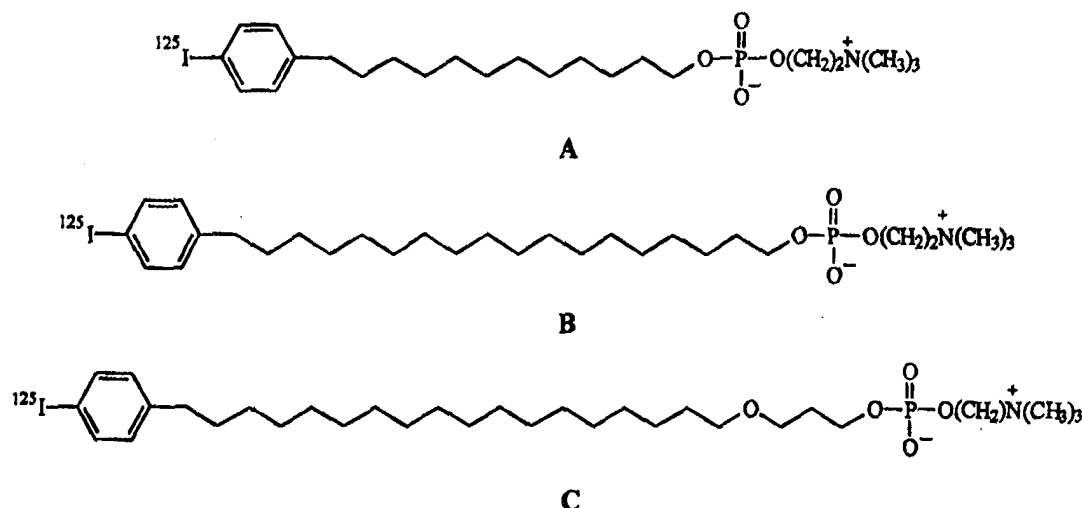




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 51/04, C07F 9/02	A1	(11) International Publication Number: WO 98/24480 (43) International Publication Date: 11 June 1998 (11.06.98)
(21) International Application Number: PCT/US96/19352 (22) International Filing Date: 4 December 1996 (04.12.96) (71) Applicant (for all designated States except US): THE REGENTS OF THE UNIVERSITY OF MICHIGAN [US/US]; Wolverine Tower, Room 2071, 3003 South State Street, Ann Arbor, MI 48109 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): COUNSELL, Raymond, E. [US/US]; 2257 Delaware Drive, Ann Arbor, MI 48103 (US). LONGINO, Marc, A. [US/US]; 2829 Carlton Drive, Ann Arbor, MI 48108 (US). PINCHUK, Anatoly, N. [RU/US]; 1373 McIntyre Drive, Ann Arbor, MI 48105 (US). RAMPY, Mark, A. [US/US]; 10224 Wild Apple Circle, Gaithersburg, MD 20879 (US). WEICHERT, Jamey, P. [RU/US]; 1230 Wines Drive, Ann Arbor, MI 48103 (US). (74) Agents: CARROLL, Peter, G. et al.; Medlen & Carroll, LLP, Suite 2200, 220 Montgomery Street, San Francisco, CA 94104 (US).		(81) Designated States: CA, JP, US. Published With international search report.

(54) Title: RADIOIODINATED PHOSPHOLIPID ETHER ANALOGS AND METHODS OF USING THE SAME



(57) Abstract

Improved radioiodinated phospholipid ether analogs are described which demonstrate significant tumor avidity and longer plasma half-life than shorter-chain analogs, providing superior imaging and visualization of neoplastic lesions and tumor-specific cytotoxic cancer therapy.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

**RADIOIODINATED PHOSPHOLIPID ETHER ANALOGS
AND METHODS OF USING THE SAME**

FIELD OF THE INVENTION

5 The present invention relates generally to the field of radiopharmaceuticals and biological probes, and more specifically to radiolabelled analogs of phospholipid ethers useful in cancer diagnosis and treatment.

BACKGROUND OF THE INVENTION

10 The early detection of cancer has been one of the primary goals of modern imaging technology, since the identification of a suspected tumor in a localized stage significantly improves the chances for successful treatment and elimination of the cancerous tissue. A large number of imaging strategies have therefore been designed, using a variety of techniques and modalities, to aid the physician in making an accurate diagnosis as early as possible.

15 Unfortunately, conventional imaging techniques such as computerized tomography (CT) and MRI (magnetic resonance imaging) are limited in their ability to afford a conclusive diagnosis of a suspected lesion, since they are only capable of observing differences in the density or morphology of tissues. A more invasive and costly biopsy procedure is often necessary to provide a definitive diagnosis. In contrast, nuclear medicine techniques such as
20 positron emission tomography (PET) and single photon emission tomography (SPECT) can provide functional or biochemical information about a particular organ or area of interest. However, the success of these nuclear imaging techniques depends in large part on the selective uptake and detection of appropriate radiopharmaceuticals. Selective uptake, in turn, depends upon the development of radiopharmaceuticals with a high degree of specificity for
25 the target tissue. Unfortunately, the tumor-localizing agents developed thus far for oncological applications have had only limited application.

 For example, one of these prior art compounds, ⁶⁷Ga gallium citrate, was originally identified for its ability to accumulate in tumor tissue. Unfortunately, ⁶⁷Ga gallium citrate is taken up by a variety of other non-tumorous lesions as well, including inflammatory lesions,
30 and unacceptable amounts of radioactivity can also accumulate in liver and spleen tissue. The rapid buildup of a radiopharmaceutical in these organs can seriously interfere with the imaging of nearby lesions, and also negatively impacts the dosage that can safely be given to a patient.

An alternative approach has been to develop radiolabelled monoclonal antibodies (Mabs) directed to tumor-specific antigens. However, these monoclonal antibodies are specific only to the particular tumor tissue for which they have been produced, and therefore will not localize generally in neoplastic tissue. Moreover, the use of Mabs for diagnostic imaging has lead to additional problems, including varying degrees of antigen expression, low tumor uptake, non-specific binding and adverse immunogenic reactions.

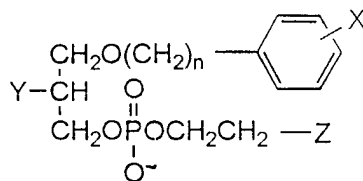
In an attempt to address these problems, the present inventors have recently identified and developed a series of novel compounds demonstrating useful tumor specificity. *See, e.g.*, U.S. Patent Nos. 4,925,649; 4,965,391; 5,087,721; and 5,347,030; all of which are herein incorporated by reference. It is believed that these radioiodinated phospholipid ether analogs take advantage of a unique biochemical characteristic of malignant tumor cells: *i.e.* the large concentration of naturally-occurring ether lipids in the cell membranes relative to corresponding normal tissues. Although the precise mechanism of action is not fully understood, the prevailing hypothesis is that the phospholipid ether analogs become entrapped in tumor membranes. Accordingly, these compounds localize in tumor tissue and remain in place for diagnostic and/or therapeutic applications.

The selective retention of the radiolabelled phospholipid ether analogs described in the above patents has been demonstrated in a variety of rodent and animal tumors. Unfortunately, the data obtained from these studies has also demonstrated a relatively rapid clearance of the radiopharmaceutical compound from the blood, and an undesirable accumulation by non-target tissues. As noted above, non-target tissue uptake can decrease the efficacy of radiodiagnostic imaging by creating high background activity, or by causing excessive exposure of radiosensitive tissues to the injected radioactivity.

Accordingly, there remains a significant need in the art for radiopharmaceuticals which exhibit a rapid clearance from non-target tissues as well as an extended half-life in the blood plasma, while still retaining its specificity and avidity for neoplastic tissue. Such an agent should not only assist in the non-invasive imaging of primary tumors and metastases, but should also provide a potential cytotoxic agent for site-specific eradication of the tumor tissue.

SUMMARY OF THE INVENTION

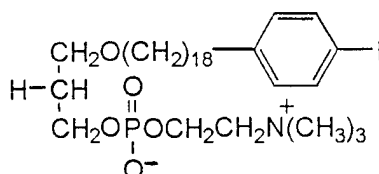
The present invention solves the problems present in the prior art through the provision of improved radiolabelled phospholipid ether analogs of naturally-occurring phospholipid ether compounds, having the general Formula I:



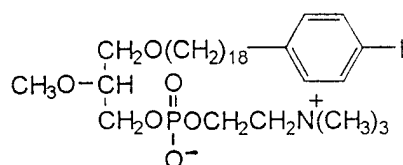
where X is a radioactive isotope and n is an integer between 16 and 30. In a preferred embodiment of the present invention, X is a radioactive isotope of iodine, preferably selected from the group comprising ^{123}I , ^{125}I , and ^{131}I . It is further contemplated that X can be substituted at the *ortho*, *meta* or *para* position on the aromatic ring. In a preferred embodiment, the radioactive isotope is substituted at the *para* position.

