Supporting Information

Identification of stable *S*-adenosylmethionine (SAM) analogues derivatised with bioorthogonal tags: Effect of ligands on the affinity of the *E. coli* methionine repressor, MetJ, for its operator DNA

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CONTENTS

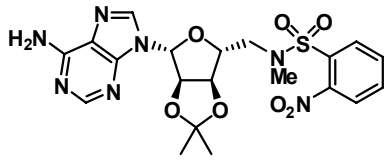
General Experimental

THF was freshly distilled from sodium with benzophenone as an indicator. Dichloromethane was distilled from CaH_2. Ether refers to diethyl ether and petrol refers to petroleum spirit (b.p. 40-60 °C), unless otherwise stated. HPLC methanol and acetonitrile were used without distillation unless stated as dry. Dry methanol and DMF were obtained from a Sure-Seal bottle, stored under nitrogen. Saturated ammonia in methanol was prepared by bubbling ammonia gas through methanol for a minimum of 30 min. All other solvents and reagents were of analytical grade and used as supplied. Commercially available starting materials were obtained from Sigma–Aldrich, Fisher, Lancaster or Alfa Aesar. PyBOP was recyrstallised from CH_2Cl_2–ether. All non-aqueous reactions were carried out using oven or flame dried glassware, under nitrogen unless otherwise stated. Solvents were removed under reduced pressure using a Büchi rotary evaporator attached to a Vacuubrand Vario CVC 2000 pump.

Flash column chromatography was carried out using silica (35-70 μm particles), according to the method of Still, Kahn and Mitra.[1] Thin layer chromatography was carried out on commercially available pre-coated plates (Merck silica Kieselgel $60F_{254}$). Ion exchange chromatography was carried out using Supelco DSC-SCX resin. Chemical shifts are quoted in parts per million downfield of tetramethyl silane and values of coupling constants *J* are given in Hz.

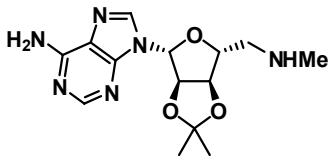
Proton and carbon NMR spectra were recorded on a Bruker Advance DPX 300, Advance 500 or DRX500 spectrophotometer using an internal deuterium lock. Carbon NMR spectra were recorded with composite pulse decoupling using the waltz 16 pulse sequence. DEPT, COSY, HMQC and HMBC pulse sequences were routinely used to aid the assignment of spectra. Chemical shifts are quoted in parts per million downfield of tetramethylsilane, and coupling constants (*J*) are given in Hz. NMR spectra were recorded at 300 K unless otherwise stated. Infra-red spectra were recorded using a Perkin Elmer spectrum one FT-IR spectrophotometer. Melting points were recorded on a Reichert hot stage microscope and are uncorrected. Microanalyses were carried out by staff in the School of Chemistry at the University of Leeds using a Carlo Erba 1108 automatic analyser. Optical activity measurements were recorded at room temperature on an AA-1000 polarimeter; units for $[\alpha]_D$ are 10^{-1} deg cm^2 g^{-1} and are omitted. Mass spectra were recorded on a Micromass LCT-KA111 or Bruker MicrOTOF focus electrospray mass spectrometer. Isotopic distributions were as expected. Accurate molecular weights were generally obtained by staff in the School of Chemistry at the University of Leeds using electrospray mass spectrometry using reserpine as the lock mass and sodium iodide as the standard. Analytical LC-MS was performed using a Waters X-Terra achiral column (MS C18, 5 μm, 50 × 4.6 mm) with a Waters 2525 pump, Waters 2996 photodiode array detector and a Waters Micromass ZQ mass spectrometer as the detector: (Method Ultraquick:1.2 ml/min; H_2O:MeCN with 0.5% formic acid; 2.5 min, 95.5, 3.5 min, 5:95, 5 min, 8:2.

Experimental



5'-Deoxy-5'-*N*-methyl-*N'*-*o*-nitrobenzenesulfonylamino-2',3'-*O,O*-(1-methylethylidene)adenosine S1

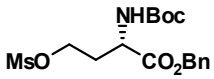
Diethyl azodicarboxylate (13.3 ml, 72.6 mmol) was added to a solution of 2',3'-*O,O*-(1-methylethylidene)adenosine[2] (20.3 g, 66.0 mmol), 2-nitrobenzenesulfonic acid methylamide (15.7 g, 72.6 mmol) and triphenylphosphine (19.1 g, 72.6 mmol) in THF (600 ml) at 0 ºC and stirred for 22 h, warming to room temperature. Triphenylphoshine (9.5 g, 39.6 mmol) and diethyl azodicarboxylate (6.70 ml, 39.6 mmol) were added, the reaction mixture stirred for four h and the solvent removed *in vacuo* to yield a crude solid which was recrystallised from hot methanol and further purified by trituration with ether to yield the *sulfonamide* (**S1**) (19.7 g, 59%) as an amorphous yellow solid, (Found: C, 47.3; H, 4.5; N, 19.5; S, 6.2; $C_{20}H_{23}N_7O_7S$ requires C, 47.5; H, 4.6; N, 19.4; S, 6.3%); R_f 0.39 (5% methanol in CH_2Cl_2); m.p. 240-241 ºC; $[\alpha]_D^{20}$ + 30.4 (*c*. 1.00 in chloroform); ν_{max}/cm^{-1} (film); 3583, 3335 (NH_2), 3186 (NH_2), 2983 (CH), 2917 (CH), 2846, 1709 and 1643 (NH_2); δ_H (500 MHz; $CDCl_3$); 8.36 (1H, s, 8-H), 7.87-7.82 (2H, m, 2-H and Ar-H), 7.63-7.48 (3H, m, Ar-H), 6.03 (1H, d, *J* 2.1, 1'-H), 5.57 (2H, br. s, NH_2), 5.46 (1H, dd, *J* 6.4 and 2.1, 2'-H), 5.14 (1H, dd, *J* 6.4 and 3.3, 3'-H), 4.47-4.40 (1H, m, 4'-H), 3.68 (1H, dd, *J* 14.9 and 4.9, 5-H_A), 3.51 (1H, dd, *J* 14.9 and 7.9, 5-H_B), 2.86 (3H, s, NMe), 1.61 (3H, s, Me) and 1.48 (3H, s, Me); δ_C (75 MHz; *d*-DMSO); 156.5 (C-6), 153.1 (C-2), 149.0 (C-4), 148.1 (C-Ns), 140.5 (C-8), 134.9 (C-Ns), 132.6 (C-Ns), 130.7 (C-Ns), 130.1 (C-Ns), 124.6 (C-Ns), 119.5 (C-5), 113.9 (Me_2C), 89.2 (C-1'), 84.7 (C-4'), 83.5 (C-2'), 82.1 (C-3'), 51.7 (C-5'), 36.0 (NMe), 27.3 (Me) and 25.5 (Me); *m/z* (ES) 506 (100%, MH^+); (Found MH^+ 506.1456, $C_{20}H_{23}N_7O_7S$ requires MH^+ 506.1452).



5'-Deoxy-5'-methylamino-2',3'-*O,O*-(1-methylethylidene)adenosine 4

Thiophenol (4.31 ml, 0.0420 mol) was added to a suspension of 5'-deoxy-5'-*N*-methyl,-*N*'-*o*-nitrobenzenesulfonylamino-2',3'-*O,O*-(1-methylethylidene)adenosine (**S1**) (10.5 g, 0.0210

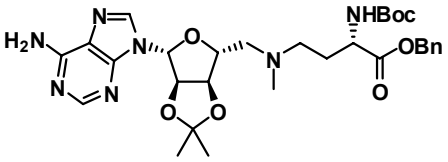
mol) and cesium carbonate (13.7 g, 0.0420 mol) in acetonitrile (350 ml), the mixture was stirred for 18 h then partitioned between CH_2Cl_2 and 1M aqueous sodium hydroxide (100 ml). The aqueous fraction was extracted with CH_2Cl_2 (3 × 300 ml) and the combined organic fractions dried ($MgSO_4$) and concentrated *in vacuo* to yield a crude product which was purified by column chromatography, eluting with CH_2Cl_2 and then 6% saturated ammonia in methanol in CH_2Cl_2 to afford the amine[3] (**4**), (5.62 g, 84%) as a yellow foam, (Found: C, 52.8; H, 6.00; N, 26.5; $C_{14}H_{20}N_6O_3$ requires C, 52.5; H, 6.30; N 26.2%), R_f 0.39 (5% methanol in CH_2Cl_2); $[\alpha]_D^{20}$ – 19.6 (*c*. 1.00 in MeOH); ν_{max}/cm^{-1} (film); 3329 (NH_2), 3184 (NH_2), 2987 (CH), 2937 (CH), 1648 (NH_2) and 1600; δ_H (300 MHz; $CDCl_3$); 8.36 (1H, s, 2-H), 7.91 (1H, s, 8-H), 6.01 (1H, d, *J* 3.3, 1'-H), 5.62 (2H, br. s, NH_2), 5.47 (1H, dd, *J* 6.4 and 3.3, 2'-H), 5.03 (1H, dd, *J* 6.4 and 3.3, 3'-H), 4.40-4.34 (1H, m, 4'-H), 2.87 (2H, app. t, *J* 4.4, 5'-H_2), 2.44 (3H, s, NMe), 1.64 (3H, s, Me) and 1.38 (3H, s, Me); *m/z* (ES) 321 (100%, MH^+).



***N*-(*tert*-Butoxycarbonyl)-*O*-methanesulfonyl-*L*-homoserine benzyl ester 6**

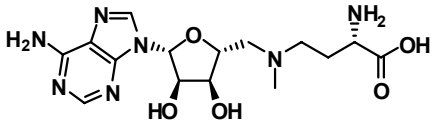
N-Methylmorpholine (1.63 ml, 14.8 mmol), followed by isobutylchloroformate (1.92 ml, 14.8 mmol) were added dropwise to a solution of Boc-Asp-OBzl (4.78 g, 14.8 mmol) in THF (73 ml) at –20 ºC, the reaction was stirred for 10 min, $NaBH_4$ (1.20 g, 44.4 mmol) was added portionwise, followed by MeOH (148 ml) dropwise and the reaction was stirred for a further 10 min. Aqueous HCl solution (1.0 M, 29.6 ml) was added dropwise over 5 min, the reaction stirred for 5 min and the solvent removed *in vacuo* to yield a solid which was partitioned between EtOAc (70 ml) and water (10 ml), the organic layer was washed with water (2 × 45 ml), 5% $NaHCO_3$ solution (46 ml), water (2 × 45 ml), brine (45 ml), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting intermediate alcohol was dissolved in CH_2Cl_2 (200 ml), triethylamine (4.11 ml, 29.5 mmol) was added at 0 ºC, followed by methanesulfonic anhydride (6.45 g, 37.0 mmol) and the reaction stirred for 20 min, warming to room temperature. The reaction was concentrated *in vacuo* and the resulting oil purified by column chromatography, eluting with 30% EtOAc in petrol to afford *the mesylate* (**6**) (2.98 g, 52%) as a colourless oil; R_f 0.40 (50% EtOAc in petrol); $[\alpha]_D^{20}$ –17.6 (*c*. 1.00 in MeOH); ν_{max}/cm^{-1} (film); 3378 (br. NH_2), 2978 (CH), 2937 (CH), 1782, 1741(CO) and 1713 (NH); δ_H (300 MHz; $CDCl_3$); 7.37 (5H, br. s, Bn), 5.24 (1H, br. d, NH), 5.18 (2H, s, Bn CH_2), 4.53-4.42 (1H, m, 2-H), 4.36-4.23 (2H, m, 4-H_2), 2.96 (3H, s, Me), 2.41-2.28 (1H, m, 3-H_A), 2.19-2.03 (1H, m, 3-H_B) and 1.44 (9H, s, Boc); δ_C (75 MHz; $CDCl_3$); 172.0 (*C*O_2Bn), 155.7 (*C*$O_2{}^t$Bu), 135.4 (Bn), 129.1 (Bn), 128.9 (Bn), 80.8 (*C*Me_3), 68.0 (Bn*C*H_2), 66.2 (C-4), 50.8 (C-2), 37.6

(Me), 32.3 (C-3), 28.7 (C*Me*$_3$) and one signal missing or overlapped; *m/z* (ES) 288 (100% [MH-Boc]$^+$); Found MNa$^+$ 410.1227, $C_{17}H_{25}NNaO_7S_1$ requires MNa$^+$ 410.1244).



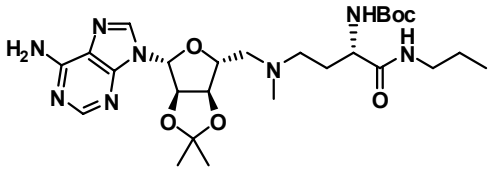
5'-Deoxy-5'-*N*-methyl-*N*'-[4-{(*S*)-2-(*N*-*tert*-butoxycarbonyl)amino-butyric acid}] benzyl ester-2',3'-*O,O*-(1-methylethylidene)adenosine 5

A solution of 5'-deoxy-5'-methylamino-2',3'-*O,O*-(1-methylethylidene)adenosine (**4**) (1.10 g, 3.44 mmol) in acetonitrile (20 ml) was added to a solution of *N*-(*tert*-butoxycarbonyl)-*O*-methanesulfonyl-$_L$-homoserine benzyl ester (**6**) (794 mg, 2.05 mmol), tetrabutylammonium iodide (931 mg, 2.52 mmol) and *N,N'*-diisopropylethylamine (493 µl, 2.52 mmol) in acetonitrile (20.0 ml), the reaction was heated to 65 ºC for 13 h and the solvent removed *in vacuo*, to yield a crude solid which was purified by column chromatography, eluting with 5% MeOH in CH_2Cl_2 to afford the *benzyl ester* (**5**) (875 mg, 70%) as a yellow glass; R_f 0.53 (10% MeOH in CH_2Cl_2); $[\alpha]_D^{20}$ –17.6 (*c*. 1.00 in methanol); ν_{max}/cm^{-1} (film); 3318 (NH_2), 3258 (NH_2), 3173, 2974 (CH), 2923 (CH), 2846, 2796, 1733 (NH_2), 1704 (C=O) and 1641; δ_H (300 MHz; MeOD); 8.16 (1H, s, 8-H), 8.11 (1H, s, 2-H), 7.29-7.14 (5H, m, Bn), 6.05 (1H, d, *J* 2.6, 1'-H), 5.32 (1H, dd, *J* 6.7 and 2.6, 2'-H), 5.14-4.95 (2H, m, Bn CH_2), 4.91-4.86 (1H, m, 3'-H), 4.25-4.18 (1H, m, 4'-H), 4.16-4.10 (1H, m, butyric acid 2-H), 2.59 (1H, dd, *J* 13.1 and 5.9, 5'-H_A), 2.42 (1H, dd, *J* 13.1 and 7.7, 5'-H_B), 2.38-2.22 (2H, m, butyric acid 4-H_2), 2.08 (3H, s, NMe), 1.89-1.75 (1H, m, butyric acid 3-H_A), 1.70-1.54 (1H, m, butyric acid 3-H_B), 1.48 (3H, s, Me), 1.30 (9H, s, ^{t}Bu) and 1.27 (3H, s, Me); δ_C (75 MHz; $CDCl_3$); 174.4 (*C*O_2Bn), 158.3 (*C*$O_2{}^t$Bu), 157.3 (C-6), 154.3 (C-2), 150.5 (C-4), 142.4 (C-8), 137.6 (Bn), 129.8 (Bn), 129.6 (Bn), 129.6 (Bn), 128.3 (Bn), 120.9 (C-5)115.9 (*C*Me$_2$), 91.9 (C-1'), 86.4 (C-4'), 85.3 (C-2'), 84.9 (C-3'), 80.9 (*C*Me$_3$), 68.1 (Ph*C*H$_2$), 60.7 (C-5'), 55.6 (butyric acid C-4), 54.0 (butyric acid C-2), 43.3 (NMe), 29.9 (butyric acid C-3), 29.0 (C*Me$_3$*), 27.8 (Me) and 25.9 (Me); *m/z* (ES) 612 (100%, MH$^+$) and 439; (Found MH$^+$ 612.3128; $C_{30}H_{41}N_7O_7$ requires MH$^+$ 612.3140).



5'-Deoxy-5'-*N*-methyl-*N*'-{4-(*S*)-(2-amino-butyric acid)} adenosine (aza-SAM) 2

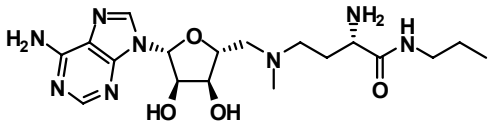
5'-Deoxy-5'-*N*-methyl-*N*'-[4-{(*S*)-2-(*N*-*tert*-butoxycarbonyl)amino-butyric acid}] benzyl ester-2',3'-*O,O*-(1-methylethylidene)adenosine (**5**) (250 mg, 410 mmol) was treated with aqueous sodium hydroxide solution (6.0 ml of a 2.0 M solution) and acetonitrile (1 ml) for 3 h 20 min, the pH was adjusted to 7.0 with 5 M aqueous HCl solution and the reaction stirred for 1 h. 5 M aqueous HCl solution (11.0 ml) was added, the reaction stirred for 10 min, neutralised by addition of 4 M aqueous sodium hydroxide solution. The resulting solution was applied directly to an ion exchange column (SCX) and eluting with saturated ammonia in methanol solution gave the amino acid[2] (**2**) (69 mg, 44 %) as an amorphous yellow solid; R_t (Method: Analysis F) 0.67 min; $[\alpha]_D^{20}$ + 20.8 (*c*. 1.00 in 5.0 M HCl); ν_{max}/cm^{-1} (film); 3324 (NH_2), 3163 (OH), 2939 (CH), 2851, 1646 (NH_2) and 1599 (C=O); δ_H (300 MHz; D_2O); 8.08 (1H, s, Ar-H), 7.95 (1H, s, Ar-H), 5.89 (1H, d, *J* 4.6, 1'-H), 4.56 (1H, app. t, *J* 5.1, 2'-H), 4.20-4.13 (1H, m, 3'-H), 4.09 (1H, app. t, *J* 5.6, 4'-H), 3.23-3.16 (1H, m, butyric acid 2-H), 2.74-2.66 (2H, m, 5'-H_2), 2.51-2.40 (2H, m, butyric acid 4-H_2), 2.18 (3H, s, Me) and 1.88-1.55 (2H, m, butyric acid 3-H_2); *m/z* (ES) 382 (100%, MH^+).



5'-Deoxy-5'-*N*-methyl-*N*'-propyl-[4-{(*S*)-2-(*N*-*tert*-butoxycarbonyl)amino butyramide}]-2',3'-*O,O*-(1-methylethylidene)adenosine S2

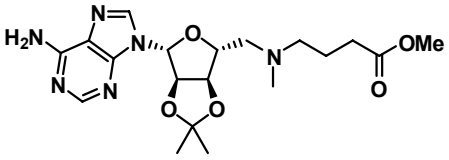
5'-Deoxy-5'-*N*-methyl-*N*'-[4-{(*S*)-2-(*N*-*tert*-butoxycarbonyl)amino-butyric acid}] methyl ester-2',3'-*O,O*-(1-methylethylidene)adenosine (**5**) (88 mg, 0.16 mmol) was dissolved in *N*-propylamine (2.4ml, 0.29 mol) and heated to 47 °C for 23 h. The solvent was removed *in vacuo* and the resulting crude product purified by ion exchange chromatography (SCX) eluting with saturated ammonia in methanol solution to yield the *amide* (**S2**) (89 mg, 99%) as a yellow glass, R_f 0.50 (10% methanol in CH_2Cl_2); $[\alpha]_D^{20}$ –52.0 (*c*. 1.00 in methanol); ν_{max}/cm^{-1} (film); 3327 (NH_2), 3109 (NH), 2970 (CH), 2876, 2478, 2237, 2135, 2067, 1695 (CO), 1651 (NH_2) and 1615 (NH); δ_H (500 MHz; MeOD); 8.31 (1H, s, 8-H), 8.25 (1H, s, 2-H), 6.20 (1H, d, *J* 2.1, 1'-H), 5.52-5.47 (1H, m, 2'-H), 5.04-4.99 (1H, m, 3'-H), 4.39-4.34 (1H, m, 4'-H),

4.04 (1H, app. t, *J* 6.0, butyramide 2-H), 3.18 (1H, td, *J* 13.7 and 7.3, propyl 1-H_A), 3.13 (1H, td, *J* 13.7 and 7.3, propyl 1-H_B), 2.74-2.61 (2H, m, 5'-H_2), 2.52-2.41 (2H, m, butyramide 4-H_2), 2.25 (3H, s, NMe), 1.93-1.84 (1H, m, butyramide 3-H_A), 1.75-1.65 (1H, m, butyramide 3-H_B), 1.62 (3H, s, Me), 1.56-1.47 (2H, m, propyl 2-H_2), 1.45 (9H, s, CMe_3), 1.40 (3H, s, Me), 0.92 (3H, t, *J* 6.4, propyl 3-H_3); δ_C (75 MHz; $CDCl_3$); 175.0 (butyramide C-1), 157.7 (C-6), 154.3 (C-2), 150.6 (C-4), 142.2 (C-8), 120.9 (C-5), 115.9, (*C*Me_2), 91.7 (C-1'), 86.3 (C-4'), 85.0 (C-2'), 84.9 (C-3'), 80.9 (*C*Me_3), 61.0 (C-5'), 55.9 (butyramide C-4), 55.2 (butyramide C-2), 43.9 (NMe), 42.4 (propyl C-1), 30.7 (butyramide C-3), 29.0 (C*Me*$_3$), 27.8 (Me), 25.9 (Me), 23.9 (propyl C-2), 12.0 (propyl C-3) and one signal missing or overlapped; *m/z* (ES) 564 (100%, MH^+); (Found MH^+ 563.3299; $C_{26}H_{42}N_8O_6$ requires MH^+ 563.3300).



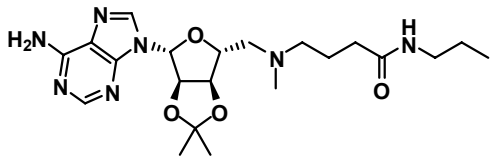
5'-Deoxy-5'-*N*-methyl-*N*'-propyl-{4-[(*S*)-2-(*N*-*tert*-butoxycarbonyl)amino butyramide}]-adenosine 7

5'-Deoxy-5'-*N*-methyl-*N*'-propyl-[4-{(*S*)-2-(*N*-*tert*-butoxycarbonyl)amino butyramide}]-2',3'-*O,O*-(1-methylethylidene)adenosine (**S2**) (32 mg, 0.057 mmol) was treated with 5 M aqueous HCl solution (1 ml) for 5 min, carefully neutralised to pH 7.8 by addition of 5 M aqueous KOH solution and the solution applied directly to an ion exchange column (SCX) eluting with saturated ammonia in methanol solution to yield the *adenosine derivative* (**7**) (22 mg, 91%) as a colourless glass, R_t (Method: Ultraquick) 2.90 min; $[\alpha]_D^{20}$ +7.6 (*c*. 1.00 in methanol); ν_{max}/cm^{-1} (film); 3326 (NH_2), 3269 (OH), 3197 (OH), 2962 (CH), 2928 (CH), 2868, 2808, 1648 (NH_2) and 1600 (C=O); δ_H (500 MHz; MeOD); 8.18 (1H, s, 8-H), 8.11 (1H, s, 2-H), 5.89 (1H, d, *J* 4.4, 1'-H), 4.60 (1H, app. t, *J* 4.9, 2'-H), 4.13 (1H, app. t. *J* 5.6, 3'-H), 4.11-4.05 (1H, m, 4'-H), 3.18 (1H, t, *J* 6.8, butyramide 2-H), 3.01 (2H, td, *J* 7.3 and 4.3, propyl 1-H_2), 2.71-2.66 (2H, m, 5'-H_2), 2.46-2.39 (2H, m, butyramide 4-H_2), 2.20 (3H, s, Me), 1.78-1.68 (1H, m, butyramide 3-H_A), 1.61-1.51 (1H, m, butyramide 3-H_B), 1.38 (2H, tq, *J* 7.3 and 7.3, propyl 2-H_2), 0.79 (3H, t, *J* 7.3, propyl 3-H_3); δ_C (75 MHz; $CDCl_3$); 177.6 (butyramide C-1), 157.7 (C-6), 154.2 (C-2), 150.9 (C-4), 141.8 (C-8), 120.9 (C-5), 90.0 (C-1'), 83.6 (C-4'), 75.0 (C-2'), 74.0 (C-3'), 61.3 (C-5'), 56.1 (butyramide C-4), 55.1 (butyramide C-2), 43.4 (Me), 42.4 (propyl C-1), 33.7 (butyramide C-3), 23.9 (propyl C-2) and 12.0 (propyl C-3); *m/z* (ES) 423 (100%, MH^+); (Found MH^+ 423.2449; $C_{18}H_{30}N_8O_4$ requires MH^+ 423.2463).



