Synthesis of Alkyl Chain-Modified Ether Lipids and Evaluation of Their *in Vitro* Cytotoxicity[1]

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A series of alkyl lysophospholipid (ALP) analogs of ET-18-OCH_3 (1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine) containing modifications in the long C-1 chain has been synthesized and evaluated in human tumor cell line cytotoxicity assays. The compounds have also been evaluated in platelet activating factor (PAF) receptor agonism and hemolysis tests. Two modifications have been studied, introduction of a carbonyl group at different positions of the C-1 chain and branching of this chain, in some compounds with incorporation of a phenyl group. Several compounds showed a cytotoxic potency comparable to that of the reference compound ET-18-OCH_3, associated with reduced proaggregating and hemolytic effects. The two enantiomers of 1-*O*-(7-oxooctadecyl)-2-*O*-methyl-*rac*-glycero-3-phosphocholine (**2**) showed the same level of cytotoxicity or antiproliferative activity, with the PAF-agonistic effect confined to *R*-**2**. The very low stereoselectivity found in the *in vitro* cytotoxicity confirms earlier results and indicates a lack of stereospecific interactions with a macromolecular target.

Ether-linked analogs of the natural membrane constituent lysophosphatidylcholine (alkyl lysophospholipids, ALPs) are membrane-targeted, DNA noninteracting cytotoxic agents. The antitumoral activity of this class of compounds has been shown both in *in vitro* and *in vivo* experiments.[2] Some representative members of this family, such as ET-18-OCH_3 (edelfosine) and BM-41440 (ilmofosine), are currently under clinical evaluation.[3] A simplified analog, hexadecylphosphocholine (miltefosine), has been recently introduced as a topical treatment of skin metastases secondary to breast cancer.[4]

A large number of modifications of the ALP structure have been explored over the past 15 years. Most of the efforts were directed toward improving potency through changes in the polar head and glycerol backbone, i.e., the hydrophilic portion of the ALP molecule. Little attention has been paid, however, to the lipophilic, long alkyl chain, except for some studies directed to the optimization of its length.[5-7a] In general, a minimum of 14–16 carbon atoms is required for acceptable cytotoxic or antitumor activity. This is also true for compounds lacking the phosphodiester moiety.[8-10] The contribution of the alkyl chain seems to be mainly to the overall lipophilicity of the molecule, since compounds bearing two medium-length chains maintain the antitumor activity.[7a] Moreover, the position of the alkyl chain on the glycerol backbone is not crucial for cytotoxicity.[11]

Physicochemical perturbation of the membrane lipid bilayer may be responsible for the unspecific membranolytic effect shown by ALPs at high concentrations (detergent-like action).[12] On the other hand, alteration of the activity of membrane-associated enzymes such as protein kinase C,[13] phospholipase C,[14] phosphatidylinositol-3-kinase,[15] Na^+,K^+-ATPase,[16] or transacylases[17] has been proposed as a possible mechanism of action for ALPs. Finally, a direct agonist action of many ALPs on the platelet activating factor (PAF) receptor could give rise to undesired proaggregating and hypotensive effects, whose *in vivo* relevance is yet to be ascertained.

We describe here the synthesis and *in vitro* biological evaluation of a number of new ALP analogs, 1–**16**, in which the C-1 alkyl chain has been modified. Thus, a polar function such as a carbonyl group has been introduced at different positions, and the straight C_{18} alkyl chain has been replaced with several branched alkyl or aralkyl groups. Our aim was to ascertain if these structural modifications could maintain the cytotoxic activity while reducing the unspecific membranolytic effect, related to the intercalating ability of the molecule, and the PAF-related effect, which has been described to require the presence of a linear alkyl group.[18] Only a few precedents for these kinds of modifications were found in literature, namely the methyl-branched compound [3-[(2',2'-dimethyloctadecyl)oxy]-2-methoxypropyl]-1-phosphocholine,[19] the C-10 ketone [3-[(10'-oxohexadecyl)oxy]-2-methoxypropyl]-1-phosphocholine,[10] and a series of ALPs containing a carbonyl group in the C-2 position of the alkyl chain,[20] which includes compound **17** used here as a reference standard.

Chemistry

The compounds described here were synthesized using two different pathways which involved the use of (*R*)-(−)-, (*S*)-(+)-, or *rac*-1,2-*O*-isopropylideneglycerol (Scheme 1) and *rac*-3-*O*-benzyl-2-*O*-methylglycerol (Schemes 2, 3, and 4) as starting materials.

The syntheses of compounds **2**, **8**, and **9** are depicted in Scheme 1. Alkylation of *rac*-1,2-*O*-isopropylideneglycerol (**20**) with the appropriate dibromoalkane in DMF and sodium hydride followed by a nucleophilic substitution with sodium cyanide in DMSO gave compound **21**. After cleavage of the acetonide group with 2

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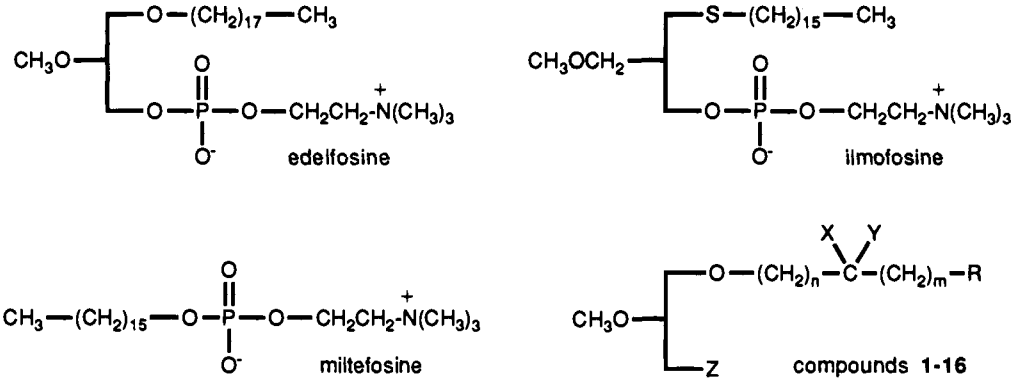
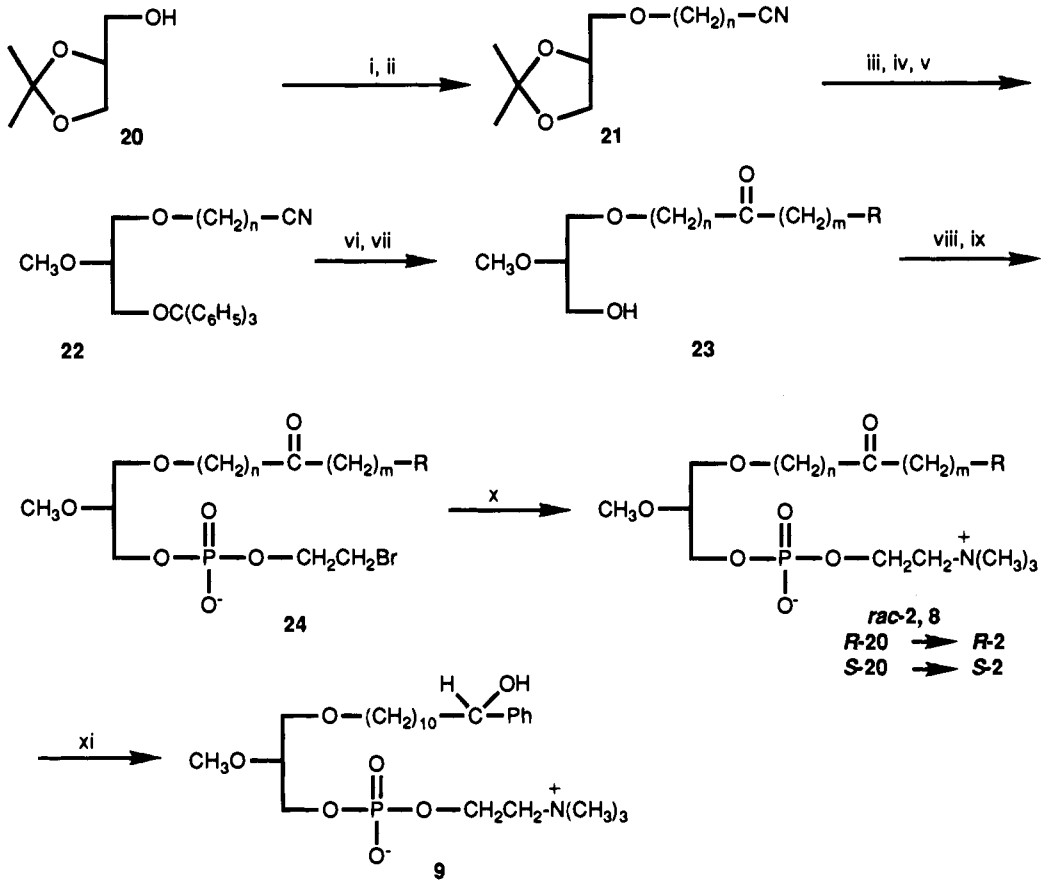
Chart 1[a]



[a] For the definitions of X, Y, Z, R, *n*, and *m*, see Table 1.

Scheme 1[a]

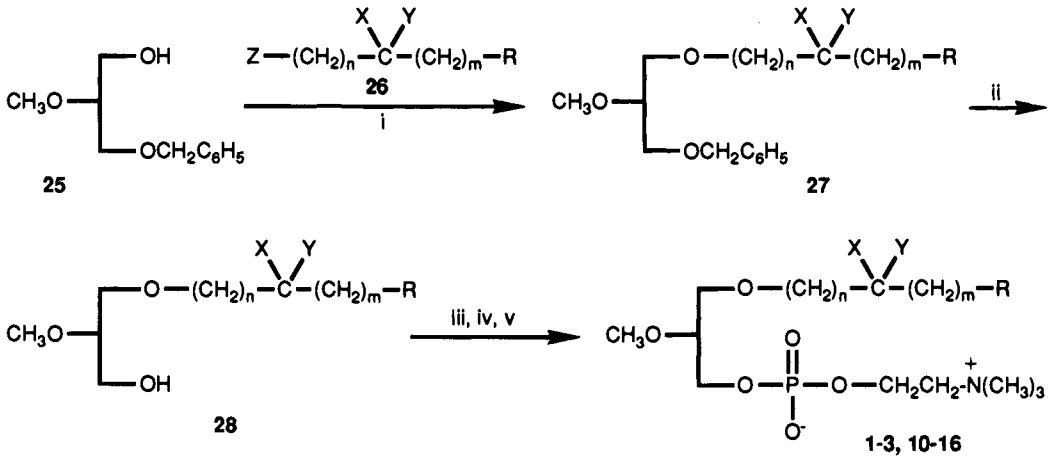


[a] (i) $Br(CH_2)_6Br$ or $Br(CH_2)_{10}Br$, NaH, DMF; (ii) NaCN, DMSO; (iii) 2 N HCl, THF; (iv) TrCl, pyridine; (v) MeI, KH, C_6H_6; (vi) $CH_3(CH_2)_{10}MgBr$ or PhMgBr, benzene; (vii) 1 N HCl, dioxane; (viii) $Cl_2P(O)OCH_2CH_2Br$, Et_3N, Et_2O; (ix) KCl, H_2O; (x) Me_3N, $CHCl_3$; (xi) H_2, $Pd(OH)_2$–C, MeOH/H_2O. Tr = trityl.

N HCl in THF, the primary hydroxyl group of the resulting diol was selectively protected with trityl chloride in pyridine and the secondary hydroxyl group was alkylated with methyl iodide and potassium hydride in benzene, to give cyanide **22**. Grignard reaction with undecylmagnesium bromide or phenylmagnesium bromide in benzene and subsequent removal of the trityl protective group with 1 N HCl in dioxane afforded alcohol **23**, which contained a ketone function in the *sn*-1 alkyl chain of glycerol. The bromoethyl intermediate **24** was obtained by reaction of alcohol **23** with 2-bromoethyl dichlorophosphate and triethylamine in diethyl ether and subsequent hydrolysis of the residual phosphochloride bond.[21] Nucleophilic displacement of bromide ion with trimethylamine in chloroform gave the phosphocholines **2** and **8**. Compound **9**, which contains a hydroxyl group, was prepared from **8** by catalytic hydrogenation of the ketone function with palladium hydroxide on charcoal.

