Nucleotides

Part LV[1])

Synthesis and Application of a Novel Linker for Solid-Phase Synthesis of Modified Oligonucleotides

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Various bifunctional amino-protecting groups such as the phthaloyl, succinyl, and glutaryl group were investigated as potential linker molecules for attachment to solid-support materials. Pentane-1,3,5-tricarboxylic acid 1,3-anhydride (**16**) offered the best properties and reacted with the amino groups of differently sugar-protected adenosine (see **20** and **22**), cytidine (see **29**), and guanosine derivatives (see **32**) to the corresponding 2-(2-carboxyethyl)glutaryl derivatives **23**, **24**, **30**, and **33**. The usefulness of the new linker-type molecules was demonstrated by the solid-support synthesis of the potentially antivirally active 3′-deoxyadenylyl-(2′–5′)-2′-adenylic acid 2′-{2-[(adenin-9-yl)methoxy]ethyl} ester (**38**) starting from the 2′-end with N^6,N^6-[2-(2-carboxyethyl)glutaryl]-9-{{2-[(4,4′-dimethoxytrityl)oxy]ethoxy}methyl}adenine (**12**).

1. Introduction. – In 1963 *Bruce Merrifield* developed the principle of solid-phase synthesis, allowing to produce chemically oligopeptides by this simple and ingenious technique [2]. Some years later, this very efficient method was applied to the synthesis of oligonucleotides [3][4] and is ever since used in the modern machine-aided approach in DNA synthesizers [5]. Today, almost all synthetic oligonucleotides are prepared by solid-phase phosphoramidite techniques [6–10] from the 3′-end towards 5′-direction due to the easy accessibility of the common 5′-*O*-(4,4′-dimethoxytrityl)nucleoside 3′-phosphoramidites as monomeric building blocks. Usually, the 3′-terminus is attached by means of a linker arm to a solid support consisting mostly of controlled pore glass (CPG) beads [9] or cross-linked polystyrene polymers [11]. The most common linkers are the succinyl [12] and oxalyl [13] residues forming an ester linkage with the sugar moiety and an amide bond to the solid support. This well-established approach works perfectly and is of general application for most purposes in oligonucleotide synthesis. In cases, however, where special modifications at the 3′-terminus are required without having an additional OH function available, such as in 2′,3′-dideoxynucleosides or (ω-hydroxyalkyl)-pyrimidines and -purines, a new type of linker system is needed to connect the first unit of the oligonucleotide sequence *via* the nucleobase to the solid phase. We have especially been interested in the automated synthesis of potential antivirally active modified (2′–5′)-oligoadenylates [14–16] since adenylyl-(2′–5′)-2′-adenylic acid 2′-{2-[(adenin-9-yl)methoxy]ethyl} ester [17][18] exhibits a broad-spectrum antiviral activity [19]. A new linker

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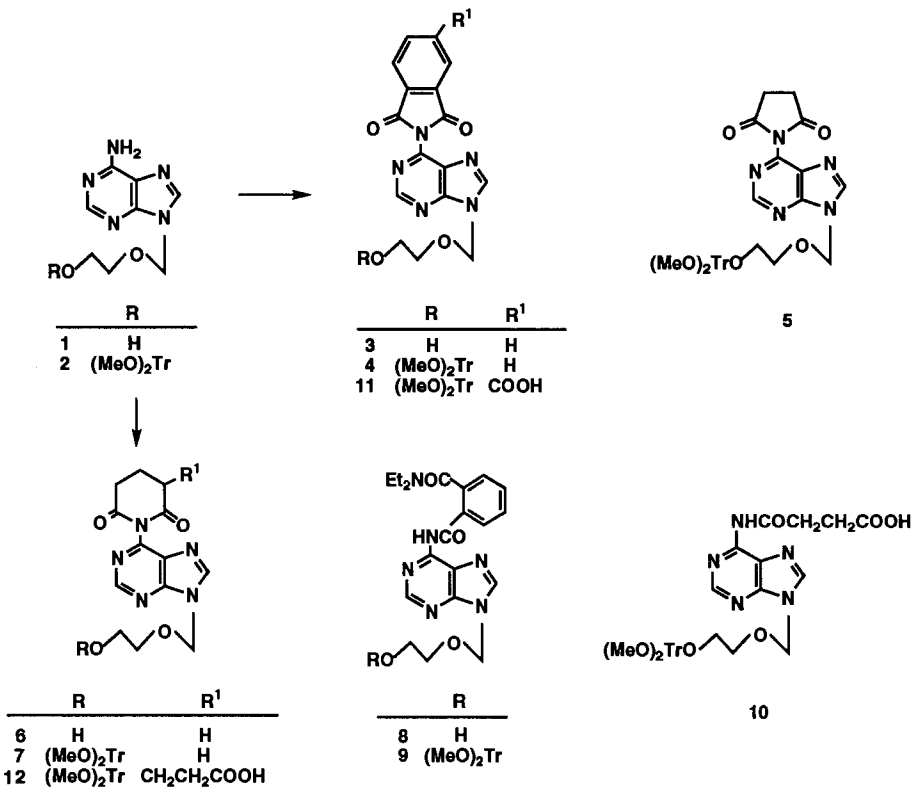
type connecting the 6-amino group of the adenine moiety at the 2′-end with the solid support had to be developed to suit the chemical requirements. Based upon studies of *Hata et al.* [20][21] who introduced the phthaloyl group into the adenosine series (→ phthalimidopurines), we found that the succinyl (→ succinimidopurines) and especially the glutaryl functions (→ glutarimidopurines) show greater chemical stabilities and are, therefore, more suitable for the anticipated purpose.

2. Synthesis and Discussion. – In model studies, 9-[(2-hydroxyethoxy)methyl]adenine (**1**) [22] was first treated with phthaloyl or glutaryl chloride to give 6-phthalimido- (**3**) and 6-glutarimido-9-[(2-hydroxyethoxy)methyl]-9*H*-purine (**6**), respectively, but reactions of 9-{{2-[(4,4′-dimethoxytrityl)oxy]ethoxy}methyl}adenine (**2**) with phthalic, succinic, or glutaric anhydride were more straightforward and led to the corresponding 6-imidopurine derivatives **4**, **5**, and **7**, respectively, in moderate-to-good yields after chromatographic workup (*Scheme 1*). Ring opening of the imido functions by Et_2NH was performed under conditions usually applied in coupling reactions to load solid-support materials and revealed that only the phthalimido derivatives **3** and **4** reacted in the expected manner to N^6-[2-(diethylcarbamoyl)benzoyl]-9-[(2-hydroxyethoxy)methyl]adenine (**8**) and its dimethoxytrityl derivative **9** whereas the succimido and glutarimido analogs **5**–**7**

Scheme 1

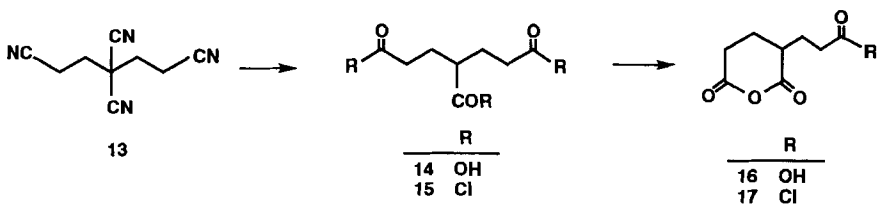


turned out to be too stable for this type of modification. The succinimido ring in **5** could be opened by a mixture of Et_3N/pyridine/H_2O 2:2:1 to give 9-{{2-[(4,4′-dimethoxytrityl)oxy]ethoxy}methyl}-N^6,N^6-succinyladenine (**10**) in almost quantitative yield. Unfortunately, all coupling experiments of the terminal carboxy group of **10** with amino functions of various solid-support materials failed since the intramolecular cyclization back to the imido structure proved to be faster and, therefore, the predominant reaction. Despite the fact that the imido derivatives could not be applied in the anticipated manner, they turned out to be valuable model substances to study the conditions of cleavage from the purine moiety, in general. Thus, 9-[2-(hydroxyethoxy)methyl]-N^6,N^6-phthaloyladenine (**3**) could be deprotected to **1** by NH_3/H_2O/MeOH 1:2:2 in a clean reaction within 10 min at room temperature, and in a similar manner, **10** was deblocked by $MeNH_2$/H_2O/MeOH 1:2:2 to give **2**.

From these results, it was obvious that either a long-chain dicarboxylic acid, which does not show intramolecular cyclization or a new type of imide carrying an additional carboxy group, will solve the linker problem. The first approach starting from octanedioic acid was discarded after some preliminary experiments, due to the fact that the 2-cyanoethyl and 2-(4-nitrophenyl)ethyl monoesters could not be prepared in pure form. More successful, however, was the reaction of **2** with trimellitic acid anhydride (= benzene-1,2,4-tricarboxylic acid 1,2-anhydride) which led in pyridine/Et_3N to N^6,N^6-(4-carboxyphthaloyl)-9-{{2-[(4,4′-dimethoxytrityl)oxy]ethoxy}methyl}adenine (**11**) in 70% isolated yield. This product could be coupled with the amino functions of a modified CPG and *TentaGel* solid-support material in the usual manner leading to a loading of 30 and 60 μmol/g, respectively. Stability tests of these loaded supports with 0.5M DUB (1,8-diazabicyclo[5.4.0]undec-7-ene) in various aprotic solvents like MeCN, CH_2Cl_2, THF, and pyridine, however, revealed, unexpectedly, that the phthaloyl residue was not stable under these conditions. Comparative model reactions of **4**, **5**, and **7** in 0.5M DBU/MeCN told us that the phthalimido derivative **4** is the most labile compound of this series showing a complete cleavage to **2** within 5 h, whereas **7** turned out to be stable, and **5** offered intermediate stability.

