Synthesis of Phosphocholine and Quaternary Amine Ether Lipids and Evaluation of *in Vitro* Antineoplastic Activity

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The *in vitro* antineoplastic activity of many phosphorus-containing (e.g., phosphocholines) and non-phosphorus-containing (e.g., quaternary ammonium salts) ether lipids has been evaluated in the HL-60 promyelocytic cell line. These compounds are analogues of ET-18-OMe (1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine). Structural modification of 1-(alkylamido)-, -(alkylthio)-, and -(alkyloxy)propyl backbones has provided further insight into the structure–activity relationships of these lipids. In this study, a long saturated C-1 chain and a three-carbon backbone with a single short C-2 substituent were preferred. At the positively charged nitrogen of phosphocholines, fewer than three substituents caused a significant loss of activity, and substituents larger than methyl decreased activity slightly. In the nonphosphorus compounds, many nitrogen heterocycles and also a sulfonium moiety were incorporated without changing the degree of activity; however, a thiazolium group decreased activity. The most active compound, **29** [*N*-[3-(hexadecyloxy)-2-methoxypropyl]-3-(hydroxymethyl)pyridinium bromide], was approximately twice as active as the reference standard, ET-18-OMe, in a trypan blue dye exclusion assay.

Introduction

Synthetic ether lipids display a wide range of biological activities including inhibition of (1) membrane-associated enzyme [protein kinase C (PKC),[1,2a] phospholipase C,[2b] and sodium/potassium ATPase[2c,2d]] activity, (2) neoplastic cell growth,[3] and (3) infectious HIV-1 replication.[4,5] Exact mechanisms of action for these inhibitory activities have not been established, but some interrelationships may occur. Both preferential PKC inhibition[1,2] and membrane fluidization[6] in the malignant cell have been proposed as mechanisms for the antineoplastic activity of ether lipids. Entry of HIV-1 into cells may depend on phosphorylation of the CD4 receptor[7] by PKC, suggesting a possible link between PKC inhibition and anti-HIV-1 activity. Further, increased membrane fluidization has also been suggested as a mechanism by which AL721, a mixture of naturally occurring lipids, inhibits HIV infectivity.[8]

In recent years, we have focused on the biological activity of ether lipids and have synthesized a variety of analogues, both type A phosphorus-containing[5a,9,10] (phosphocholines and phosphoethanolamines) and type B non-phosphorus-containing[2a,11] (primarily quaternary ammonium salts). *In vitro* biological activities of these compounds against various neoplastic cell lines,[6,9-13] membrane-linked enzymes,[2,10] and/or HIV-1[4,5] have been reported. Further structural modification of 1-(alkyloxy)-, -(alkylthio)-, and -(alkylamido)propyl phospholipids and quaternary ammonium ether lipids has been made to further define the structure–activity relationships. The present paper will describe the antineoplastic activity of these analogues against the human HL-60 promyelocytic leukemia cell line.

The standard used in all assays was 1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine, ET-18-OMe. Two assays were used to determine the *in vitro* ID_{50} values: a trypan blue dye exclusion assay (TB) and an assay measuring inhibition of incorporation of tritiated thymidine (TdR).

Chemistry

The ether lipid structures are shown in Figures 1 (type A phosphorus) and 2 (type B non-phosphorus). The type B non-phosphorus compounds constitute three series: (1) analogues with a heterocyclic (e.g., pyridine or *N*-methylpyrrole) or acyclic (e.g., trimethyl) amine directly attached to the carbon backbone; (2) analogues with an inverse choline substituent (e.g., *N*,*N*-dimethyl-3′-hydroxypropylamine) on the carbon backbone; and (3) analogues with the quaternary moiety present at the end of an alkoxy spacer group. The syntheses of many of these compounds (Figure 1, compounds **1**, **2**, **18–22**; Figure 2, compounds **23** and **24**) have been reported previously and for new compounds follow general procedures described in earlier papers.[2a,9-11,13]

Scheme I illustrates the preparation of thiolipids **3** and **5–7**. First, 3-mercapto-1,2-propanediol was alkylated with an alkyl halide and alcoholic potassium hydroxide.[14,15] The primary hydroxyl was then protected as the trityl ether.[15] For compound **3**, a dicyclohexylcarbodiimide (DCC)/dimethyl sulfoxide (DMSO) oxidation[16] gave a ketone at C-2, which was reacted with methylmagnesium iodide to give a tertiary alcohol at C-2. The general reaction sequence then continued by formation of the C-2 alkyl ether with an alkyl iodide and sodium hydride. Detritylation with *p*-toluenesulfonic acid[17] reformed the free

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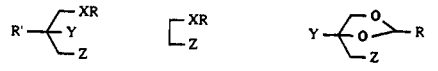
Structure A Structure B Structure C



Cmpd[a]	X	R	R'	Y	Z[b]
Reference Compounds:					
ET18OMe	O	18:0	OMe	H	PC
1[c]	NHCO	17:0	OEt	H	PC
2[d]	S	16:0	OEt	H	PC
New Compounds:					
3	S	18:0	OEt	Me	PC
4	S	$CH_2CHOMe(CH_2)_{13}CH_3$	OEt	H	PC
5	S	16:1	OEt	H	PC
6	S	16:0	OPr	H	PC
7	S	16:0	OEt	H	$OPO_3(CH_2)_2NEt_3$
8	O	18:0	OEt	H	$OPO_3(CH_2)_2NMe_2Bz$
9	O	$(CH_2)_9CO(CH_2)_5CH_3$	OEt	H	PC
10	O	16:0	OMe	OMe	PC
11[a]	-	17:0	-	H	PC
12	O	16:0	OEt	H	$OPO_3(CH_2)_2$pyridine
13	NHCO	17:0	H	H	PC
14	NHCO	17:0	H	H	$OPO_3(CH_2)_2$pyridine
15	NHCO	17:0	H	H	$OPO_3(CH_2)_2NMe_2(CH_2)_3OH$
16	NHCO	17:0	O14:0	H	PC
17	NMeCO	17:0	OMe	H	PC
18[a,e]	S	16:0	-	-	PC
19[e]	O	16:0	OEt	H	$OPO_3(CH_2)_2NH_2$isopropyl
20[e]	O	16:0	OEt	H	$OPO_3(CH_2)_2NH_2$tbutyl
21[e]	NHCO	19:0	OEt	H	PC
22[a,e]	NHCO	17:0	-	-	PC

Figure 1. Structures of type A phosphorus-containing ether lipids. (a) All compounds have structure A except for 11 which has structure C and 18 and 22 which have structure B. (b) PC = phosphocholine = $OPO_3(CH_2)_2NMe_3$; all Z groups are zwitterionic. (c) Synthesis reported in ref 10. (d) Synthesis reported in 9. (e) Synthesis reported in ref 5a.

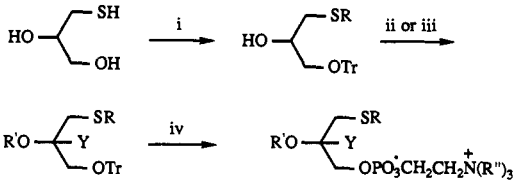
Structure A Structure B

Cmpd #[a]	X	R	R'	Y	Z[b]
Reference Compounds:					
ET18OMe	O	18:0	OMe	H	PC[c]
23[d]	S	16:0	OMe	H	$NMe_2(CH_2)_2OH$ Br^-
24[d]	S	18:0	OMe	H	$NMe_2(CH_2)_3OH$ I^-
New Compounds:					
25	O	16:0	OMe	H	$S(CH_2)_2NMe_3$ Br^-
26	O	16:0	OMe	H	$SMe(CH_2)_2OH$ OTs^-
27	O	8:0	OMe	H	NMe_3 Br^-
28	O	16:0	OMe	H	$O(CH_2)_4NMe_2(CH_2)_3OH$ Br^-
29	O	16:0	OMe	H	3-hydroxymethylpyridine Br^-
30	O	18:0	OEt	H	NMe-morpholine Br^-
31	O	18:0	OEt	H	NMe-4-hydroxypiperidine Br^-
32	S	16:0	CH_2OMe	H	NMe_3 Br^-
33	S	16:0	CH_2OMe	H	$NMe_2(CH_2)_3OH$ Br^-
34	S	16:0	OMe	H	$NMe_2CH_2CHOHCH_2OH$ Br^-
35	S	16:0	O6:0	H	$NMe_2(CH_2)_2OH$ Br^-
36	S	16:0	OMe	H	thiazole Br^-
37[a]	S	16:0	-	-	pyridine Br^-
38[a]	S	16:0	-	-	$NMe_2(CH_2)_2OH$ Br^-
39	S	12:0	OMe	H	NMe_3 Br^-
40	S	16:0	OEt	H	NMe-pyrrole Br^-
41	S	16:0	O5:0	H	NMe_3 Br^-
42	S	16:0	H	H	NMe-3-hydroxypyrrole Br^-

