Synthesis of Phospholiponucleosides

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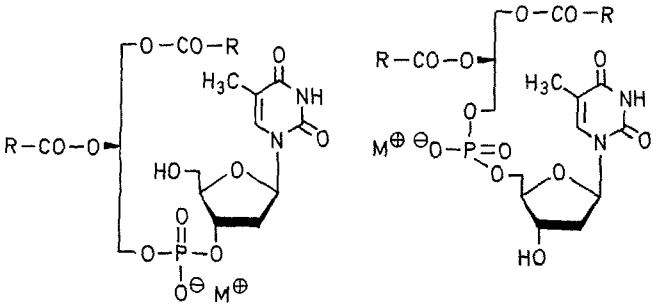
The term liponucleotides has been applied to certain coenzymes, e.g. cytidine diphosphate 1,2-diacyl-*sn*-glycerol, [$(R^1O)(HO)P_\beta(O)OP_\alpha(O)(OH)(OR^2)$, R^1 = 1,2-diacyl-*sn*-glyceryl-3; R^2 = cytidyl-5′]. These coenzymes are obligatory intermediates in the biosynthesis of important phospholipid components of biological membranes, e.g. phosphatidylglycerol[1,2] [$(R^1O)(R^3O)P(O)OH$, R^1 = 1,2-diacyl-*sn*-glyceryl-3; R^3 = *sn*-glyceryl-1]. The phospholipids result from a nucleophilic displacement by the corresponding polar head group alcohol, e.g. glycerol (R^3OH), at one of the two phosphorus atoms (P_β) of the pyrophosphate coenzyme, with elimination of cytidine 5′-monophosphate under enzymatic catalysis.

The present communication describes the nonenzymatic synthesis of two phosphatidylnucleosides, **1** and **2**. These compounds have the function $(R^1O)(R^4O)P(O)OH$ [R^1 = 1,2-diacyl-*sn*-glyceryl-3; R^4 = 2′-deoxythymidyl-3′ or 2′-deoxythymidyl-5′], respectively, and are therefore, phospholiponucleosides or liponucleotides properly. To our knowledge, compounds of this type, which are functionally related to the phospholipids rather than to the coenzymes, have not been previously described[7]. The phase transition characteristics of aqueous dispersions of **1** and **2**, and the possibility that they may form bilayers and vesicles[3], are under investigation.



1a R = $C_{13}H_{27}$, $M^\oplus$ = $(C_2H_5)_3\overset{\oplus}{N}H$

b R = $C_{13}H_{27}$, $M^\oplus$ = 0.5 $Ca^{2\oplus}$

2a R = $C_{13}H_{27}$, $M^\oplus$ = $(C_2H_5)_3\overset{\oplus}{N}H$

b R = $C_{13}H_{27}$, $M^\oplus$ = 0.5 $Ca^{2\oplus}$

The synthesis of the phosphatidyl-3′-nucleoside **1** is shown in Scheme **A**. The cyclic enediol pyrophosphate[4] **3** establishes both P—O bonds of the phosphotriester **8** without the need for additional activation. Removal of the protective group (R^1) at position C-5′ of the triester **8** leads to triester **9**, which is the first intermediate subject to purification. Removal of the protective group (acetoinyl = 3-oxo-2-butyl) at the phosphate ester function of **9** yields the desired liponucleotide in the form of its triethylammonium salt, **1a**. This salt is converted into calcium salt, **1b**, for phase transition studies[3].

The synthesis of the phosphatidyl-5′-nucleoside **2** is shown in Scheme **B**, and involves a different strategy. Now the first P—O bond is established at the diglyceride stage, and the second P—O is formed at the unprotected nucleoside stage. This scheme takes advantage of the selective attack by a primary alcohol (C-5′—OH of **11**) on the cyclic phosphotriester **10**, in the presence of an unprotected secondary alcohol (C-3′—OH of **11**). The triester **12** is the first intermediate subject to purification in this scheme. This step is followed by removal of the phosphate-protective group to give the desired liponucleotide as triethylammonium salt **2a**, and from it, the corresponding calcium salt, **2b**.

