Nucleotides

Part LXX[1])

Inhibition of HIV-1 Replication by Chemically Synthesized, Nuclease-Resistant, Nontoxic (2′ → 5′)-Oligoadenylate Agonists

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With best personal wishes dedicated to *Wolfgang Pfleiderer* on the occasion of his 75th birthday

The chemical syntheses of nuclease-resistant, nontoxic bioactive (2′-5′)agonists, 3′-deoxyadenylyl-(2′ → 5′)-3′-deoxyadenylyl-(2′ → 5′)-3′-deoxyadenylyl-(2′ → 2″)-9-[(2″-hydroxyethoxy)methyl]adenine (d^{3}A-d^{3}A-d^{3}A-etherA; **36**), 1-benzyl-3′-deoxyadenylyl-(2′ → 5′)-3′-deoxyadenylyl-(2′ → 5′)-3′-deoxyadenylyl-(2′ → 2″)-9-[2″-hydroxyethoxy)methyl]adenine (N^1-benzyl-d^{3}A-d^{3}A-d^{3}A-etherA; **37**), N^6-benzyl-3′-deoxyadenylyl-(2′ → 5′)-3′-deoxyadenylyl-(2′ → 5′)-3′-deoxyadenylyl-(2′ → 2″)-9-[(2″-hydroxyethoxy)methyl]adenine (N^6-benzyl-d^{3}A-d^{3}A-d^{3}A-etherA; **38**), N^6-benzyladenylyl-(2′ → 5′)-adenylyl-(2′ → 5′)-adenylyl-(2′ → 2″)-9-[(2″-hydroxyethoxy)-methyl]adenine (N^6-benzyl-A-A-A-etherA; **39**), as well as the biological activities of **37**, **38**, and already synthesized and published adenylyl-(2′ → 5′)-adenylyl-(2′ → 5′)-adenylyl-(2′ → 2″)-9-[(2″-hydroxyethoxy)methyl]-adenine (A-A-A-etherA; **40**), are described. The above (2′-5′)A derivatives **37** – **40** inhibit HIV-1 replication as measured by inhibition of syncytia formation, HIV-1 reverse transcriptase activity, or HIV-1 p24-antigen expression, with no evidence of cytotoxicity. Oligonucleotides **37**, **38**, and **40** were taken up intact into T cells in culture of cytoplasmic concentrations sufficient to activate the latent endoribonuclease, RNase L. N^6-Benzyl-d^{3}A-d^{3}A-d^{3}A-etherA (**38**) also exerts immunostimulatory effects by increasing expression of monocyte chemotactic protein-1 (MCP-1), and, thereby, competing with HIV-1 for binding to a critical HIV-coreceptor.

1. Introduction. – The antiviral defense mechanism in mammalian cells is induced after HIV-1 infection by the action of interferon and, subsequently, by the (2′ → 5′)-oligoadenylate synthetase (2′-5′)OAS/RNase L/p68 kinase (PKR) antiviral pathway [2][3]. The (2′-5′)OAS is an allosterically regulated enzyme activated by dsRNA to convert ATP to (2′-5′)A, which exerts its biological effects primarily by binding to and activating its target enzyme, RNase L, an 80 kDA (2′-5′)A-dependent endoribonuclease. Once activated, RNase L hydrolyses HIV-1 mRNA and subsequently inhibits HIV-1 protein synthesis [4]. HIV-1 has evolved several mechanisms to inhibit the (2′-5′)OAS/RNase L/PKR antiviral pathway. HIV-1 tat protein inhibits (2′-5′)OAS and

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PKR activity by sequestering the dsRNA HIV-1 TAR RNA required for their activation [5–7]. In HIV-1-infected cells, the (2′-5′)A needed to activate RNase L is depleted [2][4][8][9]. In addition, expression of a RNAse L inhibitor (RLI) is upregulated in HIV-1-infected cells [10].

A class of nuclease-resistant, nontoxic, structurally and configurationally modified (2′-5′)A derivatives has been developed to circumvent the HIV-1-induced blockades in the (2′-5′)OAS/RNase L antiviral defense pathways [11–18]. These (2′-5′)A derivatives were designed to augment the depleted intracellular (2′-5′)A pool in HIV-infected cells by acting distal to the HIV-1-induced blockades in the (2′-5′)OAS/RNase L antiviral pathway. They activate the latent RNase L, thereby leading to hydrolysis of HIV-1 mRNA, inhibition of HIV-1 protein synthesis, and inhibition of HIV-1 replication. A ten-fold-enhanced uptake into the cytoplasm without degradation of the phosphorothioate/phosphodiester-substituted (2′-5′)A derivatives in HIV-1-infected sup-T1 cells has been demonstrated [11]. (2′-5′)A and (2′-5′)A derivatives also directly inhibit HIV-1 reverse transcriptase (RT), by preventing HIV-1RT/primer complex formation, and inhibit DNA topoisomerase I in HIV-1-infected cells [18–21].

We report here the chemical synthesis of four new (2′-5′)A agonists **36**–**39** and the biological characterization of compounds **37**, **38**, and of the already-published **40**. The rationale for the synthesis of these compounds was based on the anti-HIV-1 properties of the enzymatically and chemically synthesized (2′-5′)A derivatives previously reported from our laboratories [11–19].

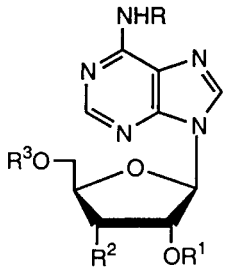
2. Synthesis. – As starting materials, 3′-deoxyadenosine (**1**) [22–24] was converted by benzylation to its 1-benzyl derivative **3**, which, on treatment with methylamine in EtOH, rearranged to the N^6-benzyl derivative **4** [25]. Both compounds were then transformed into their 5′-*O*-monomethoxytrityl derivatives **6** and **7**, respectively. In the case of the ribo series, N^6-benzyl-5′-*O*-(monomethoxytrityl)adenosine (**8**; obtained from **2**) [15] was silylated with (*tert*-butyl)dimethylsilyl chloride to give three isomers, namely 2′,3′-bis-*O* silyl, 2′-*O*-silyl, and 3′-*O*-silyl derivatives **9**–**11**, which were separated by silica-gel column chromatography and isolated in 16, 30, and 42% yield, respectively.

The fully protected phosphotriester **12** of N^6-benzyl-3′-deoxy-5′-*O*-(monomethoxytrityl)adenosine (**7**) was prepared by treatment of **7** with 2,5-dichlorophenyl bis(1*H*-1,2,4-triazol-1-yl)phosphinate) and subsequent condensation with 2-(4-nitrophenyl)ethanol. The corresponding phosphodiester **13** resulted by cleavage of the 2,5-dichlorophenyl group with a solution of 4-nitrobenzaldehyde oxime in dioxane/H_2O/Et_3N 1:1:1.

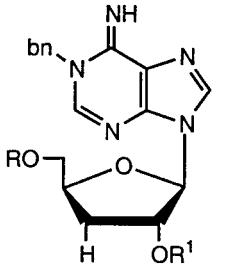
The phosphoramidites **16** [26] and **17** [27] were prepared starting from **14** and **15**, respectively, with 2-cyanoethyl tetraisopropylphosphorodiamidite [28–31] and 2-(4-nitrophenyl)ethyl tetraisopropylphosphorodiamidite [27] as the phosphitizing reagents activated by 1*H*-tetrazole.

The 9-[(2-hydroxyethoxy)methyl]adenine derivative **19** resulted from **18** [32] by benzoylation and followed by detritylation with 80% AcOH.

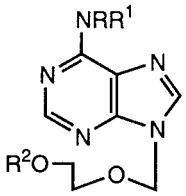
The dimers **21** and **23** were prepared by condensing the phosphoramidite **16** with N^6-benzoyl-9-[(2-hydroxyethoxy)methyl]adenine (**20**) [33][17] and the phosphoramidite **17** [33] with N^6,N^6-dibenzoyl-9-[(2-hydroxyethoxy)methyl]adenine (**19**), respectively, in presence of 1*H*-tetrazole in MeCN, followed by I_2 oxidation (*Scheme*). The



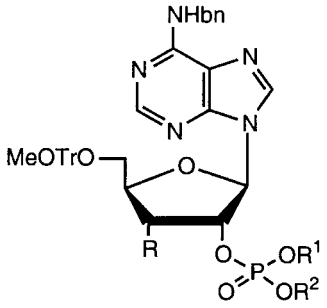
	R	R^1	R^2	R^3
1	H	H	H	H
2	H	H	OH	H
4	bn	H	H	H
5	bn	H	OH	H
7	bn	H	H	MeOTr
8	bn	H	OH	MeOTr
9	bn	tbds	Otbds	MeOTr
10	bn	tbds	OH	MeOTr
11	bn	H	Otbds	MeOTr
14	bz	H	H	$(MeO)_2Tr$
15	npeoc	H	H	MeOTr



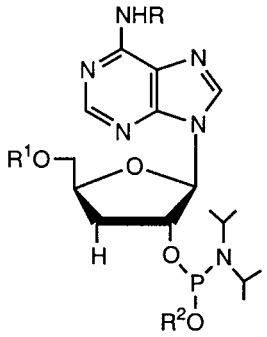
	R	R^1
3	H	H
6	MeOTr	H
33	MeOTr	$P(O)(OC_6H_3Cl_2)O^- C_5H_5NH^+$



	R	R^1	R^2
18	H	H	$(MeO)_2Tr$
19	bz	bz	H
20	bz	H	H



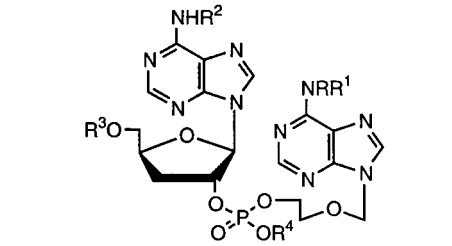
	R	R^1	R^2
12	H	npe	$2,5\text{-}Cl_2C_6H_3$
13	H	npe	Et NH
34	Otbds	$2,5\text{-}Cl_2C_6H_3$	C_5H_5NH



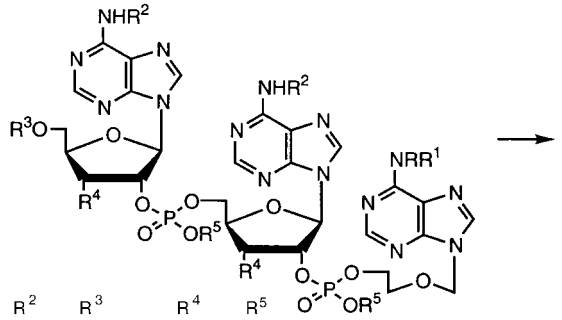
	R	R^1	R^2
16	bz	$(MeO)_2Tr$	ce
17	npeoc	MeOTr	npe

bn = benzyl, bz = benzoyl, tbds = (*tert*-butyl)dimethylsilyl, ce = 2-cyanoethyl, npe = 2-(4-nitrophenyl)ethyl, npeoc = [2-(4-nitrophenyl)ethoxy]carbonyl, MeOTr = monomethoxytrityl, $(MeO)_2Tr$ = dimethoxytrityl