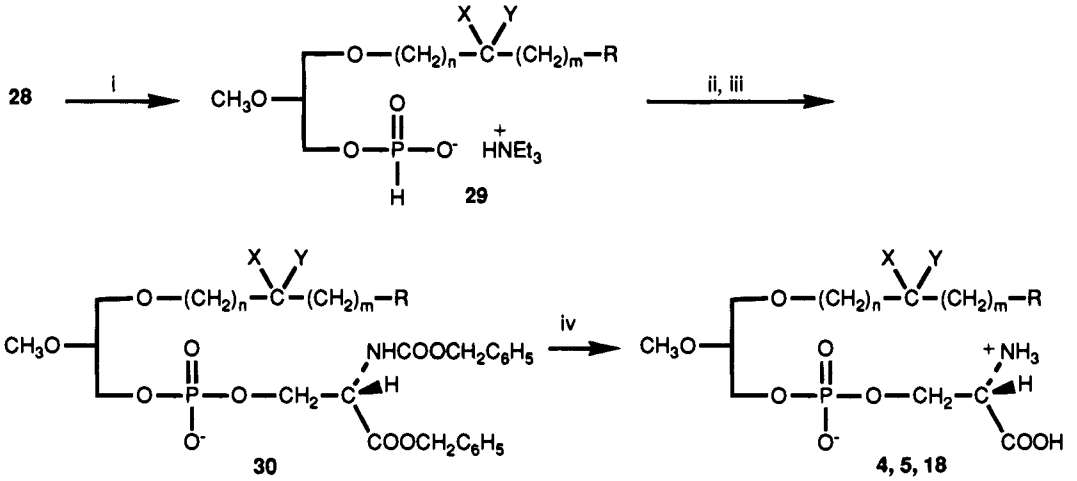
The pure enantiomers *R*-(+)-**2** and *S*-(−)-**2** were synthesized, respectively from the *R*-(−) and *S*-(+) enantiomers of isopropylideneglycerol **20**, following the same synthetic pathway described for *rac*-**2**. To evaluate their optical purity, we prepared the (+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid esters of both enantiomers of **23** and of the racemic mixture of **23** by treatment with (*R*)-(−)-MTPA chloride.[22] The diastereomeric ratio of the resulting crude mixtures was

Scheme 2[a]



[a] (i) KH, benzene; for compounds **1** and **2** KOH, toluene; for compound **12** NaH, DMF; (ii) H_2, $Pd(OH)_2$—C, $MeOH/H_2O$; for compounds **1** and **2** H_2, $Pd(OH)_2$—C, 35% HCl, $MeOH/H_2O$; (iii) $Cl_2P(O)OCH_2CH_2Br$, Et_3N, Et_2O; (iv) KCl, H_2O; (v) Me_3N, $CHCl_3$.

Scheme 3[a]



[a] (i) PCl_3, imidazole, Et_3N, CH_3CN; (ii) Z-Ser-Bzl, PVCl, pyridine; (iii) I_2, pyridine, H_2O; (iv) for compound **4** H_2, $Pd(OH)_2$—C, HCl, $MeOH/H_2O$; for compound **5** H_2, $Pd(OH)_2$—C, $MeOH/H_2O$.

analyzed by ^{1}H-NMR and HPLC, using a Chiralcel OJ column. A small difference was observed for the following signal: doublet of doublets for one of the hydrogens CH_2OMTPA (the MTPA ester of *R*-(+)-**23** had δ 4.46, J = 11.2 and 3.8 Hz; the MTPA ester of *S*-(−)-**23** had δ 4.53, J = 11.3 and 3.7 Hz). The enantiomeric excess of the alcohols was estimated as greater than 95%. This approximate ee was confirmed in the HPLC experiments: *R*-(+)-**23**, ee = 97.2%; *S*-(−)-**23**, ee = 96.7%.

Compounds **1**−**3** and **10**−**16** were synthesized according to the procedure shown in Scheme 2. Intermediates **27** were prepared by reaction of alcohol **25**[23] with the mesylates or bromides **26** in the presence of a strong base. The synthesis of compounds of formula **26** by using standard procedures is described in the Experimental Section. Alcohols **28** were obtained by hydrogenation of **27** with palladium hydroxide on charcoal. Phosphorylation using the same procedure described for the synthesis of **2** and **8** afforded the phosphocholines **1**−**3** and **10**−**16**.

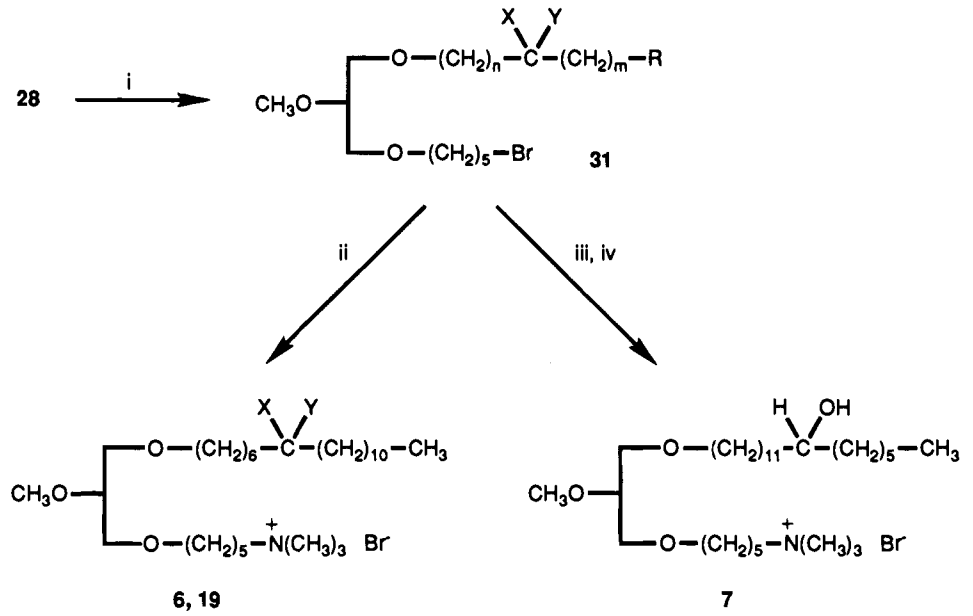
For the synthesis of serine-containing ALP analogs **4**, **5**, and the previously described compound **18**,[11b] the appropriate alcohols **28** were phosphorylated via the H-phosphonate intermediate **29**,[24] prepared by reaction with phosphorus trichloride, imidazole, and triethylamine in acetonitrile. After condensation of phosphonates **29** with *N*-(benzyloxycarbonyl)-L-serine benzyl ester in the presence of pivaloyl chloride as the coupling agent, oxidation to phosphates **30** was carried out with aqueous iodine. Finally, simultaneous removal of the protecting groups on the carboxyl, amino, and ketone functions of **30** was achieved by hydrogenation with palladium hydroxide on charcoal, to give phosphoserines **4**, **5**, and **18**.

Compounds **6**, **7**, and **19**, characterized by the absence of a phosphoryl group, were synthesized according to Scheme 4. Alkylation of alcohols **28** with 1,5-dibromopentane and sodium hydride in DMF afforded compounds **31**. Nucleophilic displacement of bromide ion with trimethylamine in chloroform gave the trimethylammonium analogs **6** and **19**. Compound **7**, which contains a hydroxy group at C-12, was prepared by reduction of the ketone function with sodium borohydride and subsequent reaction of the bromo derivative with trimethylamine.

Results and Discussion

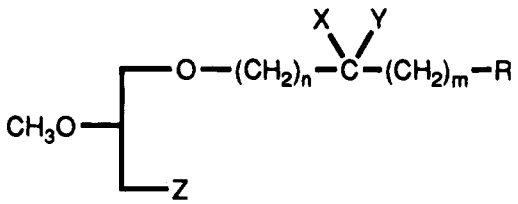
Table 1 shows the IC_{50} values for the cytotoxic activity of compounds **1**−**19** in HL-60 leukemic cells after a 24

Scheme 4[a]



[a] (i) $Br(CH_2)_5Br$, NaH, DMF; (ii) Me_3N, $CHCl_3$; (iii) $NaBH_4$, EtOH; (iv) Me_3N, $CHCl_3$.

Table 1. Biological Activities of Compounds **1**–**19** and Reference Standards



compd	Z	*n*	X	Y	*m*	R	HL-60[a] IC_{50}, μM	hemolysis[b] MNEC, μM	PAF agonism[c] IC_{50}, μM	log *P*[d] C-1 chain
1	phosphocholine	3	O		13	CH_3	4.1 ± 0.3	10	>200	5.92
2	phosphocholine	6	O		10	CH_3	9.1 ± 1.0	20	0.12 ± 0.07	5.92
R-**2**	phosphocholine	6	O		10	CH_3	11.3 ± 1.2	20	0.09 ± 0.02	5.92
S-**2**	phosphocholine	6	O		10	CH_3	5.9 ± 0.3	20	>200	5.92
3	phosphocholine	11	O		5	CH_3	45	50	2.1 ± 0.3	5.92
4	phosphoserine	6	O		10	CH_3	>160	>200	>200	
5	phosphoserine	11	O		5	CH_3	30	>200	>200	
6	$O(CH_2)_5N^+(CH_3)_3Br^-$	6	O		10	CH_3	4.2 ± 0.3	5	>200	
7	$O(CH_2)_5N^+(CH_3)_3Br^-$	11	OH	H	5	CH_3	25.6 ± 3.2	50	>200	
8	phosphocholine	10	O		0	C_6H_5	>160	>200	>200	4.29
9	phosphocholine	10	OH	H	0	C_6H_5	>160	>200	>200	4.70
10	phosphocholine	6	C_4H_9	H	3	CH_3	54	20	>200	5.51
11	phosphocholine	6	C_8H_{17}	H	7	CH_3	52	>200	69 ± 14	8.68
12	phosphocholine	15	H	H	0	C_6H_5	13.2 ± 1.1	10	20 ± 4	7.59
13	phosphocholine	6	C_8H_{17}	H	0	C_6H_5	30.7 ± 2.9	5	>200	7.13
14	phosphocholine	6	CH_3	H	10	CH_3	8.9 ± 0.9	5	>200	7.10
15	phosphocholine	6	C_6H_{13}	H	10	CH_3	50	>200	>200	9.08
16	phosphocholine	11	C_6H_{13}	H	5	CH_3	46	>200	1.5 ± 0.3	9.08
						Reference Compounds				
ET-18-OCH_3, edelfosine							6.1 ± 0.7	2	1.8 ± 0.4	6.77
hexadecylphosphocholine, miltefosine							20.4 ± 2.3	2	>200	
17	phosphocholine	1	O		15	CH_3	3.5 ± 0.4	5	>200	6.29
18	phosphoserine	16	H	H	0	CH_3	25.2 ± 2.9	>200	>200	
19	$O(CH_2)_5N^+(CH_3)_3Br^-$	16	H	H	0	CH_3	8.0 ± 0.9	5	>200	

[a] IC_{50} values were determined by dose–response curves, using the trypan blue dye exclusion assay, after a 24 h exposure to the drug. Average ± SD of two experiments performed in triplicate, except for compounds with IC_{50} > 30 μM (single experiments). [b] MNEC = maximal nonerythrolytic concentration (μM), measured in erythrocytes from rat citrated blood after exposure to the drug for 30 min at 37 °C. [c] Aggregating effect was determined in rabbit platelets after incubation with the compound for 30 min at 37 °C. Specific agonism of the PAF receptor was assessed by preincubation with the PAF antagonist WEB-2086 (5 μM, 3 min). [d] Predicted log *P* values were calculated for the C-1 chain (including the ethereal O atom) using the method of Viswanadhan et al.[27] as implemented in program Tsar (Oxford Molecular).

h incubation period. The effect was measured by trypan blue exclusion test. In the same table the results obtained in two assays directed to evaluate the undesired cellular effects of these ether lipids are collected. Membranolytic activity was determined as the maximal nonerythrolytic concentration (MNEC) in rat erythro-

cytes, and PAF agonism was measured as the IC_{50} for aggregation of washed rabbit platelets. Calculated log *P* values for the C-1 chain of the phosphocholines are also reported for comparative purposes.

Introduction of a carbonyl group into positions 4 and 7 of the C-1 alkyl chain (compounds 1 and **2**) did not modify the cytotoxicity potency (IC_{50} of 4 and 9 μM, respectively) when compared with the model compound ET-18-OCH_3 (IC_{50} = 6 μM in our assay) and the previously described[20a] C-2 ketone analog **17** (IC_{50} = 3.5 μM). On the other hand, when the carbonyl group was placed in position 12, the resulting phosphocholine **3** showed a very low cytotoxicity (IC_{50} = 45 μM). This is in good agreement with the lack of activity reported[10] for the C-10 ketone analog of ET-16-OEt.

Compounds 4 and **5**, which are the phosphoserine analogs of phosphocholines **2** and **3**, showed a markedly reduced activity. This detrimental influence of phosphoserine was also observed in compound **18**, which lacks the carbonyl function; compound **18** was 4 times less potent than ET-18-OCH_3. Replacement of the phosphocholine group of ET-18-OCH_3 with an ammonium polar head as in compound **19** maintained the cytotoxicity, an observation also reported for other series.[9,10,25,26] In the ketone-containing compounds, the pentamethylene trimethylammonium salt **6** was as potent as the corresponding phosphocholine **2**. The introduction in this series of a hydroxy group at position 12 of the C-1 chain was highly detrimental for the activity (compare **7** and **19**).

Simultaneous introduction of a polar ketone or hydroxyl group and replacement of the C_7-terminal portion of the C-1 chain with a phenyl group led to compounds **8** and **9**, which were inactive. This may be due to the position of the carbonyl group, as observed with compound **3**, or to the very low lipophilicity of the modified C-1 chain (log *P* 2-2.5 units lower than that of ET-18-OCH_3).

The C_{18} straight chain of ET-18-OCH_3 has been replaced by several branched or phenyl-bearing lipophilic chains, resulting in compounds **10**–**16**. Analogs **12**–**14**, in which the lipophilicity is similar to that of ET-18-OCH_3, were found to be the most active within this series. Compound **10**, bearing a shorter butylundecyl chain (log *P* of 5.51), was clearly less cytotoxic, and the same was observed for compounds 11, **15**, and **16**, in which the total number of carbon atoms in the C-1 chain is 23 or 24, giving calculated log *P* values greater than 8.6. In this group, the most interesting compound appears to be the 7-methyl derivative 14, which showed good cytotoxic activity (IC_{50} = 9 μM) associated with no proaggregating PAF-like effect. Introduction of a larger *n*-hexyl substituent instead of the methyl group in the same position of C-1 chain gave rise to a markedly less potent compound, **15**, and replacement with a phenyl group led to compound **13**, which has a low activity level (IC_{50} = 30 μM). However, the phenyl group is compatible with good cytotoxicity when placed at the end of the alkyl chain (compound **12**, IC_{50} = 13 μM).

