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Syntheses and Biological Activities of *N*-Alkyl- and *N*-Alkenylcarbamoyl Phospholipids

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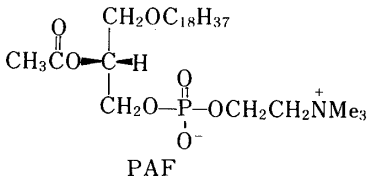
Various carbamate analogs of lysophospholipids, *N*-alkyl- and alkenylcarbamoyl phospholipids (ACPLs), were synthesized and their antimicrobial properties were examined. The ACPLs studied differed in certain aspects of their chemical structure: the polar-head base, the aliphatic nonpolar tail attached to the carbamate nitrogen and the molecular backbone, *e.g.*, 2-*O*-methylglycerol and 1,3-propanediol. In contrast to lysolecithin and lecithin, the majority of the ACPLs showed inhibitory activities against *Tetrahymena pyriformis* (protozoan) and a range of fungi. In the series of ACPLs studied, the maximal inhibitory activities were observed with 2-*O*-methyl-1-*O*-(*N*-tetradecyl)carbamoylglycero-3-phosphocholine (**3**-C_{14}-A) and its 2-demethoxy compound (**4**-C_{14}-A). In comparison with clotrimazole, these compounds showed more potent activities against *T. pyriformis*, and comparable or lower activities against human pathogenic and phytopathogenic fungi. The relationships between structure and antimicrobial activity are discussed.

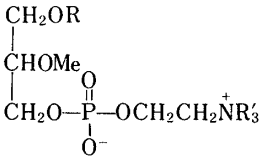
Keywords——alkyl lysophospholipid; alkylcarbamoyl phospholipid; 2-*O*-methyl-1-*O*-(*N*-tetradecyl)carbamoylglycero-3-phosphocholine; CV-3988; PAF-antagonist; *Tetrahymena pyriformis*; antifungal activity; antiprotozoal activity; antitumor activity; structure–activity relationship

Alkyl ether phospholipids of both natural and synthetic origin have attracted much attention in recent years due to their interesting biological activities. The most striking development in this field was the discovery of 1-*O*-octadecyl (and/or 1-*O*-hexadecyl)-2-*O*-acetyl-*sn*-glycero-3-phosphocholine (**1**),[1] a new and extremely potent physiological mediator, termed platelet-activating factor (PAF, Fig. 1), which has been isolated from basophils,[2] neutrophils and macrophages.[3] This mediator has also been isolated from rabbit renal medulla and called the antihypertensive polar renomedullary lipid (APRL).[4] Another interesting development in this field was the discovery of tumor-inhibitory effects of the octadecyl ether analogs of lysolecithin (**1**, Fig. 1) by Munder *et al.*[5] We have recently synthesized a wide variety of alkyl lysophospholipids[6] (ALPLs **1** and **2**, Fig. 1) and clarified their antimicrobial and antitumor properties,[6-8] including ability to induce cell differentiation of tumor cells.[9]

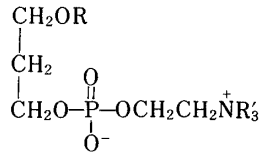
In search of a better therapeutic agent, we focused our attention on a new series of synthetic phospholipids differing in structure from ALPLs. *N*-Alkyl- and *N*-alkenylcarbamoyl phospholipids (ACPLs, **3** and **4**) with a variety of aliphatic chains and polar-head groups were synthesized with the aim of obtaining an esterase-resistant phospholipid. *N*-Substituted carbamoyl phospholipids, where a long-chain aliphatic group is introduced through a carbamoyl function onto the molecular backbone, appear to resist the action of specific



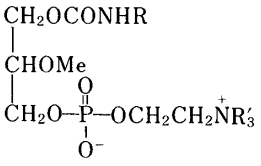
PAF



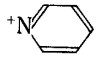
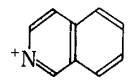
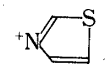
1
ALPL
(R=alkyl, C_nH_{2n+1})

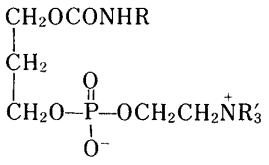


2
ALPL
(R=alkyl, C_nH_{2n+1})



3
ACPL

R	$\overset{+}{N}R'_3$	Compd. No.
alkyl, C_nH_{2n+1}	$\overset{+}{N}Me_3$	3-C_n-A
	$\overset{+}{N}H_3$	3-C_n-B
		3-C_n-C
		3-C_n-D
		3-C_n-E



4
ACPL

R	$\overset{+}{N}R'_3$	Compd. No.
alkyl, C_nH_{2n+1}	$\overset{+}{N}Me_3$	4-C_n-A
	$\overset{+}{N}H_3$	4-C_n-B
alkenyl, C_nH_{2n-3}	$\overset{+}{N}Me_3$	4-$C_{n:2}$-A
	$\overset{+}{N}H_3$	4-$C_{n:2}$-B
C_nH_{2n-5}	$\overset{+}{N}Me_3$	4-$C_{n:3}$-A
	$\overset{+}{N}H_3$	4-$C_{n:3}$-B

Fig. 1

phospholipases and therefore may possess characteristic biological properties. In fact, potent antitumor activities of ACPLs have been found in several experimental tumor systems in our laboratories.[8] Subsequently, 2-methoxy-3-(*N*-octadecyl)carbamoyloxypropyl 2-thiazolioethyl phosphate (**3**, R=$C_{18}H_{37}$, $\overset{+}{N}R'_3$=thiazolio,[10] CV-3988, Fig. 1), the first PAF-specific antagonist,[11] was found among these ACPLs. This compound shows specific inhibition of PAF-induced platelet aggregation and hypotension.

Antimicrobial activities of phospholipids and their modified analogs had not been adequately studied before our previous publications.[6,12] Several ACPLs were shown, in our laboratories, to possess broad-spectrum inhibitory activity against eukaryotic microorganisms. This report deals with the syntheses and the structure–antimicrobial activity relationships of ACPLs.

Chemistry

N-Alkyl and *N*-alkenylcarbamoyl phospholipids (ACPLs), a new series of synthetic lysophospholipids, structurally differ from lysolecithin in that they have a long chain alkyl or alkenylcarbamoyl group in place of the fatty acid ester chain, together with a modified glycerol moiety and a modified polar-head base. As for the dialkylcarbamoyl esters, there has been only one report which describes the synthesis of 1,2-di-*O*-(*N*-pentadecyl)carbamoyl-*sn*-glycero-3-phosphocholine and its complete resistance to the action of phospholipase A_2 from *Crotalus adamanteus*.[13] Based on this information, ACPLs are expected to be resistant to phospholipases A_1 and A_2 and therefore may possess several important biological activities

