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Synthesis and Antiproliferative Potency of 9-β-D-Arabinofuranosyl-2-fluoroadenine Phospholipid Adducts

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Abstract—Three novel alkylphospholipid and four novel *O*-alkylglycerophospholipid derivatives of fludarabine (F-ara-AMP), known as a drug for the clinical treatment of chronic lymphocytic leukemia, were synthesized. The antiproliferative activity was determined in comparison to the parent nucleoside fludarabine in an immortalized but nontumorigenic human mammary epithelial cell line (H 184 A1N4), in two human breast tumor cell lines (MaTu and MCF7), and in two leukemic cell lines (HL 60 and Daudi). Fludarabine inhibited the growth of the leucemic cell lines very effectively. The breast tumor cell lines responded with much less sensitivity. The antiproliferative potency of the new compounds strongly depended on the chemical structure of the lipid component, and derivatives with a high effectiveness against one or both of the breast tumor cell lines were described.

Introduction

The purine derivative 9-β-D-arabinofuranosyl-2-fluoroadenine (F-ara-A), known as Fludara®, first entered into clinical trials in 1982 for clinical treatment of lymphocytic hematological malignancies. To enhance solubility, Fludara® is formulated as the monophosphate (F-ara-AMP, fludarabine) **1** (Fig. 1), which is instantaneously and quantitatively dephosphorylated to the parent nucleoside upon intravenous infusion.[1,2] Inside the cells rephosphorylation occurs which leads finally to fluoroadenine arabinoside triphosphate (F-ara-ATP), the major cytotoxic metabolite of F-ara-A.[3] This metabolite inhibits several key processes necessary for the DNA replication, i.e. enzymes required in the DNA synthesis, such as ribonucleotide reductase, DNA primase, DNA polymerase, 3′-5′ exonuclease activity of DNA polymerase δ and ε and DNA ligase I.[4] Initial preclinic studies demonstrated antitumor activity against acute myelogenous leukemia (AML). In phase I studies fatal neurotoxicity was found as dose limiting toxicity.[5] However, phase II investigations have confirmed the efficacy of fludarabine in lymphoid malignancies, e.g. non-Hodgkin's lymphoma, mycosis fungoides, and chronic lymphocytic leukemia (CLL)).[6] In 1991 fludarabine was introduced for use in the management of patients with CLL in the USA, in 1994 permission for the clinical treatment of CLL was given in Europe. Since fludarabine has a high therapeutic potential for the treatment of AML, combination therapies with cytosine-arabinoside (ara-C) are currently being developed as an effective new approach.[4,7]

Recently, several scientific groups described the preparation of phospholipid adducts with the cytostatically active nucleoside cytosine-arabinoside (ara-C), which exhibits cytostatic effectiveness superior to that of the mother compound.[8–10] This observation may be explained by the fact that two cytotoxic principles with different target sites are effective inside a neoplastic cell: (a) mitogenic signal transduction by the phospholipid component and (b) nucleic acid metabolism of the nucleoside component. The combination can result in modified properties or even synergistic effects. Moreover, increased biostability of the nucleosides in form of their phospholipid adducts may lead to improved bioavailability. Previous studies showed that the effectiveness of nucleoside phospholipid adducts depends strongly on the chemical structure of the phospholipid moiety.[11,12] Thus, corresponding compounds containing suitably substituted *O*-alkylglycerophospho groups or long-chain alkyl groups like the hexadecyl chain were found to be agents with high biological potency. Furthermore, improved effectiveness could be observed with alkylphosphono derivatives.[11,12] Based on these results, we prepared three novel alkylphospholipid adducts of Fludara®, 2-fluoro-ara-adenosine-5′-hexadecylphosphate **2**, the corresponding 5′-hexadecyldiphosphate **3** and the 5′-hexadecylphosphonophosphate **4**. In addition, four novel *O*-alkylglycerophospholipid adducts of F-ara-A: two monophosphates (**5** and **6**), one diphosphate (**7**)

