Nucleotides

Part LVI¹)

Synthesis and Biological Activity of Modified (2'-5')Triadenylates Containing 2'-Terminal 2',3'-Dideoxy-3'-fluoroadenosine Derivatives

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Some new (2'-5')triadenylates 13–16, containing at the 2'-terminal end 3'-fluoro-2',3'-dideoxyadenosine derivatives, have been synthesized by the phosphotriester method. The selectively blocked nucleosides 2, 4, 5, and 7 were synthesized from the corresponding unprotected nucleosides 1, 3, and 6. The synthesized trimers 13 and 14 were 4- and 8-fold, respectively, more stable towards phosphodiesterase from *Crotalus durissus* than the natural trimer 17. In comparison to trimer 17 the new compounds 13–15 inhibit HIV-1 reverse transcriptase (RT) activity, and 15 and 16 the HIV-1 induced syncytia formation 2–3 fold whereas none of 13–16 can improve RNase L activity.

1. Introduction. – The series of 2',5'-phosphodiester bond-linked oligoadenylate 5'triphosphates, with exception of the dimeric forms, are known as potential inhibitors of translation [2] and counteracting especially virus replication. Their mechanism of action seems to be mediated mainly through the activation of a latent endonuclease (RNase L), leading to the degradation of viral RNA and subsequent inhibition of protein synthesis [3]. But, the presence of these triphosphates in intact cells has a dramatic effect on the RNase L which is activated but is not able to discriminate between viral and cellular RNA, and hence, also degrades cellular mRNA and rRNA [4][5]. From this point of view, and, because of a high sensitivity of (2'-5')oligoadenylate 5'-triphosphates towards enzymes which degrade phosphorylated oligonucleotides [6][7], the use of the first in chemotherapy seems to be problematical. Such disadvantages have not been found for the various unphosphorylated (2'-5')oligoadenylates and its synthetic analogues. The metabolic stability of (2'-5')oligoadenylates plays also an important role for their potential activity and practical use. Many analogues of (2'-5')oligoadenylates have been synthesized to achieve new approaches to antiviral and antitumor therapy [8-16]. Previ-

¹) Part LV: [1].

ous studies have shown that a 3' modification at the 2'-terminus of (2'-5')oligoadenylates makes a major contribution to the metabolic stability and biological activity of such analogues [5][17]. Thus, cordycepin (= 3'-deoxyadenosine) trimer core [18] was found to be a biologically active compound with metabolic stability [19]; later it turned out that it is also an inhibitor of HIV-1 reverse transcriptase (RT) [20]. Recent studies with 3'-deoxy-3'-fluoroadenosine containing analogues of 5'-phosphorylated (2'-5')oligoadenylate trimer have shown high metabolic stability towards 2',5'-phosphodiesterase of mouse L cells, and the ability to bind to and to activate RNase L [21-23]. As far as each individual nucleoside residue of (2'-5')oligoadenylate may assume a different role in inhibition of RT and replication of viruses, we synthesized some new (2'-5')triadenylates with a double modification at the 2'- and 3'-position of the 2'-terminal adenosine unit, as potential metabolically stable (2'-5')oligoadenylates. The rationale for the replacement of H-atoms and OH groups of biologically significant molecules by F-atoms has been extensively reviewed [24].

2. Syntheses. – The syntheses of 2',3'-dideoxy-3'-fluoroadenosine containing trimers were achieved by the phosphotriester method, using the approach published by us earlier [25]. The starting 2',3'-dideoxy-3'-fluoroadenosine derivatives 1, 3, and 6 were also described earlier [26] [27]²), and their selective interconversion into the blocked nucleosides 2, 4, 5, and 7 were achieved by the transient protection method [28]. Thus, trimethylsilylation of 1, 3, and 6 with chlorotrimethylsilane in pyridine was followed by benzoylation and hydrolysis with dilute NH₄OH solution (in the case of 7, only with H₂O) to give the corresponding nucleosides 2, 4, 5, and 7 after isolation by column chromatography (CC, silica gel) in 91, 29, 67, and 72% yield, respectively.

Condensation of 2 or 5 with 2'-phosphodiester 8 [25] in pyridine in the presence of a mixture of 1*H*-tetrazole/2,4,6-triisopropylbenzenesulfonyl chloride (TpsCl) 3:1, followed by detritylation with 2% TsOH solution in $CH_2Cl_2/MeOH$ 4:1 in a one-pot reaction led to the 5'-OH dimers 10 and 11 in 63 and 67% yield, respectively. Similar condensation of 7 with 9 [25] in the presence of a mixture of 1-methyl-1*H*-imidazole/ TpsCl 3:1 and subsequent cleavage of the dimethoxytrityl group gave in an analogous manner dimer 12 in 77% yield.

