

64. Nucleotides

Part XLVII¹⁾

**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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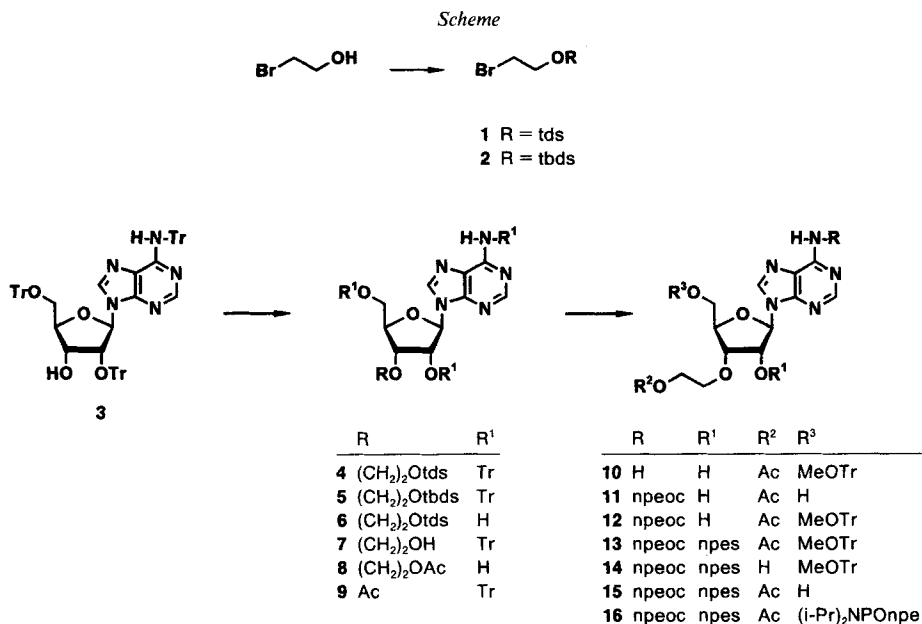
(20. XI. 95)

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 ($\rightarrow$ **20**). After cleavage of the acetyl protecting group ($\rightarrow$ **21**), cholesteryl carbonate was introduced into the OH function of the spacer ($\rightarrow$ **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 phosphodiester 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(heptyl)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,



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

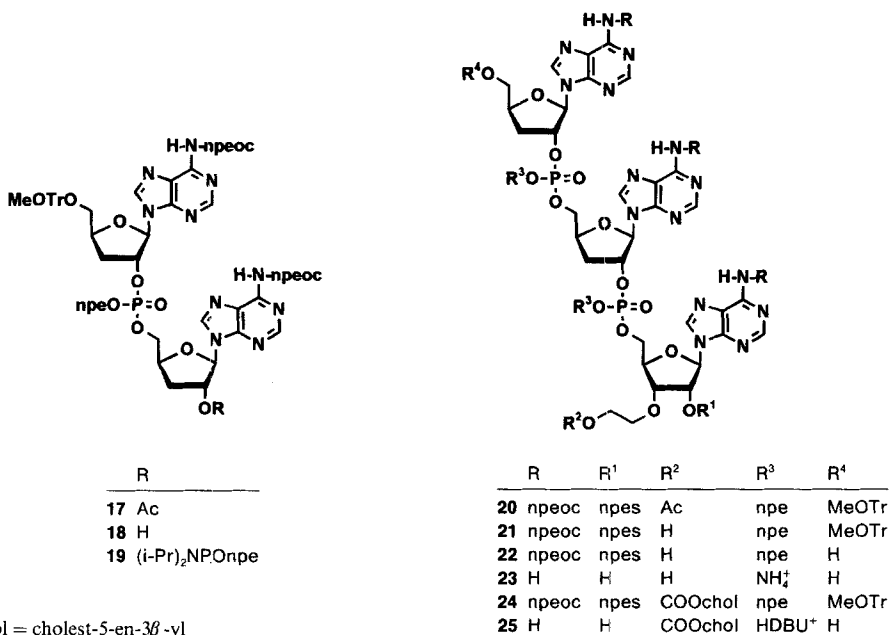
respectively. The silyl protecting groups turned out to be unstable under detritylation conditions, since treatment of **5** with ZnBr₂ [26] under aprotic conditions gave the silylated compound **6** only in 40% yield. Therefore, the silyl group in **4** or **5** was cleaved off with fluoride ions (Bu₄NF) in THF to give 3'-*O*-(2-hydroxyethyl)-*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/H₂O 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*²-[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



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 H₂O, but soluble in DMSO, various buffers, and ternary mixtures of CH₂Cl₂/MeOH/H₂O.

