Aqueous methods for the preparation of 5'-substituted guanosine derivatives

Paul Brear, Gemma R. Freeman, Mark C. Shankey, Milena Trmčić and David R. W. Hodgson*

Department of Chemistry, Durham University, Science Laboratories, South Road, Durham DH1 3LE, UK.

E-mail: d.r.w.hodgson@durham.ac.uk

This document contains experimental details of the preparation of compounds **2a-d**, **3** and **4b**. In addition, ^{1}H, ^{13}C and, where appropriate, ^{31}P NMR spectra of these compounds are also included.

Experimental

^{1}H, ^{13}C and ^{31}P NMR spectra were obtained on Varian 200, 400, 500 or 700 MHz spectrometers or on a Bruker 400 spectrometer at the frequencies given below. The samples were dissolved in either D_2O, or DMSO-d_6 as specified. Chemical shifts of ^{1}H and ^{13}C NMR spectra are referenced to residual HOD (^{1}H δ=4.65) in D_2O and DMSO-d_5 (^{1}H δ=2.50, ^{13}C δ=39.5) in DMSO-d_6. Mass spectrometry was performed using a Thermo Finnigan LTQ-FT spectrometer.

5′-Deoxy-5′-iodoguanosine 1. This material was prepared using a procedure based on that of Dean.[1]

5′-*S*-Phosphoryl-5′-deoxy-5′-thioguanosine, sodium salt 2a. 5′-Deoxy-5′-iodoguanosine **1** (76 mg, 0.193 mmol) was added to sodium hydroxide solution (2 mL, 100 mM) in a 5 mL round bottomed flask. Trisodium thiophosphate (0.36 g, 2 mmol) was added to the suspension and the mixture was stirred under an argon atmosphere at 50 °C for 2.5 h. The solution was then transferred to centrifuge tubes, the reaction mixture was allowed to cool to room temperature and then methanol (3.26 mL) was added, which caused the precipitation of a white solid. The precipitate was then removed by centrifugation and the supernatant was decanted, frozen in liquid nitrogen, placed in an ice bath and the solvent was

removed using an oil pump. The residue was then suspended in acetone (3 mL) and stirred for 30 min at room temperature. The solid was collected on a Hirsch funnel and washed with acetone (3 × 2 mL). The hygroscopic white powder was then dried overnight over silica gel in a vacuum desiccator to afford the thiophosphate **2a** (59 mg, 0.14 mmol, 72%) contaminated with small amounts of inorganic phosphate ion and 5′-deoxy-5′-thioguanosine, mp 149-150 °C (dec.); δ_H(700 MHz, D_2O) 2.91-2.94 (2 H, m, 5′-CH_2), 4.21 (1 H, m, 4′-C*H*), 4.32 (1H, dd, *J* 5.6 and 3.5, 3′-C*H*), 4.60 (1 H, t, *J* 5.6, 2′-C*H*), 5.75 (1 H, d, *J* 5.6, 1′-C*H*), 7.83 (1 H, s, 8-C*H*); δ_C(175 MHz, D_2O) 32.1 (5′-*C*H_2SP), 72.2, 73.7, 84.6 (d, $^3J_{C-P}$ 4.7, 4′-*C*H), 86.5, 117.9, 135.7, 151.9, 161.4, 168.1; δ_P(283 MHz, D_2O) 16.0; FT-MS (ES^-) *m/z* found 378.0276 ($[M + H]^-$. $C_{10}H_{13}O_7N_5P^{32}S^-$ requires 378.0278).

5′-Deoxy-5′-*N*-hydrazinoguanosine 2b. 5′-Deoxy-5′-iodoguanosine **1** (100 mg, 0.25 mmol) was placed in a 5 mL round-bottomed flask and hydrazine hydrate (1 mL of a 50% solution in water) was added. The mixture was placed under a nitrogen atmosphere at 40 °C for 16 h. The reaction mixture was then transferred to a centrifuge tube and methanol (2.5 mL) was added, which caused the precipitation of a white solid. The solid was collected by centrifugation and was then transferred to a 20 mL round-bottomed flask and heated to reflux in methanol (10 mL). When the solid had dissolved, the solution was transferred to a 15 mL centrifuge tube and allowed to cool. The white solid that precipitated was collected by centrifugation and was then dried overnight in a vacuum desicator to afford the hydrazine **2b** (30 mg, 0.1 mmol, 40%), mp 195-197 °C (dec.); δ_H(700 MHz, DMSO-d_6) 2.78-2.81 (1 H, *ABX* system, J_{AB} 11.9 and J_{AX} 4.9, 5′-C$H_A$$H_B$), 2.83-2.86 (1 H, *ABX* system, J_{AB} 12.6 and J_{BX} 6.3, 5′-C$H_A$$H_B$), 3.94 (1 H, q, *J* 4.9, 4′-CH_X), 4.02 (1 H, t, *J* 4.6, 3′-C*H*), 4.44 (1 H, t, *J* 5.25, 2′-C*H*), 5.62 (1 H, d, *J* 7, 1′-C*H*), 6.45 (2 H, s, NH_2), 7.86 (1 H, s, 8-C*H*); δ_C(175 MHz, DMSO-d_6) 57.8 (5′-*C*H_2NHNH_2), 72.3, 73.5, 83.3, 87.4, 117.6, 136.7, 151.8, 154.3, 157.3; FT-MS (ES^+) *m/z* found 298.1258 ($[M + H]^+$. $C_{10}H_{16}O_4N_7^+$ requires 298.1258).

