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Preparation of Lecithin-Type of Phospholipid Analogues and Mesomorphic States[1]

Naoki Mitsuo, Takehisa Kunieda, and Takeo Takizawa

Faculty of Pharmaceutical Sciences, University of Tokyo[2]

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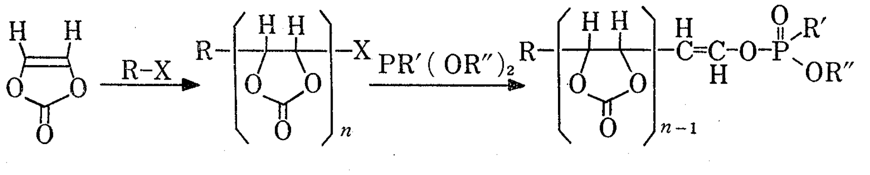
Enol phosphates (3), arising from reaction of vinylene carbonate-tetrachloromethane telomers (2) with phosphite, were readily converted by successive procedures involving hydrolytic cleavage of carbonate rings, acylation and introduction of ethylamine moieties, to the unnatural types of cephalin and lecithin analogues, which possessed saturated long ($C_{18:0}$) and short ($C_{8:0}$) chains. Two kinds of fatty acid residues were selectively introduced by monoacylation with acid anhydrides followed by treatment with acid chlorides. Use of phosphonite in place of phosphite gave the same type of enol phosphonate which may serve for phosphono-lipid preparation. Synthetic phospholipid analogues thus formed exisist in thermotropic and lyotropic liquid crystalline states within definite temperature range. Their phase transition temperatures and Craft points as well as the lipid-water binary phase diagrams were compared with those of the corresponding natural phospholipids.

Keywords——vinylene carbonate telomer; enol phosphate; lecithin; cephalin; phospholipid analogue; liquid crystal; phosphonolipid; craft point; enol phosphonate; selective monoacylation

There has been an increasing need for chemically well-defined phospho-lipid species as model compounds in the biological and physico-chemical studies, particularly in connection with the function of biological membranes.[3] Organic synthesis may be only one available source for such lipids, since it is still quite difficult to isolate them in reasonable quantities from natural sources. In the previous papers,[4] we reported the smooth and stereoselective conversion of the vinylene carbonate telomers (2) (except the $n=1$ telomers) into the *trans* enol phosphates (3) which might serve as the synthetic intermediate for phospholipid analogues. Model systems of lipids may provide the valuable informations on the relation between the structure and the biological properties in comparison with natural type phospholipids.



1 2: R = polyhalomethyl
X = Cl,Br
3: n=2,3 R' = alkyl, OR''
R''= alkyl

A synthetic route from such enol phosphate esters to lecithin- and cephalin-types of lipids

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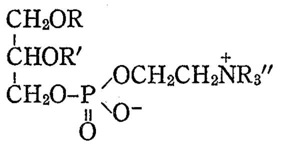
[1] This constitutes Part X in the series, "Studies on Telomers and Oligomers of Vinylene Carbonate." Part IX: T. Kunieda, T. Tamura, and T. Takizawa, *Chem. Pharm. Bull.* (Tokyo), **25**, 1749 (1977).

[2] Location: *Hongo, Bunkyo-ku, Tokyo, 113, Japan.*

[3] A.J. Slotboom and P.P.M. Bonsen, *Chem. Phys. Lipids*, **5**, 301 (1970); K. Inoue, *Biochem. Biophys. Acta*, **339**, 390 (1974).

[4] N. Mitsuo, T. Kunieda, and T. Takizawa, *Chem. Pharm. Bull.* (Tokyo), **25**, 231 (1977); Y. Nii, T. Kunieda, and T. Takizawa, *Tetrahedron Lett.*, **1976**, 2323.

has been explored as a part of studies on the synthetic utility of the $n=2$ and 3 telomers (**2**)[5] arising from free-radical telomerization of vinylene carbonate (**1**) and carbontetrachloride. It may be interesting to involve the following new type of unnatural phospholipids (**5**—**7**) in the comparative study of the liposomal property with those of natural phosphoglyceride type of lipids (**4**), since the permeability of lipid bilayers has been shown to be markedly dependent on the structural features of phospholipids.[6] Synthetic lipid analogus can be both in thermotropic and lyotropic liquid crystalline phases within certain definite temperature ranges. The forth-coming paper[7] will discuss the barrier ability of liposomal membranes composed of such synthetic analogues which can retain a water-solute like glucose to the nearly same extent as do naturally occurring lecithins.



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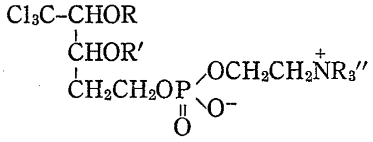

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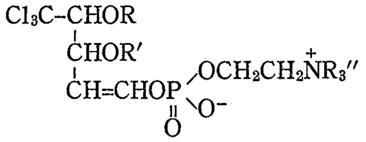
(R, R$'$=fatty acid residues
R$''$=CH_3, H)



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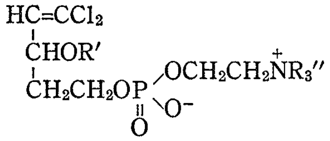

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Synthesis of DL-Lipid-analogues

