# Synthesis and Antitumor Activity of New Alkylphospholipids Containing Modifications of the Phosphocholine Moiety

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**New antitumor alkylglycerophospholipids, in which primarily the phosphocholine moiety of the platelet activating factor (PAF) molecule was modified, were synthesized from 1-alkyl-2-substituted glycerols by introducing polar head phosphoryl groups having methylene bridges of various lengths (from 2 to 14 carbons). They were tested for PAF agonistic activity and antitumor properties. In a series of 1-octadecyl-2-acetoacetylglycerophospholipids (1a—f), an increase in the length of the methylene bridge separating the phosphate and trimethylammonio group in the polar head side chain at position 3 of the glycerol backbone resulted in a progressive decrease in PAF agonistic activity and a characteristic change in antitumor activity against human promyelocytic leukemia cells (HL-60). Maximal potency was obtained with the compound having a decamethylene bridge (1e, $IC_{50}$ value = 1.5 μg/ml). Thus, alkylphospholipids possessing a decamethylene bridge and a variety of substituents at position 2 (1g—n) were synthesized. They showed potent inhibitory activity with $IC_{50}$ values ranging from 0.4 to 1.9 μg/ml, depending on the nature of the 2-substituent in the phospholipid molecule. In *in vivo* tests of the present series of alkylglycerophospholipids (1a—n), using mice bearing sarcoma 180 and mice with mammary carcinoma MM46 (both cells and compounds were given i.p.), 1-octadecyl-2-acetoacetyl-3-glyceryl ω-trimethylammoniodecyl phosphate (1e) showed the most potent life-prolonging effect. The structure–activity relationships are discussed.**

**Keywords** alkyl lysophospholipid; chemical modification; octadecylglycerophospholipid; antitumor activity; sarcoma 180; mammary carcinoma MM46; human promyelocytic leukemia cell HL-60; PAF agonistic activity; structure–activity relationship

Since the discovery of the ether-lipid structure of platelet activating factor (PAF), a mediator involved in mammalian allergic and inflammatory processes, a great deal of attention has been focused on alkyl ether phospholipids and their effects on the host defense system.[1] A typical antitumor agent in this class is 1-octadecyl-2-methylglycero-3-phosphocholine (ET-18-OMe), which has been subjected to clinical trials in West Germany.[2] However, its use in therapy has been limited by undesirable side effects, at least partly attributed to PAF agonistic action.[3] Recent reports have indicated that Ilmofosine (a sulfur-containing phospholipids, BM-41440, currently in clinical studies) is as active as ET-18-OMe in direct tumoricidal activity, but has no PAF action.[4]

We have reported the synthesis and antitumor activity of alkyl ether phospholipids where the alkyl ether chain at position 1 of the glycerol backbone, the polar head base at position 3 and the glycerol moiety itself were modified.[5-9] In the hope that appropriate structural alterations would result in an antitumor phospholipid which has elevated antitumor activity together with reduced side effects by at least removing the PAF agonistic action, we have been studying the structure–activity relationship of this important phospholipid molecule and have found that a 2-acetoacetyl analog (**1a**) of PAF showed favorable activities as an antitumor agent,[5] but its PAF agonistic action, though very weak, still remained. A recent structure–activity relationship study of PAF, directed toward separation of the blood pressure and platelet aggregation activities, showed that these biological activities can be removed by modification of the phosphocholine portion of the molecule.[10] This led us to synthesize a series of 1-alkyl-2-acetoacetylglycerophospholipids having an extended methylene bridge in the 3-polar head side chain and to clarify the influence of such structural changes on both antitumor activity and PAF agonistic action. The present report deals with the synthesis and antitumor activities of a new series of alkylglycerophospholipids in which we primarily altered the phosphocholine portion and also made modifications in other portions of the molecule.

## Chemistry

With the aim of obtaining alkylphospholipids with appropriate structural modifications which exhibit potent antitumor activity and reduced undesirable side effects, a series of 1-octadecyl-2-acetoacetylglycerophospholipids, mainly modified in the length of the methylene bridge between the phosphate and trimethylammonium group (**1a—f**), were synthesized as outlined in Charts 1 and 2.

The key intermediates, 2-benzyl-3-octadecylglyceryl ω-trimethylammonioalkyl phosphates (**7a—f**) were synthesized from 2-benzyl-3-octadecylglycerol (**6**)[10,11] by phosphorylation according to either method A or B. Thus, compounds **7a—f** were obtained by reaction with $POCl_3$ followed by treatment with an appropriate ω-hydroxyalkyl trimethylammonium tosylate[12] (**3a—f**, method A). The cationic alcohols (**3a—f**) were prepared for this study either

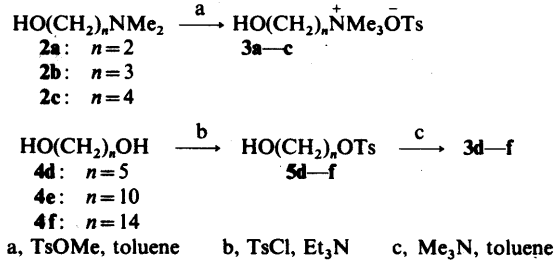
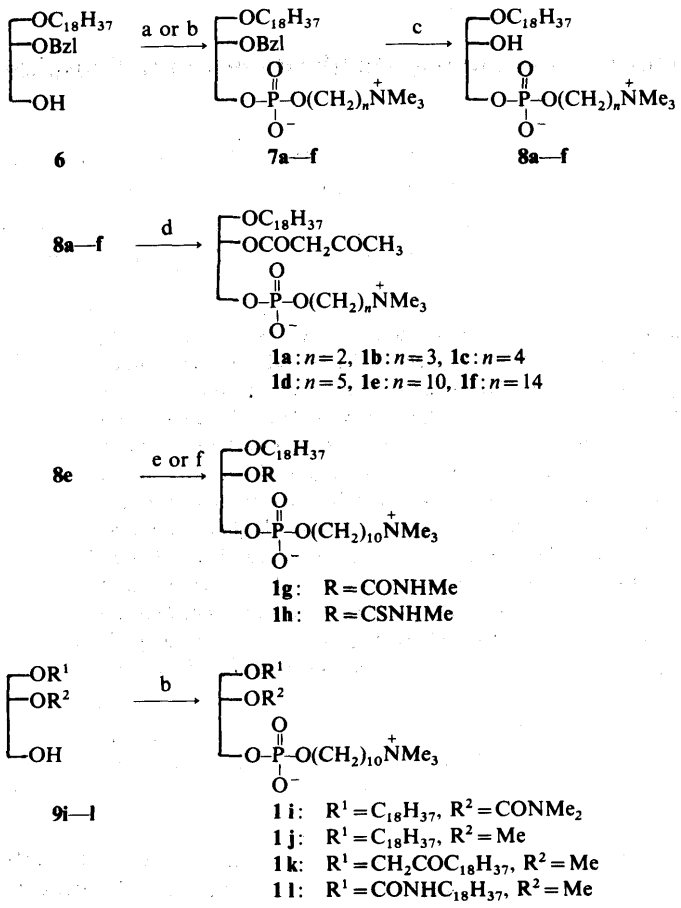



Chart 1




a: $POCl_3$, $Et_3N$, $CHCl_3$; **3a—f**, pyridine (method A) b: $Et_3N$, $Br(CH_2)_{10}OP(O)Cl_2$, toluene; $NMe_3$, toluene (method B) c: $H_2$/Pd–C, AcOH d: diketene, pyridine, $CH_2Cl_2$ e: MeNCO, pyridine f: MeNCS, pyridine

