156. Nucleotides

Part XLIV[1])

Synthesis, Characterization, and Biological Activity of Monomeric and Trimeric Cordycepin-Cholesterol Conjugates and Inhibition of HIV-1 Replication

by **Marita Wasner**[a]), **Earl E. Henderson**[b]), **Robert J. Suhadolnik**[c]), and **Wolfgang Pfleiderer**[a])*

[a]) Fakultät für Chemie, Universität Konstanz, Postfach 5560, D–78434 Konstanz
[b]) Department of Microbiology and Immunology and [c]) Department of Biochemistry, Fels Institute of Cancer Research and Molecular Biology, Temple University, School of Medicine, Philadelphia, PA 19140, USA

(11.III.94)

The antivirally active 3′-deoxyadenylyl-(2′–5′)-3′-deoxyadenylyl-(2′–5′)-3′-deoxyadenosine (cordycepin trimer core) was modified at the 2′- or 5′-terminus, by attachment of cholesterol *via* a carbonate bond (→ **15**) or a succinate linker (→ **16** and **27**) to improve cell permeability. The corresponding monomeric conjugates **4**, **7**, and **21** of cordycepin were prepared as model substances to study the applicability of the anticipated protecting groups – the monomethoxytrityl (MeOTr), the (*tert*-butyl)dimethylsilyl (tbds), and the β-eliminating 2-(4-nitrophenyl)ethyl (npe) and 2-(4-nitrophenyl)ethoxycarbonyl (npeoc) groups – for the final deblocking steps without harming the ester bonds of the conjugate trimers. The syntheses were performed in solution using phosphoramidite chemistry. The fully protected trimer conjugates **13**, **14**, and **26** as well as all intermediates were characterized by elemental analyses, UV and ^{1}H-NMR spectra. The deblocked conjugates **15**, **16**, and **27** were pure according to HPLC and showed the correct compositions by mass spectra. Comparative biological studies indicated that cordycepin-cholesterol conjugate trimers **16** and **27** were 333- and 1000-fold, respectively, more potent inhibitors of HIV-1-induced syncytia formation than cordycepin trimer core.

1. Introduction. – Interferon-treated cells and lysates of rabbit reticulocytes contain the enzyme 2–5A synthetase which produces (2′–5′)oligoadenylate 5′-triphosphates from ATP in the presence of double-stranded RNA. The trimer pppA2′p5′A2′p5′A plays the most important role in the antiviral mechanism induced by interferon [2] [3] in activating the endoribonuclease L (RNase L) in mammalian cells. Binding and activation of the specifically linked (2′–5′)A oligomers to RNase L leads to the hydrolysis of mRNA and consequently to the inhibition of protein synthesis. The lifetimes of the (2′–5′)A oligomers are severely restricted by the presence of an exonucleolytic 2′-phosphodiesterase (2′-PDE) which rapidly degrades the (2′–5′)-phosphodiester linkages of the activators. With the objective of decreasing or suppressing the hydrolytic action of 2′-PDE completely, many analogues of the natural (2′–5′)A oligomers possessing enzymatic stability but still functioning as substrates in the (2′–5′)A system were synthesized in recent years [4–11] to achieve new approaches to antiviral and antitumoral therapy. One of these analogues, cordycepin (= 3′-deoxyadenosine) trimer core d3′(A2′p5′A2′p5′A) [12], was found to be a biologically active substance with metabolic

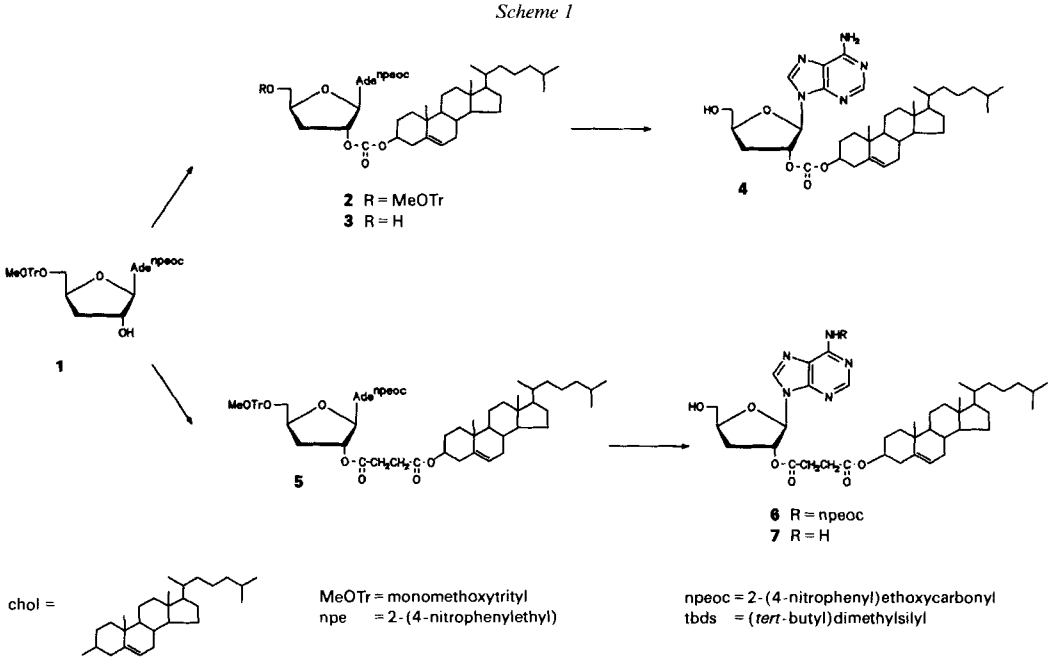
[1]) Part XLIII: [1].

stability and without toxicity to cells. Moreover, it was found to be also an inhibitor or HIV-reverse-transcriptase (RT) [13] [14] by interfering in the primer complex formation of RT to $tRNA^{Lys,3}$ *via* the anticodon region.

One of the main limitations in the application of most of the synthetically modified (2′–5′)oligoadenylates is the low permeability of the polyanion species through the cell membranes. This can, as shown by previous reports [15–17], in principle be improved by attaching lipophilic groups to various moieties of these molecules to facilitate interactions with membrane substituents and receptors, respectively. So far, the attachement was performed *via* phosphodiester bonds, whereas our recent efforts use normal ester linkages to form cholesterol (= cholest-5-en-3β-ol) conjugates at the 2′- and 5′-end of cordycepin and its trimer core in form of a carbonate or *via* a succinate linker.

2. Syntheses. – The chemical syntheses of various cordycepin conjugates were based upon 3′-deoxy-5′-*O*-(monomethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine [4] (**1**) as an universal starting material. Reaction of **1** with cholesteryl chloroformate in anhydrous CH_2Cl_2 in presence of 1-methyl-1*H*-imidazole and 4-(dimethylamino)pyridine (DMAP) gave **2** which was subsequently detritylated to the intermediate **3** in 68% yield and followed by the cleavage of the 2-(4-nitrophenyl)ethoxycarbonyl (npeoc) group using 1,8-diazabicyclo[5.3.0]undec-7-ene (DBU) in a β-elimination process to give 2′-*O*-(cholesteryloxycarbonyl)cordycepin (**4**) in 74% isolated yield. A structural analogue carrying a succinyl spacer between the cordycepin and cholesterol moieties was synthesized in a similar manner. In a one-pot reaction, **5** was obtained in 77% yield by treatment of **1** first with succinic anhydride and DMAP in CH_2Cl_2 and secondly with cholesterol in the presence of *N*-[3-(dimethylamino)propyl]-*N*′-ethylcarbodiimide hy-

