CHEMBIOCHEM

Supporting Information

CHEMBIOCHEM

Supporting Information

for

Synthesis and Properties of Aminopropyl Nucleic Acids

Ding Zhou, Irene M. Lagoja, Jef Rozenski, Roger Busson, Arthur Van Aerschot, and Piet Herdewijn*

For all reactions, analytical grade solvents were used. All moisture-sensitive reactions were carried out in oven-dried glassware (100°C) under a nitrogen atmosphere. ^{1}H NMR was determined with a Varian Unity 500 MHz spectrometer with tetramethylsilane (TMS) as internal standard and a 200 MHz Varian Gemini apparatus was used for ^{13}C NMR determination in the solvents [D_6]DMSO (39.6 ppm) or $CDCl_3$ (76.9 ppm) and using the solvent peak as reference. Exact mass measurements were performed on a quadrupole time-of-flight mass spectrometer (Q-Tof-2, Micromass, Manchester, UK) equipped with a standard electrospray-ionization (ESI) interface; samples were infused in *i*-PrOH/H_2O 1:1 at 3µl/min. TLC was performed with TLC aluminum sheets (Merck, Silica gel 60 F_{254}). The spots were examined with UV light, or sprayed with sulphuric acid / anisaldehyde or 1% potassium permanganate solution. Column chromatography was performed on ICN silica gel 60-200 60A. The names of the compounds accorded to IUPAC rules and were verified with a nomenclature program (ACD Labs, Version 4.08, Sept. 1999, Adv. Chem. Dev. Inc., Toronto, Canada). (*S)*-1,2-*O*-isopropylidene-glycerol **4,** is commercially available (Fluka, 59447; 2,2-Dimethyl-1,3-dioxolane-4-methanol).

(*R*)-1-*O*-Methylphenylsulfonyl-2,3-*O*-isopropylidene-glycerol (5): Under ice cooling *(S)*-1,2-*O*-isopropylidene-1,2,3-propanetriol **4** (20.00 g, 0.15 mol) was added in two portions to a solution of p-toluenesufonyl chloride (38.00 g, 0.20 mol) in pyridine

(200 mL). The mixture was stirred at 0°C for 4 h and left overnight at room temperature. Methanol (30 mL) was added and the mixture was stirred for another 10 min. After evaporating the volatiles, the residual oil was dissolved in CH_2Cl_2 (300 mL) and washed with water (2 x 100 mL). After drying over Na_2SO_4 the organic layer was concentrated and the residue was purified by column chromatography (silica, CH_2Cl_2 ; CH_2Cl_2/MeOH 99:1). Yield: 40.8 g (94%); R_f= 0.85 (CH_2Cl_2/MeOH 99:1); ^{1}H NMR ($CDCl_3$): d =1.30 (s, 3H, CH_3), 1.33 (s, 3H, CH_3), 2.44 (s, 3H, CH_3-Ar), 3.71-3.78 (dd, 1H, H-3'A, $J_{(3'A,, 2')}$ =5.2 Hz, $J_{(gem)}$ =8.8 Hz), 3.98-4.06 (m, 3H, H-3'B + 2H-1'), 4.21-4.33 (tt, 1H, H-2', $J_{(3',2')}$ = 5.2 Hz, $J_{(1',2')}$ = 6.0 Hz), 7.33-7.37 (d, 2H, 3,5-H-Ar, J =8.6 Hz), 7.76-7.80 (d, 2H, 2,6-H-Ar, J =8.6 Hz); ^{13}C NMR ($CDCl_3$): d = 21.3 (CH_3-Ar), 24.9, 26.3 (2 CH_3), 65.9 (C-1'), 69.4 (C-3'), 72.7 (C-2'), 109.8 (C-4'), 127.8 (C-3,5-Ar), 129.8 (C-2,6-Ar), 132.6 (C-4-Ar), 145.0 (C-1-Ar). ESMS calcd for $C_{13}H_{18}O_5S$ $[M+H]^+$: 287.0953; found: 287.0952.

(*S*)-9-[(2,2-dimethyl-1,3-dioxolan-4-yl)methyl]-9*H*-purin-6-amine (6a): A mixture of adenine (23.90 g, 0.18 mol) and 60% NaH (60% dispersion in oil, 9.40 g, 0.23 mol) was dissolved in DMF (500 mL) and stirred for 90 min at 80°C. Afterwards compound 5 (39.0 g, 0.15 mol) was added drop wise, and stirring at 100°C was continued for 15 h. After filtration through Celite and washing the Celite-residue with CH2Cl2, the combined filtrates were evaporated and purified by column chromatography (silica, 800 g, CH2Cl2/MeOH 93:7 to 9:1). Yield: 25.96 g (68%); R_f= 0.37 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR ([D_6]DMSO): d = 1.22, 1.26 (2 s, 6H, 2 CH_3), 3.71-3.78 (dd, 1H, H-3'A, $J_{(3'A,, 2')}$ =5.4 Hz, $J_{(gem)}$ = 8.8 Hz), 3.98-4.05 (dd, 1H, H-3'B, $J_{(3'B,, 2')}$ =6.8 Hz, $J_{(gem)}$ =8.8 Hz), 4.16-4.36 (m, 2H, 2H-1'), 4.45-4.50 (m, 1H, H-2'), 7.24 (br s, 2H, NH_2), 8.08 (s, 1H, H-8), 8.15 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$): d =25.2, 26.6 (2 x CH_3), 45.4 (C-1'), 66.1 (C-3'), 73.7 (C-2'), 109.1 (C-4'), 118.6 (C-5), 141.7 (C-8), 150.0 (C-4), 152.7 (C-2), 156.2 (C-6). ESMS calcd for $C_{11}H_{16}N_5O$ $[M+H]^+$: 250.1304; found: 250.1297.