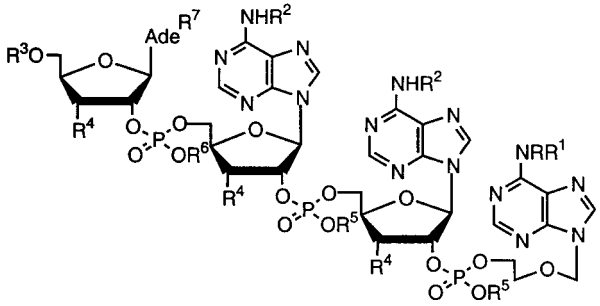
Scheme



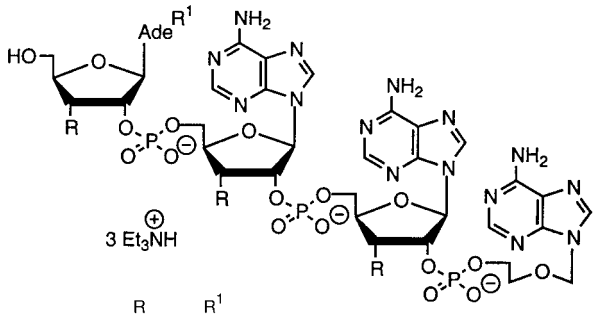
	R	R^1	R^2	R^3	R^4
21	H	bz	bz	$(MeO)_2Tr$	ce
22	H	bz	bz	H	ce
23	bz	bz	npeoc	MeOTr	npe
24	bz	bz	npeoc	H	npe



	R	R^1	R^2	R^3	R^4	R^5
25	H	bz	bz	$(MeO)_2Tr$	H	ce
26	H	bz	bz	H	H	ce
27	bz	bz	npeoc	MeOTr	H	npe
28	bz	bz	npeoc	H	H	npe
35	H	bz	npeoc	H	Otbds	npe



	R	R^1	R^2	R^3	R^4	R^5	R^6	R^7
29	H	bz	bz	$(MeO)_2Tr$	H	ce	ce	N^6-bz
30	H	bz	bz	MeOTr	H	ce	2,5-$Cl_2C_6H_3$	N^1-bn
31	bz	bz	npeoc	MeOTr	H	npe	npe	N^6-bn
32	H	bz	npeoc	MeOTr	Otbds	npe	2,5-$Cl_2C_6H_3$	N^6-bn



	R	R^1
36	H	NH_2
37	H	N^1-bn
38	H	N^6-bn
39	OH	N^6-bn
40	OH	NH_2

trityl groups were then cleaved with 2% TsOH in CH_2Cl_2/MeOH 4:1 to give the corresponding 5′-hydroxy dimers **22** and **24**, respectively.

For the chain elongation to the trimers **25** and **27**, the amidite **16** was condensed with the dimer **22** and, analogously, the amidite **17** with the dimer **24**, followed by oxidation with I_2. The free 5′-hydroxy trimers **26** and **28** were liberated by acid treatment of **25** and **27**.

Then, the fully protected four tetramers **29**, **30**, **31**, and **32** were assembled by the phosphoramidite and phosphodiester approaches. The fully protected tetramer d^3A-d^3A-d^3A-etherA **29** was synthesized from amidite **16** and the 5′-hydroxy trimer **26** in presence of the 1*H*-tetrazole activator and subsequent oxidation with I_2. The synthesis of the tetramers N^1-benzyl-d^3A-d^3A-d^3A-etherA **30**, N^6-benzyl-d^3A-d^3A-d^3A-etherA **31**, and N^6-benzyl-A-A-A-etherA **32** was based on the phosphodiester method. Thus, the monomer blocks 1-benzyl-3′-deoxy-5′-(monomethoxytrityl)adenosine (**6**) and N^6-benzyl-3′-*O*-[(*tert*-butyl)dimethylsilyl]-5′-*O*-(momomethoxytrityl)adenosine (**11**) were first converted *in situ* to their corresponding phosphodiester salts **33** and **34** (see above) by treatment with 2,5-dichlorophenyl phosphorodichloridate and 1*H*-1,2,4-triazole in pyridine, followed by hydrolysis with pyridine/H_2O. Subsequent condensation with the trimer **26** and **35** [17] in the presence of 1*H*-tetrazole/2,4,6-triisopropylbenzenesulfonyl chloride afforded the tetramers **30** and **32**. The N^6-benzyl-3′-deoxy-5′-(monomethoxytrityl)adenosine (**7**) was transformed *via* phosphotriester **12** to the phosphodiester salt **13** (see above), the latter reacting analogously with trimer **28** in the presence of 1*H*-tetrazole/2,4,6-triisopropylsulfonylchloride to give **31** in 88% yield after purification.

Finally, fully deblocked tetramers d3′A-d3′A-d3′A-etherA **36** and N^1-benzyl-d3′A-d3′A-d3′A-etherA (**37**) were obtained from **29** and **30** by treatment with ammonia to remove cyanoethyl and benzoyl groups followed by detritylation with 80% AcOH. In the case of N^6-benzyl-d3′A-d3′A-d3′A-etherA **38** and N^6-benzyl-d3′A-d3′A-d3′A-etherA **39**, the precursors **31** and **32**, respectively, were subjected to a subsequent removal of the trityl group TsOH in CH_2Cl_2/MeOH 4:1) and of 2-(4-nitrophenyl)ethyl (npe) and [2-(4-nitrophenyl)ethoxy]carbonyl (npeoc) groups by 0.5M DBU (1,8-diazabicyclo[5.4.0]undec-7-ene)/pyridine, followed by benzoyl cleavage with ammonia to give the tetramer **38**, whereas the ribo tetramer **39** needed an additional step with Bu_4NF in THF to remove the 3′-*O*-silyl groups. All four tetramers were purified by *DEAE-Sephadex A-25* column chromatography, eluting with $(Et_3NH)HCO_3$ buffer (pH 7) and lyophilized and characterized by TLC, HPLC, and UV. The synthesis of the tetramer **40** has already been published in detail [17].

3. Biological Studies. – *Inhibition of HIV-1 Replication*. The infected-centers assay was used to measure the ability of **37**–**40** to inhibit HIV-1-induced syncytia formation, and PCR-amplification and HIV-1-integrase assays were accomplished as previously described [34]. The level of HIV-1 p24 antigen was measured in cell-culture supernatents by the antigen-capture ELISA assay (*SAIC-Frederick*). Recombinant human RNase L was expressed in *E. coli* (DH5α) as a glutathione-S-transferase(GST)-RNase L fusion protein as described [11][12]. The activation of recombinant human GST-RNase L was measured as the percent of poly(U)-3′-[^{32}P]pCp hydrolyzed in the presence of p_3A_3 ($1 \cdot 10^{-10}$ to 10^{-6} M) or (2′-5′)A derivatives, as previously described

[11][12]. The effect of (2′-5′)A derivatives on HIV-1 reverse-transcriptase (RT) activity was measured as reported [34], except that test compounds (100 μM) were added 2 h prior to infection of peripheral blood mononuclear cells (PBMC) ($2 \cdot 10^5$) with HIV-1 IIIB (m.o.i.0.1).

At 72 h post-infection, cell-culture supernatant (25 μl) was incubated for 5 h at 37° with 50 ml of RT buffer. An aliquot (50 μl) was spotted onto a sheet of *Whatman DE-81* chromatography paper in a dot-blot apparatus. After washing twice with 30 mM sodium citrate (pH 7.0) and once with 95% EtOH, individual circles were excised, and the radioactivity was determined by scintillation spectrometry. Cytotoxicity was determined by colony formation in uninfected cells or by trypan-blue exclusion.

Uptake Assays. Accumulation of the (2′-5′) derivatives in SupT1 cells (**40**) and H9 cells (**37** and **38**) was determined as previously described [11]. Intracellular cytoplasmic uptake and quantitation of **37**, **38**, and **40** was determined by HPLC as described [11][17].

Expression of Monocyte Chemotatic Protein-1 (*MCP-1*). The level of MCP-1 expression was determined in the supernatent cultures of PBMCs taken 48 h after culture initiation. Cultures were initiated with PBMCs ($2 \cdot 10^6$/ml) in RPMI-1640 medium containing 10% fetal-calf serum, and were treated with medium alone or 0.1 or 1.0 μM N^6-benzyl-d3′A-d3′A-d3′A-etherA **38** in quadruplicate replicate cultures. Designated cultures received treatment with or without phytohemagglutinin (PHA) at 5 μg/ml also at culture initiation. MCP-1 Levels were determined by an enzyme-linked immunoabsorbant assay (ELISA) with 96-well plates coated with monoclonal anti-MCP-1 antibody 10F 7 (*Pharmingen Laboratories*, San Diego, CA) at a concentration of 2 μg/ml. Coated plates were blocked with 1% bovine serum albumin in phosphate-buffered saline. Samples were added to the plates in a binding medium containing 0.1M Na_2HPO_4, 0.1M NaH_2PO_4, and 0.05% *Tween-20* at pH 9.0. After washing, the captured MCP-1 was detected with biotinylated monoclonal anti-MCP-1 antibody 5D3-F 7 (*Pharmingen Laboratories*), and the intensity of the reaction was determined with horseradish-peroxidase-conjugated streptavidin in binding medium, and developed with a substrate solution containing 2,2′-azinobis-[3-ethyl-2,3-dihydro-benzothiazole-6-sulfonic acid) at 0.3 mg/ml in 0.1M citric acid (pH 4.35). The colorimetric reaction was read at 405 nm following the addition of 3% H_2O_2 solution. The concentration of MCP-1 in the supernatents was assessed with a series of standards in the ELISA consisting of recombinant MCP-1 (*Pharmingen Laboratories*).

4. Results and Discussion. – *Chemical Syntheses.* The phosphotriester and phosphoramidite approaches were used for the chemical syntheses of the (2′-5′)A derivatives d3′A-d3′A-d3′A-etherA, N^1-benzyl-d3′A-d3′A-d3′A-etherA, N^6-benzyl-d3′A-d3′A-d3′A-etherA, and N^6-benzyl-A-A-A-etherA **36**–**39**. The syntheses required the choice of appropriate protecting groups for the preparation of monomeric building blocks. Reactive functional groups that can be individually manipulated to be protected by transient and/or permanent blocking groups. Such groups have proven successful in our laboratories in the chemical synthesis of a class of structurally and stereochemically modified (2′-5′)A derivatives [11–19]. The rationale for these chemical syntheses was based on the previous demonstration of anti-HIV properties of enzymatically and chemically synthesized (2′-5′)A derivatives in our laboratories. The N^6-benzyl- and N^1-

benzyl-3′-deoxyadenylyl skeleton with the terminal etherA function was chosen to provide nonpolar groups for increased cytoplasmic uptake, stability to hydrolysis of 2′-phosphodiesterase, and stability to hydrolysis by endonucleases, respectively. The 3′-deoxyadenosine (cordycepin) trimer core derivative (2′-5′)-d^3(A-A-A) has been demonstrated to inhibit HIV-1 replication without cytotoxicity [35][36]. Inhibition by HIV-1 replication by (2′ → 5′)-oligoadenylate derivatives modified at the N^6 position of the adenosine moiety of (2′-5′)A with the benzyl group has been previously reported to be increased more than 1500-fold compared to a 3-fold increase of authentic (2′-5′)(A-A-A) trimer core [16]. The replacement of the ribosyl moiety of (2′-5′)A with an ether linkage at the 2′,3′-terminus of the tetramer core yielded A-A-A-etherA **40**.