The unspecific hemolytic effect of the above compounds parallels to a certain extent their cytotoxicity in HL-60 cells. Replacement of the phosphocholine polar head of **2** and **3** with a phosphoserine moiety led to compounds 4 and **5**, respectively, lacking of membranolytic activity. On the other hand, compound **6**, which is a pentylammonium analog of **2**, shows a strong lytic effect. Thus, the hemolytic effect seems to be related with the total ionic charge of the polar head, which is cationic in **6**, neutral in **2**, and anionic in 4. A good compromise between cytotoxic and hemolytic effects may be achieved in some cases, i.e., compounds 1 and **2**.

Calculated log *P* values for the C-1 chain of glycerophosphocholine derivatives are reported in Table 1. These values reflect the contribution of the lipophilic chain to the global log *P* of the molecule and, in general, are also related with the hemolytic activity. Thus, compounds containing C-1 chains with a log *P* around 6–7 are clearly more hemolytic than those derivatives having less lipophilic C-1 chains (**8** and **9**, log *P* of 4.29 and 4.70, respectively). Analogs **15** and **16**, whose C-1 chain has a log *P* around 9, are also devoid of lytic activity. This value of 6–7 for C-1 log *P* could indicate a better distribution of the phospholipid derivative into the cell membrane. In the C-1 ketone series, the effect of the introduction of the oxygenated function is a reduction of the membranolytic activity (compare ET-18-OCH_3 with 1–**3**). Although the log *P* values calculated for the C-1 chain of compounds 1–**3** are all identical (5.92), their hemolytic potency is decreasing in the order 1 > **2** > **3**. This may be related to a conformational perturbation of the C-1 chain (due to the 120° angle of the carbonyl function) or to the position of the hydrophilic function in the middle of the alkyl chain, both effects making more difficult the packing of the ALP in the erythrocyte membrane. The reduction in the hemolytic behavior of **2** as compared with ET-18-OCH_3 does not seem to be due to simple steric factors, since compound 14, the analog of **2** containing a methyl group instead of a carbonyl, shows a marked hemolytic activity.

As expected, only those compounds containing the phosphocholine polar head were recognized by the PAF receptor and showed a proaggregatory activity in rabbit platelets. However, most of the phosphocholine derivatives were also completely inactive as agonists at the PAF receptor level, a side effect which is present in the parent drug ET-18-OCH_3. This lack of proaggregatory behavior appear to be related with the presence of a perturbation in the C-1 chain near the glycerol backbone. Thus, compounds **17** and 1 contain a carbonyl in position 2 and 4, respectively, and compounds **10**, 11 and **13**–**15** are branched in position 7 of the C-1 chain. The presence of an alkyl chain or a carbonyl group at more distal positions (i.e., compound **16** or ketones **2** and **3**) did not significantly modify the PAF agonistic potency of ET-18-OCH_3. The *rac*-C-7 ketone **2** showed a low IC_{50} value (0.12 μM) in this assay. The enantiomer *R*-**2** had much higher PAF-like activity than *S*-**2**, confirming previous results.[28] This is not surprising, since the PAF receptor has been described to recognize only phospholipids of the natural *R* configuration.[18] There was no difference between the two enantiomers regarding the nonspecific membranolytic effect, and isomer *S*-**2** was only slightly more cytotoxic in HL-60 cells.

(Oxoalkyl)phosphocholines 1-**3** and enantiomers *R*-**2** and *S*-**2** were further evaluated in three cellular models. Human epidermal carcinoma A431 cells were exposed

Table 2. Antiproliferative Activity of ALP Compounds on Human Tumor Cell Lines.

compd	position of C=O	IC_{50}, μM HL-60[a]	K562[a]	A431[b]
edelfosine		1.0 ± 0.1	6.1 ± 1.8	13.5 ± 2.6
17	2	1.3 ± 0.4	9.0 ± 0.7	14.2 ± 2.3
1	4	1.1 ± 0.6	10.5 ± 1.8	13.5 ± 1.4
2	7	3.0 ± 0.3	41.4 ± 1.2	21.1 ± 5.1
R-**2**	7	3.0 ± 0.2	39.2 ± 3.7	21.8 ± 5.4
S-**2**	7	4.4 ± 0.3	40.2 ± 1.2	19.7 ± 3.6
3	12	12.3 ± 3.2	84.5	40.6 ± 4.5

[a] IC_{50} values were determined by dose–response curves, using the cell-counting method after a 120 h exposure to the drug. Average ± SD of two experiments performed in duplicate. [b] IC_{50} values were determined by dose–response curves, using the SRB assay after a 48 h exposure to the drug. Average ± SD of two to five experiments performed in eight replicates.

to different concentrations of the test compounds for 48 h, and the cell number was indirectly measured by the SRB assay. Growth inhibition was measured in two human leukemic cells, HL-60 and K562, by cell counting after exposure to the drugs for 120 h. The resulting IC_{50} values are collected in Table 2.

The structure–activity relationships found in all three cellular models were parallel and indicated that good activity is compatible with the presence of a carbonyl group in the C-1 chain at positions near the glycerol backbone (C-2 and C-4, compounds **17** and **1**). The C-7 ketone **2**, which was highly cytotoxic against HL-60 cells, after a 120 h incubation period, was considerably less potent against K562 and A431 cells after 120 or 48 h of treatment, respectively. As already observed in the first screening test (Table 1), the C-12 ketone **3** was weakly cytotoxic, indicating that a perturbation of hydrophobicity in the central portion of the long chain is particularly detrimental for the activity of ALPs.

No stereoselectivity was observed with enantiomers *R*-**2** and *S*-**2** in any cellular model. The IC_{50} values were not significantly different with respect to the racemic mixture **2**, even in HL-60 cells. This lack of *in vitro* stereoselectivity is in good agreement with the results reported in literature for the enantiomers of ET-18-OCH_3[7a] and ET-16-OCH_3.[7b]

In conclusion, modification of the C-1 alkyl chain of edelfosine analogs by introduction of an oxygenated function or by branching led to several compounds that were tested in different human tumor cell lines. Some of the new analogs, such as **1** and *S*-**2**, display an interesting *in vitro* biological profile, associating a good cytotoxic potency with less membranolytic and PAF-like side effects than ET-18-OCH_3. Within the ketone-containing series, the structure–activity relationships appear to be not cell dependent and controlled mainly by the position of the carbonyl group. In the alkyl-branched series, the overall lipophilicity of the molecule seems to be the crucial factor for the activity, which is also modulated by the size of the branching group. Stereoselectivity is observed for the PAF receptor agonism but not for the cytotoxic effect, confirming previous results[7,28] and suggesting the absence of a specific interaction between ALPs and a receptor or enzymatic target involved in cell proliferation.

Experimental Section

Synthetic Methods. Solvents were dried as follows: chloroform and dichloromethane were dried over calcium oxide, tetrahydrofuran and diethyl ether were refluxed over sodium/benzophenone just prior to use, acetonitrile was distilled from calcium hydride, and triethylamine was distilled from potassium hydroxide. The other solvents were absolute grade and stored over molecular sieves. Silica gel TLC plates (Merck silica gel 60 F_{254}) were visualized with 5% phosphomolybdic acid in ethanol. MN silica gel 60 (70-230 mesh; ASTM) was used for flash chromatography. Organic extracts were dried over Na_2SO_4 or $MgSO_4$. 2-Bromoethyl dichlorophosphate[29] and 3-*O*-benzyl-2-*O*-methylglycerol[23] were prepared by literature procedures. ^1H- and ^{13}C-NMR were taken on a Varian Gemini 300 instrument. Optical rotations were determined on a Perkin–Elmer 241 polarimeter. The chiral HPLC analyses were performed in a Hewlett–Packard HP 1090 apparatus, with a UV detector (λ = 240 nm), using a Chiralcel OJ column (Daicel, Japan; cellulose tris(4-methylbenzoate) on silica gel) of 25 x 0.46 cm and eluting with mixtures of hexane and 2-propanol (80:20). Elemental analyses were performed by Centro de Investigación y Desarrollo, CSIC, Barcelona (Spain). Starting materials were generally purchased from Aldrich Chemical Co. and used without further purification.

Biological Procedures. Cytotoxicity Screening Assays with HL-60 Cells. HL-60 human promyelocytic leukemia cells were continuously cultured in RPMI-1640 medium supplemented with 5% heat-inactivated fetal calf serum, 50 U/mL penicillin, 50 μg/mL streptomycin, and 2 mM L-glutamine. Cells were allowed to grow in T-flasks at 37 °C in 5% CO_2 atmosphere. The viability of cells before testing was always >90% based on trypan blue dye exclusion.

Dose–response cytotoxicity curves were assessed by trypan blue dye exclusion assay, using HL-60 cells cultured in 24-well culture plates. We incubated 10^6 cells/mL with the test compounds at 2.5, 5, 10, 20, 40, 80, and 160 μM for 24 or 120 h depending on the assay (see Tables 1 and 2). All experiments were done in triplicates, and results are expressed as the percentage of viable cells referred to total cells in the control. IC_{50} was defined as the concentration of the compound that resulted in 50% mortality.

Hemolytic Effect and PAF Agonism. Membranolytic activity was measured by a described methodology,[30] with modifications. Erythrocytes from rat citrated blood were washed with Hank's balanced salt solution (HBSS) and incubated for 30 min at 37 °C with the solution to be tested in a proportion of 9:1. Lysis was calculated spectrophotometrically at 546 nm.

PAF agonism was determined by a turbidimetric test.[31] Platelets from arterial rabbit blood were washed and adjusted in HBSS at 5 x 10^5 platelets/mL. After 30 min at 37 °C, platelets were incubated with different concentrations of the compound to be tested. If an agonistic effect was observed, we repeated the test preincubating with the PAF antagonist WEB 2086, and then we added the ALP analog in order to check if the agonistic action was PAF receptor specific.

Antiproliferative Activity. Stock solutions of the compounds were prepared in culture medium or EtOH. Stock solutions were diluted with complete growth medium prior to use. The final concentration of EtOH in the assay mixture never exceeded 0.2% and was ineffective on cell proliferation. Cells (see Table 2) were routinely grown in RPMI-1640 medium (Flow Laboratories, Irvine, CA) containing 10% heat-inactivated fetal calf serum and 2 mM L-glutamine at 37 °C in a humidified 5% CO_2/95% air atmosphere.

For the cell-counting assays, HL-60 and K562 cells (1 x 10^5 cells/mL), growing in suspension, were incubated with different concentrations of ether lipids for 120 h and then counted using a Coulter Counter.

For the sulforhodamine B assay (SRB), adherent A431 cells were collected by trypsinization and seeded (5000 cells/cm^2) in 96-well microplates. After 24 h, different concentrations of ether lipids, dissolved in complete medium, were added. After 48 h of exposure to the drug, cells were fixed with 10% trichloroacetic acid and stained with 0.4% SRB dissolved in 1% acetic acid.[32] Protein-bound dye was solubilized with 10 mM Tris, pH 10.4. Optical density at 550 nm was read with a microplate reader (EAR 400 AT; SLT-Labinstruments, Austria). All values were corrected for background, using 96-well microplates with cell free medium.

1-*O*-(6-Bromohexyl)-2,3-*O*-isopropylideneglycerol. Sodium hydride (0.188 g, 6.2 mmol of 80% oil dispersion) and 1,6-dibromohexane (2.73 g, 11.2 mmol) were placed in 20 mL of DMF under nitrogen. *rac*-1,2-*O*-Isopropylideneglycerol (0.74 g, 5.6 mmol) in 10 mL of DMF was added dropwise. The reaction mixture was stirred at room temperature for 18 h, and a mixture of EtOH and H_2O (50 mL) was added. The solution was extracted with Et_2O (2 × 50 mL), the organic phase was dried over Na_2SO_4, and the solvent was removed in vacuo. After distillation of the excess reagents (heating to 70–72 °C/0.3 Torr), the residue was purified by flash chromatography on silica gel, eluting with dichloromethane, to give 0.680 g (41%) of the alkyl bromide. ^{1}H-NMR ($CDCl_3$): δ 1.25 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.30 (m, 4H, 2 CH_2), 1.50 (m, 2H, OCH_2CH_2), 1.75 (m, 2H, CH_2CH_2Br), 3.25–3.45 (m, 6H, CH_2OCH_2 and CH_2Br), 3.63 (t, 1H, $J_{AB} = J_{BC} = 7.4$ Hz, CH_AH_BO), 3.96 (t, 1H, $J_{AB} = J_{AC} = 7.4$ Hz, CH_AH_BO), 4.17 (m, 1H, CHO).

1-*O*-(6-Cyanohexyl)-2,3-*O*-isopropylideneglycerol. A solution of NaCN (1.19 g, 24.0 mmol) in 25 mL of DMSO was heated to 90 °C and a solution of 1-*O*-(6-bromohexyl)-2,3-*O*-isopropylideneglycerol (6.0 g, 20.0 mmol) in 8 mL of DMSO was added dropwise. The reaction mixture was stirred at 90 °C for 1 h and then H_2O (60 mL) was added. The solution was extracted with Et_2O (2 × 100 mL), the organic phases were dried over Na_2SO_4, and the solvent was evaporated under reduced pressure to obtain 4.55 g (95%) of **21** ($n = 6$). ^{1}H-NMR ($CDCl_3$): δ 1.30 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.30–1.47 (m, 4H, 2 CH_2), 1.50–1.70 (m, 4H, OCH_2CH_2 and CH_2CH_2CN), 2.30 (t, 2H, $J = 7.0$ Hz, CH_2CN), 3.24–3.50 (m, 4H, CH_2OCH_2), 3.67 (dd, 1H, $J_{AB} = 8.2$ Hz, $J_{BC} = 6.4$ Hz, CH_AH_BO), 4.02 (dd, 1H, $J_{AB} = 8.2$ Hz, $J_{AC} = 6.4$ Hz, CH_ACH_BO), 4.22 (m, 1H, CHO).