(*S*)-*N*^{6}-benzoyl-9-[2,2-dimethyl-1,3-dioxolan-4-yl]methyl]-adenine (7a): To an ice-cooled solution of **6a** (14.00 g, 56.2 mmol) in dry pyridine (100 mL) benzoyl chloride (9.9 mL, 84.3 mmol) was added in one portion. Stirring at 0°C was continued for one hour, and then another portion of benzoyl chloride (9.9 mL 84.3 mmol) was added. The mixture was allowed to reach room temperature and stirring was continued overnight. Treatment of the mixture with NH_3/MeOH (40 mL) at 0°C for 30 min led to the desired mono-benzoyl derivative **7a**. After evaporation of the solvent the resulting mix-

ture was partitioned between CH_2Cl_2 (40 mL) and water (30 mL). The organic layer was dried over Na_2SO_4, evaporated and purified by column chromatography. (silica, 150 g, CH_2Cl_2/MeOH 95:5). Yield: 15.7 g (79%), R_f = 0.33 (CH_2Cl_2/MeOH 95:5); ^{1}H NMR ([D$_6$]DMSO): *d* = 1.23, 1.29 (2 s. 6H, 2 CH_3), 3.77-3.84 (dd, 1H, H-3'A, $J_{(3'A,, 2')}$ =5.2 Hz, $J_{(gem)}$ = 8.8 Hz), 4.04-4.11 (dd, 1H, H-3'B, $J_{(3'B,, 2')}$ =6.6 Hz, $J_{(gem)}$ =8.8 Hz), 4.30-4.44 (m, 2H, 2H-1'), 4.51-4.58 (m, 1H, H-2'), 7.42-7.44 (t, 2H, 3,5H-Bz, *J* = 7.5Hz), 7.58-7.61 (t, 1H, 4H-Bz, *J* = 7.5Hz), 8.03-8.06 (d, 2H, 2,6H-Bz, *J* = 7.5Hz), 8.15 (s, 1H, H-8), 8.78 (s, 1H, H-2), 11.20 (s, 1H, NH). ^{13}CNMR (CMSO-d$_6$): *d* = 25.1, 26.6 (2 CH_3), 45.9 (C-1'), 66.2 (C-3'), 73.6 (C-2'), 109.3 (C-4'), 125.3 (C-5), 128.4 (3,5CH-Bz), 128.7 (2,6CH-Bz), 132.6 (4CH-Bz), 133.7 (1C-Bz), 145.5 (C-8), 150.4 (C-4), 151.7 (C-2), 152.9 (C-6), 165.9 (C=O). ESMS calcd for $C_{18}H_{20}N_5O_3$ [*M*+H]$^+$: 354.1566; found: 354.1573.

(*S*)-*N*^{1}-[9-(2,3-dihydroxypropyl)-9*H*-purin-6-yl]benzamide (8a): Compound **7a** (15.70 g, 44.4 mmol) suspended in aqueous 75% TFA (100 mL) was stirred at room temperature overnight. The mixture was evaporated to a small volume and co-evaporated with toluene. The residue was purified by column chromatography (silica, 300 g, CH_2Cl_2/MeOH 90:10). Yield: 12.70 g (91%); R_f = 0.33 (CH_2Cl_2/MeOH 95:5), ^{1}H NMR ([D$_6$]DMSO): *d* = 3.35 – 3.47 (m, 2H, 2 H-3'), 3.88 – 3.94 (m, 1H, H-2'), 4.12 – 4.17 (m, 1H, H-1'A, $J_{(1', 2)}$ = 8.3 Hz, $J_{(gem)}$ = 13.9 Hz), 4.43 – 4.46 (dd, 1H, H-1'A, $J_{(1'A, 2)}$ = 3.4 Hz, $J_{(gem)}$ = 13.9 Hz), 4.83 – 4.85 (t, 1H, 3'OH, $J_{(3',3'OH)}$ = 5.6 Hz), 5.13 – 5.14 (d, 1H, 2'OH, $J_{(2',2'OH)}$ = 5.9 Hz), 7.53 – 7.56 (t, 2H, 3,5H-Bz, *J* = 7.3 Hz), 7.62 – 7.65 (t, 1H, 4H-Bz, *J* = 7.3 Hz), 8.04 – 8.06 (d, 2H, 2,6H-Bz, *J* = 7.3 Hz), 8.38 (s, 1H, H-2), 8.72 (s, 1H, H-8), 11.13 (s, 1H, NH), ^{13}C NMR ([D$_6$]DMSO): *d* = 46.8 (C-1'), 63.7 (C-3',) 69.6 (C-2'), 125.4 (C-5), 128.4 (3,5CH-Bz), 128.5 (2,6CH-Bz), 132.4 (4CHBz), 133.6 (1C-Bz), 145.5 (C-8), 150.0 (C-4), 151.2 (C-2), 152.7 (C-6), 165.7 (C=O). ESMS calcd for $C_{15}H_{16}N_5O_3$ [*M*+H]$^+$: 314.1253; found: 314.1246.

(*S*)-1-(2-{[*tert*-butyl(dimethyl)silyl]oxy}-3-hydroxypropyl)-5-methyl-2,4(1*H*,3*H*)-pyrimidinedione (10a): Under ice-bath cooling MMTrCl (6.30 g, 20.5 mmol) was added to a solution of **8a** (2.00 g, 17.1 mmol) in pyridine (20 mL). After stirring for 4 h, the solvent was removed and the residue was dissolved in CH_2Cl_2 (30 mL) and washed with saturated aqueous $NaHCO_3$ (2 x 20 mL). The organic layer was dried over Na_2SO_4 and concentrated. The residue was dissolved in DMF (20 mL) and under ice-cooling imidazole (3.50 g, 51.3 mmol) was added, followed by *tert*–butyl(dimethyl)-

