Synthesis of Phosphatidyl-6-D-glucose and Attempted Synthesis of Phosphatidyl-1-D-glucose

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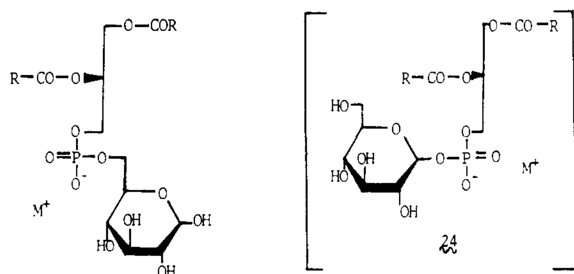
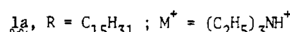
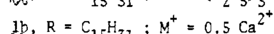
The synthesis of significant quantities of 6-*O*-(1′,2′-di-*O*-palmitoyl-*sn*-glycero-3′-phosphoryl)-D-glucose, as its calcium and sodium salts, has been achieved by two procedures, both of which are based on the cyclic enediol phosphoryl method of phosphodiester synthesis, $(R^1O)(R^2O)P(O)(OH)$. The phosphorylating reagent is bis-(1,2-dimethylethenylene) pyrophosphate. The lipid alcohol, R^1OH, is 1,2-dipalmitoyl-*sn*-glycerol in both procedures. In procedure A, the protected glycose alcohol, R^2OH, is methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside, and only the C-6-OH group is available for reaction. In procedure B, R^2OH is 2,3,4-tri-*O*-benzyl-D-glucose, and both C-1-OH and C-6-OH groups are available for reaction; the phosphorylation is selective toward the primary alcohol at C-6 in the presence of the cyclic hemiacetal at C-1. Conditions for the removal of protective groups are mild, and the phosphatidylglucose is obtained in satisfactory yield from readily available materials. The synthetic strategy also provides useful intermediates in which the phosphatidyl-6-glucose has a free C-1-OH and protected C-2,3,4 positions (procedure B) or a protected C-1 position and free C-2,3,4 positions (procedure A). These intermediates lend themselves to the construction of more complex derivatives of the basic glycophospholipid structure. The synthesis of 1′,2′-di-*O*-palmitoyl-*sn*-glycero-3′-phosphoryl 2,3,4,6-tetra-*O*-benzyl-D-glucopyranoside, as triethylammonium, sodium, and calcium salts, has also been achieved. However, all attempts to remove the benzyl protective groups from this intermediate by hydrogenation resulted in extensive decomposition. Our results cast doubt on the reported synthesis of phosphatidyl-1-glucose by a similar deprotection technique.

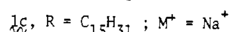
Virtually all the phospholipids present in biological membranes[1,2] are phosphodiesters, $(R^1O)(R^2O)P(O)(OH)$, derived from a hydrophobic alcohol, R^1OH, and a hydrophilic alcohol, R^2OH. In most cases, the hydrophobic alcohol is a representative of one of the two main classes of membrane lipids, namely, 1,2-diacyl-*sn*-glycerol[3] or *N*-acylsphingosine. The hydrophilic alcohol varies greatly in structure, but in all authenticated cases it contains a primary or secondary alcohol function by which it forms the second ester bond of the phosphodiester molecule. Apparently, none of the membrane phospholipids contain energy-rich phosphate bonds, of the type illustrated by enol phosphates, glucose-1-phosphate, or pyrophosphate esters. The coenzyme cytidine diphosphate 1,2-diacyl-*sn*-glycerol ("CDP diglyceride"), which is included among the phospholipids and the glycolipids in some reviews[1,4] is not a membrane component but an obligatory intermediate in the biosynthesis of many membrane phospholipids, e.g., phosphatidylinositol, phosphatidylglycerol, cardiolipin, and phosphatidylserine.

This paper describes the chemical synthesis of phosphatidyl-6-glucose (1). We have isolated the phospholipid 1 as mono- and divalent metal ion salts and have used in its synthesis the optically active form of a 1,2-diacyl-*sn*-glycerol made from a fatty acid of 99.9% purity. This is needed for studies of bilayer formation and the associated phase-transition properties[2,5] of aqueous dispersions of the phosphatidylglucoses, which is one of the goals of this investigation. A second goal of the research is to utilize the basic structure of phosphatidyl-6-glucose as the starting point for the construction of more elaborate glycophospholipids. Therefore, we have adopted a synthetic strategy that leads not only to the final phosphatidylglucose 1 but also to derivatives of 1 in which the hemiacetal C-1-OH function is protected while the alcoholic C-2,3,4-OH groups are free to undergo further condensations, or in which C-2,3,4-OH groups are protected while the C-1-OH function is free to undergo further reactions with glycoses, α-amino acids, peptides, etc.









This paper also describes unsuccessful attempts to synthesize phosphatidyl-1-glucose **24** by procedures that furnished the phosphatidyl-6-glucose isomer **1**. Our results cast doubts on previous reports of the synthesis of phosphatidyl-1-glucose (see below).

Apparently, the natural occurrence of the simple phosphatidylglucoses, 1 or 24, has not been established, although this term appears in a classification of the known phospholipids (Class A.20).[1] An early[6] report of the isolation of "phosphatidyl-6-glucose" from *Acholeplasma laidlawaii B* (formerly *Mycoplasma laidlawii B*) was later corrected,[7–9] and the structure was shown to be a glycero-3-phosphoryl derivative of a diglucosyldiglyceride. This type of compound was also obtained from *Streptococcus faecalis*.[10] An analogous type of compound, in which the glycero-3-phosphoryl moiety had been replaced by the phosphatidyl group[3] was isolated also from *S. faecalis*.[11,12] In these glycophospholipids[13] the phosphatidic acid es-

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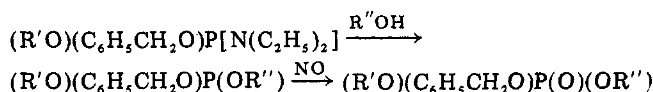
terifies the C-6-OH function of a modified D-glucose. This modified D-glucose moiety is a 3-*O*-diglucosyl-1,2-di-*O*-acyl-*sn*-glycerol. Phosphatidyl derivatives of the simpler 3-*O*-monoglucosyl-1,2-di-*O*-acyl-*sn*-glycerol have been isolated from *Pseudomonas diminuta*.[14-16] The glycophospholipids[17] are of considerable structural interest, since they combine in the same molecule structural elements of the membrane phospholipids and of the phosphorus-free glycolipids[13] related to the cerebrosides.

