Nucleotides

Part LVIII[1])

Synthesis and Biological Activity of (2′–5′)Oligoadenylate Trimers Containing a 5′-Terminal 5′-Amino-5′-deoxy- or 5′-Amino-3′,5′-dideoxyadenosine Derivative

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The new (2′–5′)oligoadenylate trimers **26**–**34** containing 5′-amino-5′-deoxyadenosine or 5′-amino-3′,5′-dideoxyadenosine or their lipophilic 5′-deoxy-5′-(hexadecanoylamino) derivatives at the 5′-terminus, and adenosine or 3′-deoxyadenosine (=cordycepin) at the penultimate and 2′-end position of the trimers were synthesized by the phosphoramidite method. The newly synthesized trimers **26**–**34** inhibited, at 100 μM concentration, HIV-1-induced syncytia formation (SYN) by 19–96% and reverse-transcriptase activity (RT) by 27–100% (see *Table*). The two hexadecanoylamino derivatives **27** and **30** which were found to be potent inhibitors of SYN and RT showed also a 73 and 49% inhibition, respectively, of expression of HIV-1 p24 antigen (p24-EX). The same compounds **27** and **30** inhibited also, with a 100% efficacy, an amplification of HIV-1 partial reverse transcripts (PCR) and HIV-1 integrase activity (INT), respectively.

1. Introduction. – The major disadvantages of the therapeutic application of (2′–5′)oligoadenylates are their polar character which does not allow them to penetrate easily through the cell membrane and their sensitivity to nucleases, leading to rapid degradation of the oligomers [2]. Many attempts have been made to overcome these problems by chemical modification of (2′–5′)oligoadenylates. Some improvement of cell uptake by incorporation of oligonucleotides into liposomes [3] or by conjugate formation [4–7] was described earlier. The attachment of a lipophilic cholesterol group [8] [9] to the (2′–5′)oligoadenylates was shown to facilitate membrane crossing [8]. On the other hand, chemical modification of (2′–5′)oligoadenylates by conjugation with vitamins, lipids, or fatty acids resulted in improvement of biological properties [10–12]. It is also known that the substitution of the 5′- or 3′(2′)-terminal OH groups of oligonucleotides [13], in general, and of (2′–5′)oligoadenylates [14] [15], in particular, by an amino group led to an enhancement of stability of these compounds towards degradation by nucleases. Since biological activity of oligonucleotides depends to a large extent on the structure of each individual nucleoside residue, we synthesized some new trimers containing 5′-amino-5′-deoxyadenosine, and 5′-amino-3′,5′-dideoxy-

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adenosine or their lipophilic 5′-deoxy-5′-(hexadecanoylamino) derivatives at the 5′-terminus, and adenosine or 3′-deoxyadenosine (= cordycepin) at the penultimate and 2′-end position of the trimers. The ability of the newly synthesized trimers to inhibit HIV-1 reverse transcriptase (RT), HIV-1-induced syncytia formation, PCR amplification, HIV-1 integrase (INT), and the post-translational expression of HIV-1 p24 antigen were investigated.

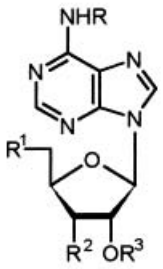
2. Syntheses. – The synthesis of the monomeric building blocks N^6-benzoyl-3′-deoxyadenosine (**1**) [16], 3′-deoxy-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**6**) and 3′-deoxy-N^6,2′-*O*-bis[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**14**) [17]; protected nucleosides **11** and **13** [18], and phosphoramidites **19** [18], **18** [19], and **20** and **21** [15] have already been described in the literature.

The syntheses of the 5′-amino-3′,5′-dideoxyadenosine derivatives **4**, **5**, **9**, and **10** were based upon N^6-benzoyl-3′-deoxyadenosine (**1**) [16] and 3′-deoxy-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**6**) [17] as starting materials. Reaction of **1** with TsCl in pyridine gave, after column chromatography (CC), tosylate **2** (87%) which was treated with LiN_3/DMF at 65° to give 5′-azido-N^6-benzoyl-3′,5′-dideoxyadenosine (**3**) in 54% isolated yield. Treatment of **3** with Ph_3P/pyridine/NH_4OH [20] afforded 5′-amino-3′,5′-dideoxyadenosine (**4**) [21] which was isolated by CC in 86% yield. Trimethylsilylation of **4** with hexamethyldisilazane (HMDS) followed by treatment with 3-methyl-1-[2-(4-nitrophenyl)ethoxycarbonyl]-1*H*-imidazol-3-ium chloride [22] in CH_2Cl_2 and hydrolysis with Et_3N/MeOH led to the selectively blocked bis[2-(4-nitrophenyl)-ethoxycarbonyl] derivative **5** in 66% yield. The overall yield of the sequence **1** → **5** was 27%. Compound **5** was also prepared by an alternative route from 3′-deoxy-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**6**). Thus, treatment of **6** with TsCl in pyridine gave the tosylate **7** (77%) which was converted to the azido derivative **8**, as described for **3**, in 86% yield. The reaction of **8** with Ph_3P in dioxane at room temperature for 24 h followed by hydrolysis with Et_3N/H_2O for 8 h led to the amino derivative **9** in 96% isolated yield. Treatment of **9** in pyridine with 2-(4-nitrophenyl)ethyl carbonochloridate [23] in CH_2Cl_2 at 4° gave **5** in 70% yield. The overall yield of **5** in this case was 43%. Subsequent treatment of **9** with hexadecanoyl chloride (= palmitoyl chloride; palmCl) in CH_2Cl_2 afforded the 5′-(hexadecanoylamino) derivative **10** in 73% yield.

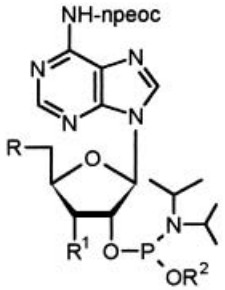
The selectively 2′,3′-di-*O*-silylated derivative **12** was obtained in 95% yield by detritylation of **11** with 2% TsOH in CH_2Cl_2/MeOH 4:1.

Reaction of the selectively protected compounds **5** and **10** with (2-cyanoethoxy)-bis(diisopropylamino)phosphane (= 2-cyanoethyl tetraisopropylphosphorodiamidite) [24] in CH_2Cl_2 in the presence of 1*H*-tetrazole resulted in the formation of the phosphoramidites **15** and **16** in 76 and 77% yield, respectively. Condensation of **12** with the phosphoramidite **19** in the presence of 1*H*-tetrazole in MeCN and subsequent oxidation with I_2 in H_2O/CH_2Cl_2/pyridine 1:1:3 followed by detritylation with 2% TsOH in CH_2Cl_2/MeOH 4:1 afforded the 5′-OH dimer **22** in 70% yield. Analogous reactions of **12** with the phosphoramidite **18** and of nucleoside **14** with phosphoramidites **17** and **18** led, after detritylation, to 5′-OH dimers **23**–**25** in 72, 79, and 70% yield, respectively.

The transformation of the dimers **22**–**25** to the trimer level involved the same techniques, *i.e.*, a condensation step with phosphoramidites **15**, **16**, **20**, and **21** and



	R	R^1	R^2	R^3
1	Bz	OH	H	H
2	Bz	Ts-O	H	H
3	Bz	N_3	H	H
4	H	NH_2	H	H
5	npeoc	npeoc-NH	H	H
6	npeoc	OH	H	H
7	npeoc	Ts-O	H	H
8	npeoc	N_3	H	H
9	npeoc	NH_2	H	H
10	npeoc	palm-NH	H	H
11	npeoc	MeOTr-O	tbds-O	tbds
12	npeoc	OH	tbds-O	tbds
13	npeoc	MeOTr-O	tbds-O	H
14	npeoc	OH	H	npeoc

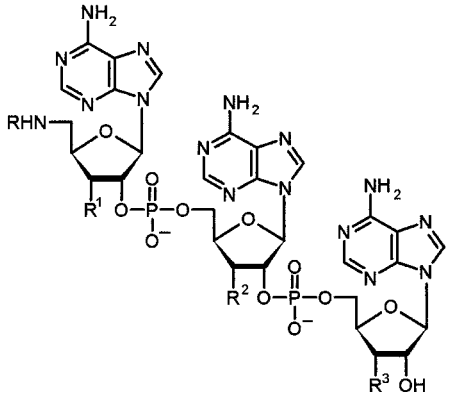


	R	R^1	R^2
15	npeoc-NH	H	ce
16	palm-NH	H	ce
17	MeOTr-O	tbds-O	ce
18	MeOTr-O	H	ce
19	MeOTr-O	tbds-O	npe
20	palm-NH	tbds-O	ce
21	npeoc-NH	tbds-O	ce