Cytotoxicity. No evidence of cytotoxicity was observed in T cells treated with **37**, **38**, and **40** (*Table*). However, etherA and A-A-etherA inhibited T-cell growth to 50 and 25%, respectively.

Table. *Inhibition of HIV-1 Replication by (2′-5′)A and (2′-5′)A Agonists*

(2′-5′)A or agonist	Conc. [μM]	Cytotoxicity[a]) [% survival]	Inhibition of syncytia formation[b]) [fold reduction]	Inhibition of HIV-1 RT activity[c]) [%]	Inhibition of HIV-1 p24-antigen expressiion[d]) [%]	Hydrolysis of poly(U)-3′-[^{32}P]pCp[e]) [%]
Control		100	0	0	0	0
p_3A_3	300	–	–	–	–	92
pA_3	300	–	–	–	–	95
A_3	300	100	67	10	–	0
EtherA	300	50	87	45	–	–
A-A-etherA	300	75	99	63	–	0
A-A-A-etherA **40**	300	100	99	72	–	87
	100			–	41	
N^6-benzyl-A-A-A-etherA **39**	100	–	63	–	87	–
N^1-benzyl-d$^{3'}$A-d$^{3'}$A-d$^{3'}$A-etherA **37**	100	100	99	67	–	–
	10		81	–	–	–
	1		59	–	–	–
	0.1		29	–	–	–
	0.01		0	–	–	–
N^6-benzyl-d$^{3'}$A-d$^{3'}$A-d$^{3'}$-A-etherA **38**	100	100	99	60	51	–
	10	0	86	–	–	–
	1	0	41	–	–	86
	0.1	–	44	–	–	**90**
	0.01	–	0			

[a]) Cytotoxicity was determined by colony formation in uninfected SupT1 cells or H9 cells or by trypan-blue exclusion. [b]) Expressed as HIV-1-induced syncytia formed/10^4 cells as determined by coculture with SupT1 cells (average of 3 independent determinations on day 4). [c]) Percent inhibition of HIV-1 reverse-transcriptase (RT) activity: Expressed as dpm · 10^3 M as determined by phosphofluorescent imaging (average of 3 independent determinations on day 3). [d]) Percent inhibition of expression of HIV-1 p24 antigen as determined by p24-antigen-capture ELISA assay (mean of triplicate determinations). [e]) Activation of GST-RNase L by (2′-5′)A or (2′-5′)A derivatives ($1 \cdot 10^{-7}$ M) was measured by the percent hydrolysis of poly(U)-3′-[^{32}P]pCp (mean of duplicate determinations with a variance of 5–10%).

Inhibition of HIV-1 Replication by **37**–**40**. The (2′-5′)A derivatives were evaluated with respect to their anti-HIV-1 activity in HIV-1-infected T cells in culture. Inhibition of HIV-1-induced syncytia formation, inhibition of HIV-1 reverse-transcriptase (RT) activity, and inhibition of p24-antigen expression were used to measure the effect of these (2′-5′)A derivatives on HIV-1 replication. Compounds **37**, **38**, and **40** completely inhibited HIV-1-induced syncytia formation at 30, 100, 300 μM, respectively, whereas **39** showed a 63% inhibition.

Compounds **38** and **40** (300 μM) also inhibited HIV-1 RT activity to 60 and 72%, respectively. On the basis of these results, we further explored the mechanism of inhibition of HIV-1 replication by N^6-benzyl-d$^{3'}$A-d$^{3'}$A-d$^{3'}$A-etherA **38** by examining key stages in the life cycle of HIV-1. PCR Analyses demonstrated amplification of all reverse transcripts tested in HIV-1-infected SupT1 cells treated with N^6-benzyl-d$^{3'}$A-d$^{3'}$A-d$^{3'}$A-etherA **38** (data not shown). Therefore, **38** did not inhibit HIV-1 replication at the level of reverse transcription. Compound **38** also did not demonstrate any inhibition of HIV-1 integrase, the enzyme that incorporates HIV-1 DNA into the DNA target cells (data not shown). This compares with 100% inhibition of HIV-1 integrase with a 5′-palmitoyl-(2′-5′)A derivative [37]. As reported earlier for the 3′-palmitoyl-(2′-5′)A derivatives [37], we also observed that **38** and **40** inhibited expression of HIV-1 p24 antigen, by 52 and 41%, respectively.

Finally, compounds **38** and **40** were able to bind and activate recombinant human RNase L. Compound **40** (at 300 μM) resulted in 87% and compound **38** (at 0.01 μM) in 90% hydrolysis of poly(U)-3′[^{32}P]pCp compared to 92% hydrolysis in the presence of authentic p_3A_3 (300 μm) (*Table*). Therefore, one mode of action of compounds **38** and **40** is to activate the (2′-5′)A-dependent RNase L distal to the HIV-1-induced blockade in the antiviral-defense pathway.

Uptake and Intracellular Concentration of A-A-A-etherA **40**, N^1*-Benzyl-d*$^{3'}$*A-d*$^{3'}$*A-d*$^{3'}$*A-etherA* **37** *and* N^6*-Benzyl-d*$^{3'}$*-d*$^{3'}$*A-d*$^{3'}$*A-etherA* **38**. To determine whether these compounds were taken up intact, SupT1 and H9 cells were incubated with **37**, **38**, or **40** at $1 \cdot 10^{-4}$ M for 4 h at 37°. CF_3COOH *Freon* extracts were prepared and analyzed by HPLC as described [11]. The cytoplasmic concentrations of compounds **37**, **38**, and **40** were determined to be $8 \cdot 10^{-7}$, $8.9 \cdot 10^{-7}$, and $4.9 \cdot 10^{-8}$ M, above the concentration required to activate RNase L. No metabolic degradation products of these compounds were detected by HPLC analysis of the cytoplasmic extracts prepared after 4-h incubations.

Inhibition of Expression of Monocyte Chemotactic Protein-1 (*MCP-1*) *by* N^6*-Benzyl-d*$^{3'}$*A-d*$^{3'}$*A-d*$^{3'}$*A-etherA* **38**. In an effort to more fully understand the inhibition of HIV-1 replication by compound **38**, we wished to determine whether this compound might exert immunomodulatory activity. We examined the capacity of **38** to alter the level of expression of monocyte chemotactic protein (MCP-1). This chemokine is a ligand for CC chemokine receptor (CCR) 2 and is involved in the development of inflammatory response [38]. It is known that CCR 2 is a coreceptor for some HIV-1 strains. A genetic variant of CCR 2 valine 64 is changed to isoleucine has been identified in certain HIV-1-infected individuals, and these patients have been found to progress to AIDS 2–4 years later than subjects homozygous for the wild-type CCR2 allele [39][40]. We treated peripheral blood mononuclear cells (PBMCs) with **38** and observed a significant elevation of MCP-1 levels (*Fig.*)

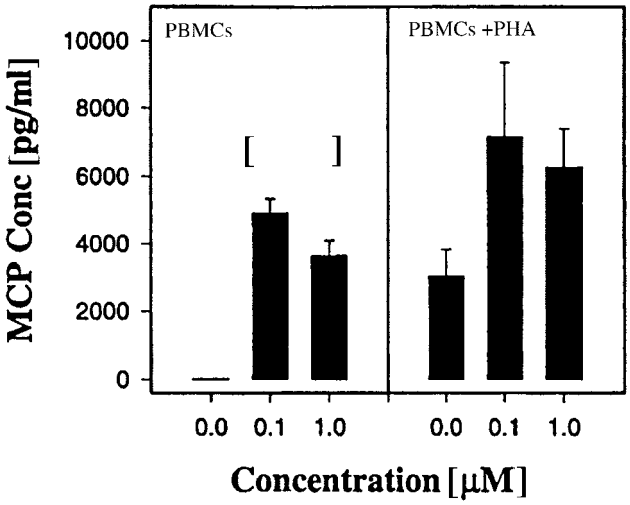


Fig. 1. *Induction of MCP-1 expression by* N^6*-benzyl-d*^{3}*A-d*^{3}*A-d*^{3}*A-etherA* **38** *in PHA-activated and non-activated PBMCs.* Supernatants from PBMC cultures were collected after 48 h of incubation with the designated concentrations of **38** in the presence or absence of PHA (5 μg/ml), and the MCP-1 level was determined by ELISA. Results represent the mean (+/− standard deviation) of values from quadruplicate cultures.

We also examined the ability of compound **38** to alter levels of MCP-1 in activated PBMCs, and observed a two-fold increase in MCP-1 levels at **38** concentrations of 0.1 and 1.0 μM. These results demonstrate that **38** exerts immunostimulatory effects on the immune system, leading to an increase in the expression of a chemokine ligand that may compete with HIV-1 binding to a critical HIV-1 coreceptor, which is required for HIV-1 to transfer its dsRNA genome into the target T cell for infection and replication.

In conclusion, the data presented clearly demonstrate that compounds **37**, **38**, and **40** have distinct anti-HIV-1 activities. (2′-5′)A Derivatives **38** and **40** upregulate the (2′-5′)A-dependent RNase L antiviral defense pathway, as evidenced by the hydrolysis of poly(U)-3′-[^{32}P]pCp. In addition, compound **38** inhibits HIV-1 replication at a critical HIV-1 coreceptor. As nuclease-resistant (2′-5′)A derivatives that are taken up intact into the cytoplasm of T cells, N^1-benzyl-d$^{3'}$A-d$^{3'}$A-d$^{3'}$A-etherA **37**, N^6-benzyl-d$^{3'}$A-d$^{3'}$A-d$^{3'}$A-etherA **38**, and A-A-A-etherA **40** have potential as inhibitors of HIV-1 replication.

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Experimental Part

General. TLC: Precoated silica gel (Sil) thin-layer sheets *F 1500 LS 254* from *Merck*; precoated cellulose (Cel) thin-layer sheets *F 1400 LS 254* from *Schleicher & Schuell*. Prep. column chromatography (CC): silica gel (*Merck 60*, 63–200 μm); ion exchange, *DEAE-Sephadex A-25* (*Pharmacia*). HPLC: *Merck-Hitachi LaChrom L7100*; diode-array detector *Merck-Hitachi L7450A*; autosampler *Merck-Hitachi L7200*, column *Merck Lichrospher-100* (*RP-18*), 0.1M (Et_3NH)OAc/MeCN gradient; t_R in min. M.p.: *Büchi B-545*; uncorrected. UV/VIS: *Perkin-Elmer Lamda-5*; λ_{max} in nm (log ε). ^{1}H-NMR: *Bruker WM-250*; δ in ppm rel. to deuterated solvent. ^{31}P-NMR: *Jeol GX-400*; δ in ppm rel. to H_3PO_4.