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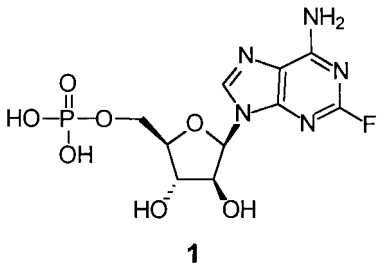

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Figure 1. Structure of F-ara-AMP (fludarabine **1**).

and one phosphonophosphate (**8**), all of them containing antiproliferative active glycerolipid components,[13,14] have been synthesized. The antiproliferative activity in vitro of all new compounds against the cell lines H 184 A1N4, MaTu, MCF7, Daudi and HL 60 was determined and compared to that of the parent nucleoside 1 revealing that the phospholipid adducts showed a higher effectiveness against the solid mammary tumor cell line MaTu whereas the parent nucleoside fludarabine preferably affected the leukemic cell lines Daudi and HL 60. The results also show that the biological activity of the compounds tested strongly depends on the chemical structure of the lipid component.

Results and Discussion

Chemistry

Compound **2** was prepared starting from fludarabine (F-ara-AMP) **1** (Fig. 2). After acetylation, condensation of the peracetylated intermediate with hexadecyl alcohol was carried out in the presence of 2,4,6-triisopropylphenylsulfonyl chloride (TPS). After removal of the protective groups from the nucleoside by treatment with methanolic ammonia and subsequent chromatographic purification, the desired product **2** was obtained in 35% yield. When the TPS-mediated condensation reaction was conducted without protection of the active groups of the starting compound a complex mixture of numerous products was obtained. On the other hand, this direct approach was successful when DCC was used instead of TPS, but the yield of compound **2** was comparatively low (12%).

For the preparation of **3** and **4** two pathways were studied: (a) the preparation of 2-fluoro-ara-adenosine-5′-phospho morpholidate and subsequent coupling with hexadecylphosphate (or hexadecylphosphonate) and (b) the preparation of the alkylphospho(no)morpholidates followed by the condensation with F-ara-AMP **1**. The latter method (Fig. 3) is preferred to the former since it led mainly to the desired compounds with a noticeable formation of side products, which could be separated chromatographically. Substance **3** was obtained with 38%, substance **4** with 27% yield.

The *O*-alkylglyceromonophospho derivatives **5** and **6** were prepared by condensation of **1** with the corresponding alcohol in the presence of TPS (Fig. 2). A lot of side products also formed during the condensation with the acetyl-protected F-ara-AMP. These side products could be successfully removed by flash chromatography on silica gel. The desired pure compounds **5** and **6** were obtained in 5–6% yield.

The substances **7** and **8** were synthesized by reaction of the new 1-*O*-alkylglycero phospho and phosphono morpholidates with **1** (Fig. 3). Both derivatives were purified by flash-chromatography and obtained in 3% yield.

The structures and the purity of the new products were confirmed by mass spectra, ^{1}H and ^{31}P NMR spectra and by TLC (see Experimental).

Biological assays

The seven new compounds have been tested for their antiproliferative activity in comparison to the parent nucleoside fludarabine (**1**) against five cell lines, the immortalized but nontumorigenic human mammary epithelial cell line H 184 A1N4 (H184), the human mammary tumor cells lines MaTu and MCF7 and the leukemic cell lines Daudi and HL 60 (see Experimental). The results are presented in Figure 4. The values given represent the concentration of the compounds resulting in 50% inhibition of cell growth (IC_{50}).

The antiproliferative potency of the compounds varied strongly depending on both their structure and the type of cell line studied. The parent nucleoside **1** preferably affected the leucemic cell lines Daudi and HL 60 and inhibited the growth of the other cell lines in the following rank order: H 184 A1N4 > MCF7 > MaTu. In contrast, most of the phospholipid nucleoside adducts (with the exception of compounds **3** and **6**) showed a