5′-Deoxy-5′-*N*-hydroxylaminoguanosine 2c. 5′-Deoxy-5′-iodoguanosine **1** (150 mg, 0.3 mmol) was placed in a 20 mL round-bottomed flask. Hydroxylamine solution (8 mL of a 50% solution in water) was added and the mixture was heated

at 60 °C with stirring. After 12 h a clear solution had formed and after 72 h the hydoxylamine solution was removed from the mixture on the rotary evaporator. The off-white solid residue was then re-crystallised from water (7 mL) and the resulting white crystals were collected on a Hirsch funnel and then dried over silica gel in a vacuum desicator to afford the hydroxylamine (74 mg, 0.25 mmol, 63%), mp 149-151 °C (dec.) (lit.,[2] 154 °C (dec.)); δ_H(700 MHz, DMSO-d_6) 2.90-2.93 (1 H, *ABX* system, J_{AB} 14 and J_{AX} 7, 5′-CH_AH$_B$), 2.98-3.01 (1 H, *ABX* system, J_{AB} 14 and J_{BX} 7, 5′-CH_AH$_B$), 4.01 (1 H, q, *J* 7, 4′-CH_X), 4.04-4.06 (1 H, m, 3′-C*H*), 4.48-4.49 (1 H, m, 2′-C*H*), 5.09 (1 H, s, 3′-O*H*), 5.37 (1 H, d, *J* 7, 2′-OH), 5.64 (1 H, d, *J* 7, 1′-C*H*), 6.4 (2H, s, NH_2), 7.90 (1 H, s, 8-C*H*); δ_C(175 MHz, DMSO-d_6) 56.7 (5′-*C*H$_2$NHOH), 72.4, 73.5, 82.3, 86.9, 117.5, 136.7, 152.1, 154.3, 157.4; FT-MS (ES$^+$) *m/z* found 299.1098 ([M + H]$^+$. $C_{10}H_{15}O_5N_6^+$ requires 299.1098).

5′-Azido-5′-deoxyguanosine 2d. Sodium azide (650 mg, 3.08 mmol) was added to a stirred suspension of 5′-deoxy-5′-iodoguanosine **1** (200 mg, 0.5 mmol) in water (5 mL) and the mixture was heated at 110 °C in an oil bath for 20 h. The solution was then transferred into a centrifuge tube and chilled for 3 days, upon which a white precipitate was formed. The suspension was centrifuged, the supernatent was decanted, and the precipitate was allowed to stand repeatedly in cold water overnight (3 × 2 mL). The precipitate was then dried overnight in a vacuum desiccator to afford the azide **2d** (64.5 mg, 42%), mp= 200 °C (dec.); δ_H(400 MHz, DMSO-d_6) 3.53 (1 H, *ABX* system, J_{AB} 13.2 and J_{AX} 4.0, 5′-CH$_A$$H_B$), 3.66 (1 H, *ABX* system, J_{AB} 13.2 and J_{BX} 6.8, 5′-CH$_A$$H_B$), 3.99-3.82 (1 H, m, 4′-CH_x), 4.07-3.99 (1 H, m, 3′-*H*), 4.58 (1 H, t, *J* 5.6, 2′-*H*), 5.40 (1 H, br s, 3′-O*H*), 5.54 (1 H, br s, 2′-O*H*), 5.72 (1 H, d, *J* 6.0, 1′-*H*), 6.56 (2 H, s, NH_2), 7.91 (1 H, s, 8-*H*); δ_C(101 MHz, DMSO-d_6) 51.8 (5′-*C*H$_2$N$_3$), 70.9, 72.6, 82.8, 86.8, 116.8, 135.8, 151.3, 153.7, 156.9; FT-MS (ES$^+$) *m/z* found 331.0875 ([M + Na]$^+$. $C_{10}H_{12}O_4N_8Na^+$ requires 331.0873).

5′-Deoxy-5′-thioguanosine 3. 5′-Deoxy-5′-iodoguanosine **1** (120 mg, 0.3 mmol) and trisodium thiophosphate (0.54 g, 3 mmol) were placed in a 10 mL round-bottomed flask, and sodium hydroxide solution (3 mL, 100 mM) was added. The

mixture was then heated at 50 °C for 2.5 h with stirring under a nitrogen atmosphere. The solution was allowed to cool and was then deoxygenated by sparging with nitrogen gas. The mixture was then acidified to approximately pH 4 by the addition of hydrochloric acid (7 mL, 1 M). The resulting solution was then stirred at room temperature for 72 h under a nitrogen atmosphere. The white precipitate that formed was collected by a centrifugation and washed with water (2 mL), ethanol (2 mL) and diethyl ether (2 mL). The solid was then dried overnight in a vacuum desicator to afford the thiol **3** (67.7 mg, 0.23 mmol, 69%) contaminated with disulfide, mp 216-218 °C (dec.) (lit.,[3] 210 °C (dec.)); δ_H(500 MHz, DMSO-d_6) 2.71-2.75 (1 H, *ABX* system, J_{AB} 13.8 and J_{AX} 6.3, 5′-CH_AH$_B$), 2.80-2.84 (1 H, *ABX* system, J_{AB} 13.5 and J_{BX} 6.5, 5′-CH_AH$_B$), 3.88-3.91 (1 H, m, *J* 3, 4′-CH_X), 4.08 (1 H, q, *J* 5.0, 3′-C*H*), 4.57 (1 H, t, *J* 5.8, 2′-C*H*), 5.69 (1 H, d, *J* 6.5, 1′-C*H*), 6.64 (2 H, s, NH_2), 7.92 (1 H, s, 8-C*H*); δ_C(125 MHz, DMSO-d_6) 27.2 (5′-*C*H$_2$SH), 72.6, 73.4, 85.9, 86.9, 117.4, 136.5, 152.2, 154.5, 157.5; FT-MS (ES$^+$) *m/z* found 322.0580 ([M + Na]$^+$. $C_{10}H_{13}O_4N_5Na^{32}S^+$ requires 322.0580).

