Potential Tumor or Organ-Imaging Agents. 30. Radioiodinated Phospholipid Ethers

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Received October 31, 1988

A radioiodinated analogue of a naturally occurring alkyl lysophospholipid (ALP) was synthesized for evaluation as a potential tumor-localizing imaging agent. *rac*-1-[12-(*m*-Iodophenyl)dodecyl]-2-methylglycero-3-phosphocholine (ET-12IP-OMe, 14) was radiolabeled with iodine-125 via an isotope-exchange procedure. Tissue distribution studies with [^{125}I]ET-12IP-OMe in tumor-bearing rats revealed an immediate tumor uptake of radioactivity. Although radioactivity was also present in nontarget tissues at this time, clearance of tracer from the tumor was much slower and thus provided a suitable tumor to nontarget tissue ratio at 24 h. As a result of this selective accumulation, it was possible to clearly delineate the tumor with γ-camera scintigraphy.

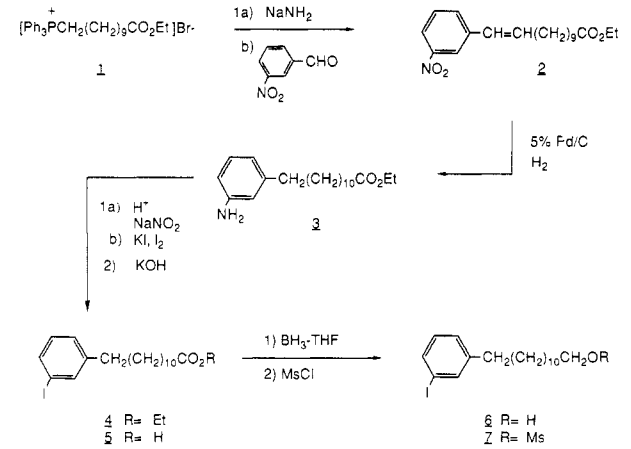
Ideally, radioisotope imaging has the potential to provide not only the detection and diagnosis of primary tumors and metastases but also to monitor the response of patients to therapy.[1] Unfortunately, the lack of appropriate tumor-localizing radiodiagnostics has hindered the achievement of these goals. A particular problem associated with the currently available agents (e.g. [^{67}Ga]gallium citrate) is poor tumor specificity.[4]

In order to achieve greater tumor specificity, numerous laboratories have explored the ability of tumor antibodies to serve as carriers for various radionuclides.[5-8] Studies in our own laboratory, on the other hand, have focused on drug molecules which have shown an ability to accumulate in tumors to serve as carriers of the radiodiagnostic probe.[9-12] The present paper represents another example illustrating the feasibility of such a pharmacological approach to radiodiagnostic design.

Analogues of the naturally occurring alkyl lysophospholipid (ALP) are known to exhibit antitumor and antimetastatic activities. These compounds, such as *rac*-1-octadecyl-2-methylglycero-3-phosphocholine (ET-18-OMe) (Figure 1), have shown a selective cytotoxicity toward a variety of human and murine tumors in both in vivo and in vitro studies.[13,14] Although the mechanism(s) for this activity is not fully understood, studies have indicated that several actions may act cooperatively. These include activation of cytotoxic macrophages,[15,16] induction of differentiation,[17] an antiinvasive activity,[18] and inhibition of Ca^{2+}-dependent protein kinase C[19] as well as a direct cytotoxicity.[14,20,21] In addition, Hoffman et al.[22] and others[23,24] have reported a selective accumulation of ALP analogues within tumor cells. It has been proposed that the direct cytotoxicity results from accumulation of ALP analogues within the tumor cells, which disrupts the natural phospholipid metabolism and eventually leads to cell death.[25]

On the basis of what is currently known about the accumulation of ALP-like molecules within cancer cells, a suitably radioiodinated ALP analogue would be expected to concentrate within neoplastic cells and thereby allow visualization via scintigraphic imaging. Accordingly, this paper describes the synthesis of radioiodinated *rac*-1-[12-(*m*-iodophenyl)dodecyl]-2-methylglycero-3-phosphocholine (ET-12IP-OMe, 14) and evaluation of this agent as a potential tumor-localizing agent.

Scheme I. Synthesis of 12-(*m*-Iodophenyl)dodecyl Methanesulfonate



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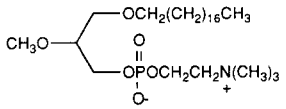


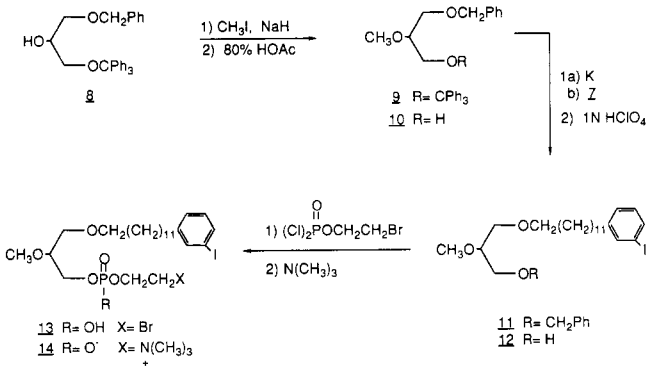
Figure 1. *rac*-1-Octadecyl-2-methylglycero-3-phosphocholine (ET-18-OMe).

Chemistry

The identification of biologically active ether phospholipids has triggered a renewed interest in the chemical synthesis of these compounds. A variety of synthetic procedures have been developed and several reviews have appeared.[26-29] In general, these synthetic methods are similar in that the ether bond is introduced early in the synthesis. An alternate approach was needed in this study, however, since the 12-(*m*-iodophenyl)dodecyl moiety required for the synthesis of the target compound (**14**) involved a multistep synthesis. Therefore, a synthetic route to **14** was designed in which the ether bond at the *sn*-1 position was established late in the sequence.