Bz = benzoyl
ce = 2-cyanoethyl
tbds = (*tert*-butyl)dimethylsilyl
npeoc = 2-(4-nitrophenyl)ethoxycarbonyl
npe = 2-(4-nitrophenyl)ethyl
palm = palmitoyl
MeOTr = monomethoxytrityl

subsequent oxidation, followed by treatment with 0.5M 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)/MeCN and 1M Bu_4NF/THF, or only with 0.5M DBU/MeCN (**28**, **29**), to remove the different protecting groups. Final purification was achieved by ion-exchange CC (*DEAE-Servacell 23-SS* (**26**–**29**) or *DEAE-Sephadex A-25* (**30**–**34**)) to give the trimers **26**–**34** in 48, 49, 44, 57, 62, 46, 30, 28, and 46% overall yield, respectively. Due to the poor solubility of the hexadecanoylamino derivatives **27** and **29**–**31** in H_2O, a solution of $(Et_3NH)HCO_3$ in MeOH/H_2O was used for ion-exchange CC. The formation of the [2-(4-nitrophenyl)ethyl]amino derivatives **32** and **34** as by-products took place, probably, because of the base-catalyzed nucleophilic addition of the 5′-amino function of the trimers to (4-nitrophenyl)ethylene which was formed on removal of the of 2-(4-nitrophenyl)ethoxycarbonyl protecting groups by DBU [23].

3. Biological Application. – Replacement of the 5′-hydroxy group of the (2′–5′)-oligoadenylate trimer core by the 5′-amino group or by the 5′-(hexadecanoylamino) (palmNH) group at either the adenylyl or the 3′-deoxyadenylyl moieties produced new



	R	R^1	R^2	R^3
26	H	H	OH	OH
27	palm	H	OH	OH
28	H	H	H	H
29	palm	H	H	H
30	palm	OH	H	OH
31	palm	OH	OH	H
32	npe	OH	H	OH
33	H	OH	H	OH
34	npe	OH	OH	H
35	H	OH	OH	OH

derivatives that inhibit HIV-1 replication. The 5′-amino-5′-deoxyadenylyl-(2′–5′)-adenylyl-(2′–5′)-adenosine (**35**) [15] inhibited HIV-1 replication by 23% as determined by HIV-1-induced syncytia formation (*Table*). This compares with a 66, 35, and 19% inhibition of syncytia formation by the 5′-amino-substituted 3′-deoxyadenylyl/adenylyl trimers **26**, **28**, and **33**. Of these four 5′-amino trimers, **26** was the best inhibitor according to HIV-1 syncytia formation. Replacement of the 5′-amino by the 5′-(hexadecanoylamino) group as in the 3′-deoxyadenylyl/adenylyl trimers **27**, **29**, **30**, and **31** resulted in 81, 89, 100, and 85% inhibition, respectively, of HIV-1-induced syncytia

Table. *Inhibition of HIV-1 Replication and Biological Activities of (2′–5′)Oligoadenylate Trimers* **26**–**35** [a])

	R	R^1	R^2	R^3	Syn[b])	RT[c])	PCR[d])	INT[e])	p24-EX[f])
26	H	H	OH	OH	66	85	–	–	–
27	palm	H	OH	OH	81	62	100	0	73
28	H	H	H	H	35	73	–	–	–
29	palm	H	H	H	89	27	–	–	–
30	palm	OH	H	OH	100	85	0	100	49
31	palm	OH	OH	H	85	60	–	–	–
32	npe	OH	H	OH	31	80	–	–	–
33	H	OH	H	OH	19	69	–	–	–
34	npe	OH	OH	H	32	69	–	–	–
35	H	OH	OH	OH	23	78	–	–	–

[a]) For the inhibition of HIV-1-induced syncytia formation, HIV-1 RT, and PCR amplification, compounds **26**–**35** were tested at 100 μM. [b]) Inhibition of HIV-1 replication as determined by HIV-1-induced syncytia formation (%) for each compounds. The number of syncytia/10^4 cells was 192 ± 12 for the control Sup T1 cells. The mean of triplicate determinations is shown; variance did not exceeded 5–10%. [c]) Percent inhibition of reverse-transcriptase (HIV-1 RT) activity. Control values for HIV-1 RT activity averaged 1430 dpm. The mean of triplicate determinations is shown; variance did not exceed 5–10%. [d]) Inhibition of HIV-1 reverse transcription was measured by PCR amplification of partial reverse transcripts. 100% inhibition indicates no amplification by any of the four primer sets; 0% inhibition indicates amplification by one or more primer sets. [e]) HIV-1 Integrase assays were done by integration by the HIV-1 genome by endonucleolytic cleavage of two terminal nucleotides from the 3′-ends of the viral DNA. Complete (100%) inhibition is based on a comparison to AZT 5′-monophosphate (1000 μM); 0% indicates no inhibition of integrase activity. Concentrations of **27** and **30** were 1000 μM. [f]) Inhibition of expression of p24 antigen was determined by Western blotting. Concentrations of **27** and **30** were 300 μM.

formation. Of these trimers, **30** was the most potent inhibitor of syncytia formation. Therefore, replacement of one H-atom at the 5′-amino group of (2′–5′)A trimer derivatives with the hydrophobic 5′-(hexadecanoylamino) group reveals a marked increase in the inhibition of HIV-1 replication. Compounds **32** and **34** which contain the [2-(4-nitrophenyl)ethyl]amino (npeNH) group at the 5′-terminus, inhibited HIV-1-induced syncytia formation only by 31 and 32%, respectively, showing no significant difference in the activity as compared to **35**. Compound **30** is so far the best candidate showing the highest inhibition of HIV-1 RT activity (85%) and inhibition of HIV-1-induced syncytia formation (100%).

Based on the 81 and 100% inhibition of HIV-1 replication as determined by inhibition of syncytia formation and RT activity, compounds **27** and **30** were selected to further examine the mechanism by which HIV-1 replication is inhibited. This was accomplished by studying PCR amplification of HIV-1 partial reverse transcripts (PCR), inhibition of HIV-1 integrase (INT), and inhibition of expression of HIV-1 p24 antigen (p24-EX). In the HIV-1 reverse transcription (PCR) assays, compound **27**, but not **30**, completely inhibited HIV-1 reverse transcription as evidenced by no amplification of the primer sets (*Table*). Therefore, the data demonstrate that the position of the adenylyl and 3′-deoxyadenylyl moieties in the (2′–5′)oligoadenylate trimer cores may be important in blocking the amplification of HIV-1 partial reverse transcripts (compare **27** with **30**). Based on these observations, it was reasoned that **27**, which is an inhibitor of HIV-1 reverse transcription as measured by PCR, would inhibit expression of HIV-1 p24 antigen. The data presented in the *Table* clearly show that there is a 73% inhibition of expression of HIV-1 p24 antigen. Because HIV-1 integrase is the enzyme that incorporates HIV-1 DNA into host cell DNA, we determined the inhibition of HIV-1 integrase by compounds **27** and **30**. Compound **30**, but not compound **27**, inhibited HIV-1 integrase. The observation that compound **30** inhibited HIV-1 integrase suggests that the expression of p24 antigen would also be inhibited as actually found by 49% in HIV-1 Sup T1 cells treated with compound **30**.

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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-Servacel 23-SS* (*Serva*), and *DEAE-Sephadex A-25* (*Pharmacia*). HPLC: *Merck-Hitachi, L-6200-Intelligent* pump, *D-2000* chromatointegrator, detection at 260 nm (*Uvicon 730 SLC*, Fa. *Kontron*); column *RP 18* (*LiChrosphes* 125 × 4 mm, 5 μm, *Merck 50943*); flow rate 1 ml/min; elution: $A = 0.1$M aq. (Et_3NH)OAc buffer (pH 7.0), $B = 0.1$M aq. (Et_3NH)OAc buffer (pH 7.0)/MeCN 1:1, C = MeCN; t_R in min. M.p.: *Gallenkamp* melting-point apparatus; no correction. UV/VIS: *Perkin-Elmer Lambda 15*; λ_{max} in nm (log ε). IR: *Perkin-Elmer FTIR-1600*; $\tilde{\nu}$ in cm^{-1}. ^{1}H-NMR: *Bruker WM-250*; δ in ppm rel. to $SiMe_4$. ^{31}P-NMR: *Jeol JM GX-400*; δ in ppm rel. to 85% H_3PO_4 soln. Fast-atom bombardment (FAB) MS (matrix dimethylformamide (DMF)/AcOH/glycerol 1:1:1): *Finnigan MAT 312/AMD-5000*.