5'-Deoxy-5'-*N*-methyl-*N*'-(4-butyric acid methyl ester)-2',3'-*O,O*-(1-methylethylidene)adenosine S3

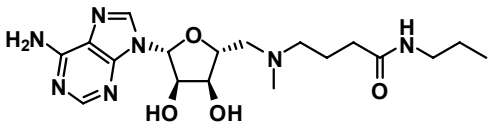
A solution of 4-iodobutyric acid methyl ester[4] (88 mg, 0.38 mmol) in acetonitrile (3 ml) was added to a solution of 5'-deoxy-5'-methylamino-2',3'-*O,O*-(1-methylethylidene)adenosine (**4**) (110 mg, 0.344 mmol) and *N*,*N*'-diisopropylethylamine (59 μL, 0.34 mmol) in acetonitrile (5 ml), heated to 70 ºC for 6.5 h, cooled to room temperature, the solvent was removed *in vacuo* and the resulting crude product purified by column chromatography, eluting with 4% saturated ammonia in methanol solution in CH_2Cl_2 to yield the *ester* (**S3**) (128 mg, 98%) as a colourless glass, R_f 0.37 (5% saturated ammonia in methanol solution in CH_2Cl_2); $[\alpha]_D^{20}$ – 6.2 (*c*. 2.00 in $CHCl_3$); ν_{max}/cm^{-1} (film); 3325 (NH_2), 3175 (NH_2), 2983 (CH), 2945 (CH), 2851, 2796, 1732 (NH_2), 1645 (CO) and 1598; δ_H (300 MHz; $CDCl_3$); 8.36 (1H, s, 2-H), 7.99 (1H, s, 8-H), 6.24 (2H, br. s, NH_2), 6.09 (1H, d, *J* 2.1, 1'-H), 5.48 (1H, dd, *J* 6.4 and 2.1, 2'-H), 4.98 (1H, dd, *J* 6.4 and 3.3, 3'-H), 4.43 (1H, td, *J* 6.9 and 3.3, 4'-H), 3.66 (3H, s, OMe), 2.70 (2H, d, *J* 6.9, 5'-H_2), 2.46 (2H, t, *J* 6.9, butyric acid 4-H_2), 2.35-2.29 (2H, m, butyric acid 3-H_2), 2.31 (3H, s, NMe), 1.77 (2H, dt, *J* 14.6 and 7.4, butyric acid 2-H_2), 1.62 (3H, s, Me) and 1.40 (3H, s, Me); δ_C (75 MHz; $CDCl_3$); 174.3 (butyric acid C-1), 156.1 (C-6), 153.5 (C-2), 149.6 (C-4), 140.4 (C-8), 120.7 (C-5), 114.8 (*C*Me_2), 91.4 (C-1'), 85.2 (C-4'), 84.3 (C-2'), 83.7 (C-3'), 59.4 (C-5'), 57.5 (butyric acid C-4), 52.0 (OMe), 42.8 (NMe), 31.9 (butyric acid C-2), 27.5 (Me), 25.8 (Me) and 12.5 (butyric acid C-3); *m/z* (ES) 421 (100% MH^+); (Found MH^+ 421.2186, $C_{19}H_{28}N_6O_5$ requires MH^+ 421.2194).



5'-Deoxy-5'-*N*-methyl-*N*'-(4-propyl butyramide)-2',3'-*O,O*-(1-methylethylidene)adenosine S4

N-Propylamine (3.80 ml, 46.0 mmol) was added to a solution of 5'-deoxy-5'-*N*-methyl-*N*'-(4-butyric acid methyl ester)-2',3'-*O,O*-(1-methylethylidene)adenosine (**S3**) (483 mg, 1.15 mmol) in methanol (5 ml) and the reaction heated to 47 °C for 5 d. The solvent was removed *in vacuo* and the resulting crude solid purified by ion exchange chromatography (SCX),

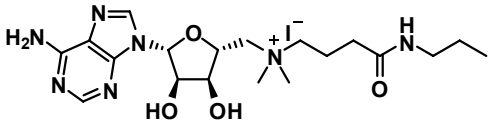
eluting with saturated ammonia in methanol to yield the *amide* (**S4**) (478 mg, 93%) as a colourless glass, R_f 0.51 (15% methanol in CH_2Cl_2); $[\alpha]_D^{20}$ + 12.0 (*c*. 1.00 in methanol); ν_{max}/cm^{-1} (film); 3576 (NH), 3318 (NH_2), 3186 (NH_2), 2962 (CH), 2928 (CH), 2868, 2796, 1645 (NH_2) and 1598 (C=O); δ_H (300 MHz; $CDCl_3$); 8.28 (1H, s, 2-H), 7.94 (1H, s, 8-H), 6.25-6.12 (3H, m, NH_2 and NH), 6.01 (1H, d, *J* 2.1, 1'-H), 5.43 (1H, dd, *J* 6.4 and 2.1, 2'-H), 4.87 (1H, dd, *J* 6.4 and 3.3, 3'-H), 4.31 (1H, dt, *J* 3.3 and 6.9, 4'-H), 3.13-3.03 (2H, m, propyl 1-H_2), 2.51 (2H, app. d, *J* 6.9, 5'-H_2), 2.39-2.22 (2H, m, butyramide 2-H_2), 2.16 (3H, s, NMe), 2.08 (2H, t, *J* 7.2, butyramide 4-H_2), 1.76-1.60 (2H, m, butyramide 3-H_2), 1.54 (3H, s, Me), 1.40 (2H, dd, *J* 14.6 and 7.4, propyl 2-H_2), 1.33 (3H, s, Me), 0.81 (3H, t, *J* 7.4, propyl 3-H_2); δ_C (75 MHz; $CDCl_3$); 173.3 (CO), 156.2 (C-6), 153.5 (C-2), 149.6 (C-4), 140.3 (C-8), 120.7 (C-5), 114.8 (*C*Me_2), 91.5 (C-1'), 85.5 (C-4'), 84.4 (C-2'), 83.8 (C-3'), 59.9 (C-5'), 57.4 (butyramide C-2), 42.8 (butyramide C-4), 41.6 (propyl C-1), 34.6 (NMe), 27.5 (Me), 25.7 (Me), 23.4 (propyl C-2 or butyramide C-3), 23.3 (propyl C-2 or butyramide C-3) and 11.8 (propyl C-3); *m/z* (ES) 448 (30%, MH^+) and 245; (Found MH^+ 448.2684; $C_{21}H_{33}N_7O_4$ requires MH^+ 448.2672).



5'-Deoxy-5'-*N*-methyl-*N*'-(4-propyl butyramide)-adenosine 8

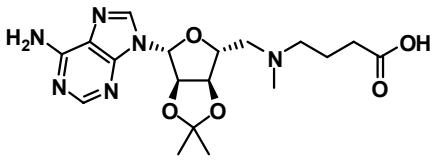
A solution of 5'-deoxy-5'-*N*-methyl-*N*'-(4-propyl butyramide)-2',3'-*O,O*-(1-methylethylidene)adenosine (**S4**) (75 mg, 0.17 mmol) in methanol (1 ml) and water (1ml) was treated with trifluoroacetic acid (0.50 ml, 6.6 mmol) and stirred for 5 min. The solvent was removed *in vacuo*, evaporation of the volatiles secured by addition and evaporation of toluene (10 ml) and the resulting crude solid was purified by ion exchange chromatography (SCX), eluting with saturated ammonia in methanol solution to yield the *amide* (**8**) (67 mg, 98%) as a colourless glass, R_f 0.15 (50% methanol in CH_2Cl_2); $[\alpha]_D^{20}$ −4.3 (*c*. 0.10 in methanol); ν_{max}/cm^{-1}; (film); 3319 (NH_2), 3269 (OH), 3192 (OH), 2962 (CH), 2928 (CH), 2868, 2802, 1646 (NH_2) and 1602 (C=O); δ_H (500 MHz; MeOD); 8.30 (1H, s, 8-H), 8.23 (1H, s, 2-H), 6.02 (1H, d, *J* 4.3, 1'-H), 4.74 (1H, app. t, *J* 5.1, 2'-H), 4.28 (1H, app.t, *J* 5.1, 3'-H), 4.21 (1H, dd, *J* 10.7 and 5.1, 4'-H), 2.97 (2H, app. t, *J* 7.3, propyl 1-H_2), 2.83-2.80 (2H, m, 5'-H_2), 2.48 (2H, app. t, *J* 7.3 butyramide 4-H), 2.31 (3H, s, Me), 2.19 (2H, app. t, *J* 7.3, butyramide 2-H), 1.84-1.76 (2H, m, butyramide 3-H_2), 1.48 (2H, app. dt, *J* 7.3 and 7.3, propyl 2-H_2) and 0.90 (3H, app.t, *J* 7.3, propyl 3-H_3); δ_C (75 MHz; $CDCl_3$); 176.0 (butyramide C-1), 157.6 (C-6), 154.2 (C-2), 150.9 (C-4), 141.8 (C-8), 120.9 (C-5), 90.9 (C-1'), 83.7 (C-4'), 75.0 (C-2'), 74.0 (C-

3'), 61.1 (C-5'), 58.7 (butyramide C-4), 43.4 (Me), 42.5 (propyl C-1), 35.1 (butyramide C-2), 24.4 (butyramide C-3), 23.9 (propyl C-2) and 12.1 (propyl C-3); *m/z* (ES) 408 (100%, MH^+); (Found MH^+ 408.2340; $C_{18}H_{29}N_7O_4$ requires MH^+ 408.2354).



5'-Deoxy-5'-*N,N'*-dimethyl-*N*'-(4-propyl butyramide)-adenosine iodide 9

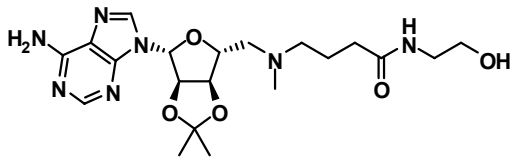
Methyl iodide (14 μl, 0.22 mmol) was added to a solution of 5'-deoxy-5'-*N*-methyl-*N*'-(4-propyl butyramide)-adenosine (**8**) (92 mg, 0.23 mmol) in acetonitrile (10 ml) and water (2 ml), stirred for six h, methyl iodide (2.1 μl, 0.035 mmol) was added and the reaction mixture stirred for 18 h. The solvent was removed *in vacuo* to yield the *quaternary ammonium salt* (**9**) (122 mg, 97%) as an off-white glass, R_f 0.15 (methanol); $[\alpha]_D^{20}$ +10.0 (*c*. 1.00 in methanol); ν_{max}/cm^{-1} (film); 3319 (NH_2), 3197 (NH_2), 2963 (CH), 2928 (CH), 2868, 1646 (NH_2), and 1600 (C=O); δ_H (500 MHz; MeOD); 8.23 (1H, s, 8-H), 8.14 (1H, s, 2-H), 5.99 (1H, d, *J* 3.3, 1'-H), 4.61 (1H, app. t, *J* 4.4, 2'-H), 4.50-4.40 (2H, m, 3'-H and 4'-H), 4.06-3.92 (1H, m, 5'-H_A), 3.72 (1H, d, *J* 14.1, 5'-H_B), 3.42-3.32 (2H, m, butyramide 4-H_2), 3.11 (3H, s, Me), 3.10 (3H, s, Me), 2.99 (2H, t, *J* 7.4, propyl 1-H_2), 2.15 (2H, t, *J* 6.9, butyramide 2-H_2), 2.02-1.90 (2H, m, butyramide 3-H_2), 1.38 (2H, tq, *J* 7.3 and 7.3, propyl 2-H_2), 0.79 (3H, t, *J* 7.4, propyl 3-H_3); δ_C (75 MHz; $CDCl_3$); 173.8 (butyramide C-1), 157.7 (C-6), 154.4 (C-2), 150.6 (C-4), 142.2 (C-8), 121.1 (C-5), 92.2 (C-1'), 79.0 (C-4'), 74.3 (C-2'), 74.0 (C-3'), 67.6 (C-5'), 66.2 (butyramide C-4), 52.9 (NMe), 42.6 (propyl C-1), 32.9 (butyramide C-2), 23.9 (butyramide C-3), 20.1 (propyl C-2) and 12.1 (propyl C-3); *m/z* (ES) 422 (100%, $[M-I^-]^+$) and 295 ($[MH-(CH_2)_3CONH(CH_2)_2CH_3]^+$); (Found $[M-I^-]^+$ 422.2497; $C_{19}H_{32}N_7O_4$ requires $[M-I^-]^+$ 422.2510).



5'-Deoxy-5'-*N*-methyl-*N*'-(4-butyric acid)-2',3'-*O,O*-(1-methylethylidene)adenosine S5

Aqueous sodium hydroxide solution (8.0 ml of a 1.0 M solution, 8.0 mmol) was added to a solution of 5'-deoxy-5'-*N*-methyl-*N*'-(4-butyric acid methyl ester)-2',3'-*O,O*-(1-methylethylidene)adenosine (**S3**) (1.63 g, 3.87 mmol) in methanol (4 ml) and the reaction mixture stirred for 2 d. The pH was raised to 8.0 by careful addition of 5 M aqueous HCl

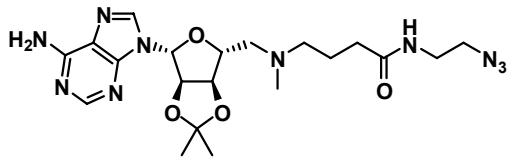
solution and loaded directly onto ion exchange column (SCX) for purification, eluting with saturated ammonia in methanol solution to yield the *acid* (**S5**) (901 mg, 57%) as a colourless glass, R_f 0.08 (50% methanol in CH_2Cl_2); $[\alpha]_D^{20}$ +41.6 (*c*. 1.00 in methanol); ν_{max}/cm^{-1}; 3336 (OH), 3191 (OH), 2989 (CH), 2600, 2250, 1957, 1707, 1650 (NH_2) and 1580 (C=O); δ_H (300 MHz; MeOD); 8.16 (1H, s, 8-H), 8.15 (1H, s, 2-H), 6.18 (1H, d, *J* 2.1, 1'-H), 5.39 (1H, dd, *J* 6.4 and 2.1, 2'-H), 5.38 (1H, dd, *J* 6.4 and 3.3, 3'-H), 4.50 (1H, dt, *J* 10.0 and 3.3, 4'-H), 3.28-3.18 (1H, m, 5'-H), 3.12-3.07 (1H, m, 5'-H), 2.73 (2H, t, *J* 6.8, butyric acid 4-H_2), 2.47 (3H, s, NMe), 2.19-2.06 (2H, m, butyric acid 2-H_2), 1.56 (2H, app. tt *J* 14.1 and 6.8, butyric acid 3-H), 1.48 (3H, s, Me) and 1.27 (3H, s, Me); δ_C (75 MHz; $CDCl_3$); 180.4 (butyric acid C-1), 157.8 (C-6), 154.4 (C-2), 150.3 (C-4), 142.7 (C-8), 121.1 (C-5), 116.2 (*C*Me_2), 92.5 (C-1'), 85.5 (C-4'), 84.7 (C-2'), 84.2 (C-3'), 59.7 (butyric acid C-4), 59.1 (C-5'), 41.6 (NMe), 36.5 (butyric acid C-2), 27.6 (Me), 25.7 (Me) and 22.0 (butyric acid C-3); *m/z* (ES) 407 (30%, MH^+) and 185; (Found MH^+ 407.2027; $C_{18}H_{26}N_6O_5$ requires MH^+ 407.2043).



5'-Deoxy-5'-*N*-methyl-*N*'-(4-[(2-hydroxyethyl)amino] butyramide)-2',3'-*O,O*-(1-methylethylidene)adenosine 10

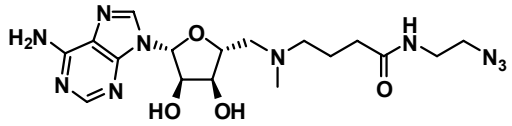
Ethanolamine (656 µl, 10.9 mmol) was added to a solution of 5'-deoxy-5'-*N*-methyl-*N*'-(4-butyric acid)-2',3'-*O,O*-(1-methylethylidene)adenosine (**S5**) (444 mg, 1.09 mmol) in DMF (8 ml) and stirred 5 min before addition of PyBOP (1.71 g, 3.28 mmol) and *N*,*N*'-di*iso*propylethylamine (571 µl, 3.28 mmol). The reaction was stirred for 30 min, filtered and the filtrate concentrated *in vacuo* to yield a crude product which was purified by column chromatography eluting with 10% saturated ammonia in methanol solution in CH_2Cl_2 to afford the *amide* (**10**) (454 mg, 93%) as a colourless glass, R_f 0.48 (20% methanol in CH_2Cl_2); $[\alpha]_D^{20}$ 1.2 (*c*. 1.00 in methanol); ν_{max}/cm^{-1} (film); 3328 (NH_2), 3192 (OH), 2983 (CH), 2939 (CH), 2873, 2851, 2796, 1646 (NH_2), 1600 (C=O); δ_H (500 MHz; MeOD); 8.19 (1H, s, 8-H), 8.13 (1H, s, 2-H), 6.08 (1H, d, *J* 2.1, 1'-H), 5.39 1H, dd, *J* 6.4 and 2.1, 2'-H), 4.88 (1H, dd, *J* 6.4 and 3.4, 3'-H), 4.25 (1H, td, *J* 9.8 and 3.4, 4'-H), 3.48 (2H, t, *J* 6.0, *CH*$_2$$CH_2OH$), 3.17 (2H, t, *J* 6.0, CH_2*CH*$_2$OH), 2.54 (2H, app. d, *J* 6.8, 5'-H_2), 2.26 (2H, t, *J* 7.3, butyramide 4-H_2), 2.13 (3H, s, NMe), 2.04 (2H, t, *J* 7.3 butyramide 2-H_2), 1.58 (2H, tt, *J* 7.3 and 7.3, butyramide 3-H_2), 1.49 (3H, s, Me), 1.28 (3H, s, Me); δ_C (75 MHz; MeOD); 176.3 (butyramide C-1), 157.7 (C-6), 154.3 (C-2), 150.6 (C-4), 142.3 (C-8), 120.9 (C-5), 115.8 (*C*Me_2), 91.9 (C-1'), 86.4 (C-4'), 85.3 (C-2'), 85.1 (C-3'), 61.9 (CH_2*C*H_2OH), 61.7 (C-5'),

60.7 (butyramide C-4), 43.3 (NMe or *C*H_2CH_2OH), 43.1 (NMe or *C*H_2CH_2OH), 35.0 butyramide C-2), 27.7 (Me), 25.9 (Me) and 24.2 (butyramide C-2); *m/z* (ES) 450 (100%, MH^+); (Found MH^+ 450.2468; $C_{20}H_{31}N_7O_5$ requires MH^+ 450.2459).



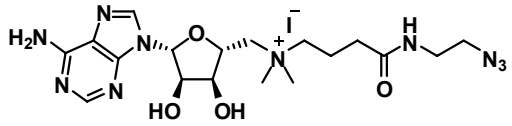
5'-Deoxy-5'-*N*-methyl-*N*'-(4-[(2-azidoethyl)amino] butyramide)-2',3'-*O,O*-(1-methylethylidene)adenosine 11

Methanesulfonic anhydride (322 mg, 1.84 mmol) was added to a solution of 5'-deoxy-5'-*N*-methyl-*N*'-(4-[(2-hydroxyethyl)amino] butyramide)-2',3'-*O,O*-(1-methylethylidene)adenosine (**10**) (332 mg, 0.739 mmol) in CH_2Cl_2 (7 ml) and triethylamine (309 μl, 2.22 mmol) at 0 °C, stirred for 30 min and the solvent removed *in vacuo*. The resulting crude mesylate was dissolved in DMSO (7 ml), sodium azide (1.44 g, 22.2 mmol) added and the mixture heated to 70 °C for 2 h. Water (10 ml) was added and the resulting solution was applied directly to an ion exchange column (SCX) eluting with saturated ammonia in methanol solution to afford the *azide* (**11**) (188 mg, 56%) as a yellow glass; R_f 0.64 (10% methanol in CH_2Cl_2); $[\alpha]_D^{20}$ –7.2 (*c*. 1.00 in methanol); ν_{max}/cm^{-1} (film); 3351 (NH_2), 2917 (CH), 2851 (CH), 2104, 2060, 1995 (N_3), 1643 (C=O); δ_H (300 MHz; MeOD); 8.20 (1H, s, 8-H), 8.15 (1H, s, 2-H), 6.09 (1H, d, *J* 2.3, 1'-H), 5.41 (1H, dd, *J* 6.7 and 2.3, 2'-H), 2.90 (1H, dd, *J* 6.7 and 3.6, 3'-H), 4.26 (1H, td, *J* 7.3 and 3.6, 4'-H), 3.29-3.20 (4H, m, CH_2 and CH_2), 2.58-2.54 (2H, m, 5'-H_2), 2.28 (2H, t, *J* 7.4, butyramide 4-H_2), 2.13 (3H, s, NMe), 2.06 (2H, t, *J* 7.4, butyramide 2-H_2), 1.67-1.55 (2H, m, butyramide 3-H_2), 1.50 (3H, s, Me) and 1.29 (3H, s, Me); δ_C (75 MHz; MeOD); 176.4 (butyramide C-1), 157.7 (C-6), 154.3 (C-2), 150.6 (C-4), 142.3 (C-8), 120.9 (C-5), 115.8 (*C*Me_2), 91.9 (C-1'), 86.4 (C-2'), 85.3 (C-2'), 85.1 (C-3'), 60.8 (C-5'), 58.6 (butyramide C-4), 51.8 (ethyl 1-C or ethyl 2-C), 43.2 (NMe), 40.2 (ethyl 1-C or ethyl 2-C), 34.9 (butyramide C-2), 27.7 (Me), 25.8 (Me) and 24.2 (butyramide C-3); *m/z* (ES) 475 (100%, MH^+); (Found MH^+ 475.2523; $C_{20}H_{30}N_{10}O_4$ requires MH^+ 475.2524).



5'-Deoxy-5'-*N*-methyl-*N*'-(4-[(2-azidoethyl)amino] butyramide)-adenosine 12

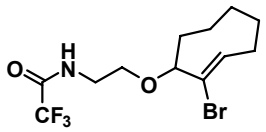
5'-Deoxy-5'-*N*-methyl-*N*'-(4-[(2-azidoethyl)amino] butyramide)-2',3'-*O,O*-(1-methylethylidene)adenosine (**11**) (125 mg, 0.263 mmol) was treated with 5M aqueous HCl (10 ml) solution for 20 min, neutralised by addition of 4M aqueous NaOH solution and the solution applied directly to an ion exchange column (SCX), eluting with saturated ammonia in methanol solution to afford the *azide* (**12**) (107 mg, 94%) as a colourless glass; R_f 0.21 (20% methanol in CH_2Cl_2); $[\alpha]_D^{20}$ –4.0 (*c*. 1.00 in methanol); ν_{max}/cm^{-1} (film); 3348 (OH), 2944 (CH), 2873 (CH), 2813, 2106 (N_3), 1644 (NH_2), 1604 (C=O); δ_H (300 MHz; MeOD); 8.17 (1H, s, 8-H), 8.10 (1H, s, 2-H), 5.89 (1H, d, *J* 3.3, 1'-H), 4.61 (1H, m, 2'-H), 4.17-4.05 (2H, m, 3'-H and 4'-H), 3.25-3.19 (4H, m, ethyl 1-H_2 and ethyl 2-H_2), 2.69 (1H, d, *J* 5.6, 5-H), 2.36 (2H, t, *J* 7.4, butyramide 4-H_2), 2.19 (3H, s, Me), 2.09 (2H, t, *J* 7.4, butyramide 4-H_2) and 1.74-1.62 (2H, m, butyramide 3-H_2); δ_C (75 MHz; MeOD); 176.4 (butyramide C-1), 157.6 (C-6), 154.2 (C-2), 150.9 (C-4), 141.8 (C-8), 121.0 (C-5), 91.0 (C-1'), 83.8 (C-4'), 75.1 (C-2'), 74.1 (C-3'), 61.2 (C-5'), 58.7 (butyramide C-4), 51.8 (ethyl C-1 or ethyl C-2), 43.4 (Me), 40.2 (ethyl C-1 or ethyl C-2), 35.1 (butyramide C-2), 24.3 (butyramide C-3); *m/z* (ES) 435 (100%, MH^+); (Found MH^+ 435.2228; $C_{17}H_{26}N_{10}O_4$ requires MH^+ 435.2211).