To our knowledge, the chemical synthesis of two derivatives of phosphatidyl-6-glucose has been claimed: the sodium salt derived from 1,2-dipalmitoyl-*sn*-glycerol[18] and the free acid derived from 1,2-distearoyl-*rac*-glycerol.[19] One derivative of phosphatidyl-6-galactose has been described:[20] the free acid derived from 1,2-distearoyl-*rac*-glycerol. In one approach,[18] a benzyl phosphotriester, $(R^1O)(R^2O)P(O)(OCH_2C_6H_5)$, was made by reaction of $(R^1O)(AgO)P(O)(OCH_2C_6H_5)$ with tetrabenzyl-6-deoxy-6-iodo-D-glucopyranose. The phosphate protective group was removed by sodium iodide in acetone; the alcohol protective groups were removed by hydrogenolysis over Pd. In another approach,[19] phosphoroamidites were converted into trialkyl phosphites, which were oxidized to phosphotriesters:

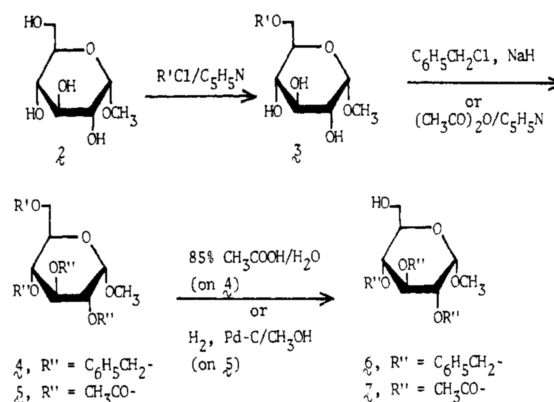


$$(R'O)(C_6H_5CH_2O)P[N(C_2H_5)_2] \xrightarrow{R''OH}$$
$$(R'O)(C_6H_5CH_2O)P(OR'') \xrightarrow{NO} (R'O)(C_6H_5CH_2O)P(O)(OR'')$$

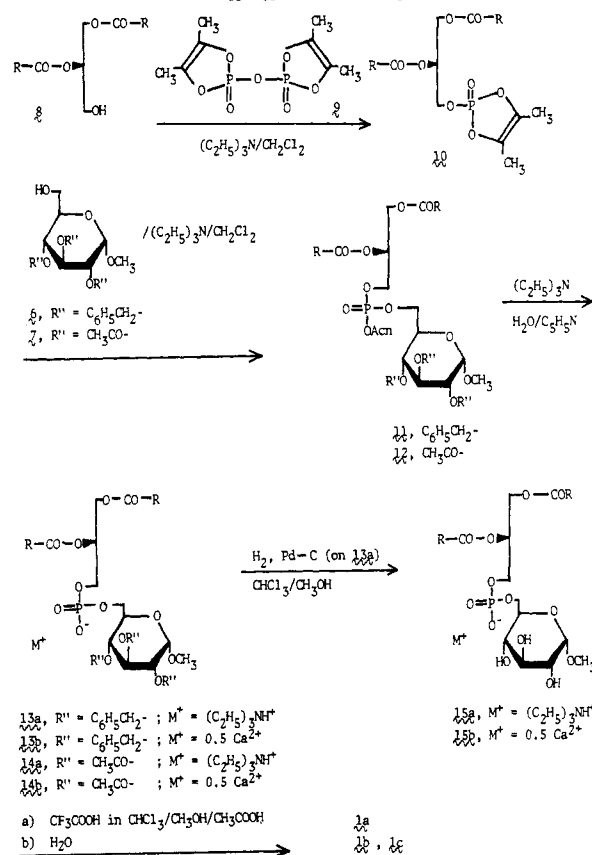
Several approaches were utilized to convert the intermediates into the desired phosphotriesters, $(R^1O)(R^2O)P(O)(OCH_2C_6H_5)$, and phosphodiesters, $(R^1O)(R^2O)P(O)(OH)$, containing the residues of the lipid and glycose alcohols, R^1OH and R^2OH, respectively. Protection of R^2OH was by acetyl groups, in which case deprotection required treatment with a hydrazine buffer,[19] or by cyclic acetals, in which case deprotection involved acid catalysis.[20] These pioneering investigations, however, have not made the phosphatidyl-6-glucose derivatives readily available in significant quantities and reasonable degrees of purity.

To the best of our knowledge, no glycophospholipid derived from phosphatidyl-1-glucose has been isolated from natural sources. However, the synthesis of the sodium salt derived from 1-myristoyl-2-oleoyl-*sn*-glycerol[21] and the sodium salt derived from 1,2-dipalmitoyl-*sn*-glycerol[22] have been claimed. Both substances were made from the silver salt $(R^1O)(AgO)P(O)(OCH_2C_6H_5)$. In one case[21] the protected glycose was tetraacetyl-D-glucopyranosyl bromide, and in the other,[22] tetrabenzyl-D-glucopyranosyl bromide. Therefore, all of these procedures

Scheme I ($R' = (p\text{-}CH_3OC_6H_4)(C_6H_5)_2C$)



Scheme II ($R = C_{15}H_{31}$; Acn = $CH_3C(O)CH(CH_3)$)



suffer from difficulties inherent in the sluggish nucleophilic displacement by a silver phosphate ester on the C-6-I or C-1-Br derivative of glycoses.