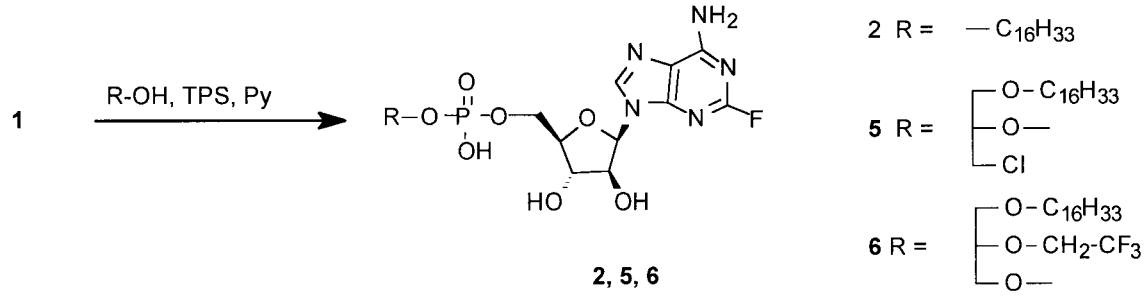


Figure 2. Synthesis and structure of the alkyl monophospholipid adduct **2** and the glycero monophospholipid adducts **5** and **6** of F-ara-A.

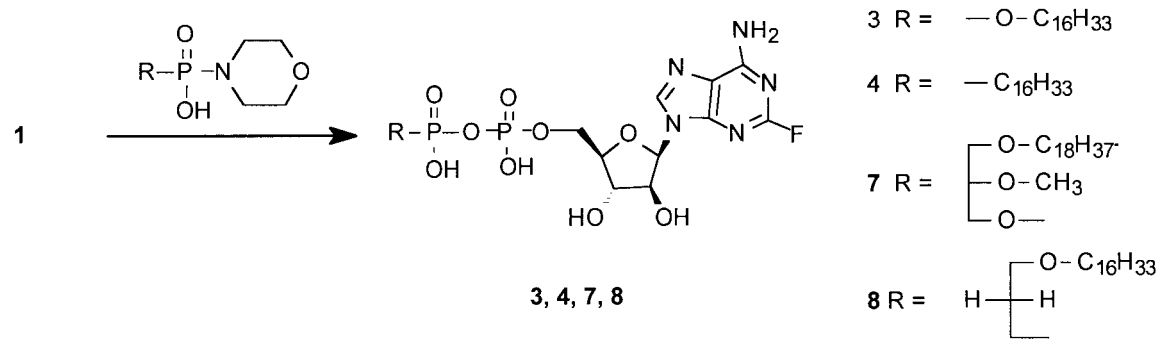
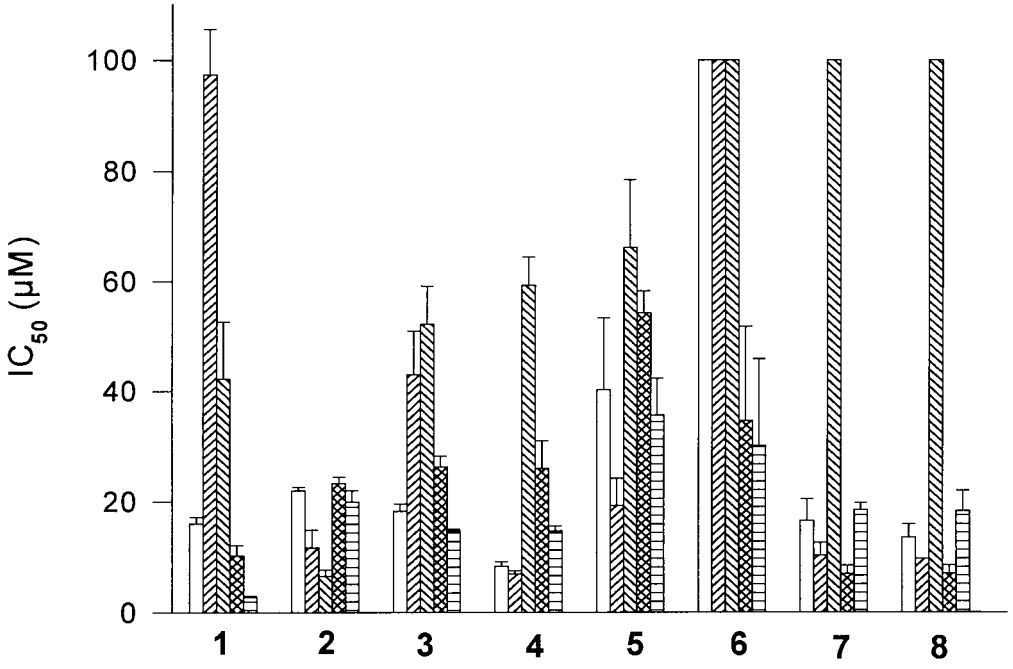


Figure 3. Synthesis and structure of the alkyl phospholipid adducts **3** and **4** and the 1-O-alkylglycero phospholipid adducts **7** and **8** of F-ara-A.