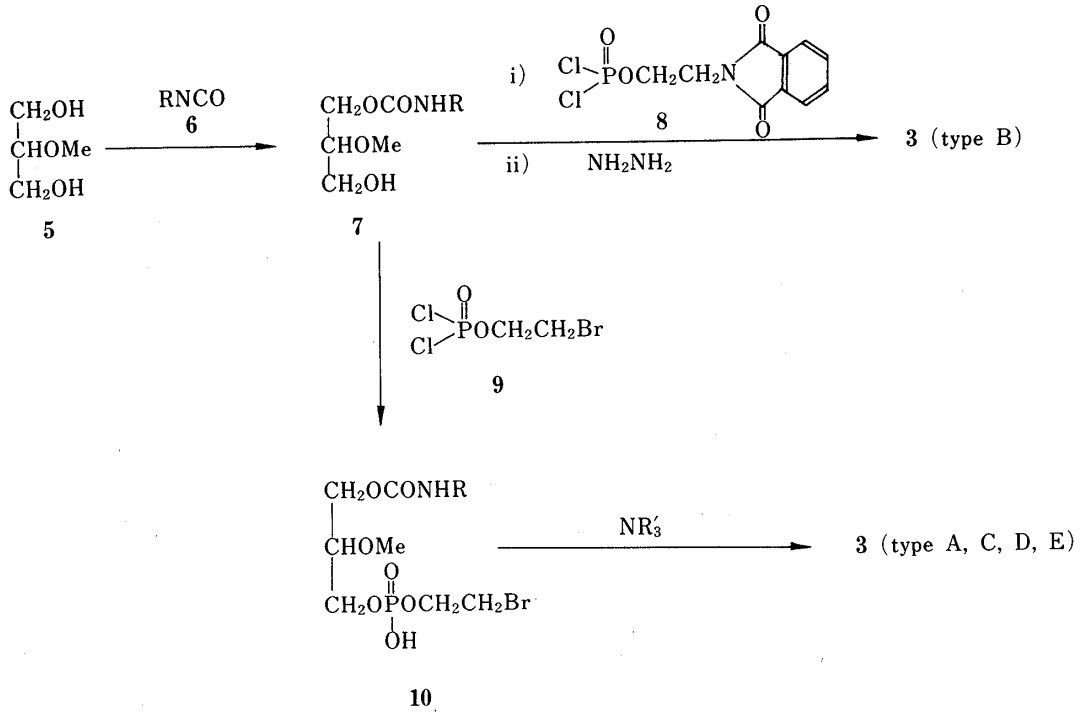


Chart 1

such as antifungal and antitumor activities, as in the case of ALPLs.

In trying to find a better therapeutic agent, we designed and synthesized a variety of ACPLs which differ in the structure of the aliphatic side chain, the chain length and its degree of unsaturation, and the structure of the phosphoryl base and the molecular backbone. ACPLs are classified here into two groups according to the structure of the molecular backbone: ACPLs possessing the 2-*O*-methylglycerol moiety (**3**) and those with the 1,3-propanediol moiety (**4**). The synthetic methods are outlined in Charts 1 and 2.

Various 2-*O*-methyl-3-*O*-(*N*-substituted)carbamoylglycerols (**7**) were synthesized from 2-*O*-methylglycerol (**5**) by carbamoylation with aliphatic isocyanates (**6**), obtained from the reaction of appropriate fatty acids with diphenyl phosphoroazidate.[14] Phosphorylation of **7** with 2-bromoethyl phosphorodichloridate (**9**) gave 2-methoxy-3-*N*-substituted carbamoyloxypropyl 2-bromoethyl phosphates (**10**), key intermediates for the synthesis of ACPLs with a variety of polar-head bases (**3**, types A, C—E). The reaction with trimethylamine gave 2-*O*-methyl-1-*O*-(*N*-substituted)carbamoylglycero-3-phosphocholines (**3**, type A), and that with a heterocycles such as pyridine, isoquinoline and thiazole gave ACPLs possessing the corresponding phosphoryl bases (**3**, types C, D and E). The reaction of **7** with 2-phthalimidoethyl phosphorodichloridate (**8**), followed by work-up, gave the phthalimidoethyl esters, which on hydrazinolysis gave ACPLs with the amino group as a polar-head base (**3**, type B).

ACPLs **3** have a chiral carbon in the glycerol moiety at position 2 and this chirality is presumed to have a profound influence upon the biological activity. However, in the present study, all of the type **3** compounds listed in Tables I and III are racemic.

Various ACPLs with the 1,3-propanediol moiety as a molecular backbone (**4**) were synthesized, in a similar way, starting from 1,3-propanediol according to the method outlined in Chart 2.

ACPLs (**3** and **4**) were purified by column chromatography and by crystallization from a suitable solvent system. The structure and purity of the products were confirmed by thin-layer chromatography (TLC), nuclear magnetic resonance (NMR) and elemental analysis. The

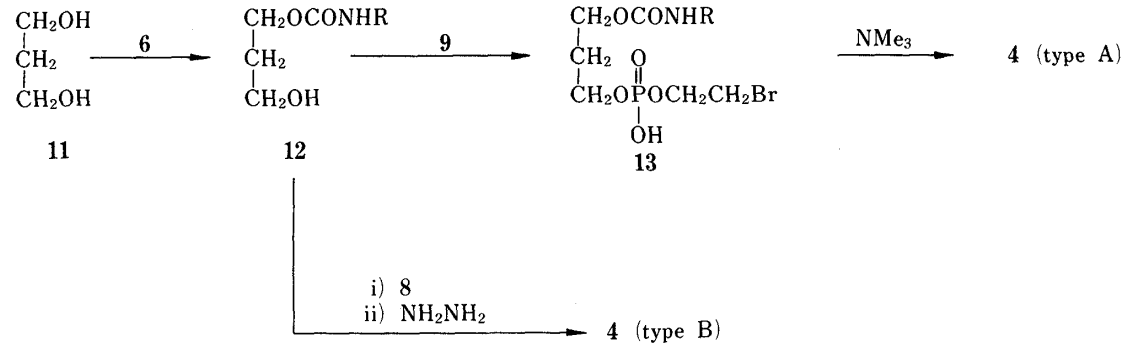


Chart 2

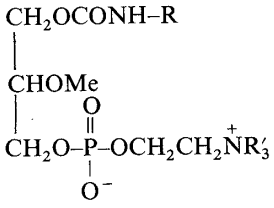
physical data are listed in Table VI. TLC on silica gel using $CHCl_3$–MeOH–H_2O (65:25:4) showed that the *Rf* values depended on the chemical structure; the *Rf* value increased as the alkylcarbamoyl chain length increased, and, in general, the *Rf* values of ACPLs with an amino group as a polar-head base were higher than those of ACPLs with the quaternary bases. ACPLs with saturated aliphatic chains were found to be difficult to fuse, with no definite melting point. ACPLs varied, depending upon the degree of the nonpolar-tail unsaturation, from high melting crystals or powder to a grease-like solid. ACPLs which have a quaternary polar-head base (**3** and **4**, types A, C, D and E) were hygroscopic, especially those with shorter alkyl chains, and were generally isolated as hydrates. They were amphiphilic and showed good solubility in a variety of solvents, which was advantageous for biological studies. However, with aliphatic chains longer than C_{18}, the water solubility decreased. In fact, 3-C_{22}-E and 4-C_{29}-A were practically insoluble in water. ACPLs with the amino function as a polar-head base (**3** and **4**, type B) crystallized readily and afforded colorless nonhygroscopic crystals. In contrast to those with the quaternary bases, the ACPLs of type B showed relatively poor solubility in water, MeOH and EtOH. The dependency of the solubility on the ACPL structure was similar to that of ALPLs reported previously.[6]

Structure and Activity Relationships

Syntheses of chemically defined ACPLs enabled us to study the structure-antimicrobial activity relationships. We examined a number of ACPLs for ability to inhibit the growth of *Tetrahymena pyriformis* and 13 species of fungi, and the results are listed in Tables I—IV. In contrast to lysolecithin and lecithin, many of the ACPLs (**3** and **4**), especially those having an aliphatic chain with C_{10-18} carbon atoms, showed potent activities in *in vitro* tests against *T. pyriformis* and a variety of human pathogenic and phytopathogenic fungi. However, ACPLs were found to be less inhibitory to Gram-positive and -negative bacteria.

