Supporting Information

N^3,5'-Cycloxanthosine, the First Natural Occurrence of a Cyclonucleoside.

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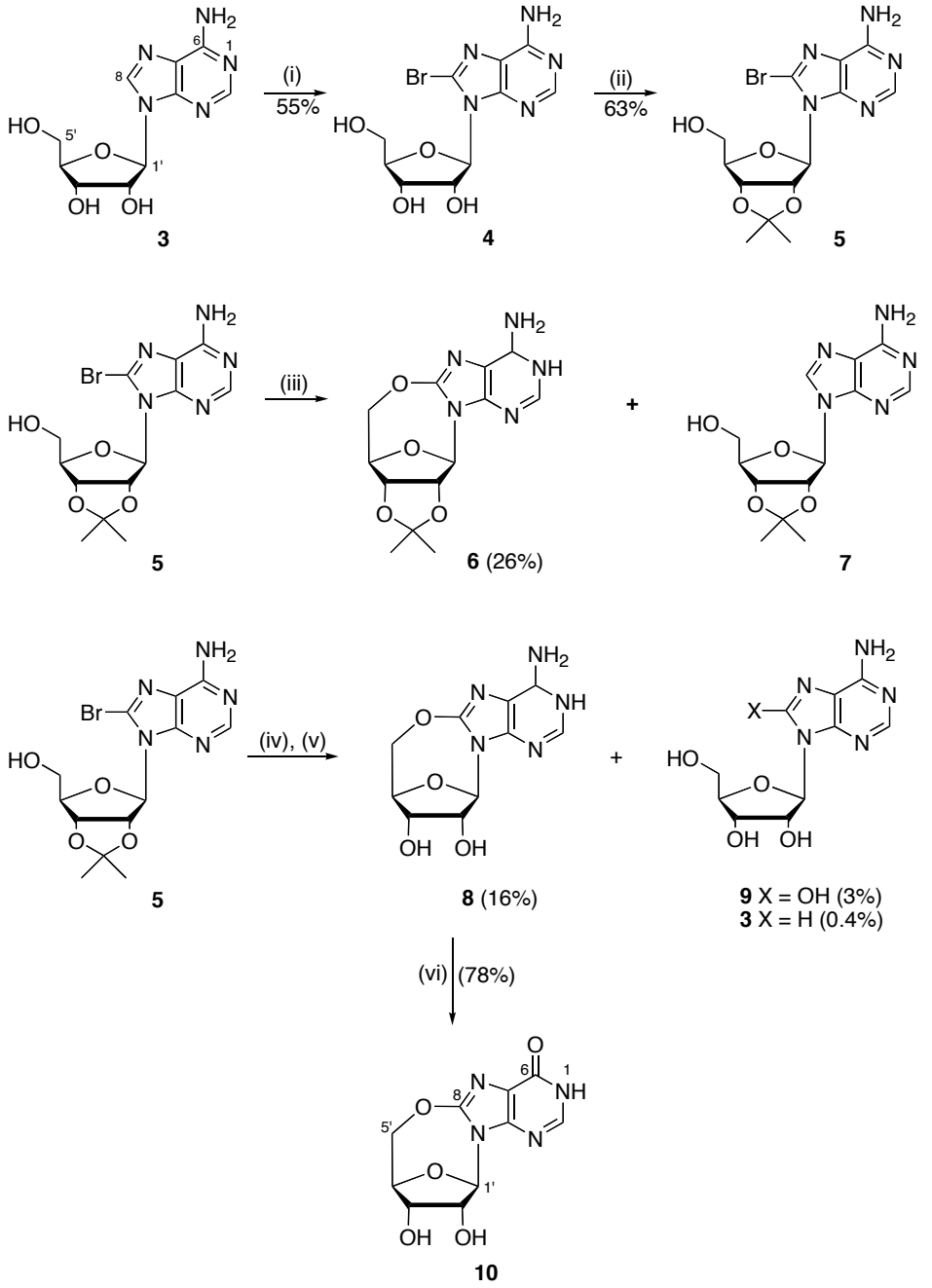
Contents

