Synthesis of Quaternary Amine Ether Lipids and Evaluation of Neoplastic Cell Growth Inhibitory Properties

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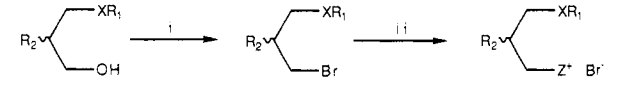
Novel quaternary amine ether lipids have been synthesized and tested for inhibition of neoplastic cell proliferation with the HL-60 promyelocytic leukemia cell line. These compounds contain a positively charged quaternary amine functional group attached either directly to the glycerol backbone or at the end of an alkoxy chain. The biological testing has identified several analogues with activity equivalent to or greater than that exhibited by the reference compound in this assay, ET-18-OMe (1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine). Among the most active analogues are compounds **11**, [*N,N,N*-triethyl-3-(hexadecyloxy)-2-ethoxy-1-propylammonium bromide] and **22** [*N*-[4-[3-(hexadecyloxy)-2-ethoxypropoxy]-1-butyl]pyridinium bromide], which are approximately 3 times as active as the reference standard.

Ether lipids derived from glycerol display a wide variety of biological activities.[1-3] One of these is the growth inhibition of various neoplastic cell lines exhibited by synthetic alkyl ether phospholipids.[4-8] The mechanism for this antineoplastic activity is not fully understood and multiple hypotheses have been proposed.[9-13] Membrane interaction[12,14,15] and protein kinase C inhibition[8,16,17] are considered to be two of the many possible modes of action which may be involved in inhibition of malignant cell growth. The alkyl ether phospholipid ET-18-OMe (1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine) has been found to both accumulate in the plasma membrane of neoplastic cells[14,15] and also to selectively inhibit protein kinase C over other kinases present in HL-60 cells.[17] Further, quite recently, ether lipids have also been shown to possess antiviral activity against human immunodeficiency virus type 1 (HIV-1).[18]

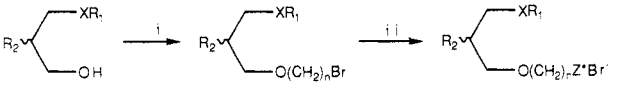
This laboratory has previously reported a series of quaternary amine ether lipids (inverse cholines) which contain an ammonium functionality but lack the phosphate group present in ether phospholipids. These analogues were found to be inhibitors of protein kinase C.[19] Since inhibition of this kinase has been linked to antineoplastic activity, this series of lipids was tested for inhibition of malignant cell proliferation. The present paper will report the results of this study on the growth inhibition of HL-60 human leukemic cells in vitro. Two additional series of quaternary amine compounds which are structurally related to the inverse cholines were also prepared and evaluated to further investigate the structural features needed for PKC inhibition and antineoplastic activity. Figure 1 shows an overview of the basic compound structures and of ET-18-OMe, the reference compound used in this assay. Each of the compound classes shares in common a long chain alkyl ether at the carbon-1 position and a short chain alkyl ether at the carbon-2 position of a three-carbon backbone. The carbon-3 position is different in each class of compounds. (1) Alkyl ether phospholipids such as the reference Et-18-OMe contain a phosphocholine group. (2) Series I quaternary amine ether lipids (inverse cholines) contain a *N,N*-dimethyl-β-hydroxyethyl or *N,N*-dimethyl-3-hydroxypropyl ammonium group. (3) Series II quaternary amine ether lipids contain an ammonium group directly attached to carbon-3 but lack the terminal hydroxyl moiety found in the inverse cholines. (4) Series III analogues contain a quaternary

Scheme I[a,b]



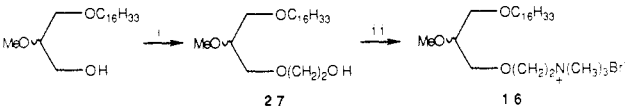
[a] (i) (1) MsCl, Et_3N, (2) LiBr, acetone or CBr_4, Ph_3P; (ii) $(CH_3)_2N(CH_2)_{2-3}OH$ or $N(CH_3)_3$ or $N(CH_2CH_3)_3$ or pyridine or 4-hydroxy-*N*-methylpiperidine. [b] X, R_1, R_2, and Z are given in Tables I–III.

Scheme II[a,b]



[a] (i) for n = 3–10, $Br(CH_2)_nBr$, NaH, NaI; (ii) $N(CH_3)_3$ or pyridine. [b] X, R_1, R_2 and Z are given in Tables I–III.

Scheme III[a]



[a] (i) (1) $BrCH_2CH_2OTHP$, NaH, (2) pTSA, $CHCl_3$, MeOH; (ii) (1) MsCl, Et_3N, (2) LiBr, acetone, (3) $N(CH_3)_3$.

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(3) (a) Lee, T.-C.; Snyder, F. In *Phospholipids and Cellular Regulations*; CRC Press: Boca Raton, FL, 1985; Vol. II, pp 2–39. (b) Snyder, F. *Annu. Rev. Med. Chem.* **1982**, *17*, 243–252.
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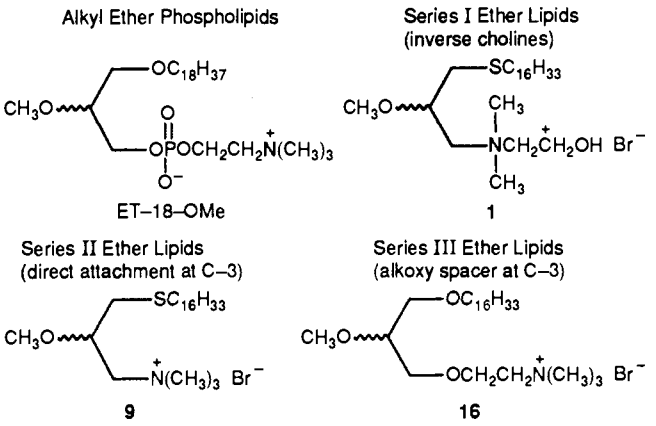


Figure 1. Overview of ether lipid structures.

amine salt at the terminal end of an alkoxy chain present at carbon-3.[2,20] Analogues containing structural variations (chain length, heteroatom, etc.) at all three positions were synthesized to study the structure–activity relationships of the series I–III ether lipids.

Chemistry

A common intermediate in the synthesis of each series of quaternary amine ether lipids is a substituted propanol (either a 1,2-dialkylglycerol, a 1-*S*-alkyl-2-*O*-alkylthioglycerol, a 3-alkoxy-1-propanol, or a 4-(hydroxymethyl)-1,3-dioxolane). The preparation of these alcohols followed standard literature methods.[7,19,21] The remaining steps in the synthesis of these ether lipids involved function-

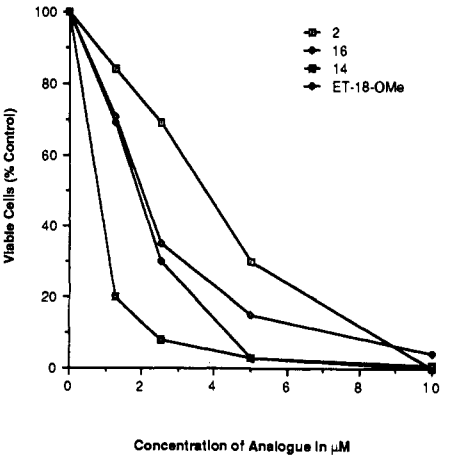
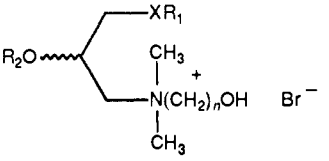
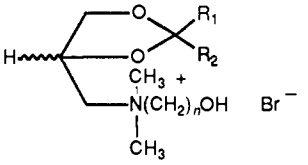


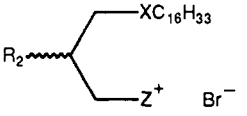
Figure 2. Inhibition of HL-60 cell growth.