Y is selected from the group comprising H, OH, COOH, $\text{O}=\text{C}-\text{OR}$, and OR, and Z is selected from the group comprising NH_2 , NR_2 , and NR_3 , wherein R is an alkyl or aralkyl substituent.

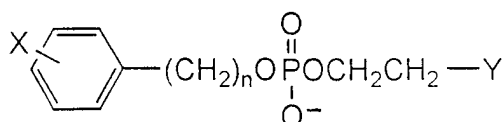
In accordance with a specific illustrative embodiment of the invention, the improved compound is 1-O-[18-(p-iodophenyl)octadecyl]-1,3-propanediol-3-phosphocholine.



In accordance with another specific illustrative embodiment, the improved compound is 1-O-[18-(p-Iodophenyl)octadecyl]-2-O-methyl-*rac*-glycero-3-phosphocholine:

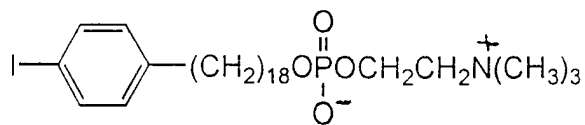


In a further embodiment of the invention, the radiolabelled aralkyl side chain may be substituted directly onto the alkyl phosphocholine moiety in accordance with general Formula II:



where X is a radioactive isotope substituted at the *ortho*, *meta* or *para* position, preferably of iodine, n is an integer from 16 to 30, and Y is selected from the group comprising NH_2 , NR_2 and NR_3 , wherein R is an alkyl or aralkyl substituent.

In accordance with a specific illustrative embodiment of the invention, the improved compound is 18-(*p*-iodophenyl)octadecyl phosphocholine.



BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is an illustrative preparatory scheme for 18-(*p*-iodophenyl)octadecanol;

Figure 2 is an illustrative preparatory scheme for 18-(*p*-iodophenyl)octadecyl phosphocholine;

Figure 3 is an illustrative preparatory scheme for 1-*O*-[18-(*p*-iodophenyl)octadecyl]-1,3-propanediol-3-phosphocholine;

Figure 4 is an illustrative preparatory scheme for 1-*O*-[18-(*p*-iodophenyl)octadecyl]-2-*O*-methyl-*rac*-glycero-3-phosphocholine;

Figures 5A-5C provide the chemical structures of the alkyl chain length analogs 12-(*p*-iodophenyl)dodecyl phosphocholine (Figure 5A), 15-(*p*-iodophenyl)pentadecyl phosphocholine (Figure 5B), and 18-(*p*-iodophenyl)octadecyl phosphocholine (Figure 5C) used in biodistribution studies with rats bearing Walker-256 tumor;

Figure 6 provides a line graph illustrating the blood clearance profile of 12-(*p*-iodophenyl)dodecyl phosphocholine (**C-12**), 15-(*p*-iodophenyl)pentadecyl phosphocholine (**C-15**) and 18-(*p*-iodophenyl)dodecylphosphocholine (**C-18**) in rats bearing Walker-256 tumors;

Figures 7A-7C provide the chemical structures of the alkyl chain length analogs 12-(*p*-iodophenyl)dodecyl phosphocholine (Figure 7A), 18-(*p*-iodophenyl)octadecyl phosphocholine (Figure 7B), and 1-*O*-[18-(*p*-iodophenyl)octadecyl]-1,3-propanediol-3-phosphocholine used in biodistribution studies with rats bearing Dunning (MATLyLu) prostate tumors;

Figures 8A-bC are depictions of whole-body gamma-camera scintigraphy scans of Dunning (MATLyLu) prostate tumor-bearing using 18-(*p*-iodophenyl)octadecyl phosphocholine (Figure 8A), 12-(*p*-iodophenyl)dodecyl phosphocholine (Figure 8B) and 1-*O*-[18-(*p*-iodophenyl)octadecyl]-1,3-propanediol-3-phosphocholine (Figure 8C).

GENERAL DESCRIPTION OF THE INVENTION

The present invention represents a significant improvement of the radiolabelled phospholipid ether analogs previously described in the prior art, providing a series of radiopharmaceutical compounds exhibiting greatly increased plasma half-life and a

significantly lower accumulation in non-target organs. Thus, these improved radiopharmaceuticals provide superior tumor imaging capabilities by reducing the amount of background radiation from non-target tissues, and the rapid clearance of the compounds from non-target organs also allows for an increase in the radiation dosimetry of the radiopharmaceutical, for further enhancement of tumor imaging capabilities and cytotoxic cancer therapy.

Surprisingly, the nature of the phospholipid ether compounds which exhibit these enhanced capabilities are compounds having an extension of the carbon chain bearing the radiolabelled phenyl group. Previous studies with related alkyl phosphocholine analogs had demonstrated that blood levels actually decreased with increasing chain length, while tumor levels increased. *See, e.g., Kotting et al., "Alkylphosphocholines: influence of structural variation on biodistribution at antineoplastically active concentrations." Cancer Chemother. Pharm. 30:105-112 (1992).* In contrast, the improved compounds of the present invention have displayed a propensity to remain in the circulation much longer than the original shorter chain analogs, and the delayed clearance from the blood plasma advantageously results in additional opportunities for uptake of the radiopharmaceuticals by tumor tissue as they continuously circulate through the vasculature.

Although an understanding of the underlying mechanism is not essential to the beneficial use of the improved compounds provided by the present invention, the inventors believe that the uptake and transport of the radiolabelled analogs by plasma components may be an important factor relating to the tumor retention of the compounds. Certainly, increasing the length of the carbon chain results in a corresponding increase in the lipophilicity of the analogs, and greater lipophilicity would presumably increase the affinity of these compounds for the cell membrane. There is also evidence (*not shown*) to indicate that the longer carbon chain alters the binding affinity of these compounds for plasma components such as plasma albumin.

The differences in the clearance and quantity of radioactivity in non-target tissues observed with the improved compounds of the present invention significantly enhances the chances for the imaging of tumors in human patients. Moreover, it should also be noted that the improved phospholipid ether analogs of the present invention are cytotoxic to tumor cells, even without the presence of a radioactive isotope in the compound. Therefore, the inclusion of a long-lived radioactive isotope of iodine, for example, yields tumor-specific radiopharmaceuticals which are tissue-destructive by more than one mode.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The following examples relate to specific embodiments and methods of using the improved radiolabelled phospholipid analogs of the present invention, and include illustrative methods for synthesizing the analogs. All starting materials and reagents were obtained from Aldrich Chemical Company, Milwaukee, Wisconsin.

In the experimental disclosure which follows, the following abbreviations apply: eq (equivalents); M (Molar); μ M (micromolar); N (Normal); mol (moles); mmol (millimoles); μ mol (micromoles); nmol (nanomoles); g (grams); mg (milligrams); kg (kilograms); μ g (micrograms); L (liters); ml (milliliters); μ l (microliters); cm (centimeters); mm (millimeters); μ m (micrometers); nm (nanometers); $^{\circ}$ C (degrees Centigrade); h (hours); min (minutes); sec (seconds); msec (milliseconds); Ci (Curies) mCi (milliCuries); μ Ci (microCuries); TLC (thin layer chromatography); Ts (tosyl); Bn (benzyl); Ph (phenyl); Ms (mesyl); Et (ethyl), Me (methyl).

EXAMPLE 1

The synthesis of 18-(p-Iodophenyl)octadecanol (Compound **VI**), which is a preliminary compound for the illustrative preparatory schemes for the phospholipid ether analogs discussed in detail in Examples 2 through 4 below, was accomplished from commercially-available 15-(p-iodophenyl) pentadecanoic acid (Compound **I**) in accordance with the illustrative preparatory scheme shown in Figure 1.