The consequence of these results was the plan to protect **2** with pentane-1,3,5-tricarboxylic acid 1,3-anhydride (**16**), *i.e.*, as imide **12** carrying, like **11**, also an additional carboxy group. Anhydride **16** was prepared from pentane-1,3,3,5-tetracarbonitrile (**13**) *via* pentane-1,3,5-tricarboxylic acid (**14**) [23] (*Scheme 1*). The cyclization of **14** into anhydride **16** could, however, not been achieved by vacuum sublimation as described in the literature, but treatment with 1.1 mol-equiv. of $SOCl_2$ worked well (88% yield of **16**). Excess of $SOCl_2$ led to 2-(3-chloro-3-oxopropyl)pentanedioic acid 1,5-anhydride (**17**), and from its further reaction with PCl_5, pentane-1,3,5-tricarbonyl trichloride (**15**) [24]

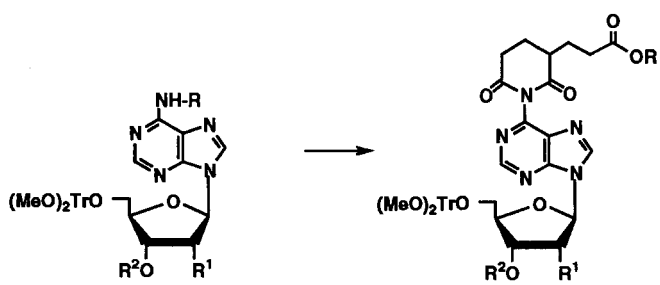
Scheme 2



could be obtained. Finally, acylation of **2** with **16** was a straightforward reaction leading to the new linker N^6,N^6-[2-(2-carboxyethyl)glutaryl]-9-{{2-(4,4'-dimethoxytrityl)oxy]-ethoxy}methyl}adenine (**12**) in 86% yield.

The new linker system was then also applied to adenosine, 2'-deoxyadenosine, 2'-deoxycytidine, and 2'-deoxyguanosine, protecting the free amino group in a similar manner. The adenosine-derived linker was 2',3'-di-*O*-benzoyl-N^6,N^6-[2-(2-carboxyethyl)glutaryl]-5'-*O*(4,4'-dimethoxytrityl)adenosine (**23**) which was synthesized from 5'-*O*-(4,4'-dimethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**18**) [25] first by benzoylation (→ **19**), then deblocking of the npeoc group by DBU (→ **20**), and final reaction with **16** (→ **23**; *Scheme 3*). Similarly, 3'-*O*-[(*tert*-butyl)dimethylsilyl]-2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**21**) was first deprotected by DBU to **22** and then treated by **16** to form the linker molecule **24** in 76% isolated yield. The latter was furthermore desilylated by Bu_4NF in THF yielding **25** which reacted with 3-methyl-1-[2-(4-nitrophenyl)ethoxycarbonyl]-1*H*-imidazolium chloride in presence of DABCO (1,4-diazabicyclo[2.2.2]octane) to 2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-3'-*O*-[2-(4-nitrophenyl)ethoxycarbonyl]-N^6,N^6-{2-{3-[2-(4-nitrophenyl)-ethoxy]-3-oxopropyl}glutaryl}adenosine (**26**).

Scheme 3



	R	R^1	R^2
18	npeoc	OH	H
19	npeoc	Obz	bz
20	H	Obz	bz
21	npeoc	H	tbds
22	H	H	tbds

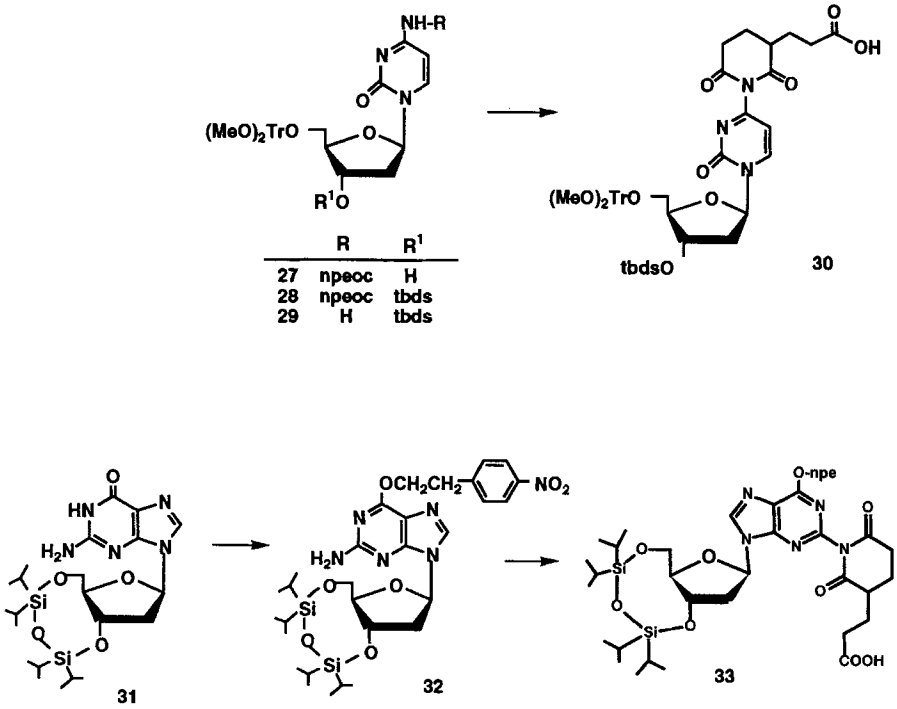
	R	R^1	R^2
23	H	Obz	bz
24	H	H	tbds
25	H	H	H
26	npe	H	npeoc

npeoc = [2-(4-nitrophenyl)ethoxy]carbonyl, tbds = (*tert*-butyl)dimethylsilyl, bz = benzoyl

In the 2'-deoxycytidine series, 2'-deoxy-N^4-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine [27] was first dimethoxytritylated to 2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-N^4-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine (**27**) (*Scheme 4*). Then, silylation by (*tert*-butyl)dimethylsilyl chloride gave **28**, and the npeoc group was deblocked by DBU. The resulting **29** was finally acylated with **16** to afford the linker molecule **30**.

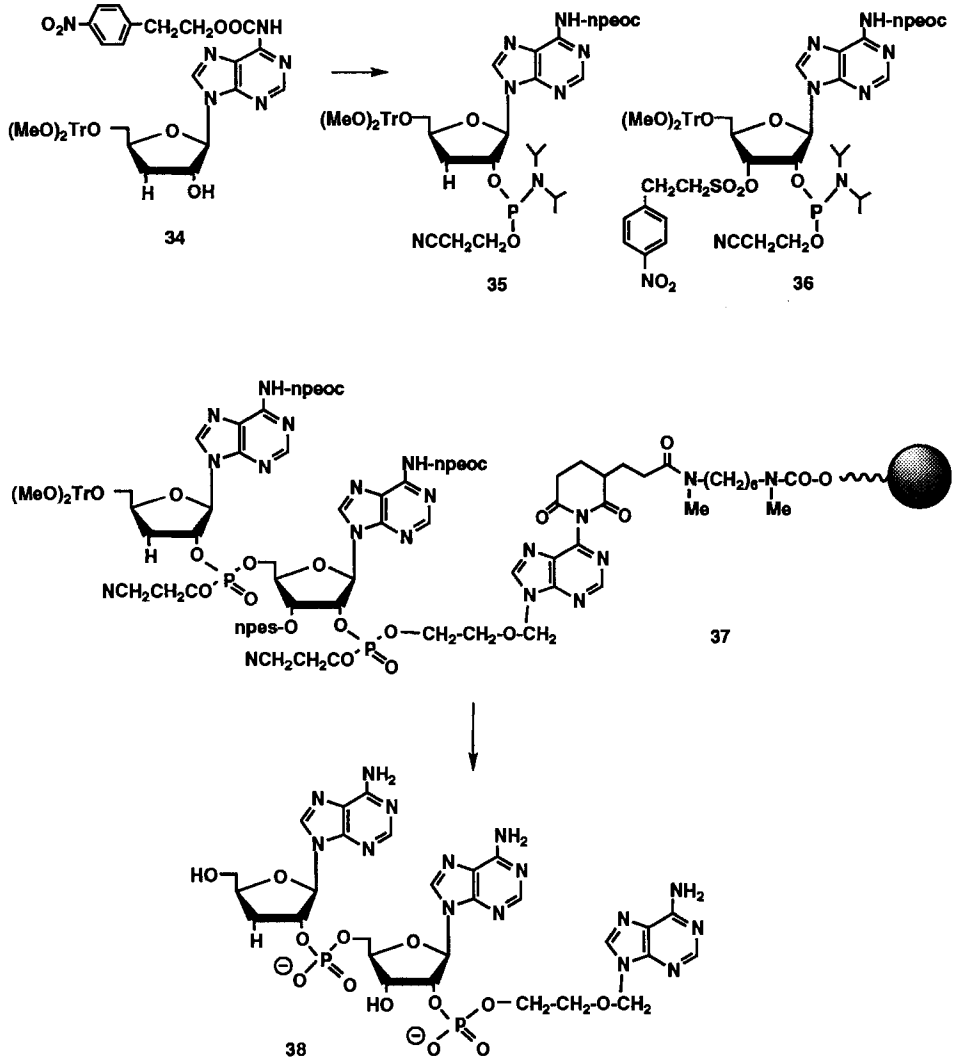
The fully protected 2'-deoxyguanosine linker molecule **33** was synthesized from 2'-deoxy-3',5'-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)guanosine (**31**) which was treated with 2-(4-nitrophenyl)ethanol in a *Mitsunobu* reaction leading under O^6-alkylation to **32** (*Scheme 3*). Reaction of **32** with **16** afforded N^2,N^2-[2-(2-carboxyethyl)glutaryl]-2'-deoxy-O^6-[2-(4-nitrophenyl)ethyl]-3',5'-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-guanosine (**33**) in 58% yield.