Bioassay. Assays measuring HIV-1 induced syncytia formation and HIV-1 reverse transcriptase activity were accomplished as previously described [10]. Primers for PCR amplification were synthesized by *Ransom Hill Bioscience*. The sequences of the HIV-1 specific primers are: GGCTAACTAGGGAACCCACTGCTT, CTGCTAGAGATTTTCCACACTGAC, CACACACAAGGCTACTTCCCT, GCGAGAGCGTCAGTATTAAGCGGGG, GGCTGACCTGATTGCTGTGTCCTG, CCGAGTCCTGCGTCGAGAGAGC. PCR amplifications were performed as described by *Trono* [25], using 75 ng of each primer, in a 50-μl reaction mixture

containing 200 μM of each of the four dNTPs, 50 mM KCl, 10 mM *Tris*·HCl (2-amino-2-(hydroxymethyl)propane-1,3-diol hydrochloride; pH 8.0), 1.5 mM $MgCl_2$, 0.1% *Triton X-100*, 10 ng of cellular DNA from Sup T1 cells, and 5U of *Taq* DNA polymerase (*Promega*). The reaction mixture was covered with mineral oil and subjected to 30 cycles in a *MJ Research Programmable Thermal Controller.* Each cycle consisted of denaturation for 1 min at 94°, primer annealing for 2 min at 55°, and polymerization for 3 min at 72°, with a final 7-min extension step at 72° [25].

Integration of HIV-1 genome through the endonucleolytic cleavage of two terminal nucleotides from the 3′-ends of the viral DNA is essential for a productive infection to occur. Two synthetic, complementary oligonucleotides corresponding to the U5 region of the HIV-1 LTR were purchased from *Ransom Hill Bioscience:* oligomer 1, 5′-GTGTGG-AAAATCTCTAGCAGT-3′, and oligomer 2, 5′-ACTGCTAGAGA-TTTTCCACAC-3′. Oligomer 1 (100 pmol) was 5′-end labelled with the aid of *T4* polynucleotide kinase in the presence of 50 μCi of [γ-^{32}P]ATP (*NEN Life Science*, 60000 Ci/mmol), purified by passage through a *G-10 Sephadex (Pharmacia Biotech)* spin column, and annealed by heating at 80° for 3 min and cooling slowly to r. t. End processing reaction were performed in 20-μl final volume containing 1M HEPES (4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid; pH 7.5), 50 mM NaCl, 50 μM EDTA (ethylenediaminetetraacetic acid), 50 μM DTT (DL-dithiothreitol), 20% glycerol, (*v*/*v*), 7.5 mM $MnCl_2$, 0.1 mg/ml bovine serum albumin, 10 mM 2-mercaptoethanol, 10% dimethyl sulfoxide (*v*/*v*), 25 mM MOPS (morpholine-4-propanesulfonic acid), pH 7.2, 0.5 μM HIV-1 integrase, and 0.1 pmol of oligomer 1/oligomer 2 complex. Reaction mixtures were incubated at 37° for 1 h, mixed with an equal volume of gel loading buffer, and submitted to electrophoresis (20% denaturing polyacrylamide gel). Following electrophoresis, gels were exposed to a phosphorimager screen (*Fuji BAS 2000 Phosphorimager.*)

Western blotting procedure for HIV-1 p24 antigen: Translation as well as polyprotein processing were evaluated through Western blot analyses. *NP-40* Extractions were performed on (2′–5′)A derivatives treated, HIV-1 IIIB infected SupT1 cells 48 h post infection. Then, the protein (25 μg) was submitted to electrophoresis (10% *SDS* polyacrylamide gel) and transferred to nitro-cellulose membranes (*Pharmacia Biotech*) using the *Bio-Rad Trans-blot Semi-Dry Transfer Cell* apparatus. Nitro-cellulose blots were blocked overnight at 4° in 5% dry milk blocking soln. (10 ml) prepared in *TBS-T* buffer (10 mM *Tris*·HCl, pH 7.6, 136 mM NaCl, 0.1% *Tween-20*). Human sera obtained from HIV-1 infected individuals diluted 1:1000 in 5% *TBS-T* blocking soln. served as the primary antibody. Detection of HIV-1 p24 antigen was performed with horseradish peroxidase-labelled goat anti-human *IgG* secondary antibody (*Pierce*) developed in the *Amersham* enhanced chemiluminescent soln. and visualized by multiple exposures to *Fuji RX Medical X-ray* film.

N^6-*Benzoyl-3′-deoxy-5′*-O-*[(*p-*toluyl)sulfonyl]adenosine* (**2**). A soln. of 3′-deoxy-*N*^{6}-benzoyladenosine (**1**) [16] (0.8 g, 2.24 mmol) and TsCl (0.68 g, 3.56 mmol) in dry pyridine (20 ml) was stirred at r.t. for 18 h. The mixture was diluted with $CHCl_3$ (100 ml) and washed with sat. $NaHCO_3$ soln. (2 × 100 ml). The org. layer was dried (Na_2SO_4), evaporated, and co-evaporated with toluene, and the residue purified by CC (silica gel, 10 × 3.5 cm, $CHCl_3$, then $CHCl_3$/MeOH 19:1): 1.0 g (87%) of **2**. Solid foam. UV (MeOH): 221 (4.56), 278 (4.30). ^{1}H-NMR ((D$_6$)DMSO): 11.23 (*s*, N*H*Bz); 8.69, 8.45 (2*s*, H–C(2), H–C(8)); 8.06–7.26 (*m*, arom. H); 5.96 (*d*, H–C(1′)); 5.79 (*d*, OH–C(2′)); 4.71 (*m*, H–C(2′)); 4.52 (*m*, H–C(4′)); 4.30 (*m*, 2 H–C(5′)); 2.32 (*m*, 1 H–C(3′)); 2.30 (*s*, Me); 2.03 (*m*, 1 H–C(3′)). Anal. calc. for $C_{24}H_{23}N_5O_6S$ (509.5): C 56.57, H 4.54, N 13.74; found: C 56.47, H 4.50, N 13.69.

*5′-Azido-*N^6*-benzoyl-3′,5′-dideoxyadenosine* (**3**). A mixture of **2** (1.0 g, 1.96 mmol), LiN_3 (0.7 g, 14.3 mmol), and DMF (6 ml) was stirred at 65° for 2 h, evaporated, and co-evaporated with toluene (3 × 20 ml). The residue was treated with H_2O (100 ml) and extracted with $CHCl_3$ (4 × 70 ml). The org. layer was purified by CC (silica gel, 7 × 3.5 cm, $CHCl_3$, then $CHCl_3$/MeOH 24:1): 0.4 g (54%) of **3**. Solid foam. UV (MeOH): 229 (4.20), 278 (4.31). IR (KBr): 2110 (N_3). ^{1}H-NMR ((D$_6$)DMSO): 11.19 (*s*, N*H*Bz); 8.76, 8.62 (2*s*, H–C(2), H–C(8)); 8.12–7.54 (arom. H); 6.04 (*d*, H–C(1′)); 5.81 (*d*, OH–C(2′)); 4.81 (*m*, H–C(2′)); 4.51 (*m*, H–C(4′)); 3.61 (*d*, 2 H–C(5′)); 2.37, 2.08 (2*m*, 2 H–C(3′)). Anal. calc. for $C_{17}H_{16}N_8O_3$ (380.4): C 53.68, H 4.23, N 29.45; found: C 53.55, H 4.20, N 29.21.

5′-Amino-3′,5′-dideoxyadenosine (**4**) [21]. A mixture of **3** (0.75 g, 1.96 mmol), Ph_3P (1.01 g, 3.85 mmol), pyridine (12 ml), and conc. NH_4OH (12 ml) was stirred at r.t. for 5 h, treated with H_2O (100 ml), and extracted with $CHCl_3$ (2 × 50 ml). The aq. layer was evaporated and the residue purified by CC (silica gel, 5 × 2.5 cm, $CHCl_3$/MeOH 9:1, then $CHCl_3$/MeOH/Et_3N 9:1:0.5) and finally crystallized from H_2O: 0.42 g (86%) of **4**. M.p. 215–217° (dec.). UV (MeOH): 259 (4.22). ^{1}H-NMR ((D$_6$)DMSO):8.32, 8.12 (2*s*, H–C(2), H–C(8)); 7.25 (*s*, NH_2–C(6)); 5.82 (*d*, H–C(1′)); 5.61 (*d*, OH–C(2′)); 4.61 (*m*, H–C(2′)); 4.23 (*m*, H–C(4′)); 2.72 (*m*, 2 H–C(5′)); 2.21, 1.92 (2*m*, 2 H–C (3′)); 1.47 (br. *s*, NH_2–C(5′)). Anal. calc. for $C_{10}H_{14}N_6O_2$ (250.3): C 47.99, H 5.63, N 33.58; found: C 47.69, H 5.65, N 33.38.

