79. Nucleotides

Part LIII[1])

6-Aminohexanoyl-Linked Conjugates of Monomeric and Trimeric Cordycepin

by **Marita Wasner**[a]), **Robert J. Suhadolnik**[b, d]), **Susan E. Horvath**[b]), **Martin E. Adelson**[d]), **Ning Kon**[b]), **Ming-Xu Guan**[c]), **Earl E. Henderson**[c, d]), and **Wolfgang Pfleiderer**[a])*

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

(24.I.97)

To improve cell permeability, monomeric 3′-deoxyadenosine (cordycepin) and antivirally active trimeric 3′-deoxyadenylyl-(2′–5′)-3′-deoxyadenylyl-(2′–5′)-3′-deoxyadenosine (2′–5′)d$^{3'}$(A-A-A); cordycepin-trimer core) were modified at the 2′-*O*- or 5′-*O*-position by myristic, cholic, and folic acid = tetradecanoic, 3α,7α,12α-trihydroxy-5β-cholan-24-oic, and *N*-{4-{[(2-amino-3,4-dihydro-4-oxopteridin-6-yl)methyl]amino}benzoyl}-L-glutamic acid, resp., linked by a 6-aminohexanoyl spacer. Syntheses of the trimeric educts **21**, **27**, and **28** were performed by phosphoramidite chemistry, using β-eliminating 2-(4-nitrophenyl)ethyl (npe), 2-(4-nitrophenyl)ethoxycarbonyl (npeoc) and (9*H*-fluoren-9-yl)methoxycarbonyl (fmoc) groups which allow a deprotection by DBU in an aprotic solvent without harming the ester-bounded conjugates, to give the products **24**–**26** and **31**–**33**. The metabollically stable (2′–5′)A derivatives **26** and **33**, covalently linked to folic acid either at the 2′-terminus or at the 5′-terminus of (2′–5′)d$^{3'}$(A-A-A), respectively, are a new class of the anti-HIV-1 compounds. Inhibition of HIV-1 reverse transcriptase (RT) activity by **26** and **33** was 45 and 81%, respectively. Only **33** activated recombinant, human RNase L by 35%.

Introduction. – It is well established that the natural antiviral pathways ubiquitous in mammalian cells – the (2′–5′)oligo(A) synthetase/RNase L cascade and the PKR antiviral pathway – are activated on virus infections, including HIV-1 [2–5]. Both pathways require dsRNA, which on the one hand activates (2′–5′)oligo(A) synthetase converting ATP into 2′–5′-linked oligoadenylates with a 5′-terminal triphosphate function. These unusual oligonucleotides bind and activate RNase L leading to the degradation of viral RNA and subsequent inhibition of protein synthesis [3] [4]. On the other hand, dsRNA-dependent PKR (p68 kinase) undergoes autophosphorylation and catalyzes phosphorylation of the α-subunit of eIF-2, thereby inhibiting initiation of protein synthesis [6]. Although, (2′–5′)oligoadenylates are rapidly inactivated by two different nucleases, the (2′–5′)oligo(A)system represents a chemotherapeutic possibility for the control of virus and cell growth. Many (2′–5′)oligo(A) derivatives have been synthesized with the aim to

[1]) ‚Part LII: [1].

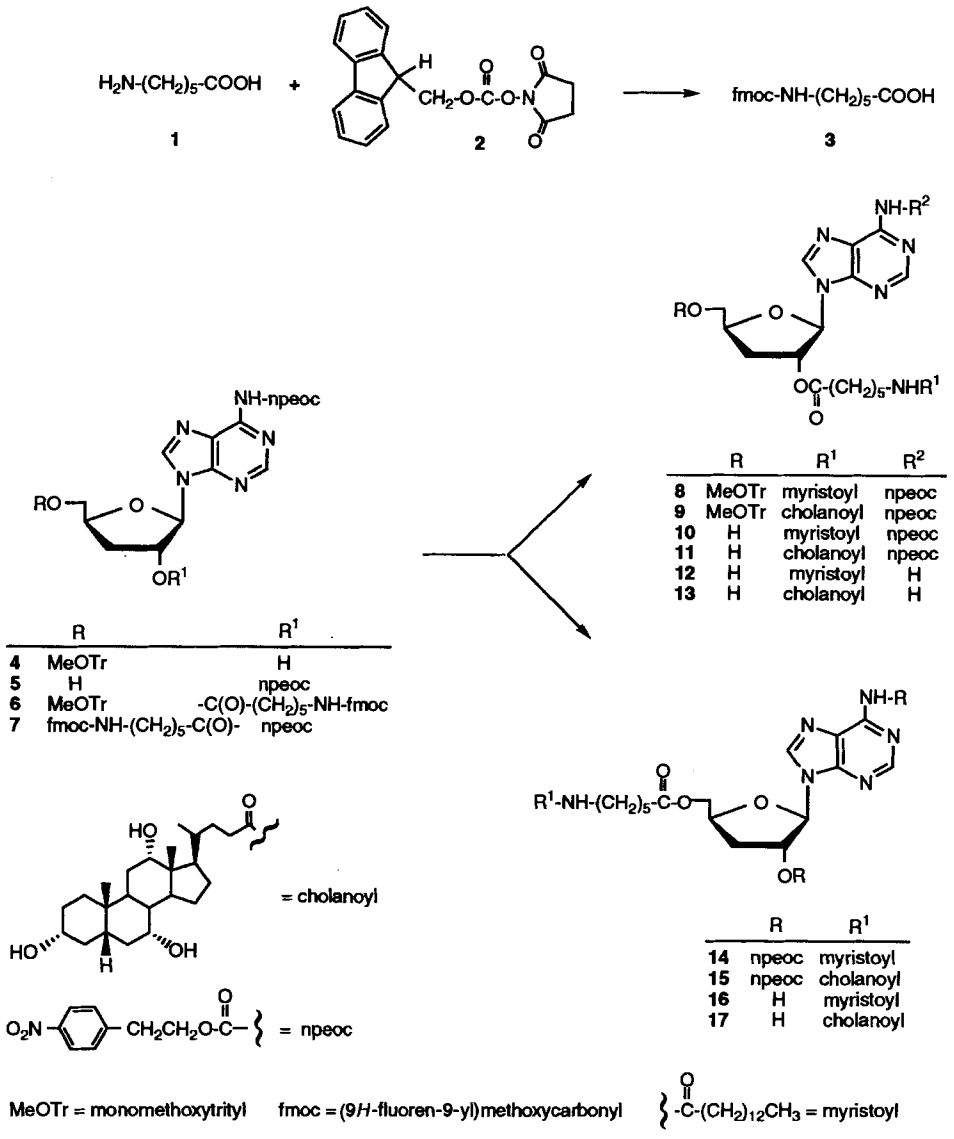
increase biological activity and to avoid premature decomposition of the antivirally active substance. One of the modified (2′–5′)oligo(A) analogues is the cordycepin-trimer core (2′–5′)d$^{3'}$(A-A-A) [7] [8] which was found to be biologically active, metabolically stable, and not toxic to cells [9]. Although, the trimer-cordycepin core effects no activation of RNase L [10]; this substance inhibits HIV-1 production [11] probably by weakening the complex formation of primer tRNA^{Lys,3} to HIV-1 reverse transcriptase [5] [12] [13].

One of the major problems in application of oligonucleotides to cells and biological systems is their polyanionic structure which renders them difficult to penetrate cell membranes. Numerous efforts have been made to improve the cellular uptake of oligonucleotides, *e.g.*, incorporation in liposomes [14] or syntheses of lipophilic conjugates [15–17]. The attachment of cholesterol, various vitamins, and lipids to the 2′-*O*- and 5′-*O*-position of the sugar moiety of monomeric and trimeric cordycepin has already been published [18–20]. The aim of conjugation of cordycepin derivatives with myristic, cholic, and folic acid (= tetradeconoic, 3α,7α,12α-trihydroxy-5β-cholan-24-oic, and *N*-{4-{[(2-amino-3,4-dihydro-4-oxopteridin-6-yl)methyl]amino}benzoyl}-L-glutamic acid, resp.) is to compare lipophilic with hydrophilic residues. Therefore, the 6-aminohexanoyl linkage serves as a biodegradable ester-bound spacer, which should be cleaved off from the conjugate by innercellular esterases.

Syntheses. – The synthesis of cordycepin [21], its protected derivatives **4** and **5** [8], and the cordycepin-trimer derivative **27** [20] have already been described in the literature. For the protection of the spacer, 6-aminohexanoic acid (**1**) was blocked at the amino function by reaction with *N*-{{[(9*H*-fluoren-9-yl)methoxy]carbonyl}oxy}succinimide (**2**) in a 9% aqueous Na_2CO_3 solution and DMF to give compound **3** in 78% yield (*Scheme 1*). Esterification of the cordycepin derivatives **4** and **5** with **3** worked well with the carbodiimide method applying *N*-[3-(dimethylamino)propyl]-*N*-ethylcarbodiimide hydrochloride (EDC) and 4-(dimethylamino)pyridine (DMAP) as condensing agents to form the monomeric educts **6** and **7** in 90 and 80% yield, respectively. The 2′-*O*-conjugates **8** and **9** were prepared first by cleavage of the (9*H*-fluoren-9-yl)methoxycarbonyl (fmoc) protecting group from **6** with 3% piperidine in dry DMF, followed by acylation with myristic and cholic acid, respectively, in the presence of *O*-{[cyano(ethoxycarbonyl)-methyliden]amino}-1,1,3,3-tetramethyluronium tetrafluoroborate (TOTU) and *N*-methylmorpholine. Detritylation to **10** and **11** proceeded in good yields (88 and 87%), and subsequent elimination of the [2-(4-nitrophenyl)ethoxy]carbonyl (npeoc) group resulted in 86% yield of the myristic-acid conjugate **12** and in 78% yield of the cholic-acid conjugate **13**. The structural analogues conjugated at the 5′-*O*-position were synthesized in a similar manner first by deblocking the amino function in compound **7** and then by TOTU-activated amidation with myristic and cholic acid, respectively, to give **14** in 70% and **15** in 76% yield. Deprotection with 1,8-diazabicylo[5.4.0]undec-7-ene (DBU) gave **16** in 86% and **17** in 67% yield, respectively.

