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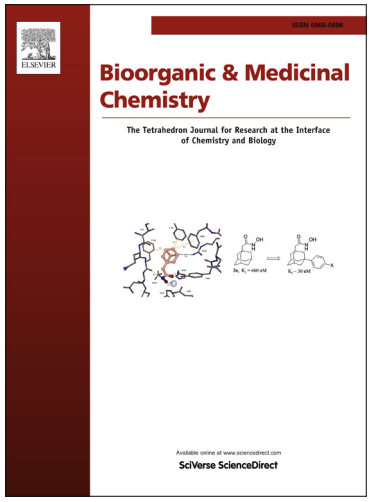

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Synthesis and *in vitro* cytostatic activity of 1,2- and 1,3-diacylglycerophosphates of clofarabine

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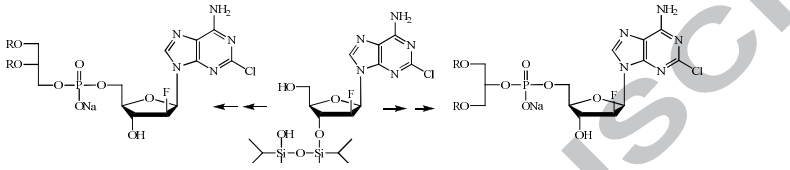

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ABSTRACT

The conjugates of anticancer nucleoside clofarabine [2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine] with 1,2- and 1,3-diacylglycerophosphates have been prepared by the phosphoramidite method using a combination of 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl protecting group for the sugar moiety of the nucleoside and 2-cyanoethyl protection for the phosphate fragment. Some of the synthesized conjugates exhibited cytostatic activity against HL-60, A-549, MCF-7, and HeLa tumor cell lines.

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1. Introduction

Nucleoside and nucleotide analogues play an important role as anticancer agents.[1] It is known, however, that such compounds often have a number of drawbacks including low bioavailability, poor pharmacokinetic properties, and toxic effects connected with insufficiently selective delivery of the agent to the tumor tissue. It is also shown that the conjugation of these nucleosides with lipid derivatives can give the prodrugs characterized by improved pharmacokinetics, different pharmacological profiles, and fewer side effects as compared with the parent compounds.[2]

This approach was firstly employed for preparing the prodrugs of 1-*β*-D-aravinofuranosylcytosine (ara-C). In the 1970s, several groups of researchers started the investigations aimed at the preparation of the conjugates of ara-C with natural or synthetic phospholipids and evaluation of their cytotoxicity.[3, 4] A number of 1-*β*-D-arabinofuranosylcytosine 5'-diphosphate-1,2-diacylglycerols demonstrated promising antiproliferative properties, especially in the *in vivo* experiments.[5-7] 1-β-D-arabinofuranosylcytosine 5'-monophosphate-L-1,2-dipalmitin exhibited *in vivo* and *in vitro* cytotoxicity against L1210 lymphoid leukemia.[5] A series of oxy- and thioether phospholipid derivatives of ara-C also demonstrated significant antitumor activity against various leukemias in animals.[8, 9] The lipid prodrugs of other antitumor nucleoside analogs, such as 5'-fluoro-2'-deoxyuridine, fludarabine, gemcitabine, have been synthesized.[10-12] The results of the above mentioned studies indicate that the *in vitro* activity of the lipid derivatives of antitumor nucleosides is often lower than that of nucleoside alone. Contrary to this, when tested *in vivo*, the same conjugates tend to be equally or more active than the parent drug. It was shown that the ability of these compounds to form micelles is important for improving their activity[13]. The incorporation of the conjugates into liposomes often leads to the enhanced antitumor effect[14].

The metabolic pathways of nucleoside 1,2-diacylglyceromonophosphates were clarified by the example of corresponding AZT (3'-azidothymidine) derivatives. It was shown that, in the cells, these compounds undergo deacylation catalyzed by phospholipases A and lysophospholipases. The produced AZT glycerophosphates are further hydrolyzed by phosphodiesterases to liberate the nucleoside/5'-nucleotide moiety which, after phosphorylation by kinases, give AZT 5'-triphosphate, an active metabolite.[15] Recently we have synthesized the conjugates of antiviral nucleoside ribavirin [1-(*β*-D-ribofuranosyl)-1H-1,2,4-triazole-3-carboxamide] with 1,2- and 1,3-diacylglycerophosphates and demonstrated that 1,3-isomers, in principle, are also able to enter the first stage of the above described metabolic pathway catalyzed by phospholipases.[16] However, the hydrolysis of 1,3-diacyl derivatives by porcine pancreatic phospholipase A_2 was essentially decelerated as compared with 1,2-isomers containing natural phosphatidyl residues, similar to the phenomenon previously observed for isomeric 1,2- and 1,3-diacylglycerophosphocholines.[17] Thus, 1,3-diacylglyceromonophosphate derivatives of biologically active nucleosides may exhibit different pharmacokinetic properties and increased stability in gastrointestinal tract in comparison with their 1,2-counterparts. We believe that such compounds deserve further investigation as prodrug forms of biologically active nucleosides.

Clofarabine [2-chloro-9-(2-deoxy-2-fluoro-*β*-D-arabinofuranosyl)adenine, C1-F-ara-A] is a purine nucleoside used in the therapy of pediatric acute myeloid leukemia.[1] An evaluation of this compound against various tumor systems *in vitro* and in mice has demonstrated that C1-F-ara-A exhibits significant cytotoxicity against human tumor cell lines including leukemias and several types of solid tumors.[18]

The usage of C1-F-ara-A is also limited by some disadvantages such as narrow therapeutic ratio, short biological half-life, bone marrow toxicity.[19] There are few data on the preparation and properties of lipid derivatives of clofarabine in the literature. Several dialkylglycerophosphate derivatives of C1-F-ara-A described to date exhibited higher plasma exposure, increased terminal half-life, and high efficacy in a variety of solid tumor xenograft models.[20]

The present work focuses on the synthesis of clofarabine conjugates with natural 1,2-diacylglycerophosphates and their synthetic 1,3-counterparts as well as on early evaluation of their *in vitro* activity in promyelocytic leukemia HL-60, lung carcinoma A-549, breast carcinoma MCF-7, and cervix carcinoma HeLa cell lines.