*3′,5′-Dideoxy-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]-5′-{[2-(4-nitrophenyl)ethoxycarbonyl]amino}adenosine* (**5**). *a*) A mixture of **4** (0.12 g, 0.48 mmol), HMDS (2 ml), and dioxane (2 ml) was refluxed in the presence of a cat. amount of NH_4Cl for 30 min, and then evaporated. The residue was dissolved in CH_2Cl_2 (5 ml), treated with 3-methyl-1-[2-(4-nitrophenyl)ethoxycarbonyl]-1*H*-imidazol-3-ium chloride [22] (0.59 g, 1.92 mmol) and stirred at r.t. for 18 h. The precipitate was filtered off and the soln. evaporated. The residue was treated with MeOH (20 ml) and Et_3N (3 ml), the mixture stirred at r.t. for 18 h and then evaporated, and the residue purified by CC (silica gel, 15×2.5 cm, $CHCl_3$ and then $CHCl_3$/MeOH 19:1) and finally crystallized from MeCN: 0.2 g (66%) of **5**. M.p. 112–114°. UV (MeOH): 205 (4.47), 267 (4.41). ^{1}H-NMR ((D$_6$)DMSO): 10.58 (*s*, NH–C(6)); 8.58, 8.53 (2*s*, H–C(2), H–C(8)); 8.15, 8.12 (2*d*, 4 H *o* to NO_2); 7.61, 7.52 (2*d*, 4 H *m* to NO_2); 7.43 (*m*, NH–C(5′)); 5.91 (*d*, H–C(1′)); 5.70 (*d*, OH–C(2′)); 4.69 (*m*, H–C(2′)); 4.38 (*m*, H–C(4′), 1 OCH_2CH$_2$); 4.21 (*t*, 1 OCH_2CH$_2$); 3.25 (*m*, 2 H–C(5′)); 3.10, 2.99 (2*t*, 2 OCH$_2$CH_2); 2.10, 1.97 (2*m*, 2 H–C(3′)). Anal. calc. for $C_{28}H_{28}N_8O_{10}$ (636.6): C 52.83, H 4.43, N 17.60; found: C 52.89, H 4.40, N 17.41.

b) A soln. of 2-(4-nitrophenyl)ethyl carbonochloridate [23] (0.43 g, 1.89 mmol) in CH_2Cl_2 (10 ml) was added dropwise to a soln. of **8** (0.6 g, 1.35 mmol) in pyridine (8 ml) at 4° within 1.5 h. Then the mixture was stirred at 4° for 2 h, diluted with $CHCl_3$ (100 ml), and washed with sat. $NaHCO_3$ soln. (70 ml). The org. layer was dried (Na_2SO_4), evaporated, and co-evaporated with toluene (2×50 ml). The residue was purified by CC (silica gel, 15×2.5 cm, $CHCl_3$, then $CHCl_3$/MeOH 50:1): 0.6 g (70%) of **5**.

*3′-Deoxy-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]-5′-*O*-[(*p*-toluyl)sulfonyl]adenosine* (**7**). As described for **2**, with **6** [17] (1.2 g, 2.7 mmol), TsCl (0.72 g, 3.78 mmol), and pyridine (20 ml). After dilution with $CHCl_3$ (100 ml) and workup as described for **2**, drying under vacuum at 40° gave 1.2 g (74%) of **7**. Solid foam. UV (MeOH): 211 (4.50), 266 (4.40). ^{1}H-NMR ((D$_6$)DMSO): 10.62 (*s*, N*H*npeoc); 8.56, 8.42 (2*s*, H–C(2), H–C(8)); 8.16 (*d*, 2 H *o* to NO_2); 7.62 (*m*, arom. H (Ts)); 7.24 (*d*, 2 H *m* to NO_2); 5.92 (*d*, H–C(1′)); 5.77 (*d*, OH–C(2′)); 4.68 (*m*, H–C(2′)); 4.49 (*m*, H–C(4′)); 4.40 (*t*, OCH_2CH$_2$); 4.28 (*m*, 2 H–C(5′)); 3.12 (*t*, OCH$_2$CH_2); 2.30 (*m*, 1 H–C(3′)); 2.28 (*s*, Me); 2.01 (*m*, 1 H–C(3′)). Anal. calc. for $C_{26}H_{26}N_6O_9S$ (598.6): C 52.16, H 4.37, N 14.03; found: C 52.11, H 4.35, N 14.13.

*5′-Azido-3′,5′-dideoxy-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**8**). As described for **3**, with **7** (3.27 g, 5.46 mmol), LiN_3 (1 g, 20.4 mmol), and DMF (20 ml; 2.5 h). Crystallization from EtOH gave 2.2 g (86%) of **8**. M.p. 88–90°. UV (MeOH): 210 (4.50), 266 (4.41), 273 (sh, 4.35). IR (KBr): 2110 (N_3). ^{1}H-NMR ((D$_6$)DMSO): 10.59 (*s*, N*H*npeoc); 8.63, 8.59 (2*s*, H–C(2), H–C(8)); 8.16 (*d*, 2 H *o* to NO_2); 7.61 (*d*, 2 H *m* to NO_2); 6.00 (*d*, H–C(1′)); 5.78 (*d*, OH–C(2′)); 4.79 (*m*, H–C(2′)); 4.50 (*m*, H–C(4′)); 4.38 (*t*, OCH_2CH$_2$); 3.58 (*m*, 2 H–C(5′)); 3.10 (*t*, OCH$_2$CH_2); 2.35, 2.04 (2*m*, 2 H–C(3′)). Anal. calc. for $C_{19}H_{19}N_9O_6$ (469.4): C 48.61, H 4.07, N 26.85; found: C 48.52, H 4.08, N 26.94.

*5′-Amino-3′,5′-dideoxy-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**9**). A mixture of **8** (0.46 g, 0.98 mmol) and Ph_3P (1.31 g, 5 mmol) in dioxane (7 ml) was stirred at r.t. for 24 h, treated with H_2O (1 ml) and Et_3N (0.1 ml), and stirred at r.t. for 8 h. The mixture was evaporated and the residue purified with CC (silica gel, 10×3.5 cm, $CHCl_3$/MeOH 9:1 and then $CHCl_3$/MeOH/Et_3N 4:1:0.05): 0.28 g (96%) of **9**. Solid foam. UV (MeOH): 210 (4.47), 267 (4.39). ^{1}H-NMR ((D$_6$)DMSO/D$_2$O 10:1): 8.68, 8.61 (2*s*, H–C(2), H–C(8)); 8.15 (*d*, 2 H *o* to NO_2); 7.61 (*d*, 2 H *m* to NO_2); 5.95 (*d*, H–C(1′)); 4.67 (*m*, H–C(2′)); 4.38 (*t*, OCH_2CH$_2$); 4.30 (*m*, H–C(4′)); 3.10 (*t*, OCH$_2$CH_2); 2.78 (*m*, 2 H–C(5′)); 2.24, 1.95 (2*m*, 2 H–C(3′)). Anal. calc. for $C_{19}H_{21}N_7O_6$ (443.4): C 51.46, H 4.77, N 22.11; found: C 51.25, H 4.69, N 21.95.

