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Chemical Syntheses of the 2′-5′-Cordycepin-Trimer Core

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The chemical synthesis of 3′-deoxyadenylyl-(2′-5′)-3′-deoxyadenylyl-(2′-5′)-3′-deoxyadenosine (**30**; trimeric cordycepin) is described by three different routes using various protecting groups and applying the phosphotriester approach. The intermediates have been isolated and characterized by elemental analyses and spectroscopic means. High yields of **30** have been obtained on deprotection making this biologically very active compound available in preparative scale.

Introduction. – Recently, much attention has been paid to a low-molecular-weight inhibitor of cell-free protein synthesis [2] [3] which is formed by an interferon-induced enzyme, 2′-5′A synthetase, from ATP and possessing the unusual 5′-*O*-triphosphoryl-adenylyl-(2′-5′)-adenylyl-(2′-5′)-adenosine (pppA2′p5′A2′p5′A) structure [4]. It activates a latent endo-ribonuclease, RNase L, which is finally responsible for the cleavage of messenger and ribosomal RNA's. The antiviral and antitumor activity of interferon is illustrated by this cascade of reactions [5–7], but it is also limited by the fact that interferon triggers simultaneously the increase of a specific 2′-phosphodiesterase which degrades the 2′-5′-phosphodiester bond of the enzyme activator. Besides the exploration of the biological role of the 2′-5′A system [6] by applying several synthetic analogs modified at the base [8–13] and sugar moieties [14–19] as well as the internucleotidic linkage [20], some efforts have been made to extend the biological half-life of the 2′-5′A system and potentiating thereby significantly the biological activity [21] [22]. Since the 2′-5′A-trimer core reveals almost the same biological activity as its 5′-triphosphate, it was obvious to synthesize the cordycepin trimer core 3′d(A2′p5′A2′p5′A) (**30**) as a closely related structural analog of the naturally occurring inhibitor from the 3′-deoxyadenosine (= cordycepin; **1**). Screening of the 2′-5′(cordcycepin)$_3$ indicated an extended metabolic stability without toxicity to cells and a broad spectrum of biological acitivities characterized by a more potent inhibition of protein synthesis than was 2′-5′A$_3$ in lysed rabbit reticulocytes [23], the prevention of the transformation of *Epstein-Barr* virus infected lymphocytes [24], the synthesis of EBV-induced nuclear antigen [25], tabacco mosaic virus replication in tabacco plants [26], and the chondro sarcoma growth in animals [27]. Since there are also some controversal reports [28] [29] regarding the bounding to and activation of the 2′-5′-A-dependent endo-ribonuclease by the cordycepin-trimer analog, more studies are necessary on these lines to clarify the situation. In order to provide the

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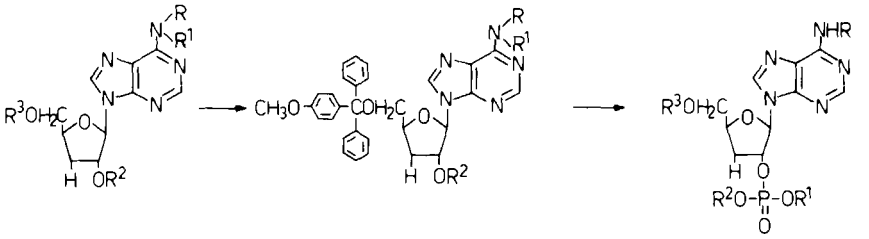
synthetic material, three different syntheses of 3'd(A2'p5'A2'p5'A) [30] have been performed, applying the phosphotriester approach and using various combinations of blocking groups.

2. Syntheses. – In the first chemical synthesis [21] of the cordycepin trimer core **30**, 3'-deoxyadenosine (**1**) [31] was converted initially by benzoylation and subsequent base treatment *via* the intermediary N^6,N^6,2'-*O*,5'-*O*-tetrabenzoyl derivative **2** into the N^6-benzoyl-3'-deoxyadenosine (**3**) [32]. Monomethoxytritylation at the 5'-position proceeded almost quantitatively to N^6-benzoyl-3'-deoxy-5'-*O*-(monomethoxytrityl)-adenosine (**4**) as a key intermediate. Reaction of **4** with a small excess of 2-chlorophenyl phosphorodi(triazolide) in pyridine followed by subsequent addition of 3-hydroxypropiononitrile afforded the corresponding 2'-phosphotriester **5** in an isolated yield of 79%, after column chromatography on silica gel. This building block was used on one hand for quantitative deblocking of the cyanoethyl group by Et_3N at r.t. to yield the protected 3'-deoxyadenosine 2'-phosphodiester **6** in form of its triethylammonium salt and on the other hand for detritylation by 2% CF_3COOH in $CHCl_3$ yielding the 5'-deprotected phosphotriester **7** as the second component for the condensation reactions. The third component finally, providing the 2'-terminus, was prepared from **4** by exhaustive benzoylation to N^6,N^6,2'-*O*-tribenzoyl-3'-deoxy-5'-*O*-(monomethoxytrityl)adenosine (**8**) and its 5'-deprotection to N^6,N^6,2'-*O*-tribenzoyl-3'-deoxyadenosine (**9**).

The phosphodiester **6** was then condensed with the phosphotriester **7**, after activation with 3-nitro-1-(2,4,6-triisopropylbenzenesulfonyl)-1,2,4-triazole (NTPST) [33] to give, after 20 h and the usual workup including separation and purification by column chromatography on silica gel, the fully protected dinucleoside diphosphotriester **10** in 58% yield. The extension of the chain was then achieved by eliminating the cyanoethyl group by Et_3N in pyridine and further condensation of the resulting terminal phosphodiester **11** with **4** in presence of NTPST to the fully blocked trinucleoside diphosphotriester **12** which was isolated in 79% yield.

The second sequence of reaction was performed from the 2'-terminus of the oligonucleotide chain and with the 2-(*p*-nitrophenyl)ethyl group (npe) as a new versatile phosphate-protecting group [34–37]. N^6-Benzoyl-2'-*O*[(*tert*-butyl)dimethylsilyl]-3'-deoxyadenosine (**13**) was prepared from **4** first by silylation with (*tert*-butyl)dimethylsilyl chloride/imidazole in pyridine to give **14** and then by detritylation with 1% CF_3COOH in $CHCl_3$, with an overall yield of 71%. Furthermore, **4** was also used as starting material for the preparation of the phosphodiester **15**, which resulted from phosphorylation to the 2,5-dichlorophenyl 2-(*p*-nitrophenyl)ethyl phosphotriester **16** with 2,5-dichlorophenyl phosphorodichloridate/1,2,4-triazole and 2-(*p*-nitrophenyl)ethanol (88% yield) and subsequent oximate treatment [38] to remove the 2,5-dichlorophenyl protecting group (*ca.* 100% yield). The condensation of **13** and **17** to the dinucleoside phosphotriester **18** proceeded again in presence of NTPST in 82% isolated yield. The two final steps to the fully blocked 2'-5'cordycepintrimer **19** were accomplished by detritylation first to **20** and its subsequent analogous condensation with a small molar excess of **17** in presence of NTPST and at a slightly elevated temperature (overall yield 83%).