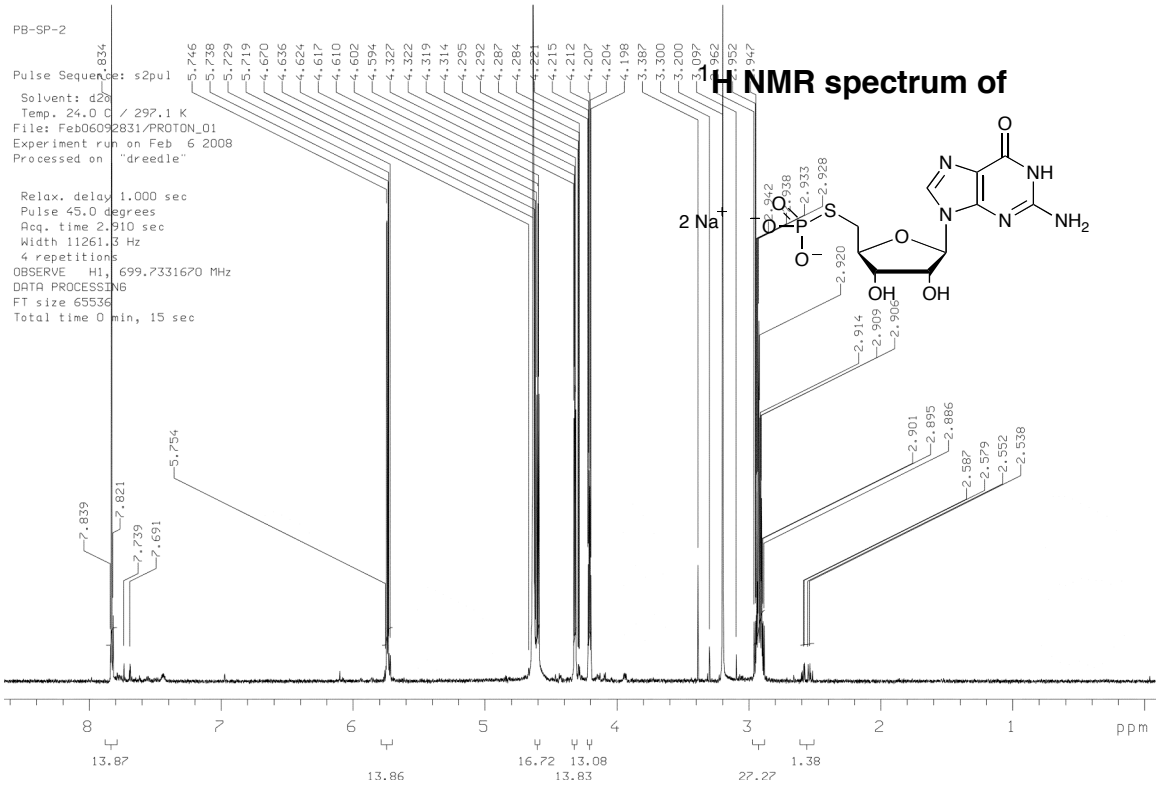
5′-Amino-5′-deoxyguanosine 4b. Sodium thiophosphate (518 mg, 2.88 mmol) was added to a stirred suspension of 5′-azido-5′-deoxyguanosine **2d** (740 mg, 2.4 mmol) in water (27.75 mL). The mixture was heated at 110°C in oil bath for 1 h to give a pale orange solution. The solution was then transferred into a centrifuge tube and chilled overnight. The beige precipitate was collected by centrifugation and washed with cold water (~5×10 mL) until the washings were colourless. The product was dried in a vacuum desiccator overnight to afford the amine **4b** (470 mg, 70 %) as a beige powder, mp= 210 °C (dec.) (lit.,[1] 219-220 ºC (from water) and lit.,[4] 221 °C); δ_H(400 MHz, DMSO-d_6) 2.75 (1 H, *ABX* system, J_{AB} 13.2 and J_{AX} 5.2, 5′-CH$_A$H$_B$), 2.77 (1 H, *ABX* system, J_{AB} 13.2 and J_{BX} 4.4, 5′-CH$_A$H$_B$), 3.77-3.83 (1 H, m, 4′-H$_x$), 4.09 (1 H, t, *J* 4.8, 3′-*H*), 4.46 (1 H, t, *J* 5.6, 2′-*H*), 5.67 (1 H, d, *J* 6.0, 1′-*H*), 6.56 (2 H, s, NH_2), 7.93 (1 H, s, 8-*H*); δ_C(101 MHz, DMSO-d_6) 43.5 (5′-*C*H$_2$NH$_2$), 70.7, 73.2, 85.5, 86.4, 116.8, 135.8, 151.4, 153.9, 157.2; FT-MS (ES$^+$) *m/z* 283.1151 ([M + H]$^+$. $C_{10}H_{15}O_4N_6^+$ requires 283.1149).

References

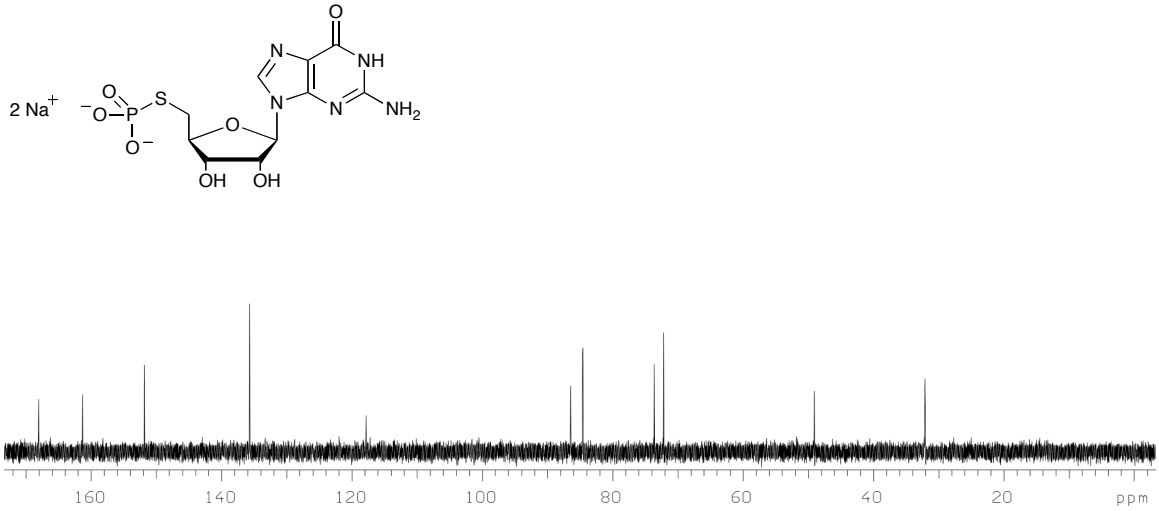
1. D. K. Dean, *Synth. Commun.*, 2002, **32**, 1517-1521.
2. H. Li and M. J. Miller, *J. Org. Chem.*, 1999, **64**, 9289-9293.
3. J. H. Marriott, M. Mottahedeh and C. B. Reese, *Tetrahedron Lett.*, 1990, **31**, 7485-7488.
4. K. Schattka and B. Jastorff, *Chem. Ber. Recl.*, 1972, **105**, 3824-3832.