*3′,5′-Dideoxy-5′-(hexadecanoylamino)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**10**). A soln. of hexadecanoyl chloride (palmCl; 0.74 g, 0.82 ml, 2.7 mmol) in CH_2Cl_2 (10 ml) was added dropwise to a soln. of **9** (0.8 g, 1.8 mmol) in pyridine (10 ml) at 4° within 1 h. Then the mixture was stirred at 4° for 18 h, diluted with $CHCl_3$ (100 ml), and washed with sat. $NaHCO_3$ soln. (70 ml). The org. layer was dried (Na_2SO_4), evaporated, and co-evaporated with toluene (2×50 ml). The residue was purified by CC (silica gel, 10×3.5 cm, $CHCl_3$, then $CHCl_3$/MeOH 25:1) and finally crystallized from EtOH: 0.9 g (73%) of **10**. M.p. 142–143°. UV (MeOH): 207 (4.50), 267 (4.48). ^{1}H-NMR ((D$_6$)DMSO): 10.58 (*s*, NH–C(6)); 8.61, 8.57 (2*s*, H–C(2), H–C(8)); 8.15 (*d*, 2 H *o* to NO_2); 7.98 (*m*, NH–C(5′)); 7.61 (*d*, 2 H *m* to NO_2); 5.91 (*d*, H–C(1′)); 5.70 (*d*, OH–C(2′)); 4.70 (*m*, H–C(2′)); 4.38 (*m*, H–C(4′), OCH_2CH$_2$); 3.32 (*m*, 2 H–C(5′)); 3.11 (*m*, OCH$_2$CH_2); 2.05 (*m*, 2 H–C(3′), COCH$_2$ of palm); 1.45 (*m*, 1 CH$_2$ of palm); 1.19 (*m*, 12 CH$_2$ of palm); 0.83 (*t*, Me of palm). Anal. calc. for $C_{35}H_{51}N_7O_7$ (681.8): C 61.65, H 7.53, N 14.37; found: C 61.90, H 7.48, N 14.08.

*2′,3′-Bis-*O*-[(*tert*-butyl)dimethylsilyl]-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**12**). A soln. of **11** [18] (1.5 g, 1.56 mmol) in 2% TsOH in CH_2Cl_2/MeOH 4:1 (50 ml) was kept at r.t. for 30 min. The mixture was diluted with $CHCl_3$ (100 ml) and washed with sat. $NaHCO_3$ soln. (100 ml). The org. layer was dried (Na_2SO_4) and evaporated and the residue purified by CC (silica gel, 20×3.5 cm, $CHCl_3$): 1.02 g (95%) of **12**. Solid foam. UV (MeOH): 267 (4.43), 273 (sh, 4.40). ^{1}H-NMR ((D$_6$)DMSO): 10.62 (*s*, NH–C(6)); 8.72, 8.62 (2*s*, H–C(2),

H–C(8)); 8.15 (*d*, 2 H *o* to NO_2); 7.61 (*d*, 2 H *m* to NO_2); 6.00 (*d*, H–C(1′)); 5.29 (*t*, OH–C(5′)); 4.86 (*dd*, H–C(2′)); 4.38 (*t*, OCH_2CH_2); 4.34 (*dd*, H–C(3′)); 3.99 (br. *s*, H–C(4′)); 3.73 (*m*, 2 H–C(5′)); 3.10 (*t*, OCH_2CH_2); 0.91, 0.67 (2*s*, *t*-BuSi); 0.11, 0.09, −0.13, −0.45 (4*s*, MeSi). Anal. calc. for $C_{31}H_{48}N_6O_8Si_2$ (688.9): C 54.04, H 7.02, N 12.19; found: C 53.90, H 7.12, N 11.89.

*3′,5′-Dideoxy-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]-5′-{[2-(4-nitrophenyl)ethoxycarbonyl]amino}adenosine 2′-*O*-[2-Cyanoethyl Diisopropylphosphoramidite]* (**15**). To a soln. of **5** (0.13 g, 0.2 mmol) and 1*H*-tetrazole (4.2 mg, 0.06 mmol) in CH_2Cl_2 (2 ml), 2-cyanoethyl tetraisopropylphosphorodiamidite (0.72 g, 0.24 mmol) was added under N_2. After stirring at r.t. for 18 h, the mixture was diluted with $CHCl_3$ (70 ml) and washed with sat. $NaHCO_3$ soln. (30 ml). The org. layer was dried (Na_2SO_4) and evaporated. The residue was purified by CC (silica gel, 10 × 2.5 cm, petroleum ether/acetone 3:1, then 1:1): 0.13 g (76%) of **15**. Solid foam. UV (MeOH): 206 (4.61), 267 (4.54). ^{31}P-NMR ($CDCl_3$): 149.76, 150.75 (2 diastereoisomers). Anal. calc. for $C_{37}H_{45}N_{10}O_{11}P$ (836.8): C 53.10, H 5.42, N 16.73; found: C 52.95, H 5.46, N 16.90.

*3′,5′-Dideoxy-5′-(hexadecanoylamino)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 2′-*O*-[2-Cyanoethyl Diisopropylphosphoramidite]* (**16**). As described for **15**, with **10** (0.6 g, 0.88 mmol), 1*H*-tetrazole (18 mg, 0.26 mmol), CH_2Cl_2 (8 ml), and 2-cyanoethyl tetraisopropylphosphorodiamidite (0.32 g, 1.06 mmol). Workup (washing with sat. $NaHCO_3$ soln. (30 ml)) and purification by CC gave 0.7 g (77%) of **16**. Solid foam. UV (MeOH): 207 (4.58), 267 (4.47). ^{31}P-NMR ($CDCl_3$): 149.9, 150.76 (2 diastereoisomers). Anal. calc. for $C_{44}H_{68}N_9O_8P$ (882.0): C 59.91, H 7.77, N 14.29; found: C 60.11, H 7.69, N 14.01.

*3′-*O*-[(*tert*-Butyl)dimethylsilyl]-5′-*O*-(monomethoxytrityl)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 2′-[2-Cyanoethyl Diisopropylphosphoramidite]* (**17**). As described for **15**, with **13** [18] (4.9 g, 5.8 mmol), 1*H*-tetrazole (0.16 g, 2.3 mmol), CH_2Cl_2 (30 ml), and 2-cyanoethyl tetraisopropylphosphorodiamidite (2.1 g, 6.9 mmol). Workup (washing with sat. $NaHCO_3$ soln. (100 ml)) and purification by CC gave 5.0 g (82%) of **17**. Solid foam. UV (MeOH): 234 (4.30), 267 (4.53) 272 (sh, 4.48). ^{31}P-NMR ($CDCl_3$): 153.78, 152.01. Anal. calc. for $C_{54}H_{67}N_8O_{10}PSi$ (1047.2): C 61.93, H 6.44, N 10.69; found: C 61.55, H 6.50, N 10.39.

*3′-*O*-[(*tert*-Butyl)dimethylsilyl]-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-[2′-[O^P-2-(4-nitrophenyl)-ethyl]-5′]-2′,3′-bis-*O*-[(*tert*-butyl)dimethylsilyl]-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**22**). To a soln. of **12** (0.6 g, 0.87 mmol) in abs. MeCN (10 ml), **19** [18] (1.4 g, 1.22 mmol) and 1*H*-tetrazole (0.42 g, 6.0 mmol) were added under Ar. The mixture was stirred at r.t. for 18 h, oxidized with I_2 (0.5 g) in CH_2Cl_2/H_2O/pyridine 1:1:3 (5 ml), then stirred for another 20 min, diluted with $CHCl_3$ (100 ml), and washed with sat $Na_2S_2O_3$ soln. (2 × 50 ml). The org. layer was dried (Na_2SO_4), evaporated, and co-evaporated with toluene (2 × 50 ml). The residue was dissolved in 2% TsOH in CH_2Cl_2/MeOH 4:1 (70 ml), stirred at r.t. for 30 min, diluted with $CHCl_3$ (100 ml), and washed with sat. $NaHCO_3$ soln. (2 × 70 ml). The org. layer was dried (Na_2SO_4) and evaporated, and the residue purified by CC (silica gel, 20 × 3.5 cm, $CHCl_3$, then $CHCl_3$/MeOH 50:1): 1.03 g (70%) of **22**. Solid foam. UV (MeOH): 266 (4.69). Anal. calc. for $C_{64}H_{88}N_{13}O_{20}PSi_3$ (1474.7): C 52.12, H 6.01, N 12.34; found: C 51.98, H 6.07, N 12.17.

*3′-Deoxy-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-[2′-[O^P-(2-cyanoethyl)]-5′]-2′,3′-bis-*O*-[(*tert*-butyl)-dimethylsilyl]-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**23**). As described for **22**, with **12** (0.4 g, 0.58 mmol), **18** [19] (0.7 g, 0.76 mmol), MeCN (10 ml), and 1*H*-tetrazole (0.27 g, 3.85 mmol). Workup, oxidizing, and purification, as described for **22**, gave 0.52 g (72%) of **23**. Solid foam. UV (MeOH): 266 (4.68). Anal. calc. for $C_{53}H_{70}N_{13}O_{17}PSi_2$ (1248.3): C 50.99, H 5.65, N 14.58; found: C 51.11, H 5.59, N 14.42.