Figure 2. Structures of type B non-phosphorus-containing ether lipids. (a) All compounds have structure A except for 37 and 38 which have structure B. (b) All Z groups contain a positively charged quaternary nitrogen atom except for compound 26 which has a positively charged sulfur atom. (c) PC = phosphocholine = $OPO_3(CH_2)_2NMe_3$; all Z groups are zwitterionic. (d) Synthesis reported in ref 2a.

primary hydroxyl. Phosphocholines were prepared by reaction with phosphorus oxychloride followed by choline tosylate.[18] For compound 7, (*N,N,N*-triethylamino)-ethanol tosylate replaced choline tosylate, and, for phospholipid 8, (*N,N*-dimethyl-*N*-benzylamino)ethanol bromide was used in this phosphorylation step.

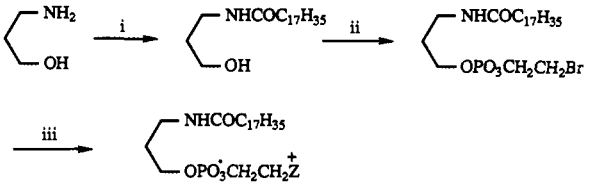
Scheme I[a,b]



3, 5-7

[a] (i) (1) RX, KOH, (2) TrCl; (ii) (1) DMSO, DCC, (2) MeMgI (Y = Me), (3) EtI, NaH (R' = Et); or (iii) (1) R'I, NaH (Y = H, R' = Et or Pr); (iv) (1) pTSA, (2) $POCl_3$, (3) choline tosylate (R'' = Me), or (2) $Cl_2PO_2CH_2CH_2Br$, (3) NEt_3 (R'' = Et).[b] R, R'O, Y as given in Figure 1 for compounds 3 and 5–7.

Scheme II[a]



13-15

[a] (i) $C_{17}H_{35}COCl$; (ii) $Cl_2PO_2CH_2CH_2Br$; (iii) Z = NMe_3 for compound 13, pyridine for compound 14, $Me_2NCH_2CH_2CH_2OH$ for compound 15.

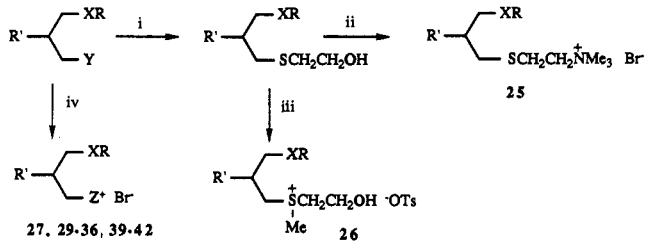
In the synthesis of compound 4, preparation of the β-methoxy-substituted C-1 alkyl chain required additional steps that are not illustrated in Scheme I. 3-Mercapto-1,2-propanediol was reacted with 1,2-epoxyhexadecane and KOH; the 1,2-diol was then protected as the dimethyl ketal. After alkylation of the free side-chain hydroxyl group, the ketal was opened with acid. The remaining steps (tritylation at C-1, alkylation of C-2, detritylation, and phosphorylation) were performed as shown in Scheme I.

Compound 9 was prepared by reaction of 3-*O*-trityl-2-*O*-ethylglycerol with the mesylate of 10-oxo-1-hexadecanol and sodium hydride followed by detritylation and phosphorylation as shown in Scheme I. The keto alcohol was prepared by reaction of dihexylcadmium[19] with the acid chloride of sebacic acid monomethyl ester.[20]

Formation of the dimethoxy phosphocholine, 10, began with esterification of the primary hydroxyl of 1-*O*-hexadecylglycerol with benzoyl chloride.[16] A ketone at C-2 was generated with the DCC/DMSO oxidation shown in Scheme I and was ketalized with methanol and sulfosalicylic acid.[16] Saponification of the benzyl ester was followed by formation of the phosphocholine with 2-chloro-2-oxo-1,3,2-dioxaphospholane and trimethylamine.[21] This same phosphorylation procedure was used in preparation of compound 11 from 2-heptadecyl-4-(hydroxymethyl)-1,3-dioxolane. The latter compound was formed by transacetalation of octadecanal dimethoxy acetal with glycerol.[22]

Scheme II shows the synthesis of amido compounds 13, 14, and 15. 3-Octadecanamido-1-propanol was formed by reaction of 3-amino-1-propanol with stearoyl chloride in pyridine. This alcohol was reacted with 2-bromoethyl phosphodichloridate[23] and the isolated bromide then displaced with trimethylamine, pyridine, or (*N,N*-dimethylamino)ethanol to give the final products. This same phosphorylating reagent and reaction with pyridine gave the glycerophosphate 12. Amido compounds 16 and 17 were prepared from 3-amino-1,2-propanediol by the reaction sequence: amidation, tritylation at C-1, alkylation at C-2, detritylation, and phosphorylation with 2-bromo-

Scheme III[a,b]



[a] Y = I or Br; (i) $HSCH_2CH_2OH$, KOH; (ii) (1) CBr_4, $P(C_6H_5)_3$, (2) NMe_3 (iii) MeOTs; (iv) Z is the amine given in Figure 2.[b] X, R, R' as given in Figure 2 for compounds **25–27**, **29–36**, and **39–42**.

ethyl phosphodichloridate and trimethylamine. Alkylation of the C-2 hydroxyl of 3-octadecanamido-1-(trityloxy)-2-propanol with methyl iodide (as described in ref 10) led to a small amount (<10%) of dimethylated compound by reaction occurring also at the amide nitrogen. The dimethylated product (higher R_f) could be separated from the monomethyl compound by column chromatography after detritylation; phosphorylation then gave compound **17**. With larger alkylating groups, this side reaction in the alkylation of the C-2 hydroxyl was negligible.

The preparation of non-phosphorus lipids **25** and **26** is shown in Scheme III. 3-(Hexadecyloxy)-2-methoxy-1-iodopropane was reacted with 2-mercaptoethanol in alcoholic potassium hydroxide[14,15] to give a common intermediate for both analogues. For compound **25**, the hydroxyl was brominated with carbon tetrabromide and triphenylphosphine[24] followed by displacement with trimethylamine. The sulfonium compound, **26**, was prepared from the sulfide intermediate by reaction with methyl toluenesulfonate in refluxing acetone.[25] Methylation with methyl iodide was attempted, but the iodide salt was found to be unstable and to undergo disproportionation to the starting sulfide. The tosylate proved to be a more stable compound.

The 2,3-disubstituted 1-halopropanes shown in Scheme III (and the 2-substituted 1-haloethanes) were prepared by established synthetic procedures given in earlier papers.[2a,9,11,13] Displacement of the halide with the appropriate tertiary or aromatic amine[2a,11,13] (step iv in Scheme III) completed formation of ammonium salts **27–31** and **34–41**.

