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A New Phosphorylating Reagent. V.[1] The Preparation of α-Glycerophosphoryl Choline and Its Analogues by Means of 2-Chloromethyl-4-nitrophenyl Phosphorodichloridate

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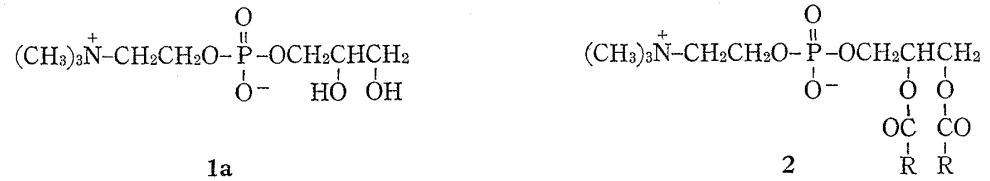
β′-Haloethyl isopropylidene-α-glycerophosphoryl 2-chloromethyl-4-nitrophenyl phosphates (**10** and **11**) were prepared by the stepwise phosphorylations of ethylene halohydrin and 1,2-isopropylidene-glycerol with 2-chloromethyl-4-nitrophenyl phosphorodichloridate (**3**), a phosphorylating reagent having an "activatable" protecting group. The protecting group of **10** and **11** was readily and selectively removed by hydrolysis through an active intermediate **14** derived from the reaction of **10** and **11** with various tertiary amines to afford the corresponding isopropylidene-α-glyceryl β′-haloethyl hydrogen phosphate (**15**) along with 2-hydroxy-5-nitrobenzyl trialkyl ammonium chloride (**16**), a chip of the active intermediate **14**. The halogen atom in the ethyl chain of the phosphate **15** reacted with tertiary amine at the same time to afford the corresponding quaternary ammonium compound **17** which was converted by subsequent hydrolysis into α-glycerophosphoryl choline (**1a**) and its analogues (**1b—d**). Thus, DL-α-glycerophosphoryl choline (**1a**), its L-isomer (**1a**-L), inner salt of β′-(DL-α-glyceryl hydrogen phosphoroxy) ethyl triethyl ammonium hydroxide (**1b**), 1-[β′-(DL-α-glyceryl) hydrogen phosphoroxy] ethyl 1-methyl piperidinium hydroxide (**1c**) and inner salt of 1-[β′-(DL-α-glyceryl) hydrogen phosphoroxy] ethyl 1,4-diazonia bicyclo [2,2,2] octane hydroxide (**1d**) were prepared in good yield. DL-Myristoyl lecithin (**18**) was prepared by acylation of **1a** with myristoyl chloride to confirm the structure of synthetic 1a.

L-α-Glycerophosphoryl choline (**1a**-L), which was one of the important intermediates for synthetic lecithin,[3] was prepared by Baer, *et al.*[4] using phenyl phosphorodichloridate as phosphorylating agent in 1948. In the same year, Aloisi reported the preparation of DL-isomer (**1a**) from the reaction of silver DL-α-glyceryl phosphate with picric acid salt of choline bromide.[5] The more convenient preparation method for **1a**-L was developed by Hanahan.[6] This method consisted of the deacylation of egg yolk lecithin with alkaline hydroxylamine and of the isolation *via* cadmium chloride complex.



1a **2**

Chart 1

1) Part IV: Y. Mushika and N. Yoneda, *Chem. Pharm. Bull.* (Tokyo), **19**, 687 (1971).
2) Location: *Kashima-cho, Higashiyodogawa-ku, Osaka.*
3) *a*) F. Kogel, G.H. de Haas and L. L. M van Deenen, *Rec. Trav. Chim.*, **79**, 661 (1960); *b*) G. H. de Haas and L. L. M. van Deenen, *Tetrahedron Letters*, **1960**, 7; *c*) E. Baer and D. Buchea, *Can. J. Biochem. and Phys.*, **37**, 953 (1959); *d*) N.H. Tattrie and C.S. McAther, *Can. J. Biochem. and Phys.*, **35**, 1165 (1957); *e*) Ph. R. Bird, G.H. de Haas, C.H. Th. Heemskerk and L.L. M. van Deenen, *Biochim. Biophys. Acta*, **98**, 566 (1965).
4) E. Baer and M. Kates, *J. Am. Chem. Soc.*, **70**, 1394 (1948).
5) M. Aloisi and P. Buffa, *Biochem. J.*, **43**, 157 (1948).
6) D. J. Hanahan, "Biochemical Preparations," Vol. 9, ed. by M. J. Coon, John Wiley and Sons, Inc., New York, N. Y., 1962, p. 55.

In the previous papers,[1,7a] the mixed dialkyl hydrogen phosphates (**6**) were easily prepared by selective hydrolyses of the corresponding dialkyl 2-chloromethyl-4-nitrophenyl phosphates (**4**) in aqueous pyridine. These results were due to the formation of active intermediates, 1-(2′-dialkyl phosphoroxy-5′-nitrobenzyl) pyridinium compound (**5**) which was derived from the reaction of **4** with pyridine. The activation of the phosphate **4** will be also possible in the cases of using the other tertiary amines in place of pyridine and the preparation of a dialkyl hydrogen phosphate containing quaternary ammonium group in alkyl chain such as α-glycerophosphoryl choline (**1a**) is expected when alkyl group of the phosphate **4** is substituted with an active functional group to the tertiary amines.