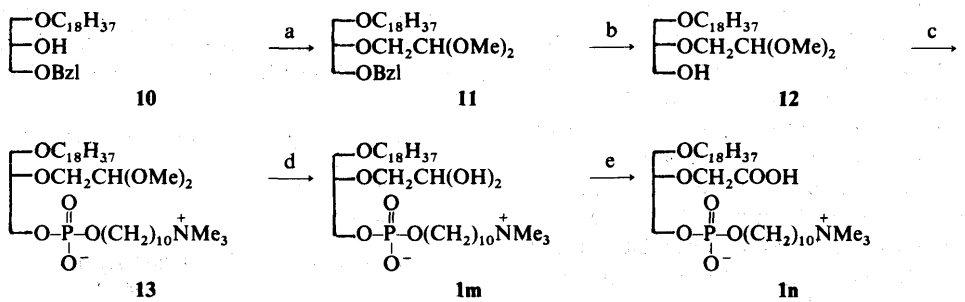
Chart 2

by reaction of an appropriate $\omega$-dimethylamino alcohol (**2a—c**) with methyl tosylate or by reaction of the appropriate alkylenediol monotosylates (**5d—f**) with trimethylamine. With respect to compound **7e** ($n=10$), the alternative synthesis involved condensation of the disubstituted glycerol **6** with 10-bromodecyl phosphodichloridate[13] and subsequent treatment of the intermediary 10-bromodecyl glyceryl phosphate with trimethylamine (method B). The benzyl ether groups were removed from **7a—f** by hydrogenolysis using a palladium catalyst. The resulting 1-octadecyllysophospholipids (**8a—f**) which had 3-methylene bridges of various lengths were novel except for **8a**.[9,11] The reaction of **8a—f** with diketene led to a new series of phospholipids, 2-acetoacetyl-3-octadecylglyceryl $\omega$-trimethylammonioalkyl phosphates (**1a—f**).

As described below, we found that, among the congeners (**1a—f**), a 2-acetoacetylglycerophospholipid with a decamethylene bridge (**1e**, the decamethylene bridge compound) had the greatest antitumor activity; this led us to synthesize further analogs having a decamethylene bridge as a structural feature. To clarify the contribution of the nature of the substituent at position 2, we synthesized a variety of 1-octadecyl-2-substituted glycerophospholipids with a decamethylene bridge in the polar head side chain (Chart 2). The 2-methylcarbamoyl (**1g**) and 2-methylthiocarbamoyl (**1h**) derivatives were prepared by treating compound **8e** with methyl isocyanate and methyl isothiocyanate, respectively. The 2-dimethylcarbamoyl and 2-methyl compounds (**1i** and **1j**) were prepared from the 3-octadecyl-2-substituted glycerols, (**9i** and **9j**), by esterification with 10-bromodecyl phosphodichloridate. Compounds **9i** and **9j** were synthesized by the methods previously reported by us.[9,14] To synthesize the 2-formylmethyl compound with a 10-trimethylammoniodecyl phosphate group (**1m**), 1-benzyl-3-octadecylglycerol (**10**)[12] was first etherified with bromoacetoaldehyde dimethyl acetal using a phase transfer catalyst (Chart 3). The resulting ether (**11**), after debenzylation by catalytic hydrogenation, gave 2-(2,2-dimethoxyethyl)-3-octadecylglycerol (**12**). Reaction with $POCl_3$, followed by reaction with a cationic decanol (**3e**), gave the dimethylacetal (**13**) which, on treatment with a cation-exchange resin (Amberlite IR-120, H-form), yielded 1-octadecyl-2-formylmethylphospholipid (**1m**). However, the product isolated was always contaminated by a small amount of the 2-carboxymethyl compound (**1n**), formed from **1m** by inevitable exposure to air.

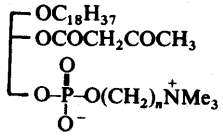
Accordingly, to obtain a stable test compound, **1m** was converted to 1-octadecyl-2-carboxymethylglycerolipid (**1n**) in a pure form by oxidation with air.

To examine the effect of structural changes in the alkyl portion (nonpolar tail) of the present series of phospholipids on the antitumor properties, further modification of compound **1j** was undertaken. 1-(2-Oxoicosyl)-2-methylglycero- and 1-octadecylcarbamoyl-2-methylglycerophospholipids (**1k** and **1l**) were synthesized by the method shown in Chart 2. The intermediates (**9k** and **9l**) were synthesized by the methods reported by us.[15,16] These novel




a: $BrCH_2CH(OMe)_2$, $C_{16}H_{33}NMe_3Cl$, 50% NaOH b: $H_2$/Pd–C, EtOH c: $POCl_3$, $Et_3N$, $CHCl_3$; **3e**, pyridine d: Amberlite IR-120 [H], $THF-H_2O$ (4:1) e: air/charcoal, acetone

TABLE I. Biological Activity of Compounds **1a—f**



| Compound | $n$ | Antitumor activity, T/C (%) HL-60 $IC_{50}$ ($\mu$g/ml) | S 180 Day−4[a] 1 mg/mouse[d] | S 180 Days 0—2 0.33 mg/mouse | S 180 Days 0—2 1 mg/mouse | MM 46 Days 2—5 0.25 mg/mouse | PAF-Agonistic and antagonistic activities on platelets: Platelet aggregation[b] $3\times10^{-5}$ M | Platelet aggregation[b] $1\times10^{-5}$ M | Inhibition on PAF-induced platelet aggregation[c] $IC_{50}\times10^{5}$ (M) |
|---|---|---|---|---|---|---|---|---|---|
| **1a** | 2 | 6.7 | 200 (0/5)[e] | 229 (2/5) | 210 (2/5) | 289 (3/5) | 56.8 | 0 | — |
| **1b** | 3 | 11.0 | 183 (0/5) | 256 (1/5) | 348 (1/5) | (5/5) | 33.3 | 0 | — |
| **1c** | 4 | 3.3 | 176 (0/5) | 207 (0/5) | | | 0 | 0 | 0.8 |
| **1d** | 5 | 6.5 | 177 (0/4) | 169 (0/4) | | | 0 | — | 1.4 |
| **1e** | 10 | 1.5 | 146 (1/5) | 326 (2/5) | 408 (0/5) | 122 (4/5) | 0 | — | 2.3 |
| **1f** | 14 | 8.7 | 180 (2/5) | 128 (0/4) | | | 0 | — | >3 |

*a*) Schedule of drug administration. *b*) Reference 19. *c*) Reference 20. *d*) Dose: a test sample was administered i.p. once a day on the days designated. *e*) The number of treated mice still alive on day 60/the total number of treated mice.

alkylphospholipids possessing a decamethylene bridge in the polar head side chain (**1g—n**) were tested for their antitumor activity as described below.

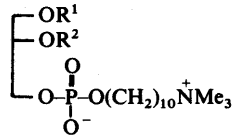
## Biological Results and Discussion

For examination of the antitumor activity of this series of phospholipids, we used human promyelocytic leukemia cells (HL-60) for *in vitro* tests and mouse sarcoma 180 and mammary carcinoma MM46 for *in vivo* tests. The results are shown in Tables I and II.

All of the fourteen alkylglycerophospholipids (**1a—l, n**), in which the number of carbons in the methylene bridge of the polar head side chain varied from 2 to 14, showed distinct cytotoxic activity against HL-60 cells with $IC_{50}$ values ranging from 0.4 to 11 $\mu$g/ml. Among 1-octadecyl-2-acetoacetylglycerophospholipids, the most active was the congener with the decamethylene bridge at position 3 (**1e**, the $IC_{50}$ value = 1.5 $\mu$g/ml). This was followed by the 3-tetra- (**1c**), penta- (**1d**), di- (**1a**), tetradeca- (**1f**), and trimethylene (**1b**) compounds in order of decreasing activity. These results show that the length of the methylene bridge between the phosphate and trimethylammonium group is an important factor in modulating the *in vitro* activity (mainly direct cytotoxicity) against HL-60 cells. However, no simple correlation was observed between the length of the methylene bridge and the $IC_{50}$ value of the corresponding phospholipid. It would be expected that the presence of the ten-carbon chain at position 3 in compound **1e** gives the molecule conformational flexibility which allows the spatial arrangement of the three glyceryl substituents (especially their putative key atoms), in a favorable way for access and binding to the active site of an essential membrane protein of tumor cells. Thus, among the congeners, the phospholipid (**1e**) may have the most suitable three-dimension structure to interact with membrane protein, and this is assumed to endow it with marked ability to kill tumor cells. The exact nature of the above-mentioned relation between the potency of the activity and the length of the methylene bridge remains to be clarified.