1-*O*-(6-Cyanohexyl)glycerol. A solution of 1-*O*-(6-cyanohexyl)-2,3-*O*-isopropylideneglycerol (4.0 g, 16.6 mmol) in 500 mL of THF was mixed with 200 mL of 2 N HCl. After stirring at room temperature for 2 h, the solution was neutralized with $NaHCO_3$. The two phases were separated and the aqueous layer was extracted with EtOAc (3 × 100 mL). The combined organic extracts were dried and evaporated under reduced pressure to give 2.78 g (83%) of the (cyanoalkyl)glycerol. ^{1}H-NMR ($CDCl_3$): δ 1.15–1.35 (m, 4H, 2 CH_2), 1.35–1.55 (m, 4H, OCH_2CH_2 and CH_2CH_2CN), 2.20 (t, 2H, $J = 7.2$ Hz, CH_2CN), 3.30 (m, 4H, CH_2OCH_2), 3.38 (dd, 1H, $J_{AB} = 11.4$ Hz, $J_{BC} = 6.2$ Hz, CH_ACH_BOH), 3.48 (dd, 1H, $J_{AB} = 11.4$ Hz, $J_{AC} = 3.7$ Hz, CH_ACH_BOH), 3.67 (m, 1H, CHOH).

1-*O*-(6-Cyanohexyl)-3-*O*-tritylglycerol. Trityl chloride (7.24 g, 26 mmol) was added to a solution of 1-*O*-(6-cyanohexyl)glycerol (2.6 g, 13.0 mmol) in 60 mL of pyridine. After the solution was stirred at room temperature for 22 h, it was poured into an ice–water mixture and extracted with Et_2O (3 × 100 mL). The organic phase was washed with 0.1 N HCl to acid pH and then washed with 5% $NaHCO_3$ solution and H_2O. After drying, the solvent was evaporated to obtain a crude product which was purified by flash chromatography on silica gel, eluting with mixtures of petroleum ether:Et_2O of increasing polarity, to give 3.98 g (69%) of the secondary alcohol. ^{1}H-NMR ($CDCl_3$): δ 1.25–1.70 (m, 8H, 4 CH_2), 2.27 (t, 2H, $J = 7.2$ Hz, CH_2CN), 2.40 (b s, 1H, OH), 3.15 (m, 2H, CH_2OCPh_3), 3.36–3.55 (m, 4H, CH_2OCH_2), 3.93 (m, 1H, CHOH), 7.15–7.45 (m, 15H, ArH).

1-*O*-(6-Cyanohexyl)-2-*O*-methyl-3-*O*-tritylglycerol. A solution of 1-*O*-(6-cyanohexyl)-3-*O*-tritylglycerol (3.3 g, 7.45 mmol) in 10 mL of C_6H_6 was added to a suspension of KH (1.78 g, 8.94 mmol of 20% dispersion in mineral oil) in 50 mL of dry C_6H_6. After stirring for 30 min at room temperature, a solution of CH_3I (4.22 g, 29.0 mmol) in C_6H_6 was added. The reaction mixture was stirred at room temperature for 3 h, diluted with H_2O (40 mL), extracted with Et_2O (3 × 50 mL), and dried. The solvent was removed in vacuo to obtain a crude product which was purified by silica gel chromatography. On elution with mixtures of petroleum ether:Et_2O of increasing polarity, 2.83 g (83%) of **22** ($n = 6$) was obtained. ^{1}H-NMR ($CDCl_3$): δ 1.25–1.65 (m, 8H, 4 CH_2), 2.27 (t, 2H, $J = 7.2$ Hz, CH_2CN), 3.17 (m, 2H, CH_2OCPh_3), 3.38 (s, 3H, CH_3O), 3.35–3.60 (m, 5H, CH_2OCH_2 and $CHOCH_3$), 7.15–7.47 (m, 15H, ArH).

1-*O*-(7-Oxooctadecyl)-2-*O*-methylglycerol. Magnesium (0.260 g, 10.7 mmol) and a crystal of I_2 in dry Et_2O were placed in a three-necked flask, and then a solution of 1-bromoundecane (2.06 g, 8.75 mmol) in 20 mL of Et_2O was added dropwise. The reaction mixture was refluxed for 1 h, and then the Et_2O was evaporated under reduced pressure and 10 mL of dry C_6H_6 was added. A solution of 1-*O*-(6-cyanohexyl)-2-*O*-methyl-3-*O*-tritylglycerol (2.0 g, 4.38 mmol) in 30 mL of dry C_6H_6 was added dropwise. The mixture was refluxed for 6 h and stirred at room temperature for 3 days. The solvent was evaporated at reduced pressure, and then 100 mL of dioxane and 30 mL of 1 N HCl were added. The mixture was refluxed for 2 h, neutralized with 5% $NaHCO_3$ solution, and extracted with Et_2O (3 × 100 mL). After drying and evaporating the solvent, the residue was purified by flash chromatography on silica gel, using mixtures of petroleum ether:Et_2O of increasing polarity as eluant, giving 0.575 g (35%) of **23** ($n = 6$, $m = 10$). ^{1}H-NMR ($CDCl_3$): δ 0.82 (t, 3H, terminal CH_3), 1.20 (m, 20H, 10 CH_2), 1.50 (m, 6H, $CH_2CH_2COCH_2CH_2$ and OCH_2CH_2), 2.30 (t, 4H, $J = 7.1$ Hz, CH_2COCH_2), 3.40 (s, 3H, CH_3O), 2.38 (b s, 1H, OH), 3.32–3.42 (m, 3H, $CHOCH_3$ and CH_2OCH_2), 3.47 (m, 2H, CH_2OCH_2), 3.55 (dd, 1H, $J_{AB} = 11.5$ Hz, $J_{BC} = 5.5$ Hz, CH_ACH_BOH), 3.69 (dd, 1H, $J_{AB} = 11.5$ Hz, $J_{AC} = 3.9$ Hz, CH_ACH_BOH). ^{13}C-NMR ($CDCl_3$): δ 14.12 (terminal CH_3), 22.72–31.99 (13 CH_2), 42.72 and 42.88 (CH_2COCH_2), 57.90 (CH_3O), 62.35 (CH_2OH), 70.59 (CH_2OCH_2), 71.81 (CH_2OCH_2), 80.63 ($CHOCH_3$), 212.36 (C=O).

1-*O*-(7-Oxooctadecyl)-2-*O*-methylglycero-3-(2-bromoethyl)phosphate. A solution of 1-*O*-(7-oxooctadecyl)-2-*O*-methylglycerol (0.125 g, 0.3 mmol) in 5 mL of dry Et_2O was added at 0 °C to a solution of 2-bromoethyl dichlorophosphate (0.134 g, 0.55 mmol) and triethylamine (0.15 mL, 1.098 mmol) in 8 mL of dry Et_2O. The mixture was stirred at room temperature for 24 h, and a solution of 0.54 mL of 0.1 M KCl was added. After stirring at room temperature for 1.5 h, the reaction mixture was extracted with Et_2O (3 × 10 mL) and the combined extracts were dried. The solvent was removed in vacuo, giving 0.15 g (quantitative yield) of **24** ($n = 6$, $m = 10$). ^{1}H-NMR ($CDCl_3$): δ 0.82 (t, 3H, terminal CH_3), 1.20 (m, 20H, 10 CH_2), 1.50 (m, 6H, $CH_2CH_2COCH_2CH_2$ and OCH_2CH_2), 2.35 (t, 4H, $J = 7.1$ Hz, CH_2COCH_2), 3.41 (s, 3H, CH_3O), 3.35–3.57 (m, 7H, $CHOCH_3$, CH_2OCH_2 and CH_2Br), 4.00–4.40 (m, 4H, $CH_2OP(O)(OH)OCH_2$).

1-*O*-(7-Oxooctadecyl)-2-*O*-methyl-*rac*-glycero-3-phosphocholine (*rac*-2). A solution of 1-*O*-(7-oxooctadecyl)-2-*O*-methylglycero-3-(2-bromoethyl)phosphate (0.135 g, 0.24 mmol) in 5 mL of dry $CHCl_3$ was transferred to a thick-walled glass flask. Dry trimethylamine (1 mL) was added while cooling the flask in dry ice. The flask was sealed and heated to 65 °C for 18 h. After cooling, the solution was evaporated to dryness to obtain a crude product which was purified by silica gel chromatography, eluting with $CHCl_3$:MeOH (65:25) and subsequently with $CHCl_3$:MeOH:H_2O (65:25:4), to give 0.73 g (45%, from the primary alcohol) of *rac*-**2**. ^{1}H-NMR ($CDCl_3$): δ 0.82 (t, 3H, terminal CH_3), 1.20 (m, 20H, 10 CH_2), 1.50 (m, 6H, $CH_2CH_2COCH_2CH_2$ and OCH_2CH_2), 2.34 (t, 4H, $J = 7.1$ Hz, CH_2COCH_2), 3.31 (s, 9H, $N^+(CH_3)_3$), 3.37 (s, 3H, CH_3O), 3.30–3.50 (m, 5H, $CHOCH_3$ and CH_2OCH_2), 3.75 (m, 2H, CH_2N^+), 3.77–3.92 (m, 2H, CH_2OP), 4.25 (m, 2H, $POCH_2$). ^{13}C-NMR ($CDCl_3$): δ 14.27 (terminal CH_3), 22.84–32.09 (13 CH_2), 42.89 and 43.12 (CH_2COCH_2), 54.59 ($N^+(CH_3)_3$), 58.04 (CH_3O), 59.50 (d, $J_{C\text{-}P} = 4.5$ Hz, $POCH_2$), 65.02 (d, $J_{C\text{-}P} = 5.4$ Hz, CH_2OP), 66.60 (d, $J_{C\text{-}P} = 6.4$ Hz, CH_2N^+), 70.60 (CH_2OCH_2), 71.86 (CH_2OCH_2), 80.06 (d, $J_{C\text{-}P} = 7.9$ Hz, $CHOCH_3$), 212.54 (C=O). Anal. ($C_{27}H_{56}NO_7P \cdot 5H_2O$) C, H, N, P. Compound *rac*-**2** was also obtained in comparable yields following the reaction pathway depicted in Scheme 2 (see, below, the synthesis of 1).

2-*O*-Methyl-1-*O*-(7-oxooctadecyl)-*sn*-glycero-3-phosphocholine (*R*-(+)-2). Alcohol *S*-(−)-**23** ($n = 6$, $m = 10$, R = CH_3) was obtained from *R*-(−)-1,2-*O*-isopropylideneglycerol, using the same procedure described for the synthesis of *rac*-**23**. Compound *R*-(+)-**2** was obtained in 36% yield from *S*-(−)-**23**, using the same procedure described above for *rac*-**2**. $[\alpha]^{25}_D$ +1.1° (c 2.4, $CHCl_3$). Anal. ($C_{27}H_{56}NO_7P \cdot 2H_2O$) C, H, N, P.

2-*O*-Methyl-3-*O*-(7-oxooctadecyl)-*sn*-glycero-1-phosphocholine (*S*-(−)-**2**). Alcohol *R*-(+)-**23** ($n = 6$, $m = 10$, R = CH_3) was obtained from (*S*)-(+)-1,2-*O*-isopropylideneglycerol, using the same procedure described for the synthesis of *rac*-**23**. Compound *S*-(−)-**2** was obtained in 56% yield from *R*-(+)-**23**, by the same method described above for *rac*-**2**. $[\alpha]^{25}_D$ −0.9° (*c* 0.3, $CHCl_3$). Anal. ($C_{27}H_{56}NO_7P \cdot 4H_2O$) C, H, N.

General Procedure for Preparation of Mosher Esters of Alcohols *rac*-23, *S*-(−)-23, and *R*-(+)-23 ($n = 6$, $m = 10$, R = CH_3). Thionyl chloride (4 mL) was added to (*R*)-(+)-MTPA (0.1 g, 0.43 mmol). The mixture was refluxed for 5 h, and then the excess of $SOCl_2$ was removed under reduced pressure to give the Mosher acid chloride, which was used without further purification. To a solution of the appropriate alcohol **23** (0.057 g, 0.15 mmol) in 1 mL of dry $CHCl_3$ were added (*R*)-(−)-MTPA chloride (0.078 g, 0.3 mmol) and 3 drops of pyridine. The mixture was stirred at room temperature for 4 h, and 5 mL of $CHCl_3$ was added. The solution was washed with 0.1 N HCl, dried, and evaporated to give a crude product which was analyzed by ^{1}H-NMR and HPLC using a Chiralcel OJ column to evaluate their enantiomeric excess. The crude product was purified by preparative TLC on silica gel, eluting with a mixture of petroleum ether:Et_2O (2:8), in order to describe all the signals of the ^{1}H-NMR spectrum of the Mosher esters *S*-(−)-**23** and *R*-(+)-**23**. ^{1}H-NMR for the Mosher ester of *S*-(−)-**23** ($CDCl_3$): δ 0.84 (t, 3H, terminal CH_3), 1.22 (s, 20H, 10 CH_2), 1.45−1.65 (m, 6H, $CH_2CH_2COCH_2CH_2$ and OCH_2CH_2), 2.36 (t, 4H, J = 7.3 Hz, CH_2COCH_2), 3.30−3.45 (m, 5H, $CHOCH_3$ and CH_2OCH_2), 3.35 (s, 3H, CH_3O), 3.53 (s, 3H, CH_3O in MTPA), 4.34 (dd, 1H, J_{AB} = 11.3 Hz, J_{BC} = 5.5 Hz, CH_ACH_BO), 4.53 (dd, 1H, J_{AB} = 11.3 Hz, J_{AC} = 3.7 Hz, CH_ACH_BO), 7.35−7.40 (m, 3H, ArH), 7.47−7.59 (m, 2H, ArH). ^{1}H-NMR for the Mosher ester of *R*-(+)-**23** ($CDCl_3$): δ 4.46 (dd, 1H, J_{AB} = 11.2 Hz, J_{AC} = 3.8 Hz, CH_ACH_BO).