A difference in the structure of the long aliphatic chain in ACPLs had a large effect on the antimicrobial activity. As shown in the tables, maximal potency and broad-spectrum inhibition against these microorganisms could be seen with *N*-tridecyl- and tetradecylcarbamoyl phospholipids (**3**-C_{13}-A, **3**-C_{14}-A, **4**-C_{13}-A and **4**-C_{14}-A). The minimum inhibitory concentration (MIC) values of these compounds were 0.1 μg/ml against *T. pyriformis* and 3.12 μg/ml against human pathogenic *Trichophyton rubrum* IFO 5467. Compared with clotrimazole, a clinically useful antimycotic drug, these tridecyl and tetradecylcarbamates were more active or comparably active against *T. pyriformis, Candida albicans, Trichophyton* spp. and *Saccharomyces cerevisiae*, but less active against nine other species of phytopathogenic fungi. *N*-Pentadecylcarbamoyl (**3**-C_{15}-A and **3**-C_{15}-E) and *N*-hexadecylcarbamoyl compounds (**3**-C_{16}-A and **3**-C_{16}-E) were slightly less active than *N*-tetradecylcarbamates against *T. pyriformis* but almost equally active against the above three human pathogenic fungi. The antimicrobial activities and spectrum of *N*-tetradecylcarbamate (**3**-C_{14}-A) were comparable to

TABLE I. Inhibitory Activities of *N*-Alkylcarbamoyl Phospholipids (**3**) against *T. pyriformis* and Human Pathogenic Fungi



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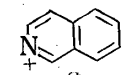
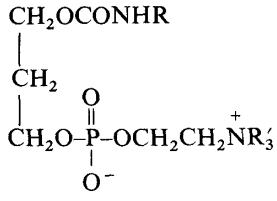
Compd. No.	R	$\overset{+}{N}R'_3$	MIC (μg/ml) *T. pyriformis*	*Candida albicans* TA	*Trichophyton mentagrophytes* IFO 5809	*Trichophyton rubrum* IFO 5467
3-C_{10}-A	$C_{10}H_{21}$	$\overset{+}{N}Me_3$	2	>100		
3-C_{10}-B	$C_{10}H_{21}$	$\overset{+}{N}H_3$	2	>100		
3-C_{12}-A	$C_{12}H_{25}$	$\overset{+}{N}Me_3$	0.2	50	12.5	3.12
3-C_{13}-A	$C_{13}H_{27}$	$\overset{+}{N}Me_3$	0.1	25	12.5	3.12
3-C_{14}-A	$C_{14}H_{29}$	$\overset{+}{N}Me_3$	0.1	12.5	6.25	3.12
3-C_{14}-B	$C_{14}H_{29}$	$\overset{+}{N}H_3$	1	12.5	12.5	6.25
3-C_{14}-E	$C_{14}H_{29}$		0.4	12.5	6.25	3.12
3-C_{15}-A	$C_{15}H_{31}$	$\overset{+}{N}Me_3$	0.2	6.25	3.12	3.12
3-C_{15}-E	$C_{15}H_{31}$		0.4	25	6.25	1.56
3-C_{16}-A	$C_{16}H_{33}$	$\overset{+}{N}Me_3$	0.4	25	3.12	1.56
3-C_{16}-E	$C_{16}H_{33}$		0.4	12.5	6.25	1.56
3-C_{17}-A	$C_{17}H_{35}$	$\overset{+}{N}Me_3$	0.4	12.5	12.5	3.12
3-C_{17}-E	$C_{17}H_{35}$		2	50	6.25	3.12
3-C_{18}-A	$C_{18}H_{37}$	$\overset{+}{N}Me_3$	2—4	>100	12.5	6.25
3-C_{18}-B	$C_{18}H_{37}$	$\overset{+}{N}H_3$	>4	>100		
3-C_{18}-C	$C_{18}H_{37}$		4	25	12.5	6.25
3-C_{18}-D	$C_{18}H_{37}$		≧4	>100	12.5	6.25
3-C_{18}-E	$C_{18}H_{37}$		2—4	>100	>100	50
3-C_{22}-E	$C_{22}H_{45}$		>4	100	>100	>100

those of tetradecyl lysophospholipid, a representative compound in a series of ALPLs.[6] MIC values of ACPLs had a chain length dependence quite similar to that displayed by ALPLs.[6] Increasing as well as decreasing the chain length from that of the tetradecyl group tended to

TABLE II. Inhibitory Activities of *N*-Alkyl- and *N*-Alkenylcarbamoyl Phospholipids (**4**) against *T. pyriformis* and Human Pathogenic Fungi



4

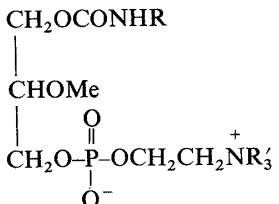
Compd. No.	R	$\overset{+}{N}R'_3$	MIC (μg/ml) *T. pyriformis*	*Candida albicans* TA	*Trichophyton mentagrophytes* IFO 5809	*Trichophyton rubrum* IFO 5467
4-C_{12}-A	$C_{12}H_{25}$	$\overset{+}{N}Me_3$	0.2	25	25	3.12
4-C_{12}-B	$C_{12}H_{25}$	$\overset{+}{N}H_3$	0.2	25	50	25
4-C_{13}-A	$C_{13}H_{27}$	$\overset{+}{N}Me_3$	0.1	12.5	12.5	3.12
4-C_{14}-A	$C_{14}H_{29}$	$\overset{+}{N}Me_3$	0.1	12.5	12.5	3.12
4-C_{14}-B	$C_{14}H_{29}$	$\overset{+}{N}H_3$	1	25	25	12.5
4-C_{17}-A	$C_{17}H_{35}$	$\overset{+}{N}Me_3$	2	50	12.5	6.25
4-$C_{17:2}$-A	$C_{17}H_{31}$	$\overset{+}{N}Me_3$	2	25		
4-$C_{17:2}$-B	$C_{17}H_{31}$	$\overset{+}{N}H_3$	0.4	50		
4-$C_{17:3}$-A	$C_{17}H_{29}$	$\overset{+}{N}Me_3$	0.2	25	6.25	3.12
4-$C_{17:3}$-B	$C_{17}H_{29}$	$\overset{+}{N}H_3$	0.2	>100		
Clotrimazole			4	25	6.25	3.12

lower the inhibitory activity of the resulting ACPL. In order to examine the effect of the aliphatic chain unsaturation, we synthesized *N*-alkenylcarbamoyl phospholipids which include *cis* double bonds. Tables II and IV suggest that the unsaturation does not drastically change the inhibitory properties, although *N*-8,11,14(*Z,Z,Z*)-heptadecatrienylcarbamate (**4**-$C_{17:3}$-A) was slightly more inhibitory than the corresponding heptadecanylcarbamate (**4**-C_{17}-A) and the *N*-8,11(*Z,Z*)-heptadecadienylcarbamate (**4**-$C_{17:2}$-A).