*3′-*O*-[(*tert*-Butyl)dimethylsilyl]-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-[2′-[O^P-(2-cyanoethyl)]-5′]-3′-deoxy-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**24**). As described for **22**, with **14** [17] (0.78 g, 1.22 mmol), **17** (1.78 g, 1.7 mmol), MeCN (20 ml), and 1*H*-tetrazole (0.6 g, 8.57 mmol). Workup, oxidizing, and purification, as described for **22**, gave 1.28 g (79%) of **24**. Solid foam. UV (MeOH): 267 (4.69). Anal. calc. for $C_{56}H_{63}N_{14}O_{21}PSi$ (1327.2): C 50.67, H 4.78, N 14.77; found: C 50.81, H 4.69, N 14.58.

*3′-Deoxy-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-[2′-[O^P-(2-cyanoethyl)]-5′]-3′-deoxy-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**25**). As described for **22**, with **14** [17] (0.19 g, 0.29 mmol), **18** [19] (0.35 g, 0.38 mmol), MeCN (5 ml), and 1*H*-tetrazole (0.13 g, 1.85 mmol). Workup, oxidizing, and purification, as described for **22**, gave 0.3 g (70%) of **25**. Solid foam. UV (MeOH): 267 (4.69). Anal. calc. for $C_{50}H_{49}N_{14}O_{20}P$ (1197.0): C 50.17, H 4.12, N 16.38; found: C 49.98, H 4.10, N 16.18.

5′-Amino-3′,5′-dideoxyadenylyl-(2′–5′)-adenylyl-(2′–5′)-adenosine Bis(ammonium) Salt (**26**·2 NH_4^+). A soln. of **15** (0.1 g, 0.12 mmol) and **22** (0.12 g, 0.08 mmol) in MeCN (2 ml) in the presence of 1*H*-tetrazole (34 mg, 0.48 mmol) was stirred at r.t. for 18 h under N_2. The mixture was oxidized with I_2 (0.1 g) in CH_2Cl_2/H_2O/pyridine 1:1:3 (1 ml), stirred for 15 min, and diluted with $CHCl_3$ (80 ml). The soln. was washed with sat. $Na_2S_2O_3$ soln. (2 × 15 ml). The org. layer was dried (Na_2SO_4) and evaporated. The residue was dissolved in 0.5M DBU/MeCN (18 ml) and stirred for 48 h. Then the soln. was neutralized with 1M AcOH/MeCN (9 ml),

evaporated, and co-evaporated with pyridine (3 × 20 ml). The residue was dissolved in 1M Bu_4NF/THF (9 ml) and stirred at r.t. for 72 h. After evaporation, the residue was taken up in $CHCl_3/H_2O$ 1:1 (200 ml). The aq. phase was applied onto a *DEAE-Servacel-23-SS* column (15 × 2.5 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 NH_4OH soln. (3 × 20 ml) and then with MeOH (3 × 20 ml). The residual NH_4^+ salt was lyophilized (H_2O): 36.3 mg (48%) of **26** · 2 NH_4^+. HPLC (*A* = 0.1M aq. $(Et_3NH)OAc$ buffer (pH 7.0), *B* = 0.1M aq. $(Et_3NH)OAc$ buffer (pH 7.0)/MeCN 1:1; gradient: *A/B* 1:1 within 0–5 min, then *A/B* 6:4 within 5–35 min): t_R 17.24 UV (H_2O): 257 (4.55). ^{1}H-NMR ((D_6)DMSO): 8.42, 8.38, 8.29 (3*s*, H–C(2), H–C(8)); 8.12 (*s*, H–C(2), H–C(8)); 8.09 (br. *s*, NH_2–C(5′)); 8.04 (*s*, H–C(2), H–C(8)); 7.44–7.25 (*m*, 9 H, 3 NH_2–C(6); 3 OH–C(2′) or OH–C(3′)); 6.06 (*d*, H–C(1′)); 6.05 (*s*, H–C(1′) of cord.); 5.86 (*d*, H–C(1′)); 2.85 (*m*, 2 H–C(3′) of cord.). FAB-MS: 909 (*M*H$^+$ for free acid).

3′,5′-Dideoxy-5′-(hexadecanoylamino)adenylyl-(2′–5′)-adenylyl-(2′–5′)-adenosine Bis(ammonium) Salt (**27** · 2 NH_4^+). As described for **26**, with **16** (0.12 g, 0.13 mmol), **22** (0.13 g, 0.09 mmol), MeCN (2 ml), 1*H*-tetrazole (46 mg, 0.66 mmol), I_2 (0.1 g) in CH_2Cl_2/H_2O/pyridine 1:1:3 (1 ml), $CHCl_3$ (100 ml), sat. $Na_2S_2O_3$ soln. (2 × 15 ml), 0.5M DBU/MeCN (16 ml), 1M AcOH/MeCN (8 ml), and 1M Bu_4NF/THF (10 ml). The residue in MeOH/H_2O 1:1 (100 ml) was applied onto a *DEAE-Servacel-23-SS* column (30 × 2.5 cm, linear gradient of 0.005–0.2M $(Et_3NH)HCO_3$ buffer (pH 7.5) in MeOH/H_2O 1:1). The product fractions were evaporated and co-evaporated with NH_4OH (3 × 25 ml) and then with MeOH (3 × 25 ml). The residual NH_4^+ salt was lyophilized (MeOH/H_2O 1:1): 51 mg (49%) of **27** · 2 NH_4^+. HPLC (*A* = 0.1M aq. $(Et_3NH)OAc$ buffer (pH 7.0), *B* = 0.1M aq. $(Et_3NH)OAc$ buffer (pH 7.0)/MeCN 1:1, *C* = MeCN; gradient: *A* within 0–2 min, *A* → *B* within 2–22 min, *B* → *C* within 22–35 min): t_R 28.05. UV (MeOH/H_2O 1:1): 257 (4.55). ^{1}H-NMR ((D_6)DMSO/D_2O 10:1): 8.38, 8.34, 8.20 (3*s*, H–C(2), H–C(8)); 8.10 (*s*, H–C(2), H–C(8)); 8.06 (*s*, H–C(2), H–C(8)); 6.00 (*d*, H–C(1′)); 5.99 (*s*, H–C(1′) of cord.); 5.83 (*d*, H–C(1′)); 2.20 (*m*, 2 H–C(3′) of cord.); 2.00 (*m*, $COCH_2$ of palm); 1.40 (*m*, 1 CH_2 of palm); 1.17 (*m*, 12 CH_2 of palm); 0.80 (*t*, Me of palm). FAB-MS: 1148 (*M*H$^+$ for free acid).

5′-Amino-3′,5′-dideoxyadenylyl-(2′–5′)-3′-deoxyadenylyl-(2′–5′)-3′-deoxyadenosine Bis(ammonium) Salt (**28** · 2 NH_4^+). A soln. of **15** (0.2 g, 0.24 mmol) and **25** (0.23 g, 0.19 mmol) in MeCN/CH_2Cl_2 7:1 (8 ml) in the presence of 1*H*-tetrazole (84 mg, 1.2 mmol) was stirred at r.t. for 18 h under N_2. The mixture was oxidized with I_2 (0.2 g) in CH_2Cl_2/MeOH/pyridine 1:1:3 (2 ml), stirred for 20 min, and diluted with $CHCl_3$ (100 ml). The soln. was washed with sat. $Na_2S_2O_3$ soln. (2 × 20 ml), the org. layer dried (Na_2SO_4) and evaporated, and the residue dissolved in 0.5M DBU/MeCN (26 ml) and stirred for 48 h. Then the soln. was neutralized with 1M AcOH/MeCN (13 ml) and evaporated. The residue was taken up in $CHCl_3/H_2O$ 1:1 (100 ml). The aq. phase was applied onto a *DEAE-Servacel 23-SS* column (30 × 2.5 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 NH_4OH (3 × 20 ml) and then with MeOH (3 × 20 ml). The residual NH_4^+ salt was lyophilized (H_2O): 57.8 mg (44%) of **28** · 2 NH_4^+. HPLC (*A* = 0.1M aq. $(Et_3NH)OAc$ buffer (pH 7.0), *B* = 0.1M aq. $(Et_3NH)OAc$ buffer (pH 7.0)/MeCN 1:1; gradient: *A/B* 1:1 within 0–5 min, then *A/B* 6:4 within 5-35 min): t_R 13.16. UV (H_2O): 257 (4.52). ^{1}H-NMR ((D_6)DMSO/D_2O 10:1): 8.32, 8.27, 8.23, 8.12 (4*s*, H–C(2), H–C(8)); 8.03 (*s*, H–C(2), H–C(8)); 6.08, 5.96, 5.79 (3 br. *s*, 3 H–C(1′)); 4.98 (*m*, 2 H–C(2′)); 4.46 (*m*, H–C(2′), H–C(4′)); 4.35 (*m*, 2 H–C(4′)); 2.45 (*m*, H–C(3′)); 2.22 (*m*, 4 H–C(3′)); 1.88 (*m*, H–C(3′)). FAB-MS: 877 (*M*H$^+$ for free acid).

