Nucleotides

Part LVI[1])

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

by **Evgeny I. Kvasyuk**[a]), **Tamara I. Kulak**[a]), **Olga V. Tkachenko**[a]), **Svetlana L. Sentyureva**[a]), **Igor A. Mikhailopulo**[a]), **Robert J. Suhadolnik**[b])[d]), **Earl E. Henderson**[c])[d]), **Susan E. Horvath**[b]), **Ming-Xu Guan**[c]), and **Wolfgang Pfleiderer**[c])*

[a]) Institute of Bioorganic Chemistry, Byelorussian Academy of Sciences, Minsk 220141, Belarus

[b]) Department of Biochemistry, [c]) Department of Microbiology and Immunology,

[d]) Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, Philadelphia, PA 19140, USA

[e]) Fakultät für Chemie, Universität Konstanz, Postfach 5560, D-78434 Konstanz

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-

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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 NH_4OH solution (in the case of **7**, only with H_2O) 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/H_2O/Et_3N 1:1:1 (**15**), and finally with conc. NH_4OH 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 H_2O/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

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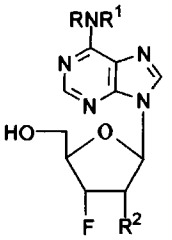
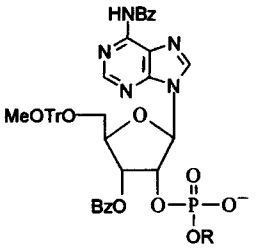
[2]) The 2′-chloro-2′,3′-dideoxy-3′-fluoroadenosine (**3**) has been obtained as a gift from Dr. *Tamara Pricota* (Institute of Bioorganic Chemistry, Byelorussian Academy of Sciences, Minsk).

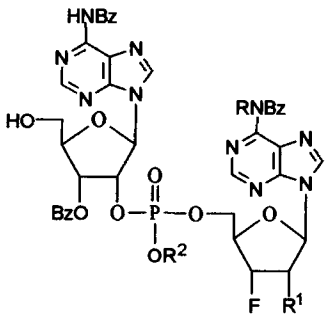


	R	R^1	R^2
1	H	H	N_3
2	H	Bz	N_3
3	H	H	Cl
4	Bz	Bz	Cl
5	H	Bz	Cl
6	H	H	H
7	Bz	Bz	H

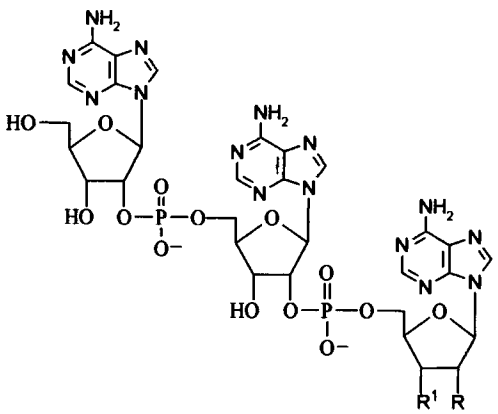


	R
8	Npe
9	*o*-ClC_6H_4

Bz = benzoyl
o-ClC_6H_4 = 2-chlorophenyl
MeOTr = monomethoxytrityl
Npe = 2-(4-nitrophenyl)ethyl



	R	R^1	R^2
10	H	N_3	Npe
11	H	Cl	Npe
12	Bz	H	*o*-ClC_6H_4



	R	R^1
13	N_3	F
14	Cl	F
15	H	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, N_3, or NH_2 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.

Table. *Inhibition of HIV-1 Replication and Biological Activities of (2′–5′)Oligoadenylate Trimers* **13**–**17**[a])

	R	R[1]	Syn[b])	RT[c])	RNase L[d])
13	N_3	F	–	99.7	0
14	Cl	F	2.0	99.7	0
15	H	F	7.0	99.7	0
16	NH_2	F	9.0	–	0
17	OH	OH	3.0	33	50

[a]) Compounds were tested at 300 μM. [b]) Inhibition of HIV-1 replication was determined by HIV-1-induced syncytia formation (fold reduction) for each compound. The number of syncytia/10^4 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′-[^{32}P]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, N_3, or NH_2 substituent, recombinant human GST-RNase L is not activated to hydrolyze poly(U)-3′-[^{32}P]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). ^{1}H-NMR: *Bruker WM-360*; δ in ppm rel. to $SiMe_4$.

