64. Nucleotides

Part XLVII[1])

Synthesis of 3′-Deoxyadenylyl-(2′–5′)-3′-deoxyadenylyl-(2′–5′)-3′-*O*-(2-hydroxyethyl)adenosine and 3′-Deoxyadenylyl-(2′–5′)-3′-deoxyadenylyl-(2′–5′)-3′-*O*-{2-[(cholest-5-en-3*β*-yloxy)carbonyloxy]ethyl}adenosine: A New Type of (2′–5′)Adenylate Trimer Conjugate

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(20.XI.95)

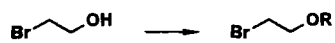
A suitably protected adenosine derivative bearing an acetyl-protected 2-hydroxyethyl moiety in 3′-*O*-position was attached to the 2′-terminus of a cordycepin (3′-deoxyadenosine) dimer. Coupling was performed by phosphoramidite chemistry using two alternative approaches – condensation of 5′-phosphoramidite **16** with 2′-OH cordycepin dimer **18** and condensation of dimeric cordycepin phosphoramidite **19** with 5′-OH adenosine derivative **15** – of which the latter synthesis worked best (→**20**). After cleavage of the acetyl protecting group (→**21**), cholesteryl carbonate was introduced into the OH function of the spacer (→**24**). Final deblocking of trimer **24** with, or trimer **21** without the cholesterol moiety afforded the modified cordycepin conjugates **25** and **23**, respectively.

1. Introduction. – The discovery of the naturally occurring (2′–5′)adenylates [2] [3] is closely connected to the elucidation of interferon action, the cellular response to virus infection. In 1981, the antiviral activity and enhanced metabolic stability of the 3′-deoxy analogue (= cordycepin) trimer was first reported by *Doetsch et al.* [4]. Since then, more details of the antiviral properties of the cordycepin trimer have been published [5–8]. Its most striking feature is the inhibition of HIV-1 RT (reverse transcriptase) [9] by interfering with the anticodon region of $tRNA^{Lys,3}$, the primer of RT [10] [11]. More recently, it was shown [12] that the antiviral activity of the cordycepin trimer core 3′d(A2′p5′A2′p5′A) can be augmented up to 1000-fold by the introduction of lipophilic moieties such as cholesterol (cholest-5-en-3*β*-ol). Cholesterol improves most likely cell penetration of the polyanionic oligonucleotides [13–20]. Contrary to the wide-spread practice using phosphordiester bonds for conjugate coupling [21], *Wasner et al.* [12] attached the cholesterol moiety *via* ester linkages to the 2′- and 5′-end of the cordycepin trimer core. To investigate the antiviral properties of cordycepin trimer core derivatives with an ether-bound spacer, we wish to report on the synthesis of the title compounds **23** and **25** bearing a 2-hydroxyethyl spacer attached to the 3′-hydroxy function of the 2′-terminal adenosine moiety.

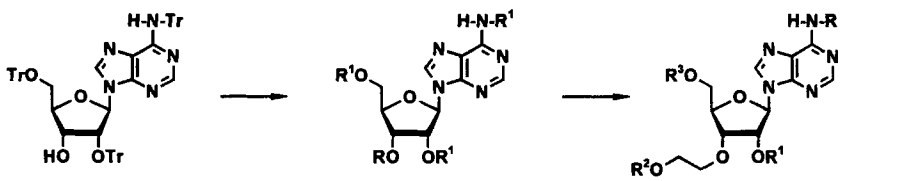
[1]) Part XLVI: [1].

2. Syntheses. – The synthesis of the title compounds **23** and **25** was the result of a multistep procedure involving as the key part the condensation of an appropriately protected cordycepin dimer (**18**, **19**) and the 3′-*O*-(acetoxyethyl)adenosine derivatives **15** and **16**, respectively. The latter compound was synthesized from adenosine which was first converted into its N^6,2′-*O*,5′-*O*-tris(trityl) derivative **3** by a known procedure [22–24]. The alkylations of **3** were performed with 1-bromo-2[dimethyl(thexyl)silyloxy]- (**1**) and 1-bromo-2-[(*tert*-butyl)dimethylsilyloxy]ethane (**2**) [25] to give, under *Williamson* conditions using NaH as base, the 3′-*O*-alkyl ethers **4** and **5** in 68 and 81% yield,

Scheme



1 R = tds
2 R = tbds



3

	R	R¹
4	$(CH_2)_2$Otds	Tr
5	$(CH_2)_2$Otbds	Tr
6	$(CH_2)_2$Otds	H
7	$(CH_2)_2$OH	Tr
8	$(CH_2)_2$OAc	H
9	Ac	Tr

	R	R¹	R²	R³
10	H	H	Ac	MeOTr
11	npeoc	H	Ac	H
12	npeoc	H	Ac	MeOTr
13	npeoc	npes	Ac	MeOTr
14	npeoc	npes	H	MeOTr
15	npeoc	npes	Ac	H
16	npeoc	npes	Ac	$(i\text{-Pr})_2$NPOnpe

tds = dimethyl(thexyl)silyl, tbds = (*tert*-butyl)dimethylsilyl, Tr = trityl, MeOTr = monomethoxytrityl, npeoc = 2-(4-nitrophenyl)ethyl, npes = 2-(4-nitrophenyl)ethoxysulfonyl, i-Pr = isopropyl

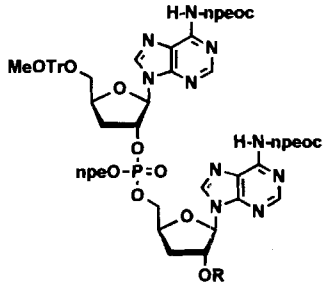
respectively. The silyl protecting groups turned out to be unstable under detritylation conditions, since treatment of **5** with $ZnBr_2$ [26] under aprotic conditions gave the silylated compound **6** only in 40% yield. Therefore, the silyl group in **4** or **5** was cleaved off with fluoride ions (Bu_4NF) in THF to give 3′-*O*-(2-hydroxylethyl)-N^6,2′-*O*,5′-*O*-tris(trityl)-adenosine (**7**) in 88 and 77% yield respectively. Then, compound **7** was acetylated and subsequently detritylated with 80% AcOH/H_2O to afford intermediate **8** in 81% overall yield. Attempts to alkylate **3** with 2-bromoethyl acetate directly led only to the 3′-*O*-acetyl derivative **9** in 81% yield.

