

93. Nucleotides

Part XLVIII¹⁾

Synthesis of 2'-Amino-2'-deoxyarabinonucleoside Phosphoramidite Building Blocks

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Chemical syntheses of 2'-amino-2'-deoxyarabinonucleosides of uracil, thymine, cytosine, adenine, and guanine and their conversion into suitably protected 3'-phosphoramidite building blocks **24–28** for oligonucleotide synthesis are described. The 2-(4-nitrophenyl)ethoxycarbonyl (npeoc) group was used for protection of the aglycon and the 2'-amino functions.

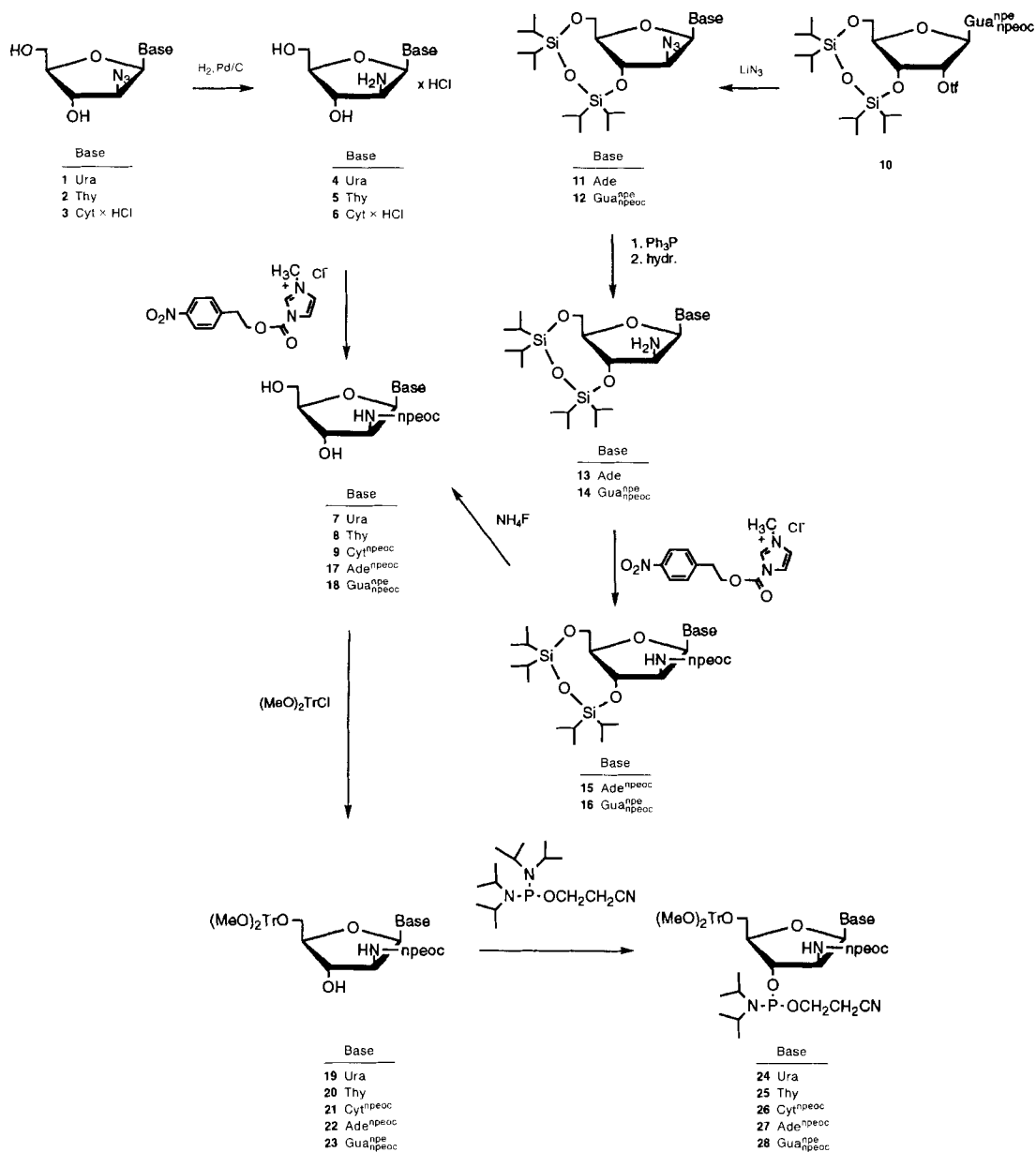
1. Introduction. – Various arabinonucleosides, especially arabinosylcytosine (aC) and arabinosyladenine (aA) have interesting antiviral and antitumor activities [2]. Although aC is a potent drug against human acute myeloblastic leukemia [3–5], its usefulness is limited by its deficient stability against deamination by cytidine deaminase, forming inactive arabinosyluracil (aU). It has been found that 1-(2-azido-2-deoxy- β -D-arabinofuranosyl)cytosine and 1-(2-amino-2-deoxy- β -D-arabinofuranosyl)cytosine are stable against cytidine deaminase, showing similar biological activities as aC [6–8]. Due to the significance of these nucleosides, it may be of interest to introduce 2'-amino-2'-deoxyarabinonucleosides into oligonucleotides, especially because the introduction of 2'-amino-2'-deoxyribonucleosides of uracil and cytosine led to increased resistance against enzymatic degradation by snake-venom phosphodiesterase [9]. On the other hand, this structural modification is, however, also associated with a reduced hybridization of the oligonucleotide strands [10]. So 2'-amino-2'-deoxyarabinooligonucleotides may combine the higher stability against exonucleases with improved ability of hybridization.

For this reason, we would like to report the syntheses of suitably protected 2'-amino-2'-deoxyarabinonucleoside phosphoramidite building blocks. The synthesis of the starting 2'-amino-2'-deoxy- β -D-arabinonucleosides were performed according to literature. Protection of the aglycons and the 2'-amino function was achieved in the usual manner by the 2-(4-nitrophenyl)ethyl (npe) and the 2-(4-nitrophenyl)ethoxycarbonyl (npeoc) group which have already been proven to be very efficient for the synthesis of oligodeoxynucleotides [11] and oligoarabinonucleotides [12].