silyl chloride (5.40 g, 34.2 mmol). The solution was kept at room temperature overnight. The reaction was quenched with ice and the solvent was evaporated. The residue was dissolved in saturated aqueous $NaHCO_3$ (30 mL), and extracted with CH_2Cl_2 (2 x 30 mL). After drying with Na_2SO_4 and concentrating the organic layer, the obtained residue was treated with 4% p-toluenesulfonic acid (30 mL) on an ice bath for two hours followed by addition of saturated aqueous $NaHCO_3$ (30 mL) and extraction with CH_2Cl_2 (2 x 30 mL). The organic layer was dried over Na_2SO_4 and concentrated and further purified by column chromatography (silica, 200 g, CH_2Cl_2). Yield: 1.78 g (54%); R_f = 0.33 (CH_2Cl_2/MeOH 95:5), ^{1}H NMR ($CDCl_3$): d = -0.18, -0.08 (2 s, 6H, 2 Si-CH_3), 0.76 (s, 9H, 3 CH_3), 3.08–3.18 (dd, 1H, H-3'A, $J_{(3'A, 2')}$ = 8.0 Hz, $J_{(gem)}$ = 12.0 Hz), 3.43–3.51 (dd, 1H, H-3'B, $J_{(3'B, 2')}$ = 3.3 Hz, $J_{(gem)}$ = 12.0 Hz), 4.08 (m, 1H, H-2'), 4.17 – 4.26 (dd, 1H, H-1'A, $J_{(1'A, 2)}$ = 3.4 Hz, $J_{(gem)}$ = 14.3 Hz), 4.45 – 4.54 (dd, 1H, H-1'B, $J_{(1'B, 2)}$ = 4.6 Hz, $J_{(gem)}$ = 14.3 Hz), 7.36–7.51 (m, 3H, 3,4,5H-Bz), 7.93 (s, 1H, H-8), 7.96–7.99 (d, 2H, 2,6H-Bz, J = 9.0 Hz), 8.66 (s, 1H, H-2), 9.71 (s, 1H, NH). ^{13}C NMR ([D_6]DMSO): d = -5.5, -5.1 (2 Si-CH_3), 17.5 (Si-C), 25.4 (3 CH_3), 46.4 (C-1'), 62.3 (C-3',) 70.2 (C-2'), 122.4 (C-5), 127.8, 128.5 (2,3,5,6CH-Bz), 132.5 (4CH-Bz), 133.5 (1C-Bz), 144.4 (C-8), 149.7 (C-4), 152.2 (C-2), 164.9 (C=O). ESMS calcd for $C_{21}H_{30}N_5O_3Si$ [M+H]$^+$: 428.2118; found: 428.2101.

***N*-[9-((*S*)-3-azido-2-{[*tert*-butyl(dimethyl)silyl]oxy}propyl)-9*H*-purin-6-yl]benzamide (12a)**: Under ice cooling dry triethylamine (2 mL) was added to a solution of **10a** (2.95 g, 6.9 mmol) in CH_2Cl_2 (20 mL) followed by addition of methanesulfonyl chloride (0.86 g, 7.6 mmol). The mixture slowly was allowed to come to room temperature. After 2 hours water (20 mL) was added and the mixture was extracted with CH_2Cl_2 (2 x 20 mL). The organic layer was dried over Na_2SO_4 and concentrated. The residue was dissolved in dry DMF (20 mL) and sodium azide (0.58 g, 9.0 mmol) was added. After heating at 100°C overnight, the reaction mixture was allowed to cool to room temperature and concentrated. The residue was diluted with water (15mL) and extracted with ethyl acetate (2 x 20 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to a yellow viscous oil, which was further purified by column chromatography (silica, 160 g, CH_2Cl_2/MeOH 95:5). Yield: 1.78 g (57%); R_f = 0.33 (EE:PE = 7: 1), ^{1}H NMR ([D_6]DMSO): d = -0.57, -0.09 (2 s, 6H, 2 Si-CH_3), 0.66 (s, 9H, 3 CH_3), 3.22–3.29 (dd, 1H, H-3'A, $J_{(3'A, 2')}$ = 3.6 Hz, $J_{(gem)}$ = 11.8 Hz), 3.51–3.58 (dd, 1H, H-3'B, $J_{(3'B, 2')}$ = 3.0 Hz, $J_{(gem)}$ = 11.8 Hz), 4.34 (m, 3H, H-1', H-2'), 7.49–7.68 (m, 3H, 3,4,5H-Bz), 7.97–8.00 (d, 2H, 2,6H-Bz, J = 6.8 Hz), 8.36 (s, 1H, H-8), 8.69 (s, 1H, H-2);

^{13}C NMR ([D$_6$]DMSO): *d* = -5.8, -5.0 (2 Si-CH_3), 17.4 (Si-C), 25.5 (3 CH_3), 47.0 (C-1'), 54.2 (C-3',) 69.6 (C-2'), 125.5 (C-5), 128.6 (2,3,5,6CH-Bz), 132.6 (4CH-Bz), 133.7 (1C-Bz), 145.4 (C-8), 150.4 (C-4), 151.6 (C-2), 152.9 (C-6). ESMS calcd for $C_{21}H_{29}N_8O_2Si$ [*M*+H]$^+$: 453.2183; found: 453.2178.

(*R*)-*N*-[9-(3-amino-2-{[*tert*-butyl(dimethyl)silyl]oxy}propyl)-9*H*-purin-6-yl]benzamide (13a) and its (*S*)-enantiomer (23a): To **12a** and **22a** (1.50 g, 3.32 mmol), respectively, dissolved in dry THF (15 mL), triphenylphosphine (1.13 g, 4.31 mmol) was added. After stirring overnight, water (10 mL) was added and stirring was continued for another 10 h. The reaction mixture was concentrated and further purified by column chromatography (silica, 100 g, CH_2Cl_2/MeOH 9:1). Yield: 1.13 g (80%); R_f = 0.25 (CH_2Cl_2/MeOH 9:1), ^{1}H NMR ([D$_6$]DMSO): *d* = -0.43, -0.10 (2 s, 6H, 2 Si-CH_3), 0.73 (s, 9H, 3 CH_3), 3.36 (br s, NH_2), 4.05 (m, 1H, H-2'), 2.55– 2.69 (m, 2H, H-3'), 4.26 (m, 2H, H-1'), 7.51–7.68 (m, 3H, 3,4,5H-Bz), 8.03–8.07 (d, 2H, 2,6H-Bz, *J* = 7.0 Hz), 8.36 (s, 1H, H-8), 8.37 (s, 1H, H-2); ^{13}C NMR ([D$_6$]DMSO): *d* = -5.2, -4.4 (2 Si-CH_3), 18.0 (Si-C), 26.1 (3 CH_3), 45.6 (C-1'), 48.5 (C-3',) 72.2 (C-2'), 125.2 (C-5), 129.1 (3,5,CH-Bz), 129.4 (2,6CH-Bz), 133.4 (4CH-Bz), 134.1 (1C-Bz), 146.3 (C-8), 150.5 (C-4), 152.2 (C-2), 153.4 (C-6), 166.8 (C=O). ESMS calcd for $C_{21}H_{29}N_8O_2Si$ [*M*+H]$^+$: 427.2278; found: 427.2279.