Modification of the polar-head group was found to exert a striking influence on the antimicrobial activity of ACPLs. Comparison of the MICs of different types of *N*-octadecylcarbamoyl phospholipids against *T. pyriformis* showed that the trimethylammonio group (**3**-C_{18}-A) was most preferable as a polar-head base, and the thiazolio (**3**-C_{18}-E), pyridinio (**3**-C_{18}-C), isoquinolinio (**3**-C_{18}-D) and ammonio group (**3**-C_{18}-B) exerted a decreasing inhibitory effect in that order. Against human pathogenic fungi, the most inhibitory polar-head base in the ACPLs with the C_{12-18} alkyl group was the trimethylammonio group, followed by the thiazolio group. Replacing the trimethylammonio group of *N*-octadecylcarbamoyl phospholipid (**3**-C_{18}-A) with various other polar-head bases did not improve the activity against phytopathogenic fungi (Table III).

A difference in the structure of the molecular backbone, glycerol or 1,3-propanediol, produced no detectable effect upon the inhibitory activity of ACPLs. Comparison of the activities of 1-*O*-(*N*-alkyl)carbamoyl-2-*O*-methylglycero-3-phosphocholines with a carbon chain from C_{12} to C_{14} and C_{17} (**3**-C_{12-14}-A and **3**-C_{17}-A) with those of the corresponding 2-demethoxy compounds (**4**-C_{12-14}-A and **4**-C_{17}-A) showed that ACPLs **3** and **4** possessed

TABLE III. Inhibitory Activities of *N*-Alkylcarbamoyl Phospholipids (**3**) against a Variety of Fungi



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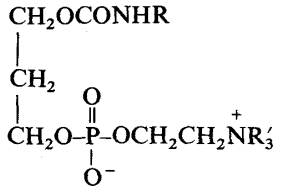
Compd. No.	MIC (μg/ml)									
	Aspergillus niger IFO 6341	*Penicillium citrinum* IFO 6352	*Mucor spinescens* IFO 6350	*Rhodotorula rubra* IFO 0907	*Saccharomyces cerevisiae* IFO 0209	*Pyricularia oryzae* IFO 5279	*Helmin-thosporium oryzae* IFO 7503	*Botrytis cinerea* IFO 5365	*Helmin-thosporium sigmoideum* IFO 4867	*Collectrichum lagenarium* IFO 6207
3-C_{10}-A	>100	>100	>100	>100	>100	100	100	100	100	50
3-C_{12}-A	25	25	25	50	12.5	12.5	25	12.5	25	6.25
3-C_{13}-A	25	12.5	12.5	25	12.5	6.25	25	12.5	12.5	6.25
3-C_{14}-A	12.5	12.5	12.5	25	6.25	3.12	12.5	3.12	6.25	6.25
3-C_{14}-B	25	25	>100	50	25	6.25	>100	6.25	100	100
3-C_{15}-A	>100	12.5	25	>100	12.5	1.56	12.5	1.56		100
3-C_{15}-E	>100	50	>100	>100	25	1.56	>100	12.5		100
3-C_{16}-A	>100	100	>100	>100	>100	1.56	25	3.12		100
3-C_{16}-E	>100	>100	>100	>100	>100	3.12	>100	25		>100
3-C_{17}-A	>100	>100	>100	>100	>100	1.56	100	6.25		100
3-C_{17}-E	>100	>100	>100	>100	>100	3.12	>100	100		>100
3-C_{18}-A	>100	>100	>100	>100	>100	3.12	>100	25	12.5	>100
3-C_{18}-B	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
3-C_{18}-C	>100	>100	>100	>100	>100	6.25	>100	25	12.5	>100
3-C_{18}-D	>100	>100	>100	>100	>100	6.25	>100	6.25	25	>100
3-C_{18}-E	>100	>100	>100	>100	>100	12.5	>100	50	50	>100

TABLE IV. Inhibitory Activities of *N*-Alkyl- and *N*-Alkenylcarbamoyl Phospholipids (**4**) against a Variety of Fungi



4

Compd. No.	MIC (μg/ml)									
	Aspergillus niger IFO 6341	*Penicillium citrinum* IFO 6352	*Mucor spinescens* IFO 6350	*Rhodotorula rubra* IFO 0907	*Saccharomyces cerevisiae* IFO 0209	*Pyricularia oryzae* IFO 5279	*Helminthosporium oryzae* IFO 7503	*Botrytis cinerea* IFO 5365	*Helminthosporium sigmoideum* IFO 4867	*Collectrichum lagenarium* IFO 6207
4-C_{12}-A	12.5	12.5	12.5	25	12.5	12.5	12.5	12.5	25	3.12
4-C_{12}-B	25	25	100	50	25	25	50	25	50	25
4-C_{13}-A	12.5	12.5	12.5	12.5	12.5	6.25	12.5	6.25	12.5	6.25
4-C_{14}-A	12.5	12.5	12.5	25	6.25	3.12	25	12.5	6.25	6.25
4-C_{14}-B	50	25	>100	25	50	25	100	50	100	100
4-C_{17}-A	>100	>100	>100	>100	>100	6.25	>100	12.5	50	50
4-$C_{17:2}$-A	100	100	>100	>100	>100	12.5	50	25	>100	50
4-$C_{17:2}$-B	>100	>100	>100	>100	>100	100	>100	50	50	>100
4-$C_{17:3}$-A	50	50	100	100	100	12.5	25	12.5	50	25
4-$C_{17:3}$-B	>100	>100	>100	>100	>100	50	100	50	100	100
Clotrimazole	6.25	3.12	<0.78	1.56	12.5	0.2	3.12	0.78	0.2	0.78

substantially the same activity against *T. pyriformis* and a number of fungi and very similar antimicrobial spectra (Tables I—IV).

Observation under a phase-contrast microscope indicated that there was no appreciable change in the shape, size and surface appearance of *Tetrahymena* cells, when they died after exposure to ACPLs (*e.g.*, **3**-C_{14}-A) for several hours. This suggests that cell death preceded osmotic swelling or rupture of the cell membranes. Although the mechanism of antimicrobial action remains to be clarified, the ACPL action site is assumed to be the cell membranes. In view of recent work on the analysis of cytolytic and membrane-perturbing properties of lysophosphatidylcholine and its analogues,[15,16] we assumed that the first step in the interaction with eukaryotic cells is the incorporation of ACPL molecules into the lipid layer of the cell membranes, and, due to the resistance of the carbamate linkage of the phospholipid analogs to phospholipases A_1 and A_2,[13] accumulation and retention of ACPL molecules in the lipid bilayer occurs as the second step. ACPLs can be considered to have a preferential modulating activity towards biomembranes (*e.g.*, membrane proteins), like lysolecithin.[15-17] This presumably causes a change in enzymatic function, through a change in the membrane microenvironments, leading to a disturbance of cellular metabolism (*e.g.*, disturbance of cell-membrane permeability to ions and small molecules) and finally results in cell death. Further study is needed to elucidate the growth inhibitory mechanism of ACPLs.