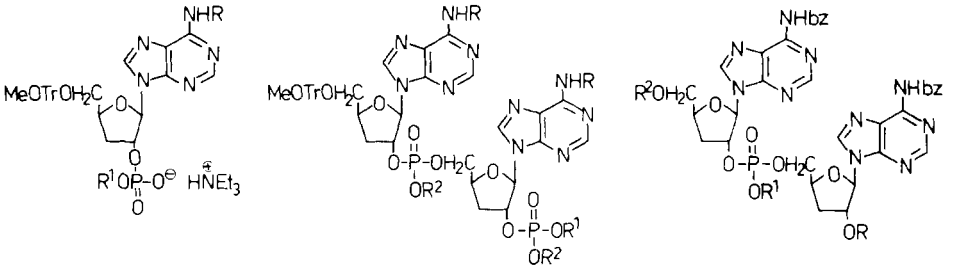
The third synthetic approach to the cordycepin trimer makes use of a generally more simplified blocking-group strategy [39] [40] using in principle, with exception of the 5'-OH group, one type of protection for the amino, hydroxy, and phosphate groups in



	R	R^1	R^2	R^3
1	H	H	H	H
2	bz	bz	bz	bz
3	bz	H	H	H
9	bz	bz	bz	H
13	bz	H	tbds	H
21	npeoc	H	H	H
25	npeoc	H	npeoc	H

	R	R^1	R^2
4	bz	H	H
8	bz	bz	bz
14	bz	H	tbds
22	npeoc	H	H
23	npeoc	H	npeoc

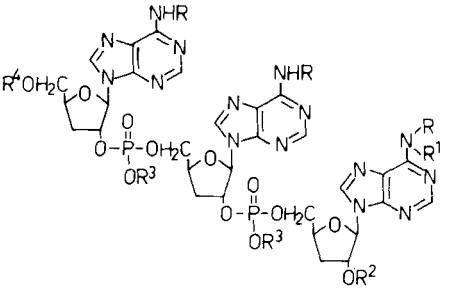
	R	R^1	R^2	R^3
5	bz	CH_2CH_2CN	2-Cl–C_6H_4	MeOTr
7	bz	CH_2CH_2CN	2-Cl–C_6H_4	H
16	bz	2,5-$Cl_2C_6H_3$	npe	MeOTr
24	npeoc	2,5-$Cl_2C_6H_3$	npe	MeOTr
26	npeoc	2,5-$Cl_2C_6H_3$	npe	H



	R	R^1
6	bz	2-Cl–C_6H_4
17	bz	npe
15	npeoc	npe

	R	R^1	R^2
10	bz	CH_2CH_2CN	2-Cl–C_6H_4
11	bz	$HNEt_3$	2-Cl–C_6H_4
27	npeoc	2,5-$Cl_2C_6H_3$	npe
28	npeoc	$HNEt_3$	npe

	R	R^1	R^2
18	tbds	npe	MeOTr
20	tbds	npe	H



	R	R^1	R^2	R^3	R^4
12	bz	bz	bz	2-Cl–C_6H_4	MeOTr
19	bz	H	tbds	npe	MeOTr
29	npeoc	H	npeoc	npe	MeOTr
30	H	H	H	$HNEt_3$	H

bz = benzoyl; MeOTr = monomethoxytrityl; tbds = (*tert*-butyl)dimethylsilyl; npe = 2-(*p*-nitrophenyl)ethyl; npeoc = 2-(*p*-nitrophenyl)ethoxycarbonyl

applying the 2-(*p*-nitrophenyl)ethyl (npe) and 2-(*p*-nitrophenyl)ethoxycarbonyl (npeoc) group to block these functionalities. The 3'-deoxyadenosine (**1**) was first transformed in a one-pot reaction *via* trimethylsilylation [41] and acylation with 1-methyl-3-[2-(*p*-nitrophenyl)ethoxycarbonyl]imidazolium chloride [39] into crystalline 3'-deoxy-N^6-[2-(*p*-nitrophenyl)ethoxycarbonyl]adenosine (**21**) (91% overall yield). Monomethoxytritylation gave **22** which was acylated with the imidazolium reagent to give 3'-deoxy-5'-*O*-(monomethoxytrityl)-N^6,2'-*O*-bis[2-(*p*-nitrophenyl)ethoxycarbonyl]adenosine (**23**) and phosphorylated with 2,5-dichlorophenyl phosphorodichloridate/1,2,4-triazole and 2-(*p*-nitrophenyl)ethanol to form the corresponding phosphotriester **24** in 81% yield. The monomeric building blocks **25**, **26**, and **15** for the chain formation of the oligonucleotides resulted from the detritylation of **23** and **24** and the oximate cleavage of **24** with *p*-nitrobenzaldoxime/Et_3N in pyridine, respectively. The condensation of the phosphodiester **15** with the phosphotriester **26** worked very well in presence of 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl) and *N*-methylimidazole to give the dimer **27** in 88% yield. Subsequent oximate treatment converted the 2'-terminal phosphotriester function into the corresponding phosphodiester **28** which was coupled with **25** leading to the fully protected cordycepin trimer **29** (91% yield).

The final total deprotection of the various blocking groups to form the cordycepin-trimer core as its triethylammonium salt **30** afforded 4 steps with **19**, 3 with **12**, but only 2 with **29**. Treatment of **12** with tetramethylguanidinium pyridine-4-carbaldehydeoximate in aqueous dioxane to cleave the *o*-chlorophenyl group, by conc. NH_3 to remove the benzoyl groups and by 80% AcOH to split off the monomethoxytrityl group gave, on purification *via DEAE-Sephadex* chromatography using a linear gradient of triethylammonium hydrogen carbonate buffer (Et_3NHCO_3) **30** in 80% yield. Similarly, **19** afforded **30** in 87% yield, after treatment with DBU (1,8-diazabicyclo[5.4.0]undec-7-ene in pyridine to eliminate the 2-(*p*-nitrophenyl)ethyl group, with Bu_4NF for removal of the (*tert*-butyl)dimethylsilyl group, and again with NH_3 and AcOH to achieve deacylation and detritylation, respectively. The deprotection of **29** turned out to be most simple and straightforward. It was carried out with 0.5M DBU in pyridine (48 h) and subsequent treatment in 80% AcOH followed by *DEAE-Sephadex* chromatography to give **30** in 85% yield.

3. Physical Data. – The protected nucleosides and nucleotides were characterized by elemental analyses and the UV spectra as a quantitative measure of an easily determinable molecular property. The λ_{max} values and the molecular extinction coefficients are listed in the *Table*. The ^{1}H-NMR spectra are in general of complex nature due to many overlapping signals and to the fact that the protected phosphotriesters always consist of diastereoisomeric mixtures derived from the chiral P-center. Some distinct signals like those of the protons of the aglycons, of the anomeric protons, and of CH_3O of the monomethoxytrityl group are reported to help finding the right reaction product during the isolation procedures.