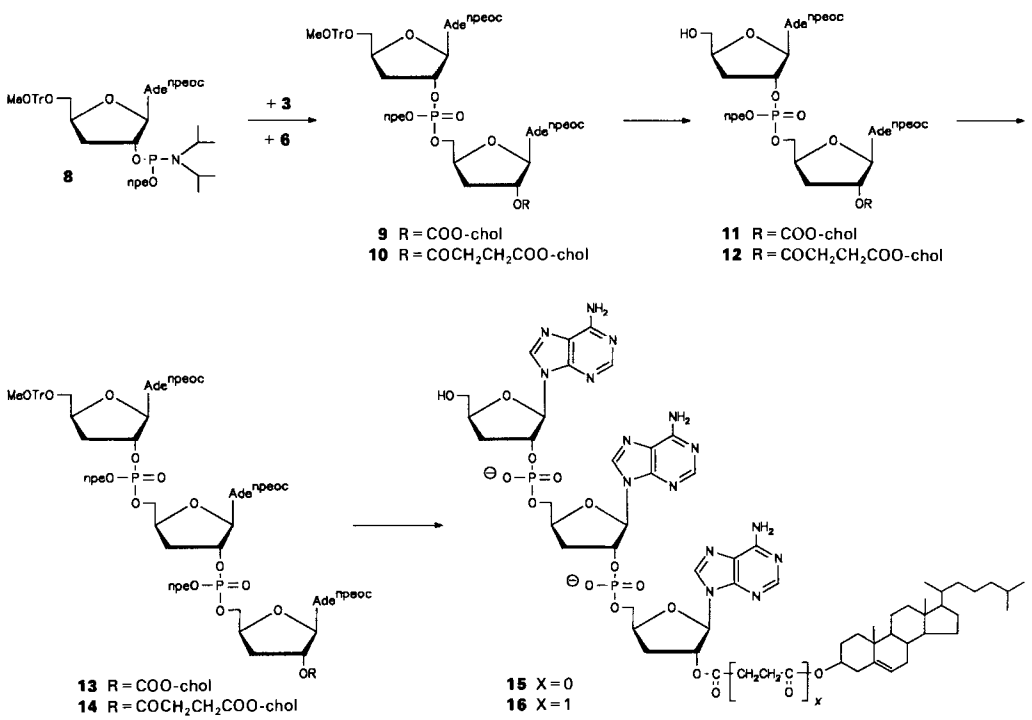
Scheme 1



drochloride (EDC). Detritylation to **6** proceeded almost quantitatively and subsequent elimination of the npeoc group resulted in 57% yield of 2′-*O*-[2-(cholesteryloxycarboxyl)propionyl]-3′-deoxyadenosine (**7**). The relatively low yields of monomeric cordycepin conjugates **4** and **7** are due to their unusual physical properties derived from the combination of the hydrophobic cholesterol and the hydrophilic cordycepin moieties.

The corresponding oligonucleotide conjugates were obtained by stepwise syntheses starting from the partially deprotected monomers **3** and **6**, respectively, which were condensed with 3′-deoxy-5′-*O*-(monomethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 2′-[2-(4-nitrophenyl)ethyl *N*,*N*-diisopropylphosphoramidite] [18] (**8**) to give, on subsequent oxidation of the intermediary phosphite triester, the fully protected dimers **9** and **10** in 88 and 91% yield, respectively (*Scheme 2*). Detritylation of these dimers generated the 5′-OH building blocks **11** and **12** which, on further condensation with the phosphoramidite **8** and I_2 oxidation, led to the cordycepin trimers **13** in 78% and **14** in 94% yield, respectively. The final deprotection of the various blocking groups was achieved subsequently without purification of intermediates by 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) and AcOH treatment to give the cordycepin trimer conjugates **15** and **16** as colorless powders.

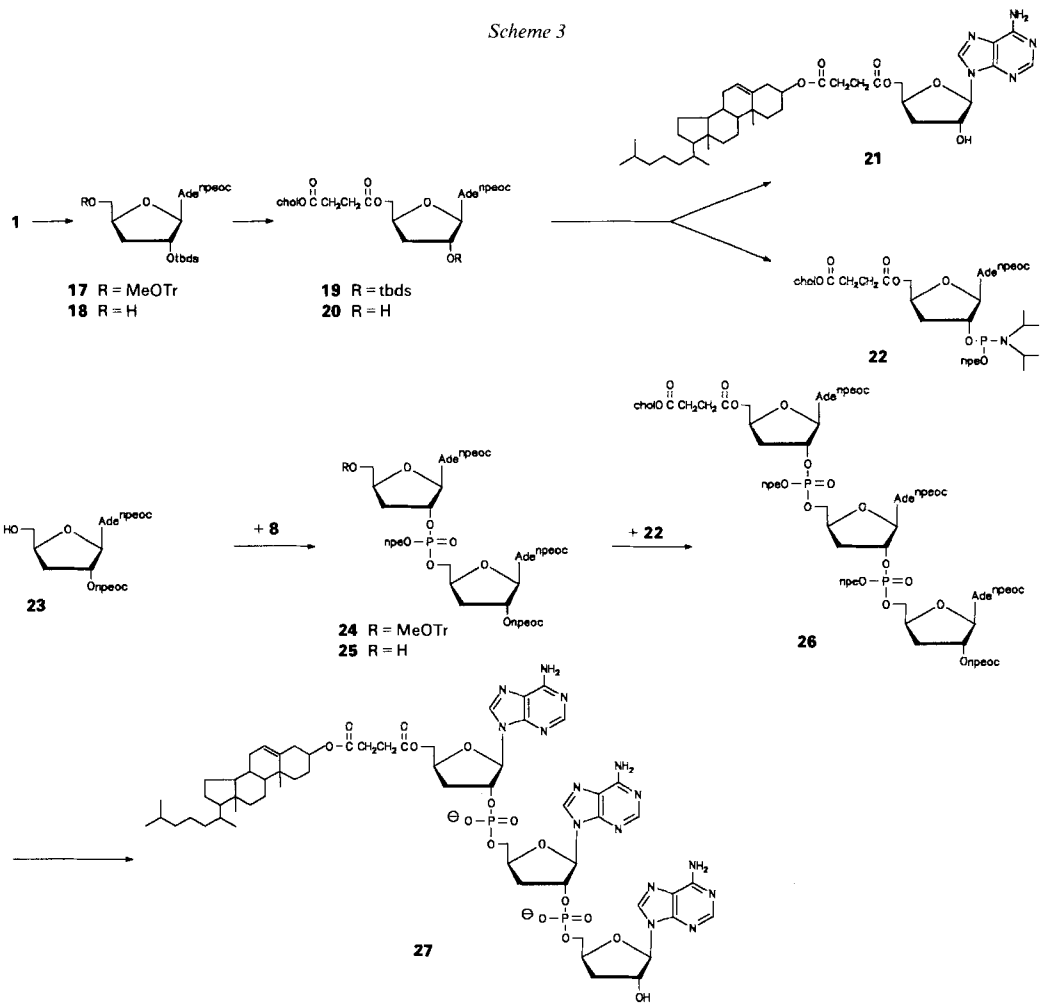
Scheme 2



To prepare a 5′-*O*-cholesteryl-cordycepin conjugate, the starting compound **1** was firstly protected at the 2′-OH group by (*tert*-butyl)dimethylsilyl chloride to give **17** in 77% yield followed by detritylation to afford 2′-*O*-[(*tert*-butyl)dimethylsilyl]-N^6-[2-(4-ni-

trophenyl)ethoxycarbonyl]cordycepin (**18**) almost quantitatively (*Scheme 3*). In a one-pot reaction, **18** was converted in three steps into 5′-*O*-{2-[(cholesteryloxycarbonyl)ethyl]carbonyl} derivative **20**, first by treatment with succinic anhydride and DMAP (→corresponding succinate intermediate), then by esterification with cholesterol using the carbodiimide method (EDC; → **19**) and finally by desilylation with fluoride ion (Bu_4NF) in THF, buffered by AcOH to avoid deblocking of the npeoc group. Compound **20** was then either deprotected to 5′-*O*-{[2-(cholesteryloxycarbonyl)ethyl]carbonyl}-cordycepin (**21**) by DBU in 69% yield or phosphitylated by bis(*N*,*N*-diisopropyl)[2-(4-nitrophenyl)ethoxy]phosphane [18] to the diastereoisomeric mixture **22** of the phosphoramidite which is an essential building block for the cordycepin trimer formation. The second component for this approach was obtained from 3′-deoxy-N^6,2′-*O*-bis-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine [3] (**23**) and the phosphoramidite **8** [18] in a normal condensation and oxidation process yielding dimer **24** (87%) and on subsequent detritylation **25** (85%; *Scheme 3*). The final condensation of **22** and **25** followed by oxidation to

Scheme 3



the fully blocked trimer conjugate **26** did not work so well as expected, but besides 53% of **26** also 43% of the starting dimer **25** could be recovered. Deprotection of the npe and npeoc groups *via* β-elimination by DBU and usual workup with MeCN extraction and lyophilization from H_2O led to the conjugate trimer **27**.

The purity of the newly synthesized cordycepin trimer conjugates **15**, **16**, and **27** was checked by HPLC and the composition proven by FAB-MS. The ^{1}H-NMR spectra (see *Exper. Part*) are of complex nature but show at least some distinct signals as further proof of the molecular structures.