The bromide precursor (3-(hexadecylthio)-2-(methoxymethyl)-1-bromopropane) for compounds **32** and **33** was prepared from diethyl bis(hydroxymethyl)malonate by a slight modification of the method of Bosies *et al.*[26] The alcohol intermediate for compound **42** was prepared by reaction of hexadecyl mercaptan with 3-bromopropanol.[14,15]

Results and Discussion

Tables I (type A phosphorus) and II (type B non-phosphorus) show the *in vitro* ID_{50} values found for these ether lipid analogues in the HL-60 leukemic cell system. The trypan blue dye exclusion assay (TB) was carried out for 48 h. The standard thymidine incorporation assay (TdR) was also run for 48 h and involved a 24-h pulse of [^{3}H]thymidine; in some instances, it was performed for 48 h with an 8-h pulse of [^{3}H]thymidine (TdR8) (Tables I and II and Experimental Section). Comparison of the TB and TdR data shows values, in general, of the same magnitude. It should be noted, however, that these two assays measure different endpoints: the TB assay measures cytotoxicity or cell kill only; the TdR assay measures a combination of cytotoxicity and cytostasis. Since the TdR assay was not used in earlier papers,[6,9–12] data are also included for certain previously reported compounds (Table I, **1**[10] and **2**;[6,9,12] Table II, **23**[11] and **24**[11]).

Table I. ID_{50} Values (μM) for Type A Phosphorus-Containing Ether Lipids

compd	TB[a] ID_{50} ± SD[d]	TdR[b] ID_{50} ± SD[d]	TdR8[c] ID_{50} ± SD[d]
	Standard Compound		
Et-18-OMe	2.48 ± 0.31 (33)	2.38 ± 0.40 (21)	2.41 ± 0.62 (16)
	Previously Reported Compounds		
1[e]	1.64 ± 0.19 (4)	3.30 ± 0.37 (5)	ND[f]
2[g]	2.25 ± 0.17 (1)	2.19 ± 0.44 (3)	3.11 ± 0.10 (3)
	New Compounds		
3	3.83 (2)	ND	ND
4	3.39 ± 0.12 (3)	2.47 (2)	ND
5	6.75 (1)	ND	ND
6	2.73 ± 0.21 (3)	2.19 (2)	ND
7	3.31 (1)	ND	ND
8	3.65 (1)	ND	ND
9	13.04 (2)	21.92 (2)	ND
10	3.38 (1)	ND	ND
11	7.41 (1)	6.45 (2)	ND
12	2.64 (1)	2.56 ± 0.61 (3)	ND
13	5.48 ± 0.71 (3)	6.08 ± 0.14 (3)	ND
14	4.16 ± 0.63 (3)	4.33 ± 0.23 (3)	ND
15	7.23 ± 1.28 (3)	6.24 ± 0.22 (3)	ND
16	ND	>100 (3)	ND
17	3.97 ± 0.73 (3)	4.02 (2)	3.44 ± 0.40 (4)
18	4.15 (1)	5.16 (1)	ND
19	4.70 (1)	ND	ND
20	7.50 (1)	ND	ND
21	3.17 ± 0.25 (3)	3.39 (2)	2.71 ± 1.08 (4)
22	4.56 (2)	7.02 (2)	5.73 ± 1.32 (4)

[a] Trypan blue dye exclusion 48-h incubation, triplicate determinations. [b] [^{3}H]Thymidine incorporation 48-h incubation, 24-h pulse, quadruplicate determinations. [c] [^{3}H]Thymidine incorporation 48-h incubation, 8-h pulse, quadruplicate determinations. [d] SD = standard deviation; numbers in parentheses = number of experiments; all values are reported as μM. [e] Previously reported in ref 10. [f] ND = not determined. [g] Previously reported in refs 6, 9, and 12.

Table II. ID_{50} Values (μM) for Type B Non-Phosphorus-Containing Ether Lipids

compd	TB[a] ID_{50} ± SD[d]	TdR[b] ID_{50} ± SD[d]	TdR8[c] ID_{50} ± SD[d]
	Standard Compound		
ET-18-OMe	2.48 ± 0.31 (33)	2.38 ± 0.40 (21)	2.41 ± 0.62 (16)
	Previously Reported Compounds[b]		
23[e]	3.86 ± 0.46 (4)	4.34 ± 0.55 (5)	3.37 ± 0.50 (3)
24[e]	2.93 ± 0.34 (9)	3.51 ± 0.37 (6)	2.46 ± 0.78 (7)
	New Compounds		
25	2.23 ± 0.91 (3)	2.40 (2)	1.85 ± 0.39 (4)
26	2.49 ± 0.29 (3)	2.01 ± 0.18 (3)	2.03 ± 0.85 (5)
27	21.15 ± 5.40 (3)	ND[f]	27.03 ± 12.2 (3)
28	1.83 ± 0.08 (3)	1.98 (2)	1.21 ± 0.31 (4)
29	1.07 ± 0.30 (4)	1.68 (2)	0.85 ± 0.26 (4)
30	2.92 (1)	3.92 (2)	ND
31	ND	4.30 (2)	ND
32	ND	3.52 (1)	ND
33	ND	3.46 (1)	ND
34	3.62 ± 0.04 (3)	3.05 ± 0.83 (5)	1.90 ± 0.94 (6)
35	ND	7.15 (1)	ND
36	8.75 (1)	7.17 (2)	ND
37	ND	2.28 (1)	ND
38	ND	3.95 (1)	ND
39	ND	2.26 (1)	ND
40	2.69 (1)	2.09 (1)	1.90 (1)
41	6.14 (1)	4.97 (1)	5.02 (1)
42	ND	3.77 (1)	ND

[a–d] See Table I. [e] Previously reported in ref 11. [f] ND = not determined.

Modifications at C-1 gave the following results. Placement of a ketone group in the middle of a 1-(alkyloxy) chain (phosphocholine **9**) abolished activity (inactivity is defined in these assays as $ID_{50} > 10\ \mu M$). A *cis*-9,10 double bond decreased activity 2–3-fold; compare unsaturated thiophosphocholine **5** to its saturated analogue, **2**. Cytotoxicity as measured by the TB assay was reduced slightly by addition of a C-1 β methoxy group in a thiophosphocholine; compare **4** to **2**, or by methylation of the nitrogen in a 1-(alkylamido) phosphocholine; compare **17** (ID_{50} = 4.0 μM, TB, Table I) with its secondary amide analogue (ID_{50} = 2.4 μM, TB, ref 10).

In the propylamido phosphocholine series, increasing the C-1 chain length from C-18 (**1**) to C-20 (**21**) caused little difference in the TdR assay and decreased activity in the TB assay. In the type B non-phosphorus ether lipids, **27** with a short octyloxy C-1 chain was inactive; however, **39** with a dodecylthio C-1 chain showed activity comparable to the standard, ET-18-OMe. Short C-1 alkyl chains in phosphocholines also lead to inactive compounds.[27]

Fairly extensive changes at C-2 caused these results. Complete omission of C-2 decreased activity approximately 2-fold. In the thiophosphocholines, compare **18** (2C backbone) with **2** (3C), and in the amidophosphocholines, compare **22** (2C) with **1** (3C).

Substitution of a hydrogen (**13**) for the ethoxy group (**1**) at C-2 of a 1-(alkylamido)propyl phosphocholine substantially reduced activity. Noseda *et al.*[6] also observed this decreased activity on comparison of ET-18-OMe with ET-18-H.

Formation of a quaternary carbon at C-2 as in phosphocholines **3** (ethoxy and methyl substituents) and **10** (two methoxy groups) slightly decreased activity relative to ET-18-OMe. [3-(Octadecylthio)-2-ethoxypropyl]phosphocholine, a tertiary C-2 derivative of **3**, is also more active (ID_{50} = 1.3 μM, TB, ref 6) than **3** (ID_{50} = 3.8 μM, TB, Table I).