Table I. ID_{50} Values for Series I Ether Lipids (Inverse Cholines) Tested for HL-60 Growth Inhibition

no.	X	R_1	R_2	n	ID_{50},[a-c] μM	N[d]
						
1	S	$C_{16}H_{33}$	Me	2	4.66 ± 0.27	4
2	S	$C_{16}H_{33}$	Et	2	3.72 ± 0.05	4
3	S	$C_{16}H_{33}$	Et	3	2.95 ± 0.36	4
4[e]	S	$C_{18}H_{37}$	Me	3	3.35 ± 0.22	4
5[e]	O	$C_{16}H_{33}$	Et	*f*	2.72 ± 0.24	4
6	O	$C_{10}H_7$[g]	Me	2	>10	4
						
7		$C_{17}H_{35}$	H	3	6.47 ± 0.24	4
8		Me	Me	2	>10	4

[a] ET-18-OMe was used as a positive control for each assay. The mean ID_{50} value for this reference compound was 2.01 ± 0.22 μM over 32 determinations. [b] The standard deviations were calculated from the averaged ID_{50} values. [c] A compound with an ID_{50} value greater than 10 μM is considered inactive for the present study. [d] N = number of determinations. [e] These compounds were prepared as the iodide salts. [f] The ammonium group for this compound was *N*-methyl-4-hydroxypiperidinium. [g] $C_{10}H_7$ = 1-naphthyl.

Table II. ID_{50} Values for Series II Ether Lipids Tested for HL-60 Growth Inhibition



no.	X	R_2	Z	ID_{50},[a-c] μM	N[d]
9	S	OMe	NMe_3	2.20 ± 0.30	8
10	O	OMe	NMe_3	1.59 ± 0.17	4
11	O	OEt	NEt_3	0.68 ± 0.11	12
12	O	OEt	NC_5H_5	1.01 ± 0.06	4
13	O	H	NMe_3	1.61 ± 0.10	8
14	O	H	NC_5H_5	0.82 ± 0.24	20

[a-d] See Table I.

alization of the alcohol and formation of the quaternary amine group. Scheme I gives a representative synthetic methodology for the series I and II analogues. Schemes

(9) (a) Munder, P. F.; Modolell, M.; Bausert, W.; Oettgen, H. F.; Westphal, O. In *Augmenting Agents in Cancer Therapy*; Raven: New York, 1981; pp 441–458. (b) Berdel, W. E.; Bausert, W. R.; Weltzien, H. U.; Modolell, M. L.; Widman, K. H.; Munder, P. G. *Eur. J. Cancer* **1980**, *16*, 1199–1204. (c) Talmadge, J. E.; Schneider, M.; Lenz, B.; Phillips, H.; Long, C. *Lipids* **1987**, *22*, 871–877.

(10) (a) Soodsma, J. F.; Piantadosi, C.; Snyder, F. *Cancer Res.* **1970**, *30*, 309–311. (b) Berdel, W. E.; Greiner, E.; Fink, J.; Stavrou, D.; Reichert, A.; Rastetter, J.; Hoffman, D. R.; Snyder, F. *Cancer Res.* **1983**, *43*, 541–545. (c) Hoffman, D. R.; Hoffman, L. H.; Snyder, F. *Cancer Res.* **1986**, *46*, 5803–5809.

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(12) Berdel, W. E.; Bausert, W. R.; Fink, J.; Rastetter, J.; Munder, P. G. *Anticancer Res.* **1981**, *1*, 345–352.

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(16) Parker, J.; Daniel, L. W.; Waite, M. *J. Biol. Chem.* **1987**, *262*, 5385.

(17) Helfman, C. M.; Barnes, K. C.; Kinkade, J. M.; Vogler, W. R.; Shoji, M.; Kuo, J. F. *Cancer Res.* **1983**, *43*, 2955–2961.

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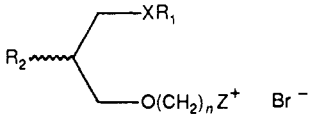
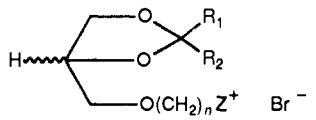
(20) (a) Compounds of this general structure type have been reported in European Patent Application Nos. 84307720.7 (1984) and 84307721.5 (1984) by Ono Pharmaceutical Co. (b) Piwinski, J. J.; Kreutner, W.; Green, J. J. *Annu. Rep. Med. Chem.* **1987**, *22*, 73–84.

(21) Crumpton, S. C.; Goz, B.; Ishaq, K. S. *Anticancer Res.* **1988**, *8*, 1361–1366.

Table III. ID_{50} Values for Series III Ether Lipids Tested for HL-60 Growth Inhibition

no.	X	R_1	R_2	n	Z	ID_{50},[a-c] μM	N[d]
							
15	S	$C_{16}H_{33}$	OEt	4	NMe_3	1.83 ± 0.04	4
16	O	$C_{16}H_{33}$	OMe	2	NMe_3	1.85 ± 0.15	4
17	O	$C_{16}H_{33}$	OMe	3	NMe_3	1.20 ± 0.08	4
18	O	$C_{16}H_{33}$	OMe	4	NMe_3	2.30 ± 0.37	4
19	O	$C_{16}H_{33}$	OMe	6	NMe_3	1.75 ± 0.35	4
20	O	$C_{16}H_{33}$	OMe	10	NMe_3	3.79 ± 0.13	4
21	O	$C_{16}H_{33}$	OEt	4	NMe_3	1.86 ± 0.04	4
22	O	$C_{16}H_{33}$	OEt	4	NC_5H_5	0.78 ± 0.02	12
23	O	$C_{18}H_{37}$	OEt	4	NMe_3	1.26 ± 0.12	4
24	O	$C_{16}H_{33}$	H	4	NMe_3	1.56 ± 0.10	4
							
25		$C_{17}H_{35}$	H	4	NMe_3	2.82 ± 0.42	4

[a-d] See Table I.

II and III show the preparation of the series III compounds.

Conversion of the alcohol at carbon-3 to the bromide was accomplished in one of two ways: (1) a two-step sequence involving reaction first with methanesulfonyl chloride to form the mesylate, which was then displaced with an excess of lithium bromide,[19] or (2) a direct route using reaction with triphenylphosphine and carbon tetrabromide. The inverse cholines were formed by amination with 2-(dimethylamino)ethanol or 3-(dimethylamino)-1-propanol in dimethylformamide.[21] Series II compounds where the ammonium group is directly attached at carbon-3 were also prepared from this bromide by reaction with trimethylamine (gaseous or aqueous), triethylamine, or pyridine.