5'-Deoxy-5'-*N,N'*-dimethyl-*N*'-(4-[(2-azidoethyl)amino] butyramide)-adenosine iodide 15

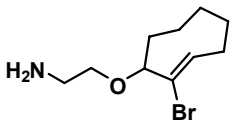
Methyl iodide (6.9 µl, 0.11 mmol) was added to a solution of 5'-deoxy-5'-*N*-methyl-*N*'-(4-[(2-azidoethyl)amino] butyramide)- adenosine (**12**) (48 mg, 0.11 mmol) in acetonitrile (0.3 ml) and water (0.1 ml), stirred 24 h, methyl iodide (1.0 µl, 0.016 mmol) was added, stirred 2 d and methyl iodide (1.2 µl, 0.020 mmol) was added. The reaction was stirred for 24 h and concentrated *in vacuo* to afford the *azide* (**15**) (56 mg, 88%) as a colourless glass; R_t (Method: Analysis G) 4.60 min; $[\alpha]_D^{20}$ + 1.0 (*c*. 4.00 in MeOH); ν_{max}/cm^{-1} (film); 3321 (NH_2), 3192 (NH_2), 2939 (CH), 2105 (N_3), 1650 (NH_2) and 1601 (C=O); δ_H (500 MHz; MeOD); 8.21 (1H, s, 8-H), 8.15 (1H, s, 2-H), 5.98 (1H, app. s, 1'-H), 4.61 (1H, app. s, 2'-H), 4.44 (2H, app, s, 3'-H and 4'-H), 4.03-3.93 (1H, m, 5'-H_A), 3.71 (1H, app. d, *J* 14.1, 5'-H_B), 3.44-3.31 (2H, m,

butyramide 4-H_2), 3.29-3.22 (2H, m, CH_2), 3.22-3.19 (2H, m, CH_2), 3.10 (3H, s, NMe), 3.09 (3H, s, NMe), 2.20-2.13 (2H, m, butyramide 2-H_2), 2.02-1.90 (2H, m, butyramide 3-H_2); δ_C (75 MHz; MeOD); 174.2 (butyramide C-1), 157.8 (C-6), 154.4 (C-2), 150.6 (C-4), 142.2 (C-8), 121.2 (C-5), 92.4 (C-1'), 79.0 (C-4'), 74.3 (C-2' or C-3'), 74.1 (C-2' or C-3'), 67.7 (C'-5'), 66.2 (butyramide C-4), 52.9 (2 NMe), 51.8 (CH_2), 43.4 (CH_2), 32.8 (butyramide C-2), 20.0 (butyramide C-3); m/*z* (ES) 449.2 (100% $[M-I^-]^+$); (Found $[M-I^-]^+$ 449.2365; $C_{18}H_{29}N_{10}O_4I$ requires $[M-I^-]^+$ 449.2368).



(*Z*)-*N*-(2-(2-bromocyclooct-2-enyloxy)ethyl)-2,2,2-trifluoroacetamide S6

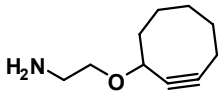
Silver trifluoromethanesulfonate (12.3 g, 47.7 mmol) was added to a solution of 8,8-dibromo-bicyclo[5.1.0]octane[5] (4.27 g, 15.9 mmol) and *N*-(2-hydroxyethyl)-2,2,2-trifluoroacetamide (18.7 ml, 168 mmol) in toluene (20 ml) in a flame-dried, foil-lined flask. The reaction was stirred for 18 h, concentrated *in vacuo* and purified by column chromatography, eluting with 20-30% EtOAc in petrol to afford the *alkene* (**S6**) (3.15 g, 58%); >95:<2 *trans*:*cis* as a brown oil; R_f 0.52 (20% EtOAc in petrol); ν_{max}/cm^{-1} (film); 3324, 3102 (alkene CH), 2933 (CH), 2859 (CH), 1788 and 1715 (C=O); δ_H (500 MHz; $CDCl_3$); 7.05 (1H, br. s, NH), 6.23 (1H, dd, *J* 12.0 and 3.9, cyclooctenyl 2-H), 3.89 (1H, dd, *J* 10.3 and 4.7, cyclooctenyl 8-H), 3.69-3.47 (4H, m, 1-H_2 and 2-H_2), 2.73 (1H, app. qd, *J* 11.6 and 5.1, cyclooctenyl 3-H_A), 2.35-2.28 (1H, m, cyclooctenyl 3-H_B), 2.00-1.84 (2H, m, CH), 1.77-1.68 (2H, m, CH), 1.55-1.43 (1H, m, CH), 1.33-1.20 (1H, m, CH), 0.90-0.75 (2H, m, CH); δ_C (75 MHz; $CDCl_3$); 157.0 (C=O), 132.5 (cyclooctenyl C-1), 132.1 (cyclooctenyl C-2), 121.6 (CF_3), 84.8 (cyclooctenyl C-8), 66.3 (C-2), 39.8 (C-1 or CH_2), 39.7 (C-1 or CH_2), 36.5 (CH_2), 33.4 (CH_2), 28.2 (CH_2), 26.3 (CH_2); m/*z* (ES) 366.0 (100% MNa^+ ^{79}Br); (Found MNa^+ ^{79}Br 366.0276; $C_{12}H_{17}BrF_3NO_2$ requires MNa^+ ^{79}Br 366.0287). The *trans* isomer gradually equilibrated to the *cis* isomer on standing.



(*Z*)-2-(2-bromocyclooct-2-enyloxy)ethanamine S7

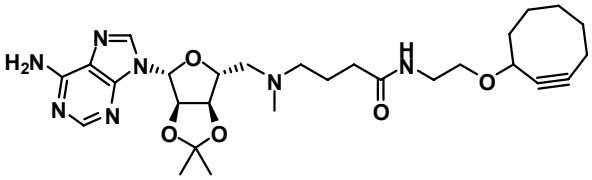
Aqueous sodium hydroxide solution (1.00 ml of a 1.0 M solution, 1.00 mmol) was added to a solution of (*Z*)-*N*-(2-(2-bromocyclooct-2-enyloxy)ethyl)-2,2,2-trifluoroacetamide (**S6**) (113

mg, 0.328 mmol) in methanol (4 ml). The reaction was heated to 50 °C for 1 h, neutralised by addition of 5 M aqueous HCl solution and the solution applied directly to an ion exchange column (SCX), eluting with saturated ammonia in methanol solution to afford the *alkene* (**S7**) (974 mg, 52%); >95:<2 *trans*:*cis* as a brown oil; R_f 0.54 (10% saturated ammonia in methanol solution in CH_2Cl_2); ν_{max}/cm^{-1} (film); 3365 (NH_2), 2929 (CH), 2854 (CH), 2156, 1630; δ_H (500 MHz; MeOD); 6.16 (1H, dd, *J* 12.0 and 4.3, cyclooctenyl 2-H), 3.81 (1H, dd, *J* 10.3 and 4.7, cyclooctenyl 8-H), 3.41-3.33 (1H, m, 2-H_A), 3.22-3.16 (2H, m, 1-H_A and 2-H_B), 2.67-2.51 (3H, m, 1-H_B, cyclooctenyl 3-H_A and CH), 2.16-2.10 (1H, m, cyclooctenyl 3-H_B), 1.89-1.64 (4H, m, CH), 1.44-1.33 (1H, m, cyclooctenyl 7-H_A), 1.19-1.10, 1H, m, cyclooctenyl 7-H_A), 0.79-0.68 (1H, m, CH); δ_C (75 MHz; MeOD); 134.5 (cyclooctenyl C-1), 133.2 (cyclooctenyl C-2), 86.4 (cyclooctenyl C-8), 71.6 (C-2), 42.6 (C-1), 41.0 (CH_2), 37.7 (CH_2), 34.5 (cyclooctenyl C-3), 29.3 (CH_2), 27.8 (CH_2); m/*z* (ES) 247.9 (80% MH^+ ^{79}Br) and 289.0 (80% $[M + MeCN]H^+$ ^{79}Br); (Found MH^+ ^{79}Br 248.0641; $C_{10}H_{18}BrNO$ requires MH^+ ^{79}Br 248.0645). The *trans* isomer gradually equilibrated to the *cis* isomer on standing.



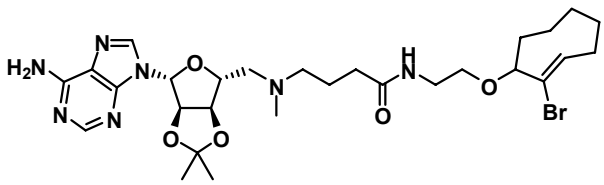
2-(Cyclooct-2-ynyloxy)ethanamine S8

Sodium hydride (60% dispersion in mineral oil, 72 mg, 1.8 mmol) was added to a solution of (*Z*)-2-(2-bromocyclooct-2-enyloxy)ethanamine (**S7**) (89 mg, 0.36 mmol) in DMF (1 ml) and THF (1 ml). The reaction was cooled to 0 °C, neutralised by addition of 1M aqueous HCl solution and the solution applied directly to an ion exchange column (SCX), eluting with saturated ammonia in methanol solution to afford the *alkyne* (**S8**) (51 mg, 85%) as a colourless oil; R_f 0.74 (10% saturated ammonia in methanol solution in CH_2Cl_2); ν_{max}/cm^{-1} (film); 3366 (NH_2), 3291 (CH_2), 2926 (CH), 2852 (CH), 2206 and 1637; δ_H (500 MHz; MeOD); 4.12-4.05 (1H, m, cyclooctynyl 1-H), 3.48 (1H, dt, *J* 9.4 and 5.6, 2-H_A), 3.25 (1H, dt, *J* 10.7 and 5.6, 2-H_B), 2.67 (2H, t, *J* 5.1, 1-H_2), 2.20-1.96 (3H, m, CH), 1.89-1.67 (4H, m, 6-H_2 and CH), 1.63-1.48 (2H, m, CH), 1.42-1.33 (1H, m, CH); δ_C (75 MHz; MeOD); 101.3 (C≡C), 94.2 (C≡C), 77.9 (C-2), 71.9 (cyclooctynyl C-1), 43.9 (cyclooctynyl C-8), 42.8 (C-1), 35.9 (CH_2), 32.0 (CH_2), 31.2 (CH_2), 21.7 (CH_2); m/*z* (ES) 168.1 (100% MH^+); (Found MH^+ 168.1384; $C_{10}H_{17}NO$ requires MH^+ 168.1383).



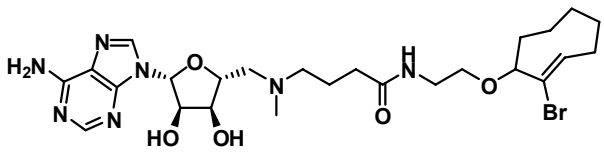
5'-Deoxy-5'-*N*-methyl-*N*'-{4-[2-(cyclooct-2-ynyloxy)ethanamine] butyramide}-2',3'-*O,O*-(1-methylethylidene)adenosine S9

PyBOP (183 mg, 0.351 mmol) and DIPEA (61.0 µl) were added to a solution of 5'-deoxy-5'-*N*-methyl-*N*'-(4-butyric acid)-2',3'-*O,O*-(1-methylethylidene)adenosine (**72**) (114 mg, 0.281 mmol) and 2-(cyclooct-2-ynyloxy)ethanamine (**S8**) (39.2 mg, 0.234 mmol) in DMF (2 ml). The reaction was stirred for 20 min and the solution applied directly to an ion exchange column (SCX), eluting with saturated ammonia in methanol solution, followed by column chromatography, eluting with 3.5% saturated ammonia in methanol solution in CH_2Cl_2 to afford the *alkyne* (**S9**) (123 mg, 94%) as a colourless glass; R_f 0.17 (5% saturated ammonia in methanol solution in CH_2Cl_2); $[\alpha]_D^{20}$ –5.4 (*c*. 4.00 in MeOH); ν_{max}/cm^{-1} (film); 3324 (OH), 3181 (NH_2), 2933 (CH), 2853 (CH), 2800 (CH), 2479, 2207, 2064, 1647 (C=O); δ_H (500 MHz; MeOD); 8.19 (1H, s, adenosine 8-H), 8.13 (1H, s, adenosine 2-H), 6.07 (1H, app. s, adenosine 1'-H), 5.39 (1H, app. s, adenosine 2'-H), 4.90-4.86 (1H, m, adenosine 3'-H), 4.27-4.21 (1H, m, adenosine 4'-H), 4.09-4.03 (1H, m, cyclooctynyl 1-H), 3.49-3.43 (1H, m, ethyl 2-H_A), 3.29-3.14 (3H, m, ethyl 2-H_B and ethyl 1-H_2), 2.58-2.46 (2H, m, adenosine 5'-H_2), 2.26 (2H, t, *J* 6.8, butyramide 4-H_2), 2.12 (3H, s, NMe), 2.10-1.92 (4H, m, butyramide 2-H_2 and CH_2), 1.84-1.64 (6H, m, 3 CH_2), 1.64-1.51 (3H, m, butyramide 2-H_2 and CH), 1.49 (3H, s, Me), 1.36-1.28 (1H, m, CH), 1.27 (3H, s, Me); δ_C (75 MHz; MeOD); 173.2 (butyramide C-1), 157.7 (adenosine C-6), 154.4 (adenosine C-2), 150.6 (adenosine C-4), 142.3 (adenosine C-8), 121.0 (adenosine C-5), 115.8 (CMe_2), 101.2 (C≡C), 93.9 (C≡C), 91.9 (adenosine C-1'), 86.5 (adenosine C-4'), 85.3 (adenosine C-2'), 85.1 (adenosine C-3'), 74.0 (cyclooctynyl C-1), 68.9 (ethyl C-2), 60.8 (adenosine C-5'), 58.5 (butyramide C-4), 43.7 (CH_2), 43.2 (NMe), 40.7 (ethyl C-1), 35.7 (CH_2), 35.0 (CH_2), 31.2 (CH_2), 27.7 (Me), 25.9 (Me), 24.3 (butyramide C-3), 21.2 (CH_2) and one signal missing or overlapped; m/*z* (ES) 556 (100%, $[MH]^+$); (Found $[MH]^+$ 556.3255; $C_{28}H_{41}N_7O_5$ requires $[MH]^+$ 556.3242).



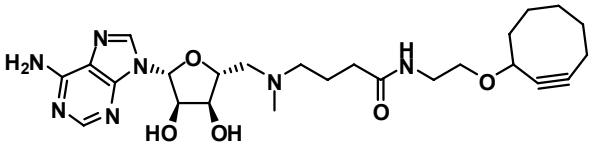
5'-Deoxy-5'-*N*-methyl-*N*'-{4-[(*Z*)-2-(2-bromocyclooct-2-enyloxy)ethanamino] butyramide}-2',3'-*O,O*-(1-methylethylidene)adenosine 13

PyBOP (2.64 g, 5.07 mmol) and DIPEA (884 µl, 5.07 mmol) were added to a solution of 5'-deoxy-5'-*N*-methyl-*N*'-(4-butyric acid)-2',3'-*O,O*-(1-methylethylidene)adenosine (**S5**) (619 mg, 1.52 mmol) and (*Z*)-2-(2-bromocyclooct-2-enyloxy)ethanamine (**S7**) (420 mg, 1.69 mmol) in DMF (12 ml). The reaction was stirred for 30 min and the solution applied directly to an ion exchange column, eluting with saturated ammonia in methanol solution, followed by column chromatography, eluting with 5% saturated ammonia in methanol solution in CH_2Cl_2 to afford the *alkene* (**13**) (774 mg, 80%); >95:<2 *trans*:*cis* as a colourless oil; R_f 0.33 (5% saturated ammonia in methanol in CH_2Cl_2); $[\alpha]_D^{20}$ –4.6 (*c*. 4.00 in MeOH); ν_{max}/cm^{-1} (film); 3325 (NH_2), 3182 (NH_2), 2933 (CH), 2853 (CH), 2796, 1649 (NH_2), 1599 (C=O); δ_H (500 MHz; MeOD); 8.32 (1H, s, adenosine 8-H or 2-H), 8.26 (1H, s, adenosine 8-H or 2-H), 6.30 (1H, dd, *J* 11.5 and 3.9, C=CH), 6.20 (1H, d, *J* 1.7, adenosine 1'-H), 5.39 (1H, dd, *J* 6.4 and 1.7, adenosine 2'-H), 4.89 (1H, dd, *J* 6.4 and 3.9, adenosine 3'-H), 4.28-4.23 (1H, m, adenosine 4'-H), 3.87 (1H, dd, *J* 10.3 and 4.7, cyclooctenyl 8-H), 3.48-3.39 (1H, m, ethyl 2-H_A), 3.28-3.23 (1H, m, ethyl 2-H_B), 3.22-3.19 (2H, m, ethyl 1-H_2), 2.65-2.49 (3H, m, CH and adenosine 5'-H_2), 2.27 (2H, t, *J* 7.3, butyramide 4-H_2), 2.20-2.14 (1H, m, CH), 2.12 (3H, s, NMe), 2.05 (2H, t, *J* 7.3, butyramide 2-H_2), 1.96-1.66 (4H, m, CH), 1.65-1.54 (3H, m, butyramide 3-H_2 and CH_2), 1.50 (3H, s, Me), 1.46-1.37 (1H, m, CH), 1.29 (3H, s, Me), 1.24-1.13 (1H, m, CH), 0.83-0.73 (1H, m, CH); δ_C (75 MHz; MeOD); 176.1 (butyramide C-1), 157.6 (adenosine C-6), 154.4 (adenosine C-2), 150.6 (adenosine C-4), 142.2 (adenosine C-8), 134.4 (cyclooctenyl C-2), 133.4 (cyclooctenyl C-1), 121.0 (adenosine C-5), 115.9 (*C*Me_2), 93.3 (adenosine C-1'), 86.5 (adenosine C-4'), 86.3 (cyclooctenyl C-8), 85.4 (adenosine C-2'), 85.1 (adenosine C-3'), 68.5 (ethyl C-2), 60.8 (adenosine C-5'), 58.6 (butyramide C-4), 43.2 (NMe), 40.9 (ethyl C-1), 40.6 (CH_2), 37.7 (CH_2), 35.7 (CH_2), 34.5 (butyramide C-2), 29.3 (CH_2), 27.9 (Me or CH_2), 27.8 (Me or CH_2), 25.9 (Me), 24.3 (butyramide C-3); m/*z* (ES) 636.2 (100% MH^+ ^{79}Br); (Found MH^+ ^{79}Br 636.2505; $C_{28}H_{42}BrN_7O_5$ requires MH^+ ^{79}Br 636.2504). The *trans* isomer gradually equilibrated to the *cis* isomer on standing.



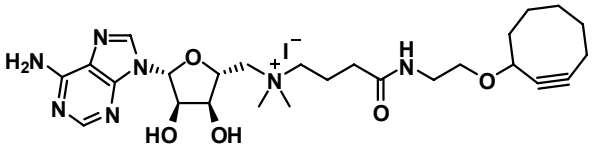
5'-Deoxy-5'-*N*-methyl-*N*'-{4-[(*Z*)-2-(2-bromocyclooct-2-enyloxy)ethanamino] butyramide}-adenosine S10

5'-Deoxy-5'-*N*-methyl-*N*'-{4-[(*Z*)-2-(2-bromocyclooct-2-enyloxy)ethanamino] butyramide}-2',3'-*O,O*-(1-methylethylidene)adenosine (**13**) (17 mg, 0.027 mmol) was treated with 5M HCl (0.5 ml) for 6 min, neutralised by addition of 1 M aqueous sodium hydroxide solution and the solution applied directly to an ion exchange column (SCX), eluting with saturated ammonia in methanol solution to afford the *alkene* **S10** (12 mg, 75%); >95:<2 *trans*:*cis* as a colourless glass; R_f 0.15 (10% saturated ammonia in methanol in CH_2Cl_2); $[\alpha]_D^{20}$ –5.2 (*c*. 4.00 in MeOH); ν_{max}/cm^{-1} (film); 3343 (OH), 2933 (CH), 2868 (CH), 2518, 1645 (C=O); δ_H (500 MHz; MeOD); 8.18 (1H, s, adenosine 8-H), 8.11 (1H, s, adenosine 2-H), 6.19 (1H, app. d, 11.5, cyclooctenyl 2-H), 5.89 (1H, d, *J* 4.3, adenosine 1'-H), 4.59 (1H, app. t, *J* 4.7, adenosine 2'-H), 4.14 (1H, app. t, *J* 5.6, adenosine 3'-H), 4.08 (1H, dd, *J* 11.5 and 6.0, adenosine 4'-H), 3.85 (1H, dd, *J* 10.7 and 4.7, cyclooctenyl 8-H), 3.44-3.38 (1H, m, ethyl 2-H_A), 3.26-3.23 (1H, m, ethyl 2-H_B), 3.22-3.19 (2H, m, ethyl 1-H_2), 2.69 (2H, app. d, *J* 5.6, adenosine 5'-H_2), 2.64-2.52 (1H, m, CH), 2.37 (2H, t, *J* 7.7, butyramide 4-H_2), 2.21 (3H, s, NMe), 2.21-2.13 (1H, m, CH), 2.10 (2H, t, *J* 7.7, butyramide 2-H_2), 1.93-1.85 (1H, m, CH), 1.85-1.64 (5H, m, butyramide 3-H_2 and CH), 1.63-1.53 (1H, m, CH), 1.46-1.36 (1H, m, CH), 1.20-1.10 (1H, m, CH), 0.82-0.72 (1H, m, CH); δ_C (75 MHz; MeOD); 176.1 (butyramide C-1), 157.7 (adenosine C-6), 154.2 (adenosine C-2), 150.9 (adenosine C-4), 141.8 (adenosine C-8), 134.2 (cyclooctenyl C-1), 133.4 (cyclooctenyl C-2), 121.1 (adenosine C-5), 90.9 (adenosine C-1'), 86.2 (cyclooctenyl C-8), 83.8 (adenosine C-4'), 75.1 (adenosine C-2'), 74.0 (adenosine C-3'), 68.4 (ethyl C-2), 61.1 (adenosine C-5'), 58.7 (butyramide C-4), 43.5 (NMe), 40.9 (CH_2), 40.6 (ethyl C-1), 37.7 (CH_2), 35.1 (butyl C-2), 34.5 (CH_2), 29.3 (CH_2), 27.8 (CH_2), 24.4 (butyramide C-3); m/*z* (ES) 596.2 (100% MH^+ ^{79}Br); (Found MH^+ ^{79}Br 596.2191; $C_{25}H_{38}BrN_7O_5$ requires MH^+ ^{79}Br 596.2191). The *trans* isomer gradually equilibrated to the *cis* isomer on standing.



