Supplementary Data

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Stereoselective preparation of (R_P)-8-hetaryladenosine-3',5'-cyclic phosphorothioic acids.

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(S_P)-2'*O*-(*tert*-Butyldimethylsilyl)-8-(*N*-methylpyrrol-2-yl)adenosine-3',5'-cyclic *N*-phenylphosphoramidate (11b)

A solution of $Pd(OAc)_2$ (0.037 g, 0.166 mmol) and PPh_3 (0.091 g, 0.348 mmol) in DMF (DMF, 5 mL) was stirred at 50 °C until the solution had turned dark red. (S_P)-8-Bromoadenosine-2'*O*-(*tert*-butyldimethylsilyl)-3',5'-cyclic-*N*-phenylphosphoramidate (**6**) (0.500 g, 0.83 mmol) in DMF (2 mL) and 2-(tri-*n*-butylstannyl)-1-methylpyrrole (0.461 g, 1.24 mmol) were added. The reaction mixture was stirred at 90 °C for 4 h, and was allowed to cool to room temperature. The DMF was removed at reduced pressure, and the residual material was subjected to flash chromatography on silica gel using 7.5% MeOH in CH_2Cl_2. The product was isolated as a white solid material which contained traces of organotin residues. These were effectively removed by dissolving the crude product in CH_2Cl_2 and precipitation by hexane; yield 0.350 g (70%). HRMS

(electrospray, TOF ES$^+$): M+H 598.2339. Calc. for [$C_{27}H_{36}N_7O_5PSi+H^+$]: 598.2357. ^{31}P NMR ($CDCl_3$), 121 MHz): δ 2.54 ppm; ^{1}H NMR ($CDCl_3$, 300 MHz): δ -0.13 (6H, s, 2 × CH_3), 0.66 (9H, s, $C(CH_3)_3$), 3.73 (3H, s, NCH_3), 4.35 – 4.41 (1H, m, H-4'), 4.50 – 4.66 (2H, m, OCH_2), 5.06 (1H, d, *J* 5.3, H-2'), 5.80 – 5.85 (1H, m, H-3'), 5.99 (1H, s, H-1'), 6.19 – 6.21 (1H, m, H-pyr.), 6.57 – 6.59 (1H, m, H-pyr), 6.65 (2H, bs, NH_2), 6.76 – 6.78 (1H, m, H-pyrrol), 6.95 (1H, t, *J* 7.3 Hz, H-Ph), 7.05 (2H, d, *J* 7.6 Hz, 2 × H-Ph), 7.17 (2H, t, *J* 7.9 Hz, 2 × H-Ph), 7.83 (1H, d, *J* 9.5 Hz, NH), 8.31 (1H, s, H-2); ^{13}C NMR ($CDCl_3$, 75 MHz): δ -5.5 and -4.9 (2 × CH_3), 17.9 (Si-C), 25.5 (3 × CH_3), 36.3 (N-CH_3), 68.9 (d, *J* 6.6 Hz, OCH_2), 70.5 (d, *J* 4.0 Hz, CH-4'), 72.8 (d. *J* 8.5 Hz, CH-2'), 77.5 (d, *J* 3.7 Hz, CH-3'), 94.2 (CH-1'), 108.4, 114.2, 119.4, 119.9, 119.9, 122.7, 126.8, 129.2, 129.2, 138.6, 144.2, 149.8, 152.9, 155.5, 162 .5.

(R_P)-8-(3-Pyridinyl)adenosine-3',5'-cyclic phosphorothioic acid tri-*n*-butylammonium salt (14c)

A mixture of (S_P)-2'*O*-(*tert*-butyldimethylsilyl)-8-(3-pyridinyl)adenosine-3',5'-cyclic *N*-phenylphosphoramidate (**11c**) (0.300 g, 0.5 mmol) and potassium *tert*-butoxide (0.62 mL, 0.62 mmol, 1 M in THF) in THF (6 mL) under argon was stirred at room temperature for 1 h before carbon disulfide (0.09 mL, 1.5 mmol) was added. The reaction mixture was stirred at room temperature for 3 h. The solvent was partially removed at reduced pressure, and hexane was added until precipitation was complete. The precipitate was dissolved in water (9 mL) and 1.2 M HCl (1.25 mL) was added at 0 ^{o}C. The precipitated silylated product, (R_P)-2'*O*-(*tert*-butyldimethylsilyl)-8-(3-pyridinyl)adenosine-3',5'-cyclic phosphorothioic acid (**12c**) was filtered off and dried overnight at high vacuum.