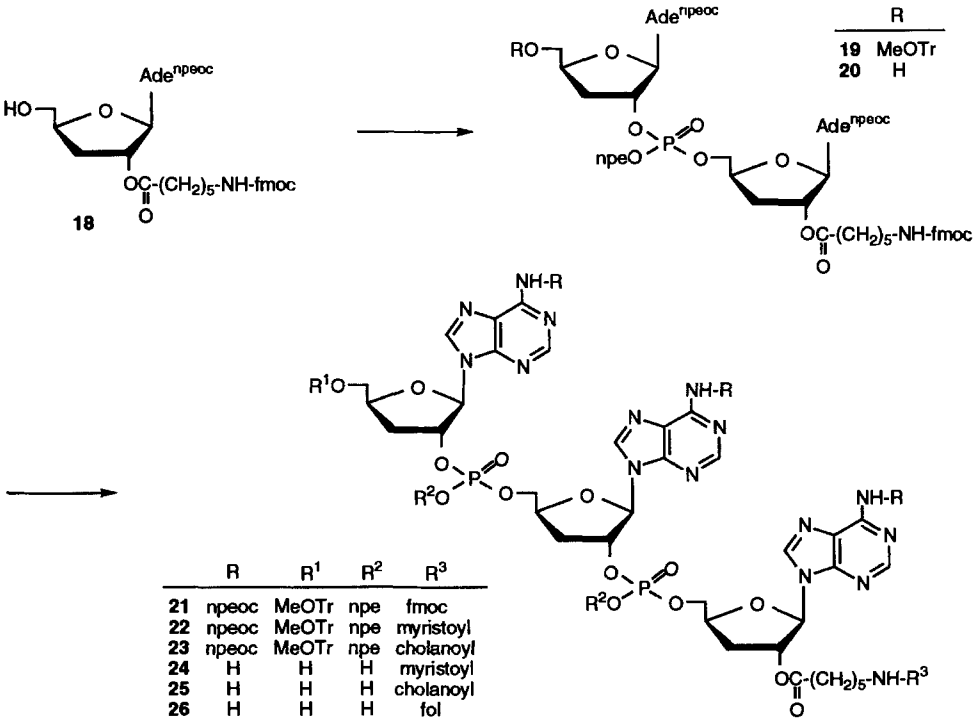
Starting material for the trimeric 2′-*O*-conjugates was compound **18**, which was prepared by acid treatment of **6**. Stepwise condensation with 3′-deoxy-5′-*O*-(monomethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 2′-[2-(4-nitrophenyl)ethyl diisopropylphosphoramidite] [22] gave, on subsequent oxidation of the intermediary phosphite triester, the fully protected dimer **19** in 89% yield. Quantitative detritylation

Scheme 1



to the dimer **20** and further condensation and oxidation generated the trimer **21** in 94% yield. Similarly to the one-pot reaction of the monomers, the fmoc group in compound **21** was cleaved off by treatment with 3% piperidine in dry DMF, and subsequent reaction with myristic or cholic acid by TOTU activation proceeded to the fully protected trimer conjugates **22** and **23** in 73 and 69% yield, respectively. Treatment with 2% TsOH in CH_2Cl_2/MeOH 4:1 and further β-elimination of the npe/npeoc groups with DBU in dry pyridine gave the desired cordycepin-trimer conjugates **24** (78%) and **25** (80%).

Scheme 2

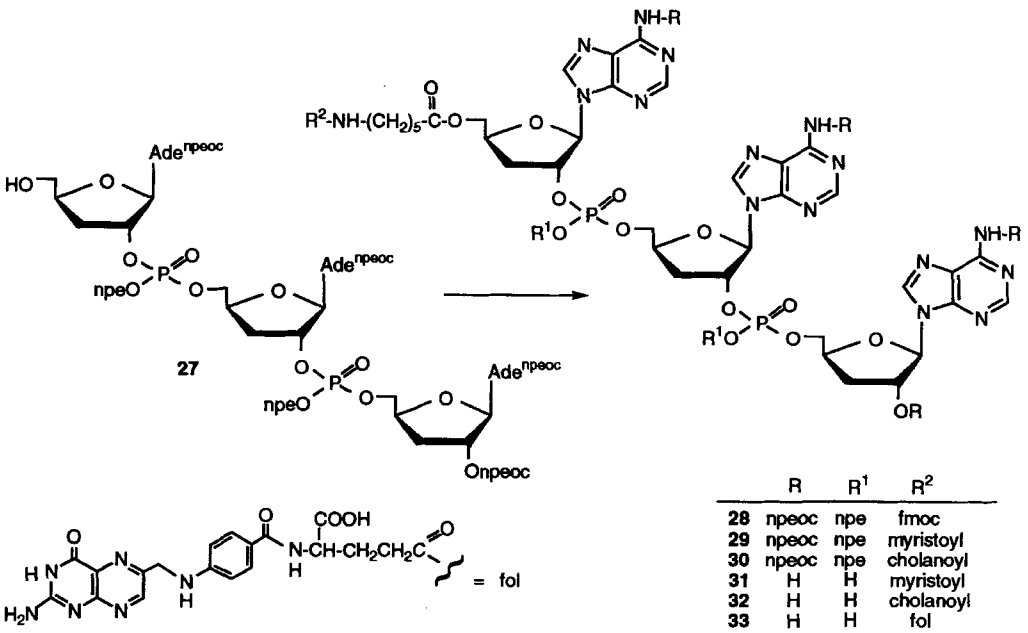


Synthesis of the corresponding 5′-*O*-conjugates of cordycepin trimer was achieved by carbodiimide-activated esterification of the trimeric educt **27** [20] with the fmoc-protected amino acid **3** to give compound **28** in 79% yield. Subsequent deblocking of the amino group and acylation with myristic and cholic acid gave conjugates **29** and **30** in 69 and 75% yield, respectively. β-Elimination of the npe and npeoc groups by DBU treatment generated the deblocked cordycepin-trimer 5′-*O*-conjugates **31** and **32** in 95 and 96% yield, respectively.

The approach to the folic-acid conjugates **26** and **33** did not allow to isolate the intermediary products due to their unusual and inconvenient physical properties. Both trimeric educts, compound **21** and **28**, respectively, were treated in the first step with 3% piperidine in dry DMF and then successively condensed with 6 equiv. of EDC-activated folic acid. After evaporation, the crude product was treated with Et_2O and CH_2Cl_2 and then deprotected by DBU in dry pyridine. Finally, the mixture was neutralized with AcOH and evaporated, and the residue treated with MeCN, and subsequently with 80% $AcOH/H_2O$, and centrifuged. The supernatant was evaporated and submitted to final HPLC purification providing the desired conjugates **26** and **33** in relatively low yields.

Biochemical Application. – Covalent linkage of folic acid to the cordycepin-trimer core ((2′–5′)d^3(A-A-A)) produced a new group of inhibitors of HIV-1 replication (*Table*). It has recently been proposed that cellular uptake of a compound (*i.e.*, (2′–5′)A)

Scheme 3



	R	R¹	R²
28	npeoc	npe	fmoc
29	npeoc	npe	myristoyl
30	npeoc	npe	cholanoyl
31	H	H	myristoyl
32	H	H	cholanoyl
33	H	H	fol

covalently linked to folic acid can be enhanced *via* receptor-mediated endocytosis [23]. The three studies performed to determine the anti-HIV-1 activity of each (2′–5′)A derivative were *i*) inhibition of HIV-1-induced syncytia formation, *ii*) inhibition of HIV-1 reverse-transcriptase (RT) activity, and *iii*) activation of recombinant human GST-RNase L. Inhibition of HIV-1 replication was 2.4- and 7.2-fold with **26** and **33**, respectively, compared with 4.8- and 1.5-fold reduction in HIV-1-induced syncytia formation with (2′–5′)d$^{3'}$(A-A-A) and cordycepin, respectively (*Table*).

We hypothesize that the inhibition of HIV-1 replication with **26** and **33** could be a consequence of the inhibition of HIV-1 reverse transcriptase (HIV-1 RT) activity and/or the activation of a (2′–5′)A-dependent ribonuclease, RNAse L, which functions to degrade single-stranded RNA. Inhibition of HIV-1 RT activity with **26** and **33** was 45 and 81%, respectively, compared to 96 and 13% inhibition of HIV-1 RT activity by (2′–5′)d$^{3'}$(A-A-A) and cordycepin, respectively. When folic acid is covalently linked to C(2′) at the 2′-terminus of the cordycepin-trimer core as in **26**, GST-RNase L is not activated; however, covalent linkage to C(5′) at the 5′-terminus as in **33** activates GST-RNase L by 35%. These data suggest that the inhibition of HIV-1 replication by **26** is due to the inhibition of HIV-1 RT; however, the 7.5-fold reduction in syncytia formation by **33** is in part attributed to the inhibition of HIV-1 RT activity and the activation of RNase L.

Table. *Inhibition of HIV-1-Replication and Biological Activities of Cordycepin-Trimer-Folate Conjugates*

		Inhibition of syncytia formation[a])	Inhibition of HIV-1 RT activity[b]) [%]	Activation of RNase L[c]) [%]
26	(2′–5′)d$^{3'}$[A-A-A(base-fol)$^{2'}$]	2.4	45	0
33	(2′–5′)d$^{3'}$*[(fol-base)$^{5'}$A-A-A]	7.2	81	35
	(2′–5′)d$^{3'}$(A-A-A)	4.8	96	12
	Cordycepin (d$^{3'}$A)	1.5	13	[d])

[a]) Inhibition of HIV-1 replication was determined by HIV-1-induced syncytia formation (fold reduction in infection) for each cordycepin-trimer-folate derivative or (2′–5′)A (300 μM). The number of syncytia/10^4 cells was 144 for the control Sup T1 cells. The mean of triplicate determinations is shown; variance did not exceed 5–10%.