Synthesis of Phospholiponucleosides:

All reactions involving enediol cyclophosphoryl derivatives are carried out under anhydrous conditions. The nucleosides, **4** and **11**, are dehydrated by repeated evaporations from dry pyridine. Triethylamine and dichloromethane are distilled from sodium and phosphorus pentox-

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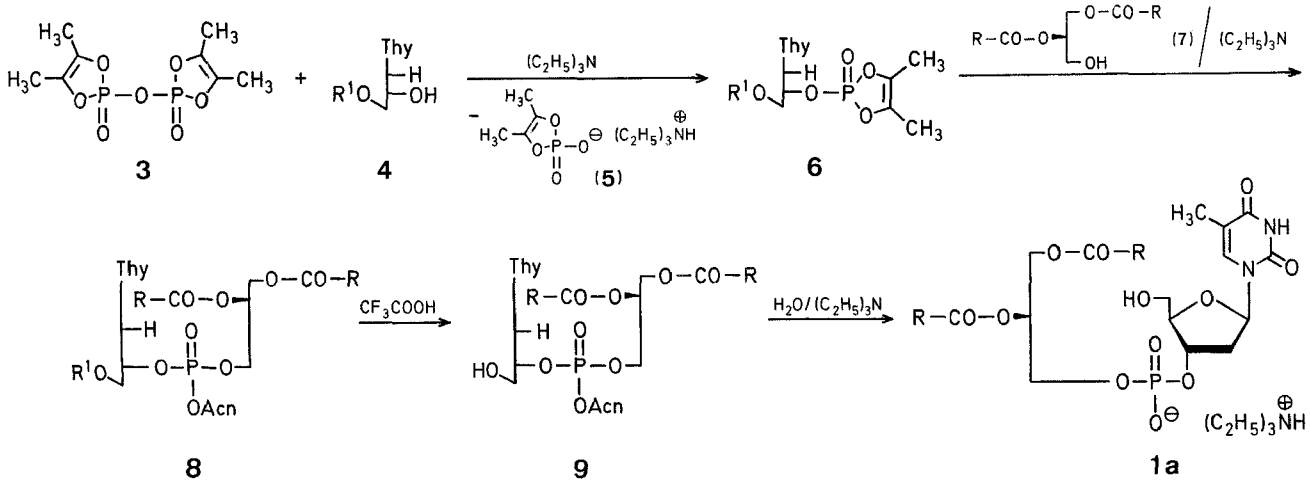
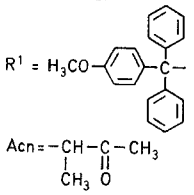
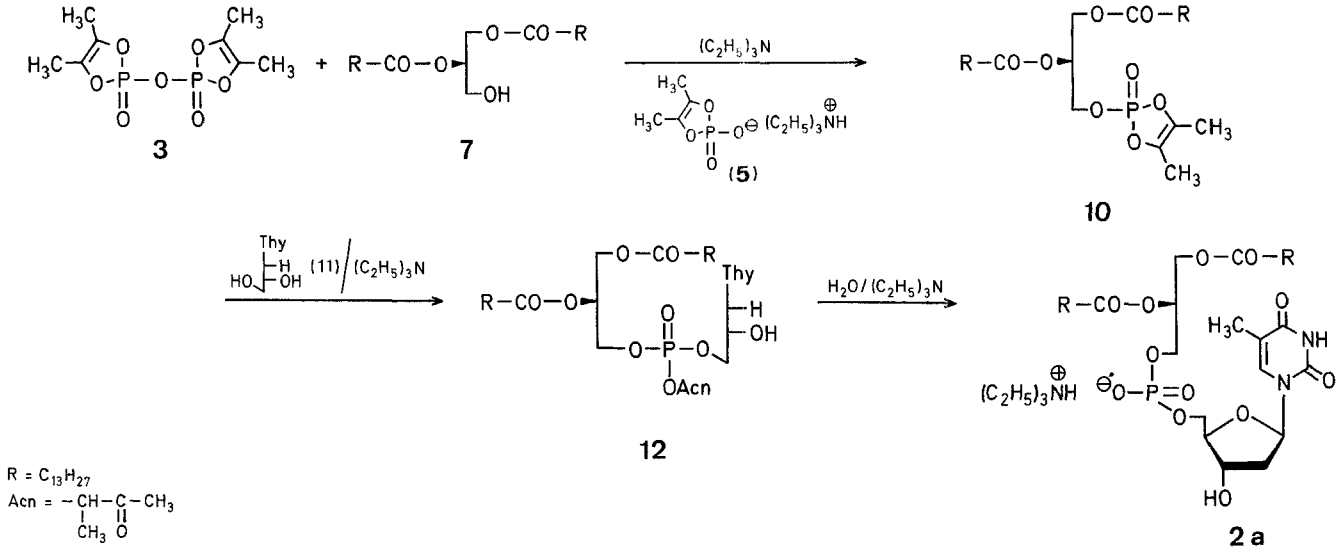