In a series of steps, compound **8** was then converted into the monomeric building block **16**. Monomethoxytritylation of **8** gave **10** only in 54% yield. Better results were obtained when the 6-amino function of the base was protected first by the 2-(4-nitrophenyl)ethoxycarbonyl (npeoc) group [27–29] using 3-methyl-1-[2-(4-nitrophenyl)-

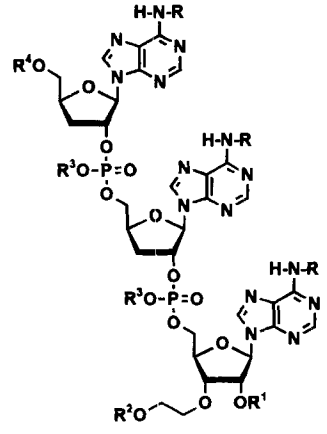
ethoxycarbonyl]-1*H*-imidazolium chloride under activation with 4-(dimethylamino)-pyridine (DMAP) to give compound **11** in 78% yield. Selective monomethoxytritylation afforded derivative **12** then in 81% yield. The 2′-OH position was finally protected by the 2-(4-nitrophenyl)ethoxysulfonyl (npes) group [30] leading to **13** in 85% yield. The npes group was chosen in preference to the npeoc blocking group due to its greater stability under deacetylation conditions. This was verified by treatment of **13** with K_2CO_3/MeOH to give **14** in 63% yield. Furthermore, detritylation of **13** with 2% toluene-4-sulfonic acid in $CHCl_3$/MeOH led to **15** in 94% yield, and its conversion into the corresponding 5′-phosphoramidite **16** could be performed with bis(diisopropylamino)[2-(4-nitrophenyl)ethoxy]phosphane [31] in the presence of 1*H*-tetrazole in 59% yield.

The second component for the trimer formation was prepared from 3′-deoxy-5′-*O*-(monomethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]}→5′}-2′-*O*-acetyl-3′-deoxy-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**17**) [32] yielding **18** by deacetylation with K_2CO_3/MeOH in 80%. Analogously to the synthesis of **16**, the dimeric phosphoramidite **19** was obtained again in 80% yield from **18**.

The synthesis of trimer **20** was realized by two different routes – coupling either the 5′-phosphoramidite **16** with the 2′-OH group of cordycepin dimer **18** or the dimeric cordycepin phosphoramidite **19** with the 5′-OH function of adenosine derivative **15** – in yields of 78 and 87%, respectively. Cleavage of the acetyl group in **20** with K_2CO_3 gave intermediate **21** in 60% yield. Total deprotection of **21** was achieved by subsequent acid treatment to form compound **22** in 90% yield, and DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) treatment leading finally to **23** in 59% yield. On the other hand, intermediate **21** gave, on reaction with cholesteryl chloroformate in presence of 1-methyl-1*H*-imidazole



	R
17	Ac
18	H
19	(i-Pr)$_2$NPOnpe



	R	R^1	R^2	R^3	R^4
20	npeoc	npes	Ac	npe	MeOTr
21	npeoc	npes	H	npe	MeOTr
22	npeoc	npes	H	npe	H
23	H	H	H	NH_4^+	H
24	npeoc	npes	COOchol	npe	MeOTr
25	H	H	COOchol	HDBU$^+$	H

chol = cholest-5-en-3β-yl

and DMAP, the fully protected conjugate **24** in 78% yield, and subsequent stepwise deblocking with DBU and acid without purification of intermediates afforded the conjugate **25** in 85% yield as colorless powder. Contrary to the deblocked trimer **23** the cholesterol conjugate **25** was insoluble in H_2O, but soluble in DMSO, various buffers, and ternary mixtures of CH_2Cl_2/MeOH/H_2O.

Experimental Part

General. TLC: Precoated silica gel TLC sheets *F 1500 LS 254* from *Schleicher & Schüll.* Prep. TLC: silica gel *60 PF_{254}* (*Merck*). Prep. column flash chromatography (FC): silica gel for flash chromatography (*Baker*); 0.2 bar. Ion-exchange chromatography: *Pharmacia* (detection at 260 nm; flow rate 1 ml/min); column *XK 16/70*, packed with *DEAE Sephadex A 25* (HCO_3^-; *Pharmacia*). HPLC: *Merck-Hitachi L 6200, L-3000* photo diode array detector; column *RP 18, LiChrosphere* 125 × 4 mm, 5 μm, *Merck*; flow rate 1 ml/min. UV/VIS: *Perkin Elmer Lambda 5*; λ_{max} in nm (log ε). ^{1}H-NMR: *Bruker AC 250*; δ in ppm rel. to $CHCl_3$ ((D_6)DMSO). ^{31}P-NMR: *Jeol JM 6X-400*; δ in ppm rel. to 85% H_3PO_4 soln.

2-Bromo-1-[dimethyl(1,1,2-trimethylpropyl)silyloxy]ethane (**1**). To an ice-cooled mixture of dimethyl(thexyl)silyl chloride (dimethyl(1,1,2-trimethylpropyl)silyl chloride; 50 ml, 0.25 mol) and 1*H*-imidazole (23 g, 0.34 mol) in abs. DMF (100 ml) was added dropwise a soln. of 2-bromoethanol (20 ml, 0.28 mol) in abs. DMF (100 ml). The mixture was stirred overnight, diluted with light petroleum ether (250 ml), and washed with H_2O (2 × 250 ml). The aq. phases were reextracted with light petroleum ether (3 × 250 ml), and the combined org. layer was dried (Na_2SO_4) and evaporated. The residue was submitted to vacuum destillation (73°/0.4 mbar): 53 g (80%) of **1**. Colorless oil. ^{1}H-NMR ($CDCl_3$): 3.85 (*t*, CH_2O); 3.37 (*t*, CH_2Br); 1.60–1.50 (*m*, CH); 0.85 (*m*, 4 MeC); 0.10 (*s*, 2 MeSi). Anal. calc. for $C_{10}H_{23}BrOSi$ (267.3): C 44.94, H 8.67; found: C 45.22, H 8.58.

3′-O-{2-[Dimethyl(1,1,2-trimethylpropyl)silyloxy]ethyl}-N^6,2′-O,5′-O-tris(triphenylmethyl)adenosine (**4**). A mixture of *N^6,2′-O,5′-O*-tris(triphenylmethyl)adenosine (**3**) [22–24] (18.7 g, 19 mmol) and 80% oil-immersed NaH (5.0 g, 170 mmol) in abs. MeCN (500 ml) was stirred at r.t. for 20 min, then NaI (1.5 g, 10 mmol) was added. A soln. of **1** (30.0 g, 110 mmol) in abs. MeCN (10 ml) was added dropwise, and the mixture was kept overnight. Then it was diluted with AcOEt (250 ml) and washed with phosphate buffer pH 7 (250 ml) and sat. NaCl soln. (3 × 250 ml). The aq. phases were reextracted with AcOEt (3 × 250 ml), and the combined org. layer was dried (Na_2SO_4) and evaporated, and the residue purified by FC (silica gel, 6 × 30 cm, light petroleum ether → light petroleum ether/AcOEt 2:1): 15.4 g (68%) of **4**. Amorphous solid. UV (MeOH): 282 (sh, 4.18), 274 (4.32), 270 (sh, 4.31). ^{1}H-NMR ($CDCl_3$): 7.70, 7.66 (2*s*, H–C(2), H–C(8)); 7.38–7.00 (*m*, 45 H of Tr); 6.90 (s, NH); 6.04 (*d*, *J* = 7.4, H–C(1′)); 5.13 (*dd*, H–C(2′)); 4.12 (*m*, H–C(4′)); 3.69–3.63 (*m*, CH_2Otds); 3.30–3.00 (*m*, CH_2O–C(3′), 2 H–C(5′)); 2.79 (‘*d*’, H–C(3′)); 1.60–1.50 (*m*, CH); 0.87–0.82 (*m*, 4 MeC); 0.11, 0.08 (2s, 2 MeSi, diast.) Anal. calc. for $C_{77}H_{77}N_5O_5Si$ (1180.6): C 78.34, H 6.57, N 5.93; found: C 78.13, H 6.67, N 5.80.