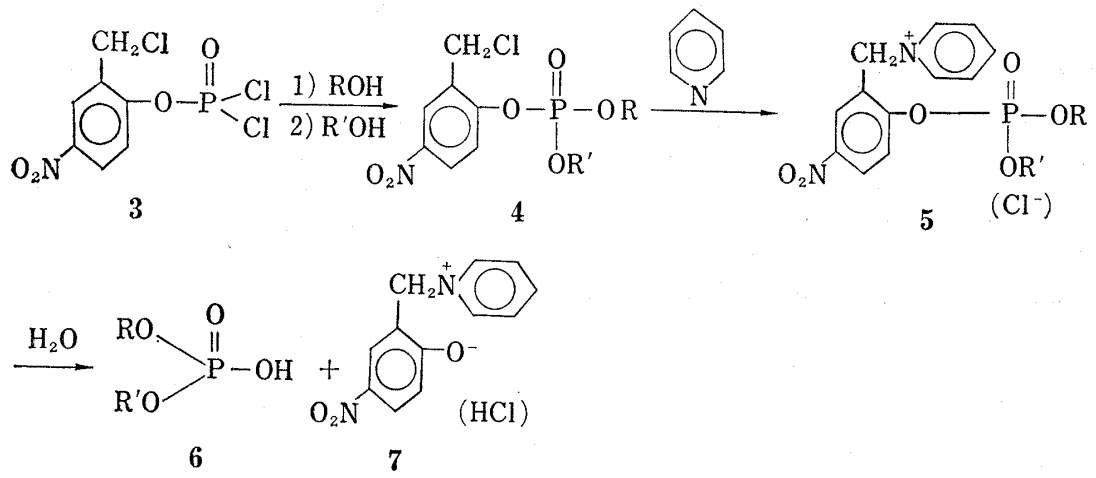


Chart 2

The present work describes a convenient method for the preparation of α-glycerophosphoryl choline (**1a**) and its analogues by the reaction of β′-haloethyl isopropylidene-α-glyceryl 2-chloromethyl-4-nitrophenyl phosphate (**10** and **11**) with various amines.

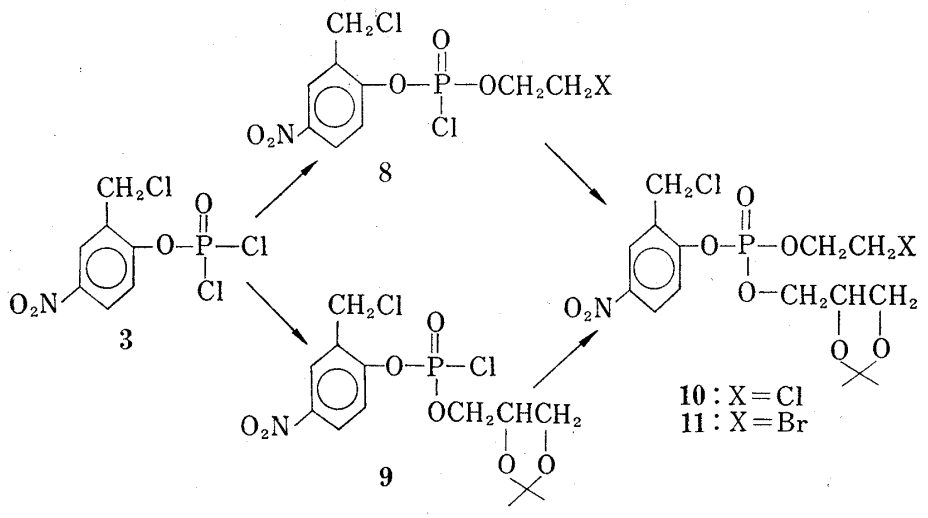


Chart 3

The preparation of β′-chloromethyl isopropylidene-DL-α-glyceryl 2-chloromethyl-4-nitrophenyl phosphate (**10**) was achieved by stepwise phosphorylation of ethylene chlorohydrin and 1,2-isopropylidene-DL-α-glycerol with the phosphorodichloridate **3** as shown in

7) *a*) Y. Mushika, T. Hata and T. Mukaiyama, *Bull. Chem. Soc. Japan*, **44**, 232 (1971); *b*) T. Hata, Y. Mushika and T. Mukaiyama, *J. Am. Chem. Soc.*, **91**, 4532 (1969).

Chart 3. β'-Chloroethyl 2-chloromethyl-4-nitrophenyl phosphorochloridate (**8**) was obtained when 1 mole of ethylene chlorohydrin was phosphorylated with 1 mole of the phosphorodichloridate **3** in tetrahydrofuran (THF) at —20——30° in the presence of 1 mole of pyridine. The phosphorochloridate **8** was too unstable to isolate purely from the reaction mixture. Then, further phosphorylation with **8** was carried out by using 3 moles of 1,2-isopropylidene-DL-α-glycerol and 1 mole of pyridine at room temperature in the above reaction mixture. The phosphate **10**, thus obtained as pale yellow viscous oil, was contaminated with a trace of two kinds of by–products, which were detected as two spots (*Rf* 0.65 and 0.41) on thin–layer chromatography with silicagel developed with a mixture of benzene and ethyl acetate (4:1 v/v) (*Rf* of main product was 0.55), even after purification by means of a column of silica gel. Therefore, the analytically pure **10** was not obtained, but the structure of **10** was supported by infrared (IR) and ultraviolet (UV) spectra which showed a maximum absorption band at 270 mμ (in EtOH) for dialkyl 2-chloromethyl-4-nitrophenyl phosphate.[7a] The two by–products were identified by thin–layer chromatography (TLC) with authentic samples, bis(β-chloroethyl) 2-chloromethyl-4-nitrophenyl phosphate (**12**) and bis(isopropylidene-DL-α-glyceryl) 2-chloromethyl-4-nitrophenyl phosphate (**13**), which were prepared by the phosphorylation of 2 moles of the corresponding alcohols with the phosphorodichloridate **3** as shown in Chart 4. *Rf*-Values of the compounds **12** and **13** on TLC by using the same solvent system described above were 0.61 and 0.41, and UV spectra in ethanolic solution of **12** and **13** showed a maximum absorption band at 269 mμ and 271 mμ, respectively. The crude phosphate **10** was also prepared in high yield by the phosphorylation of 1,2-isopropylidene-DL-glycerol and ethylene chlorohydrin with the phosphorodichloridate **3** in the reverse order described above.

