54. Nucleosides

Part LX[1])

Synthesis and Characterization of Monomeric Cordycepin-Vitamin and Cordycepin-Lipid Conjugates Model Substances for Biodegradable Ester and Carbonate Linkages in Conjugates and Potential Inhibitors of HIV-1 Replication

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Dedicated to Prof. Dr. Dr. h. c. *Hans-Jürgen Bestmann* on the occasion of his 70th birthday and in admiration to his interesting contribution to organic chemistry

(2.I.96)

Monomeric 3′-deoxyadenosine (cordycepin) was modified at the 2′-*O*- (**13**–**18**) and 5′-*O*-position (**25**–**29**) by the vitamins E, D_2, and A and by the two lipids 1,2-di-*O*-palmitoylglycerol and 1,2-di-*O*-hexadecylglycerol *via* succinate or carbonate linkages. These base-labile conjugates afforded protection groups like the 2-(4-nitrophenyl)ethoxycarbonyl (npeoc) and monomethoxytrityl group (MeOTr) that are cleavable without harming the ester and carbonate bonds, respectively. Monomeric conjugates of cordycepin and vitamin E, vitamin D_2, 1,2-di-*O*-palmitoylglycerol, and 1,2-di-*O*-hexadecylglycerol (see **13**, **14**, **17**, **18**, **25**, **26**, **28**, and **29**) inhibited HIV-1-induced syncytia formation 1.7 to 6.2 fold compared to 1.5-fold for cordycepin (see *Table*); IC_{50} values for **25** and **28** were 257 and 267 μM, respectively. In addition, the monomeric cordycepin-vitamin and -lipid conjugates inhibited HIV-1 RT activity 28–49% which compares with a 13% inhibition of HIV-1 RT observed for cordycepin. The minimal inhibition of HIV-1-induced syncytia formation and HIV-1 RT activity did not proceed by the activation of RNase L. The monomeric conjugates tested (**13**, **14**) increased PKR expression.

1. Introduction. – The 3′-deoxyadenosine (cordycepin) was discovered by isolation from *Cordyceps militaris* [2]. It shows some inhibitory activity against *Bacillus subtilis*, avian tubercle bacillus, and *Ehrlich* ascites tumor cells [3]. Recently, cordycepin trimer core d$^{3'}$(A2′p5′A2′p5′A) [4] was found to be an inhibitor of HIV-1 reverse-transcriptase (RT) [5] [6] by interfering in the primer complex formation of RT to $rRNA^{Lys,3}$ *via* the anticodon region.

However, the polarity of nucleosides and oligonucleotides is a limiting factor for penetration through cell membranes and, therefore, many attempts of conjugate formation are reported in literature to overcome this problem [7–9]. The attachment of cholesterol to the 2′-*O*- and 5′-*O*-position of the sugar moiety of monomeric and trimeric

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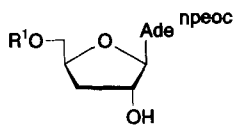
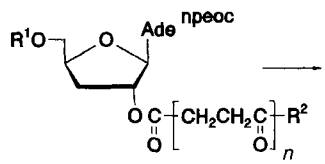
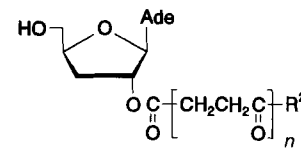
cordycepin was already shown to facilitate membrane crossing [10]. So other lipophilic conjugates of monomeric cordycepin were prepared as model substances for testing reaction conditions to synthesize the trimeric analogues. It is the aim to link the lipophilic residues to the cordycepin moiety by ester and carbonate functions, respectively, since this kind of binding will guarantee the anticipated degradation of the pro-drug in the cell after membrane crossing.

2. Syntheses. – The syntheses of cordycepin [11] and its protected derivatives **1** [12], **2** [13], and **19** [12] were already described in the literature. Starting material for the 2′-*O*-conjugates were compounds **1** and **2**, respectively. Reaction of freshly prepared 2-*ambo*-α-tocopheryl chloroformate with compound **1** in abs. CH_2Cl_2 in the presence of 1-methyl-1*H*-





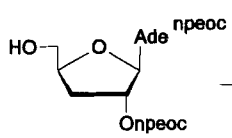


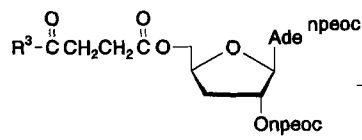
	R^1
1	MeOTr
2	npeoc

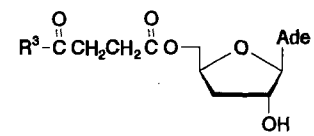
	R^1	R^2	*n*
3	MeOTr	VitE	0
4	MeOTr	VitE	1
5	npeoc	$VitD_2$	1
6	npeoc	VitA	1
7	MeOTr	PL	1
8	MeOTr	EL	1

	R^1	R^2	*n*
9	H	VitE	0
10	H	VitE	1
11	H	PL	1
12	H	EL	1

	R^2	*n*
13	VitE	0
14	VitE	1
15	$VitD_2$	1
16	VitA	1
17	PL	1
18	EL	1





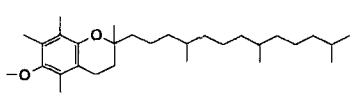


19

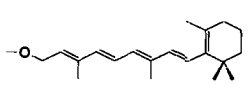
	R^3
20	VitE
21	$VitD_2$
22	VitA
23	PL
24	EL

	R^3
25	VitE
26	$VitD_2$
27	VitA
28	PL
29	EL

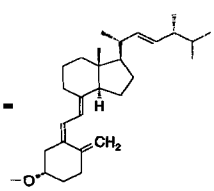
VitE =



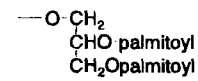
Vit A =



Vit D_2 =



PL =



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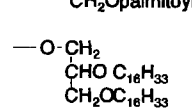

imidazole and 4-(dimethylamino)pyridine (DMAP) gave the fully protected vitamin E conjugate **3** in 65% yield bearing a carbonate moiety. The structural analogue carrying a succinyl spacer between cordycepin and vitamin E (= 2-*ambo*-α-tocopherol) moieties was synthesized by a one-pot reaction starting from the 2′-OH component **1** which was first treated with succinic anhydride and DMAP and second with vitamin E in the presence of *N*-[3-(dimethylamino)propyl]-*N*′-ethylcarbodiimide hydrochloride (EDC) to give the corresponding conjugate **4** in 74% yield. Detritylation of the conjugates **3** and **4** by 2% TsOH in CH_2Cl_2/MeOH 4:1 afforded the intermediates **9** and **10**, respectively, in excellent yields. Because of the acid lability of the vitamins D_2 and A, the monomethoxytrityl protecting group had to be replaced in the starting material by the base-labile β-eliminating 2-(4-nitrophenyl)ethoxycarbonyl (npeoc) group [14]. Compound **2** [13] was reacted with succinic anhydride and DMAP to give the corresponding 3′-deoxy-N^6,5′-*O*-bis[2-(4-nitrophenyl)ethoxycarbonyl]-2′-*O*-succinyladenosine which was isolated for subsequent esterification by the carbodiimide method (EDC) with vitamin D_2 (ergocalciferol) in the absence of O_2 and light, to give **5** in 56% yield. Conjugate formation of **2** with A (retinol) took place in a similar manner, but without isolation of the succinyl derivative to give **6** in 74% yield. In the case of the succinyl ester conjugates with 1,2-di-*O*-palmitoylglycerol [15] [13] and 1,2-di-*O*-hexadecylglycerol [16] [13], the fully protected conjugates **7** and **8** were detritylated by 2% TsOH in CH_2Cl_2/MeOH 4:1 to give **11** (78%) and **12** (56%). The final deblocking was achieved by β-elimination with 0.5M 1,8-diazabicyclo[5.3.0]undec-7-ene (DBU) in abs. pyridine to result in the compounds **13**–**18**.

The syntheses of the 5′-*O*-conjugates were performed by one-pot reactions starting from N^6,2′-bis[2-(4-nitrophenyl)ethoxycarbonyl]-3′-deoxyadenosine (**19**) [13] which was treated first with succinic anhydride and DMAP, followed by carbodiimide-activated esterification with the different lipophilic alcohols to give **20**–**24**. β-Elimination of the npeoc groups with 0.5M DBU in abs. pyridine resulted in the 5′-*O*-conjugates **25**–**29**. It was noticed that the deprotected vitamin A conjugates **16** and **27** are extremely instable to light and O_2 in comparison to their precursors **6** and **22**, respectively. Correct elemental analyses could, therefore, not be obtained for **16** and **27**.