Scheme 4



These new linker molecules **12**, **23**, **24**, **30**, and **33** can be used as alternatives to start oligonucleotide syntheses on solid-support materials and have the potential of additional modifications at the sugar moieties as, *e.g.*, conjugate formation at the 3′-OH position which is commonly needed for linker bonding. To utilize the new strategy, a potentially antivirally active modified (2′–5′)-oligoadenylate was synthesized. In the first step, compound **12** was coupled onto a glyceryl-CPG support, which was modified by the [hexane-1,6-diylbis(methylimino)] spacer, using *O*-{[(2-cyanoethoxycarbonyl)methylidene]-amino}-1,1,3,3-tetramethyluronium tetrafluoroborate (TOTU) as condensing agent. This material was then treated in a 10-μmol scale in a DNA synthesizer subsequently with 5′-*O*-(4,4′-dimethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]-3′-*O*-[2-(4-nitrophenyl)ethylsulfonyl]adenosine 2′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (**36**) [26] and 3′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]-adenosine 2′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (**35**; prepared from **34**) in the usual manner leading to the fully protected trimer **37** (*Scheme 5*). Thereafter, deprotection was achieved by CCl_3COOH treatment in CH_2Cl_2 to cleave off the dimethoxytrityl group followed by removal of the npeoc groups by DBU in a β-elimination process. After these procedures, the (2′–5′)-trimer was still attached to the support and could be washed to get rid of the cleaved protecting groups and reagents. Finally, the oligomer was split off the support by treatment with aqueous $MeNH_2$ solution to give, after lyophilization, 89% of crude 3′-deoxyadenylyl-(2′–5′)-2′-adenylic acid 2′-{2-[(adenin-9-yl)methoxy]ethyl} ester (**38**) which turned out to be > 95% pure according to HPLC (*Fig.*)

Scheme 5



Experimental Part

General. DNA Synthesizer *ABI 380* from *Applied Biosystems*. Solid phase: CPG (*BIORAN*, 46.6 nm, LCAMA version). High vacuum = h.v. Column chromatography = CC. Flash chromatography = FC. TLC: precoated silica gel thin-layer sheets *F 15500 LS 254* from *Schleicher & Schüll*. Prep. TLC: silica gel *60 PF245* (*Merck*). Prep. column chromatography: silica gel *Merck 60* (0.063–0.2 mesh). M.p.: *Büchi* apparatus, model Dr. *Tottoli*, no corrections. HPLC: *Merck-Hitachi L6200* and *L4000*, column *RP 18* (*Merck*, 125 × 4 mm, 5 µm), flow rate 1 ml/min, mobile phase 0.1M $AcONH_4$/MeCN. UV/VIS: *Lambda 5 Perkin-Elmer*; λ_{max} in nm (lg ε). ^{1}H-NMR: *Bruker AC-250*; δ in ppm rel. to $SiMe_4$. ^{31}P-NMR: *Jeol-400*; δ in ppm rel. to H_3PO_4.

1. *9-{{2-[(4,4'-Dimethoxytrityl)oxy]ethoxy}methyl}adenine* (**2**). 9-[(2-Hydroxyethoxy)methyl]adenine (**1**) [22] (4.3 g, 20 mmol) was suspended in anh. pyridine (200 ml). After the addition of 4,4'-dimethoxytrityl chloride

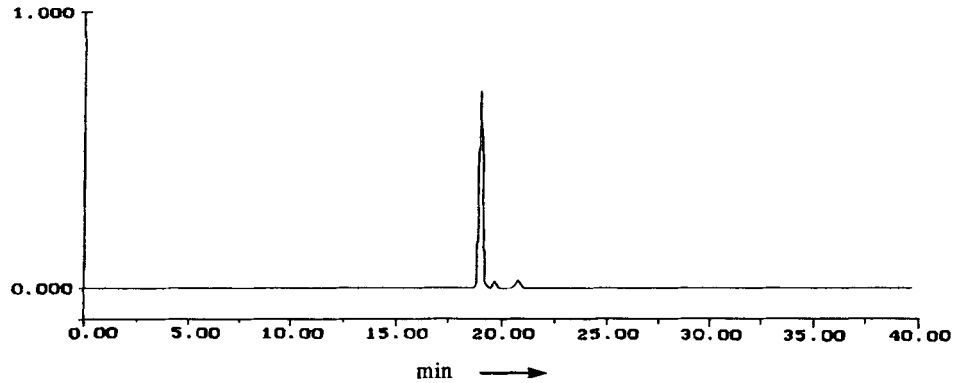


Figure. *HPLC of* **38** *on a* RP-18 *column.* Condition, see *Exper. Part.*

(7.3 g, 22 mmol), the mixture was stirred for 8 h at 30°, then evaporated, and co-evaporated 3 times with toluene. The residue was distributed between CH_2Cl_2 and $NaHCO_3$ soln. and the org. phase dried and evaporated. Recrystallization from AcOEt (150 ml) yielded 8.8 g and 0.5 g from the filtrate (91%). Colorless crystals. M.p. 124°. R_f (AcOEt/MeOH 10:1) 0.77. UV (CH_2Cl_2): 263 (4.14), 238 (4.34). ^{1}H-NMR ($CDCl_3$): 3.22 (*t*, OCH_2CH_2O); 3.70 (*t*, OCH_2CH_2O); 3.77 (*s*, MeO); 5.67 (*s*, OCH_2N); 5.88 (*s*, NH_2); 6.76–6.82 (*m*, H_o to MeO); 7.23–7.42 (*m*, arom. H); 7.96 (*s*, H–C(8)); 8.38 (*s*, H–C(2)). Anal. calc. for $C_{29}H_{29}N_5O_4$ (511.6): C 68.08, H 5.71, N 13.68; found: C 67.82, H 5.67, N 13.59.

2. *9-[(2-Hydroxyethoxy)methyl]-*N^6,N^6*-phthaloyladenine* (= *2-{9-[(2-Hydroxyethoxy)methyl]-9*H*-purin-1-yl}-1*H*-isoindole-1,3(2*H*)-dione*; **3**). To a chilled soln. of **1** (420 mg, 2 mmol) anh. pyridine (5 ml), Me_3SiCl (0.6 ml, 5 mmol) was added and the mixture stirred for 15 min. Then phthaloyl dichloride (0.4 ml, 2.8 mmol) was added and stirring continued for further 12 h. After addition of ice (2 g), the slurry was extracted with AcOEt (100 ml) and the org. layer washed twice with brine (50 ml) and evaporated. Traces of pyridine were removed by co-evaporation with toluene. Purification by CC (silica gel, 1.5 × 20 cm, AcOEt) gave, after drying under h.v., 400 mg (59%) of **3**. Yellowish foam. R_f (CH_2Cl_2/MeOH 9:1) 0.56. UV (CH_2Cl_2): 270 (4.12), 228 (4.36). ^{1}H-NMR ($CDCl_3$): 2.31 (*s*, OH); 3.76–3.78 (*m*, OCH_2CH_2O); 5.81 (*s*, OCH_2N); 7.83–8.05 (*m*, pht); 8.33 (*s*, H–C(8)); 9.09 (*s*, H–C(2)). Anal. calc. for $C_{16}H_{13}N_5O_4$ (339.3): C 56.63, H 3.86, N 20.64; found: C 56.15, H 3.84, N 19.47.

3. *9-{{2-[(4,4'-Dimethoxytrityl)oxy]ethoxy}methyl}-*N^6,N^6*-phthaloyladenine* (= *2-{9-{{2-[(4,4'-Dimethoxytrityl)oxy]ethoxy}methyl}-9*H*-purin-6-yl}-1*H*-isoindole-1,3(2*H*)-dione*; **4**). A mixture of **2** (255 mg, 0.5 mmol), Et_3N (0.2 ml), and phthalic anhydride (0.74 g, 5 mmol) in anh. pyridine (5 ml) was kept for 3 h at 90°. The mixture was chilled, and ice (1 g) and then CH_2Cl_2 (100 ml) were added. The org. layer was washed twice with $NaHCO_3$ soln. (20 ml), dried ($NaSO_4$) and evaporated. Traces of pyridine were removed by co-evaporation with toluene. Purification by CC (silica gel, 1.5 × 20 cm, $CHCl_3$) gave, after drying under h.v., 170 mg (53%) of **4**. Colorless foam. R_f (toluene/AcOEt 1:5) 0.32. UV (CH_2Cl_2): 271 (4.21), 228 (4.61). ^{1}H-NMR ($CDCl_3$): 3.28 (*t*, OCH_2CH_2O); 3.72 (*t*, OCH_2CH_2O); 3.78 (*s*, MeO); 5.83 (*s*, OCH_2N); 6.82 (*d*, H_o to MeO); 7.23–7.45 (*m*, arom. H); 7.56 (*m*, H_m, pht); 8.02 (*m*, H_o, pht); 8.33 (*s*, H–C(8)); 9.08 (*s*, H–C(2)). Anal. calc. for $C_{37}H_{31}N_5O_6 \cdot 0.5\ CH_2Cl_2$ (684.1): C 65.83, H 4.71, N 10.27; found: C 65.61, H 4.64, N 9.58.