[b]) Percent inhibition of HIV-1 reverse-transcriptase (HIV-1 RT) activity was measured for each cordycepin-trimer-folate derivative or (2′–5′)A at 300 μM. Control values for HIV-1 RT activity ranged from 24000 to 33000 cpm. The mean of duplicate determinations is shown; variance did not exceed 5–10%.

[c]) The activation of recombinant human RNase L was measured as the percent hydrolysis of poly(U)-3′-[^{32}P]pCp in the presence of the cordycepin-trimer-folate derivative or (2′–5′)A (10 μM). The mean of a duplicate determination is shown; variance did not exceed 5–10%.

[d]) Not tested.

Experimental Part

General. TLC: Precoated silica gel TLC sheets *F1500 LS 254* from *Schleicher & Schüll.* Prep. TLC: silica gel *60 PF_{254}* (*Merck*). Prep. column flash chromatography (FC): silica gel for flash chromatography (*Baker*). HPLC: *Merck-Hitachi L-6200, L-3000* photo diode array; detector; column *RP18*, 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 DMSO.

Bioassay. Assays measuring HIV-1-induced syncytia formation, HIV-1 reverse-transcriptase activity, and activation of recombinant, human RNase L were accomplished as previously described.

1. *6-{{[(9H-Fluoren-9-yl)methoxy]carbonyl}amino}hexanoic Acid* (**3**). To a soln. of 6-aminohexanoic acid (**1**; 144 mg, 1.1 mmol) in 9% aq. Na_2CO_3 soln. (2.4 ml) was added a soln. of (9*H*-fluoren-9-yl)methyl succinimidyl carbonate (**2**; 337 mg, 1 mmol) in DMF (2.5 ml). After stirring for 1 h at r.t., the mixture was diluted with H_2O (50 ml) and extracted twice with Et_2O (2 × 20 ml). Then, the H_2O phase was acidified with conc. HCl soln. to pH 2 and extracted with AcOEt (5 × 30 ml). The org. layer was dried ($MgSO_4$) and evaporated. The residue was crystallized in $CHCl_3$/petroleum ether: 306 mg (86%) of **3**. Colorless crystals. M.p. 116°. ^{1}H-NMR ($CDCl_3$): 7.80–7.55 (2*m*, H–C(1)(fmoc), H–C(4)(fmoc), H–C(5)(fmoc), H–C(8)(fmoc)); 7.45–7.25 (*m*, H–C(2)(fmoc), H–C(3)(fmoc), H–C(6)(fmoc), H–C(7)(fmoc)); 4.45 (*m*, CH_2O)(fmoc)); 4.2 (*m*, H–C(9)(fmoc)); 3.25–3.0, 2.4, 1.8–1.3 (3*m*, NH(CH_2)$_5$CO). Anal. calc. for $C_{21}H_{23}NO_4$ (353.42): C 71.37, H 6.56, N 3.96; found: C 71.35, H 6.65, N 3.93.

2. *3′-Deoxy-2′-O-{6-{{[(9H-fluoren-9-yl)methoxy]carbonyl}amino}hexanoyl}-5′-O-(monomethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**6**). A mixture of 3′-deoxy-5′-*O*-(monomethoxytrityl)-*N*^{6}-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**4**; 215 mg, 0.3 mmol) [8], **3** (120 mg, 0.3 mmol), *N*-[3-(dimethylamino)propyl]-*N*′-ethylcarbodiimide hydrochloride (EDC; 58 mg, 0.3 mmol), and 4-(dimethylamino)pyridine (DMAP; 37 mg, 0.3 mmol) in dry CH_2Cl_2 (4 ml) was stirred at r.t. for 3 h, diluted with CH_2Cl_2 (60 ml), and washed with sat. $NaHCO_3$/$NaCO_3$ soln. (25 ml). The aq. phase was reextracted with CH_2Cl_2 (40 ml), the combined org. layer extracted with 10% citric acid soln., the acid phase reextracted with CH_2Cl_2, the combined org. layer dried ($MgSO_4$) and evaporated, and the residue purified by FC (silica gel, 14.5 × 2 cm, toluene/AcOEt 1:1 then 1:1 + 3% MeOH): 292 mg (*ca.* 93%) of **6** which was already contaminated with amino acid. A pure sample of **6**, was obtained by prep. TLC (silica gel, 20 × 40 cm, $CHCl_3$ + 4% MeOH) of 138 mg of the crude product: 117 mg of **6**. UV (CH_2Cl_2): 300 (4.00), 286 (sh, 4.14), 272 (sh, 4.58), 266 (4.66), 237 (sh, 4.36). ^{1}H-NMR ($CDCl_3$): 8.61, 8.58, 8.17–8.13 (3*s*, *d*, H–C(8), H–C(2), NH, 2 H *o* to NO_2); 7.76–7.16 (*m*, 8 H of fmoc, 2 H *m* to NO_2, 12 H of MeO*Tr*); 6.78 (*d*, 2 H *o* to MeO); 6.13 (*d*, H–C(1′)); 5.73 (*m*, H–C(2′)); 4.89–4.15 (*m*, N*H*(CH_2)$_5$CO, H–C(4′), OCH_2CH_2, H–C(9)(fmoc), CH_2O(fmoc)); 3.76 (*s*, MeO); 3.5–3.35 (*m*, 2 H–C(5′)); 3.35–3.15 (*m*, OCH_2CH_2,

1 CH_2 of $NH(CH_2)_5CO$); 2.65 (*m*, H–C(3')); 2.39 (*t*, 1 CH_2 of $NH(CH_2)_5CO$); 1.7–1.2 (*m*, 3 CH_2 of $NH(CH_2)_5CO$). Anal. calc. for $C_{60}H_{57}N_7O_{11}$ (1052.15): C 68.49, H 5.46, N 9.32; found: C 68.56, H 5.67, N 9.12.

3. *3'-Deoxy-5'-O-{6-{{[(9H-fluoren-9-yl)methoxy]carbonyl}amino}hexanoyl}-N⁶,2'-O-bis[2-(4-nitrophenyl)-ethoxycarbonyl]adenosine* (**7**). As described in *Exper. 2*, with 3'-deoxy-*N*⁶,2'-*O*-bis[2-(-nitrophenyl)ethoxycarbonyl]adenosine (**5**; 1.912 g, 3 mmol) [8], **3** (1.166 g, 3.3 mmol), EDC (633 mg, 3.3 mmol), DMAP (403 mg, 3.3 mmol), and anh. CH_2Cl_2 (10 ml; 3.5 h). Workup with CH_2Cl_2 (150 ml), $NaHCO_3$ soln. and 10% citric acid soln. (80 ml) and purification by FC (silica gel, 7.5 × 4.5 cm, toluene/AcOEt 1:1, then 1:1 + 2% MeOH) gave 2.35 g (80%) of **7**. Amorphous solid. UV (CH_2Cl_2): 297 (4.12), 286 (sh, 4.31), 272 (sh, 4.66), 266 (4.71). ^{1}H-NMR ($CDCl_3$): 9.3–8.0 (*m*, H–C(8), H–C(2), NH, 4 H *o* to NO_2); 7.75–7.3 (*m*, 8 H of fmoc, 4 H *m* to NO_2); 6.12 (*s*, H–C(1')); 5.71 (*m*, H–C(2')); 5.18 (*t*, $NH(CH_2)_5CO$); 4.65–4.15 (*m*, H–C(4'), 2 OCH_2CH_2, H–C(9)(fmoc), CH_2O(fmoc), 2 H–C(5')); 3.2–3.0 (*m*, 2 OCH_2CH_2, 1 CH_2 of $NH(CH_2)_5CO$); 2.65 (*m*, H–C(3')); 2.25 (*m*, H–C(3'), 1 CH_2 of $NH(CH_2)_5CO$); 1.65–1.15 (*m*, 3 CH_2 of $NH(CH_2)_5CO$). Anal. calc. for $C_{49}H_{48}N_8O_{14}$ (972.97): C 60.49, H 4.97, N 11.52; found: C 60.83, H 5.08, N 11.15.

4. *3'-Deoxy-5'-O-(monomethoxytrityl)-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]-2'-O-[6-(tetradecanoylamino)-hexanoyl]adenosine* (**8**). For acid-activation, a mixture of tetradecanoic acid (128 mg, 0.56 mmol), TOTU (184 mg, 0.56 mmol), and *N*-methylmorpholine (57 mg, 0.56 mmol) in abs. DMF (4 ml) was kept at r.t. for 1.5 h. Then, **6** (490 mg, 0.466 mmol) was treated with 3% piperidine in abs. DMF (7 ml; 10 min) and evaporated. The above-mentioned soln. was added and the mixture stirred for 3 h at r.t. and evaporated. The residue was diluted with AcOEt (180 ml), washed with $NaHCO_3$ soln. (2 × 80 ml), the aq. phase reextraced with AcOEt (80 ml), the combined org. layer dried ($MgSO_4$) and evaporated, and the residue purified by FC (silica gel, 11 × 2 cm, toluene/AcOEt 1:1, then 1:1 + 2% MeOH, 1:1 + 3% MeOH): 408 mg (84%) of **8**. Amorphous solid. UV (CH_2Cl_2): 272 (sh, 4.39), 267 (4.44), 237 (sh, 4.30). ^{1}H-NMR ($CDCl_3$): 8.38, 8.18 (2*s*, *m*, H–C(8), H–C(2), 2 H *o* to NO_2, NH(ade)); 7.45–7.20 (*m*, 2 H *m* to NO_2, 12 H of MeO*Tr*); 6.79 (*d*, 2 H *o* to MeO); 6.14 (*d*, H–C(1')); 5.73 (*m*, H–C(2')); 5.50 (*t*, $NH(CH_2)_5CO$); 4.55 (*m*, *t*, H–C(4'), OCH_2CH_2); 3.78 (*s*, MeO); 3.41 (*m*, 2 H–C(5')); 3.20 (*q*, 1 CH_2 of $NH(CH_2)_5CO$); 3.15 (*t*, OCH_2CH_2); 2.6, 2.2 (2*m*, 2 H–C(3')); 2.39, 2.14 (2*m*, 2 CH_2 of $NH(CH_2)_5CO$, 2 H of $Me(CH_2)_{12}CO$); 1.7–1.15 (*m*, 28 H, 2 CH_2 of $NH(CH_2)_5CO$, $Me(CH_2)_{12}CO$); 0.87 (*t*, $Me(CH_2)_2CO$). Anal. calc. for $C_{59}H_{73}N_7O_{10} \cdot {}^1/_2\ H_2O$ (1040.27): C 67.52, H 7.06, N 9.34; found: C 67.25, H 7.20, N 9.33.