The transformations of the dimers 10-12 to the trimer level required the same techniques consisting of a condensation step, followed by successive treatment with 2% TsOH solution and either 1M 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)/pyridine (13, 14) or with a solution of 4-nitrobenzaldehyde oxime in dioxane/H₂O/Et₃N 1:1:1 (15), and finally with conc. NH₄OH solution to remove the different protecting groups. Final purification was done by ion-exchange CC (*DEAE-Servacell 23-SS*) to give the trimers 13-15 in 51, 44, and 53% overall yield, respectively. The catalytic hydrogenolysis of the azido derivative 13 in the presence of 10% Pd/C in H₂O/EtOH 1:1, followed by ion exchange CC led to the trimer 16 in 71% yield.

3. Biological Application. – The stability of the newly synthesized trimers **13** and **14** towards phosphodiesterase from *Crotalus durissus* and comparison with the naturally

²) The 2'-chloro-2',3'-dideoxy-3'-fluoroadenosine (3) has been obtained as a gift from Dr. *Tamara Pricota* (Institute of Bioorganic Chemistry, Byelorussian Academy of Sciences, Minsk).





Bz = benzoyl o-ClC₆H₄ = 2-chlorophenyl MeOTr = monomethoxytrityl Npe = 2-(4-nitrophenyl)ethyl



	R	<u>R¹</u>	<u>R</u> ²
10	Н	N ₃	Npe
11	н	Ci	Npe
12	Bz	н	o-ClC ₆ H ₄



	R	R ¹	
13	N ₃	F	
14	ĊĨ	F	
15	Н	F	
16	NH ₂	F	
17	OH	OH	

occurring (2'-5')A trimer core 17 was studied by means of prep. TLC. The calculated half-life for the trimers 13, 14, and 17 was found to be 126, 205, and 27 min, respectively.

Replacement of the OH group at the 2',3'-terminus of trimer 17 with either F, Cl, N₃, or NH₂ substituents produced a new type of inhibitors of HIV-1 replication (*Table*). Three separate studies were performed to determine the antiviral activity of these (2'-5')-oligoadenylate analogues: *i*) inhibition of HIV-1-induced syncytia formation, *ii*) inhibition of HIV-1 RT activity, and *iii*) activation of recombinant human GST-RNase L.

	R	R ¹	Syn ^b)	RT ^c)	RNase L ^d)
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13	N ₃	F	-	99.7	0
14	C1	F	2.0	99.7	0
15	Н	F	7.0	99.7	0
16	NH ₂	F	9.0	-	0
17	OH	OH	3.0	33	50

Table. Inhibition of HIV-1 Replication and Biological Activities of (2'-5')Oligoadenylate Trimers 13-17*)

^a) Compounds were tested at 300 μ M. ^b) Inhibition of HIV-1 replication was determined by HIV-1-induced syncytia formation (fold reduction) for each compound. The number of syncytia/10⁴ cells was 121 ± 16 for the control Sup T1 cells. The mean of triplicate determinations is shown; variance did not exceed 5–10%. ^c) Percent inhibition of reverse transcriptase (HIV-1 RT) activity. Control values for HIV-1 RT activity ranged from 15,000 to 16,000 cpm. The mean of duplicate determinations is shown; variance did not exceed 5–10%. ^d) The activation of recombinant human RNase L was measured as the percent hydrolysis of poly(U)-3'-[³²P]pCp in the presence of the trimers 13–17. The mean of duplicate determinations is shown; variance did not exceed 5–10%.

Compounds 14-16 inhibited induced syncytia formation 2.0, 7.0, and 9.0 fold, respectively, compared to 3.0 fold reduction with unmodified trimer 17. Trimers 13-15 inhibited HIV-1 RT activity to 99.7%, which compares with a 33% inhibition of HIV-1 RT by the compound 17. The previously obtained data about a 96% inhibition of HIV-1 RT by the cordycepin trimer core [29] and the results presented here show that OH groups at either the C(2') or C(3') position of the trimer 17 are not essential for the inhibition of HIV-1 RT activity. When (2'-5') oligoadenylate 17 is modified at the C(3')position with the F-atom and at the C(2') position either by a H, Cl, N₃, or NH₂ substituent, recombinant human GST-RNase L is not activated to hydrolyze poly(U)-3'-[³²P]pCp compared to a 50% activation of GST-RNase L by the trimer 17. These results are in agreement with previous data showing that interaction of 3'-deoxy-3'-fluoro analogues of 5'-phosphorylated (2'-5') oligoadenylate trimers with RNase L from mouse L cells and rabbit reticulocytes [21], their ability to stimulate activation of mouse and human RNase L [22], and a 12% activation of GST-RNase L by both the cordycepin trimer core and its conjugate with vitamin E at the 2'-terminus of the trimer [29], required the OH group at the C(2') position of the 2',3'-terminus as a feature essential for the activation of GST-RNase L.