3′,5′-Dideoxy-5′-(hexadecanoylamino)adenylyl-(2′–5′)-3′-deoxyadenylyl-(2′–5′)-3′-deoxyadenosine Bis(ammonium) Salt (**29** · 2 NH_4^+). As described for **28**, with **16** (0.46 g, 0.52 mmol), **25** (0.45 g, 0.38 mmol), MeCN (10 ml), 1*H*-tetrazole (0.18 g, 2.57 mmol), I_2 (0.4 g) in CH_2Cl_2/H_2O/pyridine 1:1:3 (4 ml), $CHCl_3$ (150 ml), sat. $Na_2S_2O_3$ soln. (2 × 30 ml), 0.5M DBU/MeCN (56 ml), and 1M AcOH/MeCN (28 ml). The residue in MeOH/H_2O 1:1 (200 ml) was applied onto a *DEAE-Servacel-23-SS* column (30 × 2.5 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 NH_4OH (3 × 20 ml) and then with MeOH (3 × 20 ml). The residual NH_4^+ salt was lyophilized (MeOH/H_2O 1:1): 115.6 mg (57%) of **29** · 2 NH_4^+. HPLC (*A* = 0.1M aq. $(Et_3NH)OAc$ buffer (pH 7.0), *B* = 0.1M aq. $(Et_3NH)OAc$ buffer (pH 7.0)/MeCN 1:1; *C* = MeCN; gradient: *A* within 0–2 min, *A* → *B* within 2–22 min, *B* → *C* within 22–35 min): t_R 28.05. UV (H_2O/MeOH 1:1): 257 (4.55). ^{1}H-NMR ((D_6)DMSO): 8.34, 8.31, 8.27, 8.13, 8.11, 8.09 (6*s*, H–C(2), H–C(8)); 8.07 (*m*, N*H*palm); 7.42, 7.40, 6.33 (3 br. *s*, 7 H, 3 NH_2, OH–C(2′)); 6.02 (*s*, 2 H–C(1′)); 5.84 (*s*, H–C(1′)); 5.07 (*m*, 2 H–C(2′)); 4.52 (*m*, H–C(2′)); 4.35 (*m*, 3 H–C(4′)); 2.20 (*m*, 2 H–C(3′)); 2.04 (*m*, $COCH_2$ of palm); 1.43 (*m*, 1 CH_2 of palm); 1.20 (*m*, 12 CH_2 of palm); 0.83 (*t*, Me of palm). FAB-MS: 1116 (*M*H$^+$ for free acid).

5′-Deoxy-5′-(hexadecanoylamino)adenylyl-(2′–5′)-3′-deoxyadenylyl-(2′–5′)-adenosine Bis(ammonium) Salt (**30** · 2 NH_4^+). As described for **26**, with **20** (0.1 g, 0.1 mmol), **23** (0.1 g, 0.08 mmol), MeCN (3 ml), 1*H*-tetrazole (34 mg, 0.48 mmol), I_2 (0.1 g) in CH_2Cl_2/H_2O/pyridine 1:1:3 (1 ml), $CHCl_3$ (80 ml), sat. $Na_2S_2O_3$ soln.

(2 × 15 ml), 0.5M DBU/MeCN (12 ml), 1M AcOH/MeCN (6 ml), and 1M Bu_4NF/THF (5.5 ml). The residue in MeOH/H_2O 1:1 (100 ml) was applied onto a *DEAE-Sephadex-A-25* column (60 × 1.5 cm, linear gradient of 0.005–0.25M $(Et_3NH)HCO_3$ buffer (pH 7.5) in MeOH/H_2O 1:1). The product fractions were evaporated and co-evaporated with NH_4OH (3 × 25 ml) and then with MeOH (25 ml). The residual NH_4^+ salt was lyophilized (MeOH/H_2O 1:1): 58.7 mg (62%) of **30** · 2 NH_4^+. HPLC (*A* = 0.1M aq. (Et_3NH)OAc buffer (pH 7.0), *B* = 0.1M aq. (Et_3NH)OAc buffer (pH 7.0)/MeCN 1:1, *C* = MeCN; gradient: *A* within 0–2 min, *A* → *B* within 2–22 min, *B* → *C* within 22–35 min): t_R 26.45. UV (MeOH/H_2O 1:1): 258 (4.54). ^{1}H-NMR ((D_6)DMSO/D_2O 10:1): 8.40, 8.29, 8.24, 8.12, 8.05 (2 H) (5*s*, 6 H, H–C(2), H–C(8)); 5.96 (*m*, 2 H, H–C(1′)); 5.85 (*s*, H–C(1′)); 2.10 (*m*, 2 H, H–C(3′) of cord.); 2.02, 1.75 (2*m*, 4 H of palm); 1.16 (*m*, 12 CH_2 of palm); 0.78 (*t*, Me of palm). FAB-MS: 1148 (*M*H$^+$ for free acid).

5′-Deoxy-5′-(hexadecanoylamino)adenylyl-(2′–5′)-adenylyl-(2′–5′)-3′-deoxyadenosine Bis(ammonium) Salt (**31** · 2 NH_4^+). As described for **26**, with **20** (130 mg, 0.128 mmol), **24** (132 mg, 0.1 mmol), MeCN (4 ml), 1*H*-tetrazole (42 mg, 0.6 mmol), I_2 (0.1 g) in CH_2Cl_2/H_2O/pyridine 1:1:3 (1 ml), $CHCl_3$ (100 ml), sat. $Na_2S_2O_3$ soln. (2 × 20 ml), 0.5M DBU/MeCN (21 ml), 1M AcOH/MeCN (10.5 ml), and 1M Bu_4NF/THF (5 ml). The residue in MeOH/H_2O 1:1 (100 ml) was applied onto a *DEAE-Sephadex-A-25* column (60 × 1.5 cm, linear gradient of 0.005–0.4M $(Et_3NH)HCO_3$ buffer (pH 7.5) in MeOH/H_2O 1:1). The product fractions were evaporated and co-evaporated with NH_4OH (3 × 20 ml) and then with MeOH (25 ml). The residual NH_4^+ salt was lyophilized (MeOH/H_2O 1:1): 53.7 mg (46%) of **31** · 2 NH_4^+. HPLC (*A* = 0.1M aq. (Et_3NH)OAc buffer (pH 7.0), *B* = 0.1M aq. (Et_3NH)OAc buffer (pH 7.0)/MeCN 1:1, *C* = MeCN; gradient: *A* within 0–2 min, *A* → *B* within 2–22 min, *B* → *C* within 22–35 min): t_R 26.68. UV (MeOH/H_2O 1:1): 258 (4.55). ^{1}H-NMR ((D_6)DMSO/D_2O 10:1): 8.38, 8.30, 8.23, 8.10, 8.07 (2 H) (5*s*, 6 H, H–C(2), H–C(8)); 5.99 (*d*, 2 H, H–C(1′)); 5.77 (*d*, H–C(1′)); 2.04, 1.37 (2*m*, 4 H of palm); 1.19 (*m*, 12 CH_2 of palm); 0.80 (*t*, Me of palm). FAB-MS: 1148 (*M*H$^+$ for free acid).

5′-Deoxy-5′-[2-(4-nitrophenyl)ethylamino]adenylyl-(2′–5′)-3′-deoxyadenylyl-(2′–5′)-adenosine Bis(ammonium) Salt (**32** · 2 NH_4^+) *and 5′-Amino-5′-deoxyadenylyl-(2′–5′)-3′-deoxyadenylyl-(2′–5′)-adenosine Bis(ammonium) Salt* (**33** · 2 NH_4^+). As described for **26**, with **21** (220 mg, 0.22 mmol), **23** (0.2 g, 0.16 mmol), MeCN (4 ml), 1*H*-tetrazole (77 mg, 1.1 mmol), I_2 (0.2 g) in CH_2Cl_2/H_2O/pyridine 1:1:3 (2 ml), $CHCl_3$ (100 ml), sat. $Na_2S_2O_3$ soln. (2 × 30 ml), 0.5M DBU/MeCN (34 ml), 1M AcOH/MeCN (17 ml), and 1M Bu_4NF/THF (12 ml). The residue was applied onto a *DEAE-Sephadex-A-25* column (60 × 1.5 cm, linear gradient of 0.005–0.3M $(Et_3NH)HCO_3$ buffer (pH 7.5)). The product fractions were evaporated and co-evaporated with NH_4OH (3 × 20 ml) and then with MeOH (15 ml). The residual NH_4^+ salts were lyophilized: 50.8 mg (30%) of **32** · 2 NH_4^+, and 42.2 mg (28%) of **33** · 2 NH_4^+.