4. N^6,N^6*-(4-Carboxyphthaloyl)-9-{{2-[(4,4'-dimethoxytrityl)oxy]ethoxy}methyl}adenine* (= *2-{9-{{2-[(4,4'-Dimethoxytrityl)oxy]ethoxy}methyl}-9*H*-purin-6-yl}-2,3-dihydro-1,3-dioxo-1*H*-isoindole-5-carboxylic Acid*; **11**). A mixture of **2** (512 mg, 1 mmol), Et_3N (1 ml), and benzene-1,2,4-tricarboxylic acid 1,2-anhydride (0.76 g, 4 mmol) was stirred in anh. pyridine (5 ml) for 5 h at 90° (→ dark orange). After evaporation, the residue was diluted with CH_2Cl_2 (200 ml), then washed with 10% citric-acid soln. at 5° and with ice-water. After drying (Na_2SO_4), the org. layer was evaporated and the residue dried under h.v.: 0.48 g (70%) of **11**. Colorless foam which can be stored at 0°. R_f (CH_2Cl_2/MeOH 9:1) 0.5. UV (CH_2Cl_2): 270 (4.26), 228 (4.76). ^{1}H-NMR ($CDCl_3$): 3.32 (*t*, OCH_2CH_2O); 3.77–3.78 (*s*, MeO, OCH_2CH_2O); 5.88 (*s*, OCH_2N); 6.81–6.85 (*m*, H_o to MeO); 7.23–7.46 (*s*, arom. H); 8.00 (*d*, arom. H); 8.30 (*d*, arom. H); 8.42 (*d*, arom. H); 8.49 (*s*, H–C(8)); 9.14 (*s*, H–C(2)). Anal. calc. for $C_{38}H_{31}N_5O_8 \cdot H_2O$ (703.7): C 64.86, H 4.72, N 9.95; found: C 64.66, H 4.68, N 9.71.

5. *9-{{2-[(4,4'-Dimethoxytrityl)oxy]ethoxy}methyl}-*N^6,N^6*-succinyladenine* (= *1-{9-{{2-[(4,4'-Dimethoxytrityl)oxy]ethoxy}methyl}-9*H*-purin-6-yl}pyrrolidine-2,5-dione*; **5**). A mixture of **2** (580 mg, 1.13 mmol), Et_3N

(0.2 ml) and succinic anhydride (1 g, 10 mmol) in anh. pyridine (5 ml) was reacted as described in *Exper. 3*: 490 mg (73%) of **5**. Colorless foam. R_f (MeOH/AcOEt 1:10) 0.77. UV (CH_2Cl_2): 268 (4.09), 236 (4.34). ^{1}H-NMR ($CDCl_3$): 3.04 (*s*, 4 H, suc); 3.28 (*t*, OCH_2CH_2O); 3.70 (*t*, OCH_2CH_2O); 3.78 (*s*, MeO); 5.80 (*s*, OCH_2N); 6.80–6.84 (*m*, H_o to MeO); 7.26–7.34 (*m*, arom. H); 8.32 (*s*, H–C(8)); 9.04 (*s*, H–C(2)). Anal. calc. for $C_{33}H_{31}N_5O_6 \cdot 0.5\,H_2O$ (602.6): C 65.77, H 5.35, N 11.62; found: C 65.84, H 5.33, N 11.43.

6. *N^6,N^6-Glutaryl-9-[(2-hydroxyethoxy)methyl]adenine* (= *1-{9-[(2-Hydroxyethoxy)methyl]-9H-purin-6-yl}piperidine-2,6-dione*; **6**). To a chilled soln. of **1** (420 mg, 2 mmol) in anh. pyridine (10 ml), Me_3SiCl (0.6 ml, 5 mmol) was added and the mixture stirred for 15 min. Glutaryl dichloride (0.34 ml, 2.8 mmol) was added and the mixture stirred at r.t. for 18 h. After addition of ice (2 g), the slurry was extracted with AcOEt (100 ml), and workup as described in *Exper. 2* yielded 0.37 g (60%) of **6**. Hygroscopic foam. R_f (CH_2Cl_2/MeOH) 9:1) 0.50. UV (CH_2CH_2): 266 (3.96). ^{1}H-NMR ($CDCl_3$): 2.22 (*m*, 2 H, glut); 2.60 (*s*, OH); 2.87–2.90 (*m*, 4 H, glut); 3.73–3.75 (*m*, OCH_2CH_2O); 5.76 (*s*, OCH_2N); 8.25 (*s*, H–C(8)); 9.02 (*s*, H–C(2)). Anal. calc. for $C_{13}H_{15}N_5O_4$ (305.3): C 51.14, H 4.95, N 22.94; found: C 51.63, H 5.13, N 21.28.

7. *9-{{[2-(4,4'-Dimethoxytrityl)oxy]ethoxy}methyl]-N^6,N^6-glutaryladenine* (= *1-{9-{{2-[(4,4'-Dimethoxytrityl)oxy]ethoxy}methyl}-9H-purin-6-yl}piperidine-2,6-dione*; **7**). A mixture of **2** (255 mg, 0.5 mmol), Et_3N (0.2 ml), and glutaric anhydride (0.34 g, 3 mmol) in anh. pyridine (5 ml) was reacted described for **4**: 0.15 g (50%) of **7**. Colorless foam. R_f (toluene/AcOEt 1:5) 0.23. UV (CH_2Cl_2): 265 (4.06), 236 (4.36). ^{1}H-NMR ($CDCl_3$): 2.22 (*m*, 2 H, glut); 2.92 (*t*, 4 H, glut); 3.30 (*t*, OCH_2CH_2O); 3.74 (*t*, OCH_2CH_2O); 3.78 (*s*, MeO); 5.79 (*s*, OCH_2N); 6.80–6.84 (*m*, H_o to MeO); 7.23–7.46 (*m*, arom. H); 8.26 (*s*, H–C(8)); 9.01 (*s*, H–C(2)). Anal. calc. for $C_{34}H_{33}N_5O_6 \cdot 0.5\,H_2O$ (616.7): C 66.22, H 5.55, N 11.35; found: C 66.52, H 5.54, N 11.43.

8. *N^6,N^6-[2-(2-Carboxyethyl)glutaryl]-9-{{2-[(4,4'-dimethoxytrityl)oxy]ethoxy}methyl}adenine* (= *1-{9-{{2-[(4,4'-Dimethoxytrityl)oxy]ethoxy}methyl}-9H-purin-6-yl}-2,6-dioxopiperidine-3-propanoic Acid*; **12**). A mixture of **2** (512 mg, 1 mmol), Et_3N (0.5 ml), and pentane-1,3,5-tricarboxylic acid 1,3-anhydride (**16**; 0.76 g, 4 mmol) was stirred in anh. pyridine (5 ml) for 6 h at 90°. Workup as described for **11** yielded 0.59 g (86%) of **12**. Brownish foam. R_f ($CHCl_3$) 0.5–0.65. UV (CH_2Cl_2): 266 (4.06), 236 (4.34). ^{1}H-NMR ($CDCl_3$): 2.27 (*m*, 4 H, glut); 2.56 (*m*, 2 H, glut); 3.00 (*m*, 3 H, glut); 3.29 (*m*, OCH_2CH_2O); 3.72 (*m*, OCH_2CH_2O); 3.78 (*s*, 2 MeO); 5.80 (*s*, OCH_2N); 6.82 (*d*, H_o to MeO); 7.15–7.45 (*m*, arom. H); 8.36 (*s*, H–C(8)); 9.03 (*s*, H–C(2)). Anal. calc. for $C_{37}H_{37}N_5O_8$ (679.72): C 65.38, H 5.48, N 10.30; calc. with 0.25 equiv. of H_2O: C 64.95, H 5.52, N 10.23; found: C 64.87, H 5.69, N 9.21.

9. *N^6-[2-(Diethylcarbamoyl)benzoyl]-9-[(2-hydroxyethoxy)methyl]adenine* (= *N^1,N^1-Diethyl-N^2-{9-[(2-hydroxyethoxy)methyl]-9H-purin-6-yl}benzene-1,2-dicarboxamide*; **8**). A soln. of **3** (130 mg, 0.38 mmol) in CH_2Cl_2 (10 ml) was treated with Et_2NH (10 ml) for 70 h at r.t. The mixture was evaporated and the resulting oil purified by CC (0.5 × 25 cm, MeOH/AcOEt 1:10) to give 0.11 g (70%) of **8**. Colorless foam. R_f (CH_2Cl_2/MeOH 9:1) 0.56. UV (CH_2Cl_2): 280 (4.22), 229 (4.12). ^{1}H-NMR ($CDCl_3$): 1.11 (*m*, 2 Me); 3.20 (*q*, CH_2N); 3.54 (*q*, CH_2N); 3.67–3.78 (*m*, CH_2CH_2); 5.68 (*s*, OCH_2N); 7.29–7.33 (*m*, H_o, pht); 7.47–7.61 (*m*, H_m, pht); 7.95 (*m*, H_o, pht); 8.13 (*s*, H–C(8)); 8.85 (*s*, H–C(2)). Anal. calc. for $C_{20}H_{24}N_6O_4 \cdot H_2O$ (430.4): C 55.80, H 6.08, N 19.52, found: C 55.82, H 5.84, N 19.65.