1-*O*-(11-Phenyl-11-oxoundecyl)-2-*O*-methylglycero-3-phosphocholine (**8**). This compound was prepared in 21% yield from the appropriate alcohol **23**, following the methods described above for **2**, starting from 1,10-dibromodecane and 1,2-*O*-isopropylideneglycerol. ^{1}H-NMR (CD_3OD): δ 1.20 (m, 12H, 6 CH_2), 1.50 (m, 2H, CH_2CH_2CO), 1.67 (m, 2H, OCH_2CH_2), 2.90 (t, 2H, CH_2CO), 3.25 (s, 9H, $N^+(CH_3)_3$), 3.38 (s, 3H, CH_3O), 3.30−3.50 (m, 5H, $CHOCH_3$ and CH_2OCH_2), 3.67 (m, 2H, CH_2N^+), 3.72−3.92 (m, 2H, CH_2OP), 4.23 (m, 2H, $POCH_2$), 7.35−7.55 (m, 3H, ArH), 7.90 (d, 2H, ArH). ^{13}C-NMR (CD_3OD): δ 25.79−30.95 (8 CH_2), 39.72 (CH_2CO), 54.99 (t, $J_{C\text{-}N}$ = 3.6 Hz, $N^+(CH_3)_3$), 58.55 (CH_3O), 60.68 (d, $J_{C\text{-}P}$ = 5.2 Hz, $POCH_2$), 66.50 (d, $J_{C\text{-}P}$ = 6.0 Hz, CH_2OP), 67.82 (m, $J_{C\text{-}P}$ = 7.0 Hz, $J_{C\text{-}N}$ = 3.6 Hz, CH_2N^+), 71.40 (CH_2OCH_2), 73.06 (CH_2OCH_2), 81.25 (d, $J_{C\text{-}P}$ = 8.2 Hz, $CHOCH_3$), 129.40−134.40 (Ar), 203.0 (C=O). Anal. ($C_{26}H_{46}NO_7P \cdot 3H_2O$) C, H, N.

1-*O*-(11-Hydroxy-11-phenylundecyl)-2-*O*-methylglycero-3-phosphocholine (**9**). A mixture of phosphocholine **8** (15 mg, 0.029 mmol), 20% $Pd(OH)_2$ on charcoal (8.0 mg), 9 mL of MeOH, and 1 mL of water was hydrogenated at room temperature and atmospheric pressure, until no more H_2 was consumed. Filtration and evaporation yielded 12 mg (67%) of **9**. ^{1}H-NMR ($CDCl_3$): δ 4.58 (t, 1H, $CHOH$). ^{13}C-NMR ($CDCl_3$): δ 25.99−29.92 (8 CH_2), 39.53 ($CH_2CH(OH)Ph$), 54.56 ($N^+(CH_3)_3$), 58.04 (CH_3O), 59.60 (d, $POCH_2$), 65.30 (d, CH_2OP), 66.60 (d, CH_2N^+), 70.40 (CH_2OCH_2), 72.04 (CH_2OCH_2), 74.55 (CH(OH)Ph), 80.00 (d, $CHOCH_3$), 130.40−146.13 (Ar). Anal. ($C_{26}H_{48}NO_7P \cdot 3H_2O$) C, H, N.

General Procedure for the Preparation of Mesyl Derivatives 26. 7-(Ethylenedioxy)-1-octadecyl Methanesulfonate (Z = OMs, $n = 6$, $m = 10$, XY = OCH_2CH_2O, R = CH_3). NaH (1.5 g, 50.0 mmol of 80% oil dispersion) and 1,6-dibromohexane (25.0 g, 100.0 mmol) were placed in 100 mL of dry THF under nitrogen. The reaction mixture was refluxed, and a solution of benzyl alcohol (4.32 g, 40.0 mmol) in 40 mL of THF was added dropwise. At the end of the addition, reflux was continued for 1 h, and the solution was poured into an ice−water mixture and extracted with Et_2O (3 × 100 mL). The combined organic phases were dried and evaporated under reduced pressure to obtain a crude product, which was purified by distillation at 86 °C/1 Torr to give 4.5 g (35%) of 1-(benzyloxy)-6-bromohexane. ^{1}H-NMR ($CDCl_3$): δ 1.42 (m, 4H, 2 CH_2), 1.62 (m, 2H, OCH_2CH_2), 1.85 (m, 2H, CH_2CH_2Br), 3.37 (t, 2H, CH_2Br), 3.45 (t, 2H, CH_2OCH_2Ph), 4.50 (s, 2H, OCH_2Ph), 7.35 (m, 5H, ArH).

A solution of NaCN (0.576 g, 11.7 mmol) in 15 mL of DMSO was heated to 90 °C, and a solution of 1-(benzyloxy)-6-bromohexane (3.0 g, 11.7 mmol) in 5 mL of DMSO was added dropwise. The reaction mixture was stirred at 90 °C for 16 h, and then water (30 mL) was added. The solution was extracted with Et_2O (3 × 50 mL). The combined organic phases were dried and the solvent was evaporated under reduced pressure to obtain 2.053 g (87%) of 7-(benzyloxy)-heptanenitrile. This product was used without further purification in the next step. ^{1}H-NMR ($CDCl_3$): δ 1.40 (m, 4H, 2 CH_2), 1.57 (m, 4H, OCH_2CH_2 and CH_2CH_2CN), 2.20 (t, 2H, CH_2CN), 3.40 (t, 2H, CH_2OCH_2Ph), 4.45 (s, 2H, OCH_2Ph), 7.30 (m, 5H, ArH).

Magnesium (0.32 g, 13.16 mmol) and a crystal of I_2 in dry ethyl ether were placed in a three-necked flask, and a solution of 1-bromoundecane (2.7 g, 11.5 mmol) in 25 mL of Et_2O was added dropwise. The reaction mixture was refluxed for 1 h. A solution of the above nitrile (1.0 g, 4.6 mmol) in 40 mL of dry C_6H_6 was added dropwise. After the mixture was refluxed for 6 h, a cold 10% H_2SO_4 solution was added, and reflux was maintained for an additional hour. After separation, the organic phase was washed with 5% $NaHCO_3$ solution, dried, and evaporated to give a crude product which was purified by flash chromatography on silica gel. On elution with mixtures of petroleum ether:Et_2O of increasing polarity, 0.716 g (41%) of 1-(benzyloxy)-7-octadecanone was obtained. ^{1}H-NMR ($CDCl_3$): δ 0.80 (t, 3H, terminal CH_3), 1.20 (m, 20H, 10 CH_2), 1.50 (m, 6H, OCH_2CH_2 and $CH_2CH_2COCH_2CH_2$), 2.25 (t, 4H, CH_2COCH_2), 3.37 (t, 2H, CH_2OCH_2Ph), 4.40 (s, 2H, OCH_2Ph), 7.20 (m, 5H, ArH).

A mixture of the above ketone (2.7 g, 7.22 mmol), ethylene glycol (15.2 g, 245.0 mmol), *p*-toluenesulfonic acid (1.82 g, 9.6 mmol), and 120 mL of anhydrous C_6H_6 was refluxed for 24 h in a Dean−Stark apparatus. The reaction mixture was washed first with a 10% NaOH solution and then with H_2O. The organic phase was dried, and the solvent was removed in vacuo to obtain 2.51 g (83%) of 1-(benzyloxy)-7-(ethylenedioxy)-octadecane. This product was used without further purification in the next step. ^{1}H-NMR ($CDCl_3$): δ 0.85 (t, 3H, terminal CH_3), 1.30 (m, 18H, 9 CH_2), 1.55 (m, 6H, $CH_2CH_2C(OCH_2CH_2O)CH_2$), 3.42 (t, 2H, CH_2OCH_2Ph), 3.90 (s, 4H, OCH_2CH_2O), 4.47 (s, 2H, OCH_2Ph), 7.20−7.35 (m, 5H, ArH).

A mixture of the above compound (1.25 g, 3.0 mmol), 20% $Pd(OH)_2$ on charcoal (0.81 g), 70 mL of MeOH, and 10 mL of H_2O was hydrogenated at room temperature and atmospheric pressure until no more H_2 was consumed. Filtration and evaporation yielded 0.95 g (97%) of 7-(ethylenedioxy)-1-octadecanol, which was used without purification in the next step. ^{1}H-NMR ($CDCl_3$): δ 0.75 (t, 3H, terminal CH_3), 1.15−1.25 (m, 24H, 12 CH_2), 1.50 (m, 6H, CH_2CH_2OH and $CH_2C(OCH_2CH_2O)CH_2$), 2.52 (b s, 1H, OH), 3.50 (t, 2H, CH_2OH), 3.80 (s, 4H, OCH_2CH_2O).

Triethylamine (1.45 mL, 10.74 mmol) was added to a solution of the above alcohol (1.85 g, 5.37 mmol) in 30 mL of dry CH_2Cl_2. The mixture was cooled to 0 °C, and a solution of methanesulfonyl chloride (1.23 g, 10.74 mmol) in 15 mL of dichloromethane was added dropwise. The reaction mixture was maintained at 0 °C for 4 h and washed with H_2O, and the organic phase was dried. The solvent was removed in vacuo to obtain a crude product which was purified by flash chromatography on silica gel, eluting with mixtures of petroleum ether:Et_2O of increasing polarity to give 1.79 g (79%) of 7-(ethylenedioxy)-1-octadecyl methanesulfonate. ^{1}H-NMR ($CDCl_3$): δ 0.82 (t, 3H, terminal CH_3), 1.15−1.40 (m, 24H, 12 CH_2), 1.52 (m, 4H, $CH_2C(OCH_2CH_2O)CH_2$), 1.72 (m, 2H, OCH_2CH_2), 2.95 (s, 3H, CH_3SO_3), 3.87 (s, 4H, OCH_2CH_2O), 4.18 (t, 2H, CH_2OMs).

4-(Ethylenedioxy)-1-octadecyl Methanesulfonate (26, Z = OMs, $n = 3$, $m = 13$, XY = OCH_2CH_2O, R = CH_3). Using the above procedure and starting from 1-(benzyloxy)-3-bromopropane, this compound was prepared in 9% overall yield; the ^{1}H-NMR is essentially identical to that of the 7-ethylenedioxy isomer.

12-Oxo-1-octadecyl Methanesulfonate (26, Z = OMs, n = 11, m = 5, XY = O, R = CH_3). This compound was prepared in 50% yield (last step) by the above-described procedure, starting from 1-(benzyloxy)-11-bromoundecane. ^{1}H-NMR ($CDCl_3$): δ 0.80 (t, 3H, terminal CH_3), 1.15–1.35 (m, 14H, 7 CH_2), 1.48 (m, 4H, $CH_2CH_2COCH_2CH_2$), 1.67 (m, 2H, CH_2CH_2-OMs), 2.32 (t, 4H, CH_2OCH_2), 2.92 (s, 3H, CH_3SO_3), 4.15 (t, 2H, CH_2OMs).

7-Methyl-1-octadecyl Methanesulfonate (26, Z = OMs, n = 6, m = 10, X = H, Y = CH_3, R = CH_3). Methyllithium (6 mL of 1.6 M solution in Et_2O) was added dropwise at 0 °C to a solution of 1-(benzyloxy)-7-octadecanone (1.4 g, 3.74 mmol) in 5 mL of dry C_6H_6. The reaction mixture was stirred at room temperature for 10 h, and a mixture of MeOH and H_2O (50 mL) was added. The solution was extracted with $CHCl_3$ (3 × 25 mL). The organic phase was dried, and the solvents were removed in vacuo to obtain 1.84 g (96%) of 1-(benzyloxy)-7-methyloctadecan-7-ol. This compound was used without further purification in the next step.

A mixture of the above alcohol (1.84 g, 4.71 mmol), PtO_2 (76 mg), and 12 mL of trifluoroacetic acid was hydrogenated at room temperature and atmospheric pressure until no more H_2 was consumed. Filtration and evaporation yielded 1.5 g (84%) of 7-methyloctadecyl trifluoroacetate, which was dissolved in 15 mL of MeOH containing a few drops of 35% HCl. After the solution was heated at reflux for 2 h, the solvent was evaporated under reduced pressure to give 1.043 g (78% from tertiary alcohol) of 7-methyl-1-octadecanol. Triethylamine (0.559 g, 5.52 mmol) was added to a solution of the above primary alcohol (1.46 g, 3.68 mmol) in 20 mL of dry CH_2Cl_2. The mixture was cooled to 5 °C, and a solution of methanesulfonyl chloride (0.632 g, 5.52 mmol) in 10 mL of CH_2Cl_2 was added dropwise. The reaction mixture was maintained at 5 °C for 1 h and washed with 2 N HCl and then with 10% $NaHCO_3$ solution. The organic phase was dried, and the solvent was evaporated to obtain 1.039 g (78%) of 7-methyl-1-octadecyl methanesulfonate. ^{1}H-NMR ($CDCl_3$): δ 0.75–0.82 (b s, 6H, 2 CH_3), 1.00–1.39 (m, 29H, 14 CH_2 and CH), 1.65 (m, 2H, CH_2CH_2OMs), 2.91 (s, 3H, CH_3SO_3), 4.13 (t, 2H, CH_2-OMs).