The sterically constrained dioxolane ring compound, **11**, was 2–3-fold less active than ET-18-OMe in both the TB and TdR assays. Rotationally restricted type B non-phosphorus-containing quaternary ammonium ether lipids are also less active than their open 3-carbon analogues.[2a]

In the thiophosphocholines, an increase in the C-2 alkoxy group from ethoxy (**2**) to propoxy (**6**) caused no difference in activity, while increasing the alkoxy group to 14 carbons in amidophosphocholine **16** abolished antineoplastic activity. Two type B non-phosphorus compounds (**32** and **33**) with a methoxymethyl at C-2 showed similar activity in the TdR assay to compounds **23** and **24** with methoxy groups. However, an inverse choline with a six-carbon alkoxy group at C-2 (**35**) showed 2-fold less activity in the TdR assay than the corresponding compound (**23**) with a methoxy group. Also, a 3-fold decrease in activity was found in the TB assay for compound **41** (ID_{50} = 6.1 μM, C-2 pentoxy) compared with its C-2 methoxy analog reported in ref 11 (ID_{50} = 2.2 μM).

Structural modifications were also performed at C-3. A type B compound, **25**, with a thioether spacer between the backbone and the quaternary ammonium group, had the same activity (ID_{50} = 2.2 μM, TB, Table II) as its previously reported oxyether analogue (ID_{50} = 1.8 μM, TB, ref 11). Type B compound **26** has a positively charged sulfur rather than a positively charged nitrogen. This sulfonium analogue was as active as the standard glycerophosphocholine, ET-18-OMe, and more active than a similar ammonium compound, **23**.

In type A phospholipids, larger ammonium groups (compare **7**, triethyl, with **2**, trimethyl, or **8**, dimethylbenzyl, with ET-18-OMe, trimethyl) decreased activity slightly. Decreased activity relative to ET-18-OMe was also found with phospholipids **19** and **20**, which contain only a single isopropyl or *tert*-butyl group rather than three methyls on the nitrogen.

In many type A and type B compounds, activity was not affected by addition of a hydroxyl or hydroxyalkyl group to the quaternary nitrogen substituent, compare **15** with **13**, or **28** (ID_{50} = 1.8 μM, TB, Table II, TB) with its trimethyl analog (ID_{50} = 2.3 μM, TB, ref 11), or **29** (ID_{50} = 1.1 μM, TB, Table II) with its nonsubstituted pyridine analog (ID_{50} = 1.0 μM, TB, ref 11). Similarly, an inverse choline with two hydroxy groups (**34**) had similar activity to those with one hydroxy group (e.g., **23** and **24**).

The substitution of pyridinium for an acyclic trialkylammonium, in general, increased activity. In amidopropyl phospholipids, the order of activity with different charged nitrogen groups was **14** (pyridine) > **13** (trimethylamine) = **15** (dimethyl(3-hydroxypropyl)amine). However, glycerol pyridinoethyl phosphate **12** was equipotent to glycerophosphocholine ET-18-OMe. In the type B series, a pyridinium compound (**37**) was also more active than its inverse choline analog (**38**). Interestingly, in a series of alkyl ethylene phospholipids, Honma *et al.*[28] found that a pyridinoethyl group caused greater induction of differentiation in HL-60 cells than did a choline group.

In type B lipids, different charged nitrogen moieties showed the general order of activity: **29** (3-(hydroxymethyl)pyridine) > ET-18-OMe = **40** (*N*-methylpyrrole) > **42** (3-hydroxy-*N*-methylpyrrole) = **30** (*N*-methylmorpholine) = **31** (4-hydroxy-*N*-methylpiperidine) = **23**, **24** (inverse cholines) ≫ **36** (thiazole).

Conclusions

In conclusion, our present data support the current generalization that straight long-chain alkyl groups at the first heterogroup of the C-1 position and short-chain alkyl groups at the heteroatom of the C-2 position of an ether lipid molecule lead to optimal antineoplastic activity. This is supported by the following results. Compounds with only eight carbons at C-1 or with a ketone in the middle of the C-1 chain were not active, and unsaturation in the C-1 chain decreased activity. A three-carbon backbone with a single substituent at the C-2 position was preferred over an ethyl or a 2,2-disubstituted propyl backbone. However, increasing the size of the C-2 alkoxy substituent to five or six carbons decreased activity and to 14 carbons abolished activity. Also, imposing the steric constraint of a ring system between C-1 and C-2 ethers decreased antineoplastic inhibition. This study also found that many different ammonium groups can be present in active phosphorus or non-phosphorus lipids without changing the degree of activity. However, the activity of phospholipids was decreased slightly by large nitrogen substituents and more significantly by nonquaternary amines. Also, pyridinium or sulfonium moieties can lead to enhanced activity. Work will be continued with both phosphorus and non-phosphorus ether lipids to confirm and extend these structure–activity studies.

Experimental Section

Growth Inhibition of HL-60 Cells. HL-60 human promyelocytic leukemia cells were maintained as suspension cultures

in RPMI 1640 medium supplemented with 10% fetal bovine serum (heat inactivated), 100 units/mL penicillin, 100 μg/mL streptomycin, 2 mM L-glutamine, and 0.22% $NaHCO_3$. The cells were subcultured at 2–3-day intervals and maintained in a humidified atmosphere of 5% CO_2 at 37 °C. 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 or 10 (for less active compounds) 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. For the trypan blue exclusion assay (cytotoxicity assay), trypan blue dye (1.0 mL) in PBS was added after 48 h, and viable cell numbers (cells retaining a cell membrane permeability barrier against dye intrusion) were determined by hemocytometer counting. The combined cytotoxic/cytostatic activities of the compounds were assessed in the leukemic cells by inhibition of tritiated thymidine incorporation. Cells in log phase growth were aliquoted 5×10^4/well in 96-well microtiter plates, and compound dilutions and controls were added. After 24 h, the cells were pulsed with 1.0 μCi [*methyl*-^{3}H]-thymidine (6.7 Ci/mmol) and incubated for an additional 24 h. (In the TdR8 assay, the cells were pulsed for the last 8 h of a 48-h time period). The cells were then harvested onto glass fiber filters (MASH II, Whittaker M.A. Bioproducts) and dried overnight. The filter disks were counted by liquid scintillation and incorporation is expressed as a percent of controls. The antiproliferative, cytotoxic action of the inhibitors was measured as a decrease of [^{3}H]thymidine incorporation into the cells.

Synthetic Methods. All chemicals were used as provided by the supplier without further purification unless otherwise indicated. Column chromatography was performed with silica gel 60 (230–400 or 70–230 mesh). The final compounds were, in general, very hygroscopic solids, and no melting points were taken; when reported, all melting points were obtained on a Hoover Meltemp apparatus and are uncorrected. Proton nuclear magnetic resonance spectra were obtained on either a Varian 80-MHz, a Bruker 300-MHz, or a Varian 400-MHz spectrometer as solutions in $CDCl_3$ with Me_4Si as an internal standard. Elemental analyses of final products were performed by Atlantic Microlabs, Inc. FAB mass spectra were run on a VG 70S mass spectrometer. References 2a and 9 provide synthetic details for the preparation of dialkylglycerols, thioglycerols, and halides. Thin-layer chromatography of compounds 3–17 in $CHCl_3$–MeOH–NH_4OH (70:35:7) or $CHCl_3$–MeOH (2:1) gave a single spot, which gave a blue color with a modified Dittmer–Lester reagent.[29] Compounds 25–42 gave a single spot in $CHCl_3$–MeOH (4:1).

[3-(Octadecylthio)-2-ethoxy-2-methylpropyl]-1-phosphocholine (3). DMSO (3.3 mL) and dicyclohexylcarbodiimide (14.7 g) were dissolved in 300 mL of anhydrous Et_2O. After the mixture was stirred for 30 min, 3-(octadecylthio)-1-(trityloxy)-2-propanol (10 g, 0.017 mol) and pyridine (0.7 mL) in 80 mL of Et_2O were added dropwise. The reaction mixture was cooled to 4 °C for 30 min and then warmed to room temperature. Trifluoroacetic anhydride (0.7 mL) was added, and stirring was continued overnight, resulting in the formation of a white precipitate. Oxalic acid (4.2 g) in 70 mL of MeOH was then added. After 30 min, the dicyclohexylurea was filtered, and the filtrate was washed with saturated $NaHCO_3$ and H_2O. The organic solution was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was taken up in 150 mL of acetone, refiltered, and then cooled to −20 °C. The precipitate was then recrystallized again from hexanes, giving 5.9 g of a white solid, melting at 51–53 °C (9.8 mmol, 59% yield). IR: 3060, 2910, 2850, 1700, 1440, 1090, 700 nm. ^{1}H NMR ($CDCl_3$): 0.87 (t, 3H, terminal CH_3), 1.0–1.7 {m, 32H, $(CH_2)_{16}$}, 2.5 (t, 2H, SCH_2CH_2), 3.35 (s, 2H, $COCH_2S$), 3.95 (s, 2H, CH_2-OTr), 7.1–7.5 (m, 15H, OTr).