The synthesis of 12-(*m*-iodophenyl)dodecyl methanesulfonate (**7**) was accomplished as outlined in Scheme I. Although 1-arylalkanoic acids have been prepared by Wittig reactions,[30,31] Friedel–Crafts acylations,[32,33] copper-catalyzed Grignard reactions,[34] and other organometallic reactions,[35,36] the Wittig reaction was selected on the basis that it provided a convenient method for introducing a *m*-nitrophenyl group which could be ultimately converted to the desired *m*-iodophenyl fatty acid(s).

Scheme II. Synthesis of ET-12IP-OMe



The Wittig reaction involving (11-carbethoxyundecyl)-triphenylphosphonium bromide[31] with *m*-nitrobenzaldehyde was performed by following the "instant ylide" procedure reported by Schlosser et al.[37] This procedure required mixing the dry Wittig salt with $NaNH_2$ under anhydrous conditions prior to the addition of solvent at room temperature. The final step in the procedure was the addition of aldehyde. This Wittig procedure provided a shorter reaction time and an improved yield over the method previously reported for the synthesis of **2**.[31] The alternate Wittig approach to olefin **2** involving ethyl 11-oxoundecanoate and (*m*-nitrobenzyl)triphenylphosphonium bromide was also investigated. However, difficulty was experienced in preparing[30] and storing large quantities of ethyl 11-oxoundecanoate. Therefore this route was not pursued further.

The conversion of **2** to amino ester **3** was readily accomplished by catalytic hydrogenation with 5% Pd/C in EtOAc. Compound **3** was then converted to the corresponding *m*-iodo derivative **4** via the diazonium salt by utilizing a procedure similar to that reported by Coffen and co-workers.[38] This approach was selected for formation of the aryl iodide since only one isomer is obtained. In order to facilitate purification of the iodinated product, iodination was performed with the ethyl ester. The ester, unlike the corresponding acid **5**, was considerably less polar than the accompanying side products and was easily purified by column chromatography.

The synthesis of alcohol **6** was completed by saponification of **4** and reduction of the resulting acid with BH_3–THF following conditions reported by Yoon and co-workers.[39] The reduction proceeded rapidly and afforded a quantitative yield of **6**. Compound **7** was formed by treatment of **6** with methanesulfonyl chloride in the presence of pyridine.

The remaining steps in the synthesis of the ALP analogue **14** are presented in Scheme II. The glycerol derivative **10** was prepared by using a method similar to one reported by Holy.[40] The secondary alcohol of the diether **8** was converted to the methyl ether. Detritylation was then achieved by refluxing **9** in 80% HOAc. Etherification of the resulting alcohol **10** to afford **11** was accomplished by alkylating with mesylate **7** in the presence of K metal and benzene. The next step in the reaction sequence was

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Table I. Distribution of Radioactivity following Intravenous Administration of [^{125}I]ET-12IP-OMe in Normal and Tumor-Bearing Rats[a]

tissue	6 h	24 h	48 h
	Normal Rats		
adrenal	0.23 ± 0.00	0.48 ± 0.02	0.31 ± 0.03
blood	0.10 ± 0.01	0.08 ± 0.00	0.04 ± 0.00
kidney	0.65 ± 0.11	0.43 ± 0.03	0.20 ± 0.03
liver	1.74 ± 0.12	0.68 ± 0.02	0.27 ± 0.01
lung	0.36 ± 0.01	0.22 ± 0.01	0.10 ± 0.00
muscle	0.02 ± 0.00	0.03 ± 0.00	0.02 ± 0.00
plasma	0.15 ± 0.02	0.12 ± 0.00	0.05 ± 0.00
thyroid	2.76 + 0.48	24.03 + 4.93	34.09 + 8.31
	Tumor-Bearing Rats		
adrenal	0.52 ± 0.02	0.44 ± 0.06	0.30 ± 0.04
blood	0.23 ± 0.01	0.10 ± 0.02	0.05 ± 0.02
kidney	1.32 ± 0.09	0.46 ± 0.11	0.04 ± 0.10
liver	3.59 ± 0.19	0.74 ± 0.10	0.35 ± 0.07
lung	0.87 ± 0.08	0.30 ± 0.02	0.13 ± 0.01
muscle	0.05 ± 0.00	0.03 ± 0.01	0.02 ± 0.00
plasma	0.32 ± 0.02	0.12 ± 0.04	0.07 ± 0.02
thyroid	4.01 ± 0.08	37.34 ± 6.06	36.62 ± 6.34
tumor	1.12 + 0.02	1.12 + 0.02	0.63 + 0.13

[a] Expressed as percent dose per gram of tissue ± SEM; n = 3 or 4; Walker 256 sarcoma was implanted in the thigh.

the cleavage of the benzyl ether. Catalytic hydrogenation was unacceptable since such conditions would also effect hydrogenolysis of the aryl iodide. Debenzylation was achieved in moderate yields by heating **11** in a solution of 1 N $HClO_4$ in 1,4-dioxane.

Many procedures have been described in the literature for accomplishing the addition of the phosphocholine moiety. With use of conditions developed by Hansen and co-workers,[41] the glycerol derivative **12** was treated with 2-bromoethyl dichlorophosphate to form **13**. This was followed by displacement of the bromide with trimethylamine to afford the desired ALP analogue **14**. Radioiodination of **14** with iodine-125 was accomplished with an isotope exchange in a melt of pivalic acid.[42]

Biological Studies

Tissue distribution studies were performed with the radioiodinated ALP analogue **14** in both normal and tumor-bearing female Sprague–Dawley rats. The tumor selected for this investigation was the Walker 256 carcinosarcoma, which was maintained as a solid tumor by serial intramuscular (im) passage. Approximately 10 days prior to initiating a study, rats received an im transplant of viable tumor cells in the thigh.

