Electronic Supplementary Information

2'-*O*-,3'-*O*-,5'-*O*-triacetyladenosine 6.

4.0 g adenosine **5** (15 mmol) were coevaporated twice with dried pyridine (20 mL) and then suspended in 50 mL of dried pyridine. The mixture was cooled in an ice bath and 30 mg 4-dimethylaminopyridine (0.2 mmol) followed by 15 mL of acetanhydride (61 mmol) were added. After some minutes the ice bath was removed. The clear solution was stirred overnight. The next day water was added and the solvent was removed *in vacuo*. The residue was dissolved in 50 mL of $CHCl_3$. The organic phase was washed with saturated $NaHCO_3$-solution and brine and dried over Na_2SO_4. The solvent was removed *in vacuo*. The tetraacetylated byproduct was removed chromatographically (solvent chloroform:methanol = 95:5) and left 2.9 g of **6** as a colourless solid (7.5 mmol, 50%); δ_H(400 MHz; DMSO-d_6; Me_4Si) 2.02, 2.05, 2.12 [9 H, 3 x s, 3 x (C=O)-CH_3], 4.25 (1 H, dd, *J* 11.7 and 5.3, *H*-5'), 4.35-4.44 (2 H, m, *H*-4', *H*-5'), 5.64 (1 H, m, *H*-3'), 6.04 (1 H, m, *H*-2'), 6.21 (1 H, d, *J* 5.4, *H*-1'), 7.36 (2 H, s, NH_2), 8.18 (1 H, s, *H*-2), and 8.35 (1 H, s, *H*-8); δ_C(400 MHz; DMSO-d_6; Me_4Si) 20.1, 20.2, 20.3 [3 x (C=O)-*C*H_3], 62.7 (*C*-5'), 70.0 (*C*-3'), 71.8 (*C*-2'), 79.3 (*C*-4'), 85.5 (*C*-1'), 119.9 (*C*-5), 139.9 (*C*-8), 149.0 (*C*-4), 152.7 (*C*-2), 156.1 (*C*-6), 169.1, 169.3, and 169.9 [3 x (*C*=O)-CH_3]; FAB^+-MS: calculated for $C_{16}H_{19}N_5O_7$ [M + H^+]: 394.3; found: 394.1.

DMT-hexaethylene glycol.

13 mL of hexaethylene glycol (14 g, 51 mmol) were coevaporated (20 mL) and dissolved in 100 mL of dried pyridine. 6 g 4-dimethylaminopyridine (5 mmol) and 50 mL triethylamine (40 g, 390 mmol) were added, followed by 19 g 4,4'-dimethoxytrityl chloride which were dissolved in 50 mL dried pyridine. The solution was stirred overnight and 50 mL H_2O were added. Workup and chromatographic purification ($CHCl_3$ → $CHCl_3$:MeOH = 95:5) were carried out as described for **12**. Mono-DMT-protected hexaethyene glycol was obtained as a yellow oil (13 g, 21 mmol, 41%); $\mathbf{R_f}$ [$CHCl_3$:MeOH = 95:5] = 0.5

N^2- isobutyrylguanosine 17.