3′-O-{2-[(tert-Butyl)dimethylsilyloxy]ethyl}-N^6,2′-O,5′-O-tris(triphenylmethyl)adenosine (**5**). As described for **4**, with **3** (1.00 g, 1.0 mmol), 80% oil-immersed NaH (0.29 g, 9.6 mmol), abs. MeCN (30 ml), NaI (0.12 g, 0.8 mmol), and 2-bromo-1-[(*tert*-butyl)dimethylsilyloxy]ethane (**2**) [22]. Workup with AcOEt (3 × 50 ml) and sat. NaCl soln. (3 × 50 ml) and purification by FC (silica gel, 3 × 8 cm, light petroleum ether → light petroleum ether/AcOEt 3:1) gave 0.92 g (81%) of **5**. Amorphous solid. UV (MeOH): 280 (sh, 4.24), 274 (4.33), 270 (sh, 4.32). ^{1}H-NMR ($CDCl_3$): 7.70, 7.67 (2*s*, H–C(2), H–C(8)); 7.38–6.92 (*m*, 45 H of Tr, NH); 6.05 (*d*, *J* = 7.3, H–C(1′)); 5.10 (*dd*, H–C(2′)); 4.13 (*m*, H–C(4′)); 3.73–3.60 (*m*, CH_2Otbds); 3.25–3.05 (*m*, CH_2O–C(3′), 2 H–C(5′)); 2.79 (*dd*, H–C(3′)); 1.56 (*s*, ½ H_2O); 0.87 (*s*, 3 MeC); 0.07, 0.04 (2*s*, 2 MeSi, diast.). Anal. calc. for $C_{75}H_{73}N_5O_5Si \cdot$ ½ H_2O (1161.5): C 77.56, H 6.41, N 6.03; found: C 77.33, H 6.48, N 6.21.

3′-O-{2-[(tert-Butyl)dimethylsilyloxy]ethyl}adenosine (**6**). A mixture of **5** (0.20 g, 0.17 mmol), dry $ZnBr_2$ (0.21 g, 0.94 mmol), abs. toluene (1.5 ml), and abs. nitromethane (3.5 ml) was kept 5 h at 70°. Then the mixture was diluted with AcOEt (50 ml) and washed with phosphate buffer pH 7 (3 × 50 ml). The aq. phases were reextracted with AcOEt (3 × 50 ml), and the combined org. phase was dried (Na_2SO_4) and evaporated. The residue was purified by FC (silica gel, 2 × 10 cm, toluene/AcOEt 1:1 → 1:1 + 10% MeOH): 0.03 g (40%) of **6**. Amorphous solid. UV (MeOH): 259 (4.18). ^{1}H-NMR ((D_6)DMSO): 8.36, 8.14 (2*s*, H–C(2), H–C(8)); 7.39 (br. *s*, NH_2); 5.88 (*d*, *J* = 6.1, H–C(1′)); 5.50 (br. *s*, OH–C(5′)); 5.41 (*d*, OH–C(2′)); 4.33 (*dd*, H–C(2′)); 4.04 (*m*, H–C(3′), H–C(4′)); 3.75–3.61 (*m*, CH_2Otbds, CH_2O–C(3′), 2 H–C(5′)); 3.38 (br. *s*, ½ H_2O); 0.91 (*s*, 3 MeC); 0.13 (*s*, 2 MeSi). Anal. calc. for $C_{18}H_{31}N_5O_5Si \cdot$ ½ H_2O (434.6): C 49.75, H 7.19, N 16.11; found: C 49.60, H 7.57, N 16.01.

3′-O-{2-(Hydroxyethyl)-N⁶,2′-O,5′-O-tris(triphenylmethyl)adenosine (**7**). *a*) A mixture of **4** (15.4 g, 13 mmol), $Bu_4NF \cdot 3\,H_2O$ (4.5 g, 14 mmol), and abs. THF (30 ml) was kept at r.t. for 4 h. Then, it was diluted with AcOEt (150 ml) and washed with H_2O (3 × 100 ml). The aq. phases were reextracted with AcOEt (2 × 100 ml), the combined org. layer was dried (Na_2SO_4) and evaporated, and the residue purified by FC (silica gel, 5 × 13 cm, toluene → toluene/AcOEt 1:1): 11.9 g (88%) of **7**. Amorphous solid. UV (MeOH): 284 (sh, 4.16), 274 (4.34), 268 (sh, 4.33). ^{1}H-NMR ((D$_6$)DMSO): 8.35 (br. *s*, NH); 7.50, 7.49 (2*s*, H–C(2), H–C(8)); 7.38–7.00 (*m*, 45 H of Tr); 6.14 (*d*, *J* = 7.7, H–C(1′)); 5.11 (*dd*, H–C(2′)); 4.61 (*t*, OH); 4.04 (*m*, H–C(4′)); 3.49–2.88 (*m*, CH_2OH, CH_2O–C(3′), 2 H–C(5′)); 3.34 (*s*, H_2O); 2.45 (*m*, H–C(3′)). ^{1}H-NMR ($CDCl_3$): 7.80, 7.74 (2*s*, H–C(2), H–C(8)); 7.38–6.99 (*m*, 45 H of Tr, NH); 6.13 (*d*, *J* = 7.4, H–C(1′)); 5.18 (*dd*, H–C(2′)); 4.08 (*m*, H–C(4′)); 3.63 (*m*, CH_2OH); 3.33–3.23, 3.07–2.95 (*m*, CH_2O–C(3′), 2 H–C(5′)); 2.74 ('*d*', H–C(3′)); 1.70 (br. *s*, H_2O). Anal. calc. for $C_{69}H_{59}N_5O_5 \cdot H_2O$ (1056.3): C 78.46, H 5.63, N 6.63; found: C 78.60, H 5.74, N 6.72.

b) As described in *a*, with **5** (2.6 g, 2.3 mmol), $Bu_4NF \cdot 3\,H_2O$ (0.7 g, 2.3 mmol), and abs. THF (5 ml). Workup and purification by FC gave 1.8 g (77%) of **7**. Amorphous solid.