5. *3'-Deoxy-5'-O-(monomethoxytrityl)-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]-2'-O-{6-[(3α,7α,12α-trihydroxy-5β-cholan-24-oyl)amino]hexanoyl}adenosine* (**9**). As described in *Exper. 4*, with cholic acid (450 mg, 1.1 mmol) and TOTU (361 mg, 1.1 mmol) in abs. DMF (5 ml; 1 h). Deblocking of **6** (1.052 g, 1 mmol) with 3% piperidine in abs. DMF (15 ml; 10 min) and addition of the above-mentioned soln. (r.t., 1.5 h). Workup with AcOEt (200 ml) and $NaHCO_3$ soln. (2 × 100 ml), reextraction, and purification by FC (silica gel, 14 × 3.5 cm, $CHCl_3$, then $CHCl_3$/MeOH 98:2, 96:4, 93:7, 90:10) gave 944 mg (77%) of **9**. Amorphous solid. UV (CH_2Cl_2): 272 (sh, 4.42), 267 (4.47), 237 (sh, 4.31). ^{1}H-NMR ($CDCl_3$): 9.12 (br., NH(ade)); 8.68–8.11 (2*s*, *d*, H–C(8), H–C(2), 2 H *o* to NO_2); 7.48–7.12 (*m*, 2 H *m* to NO_2, MeO*Tr*); 6.8 (*d*, 2 H *o* to MeO); 6.1 (*s*, H–C(1')); 5.88 (*t*, $NH(CH_2)_5CO$); 5.63 (*m*, H–C(2')); 4.6 (*m*, H–C(4')); 4.51 (*t*, OCH_2CH_2); 3.98–3.7 (*s*, *m*, MeO, 2 H of chol.); 3.55–3.1 (*m*, 8 H, 2 H–C(5'), OCH_2CH_2 1 CH_2 of $NH(CH_2)_5CO$, 2 H of chol.); 2.65–0.6 (*m*, 2 H–C(3'), 4 CH_2 of $NH(CH_2)_5CO$, 33 H of chol.). Anal. calc. for $C_{69}H_{85}N_7O_{13}$ (1220.47): C 67.90, H 7.02, N 8.03; found: C 67.74, H 7.26, N 7.65.

6. *3'-Deoxy-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]-2'-O-[6-(tetradecanoylamino)hexanoyl]adenosine* (**10**). Compound **8** (330 mg, 0.317 mmol) was stirred at r.t. in CH_2Cl_2/MeOH 4:1 (6 ml) containing 2% of TsOH · H_2O for 15 min. Then the mixture was diluted with CH_2Cl_2 (70 ml) and washed with sat. $NaHCO_3$ soln. (2 × 40 ml), the aq. phase reextracted with CH_2Cl_2, the combined org. layer dried ($MgSO_4$) and evaporated, and the residue purified by FC (silica gel, 12 × 2 cm, $CHCl_3$, $CHCl_3$ + 5% MeOH). The obtained oily substance was treated with small amounts of Et_2O/MeCN 2:1 to give 215 mg (88%) of **10**. Amorphous solid. UV (CH_2Cl_2): 272 (sh, 4.36), 267 (4.41). ^{1}H-NMR ($CDCl_3$): 8.71, 8.21–8.15 (2*m*, H–C(8), H–C(2), 2 H *o* to NO_2, NH(ade)); 7.45 (*d*, 2 H *m* to NO_2); 6.03 (*d*, H–C(1')); 5.58 (*m*, H–C(2')); 5.55 (br., $NH(CH_2)_5CO$); 5.0 (br., OH–C(5')); 4.65–4.50 (*m*, *t*, H–C(4'), OCH_2CH_2); 4.12, 3.75 (2*m*, 2 H–C(5')); 3.25 (*q*, 1 CH_2 of $NH(CH_2)_5CO$); 3.15 (*t*, OCH_2CH_2); 2.9, 2.25 (2*m*, 2 H–C(3')); 2.35, 2.15 (2*t*, 2 CH_2 of $NH(CH_2)_5CO$, 2 H of $Me(CH_2)_{12}CO$); 1.7–1.15 (*m*, 28 H, 2 CH_2 of $NH(CH_2)_5CO$, $Me(CH_2)_{12}CO$); 0.87 (*t*, $Me(CH_2)_{12}CO$). Anal. calc. for $C_{39}H_{57}N_7O_9 \cdot {}^1/_2\ H_2O$ (782.02): C 59.90, H 7.41, N 12.54; found: C 60.27, H 7.66, N 12.06.

7. *3'-Deoxy-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]-2'-O-{6-[(3α,7α,12α-trihydroxy-5β-cholan-24-oyl)amino]-hexanoyl}adenosine* (**11**). As described in *Exper. 6*, with **9** (860 mg, 0.705 mmol) and CH_2Cl_2/MeOH 4:1 (14 ml) containing 2% of TsOH · H_2O (15 min). Workup with CH_2Cl_2 (70 ml) and sat. $NaHCO_3$ soln. (2 × 40 ml), and reextraction, and purification by precipitation of the crude product in Et_2O (100 ml) gave 578 mg (87%) of **11**. UV (MeOH): 272 (sh, 4.39), 267 (4.43). ^{1}H-NMR ($CDCl_3$): 9.70 (*s*, NH(ade)); 8.73, 8.44 (2*s*, H–C(8), H–C(2));

8.17 (*d*, 2 H *o* to NO_2); 7.45 (*d*, 2 H *m* to NO_2); 6.18 (*m*, N*H*(CH_2)$_5$CO); 6.10 (*d*, H–C(1′)); 5.57 (*m*, H–C(2′)); 5.05 (br., OH–C(5′)); 4.55–4.45 (*m*, H–C(4′), O*C*H_2CH$_2$); 4.1–3.65 (*m*, 2 H of chol., 2 H–C(5′)); 3.5–2.7 (*m*, 4 H of chol., OCH$_2$C*H*$_2$, 1 CH_2 of NH(CH_2)$_5$CO, H–C(3′)); 2.45–0.6 (*m*, H–C(3′), 4 CH_2 of NH(CH_2)$_5$CO, 33 H of chol.). Anal. calc. for $C_{49}H_{69}N_7O_{12}$ (948.13): C 62.07, H 7.34, N 10.34; found: C 61.54, H 7.41, N 9.92.

8. *3′-Deoxy-2′-O-[6-(tetradecanoylamino)hexanoyl]adenosine* (**12**). Compound **10** (160 mg, 0.208 mol) was co-evaporated twice with abs. pyridine and then dissolved in abs. pyridine (2 ml). DBU (158 mg, 1.04 mmol) was added, the mixture kept at r.t. for 18 h, then AcOH (62 mg, 1.04 mmol) added, and the soln. evaporated. The residue was diluted with $CHCl_3$ (80 ml) and washed with a 10% citric acid 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 precipitated by MeOH and washed with Et_2O: 103 mg (86%) of **12**. UV (CH_2Cl_2): 259 (4.16). ^{1}H-NMR ((D$_6$)DMSO): 8.33, 8.13 (2*s*, H–C(8), H–C(2)); 7.68 (*t*, N*H*(CH_2)$_5$CO); 7.25 (*s*, NH_2); 6.05 (*d*, H–C(1′)); 5.60 (*m*, H–C(2′)); 5.11 (*t*, OH–C(5′)); 4.32 (*m*, H–C(4′)); 3.65, 3.47 (2*m*, 2 H–C(5′)); 2.95 (*q*, 1 CH_2 of NH(CH_2)$_5$CO); 2.6, 2.15 (2*m*, 2 H–C(3′)); 2.34, 2.00 (2*t*, 2 CH_2 of NH(CH_2)$_5$CO, 2 H of Me(C*H*$_2$)$_{12}$CO); 1.65–1.15 (*m*, 28 H, 2 CH_2 of NH(CH_2)$_5$CO, Me(C*H*$_2$)$_{12}$CO); 0.83 (*t*, *Me*(CH_2)$_{12}$CO). Anal. calc. for $C_{30}H_{50}N_6O_5 \cdot ½\ H_2O$ (583.76): C 61.72, H 8.81, N 14.39; found: C 62.04, H 9.07, N 13.85.

9. *3′-Deoxy-2′-O-{6-[(3α,7α,12α-trihydroxy-5β-cholan-24-oyl)amino]hexanoyl}adenosine* (**13**). As described in *Exper. 8*, with **11** (430 mg, 0.454 mmol), 0.5M DBU in abs. pyridine (6.7 ml; 20 h), and AcOH (250 mg, 4.16 mmol). Workup with $CHCl_3$ (200 ml) including a small amount of MeOH, H_2O (60 ml), and sat. $NaHCO_3$ soln. (80 ml), reextraction, purification by FC (silica gel, 4 × 3 cm, $CHCl_3$/MeOH 9:1, then 4:1), and precipitation of the product in petroleum ether gave 269 mg of **13** (78%). UV (MeOH): 259 (4.16). ^{1}H-NMR ((D$_6$)DMSO): 8.33, 8.13 (2*s*, H–C(8), H–C(2)); 7.71 (*t*, N*H*(CH_2)$_5$CO); 7.31 (*s*, NH_2(ade)); 6.05 (*d*, H–C(1′)); 5.60 (*m*, H–C(2′)); 5.12 (*t*, OH–C(5′)); 4.31 (*m*, H–C(4′), OH-chol.); 4.07 (*d*, OH-chol.); 3.98 (*d*, OH-chol.); 3.77, 3.55, 3.15 (3*m*, 3 H of chol.); 3.65, 3.47 (*m*, 2 H–C(5′)); 2.98 (*m*, 1 CH_2 of NH(CH_2)$_5$CO); 2.6–0.55 (*m*, 2 H–C(3′), 4 CH_2 of NH(CH_2)$_5$CO, 33 H of chol.). Anal. calc. for $C_{40}H_{62}N_6O_8 \cdot ½\ H_2O$ (763.98): C 62.89, H 8.31, N 11.00; found: C 62.63, H 8.28, N 11.26.