General Experimental Procedures

Chiroptical measurements ($[\alpha]_D$), given in 10^{-1} deg cm^2 g^{-1}, were obtained on a Jasco P-1010 Intelligent Remote Module type polarimeter, in a 100 × 10 mm cell at 22 °C. UV-VIS absorption spectra were recorded on a CARY3 UV-visible Spectrophotometer. ^{1}H and ^{13}C NMR spectra were acquired at 298K on a Varian Inova 400, a Varian Unity 400 plus, a Bruker Avance 500 or a Bruker Avance 600 Spectrometer, in the solvents indicated, and referenced to residual ^{1}H and ^{13}C signals in the deuterated solvents. Electrospray Ionisation Mass Spectra (ESI-MS) were acquired using either a Waters 2790 Separations Module equipped with a Micromass ZMD mass detector *or* an Agilent 1100 Series Separations Module equipped with an Agilent 1100 Series LC/MSD mass detector. High resolution (HR) ESI-MS measurements were obtained on a Bruker BioApex 47E FT mass spectrometer at a cone voltage of 100 kV while EI-MS measurements were obtained on a Kratos MS25RFA mass spectrometer at 70 eV. Normal-phase column chromatography (flash) was performed using Merck silica gel 60. Analytical TLC was performed on Merck Silica 60 F_{254} sheets. Plates were visualised with 254 nm UV light and / or heating of the plate that had been dipped in *p*-anisaldehyde. High performance liquid chromatography (HPLC) was performed using either a Waters 2790 Separations Module equipped with a Waters 996 Photodiode Array Detector, Alltech 500 Evaporative Light Scattering Detector and a Waters Fraction Collector II, running Waters Millennium software *or* an Agilent 1100 Series Separations Module equipped with a six column switching capability, Agilent 1100 Series Diode Array and / or Multiple Wavelength Detectors, Polymer Laboratories PL-ELS1000 Evaporative Light Scattering Detector (ELSD) and an Agilent 1100 Series Fraction Collector and running ChemStation

Rev.A.10.0 and Purify version A.1.2 software. Unless otherwise specified, a constant level of 0.01% TFA was used in all HPLC separations. Solvents were dried according to standard techniques.

Scheme 1. Synthesis of 8,5'-*O*-cycloinosine (**10**)



Reagents and Conditions: (i) Br_2 (aq), 0.5M NaOAc (aq) buffer (pH 4), RT, 3 h (55%); (ii) *p*-TsOH.H_2O, acetone, RT, 45 min (63%); (iii) NaH (95%), 1,4-dioxane, N_2, RT, 24 h (26% of **6**); (iv) NaH (95%), 1,4-dioxane, N_2, RT, 21 h; (v) 1M H_2SO_4 (aq), 60 ^oC, 3 h (16% of **8**; 3% of **9**; 0.4% of **3**); (vi) 4M $NaNO_2$ (aq), 2M acetic acid (aq), RT, 65 h (78%).

Experimental: Synthesis of 8,5'-*O*-Cycloinosine (10) from Adenosine (3)

8-Bromoadenosine[1,2] (**4**). Saturated bromine water (0.15 L, 28.16 mmol) was slowly added to a mixture of adenosine **3** (4.90 g, 18.33 mmol) in 0.5 M aqueous sodium acetate buffer (0.12 L, pH 4) and the resultant solution stirred at room temperature for 3 h. 5M aqueous sodium hydrogensulfite (50 mL) was added to the resultant mixture to discharge the colour and the pH adjusted to 7 with 5M aqueous sodium hydroxide. The reaction mixture was concentrated to approximately half its volume *in vacuo*, cooled at 4 °C for 17 h, then the precipitate filtered and successively washed with water and acetone to afford *8-bromoadenosine* **4** (3.48 g, 55%) as a pale yellow solid: ^{1}H NMR (d_6-DMSO, 500 MHz) δ 8.12 (s, H-2), 7.56 (br s, 6-NH_2), 5.84 (d, J = 5.4 Hz, H-1'), 5.33 (br m, 2'-, 3'-, 5'-OH), 5.06 (br m, H-2'), 4.20 (br m, H-3'), 4.98 (br m, H-4'), 3.68 (d, J = 9.9 Hz, H-5'a), 3.53 (d, J = 9.2 Hz, H-5'b). The addition of 1 drop of D_2O to the NMR sample saw the loss of the signals (exchangeable protons) at δ 7.56 and 5.33; ^{13}C NMR (d_6-DMSO, 125 MHz) δ 155.1 (C, C-6), 152.3 (CH, C-2), 149.8 (C, C-4), 127.1 (C, C-8), 119.7 (C, C-5), 90.4 (CH, C-1'), 86.7 (CH, C-4'), 71.1 (CH, C-2' or C-3'), 70.8 (CH, C-3' or C-2'), 62.1 (CH_2, C-5'); ESI(-)MS (50V) m/z 693 [2M-H; ^{81}Br^{81}Br isotopes]$^-$, 691 [2M-H; ^{81}Br^{79}Br isotopes]$^-$, 689 [2M-H; ^{79}Br^{79}Br isotopes]$^-$.