The 2-acetoacetyl compounds (**1a—f**) were tested for *in vivo* activity by means of a life extension assay using S180 and mammary carcinoma MM46. When tumor cells were inoculated intraperitoneally (i.p.) and the compounds were administered i.p., under the given experimental conditions, antitumor activity was observed as shown in Table I. Again, the most potent activity was shown by the 3-decamethylene compound (**1e**). The T/C values induced by compound **1e** (i.p.) were 326% (2/5) against S180, and 122% (4/5) against MM46. Sixty-day survival was achieved in 2 out of 5 mice and 4 out of 5 mice, respectively. The survivors (given in parentheses in Tables I and II) were not included in the calculation of T/C for convenience. Compound **1e** was followed by the 3-trimethylene (**1b**), and 3-dimethylene compounds (**1a**). The di- and trimethylene compounds (**1a** and **1b**) showed *in vivo* activities almost comparable to those of **1e** in spite of their surprisingly low *in vitro* activities ($IC_{50}$ values of 6.7 and 11 $\mu$g/ml, respectively). With respect to the tetra- (**1c**), penta- (**1d**) and tetradecamethylene compounds (**1f**), the *in vivo* activities

TABLE II. Antitumor Activity of Compounds **1e, g—l** and **n**



| Compound | $R^1$ | $R^2$ | $IC_{50}$ ($\mu$g/ml) HL-60 | T/C (%) S 180[a] | T/C (%) MM 46[b] |
|---|---|---|---|---|---|
| **1e** | $-C_{18}H_{37}$ | $-COCH_2COMe$ | 1.5 | 297 (2/5)<br>326 (2/5) | 122 (4/5)<br>(4/4) |
| **1g** | $-C_{18}H_{37}$ | $-CONHMe$ | 0.8 | 133 (0/5) | — |
| **1h** | $-C_{18}H_{37}$ | $-CSNHMe$ | 1.1 | 187 (0/5) | 170 (2/5) |
| **1i** | $-C_{18}H_{37}$ | $-CONMe_2$ | 0.9 | 155 (0/4) | — |
| **1j** | $-C_{18}H_{37}$ | $-Me$ | 0.4 | 175 (1/5) | 249 (2/5) |
| **1k** | $-CH_2COC_{18}H_{37}$ | $-Me$ | 0.75 | 242 (0/5) | 145 (4/5) |
| **1l** | $-CONHC_{18}H_{37}$ | $-Me$ | 0.8 | 253 (0/5) | 185 (2/5) |
| **1n** | $-C_{18}H_{37}$ | $-CH_2CO_2H$ | 1.9 | 335 (0/5) | 287 (1/5) |

*a*) Dose: 0.33 mg/mouse, administered once a day on days 0—2. *b*) Dose: 0.25 mg/mouse, administered once a day on days 2—5.

were unexpectedly modest, considering that their *in vitro* activities were more potent than those of the dimethylene (**1a**) and trimethylene compounds (**1b**). The tetradecamethylene compound (**1f**) showed marginal activity in both *in vitro* and *in vivo* tests. These results show that the *in vitro* activity in many cases does not reflect *in vivo* activity and that there is no direct relation between the *in vitro* and *in vivo* activities of these compounds.

Based on these considerations, it is unlikely that a simple *in vitro* screen can be used to select agents with favorable efficacy against *in vivo* tumors, and a strategic approach must consider the physiological environment of the tumor cells.

The presence of the 3-decamethylene bridge in a molecule appeared to be associated with high antitumor efficacy and, as described below, loss of PAF-like toxicity. This prompted us to synthesize further 3-decamethylene analogs having a variety of substituents at position 2 (**1g—n**). All of this class of compounds (seven analogs) showed potent *in vitro* activity against HL-60 cells with $IC_{50}$ values ranging from 0.4 to 1.9 $\mu$g/ml, the 2-methyl compound (**1j**) being the most potent and twice as potent as ET-18-OMe ($IC_{50}$ = 1.0 $\mu$g/ml, Table II). After i.p. administration to mice inoculated with S180 and MM46, favorable T/C values were obtained with compounds possessing 2-methyl and 2-carboxymethyl groups (**1j, n**), though the potency did not appear to exceed that of the 2-acetoacetyl compound (**1e**). Again, the therapeutic effects of 2-substituted 3-decamethylene compounds did not appear to correlate with their *in vitro* cytotoxic activity. With respect to the 2-methyl-3-decamethylene compound (**1j**) which is most potently cytotoxic in this series, further modification of the hydrophobic group (nonpolar tail) at position 1 and clarification of the effect of a change in the nature of its linkage to the glyceryl backbone were attempted. It has been reported that the hydrophobicity of the alkyl chain is a key characteristics for activity[8] and compounds having a $C_{16-18}$ chain are optimal for antitumor activity.[5] As shown in Table II, the substitution by a 2-oxo-icosyl or *N*-octadecylcarbamoyl group at position 1 resulted in compounds with potent antileukemic activity ($IC_{50}$ = 0.75 and 0.8 $\mu$g/ml) and relatively favorable *in vivo* antitumor effects. However, these compounds were still not superior to compound **1e**.

The acetoacetyl compounds, **1a, e**, were tested for their effects on macrophage activation. These compounds (0.3 mg/mouse) were intraperitoneally injected into CDF1 mice, and the peritoneal exudate macrophage cells on day 4 were harvested and assayed for their cytostatic activity.[17] Significant cytostatic activity was observed against EL-4 cells in peritoneal exudate cells stimulated with these compounds. The activity of **1a** was found to be a little more potent than that of **1e** and comparable to that of ET-18-OMe.[5,18] The detailed data will be reported elsewhere. This result suggests that the antitumor activity of **1a** and **1e** is partly mediated by cytostatic peritoneal macrophages *in vivo*.

The compounds were subsequently tested for PAF agonistic activity (platelet aggregation and hypotension), which causes undesirable side effects in the host. They did not show any PFA agonistic activity in rabbit platelet-rich plasma at concentrations up to $3 \times 10^{-5}$ M, except for the compounds having the di- and trimethylene bridges (**1a, b**) (Table I). The aggregating effect (PAF agonistic activity) of the latter two (**1a, b**) at $3 \times 10^{-5}$ M was lower than that of ET-18-OMe (62.7% at $3 \times 10^{-5}$ M). In the blood pressure assay, compound **1a** exhibited only weak hypotensive activity, comparable to that of ET-18-OMe, in SD rats (single dose at 0.3 mg/kg, i.v.).[20] As shown in Table I, the platelet-aggregating activity progressively decreased with increase in the length of the methylene bridge of the 3-polar head side chain. This result is consistent with the view that the distance between the phosphate group and the quaternary nitrogen is critical for the platelet activation.[10] The tetramethylene (**1c**) and higher methylene congeners, including compound **1e**, did not cause any aggregation of rabbit platelets at concentrations of up to 100 $\mu$M. These results suggest that these antitumor phospholipids have no undesirable PAF agonistic side effects.

It is apparent from all of these results that the *in vivo* antitumor activity of alkylglycerophospholipids is greatest, where there is a decamethylene bridge between the phosphate and trimethylammonio group at position 3, where there is a 2-acetoacetyl group, and where the nonpolar tail is 1-octadecyl which is bound to the glycerol backbone by an ether linkage. However, the limited number of tumor cell types and experimental conditions (tumor cell-inoculation and drug-administration routes and schedules) used in the present study preclude definitive conclusions, and the situation remains to be clarified by further studies.