In general terms, aliphatic chain elongation from C-15 to C-18 was achieved by Li_2CuCl_4 -catalyzed cross-coupling between Grignard reagent (Compound **IV**) prepared from benzyl 3-bromopropyl ether and 15-(p-iodophenyl) pentadecyl tosylate (Compound **III**). See, e.g., Fouquet and Schlosser, "Improved carbon-carbon linking by controlled copper catalysis," *Angew. Chem. Int. Ed.* 13:82-83 (1974); Schlosser and Bossert, "The 'two-fold reaction' benchmark applied to the copper catalyzed assembling of 1, ω -difunctional hydrocarbon chains," *Tetrahedron* 47:6287-92 (1991). Cleavage of the benzyl protective group in Compound **V** by anhydrous aluminum chloride-anisole (see, e.g., Akiyama *et al.*, "AlCl₃ - N,N-dimethylaniline: a novel benzyl and allyl ether cleavage reagent," *Bull. Chem. Soc. Jpn.* 65:1932-38 (1992)) afforded alcohol **VI** with 18 carbon atoms in aliphatic chain.

Specifically, to a solution of 15-(p-iodophenyl)dodecanoic acid (Compound **I**) (210 mg; 0.47 mmol) in 3 ml of tetrahydrofuran was added $\text{BH}_3 \cdot \text{THF}$ complex (1 ml of 1.0 M solution; 1 mmol) dropwise at 0°C . The reaction mixture was then stirred at room temperature

under anhydrous conditions for 10 h. It was again cooled to 0°C and quenched with water. Ethyl acetate and additional water were added. The organic layer was separated, washed with water and dried (Na₂SO₄). Evaporation of solvent gave the white solid Compound **II**, 15-(p-iodophenyl)pentadecanol (204 mg, 100 %). Compound **II** was used in the next step without purification.

A mixture of 15-(p-iodophenyl)pentadecanol (Compound **II**) (200 mg; 0.47 mmol), tosyl chloride (102 mg; 0.53 mmol) and 4-dimethylaminopyridine (66 mg; 0.53 mmol) in dichloromethane (3 ml) was stirred at room temperature for 12 h. The reaction mixture was then partitioned between chloroform (40 ml), methanol (40 ml) and 0.1 N hydrochloric acid (36 ml). The chloroform layer was separated and extraction was repeated (2 x 40 ml of chloroform). Extracts were combined, dried (Na₂SO₄) and evaporated. The residue was chromatographed on silica gel in hexane-chloroform (from 95:5 to 40:60) to give Compound **III**, 15-(p-iodophenyl)pentadecyl tosylate (259 mg; 95 %).

To a solution of 3-benzyloxypropanol (5.28 g; 31.8 mmol) and carbon tetrabromide (13.1 g; 39.6 mmol) in dichloromethane (50 ml) was slowly added triphenylphosphine (10.4 g; 39.7 mmol) at 0°C. The reaction mixture was stirred at 0°C for 30 min and at room temperature for 1 h. By that time TLC in chloroform showed completion of the reaction. Most of the solvent was evaporated, the residue was diluted with hexane (200 ml) and the precipitate of triphenylphosphin oxide was removed by filtration. Evaporation of the filtrate gave an oily residue which was purified by chromatography in 1 % ether in hexane. This afforded Compound **IV**, benzyl 3-bromopropyl ether (5.61 g; 77 %).

To a suspension of magnesium powder (60 mg; 2.5 mmol) in tetrahydrofuran (1.5 ml) was added dibromoethane (0.02 ml) for activation. After the reaction with dibromoethane had ceased, the solution was removed by syringe and replaced with fresh tetrahydrofuran (2.5 ml). Then, benzyl 3-bromopropyl ether (Compound **IV**) (0.12 ml; 0.67 mmol) was added dropwise to the stirred suspension of magnesium for 30 min. When all the halide had been added, stirring was continued for an additional 2 h at room temperature. The green-gray solution of Grignard reagent was carefully withdrawn by syringe and transferred to 25 ml flask, which was then cooled in a dry ice bath to -78°C. A solution of Li₂CuCl₄ (0.0067 mmol/ml) in tetrahydrofuran (0.5 ml; 0.0034 mmol) was added to the Grignard reagent under stirring, followed by Compound **III**, 15-(p-iodophenyl)pentadecyl tosylate (260 mg; 0.44 mmol) in 3 ml of tetrahydrofuran.

The reaction mixture was allowed to warm to room temperature during a 2 h period, and stirring was continued for an additional 12 h. The reaction was quenched by ammonium chloride solution and extracted with ethyl acetate. The extract was washed with water, dried (Na_2SO_4) and evaporated. Silica gel chromatography with 2% ether in hexane provided

5 Compound V. 1-Benzoyloxy-18-(p-iodophenyl)octadecane (190 mg; 76 %).

Finally, to a solution of 1-benzoyloxy-18-(p-iodophenyl)octadecane (Compound V) (185 mg; 0.33 mmol) and anisole (0.15 ml; 1.32 mmol) in dichloromethane (3 ml) was added anhydrous aluminum chloride (135 mg; 1 mmol) at the room temperature. Stirring was continued for 2 h. The reaction was quenched by diluted hydrochloric acid and extracted by
10 chloroform. Extract was dried (Na_2SO_4) and evaporated. The residue was chromatographed in hexane - ethyl acetate (gradient from 95:5 to 85:15) to give 18-(p-iodophenyl) octadecanol, Compound VI (123 mg; 77 %).

EXAMPLE 2

15 Further conversion of 18-(p-iodophenyl) octadecanol into the desired phosphocholines was performed as described in detail for the C-12 analogs in Rampy *et al.*, "Synthesis and biological evaluation of radio-iodinated phospholipid ether analogs," *Nucl. Med. Biol.* 22, 505-512 (1995). In one preferred embodiment, the improved phospholipid ether analog contemplated by the present invention is a simple straight chain alkyl phospholipid, 18-(p-
20 iodophenyl)octadecyl phosphocholine (Compound XVI). The synthesis of Compound XVI was accomplished according to the illustrative preparatory scheme shown in Figure 2.

As illustrated in Figure 2, 2-chloro-2-oxo-1,3,2-dioxaphospholane (0.025 ml; 0.27 mmol) was added to the stirred solution of 18-(p-iodophenyl)octadecanol (Compound VI) (115 mg; 0.24 mmol) in dry benzene (3 ml) containing triethylamine (0.042 ml; 0.29 mmol).
25 Stirring was continued overnight. The precipitated triethylamine hydrochloride was filtered off and the solvent was removed *in vacuo*. The residue was transferred into a pressure bottle. A solution of trimethylamine in acetonitrile (5 ml; 25 % w/v) was added. The bottle was sealed and heated at 75°C for 24 h. The acetonitrile was then evaporated and the residue was chromatographed on silica gel with chloroform-methanol (gradient from 10:0 to 5:5), followed
30 by final elution with chloroform-methanol-water (65:25:4). After evaporation of the solvent, the product was precipitated by addition of acetone to give a white solid (130 mg; 84 %).

EXAMPLE 3

In another preferred embodiment, the improved phospholipid ether analogs contemplated by the present invention are constructed using a propanediol backbone. In this example, the synthesis of 1-*O*-[18-(*p*-Iodophenyl)octadecyl]-1,3-propanediol-3-phosphocholine (Compound **XIV**) was accomplished according to the illustrative preparatory scheme shown in Figure 3.

To a solution of 18-(*p*-iodophenyl)octadecanol (Compound **VI**) (150 mg; 0.317 mmol) and triethylamine (0.07 ml; 0.475 mmol) in dry methylene chloride (2 ml) was added methane sulfonyl chloride (0.03 ml; 0.38 mmol) at 0°C. Stirring was continued for 40 min and the reaction was quenched by addition of water. The reaction mixture was diluted with chloroform and washed several times with water. The chloroform layer was dried (Na₂SO₄) and evaporated. The residue was chromatographed in hexane - ethyl acetate (9:1). This afforded pure Compound **VII**, 18-(*p*-Iodophenyl)octadecyl methanesulfonate (142 mg; 82 % yield).

To a solution of 3-benzyloxy propanol (Compound **VIII**) (0.03 ml; 0.18 mmol) and 18-(*p*-iodophenyl) octadecyl methanesulfonate (Compound **VII**) (66 mg; 0.12 mmol) in dry dimethylformamide (3 ml) was added sodium hydride (8 mg of 60% suspension in oil; 0.2 mmol) at the room temperature. The reaction mixture was stirred for 12 hr, quenched by water and extracted with ethyl acetate. The extract was washed with brine, dried and evaporated. Column chromatography in hexane-ethyl acetate (gradient from 95:5 to 85:15) afforded Compound **X**, 1-*O*-[18-(*p*-Iodophenyl) octadecyl]-3-*O*-benzyl-1,3-propanediol (60 mg; 81 % yield).