10. *3′-Deoxy-5′-O-[6-(tetradecanoylamino)hexanoyl]adenosine* (**16**). 10.1. As described in *Exper. 4*, with tetradecanoic acid (151 mg, 0.66 mmol), TOTU (67 mg, 0.66 mmol), and *N*-methylmorpholine (67 mg, 0.66 mmol) in abs. DMF (3 ml; 1 h). Deblocking of **7** (584 mg, 0.6 mmol) with 3% piperidine in abs. DMF (6 ml; 10 min) and addition of the above-mentioned soln. (r.t., 2.5 h). Workup with $CHCl_3$ (150 ml) and $NaHCO_3$ soln. (2 × 70 ml) and reextraction. Purification by FC (silica gel, 12 × 3 cm, $CHCl_3$, then $CHCl_3$/MeOH 98:2, 95:5): 405 mg (70%) of **14**. Colorless oil. ^{1}H-NMR ($CDCl_3$): 8.73–8.15 (*m*, H–C(8), H–C(2), 4 H *o* to NO_2, NH(ade)); 7.47–7.38 (*m*, 4 H *m* to NO_2); 6.12 (*d*, H–C(1′)); 5.73 (*m*, H–C(2′)); 5.57 (*t*, N*H*(CH_2)$_5$CO); 4.68 (*m*, H–C(4′)); 4.54, 4.43 (2*t*, 2 OC*H*$_2$CH$_2$); 4.4–4.2 (*m*, 2 H–C(5′)); 3.7–3.5 (*m*, 1 CH_2 of NH(CH_2)$_5$CO); 3.25–3.1 (*m*, 2 OCH$_2$C*H*$_2$, 1 CH_2 of NH(CH_2)$_5$CO); 2.65, 2.25 (2*m*, 2 H–C(3′)); 2.27, 2.15 (2*t*, 2 CH_2 of NH(CH_2)$_5$CO, 1 CH_2 of Me(CH_2)$_{12}$CO); 1.7–1.15 (*m*, 3 CH_2 of NH(CH_2)$_5$CO, 11 CH_2 of Me(CH_2)$_{12}$CO); 0.87 (*t*, *Me*(CH_2)$_{12}$CO).

10.2. As described in *Exper. 8*, with crude **14** (392 mg, 0.408 mmol), 0.5M DBU in abs. pyridine (12 ml; 20 h), and AcOH (400 mg, 6.66 mmol). Workup with CH_2Cl_2 (120 ml) and H_2O (2 × 60 ml), reextraction, co-evaporation of the residue with toluene, and purification by precipitation of the product in petroleum ether gave 202 mg of **16** (86%). Colorless powder. UV (MeOH): 259 (4.16). ^{1}H-NMR ($CDCl_3$): 8.22, 8.13 (2*s*, H–C(8), H–C(2)); 7.68 (br., N*H*(CH_2)$_5$CO); 7.26 (br., NH_2(ade)); 5.90 (*s*, H–C(1′)); 5.76 (br., OH–C(2′)); 4.69 (*m*, H–C(2′)); 4.53 (*m*, H–C(4′)); 4.3–4.1 (*m*, 2 H–C(5′)); 3.0 (*m*, 1 CH_2 of NH(CH_2)$_5$CO); 2.35–2.0 (2*m*, 2*t*, 2 H–C(3′), 1 CH_2 of NH(CH_2)$_5$CO, 1 CH_2 of Me(CH_2)$_{12}$CO); 1.5–1.1 (*m*, 3 CH_2 of NH(CH_2)$_5$CO, 11 CH_2 of Me(CH_2)$_{12}$CO); 0.83 (*t*, *Me*(CH_2)$_{12}$CO). Anal. calc. for $C_{30}H_{50}N_6O_5$ (574.76): C 62.69, H 8.77, N 14.62; found: C 62.32, H 8.68, N 14.55.

11. *3′-Deoxy-5′-O-{6-[(3α,7α,12α-trihydroxy-5β-cholan-24-oyl)amino]hexanoyl}adenosine* (**17**). 11.1. As described in *Exper. 4*, with cholic acid (270 mg, 0.66 mmol), TOTU (67 mg, 0.66 mmol), and *N*-methylmorpholine (67 mg, 0.66 mmol) in abs. DMF (3 ml; 1 h). Deblocking of **7** (584 mg, 0.6 mmol) with 3% piperidine in abs. DMF (6 ml; 10 min) and addition of the above-mentioned soln. (r.t., 1.5 h). Workup with $CHCl_3$ (200 ml) and $NaHCO_3$ soln. (2 × 100 ml), reextraction, and purification by FC (silica gel, 8 × 3 cm, $CHCl_3$/MeOH 95:5, 93:7) gave 518 mg (*ca.* 76%) of **15**. Amorphous solid, contaminated with cholic acid. ^{1}H-NMR ($CDCl_3$): 9.35 (br., NH(ade)); 8.73, 8.3–8.15 (*s*, *m*, H–C(8), H–C(2), 4 H *o* to NO_2); 7.47–740 (*m*, 4 H *m* to NO_2); 6.15 (*s*, H–C(1′)); 5.92 (*t*, N*H*(CH_2)$_5$CO); 5.75 (*m*, H–C(2′)); 4.63 (*m*, H–C(4′)); 4.6–4.2 (*m*, 2 OC*H*$_2$CH$_2$, 2 H–C(5′)); 4.0–3.25 (*m*, 6 H of chol.); 3.22–3.1 (*m*, 2 OCH$_2$C*H*$_2$, 1 CH_2 of NH(CH_2)$_5$CO); 2.7, 2.5–0.65 (*m*, 2 H–C(3′), 4 CH_2 of NH(CH_2)$_5$CO, 33 H of chol.).

11.2. As described in *Exper. 8*, with crude **15** (447 mg, 0.392 mmol), 0.5M DBU in abs. pyridine (11.5 ml; 20 h), and AcOH (380 mg, 6.32 mmol). Workup with $CHCl_3$ (200 ml) including a small amount of MeOH, H_2O

(60 ml), and sat. $NaHCO_3$ soln., reextraction, co-evaporation of the residue with toluene, purification by FC (silica gel, 4 × 3 cm, $CHCl_3$, then $CHCl_3$/MeOH 9:1, 8:2), and precipitation of the product in petroleum ether: 197 mg of **17** (67%). Colorless powder. UV (MeOH): 259 (4.16). ^{1}H-NMR ((D_6)DMSO): 8.22, 8.13 (2*s*, H–C(8), H–C(2)); 7.70 (*t*, N*H*$(CH_2)_5$CO); 7.28 (*s*, NH_2(ade)); 5.90 (*d*, H–C(1′)); 5.72 (*m*, OH–C(2′)); 4.67 (br., H–C(2′)); 4.50 (*m*, H–C(4′)); 4.29, 4.07, 3.99 (3*m*, 3 OH-chol.); 4.25–4.17 (*m*, 2 H–C(5′)); 3.77, 3.59, 3.17 (3M, 3 H of chol.); 3.0 (*q*, 1 CH_2 of NH$(CH_2)_5$CO); 2.3–0.55 (*m*, 2 H–C(3′), 4 CH_2 of NH$(CH_2)_5$CO, 33 H of chol.). Anal. calc. for $C_{40}H_{62}N_6O_8 \cdot H_2O$ (763.98): C 62.15, H 8.35, N 10.87; found: C 62.36, H 8.27, N 10.65.

12. *3′-Deoxy-2′-*O*-{6-{{[(9*H*-fluoren-9-yl)methoxy]carbonyl}amino}hexanoyl}-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**18**). To a soln. of **6** (1.052 g, 1 mmol) in abs. CH_2Cl_2 (20 ml) was added CF_3COOH (0.4 ml) and stirred at r.t. for 1 h. Then, MeOH (5 ml) was added, the mixture evaporated and co-evaporated with toluene/MeOH, and the residue purified by FC (silica gel, 14.5 × 3.5 cm, CH_2Cl_2, then CH_2Cl_2/MeOH 98:2, 97:3, 95:5, 90:10, 85:15): 535 mg (69%) of **18**. Amorphous solid. UV (CH_2Cl_2): 299 (4.00), 286 (sh, 4.10), 272 (sh, 4.55), 266 (4.62). ^{1}H-NMR ($CDCl_3$): 8.72–8.10 (3*s*, *d*, H–C(8), H–C(2), NH, 2 H *o* to NO_2); 7.77–7.27 (*m*, 8 H of fmoc, 2 H *m* to NO_2); 5.96 (*d*, H–C(1′)); 5.65 (*m*, H–C(2′)); 4.90–4.80 (*m*, N*H*$(CH_2)_5$CO, OH–C(5′)); 4.56–4.22 (*m*, H–C(4′), O*C*H_2CH$_2$, H–C(9)(fmoc), CH_2O(fmoc)); 4.15, 3.72, (2*m*, 2 H–C(5′)); 3.25–3.05 (*m*, OCH$_2$C*H*$_2$, 1 CH_2 of NH$(CH_2)_5$CO); 2.90, 2.30 (2*m*, 2 H–C(3′)); 2.36 (*t*, 1 CH_2 of NH$(CH_2)_5$CO); 1.9–1.35 (*m*, 1 CH_2 of NH$(CH_2)_5$CO). Anal. calc. for $C_{40}H_{41}N_7O_{10}$ (779.81): C 61.61, H 5.30, N 12.57; found: C 61.65, H 5.37, N 12.53.