Most of this material (0.180 g, 0.33 mmol) was dissolved in dry DMF (1.5 mL) under argon, and ammonium fluoride (0.075 g, 2 mmol) was added. The mixture was stirred at room temperature for 5 d and filtered. Tri-*n*-butylamine (0.111 g, 0.6 mmol) was added to the filtrate before evaporation at reduced pressure. The residual material was triturated with hexane to remove excess of tri-*n*-butylamine and was then subjected to flash chromatography on silica gel using CH_2Cl_2:MeOH: nBu_3N [100:10:1]. The tri-*n*-butylammonium salt, which contained small amounts of tri-*n*-butylamine, was further purified by dissolution in CH_2Cl_2 and reprecipitation by addition of hexane; yield 0.100 g (34% from **11c**) of a white solid. HRMS (electrospray, TOF ES-): M-$NHBu_3$ 421.0492. Calc. for $C_{15}H_{14}N_6O_5PS]^-$: 421.0489. ^{31}P ($CDCl_3$, 121 MHz): δ 57.17; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.93 (9H, t, *J* 7.2 Hz, 3 × CH_3), 1.29 – 1.43 (6H, m, 3 × CH_2), 1.67 – 1.79 (6H, m, 3 × CH_2), 2.98 3.06 (6H, m, 3 × CH_2), 4.30 – 4.44 (3H, m, H-4' and OCH_2), 5.14 (1H, d, *J* 5.2 Hz, H-2'), 5.50 – 5.57 (1H, m, H-3'), 5.71 (1H, s, H-1'), 6.00 (2H, bs, NH_2), 7.38 – 7.45 (1H, m, H-pyrid), 8.03 – 8.09 (1H, m, H-pyrid), 8.19 (1H, s, H-2), 8.70 – 8.73 (1H, m, H-pyrid), 9.01 (1H, d, *J* 1.6 Hz, H-pyrid); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 13.6 (3 × CH_3), 20.1 (3 × CH_2), 25.2 (3 × CH_2), 51.9 (3 × CH_2), 67.2 (d, *J* 9.7 Hz, OCH_2), 71.6 (d, *J* 7.4 Hz, CH-4'), 71.8 (d. *J* 6.5 Hz, CH-2'), 77.2 (d, *J* 6.2 Hz, CH-3'), 92.6 (CH-1'), 120.1, 124.0, 125.7, 137.3, 149.3, 150.6, 151.1, 151.7, 153.3, 155.8.

(R_P)-Adenosine-3',5'-cyclic phosphorothioic acid tri-*n*-butylammonium salt (14d)

A 1.6 M solution of *n*-BuLi in hexane (0.86 mL, 1.39 mmol) was added to a solution of (S_P)-2'*O*-(*tert*-butyldimethylsilyl)adenosine-3',5'-cyclic *N*-benzylphosphoramidate (**8**) (0.740 g, 1.39 mmol) in dry THF (10 mL) at -78 °C. The mixture was stirred under argon,

at this temperature for 10 min before CS_2 (0.24 mL, 4 mmol) was added. The cooling bath was removed after 10 min. The reaction mixture was stirred at room temperature for 3 h and then was concentrated to a small volume at reduced pressure. Hexane was added until precipitation was completed. The precipitate was dissolved in water (5 mL, and 1 M HBr was added at 0 ^{o}C to pH 2-3. The product, (R_P)-2'*O*-(*tert*-butyldimethylsilyl) adenosine-3',5'-cyclic phosphorothioic acid (**12d**), was filtered off and dried overnight at high vacuum.

A solution of the thioic acid **12d** (0.500 g, 1.2 mmol) and NH_4F (0.3 g, 8 mmol) in DMF (5 mL) was stirred together at room temperature for 5 d. The reaction mixture was filtered, *n*-Bu_3N (0.4 g, 2.4 mmol) was added to the filtrate, and the solution was evaporated at reduced pressure. The residue was triturated with hexane to remove excess of *n*-Bu_3N before flash chromatography on silica gel using CH_2Cl_2:CH_3OH: nBu_3N [100:10:1]. The tri-*n*-butylammonium salt, which contained some *n*-Bu_3N, was further purified by dissolution in CH_2Cl_2 and precipitation by hexane addition; yield 0.32 g (41% from **8**) of a white solid. HRMS (electrospray, TOF ES^-): 344.0231. Calc. for $[C_{10}H_{11}N_5O_5PS]^-$: 344.0224. ^{31}P NMR (MeOH-d_4, 121 MHz): δ 58.16; ^{1}H NMR (MeOH-d_4, 200 MHz): 0.95 – 1.02 (9H, t), 1.35 – 1.40 (6H, m), 1.60 – 1.72 (6H, m), 3.08 – 3.16 (6H, m), 4.31 – 4.37 (3H, m), 4.68 – 4.85 (2H, m), 6.03 (1H, s), 8.20 (1H, s,), 8.22 (1H, s, H-2); ^{13}C NMR (MeOH-d_4, 50 Mz): 13.9, 20.9, 26.7, 53.8, 68.3, 73.1, 74.1|, 77.6, 93.0, 120.4, 141.05, 150.3, 154.2, 157.3.