The radiolabeled analogue was administered intravenously (iv) in a 2–3% Tween 20/saline vehicle and the animals were sacrificed at various time periods. Tissue samples were removed and the radioactivity present within the sample was determined. The amount of radioactivity was expressed as the percentage of the administered dose per gram of tissue. A total of 13 tissues including the tumor were examined. However, only the data obtained from nine tissues are included in Table I. Fat, heart, ovary, and spleen generally contained low levels of radioactivity and were, therefore, not tabulated.

In order to determine the macromolecules (lipoprotein, plasma protein) involved in transport of the radiolabeled analogue to the tissues, plasma samples were subjected to polyacrylamide gel electrophoresis (PAGE). In addition, lipid extraction of the liver, plasma, and tumor was performed in order to assess whether metabolism of the analogue was occurring. Table II summarizes the percent of the lipid-extractable material present in the plasma, liver, and tumor, as well as the amount of radioactivity found to comigrate on thin-layer chromatography with the parent compound.

Table II. Analysis of Lipid-Soluble Radioactivity Extracted from Rat Liver, Plasma, and Tumor following Intravenous Administration of [^{125}I]ET-12IP-OMe[a]

	% $CHCl_3/CH_3OH$ extractable		% parent compd as detmnd by TLC	
tissue	control	tumored	control	tumored
		6 h		
liver	82.0 ± 3.9	84.7 ± 1.6	93.1 ± 2.2	90.9 ± 4.8
plasma	28.3 ± 7.9	49.3 ± 9.2	69.6 ± 2.9	32.7 ± 11.1
tumor		84.1 ± 2.7		82.4 ± 6.2
		24 h		
liver	76.7 ± 0.2	81.2 ± 1.6	63.1 ± 9.5	90.0 ± 0.4
plasma	56.3 ± 14.7	32.1 ± 10.4	22.1 ± 7.5	51.0 ± 4.2
tumor		88.6 ± 0.1		92.0 ± 1.2
		48 h		
liver	81.9 ± 1.2	78.7 ± 1.2	31.0 ± 4.4	46.6 ± 2.1
plasma	81.2 ± 3.1	68.3 ± 7.6	15.1 ± 4.7	19.6 ± 1.7
tumor		90.0 ± 1.6		78.9 ± 8.6

[a] Percent ± SEM; n = 3 or 4; tumored animals had Walker 256 sarcoma implanted in the thigh.

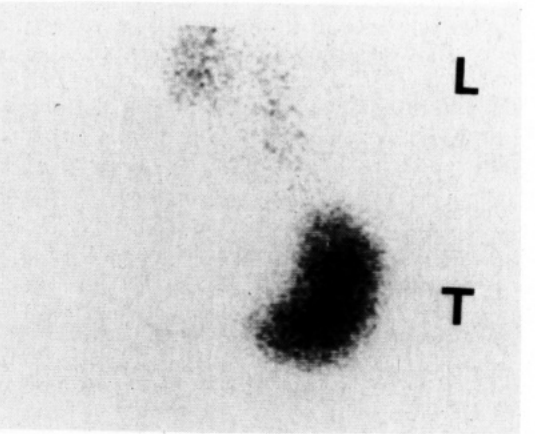

Figure 2. γ-camera scintigram of a rat bearing a Walker 256 tumor implanted in the thigh at 24 h following administration of [^{125}I]ET-12IP-OMe. The view is of the animal's abdomen (L = liver, T = tumor).

To evaluate the potential of [^{125}I]ET-12IP-OMe (**14**) as a radiodiagnostic agent, an image obtained at 24 h following administration of [^{125}I]ET-12IP-OMe to a tumor-bearing rat is shown in Figure 2.

Results and Discussion

The tissue distribution of radioactivity following administration of [^{125}I]ET-12IP-OMe (**14**) was surveyed at five time intervals (30 min, 3, 6, 24, and 48 h). On the basis of this preliminary survey, subsequent experiments involving multiple animals (n = 3 or 4) were performed at 6, 24 and 48 h. The results for these experiments are tabulated in Table I. In general, the profiles of radioactivity in the tumor-bearing and normal rats were similar at the early time points of 30 min and 3 h (data not shown). By 6 h, a difference in clearance of radioactivity was clearly evident between the two animal groups. A more rapid clearance was observed from the tissues of the normal rats. Such a difference resulted in approximately a 2-fold lower concentration of radioactivity in the tissues of the normal animals. This distinction disappeared by 24 h and no major differences were noted during the rest of the study.

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One exception to this trend was the accumulation in the thyroid, which was used as an indicator of in vivo deiodination. Only moderate levels of radioactivity were present in the thyroid. This indicated that the [^{125}I]14 was reasonably stable to metabolic deiodination, suggesting that clearance of radioactivity from the liver and other tissues were not due to this type of metabolism.

The tumor exhibited a high level of radioactivity at 30 min (0.9% dose per gram of tissue). Unlike the nontarget tissues in the tumor-bearing rats, which exhibited reduction of radioactivity after 6 h, radioactivity in the tumor remained relatively constant for 24 h. However, this level was found to decline by approximately 50% at 48 h. This difference in clearance of radioactivity between the tumor and the nontarget tissue resulted in a favorable tumor to blood ratio (12.88 ± 2.61). Such results suggested that background interference due to radioactivity present in the blood would be minimal during scintigraphic imaging.