8-Bromo-2',3'-*O*-isopropylideneadenosine[3,4] (**5**). *p*-Toluenesulfonic acid monohydrate (3.73 g, 19.61 mmol) was added in one portion to a suspension of 8-bromoadenosine **4** (2.24 g, 6.47 mmol) in acetone (40 mL), and the reaction mixture stirred for 45 min at room temperature. The resultant solution was carefully neutralised with saturated

aqueous sodium carbonate (100 mL) and extracted with chloroform (4 × 100 mL). The combined organic extracts were dried ($MgSO_4$), filtered, concentrated to dryness *in vacuo* and the ensuing solid residue washed with diethyl ether to yield *8-bromo-2',3'-O-isopropylideneadenosine* **5** (1.57 g, 63%) as a white solid: ^{1}H NMR (d_6-DMSO, 500 MHz) δ 8.15 (s, H-2), 7.53 (br s, 6-NH_2), 6.03 (br m, H-1'), 5.66 (br m, H-2'), 5.13 (br m, 5'-OH), 5.04 (br m, H-3'), 4.18 (br m, H-4'), 3.49 (br m, H_2-5'), 1.54 (s, CCH_3), 1.32 (s, CCH_3); ^{13}C NMR (d_6-DMSO, 125 MHz) δ 155.1 (C, C-6), 152.8 (CH, C-2), 149.8 (C, C-4), 126.3 (C, C-8), 119.3 (C, C-5), 113.2 [C, $C(CH_3)_2$], 91.0 (CH, C-1'), 87.1 (CH, C-4'), 81.9 (CH, C-2' or C-3'), 81.6 (CH, C-3' or C-2'), 61.5 (CH_2, C-5'), 27.1 (CH_3, CCH_3), 25.2 (CH_3, CCH_3); ESI(+)MS (30V) *m/z* 388 [M+H; ^{81}Br isotope]$^+$, 386 [M+H; ^{79}Br isotope]$^+$.

8,5'-*O*-Cyclo-2',3'-*O*-isopropylideneadenosine[5] (**6**). Sodium hydride (95%) (0.065 g, 2.57 mmol) was added portionwise to a solution of 8-bromo-2',3'-*O*-isopropylideneadenosine **5** (0.49 g, 1.27 mmol) in anhydrous 1,4-dioxane (20 mL), under an atmosphere of nitrogen. The initially effervescent reaction mixture was stirred at room temperature for 24 h, quenched with water (50 mL), extracted with chloroform (3 × 100 mL), dried ($MgSO_4$), filtered and concentrated to dryness *in vacuo*. Partial purification of the resultant residue by silica gel column chromatography (22 g SiO_2; a one step gradient elution from ethyl acetate to MeOH : DCM (5 : 95)) afforded a mixture of 8,5'-*O*-cyclo-2',3'-*O*-isopropylideneadenosine **6** and tentatively 2',3'-*O*-isopropylideneadenosine[6] **7** (0.36 g). Slow crystallisation of the mixture from ethanol at –30 °C afforded *8,5'-O-cyclo-2'3'-O-isopropylidineadenosine* **6** (0.10 g, 26%) as a pale yellow solid: ^{1}H NMR (d_6-DMSO, 500 MHz) δ 8.12 (br s, H-2), 7.08 (br s, 6-NH_2), 6.05 (br s, H-1'), 5.10 (br m,