14.2 g guanosine **16** (50 mmol) were coevaporated twice with 200 mL of dry pyridine and suspended in 500 mL of dry pyridine. The suspension was cooled in an ice bath and 50 mL of trimethylchlorosilane (43 g, 400 mmol) were added. After 1 h, 80 mL of isobutyric anhydride (76 g, 480 mmol) were added, and the mixture was stirred for 2 h. Then, 100 mL of ice were added, and after further 15 min 100 mL of aqueous ammonium hydroxide (25%). The solution was extracted with diethylether (2 x 500 mL) and concentrated *in vacuo* to 100 mL. **17** crystallised at 4 °C and was filtered off to yield 4.6 g (13 mmol, 26%) of colourless crystals; δ_H(400 MHz; DMSO-d_6; Me_4Si) 1.11 [6 H, d, *J* 6.8, (C=O)-CH-(CH_3)$_2$], 2.79 [1 H, sept., *J* 6.8, (C=O)-C*H*-(CH_3)$_2$], 3.54 (1 H, dd, *J* 11.9 and 4.1, *H*-5'), 3.63 (1 H, dd, *J* 11.9 and 4.1, *H*-5'), 3.90-3.93 (m, 1 H, *H*-4'), 4.14-4.16 (1 H, m, *H*-3'), 4.43-4.45 (1 H, m, *H*-2'), 5.80 (1 H, d, *J* 6.0, *H*-1'), and 8.27 (1 H, s, *H*-8); δ_C(400 MHz; DMSO-d_6; Me_4Si) 18.8 (2 x *C*H_3), 34.7 [(C=O)-*C*H-(CH_3)$_2$], 61.0 (*C*-5'), 70.1 (*C*-2'), 73.9 (*C*-3'), 85.2 (*C*-4'), 86.5 (*C*-1'), 119.9 (*C*-5), 137.6 (*C*-8), 148.0 (*C*-4), 148.7 (*C*-2), 155.0 (*C*-6), and 180.1 [(*C*=O)-CH-(CH_3)$_2$]; FAB^+-MS: calculated for $C_{14}H_{19}N_5O_6$ [M + H^+]: 354.3; found: 354.1.

5'-*O*-(4,4'-dimethoxytrityl)-N^6-(3,6-dioxa-8-*N*'-BOC-aminooctyl)adenosine 11.

1.0 g **8** (2.2 mmol) were coevaporated with 10 mL of dried pyridine and dissolved in 20 mL of dried pyridine. 0.6 mL triethylamine (0.9 g, 8 mmol) and 30 mg 4-dimethylaminopyridine (0.2 mmol) were added. 0.7 g 4,4'-dimethoxytrityl chloride (3 mmol) were dissolved in 15 mL of dry pyridine and added drop by drop. The solution was stirred overnight at room temperature. The solvent was removed, the residue was worked up as described for **6**, and purified chromatographically (silica gel, chloroform → chloroform:methanol = 95:5). 5'-*O*-(4,4'-dimethoxytrityl)-N^6-(3,6-dioxa-8-*N*'-BOC-aminooctyl)adenosine **11** was obtained as a colourless oil (1.2 g, 1.5 mmol, 76%); δ_H(400 MHz; DMSO-d_6; Me_4Si) 1.36 (9 H, s, BOC), 3.08, 3.23, 3.35, 3.50, 3.54, 3.61 (14 H, 6 x m, ether-*H*, *H*-5'), 3.73 (6 H, s, 2 x phenyl-O-CH_3), 4.08 (1 H, m, *H*-4'), 4.32 (1 H, m, *H*-3'), 4.70 (1 H, m, *H*-2'), 5.19 (1 H, d, *J* 5.8, O*H*), 5.51 (1 H, d, *J* 5.6, O*H*), 5.94 (1 H, d, *J* 4.5, *H*-1'), 6.81-6.83 (4 H, m, phenyl-*H*), 7.20-7.25 (7 H, m, phenyl-*H*), 7.34-7.36 (2 H, m, phenyl-*H*),

8.26 (1 H, s, *H*-2), and 8.31 (1 H, s, *H*-8); δ_C(400 MHz; DMSO-d_6; Me_4Si) 28.1 [BOC-(C=O)-O-(*C*H_3)$_3$], 54.9 (-O-*C*H_3), 63.6 (*C*-5'), 68.7, 69.1, 69.4 (ether-*C*), 70.2 (*C*-3'), 72.9 (*C*-2'), 79.1 (C_q-BOC), 82.9 (*C*-4'), 85.3 (C_q-DMT), 87.9 (*C*-1'), 113.0 (phenyl), 123.7 (*C*-5), 126.5, 127.6, 129.6, 135.4, 135.5 (phenyl), 139.4 (*C*-8), 144.7 (phenyl), 149.7 (*C*-4), 152.4 (*C*-2), 155.5 (*C*-6), and 2 x 157.9 [BOC-(*C*=O)-O-(CH_3)$_3$, phenyl]; MALDI$^+$-MS: calculated for $C_{42}H_{52}N_6O_{10}$ [MH^+]: 800.9; found: 801.6.