Lipid extraction of the liver from either normal or tumored animals at 6, 24, and 48 h demonstrated that most of the radioactivity present was lipid soluble (Table II). With time, the percent of radioactivity associated with the parent compound was observed to decline, indicating that metabolism was occurring. The absence of large quantities of radiolabeled metabolites in the liver at 6 and 24 h suggests that any hepatic metabolites formed were rapidly cleared. This may also explain the much lower level of lipid extractable radioactivity from the plasma and the decreased amount of radioactivity which comigrated with the parent compound on TLC analysis. PAGE of the plasma revealed most of the radioactivity to be largely associated with albumin. These data suggest that ET-12IP-OMe exhibits an affinity toward albumin similar to that reported for natural lysophospholipids[43] and ET-18-OMe.[44] Similar to the liver, most of the radioactivity in the tumor was lipid extractable. In addition, most of this radioactivity remained associated with the parent compound. These facts combined with the slow clearance of radioactivity from the tumor suggests that metabolism of ET-12IP-OMe is slower in the tumor than in normal tissues.

As would be anticipated on the basis of the tissue distribution studies, excellent images of the tumor were obtained upon γ-camera scintigraphy following administration of [^{125}I]ET-12IP-OMe (Figure 2).

Iodine-125, which possesses a long half-life (60 days) and emits weak γ-photons (35 KeV), was an appropriate choice for these initial tissue distribution and scintigraphic studies. The weak γ-photons, however, limits its scintigraphic use to small animals such as rats. Future studies with larger animals or human subjects will require the use of iodine-123 ($T_{1/2}$ = 13 h; 159 KeV), which can be readily substituted for iodine-125 in the synthetic scheme.

In summary, a radioiodinated analogue of ALP was shown to be taken up and retained within the Walker 256 carcinosarcoma. In addition, the clearance rate of radioactivity from the tumor versus nontarget tissue was sufficiently different so as to provide clear images of the tumor. Additional studies with 14 in other animal tumor models are required, however, as well as a determination of its tumor specificity in order to fully evaluate the clinical potential of this new agent.

Experimental Section

Synthetic Methods. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. IR spectra (IR) were recorded in $CHCl_3$ solutions on either a Perkin-Elmer 281 spectrophotometer or a Nicolet 5DXT Fourier transform spectrophotometer. ^{1}H NMR spectra were recorded either on a Bruker WM360 or on a Bruker WP270SY. Chemical shifts were reported in parts per million (δ) relative to tetramethylsilane. Chemical-ionization mass spectra (CIMS) were performed on a VG Analytical 70-250-S with isobutane as the carrier gas. Elemental analyses were performed by Midwest Microlab, Ltd., Indianapolis, IN. Thin-layer chromatography (TLC) was performed on either Merck silica gel-60 F_{254} polyethylene-backed plates or Analtech silica gel GHLF glass-backed plates. Visualization was achieved by UV, I_2, and charring after spraying with 50% H_2SO_4. Molybdenum Blue and Dragendorff's spray (Sigma Chemical Co., St. Louis, MO) were used to visualize the phospholipids. TLC analyses of radioiodinated compounds were performed with Merck silica gel 60 plates and were scanned with a Vanguard 930 autoscanner. Column chromatography was carried out with Davisil 62 silica gel from Grace, Davison Chemical, Baltimore, MD. Radioiodinated compounds were purified by column chromatography with Merck silica gel 60 (230–400 mesh). Radioiodination reactions were performed inside a Plexiglas glovebox. The glovebox was vented with a model RIT-140 radioiodine trap (HiQ Filter Products, La Jolla, CA). Anhydrous solvents were prepared by standard methods.[45] EtOH-free $CHCl_3$ was prepared by extraction with concentrated H_2SO_4 and then H_2O until the solvent was neutral.[45] The $CHCl_3$ was dried (K_2CO_3), distilled, and stored over molecular sieves. Starting materials were purchased from Aldrich Chemical Co., Milwaukee, WI, unless otherwise stated. Trimethylamine (45–55% aqueous solution) was purchased from Fluka Chemical Corp., Ronkonkoma, NY.

Ethyl 12-(*m*-Nitrophenyl)-11-dodecenoate (2). Sodium amide (1.80 g, 43.8 mmol) was weighed directly into a flame-dried 250-mL round-bottom flask charged with N_2. (11-Carbethoxyundecyl)triphenylphosphonium bromide (1)[31] (20.0 g, 36.0 mmol) followed by anhydrous THF (50 mL) was added. After the reaction mixture was stirred for 50 min at room temperature, a solution of *m*-nitrobenzaldehyde (5.00 g, 33.1 mmol) in anhydrous THF (20 mL) was added dropwise. The reaction mixture was stirred for 4.5 h and then cooled to 0 °C before H_2O (2 mL) was cautiously added to destroy the residual base. Following filtration of the reaction mixture, the solvent was removed from the filtrate under reduced pressure and the resulting residue was dissolved in Et_2O. The ether solution was extracted with H_2O, 1 N HCl, H_2O, and brine and then dried ($MgSO_4$). Filtration and concentration of the filtrate under reduced pressure yielded an oily residue, which was dissolved in petroleum ether (bp 30–60 °C) and EtOAc and cooled to 0 °C for 24 h. The precipitated triphenylphosphine oxide was filtered and the filtrate evaporated to dryness in vacuo. Purification by column chromatography (160 g silica gel, hexanes/EtOAc, 10:1) gave the nitro compound 2: 6.86 g, 60%; IR ($CHCl_3$) 1730 (ester C=O), 1540 (assym N=O), 1360 (sym N=O) cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.12 (m, 2 H, ArH), 7.52 (m, 2 H, ArH), 6.50–6.38 (m, 1.2 H, trans ArC*H*=C*H*R and cis ArC*H*=CHR), 5.84 (dt, 0.8 H, cis ArCH=C*H*R), 4.12 (q, 2 H, CO_2CH_2), 2.31 (m, 4 H, CH=CHC*H*$_2$ and CH_2CO_2), 1.36 (m, 17 H, $(CH_2)_7$ and CH_3). Anal. ($C_{20}H_{29}NO_4$) C, H, N.