The series III derivatives containing an alkoxy spacer group could be prepared in two ways. First, a 1,2-dialkylglycerol was alkylated with the tetrahydropyranyl ether of 2-bromoethanol in the presence of sodium hydride. The hydroxyl group was then regenerated with *p*-toluenesulfonic acid in chloroform/methanol. Mesylation followed by bromination as in the series I compounds gave the terminal bromide. The quaternary amine was formed by reaction with trimethylamine. Second, the alkoxy-ω-bromide was prepared directly by reaction of the 1,2-dialkylglycerol with an excess of 1,ω-dibromoalkane, sodium hydride, and a catalytic amount of sodium iodide in dimethylformamide.[22] The desired ammonium compound was then formed by displacement of the terminal bromide with the appropriate amine.

Results and Discussion

All of the series I–III derivatives were tested for malignant cell growth inhibition in the HL-60 promyelocytic leukemia cell line. Dose–response curves are shown in Figure 2 for a representative series I (**2**), series II (**14**), and series III (**16**) compound as compared with the reference ether lipid analogue ET-18-OMe. Table I lists the ID_{50} values found for each series I (inverse choline) compound and Table II and Table III, respectively, give the results for the series II and III lipids. Promising in vitro activity is exhibited by many of these analogues. Fifteen of the 25 analogues synthesized and tested show inhibition of HL-60 cell growth equal or superior to that exhibited by the reference compound ET-18-OMe. Some of the relationships that can be found within and between the three series of quaternary amine ether lipids in this preliminary study are discussed below.

First, the inverse cholines (series I) are, in general, less active than the series II or III analogues. None of the eight inverse cholines synthesized have ID_{50} values comparable to that of the reference compound ET-18-OMe. All of the series II and III derivatives except **20** and **25**, however, do show activity comparable to or better than ET-18-OMe. Two direct comparisons of the two series can be made. Compound **9**, a series II ether lipid which contains a trimethyl ammonium group but lacks a terminal hydroxyl moiety, exhibits greater activity than compound **1**, the inverse choline with corresponding structural features at carbons 1 and 2. Similarly, compound **7**, a dioxolane inverse choline, has an ID_{50} of 6.47 μM whereas compound **25**, a dioxolane series III compound, has an ID_{50} $^1/_2$ that value.[23]

Structural modifications at the carbon-1 position give the following results. The effect of the heteroatom at the carbon-1 ether linkage (sulfur or oxygen) has not been fully established and may vary with series type. In series III, compounds **15** (S) and **21** (O) have identical ID_{50} values. However, with series II derivatives, a sulfur analogue (**9**) shows somewhat lower activity than the corresponding oxygen-containing compound (**10**). Straight chain alkyl ethers of only 16 or 18 carbons were synthesized since previous experimentation with 1,2-dialkylglycero-3-phosphocholines has shown compounds with chains of shorter lengths to have reduced activity in HL-60 cell growth inhibition.[24] The size of the alkyl chain does seem to affect the activity shown by the present analogues. For example, dimethyl acetal inverse choline **8** has an ID_{50} value greater than 10 μM and (in this publication) is considered to be inactive while a long chain acetal inverse choline (**7**) does exhibit activity in the concentration range tested. Another compound without a long chain alkyl group at carbon-1, naphthyl ether inverse choline **6**, also shows no inhibition of cell growth at concentrations below 10 μM. Reducing the rotational degrees of freedom associated with the glycerol backbone by incorporating the carbon-1 and carbon-2 substituents into a dioxolane ring appears to decrease activity (in the series II lipids, compare **25** to **23**, and in the series I compounds, compare **7** to **3** or **4**).

In the series II analogues, the substituent at carbon-2 of the backbone can be either methoxy (**18**) or ethoxy (**21**) without a significant change in ID_{50} values. Substitution of a hydrogen (**24**) for the alkoxy group causes a slight increase in activity. Also, as shown in Table II, compounds containing a methoxy group (**10**) or a hydrogen (**13**) at carbon-2 and a trimethyl ammonium group directly attached at carbon-3 inhibit cell growth to an equivalent degree as do pyridinium analogues with either a hydrogen (**14**) or an ethoxy moiety (**12**). In contrast, however, replacing the carbon-2 alkoxy group with a hydrogen in alkyl ether phospholipid compounds leads to a reduction in neoplastic cell growth inhibition.[11,24]

Variations in structure at the carbon-3 position include the change from the inverse choline (series I) structure to

(22) Wissner, A.; Sum, P.-E.; Schuab, R. E.; Kohler, C. A.; Goldstein, B. M. *J. Med. Chem.* **1986**, *29*, 328–333.

(23) The dioxolane compounds were synthesized and tested as a mixture of cis/trans isomers.

(24) Berdel, W. E.; Von Hoff, D. D.; Unger, C.; Schick, H. D.; Fink, U.; Reichert, A.; Eibl, H.; Rastetter, J. *Lipids* **1986**, *21*, 301–304.

the series II or III ether lipid configuration which has already been discussed. Within the series II and III analogues, the length of the spacing between carbon-3 and the charged nitrogen can also be varied. Compound **13**, which has the trimethyl ammonium salt directly attached at carbon-3 (Table II), and compound **24**, which has a butoxy spacer between these two groups (Table III), show identical activities. In compounds with a methoxy group at carbon-2 and a hexadecyloxy group at carbon-1, the length of this separation has been extended from no spacing (Table II: **10**, direct attachment) to an *O*-alkylene chain of two to 10 carbons (Table III: **16–20**). No distinct trend is seen with the increase in chain length. In this group of compounds, maximum activity is observed with a carbon spacing of three between the oxygen ether and the trimethyl ammonium salt and the least activity is seen at a length of 10 carbons. The remaining analogues (zero, two, four, six carbons) show activity comparable to each other and to the reference compound ET-18-OMe.

A final change is in the presence or nature of the quaternary amine group. Compound **27** (an intermediate in the synthesis of **16**, Scheme III), which contains a β-hydroxyethoxy group at carbon-3 and does not have a charged nitrogen functionality, shows no inhibition of HL-60 cell growth below 10 μM. Both the corresponding series I analogue (**1**) which has a *N,N*-dimethylammonium group in place of the oxygen ether and especially the series III analogue (**16**) where the hydroxyl is replaced by a *N,N,N*-trimethylammonium group do show growth inhibition. A second uncharged compound, *N*-methyl-*N*-[3-(hexadecyloxy)-2-ethoxy-1-propyl]-β-hydroxyethylamine (**26**), was also found to be essentially inactive (ID_{50} = 9.8 μM). This compound is comparable in structure to the series I inverse cholines except that the nitrogen bears only one methyl (tertiary amine) instead of two (quaternary amine). The form of the charged nitrogen also affects activity. A pyridinium or a triethyl ammonium salt shows greater activity than the corresponding trimethyl ammonium salt (in Table III, compare **21** to **22**, and in Table II, compare **13** to **14** and also compare **10** to **11** or to **12**). It should also be noted that the activity shown by all four compounds containing either a pyridinium or triethyl ammonium group is greater than the activity exhibited by the reference compound ET-18-OMe.

In the series I (inverse choline) compounds, a compound containing the (hydroxypropyl)ammonium group (**3**) is slightly more active than the corresponding (hydroxyethyl)ammonium salt (**2**). One compound in this series, **5**, was made with the nitrogen contained in a heterocyclic ring structure. This *N*-methyl-4-hydroxypiperidinium analogue shows activity similar to that of compounds containing a *N,N*-dimethyl-3-hydroxypropyl group (**3** or **4**).