3. Biochemical Application. – The infected-centers assay was used to measure the ability of the trimeric cordycepin-cholesterol conjugates **16** and **27** to inhibit HIV-1-induced syncytia formation, an indicator of HIV-1 replication in T-cells. Authentic (2′–5′)A trimer core inhibited syncytia formation 9-fold, whereas cordycepin trimer core inhibited syncytia formation 18-fold. Both cordycepin-cholesterol trimer conjugates **16** and **27** were 333- and 1000-fold, respectively, more potent inhibitors of HIV-1-induced syncytia formation than cordycepin trimer core (*Table*). Cordycepin-cholesterol conjugate **27** showed the highest activity and inhibited HIV-1-induced syncytia formation 18000-fold. Neither DMSO, cholesterol, cordycepin, nor adenin exhibited any inhibitory activity. The increased anti-HIV-1 activity of the cordycepin-cholesterol conjugates relative to cordycepin trimer core may be attributed to increased cellular uptake *via* membrane fluidization or by receptor-mediated endocytosis. Experiments are underway to determine the mechanism of uptake of these interesting new cordycepin conjugates.

Table. *Inhibition of HIV-1 Replication in Peripheral Blood Lymphocytes by Cordycepin-Cholesterol Conjugates*

Test compound[a])	Syncytia/plated cell (*m.o.i.* (IIIB) = 0.10)	Fold reduction in infection
Control (vehicle)	18/200	1
(2′–5′)A Trimer core	2/200	9
Cordycepin trimer core	1/200	18
Cordycepin-cholesterol trimer conjugate **16**	3/200,000	6000
Cordycepin-cholesterol trimer conjugate **27**	1/200,000	18000
DMSO (control)	20/200	0.9
Cholesterol	10/200	1.8
Cordycepin	12/200	1.5
Adenine	14/200	1.3

[a]) Compounds were tested at 100 μM, with the exception that cordycepin and adenine were tested at 300 μM and DMSO was tested at 1.7%.

These investigation were supported by the *Fonds der chemischen Industrie* and the *U.S. Public Health Service* grant P30-CA 12227.

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 FC (*Baker*). HPLC: *Merck-Hitachi L-6200, L-3000* photo diode array detector; column RP *18*, 125 × 4 mm, 5 μm, *Merck*; flow rate 1 ml/min, mobile phase: *A*; 0.1M (Et_3NH)OAc buffer (pH 7) MeCN 1:1; *B*, MeCN; gradient: 0 min, 100% *A*; 5 min, 100% *A*; 35 min, 100% *B*; 40 min, 100% *B*. UV/VIS: *Perkin Elmer Lambda 5*; λ_{max} in nm (log ε). ^{1}H-NMR: *Bruker AC 250*;

δ in ppm rel. to DMSO. Californium-252 plasma-desorption (PD) MS: *Bio-Ion 20 PDMS* from *Bio-Ion Nordic* (Uppsala, Sweden). Fast-atom bombardment (FAB) MS: *Finnigan MAT 312/AMD-5000*.

Bioassay. Sup T1 cells were obtained from the 'AIDS Research and Reference Reagent Program' and were maintained in RPMI-1640 medium supplemented with 10% fetal calf serum as described [19]. The infected-centers assay was used to measure the ability of cordycepin-cholesterol conjugates to inhibit HIV-1-induced syncytia formation in human cells as previously described [19]. Briefly, freshly isolated peripheral blood lymphocytes (PBL) were treated with (2′–5′)A trimer or derivatives for 2 h and infected with HIV-1 strain IIIB at a *m.o.i.* of *ca.* 0.1. The infected PBL were maintained in RPMI-1640 medium supplemented with 10% (*v*/*v*) heat-inactivated fetal calf serum at 37° in a humidified 5% CO_2 in air atmosphere. After 48 h, the cells were washed twice in *Hank*'s balanced salt soln. serially diluted, and seeded into multiple wells of a 96-well microtiter plate. Immediately, $2 \cdot 10^5$ exponentially growing Sup T1 cells were added to each well; Sup T1 cells readily form a syncytium with a cell which is productively infected with HIV-1. The wells were examined daily for the presence of syncytia, using an inverted tissue culture microscope. The first signs of syncytia formation can be seen in 12 h, with some complete syncytia developing by 24 h. Final results were read at 96 h. Each syncytium was counted as a single infected cell. The number of syncytia per seeded cell was determined and expressed as an infected center per plated cell.

1. *2′-O-(Cholest-5-en-3β-yloxycarbonyl)-3′-deoxy-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**3**). To an ice-cooled soln. of 3′-deoxy-5′-*O*-(monomethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine [4] (**1**; 1 g, 1.4 mmol) in abs. CH_2Cl_2 (10 ml) were given 1-methyl-1*H*-imidazole (286 mg, 3.5 mmol) and catalytic amounts of DMAP. A soln. of cholesteryl chloroformate (= cholest-5-en-3β-yl chloroformate; *Fluka*; 1.57 g, 3.5 mmol) in abs. CH_2Cl_2 (15 ml) was added dropwise. The mixture was kept at r.t. for 32 h and evaporated, the residue diluted with AcOEt (250 ml), the extract washed with sat. aq. NaCl soln. (3 × 60 ml), the aq. phase reextracted with AcOEt, and the combined org. layer dried ($MgSO_4$) and evaporated to give *2′-O-(cholest-5-en-3β-yloxycarbonyl)-3′-deoxy-5′-O-(monomethoxytrityl)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**2**), which was detritylated without further purification as follows: The residue (2.20 g) was dissolved in 30 ml of CH_2Cl_2/MeOH 4:1 containing 2% of TsOH·H_2O and kept at r.t. for 20 min. The mixture was diluted with $CHCl_3$ (150 ml), washed with sat. $NaHCO_3$ soln. (2 × 120 ml), the aq. phase reextracted with $CHCl_3$, the combined org. layer dried ($MgSO_4$) and evaporated and the residue purified by FC (silica gel, 18 × 3 cm, toluene/AcOEt 1:1→1:1 + 2% MeOH): 923 mg (77%) of **3**. Amorphous solid. UV (MeOH): 298 (sh, 4.38), 267 (4.41). ^{1}H-NMR ((D$_6$)DMSO): 10.61 (*s*, NH); 8.65, 8.61 (2*s*, H–C(8), H–C(2)); 8.15 (*d*, 2 H *o* to NO_2); 7.60 (*d*, 2 H *m* to NO_2); 6.23 (*s*, H–C(1′)); 5.59 (*m*, H–C(2′)); 5.31 (*m*, olef. H of chol); 5.07 (*t*, OH–C(5′)); 4.38 (*m*, H–C(4′), $OCH_2CH_2(N^6)$, H–C(3) of chol); 3.68 (*m*, 2 H–C(5′)); 3.10 (*t*, OCH_2CH_2); 2.58–0.62 (*m*, 45 H, 2 H–C(3′), chol). Anal. calc. for $C_{47}H_{64}N_6O_9 \cdot H_2O$ (875.1): C 64.51, H 7.60, N 9.60; found: C 64.84, H 7.68, N 9.51.

2. *2′-O-(Cholest-5-en-3β-yloxycarbonyl)-3′-deoxyadenosine* (**4**). To **3** (200 mg, 0.23 mmol) was added 0.5M DBU in dry pyridine (9.2 ml). The mixture was stirred at r.t. for 20 h, neutralized with 1M AcOH in pyridine (4.6 ml), and evaporated. The residue was diluted with $CHCl_3$ (50 ml), extracted with H_2O (3 × 30 ml), the aq. phase reextracted with $CHCl_3$, the combined org. layer dried ($MgSO_4$), evaporated, and co-evaporated with toluene, and the residue treated with hot MeOH (10 ml). After filtration, 95 mg (61%) of a solid was obtained. More **3** (20 mg, 13%) was isolated from the mother liquor. UV (MeOH): 259 (4.15). ^{1}H-NMR ($CDCl_3$): 8.33, 7.87 (2*s*, H–C(8), H–C(2)); 5.98 (*m*, H–C(1′), OH–C(5′)); 5.75 (br. NH_2); 5.57 (*m*, H–C(2′)); 5.38 (*m*, olef. H of chol); 4.59 ('*t*', H–C(5′)); 4.44 (*m*, H–C(3) of chol); 4.10 (*m*, H–C(4′)); 3.65 ('*t*', H–C(5′)); 2.99 (*m*, H–C(3′)); 2.37–0.67 (*m*, 44 H, H–C(3′), chol). Anal. calc. for $C_{38}H_{57}N_5O_5$ (663.9): C 68.75, H 8.65, N 10.55; found: C 68.24, H 8.62, N 10.59.