In Chart 1 are outlined the synthetic routes from the $n=2$ telomers to lecithin (cholin)- and cephalin (ethanolamine)-type of unnatural lipids *via* mono- and di-hydroxy derivatives. Treatment of the $n=2$ telomers **8** ("*syn*" and "*anti*")[8] with trimethyl phosphite gave *trans* enol phosphate **9** (65% and 72%, respectively) which was catalytically hydrogenated on palladium-charcoal to **10**.[4] Saturated and unsaturated phosphate diols **11** arisen by mild hydrolysis of **9** and **10** with sodium borohydride were acylated with acid chlorides to give the diacylphosphates **13**, while selective monoacylation could be achieved with fatty acid anhydrides to give **12**, presumably due to a steric repulsion between a bulky trichloromethyl group and the agent, though smaller agent like acetic anhydride afforded the diacetate in high yield. Hence, a regioselective introduction of unlike fatty acid moieties has become feasible by successive treatment with fatty acid anhydride and acid chlorides. Thus, treatment of **11i** with stearic anhydride gave 50% yield of mono-acylated **12** (R_1CO=stearoyl) (in addition to the distearate (6%)) which was then acylated by caprylyl chloride to afford **13c** (R_1CO=stearoyl, R_2CO=caprylyl) in 90% yield. The structure of **12** was established by the conversion of **13c** to **13e** on treatment with activated zinc in acetic acid as well as the nuclear magnetic resonance (NMR) spectrum showing a triplet peak at δ5.80 attributable to Ha. In this work, stearic (C_{18}) and caprylic (C_8) acids have been employed as model fatty acids for long and short chains. Direct acylation of the enol phosphates **9** with anhydrides has been unsuccessful under the conditions (tetrabutylammonium bromide[9] as a catalyst in dimethylformamide (DMF) at 140°) where unsubstituted ethylene carbonate could be smoothly converted to ethylene glycol diacetate (quantitatively) and the distearate (26%). Treatment with alkyl-lithium

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5) T. Tamura, T. Kunieda, and T. Takizawa, *J. Org. Chem.*, **39**, 38 (1974). For a review, T. Kunieda, and T. Takizawa, *Heterocycles*, **8**, 661 (1977).
6) T. Kitagawa, K. Inoue, and S. Nojima, *J. Biochem.*, **79**, 1147 (1976). and the references cited therein.
7) N. Mitsuo, T. Kunieda, and T. Takizawa, *Chem. Pharm. Bull.* (Tokyo), **26**, 1501 (1978).
8) H. Takahata, T. Kunieda, and T. Takizawa, *Chem. Pharm. Bull.* (Tokyo), **23**, 3017 (1975).
9) A. Hilt, J. Trivedi, and K. Hamann, *Makromolecular Chemie*, **89**, 177 (1965).

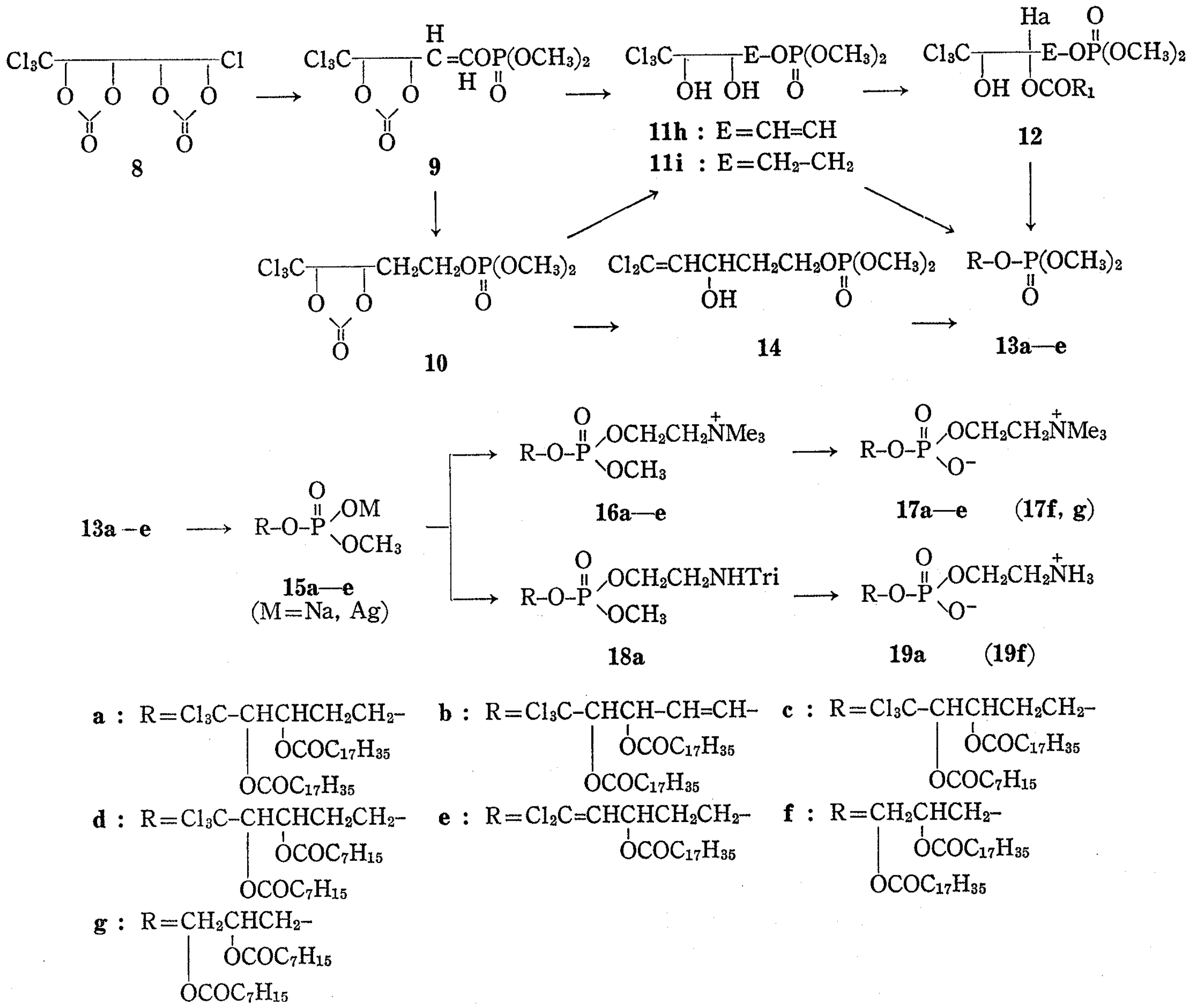

Chart 1

and -magnesium agents (—78°) resulted in a regio-isometic mixture of half-acylated products which were difficult to separate by chromatographic means.