A full tabulation and discussion of the results found in a protein kinase C inhibition assay for the inverse choline (series I) compounds, compound **9** (series II), and compound **16** (series III) have been previously described.[19] Six additional compounds from the latter two series (series II, **10**, **13**, **14** and series III, **18**, **19**, **21**) have been tested in a limited number of trials. Table IV shows the results in both the PKC and HL-60 assays (only compounds **4** and **7** from series I have been included here). The most active PKC inhibitors, **4** and **7**, were both series I compounds and both were among the least active analogues in the HL-60 assay. In contrast, the series II and III compounds showed more activity in the HL-60 assay but were less active in the PKC assay with only **9** and **16** having IC_{50} values in the concentration range tested. On the basis of only these results, it would appear that within these series of compounds, PKC activity does not parallel HL-60 activity. However, the assay systems are not equivalent—PKC being an isolated enzyme system and HL-60 being intact cells. The incorporation and passage of these analogues may vary in whole cells and thus affect the correlation between PKC inhibition and HL-60 cell growth. Therefore, it is evident that the mechanism of action of these compounds is not known. PKC inhibition, membrane interactions, or other modes of action may also be involved in the antineoplastic activity shown by these quaternary amine lipids.

Table IV. IC_{50} Values for Ether Lipids Tested for Protein Kinase C Inhibition and HL-60 Growth Inhibition

compound[a]	series	IC_{50}, μM PKC	IC_{50}, μM HL-60
ET-18-OMe	reference	12[b]	2.01
4	I	5[b]	3.35
7	I	13[b]	6.47
9	II	25[b]	2.20
10	II	>40	1.59
13	II	>40	1.61
14	II	>40	0.82
16	III	31[b]	1.85
18	III	>40	2.30
19	III	>40	1.75
21	III	>40	1.86

[a] See Tables I (Series I), II (Series II), and III (Series III) for compound structures. [b] These results have been reported previously in ref 19. PKC testing was done in duplicate in three separate trials using a fresh preparation of PKC each time. The remaining six analogues were tested only once in duplicate.

In summary, the majority of series II and series III compounds display HL-60 cell growth inhibition comparable to that shown by the reference phospholipid ET-18-OMe with several analogues exhibiting enhanced activity. Inverse cholines (series I), however, display less inhibition of malignant cell growth than either the reference compound or the series II or III analogues. Many of the compounds in this study exhibit encouraging in vitro antineoplastic activity, but the structure–activity relationships are only preliminary. Other analogues of these quaternary amine ether lipids will be prepared and further testing will be done to more fully understand the structural features contributing to the antineoplastic activity.

Experimental Section

All chemicals were used as provided by the supplier without further purification unless otherwise indicated. Column chromatography was performed with use of silica gel 60 (230–400 mesh). All melting points were obtained on a Hoover Meltemp apparatus and are uncorrected. Proton nuclear magnetic resonance spectra were obtained on either a JEOLCO 60-MHz, Varian 80-MHz, or Varian 400-MHz spectrometer as solutions in $CDCl_3$ with internal Me_4Si as standard. Elemental analyses of final products were performed by Atlantic Microlabs, Inc. References 7, 19, 21 provide synthetic details for the preparation of the dialkylglycerols and thioglycerols.

Growth Inhibition of HL-60 Cells. HL-60 human promyelocytic leukemia cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 units/mL streptomycin, 0.22% $NaHCO_3$, and 2 mM glutamine. The cells were subcultured at 2–3-day intervals and maintained in an atmosphere of 5% CO_2. Cell numbers in the stock cultures were maintained in the range 5×10^5–1.5×10^6. To determine the effects of inhibitors on cell proliferation, the cells were incubated at 5×10^5 cells/mL with various concentrations of the inhibitors, which were added in a small volume of ethanol. Stock solutions of the lipid inhibitors were made at 1 mg/mL in ethanol and diluted into cell culture medium immediately prior to use. The concentrations of ethanol used were found to have no effect on

cell proliferation. After 48 h, viable cell numbers were determined by hemocytometer counting of trypan blue treated cells.

Assay of Protein Kinase C Activity. The compounds listed in Table IV were tested for inhibition of partially purified PKC isolated from HL-60 cells. The enzyme employed was prepared as described in ref 8. The enzyme assays were done at pH 7.5 in a total volume of 0.25 mL and all tubes contained 25 mM Tris, 10 mM $MgCl_2$, 40 μg/mL histone, 10 μM ATP (including 1 μCi of [γ-^{32}P]ATP), 0.1 mM $CaCl_2$, 20 μg/mL phosphatidylserine, 0.05 mL of the PKC preparation, and 2.5 μM of oleoylacetylglycerol (OAG). Enzymatic activity was determined as the incorporation of ^{32}P from [γ-^{32}P]ATP into histone in the presence of Ca^{2+}, phosphatidylserine, and OAG. Reactions were initiated by the addition of the enzyme preparation and halted after 20 min at 30 °C by the addition of 0.05 mL of bovine serum albumin (10 mg/mL) and 1 mL of 25% ice-cold trichloroacetic acid. The tubes were kept on ice and then filtered in a Millipore vacuum box using Millipore HA filters and washed with 25% trichloroacetic acid. The radioactivity bound to the filters was determined by scintillation spectrometry in 5 mL of Budget Solve. The amount of enzyme used was shown to result in linear activity for at least 20 min and the assay was linearly dependent on the amount of enzyme used. The analogue to be tested was dissolved in ethanol and added directly into the reaction mixture before the addition of PKC. As a control, 0.1% ethanol was included in the samples with the enzyme but with no inhibitor.

Preparation of Bromides. (±)-1-(Hexadecyloxy)-2-methoxy-3-bromopropane. Methanesulfonyl chloride (0.2 mL, 1.2 g, 0.003 mol) was added to a solution of 1-*O*-hexadecyl-2-*O*-methylglycerol (0.66 g, 0.002 mol) in 70 mL of methylene chloride and 0.4 mL of triethylamine. The reaction mixture was stirred at room temperature for 24 h. The solution was then extracted with water (3 × 50 mL). The organic layer was dried over anhydrous sodium sulfate and then evaporated in vacuo to give an oil. This oil was used in the next reaction without purification. The mesylate was dissolved in 50 mL of acetone, lithium bromide (0.870 g, 0.01 mol) was added, and the reaction was heated to reflux for 9 h. A second portion of lithium bromide (0.5 g, 0.006 mol) was added and reflux continued for a total of 36 h. After cooling, the sodium mesylate was filtered and the acetone was removed on a rotary evaporator. The residue was dissolved in 100 mL of diethyl ether and extracted with water (3 × 50 mL). The organic phase was dried over sodium sulfate, the ether was removed in vacuo, and the resulting oil was chromatographed on silica gel using hexane/ethyl acetate (9:1) as eluant. The bromide (0.6 g) was obtained in 75% yield from the 1,2-dialkylglycerol. NMR ($CDCl_3$): δ 0.87 (t, 3 H, terminal CH_3), 1.2–1.4 (m, 26 H, $(CH_2)_{13}$), 1.6 (m, 2 H, OCH_2CH_2), 3.45 (s, 3 H, OCH_3). 3.4–3.6 (overlapping m, 7 H, CH_2OCH_2, CH, CH_2Br).

The other 1-alkoxy[or (alkylthio)]-2-alkoxy-3-bromopropanes were prepared by a comparable procedure. 1-(Octadecylthio)-2-methoxy-3-iodopropane and 1-(hexadecyloxy)-2-methoxy-3-iodopropane used in the preparation of compounds 4 and 5 were prepared as above but with sodium iodide as the halide source.