(R_P)-8-(*N*-Methylpyrrol-2-yl)adenosine-3',5'-cyclic phosphorothioic acid sodium salt (15b)

(R_P)-8-(*N*-Methylpyrrol-2-yl)-3',5'-cyclic phosphorothioic acid tri-*n*butylammonium salt (**14b**) (0.100 g, 0.16 mmol) was dissolved in 0.1 M NaOH in MeOH (1.7 mL). The sodium salt was precipitated by addition of hexane and collected by filtration; yield 0.060 g (84%) of a white solid material. HRMS (electrospray, TOF ES-): M-Na 423.0630. Calc. for $[C_{15}H_{16}N_6O_5PS]^-$: 423.0646. ^{31}P NMR (MeOH-d_4) δ 58.06; (D_2O): δ 56.42; ^{1}H NMR (MeOH-d_4, 300 MHz): δ 3.86 (3H, s, N-CH_3), 4.22 – 4.41 (3H, m, H-4' and OCH_2), 5.04 (1H, d, *J* 5.3 Hz, H-2'), 5.49 – 5.56 (1H, m, H-3'), 5.93 (1H, s, H-1'), 6.25 – 6.29 (1H, m, H-Pyr), 6.67 – 6.70 (1H, m, H-Pyr), 7.01 – 7.03 (1H, m, H-Pyr), 8.22 (1H, s, H-2); ^{13}C NMR (MeOH-d_4, 75 MHz): δ 35.7 (N-CH_3), 68.5 (d, *J* 9.8 Hz, OCH_2), 72.8 (d, *J* 7.6 Hz, CH-4'), 73.1 (d. *J* 5.7 Hz, CH-2'), 77.7 (d, *J* 6.6 Hz, CH-3'), 94.2 (CH-1'), 109.4, 115.6, 120.1, 121.1, 128.4, 146.3, 151.2, 153.6, 156.8.

(R_P)-8-(3-Pyridinyl)adenosine-3',5'-cyclic phosphorothioic acid sodium salt (15c)

(R_P)-8-(3-Pyridinyl)adenosine-3',5'-cyclic phosphorothioic acid tri-*n*-butylammonium salt (**14c**) (0.100 g, 0.16 mmol) was dissolved in 0.1 M NaOH in MeOH (1.7 mL). Addition of hexane precipitated the sodium salt; yield 0.057 g (80%) of a white solid material. HRMS (electrospray, TOF ES^-): M-Na 421.0495. Calc. for $[C_{15}H_{14}N_6O_5PS]$: 421.0489. ^{31}P (MeOH-d_4, 121 MHz): δ 58.08 ppm; ^{1}H NMR (MeOH-d_4, 300 MHz): δ 4.16 – 4.31 (3H, m, H-4' and OCH_2), 5.03 (1H, d, *J* 5.1 Hz, H-2'), 5.41 – 5.45 (1H, m, H-3'), 5.64 (1H, s, H-1'), 7.57 – 7.61 (1H, m, H-pyrid), 8.18 – 8.22 (1H, m, H-pyrid), 8.19 (1H, s, H-2), 8.68 – 8.71 (1H, m, H-pyrid), 8.92 (1H, d, *J* 1.4 Hz, H-pyrid); ^{13}C NMR (MeOH-d_4, 75 MHz): δ 68.3 (d, *J* 9.4 Hz, OCH_2), 72.7 (d, *J* 7.7 Hz, CH-4'), 73.3 (d. *J*

5.6 Hz, CH-2'), 77.6 (d, *J* 6.4 Hz, CH-3'), 94.2 (CH-1'), 120.3, 125.3, 127.2, 138.9, 149.7, 150.6, 151.8, 151.9, 154.3, 157.3.

(R_P)-8-Adenosine-3',5'-cyclic phosphorothioic acid sodium salt (15d)

(R_P)-Adenosine-3',5'-cyclic phosphorothioic acid tri-*n*-butylammonium salt (**14d**) (0.300 g, 0.56 mmol) was dissolved in 0.1 M NaOH in MeOH (6.1 mL). The sodium salt was precipitated by addition of diethyl ether and collected by filtration; yield (0.19 mg, 90%) of a white, solid material. HRMS (electrospray, TOF ES^-): M-Na 344.0211. Calc. for $[C_{10}H_{11}N_5O_5PS]^-$: 344.0224. ^{31}P NMR (MeOH-d_4, 121 MHz): δ 58.39. ^{31}P NMR (D_2O, 121 MHz): δ 57.02; ^1H NMR (D_2O, 200 MHz): 4.24 – 4.37 (3H, m), 4.54 – 4.65 (2H, m), 5.93 (1H, s), 7.98 (s, 1H, s), 8.02 (1H, s, H-2); ^{13}C NMR (D_2O, 50 Mz): δ 68.1, 71.8, 72.7, 76.4, 91.3, 118.8, 139.9, 148.35, 153.1, 155.6.