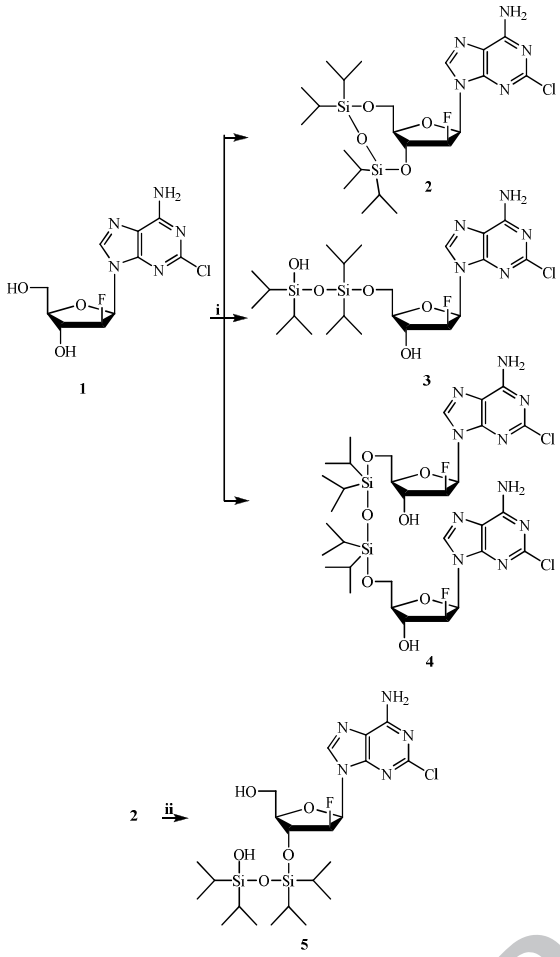
2. Results and Discussion

2.1. Synthesis

The phosphoramidite approach[21] widely used in oligonucleotide chemistry was chosen for the preparation of diacylglycerophosphates of clofarabine. The method included the formation of P-O bond via condensation of 3'-O-protected nucleoside with 1,2- or 1,3-diacylglyceryl phosphoramidites to obtain phosphite intermediates which were further oxidized with iodine.

To provide C1-F-ara-A derivative bearing free 5'-OH group appropriated for the condensation with 1,2- and 1,3-diacylglyceryl phosphoramidites, we employed 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl (TIPDS) protecting group previously used for temporarily masking the hydroxyl functions of various nucleosides.[22]

Scheme 1. Synthesis of 3'-O-TIPDS derivative **5.** Reagents and condition: **(i)**: $TIPDSiCl_2$/Py, **(ii)**: TFA/H_2O/THF



The treatment of clofarabine (**1**) with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane ($TIPDSiCl_2$) (1.2 molar equiv.) in pyridine resulted in the formation of cyclic 3',5'-O-TIPDS clofarabine derivative **2** (72%), along with two minor compounds, 5'-O-silylated nucleoside **3** (22%) and *bis*(nucleoside)siloxane **4** (3%).

The structure and purity of clofarabine derivatives **2**-**4** have been confirmed by means of ^{1}H, ^{13}C, and ^{19}F NMR spectroscopy, mass spectrometry, and elemental analysis. The peaks at 6.02 and 5.15 ppm observed in ^{1}H NMR spectrum of clofarabine (**1**) in DMSO-d_6, corresponding to the protons of 3'-OH and 5'-OH groups, disappeared in the spectrum of cyclic 3',5'-O-TIPDS derivative **2**; instead, a group of signals appears at 0.96-1.32 ppm due to the presence of 28 isopropyl protons of TIPDS fragment. A similar group of signals is observed at 0.78-1.03 ppm in ^{1}H NMR spectrum of nucleoside **3**; however, in this case there are also a dublet at 6.06 ppm and a singlet at 6.14 ppm attributed, correspondingly, to the proton of 3'-OH group of sugar moiety and hydroxyl group of siloxane fragment.

The introduction of TIPDS group into the 3',5'-diol fragment of C1-F-ara-A molecule causes a significant low-field shift of H-3' and H-5',H-5" resonance. In the spectrum of clofarabine (**1**), the signals from these protons are observed at 4.43 and 3.62-3.72 ppm, whereas in the case of nucleoside **2** the corresponding peaks are located at 5.05 and 3.96-4.17 ppm. In ^{1}H NMR spectrum of 5'-O-silylated derivative **3,** a shift to the low field is observed only for H-5',H-5" resonance (multiplet centered at 3.98 ppm). The location of the peaks from nucleoside protons of compound **4** coincides with those of 5'-O-TIPDS derivative **3**; however, in the spectrum of **4** we do not observe a peak from OH group of siloxane fragment characteristic for nucleoside **3**. Moreover, a comparison of integral intensity of the signals from nucleoside and isopropyl protons suggests that in the molecule of compound **4** there are two nucleoside residues connected to TIPDS fragment. The data of mass-spectrometry ($[MH]^+$: 849) confirm the dimeric structure of compound **4**.

The 3'-O-silylated nucleoside **5** having free 5'-OH group suitable for further coupling with lipid phosphoramidites was prepared in 90% yield by the selective deblocking of 5'-OH function of cyclic 3',5'-O-TIPDS derivative **2** under the treatment with a mixture of trifluoroacetic acid/water/THF (1:1:4, v/v)[23] for 3 h at 0° C.

Phosphoramidite derivatives of 1,2-diacyl glycerols (**6**, **7**) and their 1,3-diacyl regioisomers **8**, **9** were prepared as described previously[16].

The condensation of 3'-O-TIPDS-protected nucleoside **5** with phosphoramidites **6-9** in CH_2Cl_2 in the presence of tetrazole followed by I_2/H_2O oxidation gave the phosphotriesters **10-13** isolated in 82-88% yields.

Compounds **10-13** were treated with a mixture of Py/NEt_3 (1:1, v/v) for removing β-cyanoethyl protecting group from the phosphate and then with 1M tetrabutylammonium fluoride in THF to remove 3'-O-TIPDS protecting group of the nucleoside fragment. The deprotected lipid clofarabine derivatives were purified by silica gel column chromatography and isolated as sodium salts **14-17** after the sequential treatment with HCl and NaOH solutions in 60-97% overall yields.