Compound **X** was debenzylated by AlCl₃-anisole as described for the synthesis of Compound **VI**, to produce Compound **XII**, 1-*O*-[18-(*p*-Iodophenyl)octadecyl]-1,3-propanediol (50 mg; 0.08 mmol). (42 mg, 99 % yield). Finally, the desired phosphocholine **XIV** was synthesized above from the alcohol **XII** (42 mg; 79 mmol) in an analogous manner to that described in Example 2 for Compound **XVI**. (45 mg, 55 % yield).

EXAMPLE 4

This example illustrates yet another preferred embodiment of the improved phospholipid ether analogs contemplated by the present invention, wherein the hydrogen located at the 2-position of Compound **XIV** is replaced with an *O*-methyl group. The synthesis of 1-*O*-[18-(*p*-Iodophenyl)octadecyl]-2-*O*-methyl-*rac*-glycero-3-phosphocholine

(Compound **XV**) was accomplished according to the illustrative preparatory scheme shown in Figure 4.

Compound **XI**, 1-*O*-[18-(*p*-Iodophenyl)octadecyl]-2-*O*-methyl-3-*O*-benzyl-*rac*-glycerol, was synthesized as described above in Example 3 above for Compound **X**, from 18-(*p*-iodophenyl) octadecyl methanesulfonate (Compound **VII**) (67 mg) and 1-*O*-benzyl-2-*O*-methyl-*rac*-glycerol (Compound **IX**) (36 mg). (62 mg, 78% yield). The synthesis of Compound **IX** is known in the art and is described in detail in Pinchuk *et al.*, *Chem. Phys. Lipids*, 59:263-65 (1991). Briefly, to 2-*O*-methyl-1,2-*O*,*O*-benzylideneglycerol (2.4 g, 12.4 mmol) cooled in an ice bath was added 18 ml of 1.0 M borane-THF solution (18 ml, 18 mmol) with stirring. After 10 minutes the mixture was left at room temperature for 12 h, then for 48 h at 40-45°C until TLC (hexane/ethyl acetate 6:4) showed no starting material remained. The mixture was cooled and water was added drop-wise to destroy the borane excess. The solvent was evaporated, the residue was dissolved in water, extracted with ether (3 x 100 ml), extracts were washed with water, dried (Na₂SO₄), and evaporated to give 2.32 g (96%) of a slightly yellow oil. The compound was used for the following step without any further purification.

Compound **XIII**, 1-*O*-[18-(*p*-Iodophenyl)octadecyl]-2-*O*-methyl-*rac*-glycerol, was synthesized from the benzyl ether (Compound **XI**) (58 mg, 0.09 mmol) by the procedure described for Compound **VI** in Example 1. (40 mg, 80% yield). The desired phosphocholine **XV** was synthesized from the alcohol **XIII** (33 mg, 0.06 mmol) by the procedure described in Example 3 above for Compound **XVI**. (32 mg 75% yield).

EXAMPLE 5

Radioiodination of Phospholipid Ether Analogs

For certain uses, such as scintigraphy or experimental evaluation of tissue distribution, it is desirable to create radioactive compounds. Radioiodination of the iodinated phospholipid ether analogues disclosed herein, or one of the intermediates in the synthesis pathway, can be accomplished by a variety of techniques, some of which are known in the art. For example, aromatic compounds with electron donating groups (such as anilines) can be radiolabelled by electrophilic iodination in the presence of radioiodine, iodine monochloride, chloramine-T, iodogen, etc. Unactivated aromatic rings can be radioiodinated by exchange of a leaving group, such as aryl boronic acids, aryl thallium trifluoroacetates, triazenes or metallated arenes with radioiodine. Direct electrophilic radioiodination of a phenyl ring is yet another

alternative, but may produce isomeric mixtures which are difficult to separate. Iodine exchange of aryl iodides with radioiodine may be a preferable approach insofar as no complex separation techniques are necessary, since the substrate and radioiodinated product are chemically identical.

5 In a preferred embodiment of the invention, an isotope exchange-type technique is utilized wherein the substrate and radioiodine are reacted at an elevated temperature in a "melt." The molten reaction medium possesses a sufficiently high dielectric constant to solubilize both the substrate and radioiodide. Examples of reaction media currently in use are benzoic acid (mp 122°C., bp 249°C.) and acetamide (mp 182°C., bp 221°C.). In a specific
10 preferred embodiment, an acidic exchange medium comprising pivalic acid, a homolog of acetic acid, also known as trimethyl acetic acid, can be used. Pivalic acid has a melting point of 33°C. and a boiling point of 164°C.

The phospholipid ether analogs discussed herein were made via isotope exchange in pivalic acid. This technique is described in detail in Weichert *et al.*, "Radioiodination via
15 isotope exchange in pivalic acid," *Appl. Radiat. Isot.* 37:907-13 (1986). Briefly, the unlabeled compound (0.5 mg) was placed in a 300- μ l V-vial (Wheaton, Millville, NJ) fitted with teflon faced seal and screw cap. Absolute ethanol (20 μ l) was added via a microliter syringe, followed by aqueous Na¹²⁵I (0.5-3.0 mCi, 2-10 μ l) (no-carrier-added in reductant-free 0.1 N NaOH from Amersham Radiochemicals). The vial was gently swirled to dissolve the contents
20 and ensure homogeneity.

Inlet and outlet cannuli were inserted and a gentle stream of nitrogen was applied to remove the solvents. Two successive in-line charcoal traps were placed on the outlet side in order to trap any volatile radioiodine present in the reaction vial. Once dry, solid pivalic acid (5 mg), previously dried by azeotropic removal of water with toluene and distilled under
25 nitrogen, was added. The vial was sealed and heated at 160°C in a preheated single well aluminum heating block containing 1 cm of sand in the bottom of the well. After 1 hr., the reaction vial was removed from the heating block and allowed to cool to room temperature. Absolute ethanol (70 μ l) was added through a micro syringe followed by gentle agitation and subsequent removal of a TLC sample (1-2 μ l).

30 The entire contents of the reaction vial were then injected directly onto a silica gel HPLC column eluted with hexane/isopropanol/water (40:52:8) at 0.8 ml/min. Peaks were analyzed by both UV (230 and 254 nm) and radiodetection. After pooling appropriate fractions, the radiochemical purity of the final product was monitored by TLC (gamma and

UV detection) and by HPLC (UV at 230/254 nm and radiochemical detector). Fractions were combined and the solvent was removed with a gentle stream of nitrogen. HPLC analysis of the final compound confirmed both chemical (UV at 230/254 nm) and radiochemical (radioactivity) purity.

5 Of course, any isotope of iodine such as the clinically used isotopes, ^{122}I , ^{123}I , ^{125}I and ^{131}I can be used. ^{125}I is preferred for *in vitro* work in the laboratory due to its relatively long half-life. For radiodiagnostic purposes in humans, ^{123}I or ^{131}I are preferred due to their shorter half-lives and favorable imaging energies. Thus, the radioiodination procedure described above may be modified, as known by those of skill in the art, to compensate for the
10 difference in half-life.

 It is further contemplated that the radioiodinated phospholipid ether analogs of the present invention may be solubilized in a suitable transport agent or carrier vehicle, and administered to mammalian subjects as radiologic agents by any known manner, preferably parentally such as intravenously or intraperitoneally. It is not intended that the present
15 invention be limited by the particular nature of the therapeutic preparation. For example, such compositions can be provided together with physiologically tolerable liquid, gel or solid carriers, diluents, adjuvants and excipients.

 These therapeutic preparations can be administered to mammals for veterinary use, such as with domestic animals, and clinical use in humans in a manner similar to other
20 therapeutic agents. In general, the dosage required for therapeutic efficacy will vary according to the type of use and mode of administration, as well as the particularized requirements of individual hosts.

 Such compositions are typically prepared as liquid solutions or suspensions, or in solid forms. Oral formulations for cancer usually will include such normally employed additives
25 such as binders, fillers, carriers, preservatives, stabilizing agents, emulsifiers, buffers and excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations, or powders, and typically contain 1%-95% of active ingredient, preferably 2%-70%.

30 The compositions are also prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The radioiodinated compounds of the present invention are often mixed with diluents or excipients which are physiologically tolerable and compatible.

Suitable diluents and excipients are, for example, water, saline, dextrose, glycerol, or the like, and combinations thereof. In addition, if desired the compositions may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, stabilizing or pH buffering agents.

Additional formulations which are suitable for other modes of administration, such as topical administration, include salves, tinctures, creams, lotions, and, in some cases, suppositories. For salves and creams, traditional binders, carriers and excipients may include, for example, polyalkylene glycols or triglycerides.