3. *2′-O-{[2-(Cholest-5-en-3β-yloxycarbonyl)ethyl]carbonyl}-3′deoxy-5′-O-(monomethoxytrityl)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**5**). A mixture of **1** [3] (1 g, 1.4 mmol) succinic anhydride (168 mg, 1.68 mmol) and DMAP (222 mg, 1.82 mmol) in abs. CH_2Cl_2 (7 ml) was kept at r.t. for 2.5 h. Then *N*-[3-(dimethylamino)propyl]-*N*′-ethylcarbodiimide hydrochloride (EDC; 349 mg, 1.82 mmol), cholesterol (= cholest-5-en-3β-ol) (758 mg, 1.96 mmol), and cat. amounts of DMAP were added. The mixture was kept at r.t. for 1.5 h, then diluted with $CHCl_3$ (100 ml), washed with sat. $NaHCO_3$ soln. (2 × 50 ml), the aq. phase reextracted with $CHCl_3$, the combined org. layer dried ($MgSO_4$) and evaporated, and the crude product purified by FC (silica gel, 14 × 3 cm, toluene→toluene/AcOEt 1:1): 1.11 g (67%) of **5**. Amorphous solid. UV (MeOH): 298 (sh, 3.57), 272 (sh, 4.41), 267 (4.44), 235 (4.30). ^{1}H-NMR ($CDCl_3$): 8.69, 8.21–8.12 (3*s*, *d*, H–C(8), H–C(2), NH, 2 H *o* to NO_2); 7.46–7.20 (*m*, 14 H, 2 H *m* to NO_2, MeO *Tr*); 6.80 (*d*, 2 H *o* to MeO); 6.15 (*s*, H–C(1′)); 5.77 (*d*, H–C(2′)); 5.35 (*m*, olef. H of chol); 4.62–4.51 (*m*, H–C(4′), H–C(3) of chol); 4.53 (*t*, OCH_2CH_2 (N^6)); 3.78 (*s*, MeO); 3.41–3.37 (*m*, 2 H–C(5′)); 3.16 (*t*, OCH_2CH_2 (N^6)); 2.69–2.64 (*m*, $OOCCH_2CH_2COO$, H–C(3′)); 2.32–0.67 (*m*, 44 H, H–C(3′), chol). Anal. calc. for $C_{70}H_{84}N_6O_{11} \cdot H_2O$ (1203.6): C 69.68, H 7.20, N 6.98; found: C 69.92, H 7.23, N 6.67.

4. *2′-O-{[2-(Cholest-5-en-3β-yloxycarbonyl)ethyl]carbonyl}-3′-deoxy-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**6**). Compound **5** (1.19 g, 1 mmol) was stirred at r.t. in CH_2Cl_2/MeOH 4:1 (20 ml) containing 2% of TsOH·H_2O for 15 min. Then the mixture was diluted with $CHCl_3$ (130 ml), washed with sat. $NaHCO_3$ soln. (3 × 50 ml), the aq. phase reextracted with $CHCl_3$, the combined org. layer dried ($MgSO_4$) and evaporated, and the residue purified by FC (silica gel, 14 × 3 cm, toluene→toluene/AcOEt 3:2→1:1 + 5% MeOH): 886 mg (97%) of **6**. Amorphous solid. UV (MeOH): 296 (sh, 3.64), 272 (sh, 4.40), 267 (4.44). ^{1}H-NMR ($CDCl_3$): 8.71, 8.08 (2*s*, H–C(8), H–C(2)); 8.44 (br. NH); 8.17 (*d*, 2 H *o* to NO_2); 7.44 (*d*, 2 H *m* to NO_2); 6.00 (*d*, H–C(1′)); 5.53 (*m*, H–C(2′)); 5.36 (*m*, olef. H of chol); 4.55 (*m*, H–C(4′), $OCH_2CH_2(N^6)$, H–C(3) of chol); 4.10 (*m*, H–C(5′)); 3.68 (*m*, H–C(5′)); 3.15 (*t*, $OCH_2CH_2(N^6)$); 2.89 (*m*, 1 H–C(3′)); 2.63 (*s*, $OOCCH_2CH_2COO$); 2.32–0.68 (*m*, 44 H, H–C(3′), chol). Anal. calc. for $C_{50}N_{68}N_6O_{10}\cdot H_2O$ (931.2): C 65.12, H 7.54, N 9.11; found: C 64.86, H 7.62, N 9.12.

5. *2′-O-{[2-(Cholest-5-en-3β-yloxycarbonyl)ethyl]carbonyl}-3′-deoxyadenosine* (**7**). To **6** (183 mg, 0.2 mmol), which was co-evaporated twice in abs. pyridine, was added 0.5M DBU in dry pyridine (7.9 ml). The mixture was stirred at r.t. for 18 h, neutralized with 1M AcOH in pyridine (3.85 ml), and evaporated. The residue was diluted with $CHCl_3$ (60 ml), extracted with H_2O (3 × 25 ml), the aq. phase reextracted with $CHCl_3$, the combined org. layer dried ($MgSO_4$), evaporated, and co-evaporated with toluene, and the residue crystallized in EtOH (15 ml): 72 mg (50%) of colorless crystals, m.p.: 208°. More **7** (10 mg, 7%) was isolated from the mother liquor. UV (MeOH): 259 (4.15). ^{1}H-NMR ((D$_6$)DMSO): 8.32, 8.11 (2*s*, H–C(8), H–C(2)); 7.31 (*s*, NH_2); 6.06 (*d*, H–C(1′)); 5.60 (*d*, H–C(2′)); 5.30 (*m*, olef. H of chol); 5.13 (*t*, OH–C(5′)); 4.43 (*m*, H–C(3) of chol); 4.24 (*m*, H–C(4′)); 3.63 (*m*, 2 H–C(5′)); 2.55 (*m*, H–C(3′), $OOCCH_2CH_2COO$); 2.24–0.63 (*m*, 44 H, H–C(3′), chol). Anal. calc. for $C_{41}H_{61}N_5O_6\cdot H_2O$ (738.0): C 67.50, H 8.57, N 9.61; found: C 67.60, H 8.55, N 9.41.

6. *3′-Deoxy-5′-O-(monomethoxytrityl)-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 2′-[2-(4-Nitrophenyl)ethyl N,N-Diisopropylphosphoramidite]* [18] (**8**). A mixture of **1** [4] (1.8 g, 2 mmol), bis(diisopropylamino)[2-(4-nitrophenyl)ethoxy]phosphane [18] (1.59 g, 4 mmol), and 1*H*-tetrazole (70 mg, 1 mmol) in anh. MeCN (10 ml) was kept at r.t. for 2 h. Then it was diluted with $CHCl_3$ (100 ml) and extracted with NaCl/$NaHCO_3$ soln. 4:1, the org. phase reextracted with $CHCl_3$, the combined org. layer dried ($MgSO_4$) and evaporated, and the residue purified by FC (silica gel, 15 × 2 cm, toluene/AcOEt 1:1): 1.82 g (90%) of **8**. Amorphous solid. UV (MeOH): 268 (4.56), 233 (4.35). ^{1}H-NMR ($CDCl_3$): 8.69, 8.21–8.00 (*s*, *m*, NH, H–C(8), H–C(2), 4 H *o* to NO_2); 7.47–7.16 (*m*, 4 H *m* to NO_2, 12 H of MeOTr); 6.82 (2*d*, 2 H *o* to MeO); 6.15 (*s*, H–C(1′)); 4.92 (*m*, H–C(2′)); 4.62 (*m*, H–C(4′)); 4.53 (*m*, OCH_2CH_2); 3.96–3.81 (*m*, OCH_2CH_2); 3.79 (2*s*, MeO); 3.55 (*m*, Me_2CH); 3.43 (*m*, H–C(5′)); 3.35 (*m*, H–C(5′)); 3.16 (2*t*, OCH_2CH_2); 2.97 (*m*, OCH_2CH_2); 2.29 (*m*, H–C(3′)); 2.10 (*m*, H–C(3′)); 1.11 (*m*, 2 Me_2CH). ^{31}P-NMR ($CDCl_3$): 149.54; 148.62. Anal. calc. for $C_{53}H_{57}N_8O_{11}P$ (1013.1): C 62.84, H 5.67, N 11.06; found: C 62.60, H 5.82, N 10.82.