It can be concluded from the enzymatic hydrolysis of 3'd(A2'p5'A2'p5'A) (**30**) that the trimer prefers a stacked conformation in solution expressed by a hypochromicity of 26% based on an extinction coefficient of 3'-deoxyadenosine (**1**) of $\varepsilon = 15200$. The corresponding natural ribo-trimer (2'-5')A_3 core is less fixed as seen from a 21% hypochromicity. A detailed conformational analysis of **30** by 500-MHz high-resolution NMR

Table. *Physical Data of 3'-Deoxyadenosine Derivatives*

	UV spectra (MeOH)						^{1}H-NMR spectra ($CDCl_3$, δ [ppm])		
	λ_{max} [nm]			lg ε			H–C(2)	H–C(8)	H–C(1')
2	239	252 (sh)	271	4.04	4.41 (sh)	4.33	8.55 (*s*)	8.23 (*s*)	6.26 (*d*)
3			280			4.46	8.73(*s*)[a])	8.70(*s*)[a])	6.02(*d*)[a])
4	230		279	4.46		4.29	8.71 (*s*)	8.35 (*s*)	6.10 (*d*)
5	230		278	4.42		4.31	8.70 (*m*)	8.16 (*s*)	6.12 (*d*), 6.07 (*d*)
6	230		278	4.42		4.29	8.72 (*s*)	8.47 (*s*)	6.32 (*d*)
7	231		278	4.12		4.30	8.65 (*s*)	8.34 (*m*)	6.16 (*d*), 6.11 (*d*)
8	229		270	4.72		4.36	8.62 (*s*)	8.28 (*s*)	6.28 (*d*)
9	230	250	272	4.60	4.50	4.40	8.86 (*s*)[a])	8.69 (*s*)[a])	6.43 (*d*)[a])
10	230		278	4.58		4.55	8.65 (*m*)	8.14 (*m*)	6.25 (*d*) 6.21 (*d*)
11	230		278	4.57		4.55	8.63 (*m*)	8.14 (*m*)	6.26 (*d*) 6.18 (*d*)
12	230		278	4.70		4.90	8.62 (*m*, 3 H)	8.31 (*m*, 3 H)	6.18 (*d*) 5.91 (*m*)
13			278			4.31	8.78 (*s*)	8.09 (*s*)	5.68 (*d*)
15	235		268	4.33		4.56	8.62 (*s*)	8.16 (*s*)	6.28 (*d*)
16			278			4.47	8.66 (*s*)	8.19 (*s*)	6.25 (*d*)
17			278			4.47	8.65 (*s*)	8.19 (*s*)	6.28 (*d*)
18			278			4.69	8.67 (*m*, 2 H)	8.25 (*m*, 2 H)	6.22 (*s*) 5.93 (*d*)
19			278			4.85	8.55 (*m*, 3 H)	8.20 (*m*, 3 H)	6.16 (*s*) 6.08 (*s*)
20			278			4.68	8.71 (*m*, 2 H)	8.30 (*m*, 2 H)	6.07 (*d*) 5.95 (*s*)
21			267			4.43	8.68 (*s*)[a])	8.60 (*s*)[a])	5.99 (*d*)[a])
22	233		268	4.30		4.46	8.63 (*s*)	8.28 (*s*)	6.00 (*d*)
23	235		267	4.33		4.56	8.67 (*s*)	8.12 (*s*)	6.16 (*d*)
24			267			4.59	8.61 (2*s*)	8.11 (*s*)	6.20 (*d*)
25			267			4.56	8.71 (*s*)	8.02 (*s*)	5.97 (*d*)
26			267			4.58	8.68 (2*s*)	8.12 (*s*)	6.00 (*d*), 5.98 (*d*)
27			268			4.82	8.61 (*m*)	8.12 (*m*)	6.15 (*d*) 6.10 (*d*)
28			268			4.82	8.59 (*m*)	8.11 (*m*)	6.23 (*d*) 6.15 (*d*)
29			268			4.99	8.66 (*m*, 3 H)	8.15 (*m*, 3 H)	6.16 (*s*) 6.10 (*s*) 6.04 (*s*)

[a]) In (D_6)DMSO.

studies [42] is reported elsewhere and shows a complete ^{1}H-NMR spectral assignment of all sugar ring proton signals, information about the N-N-N-stacked state at low temperature as well as the backbone geometry. The CD spectra are also in agreement with the proposed molecular features.

Experimental Part

General. Enzyme: snake-venom phosphodiesterase. TLC: precoated silica-gel thin-layer sheets *F 1500 LS 254* and cellulose thin-layer sheets *F 1440* from *Schleicher & Schüll.* Prep. TLC: silica gel *60 PF_{254}* (*Merck*). Prep. column chromatography: silica gel (*Merck 60*, 0.063-0.2 mesh). Paper chromatography (PC): sheets (58 × 60 cm) from *Schleicher & Schüll.* Ion-exchange chromatography: *DEAE Sephadex A-25 (Pharmacia).* M.p.: *Büchi* apparatus, model Dr. *Tottoli;* no corrections. UV/VIS: *Cary* Recording Spectrometer, model *118*, *Applied Phys. Corp.*, and *Uvikon 820, Kontron*; λ_{max} in nm (lg ε). ^{1}H-NMR: *Bruker WM 250*; in δ (ppm) relative to TMS.

1. N^6,N^6,*2'*-O,*5'*-O-*Tetrabenzoyl-3'-deoxyadenosine* (**2**). A soln. of 1.13 g (5 mmol) of 3'-deoxyadenosine (**1**) in abs. pyridine is coevaporated 3 times. To the residue in 20 ml of abs. pyridine, 5 ml of benzoyl chloride are added and stirred at r.t. for 2 h. Ice is slowly added with stirring, the mixture extracted thrice with 20 ml of $CHCl_3$ and

washed with H_2O, the org. layer dried (Na_2SO_4) and then evaporated to dryness. The residue is coevaporated twice with toluene and then recrystallized from EtOH/Et_2O to give 2.64 g (86%) of colourless crystals. M.p. 178–179°. UV(MeOH): 239 (4.64), 252 (sh, 4.41), 271 (4.33). Anal. calc. for $C_{38}H_{29}N_5O_7$ (667.7): C 68.36, H 4.38, N 10.50; found: C 68.30, H 4.40, N 10.33.

2. N^6-*Benzoyl-3'-deoxyadenosine* (**3**) [32]. In 100 ml of EtOH/pyridine 1:1, 1.9 g (2.8 mmol) of **2** are dissolved and 20 ml of 1N NaOH added. After stirring for 45 min at r.t., the soln. is neutralized with *Dowex-1* (pyridinium form), filtered, and the filtrate evaporated. The residue is recrystallized from MeOH: 0.8 g (84%) of **2**. Colourless crystals. M.p. 202–204°. Anal. calc. for $C_{17}H_{17}N_5O_4$ (355.4): C 57.46, H 4.82, N 19.71; found: C 57.54, H 4.86, N 19.59.

3. N^6-*Benzoyl-3'-deoxy-5'*-O-*(monomethoxytrityl)adenosine* (**4**). A soln. of 0.77 g (0.21 mmol) of **3** in 15 ml of abs. pyridin is evaporated. To the residue in 20 ml of abs. pyridine, 0.15 g (0.5 mmol) of *p*-monomethoxytrityl chloride are added and stirred for 24 h at r.t. Then, 2 ml of MeOH are added, the mixture is stirred for 30 min, evaporated to 1/4 of the volume and diluted with 50 ml of $CHCl_3$. The soln. is washed twice with 30 ml of H_2O, the org. layer evaporated and coevaporated 3 times with 10 ml of toluene, and the residue in little $CHCl_3$ chromatographed on a silica-gel column (40 × 2 cm) with $CHCl_3$/MeOH 99:1. The product is dried at 40°: 1.06 g (81%) of an amorphous powder. Anal. calc. for $C_{37}H_{33}N_5O_5$ (627.7): C 70.79, H 5.29, N 11.15; found: C 70.57, H 5.38, N 10.98.