Results and Discussion

Synthesis of Phosphatidyl-6-glucose (1). The 6-isomer 1 was made by two procedures. Procedure A is outlined in Schemes I and II. Procedure B is summarized in Scheme III. The properties of the final product (1) and its precursors are given in Table I (see Table II for NMR data). Procedure A defines the structure of the 6-isomer, 1, unequivocally, since the second phosphorus–oxygen bond is established by condensation of a glycose intermediate, 6 or 7, which has only one unprotected hydroxyl group (at the C-6 position). Procedure B, however, is the method of choice, since it involves a smaller number of steps from available starting materials, and affords the final product 1 in higher yield. In procedure B there is an element of ambiguity, since the second phosphorus–oxygen bond is established on a glycose intermediate, 18,

(13) The term "glycolipid" has been applied to compounds with and without phosphorus. The best known glycolipids are cyclic acetals, R^1OR^2, in which the lipid, R^1OH = *N*-acylsphingosine (in cerebrosides) or 1,2-diacyl-*sn*-glycerol, and the glycose, R^2OH = a mono- or polysaccharide linked at the anomeric carbon, C-1. Phosphatidylinositol and its mono- and diphosphorylated derivatives, which are membrane phospholipids, are discussed among the glycolipids because *myo*-inositol is vaguely related to the sugar alcohols.

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Table I. Properties of Dipalmitoylphosphatidyl-6-D-glucose (1) and Its Precursors

no.	compound[a]	mp,[b] °C	R_f (solvent)[c]	$[\alpha]^{20}{}_D$,[d] deg (c, %)	molecular formula (M_r)	calcd C	calcd H	calcd Ca	found C	found H	found Ca
3	1-Me-6-mmt-α-D-Glcp	90–92	0.25 (B)	52.1 (8.2)							
4	1-Me-2,3,4-Bn-6-mmt-α-D-Glcp	51–54	0.77 (A)	18.6 (3.6)							
5	1-Me-2,3,4-Ac-6-mmt-α-D-Glcp	108–111	0.75 (B)	100.1 (9.1)	$C_{33}H_{36}O_{10}$ (592.62)	66.89	6.08		66.95	6.14	
6	1-Me-2,3,4-Bn-α-D-Glcp	e	0.30 (A)	17.5 (8.9)							
13b	DPP-6-(1-Me-2,3,4-Bn)-α-D-Glcp·0.5Ca[f]	68–71 dec	0.84 (C)	15.4 (5.7)	$C_{126}H_{196}O_{26}P_2Ca·2H_2O$ (2264.90)	66.81	8.90	1.77	66.80	8.68	1.64
14b	DPP-6-(1-Me-2,3,4-Ac)-α-D-Glcp·0.5Ca[g]	(80) 112–116 dec	0.41 (C)	49.6 (3.8)	$C_{96}H_{172}O_{32}P_2Ca$ (1940.37)	59.19	9.12		59.42	8.94	
15b	DPP-6-(1-Me)-α-D-Glcp·0.5Ca[h]	170–172 dec	0.41 (C)	34.4 (5.6)	$C_{84}H_{160}O_{26}P_2Ca·2H_2O$ (1724.19)	58.51	9.59	2.32	58.07	9.10	2.35
20b	DPP-6-(2,3,4-Bn)-D-Glc·0.5Ca[i]	(70) 180–184 dec	0.78 (C)	17.7 (3.9)	$C_{124}H_{192}O_{26}P_2Ca·2H_2O$ (2236.84)	66.58	8.83	1.79	66.08	9.06	1.87
1b	DPP-6-D-Glc·0.5Ca[j]	(155) 171–173 dec	0.23 (C)	16.0 (3.2)	$C_{82}H_{156}O_{26}P_2Ca·2H_2O$ (2236.84)	58.62	9.60	2.39	59.04	9.89	2.05
1c	DPP-6-D-Glc·Na	(152) 172–176 dec	0.22 (C)	12.2 (1.8)	$C_{41}H_{78}O_{13}PNa·H_2O$ (851.03)	57.86	9.48	k	58.26	9.68	
23b	DPP-1-(2,3,4,6-Bn)-D-Glcp·0.5Ca[l]	145–149 dec	0.88 (C)	35.5 (2.0)	$C_{138}H_{204}O_{26}P_2Ca·2H_2O$ (2417.08)	68.57	8.67	1.66	68.62	8.81	1.51
23c	DPP-1-(2,3,4,6-Bn)-D-Glcp·Na^+	70–72	0.76 (C)	36.0 (2.3)	$C_{69}H_{102}O_{13}PNa·H_2O$	68.40	8.65	m	68.65	8.95	

[a] Abbreviations: Me = methyl; Bn = benzyl; Ac = acetyl; mmt = monomethoxytrityl; Glc = glucose; Glcp = glucopyranose; DPP = dipalmitoylphosphatidyl. [b] Samples sinter at the temperatures given in parenthesis. [c] Solvent A, chloroform; solvent B, chloroform/methanol (9:1); solvent C, chloroform/methanol/water (65:25:2); solvent D, chloroform/methanol/concentrated NH_4OH (3:5:1). All compounds except 1 were chromatographed on Merck HPTLC 60 F-254, 1 on plates made from silica gel H. [d] All optical rotations in chloroform/methanol (2:1, v/v). [e] Gummy solid. [f] [DPP-6-(1-Me-2,3,4-Bn)-α-D-Glcp]$^-$$(C_2H_5)_3NH^+$ (13a), R_f 0.87 (C). [g] [DPP-6-(1-Me-2,3,4-Ac)-α-D-Glcp]$^-$$(C_2H_5)_3NH^+$ (14a), $[\alpha]^{20}{}_D$ 51.7 (c, 8.5). [h] (DPP-6-(1-Me)-α-D-Glcp]$^-$$(C_2H_5)_3NH^+$ (15a), R_f 0.42 (C). [i] [DPP-6-(2,3,4-Bn)-D-Glc]$^-$$(C_2H_5)_3NH^+$ (20a), R_f 0.74 (C). [j] [DPP-6-D-Glc]$^-$$(C_2H_5)_3NH^+$ (1a), R_f 0.23 (C). [k] Na: calcd, 2.70; found, 2.52. [l] [DPP-1-(2,3,4,6-Bn)-D-Glcp]$^-$$(C_2H_5)_3NH^+$ (23a); R_f 0.84 (C), 0.56 (D). [m] Na: calcd, 1.90; found, 1.96.