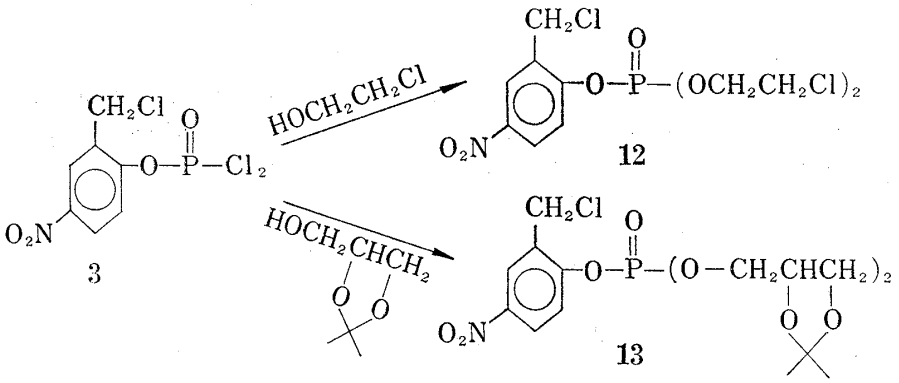


Chart 4

In the similar manner, β'-chloroethyl isopropylidene-L-α-glyceryl 2-chloromethyl-4-nitrophenyl phosphate (**10**-L) was prepared by using 1,2-isopropylidene-L-glycerol, which was synthesized by glycol fission of 1,2,5,6-diisopropylidene-D-mannitol with sodium metaperiodate and reduction with sodium borohydride, in place of 1,2-isopropylidene-DL-glycerol. β'-Bromoethyl isopropylidene-DL-α-glyceryl 2-chloromethyl-4-nitrophenyl phosphate (**11**) was also prepared by the use of ethylene bromohydrin instead of ethylene chlorohydrin.

In the next place, the activation of the stable protecting group, 2-chloromethyl-4-nitrophenyl, and the reaction of tertiary amine with β-halogen atom in the ethyl chain of the phosphates **10** and **11** were investigated by using various tertiary amines such as trimethylamine, triethylamine, tributylamine, 1-methylpiperidine and 1,4-diaza bicyclo [2,2,2] octane (DABCO) as shown in Chart 5. For example, when a suspension of the phosphate **10** in a large excess of 30% aqueous trimethylamine solution was kept standing at room temperature for 1 hr, the reaction mixture became to a yellow homogeneous solution. This observation showed that **10** changed into an active intermediate, 2-(β'-chloroethyl-DL-α-glyceryl phosphoroxy)-5-nitrobenzyl trimethyl ammonium salt (**14**). Then, the homogeneous solution was warmed at 45—

