

Synthesis of 3'(2')-*O*-Lysophosphatidyl nucleosides – a Further Application of a Chemoenzymatic Strategy

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Mono[(2*R*)-2,3-dihydropropyl] esters of the four 3'-nucleotides of DNA, prepared from protected nucleoside phosphoramidites and [(4*S*)-2,2-dimethyl-1,3-dioxolan-4-yl]methanol, were regioselectively acylated at the C-1 hydroxyl of the glycerol moiety by a lipase-catalyzed transacylation with activated palmitic acid ester in organic solvent, giving the relevant 3'-*O*-lysophosphatidyl-2'-deoxynucleosides. The synthesis was also adapted for the preparation of 3'-*O*-lyso-

phosphatidyl derivatives of 5'-deoxy-5-fluorouridine and 5'-deoxy-5'-(methylthio)adenosine, with the 2'-*O*-isomer of the latter compound also being prepared. The enhanced ability of lysophosphatidyl compounds to interact with lipid monolayers was also tested in comparison with that of the relevant free nucleosides.

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Introduction

The constant quest to produce biologically active compounds with ever better capabilities to interact with cell membranes has recently encouraged us to develop a general synthetic preparation of 5'-*O*-lysophosphatidyl conjugates of nucleosides, some of these being nucleoside analogues of pharmacological interest.^[1] The basic idea behind this approach is that lipophilic moieties conjugated to nucleosides may significantly enhance the cellular uptake of these polar molecules, as a consequence of the affinity of the lipophilic moieties for membrane structures.

In a two-step chemoenzymatic strategy, mono[(2*R*)-2,3-dihydroxypropyl] esters of 5'-nucleotides (5'-GPNs) were first prepared from a phosphoramidite of [(4*S*)-2,2-dimethyl-1,3-dioxolan-4-yl]methanol and the appropriately protected nucleosides. Selective acylation at 1-OH of the glycerol moiety of the 5'-GPNs was then achieved by a lipase-catalyzed (Lipozyme) transacylation with activated fatty acid esters in organic solvents.

By this procedure we succeeded in synthesizing 5'-*O*-lysophosphatidyl derivatives of some deoxyribo- and ribonucleosides that normally occur in nucleic acids, along with lysophosphatidyl conjugates of two nucleoside analogues of wide use in current antiviral therapy: AZT and acyclovir.

As other nucleosides showing antineoplastic activity are of great interest in medicinal chemistry, we also thought it

useful to prepare the lysophosphatidyl conjugates of these compounds. The covalent attachment of lipophilic moieties to such nucleoside analogues is particularly useful, since low-density lipoproteins (LDL) have been claimed as attractive carriers for selective and efficient intracellular delivery of antineoplastic drugs to tumor cells. Indeed, this therapeutic approach has already been explored for some conventional lipophilic antitumor drugs,^[2,3] and the availability of lysophosphatidyl conjugates of antitumor nucleoside analogues could then be of great utility for further pharmacological studies in this field.

However, not all nucleoside compounds known to possess antiproliferative properties bear a 5'-hydroxyl group at their sugar (or sugar-like) moiety [e.g., 5'-deoxy-5-fluorouridine (DFUR) or 5'-deoxy-5'-(methylthio)adenosine (MTA)], thus denying the possibility of obtaining the relevant 5'-*O*-lysophosphatidyl conjugates. In these cases, the covalent attachment of the lysophosphatidyl moiety at the 3'-(or 2')-hydroxy group of the sugar residue may be an alternative and reasonably simple way.

We therefore also now wish to report a procedure for the preparation of 3'(2')-*O*-lysophosphatidyl conjugates of 2'-deoxyribo- or ribonucleosides, by which the synthesis of lysophosphatidyl derivatives of DFUR and MTA has also been achieved.

Results and Discussion

To achieve the preparation of 3'-*O*-lysophosphatidyl nucleosides we planned to follow the same two-step chemoenzymatic strategy as previously used for the preparation of 5'-*O*-lysophosphatidyl nucleosides.^[1] Therefore, mono[(2*R*)-2,3-dihydroxypropyl] esters of 3'-nucleotides (3'-GPNs) had

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to be prepared first, possibly with modification of the chemical step. These would subsequently be acylated by Lipzyme in organic solvent. All this was of course based on the assumption that 3'-GPNs could also be recognized by Lipzyme as substrates.

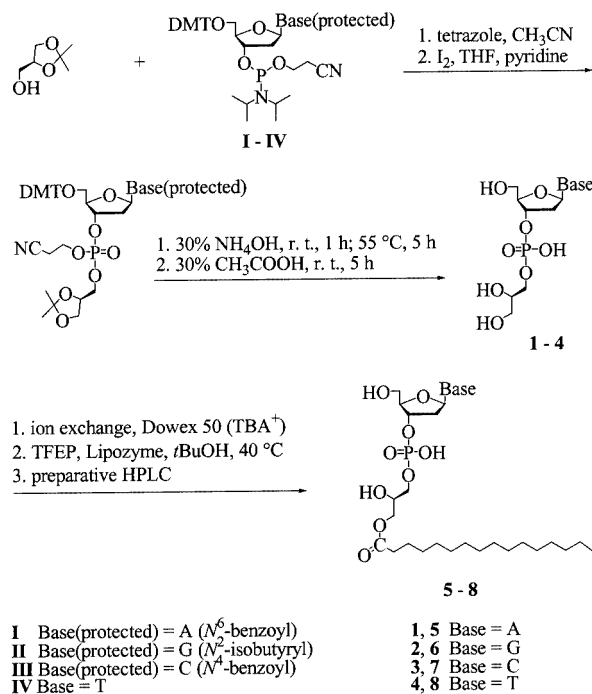
Synthesis of 3'-*O*-Lysophosphatidyl-2'-deoxynucleosides

The synthetic plan was first applied to the preparation of 3'-*O*-lysophosphatidyl derivatives of the four 2'-deoxynucleosides that normally occur in DNA.

As shown in Scheme 1, protected mono[(2*R*)-2,3-dihydroxypropyl] esters of 3'-(2'-deoxy)nucleotides (3'-GPdNs) were prepared from [(4*S*)-2,2-dimethyl-1,3-dioxolan-4-yl]-methanol and the relevant suitably protected 2'-deoxynucleoside phosphoramidites **I–IV**. For this purpose, nucleoside phosphoramidites currently used in DNA solid-phase synthesis were employed. Then, after oxidation by iodine and removal of all protective groups by consecutive treatment with ammonia and weak acid, free 3'-GPdNs (**1–4**) were obtained. Finally, the enzymatic acylation of each 3'-GPdN [as a tetrabutylammonium (TBA) salt] was achieved by use of *Rhizomucor miehei* lipase (Lipozyme) in *tert*-butyl alcohol and in the presence of trifluoroethyl palmitate (TFEP). At the end of the synthetic run, HPLC analyses of the crude reaction mixtures in each case revealed the presence of only one acylation product (**5–8**), which was isolated and characterized on the basis of its spectroscopic features (FAB-MS, NMR). The ^1H and ^{13}C NMR spectra of compounds **1–8** are reported in the Exp. Sect. The complete assignments of individual resonances were performed with the help of ^1H – ^1H COSY, APT, and HSQC experiments, while 1D decoupling experiments, when necessary, allowed the ^1H – ^1H coupling constants to be measured.

In the ^{13}C NMR spectra of compounds **5–8**, the resonances of the C-1 and C-2 carbon atoms of the 3-phosphoryl-*sn*-glycerol moiety were shifted downfield ($\Delta\delta = 1.2$ ppm for pyrimidine and 1.7 ppm for purine nucleotides) and upfield ($\Delta\delta = -3.65 \pm 0.5$ ppm), respectively, from the corresponding resonances of the relevant 3'-GPdNs. From the α - and β -effects of the acylation, these results clearly indicate that each 3'-GPdN had undergone regioselective acylation at the primary hydroxyl group of its glycerol moiety. Accordingly, in the ^1H NMR spectra of these compounds, the C-1 methylene protons of the 3-phosphoryl-*sn*-glycerol moiety were shifted downfield ($\Delta\delta = 0.46 \pm 0.1$ ppm) in comparison with the spectra of the pertinent 3'-GPdNs. In conclusion, the corresponding 3'-*O*-lysophosphatidyl derivative was formed, whichever the starting 2'-deoxynucleoside had been. Confirmatory evidence for the assigned structures was also provided by HRFAB-MS(–) spectra obtained for each compound (see Exp. Sect.).

It has been reported that various lipases (including Lipozyme) in organic solvents in the presence of substrates bearing different kinds of alcoholic functions preferentially acylate the primary ones.^[4,5] Thus, in the case of 5'-GPdNs,^[1] bearing only one primary and two secondary hydroxyls, it had not been surprising to find that the enzyme had been



Scheme 1

able to acylate the primary hydroxyl of the glycerol moiety selectively. In 3'-GPdNs there are two primary hydroxyls – both, in principle, susceptible to enzymatic acylation – present in the molecule, in addition to a secondary hydroxyl. Actually, though, the acylation once again occurred regioselectively at the primary hydroxyl of the glycerol moiety and not at the free 5'-OH of the sugar ring. A preference of *R. miehei* lipase for recognition of that side of 3'-GPdNs molecules which is structurally related to natural glycerides is evident.

Table 1 reports the conversion values obtained after 48 h enzymatic acylation of the four 3'-GPdNs, in comparison with those previously found, under the same experimental conditions, for the 5'- counterparts. From these data it appears that, whereas both the two pyrimidine 3'-GPdNs and their 5'-isomers undergo enzymatic acylation essentially to the same extent, the degrees of conversion of purine 5'-

Table 1. Lipase-catalyzed palmitoylation of 3'- and 5'-GPdNs

Substrate	Conversion ^[a] [%]
3'-GPdA	63
5'-GPdA	40
3'-GPdG	32
5'-GPdG	25
3'-GPdC	40
5'-GPdC	37
3'-GPdT	58
5'-GPdT	55

^[a] After 48 h incubation, according to the general procedure reported under Exp. Sect. for the synthesis of compounds **5–8**.

GPdNs are decidedly lower than those of the relevant 3'-compounds.

These findings may be accounted for by the supposition that, in purine 5'-GPdNs (and unlike in 3'-GPdNs), a conformational preference could compel the glycerol moiety to be positioned close to the purine nucleobase, making it not entirely available for the catalytic site of the enzyme.

Preliminary indirect evidence in support of this hypothesis was found by comparison of NMR spectroscopic data (D_2O) of purine 5'-GPdNs with those of their 3'-counterparts. In the 1H NMR spectra of the former compounds, the resonances of H-1_a and H-1_b of the glycerol moiety appeared as double doublets at $\delta = 3.37$ and 3.47 ppm, respectively, for the adenosine derivative, and at $\delta = 3.43$ and 3.52 ppm for the guanosine one. In contrast, the resonances of these two protons in the 1H NMR spectra of purine 3'-GPdNs appeared at $\delta = 3.67$ and 3.74 ppm both for the adenosine and for the guanosine derivatives. The upfield values found for the H-1_a and H-1_b resonances of the two purine 5'-GPdNs indicated that some constraint in these compounds causes the C-1 methylene protons of the glycerol moiety to lie inside the shielding cone of the relevant purine rings. A ROE correlation between the H-8 of the base and the H-2 or H-3_b (overlapped signals) of the glycerol moiety, observable in ROESY experiments on both purine 5'-GPdNs, seemed to support this hypothesis.