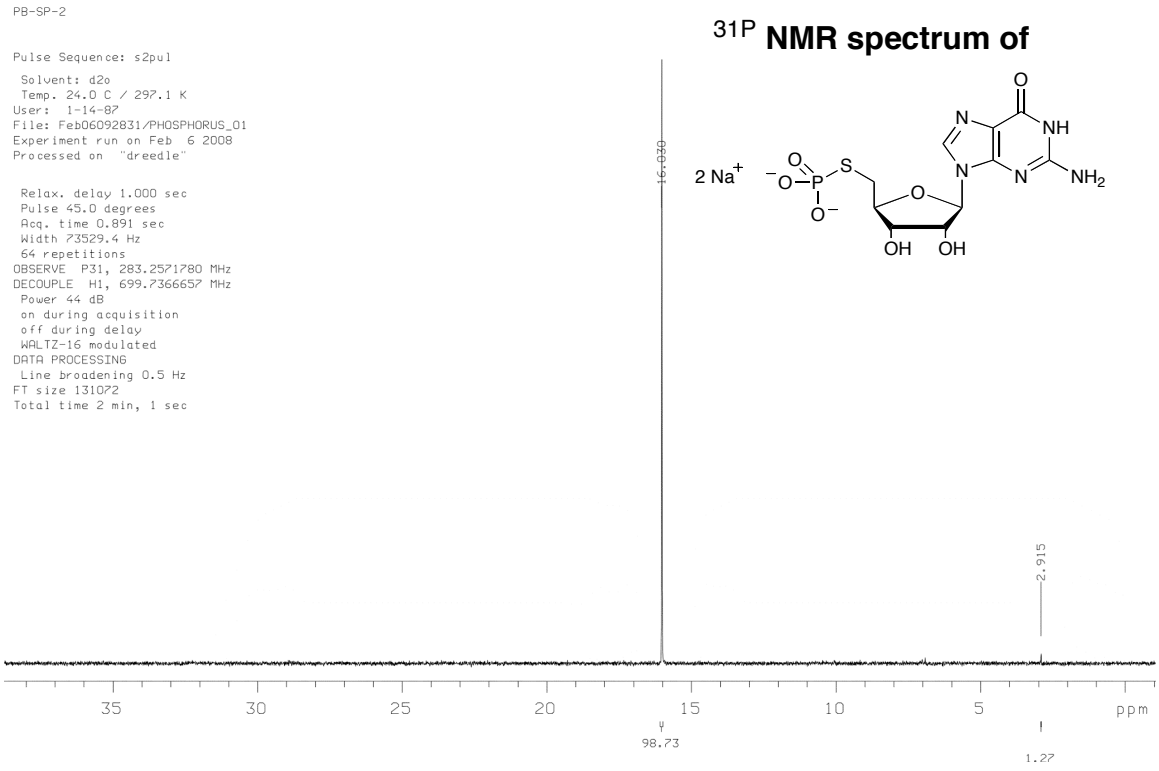
NMR Spectra of Compounds

^{1}H NMR spectrum of

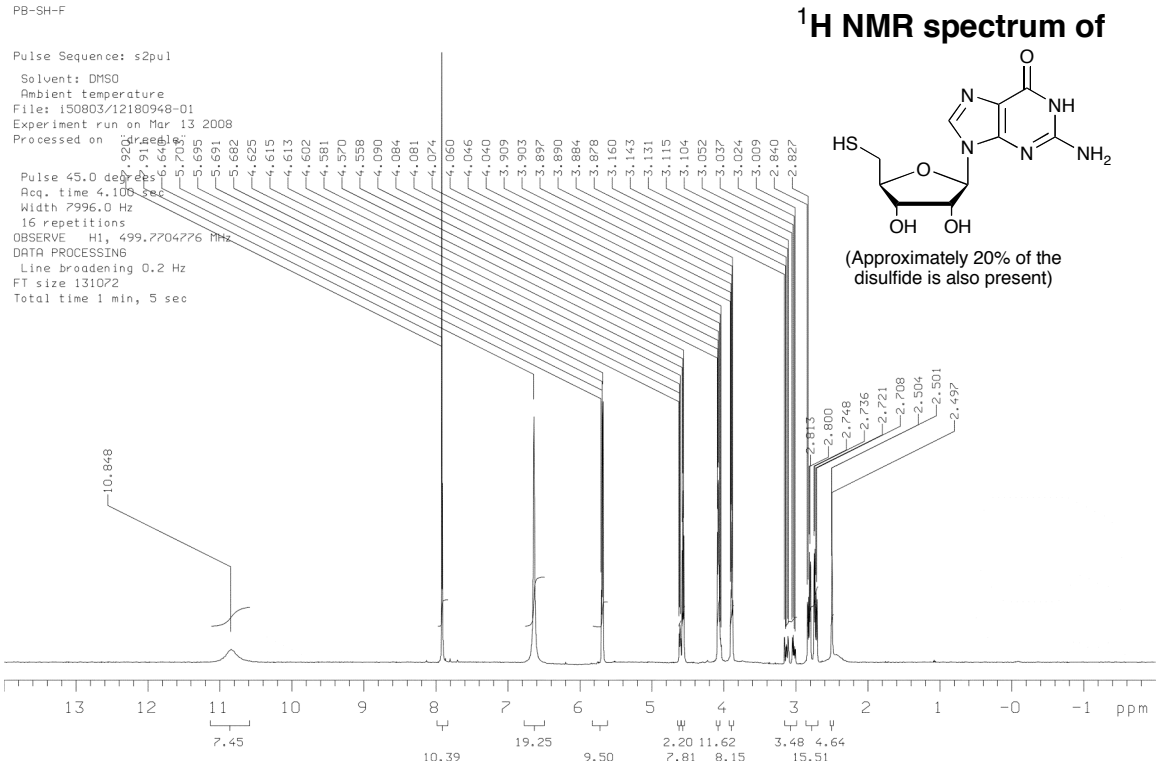


^{13}C NMR spectrum of





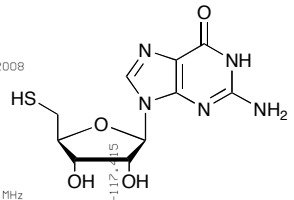
^{31}P NMR spectrum of