N^2-isobutyryl-5'-*O*-(4,4'-dimethoxytrityl)guanosine 18.

4.6 g N^2-isobutyrylguanosine **17** (13 mmol) were coevaporated with dry pyridine (3 x 45 mL) and subsequently dissolved in 80 mL of dry pyridine. The reaction was performed in analogy to the synthesis of **11** by adding 3 mL triethylamine (2.8 g, 17 mmol) and 90 mg 4-dimethylaminopyridine (0.07 mmol), and finally 5.4 g dimethoxytrityl chloride (16 mmol, dissolved in 20 mL of dry pyridine). Workup and chromatographic purification as described for **11** produced **18** as a colourless foam (3.5 g, 5.3 mmol, 41%); δ_H(400 MHz; DMSO-d_6; Me_4Si) 1.18 (6 H, dd, *J* 6.8 and 1.4, 2 x C*H*$_3$-iBu), 2.81 (1 H, sept., *J* 6.8, C*H*-iBu), 3.23 (1 H, dd, *J* 10.4 and 2.8, *H*-5'), 3.32 (1 H, dd, *J* 10.4 and 5.9, *H*-5'), 4.08-4.11 (1 H, m, *H*-4'), 4.28 (1 H, m, *H*-3'), 4.61 (1 H, m, *H*-2'), 5.29 (1 H, d, *J* 5.6, O*H*), 5.71 (1 H, d, *J* 5.5, O*H*), 5.92 (1 H, d, *J* 4.6, *H*-1'), 6.87 (4 H, m, phenyl), 7.26 (7 H, m, phenyl), 7.39 (2 H, m, phenyl), 8.18 (1 H, s, NH), and 8.36 (1 H, s, *H*-8); δ_C(400 MHz; DMSO-d_6; Me_4Si) 19.3, 19.4 (2 x *C*H_3), 35.2 (*C*H-N^2-iBu), 55.5 (O-*C*H_3), 64.4 (*C*-5'), 70.8 (*C*-2'), 73.8 (*C*-3'), 83.7 (C_q-DMT), 85.9 (*C*-4'), 87.8 (*C*-1'), 113.5 (phenyl), 120.9 (*C*-5), 127.1, 128.1, 128.2, 130.1, 130.2, 135.9 (phenyl), 138.1 (*C*-8), 145.2 (phenyl), 148.5 (*C*-4), 149.2 (*C*-2), 155.3 (*C*-6), 158.5 (phenyl), and 180.6 [ibu-(*C*=O)]; FAB$^+$-MS: calculated for $C_{35}H_{37}N_5O_8$ [M + H^+]: 655.7; found: 656.2.

2'-*O*-,3'-*O*-diisobutyryl-5'-*O*-(4,4'-dimethoxytrityl)-N^6-(3,6-dioxa-8-*N*'-BOC-aminooctyl)adenosine 12.

0.6 g 5'-*O*-(4,4'-dimethoxytrityl)-N^6-(3,6-dioxa-8-*N*'-BOC-aminooctyl)adenosine **11** (0.8 mmol) were dissolved in 5 mL of dry pyridine and added to an ice-cold solution of 1.6 mL isobutyric anhydride (1.5 g, 9.6 mmol) in 10 mL of dry pyridine.