10. *N^6-[2-(Diethylcarbamoyl)benzoyl]-9-{{2-[(4,4'-dimethyltrityl)oxy]ethoxy}methyl}adenine* (= *N^1-{9-{{2-[(4,4'-Dimethoxytrityl)oxy]ethoxy}methyl}-N^2,N^2-diethylbenzenedicarboxamide*; **9**). To a soln. of **4** (85 mg, 0.13 mmol) in CH_2Cl_2 (20 ml), Et_2NH (10 µl, 1 mmol) was added and the mixture stirred at r.t. for 90 h and then evaporated. The residue was purified by prep. TLC (20 × 20 × 0.2 cm, $CHCl_3$/MeOH 24:1; product band at R_f 0.5): 75 mg (90%) of **9**. Solid foam. UV (CH_2Cl_2): 280 (4.31), 232 (4.47). ^{1}H-NMR ($CDCl_3$): 1.11 (*m*, 2 Me); 3.20 (*q*, CH_2N); 3.54 (*q*, CH_2N); 3.72 (*m*, OCH_2CH_2O); 5.68 (*s*, OCH_2N); 7.31 (*m*, H_o, pht); 7.54 (*m*, 2 H_m, pht); 7.95 (*m*, 1 H_o, pht); 8.13 (*s*, H–C(8)); 8.85 (*s*, H–C(2)). Anal. calc. for $C_{41}H_{42}N_6O_6 \cdot H_2O$ (732.8): C 67.19, H 6.05, N 11.46; found: C 66.91, H 5.95, N 11.12.

11. *9-{{2-[(4,4'-Dimethoxytrityl)oxy]ethoxy}methyl}-N^6-succinyladenine* (= *4-{{9-{{2-[(4,4'-Dimethoxytrityl)oxy]ethoxy}methyl}-9H-purin-6-yl}amino}-4-oxobutanoic Acid*; **10**). A soln. of **2** (150 mg, 0.29 mmol) in Et_3N/pyridine/H_2O 2:2:2 (15 ml) was stirred for 2 h at 40°. The mixture was then evaporated and distributed between CH_2Cl_2 and 10% citric-acid soln. at 5°. The org. layer was washed twice with ice-water, dried (Na_2SO_4), and evaporated under h.v.: 0.15 g (96%) of **10**. Colorless foam. R_f ($CHCl_3$/MeOH) 0.04. UV (CH_2Cl_2): 270 (4.22), 236 (4.36). ^{1}H-NMR ($CDCl_3$): 2.84 (*t*, suc); 3.13 (*t*, suc); 3.21 (*t*, OCH_2CH_2O); 3.76 (*t*, OCH_2CH_2O); 3.77 (*s*, MeO); 5.72 (*s*, OCH_2N); 6.76–6.79 (*m*, H_o to MeO); 7.26–7.34 (*m*, arom. H); 8.32 (*s*, H–C(8)); 9.04 (*s*, H–C(2)); 10.6 (*s*, COOH). Anal. calc. for $C_{33}H_{33}N_5O_7 \cdot H_2O$ (629.7): C 63.86, H 5.52, N 11.28; found: C 64.05, H 5.58, N 11.09.

12. *Pentane-1,3,5-tricarboxylic Acid 1,3-Anhydride* (= *Tetrahydro-2,6-dioxo-2H-pyran-3-propanoic Acid*; **16**). A mixture of pentane-1,3,5-tricarboxylic acid (**14**) [23] (5 g, 24.5 mmol) and thionyl chloride (2 ml, 27 mmol) in

anh. 1,2-dichloroethane (50 ml) was heated under reflux for 5 h till a clear soln. was obtained. After cooling to r.t., hexane (20 ml) was added in small portions with stirring. The resulting precipitate was filtered off, washed once with hexane, and dried *in vacuo*: 4.04 g (88%) of **16**. Colorless crystals. M.p. 104°. ^{1}H-NMR ((D$_6$)DMSO): 1.63–2.10 (*m*, CH_2(2), CH_2(4)); 2.33 (*t*, CH_2(5)); 2.74–2.80 (*m*, CH_2(1), CH(3)); 12.10 (*s*, COOH). Anal. calc. for $C_8H_{10}O_5$ (186.2): C 51.61, H 5.41; found: C 51.32, H 5.45.

13. *2-(3-Chloro-3-oxopropyl)pentanedioic Acid 1,5-Anhydride* (= *Tetrahydro-2,6-dioxo-2*H*-pyran-3-propanoyl Chloride*; **17**). A mixture of **14** (10 g, 49 mmol) and thionyl chloride (30 ml) was heated under reflux for 6 h and then evaporated. The resulting oil was kept for 2 h at r.t. under h.v.: 10 g (99%) of **17**. This crude yellowish oil was not further purified since attempted distillation resulted in decomposition. ^{1}H-NMR ($CDCl_3$): 1.79–2.43 (*m*, CH_2(3), CH_2CH_2COCl); 2.62–3.02 (*m*, CH(2), CH_2(4)); 3.21 (*t*, CH_2CH_2COCl). Anal. calc. for $C_9H_9ClO_4$ (204.6): C 46.96, H 4.43; found: C 44.86, H 4.21.

14. *2′,3′-Di-*O*-benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**19**). 5′-*O*-(4,4′-Dimethoxytrityl)-*N*^{6}-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**18**) [25] (0.763 mg, 1 mmol) was dissolved in anh. MeCN (15 ml), then benzoyl cyanide (0.33 g, 2.5 mmol) and Bu_3N (50 ml) were added and stirred for 2 h at r.t. The mixture was evaporated, the residue redissolved in CH_2Cl_2 (100 ml), the soln. washed twice with $NaHCO_3$ soln., and then the org. layer dried (Na_2SO_4) and evaporated. Purification by CC (1.5 × 25 cm, toluene/AcOEt 7:3) gave 0.90 g (93%) of **19**. Colorless foam. R_f (toluene/AcOEt 1:5) 0.71. UV (CH_2Cl_2): 266 (4.49), 234 (4.68). ^{1}H-NMR ($CDCl_3$): 3.12 (*t*, CH_2CH_2); 3.62 (*m*, 2 H–C(5′)); 3.76 (*s*, MeO); 4.52 (*t*, OCH_2CH_2); 4.61 (*d*, H–C(4′)); 6.11 (*m*, H–C(3′)); 6.43 (*t*, H–C(2′)); 6.57 (*d*, H–C(1′)); 6.82 (*d*, H_o to MeO); 7.17–7.58 (*m*, arom. H); 7.89 (*d*, H_m to NO_2); 7.99 (*d*, H_o to NO_2); 8.22 (*s*, H–C(8)); 8.32 (*s*, NH); 8.71 (*s*, H–C(2)). Anal. calc. for $C_{54}H_{46}N_6O_{12}$ (971.0): C 66.79, H 4.77, N 8.65; found: C 66.83, H 4.97, N 8.44.

15. *2′,3′-Di-*O*-benzoyl-5′-*O*-(4,4′-dimethoxytrityl)adenosine* (**20**). To a soln. of **19** (400 mg, 0.41 mmol) in anh. pyridine (40 ml), DBU (3.2 ml) was added. The mixture was stirred for 18 h, then evaporated, and co-evaporated twice with toluene (20 ml). The residue was dissolved in $CHCl_3$ (150 ml), the soln. washed twice with $NaHCO_3$ soln. (20 ml) and the org. layer dried (Na_2SO_4) and again evaporated. Purification was achieved by CC (1.5 × 25 cm, toluene/AcOEt 7:3): 0.3 g (95%) of **20**. Colorless foam. R_f (toluene/AcOEt, 1:5) 0.33. UV (CH_2Cl_2): 233 (4.66). ^{1}H-NMR ($CDCl_3$): 3.61 (*m*, 2 H–C(5′)); 3.76 (*s*, MeO); 4.58 (*d*, H–C(4′)); 5.72 (*s*, NH_2); 6.11 (*m*, H–C(3′)); 6.42 (*t*, H–C(2′)); 6.55 (*d*, H–C(1′)); 6.81 (*d*, H_o to MeO); 7.17–7.58 (*m*, arom. H); 7.89 (*d*, H_m to NO_2); 7.99 (*d*, H_o to NO_2); 8.06 (*s*, H–C(8)); 8.33 (*s*, H–C(2)). Anal. calc. for $C_{45}H_{39}N_5O_8 \cdot H_2O$ (795.8): C 67.91, H 5.19, N 8.79; found: C 67.52, H 4.95, N 8.61.

16. *3′-*O*-[(*tert*-Butyl)dimethylsilyl]-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**21**). A soln. of 2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-*N*^{6}-[2-(4-nitrophenyl)ethoxycarbonyl]-adenosine [27] (5.2 g, 6.9 mmol) in anh. pyridine (60 ml) was treated by (*tert*-butyl)dimethylsilyl chloride (3.2 g, 20 mmol) and 1*H*-imidazole (2.9 g, 40 mmol) with stirring for 18 h at r.t. The reaction was quenched by addition of MeOH (15 ml) and the mixture stirred for 15 min, then evaporated, and co-evaporated twice with toluene (20 ml). The residue was dissolved in $CHCl_3$ (150 ml), the soln. washed twice with $NaHCO_3$ soln. and the org. layer dried (Na_2SO_4) and again evaporated. Purification by CC (4 × 45 cm, $CHCl_3$) gave 5 g (84%) of **21**. Colorless solid. R_f ($CHCl_3$/MeOH, 24:1) 0.7. UV (CH_2Cl_2): 267 (4.48), 238 (4.45), 226 (4.44). ^{1}H-NMR ($CDCl_3$): −0.05 (*s*, MeSi); 0.00 (*s*, MeSi); 0.86 (*s*, *t*-Bu); 2.51–2.91 (*m*, 2 H–C(2′)); 3.14 (*t*, CH_2CH_2); 3.40–3.52 (*m*, 2 H–C(5′)); 3.77 (*s*, MeO); 3.89 (*m*, H–C(4′)); 4.50 (*t*, OCH_2CH_2); 4.72 (*m*, H–C(3′)); 6.44 (*t*, H–C(1′)); 6.80 (*d*, H_o to MeO); 7.15–7.44 (*m*, arom. H); 8.16 (*d*, H_o to NO_2); 8.20 (*s*, H–C(8)); 8.66 (*s*, H–C(2)); 8.76 (*s*, NH). Anal. calc. for $C_{46}H_{53}N_6O_9Si$ (862.0): C 64.09, H 6.19, N 9.74; found: C 64.05, H 6.20, N 9.65.