3′-O-(2-Acetoxyethyl)adenosine (**8**). A soln. of **7** (1.8 g, 1.7 mmol) and Ac_2O (8.2 ml, 87 mmol) in abs. pyridine (30 ml) was stirred at r.t. for 1 h. Toluene was added and the soln. evaporated. The residue was dried overnight, then 80% AcOH/H_2O (20 ml) was added. The mixture was kept 1 h at 100°, evaporated, co-evaporated with H_2O and MeOH, and purified by FC (silica gel, 2 × 7 cm, CH_2Cl_2 → CH_2Cl_2/MeOH 95:5): 0.50 g (81%) of **8**. Amorphous solid. UV (MeOH): 258 (4.17). ^{1}H-NMR ((D$_6$)DMSO): 8.36, 8.14 (2*s*, H–C(2), H–C(8)); 7.37 (*s*, NH_2); 5.87 (*d*, *J* = 6.4, H–C(1′)); 5.53–5.48 (*m*, OH–C(2′), OH–C(5′)); 4.75 (*dd*, H–C(2′)); 4.18 (*t*, CH_2OAc); 4.03 (*m*, H–C(3′), H–C(4′)); 3.91–3.55 (*m*, CH_2O–C(3′), 2 H–C(5′)); 2.03 (*s*, Me). Anal. calc. for $C_{14}H_{19}N_5O_6$ (353.3): C 47.59, H 5.42, N 19.82; found: C 47.86, H 5.68, N 19.54.

3′-O-Acetyl-N⁶,2′-O,5′-O-tris(triphenylmethyl)adenosine (**9**). As described for **4**, with **3** (0.22 g, 0.22 mmol), 80% oil-immersed NaH (0.04 g, 1.2 mmol), abs. MeCN (10 ml), NaI (0.005 g, 0.03 mmol), and 2-bromoethyl acetate (0.12 g, 0.71 mmol). Workup and purification by FC yielded 0.19 g (81%) of **9**. Amorphous solid. UV (MeOH): 285 (sh, 4.13), 274 (4.34), 270 (sh, 4.33). ^{1}H-NMR ((D$_6$)DMSO): 8.41 (*s*, NH); 7.62, 7.49 (2*s*, H–C(2), H–C(8)); 7.49–6.95 (*m*, 45 H of Tr); 6.32 (*d*, *J* = 8.0, H–C(1′)); 5.28 (*dd*, H–C(2′)); 3.95 (*m*, H–C(4′)); 3.49 ('*d*', H–C(3′)); 3.03, 2.95 (*m*, 2 H–C(5′)); 1.96 (*s*, Me). Anal. calc. for $C_{69}H_{57}N_5O_5$ (1036.2): C 79.98, H 5.54, N 6.76; found: C 79.73, H 5.18, N 6.51.

3′-O-(2-Acetoxyethyl)-5′-O-[(4-methoxyphenyl)diphenylmethyl]adenosine (**10**). After co-evaporation with dry pyridine (2 × 5 ml), **8** (0.37 g, 1.0 mmol) and monomethoxytrityl chloride (MeOTrCl; 0.39 g, 1.3 mmol) were stirred in dry pyridine (10 ml) at r.t. for 3 d. Then, more MeOTrCl (0.17 g, 0.5 mmol) was added and the soln. stirred for another 3 d. The mixture was evaporated and co-evaporated with toluene (3 × 10 ml). The residue was dissolved in AcOEt (50 ml), the soln. washed with sat. $NaHCO_3$ soln. (3 × 50 ml), and the aq. phase reextracted with AcOEt (3 × 50 ml). The combined org. layer was dried (Na_2SO_4) and evaporated, and the residue purified by FC (silica gel, 3 × 7 cm, CH_2Cl_2 → CH_2Cl_2/MeOH 95:5): 0.35 g (54%) of **10**. Amorphous solid. UV (MeOH): 259 (4.20), 233 (4.25). ^{1}H-NMR ((D$_6$)DMSO): 8.26, 8.08 (2*s*, H–C(2), H–C(8)); 7.34–7.18 (*m*, 12 H of MeOTr, NH); 6.77 (*d*, 2 H *o* to MeO); 5.90 (*d*, *J* = 4.6, H–C(1′)); 5.58 (*d*, OH–C(2′)); 4.88 (*dd*, H–C(2′)); 4.22 ('*t*', H–C(3′)); 4.14 (*m*, CH_2OAc, H–C(4′)); 3.83–3.62 (*m*, MeO, CH_2O–C(3′)); 3.23 (*m*, 2 H–C(5′)); 1.91 (*s*, Me). Anal. calc. for $C_{34}H_{35}N_5O_7$ (625.7): C 65.27, H 5.64, N 11.19; found: C 65.06, H 5.81, N 10.54.

3′-O-(2-Acetoxyethyl)-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**11**). A mixture of **8** (1.84 g, 5.2 mmol), hexamethyldisilazane (13 ml), abs. dioxane (15 ml), and a catalytic amount of $(NH_4)_2SO_4$ was refluxed for 4 h and then evaporated. The residue was dissolved in toluene (50 ml). After filtration, the soln. was evaporated. Then 3-methyl-1-[2-(4-nitrophenyl)ethoxycarbonyl]-1*H*-imidazolium chloride [27] (3.2 g, 10 mmol) and CH_2Cl_2 (100 ml) were added. The mixture was kept overnight and filtered, and the filtrate evaporated. To the residue, MeOH (100 ml) and Et_3N (10 ml) were added, and 2.21 g (78%) of **11** crystallized. Colorless crystals. M.p. 165°. UV (MeOH): 266 (4.45). ^{1}H-NMR ((D$_6$)DMSO): 10.64 (*s*, NH); 8.69, 8.63 (2*s*, H–C(2), H–C(8)); 8.17 (*d*, 2 H *o* to NO_2); 7.62 (*d*, 2 H *m* to NO_2); 5.99 (*d*, *J* = 5.6, H–C(1′)); 5.61 (*d*, OH–C(2′)); 5.22 (*t*, OH–C(5′)); 4.77 (*dd*, H–C(2′)); 4.40 (*t*, CH_2O of npeoc); 4.18 (*m*, CH_2OAc); 4.06 (br. *s*, H–C(3′), H–C(4′)); 3.90–3.50 (*m*, CH_2O–C(3′), 2 H–C(5′)); 3.13 (*t*, CH_2C of npeoc); 2.03 (*s*, Me). Anal. calc. for $C_{23}H_{26}N_6O_{10}$ (546.5): C 50.55, H 4.79, N 15.38; found: C 50.55, H 4.94, N 14.86.