The above ketone (3 g, 5 mmol) was reacted with MeMgI (prepared from 500 mg of Mg turnings and 1.3 mL of MeI) in 50 mL of Et_2O. The reaction was refluxed for 8 h, cooled, and hydrolyzed with saturated NH_4Cl. The organic solution was washed with H_2O, dried over Na_2SO_4, and concentrated *in vacuo*. Column chromatography on silica gel using hexanes–EtOAc (100:0 to 95:5) as eluant gave 2 g (3 mmol, 60% yield) of pure product and 1 g of impure alcohol. IR: 3500, 3060, 2910, 2850, 1440, 1070, 695 nm. ^{1}H NMR ($CDCl_3$): 0.87 (t, 3H, terminal CH_3), 1.0–1.7 {m, 35H, $(CH_2)_{16}$, $HOCCH_3$}, 2.5 (t, 2H, SCH_2CH_2), 2.75 (s, 2H, CH_2S), 3.1 (s, 2H, CH_2OTr), 7.1–7.5 (m, 15H, OTr).

The pure alcohol in THF (40 mL) was added dropwise to NaH (200 mg, 80% oil dispersion) in THF (3 mL). The mixture was refluxed for 1 h and then cooled to room temperature. EtI (0.2 mL) was added, and the reaction mixture was refluxed for 2 h and stirred overnight at room temperature. At this time, the reaction was incomplete by TLC, and additional aliquots of NaH and EtI were added. After another 16-h reflux, the mixture was cooled, and 40 mL of Et_2O followed by 40 mL of H_2O was added. The organic layer was separated, washed again with H_2O, dried over Na_2SO_4, and concentrated *in vacuo*. The crude product was dissolved in 6 mL of MeOH and 50 mL of Et_2O, and *p*-toluenesulfonic acid (200 mg) was added. After 24 h, the solution was washed with saturated $NaHCO_3$ and H_2O, dried over Na_2SO_4, and evaporated. The residue was combined with the material from reaction of the impure tertiary alcohol and chromatographed on silica gel with 95:5 hexanes–EtOAc to give 1 g of primary alcohol (2.5 mmol, 50% overall yield from ketone). ^{1}H NMR ($CDCl_3$): 0.87 (t, 3H, terminal CH_3), 1.0–1.7 {m, 38H, $(CH_2)_{16}$, $CH_3CH_2OCCH_3$}, 2.5 (t, 2H, SCH_2CH_2), 2.6 (s, 2H, CH_2S), 3.2–3.6 (overlapping peaks, 4H, CH_3CH_2O, CH_2OH).

The primary alcohol (0.5 g, 1.2 mmol) and Et_3N (0.23 mL) in 13 mL of alcohol-free $CHCl_3$ were added dropwise to $POCl_3$ (0.15 mL) at 0 °C. The reaction mixture was refluxed for 1 h and cooled to room temperature, and 1 mL of pyridine and 0.93 g of solid choline tosylate were added. After stirring overnight, the organic solution was washed with 3% Na_2CO_3, 5% HCl, and H_2O using MeOH to break the emulsions that formed. The solution was dried over Na_2SO_4, and the solvent was then removed *in vacuo*. The crude product was precipitated from $CHCl_3$–acetone (3:50) and then chromatographed on silica gel using $CHCl_3$–MeOH–NH_4OH (70:35:1 to 70:35:7). Pure product (140 mg, 0.25 mmol, 20% yield) was obtained as a hygroscopic solid. ^{1}H NMR ($CDCl_3$): 0.87 (t, 3H, terminal CH_3), 1.15 (t, 3H, CH_3CH_2O), 1.2–1.4 {m, 33H, $(CH_2)_{15}$, $CH_3CH_2OCCH_3$}, 1.55 (m, 2H, SCH_2CH_2), 2.52 (t, 2H, SCH_2CH_2), 2.72 (s, 2H, CH_2S), 3.41 {s, 9H, $N(CH_3)_3$}, 3.45 (q, 2H, CH_3CH_2O), 3.85 (m, 4H, CH_2OP, CH_2N), 4.32 (m, 2H, $POCH_2$). Anal. ($C_{29}H_{62}NO_5SP \cdot H_2O$) C, H, N, S.

3-((2′-Methoxyhexadecyl)thio)-2-(ethoxypropyl)-1-phosphocholine (4). 1,2-Epoxyhexadecane (12 g, 0.05 mol) was added to 3-mercapto-1,2-propanediol (5.5 g, 0.05 mol) and KOH (2.8 g, 0.05 mol) in 100 mL of 95% EtOH. After 24 h at room temperature, water (500 mL) was added, and the precipitate was filtered. Recrystallization from MeOH and 2-propanol gave 10.5 g (0.030 mol, 60% yield) of a white solid (mp 82–84 °C). ^{1}H NMR ($CDCl_3$): 0.87 (t, 3H, terminal CH_3), 1.2–1.6 {m, 26H, $(CH_2)_{13}$}, 2.45–2.85 (m, 4H, CH_2SCH_2), 3.5–3.9 (m, 4H, CH_2OH, $CHOH$, $CHOH$).

Reference 2a gives details for the ketalization with acetone and concentrated H_2SO_4. The side-chain hydroxyl was methylated with MeI and NaH as given above.

This ketal was dissolved in 30 mL of MeOH and 4 mL of 10% HCl, refluxed for 3 h, and stirred at room temperature overnight. Et_2O (100 mL) was added, and the mixture was washed with saturated $NaHCO_3$ and H_2O, which were reextracted with 75 mL of Et_2O. The aqueous phase was reduced in volume *in vacuo* and extracted again with 25 mL of Et_2O. The ether extracts were combined and dried over Na_2SO_4, and the solvent was removed. The diol was obtained in a 40% yield in three steps from the triol (4.4 g, 0.012 mol). IR: 3400, 2910, 2850, 1460, 1100 nm. ^{1}H NMR ($CDCl_3$): 0.87 (t, 3H, terminal CH_3), 1.2–1.6 {m, 26H, $(CH_2)_{13}$}, 2.45–2.85 (m, 4H, CH_2SCH_2), 3.4 (s, 3H, OCH_3), 3.5–3.9 (m, 4H, CH_2OH, $CHOH$, $CHOCH_3$).

Tritylation with triphenylmethyl chloride,[9] ethylation with EtI and NaH, detritylation with BF_3–MeOH,[9] and phosphorylation with $POCl_3$ and choline tosylate follow standard procedures given in ref 9 and as above for 3. ^{1}H NMR ($CDCl_3$): 0.87 (t, 3H, terminal CH_3), 1.15 (t, 3H, CH_3CH_2O), 1.2–1.4 {m, 24H, $(CH_2)_{12}$}, 1.55 (m, 2H, SCH_2CHCH_2), 2.6–2.85 (m, 4H, CH_2SCH_2), 3.3 (s and m, 4H, $CHOCH_3$), 3.4 {s, 9H, $N(CH_3)_3$}, 3.6 (m, 3H, CH_3CH_2-

OC*H*), 3.85 (m, 2H, CH_2N), 3.95 (m, 2H, CH_2OP), 4.32 (m, 2H, $POCH_2$). FAB MS: *m/e* 556 (MH^+). Anal. ($C_{27}H_{58}NO_6SP\cdot 2H_2O$) C, H, N, S.