7-Phenyl-1-pentadecyl Methanesulfonate (26, Z = OMs, n = 6, m = 0, X = C_6H_{17}, Y = H, R = C_6H_5). Magnesium (1.17 g, 48.32 mmol), a crystal of I_2, and 2 mL of dry THF were placed into a three-necked flask, and a solution of 1-bromooctane (8.75 g, 45.3 mmol) in 10 mL of THF was added dropwise. After the reaction mixture was refluxed for 2 h, a solution of 7-(benzyloxy)-1-phenyl-1-heptanone (8.947 g, 30.2 mmol) in 10 mL of THF was added dropwise. The mixture was refluxed for 4 h, and then saturated NH_4OH solution was added. The mixture was extracted with Et_2O (3 × 25 mL); the combined organic extracts were dried and evaporated under reduced pressure to give a crude product, which was purified by flash chromatography on silica gel, eluting with mixtures of petroleum ether:Et_2O of increasing polarity, to give 6.0 g (59%) of 1-(benzyloxy)-7-phenylpentadecan-7-ol. A mixture of the alcohol (0.5 g, 1.22 mol), 10% Pd on charcoal (60 mg), and 20 mL of EtOH was hydrogenated at room temperature and atmospheric pressure until no more H_2 was consumed. Filtration and evaporation yielded 0.338 g (91%) of 7-phenyl-1-pentadecanol. The primary alcohol was mesylated as indicated above to give 7-phenyl-1-pentadecyl methanesulfonate. ^{1}H-NMR ($CDCl_3$): δ 0.84 (t, 3H, CH_3), 1.18 (m, 18H, 9 CH_2), 1.50–1.70 (m, 6H, CH_2CH_2OMs and 2 CH_2), 2.44 (m, 1H, CH), 2.93 (s, 3H, CH_3SO_3), 4.14 (t, 2H, CH_2OMs), 7.08–7.30 (m, 5H, ArH).

7-Hexyl-1-octadecyl Methanesulfonate (26, Z = OMs, n = 6, m = 10, X = C_6H_{13}, Y = H, R = CH_3) and 12-Hexyl-1-octadecyl Methanesulfonate (26, Z = OMs, n = 11, m = 5, X = C_6H_{13}, Y = H, R = CH_3). These compounds were prepared by the same procedure as described above for 7-methyl-1-octadecyl methanesulfonate and 7-phenyl-1-pentadecyl methanesulfonate, in 61 and 57% yields, respectively (calculated from the appropriate ketone).

7-Butyl-1-undecyl Methanesulfonate (26, Z = OMs, n = 6, m = 3, X = C_4H_9, Y = H, R = CH_3). Magnesium (1.22 g, 50.0 mmol) and a crystal of I_2 in 10 mL of dry Et_2O were placed into a three-necked flask, and a solution of 1-bromobutane (7.54 g, 55.0 mmol) in 25 mL of Et_2O was added dropwise. The reaction mixture was refluxed for 1 h and cooled, and a solution of ethyl 7-bromoheptanoate (4.74 g, 20.0 mmol) in 25 mL of Et_2O was added dropwise. Reflux was continued for 1 h and then the solution was poured into 100 mL of ice–NH_4Cl saturated solution. The two phases were separated, and the aqueous layer was extracted with Et_2O (3 × 100 mL). The organic phase was dried, and the solvent was removed in vacuo. The crude product was purified by distillation under vacuum (160–165 °C/0.1 Torr) to give 5.05 g (82%) of 11-bromo-5-butylundecan-5-ol.

The above alcohol (4.45 g, 14.5 mmol) and a few crystals of *p*-toluenesulfonic acid were placed in a three-necked flask. The mixture was stirred under nitrogen at 130 °C for 2 h. The crude product was purified by distillation under high vacuum (150 °C/0.1 Torr) to give 3.98 g (95%) of a mixture of 1-bromo-7-butyl-7-undecene and 1-bromo-7-butyl-6-undecene.

Sodium acetate (0.656 g, 8.0 mmol) in 6 mL of dry DMF was placed into a three-necked flask, and a solution of the above alkene mixture (0.578 g, 2.0 mmol) and benzyltriethylammonium chloride (0.068 g, 0.2 mmol) in 9 mL of DMF was added dropwise. The mixture was stirred at 70 °C for 15 h, and then the solution was poured into an ice–water mixture and extracted with Et_2O (3 × 25 mL). The combined extracts were dried, and the solvent was removed in vacuo to give 0.514 g (96%) of an acetate mixture.

A solution of this mixture (0.536 g, 2.0 mmol) in 15 mL of MeOH was added to a solution of KOH (1.6 g of 80%) in 8 mL of H_2O. The mixture was refluxed for 30 min and evaporated under reduced pressure, and then H_2O (20 mL) was added. The solution was extracted with Et_2O, the combined extracts were dried, and the solvent was removed in vacuo to give a crude product, which was purified by vacuum distillation (150 °C/0.1 Torr) to obtain 0.402 g (89%) of a mixture of 7-butyl-7-pentadecen-1-ol and 7-butyl-6-pentadecen-1-ol.

A mixture of 10% Pd on charcoal (0.1 g), the above alkenols (1.0 g, 4.42 mmol), and 25 mL of MeOH was hydrogenated at room temperature and atmospheric pressure until no more H_2 was consumed. Filtration and evaporation yielded a crude product, which was purified by vacuum distillation (120 °C/0.1 Torr) to obtain 0.812 g (81%) of 7-butyl-1-undecanol. Triethylamine (0.178 g, 1.76 mmol) was added to a solution of this alcohol (0.267 g, 1.17 mmol) in 5 mL of dry CH_2Cl_2. The mixture was cooled to 0 °C, and a solution of methanesulfonyl chloride (0.202 g, 1.76 mmol) in 5 mL of CH_2Cl_2 was added dropwise. The reaction mixture was mantained at 0 °C for 5 h and washed with 2 N HCl and then with $NaHCO_3$ saturated solution. The organic phase was dried, and the solvent was evaporated to obtain 0.333 g (93%) of 7-butyl-1-undecyl methanesulfonate.

7-Octyl-1-pentadecyl Methanesulfonate (26, Z = OMs, n = 6, m = 7, X = C_6H_{17}, Y = H, R = CH_3). This compound was prepared following the reaction sequence described for 7-butyl-1-undecyl methanesulfonate in 43% yield (calculated from 7-bromoheptanoate). ^{1}H-NMR ($CDCl_3$): similar to that of 7-methyl-1-octadecyl methanesulfonate.

General Method for the Preparation of Phosphocholines 3, 10, 11, and 13-16. 1-*O*-(12-Oxooctadecyl)-2-*O*-methylglycero-3-phosphocholine (3). A solution of 3-*O*-benzyl-2-*O*-methylglycerol (**25**) (1.03 g, 5.25 mmol) in 10 mL of dry C_6H_6 was added dropwise to a suspension of KH (9.63 mmol, 1.92 g of 20% dispersion in oil) in 100 mL of dry C_6H_6. The mixture was stirred at room temperature for 1 h, and then a solution of 12-oxo-1-octadecyl methanesulfonate (1.6 g, 4.38 mmol) in 20 mL of dry C_6H_6 was added dropwise. The reaction mixture was stirred at room temperature for 3 h. After addition of H_2O, the reaction mixture was extracted with Et_2O (3 × 50 mL). The combined organic extracts were dried and evaporated under reduced pressure to obtain a crude product, which was purified by flash chromatography on silica gel, eluting with mixtures of petroleum ether:Et_2O of increasing polarity, to give 0.69 g (34%) of 1-*O*-(12-oxooctadecyl)-2-*O*-methyl-3-*O*-benzylglycerol (**27**). ^{1}H-NMR ($CDCl_3$): δ 0.85 (t, 3H, CH_3), 1.22 (m, 20H, 10 CH_2), 1.65 (m, 6H, CH_2CH_2-$COCH_2CH_2$ and OCH_2CH_2), 2.35 (t, 4H, J = 7.5 Hz,

CH_2COCH_2), 3.43 (s, 3H, CH_3O), 3.35–3.57 (m, 7H, CH_2OCH_2-Ph, CH_2OCH_2 and $CHOCH_3$), 4.50 (s, 2H, OCH_2Ph), 7.30 (m, 5H, ArH).

A mixture of the above glycerol derivative (0.84 g, 1.81 mmol), 20% $Pd(OH)_2$ on charcoal (0.488 g), 45 mL of MeOH, and 5 mL of H_2O was hydrogenated at room temperature and atmospheric pressure until no more H_2 was consumed. Filtration and evaporation yielded 0.573 g (85%) of 1-*O*-(12-oxooctadecyl)-2-*O*-methylglycerol. ^{1}H-NMR ($CDCl_3$): essentially identical with that of **23** ($n = 6$, $m = 10$).

From this alcohol the phosphocholine **3** was prepared in 25% yield by reaction with 2-bromoethyl dichlorophosphate and Me_3N as described for compound **2**. ^{1}H-NMR ($CDCl_3$): δ 0.80 (t, 3H, CH_3), 1.17 (m, 20H, 10 CH_2), 1.47 (m, 6H, CH_2CH_2-$COCH_2CH_2$ and OCH_2CH_2), 2.30 (t, 4H, J = 7.2 Hz, CH_2-$COCH_2$), 3.34 (s, 9H, $N^+(CH_3)_3$), 3.35 (s, 3H, CH_3O), 3.27–3.47 (m, 5H, CH_2OCH_2 and $CHOCH_3$), 3.70–3.90 (m, 4H, CH_2N^+ and CH_2OP), 4.25 (m, 2H, $POCH_2$). ^{13}C-NMR ($CDCl_3$): δ 14.07 (CH_3), 22.52–31.67 (13 CH_2), 43.06 (CH_2COCH_2), 54.59 ($N^+(CH_3)_3$), 57.99 (CH_3O), 59.70 (d, $J_{C\text{-}P}$ = 5.1 Hz, $POCH_2$), 65.52 (d, $J_{C\text{-}P}$ = 5.5 Hz, CH_2OP), 66.45 (d, $J_{C\text{-}P}$ = 5.6 Hz, CH_2N^+), 70.06 (CH_2OCH_2), 72.01 (CH_2OCH_2), 79.70 (d, $J_{C\text{-}P}$ = 7.9 Hz, $CHOCH_3$), 213.0 (C=O). Anal. ($C_{27}H_{56}NO_7P\cdot 3H_2O$) C, H, N.

Phospholipids **10**, 11, and **13**–**16** were prepared from 3-*O*-benzyl-2-*O*-methylglycerol and the appropriate mesylate **26** using the same sequence of reactions described for compound **3**. Yields, ^{1}H- and ^{13}C-NMR spectra, and elemental analyses for each compound are given below.

1-*O*-(7-Butylundecyl)-2-*O*-methylglycero-3-phosphocholine (10). Yield: 73% (from the appropriate alcohol **28**). ^{1}H-NMR ($CDCl_3$): δ 0.79 (t, 6H, 2 CH_3), 1.09–1.23 (m, 21H, 10 CH_2 and CH), 1.46 (m, 2H, OCH_2CH_2), 3.30 (s, 9H, $N^+(CH_3)_3$), 3.34 (m, 5H, CH_2OCH_2 and CH_3O), 3.36–3.49 (m, 3H, CH_2OCH_2 and $CHOCH_3$), 3.75 (m, 3H, CH_2N^+ and CH_AH_B-OP), 3.81 (m, 1H, CH_AH_BOP), 4.20 (m, 2H, $POCH_2$). ^{13}C-NMR (CD_3OD): δ 14.32 (terminal CH_3), 23.32–33.88 (11 CH_2), 37.54 (CH), 54.52 ($N^+(CH_3)_3$), 55.03 (CH_3O), 59.53 (d, $J_{C\text{-}P}$ = 4.58 Hz, $POCH_2$), 65.14 (d, $J_{C\text{-}P}$ = 5.73 Hz, CH_2OP), 66.53 (d, $J_{C\text{-}P}$ = 6.36 Hz, CH_2N^+), 70.69 (CH_2OCH_2), 72.12 (CH_2OCH_2), 80.08 (d, $J_{C\text{-}P}$ = 8.02 Hz, $CHOCH_3$). Anal. ($C_{24}H_{52}NO_6P\cdot 3.5H_2O$) C, H, N, P.

1-*O*-(7-Octylpentadecyl)-2-*O*-methylglycero-3-phosphocholine (11). Yield: 28% (from the appropriate alcohol **28**). ^{1}H-NMR (500 MHz, CD_3OD): δ 0.99 (t, 6H, 2 CH_3), 1.32–1.44 (m, 39H, 19 CH_2 and CH), 1.66 (m, 2H, OCH_2CH_2), 3.32 (s, 9H, $N^+(CH_3)_3$), 3.55 (m, 5H, CH_2OCH_2 and CH_3O), 3.60 (m, 1H, $CH_AH_BOCH_2$), 3.65 (m, 3H, $CH_AH_BOCH_2$ and $CHOCH_3$), 3.75 (m, 2H, CH_2N^+), 4.01 (m, 1H, CH_AH_BOP), 4.09 (m, 1H, CH_AH_BOP), 4.41 (m, 2H, $POCH_2$). Anal. ($C_{32}H_{68}NO_6P\cdot 4.5H_2O$) C, H, N.