13. *3′-Deoxy-5′-*O*-(monomethoxytrityl)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]} → 5′}-3′-deoxy-2′-*O*-{6-{{[(9*H*-fluoren-9-yl)methoxy]carbonyl}amino}hexanoyl}-*N^6*-(4-nitrophenyl)-ethoxycarbonyl]adenosine* (**19**). A mixture of **18** (1.46 g, 1.87 mmol), 3′-deoxy-5′-*O*-(monomethoxytrityl)-*N*^{6}-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 2′-[2-(4-nitrophenyl)ethyl diisopropylphosphoramidite] [22] (2.28 g, 2.25 mmol) and 1*H*-tetrazole (656 mg, 9.36 mmol) was stirred in dry MeCN (7 ml) and a few drops of dry CH_2Cl_2 under N_2 at r.t. for 2.5 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 $CHCl_3$ (200 ml), and washed with $Na_2S_2O_3$/NaCl soln. (100 ml) and sat. $NaHCO_3$ soln., 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, CH_2Cl_2, CH_2Cl_2/MeOH 98:2, 97:3, 96:4). Purification had to be repeated for contaminated fractions: 2.858 g (89%) of **19**. Amorphous solid. UV (CH_2Cl_2): 299 (4.26), 286 (sh, 4.47), 272 (sh, 4.83), 266 (4.89), 239 (sh, 4.52). ^{1}H-NMR ($CDCl_3$): 8.69–8.10 (*m*, 2 H–C(8), 2 H–C(2), 2 NH, 6 H *o* to NO_2); 7.76–7.20 (*m*, 8 H of fmoc, 6 H *m* to NO_2, 12 H of MeO*Tr*); 6.80 (*d*, 2 H *o* to MeO); 6.19, 6.02 (*d*, *s*, 2 H–C(1′)); 5.66, 5.5–5.3 (2*m*, 2 H–C(2′)); 4.95 (*m*, N*H*$(CH_2)_5$CO); 4.55–4.15 (*m*, 2 H–C(4′), 3 O*C*H_2CH$_2$, H–C(9)(fmoc), CH_2O(fmoc), 2 H–C(5′)); 3.77 (*s*, MeO); 3.5–3.25 (2*m*, 2 H–C(5′)); 3.19–3.00 (*m*, 3 OCH$_2$C*H*$_2$, 1 CH_2 of NH$(CH_2)_5$CO); 2.8–2.15 (*m*, 4 H–C(3′), 1 CH_2 of NH$(CH_2)_5$CO); 1.85–1.3 (*m*, 3 CH_2 of NH$(CH_2)_5$CO). Anal. calc. for $C_{87}H_{83}N_{14}O_{22}P$ (1707.67): C 61.19, H 4.90, N 11.48; found: C 60.99, H 4.98, N 11.20.

14. *3′-Deoxy-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{*O^P*-[2-(4-nitrophenyl)ethyl]} → 5′}-3′-deoxy-2′-*O*-{6-{{[(9*H*-fluoren-9-yl)methoxy]carbonyl}amino}hexanoyl}-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**20**). As described in *Exper. 6*, with **19** (2.36 g, 1.38 mmol) and CH_2Cl_2/MeOH 4:1 (28 ml) containing 2% of TsOH · H_2O (45 min). Workup with $CHCl_3$ (150 ml) and sat. $NaHCO_3$ soln. (100 ml), reextraction, and purification by FC (silica gel, 14 × 3.5 cm, $CHCl_3$, then $CHCl_3$/MeOH 96:4, 95:5, 94:6, 92:8) gave 1.995 g (quant.) of **20**. Amorphous solid. UV (CH_2Cl_2): 299 (4.25), 286 (sh, 4.48), 272 (sh, 4.83), 266 (4.88). ^{1}H-NMR ((D_6)DMSO): 10.61 (*s*, 2 NH); 8.62–8.53 (*m*, 2 H–C(8), 2 H–C(2)); 8.15–7.23 (*m*, 6 H *o* to NO_2, 8 H of fmoc, 6 H *m* to NO_2); 6.19–6.11 (*m*, 2 H–C(1′)); 5.69, 5.15 (2*m*, 2 H–C(2′)); 5.10 (*t*, OH–C(5′)); 4.45–4.05 (*m*, N*H*$(CH_2)_5$CO, 2 H–C(4′), 3 O*C*H_2CH$_2$, H–C(9)(fmoc), CH_2O(fmoc), 2 H–C(5′)); 3.65–3.45 (2*m*, 2 H–C(5′)); 3.25–2.85 (*m*, 3 OCH$_2$C*H*$_2$, 1 CH_2 of NH$(CH_2)_5$CO); 2.8–2.0 (*m*, 4 H–C(3′), 1 CH_2 of NH$(CH_2)_5$CO); 1.7–1.25 (*m*, 3 CH_2 of NH$(CH_2)_5$CO). Anal. calc. for $C_{67}H_{66}N_{14}O_{21}P$ (1434.32): C 56.11, H 4.64, N 13.67; found: C 55.99, H 4.75, N 13.34.

15. *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′}-3′-deoxy-2′-*O*-{6-{{[(9*H*-fluoren-9-yl)methoxy]carbonyl}amino}hexanoyl}-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**21**). As described in *Exper. 13*, with **20** (2.0 g, 1.39 mmol), 3′-deoxy-5′-*O*-(monomethoxytrityl)-*N*^{6}-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 2′-[2-(4-nitrophenyl)ethyl diisopropylphosphoramidite] [22] (1.766 g, 1.74 mmol), 1*H*-tetrazole (478 mg, 6.95 mmol), anh. MeCN (7 ml; 2 h), and I_2 soln. Workup with $CHCl_3$ (150 ml) and $Na_2S_2O_3$/NaCl soln. (2 × 80 ml), reextraction, and purification by FC (silica gel, 7 × 4.5 cm, $CHCl_3$, then $CHCl_3$/MeOH 97:3, 96:4 gave 3.092 g (94%) of **21**. Amorphous solid. UV (CH_2Cl_2): 297 (4.45),

286 (sh, 4.67), 272 (sh, 5.01), 266 (5.05). ^{1}H-NMR ((D_6)DMSO): 9.1–8.0 (*m*, 3 NH, 3 H–C(8), 3 H–C(2), 10 H *o* to NO_2); 7.75–7.15 (*m*, 8 H of fmoc, 10 H *m* to NO_2, 12 H of MeO*Tr*); 6.78 (*d*, 2 H *o* to MeO); 6.19–6.01 (*m*, 3 H–C(1')); 5.73, 5.45, 5.32 (*m*, 3 H–C(2')); 5.0 (*m*, N*H*$(CH_2)_5$CO); 4.6–4.1 (*m*, 3 H–C(4'), 5 OCH_2CH$_2$, H–C(9)(fmoc), CH_2O(fmoc), 4 H–C(5')); 3.76 (*s*, MeO); 3.45, 3.3 (2*m*, 2 H–C(5')); 3.25–2.95 (*m*, 5 OCH$_2$CH_2, 1 CH_2 of NH$(CH_2)_5$CO); 2.8–1.9 (*m*, 6 H–C(3'), 1 CH_2 of NH$(CH_2)_5$CO); 1.7–1.3 (*m*, 3 of NH$(CH_2)_5$CO). Anal. calc. for $C_{114}H_{109}N_{21}O_{33}P_2$ (2363.19): C 57.94, H 4.65, N 12.44; found: C 57.71, H 4.69, N 12.27.

16. *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'}-3'-deoxy-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]-2'-*O*-[6-(tetradecanoylamino)hexanoyl]adenosine* (**22**). As described in *Exper. 4*, with tetradecanoic acid (38 mg, 0.165 mmol), TOTU (54 mg, 0.165 mmol), and *N*-methylmorpholine (17 mg, 0.165 mmol) in abs. DMF (1.5 ml; 1 h). Deblocking of **21** (354 mg, 0.15 mmol) with 3% piperidine in abs. DMF (2 ml; 10 min) and addition of the above-mentioned soln. (r.t., 3 h), more preactivated tetradecanoic acid (38 mg, 0.165 mmol), TOTU (54 mg, 0.165 mmol), and *N*-methylmorpholine (17 mg, 0.165 mmol) in abs. DMF (1.5 ml; 1.5 h). Workup with AcOEt (100 ml) and $NaHCO_3$ soln. (2 × 50 ml), reextraction, and purification by FC (2 ×, silica gel, 12 × 3 cm, $CHCl_3$, then $CHCl_3$/MeOH 98:2, 97:3, 96:4, 95:5) gave 257 mg (73%) of **22**. Amorphous solid. UV (CH_2Cl_2): 272 (sh, 4.92), 267 (4.95), 239 (sh, 4.60). ^{1}H-NMR ($CDCl_3$): 8.65–8.0 (*m*, 3 H–C(8), 3 H–C(2), 10 H *o* to NO_2); 7.5–7.15 (*m*, 10 H *m* to NO_2, 12 H of MeO*Tr*); 6.78 (*d*, 2 H *o* to MeO); 6.21–5.98 (*m*, 3 H–C(1')); 5.75–5.25 (*m*, 3 H–C(2')); 4.65–4.1 (*m*, 3 H–C(4'), 5 OCH_2CH$_2$, 4 H–C(5'), N*H*$(CH_2)_5$CO); 3.78 (*s*, MeO); 3.7–0.85 (*m*, 2 H–C(5'), 5 OCH$_2$CH_2, NH$(CH_2)_5$CO, 6 H–C(3'), *Me*$(CH_2)_{12}$CO). Anal. calc. for $C_{113}H_{125}N_{21}O_{32}P_2$ (2351.31): C 57.72, H 5.36, N 12.51; found: C 57.58, H 5.43, N 12.51.