Ethyl 12-(*m*-Aminophenyl)dodecanoate (3). Compound 3 was prepared by using a modified procedure reported by Weichert et al.[31] A solution of 2 (9.76 g, 28.1 mmol) in EtOAc (55 mL) was hydrogenated for 4 h over 5% Pd/C (0.346 g) at room temperature and at an initial pressure of 45 psi. Workup as previously described[31] afforded the reduced compound 3: 8.75 g, 97.5%; IR ($CHCl_3$) 3460, 3380 (amine NH), 1725 (ester C=O) cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.05 (t, 1 H, ArH), 6.58 (d, 1 H, ArH), 6.51 (m, 2 H, ArH), 4.12 (q, 2 H, CO_2CH_2), 3.59 (br s, 2 H, NH_2), 2.50 (t, 2 H, $ArCH_2$), 2.28 (t, 2 H, CH_2CO_2), 1.58 (m, 4 H, $ArCH_2CH_2$ and $CH_2CH_2CO_2$), 1.25 (m, 17 H, $(CH_2)_7$ and CH_3). Anal. ($C_{20}H_{33}NO_2$) C, H, N.

Ethyl 12-(*m*-Iodophenyl)dodecanoate (4). Glacial acetic acid (4 mL) and concentrated HCl (2.5 mL) were added to a 50-mL Erlenmeyer flask containing amine 3 (3.92 g, 12.3 mmol). The

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mixture was cooled in a NaCl/ice bath prior to the addition of a cold aqueous solution of $NaNO_2$ (4 mL, 3.1 M). The reaction mixture was stirred for 40 min at 0 °C before a cold, aqueous solution of KI/I_2 (2.31 g/1.78 g, 7 mL) was cautiously added. Following this addition, the reaction mixture was allowed to slowly warm to room temperature and was stirred overnight. Et_2O was added. The Et_2O layer was separated and extracted with H_2O, 10% $Na_2S_2O_3$, H_2O, saturated $NaHCO_3$ (until basic), and brine. The organic layer was dried ($MgSO_4$) and filtered. The solvent was removed under reduced pressure to give a reddish oil, which was purified by column chromatography (120 g silica gel, hexanes/EtOAc, 20:1) to yield a clear oil, **4**: 5.28 g, 57%; IR ($CHCl_3$) 1720 (ester C=O) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.53 (m, 2 H, ArH), 7.14 (d, 1 H, ArH), 7.00 (t, 1 H, ArH), 4.12 (q, 2 H, CO_2CH_2), 2.53 (t, 2 H, $ArCH_2$), 2.28 (t, 2 H, CH_2CO_2), 1.60 (m, 4 H, $CH_2CH_2CO_2$ and $ArCH_2CH_2$), 1.25 (m, 17 H, $(CH_2)_7$ and CH_3). Anal. ($C_{20}H_{31}IO_2$) C, H, I.

12-(*m*-Iodophenyl)dodecanoic Acid (5). Ethyl ester **4** (7.17 g, 16.7 mmol), 95% EtOH (100 mL) and KOH (2.00 g, 30.3 mmol) were placed into a 250-mL round-bottom flask fitted with a reflux condenser, and the reaction mixture was refluxed overnight. The EtOH was removed in vacuo and H_2O was added. The aqueous solution was acidified with 1 N HCl and then extracted with Et_2O. The ether layer was dried ($MgSO_4$) and filtered. Concentration of the filtrate under reduced pressure yielded a yellow solid, which was recrystallized with 95% EtOH to provide a white, flaky solid, **5**: 5.90 g, 88%; mp 55–57 °C; IR ($CHCl_3$) 3300–2800 (acid OH), 1709 (acid C=O) cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.51 (m, 2 H, ArH), 7.12 (d, 1 H, ArH), 6.99 (t, 1 H, ArH), 2.53 (t, 2 H, $ArCH_2$), 2.34 (t, 2 H, CH_2CO_2), 1.64–1.55 (m, 4 H, $ArCH_2CH_2$ and $CH_2CH_2CO_2$), 1.27 (br s, 14 H, $(CH_2)_7$). Anal. ($C_{18}H_{27}IO_2$) C, H, I.

12-(*m*-Iodophenyl)dodecanol (6). A solution of **5** (3.30 g, 8.21 mmol) in anhydrous THF (20 mL) was added to a flame-dried 100-mL two-neck round-bottom flask charged with N_2. The solution was cooled to 0 °C before BH_3–THF (15.0 mL, 1 M) was added dropwise. The reaction mixture was then stirred at room temperature under anhydrous conditions for 20 h. The reaction mixture was again cooled to 0 °C and quenched with H_2O. Et_2O and additional H_2O were added. The ether layer was separated, extracted with H_2O, saturated $NaHCO_3$, and H_2O, dried ($MgSO_4$), and then filtered. The solvent was removed under reduced pressure to yield a yellow oil, which was purified by column chromatography (90 g of silica gel, hexanes/EtOAc, 8:1) to furnish a white solid, **6**: 3.14 g, 98%; mp 30–32 °C; IR ($CHCl_3$) 3620, 3460 (alcohol OH), cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.53 (s, 1 H, ArH), 7.50 (d, 1 H, ArH), 7.12 (d, 1 H, ArH), 6.99 (t, 1 H, ArH), 3.63 (t, 2 H, CH_2OH), 2.53 (t, 2 H, $ArCH_2$), 1.56 (m, 4 H, CH_2CH_2OH and $ArCH_2CH_2$), 1.28 (br s, 16 H, $(CH_2)_8$). Anal. ($C_{18}H_{29}IO$) C, H, I.