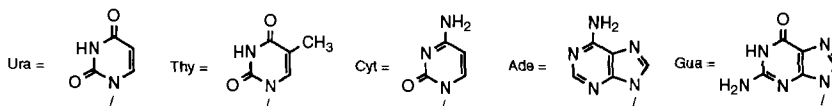
2. Syntheses. – The synthetic approach towards the two series of 2'-amino-2'-deoxyarabinosylpyrimidines and -purines was based on two different strategies. The modified

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Scheme



(MeO)₂Tr = dimethoxytrityl; npe = 2-(4-nitrophenyl)ethyl; npeoc = 2-(4-nitrophenyl)ethoxycarbonyl; tf = trifluoromethylsulfonyl



pyrimidine nucleosides **4–6** were prepared from the corresponding ribonucleosides which were first converted by the procedure of *Matsuda et al.* [13] [14] into 1-(2-azido-2-deoxy- β -D-arabinofuranosyl)uracil (**1**), 1-(2-azido-2-deoxy- β -D-arabinofuranosyl)thymine (**2**), and 1-(2-azido-2-deoxy- β -D-arabinofuranosyl)cytosine (**3**), respectively. The azido group of the latter was then reduced by hydrogenation over Pd/C in HCl/MeOH to yield **4–6** as crystalline products [6] [13] [15]. The subsequent introduction of the 2-(4-nitrophenyl)ethoxycarbonyl (npeoc) group worked best with 3-methyl-1-[2-(4-nitrophenyl)ethoxycarbonyl]-1*H*-imidazol-3-ium chloride [16] as acylating agent. In DMF solution and under 4-(dimethylamino)pyridine catalysis, this reagent protected the 2'-amino group selectively leading to **7** and **8** in yields of 75 and 79%, respectively. In the case of 1-(2-amino-2-deoxy- β -D-arabinofuranosyl)cytosine (**6**), the amino group of the aglycon and the sugar moiety could be blocked simultaneously, if the 3'- and 5'-hydroxy functions were silylated first by refluxing in hexamethyldisilazane (HMDS) to achieve a transient protection [17]. After acylation of the amino groups, the OH functions were desilylated with NH_4F yielding 1-{2-deoxy-2-[2-(4-nitrophenyl)ethoxycarbonylamino]- β -D-arabinofuranosyl}-*N*⁴-[2-(4-nitrophenyl)ethoxycarbonyl]cytosine (**9**) in 65% yield.

The syntheses of the 2'-amino-2'-deoxy- β -D-arabino-*purine* nucleosides were performed from appropriately protected precursors. The *N*²-[2-(4-nitrophenyl)ethoxycarbonyl]-*O*⁶-[2-(4-nitrophenyl)ethyl]-2'-*O*-(trifluoromethylsulfonyl)-3',5'-*O*-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)guanosine (**10**) [18] and 9-[2-azido-2-deoxy-3,5-*O*-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)- β -D-arabinofuranosyl]adenine (**11**) [19] resulted from multistep procedures already described in literature. The trifluoromethylsulfonyl group of **10** was displaced by nucleophilic attack of LiN_3 in DMF yielding 9-[2-azido-2-deoxy-3,5-*O*-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)- β -D-arabinofuranosyl]-*N*²-[2-(4-nitrophenyl)ethoxycarbonyl]-*O*⁶-[2-(4-nitrophenyl)ethyl]guanine (**12**) in 92% yield. Reduction of the azido group of **11** and **12** was performed by *Staudinger* reaction with Ph_3P in THF leading to the iminophosphorane. The following hydrolysis at elevated temperatures gave the 2'-amino compounds **13** and **14**. However, **14** could not be isolated in pure form without contamination of triphenylphosphine oxid. The 2'-amino functions of **13** and **14** were blocked by treatment with 3-methyl-1-[2-(4-nitrophenyl)ethoxycarbonyl]-1*H*-imidazol-3-ium chloride in CH_2Cl_2 to form **15** and **16**. Deprotection of the 3',5'-hydroxy groups with NH_4F in MeOH at 60° led to **17** and **18** in yields of 91 and 79%, respectively.

Reaction of **7–9**, **17**, and **18** with dimethoxytrityl chloride in pyridine gave selectively the 5'-tritylated compounds **19–23** in yields of 80–90%. Conversion into the corresponding 3'-(2-cyanoethyl *N,N*-diisopropylphosphoramidites) **24–28** was achieved by reaction with (2-cyanoethoxy)bis(diisopropylamino)phosphine [20] under 1*H*-tetrazole activation in high yields.

3. Physical Data. – All newly synthesized compounds were characterized in the usual manner by elemental analysis, and UV and ¹H-NMR spectra (see *Exper. Part*).

Experimental Part

General. Products were dried under high vacuum. TLC: precoated silica gel thin-layer sheets 60 F254 from Merck. Flash chromatography (FC): silica gel (Baker, 30–60 μm), 0.3–0.4 bar. M.p.: Gallenkamp melting-point apparatus; no corrections. UV/VIS: Perkin-Elmer, Lambda 15; λ_{max} in nm (log ϵ). $^1\text{H-NMR}$: Bruker AC 250; δ in ppm rel. to Me_4Si .

1. *1-(2-Amino-2-deoxy- β -D-arabinofuranosyl)uracil Hydrochloride (4)* [15]. A suspension of 1-(2-azido-2-deoxy- β -D-arabinofuranosyl)uracil (**1**; 2.69 g, 10 mmol) [14] [15] and 5% Pd/C (200 mg) in halfsat. methanolic HCl (50 ml) was hydrogenated in a shaking apparatus at 1 atm. After 16 h, the mixture was filtered through Celite. The catalyst was washed with $\text{H}_2\text{O}/\text{MeOH}$ 1:1 (3×20 ml). The product precipitated from the combined filtrates as colorless needles. The needles were washed with EtOH and Et_2O : 2.40 g (86%) of **4**. M.p. 200° (dec.). UV (H_2O): 260 (3.98). $^1\text{H-NMR}$ ((D_6) DMSO): 11.40 (s, H–N(3)); 8.55 (s, NH_3^+ –C(2')); 7.90 (d, H–C(6)); 6.12 (s, OH–C(3')); 6.01 (d, H–C(1')); 5.63 (d, H–C(5)); 4.23 (t, H–C(2')); 3.9–3.6 (m, H–C(3'), H–C(4'), 2 H–C(5')). Anal. calc. for $\text{C}_9\text{H}_{13}\text{N}_3\text{O}_5 \cdot \text{HCl} \cdot \text{H}_2\text{O}$ (297.7): C 36.31, H 5.42, N 14.12; found: C 36.26, H 5.44, N 14.10.