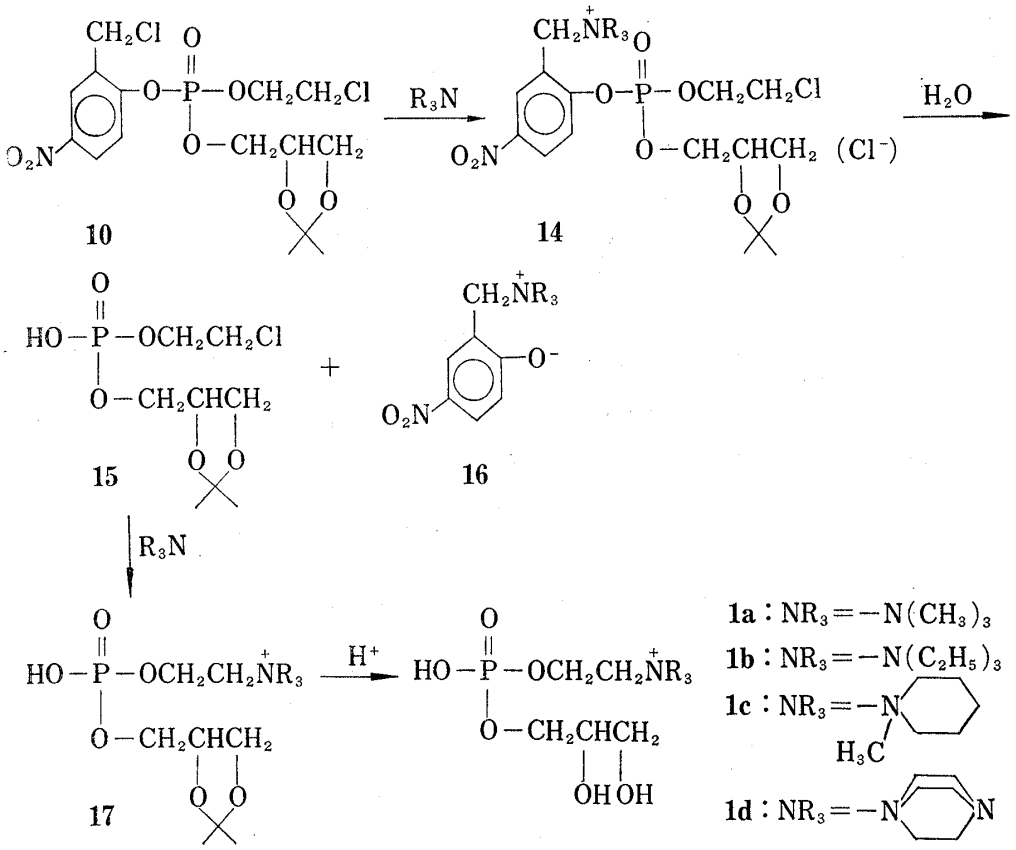


Chart 5

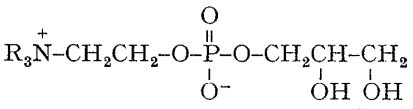
50° for 3 hr. The fresh generation of four compounds, of which *Rf* were 0.75, 0.68, 0.61 and 0.14, respectively, were detected by paper chromatography developed with a solvent A. The compound of *Rf* 0.68 was isolated in 92% yield from the reaction mixture as its hydrochloride and confirmed to be 2-hydroxy-5-nitrobenzyl trimethyl ammonium chloride (**16a**) by identification with an authentic specimen. The spot of *Rf* 0.75 was colored blue with Hanes–Isherwood agent[8] for phosphoric esters and was negative to modified Dragendorff agent[9] for quaternary ammonium compounds. This spot, which did not absorb UV light, disappeared gradually and the intensity of the other spot (*Rf* 0.61) increased when the above reaction mixture was warmed at 45—50° for longer period. The former spot disappeared perfectly after 24 hr. The spot of *Rf* 0.61 was both positive to Hanes–Isherwood and Dragendorff agents. From these facts, it was guessed that the two compounds were *β'*-chloroethyl isopropylidene-DL-α-glyceryl hydrogen phosphate (**15**) (*Rf* 0.75) and isopropylidene-DL-α-glycerophosphoryl choline (**17a**) (*Rf* 0.61) respectively. After removal of the crystalline compound **16a**, the reaction mixture was treated with a suitable amount of Amberlite IR 120 resin (H form) to remove trimethyl ammonium chloride. When the strongly acidic eluent was allowed to stand at room temperature for 2 hr, a spot of *Rf* 0.39, which was both positive to Hanes–Isherwood and Dragendorff reagents, appeared freshly in place of the spot of *Rf* 0.61. This observation suggests that the isopropylidene derivative **17a** is hydrolyzed in acidic solution into the desired DL-α-glycerophosphoryl choline (**1a**). From this eluent, **1a** was obtained as very hygroscopic white crystalls (mp 152—153°) in 47% yield based on the starting material **3**. The structure of **1a** was supported by elementary analysis and nuclear magnetic resonance (NMR) spectrum (in D_2O) which showed a singlet peak at 6.65 τ for $N(CH_3)_3$.[10]

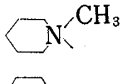
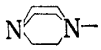
8) C. S. Hanes and F. A. Isherwood, *Nature*, **164**, 1107 (1949).
9) H. M. Bregoff, E. Roberts and C. C. Delwiche, *J. Biol. Chem.*, **205**, 565 (1953).
10) It was reported by Chapman, *et al.* that a singlet peak for $N(CH_3)_3$ of $CdCl_2$ complex of L-isomer (**1a**-L) appeared at 6.75 τ. We also detected that **1a**-L (free form) showed at 6.73 (9H, singlet, $N(CH_3)_3$). D. Chapman and H. Morrison, *J. Biochem.*, **241**, 5044 (1966).

In the similar way, the optically active L-isomer(**1a**-L) having specific rotation of −2.64° (lit. −2.89°)[11] was prepared in the yield of 56% based on the phosphorodichloridate **3** *via* the reaction of the neutral phosphate **10**-L with trimethylamine as hygroscopic viscous oil.

A few analogues of **1a** were also prepared in the similar manner mentioned above by using the phosphates **10** and **11**. In the cases of the use of easily water-soluble amines, such as 1-methylpiperidine and DABCO, the reactions proceeded successfully to afford the inner salts of 1-[*β*'-(DL-*α*-glyceryl) hydrogen phosphoroxyl] ethyl 1-methyl piperidinium hydroxide (**1c**) and inner salt of 1-[*β*'-(DL-*α*-glyceryl) hydrogen phosphoroxy] ethyl 1,4-diazonia bicyclo [2,2,2] octane hydroxide (**1d**) as pale yellow hygroscopic viscous oil along with 1-(2'-hydroxy-5'-nitrobenzyl)-1-methylpiperidinium chloride (**16c**) and 1-(2'-hydroxy-5'-nitrobenzyl) 1,4-diazonia bicyclo [2,2,2] octane chloride (**16d**), respectively. Although DABCO was bifunctional amine, monosubstituted compounds **1d** and **16d** were obtained from this reaction because of using a large excess amount of DABCO to avoid complicated reactions.

TABLE I. The Preparation of *α*-Glycerophosphoryl Choline (**1a**) and Its Analogues (**1b**—**e**)



Compd.	R_3N–	Configuration	Yield (%)[a]	Reaction condition: Starting material	Reaction condition: Tertiary amine[b] (mole ratio to 10, 11)	Reaction condition: Reaction temp. (°C)	Reaction condition: Reaction time (hr)
1a	$(CH_3)_3N$–	DL	46.6	Compd. **10**	$(CH_3)_3N$ (4/1)	45—50	24
1a-L	$(CH_3)_3N$–	L	55.6	**10**-L	$(CH_3)_3N$ (8/1)	45—50	24
1b	$(C_2H_5)_3N$–	DL	5.7	**10**	$(C_2H_5)_3N$ (12/1)	70—75	24
			10.4	**11**	$(C_2H_5)_3N$ (12/1)	45—50	24
1c		DL	55.0	**10**	(8/1)	80—85	20
1d		DL	51.6	**10**	 (10/1)	45—50	20
1e	$(C_4H_9)_3N$–	DL	≒0	**10**	$(C_4H_9)_3N$ (8/1)	45—50	24
			≒0	**10**	$(C_4H_9)_3N$ (8/1)	80—85	24

a) Yields of **1** were based on 2-chloromethyl-4-nitrophenyl phosphorodichloridate (**3**).
b) Thirty percent aqueous solution of tertiary amines (g/v) were used.