1. *1-Benzyl-9-(3′-deoxy-β-*D*-ribofuranosyl)1,9-dihydro-6*H*-purine-6-imine* (=*1-Benzylcordycepin*; **3**). A soln. of 3′-deoxyadenosine (**1**) [22–24] (0.84 g, 3.36 mmol) in anh. DMF (11 ml) was treated with benzyl bromide (1.28 ml, 7.4 mmol) and stirred at r.t. for 48 h. The mixture was added to acetone (200 ml) to precipitate a colorless solid (hydrobromide salt), which was stored in a refrigerator overnight. The clear supernatant soln. was decanted, and the precipitate was dissolved in H_2O (30 ml) and treated with 25% ammonia (30 ml), whereby a white precipitate separated. The mixture was stirred at 50° for 3 h, and, after cooling to r.t., the product was collected by filtration and washed with Et_2O: 0.73 g (63%) of **3**. Colorless solid. M.p. 217°. TLC (Sil, $CHCl_3$/MeOH 4:1): R_f 0.21. UV (MeOH): 258 (4.14), 266 (sh, 4.06), 301 (sh, 3.40). ^{1}H-NMR ((D$_6$)DMSO): 8.24, 8.15 (2*s*, H–C(2), H–C(8)); 7.34 (*m*, Ph); 7.00 (*m*, NH); 5.75 (*d*, H–C(2′)); 5.65 (*m*, OH–C(2′)); 5.23 (*s*, CH_2); 5.02 (*t*, OH–C(5′)); 2.10, 1.85 (2*m*, 2 H–C(3′)). Anal. calc. for $C_{17}H_{19}N_5O_3$ (341.4): C 59.81, H 5.61, N 20.51; found: C 59.34, H 5.52, N 20.32.

2. N^6-*Benzyl-3′-deoxyadenosine* (=N^6-*Benzylcordycepin*; **4**) [25]. A soln. of **1** (0.91 g, 3.6 mmol) in dry DMF (25 ml) was treated with benzyl bromide (1.25 g, 7.2 mmol) and stirred at r.t. for 20 h. The solvent was concentrated to *ca.* 1 ml and the remainder added dropwise to dry acetone. The white precipitate was filtered and dried. The precipitate was taken up in EtOH (25 ml) and treated with 33% of Me_2NH in EtOH (25 ml). After standing at r.t. for 24 h, **4** had separated from the clear soln. The crystals were washed with Et_2O and dried: 0.69 g (60%) of **4**. M.p. 185°. TLC (Sil, $CHCl_3$/MeOH 9:1): R_f 0.38. UV (MeOH): 271 (4.32), 267 (sh, 4.32). ^{1}H-NMR ($CDCl_3$): 8.37 (*s*, *m*, 3 H, H–C(8), Ph); 8.19 (*s*, H–C(2)); 7.33–7.19 (*m*, 3 H, Ph); 5.87 (*s*, H–C(1′)); 5.64 (*d*, OH–C(3′)); 5.10 (*t*, OH–C(5′)); 4.70 (br. *s*, CH_2); 4.57 (*m*, H–C(2′)); 4.34 (*m*, H–C(4′)); 3.70–3.66 (2*m*, 2 H–C(5′)); 2.27, 1.90 (2*m*, 2 H–C(3′)).

3. *1-Benzyl-9-[3′-deoxy-5′-*O*-(monomethoxytrityl)-β-*D*-ribofuranosyl]-1,9-dihydro-6*H*-purin-6-imine* (**6**) *and* N^6*-Benzyl-3′-deoxy-5′-*O*-(monomethoxytrityl)adenosine* (**7**). Compound **3** or **4** (0.68 g, 2 mmol), resp., was co-evaporated with anh. pyridine (3 × 10 ml), taken up in anh. pyridine (15 ml), and treated with monomethoxytrityl chloride (0.613 g, 2 mmol) and a small amount of *N,N*-dimethylpyridin-4-amine (DMAP). After stirring for 20 h at r.t., MeOH (1 ml) was added to the mixture and the latter concentrated to 1/4 volume. The residue was taken up in CH_2Cl_2 (50 ml) and washed with sat. $NaHCO_3$ soln. (2 × 25 ml), dried (Na_2SO_4), evaporated, and co-evaporated with toluene (2 × 20 ml). In the case of **6**, the pure product was crystallized from AcOEt to give 48% and, from the mother liquid on purification by CC (silica gel, $CHCl_3$, $CHCl_3$/MeOH 50:6), another 7% of **6**. Crude **7** was purified by CC (silica gel, CH_2Cl_2, CH_2Cl_2/MeOH 50:1 → 50:2) give, after drying, 1.142 g (92%) of **7**. Colorless foam.

Data of **6**: TLC (Sil, $CHCl_3$/MeOH 95:5): R_f 0.14. UV (MeOH): 296 (sh, 4.10), 268 (sh, 4.10), 259 (4.17), 234 (sh, 4.28). ^{1}H-NMR ((D$_6$)DMSO)): 8.20, 7.98 (2*s*, H–C(2), H–C(8)); 7.39–7.17 (*m*, arom. H); 7.04 (*m*, NH); 6.84, 6.81 (*d*, 2 H *o* to MeO); 5.81 (*s*, H–C(1′)); 5.86 (*d*, OH–C(2′)); 5.23 (*s*, CH_2); 4.64 (*m*, H–C(2′)); 4.47 (*m*, H–C(4′)); 3.70 (*s*, MeO); 3.48 (*m*, 2 H–C(5′)); 2.48, 1.93 (2*m*, 2 H–C(3′)). Anal. calc. for $C_{37}H_{35}N_5O_4 \cdot H_2O$ (613.7): C 70.34, H 5.90, N 11.08; found: C 69.99, H 5.88, N 10.86.

Data of **7**: Colorless foam. TLC (Sil, $CHCl_3$/MeOH 85:15): R_f 0.25. UV (MeOH): 268 (4.29), 233 (4.22), 206 (4.76). ^{1}H-NMR ($CDCl_3$): 8.53 (*m*, NH); 8.29 (*s*, *m*, H–C(8)); 7.96 (*s*, H–C(2)); 7.37–7.12 (*m*, arom. H); 6.78 (*d*, 2 H *o* to MeO); 5.79 (*d*, H–C(1′)); 5.43 (*d*, OH–C(3′)); 4.78 (br. *s*, CH_2); 4.73 (*m*, H–C(2′)); 4.58 (*m*, H–C(4′)); 3.68 (*s*, MeO); 3.29, 3.17 (2*m*, 2 H–C(5′)); 2.18–2.11 (2*m*, 2 H–C(3′)). Anal. calc. for $C_{37}H_{35}N_5O_4$ (613.7): C 72.41, H 5.74, N 11.41; found: C 71.94, H 5.90, N 11.01.

4. N^6*-Benzyl-2′,3′-bis-*O*-[(*tert*-butyl)dimethylsilyl]-5′-*O*-(monomethoxytrityl)adenosine* (**9**), N^6*-Benzyl-2′-*O*-[(*tert*-butyl)dimethylsilyl]-5′-*O*-(monomethoxytrityl)adenosine* (**10**), *and* N^6*-Benzyl-3′-*O*-[(*tert*-butyl)dimethylsilyl)]-5′-*O*-(monomethoxytrityl)adenosine* (**11**). Compound **8** [15] (1.89 g, 3 mmol) was co-evaporated with anh. pyridine, then dissolved in pyridine (15 ml), and treated with 1*H*-imidazole (0.48 g, 7.09 mmol) and (*tert*-butyl)chlorodimethylsilane (0.542 g, 3.6 mmol). After stirring at r.t. for 20 h, MeOH (2 ml) was added and the mixture concentrated to 1/4 of its volume. The mixture was taken up in CH_2Cl_2 (50 ml) and washed with H_2O (2 × 25 ml), the org. phase dried and evaporated, and the residue co-evaporated with toluene (2 × 10 ml) and purified by CC (silica gel, 20 × 2.5 cm). Elution with toluene/AcOEt 9:1, 8:2, and 7:3 gave 0.42 g (16%) of **9**, elution with toluene/AcOEt 65:35 gave 0.67 g (30%) of **10**, and elution with toluene/AcOEt 3:2 gave 0.94 g (42%) of **11** as colorless foams.

Data of **9**: TLC (Sil, toluene/AcOEt 1:1): R_f 0.79. UV (MeOH): 268 (4.34), 232 (sh, 4.25), 203 (4.87). ^{1}H-NMR ($CDCl_3$): 8.30 (*s*, H–C(2)); 8.00 (*d*, H–C(8)); 7.40–7.10 (*m*, 12 arom. H); 6.83 (*d*, 2 H *o* to MeO); 6.00 (*t*, N*H*bn); 5.96 (*d*, H–C(1′)); 4.85 (*m*, CH_2, H–C(2′)); 4.25 (*m*, H–C(3′), H–C(4′)); 3.78 (*s*, MeO); 3.52–3.31 (*m*, 2 H–C(5′)); 0.85, 0.78 (2*s*, 2 ^{t}Bu); 0.04, –0.02, –0.06, –0.26 (4*s*, 2 Me_2Si). Anal. calc. for $C_{49}H_{43}N_5O_5Si_2$ (792.1): C 68.57, H 7.39, N 8.16; found: C 68.64, H 7.19, N 8.06.

Data of **10**: TLC (Sil, toluene/AcOEt 1:1): R_f 0.62. UV (MeOH): 266 (4.31), 233 (sh, 4.24), 205 (4.80). ^{1}H-NMR ($CDCl_3$): 8.32 (*m*, *s*, NH, H–C(2)); 7.94 (*d*, H–C(8)); 7.47–7.21 (*m*, arom. H); 6.81 (*d*, 2 H *o* to MeO); 6.02 (*t*, N*H*bn); 6.00 (*d*, H–C(1′)); 5.00 (*m*, H–C(2′)); 4.90 (*m*, CH_2); 4.35 (*m*, H–C(3′)); 4.25 (*m*, H–C(4′)); 3.78 (*s*, MeO); 3.51–3.40 (2*m*, 2 H–C(5′)); 2.71 (*d*, OH–C(3′)); 0.84 (*s*, ^{t}Bu); –0.12, –0.07 (2*s*, Me_2Si). Anal. calc. for $C_{43}H_{49}N_5O_5Si$ (744.0): C 69.42, H 6.63, N 9.41; found: C 69.68, H 6.67, N 8.94.