5'-Deoxy-5'-*N*-methyl-*N*'-{4-[2-(cyclooct-2-ynyloxy)ethanamine] butyramide}-adenosine 14

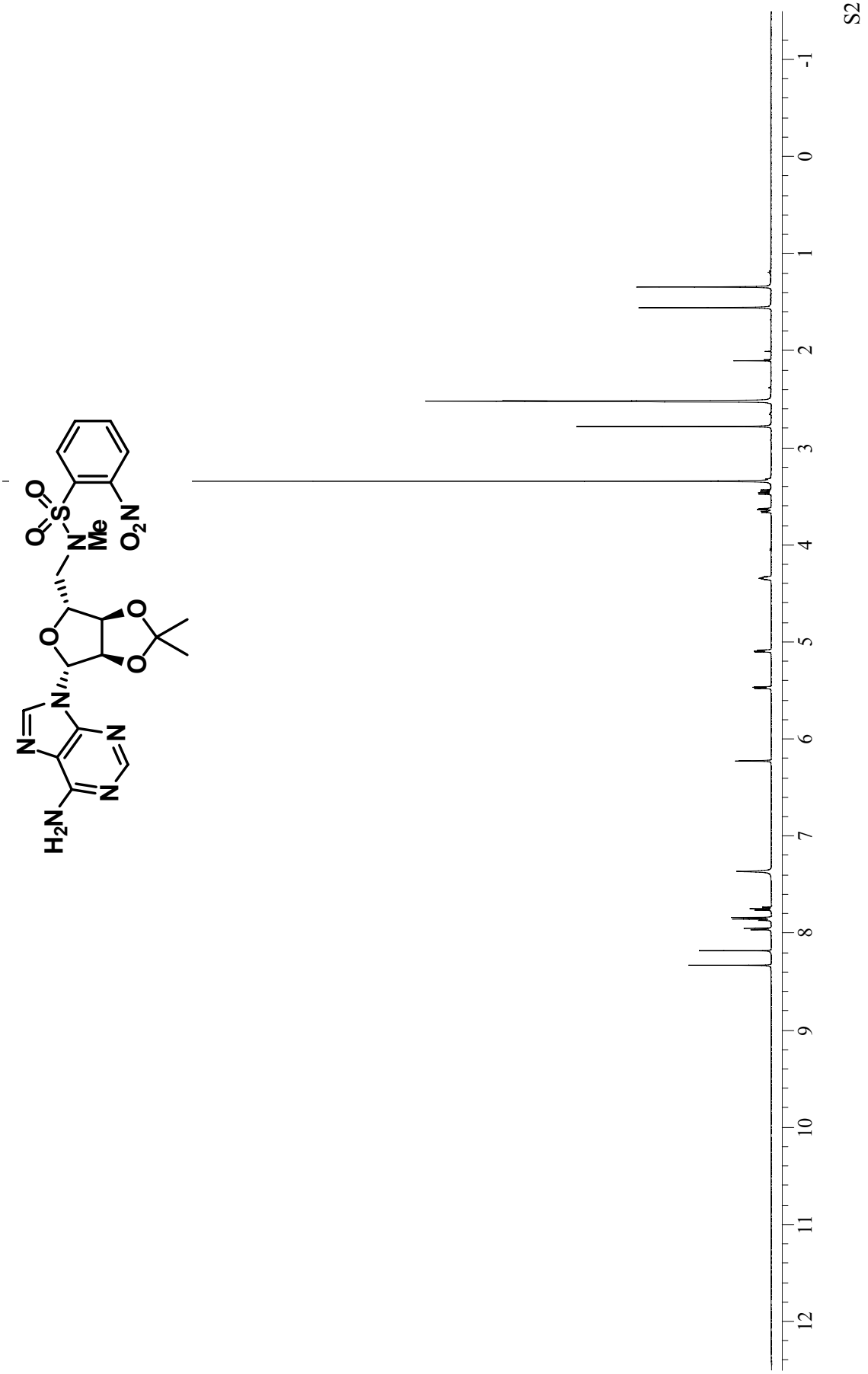
5'-Deoxy-5'-*N*-methyl-*N*'-{4-[(*Z*)-2-(2-bromocyclooct-2-enyloxy)ethanamino] butyramide}-2',3'-*O,O*-(1-methylethylidene)adenosine (**S10**) (770 mg, 1.21 mmol) was treated with 5 M aqueous HCl solution for 7 min, cooled to 0 °C, neutralised by addition of 4 M aqueous NaOH solution and and the solution applied directly to an ion exchange column (SCX), eluting with saturated ammonia in methanol solution. The resulting solid was dissolved in DMF (3 ml) and THF (3 ml) and sodium hydride (242 mg, 6.05 mmol) was added. The reaction was stirred for 40 min cooled to 0 °C, neutralised by addition of 1 M aqueous HCl solution and the solution applied directly to an ion exchange column (SCX), eluting with saturated ammonia in methanol solution to afford the *alkyne* (**14**) (592 mg, 95%) as a yellow glass; R_f 0.10 (10% saturated ammonia in methanol in CH_2Cl_2); $[\alpha]_D^{20}$ –6.4 (*c*. 4.00 in MeOH); ν_{max}/cm^{-1} (film); 3326 (OH), 3269 (OH), 3192 (NH_2), 2931 (CH), 2857, 2802, 1649 (NH_2), 1602 (C=O); δ_H (500 MHz; MeOD); 8.30 (1H, s, adenosine 8-H), 8.24 (1H, s, adenosine 2-H), 6.02 (1H, d, *J* 4.3, adenosine 1'-H), 4.75 (1H, app. t, *J* 4.7, adenosine 2'-H), 4.28 (1H, app. t, *J* 5.6, adenosine 3'-H), 4.22 (1H, dd, *J* 11.3 and 5.8, adenosine 4'-H), 4.20-4.15 (1H, m, cyclooctenyl 1-H), 3.61-3.54 (1H, m, ethyl 2-H_A), 3.38-3.27 (3H, ethyl 2-H_B and ethyl 1-H_2), 3.19-3.13 (1H, m, cyclooctenyl 8-H), 2.84 (2H, app. d, *J* 5.6, adenosine 5'-H_2), 2.50 (2H, t, *J* 7.5, butyramide 4-H_2), 2.33 (3H, s, NMe), 2.25-2.02 (4H, m, butyramide 2-H_2 and CH_2), 1.95-1.74 (6H, m, butyramide 3-H_2 and CH_2), 1.71-1.53 (2H, m, CH_2), 1.47-1.28 (1H, m, CH); δ_C (75 MHz; MeOD); 176.1 (butyramide C-1), 157.6 (adenosine C-6), 154.2 (adenosine C-2), 150.9 (adenosine C-4), 141.8 (adenosine C-8), 121.0 (adenosine C-5), 101.3 (C≡C), 93.9 (C≡C), 91.0 (adenosine C-1'), 83.7 (adenosine C-4'), 77.5 (adenosine C-2'), 74.0 (adenosine C-3' and cyclooctenyl C-8), 68.9 (ethyl C-2), 61.1 (adenosine C-5'), 58.7 (butyramide C-4), 43.7 (cyclooctenyl C-7), 43.4 (NMe), 40.7 (CH_2), 35.7 (CH_2), 35.0 (butyramide C-2), 31.8 (CH_2), 28.4 (CH_2), 24.9 (CH_2), 21.6 (CH_2); m/*z* (ES) 516.3 (100% MH^+); (Found MH^+ 516.2941; $C_{25}H_{38}N_7O_5$ requires MH^+ 516.2929).



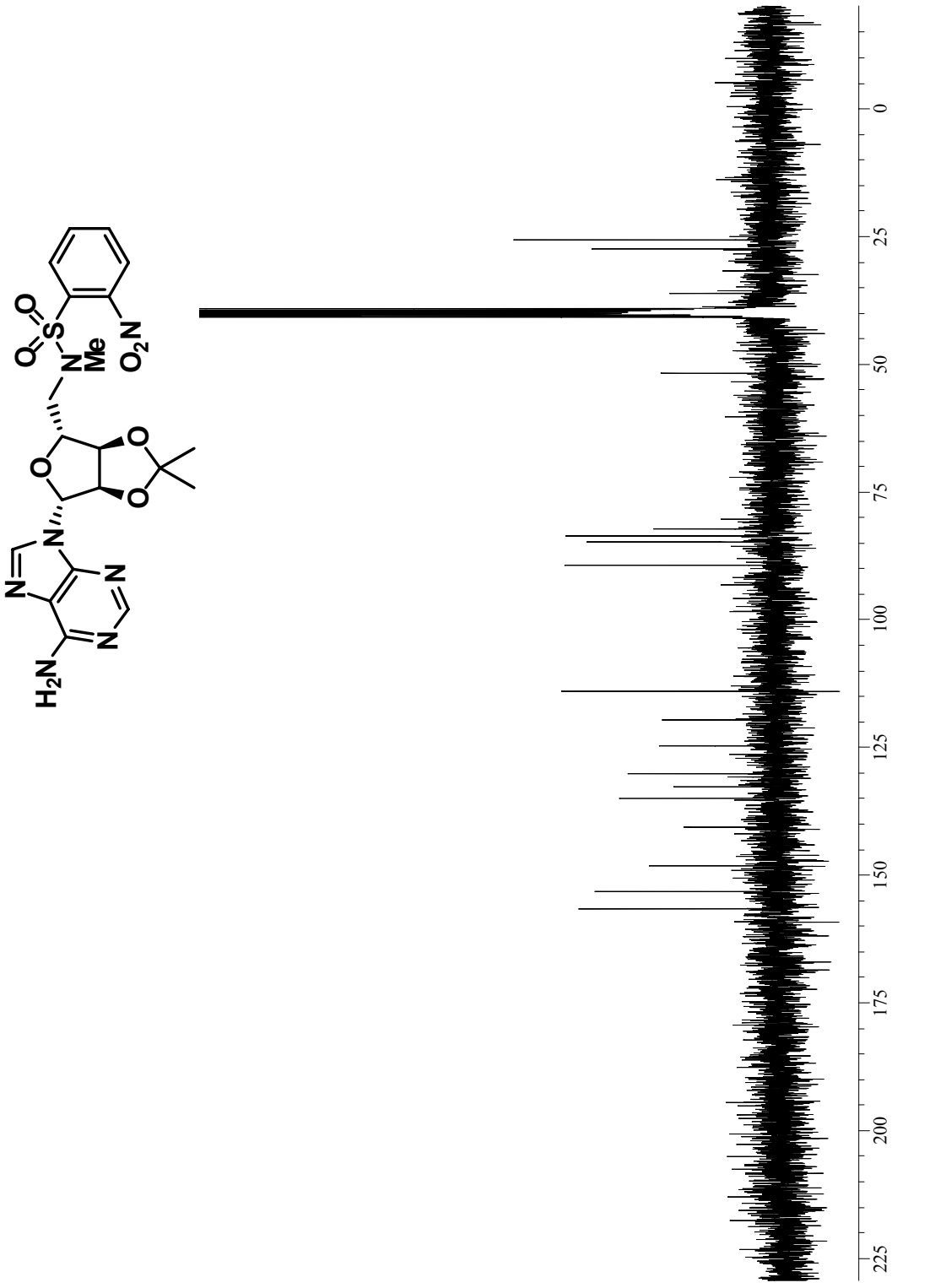
5'-Deoxy-5'-*N,N'*-dimethyl-*N'*-{4-[2-(cyclooct-2-ynyloxy)ethanamine] butyramide}-adenosine iodide 16

Methyl iodide (3.4 μl, 0.055 mmol) was added to a solution of 5'-deoxy-5'-*N*-methyl-*N'*-{4-[2-(cyclooct-2-ynyloxy)ethanamine] butyramide}-adenosine (**14**) (28 mg, 0.055 mmol) in acetonitrile (0.3 ml) and water (0.1 ml), stirred for 17 h, methyl iodide (1.5 μl, 0.024 mmol) was added, stirred for 1.5 d and concentrated *in vacuo* to afford the *alkyne* (**16**) (31 mg, 86%) as a colourless glass; R_t (Method: Analysis G) 5.61 min; $[\alpha]_D^{20}$ +6.8 (*c*. 2.00 in MeOH); ν_{max}/cm^{-1} (film); 3322 (NH_2), 3197 (NH_2), 2930 (CH), 2851 (CH), 1648 (NH_2) and 1601 (C=O); δ_H (500 MHz; MeOD); 8.20 (1H, s, adenosine 8-H), 8.15 (1H, s, adenosine 2-H), 5.98 (1H, d, *J* 3.4, adenosine 1'-H), 4.61 (1H, app. t, *J* 3.9, adenosine 2'-H), 4.47-4.41 (2H, m, adenosine 3'-H and adenosine 4'-H), 4.11-4.06 (1H, m, cyclooctenyl 1-H), 3.97 (1H, dd, *J* 14.1 and 8.9, adenosine 5'-H_A), 3.71 (1H, app. d, *J* 14.1, adenosine 5'-H_B), 3.51-3.44 (1H, m, ethyl 2-H_A), 3.42-3.31 (2H, m, CH_2), 3.31-3.24 (1H, m, ethyl 2-H_B), 3.22-3.19 (2H, m, butyramide 4-H_2), 3.13-3.03 (1H, m, CH), 3.10 (3H, s, NMe), 3.09 (3H, s, NMe), 2.17 (2H, t, *J* 6.8, butyramide 2-H_2), 2.14-2.03 (1H, m, CH), 2.02-1.92 (2H, m, butyramide 3-H_2), 1.86-1.65 (5H, m, CH), 1.62-1.43 (2H, m, CH_2), 1.37-1.25 (1H, m, CH); δ_C (75 MHz; MeOD); 173.9 (butyramide C-1), 157.8 (adenosine C-6), 154.4 (adenosine C-2), 150.6 (adenosine C-4), 142.2 (adenosine C-8), 121.2 (adenosine C-5), 101.4 (C≡C), 93.9 (C≡C), 92.4 (adenosine C-1'), 79.0 (adenosine C-4'), 74.3 (adenosine C-2' or adenosine C-3' or cyclooctenyl C-1), 74.1 (adenosine C-2' or adenosine C-3' or cyclooctenyl C-1), 74.0 (adenosine C-2' or adenosine C-3' or cyclooctenyl C-1), 68.8 (ethyl C-2), 67.7 (adenosine C-5'), 66.2 (butyramide C-4), 52.9 (NMe), 43.6 (CH_2), 40.7 (CH_2), 35.7 (butyramide C-2), 32.8 (CH_2), 31.2 (CH_2), 27.8 (CH_2), 21.5 (CH_2), 20.0 (CH_2) and one signal missing or overlapped; m/*z* (ES) 530.3 (100% $[M-I^-]^+$); (Found $[M-I^-]^+$ 530.3081; $C_{26}H_{40}N_7O_5I$ requires $[M-I^-]^+$ 530.3085).

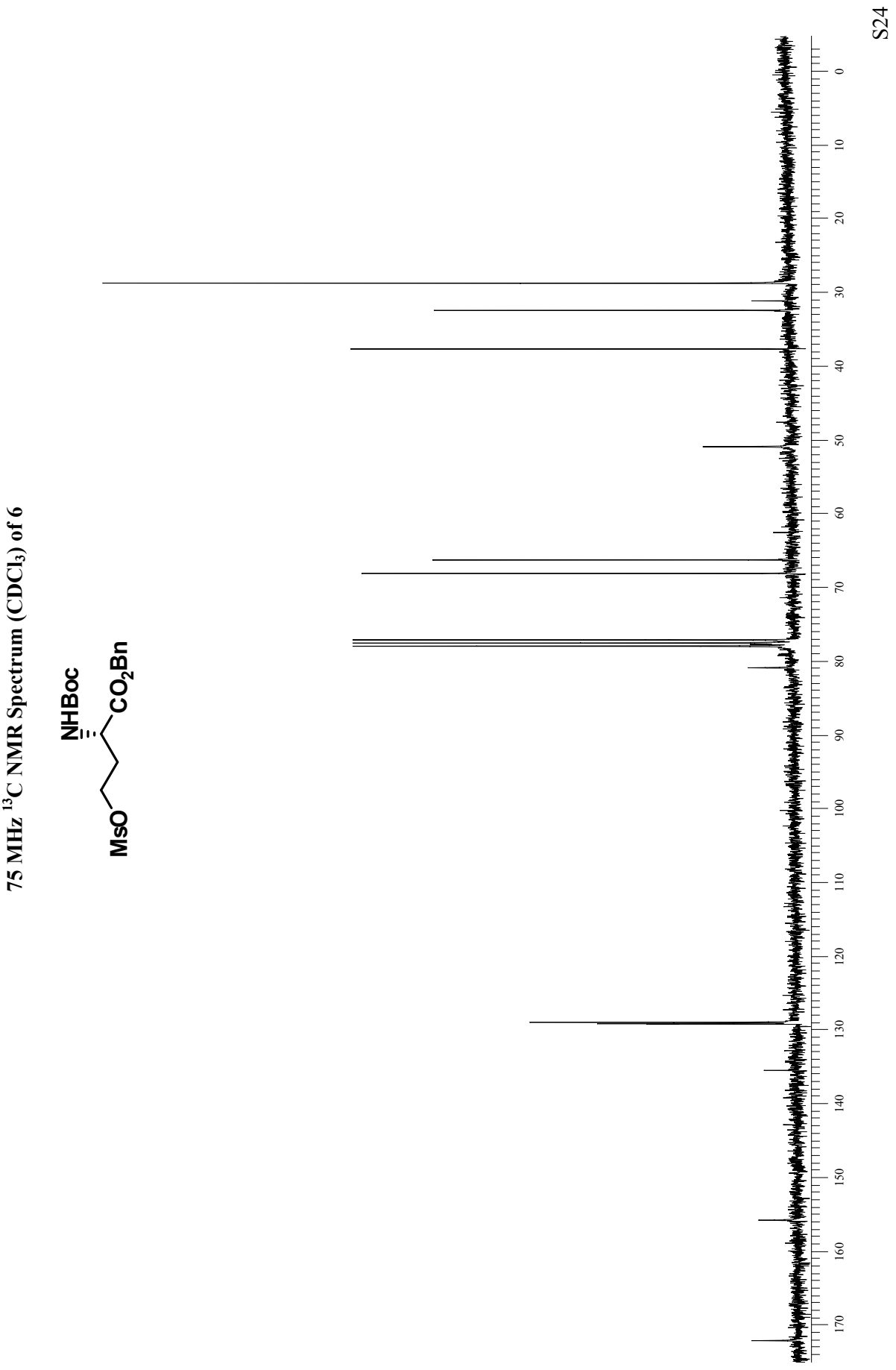
500 MHz ^{1}H NMR Spectrum ($CDCl_3$) of S1



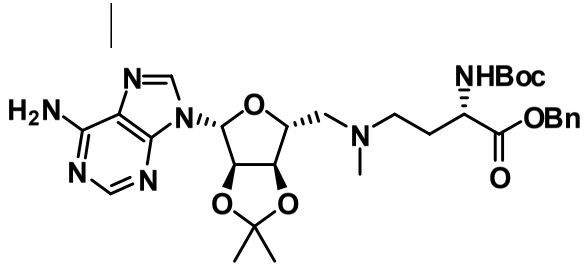
75 MHz ^{13}C NMR Spectrum (*d*-DMSO) of S1