(*R*)-*N*^{1}-[9-(2-hydroxy-3-[(4-methoxyphenyl)(diphenyl)methyl]-aminopropyl)-9*H*-purin-6-yl]benzamide (3a) and its (*S*)-entantiomer (15a): A mixture of **13a** and **23a** (0.87 g, 2.78 mmol), respectively, and monomethoxytrityl chloride (1.20 g, 3.81 mmol) in pyridine (20 mL) was stirred at room temperature overnight. The mixture was concentrated, diluted with CH_2Cl_2 (30 mL) and washed with saturated $NaHCO_3$ (2 x 20 mL) and H_2O (2 x 20 mL). After drying, the volatiles were removed, and the residue was dissolved in 1M TBAF (5.6 mL, 5.6 mmol) and stirred for two hours. Then the reaction mixture was evaporated and purified by column chromatography (silica, 85 g, CH_2Cl_2/MeOH: Et_3N 95: 4.5: 0.5). Yield: 0.91 g (71%); R_f = 0.73 (CH_2Cl_2/MeOH 95: 5), ^{1}H NMR ([D$_6$]DMSO + D_2O): *d* = 1.85–2.05 (m, 2H, H-3'), 3.66 (s, 3H, O-CH_3), 4.06 (m, 1H, H-2'), 4.32–4.35 (m, 2H, H-1'), 6.75–6.80 (t, 2H, 2 H-4-MMTr, $J_{(3,4)}$= 8.8 Hz), 7.09–7.31 (m, 12H, MMTr), 7.49–7.66 (m,3H, 3,4,5H-Bz), 7.98–8.02 (d, 2H, 2,6-H-Bz, *J*= 6.6 Hz), 8.28 (s, 1H, H-8), 8.60 (s, 1H, H-2), 11.15 (s, 1H, NH); ^{13}C NMR ([D$_6$]DMSO): *d* = 47.5 (C-1'), 55.0 (C-3'), 59.8 (NH-C-MMTr), 68.7 (O-CH_3), 69.8 (C-2'), 113.2 (MMTrC-3,5), 125.3 (C-5), 126.2 (MMTRC-3',5',3'',5'',), 127.8 (MMTrC-4',4''),

128.4 (3,5CH-Bz), 128.6 (2,6CH-Bz), 129.7 (MMTrC-2',6',2'',6''), 132.5 (4CH-Bz), 133.7 (1C-Bz), 137.9 (MMTrC2,3,5,6), 145.6 ()MMTrC-1',1''), 146.4 (C-8), 150.1 (C-4), 151.3 (C-2), 152.8 (C-6), 157.6 (MMTrC-4). ESMS calcd for $C_{35}H_{34}N_6O_3$ $[M+H]^+$: 586.2692; found: 586.2666.

(*S*)-1-(2,3-dihydroxypropyl)-5-methyl-2,4(1*H*,3*H*)-pyrimidinedione (8b): *(S)*-1,2-*O*-isopropylidene-1,2,3-propanetriol **4** (9.00 g, 68.2 mol), N^3-benzoylthymine (20.00 g, 95.4 mmol) and triphenylphosphine (36.60 g, 140 mmol) were dissolved in dry THF (100 mL). Under ice-cooling DIAD (29.0 mL, 140 mmol) in THF (20 mL) was added dropwise over 1 h. The reaction was allowed to warm to room temperature and stirred for two more hours. After concentrating the reaction mixture, the residue was dissolved in CH_2Cl_2 (40 mL) and washed with water (2 x 30 mL). After drying the organic layer over Na_2SO_4 and concentrating, the residual oil was treated with NH_3/MeOH (20 mL) for 3 h at room temperature. The volatiles were removed and the residue **7b** was treated with 75% aqueous TFA solution (15 mL) overnight. The reaction was concentrated and the residue was purified by column chromatography (silica, 400 g, CH_2Cl_2/MeOH 94: 6). Yield: 8.29 g (61%); ^{1}H NMR ($CDCl_3$) d = 1.38 (s, 3H, CH_3-T), 3.07-3.21 (m, 2H, H-3'), 3.37-3.50 (m, 3H, H-1', H-2'), 6.79 (s, 1H, H-6-T), 10.55 (s, 1H, NH-T); ^{13}C NMR ($CDCl_3$): d = 11.1 (CH_3-T), 49.5 (C-1'), 62.4 (C-3'), 68.8 (C-2'), 107.9 (C-5), 141.3 (C-6), 150.8 (C-2), 163.9 (C-4). ESMS Calcd for $C_8H_{13}N_2O_4$ $[M+H]^+$: 201.0875; found: 201.0879.