Data of **32** · 2 NH_4^+: UV (H_2O): 259 (4.57). HPLC (*A* = 0.1M aq. (Et_3NH)OAc buffer (pH 7.0), *B* = 0.1M aq. (Et_3NH)OAc buffer in H_2O/MeCN 1:1, *C* = MeCN; gradient: *A* within 0–2 min, *A* → *B* within 2–22 min, *B* → *C* within 22–35 min): t_R 11.23 min. ^{1}H-NMR ((D_6)DMSO/D_2O 10:1): 8.39, 8.30, 8.24, 8.13, 8.06, 8.02 (6*s*, 6 H, H–C(2), H–C(8)); 7.95 (*d*, 2 H, *o* to NO_2); 7.36 (*d*, 2 H, *m* to NO_2); 6.09 (*d*, H–C(1′)); 5.95 (*s*, H–C(1′)); 5.85 (*d*, H–C(1′)); 2.20 (*m*, 2 H H–C(3′)). FAB-MS: 1059 (*M*H$^+$ for free acid).

Data of **33** · 2 NH_4^+: UV (H_2O): 259 (4.58). HPLC (*A* = 0.1M aq. (Et_3NH)OAc buffer (pH 7.0), *B* = 0.1M aq. (Et_3NH)OAc buffer (pH 7.0)/MeCN 1:1; gradient: *A*/*B* 1:1 within 0–5 min, then *A*/*B* 6:4 within 5–35 min): t_R 11.84. ^{1}H-NMR ((D_6)DMSO/D_2O 10:1): 8.40, 8.32, 8.26, 8.14, 8.05, 8.02 (6*s*, 6 H, H–C(2), H–C(8)); 5.99 (*d*, H–C(1′)); 5.95 (*s*, H–C(1′)); 5.85 (*d*, H–C(1′)); 2.18 (*m*, 2 H, H–C(3′)). FAB-MS: 909 (*M*H$^+$ for free acid).

5′-Deoxy-5′-[2-(4-nitrophenyl)ethylamino]adenylyl-(2′–5′)-adenylyl-(2′–5′)-3′-deoxyadenosine Bis(ammonium) Salt (**34** · 2 NH_4^+). As described for **26**, with **21** (220 mg, 0.22 mmol), **24** (0.2 g, 0.16 mmol), MeCN (4 ml), 1*H*-tetrazole (77 mg, 1.1 mmol), I_2 (0.2 g) in CH_2Cl_2/H_2O/pyridine 1:1:3 (2 ml), $CHCl_3$ (80 ml), sat. $Na_2S_2O_3$ soln. (2 × 25 ml), 0.5M DBU/MeCN (22 ml), 1M AcOH/MeCN (11 ml), and 1M Bu_4NF/THF (4.6 ml). The residue was applied onto a *DEAE-Sephadex-A-25* column (60 × 1.5 cm, linear gradient of 0.005–0.3M $(Et_3NH)HCO_3$ buffer (pH 7.5)). The product fractions were evaporated and co-evaporated with NH_4OH (3 × 15 ml) and then with MeOH (20 ml). The residual NH_4^+ salts were lyophilized: 76.4 mg (46%) of **34** · 2 NH_4^+. UV (H_2O): 259 (4.57). HPLC (*A* = 0.1M aq. (Et_3NH)OAc buffer (pH 7.0), *B* = 0.1M aq. (Et_3NH)OAc buffer (pH 7.0)/MeCN 1:1; *C* = MeCN; gradient: *A* within 0–2 min, *A* → *B* within 2–22 min, *B* → *C* within 22–35 min): t_R 14.57. ^{1}H-NMR ((D_6)DMSO/D_2O 10:1): 8.40, 8.27, 8.09, 8.04 (2 H); 7.80 (5*s*, 6 H, H–C(2), H–C(8)); 7.82 (*d*, 2 H, *o* to NO_2); 7.24 (*d*, 2 H, *m* to NO_2); 6.07 (*d*, H–C(1′)); 6.03 (*d*, H–C(1′)); 5.69 (*s*, H–C(1′)); 1.80 (*m*, 2 H, H–C(3′)). FAB-MS: 1059 (*M*H$^+$ for free acid).

REFERENCES

[1] B. Greiner, W. Pfleiderer, *Helv. Chim. Acta* **1998**, *81*, 1528.
[2] P. F. Torrence, in 'Biological Response Modifiers. New Approaches to Disease Intervention', Academic Press, Orlando 1985, p. 77.
[3] K. Renneisen, L. Leserman, E. Matthes, H. C. Schröder, W. E. G. Müller, *J. Biol. Chem*. **1990**, *265*, 16337.
[4] J. Goodchild, *Bioconjugate Chem*. **1990**, *1*, 165.
[5] M. Manoharan, C. J. Guinosso, P. D. Cook, *Tetrahedron Lett*. **1991**, *32*, 7171.
[6] D. W. Will, T. Brown, *Tetrahedron Lett*. **1992**, *33*, 2729.
[7] S. L. Beaucage, R. P. Iyer, *Tetrahedron* **1993**, *49*, 1925.
[8] M. Wasner, E. E. Henderson, R. J. Suhadolnik, W. Pfleiderer, *Helv. Chim. Acta* **1994**, *77*, 1757.
[9] C. Hörndler, W. Pfleiderer, *Helv. Chim. Acta* **1996**, *79*, 718.
[10] M. Wasner, R. J. Suhadolnik, S. E. Horvath, M. E. Adelson, N. Kon, M.-X. Guang, E. E. Henderson, W. Pfleiderer, *Helv. Chim. Acta* **1996**, *79*, 609.
[11] M. Wasner, R. J. Suhadolnik, S. E. Horvath, M. E. Adelson, N. Kon, M.-X. Guang, E. E. Henderson, W. Pfleiderer, *Helv. Chim. Acta* **1996**, *79*, 619.
[12] M. Wasner, R. J. Suhadolnik, S. E. Horvath, M. E. Adelson, N. Kon, M.-X. Guang, E. E. Henderson, W. Pfleiderer, *Helv. Chim. Acta* **1997**, *80*, 1061.
[13] P. Herdewijn, E. DeClercq, in 'Medical Chemistry for 21st Century', Eds. C. G. Wermuth, N. Koga, H. Konig, and B. W. Metcalf, Blackwell Science, Oxford, 1992, p. 45.
[14] W. Pfleiderer, F. Himmelsbach, R. Charubala, *Bioorg. Med. Chem. Lett*. **1994**, *8*, 1047.
[15] E. I. Kvasyuk, T. I. Kulak, I. A. Mikhailopulo, R. Charubala, W. Pfleiderer, *Helv. Chim. Acta* **1995**, *78*, 1777.
[16] R. J. Suhadolnik, T. Uematsu, *Carbohydr. Res.* **1982**, *61*, 545.
[17] R. Charubala, E. Uhlmann, F. Himmelsbach, W. Pfleiderer, *Helv. Chim. Acta* **1987**, *70*, 2028.
[18] H. Schirmeister, W. Pfleiderer, *Helv. Chim. Acta* **1994**, *77*, 10.
[19] R. W. Sobol, R. Charubala, W. Pfleiderer, R. J. Suhadolnik, *Nucleic Acids Res.* **1993**, *21*, 2437.
[20] W. S. Mungal, G. L. Greene, G. A. Heavner, R. J. Letsinger, *J. Org. Chem*. **1975**, *40*, 1659.
[21] P. Herdewijn, J. Balzarini, R. Pauwels, G. Janssen, A. van Aerschot, E. De Clercq, *Nucleosides Nucleotides* **1989**, *8*, 1231.
[22] F. Himmelsbach, W. Pfleiderer, *Tetrahedron Lett*. **1983**, *24*, 3583.
[23] F. Himmelsbach, B. S. Schulz, T. Trichtinger, R. Charubala, W. Pfleiderer, *Tetrahedron* **1984**, *40*, 59.
[24] R. E. Sobol, E. E. Henderson, N. Kon, J. Shao, P. Hitzges, E. Mordechai, N. L. Reichenbach, R. Charubala, H. Schirmeister, W. Pfleiderer, R. J. Suhadolnik, *J. Biol. Chem*. **1995**, *270*, 5963.
[25] D. Trono, *J. Virol.* **1992**, *66*, 4893.

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