Phospholipids **5**, **6**, and **7** were prepared from 3-mercapto-1,2-propanediol by the sequence shown in Scheme I using the appropriate reagents. The procedures were as given in refs 2a and 9 and as above. ^1H NMR and elemental analyses are supplied in the supplementary material.

3-(Octadecyloxy)-2-(ethoxypropyl)-1-phospho-*N,N*-dimethyl-*N*-benzylethanolamine (8). N,N-Dimethyl-*N*-benzylethanolamine bromide was prepared by reaction of benzyl bromide (0.6 g, 0.003 mol) and *N,N*-dimethylethanolamine in 15 mL of Et_2O at room temperature for 16 h. The resulting precipitate (1 g, mp 126–129 °C) was filtered. ^1H NMR ($CDCl_3$): 3.3 (s, 6H, NMe_2), 3.8 (m, 2H, CH_2OH), 4.3 (m, 2H, CH_2NBz), 4.9 (s, 2H, $CH_2C_6H_5$), 7.45 and 7.6 (m, 3H and 2H, aromatic H). 3-(Octadecyloxy)-2-ethoxy-1-propanol (0.74 g, 0.002 mol) was reacted with $POCl_3$ (0.5 mL) and *N,N*-dimethyl-*N*-benzylethanolamine bromide (0.9 g) in an analogous manner to the reaction of 3-(octadecylthio)-2-ethoxy-2-methyl-1-propanol with $POCl_3$ and choline tosylate as described above. Crude product was precipitated from $CHCl_3$–acetone (10:1), chromatographed with $CHCl_3$–MeOH–NH_4OH (70:35:1 to 70:35:7), and then reprecipitated and rechromatographed with $CHCl_3$–MeOH (3:2). Pure product (100 mg, 0.1 mmol) was obtained in an 8% yield as a hygroscopic solid. ^1H NMR ($CDCl_3$): 0.85 (t, 3H, terminal CH_3), 1.1 (t, 3H, CH_3CH_2O), 1.26 {m, 30H, $(CH_2)_{15}$}, 1.55 (m, 2H, OCH_2CH_2), 3.25 {s, 6H, $N(CH_3)_2$}, 3.3–3.5 (m, 5H, CH_2OCH_2, OCH), 3.55 (q, 2H, CH_3CH_2O), 3.8–3.9 (m, 4H, CH_2N, CH_2OP), 4.45 (m, 2H, $POCH_2$), 4.8 (broad s, 2H, NCH_2Bz), 7.3–7.7 (m, 5H, C_6H_5). Anal. ($C_{34}H_{64}NO_6P\cdot 0.5H_2O$) C, H, N.

[3-[(10′-Oxohexadecyl)oxy]-2-ethoxypropyl]-1-phosphocholine (9). Sebacic acid monomethyl ester was prepared from sebacic acid and MeOH as described in ref 20. The acid chloride was formed with $SOCl_2$ and added to dihexylcadmium as detailed in ref 19. The resulting 10-oxohexadecanoic acid was protected as the ethylene ketal, reduced with LAH, and mesylated with methanesulfonyl chloride (deprotection of the ketal occurred during acid workup) using standard literature procedures. This mesylate was reacted with 3-(trityloxy)-2-ethoxy-1-propanol and NaH. The detritylation and formation of the phosphocholine followed the reactions described for **3**. ^1H NMR ($CDCl_3$): 0.85 (t, 3H, terminal CH_3), 1.1 (t, 3H, CH_3CH_2O), 1.26 {m, 16H, $(CH_2)_3$ and $(CH_2)_5$}, 1.55 (m, 6H, OCH_2CH_2, $CH_2CH_2COCH_2CH_2$), 2.35 (m, 4H, CH_2COCH_2), 3.34 {s, 9H, $N(CH_3)_3$}, 3.3–3.5 (m, 5H, CH_2OCH_2, OCH), 3.56 (q, 2H, CH_3CH_2O), 3.75–3.9 (m, 4H, CH_2N, CH_2OP), 4.27 (m, 2H, $POCH_2$). Anal. ($C_{26}H_{54}NO_7P\cdot H_2O$) C, H, N.

[3-(Hexadecyloxy)-2,2-dimethoxypropyl]-1-phosphocholine (10). 3-(Hexadecyloxy)-2,2-dimethoxy-1-propanol (2.2 g, 6.0 mmol, prepared as detailed in ref 16) and Et_3N (0.7 mL, 0.007 mol) were dissolved in 125 mL of benzene and cooled to 0 °C. A solution of 2-chloro-2-oxo-1,3,2-dioxaphospholane (1 g, 0.007 mol) in benzene was added dropwise. After stirring overnight, the mixture was filtered and then concentrated *in vacuo*. The residue was dissolved in 75 mL of CH_3CN, placed in a glass bomb, and cooled to −78 °C. Condensed Me_3N (2 g) was then added. After removal of the cooling bath, the solution was refluxed for 24 h. The phosphocholine precipitated on cooling and was removed by filtration. Pure product (2.2 g, 4.2 mmol, 70% yield) was obtained after two precipitations from acetone and one time from Et_2O. ^1H NMR ($CDCl_3$): 0.85 (t, 3H, terminal CH_3), 1.2 {m, 26H, $(CH_2)_{13}$}, 1.55 (m, 2H, OCH_2CH_2), 3.2 (s, 6H, OCH_3), 3.3 {s, 9H, $N(CH_3)_3$}, 3.3–3.5 (m, 4H, CH_2OCH_2), 3.8–3.9 (m, 4H, CH_2N, CH_2OP), 4.2 (m, 2H, $POCH_2$). Anal. ($C_{28}H_{56}NO_7P\cdot H_2O$) C, H, N.

[(2-Heptadecyl-1,3-dioxol-4-yl)methyl]phosphocholine (11). 2-Heptadecyl-4-(hydroxymethyl)-1,3-dioxolane was synthesized according to the method of Piantadosi et al.[22] The phosphocholine was formed with 2-chloro-2-oxo-1,3,2-dioxaphospholane and Me_3N as described for **10**. ^1H NMR ($CDCl_3$): 0.85 (t, 3H, terminal CH_3), 1.2–1.6 {m, 32H, $(CH_2)_{16}$}, 3.3 {s, 9H, $N(CH_3)_3$}, 3.7–4.4 (overlapping m, 9H, $CH_2CHCH_2OPO_3CH_2CH_2N$), 4.7 and 4.9 (2 t, total 1H, OCHO). Anal. ($C_{28}H_{54}NO_6P\cdot 2.5H_2O$) C, H, N.

2′-Pyridylethyl 3-(Hexadecyloxy)-2-ethoxypropyl Phosphate (12). This compound was prepared by reaction of 3-(hexadecyloxy)-2-ethoxy-1-propanol with 2-bromoethyl dichlorophosphate and pyridine as given below for **13**. ^1H NMR ($CDCl_3$): 0.87 (t, 3H, terminal CH_3), 1.13 (t, 3H, OCH_2CH_3), 1.25 {m, 26H, $(CH_2)_{13}$}, 1.52 (m, 2H, OCH_2CH_2), 3.4 (m, 2H, OCH_2CH_2), 3.4–3.55 (m, 2H, $CH_2OCH_2CH_2$), 3.6 (m, 3H, $CHOCH_2CH_3$), 3.8 (m, 2H, CH_2OP), 4.3 (m, 2H, $POCH_2$), 5.0 (m, 2H, $CH_2NC_5H_5$), 8.05 (t, 2H, 3′ aromatic H), 8.45 (t, 1H, 4′ aromatic H), 9.25 (d, 2H, 2′ aromatic H). Anal. ($C_{28}H_{52}NOP\cdot H_2O$) C, H, N.