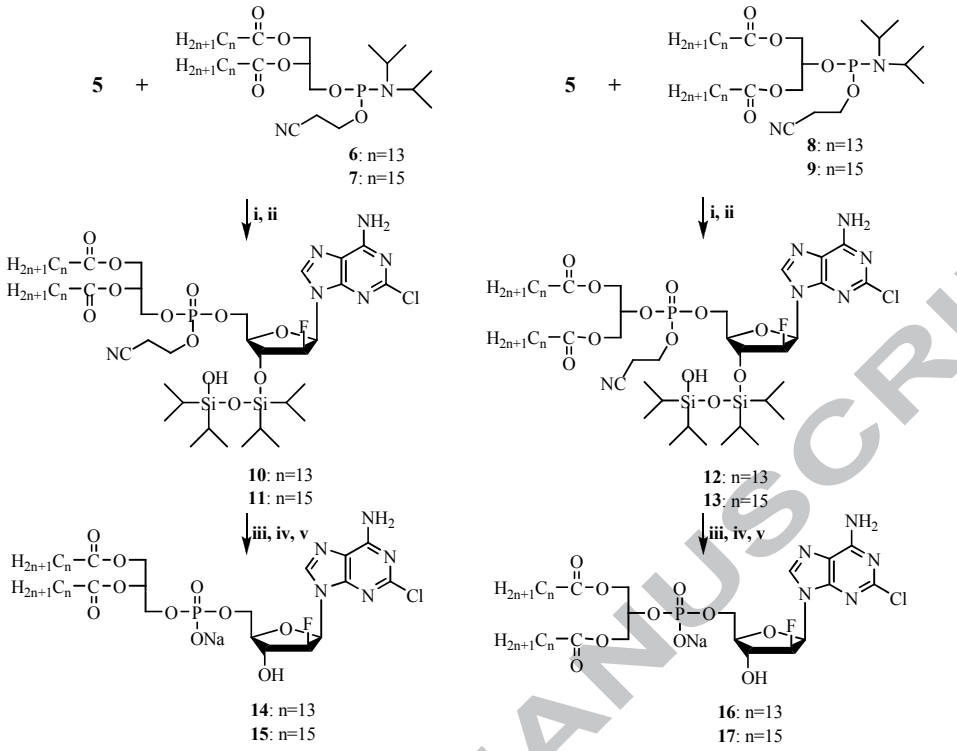
The structure of the synthesized clofarabine conjugates are confirmed by the data of NMR spectroscopy including 2D NMR methods. The data of elemental analysis and mass-spectrometry are in good accordance with the composition of the lipid-clofarabine derivatives.

2.2. *In vitro* cytostatic activity of diacylglycerophosphates of clofarabine

In vitro cytostatic activity of clofarabine derivatives **14**-**17** against hematologic and solid tumor cell lines (promyelocytic leukemia HL-60, lung carcinoma A-549, breast carcinoma MCF-7, cervix carcinoma HeLa) was evaluated in comparison with parent nucleoside clofarabine (**1**). The obtained results are given in Table 1.

Compound **14** inhibited HL-60 cell line at micromolar concentrations (IC_{50} 0.8 μM). Compounds **15** and **17** were weaker inhibitors of HL-60 cells, while conjugate **16** did not exhibit noticeable cytotoxicity. The antiproliferative activity of compounds **14**, **16**, **17** against breast carcinoma MCF-7 and the cytotoxicity of conjugate **14** in HeLa cell line were of the same order of magnitude as that of parent nucleoside **1**. The tested conjugates were only weak inhibitors of lung carcinoma A-549.

As mentioned above, the *in vivo* activity of lipid nucleoside derivatives can be substantially higher in comparison with *in vitro* experiments. Hence, further *in vivo* investigation of antiproliferative properties of the synthesized clofarabine derivatives would be helpful for estimating their prospects as nucleoside prodrugs.



Scheme 2. Synthesis of clofarabine conjugates **14** - **17**. Reagents and conditions: (**i**): CH_2Cl_2, tetrazole; (**ii**): I_2/Py/CH_2Cl_2/H_2O; (**iii**): Py/$(C_2H_5)_3N$ = 1/1; (**iv**): 1M TBAF/THF; (**v**): HCl/$CHCl_3$, NaOH/MeOH

Table 1. *In vitro* cytostatic activity of clofarabine conjugates, IC_{50}, μM

Cell lines	Compound				
	1	**14**	**15**	**16**	**17**
HL-60	0.1	0.8	10.0	>100	55.0
A-549	8.0	>100	>100	>100	90.0
MCF-7	50.0	40.0	>100	40.0	30.0
HeLa	50.0	50.0	>100	>100	100.0

3. Conclusions

The method proposed in this work for the synthesis of lipid clofarabine derivatives is based on a combination of TIPDS protecting group for the carbohydrate moiety of nucleoside and cyanoethyl group for the phosphate fragment of lipid-nucleoside conjugates. It allows obtaining of the desired phospholipid clofarabine derivatives in reasonable yields through a limited number of synthetic steps.

Diacylglycerophosphate clofarabine derivatives **14**-**17** deserve consideration as clofarabine prodrugs for further *in vivo* investigation. The study on the biophysical and biochemical properties of the synthesized conjugates is now in progress.

4. Experimental

Clofarabine was kindly provided by Dr. G.G. Sivets[24]. TLC was performed on silica gel sheets Merck 60 F 254. Lipid derivatives were visualized on TLC by spraying with 30 % H_2SO_4 and charring. Preparative column chromatography was performed on silica gel Merck 60 (70-230 μm). Elemental analysis was done with the use of CHNS-O Analyser EA-3000 (EuroVector). UV spectra were recorded using UV-VIS spectrometer Cary 100 (Varian). NMR spectra were registered on Avance 500 (Bruker). In ^{1}H- and ^{13}C NMR spectra, chemical shifts (δ) are given in ppm related to internal $SiMe_4$, coupling constants (*J*) in Hz. In ^{31}P NMR spectra, chemical shifts are given in ppm related to external H_3PO_4. Assignment of the signals in ^{1}H and ^{13}C NMR spectra was carried out with the use of ^{1}H-^{1}H and ^{1}H-^{13}C correlation spectroscopy methods.

4.1. Cytostatic activity

The samples of compounds **14**-**17** for *in vitro* testing were prepared by sonication of drug suspensions in 0.9% NaCl solution (3 × 1 min) using an ultrasonic dispergator UZDN-A with working frequency 22 kHz and power up to 100 W/cm^2.

Human cell lines were obtained from the Institute of Cytology, Russian Academy of Sciences. The cell lines studied were maintained in RPMI 1640 medium (AppliChem, Darmstadt, Germany) supplemented with 20% (HL-60) fetal calf serum (HyClone, Cramlington, UK), in DMEM (A 549) and Eagle's

MEM (AppliChem) (MCF7, HeLa) with 10% fetal calf serum (HyClone). Cells were cultivated at 37°C in a humidified atmosphere of 5% CO_2. Cellular sensitivities to clofarabine and its lipid derivatives were measured with the MTT assay. Cells were plated in triplicate in 96-well plates (10^5 cells/mL for suspended cell cultures and 5×10^3 cells/ mL for monolayer cultures); at the same day (suspended cell cultures) or on the following day (monolayer cultures), compounds were added at the appropriate dilutions. Plates were incubated under standard conditions for 48 h. Thereafter, 10 μL MTT (Sigma) in phosphate buffered saline (5 mg/mL) were added. The plates were incubated for an additional 4 h and 150 μL dimethylsulphoxide (DMSO) were added to dissolve the formazan crystals. The optical density was read on Infinite M 200 plate reader (Tecan, Switzerland) at 540 nm. The antiproliferative effects were expressed as IC_{50}.