^{1}H NMR spectrum of

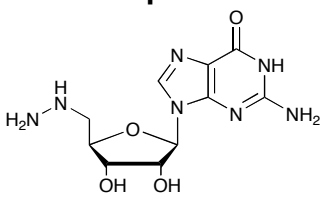
(Approximately 20% of the disulfide is also present)

^{13}C NMR spectrum of

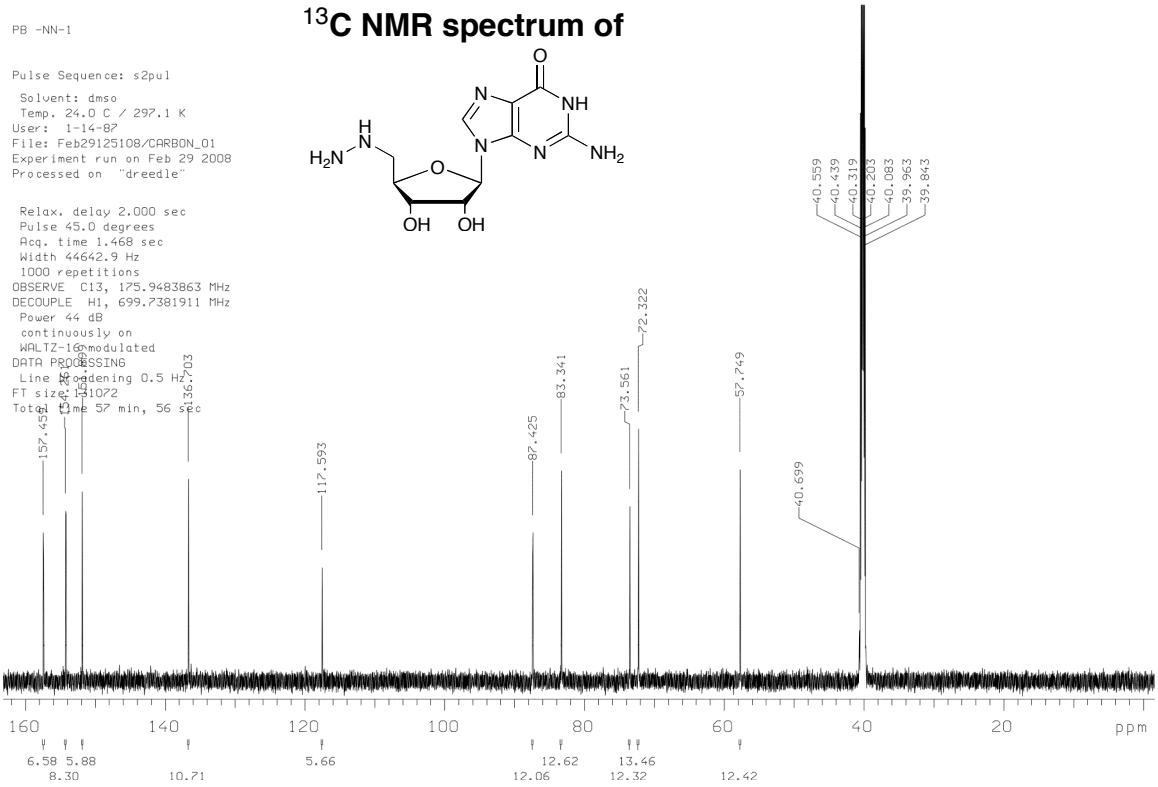


(Approximately 20% of the disulfide is also present)