Experimental Part

General. TLC: Precoated silica gel TLC sheets *F 1500 LS 254* from *Schleicher & Schüll*. Prep. TLC: silica gel *60 PF₂₅₄* (*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* (HCO₃⁻; *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). ¹H-NMR: *Bruker AC 250*; δ in ppm rel. to CHCl₃ ((D₆)DMSO). ³¹P-NMR: *Jeol JM 6X-400*; δ in ppm rel. to 85% H₃PO₄ soln.

2-Bromo-1-[dimethyl(1,1,2-trimethylpropyl)silyloxy]ethane (1). To an ice-cooled mixture of dimethyl-(hexyl)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 H₂O (2 × 250 ml). The aq. phases were reextracted with light petroleum ether (3 × 250 ml), and the combined org. layer was dried (Na₂SO₄) and evaporated. The residue was submitted to vacuum distillation (73°/0.4 mbar): 53 g (80%) of **1**. Colorless oil. ¹H-NMR (CDCl₃): 3.85 (*t*, CH₂O); 3.37 (*t*, CH₂Br); 1.60–1.50 (*m*, CH); 0.85 (*m*, 4 MeC); 0.10 (*s*, 2 MeSi). Anal. calc. for C₁₀H₂₂BrOSi (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}-N⁶,2'-O,5'-O-tris(triphenylmethyl)adenosine (4). A mixture of N⁶,2'-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 (Na₂SO₄) 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). ¹H-NMR (CDCl₃): 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*, CH₂Otds); 3.30–3.00 (*m*, CH₂O–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 (2*s*, 2 MeSi, diast.) Anal. calc. for C₇₇H₇₇N₅O₅Si (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}-N⁶,2'-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). ¹H-NMR (CDCl₃): 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*, CH₂Otds); 3.25–3.05 (*m*, CH₂O–C(3'), 2 H–C(5')); 2.79 (*dd*, H–C(3')); 1.56 (*s*, ½ H₂O); 0.87 (*s*, 3 MeC); 0.07, 0.04 (2*s*, 2 MeSi, diast.). Anal. calc. for C₇₅H₇₃N₅O₅Si · ½ H₂O (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 ZnBr₂ (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 (Na₂SO₄) 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). ¹H-NMR ((D₆)DMSO): 8.36, 8.14 (2*s*, H–C(2), H–C(8)); 7.39 (br. *s*, NH₂); 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*, CH₂Otds, CH₂O–C(3'), 2 H–C(5')); 3.38 (br. *s*, ½ H₂O); 0.91 (*s*, 3 MeC); 0.13 (*s*, 2 MeSi). Anal. calc. for C₁₈H₃₁N₅O₅Si · ½ H₂O (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), Bu₄NF · 3 H₂O (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 H₂O (3 × 100 ml). The aq. phases were reextracted with AcOEt (2 × 100 ml), the combined org. layer was dried (Na₂SO₄) 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). ¹H-NMR ((D₆)DMSO): 8.35 (br. s, NH); 7.50, 7.49 (2s, 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, CH₂OH, CH₂O-C(3'), 2 H-C(5')); 3.34 (s, H₂O); 2.45 (m, H-C(3')). ¹H-NMR (CDCl₃): 7.80, 7.74 (2s, 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, CH₂OH); 3.33–3.23, 3.07–2.95 (m, CH₂O-C(3'), 2 H-C(5')); 2.74 ('d', H-C(3')); 1.70 (br. s, H₂O). Anal. calc. for C₆₉H₅₉N₅O₅ · H₂O (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), Bu₄NF · 3 H₂O (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 Ac₂O (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/H₂O (20 ml) was added. The mixture was kept 1 h at 100°, evaporated, co-evaporated with H₂O and MeOH, and purified by FC (silica gel, 2 × 7 cm, CH₂Cl₂ → CH₂Cl₂/MeOH 95:5): 0.50 g (81%) of **8**. Amorphous solid. UV (MeOH): 258 (4.17). ¹H-NMR ((D₆)DMSO): 8.36, 8.14 (2s, H-C(2), H-C(8)); 7.37 (s, NH₂); 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, CH₂OAc); 4.03 (m, H-C(3'), H-C(4')); 3.91–3.55 (m, CH₂O-C(3'), 2 H-C(5')); 2.03 (s, Me). Anal. calc. for C₁₄H₁₉N₅O₆ (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). ¹H-NMR ((D₆)DMSO): 8.41 (s, NH); 7.62, 7.49 (2s, 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 C₆₉H₅₇N₅O₅ (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]diphenylmethyladenosine (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. NaHCO₃ soln. (3 × 50 ml), and the aq. phase reextracted with AcOEt (3 × 50 ml). The combined org. layer was dried (Na₂SO₄) and evaporated, and the residue purified by FC (silica gel, 3 × 7 cm, CH₂Cl₂ → CH₂Cl₂/MeOH 95:5): 0.35 g (54%) of **10**. Amorphous solid. UV (MeOH): 259 (4.20), 233 (4.25). ¹H-NMR ((D₆)DMSO): 8.26, 8.08 (2s, 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, CH₂OAc, H-C(4')); 3.83–3.62 (m, MeO, CH₂O-C(3')); 3.23 (m, 2 H-C(5')); 1.91 (s, Me). Anal. calc. for C₃₄H₃₃N₅O₇ (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 (NH₄)₂SO₄ 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]-1H-imidazolium chloride [27] (3.2 g, 10 mmol) and CH₂Cl₂ (100 ml) were added. The mixture was kept overnight and filtered, and the filtrate evaporated. To the residue, MeOH (100 ml) and Et₃N (10 ml) were added, and 2.21 g (78%) of **11** crystallized. Colorless crystals. M.p. 165°. UV (MeOH): 266 (4.45). ¹H-NMR ((D₆)DMSO): 10.64 (s, NH); 8.69, 8.63 (2s, H-C(2), H-C(8)); 8.17 (d, 2 H *o* to NO₂); 7.62 (d, 2 H *m* to NO₂); 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, CH₂O of npeoc); 4.18 (m, CH₂OAc); 4.06 (br. s, H-C(3'), H-C(4')); 3.90–3.50 (m, CH₂O-C(3'), 2 H-C(5')); 3.13 (t, CH₂C of npeoc); 2.03 (s, Me). Anal. calc. for C₂₃H₂₆N₆O₁₀ (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 CHCl₃ (100 ml) and washed with sat. NaHCO₃ soln. (3 × 100 ml), the aq. phase reextracted with CHCl₃ (3 × 50 ml), the combined org. layer dried (Na₂SO₄) and evaporated, and the residue purified by FC (silica