EXAMPLE 6

Biodistribution Studies With Walker-256 Carcinosarcoma

The three radiolabelled compounds illustrated in Figure 5, including the improved C-18 analog 18-(*p*-iodophenyl)octadecyl phosphocholine (Compound XVI) described in Example 2, were prepared and administered to two sets of female Sprague Dawley rats (Charles River, Portage, Michigan). One normal set was used as a control, and one set was inoculated with Walker-256 carcinosarcoma cells (5×10^6 cells) in 0.2 ml saline in the right hindlimb. The Walker carcinoma cell line was provided by Dr. James Varani of the Department of Pathology at the University of Michigan, and is an accepted cell line representative of effect in humans. See, e.g. Tayck *et al.*, "Influence of the Walker 256 Carcinosarcoma on Muscle, Tumor, and Whole-Body Protein Synthesis and Growth Rate in the Cancer-Bearing Rat, *Cancer Res.* 46:5649-54 (1986).

The animals were used 6-8 days later when the tumor weight averaged 10 g. The radiolabelled compounds were dissolved in absolute ethanol (50-500 μ l) and Tween-20 (0.1 ml/mg of compound) was added to the solution. Ethanol was removed by evaporation under a stream of nitrogen. Physiological saline or sterile water was added, to give a 2-3% Tween-20 solution which was subsequently mixed by vortex. The solubilized radiolabelled compounds (5-10 μ Ci, 0.3 ml) were administered intravenously via the tail vein to tumor bearing rats, and the animals were sacrificed by exsanguination while under ether anesthesia at the various time points. Blood samples were collected through cardiac puncture and selected tissues (liver, kidney, etc.) were removed, trimmed, blotted to remove excess blood and weighed. Large organs were thoroughly minced with scissors to obtain random representative tissue samples.

For the biodistribution experiments with 12-(*p*-iodophenyl)dodecyl phosphocholine, weighed tissue samples were counted with a well scintillation counter (85% counting efficiency). The concentration of radioactivity in each tissue was expressed as a percentage of administered dose per gram of tissue.

Tissue distribution was assessed at various times following administration of the radioiodinated chain length isomers. An illustration of the data for each analog is set forth in Table 1 below. The results are expressed as the mean % administered dose per gram $\pm$ SEM (% Dose/g $\pm$ SEM).

Tissue	C-12 Analog		C-15 Analog		C-18 Analog	
	24hr	48hr	24hr	48hr	24hr	48hr
Adrenal	0.33 $\pm$ 0.01	0.29 $\pm$ 0.01	0.73 $\pm$ 0.08	0.49 $\pm$ 0.05	0.85 $\pm$ 0.04	0.92 $\pm$ 0.06
Blood	0.27 $\pm$ 0.04	0.27 $\pm$ 0.03	0.16 $\pm$ 0.01	0.14 $\pm$ 0.01	0.96 $\pm$ 0.00	0.64 $\pm$ 0.07
Duodenum	1.35 $\pm$ 0.13	0.77 $\pm$ 0.06	1.13 $\pm$ 0.13	1.38 $\pm$ 0.24	0.69 $\pm$ 0.05	0.68 $\pm$ 0.04
Kidney	4.10 $\pm$ 0.44	3.44 $\pm$ 0.08	1.14 $\pm$ 0.11	0.91 $\pm$ 0.04	0.65 $\pm$ 0.00	0.59 $\pm$ 0.04
Liver	1.62 $\pm$ 0.17	1.37 $\pm$ 0.01	1.29 $\pm$ 0.10	0.83 $\pm$ 0.04	0.55 $\pm$ 0.04	0.59 $\pm$ 0.05
Lung	1.06 $\pm$ 0.04	0.53 $\pm$ 0.01	0.97 $\pm$ 0.03	0.81 $\pm$ 0.04	0.88 $\pm$ 0.06	0.76 $\pm$ 0.07
Plasma	0.39 $\pm$ 0.05	0.39 $\pm$ 0.05	0.16 $\pm$ 0.01	0.15 $\pm$ 0.01	1.47 $\pm$ 0.03	0.95 $\pm$ 0.11
Tumor	2.99 $\pm$ 0.34	1.84 $\pm$ 0.09	1.47 $\pm$ 0.10	1.65 $\pm$ 0.23	0.98 $\pm$ 0.07	1.14 $\pm$ 0.01

As the above data clearly demonstrates, the clearance of the C18 analog from non-target tissues was much more rapid than from tumor, and the quantities of radioactivity detected in the liver, kidney and duodenum were significantly lower following administration of the C18 analog, as compared to the same organs in the C15 and C12 analog studies. In addition, the C18 analog was retained in the circulation to a much greater extent than the other chain length isomers surveyed. As Table 1 illustrates, the plasma level for the C-18 analog was significantly higher than the plasma levels for both the C-12 and the C-15 analog. Moreover, even at 120 hours, blood levels for the C18 analog were 0.60 ± 0.01 (% Dose/g $\pm$ SEM), as compared to levels of 0.07 ± 0.01 and 0.22 ± 0.03 for the C15 and C12 analogs,

respectively. This effect is illustrated in Figure 6, which shows the blood clearance profile of all three analogs up to 120 hours post-injection.

While the rapid decline in radiation levels in non-target tissue was accompanied by a much more subtle reduction in the radioactivity present in the tumor, the C18 analog still retained a beneficial degree of tumor specificity in this tumor model. In fact, the level of C18 in the tumor increased significantly over a longer time period, most likely as a result of the longer plasma half-life.

EXAMPLE 7

Biodistribution Studies With Dunning Prostate Tumor

The three radiolabelled compounds illustrated in Figures 7A-7C, including the improved C-18 analogs 18-(*p*-iodophenyl)octadecyl phosphocholine (Compound **XVI** - Figure 7B) and 1-*O*-[18-(*p*-iodophenyl)octadecyl]-1,3-propanediol-3-phosphocholine (Compound **XIV** - Figure 7C) described in Examples 2 and 3, respectively, were prepared and administered to two sets of male Copenhagen rats (Harlan Sprague-Dawley, Indianapolis, IN). One group of normal animals was used as a control, and another group was inoculated with Dunning prostate tumor cells (1×10^6 MAT-LyLu cells) in 0.2 ml saline in the right hindlimb. The MAT-LyLu subline of the Dunning (R-3327) adenocarcinoma prostate tumor was provided by Dr. Ken Pienta of the Department of Urology at the University of Michigan, and is an accepted cell line representative of effect in humans. *See, e.g., Pienta et al., "Inhibition of Spontaneous Metastasis in a Rat Prostrate Cancer Model by Oral Administration of Modified Citrus Pectin, J. Natl. Cancer Inst. 87:348-53 (1995).*

The animals were maintained with free access to food and water until day 8-10. The radioiodinated phospholipid ether analog formulated in 2% Tween 20 - sterile water (5-10 μ Ci, 0.3-0.4 ml, described above in Example 6) was administered intravenously into the tail vein of the tumor-bearing animals under metofane anesthesia. The animals were euthanized at selected time points after injection ($n=3$ per time point) and samples of blood, plasma and a variety of tissues including tumor and prostate are collected and analyzed for radioiodine content. Biodistribution values are calculated as the mean of the individual % administered dose per gram of tissue and % dose per organ values. An illustration of the data for each analog is set forth in Table 2 below. These results are also expressed as the mean % administered dose per gram $\pm$ SEM (% Dose/g $\pm$ SEM).

	C-12 Analog		Compound XIV		Compound XVI	
Tissue	24hr	48hr	24hr	48hr	24hr	48hr
Adrenal	0.29 ± 0.01	0.23 ± 0.01	1.37 ± 0.03	1.04 ± 0.07	0.88 ± 0.02	0.76 ± 0.03
Blood	0.23 ± 0.02	0.22 ± 0.01	0.41 ± 0.01	0.29 ± 0.01	0.88 ± 0.04	0.64 ± 0.05
Kidney	4.45 ± 0.27	3.38 ± 0.43	0.73 ± 0.02	0.65 ± 0.01	0.59 ± 0.02	0.51 ± 0.03
Liver	1.27 ± 0.02	1.05 ± 0.03	0.57 ± 0.02	0.36 ± 0.01	0.57 ± 0.02	0.40 ± 0.03
Lung	0.48 ± 0.03	0.33 ± 0.02	0.89 ± 0.07	0.78 ± 0.02	0.84 ± 0.01	0.69 ± 0.05
Plasma	0.32 ± 0.03	0.32 ± 0.01	0.58 ± 0.01	0.42 ± 0.03	1.43 ± 0.06	0.99 ± 0.11
Tumor	0.37 ± 0.03	0.30 ± 0.01	0.43 ± 0.05	0.39 ± 0.01	0.75 ± 0.04	0.81 ± 0.04

As with the Walker-256 tumor in Example 6, the C-18 analogs again demonstrated kidney and liver levels several times lower than the C-12 analog in the prostate tumor model. Importantly, however, the longer chain analogs also demonstrated a superior tumor avidity, with tumor levels of Compound **XVI** more than twice as high as the shorter chain analog. Thus, the improved compounds of the present invention may find particularly advantageous use in the imaging and/or treatment of prostate tumors.