On the other hand, the use of sparingly water-soluble amines afforded the worse results; for example, when the reaction of the phosphate **10** with triethylamine was carried out at 40—45° in the presence of water, the formation of a trace of the desired compound, the inner salt of *β*'-(DL-*α*-glyceryl hydrogen phosphoroxy) ethyl triethyl ammonium hydroxide (**1b**) was detected by paper chromatography as a spot of *Rf* 0.47. Then, the reaction temperature raised to 70—75° (24 hr), but the yield of **1b** was only 6% based on the phosphorodichloridate **3**. Even in the case of using the bromine derivative **11** instead of **10**, the yield of **1b** was about 10%. The use of tributylamine gave little or no amount of the inner salt of *β*'-(DL-*α*-glyceryl hydrogen phosphoroxy) ethyl tributyl ammonium hydroxide (**1e**). The lower reactivities of such amines were probably due to their lower solubilities in water and their bulkiness. The results are summarized in Table I and II.

In connection with the above reaction, authentic specimens of 2-hydroxy-5-nitrobenzyl trialkyl ammonium chloride (**16**) were prepared by the reaction of 2-hydroxy-5-nitrobenzyl

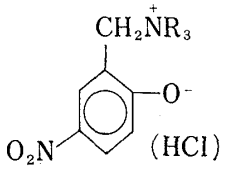
11) N. H. Tattrie and C. S. McArther, "Biochemical Preparations," Vol. 6, ed. by C. S. Vestling, John Wiley and Sons, Inc., New York, N. Y., 1958, p. 16.

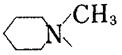
TABLE II. α-Glycerophosphoryl Choline and Its Analogues (1)

Compd.	Appearance	*Rf* value[a] Solvent A	*Rf* value[a] Solvent B	Formula	Elementary analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
1a	white powder mp 152—153°	0.39	0.53	$C_8H_{20}O_6NP$	37.35	7.84	5.45	37.55	7.87	5.64
1a-L	colorless viscous oil	0.39	0.53	$C_8H_{20}O_6NP\cdot{}^1/_2H_2O$	36.09	7.95	5.24	35.82	7.92	4.82
1b	pale yellow viscous oil	0.47	0.60	$C_{11}H_{26}O_6NP\cdot H_2O$	41.63	8.89	4.41	41.60	8.74	4.21
1c	pale yellow viscous oil	0.45	0.57	$C_{11}H_{24}O_6NP\cdot{}^1/_2H_2O$	43.13	8.23	4.57	43.02	8.47	4.34
1d	pale yellow viscous oil	0.35	0.50	$C_{11}H_{23}O_6N_2P\cdot{}^3/_4H_2O$	40.80	7.63	8.65	40.22	7.77	9.10

a) Paper chromatographies were carried out by ascending technique using Toyo Roshi No. 51A paper. Solvent systems used were isopropanol–conc. aq. ammonia–water (7:1:2 v/v) (solvent A) and (6:3:1 v/v) (solvent B).

TABLE III. 2-Hydroxy-5-nitrobenzyl Trialkyl Ammonium Chloride (**16**) from the Reaction of 2-Hydroxy-5-nitrobenzyl Chloride (**18**) with Tertiary Amine



Compd.	R_3N–	Yield (%)[a]	mp (°C) (Recryst. solvt)	*Rf*[b]	Formula	Elementary analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
16a	$(CH_3)_3N$–	81.3	251—253 ($EtOH–H_2O$)	0.68	$C_{10}H_{14}O_3N_2\cdot HCl$	48.69	6.13	11.36	48.56	6.26	11.21
16b	$(C_2H_5)_3N$–	73.5	163—164 (EtOH)	0.75	$C_{13}H_{20}O_3N_2\cdot HCl$	54.07	7.33	9.70	53.95	7.43	9.64
16c		75.5	193—195 ($EtOH–H_2O$)	0.72	$C_{13}H_{18}O_3N_2\cdot HCl$	54.45	6.68	9.77	54.90	6.78	9.65
16d		70.8	>240 ($EtOH–H_2O$)	0.60	$C_{13}H_{17}O_3N_3\cdot 2HCl\cdot H_2O$	44.08	5.98	11.86	44.15	5.89	12.11

a) Yields were based on 2-hydroxy-5-nitrobenzyl chloride (**18**).
b) Paper chromatographies were performed by ascending techniqueusing Toyo Roshi No. 51A paper. Solvent system used was isopropanol– conc. aq. ammonia– water (7:1:2 v/v) (solvent A).

chloride (**18**) with various tertiary amines as shown in Chart 6. The results are listed in Table III.

In the next, the acylation of DL-α-glycerophosphoryl choline (**1a**) with myristoyl chloride was tried to confirm furthermore the structure of **1a** according to Kogel.[3a] When the reaction of **1a** with an excess amount of myristoyl chloride was carried out at room temperature for 1 week in the absence of hydrochloric acid catcher, DL-myristoyl lecithin (**2a**) was obtained as white powder in the yield of 30%. The structure of **18** was supported by IR spectrum, which showed absorption bands at 3350 and 1650 (OH, water of crystallization), 1737 (C=O), 1235 (P=O), 1170, 1085 and 1060 cm^{-1} (P-O-C, organo phosphoric salt), and elementary analysis, of which the results agreed to the composition having 1 mole of water of crystallization.