3. Biological Application. – The 2′-*O*- and 5′-*O*-monomeric cordycepin-vitamin and -lipid conjugates were evaluated with respect to give biological activities: *i*) activation of RNase L, *ii*) HIV-1-induced syncytia formation, *iii*) HIV-1 reverse-transcriptase activity, *iv*) PKR expression, and *v*) PKR activity (*Table*). Cordycepin inhibited HIV-1-induced syncytia formation 1.5-fold, compared with a 1.7- to 6.2-fold inhibition observed with the monomeric cordycepin-vitamin and -lipid conjugates **13**, **14**, **17**, **18**, **25**, **26**, **28**, and **29**. IC_{50} Values for monomeric conjugates **25** and **28** were 254 and 267 μM, respectively. The monomeric conjugates (300 μM) inhibited HIV-1 RT in the range 28–49%. None of the monomeric cordycepin-vitamin and -lipid conjugates activated recombinant, human GST-RNase L. In view of the inhibition of syncytia formation and HIV-1 RT activity observed in the absence of RNase L activation, the effect of the (2′–5′)A conjugates on PKR expression was measured. The two monomeric conjugates **13** and **14** tested resulted in an increase in PKR expression of 65 and 92%, respectively. Conjugate **14** with a succinyl linker showed a greater increase in PKR expression than did conjugate **13** with a carbonyl linker. The monomeric cordycepin conjugates were used as model substances

Table. *Inhibition of HIV-1 Replication and Biological Activities of Monomeric Cordycepin-Vitamin and -Lipid Conjugates* **13**, **14**, **17**, **18**, **25**, **26**, **28**, *and* **29**[a])

		Inhibition of syncytia formation[b])	Inhibition of HIV-1 RT activity [%][c])	Activation of RNase L[d])	PKR Expression[e]) ([%] change)
13	Cordycepin 2′-(vitamin E carbonate)[f])	1.9	49	0	+65
14	Cordycepin 2′-(vitamin E succinate)[f])	1.8	46	0	+92
17	Cordycepin 2′-(2,3-di-*O*-palmitoyl-glyceryl succinate)[f])	3.9	36	0	n.t.[g])
18	Cordycepin 2′-(2,3-di-*O*-hexadecyl-glyceryl succinate)[f])	3.7	28	0	n.t.[g])
25	Cordycepin 5′-(vitamin E succinate)[f])	6.2	44	0	n.t.[g])
26	Cordycepin 5′-(vitamin D_2 succinate)[f])	4.2	30	0	n.t.[g])
28	Cordycepin 5′-(2,3-di-*O*-palmitoyl-glyceryl succinate)[f])	2.6	31	0	n.t.[g])
29	Cordycepin 5′-(2,3-di-*O*-hexadecyl-glyceryl succinate)[f])	1.7	43	0	n.t.[g])
	Vitamin D_2	n.t.[g])	21	n.t.[g])	n.t.[g])
	Cordycepin	1.5	13	n.t.[g])	−27
	Adenine	1.3	9.8	n.t.[g])	n.t.[g])

[a]) Compounds were tested at 300 μM.

[b]) Inhibition of HIV-1 replication was determined by syncytia formation (fold reduction in infection). The mean of triplicate determinations is shown; variance did not exceed 5–10%.

[c]) Percent inhibition of HIV-1 reverse transcriptase (HIV-1 RT) activity. Control values for HIV-1 RT activity ranged from 24000 to 33000 cqm. The mean of duplicate determinations is shown; variance did not exceed 5–10%.

[d]) Activation of recombinant RNase L was measured as the percent hydrolysis of [^{32}P]poly(U) in the presence of cordycepin 2′-*O*- and 5′-*O*-vitamin and -lipid conjugates (10 μM). The mean of duplicate determinations is shown; variance did not exceed 5–10%.

[e]) PKR activity was measured as described in the *Exper. Part.* At 20 h post-infection, the quantity of PKR observed varied by < 1%, irrespective of HIV-1 infection.

[f]) Test compounds were dissolved in MeOH; final concentration of MeOH in the assays was 10%. Data were normalized to a 10% MeOH control.

[g]) n.t. = not tested.

for the synthesis and characterization of trimeric cordycepin-vitamin and -lipid conjugates.

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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. EL = 'Ether lipid'.

Bioassay. Recombinant, human RNase L was expressed in *E. coli* (DH5α) as a fusion protein glutathione-*S*-transferase(GST)-RNase L as described [17]. The activation of recombinant, human GST-RNase L was measured as the percent of poly(U)[^{32}P]pCp hydrolysed in the presence of authentic p_3A_3 (10^{-10}–10^{-6}) or (2′–5′)A derivative

as previously described [17]. The infected-centers assay was used to measure the ability of (2′–5′)A derivatives (300 μM) to inhibit HIV-1-induced syncytia formation in human cells as previously described [10]. The effect of (2′–5′)A derivatives (300 μM) on HIV-1 reverse transcriptase (RT) activity was measured as previously described [18]. (2′–5′)A derivatives or controls (in 25 μl) were added to 50 μl of supernatant from HIV-1 infected Molt4 IIIB cells containing RT activity and incubated for 2 h at 37° in the presence of a cocktail containing 50 mM *Tris* (pH 8.0), 20 mM DTT (dithiothreitol), 10 mM $MgCl_2$, 60 mM NaCl, 0.05% NP-40, 5 μg/ml of oligodeoxythymidylic acid, 10 μg/ml of polyriboadenylic acid, 10 mM TTP, and 1 μCi of [α-^{32}P]TTP. 50 μl of the cocktail were then spotted onto *DEAE* paper, dried, washed in *2X SSC* soln. (3×, 10 min each), dried, and exposed to radiographic film for 18 to 24 h at −80°. The filters were cut, and final quantification (cqm) was determined by scintillation spectrometry. Test compounds were added at 100 μm 2 h prior to infection of $1 \cdot 10^6$SupT1 cells with HIV-1 IIIB at a *m.o.i.* of 0.1. NP-40 extracts were prepared 20 h post-infection, and PKR expression was measured as described [19].

1. *3′-Deoxy-*N^6,*5′-*O-*bis[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**2**) [13]. To a soln. of *3′-deoxy-*N^6-*[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* [12] (1 g, 2.25 mmol) in abs. pyridine (8 ml) at −30° was added dropwise 2-(4-nitrophenyl)ethyl chloroformate [14] (1.5 g, 6.5 mmol) in abs. CH_2Cl_2 (4 ml) within 30 min. Then the mixture was stirred at −10° for 30 min. More 2-(4-nitrophenyl)ethyl chloroformate [14] (800 mg, 3.5 mmol) in abs. CH_2Cl_2 (4 ml) was added at −10°. After 70 min, the mixture was diluted with $CHCl_3$ (100 ml) and washed with sat. $NaHCO_3$ soln. (3 × 50 ml), the aq. phase re-extracted with $CHCl_3$ (2 × 50 ml), the combined org. layer dried ($MgSO_4$), evaporated, and co-evaporated with toluene, and the residue purified by FC (silica gel, 15 × 3.5 cm, toluene→toluene/AcOEt 4:1→1:1→2:3→1:1 + 1% MeOH→1:1 + 4% MeOH→1:1 + 25% MeOH): 1.05 g (73%) of **2**. Amorphous solid. UV (MeOH): 266 (4.55). ^{1}H-NMR ((D$_6$)DMSO): 10.58 (*s*, NH); 8.62, 8.49 (2*s*, H–C(8), H–C(2)); 8.13 (2*d*, 4 H *o* to NO_2); 7.55 (2*d*, 4 H *m* to NO_2); 5.99 (*d*, H–C(1′)); 5.79 (*d*, OH–C(2′)); 4.70 (*m*, H–C(2′)); 4.51 (*m*, H–C(4′)); 4.41–4.21 (*m*, 6 H, 2 H–C(5′), OCH_2CH_2); 3.12–3.00 (2*t*, 4 H, OCH_2CH_2); 2.25 (*m*, H–C(3′)); 2.03 (*m*, H–C(3′)). Anal. calc. for $C_{28}H_{27}N_7O_{11}$ (637.6): C 52.75, H 4.27, N 15.38; found: C 52.66, H 4.29, N 15.34.

2. *3′-Deoxy-5′-*O-*(monomethoxytrityl)-*N^6-*[2-(4-nitrophenyl)ethoxycarbonyl]-2′-*O-*(2-*ambo-α-*tocopheryloxycarbonyl)adenosine* (**3**). The 2-*ambo*-α- tocopheryl chloroformate was synthesized by adding dropwise 2-*ambo*-α-tocopherol (431 mg, 1 mmol) and Et_3N (0.14 ml, 1 mmol) in anh. THF to an ice-cooled soln. of trichloromethyl chloroformate (*Fluka*; 0.181 ml, 1.5 mmol) in anh. THF (4 ml). After stirring (30 min) at 0°, small amounts of charcoal were added, and the mixture was kept for further 15 min. Then the precipitate was filtered twice, and the filtrate was evaporated to a yellow oil (480 mg, 97%).