2′-Bromoethyl 3-Octadecanamidopropyl Phosphate. Amidophosphocholines **13**–**15** were prepared from a common precursor. First, 3-amino-1-propanol (10 g, 0.133 mol) was dissolved in pyridine (50 mL) and DMF (150 mL). Stearoyl chloride (30 g, 0.10 mol) in 100 mL of DMF was added dropwise. After 24 h at room temperature, the product was removed by filtration, washed with H_2O, and recrystallized from MeOH and from $CHCl_3$. The amide was obtained in a 49% yield (16.6 g, 0.049 mol, mp 96–97.5 °C). ^1H NMR ($CDCl_3$): 0.85 (t, 3H, terminal CH_3), 1.25 (m, 28H, $(CH_2)_{14}$}, 1.6 (m, 4H, $NHCOCH_2CH_2$, $HNCH_2CH_2CH_2OH$), 2.2 (t, 2H, $NHCOCH_2$), 3.45 (m, 2H, CH_2OH), 3.5 (m, 2H, CH_2NH), 5.8 (m, 1H, NH).

2-Bromoethyl dichlorophosphate (2.3 mL, 3.5 g, 0.015 mol) was dissolved in 100 mL of Et_2O and cooled to 0 °C. Pyridine (6 mL) was added followed by the above amido alcohol (1.7 g, 0.005 mol) in 125 mL of anhydrous THF. The mixture was refluxed for 3 h and cooled, and H_2O (7 mL) was added. After stirring overnight, the solvent was removed *in vacuo*, and the residue was dissolved in 100 mL of $CHCl_3$–MeOH (4:1). The solution was extracted with H_2O and backextracted with 2 × 50 mL of 4:1 $CHCl_3$–MeOH. The combined organic fractions were dried over Na_2SO_4, evaporated, and chromatographed on silica gel using 9:1 $CHCl_3$–MeOH. The desired bromoethyl phosphate (1.6 g, 3.0 mmol) was obtained in a 20% yield. ^1H NMR ($CDCl_3$): 0.85 (t, 3H, terminal CH_3), 1.25 (m, 28H, $(CH_2)_{14}$}, 1.55 (m, 2H, $NHCOCH_2CH_2$), 1.85 (m, 2H, $NCH_2CH_2CH_2OH$), 2.2 (t, 2H, $NHCOCH_2$), 3.35 and 3.5 (2 m, 4H, CH_2NHCO, CH_2Br), 4.0–4.2 (m, 4H, $CH_2OPO_3CH_2$), 6.8 (m, 1H, NH).

This intermediate was reacted with aqueous Me_3N in $CHCl_3$–2-propanol–DMF (5:3:5) at 65 °C for 4 h (compound **13**, 41% yield), with pyridine (neat) at reflux (compound **14**, 12% yield), and with *N,N*-dimethyl-3-aminopropanol (neat) at 65 °C for 4 days (compound **15**, 6% yield). After removal of solvent *in vacuo*, purification was performed by precipitation from $CHCl_3$–acetone (10:1) and chromatography on silica gel using $CHCl_3$–MeOH–NH_4OH (70:35:1 to 70:35:7). ^1H NMR and elemental analyses for each compound are given below.

(3-Octadecanamidopropyl)-1-phosphocholine (13). ^1H NMR ($CDCl_3$): 0.85 (t, 3H, terminal CH_3), 1.25 {m, 28H, $(CH_2)_{14}$}, 1.55 and 1.7 (2 m, 4H, $NHCOCH_2CH_2$, $NCH_2CH_2CH_2OP$), 2.2 (t, 2H, $NHCOCH_2$), 3.3 {s and m, 11H, $N(CH_3)_3$, CH_2NHCO}, 3.8–3.9 (m, 4H, CH_2OP, CH_2NMe_3), 4.2 (m, 2H, $POCH_2$), 7.5 (m, 1H, NH). Anal. ($C_{26}H_{55}N_2O_5P\cdot 1.5H_2O$) C, H, N.

2′-Pyridylethyl 3-Octadecanamidopropyl Phosphate (14). ^1H NMR ($CDCl_3$): 0.85 (t, 3H, terminal CH_3), 1.25 {m, 28H, $(CH_2)_{14}$}, 1.55 and 1.65 (m, 4H, $NHCOCH_2CH_2$, $NCH_2CH_2CH_2OP$), 2.15 (t, 2H, $NHCOCH_2$), 3.2 (m, 2H, CH_2NHCO), 3.8 (m, 2H, CH_2OP), 4.4 (m, 2H, $POCH_2$), 5.1 (m, 2H, $CH_2NC_5H_5$), 7.35 (t, 1H, NH), 8.05 (t, 2H, 3′ aromatic H), 8.45 (t, 1H, 4′ aromatic H), 9.25 (d, 2H, 2′ aromatic H). Anal. ($C_{28}H_{51}N_2O_5P\cdot 2.25H_2O$) C, H, N.

3-(Octadecanamidopropyl)-1-phospho-*N,N*-dimethyl-*N*-(3′-hydroxypropyl)propanolamine (15). ^1H NMR ($CDCl_3$): 0.85 (t, 3H, terminal CH_3), 1.25 {m, 28H, $(CH_2)_{14}$}, 1.55 (m, 2H, $NHCOCH_2CH_2$), 1.8 and 1.95 (2 m, 4H, $HNCH_2CH_2CH_2OP$, $NCH_2CH_2CH_2OH$), 2.2 (t, 2H, $NHCOCH_2$), 2.45 (t, 1H, OH), 3.3 (s, overlapping m, 8H, $N(CH_3)_2$, CH_2NH}, 3.65 (m, 4H, $NCH_2CH_2CH_2OH$), 3.8–4.0 (m, 4H, $CH_2OPOCH_2CH_2N$), 4.4 (m, 2H, $POCH_2$), 7.6 (m, 1H, NH). Anal. ($C_{28}H_{59}N_2O_6P\cdot 1.75H_2O$) C, H, N.

[3-Octadecanamido-2-(tetradecyloxy)propyl]-1-phosphocholine (16). 3-Octadecanamido-1-(trityloxy)-2-propanol, synthesized as described in ref 10, was alkylated with tetradecyl bromide and detritylated with *p*-toluenesulfonic acid as described for **3**. The phosphocholine was prepared by reaction with 2-bromoethyl dichlorophosphate and Me_3N as described for **13** and in ref 5a. ^1H NMR ($CDCl_3$): 0.85 (t, 6H, terminal CH_3), 1.25

{m, 50H, $(CH_2)_{14}$, $(CH_2)_{11}$}, 1.55 (m, 4H, $NHCOCH_2CH_2$, OCH_2CH_2), 2.2 (t, 2H, $NHCOCH_2$), 3.3 {s, 9H, $N(CH_3)_3$}, 3.3–3.8 (overlapping m, 5H, $NHCH_2CHOCH_2$), 3.8–3.9 (4H, CH_2OP, CH_2NMe_3), 4.2 (m, 2H, $POCH_2$), 6.8 (m, 1H, NH). FAB MS: *m/e* 719 (MH^+).

[3-(*N*-Methyloctadecanamido)-2-methoxypropyl]-1-phosphocholine (17). Preparation of 3-octadecanamido-2-methoxy-1-propanol as described in ref 10 gave as a minor side product the N,O-dimethylated compound, which could be separated by silica gel chromatography. Anal. ($C_{23}H_{47}NO_3$) C, H, N. The phosphocholine was prepared as described for **13**. ^1H NMR ($CDCl_3$): 0.85 (t, 3H, terminal CH_3), 1.25 {m, 28H, $(CH_2)_{14}$}, 1.55 (m, 2H, $NHCOCH_2CH_2$), 2.2 (t, 2H, $NHCOCH_2$), 3.05 (s, 3H, NCH_3), 3.3 {s, 12H, $N(CH_3)_3$, OCH_3}, 3.2–3.6 (overlapping m, 3H, CH_3NCH_2CH), (3.8–3.9, 4H, CH_2OP, CH_2NMe_3), 4.2 (m, 2H, $POCH_2$). Anal. ($C_{28}H_{59}N_2O_6P$) C, H, N.