R = $C_{13}H_{27}$



Scheme A

dichloromethane solution (1 ml) of **6** containing 2 mol-equivalents of triethylamine (0.28 ml), at 25 °C. After 10 min at 25 °C, 16 h at 0 °C, and 2 h at 25 °C, the solution is evaporated to give **8**. A solution of **8** (1.00 g, 0.86 mmol) in dichloromethane (150 ml) is cooled to 0 °C, and is added to a stirred 0.026 molar dichloromethane solution of trifluoroacetic acid (650 ml) at 0 °C. After 30 min at 0 °C, this solution is treated with pyridine (18 ml) in dichloromethane (60 ml). Product **9** is isolated in pure form by column chromatography on silica gel [elu-



R = $C_{13}H_{27}$

Scheme B

ide, respectively. The progress of all reactions is followed by analytical T.L.C. on precoated silica gel plates (0.25 mm thick; HPTLC 60F-254, Merck Cat. No. 5760). Purifications are carried out by preparative T.L.C. on 20 × 20 cm precoated silica gel plates (2 mm thick, PLC 60F-254, Merck Cat. No. 5766). Solvents: *A*, chloroform/methanol, 9/1; *B*, chloroform/methanol, 4/1; *C*, chloroform/methanol/conc. NH_4OH, 30/15/1; *D*, ethyl acetate/acetone/water, 7/3/1; (v/v). Samples are dried for 18 h at 20 °C/0.2 torr prior to microanalyses. All evaporations are performed under vacuum. The esters and triethylammonium salts prepared are too hygroscopic for microanalyses.

3′-*O*-(1,2-Di-*O*-myristoyl-*sn*-glycero-3-phosphoryl)-2′-deoxythymidine (1):

A solution of 5′-*O*-*p*-methoxytritylthymidine[5] (**4**; 0.514 g, 1 mmol) in dichloromethane (2 ml) is added to a stirred dichloromethane solution (1 ml) of bis[2-butene-2,3-diyl] pyrophosphate[4] (**3**; 0.282 g, 1 mmol), containing triethylamine (0.14 ml) at 25 °C. After 2 h at 25 °C, the solution is evaporated to give **6**. A solution of 1,2-di-*O*-myristoyl-*sn*-glycerol[6] (**7**; 0.512 g, 1 mmol) in dichloromethane (2 ml) is added to a

tion with solvent *A*] followed by preparative T.L.C. [elution with solvent *D*; extraction from the silica by solvent *B*]; yield: 52% (based on nucleoside **4**); R_f: 0.63 (solvent *D*).