Because eukaryotic microorganisms and mammalian cells[18] resemble each other morphologically and metabolically, *Tetrahymena* and certain species of fungi have been used as a preliminary test system for screening cytotoxic antitumor agents.[19] Although prediction of the *in vivo* activity of compounds is beyond its scope, this prescreening system appears to be useful for detecting *in vitro* antitumor activity due to the simplicity of the procedure and the reproducibility of the results. In fact, we found that most of the ACPLs to which *T. pyriformis* and certain species of fungi were sensitive definitely inhibited the growth of myeloid leukemia cells at concentrations of the same order as the MICs; those ACPLs that did not inhibit the eukaryotic microorganisms had no antiproliferative effect upon tumor cells.[12]

In contrast to octadecyl lysophospholipid (ALPL, **1**, $R=C_{18}H_{37}$, $R'=CH_3$),[20] none of the ACPLs caused rabbit platelet aggregation or hypotension in rats. Certain ACPLs were shown to possess essentially the same *in vivo* antitumor activity but much less toxicity as compared with the octadecyl ether (ALPL, **1**). Details will be reported elsewhere in the near future. Further studies on the evaluation of biologically important ACPLs are in progress in our laboratories.

Experimental

Infrared (IR) spectra were recorded on a Hitachi EPI-G2 spectrometer. NMR spectra were measured with a Varian T-60 spectrometer. Chemical shifts are expressed as δ (ppm), using TMS as an internal standard. TLC was performed using precoated silica gel plates (Kieselgel 60, F-254, Merck) in a solvent system of $CHCl_3$–MeOH–H_2O (65 : 25 : 4). A 10% solution of phosphomolybdic acid in EtOH was used for detection.

Materials——Clotrimazole used as a reference standard was obtained from a commercial source.

MIC——a) MICs of ACPLs against fungi were determined by the agar dilution method after incubation at 28 °C for 3 d. The assay medium used for phytopathogenic fungi, saprophytic fungi and yeast had the following composition: 1.0% glucose, 1.0% meat extract, 1.0% peptone, 0.25% NaCl and 2.5% agar in water. In the case of *Trichophyton*, a medium containing 1% peptone, 4% glucose and 2.5% agar was used. For *Candida*, 4% Tripticase soy agar (BBL) was used.

b) MIC against *Tetrahymena pyriformis* was determined by the broth dilution method described by Tanida *et al.*[21]

3-*O*-*N*-Alkylcarbamoyl-2-*O*-methylglycerol (7, General Procedure)——The 2-*O*-methylglyceryl carbamate (**7**) was prepared by treating 2-*O*-methylglycerol in an inert solvent with an appropriate alkyl isocyanate prepared from the corresponding carboxylic acid by the method of Shioiri.[14] Typical examples follow.

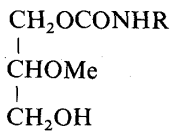
2-*O*-Methyl-3-*O*-(*N*-octadecyl)carbamoylglycerol (**7**, $R=C_{18}H_{37}$-): *n*-Octadecyl isocyanate (9.7 g, 33 mmol) and 2-*O*-methylglycerol (3.5 g, 33 mmol) were dissolved in pyridine (20 ml). The mixture was stirred at room tem-

perature overnight, poured into water, neutralized with HCl and extracted with ether. The extract was washed with water, dried and concentrated. The residue was chromatographed on silica gel (200 g) using $CHCl_3$–Et_2O (1 : 1) as an eluent. After work-up, the desired compound was obtained as colorless leaflets (8.2 g). IR ν_{max}^{Nujol} cm^{-1}: 3340, 1687. MS *m/e*: 401 (M^+).

2-*O*-Methyl-3-*O*-(*N*-pentadecyl)carbamoylglycerol (**7**, R = $C_{15}H_{31}$–): A mixture of *n*-hexadecanoic acid (5.13 g, 20 mmol), diphenylphosphoroazidate (DPPA, 5.5 g, 20 mmol) and triethylamine (3 ml) in toluene (50 ml) was stirred at room temperature for 2 h, then refluxed for 1 h. After cooling, CH_2Cl_2 (20 ml) and 2-*O*-methylglycerol (4.2 g, 40 mmol) in CH_2Cl_2 (20 ml) were added to the mixture. The combined solution was refluxed for 2 h and water (200 ml) was added. The mixture was stirred vigorously and the organic layer was separated, dried and concentrated. The residue was chromatographed on silica gel (60 g) using ethyl acetate–*n*-hexane (1 : 1) as an eluent. After work-up, the desired product was obtained as colorless leaflets (5.25 g). IR ν_{max}^{KBr} cm^{-1}: 3350, 2920, 2850, 1690. NMR ($CDCl_3$) δ: 0.88 (3H, t), 1.27 (26H), 2.30 (1H, br), 3.0 (2H, t), 3.46 (3H), 3.53 (2H, d), 3.65 (1H, m), 4.20 (2H, d), 4.83 (1H, br).

The following 3-*O*-(*N*-alkyl)carbamoyl-2-*O*-methylglycerols (**7**) were synthesized in a similar way. Their melting points are listed in Table V.

TABLE V. Melting Points of 3-*O*-(*N*-Alkyl)carbamoyl-2-*O*-methylglycerols (**7**)



7

R	$C_{10}H_{21}$	$C_{12}H_{25}$	$C_{13}H_{27}$	$C_{14}H_{29}$	$C_{15}H_{31}$	$C_{16}H_{33}$
mp	Oil	35—36 °C	31—32 °C	38—39 °C	42.5—43 °C	47.5—48 °C
R	$C_{17}H_{35}$	$C_{18}H_{37}$	$C_{22}H_{45}$			
mp	52—52.5 °C	55—56 °C	69—70 °C			

3-(*N*-Substituted carbamoyloxy)propan-1-ol (12, General Procedure)——A series of 3-(*N*-substituted carbamoyloxy)propanols was synthesized by a method similar to that described for 3-*O*-(*N*-alkyl)carbamoyl-2-*O*-methylglycerols. Typical examples follow.

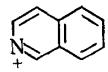
3-(*N*-Heptadecylcarbamoyloxy)propan-1-ol (**12**, R = $C_{17}H_{35}$–): A mixture of stearic acid (5.68 g, 20 mmol), DPPA (5.5 g, 20 mmol) and triethylamine (3.0 ml) in toluene (50 ml) was stirred at room temperature for 2 h. After addition of ether (100 ml), the separated organic layer was dried, concentrated (to 40 ml) and refluxed for 2 h. The solvent was removed and 1,3-propanediol (5.7 g, 75 mmol) and CH_2Cl_2 (30 ml) were added to the residue. After the resulting mixture had been stirred at room temperature overnight, water (20 ml) and $CHCl_3$ (20 ml) were added. The separated organic layer was dried and concentrated. The residue was chromatographed on silica gel using chloroform–water (93 : 3) as an eluent. After work-up, the desired product was obtained as colorless leaflets (3.0 g). mp 77—78 °C. IR ν_{max}^{film} cm^{-1}: 3450, 2920, 2850, 1695, 1640, 1530.