4. N^6-*Benzoyl-3'-deoxy-5'*-O-*(monomethoxytrityl)adenosine 2'-[2-Chlorophenyl 2-Cyanoethyl Phosphate]* (**5**). To a soln. of 0.354 g (5.2 mmol) of 1,2,4-triazole in 6 ml of abs. pyridine, 0.73 g (2.9 mmol) of 2-chlorophenyl phosphorodichloridate are added and stirred for 30 min at r.t. Then, a soln. of 0.93 g (1.48 mmol) of **4** in 4 ml of abs. pyridine is added slowly and dropwise with stirring. After *ca.* 30 min (no **4** is left), 0.25 ml of 2-hydroxypropionitrile are added and stirred for 4 h. After evaporated to 1/4 of the volume, 50 ml of $CHCl_3$ are added, the org. phase is washed twice with 10 ml of H_2O, dried (Na_2SO_4), and evaporated, and the residue in little $CHCl_3$ chromatographed on a silica-gel column (25 × 2.5 cm) with $CHCl_3$/MeOH 95:5. The residue of the main fraction in 5 ml of $CHCl_3$ is added dropwise with stirring into 50 ml of hexane and the amorphous colourless precipitate dried at 50°/*in vacuo* to give 1.025 g (79%) of **5**. M.p. 108–110°. Anal. calc. for $C_{46}H_{40}ClN_6O_8P \cdot 2H_2O$ (871.3): C 60.89, H 4.88, N 9.26; found: C 60.51, H 4.50, N 9.31.

5. N^6-*Benzoyl-3'-deoxy-5'*-O-*(monomethoxytrityl)adenosine 2'-[2-Chlorophenyl Triethylammonium Phosphate]* (**6**). In 2.5 ml of abs. pyridine/Et_3N 1:1, 0.783 g (0.9 mmol) of **5** are dissolved and stirred for 3 h at r.t. The soln. is evaporated and coevaporated 3 times with each 3 ml of abs. pyridine and the residue in 5 ml of $CHCl_3$ chromatographed on a silica-gel column (13 × 2.5 cm) with $CHCl_3$/MeOH/Et_3N 7:1:1. The residue of the main fraction in 4 ml of $CHCl_3$ is added dropwise with stirring to 50 ml of hexane. The colourless precipitate is dried at 50°/*in vacuo*: 0.784 g (95%) of **6**.

6. N^6-*Benzoyl-3'-deoxyadenosine 2'-[2-Chlorophenyl 2-Cyanoethyl Phosphate]* (**7**). In 4.5 ml of 2% CF_3COOH in $CHCl_3$, 0.375 g (0.43 mmol) of **5** are dissolved and stirred for 30 min at r.t. MeOH is added dropwise till the yellow colour has almost disappeared. After 10 min, the soln. is diluted with 20 ml of $CHCl_3$ and then extracted with 5 ml of phosphate buffer (pH 7). The org. layer is dried (Na_2SO_4), evaporated to a small volume, and the residue separated on 2 prep. silica-gel plates (40 × 20 × 0.2 cm) with $CHCl_3$/MeOH 97:3. The main band is eluted with $CHCl_3$/MeOH 1:1 and the product in little $CHCl_3$ added dropwise with stirring into 50 ml of hexane. The colourless precipitate is dried at 50°/*in vacuo* to yield 0.205 g (80%) of **7**. Anal. calc. for $C_{26}H_{24}ClN_6O_7P$ (598.9): C 52.14, H 4.04, N 14.03; found: C 52.59, H 3.87, N 13.66.

7. N^6,N^6,*2'*-O-*Tribenzoyl-3'-deoxy-5'*-O-*(monomethoxytrityl)adenosine* (**8**). In 10 ml of pyridine, 0.313 g (0.5 mmol) of **4** and 0.5 ml of benzoyl chloride are dissolved and stirred at r.t. for 2 h. The reaction is stopped by addition of ice followed by several extractions with $CHCl_3$. The org. layer is washed with H_2O, separated, dried (Na_2SO_4), evaporated, and coevaporated twice with benzene. The residue in little $CHCl_3$ is chromatographed on 2 prep. silica-gel plates (40 × 20 × 0.2 cm) with $CHCl_3$/MeOH 98:2. The main band is eluted with 200 ml of $CHCl_3$/MeOH 1:1 and yields, after drying at 40° *in vacuo*, 0.384 g (92%) of an amorphous solid. Anal. calc. for $C_{51}H_{41}N_5O_7$ (835.9): C 73.28, H 4.94, N 8.38; found: C 73.02, H 4.88, N 8.36.

8. N^6,N^6,*2'*-O-*Tribenzoyl-3'-deoxyadenosine* (**9**). To 3 ml of a 2% soln. of CF_3COOH in $CHCl_3$ are added 0.21 g (0.25 mmol) of **8**, and after stirring for 30 min, first 0.5 ml of MeOH, and after 5 min, 5 ml of phosphate buffer (pH 7). The mixture is extracted with 30 ml of $CHCl_3$, the org. layer dried (Na_2SO_4) and evaporated, and the residue purified by prep. TLC on silica gel with $CHCl_3$/MeOH 95:5. The main band is eluted with $CHCl_3$/MeOH 1:1, the soln. evaporated, and the residue in 2 ml of $CHCl_3$ added dropwise to 30 ml of hexane with stirring. The colourless powder is dried *in vacuo*: 0.108 g (77%) of **9**. Anal. calc. for $C_{31}H_{25}N_5O_6 \cdot 1\ H_2O$: C 64.02, H 4.67, N 12.04; found: C 64.12, H 4.40, N 11.90.

9. N^6-*Benzoyl-3'-deoxy-5'-O-(monomethoxytrityl)adenylyl-{2'-[O^P-(2-chlorophenyl)]→5'}-N^6-benzoyl-3'-deoxyadenosine 2'-[2-Chlorophenyl 2-Cyanoethyl Phosphate]* (**10**). A soln. of 0.229 g (0.25 mmol) of **6** and 0.15 g (0.25 mmol) of **7** in 4 ml of abs. pyridine is evaporated. This process is repeated and then the residue dissolved in 2.5 ml of abs. pyridine. After addition of 0.19 g (0.5 mmol) of 3-nitro-1-(2,4,6-triisopropylbenzenesulfonyl)-1,2,4-triazole (NTPST), the mixture is stirred for 20 min at r.t., then concentrated, diluted with 40 ml of $CHCl_3$, and washed 3 times with 10 ml of H_2O. The org. layer is dried (Na_2SO_4), evaporated, and coevaporated with toluene several times. The residue in little $CHCl_3$ is chromatographed on 2 prep. silica-gel plates (40 × 20 × 0.2 cm) with $CHCl_3$/MeOH 95:5. The main band is eluted with $CHCl_3$/MeOH 1:1 and its residue in 2 ml of $CHCl_3$ added slowly and dropwise with stirring to 50 ml of hexane. The colourless precipitate is dried at 40°/*in vacuo*: 0.205 g (58%) of **10**. Anal. calc. for $C_{69}H_{59}Cl_2N_{11}O_{14}P_2 \cdot 2\,H_2O$ (1399.1): C 57.74, H 4.42, N 10.73; found: C 57.82, H 4.68, N 10.21.

10. N^6-*Benzoyl-3'-deoxy-5'-O-(monomethoxytrityl)adenylyl-{2'-[O^P-(2-chlorophenyl)]→5'}-N^6-benzoyl-3'-deoxyadenosine 2'-(2-Chlorophenyl Triethylammonium Phosphate)* (**11**). To 2 ml of abs. pyridine/Et_3N 1:1 0.139 g (0.1 mmol) of **10** are added and stirred at r.t. for 4 h. The mixture is evaporated and coevaporated twice with 4 ml of abs. pyridine and twice with 4 ml of benzene. The residue in 2 ml of $CHCl_3$ is then added dropwise with stirring to 50 ml of hexane and the colourless precipitate dried at 40°/*in vacuo*: 0.13 g (90%) of **11**.