	1	2	3	4	5	6	7	8
H 184[a)]	16.0± 1.2	21.8±1.0	18.2±1.0	08.3±1.0	40.2±13.2	>100	16.5±4.0	13.5±2.4
MaTu [a)]	97.3± 8.2	11.5±3.0	36.0±5.0	07.0±1.0	19.3±4.9	>100	10.3±2.3	39.7±6.0
MCF 7[a)]	42.2±10.5	6.6±1.1	52.2±6.9	59.3±5.2	66.2±12.2	>100	>100	>100
Daudi [b)]	10.3± 1.8	23.3±1.2	26.3±2.0	26.0±5.1	54.3±4.0	34.7±17.1	7.0±1.5	7.0±1.5
HL 60 [b)]	2.8± 0.3	14.7±0.3	14.7±0.9	20.0±2.0	35.7±6.6	30.2±15.7	18.5±1.3	18.2±3.8

[a]Determined by XTT assay.
[b]Determined by cell counting.

Figure 4. Growth inhibitory potencies (IC_{50}; μM) of fludarabine **1** and the phospholipid adducts **2–8** in an immortalized human mammary epithelial cell line (H 184), breast tumor cell lines (MaTu, MCF7), the B lymphoblast cell line Daudi and the leukemic promyelocytic cell line HL 60.

high antiproliferative potency against MaTu cells, a tumor cell line derived from a solid human mammary carcinoma.[19] The IC_{50} values were in the same range as those of fludarabine in HL 60 cells. All of the derivatives, except compounds **5** and **6**, showed a good activity against the immortalized mammary epithelial cell line H 184 A1N4. Comparing the two breast tumor cell lines it was found that MCF7 cells, itself derived from a pleural effusion from breast cancer were more sensitive than MaTu cells only in response to fludarabine and compound 2. The opposite is true in the case of the other derivatives. With compound **4**, and especially with the compounds **7** and **8**, the greatest difference between the two breast tumor cell lines has been observed because MCF7 cells did not respond to compounds **6**, **7**, and **8**. Fludarabine has been successfully used in clinical trials against lymphoid leukemic malignancies. In our results this is reflected by the fact that it has its strongest effects on leukemic cell lines used, but both phospholipid adducts **7** and **8** show similarly high activities on the HL 60 cell line. To what extent the fludara phospholipid adducts will be helpful for the treatment of solid tumors, as the results with the MaTu cell line indicate, should be further evaluated.

Experimental

Compounds

Fludarabine (F-ara-AMP) was obtained from the Schering AG (Berlin, Germany). Hexadecylphosphate was obtained by reaction of hexadecyl alcohol and phosphorus oxychloride in hexane in the presence of triethylamine according to known procedures.[15] Hexadecylphosphonate was prepared by the Michaelis–Arbuzov reaction of hexadecyl bromide with triethyl phosphite and conversion of the obtained diethyl ester into the bis(trimethylsilyl) ester followed by hydrolysis of the latter.[11] 1-Chloro-1-deoxy-3-*O*-hexadecylglycerol was synthesized from epichlorohydrin and hexadecanol in the presence of stannic chloride.[15] 1-*O*-Hexadecyl-2-*O*-(2,2,2-trifluoroethyl)glycerol was prepared according to Ref. 16 and the hexadecyloxypropyl phosphonate according to Ref. 11. 2-*O*-Methyl-1-*O*-octadecyl-glycero-3-phosphate was obtained by phospholipase D-catalyzed hydrolysis of the corresponding glycerophosphocholine.[17] Pyridine was distilled and stored over calcium hydride. Dry ethanol-free chloroform was obtained by chromatography on Al_2O_3. All other solvents were distilled prior to use. All reactions were carried out at room temperature unless otherwise stated.

