Chemoenzymatic Synthesis of Lysophosphatidylnucleosides†

Rosa Chillemi, Domenico Russo, and Sebastiano Sciuto*

Dipartimento di Scienze Chimiche, Università di Catania, viale A. Doria 6, 95125 Catania, Italy

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5′-*O*-Lysophosphatidylnucleosides [5′-*O*-(1-*O*-acyl-*sn*-glycero-3-phosphoryl)nucleosides] were obtained by a two-step chemoenzymatic synthesis. 5′-*O*-(*sn*-Glycero-3-phosphoryl)nucleosides (5′-GPNs) were first prepared from a phosphoramidite of 1,2-*O*-isopropylidene-*sn*-glycerol and appropriately protected nucleosides, applying the phosphoramidite methodology on the solid phase or in solution. In a following step, the regioselective acylation at the C-1 hydroxyl of the glycerol moiety of 5′-GPNs was achieved by a lipase-catalyzed transacylation with activated fatty acid esters in organic solvent. Some deoxyribo- and ribonucleosides, as well, were converted into the corresponding lysophosphatidyl derivatives utilizing either saturated or unsaturated fatty acid esters with different length alkyl chains. The synthesis was also applied to the preparation of *O*-(1-*O*-palmitoyl-*sn*-glycero-3-phosphoryl) conjugates of Acyclovir and AZT, of potential pharmacological interest.

Introduction

Liponucleoside conjugates are compounds of high significance in some areas of medicinal chemistry, showing antineoplastic[1−3] and antiviral activity.[4,5] The presence of a lipophilic moiety in the molecular structure of these compounds allows them to permeate intact cells better than free nucleosides, and in effect, the conjugation of hydrophobic groups to pharmacologically active nucleosides has shown its efficacy at markedly improving their activity.[3,6,7]

One of the most interesting lipophilic groups useful in this regard is the phosphatidyl group, on account of its widespread occurrence in the molecular structure of many lipid constituents of cell membranes. Accordingly, in the past decade, some phosphatidylnucleosides were prepared chemically, and the first 5′-phosphatidylthymidine was synthesized in 1982 by the phosphotriester method.[8] However, the method was difficult to apply to the synthesis of those 5′-phosphatidylnucleosides having the base moiety other than thymine or which have ribose or arabinose as the sugar moiety.[9] Therefore, a few years later, Shuto and co-workers proposed a novel method for preparing 5′-*O*-phosphatidylnucleosides from phosphatidylcholines and nucleosides in a one-step phospholipase-D-catalyzed transphosphatidylation.[10]

To our knowledge, the closely related 5′-*O*-lysophosphatidylnucleosides, which differ from the phosphatidyl ones in that only one fatty acid residue is attached to their glycerol moiety, have not been synthesized so far. In principle, these compounds could be prepared following the above synthetic approach,[10] using lysophosphatidylcholines and nucleosides. However, this way may greatly depend on the availability and/or expensiveness of the relevant lysophosphatidylcholines and phospholipase-D.

So, we thought it useful to search for a new general synthetic method for preparing these compounds, and we report here a chemoenzymatic synthesis of a variety of 5′-*O*-lysophosphatidyl derivatives of nucleosides, including some nucleoside analogues of pharmacological interest.

Results and Discussion

The direct chemical attachment of a lysophosphatidyl group to a nucleoside, following the phosphoramidite chemistry, suffers from the lability of the carboester bond in the lysophosphatidyl group under the strongly basic conditions routinely used to remove classical amino protecting groups of nucleosides. To overcome this problem, we used the phosphoramidite procedure to first attach a suitably protected glycerophosphoryl group at the 5′-OH of a protected nucleoside. Then, after removal of all protecting groups, the primary alcoholic function of the conjugated glycerol moiety was esterified using activated fatty acyl donors in the presence of lipases working in organic solvents (Schemes 1 and 2).

Synthesis of 5′-*O*-Lysophosphatidyl-dC, -dG, -dA, and -dT. We first achieved the synthesis of 5′-*O*-lysophosphatidyl derivatives of the four deoxynucleosides normally occurring in DNA, namely 5′-*O*-(1-*O*-palmitoyl-*sn*-glycero-3-phosphoryl) derivatives of 2′-deoxycytidine, -guanosine, -adenosine, and -thymidine (**7**−**10**).

5′-*O*-(*sn*-Glycero-3-phosphoryl)-2′-deoxynucleosides (5′-GPdNs) (**2**−**5**), which would have been subsequently used

† Dedicated to Professor Mario Piattelli on the occasion of his 70th birthday.