4.2. Chemistry

4.2.1. 2-Chloro-9-[2-deoxy-2-fluoro-3,5-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]adenine, 2

To a solution of **1** (225 mg, 0.74 mmol) in pyridine (1 mL), 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (0.28 mL, 0.89 mmol) was added, and the mixture was stirred at room temperature for 24 h. After that the mixture was evaporated and the residue partitioned between chloroform (100 mL) and water (30 mL). The chloroform layer was dried over anhydrous sodium sulfate, evaporated, and co-evaporated with toluene. The residue was applied on silica gel column (60 cm^3), and the products were eluted using a gradient of CH_3OH (0→20%) in $CHCl_3$. The appropriate fractions were combined and evaporated to give compounds **2** (352 mg, 87%), **3** (36 mg, 9%), and **4** (17 mg, 2%) as solid foams.

2: ^{1}H NMR (DMSO-d_6): δ 8.17 (s, 1H, H-8), 7.91 (br s, 2H, NH_2), 6.36 (dd, 1H, $J_{1',2'}$ 6.5 Hz, $J_{1',F}$ 4.5 Hz, H-1'), 5.57 (m, 1H, $J_{2',F}$ 54.0 Hz, H-2'), 5.05 (m, 1H, $J_{3',F}$ 21.5 Hz, H-3'), 4.17 (dd, 1H, $J_{5',4'}$ 5.5 Hz, $J_{5',5"}$ 12.5 Hz, H-5'), 3.96 (dd, 1H, $J_{5",4'}$ 2.5 Hz, $J_{5",5'}$ 12.5 Hz, H-5"), 3.89 (m, 1H, H-4'), 1.32-0.96 (m, 28H, 4[i-Pr]). ^{13}C NMR (DMSO-d_6): δ 156.83, 153.22, 150.13, 139.89, 117.93 (Ade), 95.00 (d, $J_{C,F}$ 196.0 Hz, C(2')), 79.71 (d, $J_{C,F}$ 17.0 Hz, C(1')), 78.55 (d, $J_{C,F}$ 4.0 Hz, C(4')), 74.69 (d, $J_{C,F}$ 21.0 Hz, C(3'),), 62.00 (C(5')), 17.28, 17.11, 16.80, 16.71, 12.52, 12.39, 12.07, 11.98 (i-Pr). ^{19}F NMR (DMSO-d_6): δ -200.33. Anal. calcd for $C_{22}H_{37}ClFN_5O_4Si_2$ (546.183), %: C 48.38, H 6.83, N 12.82; found, %: C 48.81, H 6.88, N 12.58. ESI-MS: 546 $[M+H]^+$. UV (EtOH), λ_{max}, nm (lg ε): 212 (4.39), 263 (4.16).

4.2.2. 2-Chloro-9-[2-deoxy-2-fluoro-5-O-(1-hydroxy-1,1,3,3-tetraisopropyldisiloxane-3-yl)-β-D-arabinofuranosyl]adenine, 3

^{1}H NMR (DMSO-d_6): δ 8.14 (s, 1H, H-8), 7.90 (br s, 2H, NH_2), 6.35 (dd, 1H, $J_{1',2'}$ 4.5 Hz, $J_{1',F}$ 13.0 Hz, H-1'), 6.14 (s, 1H, SiOH), 6.06 (d, 1H, $J_{OH,3'}$ 5.5 Hz, 3'-OH), 5.27 (m, 1H, $J_{2',F}$ 52.5 Hz, H-2'), 4.49 (m, 1H, $J_{3',F}$ 19.5 Hz, H-3'), 3.98 (m, 2H, H-5', H-5"), 3.92 (m, 1H, H-4'), 1.03-0.78 (m, 28H, 4[i-Pr]). ^{13}C NMR (DMSO-d_6): δ 156.77, 153.30, 150.12, 139.62, 117.35 (Ade), 95.29 (d, $J_{C,F}$ 193.0 Hz, C(2')), 82.61 (d, J 6.0 Hz, C(4')), 81.34 (d, $J_{C,F}$ 17.0 Hz, C(1')), 72.60 (d, $J_{C,F}$ 23.0 Hz, C(3')), 61.66 (C(5')), 17.19, 17.18, 17.08, 17.05, 17.02, 12.95, 12.37, 12.33 (i-Pr). ^{19}F NMR (DMSO-d_6): δ -198.28. Anal. calcd for $C_{22}H_{39}ClFN_5O_5Si_2$ (564.198), %: C 46.83, H 6.97, N 12.41; found, %: C 47.14, H 6.96, N 11.88. ESI-MS: 564 $[M+H]^+$. UV (EtOH), λ_{max}, nm (lg ε): 212 (4.35), 263 (4.13).

4.2.3. 1,1,3,3-Tetraisopropyldisiloxane-1,3-di{[2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine]-5'-yl}, 4

^{1}H NMR (DMSO-d_6): δ 8.12 (d, 2H, J 1.5 Hz, H-8), 7.89 (br s, 4H, NH_2), 6.34 (dd, 2H, $J_{1',2'}$ 5.0 Hz, $J_{1',F}$ 13.0 Hz, H-1'), 6.06 (d, 2H, $J_{OH,3'}$ 5.0 Hz, 3'-OH), 5.27 (m, 2H, $J_{2',F}$ 52.5 Hz, H-2'), 4.50 (m, 2H, $J_{3',F}$ 19.5 Hz, H-3'), 3.99 (m, 4H, H-5', H-5"), 3.91 (m, 2H, H-4'), 1.03 - 0.78 (m, 28H, 4[i-Pr]). ^{13}C NMR (DMSO-d_6): δ 156.81, 153.31, 150.14, 139.69, 117.45 (Ade), 95.35 (d, $J_{C,F}$ 193.0 Hz, C(2')), 82.66 (d, $J_{C,F}$ 6.0 Hz, C(4')), 81.31 (d, $J_{C,F}$ 17.0 Hz, C(1')), 72.62 (d, $J_{C,F}$ 23.0 Hz, C(3')), 61.91 (C(5')), 17.04, 12.28, 12.25 (i-Pr). ^{19}F NMR (DMSO-d_6): δ -198.53. Anal. calcd for $C_{32}H_{48}Cl_2F_2N_{10}O_7Si_2$ (849.860), %: C 45.22, H 5.69, N 16.48; found, %: C 44.95, H 5.63, N 16.32. ESI-MS: $[MH]^+$: 849. UV (EtOH), λ_{max}, nm (lg ε): 212 (4.59), 263 (4.38).