17. *3′-*O*-[(*tert*-Butyl)dimethylsilyl]-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)adenosine* (**22**). A soln. of **21** (4.75 g, 5.5 mmol) in anh. pyridine (90 ml) was treated with DBU (7.6 ml) at r.t. with stirring for 18 h. Workup as described for **20** and purification by CC (4 × 45 cm, $CHCl_3$/MeOH 24:1) gave 3.56 g (93%) of **22**. Colorless solid. R_f ($CHCl_3$/MeOH 24:1) 0.34. UV (CH_2Cl_2): 237 (4.37). ^{1}H-NMR ($CDCl_3$): 0.08 (*s*, MeSi); 0.11 (*s*, MeSi); 0.86 (*s*, *t*-Bu); 2.37–2.85 (*m*, 2 H–C(2′)); 3.27–3.48 (*m*, H–C(5′)); 3.77 (*s*, MeO); 4.15 (*m*, H–C(4′)); 4.53 (*m*, H–C(3′)); 5.91 (*s*, NH_2); 6.40 (*t*, H–C(1′)); 6.80 (*d*, H_o to MeO); 7.12–7.40 (*m*, arom. H); 8.02 (*s*, H–C(8)); 8.30 (*s*, H–C(2)). Anal. calc. for $C_{37}H_{45}N_6O_5Si \cdot H_2O$ (667.9): C 64.79, H 6.80, N 10.21; found: C 64.85, H 6.72, N 10.08.

18. *2′,3′-Di-*O*-benzoyl-*N^6*,*N^6*-[2-(2-carboxyethyl)glutaryl]-5′-*O*-(4,4′-dimethoxytrityl)-adenosine* (= *1-{9-[2′,3′-Di-*O*-benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-β-*D*-ribofuranosyl]-9*H*-purin-6-yl}-2,6-dioxopiperidine-3-propanoic Acid*; **23**). A mixture of **20** (300 mg, 0.4 mmol) and **16** (0.6 g, 3.2 mmol) was stirred in anh. pyridine (5 ml) for 6 h at 90°. Workup as described for **11** and purification by CC (1.5 × 23 cm, $CHCl_3$) gave 0.3 g (80%) of **23**. Brownish foam. Anal. pure and colorless material was obtained by prep. TLC ($CHCl_3$/MeOH 9:1). R_f ($CHCl_3$/MeOH 9:1) 0.58. UV (CH_2Cl_2): 265 (4.11), 233 (4.63). ^{1}H-NMR ($CDCl_3$): 1.67–3.15 (*m*, $COCH_2CH_2CHCO$,

CH_2CH_2 COOH); 3.61 (*m*, 2H–C(5')); 3.75 (*s*, MeO); 4.59 (*d*, H–C(4')); 6.12 (*m*, H–C(3')); 6.43 (*t*, H–C(2')); 6.55 (*d*, H–C(1')); 6.81 (*d*, H_o to MeO); 7.17–7.58 (*m*, arom. H); 7.89 (*d*, H_m to NO_2); 7.99 (*d*, H_o to NO_2); 8.42 (*s*, H–C(8)); 8.91 (*s*, H–C(2)). Anal. calc. for $C_{53}H_{47}N_5O_{12} \cdot H_2O$ (982.0): C 64.82, H 5.23, N 7.13; found: C 64.94, H 5.24, N 6.92.

19. *3'-O-[(*tert*-Butyl)dimethylsilyl]-*N^6,N^6-*[2-(2-carboxyethyl)glutaryl]-2'-deoxy-5'-O-(4,4'-dimethoxytrityl)adenosine* (= *1-{9-{3'-O-[(*tert*-Butyl)dimethylsilyl]-2'-deoxy-5'-O-(4,4'-dimethoxytrityl)-β-*D*-ribofuranosyl}-9H-purin-6-yl}-2,6-dioxopiperidine-3-propanoic Acid*; **24**). A mixture of **22** (3 g, 4.3 mmol) and **16** (6 g, 32 mmol) was stirred in anh. pyridine (50 ml) for 6 h at 90°. Workup as described for **11** and purification by CC (4 × 30 cm, $CHCl_3$/MeOH 50:1) gave 2.76 g (76%) of **24**. Colorless foam. R_f ($CHCl_3$/MeOH 9:1) 0.65. UV (CH_2Cl_2): 266 (4.10), 236 (4.34). ^{1}H-NMR ($CDCl_3$): –0.01 (*s*, MeSi); 0.02 (*s*, MeSi); 0.87 (*s*, *t*-Bu); 1.60–3.15 (*m*, 2 H–C(2'), $COCH_2CH_2CHCO$, CH_2CH_2COOH); 3.30–3.53 (*m*, 2 H–C(5')); 3.77 (*s*, MeO); 4.16 (*m*, H–C(4')); 4.56 (*m*, H–C(3')); 6.46 (*m*, H–C(1')); 6.80 (*d*, H_o to MeO); 7.12–7.40 (*m*, arom. H); 8.42–8.52 (*m*, H–C(8)); 8.92 (*s*, H–C(2)). Anal. calc. for $C_{45}H_{53}N_5O_9Si \cdot 0.5\,H_2O$ (845.0): C 63.96, H 6.44, N 8.28; found: C 64.09, H 6.64, N 7.64.

20. *N^6,N^6-[2-(2-Carboxyethyl)glutaryl]-2'-deoxy-5'-O-(4,4'-dimethoxytrityl)adenosine* (= *1-{9-[2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-β-*D*-ribofuranosyl]-9H-purin-6-yl}-2,6-dioxopiperidine-3-propanoic Acid*; **25**). A soln. of **24** (2.2 g, 2.58 mmol) in THF (80 ml) was treated with Bu_4NF (1.7 g, 5.2 mmol) by stirring at r.t. for 30 min. The mixture was concentrated, diluted with CH_2Cl_2 (200 ml), washed with 10% citric-acid soln. at 5° and ice-water, dried (Na_2SO_4), evaporated, and dried under h.v. Purification by CC (4 × 30 cm, $CHCl_3$/MeOH 20:1) gave 1.63 g (86%) of **25**. Colorless foam which is stable at r.t. and below. R_f ($CHCl_3$/MeOH 24:1) 0.2. UV (CH_2Cl_2): 266 (4.08), 236 (4.34). ^{1}H-NMR ($CDCl_3$): 1.52–3.15 (*m*, 2 H–C(2'), $COCH_2CH_2CHCO$, CH_2CH_2COOH); 3.38–3.53 (*m*, 2 H–C(5')); 3.76 (*s*, MeO); 4.16 (*m*, H–C(4')); 4.65 (*m*, H–C(3')); 6.46 (*m*, H–C(1')); 6.80 (*d*, H_o to MeO); 7.12–7.40 (*m*, arom. H); 8.32–8.52 (*m*, H–C(8)); 8.87 (*s*, H–C(2)). Anal. calc. for $C_{39}H_{39}N_5O_9 \cdot 0.5\,CH_2Cl_2$ (764.2): C 62.08, H 5.28, N 9.17; found: C 61.85, H 5.42, N 9.00.

21. *2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-3'-O-[2-(4-nitrophenyl)ethoxycarbonyl]-*N^6,N^6-*{2-{3-[2-(4-nitrophenyl)ethoxy]-3-oxopropyl}glutaryl}adenosine* (= *2-(4-Nitrophenyl)ethyl 1-{9-{2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-3'-O-[2-(4-nitrophenyl)ethoxycarbonyl]-β-*D*-ribofuranosyl}-9H-purin-6-yl}-2,6-dioxopiperidine-3-propanoate*; **26**). A mixture of **25** (1.2 g, 1.66 mmol), DABCO (222 mg, 2.6 mmol), and 3-methyl-1-[(4-nitrophenyl)ethoxycarbonyl]-1*H*-imidazolium chloride (1.5 g, 3.2 mmol) was stirred in anh. CH_2Cl_2 (200 ml) for 2 h at r.t. The org. layer was washed with 10% citric-acid soln. at 5° and ice-water, dried (Na_2SO_4), evaporated, and dried under h.v. Purification by CC (4 × 45 cm, $CHCl_3$/MeOH 40:1) gave 1.14 g (65%) of **26**. Colorless foam. R_f ($CHCl_3$/MeOH 24:1) 0.54. UV (CH_2Cl_2): 268 (4.49), 237 (4.44), 226 (4.44). ^{1}H-NMR ($CDCl_3$): 1.83–3.20 (*m*, 2 H–C(2'), $COCH_2CH_2CHCO$, CH_2CH_2COO, 2 OCH_2CH_2); 3.38–3.53 (*m*, 2 H–C(5')); 3.76 (*s*, MeO); 3.91–4.08 (*m*, H–C(4')); 4.29–4.49 (*m*, 2 OCH_2CH_2); 4.39–4.50 (*m*, H–C(3')); 6.47–6.60 (*m*, H–C(1')); 6.80 (*d*, H_o to MeO); 7.12–7.50 (*m*, arom. H); 8.12–8.30 (*m*, H–C(8), H_o to NO_2); 8.89 (*s*, H–C(2)). Anal. calc. for $C_{56}H_{53}N_7O_{15} \cdot H_2O$ (1082.1): C 62.15, H 5.12, N 9.06; found: C 62.71, H 5.25, N 8.80.