Experimental Part

General. TLC: Precoated silica gel thin-layer sheets 60 F 254 from Merck. Prep. column chromatography (CC): silica gel (Merck 60, 63–200 µm). Ion-exchange chromatography: DEAE-Servacell-23-SS (Serva). M.p.: Gallenkamp melting-point apparatus; no correction. UV/VIS: Specord UV-VIS (Carl Zeiss, Germany); λ_{max} in nm (log ε). ¹H-NMR: Bruker WM-360; δ in ppm rel. to SiMe₄.

Bioassay. The stability of the trimers 13 and 14 towards phosphodiesterase from *Crotalus durissus* was determined as described [15]. Assays measuring HIV-1 induced syncytia formation, HIV-1 reverse transcriptase activity, and activation of RNase L were accomplished by known methods [29].

2'-Azido-N⁶-benzoyl-2', 3'-dideoxy-3'-fluoroadenosine (2). A mixture of 1 (0.1 g, 0.34 mmol) and chlorotrimethylsilane (0.37 g, 0.42 ml, 3.4 mmol) in pyridine (3 ml) was stirred at r.t. for 4 h and then treated with benzoyl chloride (0.09 g, 0.7 mmol). After stirring at r.t. for 0.5 h, the mixture was treated with H_2O (0.5 ml) and conc. NH₄OH soln. (1 ml) and evaporated. The residue was purified by CC (silica gel, 10 × 2.5 cm, CHCl₃ and then CHCl₃/MeOH 24:1) and finally crystallized from EtOH: 123 mg (91%) of **2**. M.p. 125–126°. UV (MeOH): 230 (4.32), 280 (4.56). ¹H-NMR (CDCl₃): 9.18 (*s*, NH); 8.80, 8.11 (2*s*, H–C(2), H–C(8)); 8.05–7.51 (*m*, 5 arom. H); 6.20 (*dd*, OH–C(5')); 6.00 (*d*, H–C(1')); 5.45 (*dd*, H-C(3')); 4.95 (*ddd*, H–C(2')); 4.61 (*d*, H–C(4')); 3.95 (*m*, 2 H–C(5')). Anal. calc. for $C_{17}H_{15}FN_8O_3$ (398.3): C 51.25, H 3.79, N 28.12; found: C 51.22, H 3.80, N 28.14.

 N^6 , N^6 -Dibenzoyl-2'-chloro-2', 3'-dideoxy-3'-fluoroadenosine (4) and N^6 -Benzoyl-2'-chloro-2', 3'-dideoxy-3'-fluoroadenosine (5). As described for 2, with 3 (0.1 g, 0.34 mmol), trimethylchlorosilane (0.37 g, 0.42 ml, 0.34 mmol), pyridine (3 ml) and benzoyl chloride (0.9 g, 0.7 mmol); then treatment with H_2O (0.6 ml) and conc. NH_4OH soln. (1 ml). CC (silica gel, 15×2.5 cm, CHCl₃ and then CHCl₃/MeOH 49:1) gave 50 mg (29%) of 4 and 91 mg (67%) of 5.

Data of **4**: Colourless foam. UV (MeOH): 230 (4.50), 280 (4.40). ¹H-NMR (CDCl₃): 8.65, 8.17 (2*s*, H–C(2), H–C(8)); 7.90–7.32 (*m*, 10 arom. H); 6.07 (*d*, H–C(1')); 5.86 (*dd*, OH-C(5')); 5.30 (*dd*, H–C(2')); 5.17 (*dd*, H–C(3')); 4.62 (*m*, H–C(4')); 3.92 (*m*, 2 H–C(5')). Anal. calc. for $C_{24}H_{19}CIFN_5O_4$ (495.9): C 58.12, H 3.86, N 14.12; found: C 58.24, H 3.90, N 14.03.