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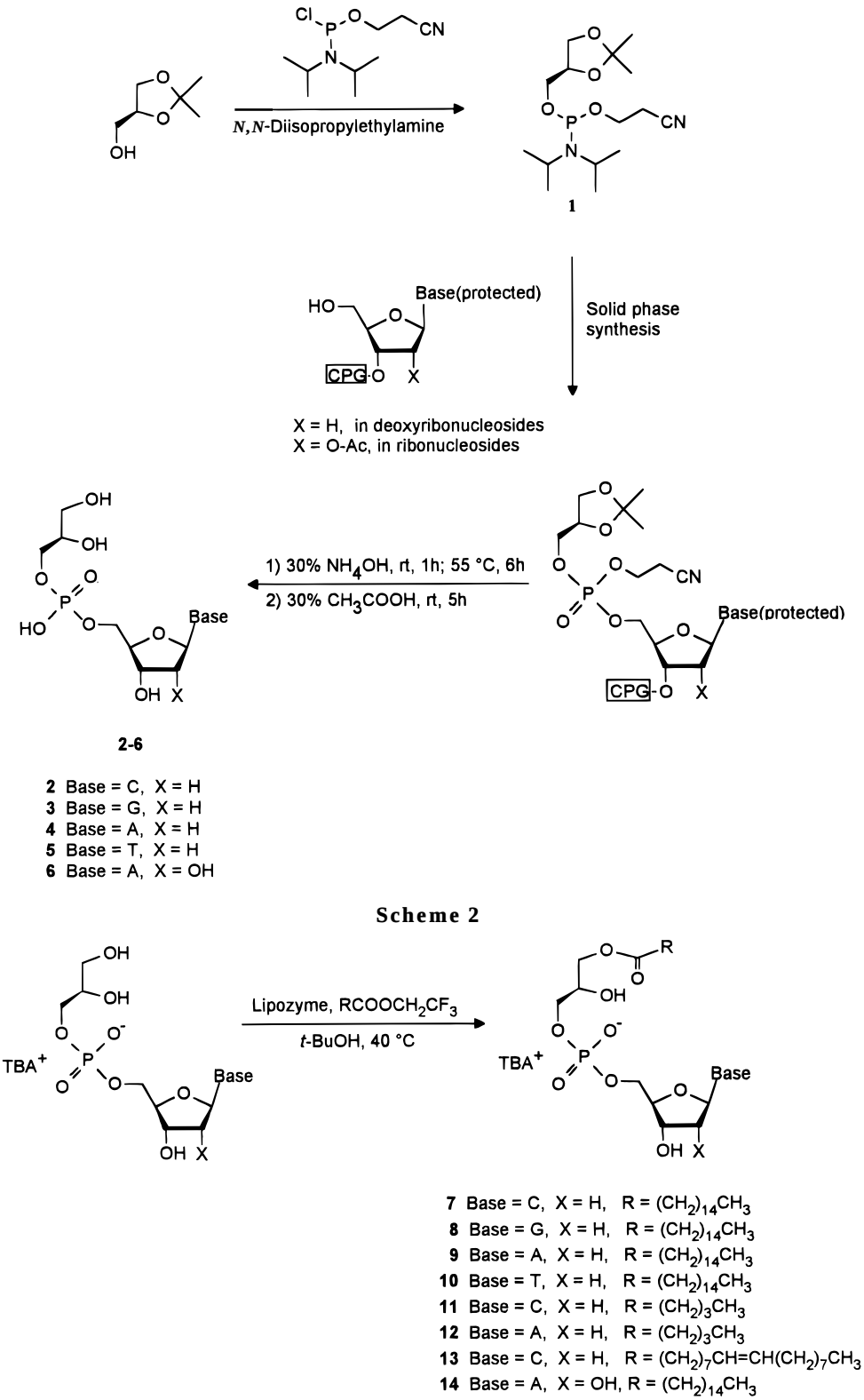
Scheme 1



2 Base = C, X = H
3 Base = G, X = H
4 Base = A, X = H
5 Base = T, X = H
6 Base = A, X = OH

Scheme 2

7 Base = C, X = H, R = $(CH_2)_{14}CH_3$
8 Base = G, X = H, R = $(CH_2)_{14}CH_3$
9 Base = A, X = H, R = $(CH_2)_{14}CH_3$
10 Base = T, X = H, R = $(CH_2)_{14}CH_3$
11 Base = C, X = H, R = $(CH_2)_3CH_3$
12 Base = A, X = H, R = $(CH_2)_3CH_3$
13 Base = C, X = H, R = $(CH_2)_7CH{=}CH(CH_2)_7CH_3$
14 Base = A, X = OH, R = $(CH_2)_{14}CH_3$

as a substrate for the enzymatic acylation, were synthesized by accomplishing on the solid phase a coupling cycle between the relevant CPG(controlled pore glass)-3′-linked protected 2′-deoxynucleoside and 1,2-*O*-isopropylidene-*sn*-glycero-3-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (**1**). The required **1** was prepared from 1,2-*O*-isopropylidene-*sn*-glycerol and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (Scheme 1). The stereochemistry at the chiral center of the glycerol moiety of **1** was chosen the same as that normally found in naturally occurring phospholipids.

After the ammonia treatment, usually employed to detach deoxynucleotides from CPG and to remove all base and phosphate protecting groups, the desired 5′-GPdNs were obtained by removing the isopropylidene group by acetic acid hydrolysis, followed by HPLC. 5′-GPdN structures were then confirmed on the basis of their spectroscopic properties.

Table 1. Influence of the Alkyl Chain Length of Acyl Donors on Enzymatic Acylation of 2 and 4

substrate	acyl donor[a]	product	yield[b] (%)
2	valerate	**11**	75
2	palmitate	**7**	37
4	valerate	**12**	82
4	palmitate	**9**	40

[a] As trifluoroethyl ester. [b] After 48 h incubation with Lipozyme in *tert*-butyl alcohol at 40 °C.

5′-GPdNs are insoluble in the organic solvents commonly used for enzymatic acylations, and therefore, they were converted into the corresponding tetrabutylammonium (TBA) salts, which show a slight solubility in chloroform but sufficient solubility in *tert*-butyl alcohol. The regioselective acylation of 5′-GPdNs was then achieved in the latter solvent by treatment with trifluoroethyl palmitate in the presence of immobilized lipase from the fungus *Rhizomucor miehei* (Lipozyme) as enzyme catalyst (Scheme 2). The treatment was prolonged for 2 days at the optimal temperature of 40 °C, and each reaction course was followed by monitoring by HPLC the rising amount of the relevant 5′-*O*-lysophosphatidyldeoxynucleoside.