gel, 3×7 cm, toluene/AcOEt 1:1 $\rightarrow$ 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). $^1\text{H-NMR}$ ((D_6) DMSO): 10.65 (s, NH); 8.58, 8.55 (2s, H-C(2), H-C(8)); 8.16 (d, 2 H *o* to NO₂); 7.62 (d, 2 H *m* to NO₂); 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, CH₂O of npeoc); 4.25 (t', H-C(3')); 4.13 (m, CH₂OAc, H-C(4')); 3.84–3.71 (m, CH₂O-C(3'), MeO); 3.25 (m, 2 H-C(5')); 3.10 (t, CH₂C of npeoc); 1.92 (s, Me). Anal. calc. for C₄₃H₄₂N₆O₁₁ (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]-N⁶-[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 CHCl₃ (100 ml) and washed with sat. NaHCO₃ soln. (3×100 ml), the aq. phase reextracted with CHCl₃ (3×50 ml), the combined org. layer dried (Na₂SO₄) and evaporated, and the residue purified by FC (silica gel, 2.5×12 cm, toluene/AcOEt 1:1 $\rightarrow$ toluene/AcOEt/MeOH 25:25:1): 0.28 g (85%) of **13**. Amorphous solid. UV (MeOH): 267 (4.58), 235 (4.36). $^1\text{H-NMR}$ ((D_6) DMSO): 10.72 (s, NH); 8.60, 8.49 (2s, H-C(2), H-C(8)); 8.15 (m, 4 H *o* to NO₂); 7.62, 7.49 (2d, 4 H *m* to NO₂); 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, CH₂O of npeoc); 4.19 (m, H-C(4')); 4.10 (m, CH₂OAc); 3.90–3.70 (m, CH₂O-C(3'), CH₂O of npes); 3.71 (s, MeO); 3.38–3.20 (m, 2 H-C(5')); 3.10 (m, CH₂C of npeoc, CH₂C of npes); 1.86 (s, Me). Anal. calc. for C₅₁H₄₉N₇O₁₅ (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]-N⁶-[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 K₂CO₃ in MeOH/CH₂Cl₂ 2:1 (7.5 ml) was stirred at r.t. for 2 h. The mixture was diluted with CHCl₃ (100 ml) and washed with 10% citric acid (100 ml) and sat. NaCl soln. (2×100 ml), the aq. phase reextracted with CHCl₃ (3×50 ml), the org. layer dried (Na₂SO₄) and evaporated, and the residue purified by FC (silica gel, 2.5×10 cm, toluene/AcOEt 1:1 $\rightarrow$ toluene/AcOEt/MeOH 25:25:1): 0.07 g (63%) of **14**. Amorphous solid. UV (MeOH): 267 (4.59), 235 (4.37). $^1\text{H-NMR}$ ((D_6) DMSO): 10.69 (s, NH); 8.69, 8.50 (2s, H-C(2), H-C(8)); 8.13 (t', 4 H *o* to NO₂); 7.62, 7.48 (2d, 4 H *m* to NO₂); 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, CH₂O of npeoc); 4.20 (m, H-C(4')); 3.50 (m, CH₂OH); 3.71 (s, MeO); 3.70–3.20 (m, CH₂O-C(3'), CH₂O of npes, 2 H-C(5')); 3.10 (m, CH₂C of npeoc, CH₂C of npes). Anal. calc. for C₄₉H₄₇N₇O₁₄S (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)-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]-2'-O-[2-(4-nitrophenyl)ethoxysulfonyl]adenosine (**15**). A soln. of **13** (1.95 g, 1.9 mmol) in MeOH/CHCl₃ 4:1 (30 ml) containing 2% of TsOH was stirred at r.t. for 15 min. The mixture was diluted with CHCl₃ (100 ml) and washed with sat. NaHCO₃ soln. (3×100 ml), the aq. phase reextracted with CHCl₃ (3×100 ml), the combined org. layer dried (Na₂SO₄) and evaporated, and the residue purified by FC (silica gel, 3×15 cm, CH₂Cl₂ $\rightarrow$ CH₂Cl₂/MeOH 95:5): 1.35 g (94%) of **15**. Amorphous solid. UV (MeOH): 266 (4.51). $^1\text{H-NMR}$ ((D_6) DMSO): 10.69 (s, NH); 8.71, 8.57 (2s, H-C(2), H-C(8)); 8.18–8.12 (m, 4 H *o* to NO₂); 7.62, 7.48 (2d, 4 H *m* to NO₂); 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'), CH₂O of npeoc); 4.14 (m, H-C(4'), CH₂OAc); 3.90–3.60 (m, CH₂O-C(3'), CH₂O of npes, 2 H-C(5')); 3.08–3.14 (m, CH₂C of npeoc, CH₂C of npes); 1.98 (s, Me). Anal. calc. for C₃₁H₃₃N₇O₁₄S (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)-N⁶-[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 N₂ at r.t. for 3 h. The mixture was evaporated, the residue diluted with CHCl₃ (100 ml) and washed with sat. NaHCO₃ soln. (100 ml), the aq. phase reextracted with CHCl₃ (3×100 ml), the combined org. layer dried (Na₂SO₄) and evaporated, and the residue purified by FC (silica gel, 2.5×10 cm, toluene $\rightarrow$ toluene/AcOEt 3:1): 0.33 g (59%) of **16**. Amorphous solid. UV (CH₂Cl₂): 267 (4.68). $^1\text{H-NMR}$ (CDCl₃): 8.64, 8.34 (4s, H-C(2), H-C(8), diast.); 8.20–8.05 (m, NH, 6 H *o* to NO₂); 7.48–7.30 (m, 6 H *m* to NO₂); 6.25 (m, H-C(1'), diast.); 5.53 (m, H-C(2'), diast.); 4.52 (m, CH₂O of npe); 4.30–4.20 (m, H-C(3'), H-C(4'), CH₂OAc); 3.95–3.45 (m, CH₂O of npeoc, CH₂O-C(3'), CH₂O of npes, 2 H-C(5'), 2 Me₂CH); 3.23–2.95 (m, CH₂C of npe, CH₂C of npeoc, CH₂C of npes); 2.02 (2s, MeCO, diast.); 1.10 (m, 2 Me₂CH). $^{31}\text{P-NMR}$ (CDCl₃): 149. Anal. calc. for C₄₅H₅₄N₉O₁₇PS (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]-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-[2'-{O^P-[2-(4-nitrophenyl)ethyl]} $\rightarrow$ 5'}-3'-deoxy-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**18**). As described for **14**, with 3'-deoxy-5'-O-[(4-methoxyphenyl)diphenylmethyl]-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-[2'-{O^P-[2-(4-nitrophenyl)ethyl]} $\rightarrow$ 5'}-2'-O-acetyl-3'-deoxy-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**17**) [32]