Data of 5: M.p. 198–200° (from EtOH). UV (MeOH): 230 (4.12), 281 (4.32). ¹H-NMR (CDCl₃): 9.16 (s, NH); 8.78, 7.98 (2s, H–C(2), H–C(8)); 8.06–7.50 (m, 5 arom. H): 6.23 (dd, OH–C(5')); 6.06 (d, H–C(1')); 5.45–5.12 (m, H–C(2'), H–C(3')); 4.62 (m, H–C(4')); 3.95 (m, 2 H–C(5')). Anal. calc. for $C_{17}H_{15}CIFN_5O_3$ (391.8): C 52.11, H 3.85, N 17.87; found: C 52.30, H 3.81, 17.69.

N⁶,N⁶-*Dibenzoyl-2'*,3'-*dideoxy-3'-fluoroadenosine* (7). As described for **2**, with **6** (13 mg, 0.05 mmol), chlorotrimethylsilane (43 mg, 50 μl, 0.39 mmol), pyridine (0.5 ml; 3 h), and benzoyl chloride (35 mg, 29 μl, 0.25 mmol); then treatment with H₂O (0.1 ml). CC (silica gel, 10×1 cm, CHCl₃/MeOH 24:1) gave 17 mg (72%) of **7**. Colourless foam. UV (MeOH): 250 (4.25), 278 (4.50). ¹H-NMR (CDCl₃): 8.44, 8.18 (2*s*, H–C(2), H–C(8)); 7.86–7.30 (*m*, 10 arom. H); 6.40 (*d*, H–C(1')); 5.73 (br. *s*, OH–C(5')); 5.48 (*dd*, H–C(3')); 4.51 (*m*, H–C(4')); 3.88 (*m*, 2 H–C(5')); 3.11, 2.64 (2*m*, H–C(2')). Anal. calc. for C₂₄H₂₀ClFN₅O₄ (461.4): C 62.46, H 4.36, N 15.17; found: C 62.29, H 4.33, N 15.01.

N⁶,3'-O-Dibenzoyladenylyl-{2'-{O^P-[2-(4-nitrophenyl)ethyl]} → 5'}-2'-azido-N⁶-benzoyl-2',3'-dideoxy-3'-fluoroadenosine (**10**). To a soln. of **2** (100 mg, 0.25 mmol) and **8** (325 mg, 0.3 mmol) in pyridine (2.8 ml), 1*H*-tetrazole (126 mg, 1.8 mmol) and TpsCl (273 mg, 0.9 mmol) were added. The mixture was stirred at r.t. for 20 h, diluted with CHCl₃ (100 ml), and washed with 0.05M (Et₃NH)HCO₃ (2 × 75 ml). The org. phase was dried (Na₂SO₄), evaporated, and co-evaporated with toluene (30 ml). The residue was dissolved in 2% TsOH soln. in CH₂Cl₂/MeOH 4:1 (15 ml), stirred for 10 min, diluted with CHCl₃ (100 ml), and washed with 0.05M (Et₃NH)HCO₃ (2 × 50 ml). The org. phase was dried (Na₂SO₄) and evaporated. The residue was purified by CC (silica gel, 10 × 1.5 cm, CHCl₃ and then CHCl₃/MeOH 24:1): 172 mg (63%) of **10**. Colourless foam. UV (MeOH): 230 (4.54), 280 (4.55). Anal. calc. for C₄₉H₄₂FN₁₄O₁₃P (1084.9): C 54.24, H 3.90, N 18.07; found: C 54.40, H 3.85, N 17.96.

N⁶,3'-O-Dibenzoyladenylyl-{2'-{O^P-[2-(4-nitrophenyl)ethyl]} → 5'}-N⁶-benzoyl-2'-chloro-2',3'-dideoxy-3'-fluoroadenosine (11). As described for 10, with 5 (47 mg, 1.12 mmol), 8 (162 mg, 0.15 mmol), 1H-tetrazole (64 mg, 0.92 mmol), and TpsCl (139 mg, 0.46 mmol) in pyridine (1.5 ml; then treatment with 0.05M (Et₃NH)HCO₃ (50 ml) and 2% TsOH soln. in CH₂Cl₂/MeOH 4:1 (6 ml)). CC (silica gel, 15 × 1.5 cm, CHCl₃ and then CHCl₃/MeOH 49:1) gave 87 mg (67%) of 11. Colourless foam. UV (MeOH): 230 (4.50), 278 (4.56). Anal. calc. for C₄₉H₄₂ClFN₁₁O₁₃P (1078.4): C 54.57. H 3.92, N 14.28; found: C 54.67, H 3.88, N 14.09.