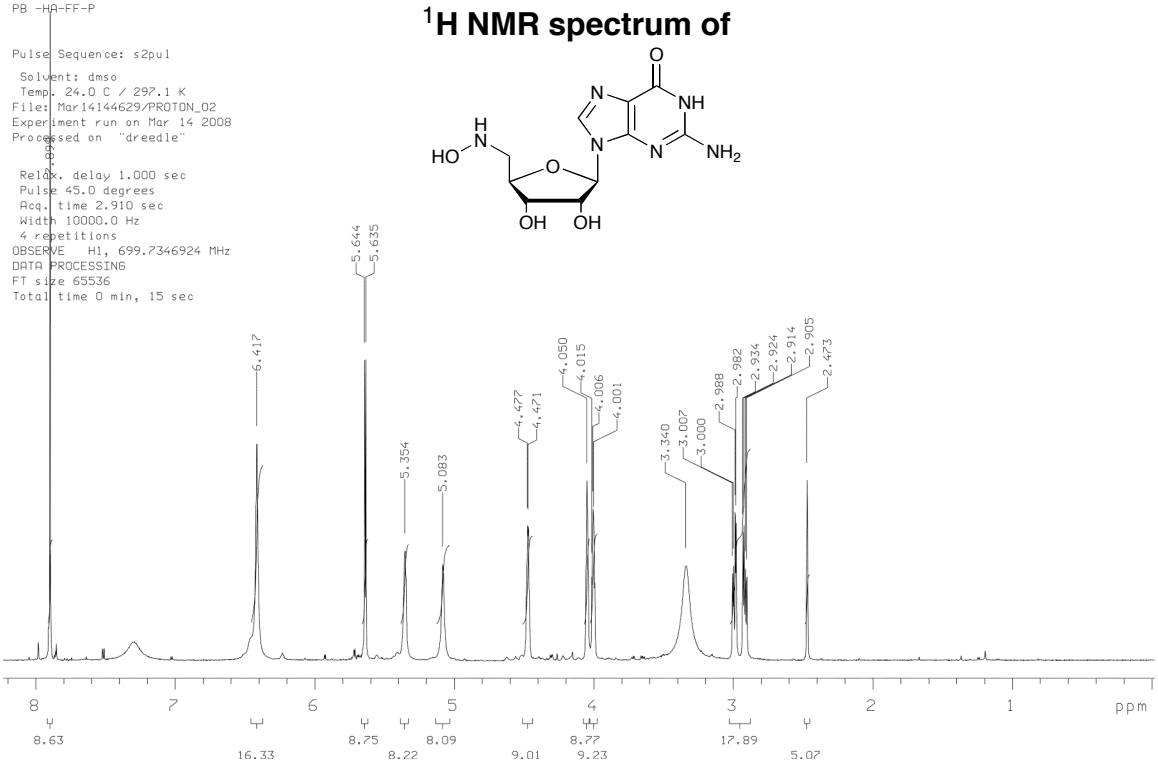
^{1}H NMR spectrum of

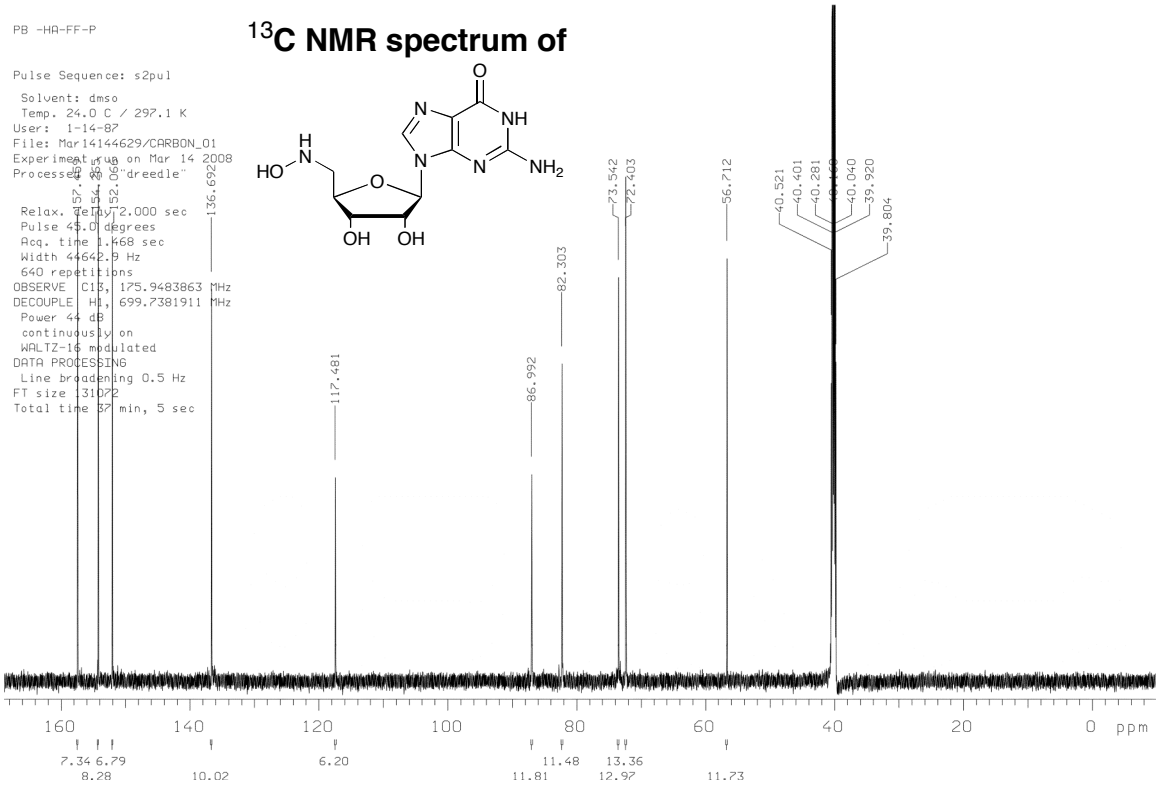


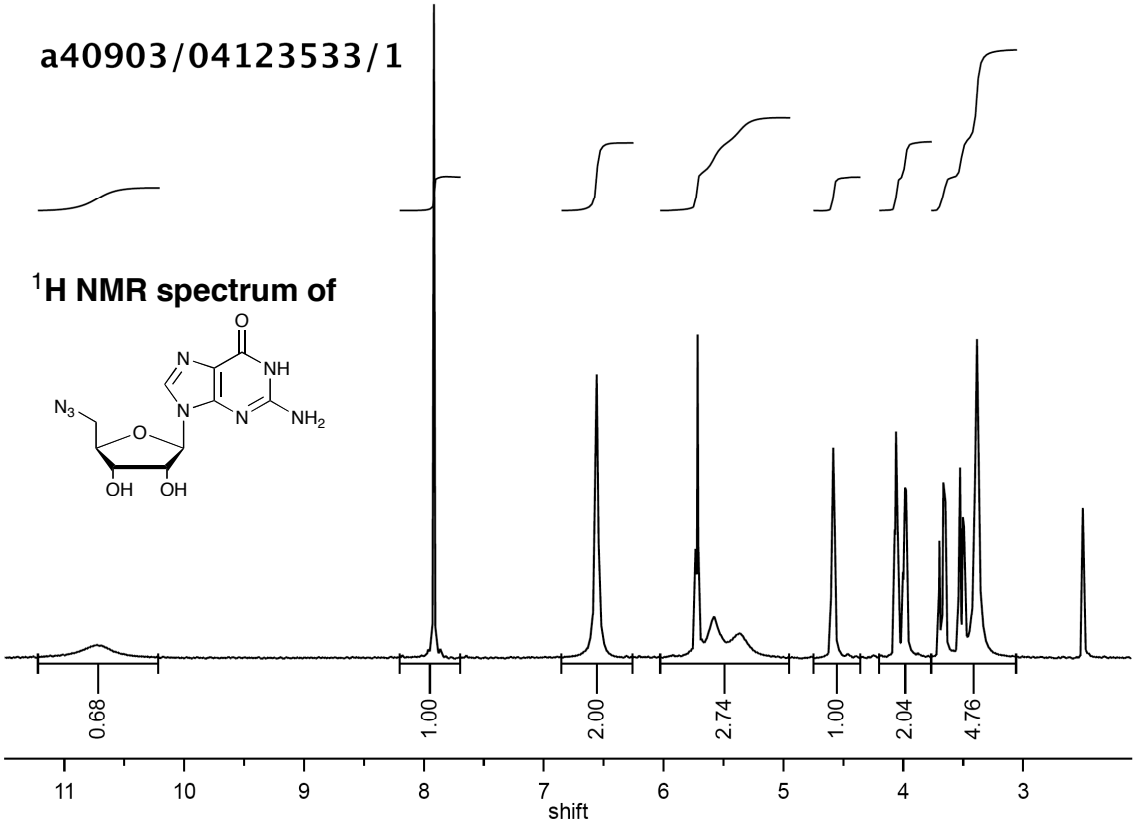
^{13}C NMR spectrum of

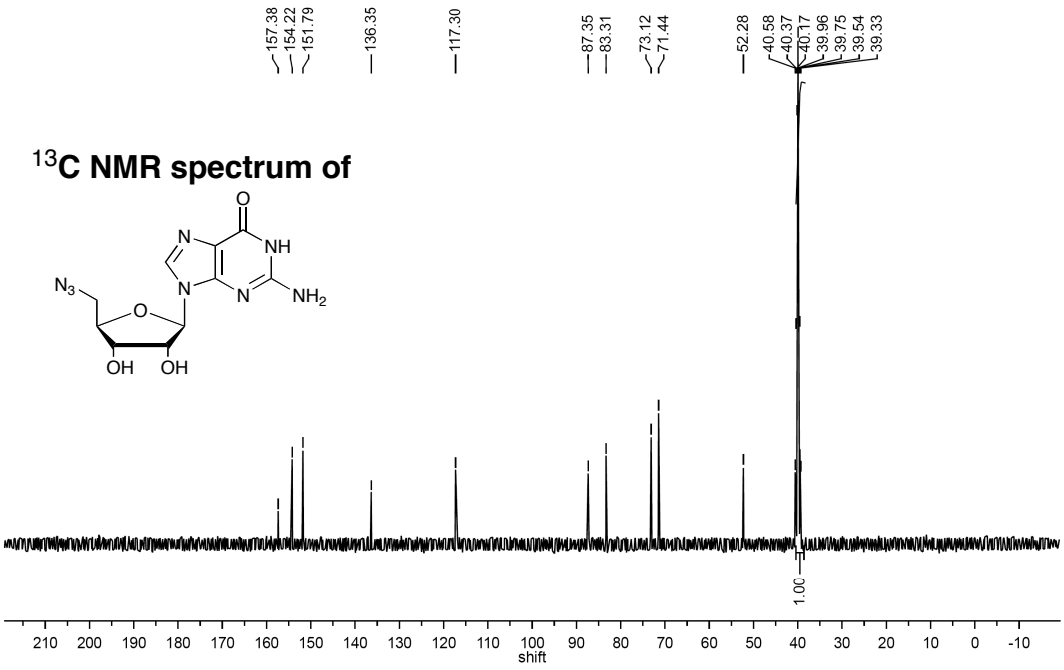


^{1}H NMR spectrum of

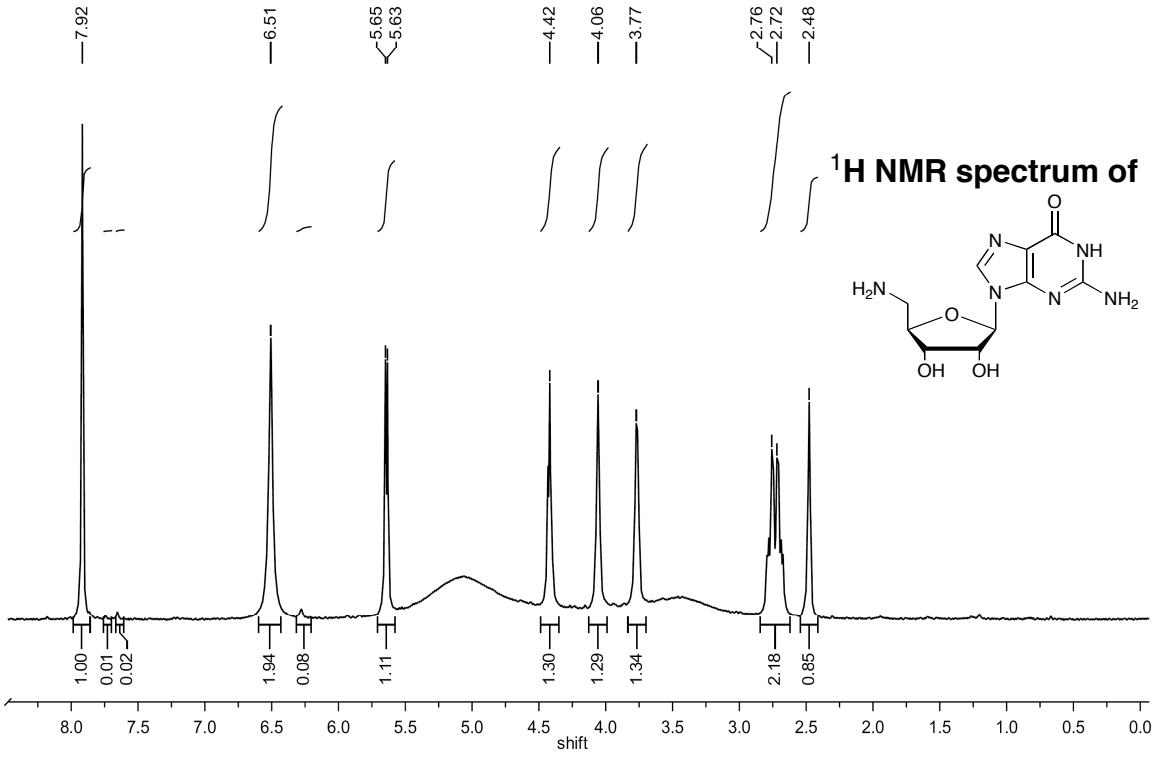



13C NMR spectrum of
PB -HA-FF-P
Pulse Sequence: s2pul
Solvent: dmso
Temp. 24.0 C / 297.1 K
User: 1-14-87
File: Mar14144629/CARBON_01
Experiment run on Mar 14 2008