(*S*)-1-(2-{[*tert*-butyl(dimethyl)silyl]oxy}-3-hydroxypropyl)-5-methyl-2,4(1*H*,3*H*)-pyrimidinedione (10b): After coevaporation with dry pyridine (2 x 30 mL) **8b** (4.60 g, 23 mmol) was dissolved in dry pyridine (40 mL). MMTrCl (9.20 g, 30 mmol) was added and the reaction was stirred at room temperature for three hours. The reaction was quenched with MeOH (23 mL) and the solvent was removed. Following coevaporation with toluene (2 x 10 mL), the residue was dissolved in CH_2Cl_2 (30 mL) and washed with saturated aqueous $NaHCO_3$ (2 x 20 mL). The organic layer was dried, concentrated and dissolved in dry DMF (30 mL). Imidazole (3.20 g, 46 mmol) was added, followed by the addition of *t*-butyl(dimethyl)silylchloride (4.20 g, 27.6 mmol). The reaction was stirred overnight and worked up by the addition of MeOH (20 mL). After removal of the volatiles, the residue was dissolved in CH_2Cl_2 (30 mL) and washed with saturated aqueous $NaHCO_3$ (2 x 20 mL). The organic layer was dried over Na_2SO_4 and concentrated. Under ice-cooling the residual oil was treated with 2% pTsOH in

CH_2Cl_2/MeOH (2:1) (50 mL) for a few hours (TLC monitoring). The solvent was removed and the residue was purified by column chromatography (silica, 100 g, CH_2Cl_2/MeOH 97: 3). Yield: 3.40 g (47%); ^{1}H NMR ($CDCl_3$) d = 0.02, 0.06 (2 s, 6H, 2 Si-CH_3), 0.87 (s, 9H, 3 CH_3), 1.89 (s, 3H, CH_3-T), 2.24-2.26 (m, 2H, H-3'), 3.35-3.44 (dd, 1H, H-1'A, $J_{(1'A,2')}$ = 6.4 Hz, $J_{(gem)}$ = 12.0 Hz), 3.49-3.57 (dd, 1H, H-1'B, $J_{(1'B,2')}$ = 3.7 Hz, $J_{(gem)}$ = 12.0 Hz), 3.66-3.77 (dd, 1H, H-3'A, $J_{(3'A,2')}$ = 4.9 Hz, $J_{(gem)}$ = 15.7 Hz), 3.96-4.07 (m, 2H, H-3'B, H-2'), 7.07 (s, 3H, CH_3-T); ^{13}C NMR ([D_6]DMSO): d = -5.3, -4.9 (2 Si-CH_3), 11.9 (CH_3-T), 17.7 (Si-C), 25.5 (3 CH_3), 45.8 (C-1'), 62.8 (C-3',) 70.3 (C-2'), 110.0 (C-5), 142.7 (C-6), 152.1 (C-2),164.6 (C-4). ESMS Calcd for $C_{14}H_{27}N_2O_4Si$ [M+H]$^+$:315.1740; found: 315.1730.

(*S*)-1-(3-azido-2-{[*tert*-butyl(dimethyl)silyl]oxy}propyl)-5-methyl-2,4(1*H*,3*H*)-pyrimidine-dione (12b): Under ice-cooling compound **10b** (3.10 g, 9.8 mmol) was added to a stirred solution of methanesulfonyl chloride (1.35 g, 11.8 mmol) in pyridine (20 mL). After stirring at 0°C for 4 h, methanol (30 mL) was added and the solvents were evaporated. The residual oil was dissolved in CH_2Cl_2 (20 mL) and washed with water (2 x 15 mL). After drying over Na_2SO_4, the organic layer was evaporated and the residue was dissolved in dry DMF (20 mL) and NaN_3 (0.95 g, 14.7 mmol) was added. The mixture was heated to 100°C and stirring was continued overnight. After removal of the volatiles the residue was dissolved in CH_2Cl_2 (20 mL) and washed with H_2O (2 x 15 mL). The organic layer was dried over Na_2SO_4, concentrated and further purified by column chromatography (silica, 100 g, MeOH gradient from 0% to 2% in CH_2Cl_2). Yield: 2.80 g (84 %); ^{1}H NMR ($CDCl_3$) d = -0.11, -0.07 (s, 6H, 2 Si-CH_3), 0.83 (s, 9H, 3 C-CH_3), 1.84 (s, 3H, CH_3-T), 3.07-3.15 (dd, 1H, H-3'A, $J_{(3'A,2')}$ = 3.8 Hz, $J_{(gem)}$ = 13.0Hz), 3.35-3.44 (dd, 1H, H-3'B, $J_{(3'B,2')}$ = 4.0Hz, $J_{(gem)}$ = 13.0Hz), 3.45-3.56 (dd, 1H, H-1'A, $J_{(1'A,2')}$ = 8.1 Hz, $J_{(gem)}$ = 13.8 Hz), 3.87-3.95 (dd, 1H, H-1'B, $J_{(1'B,2')}$ = 3.6Hz, $J_{(gem)}$ = 13.8 Hz) , 4.14–4.16 (m, 1H, H-2'), 6.99 (s, 1H, H-6-T), 9.93 (s, 1H, NH-T). ^{13}C NMR ($CDCl_3$):d = -5.6, -5.1 (2xSi-CH_3), 11.8 (CH_3-T), 17.5 (Si-C-CH_3), 25.4 (C-CH_3), 52.0 (C-3'), 54.2 (C-1'), 68.8 (C-2'), 109.8 (C-5), 142.2 (C-6), 151.2 (C-2), 162.5 (C-4), 164.5 (C=O). ESMS calcd for $C_{14}H_{26}N_5O_3Si$ [M+H]$^+$: 340.1805; found: 340.1817.

(*R*)-1-(3-amino-2-{[*tert*-butyl(dimethyl)silyl]oxy}propyl)-5-methyl-2,4(1*H*,3*H*)pyrimidine-dione (13b) and its (*S*)-enantiomer (23b): Compound **12b** and **22b** (1.00 g, 2.94 mmol), respectively, was dissolved in dry THF (15 mL) and triphenylphosphine (1.54 g, 5.88 mmol) was added. The reaction was stirred at room temperature for 5 h.