7. *3′-Deoxy-5′-O-(monomethoxytrityl)-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]}→5′}-2′-O-(cholest-5-en-3β-yloxycarbonyl)-3′-deoxy-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**9**). A mixture of **3** (470 mg, 0.55 mmol), **8** [18] (1.11 g, 1.1 mmol), and 1*H*-tetrazole (191 mg, 2.74 mmol) was stirred in dry MeCN/CH_2Cl_2 4:1 (5 ml) 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_2 (1 ml), and H_2O (1 ml)) until no change of color was detected. The mixture was stirred for 15 min, diluted with $CHCl_3$ (100 ml), and washed with a $Na_2S_2O_3$/NaCl soln. (2 × 50 ml), the aq. phase reextracted with $CHCl_3$, the combined org. layer dried ($MgSO_4$), evaporated, and co-evaporated with toluene, and the residue purified by FC (silica gel, 16 × 3 cm, toluene/AcOEt 1:1 + 4% MeOH→1:1 + 5% MeOH): 863 mg (88%) of an amorphous solid. UV (MeOH): 296 (sh, 4.11), 274 (sh, 4.73), 267 (4.79), 237 (sh, 4.47). ^{1}H-NMR ($CDCl_3$): 8.70–8.00 (*m*, 2 NH, 2 H–C(8), 2 H–C(2), 6 H *o* to NO_2); 7.45–7.10 (*m*, 6 *m* to NO_2, 12 H of MeOTr); 6.79 (*d*, 2 H *o* to MeO); 6.19–6.10 (*m*, H–C(1′)); 5.64 (*m*, H–C(2′)); 5.40 (*m*, H–C(2′), olef. H of chol); 4.60–4.20 (*m*, 2 H–C(4′), H–C(3) of chol, 3 OCH_2CH_2, 2 H–C(5′)); 3.77 (*s*, MeO); 3.40 (*m*, 2 H–C(5′)); 3.22–0.67 (*m*, 3 OCH_2CH_2, 4 H–C(3′), 43 H of chol). Anal. calc. for $C_{94}H_{106}N_{13}O_{21}P\cdot H_2O$ (1802.9): C 62.62, H 6.04, N 10.10; found: C 62.44, H 6.03, N 9.67.

8. *3′-Deoxy-5′-O-(monomethoxytrityl)-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]}→5′}-2′-O-{[2-(cholest-5-en-3β-yloxycarbonyl)ethyl]carbonyl}-3′-deoxy-N⁶-[2-(4-nitrophenyl)-ethoxycarbonyl]adenosine* (**10**). As described in *Exper. 7*, with **6** (456 mg, 0.5 mmol) **8** [18] (709 mg, 0.7 mmol), 1*H*-tetrazole (140 mg, 2 mmol), anh. CH_2Cl_2 (2.5 ml; 1 h), more **6** (305 mg, 0.3 mmol; 4 h), and I_2 soln. Workup with $CHCl_3$ (80 ml) and $Na_2S_2O_3$/NaCl soln. (2 × 30 ml), FC (silica gel, 17 × 3 cm, toluene/AcOEt 1:1 + 6% MeOH) gave 840 mg (91%) of **10**. Amorphous solid. UV (MeOH): 296 (sh, 4.13), 272 (sh, 4.73), 267 (4.76), 238 (sh, 4.45). ^{1}H-NMR ($CDCl_3$): 8.70–8.62, 8.20–8.06 (4*s*, 2 NH, 2 H–C(8), 2 H–C(2), 6 H *o* to NO_2); 7.47–7.19 (*m*, 6 H *m* to NO_2, 12 H of MeOTr); 6.80 (*d*, 2 H *o* to MeO); 6.20 (*d*, H–C(1′)); 6.05 (*s*, H–C(1′)); 5.60 (*m*, H–C(2′)); 5.45

(*m*, H–C(2′)); 5.35 (*m*, olef. H of chol); 4.65–4.50 (*m*, 2 H–C(4′), H–C(3) of chol, 2 OCH_2CH_2); 4.45–4.22 (*m*, OCH_2CH_2, 2 H–C(5′)); 3.78 (*s*, MeO); 3.42–3.30 (*m*, 2 H–C(5′)); 3.20–3.00 (*m*, 3 OCH_2CH_2); 2.67–0.67 (*m*, $OOCCH_2CH_2COO$), 4 H–C(3′), 43 H of chol). Anal. calc. for $C_{97}H_{110}N_{13}O_{22}P \cdot H_2O$ (1859.1): C 62.67, H 6.07, N 9.79; found: C 62.42, H 6.03, N 9.63.

9. *3′-Deoxy-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{*O^P*-[2-(4-nitrophenyl)ethyl]}→5′}-2′-O-(cholest-5-en-3β-yloxycarbonyl)-3′-deoxy-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**11**). As described in *Exper. 4*, with **9** (863 mg, 0.48 mmol) and CH_2Cl_2/MeOH 4:1 (9.7 ml) containing 2% of $TsOH \cdot H_2O$ (1 h). Workup with $CHCl_3$ (120 ml) and sat. $NaHCO_3$ soln. (2 × 50 ml). FC (silica gel, 16.5 × 3 cm, toluene/AcOEt 1:1 + 4% MeOH→toluene/AcOEt 1:1 + 15% MeOH) gave 600 mg (82%) of **11**. Amorphous solid. UV (MeOH): 296 (sh, 4.07), 274 (sh, 4.71), 267 (4.76). ^{1}H-NMR ((D_6)DMSO): 10.61 (br., 2 NH); 8.63–8.53 (*m*, 2 H–C(8), 2 H–C(2)); 8.16–8.02 (*m*, 6 H *o* to NO_2); 7.62–7.37 (*m*, 6 H *m* to NO_2); 6.23–6.15 (*m*, 2 H–C(1′)); 5.62 (*m*, H–C(2′)); 5.31 (*m*, olef. H of chol); 5.26 (*m*, H–C(2′)); 5.11 (*m*, OH–C(5′)); 4.50–4.10 (*m*, 2 H–C(4′), H–C(3) of chol, 3 OCH_2CH_2, 2 H–C(5′)); 3.45 (*m*, 2 H–C(5′)); 3.10 (*m*, 2 OCH_2CH_2); 2.93 (*m*, OCH_2CH_2); 2.65–0.62 (*m*, 4 H–C(3′), 43 H of chol). Anal. calc. for $C_{74}H_{90}N_{13}O_{20}P$ (1512.6): C 58.76, H 6.00, N 12.04; found: C 58.58, H 6.05, N 12.02.

10. *3′-Deoxy-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{*O^P*-[2-(4-nitrophenyl)ethyl]}→5′}-2′-O-{[2-(cholest-5-en-3β-yloxycarbonyl)ethyl]carbonyl}-3′-deoxy-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**12**). As described in *Exper. 4*, with **10** (565 mg, 0.3 mmol) and CH_2Cl_2/MeOH 4:1 (6 ml) containing 2% of $TsOH \cdot H_2O$ (1 h). Workup with $CHCl_3$ (80 ml) and sat. $NaHCO_3$ soln. (2 × 30 ml). FC (silica gel, 11 × 3 cm, toluene/AcOEt 1:1 + 4% MeOH→1:1 + 12% MeOH) gave 437 mg (91%) of amorphous solid. UV (CH_2Cl_2): 298 (sh, 4.74), 272 (sh, 4.17), 267 (4.78). ^{1}H-NMR ((D_6)DMSO): 10.5 (br., 2 NH); 8.62–8.54 (*m*, 2 H–C(8), 2 H–C(2)); 8.33–8.02 (*m*, 6 H *o* to NO_2); 7.61–7.37 (*m*, 6 H *m* to NO_2); 6.19–6.14 (*m*, 2 H–C(1′)); 5.70 (*m*, H–C(2′)); 5.23 (*m*, H–C(2′), olef. H of chol); 5.10 (*t*, OH–C(5′)); 4.45–4.15 (*m*, 2 H–C(4′), H–C(3) of chol, 3 OCH_2CH_2, 2 H–C(5′)); 3.69 (*m*, H–C(5′)); 3.50 (*m*, H–C(5′)); 3.10 (*m*, 2 OCH_2CH_2); 2.92 (*t*, OCH_2CH_2); 2.55 (*m*, $OOCCH_2CH_2COO$); 2.48–0.60 (*m*, 4 H–C(3′), 43 H of chol). Anal. calc. for $C_{77}H_{94}N_{13}O_{21}P$ (1568.7): C 58.96, H 6.04, N 11.61; found: C 58.64, H 6.00, N 11.64.