To an ice-cooled soln. of 2-*ambo*-α- tocopheryl chloroformate (247 mg, 0.5 mmol) and 1-methyl-1*H*-imidazole (41 mg, 0.5 mmol) in abs. CH_2Cl_2 (2 ml) were added some pearls of molecular sieve (4 Å), **1** [12] (179 mg, 0.25 mmol), and catalytic amounts of DMAP. The mixture was kept at r.t. for 2 d, then 2-*ambo*-α-tocopheryl chloroformate (123 mg, 0.25 mmol) and 1-methyl-1*H*-imidazole (21 mg, 0.25 mmol) were added, and the mixture was kept at r.t. for another 16 h. FC (silica gel, 16.5 × 2 cm, toluene→toluene/AcOEt 2:1→3:2→1:1) gave 230 mg of a colourless foam which was dissolved in CH_2Cl_2 (2 ml) and precipitated from petroleum ether (100 ml): 190 mg (65%) of **3**. Colourless powder. UV (CH_2Cl_2): 273 (sh, 4.47), 267 (4.51), 236 (sh, 4.47). ^{1}H-NMR ((D$_6$)DMSO): 10.64 (*s*, NH); 8.62, 8.59 (2*s*, H–C(8), H–C(2)); 8.14 (*d*, 2 H *o* to NO_2); 7.59 (*d*, 2 H *m* to NO_2); 7.30–7.13 (*m*, 12 H, MeO*Tr*); 6.78 (*d*, 2 H *o* to MeO); 6.37 (*d*, H–C(1′)); 5.91 (*m*, H–C(2′)); 4.57 (*m*, H–C(4′)); 4.37 (*t*, OCH_2CH_2); 3.69 (*s*, MeO); 3.21 (*m*, 2 H–C(5′)); 3.08 (*t*, OCH_2CH_2); 2.88–0.7 (*m*, 2 H–C(3′), tocopheryl). Anal. calc. for $C_{69}H_{85}N_6O_{11}$ (1174.5): C 70.63, H 7.22, N 7.16; found: C 70.48, H 7.18, N 7.16.

3. *3′-Deoxy-5′-*O-*(monomethoxytrityl)-*N^6-*[2-(4-nitrophenyl)ethoxycarbonyl]-2′-*O-{*[2-(2-*ambo-α-*tocopheryloxycarbonyl)ethyl]carbonyl}adenosine* (**4**). A mixture of **1** [12] (717 mg, 1 mmol) succinic anhydride (120 mg, 1.2 mmol), and DMAP (159 mg, 1.3 mmol) in abs. CH_2Cl_2 (5 ml) was kept at r.t. for 4.5 h. Then *N*-[3-(dimethylamino)propyl]-*N′*-ethylcarbodiimide hydrochloride (EDC; 250 mg, 1.4 mmol) and 2-*ambo*-α-tocopherol (603 mg, 1.4 mmol) were added. The mixture was kept at r.t. in the dark for 2 h, then diluted with $CHCl_3$ (120 ml), washed with 5% citric acid (60 ml) and sat. $NaHCO_3$ soln. (60 ml), the aq. phase re-extracted with $CHCl_3$, the combined org. layer dried ($MgSO_4$) and evaporated, and the crude product purified by FC (silica gel, 16.5 × 3 cm, toluene→toluene/AcOEt 1:1→1:1 + 2% MeOH→1:1 + 3% MeOH): 911 mg (74%) of **4**. Amorphous solid. UV (CH_2Cl_2): 273 (sh, 4.47), 267 (4.52), 236 (sh, 4.41). ^{1}H-NMR ((D$_6$)DMSO): 10.64 (*s*, NH); 8.57, 8.56 (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); 7.28–7.12 (*m*, MeO*Tr*); 6.76 (*d*, 2 H *o* to MeO); 6.24 ('*s*', H–C(1′)); 5.84 (*m*, H–C(2′)); 4.46 (*m*, H–C(4′)); 4.38 (*t*, OCH_2CH_2); 3.68 (*s*, MeO); 3.2–3.06, 3.0–2.7 (*m*, 2 H–C(5′), OCH_2CH_2, C(O)CH_2CH_2C(O)); 2.2–0.75 (*m*, 2 H–C(3′), tocopheryl). Anal. calc. for $C_{72}H_{88}N_6O_{12}$ (1229.5): C 70.34, H 7.21, N 6.84; found: C 70.24, H 7.31, N 6.82.

4. *3′-Deoxy-2′-O-{[2-(ergocalciferyloxycarbonyl)ethyl]carbonyl}-N⁶,5′-O-bis[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**5**). First, *3′-deoxy-N⁶,5′-O-bis[2-(4-nitrophenyl)ethoxycarbonyl]-2′-O-succinyladenosine* was synthesized by stirring **2** [13] (512 mg, 0.8 mmol), succinic anhydride (480 mg, 4.8 mmol), and DMAP (635 mg, 5.2 mmol) in abs. CH_2Cl_2/DMF 4:1 (15 ml) at r.t. for 2 d. Then MeOH was added, the mixture kept at r.t. for 2 h and evaporated, and the residue purified by FC (silica gel (30 g), $d = 3$ cm, $CHCl_3 \rightarrow CHCl_3 + 5\%$ MeOH $\rightarrow CHCl_3 + 10\%$ MeOH) to give a colourless oil which was lyophilized in dry dioxane: 529 mg (90%). Amorphous solid. UV (MeOH): 272 (sh, 4.50), 267 (4.53). ^{1}H-NMR ((D$_6$)DMSO): 12.25 (br. COOH); 10.62 (br. NH); 8.62, 8.53 (2*s*, H–C(8), H–C(2)); 8.15 (*m*, 4 H *o* to NO_2); 7.55 (2*d*, 4 H *m* to NO_2); 6.20 (*d*, H–C(1′)); 5.73 (*d*, H–C(2′)); 4.55 (*m*, H–C(4′)); 4.4–4.2 (*m*, 2 OCH_2CH$_2$, 2 H–C(5′)); 3.10, 3.03 (2*t*, 2 OCH$_2$CH_2); 2.7–2.2 (*m*, C(O)CH_2CH_2C(O), 2 H–C(3′)). Anal. calc. for $C_{32}H_{31}N_7O_{14}\cdot 1/2$ dioxane (781.1): C 52.24, H 4.51, N 12.54; found: C 51.66, H 4.47, N 12.13.

A mixture of 3′-deoxy-*N*⁶,5′-*O*-bis[2-(4-nitrophenyl)ethoxycarbonyl]-2′-*O*-succinyladenosine (295 mg, 0.4 mmol), EDC (92 mg, 0.48 mmol), DMAP (59 mg, 0.48 mmol), and vitamin D_2 (190 mg, 0.48 mmol) in abs. CH_2Cl_2 (8 ml) was kept at r.t. under N_2 at darkness for 3 h. Then the mixture was diluted with CH_2Cl_2 (80 ml) and washed with sat. $NaHCO_3$ soln. (2 × 30 ml), the aq. phase re-extracted with $CHCl_3$, the combined org. layer dried ($MgSO_4$) and evaporated, and the residue purified by FC (silica gel, 9 × 2 cm, toluene → toluene/AcOEt 1:1 + 2% MeOH → 1:1 + 3% MeOH): 250 mg (56%) of **5**. Amorphous solid. UV (MeOH): 272 (sh, 4.68), 266 (4.70). ^{1}H-NMR ($CDCl_3$): 8.77, 8.66 (2*s*, H–C(8), H–C(2)); 8.14–8.07 (*m*, 4 H *o* to NO_2, NH); 7.41–7.33 (*m*, 4 H *m* to NO_2); 6.10 (*d*, H–C(1′)); 6.16–6.00 (*dd*, H–C(6)(VitD$_2$), H–C(7)(VitD$_2$)); 5.66 (*m*, H–C(2′)); 5.15 (*m*, H–C(22)(VitD$_2$), H–C(23)(VitD$_2$)); 5.0, 4.75 (2*m*, 2 H–C(19)(VitD$_2$)); 4.82 (*m*, H–C(3)(VitD$_2$)); 4.55 (*m*, H–C(4′)); 4.5–4.2 (*m*, 2*t*, 2 OCH_2CH$_2$, 2 H–C(5′)); 3.12, 3.06 (2*t*, 2 OCH$_2$CH_2); 2.8–0.5 (*m*, C(O)CH_2CH_2C(O), 2 H–C(3′), 36 H (VitD$_2$)). Anal. calc. for $C_{60}H_{73}N_7O_{14}$ (1116.3): C 64.56, H 6.59, N 8.78; found: C 64.19, H 6.63, N 8.76.