3-(*N*-8,11(*Z,Z*)-Heptadecadienylcarbamoyloxy)propan-1-ol (**12**, R = $C_{17}H_{31}$–): A mixture of linoleic acid (7.01 g, 25 mmol), DPPA (6.875 g, 25 mmol) and triethylamine (3.75 ml) in toluene (63 ml) was stirred at room temperature for 3 h, then refluxed for 2 h. 1,3-Propanediol (8 g, 105 mmol) in pyridine (125 ml) was added and stirring of the mixture was continued at room temperature for 3 d. The reaction mixture was evaporated and the residue was chromatographed on silica gel (70 g) using $CHCl_3$–MeOH (98 : 2) as an eluent. After work-up, the desired compound was obtained as a colorless oil (3.6 g). IR ν_{max}^{film} cm^{-1}: 3350, 3010, 2930, 1850, 1700, 1540, 1465, 1265, 1145, 1060. NMR (DMSO-d_6) δ: 0.92 (3H, t), 1.30 (14H, s), 1.72 (2H, m), 2.07 (4H, m), 2.97 (4H, m), 3.53 (2H, q), 4.05 (2H, t), 4.47 (1H, t), 5.45 (4H, m), 7.03 (1H, br).

3-(*N*-Alkyl)carbamoyloxy-2-methoxypropyl 2-*N*-Trisubstituted Ammonioethyl Phosphate[22] (3-C*n*-A, C, D and E, General Procedure)——An appropriate 3-*O*-(*N*-alkyl)carbamoyl-2-*O*-methylglycerol (**7**) was treated with 2-bromoethyl phosphorodichloridate (**9**) in an anhydrous solvent containing pyridine at room temperature for several hours. Evaporation of the reaction mixture afforded the residue of the diesterified phosphorochloridate, which, after being treated with water, gave the intermediary 3-(*N*-alkyl)carbamoyloxy-2-methoxypropyl 2-bromoethyl phosphate (**10**). This was treated with an excess of a selected amine to obtain the corresponding *N*-alkylcarbamoyl phospholipid (**3**). Thus, **3**-C_n-A, **3**-C_n-C, **3**-C_n-D and **3**-C_n-E were synthesized by the reaction using trimethylamine, pyridine, isoquinoline and thiazole, respectively. Their physical data are shown in Table VI. Typical examples follow.

2-Methoxy-3-(*N*-pentadecyl)carbamoyloxypropyl 2-Trimethylammonioethyl Phosphate (**3**-C_{15}-A): Pyridine (1.6 g, 20 mmol) was added to a solution of 2-*O*-methyl-3-*O*-(*N*-pentadecyl)carbamoylglycerol (**7**, R = $C_{15}H_{31}$–, 4.31 g, 12 mmol) and 2-bromoethyl phosphorodichloridate (**9**, 4.93 g, 20 mmol) in toluene (24 ml) with stirring under ice-cooling. The mixture was stirred at room temperature for 3 h and evaporated *in vacuo*. The residue was

TABLE VI. Physicochemical Properties of

Compd. No.	R	NR_3'	Appearance	TLC *Rf*	Formula
3-C_{10}-A	$C_{10}H_{21}$	$\overset{+}{N}Me_3$	*a)*	0.15	$C_{20}H_{43}N_2O_7P \cdot H_2O$
3-C_{10}-B	$C_{10}H_{21}$	$\overset{+}{N}H_3$	*b)*	0.47	$C_{17}H_{37}N_2O_7P$
3-C_{12}-A	$C_{12}H_{25}$	$\overset{+}{N}Me_3$	*a)*	0.15	$C_{22}H_{47}N_2O_7P \cdot 0.5H_2O$
4-C_{12}-A	$C_{12}H_{25}$	$\overset{+}{N}Me_3$	*a)*	0.15	$C_{21}H_{45}N_2O_6P \cdot 2H_2O$
4-C_{12}-B	$C_{12}H_{25}$	$\overset{+}{N}H_3$	*b)*	0.50	$C_{18}H_{39}N_2O_6P$
3-C_{13}-A	$C_{13}H_{27}$	$\overset{+}{N}Me_3$	*a)*	0.17	$C_{23}H_{49}N_2O_7P \cdot 0.5H_2O$
4-C_{13}-A	$C_{13}H_{27}$	$\overset{+}{N}Me_3$	*a)*	0.16	$C_{22}H_{47}N_2O_6P \cdot 0.5H_2O$
3-C_{14}-A	$C_{14}H_{29}$	$\overset{+}{N}Me_3$	*a)*	0.17	$C_{24}H_{51}N_2O_7P \cdot H_2O$
3-C_{14}-B	$C_{14}H_{29}$	$\overset{+}{N}H_3$	*b)*	0.50	$C_{21}H_{45}N_2O_7P$
3-C_{14}-E	$C_{14}H_{29}$		*c)*	0.21	$C_{24}H_{45}N_2O_7PS \cdot 2H_2O$
4-C_{14}-A	$C_{14}H_{29}$	$\overset{+}{N}Me_3$	*a)*	0.16	$C_{23}H_{49}N_2O_6P \cdot H_2O$
4-C_{14}-B	$C_{14}H_{29}$	$\overset{+}{N}H_3$	*b)*	0.50	$C_{20}H_{43}N_2O_6P$
3-C_{15}-A	$C_{15}H_{31}$	$\overset{+}{N}Me_3$	*a)*	0.16	$C_{25}H_{53}N_2O_7P \cdot 0.5H_2O$
3-C_{15}-E	$C_{15}H_{31}$		*c)*	0.22	$C_{25}H_{47}N_2O_7PS \cdot H_2O$
3-C_{16}-A	$C_{16}H_{33}$	$\overset{+}{N}Me_3$	*a)*	0.17	$C_{26}H_{55}N_2O_7P \cdot 0.5H_2O$
3-C_{16}-E	$C_{16}H_{33}$		*c)*	0.22	$C_{26}H_{49}N_2O_7PS \cdot H_2O$
3-C_{17}-A	$C_{17}H_{35}$	$\overset{+}{N}Me_3$	*a)*	0.17	$C_{27}H_{57}N_2O_7P \cdot 0.5H_2O$
3-C_{17}-E	$C_{17}H_{35}$		*c)*	0.22	$C_{27}H_{51}N_2O_7PS \cdot 1.5H_2O$
4-C_{17}-A	$C_{17}H_{35}$	$\overset{+}{N}Me_3$	*a)*	0.17	$C_{26}H_{55}N_2O_6P \cdot H_2O$
4-$C_{17:2}$-A	$C_{17}H_{31}$	$\overset{+}{N}Me_3$	*d)*	0.18	$C_{26}H_{51}N_2O_6P \cdot 2H_2O$
4-$C_{17:2}$-B	$C_{17}H_{31}$	$\overset{+}{N}H_3$	*d)*	0.52	$C_{23}H_{45}N_2O_6P \cdot 0.2H_2O$
4-$C_{17:3}$-A	$C_{17}H_{29}$	$\overset{+}{N}Me_3$	*d)*	0.18	$C_{26}H_{49}N_2O_6P \cdot H_2O$
4-$C_{17:3}$-B	$C_{17}H_{29}$	$\overset{+}{N}H_3$	*d)*	0.52	$C_{23}H_{43}N_2O_6P \cdot H_2O$
3-C_{18}-A	$C_{18}H_{37}$	$\overset{+}{N}Me_3$	*a)*	0.18	$C_{28}H_{59}N_2O_7P \cdot 1.5H_2O$
3-C_{18}-B	$C_{18}H_{37}$	$\overset{+}{N}H_3$	*b)*	0.53	$C_{25}H_{53}N_2O_7P \cdot 0.5H_2O$
3-C_{18}-C	$C_{18}H_{37}$		*a)*	0.24	$C_{30}H_{55}N_2O_7P \cdot 0.5H_2O$
3-C_{18}-D	$C_{18}H_{37}$		*c)*	0.60	$C_{34}H_{57}N_2O_7P \cdot 2.5H_2O$
3-C_{18}-E	$C_{18}H_{37}$		*c)*	0.24	$C_{28}H_{53}N_2O_7PS \cdot 1.5H_2O$
3-C_{22}-E	$C_{22}H_{45}$		*c)*	0.25	$C_{32}H_{61}N_2O_7PS \cdot 2H_2O$
3-C_{29}-A	$C_{29}H_{59}$	$\overset{+}{N}Me_3$	*c)*	0.22	$C_{39}H_{81}N_2O_7P \cdot H_2O$

a) Colorless powder, hygroscopic. *b)* Colorless crystalline powder. *c)* Pale yellow powder, hygroscopic.