Acylated compounds were finally purified by semipreparative HPLC and their structure assigned on the basis of a detailed examination of their spectroscopic properties.

It is worth noting that, when parallel experiments were carried out under the same experimental conditions but in the absence of the enzyme, no acylation took place.

FAB-MS(−) spectra of compounds **7**−**10** show the expected molecular ion $(M - H)^-$. In the ^{13}C NMR spectra of **7**−**10**, the resonances of the C-1 and C-2 carbon atoms of the 3-phosphoryl-*sn*-glycerol moiety shift downfield ($\Delta\delta = 1.3 \pm 0.1$ ppm) and upfield ($\Delta\delta = -3.7 \pm 0.1$ ppm), respectively, as compared to the corresponding resonances of the relevant 5′-GPdN. On account of the α- and the *β*-effect of the acylation, these results clearly indicate that 5′-GPdNs undergo regioselective acylation at the primary hydroxyl group of the glycerol moiety. Accordingly, in the ^1H NMR spectra of these compounds, the C-1 methylene protons of the 3-phosphoryl-*sn*-glycerol moiety shift downfield ($\Delta\delta = 0.56$ and 0.65 ± 0.02 ppm for pyrimidine and purine nucleotides, respectively), in comparison with the spectra of the pertinent 5′-GPdNs.

To investigate the influence of the alkyl chain length of activated fatty acids on the enzymatic acylation of 5′-GPdNs, compounds **2** and **4** were reacted with trifluoroethyl valerate in the presence of Lipozyme. In both cases, the relevant 5′-*O*-(1-*O*-valeryl-*sn*-glycero-3-phosphoryl)-2′-deoxynucleoside (**11** and **12**) was obtained in much higher yields than the longer palmitoyl derivatives **7** and **9** (Table 1), thus indicating that the alkyl chain length plays a role in determining the extent of the enzymatic acylation.

In parallel experiments, enzymatic acylations of **2** with trifluoroethyl valerate were also carried out using lipases from *Candida antarctica*, *Candida cylindracea*, and *Pseudomonas cepacea*. *C. antarctica* lipase worked less efficiently than Lipozyme, while the use of the other two lipases was unsuccessful (Table 2).

The possibility of preparing 5′-*O*-lysophosphatidyldeoxynucleosides bearing an unsaturated fatty acid residue was also tested by reacting trifluoroethyl oleate and the TBA salt of **2** in the presence of Lipozyme. As expected,

Table 2. Acylation of 2 by Different Enzymes Using Trifluoroethyl Valerate as Acyl Donor

lipase source	yield (%) of **11**[a]
Rhizomucor miehei (Lipozyme)	75
Candida antarctica	42
Candida cylindracea	0
Pseudomonas cepacea	0

[a] After 48 h incubation in *tert*-butyl alcohol at 40 °C.

5′-*O*-(1-*O*-oleoyl-*sn*-glycero-3-phosphoryl)-2′-deoxycytidine (**13**) was obtained and identified on the basis of its spectroscopic properties.

Finally, the above-described procedure was also applied to prepare 5′-*O*-lysophosphatidylnucleosides bearing ribose instead of 2′-deoxyribose as the sugar moiety, the synthesis of 5′-*O*-(1-*O*-palmitoyl-*sn*-glycero-3-phosphoryl)-adenosine (**14**) providing a typical example. To this purpose, 5′-*O*-(*sn*-glycero-3-phosphoryl)adenosine (**6**) was first synthesized by the above-described synthetic route starting from **1** and a CPG-linked protected adenosine usually employed in the RNA synthesis. The TBA salt of **6** was then reacted with trifluoroethyl palmitate in the presence of Lipozyme to give the desired **14**.

CD Spectroscopic Features. Yanagawa et al. reported that 5′-*O*-(1,2-*O*-dipalmitoyl-*sn*-glycero-3-phosphoryl)-2′-deoxycytidine spontaneously assembles to form circular and linear helical strands.[11] The authors also found[12] that the CD spectrum of an aqueous solution of the corresponding dimyristoyl derivative, which was shown to self-assemble forming superhelical strands, displays at 25 °C a weak single peak with a maximum at 274 nm, while a drastic increase in the positive Cotton effect can be observed after the solution is cooled at 15 °C. This observation, in addition to the reversibility of the cooling profile upon heating, indicated that the compound has essentially the molecular characteristics of helicity, which is stabilized by both hydrophobic interactions between the long alkyl-chain moieties and stacking between the nucleic acid bases.

On the basis of these reports, we thought it interesting to examine the CD spectrum of an aqueous solution of 5′-*O*-(1-*O*-palmitoyl-*sn*-glycero-3-phosphoryl)-2′-deoxycytidine (**7**), recorded under the same experimental conditions used by Yanagawa. The CD spectrum of **7**, which was quite superimposable on that of deoxycytidylic acid (Figure 1), did not show any drastic variation in the Cotton effect even by cooling to 5 °C. This result indicates that, in the experimental conditions used, compound **7** does not self-assemble, and it would thus follow that the observed ability of phosphatidyldeoxynucleosides[11−13] to form supramolecular structures strictly depends on the contemporary presence of the two long alkyl chains in their molecules.