12-(*m*-Iodophenyl)dodecyl Methanesulfonate (7). Alcohol **6** (5.88 g, 15.2 mmol) and anhydrous pyridine (30 mL) were placed into a flame-dried three-neck 100-mL round-bottom flask equipped with a reflux condenser and charged with N_2. The mixture was cooled to 0 °C before freshly distilled methanesulfonyl chloride (2.0 mL, 26.0 mmol) was added dropwise. After the reaction mixture had been stirred at room temperature for several hours, it was poured into ice-cold H_2O and the resulting precipitate was filtered. The solid was dissolved in Et_2O and the solution was extracted with H_2O, 1 N HCl, and H_2O. The ether layer was dried ($MgSO_4$) and filtered. The solvent was evaporated in vacuo and the residual solid was recrystallized with a hexanes and EtOAc mixture to yield the pure mesylate **7**: 4.95 g, 71%; mp 35.5–37.0 0°C; IR ($CHCl_3$) 1353 (assym S=O), 1171 (sym S=O) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.56 (m, 2 H, ArH), 7.12 (d, 1 H, ArH), 7.01 (t, 1 H, ArH), 4.22 (t, 2 H, CH_2O), 3.00 (s, 3 H, SCH_3), 2.53 (t, 2 H, $ArCH_2$), 1.75 (m, 2 H, CH_2CH_2O), 1.59 (m, 2 H, $ArCH_2CH_2$), 1.32 (m, 16 H, $(CH_2)_8$). Anal. ($C_{19}H_{31}IO_3S$) C, H.

***rac*-1-*O*-Benzyl-2-*O*-methyl-3-*O*-tritylglycerol (9). Method A.** $NaNH_2$ (0.616 g, 15.0 mmol) was added to a flame-dried two-neck 50-mL round-bottom flask containing a solution of *rac*-1-*O*-benzyl-3-*O*-tritylglycerol (**8**)[46] (5.30 g, 12.5 mmol) in anhydrous 1,4-dioxane (30 mL). The reaction mixture was heated at reflux for 1 h under N_2 before CH_3I (3.55 g, 25.0 mmol) was added dropwise. The reaction mixture was heated at reflux overnight and then cooled to room temperature prior to the addition of Et_2O and H_2O. The Et_2O layer was removed, extracted with 1 N HCl, H_2O, saturated $NaHCO_3$, H_2O, and brine, dried ($MgSO_4$), and filtered. The solvent was removed in vacuo to give a yellow oil, which solidified upon standing. The crude solid was recrystallized with hexanes to yield the ether compound **9**: 4.32 g, 79%; mp 52–54 °C; ^1H NMR (270 MHz, $CDCl_3$) δ 7.46–7.18 (m, 20 H, ArH), 4.53 (d, 2 H, $ArCH_2O$), 3.62 (m, 2 H, $ArCH_2OCH_2$), 3.52 (m, 1 H, $CHOCH_3$), 3.42 (s, 3 H, OCH_3), 3.23 (d, 2 H, Ar_3COCH_2). Anal. ($C_{30}H_{30}O_3$) C, H.

Method B. Anhydrous THF (30 mL) and NaH (0.467 g, 19.45 mmol) were added to a flame-dried three-neck 200-mL round-bottom flask equipped with a reflux condenser and charged with N_2. The mixture was heated to 55 °C before CH_3I (1.7 mL, 27.07 mmol) was added dropwise. A solution of **8** (6.60 g, 15.6 mmol) in anhydrous THF (20 mL) was then added slowly and the reaction mixture was stirred for 4 h at 55 °C. The heat was removed and the reaction mixture was cooled to 0 °C before H_2O was cautiously added. The solvent was removed in vacuo. The residue was dissolved in Et_2O and the solution was extracted with H_2O, dried ($MgSO_4$), and filtered. The solvent was evaporated to dryness under reduced pressure to yield the crude product **9** (6.73 g). The compound was not purified further but used directly in the next reaction.

***rac*-1-*O*-Benzyl-2-*O*-methylglycerol (10).** Compound **10** was prepared by a modified procedure of a synthesis reported by Holy.[40] Trityl ether **9** (6.73 g), and 80% HOAc (50 mL) were combined in a 100-mL round-bottom flask and heated at reflux for 3 h. The heat was removed and the reaction mixture was allowed to cool to room temperature prior to neutralization with 10% KOH and extraction with Et_2O. The ether layer was separated, dried ($MgSO_4$), and filtered. The solvent was removed in vacuo. Purification of the residue was obtained by column chromatography (150 g of silica gel, hexanes/EtOAc, gradient 5:1–0:1). The pure compound **10** was obtained as a clear oil: 1.84 g, 60% overall yield from compound **8**; IR ($CHCl_3$) 3590, 3460 (alcohol OH) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.29 (m, 5 H, ArH), 4.46 (s, 2 H, $ArCH_2O$), 3.60 (2 dd, 2 H, CH_2OH), 3.50 (d, 2 H, OCH_2CH), 3.38 (m, 4 H, $CHOCH_3$), 2.28 (br s, 1 H, OH).