5. *3′-Deoxy-N⁶,5′-O-bis[2-(4-nitrophenyl)ethoxycarbonyl]-2′-O-{[2-(retinyloxycarbonyl)ethyl]carbonyl}-adenosine* (**6**). As described in *Exper. 3*, with **2** [12] (287 mg, 0.45 mmol), succinic anhydride (54 mg, 0.54 mmol), and DMAP (71 mg, 0.59 mmol) in abs. CH_2Cl_2 (3 ml; 18 h). Then EDC (113 mg, 0.59 mmol) and vitamin A (169 mg, 0.59 mmol; 5 h, N_2, darkness). Workup with CH_2Cl_2 (80 ml) and sat. $NaHCO_3$ soln. (30 ml). FC (silica gel, 13.5 × 2 cm, toluene/AcOEt 1:1 → 1:1 + 2% MeOH → 1:1 + 3% MeOH → 1:1 + 4% MeOH): 337 mg (74%) of **6**. Amorphous solid. UV (CH_2Cl_2): 330 (4.67), 318 (sh, 4.62), 272 (sh, 4.61), 267 (4.63). ^{1}H-NMR ($CDCl_3$): 8.71, 8.18–8.10 (*s*, br. *m*, H–C(8), H–C(2), 4 H *o* to NO_2, NH); 7.45–7.36 (*m*, 4 H *m* to NO_2); 6.63, 6.27–6.04 (*m*, H–C(1′), 5 CH=C(retinyl)); 5.68 (*m*, H–C(2′)); 5.57 (*t*, H–C(14)(retinyl)); 4.73 (*d*, 2 H–C(15)); 4.63 (*m*, H–C(4′)); 4.53, 4.44 (2*t*, 2 OCH_2CH$_2$); 4.45–4.26 (*m*, 2 H–C(5′)); 3.15, 3.06 (2*t*, 2 OCH$_2$CH_2); 2.67 (*s*, *m*, H–C(3′), C(O)CH_2CH_2C(O)); 2.25 (*dd*, H–C(3′)); 2.1–1.5 (*m*, 3*s*, 2 H–C(4)(retinyl), 2 H–C(2)(retinyl), 2 H–C(3)(retinyl), Me–C(9)(retinyl), Me–C(13)(retinyl), Me–C(5)(retinyl)); 1.00 (*s*, 2 Me–C(1)(retinyl)). Anal. calc. for $C_{52}H_{59}N_7O_{14}$ (1006.1): C 62.08, H 5.91, N 9.75; found: C 62.06, H 6.06, N 9.63.

6. *3′-Deoxy-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]-2′-O-(2-ambo-α-tocopheryloxycarbonyl)adenosine* (**9**). Compound **3** (704 mg, 0.6 mmol) was stirred at r.t. in CH_2Cl_2/MeOH 4:1 (12 ml) containing 2% of TsOH · H_2O for 10 min. Then the mixture was diluted with $CHCl_3$ (150 ml) and washed with sat. $NaHCO_3$ soln. (2 × 70 ml), the aq. phase re-extracted with $CHCl_3$, and the combined org. layer dried ($MgSO_4$) and evaporated. FC (silica gel, 16.5 × 3 cm, $CHCl_3 \rightarrow CHCl_3 + 5\%$ MeOH) gave 484 mg of **9** which was dissolved in CH_2Cl_2 (2 ml) and precipitated in petroleum ether (100 ml) and co-evaporated with CH_2Cl_2: 428 mg (79%) of **9**. Amorphous solid. UV (CH_2Cl_2): 273 (sh, 4.38), 267 (4.43). ^{1}H-NMR ((D$_6$)DMSO): 10.62 (*s*, NH); 8.68, 8.62 (2*s*, *d*, H–C(8), H–C(2)); 8.14 (*d*, 2 H *o* to NO_2); 7.60 (*d*, 2 H *m* to NO_2); 6.32 (*d*, H–C(1′)); 5.72 (*m*, H–C(2′)); 5.11 (*t*, OH–C(5′)); 4.42 (*m*, H–C(4′)); 4.38 (*t*, OCH_2CH$_2$); 3.7, 3.55 (2*m*, 2 H–C(5′)); 3.09 (*t*, OCH$_2$CH_2); 2.65, 2.3 (2*m*, 2 H–C(3′)); 2.0–0.75 (*m*, 49 H (tocopheryl)). Anal. calc. for $C_{48}H_{69}N_6O_{10}$ (902.1): C 65.31, H 7.61, N 9.33; found: C 65.29, H 7.49, N 9.23.

7. *3′-Deoxy-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]-2′-O-{[2-(2-ambo-α-tocopheryloxycarbonyl)ethyl]carbonyl}adenosine* (**10**). As described in *Exper. 6*, with **4** (700 mg, 0.57 mmol) and CH_2Cl_2/MeOH 4:1 (11 ml) containing 2% of TsOH · H_2O (20 min). Workup with $CHCl_3$ (120 ml) and sat. $NaHCO_3$ soln. (2 × 60 ml). FC (silica gel, 15 × 2 cm, $CHCl_3 \rightarrow CHCl_3 + 4\%$ MeOH): 509 mg (93%) of **10**. Amorphous solid. UV (CH_2Cl_2): 273 (sh, 4.39), 267 (4.44). ^{1}H-NMR ((D$_6$)DMSO): 10.62 (*s*, NH); 8.65, 8.60 (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); 6.19 ('*s*', H–C(1′)); 5.86 (*m*, H–C(2′)); 5.06 (*t*, OH–C(5′)); 4.38 (*m*, *t*, H–C(4′), OCH_2CH$_2$); 3.65, 3.55 (2*m*, 2 H–C(5′)); 3.10 (*t*, OCH$_2$CH_2); 2.9, 2.75 (*m*, C(O)CH_2CH_2C(O)); 2.55, 2.1 (2*m*, 2 H–C(3′)); 2.0–0.75 (*m*, 49 H (tocopheryl)). Anal. calc. for $C_{52}H_{72}N_6O_{11}$ (957.2): C 65.25, H 7.58, N 8.78; found: C 65.12, H 7.62, N 8.83.

8. *3′-Deoxy-2′-*O*-{[2-(2,3-di-*O*-palmitoylglycer-1-yloxycarbonyl)ethyl]carbonyl}-N⁶-[2-(4-nitrophenyl)-ethoxycarbonyl]adenosine* (**11**). A mixture of **1** [12] (335 mg, 0.466 mmol), succinic anhydride (56 mg, 0.56 mmol), and DMAP (74 mg, 0.61 mmol) in abs. CH_2Cl_2 (2 ml) was kept at r.t. for 18 h. Then EDC (116 mg, 0.61 mmol) and 1,2-di-*O*-palmitoylglycerol [13] [15] (344 mg, 0.61 mmol) in abs. CH_2Cl_2 (2 ml) were added. The mixture was kept at r.t. for 5 h, then diluted with $CHCl_3$ (100 ml), and washed with sat. $NaHCO_3$ soln. (60 ml) and 10% citric acid (50 ml), the aq. phase re-extracted with $CHCl_3$, the combined org. layer dried ($MgSO_4$) and evaporated, and the crude product purified by FC (silica gel, 11 × 2 cm, toluene/AcOEt 1:1): **7**, contaminated with some 1,2-di-*O*-palmitoylglycerol. The crude **7** was then treated with CH_2Cl_2/MeOH 4:1 (14 ml) containing 2% of TsOH · H_2O for 25 min. Then the mixture was diluted with CH_2Cl_2 (100 ml) and washed with sat. $NaHCO_3$ soln. (2 × 50 ml), the aq. phase re-extracted with CH_2Cl, the combined org. layer dried ($MgSO_4$) and evaporated and the residue purified by FC (silica gel, 9 × 2 cm, toluene/AcOEt 1:1→1:1 + 7% MeOH): 400 mg (78%) of **11**. Amorphous solid. UV (CH_2Cl_2): 272 (sh, 4.37), 267 (4.42). ^{1}H-NMR ($CDCl_3$, 2 rotamers): 8.74, 8.33, 8.19, 8.07 (*s*, 3*d*, H–C(8), H–C(2), 2 H *o* to NO_2, NH); 7.45 (*d*, 2 H *m* to NO_2); 5.98 (*m*, H–C(1′)); 5.65 (*m*, H–C(2′)); 5.25 (*q*, H–C(2)(Glyc)); 5.05 (*m*, OH–C(5′)); 4.65–4.5 (*m*, H–C(4′), OCH_2CH_2); 4.35–4.05 (*m*, 2 H–C(1)(Glyc), 2 H–C(3)(Glyc), H–C(5′)); 3.65 (*m*, H–C(5′)); 3.17 (*t*, OCH_2CH_2); 2.93 (*m*, H–C(3′)); 2.65 (*m*, $C(O)CH_2CH_2C(O)$); 2.4–2.25 (*m*, 2 $CH_2(\alpha)$(Palm), H–C(3′)); 1.65 (2 $CH_2(\beta)$(Palm)); 1.25 (*m*, 48 H(Palm)); 0.88 (*t*, 2 Me(Palm)). Anal. calc. for $C_{58}H_{90}N_6O_{14}$ (1095.4): C 63.60, H 8.28, N 7.67; found: C 63.14, H 8.16, N 7.58.