The resulting acyl-phospho triesters **13a—e** could be readily converted into the corresponding lecithin and cephalin analogues by the application of well-established procedures[10] involving demethylation with sodium iodide in 2-butanone followed by replacement of sodium by silver ion and subsequent condensation of 2-aminoethyl moieties using bromocholine picrate and tritylaminoethyl iodide. Vinylphosphate bond which resisted the hydrolytic cleavage as pointed out previously,[4] survived under the demethylation conditions to permit the preparation of enol type of lipids (**17b**). Monoacyl-phosphate (**13e**) was prepared as a source of mono-chain synthetic lecithin (**17e**) by the reductive ring-opening of **10** with zinc-acetic acid followed by acylation. Synthetic lipids were all characterized as cadmium chloride adduct (monohydrate) and their over-all yields were routinely of 4—15%.

Tri- and tetra-hydroxy compounds derived from the $n=3$ telomers may be expected to give tri- and tetra-fatty acid chain phospholipids. However, some difficulty became apparent in complete acylation of the tetraol **21** with stearoyl chloride and, even on the repeated treatments, the major product was the tri-stearate (41%), presumably **22**.[11]

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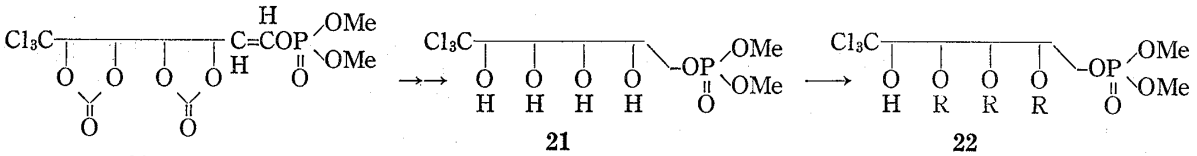
10) J.D. Billimoria and K.O. Lewis, *J. Chem. Soc.* (*C*), **1968**, 1404.

11) The hydroxy group adjacent to trichloromethyl group is difficult to be acylated by long chain acylating reagent because of steric hindrance. The NMR spectrum is not contradictory to the presumed structure of **22**.

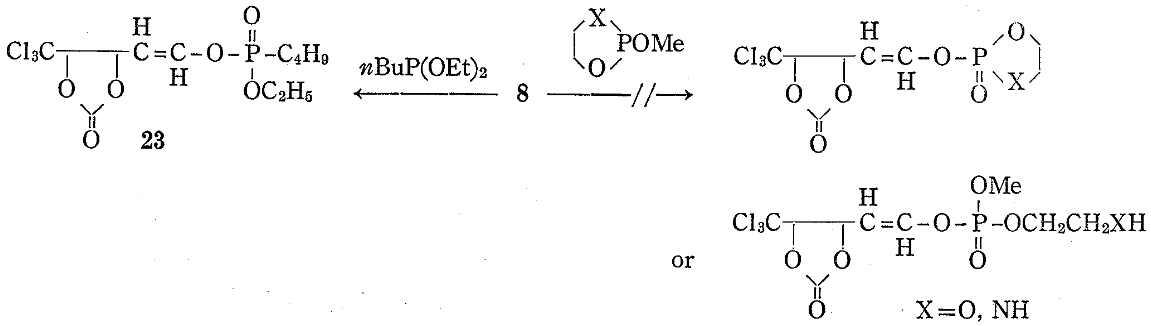


20 **21** **22**

R=stearoyl

Model reaction of the telomer **8** with diethyl *n*-butylphosphonite in the place of phosphite resulted in the similar conversion to *trans*-enol phosphonites (**23**) (27%) containing carbon-phosphorus bond. This may provide a route to phosphonolipids wihch have received an extensive attention mainly because of the biological significance such as the inhibitory action on various phospholipases.[12]

Tervalent phosphorus agents like five-membered cyclic phosphites and the amide analogues which smoothly underwent the Perkon-type reaction with chloral[13] failed to react with the telomers, probably due to the low nucleophilicity caused by ring-strain.



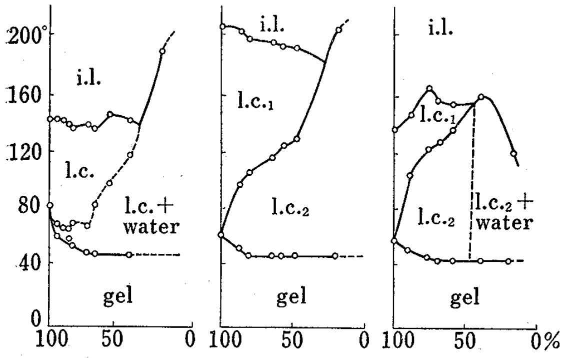
23

or

X=O, NH

Mesomorphic State (Liquid Crystals)

The synthetic phospholipids thus obtained are able to form the thermotropic and lyotropic liquid-crystals (l. c.) as expected and the phase transition temperatures, T_1 (gel→l. c.) and T_2 (l.c.→isotropic liquid) as well as the Craft point (Tc) obtained from the synthetic lipid-water binary phase-diagrams are shown in Table I together with those of natural lecithin[14]



19a **17a** **17b**

Fig. 1. Phase Diagrams of Synthetic Phospholipids (**19a**, **17a**, and **17b**)

i.l: isotropic liquid, l.c.: liquid crystal.