EXAMPLE 8

Gamma Camera Scintigraphy Studies With Dunning R3327 Prostate Tumor

Comparative *in vivo* scintigraphic studies were also conducted using the C-12 analog 12-(*p*-iodophenyl)dodecyl phosphocholine and the C-18 analogs 18-(*p*-iodophenyl)octadecyl phosphocholine (Compound **XVI**) and 1-*O*-[18-(*p*-iodophenyl) octadecyl]-1,3-propanediol-3-phosphocholine (Compound **XIV**) utilized in Example 7. The imaging studies were performed on Dunning R3327 prostate tumor-bearing animals injected with > 40 μ Ci of the radioiodinated phospholipid ether formulations described above. The anesthetized animals were imaged at selected times after injection on a gamma camera set to the ^{125}I window and outfitted with a low energy collimator. Static fifteen-minute images were obtained for each animal at the specified time. The results are illustrated in Figures 8A-8C.

As illustrated in Figures 8A-8C for 18-(*p*-iodophenyl)octadecyl phosphocholine (Compound **XVI**), 12-(*p*-iodophenyl)dodecyl phosphocholine and 1-*O*-[18-(*p*-iodophenyl)octadecyl]-1,3-propanediol-3-phosphocholine (Compound **XIV**), respectively, the mass designated as T corresponds to the tumor tissue. Administration of the C-18 compounds
5 resulted in excellent tumor localization in comparison with the C-12 analog, wherein the tumor was barely visible.

In addition to the foregoing specifically mentioned uses of the inventive compounds, the compounds of the present invention may find applicability as carrier molecules for
10 radiosensitizers. Radiosensitizers are agents administered to sensitize tumor tissue to the effects of externally applied radiation. Well known radiosensitizers, such as misonidazole and metronidazole are substituted nitroimidazoles. Substitution of an electron-capturing moiety, such as nitroimidazole, for the iodophenyl moiety in the phospholipid ether analogues of the present invention would permit tumor-localized sensitization for radiation therapy.

15 In yet another proposed use, the phospholipid analogues of the present invention could incorporate boron containing substituents for use as boron-neutron activation therapeutic agents. These therapeutic agents are administered using the stable isotope of the electron-capturing boron. External radiation activates the boron to create tissue destructive activity.

Although the invention has been described in terms of specific embodiments and
20 applications, persons skilled in this art can, in light of this teaching, generate additional embodiments without exceeding the scope or departing from the spirit of the claimed invention. In particular, the methods of synthesis are merely illustrative and can be modified by those of skill in the art for the production of various substituted phospholipid ether analogues in accordance with the invention. Moreover, other techniques of radio-tagging the
25 analogues may be employed. Of course, the invention contemplates any one of the ortho-, meta- and para-isomers of iodobenzyl as the iodine-bearing moiety.

In addition, while certain preferred embodiments of the improved phospholipid ether
analog described herein incorporate an 18-carbon alkyl chain into the compound, both longer and shorter chains are also contemplated to be within the scope of the present invention. In
30 their association with the plasma membrane of cells, the long aliphatic chain of the phospholipid ether analogs of the present invention presumably become buried in the hydrophobic phospholipid bilayer. Both NMR and x-ray diffraction studies have shown that the thickness of this hydrophobic phase of the lipid bilayer is between 35 and 40 Angstroms

depending on the type of phospholipid making up the membrane. *See* Yeagle, P., Ed., "The Structure of Biological Membranes," page 345, CRC Press, Boca Raton, FL (1991).

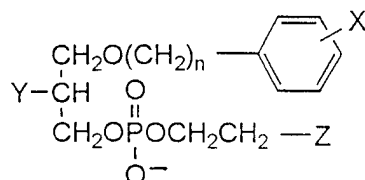
Accordingly, based on the expectation that hydrophobic chains as long as 40 Angstroms can be expected to intercalate into such lipid bilayers, the present invention contemplates that the extended alkyl chain can be up to 30 carbons in length.

Accordingly, it is to be understood that the drawings and descriptions in this disclosure are proffered to facilitate the comprehension of the invention and should not be construed to limit the scope thereof. Other improvements and modifications which become apparent to persons of ordinary skill in the art only after reading this disclosure, the drawings and the following claims are deemed within the spirit and scope of the present invention.

C L A I M S

We claim:

1. A compound of the general formula:



where X is selected from the group consisting of radioactive isotopes of iodine; n is an integer between 16 and 30; Y is selected from the group consisting of H, OH, COOH, $\text{O} \begin{array}{c} \text{O} \\ || \\ \text{O} \end{array} \text{CR}$, and OR, and Z is selected from the group consisting of NH_2 , NR_2 , and NR_3 , wherein R is an alkyl or aralkyl substituent.

2. The compound of Claim 1 wherein X is selected from the group of radioactive isotopes of iodine consisting of ^{123}I , ^{125}I , and ^{131}I .

3. A method of radioimaging a host, comprising the steps of administering to the body of said host an effective amount of a radiopharmaceutical compound as claimed in Claim 1 and subsequently scanning said host.

4. A method of therapeutically treating a living being, comprising administering to the living being a physiologically effective amount of a radiopharmaceutical compound as claimed in Claim 1.

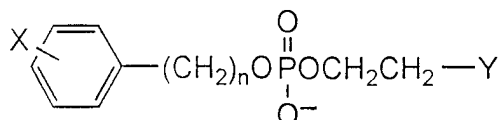
5. 1-O-[18-(p-Iodophenyl)octadecyl]-1,3-propanediol-3-phosphocholine, wherein iodine is in the form of a radioactive isotope.

6. The compound of Claim 5 wherein the radioactive isotope of iodine is selected from the group consisting of ^{123}I , ^{125}I , and ^{131}I .

7. 1-*O*-[18-(*p*-Iodophenyl)octadecyl]-2-*O*-methyl-*rac*-glycero-3-phosphocholine, wherein iodine is in the form of a radioactive isotope.

8. The compound of Claim 7 wherein the radioactive isotope of iodine is selected from the group consisting of ^{123}I , ^{125}I , and ^{131}I .

9. A compound of the general formula:



where X is selected from the group consisting of radioactive isotopes of iodine; n is an integer between 16 and 30; and Y is selected from the group comprising NH_2 , NR_2 and NR_3 , wherein R is an alkyl or aralkyl substituent.

10. The compound of Claim 9 wherein X is selected from the group of radioactive isotopes of iodine consisting of ^{123}I , ^{125}I , and ^{131}I .

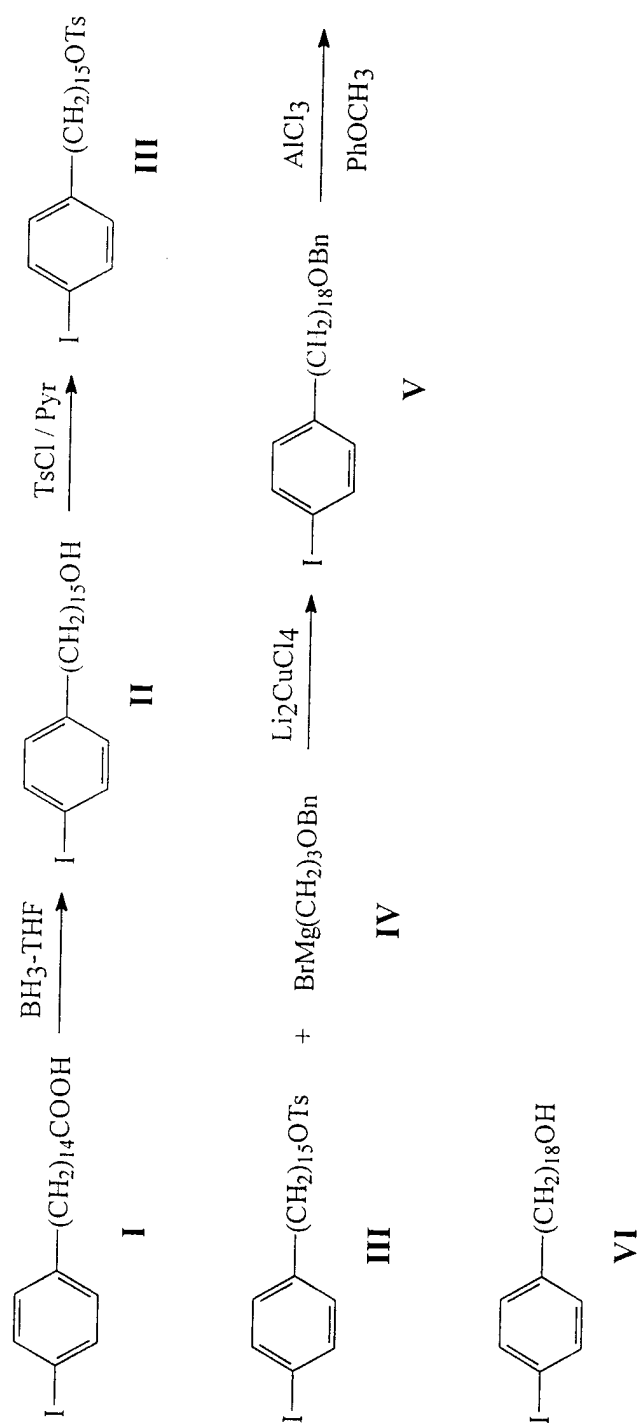
11. A method of radioimaging a host, comprising the steps of administering to the body of said host an effective amount of a radiopharmaceutical compound as claimed in Claim 9 and subsequently scanning said host.