As the enzymatic acylations of GPdNs had been performed in *tert*-butyl alcohol, 1H NMR experiments on 3'- and 5'-GPdAs (as TBA salts) were also carried out in $(CD_3)_3COD$ (see Exp. Sect.). In this solvent, unlike in D_2O , the resonances of the relevant protons of the glycerol moieties of the two compounds had similar chemical shifts, the only observable difference being that the two H-1 and the two H-3 protons in the 3'-GPdA spectrum were isochronous whereas in the 5'-GPdA spectrum they were not. More interestingly, NOE difference spectra of 5'-GPdA showed diagnostic mutual NOEs between H-8 of the base and H₂-3 of the glycerol moiety, indicating a certain proximity of these protons in space. As was to be expected from the high viscosity of the solvent, all the observed NOE enhancements were negative.^[6] These data gave further evidence of a conformational preference in which the glycerol moiety lies close to the purine nucleobase in purine 5'-GPdNs.

Before we applied the synthetic procedure to the preparation of 3'-*O*-lysophosphatidyl derivatives of DFUR and MTA, we thought it necessary to optimize the experimental conditions used for enzymatic acylation of 3'-GPdNs, to enhance the yield of this synthetic step significantly. After various trials on some 3'-GPdNs as model substrates, it was decided to maintain the amount of GPN substrate and the concentration of acyl donor unchanged from the previously used experimental conditions, but at the same time to increase the total reaction volume and the amount of enzyme (four- and elevenfold, respectively), in addition to which the temperature setting was lowered to 38 °C. As a result, it proved possible to prolong the reaction time up to 5 days and to achieve a 90% final yield when **1** was used as substrate (similar results were obtained with compounds **2–4**).

These optimized experimental conditions were then followed in the course of the syntheses of lysophosphatidyl derivatives of DFUR and MTA discussed below.

Synthesis of 3'-*O*-Lysophosphatidyl-5'-deoxy-5-fluorouridine

The required intermediate for the synthesis of the title compound is 3'-(5'-deoxy-5-fluoro)uridylic acid mono-[(2*R*)-2,3-dihydroxypropyl] ester (**12**), which was prepared first. Unlike the 2'-deoxynucleosides, DFUR bears two secondary hydroxy groups at the ribose ring, and so prior protection of the 2'-hydroxy was necessary (Scheme 2).

For this, DFUR was first treated with two molar equivalents of *tert*-butyldimethylsilyl chloride (TBDMSCl) to afford, after purification, near equimolar amounts of 3'- and 2'-*O*-(*tert*-butyldimethylsilyl)-5'-deoxy-5-fluorouridine (**10**, **11**), which were not separated. The two products were then treated with 2-cyanoethyl [(4*R*)-2,2-dimethyl-1,3-dioxolan-4-yl]methyl diisopropylamidophosphite (**9**) and converted into a mixture of 3'-[2'-*O*-(*tert*-butyl(dimethylsilyl))-5'-deoxy-5-fluoro]uridylic acid mono[(4*R*)-2,2-dimethyl-1,3-dioxolan-4-yl]methyl ester (**V**) and the relevant 2'-isomer, after oxidation by iodine and subsequent ammonia hydrolysis. Compound **V**, isolated by chromatography, then gave the desired **12**, after mild acid hydrolysis. Enzymatic acylation of **12**, with TFEP as acyl donor, afforded the 3'-*O*-lysophosphatidyl derivative of 5'-deoxy-5-fluorouridine (**13**) in good yield. The spectroscopic features (NMR and MS) of the isolated compound were reasonably consistent with the assigned structure (see Exp. Sect.).

Synthesis of 3'- and 2'-*O*-Lysophosphatidyl-5'-deoxy-5'-(methylthio)adenosine

After *N*⁶-benzoyl-5'-deoxy-5'-(methylthio)adenosine (**14**) had been prepared from commercially available MTA, compounds **17** and **18** were obtained from **14** by the same synthetic approach as used for the preparation of **12**. In this case, however, the two compounds were isolated and each of them was separately subjected to the enzymatic acylation. In this manner, compounds **19** and **20** were obtained (Scheme 3), their molecular structures being confirmed on the basis of their spectroscopic characteristics (see Exp. Sect.).

Here, once again, a difference was found between the enzymatic acylation rates of compounds **17** and **18**. After an incubation time of 48 h, in fact, 41% conversion was observed for compound **18**, compared to 60% conversion found for the 3'- isomer **17**. Also in this case a comparison of 1H NMR spectroscopic data (D_2O) of **18** with those of the 3'-counterpart showed that the resonances of H-1_a and H-1_b of the glycerol moiety appeared in the spectrum of the former as double doublets at $\delta = 3.39$ and 3.45 ppm, respectively (the similarity of these values with those found for purine 5'-GPdNs is striking), while these two protons in the 1H NMR spectrum of **17** gave signals at $\delta = 3.68$ and 3.75 ppm. The upfield-shifted values found for the H-1 resonances of the glycerol moiety of **18** were indicative of



a prevalent conformation in which these protons lie inside the adenine shielding cone. When the ^1H NMR spectra of **17** and **18** were recorded (as TBA salts) in $(\text{CD}_3)_3\text{COD}$, all the glycerol moiety protons of the latter compound appeared shielded relative to the relevant ones of **17** (see Exp. Sect.). Furthermore, NOE difference spectra of **18** obtained in the same solvent showed diagnostic enhancements to H-8 when H₂-1, H-2, or H₂-3 of the glycerol moiety were irradiated. Similar NOEs could not be obtained with **17**. These data as a whole indicate that a conformational preference exists in **18**, but not in **17**, in which the glycerol moiety lies close to the purine nucleobase. It is not unlikely that this finding may, as has been proposed for purine 5'-GpDNs, be related to a poorer ability of the lipase to recognize **18** as a substrate.

Lipoaffinity of 3'-O-Lysophosphatidyl nucleosides

Bearing in mind the aim of this work, the tendency of the newly synthesized lysophosphatidyl compounds to permeate lipophilic areas of biomembranes was indirectly investigated by evaluation of their interactions with lipid monolayers,^[7,8] in comparison with those of the relevant free nucleosides. In this approach, interactions between a pharmacologically active substance and lipids are investigated by injection of the drug into the subphase beneath a lipid monolayer at a constant surface area. The penetration of the drug can be estimated by the surface pressure increase of the lipid film, which can be measured by the Wilhelmy plate method.^[8] Figure 1 gives the results of this test when applied to compound **13** in comparison with DFUR; the enhanced ability of **13** to penetrate the phosphatidylcholine monolayer over that of the free nucleoside is clear. Similar results, not shown here for brevity, were obtained from compound **19** in comparison with MTA.

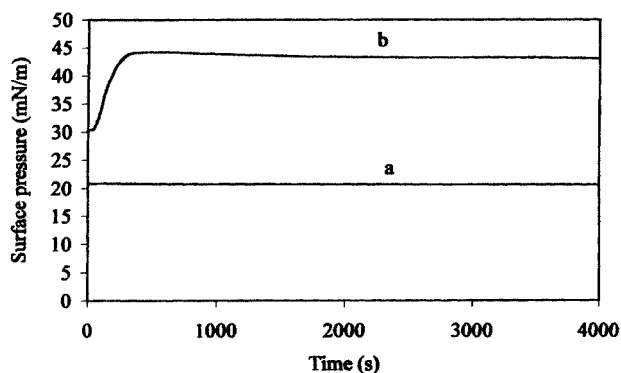


Figure 1. Kinetics of surface pressure increase of phosphatidylcholine monolayers after dissolving into the aqueous subphase DFUR (a) [10.1 μM] and compound **13** (b) [0.40 μM] (see also Exp. Sect. under General)

At present, DFUR is widely used, often in combination with other drugs, in the chemotherapeutic treatment of tumors.^[9–12] MTA, a potent inhibitor of protein carboxyl-methyltransferase, is able to induce apoptosis in leukemia U937 cells.^[13] As an inhibitor of protein tyrosine kinase activity stimulated by basic fibroblast growth factor, MTA has

also been shown to display antiproliferative activity on glioma cell lines.^[14]

Lysophosphatidyl derivatives of both these compounds, which may be viewed as intermediate metabolites of compounds themselves, have pro-drug features and are potentially better able than free drugs to permeate cell membranes.

Finally, it should be expected that, for a given lipid membrane composition, a specific fatty acid chain in the lysophosphatidyl compounds should show the best lipophilic interactions with membranes. Accordingly, lysophosphatidyl derivatives of nucleoside drugs may be prepared with ever higher efficiency in targeting drugs against specific tumor cells by a opportune choice of the acyl donor in the enzymatic step of the synthesis. Further work is in progress in this area.

Experimental Section

General: NMR spectra were recorded on a Varian Unity Inova spectrometer at 500 MHz (^1H) and 125.7 MHz (^{13}C). The chemical shifts are given in ppm and referenced to: (a) TMS as internal standard, for the experiments in C_6D_6 , CDCl_3 , and CD_3OD ; (b) the residual HOD signal ($\delta = 4.82$ ppm), for ^1H experiments in D_2O ; (c) the signal of appropriately added CD_3OD ($\delta = 49.0$ ppm), for ^{13}C experiments in D_2O ; and (d) the signal of the residual partially deuterated solvent ($\delta = 1.30$ ppm) for ^1H experiments in $(\text{CD}_3)_3\text{COD}$. ROESY experiments were carried out in D_2O at 27 $^\circ\text{C}$, with a mixing time of 300 ms. NOE difference spectra were obtained in $(\text{CD}_3)_3\text{COD}$ at 30 $^\circ\text{C}$. High-resolution fast atom bombardment mass spectra (HRFAB-MS) were recorded with a Fisons ZAB 2SE spectrometer with glycerol as matrix. Column chromatography was performed on silica gel (40–63 μm , Merck), and TLC was carried out on silica gel 60 F_{254} precoated glass plates (0.25 mm, Merck). HPLC was performed on a Hewlett–Packard 1050 chromatograph equipped with a UV detector set at 260 nm, on LiChrospher-100 ODS (5 μm ; 4 $\times$ 250 mm) and Ultrasphere ODS (5 μm ; 10 $\times$ 250 mm) or Zorbax SB-C18 (5 μm ; 9.4 $\times$ 250 mm) columns 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 3'-nucleotides, DFUR, and MTA. Commercially purchased solvents and reagents were all of reagent quality. Triethylamine (TEA), petroleum ether, *t*BuOH, dichloromethane, and diethyl ether were freshly dried and stored under argon as previously reported.^[1] TFEP was prepared by a reported procedure,^[15] and its purity was checked by its chromatographic and spectroscopic properties. Lipase from *Rhizomucor miehei* (LipozymeTM, immobilized) was from Novo-Nordisk, Copenhagen, Denmark. Before use the enzyme was allowed to stand for 45 min in dried *t*BuOH. For the monolayer penetration experiments, egg yolk phosphatidylcholine (Fluka), dissolved in chloroform (0.2 mg/mL) was spread (30 μL) on the aqueous subphase, which consisted of 0.10 M NaCl and 0.05 M phosphate buffer solution (pH 7.4). Surface pressure measurements were carried out at 25 $^\circ\text{C}$ with a NIMA PS4 surface pressure sensor (NIMA Technology LTD).