(±)-1-(Hexadecyloxy)-2-methoxy-3-(β-bromoethoxy)propane. The synthesis of this compound from 1-*O*-hexadecyl-2-*O*-methylglycerol and tetrahydro-2-(β-bromoethoxy)-2*H*-pyran is given in detail in ref 19.

(±)-1-(Hexadecyloxy)-2-methoxy-3-(4-bromobutoxy)propane. Sodium hydride (0.2 g, 0.007 mol, 80% oil dispersion), sodium iodide (50 mg, 0.33 mmol), and 1,4-dibromobutane (2.6 g, 0.012 mol) were placed in 25 mL of DMF under nitrogen. 1-*O*-Hexadecyl-2-*O*-methylglycerol (1.0 g, 0.0033 mol) in 10 mL of DMF was added dropwise. The reaction was allowed to proceed at room temperature for 32 h. Additional aliquots of sodium hydride (200 mg) and 1,4-dibromobutane (0.7 mL) were added at 7 and 24 h. The reaction mixture was then partitioned between diethyl ether (50 mL) and water (50 mL). The organic phase was dried over sodium sulfate and the solvent was removed in vacuo. The residue was chromatographed on silica gel with a gradient of hexane/ethyl acetate (95:5 to 4:1). Three products were isolated. These were identified by NMR analysis as olefin, 4-[3-(hexadecyloxy)-2-methoxypropoxy]-1-butene; bromide, 1-(hexadecyloxy)-2-methoxy-3-(4-bromobutoxy)propane; and bis-addition product, 1,4-bis[3-(hexadecyloxy)-2-methoxypropoxy]butane in order of highest to lowest R_f in 4:1 hexane/ethyl acetate. The desired product (bromide) was obtained in 46% yield (0.7 g). NMR ($CDCl_3$): δ 0.85 (t, 3 H, terminal CH_3), 1.2–1.4 (m, 26 H, $(CH_2)_{13}$), 1.6 (m, 2 H, OCH_2CH_2), 1.75 and 1.95 (m, 4 H, $OCH_2CH_2CH_2CH_2Br$), 3.45 (s, 3 H, OCH_3), 3.4–3.6 (overlapping m, 11 H, CH_2OCH_2, CH, CH_2OCH_2, CH_2Br).

The other 1-alkoxy[or (alkylthio)]-2-alkoxy-3-(ω-bromoalkoxy)propanes were prepared from the substituted alcohol and the appropriate 1,ω-dibromoalkane.

1-(Hexadecyloxy)-3-bromopropane. 3-(Hexadecyloxy)-1-propanol (4.7 g, 0.016 mol) and carbon tetrabromide (11.2 g, 0.034 mol) were dissolved in 70 mL of methylene chloride. The solution was cooled to 0 °C and triphenylphosphine (10.7 g, 0.041 mol) was added in 2-g portions over 30 min. The reaction mixture was warmed to room temperature after 45 min and then allowed to stir for 1 h. The solvent was removed on a rotary evaporator, 150 mL of diethyl ether was added, and the reaction was stirred at room temperature for 1 h. The precipitate (triphenylphosphine oxide) was filtered and the filtrate was concentrated to dryness. The residue was chromatographed on silica gel using hexanes/ethyl acetate (8:1) as eulant. Pure bromide (4.9 g, 86% yield) was obtained as an oil. NMR ($CDCl_3$): δ 0.89 (t, 3 H, terminal CH_3), 1.04–1.79 (m, 28 H, $(CH_2)_{14}$), 2.09 (m, 2 H, $OCH_2CH_2CH_2Br$), 3.14–3.34 (overlapping m, 6 H, CH_2OCH_2, CH_2Br).

Preparation of Series I (Inverse Choline) Quaternary Amine Ether Lipids. The synthesis of this series of compounds from the appropriate halide and 2-(dimethylamino)ethanol or 3-(dimethylamino)-1-propanol is detailed in ref 19. The experimental procedure for compound 5 is illustrative of the general method.

(±)-*N*-Methyl-*N*-[3-(hexadecyloxy)-2-ethoxy-1-propyl]-4-hydroxypiperidinium Iodide (5). 1-(Hexadecyloxy)-2-ethoxy-3-iodopropane (1.0 g, 0.0017 mol) and *N*-methyl-4-hydroxypiperidine (0.2 mL, 0.0019 mol) were placed in 7 mL of DMF. The solution was heated to 55 °C and maintained at this temperature for 72 h. The reaction mixture was then cooled to room temperature. The solid obtained after precipitation with diethyl ether (50 mL) was purified by column chromatography using $CHCl_3$/MeOH (9:1 to 4:1) as eluant. The yield was 21% (244 mg). NMR ($CDCl_3$): δ 0.87 (t, 3 H, terminal CH_3), 1.2–1.6 (m, 35 H, $(CH_2)_{14}$, CH_3CH_2O, piperidinium C-3 and C-5 H), 3.25–3.75 (overlapping m, 17 H, CH_3CH_2OCH, CH_2OCH_2, CH_3N, CH_2N, piperidinium C-2 and C-6 H, CHOH). Anal. ($C_{27}H_{56}NO_3I$) C, H, N.

Preparation of Series II Quaternary Amine Ether Lipids. These quaternary amine salts were prepared by displacement of the appropriate bromide with either trimethyl- or triethylamine or pyridine. A representative experimental procedure is given for each set of reaction conditions followed directly by the NMR and elemental analysis data for each compound prepared by the same method. These salts were, in general, very hygroscopic solids and no melting points were taken. Yields are given after the compound name. In some syntheses, the starting bromide contained an undetermined amount of alkene side product from the previous reaction (see the experimental method for 1-(hexadecyloxy)-2-methoxy-3-(4-bromobutoxy)propane). This olefin did not react in the amination reaction but a yield for this step could not be quantified.

(±)-*N*,*N*,*N*-Trimethyl-*N*-[3-(hexadecyloxy)-2-methoxy-1-propyl]ammonium Bromide (10). 1-(Hexadecyloxy)-2-methoxy-3-bromopropane (0.60 g, 0.0015 mol) was dissolved in 50 mL of acetonitrile and cooled to −10 °C. An excess of condensed trimethylamine gas was added, the reaction vessel was sealed, and the reaction was stirred at room temperature for 24 h. The solvent was removed in vacuo and the product was isolated by chromatography on silica gel with $CHCl_3$/MeOH (9:1) as eluant. A white solid (300 mg, 50% yield) melting at 210–212 °C was obtained. NMR ($CDCl_3$): δ 0.85 (t, 3 H, terminal CH_3), 1.2–1.4 (m, 26 H, $(CH_2)_{13}$), 1.55 (m, 2 H, OCH_2CH_2), 3.45 (s, 3 H, OCH_3), 3.50 (s, 9 H, $N(CH_3)_3$), 3.4–3.6 (overlapping m, 6 H, CH_2OCH_2, CH_2N), 4.04 (m, 1 H, CH). Anal. ($C_{23}H_{50}NO_2Br \cdot 0.5H_2O$) C, H, N.