Compound **9** (0.320 g, 0.36 mmol) is mixed with pyridine (4 ml), water (4 ml), and triethylamine (0.25 ml) at 25 °C. The mixture is stirred for 48 h at 25 °C and is freeze-dried. Product **1a** is obtained in pure form by preparative T.L.C. [elution with solvent *B*; extraction with chloroform/methanol, 2/1; precipitation as free-flowing powder from minimum of chloroform upon addition of acetone]; yield: 60% (based on triester **9**); m.p. 195–196 °C (shrinks at ~160 °C); R_f: 0.65 (solvent *C*).

The conversion of the triethylammonium salt, **1a**, into the calcium salt, **1b**, is performed as follows. A solution of **1a** (0.116 g) in chloroform/methanol, 2/1 (30 ml) is mixed with chloroform/methanol/2 molar aqueous calcium chloride, 3/48/47 (20 ml). The upper phase is discarded, and the procedure is repeated two additional times with the lower phase. The final lower phase is washed twice with chloroform/methanol/water, 3/48/47 (20 ml), and evaporated. The residue is kept for 18 h/0.2 torr to give **1b**; yield: 0.098 g (94% based on **1a**); $[\alpha]_D^{25}$: +8.5° (*c* 4.91, chloroform/methanol, 2/1).

$C_{82}H_{144}CaO_{13}N_4P_2 \cdot 2H_2O$		calc.	C 57.66	H 8.73	Ca 2.35
(1708.1)		found	57.69	8.68	2.47

U.V. (chloroform/methanol, 2/1): $\lambda_{max} = 268$ nm ($\varepsilon = 18500$).

5'-*O*-(1,2-Di-*O*-myristoyl-*sn*-glycero-3-phosphoryl)-2'-deoxythymidine (2):

A dichloromethane solution (2 ml) of 1,2-di-*O*-myristoyl-*sn*-glycerol (**7**; 0.512 g, 1 mmol) is added to a stirred dichloromethane solution (1 ml) of bis[2-benzene-2,3-diyl]pyrophosphate[4] (**3**; 0.282 g, 1 mmol), containing triethylamine (0.14 ml), at 25°C. After 2 h at 25°C, the solution is evaporated to give **10**. A solution of compound **10** (0.644 g, 1.0 mmol) in dichloromethane (4.5 ml), containing triethylamine (0.28 ml) is added to a solution of thymidine (**11**; 0.242 g, 1.0 mmol) in dimethylformamide (1 ml), at 25°C. After 10 min at 25°C, 15 h at 0°C, and 4 h at 25°C, the solution is evaporated to give product **12**. Compound **12** is obtained in pure form by preparative T.L.C. [elution with solvent *C*; extraction with solvent *B*]; yield: 40% (based on diglyceride **7**); R_f 0.65 (solvent *D*).

Compound **12** (0.250 g, 0.3 mmol) is mixed with pyridine (4 ml), water (4 ml), and triethylamine (0.25 ml) at 25°C. The mixture is stirred for 48 h at 25°C and is freeze-dried. Product **2a** is obtained in pure form by preparative T.L.C. [elution with solvent *B*; extraction with chloroform/methanol 2/1; precipitation as free-flowing powder from minimum of chloroform upon addition of acetone]; yield: 70% (based on triester **12**); m.p. 200–202°C (sinters at ~120°C); R_f: 0.23 (solvent *B*), 0.55 (solvent *C*).

The conversion of the triethylammonium salt, **2a**, into the calcium salt **2b** is performed as before; yield: 90%; $[\alpha]_D^{25}$: +5.7° (*c* 4.41, chloroform/methanol, 2/1).

$C_{82}H_{144}CaO_{13}N_4P_2 \cdot 2H_2O$		calc.	C 57.66	H 8.73	Ca 2.35
(1708.1)		found	57.98	8.85	2.33

U.V. (chloroform/methanol, 2/1): $\lambda_{max} = 268$ nm ($\varepsilon = 18900$).

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