General Procedure for the Synthesis of Compounds 1–4: Solutions of [(4*S*)-2,2-dimethyl-1,3-dioxolan-4-yl]methanol (22 μL , 0.18 mmol) and 2.5% tetrazole (1 mL) in anhydrous CH_3CN

(3 mL) were alternately added dropwise to a stirred solution of 0.12 mmol of compounds **I**, or **II**, **III**, **IV** in the same solvent. The magnetically stirred mixture was left at room temperature for 1 h and then treated with an excess of iodine solution (0.1 M) in THF/H₂O/pyridine (9:1:0.1). After the reaction mixture had been taken to dryness in vacuo, the residue was dissolved in 20 mL of CHCl₃/*n*BuOH (9:1) and the solution was extracted with freshly prepared 5% aqueous sodium metabisulfite to remove the excess of iodine. The organic layer was washed with H₂O and the solvents were evaporated in vacuo. The residue was taken up in concd. ammonia (10 mL) and the suspension was stirred at room temperature for 1 h, then at 55 °C for 5 h (this step was omitted for **4**). After the reaction mixture had been taken to dryness in vacuo, the residue was dissolved in 30% aqueous HOAc (10 mL) and the solution was allowed to stand at room temperature for 5 h. After evaporation of the solvent in vacuo, the residue was taken up in H₂O and extracted with CH₂Cl₂. The aqueous layer was then evaporated in vacuo and the totally deprotected product was purified by column chromatography on silica gel (*i*PrOH/H₂O/concd. ammonia, 87:10:3). Pure compounds (**1–4**) were obtained as colourless oils.

3'-(2'-Deoxy)adenylic Acid Mono[(2R)-2,3-dihydroxypropyl] Ester (1): 33.1 mg (68%). ¹H NMR (D₂O): δ = 2.80 (ddd, *J*_{2'',2'} = −14.2, *J*_{2'',1'} = 6.1, *J*_{2'',3'} = 2.7 Hz, 1 H, H-2''), 2.92 (ddd, *J*_{2',2''} = −14.2, *J*_{2',1'} = 7.9, *J*_{2',3'} = 5.9 Hz, 1 H, H-2'), 3.67 (dd, *J*_{1a,1b} = −11.8, *J*_{1a,2} = 6.0 Hz, 1 H, H-1a of glycerol), 3.74 (dd, *J*_{1b,1a} = −11.8, *J*_{1b,2} = 4.3 Hz, 1 H, H-1b of glycerol), 3.87 (dd, *J*_{5'',5'} = −12.7, *J*_{5'',4'} = 4.2 Hz, 1 H, H-5''), 3.91 (dd, *J*_{5',5''} = −12.7, *J*_{5',4'} = 3.2 Hz, 1 H, H-5'), 3.94–4.05 (partially overlapped multiplets, 3 H, H-2 and H-3 of glycerol), 4.41 (ddd, *J*_{4',3'} = 2.4, *J*_{4',5'} = 3.2, *J*_{4',5''} = 4.2 Hz, 1 H, H-4'), 5.01 (dddd, *J*_{3',2'} = 5.9, *J*_{3',2''} = 2.7, *J*_{3',4'} = 2.4, *J*_{3',P} = 7.1 Hz, 1 H, H-3'), 6.51 (dd, *J*_{1',2'} = 7.9, *J*_{1',2''} = 6.1 Hz, 1 H, H-1'), 8.26 (s, 1 H, H-2), 8.39 (s, 1 H, H-8) ppm. ¹H NMR (TBA salt, (CD₃)₃COD): δ = 1.13 (t, *J* = 7.3 Hz, 12 H, *N*-CH₂CH₂CH₂CH₃), 1.58 (m, 8 H, *N*-CH₂CH₂CH₂CH₃), 1.79 (m, 8 H, *N*-CH₂CH₂CH₂CH₃), 2.78 (ddd, *J*_{2'',2'} = −13.3, *J*_{2'',1'} = 5.9, *J*_{2'',3'} = 3.4 Hz, 1 H, H-2''), 2.91 (ddd, *J*_{2',2''} = −13.3, *J*_{2',1'} = 7.1, *J*_{2',3'} = 5.7 Hz, 1 H, H-2'), 3.41 (br. t, 8 H, *N*-CH₂CH₂CH₂CH₃), 3.71 (d, *J*_{1,2} = 5.9 Hz, 2 H, H-1 of glycerol), 3.85 (tt, *J*_{2,1} = 5.9, *J*_{2,3} = 4.4 Hz, 1 H, H-2 of glycerol), 3.95 (dd partially overlapping with next signal, *J*_{5'',5'} = −12.6, *J*_{5'',4'} = 2.9 Hz, 1 H, H-5''), 3.97 (dd partially overlapping with previous signal, *J*_{5',5''} = −12.6, *J*_{5',4'} = 2.3 Hz, 1 H, H-5'), 4.08 (dd, *J*_{3,2} = 4.4, *J*_{3,P} = 9.9 Hz, 2 H, H-3 of glycerol), 4.35 (dt, *J*_{4',3'} = 2.9, *J*_{4',5'} = 2.3, *J*_{4',5''} = 2.9 Hz, 1 H, H-4'), 5.15 (dddd, *J*_{3',2'} = 5.7, *J*_{3',2''} = 3.4, *J*_{3',4'} = 2.9, *J*_{3',P} = 7.3 Hz, 1 H, H-3'), 6.60 (dd, *J*_{1',2'} = 7.1, *J*_{1',2''} = 5.9 Hz, 1 H, H-1'), 8.29 (s, 1 H, H-2), 8.63 (s, 1 H, H-8) ppm. ¹³C NMR (D₂O): δ = 41.2 (d, *J*_{CCOP} = 2.5 Hz, C-2'), 64.5 (C-5'), 64.9 (C-1 of glycerol), 69.3 (d, *J*_{COP} = 6.0 Hz, C-3 of glycerol), 73.5 (d, *J*_{CCOP} = 6.9 Hz, C-2 of glycerol), 78.7 (d, *J*_{COP} = 5.4 Hz, C-3'), 87.7 (C-1'), 89.5 (d, *J*_{CCOP} = 5.3 Hz, C-4'), 121.6 (C-5), 143.5 (C-8), 151.0 (C-4), 153.9 (C-2), 157.3 (C-6) ppm. HRFAB-MS(−) calcd. for C₁₃H₁₉N₅O₈P [M − H][−] 404.0971, found 404.0963.

3'-(2'-Deoxy)guanylic Acid Mono[(2R)-2,3-dihydroxypropyl] Ester (2): 31.9 mg (63%). ¹H NMR (D₂O): δ = 2.73 (ddd, *J*_{2'',2'} = −14.1, *J*_{2'',1'} = 6.1, *J*_{2'',3'} = 2.7 Hz, 1 H, H-2''), 2.90 (ddd, *J*_{2',2''} = −14.1, *J*_{2',1'} = 8.2, *J*_{2',3'} = 6.1 Hz, 1 H, H-2'), 3.67 (dd, *J*_{1a,1b} = −11.7, *J*_{1a,2} = 5.7 Hz, 1 H, H-1a of glycerol), 3.74 (dd, *J*_{1b,1a} = −11.7, *J*_{1b,2} = 3.9 Hz, 1 H, H-1b of glycerol), 3.85 (dd, *J*_{5'',5'} = −12.4, *J*_{5'',4'} = 4.4 Hz, 1 H, H-5''), 3.89 (dd, *J*_{5',5''} = −12.4, *J*_{5',4'} = 3.6 Hz, 1 H, H-5'), 3.92–4.04 (partially overlapped multiplets, 3 H, H-2 and H-3 of glycerol), 4.36 (ddd, *J*_{4',3'} = 2.4, *J*_{4',5'} = 3.6,

*J*_{4',5''} = 4.4 Hz, 1 H, H-4'), 4.99 (dddd, *J*_{3',2'} = 6.1, *J*_{3',2''} = 2.7, *J*_{3',4'} = 2.4, *J*_{3',P} = 7.0 Hz, 1 H, H-3'), 6.36 (dd, *J*_{1',2'} = 8.2, *J*_{1',2''} = 6.1 Hz, 1 H, H-1'), 8.04 (s, 1 H, H-8) ppm. ¹³C NMR (D₂O): δ = 40.7 (d, *J*_{CCOP} = 2.2 Hz, C-2'), 64.5 (C-5'), 65.0 (C-1 of glycerol), 69.3 (d, *J*_{COP} = 4.7 Hz, C-3 of glycerol), 73.6 (d, *J*_{CCOP} = 7.7 Hz, C-2 of glycerol), 78.6 (d, *J*_{COP} = 5.4 Hz, C-3'), 87.1 (C-1'), 89.2 (d, *J*_{CCOP} = 6.1 Hz, C-4'), 119.5 (C-5), 141.1 (C-8), 154.4 (C-4), 156.6 (C-2), 161.7 (C-6) ppm. HRFAB-MS(−) calcd. for C₁₃H₁₉N₅O₉P [M − H][−] 420.0920, found 420.0938.

3'-(2'-Deoxy)cytidilic Acid Mono[(2R)-2,3-dihydroxypropyl] Ester (3): 32.5 mg (71%). ¹H NMR (D₂O): δ = 2.43 (ddd, *J*_{2'',2'} = −14.3, *J*_{2'',1'} = 7.3, *J*_{2'',3'} = 6.4 Hz, 1 H, H-2''), 2.68 (ddd, *J*_{2',2''} = −14.3, *J*_{2',1'} = 6.3, *J*_{2',3'} = 3.2 Hz, 1 H, H-2'), 3.66 (dd, *J*_{1a,1b} = −11.7, *J*_{1a,2} = 6.0 Hz, 1 H, H-1a of glycerol), 3.73 (dd, *J*_{1b,1a} = −11.7, *J*_{1b,2} = 4.3 Hz, 1 H, H-1b of glycerol), 3.84 (dd, *J*_{5'',5'} = −12.5, *J*_{5'',4'} = 4.9 Hz, 1 H, H-5''), 3.90 (dd, partially obscured by next signal, *J*_{5',5''} = −12.5, *J*_{5',4'} = 3.5 Hz, 1 H, H-5'), 3.91–4.01 (partially overlapped multiplets, 3 H, H-2 and H-3 of glycerol), 4.30 (ddd, *J*_{4',3'} = 3.2, *J*_{4',5'} = 3.5, *J*_{4',5''} = 4.9 Hz, 1 H, H-4'), 6.17 (d, *J*_{5,6} = 7.6 Hz, 1 H, H-5), 6.35 (dd, *J*_{1',2'} = 7.3, *J*_{1',2''} = 6.3 Hz, 1 H, H-1'), 7.98 (d, *J*_{6,5} = 7.6 Hz, 1 H, H-6) ppm, the H-3' resonance is obscured by the residual HOD signal. ¹³C NMR (D₂O): δ = 41.1 (d, *J*_{CCOP} = 3.3 Hz, C-2'), 64.0 (C-5'), 65.0 (C-1 of glycerol), 69.3 (d, *J*_{COP} = 5.7 Hz, C-3 of glycerol), 73.6 (d, *J*_{CCOP} = 7.6 Hz, C-2 of glycerol), 77.9 (d, *J*_{COP} = 4.3 Hz, C-3'), 88.8 (d, *J*_{CCOP} = 5.9 Hz, C-4'), 89.1 (C-1'), 98.9 (C-5), 145.0 (C-6), 158.5 (C-2), 167.7 (C-4) ppm. HRFAB-MS(−) calcd. for C₁₂H₁₉N₃O₉P [M − H][−] 380.0859, found 380.0865.