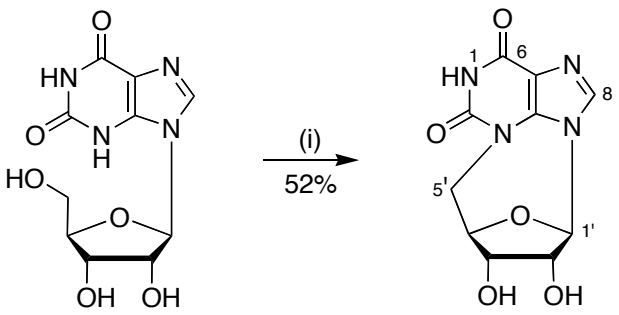
H-3'), 4.90 (br m, H-2'), 4.75 (br s, H-4'), 4.65 (br m, H-5'a), 4.65 (br m, H-5'b), 1.47 (s, CCH_3), 1.30 (s, CCH_3); ^{13}C NMR (d_6-DMSO, 125 MHz) δ 154.7 (C, C-6 or C-8), 153.0 (C, C-8 or C-6), 152.0 (CH, C-2), 147.6 (C, C-4), 114.4 (C, C-5), 111.9 [C, $C(CH_3)_2$], 85.8 (CH, C-1'), 85.2 (CH, C-4'), 84.8 (CH, C-2'), 80.9 (CH, C-3'), 74.2 (CH_2, C-5'), 25.9 (CH_3, CCH_3), 24.3 (CH_3, CCH_3); ESI(+)MS (30V) *m/z* 306 $[M+H]^+$.

8,5'-*O*-Cycloadenosine[5] (**8**). Sodium hydride (95%) (0.44 g, 17.42 mmol) was added portionwise to a solution of 8-bromo-2',3'-*O*-isopropylideneadenosine **5** (3.32 g, 8.60 mmol) in anhydrous 1,4-dioxane (110 mL), under an atmosphere of nitrogen. The initially effervescent reaction mixture was stirred at room temperature for 21 h, quenched with water (100 mL), extracted with chloroform (3 × 150 mL), dried ($MgSO_4$), filtered and concentrated to dryness *in vacuo*. The resultant solid residue (2.71 g) was dissolved in 1M aqueous sulphuric acid (81 mL, 81 mmol), heated at 60 °C for 3 h, cooled to room temperature, neutralised with 30% aqueous ammonia and the solvent removed. The ensuing residue was dissolved in warm water, filtered through a 0.45 μm nylon acrodisc filter and the filtrate subjected to preparative C_8 HPLC (21.2 mL min^{-1} 8-30% gradient CH_3CN/H_2O elution through a 5 μm Agilent Zorbax RX-C8 125 × 21.2 mm column) to yield, in order of elution, *8-hydroxyadenosine*[5] **9** (0.071 g, 3%), *adenosine* **3** (0.010 g, 0.4%) and *8,5'-O-cycloadenosine* **8** (0.37 g, 16%), all as white solids. 8,5'-*O*-Cycloadenosine (**8**): ^{1}H NMR (d_6-DMSO, 500 MHz) δ 8.12 (s, H-2), 7.05 (br s, 6-NH_2), 5.98 (s, H-1'), 5.63 (br s, OH), 5.34 (br s, OH), 4.59 (d, J = 12.9 Hz, H-5'a), 4.56 (s, H-4'), 4.46 (d, J = 6.1 Hz, H-3'), 4.25 (d, J = 6.0 Hz, H-2'), 4.09 (d, J = 12.8 Hz, H-5'b). The addition of 1 drop of D_2O to the NMR sample saw the loss of the signals (exchangeable