3′-O-(2-Acetoxyethyl)-5′-O-[(4-methoxyphenyl)diphenylmethyl]-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]-adenosine (**12**). After co-evaporation with dry pyridine (2 × 5 ml), **11** (0.85 g, 1.6 mmol) and MeOTrCl (0.58 g, 1.9 mmol) were stirred in dry pyridine (20 ml) at r.t. for 1 d. Then, more MeOTrCl (0.10 g, 0.3 mmol) was added and the soln. stirred for another 2 d. The mixture was evaporated and co-evaporated with toluene (3 × 10 ml). The residue was dissolved in $CHCl_3$ (100 ml) and washed with sat. $NaHCO_3$ soln. (3 × 100 ml), the aq. phase reextracted with $CHCl_3$ (3 × 50 ml), the combined org. layer dried (Na_2SO_4) and evaporated, and the residue purified by FC (silica

gel, 3 × 7 cm, toluene/AcOEt 1:1→toluene/AcOEt/MeOH 10:10:1): 1.0 g (81%) of **12**. Amorphous solid. UV (MeOH): 272 (sh, 4.43), 266 (4.47), 231 (4.33). ^{1}H-NMR ((D$_6$)DMSO): 10.65 (*s*, NH); 8.58, 8.55 (2*s*, H–C(2), H–C(8)); 8.16 (*d*, 2 H *o* to NO$_2$); 7.62 (*d*, 2 H *m* to NO$_2$); 7.36–7.18 (*m*, 12 H of MeOTr); 6.84 (*d*, 2 H *o* to MeO); 6.01 (*d*, J = 4.7, H–C(1′)); 5.67 (*d*, OH–C(2′)); 4.95 (*dd*, H–C(2′)); 4.41 (*t*, CH$_2$O of npeoc); 4.25 (*t*′, H–C(3′)); 4.13 (*m*, C*H*$_2$OAc, H–C(4′)); 3.84–3.71 (*m*, CH$_2$O–C(3′), MeO); 3.25 (*m*, 2 H–C(5′)); 3.10 (*t*, CH$_2$C of npeoc); 1.92 (*s*, Me). Anal. calc. for $C_{43}H_{42}N_6O_{11}$ (818.8): C 63.07, H 5.17, N 10.26; found: C 62.54, H 5.04, N 10.19.

*3′-O-(2-Acetoxyethyl)-5′-O-[(4-methoxyphenyl)diphenylmethyl)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]-2′-O-[2-(4-nitrophenyl)ethoxysulfonyl]adenosine* (**13**). To **12** (0.26 g, 0.32 mmol), which was co-evaporated in abs. pyridine (2 × 5 ml), 2-(4-nitrophenyl)ethoxysulfonyl chloride [33] (0.17 g, 0.67 mmol) and dry pyridine (5 ml) were added. The mixture was kept at r.t. for 4 h, then evaporated and co-evaporated with toluene (3 × 10 ml). The residue was dissolved in $CHCl_3$ (100 ml) and washed with sat. $NaHCO_3$ soln. (3 × 100 ml), the aq. phase reextracted with $CHCl_3$ (3 × 50 ml), the combined org. layer dried (Na_2SO_4) and evaporated, and the residue purified by FC (silica gel, 2.5 × 12 cm, toluene/AcOt 1:1→toluene/AcOEt/MeOH 25:25:1): 0.28 g (85%) of **13**. Amorphous solid. UV (MeOH): 267 (4.58), 235 (4.36). ^{1}H-NMR ((D$_6$)DMSO): 10.72 (*s*, NH); 8.60, 8.49 (2*s*, H–C(2), H–C(8)); 8.15 (*m*, 4 H *o* to NO$_2$); 7.62, 7.49 (2*d*, 4 H *m* to NO$_2$); 7.32–7.20 (*m*, 12 H of MeOTr); 6.82 (*d*, 2 H *o* to MeO); 6.40 (*d*, J = 3.2, H–C(1′)); 6.02 (*dd*, H–C(2′)); 4.75 (*m*, H–C(3′)); 4.40 (*t*, CH$_2$O of npeoc); 4.19 (*m*, H–C(4′)); 4.10 (*m*, C*H*$_2$OAc); 3.90–3.70 (*m*, CH$_2$O–C(3′), CH$_2$O of npes); 3.71 (*s*, MeO); 3.38–3.20 (*m*, 2 H–C(5′)); 3.10 (*m*, CH$_2$C of npeoc, CH$_2$C of npes); 1.86 (*s*, Me). Anal. calc. for $C_{51}H_{49}N_7O_{15}$ (1032.1): C 59.35, H 4.79, N 9.50; found: C 58.95, H 4.70, N 9.68.

*3′-O-(2-Hydroxyethyl)-5′-O-[(4-methoxyphenyl)diphenylmethyl)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]-2′-O-[2-(4-nitrophenyl)ethoxysulfonyl]adenosine* (**14**). A mixture of **13** (0.12 g, 0.11 mmol) and catalytic amounts of K_2CO_3 in MeOH/CH_2Cl_2 2:1 (7.5 ml) was stirred at r.t. for 2 h. The mixture was diluted with $CHCl_3$ (100 ml) and washed with 10% citric acid (100 ml) and sat. NaCl soln. (2 × 100 ml), the aq. phase reextracted with $CHCl_3$ (3 × 50 ml), the org. layer dried (Na_2SO_4) and evaporated, and the residue purified by FC (silica gel, 2.5 × 10 cm, toluene/AcOEt 1:1→toluene/AcOEt/MeOH 25:25:1): 0.07 g (63%) of **14**. Amorphous solid. UV (MeOH): 267 (4.59), 235 (4.37). ^{1}H-NMR ((D$_6$)DMSO): 10.69 (*s*, NH); 8.69, 8.50 (2*s*, H–C(2), H–C(8)); 8.13 (*t*′, 4 H *o* to NO$_2$); 7.62, 7.48 (2*d*, 4 H *m* to NO$_2$); 7.33–7.20 (*m*, 12 H of MeOTr); 6.82 (*d*, 2 H *o* to MeO); 6.38 (*d*, J = 2.7, H–C(1′)); 6.03 (*dd*, H–C(2′)); 4.74 (*m*, H–C(3′)); 4.69 (*t*, OH); 4.40 (*m*, CH$_2$O of npeoc); 4.20 (*m*, H–C(4′)); 3.50 (*m*, C*H*$_2$OH); 3.71 (*s*, MeO); 3.70–3.20 (*m*, CH$_2$O–C(3′), CH$_2$O of npes, 2 H–C(5′)); 3.10 (*m*, CH$_2$C of npeoc, CH$_2$C of npes). Anal. calc. for $C_{49}H_{47}N_7O_{14}S$ (990.0): C 59.45, H 4.79, N 9.90; found: C 59.01, H 4.93, N 9.52.

*3′-O-(2-Acetoxyethyl)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]-2′-O-[2-(4-nitrophenyl)ethoxysulfonyl]-adenosine* (**15**). A soln. of **13** (1.95 g, 1.9 mmol) in MeOH/$CHCl_3$ 4:1 (30 ml) containing 2% of TsOH was stirred at r.t. for 15 min. The mixture was diluted with $CHCl_3$ (100 ml) and washed with sat. $NaHCO_3$ soln. (3 × 100 ml), the aq. phase reextracted with $CHCl_3$ (3 × 100 ml), the combined org. layer dried (Na_2SO_4) and evaporated, and the residue purified by FC (silica gel, 3 × 15 cm, CH_2Cl_2→CH_2Cl_2/MeOH 95:5): 1.35 g (94%) of **15**. Amorphous solid. UV (MeOH): 266 (4.51). ^{1}H-NMR ((D$_6$)DMSO): 10.69 (*s*, NH); 8.71, 8.57 (2*s*, H–C(2), H–C(8)); 8.18–8.12 (*m*, 4 H *o* to NO$_2$); 7.62, 7.48 (2*d*, 4 H *m* to NO$_2$); 6.36 (*d*, J = 4.0, H–C(1′)); 5.77 (*dd*, H–C(2′)); 5.35 (*t*, OH–C(5′)); 4.40–4.30 (*m*, H–C(3′), CH$_2$O of npeoc); 4.14 (*m*, H–C(4′), C*H*$_2$OAc); 3.90–3.60 (*m*, CH$_2$O–C(3′), CH$_2$O of npes, 2 H–C(5′)); 3.08–3.14 (*m*, CH$_2$C of npeoc, CH$_2$C of npes); 1.98 (*s*, Me). Anal. calc. for $C_{31}H_{33}N_7O_{14}S$ (759.7): C 49.01, H 4.38, N 12.91; found: C 48.71, H 4.51, N 12.49.