3'-(2'-Deoxy)thymidilic Acid Mono[(2R)-2,3-dihydroxypropyl] Ester (4): 34.7 mg (73%). ¹H NMR (D₂O): δ = 1.95 (d, *J* = 1.0 Hz, 3 H, CH₃), 2.48 (ddd, *J*_{2'',2'} = −14.3, *J*_{2'',1'} = 7.7, *J*_{2'',3'} = 6.4 Hz, 1 H, H-2''), 2.61 (ddd, *J*_{2',2''} = −14.3, *J*_{2',1'} = 6.2, *J*_{2',3'} = 3.2 Hz, 1 H, H-2'), 3.66 (dd, *J*_{1a,1b} = −11.7, *J*_{1a,2} = 5.8 Hz, 1 H, H-1a of glycerol), 3.73 (dd, *J*_{1b,1a} = −11.7, *J*_{1b,2} = 4.2 Hz, 1 H, H-1b of glycerol), 3.85 (dd, *J*_{5'',5'} = −12.6, *J*_{5'',4'} = 4.9 Hz, 1 H, H-5''), 3.91 (dd, partially obscured by next downfield signal, *J*_{5',5''} = −12.6, *J*_{5',4'} = 3.4 Hz, 1 H, H-5'), 3.92–4.01 (overlapped multiplets, 3 H, H-2 and H-3 of glycerol), 4.26 (ddd, *J*_{4',3'} = 3.2, *J*_{4',5'} = 3.4, *J*_{4',5''} = 4.9 Hz, 1 H, H-4'), 4.85 (ddt partially obscured by the residual HOD signal, *J*_{3',2'} = 6.4, *J*_{3',2''} = 3.2, *J*_{3',4'} = 3.2, *J*_{3',P} = 7.3 Hz, 1 H, H-3'), 6.37 (dd, *J*_{1',2'} = 7.7, *J*_{1',2''} = 6.2 Hz, 1 H, H-1'), 7.71 (q, *J* = 1.0 Hz, 1 H, H-6) ppm. ¹³C NMR (D₂O): δ = 14.4 (CH₃), 40.5 (d, *J*_{CCOP} = 2.6 Hz, C-2'), 63.9 (C-5'), 64.9 (C-1 of glycerol), 69.3 (d, *J*_{COP} = 5.6 Hz, C-3 of glycerol), 73.5 (d, *J*_{CCOP} = 7.9 Hz, C-2 of glycerol), 77.8 (d, *J*_{COP} = 5.1 Hz, C-3'), 87.9 (C-1'), 88.5 (d, *J*_{CCOP} = 6.1 Hz, C-4'), 114.4 (C-5), 140.4 (C-6), 154.6 (C-2), 169.4 (C-4) ppm. HRFAB-MS(−) calcd. for C₁₃H₂₀N₂O₁₀P [M − H][−] 395.0855, found 395.0838.

2-Cyanoethyl [(4R)-2,2-Dimethyl-1,3-dioxolan-4-yl]methyl Diisopropylamidophosphite (9): The synthesis and the purification of compound **9** were carried out as previously reported.^[1] Its purity was checked by its chromatographic and spectroscopic properties.

5'-(2'-Deoxy)adenylic Acid Mono[(2R)-2,3-dihydroxypropyl] Ester: The synthesis and the purification of the title compound were carried out as previously reported.^[1] ¹H NMR [TBA salt, (CD₃)₃COD]: δ = 1.11 (t, *J* = 7.3 Hz, 12 H, *N*-CH₂CH₂CH₂CH₃), 1.56 (m, 8 H, *N*-CH₂CH₂CH₂CH₃), 1.76 (m, 8 H, *N*-CH₂CH₂CH₂CH₃), 2.62 (ddd, *J*_{2'',2'} = −13.3, *J*_{2'',1'} = 6.7, *J*_{2'',3'} = 3.8 Hz, 1 H, H-2''), 2.87 (ddd, *J*_{2',2''} = −13.3, *J*_{2',1'} = 6.7, *J*_{2',3'} = 6.1 Hz, 1 H, H-2'), 3.39 (br. t, 8 H, *N*-CH₂CH₂CH₂CH₃), 3.70 (dd partially overlapping with next sig-

nal, $J_{1a,1b} = -11.5$, $J_{1a,2} = 5.7$ Hz, 1 H, H-1a of glycerol), 3.73 (dd partially overlapping with previous signal, $J_{1b,1a} = -11.5$, $J_{1b,2} = 6.3$ Hz, 1 H, H-1b of glycerol), 3.85 (dddd, $J_{2,1a} = 5.7$, $J_{2,1b} = 6.3$, $J_{2,3a} = 3.8$, $J_{2,3b} = 4.7$ Hz, 1 H, H-2 of glycerol), 4.07 (ddd partially overlapping with next signal, $J_{3a,3b} = -11.4$, $J_{3a,2} = 3.8$, $J_{3a,P} = 10.3$ Hz, 1 H, H-3a of glycerol), 4.10 (ddd partially overlapping with previous signal, $J_{3b,3a} = -11.4$, $J_{3b,2} = 4.7$, $J_{3b,P} = 10.0$ Hz, 1 H, H-3b of glycerol), 4.16–4.22 (overlapping multiplets, 2 H, H-5' and H-5''), 4.25 (m, 1 H, H-4'), 4.81 (ddd, $J_{3',2'} = 6.1$, $J_{3',2''} = 3.8$, $J_{3',4'} = 3.4$ Hz, 1 H, H-3'), 6.65 (t, $J_{1',2'} = J_{1',2''} = 6.7$ Hz, 1 H, H-1'), 8.30 (s, 1 H, H-2), 8.67 (s, 1 H, H-8) ppm.

3'-(5'-Deoxy-5-fluoro)uridylic Acid Mono[(2R)-2,3-dihydroxypropyl] Ester (12): A mixture of the 2'- and 3'-*O*-(*tert*-butyldimethylsilyl) derivatives of DFUR was first obtained by adaptation of the procedure reported by Usman et al.^[16] DFUR (60 mg, 0.24 mmol) was dissolved in anhydrous DMF (0.5 mL) under an argon atmosphere, and imidazole (45 mg, 0.66 mmol) and TBDMSCl in THF (1 M, 0.48 mL) were then added to the magnetically stirred solution. After the mixture had been kept at room temperature for 2 h, the reaction was quenched with 5% aqueous NaHCO₃ (7 mL) and the mixture was concentrated in vacuo. The residue was coevaporated with toluene, taken up in CH₂Cl₂, and extracted with H₂O. The organic layer was taken to dryness in vacuo and the residue was purified by column chromatography on silica gel; elution with a gradient of *i*PrOH in CH₂Cl₂/TEA (99:1) from 0 to 9% afforded a mixture of 3'-*O*-(*tert*-butyldimethylsilyl)-5'-deoxy-5-fluorouridine (**10**) and 2'-*O*-(*tert*-butyldimethylsilyl)-5'-deoxy-5-fluorouridine (**11**) (62.3 mg; 72% yield, altogether). Selected ¹H NMR (CDCl₃) data referring to the *tert*-butyldimethylsilyl moiety: **10**, $\delta = 0.16$ and 0.17 (singlets, 6 H altogether, CH₃), 0.96 [s, 9 H, C(CH₃)₃] ppm; **11**, $\delta = 0.02$ and 0.03 (singlets, 6H altogether, CH₃), 0.89 [s, 9 H, C(CH₃)₃] ppm.

A solution of compound **9** (110 mg, 0.33 mmol) in anhydrous CH₃CN (1 mL) and a 2.5% tetrazole solution (1.2 mL) in the same solvent were alternately added dropwise to the mixture of **10** and **11** (61 mg, 0.17 mmol) dissolved in anhydrous CH₃CN (6 mL). The reaction mixture was then treated according to the general procedure above, as reported for compound **4**. After ammonia treatment at room temperature, the solution was evaporated in vacuo and the residue was chromatographed on a silica gel column (*i*PrOH/concd. ammonia, 95:5). Fractions containing compound **V** ($R_f = 0.20$) were taken to dryness in vacuo, the residue was dissolved in 30% aqueous HOAc (15 mL), and the solution was allowed to stand for 7 h at room temperature, to remove the isopropylidene group and to accomplish the 2' desilylation. The solution was then evaporated in vacuo and the residue was purified by column chromatography on silica gel (*i*PrOH/H₂O/concd. ammonia, 85:10:5 to afford pure **12** as a colourless oil (23.8 mg, 35% yield from the mixture of **10** and **11**): $R_f = 0.40$. ¹H NMR (D₂O): $\delta = 1.51$ (d, $J_{5',4'} = 6.3$ Hz, 3 H, H-5'), 3.66 (dd, $J_{1a,1b} = -11.8$, $J_{1a,2} = 5.8$ Hz, 1 H, H-1a of glycerol), 3.73 (dd, $J_{1b,1a} = -11.8$, $J_{1b,2} = 4.3$ Hz, 1 H, H-1b of glycerol), 3.94–4.05 (partially overlapped multiplets, 3 H, H-2 and H-3 of glycerol), 4.37–4.43 (partially overlapped multiplets, 2 H, H-3', H-4'), 4.52 (t, $J_{2',1'} = 4.9$, $J_{2',3'} = 4.9$ Hz, 1 H, H-2'), 5.94 (dd, $J_{1',2'} = 4.9$, $J_{1',F} = 1.1$ Hz, 1 H, H-1'), 7.91 (d, $J_{6,F} = 6.3$ Hz, 1 H, H-6) ppm. ¹³C NMR (D₂O): $\delta = 20.7$ (C-5'), 65.0 (C-1 of glycerol), 69.4 (d, $J_{COP} = 3.8$ Hz, C-3 of glycerol), 73.6 (d, $J_{CCOP} = 7.9$ Hz, C-2 of glycerol), 75.5 (d, $J_{CCOP} = 2.4$ Hz, C-2'), 80.3 (d, $J_{COP} = 4.5$ Hz, C-3'), 82.5 (d, $J_{CCOP} = 3.9$ Hz, C-4'), 92.4 (C-1'), 128.4 (d, $J_{CCF} = 34.4$ Hz, C-6), 143.7 (d, $J_{CF} = 233.4$ Hz, C-5), 153.0 (C-2), 162.3 (d, $J_{CCF} = 26.6$ Hz, C-4) ppm. HRFAB-MS(–) calcd. for C₁₂H₁₇FN₂O₁₀P [M – H][–] 399.0605, found 399.0613.