2. *1-(2-Amino-2-deoxy- β -D-arabinofuranosyl)thymine Hydrochloride (5)*. As described in *Exper. 1*, with 1-(2-azido-2-deoxy- β -D-arabinofuranosyl)thymine (**2**; 850 mg, 3.0 mmol) [14], 5% Pd/C (40 mg) in MeOH/HCl (40 ml). After 16 h, H_2O (30 ml) was added. After removal of catalyst and solvent, the residue was crystallized from *i*-PrOH: 852 mg (86%) of **5**. M.p. 225° (dec.). UV (H_2O): 266 (3.97). $^1\text{H-NMR}$ ((D_6) DMSO): 11.39 (s, H–N(3)); 8.50 (s, NH_3^+ –C(2')); 7.79 (s, H–C(6)); 6.10 (s, OH–C(3')); 6.05 (d, H–C(1')); 5.72 (s, OH–C(5')); 4.26 (t, H–C(2')); 3.9–3.6 (m, H–C(3'), H–C(4'), 2 H–C(5')); 1.80 (s, Me). Anal. calc. for $\text{C}_{10}\text{H}_{15}\text{N}_3\text{O}_5 \cdot \text{HCl}$ (293.7): C 40.89, H 5.49, N 14.31; found: C 40.90, H 5.55, N 14.06.

3. *1-(2-Amino-2-deoxy- β -D-arabinofuranosyl)cytosine Dihydrochloride (6)* [6] [13]. As described in *Exper. 1*, with 1-(2-azido-2-deoxy- β -D-arabinofuranosyl)cytosine hydrochloride (**3**; 1.60 g, 5.3 mmol) [14], 5% Pd/C (100 mg) in MeOH/HCl (50 ml) and H_2O (20 ml). After 20 h, H_2O (40 ml) was added. After removal of catalyst and solvent, the residue was crystallized from EtOH: 1.38 g (83%) of **6**. M.p. 195° (dec.; [13]: m.p. 192°). UV (H_2O): 269 (3.96), 226 (3.93). $^1\text{H-NMR}$ ((D_6) DMSO): 9.86 (s, NH–C(4)); 8.79 (s, NH–C(4)); 8.63 (s, NH_3^+ –C(2')); 8.25 (d, H–C(6)); 6.20 (d, H–C(5)); 6.00 (d, H–C(1')); 4.23 (t, H–C(2')); 3.97–3.60 (m, H–C(3'), H–C(4'), 2 H–C(5')). Anal. calc. for $\text{C}_9\text{H}_{14}\text{N}_4\text{O}_4 \cdot 2 \text{HCl}$ (315.2): C 34.30, H 5.12, N 17.77; found: C 33.99, H 5.07, N 17.25.

4. *1-{2-Deoxy-2-[2-(4-nitrophenyl)ethoxycarbonylamino]- β -D-arabinofuranosyl]uracil (7)*. A soln. of **4** (1.49 g, 5.0 mmol) and 4-(dimethylamino)pyridine (610 mg, 5.0 mmol) in dry DMF (20 ml) was treated with 4 Å molecular sieve by stirring for 20 min. Then 3-methyl-1-[2-(4-nitrophenyl)ethoxycarbonyl]-1H-imidazol-3-ium chloride (1.71 g, 6.0 mmol) [16] was added, and the mixture was stirred at r.t. for 20 h. The precipitate was filtered off and washed with dry DMF. The combined filtrates were evaporated and purified by FC (toluene/AcOEt 1:1 to toluene/AcOEt/MeOH 5:5:1). The product fractions were concentrated to 50 ml to yield a precipitate: 1.63 g (75%) of **7**. UV (MeOH): 265 (4.31). $^1\text{H-NMR}$ ((D_6) DMSO): 11.30 (s, H–N(3)); 8.21 (d, 2 H *o* to NO_2); 7.72 (d, H–C(6)); 7.60, 7.22 (2d, NH–C(2')); 7.51 (d, 2 H *m* to NO_2); 6.06 (d, H–C(1')); 5.58 (m, H–C(5), OH–C(3')); 5.39 (t, OH–C(5')); 4.3–4.0 (m, OCH_2CH_2 , H–C(2'), H–C(3')); 3.8–3.6 (m, H–C(4'), 2 H–C(5')); 2.98 (m, OCH_2CH_2). Anal. calc. for $\text{C}_{18}\text{H}_{20}\text{N}_4\text{O}_9$ (436.4): C 49.54, H 4.62, N 12.84; found: C 49.38, H 4.88, N 12.38.

5. *1-{2-Deoxy-2-[2-(4-nitrophenyl)ethoxycarbonylamino]- β -D-arabinofuranosyl]thymine (8)*. As described in *Exper. 4*, with **5** (174 mg, 0.6 mmol), 4-(dimethylamino)pyridine (73 mg, 0.6 mmol) and 3-methyl-1-[2-(4-nitrophenyl)ethoxycarbonyl]-1H-imidazol-3-ium chloride (224 mg, 0.72 mmol). FC (toluene/AcOEt 1:1 to toluene/AcOEt/MeOH 5:5:1). The product fractions were concentrated to 20 ml to yield a precipitate: 213 mg (79%) of **8**. UV (MeOH): 269 (4.30). $^1\text{H-NMR}$ ((D_6) DMSO): 11.24 (s, H–N(3)); 8.20 (d, 2 H *o* to NO_2); 7.62 (s, H–C(6)); 7.60–7.41 (m, 2 H *m* to NO_2 , NH–C(2')); 6.02 (d, H–C(1')); 5.54 (d, OH–C(3')); 5.37 (t, OH–C(5')); 4.25–3.98 (m, H–C(2'), H–C(3'), OCH_2CH_2); 3.79–3.54 (m, H–C(4'), 2 H–C(5')); 2.92 (m, OCH_2CH_2); 1.71 (s, Me). Anal. calc. for $\text{C}_{19}\text{H}_{22}\text{N}_4\text{O}_9$ (450.4): C 50.67, H 4.92, N 12.44; found: C 50.52, H 5.04, N 12.27.