Processed at 10:06 "dreedle"
Relax. delay 2.000 sec
Pulse 45.0 degrees
Acq. time 1.468 sec
Width 44642.9 Hz
640 repetitions
OBSERVE C13, 175.9483863 MHz
DECOUPLE H1, 699.7381911 MHz
Power 44 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 131072
Total time 37 min, 5 sec
136.692
117.481
86.992
82.303
73.542
72.403
56.712
40.521
40.401
40.281
40.040
39.920
39.804
160
140
120
100
80
60
40
20
0 ppm
7.34 6.79
8.28
10.02
6.20
11.81
11.48
12.97
13.36
11.73

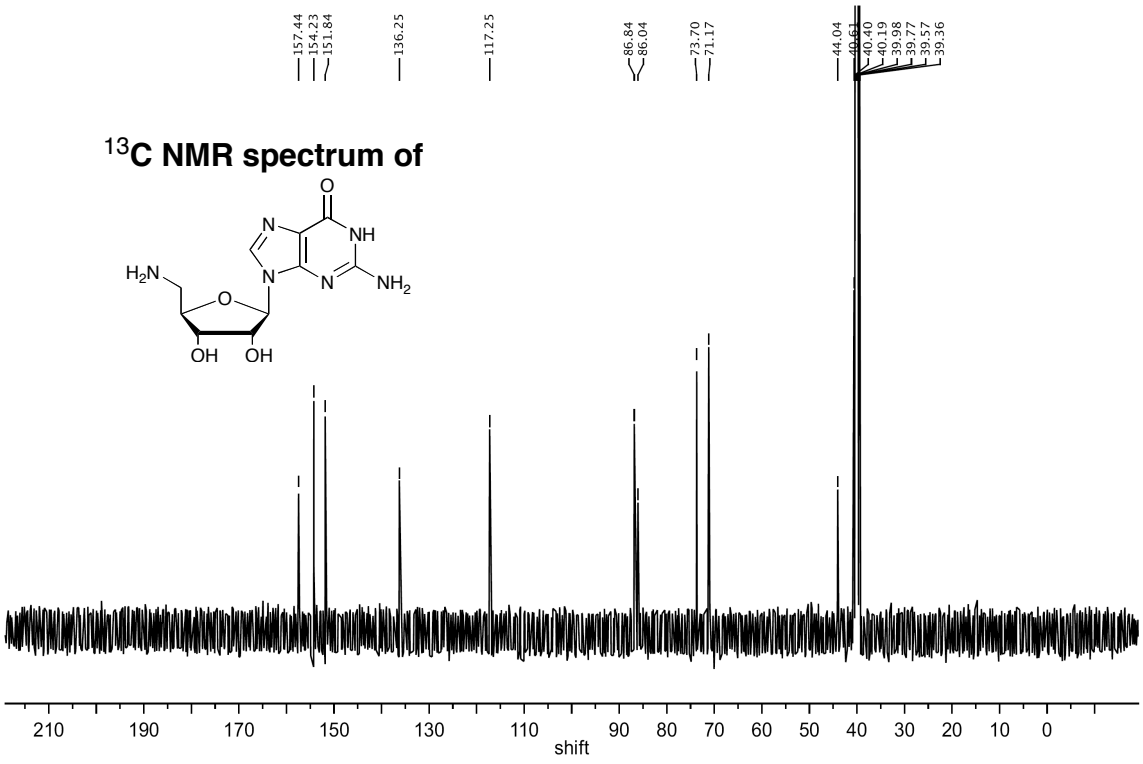

a40903/04123533/1
1H NMR spectrum of
0.68
1.00
2.00
2.74
1.00
2.04
4.76
11
10
9
8
7
6
5
4
3
shift



^{13}C NMR spectrum of



^{1}H NMR spectrum of


157.44
154.23
151.84
136.25
117.25
86.84
86.04
73.70
71.17
44.04
40.61
40.40
40.19
39.98
39.77
39.57
39.36
13C NMR spectrum of
O
N
NH
H2N
N
N
NH2
O
OH
OH
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
shift