1-*O*-(7-Phenylpentadecyl)-2-*O*-methylglycero-3-phosphocholine (13). Yield: 56% (from the appropriate alcohol **28**). ^{1}H-NMR ($CDCl_3$): δ 0.89 (t, 3H, terminal CH_3), 1.15 (m, 18H, 9 CH_2), 1.32–1.59 (m, 6H, OCH_2CH_2 and CH_2CHCH_2), 2.39 (m, 1H, CHPh), 3.21 (s, 9H, $N^+(CH_3)_3$), 3.23–3.45 (m, 8H, CH_2OCH_2 and $CHOCH_3$), 3.63 (m, 2H, CH_2N^+), 3.70 (m, 1H, CH_AH_BOP), 3.82 (m, 1H, CH_AH_BOP), 4.19–4.28 (m, 2H, $POCH_2$), 7.02–7.24 (m, 5H, ArH). ^{13}C-NMR ($CDCl_3$): δ 14.29 (terminal CH_3), 22.86–37.31 (12 CH_2), 46.40 (CHPh), 54.52 ($N^+(CH_3)_3$), 58.06 (CH_3O), 59.58 (d, $J_{C\text{-}P}$ = 4.70 Hz, $POCH_2$), 65.29 (d, $J_{C\text{-}P}$ = 5.47 Hz, CH_2OP), 66.51 (d, $J_{C\text{-}P}$ = 6.24 Hz, CH_2N^+), 70.54 (CH_2OCH_2), 72.08 (CH_2OCH_2), 79.87 (d, $J_{C\text{-}P}$ = 8.02 Hz, $CHOCH_3$), 126.29–146.89 (Ar). Anal. ($C_{30}H_{56}$-$NO_6P\cdot 4.5H_2O$) C, H, N, P.

1-*O*-(7-Methyloctadecyl)-2-*O*-methylglycero-3-phosphocholine (14). Yield: 35% (from the appropriate alcohol **28**). ^{1}H-NMR (CD_3OD): δ 0.82–0.93 (m, 6H, 2 CH_3), 1.15–1.40 (m, 29H, 14 CH_2 and CH), 1.56 (m, 2H, OCH_2CH_2), 3.23 (s, 9H, $N^+(CH_3)_3$), 3.46 (m, 5H, CH_2OCH_2 and CH_3O), 3.51–3.59 (m, 3H, CH_2OCH_2 and $CHOCH_3$), 3.61–3.63 (m, 2H, CH_2N^+), 3.81–4.01 (m, 2H, CH_2OP), 4.26 (m, 2H, $POCH_2$). ^{13}C-NMR (CD_3OD): δ 14.63 (terminal CH_3), 20.30 ($CHCH_3$), 23.84–38.32 (15 CH_2), 34.03 (CH), 54.71 (t, $J_{C\text{-}N}$ = 3.76 Hz, $N^+(CH_3)_3$), 58.32 (CH_3O), 60.41 (d, $J_{C\text{-}P}$ = 4.84 Hz, $POCH_2$), 66.20 (d, $J_{C\text{-}P}$ = 5.72 Hz, CH_2OP), 67.43 (m, CH_2N^+), 71.16 (CH_2OCH_2), 72.73 (CH_2OCH_2), 80.89 (d, $J_{C\text{-}P}$ = 8.03 Hz, $CHOCH_3$). Anal. ($C_{28}H_{60}$-$NO_6P\cdot 4H_2O$) C, H, N.

1-*O*-(7-Hexyloctadecyl)-2-*O*-methylglycero-3-phosphocholine (15). Yield: 24% (from the appropriate alcohol **28**). ^{1}H-NMR (CD_3OD): δ 0.89 (t, 6H, 2 CH_3), 1.20–1.41 (m, 39H, 19 CH_2 and CH), 1.57 (m, 2H, OCH_2CH_2), 3.29 (s, 9H, $N^+(CH_3)_3$), 3.46 (m, 5H, CH_2OCH_2 and CH_3O), 3.55 (m, 3H, CH_2OCH_2 and $CHOCH_3$), 3.65 (m, 2H, CH_2N^+), 3.88 (m, 1H, CH_AH_BOP), 3.94 (m, 1H, CH_AH_BOP), 4.28 (m, 2H, $POCH_2$). Anal. ($C_{33}H_{70}NO_6P\cdot 1H_2O$) C, H, N, P.

1-*O*-(12-Hexyloctadecyl)-2-*O*-methylglycero-3-phosphocholine (16). Yield: 34% (from the appropriate alcohol **28**). ^{1}H-NMR ($CDCl_3$): δ 0.82 (t, 6H, 2 CH_3), 1.11–1.28 (m, 39H, 19 CH_2 and CH), 1.49 (m, 2H, OCH_2CH_2), 3.35 (s, 9H, $N^+(CH_3)_3$), 3.39 (m, 5H, CH_2OCH_2 and CH_3O), 3.40–3.46 (m, 3H, CH_2OCH_2 and $CHOCH_3$), 3.69 (m, 3H, CH_2N^+ and CH_AH_BOP), 3.83 (m, 1H, CH_AH_BOP), 4.23 (m, 2H, $POCH_2$). Anal. ($C_{33}H_{60}NO_6P\cdot 4.5H_2O$) C, H, N, P.

1-*O*-(16-Phenylhexadecyl)-2-*O*-methylglycero-3-phosphocholine (12). Sodium hydride (0.459 g, 15.3 mmol of a 80% suspension in mineral oil) was washed with dry petroleum ether under a nitrogen atmosphere prior to addition of 15 mL of dry DMF. A solution of alcohol **25** (0.6 g, 3.06 mmol) in 15 mL of DMF was added dropwise, and the reaction mixture was stirred at room temperature for 2 h. A solution of 1-bromo-16-phenylhexadecane (1.748 g, 4.59 mmol) in 20 mL of DMF was added dropwise. After the reaction mixture was stirred at room temperature for 24 h, EtOH and H_2O were added, and the mixture was extracted with Et_2O (3 × 100 mL). The combined organic extracts were dried and evaporated under reduced pressure to obtain a crude product, which was purified by flash chromatography on silica gel, eluting with mixtures of petroleum ether:Et_2O of increasing polarity, to give 0.38 g (25%) of 1-*O*-(16-phenylhexadecyl)-2-*O*-methyl-3-*O*-benzylglycerol. ^{1}H-NMR ($CDCl_3$): δ 1.25 (b s, 24H, 12 CH_2), 1.55 (m, 4H, OCH_2CH_2 and CH_2CH_2Ph), 2.57 (t, J = 7.5 Hz, 2H, CH_2Ph), 3.45 (s, 3H, CH_3O), 3.35–3.60 (m, 7H, CH_2OCH_2, CH, and CH_2OCH_2Ph), 4.52 (s, 2H, OCH_2Ph), 7.12–7.35 (m, 10H, ArH).

The debenzylation and formation of the phosphocholine were carried out as described for compound **3** to yield phosphocholine **12**. Yield: 45% (from the appropriate alcohol **28**). ^{1}H-NMR ($CDCl_3$): δ 1.20 (b s, 24H, 12 CH_2), 1.52 (m, 4H, OCH_2CH_2 and CH_2CH_2Ph), 2.52 (t, 2H, J = 7.5 Hz, CH_2Ph), 3.30 (s, 9H, $N^+(CH_3)_3$), 3.35 (s, 3H, CH_3O), 3.30–3.50 (m, 5H, CH_2OCH_2 and $CHOCH_3$), 3.77 (m, 2H, CH_2N^+), 3.87 (m, 2H, CH_2OP), 4.25 (m, 2H, $POCH_2$), 7.10–7.30 (m, 5H, ArH). ^{13}C-NMR ($CDCl_3$): δ 26.33–31.78 (14 CH_2), 36.23 (CH_2Ph), 54.61 ($N^+(CH_3)_3$), 58.05 (CH_3O), 59.63 (b s, $POCH_2$), 65.34 (b s, CH_2OP), 66.58 (d, $J_{C\text{-}P}$ = 6.20 Hz, CH_2N^+), 70.46 (CH_2OCH_2), 72.15 (CH_2OCH_2), 79.90 (d, $J_{C\text{-}P}$ = 8.09 Hz, $CHOCH_3$), 126.10–128.96 and 143.57 (Ar). Anal. ($C_{31}H_{58}NO_6P\cdot 3H_2O$) C, H, N.

1-*O*-(4-Oxooctadecyl)-2-*O*-methylglycero-3-phosphocholine (1). A solution of alcohol **25** (0.580 g, 2.9 mmol) in 10 mL of dry toluene was added to a suspension of powdered KOH (0.684 g, 12.2 mmol) in 24 mL of dry toluene. The reaction mixture was refluxed for 1 h in a Dean–Stark apparatus, and then a solution of 4-(ethylenedioxy)-1-octadecyl methanesulfonate (1.0 g, 2.4 mmol) in 16 mL of toluene was added dropwise. The reaction mixture was refluxed in the Dean–Stark apparatus for an additional 5 h period, and then the toluene was evaporated under reduced pressure and some H_2O was added. The resulting solution was extracted with Et_2O (3 × 50 mL). The combined organic extracts were dried and evaporated under reduced pressure to obtain a crude product, which was purified by flash chromatography on silica gel, eluting with mixtures of petroleum ether:Et_2O of increasing polarity, to give 0.569 g (46%) of 1-*O*-[4-(ethylenedioxy)-octadecyl]-2-*O*-methyl-3-*O*-benzylglycerol. ^{1}H-NMR ($CDCl_3$): δ 0.85 (t, 3H, terminal CH_3), 1.20 (b s, 24H, 12 CH_2), 1.50–1.62 (m, 6H, $CH_2CH_2C(OCH_2CH_2O)CH_2$), 3.40 (s, 3H, CH_3O), 3.35–3.57 (m, 7H, CH_2OCH_2, $CHOCH_3$, and CH_2OCH_2Ph), 3.90 (s, 4H, OCH_2CH_2O), 4.52 (s, 2H, OCH_2Ph), 7.20–7.35 (m, 5H, ArH).

A mixture of the above intermediate (0.648 g, 1.28 mmol), 0.3 mL of 35% HCl, 20% $Pd(OH)_2$ on charcoal (0.345 g), 27

mL of MeOH, and 3 mL of H_2O was hydrogenated at room temperature and atmospheric pressure until no more H_2 was consumed. The solution was filtered and evaporated to dryness to give 0.425 g (89%) of 1-*O*-(4-oxooctadecyl)-2-*O*-methylglycerol. This product was used without further purification in the next step. ^{1}H-NMR ($CDCl_3$): δ 0.82 (t, 3H, terminal CH_3), 1.20 (b s, 22H, 11 CH_2), 1.50 (m, 2H, $COCH_2CH_2$), 1.79 (m, 2H, CH_2CH_2CO), 2.34 (t, 2H, J = 7.3 Hz, CH_2COCH_2), 2.42 (t, 2H, J = 7.2 Hz, CH_2COCH_2), 3.40 (s, 3H, CH_3O), 3.30–3.42 (m, 3H, $CHOCH_3$ and CH_2OCH_2), 3.45 (m, 2H, CH_2OCH_2), 3.57 (dd, 1H, J_{AB} = 11.5 Hz, J_{BC} = 5.4 Hz, CH_ACH_BOH), 3.68 (dd, 1H, J_{AB} = 11.5 Hz, J_{AC} = 3.9 Hz, CH_ACH_BOH).

Phosphocholine **1** was prepared in 54% yield by reaction of this alcohol with 2-bromoethyl dichlorophosphate and then with Me_3N, as described for compound **2**. ^{1}H-NMR ($CDCl_3$): δ 0.80 (t, 3H, terminal CH_3), 1.10–1.25 (m, 22H, 11 CH_2), 1.43 (m, 2H, $COCH_2CH_2$), 1.72 (m, 2H, CH_2CH_2CO), 2.29 (t, 2H, J = 7.4 Hz, $COCH_2$), 2.35 (t, 2H, J = 7.4 Hz, CH_2CO), 3.32 (s, 9H, $N^+(CH_3)_3$), 3.36 (s, 3H, CH_3O), 3.25–3.47 (m, 5H, $CHOCH_3$ and CH_2OCH_2), 3.75 (m, 4H, CH_2N^+ and CH_2OP), 4.21 (m, 2H, $POCH_2$). ^{13}C-NMR ($CDCl_3$): δ 14.25 (terminal CH_3), 22.83–32.10 (9 CH_2), 39.34 (CH_2CH_2O), 43.12 (CH_2COCH_2), 54.50 ($N^+(CH_3)_3$), 58.05 (CH_3O), 59.55 (d, J_{C-P} = 4.3 Hz, $POCH_2$), 64.78 (d, J_{C-P} = 5.4 Hz, CH_2OP), 66.51 (d, J_{C-P} = 6.9 Hz, CH_2N^+), 70.60 (CH_2OCH_2), 70.92 (CH_2OCH_2), 79.98 (d, J_{C-P} = 8.0 Hz, $CHOCH_3$), 211.91 (C=O). Anal. ($C_{27}H_{56}NO_7P\cdot3.5H_2O$) C, H, N.