TABLE I

Phospholipids	T_1	T_2	Tc
17a	64°	206.5°	49°
17b	61	137.5	48.5
17c	<rt	187.5	<rt
17d	<rt	160	<rt
17e	<rt	189.5	<rt
17f	90	231	58
17g	<20	231	<0
19a	80	140	50.5
19f	130	182	

rt=room temperature (~20°)

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12) A.F. Rosenthal and M. Pousada, *Biochem. Biophys. Acta*, **125**, 265 (1966). *cf*) J.S. Kittredge and E. Roberts, *Science*, **164**, 37 (1969).

13) J.F. Allen and O.H. Johnson, *J. Am. Chem. Soc.*, **77**, 2871 (1955).

14) E. Baer, D. Buchnea, and A.G. Newcomb, *J. Am. Chem. Soc.*, **78**, 232 (1956); D. Chapman, "Form and Function of Phospholipids," ed. by G.B. Ausell, R.M.C. Daeson, and J.N. Hawthorne, Elsevier, Amsterdam, 1973, p. 117.

and phosphatidyl ethanolamine[15] Synthetic analogues **17c,d** and **e** exist in the liquid crystalline state at room temperature. The Craft points of **17a,b** are in temperature range suitable for the liposome experiments.[7] Phospho-ethanolamine (**19a**) shows one cloudy liquid crystalline phase, while two different phases of clear liquid crystals are observed in the series of lecithins. Such observation is in accord with that of natural type of lipids.[16] Transition temperatures (T_1, T_2 and Tc) of the synthetic long-chain analogues **17a,b** and **19a** are lower than those of the corresponding natural lipids **18f** and **20f** and in particular, it may deserve the attention that T_2 point (138°) of **17b** is far lower as compared to that (231°) of natural lipid (**18f**), reflecting a unique property of *trans*-enol geometry.

Experimental

Melting points were taken on a Yanaco micro-melting point apparatus and are uncorrected. Infrared (IR) spectra were obtained on a JASCO DS-402G IR spectrophotometer. Nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ on a 100 MHz JEOL PS-100 NMR spectrometer, using internal tetramethylsilane (TMS), and coupling constants (J) were given in Herz. Mass spectra (MS) were recorded on a JEOL JMS-01SG-2.

Dimethyl 2-(5-Trichloromethyl-2-oxo-1,3-dioxolan-4-yl)ethyl Phosphate (10)——Enol phosphate **9** (9.0 g, 25 mmol) dissolved in ethyl acetate (100 ml) was catalytically hydrogenated in the presence of 10% palladium on carbon (1.0 g) under 2 atm hydrogen for 14 hr. The catalyst was filtered off and the filtrate was evaporated *in vacuo*. The oily products were chromatographed on silica gel using methylene chloride/acetone as eluting solvents to give the saturated phosphate **10** (4.4 g, 49%, corrected yield, 65%) in addition to the starting material (2.2 g). The product **10** was characterized by the following spectral data, IR (neat) 1830, 1280, 1170 cm^{-1}; NMR δ: 2.20 (2H, m), 3.80 (6H, d, $J=11$), 4.25 (2H, m), 4.85 (2H, m); mass Calcd. for $C_8H_{12}Cl_3O_7P$: M^+, 356, M^++2, 358, M^++4, 360 (3:3:1). Found: M^+, 356, M^++2, 358, M^++4, 360 (3:3:1).

Dimethyl 5,5,5-Trichloro-3,4-dihydroxy-1-pentyl Phosphate (11i)——A solution of **9** (4.4 g, 12 mmol) in 95% methanol (100 ml) was treated with sodium borohydride (1.85 g, 49 mmol) under ice cooling for 1 hr and kept at room temperature for another hour. The reaction mixture was neutralized with a cation exchange resin (IR-120 H^+). Removal of the solvent followed by repeated flash-evaporation with methanol gave the crude **11i**, which was purified by silica gel chromatography (methylene chloride/acetone). The title compound (3.63 g, 89%) thus obtained gave the satisfactory spectral data as follows, IR (neat) 3400, 1260, 1040 cm^{-1}; NMR δ: 1.90 (2H, q, $J=7$), 3.70 (6H, d, $J=11$), 3.95—4.65 (4H, m), 4.25 (2H, s, disappeared when D_2O was added).

Dimethyl 5,5,5-Trichloro-3,4-dihydroxy-1-penten-1-yl Phosphate (11h)——Analogous treatment of **9** as above gave the olefinic diol in 43% yield, mp 88° from carbon tetrachloride. IR (nuj) 3320, 1680, 1270, 1040 cm^{-1}; NMR δ 3.80 (6H, d, $J=11$), 3.95 (2H, m, when D_2O was added, 1H, d, $J=2$), 4.75 (2H, m, when D_2O was added, 1H, m), 5.60 (1H, d-d, $J_1=12$, $J_2=7.5$), 6.70 (1H, d-d, $J_1=12$, $J_2=6.5$). *Anal.* Calcd. for $C_7H_{12}Cl_3O_6P$: C, 25.52; H, 3.67. Found: C, 25.46; H, 3.77.

Dimethyl 5,5-Dichloro-3-hydroxy-4-penten-1-yl Phosphate (14)——To a methanol (15 ml) solution of **10** (1.37 g, 3.8 mmol) was added the acid-washed zinc powder (2.7 g), and the mixture was kept under vigorous stirring at room temperature overnight. After removal of the insoluble materials by filtration, the filtrate was extracted with benzene twice. The extract was evaporated *in vacuo* to leave an oily product whose chromatography on silica gel gave **14** (0.79 g, 80%) as an oil, IR (neat) 3360, 1620, 1260 cm^{-1}; NMR δ 1.95 (2H, q, $J=6$), 3.60 (broad, disappeared when D_2O was added), 3.80 (6H, d, $J=11$), 4.25 (2H, t-d, $J_1=6$, $J_2=8$), 5.95 (1H, d, $J=8$).