## Experimental

Melting points were determined using a Yanagimoto melting point apparatus and are uncorrected. Infrared (IR) spectra were measured with a Hitachi 215 spectrometer. Proton nuclear magnetic resonance ($^1$H-NMR) spectra were taken on a Varian T-60 (60 MHz) or a Varian EM-390 (90 MHz) spectrometer. In the NMR spectra, chemical shifts are given on the $\delta$ (ppm) scale with tetramethylsilane as an internal standard, and coupling constants ($J$) are given in hertz (Hz). The following abbreviations are used: s = singlet, d = doublet, t = triplet, m = multiplet. All the compounds prepared are racemic.

**3-Hydroxypropyltrimethylammonium Tosylate (3b)** A toluene solution (30 ml) of methyl *p*-toluenesulfonate (18.6 g, 0.10 mol) was added dropwise at room temperature to a solution of 3-dimethylamino-1-propanol (10.3 g, 0.10 mol) in toluene (50 ml) over a period of 0.5 h with stirring. The resulting mixture was allowed to react at room temperature for 1 h. The precipitated crystals were collected by filtration, washed successively with toluene and ether, and dried *in vacuo* to give the desired product (**3b**) as colorless needles. Yield: 26.8 g (93%). mp 80 °C. $^1$H-NMR ($CDCl_3$ + $CD_3OD$) $\delta$: 1.80—2.11 (2H, m), 2.37 (3H, s), 3.13 (9H, s), 3.49—3.73 (2H, m), 3.97 (2H, m), 7.21 (2H, d, $J$ = 8 Hz), 7.75 (2H, d, $J$ = 8 Hz). IR (KBr): 3350, 1625, 1485, 1205, 1190, 1130, 1070, 1035, 1010, 915, 815, 690 cm$^{-1}$.

**4-Hydroxybutyltrimethylammonium Tosylate (3c)** In a similar way, the title compound was synthesized as colorless prisms from 4-dimethylamino-1-butanol (**2c**, 8.0 g, 68 mmol). Yield: 19.0 g (92%). mp 109—110 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.33—1.90 (4H, m), 2.33 (3H, s), 3.06 (9H, s), 3.20—3.56 (4H, m), 4.60 (1H, t, $J$ = 5 Hz), 7.16 (2H, d, $J$ = 8 Hz), 7.56 (2H, d, $J$ = 8 Hz). IR (KBr): 3400, 1640, 1490, 1190, 1125, 1035, 1010, 915, 815, 685 cm$^{-1}$.

**5-Hydroxypentyltrimethylammonium Tosylate (3d)** A mixture of triethylamine (4.4 ml, 43 mmol), 1,5-pentanediol (**4d**, 6.2 g, 60 mmol), and tosyl chloride (5.7 g, 30 mmol) was stirred at room temperature overnight. The reaction mixture was evaporated to dryness *in vacuo* to give the residue. This was dissolved in $CH_2Cl_2$ (100 ml) and washed successively with water (30 ml), 1 N HCl (15 ml), and water (30 ml). The organic layer was separated, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on a column of silica gel (50 g) with $CH_2Cl_2$–MeOH (96 : 4) as the eluent. The eluate containing the desired product was collected and concentrated *in vacuo* to give 4.1 g (53%) of 1,5-pentanediol monotosylate (**5d**) as a colorless oil. $^1$H-NMR ($CDCl_3$) $\delta$: 1.20—1.70 (6H, m), 2.03 (1H, s), 2.43 (3H, s), 3.53 (2H, t, $J$ = 6.5 Hz), 4.00 (2H, t, $J$ = 6.5 Hz), 7.32 (2H, d, $J$ = 8 Hz), 7.76 (2H, d, $J$ = 8 Hz). IR (neat):

3330, 2930, 1590, 1350, 1185, 1170, 950, 810 $cm^{-1}$. The tosylate **5d** (2.0 g, 7.8 mmol) was allowed to react with trimethylamine (18% in toluene, 7 ml) at room temperature for 3 d. Filtration of the precipitated crystals followed by washing with toluene and drying *in vacuo* gave the desired **3d** as colorless needles. Yield: 2.3 g (95%). mp 143—144 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.20—1.80 (6H, m), 2.29 (3H, s), 3.03 (9H, s), 3.17—3.50 (4H, m), 4.40 (1H, t, $J$=5 Hz), 7.11 (2H, d, $J$=8 Hz), 7.50 (2H, d, $J$=8 Hz). IR (KBr): 3400, 2950,1485, 1215, 1190, 1170, 1115, 1027, 1005, 820, 680 $cm^{-1}$.

**10-Hydroxydecyltrimethylammonium Tosylate (3e)** By a similar procedure to that described above, 1,10-decanediol monotosylate (**5e**) was synthesized from 1,10-decanediol (**4e**, 17.4 g, 100 mmol). Yield, 7.56 g (46%). $^1$H-NMR ($CDCl_3$) $\delta$: 1.25 (14H, s), 1.42—1.63 (2H, m), 2.45 (3H, s), 3.62 (2H, t, $J$=6 Hz), 4.01 (2H, t, $J$=6 Hz), 7.31 (2H, d, $J$=8 Hz), 7.78 (2H, d, $J$=8 Hz). **3e** was synthesized as a colorless solid from **5e** (10.6 g, 32 mmol) in a similar way to that described for **3d**. Yield, 10.3 g (82%). $^1$H-NMR ($CDCl_3$+$CD_3OD$) $\delta$: 1.30 (14H, s), 1.43—1.83 (2H, m), 2.33 (3H, s), 3.12 (9H, s), 3.33 (2H, m), 3.53 (2H, t, $J$=6 Hz), 7.15 (2H, d, $J$=8 Hz), 7.71 (2H, d, $J$=8 Hz).

**14-Hydroxytetradecyltrimethylammonium Tosylate (3f)** In the same way, 1,14-tetradecanediol monotosylate (**5f**) was synthesized as a colorless oil from 1,14-tetradecanediol (**4f**, 18 g, 84 mmol). Yield, 6.4 g (40%). $^1$H-NMR ($CDCl_3$) $\delta$: 1.23 (22H, s), 1.48—1.70 (2H, m), 2.45 (3H, s), 3.58 (2H, t, $J$=6 Hz), 4.02 (2H, t, $J$=6 Hz), 7.35 (2H, d, $J$=9 Hz), 7.79 (2H, d, $J$=9 Hz). **3f** was synthesized as a colorless solid from **5f** (2.5 g, 6.5 mmol) in a similar way to that described for **3d**. Yield, 1.97 g (68%). $^1$H-NMR ($CDCl_3$+$CD_3OD$) $\delta$: 1.26 (22H, s), 1.60 (2H, m), 2.34 (3H, s), 3.13 (9H, s), 3.24 (2H, m), 3.55 (2H, t, $J$=6 Hz), 7.15 (2H, d, $J$=8 Hz), 7.71 (2H, d, $J$=8 Hz).