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-N^6*-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. NH_4OH soln. (1 ml) and evaporated. The residue was purified by CC (silica gel, 10 × 2.5 cm, $CHCl_3$ and then $CHCl_3$/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). ^{1}H-NMR ($CDCl_3$): 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, $CHCl_3$ and then $CHCl_3$/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). ^{1}H-NMR ($CDCl_3$): 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 $C_{24}H_{19}ClFN_5O_4$ (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). ^{1}H-NMR ($CDCl_3$): 9.16 (*s*, NH); 8.78, 7.98 (2*s*, 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 $C_{17}H_{15}ClFN_5O_3$ (391.8): C 52.11, H 3.85, N 17.87; found: C 52.30, H 3.81, 17.69.

N^6,N^6*-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 H_2O (0.1 ml). CC (silica gel, 10 × 1 cm, $CHCl_3$/MeOH 24:1) gave 17 mg (72%) of **7**. Colourless foam. UV (MeOH): 250 (4.25), 278 (4.50). ^{1}H-NMR ($CDCl_3$): 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 $C_{24}H_{20}ClFN_5O_4$ (461.4): C 62.46, H 4.36, N 15.17; found: C 62.29, H 4.33, N 15.01.

N^6,*3'*-O-*Dibenzoyladenylyl-{2'-{*O^P*-[2-(4-nitrophenyl)ethyl]} → 5'}-2'-azido-*N^6*-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 $CHCl_3$ (100 ml), and washed with 0.05M $(Et_3NH)HCO_3$ (2 × 75 ml). The org. phase was dried (Na_2SO_4), evaporated, and co-evaporated with toluene (30 ml). The residue was dissolved in 2% TsOH soln. in CH_2Cl_2/MeOH 4:1 (15 ml), stirred for 10 min, diluted with $CHCl_3$ (100 ml), and washed with 0.05M $(Et_3NH)HCO_3$ (2 × 50 ml). The org. phase was dried (Na_2SO_4) and evaporated. The residue was purified by CC (silica gel, 10 × 1.5 cm, $CHCl_3$ and then $CHCl_3$/MeOH 24:1): 172 mg (63%) of **10**. Colourless foam. UV (MeOH): 230 (4.54), 280 (4.55). Anal. calc. for $C_{49}H_{42}FN_{14}O_{13}P$ (1084.9): C 54.24, H 3.90, N 18.07; found: C 54.40, H 3.85, N 17.96.