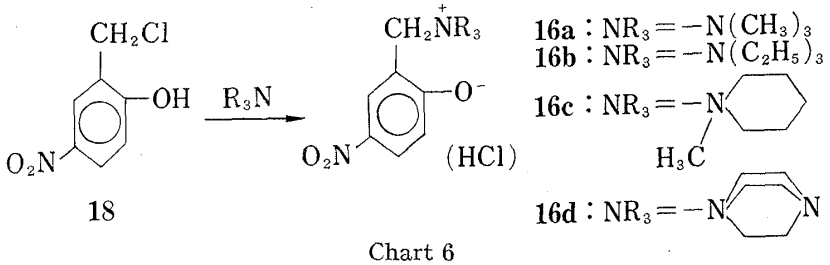


Chart 6

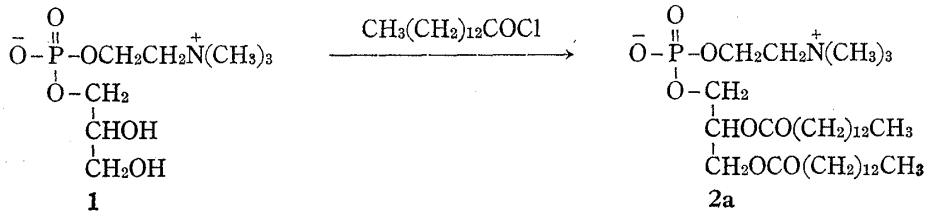


Chart 7

In conclution, it was established that the phosphorodichloridate **3** having the stable protecting group phosphorylated stepwise various alcohols such as ethylene chlorohydrin, ethylene bromohydrin, 1,2-isopropylidene DL-glycerol and its L-isomer to afford the mixed trialkyl phosphates **10** and **11** in the similar manner of the phosphorylation of simple alcohol described in the previous paper.[7a] The protecting groups of **10** and **11** were readily and selectively removed by hydrolyses through the active intermediates (**14**) derived from various tertiary amines to give the corresponding dialkyl hydrogen phosphates (**15**). The phosphates **15** were converted into α-glycerophosphoryl choline (**1a**) and its derivatives **1b**,**c** and **d** by the quarternalization of tertiary amines with halogen atom in ethyl chain of **15** and subsequent hydrolysis. For this purpose, the use of an easily water-soluble tertiary amine was more profitable. By this method, a few of the phosphates **1**, which were not existent in nature, were synthesized as well as L-glycerophosphoryl choline (**1a**-L). Myristoyl DL-lecithin (**2a**) was prepared to confirm the structure of **1a**. The other lecithin-like compounds may be prepared by using the derivatives **1b**, **c** and **d**.

Experimental

IR spectra were recorded with a Hitachi EPI-S2 spectrophotometer. UV spectra were obtained by using a Hitachi EP5-2U recording spectrophotometer. NMR spectra were measured by a Hitachi R20A resolution nuclear magnetic resonance spectrometer. Melting points were determined with Yamato MP-1 melting point apparatus. The paper chromatographies (PPC) were carried out by ascending method using Toyo Roshi No. 51A paper. Solvent systems used were: isopropanol, conc. aq. ammonia and water (7:1:2 v/v) (solvent A) and (6:3:1 v/v) (solvent B). The thin-layer chromatographic (TLC) analyses were performed by using thin-layer plate prepared from Silica gel GF_{254} (E. Merck). Solvent systems used were: benzene, ethyl acetate (4:1 v/v) (solvent C), chloroform and methanol (4:1 v/v) (solvent D).

Materials——Ethylene chlorohydrin, ethylene bromohydrin, pyridine and tetrahydrofuran were purified and dried by ordinary procedure. Commercial-available tertiary amines (30% aqueous trimethylamine solution, triethylamine, tributyl amine, 1-methylpiperidine and diazabicyclo [2,2,2] octane (DABCO)) were used without purification. Myristoyl chloride (bp 115—117° (0.4 mmHg)) was prepared from thionyl chloride and the gas chromatographically homogenious myristic acid, which was purified by several times fractional distillation of commercial-available reagent. 2-Chloromethyl-4-nitrophenyl phosphorodichloridate (3) was prepared from the phosphorylation of 2-chloromethyl-4-nitrophenol (**18**) with phosphoryl chloride according to the previous paper.[7b] 1,2-Isopropylidene-DL-glycerol (bp 79—80° (12 mmHg)) was prepared by ordinary procedure using zinc chloride as dehydrating agent. 1, 2-Isopropylidene-D-glycerol (bp 82—83.5° (12 mmHg), $[\alpha]_D^{25}$ 11.7° (c=10.0, methanol)) was prepared by the reduction of 1,2-isopropylidene-D-glyceroaldehyde, which was obtained by the ordinary glycol fission of 1,2,5,6-diisopropylidene-D-mannitol,[12] with sodium borohydride.

12) E. Baer, *J. Am. Chem. Soc.*, **67**, 338 (1945).

β′-Chloroethyl Isopropylidene-DL-α-glyceryl 2-Chloromethyl-4-nitrophenyl Phosphate (10)——a) To a solution of 3.04 g (0.01 mole) of 2-chloromethyl-4-nitrophenyl phosphorodichloridate (3) in 20 ml of tetrahydrofuran (THF) was added, dropwise, a solution of ethylene chlorohydrin (0.67 ml, 0.01 mole) and pyridine (0.80 ml, 0.01 mole) in 10 ml of THF with stirring at −30——20° over a period of about 1 hr. The stirring was continued for 4 hr under cooling and then a solution of 1,2-isopropylidene DL-α-glycerol (2.64 g, 0.02 mole) and pyridine (0.80 ml, 0.01 mole) in 10 ml of THF was added to the reaction mixture in one portion. The stirring was additionally continued at a temperature below 0° for 1 hr and at room temperature for 3 hr. After removal of white precipitate by filtration, the filtrate was concentrated to dryness *in vacuo* and the residue was dissolved in 60 ml of benzene. The solution was washed with two 20 ml portions of water and dried over anhydrous sodium sulfate. Benzene was removed by evaporation to afford 3.93 g (90.8%) of the crude phosphate **10** as pale yellow viscous oil. TLC *Rf* 0.55 (a trace of by products, *Rf* 0.65 and 0.41) (solvent C), *Rf* 0.95 (solvent D). UV λ_{max}^{EtOH} mμ (log ε): 270 (3.95). IR ν_{max}^{film} cm^{-1} :2980 and 2880 (CH_2 and CH_3), 1524 and 1345 (NO_2), 1240 (P=O) (broad), 1190 (P–O–C_{aryl}), 1080 (C–O–C), 1025 (P–O–C_{alkyl}).

b) To a solution of 3.04 g (0.01 mole) of 2-chloromethyl-4-nitrophenyl phosphorodichloridate (3) in 20 ml of THF was added, dropwise, a solution of 1,2-isopropylidene-DL-α-glycerol (1.32 g, 0.01 mole) and pyridine (0.80 ml, 0.01 mole) in 10 ml of THF with stirring at −30——20° over a period of about 1 hr. After stirring for 4 hr under cooling, a solution of ethylene chlorohydrin (1.34 ml, 0.02 mole) and pyridine (0.80 ml, 0.01 mole) in 10 ml of THF was added to the reaction mixture in one portion. The stirring was continued at room temperature for 6 hr. The reaction mixture was worked up in the same manner described above to afford 3.75 g (86.7%) of the crude phosphate **10** as pale yellow viscous oil.