9. *3′-Deoxy-2′-*O*-{[2-(2,3-di-*O*-hexadecylglycer-1-yloxycarbonyl)ethyl]carbonyl}-N⁶-[2-(4-nitrophenyl)-ethoxycarbonyl]adenosine* (**12**). As described in *Exper. 8*, with **1** [12] (501 mg, 0.7 mmol), succinic anhydride (84 mg, 0.84 mmol), and DMAP (111 mg, 0.91 mmol) in abs. CH_2Cl_2 (5 ml; 18 h). Then EDC (174 mg, 0.91 mmol) and 1,2-di-*O*-hexadecylglycerol [13] [16] (493 mg, 0.91 mmol) in abs. CH_2Cl_2 (5 ml; 5 h). Workup with $CHCl_3$ (120 ml), sat. $NaHCO_3$ soln. (60 ml), and 10% citric acid (80 ml). Purification by FC (silica gel, 5 × 3.5 cm, toluene/AcOEt 1:1→1:1 + 2% MeOH→1:1 + 3% MeOH): **8**, contaminated with some 1,2-di-*O*-hexadecylglycerol. The crude **8** was then treated with CH_2Cl_2/MeOH 4:1 (14 ml) containing 2% of TsOH · H_2O for 15 min. Workup with CH_2Cl_2 (150 ml) and sat. $NaHCO_3$ (2 × 100 ml). The residue was purified by FC (silica gel, 9 × 2 cm, toluene/AcOEt 1:1→1:1 + 2% MeOH→1:1 + 4% MeOH): 421 mg of **12** (56%). Amorphous solid. UV (CH_2Cl_2): 272 (sh, 4.38), 267 (4.44). ^{1}H-NMR ($CDCl_3$): 8.73, 8.21–8.07 (2*s*, *m*, H–C(8), H–C(2), 2 H *o* to NO_2, NH); 7.45 (*d*, 2 H *m* to NO_2); 5.99 (*m*, H–C(1′)); 5.62 (*m*, H–C(2′)); 5.00 (*m*, OH–C(5′)); 4.62–4.50 (*m*, H–C(4′), OCH_2CH_2); 4.35–4.05 (*m*, 2 H–C(5′), H–C(2)(Glyc)); 3.7–3.35, 3.15 (*m*, OCH_2CH_2, 2 H–C(1)(Glyc), 2 H–C(3)(Glyc), 2 $CH_2(\alpha)$(EL)); 2.95, 2.25 (2*m*, 2 H–C(3′)); 2.65 (*m*, $C(O)CH_2CH_2C(O)$); 1.7, 1.55, 1.3 (3*m*, 56 H(EL)); 0.85 (*t*, 2 Me(EL)). Anal. calc. for $C_{58}H_{94}N_6O_{12}$ (1067.4): C 65.26, H 8.88, N 7.87; found: C 65.06, H 8.76, N 7.65.

10. *3′-Deoxy-2′-*O*-(2-*ambo*-α-tocopheryloxycarbonyl)adenosine* (**13**). Compound **9** (318 mg, 0.35 mmol) was co-evaporated twice with abs. pyridine and then dissolved in abs. pyridine (3.5 ml). DBU (268 mg, 1.76 mmol) was added, the mixture kept at r.t. for 18 h, then AcOH (106 mg, 1.76 mmol) added, and the soln. evaporated. The residue was diluted with $CHCl_3$ (60 ml) and washed with sat. NaCl soln. (2 × 30 ml), the aq. phase re-extracted 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 × 2 cm, $CHCl_3$→$CHCl_3$ + 5% MeOH): 200 mg (94%) of **13**. Amorphous solid. UV (CH_2Cl_2): 275 (sh, 3.81), 260 (4.12). ^{1}H-NMR ((D_6)DMSO): 8.36, 8.13 (2*s*, H–C(8), H–C(2)); 7.34 (*s*, NH_2); 6.20 (*d*, H–C(1′)); 5.69 (*m*, H–C(2′)); 5.20 (*t*, OH–C(5′)); 4.40 (*m*, H–C(4′)); 3.69, 3.54 (2*m*, 2 H–C(5′)); 2.69, 2.40 (2*m*, 2 H–C(3′)); 2.10–0.75 (*m*, 49 H(tocopheryl)). Anal. calc. for $C_{40}H_{61}N_5O_6$ (708.0): C 67.86, H 8.68, N 9.89; found: C 67.76, H 8.59, N 9.74.

11. *3′-Deoxy-2′-*O*-{[2-(2-*ambo*-α-tocopheryloxycarbonyl)ethyl]carbonyl}adenosine* (**14**). As described in *Exper. 10*, with **10** (248 mg, 0.26 mmol), and DBU (197 mg, 1.3 mmol) in abs. pyridine (3 ml; 18 h), then AcOH (78 mg, 1.3 mmol). Workup with $CHCl_3$ (50 ml) and sat. NaCl soln. (2 × 20 ml). Purification by FC (silica gel, 16 × 2 cm, $CHCl_3$→$CHCl_3$ + 3% MeOH→$CHCl_3$ + 5% MeOH): 143 mg (72%) of **14**. Amorphous solid. UV (CH_2Cl_2): 275 (sh, 3.80), 259 (4.13). ^{1}H-NMR ((D_6)DMSO): 8.32, 8.12 (2*s*, H–C(8), H–C(2)); 7.32 (*s*, NH_2); 6.08 (*d*, H–C(1′)); 5.64 (*m*, H–C(2′)); 5.12 (*t*, OH–C(5′)); 4.31 (*m*, H–C(4′)); 3.61, 3.50 (2*m*, 2 H–C(5′)); 2.91, 2.74 (*m*, $C(O)CH_2CH_2C(O)$); 2.65–0.75 (*m*, 2 H–C(3′), 49 H (tocopheryl)). Anal. calc. for $C_{43}H_{65}N_5O_7$ (764.0): C 67.60, H 8.58, N 9.17; found: C 67.55, H 8.44, N 8.96.

12. *3′-Deoxy-2′-*O*-{[2-(ergocalciferyloxycarbonyl)ethyl]carbonyl}adenosine* (**15**). As described in *Exper. 10*, with **5** (180 mg, 0.16 mmol) and DBU (491 mg, 3.22 mmol) in abs. pyridine (6.5 ml; 17 h, N_2, darkness), then AcOH (193 mg, 3.22 mmol). Workup with CH_2Cl_2 (80 ml) and sat. $NaHCO_3$ soln. (2 × 40 ml). Purification by FC (silica gel, 5.5 × 2 cm, toluene/AcOEt 1:1→1:1 + 4% MeOH→1:1 + 6% MeOH): 104 mg (88%) of **15**. Amorphous solid. UV (MeOH): 262 (4.39). ^{1}H-NMR ((D_6)DMSO): 8.31, 8.12 (2*s*, H–C(8), H–C(2)); 7.31 (br. NH_2); 6.06 (*d*,

H–C(1′)); 6.20–5.95 (*dd*, H–C(6)(VitD$_2$), H–C(7)(VitD$_2$)); 5.60 (*m*, H–C(2′)); 5.20 (*m*, H–C(22)(VitD$_2$), H–C(23)(VitD$_2$), OH–C(5′)); 5.06, 4.7 (2*m*, 2 H–C(19)(VitD$_2$)); 4.8 (*m*, H–C(3)(VitD$_2$)); 4.31 (*m*, H–C(4′)); 3.65–3.45 (*m*, 2 H–C(5′)); 2.85–0.5 (*m*, C(O)CH_2CH_2C(O), 2 H–C(3′), 36 H(VitD$_2$)). Anal. calc. for $C_{42}H_{59}N_5O_6$ (730.0): C 69.11, H 8.15, N 9.59; found: C 68.57, H 8.16, N 9.41.