Synthesis of *O*-Lysophosphatidyl Derivatives of Nucleosides of Pharmacological Interest. In the second part of our work, we tested the possibility of applying the above chemoenzymatic strategy for synthesizing lysophosphatidyl derivatives of pharmacologically active nucleoside analogues. Because of their wide use in current antiviral therapy, 3′-azido-2′,3′-dideoxythymi-

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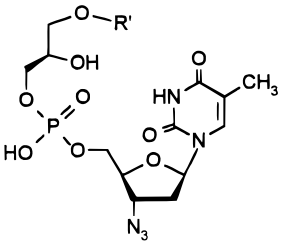
dine (AZT) and acycloguanosine (Acyclovir) were chosen for this purpose. AZT has been reported to be of marked benefit in the treatment of AIDS and AIDS-related complex,[14] and it remains one of the few clinically approved drugs against HIV infection. Acyclovir is an acyclic nucleoside analogue that has shown a potent antiviral activity, and it is known to inhibit the replication of *Herpes simplex* virus (HSV).

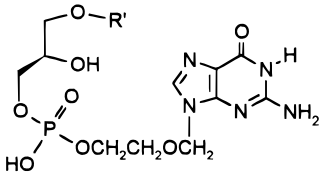
Since it is not possible to prepare CPG-linked Acyclovir or AZT suitable for solid-phase synthesis, the conversion of these compounds into the corresponding glycerophosphoryl derivatives was achieved by applying the phosphoramidite methodology in solution.

Considering that AZT has thymine as its base moiety and only one hydroxy group at the C-5′ of the sugar moiety, no protecting group was necessary. AZT was then reacted with **1** following the usual phosphoramidite sequency, giving the corresponding 5′-*O*-glycerophosphoryl derivative **15**. The subsequent lipase-catalyzed acylation with trifluoroethyl palmitate afforded the desired 5′-*O*-(1-*O*-palmitoyl-*sn*-glycero-3-phosphoryl)AZT (**16**).



15 R′ = H
16 R′ = C(=O)–$(CH_2)_{14}CH_3$

In the case of Acyclovir, the presence in the molecule of guanine as the base moiety has required a preliminary protection of the amino group. To this end, N^2-isobutyryl-Acyclovir (**17**) was first synthesized, which was then converted into the corresponding glycerophosphoryl derivative **18**, following the above-described procedure. Further reaction with trifluoroethyl palmitate in the presence of Lipozyme produced the desired *O*-(1-*O*-palmitoyl-*sn*-glycero-3-phosphoryl)Acyclovir (**19**).



18 R′ = H
19 R′ = C(=O)–$(CH_2)_{14}CH_3$

It has been reported that some HSVs are resistant to treatment with Acyclovir; therefore, some efforts have been made to synthesize Acyclovir derivatives [5,15] that can really be active against these viruses; one of these derivatives, namely Acyclovir diphosphate dimyristoylglycerol, has been recently proposed [5] as a useful prodrug in treating thymidine kinase deficient mutant and wild-type strains of HSV. It is not unlikely that the structurally related compound **19** may exert similar pharmacological properties.

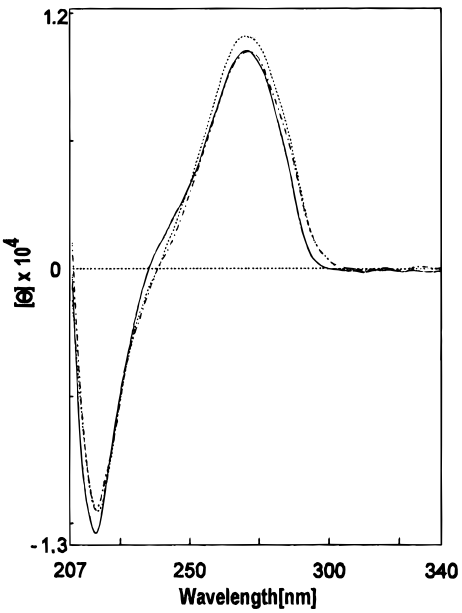


Figure 1. CD spectra of **7** at room temperature (—·—) and at 5 °C (···), superimposed to that of deoxycytidylic acid (—) run at room temperature. The concentration was 1.2×10^{-4} M in 50 mM Tris-HCl buffer, pH 8.

Experimental Section

General Methods. NMR spectra were recorded at 200 MHz (^{1}H) and 50 MHz (^{13}C). The chemical shifts are reported as δ (ppm) referenced to the following: (a) TMS as the internal standard, for experiments in $CDCl_3$, CD_3OD, and C_6D_6; (b) the residual HOD signal (δ 4.82 ppm), for ^{1}H experiments in D_2O; and (c) the signal of appropriately added CD_3OD (δ 49.0 ppm) for ^{13}C experiments in D_2O. Definitive assignments of individual ^{13}C resonances were supported by DEPT experiments and analysis of ^{13}C—^{31}P coupling constants, while ^{1}H—^{1}H—COSY and/or 1D decoupling experiments were carried out for ^{1}H resonance assignments. High-resolution fast atom bombardment (HRFAB) spectra were obtained using glycerol as matrix. 5′-GPdNs were synthesized on a Cyclone DNA synthesizer. Column chromatography was performed on silica gel (63—200 μm), and TLC was carried out on silica gel 60 F_{254} precoated glass plates (0.25 mm). HPLC was performed on a chromatograph equipped with UV detector set at 260 nm; Lichrospher-100 ODS (5 μm; 4 × 250 mm) and Ultrasphere ODS (5 μm; 10 × 250 mm) columns were used for analytical and semipreparative runs, respectively. Modified nucleosides dissolved in water or methanol were spectroscopically quantified by attributing them the molar extinction coefficients reported in the literature for the relevant 5′-nucleotides. CD spectra were recorded on a thermoprogrammable spectropolarimeter with a step size of 0.4 nm, bandwidth of 2 nm, and signal averaging time of 1.0 s. Measurements were performed in 1 cm path length cuvettes, repeating each scan three times.