12. A method of therapeutically treating a living being, comprising administering to the living being a physiologically effective amount of a radiopharmaceutical compound as claimed in Claim 9.

13. 18-(*p*-Iodophenyl)octadecyl phosphocholine, wherein iodine is in the form of a radioactive isotope.

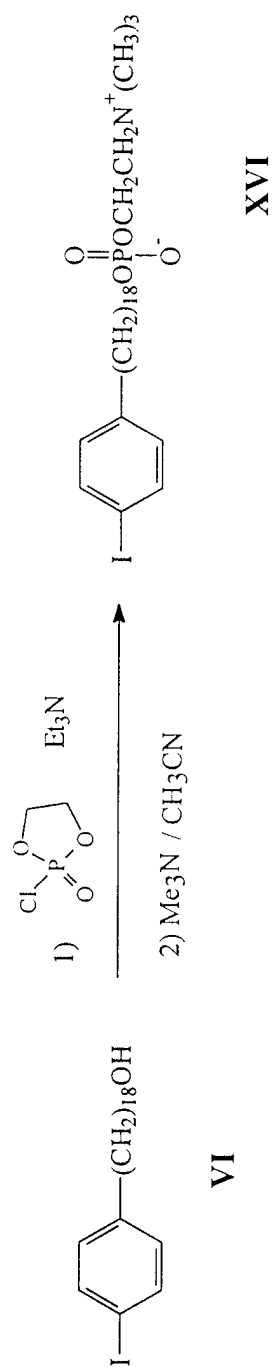
14. The compound of Claim 9 wherein the radioactive isotope of iodine is selected from the group consisting of ^{123}I , ^{125}I , and ^{131}I .