**2-Benzyloxy-3-octadecyloxypropyl 3-Trimethylammoniopropyl Phosphate (7b) Method A** An ethanol-free $CHCl_3$ solution (40 ml) of 2-benzyloxy-3-octadecyloxypropanol (**6**, 1.5 g, 5.8 mmol)[11] was added dropwise at 0 °C to a mixture of $CHCl_3$ (20 ml), phosphorus oxychloride (0.91 g, 5.9 mmol) and triethylamine (3.9 ml, 29 mmol) over a period of 0.5 h with stirring, and allowed to react at room temperature for 1 h, then cooled. To this mixture, a pyridine solution (80 ml) of 3-hydroxypropyltrimethylammonium tosylate (**3b**, 2.3 g, 8.4 mmol) was added at 0 °C. After constant stirring for 3 d at room temperature, the reaction mixture was treated with saturated aqueous sodium hydrogencarbonate (46 mmol) and evaporated to dryness, leaving a residue which was extracted with $CHCl_3$–toluene (1 : 1) (100 ml). After evaporation of the extract, the residue was re-extracted with $CHCl_3$ (70 ml) and the extract, after evaporation, gave a brownish paste. This was chromatographed on a silica gel (60 g) column with $CHCl_3$–MeOH–$H_2O$ (65 : 25 : 4) as the eluent. The eluate containing the desired product was collected and evaporated to give the phosphate (**7b**) as a colorless solid. Yield, 2.3 g (65%). $^1$H-NMR ($CDCl_3$) $\delta$: 0.87 (3H, t, $J$=6 Hz), 1.24 (30H, s), 1.45 (2H, m), 1.97 (2H, m), 3.05 (9H, s), 3.26—4.33 (11H, m), 4.63 (2H, s), 7.29 (5H, m). IR (KBr): 3420, 2920,2850, 1620, 1480, 1465, 1260, 1120, 1050, 935, 840, 730 $cm^{-1}$.

**2-Benzyloxy-3-octadecyloxypropyl 4-Trimethylammoniobutyl Phosphate (7c)** This compound was synthesized as a colorless solid starting from **6** (10.9 g, 25 mmol) as described for **7b**, except for replacement of **3b** with 4-hydroxybutyltrimethylammonium tosylate (**3c**, 7.4 g, 26 mmol). Yield, 4.5 g (29%). $^1$H-NMR ($CDCl_3$+$CD_3OD$) $\delta$: 0.88 (3H, t, $J$=6 Hz), 1.27 (30H, s), 1.42 (2H, m), 1.74 (4H, m), 3.06 (9H, s), 3.30—3.93 (11H, m), 4.70 (2H, s), 7.32 (5H, m). IR (KBr): 3400, 2930, 2855, 1465, 1240, 1230, 1095, 1065, 740 $cm^{-1}$.

**2-Benzyloxy-3-octadecyloxypropyl 5-Trimethylammoniopentyl Phosphate (7d)** This compound was synthesized in the same manner as described above, except for replacement of **3c** with 5-hydroxypentyltrimethylammonium tosylate (**3d**, 2.3 g, 7.6 mmol) as the starting compound. Yield, 2.8 g (76%). $^1$H-NMR ($CDCl_3$) $\delta$: 0.87 (3H, t, $J$=6 Hz), 1.25 (30H, s), 1.46—1.90 (8H, m), 3.10 (9H, s), 3.27—3.53 (4H, m), 3.70—3.95 (4H, m), 4.15 (3H, m), 4.66 (2H, s), 7.30 (5H, m). IR (KBr): 3400, 2920, 2850, 1465, 1235, 1115, 1100, 1067, 820 $cm^{-1}$.

**2-Benzyloxy-3-octadecyloxypropyl 10-Trimethylammoniodecyl Phosphate (7e)** 1) This compound was synthesized from **6** (2.34 g, 5.4 mmol) and **3e** (3.0 g, 8.07 mmol) in the same manner as described above. Yield, 1.32 g (34%) as a colorless solid. $^1$H-NMR ($CDCl_3$+$CD_3OD$) $\delta$: 0.87 (3H, t, $J$=6 Hz), 1.10—2.00 (48H, m), 3.22 (9H, s), 3.30—4.00 (11H, m), 4.68 (2H, s), 7.32 (5H, s). IR (KBr): 3400, 2920, 2850, 1620, 1465, 1210, 1090, 1050 $cm^{-1}$.

2) Method B: A solution of **6** (4.35 g, 10 mmol) in toluene (20 ml) was added to a mixture of 10-bromodecyl dichlorophosphate (5.3 g, 15 mmol)[13] and triethylamine (3.5 ml, 25 mmol) in toluene (30 ml) at 0 °C with stirring. The mixture was allowed to react at room temperature for 3 h, and after addition of 2 N HCl (20 ml), the reaction mixture was kept at 50 °C for 1 h under stirring, then extracted with ether. The organic layer was washed with water, dried with $MgSO_4$, and evaporated *in vacuo*. The residue was dissolved in 20% (w/w) trimethylamine–toluene (50 ml) and allowed to react at room temperature for 3 d. The reaction mixture was evaporated to dryness *in vacuo* and the residue was chromatographed on a column of silica gel (75 g). Elution with $CHCl_3$–MeOH–$H_2O$ (65 : 25 : 4) gave fractions containing the desired product. After work-up in the usual way, the phosphate (**7e**) was obtained as a colorless solid. Yield, 3.37 g (47%).

**2-Benzyloxy-3-octadecyloxypropyl 14-Trimethylammoniotetradecyl Phosphate (7f)** This compound was synthesized from **6** (1.48 g, 3.42 mmol) and **3f** (1.97 g, 4.44 mmol) by a procedure similar to that described for **7b**. Yield, 1.44 g (55%) as a colorless solid. $^1$H-NMR ($CDCl_3$) $\delta$: 0.87 (3H, t, $J$=6 Hz), 1.10—2.00 (56H, m), 3.19 (9H, s), 3.20—4.00 (11H, m), 4.69 (2H, s), 7.30 (5H, m). IR (KBr): 3420, 2925, 2860, 1640, 1470, 1225, 1095, 1065, 910, 845, 735, 700 $cm^{-1}$.

**2-Hydroxy-3-octadecyloxypropyl Trimethylammoniopropyl Phosphate (8b)** A mixture of 2-benzyloxy-3-octadecyloxypropyl trimethylammoniopropyl phosphate (**7b**, 2.0 g, 3.3 mmol) and 10% Pd–C (0.50 g) in 70% AcOH (35 ml) was vigorously stirred under a hydrogen atmosphere (1 atm) at room temperature for 3 h. After removal of the catalyst by filtration, the filtrate was evaporated to dryness *in vacuo*, leaving a residue. This was subjected to chromatography on a column of silica gel (30 g). Elution with $CHCl_3$–MeOH–$H_2O$ (65 : 50 : 8) gave the eluate containing the required compound. After work-up, the desired phosphate (**8b**) was obtained as a colorless solid. Yield, 1.4 g (82%). $^1$H-NMR ($CDCl_3$+$CD_3OD$) $\delta$: 0.86 (3H, t, $J$=6 Hz), 1.26 (30H, s), 1.53 (2H, m), 2.10 (2H, m), 3.21 (9H, s), 3.33—4.06 (11H, m). IR (KBr): 3410, 2920, 2845, 1630, 1465, 1220, 1115, 1150, 945, 850, 715, 680 $cm^{-1}$.

**2-Hydroxy-3-octadecyloxypropyl 4-Trimethylammoniobutyl Phosphate (8c)** By a similar procedure to that described above, this compound was synthesized from **7c** (4.0 g, 6.4 mmol). Yield, 3.54 g (94%). $^1$H-NMR ($CDCl_3$+$CD_3OD$) $\delta$: 0.87 (3H, t, $J$=6 Hz), 1.27 (30H, s), 1.54 (2H, m), 1.71 (4H, m), 3.13 (9H, s), 3.43 (6H, m), 3.92 (5H, m). IR (KBr): 3400, 2925, 2855, 1465, 1215, 1120, 1075, 1060, 1010, 900 $cm^{-1}$.

**2-Hydroxy-3-octadecyloxypropyl 5-Trimethylammoniopentyl Phosphate (8d)** In the same manner as described above, this compound was synthesized as a colorless solid starting from **7d** (2.5 g, 3.9 mmol). Yield, 1.8 g (84%). $^1$H-NMR ($CDCl_3$) $\delta$: 0.87 (3H, t, $J$=6 Hz), 1.26 (30H, s), 1.40—2.03 (8H, m), 3.23 (9H, s), 3.30—3.56 (5H, m), 3.70—3.97 (4H, m), 4.20—4.67 (3H, m). IR (KBr): 3400, 3230, 2920, 2850, 1490, 1467, 1210, 1115, 1090, 1065, 1007 $cm^{-1}$.