4.2.4. 2-Chloro-9-[2-deoxy-2-fluoro-3-O-(1-hydroxy-1,1,3,3-tetraisopropyldisiloxane-3-yl)-β-D-arabinofuranosyl]-adenine, 5

To a solution of nucleoside **2** (352 mg, 0.64 mmol) in THF (2 mL), a mixture of trifluoroacetic acid/water/THF (1:1:4, v:v; 1 mL) was added at 0° C. After stirring for 3 h, the reaction mixture was evaporated to dryness. The residue was evaporated with toluene (1 mL) and purified on silica gel column (50 cm^3) using a gradient of CH_3OH (0→2%) in $CHCl_3$. The appropriate fractions were combined and evaporated to give a solid foam of compound **5** (327 mg, 90%); ^{1}H NMR (DMSO-d_6): δ 8.25 (d, 1H, J 1.5 Hz, H-8), 7.90 (br s, 2H, NH_2), 6.34 (dd, 1H, $J_{1',2'}$ 4.5 Hz, $J_{1',F}$ 15.0 Hz, H-1'), 6.14 (s, 1H, SiOH), 5.30 (m, 1H, $J_{2',F}$ 50.0 Hz, H-2'), 5.25 (m, 1H, 5'-OH), 4.78 (m, 1H, $J_{3',F}$ 17.0 Hz, H-3'), 3.95 (m, 1H, H-4'), 3.72 (m, 1H, $J_{5',4'}$ 4.5 Hz, $J_{5',5"}$ 12.0 Hz, H-5'), 3.66 (m, 1H, $J_{5",4'}$ 5.5 Hz, $J_{5",5'}$ 12.0 Hz, H-5"), 0.81-1.04 (m, 28H, 4[i-Pr]). ^{13}C NMR (DMSO-d_6): δ 156.78, 153.30, 150.16, 139.86, 117.37 (Ade), 95.01 (d, $J_{C,F}$ 194.0 Hz, C(2')), 84.23 (C(4')), 81.75 (d, $J_{C,F}$ 17.0 Hz, C(1')), 73.56 (d, $J_{C,F}$ 25.0 Hz, C(3')), 60.09 (C(5')), 17.26, 17.23, 17.00, 16.96, 13.01, 12.47, 12.39 (i-Pr). ^{19}F NMR (DMSO-d_6): δ -197.81. Anal. calcd for $C_{22}H_{39}ClFN_5O_5Si_2$, (564.198), %: C 46.83, H 6.97, N 12.41; found, %: C 46.38, H 6.85, N 12.12. ESI-MS: 564 $[M+H]^+$. UV (EtOH), λ_{max}, nm (lg ε): 212 (4.35), 263 (4.13).

4.2.5. General procedure for the preparation of conjugates 10-13

A solution of lipid phosphoramidite **6-9** (0.39 mmol) in CH_2Cl_2 (2 mL) and 0.45 M solution of tetrazole (1.70 mmol) in CH_3CN (3.8 mL) were added to the solution of nucleoside **5** (0.25 mmol) in CH_2Cl_2 (2 mL) under argon. After stirring for 24 h, a solution of I_2 (0.39 mmol) in Py/H_2O/CH_2Cl_2 (3:1:1, v/v/v) was added. The mixture was diluted with $CHCl_3$ (200 mL) and extracted with 2% solution of sodium thiosulfate in brine (60 mL), the organic layer was dried (Na_2SO_4) and evaporated. The residue was purified by column chromatography (silica gel, 100 cm^3) using a gradient of CH_3OH (0→6.25%) in $CHCl_3$. The compounds **10-13** were isolated in yields ranging from 82% to 88%.

4.2.6. {2-Chloro-9-[2-deoxy-2-fluoro-3-O-(1-hydroxy-1,1,3,3-tetraisopropyldisiloxane-3-yl)-β-D-arabinofuranosyl]-adenine}-5'-[(1,2-di-O-myristoyl-*sn*-glycer-3-yl)-(2-cyanoethyl)phosphate], 10, a mixture of diastereomers

^{1}H NMR ($CDCl_3$): δ 8.14 (d, J 3.0 Hz, H-8), 8.11 (d, J 3.0 Hz, H-8;), 6.58 (dd, $J_{1',2'}$ 3.0 Hz, $J_{1',F}$ 22.5 Hz, H-1'), 6.52 (dd, $J_{1',2'}$ 3.0 Hz, $J_{1',F}$ 22.0 Hz, H-1'), 6.15 (br s, NH_2), 5.24 (m, H-2 Gly), 5.11 (m, $J_{2',F}$ 51.0 Hz, H-2'), 4.81 (m, $J_{3',F}$ 18.5 Hz, H-3'), 4.69 (br s, Si-OH), 4.42-4.15 (m, H-1 Gly, H-3 Gly, H-4', H-5', H-5", CH_2CH_2CN), 2.77 (m, CH_2CH_2CN), 2.33-2.31 (m,

$CH_3(CH_2)_{11}CH_2CO$), 1.59 (m, $CH_3(CH_2)_{10}CH_2CH_2CO$), 1.30-1.25 (m, $CH_3(CH_2)_{10}C_2H_4CO$), 1.09-0.95 (m, i-Pr), 0.88 (t, $CH_3(CH_2)_{12}CO$). ^{13}C NMR ($CDCl_3$): δ 173.33 ($CH_2OC(O)$), 172.93 ($CHOC(O)$), 156.11, 154.40, 150.70, 140.53 (Ade: C(6), C(2), C(4), C(8)), 117.99, 116.16 (C(5), CH_2CH_2CN), 94.47 (d, $J_{C,F}$ 193.0 Hz, C(2')), 84.50 (m, C(4')), 83.76 (d, $J_{C,F}$ 16.5 Hz, C(1')), 83.70 (d, $J_{C,F}$ 16.0 Hz, C(1')), 75.08 (d, $J_{C,F}$ 26.0 Hz, C(3')), 69.23 (d, J 7.0 Hz, C-2 Gly), 66.85 (br s, C(5')), 66.33 (d, CH_2CH_2CN), 66.27 (d, CH_2CH_2CN), 62.42, 61.50 (C-1 Gly, C-3 Gly), 34.13, 34.00 (CH_2CO), 31.92, 29.70, 29.49, 29.37, 29.29, 29.13, 29.09, 24.93, 24.82, 22.66 ($CH_3(CH_2)_{11}CH_2CO$), 19.64 (d, CH_2CH_2CN), 17.29, 17.24, 17.16, 17.10, 14.10, 13.41, 12.99, 12.80 (i-Pr, $CH_3(CH_2)_{12}CO$). ^{31}P NMR ($CDCl_3$): δ -0.41 (m). ESI-MS: $[MH]^+$: 1192.