After addition of water (15 mL) stirring was continued overnight. The mixture was concentrated and the residue was purified by column chromatography (silica, 50 g, CH_2Cl_2/MeOH 99: 1). Yield: 0.74 g (80%); ^{1}H NMR ([D_6]DMSO + D_2O) d = -0.14, -0.00 (s, 6H, 2xSi-CH_3), 0.81 (s, 9H, 3xC-CH_3), 1.72 (s, 3H, CH_3-T), 2.50-2.56 (m, 2H, H-3'), 3.42-3.53 (dd, 1H, H-1'A, $J_{(1'A,2')}$ = 8.0 Hz, $J_{(gem)}$ = 13.0 Hz), 3.81-3.85 (m, 1H, H-2'), 3.88-3.95 (dd, 1H, H-1'B), 7.35 (s, 1H, H-6-T). ^{13}C NMR ([D_6]DMSO): d = -5.1, -4.4 (2 Si-CH_3), 12.2 (CH_3-T), 17.5 (Si-<u>C</u>-CH_3), 26.0 (<u>C</u>-CH_3), 45.6 (C-3'), 51.9 (C-1'), 71.4 (C-2'), 108.2 (C-5), 143.9 (C-6), 157.6 (C-2), 165.0 (C-4). ESMS Calcd for $C_{14}H_{28}N_3O_3Si$ [M+H]$^+$: 314.1900; found: 314.1897.

(*R*)-1-(2-hydroxy-3-{[(4-methoxyphenyl)(diphenyl)methyl]amino}propyl)-5-methyl-2,4(1*H*,3*H*)-pyrimidinedione (3b) and its (*S*)-enantiomer (15b): Compound **13b** and **23b** (0.70 g, 2.22 mmol), respectively, were co-evaporated with dry pyridine (2 x 10 mL) and the residue was dissolved in dry pyridine (15 mL). MMTrCl (1.03 g, 3.34 mmol) was added and the reaction was stirred for three hours. The reaction was quenched with MeOH and the solvent was removed. The residue was coevaporated with toluene (2 x 10 mL), dissolved in CH_2Cl_2 (20 mL) and washed with saturated aqueous $NaHCO_3$ (2 x 20 mL). The organic layer was dried and concentrated. The residue was treated with 1M TBAF in THF (8 mL) for five hours. The solvent was removed and the residue was purified by column chromatography (silica, 40 g, CH_2Cl_2/MeOH 97: 3). Yield: 0.82 g (79%); ^{1}H NMR ($CDCl_3$) d = 1.84 (s, 3H, CH_3-T), 2.24-2.26 (m, 2H, H-3'), 3.42-3.53 (dd, 1H, H-1'A, $J_{(1'A,2')}$ = 8.8 Hz, $J_{(gem)}$ = 14.4Hz), 3.98-4.03 (m, 2H, H-2', H-1'B), 6.77-6.81 (d, 2H, H-MMTr), 7.10-7.44 (m, 13H, H-6-T, H-MMTr); ^{13}C NMR ($CDCl_3$): d = 12.0 (CH_3-T), 46.8 (C-3'), 52.3 (C-1'), 55.1 (CH_3-MMTr), 69.4 (C-MMTr,), 70.0 (C-2'), 109.7 (C-5), 113.2 (C-MMTr), 126.4, 127.9, 128.5, 129.8, 137.7, 145.9 (C-MMTr), 142.2 (C-6), 151.4 (C-2), 164.5 (C-4). ESMS calcd for $C_{28}H_{30}N_3O_4$ [M+H]$^+$: 472.2236; found: 472.2239.

(*S*)-3-azido-1,2-propanediol (17): To a stirred solution of **5** (20.20 g, 70.8 mmol) in DMF (80 mL) sodium azide (6.90 g, 92.0 mmol) dissolved in water (5 mL) was added. After heating the mixture at 100°C overnight, TLC-analysis showed complete consumption of the starting material. The reaction mixture was allowed to cool to room temperature and concentrated. The residue was partitioned between water and ethyl acetate. The organic layer was separated and the aqueous layer was extracted three times with ethyl acetate (3 x 50 mL). The combined organic layers were dried over

Na_2SO_4 and evaporated to afford yellow viscous oil, which was treated with 80% TFA in water (30 mL). Stirring at room temperature was continued for 10 h. After concen-tration and co-evaporation with toluene (2 x 10 mL) the residue was dissolved in CH_2Cl_2 (40 mL) and washed with saturated aqueous $NaHCO_3$ (2 x 20 mL). After drying over Na_2SO_4 and concentrating the residue was purified by column chroma-tography (silica, CH_2Cl_2/MeOH 95:5). Yield: 2.70 g (33 %), R_f = 0.51 (CH_2Cl_2/MeOH 95:5), NMR and MS are in accordance with reported data.[1]

(*S*)-3-azido-2-{[*tert*-butyl(dimethyl)silyl]oxy}-1-propanol (19): A solution of **17** (2.00 g, 17.1 mmol) in pyridine (20 mL) was cooled to 0°C, MMTrCl (6.30 g, 20.53 mmol) was added and the stirring was continued for four hours. After removal of the solvent the residue was dissolved in CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ (2 x 20 mL). The organic layer was dried over Na_2SO_4 and concentrated. The residue was dissolved in DMF (20 mL) under cooling with an ice-bath, imidazole (3.50 g, 51.3 mmol) was added, followed by *tert*-butyl(dimethyl)silyl chloride (5.40 g, 34.2 mmol). Stirring at room temperature was continued overnight. The reaction was quenched with ice and the solvent was evaporated. The residue was dissolved in saturated aqueous $NaHCO_3$, and extracted with CH_2Cl_2 (2 x 30 mL). The organic layer was dried with Na_2SO_4 and concentrated. The residue was treated with 4% *p*-toluene-sulfonic acid (30 mL) on an ice bath for two hours. The reaction was worked up by addition of a saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (2 x 30 mL). After drying the organic layer over Na_2SO_4 and concentrating, the residue was purified by column chromatography (silica, 200 g, CH_2Cl_2). Yield: 1.45 g (37%), ^{1}HNMR ($CDCl_3$): d = -0.11, 0.14 (s, 2xSi-CH_3), 0.91 (s, 9H, 3xC-CH_3), 3.24–3.30 (dd, 1H, H-3'A, $J_{(2',3'A)}$ = 6.0Hz, $J_{(gem)}$ =12.6 Hz), 3.34-3.43 (dd, 1H, H-3'B, $J_{(2',3'B)}$ = 5.2 Hz, $J_{(gem)}$ =12.6 Hz), 3.55–3.65 (m, 2H, H-1'), 3.81–3.90 (m, 1H, H-2'); ^{13}CNMR ([D_6]DMSO): d = -5.0, -5.1 (2xSi-CH_3), 17.4 (Si-C), 25.6 (3xC-CH_3), 53.7 (C-3'), 64.1 (C-1'), 71.7 (C-2'). ESMS Calcd for $C_9H_{22}N_3O_2Si$ [M+H]$^+$: 232.1481; found: 232.1475.