6. *1-{2-Deoxy-2-[2-(4-nitrophenyl)ethoxycarbonylamino]- β -D-arabinofuranosyl]-N^d-[2-(4-nitrophenyl)ethoxycarbonyl]cytosine (9)*. A suspension of **6** (315 mg, 1.0 mmol) in hexamethyldisilazane (10 ml) was refluxed for 6 h. After evaporation, the residue was co-evaporated with dry toluene and dissolved in dry CH_2Cl_2 . After addition of 1.24 g (4.0 mmol) of 3-methyl-1-[2-(4-nitrophenyl)ethoxycarbonyl]-1H-imidazol-3-ium chloride, the mixture was stirred at r.t. for 15 h. The precipitate was filtered off and washed with dry CH_2Cl_2 . The combined filtrates were evaporated. The residue was dissolved in MeOH (10 ml), NH_4F (222 mg, 6.0 mmol) added, and after 1 h the solvent evaporated. The residue was purified by FC (toluene/AcOEt 1:1 to AcOEt/MeOH 1:2). The product

fractions were concentrated to 20 ml to yield a precipitate: 410 mg (65%) of **9**. UV (MeOH): 271 (4.39), 250 (4.35). ¹H-NMR ((D₆)DMSO): 11.44 (s, H–N(4)); 8.1 (m, 2 H *o* to NO₂, H–C(6)); 7.59 (d, 2 H *m* to NO₂); 7.48 (d, 2 H *m* to NO₂); 7.01 (s, NH–C(2')); 6.88 (d, H–C(5)); 6.19 (d, H–C(1')); 5.43 (s, OH–C(3')); 5.21 (s, OH–C(5')); 4.39 (t, OCH₂CH₂); 4.24–4.01 (m, H–C(2'), H–C(3'), OCH₂CH₂); 3.82–3.60 (m, H–C(4'), 2 H–C(5')); 3.10 (t, OCH₂CH₂); 2.94 (t, OCH₂CH₂). Anal. calc. for C₂₇H₂₈N₆O₁₂ (628.6): C 51.59, H 4.49, N 13.37; found: C 51.76, H 4.61, N 13.74.

7. 9-[2-Azido-2-deoxy-3,5-O-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)-β-D-arabinofuranosyl]-N²-[2-(4-nitrophenyl)ethoxycarbonyl]-O⁶-[2-(4-nitrophenyl)ethyl]guanine (**12**). A soln. of **10** [18] (13.8 g, 13.8 mmol) in dry DMF (200 ml) was treated with LiN₃ (2.66 g, 54.3 mmol) with stirring at r.t. for 48 h. The solvent was evaporated, the residue dissolved in AcOEt (250 ml), washed with sat. NaHCO₃ soln. (40 ml) and sat. NaCl soln. (40 ml), dried (Na₂SO₄), and evaporated. Purification of the residue by FC (toluene/AcOEt 2:1) gave 11.3 g (92%) of **12**. Amorphous solid. UV (MeOH): 269 (4.54). ¹H-NMR (CDCl₃): 8.21 (m, 4 H *o* to NO₂); 8.00 (s, H–C(8)); 7.48 (d, 2 H *m* to NO₂); 7.39 (d, 2 H *m* to NO₂); 7.27 (s, NH–C(2)); 6.39 (d, H–C(1')); 4.81 (t, OCH₂CH₂); 4.39 (m, OCH₂CH₂, H–C(2'), H–C(3')); 4.12 (m, 2 H–C(5')); 3.85 (m, H–C(4')); 3.29 (t, OCH₂CH₂); 3.13 (t, OCH₂CH₂); 1.2–1.0 (m, 4 Me₂CH). Anal. calc. for C₃₉H₅₂N₁₀O₁₁Si₂ (893.1): C 52.45, H 5.87, N 15.68; found: C 52.30, H 5.93, N 15.35.

8. 9-[2-Amino-2-deoxy-3,5-O-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)-β-D-arabinofuranosyl]adenine (**13**). To a soln. of **11** [19] (4.06 g, 7.6 mmol) in THF (100 ml) was added with stirring Ph₃P (4.97 g, 19.0 mmol) which led to a spontaneous formation of N₂. After 2½ h, H₂O (5 ml) was added and stirring continued at 50° for 24 h. The mixture was diluted with AcOEt (150 ml), dried (Na₂SO₄), and evaporated. Hexane (15 ml) was added to the residue. Triphenylphosphine oxid crystallized as colorless needles. After 4 h, the needles were filtered off and washed with cold hexane. The combined filtrate was evaporated and the residue purified by FC (toluene/AcOEt 3:1 to AcOEt/MeOH 9:1): 3.18 g (82%) of **13**. Amorphous solid. UV (MeOH): 259 (4.17). ¹H-NMR ((D₆)DMSO): 8.11, 8.05 (2s, H–C(2), H–C(8)); 7.29 (s, NH₂–C(6)); 6.11 (d, H–C(1')); 4.68 (d, H–C(3')); 4.06 (dd, H–C(2')); 3.83 (dd, H–C(5')); 3.75 (m, H–C(4'), H–C(5')); 1.56 (s, NH₂–C(2')); 1.2–1.0 (m, 4 Me₂CH). Anal. calc. for C₂₂H₄₀N₆O₄Si₂ (508.8): C 51.94, H 7.92, N 16.52; found: C 51.49, H 7.80, N 16.49.

9. 9-{2-Deoxy-2-[2-(4-nitrophenyl)ethoxycarbonylamino]-3,5-O-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)-β-D-arabinofuranosyl]-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenine (**15**). A mixture of **13** (3.05 g, 6.0 mmol), 4-(dimethylamino)pyridine (732 mg, 6.0 mmol), and 3-methyl-1-[2-(4-nitrophenyl)ethoxycarbonyl]-1H-imidazol-3-ium chloride (7.48 g, 24.0 mmol) was stirred in dry CH₂Cl₂ (150 ml) at r.t. for 24 h. The solid was filtered off, the filtrate washed with sat. NaHCO₃ soln. (30 ml) and sat. NaCl soln. (30 ml), dried (Na₂SO₄), and evaporated. Purification of the residue by FC (toluene/AcOEt 3:2 to 2:3) gave 3.93 g (73%) of **15**. Amorphous solid. UV (MeOH): 267 (4.63). ¹H-NMR (CDCl₃): 8.68 (s, H–C(2) or H–C(8)); 8.18 (d, H–C(2) or H–C(8), 2 H *o* to NO₂); 8.06 (d, NH–C(6), 2 H *o* to NO₂); 7.46 (d, 2 H *m* to NO₂); 7.12 (d, 2 H *m* to NO₂); 6.22 (d, H–C(1')); 5.21 (d, NH–C(2')); 5.07 (t, H–C(2')); 4.83 (t, H–C(3')); 4.56 (t, OCH₂CH₂); 4.18 (t, OCH₂CH₂); 4.0–3.8 (m, H–C(4'), 2 H–C(5')); 3.19 (t, OCH₂CH₂); 2.86 (t, OCH₂CH₂); 1.2–1.0 (m, 4 Me₂CH). Anal. calc. for C₄₀H₅₄N₈O₁₂Si₂ (895.1): C 53.67, H 6.08, N 12.52; found: C 53.36, H 6.08, N 12.38.