1-*O*-(12-Oxooctadecyl)-2-*O*-methylglycero-3-phosphoserine (5). To a stirred and ice–water-cooled solution of imidazole (0.627 g, 9.22 mmol, evaporated from dry CH_3CN) in 8 mL of CH_3CN was added dropwise PCl_3 (0.242 mL, 2.77 mmol) and triethylamine (1.35 mL, 9.73 mmol). After 15 min of stirring, a solution of 1-*O*-(12-oxooctadecyl)-2-*O*-methylglycerol (0.24 g, 0.64 mmol) in 8 mL of CH_3CN was added dropwise. The ice–water bath was removed, and the mixture was stirred for 4 h at room temperature. Water (5 mL) was added, and the resulting solution was stirred at room temperature for 30 min. The mixture was first evaporated to dryness and then coevaporated with 11 mL of a pyridine:Et_3N (4:1) mixture. After evaporation to dryness the residue was dissolved in 200 mL of $CHCl_3$. The organic phase was washed with water and dried, and the solvent was evaporated to obtain the triethylammonium salt of 1-*O*-(12-oxooctadecyl)-2-*O*-methylglycero-3-*H*-phosphonic acid (quantitative yield). The product was used without purification in the next step. ^{1}H-NMR ($CDCl_3$): δ 0.65 (t, 3H, terminal CH_3), 0.95–1.15 (m, 33H, 12 CH_2 and $HN^+(CH_2CH_3)_3$), 1.35 (m, 6H, OCH_2CH_2 and $CH_2CH_2COCH_2CH_2$), 2.15 (t, 4H, CH_2COCH_2), 2.85 (q, 6H, $HN^+(CH_2CH_3)_3$), 3.20 (s, 3H, CH_3O), 3.10–3.40 (m, 5H, $CHOCH_3$ and CH_2OCH_2), 3.75–4.00 (m, 2H, CH_2OP), 6.62 (d, 1H, J_{P-H} = 618.7 Hz, PH).

A mixture of the above phosphonate (evaporated from dry pyridine), *N*-(benzyloxycarbonyl)-L-serine benzyl ester (0.34 g, 1.03 mmol, evaporated from dry pyridine), 5 mL of pyridine, and pivaloyl chloride (0.158 mL, 1.29 mmol) was stirred at room temperature for 1.5 h. Iodine (0.327 g, 1.29 mmol) and H_2O (0.256 mL) were added to the reaction mixture, which was stirred at room temperature for 25 min. Then 100 mL of $CHCl_3$ and 20 mL of 5% aqueous $NaHSO_3$ solution were added. The organic phase was dried, and the solvent was evaporated under reduced pressure to obtain a crude product, which was purified by flash chromatography on silica gel, eluting with petroleum ether:$CHCl_3$ mixtures of increasing polarity and then with $CHCl_3$:MeOH mixtures of increasing polarity, to give 40 mg (8%) of 1-*O*-(12-oxooctadecyl)-2-*O*-methylglycero-3-phosphobenzyl-*N*-(carbobenzyloxy)serine. ^{1}H-NMR ($CDCl_3$): δ 0.85 (t, 3H, terminal CH_3), 1.20 (m, 24H, 12 CH_2), 1.52 (m, 6H, OCH_2CH_2 and $CH_2CH_2COCH_2CH_2$), 2.35 (t, 4H, CH_2COCH_2), 3.40 (s, 3H, CH_3O), 3.30–3.55 (m, 5H, $CHOCH_3$ and CH_2OCH_2), 3.92–4.17 (m, 2H, CH_2OP), 4.30 (m, 1H, serine), 4.44 (m, 1H, serine), 4.58 (m, 1H, serine), 5.05–5.22 (m, 4H, 2 OCH_2Ph), 7.20–7.35 (m, 10H, ArH). ^{13}C-NMR ($CDCl_3$): δ 14.10 (terminal CH_3), 22.20–32.00 (13 CH_2), 43.00 (CH_2COCH_2), 58.39 (CH_3O), 54.85 (d, J_{C-P} = 7.4 Hz, CH serine), 66.97 (d, J_{C-P} = 5.6 Hz, $POCH_2$), 67.49 and 68.09 (2 OCH_2Ph), 69.47 (s, CH_2OP), 69.75 (CH_2OCH_2), 72.26 (CH_2OCH_2), 79.16 (d, J_{C-P} = 6.8 Hz, $CHOCH_3$), 128.20–129.50, 135.90 and 136.90 (Ar), 157.20 (NHCOO), 170.00 ($COOCH_2Ph$), 212.80 (C=O).

A mixture of the above intermediate (5 mg, 0.0065 mmol), 20% $Pd(OH)_2$ on charcoal (5.0 mg), 4.5 mL of MeOH, and 0.5 mL of H_2O was hydrogenated at room temperature and atmospheric pressure until no more H_2 was consumed. The catalyst was removed by filtration, and the solvents were evaporated under reduced pressure to obtain 2 mg (57%) of **5**. R_f 0.11 ($CHCl_3$/MeOH/H_2O, 65:25:4, v/v/v). Anal. ($C_{25}H_{50}NO_9P\cdot1H_2O$) C, H, N.

1-*O*-(7-Oxooctadecyl)-2-*O*-methylglycero-3-phosphoserine (4). The intermediate 1-*O*-[7-(ethylenedioxy)octadecyl]-2-*O*-methylglycerol was obtained in 85% yield from 1-*O*-[7-(ethylenedioxy)octadecyl]-2-*O*-methyl-3-*O*-benzylglycerol, using the same procedure described for the synthesis of 1, except for the acidic treatment. ^{1}H-NMR ($CDCl_3$): δ 0.82 (t, 3H, terminal CH_3), 1.20–1.35 (m, 24H, 12 CH_2), 1.52 (m, 6H, $CH_2C(OCH_2CH_2O)CH_2$ and OCH_2CH_2), 3.42 (s, 3H, CH_3O), 3.35–3.42 (m, 3H, $CHOCH_3$ and OCH_2), 3.47 (m, 2H, CH_2O), 3.59 (dd, 1H, J_{AB} = 11.2 Hz, J_{BC} = 5.5 Hz), 3.71 (dd, 1H, J_{AB} = 11.2 Hz, J_{AC} = 3.9 Hz), 3.90 (s, 4H, OCH_2CH_2O). ^{13}C-NMR ($CDCl_3$): δ 14.29 (terminal CH_3), 22.88–32.14 (13 CH_2), 37.30 and 37.40 ($CH_2C(OCH_2CH_2O)CH_2$), 58.10 (CH_3O), 62.95 (CH_2OH), 65.23 (OCH_2CH_2O), 70.94 (CH_2OCH_2), 72.22 (CH_2OCH_2), 80.25 ($CHOCH_3$), 112.36 ($C(OCH_2CH_2O)$).

The phosphoserine **4** was obtained in 18% yield from 1-*O*-[7-(ethylenedioxy)octadecyl]-2-*O*-methylglycerol, using the same procedure described for the synthesis of **5**, but in this case the simultaneous removal of the protecting groups was achieved in the presence of HCl. R_f 0.09 ($CHCl_3$/MeOH/H_2O, 65:25:4, v/v/v). Anal. ($C_{25}H_{50}NO_9P\cdot1H_2O$) C, H, N.

***N*-[5-[2-Methoxy-3-[(7-oxooctadecyl)oxy]propoxy]-1-pentyl]-*N,N,N*-trimethylammonium Bromide (6).** Sodium hydride (0.162 g, 5.64 mmol of 80% oil dispersion), NaI (0.027 g, 0.18 mmol), and 1,5-dibromopentane (2.48 g, 10.8 mmol) were placed in 25 mL of dry DMF under nitrogen. A solution of 1-*O*-(7-oxooctadecyl)-2-*O*-methylglycerol (0.7 g, 1.8 mmol) in 10 mL of DMF was added dropwise. The reaction mixture was stirred at room temperature for 24 h, and a small amount of EtOH and then H_2O (50 mL) were added. The solution was extracted with Et_2O (3 × 50 mL), the organic phase was dried, and the solvent was removed in vacuo. The crude product was purified by flash chromatography on silica gel, eluting with mixtures of petroleum ether:Et_2O of increasing polarity, to give 0.192 g (20%) of 1-[(7-oxooctadecyl)oxy]-2-methoxy-3-[(5-bromopentyl)oxy]propane. ^{1}H-NMR ($CDCl_3$): δ 0.79 (t, 3H, terminal CH_3), 1.17 (m, 20H, 10 CH_2), 1.48 (m, 10H, 5 CH_2), 1.80 (m, 2H, CH_2CH_2Br), 2.30 (t, 4H, CH_2COCH_2), 3.30–3.48 (m, 14H, CH_2OCH_2, $CHOCH_3$, and $CH_2OCH_2(CH_2)_3CH_2Br$).

A solution of the above intermediate (0.192 g, 0.36 mmol) in 10 mL of dry $CHCl_3$ was transferred into a thick-walled glass flask. Dry trimethylamine (0.5 mL) was added while cooling the flask in dry ice. The flask was sealed and heated to 65 °C for 24 h. After the mixture was cooled, the solution was evaporated to dryness to give a crude product which was purified by flash chromatography on silica gel, eluting with mixtures of petroleum ether:$CHCl_3$ of increasing polarity and subsequently with mixtures of $CHCl_3$:MeOH of increasing polarity, to give 0.15 g (70%) of **6**. ^{1}H-NMR (CD_3OD): δ 0.85 (t, 3H, terminal CH_3), 1.33 (m, 20H, 10 CH_2), 1.46–1.65 and 1.72 and 1.90 (3 m, 12H, $OCH_2CH_2CH_2CH_2CH_2N^+$, OCH_2CH_2, and $CH_2CH_2COCH_2CH_2$), 2.75 (t, 4H, CH_2COCH_2), 3.21 (s, 9H, $N^+(CH_3)_3$), 3.47 (s, 3H, CH_3O), 3.43–3.61 (m, 11H, CH_2OCH_2, $CHOCH_3$, and $CH_2OCH_2(CH_2)_3CH_2N^+$). Anal. ($C_{30}H_{62}NO_4Br\cdot4H_2O$) C, H, N.

***N*-[5-[2-Methoxy-3-(octadecyloxy)propoxy]-1-pentyl]-*N,N,N*-trimethylammonium Bromide (19).** The intermediate 1-*O*-octadecyl-2-*O*-methylglycerol was obtained in 32% yield from 3-*O*-benzyl-2-*O*-methylglycerol and 1-octadecyl methanesulfonate by the sequence described for the synthesis of **3** (Scheme 2). ^{1}H-NMR ($CDCl_3$): δ 0.82 (t, 3H, terminal CH_3), 1.21 (m, 28H, 14 CH_2), 1.52 (m, 2H, OCH_2CH_2), 3.43 (s, 3H, CH_3O), 3.36–3.45 (m, 3H, $CHOCH_3$ and CH_2OCH_2), 3.49 (m, 2H, CH_2OCH_2), 3.61 (dd, 1H, CH_ACH_BOH), 3.72 (dd, 1H, CH_ACH_BOH).

The ammonium salt **19** was prepared in 45% yield from the appropriate bromide (obtained from the primary alcohol in 15% yield) by reaction with trimethylamine. ^{1}H-NMR (CD_3OD): δ 0.93 (t, 3H, terminal CH_3), 1.30 (m, 30H, 15 CH_2), 1.46 and 1.56 and 1.67 and 1.84 (4 m, 8H, $OCH_2(CH_2)_3CH_2N^+$ and OCH_2CH_2), 3.16 (m, 9H, $N^+(CH_3)_3$), 3.44 (s, 3H, CH_3O), 3.35–3.55 (m, 11H, CH_2OCH_2, $CHOCH_3$, and $CH_2OCH_2(CH_2)_3CH_2N^+$). Anal. ($C_{30}H_{64}NO_3Br$) C, H, N.

***N*-[5-[3-[(12-Hydroxyoctadecyl)oxy]-2-methoxypropoxy]-1-pentyl]-*N,N,N*-trimethylammonium Bromide (7).** The compound 1-*O*-(12-oxooctadecyl)-2-*O*-methylglycerol was used as a common intermediate in the preparation of compounds **3** and **5**. The reaction of this primary alcohol with 1,5-dibromopentane, as described for the synthesis of **6**, gave 1-[(12-oxooctadecyl)oxy]-2-methoxy-3-[(5-bromopentyl)oxy]propane in 31% yield. ^{1}H-NMR ($CDCl_3$): δ 0.84 (t, 3H, terminal CH_3), 1.17 (m, 20H, 10 CH_2), 1.42–1.62 (m, 10H, 5 CH_2), 1.84 (m, 2H, CH_2CH_2Br), 2.35 (t, 4H, CH_2COCH_2), 3.43 (s, 3H, CH_3O), 3.34–3.50 (m, 11H, CH_2OCH_2, $CHOCH_3$, and $CH_2OCH_2(CH_2)_3CH_2Br$).

Sodium borohydride (2.0 mg, 0.0057 mmol) was placed in 4 mL of anhydrous EtOH under nitrogen. The above bromide (0.03 g, 0.0057 mmol) in 4 mL of EtOH was added dropwise. The reaction mixture was stirred at room temperature for 6 h, and then H_2O (8 mL) was added. The solution was extracted with Et_2O (3 × 10 mL). The organic phase was dried, and the solvent was evaporated under reduced pressure to obtain 0.027 g (90%) of 1-[(12-hydroxyoctadecyl)oxy]-2-methoxy-3-[(5-bromopentyl)oxy]propane. This compound was used without purification in the next step. ^{1}H-NMR ($CDCl_3$): δ 0.85 (t, 3H, terminal CH_3), 1.20–1.60 (m, 18H, 9 CH_2), 1.84 (m, 2H, CH_2CH_2Br), 3.42 (s, 3H, CH_3O), 3.32–3.55 (m, 12H, CH_2OCH_2, $CHOH$, $CHOCH_3$, and $CH_2OCH_2(CH_2)_3CH_2Br$).

The ammonium salt **7** was prepared in 42% yield by reaction of the above intermediate with trimethylamine as described above. ^{1}H-NMR ($CDCl_3$ + CD_3OD): δ 0.75 (t, 3H, terminal CH_3), 1.12–1.75 (m, 36H, 18 CH_2), 3.10 (m, 9H, $N^+(CH_3)_3$), 3.29 (s, 3H, CH_3O), 3.20–3.44 (m, 12H, CH_2OCH_2, $CHOH$, $CHOCH_3$, and $CH_2OCH_2(CH_2)_3CH_2N^+$). Anal. ($C_{30}H_{64}NO_4Br$) C, H, N.

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