(*S*)-2-azido-1-{[*tert*-butyl(dimethyl)silyl]oxyethyl}-4-methylsulfonate (20): To a stirred solution of methanesulfonyl chloride (0.70 g, 6.19 mmol) in pyridine (10 mL) compound **19** (1.30 g, 5.63 mol) was added at 0°C. Stirring at 0°C was continued for 4 h. Methanol (30 mL) was added and the solvents were evaporated. The residual oil was dissolved in CH_2Cl_2 (20 mL) and washed with water (2 x 20 mL). After drying over

[1] S.F. Martin, Y.L.Wong, A.S. Wagman, *J. Org. Chem*. **1994,** *59*, 4821-4881.

Na_2SO_4, the organic layer was evaporated and the residue was purified by column chromatography (silica, 50 g, MeOH gradient from 0% to 1% in CH_2Cl_2). Yield: 1.40 g (81%); ^{1}H NMR ($CDCl_3$) d = 0.12 (s, 2xSi-CH_3), 0.90 (s, 9H, CH_3), 3.02 (s, 3H, Ms-CH_3), 3.20-3.29 (dd, 1H, H-3'A, $J_{(3'A,2')}$ = 4.6 Hz, $J_{(gem)}$ = 12.8 Hz), 3.38-3.46 (dd, 1H, H-3'B, $J_{(3'B,2')}$ = 3.6 Hz, $J_{(gem)}$ = 12.8 Hz), 4.02-4.09 (m, 1H, H-2'), 4.14–4.18 (m, 2H, H-1'). ^{13}C NMR ($CDCl_3$):d = -5.0, -4.9 (2xSi-CH_3), 17.8 (Si-C), 25.5 (3xC-CH_3), 37.4 (Mes-CH_3), 53.5 (C-3'), 69.5 (C-2'), 76.3 (C-1'). Exact mass calcd for $C_{10}H_{24}N_3O_4SiS$ $[M+H]^+$: 310.1257; found: 310.1251.

(*R*)-9-(3-azido-2-{[*tert*-butyl(dimethyl)silyl]oxy}propyl)-9*H*-purin-6-ylamine (21): A mixture of adenine (0.68 g, 5.0 mmol) and NaH (60% dispersion in oil, 0.20 g, 5.0 mmol) was dissolved in DMF (15 mL) and stirred for 90 min at 80°C. Compound **20** (1.30 g, 4.2 mmol) in DMF (2 mL) was added drop wise and the mixture was stirred at 100°C for 15 hours. Following filtration through Celite, the solvent was evaporated. The obtained residue was purified by column chromatography (silica, 50 g, CH_2Cl_2). Yield: 0.80 g (46 %) ^{1}H NMR ([D_6]DMSO): d = -0.49, -0.06 (s, 6H, 2xSi-CH_3), 0.74 (s, 9H, 3xCH_3), 3.22-3.31 (dd, 1H, H-3', $J_{(3'A,2')}$ = 4.4 Hz, $J_{(gem)}$ = 12.8 Hz), 3.51-3.60 (dd, 1H, H-3'B, $J_{(3'B,2')}$ =3.6 Hz, $J_{(gem)}$ = 12.8 Hz), 4.20-4.36 (m, 3H, H-1',2'), 7.22 (s, 2H, NH_2), 8.05 (s, 1H, H-2), 8.14 (s, 1H, H-6); ^{13}C NMR ([D_6]DMSO): d = -5.9, -5.1 (2 Si-CH_3), 17.4 (Si-C), 25.5 (3 C-CH_3), 46.8 (C-1'), 54.2 (C-3'), 69.6 (C-2'), 119.0 (C-5), 141.7 (C-8), 149.9 (C-2), 152.6 (C-6), 156.2 (C-4). ESMS calcd for $C_{14}H_{25}N_8OSi$ $[M+H]^+$: 349.1920; found: 349.1927.

(*R*)-*N*-[9-(3-azido-2-{[*tert*-butyl(dimethyl)silyl]oxy}propyl)-9*H*-purin-6-yl]benzamide (22a): Compound **21** (0.75 g, 2.15 mmol) was dissolved in pyridine (15 mL) and cooled to 0°C and benzoyl chloride (4.90 mL, 1.20 mmol) was added. Stirring at 0°C was continued for 1 h after which the reaction was allowed to warm to room temperature overnight. After quenching the reaction with methanol, the volatiles were removed. The residue was cooled and treated with saturated NH_3/MeOH (20 mL) for 30 min. After evaporation of the solvent, the remaining residue was dissolved in CH_2Cl_2 (20 mL) and washed with saturated aqueous $NaHCO_3$ (2 x 10 mL). The organic layer was dried over Na_2SO_4, concentrated and purified by column chromatography (silica, 10 g, CH_2Cl_2). Yield: 0.80 g (82 %), ^{1}H NMR ($CDCl_3$): d = -0.30, -0.02 (2xs, 6H, 2xSi-CH_3), 0.86 (s, 9H, 3xCH_3), 3.18-3.25 (dd, 1H, H-3'A, $J_{(3'A,2')}$ = 3.6 Hz, $J_{(gem)}$ = 13.0 Hz), 3.39-3.48 (dd, 1H, H-3'B, $J_{(3'B,2')}$ = 4.4 Hz, $J_{(gem)}$ = 13.0 Hz), 4.37–4.39