10. 9-{2-Deoxy-2-[2-(4-nitrophenyl)ethoxycarbonylamino]-3,5-O-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)-β-D-arabinofuranosyl]-N²-[2-(4-nitrophenyl)ethoxycarbonyl]-O⁶-[2-(4-nitrophenyl)ethyl]guanine (**16**). A soln. of **12** (4.47 g, 5.0 mmol) and Ph₃P (3.28 g, 12.5 mmol) in THF (50 ml) reacted under spontaneous formation of N₂. After stirring for 2 h, H₂O (5 ml) was added and the mixture refluxed for 24 h. The mixture was diluted with AcOEt (70 ml), dried (Na₂SO₄), and evaporated. The residue was purified by FC (toluene/AcOEt) to give on evaporation crude **14**, which was dried under high vacuum. To a soln. of crude **14** in dry CH₂Cl₂ (100 ml), 4-(dimethylamino)pyridine (610 mg, 5.0 mmol) and 3-methyl-1-[2-(4-nitrophenyl)ethoxycarbonyl]-1H-imidazol-3-ium chloride (2.5 g, 8.0 mmol) were added. The mixture was stirred at r.t. for 17 h, then washed with sat. NaHCO₃ soln. (20 ml) and sat. NaCl soln. (20 ml), dried (Na₂SO₄), and evaporated. Purification of the residue by FC (toluene toluene/AcOEt 1:2) gave 4.2 g (79%) of **16**. Amorphous solid. UV (MeOH): 269 (4.63). ¹H-NMR ((D₆)DMSO): 10.51 (s, NH–C(2)); 8.12 (m, 4 H *o* to NO₂, H–C(8)); 7.98 (d, 2 H *o* to NO₂); 7.58 (m, NH–C(2'), 4 H *m* to NO₂); 7.23 (d, 2 H *m* to NO₂); 6.22 (d, H–C(1')); 4.8–3.8 (m, 3 OCH₂CH₂, H–C(2'), H–C(3'), H–C(4'), 2 H–C(5')); 3.30 (t, OCH₂CH₂); 3.14 (t, OCH₂CH₂); 2.82 (t, OCH₂CH₂); 1.2–0.8 (m, 4 Me₂CH). Anal. calc. for C₄₈H₆₁N₉O₁₃Si₂ (1060.3): C 54.37, H 5.80, N 11.89; found: C 54.14, H 5.88, N 11.89.

11. 9-{2-Deoxy-2-[2-(4-nitrophenyl)ethoxycarbonylamino]-β-D-arabinofuranosyl]-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenine (**17**). To a soln. of **15** (895 mg, 1.0 mmol) in MeOH (40 ml), NH₄F (520 mg, 14 mmol) was

added and the mixture stirred at 60° for 90 min. The solvent was evaporated, the residue treated with DMF (15 ml), the insoluble material filtered off, and the filtrate dropped into ice-water (100 ml) to yield a fine, colorless solid. After filtration, the product was washed several times with H₂O and dried *in vacuo* over P₄O₁₀: 592 mg (91%). UV (MeOH): 267 (4.59). ¹H-NMR ((D₆)DMSO): 10.56 (s, NH–C(6)); 8.54 (s, H–C(2), H–C(8)); 8.15 (d, 2 H *o* to NO₂); 8.03 (d, 2 H *o* to NO₂); 7.59 (d, 2 H *m* to NO₂); 7.50 (d, NH–C(2')); 7.21 (d, 2 H *m* to NO₂); 6.41 (d, H–C(1')); 5.56 (d, OH–C(3')); 5.27 (t, OH–C(5')); 4.40 (m, 2 OCH₂CH₂); 4.16 (m, H–C(2')); 4.00 (m, H–C(3')); 3.8–3.6 (m, H–C(4'), 2 H–C(5')); 3.13 (t, OCH₂CH₂); 2.75 (m, OCH₂CH₂). Anal. calc. for C₂₈H₂₈N₈O₁₁ (652.6): C 51.53, H 4.32, N 17.17; found: C 51.22, H 4.44, N 17.18.

12. 9-[2-Deoxy-2-[2-(4-nitrophenyl)ethoxycarbonylamino]-β-D-arabinofuranosyl]-N²-[2-(4-nitrophenyl)ethoxycarbonyl]-O⁶-[2-(4-nitrophenyl)ethyl]guanine (18). A soln. of 4.2 g (3.69 mmol) of 16 in MeOH (50 ml) was treated with NH₄F (888 mg, 24 mmol) by stirring at 60° for 2 h. The solvent was evaporated and the residue purified by FC (toluene/AcOEt 1:1 to AcOEt/MeOH 10:1). The product fractions were concentrated to 50 ml to yield a light yellowish precipitate: 2.56 g (79%) of 18. UV (MeOH): 269 (4.60). ¹H-NMR ((D₆)DMSO): 10.42 (s, NH–C(2)); 8.28 (s, H–C(8)); 8.11 (m, 4 H *o* to NO₂); 7.99 (d, 2 H *o* to NO₂); 7.61 (m, 4 H *m* to NO₂); 7.40 (m, NH–C(2')); 7.23 (d, 2 H *m* to NO₂); 6.21 (d, H–C(1')); 5.54 (d, OH–C(3')); 5.15 (t, OH–C(5')); 4.78 (t, OCH₂CH₂); 4.33 (m, 2 OCH₂CH₂); 4.09 (m, H–C(2')); 3.93 (m, H–C(3')); 3.7–3.5 (m, H–C(4'), 2 H–C(5')); 3.31 (t, OCH₂CH₂); 3.11 (t, OCH₂CH₂); 2.75 (t, OCH₂CH₂). Anal. calc. for C₃₆H₃₅N₉O₁₄ (817.7): C 52.88, H 4.31, N 15.42; found: C 52.62, H 4.36, N 14.96.