17. *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'}-3'-deoxy-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]-2'-*O*-{6-[(3α,7α,12α-trihydroxy-5β-cholan-24-oyl)amino]hexanoyl}adenosine* (**23**). As described in *Exper. 4*, with cholic acid (67 mg, 0.165 mmol), TOTU (54 mg, 0.165 mmol), and *N*-methylmorpholine (17 mg, 0.165 mmol) in abs. DMF (1.5 ml; 1 h). Deblocking of **21** (354 mg, 0.15 mmol) with 3% piperidine in abs. DMF (2 ml; 10 min) and addition of the above-mentioned soln. (r.t., 3 h), more preactivated cholic acid (34 mg, 82.5 μmol), TOTU (27 mg, 82.5 μmol), and *N*-methylmorpholine (9 mg, 82.5 μmol) in abs. DMF (1 ml; 1.5 h). Workup with $CHCl_3$ (100 ml) and $NaHCO_3$ soln. (50 ml), reextraction and purification by FC (several times, silica gel, $CHCl_3$, then $CHCl_3$/MeOH 98:2, 95:5, 93:7, 90:10, 85:15) gave 260 mg (69%) of **23**. Amorphous solid. UV (CH_2Cl_2): 272 (sh, 4.90), 267 (4.94) 240 (sh, 4.58). Anal. calc. for $C_{123}H_{137}N_{21}O_{35}P_2$ (2531.51): C 58.36, H 5.45, N 11.62; found: C 58.35, H 5.75, N 10.91.

18. *3'-Deoxyadenylyl-(2'–5')-3'-deoxyadenylyl-(2'–5')-3'-deoxy-2'-*O*-[6-(tetradecanoylamino)hexanoyl]adenosine* (**24**). A mixture of **22** (80 mg, 34 μmol) in CH_2Cl_2/MeOH 4:1 (4 ml) containing 2% TsOH · H_2O was stirred at r.t. for 20 min. Then the mixture was diluted with $CHCl_3$ (30 ml) and washed with sat. $NaHCO_3$ soln. (2 × 10 ml), the aq. phase reextracted with $CHCl_3$, and the combined org. layer dried ($MgSO_4$) and evaporated. The crude product was diluted with a small amount of $CHCl_3$ and precipitated from Et_2O (15 ml), centrifugated, and dried. For further deblocking, the precipitate was co-evaporated twice with abs. pyridine, then 0.5M DBU in abs. pyridine was added and the mixture stirred at r.t. for 2 d. Then AcOH (60 mg, 1 mmol) was added, the mixture evaporated and co-evaporated with abs. dioxane, and the product precipitated from dioxane/Et_2O 1:3, washed, and centrifugated several times with dioxane/Et_2O: 35 mg of **24** (882 *OC*). Colorless powder. HPLC (0–100% MeCN (0–20 min) in 0.1M (Et_3NH)OAc buffer (pH 7)); t_R 14.94 min.

19. *3'-Deoxyadenylyl-(2'–5')-3'-deoxyadenylyl-(2'–5')-3'-deoxy-2'-*O*-{6-[(3α,7α,12α-trihydroxy-5β-cholan-24-oyl)-amino]hexanoyl}adenosine* (**25**). As described in *Exper. 18*, with **23** (90 mg, 36 μmol) and CH_2Cl/MeOH 4:1 containing 2% TsOH · H_2O (4 ml; 20 min). Workup with $CHCl_3$ (30 ml) and sat. $NaHCO_3$ soln. (2 × 10 ml), reextraction, and precipitaion from Et_2O (15 ml) gave a colorless powder. Treatment with 0.5M DBU in abs. pyridine (1 ml; 2 d) and AcOH (60 mg, 1 mmol), workup with abs. dioxane, and precipitation with abs. MeCN/Et_2O gave 45 mg of **25** (965 *OD*). Colorless powder. HPLC (0–100% MeCN (0–20 min) in 0.1M (Et_3NH)OAc buffer (pH 7)); t_R 11.81 min.

20. *3'-Deoxyadenylyl-(2'–5')-3'-deoxyadenylyl-(2'–5')-2'-*O*-{6-{{N-{4-{[(2-amino-3,4-dihydro-4-oxopteridin-6-yl)methyl]amino}benzoyl}-*L*-γ-glutamyl}amino}hexanoyl}-3'deoxyadenosine* (**26**). For acid-activation, a mixture of folic acid (3 × 44 mg, 0.1 mmol), EDC (3 × 23 mg, 0.12 mmol), and DMAP (3 × 15 mg, 0.12 mmol) in abs. DMF (3 × 2.5 ml) was kept at r.t. for 3 h. Trimer **21** (115 mg, 0.05 mmol) was treated with a soln. of 3% piperidine in abs. DMF (2 ml; 15 min) and then evaporated. The above-mentioned soln. was added in 3 portions within 3 h and further stirred for 1 h at r.t., then the mixture was evaporated. The residue was treated with Et_2O/CH_2Cl_2 and the yellow residue washed and centrifugated with CH_2Cl_2 and Et_2O. Then the residue was co-evaporated with

abs. pyridine and dissolved in abs. pyridine (5 ml). DBU (380 mg, 2.5 mmol) was added and the mixture stirred at r.t. for 18 h. Then AcOH (300 mg, 5 mmol) was added, the mixture evaporated, and the residue treated with 80% $AcOH/H_2O$ (10 ml, 17 h). The mixture was centrifugated, the supernatant evaporated, and the residue treated with MeCN. The obtained yellow crude product (27 mg) was purified by prep. HPLC (*Lichrospher 100 RP 18*, 10 μm, 25 × 2 cm, 10% MeCN (0–5 min), 10–35% MeCN (5–40), 35–50% (40–45 min), 50% MeCN (45–50 min) in 0.1M $(Et_3NH)OAc$ buffer (pH 7), 7 ml/min): 9 mg (228 *OD*) of **26**. Yellow powder. HPLC (0–50% MeCN (2–32 min) in 0.1M $(Et_3NH)OAc$ buffer (pH 7)): t_R 16.00 min. FAB-MS (matrix glycerol/3-nitrobenzyl alcohol 1:1): (MH^+; calc. 1414.5).

21. *3′-Deoxy-5′*-O-{*6*-{{*[(9H-fluorenyl-9-methoxy] carbonyl}amino}hexanoyl*}-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′-deoxy*-N^6,*2′*-O-*bis[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**28**). As described in *Exper. 2*, with 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′-deoxy-N^6,2′-*O*-bis[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**27**; 2.533 g, 1.3 mmol) [20], **3** (505 mg, 1.43 mmol), EDC (274 mg, 1.43 mmol), DMAP (175 mg, 1.43 mmol), and CH_2Cl_2 (18 ml; 2.5 h), then more **3** (124 mg, 0.35 mmol), ECD (67 mg, 0.35 mmol), and DMAP (43 mg, 0.35 mmol; 3 h). Workup with CH_2Cl_2 (200 ml) and 10% citric acid soln. (100 ml), reextraction, sat. $NaHCO_3$ soln. (100 ml), reextraction and purification by FC (silica gel, $CHCl_3$ + 3% MeOH) gave 2.347 g (79%) of **28**. Amorphous solid. UV (CH_2Cl_2): 298 (sh, 4.46), 285 (sh, 4.71), 272 (sh, 5.01), 267 (5.06). ^{1}H-NMR ($CDCl_3$): 8.65–8.0 (*m*, 3 H–C(8), 3 H–C(2), 12 H *o* to NO_2); 7.75–7.2 (*m*, 8 H of fmoc, 12 H *m* to NO_2); 6.15–5.98 (*m*, 3 H–C(1′)); 5.7–5.15 (*m*, 3 H–C(2′)); 4.65–4.05 (*m*, $NH(CH_2)_5CO$, 3 H–C(4′), 6 OCH_2CH_2, H–C(9)(fmoc), CH_2O(fmoc), 6 H–C(5′)); 3.2–2.9 (*m*, 6 OCH_2CH_2, 1 CH_2 of $NH(CH_2)_5CO$); 2.7 (*m*, H–C(3′)); 2.4–2.1 (*m*, 5 H–C(3′), 1 CH_2 of $NH(CH_2)_5CO$); 1.65–1.25 (*m*, 3 CH_2 of $NH(CH_2)_5CO$). Anal. calc. for $C_{103}H_{100}N_{22}O_{36}P_2$ (2284.00): C 54.17, H 4.41, N 13.49; found: C 54.25, H 4.53, N 13.03.

22. *3′-Deoxy*-N^6-*[2-(4-nitrophenyl)ethoxycarbonyl]*-*5′*-O-*[6-(tetradecanoylamino)hexanoyl]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′-deoxy*-N^6,*2′*-O-*bis[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**29**). As described in *Exper. 4*, with tetradecanoic acid (40 mg, 0.176 mmol), TOTU (56 mg, 0.176 mmol), and *N*-methylmorpholine (18 mg, 0.176 mmol) in abs. DMF (1.5 ml; 1 h). Deblocking of **28** (265 mg, 0.16 mmol) with 3% piperidine in abs. DMF (4 ml; 15 min) and addition of the above-mentioned soln. (r.t., 1.5 h), more pre-activated tetradecanoic acid (40 mg, 0.176 mmol), TOTU (58 mg, 0.176 mmol), and *N*-methylmorpholine (18 mg, 0.176 mmol) in abs. DMF (1.5 ml; 1.5 h). Workup with $CHCl_3$ (100 ml) and $NaHCO_2$ soln. (50 ml), reextraction, and purification by FC (2 ×, silica gel, 13.5 × 2 cm, $CHCl_3$, then $CHCl_3$/MeOH 96:4, 94:6) gave 272 mg (75%) of **29**. Amorphous solid. UV (CH_2Cl_2): 272 (sh, 4.96), 267 (4.99). ^{1}H-NMR ($CDCl_3$): 9.15–8.05 (*m*, 3 NH(ade), 3 H–C(8), 3 H–C(2), 12 H *o* to NO_2); 7.5–7.2 (*m*, 12 H *m* to NO_2); 6.15–5.95 (*m*, 3 H–C(1′)); 5.7–5.25 (*m*, 3 H–C(2′)); 4.65–4.1 (*m*, 3 H–C(4′), 6 OCH_2CH_2, 6 H–C(5′), $NH(CH_2)_5CO$); 3.3–3.0 (*m*, 6 OCH_2CH_2, 4 CH_2 of $NH(CH_2)_5CO$); 3.0–2.0 (*m*, 6 H–C(3′), 1 CH_2 of $Me(CH_2)_{12}CO$); 1.7–1.2 (*m*, 3 CH_2 of $NH(CH_2)_5CO$, 10 CH_2 of $Me(CH_2)_{12}CO$); 0.87 (*t*, *Me*$(CH_2)_{12}CO$). Anal. calc. for $C_{102}H_{116}N_{22}O_{35}P_2$ (2272.12): C 53.92, H 5.15, N 13.56; found: C 53.72, H 5.19, N 13.41.