Analytic methods

TLC (thin layer chromatography) was carried out on Merck silicagel 60 F_{254} plates using solvent mixtures containing $CHCl_3$:MeOH:NH_4OH 70:30:4 (solvent A), 70:40:10 (solvent B), 60:30:10 (solvent C) or $CHCl_3$/MeOH:H_2O:acetic acid 50:30:8:4 (solvent D). Phosphorus containing compounds were visualized with Zinzade's reagent modified according to Beiss,[18] long chain alkyl or 1-*O*-alkylglycero containing components were detected by fluorescent spots (366 nm) on the TLC plate after spraying with 2,7-dichlorofluorescein in EtOH (0.2%), and the nucleosides could be visualized by ultraviolet absorption (254 nm). Column chromatography was performed with Merck silica gel 60 (0.015–0.040 mm). The eluents were solvent A, C, solvent E ($CHCl_3$:MeOH:NH_4OH 80:20:4) or as described otherwise. ^{1}H NMR spectra were recorded on a Varian Gemini 300 spectrometer operating at 300 MHz, ^{31}P NMR spectra on a UNITYplus-300 operating at 122 MHz in DMSO-d_6 unless otherwise stated, chemical shifts (δ) are reported in ppm and referenced to tetramethylsilane. Electrospray ionization mass spectra (ESI MS) or fast atom bombardement mass spectra (FAB MS) were recorded in positive ion mode on a Varian MAT 95 mass spectrometer.

In vitro studies

We used an immortalized (by benzpyrene), but non-tumorigenic human mammary epithelial cell line (H 184 A1N4) (obtained from Dr. M. Stampfer, Lawrence Berkeley Laboratory, University of California, Berkeley, USA), the human breast cancer cell lines MaTu[19] and MCF7, the B lymphoblast cell line Daudi and the leukemic promyelocytic cell line HL 60.

The H 184 A1N4 and the MaTu cell lines were grown in α-MEM/HAMS F12 (1:1, v/v) medium as described.[14] MCF7, Daudi and HL 60 cells were cultured in RPMI medium containing 10 % FCS. The antiproliferative potency of the nucleoside derivatives in the mammary epithelial cell lines was determined using the XTT assay (Cell Proliferation Kit II, Boehringer, Mannheim). Cells were seeded in microtiter plates (Costar) and incubated for 48 h at 37 °C and 5% CO_2. The compounds were added, and the culture was continued further for 48 h. After incubation with the XTT labeling mixture for 4 h at 37 °C plates were measured in an ELISA reader (SLT Labinstruments) with 450 nm and a reference wavelength of 620 nm.

Because the XTT assay was not suitable for Daudi and HL 60 cells, the cytostatic potency of the nucleoside derivatives was estimated by automatic cell counting. The concentration (μM) of the compounds resulting in 50% inhibition of cell growth was estimated. The values are given as the mean ± S.E.M. of at least 3 separate experiments.