All the solvents and reagents were commercially purchased and of reagent quality. Some solvents were freshly dried by distillation over the following drying agents and stored under nitrogen: CaH_2 (triethylamine), $CaCl_2$ (petroleum ether), K_2CO_3 (*t*-BuOH and dichloromethane), and Na (diethyl ether).

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Valeric, palmitic, and oleic acid trifluoroethyl esters were prepared according to a reported procedure,[16] and their purity was checked on the basis of their chromatographic and spectroscopic properties.

Lipases from *Rhizomucor miehei* (Lipozyme, immobilized), *C. antarctica* (immobilized), *P. cepacea*, and *C. cylindracea* were obtained from Novo-Nordisk, Copenhagen, Denmark.

1,2-*O*-Isopropylidene-*sn*-glycero-3-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (1). Synthesis of **1** was carried out by a slight modification of McBride and Caruthers' procedure.[17] 1,2-*O*-Isopropylidene-*sn*-glycerol (196 mg, 1.48 mmol) was dissolved in a dried solution of *N*,*N*-diisopropylethylamine (0.860 mL, 4.93 mmol) in CH_2Cl_2 (5 mL), and then 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (544 mg, 2.3 mmol) was added to the magnetically stirred solution. After 15 min, EtOAc (100 mL, prewashed with saturated aqueous $NaHCO_3$) was added to the mixture, and the resulting solution was extracted with 100 mL of saturated aqueous $NaHCO_3$. Concentration of organic layer in vacuo and liquid chromatography of the residue on silica gel (petroleum ether/diethyl ether/*t*-BuOH/triethylamine 85:12:2:1) afforded compound **1** as a colorless oil (90% yield): R_f 0.40; ^{1}H NMR (C_6D_6) δ 4.12 (m, 1H, H-2 of glycerol), 3.85−3.73 (partially overlapped multiplets, 2H, H-3 of glycerol), 3.72−3.53 (partially overlapped multiplets, 2H, H-1 of glycerol), 3.52−3.44 (partially overlapped multiplets, 2H, Me_2CH), 3.35−3.21 (partially overlapped multiplets, 2H, OCH_2CH_2CN), 1.76−1.63 (partially overlapped multiplets, 2H, OCH_2CH_2CN), 1.40 and 1.27 (s, 3H each, Me_2C), 1.09, 1.07 and 1.05 (d, J = 6.9, 6.5, 6.5 Hz, respectively, 12H, ratio 2:1:1, Me_2CH); ^{13}C NMR (C_6D_6) δ 117.5 (CN), 109.4 (Me_2C), 75.6 (d, J_{CCOP} = 7.6 Hz, C-2 of glycerol), 67.0 (C-1 of glycerol), 64.7 (d, J_{COP} = 15.1 Hz, C-3 of glycerol), 58.7 (d, J_{COP} = 18.2 Hz, OCH_2CH_2CN), 43.3 (d, J_{CNP} = 12.3 Hz, Me_2CH), 27.1 and 25.5 (Me_2C), 24.6 and 24.5 (Me_2CH), 20.0 (d, J_{CCOP} = 6.0 Hz, OCH_2CH_2CN); EI-MS (70 eV, 210 °C) *m*/*z* 332 (M^+, 3.5), 317 (11), 262 (10), 232 (18), 219 (100), 203 (53), 201 (38), 174 (71).

General Procedure for the Synthesis of 5′-*O*-(*sn*-Glycero-3-phosphoryl)nucleosides (2−6). Compounds **2**−**5** were synthesized by accomplishing on Cyclone DNA synthesizer a single coupling cycle (2 × 15 μmol scale) between the appropriate 3′-CPG- linked protected nucleoside (5′-*O*-(4,4′-dimethoxytrityl)-N^4-benzoyl-2′-deoxycytidine, 5′-*O*-(4,4′-dimethoxytrityl)-N^2-isobutyryl-2′-deoxyguanosine, 5′-*O*-(4,4′-dimethoxytrityl)-N^6-benzoyl-2′-deoxyadenosine, 5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxythymidine), and compound **1**. After detachment of the condensation products from the CPG column by treatment with concentrated ammonia at room temperature, the ammonia solution was kept at 55 °C for 6 h (this step was omitted for **5**) and then taken to dryness in vacuo. The residue was dissolved in 30% aqueous AcOH and allowed to stand at room temperature for 5 h. After evaporation of the solvent, the totally deprotected product was purified by HPLC on a reversed-phase column eluting with a gradient of CH_3CN in 0.1 M triethylammonium acetate (pH 7.0) from 0 to 20% in 30 min, at a flow rate of 2.5 mL min^{-1}. Pure compounds were obtained as colorless oils. Compound **6** was prepared starting from 3′(or 2′)-CPG-linked 2′(or 3′)-*O*-acetyl-5′-*O*-(4,4′-dimethoxytrityl)-N^6-benzoyladenosine and **1** by running the above general procedure, since the acetyl group at the sugar moiety was removable under the ammonia treatment used to free the base amino function.