The reaction mixture was stirred at room temperature overnight. The solvent was removed *in vacuo* and the residue was worked up as described for **6**. Chromatographic purification with chloroform:methanol = 99:1 → 9:1 produced **12** as a colourless oil (0.7 g, 0.7 mmol, 93%); δ_H(400 MHz; DMSO-d_6; Me_4Si) 1.03-1.12 [12 H, m, 2 x (C=O)-CH-(CH_3)$_2$], 1.36 (9 H, s, BOC), 2.42 [1 H, sept., *J* 7.0, (C=O)-C*H*-(CH$_3$)$_2$], 2.57 [1 H, m, (C=O)-C*H*-(CH$_3$)$_2$], 3.07, 3.28, 3.37, 3.50, 3.54, 3.61 (14 H, 6 x m, ether-*H*, *H*-5'), 3.73 (6 H, s, 2 x phenyl-O-CH_3), 4.25 (1 H, m, *H*-4'), 5.76 (1 H, m, *H*-3'), 6.13 (1 H, m, *H*-2'), 6.20 (1 H, d, *J* 4.9, *H*-1'), 6.81-6.84 (4 H, m, phenyl-H), 7.20-7.25 (7 H, m, phenyl-*H*), 7.34-7.36 (2 H, m, phenyl-*H*), 8.29 (1 H, s, *H*-2), and 8.31 (1 H, s, *H*-8); δ_C(400 MHz; DMSO-d_6; Me_4Si) 18.3, 18.8 [ibu-(*C*H$_3$)$_2$], 28.1 [BOC-(*C*H$_3$)$_3$], 30.0 (ether), 33.0 [ibu-(*C*H)-(CH$_3$)$_2$], 54.9 (phenyl-O-*C*H$_3$), 62.4 (*C*-5'), 69.4 (ether-*C*), 70.0 (*C*-3'), 72.0 (*C*-2'), 79.1 (BOC-*C*$_q$), 80.7 (*C*-4'), 85.6, 85.9 (*C*-1', DMT-C$_q$),113.0 (DMT-O-*C*H$_3$), 123.7 (*C*-5), 126.5, 127.5, 127.6, 129.5, 129.6, 135.1, 135.2 (phenyl), 139.7 (*C*-8), 144.5 (phenyl), 149.5 (*C*-4), 152.6 (*C*-2), 155.4 (*C*-6) 158.0 [BOC-(*C*=O)-O-(CH$_3$)$_3$, phenyl], 174.7, and 177.6 [2 x ibu-(*C*=O)]; MALDI$^+$-MS: calculated for $C_{50}H_{64}N_6O_{12}$ [MH$^+$]: 941.1; found: 941.6.

2'-*O*, 3'-*O*,*N*^{2}-triisobutyryl-5'-*O*-(4,4'-dimethoxytrityl)guanosine 19.

3.3 g *N*^{2}-isobutyryl-5'-*O*-dimethoxytritylguanosine **18** (5.3 mmol) in 50 mL dry pyridine were added to 8.3 mL of isobutyric anhydride (7.2 g, 50 mmol) in 20 mL dry pyridine, stirred overnight, worked up, and purified chromatographically as described for **12** (chloroform → chloroform:methanol = 96:4). The product was obtained as a colourless oil (3.3 g, 4.1 mmol, 78%); δ_H(400 MHz; DMSO-d_6; Me_4Si) 1.02, 1.05 [2 x 3 H, 2 x d, *J* 6.8, 3'-O-CH-(CH_3)$_2$], 1.10-1.14 [12 H, m, 2'-O-CH-(CH_3)$_2$, NH-CH-(CH_3)$_2$], 2.53-2.62 [2 H, m, 3'-O-C*H*-(CH$_3$)$_2$, 2'-O-C*H*-(CH$_3$)$_2$], 2.76 [1 H, sept., *J* 7.0, NH-C*H*-(CH$_3$)$_2$], 3.46-3.51 (1 H, m, *H*-5'), 4.20-4.23 (1 H, m, *H*-4'), 5.48 (1 H, dd, *J* 5.7 and 3.7, *H*-3'), 5.96-5.99 (1 H, m, *H*-2'), 6.09 (1 H, d, *J* 5.7, *H*-1'), 6.79-6.84 (4 H, m, phenyl), 7.19-7.26 (7 H, m, phenyl), 7.33-7.36 (2 H, m, phenyl), 8.13 (1 H, s, *H*-2), and 8.31 (1 H, s, *H*-8); δ_C(400 MHz; DMSO-d_6; Me_4Si) 18.2-18.7 [O-ibu-CH-(*C*H$_3$)$_2$, N-ibu-CH-(*C*H$_3$)$_2$], 32.8, 32.9 [O-ibu-*C*H-(CH$_3$)$_2$], 34.6 [N-(C=O)-*C*H-(CH$_3$)$_2$], 63.4 (*C*-5'), 70.5 (*C*-3'), 72.0 (*C*-2'), 81.9, 85.7 (*C*-4, *C*-1'), 113.0, 120.4 (*C*-5), 126.6, 127.5, 127.6, 129.5,