ml) and MeOH (3 × 5 ml). The residue was purified by prep. TLC (silica gel, 20 × 20 cm, CH₂Cl₂/MeOH 95:5) to give 11 mg (90%) of **22**. Amorphous solid. UV (CH₂Cl₂): 267 (5.00). ¹H-NMR ((D₆)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 NO₂); 6.36–6.14 (*m*, 3 H–C(1')); 5.79 (*s*, ½ CH₂Cl₂); 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 CH₂O of npe, 3 CH₂O of npeoc, 3 H–C(4'), 4 H–C(5')); 3.95–3.40 (*m*, CH₂OH, 2 H–C(5'), CH₂O–C(3'), CH₂O of npes); 3.14–2.95 (*m*, 2 CH₂C of npe, 3 CH₂C of npeoc, CH₂C of npes); 2.50–1.96 (*m*, 4 H–C(3')). Anal. calc. for C₈₃H₈₃N₂₁O₃₅P₂S · ½ CH₂Cl₂ (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 Diammonium 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 H₂O (10 ml) and applied on a DEAE-Sephadex column A25 (2 × 40 cm) using first H₂O (200 ml), followed by a linear gradient of (Et₃NH)HCO₃ buffer (pH 7.5; 0.0 → 0.2M) within 4000 ml. The eluted product fractions were evaporated and co-evaporated with H₂O (4 × 10 ml), sat. NH₃ soln. (3 × 10 ml), and H₂O (3 × 10 ml). The residual NH₄⁺ salts were lyophilized (H₂O): 5.2 mg (59%) of **23**. Colorless powder. HPLC (*A*: 0.1M (Et₃NH)OAc buffer (pH 7); *B*: 0.1M (Et₃NH)OAc buffer/MeCN 1:1; gradient: 0 min 95% *A*, 5 min 95% *A*, 30 min 60% *A*): *t*_R 16.19 min. ¹H-NMR (D₂O): 8.09, 7.98, 7.88, 7.82, 7.74 (*5s*, 3 H–C(2), 3 H–C(8)); 6.00, 5.76 (*2s*, 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*, CH₂OH, CH₂O–C(3'), 2 H–C(5')); 2.50–2.30 (*m*, 4 H–C(3')).