General Procedure for Acylation with Fatty Acid Chloride and Anhydride——To a solution of the di- and mono-hydroxyphosphates (**11** and **14**) in chloroform were added freshly distilled stearoyl or caprylyl chloride and pyridine under ice-cooling, and it was allowed to stand at room temperature overnight. The mixture was diluted with methylene chloride and washed with 0.2 N sulfuric acid and water. The organic layer was dried (sodium sulfate) and evaporated *in vacuo* to dryness. The products were purified by chromatography on silica gel using methylene chloride as an eluting solvent.

In the same procedure, acylation of **11i** with stearic anhydride was performed to give the mono-acylate **12**.

Compounds thus prepared were characterized by the following IR and NMR spectral data (as well as elemental composition).

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15) E. Baer and D. Buchnea, *J. Am. Chem. Soc.*, **81**, 2494 (1959).
16) D.M. Small, *J. of Lipid Research*, **8**, 551 (1967).

Compound	IR (cm^{-1})	NMR (δ)
12	(neat) 3400	0.90 (3H, t, $J=6$) 1.20 (28H, s) 1.60 (2H, m)
	1740	2.15 (2H, q, $J=6.5$) 2.35 (2H, t, $J=7.5$) 3.75 (6H, d, $J=11$)
	1265	4.15 (4H, m) 5.80 (1H, t, $J=6.5$)
	1170	
13a[a]	(KBr) 1755	0.95 (6H, t, $J=6$) 1.25 (56H, s) 1.40—2.10 (6H, m)
	1265	2.30 (2H, t, $J=7.5$) 2.45 (2H, t, $J=7.5$) 3.70 (6H, d, $J=11$)
	1175	4.00 (2H, q, $J=6$) 5.50 (1H, d, $J=2$) 5.90 (1H, t-d, $J_1=6$, $J_2=2$)
13b[b]	(KBr) 1755	0.90 (6H, t, $J=6$) 1.25 (60H, s) 1.40—1.90 (4H, m)
	1673	2.30 (2H, t, $J=7.5$) 2.45 (2H, t, $J=7.5$) 3.75 (6H, d, $J=11$)
	1284	5.40 (1H, d-d, $J_1=12$, $J_2=9$) 5.55 (1H, d, $J=4$)
	1155	6.00 (1H, d-d, $J_1=9$, $J_2=4$) 6.75 (1H, d-d, $J_1=12$, $J_2=7$)
13c	(neat) 1750	0.90 (6H, t, $J=5$) 1.25 (32H, s) 1.25—2.20 (10H, m)
	1275	2.35 (2H, t, $J=7$) 2.50 (2H, t, $J=7$) 3.80 (6H, d, $J=11$)
	1150	4.05 (2H, q, $J=6.5$) 5.60 (1H, d, $J=2$) 5.95 (1H, t-d, $J_1=6.5$, $J_2=2$)
13d	(neat) 1753	0.85 (6H, t, $J=4.5$) 1.25 (20H, s) 1.95 (2H, q, $J=6.5$)
	1275	2.30 (2H, t, $J=6.5$) 2.50 (2H, t, $J=6.5$) 3.70 (6H, d, $J=11$)
	1150	4.05 (2H, q, $J=7$) 5.50 (1H, d, $J=2$) 5.90 (1H, t-d, $J_1=7$, $J_2=2$)
13e	(neat) 1745	0.90 (3H, t, $J=5.5$) 1.30 (24H, s) 1.30—2.50 (10H, m)
	1625	3.85 (6H, d, $J=11$) 4.15 (2H, q, $J=6.5$)
	1280	5.65 (1H, t-d, $J_1=6$, $J_2=8$) 5.90 (1H, d, $J=8$)
	1165	

[a] mp 43—44° from methanol/methylene chloride, *Anal.* Calcd. for $C_{43}H_{82}Cl_3O_8P$: C, 59.74; H, 9.57. Found: C, 59.57; H, 9.67.

[b] mp 66—67° from methanol/methylene chloride, *Anal.* Calcd. for $C_{43}H_{80}O_8Cl_3P$: C, 59.88; H, 9.35. Found: C, 60.13; H, 9.44.

Dimethyl 5,5-Dichloro-3-stearoyloxy-4-penten-1-yl Phosphate (13e)——This product was obtainable from the diacylate **13a** (0.07 g) by treatment with activated zinc powder (0.2 g) in acetic acid (3 ml) at 40° for 1.5 hr. The mixture was filtered and the filtrate was evaporated *in vacuo*. Benzene extraction and removal of the solvent followed by purification by silica gel chromatography gave an oily product (0.05 g), which was identical with the compound **13e** prepared above with regard to the IR and NMR data, and chromatographic behavior.

General Procedure for Demethylation (Preparation of Silver Salt 15a—e)——A solution of the phosphoric acid triesters and sodium iodide in 2-butanone was refluxed for 3—4 hr. Upon cooling to room temperature, the sodium salts were precipitated as colorless crystals and collected by prompt filtration. Certain sodium salts which could be purified by recrystallization were too hygroscopic to get the satisfactory elemental combustion data.

The sodium salts thus obtained were dissolved in acetone and converted to the silver salt by adding silver nitrate under refluxing. After kept at this temperature for 15 min, the mixture was allowed to stand at room temperature overnight. The silver salts deposited were isolated by filtration and recrystallized from benzene–acetone to give the analytical samples.