Scheme III (R″ = $C_6H_5CH_2$; Acn = $CH_3C(O)CH(CH_3)$)

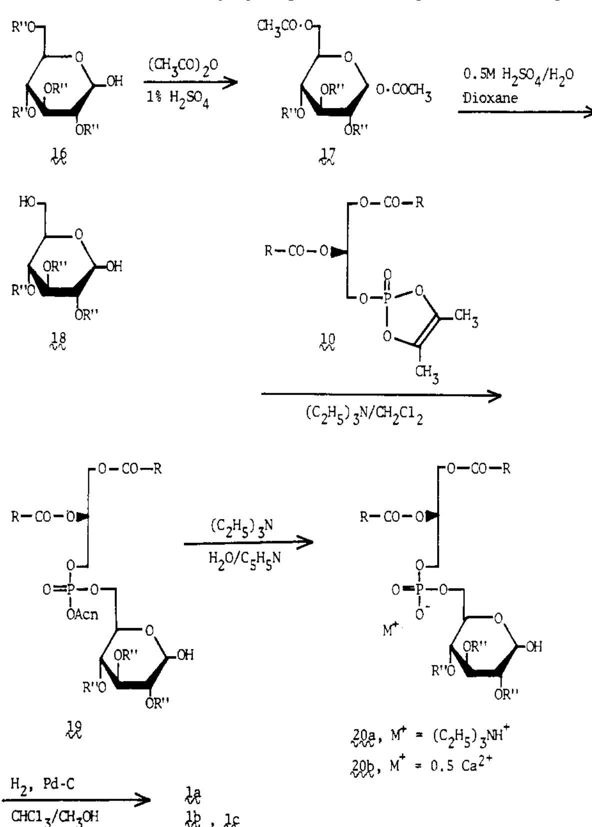

Table II. ^{1}H NMR Data[a]

compd no.	δ
3	3.40 (C-1-OCH_3); 3.76 (aromatic OCH_3)
4	3.45 (C-1-OCH_3); 3.78 (aromatic OCH_3); 4.2–5.0 (3 × $CH_2C_6H_5$)
5	1.82, 2.10, 2.20 (3 × CH_3CO); 3.55 (C-1-OCH_3); 3.86 (aromatic OCH_3)
6	3.34 (C-1-OCH_3); 4.35–5.0 (3 × $CH_2C_6H_5$)
7	2.15 (3 × CH_3CO); 3.50 (C-1-OCH_3)
13a	phosphatidyl CH_2 centered at 1.3; 3.45 (C-1-OCH_3)[b]
14a	phosphatidyl CH_2 centered at 1.25; 2.05, 2.10, 2.20 (3 × CH_3CO); 3.45 (C-1-OCH_3)[b]

[a] All compounds in $CDCl_3$; chemical shifts vs. Me_4Si = 0. Integration of the signals agree with structural assignments. [b] NMR spectra of triethylammonium salt instead of calcium salt for solubility reasons.

which has two unprotected hydroxyl groups (at C-1 and C-6). Therefore, the two procedures complement each other and show that the condensation of the dialcohol **18** with the cyclic phosphotriester **10** is regiospecific and involves only the primary alcohol group at C-6 with formation of the acyclic phosphotriester **19** (Scheme III).

Procedure A is of additional value since one of the intermediates, **15** (Scheme II), has a protected C-1 position and unprotected C-2,3,4 positions. The free secondary alcohols can, in principle, be condensed with further molecules of glycoses, α-amino acids, etc., to give more complex glycolipids of possible natural origin. Intermediate **20** in procedure B is also of synthetic value. The unprotected anomeric position in **20** can, in principle, be functionalized to give more complicated glycophospholipids without interference from the protected secondary alcohols.

The steps outlined in Scheme I are adaptations of known reactions and require no further comment. We explored two techniques of glycose protection, benzylation to give **4** and **6** and acetylation to give **5** and **7**. Both methods had been previously utilized in other approaches to phosphatidylglucoses.[18-22] As will be seen below, the acetylation route failed in the present work, since removal of the acetyl group from the appropriate intermediate, **14**, (Scheme II) utilizing a hydrazine buffer[19,21] did not yield the desired intermediate **15** in acceptable yield and in a reproducible manner.

The key phosphorylation reactions in Schemes II and III are identical and involve bis(1,2-dimethylethenylene) pyrophosphate (**9**)[23] as reagent. The establishment of the first phosphorus–oxygen bond involves the reaction of the pyrophosphate **9** with the diacylglycerol **8** to give the cyclic phosphotriester **10** in quantitative yield. The second phosphorus–oxygen bond is formed in a reaction of the cyclic phosphotriester **10** with the primary alcohol of the glycose (**6** or **7** in Scheme II; **18** in Scheme III) to yield the acyclic phosphotriester (**11** or **12**, Scheme II; **19**, Scheme III). The acyclic triester is not purified but is converted into the phosphodiester (**13** or **14**, Scheme II; **20**, Scheme III). The removal of the phosphate protective group, i.e., the 3-oxo-2-butyl (acetoinyl) group is carried out in a very mild reaction that depends on the rapid basic hydrolysis of neutral esters of phosphoacetoin.[24] The phosphodiesters are purified and submitted to the appropriate deprotection steps. The removal of benzyl protection by hydrogenolysis is successful in both procedures A and B: cf. **13** → **15** (Scheme II) and **20** → **1** (Scheme III).