 N^{6} ,3'-O-Dibenzoyladenylyl-{2'-[O^P-(2-chlorophenyl)] \rightarrow 5'}-N⁶,N⁶-dibenzoyl-2',3'-dideoxy-3'-fluoroadenosine (12). To a soln. of 7 (16 mg, 0.035 mmol) and 9 (72 mg, 0.069 mmol) in pyridine (0.5 ml), 1-methyl-1*H*imidazole (32 mg, 0.39 mmol), and TpsCl (42 mg, 0.138 mmol) were added. The mixture was stirred at r.t. for 20 h, diluted with CHCl₃ (50 ml), and washed with 0.05M (Et₃NH)HCO₃ (2 × 20 ml). The org. phase was dried (Na₂SO₄), evaporated, and co-evaporated with toluene (20 ml). The residue was dissolved in 2% TsOH soln. in CH₂Cl₂/MeOH 4:1 (3 ml), stirred for 10 min, diluted with CHCl₃ (50 ml), and washed with 0.05M (Et₃NH)HCO₃ (2 × 20 ml). The org. phase was dried (Na₂SO₄) and evaporated and the residue purified by CC (silica gel, 10 × 1 cm, CHCl₃ and then CHCl₃ MeOH 90:1): 29.6 mg (77%) of 12. Colourless foam. UV (MeOH): 233 (4.42), 278 (4.44). Anal. calc. for $C_{53}H_{43}ClFN_{10}O_{12}P$ (1109.4): C 58.46, H 3.90, N 12.62; found: C 58.29, H 3.87, N 12.79.

Adenylyl-(2'-5')-adenylyl-(2'-5')-2'-azido-2',3'-dideoxy-3'-fluoroadenosine Bis(triethylammonium) Salt (13 · 2 Et₃NH⁺). A mixture of **8** (167 mg, 0.155 mmol) and **10** (140 mg, 0.129 mmol) in pyridine (1.5 ml) in the presence of TpsCl (141 mg, 0.129 mmol) and 1*H*-tetrazole (65 mg, 0.93 mmol) was stirred at r.t. for 20 h, diluted with CHCl₃ (100 ml), and washed with 0.05M (Et₃NH)HCO₃ (2 × 30 ml). The org. phase was dried (Na₂SO₄), evaporated, and co-evaporated with toluene (20 ml). The residue was dissolved in 2% TsOH soln. in CH₂Cl₂/MeOH 4:1 (9 ml), stirred for 10 min, diluted with CHCl₃ (100 ml), and washed with 0.05M (Et₃NH)HCO₃ (2 × 30 ml). The org. phase was dried (Na₂SO₄) and evaporated. The residue was dissolved in 1M DBU/pyridine (15 ml), stirred at r.t. for 24 h, neutralized with 1M AcOH/pyridine (15 ml), evaporated, and co-evaporated with toluene (20 ml). The residue was applied onto a *DEAE-Servacell-23-SS* column (20 × 1.5 cm, linear gradient of 0.005-0.2M (Et₃NH)HCO₃ buffer (pH 7.5)). The product fractions were evaporated, and co-evaporated with MeOH (2 × 20 ml). The residual Et₃NH⁺ salt was lyophilized (H₂O): 76 mg (51%) of **13** · 2 Et₃N⁺. UV (H₂O): 260 (4.58). ¹H-NMR (D₂O): 8.21, 8.13, 8.08, 7.91, 7.88, 7.75 (6s, H-C(2), H-C(8)); 6.10, 6.00 (2d, 2 H-C(1')); 5.90 (s, H-C(1')). Anal. calc. for C₄₂H₆₅FN₂₀O₁₄P (1155.0): C 43.67, H 5.67, N 24.25; found: C 43.29, H 5.42, N 23.97.