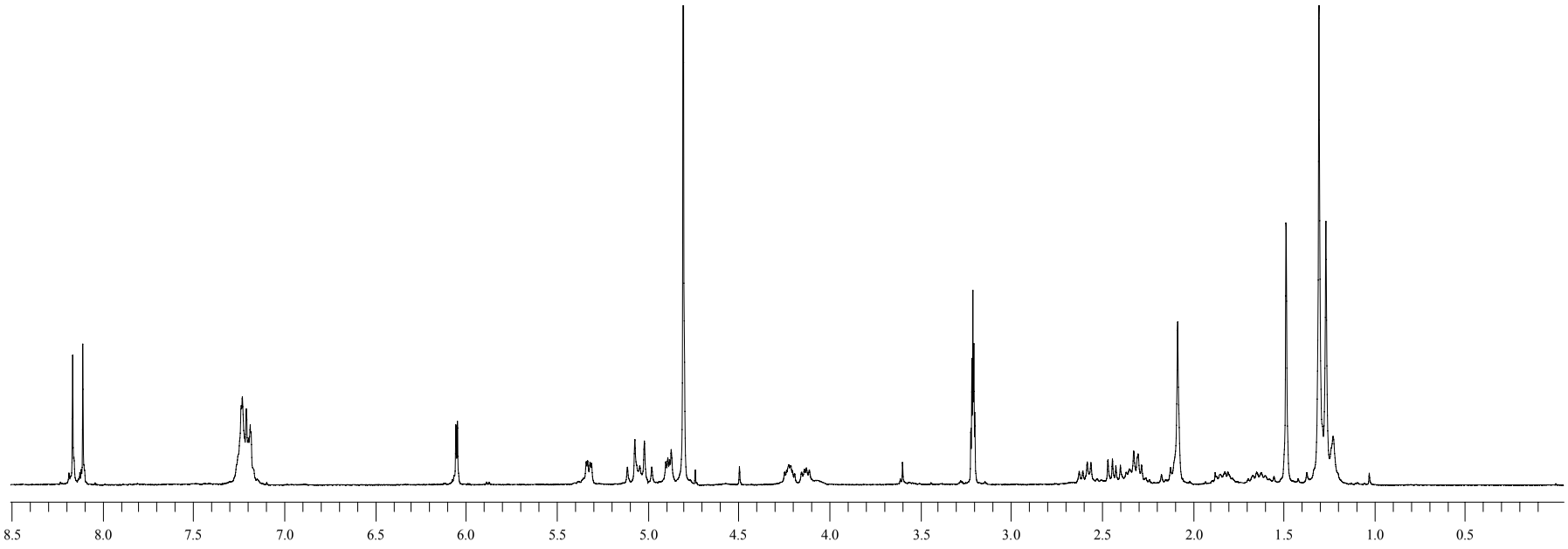


300 MHz ^{1}H NMR Spectrum ($CDCl_3$) of 6

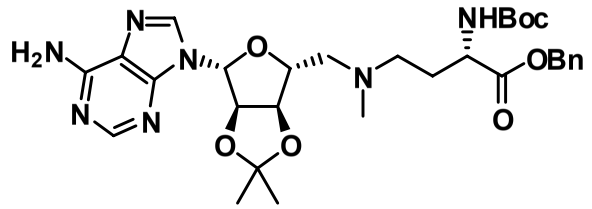

75 MHz ^{13}C NMR Spectrum ($CDCl_3$) of 6
NHBoc
CO_2Bn
MsO

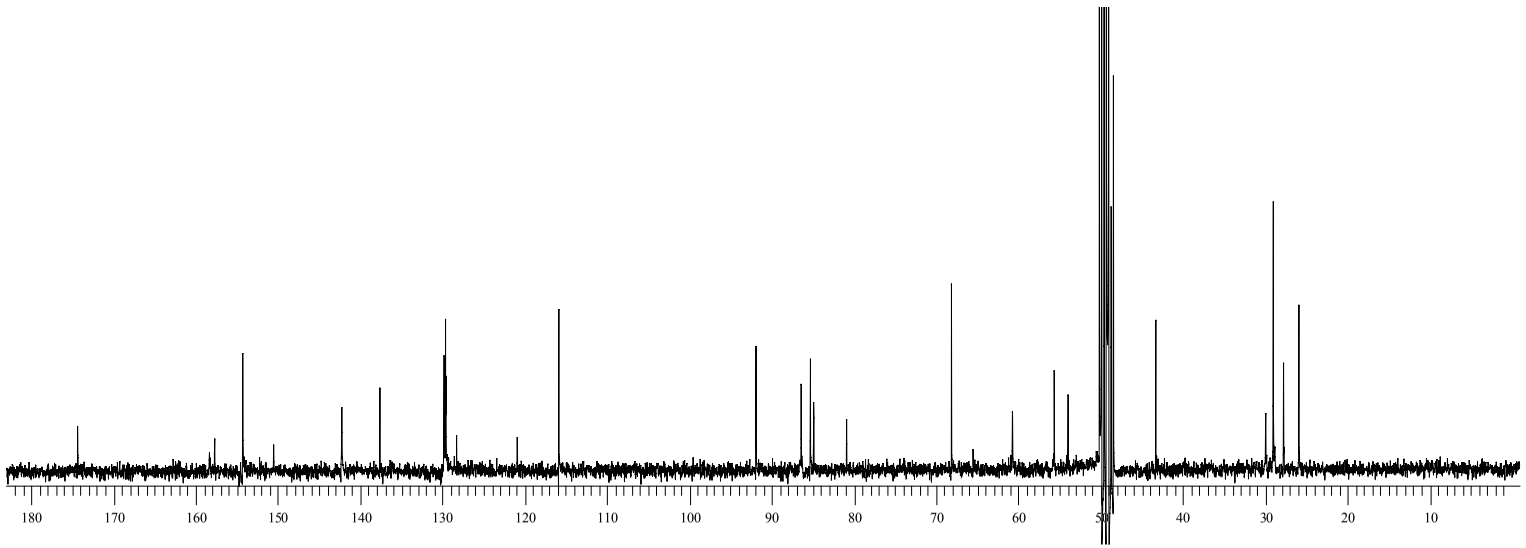
300 MHz ^{1}H NMR Spectrum (MeOD) of 5



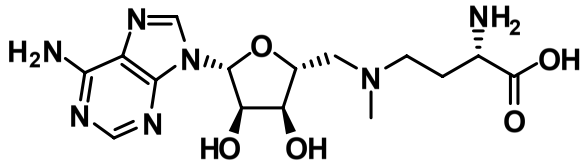


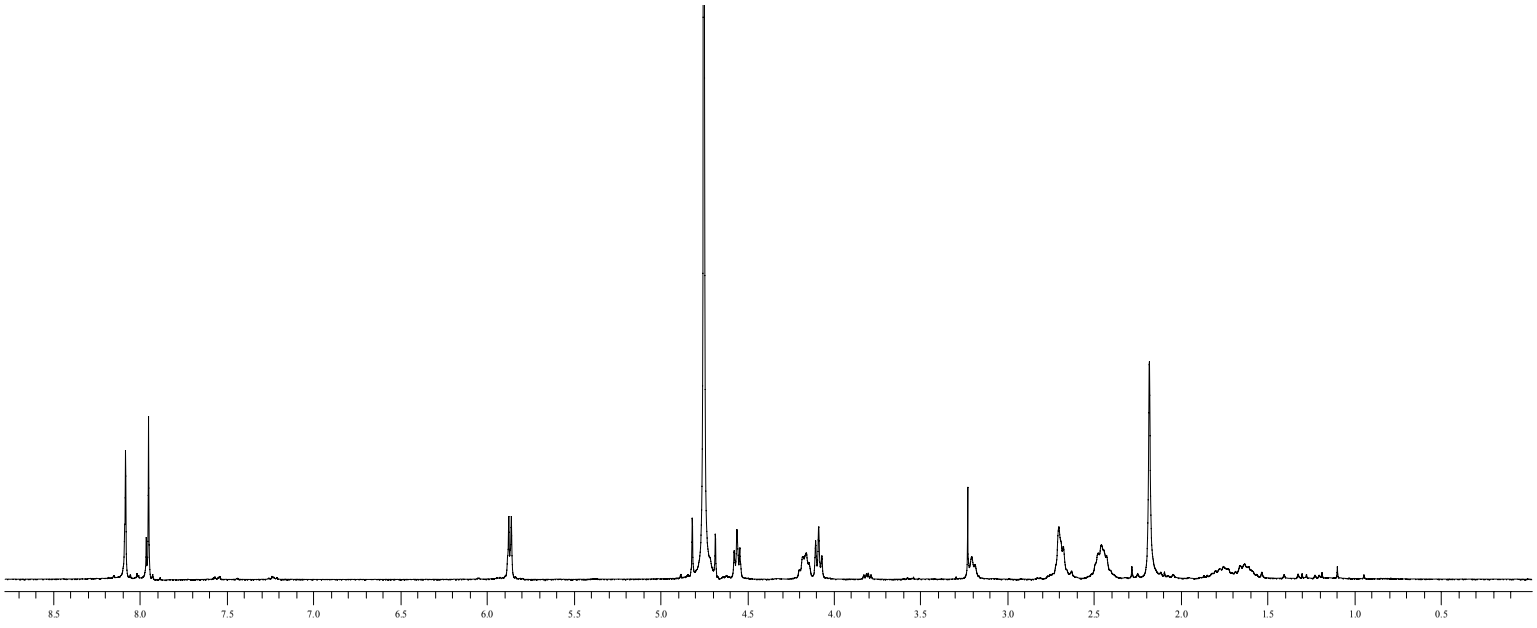
75 MHz ^{13}C NMR spectrum (MeOD) of 5

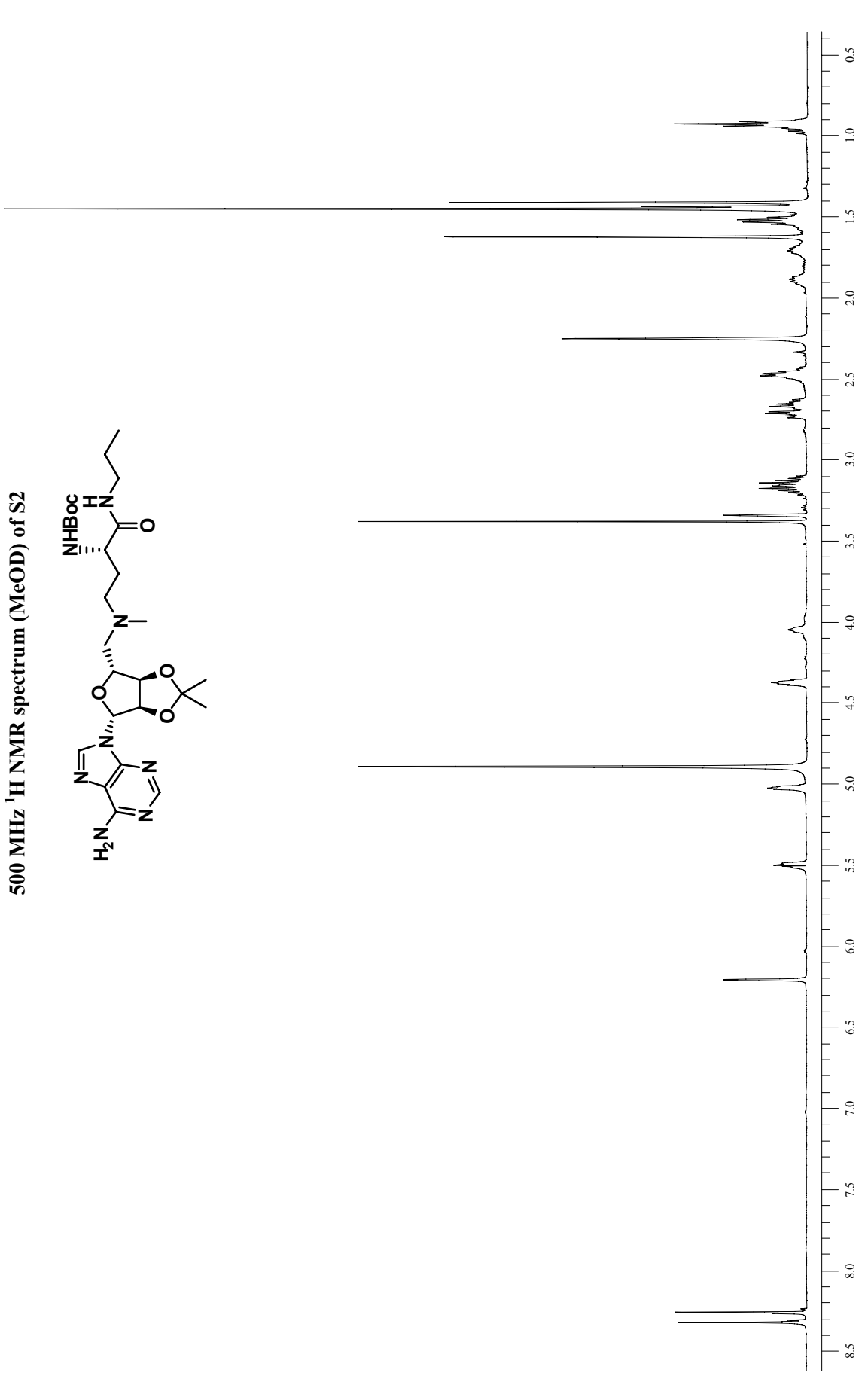




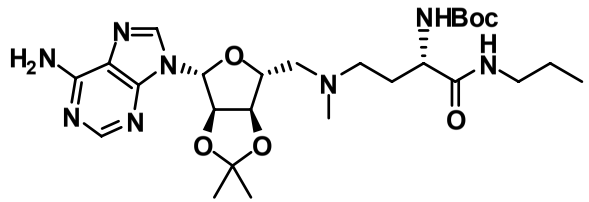
MHz ^{1}H NMR spectrum (D_2O) of 2

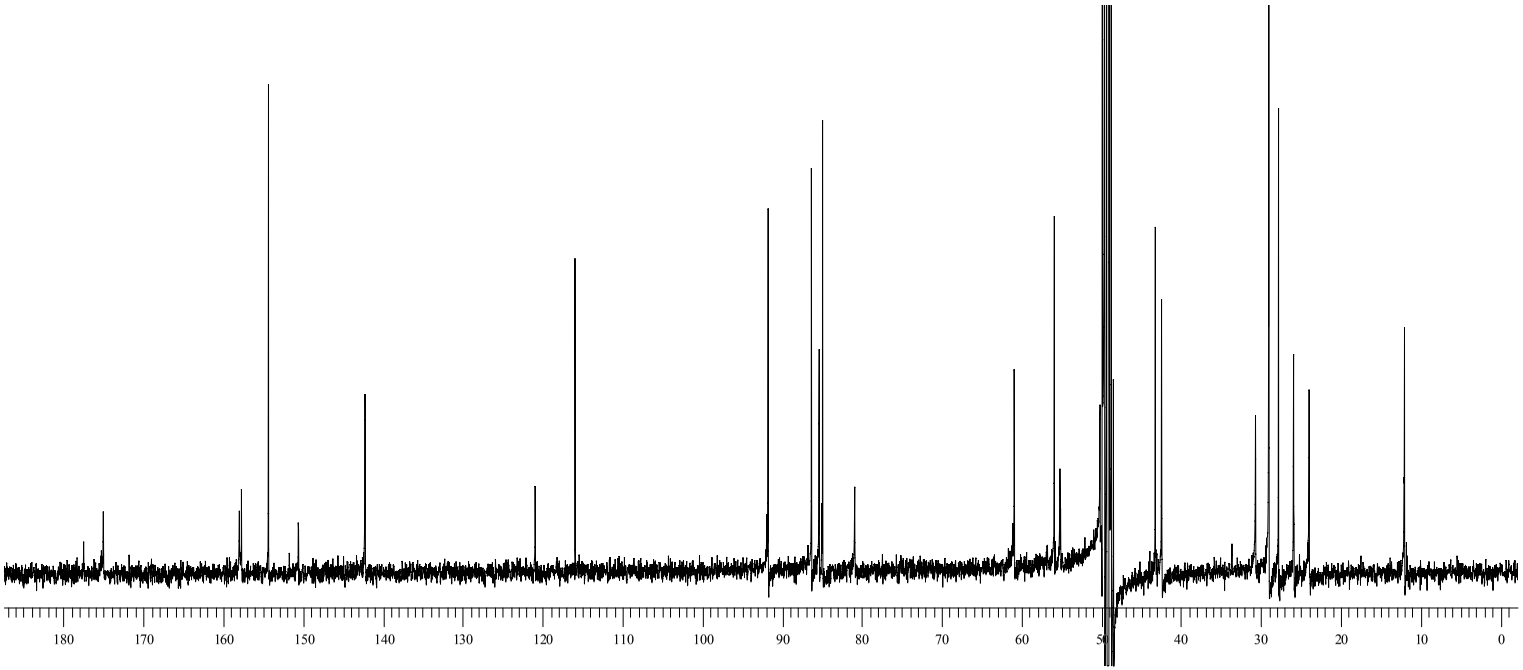




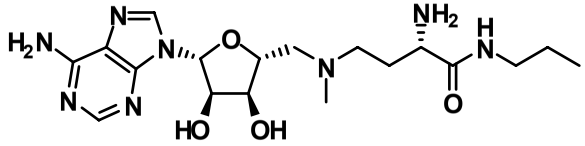

500 MHz ^{1}H NMR spectrum (MeOD) of S2
NHBoc
H
N
O
N
O
O
N
N
N
H_2N
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

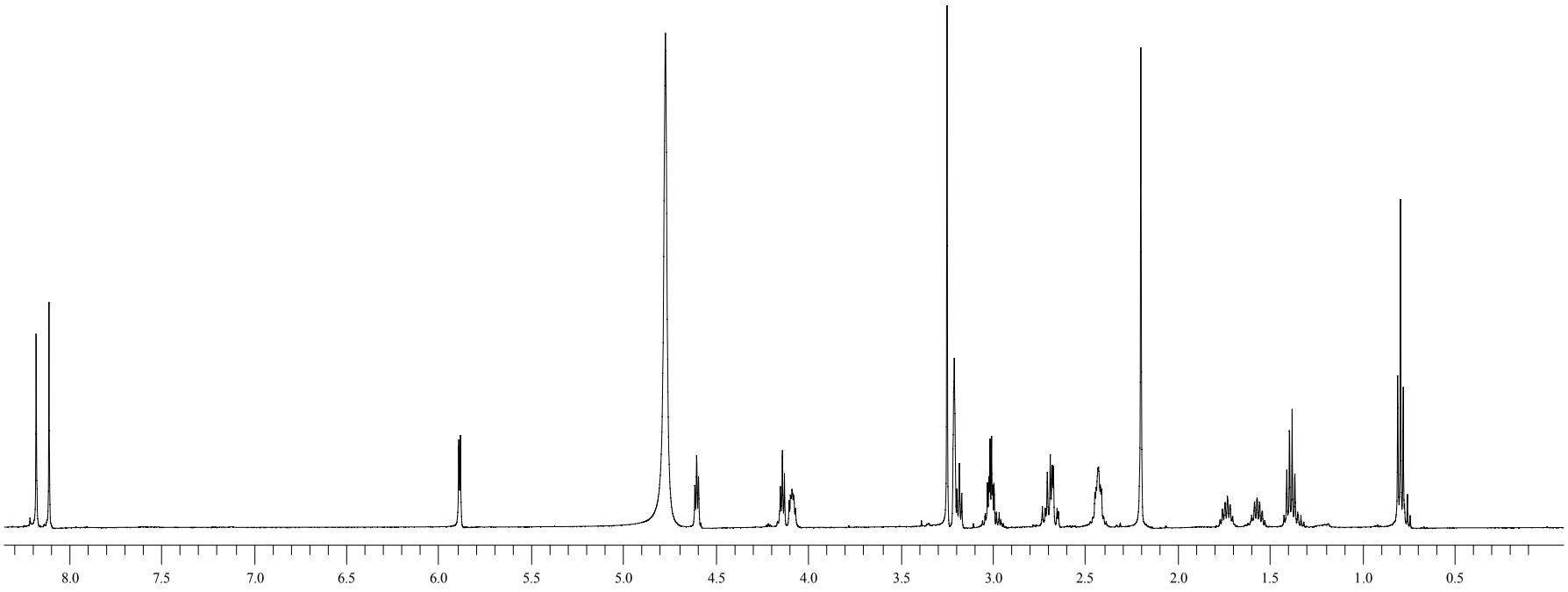
75 MHz ^{13}C NMR spectrum (MeOD) of S2



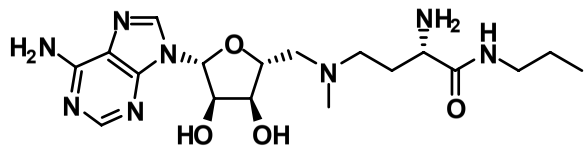


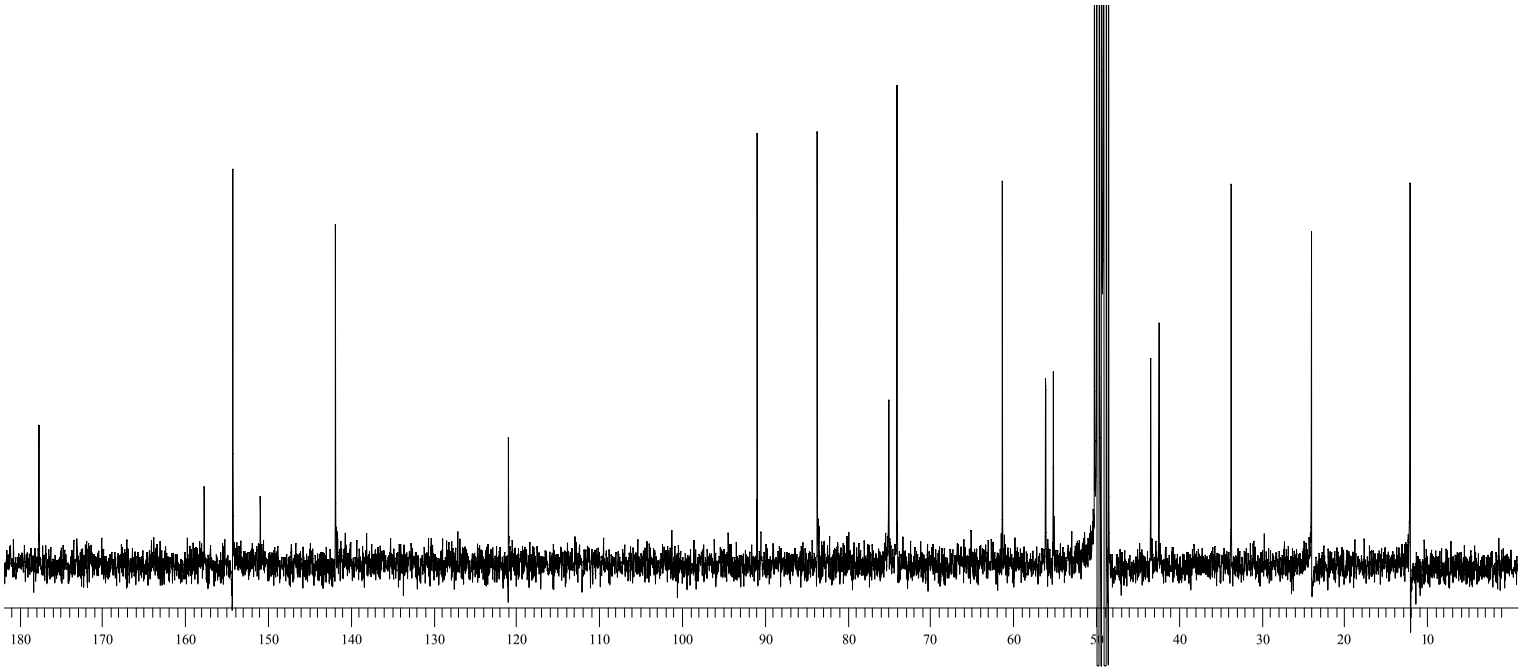
500 MHz ^{1}H NMR spectrum (MeOD) of 7



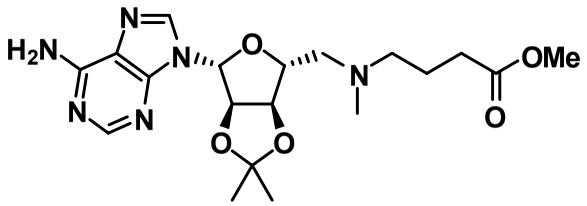


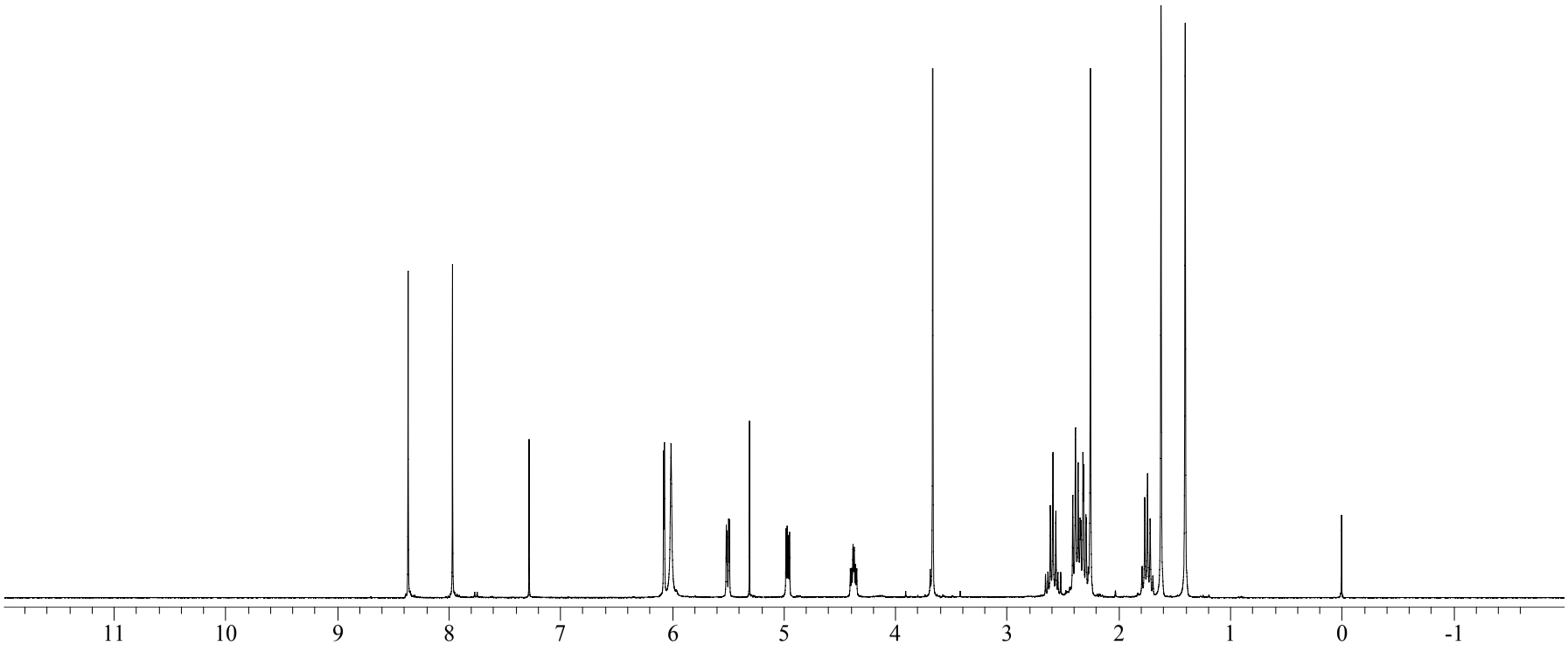
75 MHz ^{13}C NMR spectrum (MeOD) of 7



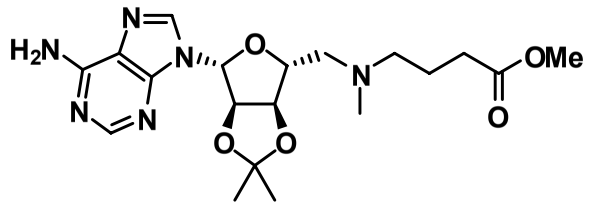


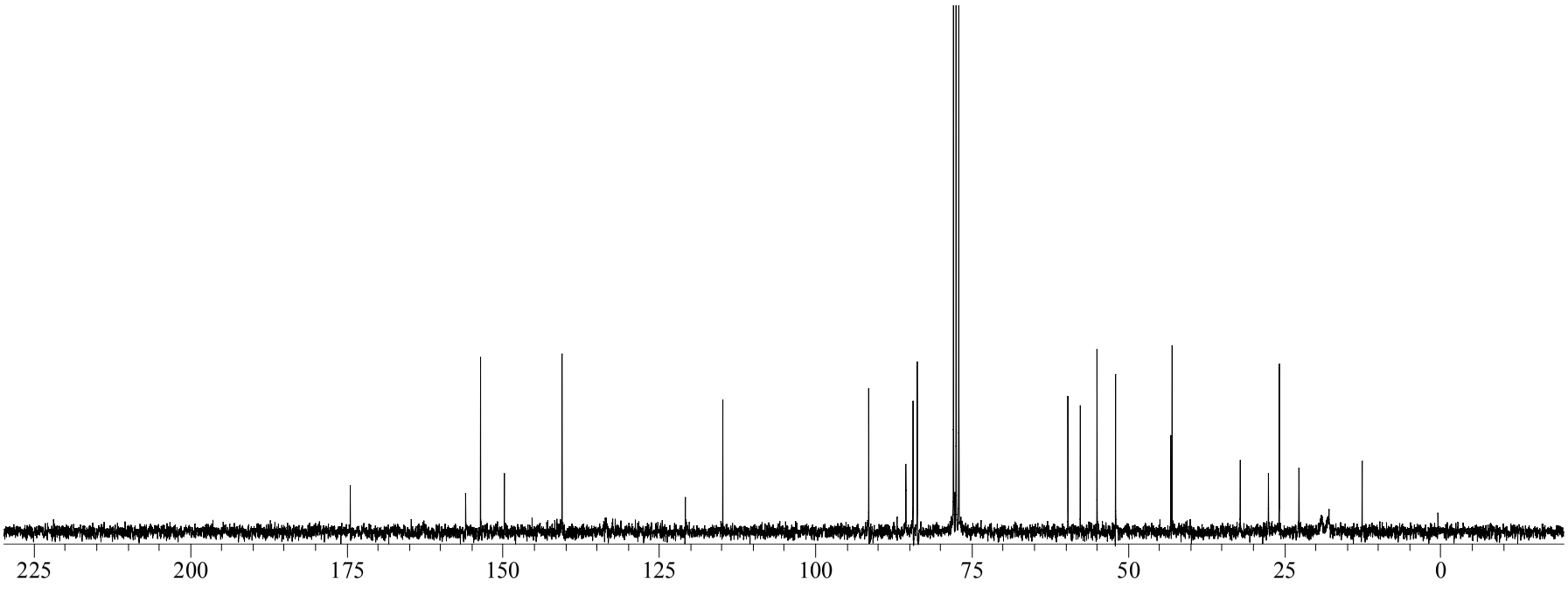
300 MHz ^{1}H NMR spectrum ($CDCl_3$) S3



