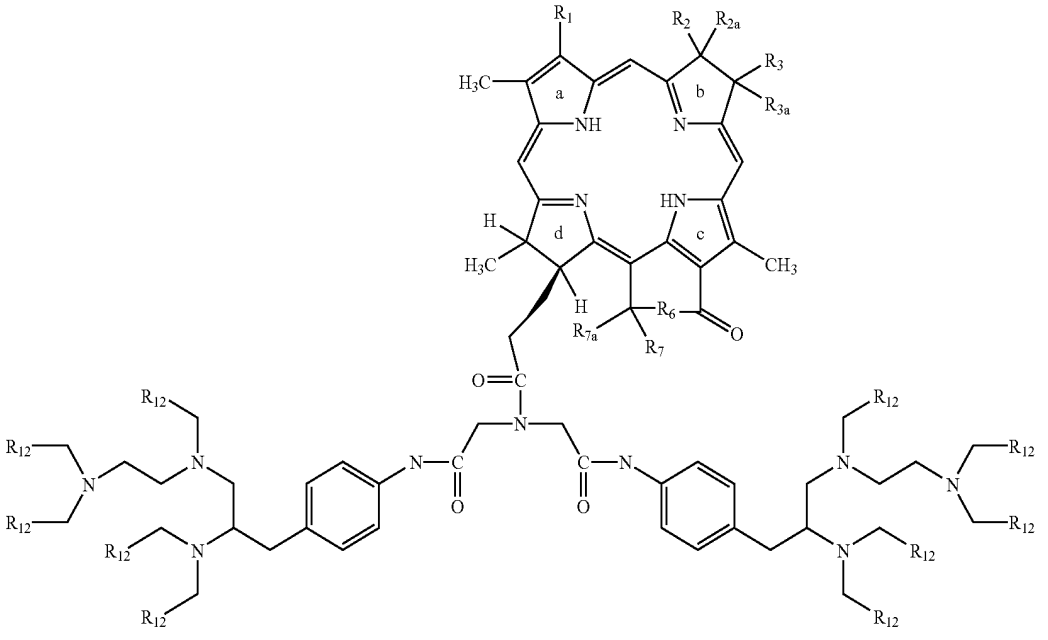
7. The compound of claim 6 where R_8 is —CH_2CH_2CON(CONHphenyleneCH_2DTPA)$_2$.

8. The compound of claim 7 wherein R_2, R_{2a}, R_3, R_{3a}, R_4, R_5, R_{5a}, R_7, and R_{7a} are independently hydrogen or lower alkyl of 1 through 4 carbon atoms, R_1 is

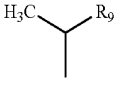


and R_9 is —O-hexyl.

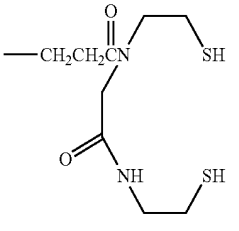
9. A compound of the formula:



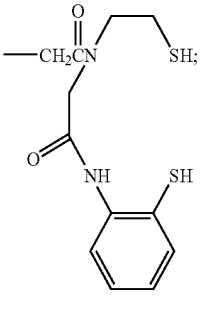
where R_1 is —CH═CH_2, —CHO, COOH, or



where R_9=—OR_{10} where R_{10} is lower alkyl of 1 through 8 carbon atoms, —$((CH_2)_2O)_nCH_3$ where n is 0 through 3, or —$(CH_2)_2$CONHphenyleneCH$_2$DTPA; R_2, R_{2a}, R_3, R_{3a}, R_7, and R_{7a} are independently hydrogen, lower alkyl or substituted lower alkyl so as to contain a functional group that will combine with a radionuclide element is selected from the group consisting of Technetium^{99}, Indium^{111}, radioactive iodine and a Lanthanum series element including Gadolinium or two R_2, R_{2a}, R_3, R_{3a}, R_7, and R_{7a} groups on adjacent carbon atoms may be taken together to form a covalent bond or two R_2, R_{2a}, R_3, R_{3a}, R_7, and R_{7a} groups on the same carbon atom may form a double bond to a divalent pendant group; and R_6 is —CH_2—, —NR_{11}— or a covalent bond where R_{11} is lower alkyl of 1 through 6 carbon atoms; and R_{12} is —COORa where Ra is hydrogen or lower alkyl of 1 through 8 carbon atoms; and dinuclide complexes thereof; wherein the functional groups that will complex or combine with an MR imaging enhancing element or ion are selected from the group consisting of —$(CH_2)_2$CONHphenyleneCH$_2$DTPA; —CH_2CH_2CON(CONHphenylene-CH_2 DTPA)$_2$;



and



10. The compound of claim 9 where the compound is a digadolinium III complex.

11. The compound of claim 9 where R_2 is —CH_3 and R_3 is —CH_2CH_3.

12. The compound or complex of claim 9 where R_6 is —NR_{11}— where R_{11} is hexyl.

13. A di-technetium^{99m} complex of the compound of claim 1.

14. A di-indium^{111} complex of the compound of claim 1.

15. The compound of claim 1 that is a di-^{99m}Tc complex of a bisaminoethanethiol derivative of HPPH.

16. The compound of claim 1 that is HPPH-di-Gd(III)di-aminophenylDTPA.

17. The compound of claim 1 that is purpurin 18 imide-di-Gd(III)di-aminophenylDTPA.

18. The compound of claim 1 that is a di-Gd(III)di-aminophenylDTPA derivative of bacteriochlorin.

19. The compound of claim 5 wherein the compound is a dicomplex of radionuclide element technetium^{99m}, indium^{111} or gadolinium III.