13. *3′-Deoxy-2′-*O*-{[2-(retinyloxycarbonyl)ethyl]carbonyl}adenosine* (**16**). As described in *Exper. 10*, with **6** (151 mg, 0.15 mmol) and 0.5M DBU in abs. MeCN (4.56 ml; 18 h), then 2M AcOH (1.14 ml). Workup with $CHCl_3$ (80 ml) and H_2O (2 × 30 ml). Purification by FC (silica gel, 10 × 1 cm, $CHCl_3$→$CHCl_3$ + 2% MeOH→$CHCl_3$ + 5% MeOH→$CHCl_3$ + 10% MeOH): 83 mg (89%) of **16**. Amorphous solid. UV (CH_2Cl_2): 330 (4.60), 320 (sh, 4.56), 259 (4.28). ^{1}H-NMR ($CDCl_3$): 8.25, 7.89 (2*s*, H–C(8), H–C(2)); 6.65–5.5 (*m*, H–C(1′), H–C(2′), 5 CH=C(retinyl), OH–C(5′), H–C(14)(retinyl)); 4.75 (*d*, 2 H–C(15)); 4.55 (*m*, H–C(4′)); 4.60, 3.65 (*m*, 2 H–C(5′)); 2.65 (br. C(O)CH_2CH_2C(O)); 2.95, 2.15 (2*m*, 2 H–C(3′)); 2.05–1.5 (*m*, 2*s*, 2 H–C(4)(retinyl), 2 H–C(2)(retinyl), Me–C(3)(retinyl), Me–C(9)(retinyl), Me–C(13)(retinyl), Me–C(5)(retinyl)); 1.00 (*s*, 2 Me–C(1)(retinyl)). Anal. calc. for $C_{34}H_{45}N_5O_6 \cdot 1/8\ CHCl_3$ (634.7): C 64.58, H 7.12, N 11.03; found: C 64.21, H 7.23, N 10.59; a correct C, H, N anal. could not be obtained, due to some instability of **16** against light and oxidation.

14. *3′-Deoxy-2′-*O*-{[2-(2,3-di-*O*-palmitoylglycer-1-yloxycarbonyl)ethyl]carbonyl}adenosine* (**17**). As described in *Exper. 10*, with **11** (340 mg, 0.31 mmol) and DBU (236 mg, 1.55 mmol) in abs. pyridine (3 ml; 18 h), then AcOH (93 mg, 1.55 mmol). Workup with $CHCl_3$ (100 ml) and sat. NaCl soln. (2 × 50 ml). Purification by FC (silica gel, 10.5 × 2 cm, $CHCl_3$→$CHCl_3$ + 6% MeOH): 256 mg (92%) of **17**. Amorphous solid. UV (CH_2Cl_2): 259 (4.11). ^{1}H-NMR ($CDCl_3$, 2 rotamers): 8.27, 7.84 (2*s*, H–C(8), H–C(2)); 5.90 (*m*, H–C(1′)); 5.82 (br. NH_2); 5.62 (*m*, H–C(2′)); 5.24 (*q*, H–C(2)(Glyc)); 4.54 (*t*, OH–C(5′)); 4.29–4.05 (*m*, H–C(4′), 2 H–C(1)(Glyc), 2 H–C(3)(Glyc), H–C(5′)); 3.65 (*m*, H–C(5′)); 2.90 (*m*, H–C(3′)); 2.61 (*m*, C(O)CH_2CH_2C(O)); 2.28 (*m*, 2 CH_2(α)(Palm), H–C(3′)); 1.57 (*m*, 2 CH_2(β)(Palm)); 1.22 (*m*, 48 H(Palm)); 0.85 (*t*, 2 Me(Palm)). Anal. calc. for $C_{49}H_{83}N_5O_{10}$ (902.2): C 65.23, H 9.27, N 7.76; found: C 65.58, H 9.12, N 7.56.

15. *3′-Deoxy-2′-*O*-{[2-(2,3-di-*O*-hexadecylglycer-1-yloxycarbonyl)ethyl]carbonyl}adenosine* (**18**). As described in *Exper. 10*, with **12** (300 mg, 0.28 mmol) and DBU (320 mg, 2.1 mmol) in abs. MeCN/pyridine 2:1 (4.2 ml; 18 h), then AcOH (126 mg, 2.1 mmol). Workup with $CHCl_3$ (100 ml) and 10% citric acid soln. (50 ml). Purification by FC (silica gel, 13.5 × 2 cm, $CHCl_3$→$CHCl_3$ + 5% MeOH): 202 mg (82%) of **18**. Amorphous solid. UV (CH_2CH_2): 259 (4.14). ^{1}H-NMR ($CDCl_3$): 8.31, 7.87 (2*s*, H–C(8), H–C(2)); 6.0–5.8 (*m*, H–C(1′), OH–C(5′), NH_2); 5.64 (*m*, H–C(2′)); 4.57 (*m*, H–C(4′)); 4.2–4.0 (*m*, 2 H–C(5′), H–C(2)(Glyc)); 3.65–3.3 (*m*, 2 H–C(1)(Glyc), 2 H–C(3)(Glyc), 2 CH_2(α)(EL)); 2.95, 2.15 (2*m*, 2 H–C(3′)); 2.65 (*m*, C(O)CH_2CH_2C(O)); 1.7–1.2 (*m*, 56 H(EL)); 0.88 (*t*, 2 Me(EL)). Anal. calc. for $C_{49}H_{87}N_5O_8$ (874.3): C 67.32, H 10.03, N 8.01; found: C 67.33, H 9.85, N 8.09.

16. *3′-Deoxy-*N^6*,2′-*O*-bis[2-(4-nitrophenyl)ethoxycarbonyl]-5′-*O*-{[2-(2-*ambo*-α-tocopheryloxycarbonyl)-ethyl]carbonyl}adenosine* (**20**). As described in *Exper. 3*, with **19** [12] (192 mg, 0.3 mmol), succinic anhydride (36 mg, 0.36 mmol), and DMAP (48 mg, 0.39 mmol) in abs. CH_2Cl_2 (3 ml; 18 h). Then EDC (75 mg, 0.39 mmol) and vitamin E (181 mg, 0.44 mmol; 5 h), more EDC (29 mg, 0.15 mmol), vitamin E (65 mg, 0.15 mmol), and DMAP (18 mg, 0.15 mmol; 18 h). Workup with $CHCl_3$ (70 ml) and 10% citric acid soln. (40 ml). FC (silica gel, 13.5 × 2 cm, toluene/AcOEt 1:1→1:1 + 4% MeOH→1:1 + 5% MeOH): 260 mg (75%) of **20**. Amorphous solid. UV (CH_2Cl_2): 272 (sh, 4.51), 266 (4.54). ^{1}H-NMR ($CDCl_3$): 8.73, 8.31, 8.20–8.13 (2*s*, *m*, H–C(8), H–C(2), 4 H *o* to NO_2, NH); 7.45–7.38 (*m*, 4 H *m* to NO_2); 6.10 ('*s*', H–C(1′)); 5.67 (*m*, H–C(2′)); 4.65 (*m*, H–C(4′)); 4.5–4.25 (*m*, 2 OCH_2CH_2, 2 H–C(5′)); 3.2–2.5 (*m*, 2 OCH_2CH_2, C(O)CH_2CH_2C(O)); 2.25 (*m*, H–C(3′)); 2.05–0.85 (*m*, H–C(3′), 49 H(tocopheryl)). Anal. calc. for $C_{61}H_{79}N_7O_{15}$ (1150.3): C 63.69, H 6.92, N 8.52; found: C 63.60, H 7.00, N 8.17.