**2-Hydroxy-3-octadecyloxypropyl 10-Trimethylammoniodecyl Phosphate (8e)** By a procedure similar to that described above, this compound was synthesized from **7e** (1.3 g, 1.8 mmol). Yield, 0.99 g (87%). $^1$H-NMR ($CDCl_3$+$CD_3OD$) $\delta$: 0.89 (3H, t, $J$=6 Hz), 1.10—2.00 (48H, m), 3.13 (9H, s), 3.20—3.52 (6H, m), 3.78—3.93 (4H, m), 4.12 (1H, s). IR (KBr): 3400, 2920, 2850, 1620, 1465, 1210, 1090, 1050 $cm^{-1}$.

**2-Hydroxy-3-octadecyloxypropyl 14-Trimethylammoniotetradecyl Phosphate (8f)** By the same procedure as described above, this compound was synthesized from **7f** (1.44 g, 1.87 mmol). Yield, 1.24 g (98%). $^1$H-NMR ($CDCl_3$+$CD_3OD$) $\delta$: 0.87 (3H, t, $J$=6 Hz), 1.10—1.90 (56H, m), 3.12 (9H, s), 3.20—4.10 (11H, m). IR (KBr): 3420, 2925, 2860, 1640, 1470, 1225, 1095, 1065, 910, 845, 735, 700 $cm^{-1}$.

**2-Acetoacetyloxy-3-octadecyloxypropyl Trimethylammonioethyl Phosphate (1a)** Diketene (3 ml, 38 mmol) was added dropwise to a solution of 2-hydroxy-3-octadecyloxypropyl 2-trimethylammonioethyl phosphate (**8a**, 1.0 g, 2.0 mmol)[11] in a mixture of pyridine (20 ml) and $CH_2Cl_2$ (20 ml) over a period of 0.5 h at 40 °C with stirring. The mixture was stirred at the same temperature for 2 h and concentrated to dryness *in vacuo*. The residue was chromatographed on a column of silica gel (15 g) with $CHCl_3$–MeOH–$H_2O$ (65 : 25 : 4). The eluate containing the desired compound was collected and evaporated to dryness *in vacuo*. The residue was triturated with acetone and the precipitate was collected by filtration, washed with acetone and dried *in vacuo* to give the desired phosphate (**1a**) as a pale yellow solid. Yield, 0.81 g (69%). *Anal.* Calcd for $C_{30}H_{60}NO_8P \cdot 2H_2O$: C, 57.21; H, 10.24; N, 2.22; P, 4.92. Found: C, 57.42; H, 10.30; N, 2.14; P, 4.63. $^1$H-NMR ($CDCl_3$) $\delta$: 0.87 (3H, t, $J$=6 Hz), 1.27 (30H, s), 1.50 (2H, m), 2.26 (3H, s), 3.33 (9H, s), 3.40—4.30 (12H, m), 5.20 (1H, m). IR (KBr): 3440, 2925, 2855, 1740, 1715, 1465, 1245, 1090, 1060, 970 $cm^{-1}$.

**2-Acetoacetyloxy-3-octadecyloxypropyl 3-Trimethylammoniopropyl Phosphate (1b)** This compound was prepared from **8b** (0.80 g, 1.53 mmol) by a procedure similar to that described above. Yield, 0.73 g (77%). *Anal.*

Calcd for $C_{31}H_{62}NO_8P \cdot 1.5H_2O$: C, 58.65; H, 10.32; N, 2.21; P, 4.88. Found: C, 58.65, H, 10.53; N, 2.38; P, 4.70. $^1$H-NMR ($CDCl_3$) $\delta$: 0.87 (3H, t, $J$=6 Hz), 1.26 (30H, s), 1.53 (2H, m), 2.13 (2H, m), 2.26 (3H, s), 3.30 (9H, s), 3.40—4.03 (12H, m), 5.20 (1H, m). IR (KBr): 3420, 2920, 2840, 1740, 1715, 1465, 1235, 1090, 1055, 840 cm$^{-1}$.

**2-Acetoacetyloxy-3-octadecyloxypropyl 4-Trimethylammoniobutyl Phosphate (1c)** This compound was synthesized from **8c** (1.0 g, 1.86 mmol) in the same manner as described for **1a**. Yield, 0.60 g (52%). *Anal.* Calcd for $C_{32}H_{64}NO_8P \cdot 0.5H_2O$: C, 60.93; H, 10.39; N, 2.22. Found: C, 61.00; H, 10.75; N, 2.17. $^1$H-NMR ($CDCl_3+CD_3OD$) $\delta$: 0.87 (3H, t, $J$=6 Hz), 1.27 (30H, s), 1.45—2.02 (6H, m), 2.27 (3H, s), 3.10 (9H, s), 3.43—3.91 (12H, m), 5.20 (1H, m). IR (KBr): 3410, 2920, 2850, 1740, 1715, 1630, 1465, 1225, 1090, 1065, 830 cm$^{-1}$.

**2-Acetoacetyloxy-3-octadecyloxypropyl 5-Trimethylammoniopentyl Phosphate (1d)** This compound was synthesized from **8d** (0.80 g, 1.5 mmol) in the same way as described for **1a**. Yield, 0.75 g (81%). *Anal.* Calcd for $C_{33}H_{66}NO_8P \cdot H_2O$: C, 60.62; H, 10.48; N, 2.14; P, 4.74. Found: C, 60.21; H, 10.62; N, 2.07; P, 4.57. $^1$H-NMR ($CDCl_3$) $\delta$: 0.90 (3H, t, $J$=6 Hz), 1.26 (30H, s), 1.46 (2H, m), 1.60 (4H, m), 1.90 (2H, m), 2.26 (3H, s), 3.30 (9H, s), 3.40—4.00 (12H, m), 5.23 (1H, m). IR (KBr): 3400, 2920, 2850, 1740, 1715, 1665, 1235, 1070, 835 cm$^{-1}$.

**2-Acetoacetyloxy-3-octadecyloxypropyl 10-Trimethylammoniodecyl Phosphate (1e)** This compound was synthesized from **8e** (0.99 g, 0.60 mmol) in the same way as described for **1a**. Yield, 0.84 g (75%). *Anal.* Calcd for $C_{38}H_{76}NO_8P \cdot 0.5H_2O$: C, 63.83; H, 10.86; N, 1.96. Found: C, 63.71; H, 11.19; N, 2.04. $^1$H-NMR ($CDCl_3+CD_3OD$) $\delta$: 0.88 (3H, t, $J$=6 Hz), 1.25—1.90 (48H, m), 2.27 (3H, s), 3.11 (9H, s), 3.23—4.02 (12H, m), 5.20 (1H, m). IR (KBr): 3420, 2930, 2860, 1745, 1725, 1635, 1465, 1230, 1075, 840 cm$^{-1}$.

**2-Acetoacetyloxy-3-octadecyloxypropyl 14-Trimethylammoniotetradecyl Phosphate (1f)** This compound was synthesized from **8f** (1.20 g, 1.77 mmol) in the same way as described for **1a**. Yield, 1.21 g (90%). *Anal.* Calcd for $C_{42}H_{84}NO_8P \cdot 1.5H_2O$: C, 63.93; H, 11.11; N, 1.77. Found: C, 64.19; H, 11.29; N, 1.67. $^1$H-NMR ($CDCl_3+CD_3OD$) $\delta$: 0.88 (3H, t, $J$=6 Hz), 1.15—1.90 (56H, m), 2.27 (3H, s), 3.13 (9H, s), 3.20—4.03 (12H, m), 5.20 (1H, m). IR (KBr): 3450, 2925, 2860, 1745, 1720, 1635, 1465, 1250, 1070, 835 cm$^{-1}$.