N-Alkyl- and *N*-Alkenylcarbamoyl Phospholipids

Analysis (%)							
Calcd				Found			
C	H	N	P	C	H	N	P
50.83	9.60	5.93	6.55	50.46	9.69	6.04	6.82
49.50	9.04	6.79	7.51	49.48	8.66	6.86	7.61
53.58	9.99	5.63	6.21	53.75	9.84	5.70	6.30
51.62	10.10	5.73	6.34	51.57	9.88	5.75	6.63
52.67	9.58	6.82	7.55	52.59	9.39	6.96	7.54
54.46	9.97	5.54	6.12	54.21	9.76	5.78	6.07
55.56	10.17	5.89	6.51	55.32	10.34	5.81	6.51
54.52	10.11	5.30	5.86	54.48	10.06	5.15	5.88
53.83	9.68	5.98	6.61	53.61	9.59	5.95	6.68
50.33	8.27	4.89	5.40	50.35	8.20	4.76	5.38
55.40	10.31	5.62	6.21	55.10	10.51	5.41	6.36
54.78	9.88	6.39	7.06	54.52	10.08	6.19	7.13
56.26	10.28	5.25	5.80	56.24	10.11	5.22	5.75
52.80	8.68	4.93	5.45	52.56	9.17	4.93	5.36
57.02	10.31	5.11	5.66	57.04	10.44	5.04	5.63
53.59	8.82	4.81	5.32	53.69	9.08	4.90	5.35
57.73	10.41	4.99	5.51	57.67	10.37	4.93	5.71
53.54	8.98	4.62	5.11	53.42	9.14	4.61	5.22
57.75	10.63	5.18	5.73	57.60	10.61	5.32	5.53
56.29	9.99	5.05	5.58	56.22	9.53	5.18	5.46
57.53	9.53	5.83	6.45	57.54	9.60	5.96	6.45
58.41	9.61	5.24	5.79	58.16	9.63	5.23	5.75
56.08	9.21	5.69	6.29	56.08	8.92	5.91	6.07
56.63	10.52	4.72	5.22	56.37	10.70	4.91	5.27
56.26	10.20	5.25	5.80	56.27	10.62	5.29	5.88
60.48	9.48	4.70	5.20	60.20	9.28	4.77	5.30
59.89	9.17	4.11	4.54	59.92	9.00	4.33	4.68
54.26	9.11	4.52	5.00	54.30	8.90	4.71	5.03
56.12	9.57	4.09	4.52	56.14	9.47	4.07	4.68
63.38	11.32	3.79	4.19	63.51	11.45	4.01	3.98

d) Viscous compound.

treated with water (100 ml) at room temperature for 1 h, refluxed for 1 h, then cooled and extracted with ether. The extract was washed with water, dried and evaporated to dryness to give the intermediary 2-bromoethyl phosphate (**10**, R = $C_{15}H_{31}$–, 6.56 g). A portion (1.64 g) of the intermediate was dissolved in a toluene solution (20 ml) of trimethylamine (4 g), and the mixture was allowed to stand at room temperature for 2 d, then evaporated to dryness. The residue was chromatographed on a column of silica gel (15 g) using $CHCl_3$–MeOH (65 : 25), followed by $CHCl_3$–MeOH–H_2O (65 : 25 : 4). After work-up, the desired product was obtained as an amorphous solid (818 mg). IR ν_{max}^{film} cm^{-1}: 3350, 2920, 2850, 1700, 1540, 1465, 1240, 1090, 1060, 970, 910, 735. NMR ($CDCl_3$) δ: 0.88 (3H, t), 1.27 (26H), 3.10 (2H, m), 3.35 (9H, s), 3.42 (3H, s), 3.58 (1H, m), 3.83 (2H, m), 4.20 (6H, m), 5.93 (1H, br).

2-Methoxy-3-(*N*-pentadecyl)carbamoyloxypropyl 2-Thiazolioethyl Phosphate (**3**-C_{15}-E): The intermediary 2-methoxy-3-(*N*-pentadecyl)carbamoyloxypropyl 2-bromoethyl phosphate (**10**, R = $C_{15}H_{31}$–), described above (4.92 g), was dissolved in a mixture of thiazole (3.8 g) and benzene (5 ml). The solution was warmed at 60 °C for 13 h, refluxed for 4 h, then evaporated to dryness. The residue was chromatographed on a column of silica gel (45 g) using $CHCl_3$–MeOH (4 : 1), followed by $CHCl_3$–MeOH–H_2O (65 : 25 : 4) to give the desired compound as a pale yellow solid (1.05 g). IR ν_{max}^{film} cm^{-1}: 3350, 2920, 2850, 1700, 1550, 1465, 1240, 1095, 1060, 910. NMR ($CDCl_3$) δ: 0.88 (3H, t), 1.22 (26H), 3.10 (2H, m), 3.38 (3H, s), 3.55 (1H, m), 3.73 (2H, m), 4.13 (6H, m), 4.95 (2H, m), 6.00 (1H, br), 8.33 (1H), 8.60 (1H), 10.53 (1H).

2-Methoxy-3-(*N*-octadecyl)carbamoyloxypropyl 2-Pyridinioethyl Phosphate (**3**-C_{18}-C): Pyridine (0.84 g, 6 mmol) was added dropwise to a solution of 2-*O*-methyl-3-*O*-(*N*-octadecyl)carbamoylglycerol (**7**, R = $C_{18}H_{37}$–, 2.0 g, 5 mmol) and the phosphorodichloridate (**9**, 1.45 g, 6 mmol) in benzene (25 ml) under ice-cooling. The mixture was stirred at room temperature for 6 h, then concentrated *in vacuo*. After addition of water (20 ml), the whole was refluxed for 1 h, then cooled and extracted with ether. The extract was washed with water, dried and concentrated. The residue, 2-methoxy-3-(*N*-octadecyl)carbamoyloxypropyl 2-bromoethyl phosphate (**10**, R = $C_{18}H_{37}$–), was dissolved in pyridine (30 ml) and the solution was warmed at 60 °C for 14 h, then evaporated to dryness. Ag_2CO_3 (3 g) and MeOH (50 ml) were added to the residue. The mixture was refluxed for 2 h, then filtered and evaporated to dryness. The residue was chromatographed on a column of silica gel (30 g) using $CHCl_3$–MeOH–H_2O (65 : 25 : 4) as an eluent. After work-up, the desired product was obtained as a colorless solid (0.90 g). IR ν_{max}^{KBr} cm^{-1}: 3340, 1698, 1540, 1470, 1255, 1075, 1050. NMR ($CDCl_3$) δ: 0.7—1.8 (35H), 3.44 (3H, s), 2.9—4.8 (9H, m), 5.20 (2H, br), 6.16 (1H, br), 8.0—8.8 (3H, m), 9.58 (2H, m).