5′-*O*-(*sn*-Glycero-3-phosphoryl)-2′-deoxycytidine (2): 86% yield; ^{1}H NMR (D_2O) δ 7.91 (d, $J_{6,5}$ = 7.6 Hz, 1H, H-6), 6.27 (dd, $J_{1',2'}$ = 6.9 Hz, $J_{1',2''}$ = 6.2 Hz, 1H, H-1′), 6.10 (d, $J_{5,6}$ = 7.6 Hz, 1H, H-5), 4.53 (ddd, $J_{3',2'}$ = 6.2 Hz, $J_{3',2''}$ = 3.7 Hz, $J_{3',4'}$ = 3.3 Hz, 1H, H-3′), 4.16 (m, 1H, H-4′), 4.07 (m, 2H, H-5′), 3.93−3.75 (overlapped multiplets, 3H, H-3 and H-2 of glycerol), 3.62 (dd, $J_{1a,1b}$ = −11.8, $J_{1a,2}$ = 4.3 Hz, 1H, H-1a of glycerol), 3.55 (dd, $J_{1b,1a}$ = −11.8 Hz, $J_{1b,2}$ = 6.2 Hz, 1H, H-1b of glycerol), 2.42 (ddd, $J_{2'',2'}$ = −14.0 Hz, $J_{2'',1'}$ = 6.2 Hz, $J_{2'',3'}$ = 3.7 Hz, 1H, H-2″), 2.28 (ddd, $J_{2',2''}$ = −14.0 Hz, $J_{2',1'}$ = 6.9 Hz, $J_{2',3'}$ = 6.2 Hz, 1H, H-2′); ^{13}C NMR (D_2O) δ 168.3 (C-4), 159.4 (C-2), 144.6 (C-6), 99.2 (C-5), 89.0 (C-1′), 88.3 (d, J_{CCOP} = 8.4 Hz, C-4′), 73.6 (C-3′), 73.5 (d, J_{CCOP} = 7.5 Hz, C-2 of glycerol), 69.2 (d, J_{COP} = 5.3 Hz, C-3 of glycerol), 67.7 (d, J_{COP} = 4.6 Hz, C-5′), 64.9 (C-1 of glycerol), 42.3 (C-2′); HRFAB-MS(−) calcd for $C_{12}H_{19}N_3O_9P$ $(M - H)^-$ 380.0859, found 380.0861.

5′-*O*-(*sn*-Glycero-3-phosphoryl)-2′-deoxyguanosine (3): 84% yield; HRFAB-MS(−) calcd for $C_{13}H_{19}N_5O_9P$ $(M - H)^-$ 420.0920, found 420.0940.

5′-*O*-(*sn*-Glycero-3-phosphoryl)-2′-deoxyadenosine (4): 85% yield; HRFAB-MS(−) calcd for $C_{13}H_{19}N_5O_8P$ $(M - H)^-$ 404.0971, found 404.0952.

5′-*O*-(*sn*-Glycero-3-phosphoryl)-2′-deoxythymidine (5): 86% yield; HRFAB-MS(−) calcd for $C_{13}H_{20}N_2O_{10}P$ $(M - H)^-$ 395.0855, found 395.0830.

5′-*O*-(*sn*-Glycero-3-phosphoryl)adenosine (6): 81% yield; HRFAB-MS(−) calcd for $C_{13}H_{19}N_5O_9P$ $(M - H)^-$ 420.0920, found 420.0915.

5′-*O*-(*sn*-Glycero-3-phosphoryl)-3′-azido-2′,3′-dideoxythymidine (15). To a stirred solution of **1** (398 mg, 1.2 mmol) in anhyd CH_3CN (4 mL) were alternately added a solution of AZT (267 mg, 1 mmol) in anhyd CH_3CN (3 mL) and a 2.5% tetrazole solution in the same solvent (4 mL) dropwise. The stirred mixture was allowed to react for 10 min and was then treated with an excess of 0.1 M iodine solution in pyridine/THF/H_2O (0.1:9:1). The solvent was then evaporated in vacuo and the residue dissolved in *n*-BuOH/$CHCl_3$ (1:9). The solution was extracted with freshly prepared 5% aqueous sodium metabisulfite to remove the excess of iodine. The organic layer was washed with H_2O and concentrated in vacuo. The residue was taken up in concd ammonia (25 mL) and the suspension stirred at room temperature for 2 h. After the reaction mixture was taken to dryness in vacuo, the residue was dissolved in 30% aqueous AcOH (25 mL) and the solution allowed to stand for 5 h at room temperature. Following evaporation of the solvent in vacuo, the residue was purified by column chromatography on silica gel (*i*-PrOH/H_2O/concd ammonia, 85:10:5) to afford pure **15** as a colorless oil (68% yield): R_f 0.33; ^{1}H NMR (D_2O) δ 7.72 (q, J = 1.1 Hz, 1H, H-6), 6.27 (t, J = 6.6 Hz, 1H, H-1′), 4.52 (dt, $J_{3',2'}$ = 5.7 Hz, $J_{3',4'}$ = 4.1 Hz, 1H, H-3′), 4.20−4.05 (partially overlapped multiplets, 3H, H-4′, H-5′), 3.95−3.82 (overlapped multiplets, 3H, H-3 and H-2 of glycerol), 3.65 (dd, $J_{1a,1b}$ = −11.8 Hz, $J_{1a,2}$ = 4.2 Hz, 1H, H-1a of glycerol), 3.59 (dd, $J_{1b,1a}$ = −11.8 Hz, $J_{1b,2}$ = 5.7 Hz, 1H, H-1b of glycerol), 2.51 (dd, $J_{2',1'}$ = 6.6 Hz, $J_{2',3'}$ = 5.7 Hz, 2H, H-2′), 1.93 (d, J = 1.1 Hz, 3H, CH_3); ^{13}C NMR (D_2O) δ 169.4 (C-4), 154.6 (C-2), 140.2 (C-6), 114.6 (C-5), 87.9 (C-1′), 85.8 (d, J_{CCOP} = 8.6 Hz, C-4′), 73.6 (d, J_{CCOP} = 7.9 Hz, C-2 of glycerol), 69.2 (d, J_{COP} = 5.6 Hz, C-3 of glycerol), 67.9 (d, J_{COP} = 5.0 Hz, C-5′), 65.0 (C-1 of glycerol), 63.4 (C-3′), 39.2 (C-2′), 14.6 (CH_3); HRFAB-MS-(−) calcd for $C_{13}H_{19}N_5O_9P$ $(M - H)^-$ 420.0920, found 420.0942.