**2-Methylcarbamoyloxy-3-octadecyloxypropyl 10-Trimethylammoniodecyl Phosphate (1g)** A mixture of **8e** (0.35 g, 0.56 mmol), methyl isocyanate (2 ml), and pyridine (5 ml) was allowed to react at 50 °C for 4 h under stirring. The reaction mixture was evaporated to dryness and the residue was chromatographed on a column of silica gel (10 g) with $CHCl_3$–MeOH–$H_2O$ (65 : 25 : 4) as the eluent. Work-up in the usual way afforded the desired phosphate (**1g**) as a colorless powder. Yield, 0.27 g (71%). *Anal.* Calcd for $C_{36}H_{75}N_2O_7P \cdot 2H_2O$: C, 60.47; H, 11.14; N, 3.92; P, 4.33. Found: C, 60.59; H, 11.01; N, 4.10; P, 4.44. $^1$H-NMR ($CDCl_3$) $\delta$: 0.86 (3H, t, $J$=6 Hz), 1.20—2.10 (48H, m), 2.69 (3H, d, $J$=5 Hz), 3.15 (9H, s), 3.10—4.20 (10H, m), 4.87 (1H, m), 6.29 (1H, br s). IR (KBr): 3400, 2920, 2850, 1710, 1460, 1230, 1065 cm$^{-1}$.

**2-Methylthiocarbamoyloxy-3-octadecyloxypropyl 10-Trimethylammoniodecyl Phosphate (1h)** A mixture of **8e** (0.35 g, 0.56 mmol), methyl isothiocyanate (2 ml), and pyridine (5 ml) was stirred at 50 °C for 4 h. The reaction mixture was evaporated to dryness and the resulting residue was chromatographed on a column of silica gel (10 g) with $CHCl_3$–MeOH–$H_2O$ (65 : 25 : 4) as the eluent. Work-up in the usual way gave the desired phosphate (**1h**) as a colorless solid. Yield, 0.27 g (71%). *Anal.* Calcd for $C_{36}H_{75}N_2O_6PS \cdot 0.5H_2O$: C, 61.42; H, 10.88; N, 3.98; P, 4.40. Found: C, 61.26; H, 10.98; N, 3.77; P, 4.46. $^1$H-NMR ($CDCl_3$) $\delta$: 0.86 (3H, t, $J$=6 Hz), 1.10—2.10 (48H, m), 3.00 (3H, d, $J$=5 Hz), 3.28 (9H, s), 3.10—4.10 (11H, m), 5.60 (1H, br s). IR (KBr): 3400, 2920, 2850, 1460, 1230, 1065 cm$^{-1}$.

**2-Methoxy-3-octadecyloxypropyl 10-Trimethylammoniodecyl Phosphate (1j)** This compound was synthesized by the reaction of 2-methoxy-3-octadecyloxypropanol (**9j**, 2.15 g, 6.0 mmol)[9] with 10-bromodecyl dichlorophosphate (3.17 g, 9.0 mmol) in the same manner as described for **7e** (method B). Yield, 2.47 g (65%). *Anal.* Calcd. for $C_{35}H_{74}NO_6P \cdot 0.5H_2O$: C, 65.18; H, 11.72; N, 2.17; P, 4.82. Found: C, 65.43; H, 11.99; N, 2.17; P, 4.86. $^1$H-NMR ($CDCl_3$) $\delta$: 0.85 (3H, t, $J$=6 Hz), 1.10—1.90 (48H, m), 3.31 (9H, s), 3.40 (3H, s), 3.20—4.00 (11H, m). IR (KBr): 3400, 2920, 2855, 1640, 1465, 1230, 1070 cm$^{-1}$.

**2-Methoxy-3-(2-oxoicosyloxy)propyl 10-Trimethylammoniodecyl Phosphate (1k)** By a procedure similar to that described above, this compound was synthesized as a colorless solid starting from 2-methoxy-3-(2-oxoicosyloxy)propanol (**9k**, 1.6 g, 3.4 mmol).[15] Yield, 0.70 g (26%). *Anal.* Calcd for $C_{37}H_{76}NO_7P \cdot H_2O$: C, 63.85; H, 11.30; N, 2.01; P, 4.45. Found: C, 63.84; H, 11.98; N, 2.02; P, 4.29. $^1$H-NMR ($CDCl_3$) $\delta$: 0.87 (3H, t, $J$=6 Hz), 1.20—1.90 (48H, m), 2.73 (2H, $J$=7 Hz), 3.30 (9H, s), 3.43 (3H, s), 3.20—3.90 (9H, m), 4.09 (2H, s). IR (KBr): 3400, 2920, 2850, 1720, 1460, 1220, 1060 cm$^{-1}$.

**2-Dimethylcarbamoyloxy-3-octadecyloxypropyl 10-Trimethylammoniodecyl Phosphate (1i)** This compound was synthesized as a colorless solid starting from 2-dimethylcarbamoyloxy-3-octadecyloxypropanol (**9i**, 2.49 g, 6.0 mmol)[14] in the same way as described for **1j**. Yield, 2.55 g (61%). *Anal.* Calcd for $C_{37}H_{77}N_2O_7P$: C, 62.50; H, 11.20; N, 3.94; P, 4.36. Found: C, 62.84; H, 11.49; N, 3.93; P, 4.53. $^1$H-NMR ($CDCl_3$) $\delta$: 0.87 (3H, t, $J$=6 Hz), 1.10—1.90 (48H, m), 2.87 (6H, s), 3.33 (9H, s), 3.10—4.00 (11H, m). IR (KBr): 3450, 2920, 2855, 1700, 1495, 1460, 1400, 1200, 1070 cm$^{-1}$.

**3-Octadecylcarbamoyloxy-2-methoxypropyl 10-Trimethylammoniodecyl Phosphate (1l)** This compound was synthesized as a colorless solid starting from 3-octadecylcarbamoyloxy-2-methoxypropanol (**9l**, 0.67 g, 1.7 mmol)[16] in the same way as described for **1j**. Yield, 0.59 g (52%). *Anal.* Calcd for $C_{36}H_{75}N_2O_7P$: C, 60.47; H, 11.14; N, 3.92; P, 4.33. Found: C, 60.69; H, 11.60; N, 4.10; P, 4.34. $^1$H-NMR ($CDCl_3+CD_3OD$) $\delta$: 0.87 (3H, t, $J$=6 Hz), 1.25 (32H, m), 1.35 (16H, m), 2.95—3.20 (2H, m), 3.11 (9H, s), 3.43 (3H, s), 3.30—4.05 (7H, m), 4.11 (2H, m). IR (KBr): 3400, 2920, 2850, 1700, 1635, 1460, 1230, 1070 cm$^{-1}$.

**3-Benzyloxy-2-(2,2-dimethoxyethoxy)propyl Octadecyl Ether (11)** A mixture of 3-benzyloxy-2-hydroxypropyl octadecyl ether (**10**, 13.1 g, 30 mmol),[11] bromoacetaldehyde dimethyl acetal (8.5 g, 50 mmol), cetyltrimethylammonium chloride (192 mg, 0.60 mmol), and 50% sodium hydroxide (12 g, 150 mmol) was stirred vigorously at 85 °C for 20 h. After being cooled to room temperature the reaction mixture was extracted with hexane. The organic layer was separated, washed with water, dried with $MgSO_4$, and evaporated to dryness. The residue was chromatographed on a column of silica gel (300 g) with hexane–ethyl acetate–acetone (24 : 1 : 1) as the eluent. The eluate containing the desired product was collected and evaporated to dryness, giving the ether as a colorless oil. Yield, 10.1 g (64%). $^1$H-NMR ($CDCl_3$) $\delta$: 0.87 (3H, t, $J$=6 Hz), 1.27 (32H, s), 3.33 (6H, s), 4.40—3.90 (9H, m), 4.50 (1H, t, $J$=7 Hz), 4.53 (2H, s), 7.27 (5H, s). IR (KBr): 2920, 2850, 1465, 1205, 1115 cm$^{-1}$.