In the similar manner, β′-chloroethyl isopropylidene-L-α-glyceryl 2-chloromethyl-4-nitrophenyl phosphate (**10**-L) and β′-bromoethyl isopropylidene-DL-α-glyceryl 2-chloromethyl-4-nitrophenyl phosphate (**11**) were prepared from the reaction of the corresponding ethylene halohydrin and 1,2-isopropylidene glycerol with 2-chloromethyl-4-nitrophenyl phosphorodichloridate (**3**). It was detected by PPC that the phosphates, **10**-L and **11** were also contaminated with a trace of by products.

Bis (β′-chloroethyl) 2-Chloromethyl-4-nitrophenyl Phosphate (12)——To a solution of 3.04 g (0.01 mole) of 2-chloromethyl-4-nitrophenyl phosphorodichloridate (**3**) in 20 ml of THF was added, dropwise, a solution of ethylene chlorohydrin (2.68 ml, 0.04 mole) and pyridine (1.60 ml, 0.02 mole) in 10 ml of THF with stirring under ice cooling. The stirring was continued under cooling for 1 hr and additionally at room temperature for 5 hr. After removing a white precipitate by filtration, the filtrate was concentrated to dryness in the diminished pressure and the residual oil was dissolved in 50 ml of benzene. The solution was washed with two 10 ml portions of water and dried over anhydrous sodium sulfate. The solution was decolorized with charcoal and then the solvent was removed by evaporation *in vacuo* to afford 3.50 g (88.9%) of the phosphate **12** as pale yellow viscous oil. TLC, *Rf* 0.65 (solvent C), 0.95 (solvent D). UV λ_{max}^{EtOH} mμ (log ε): 269 (3.97). IR ν_{max}^{film} cm^{-1}: 2960 (CH_2), 1523 and 1348 (NO_2), 1242 (P=O), 1025 (P–O–C_{alkyl}). *Anal.* Calcd. for $C_{11}H_{13}O_6NCl_3P$: C, 33.66; H, 3.34; N, 3.57; Cl, 27.10. Found: C, 34.22; H, 3.54; N, 3.80; Cl, 27.86.

Bis (isopropylidene-DL-α-glyceryl) 2-Chloromethyl-4-nitrophenyl Phosphate (13)——To a solution of 3.04 g (0.01 mole) of 2-chloromethyl-4-nitrophenyl phosphorodichloridate (**3**) in 20 ml of THF was added, dropwise, a solution of 1,2-isopropylidene-DL-glycerol (5.28 g, 0.04 mole) and pyridine (1.60 ml, 0.02 mole) in 10 ml of THF with stirring under ice cooling. The stirring was continued under cooling for 1 hr and additionally at room temperature for 5 hr. The reaction mixture was worked up in the similar manner described in the case of the phosphate **12** to afford 2.85 g (57.5%) of the phosphate **13** as pale yellow viscous oil. TLC, *Rf* 0.41 (solvent C), 0.95 (solvent D). UV λ_{max}^{EtOH} mμ (log ε): 271 (3.95). IR ν_{max}^{film} cm^{-1}: 2980 and 2880 (CH_3, CH_2 and CH), 1525 and 1343 (NO_2), 1290—1215 (broad) (P=O and C–O–C), 1085—1020 (broad) (P–O–C and C–O–C). *Anal.* Calcd. for $C_{19}H_{27}O_{10}NClP$: C, 46.02; H, 5.49; N, 2.82; Cl, 7.15. Found: C, 45.95; H, 5.45; N, 3.36; Cl, 7.98.

DL-α-Glycerophosphoryl Choline (1a)——A suspension of β′-chloroethyl isopropylidene-DL-α-glyceryl 2-chloromethyl-4-nitrophenyl phosphate (**10**), which was prepared from 6.08 g (0.02 mole) of 2-chloromethyl-4-nitrophenyl phosphorodichloridate (**3**), 1.34 ml (0.02 mole) of ethylene chlorohydrin and 5.28 g (0.04 mole) of 1,2-isopropylidene-DL-glycerol, in 20 ml of 30% aqueous trimethylamine solution was stirred at room temperature for ½ hr and then warmed at 45—50° for 24 hr. After concentrating to dryness *in vacuo*, 40 ml of ethanol was added to the residue and insoluble yellow precipitate, 2-hydroxy-5-nitrobenzyl trimethyl ammonium chloride (**16a**) (2.25g, 91.5%) was filtered off. The filtrate was diluted with 50 ml of water and aqueous solution was passed through a column of Amberlite IR 120 resin (H form) (charged with 30 ml of resin). The column was washed with water until the washings were neutral to litmus. The combined original eluent and washings were kept standing at room temperature for 2 hr, and then passed through a column of Amberlite IR 45 resin (OH form) (charged with 30 ml of resin). The column was washed with 100 ml of water. The combined original eluent and washings were decolorized with charcoal and evaporated to driness *in vacuo*. A suspension of the resulted oil in a mixture of acetone and ethanol was allowed to stand at room temperature for 5 days to afford 2.40 g (46.6%) of the crystalline phosphate **1a**, mp 145—148°. Recrystallization from 95% ethanol afforded the chromatographically homogeneous phosphate **1a** as very hygroscopic white powder, mp 152—153°. NMR (in D_2O) τ: 6.67 (9H, singlet, $(CH_3)_3N$), 5.80—6.41 (9H, multiplet,

NCH_2CH_2O and $OCH_2CH(O-)CH_2O$), 5.58 (2H, broad singlet, C(OH)–C(OH)). The other data are listed in Table II.