Compound	mp	IR (cm^{-1})	NMR (δ)	*Anal.*
15a: Na salt	51—53°	1756	0.85 (6H, t, $J=6$) 1.20 (52H, s)	Calcd. for
	from	1268	1.50—2.00 (6H, m) 2.30 (2H, t, $J=7$)	$C_{42}H_{79}Cl_3NaO_8P$
	benzene/	1170	2.45 (2H, t, $J=7$) 2.80 (3H, s)	C, 57.82; H, 9.13
	acetone		3.50 (3H, d, $J=10$) 3.80 (2H, m)	Found:
			5.55 (1H, s) 5.90 (1H, m)	C, 57.26; H, 9.20
15a: Ag salt	66—67°	(KBr)	0.85 (6H, t, $J=5$) 1.20 (56H, s)	Calcd. for
	from	1757	1.50—2.20 (8H, m) 2.30 (2H, t, $J=7$)	$C_{42}H_{79}AgCl_3O_8P$:
	benzene/	1170	2.45 (2H, t, $J=7.5$) 3.60 (3H, d, $J=11$)	C, 52.70: H, 8.32
	acetone		3.90 (2H, m) 5.55 (1H, s) 5.90 (1H, s)	Found:
				C, 52.84: H, 8.60
15b: Ag salt	67—70°	(KBr)		Calcd. for
	from	1760		$C_{42}H_{77}AgCl_3O_8P$:
	chloroform/	1675		C, 52.81; H, 8.12
	acetone	1160		Found:
				C, 52.55; H, 8.22

15e:	Ag salt	59—60° from chloroform/ acetone	(KBr) 1742 1628 1168	0.90 (3H, t, $J=5.5$) 1.20 (28H, s) 1.20—1.80 (2H, m) 2.00 (2H, m) 2.25 (2H, t, $J=7$) 3.70 (3H, d, $J=11$) 3.95 (2H, m) 5.60 (1H, t-d, $J_1=7$, $J_2=8$) 5.85 (1H, d, $J=8$)	Calcd. for $C_{24}H_{44}AgCl_2O_6P$: C, 45.16; H, 6.95 Found: C, 44.34; H, 7.10
15c:	Ag salt		(neat) 1745 1150	0.90 (6H, t, $J=5.5$) 1.25 (36H, s) 1.25—2.80 (10H, m) 3.65 (3H, d, $J=11$) 4.00 (2H, m) 5.60 (1H, d, $J=2$) 5.95 (1H, t-d, $J_1=6$, $J_2=2$)	
15d:	Ag salt		(neat) 1745 1150	0.90 (6H, t, $J=5$) 1.25 (16H, s) 1.25—2.10 (6H, m) 2.20—2.70 (4H, m) 3.65 (3H, d, $J=11$) 3.85 (2H, m) 5.60 (1H, d, $J=1.5$) 5.90 (1H, t-d, $J_1=6$, $J_2=1.5$)	

Preparation of Lecithin-type Phospholipids (17a—e)——A solution of the above silver salts **15** and bromocholine picrate in tetrahydrofuran was heated under reflux for 17 hr in the dark. The insoluble materials were filtered off and the filtrate was evaporated *in vacuo*. The products were extracted with benzene, and the extract was treated with sodium iodide in hot 2-butanone for 3.5 hr. The solvent was removed *in vacuo*, and the residue was dissolved in acetone. Upon cooling to 0°, the sodium salts precipitated were separated by decantation and deionized by passing through the ion exchange column (Amberlite IRC-50-H^+ and IR45-OH^-) using 90% ethanol as an eluting solvent. Purification was performed by silica gel chromatography (methylene chloride/methanol) and recrystallization.

5,5,5-Trichloro-3,4-distearoyloxypentyl 2′-(Trimethylammonium)ethyl Phosphate (17a)——mp 62—66°, 206—207° (from chloroform/acetone) yield 25%, IR (KBr) 3400, 1755, 1640, 1175 cm^{-1}; NMR δ: 0.85 (6H, t, $J=6$), 1.25 (56H, s), 1.50—2.10 (6H, m), 2.30 (2H, t, $J=7$), 2.45 (2H, t, $J=7$), 3.40 (9H, s), 3.95 (4H, m), 4.40 (2H, m), 5.50 (1H, d, $J=1$), 5.90 (1H, t-d, $J_1=5.5$, $J_2=1$), 6.35 (2H, m). $CdCl_2$ adduct *Anal.* Calcd. for $(C_{46}H_{91}Cl_3NO_9P)_2(CdCl_2)_3$: C, 45.49; H, 7.55; N, 1.15. Found: C, 44.93; H, 7.62; N, 1.14.

5,5,5-Trichloro-3,4-distearoyloxy-1-penten-1-yl 2′-(Trimethylammonium)ethyl Phosphate (17b)——50% yield, and mp 61—65°, 136—139° (from chloroform/acetone), IR (KBr) 3300, 1757, 1738, 1675, 1272, 1170, 969 cm^{-1}; NMR δ: 0.90 (6H, t, $J=6$), 1.25 (56H, s), 1.40—1.80 (4H, m), 2.25 (2H, t, $J=7$), 2.45 (2H, t, $J=7$), 3.30 (9H, s), 3.80 (2H, m), 4.30 (2H, m), 5.00 (3H, m), 5.50 (1H, d, $J=4$), 5.90 (1H, d-d, $J_1=12$, $J_2=7$); $CdCl_2$ adduct. *Anal.* Calcd. for $(C_{46}H_{89}Cl_3NO_9P)_2(CdCl_2)_3$: C, 45.46; H, 7.40; N, 1.16. Found: C, 45.81; H, 7.40; N, 1.18.