Data of **11**: TLC (Sil, toluene/AcOEt 1:1): R_f 0.43. UV (MeOH): 268 (4.32), 232 (sh, 4.23), 203 (4.85). ^{1}H-NMR ($CDCl_3$): 8.36 (*m*, *s*, NH, H–C(2)); 7.97 (*d*, H–C(8)); 7.54–7.19 (*m*, arom. H); 6.78 (*d*, 2 H *o* to MeO); 6.41 (*t*, N*H*bn); 6.00 (*d*, H–C(1′)); 4.84 (*m*, CH_2); 4.35 (*m*, H–C(3′)); 4.59 (*m*, H–C(2′)); 4.19 (*m*, H–C(4′)); 3.76 (*s*, MeO); 3.47–3.21 (2*m*, OH–C(3′), 2 H–C(5′)); 0.88 (*s*, ^{t}Bu); 0.085, 0.06 (2*s*, Me_2Si). Anal. calc. for $C_{43}H_{49}N_5O_5Si$ (744.0): C 69.42, H 6.63, N 9.41; found: C 69.37, H 6.54, N 9.17.

5. N^6-*Benzyl-3′-deoxy-5′-*O-*(monomethoxytrityl)-2′-adenylic Acid 2,5-Dichlorophenyl 2-(4-Nitrophenyl)ethyl Ester* (**12**). A soln. of 1*H*-1,2,4-triazole (0.31 g, 0.45 mmol) and 2,5-dichlorophenyl phosphorodichloridate (0.57 g, 2.13 mmol) in anh. pyridine (4 ml) was stirred in an ice-bath for 30 min. To this mixture, **7** (0.61 g, 1 mmol), which was previously co-evaporated with pyridine and dissolved in anh. pyridine (4 ml), was added dropwise. After 30 min stirring (TLC control), 2-(4-nitrophenyl)ethanol (0.47 g, 2.82 mmol) was added and stirring continued at r.t. for 24 h. After dilution with CH_2Cl_2 (150 ml), the mixture was washed with H_2O (2 × 50 ml), dried (Na_2SO_4) and evaporated, and the residue co-evaporated with toluene (2 × 50 ml) to remove pyridine and purified by CC (silica gel, 15 × 3 cm, $CHCl_3$, $CHCl_3$/MeOH 25:1): 0.79 g (80%) of **12**. Colorless foam. TLC (Sil, Et_2O): R_f 0.20. UV (MeOH): 267 (4.49), 229 (4.46). ^{1}H-NMR ($CDCl_3$): 8.13–8.07 (*m*, H–C(2), 2 H *o* to NO_2); 7.40–7.08 (*m*, arom. H, 2 H *m* to NO_2, H–C(8)); 6.81 (*d*, 2 H *o* to MeO); 6.20 (*d*, H–C(1′)); 4.80 (*m*, H–C(2′)); 4.54 (*t*, *m*, CH_2 (npe), CH_2(bn)); 3.78 (*s*, MeO); 3.60 (2*m*, 2 H–C(5′)); 3.13 (*m*, CH_2 (npe)); 2.52–2.30 (2*m*, 2 H–C(3′)). Anal. calc. for $C_{51}H_{45}Cl_2N_6O_9P$ (987.8): C 62.01, H 4.59, N 8.50; found: C 62.07, H 4.57, N 8.20.

6. N^6-*Benzyl-3′-deoxy-5′-*O-*(monomethoxytrityl)-2′-adenylic Acid 2-(4-Nitrophenyl)ethyl Ester Triethylammonium salt* (**13**). A soln. of 4-nitrobenzaldehyde oxime (1.67 g, 10 mmol) in Et_3N/dioxane/H_2O 1:1:1 (60 ml) was stirred at r.t. for 30 min. Then **12** (0.79 g, 0.8 mmol) was added and stirred for 3 h at r.t. Pyridine (10 ml) was added and evaporated and the residue co-evaporated with pyridine (2 × 10 ml) and toluene (2 × 10 ml) and purified by CC (silica gel, 10 × 3 cm, $CHCl_3$, $CHCl_3$/MeOH 25:1, and $CHCl_3$/MeOH/Et_3N 100:3:3): 0.70 g (92%) of **13**. Colorless foam. TLC (Sil, $CHCl_3$/MeOH/Et_3N 95:4:4): R_f 0.49. UV (MeOH): 267 (4.45), 233 (4.21), 203 (4.91). ^{1}H-NMR ($CDCl_3$): 12.31 (*m*, NH); 8.08, 8.04 (2*s*, H–C(8), H–C(2)); 7.42–7.19 (*m*, 19 arom. H); 6.78 (*d*, 2 H *o* to MeO); 6.24 (*m*, H–C(1′)); 5.13 (*d*, H–C(2′)); 4.78 (br. *s*, CH_2); 4.58 (*m*, H–C(4′)); 4.18 (*t*, C*H*$_2$CH$_2$ (npe)); 3.76 (*s*, *m*, MeO, 1 H–C(5′)); 3.50 (*m*, 1 H–C(5′)); 3.34 (*t*, CH$_2$C*H*$_2$ (npe)); 3.05 (*m*, Et_3N); 2.34, 1.92 (2*m*, 2 H–C(3′)); 1.32 (*t*, 9 H, Et_3N). Anal. calc. for $C_{51}H_{58}N_7O_9P \cdot 2\,H_2O$ (980.2): C 62.49, H 6.37, N 10.00; found: C 62.15, H 6.39, N 9.43.

7. N^6-*Benzoyl-3′-deoxy-5′-*O-*(4,4′-dimethoxytrityl)adenosine* (**14**). In a one-pot procedure: Compound **1** (2.51 g, 10 mmol) was co-evaporated with anh. pyridine (2 × 10 ml), taken up in pyridine (50 ml), and treated with 4,4′-dimethoxytrityl chloride (5.08 g, 15 mmol) and *Hünig*'s base (1.29 g, 10 mmol). After stirring at r.t. for 3 h, trimethyl chloro silane (6.4 ml, 50 mmol) was added, and, after another 30 min stirring at r.t., the resulting pyridinium chloride was filtered off and benzoyl chloride (5.8 ml, 50 mmol) added to the mixture, which was stirred for further 16 h at r.t. Again, pyridinium chloride was removed by filtration, and the filtrate was cooled in an ice-bath (0°) before 25% ammonia (50 ml) was added. The mixture was brought to r.t., stirred for 30 min, and evaporated. The crude product was extracted with $CHCl_3$ (250 ml), the extract washed with sat. NaCl soln. (2 × 100 ml), dried (Na_2SO_4), and evaporated, and the residue purified by FC (silica gel, 17.5 × 2.5 cm, toluol/AcOEt 2:1 and 1:1, toluene/AcOEt/MeOH 49:49:2, 48.5:48.5:3, and 48:48:4). The product was dried *in vacuo:* 4.5 g (69%) of **14**. TLC (Sil, $CHCl_3$/MeOH 95:5): R_f 0.57. UV (MeOH): 280 (4.33), 233 (4.51). ^{1}H-NMR ($CDCl_3$): 9.10 (*m*, NH); 8.77–8.29 (4*s*, H–C(8), H–C(2)); 8.00 (*d*, 2 H *o* to CO); 7.65–7.48 (*m*, 3 H *m* and *p* to CO); 7.35–7.10 (*m*, arom. H); 6.77 (*d*, 4 H *o* to MeO); 5.99 (*s*, H–C(1′)); 4.95 (*m*, H–C(2′), OH–C(2′)); 4.69 (*m*, H–C(4′)); 3.76 (*s*, 2 MeO); 3.42–3.25 (*m*, 2 H–C(5′)); 2.25 (*m*, 2 H–C(3′)). Anal. calc. for $C_{38}H_{35}N_5O_6$ (657.7): C 69.89, H 5.36, N 10.65; found: C 69.84, H 5.49, N 10.20.

8. N^6-*Benzoyl-3′-deoxy-5′-*O-*(4,4′-dimethoxytrityl)adenosine 2′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**16**) [26]. Compound **14** (3.24 g, 4.93 mmol) was co-evaporated with toluene (2 × 10 ml) and then dissolved in anh. MeCN (10 ml) under Ar. After addition of 1*H*-tetrazole (0.175 g, 5 mmol) and 2-cyanoethyl tetraisopropylphosphoradiamidite [28–31] (1.81 g, 6 mmol), the mixture was stirred at r.t. for 2 h, then diluted with $CHCl_3$ (200 ml) and washed with sat. $NaHCO_3$ soln. (2 × 80 ml), dried (Na_2SO_4), and evaporated. The residue was dissolved in a small amount of $CHCl_3$ and precipitated with hexane to give, after drying *in vacuo*, 3.865 g (90%) of **16**. Colorless powder. TLC (toluene/AcOEt 1:1): R_f 0.51, 0.45. UV (MeOH): 280 (4.32), 234

(4.49). ^{1}H-NMR ($CDCl_3$): 9.00 (*m*, NH); 8.77–8.29 (4*s*, H–C(8), H–C(2)); 8.00 (*d*, 2 H *o* to CO); 7.65–7.15 (*m*, arom. H); 6.81 (*d*, 4 H *o* to MeO); 6.29, 6.22 (2*s*, H–C(1′)); 5.03 (*m*, H–C(2′)); 5.23 (*m*, H–C(2′)); 4.67 (*m*, H–C(4′)); 3.78 (*s*, 2 MeO); 3.90–3.30 (*m*, 2 Me_2CH, CH_2CH_2CN, 2 H–C(5′)); 2.64 (*t*, CH_2CH_2CN); 2.50–2.05 (2*m*, 2 H–C(3′)); 1.30–1.10 (*m*, 2 Me_2CH). ^{31}P-NMR ($CDCl_3$): 150.75; 149.88. Anal. calc. for $C_{47}H_{52}N_7O_7P$ (857.9): C 65.80, H 6.11, N 11.43; found: C 65.81, H 6.10, N 11.06.

9. N^6,N^6-*Dibenzoyl-9-[2-(hydroxyethoxy)methyl]adenine* (= N-*Benzoyl*-N-*{9-[(2-hydroxyethoxy)methyl]-9*H-*purin-6-yl}benzamide*; **19**). A soln. of 9-{[2-[(4,4′-dimethoxytrityl)oxy]ethoxy}methyl}adenine (**18**) [32] (0.58 g, 1.14 mmol) was dissolved in anh. pyridine (10 ml) and treated with benzoyl chloride (0.42 g, 3 mmol). After stirring at r.t. for 20 h, the mixture was poured onto ice and extracted with CH_2Cl_2 (100 ml), the extract washed with H_2O (2 × 50 ml), dried (Na_2SO_4), and evaporated and the residue co-evaporated with toluene (2 × 10 ml). The residue was then treated with 80% AcOH (10 ml), and, after 30 min stirring, evaporated and co-evaporated with MeOH (3 × 10 ml). The residue was purified by CC (silica gel, 10 × 2 cm, $CHCl_3$, $CHCl_3$/MeOH 25:1): 0.43 g (90%) of **19**. Colorless foam. TLC (Sil, $CHCl_3$/MeOH 9:1). R_f 0.38. UV (MeOH): 274 (4.24), 246 (4.38). ^{1}H-NMR ((D$_6$)DMSO)): 8.75 (*s*, H–C(8)); 8.71 (*s*, H–C(2)); 7.79 (*m*, 4 H *o* to CO); 7.59–7.42 (2*m*, arom. H); 5.69 (*s*, OCH_2N); 4.70 (*t*, OH–C(5′)); 3.55 (*m*, CH_2O); 3.47 (*m*, CH_2). Anal. calc. for $C_{22}H_{19}N_5O_4 \cdot 0.5\ H_2O$ (426.4): C 61.96, H 4.72, N 16.42; found: C 62.16, H 4.54, N 16.17.