20. A method for the detection of tumors that comprises injecting from about 5 to about 20 μmol/kg of body weight of the compound of claim 1 into a mammal followed by MR imaging of the mammal to locate and visualize the tumors.

21. The method of claim 20 wherein the tumor is exposed to light at the absorption frequency of the compound after MR imaging at a sufficient intensity to cause tumor necrosis.

22. A method for the detection of tumors that comprises injecting from about 5 to about 20 μmol/kg of body weight of the compound of claim 3 into a mammal followed by MR imaging of the mammal to locate and visualize the tumors.

23. The method of claim 22 wherein the tumor is exposed to light at the absorption frequency of the compound after MR imaging at a sufficient intensity to cause tumor necrosis.

24. A method for the detection of tumors that comprises injecting from about 5 to about 20 μmol/kg of body weight of the compound of claim 5 into a mammal followed by MR imaging of the mammal to locate and visualize the tumors.

25. The method of claim 24 wherein the tumor is exposed to light at the absorption frequency of the compound after MR imaging at a sufficient intensity to cause tumor necrosis.

26. A method for the detection of tumors that comprises injecting from about 5 to about 20 μmol/kg of body weight of the compound of claim 6 into a mammal followed by MR imaging of the mammal to locate and visualize the tumors.

27. The method of claim 26 wherein the tumor is exposed to light at the absorption frequency of the compound after MR imaging at a sufficient intensity to cause tumor necrosis.

28. A method for the detection of tumors that comprises injecting from about 5 to about 20 μmol/kg of body weight of the compound of claim 7 into a mammal followed by MR imaging of the mammal to locate and visualize the tumors.

29. The method of claim 28 wherein the tumor is exposed to light at the absorption frequency of the compound after MR imaging at a sufficient intensity to cause tumor necrosis.

30. A method for the detection of tumors that comprises injecting from about 5 to about 20 μmol/kg of body weight of the compound of claim 8 into a mammal followed by MR imaging of the mammal to locate and visualize the tumors.

31. The method of claim 30 wherein the tumor is exposed to light at the absorption frequency of the compound after MR imaging at a sufficient intensity to cause tumor necrosis.

32. A method for the detection of tumors that comprises injecting from about 5 to about 20 μmol/kg of body weight of the compound of claim 9 into a mammal followed by MR imaging of the mammal to locate and visualize the tumors.

33. The method of claim 32 wherein the tumor is exposed to light at the absorption frequency of the compound after MR imaging at a sufficient intensity to cause tumor necrosis.

34. A method for the detection of tumors that comprises injecting from about 5 to about 20 μmol/kg of body weight of the compound of claim 10 into a mammal followed by MR imaging of the mammal to locate and visualize the tumors.

35. The method of claim 34 wherein the tumor is exposed to light at the absorption frequency of the compound after MR imaging at a sufficient intensity to cause tumor necrosis.

36. A method for the detection of tumors that comprises injecting from about 5 to about 20 μmol/kg of body weight of the compound of claim 11 into a mammal followed by MR imaging of the mammal to locate and visualize the tumors.

37. The method of claim 36 wherein the tumor is exposed to light at the absorption frequency of the compound after MR imaging at a sufficient intensity to cause tumor necrosis.

38. A method for the detection of tumors that comprises injecting from about 5 to about 20 μmol/kg of body weight of the compound of claim 11 into a mammal followed by MR imaging of the mammal to locate and visualize the tumors.

39. The method of claim 38 wherein the tumor is exposed to light at the absorption frequency of the compound after MR imaging at a sufficient intensity to cause tumor necrosis.

40. A method for the detection of tumors that comprises injecting from about 5 to about 20 μmol/kg of body weight of the compound of claim 12 into a mammal followed by MR imaging of the mammal to locate and visualize the tumors.

41. The method of claim 40 wherein the tumor is exposed to light at the absorption frequency of the compound after MR imaging at a sufficient intensity to cause tumor necrosis.

42. A method for the detection of tumors that comprises injecting from about 5 to about 20 μmol/kg of body weight of the compound of claim 16 into a mammal followed by MR imaging of the mammal to locate and visualize the tumors.

43. The method of claim 42 wherein the tumor is exposed to light at the absorption frequency of the compound after MR imaging at a sufficient intensity to cause tumor necrosis.

44. A method for the detection of tumors that comprises injecting from about 5 to about 20 μmol/kg of body weight of the compound of claim 17 into a mammal followed by MR imaging of the mammal to locate and visualize the tumors.

45. The method of claim 44 wherein the tumor is exposed to light at the absorption frequency of the compound after MR imaging at a sufficient intensity to cause tumor necrosis.

46. A method for the detection of tumors that comprises injecting from about 5 to about 20 μmol/kg of body weight of the compound of claim 18 into a mammal followed by MR imaging of the mammal to locate and visualize the tumors.

47. The method of claim 46 wherein the tumor is exposed to light at the absorption frequency of the compound after MR imaging at a sufficient intensity to cause tumor necrosis.

48. The compound of claim 1 where the compound is a digadolinium III complex.

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