129.6, 135.1 (phenyl), 137.5 (*C*-8), 144.4 (phenyl),148.2 (*C*-4), 148.5 (*C*-2), 154.6 (*C*-6), 158.0 (phenyl), 174.2, 174.8 [2'-iBu-(*C*=O), 3'-iBu-(*C*=O)], and 179.9 [*N*-iBu-(*C*=O)]; FAB$^+$-MS: calculated for $C_{35}H_{37}N_5O_8$ [M + H$^+$]: 655.7; found: 656.2.

2'-*O*-,3'-*O*-diisobutyryl-*N*^{6}-(3,6-dioxa-8-*N*'-BOC-aminooctyl)adenosine 13.

A solution of 0.8 g trichloroacetic acid (4.9 mmol) in 20 mL chloroform (113 mmol) was added to 0.7 g of **12** (0.7 mmol). The solution was stirred for 20 min, neutralised with a saturated aqueous solution of sodium hydrogen carbonate, worked up and purified chromatographically ($CHCl_3$ → $CHCl_3$:MeOH = 95:5) as described for **6** (0.36 g, 0.5 mmol, 73%); δ_H(400 MHz; DMSO-d_6; Me_4Si) 0.96, 1.00 [2 x 3 H, 2 x d, *J* 6.8, 3'-O-(C=O)-CH-(C*H*$_3$)$_2$], 1.14, 1.16 [2 x 3 H, 2 x d, *J* 6.8, 2'-O-(C=O)-CH-(C*H*$_3$)$_2$], 1.34 (9 H, s, BOC), 2.46 [1 H, sept., *J* 6.8, 3'-O-(C=O)-C*H*-(CH$_3$)$_2$], 2.64 [1 H, sept., *J* 6.8, 2'-O-(C=O)-C*H*-(CH$_3$)$_2$], 3.04, 3.35, 3.47, 3.52, 3.59, 3.61 (12 H, 6 x m, ether-H), 3.65 (1 H, m, *H*-5'), 3.72 (1 H, m, *H*-5'), 4.23 (1 H, m, *H*-4'), 5.52 (1 H, m, *H*-3'), 5.71 (1 H, m, 5'-O*H*), 5.90 (1 H, m, *H*-2'), 6.19 (1 H, d, *J* 6.8, *H*-1'), 8.30 (1 H, s, *H*-2), and 8.39 (1 H, s, *H*-8); δ_C(400 MHz; DMSO-d_6; Me_4Si) 18.3, 18.8 [ibu-(*C*H$_3$)$_2$], 28.1 [BOC-(*C*H$_3$)$_3$], 32.8-33.0 [ibu-(*C*H)-(CH$_3$)$_2$], 38.0 (ether), 61.2 (*C*-5'), 69.1, 69.4 (ether), 71.3 (*C*-3'), 72.5 (*C*-2'), 79.1 (BOC-C$_q$) 84.0 (*C*-4'), 85.5 (*C*-1'), 139.5 (*C*-8), 148.0 (*C*-4), 152.5 (*C*-2), 154.5 (*C*-6), 155.5 [BOC-(*C*=O)], 174.6, and 175.0 [2 x ibu-(*C*=O)]; FAB$^+$-MS: calculated for $C_{29}H_{46}N_6O_{10}$ [M + H$^+$]: 638.7; found: 639.3.