3'-Deoxy-5'-O-[(4-methoxyphenyl)diphenylmethyl]-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2'-{OP-[2-(4-nitrophenyl)ethyl]} → 5'}-3'-deoxy-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2'-{OP-[2-(4-nitrophenyl)ethyl]} → 5'}-3'-O-{2-[(cholest-5-en-3β-yloxy)carbonyloxy]ethyl}-N⁶-[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-1H-imidazole (30 μl, 0.38 mmol), and abs. CH₂Cl₂ (4 ml) was stirred at r.t. for 40 h, then diluted with CHCl₃ (20 ml), and washed with sat. NaCl soln. (3 × 20 ml). The aq. phase was reextracted with CHCl₃ (3 × 20 ml), the combined org. layer dried (Na₂SO₄) and evaporated, and the residue purified by FC (silica gel, 1.5 × 13 cm, CH₂Cl₂ → CH₂Cl₂/MeOH 96:4): 89 mg (78%) of **24**. Amorphous solid. UV (CH₂Cl₂): 267 (5.02). ¹H-NMR ((D₆)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 NO₂); 7.62–7.32 (*m*, 12 H *m* to NO₂); 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 CH₂O of npe, 3 CH₂O of npeoc, CH₂OCOchol, H–C(4'), 4 H–C(5'), H–C(3) of chol); 3.78–3.95 (*m*, CH₂O–C(3'), CH₂O of npes); 3.68 (*s*, MeO); 3.20–2.70 (*m*, 2 H–C(5'), 2 CH₂C of npe, 3 CH₂C of npeoc, CH₂C of npes); 2.30–0.50 (*m*, 4 H–C(3'), 43 H of chol). Anal. calc. for C₁₃₁H₁₄₃N₂₁O₃₈P₂S (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 CHCl₃ (100 ml), and washed with H₂O (3 × 100 ml). The aq. phase was reextracted with CHCl₃ (2 × 50 ml), and the combined org. layer dried (Na₂SO₄) and evaporated. To the residue, 80% AcOH/H₂O (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, H₂O, MeOH, and Et₂O: 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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