11. *3′-Deoxy-5′-O-(monomethoxytrityl)-*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′}-2′-O-(cholest-5-en-3β-yloxycarbonyl)-3′-deoxy-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**13**). As described in *Exper. 7*, with **11** (600 mg, 0.4 mmol), **8** [18] (800 mg, 0.79 mmol), 1*H*-tetrazole (140 mg, 2 mmol), anh. MeCN/CH_2Cl_2 3:1 (4 ml), and I_2 soln. Workup with $CHCl_3$ (100 ml) and $Na_2S_2O_3$/NaCl soln. (2 × 40 ml). FC (silica gel, 14 × 3 cm, $CHCl_3$ + 1→5% MeOH): gave 748 mg (78%) of **13** and 208 mg (21%) of an impure fraction as amorphous solids. UV (MeOH): 272 (sh, 4.90), 267 (4.93), 238 (sh, 4.55). ^{1}H-NMR ($CDCl_3$): 9.62–7.85 (*m*, 3 NH, 3 H–C(8), 3 H–C(2), 10 H *o* to NO_2); 7.45–7.12 (*m*, 10 H *m* to NO_2, 12 H of MeOTr); 6.79 (*d*, 2 H *o* to MeO); 6.15 (*m*, 2 H–C(1′)); 6.00 (*d*, H–C(1′)); 5.68 (*m*, H–C(2′)); 5.60–5.20 (*m*, 2 H–C(2′), olef. H of chol); 4.60–4.12 (*m*, 3 H–C(4′), H–C(3) of chol, 5 OCH_2CH_2, 4 H–C(5′)); 3.77 (*s*, MeO); 3.42 (*dd*, H–C(5′)); 3.37 (*dd*, H–C(5′)); 3.20–3.00 (*m*, 5 OCH_2CH_2); 2.75 (*m*, H–C(3′)); 2.55 (*m*, H–C(3′)); 2.50–0.68 (*m*, 4 H–C(3′), 43 H of chol). Anal. calc. for $C_{121}H_{132}N_{20}O_{32}P_2 \cdot H_2O$ (2440.4): C 59.55, H 5.45, N 11.48; found: C 59.03, H 5.36, N 11.39.

12. *3′-Deoxy-5′-O-(monomethoxytrityl)-*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′}-2′-O-{[2-(cholest-5-en-3β-yloxycarbonyl)ethyl]carbonyl}-3′-deoxy-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**14**). As described in *Exper. 7*, with **12** (157 mg, 0.1 mmol), **8** [18] (204 mg, 0.4 mmol), 1*H*-tetrazole (35 mg, 0.5 mmol), anh. MeCN (1 ml), anh. CH_2Cl_2 (0.15 ml), and I_2 soln. Workup with $CHCl_3$ (40 ml) and $Na_2S_2O_3$/NaCl soln. (2 × 10 ml). FC (silica gel, 13 × 2 cm, $CHCl_3$ + 1→ + 3% MeOH) gave **14** (234 mg, 94%). Amorphous solid. UV (MeOH): 296 (sh, 4.93), 274 (sh, 4.87), 267 (4.93), 235 (sh, 4.53). ^{1}H-NMR ($CDCl_3$): 8.69–8.51, 8.26–8.03 (*m*, 3 NH, 3 H–C(8), 3 H–C(2), 10 H *o* to NO_2); 7.46–7.16 (*m*, 10 H *m* to NO_2, 12 H of MeOTr); 6.79 (*d*, 2 H *o* to MeO); 6.18 (*d*, H–C(1′)); 6.08 (*d*, H–C(1′)); 5.99 (*d*, H–C(1′)); 5.75 (*m*, H–C(2′)); 5.60–5.30 (*m*, 2 H–C(2′), olef. H of chol); 4.65–4.20 (*m*, 3 H–C(4′), H–C(3) of chol, 5 OCH_2CH_2, 4 H–C(5′)); 3.77 (*s*, MeO); 3.47 (*m*, H–C(5′)); 3.35 (*m*, H–C(5′)); 3.20–3.00 (*m*, 5 OCH_2CH_2); 2.47 (br., $OOCCH_2CH_2COO$); 2.35–0.67 (*m*, 6 H–C(3′), 43 H of chol). Anal. calc. for $C_{124}H_{136}N_{20}O_{33}P_2$ (2496.6): C 59.66, H 5.49, N 11.22; found: C 59.75, H 5.52, N 11.00.

13. *3′-Deoxyadenylyl-(2′ → 5′)-3′-deoxyadenylyl-(2′ → 5′)-2′-O-(cholest-5-en-3β-yloxycarbonyl)-3′-deoxyadenosine* (**15**). A mixture of dry **13** (24.4 mg, 10 μmol) and DBU (190 mg, 1.25 mmol) in dry MeCN (2.5 ml) was kept at r.t. for 20 h. Then AcOH (75 mg, 1.25 mmol) was added, the mixture evaporated, then diluted with $CHCl_3$ (35 ml), and washed with H_2O (10 ml). The aq. phase was reextracted with $CHCl_3$ and the combined org. layer

dried ($MgSO_4$) and evaporated. To the residue was added 80% AcOH (5 ml). The mixture was kept at r.t. for 19 h and then lyophilized. The residue was washed and centrifugated several times with H_2O and Et_2O: **15** (10 mg). Colorless powder. HPLC: t_R 23.95 min. FAB-MS (matrix DMSO/3-nitrobenzyl alcohol): 1291 (MH^+). ^{252}Cf-PD-MS (accelerating voltage 16 kV, 10 Mio counts): 1291.1 (MH^+).

14. *3′-Deoxyadenylyl-(2′→5′)-3′-deoxyadenylyl-(2′→5′)-2′-O-{[2-(cholest-5-en-3β-yloxycarbonyl)ethyl]-carbonyl}-3′-deoxyadenosine* (**16**). As described in *Exper. 13*, with dry **14** (25 mg, 10 μmol), DBU (152 mg, 1 mmol), dry MeCN (2 ml; 24 h), and AcOH (60 mg, 1 mmol). Workup with $CHCl_3$ (20 ml) and H_2O (2 × 10 ml). After treatment with 80% AcOH (5 ml; 18 h), lyophilization and centrifugation, the residue was washed several times with H_2O, MeCN, Et_2O/EtOH 4:1, and Et_2O: **16** (14 mg). Colorless powder. HPLC: 26.03 min.

15. *2′-O-[(*tert*-Butyl)dimethylsilyl]-3′-deoxy-5′-O-(monomethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**17**). A mixture of **1** [4] (2.15 g, 3 mmol) and 1*H*-imidazole (490 mg, 7.2 mmol) was co-evaporated with abs. pyridine (2 × 15 ml). The residue was dissolved in abs. pyridine (20 ml) and (*t*-Bu)Me_2SiCl (543 mg, 3.6 mmol) added. The mixture was kept at r.t. for 22 h, diluted with $CHCl_3$ (100 ml), and washed with sat. $NaHCO_3$ soln. (3 × 40 ml). The aq. phase was reextracted with $CHCl_3$, the combined org. layer dried ($MgSO_4$), evaporated, and co-evaporated with toluene, and the residue purified by FC (silica gel, toluene/AcOEt 4:1→3:1): 1.92 g (77%) of **17**. Amorphous solid. UV (MeOH): 273 (sh, 4.40), 267 (4.45), 234 (4.29). ^{1}H-NMR ($CDCl_3$): 8.72, 8.25–8.17 (*s*, *m*, H–C(8), H–C(2), NH, 2 H *o* to NO_2); 7.46–7.23 (*m*, 14 H, 2 H *m* to NO_2, MeOTr); 6.83 (*d*, 2 H *o* to MeO); 6.03 (*s*, H–C(1′)); 4.81 (*d*, H–C(2′)); 4.68 (*m*, H–C(4′)); 4.53 (*t*, OCH_2CH_2 (N^6)); 3.79 (*s*, MeO); 3.48 (*dd*, H–C(5′)); 3.16 (*t*, OCH_2CH_2 (N^6)); 2.19 (*m*, H–C(3′)); 1.95 (*m*, H–C(3′)); 0.90 (*s*, *t*-Bu); 0.14 (*s*, MeSi); 0.09 (*s*, MeSi). Anal. calc. for $C_{45}H_{50}N_6O_8Si$ (831.1): C 65.04, H 6.06, N 10.11; found: C 65.28, H 6.16, N 9.80.

16. *2′-O-[(*tert*-Butyl)dimethylsilyl]-3′-deoxy-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**18**). As described in *Exper. 4*, with **17** (831 mg, 1 mmol) and CH_2Cl_2/MeOH 4:1 (20 ml) containing 2% of TsOH·H_2O. Workup with AcOEt (130 ml) and sat. $NaHCO_3$ soln. (2 × 60 ml). FC (silica gel, 11.5 × 3 cm, toluene/AcOEt 1:1 + 6% MeOH) gave 530 mg (95%) of **18**. Amorphous solid. UV (MeOH): 298 (sh, 3.58), 274 (sh, 4.38), 268 (4.44). ^{1}H-NMR ((D$_6$)DMSO): 10.59 (*s*, NH); 8.70, 8.61 (2*s*, H–C(8), H–C(2)); 8.15 (*d*, 2 H *o* to NO_2), 7.61 (*d*, 2 H *m* to NO_2); 5.96 (*s*, H–C(1′)); 5.15 (*t*, OH–C(5′)); 4.74 (*m*, H–C(2′)); 4.38 (*m*, H–C(4′), OCH_2CH_2 (N^6)); 3.73 (*m*, H–C(5′)); 3.58 (*m*, H–C(5′)); 3.10 (*t*, OCH_2CH_2); 2.27 (*m*, H–C(3′)); 1.90 (*m*, H–C(3′)); 0.82 (*s*, *t*-Bu); 0.00 (*s*, Me_2Si). Anal. calc. for $C_{25}H_{34}N_6O_7Si$ (558.7): C 53.75, H 6.13, N 15.04; found: C 53.98, H 6.12, N 14.80.