11. N^6-*Benzoyl-3'-deoxy-5'-O-(monomethoxytrityl)adenylyl-{2'-[O^P-(2-chlorophenyl)]→5'}-N^6-benzoyl-3'-deoxyadenylyl-{2'-[O^P-(2-chlorophenyl)]→5'}-N^6,N^6,2'-O-tribenzoyl-3'-deoxyadenosine* (**12**). A soln. of 0.101 g (0.07 mol) of **11** and 0.04 g (0.07 mmol) of **9** in 4 ml of abs. pyridine is evaporated. This process is repeated 3 times. To the residue in 1 ml of abs. pyridine, 0.053 g (0.14 mmol) of NTPST are added, and the mixture is stirred for 20 h at r.t. After evaporation to a small volume, 30 ml of $CHCl_3$ are added, the mixture is washed with H_2O twice, and the $CHCl_3$ layer dried (Na_2SO_4), evaporated, and coevaporated twice with toluene. The residue in little $CHCl_3$ is chromatographed on 2 silica-gel plates (40 × 20 × 0.2 cm) with $CHCl_3$/MeOH 95:5. The main band is eluted with 200 ml of $CHCl_3$/MeOH 1:1 and its residue redissolved in 2 ml of $CHCl_3$ and added slowly and dropwise with stirring into hexane. The colourless precipitate is dried under high vacuum to give 0.075 g (79%) of **12**. Anal. calc. for $C_{97}H_{79}Cl_3N_{15}O_{20}P_2 \cdot 2\,H_2O$ (1943.6): C 58.37, H 3.89, N 10.73; found: C 58.97, H 4.03, N 10.39.

12. N^6-*Benzoyl-2'-O-[(*tert*-butyl)dimethylsilyl]-3'-deoxyadenosine* (**13**). In 5 ml of abs. pyridine are dissolved 0.94 g (1.5 mmol) of **4**, 0.34 g (2.25 mmol) of (*tert*-butyl)dimethylsilyl chloride, and 0.255 g (3.75 mmol) of imidazole. The mixture is stirred for 20 h at r.t., then diluted with 40 ml of $CHCl_3$, and extracted with 15 ml of H_2O. The org. layer is dried (Na_2SO_4), evaporated, and coevaporated several times with toluene to yield a colourless foam which is treated with 50 ml of 1% CF_3COOH in $CHCl_3$ for 10 min the cleave the monomethoxytrityl group. The orange soln. is treated dropwise with EtOH till the colour turns yellowish. After stirring for 20 min, 25 ml of 0.1M Et_3NHCO_3 buffer are added. The org. layer is dried (Na_2SO_4), evaporated to a small volume, and chromatographed on a silica-gel column (10 × 4.5 cm) with $CHCl_3$ and $CHCl_3$/MeOH 99:1. The amorphous product (541 mg) in 10 ml of Et_2O is added dropwise with stirring into 70 ml of hexane to yield 0.5 g (71%) of **13**. Colourless amorphous powder. Anal. calc. for $C_{23}H_{31}N_5O_4Si$ (469.6): C 58.83, H 6.65, N 14.91; found: C 58,52, H 6.73, N 14.73.

13. N^6-*Benzoyl-2'-O-[(*tert*-butyl)dimethylsilyl]-3'-deoxy-5'-O-(monomethoxytrityl)adenosine* (**14**). To a soln. of 0.94 g (1.5 mmol) of **4**, 5 ml of abs. pyridine are added, followed by 0.34 g (2.25 mmol) of (*tert*-butyl)dimethylsilyl chloride and 0.25 g (3.75 mmol) of imidazole. After stirring for 20 h at r.t., the mixture is diluted with 40 ml of $CHCl_3$ and then washed with 15 ml of H_2O. the org. layer is dried (Na_2SO_4), evaporated, and coevaporated with toluene to yield an amorphous foam which is chromatographically pure for further reaction.

14. *3'-Deoxy-5'-O-(monomethoxytrityl)-N^6-[2-(p-nitrophenyl)ethoxycarbonyl]adenosine 2'-[2-(p-Nitrophenyl)ethyl Triethylammonium Phosphate]* (**15**). To 60 ml of dioxane/Et_3N/H_2O 1:1:1, 1.67 g (10 mmol) of 4-nitrobenzaldehyde oxime are added and stirred for 30 min at r.t. Then, 1.1 g (1 mmol) of **24** are added and stirred for another 60 min. The mixture is evaporated 25–30° and the residue coevaporated twice with each 40 ml of abs. pyridine and twice with 40 ml of toluene. The residue is chromatographed on a silica-gel column (15 × 2.5 cm) with $CHCl_3$, $CHCl_3$/MeOH 100:3, and $CHCl_3$/MeOH/Et_3N 100:3:3. The product fractions are evaporated and coevaporated several times with $CHCl_3$ and CH_2Cl_2 to yield, after drying at 40°/*in vacuo*, 0.985 g (94%) of a solid foam. Anal. calc. for $C_{53}H_{59}N_8O_{13}P \cdot H_2O$ (1065.1): C 59.58, H 5.56, N 10.54; found: C 59.77, H 5.77, N 10.52.

15. N^6-*Benzoyl-3'-deoxy-5'-O-(monomethoxytrityl)adenosine 2'-[2,5-Dichlorophenyl 2-(p-Nitrophenyl)ethyl Phosphate]* (**16**). To a soln. of 0.236 g (3.4 mmol) of 1,2,4-triazole in 2 ml of abs. pyridine, 0.48 g (1.7 mmol) of 2,5-dichlorophenyl phosphorodichloridate are added and stirred for 15 min at r.t. The mixture is cooled with ice/H_2O and a soln. of 0.766 g (1.22 mmol) of **4** in 2.5 ml of abs. pyridine added dropwise within 30 min. Stirring is

continued for 30 min, then 0.368 g (2.2 mmol) of 2-(*p*-nitrophenyl)ethanol are added and stirred for 2.5 h at 2°. The soln. is diluted with 30 ml of $CHCl_3$, extracted with 10 ml of H_2O, the org. layer dried (Na_2SO_4) and evaporated, and the residue separated on a silica-gel column (6 × 4.5 cm) with CH_2Cl_2, 200 ml of CH_2Cl_2/MeOH 99:1, and 500 ml of CH_2Cl_2/MeOH 98:2, yielding a solid foam which is dissolved in little CH_2Cl_2 and added dropwise with stirring into 60 ml of hexane. The colourless precipitate is dried at 50°/*in vacuo*: 1.08 g (88%) of **16**. Anal. calc. for $C_{51}H_{43}Cl_2N_6O_{10}P$ (1001.8): C 61.14, H 4.33, N 8.39; found: C 61.03, H 4.41, N 8.28.