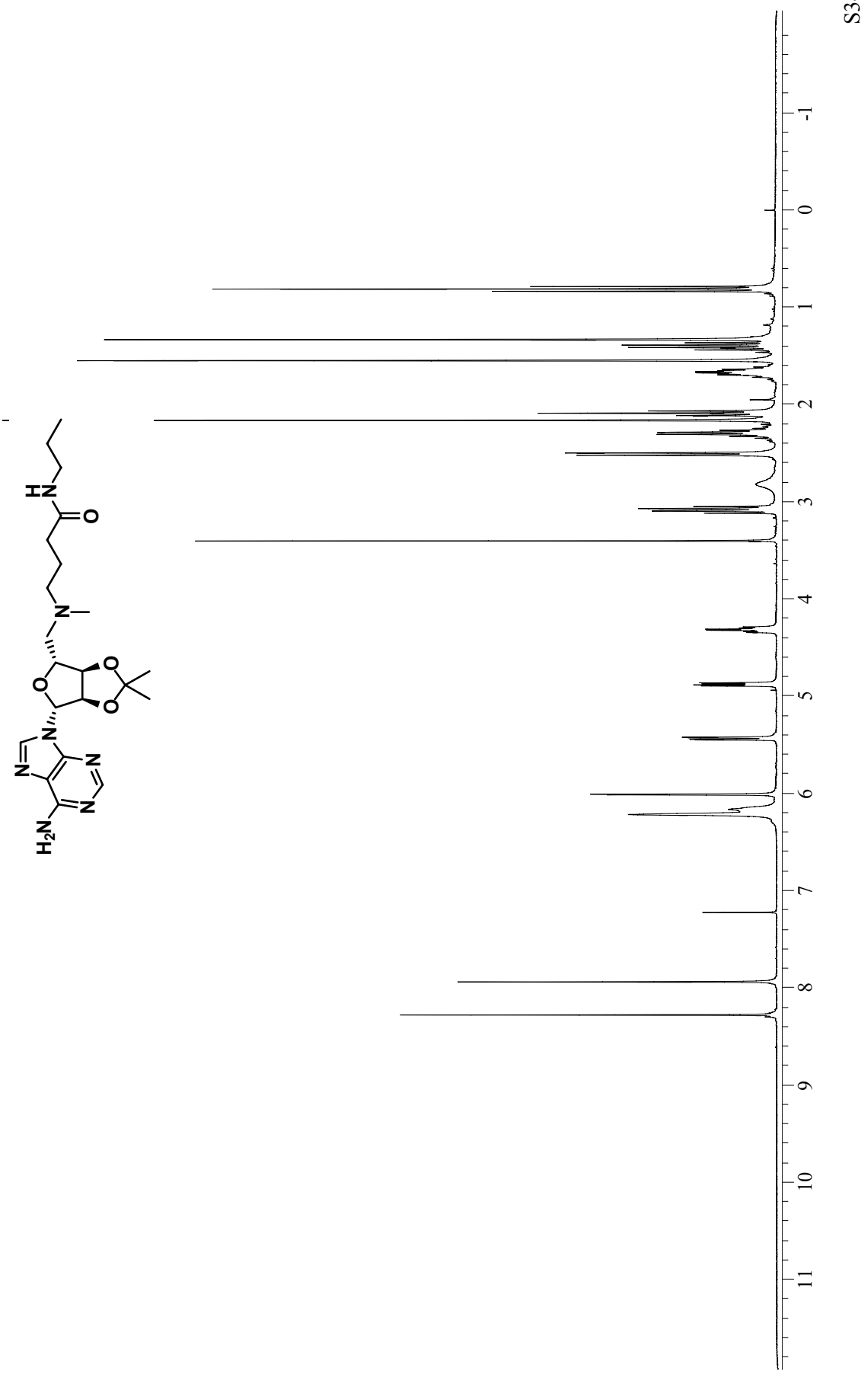


75 MHz ^{13}C NMR spectrum ($CDCl_3$) of S3

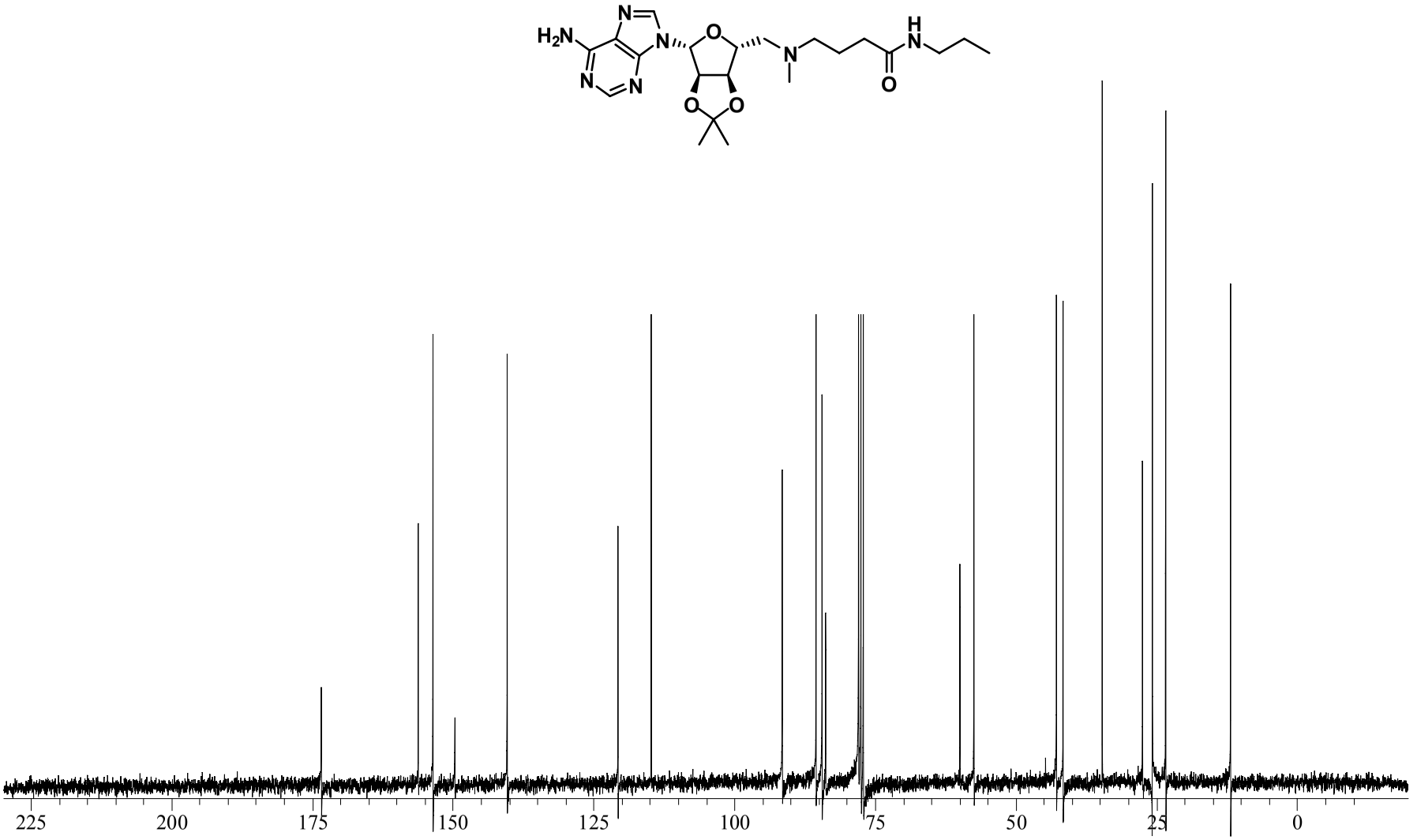




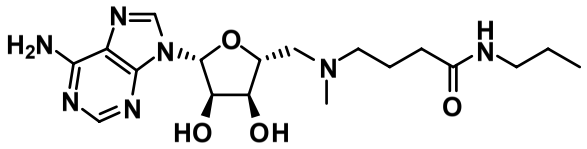
300 MHz ^{1}H NMR spectrum ($CDCl_3$) of S4

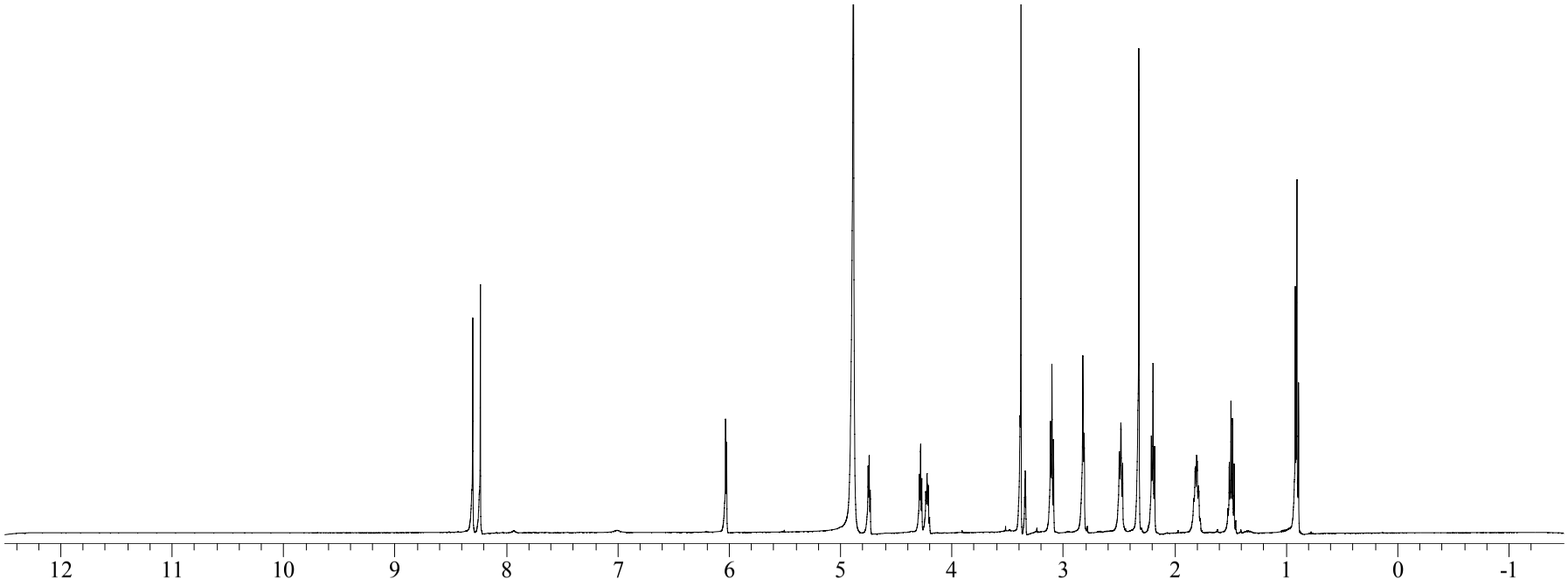


75 MHz ^{13}C NMR spectrum ($CDCl_3$) of S4

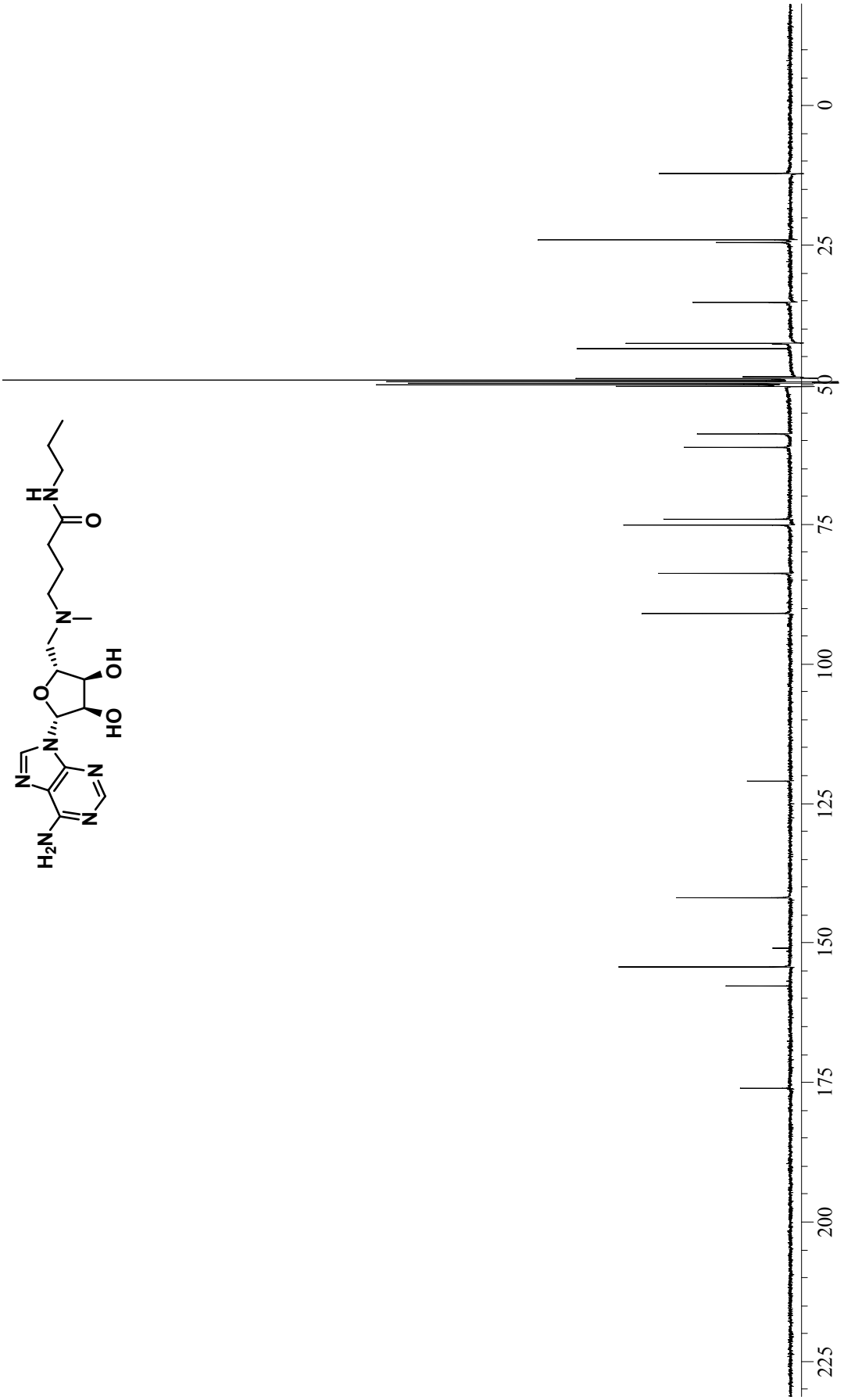


500 MHz ^{1}H NMR spectrum (MeOD) of 8

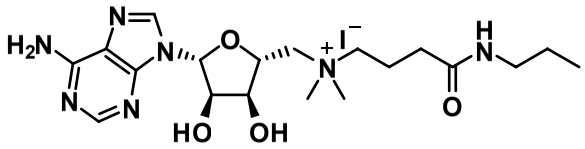


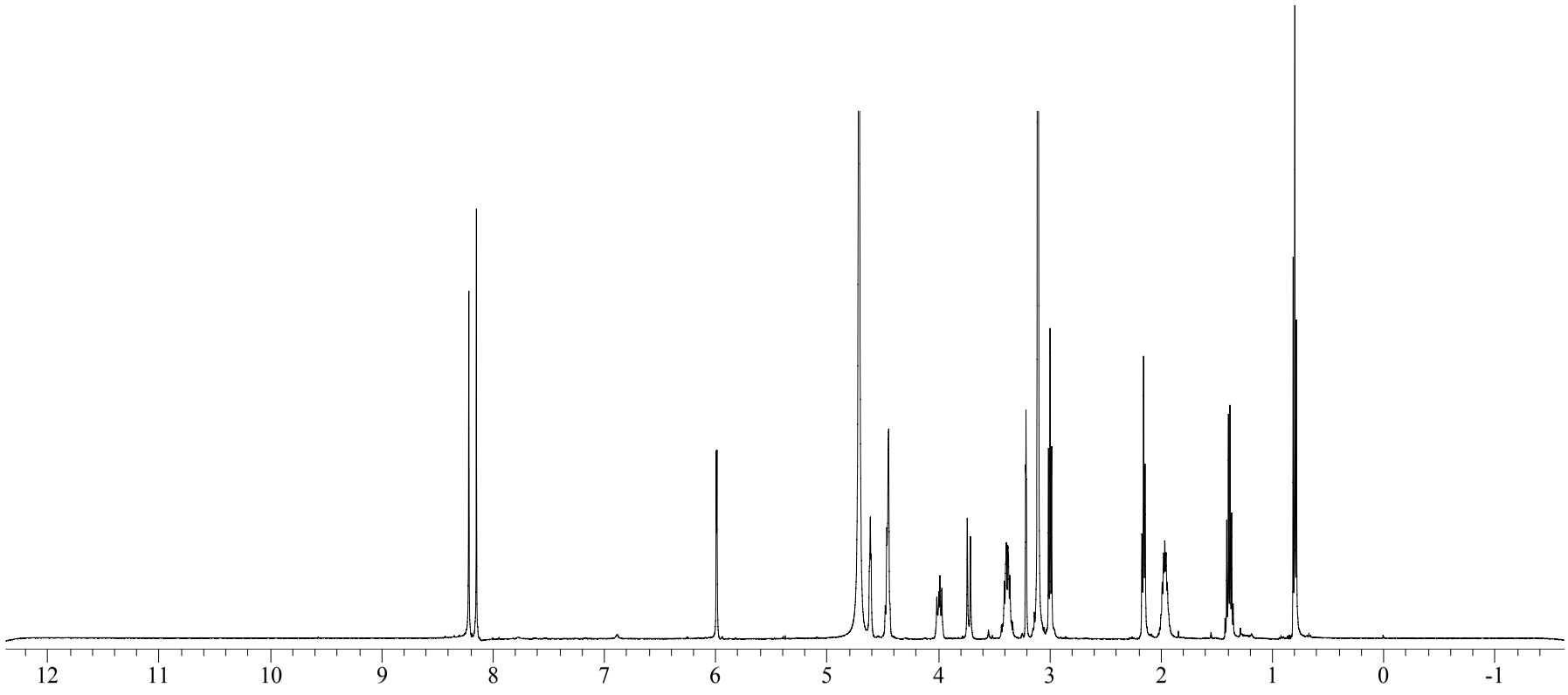


75 MHz ^{13}C NMR spectrum (MeOD) of 8

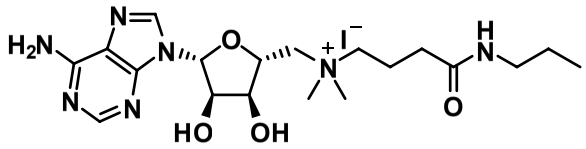


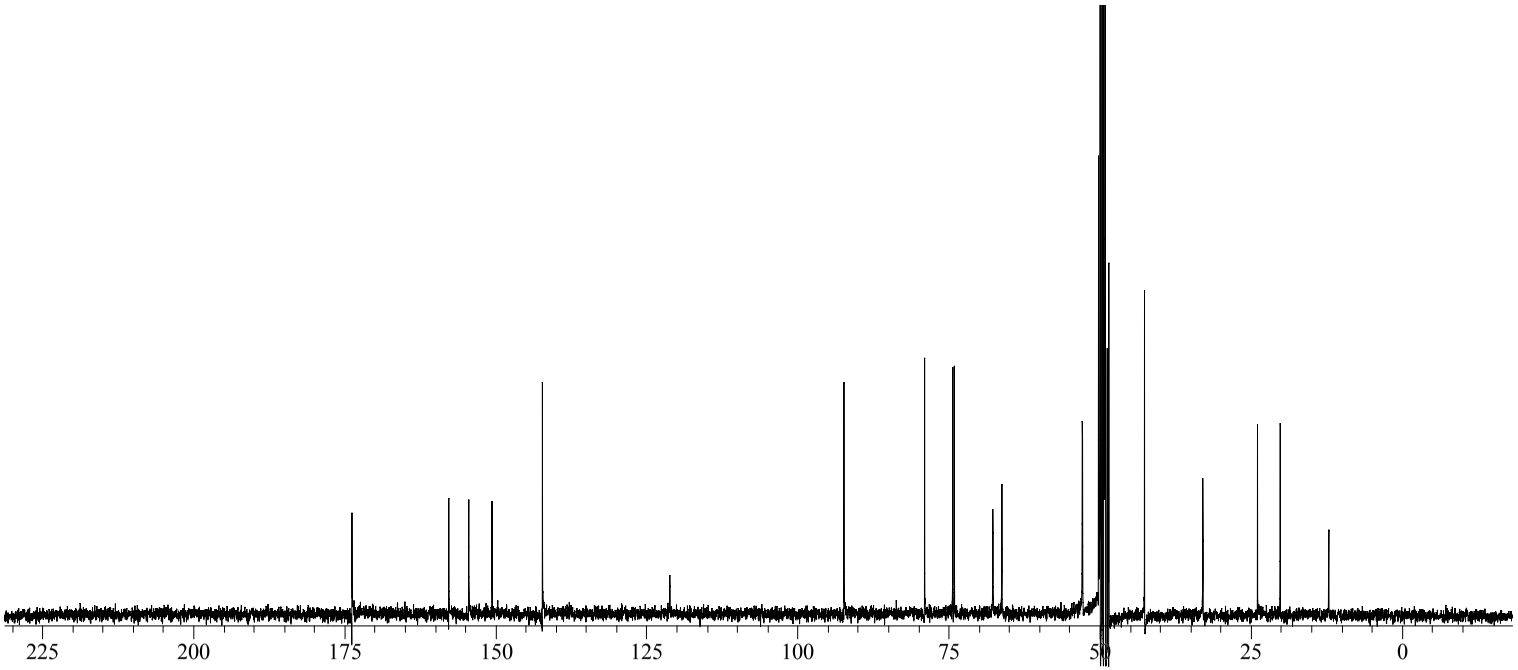
500 MHz ^{1}H NMR spectrum (MeOD) of 9



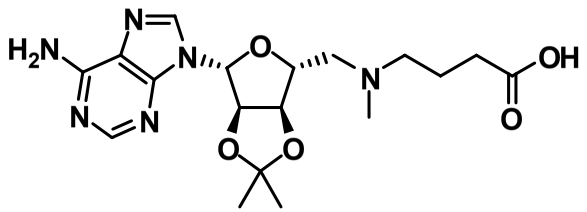


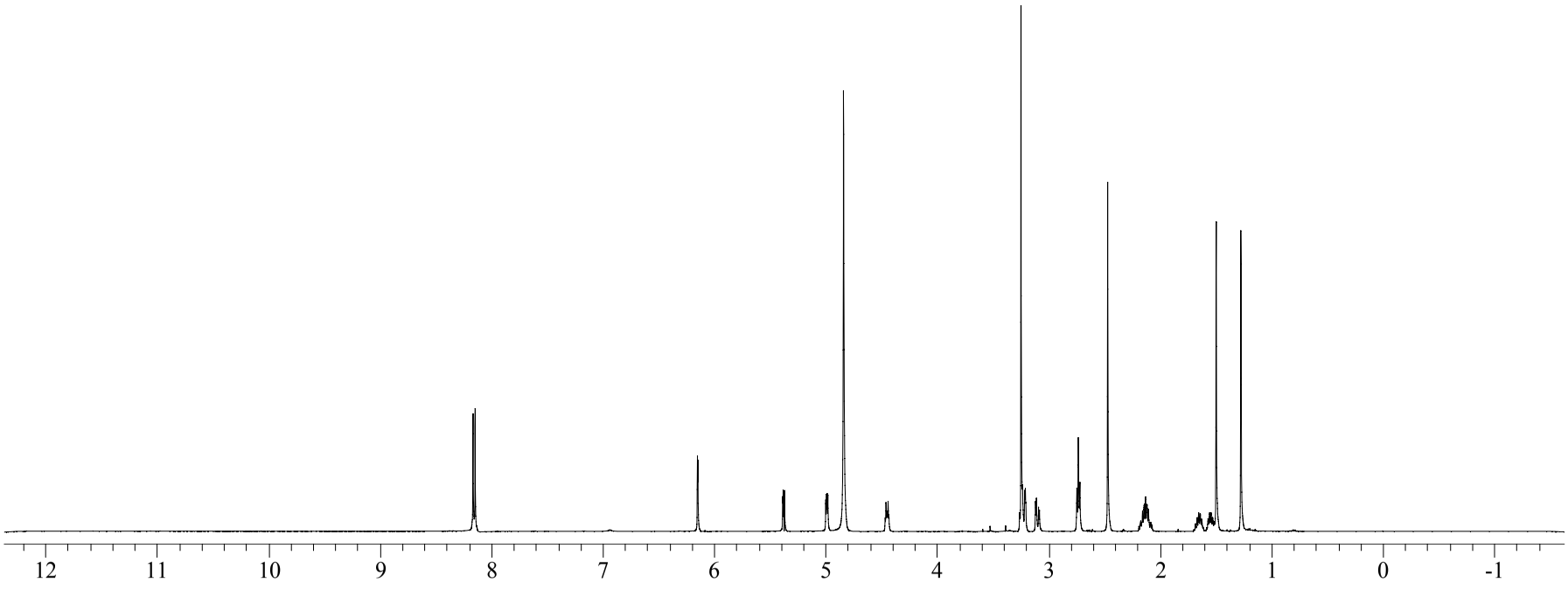
75 MHz ^{13}C NMR spectrum (MeOD) of 9



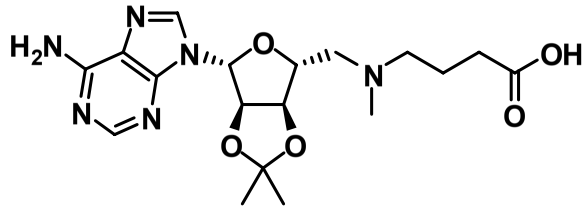


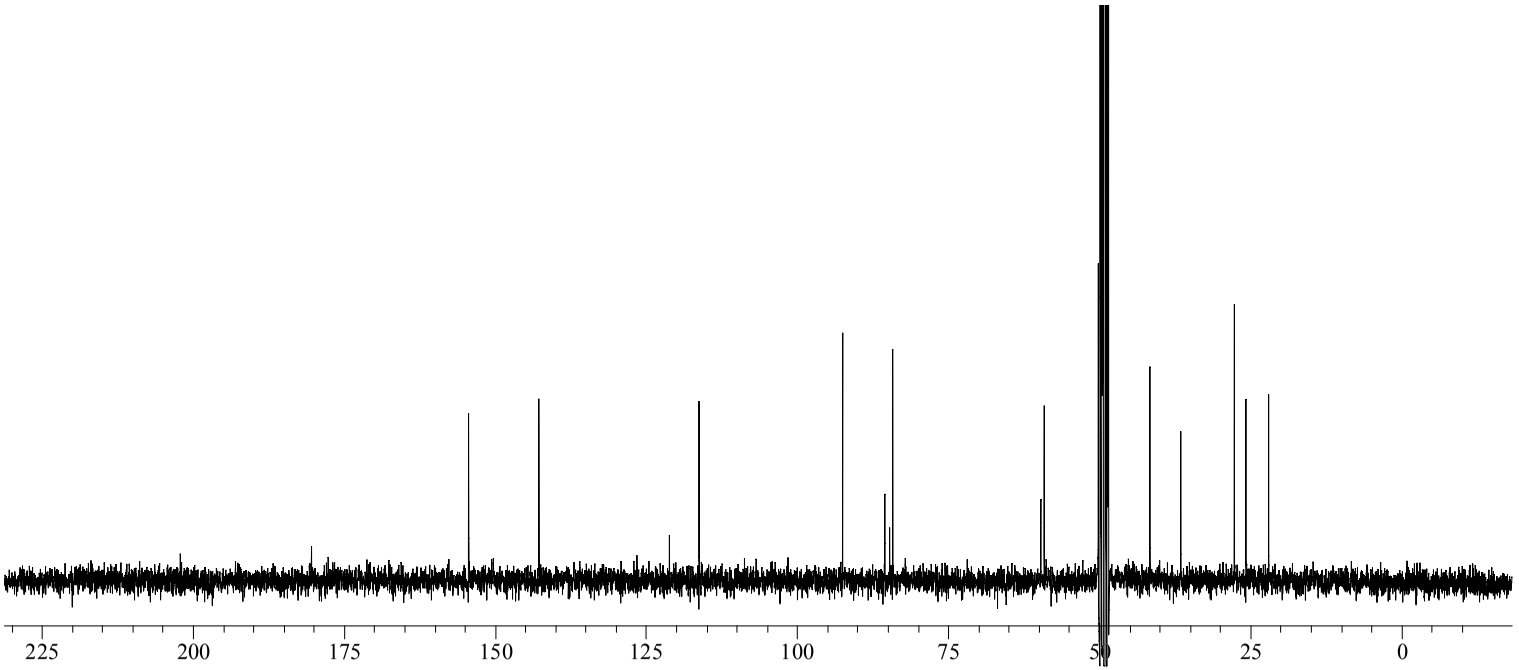
500 MHz ^{1}H NMR spectrum ($CDCl_3$) of S5