An important step in procedure A is the liberation of the glycose **1** from the methyl glycoside **15**. This was achieved at 0 °C by means of dilute trifluoroacetic acid in a mixed solvent containing acetic acid. An analogous reaction on the much simpler methyl 2,3,4,6-tetra-*O*-benzyl-*α*-D-glucopyranoside had been carried out by means of hydrochloric acid[25] in acetic acid at higher temperatures, conditions that are too severe for the sensitive phosphatidyl-6-glucose.

Attempted Synthesis of Phosphatidyl-1-glucose 24. Our approach to the synthesis of this compound is shown in Scheme IV. The first phosphorus–oxygen bond is established by reaction of the hemiacetal C-1-OH group of the protected glycose **16** with the pyrophosphate reagent **9** to yield the cyclic phosphotriester **21**. The second phosphorus–oxygen bond is established in the mild reaction of the 1,2-diacyl-*sn*-glycerol with the cyclic phosphotriester **21** to give the acyclic phosphotriester **22**. Without purification, this triester **22** is converted into the phosphodiester **23** as before. The analytical data in support of these structures are summarized in Table I. All attempts to obtain phosphatidyl-1-glucose **24** by removal of the benzyl protective groups upon hydrogenation, utilizing the techniques that were successful in the synthesis of 6-isomer **1**, failed in the case of the 1-isomer **24**. The hydrogenation was carried out on the triethylammonium, calcium, and sodium salts, **23a–c**, and in all cases the product of the reaction was insoluble in chloroform and sparingly soluble in methanol and in chloroform/methanol. Exhaustive transesterification of the hydrogenation product with methanolic HCl followed by quantitative analysis of the resulting methyl palmitate led to erratic results inconsistent with formula **24**. Elemental analyses were also inconsistent with the formula. We conclude that hydrogenation of the high-energy-protected derivative of phosphatidyl-1-glucose **23** is accompanied by extensive decomposition, probably initiated by hydrogenolysis of the activated acetal phosphate C1–O bonds. Our results are inconsistent with those of Russian investigators.[22]

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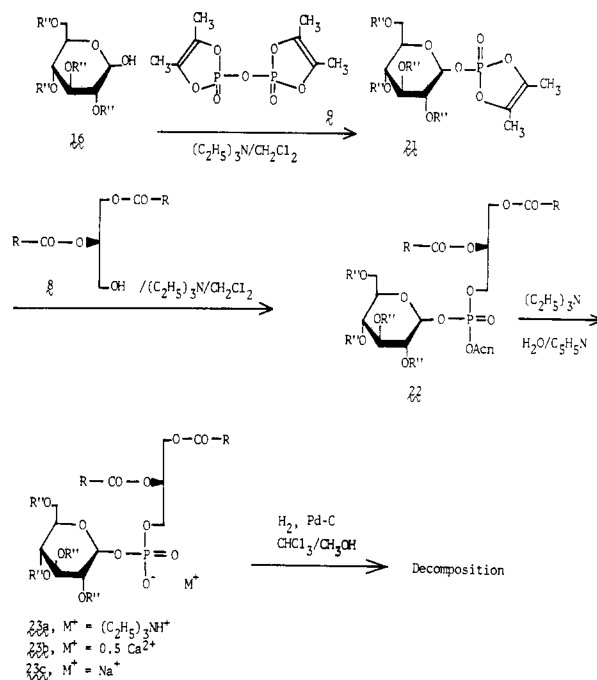
Scheme IV ($R = C_{15}H_{31}$; $R'' = C_6H_5CH_2$; Acn = $CH_3C(O)CH(CH_3)$)



Experimental Section

All reactions involving enediol cyclophosphoryl derivatives were carried out under anhydrous conditions. Triethylamine and dichloromethane were distilled from sodium and phosphorus pentoxide, respectively. All purifications were carried out by column chromatography; silica gel 60 was packed by using chloroform or ether and the crude compound was dissolved in the minimum volume of chloroform for application to the column. All evaporations were performed under vacuum. Samples were dried for 18 h at 20 °C (0.2 torr) prior to elemental analyses, which were performed by Galbraith Lab., Knoxville, TN. The properties of the new compounds are given in Table I.

Methyl 6-*O*-Monomethoxytrityl-*α*-D-glucopyranoside (3). Monomethoxytrityl chloride (16.5 g, 53 mmol) was added in portions to a solution of methyl *α*-D-glucopyranoside (**2**; 9.7 g, 50 mmol; Sigma Chemical Co.) in dry pyridine (50 mL) at 25 °C. After 36 h at 25 °C, the solution was evaporated, and the residue was purified (500 g of silica gel; elution with (1) 1 L of chloroform, (2) 500 mL of chloroform/methanol (9:1), (3) 80 mL of the same solvent, and (4) 170 mL of the same solvent). Fraction 4 contained pure product **3**. Fractions 2 and 3 were combined and repurified (elution with (1) 1.5 L of chloroform, (2) 200 mL of chloroform/methanol (9:1), and (3) 500 mL of the same solvent). Fraction 3 contained pure **3**. The combined solutions containing **3** were evaporated, and the residue was treated with ether to yield the 6-protected glucoside **3** (18.80 g, 81% yield).

Methyl 2,3,4-Tri-*O*-benzyl-6-*O*-monomethoxytrityl-*α*-D-glucopyranoside (4). Sodium hydride (5 g) was added to a solution of the glucoside **3** (2.5 g, 5.4 mmol) in benzyl chloride (60 mL) at 25 °C.[26] The mixture was stirred for 3.5 h at 150 °C, cooled to 25 °C, and cautiously treated with water (100 mL). The cooled mixture was extracted with ether, and the extract was evaporated. The residue was freed from unreacted benzyl chloride at 0.1 torr (40 °C). The residue was dissolved in chloroform (200 mL), and the solution was percolated through a short column of

(26) Tate, M. E.; Bishop, C. T. *Can. J. Chem.* **1963**, *41*, 1801.

silica gel. The solution was evaporated and the residue was purifed (100 g of silica gel; elution with (1) 500 mL petroleum ether, (2) 200 mL of petroleum ether/ether (4:1), (3) 225 mL of the same solvent, and (4) 220 mL of the same solvent). Fraction 4 was evaporated to yield the fully protected glucoside 4 (2.30 g, 58% yield).