4.2.7. {2-Chloro-9-[2-deoxy-2-fluoro-3-O-(1-hydroxy-1,1,3,3-tetraisopropyldisiloxane-3-yl)-β-D-arabinofuranosyl]-adenine}-5'-[(1,2-di-O-palmitoyl-*sn*-glycer-3-yl)-(2-cyanoethyl)phosphate], 11, a mixture of diastereomers

^1H NMR ($CDCl_3$): δ 8.14 (d, J 3.0 Hz, H-8), 8.11 (d, J 3.0 Hz, H-8), 6.55 (dd, $J_{1',2'}$ 3.0 Hz, $J_{1',F}$ 22.5 Hz, H-1'), 6.52 (dd, $J_{1',2'}$ 3.0 Hz, $J_{1',F}$ 22.0 Hz, H-1'), 6.03 (br s, NH_2), 5.25 (m, H-2 Gly), 5.10 (m, $J_{2',F}$ 51.0 Hz, H-2'), 4.80 (m, $J_{3',F}$ 18.0 Hz, H-3'), 4.68 (br s, Si-OH), 4.42-4.14 (m, H-1 Gly, H-3 Gly, H-4', H-5', H-5", CH_2CH_2CN), 2.76 (m, CH_2CH_2CN), 2.34-2.28 (m, $CH_3(CH_2)_{13}CH_2CO$), 1.59 (m, $CH_3(CH_2)_{12}CH_2CH_2CO$), 1.30-1.25 (m, $CH_3(CH_2)_{12}C_2H_4CO$), 1.09-0.95 (m, i-Pr), 0.88 (t, $CH_3(CH_2)_{14}CO$). ^{13}C NMR ($CDCl_3$): δ 173.31 ($CH_2OC(O)$), 172.91 ($CHOC(O)$), 156.09, 154.37, 150.68, 140.56 (Ade: C(6), C(2), C(5), C(8)), 117.97, 116.13 (C(4), CH_2CH_2CN), 94.44 (d, $J_{C,F}$ 193.0 Hz, C(2')), 85.56 (m, C(4')), 83.78 (d, $J_{C,F}$ 16.0 Hz, C(1')), 83.71 (d, $J_{C,F}$ 16.0 Hz, C(1')), 75.06 (d, $J_{C,F}$ 26.0 Hz, C(3')), 69.21 (d, J 7.0 Hz, C-2 Gly), 66.83 (br s, C(5')), 66.32 (d, CH_2CH_2CN), 66.27 (d, CH_2CH_2CN), 62.42, 61.50 (C-1 Gly, C-3 Gly), 34.13, 33.98 (CH_2CO), 31.92, 29.70, 29.50, 29.36, 29.29, 29.13, 29.09, 24.93, 24.82, 22.68 ($CH_3(CH_2)_{13}CH_2CO$), 19.61 (d, J 7.0 Hz, CH_2CH_2CN), 17.32, 17.26, 17.19, 17.13, 14.13, 13.43, 13.02, 12.83 (i-Pr, $CH_3(CH_2)_{14}CO$). ^{31}P NMR ($CDCl_3$): δ -0.41 (m). ESI-MS: $[MH]^+$: 1248.

4.2.8. {2-Chloro-9-[2-deoxy-2-fluoro-3-O-(1-hydroxy-1,1,3,3-tetraisopropyldisiloxane-3-yl)-β-D-arabinofuranosyl]-adenine}-5'-[(1,3-di-O-myristoyl-*sn*-glycer-2-yl)-(2-cyanoethyl)phosphate], 12, a mixture of diastereomers

^1H NMR ($CDCl_3$): δ 8.14 (d, J 3.0 Hz, H-8), 8.13 (d, J 3.0 Hz, H-8), 6.52 (dd, $J_{1',2'}$ 2.5 Hz, $J_{1',F}$ 22.5 Hz, H-1'), 6.51 (dd, $J_{1',2'}$ 2.5 Hz, $J_{1',F}$ 22.5 Hz, H-1'), 6.44 (br s, NH_2), 5.12 (m, $J_{2',F}$ 51.0 Hz, H-2'), 4.82-4.77 (m, H-3', SiOH), 4.45-4.19 (m, CH_2CH_2CN, H-1 Gly, H-2 Gly, H-3 Gly, H-4', H-5', H-5"), 2.77 (m, CH_2CH_2CN), 2.33 (m, $CH_3(CH_2)_{11}CH_2CO$), 1.60 (m, $CH_3(CH_2)_{10}CH_2CH_2CO$), 1.26 (m, $CH_3(CH_2)_{10}C_2H_4CO$), 1.09-0.95 (m, i-Pr), 0.88 (t, $CH_3(CH_2)_{12}CO$). ^{13}C NMR ($CDCl_3$): δ 173.23 ($CH_2OC(O)$), 156.20, 154.36, 150.56, 140.46 (Ade, C(6), C(2), C(4), C(8)), 117.87, 116.09 (C(5), CH_2CH_2CN), 94.41 (d, $J_{C,F}$ 192.5 Hz, C(2')), 94.36 (d, $J_{C,F}$ 193.0 Hz, C(2')), 84.51 (m, C(4')), 83.80 (d, $J_{C,F}$ 15.5 Hz, C(1')), 75.15, 74.93 (C(3'), C-2 Gly), 66.80 (m, C(5')), 62.41 (m, C-1 Gly, C-3 Gly), 33.95, 33.93 (CH_2CO), 31.89, 29.62, 29.44, 29.32, 29.25, 29.10, 24.75, 22.65 ($CH_3(CH_2)_{11}CH_2CO$), 19.55 (d, J 7.0 Hz, CH_2CH_2CN), 17.27, 17.21, 17.09, 14.07, 13.39, 12.97, 12.78 (i-Pr, $CH_3(CH_2)_{12}CO$). ^{31}P NMR ($CDCl_3$): δ -0.40 (m). ESI-MS: $[MH]^+$: 1192.

4.2.9. {2-Chloro-9-[2-deoxy-2-fluoro-3-O-(1-hydroxy-1,1,3,3-tetraisopropyldisiloxane-3-yl)-β-D-arabinofuranosyl]-adenine}-5'-[(1,3-di-O-palmitoyl-*sn*-glycer-2-yl)-(2-cyanoethyl)phosphate], 13, a mixture of diastereomers