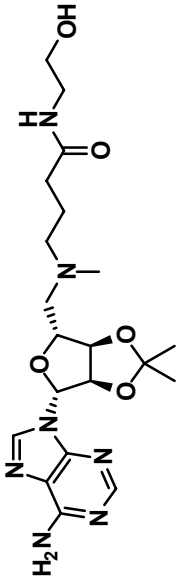


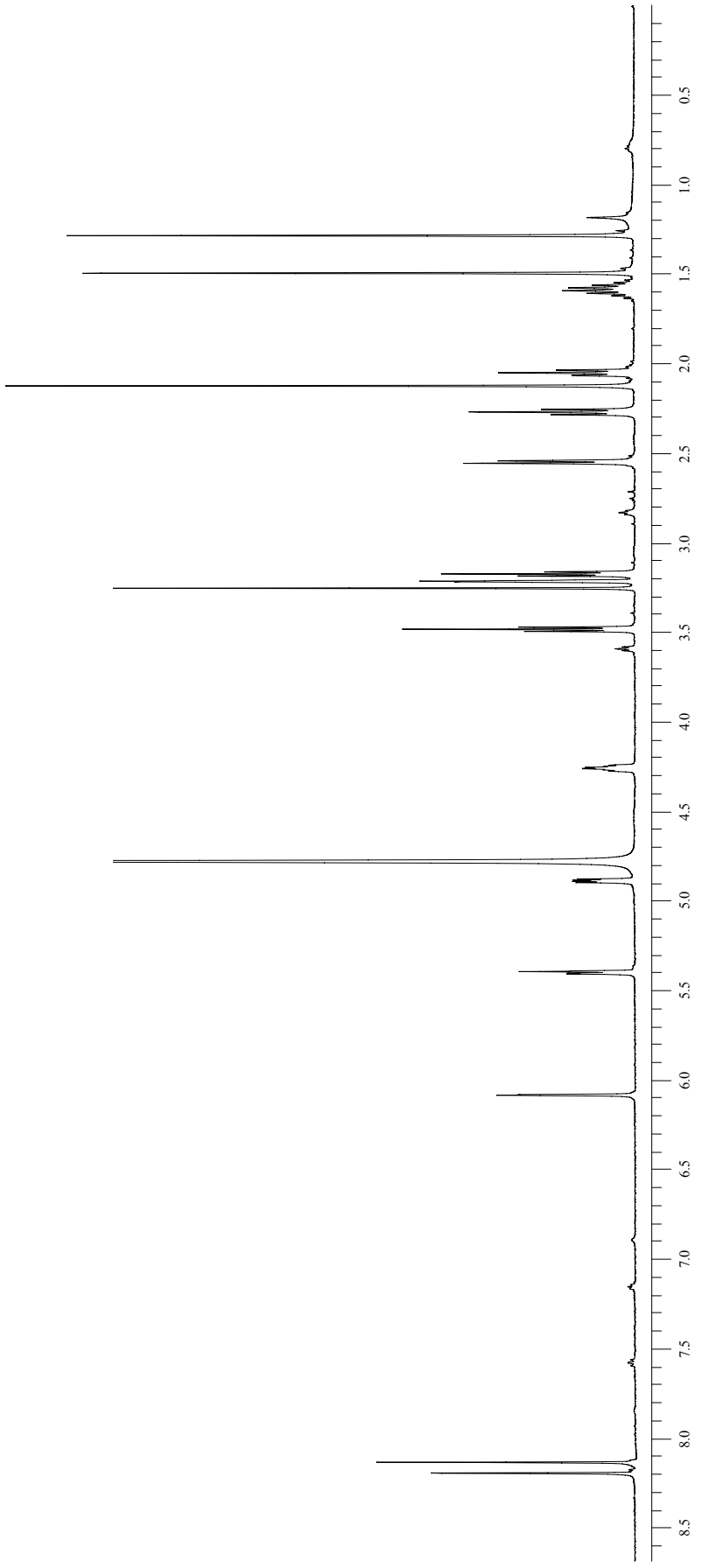
75 MHz ^{13}C NMR spectrum (MeOD) of S5



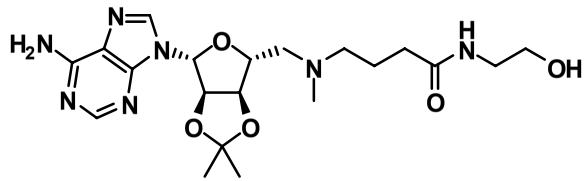


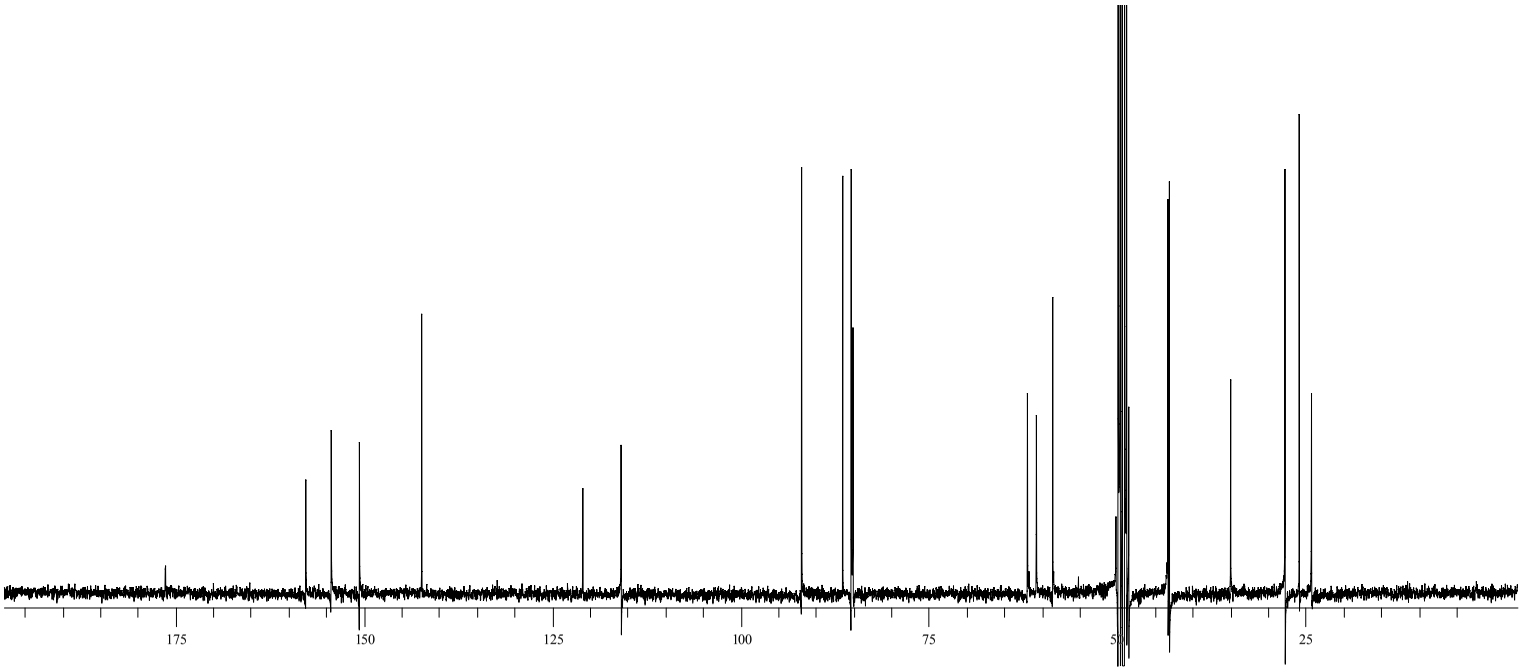
500 MHz ^{1}H NMR spectrum (MeOD) of 10



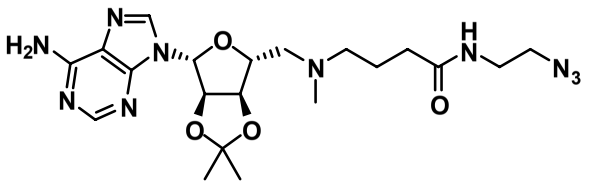


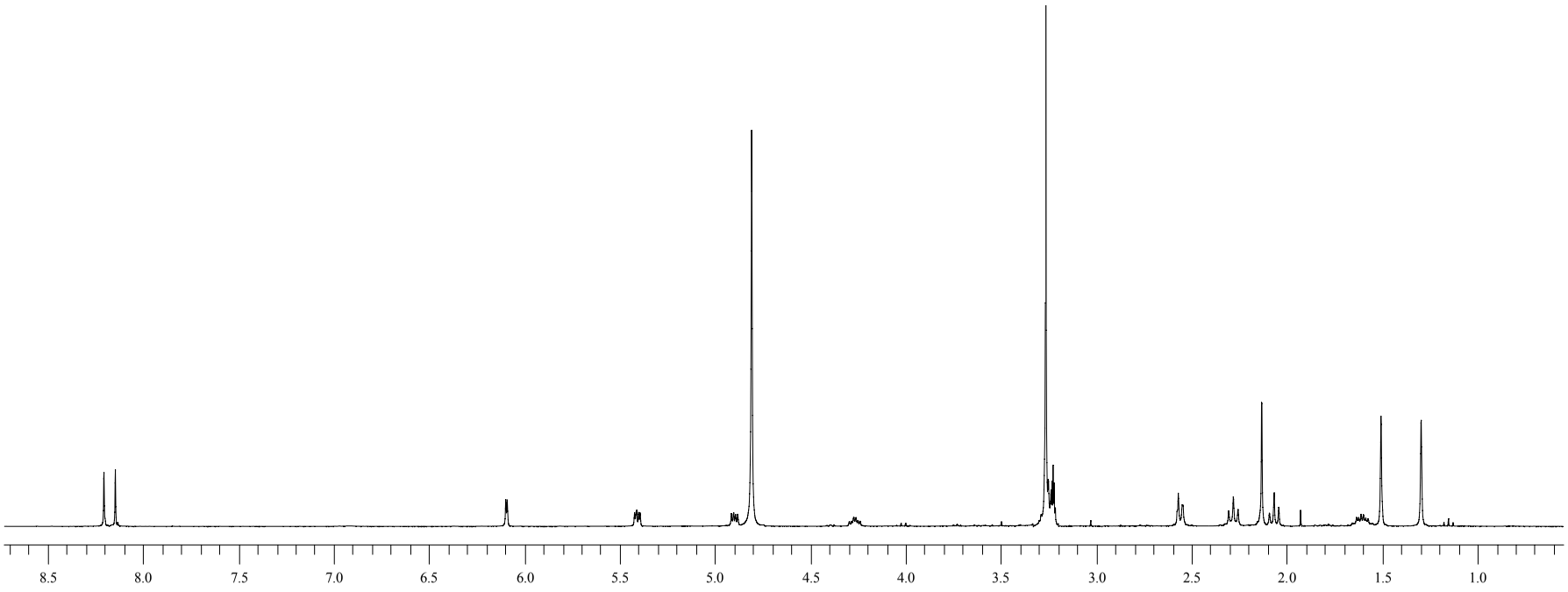
75 MHz ^{13}C NMR spectrum (MeOD) of 10



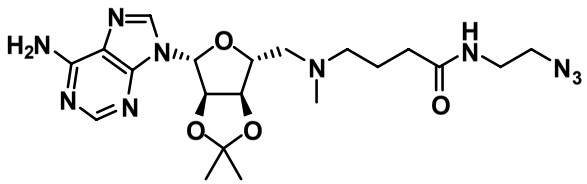


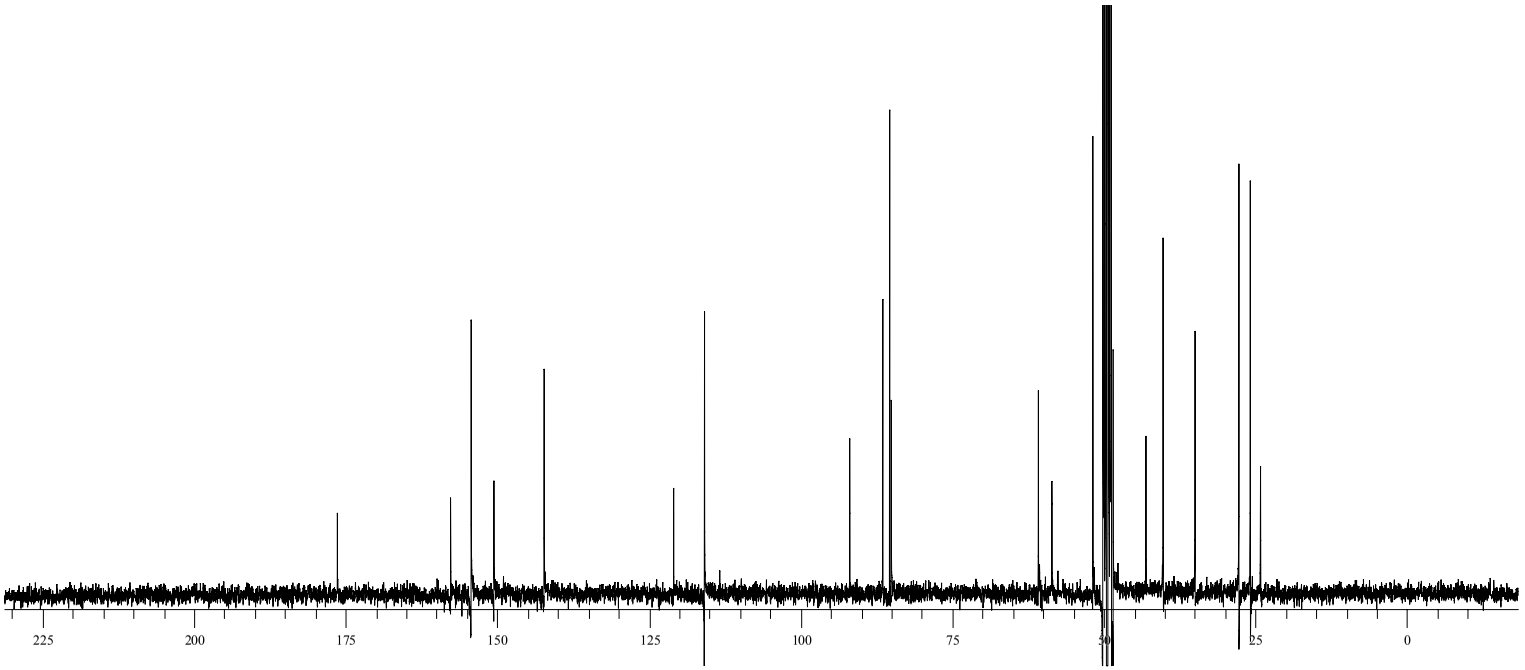
300 MHz ^{1}H NMR spectrum (MeOD) of 11



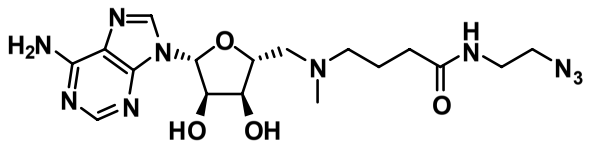


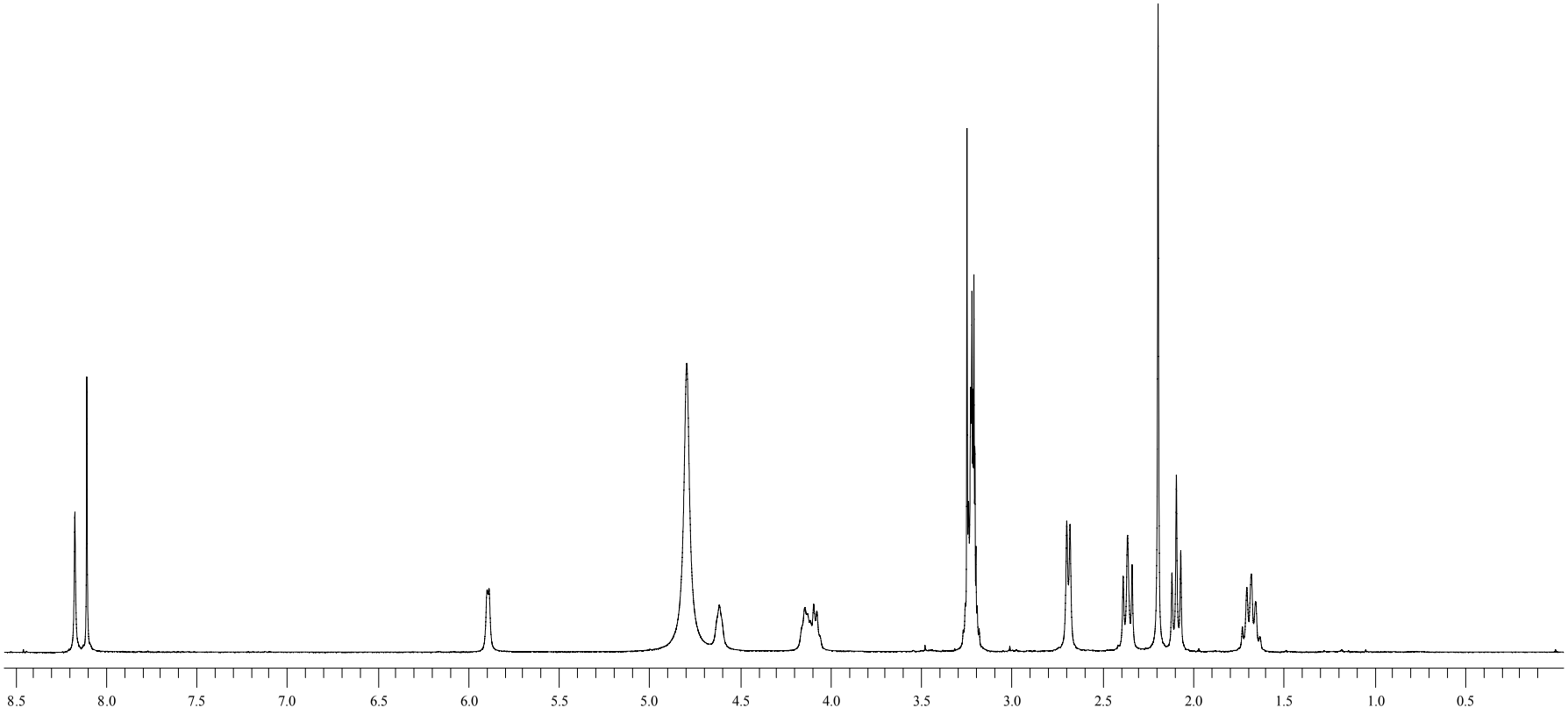
75 MHz ^{13}C NMR spectrum (MeOD) of 11



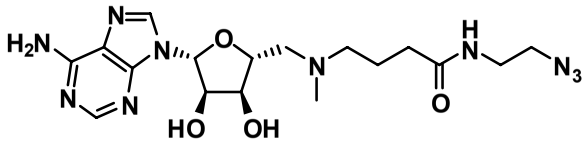


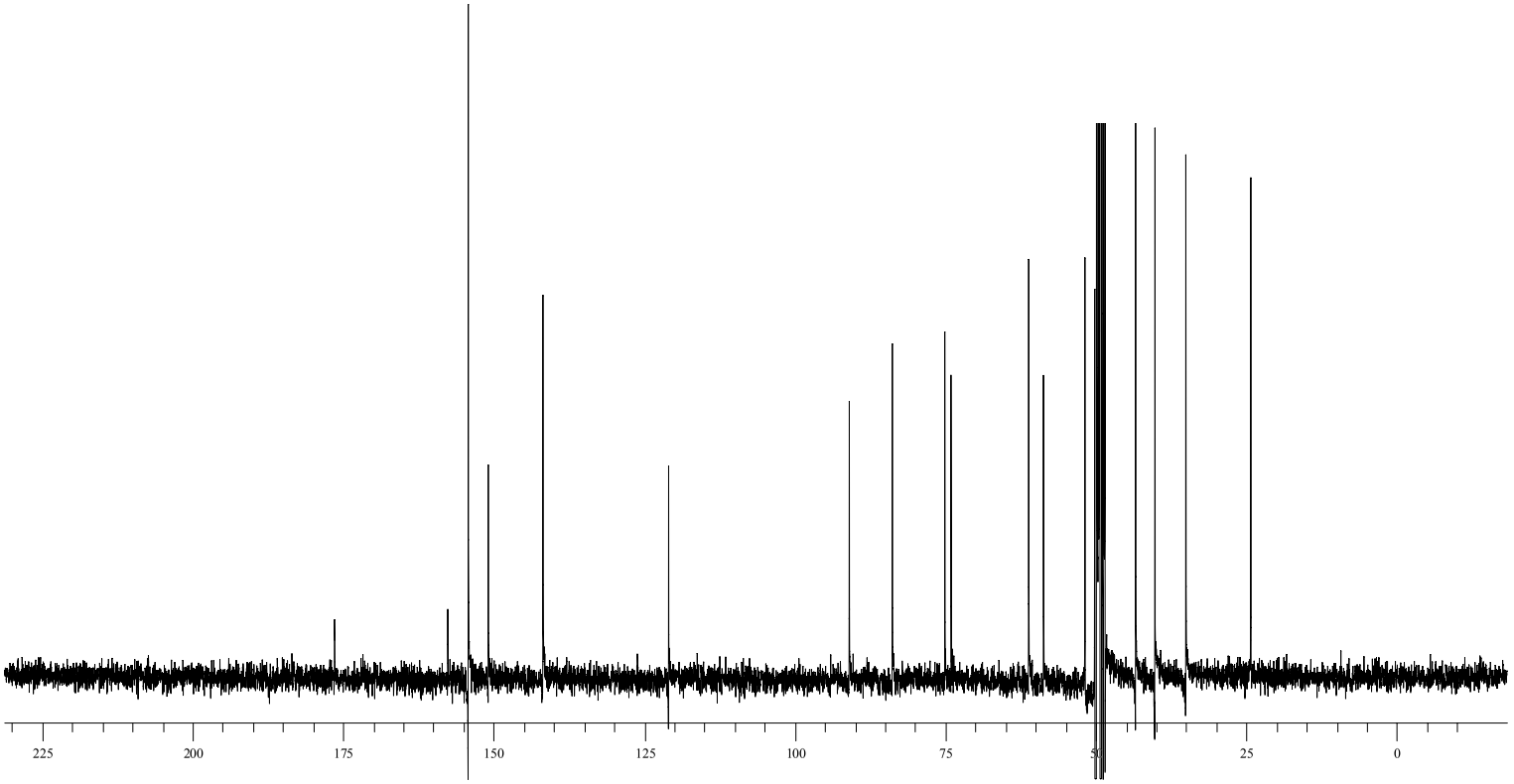
300 MHz ^{1}H NMR spectrum (MeOD) of 12



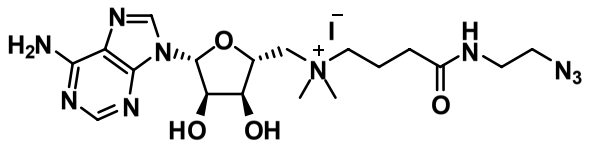