FIGURE 1



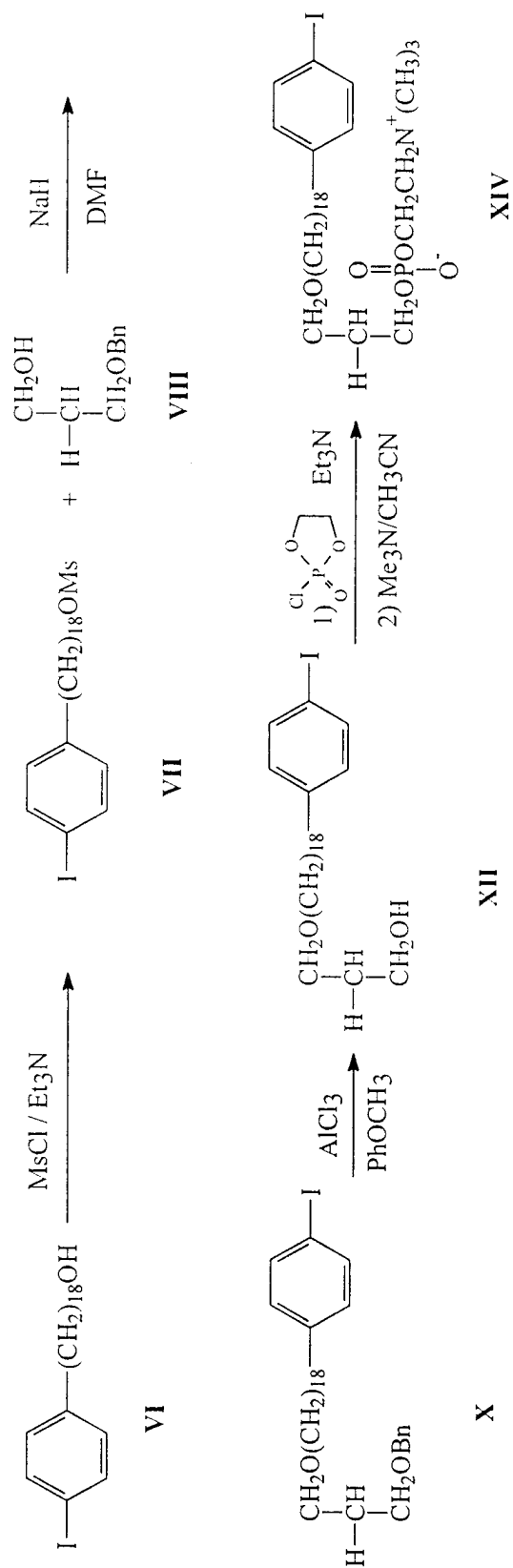
2/8

FIGURE 2



3/8

FIGURE 3



4/8

FIGURE 4

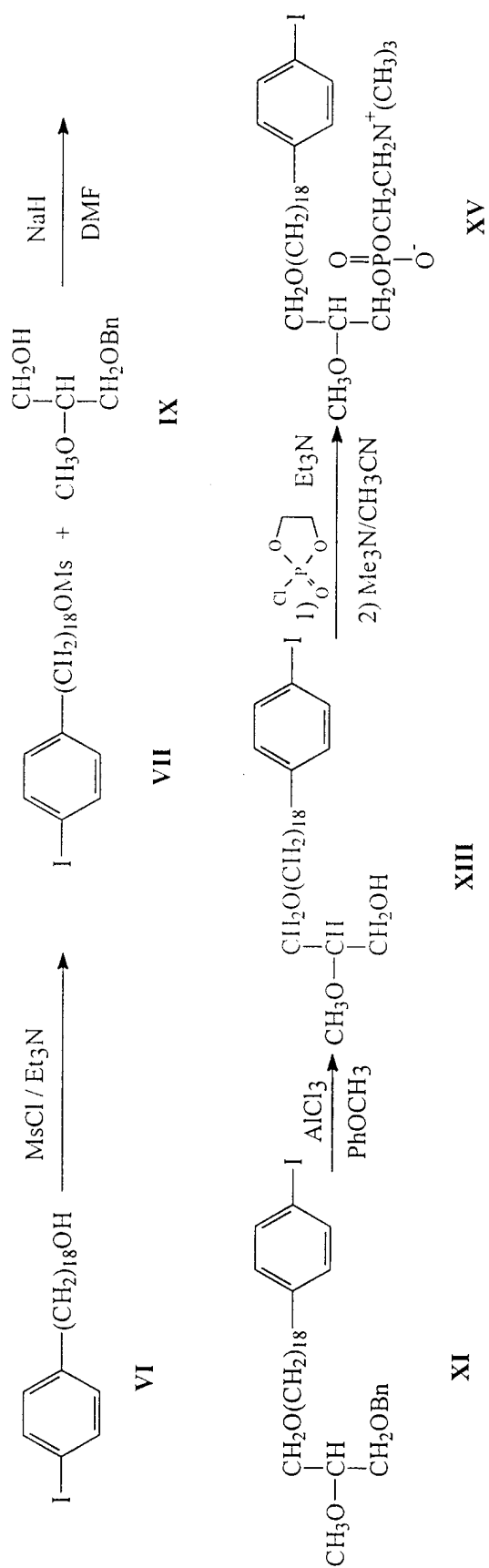


Figure 5

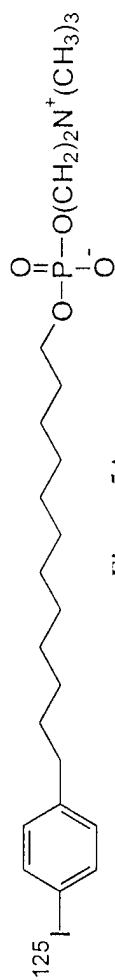


Figure 5A

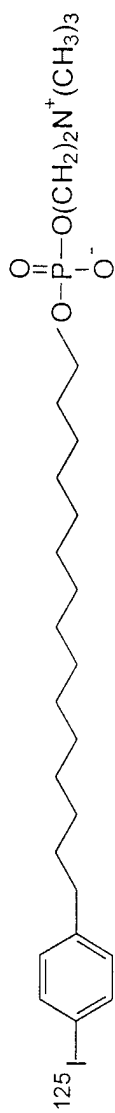


Figure 5B



Figure 5C

6/8

FIGURE 6

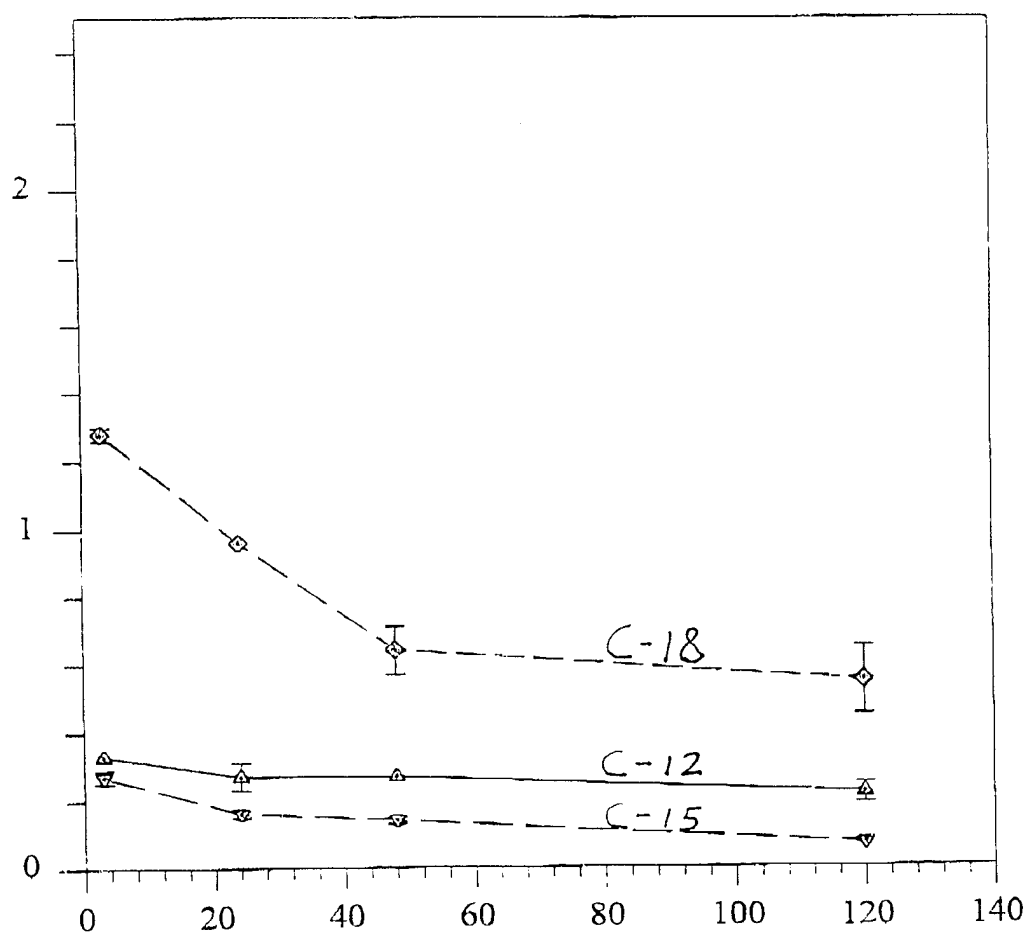
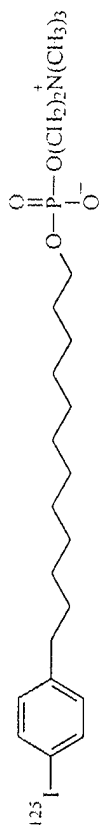
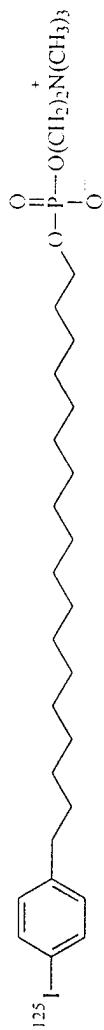


Figure 7**Figure 7A****Figure 7B****Figure 7C**

8/8

FIGURE 8



Figure 8C

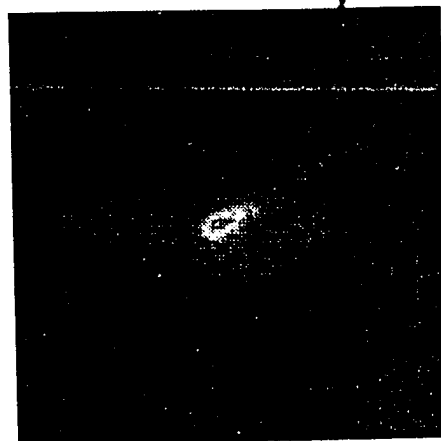


Figure 8B

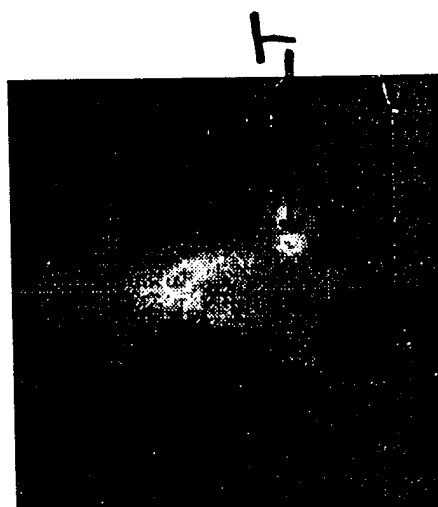


Figure 8A

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/19352

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 51/04; C07F 9/02
US CL : 424/1.77, 1.85; 558/166, 169

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/1.77, 1.85; 558/166, 169

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS ONLINE-structure search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,369,097 A (SALARI et al.) 29 November 1994, columns 1-2.	1-8
Y	US 5,347,030 A (COUNSELL et al.) 13 September 1994, columns 3-5 and figures.	1-8
A	US 4,925,649 A (COUNSELL et al.) 15 May 1990, entire document.	1-14
Y	US 5,087,721 A (COUNSELL et al.) 11 February 1992, columns 3-5 and figures.	1-8
Y	US 5,030,733 A (TEGELER et al.) 09 July 1991, columns 1-3	9-14
Y	US 4,965,391 A (COUNSELL et al.) 23 October 1990, columns 3-5 and figures.	1-8

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

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
25 APRIL 1997

Date of mailing of the international search report
02 JUN 1997

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

LARA CHAPMAN KELLEY

Telephone No. (703) 308-1235

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/19352

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,778,912 A (INOUE et al.) 18 October 1988, columns 1-3.	1-8
Y	US 4,764,358 A (KNAPP et al.) 16 August 1988, see entire document.	1-14
Y	FOS, E. Synthesis of Alkyl Chain-Modified Ether Lipids and Evaluation of their In Vitro Cytotoxicity. J. Med. Chem. 1995. Vol. 38. pages 1216-1228, especially page 1216.	1-14
Y	GARCIA, M.L. A Convenient Synthesis of 1-Ether-2-Acylamido-2-Deoxy-sn-Glycerophospholipids. Synthetic Communications. 1993. Vol. 23. No. 22. pages 3165-3177, especially page 3165.	1-14
Y	KOTTING, J. Alkylphosphocholines: Influence of Structural Variation on Biodistribution at Antineoplastically Active Concentrations. Cancer Chemotherapy and Pharmacology. 1992. Vol. 30. pages 105-112, especially pages 105-106.	1-14
Y	LEWIS, R.N.A.H. Studies of the Thermotropic Phase Behavior of Phosphatidylcholines Containing 2-Alkyl Substituted Fatty Acyl Chains: A New Class of Phosphatidylcholines Forming Inverted Nonlamellar Phases. Biophysical Journal. April 1994. Vol. 66. pages 1088-1103, especially abstract.	1-14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/19352**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.