*3′-O-(2-Acetoxyethyl)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]-2′-O-[2-(4-nitrophenyl)ethoxysulfonyl]-adenosine 5′-[2-(4-Nitrophenyl)* N,N*-Diisopropylphosphoramidite]* (**16**). A soln. of **15** (0.40 g, 0.5 mmol), bis(diisopropylamino)[2-(4-nitrophenyl)ethoxy]phosphane [31] (0.42 g, 1.1 mmol) and 1*H*-tetrazole (18 mg, 0.3 mmol) in abs. MeCN (5 ml) was stirred under N_2 at r.t. for 3 h. The mixture was evaporated, the residue diluted with $CHCl_3$ (100 ml) and washed with sat. $NaHCO_3$ soln. (100 ml), the aq. phase reextracted with $CHCl_3$ (3 × 100 ml), the combined org. layer dried (Na_2SO_4) and evaporated, and the residue purified by FC (silica gel, 2.5 × 10 cm, toluene→toluene/AcOEt 3:1): 0.33 g (59%) of **16**. Amorphous solid. UV (CH_2Cl_2): 267 (4.68). ^{1}H-NMR ($CDCl_3$): 8.64, 8.34 (4*s*, H–C(2), H–C(8), diast.); 8.20–8.05 (*m*, NH, 6 H *o* to NO$_2$); 7.48–7.30 (*m*, 6 H *m* to NO$_2$); 6.25 (*m*, H–C(1′), diast.); 5.53 (*m*, H–C(2′), diast.); 4.52 (*m*, CH$_2$O of npe); 4.30–4.20 (*m*, H–C(3′), H–C(4′), C*H*$_2$OAc); 3.95–3.45 (*m*, CH$_2$O of npeoc, CH$_2$O–C(3′), CH$_2$O of npes, 2 H–C(5′), 2 Me$_2$C*H*); 3.23–2.95 (*m*, CH$_2$C of npe, CH$_2$C of npeoc, CH$_2$C of npes); 2.02 (2*s*, MeCO, diast.); 1.10 (*m*, 2 *Me*$_2$CH). ^{31}P-NMR ($CDCl_3$): 149. Anal. calc. for $C_{45}H_{54}N_9O_{17}PS$ (1056.0): C 51.18, H 5.15, N 11.94; found: C 51.31, H 5.35, N 11.52.

*3′-Deoxy-5′-O-[(4-methoxyphenyl)diphenylmethyl]-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{*O^P*-[2-(4-nitrophenyl)ethyl]}→5′}-3′-deoxy-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**18**). As described for **14**, with 3′-deoxy-5′-*O*-[(4-methoxyphenyl)diphenylmethyl]-*N*^{6}-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{*O*^{P}-[2-(4-nitrophenyl)ethyl]}→5′}-2′-*O*-acetyl-3′-deoxy-*N*^{6}-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**17**) [32]

(4.4 g, 3.1 mmol) and K_2CO_3 (70 mg) in MeOH/CH_2Cl_2 3:2 (50 ml). Workup after 3 h reaction time with $CHCl_3$ (4 × 200 ml), 10% citric acid (200 ml), and sat. NaCl soln. (2 × 100 ml), and purification by FC (silica gel, 4 × 14 cm, CH_2Cl_2→CH_2Cl_2/MeOH 95:5) gave 3.5 g (80%) of **18**. Amorphous solid. UV (CH_2Cl_2): 267 (4.80), 239 (sh, 4.49). ^{1}H-NMR ((D$_6$)DMSO): 10.59, 10.57 (2*s*, 2 NH); 8.57 (*m*, 2 H–C(2), 2 H–C(8)); 8.13, 8.03 (2*t*, 6 H *o* to NO_2); 7.59, 7.40 (2*t*, 6 H *m* to NO_2); 7.20 (*m*, 12 H of MeOTr); 6.77 (*d*, 2 H *o* to OMe); 6.26 (2*s*, H–C(1′), diast.); 5.92 (2*s*, H–C(1′), diast.), 5.70 (*t*, OH–C(2′)); 6.76 (*s*, ½ CH_2Cl_2); 5.51 (*dd*, H–C(2′)); 4.68 (br. *s*, H–C(2′)); 4.45–4.35 (*m*, 2 H–C(4′), 2 CH_2O of npeoc, CH_2O of npe, 2 H–C(5′)); 3.69 (*s*, MeO); 3.19, 2.93 (*m*, 2 CH_2C of npeoc, CH_2C of npe, 2 H–C(5′)); 2.60, 2.20, 1.98 (*m*, 4 H–C(3′)). Anal. calc. for $C_{66}H_{62}N_{13}O_{20}O \cdot ½CH_2Cl_2$ (1414.7): C 56.46, H 4.49, N 12.87; found: C 56.15, H 4.49, N 12.63.

*3′-Deoxy-5′-O-[(4-methoxyphenyl)diphenylmethyl]-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]}→5′}-3′-deoxy-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 2′-[2-(4-Nitrophenyl)-ethyl* N,N-*Diisopropylphosphoramidite]* (**19**). As described for **16**, with **18** (0.51 g, 0.36 mmol), bis(diisopropyl-amino)[2-(4-nitrophenyl)ethoxy]phosphane [31] (0.31 g, 0.78 mmol), and 1*H*-tetrazole (14 mg, 0.20 mmol) in MeCN (10 ml). Workup with $CHCl_3$ (4 × 50 ml) and sat. NaCl/$NaHCO_3$ soln. (50 ml) and purification by FC (silica gel, 2.5 × 8 cm, AcOEt→AcOEt/acetone 1:1) gave 0.48 g (80%) of **19**. Amorphous solid. UV (CH_2Cl_2): 267 (4.86), 240 (sh, 4.54). ^{1}H-NMR ($CDCl_3$): 8.30 (br. *s*, 2 NH); 8.65–8.55, 8.15–7.90 (2*m*, 2 H–C(2), 2 H–C(8), 8 H *o* to NO_2); 7.40–7.16 (*m*, 8 H *m* to NO_2, 12 H of MeOTr); 6.76 (*d*, 2 H *o* to MeO); 6.17, 6.02 (*m*, 2 H–C(1′)); 5.43, 4.87 (*m*, 2 H–C(2′)); 4.60–4.10 (*m*, 2 H–C(4′), 2 CH_2O of npeoc, 2 CH_2O of npe); 3.90–3.80 (*m*, 2 Me_2CH); 3.72 (2*s*, MeO, diast.); 3.58–3.28 (*m*, 4 H–C(5′)); 3.18–2.90 (*m*, 2 CH_2C of npeoc, 2 CH_2C of npe); 2.50–2.00 (*m*, 4 H–C(3′)); 1.10–1.00 (*m*, 4 Me, diast.). ^{31}P-NMR ($CDCl_3$): 150 (2*s*); 149 (2*s*); −1 (2*s*). Anal. calc. for $C_{80}H_{83}N_{15}O_{12}P_2$ (1668.6): C 57.59, H 5.01, N 12.59; found: C 57.18, H 4.88, N 12.56.