10. N^6-*Benzoyl-3′-deoxy-5′*-O-*(4,4′-dimethoxytrityl)adenylyl-{2′-[O^P-(2-cyanoethyl)] → 2″}*-N^6-*benzoyl-9-[(2″-hydroxyethoxy)methyl]adenine* (= N^6-*Benzoyl-3′-deoxy-5′*-O-*(4,4′-dimethoxytrityl)-2′-adenylic Acid 2-{[6-(Benzoylamino)-9*H-*purin-9-yl]methoxy}ethyl] 2-Cyanoethyl Ester*; **21**). A soln. of **16** (0.53 g, 0.57 mmol) and *N*^{6}-benzoyl-9-[2-(hydroxyethoxy)methyl]adenine (= *N*-{9-[(2-hydroxyethoxy)methyl]-9*H*-purin-6-yl}benzamide; **20**) [17][33] (0.119 g, 0.4 mmol) and 1*H*-tetrazole (0.08 g, 1.15 mmol) in anh. MeCN (2 ml) was stirred for 3 h under Ar. Then, I_2 in CH_2Cl_2/pyridine/H_2O 1:5:1 was added until the brown color persisted. After stirring at r.t. for 30 min, the mixture was diluted with CH_2Cl_2 (50 ml), washed with $Na_2S_2O_3$/NaCl soln. (2 × 25 ml), dried (Na_2SO_4) and evaporated. The residue was co-evaporated with toluene (2 × 20 ml) to remove pyridine and purified by CC (silica gel, 10 × 2 cm, $CHCl_3$, $CHCl_3$/MeOH 25:1): 0.42 g (91%) of **21**. Colorless foam. TLC (Sil, $CHCl_3$/MeOH 9:1): R_f 0.50. UV (MeOH): 278 (4.41), 232 (4.46). ^{1}H-NMR ($CDCl_3$): 8.94 (*s*, NH); 8.77–8.19 (4*s*, H–C(8), H–C(2)); 8.00 (*m*, 4 H *o* to CO); 7.65–7.19 (*m*, arom. H); 6.79 (*d*, 4 H *o* to MeO); 6.30 (*s*, H–C(1′)); 5.69 (*s*, OCH_2N); 5.53 (*t*, H–C(2′)); 4.66 (*m*, H–C(4′)); 4.32 (*m*, CH_2CH_2CN, CH_2O); 3.78 (*s*, 2 MeO); 3.44 (2*m*, 2 H–C(5′)); 2.79 (*m*, CH_2CH_2CN); 2.57–2.37 (2*m*, 2 H–C(3′)). Anal. calc. for $C_{56}H_{52}N_{11}O_{11}P \cdot 2\ H_2O$ (1122.1): C 59.94, H 5.03, N 13.73; found: C 60.26, H 4.79, N 13.28.

11. N^6-*Benzoyl-3′-deoxyadenylyl-{2′-[O^P-(2-cyanoethyl)] → 2″}*-N^6-*benzoyl-9-[(2″-hydroxyethoxy)methyl]adenine* (= N^6-*Benzoyl-3′-deoxy-2′-adenylic Acid 2-{[6-(Benzoylamino)-9*H-*purin-9-yl]methoxy}ethyl 2-Cyanoethyl Ester*; **22**). A soln. of **21** (0.38 g, 0.33 mmol) was stirred with 2% TsOH in CH_2Cl_2/MeOH 4:1 (7 ml) at r.t. for 45 min. The mixture was diluted with CH_2Cl_2 (50 ml), washed with H_2O (2 × 20 ml), dried (Na_2SO_4), and evaporated. The crude product was purified by CC (silica gel, 10 × 2 cm, $CHCl_3$, $CHCl_3$/MeOH 10:1): 0.21 g (79%) of **22**. Colorless foam. TLC (Sil, $CHCl_3$/MeOH 9:1): R_f 0.30. UV (MeOH): 278 (4.54), 229 (4.37). ^{1}H-NMR ($CDCl_3$): 8.71 (4*s*, diastereoisomers, H–C(8), H–C(2)); 8.55 (*s*, NH)); 8.31 (*s*, NH); 8.00 (2*d*, *m*, H–C(2), H–C(8), 4 H *o* to CO); 7.52–7.43 (*m*, arom. H); 6.15 (*s*, H–C(1′)); 5.65 (*m*, OH–C(5′), OCH_2N); 5.40 (*t*, H–C(2′)); 4.54 (*t*, H–C(4′)); 4.15 (*m*, CH_2CH_2CN, CH_2O); 3.98–3.65 (3*m*, OCH_2, 2 H–C(5′)); 2.71 (*m*, 1 H–C(3′), CH_2CH_2CN); 2.66 (*m*, 1 H–C(3′)).

12. *3′-Deoxy-5′*-O-*(monomethoxytrityl)*-N^6-*{[2-(4-nitrophenyl)ethoxy]carbonyl}adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]} → 2″-}*-N^6,N^6-*dibenzoyl-9-[(2″-hydroxyethoxy)methyl]adenine* (= *3′-Deoxy-5′*-O-*(monomethoxytrityl)*-N^6-*{[2-(4-nitrophenyl)ethoxy]carbonyl}-2′-adenylic Acid 2-{[6-(Dibenzoylamino)-9*H-*purin-9-yl]methoxy}ethyl 2-(4-Nitrophenyl)ethyl Ester*; **23**). As described for **21**, with 3′-deoxy-5′-*O*-(monomethoxytrityl)-*N*^{6}-{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine 2′-[2-(4-nitrophenyl)ethyl diisopropylphosphoramidite] (**17**) [27] (0.405 g, 0.4 mmol), **19** (0.089 g, 0.3 mmol), 1*H*-tetrazole (0.14 g, 2 mmol), and MeCN (0.5 ml) (workup with CH_2Cl_2 (50 ml) and H_2O (2 × 25 ml). CC (silica gel, 15 × 3 cm, $CHCl_3$, $CHCl_3$/MeOH 50:1 gave 0.35 g (86%) of **23**. Colorless foam. TLC (Sil, $CHCl_3$/MeOH 9:1): R_f 0.64. UV (MeOH): 276 (sh, 4.67), 266 (4.76), 254 (sh, 4.70), 238 (4.70). Anal. calc. for $C_{69}H_{61}N_{12}O_{16}P \cdot 2\ H_2O$ (1381.4): C 59.99, H 4.74, N 12.16; found: C 59.39, H 4.39, N 11.79.

13. *3′-Deoxy*-N^6-*{[2-(4-nitrophenyl)ethoxy]carbonyl}adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]} → 2″}*-N^6,N^6-*dibenzoyl-9-[(2″-hydroxyethoxy)methyl]adenine* (= *3′-Deoxy*-N^6-*{[2-(4-nitrophenyl)ethoxy]carbonyl}-2′-adenylic Acid 2-{[6-(Dibenzoylamino)-9*H-*purin-9-yl]methoxy}ethyl 2-(4-Nitrophenyl)ethyl Ester*; **24**). As described for **22**, with **23** (0.4 g, 0.30 mmol) and 2% TsOH in CH_2Cl_2/MeOH 4:1 (10 ml). CC (silica gel, 10 × 2.5 cm, CH_2Cl_2, CH_2Cl_2/MeOH 25:1) gave 0.28 g (85%) of **24**. Colorless foam. TLC (Sil, $CHCl_3$/MeOH 9:1): R_f 0.28. UV (MeOH): 276 (4.64), 266 (4.71), 250 (sh, 4.64). ^{1}H-NMR ($CDCl_3$): 8.72, 8.66 (2*s*, H–C(8),

H–C(2)); 8.50 (*m*, NH); 8.28–8.10 (*m*, H–C(2), H–C(8), 4 H *o* to NO_2); 7.83 (*d*, 2 H *o* to CO); 7.51–7.26 (*m*, 14 arom. H); 6.05 (*t*, H–C(1′)); 5.66 (*m*, OH–C(5′), OCH_2N); 5.41 (*m*, H–C(2′)); 4.50 (*m*, CH_2(npeoc), H–C(4′)); 4.20 (*m*, 6 H, CH_2(npe), H–C(5′)); 3.70 (2*m*, 4 H, CH_2); 3.13 (*t*, CH_2(npeoc)); 3.00 (*m*, CH_2(npe)); 2.60–2.25 (2*m*, 2 H–C(3′)). Anal. calc. for $C_{49}H_{46}N_{12}O_{15}P \cdot 2H_2O$ (1091.9): C 53.02, H 4.54, N 15.39; found: C 52.86, H 4.08, N 14.82.

14. N^6-*Benzoyl-3′-deoxy-5′*-O-*(4,4′-dimethoxytrityl)adenylyl-{2′-[O^P-(2-cyanoethyl)]→5′}*-N^6-*benzoyl-3′-deoxyadenylyl-{2′-[O^P-(2-cyanoethyl)]→2′′}*-N^6-*benzoyl-9-[(2′′-hydroxyethoxy)methyl]adenine* (= N^6-*Benzoyl*-O^P-*(2-cyanoethyl)-3′-deoxy-5′*-O-*(4,4′-dimethoxytrityl)adenylyl-(2′→5′)*-N^6-*benzoyl-3′-deoxy-2′-adenylic Acid 2-{[6-(Benzoylamino)-9*H*-purin-9-yl]methoxy}ethyl 2-Cyanoethyl Ester*; **25**). As described for **21**, with **16** (0.37 g, 0.40 mmol), **22** (0.20 g, 0.23 mmol), 1*H*-tetrazole (0.056 g, 0.8 mmol), and MeCN (3 ml). CC (silica gel, 10 × 2 cm, $CHCl_3$, $CHCl_3$/MeOH 9:1) gave 0.38 g (82%) of **25**. Colorless foam. TLC (Sil, $CHCl_3$/MeOH 9:1): R_f 0.35. UV (MeOH): 278 (4.65), 231 (4.65). ^{1}H-NMR ($CDCl_3$): 9.10, 8.98, 8.76 (3*s*, NH); 8.77, 8.29 (2*m*, H–C(8), H–C(2)); 7.98 (*m*, H *o* to CO); 7.65–7.16 (*m*, arom. H); 6.79 (*dd*, 2 H *o* to MeO); 6.20 (2*m*, H–C(1′)); 5.69 (2*t*, OCH_2N, H–C(2′)); 4.66 (*m*, H–C(4′)); 4.32 (*m*, CH_2CH_2CN, CH_2O); 3.82 (*m*, CH_2O); 3.78 (2*s*, MeO); 3.45 (2*m*, 2 H–C(5′)); 2.76 (*t*, CH_2CH_2CN); 2.43 (2*m*, 2 H–C(3′)).