22. *2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-*N^4*-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine* (**27**). 2'-Deoxy-N^4-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine [27] (4.2 g, 10 mmol) was co-evaporated twice with anh. pyridine (50 ml). The residue was dissolved in the same solvent (100 ml), then 4,4'-dimethoxytrityl chloride (4.1 g, 12 mmol), Et_3N (2 ml), and 4-(dimethylamino)pyridine (DMAP, 61 mg) were added. After 5 h stirring at r.t., MeOH was added, stirred for 30 min, and then evaporated. The residue was dissolved in CH_2Cl_2 (200 ml), the soln. treated with phosphate buffer pH 7 (2 × 400 ml), the aq. phase extracted with CH_2Cl_2 (3 × 50 ml), and the united org. phase dried (Na_2SO_4), evaporated, and co-evaporated with toluene (2 × 50 ml). The residue was again dissolved in CH_2Cl_2 (20 ml) and purified by CC (silica gel, 3 × 30 cm, CH_2Cl_2, CH_2Cl_2/MeOH): 6.22 g (86%) of **27**. Colorless solid. R_f ($CHCl_3$/MeOH 95:5) 0.36. UV (MeOH): 280 (sh, 4.25), 275 (4.25), 235 (4.56). ^{1}H-NMR ($CDCl_3$): 2.21 (*m*, 1 H–C(2')); 2.75 (*m*, 1 H–C(2')); 3.09 (*t*, OCH_2CH_2); 3.36–3.60 (*m*, OH–C(3'), 2 H–C(5')); 3.78 (*s*, MeO); 4.15 (*m*, H–C(4')); 4.41 (*t*, OCH_2CH_2); 4.51 (*m*, H–C(3')); 6.29 (*m*, H–C(1')); 6.84 (*m*, 4 H_o to MeO); 6.95 (*m*, H–C(5)); 7.19–7.42 (*m*, 11 arom. H); 8.15–8.25 (*m*, 2 H_o to NO_2, H–C(6), NH). Anal. calc. for $C_{39}H_{38}N_4O_{10}$ (722.8): C 64.81, H 5.30, N 7.75; found: C 64.89, H 5.56, N 7.67.

23. *3'-O-[(*tert*-Butyl)dimethylsilyl]-2'-deoxy-5'-O-(4,4'-dimethoxytrityl)-*N^4*-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine* (**28**). As described for **21**, **27** (5.4 g, 7.07 mmol) was silylated. Purification by CC (4 × 45 cm, $CHCl_3$/MeOH 50:1) gave 5.58 g (98%) of **28**. Colorless foam. R_f ($CHCl_3$/MeOH 24:1) 0.71. UV (CH_2Cl_2): 276 (4.22), 236 (4.53). ^{1}H-NMR ($CDCl_3$): –0.06 (*s*, MeSi); –0.01 (*s*, MeSi); 0.79 (*s*, *t*-Bu); 2.21 (*m*, 1 H–C(2')); 2.75 (*m*, 1 H–C(2')); 3.09 (*t*, OCH_2CH_2); 3.36–3.43 (*m*, 2H–C(5')); 3.78 (*s*, MeO); 4.15 (*m*, H–C(4')); 4.37 (*t*, OCH_2CH_2); 4.51 (*m*, H–C(3')); 6.29 (*m*, H–C(1')); 6.83 (*d*, H_o to MeO); 6.97 (*d*, H–C(5)); 7.19–7.42 (*m*, arom. H); 7.59 (*d*, H_m to NO_2); 8.15–8.25 (*m*, H_o to NO_2, H–C(6), NH). Anal. calc. for $C_{45}H_{53}N_4O_{10}Si$ (838.0): C 64.49, H 6.37, N 6.68; found: C 64.37, H 6.35, N 6.62.

24. *3'-O-[(*tert*-Butyl)dimethylsilyl]-2'-deoxy-5'-O-(4,4'-dimethoxytrityl)cytidine* (**29**). A soln. of **28** (5.8 g, 6.9 mmol) in anh. pyridine (100 ml) was treated with DBU (7.6 ml) by stirring for 18 h. Workup as described for **20** and purification by CC (4 × 45 cm, $CHCl_3$/MeOH 24:1) gave 4.32 g (97%) of **29**. Colorless foam. R_f ($CHCl_3$/MeOH 24:1) 0.25. UV (CH_2Cl_2): 281 (4.01), 230 (4.40). ^{1}H-NMR ($CDCl_3$): −0.02 (*s*, MeSi); 0.06 (*s*, MeSi); 0.86 (*s*, *t*-Bu); 2.24 (*m*, 1 H−C(2')); 2.50 (*m*, 1 H−C(2')); 3.34 (*m*, 1 H−C(5')); 3.58 (*m*, 1 H−C(5')); 3.85 (*s*, MeO); 4.00 (*m*, H−C(4')); 4.51 (*m*, H−C(3')); 5.50 (*d*, H−C(5)); 6.32 (*m*, H−C(1')); 6.90 (*d*, H_o to MeO); 7.27–7.49 (*m*, arom. H); 8.08 (*d*, H−C(6)). Anal. calc. for $C_{36}H_{45}N_3O_6Si \cdot 1.5\ H_2O$ (670.9). C 64.45, H 7.21, N 6.32; found: C 64.35, H 6.79, N 6.32.

25. *3'-O-[(*tert*-Butyl)dimethylsilyl]-N^6,N^6-[2-(2-carboxyethyl)glutaryl]-2'-deoxy-5'-O-(4,4'-dimethoxytrityl)cytidine* (= *1-{4-{3'-O-[(*tert*-Butyl)dimethylsilyl]-2'-deoxy-5'-O-(4,4'-dimethoxytrityl)-β-*D*-ribofuranosyl}-2-oxopyrimidin-1(2H)-yl}-2,6-dioxopiperidine-3-propanoic Acid;* **30**). A mixture of **29** (2 g, 3.1 mmol) and **16** (4 g, 21 mmol) was stirred in anh. pyridine (40 ml) for 6 h at 90°. Workup as described for **11** and purification by CC (4 × 30 cm, $CHCl_3$/MeOH 50:1) gave 1.86 g (75%) of **30**. Colorless foam. An anal. pure sample was obtained by prep. TLC ($CHCl_3$/MeOH 24:1). R_f ($CHCl_3$/MeOH 24:1) 0.2–0.32. UV (CH_2Cl_2): 307 (3.81), 234 (4.34). ^{1}H-NMR ($CDCl_3$): −0.06 (*s*, MeSi); −0.01 (*s*, MeSi); 0.79 (*s*, *t*-Bu); 1.65–2.94 (*m*, 2 H−C(2'), $COCH_2CH_2CHCO$, CH_2CH_2COOH); 3.29–3.40 (*m*, 1 H−C(5')); 3.55–3.85 (*m*, 1 H−C(5'), MeO); 4.00 (*m*, H−C(4')); 4.37–4.54 (*m*, H−C(3')); 5.88 (*d*, H−C(5)); 6.16 (*m*, H−C(1')); 6.83 (*d*, H_o to MeO); 7.22–7.37 (*m*, arom. H); 8.30–8.70 (*m*, H−C(6), COOH). Anal. calc. for $C_{44}H_{53}N_3O_{10}Si$ (812.0): C 65.08, H 6.57, N 5.17; found: C 65.09, H 6.54, N 5.01.

26. *2'-Deoxy-3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)guanosine* (**31**). A suspension of 2'-deoxyguanosine monohydrate (8 g, 28 mmol) in dry DMF (100 ml) was heated to 80° and then evaporated to remove the crystal water. The 1*H*-imidazole (7.6 g, 0.115 mol) was added to the residue and the mixture co-evaporated with anh. DMF (2 × 20 ml). The resulting residue was suspended in dry DMF (100 ml) and cooled to 0°, and then 1,3-dichloro-1,1,3,3-tetraisopropyl-1,3-disiloxane (9.65 ml, 31 mmol) was added dropwise with stirring. The suspension was stirred overnight to give a turbid soln. which was poured onto ice (800 g). The resulting precipitate was collected and washed with little H_2O, then heated in MeOH for a few min, the mixture cooled, and the precipitate collected, washed with Et_2O and dried; 13.5 g (95%) of **31**. Colorless crystal powder. M.p. > 350°. R_f (CH_2Cl_2/MeOH 9:1) 0.52. UV (MeOH): 272 (sh, 4.00), 255 (4.17). ^{1}H-NMR ((D$_6$)DMSO): 0.90–1.20 (*m*, 28 H, i-Pr); 2.50 (*m*, 1 H−C(2')); 2.65 (*m*, 1 H−C(2')); 3.78 (*m*, H−C(4')); 3.95 (*m*, 2 H−C(5')); 4.68 (*m*, H−C(3')); 6.04 (*dd*, H−C(1')); 6.47 (br. *s*, NH_2); 7.81 (*s*, H−C(8)); 10.63 (br. *s*, NH). Anal. calc. for $C_{22}H_{39}N_5O_5Si_2$ (509.7): C 51.84, H 7.71, N 13.74; found: C 51.41, H 7.42, N 13.71.