***rac*-1-*O*-[12-(*m*-Iodophenyl)dodecyl]-2-*O*-methyl-3-*O*-benzylglycerol (11).** Anhydrous benzene (5 mL) and potassium metal (0.0748 g, 1.91 mmol) were added to flame-dried three-neck 50-mL round-bottom flask equipped with a reflux condenser and a N_2 atmosphere. The mixture was heated at reflux. After the potassium metal had melted, a solution of **10** (0.423 g, 2.15 mmol) in anhydrous benzene (5 mL) was added dropwise via a syringe. The reaction mixture was heated at reflux for 1 h before a solution of mesylate **7** (0.868 g, 1.86 mmol) in dry benzene (4 mL) was also added and the heating was continued overnight. The heat was removed and the reaction mixture was cooled to 0 °C before H_2O was slowly added. The organic layer was separated, extracted with H_2O, 1 N H_2SO_4, and H_2O, dried ($MgSO_4$), and then filtered. The removal of the solvent under reduced pressure provided the crude product, which was purified by column chromatography (26 g of silica gel, hexanes/EtOAc, 8:1) to yield the pure ether **11**: 349 mg, 33%; ^1H NMR (270 MHz, $CDCl_3$) δ 7.52 (t, 2 H, ArH), 7.34 (m, 5 H, ArH), 7.13 (d, 1 H, ArH), 7.00 (t, 1 H, ArH), 4.55 (s, 2 H, $ArCH_2O$), 3.64–3.50 (m, 5 H, CH_2CHCH_2), 3.50–3.40 (m, 5 H, OCH_3 and CH_2O), 2.53 (t, 2 H, $ArCH_2CH_2$), 1.56 (m, 4 H, $ArCH_2CH_2$ and CH_2CH_2O), 1.25 (br s, 16 H, $(CH_2)_8$). Anal. ($C_{29}H_{44}IO_3$) C, H, I.

***rac*-1-*O*-[12-(*m*-Iodophenyl)dodecyl]-2-*O*-methylglycerol (12).** Ether **11** (840 mg, 1.48 mmol) and a 1 N solution of $HClO_4$ in 1,4-dioxane (11 mL) were combined in a 25-mL round-bottom flask equipped with a reflux condenser. The reaction mixture was heated at 80–100 °C for 10 h. The solvent was removed in vacuo and Et_2O was added. The ether solution was extracted with H_2O, saturated $NaHCO_3$, and H_2O. After the organic layer was dried ($MgSO_4$) and filtered, the solvent was removed under reduced pressure to yield the crude product, which was purified by column chromatography (90 g of silica gel, hexanes/EtOAc 4:1) to obtain pure alcohol **12**: 470 mg, 66%; IR ($CHCl_3$) 3582, 3500 (alcohol OH) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.52 (m, 2 H, ArH), 7.14 (d, 1 H, ArH), 7.00 (t, 1 H, ArH), 3.70 (m, 2 H,

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CH_2OH), 3.56 (m, 2 H, CH_2O), 3.50–3.41 (m, 6 H, CH_2OCH_2CH and OCH_3), 2.53 (t, 2 H, $ArCH_2$), 2.23 (br s, 1 H, OH), 1.56 (m, 4 H, $ArCH_2CH_2$ and CH_2CH_2O), 1.25 (br s, 16 H, $(CH_2)_8$). Anal. ($C_{22}H_{37}IO_3$) C, H.

***rac*-1-*O*-[12-(*m*-Iodophenyl)dodecyl]-2-*O*-methylglycerol 3-(2′-Bromoethyl phosphate) (13).** A solution of 2-bromoethyl dichlorophosphate[41] (0.277 g, 1.14 mmol) in anhydrous Et_2O (7 mL) was placed into a flame-dried three-neck 50-mL round-bottom flask equipped with a reflux condenser and charged with N_2. The solution was cooled to 0 °C before dry pyridine (0.40 mL, 3.40 mmol) was added. A solution of alcohol **12** (150 mg, 0.315 mmol) in anhydrous Et_2O (1.5 mL) was added. The reaction mixture was stirred at 0 °C for 10 min before it was heated at a gentle reflux for 4.5 h. The heat was removed and H_2O (0.5 mL) was added to the cooled reaction mixture. After the reaction mixture had been stirred for 30 min, the solvent was removed in vacuo. The residue was dissolved in $CHCl_3$ and the solution was extracted with H_2O, 1 N HCl, and H_2O. MeOH was used to disrupt the emulsions which formed. The solvent was removed in vacuo and the residual H_2O was removed as an azeotrope with benzene. The crude product was purified by column chromatography (19 g of silica gel, $CHCl_3$/MeOH, 9:1) to obtain compound **13**: 87 mg, 42%; IR ($CHCl_3$), 1250 (ester P=O) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.47 (m, 2 H, ArH), 7.10 (d, 1 H, ArH), 6.95 (t, 1 H, ArH), 4.22 (br s, 2 H, $POCH_2$), 4.00 (m, 2 H, $CHCH_2OP$), 3.69–3.33 (m, 10 H, CH_2OCH_2CH, OCH_3, and CH_2Br), 2.53 (t, 2 H, $ArCH_2$), 2.20 (br s, H_2O), 1.56 (m, 4 H, $ArCH_2CH_2$ and CH_2CH_2O), 1.23 (br s, 16 H, $(CH_2)_8$); CIMS *m/e* 583 (M − 80).