13. 1-[2-Deoxy-5-O-(dimethoxytrityl)-2-[2-(4-nitrophenyl)ethoxycarbonylamino]-β-D-arabinofuranosyl]-uracil (19). In dry pyridine, 7 (873 mg, 2.0 mmol) was co-evaporated 3 times and then dissolved in dry pyridine (30 ml). After addition of (MeO)₂TrCl (709 mg, 2.1 mmol), the mixture was stirred at r.t. for 24 h. The solvent was evaporated, the residue diluted with AcOEt (70 ml), and the soln. washed with sat. NaHCO₃ soln. (20 ml) and sat. NaCl soln. (20 ml), dried (Na₂SO₄), and evaporated. Purification by FC (toluene to toluene/AcOEt/MeOH 5:5:1) and co-evaporation with CH₂Cl₂ gave 1.26 g (85%) of 19. Amorphous solid. UV (MeOH): 266 (4.35), 233 (4.46). ¹H-NMR (CDCl₃): 10.1, 9.6 (2s, H–N(3)); 8.1, 8.0 (2d, 2 H *o* to NO₂); 7.82 (d, H–C(6)); 7.4–7.1 (m, 9 H of Tr, 2 H *m* to NO₂); 6.81 (m, 4 H *o* to MeO); 6.31 (m, H–C(1')); 6.09 (m, NH–C(2')); 5.30 (m, H–C(5)); 4.4–4.1 (m, H–C(2'), H–C(3'), OCH₂CH₂); 3.91 (m, H–C(4')); 3.8–3.5 (m, 2 MeO, 2 H–C(5')); 3.03 (t, OH–C(3')); 2.86 (m, OCH₂CH₂). Anal. calc. for C₃₉H₃₈N₄O₁₁ (738.8): C 63.41, H 5.18, N 7.58; found: C 63.11, H 5.43, N 7.49.

14. 1-[2-Deoxy-5-O-(dimethoxytrityl)-2-[2-(4-nitrophenyl)ethoxycarbonylamino]-β-D-arabinofuranosyl]-thymine (20). As described in *Exper. 13*, with 8 (950 mg, 2.11 mmol) and (MeO)₂TrCl (749 mg, 2.21 mmol) in dry pyridine (30 ml). FC (toluene to toluene/AcOEt 1:3) gave 1.41 g (89%) of 20. Amorphous solid. UV (MeOH): 271 (4.29), 232 (4.39). ¹H-NMR ((D₆)DMSO): 11.30 (s, H–N(3)); 8.19 (d, 2 H *o* to NO₂); 7.68 (d, NH–C(2')); 7.51–7.24 (m, Tr, *m* to NO₂, H–C(6)); 6.90 (d, 4 H *o* to MeO); 6.18 (d, H–C(1')); 5.60 (d, OH–C(3')); 4.35–4.11 (m, H–C(2'), H–C(3'), OCH₂CH₂); 3.83 (m, H–C(4')); 3.75 (s, 2 MeO); 3.32 (m, 2 H–C(5')); 2.99 (m, OCH₂CH₂); 1.56 (s, Me). Anal. calc. for C₄₀H₄₀N₄O₁₁ (752.8): C 63.82, H 5.36, N 7.44; found: C 63.76, H 5.31, N 7.14.

15. 1-[2-Deoxy-5-O-(dimethoxytrityl)-2-[2-(4-nitrophenyl)ethoxycarbonylamino]-β-D-arabinofuranosyl]-N⁴-[2-(4-nitrophenyl)ethoxycarbonyl]cytosine (21). As described in *Exper. 13*, with 9 (319 mg, 0.5 mmol) and (MeO)₂TrCl (177 mg, 0.525 mmol) in dry pyridine (10 ml). FC (toluene/AcOEt 1:1 to toluene/AcOEt/MeOH 10:10:1) gave 370 mg (85%) of 21. Amorphous solid. UV (MeOH): 273 (4.40), 237 (4.50). ¹H-NMR ((D₆)DMSO): 10.78 (s, NH–C(4)); 8.15 (m, 4 H *o* to NO₂, H–C(6)); 7.6–7.2 (m, 9 H of Tr, NH–C(2'), 2 H *m* to NO₂); 6.90 (d, 4 H *o* to MeO); 6.75 (d, H–C(5)); 6.21 (d, H–C(1')); 5.62 (s, OH–C(3')); 4.35 (m, H–C(2'), 2 OCH₂CH₂); 4.04 (m, H–C(3')); 3.82 (m, H–C(4')); 3.73 (s, 2 MeO); 3.32 (m, 2 H–C(5')); 3.06 (t, OCH₂CH₂); 2.92 (m, OCH₂CH₂). Anal. calc. for C₄₈H₄₆N₆O₁₄ (930.9): C 61.93, H 4.98, N 9.03; found: C 61.87, H 5.02, N 9.13.

16. 9-[2-Deoxy-5-O-(dimethoxytrityl)-2-[2-(4-nitrophenyl)ethoxycarbonylamino]-β-D-arabinofuranosyl]-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenine (22). As described in *Exper. 13*, with 17 (2.0 g, 3.06 mmol) and (MeO)₂TrCl (1.09 g, 3.22 mmol) in dry pyridine (30 ml). FC (toluene/AcOEt 2:1 to toluene/AcOEt/MeOH 5:5:1) gave 2.34 g (83%) of 22. Amorphous solid. UV (MeOH): 267 (4.58), 235 (4.47). ¹H-NMR (CDCl₃): 8.67 (s, H–C(2) or H–C(8)); 8.23 (m, H–C(2) or H–C(8), 2 H *o* to NO₂); 8.11 (s, NH–C(6)); 8.02 (d, 2 H *o* to NO₂); 7.5–7.2 (m, 9 H of Tr, 2 H *m* to NO₂); 7.06 (d, 2 H *m* to NO₂); 6.76 (m, 4 H *o* to MeO); 6.38 (d, H–C(1')); 5.85 (d, NH–C(2')); 4.79 (m, H–C(2')); 4.66 (m, H–C(3'), OCH₂CH₂); 4.03 (m, OCH₂CH₂, H–C(4')); 3.78 (s, 2 MeO); 3.52 (m, 2 H–C(5')); 3.17 (t, OH–C(3'), OCH₂CH₂); 2.78 (t, OCH₂CH₂). Anal. calc. for C₄₉H₄₆N₈O₁₃ (955.0): C 61.63, H 4.86, N 11.73; found: C 61.71, H 4.90, N 11.52.