17. *3′-Deoxy-5′-*O*-{[2-(ergocalciferyloxycarbonyl)ethyl]carbonyl}-*N^6*,2′-*O*-bis-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**21**). As described in *Exper. 3*, with **19** [12] (255 mg, 0.4 mmol), succinic anhydride (48 mg, 0.48 mmol), and DMAP (64 mg, 0.52 mmol) in abs. CH_2Cl_2 (8 ml; 18 h). Then EDC (100 mg, 0.52 mmol) and vitamin D_2 (206 mg, 0.52 mmol; 3 h, darkness). Workup with $CHCl_3$ (80 ml) and sat. $NaHCO_3$ soln. (40 ml). Purification by FC (silica gel, toluene/AcOEt 1:1→1:1 + 4% MeOH→1:1 + 5% MeOH): 354 mg (79%) of **21**. Amorphous solid. UV (CH_2Cl_2): 272 (sh, 4.66), 267 (4.68). ^{1}H-NMR ($CDCl_3$): 8.74, 8.21–8.14 (*s*, *m*, H–C(8), H–C(2), 4 H *o* to NO_2, NH); 7.46–7.38 (*m*, 4 H *m* to NO_2); 6.12 (*d*, H–C(1′)); 6.20, 6.00 (2*d*, H–C(6)(VitD$_2$), H–C(7)(VitD$_2$)); 5.70 (*m*, H–C(2′)); 5.19 (*m*, H–C(22)(VitD$_2$), H–C(23)(VitD$_2$)); 5.04, 4.82 (2*m*, 2 H–C(Vit19)(D$_2$)); 4.92 (*m*, H–C(3)(VitD$_2$)); 4.66–4.25 (*m*, H–C(4′), 2 OCH_2CH_2, 2 H–C(5′)); 3.25–3.1 (*m*, 2 OCH_2CH_2); 2.9–0.5 (*m*, C(O)CH_2CH_2C(O), 2 H–C(3′), 36 H(VitD$_2$)). Anal. calc. for $C_{60}H_{73}N_7O_{14}$ (1116.28): C 64.56, H 6.59, N 8.78; found: C 64.18, H 6.55, N 8.89.

18. *3′-Deoxy-5′-*O*-{[2-(retinyloxycarbonyl)ethyl]carbonyl}-*N^6*,2′-*O*-bis[2-(4-nitrophenyl)ethoxycarbonyl]-adenosine* (**22**). As described in *Exper. 3*, with **19** [12] (287 mg, 0.45 mmol), succinic anhydride (54 mg, 0.54 mmol), and DMAP (71 mg, 0.59 mmol) in abs. CH_2Cl_2 (3 ml; 18 h). Then EDC (113 mg, 0.59 mmol) and vitamin A (169 mg, 0.59 mmol; 5 h, N_2, darkness). Workup with CH_2Cl_2 (80 ml) and sat. $NaHCO_3$ soln. (30 ml). FC (silica gel, 13.5 × 2 cm, toluene/AcOEt 1:1→1:1 + 2% MeOH→1:1 + 3% MeOH): 293 mg (65%) of **22**. Amorphous solid. UV (CH_2Cl_2): 331 (4.67), 318 (sh, 4.62), 272 (sh, 4.61), 267 (4.63). ^{1}H-NMR ($CDCl_3$): 8.72, 8.18–8.13 (*s*, *m*, H–C(8), H–C(2), 4 H *o* to NO_2); 7.43–7.35 (*m*, 4 H *m* to NO_2); 6.10 (*d*, H–C(1′)); 5.67 (*m*, H–C(2′)); 6.61, 6.15, 6.1–6.0 (*m*, 5 CH=C(retinyl)); 5.55 (*t*, H–C(14)(retinyl)); 4.70 (*d*, 2 H–C(15)); 4.60 (*m*, H–C(4′)); 4.51, 4.40 (2*t*, 2 OCH_2CH_2); 4.45–4.25 (*m*, 2 H–C(5′)); 3.15–3.0 (*m*, 2 OCH_2CH_2); 2.65–2.5 (*m*, H–C(3′), $C(O)CH_2CH_2C(O)$); 2.2 (*dd*, H–C(3′)); 2.0–1.8 (*m*, 2*s*, 2 H–C(4)(retinyl), 2 H–C(2)(retinyl), 2 H–C(3)(retinyl), Me–C(9)(retinyl), Me–C(13)(retinyl), Me–C(5)(retinyl)); 0.99 (*s*, 2 Me–C(1)(retinyl)). Anal. calc. for $C_{52}H_{59}N_7O_{14}$ (1006.1): C 62.08, H 5.91, N 9.75; found: C 62.08, H 5.90, N 9.46.

19. *3′-Deoxy-*N^6*,2′-*O*-bis[2-(4-nitrophenyl)ethoxycarbonyl]-5′-*O*-{[2-(2,3-di-*O*-palmitoylglycer-1-yloxycarbonyl)ethyl]carbonyl}adenosine* (**23**). As described in *Exper. 3*, with **19** [12] (383 mg, 0.6 mmol), succinic anhydride (72 mg, 0.72 mmol), and DMAP (95 mg, 0.78 mmol) in abs. CH_2Cl_2 (5 ml; 18 h). Then EDC (150 mg, 0.78 mmol) and 1,2-di-*O*-palmitoylglycerol [13] [15] (478 mg, 0.84 mmol; 2 h). Workup with $CHCl_3$ (100 ml) and 10% citric acid soln. (50 ml). FC (silica gel, 13 × 2 cm, toluene/AcOEt 1:1→1:1 + 5% MeOH): 575 mg (74%) of **23**. Amorphous solid. UV (CH_2Cl_2): 272 (sh, 4.50), 267 (4.54). ^{1}H-NMR ($CDCl_3$): 8.74, 8.22–8.15 (*s*, *m*, H–C(8), H–C(2), 4 H *o* to NO_2, NH); 7.47–7.39 (*m*, 4 H *m* to NO_2); 6.13 (*m*, H–C(1′)); 5.69 (*m*, H–C(2′)); 5.26 (*q*, H–C(2)(Glyc)); 4.65 (*m*, H–C(4′)); 4.54–4.10 (*m*, 2*t*, 2 OCH_2CH_2, 2 H–C(1)(Glyc), 2 H–C(3)(Glyc), 2 H–C(5′)); 3.19–3.09 (*m*, 2 OCH_2CH_2); 2.7–2.6 (*m*, $C(O)CH_2CH_2C(O)$, H–C(3′)); 2.4–2.25 (*m*, 2 $CH_2(\alpha)$(Palm), H–C(3′)); 1.8–1.6 (*m*, 2 $CH_2(\beta)$(Palm)); 1.25 (*m*, 48 H(Palm)); 0.88 (*t*, 6 H, Me(Palm)). Anal. calc. for $C_{67}H_{97}N_7O_{18}$ (1288.5): C 62.45, H 7.59, N 7.61; found: C 62.40, H 7.61, N 7.58.

20. *3′-Deoxy-2′-*O*-{[2-(2,3-di-*O*-hexadecylglycer-1-yloxycarbonyl)ethyl]carbonyl}-*N^6*,2′-*O*-bis[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**24**). As described in *Exper. 3*, with **19** [12] (319 mg, 0.5 mmol), succinic anhydride (60 mg, 0.6 mmol), and DMAP (79 mg, 0.65 mmol) in abs. CH_2Cl_2 (5 ml; 18 h). Then EDC (125 mg, 0.65 mmol) and 1,2-di-*O*-hexadecylglycerol [13] [16] (352 mg, 0.65 mmol) in abs. CH_2Cl_2 (5 ml; 5 h). Workup with $CHCl_3$ (120 ml), sat. $NaHCO_3$ soln. (60 ml) and 10% citric acid soln. (80 ml). FC (silica gel, 13 × 2 cm, toluene/AcOEt 1:1→1:1 + 2% MeOH→1:1 + 4% MeOH): 385 mg (61%) of **24**. Amorphous solid. UV (CH_2Cl_2): 272 (sh, 4.51), 267 (4.55). ^{1}H-NMR ($CDCl_3$): 8.74, 8.21–8.15 (*s*, *m*, H–C(8), H–C(2), 4 H *o* to NO_2, NH); 7.45 (2*d*, 4 H *m* to NO_2); 6.13 (‘*s*’, H–C(1′)); 5.69 (*m*, H–C(2′)); 4.62 (*m*, H–C(4′)); 4.54, 4.46 (2*t*, 2 OCH_2CH_2); 4.35–4.0 (*m*, 2 H–C(5′), H–C(2)(Glyc)); 3.65–3.4 (*m*, 2 H–C(1)(Glyc), 2 H–C(3)(Glyc), 2 $CH_2(\alpha)$(EL)); 3.2–3.05 (*m*, 2 OCH_2CH_2); 2.7–2.55 (*m*, H–C(3′), $C(O)CH_2CH_2C(O)$); 2.25 (*m*, H–C(3′)); 1.75, 1.5, 1.25 (3*m*, 56 H(EL)); 0.88 (*t*, 2 Me(EL)). Anal. calc. for $C_{67}H_{101}N_7O_{16}$ (1260.6): C 63.84, H 8.08, N 7.78; found: C 63.97, H 8.12, N 7.75.