16. N^6-*Benzoyl-3'-deoxy-5'*-O-*(monomethoxytrityl)adenosine 2'[2-(*p*-Nitrophenyl)ethyl Triethylammonium Phosphate]* (**17**). A soln. of 1.67 g (10 mmol) of *p*-nitrobenzaldehyde oxime in 60 ml of Et_3N/dioxane/H_2O 1:1:1 is stirred for 30 min at r.t. Then, 1.0 g (1 mmol) of **16** is added and stirred for 1 h. The mixture is evaporated to a sirup which is coevaporated 4 times with abs. pyridine and 3 times with benzene. The solid residue in 30 ml of $CHCl_3$ is chromatographed on a silica-gel column (5 × 2.5 cm) with 300 ml of $CHCl_3$, 200 ml of $CHCl_3$/MeOH 96:4, and 200 ml of $CHCl_3$/MeOH/Et_3N 7:1:2 which elutes the product. The solid residue is coevaporated twice with abs. pyridine and twice with toluene. The resulting solid foam in little CH_2Cl_2 is added dropwise with stirring to 70 ml of Et_2O/hexane 3:4. The precipitate is dried at 50°/*in vacuo* yielding 0.934 g (96%) of a colourless powder. Anal. calc. for $C_{51}H_{56}N_7O_{10}P \cdot H_2O$ (976.0): C 62.76, H 5.99, N 10.04; found: C 62.89, H 5.88, N 9.88.

17. N^6-*Benzoyl-3'-deoxy-5'*-O-*(monomethoxytrityl)adenylyl-{2'-[*O^P*-(2-(*p*-nitrophenyl)ethyl)]→5'}-*N^6-*benzoyl-2'*-O-*[(*tert*-butyldimethyl)silyl]-3'-deoxyadenosine* (**18**). A soln. of 0.92 g (1.18 mmol) of **17** in 5 ml of abs. pyridine is evaporated and coevaporated 3 times more with abs. pyridine. To the residue are given 0.376 g (0.8 mmol) of **13** and 10 ml of abs. pyridine, and the soln. is evaporated. To the residue in 12 ml of abs. pyridine, 0.46 g (1.2 mmol) of NTPST are added. The mixture is concentrated to *ca.* 8 ml and stirred at r.t. for 20 h. More NTPST (0.09 g) is added and the reaction continued for 1 h. The mixture is cooled with ice/H_2O, diluted with 30 ml of $CHCl_3$ and extracted with 15 ml of H_2O. The org. layer is dried (Na_2SO_4), evaporated, and coevaporated first with pyridine and then 3 times with toluene. The resulting solid foam in little $CHCl_3$ is chromatographed on a silica-gel column (10 × 2.5 cm) with $CHCl_3$, $CHCl_3$/MeOH 99:1, and $CHCl_3$/MeOH 98:2. The main fraction ist evaporated and the solid foam in little CH_2Cl_2 added dropwise to 60 ml of Et_2O/hexane 1:2. The colourless precipitate is dried at 50°/*in vacuo:* 0.86 g (82%) of **18**. Anal. calc. for $C_{68}H_{70}N_{11}O_{13}PSi$ (1308.4): C 62.42, H 5.39, N 11.77; found: C 62.31, H 5.33, N 11.58.

18. N^6-*Benzoyl-3'-deoxy-5'*-O-*(monomethoxytrityl)adenylyl-{2'-[*O^P*-(2-(*p*-nitrophenyl)ethyl)]→5'}-*N^6-*benzoyl-3'-deoxyadenylyl-{2'-[*O^P*-(2-(*p*-nitrophenyl)ethyl)]→5'}-*N^6-*benzoyl-2'*-O-*[(*tert*-butyldimethyl)silyl]-3'-deoxyadenosine* (**19**). A soln. of 0.46 g (0.47 mmol) of **17** and 0.414 g (0.4 mmol) of **20** in 10 ml of abs. pyridine is evaporated. This process is repeated once. To the residue in 4 ml of abs. pyridine, 0.23 g (0.6 mmol) of NTPST are added and stirred at 26–27° for 24 h. The soln. is cooled with ice, diluted with 30 ml of $CHCl_3$, and extracted twice with 15 ml of H_2O. The org. layer is dried (Na_2SO_4), filtered, evaporated, and coevaporated 5 times with toluene. The residue in little $CHCl_3$ is chromatographed on a silica-gel column (10 × 2 cm) with 100 ml of $CHCl_3$, 100 ml of $CHCl_3$/acetone 4:1, 100 ml of $CHCl_3$/acetone 7:3, and 800 ml of a $CHCl_3$/MeOH gradient 99:1→96:4. The product is eluted with the last fraction and its soln. in 6 ml of CH_2Cl_2 added tropwise to 60 ml of Et_2O/hexane 1:2. The precipitate is dried *in vacuo*: 0.623 g (83%) of **19**. Anal. calc. for $C_{93}H_{93}N_{17}O_{21}P_2Si$ (1874.9): C 59.58, H 5.00, N 12.70; found: C 59.03, H 4.70, N 12.53.

19. N^6-*Benzoyl-3'-deoxyadenylyl-{2'-[*O^P*-(2-(*p*-nitrophenyl)ethyl)]→5'}-*N^6-*benzoyl-2'*-O-*[(*tert*-butyldimethyl)silyl]-3'-deoxyadenosine* (**20**). To 25 ml of 1% CF_3COOH in $CHCl_3$, 0.707 g (0.54 mmol) of **18** are added and stirred for 25 min at r.t. The reaction is stopped by addition of 10 ml of 0.1M TBK buffer and shaking till the yellow colour has disappeared. The org. layer is separated, dried (Na_2SO_4), and evaporated. The residue in little $CHCl_3$ is chromatographed on a silica gel column (6 × 2 cm) with $CHCl_3$ and then with gradient of $CHCl_3$/MeOH till 97:3. The product is eluted at the end, dissolved in little $CHCl_3$, and added dropwise to 50 ml of Et_2O/hexane 1:2. The colourless precipitate is dried *in vacuo*: 0.486 g (81%) of **20**. Anal. calc. for $C_{48}H_{54}N_{11}O_{12}PSi$ (1036.1): C 55.64, H 5.25, N 14.87; found: C 55.35, H 5.39, N 14.74.

20. *3'-Deoxy-*N^6-*[2-*(*p*-*nitrophenyl)ethoxycarbonyl]adenosin* (**21**). To a mixture of 30 ml of hexamethyldisilazane (HMDS) and 30 ml of anh. dioxane are added 5.38 g (20 mmol) of **1** and a few crystals of $(NH_4)_2SO_4$. After refluxing for 3 h, the mixture is evaporated, the residue dissolved in 120 ml of toluene, filtered, and again evaporated. To the residue in 400 ml of anh. CH_2Cl_2, 12.5 g (40 mmol) of 1-methyl-3-{[2-(*p*-nitrophenyl)ethoxy]carbonyl}imidazolium chloride [39] are added and stirred for 18 h at r.t. The precipitate is filtered off, the filtrate evaporated, and the residue in 200 ml of MeOH and 50 ml of Et_3N stirred over night. The crystalline precipitate is washed with cold MeOH and dried at 60°: 8.33 g (94%) of **21**. M.p. 124°. Anal. calc. for $C_{19}H_{20}N_6O_7 \cdot H_2O$ (462.4): C 49.35, H 4.80, N 18.17; found: C 49,15, H 4.45, N 17.85.