17. 9-[2-Deoxy-5-O-(dimethoxytrityl)-2-[2-(4-nitrophenyl)ethoxycarbonylamino]-β-D-arabinofuranosyl]-N²-[2-(4-nitrophenyl)ethoxycarbonyl]-O⁶-[2-(4-nitrophenyl)ethyl]guanine (23). As described in *Exper. 13*, with

18 (2.04 g, 2.5 mmol) and (MeO)₂TrCl (890 mg, 2.63 mmol) in dry pyridine (50 ml). FC (toluene/AcOEt 3:1 to AcOEt) gave 2.52 g (90%) of **23**. Amorphous solid. UV (MeOH): 269 (4.63), 236 (4.51). ¹H-NMR (CDCl₃): 8.0 (*m*, 4 H *o* to NO₂); 7.87 (*m*, H–C(8), 2 H *o* to NO₂); 7.43 (*s*, NH–C(2)); 7.38 (*d*, 2 H *m* to NO₂); 7.2–6.9 (*m*, 9 H of Tr, 4 H *m* to NO₂); 6.59 (*m*, 4 H *o* to MeO); 6.45 (*d*, NH–C(2')); 6.12 (*d*, H–C(1')); 5.12 (*m*, H–C(2')); 4.6 (*m*, H–C(3'), OCH₂CH₂); 4.29 (*t*, OCH₂CH₂); 4.0–3.8 (*m*, H–C(4'), OCH₂CH₂); 3.61 (*s*, 2 MeO); 3.2 (*m*, 2 H–C(5'), OCH₂CH₂); 2.90 (*m*, OH–C(3'), OCH₂CH₂); 2.68 (*t*, OCH₂CH₂). Anal. calc. for C₅₇H₅₃N₉O₁₆ (1120.1): C 61.12, H 4.77, N 11.25; found: C 60.81, H 4.88, N 11.05.

18. 1-{2-Deoxy-5-O-(dimethoxytrityl)-2-[2-(4-nitrophenyl)ethoxycarbonylamino]-β-D-arabinofuranosyl}-uracil 3'-(2-Cyanoethyl N,N-Diisopropylphosphoramidite) (**24**). A mixture of **19** (960 mg, 1.3 mmol), (2-cyanoethoxy)bis(diisopropylamino)phosphine (588 mg, 1.95 mmol), and 1*H*-tetrazole (45 mg, 0.65 mmol) was stirred in dry CH₂Cl₂ (20 ml) under N₂ at r.t. for 24 h. After dilution with CH₂Cl₂ (60 ml), the soln. was washed with sat. NaHCO₃ soln. (20 ml) and sat. NaCl soln. (20 ml), dried (Na₂SO₄), and evaporated. The residue was purified by FC (toluene to toluene/AcOEt 1:1 with addition of 0.2% of Et₃N). The product fractions were evaporated and co-evaporated with dry CH₂Cl₂: 1.06 g (87%) of **24**. Amorphous solid. UV (MeOH): 265 (4.33), 234 (4.40). ¹H-NMR (CDCl₃): 10.3, 9.1 (2*s*, H–N(3)); 8.1 (2*d*, 2 H *o* to NO₂); 7.92, 7.79 (2*d*, H–C(6)); 7.4–7.2 (*m*, 9 H of Tr, 2 H *m* to NO₂); 6.86 (*m*, 4 H *o* to MeO); 6.54, 6.41 (2*d*, H–C(1')); 6.18 (*m*, NH–C(2')); 5.28 (*m*, H–C(5)); 4.7–4.4 (*m*, H–C(2'), H–C(3')); 4.2 (*m*, OCH₂CH₂); 4.0 (*m*, H–C(4')); 3.8–3.3 (*m*, 2 MeO, 2 H–C(5'), 2 Me₂CH, OCH₂CH₂CN); 3.1–2.9 (*m*, OCH₂CH₂); 2.62, 2.39 (2*t*, OCH₂CH₂CN); 1.3–0.9 (*m*, 2 Me₂CH). Anal. calc. for C₄₈H₅₅N₆O₁₂P (939.0): C 61.40, H 5.90, N 8.95; found: C 60.98, H 6.11, N 9.08.

19. 1-{2-Deoxy-5-O-(dimethoxytrityl)-2-[2-(4-nitrophenyl)ethoxycarbonylamino]-β-D-arabinofuranosyl}-thymine 3'-(2-Cyanoethyl N,N-Diisopropylphosphoramidite) (**25**). As described in *Exper. 18*, with **20** (1.43 g, 1.9 mmol), (2-cyanoethoxy)bis(diisopropylamino)phosphine (900 mg, 3.0 mmol), and 1*H*-tetrazole (70 mg, 1.0 mmol) in dry CH₂Cl₂ (15 ml). FC (toluene to toluene/AcOEt 1:2 with addition of 0.2% of Et₃N) gave 1.54 g (85%) of **25**. Amorphous solid. UV (MeOH): 269 (4.31), 232 (4.41). ¹H-NMR (CDCl₃): 8.61 (*s*, H–N(3)); 8.1, 8.0 (2*d*, 2 H *o* to NO₂); 7.51 (*s*, H–C(6)); 7.4–7.1 (*m*, 9 H of Tr, 2 H *m* to NO₂); 6.82 (*m*, 4 H *o* to MeO); 6.08 (*d*, H–C(1')); 6.0 (*m*, NH–C(2')); 4.5–4.0 (*m*, H–C(2'), H–C(3'), H–C(4'), OCH₂CH₂); 3.79 (*s*, 2 MeO); 3.8–3.3 (*m*, 2 H–C(5'), 2 Me₂CH, OCH₂CH₂CN); 3.11, 2.92 (2*t*, OCH₂CH₂); 2.58, 2.39 (2*t*, OCH₂CH₂CN); 1.65 (*s*, Me–C(5)); 1.3–0.9 (*m*, 2 Me₂CH). Anal. calc. for C₄₉H₅₇N₆O₁₂P (953.0): C 61.76, H 6.03, N 8.82; found: C 61.91, H 6.07, N 8.60.