Methyl 2,3,4-Tri-*O*-acetyl-6-*O*-monomethoxytrityl-α-D-glucopyranoside (5). Acetic anhydride (3 mL) was added to a solution of the glucoside 3 (3 g, 6.4 mmol) in pyridine (15 mL) at 0 °C.[27] After 24 h at 0 °C, the mixture was poured into ice-water. The product was filtered and dried in vacuum to yield virtually pure fully protected glucoside 5 (3.78 g, 99% yield).

Methyl 2,3,4-Tri-*O*-benzyl-α-D-glucopyranoside (6). A solution of the fully protected glucoside 4 (1.0 g, 1.36 mmol) in 85% aqueous acetic acid (25 mL) was kept for 5.5 h at 40 °C. The solution was evaporated, and the residue was purified (100 g of silica gel; elution with (1) 600 mL of petroleum ether/ether (3:1), (2) 200 mL of chloroform). Fraction 2 was evaporated to yield the 6-unprotected glucoside 6 (0.60 g, 95% yield).

Methyl 2,3,4-Tri-*O*-acetyl-α-D-glucopyranoside (7). A solution of the fully protected glucoside 5 (2 g, 3.37 mmol) in methanol (50 mL) was hydrogenated (40 psi of H_2) in the presence of 10% Pd/C (1 g; Johnson Matthey, Inc., Malvern, PA). The solution was filtered and evaporated. The residue was purified (60 g of silica gel; elution with (1) 200 mL of chloroform and (2) 200 mL of chloroform/methanol (9:1)). Fraction 2 was evaporated to yield the 6-unprotected glucoside 7 (0.80 g, 74% yield).

Methyl 2,3,4-Tri-*O*-benzyl-6-*O*-(1′,2′-di-*O*-palmitoyl-*sn*-glycero-3′-phosphoryl)-α-D-glucopyranoside Triethylammonium Salt (13a) and Calcium Salt (13b). A dichloromethane solution (2 mL) of 1,2-di-*O*-palmitoyl-*sn*-glycerol (8, 0.568 g, 1 mmol) was added to a stirred dichloromethane solution (2 mL) of bis(1,2-dimethylethenylene) pyrophosphate[28] (9, 0.282 g, 1 mmol) containing triethylamine (0.14 mL, ca. 1 mmol). After 2.5 h at 25 °C, the solution was evaporated to give the cyclic phosphate 10 (this compound is quite sensitive to moisture). A solution of the 6-unprotected glucoside (6, 0.464 g, 1 mmol; previously dried by evaporation with 3 × 3 mL of pyridine) in dichloromethane (2 mL) was added to a dichloromethane solution (1 mL) of the cyclic phosphate 10 containing triethylamine (0.28 mL, 2 mmol) at 25 °C. After being stirred for 24 h at 25 °C, the solution was evaporated. The residue was dissolved in chloroform (7 mL), the solution was put through a short column of silica gel, and the column was eluted with ether (400 mL) to give the impure triester 11. The solvent was evaporated, the residue was mixed with pyridine (30 mL), water (30 mL), and triethylamine (0.6 mL), and the turbid solution was warmed at 35–38 °C for 5 min, stirred for 26 h at 25 °C, and then evaporated. The residue was purified (120 g of silica gel; elution with (1) 500 mL of ether, (2) 200 mL of chloroform/methanol (9:1) and (3) 55 mL of the same solvent). Fraction 3 contained the pure triethylammonium salt of the phosphodiester 13a. The solution was evaporated, and the residue was dissolved in the minimum (ca. 2 mL) of chloroform/methanol (2:1). Acetone (20 mL) was added to this solution, and the mixture was kept for 5 h at 0 °C to obtain the solid salt 13a, which was filtered and dried in vacuum [0.586 g, 48% of 13a based on 1,2-dipalmitoyl-*sn*-glycerol (8)].

A solution of the phosphodiester triethylammonium salt 13a (0.070 g) in chloroform/methanol (2:1) (35 mL) was mixed with 15 mL of a solution containing 3 mL of chloroform, 48 mL of methanol, and 47 mL of 2 M aqueous calcium chloride. The mixture was stirred gently, and after phase separation, the upper phase was discarded. The procedure was repeated two additional times with the lower phase. The final lower organic phase was washed twice with chloroform/methanol/water (3:48:47) (10 mL) and evaporated. The solid was dried for 6 h at 25 °C (1 torr) to yield the phosphodiester calcium salt 13b (0.063 g).

Methyl 2,3,4-Tri-*O*-acetyl-6-*O*-(1′,2′-di-*O*-palmitoyl-*sn*-glycero-3′-phosphoryl)-α-D-glucopyranoside Triethylammonium Salt (14a) and Calcium Salt (14b). A procedure analogous to that described above was employed by utilizing the following quantities of reagents: 1,2-di-*O*-palmitoyl-*sn*-glycerol (8, 1.136 g, 2 mmol), bis(1,2-dimethylethenylene) pyrophosphate (9, 0.564 g, 2 mmol), 6-unprotected glucoside (7, 0.640 g, 2 mmol), and triethylamine 0.56 mL, 6 mmol). The crude phosphotriester 12 was obtained as in the previous experiment and was converted into the triethylammonium salt of the phosphodiester 14a as described (30% yield of 14a based on 1,2-dipalmitoyl-*sn*-glycerol (8)).

The phosphodiester calcium salt 14b was prepared from the corresponding triethylammonium salt 14a as described above.