15. N^6-*Benzoyl-3′-deoxyadenylyl-{2′-[O^P-(2-cyanoethyl)]→5′}*-N^6-*benzoyl-3′-deoxyadenylyl-{2′-[O^P-(2-cyanoethyl)]→2′′}*-N^6-*benzoyl-9-[(2′′-hydroxyethoxy)methyl]adenine* (= N^6-*Benzoyl*-O^P-*(2-cyanoethyl)-3′-deoxyadenylyl-(2′→5′)*-N^6-*benzoyl-3′-deoxy-2′-adenylic Acid 2-{[6-(Benzoylamino)-9*H*-purin-9-yl]methoxy}ethyl 2-Cyanoethyl Ester*; **26**). A soln. of **25** (0.31 g, 0.19 mmol) in 80% AcOH (5 ml) was stirred at r.t. for 30 min. Then the solvent was removed and the residue co-evaporated with MeOH (4 × 10 ml). The residue was treated with Et_2O (25 ml) to remove dimethoxytrityl alcohol, and, after decanting the Et_2O phase, the residue was dried *in vacuo:* 0.2 g (79%) of **26**. TLC (Sil, $CHCl_3$/MeOH 9:1): R_f 0.31.

16. *3′-Deoxy-5′*-O-*(monomethoxytrityl)*-N^6-*{[2-(4-nitrophenyl)ethoxy]carbonyl}adenylyl-{2′-[O^P-[2-(4-nitrophenyl)ethyl]]}→5′}-3′-deoxy*-N^6-*{[2-(4-nitrophenyl)ethoxy]carbonyl}adenylyl-{2′-[O^P-[2-(4-nitrophenyl)-ethyl]]}→2′′}*-N^6,N^6-*dibenzoyl-9-[(2′′-hydroxyethoxy)methyl]adenine* (= *3′-Deoxy-5′*-O-*(monomethoxytrityl)*-N^6-*{[2-(4-nitrophenyl)ethoxy]carbonyl}*-O^P-*[2-(4-nitrophenyl)ethyl]adenylyl-(2′→5′)-3′-deoxy*-N^6-*{[2-(4-nitrophenyl)ethoxy]carbonyl}-2′-adenylic Acid 2-{[6-(Dibenzoylamino)-9*H*-purin-9-yl]methoxy}ethyl 2-(4-Nitrophenyl)ethyl Ester*; **27**). As described for **21**, with **17** [27] (0.405 g, 0.4 mmol), **24** (0.089 g, 0.3 mmol), 1*H*-tetrazole (0.14 g, 2 mmol), and MeCN (0.5 ml) (workup with CH_2Cl_2 (50 ml) and H_2O (2 × 25 ml). CC (silica gel, 15 × 3 cm, $CHCl_3$, $CHCl_3$/MeOH 50:1) gave 0.35 g (86%) of **27**. Colorless foam. TLC (Sil, $CHCl_3$/MeOH 9:1): R_f 0.64.

17. *3′-Deoxy*-N^6-*{[2-(4-nitrophenyl)ethoxy]carbonyl}adenylyl-{2′-[O^P-[2-(4-nitrophenyl)ethyl]]}→5′}-3′-deoxy*-N^6-*{[2-(4-nitrophenyl)ethoxy]carbonyl}adenylyl-{2′-[O^P-[2-(4-nitrophenyl)ethyl]]}→2′′}*-N^6,N^6-*dibenzoyl-9-[(2′′-hydroxyethoxy)methyl]adenine* (= *3′-Deoxy*-N^6-*{[2-(4-nitrophenyl)ethoxy]carbonyl}*-O^P-*[2-(4-nitrophenyl)ethyl]adenylyl-(2′→5′)-3′-deoxy*-N^6-*{[2-(4-nitrophenyl)ethoxy]carbonyl}-2′-adenylic Acid 2-{[6-(Dibenzoylamino)-9*H*-purin-9-yl]methoxy}ethyl 2-(4-Nitrophenyl)ethyl Ester*; **28**). As described for **22**, with **27** (0.4 g, 0.30 mmol) and 2% TsOH in CH_2Cl_2/MeOH 4:1 (10 ml). CC (silica gel, 10 × 2.5 cm, CH_2Cl_2, CH_2Cl_2/MeOH 25:1) gave 0.28 g (85%) of **28**. Colorless foam. TLC (Sil, $CHCl_3$/MeOH 9:1): R_f 0.45. UV (MeOH): 266 (4.79), 276 (sh, 4.71).

18. N^6-*Benzoyl-3′-deoxy-5′*-O-*(4,4′-dimethoxytrityl)adenylyl-{2′-[O^P-(2-cyanoethyl)]→5′}*-N^6-*benzoyl-3′-deoxyadenylyl-{2′-[O^P-(2-cyanoethyl)]→5′}*-N^6-*benzoyl-3′-deoxyadenylyl-{2′-[O^P-(2-cyanoethyl)]→2′′}*-N^6-*benzoyl-9-[(2′′-hydroxyethoxy)methyl]adenine* (= N^6-*Benzoyl*-O^P-*(2-cyanoethyl)-3′-deoxy-5′*-O-*(4,4′-dimethoxytrityl)adenylyl-(2′→5′)*-N^6-*benzoyl*-O^P-*(2-cyanoethyl)-3′-deoxyadenylyl-(2′→5′)*-N^6-*benzoyl-3-deoxy-2′-adenylic Acid 2-{[6-(Benzoylamino)-9*H*-purin-9-yl]methoxy}ethyl 2-Cyanoethyl Ester*; **29**. As described for **21**, with **16** (0.18 g, 0.20 mmol), **26** (0.12 g, 0.1 mmol), 1*H*-tetrazole (0.0528 g, 0.4 mmol), and MeCN (2 ml). CC (silica gel, 10 × 2 cm, $CHCl_3$, $CHCl_3$/MeOH 50:1.5) gave 0.16 (83%) of **29**). Colorless foam. TLC (Sil, $CHCl_3$/MeOH 9:1): R_f 0.40. UV (MeOH): 279 (4.83), 230 (4.82).

19. *1-Benzyl-3′-deoxy-5′*-O-*(monomethoxytrityl)adenylyl-{2′-[O^P-(2,5-dichlorophenyl)]→5′}*-N^6-*benzoyl-3′-deoxyadenylyl-{2′-[O^P-(2-cyanoethyl)]→5′}*-N^6-*benzoyl-3′-deoxyadenylyl-{2′-[O^P-(2-cyanoethyl)]→2′′}*-N^6-*benzoyl-9-[(2′′-hydroxyethoxy)methyl]adenine* (= *1-Benzyl-3′-deoxy*-O^P-*(2,5-dichlorophenyl)-5′*-O-*(monomethoxytrityl)adenylyl-(2′→5′)*-N^6-*benzoyl*-O^P-*(2-cyanoethyl)-3′-deoxyadenylyl-(2′→5′)*-N^6-*benzoyl-3′-deoxy-2′-adenylic Acid 2-{[6-(Benzoylamino)-9*H*-purin-9-yl]methoxy}ethyl 2-Cyanoethyl Ester*; **30**). To an ice-cold soln. of 2,5-dichlorophenyl phosphorodichloridate (0.113 g, 0.4 mmol) in anh. pyridine (1.5 ml), 1*H*-1,2,4-triazole (0.054 g, 0.78 mmol) was added. After stirring for 30 min, **6**, which was previously co-evaporated with anh. pyridine (3 × 5 ml) and dissolved in pyridine (1.5 ml), was added dropwise. Stirring was continued for 30 min at r.t. (TLC: complete conversion). The mixture was then cooled again in an ice-bath and treated with

90% aq. pyridine (0.35 ml) for 15 min. The soln. was diluted with CH_2Cl_2 (25 ml), washed with H_2O (2 × 20 ml), dried (Na_2SO_4), and evaporated. To the obtained diester salt **33**, **26** (0.12 g, 0.096 mmol) was added. This mixture was co-evaporated with anh. pyridine (2 × 5 ml), then dissolved in pyridine (1.5 ml), and treated with 2,4,6-triisopropylbenzene sufonyl chloride (0.15 g, 0.5 mmol) and 1*H*-tetrazole (0.11 g, 1.5 mmol). After stirring at r.t. for 2 h, the mixture was diluted with CH_2Cl_2 (25 ml), washed with H_2O (2 × 20 ml), dried (Na_2SO_4), and evaporated. The residue was co-evaporated with toluene (2 × 10 ml) to remove pyridine and purified by CC (silica gel, 10 × 2 cm, CH_2Cl_2/MeOH 90:5): 0.16 g (80%) of **30**. Colorless foam. TLC (Sil, toluene/AcOEt/MeOH 5:4:1): R_f 0.20.

20. N^6-*Benzyl-3′-deoxy-5′*-O-*(monomethoxytrityl)adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]} → 5′}-3′-deoxy*-N^6-*{2-(4-nitrophenyl)ethoxy]carbonyl}adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]} → 5′}-3′-deoxy*-N^6-*{[2-(4-nitrophenyl)ethoxy]carbonyl]adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]} → 2″}*-N^6,N^6-*dibenzoyl-9-[(2″-hydroxyethoxy)methyl]adenine* (= N^6-*Benzoyl-3′-deoxy-5′*-O-*(monomethoxytrityl)*-O^P-*[2-(4-nitrophenyl)ethyl]adenylyl-(2′ → 5′)-3′-deoxy*-N^6-*{[2-(4-nitrophenyl)ethoxy]carbonyl}*-O^P-*[2-(4-nitrophenyl)ethyl]adenylyl-(2′ → 5′)-3′-deoxy*-N^6-*{[2-(4-nitrophenyl)ethoxy]carbonyl}-2′-adenylic Acid 2-{[6-(Dibenzoylamino)-9*H*-purin-9-yl]-methoxy}ethyl 2-(4-Nitrophenyl)ethyl Ester*; **31**). A mixture of the phosphodiester salt **13** (0.094 g, 0.1 mmol) and **28** (0.075 g, 0.04 mmol) was co-evaporated twice with anh. pyridine and then dissolved in pyridine (0.8 ml). After addition of 1*H*-tetrazole (0.042 g, 0.6 mmol) and 2,4,6-triisopropylbenzenesulfonyl chloride (0.061 g, 0.2 mmol), the mixture was stirred at r.t. for 24 h. The mixture was diluted with CH_2Cl_2 (50 ml), washed with H_2O (2 × 20 ml), dried (Na_2SO_4), and evaporated. The residue was co-evaporated twice with toluene, purified by CC (silica gel, 10 × 2.5 cm, $CHCl_3$, $CHCl_3$/MeOH 25:1), and dried *in vacuo:* 0.09 g (88%) of **31**. Colorless foam. TLC (Sil, $CHCl_3$/MeOH 9:1): R_f 0.63. UV (MeOH): 276 (sh, 4.84), 266 (4.91).