(±)-*N*,*N*,*N*-Trimethyl-*N*-[3-[3-(hexadecyloxy)-2-methoxypropoxy]-1-propyl]ammonium Bromide (17). Mp: 225 °C dec. NMR ($CDCl_3$): δ 0.87 (t, 3 H, terminal CH_3), 1.2–1.4 (m, 26 H, $(CH_2)_{13}$), 1.55 (m, 2 H, OCH_2CH_2), 2.10 (m, 2 H, $OCH_2CH_2CH_2N$), 3.40 (s, 3 H, OCH_3), 3.45 (s, 9 H, $N(CH_3)_3$),

3.4–3.8 (overlapping m, 11 H, $CH_2OCH_2CHCH_2OCH_2CH_2CH_2N$). Anal. ($C_{26}H_{56}NO_3Br \cdot 1.0H_2O$) C, N; H: calcd, 11.05; found, 10.61.

(±)-*N*,*N*,*N*-Trimethyl-*N*-[6-[3-(hexadecyloxy)-2-methoxypropoxy]-1-hexyl]ammonium Bromide (19). Yield: 25%. NMR ($CDCl_3$): δ 0.85 (t, 3 H, terminal CH_3), 1.2–1.4 (m, 26 H, $(CH_2)_{13}$), 1.45 and 1.55 and 1.75 (3 m, 10 H, OCH_2CH_2, $OCH_2(CH_2CH_2)_2CH_2N$), 3.45 (s, 12 H, OCH_3, $N(CH_3)_3$), 3.4–3.7 (overlapping m, 11 H, $CH_2OCH_2CHCH_2OCH_2(CH_2CH_2)_2CH_2N$). Anal. ($C_{29}H_{62}NO_3Br \cdot 1.0H_2O$) C, H, N.

(±)-*N*,*N*,*N*-Trimethyl-*N*-[4-[3-(octadecyloxy)-2-ethoxypropoxy]-1-butyl]ammonium Bromide (23). Yield: 34% NMR ($CDCl_3$): δ 0.85 (t, 3 H, terminal CH_3), 1.2 (t, 3 H, OCH_2CH_3), 1.2–1.4 (m, 30 H, $(CH_2)_{15}$), 1.55 (m, 2 H, OCH_2CH_2), 1.70 and 1.90 (m, 4 H, $OCH_2CH_2CH_2CH_2N$), 3.45 (s, 9 H, $N(CH_3)_3$), 3.4–3.8 (overlapping m, 13 H, $CH_2OCH_2CHCH_2OCH_2CH_2CH_2CH_2N$, OCH_2CH_3). Anal. ($C_{30}H_{64}NO_3Br \cdot 2.0H_2O$) C, H, N.

(±)-*N*,*N*,*N*-Trimethyl-*N*-[4-[(2-Heptadecyl-1,3-dioxolan-4-yl)methoxy]-1-butyl]ammonium Bromide (25). This synthesis was performed at 55 °C. NMR ($CDCl_3$): δ 0.87 (t, 3 H, terminal CH_3), 1.2–1.3 (m, 32 H, $(CH_2)_{16}$), 1.4–1.6 (m, 4 H, $OCH_2CH_2CH_2CH_2N$), 3.25 (s, 9 H, $N(CH_3)_3$), 3.5–4.0 (overlapping m, 9 H, $OCH_2CHCH_2OCH_2$, CH_2N), 4.4 (m, 1 H, OCHO). Anal. ($C_{28}H_{58}NO_3Br \cdot 0.5H_2O$) C, H, N.

(±)-*N*,*N*,*N*-Trimethyl-*N*-[4-[3-(hexadecylthio)-2-ethoxypropoxy]-1-butyl]ammonium Bromide (15). 1-(Hexadecylthio)-2-ethoxy-3-(4-bromobutoxy)propane (0.6 g, 0.001 mol) was added to 25 mL of acetonitrile. Aqueous trimethylamine (40%, 10 mL) was added, causing an immediate clouding of the solution which cleared with time. The reaction was allowed to proceed overnight at room temperature. The solvent was removed and two silica gel columns were run with $CHCl_3$/MeOH (9:1) to give a very hygroscopic solid in a 50% yield. No melting point was determined. NMR ($CDCl_3$): δ 0.85 (t, 3 H, terminal CH_3), 1.2 (t, 3 H, OCH_2CH_3), 1.2–1.4 (m, 26 H, $(CH_2)_{13}$), 1.45 (m, 2 H, OCH_2CH_2), 1.65 and 1.85 (m, 4 H, $OCH_2CH_2CH_2N$), 2.51 (t, 2 H, SCH_2), 2.63 (overlapping d of d, 2 H, $CHCH_2S$), 3.45 (s, 9 H, $N(CH_3)_3$), 3.4–3.7 (overlapping m, 9 H, $CHCH_2OCH_2CH_2CH_2CH_2N$, OCH_2CH_3). Anal. ($C_{28}H_{60}NSO_2Br \cdot H_2O$) C, H, N.

(±)-*N*,*N*,*N*-Trimethyl-*N*-[10-[3-(hexadecyloxy)-2-methoxypropoxy]-1-decyl]ammonium Bromide (20). NMR ($CDCl_3$): δ 0.85 (t, 3 H, terminal CH_3), 1.2–1.4 (m, 34 H, $(CH_2)_{13}$, $O(CH_2)_3(CH_2CH_2)_2(CH_2)_3N$), 1.55 and 1.75 and 1.90 (3 m, 10 H, OCH_2CH_2, $OCH_2CH_2CH_2(CH_2CH_2)_2CH_2CH_2CH_2N$), 3.45 (s, 12 H, OCH_3, $N(CH_3)_3$), 3.4–3.7 (overlapping m, 11 H, $CH_2OCH_2CHCH_2OCH_2(CH_2CH_2)_4CH_2N$). Anal. ($C_{33}H_{70}NO_3Br \cdot 0.50H_2O$) C, H, N.

(±)-*N*,*N*,*N*-Trimethyl-*N*-[4-[3-(hexadecyloxy)-2-ethoxypropoxy]-1-butyl]ammonium Bromide (21). NMR ($CDCl_3$): δ 0.85 (t, 3 H, terminal CH_3), 1.2 (t, 3 H, OCH_2CH_3), 1.2–1.4 (m, 26 H, $(CH_2)_{13}$), 1.55 (m, 2 H, OCH_2CH_2), 1.70 and 1.90 (m, 4 H, $OCH_2CH_2CH_2CH_2N$), 3.45 (s, 9 H, $N(CH_3)_3$), 3.4–3.8 (overlapping m, 13 H, $CH_2OCH_2CHCH_2OCH_2CH_2CH_2CH_2N$, OCH_2CH_3). Anal. ($C_{28}H_{60}NO_3Br \cdot 0.5H_2O$) C, H, N.

***N*,*N*,*N*-Trimethyl-*N*-[3-(hexadecyloxy)-1-propyl]ammonium Bromide (13).** 1-(Hexadecyloxy)-3-bromopropane (0.5 g, 0.001 mol) was dissolved in 13 mL of $CHCl_3$/2-propanol/DMF (3:5:5), and 1 mL of 40% aqueous trimethylamine was added. The reaction mixture was heated at 60 °C and then stirred at room temperature overnight. Thin-layer chromatography indicated an incomplete reaction and an additional 0.5 mL of trimethylamine was added and the reaction continued at 60 °C for 2 h. After cooling, the solvent was removed and the product was purified by repeated recrystallization with ether. The salt was obtained as a white solid in 75% yield, decomposing at 200–203 °C. NMR ($CDCl_3$): δ 0.87 (t, 3 H, terminal CH_3), 1.2–1.3 (m, 26 H, $(CH_2)_{13}$), 1.53 (m, 2 H, OCH_2CH_2), 2.08 (m, 2 H, $OCH_2CH_2CH_2N$), 3.2–3.48 (overlapping m, 13 H, CH_2OCH_2, $N(CH_3)_3$), 3.66 (m, 2 H, CH_2N). Anal. ($C_{22}H_{48}NOBr$) C, H, N.