21. *3'-Deoxy-5'-O-(monomethoxytrityl)* N^6-*[2-(*p*-nitrophenyl)ethoxycarbonyl]adenosine* (**22**). A soln. of 8.0 g (18 mmol) of **21** in 40 ml of abs. pyridine is evaporated. To the residue in 90 ml of abs. pyridine, 6.78 g (22 mmol) of *p*-monomethoxytrityl chloride are added and stirred at r.t. for 18 h. After evaporation, the residue is dissolved in 200 ml of $CHCl_3$ and extracted with 200 ml of phosphate buffer (pH 7). The aq. phase is extracted 3 times with 30 ml of $CHCl_3$ each, the org. phase dried (Na_2SO_4) and evaporated, and the residue thrice coevaporated with each 50 ml of toluene. The residue in CH_2Cl_2 is chromatographed on a short silica-gel column (200 g of silica gel) with CH_2Cl_2, $CH_2Cl_2/CHCl_3$ 1:1, and $CHCl_3$, yielding 11.6 g (90%) of an amorphous solid. Anal. calc. for $C_{39}H_{36}N_6O_8$ (716.8): C 65.35, H 5.06, N 11.73; found: C 65.41, H 5.02, N 11.54.

22. *3'-Deoxy-5'-O-(monomethoxytrityl)-*N^6,*2'-O-bis[2-(*p*-nitrophenyl)ethoxycarbonyl]adenosine* (**23**). To a soln. of 0.717 g (1 mmol) of **22** and 36 mg (0.3 mmol) of 4-(dimethylamino)pyridine in 10 ml of CH_2Cl_2, 0.624 g (2 mmol) of 1-methyl-3-{[2-(*p*-nitrophenyl)ethoxy]carbonyl}imidazolium chloride [39] are added and stirred for 9 h at r.t. After dilution with $CHCl_3$, the org. phase is washed twice with H_2O, dried (Na_2SO_4) and evaporated. The residue in CH_2Cl_2 is chromatographed on a silica-gel column (24 × 2.5 cm) with CH_2Cl_2, $CH_2Cl_2/CHCl_3$ 1:1, and $CHCl_3$, yielding 0.81 g (89%) of an amorphous solid foam. Anal. calc. for $C_{48}H_{43}N_7O_{12}$ (909.9): C 63.36, H 4.76, N 10.78; found: C 63.00, H 4.99, N 10.71.

23. *3'-Deoxy-5'-O-(monomethoxytrityl)-*N^6*[2-(*p*-nitrophenyl)ethoxycarbonyl]adenosine 2'-[2,5-Dichlorphenyl 2-(*p*-Nitrophenyl)ethyl Phosphate]* (**24**). In 23 ml of abs. pyridine are dissolved 1.28 g (18.6 mmol) of 1,2,4-triazole and 2.32 g (8.7 mmol) of 2,5-dichlorophenyl phosphorodichloridate. After stirring for 30 min at r.t., the mixture is cooled to 0° and a soln. of 4.2 g (5.8 mmol) of **22** in 34 ml of pyridine added dropwise and stirred for 1 h. Then, 2.9 g (17.4 mmol) of 2-(*p*-nitrophenyl)ethanol is added and stirred for 16 h at r.t. The reaction is stopped by addition of 300 ml of phosphate buffer (pH 7) and then the product extracted 3 times with each 150 ml of $CHCl_3$. The org. layers are again treated with phosphate buffer and H_2O. The combined extract is dried (Na_2SO_4), evaporated, and coevaporated 3 times with each 40 ml of toluene. The residue is chromatographed on a silica-gel column (50 × 4.5 cm) with CH_2Cl_2, $CHCl_3$ and $CHCl_3$/MeOH 200:1. The main fraction yields 5.1 g (81%) of **24**. Colourless solid foam. Anal. calc. for $C_{53}H_{46}Cl_2N_7O_{13}P$ (1090.9): C 58.36, H 4.25, N 8.99; found: C 58.15, H 4.29, N 9.03.

24. *3'-Deoxy-*N^6,*2'-O-bis[2-(*p*-nitrophenyl)ethoxycarbonyl]adenosine* (**25**). To 40 ml of 1% TsOH in CH_2Cl_2/MeOH 4:1, 0.91 g (1 mmol) of **23** are added and stirred for 15 min at r.t. The mixture is diluted with 100 ml of $CHCl_3$ and then extracted 3 times with 100 ml of phosphate buffer (pH 7). The org. layer is dried (Na_2SO_4), filtered, and evaporated and the residue in little $CHCl_3$ chromatographed on a silica-gel column (22 × 2.5 cm) with $CHCl_3$ and then with $CHCl_3$/MeOH 100:1, yielding 0.57 g (89%) of a colourless amorphous powder. Anal. calc. for $C_{28}H_{27}N_7O_{11}$ (637.6): C 52.75, H 4.27, N 15.38; found: C 52.77, H 3.93, N 15.34.

25. *3'-Deoxy-*N^6*-[2-(*p*-nitrophenyl)ethoxycarbonyl]adenosine 2'-[2,5-Dichlorophenyl 2-(*p*-Nitrophenyl)ethyl Phosphate]* (**26**). To 40 ml of 1% TsOH in CH_2Cl_2/MeOH 4:1 are added 1.09 g (1 mmol) of **24** and stirred for 15 min at r.t. The soln. is diluted with 60 ml of CH_2Cl_2 and then treated 3 times with each 100 ml of phosphate buffer (pH 7). The org. layer is dried (Na_2SO_4), filtered, and evaporated and the residue in little $CHCl_3$ chromatographed on a silica-gel column (20 × 2.5 cm) with $CHCl_3$ and $CHCl_3$/MeOH 100:1, yielding, after drying *in vacuo* 0.75 g (92%) of **26** as a solid foam. Anal. calc. for $C_{33}H_{30}Cl_2N_7O_{12}P$ (818.5): C 48.42, H 3.69, N 11.98; found: C 48.32, H 3.97, N 11.98.

26. *3'-Deoxy-5'-O-(monomethoxytrityl)-*N^6*-[2-(*p*-nitrophenyl)ethoxycarbonyl]adenylyl-{2'-[*O^P*-(2-(*p*-nitrophenyl)ethyl)]→5'}-3'-deoxy-*N^6*-[2-(*p*-nitrophenyl)ethoxycarbonyl]adenosine 2'-[2,5-Dichlorophenyl 2-(*p*-Nitrophenyl)ethyl Phosphate]* (**27**). A soln. of 1.05 g (1 mmol) of **15** and 0.655 g (0.08 mmol) of **26** in 5 ml of abs. pyridine is evaporated. This process is repeated 3 times. Then, 0.46 ml (6 mmol) of 1-methylimidazole and 0.606 g (2 mmol) of 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl) are added to the residue in 8 ml of abs. pyridine and stirred for 2 h at r.t. The reaction is stopped by addition of 100 ml of phosphate buffer (pH 7) and the product extracted 4 times with 50 ml of CH_2Cl_2. The united org. phase is washed with 200 ml of phosphate buffer, dried (Na_2SO_4), evaporated, and coevaporated twice with 40 ml of toluene. The residue in $CHCl_3$ is chromatographed on a silica-gel column (22 × 2 cm) with $CHCl_3$ and $CHCl_3$/MeOH 100:1 to 100:2, yielding 1.23 g (88%) of **27** as a solid foam. Anal. calc. for $C_{80}H_{72}Cl_2N_{14}O_{24}P_2$ (1746.2): C 55.02, H 4.16, N 11.23; found: C 54.68, H 4.06, N 11.21.