5,5-Dichloro-3-stearoyloxy-4-pentenyl 2′-(Trimethylammonium)ethyl Phosphate (17e)——21% yield, and recrystallized from chloroform/acetone, mp 188—191°. This was in liquid crystalline phase at room temperature, IR (KBr) 3400, 1742, 1630, 1236, 1170 cm^{-1}; NMR δ: 0.90 (3H, t, $J=5.5$), 1.25 (28H, s), 1.60 (2H, m), 2.00 (2H, m), 2.30 (2H, t, $J=6.5$), 3.40 (9H, s), 3.90 (4H, m), 4.40 (2H, m), 5.55 (1H, t-d, $J_1=7$, $J_2=8.5$) 5.85 (1H, d, $J=8.5$) 5.95 (2H, s). $CdCl_2$ adduct. *Anal.* Calcd. for $(C_{28}H_{56}Cl_2NO_7P)_2(CdCl_2)_3$: C, 37.55; N, 6.30; N, 1.56. Found: C, 37.27; H, 6.12; N, 1.80.

5,5,5-Trichloro-4-caprylyloxy-3-stearoyloxypentyl 2′-(Trimethylammonium)ethyl Phosphate (17c)——17% yield, and recrystallized at −20°, mp 187—188°, IR (neat) 3350, 1750, 1240, 1150 cm^{-1}, NMR δ: 0.90 (6H, t, $J=6$), 1.25 (36H, s), 1.25—2.00 (6H, m), 2.30 (2H, t, $J=7.5$), 2.50 (2H, t, $J=7.5$), 3.30 (9H, s), 3.70 (4H, m), 3.95 (6H, s), 4.20 (2H, m), 5.55 (1H, d, $J=1.5$), 5.90 (1H, t-d, $J_1=6$, $J_2=1.5$). $CdCl_2$ adduct *Anal.* Calcd. for $(C_{36}H_{71}Cl_3NO_9P)_2(CdCl)_3$: C, 40.25; H, 6.66; N, 1.30. Found: C, 40.01; H, 6.45; H, 1.42.

5,5,5-Trichloro-3,4-dicaprylyloxypentyl 2′-(Trimethylammonium)ethyl Phosphate (17d)——27% yield, mp 158—162°, IR (neat) 3350, 1750, 1245, 1145 cm^{-1}, NMR δ: 0.90 (6H, t, $J=6$), 1.30 (16H, s), 1.50—2.00 (6H, m), 2.30 (2H, t, $J=7.5$), 2.50 (2H, t, $J=7$), 3.35 (9H, s), 3.80 (4H, m), 4.15 (4H, s), 4.25 (2H, m), 5.55 (1H, d, $J=1.5$), 5.90 (1H, t-d, $J_1=6.5$, $J_2=1.5$). $CdCl_2$ adduct. *Anal.* Calcd. for $(C_{26}H_{51}Cl_3NO_9P)_2(CdCl_2)_3$: C, 33.43; H, 5.50; N, 1.50. Found: C, 33.47; H, 5.38; N, 1.39.

Preparation of Cephalin-type Lipid (19a)——A solution of **15a** (M=Ag) (0.19 g, 0.2 mmol) and tritylaminoethyl iodide (0.08 g) in benzene (5 ml) was heated under reflux for 2 hr in the dark. Silver iodide was filtered off and the solvent was evaporated *in vacuo*. Chromatography on silica gel gave **18a** (0.16 g, 72%) as an oily product. The triester (0.13 g, 0.12 mmol) was treated with sodium iodide (0.02 g) in 2-butanone at 80° for 3.5 hr. The solvent was removed *in vacuo*, and the crude product was dissolved in acetone. On cooling to −20°, the sodium salt was deposited. Usual work up as above gave sodium tritylaminoethyl 5,5,5-trichloro-3,4-distearoyloxypentyl phosphate (0.09 g, 69%), which was dissolved in 90% acetic acid and heated under reflux for only 3 min. The solvent was removed *in vacuo*, and then to the methanolic solution of the residue was added hydrochloric acid (1 eq). Upon cooling to 0°, the phosphatidyl ethanolamine deposited was collected by filtration. Recrystallization from chloroform/methanol gave 2-aminoethyl 5,5,5-trichloro-3,4-distearoyloxypentyl phosphate (**19a**) (74%), mp 78—82°, 139—141°, IR (KBr) —2900 (broad), 1755, 1638, 1210, 1170 cm^{-1}, NMR δ: 0.90 (6H, t, $J=6$), 1.25 (56H, s), 1.65 (4H, m), 1.90 (2H, m), 2.30 (2H, t, $J=7$), 2.50 (2H, t, $J=7$), 3.20 (2H, m), 3.85 (2H, m), 4.05 (2H, m), 5.55 (1H, d, $J=2$), 5.90 (1H, m), 8.40

(3H, broad). *Anal.* Calcd. for $C_{43}H_{83}Cl_3NO_8P$: C, 58.73; H, 9.51; N, 1.59. Found: C, 58.83; H, 9.75; N, 1.43.

Attempt to prepare Tetrachain-phospholipid——The $n=3$ telomer **2** ($R=CCl_3$, $X=Cl$, *anti-syn*)[8] (1.85 g) and trimethyl phosphite (4.2 g) were dissolved in toluene (150 ml) and refluxed under nitrogen atmosphere for 3 days. Removal of the solvent *in vacuo* followed by silica gel chromatography using methylene chloride and acetone as eluting solvents gave the enol phosphate **20** (1.44 g, 72%). It was recrystallized from carbon tetrachloride/chloroform, mp 197—199°, IR (KBr) 2970, 1835, 1680, 1270, 948 cm^{-1}, NMR (CH_3CN), δ: 3.85 (6H, d, $J=11$), 4.75 (1H, d-d, $J_1=6$, $J_2=1.5$), 5.15 (1H, d-d, $J_1=4$, $J_2=1.5$), 5.20 (1H, d-d, $J_1=9$, $J_2=6$), 5.40 (1H, d, $J=4$), 5.60 (1H, d-d, $J_1=12$, $J_2=9$), 6.90 (1H, d-d, $J_1=12$, $J_2=7.5$). *Anal.* Calcd. for $C_{11}H_{12}Cl_3O_{10}P$: C, 29.92; H, 2.74. Found: C, 30.02; H, 2.68.