(±)-*N*,*N*,*N*-Trimethyl-[4-[3-(hexadecyloxy)-2-methoxypropoxy]-1-butyl]ammonium Bromide (18). NMR ($CDCl_3$): δ 0.85 (t, 3 H, terminal CH_3), 1.2–1.4 (m, 26 H, $(CH_2)_{13}$), 1.55 (m, 2 H, OCH_2CH_2), 1.70 and 1.90 (m, 4 H, $OCH_2CH_2CH_2CH_2N$), 3.45 (s, 12 H, OCH_3, $N(CH_3)_3$), 3.4–3.8 (overlapping m, 11 H, $CH_2OCH_2CHCH_2OCH_2CH_2CH_2CH_2N$). Anal. ($C_{27}H_{58}NO_3Br \cdot 0.5H_2O$) C, H, N.

(±)-*N*,*N*,*N*-Trimethyl-*N*-[4-[3-(hexadecyloxy)propoxy]-1-butyl]ammonium Bromide (24). Yield: 52% NMR ($CDCl_3$): δ 0.87 (t, 3 H, terminal CH_3), 1.2–1.3 (m, 26 H, $(CH_2)_{13}$), 1.46–1.67 (m, 4 H, $(CH_2)_{13}CH_2CH_2OCH_2CH_2$), 1.82 (m, 4 H, $OCH_2CH_2CH_2CH_2N$), 3.2–3.4 (overlapping m, 17 H, CH_2OCH_2, CH_2OCH_2, $N(CH_3)_3$), 3.64 (m, 2 H, CH_2N). Anal. ($C_{26}H_{56}NO_2Br \cdot 1.5H_2O$) C, H, N.

(±)-*N*,*N*,*N*-Triethyl-*N*-[3-(hexadecyloxy)-2-ethoxy-1-propyl]ammonium Bromide (11). 1-(Hexadecyloxy)-2-ethoxy-3-bromopropane (0.5 g, 0.002 mol) and triethylamine (10 mL) were placed in 20 mL of acetonitrile and heated to reflux for a total of 24 h. The solvent was removed and the residue was dissolved in a minimum volume of $CHCl_3$. Diethyl ether was added to cause precipitation, then the solution was cooled to 0 °C. After filtration, the solid was purified by chromatography on silica gel with 9:1 $CHCl_3$/MeOH. The yield was 4% (23 mg). Mp: 108–110 °C. NMR ($CDCl_3$): δ 0.85 (t, 3 H, terminal CH_3), 1.20 (t, 3 H, OCH_2CH_3), 1.2–1.4 (m, 26 H, $(CH_2)_{13}$), 1.40 (t, 9 H, $N(CH_2CH_3)_3$). 1.55 (m, 2 H, OCH_2CH_2), 3.3–3.8 (overlapping m, 14 H, CH_2OCH_2, OCH_2CH_3, $CH_2N(CH_2CH_3)_3$), 4.15 (m, 1 H, CH). Anal. ($C_{27}H_{58}NO_2Br$) C, H, N.

(±)-*N*-[3-(Hexadecyloxy)-2-ethoxy-1-propyl]pyridinium Bromide (12). 1-(Hexadecyloxy)-2-ethoxy-3-bromopropane (0.5 g, 0.002 mol) was placed in 20 mL of pyridine and the solution was heated to reflux for 8 h. The solvent was removed in vacuo and the residue was chromatographed on silica gel using $CHCl_3$/MeOH (9:1) as eluant. The solid product weighed 260 mg (52% yield). Mp: 184–185 °C. NMR (CDC_3): δ 0.85 (t, 3 H, terminal CH_3), 0.98 (t, 3 H, OCH_2CH_3), 1.2–1.3 (m, 26 H, $(CH_2)_{13}$), 1.50 (m, 2 H, OCH_2CH_2), 3.3–3.7 (overlapping m, 6 H, CH_2OCH_2, OCH_2CH_3), 4.05 (m, 1 H, CH), 4.75 and 5.50 (2 d of d, 2 H, CH_2N), 8.1 (t, 2 H, pyridinium H), 8.60 (t, 1 H, pyridinium H), 9.40 (d, 2 H, pyridinium H). Anal. ($C_{26}H_{48}NO_2Br \cdot 0.5H_2O$) C, H, N.

***N*-[3-(Hexadecyloxy)-1-propyl]pyridinium Bromide (14).** Yield: 37%. Mp: >200 °C dec. NMR ($CDCl_3$): δ 0.86 (t, 3 H, terminal CH_3), 1.2–1.3 (m, 28 H, $(CH_2)_{14}$), 2.37 (q, 2 H, $OCH_2CH_2CH_2N$), 3.31 and 3.50 (2 t, 4 H, CH_2OCH_2), 5.11 (t, 2 H, CH_2N), 8.08 (m, 2 H, pyridinium H), 8.48 (m, 1 H, pyridinium H), 9.44 (m, 2 H, pyridinium H). Anal. ($C_{24}H_{44}NOBr \cdot 0.67H_2O$) C, H, N.

(±)-*N*-[4-[3-(Hexadecyloxy)-2-ethoxypropoxy]-1-butyl]-pyridinium Bromide (22). Yield: 85%. NMR ($CDCl_3$): δ 0.85 (t, 3 H, terminal CH_3), 1.18 (t, 3 H, OCH_2CH_3), 1.2–1.3 (m, 26 H, $(CH_2)_{13}$), 1.54 (m, 2 H, OCH_2CH_2), 1.61 (m, 2 H, $OCH_2CH_2CH_2CH_2N$), 2.13 (m, 2 H, $OCH_2CH_2CH_2CH_2N$), 3.2–3.8 (overlapping m, 11 H, CH_2OCH_2, OCH_2CH_3, $CHCH_2OCH_2$), 5.10 (t, 2 H, CH_2N), 8.1 (t, 2 H, pyridinium H), 8.45 (t, 1 H, pyridinium H), 9.60 (d, 2 H, pyridinium H). Anal. ($C_{30}H_{56}NO_3Br \cdot H_2O$) C, H, N.

The syntheses of **(±)-*N*,*N*,*N*-trimethyl-*N*-[3-(hexadecylthio)-2-methoxy-1-propyl]ammonium bromide (9) and (±)-*N*,*N*,*N*-trimethyl-*N*-[2-[3-(hexadecyloxy)-2-methoxypropoxy]ethyl]ammonium bromide (16)** are reported in ref 19.