Methyl 6-*O*-(1′,2′-Di-*O*-palmitoyl-*sn*-glycero-3′-phosphoryl)-α-D-glucopyranoside Triethylammonium Salt (15a) and Calcium Salt (15b). The benzyl-protected phosphodiester triethylammonium salt 13a (0.250 g, 0.21 mmol) was dissolved in chloroform/methanol (1:1) (40 mL), and the solution was subjected to hydrogenolysis (ca. 2 h, 38 psi of H_2) in the presence of 10% Pd/C (0.35 g, Johnson Matthey Inc.). The solution was filtered and evaporated. The residue was dissolved in chloroform/methanol (1:1) (20 mL), and the solution was treated with triethylamine (0.2 mL) to ensure that all the phosphate was present as the triethylammonium salt. The solution was filtered and evaporated. The residue was dissolved in the minimum volume of chloroform/methanol (2:1). The solution was treated with 25× its volume of acetone to precipitate the pure phosphodiester triethylammonium salt 15a, which was filtered and dried (0.171 g, 88% yield of 15a based on 13a).

The conversion of the phosphodiester glucoside triethylammonium salt 15a into the corresponding calcium salt 15b was carried out as described above.

6-*O*-(1′,2′-Di-*O*-palmitoyl-*sn*-glycero-3′-phosphoryl)-D-glucose Triethylammonium Salt (1a), Calcium Salt (1b), and Sodium Salt (1c) by Method A (Methyl Glucoside Route). The methyl glucoside 15a (0.150 g, 0.162 mmol) was dissolved in a mixed solvent: chloroform (7.5 mL), methanol (15 mL), and acetic acid (7.5 mL) at 0 °C. The acetic acid contained 2% trifluoroacetic acid (the overall concentrations of trifluoroacetic acid and glucoside 15a were 0.065 M and 0.0054 M, respectively). The solution was stirred for 12 h at 0 °C and was poured into a mixture of ice and water. The mixture was extracted with chloroform (3 × 100 mL), and the chloroform extract was *very cautiously* washed with 1% aqueous sodium bicarbonate. This step may result in the formation of emulsions, which, if needed, can be broken by means of saturated aqueous NaCl. The extract was washed with water, dried over sodium sulfate, and evaporated. The residue was purified by utilizing 20 g of silica gel packed by means of chloroform into an 18 in. × 0.55 in. column surrounded by a jacket containing an ice-water mixture. Elution was carried out at a fast rate of 3 mL/min with (1) 100 mL of chloroform/methanol (8:1) and (2) 150 mL of chloroform/methanol (2.5:1). Fraction 2 was evaporated. The residue was dissolved in 5 mL of chloroform/methanol (1:2), and the solution was slowly concentrated in a rotoevaporator (30 torr) until crystallization was observed. At this point, acetone (7 mL) was added, and the mixture was kept for 2 h at −20 °C and filtered. The crystalline triethylammonium salt of phosphatidyl-6-glucose (1a) was obtained in 31% yield on the basis of 15a. This salt was converted into the calcium salt (1b) as described above. The sodium salt (1c) was prepared from the triethylammonium salt (1a) by a procedure analogous to that used to prepare the calcium salt, substituting sodium chloride for calcium chloride in the reagent.

2,3,4-Tri-*O*-benzyl-D-glucose (18). A suspension of 2,3,4,6-tetra-*O*-benzyl-D-glucose (16, 3.0 g, 6.09 mmol, Sigma Chemical Co.) in acetic anhydride (25 mL) and concentrated sulfuric acid (0.25 mL) was kept for 10 min at 25 °C.[29] The solution was poured into ice-water, and the mixture was stirred for 2 h and extracted with dichloromethane (3 × 50 mL). The organic phase was washed with sodium bicarbonate and water, dried over sodium sulfate, and evaporated to yield crude 1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzyl-D-glucopyranose (17). This material was dissolved in a mixture of dioxane and 0.5 M, aqueous sulfuric acid (10:1) (22 mL), and the solution was kept for 3 h at reflux temperature. The solution was neutralized with sodium bicarbonate and extracted

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(28) Ramirez, F.; Okazaki, H.; Marecek, J. F.; Tsuboi, H. *Synthesis* **1976**, 819.

(29) (a) Eby, R.; Sondheimer, S. J.; Schuerch, C. *Carbohydr. Res.* **1979**, *73*, 273; (b) Ponpipom, M. M. *Ibid.* **1977**, *59*, 311.

with dichloromethane (2 × 50 mL). The washed and dried organic solution was evaporated to yield the crude product **18**, which was purified (60 g of silica gel; elution with (1) 50 mL of petroleum ether, (2) 500 mL of chloroform, and (3) 250 mL of chloroform/methanol (10:1)). Fraction 3 was evaporated, and the residue was crystallized from chloroform/petroleum ether to give the 2,3,4-protected glucose **18** (1.78 g, 71% yield: mp 90–92 °C (lit.[29] mp 90–91 °C).

2,3,4-Tri-*O*-benzyl-6-*O*-(1′,2′-di-*O*-palmitoyl-*sn*-glycero-3′-phosphoryl)-D-glucose Triethylammonium Salt (20a) and Calcium Salt (20b). A dichloromethane solution (1 mL) of 1,2-di-*O*-palmitoyl-*sn*-glycerol (**8**, 0.284 g, 0.5 mmol) was added to a stirred dichloromethane solution (1 mL) of bis(1,2-dimethylethenylene) pyrophosphate (**9**, 0.141 g, 0.5 mmol) containing triethylamine (0.07 mL, ca. 0.5 mmol). After 3 h at 25 °C, the solution was evaporated to give the cyclic phosphate **10**. A solution of the 2,3,4-protected glucose **18** (0.225 g, 0.5 mmol, dried by evaporation with 3 × 2 mL of pyridine) in dichloromethane (1 mL) was added to a dichloromethane solution (1 mL) of the cyclic phosphate **10** containing triethylamine (0.14 mL, 1 mmol) at 25 °C. After being stirred for 12 h at 25 °C, the solution was put through a short column of silica gel and the column was eluted with ether (300 mL). The eluate was evaporated, and the residue was mixed with pyridine (15 mL), water (15 mL), and triethylamine (0.3 mL) at 25 °C. The turbid mixture was warmed (10 min) to 35 °C and was stirred at 25 °C for 16 h. The solution was evaporated (freeze-dried), and the residue was purified (70 g of silica gel; elution with (1) 350 mL of ether, (2) 160 mL of chloroform/methanol (8:1), (3) 6 mL of the same solvent, and (4) 20 mL of the same solvent). Fraction 4 was evaporated, and the residue was dissolved in chloroform (0.5 mL). The solution was diluted with acetone (10 mL) and kept for 2 h at 0 °C to yield the phosphodiester **20a** (0.31 g, 53% yield of **20a** based on **8**).