3'- and 2'-[5'-Deoxy-5'-(methylthio)]adenylic Acid Mono[(2R)-2,3-dihydroxypropyl] Ester (17 and 18): In an adaptation of a reported procedure,^[17] MTA (100 mg, 0.34 mmol) was first converted into the corresponding *N*⁶-benzoyl derivative (**14**), which was then purified by column chromatography on silica gel, eluting with a gradient of CH₃OH in CH₂Cl₂ from 0 to 5% ($R_f = 0.13$, CH₂Cl₂/CH₃OH, 95:5); the pure fractions, evaporated in vacuo, gave compound **14** as a colourless oil (109 mg, 80%). Selected NMR spectroscopic data referring to the benzoyl group: ¹H NMR (CD₃OD): $\delta = 7.56$ (dd, $J_{ortho} = 8.4$, $J_{ortho} = 7.5$ Hz, 2 H, H-3 and H-5), 7.66 (tt, $J_{ortho} = 7.5$, $J_{meta} = 1.2$ Hz, 1 H, H-4), 8.09 (dd, $J_{ortho} = 8.4$, $J_{meta} = 1.2$ Hz, 2 H, H-2 and H-6) ppm. ¹³C NMR (CD₃OD): $\delta = 128.8$ (C-2 and C-6), 129.2 (C-3 and C-5), 133.2 (C-4), 134.2 (C-1), 167.2 (CONH) ppm. Compound **14** (109 mg, 0.27 mmol) was then treated with TBDMSCl as described for DFUR. Column chromatography on silica gel, eluting first with a gradient of CH₂Cl₂/TEA (99:1) in hexane/TEA (99:1) from 50 to 100% and then with a gradient of *i*PrOH in CH₂Cl₂/TEA (99:1) from 0 to 3%, afforded a mixture of compounds **15** and **16** (109.5 mg, 78% yield altogether). Selected ¹H NMR (CDCl₃) data referring to the *tert*-butyldimethylsilyl moiety: **15**, $\delta = 0.22$ and 0.23 (singlets, 6 H altogether, CH₃), 0.98 [s, 9 H, C(CH₃)₃] ppm; **16**, $\delta = 0.01$ and 0.02 (singlets, 6 H altogether, CH₃), 0.88 [s, 9 H, C(CH₃)₃] ppm.

A solution of compound **9** (136 mg, 0.41 mmol) in anhydrous CH₃CN (1.5 mL) and a 2.5% tetrazole solution (1.5 mL) in the same solvent were alternately added dropwise to the mixture of **15** and **16** (109 mg, 0.21 mmol) dissolved in anhydrous CH₃CN (7 mL). The reaction mixture was then treated according to the general procedure above reported for compounds **1–3**, except that the acid hydrolysis was carried out for 7 h. After evaporation of the solvent in vacuo, the residue was dissolved in 50% aqueous CH₃OH and purified by HPLC on a Zorbax SB-C18 semipreparative column, eluting with a gradient of CH₃CN in 0.1 M triethylammonium acetate (pH 7.0) from 5 to 20% in 25 min, at the flow rate of 3.5 mL min^{–1}. Compounds **17** ($t_R = 10.6$ min) and **18** ($t_R = 13.0$ min) were obtained as colourless oils.

Compound 17: 32.2 mg (34% yield from the mixture of **15** and **16**). ¹H NMR (D₂O): $\delta = 2.17$ (s, 3 H, S–CH₃), 3.02 (dd, $J_{5'',5'} = -14.4$, $J_{5'',4'} = 6.7$ Hz, 1 H, H-5''), 3.10 (dd, $J_{5',5''} = -14.4$, $J_{5',4'} = 4.4$ Hz, 1 H, H-5'), 3.68 (dd, $J_{1a,1b} = -11.7$, $J_{1a,2} = 6.0$ Hz, 1 H, H-1a of glycerol), 3.75 (dd, $J_{1b,1a} = -11.7$, $J_{1b,2} = 4.4$ Hz, 1 H, H-1b of glycerol), 3.97–4.08 (partially overlapped multiplets, 3 H, H-2 and H-3 of glycerol), 4.61 (ddd, $J_{4',3'} = 4.0$, $J_{4',5'} = 4.4$, $J_{4',5''} = 6.7$ Hz, 1 H, H-4'), 4.85 (ddd, $J_{3',2'} = 5.3$, $J_{3',4'} = 4.0$, $J_{3',P} = 7.8$ Hz, 1 H, H-3'), 5.08 (dd, $J_{2',1'} = 5.9$, $J_{2',3'} = 5.3$ Hz, 1 H, H-2'), 6.19 (d, $J_{1',2'} = 5.9$ Hz, 1 H, H-1'), 8.32 (s, 1 H, H-2), 8.45 (s, 1 H, H-8) ppm. ¹H NMR (TBA salt, (CD₃)₃COD): $\delta = 1.13$ (t, $J = 7.3$ Hz, 12 H, *N*–CH₂CH₂CH₂CH₃), 1.58 (m, 8 H, *N*–CH₂CH₂CH₂CH₃), 1.79 (m, 8 H, *N*–CH₂CH₂CH₂CH₃), 2.22 (s, 3 H, S–CH₃), 3.04 (dd, $J_{5'',5'} = -14.3$, $J_{5'',4'} = 6.1$ Hz, 1 H, H-5''), 3.12 (dd, $J_{5',5''} = -14.3$, $J_{5',4'} = 4.5$ Hz, 1 H, H-5'), 3.43 (br. t, 8 H, *N*–CH₂CH₂CH₂CH₃), 3.70 (dd partially overlapping with next signal, $J_{1a,1b} = -11.5$, $J_{1a,2} = 5.6$ Hz, 1 H, H-1a of glycerol), 3.73 (dd partially overlapping with previous signal, $J_{1b,1a} = -11.5$, $J_{1b,2} = 6.4$ Hz, 1 H, H-1b of glycerol), 3.90 (dddd, $J_{2,1a} = 5.6$, $J_{2,1b} = 6.4$, $J_{2,3a} = 3.9$, $J_{2,3b} = 4.9$ Hz, 1 H, H-2 of glycerol), 4.12 (ddd partially overlapping with next signal, $J_{3a,3b} = -11.3$, $J_{3a,2} = 3.9$, $J_{3a,P} = 9.0$ Hz, 1 H, H-3a of glycerol), 4.18 (ddd partially overlapping with previous signal, $J_{3b,3a} = -11.3$, $J_{3b,2} = 4.9$, $J_{3b,P} = 8.9$ Hz, 1 H, H-3b of glycerol), 4.53 (ddd, $J_{4',3'} = 4.8$, $J_{4',5'} = 4.5$, $J_{4',5''} = 6.1$ Hz, 1 H, H-4'), 4.93 (ddd, $J_{3',2'} = 5.2$, $J_{3',4'} = 4.8$, $J_{3',P} = 6.5$ Hz, 1 H, H-3'), 5.01 (dd, $J_{2',1'} = 4.9$, $J_{2',3'} =$

5.2 Hz, 1 H, H-2'), 6.24 (d, $J_{1',2'} = 4.9$ Hz, 1 H, H-1'), 8.33 (s, 1 H, H-2), 8.49 (s, 1 H, H-8) ppm. ^{13}C NMR (D_2O): $\delta = 18.2$ ($\text{S}-\text{CH}_3$), 38.6 (C-5'), 65.0 (C-1 of glycerol), 69.5 (d, $J_{\text{COP}} = 5.6$ Hz, C-3 of glycerol), 73.6 (d, $J_{\text{CCOP}} = 8.0$ Hz, C-2 of glycerol), 75.5 (d, $J_{\text{CCOP}} = 4.8$ Hz, C-2'), 78.8 (d, $J_{\text{COP}} = 5.2$ Hz, C-3'), 85.7 (d, $J_{\text{CCOP}} = 3.6$ Hz, C-4'), 90.1 (C-1'), 122.1 (C-5), 143.8 (C-8), 151.9 (C-4), 153.8 (C-2), 157.1 (C-6) ppm. HRFAB-MS(–) calcd. for $\text{C}_{14}\text{H}_{21}\text{N}_5\text{O}_8\text{PS}$ [$\text{M} - \text{H}$] $^-$ 450.0848, found 450.0851.

Compound 18: 38.9 mg (41% yield from the mixture of **15** and **16**). ^1H NMR (D_2O): $\delta = 2.19$ (s, 3 H, $\text{S}-\text{CH}_3$), 3.03 (dd, $J_{5'',5'} = -14.3$, $J_{5'',4'} = 6.7$ Hz, 1 H, H-5''), 3.08 (dd, $J_{5',5''} = -14.3$, $J_{5',4'} = 5.3$ Hz, 1 H, H-5'), 3.39 (dd, $J_{1a,1b} = -11.8$, $J_{1a,2} = 6.1$ Hz, 1 H, H-1a of glycerol), 3.45 (dd, partially overlapping with next signal, $J_{1b,1a} = -11.8$, $J_{1b,2} = 4.4$ Hz, 1 H, H-1b of glycerol), 3.47 (multiplet partially obscured by previous signal, 1 H, H-3a of glycerol), 3.62 (m, 1 H, H-2 of glycerol), 3.70 (m, 1 H, H-3b of glycerol), 4.43 (ddd, $J_{4',3'} = 3.7$, $J_{4',5'} = 5.3$, $J_{4',5''} = 6.7$ Hz, 1 H, H-4'), 4.61 (dd, $J_{3',2'} = 5.5$, $J_{3',4'} = 3.7$ Hz, 1 H, H-3'), 5.33 (ddd, $J_{2',1'} = 5.9$, $J_{2',3'} = 5.5$, $J_{2',p} = 8.8$ Hz, 1 H, H-2'), 6.28 (d, $J_{1',2'} = 5.9$ Hz, 1 H, H-1'), 8.36 (s, 1 H, H-2), 8.48 (s, 1 H, H-8) ppm. ^1H NMR (TBA salt, $(\text{CD}_3)_3\text{COD}$): $\delta = 1.11$ (t, $J = 7.3$ Hz, 12 H, $\text{N}-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 1.54 (m, 8 H, $\text{N}-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 1.75 (m, 8 H, $\text{N}-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 2.20 (s, 3 H, $\text{S}-\text{CH}_3$), 2.96 (dd, $J_{5'',5'} = -14.1$, $J_{5'',4'} = 6.3$ Hz, 1 H, H-5''), 3.06 (dd, $J_{5',5''} = -14.1$, $J_{5',4'} = 4.8$ Hz, 1 H, H-5'), 3.36 (br. t, 8 H, $\text{N}-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 3.65 (d, $J_{1,2} = 5.9$ Hz, 2 H, H-1 of glycerol), 3.81 (ddt, $J_{2,1} = 5.9$, $J_{2,3a} = 5.0$, $J_{2,3b} = 4.0$ Hz, 1 H, H-2 of glycerol), 3.97 (ddd, $J_{3a,3b} = -11.1$, $J_{3a,2} = 5.0$, $J_{3a,p} = 8.8$ Hz, 1 H, H-3a of glycerol), 4.02 (ddd, $J_{3b,3a} = -11.1$, $J_{3b,2} = 4.0$, $J_{3b,p} = 8.4$ Hz, 1 H, H-3b of glycerol), 4.37 (ddd, $J_{4',3'} = 5.4$, $J_{4',5'} = 4.8$, $J_{4',5''} = 6.3$ Hz, 1 H, H-4'), 4.78 (m partially obscured by the residual HOD signal, H-3'), 5.22 (ddd, $J_{2',1'} = 4.3$, $J_{2',3'} = 5.3$, $J_{2',p} = 6.3$ Hz, 1 H, H-2'), 6.37 (d, $J_{1',2'} = 4.3$ Hz, 1 H, H-1'), 8.30 (s, 1 H, H-2), 8.55 (s, 1 H, H-8) ppm. ^{13}C NMR (D_2O): $\delta = 18.1$ ($\text{S}-\text{CH}_3$), 38.6 (C-5'), 64.7 (C-1 of glycerol), 69.1 (d, $J_{\text{COP}} = 6.0$ Hz, C-3 of glycerol), 73.3 (d, $J_{\text{CCOP}} = 7.6$ Hz, C-2 of glycerol), 74.8 (d, $J_{\text{CCOP}} = 3.6$ Hz, C-3'), 79.1 (d, $J_{\text{COP}} = 5.2$ Hz, C-2'), 86.4 (C-4'), 89.0 (d, $J_{\text{CCOP}} = 6.0$ Hz, C-1'), 121.9 (C-5), 143.5 (C-8), 152.0 (C-4), 155.8 (C-2), 158.5 (C-6) ppm. HRFAB-MS(–) calcd. for $\text{C}_{14}\text{H}_{21}\text{N}_5\text{O}_8\text{PS}$ [$\text{M} - \text{H}$] $^-$ 450.0848, found 450.0834.