2'-*O*, 3'-*O*, *N*^{2}-triisobutyrylguanosine 20.

3.3 g **19** (4.1 mmol) were reacted with 5% CCl_3COOH in CH_2Cl_2, worked up, and purified as described for **13**. **20** was obtained as a colourless foam (1.0 g, 2.0 mmol, 49%); δ_H(600 MHz; DMSO-d_6; Me_4Si) 0.99, 1.03 [2 x 3 H, 2 x d, *J* 7.0, 3'-O-CH-(C*H*$_3$)$_2$], 1.13 [6 H, d, *J* 6.7, NH-CH-(C*H*$_3$)$_2$], 1.15, 1.17 [2 x 3 H, 2 x d, *J* = 3.7, 2'-O-CH-(C*H*$_3$)$_2$], 2.47-2.52 [1 H, m, 3'-O-C*H*-(CH$_3$)$_2$], 2.64 [1 H, sept., *J* 7.0, 2'-O-C*H*-(CH$_3$)$_2$], 2.80 [1 H, sept., *J* 6.7, NH-C*H*-(CH$_3$)$_2$], 3.67-3.74 (2 H, m, *H*-5'), 4.21-4.23 (1 H, m, *H*-4'), 5.40 (1 H , t, *J* 5.1, O*H*), 5.49 (1 H, dd, *J* 5.5 and 2.2, *H*-3'), 5.78 (1 H, dd, *J* 7.1 and 5.5, *H*-2'), 6.05 (1 H, d, *J* 7.1, *H*-1'), and 8.31 (1 *H*, s, H-8); δ_C(600 MHz; DMSO-d_6; Me_4Si) 18.9-19.0 [O-(C=O)-CH-(*C*H$_3$)$_2$],

19.3 [N-(C=O)-CH-(*C*H_3)$_2$], 33.3, 33.4 [O-(C=O)-*C*H-(CH_3)$_2$], 35.2 [N-(C=O)-*C*H-(CH_3)$_3$], 61.4 (*C*-5'), 71.8 (*C*-3'), 73.7 (*C*-2'), 84.6 (*C*-4, *C*-1'), 120.6 (*C*-5), 137.8 (*C*-8), 149.3, 148.9 (*C*-2, *C*-4),155.2 (*C*-6), 175.5, 175.2 [ibu-O-(C=O)], and 180.7 [ibu-N-(*C*=O)].

General procedure for the synthesis of phosphoramidites.

An equivalent of nucleoside (0.6 mmol) starting material was coevaporated twice with 10 mL of CH_2Cl_2:pyridine = 9:1 and evacuated over KOH for at least 6 h. The nucleoside was dissolved in 2 mL of dry dichloromethane, and 7.5 equivalents of freshly distilled diisopropylethylamine were added. Finally, 2 equivalents of either (methyl-*N*,*N*-diisopropyl)chlorophosphoramidite or (cyanoethyl-*N*,*N*-diisopropyl)-chlorophosphoramidite, respectively, were added and the solution was stirred for 1 h. Then, 0.2 mL methanol were added, and the mixture was diluted with 10 mL of ethyl acetate and 0.5 mL triethylamine. The organic layer was washed twice with saturated $NaHCO_3$-solution, twice with brine, and finally dried over Na_2SO_4. The obtained phosphoramidite was directly used for coupling without further purification. To this aim, it was coevaporated twice with 10 mL of dry pyridine and stored overnight *in vacuo*.