*3′-Deoxy-5′-O-[(4-methoxyphenyl)diphenylmethyl]-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]}→5′}-3′-deoxy-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]}→5′}-3′-O-(2-acetoxyethyl)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]-2′-O-[2-(4-nitrophenyl)-ethoxysulfonyl]adenosine* (**20**): *a*) A soln. of **18** (0.28 g, 0.20 mmol), **16** (0.38 g, 0.36 mmol), and 1*H*-tetrazole (70 mg, 1.0 mmol) in abs. MeCN (5 ml) was stirred under N_2 at r.t. for 2 h. Then it was oxidized with a I_2 soln. (I_2 (500 mg) in pyridine (3 ml), CH_2Cl (1 ml), and H_2O (1 ml)) until no change of colour was detected. The mixture was stirred for 15 min, diluted with $CHCl_3$ (100 ml), and washed with sat. $Na_2S_2O_3$/NaCl soln. (3 × 100 ml). The aq. phase was reextracted with $CHCl_3$ (3 × 100 ml), the combined org. layer dried (Na_2SO_4), evaporated, and co-evaporated with toluene (3 × 10 ml). The residue was purified by FC (silica gel, 2.5 × 10 cm, CH_2Cl→CH_2Cl_2/MeOH 97:3): 0.37 g (78%) of **20**. Amorphous solid. UV (CH_2Cl_2): 267 (5.01). ^{1}H-NMR ($CDCl_3$): 9.00–8.40, 8.20–8.00 (2*m*, 3 H–C(2), 3 H–C(8), 3 NH, 12 H *o* to NO_2); 7.40–7.10 (*m*, 12 H *m* to NO_2, 12 H of MeOTr); 6.75 (*d*, 2 H *o* to MeO); 6.20–6.00 (*m*, 3 H–C(1′)); 5.50–5.30 (*m*, 3 H–C(2′)); 5.67 (*s*, ½ CH_2Cl_2); 4.60–3.85 (*m*, 2 CH_2O of npe, H–C(3′), 3 H–C(4′), CH_2OAc, 3 CH_2O of npeoc, 4 H–C(5′)); 3.73 (*s*, MeO); 3.70–3.00 (*m*, CH_2O–C(3′), CH_2O of npes, 2 H–C(5′), 2 CH_2C of npe, 3 CH_2C of npeoc, CH_2C of npes); 2.50–2.10 (*m*, 4 H–C(3′)); 2.00 (*s*, Me). Anal. calc. for $C_{105}H_{101}N_{21}O_{37}P_2S \cdot ½ CH_2Cl_2$ (2385.6): C 53.12, H 4.31, N 12.33; found: C 52.35, H 4.48, N 12.34.

b) As described in *a* with **15** (0.19 g, 0.25 mmol), **19** (0.75 g, 0.45 mmol), 1*H*-tetrazole (0.11 g, 1.5 mmol), and abs. MeCN (6 ml). Workup after 6 h reaction time and purification by FC gave 0.52 g (87%) of **20**. Amorphous solid.

*3′-Deoxy-5′-O-[(4-methoxyphenyl)diphenylmethyl]-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]}→5′}-3′-deoxy-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]}→5′}-3′-O-(2-hydroxyethyl)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]-2′-O-[2-(4-nitrophenyl)-ethoxysulfonyl]adenosine* (**21**). As described for **14**, with **20** (0.11 g, 46 μmol), catalytic amounts of K_2CO_3, and CH_2Cl_2/MeOH 3:2 (5 ml). Workup after 3 h with $CHCl_3$ (4 × 50 ml), 10% citric acid (50 ml), and sat. NaCl soln. (2 × 50 ml), and purification by FC (silica gel, 2.5 × 10 cm, CH_2Cl_2→CH_2Cl_2/MeOH 95:5) gave 0.065 g (60%) of **21**. Amorphous solid. UV (CH_2Cl_2): 267 (5.02). ^{1}H-NMR ((D$_6$)DMSO): 10.68–10.55 (*m*, 3 NH); 8.58–8.42 (*m*, 3 H–C(2), 3 H–C(8)); 8.15–8.00 (*m*, 12 H *o* to NO_2); 7.60–7.30 (*m*, 12 H *m* to NO_2); 7.25–7.10 (*m*, 12 H of MeOTr); 6.78 (*d*, 2 H *o* to MeO); 6.48–6.12 (*m*, 3 H–C(1′)); 5.95, 5.55–5.38 (*m*, 3 H–C(2′)); 5.67 (*s*, ½ CH_2Cl_2); 4.74 (*m*, OH); 4.55–4.30 (*m*, H–C(3′), 2 CH_2O of npe, 3 CH_2O of npeoc); 4.30–3.50 (*m*, CH_2OH, CH_2O–C(3′), 3 H–C(4′), 4 H–C(5′), CH_2O of npes); 3.70 (*s*, MeO); 3.15–2.90 (*m*, 2 H–C(5′), 2 CH_2C of npe, 3 CH_2C of npeoc, CH_2O of npes); 2.50–2.10 (*m*, 4 H–C(3′)). Anal. calc. for $C_{103}H_{99}N_{21}O_{36}P_2 \cdot ½ CH_2Cl_2$ (2343.5): C 53.05, H 4.30, N 12.55; found: C 52.46, H 4.40, N 12.51.