**2-(2,2-Dimethoxyethoxy)-3-octadecyloxypropanol (12)** A mixture of **11** (10 g, 19 mmol) and 10% Pd–C (2.5 g) in EtOH (150 ml) was vigorously stirred under a hydrogen atmosphere (1 atm) at room temperature for 24 h. The reaction mixture, after filtration to remove the catalyst, was evaporated to dryness *in vacuo* to give the desired alcohol as a colorless oil. Yield, 7.9 g (95%). $^1$H-NMR ($CDCl_3$) $\delta$: 0.87 (3H, t, $J$=6 Hz), 1.27 (32H, s), 2.57 (1H, br s), 3.33—3.80 (15H, m), 4.50 (1H, t, $J$=7 Hz). IR (KBr): 3430, 2920, 2850, 1465, 1110 cm$^{-1}$.

**2-(2,2-Dimethoxyethoxy)-3-octadecyloxypropyl 10-Trimethylammoniodecyl Phosphate (13)** This compound was synthesized as a colorless solid starting from **12** (1.7 g, 4.0 mmol) in a manner similar to that described for **7b**. Yield, 1.7 g (60%). $^1$H-NMR ($CDCl_3+CD_3OD$) $\delta$: 0.87 (3H, t, $J$=6 Hz), 1.23 (32H, s), 1.33 (16H, m), 3.10 (9H, s), 3.37 (6H, s), 3.40—4.00 (13H, m), 4.47 (1H, t, $J$=7 Hz). IR (KBr): 3400, 2920, 2850, 1465, 1230, 1210, 1090, 1065, 970 cm$^{-1}$.

**2-Formylmethoxy-3-octadecyloxypropyl 10-Trimethylammoniodecyl Phosphate (1m)** A suspension of **13** (0.82 g, 1.2 mmol) and an acidic cation-exchange resin (Amberlite IR-120, H form, 0.45—0.60 mm, 4 ml) in 80% (v/v) tetrahydrofuran (THF) (120 ml) was stirred at 45 °C for 42 h. After being cooled to room temperature, the mixture was filtered and the filtrate was evaporated to dryness. The residue was chromatographed on a column of silica gel (20 g) with $CHCl_3$–MeOH–$H_2O$ (65 : 25 : 4) as the eluent. The eluate containing the desired product was collected and evaporated to dryness, giving the desired phosphate as a colorless solid. However, this product was found to be contaminated by a small amount of an oxidized product (**1n**) (see below). Yield, 0.30 g (39%). $^1$H-NMR ($CDCl_3+CD_3OD$) $\delta$: 0.90 (3H, t, $J$=6 Hz), 1.23—1.75 (48H, m), 3.13 (9H, s), 3.30—4.10 (13H, m), 4.60—4.80 (1H, m). IR (KBr): 3400, 2920, 2850, 1465, 1220, 1100, 1070, 965 cm$^{-1}$.

**2-Carboxymethoxy-3-octadecyloxypropyl 10-Trimethylammoniodecyl Phosphate (1n)** The 2-formylmethoxy compound **1m**, obtained from **13** (0.82 g, 1.2 mmol) in the same manner as described above, was dissolved in acetone (200 ml). After addition of activated charcoal (1 g), air was passed continuously through the resulting mixture under stirring at room temperature for 10 h. The activated charcoal was removed by filtration, and the filtrate was evaporated to dryness to give the residue. This was chromatographed on a column of a basic anion-exchange resin, Amberlite IRA-68 (0.35—0.45 mm, 50 ml) and eluted with tetrahydrofuran–$H_2O$ (4 : 1, 150 ml) followed by tetrahydrofuran–28% ammonia–MeOH

(10 : 1 : 1, 120 ml). The eluate containing **1n** was collected and evaporated to dryness, giving the residue. Further chromatography on a column of Amberlite IR-120 (H-form, 0.45—0.60 mm, 10 ml) with THF–$H_2O$ (4 : 1) as the eluent, followed by work-up gave the desired compound as a colorless solid. Yield, 0.3 g (21%). *Anal.* Calcd for $C_{36}H_{74}NO_8P \cdot 0.5H_2O$: C, 62.76; H, 10.97; N, 2.04; P, 4.50. Found: C, 62.56; H, 11.47; N, 2.05; P, 4.36. $^1$H-NMR ($CDCl_3+CD_3OD$) $\delta$: 0.87 (3H, t, $J$=6 Hz), 1.23—1.80 (48H, m), 3.13 (9H, s), 3.24—4.10 (11H, m), 4.27 (2H, s). IR (KBr): 3400, 2920, 2850, 1720, 1465, 1230, 1195, 1045, 965 cm$^{-1}$.

**Platelet Aggregation** Blood was collected in 3.15% sodium citrate (1 ml for 9 ml of blood) by cardiac puncture from male white rabbits. Platelet-rich plasma (PRP) was obtained by centrifuging the blood at 3000 rpm for 5 s at room temperature. The platelet density was adjusted to 450000 per $\mu$l with platelet-poor plasma (PPP). Platelet aggregation was measured with a 3 channel aggregometer (Rikadenki, Japan).[20] Agonistic activities of compounds were examined as follows. The PRP (250 $\mu$l) was preincubated at 37 °C for 2 min and then test compounds (25 $\mu$l) were added. The extent of aggregation (percentage) was expressed in terms of the maximum change of light transmission, taking the difference between light transmission for PRP and PPP as 100%. When test compounds ($3 \times 10^{-5}$ M) did not show agonistic activities, antagonistic activities were examined. Namely, 2 min after the addition of test compounds, submaximal concentrations of PAF (25 $\mu$l) were added. Percent inhibition of PAF-induced aggregation by compounds was calculated by dividing the percentage of aggregation by that observed in the control. In the control, 25 $\mu$l of saline was added instead of test compounds.

**Growth Inhibition Assay (Microculture Tetrazolium Assay)** The cytotoxic potential of the compounds against human promyelocytic leukemia HL-60 cells was measured using a vital stain, tetrazolium (Sigma Chem. Co.). The HL-60 cells were maintained in RPMI-1640 medium supplemented with 15% fetal calf serum. Various concentrations of each compound were incubated with $1 \times 10^5$ cells/ml in 16 mm culture dishes. After 5 d at 37 °C in the presence of 5% $CO_2$ and 95% humidified air, viable cells were determined by tetrazolium dye exclusion.

***In Vivo* Antitumor Activity** a) Life Span Assay against Sarcoma 180: 1) Post-treatment (Day 0—2) Assay: ICR mice (18—22 g) were inoculated intraperitoneally (i.p.) with $10^5$ sarcoma 180 cells and received i.p. the test alkylphospholipid (0.33 mg or 1.0 mg/mouse, i.p.) on days 0, 1 and 2 after inoculation. Five mice were used for each dose level of the test compound. Experiments were terminated on day 60. Control mice received saline in place of the test compound. Antitumor activity was assessed on the basis of % T/C (100 × mean life span of treated mice/mean life span of control mice). Sixty-day survivors per total number of mice in each group are given in parentheses in Table I and are not included in the calculations of % T/C.

2) Pre-treatment (Day −4) Assay: A test sample (1 mg/mouse) was administered i.p. once 4 d prior to tumor transplantation. The tumor inoculation was carried out under the same conditions as described above. The activity (% T/C) was also assessed in the same way.

b) Life Span Assay against Mouse Mammary Carcinoma, MM46: A tumor inoculum of $10^4$ MM46 cells was implanted i.p. into C3H female mice on day 0. Five mice were used for each test compound (0.25 mg/mouse, i.p.) and each compound was given 4 times on days 2—5. The activity was assessed on the basis of % T/C as described above.

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