Adenylyl-(2'-5')-adenylyl-(2'-5')-2'-chloro-2',3'-dideoxy-3'-fluoroadenosine Bis(triethylammonium) Salt (14 · 2 Et₃NH⁺). As described for 13, with 8 (84 mg, 0.78 mmol), 11 (70 mg, 0.065 mmol), pyridine (1 ml), 1*H*-tetrazole (33 mg, 0.471 mmol), TpsCl (71 mg, 0.273 mmol; 20 h), 2% TsOH soln. in CH₂Cl₂/MeOH 4:1 (5 ml, 10 min), 1M DBU/pyridine (7 ml, 18 h), 1M AcOH/pyridine (7 ml), and conc. NH₄OH soln. (40 ml, 20 h). Purification by ion-exchange CC (*DEAE-Servacell-23-SS*) gave 32 mg (44%) of 14 · 2 Et₃NH⁺. UV (H₂O): 260 (4.56). ¹H-NMR (D₂O): 8.20, 8.15, 8.05, 7.95, 7.90, 7.78 (6*s*, H–C(2), H–C(8)); 6.14 (*s*, H–C(1')); 6.10, 5.94 (2*d*, 2 H–C(1')). Anal. calc. for C₄₂H₆₅CIFN₁₇O₁₄P (1148.5): C 43.92, H 5.70, N 20.73; found: C 43.52, H 5.40, N 20.34.

Adenylyl-(2'-5')-adenylyl-(2'-5')-2',3'-dideoxy-3'-fluoroadenosine Bis(triethylammonium) Salt (15 \cdot 2 Et₃NH⁺). A mixture of 9 (40 mg, 0.038 mmol) and 12 (21.5 mg, 0.019 mmol) in pyridine (1 ml) in the presence of TpsCl (24 mg, 0.08 mmol) and 1-methyl-1H-imidazole (19.2 mg, 0.018 ml, 0.234 mmol) was stirred at r.t. for 20 h, diluted with CHCl₃ (50 ml), and washed with 0.05M (Et₃NH)HCO₃ (2 \times 20 ml). The org. phase was dried (Na₂SO₄), evaporated, and co-evaporated with toluene (15 ml). The residue was dissolved in 2% TsOH soln. in CH₂Cl₂/MeOH 4:1 (1.5 ml), stirred for 10 min, diluted with CHCl₃ (50 ml), and washed with 0.05M (Et₃NH)HCO₃ (2 \times 15 ml). The org. phase was dried (Na₂SO₄), and evaporated. The residue was treated with a soln. of 4-nitrobenzaldehyde oxime (50 mg, 0.3 mmol) in Et₃N/H₂O/dioxane 1:1:1 (3 ml), kept at r.t. for 24 h, and evaporated. The residue was dissolved in 0.005–0.12M (Et₃NH)HCO₃ buffer (pH 7.5)). The product fractions were evaporated and co-evaporated with MeOH (2 \times 10 ml). The residual t₃N/H soln and salt (H₂O): 11 mg (53%) of 15 \cdot 2 Et₃NH⁺. UV (H₂O): 259 (4.59). ¹H-NMR (D₂O): 8.17, 8.12, 8.07, 7.93, 7.85, 7.75 (6s, H--C(2), H--C(8)); 6.33 (dd, H-C(1')); 6.08, 5.86 (2d, 2 H-C(1')); 2.68, 2.36 (2m, 2 H-C(2')). Anal. calc. for C₄₂H₆₆FN₁₇O₁₄P (1114.0): C 45.28, H 5.97, N 21.37; found: C 45.30, H 6.01, N 21.50.

Adenylyl-(2'-5')-adenylyl-(2'-5')-2'-amino-2',3'-dideoxy-3'-fluoroadenosine Bis(triethylammonium) Salt (16 · 2 Et₃NH⁺). A soln. of 13 (11 mg, 0.01 mmol) in H₂O/EtOH 1:1 (8 ml) in the presence of Pd/C (13 mg) was stirred under H₂ for 48 h. Then the catalyst was filtered off and washed with H₂O (6 × 1 ml). The filtrate and washings were evaporated. The residue was purified by ion exchange CC (*DEAE-Servacell-23-SS* (15 × 1.5 cm), linear gradient of 0.005-0.2M (Et₃NH)HCO₃ buffer (pH 7.5)). The product fractions were evaporated, and co-evaporated with MeOH (2 × 5 ml). The residual Et₃NH⁺ salt was lyophilized (H₂O): 8 mg (53%) of 16 · 2 Et₃NH⁺. UV (H₂O): 260 (4.60). ¹H-NMR (D₂O): 8.19, 8.14, 8.06, 7.92, 7.87, 7.76 (6 s, H-C(2), H-C(8)); 6.20, 6.07, 5.91 (3 d, 3 H-C(1')). Anal. calc. for C₄₂H₆₇FN₁₈O₁₄P · 2 H₂O (1165.1): C 43.29, H 6.14, N 21.63; found: C 42.98, H 6.01, N 21.30.

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