Syntheses

2-Fluoro-ara-adenosine-5′-hexadecylphosphate 2. (a) Fludarabine (F-ara-AMP, **1**) (365 mg, 1.0 mmol) was twice solved in pyridine and evaporated to remove sources of water. Then hexadecyl alcohol (270 mg, 1.0 mmol) and DCC (515 mg, 2.5 mmol) were added. After 15 h water (5 mL) was added to the solution and the mixture was stirred for additional 2 h. Then the mixture was evaporated to dryness. The residue was suspended in water (10 mL) and the precipitate formed was separated. After evaporation of the filtrate to complete dryness the product mixture was chromatographed (solvent A) and rechromatographed (solvent E) to give 70 mg (12%) of the title product. (b) A mixture of F-ara-AMP (365 mg, 1.0 mmol), acetanhydride (10 mL) and pyridine (20 mL) was stirred for 15 h. Then water (10 mL) was added and stirring was continued for 1 h. The mixture was evaporated to give 700 mg of peracetyl 2-fluoro-ara-adenosine-5′-phosphate as a brownish oil which was used without further purification. The crude peracetyl 2-fluoro-ara-adenosine-5′-phosphate was dissolved in pyridine (25 mL) and TPS (1.21 g, 4.0 mmol) was added. After 30 min of stirring hexadecyl alcohol (266 mg, 1.0 mmol) was given into the solution and stirring was continued for 20 h. Water (10 mL) was added and after 1 h of stirring the mixture was evaporated to dryness. The residue was suspended in diethyl ether and the precipitate was separated. The solution was evaporated and the residue was chromatographed (solvent A) yielding 298 mg of 2-fluoro-ara-adenosine-5′-hexadecylphosphate peracetate which was deprotected by treatment with 2N NH_3-MeOH (20 mL) for 5 h. After lyophilization of the deprotected substance it was chromatographed using solvent A and solvent E to give 206 mg (35%) of the pure title product. TLC: R_f 0.24 (solvent A); MS ESI pos.: $[M+H]^+$ m/z 654; ^{1}H NMR (CD_3OD:$CDCl_3$ 1:2): δ 0.88 (3H, t, J = 6.5 Hz, CH_3), 1.10–1.40 (26H, m, $(CH_2)_{13}$), 1.63 (2H, m_c, P-O-CH_2-$\underline{CH_2}$-alkyl chain); 3.87 (2H, dt, J = 6.5 and 6.5 Hz, P-O-$\underline{CH_2}$-alkyl chain), 4.03

(2H, m_c, H4′), 4.15 (2H, dd_{br}, H5′), 4.30–4.45 (2H, m, H2′, H3′), 6.28 (1H, d, $J=5$ Hz, H1′); 8.36 (1H, s, H8); ^{31}P NMR: δ 0.260.

2-Fluoro-ara-adenosine-5′-hexadecyldiphosphate 3 and 2-fluoro-ara-adenosine-5′-hexadecylphosphonophosphate 4. Hexadecylphosphoric (phosphonic) acid (1.24 mmol) and morpholine (0.42 g, 4.8 mmol) were dissolved in a mixture of water (12 mL) and *tert*-butanol (12 mL). To this solution DCC (1.0 g, 4.8 mmol) in *tert*-butanol (15 mL) was added and the reaction mixture was refluxed for 3.5 h. Then the solvent was evaporated to a volume of 5 mL and the mixture was cooled to 0–5 °C. The formed precipitate was separated and the filtrate was evaporated to dryness yielding the crude phospho (no)morpholidate. Sources of water were removed by evaporation with pyridine (2×25 mL). The remaining residue was resolved in pyridine (25 mL) and F-ara-AMP (**1**, 402 mg, 1.1 mmol) was added to the solution. The reaction mixture was stirred at 38 °C for 16 h. After the addition of water (5 mL) stirring was continued at room temperature for 1 h. After evaporation of the solvent and chromatography (solvent A) and rechromatography (solvent E) of the substance obtained, the title compounds could be isolated as a white solid.

(a) The title compound **3** was obtained in 280 mg (38%). TLC: R_f 0.20 (solvent A); MS ESI pos.: $[M+H]^+$ m/z 670; ^{1}H NMR: δ 0.85 (3H, t, $J=7$ Hz, CH_3), 1.10–1.35 (26H, m, $(CH_2)_{13}$), 1.40–1.55 (2H, m, P-O-CH_2-$\underline{CH_2}$-alkyl chain), 3.69 (2H, m_c, CH_2-O-P), 3.84 (1H, m_c, H4′), 4.00 (2H, m_c, H5′), 4.15 (1H, dd, $J=5.2$ Hz and 5.2 Hz, H3′), 4.36 (1H, dd, $J=5.2$ Hz and 5.2 Hz, H2′), 6.08 (1H, d, $J=5.2$ Hz, H1′), 7.80, (2H, bs, NH_2), 8.32 (1H, s, H8); ^{31}P NMR: δ −11.65 (P1), −11.35 (P2, AB system, $J^3_{P\text{-}P}$ 21.36 Hz).