***O*-(*sn*-Glycero-3-phosphoryl)Acyclovir (18).** By adapting a previously reported procedure,[18] Acyclovir was first converted into the corresponding N^2-isobutyryl derivative (**17**). Crude **17** was chromatographed on a silica gel column (CH_2Cl_2/CH_3OH 95:5), giving the desired compound as a colorless oil (60% yield). Selected NMR data referred to the N^2-isobutyryl group: ^{1}H NMR (CD_3OD) δ 2.72 (septet, J = 6.9 Hz, 1H, CH), 1.23 (d, J = 6.9 Hz, 6H, CH_3); ^{13}C NMR (CD_3OD) δ 181.7 (CONH), 37.0 (CH), 19.4 (CH_3).

Compound **18** was then synthesized from **17** (90 mg, 0.30 mmol) and **1** (147 mg, 0.44 mmol) and purified in the same way as reported above for **15**, but accomplishing the ammonia treatment at 55 °C for 6 h. Compound **18** was obtained as a colorless oil (71% yield from **17**): R_f (SiO_2, *i*-PrOH/H_2O/concd ammonia 85:10:5) 0.12; ^{1}H NMR (D_2O) δ 7.91 (s, 1H, H-8), 5.50 (s, 2H, N^9-CH_2), 3.97 (m, 2H, OCH_2CH_2OP), 3.78 (m, 5H, H-2 and H-3 of glycerol, OCH_2CH_2OP), 3.61 (dd, $J_{1a,1b}$ = −11.8 Hz, $J_{1a,2}$ = 4.2 Hz, 1H, H-1a of glycerol), 3.53 (dd, $J_{1b,1a}$ = −11.8 Hz, $J_{1b,2}$ = 5.9 Hz, 1H, H-1b of glycerol); ^{13}C NMR (D_2O) δ

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161.8 (C-6), 156.9 (C-2), 154.5 (C-4), 142.7 (C-8), 118.8 (C-5), 75.6 (N^9-CH_2), 73.5 (d, J_{CCOP} = 7.8 Hz, C-2 of glycerol), 71.3 (d, J_{CCOP} = 7.4 Hz, O*C*H_2CH_2OP), 69.1 (d, J_{COP} = 5.5 Hz, C-3 of glycerol), 67.4 (d, J_{COP} = 5.2 Hz, OCH_2*C*H_2OP), 64.9 (C-1 of glycerol); HRFAB-MS(−) calcd for $C_{11}H_{16}N_5O_8P$ $(M - H)^-$ 378.0815, found 378.0802.

General Procedure for the Enzymatic Synthesis of 5′-*O*-(1-*O*-Acyl-*sn*-glycero-3-phosphoryl)nucleosides (7−14, 16, and 19). Each 5′-*O*-(*sn*-glycero-3-phosphoryl)nucleoside (25 μmol) was first converted into the relevant TBA salt by dissolving it in H_2O and stirring the solution with an excess of Dowex-50 W resin (TBA form). The resin was filtered off and the filtrate taken to dryness in vacuo. The residue was then dissolved in dried *t*-BuOH (10 mL), and the solution was treated with the appropriate trifluoroethyl ester (250 μmol) and Lipozyme (20 mg). The suspension was shaken at 240 rpm for 48 h at 40 °C. After the incubation period, the enzyme was removed by filtration and the solvent evaporated in vacuo. The residue was taken up and partitioned in a pentane/H_2O (1:1) mixture to remove the excess acylating agent. The aqueous layer was concentrated in vacuo and the residue containing the acylated product purified by reversed-phase HPLC using a linear gradient of CH_3CN in 0.1 M triethylammonium acetate (pH 7) from 0 to 70% in 40 min and a flow rate of 2.5 mL min^{-1}.

5′-*O*-(1-*O*-Palmitoyl-*sn*-glycero-3-phosphoryl)-2′-deoxycytidine (7): 37% yield from **2**; ^{1}H NMR (CD_3OD) δ 8.04 (d, J = 7.5 Hz, 1H, H-6), 6.32 (dd, $J_{1',2'}$ = 6.9 Hz, $J_{1',2''}$ = 6.2 Hz, 1H, H-1′), 5.96 (d, J = 7.5 Hz, 1H, H-5), 4.47 (m, 1H, H-3′), 4.18 (dd, $J_{1a,1b}$ = −11.2 Hz, $J_{1a,2}$ = 4.5 Hz, 1H, H-1a of glycerol), 4.23−3.85 (partially overlapped multiplets, 7H, H-1b, H-2 and H-3 of glycerol, H-4′, H-5′), 2.34 (overlapped multiplets, 3H, H-2″, α-CH_2 of palmitoyl), 2.15 (ddd, $J_{2',1'}$ = 6.9 Hz, $J_{2',2''}$ = −13.7 Hz, $J_{2',3'}$ = 6.3 Hz, 1H, H-2′), 1.60 (m, 2H, β-CH_2 of palmitoyl), 1.29 (br s, 24H, from γ- to ζ-CH_2 of palmitoyl), 0.84 (t, J = 6.8 Hz, 3H, CH_3 of palmitoyl); ^{13}C NMR (CD_3OD) δ 175.3 (COO), 167.7 (C-4), 158.8 (C-2), 143.0 (C-6), 87.4 (d, J_{CCOP} = 8.5 Hz, C-4′), 87.2 (C-1′), 72.2 (C-3′), 69.7 (d, J_{CCOP} = 7.5 Hz, C-2 of glycerol), 67.6 (d, J_{COP} = 4.4 Hz, C-3 of glycerol), 66.1 (C-1 of glycerol), 66.0 (d, J_{COP} = 4.1 Hz, C-5′), 41.8 (C-2′), 34.9 (α-CH_2 of palmitoyl), 33.1 (ν-CH_2 of palmitoyl), 30.8, 30.6, 30.5, 30.2 (from γ- to μ-CH_2 of palmitoyl), 26.0 (β-CH_2 of palmitoyl), 23.7 (ζ-CH_2 of palmitoyl), 14.4 (CH_3 of palmitoyl), C-5 not observed; HRFAB-MS(−) calcd for $C_{28}H_{49}N_3O_{10}P$ $(M - H)^-$ 618.3156, found 618.3162.