^1H NMR ($CDCl_3$): δ 8.14 (d, J 3.0 Hz, H-8), 8.13 (d, J 3.0 Hz, H-8), 6.52 (dd, $J_{1',2'}$ 3.0 Hz, $J_{1',F}$ 22.5 Hz, H-1'), 6.51 (dd, $J_{1',2'}$ 3.0 Hz, $J_{1',F}$ 22.5 Hz, H-1'), 6.11 (br s, NH_2), 5.11 (m, $J_{2',F}$ 51.0 Hz, H-2'), 4.83-4.68 (m, H-3', SiOH), 4.45-4.15 (m, CH_2CH_2CN, H-1 Gly, H-2 Gly, H-3 Gly, H-4', H-5', H-5"), 2.77 (m, CH_2CH_2CN), 2.33 (m, $CH_3(CH_2)_{13}CH_2CO$), 1.60 (m, $CH_3(CH_2)_{12}CH_2CH_2CO$), 1.25 (m, $CH_3(CH_2)_{12}C_2H_4CO$), 1.09-0.95 (m, i-Pr), 0.88 (t, $CH_3(CH_2)_{14}CO$). ^{13}C NMR ($CDCl_3$): δ 173.25 ($CH_2OC(O)$), 156.08, 154.39, 150.68, 140.54 (Ade: C(6), C(2), C(4), C(8)), 118.01, 116.10 (C(5), CH_2CH_2CN), 94.45 (d, $J_{C,F}$ 193.0 Hz, C(2')), 94.40 (d, $J_{C,F}$ 191.5 Hz, C(2')), 84.53 (m, C(4')), 83.83 (d, $J_{C,F}$ 15.5 Hz, C(1')), 75.18, 74.97 (C(3'), C-2 Gly), 66.83 (m, C(5')), 62.43 (m, C-1 Gly, C-3 Gly), 33.98, 33.95 (CH_2CO), 31.94, 29.72, 29.50, 29.38, 29.30, 29.15, 24.80, 22.71 ($CH_3(CH_2)_{13}CH_2CO$), 19.59 (d, J 7.5 Hz, CH_2CH_2CN), 17.30, 17.24, 17.17, 17.11, 14.10, 13.42, 13.00, 12.81 (i-Pr, $CH_3(CH_2)_{14}CO$). ^{31}P NMR ($CDCl_3$): δ -0.40 (m). ESI-MS: $[MH]^+$: 1248.

4.2.10. General procedure for the preparation of conjugates 14-17

A solution of diacylglycerophosphates of clofarabine **10**-**13** (0.30 mmol) in Py/Et_3N mixture (1:1, v:v; 6 mL) was stirred for 24 h and evaporated. The residue was evaporated with toluene (2 mL), dissolved in THF (4 mL), and 1M TBAF in THF (0.75 mL) was added to the obtained solution. After stirring for 4 h, the reaction mixture was evaporated to dryness. The residue was evaporated with toluene (2 mL) and purified on silica gel column (50 cm^3) using a gradient of CH_3OH (0→20% + 1.5% $(C_2H_5)_3N$) in $CHCl_3$. The residues after evaporation of the appropriate fractions were dissolved in $CHCl_3$/MeOH (30 mL), H_2O (6 mL) was added, mixed well, and pH of the aqueous layer was adjusted to 1. The organic layer was separated and methanolic NaOH (0.1 M, 1.0 molar equiv.) was added, the mixture was evaporated under reduced pressure to yield the sodium salts **14**, **15**, **16**, and **17** (60, 97, 76, and 73%, correspondingly).

4.2.11. [2-Chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-adenine]-5'-(1,2-di-O-myristoyl-*sn*-glycer-3-yl)phosphate, sodium salt, 14

^1H NMR (DMSO-d_6): δ 8.26 (s, 1H, H-8), 7.89 (br s, 2H, NH_2), 6.36 (br s, 1H, 3'-OH), 6.31 (m, 1H, $J_{1',2'}$ 4.0 Hz, $J_{1',F}$ 13.0 Hz, H-1'), 5.22 (m, 1H, $J_{2',F}$ 52.5 Hz, H-2'), 5.05 (m, 1H, H-2 Gly), 4.44 (m, 1H, $J_{3',F}$ 19.0 Hz, H-3'), 4.27 (dd, 1H, $J_{1,2}$ 12.0 Hz, H-1 Gly), 4.05 (dd, 1H, $J_{1,2}$ 6.5 Hz, $J_{1,1}$ 12.0 Hz, H-1 Gly), 3.94 (m, 1H, H-4'), 3.88 (m, 2H, H-5', H-5"), 3.74 (m, 2H, 2H-3 Gly), 2.23 (m, 2H, $CH_3(CH_2)_{11}CH_2CO$), 2.21 (m, 4H, $CH_3(CH_2)_{11}CH_2CO$), 1.46 (m, 4H, 2 $CH_3(CH_2)_{10}CH_2CH_2CO$), 1.28–1.15 (m, 40H, 2 $CH_3(CH_2)_{10}C_2H_4CO$), 0.85 (t, 6H, 2 $CH_3(CH_2)_{12}CO$). ^{13}C NMR (DMSO-d_6): δ 172.53 ($CH_2OC(O)$), 172.27 ($CHOC(O)$), 156.78, 153.28, 150.16, 139.91, 117.31 (Ade), 94.95 (d, $J_{C,F}$ 193.0 Hz, C(2')), 81.89 (C(4')), 81.29 (d, $J_{C,F}$ 16.0 Hz, C(1')), 73.03 (d, $J_{C,F}$ 25.0 Hz, C(3')), 70.41 (C-2 Gly), 63.59 (d, $J_{C,P}$ 2.5 Hz, C(5')), 62.64 (C-3 Gly), 62.29 (C-1 Gly), 33.56, 33.38 (CH_2CO), 31.31 ($CH_3CH_2CH_2$), 29.05, 28.93, 28.73, 28.43, 28.42 ($CH_3(CH_2)_2(CH_2)_8(CH_2)_2CO$), 24.44, 24.40 ($CH_2CH_2CO$), 22.10 ($CH_3CH_2$), 13.93 ($CH_3$). ^{31}P NMR (DMSO-d_6): δ -0.62. Anal. calcd for $C_{41}H_{69}ClFN_5NaO_{10}P{\cdot}H_2O$, (918.444), %: C 53.62, H 7.79, N 7.63; found, %: C 53.46, H 7.99, N 7.07. ESI-MS: $[MH]^+$: 900.

4.2.12. [2-Chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-adenine]-5'-(1,2-di-O-palmitoyl-*sn*-glycer-3-yl)phosphate,