(b) One hundred ninety-four milligrams (27%) of the title compound **4** was obtained. TLC: 0.12 (solvent B); MS ESI pos.: $[M+H]^+$ m/z 654.3; ^{1}H NMR: δ 0.85 (3H, t, $J=6.5$ Hz, CH_3), 1.10–1.30 (28H, m, $(CH_2)_{13}$), 1.30–1.50 (2H, m, P-$\underline{CH_2}$-alkyl chain), 3.82 (1H, m_c, H4′), 4.00 (2H, m_c, H5′), 4.14 (1H, dd, $J=5.3$ Hz and 5.3 Hz, H3′), 4.39 (1H, dd, $J=5.3$ Hz and 5.3 Hz, H2′), 6.07 (1H, d, $J=5$ Hz, H1′), 7.80, (2H, bs, NH_2), 8.25 (1H, s, H8); ^{31}P NMR: δ −11.18 (d, $J^3_{P\text{-}P}$ 30.5 Hz), −14.40 (m_c).

2-Fluoro-ara-adenosine-5′-[1-*O*-hexadecyl-2-(2,2,2-trifluoroethyl)glycero-3] phosphate 5. A solution of **1** (1 mmol, 365.2 mg) and TPS (4 mmol, 1.2 g) in 50 mL of dry pyridine was stirred for 15 min. Then 1-*O*-hexadecyl-2-(2,2,2-trifluoroethyl) glycerol (1.5 mmol, 597.8 mg) was added and stirred for 5 days. Water (5 mL) was added, and after 1 h the mixture was evaporated to dryness. The residue was dissolved in $CHCl_3$:H_2O (70 mL, 5:2, v/v), and the $CHCl_3$-phase was washed twice with 10 mL of water. The combined $CHCl_3$ phases containing the crude product were evaporated to dryness. The residue obtained was chromatographed (solvent C) and rechromatographed using $CHCl_3$:MeOH:NH_4OH mixtures with increasing polarity (90:10:1 up to 60:30:10). Fractions containing the pure product were evaporated to dryness. The residue was crystalized by treatment with dry acetone. Pure compound **5** was obtained in the form of the pyridinium salt in an amount of 47 mg (yield 6%). TLC: $R_f=0.24$ (solvent C); MSFAB pos: $[M\text{ (pyridinium salt)}-OH]^+$ m/z 807; ^{1}H NMR: δ 0.849 (3H, t, $J=7.1$ Hz, CH_3), 1.18–1.30 (26H, m, $(CH_2)_{13}$), 1.44 (2H, m_c, O-CH_2-$\underline{CH_2}$-alkyl chain), 3.20–3.53 (6H, m, O-CH_2-alkyl chain, 1-CH_2, 3-CH_2), 3.61 (1H, m_c, 2-CH), 4.02–4.12 (2H, m, CH_2-CF_3), 4.23 (1H, m_c, H4′), 4.52 (1H, t, $J=6.1$ Hz, H3′), 4.58 (1H, q, $J=6.1$ Hz, H2′), 5.02–5.12 (2H, m, H5′), 6.24 (1H, d, $J=6.1$, H1′), 7.08 (1H, m_c, P–OH), 7.82 (2H, bs, NH_2), 8.21 (1H, d, $J=5.0$, H8); ^{31}P NMR: δ 0.32.

2-Fluoro-ara-adenosine-5′-(1-chloro-1-deoxy-3-*O*-hexadecylgycero-2) phosphate 6. As described for the synthesis of compound **5** 1-chloro-1-deoxy-3-*O*-hexadecylglycerol (1.5 mmol, 502.5 mg) was added to a solution of **1** and TPS. The mixture was stirred for 7 days and worked up as described above. Pure substance **6** was obtained in the form of the pyridinium salt in an amount of 39 mg (yield 5.2%). TLC: $R_f=0.23$ (solvent C); MS FAB pos.: $[M\text{ (pyridinium salt)}-OH]^+$ m/z 743; ^{1}H NMR: δ 0.849 (3H, t, $J=6.9$ Hz, CH_3), 1.23 (26H, m_c, $(CH_2)_{13}$), 1.37–1.46 (2H, m, O-CH_2-$\underline{CH_2}$-alkyl chain), 3.15–3.63 (6H, m, O-CH_2-alkyl chain, 1-CH_2, 3-CH_2), 3.87–4.01 (1H, m, 2-CH), 4.21–4.28 (1H, m, H4′), 4.55 (1H, q, $J=6.1$ Hz, H3′), 4.60–4.68 (1H, m, H2′), 5.01–5.12 (2H, m, H5′), 6.24 (1H, d, $J=6.1$ Hz, H1′), 7.14 (1H, d, $J=16.7$ Hz, P–OH), 7.82 (2H, bs, NH_2), 8.19 (1H, d, $J=4.2$ Hz, H8); ^{31}P NMR: δ −0.25.