23. *3′-Deoxy*-N^6-*[2(4-nitrophenyl)ethoxycarbonyl]*-*5′*-O-*{6-[(3α,7α,12α-trihydroxy-5β-cholan-24-oyl)amino]hexanoyl}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′-deoxy*-N^6,*2′*-O-*bis[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**30**). As described in *Exper. 4*, with cholic acid (54 mg, 0.132 mmol), TOTU (43 mg, 0.132 mmol), and *N*-methylmorpholine (14 mg, 0.132 mmol) in abs. DMF (1.5 ml; 1 h). Deblocking of **28** (275 mg, 0.12 mmol) with 3% piperidine in abs. DMF (2 ml; 20 min) and addition of the above-mentioned soln. (r.t., 1.5 h), more preactivated cholic acid (54 mg, 0.132 mmol), TOTU (43 mg; 0.132 mmol), and *N*-methylmorpholine (14 mg, 0.132 mmol) in abs. DMF (1 ml; 1.5 h). Workup with $CHCl_3$ (100 ml) and $NaHCO_3$ soln. (50 ml), reextraction, and purification by FC (several times, silica gel, $CHCl_3$, then $CHCl_3$/MeOH 95:5, 90:5, 85:15) gave 195 mg (69%) of **30**. Amorphous solid. UV (CH_2Cl_2): 272 (sh, 4.96), 268 (4.99). Anal. calc. for $C_{112}H_{128}N_{22}O_{38}P_2$ (2452.32): C 54.68, H 5.26, N 12.57; found: C 55.17, H 5.51, N 11.86.

24. *3′-Deoxy-5′*-O-*[6-(tetradecanoylamino)hexanoyl]adenylyl-(2′–5′)-3′-deoxyadenylyl-(2′–5′)-3′-deoxyadenosine* (**31**). Trimer **29** (85 mg, 37 μmol) was first co-evaporated with abs. pyridine. Then 0.5M DBU in abs. pyridine (1.3 ml) was added and the mixture stirred at r.t. for 2 d. Then AcOH (80 mg, 1.3 mmol) was added, the mixture evaporated and co-evaporated with abs. dioxane, and the product precipitated from dioxane/Et_2O 1:3, washed, and centrifugated with dioxane/Et_2O: 45 mg of **31** (1166 *OD*). Colorless powder. HPLC (0–100% MeCN (0–20 min) in 0.1M $(Et_3NH)OAc$ buffer (pH 7)): t_R 14.98 min.

25. *3'-Deoxy-5'-O-{6-[(3α,7α,12α-trihydroxy-5β-cholan-24-oyl)amino]hexanoyl}adenylyl-(2'–5')-3'-deoxyadenylyl-(2'–5')-3'-deoxyadenosine* (**32**). As described in *Exper. 24*, with **30** (50 mg, 20 μmol), 0.5M DBU in abs. pyridine (0.8 ml; r.t.; 2 d), and AcOH (60 mg, 1 mmol). Workup with abs. dioxane, precipitation with abs. MeCN, and washing with abs. MeCN and Et_2O: 32 mg of **32** (639 *OD*). Colorless powder. HPLC (0–100% MeCN (0–20 min) in 0.1M (Et_3NH)OAc buffer (pH 7)): t_R 11.75 min.

26. *5'-O-{6-{{N-{4-{[(2-Amino-3,4-dihydro-4-oxopteridin-6-yl)methyl]amino}benzoyl}-L-γ-glutamyl}amino}-hexanoyl}adenylyl-(2'–5')-3'-deoxyadenylyl-(2'–5')-3'-deoxyadenosine* (**33**). As described in *Exper. 20*, with folic acid (3 × 44 mg, 0.1 mmol), ECD (3 × 23 mg, 0.12 mmol), and DMAP (3 × 15 mg, 0.12 mmol) in abs. DMF (3 × 2.5 ml; r.t., 3 h). Deblocking of trimer **28** (111 mg, 0.05 mmol) with 3% piperidine in abs. DMF (2 ml; 15 min) and addition of the above-mentioned soln. (3 portions within 4 h). Workup with Et_2O/CH_2Cl_2, then washing and centrifugation with CH_2Cl_2 and Et_2O, further deblocking by DBU treatment (380 mg, 2.5 mmol) in abs. pyridine (5 ml; 18 h), addition of AcOH (300 mg, 5 mmol), evaporation, and treatment with 80% AcOH/H_2O (10 ml, 17 h). The mixture was centrifugated and the supernatant evaporated and treated with MeCN: yellow powder (19 mg). Purification by prep. HPLC (*Lichrospher 100 RP18*, 10 μm, 25 × 2 cm, 10% MeCN (0–5 min), 10–35% MeCN (5–40 min), 35–50% MeCN (40–45 min), 50% MeCN (45–50 min) in 0.1M (Et_3NH)OAc buffer (pH 7), 7 ml/min): 43 *OD* of **33**. Yellow amorphous solid. HPLC (0–50% MeCN (0–20 min), 50–75% MeCN (20–25 min) in 0.1M (Et_3NH)OAc buffer (pH 7)): t_R 11.86 min. FAB-MS (matrix glycerol/3-nitrobenzyl alcohol 1:1): 1414 (MH^+; calc. 1414.5).

REFERENCES

[1] C. Hörndler, R. J. Suhadolnik, N. F. Muto, E. E. Henderson, M.-X. Guan, W. Pfleiderer, *Helv. Chim. Acta* **1997**, *80*, 767.

[2] B. R. G. Williams, R. R. Golger, R. E. Brown, C. S. Gilbert, I. M. Kerr, *Nature (London)* **1979**, *282*, 582.

[3] P. Lengyel, *Annu. Rev. Biochem.* **1982**, *51*, 251.

[4] A. G. Hovanessian, *J. Interferon Res.* **1991**, *11*, 199.

[5] H. C. Schröder, R. J. Suhadolnik, W. Pfleiderer, R. Charubala, W. E. G. Müller, *Int. J. Biochem.* **1992**, *24*, 55.

[6] B. A. Hassell, A. Zhon, R. H. Silverman, *Cell* **1993**, *72*, 753.

[7] R. Charubala, W. Pfleiderer, *Tetrahedron Lett.* **1980**, *21*, 4077.

[8] R. Charubala, E. Uhlmann, F. Himmelsbach, W. Pfleiderer, *Helv. Chim. Acta* **1987**, *70*, 2028.

[9] P. W. Doetsch, R. J. Suhadolnik, Y. Sawada, J. D. Mosca, M. B. Flick, N. L. Reichenbach, A. Q. Dang, J. M. Wu, R. Charubala, W. Pfleiderer, E. E. Henderson, *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 6699.

[10] H. Sawai, J. Imai, K. Lesiak, M. I. Johnston, P. F. Torrence, *J. Biol. Chem.* **1983**, *258*, 1671.

[11] 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.

[12] C. Barat, V. Lullien, O. Schatz, G. Keith, M. T. Nugeyre, F. Grüninger-Leitch, F. Barré-Sinoussi, S. F. J. LeGrice, J. L. Darlix *EMBO J.* **1989**, *8*, 3279.

[13] Progress in Molecular and Subcellular Biology, Biological Response Modifiers – Interferons, Doublestranded RNA and 2',5'-Oligoadenylates', Eds. W. E. G. Müller and H. C. Schröder, Springer Verlag, Berlin, 1994, Vol. 14.

[14] K. Renneisen, L. Leserman, E. Matthes, H. C. Schröder, W. E. G. Müller, *J. Biol. Chem.* **1990**, *265*, 16337.

[15] J. Goodchild, *Bioconjugate Chem.* **1990**, *1*, 165, and ref. cit. therein.

[16] S. L. Beaucage, R. P. Iyer, *Tetrahedron* **1993**, *49*, 1925.

[17] M. Manoharan, C. J. Guinosso, P. D. Cook, *Tetrahedron Lett.* **1991**, *32*, 7171.

[18] M. Wasner, E. E. Henderson, R. J. Suhadolnik, W. Pfleiderer, *Helv. Chim. Acta* **1994**, *77*, 1757.

[19] M. Wasner, R. J. Suhadolnik, S. E. Horvath, M. E. Adelson, N. Kon, M.-X. Guan, E. E. Henderson, W. Pfleiderer, *Helv. Chim. Acta* **1996**, *79*, 609.

[20] M. Wasner, R. J. Suhadolnik, S. E. Horvath, M. E. Adelson, N. Kon, M.-X. Guan, E. E. Henderson, W. Pfleiderer, *Helv. Chim. Acta* **1996**, *79*, 619.

[21] D. G. Norman, C. B. Reese, *Synthesis* **1983**, 304.

[22] H. Schirmeister, W. Pfleiderer, *Helv. Chim. Acta* **1994**, *77*, 10.

[23] C. P. Leamon, P. S. Low, *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 5572.