27. *2'-Deoxy-O^6-[2-(4-nitrophenyl)ethyl]-3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)guanosine* (**32**). Guanosine **31** (1.8 g, 3.53 mmol) was twice co-evaporated with anh. dioxane. The suspension f **31** in dioxane (30 ml) was treated with 2-(4-nitrophenyl)ethanol (0.67 g, 4 mmol), triphenylphosphine (1.13 g, 4.3 mmol), and diisopropyl azodicarboxylate (0.88 g, 4.3 mmol) at r.t. for 1 h (→ clear soln.). After evaporation the residue was purified by CC (3 × 45 cm, $CHCl_3$/MeOH 24:1); 2.3 g (99%) of **32**. Colorless powder. An anal. pure sample of **32** was obtained by prep. TLC ($CHCl_3$/MeOH 24:1). R_f ($CHCl_3$/MeOH 9:1) 0.8. UV (CH_2Cl_2): 278 (4.32), 253 (4.19). ^{1}H-NMR ($CDCl_3$): 0.98–1.09 (*m*, i-PrSi); 2.51–2.67 (*m*, 2 H−C(2')); 3.27 (*t*, OCH_2CH_2); 3.83–4.07 (*m*, H−C(4'), 2 H−C(5')); 4.70–4.87 (*m*, OCH_2CH_2, NH_2, H−C(3')); 6.18 (*m*, H−C(1')); 7.48 (*d*, H_m to NO_2); 7.81 (*s*, H−C(8)); 8.18 (*d*, H_o to NO_2). Anal. calc. for $C_{30}H_{46}N_6O_7Si_2$ (658.9): C 54.68, H 7.03, N 12.75; found: C 54.89, H 7.09, N 12.52.

28. *N^2,N^2-[2-(2-Carboxyethyl)glutaryl]-2'-deoxy-O^6-[2-(4-nitrophenyl)ethyl]-3'-5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)guanosine* (= *1-{9-{2'-Deoxy-O^6-[2-(4-nitrophenyl)ethyl]-3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-*D*-ribofuranosyl}-9H-purin-2-yl}-2,6-dioxopiperidine-3-propanoic Acid;* **33**). A mixture of **32** (1.2 g, 1.8 mmol) and **16** (1.6 g, 8.6 mmol) in anh. pyridine (22 ml) was stirred for 4 h at 90°. Workup as described for **11** and purification by CC (4 × 30 cm, $CHCl_3$/MeOH 24:1) gave 0.87 g (58%) of **33**. Colorless foam. An anal. pure sample was obtained by prep. TLC ($CHCl_3$/MeOH 24:1). R_f ($CHCl_3$/MeOH 9:1) 0.7. UV (CH_2Cl_2): 261 (4.27). ^{1}H-NMR ($CDCl_3$): 0.91–1.25 (*m*, i-PrSi); 1.60–4.09 (*m*, 2 H−C(2'), OCH_2CH_2, H−C(4'), 2 H−C(5'), $COCH_2CH_2CHCO$, CH_2CH_2COOH); 4.73–4.51 (*m*, OCH_2CH_2, H−C(3')); 6.27 (*m*, H−C(1')); 7.52–7.79 (*m*, H_m to NO_2); 8.13–8.20 (*d*, H_o to NO_2); 8.30, 8.57 (2*s*, H−C(8)); 10.42 (br. COOH). Anal. calc. for $C_{38}H_{54}N_6O_{11}Si_2$ (827.0): C 55.18, H 6.58, N 9.49; found: C 55.01, H 6.63, N 9.49.

29. *3'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**34**). 3'-Deoxy-*N*^{6}-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine [28] (3.77 g, 8.5 mmol) was co-evaporated in anh. pyridine (2 × 20 ml) and then the residue dissolved in the same solvent (80 ml). After addition of 4,4'-dimethoxytrityl chloride (3.45 g, 10 mml), the mixture was stirred at r.t. for 24 h, then quenched with MeOH (5 ml), evaporated, and co-evaporated with toluene (2 × 20 ml). The residue was dissolved in $CHCl_3$ (150 ml), the soln. extracted with sat. $NaHCO_3$ soln.

(2 × 70 ml), the org. phase dried (Na_2SO_4) and evaporated, and the residue purified by FC (4 × 45 cm, toluene/AcOEt/MeOH 5:5:1): 4.82 g (76%) of **34**. Yellowish solid foam. R_f (toluene/AcOEt 3:7) 0.12. UV (MeOH): 276 (sh, 4.42), 267 (4.46), 234 (4.42). ^{1}H-NMR ($CDCl_3$): 2.20–2.33 (*m*, 2 H–C(3′)); 3.14 (*t*, OCH_2CH_2); 3.25 (*m*, 1 H–C(5′)); 3.41 (*m*, 1H–C(5′)); 3.76 (*s*, MeO); 4.53 (*t*, OCH_2CH_2); 4.60–4.92 (*m*, H–C(4′), H–C(2′), OH–C(3′)); 5.96 (*d*, H–C(1′)); 6.76 (*d*, 4 H_o to MeO); 7.14–7.48 (*m*, 11 arom. H); 8.15 (*d*, 2 H_o to NO_2); 8.25 (*s*, H–C(8)); 8.48 (*s*, NH); 8.68 (*s*, H–C(2)). Anal. calc. for $C_{40}H_{38}N_6O_9$ (746.8): C 64.33, H 5.13, N 11.25; found: C 64.52, H 5.19, N 10.92.

30. *3′-Deoxy-5′-O-(4,4′-dimethoxytrityl)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 2′-(2-Cyanoethyl N,N-Diisopropylphosphoramidite)* (**35**). A soln. of **34** (0.746 g, 1 mmol) in abs. MeCN (5 ml) was treated with 2-cyanoethyl tetraisopropyl phosphorodiamidite (0.6 g, 2 mmol) and 1*H*-tetrazole (35 mg, 0.5 mmol) at r.t. for 18 h with stirring. The mixture was then diluted with CH_2Cl_2 (100 ml), the soln. extracted with $NaHCO_3$ soln. (30 ml), the org. layer dried (Na_2SO_4) and evaporated, and the residue purified by FC (4 × 25 cm, toluene (300 ml), toluene/AcOEt 3:7): 0.59 g (63%) of **35**. Colorless solid foam. R_f (toluene/AcOEt 3:7) 0.35 and 0.5. UV (MeOH): 275 (sh, 4.41), 266 (4.47), 235 (4.32). ^{1}H-NMR ($CDCl_3$): 1.10–1.35 (*m*, 14 H, i-Pr); 2.15–2.50 (*m*, 2 H–C(3′)); 2.64 (*t*, CH_2CN); 3.17 (*t*, OCH_2CH_2); 3.30–3.97 (*m*, 2 H–C(5′), MeO, OCH_2CH_2); 4.53 (*t*, OCH_2CH_2); 4.62 (*m*, H–C(4′)); 5.00 (*m*, H–C(2′)); 6.21, 6.29 (2*s*, 1 H, H–C(1′)); 6.85 (*d*, 4 H_o to MeO); 7.21–7.48 (*m*, 11 arom. H); 8.17 (*m*, 3 H_o to NO_2, NH); 8.30 (*s*, H–C(8)); 8.72 (*s*, H–C(2)). ^{31}P-NMR ($CDCl_3$): 149.79, 150.59. Anal. calc. for $C_{49}H_{55}N_8O_{10}P$ (947.0): 62.14, H 5.25, N 11.83; found: C 61.76, H 5.54, N 11.59.

31. *3′-Deoxyadenylyl-(2′–5′)-2′-adenylic Acid 2′{2-[(Adenin-9-yl)methoxy]ethyl} Ester* (**38**). CPG-Solid support loaded with **12** (450 mg of *Bioran-CPG*, 9.9 μmol) was treated subsequently with solns. of 5′-*O*-(4,4′-dimethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]-3′-*O*-[2-(4-nitrophenyl)ethylsulfonyl]adenosine 2′-(2-cyanoethyl *N,N*-diisopropylphosphoramidite) (**36**) [26] and **35** (200 μmol, 20-fold excess, 0.1M in anh. MeCN) in a DNA synthesizer applying the conventional protocol. Condensation time for the 1*H*-tetrazole catalyzed reaction was 2 × 600 s, detritylation was done by 3% CCl_3COOH in CH_2Cl_2, and oxidation of the P^{III}-species by I_2, followed by capping with pyridine/Ac_2O, gave the fully protected support-attached trimer **37**. Deblocking was achieved by 0.1M DBU in MeCN in 10 h. Traces of DBU were removed by washing with 1M aq. NH_4HCO_3. Cleavage from the solid support occurred with 40% aq. $MeNH_2$ soln. The product was isolated by lyophilization. For ^{1}H-NMR investigations, **38** was dissolved and lyophilized 3 times with D_2O to give 9 mg (320 *OD*, 89%) of fluffy colorless material. HPLC: t_R 19.01 min. ^{1}H-NMR (D_2O): 2.53 (*s*, *Me*NH_3); 5.35 (*s*, OCH_2N); 5.88 (*d*, H–C(1′) (A)); 6.05 (*s*, H–C(1′) (d^3A)); 7.82, 7.92, 8.03, 8.04, 8.09, 8.10 (6*s*, H–C(2), H–C(8)). ^{31}P-NMR (D_2O): −0.31 (*s*); −1.36 (*s*). FAB-MS (neg. mode, glycerine matrix): 942 ([*M* + glycerol]$^-$), 850 (M^-), 715 ([*M* − adenine]$^-$), 659 ([*M* − [9-(ethoxymethyl)adenine]]$^-$), 617 ([*M* − d^3A]$^-$).

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