2-fluoro-ara-adenosine-5′-(2-*O*-methyl-1-*O*-octadecylglycero-3) diphosphate 7. According to the procedure described for the preparation of compounds **3** and **4** 2-*O*-methyl-1-*O*-octadecylglycero-3-phosphate (2 mmol, 877 mg) and morpholine (8 mmol, 696 mg) were refluxed. The mixture was worked up as described and used for the condensation with **1**. The condensation mixture was stirred for three days, and evaporated to dryness. The residue was dissolved in $CHCl_3$:MeOH:H_2O (70 mL, 4:2:1, v/v/v). Then formic acid was added until pH 3. The $CHCl_3$-phase was washed twice with 10 mL of water. The combined $CHCl_3$-phases containing the crude product were dried over Na_2SO_4 and evaporated to dryness. The residue obtained was chromatographed ($CHCl_3$:MeOH:H_2O:acetic acid, 60:20:4:4) and rechromatographed using $CHCl_3$:MeOH:H_2O mixtures with increasing polarity (90:10:1 up to 60:30:4). Fractions containing the pure product were evaporated to dryness. The residue was crystalized by treatment with dry acetone. Pure compound **7** was obtained in the form of the free acid in an amount of 23 mg (yield 2 %). TLC: $R_f=0.37$ (solvent D); MSFAB pos.: $[M+H+Na]^+$ m/z 808; ^{1}H NMR: δ 0.85 (3H, t, $J=6.8$ Hz, CH_2–CH_3), 1.22 (30H, m_c, $(CH_2)_{15}$), 1.46 (2H, mc, O-CH_2-$\underline{CH_2}$-alkyl chain), 1.90 (3H, s, O-CH_3), 3.15–3.50 (6H, m, O-CH_2- alkyl chain, 1-CH_2, 3-CH_2), 3.72–4.40 (6H, m, 2-CH, H5′, H4′, H3′, H2′), 6.09 (1H, d, $J=5.0$ Hz, H1′), 7.76 (2H, bs, NH_2), 8.15 (1H, s, H8).

2-Fluoro-ara-adenosine-5′-(1-*O*-hexadecyloxypropyl-3-) phosphonophosphate 8. The 1-*O*-hexadecyloxypropyl-3-

phosphono morpholidate was synthesized according to the procedure described for the preparation of compounds **3** and **4** starting from 1-*O*-hexadecyloxypropyl-3-phosphonate (1.9 mmol, 700 mg) and was used for the condensation with **1** (2.2 mmol, 806 mg). The reaction mixture was worked up as described before for substance **7**. Pure compound **8** was obtained in the form of the free acid in an amount of 40 mg (yield 3 %). TLC: $R_f = 0.31$ (solvent D); MS FAB pos: $[M+H+Na]^+$ m/z 735; ^{1}H NMR: δ 0.84 (3H, t, $J = 5.0$ Hz, CH_3), 1.21 (26H, m_c, $(CH_2)_{13}$), 1.45 (2H, m_c, P-O-CH_2-$\underline{CH_2}$-alkyl chain), 1.79 (2H, m_c, 2-CH_2), 3.15–3.65 (6H, m, P-O-CH_2- alkyl chain, 1-CH_2, 3-CH_2), 3.72–4.40 (5H, m, H5′, H4′, H3′, H2′), 6.24 (1H, d, $J = 5.0$ Hz, H1′), 7.81 (2H, bs, NH_2), 8.37 (1H, s, H8).

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