The enol phosphate **20** (0.3 g) dissolved in ethyl acetate (150 ml) was catalytically hydrogenated in the presence of 10% palladium on carbon (0.1 g) under 1.1 atm hydrogen for 4 days. The catalyst was removed by centrifugation. The solvent was removed *in vacuo* and the oily residue was chromatographed on silica gel. Saturated phosphate (0.06 g) contaminated with one carbonate-ring cleaved compound were gained as the mixture which was treated with sodium borohydride (0.06 g) in 90% methanol at 60° for 1 hr and kept at room temperature overnight. In the same work-up as described for **11i**, the tetraol **21** was obtained (0.04 g). Acylation with stearoyl chloride in pyridine gave tri-acylated compound **22** (0.05 g) with negligible amounts of fully protected compound. IR (Nuj) 3400, 1745, 1270, 1155, 1045 cm^{-1}, NMR δ: 0.85 (9H, t, $J=6$), 1.20 (80H, s), 1.20—2.00 (12H, m), 2.30 (6H, m), 3.70 (6H, d, $J=11$), 4.00 (3H, m), 5.15 (3H, m).

Ethyl 2-(5-Trichloromethyl-2-oxo-1,3-dioxolan-4-yl)vinyl *n*-Butyl Phosphonate (23)——The $n=2$ telomer **8** (1.09 g) and diethyl butylphosphonite (1.09 g) were dissolved in benzene (40 ml) and heated at 80° under nitrogen atmosphere for 14 hr. Removal of the solvent *in vacuo* followed by silica gel column chromatography (benzene/methylene chloride) gave the enol phosphonate **23** (0.30 g, 23%) in addition to the unchanged material (0.15 g). Compound **23** was recrystallized from *n*-hexane/methylene chloride as colorless crystals, mp 77—80°, IR (KBr) 3040, 2955, 2865, 1820, 1665, 1250, 975 cm^{-1}, NMR δ: 0.90 (3H, t, $J=6.5$), 1.20—2.00 (9H, m), 4.20 (2H, qui, $J=7$), 4.80 (1H, d, $J=4$), 5.10 (1H, d-d, $J_1=9$, $J_2=4$), 5.50 (1H, d-d, $J_1=12$, $J_2=9$), 7.00 (1H, d-d, $J_1=12$, $J_2=8$). *Anal.* Calcd. for $C_{12}H_{18}Cl_3O_6P$: C, 36.43; H, 4.59. Found: C, 36.41; H, 4.53.

Ethylene Diacetate——Ethylene carbonate (0.88 g, 10 mmol) and acetic anhydride (1.02 g, 10 mmol) were heated under reflux for 2.5 hr with tetrabutylammonium bromide (0.032 g, 0.1 mmol) as a catalyst. Purification by silica gel chromatography (methylene chloride) gave a quantitative yield of the diacetate as an oily product, whose IR (neat) (1730, 1375, 1220 cm^{-1}) and NMR δ: 2.00 (6H, s), 4.20 (4H, s), spectra were identical with those of the authentic compound.

Ethylene Distearate——A solution of ethylene carbonate (0.088 g, 1 mmol), stearic anhydride (0.55 g, 1 mmol), and tetrabutylammonium bromide (0.032 g) in DMF (3 ml) was heated to 140° for 5 hr. Silica gel column chromatography of the reaction mixture (benzene) gave the distearate (0.152 g, 26%), which was recrystallized from *n*-hexane, mp 67—68°, IR (KBr) 2900, 2840, 1743, 1475 cm^{-1}, NMR δ: 0.85 (6H, t, $J=6.5$), 1.25 (56H, s), 1.60 (4H, m), 2.30 (4H, t, $J=7$), 4.25 (4H, s). *Anal.* Calcd. for $C_{38}H_{74}O_4$: C, 76.71; H, 12.54. Found: C, 76.69; H, 12.59.

Attempt to prepare Cyclic Enol Phosphate or Cyclic Enol Phosphate Monoamide (24)[4]——a) A solution of the $n=2$ telomer **8** (0.27 g, 0.83 mmol) and ethyl ethylene phosphite (0.18 g) in toluene was heated to reflux under nitrogen atmosphere for 1 day. After removal of the solvent, column chromatography on silica gel recovered most of the starting telomer (0.21 g).

b) The benzene solution of **8** and 1,3,2-oxazaphospholidine (2 eq) was heated to 80° in a sealed tube for 1 day. The telomer was almost decomposed to a tary oil which was insoluble in organic solvents.

c) The telomer **8** (0.06 g) was reacted with N-methyl- or N-acetyl-1,3,2-oxazaphospholidine (0.05 g) in boiling benzene or toluene under N_2 gas for 1 day to result in the formation of precipitates which were insoluble in most organic solvents and could not be identified.

Measurement of Phase Transition Temperatures——"Melting points" of the synthetic phospholipids were measured by the use of micro-melting-point apparatus equipped with a pair of polarizers. The phase diagram of the phospholipids was determined as a function of water concentration and temperature as follows. Phospholipids (0.5—3 mg) and water (0.5—2 mg) were tightly packed into a sealed capillary tube in the weight ratios of 1 : 0 to 1 : 4. After one week-standing at room temperature each capillary was slowly heated in an oil placed between two polarizers. Phase transition temperatures (gel→l.c.), (l.c.→isotropic liquid), and (l.c.$_1$→l.c.$_2$) were determined by this method. Craft points were obtained from the lipid-water binary phase-diagrams.