General Procedure for the Enzymatic Synthesis of 3'-*O*-Lysophosphatidyl Derivatives of 2'-Deoxynucleosides (5–8): The enzymatic synthesis and the purification of the title compounds were accomplished according to previous work.^[1] Each 3'GPdN (50 μmol) was first converted into the relevant TBA salt by passing it through a column of Dowex-50 W (TBA form). The aqueous eluate was taken to dryness in vacuo and the residue was allowed to stand under reduced pressure over P_2O_5 overnight. The residue was then dissolved in dried $t\text{BuOH}$ (20 mL) and the solution was added together with TFEP (500 μmol) and Lipzyme (40 mg). The suspension was shaken at 240 rpm for 48 h at 40 °C. Each reaction course was followed by HPLC monitoring of the rising amount of the relevant 3'-*O*-lysophosphatidyldeoxynucleoside. HPLC analyses, performed on Lichrospher-100 ODS with a linear gradient of CH_3CN in 0.1 M triethylammonium acetate (pH 7) from 0 to 70% over 30 min at a flow rate of 1.0 mL min^{-1} , in each case showed only one peak other than that of the substrate. The enzyme was then filtered off and the solvent was evaporated in vacuo. The residue was taken up in a pentane/ H_2O (1:1) mixture and the aqueous layer was concentrated in vacuo. The residue containing the acylated product was then purified by semipreparative HPLC on an Ultrasphere ODS column, with 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} .

3'-*O*-Lysophosphatidyl Derivative of 2'-Deoxyadenosine (5): 20.3 mg (63% yield from **1**). ^1H NMR (CD_3OD): $\delta = 0.90$ (t, $J = 6.9$ Hz, 3 H, CH_3 of palmitoyl), 1.26 and 1.27 (br. singlets, 24 H altogether, from γ - to ξ - CH_2 of palmitoyl), 1.59 (tt, $J_{\beta,\alpha} = 7.5$, $J_{\beta,\gamma} = 7.0$ Hz, 2 H, β - CH_2 of palmitoyl), 2.33 (t, $J = 7.5$ Hz, 2 H, α - CH_2 of palmitoyl), 2.67 (ddd, $J_{2'',2'} = -13.7$, $J_{2'',1'} = 5.8$, $J_{2'',3'} = 1.8$ Hz, 1 H, H-2''), 2.91 (ddd, $J_{2',2''} = -13.7$, $J_{2',1'} = 8.4$, $J_{2',3'} = 5.7$ Hz, 1 H, H-2'), 3.83 (dd, $J_{5'',5'} = -12.5$, $J_{5'',4'} = 2.9$ Hz, 1 H, H-5''), 3.86 (dd, $J_{5',5''} = -12.5$, $J_{5',4'} = 2.6$ Hz, 1 H, H-5'), 3.90–4.01 (partially overlapped multiplets, 3 H, H-2 and H-3 of glycerol), 4.12 (dd, $J_{1a,1b} = -11.4$, $J_{1a,2} = 5.7$ Hz, 1 H, H-1a of glycerol), 4.19 (dd, $J_{1b,1a} = -11.4$, $J_{1b,2} = 4.5$ Hz, 1 H, H-1b of glycerol), 4.33 (ddd, $J_{4',3'} = 1.8$, $J_{4',5'} = 2.6$, $J_{4',5''} = 2.9$ Hz, 1 H, H-4'), 5.03 (ddt, $J_{3',2'} = 5.7$, $J_{3',2''} = 1.8$, $J_{3',4'} = 1.8$, $J_{3',p} = 6.5$ Hz, 1 H, H-3'), 6.45 (dd, $J_{1',2'} = 8.4$, $J_{1',2''} = 5.8$ Hz, 1 H, H-1'), 8.19 (s, 1 H, H-2), 8.35 (s, 1 H, H-8) ppm. ^{13}C NMR (CD_3OD): $\delta = 14.4$ (CH_3 of palmitoyl), 23.7 (ξ - CH_2 of palmitoyl), 26.0 (β - CH_2 of palmitoyl), 30.2, 30.3, 30.4, 30.5, 30.66, 30.70, 30.72 (from γ - to μ - CH_2 of palmitoyl), 33.0 (ν - CH_2 of palmitoyl), 34.9 (α - CH_2 of palmitoyl), 40.5 (d, $J_{\text{CCOP}} = 3.8$ Hz, C-2'), 63.6 (C-5'), 66.2 (C-1 of glycerol), 67.7 (d, $J_{\text{COP}} = 5.5$ Hz, C-3 of glycerol), 69.9 (d, $J_{\text{CCOP}} = 7.6$ Hz, C-2 of glycerol), 77.6 (d, $J_{\text{COP}} = 4.3$ Hz, C-3'), 87.3 (C-1'), 88.8 (d, $J_{\text{CCOP}} = 6.1$ Hz, C-4'), 121.1 (C-5), 141.7 (C-8), 150.0 (C-4), 153.5 (C-2), 157.5 (C-6), 175.4 (COO) ppm. HRFAB-MS(–) calcd. for $\text{C}_{29}\text{H}_{49}\text{N}_5\text{O}_9\text{P}$ [$\text{M} - \text{H}$] $^-$ 642.3268, found 642.3260.

3'-*O*-Lysophosphatidyl Derivative of 2'-Deoxyguanosine (6): 10.6 mg (32% yield from **2**). ^1H NMR (CD_3OD): $\delta = 0.90$ (t, $J = 6.9$ Hz, 3 H, CH_3 of palmitoyl), 1.29 (br. s, 24 H, from γ - to ξ - CH_2 of palmitoyl), 1.59 (m, 2 H, β - CH_2 of palmitoyl), 2.33 (t, $J = 7.5$ Hz, 2 H, α - CH_2 of palmitoyl), 2.61 (ddd, $J_{2'',2'} = -13.8$, $J_{2'',1'} = 6.1$, $J_{2'',3'} = 2.6$ Hz, 1 H, H-2''), 2.81 (ddd, $J_{2',2''} = -13.8$, $J_{2',1'} = 7.6$, $J_{2',3'} = 6.1$ Hz, 1 H, H-2'), 3.80 (dd, $J_{5'',5'} = -12.3$, $J_{5'',4'} = 3.5$ Hz, 1 H, H-5''), 3.84 (dd, $J_{5',5''} = -12.3$, $J_{5',4'} = 3.5$ Hz, 1 H, H-5'), 3.90–4.02 (partially overlapped multiplets, 3 H, H-2 and H-3 of glycerol), 4.12 (dd, $J_{1a,1b} = -11.3$, $J_{1a,2} = 6.1$ Hz, 1 H, H-1a of glycerol), 4.19 (dd, $J_{1b,1a} = -11.3$, $J_{1b,2} = 4.6$ Hz, 1 H, H-1b of glycerol), 4.24 (dt, $J_{4',3'} = 2.7$, $J_{4',5'} = J_{4',5''} = 3.5$ Hz, 1 H, H-4'), 5.02 (dddd, $J_{3',2'} = 6.1$, $J_{3',2''} = 2.6$, $J_{3',4'} = 2.7$, $J_{3',p} = 6.7$ Hz, 1 H, H-3'), 6.27 (dd, $J_{1',2'} = 7.6$, $J_{1',2''} = 6.1$ Hz, 1 H, H-1'), 7.94 (s, 1 H, H-8) ppm. ^{13}C NMR (CD_3OD): $\delta = 14.4$ (CH_3 of palmitoyl), 23.7 (ξ - CH_2 of palmitoyl), 26.0 (β - CH_2 of palmitoyl), 30.23, 30.36, 30.40, 30.55, 30.67, 30.72, 30.74 (from γ - to μ - CH_2 of palmitoyl), 33.0 (ν - CH_2 of palmitoyl), 34.9 (α - CH_2 of palmitoyl), 40.1 (d, $J_{\text{CCOP}} = 3.3$ Hz, C-2'), 63.6 (C-5'), 66.2 (C-1 of glycerol), 67.7 (d, $J_{\text{COP}} = 4.3$ Hz, C-3 of glycerol), 69.9 (d, $J_{\text{CCOP}} = 8.2$ Hz, C-2 of glycerol), 77.5 (d, $J_{\text{COP}} = 4.5$ Hz, C-3'), 86.8 (C-1'), 88.5 (d, $J_{\text{CCOP}} = 6.4$ Hz, C-4'), 139.2 (C-8), 153.3 (C-4), 155.6 (C-2), 160.6 (C-6), 175.4 (COO) ppm, C-5 not detectable. HRFAB-MS(–) calcd. for $\text{C}_{29}\text{H}_{49}\text{N}_5\text{O}_{10}\text{P}$ [$\text{M} - \text{H}$] $^-$ 658.3217, found 658.3222.