(±)-*N*-Methyl-*N*-[3-(hexadecyloxy)-2-ethoxy-1-propyl]-β-hydroxyethylamine (26). This compound was prepared by reaction of 1-(hexadecyloxy)-2-ethoxy-3-bromopropane (0.60 g, 0.0015 mol) with 2-(methylamino)ethanol (0.225 g, 0.003 mol) in acetonitrile at 50 °C for 24 h. After cooling, the solvent was removed and the residue was partitioned between diethyl ether and water. The ether was dried over sodium sulfate and then evaporated and the product (0.1 g, 0.0002 mol, 14% yield) was purified by column chromatography on silica gel with $CHCl_3$/MeOH (9:1) as eluant. NMR ($CDCl_3$): δ 0.87 (t, 3 H, terminal CH_3), 1.2 (t, 3 H, OCH_2CH_3) 1.2–1.3 (m, 26 H, $(CH_2)_{13}$), 1.55 (m, 2 H, OCH_2CH_2), 2.33 (s, 3 H, NCH_3), 2.5–2.6 (overlapping m, 4 H, CH_2NCH_2), 3.4–3.7 (overlapping m, 9 H, $CH_2OCH_2CHOCH_2$, CH_2OH). Anal. ($C_{24}H_{51}NO_3$) C, H, N.

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Registry No. 1, 124581-78-8; **2**, 124581-79-9; **3**, 124581-80-2; **4**, 124581-94-8; **5**, 126614-04-8; **5** (starting iodide), 126614-27-5; **6**, 124581-82-4; **7**, 126614-05-9; **8**, 124581-83-5; **9**, 124581-81-3; **10**, 126614-06-0; **10** (starting bromide), 126614-21-9; **11**, 126614-07-1; **11** (starting bromide), 126614-36-6; **12**, 126614-08-2; **13**, 126644-53-9; **13** (starting bromide), 126614-26-4; **14**, 126614-09-3; **15**, 126614-10-6; **15** (starting bromide), 126614-32-2; **16**, 124581-92-6; **17**, 126614-11-7; **17** (starting bromide), 126614-28-6; **18**, 126614-12-8; **18** (starting bromide), 126614-23-1; **19**, 126614-13-9; **19** (starting bromide), 126614-29-7; **20**, 126614-14-0; **20** (starting bromide), 126614-33-3; **21**, 126614-15-1; **21** (starting bromide), 126614-34-4; **22**, 126614-16-2; **23**, 126614-17-3; **23** (starting bromide), 126614-30-0; **24**, 126614-18-4; **24** (starting bromide), 126614-35-5; **25**, 126614-19-5; **25** (starting bromide), 126614-31-1; **26**, 126614-20-8; (±)-$MeSO_3CH_2CH(OMe)CH_2OC_{16}H_{33}$, 126614-22-0; (±)-$HOCH_2CH(OMe)CH_2OC_{16}H_{33}$, 111188-59-1; (±)-$H_2C{=}CHCH_2CH_2OCH_2CH(OMe)CH_2OC_{16}H_{33}$, 126614-24-2; $H_{33}C_{16}OCH_2CH(OMe)CH_2O(CH_2)_4OCH_2CH(OMe)CH_2OC_{16}H_{33}$, 126614-25-3; $Br(CH_2)_4Br$, 110-52-1; $HO(CH_2)_3OC_{16}H_{33}$, 23377-40-4; CBr_4, 558-13-4; $MeNHCH_2CH_2OH$, 109-83-1; *N*-methyl-4-hydroxypiperidine, 106-52-5.

Syntheses and Platelet Aggregation Inhibitory and Antithrombotic Properties of [2-[(ω-Aminoalkoxy)phenyl]ethyl]benzenes

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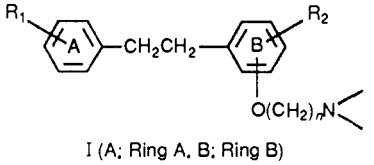
A series of [2-[(ω-aminoalkoxy)phenyl]ethyl]benzene derivatives were synthesized and evaluated for their ability to inhibit collagen-induced platelet aggregation in vitro and to protect experimental thrombosis in mice. The results showed that the compounds were in vitro inhibitors of collagen-induced platelet aggregation. Most of them were also effective in the mouse antithrombotic assay. The compounds were found to be potent antagonists to S_2 serotonergic receptor, and good correlation (r = 0.85) between their S_2 serotonergic receptor antagonism and their potency as platelet antiaggregatory drugs was observed. Among the compounds studied, mono[2-(dimethylamino)-1-[[2-[2-(3-methoxyphenyl)ethyl]phenoxy]methyl]ethyl] succinate hydrochloride (**12b**, MCI-9042) was selected for further pharmacological and toxicological evaluation.

The intravascular formation of platelet aggregates is an important pathogenic factor in the development of cardiovascular disease state such as atherosclerosis, myocardial infarction, transient ischemic attacks, and stroke.[1-4] Hence, one rational approach in the research for antithrombotic drugs is to search for inhibitors of platelet aggregation. Platelets are activated by exposed collagen on an injured vessel wall and subsequently aggregate to form a platelet plug and secrete intracellular granules containing serotonin, ADP, and some active substances. Since the first trigger of platelet aggregation in vivo is the activation by collagen of subendothelial tissues, we focused on an agent which would inhibit collagen-induced platelet aggregation.

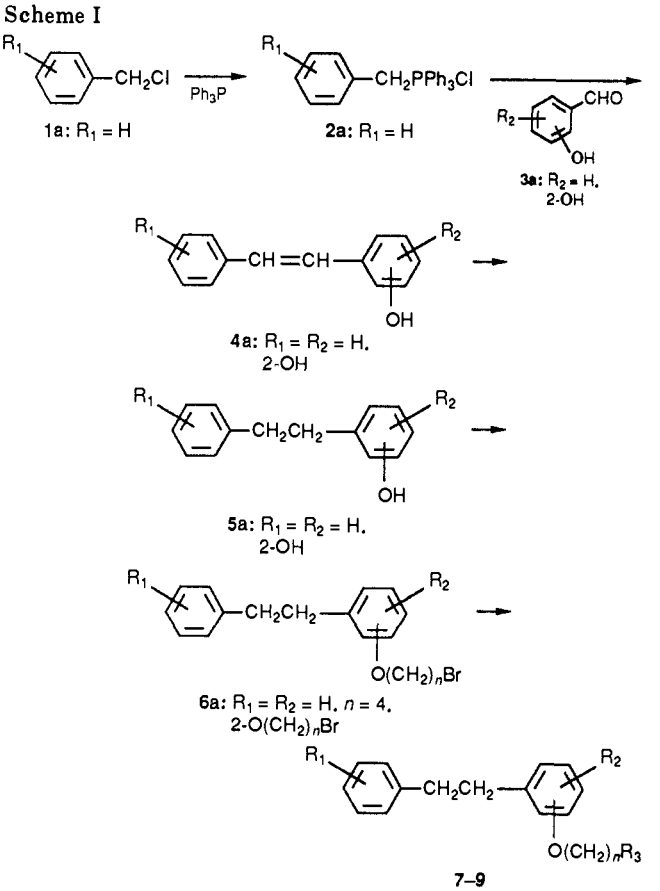
In our efforts to search for inhibitors of collagen-induced platelet aggregation, we have discovered a novel class of compounds, [2-(ω-aminoalkoxy)phenyl]ethyl]benzenes (I).



I (A: Ring A. B: Ring B)

In this paper are described the relationship between the structure of [2-[(ω-aminoalkoxy)phenyl]ethyl]benzenes and the inhibition of in vitro collagen-induced platelet aggregation and in vivo thrombus formation in mice. During the course of study with the compounds, we found that this series of compounds exhibited a potent S_2 serotonergic receptor antagonism, which highly correlated with an inhibitory effect on in vitro collagen-induced platelet aggregation.

Scheme I



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