27. *3'-Deoxy-5'-O-(monomethoxytrityl)-*N^6*-[2-(*p*-nitrophenyl)ethoxycarbonyl]adenylyl-{2'-[*O^P*-(2-(*p*-nitrophenyl)ethyl)]→5'}-3'-deoxy-*N^6*-[2-(*p*-nitrophenyl)ethoxycarbonyl]adenosine 2'-[2-(*p*-Nitrophenyl)ethyl Triethylammonium Phosphate]* (**28**). To 72 ml of dioxane/H_2O/Et_3N 1:1:1, 2.0 g (12 mmol) of 4-nitrobenzaldehyde oxime are added and stirred for 15 min. Then, 2.1 g (1.2 mmol) of **27** are added. The mixture is stirred for 40 min at

r.t., evaporated, and coevaporated with 50 ml of pyridine and twice with 50 ml of toluene. The residue is chromatographed on a silica-gel column (18 × 2.5 cm) with $CHCl_3$, $CHCl_3$/MeOH 100:3, and $CHCl_3$/MeOH/Et_3N 100:4:3. The main fraction is evaporated and coevaporated several times with $CHCl_3$ and CH_2Cl_2, yielding 1.86 g (91%) of **28** as a solid foam. Anal. calc. for $C_{80}H_{85}N_{15}O_{24}P_2 \cdot 2\,H_2O$ (1738.2): C 55.26, H 5.12, N 12.08; found: C 54.94, H 5.41, N 11.81.

28. *3'-Deoxy-*N^6*-[2-(*p*-5'-O-(monomethoxytrityl)nitrophenyl)ethoxycarbonyl]adenylyl-{2'-[*O^P*-(*p*-nitrophenyl)ethyl)]→5'}-3'-deoxy-*N^6*-[2-(*p*-nitrophenyl)ethoxycarbonyl]adenylyl-{2'-[*O^P*-2-(*p*-nitrophenyl)ethyl)]→5'}-3'-deoxy-*N^6*,2'-O-bis[2-(*p*-nitrophenyl)ethoxycarbonyl]adenosine* (**29**). A mixture of 0.851 g (0.5 mmol) of **28** and 0.287 g (0.45 mmol) of **25** in 10 ml of abs. pyridine is evaporated. This process is repeated twice. To the residue in 5 ml of abs. pyridine, 0.23 g (1 mmol) of quinoline-8-sulfonyl chloride and 0.342 g (3 mmol) of 3-nitro-1,2,4-triazole are added and stirred at r.t. for 24 h. The soln. is diluted with 100 ml of H_2O, 4 times extracted with each 50 ml of $CHCl_3$, the org. phase dried (Na_2SO_4), evaporated, and coevaporated 3 times with 40 ml of toluene. The residue in little $CHCl_3$ is chromatographed on a silica-gel column (22 × 2.5 cm) with $CHCl_3$ and a $CHCl_3$/MeOH gradient 100:1→100:3. The product in 100 ml of $CHCl_3$ is washed twice with 100 ml of H_2O and the org. layer dried (Na_2SO_4) and evaporated to a solid foam. The colourless material is dried at 40°/high vacuum: 0.91 g (91%) of **29**. Anal. calc. for $C_{102}H_{95}N_{21}O_{34}P_2$ (2220.9): C 55.76, H 4.31, N 13.24; found: C 54.92, H 4.22, N 13.01.

29. *3'-Deoxyadenylyl-(2'→5')-3'-deoxyadenylyl-(2'→5')-3'-deoxyadenosine* (**30**). a) To a mixture of 2 ml of H_2O/dioxane 1:1, 70 mg of tetramethylguanidine, and 75 mg of pyridine-4-carbaldehyde oxime, 22 mg (0.0114 mmol) of **12** are added and stirred for 5 h at r.t. The yellow soln. is evaporated, the residue in little $CHCl_3$ chromatographed on a prep. silica-gel plate (40 × 20 × 0.2 cm) with $CHCl_3$/MeOH 3:1, and the main band eluted with 100 ml of $CHCl_3$/MeOH 1:9). To its residue in 1 ml of dioxane, 5 ml of conc. NH_3 are added and stirred for 42 h at r.t. The mixture is again evaporated and the residue in 1.5 ml of 80% AcOH soln. stirred at r.t. for 90 min. After evaporation and 2 coevaporations with 5 ml of EtOH, the residue is dissolved in 10 ml of H_2O and extracted 3 times with each 5 ml of $CHCl_3$. The aq. phase is chromatographed on a *DEAE-Sephadex* column (60 × 1 cm) with a linear gradient of (0.001–0.5 mol) of Et_3NHCO_3 buffer (pH 7.4). The product fractions are evaporated and several times coevaporated with H_2O to yield 307 *OD* units (75%). HPLC (*LiChrosorb RP-18* column, 250 × 4.6 mm) with 0.1M NH_4OAc/MeCN 95:5 shows a retention time of 564 s for **30**.

b) A soln. of 14 mg of **19** in 3 ml of 0.5M DBU in pyridine is stirred for 2 h at 25–27°, then neutralized by addition of 1.5 ml of 1M AcOH and evaporated. The residue is treated with abs. pyridine, evaporated again, and then dissolved in 2 ml of 0.5M Bu_4NF. The mixture is stirred 7 h at r.t. and then quenched by addition of 2 ml of abs. MeOH. The mixture is evaporated, coevaporated twice with MeOH/toluene and then taken up in 10 ml of 25% (i-Bu)NH_2 in MeOH. The mixture is stirred for 2 days, evaporated, and the resulting residue treated with 5 ml of 80% AcOH soln. for 15 h at r.t. Some H_2O is added, the mixture evaporated, and the residue in 30 ml of H_2O extracted thrice with $CHCl_3$. The aq. layer is again evaporated and the residue in 20 ml of H_2O chromatographed on a *DEAE-Sephadex A-25* column (60 × 1 cm) with a gradient (0.001–0.3M) of TBK buffer (pH 7.5). The fractions of the main peak are evaporated and several times coevaporated with H_2O until all Et_3NHCO_3 is decomposed. Finally, a yield of 87% is calculated from the UV spectrum (256 nm; ε = 33300).

c) To 6 ml of 0.5M DBU in abs. pyridine, 22 mg (10 μmol) of **29** are added and stirred for 24 h at r.t. Neutralization is achieved by addition of 180 mg (3 mmol) of AcOH in 3 ml of pyridine. The mixture is evaporated and the residue treated with 5 ml of 80% aq. AcOH for 5 h at r.t. After evaporation and several coevaporations with H_2O till all AcOH is removed, the residue in 30 ml of H_2O is extracted 3 times with each 15 ml of $CHCl_3$ and the aq. layer evaporated to *ca.* 15 ml and chromatographed on a *DEAE-Sephadex A-25* column (60 × 1 cm) with a linear gradient (0–0.1M) of Et_3NHCO_3 buffer (pH 7.5). The fractions of the main peak are evaporated and coevaporated many times with bidistilled H_2O until the components of the buffer are removed. Finally, a yield of 82% is determined by UV (256 nm; ε = 33300). More physical data have been published elsewhere [42].

30. *Determination of the Hypochromicity of* **30**. Two batches of each 5 *OD* units of **30** in 100 μl of H_2O are incubated after addition of 5 μl of *Tris*-HCl buffer (pH 8.9), 5 μl of 0.1M $MgCl_2$ and 10 μl of snake-venom phosphodiesterase at 37°. The first batch is used to follow the progress of the enzymatic clevage chromatographically every 30 min. After complete hydrolysis (5 h) to the monomeric building blocks, the second batch is diluted to 5 ml with phosphate buffer (pH 7). This soln. is measured spectrophotometrically against a reference soln. containing the same components without the substrate. The percentage hypochromicity is calculated from the extinctions at the absorption maximum according to the equation % hypochromicity = $(1-E_{trimer}/E_{monomer}) \cdot 100$.

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