3'-*O*-Lysophosphatidyl Derivative of 2'-Deoxycytidine (7): 12.4 mg (40% yield from **3**). ^1H NMR (CD_3OD): $\delta = 0.90$ (t, $J = 7.0$ Hz, 3 H, CH_3 of palmitoyl), 1.28 (br. s, 24 H, from γ - to ξ - CH_2 of palmitoyl), 1.61 (m, 2 H, β - CH_2 of palmitoyl), 2.29 (ddd, $J_{2'',2'} = -13.8$, $J_{2'',1'} = 7.1$, $J_{2'',3'} = 6.6$ Hz, 1 H, H-2''), 2.35 (t, $J = 7.5$ Hz, 2 H, α - CH_2 of palmitoyl), 2.60 (ddd, $J_{2',2''} = -13.8$, $J_{2',1'} = 6.0$, $J_{2',3'} = 3.3$ Hz, 1 H, H-2'), 3.82 (d, $J_{5',4'} = 2.9$ Hz, 2 H, H-5'), 3.87–3.94 (partially overlapped multiplets, 2 H, H-3 of glycerol), 3.97 (m, 1 H, H-2 of glycerol), 4.11 (dd, $J_{1a,1b} = -11.2$, $J_{1a,2} = 5.9$ Hz, 1 H, H-1a of glycerol), 4.18 (dd, partially obscured by H-4' signal, $J_{1b,1a} = -11.2$, $J_{1b,2} = 4.5$ Hz, 1 H, H-1b of glycerol),

4.19 (dt, partially overlapping with the previous signal, $J_{4',3'} = 3.5$, $J_{4',5'} = 2.9$ Hz, 1 H, H-4'), 4.82 (dddd, $J_{3',2'} = 6.6$, $J_{3',2''} = 3.3$, $J_{3',4'} = 3.5$, $J_{3',P} = 7.3$ Hz, 1 H, H-3'), 6.01 (d, $J_{5,6} = 7.7$ Hz, 1 H, H-5), 6.26 (dd, $J_{1',2'} = 7.1$, $J_{1',2''} = 6.0$ Hz, 1 H, H-1'), 8.23 (d, $J_{6,5} = 7.7$ Hz, 1 H, H-6) ppm. ^{13}C NMR (CD_3OD): $\delta = 14.4$ (CH_3 of palmitoyl), 23.7 ($\xi\text{-CH}_2$ of palmitoyl), 26.0 ($\beta\text{-CH}_2$ of palmitoyl), 30.2, 30.41, 30.45, 30.6, 30.72, 30.75, 30.77 (from γ - to $\mu\text{-CH}_2$ of palmitoyl), 33.0 ($\nu\text{-CH}_2$ of palmitoyl), 34.9 ($\alpha\text{-CH}_2$ of palmitoyl), 40.9 (d, $J_{\text{CCOP}} = 3.8$ Hz, C-2'), 62.4 (C-5'), 66.2 (C-1 of glycerol), 67.6 (d, $J_{\text{COP}} = 5.6$ Hz, C-3 of glycerol), 69.9 (d, $J_{\text{CCOP}} = 7.5$ Hz, C-2 of glycerol), 76.0 (d, $J_{\text{COP}} = 3.4$ Hz, C-3'), 87.7 (C-1'), 88.1 (d, $J_{\text{CCOP}} = 6.2$ Hz, C-4'), 95.4 (C-5), 144.5 (C-6), 153.7 (C-2), 164.6 (C-4), 175.4 (COO) ppm. HRFAB-MS(–) calcd. for $\text{C}_{28}\text{H}_{49}\text{N}_3\text{O}_{10}\text{P}$ [$\text{M} - \text{H}$] $^-$ 618.3156, found 618.3163.

3'-O-Lysophosphatidyl Derivative of 2'-Deoxythymidine (8): 18.4 mg (58% yield from **4**). ^1H NMR (CD_3OD): $\delta = 0.90$ (t, $J = 7.0$ Hz, 3 H, CH_3 of palmitoyl), 1.28 (br. s, 24 H, from γ - to $\xi\text{-CH}_2$ of palmitoyl), 1.61 (tt, $J_{\beta,\alpha} = 7.5$, $J_{\beta,\gamma} = 7.0$ Hz, 2 H, $\beta\text{-CH}_2$ of palmitoyl), 1.88 (d, $J = 1.1$ Hz, 3 H, CH_3 of thymine), 2.29 (ddd, $J_{2',1'} = -13.8$, $J_{2',1''} = 7.9$, $J_{2',3'} = 6.0$ Hz, 1 H, H-2'), 2.35 (t, $J = 7.5$ Hz, 2 H, $\alpha\text{-CH}_2$ of palmitoyl), 2.48 (ddd, $J_{2'',2'} = -13.8$, $J_{2'',1'} = 5.9$, $J_{2'',3'} = 2.2$ Hz, 1 H, H-2''), 3.81 (d, $J_{5',4'} = 2.7$ Hz, 2 H, H-5'), 3.87–4.00 (partially overlapped multiplets, 3 H, H-2 and H-3 of glycerol), 4.11 (dd, $J_{1a,1b} = -11.4$ Hz, $J_{1a,2} = 5.7$ Hz, 1 H, H-1a of glycerol), 4.14 (dt, $J_{4',3'} = 2.9$, $J_{4',5'} = 2.7$ Hz, 1 H, H-4'), 4.18 (dd, $J_{1b,1a} = -11.4$, $J_{1b,2} = 4.3$ Hz, 1 H, H-1b of glycerol), 6.30 (dd, $J_{1',2'} = 7.9$, $J_{1',2''} = 5.9$ Hz, 1 H, H-1'), 7.86 (q, $J = 1.1$ Hz, 1 H, H-6) ppm, the H-3' resonance is obscured by the residual HOD signal. ^{13}C NMR (CD_3OD): $\delta = 12.5$ (CH_3 of thymine), 14.5 (CH_3 of palmitoyl), 23.8 ($\xi\text{-CH}_2$ of palmitoyl), 26.0 ($\beta\text{-CH}_2$ of palmitoyl), 30.3, 30.5, 30.7, 30.8 (from γ - to $\mu\text{-CH}_2$ of palmitoyl), 33.1 ($\nu\text{-CH}_2$ of palmitoyl), 34.9 ($\alpha\text{-CH}_2$ of palmitoyl), 40.1 (d, $J_{\text{CCOP}} = 3.5$ Hz, C-2'), 62.8 (C-5'), 66.1 (C-1 of glycerol), 67.6 (d, $J_{\text{COP}} = 5.6$ Hz, C-3 of glycerol), 69.8 (d, $J_{\text{CCOP}} = 8.3$ Hz, C-2 of glycerol), 76.8 (d, $J_{\text{COP}} = 4.9$ Hz, C-3'), 86.2 (C-1'), 87.7 (d, $J_{\text{CCOP}} = 5.7$ Hz, C-4'), 111.6 (C-5), 138.1 (C-6), 151.5 (C-2), 168.5 (C-4), 175.4 (COO) ppm. HRFAB-MS(–) calcd. for $\text{C}_{29}\text{H}_{50}\text{N}_2\text{O}_{11}\text{P}$ [$\text{M} - \text{H}$] $^-$ 633.3152, found 633.3164.

3'-O-Lysophosphatidyl Derivative of 5'-Deoxy-5-fluorouridine (13): Compound **12** (TBA salt; 50 μmol) was allowed to stand under reduced pressure over P_2O_5 overnight, was then dissolved in dried *t*-BuOH (80 mL), and the solution was added together with TFEP (2.0 mmol) and Lipozyme (440 mg). The suspension was shaken at 240 rpm for 5 days at 38 °C. The analytical monitoring of the reaction course, and the isolation and the purification of the acylated product were accomplished as described for compounds **5–8**. 28.7 mg (90% yield from **12**). ^1H NMR (CD_3OD): $\delta = 0.90$ (t, $J = 6.9$ Hz, 3 H, CH_3 of palmitoyl), 1.29 (br. s, 24 H, from γ - to $\xi\text{-CH}_2$ of palmitoyl), 1.43 (d, $J_{5',4'} = 6.3$ Hz, 3 H, H-5'), 1.61 (tt, $J_{\beta,\alpha} = 7.5$, $J_{\beta,\gamma} = 7.0$ Hz, 2 H, $\beta\text{-CH}_2$ of palmitoyl), 2.35 (t, $J = 7.5$ Hz, 2 H, $\alpha\text{-CH}_2$ of palmitoyl), 3.92–4.00 (overlapped multiplets, 3 H, H-2 and H-3 of glycerol), 4.11 (dd, $J_{1a,1b} = -11.4$, $J_{1a,2} = 5.6$ Hz, 1 H, H-1a of glycerol), 4.18 (dd, $J_{1b,1a} = -11.4$, $J_{1b,2} = 4.2$ Hz, 1 H, H-1b of glycerol), 4.23 (dq, $J_{4',3'} = 5.4$, $J_{4',5'} = 6.3$ Hz, 1 H, H-4'), 4.27 (ddd, $J_{3',2'} = 5.1$, $J_{3',4'} = 5.4$, $J_{3',P} = 6.7$ Hz, 1 H, H-3'), 4.33 (dd, $J_{2',1'} = 4.6$, $J_{2',3'} = 5.1$ Hz, 1 H, H-2'), 5.85 (dd, $J_{1',2'} = 4.6$, $J_{1',F} = 1.0$ Hz, 1 H, H-1'), 7.76 (d, $J_{6,F} = 6.5$ Hz, 1 H, H-6) ppm. ^{13}C NMR (CD_3OD): $\delta = 14.4$ (CH_3 of palmitoyl), 18.9 (C-5'), 23.7 ($\xi\text{-CH}_2$ of palmitoyl), 26.0 ($\beta\text{-CH}_2$ of palmitoyl), 30.23, 30.40, 30.44, 30.6, 30.71, 30.74, 30.76 (from γ - to $\mu\text{-CH}_2$ of palmitoyl), 33.0 ($\nu\text{-CH}_2$ of palmitoyl), 34.9 ($\alpha\text{-CH}_2$ of palmitoyl), 66.2 (C-1 of glycerol), 67.8 (d, $J_{\text{COP}} = 4.3$ Hz, C-3 of glycerol), 69.9 (d,

$J_{\text{CCOP}} = 6.8$ Hz, C-2 of glycerol), 74.4 (d, $J_{\text{CCOP}} = 3.0$ Hz, C-2'), 79.3 (d, $J_{\text{COP}} = 4.6$ Hz, C-3'), 80.2 (d, $J_{\text{CCOP}} = 5.3$ Hz, C-4'), 91.0 (C-1'), 126.0 (d, $J_{\text{CCF}} = 34.0$ Hz, C-6), 142.0 (d, $J_{\text{CF}} = 233.5$ Hz, C-5), 150.9 (C-2), 159.4 (d, $J_{\text{CCF}} = 25.9$ Hz, C-4), 175.4 (COO) ppm. HRFAB-MS(–) calcd. for $\text{C}_{28}\text{H}_{47}\text{FN}_2\text{O}_{11}\text{P}$ [$\text{M} - \text{H}$] $^-$ 637.2902, found 637.2922. Before elemental analysis, a water solution of chromatographically pure **13** was lyophilized and the resulting highly hygroscopic powder was allowed to stand overnight over P_2O_5 . $\text{C}_{28}\text{H}_{48}\text{FN}_2\text{O}_{11}\text{P}\cdot\text{H}_2\text{O}$ (656.68): calcd. C 51.21, H 7.67, N 4.27; found C 51.35, H 7.69, N 4.26.