The calcium salt of the phosphodiester (**20b**) was prepared as described above.

6-*O*-(1′,2′-Di-*O*-palmitoyl-*sn*-glycero-3′-phosphoryl)-D-glucose Triethylammonium Salt (1a) and Calcium Salt (1b) by Method B (Glucose Route). The triethylammonium salt of the protected phosphodiester (**20a**) (0.109 g, 0.09 mmol) was dissolved in chloroform/methanol (1:1) (30 mL), and the solution was subjected to hydrogenolysis (45 min, 40 psi of H_2) in the presence of 10% Pd/C (0.15 g). The solution was filtered and evaporated. The residue was dissolved in chloroform, and the solution was treated with 2–3 drops of triethylamine and evaporated. The residue was treated with acetone and filtered to yield virtually pure phosphatidyl-6-glucose **1a** (0.074 g, 90% yield).

A sample of the triethylammonium salt **1a** was converted into the calcium salt **1b** as described above. The melting point of this material and the mixture melting point with a sample of calcium salt prepared by method A were 171–173 °C. Both samples had identical R_f values, 0.22 (chloroform/methanol/water (65:25:2)) on TLC. Both samples gave virtually the same value of the specific rotation, $[\alpha]^{20}_D$ 16.5 ± 0.5° (*c* 3.2, chloroform/methanol (2:1)).

1′,2′-Di-*O*-palmitoyl-*sn*-glycero-3′-phosphoryl 2,3,4,6-Tetra-*O*-benzyl-D-glucopyranoside Triethylammonium Salt (23a), Calcium Salt (23b), and Sodium Salt (23c). A dichloromethane solution (1.5 mL) of 2,3,4,6-tetra-*O*-benzyl-D-glucose (**16**, 0.540 g, 1 mmol; previously dried by evaporation from pyridine, 3 × 4 mL) was added to a stirred dichloromethane solution (1 mL) of bis(1,2-dimethylethenylene) pyrophosphate (**9**, 0.282 g, 1 mmol) containing imidazole (0.136 g, 2 mmol). After 2 h at 25 °C, the solution was evaporated to give the cyclic phosphate **21**. 1,2-Di-*O*-palmitoyl-*sn*-glycerol (**8**, 0.568 g, 1 mmol) was introduced into a dichloromethane solution (1 mL) of the above cyclic phosphate preparation, **21**. After being stirred for 24 h at 25 °C, the solution was evaporated. The residue was dissolved in chloroform (2 mL), and the solution was put through a short column of silica gel, which was eluted with ether (350 mL). The eluate was evaporated, and the residue was mixed with pyridine (40 mL), water (40 mL), and triethylamine (0.6 mL). The turbid mixture was stirred at 40 °C for 12 h. The homogeneous solution was evaporated (freeze-dried), and the residue was purified (70 g of silica gel; elution with (1) 500 mL of ether, (2) 190 mL of chloroform/methanol (8/1), (3) 48 mL of the same solvent, and (4) 12 mL of the same solvent). Fraction 3 was evaporated; TLC analysis disclosed the presence of some 1′,2′-di-*O*-palmitoyl-3′-*O*-(1,2-di-*O*-palmitoyl-*sn*-glycerol-3-*O*-phosphoryl)glycerol or "bis(phosphatidic acid)" in this sample of the desired phosphodiester **23a**. The sample was dissolved in chloroform (1.5 mL), and the solution was applied to four precoated silica gel plates (2-mm thick, PLC 60F-254, Merck Cat. No. 5766). The plates were developed with chloroform/methanol/concentrated ammonium hydroxide (30:5:1). The diester **23a** was visualized by UV light, and the band was scraped, and extracted with chloroform/methanol (4:1) (3 × 125 mL). The combined extracts were evaporated, the residue was dissolved in chloroform (0.5 mL), and the solution was diluted with acetone (10 mL). After 6 h at −20 °C, the pure diester **23a** was filtered and dried in vacuum (0.40 g, 32% yield based on 1,2-diacyl-*sn*-glycerol **8**)

The triethylammonium salt **23a** was converted into the calcium salt **23b** and the sodium salt **23c** as described above.

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Registry No. **1a**, 85749-24-2; **1b**, 85749-25-3; **1c**, 85749-26-4; **2**, 97-30-3; **3**, 85749-09-3; **4**, 85749-10-6; **5**, 85749-11-7; **6**, 53008-65-4; **7**, 7432-72-6; **8**, 30334-71-5; **9**, 55894-94-5; **10**, 65137-73-7; **11**, 85749-12-8; **12**, 85749-16-2; **13a**, 85749-14-0; **13b**, 85749-15-1; **14a**, 85749-18-4; **14b**, 85749-19-5; **15a**, 85749-21-9; **15b**, 85749-22-0; **16**, 38768-81-9; **17**, 59433-13-5; **18**, 50272-02-1; **20a**, 85749-28-6; **20b**, 85749-29-7; **21**, 85749-30-0; **23a**, 85749-32-2; **23b**, 85749-33-3; **23c**, 38768-87-5; **24**, 53115-76-7.