5′-*O*-(1-*O*-Palmitoyl-*sn*-glycerophosphoryl)-2′-deoxyguanosine (8): 25% yield from **3**; HRFAB-MS(−) calcd for $C_{29}H_{49}N_5O_{10}P$ $(M - H)^-$ 658.3217, found 658.3220.

5′-*O*-(1-*O*-Palmitoyl-*sn*-glycero-3-phosphoryl)-2′-deoxyadenosine (9): 40% yield from **4**; HRFAB-MS(−) calcd for $C_{29}H_{49}N_5O_9P$ $(M - H)^-$ 642.3268, found 642.3275.

5′-*O*-(1-*O*-Palmitoyl-*sn*-glycero-3-phosphoryl)-2′-deoxythymidine (10): 55% yield from **5**; HRFAB-MS(−) calcd for $C_{29}H_{50}N_2O_{11}P$ $(M - H)^-$ 633.3152, found 633.3160.

5′-*O*-(1-*O*-Valeryl-*sn*-glycero-3-phosphoryl)-2′-deoxycytidine (11): 75% yield from **2**; selected ^{1}H NMR data referred to the valeryl chain (D_2O) δ 2.35 (t, J = 7.3 Hz, 2H, α-CH_2), 1.53 (tt, $J_{\beta,\alpha}$ = 7.3 Hz, $J_{\beta,\gamma}$ = 7.4 Hz, 2H, β-CH_2), 1.28 (tq, $J_{\gamma,\beta}$ = 7.4 Hz, $J_{\gamma,\delta}$ = 7.2 Hz, 2H, γ-CH_2), 0.84 (t, J = 7.2 Hz, 3H, CH_3); selected ^{13}C NMR data referred to the valeryl chain (D_2O) δ 179.8 (COO), 36.3 (α-CH_2), 29.3 (γ-CH_2), 24.4 (β-CH_2), 15.8 (CH_3); HRFAB-MS(−) calcd for $C_{17}H_{27}N_3O_{10}P$ $(M - H)^-$ 464.1434, found 464.1415.

5′-*O*-(1-*O*-Valeryl-*sn*-glycero-3-phosphoryl)-2′-deoxyadenosine (12): 82% yield from **4**; HRFAB-MS(−) calcd for $C_{18}H_{27}N_5O_9P$ $(M - H)^-$ 488.1546, found 488.1554.

5′-*O*-(1-*O*-Oleoyl-*sn*-glycero-3-phosphoryl)-2′-deoxycytidine (13): 38% yield from **2**; selected ^{1}H NMR data referred to the oleoyl chain (CD_3OD) δ 5.34 (m, 2H, CH=CH), 2.34 (m, 2H, α-CH_2), 2.03 (m, 4H, *C*H_2CH=CH*C*H_2), 1.60 (m, 2H, β-CH_2), 1.32 and 1.29 (partially overlapped br s, 20H, from γ- to ζ-CH_2 and from λ- to π-CH_2), 0.90 (t, J = 7.0 Hz, 3H, CH_3); selected ^{13}C NMR data referred to the oleoyl chain (CD_3OD) δ 175.4 (COO), 130.8 (CH=CH), 34.9 (α-CH_2), 33.1 (*o*-CH_2), 30.8, 30.6, 30.5, 30.3 and 30.2 (from γ- to ζ-CH_2 and from λ- to ζ-CH_2), 28.1 (*C*H_2−CH=CH-*C*H_2), 26.0 (β-CH_2), 23.7 (π-CH_2), 14.5 (CH_3); HRFAB-MS(−) calcd for $C_{30}H_{51}N_3O_{10}P$ $(M - H)^-$ 644.3312, found 644.3318.

5′-*O*-(1-*O*-Palmitoyl-*sn*-glycero-3-phosphoryl)adenosine (14): 45% yield from **6**; HRFAB-MS(−) calcd for $C_{29}H_{49}N_5O_{10}P$ $(M - H)^-$ 658.3217, found 658.3221.

5′-*O*-(1-*O*-Palmitoyl-*sn*-glycero-3-phosphoryl)-3′-azido-2′,3′-dideoxythymidine (16): 61% yield from **15**; HRFAB-MS(−) calcd for $C_{29}H_{49}N_5O_{10}P$ $(M - H)^-$ 658.3217, found 658.3221.

***O*-(1-*O*-Palmitoyl-*sn*-glycero-3-phosphoryl)Acyclovir (19):** 33% yield from **18**; HRFAB-MS(−) calcd for $C_{27}H_{47}N_5O_9P$ $(M - H)^-$ 616.3111, found 616.3095.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for compounds **3**−**6**, **8**−**14**, **16**, **17**, and **19** (8 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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