5'-O-Lysophosphatidyl Derivative of 5'-Deoxy-5'-(methylthio)adenosine (19): Compound **17** (TBA salt; 50 μmol) was worked up as described above for compound **12**. After incubation and isolation, pure compound **19** was obtained by HPLC on Zorbax SB-C18 semipreparative column with a linear gradient of CH_3CN in 0.1 M triethylammonium acetate (pH 7.0) from 0 to 80% in 40 min, at the flow rate of 3.5 mL min^{-1} . 31.2 mg (90% yield from **17**). ^1H NMR (CD_3OD): $\delta = 0.90$ (t, $J = 7.0$ Hz, 3 H, CH_3 of palmitoyl), 1.27 and 1.29 (br. singlets, 24 H altogether, from γ - to $\xi\text{-CH}_2$ of palmitoyl), 1.60 (m, 2 H, $\beta\text{-CH}_2$ of palmitoyl), 2.11 (s, 3 H, S- CH_3), 2.34 (t, $J = 7.5$ Hz, 2 H, $\alpha\text{-CH}_2$ of palmitoyl), 2.95 (dd, $J_{5'',5'} = -14.2$, $J_{5'',4'} = 6.6$ Hz, 1 H, H-5''), 3.02 (dd, $J_{5',5''} = -14.2$, $J_{5',4'} = 4.5$ Hz, 1 H, H-5'), 3.97–4.03 (overlapped multiplets, 3 H, H-2 and H-3 of glycerol), 4.12 (dd, $J_{1a,1b} = -11.4$, $J_{1a,2} = 5.5$ Hz, 1 H, H-1a of glycerol), 4.19 (dd, $J_{1b,1a} = -11.4$, $J_{1b,2} = 4.4$ Hz, 1 H, H-1b of glycerol), 4.45 (ddd, $J_{4',3'} = 4.3$, $J_{4',5'} = 4.5$, $J_{4',5''} = 6.6$ Hz, 1 H, H-4'), 4.80 (ddd, $J_{3',2'} = 5.2$, $J_{3',4'} = 4.3$, $J_{3',P} = 7.4$ Hz, 1 H, H-3'), 4.96 (dd, $J_{2',1'} = 5.4$, $J_{2',3'} = 5.2$ Hz, 1 H, H-2'), 6.06 (d, $J_{1',2'} = 5.4$ Hz, 1 H, H-1'), 8.22 (s, 1 H, H-2), 8.36 (s, 1 H, H-8) ppm. ^{13}C NMR (CD_3OD): $\delta = 14.4$ (CH_3 of palmitoyl), 16.7 (S- CH_3), 23.7 ($\xi\text{-CH}_2$ of palmitoyl), 26.0 ($\beta\text{-CH}_2$ of palmitoyl), 30.22, 30.32, 30.40, 30.46, 30.60, 30.75, 30.77 (from γ - to $\mu\text{-CH}_2$ of palmitoyl), 33.1 ($\nu\text{-CH}_2$ of palmitoyl), 34.9 ($\alpha\text{-CH}_2$ of palmitoyl), 37.4 (C-5'), 66.2 (C-1 of glycerol), 67.9 (d, $J_{\text{COP}} = 5.6$ Hz, C-3 of glycerol), 69.9 (d, $J_{\text{CCOP}} = 8.0$ Hz, C-2 of glycerol), 74.5 (d, $J_{\text{CCOP}} = 3.6$ Hz, C-2'), 77.6 (d, $J_{\text{COP}} = 5.2$ Hz, C-3'), 84.8 (d, $J_{\text{CCOP}} = 5.2$ Hz, C-4'), 89.6 (C-1'), 120.6 (C-5), 141.4 (C-8), 150.9 (C-4), 153.9 (C-2), 157.3 (C-6), 175.4 (COO) ppm. HRFAB-MS(–) calcd. for $\text{C}_{30}\text{H}_{51}\text{N}_5\text{O}_9\text{PS}$ [$\text{M} - \text{H}$] $^-$ 688.3145, found 688.3151. For elemental analysis purpose, pure **19** from preparative HPLC was lyophilized and the resulting dried powder was allowed to stand overnight over P_2O_5 . $\text{C}_{30}\text{H}_{52}\text{N}_5\text{O}_9\text{PS}\cdot\text{H}_2\text{O}$ (707.82): calcd. C 50.91, H 7.69, N 9.89; found C 51.01, H 7.72, N 9.91.

2'-O-Lysophosphatidyl Derivative of 5'-Deoxy-5'-(methylthio)adenosine (20): To obtain the title compound, **18** (TBA salt; 50 μmol) was treated by the same procedure as described above for the relevant 3'- isomer (**17**). 28.9 mg (84% yield from **18**). ^1H NMR (CD_3OD): $\delta = 0.90$ (t, $J = 7.0$ Hz, 3 H, CH_3 of palmitoyl), 1.28 and 1.29 (br. singlets, 24 H altogether, from γ - to $\xi\text{-CH}_2$ of palmitoyl), 1.60 (quintet, $J = 7.5$ Hz, 2 H, $\beta\text{-CH}_2$ of palmitoyl), 2.10 (s, 3 H, S- CH_3), 2.33 (t, $J = 7.5$ Hz, 2 H, $\alpha\text{-CH}_2$ of palmitoyl), 2.87 (dd, $J_{5'',5'} = -14.1$, $J_{5'',4'} = 6.3$ Hz, 1 H, H-5''), 2.96 (dd, $J_{5',5''} = -14.1$, $J_{5',4'} = 5.4$ Hz, 1 H, H-5'), 3.72 (m, 1 H, H-3a of glycerol), 3.81–3.87 (overlapped multiplets, 2 H, H-2 and H-3b of glycerol), 4.01 (dd, $J_{1a,1b} = -11.5$, $J_{1a,2} = 5.8$ Hz, 1 H, H-1a of glycerol), 4.05 (dd, $J_{1b,1a} = -11.5$, $J_{1b,2} = 4.4$ Hz, 1 H, H-1b of glycerol), 4.23 (ddd, $J_{4',3'} = 4.7$, $J_{4',5'} = 5.4$, $J_{4',5''} = 6.3$ Hz, 1 H, H-4'), 4.60 (dd, $J_{3',2'} = 5.2$, $J_{3',4'} = 4.7$ Hz, 1 H, H-3'), 5.28 (ddd, $J_{2',1'} = 4.9$, $J_{2',3'} = 5.2$, $J_{2',P} = 7.5$ Hz, 1 H, H-2'), 6.20 (d, $J_{1',2'} = 4.9$ Hz, 1 H, H-1'), 8.22 (s, 1 H, H-2), 8.36 (s, 1 H, H-8) ppm. ^{13}C NMR (CD_3OD): $\delta = 14.4$ (CH_3 of palmitoyl), 16.5 (S- CH_3), 23.7 (ξ -

CH₂ of palmitoyl), 26.0 (β-CH₂ of palmitoyl), 30.23, 30.43, 30.47, 30.58, 30.63, 30.77 (from γ- to μ-CH₂ of palmitoyl), 33.1 (ν-CH₂ of palmitoyl), 34.9 (α-CH₂ of palmitoyl), 37.4 (C-5'), 66.1 (C-1 of glycerol), 67.8 (d, $J_{\text{COP}} = 5.6$ Hz, C-3 of glycerol), 69.7 (d, $J_{\text{CCOP}} = 7.7$ Hz, C-2 of glycerol), 73.7 (d, $J_{\text{CCOP}} = 3.0$ Hz, C-3'), 78.1 (d, $J_{\text{COP}} = 5.1$ Hz, C-2'), 85.5 (C-4'), 88.7 (d, $J_{\text{CCOP}} = 6.7$ Hz, C-1'), 142.0 (C-8), 151.1 (C-4), 153.5 (C-2), 157.0 (C-6), 175.4 (COO) ppm, C-5 not detectable. HRFAB-MS(–) calcd. for C₃₀H₅₁N₅O₉PS [M – H][–] 688.3145, found 688.3155.

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- [1] R. Chillemi, D. Russo, S. Sciuto, *J. Org. Chem.* **1998**, *63*, 3224–3229.
- [2] R. A. Firestone, J. M. Pisano, J. R. Falck, M. M. McPaul, M. Krieger, *J. Med. Chem.* **1984**, *27*, 1037–1043.
- [3] G. M. Dubowchik, R. A. Firestone, *Bioconjugate Chem.* **1995**, *6*, 427–439.
- [4] Y.-F. Wang, J. J. Lalonde, M. Momongan, D. E. Bergbreiter, C.-H. Wong, *J. Am. Chem. Soc.* **1988**, *110*, 7200–7205.

- [5] F. X. Malcata, H. R. Reyes, H. S. Garcia, C. G. Hill Jr., C. H. Amundson, *Enzyme Microb. Technol.* **1992**, *14*, 426–446.
- [6] P. Amodeo, A. Motta, D. Picone, G. Saviano, T. Tancredi, P. A. Temussi, *J. Magn. Reson.* **1991**, *95*, 201–207.
- [7] N. Bourhim, M. S. Elkebbaj, Y. Gargouri, P. Giraud, J. Rietschoten, C. Olivier, R. Verger, *Colloids Surf. B* **1993**, *1*, 203–211.
- [8] R. Maget-Dana, *Biochim. Biophys. Acta* **1999**, *1462*, 109–140.
- [9] Y. Fukuda, S. Hirao, I. Koyoama, H. Takatori, M. Terakura, K. Mayumi, Y. Tsukazaki, H. Kinoshita, *Gan To Kagaku Ryoho* **2001**, *28*, 217–220.
- [10] R. Sumimoto, M. Takahashi, T. Etoh, Y. Ichiba, K. Emoto, Y. Imaoka, Y. Ishizaki, N. Tokumoto, *Gan To Kagaku Ryoho* **2001**, *28*, 83–86.
- [11] K. K. Sato, H. Hiraide, S. Tamakuma, T. Tabei, M. Maruyama, M. Touma, H. Okamura, F. Matsumoto, S. Akao, H. Ishikawa, Y. Yagi, H. Mochizuchi, *Breast Cancer* **2001**, *8*, 58–62.
- [12] H. Takahashi, Y. Maeda, K. Watanabe, K. Taguchi, F. Sasaki, S. Todo, *Int. J. Oncol.* **2000**, *17*, 1205–1211.
- [13] S.-H. Lee, Y.-D. Cho, *Exp. Cell Res.* **1998**, *240*, 282–292.
- [14] K. Miyaji, E. Tani, A. Nakano, H. Ikemoto, K. Kaba, *J. Neurosurg.* **1995**, *83*, 690–697.
- [15] W. Steglich, G. Hofle, *Angew. Chem. Int. Ed. Eng.* **1969**, *8*, 981.
- [16] N. Usman, K. K. Ogilvie, M.-Y. Jiang, R. J. Cedergren, *J. Am. Chem. Soc.* **1987**, *109*, 7845–7854.
- [17] J. Stawinski, T. Hozumi, S. A. Narang, C. P. Bahl, R. Wu, *Nucleic Acids Res.* **1977**, *4*, 353–371.

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