***rac*-1-*O*-[12-(*m*-Iodophenyl)dodecyl]-2-*O*-methylglycerol-3-*O*-phosphocholine (14).** Compound **13** (73 mg, 0.110 mmol) and $(CH_3)_3N$ (45–50% aqueous solution, 0.80 mL, 5.4 mmol) were added to a three-neck round-bottom flask containing $CHCl_3$/2-propanol/DMF (3:5:5, 6.5 mL). The reaction mixture was heated at 50 °C for 5.5 h. The heat was removed and the reaction mixture was allowed to cool to room temperature before Ag_2CO_3 (39 mg, 0.14 mmol) was added. The heat was then reapplied for 1 h. The reaction mixture was cooled and filtered and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (14 g of silica gel, $CHCl_3$/MeOH/H_2O, 65:35:4) to yield the pure phospholipid **14**: 37 mg, 52%; IR ($CHCl_3$), 1260 (ester P=O) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.45 (m, 2 H, ArH), 7.07 (d, 1 H, ArH), 6.96 (t, 1 H, ArH), 4.24 (br s, 2 H, $POCH_2$), 3.98–3.75 (m, 4 H, $CH_2OPOCH_2CH_2N$), 3.54–3.37 (m, 17 H, OCH_3, $N(CH_3)_3$, and CH_2OCH_2CH), 3.10 (br s, H_2O), 2.53 (t, 2 H, $ArCH_2$), 1.55 (m, 4 H, $ArCH_2CH_2$ and CH_2CH_2O), 1.25 (br s, 16 H, $(CH_2)_8$); CIMS *m/e* 642 (MH^+). Anal. ($C_{27}H_{49}PO_6NI\cdot H_2O$)[47] C, H, I.

Radioiodination of 14. Phospholipid **14** (1.7 mg) and pivalic acid (10 mg) were placed into a 1-mL serum vial. The vial was flushed with N_2 and sealed with a Teflon-lined rubber septum and an aluminum cap. Aqueous $Na^{125}I$ (4.5 mL, 2 mCi) was added. A stream of N_2 was used to remove the H_2O and the reaction mixture was then heated in a preheated oil bath at 150 °C for 30 min. The reaction mixture was allowed to cool to room temperature before $CHCl_3$/MeOH (1:2, 60 mL) and H_2O (60 mL) were added. The organic layer was removed and applied to a silica gel-60 column (1 × 5 cm). The column was initially eluted with $CHCl_3$/MeOH (1:1) to remove the $Na^{125}I$ and then with $CHCl_3$/MeOH/H_2O (65:25:4) to elute [^{125}I]ET-12IP-OMe. The radiochemical yield for the exchange based on TLC was 60%.

Tissue Distribution Studies. The radiolabeled compounds were dissolved in $CHCl_3$/MeOH/H_2O, 65:25:3, and Tween-20 (0.1 mL/mg of compound) was added. The solvent was evaporated with a stream of nitrogen. Physiological saline was added, and the final traces of solvent were removed by passing nitrogen over the solution until it became clear (2–3% Tween). The Walker 256 carcinosarcoma was implanted in the thigh of female Sprague–Dawley rats. These animals were used 7–10 days later when the tumor mass averaged 10.6 g. The solubilized, radiolabeled compound was administered intravenously via tail vein to normal and tumor-bearing rats (200–250 g). Groups of three to four rats were injected with 5.0–16.4 μCi per time point. The rats were sacrificed by exsanguination while under ether anesthesia at 6, 24, and 48 h. Selected tissues were removed and blotted of excess blood. Large organs were minced with scissors. Tissue samples were weighed in cellulose acetate capsules and counted with a Searle 1185 well scintillation counter (85% counting efficiency). The concentration of radioactivity in each tissue was expressed as the percentage of administered dose per gram of tissue.

Lipid Extraction of Plasma and Tissues. Radioactivity was extracted from samples of liver, plasma, and tumor by using a modified Folch procedure described previously.[49] These extracts were analyzed by TLC with $CHCl_3$/MeOH/H_2O 65:25:3 as the eluent. The plates were developed for 14.5 cm, air-dried, and then cut into 1-cm strips beginning 0.5 cm below the origin. Each strip was placed in a counting tube and assayed for radioactivity. Pure radiolabeled compound used for administration was employed as the reference standard. Results are expressed as the percentage of total radioactivity on each plate.

Plasma Electrophoresis. PAGE of plasma samples was performed by using the procedure described by Narayan.[50] The gels were then sectioned, and each section was placed in a counting tube. The tubes were assayed for radioactivity. The results are expressed as the percentage of total radioactivity comigrating with each class of lipoprotein or albumin.

γ-Camera Scintigraphy. Imaging of the tumor-bearing rat was performed with an Ohio Nuclear mobile camera with a high-sensitivity, low-energy collimator. A rat with the Walker 256 carcinosarcoma implanted in the thigh was administered 75 μCi of the radiolabeled compound intravenously via a tail vein. Twenty four hours later the rat was sedated with 87 mg/kg ketamine and 13 mg/kg xylazine im. The animal was placed supine on the collimater and an image was obtained.

This work was supported by the National Institute of Health Grant Number CA-08349 and a Dissertation Thesis Grant from the Rackham School of Graduate Studies to K.L.M. We wish to thank Dr. James Varani of the Department of Pathology for providing the Walker 256 carcinosarcoma cells, James Windak of the Department of Chemistry for performing chemical-ionization mass spectra, Dr. Milton Gross and the V.A. Medical Center Nuclear Medicine unit for assistance with the γ-camera imaging, Ms. Maria Milito and Ms. Edie Quenby for their technical assistance, and Ms. Quenby for typing the manuscript.

Registry No. 1, 102831-64-1; (*E*)-2, 121269-35-0; (*Z*)-2, 121269-44-1; 3, 102831-67-4; 4, 121269-36-1; 5, 121269-37-2; 6, 121269-38-3; 7, 121269-39-4; 8, 99881-46-6; 9, 121348-73-0; 10, 121348-74-1; 11, 121269-40-7; 12, 121269-41-8; 13, 121288-64-0; 14, 121269-42-9; [^{125}I]ET-12IP-OMe, 121269-43-0; 3-$O_2NC_6H_4CHO$, 99-61-6; $Cl_2P(O)(OCH_2CH_2Br)$, 4167-02-6.

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