protons) at δ 7.05, 5.63 and 5.34; ^{13}C NMR (d_6-DMSO, 125 MHz) δ 154.7 (C, C-6), 153.3 (C, C-8), 151.9 (CH, C-2), 147.6 (C, C-4), 114.3 (C, C-5), 88.7 (CH, C-1'), 88.0 (CH, C-4'), 77.1 (CH, C-2'), 74.6 (CH_2, C-5'), 71.0 (CH, C-3'); ESI(+)MS (80V) *m/z* 266 $[M+H]^+$. 8-Hydroxyadenosine (**9**): ^{1}H NMR (d_6-DMSO, 500 MHz) δ 10.39 (br s, 8-OH), 8.02 (s, H-2), 6.55 (br s, 6-NH_2), 5.68 (d, J = 6.1 Hz, H-1'), 5.13 (br m, 2'-, 3'-, 5'-OH), 4.87 (br m, H-2'), 4.14 (br m, H-3'), 3.87 (br m, H-4'), 3.62 (br m, H-5'a), 3.49 (br m, H-5'b). The addition of 1 drop of D_2O to the NMR sample saw the loss of the signals (exchangeable protons) at δ 10.39, 6.55 and 5.13; ^{13}C NMR (d_6-DMSO, 125 MHz) δ 151.6 (C, C-8), 150.6 (CH, C-2), 147.2 (C, C-4), 146.6 (C, C-6), 103.6 (C, C-5), 85.7 (CH, C-1'), 85.5 (CH, C-4'), 70.9 (CH, C-3' or C-2'), 70.3 (CH, C-2' or C-3'), 62.4 (CH_2, C-5'); ESI(+)MS (80V) *m/z* 306 $[M+Na]^+$, 284 $[M+H]^+$; ESI(-)MS (100V) *m/z* 282 $[M-H]^-$.

8,5'-*O*-Cycloinosine[7] (**10**). 4M Aqueous sodium nitrite (3.50 mL, 14 mmol) was added dropwise to a solution of 8,5'-*O*-cycloadenosine **8** (0.14 g, 0.53 mmol) in 2M aqueous glacial acetic acid (10 mL) at room temperature and the initially effervescent reaction was stirred for 65 h then concentrated to dryness *in vacuo*. The resultant residue was purified by preparative C_8 HPLC (21.2 mL min^{-1} 8% isocratic CH_3CN/H_2O elution through a 5 μm Agilent Zorbax RX-C8 125 × 21.2 mm column) to yield *8,5'-O-cycloinosine* **10** (0.11 g, 78%) as a yellow solid: ^{1}H NMR (d_6-DMSO, 500 MHz) see Table 1; ^{13}C NMR (d_6-DMSO, 125 MHz) see Table 1; ESI(+)MS (80V) *m/z* 555 $[2M+Na]^+$, 267 $[M+H]^+$; ESI(-)MS (80V) *m/z* 531 $[2M-H]^-$, 265 $[M-H]^-$; HR(EI)MS *m/z* 266.0651 ($[M]^+$, $C_{10}H_{10}N_4O_5$ requires 266.0649).

Scheme 2. Synthesis of N^3,5'-cycloxanthosine (**2**)



11 **2**

***Reagents and Conditions*:** (i) PPh_3, diisopropyl azodicarboxylate, DMF, 0 ^oC to RT, N_2, dark, 20.5 h (52% of **2** and 12% of **11**).

Experimental: Synthesis of N^3,5'-Cycloxanthosine (2) from Xanthosine (11)

(–)-N^3,5'-Cycloxanthosine[8,9,10] (**2**). Diisopropyl azodicarboxylate (1.05 mL, 5.33 mmol) was slowly added dropwise to a solution of xanthosine **11** (0.49 g, 1.72 mmol) and triphenylphosphine (1.38 g, 5.26 mmol) in anhydrous DMF (5 mL), under an atmosphere of nitrogen at 0 °C. The resultant mixture was warmed to room temperature and the ensuing colourless solution stirred in the absence of light for 20.5 h. The reaction mixture was then concentrated to dryness *in vacuo*, and the resultant residue washed thoroughly with diethyl ether. A solution of the diethyl ether-insoluble residue in dimethylsulfoxide (20 mL) was purified by preparative C_{18} HPLC (21.2 mL min^{-1} 4% isocratic CH_3CN/H_2O elution through a 5 μm Agilent Zorbax SB-C18 150 × 21.2 mm column) to yield, in order of elution, *cycloxanthosine* **2** (0.24 g, 52%) and *xanthosine* **11** (57 mg, 12%), both as crystalline white solids. $[\alpha]_D$, UV, ESI(±)MS, ^{1}H and ^{13}C NMR data were in agreement with data for the natural product (–)-N^3,5'-cycloxanthosine (**2**).

Reference and Notes

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