In the similar manner, L-α-glycerophosphoryl choline (**1a**-L), inner salt of β′-(DL-α-glyceryl hydrogen phosphoroxy) ethyl triethyl ammonium hydroxide (**1b**) and inner salt of 1-[β′-(DL-α-glyceryl) hydrogen phosphoxy] ethyl 1-methyl piperidinium hydroxide (**1c**) were prepared. The reaction conditions and results are summerized in Table I and II.

Inner Salt of 1-[β′-(DL-α-Glyceryl) Hydrogen Phosphoroxy]ethyl 1,4-Diazonia Bicyclo[2,2,2]octane Hydroxide (1d)——A suspension of β′-chloroethyl isopropylidene-DL-α-glyceryl 2-chloromethyl-4-nitrophenyl phosphate (**10**), which was prepared from 3.04 g (0.01 mole) of 2-chloromethyl-4-nitrophenyl phosphorodichloridate (**3**), and 11.2 g (0.1 mole) of DABCO in 20 ml of water was stirred at room temperature for 1 hr. The resulted yellow homogeneous reaction mixture was warmed at 45—50° for 20 hr. After concentrationg *in vacuo*, the residue was washed with two 50 ml portions of ether and insoluble viscous oil was treated with 20 ml of ethanol. Yellow precipitate, 1-(2′-hydroxy-5′-nitrobenzyl) 1,4-diazonia bicyclo[2,2,2]octane chloride (**16d**) was filtered off and the filtrate was acidified with 3% HCl. The acidic solution was kept standing at room temperature for 2 hr and then passed through a column of Amberlite IR 45 resin (OH form) (1.5×40 cm). The column was washed with 150 ml of water. After evaporating the combined original eluent and washing *in vacuo*, the resulted oil was washed with ether and then the insoluble oil was dissolved in 30 ml of water. The aqueous solution was treated with a column of Amberlite IRC 50 resin (H form) (1.5×70 cm) and then Amberlite IR 45 resin (OH form)(1.5 × 40 cm). The combined eluent and washings were decolorized with charcoal and evaporated *in vacuo* to afford 1.60 g (51.6%) of **1d** as pale yellow viscous oil. The result is listed in Table I and II.

Dimyristoyl DL-α-Glycerophosphoryl Choline (2a)——A suspension of 1.29 g (0.005 mole) of DL-α-glycerophosphoryl choline (**1a**) and 9.85 g (0.04 mole) of myristoyl chloride in 10 ml of chloroform was kept standing at room temperature with occasional stirring for a week. After evaporating the reaction mixture *in vacuo*, to the residue was added 80 ml of acetone and then insoluble crystal (0.23 g) was filtered off. The filtrate was concentrated to 30 ml *in vacuo* and stored in the refrigerator overnight. A white precipitate was filtered and washed with acetone. The precipitate was dissolved in 30 ml of ethanol and the solution was passed through a column of Amberlite IR 45 resin (OH form) (1 × 30 cm). The ethanolic eluent was evaporated *in vacuo* to afford 1.5 g of the crude phosphate **2a**, mp 180—210°. Recrystallization from a mixture of acetone (12 ml) and ethanol (1.2 ml) gave 1.03 g (29.6%) of the paper chromatographycally homogeneous **2a** as white powder, mp 225—227°. PPC, *Rf* 0.89 (solvent A). IR ν_{max}^{Nujol} cm^{-1}: 1735 (C=O), 1240 (broad) (P=O), 1180 (broad) and 1080 (broad) (C–O–P–O⁻). IR spectrum showed the existence of crystallin water (3350 cm^{-1}, 1650 cm^{-1}). *Anal.* Calcd for $C_{36}H_{72}O_8NP \cdot H_2O$: C, 62.13; H, 10.72; N, 2.01. Found: C, 62.56; H, 10.71; N, 2.15.

2-Hydroxy-5-nitrobenzyl Trimethyl Ammonium Chloride (16a)——To a solution of 1.87 g (0.01 mole) of 2-hydroxy-5-nitrobenzyl chloride (2-chloromethyl-4-nitrophenol) (**18**) in 30 ml of acetone was added, dropwise, 5.90 g (0.03 mole) of 30% aqueous solution of trimethylamine with stirring at room temperature. A yellow precipitate soon appeared. After stirring for 1 hr, the precipitate was filtered and washed with 50 ml of acetone and recrystallized from 90% of ethanol contained a small amount of HCl to afford 2.02 g (81.3%) of the pure compound **16a** as pale yellow prisms.

In the similar manner, 2-hydroxy-5-nitrobenzyl triethyl ammonium chloride (**16b**) and 1-(2′-hydroxy-5′-nitro)benzyl 1-methyl piperidinium chloride (**16c**) were prepared. The results are shown in Table III.

1-(2′-Hydroxy-5-nitro)benzyl 1,4-Diazonia Bicyclo[2,2,2]octane Dichloride (16d)——To a solution of 5.61 g (0.05 mole) of DABCO in 30 ml of ether was added, dropwise, a solution of 1.07 g (0.01 mole) of 2-hydroxy-5-nitrobenzyl chloride (**18**) in 10 ml of acetone with stirring at room temperature. After stirring for 1 hr, the precipitate was filtered and washed with 30 ml of ether. The solution of the precipitate in 30 ml of ethanol was acidified with ethanolic HCl and allowed to stand in the refrigerator overnight to afford 2.51 g (70.8%) of **16d**. Recrystallization from 80% ethanol afforded analytically pure sample of **16d** as pale yellow needles. The results are listed in Table III.

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