21. N^6-*Benzyl-3′*-O-*[(*tert*-butyl)dimethylsilyl]-5′*-O-*(monomethoxytrityl)adenylyl-{2′-[*O^P*-(2,5-dichlorophenyl)] → 5′}-3′*-O-*[(*tert*-butyl)dimethylsilyl]*-N^6-*{[2-(4-nitrophenyl)ethoxy]carbonyl}adenylyl-{2′-{*O^P*-[2-(4-nitrophenylethyl]} → 5′}-3′*-O-*[(*tert*-butyl)dimethylsilyl]*-N^6-*{[2-(4-nitrophenyl)ethoxy]carbonyl]adenylyl-{2′-{*O^P*-[2-(4-nitrophenyl)ethyl]} → 2″}*-N^6-*benzoyl-9-[(2″-hydroxyethoxy)methyl]adenine* (= N^6-*Benzyl-3′*-O-*[(*tert*-butyl)dimethylsilyl]*-O^P-*(2,5-dichlorophenyl)-5′*-O-*(monomethoxytrityl)adenylyl-(2′ → 5′)-3′*-O-*[(*tert*-butyl)dimethylsilyl]*-N^6-*{[2-(4-nitrophenyl)ethoxy]carbonyl}*-O^P-*[2-(4-nitrophenyl)ethyl]adenylyl-(2′ → 5′)-3′*-O-*[(*tert*-butyl)dimethylsilyl]*-N^6-*{[2-(4-nitrophenyl)ethoxy]carbonyl}-2′-adenylic Acid 2-{[6-(Benzoylamino)-9*H*-purin-9-yl]methoxy}ethyl 2-(4-Nitrophenyl)ethyl Ester*; **32**). As described for **30**, with 2,5-dichlorophenyl phosphorodichloridate (0.160 g, 0.56 mmol), pyridine (1.5 ml), 1*H*-1,2,4-triazole (0.077 g, 1.11 mmol), and **11** (0.112 g, 0.15 mmol; co-evaporated with anh. pyridine (3 × 5 ml), and pyridine (1.5 ml). Workup with 0.70 ml of 90% aq. pyridine instead of 0.35 ml. Then, as described for **38** in *Exper. 19*, with phosphodiester salt **34**, trimer **35** [17] (0.10 g, 0.066 mmol), pyridine (2 × 5 ml; co-evaporation pyridine (1.5 ml), 2,4,6-triisopropylbenzenesulfonyl chloride (0.09 g, 0.3 mmol), and 1*H*-tetrazole (0.063 g, 0.9 mmol) (stirring for 20 h instead of 2 h). CC (silica gel, 8 × 2.5 cm, CH_2Cl_2/MeOH 25:1) gave 0.16 g (75%) of **32**. Colorless foam. TLC ($CHCl_3$/MeOH 9:1): R_f 0.46. UV (MeOH): 275 (sh, 4.92), 268 (4.95).

22. *3′-Deoxyadenylyl-(2′ → 5′)-3′-deoxyadenylyl-(2′ → 5′)-3′-deoxyadenylyl-(2′ → 2″)-9-[(2″-hydroxyethoxy)methyl]adenine Tris(triethylammonium) Salt* (= *3′-Deoxyadenylyl-(2′ → 5′)-3′-deoxyadenylyl-(2′ → 5′)-3′-deoxy-2′-adenylic Acid 2-[(6-Amino-9*H*-purin-9-yl)methoxy]ethyl Ester Tris(triethylammonium) Salt*; d^{3}A-d^{3}A-d^{3}A-etherA; **36**). The fully protected tetramer **29** (0.081 g, 0.04 mmol) and 25% ammonia (30 ml) were stirred for 2 days. The solvent was evaporated, the dimethoxytrityl group cleaved with 80% AcOH (10 ml) for 3 h, the mixture evaporated and co-evaporated with MeOH to remove AcOH, and the residue dissolved in H_2O and submitted to CC (*DEAE-Sephadex A-25*, 60 × 1 cm, $(Et_3NH)HCO_3$ buffer pH 7.0). The eluate with 0.25–0.30M $(Et_3dpNH)HCO_3$ was evaporated and co-evaporated several times with H_2O and MeOH and then lyophilized: 1800 *OD* units (70%) of **36**. Colorless powder. TLC: (Cel, ^{i}PrOH/conc. NH_3 soln. /H_2O 55:10:35): R_f 0.63. UV (H_2O): 259. HPLC (*RP 18*, *Lichrospher* 125 × 4 mm, 5 μm, *Merck* 50943; flow rate 1 ml/min; *A*, 0.1M aq. $(Et_3NH)OAc$ buffer (pH 7.0), and *B*, *A*/MeCN 1:1; gradient 0–2 min 5% *B*, in 32 min 40% *B*, in 37–40 min 100% *B*). t_R 18.59 min.

23. *1-Benzyl-3′-deoxyadenylyl-(2′ → 5′)-3′-deoxyadenylyl-(2′ → 5′)-3′-deoxyadenylyl-(2′ → 2″)-9-[(2″-hydroxyethoxy)methyl]adenine Tris(triethylammonium) Salt* (= *1-Benzyl-3′-deoxyadenylyl-(2′ → 5′)-3′-deoxyadenylyl-(2′ → 5′)-3′-deoxy-2′-adenylic Acid 2-[(6-Amino-9*H*-purin-9-yl)methoxy]ethyl Ester Tris(tri-ethylammonium) Salt; N^1*-Benzyl-d^{3}A-d^{3}A-d^{3}A-etherA; **37**). A soln. of **30** (0.042 g, 0.02 mmol) in dioxane (2 ml) was treated with 25% ammonia at r.t. for 48 h. The solvent was evaporated, the residue dissolved in 80% AcOH (10 ml), and the soln. stirred for 6 h to cleave the monomethoxytrityl group. Workup as described for **36** (0.22–0.26M instead of 0.25–0.30M $(Et_3NH)HCO_3$) gave 700 *OD* units (57%) of **37**. Colorless powder. TLC, ^{i}PrOH/conc. ammonia/H_2O 55:10:35): R_f 0.78. HPLC (*RP 18*, *Lichrospher* 125 × 4 mm, 5 μm, *Merck* 50943); flow rate 1 ml/min; *A*

0.1M aq. (Et_3NH)OAc buffer (pH 7.0), and *B*,*A*/MeCN 1:1, gradient 0–2 min 5% *B* in 32 min 40% *B*, in 37 min 100% *B*, remaining till 40 min 100% *B*): t_R 19.29.

24. N^6-*Benzyl-3′-deoxyadenylyl-(2′→5′)-3′-deoxyadenylyl-(2′→5′)-3′-deoxyadenylyl-(2′→2″)-9-[(2″-hydroxyethoxy)methyl]adenine Tris(triethylammonium) Salt* (= N^6-*Benzyl-3′-deoxyadenylyl-(2′→5′)-3′-deoxyadenylyl-(2′→5′)-3′-deoxy-2′-adenylic Acid 2-[(6-Amino-9*H*-purin-9-yl)methoxy]ethyl Ester Tris(triethylammonium) Salt*; N^6-Benzyl-d^{3}A-d^{3}A-d^{3}A-etherA; **38**). A soln. of **31** (0.1 g, 0.039 mmol) and TsOH in CH_2Cl_2/MeOH (1 ml) was stirred for 1 h and then diluted with CH_2Cl_2 (25 ml) and washed with H_2O (2 × 10 ml). After evaporation, the crude product was purified by CC (silica gel, 10 × 2.5 cm, $CHCl_3$/MeOH 25:1): 0.065 g (70%) of the 5′-hydroxy tetramer. TLC ($CHCl_3$/MeOH 9:1): R_f 0.38. UV (MeOH): 276 (sh, 4.84), 266 (4.91).

The 5′-hydroxy tetramer (0.036 g, 0.015 mmol) was co-evaporated with anh. pyridine (3 × 10 ml) and treated with 0.5M DBU in pyridine (10 ml) by stirring at r.t. for 48 h. The mixture was neutralized with 1M AcOH (5 ml) and evaporated, the residue treated with sat. NH_3/MeOH (10 ml) to remove the benzoyl groups, and, after stirring at r.t. for 24 h, the soln. was again evaporated and the residue taken up in H_2O (20 ml) and washed with CH_2Cl_2 (2 × 10 ml). Then, the aq. phase was submitted to CC (*Sephadex*) and worked up as described for **36** (0.29–0.36M instead of 0.25–0.30M (Et_3NH)HCO_3): 724 *OD* units (80%) of **38**. Colorless powder. TLC (Cel, AcOEt/(^{i}PrOH/conc. ammonia/H_2O 55:10:35) 1:1): R_f 0.13. UV (H_2O): 259. HPLC (*RP 18*, *Lichrospher* 125 × 4 mm, 5 μm, *Merck* 50943); flow rate 1 ml/min; *A*, 0.1M aq. (Et_3NH)OAc buffer (pH 7.0), and *B*, *A*/MeCN 1:1; gradient 0–2 min 5% *B*, in 32 min 40% *B*, in 37–40 min 100% *B*): t_R 26.5.

25. N^6-*Benzyladenylyl-(2′→5′)-adenylyl-(2′→5′)-adenylyl-(2′→2″)-9-[(2″-hydroxyethoxy)methyl]adenine Tris(triethylammonium) Salt* (= N^6-*Benzyladenylyl-(2′→5′)-adenylyl-(2′→5′)-2′-adenylic Acid 2-[(6-Amino-9*H*-purin-9-yl)methoxy]ethyl Ester Tris(triethylammonium) Salt*; N^6-Benzyl-A-A-A-etherA, **39**). As described for **38**, with **32** (0.155 g, 0.058 mmol) TsOH, and CH_2Cl_2/MeOH (2 ml). CC (silica gel, 10 × 2.5 cm, $CHCl_3$/MeOH 50:3) gave 0.108 g (76%) of the 5′-hydroxy tetramer. TLC. ($CHCl_3$/MeOH 85:15): R_f 0.38.

Then, as described above, with the 5′-hydroxy tetramer (0.048 g, 0.022 mmol), pyridine (3 × 10 ml; co-evaporation), 0.5M DBU, and pyridine (7 ml). After neutralization with 1M AcOH (3.5 ml) and evaporation, the residue was stirred with sat. NH_3/MeOH (15 ml) to remove the benzoyl and 2,5-dichlorophenyl group, and, after stirring at r.t. for 48 h, the soln. was worked up as described for **36** (0.29–0.37M instead of 0.25–0.30M (Et_3NH)HCO_3): 738 *OD* units (60%) of **39**. Colorless powder. TLC (Cel, AcOEt/(^{i}PrOH/conc. ammonia/H_2O 55:10:35) 1:1): R_f 0.18. UV (H_2O): 259. HPLC: (*RP 18*, *Lichrospher* 125 × 4 mm, 5 μm, *Merck* 50943); flow rate 1 ml/min; *A*, 0.1M aq. (Et_3NH)OAc buffer (pH 7.0), and *B*, *A*/MeCN 1:1; gradient 0–2 min 5% *B*, in 32 min 40% *B*, in 37–40 min 100% *B*): t_R 28.70.

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