*3′-Deoxy-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]}→5′}-3′-deoxy-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]}→5′}-3′-O-(2-hydroxyethyl)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]-2′-O-[2-(4-nitrophenyl)ethoxysulfonyl]adenosine* (**22**). A soln. of **21** (14 mg, 6.0 μmol) in 80% AcOH/H_2O was stirred at r.t. for 18 h, then evaporated and co-evaporated with H_2O (3 × 5

ml) and MeOH (3 × 5 ml). The residue was purified by prep. TLC (silica gel, 20 × 20 cm, CH_2Cl_2/MeOH 95:5) to give 11 mg (90%) of **22**. Amorphous solid. UV (CH_2Cl_2): 267 (5.00). ^{1}H-NMR ((D_6)DMSO): 10.64–10.57 (*m*, 3 NH); 8.63–8.46 (*m*, 3 H–C(2), 3 H–C(8)); 8.14–7.98 (*m*, 12 H *o* to NO); 7.60–7.36 (*m*, 12 H *m* to NO_2); 6.36–6.14 (*m*, 3 H–C(1′)); 5.79 (*s*, ½ CH_2Cl_2); 5.86, 5.43–5.25 (*m*, 3 H–C(2′)); 5.11 (*m*, OH–C(5′); 4.75 (*m*, OH); 4.54–4.46 (*m*, H–C(3′)); 4.46–4.04 (*m*, 2 CH_2O of npe, 3 CH_2O of npeoc, 3 H–C(4′), 4 H–C(5′)); 3.95–3.40 (*m*, CH_2OH, 2 H–C(5′), CH_2O–C(3′), CH_2O of npes); 3.14–2.95 (*m*, 2 CH_2C of npe, 3 CH_2C of npeoc, CH_2C of npes); 2.50–1.96 (*m*, 4 H–C(3′)). Anal. calc. for $C_{83}H_{83}N_{21}O_{35}P_2S \cdot$ ½ CH_2Cl_2 (2071.2): C 48.42, H 4.09, N 14.20; found: C 48.03, H 4.24, N 13.97.

3′-Deoxyadenylyl-(2′→5′)-3′-deoxyadenylyl-(2′→5′)-3′-O-(2-hydroxyethyl)adenosine Diammonioum Salt (**23**). After co-evaporation with dry pyridine (3 × 5 ml), **22** (8.9 mg, 4.3 μmol) was dissolved in 0.5M DBU/MeCN (1.5 ml) and stirred at r.t. for 42 h. Then the mixture was neutralized with 1M AcOH and evaporated. The residue was dissolved in H_2O (10 ml) and applied on a *DEAE-Sephadex* column *A25* (2 × 40 cm) using first H_2O (200 ml), followed by a linear gradient of $(Et_3NH)HCO_3$ buffer (pH 7.5; 0.0→0.2M) within 4000 ml. The eluated product fractions were evaporated an co-evaporated with H_2O (4 × 10 ml), sat. NH_3 soln. (3 × 10 ml), and H_2O (3 × 10 ml). The residual NH_4^+ salts were lyophilized (H_2O): 5.2 mg (59%) of **23**. Colorless powder. HPLC (*A*: 0.1M (Et_3NH)OAc buffer (pH 7); *B*: 0.1M (Et_3NH)OAc buffer/MeCN 1:1; gradient: 0 min 95% *A*, 5 min 95% *A*, 30 min 60% *A*): t_R 16.19 min. ^{1}H-NMR (D_2O): 8.09, 7.98, 7.88, 7.82, 7.74 (5*s*, 3 H–C(2), 3 H–C(8)); 6.00, 5.76 (2*s*, 2 H–C(1′)); 5.68 (br. *s*, H–C(1′)); 5.05 (*s*, H–C(2′)); 4.80–4.00 (*m*, 2 H–C(2′), H–C(3′), 3 H–C(4′), 4 H–C(5′)); 3.71–3.36 (*m*, CH_2OH, CH_2O–C(3′), 2 H–C(5′)); 2.50–2.30 (*m*, 4 H–C(3′)).

3′-Deoxy-5′-O-[(4-methoxyphenyl)diphenylmethyl]-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]}→5′}-3′-deoxy-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]}→5′}-3′-O-{2-[(cholest-5-en-3β-yloxy)carbonyloxy]ethyl}-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]-2′-O-[2-(4-nitrophenyl)ethoxysulfonyl]adenosine (**24**). A mixture of **21** (98 mg, 42 μmol), catalytic amounts of DMAP, cholesteryl chloroformate (= cholest-5-en-3β-yl chloroformate; *Fluka*; 94 mg, 0.38 mmol), 1-methyl-1*H*-imidazole (30 μl, 0.38 mmol), and abs. CH_2Cl_2 (4 ml) was stirred at r.t. for 40 h, then diluted with $CHCl_3$ (20 ml), and washed with sat. NaCl soln. (3 × 20 ml). The aq. phase was reextracted with $CHCl_3$ (3 × 20 ml), the combined org. layer dried (Na_2SO_4) and evaporated, and the residue purified by FC (silica gel, 1.5 × 13 cm, CH_2Cl_2→CH_2Cl_2/MeOH 96:4): 89 mg (78%) of **24**. Amorphous solid. UV (CH_2Cl_2): 267 (5.02). ^{1}H-NMR ((D_6)DMSO): 10.66–10.58 (*m*, 3 NH); 8.65–8.42 (*m*, 3 H–C(2), 3 H–C(8)); 8.13–7.98 (*m*, 12 H *o* to NO_2); 7.62–7.32 (*m*, 12 H *m* to NO_2); 7.32–7.10 (*m*, 12 H of MeOTr); 6.75 (*d*, 2 H *o* to MeO); 6.38–6.13 (*m*, 3 H–C(1′)); 5.88, 5.49–5.37 (*m*, 3 H–C(2′)); 5.22 (*m*, H–C(6) of chol); 4.55–4.08 (*m*, H–C(3′), 2 CH_2O of npe, 3 CH_2O of npeoc, CH_2OCOOchol, H–C(4′), 4 H–C(5′), H–C(3) of chol); 3.78–3.95 (*m*, CH_2O–C(3′), CH_2O of npes); 3.68 (*s*, MeO); 3.20–2.70 (*m*, 2 H–C(5′), 2 CH_2C of npe, 3 CH_2C of npeoc, CH_2C of npes); 2.30–0.50 (*m*, 4 H–C(3′), 43 H of chol). Anal. calc. for $C_{131}H_{143}N_{21}O_{38}P_2S$ (2713.7): C 57.98, H 5.31, N 10.84; found: C 57.89, H 5.24, N 10.55.

3′-Deoxyadenylyl-(2′→5′)-3′-deoxyadenylyl-(2′→5′)-3′-O-{2-[(cholest-5-en-3β-yloxy)carbonyloxy]ethyl}-adenosine Bis(1,8-diazabicyclo[5.4.0]undec-7-enium) Salt (**25**). After co-evaporation with dry pyridine (3 × 5 ml), **24** (50 mg, 15 μmol) was dissolved in 0.5M DBU/MeCN (1.0 ml) and stirred at r.t. for 43 h, then neutralized with 1M AcOH, diluted with $CHCl_3$ (100 ml), and washed with H_2O (3 × 100 ml). The aq. phase was reextracted with $CHCl_3$ (2 × 50 ml), and the combined org. layer dried (Na_2SO_4) and evaporated. To the residue, 80% AcOH/H_2O (5 ml) was added, the mixture stirred at r.t. for 19 h and lyophilized, and the residue washed and centrifuged several times with AcOEt, H_2O, MeOH, and Et_2O: 28.5 mg (85%) of **25**. Colorless powder. HPLC (see **23** for *A* and *B*; *C*: MeCN; gradient: 0 min 90% *A*/10% *B*, 20 min 0% *A*/100% *B*, 40 min *C*): t_R 35.40 min.

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