3-(Hexadecyloxy)-2-methoxypropyl 2′-Hydroxyethyl Sulfide. This compound was used as a common intermediate in the preparation of compounds **25** and **26**. 3-(Hexadecyloxy)-2-methoxy-1-iodopropane (0.5 g, 1.1 mmol) was added to 2-mercaptoethanol (750 mg, 9.6 mmol) in 40 mL of MeOH containing 50 mg of KOH. After being stirred overnight at room temperature, the solution was partitioned between Et_2O and H_2O. The organic phase was dried over Na_2SO_4, and the solvent was removed *in vacuo*. Column chromatography on silica gel with hexanes–EtOAc (95:5 to 90:10) gave pure sulfide (0.3 g, 0.77 mmol, 80% yield). ^1H NMR ($CDCl_3$): 0.85 (t, 3H, terminal CH_3), 1.25 {m, 26H, $(CH_2)_{13}$}, 1.55 (m, 2H, OCH_2CH_2), 2.6–2.8 (t and dd, 4H, CH_2SCH_2), 3.4 (s, 3H, OCH_3), 3.4–3.8 (m, 7H, $CHCH_2OCH_2$, CH_2OH).

***N,N,N*-Trimethyl-*N*-[2-[[3-(Hexadecyloxy)-2-methoxypropyl]thio]-1-ethyl]ammonium Bromide (25).** The above product (1 g, 2.6 mmol) together with CBr_4 (1 g, 3.0 mmol) was dissolved in 50 mL of CH_2Cl_2, and the solution was cooled to 0 °C. Triphenylphosphine (0.8 g, 3.1 mmol) was added in portions over 30 min, and the mixture was warmed to room temperature over 1 h. After the solvent was removed *in vacuo*; Et_2O (50 mL) was added. The mixture was stirred for 1 h, filtered, and rewashed with ether. The ether extracts were concentrated and chromatographed on silica gel with hexanes–EtOAc (100:0 to 9:1). The pure bromide was dissolved in 25 mL of CH_3CN, aqueous Me_3N (40%, 10 mL) was added, and the mixture was stirred overnight at room temperature. After concentration, the residue was chromatographed on silica gel with $CHCl_3$–MeOH (9:1) to give the ammonium salt in 29% yield from the alcohol (390 mg, 0.76 mmol, decomposes >200 °C). ^1H NMR ($CDCl_3$): 0.87 (t, 3H, terminal CH_3), 1.24 {m, 26H, $(CH_2)_{13}$}, 1.55 (m, 2H, $CH_2OCH_2CH_2$), 2.7–3.05 (dd, 2H, $CHCH_2S$), 3.05 (t, 2H, SCH_2CH_2N), 3.4–3.6 (m, 5H, $CHCH_2OCH_2$), 3.45 (s, 3H, OCH_3), 3.51 {s, 9H, $N(CH_3)_3$}, 3.82 (m, 2H, CH_2N). Anal. ($C_{25}H_{54}NO_2SBr$) C, H, N, S.

***S*-Methyl-*S*-(2-hydroxyethyl)-*S*-[3-(hexadecyloxy)-2-methoxypropyl]sulfonium Tosylate (26).** 3-(Hexadecyloxy)-2-(methoxypropyl)-(2′-hydroxyethyl)sulfide (0.8 g, 2.1 mmol) and methyl tosylate (0.8 g, 4.3 mmol) were placed in 40 mL of acetone and refluxed for 8 h. An additional aliquot of methyl tosylate was added; the mixture was refluxed for 8 h and stirred for 48 h at room temperature. After concentration, the crude product was chromatographed on silica gel using $CHCl_3$–MeOH (9:1 to 4:1), giving pure sulfonium salt in a 20% yield (242 mg, 0.42 mmol) as a waxy solid. ^1H NMR ($CDCl_3$): 0.87 (t, 3H, terminal CH_3), 1.25 {m, 26H, $(CH_2)_{13}$}, 1.55 (m, 2H, $CHCH_2OCH_2CH_2$), 2.3 (s, 3H, $C_6H_4CH_3$), 3.1 (2 s, 3H, SCH_3), 3.4 (s, 3H, OCH_3), 3.5–4.1 (overlapping m, 11H, $CH_2OCH_2CHCH_2SCH_2CH_2$), 7.1 and 7.6 (2 d, 4H, C_6H_4). Anal. ($C_{30}H_{56}O_6S_2$) C, H, S.

3-(Hexadecylthio)-2-(methoxymethyl)-1-bromopropane. This compound was used in the synthesis of both analogues **32** and **33**. First, 3-(hexadecylthio)-2-(methoxymethyl)-1-propanol was prepared with slight modifications of the procedure of Bosies *et al.*[26] The bromide was then prepared with CBr_4 and triphenylphosphine as described in the synthesis of **25**. ^1H NMR ($CDCl_3$): 0.85 (t, 3H, terminal CH_3), 1.25 {m, 26H, $(CH_2)_{13}$}, 1.55 (m, 2H, SCH_2CH_2), 2.1 (m, 1H, CH), 2.53 (t, 2H, SCH_2CH_2), 2.63 (d, 2H, $CHCH_2S$), 3.35 (s, 3H, OCH_3), 3.45 and 3.65 (2 dd, 4H, CH_2O, CH_2Br).

***N,N,N*-Trimethyl-*N*-[3-(hexadecylthio)-2-(methoxymethyl)propyl]ammonium Bromide (32).** Compound **32** was prepared by heating (60–65 °C) the above bromide (1.0 g, 2.4 mmol) with 40% aqueous Me_3N (10 mL) in THF (25 mL) for 16 h. Concentration, precipitation from acetone, and chromatography with a $CHCl_3$–MeOH gradient (95:5 to 1:1) gave 411 mg of pure adduct (0.85 mmol, 35% yield, mp 157–159 °C) and 248 mg of impure material. ^1H NMR ($CDCl_3$): 0.85 (t, 3H, terminal CH_3), 1.25 {m, 26H, $(CH_2)_{13}$}, 1.55 (m, 2H, SCH_2CH_2), 2.4 (m, 1H, CH), 2.55 (t, 2H, SCH_2CH_2), 2.7 (d, 2H, $CHCH_2S$), 3.35 (s, 3H, OCH_3), 3.48 {s, 9H, $N(CH_3)_3$}, 3.4–3.7 (m, 4H, CH_2O, CH_2N). Anal. ($C_{24}H_{52}NOSBr$) C, H, N, S.

***N,N*-Dimethyl-*N*-(3-hydroxypropyl)-*N*-[3-(hexadecylthio)-2-(methoxymethyl)propyl]ammonium Bromide (33).** Compound **33** was also prepared from the above bromide (1.0 g, 2.4 mmol) by reaction with (*N,N*-dimethylamino)propanol (heat, 5 mL) at 60–65 °C for 16 h. Crude salt was precipitated from the reaction mixture with Et_2O at −20 °C. Chromatography as for **32** gave 518 mg of pure compound (1.0 mmol, 41% yield, mp 114–116 °C). ^1H NMR ($CDCl_3$): 0.85 (t, 3H, terminal CH_3), 1.25 {m, 26H, $(CH_2)_{13}$}, 1.55 (m, 2H, SCH_2CH_2), 2.1 (m, 2H, $NCH_2CH_2CH_2O$), 2.45 (m, 1H, CH), 2.6 (t, 2H, SCH_2CH_2), 2.7 (dd, 2H, $CHCH_2S$), 3.33 {s, 6H, $N(CH_3)_2$}, 3.38 (s, 3H, OCH_3), 3.4–3.65 (m, 4H, $CHCH_2O$, CH_2OH), 3.9 (m, 4H, CH_2NCH_2). Anal. ($C_{26}H_{56}NO_2SBr$) C, H, N, S.

The remaining ammonium salts (**27–31** and **34–42**) were prepared from the appropriate bromides (synthesized following the standard procedures described in refs 2a, 9, and 11) by reaction with an aliphatic or aromatic amine as described above or in refs 2a, 11, and 13. ^1H NMR and elemental analyses are supplied in the supplementary material.

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Supplementary Material Available: Chemical names, analytical data, and ^1H NMR data for compounds **5–7**, **27–31**, and **34–42** (3 pages). Ordering information is given on any current masthead page.

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