2-Methoxy-3-(*N*-octadecyl)carbamoyloxypropyl 2-Isoquinolinioethyl Phosphate (**3**-C_{18}-D): The intermediary 2-methoxy-3-(*N*-octadecyl)carbamoyloxypropyl 2-bromoethyl phosphate (**10**, R = $C_{18}H_{37}$–, 2.9 g), prepared as described above, was dissolved in isoquinoline (3 ml) and the solution was heated at 75 °C for 17 h, then evaporated to dryness. Ag_2CO_3 (3 g) and MeOH (50 ml) were added, and the mixture was refluxed for 1 h. Work-up as described above gave the desired product as a white solid (480 mg). IR ν_{max}^{film} cm^{-1}: 3320, 2920, 2850, 1700, 1640, 1530, 1095, 1060, 930, 820. NMR δ: 0.88 (3H), 1.27 (35H), 3.13 (3H), 3.32 (3H), 3.3—3.8 (2H), 3.7—4.4 (4H), 4.63 (2H), 5.30 (2H), 6.0 (1H), 8.0 (3H), 8.50 (1H), 8.57 (1H), 9.10 (1H), 10.70 (1H).

3-(*N*-Substituted Carbamoyloxy)propyl 2-Trisubstituted Ammonioethyl Phosphate (4-C_n-A, General Procedure)——The title phosphate was synthesized starting from an appropriate 3-[*N*-alkyl (or alkenyl) carbamoyloxy]propan-1-ol (**12**) by reaction with 2-bromoethyl phosphorodichloridate (**9**) and subsequent hydrolysis of the intermediary diesterified phosphorochloridate, followed by reaction of the produced phosphate (**13**) with trisubstituted amine as described in the previous section. A typical example follows.

3-[*N*-8,11(*Z*,*Z*)-Heptadecadienyl]carbamoyloxypropyl 2-Trimethylammonioethyl Phosphate (**4**-$C_{17;2}$-A): A mixture of 3-[*N*-8,11(*Z*,*Z*)-heptadecadienylcarbamoyloxy]propan-1-ol (**12**, R = $C_{17}H_{31}$–, 1.8 g, 5.3 mmol) and the phosphorodichloridate (**9**, 1.28 g, 5.3 mmol) in chloroform (65 ml) was refluxed for 3 h, then evaporated to dryness. Water (20 ml) was added to the residue and the mixture was refluxed for 1 h and extracted with chloroform (20 ml). The organic layer was dried and concentrated to dryness. The residue was dissolved in a toluene solution (50 ml) of trimethylamine (10 g) and the mixture was heated at 65 °C for 2 d in a sealed tube. After concentration of the mixture *in vacuo*, followed by addition of Ag_2CO_3 (1.8 g) and MeOH (25 ml), the whole was refluxed for 1 h and filtered. The filtrate was concentrated and the residue was purified by chromatography on a column of silica gel (30 g) with $CHCl_3$–MeOH–H_2O (65 : 25 : 4) as an eluent. After work-up, a colorless amorphous solid was obtained (0.5 g). IR ν_{max}^{film} cm^{-1}: 3350, 3010, 2960, 2940, 2860, 1700, 1540, 1460, 1230, 1140, 1090, 1060, 970.

3-(*N*-Alkyl)carbamoyloxy-2-methoxypropyl 2-Aminoethyl Phosphate (3-C_n-B, General Procedure)——The title compound was synthesized from an appropriate *N*-alkylcarbamoyloxyalcohol (**7**) by reaction with 2-phthalimidoethyl phosphorodichloridate (**8**) and subsequent hydrolysis of the diesterified phosphorochloridate, followed by hydrazinolysis of the intermediary diesterified phosphate. A typical example follows.

2-Methoxy-3-(*N*-octadecyl)carbamoyloxypropyl 2-Aminoethyl Phosphate (**3**-C_{18}-B): Pyridine (0.8 g, 10 mmol) was added to a solution of 2-*O*-methyl-3-*O*-(*N*-octadecyl)carbamoylglycerol (**7**, R = $C_{18}H_{37}$–, 2.05 g, 5 mmol) and 2-phthalimidoethyl phosphorodichloridate (**8**, 1.85 g, 6 mmol) in benzene (50 ml) under ice-cooling, and the mixture was stirred at room temperature for 1.5 h, then evaporated *in vacuo*. Water (4 ml) and pyridine (5 ml) were added to the residue and the mixture was warmed at 65 °C for 30 min. The reaction mixture was neutralized and extracted with ether. The extract was dried and concentrated, then the resulting residue was dissolved in an MeOH solution (50 ml)

of hydrazine hydrate (1 g). The mixture was refluxed for 1 h and concentrated. Chloroform (100 ml) was added to the residue and the precipitated phthalhydrazide was filtered off. After concentration of the filtrate, the residue was chromatographed on a silica gel column using $CHCl_3$–MeOH–H_2O (65 : 25 : 4) as an eluent. After recrystallization from MeOH, the desired compound (1.3 g) was obtained as fine needles. mp 201—203 °C. IR ν_{max}^{KBr} cm^{-1}: 2910, 2830, 1685, 1545, 1470, 1220, 1080, 1000, 920, 800.

3-(*N*-Substituted Carbamoyloxy)propyl 2-Aminoethyl Phosphate (4-C_n-B, General Procedure)——The title compound was synthesized from an appropriate *N*-substituted carbamoyloxyalcohol (**11**) by a method similar to that described above. A typical example follows.

3-(*N*-Tetradecyl)carbamoyloxypropyl 2-Aminoethyl Phosphate (**4**-C_{14}-B): Pyridine (1.1 g, 14 mmol) was added dropwise to a solution of 3-(*N*-tetradecylcarbamoyloxy)propan-1-ol (**11**, R = $C_{14}H_{29}$–, 3.0 g, 9.5 mmol) and 2-phthalimidoethyl phosphorodichloridate (**8**, 3.8 g, 12.3 mmol) in benzene (40 ml), and the mixture was stirred at room temperature for 3 h, then evaporated to dryness *in vacuo*. The residue was taken up in 70% aqueous pyridine (16 ml). The solution was warmed at 70 °C for 1.5 h, concentrated, neutralized and extracted with chloroform. The extract was dried and concentrated. Hydrazine hydrate (1.4 g) in MeOH (70 ml) was added to the residue and the mixture was refluxed for 30 min, then again concentrated. The residue was chromatographed on silica gel using $CHCl_3$–MeOH–H_2O (65 : 25 : 4) as an eluent. After work-up, the product was recrystallized from MeOH and gave needles (2.2 g). mp 211—213 °C. IR ν_{max}^{KBr} cm^{-1}: 1690, 1520, 1413, 1213, 1080, 1010.

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