(m, 3H, H-1', H-2'), 7.46-7.60 (m, 3H, 3,4,5H-Bz), 8.02-8.05 (d, 2H, 2,6H-Bz, J = 4.8 Hz), 8.81 (s, 1H, H-8), 9.25 (s, 1H, H-2); ^{13}C NMR ([D$_6$]DMSO): d = -5.6, -5.1 (2 Si-CH$_3$), 17.6 (Si-C), 25.5 (3 CH$_3$), 47.3 (C-1'), 54.1 (C-3'), 69.3 (C-2'), 122.7 (C-5), 127.9 (5,6CH-Bz) 128.6 (2,3CH-Bz), 132.7 (4CH-Bz), 133.7 (1C-Bz), 144.0 (C-8), 149.6 (C-4), 152.1 (C-2), 152.7 (C-6), 164.8 (C=O). ESMS calcd for $C_{21}H_{29}N_8O_2Si$ [M+H]$^+$: 453.2183; found: 453.2178.

(*R*)-1-(3-azido-2-{[*tert*-butyl(dimethyl)silyl]oxy}propyl)-5-methyl-2,4(1*H*,3*H*)-pyrimidine-dione (22b): Compound **19** (1.00 g, 4.33 mL), N^3-benzoylthymine (1.40 g, 6.49 mmol) and triphenylphosphine (1.36 g, 5.20 mmol) were dissolved in dry THF (20 mL) and cooled to 0 °C. DIAD (1.05 g, 5.20 mmol) in THF (5 mL) was added drop wise over 30 min. The reaction was allowed to come to room temperature and stirring was continued for two more hours. After concentrating the reaction mixture the residue was dissolved in CH_2Cl_2 (25 mL) and washed with water (2 x 20 mL). The organic layer was dried over Na_2SO_4 and the solvent was evaporated. The residual oil was treated with NH_3 / MeOH for 3 h at room temperature. After removal of the volatiles, the residue was purified by column chromatography (silica, 80 g, CH_2Cl_2/MeOH 99: 1). Yield: 1.13 g (77%); ^{1}H NMR ($CDCl_3$) d = -0.11, -0.07 (s, 6H, 2 Si-CH$_3$), 0.83 (s, 9H, 3 C-CH$_3$), 1.84 (s, 3H, CH$_3$-T), 3.07-3.15 (dd, 1H, H-3'A, $J_{(3'A,2')}$ = 3.8 Hz, $J_{(gem)}$ = 13.0 Hz), 3.35-3.44 (dd, 1H, H-3'B, $J_{(3'B,2')}$ = 4.0Hz, $J_{(gem)}$ = 13.0 Hz), 3.45-3.56 (dd, 1H, H-1'A, $J_{(1'A,2')}$ = 8.1 Hz, $J_{(gem)}$ = 13.8 Hz), 3.87-3.95 (dd, 1H, H-1'B, $J_{(1'B,2')}$ = 3.6 Hz, $J_{(gem)}$ = 13.8 Hz) , 4.14–4.16 (m, 1H, H-2'), 6.99 (s, 1H, H-6-T), 9.93 (s, 1H, NH-T); ^{13}C NMR ($CDCl_3$):d = -5.6, -5.1 (2 Si-CH$_3$), 11.8 (CH$_3$-T), 17.5 (Si-C-CH$_3$), 25.4 (C-CH$_3$), 52.0 (C-3'), 54.2 (C-1'), 68.8 (C-2'), 109.8 (C-5), 142.2 (C-6), 151.2 (C-2), 162.5 (C-4), 164.5 (C=O). ESMS calcd for $C_{14}H_{26}N_5O_3Si$ [M+H]$^+$: 340.1805; found: 340.1817.

Oligonucleotide analysis: Oligonucleotides were characterized and their purity was checked by HPLC/MS on a capillary chromatograph (CapLC, Waters, Milford, MA). Columns of 150 mm x 0.3 mm length (LCPackings, San Francisco, CA) were used. They were eluted with a triethylammonium/1,1,1,3,3,3-hexafluoro-2-propanol/acetonitrile solvent system. (Flow rate: 5 µL/min. Electrospray spectra were acquired on an orthogonal acceleration / time-of-flight mass spectrometer (Q-Tof-2, Micromass, Manchester, UK) in negative ion mode. Scan time used was 2 sec. The combined spectra from a chromatographic peak were deconvoluted using the MaxEnt algorithm of the

software (Masslynx 3.4, Micromass, Manchester, UK). Theoretical oligonucleotide masses were calculated using the monoisotopic element masses.

ESI-MS monoisotopic data of oligonucleotides containing modified monomeric units.

No.	DNA sequence[a]	calcd	Found	
			R-APNA	*S*-APNA
29	5'-CCTTTT**A*** TTTTCC-3'	3795.6	3796.5	3796.5
30	5'-CCTTTT**T*** TTTTCC-3'	3786.6	3787.5	3787.5
31	3' –GGAAAAT**A*** AAAGG-5'	4018.8	4019.6	4019.6
32	5'-CCTTTAA**T*** TTTCC-3'	3804.7	3805.5	3805.6
33	3' –GGAAAT**T*** AAAAGG-5'	4009.7	4010.6	4010.6
34	5'-CCTTTA**A*** TTTTCC-3'	3804.7	3805.5	3805.5
35	5'-CCTTTA**T*** TTTTCC-3'	3795.6	3796.5	3796.5
36	3' –GGAAAT**A***AAAAGG-5'	4018.8	4019.6	4019.6
37	5'-CCTT**T*** T**T*** T**T*** TTCC-3'	3700.6	3701.6	3701.5
38	5'-CCTT**A*** T**A*** T**A*** TTCC-3'	3727.7	3728.6	3728.5
39	5'-CACCG**T*** TGCTACC-3'	3824.7	3825.5	3825.5
40	5'-CACCG**A*** TGCTACC-3'	3833.7	3834.6	3834.5

[a] APN-units are in bold and with asterisk; DNA-units are in capital and regularly typed.