N^6,*3'*-O-*Dibenzoyladenylyl-{2'-{*O^P*-[2-(4-nitrophenyl)ethyl]} → 5'}-*N^6*-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), 1*H*-tetrazole (64 mg, 0.92 mmol), and TpsCl (139 mg, 0.46 mmol) in pyridine (1.5 ml; then treatment with 0.05M $(Et_3NH)HCO_3$ (50 ml) and 2% TsOH soln. in CH_2Cl_2/MeOH 4:1 (6 ml)). CC (silica gel, 15 × 1.5 cm, $CHCl_3$ and then $CHCl_3$/MeOH 49:1) gave 87 mg (67%) of **11**. Colourless foam. UV (MeOH): 230 (4.50), 278 (4.56). Anal. calc. for $C_{49}H_{42}ClFN_{11}O_{13}P$ (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)] → 5'}-*N^6,N^6*-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 $CHCl_3$ (50 ml), and washed with 0.05M $(Et_3NH)HCO_3$ (2 × 20 ml). The org. phase was dried (Na_2SO_4), evaporated, and co-evaporated with toluene (20 ml). The residue was dissolved in 2% TsOH soln. in CH_2Cl_2/MeOH 4:1 (3 ml), stirred for 10 min, diluted with $CHCl_3$ (50 ml), and washed with 0.05M $(Et_3NH)HCO_3$ (2 × 20 ml). The org. phase was dried (Na_2SO_4) and evaporated and the residue purified by CC (silica gel, 10 × 1 cm, $CHCl_3$ and then $CHCl_3$ 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 Et_3NH^+). 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 $CHCl_3$ (100 ml), and washed with 0.05M $(Et_3NH)HCO_3$ (2 × 30 ml). The org. phase was dried (Na_2SO_4), evaporated, and co-evaporated with toluene (20 ml).The residue was dissolved in 2% TsOH soln. in CH_2Cl_2/MeOH 4:1 (9 ml), stirred for 10 min, diluted with $CHCl_3$ (100 ml), and washed with 0.05M $(Et_3NH)HCO_3$ (2 × 30 ml). The org. phase was dried (Na_2SO_4) 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 dissolved in conc. NH_4OH soln. (60 ml), kept at r.t. for 24 h, and evaporated. The residue was taken up in $CHCl_3/H_2O$ 1:1 (100 ml). The aq. phase was applied onto a *DEAE-Servacell-23-SS* column (20 × 1.5 cm, linear gradient of 0.005–0.2M $(Et_3NH)HCO_3$ buffer (pH 7.5)). The product fractions were evaporated, and co-evaporated with MeOH (2 × 20 ml). The residual Et_3NH^+ salt was lyophilized (H_2O): 76 mg (51%) of **13** · 2 Et_3N^+. UV (H_2O): 260 (4.58). ^{1}H-NMR (D_2O): 8.21, 8.13, 8.08, 7.91, 7.88, 7.75 (6*s*, H–C(2), H–C(8)); 6.10, 6.00 (2*d*, 2 H–C(1')); 5.90 (*s*, H–C(1')). Anal. calc. for $C_{42}H_{65}FN_{20}O_{14}P$ (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 Et_3NH^+). 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 CH_2Cl_2/MeOH 4:1 (5 ml, 10 min), 1M DBU/pyridine (7 ml, 18 h), 1M AcOH/pyridine (7 ml), and conc. NH_4OH soln. (40 ml, 20 h). Purification by ion-exchange CC (*DEAE-Servacell-23-SS*) gave 32 mg (44%) of **14** · 2 Et_3NH^+. UV (H_2O): 260 (4.56). ^{1}H-NMR (D_2O): 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 $C_{42}H_{65}ClFN_{17}O_{14}P$ (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** · 2 Et_3NH^+). 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-1*H*-imidazole (19.2 mg, 0.018 ml, 0.234 mmol) was stirred at r.t. for 20 h, diluted with $CHCl_3$ (50 ml), and washed with 0.05M $(Et_3NH)HCO_3$ (2 × 20 ml). The org. phase was dried (Na_2SO_4), evaporated, and co-evaporated with toluene (15 ml).The residue was dissolved in 2% TsOH soln. in CH_2Cl_2/MeOH 4:1 (1.5 ml), stirred for 10 min, diluted with $CHCl_3$ (50 ml), and washed with 0.05M $(Et_3NH)HCO_3$ (2 × 15 ml). The org. phase was dried (Na_2SO_4), and evaporated. The residue was treated with a soln. of 4-nitrobenzaldehyde oxime (50 mg, 0.3 mmol) in Et_3N/H_2O/dioxane 1:1:1 (3 ml), kept at r.t. for 24 h, and evaporated. The residue was dissolved in conc. NH_4OH soln. and, after 24 h, evaporated. The residue was taken up in $CHCl_3/H_2O$ 1:1 (60 ml). The aq. phase was applied onto a *DEAE-Servacell-23-SS* column (15 × 1.5 cm, linear gradient of 0.005–0.12M $(Et_3NH)HCO_3$ buffer (pH 7.5)). The product fractions were evaporated and co-evaporated with MeOH (2 × 10 ml). The residual Et_3NH^+ salt was lyophilized (H_2O): 11 mg (53%) of **15** · 2 Et_3NH^+. UV (H_2O): 259 (4.59). ^{1}H-NMR (D_2O): 8.17, 8.12, 8.07, 7.93, 7.85, 7.75 (6*s*, H–C(2), H–C(8)); 6.33 (*dd*, H–C(1')); 6.08, 5.86 (2*d*, 2 H–C(1')); 2.68, 2.36 (2*m*, 2 H–C(2')). Anal. calc. for $C_{42}H_{66}FN_{17}O_{14}P$ (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 Et_3NH^+). A soln. of **13** (11 mg, 0.01 mmol) in H_2O/EtOH 1:1 (8 ml) in the presence of Pd/C (13 mg) was stirred under H_2 for 48 h. Then the catalyst was filtered off and washed with H_2O (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 $(Et_3NH)HCO_3$ buffer (pH 7.5)). The product fractions were evaporated, and co-evaporated with MeOH (2 × 5 ml). The residual Et_3NH^+ salt was lyophilized (H_2O): 8 mg (53%) of **16** · 2 Et_3NH^+. UV (H_2O): 260 (4.60). ^{1}H-NMR (D_2O): 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 $C_{42}H_{67}FN_{18}O_{14}P \cdot 2\,H_2O$ (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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