20. 1-{2-Deoxy-5-O-(dimethoxytrityl)-2-[2-(4-nitrophenyl)ethoxycarbonylamino]-β-D-arabinofuranosyl}-N⁴-[2-(4-nitrophenyl)ethoxycarbonyl]cytosine 3'-(2-Cyanoethyl N,N-Diisopropylphosphoramidite) (**26**). As described in *Exper. 18*, with **21** (343 mg, 0.368 mmol), (2-cyanoethoxy)bis(diisopropylamino)phosphine (174 mg, 0.575 mmol), and 1*H*-tetrazole (13 mg, 0.192 mmol) in dry CH₂Cl₂ (5 ml). FC (toluene/AcOEt 2:1 to 1:3 with addition of 0.2% of Et₃N) gave 347 mg (83%) of **26**. Amorphous solid. UV (MeOH): 272 (4.42), 234 (4.59). ¹H-NMR (CDCl₃): 8.15 (*d*, 2 H *o* to NO₂); 8.02 (*d*, 2 H *o* to NO₂); 7.91 (*d*, H–C(6)); 7.55 (*s*, NH–C(4)); 7.40 (*d*, 4 H *m* to NO₂); 7.25 (*m*, 9 H of Tr); 7.10 (*d*, H–C(5)); 6.80 (*d*, 4 H *o* to MeO); 6.18 (*d*, H–C(1')); 5.59 (*m*, NH–C(2')); 4.64 (*m*, H–C(2')); 4.42 (*t*, H–C(3'), OCH₂CH₂); 4.19 (*m*, H–C(4')); 4.02 (*m*, OCH₂CH₂); 3.78 (*s*, 2 MeO); 3.7–3.4 (*m*, 2 H–C(5'), 2 Me₂CH, OCH₂CH₂CN); 3.21 (*t*, OCH₂CH₂); 2.79 (*t*, OCH₂CH₂); 2.58, 2.37 (2*t*, OCH₂CH₂CN); 1.3–0.9 (*m*, 2 Me₂CH). Anal. calc. for C₅₇H₆₃N₈O₁₅P (1131.2): C 60.52, H 5.61, N 9.91; found: C 60.81, H 5.97, N 9.83.

21. 9-{2-Deoxy-5-O-(dimethoxytrityl)-2-[2-(4-nitrophenyl)ethoxycarbonylamino]-β-D-arabinofuranosyl}-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenine 3'-(2-Cyanoethyl N,N-Diisopropylphosphoramidite) (**27**). As described in *Exper. 18*, with **22** (955 mg, 1.0 mmol), (2-cyanoethoxy)bis(diisopropylamino)phosphine (452 mg, 1.5 mmol), and 1*H*-tetrazole (35 mg, 0.5 mmol) in dry CH₂Cl₂ (10 ml). FC (toluene/AcOEt 1:1 to 1:2 with addition of 0.2% of Et₃N) gave 1.03 g (89%) of **27**. Amorphous solid. UV (MeOH): 266 (4.58), 235 (4.50). ¹H-NMR (CDCl₃): 8.69 (2*s*, H–C(2) or H–C(8)); 8.2 (*m*, NH–C(6), H–C(2) or H–C(8), 2 H *o* to NO₂); 8.0 (*d*, 2 H *o* to NO₂); 7.5–7.2 (*m*, 9 H of Tr, 2 H *m* to NO₂); 7.08 (*d*, 2 H *m* to NO₂); 6.79 (*m*, 4 H *o* to MeO); 6.41 (2*d*, H–C(1')); 5.87 (2*d*, NH–C(2')); 4.82 (*m*, H–C(2')); 4.70 (*m*, H–C(3')); 4.56 (*t*, OCH₂CH₂); 4.2–4.0 (*m*, OCH₂CH₂, H–C(4')); 3.79 (*s*, 2 MeO); 3.7–3.4 (*m*, 2 H–C(5'), 2 Me₂CH, OCH₂CH₂CN); 3.20 (*t*, OCH₂CH₂); 2.78 (*t*, OCH₂CH₂); 2.56, 2.22 (2*t*, OCH₂CH₂CN); 1.2–1.0 (*m*, 2 Me₂CH). Anal. calc. for C₅₈H₆₃N₁₀O₁₄P (1155.2): C 60.31, H 5.50, N 12.13; found: C 60.07, H 5.54, N 11.84.

22. 9-{2-Deoxy-5-O-(dimethoxytrityl)-2-[2-(4-nitrophenyl)ethoxycarbonylamino]-β-D-arabinofuranosyl}-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]-O⁶-[2-(4-nitrophenyl)ethyl]guanine 3'-(2-Cyanoethyl N,N-Diisopropylphosphoramidite) (**28**). As described in *Exper. 18*, with **23** (560 mg, 0.5 mmol), (2-cyanoethoxy)bis(diisopropylamino)phosphine (226 mg, 0.75 mmol), and 1*H*-tetrazole (17 mg, 0.25 mmol) in dry CH₂Cl₂ (15 ml). FC (toluene to

toluene/AcOEt 1:2 with addition of 0.2% of Et₃N) gave 568 mg (86%) of **28**. Amorphous solid. UV (MeOH): 269 (4.64), 236 (4.53). ¹H-NMR (CDCl₃): 8.16 (t, 4 H o to NO₂); 7.96 (m, H-C(8), 2 H o to NO₂); 7.5–7.0 (m, 9 H of Tr, 6 H m to NO₂); 6.67 (m, 4 H o to MeO); 6.32 (m, H-C(1')); 6.07 (m, NH-C(2')); 5.0 (m, H-C(2')); 4.7 (m, OCH₂CH₂, H-C(3')); 4.41 (m, OCH₂CH₂); 4.1 (m, OCH₂CH₂, H-C(4')); 3.71 (s, 2 MeO); 3.6–3.4 (m, 2 H-C(5'), 2 Me₂CH, OCH₂CH₂CN); 3.30 (t, OCH₂CH₂); 3.10 (m, OCH₂CH₂); 2.71 (t, OCH₂CH₂); 2.56, 2.22 (2t, OCH₂CH₂CN); 1.15–0.95 (m, 2 Me₂CH). Anal. calc. for C₆₆H₇₀N₁₁O₁₇P (1320.3): C 60.04, H 5.34, N 11.67; found: C 59.85, H 5.37, N 11.40.

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