17. *5′-O-{[2-(Cholest-5-en-3β-yloxycarbonyl)ethyl]carbonyl}-3′-deoxy-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**20**). A mixture of **18** (560 mg, 1 mmol), succinic anhydride (120 mg, 1.2 mmol), and DMAP (160 mg, 1.3 mmol) in abs. CH_2Cl_2 (2.5 ml) was kept at r.t. for 2 h. Then the mixture was diluted with abs. CH_2Cl_2 (1 ml), and EDC (250 mg, 1.3 mmol) and cholest-5-en-3β-ol (540 mg, 1.4 mmol) were added. Then it was proceeded as described in *Exper. 3*. FC (silica gel, 14 × 3 cm, toluene/AcOEt 3:2→1:1→1:1 + 2% MeOH) gave 824 mg of crude **19**, contaminated with cholesterol. This amorphous solid was desilylated without further purification by dissolving it in abs. THF (4 ml) and treatment with AcOH (194 mg, 3.2 mmol) in abs. THF (4 ml) and $Bu_4NF \cdot 3\,H_2O$ (255 mg, 0.80 mmol). The mixture was kept at r.t. for 2 d, diluted with AcOEt (160 ml), and washed with sat. $NaHCO_3$ soln. (2 × 50 ml). The aq. phase was reextracted with AcOEt, the combined org. layer dried ($MgSO_4$) and evaporated, and the crude product purified by FC (silica gel, 14 × 3 cm, toluene/AcOEt 1:1 + 4% MeOH→toluene/AcOEt 1:1 + 6% MeOH): 558 mg (61%) of **20**. Amorphous solid. UV (MeOH): 296 (sh, 3.65), 272 (sh, 4.39), 267 (4.44), 235 (4.30). ^{1}H-NMR ((D$_6$)DMSO): 10.59 (*s*, NH); 8.61, 8.52 (2*s*, H–C(8), H–C(2)); 8.13 (*d*, 2 H *o* to NO_2); 7.60 (*d*, 2 H *m* to NO_2); 5.99 (*s*, H–C(1′)); 5.79 (*m*, OH–C(2′)); 5.25 (*m*, olef. H of chol); 4.68 (*d*, H–C(2′)); 4.55 (*m*, H–C(4′)); 4.39–4.23 (*m*, H–C(3) of chol, OCH_2CH_2 (N^6), 2 H–C(5′)); 3.09 (*t*, OCH_2CH_2 (N^6)); 2.40–0.59 (*m*, $OOCCH_2CH_2COO$, 2 H–C(3′), 43 H of chol). Anal. calc. for $C_{50}H_{68}N_6O_{10}$ (913.2): C 65.76, H 7.51, N 9.20; found: C 65.68, H 7.48, N 9.12.

18. *5′-O-{[2-(Cholest-5-en-3β-yloxycarbonyl)ethyl]carbonyl}-3′-deoxyadenosine* (**21**). To **20** (183 mg, 0.2 mmol), which was co-evaporated twice in abs. toluene, was added 0.5M DBU in dry pyridine (8 ml). The mixture was stirred at r.t. for 16 h, neutralized with 1M AcOH in pyridine (4 ml), and evaporated. The residue was diluted with $CHCl_3$ (80 ml), and washed with H_2O (3 × 25 ml), the aq. phase reextracted with $CHCl_3$, the combined org. layer dried ($MgSO_4$), evaporated, and co-evaporated with toluene, and the residue purified by FC (silica gel, 10 × 2 cm, $CHCl_3$→$CHCl_3$ + 10% MeOH): 140 mg (97%) of an amorphous solid. Crystallization in EtOH (8 ml) gave **21** (100 mg, 69%). Colorless crystals. UV (MeOH): 259 (4.13). ^{1}H-NMR ((D$_6$)DMSO): 8.23, 8.13 (2*s*, H–C(8), H–C(2)); 7.27 (*s*, NH_2); 5.89 (*s*, H–C(1′)); 5.75 (*d*, OH–C(2′)); 5.29 (*m*, olef. H of chol); 4.62 (*m*, H–C(2′)); 4.49 (*m*, H–C(4′)); 4.40 (*m*, H–C(3) of chol); 4.24 (*m*, 2 H–C(5′)); 2.52 (*m*, $OOCCH_2CH_2COO$); 2.33–0.63 (*m*, 45 H, 2 H–C(3′), chol). Anal. calc. for $C_{41}H_{61}N_5O_6$ (720.0): C 68.40, H 8.54, N 9.73; found: C 68.00, H 8.56, N 9.55.

19. *5′-O-{[2-(Cholest-5-en-3β-yloxycarbonyl)ethyl]carbonyl}-3′-deoxy-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 2′-[2-(4-Nitrophenyl)ethyl N,N-Diisopropylphosphoramidite]* (**22**). As described in *Exper. 6*, with **20** (270 mg, 0.295 mmol), bis(diisopropylamino)[2-(4-nitrophenyl)ethoxy]phosphane (235 mg, 0.59 mmol) [18], 1*H*-tetrazole (10.3 mg, 0.148 mmol), and dry MeCN/CH_2Cl_2 2:1 (1.5 ml; 2.5 h). Workup with $CHCl_3$ (30 ml) and NaCl/$NaHCO_3$ 3:1 (10 ml). FC (silica gel, 15 × 2 cm, toluene/AcOEt 1:1) gave 300 mg (84%) of **22**. Amorphous solid. UV (CH_2Cl_2): 272 (sh, 4.52), 267 (4.55). ^{1}H-NMR ($CDCl_3$): 8.71, 8.20–8.00 (*s*, *m*, NH, H–C(8), 4 H *o* to NO_2); 7.46–7.26 (*m*, H *m* to NO_2); 6.10 (*s*, H–C(1′)); 5.34 (*m*, olef. H of chol); 4.95 (*m*, H–C(2′)); 4.57–4.52 (*m*, *t*, H–C(4′), H–C(3) of chol, OCH_2CH_2); 4.40–4.37 (*m*, OCH_2CH_2); 3.92–3.75 (*m*, 2 Me_2CH); 3.59–3.53 (*m*, 2 H–C(5′)); 3.17 (*t*, OCH_2CH_2); 3.03–2.96 (*t*, OCH_2CH_2); 2.61 (*m*, $OOCCH_2CH_2COO$); 2.31–0.67 (*m*, 57 H, 2 H–C(3′), 2 Me_2CH, chol). Anal. calc. for $C_{64}H_{89}N_6O_{13}P$ (1209.4): C 63.56, H 7.42, N 9.26; found: C 63.15, H 7.60, N 8.97.

20. *3′-Deoxy-5′-O-(monomethoxytrityl)-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]}→5′-}-3′-deoxy-N⁶,2′-O-bis[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**24**). As described in *Exper. 7*, with 3′-deoxy-*N*⁶,2′-*O*-bis[2-(4-nitrophenyl)ethoxycarbonyl]adenosine [4] (**23**; 227 mg, 0.43 mmol), **8** [18] (880 mg, 0.87 mmol), 1*H*-tetrazole (152 mg, 2.17 mmol), abs. MeCN (3 ml), abs. CH_2Cl_2 (0.2 ml) and I_2 soln. Workup with $CHCl_3$ (100 ml) and $Na_2S_2O_3$/NaCl soln. (2 × 30 ml). FC (silica gel, 13 × 3 cm, toluene/AcOEt 1:1 + 4% MeOH→1:1 + 20% MeOH) gave 320 mg (47%) of **24** and 306 mg (45%) of an impure fraction as amorphous solids. UV (MeOH): 272 (sh, 4.78), 267 (4.82), 236 (sh, 4.46). ^{1}H-NMR ($CDCl_3$): 8.68–8.60, 8.24–8.02 (4*s*, *m*, 2 NH, 2 H–C(8), 2 H–C(2), 8 H *o* to NO_2); 7.46–7.16 (*m*, 8 H *m* to NO_2, 12 H of MeOTr); 6.80 (*d*, 2 H *o* to MeO); 6.18 (*d*, H–C(1′)); 5.62 (*m*, H–C(2′)); 5.42 (*m*, H–C(2′)); 4.60–4.25 (*m*, 2 H–C(4′), 4 OCH_2CH_2, 2 H–C(5′)); 3.78 (*s*, MeO); 3.46 (*m*, H–C(5′)); 3.32 (*m*, H–C(5′)); 3.20–3.02 (*m*, 4 OCH_2CH_2); 2.70 (*m*, H–C(3′)); 2.50 (*m*, H–C(3′)); 2.27 (*m*, H–C(3′)); 2.21 (*m*, H–C(3′)). Anal. calc. for $C_{75}H_{69}N_{14}O_{23}P \cdot 1/2$ toluene (1611.6): C 58.51, H 4.57, N 12.16; found: C 58.38, H 4.63, N 11.83.