sodium salt, 15

^{1}H NMR (DMSO-d_6): δ 8.22 (s, 1H, H-8), 7.84 (br s, 2H, NH_2), 6.34 (br s, 1H, 3'-OH), 6.30 (dd, 1H, $J_{1',2'}$ 4.5 Hz, $J_{1',F}$ 13.5 Hz, H-1'), 5.21 (dt, 1H, $J_{2',F}$ 53.0 Hz, H-2'), 5.04 (m, 1H, H-2 Gly), 4.44 (m, 1H, $J_{3',F}$ 18.5 Hz, H-3'), 4.28 (dd, 1H, $J_{1,2}$ 3.0 Hz, $J_{1,1}$ 12.0 Hz, H-1 Gly), 4.06 (dd, 1H, $J_{1,2}$ 7.0 Hz, $J_{1,1}$ 12.0 Hz, H-1 Gly), 3.93 (m, 1H, H-4'), 3.88 (m, 2H, H-5', H-5"), 3.74 (m, 2H, 2H-3 Gly), 2.23 (m, 2H, $CH_3(CH_2)_{13}CH_2CO$), 2.20 (m, 2H, $CH_3(CH_2)_{13}CH_2CO$), 1.47 (m, 4H, $2CH_3(CH_2)_{12}CH_2CH_2CO$), 1.28–1.15 (m, 48H, $2CH_3(CH_2)_{12}C_2H_4CO$), 0.85 (t, 6H, $2CH_3(CH_2)_{14}CO$). ^{13}C NMR (DMSO-d_6): δ 172.37 ($CH_2OC(O)$), 172.11 ($CHOC(O)$), 156.69, 153.20, 150.10, 139.75, 117.26 (Ade), 94.85 (d, $J_{C,F}$ 193.0 Hz, C(2')), 81.87 (d, J 4.0 Hz, C(4')), 81.20 (d, $J_{C,F}$ 17.0 Hz, C(1')), 73.14 (d, $J_{C,F}$ 22.0 Hz, C(3')), 70.42 (d, J 8.0 Hz, C-2 Gly), 63.42 (br s, C(5')), 62.50 (d, J 5.0 Hz, C-3 Gly), 62.27 (C-1 Gly), 33.51, 33.33 (CH_2CO), 31.19 ($CH_3CH_2CH_2$), 28.94, 28.81, 28.60, 28.33 ($CH_3(CH_2)_2(CH_2)_{10}(CH_2)_2CO$), 24.35, 24.30 ($CH_2CH_2CO$), 21.98 ($CH_3CH_2$), 13.80 ($CH_3$). ^{31}P NMR (DMSO-$d_6$): δ -0,62. Anal. calcd for $C_{45}H_{77}ClFN_5NaO_{10}P{\cdot}H_2O$, (974.551), %: C 55.46, H 8.17, N 7.19; found, %: C 55.56, H 8.19, N 7.01. ESI-MS: $[MH]^+$: 956.

4.2.13. [2-Chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-adenine]-5'-(1,3-di-O-myristoylglycer-2-yl)phosphate, sodium salt, 16

^{1}H NMR (DMSO-d_6): δ 8.22 (s, 1H, H-8), 7.88 (br s, 2H, NH_2), 6.32 (dd, 1H, $J_{1',2'}$ 5.0 Hz, $J_{1',F}$ 14.0 Hz, H-1'), 5.23 (dt, 1H, $J_{2',F}$ 53.0 Hz, H-2'), 4.42 (m, 1H, $J_{3',F}$ 17.0 Hz, H-3'), 4.30 (m, 1H, H-2 Gly), 4.08 (m, 4H, 2H-1 Gly, 2H-3 Gly), 3.97 (m, 1H, H-4'), 3.91 (m, 2H, H-5', H-5"), 2.23 (m, 4H, $2CH_3(CH_2)_{11}CH_2CO$), 1.44 (m, 4H, $2CH_3(CH_2)_{10}CH_2CH_2CO$), 1.22–1.18 (m, 40H, $2CH_3(CH_2)_{10}C_2H_4CO$), 0.84 (t, 6H, $2CH_3(CH_2)_{12}CO$). ^{13}C NMR (DMSO-d_6): δ 172.37 ($CH_2OC(O)$), 156.65, 153.15, 150.02, 139.72, 117.19 (Ade), 95.30 (d, $J_{C,F}$ 192.0 Hz, C(2')), 81.83 (br s, C(4')), 81.28 (d, $J_{C,F}$ 17.0 Hz, C(1')), 72.69 (d, $J_{C,F}$ 23.0 Hz, C(3')), 69.41 (d, J 6.0 Hz, C-2 Gly), 63.62 (C(5')), 62.97, 62.91 (C-1 Gly, C-3 Gly), 33.21 (CH_2CO), 31.17 ($CH_3CH_2CH_2$), 28.23, 28.03, 28.77, 28.69, 28.32 ($CH_3(CH_2)_2(CH_2)_8(CH_2)_2CO$), 24.24 ($CH_2CH_2CO$), 21.97 ($CH_3CH_2$), 13.79 ($CH_3$). ^{31}P NMR (DMSO-$d_6$): δ -0,62. Anal. calcd for $C_{41}H_{69}ClFN_5NaO_{10}P{\cdot}H_2O$, (918.444), %: C 53.62, H 7.79, N 7.63; found, %: C 53.56, H 8.03, N 7.67. ESI-MS: $[MH]^+$: 900.

4.2.14. [2-Chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-adenine]-5'-(1,3-di-O-palmitoylglycer-2-yl)phosphate, sodium salt, 17

^{1}H NMR (DMSO-d_6): δ 8.21 (s, 1H, H-8), 7.89 (br s, 2H, NH_2), 6.31 (dd, 1H, $J_{1',2'}$ 5.0 Hz, $J_{1',F}$ 14.0 Hz, H-1'), 5.21 (dt, 1H, $J_{2',F}$ 53.0 Hz, H-2'), 4.44 (m, 1H, $J_{3',F}$ 19.0 Hz, H-3'), 4.32 (m, 1H, H-2 Gly), 4.08 (m, 4H, 2H-1 Gly, 2H-3 Gly), 3.95 (m, 1H, H-4'), 3.93 (m, 2H, H-5', H-5"), 2.24 (m, 4H, $2CH_3(CH_2)_{13}CH_2CO$), 1.46 (m, 4H, $2CH_3(CH_2)_{12}CH_2CH_2CO$), 1.27–1.19 (m, 48H, $2CH_3(CH_2)_{12}C_2H_4CO$), 0.85 (t, 6H, $2CH_3(CH_2)_{14}CO$). ^{13}C NMR (DMSO-d_6): δ 172.49 ($CH_2OC(O)$), 156.77, 153.28, 150.15, 139.85, 117.31 (Ade), 94.89 (d, $J_{C,F}$ 192.0 Hz, C(2')), 81.92 (br s, C(4')), 81.34 (d, $J_{C,F}$ 17.0 Hz, C(1')), 73.11 (d, $J_{C,F}$ 23.0 Hz, C(3')), 69.53 (d, J 6.0 Hz, C-2 Gly), 63.74 (C(5')), 62.99, 62.93 (C-1 Gly, C-3 Gly), 33.33 (CH_2CO), 31.30 ($CH_3CH_2CH_2$), 29.01, 29.04, 28.89, 28.71, 28.44 ($CH_3(CH_2)_2(CH_2)_{10}(CH_2)_2CO$), 24.36 ($CH_2CH_2CO$), 22.09 ($CH_3CH_2$), 13.91 ($CH_3$). ^{31}P NMR (DMSO-$d_6$): δ -0,62. Anal. calcd for $C_{45}H_{77}ClFN_5NaO_{10}P{\cdot}H_2O$, (974.551), %: C 55.46, H 8.17, N 7.19; found, %: C 55.34, H 8.29, N 7.35 ESI-MS: $[MH]^+$: 956.

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