21. *3′-Deoxy-5′-*O*-{[2-(2-*ambo*-α-tocopheryloxycarbonyl)ethyl]carbonyl}adenosine* (**25**). As described in *Exper. 10*, with **20** (400 mg, 0.35 mmol) and DBU (529 mg, 3.5 mmol) in abs. pyridine (7 ml; 17 h), then AcOH (209 mg, 3.5 mmol). Workup with $CHCl_3$ (100 ml) and sat. NaCl/$NaHCO_3$ soln. (2 × 40 ml). Purification by FC (silica gel, 10 × 2 cm, $CHCl_3$→$CHCl_3$ + 5% MeOH→$CHCl_3$ + 7% MeOH): 248 mg (93%) of **25**. Amorphous solid. UV (CH_2Cl_2): 259 (4.11). ^{1}H-NMR ($CDCl_3$): 8.31, 8.10 (2*s*, H–C(8), H–C(2)); 6.05 (br. *s*, NH_2); 5.94 (‘*s*’, H–C(1′)); 5.6 (br. *s*, OH–C(2′)); 4.67 (*m*, H–C(2′), H–C(4′)); 4.40 (*m*, 2 H–C(5′)); 3.0–0.75 (*m*, $C(O)CH_2CH_2C(O)$, 2 H–C(3′), 49 H (tocopheryl)). Anal. calc. for $C_{43}H_{65}N_5O_7 \cdot 1/4H_2O$(764.0): C 67.20, H 8.59, N 9.11; found: C 67.01, H 8.36, N 9.20.

22. *3′-Deoxy-5′-*O*-{[2-(ergocalciferyloxycarbonyl)ethyl]carbonyl}adenosine* (**26**). As described in *Exper. 10*, with **21** (250 mg, 0.22 mmol) and DBU (682 mg, 4.48 mmol) in abs. pyridine (9 ml; 17 h, N_2, darkness), then AcOH (269 mg, 4.48 mmol). Workup with CH_2Cl_2 (100 ml) and sat. $NaHCO_3$ soln. (2 × 50 ml). Purification by FC (silica gel, 5 × 2 cm, toluene/AcOEt 1:1→1:1 + 6% MeOH): 156 mg (95%) of **26**. Amorphous solid. UV (MeOH): 262 (4.39). ^{1}H-NMR ($CDCl_3$): 8.31, 8.13 (2*s*, H–C(8), H–C(2)); 6.20–5.95 (*dd*, H–C(6)($VitD_2$); H–C(7)($VitD_2$)); 5.95 (*d*, H–C(1′)); 5.85 (br. *s*, NH_2); 5.60 (*m*, H–C(2′)); 5.25 (*m*, H–C(22)($VitD_2$)); H–C(23)($VitD_2$)); 5.15, 4.8 (2*m*, 2 H–C(19) ($VitD_2$)); 5.0 (*m*, H–C(3)($VitD_2$)); 4.55 (*m*, H–C(4′)); 4.1, 3.65 (2*m*, 2 H–C(5′)); 3.1–0.6 (*m*, $C(O)CH_2CH_2C(O)$, 2 H–C(3′), 36 H ($VitD_2$)). Anal. calc. for $C_{42}H_{59}N_5O_6$ (730.0): C 69.11, H 8.15, N 9.59; found: C 68.57, H 8.16, N 9.41.

23. *3′-Deoxy-5′-*O*-{[2-(retinyloxycarbonyl)ethyl]carbonyl}adenosine* (**27**). As described in *Exper. 10*, with **22** (73 mg, 72.6 μmol) and DBU (111 mg, 0.726 mmol) in abs. pyridine (1.5 ml; 18 h, N_2, darkness), then AcOH (44 mg, 0.726 mmol). Workup with $CHCl_3$ (40 ml) and H_2O (15 ml). Purification by FC (silica gel, 9 × 1 cm,

$CHCl_3 \rightarrow CHCl_3$ + 2% MeOH$\rightarrow CHCl_3$ + 5% MeOH): 38 mg (84%) of **27**. Amorphous solid. UV (CH_2Cl_2): 330 (4.59), 320 (sh, 4.55), 259 (4.28). ^{1}H-NMR ($CDCl_3$): 8.29, 8.05 (2*s*, H–C(8), H–C(2)); 6.62, 6.3–5.98 (*m*, H–C(1′), H–C(2′), 5 CH=C(retinyl), OH–C(2′)); 5.55 (*t*, H–C(14)(retinyl)); 4.78 (*m*, 2 H–C(15), H–C(4′)); 4.4 (*m*, 2 H–C(5′)); 2.65 (br. *s*, C(O)CH_2CH_2C(O)); 2.25 (*m*, 2 H–C(3′)); 2.05–1.5 (*m*, 2*s*, 2 H–C(4)(retinyl), 2 H–C(2)(retinyl), 2 H–C(3)(retinyl), Me–C(9)(retinyl), Me–C(13)(retinyl), Me–C(5)(retinyl)); 1.00 (*s*, 2 Me–C(1)(retinyl)). A correct C,H,N anal. could not be obtained, due to some instability of **27** against light and oxidation.

24. *3′-Deoxy-5′-*O*-{[2-(2,3-di-*O*-palmitoylglycer-1-yloxycarbonyl)ethyl]carbonyl}adenosine* (**28**). As described in *Exper. 10*, with **23** (400 mg, 0.31 mmol) and DBU (473 mg, 3.1 mmol) in abs. pyridine (6.2 ml; 18 h), then AcOH (186 mg, 3.1 mmol). Workup with $CHCl_3$ (100 ml) and sat. NaCl/$NaHCO_3$ soln. (2 × 40 ml). Purification by FC (silica gel, 8.5 × 2 cm, $CHCl_3 \rightarrow CHCl_3$ + 5% MeOH$\rightarrow CHCl_3$ + 8% MeOH$\rightarrow CHCl_3$ + 10% MeOH): 275 mg (98%) of **28**. Amorphous solid. UV (CH_2Cl_2): 259 (4.12). ^{1}H-NMR ($CDCl_3$): 8.32, 8.08 (2*s*, H–C(8), H–C(2)); 5.92–5.83 (*m*, H–C(1′), NH_2); 5.27 (*q*, H–C(2)(Glyc)); 4.77 (*m*, H–C(2′), H–C(4′)); 4.45–4.1 (*m*, 2 H–C(1)(Glyc), 2 H–C(3)(Glyc), 2 H–C(5′)); 2.63 (*m*, C(O)CH_2CH_2C(O)); 2.35 (*m*, 2 $CH_2(\alpha)$(Palm), 2 H–C(3′)); 1.7–1.5 (*m*, 2 $CH_2(\beta)$(Palm)); 1.25 (*m*, 48 H(Palm)); 0.88 (*t*, 2 Me(Palm)). Anal. calc. for $C_{49}H_{83}N_5O_{10}$ (902.2): C 65.23, H 9.27, N 7.76; found: C 65.22, H 9.18, N 7.55.

25. *3′-Deoxy-5′-*O*-{[2-(2,3-di-*O*-hexadecylglycer-1-yloxycarbonyl)ethyl]carbonyl}adenosine* (**29**). As described in *Exper. 10*, with **24** (303 mg, 0.24 mmol) and DBU (365 mg, 2.4 mmol) in abs. pyridine (4.8 ml; 18 h), then AcOH (144 mg, 2.4 mmol). Workup with CH_2Cl_2 (80 ml) and sat. $NaHCO_3$ soln. (2 × 40 ml). Purification by FC (silica gel, 9 × 2 cm, $CHCl_3 \rightarrow CHCl_3$ + 1% MeOH$\rightarrow CHCl_3$ + 2% MeOH$\rightarrow CHCl_3$ + 5% MeOH): 165 mg (79%) of **29**. Amorphous solid. UV (CH_2Cl_2): 259 (4.14). ^{1}H-NMR ($CDCl_3$): 8.32, 8.1 (2*s*, H–C(8), H–C(2)); 6.22 (br. *s*, NH_2); 6.03 (*m*, H–C(1′)); 4.95 (*m*, H–C(2′), OH–C(2′)); 4.45–4.15 (*m*, H–C(4′), 2 H–C(5′), H–C(2)(Glyc)); 3.7–3.35 (*m*, 2 H–C(1)(Glyc), 2 H–C(3)(Glyc), 2 $CH_2(\alpha)$(EL)); 2.67 (*m*, C(O)CH_2CH_2(O)); 2.25 (*m*, 2 H–C(3′)); 1.6–1.2 (*m*, 56 H(EL)); 0.88 (‘*t*’, 2 Me(EL)). Anal. calc. for $C_{49}H_{87}N_5O_8 \cdot 1/4H_2O$ (883.3): C 66.97, H 10.04, N 7.97; found: C 66.53, H 9.74, N 8.22.

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