21. *3′-Deoxy-5′-O-(monomethoxytrityl)-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]}→5′}-3′-deoxy-N⁶,2′-O-bis[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**25**). As described in *Exper. 4*, with **24** (320 mg, 0.2 mmol) and CH_2Cl_2/MeOH 4:1 (4 ml) containing 2% of $TsOH \cdot H_2O$ (1 h). Workup with $CHCl_3$ (40 ml) and sat. $NaHCO_3$ soln. (2 × 20 ml). FC (silica gel, 12 × 2 cm, $CHCl_3$ + 1→10% MeOH) gave 225 mg (85%) of **25**. Amorphous solid. UV (MeOH): 296 (sh, 4.20), 272 (sh, 4.80), 267 (4.84). ^{1}H-NMR ((D_6)DMSO): 10.06 (*m*, 2 NH); 8.61–7.36 (*m*, 2 H–C(8), 2 H–C(2), 8 H *o* to NO_2, 8 H *m* to NO_2); 6.18 (*m*, 2 H–C(1′)); 5.61 (*m*, H–C(2′)); 5.24 (*m*, H–C(2′)); 5.11 (*m*, OH–C(5′)); 4.40–4.04 (*m*, 2 H–C(4′), 4 OCH_2CH_2, 2 H–C(5′)); 3.65 (*m*, H–C(5′)); 3.44 (*m*, H–C(5′)); 3.09 (*m*, 3 OCH_2CH_2); 2.90 (*m*, OCH_2CH_2); 2.60 (*m*, H–C(3′)); 2.26 (*m*, 2 H–C(3′)); 2.05 (*m*, H–C(3′)). Anal. calc. for $C_{55}H_{53}N_{14}O_{22}P \cdot H_2O$ (1311.0): C 50.39, H 4.23, N 14.96; found: C 50.47, H 4.25, N 14.54.

22. *5′-O-{[2-(Cholest-5-en-3β-yloxycarbonyl)ethyl]carbonyl}-3′-deoxy-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]}→5′}-3′-deoxy-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]}→5′}-3′-deoxy-N⁶,2′-O-bis-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**26**). As described in *Exper. 7*, with **25** (171 mg, 0.133 mmol) **8** [18], 242 mg, 0.2 mmol), 1*H*-tetrazole (47 mg, 0.665 mmol) anh. MeCN (1 ml), anh. CH_2Cl_2 (0.2 ml; 8 h), and I_2 soln. Workup with $CHCl_3$ (40 ml) and $Na_2S_2O_3$/NaCl soln. (2 × 15 ml). FC (silica gel, 13 × 2 cm, $CHCl_3$→$CHCl_3$ + 4% MeOH) gave 42 mg (13%) of **26**. Amorphous solid. Another 210 mg of impure product was purified by prep. TLC (silica gel, 20 × 40 cm, $CHCl_3$ + 5% MeOH): 125 mg (39%) of **26** and 63 mg (43%) of **25**. **26**: UV (CH_2Cl_2): 285 (sh, 4.73), 272 (sh, 4.99), 267 (5.03). ^{1}H-NMR ($CDCl_3$): 8.92–7.94 (*m*, 3 H–C(8), 3 H–C(2), 12 H *o* to NO_2); 7.48–7.27 (*m*, 12 H *m* to NO_2); 6.14–6.06 (*m*, 3 H–C(1′)); 5.66–5.31 (*m*, 3 H–C(2′), olef. H of chol); 4.59–4.10 (*m*, 3 H–C(4′), H–C(3) of chol, 6 OCH_2CH_2, 6 H–C(5′)); 3.17–3.02 (*m*, 6 OCH_2CH_2); 2.59 (br., $OOCCH_2CH_2COO$); 2.30–0.66 (*m*, 6 H–C(3′), 43 H of chol). Anal. calc. for $C_{113}H_{127}N_{21}O_{36}P_2 \cdot H_2O$ (2435.3): C 55.73, H 5.34, N 12.08; found: C 55.44, H 5.40, N 11.86.

23. *5′-O-{[2-(Cholest-5-en-3β-yloxycarbonyl)ethyl]carbonyl}-3′-deoxyadenylyl-(2′→5′)-3′-deoxyadenylyl-(2′→5′)-3′-deoxyadenosine* (**27**). A mixture of dry **26** (27 mg, 11 μmol) and DBU (20 mg, 133 μmol) in dry MeCN (0.5 ml) was kept at r.t. for 2 d. Then 4 drops of AcOH were added, and the mixture was evaporated. The residue was washed several times with MeCN and lyophilized from H_2O: **27** (12 mg). Colorless powder. HPLC: t_R 24.69 min. FAB-MS (matrix DMSO/3-nitrobenzyl alcohol): 1347 (MH^+).

REFERENCES

[1] F. Bergmann, W. Pfleiderer, *Helv. Chim. Acta* **1994,** *77,* 988.
[2] P. F. Torrence, in 'Biological Response Modifers. New Approaches to Disease Intervention', Ed. P. F. Torrence, Academic Press, Orlando, FL, 1985, pp. 77–105.
[3] H. Sawai, J. Imai, K. Lesiak, M. I. Johnston, P. F. Torrence, *J. Biol. Chem.* **1983,** *258,* 1671.
[4] R. Charubala, E. Uhlmann, F. Himmelsbach, W. Pfleiderer, *Helv. Chim. Acta* **1987,** *70,* 2028.
[5] P. Herdewijn, K. Ruf, W. Pfleiderer, *Helv. Chim. Acta* **1991,** *74,* 7.
[6] P. Herdewijn, R. Charubala, W. Pfleiderer, *Helv. Chim. Acta* **1989,** *72,* 1729.
[7] S. N. Mikhailov, R. Charubala, W. Pfleiderer, *Helv. Chim. Acta* **1991,** *74,* 887.
[8] K. Ruf, W. Pfleiderer, *Carbohydr. Res.* **1991,** *216,* 421.
[9] R. Charubala, W. Pfleiderer, *Helv. Chim. Acta* **1992,** *75,* 471.
[10] K. Kariko, L. S. Wu, R. W. Sobol, Jr., R. J. Suhadolnik, R. Charubala, W. Pfleiderer, *Biochemistry* **1987,** *26,* 7136.
[11] K. Kariko, R. W. Sobol, Jr., L. Suhadolnik, L. S. Wu, N. L. Reichenbach, R. J. Suhadolnik, R. Charubala, W. Pfleiderer, *Biochemistry* **1987,** *26,* 7127.
[12] P. W. Doetsch, R. J. Suhadolnik, Y. Sawada, J. D. Mosca, M. B. Flick, N. L. Reichenbach, Dang A. Quac, J. M. Wu, R. Charubala, W. Pfleiderer, E. E. Henderson, *Proc. Natl. Acad. Sci. U. S. A.* **1981,** *78,* 6699.
[13] W. E. G. Müller, B. E. Weiler, R. Charubala, W. Pfleiderer, L. Leserman, R. W. Sobol, R. J. Suhadolnik, H. C. Schröder, *Biochemistry* **1991,** *30,* 2027.
[14] H. C. Schröder, R. J. Suhadolnik, W. Pfleiderer, R. Charubala, W. E. G. Müller, *Int. J. Biochem.* **1992,** *24,* 55.
[15] J. Goodchild, *Bioconjugate Chem.* **1990,** *1,* 165, and ref. cit. therein.
[16] S. L. Beaucage, R. P. Iyer, *Tetrahedron* **1993,** *49,* 1925, and ref. cit. therein.
[17] C. I. Hong, A. J. Kirisits, A. Nechaev, D. J. Buchheit, C. R. West, *J. Med. Chem.* **1985,** *28,* 171.
[18] H. Schirmeister, W. Pfleiderer, *Helv. Chim. Acta* **1994,** *77,* 10.
[19] E. E. Henderson, J.-Y. Yang, R.-D. Zhang, M. Bealer, *Virology* **1994,** *182,* 186.