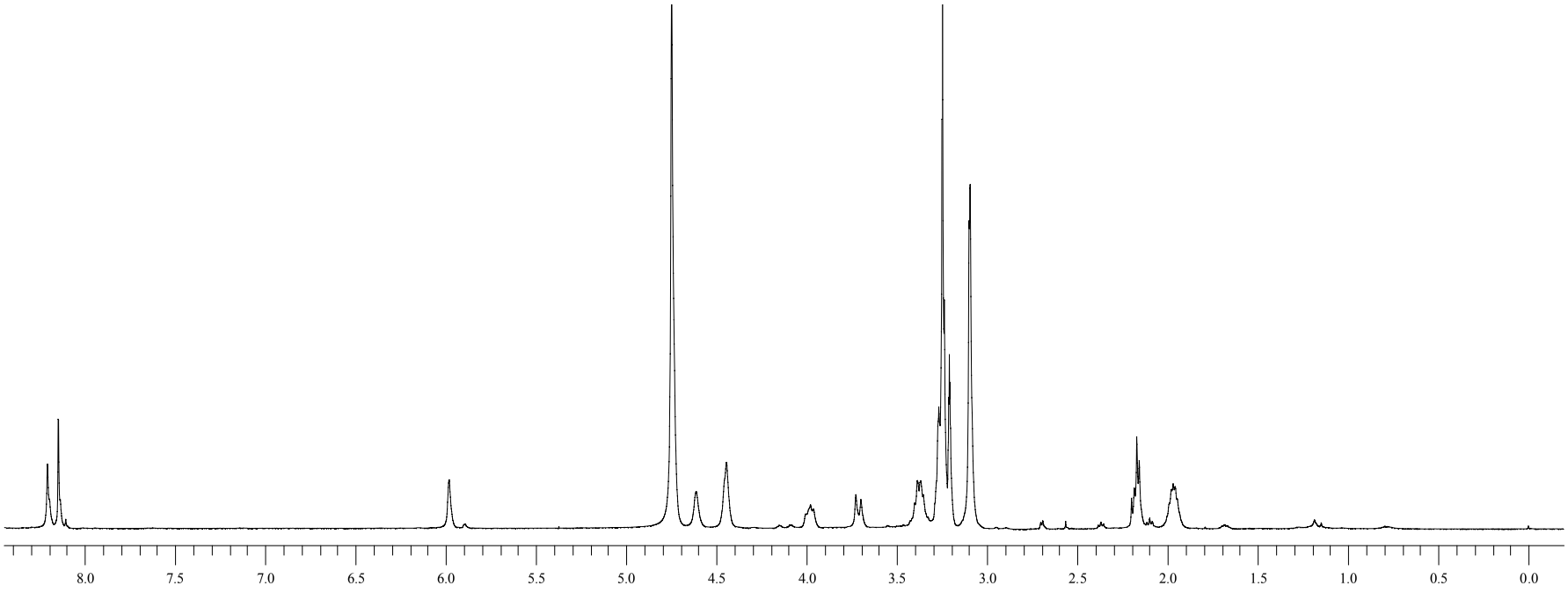
75 MHz ^{13}C NMR spectrum (MeOD) of 12



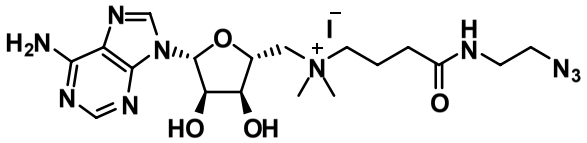


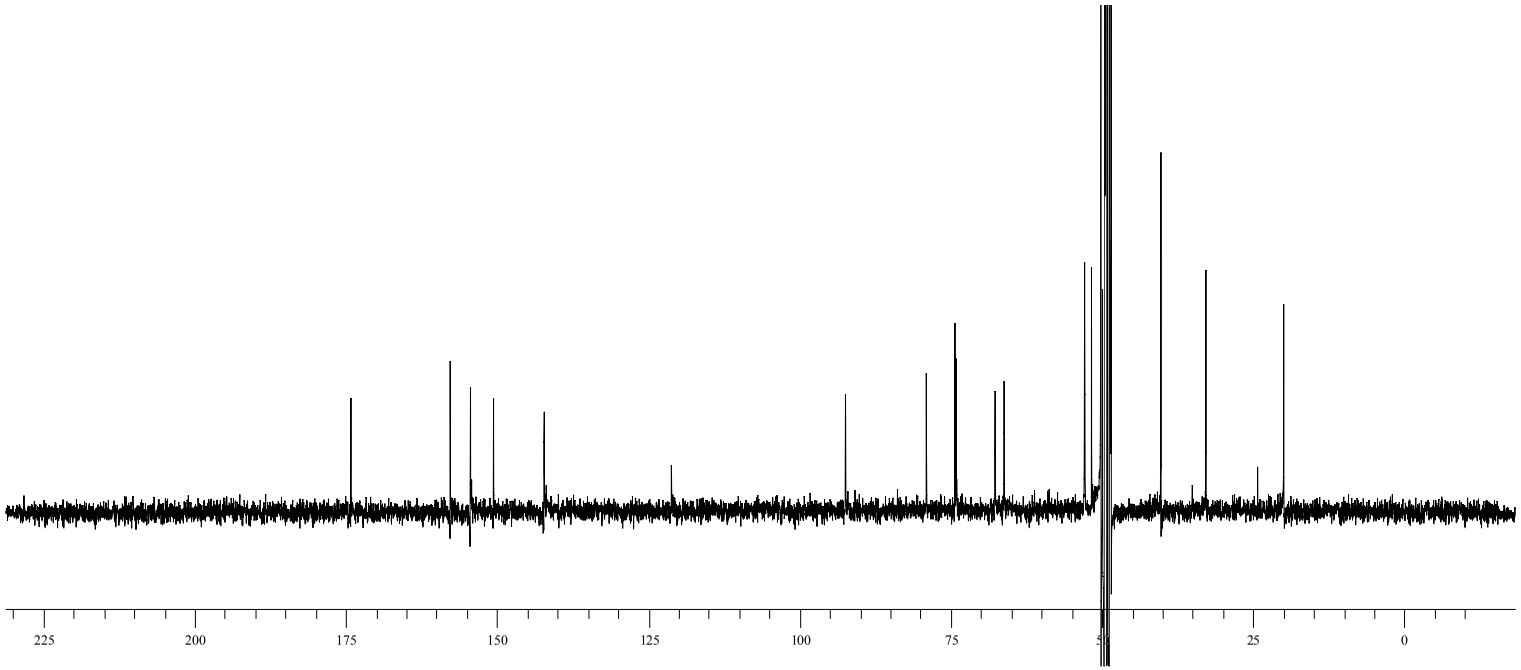
500 MHz ^{1}H NMR spectrum (MeOD) of 15



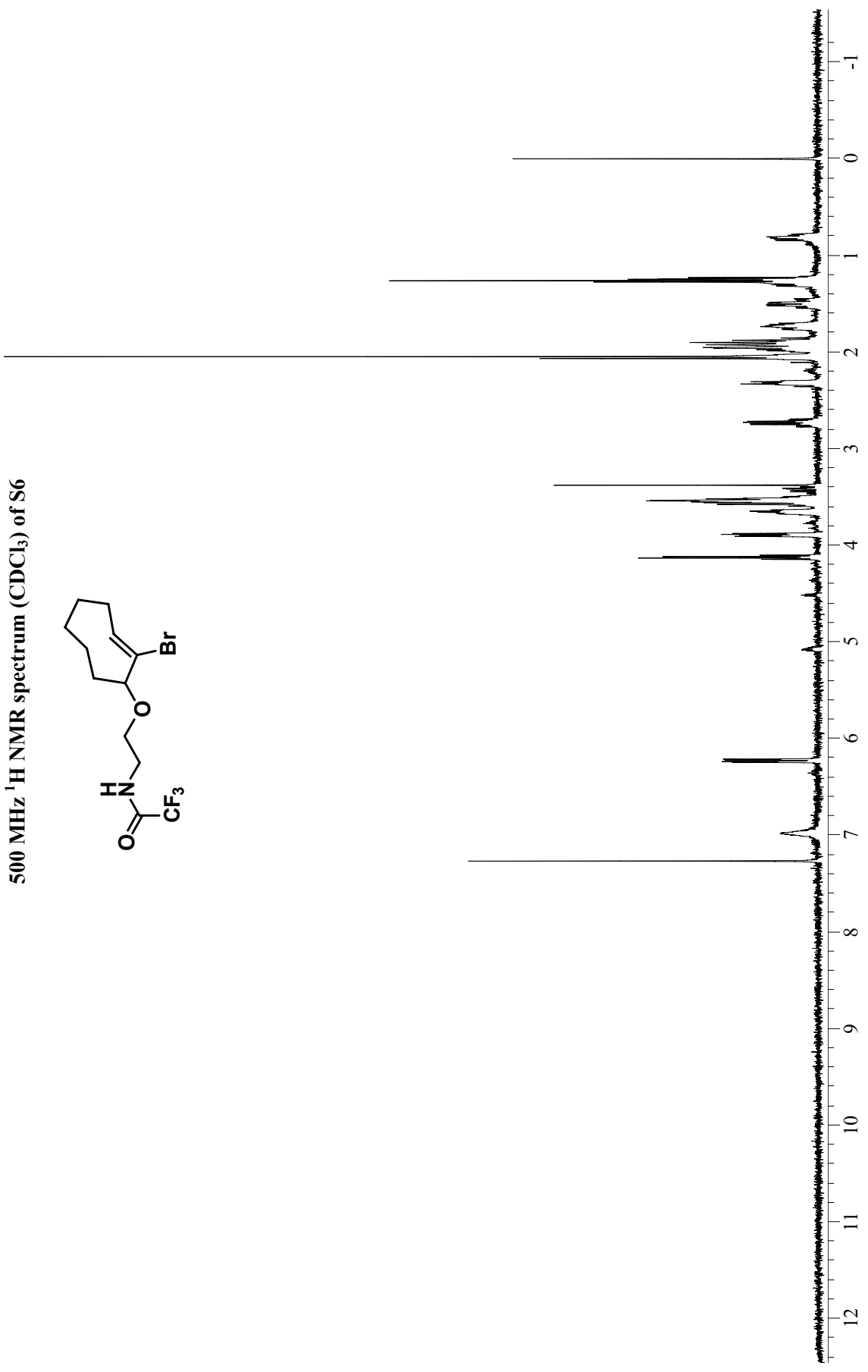


75 MHz ^{13}C NMR spectrum (MeOD) of 15

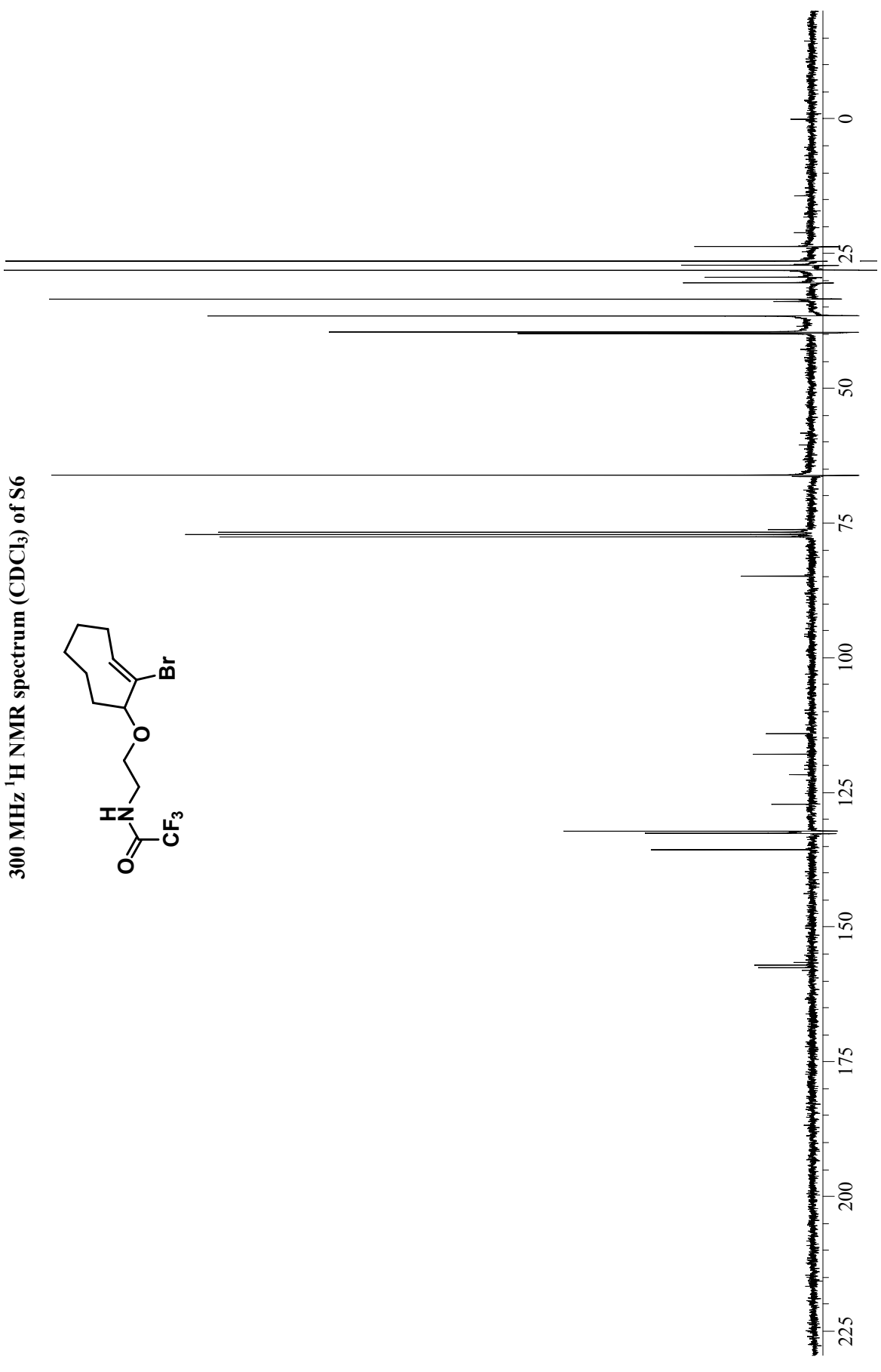




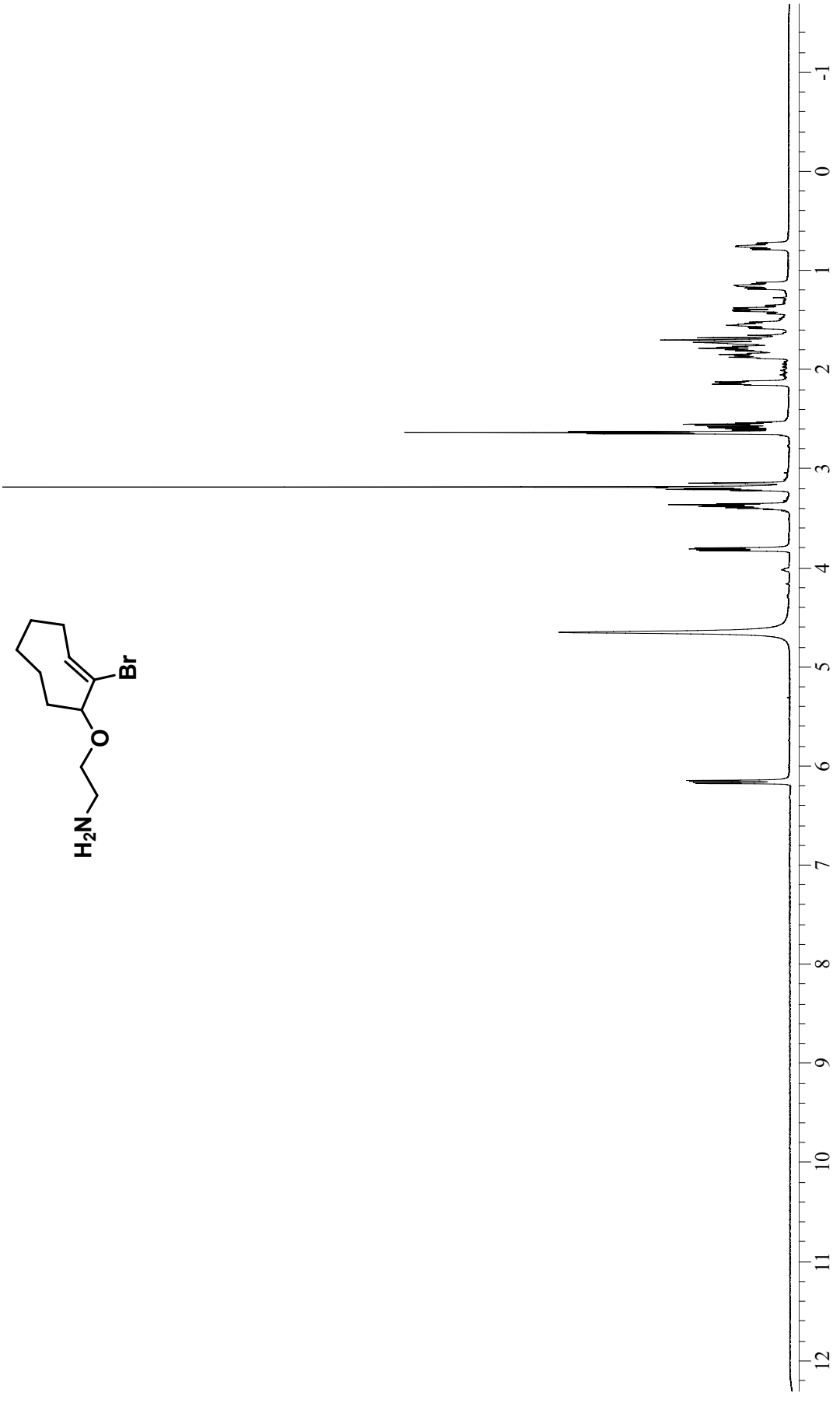
500 MHz ^{1}H NMR spectrum ($CDCl_3$) of S6



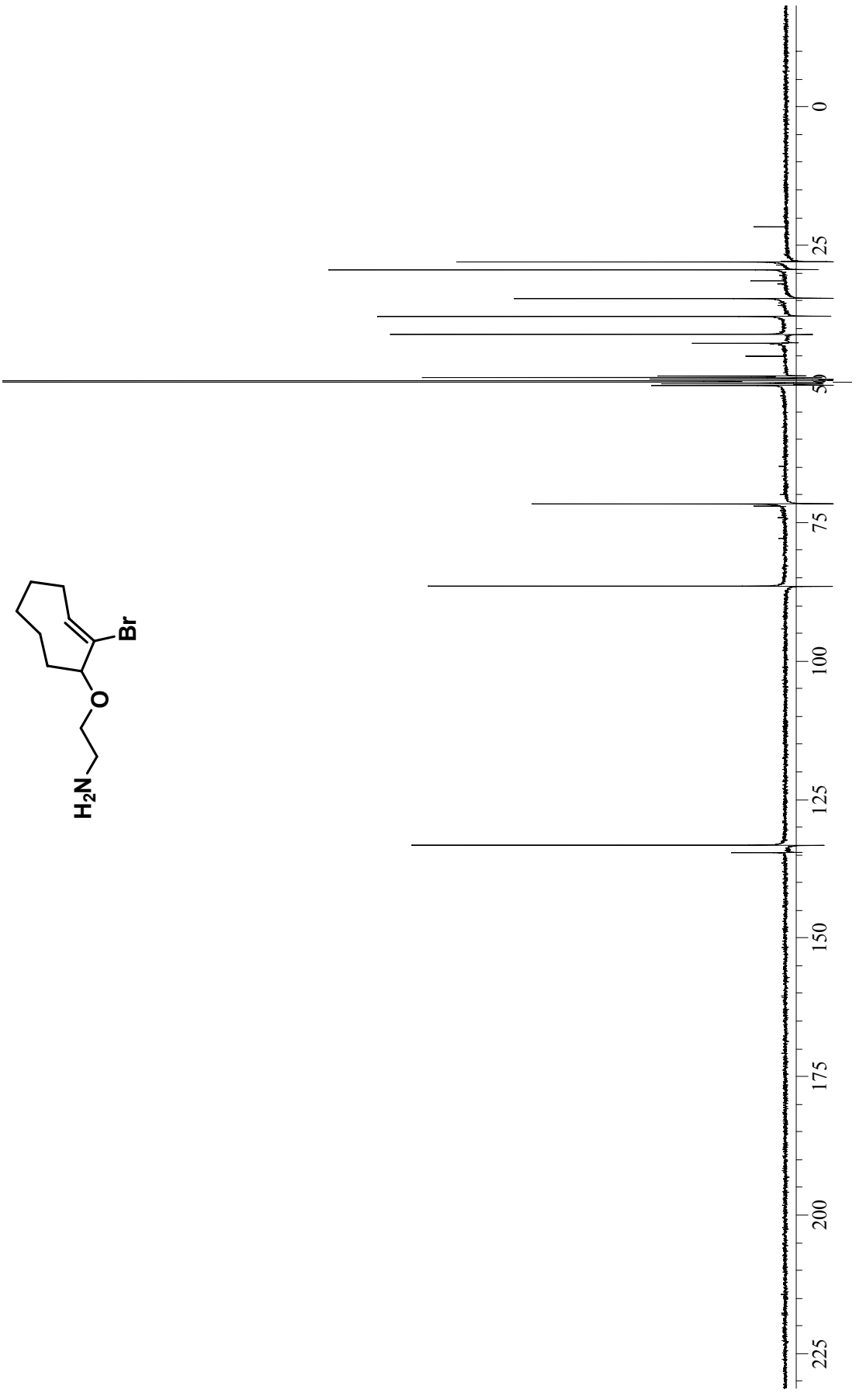
300 MHz ^{1}H NMR spectrum ($CDCl_3$) of S6



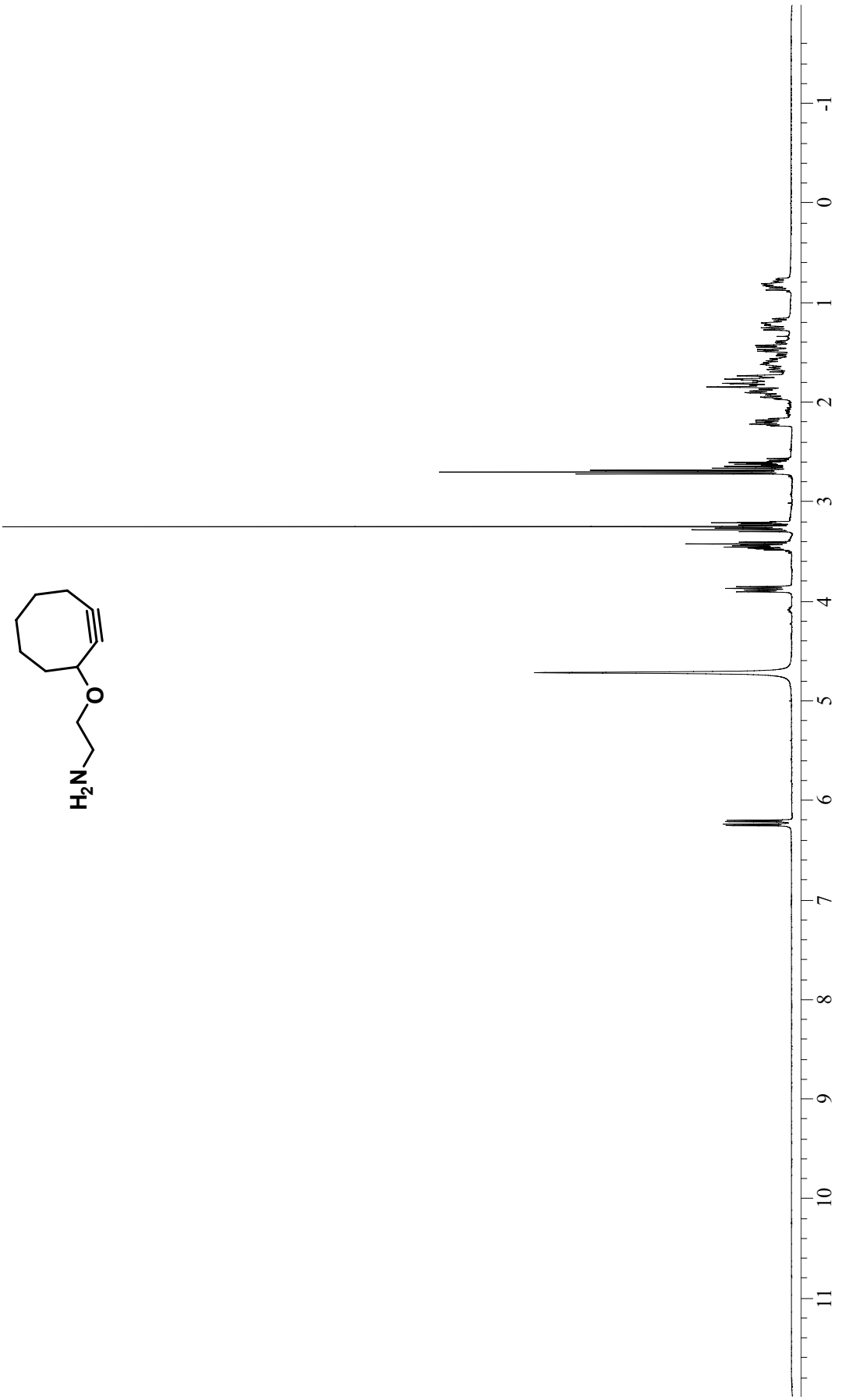
500 MHz ^{1}H NMR spectrum (MeOD) of S7



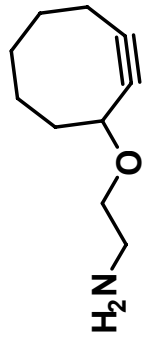
300 MHz ^{1}H NMR spectrum (MeOD) of S7



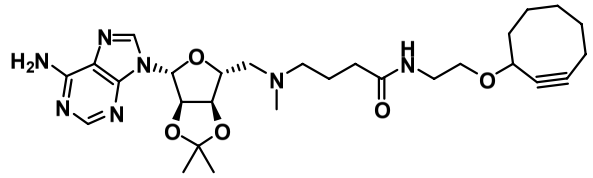
500 MHz ^{1}H NMR spectrum (MeOD) of S8

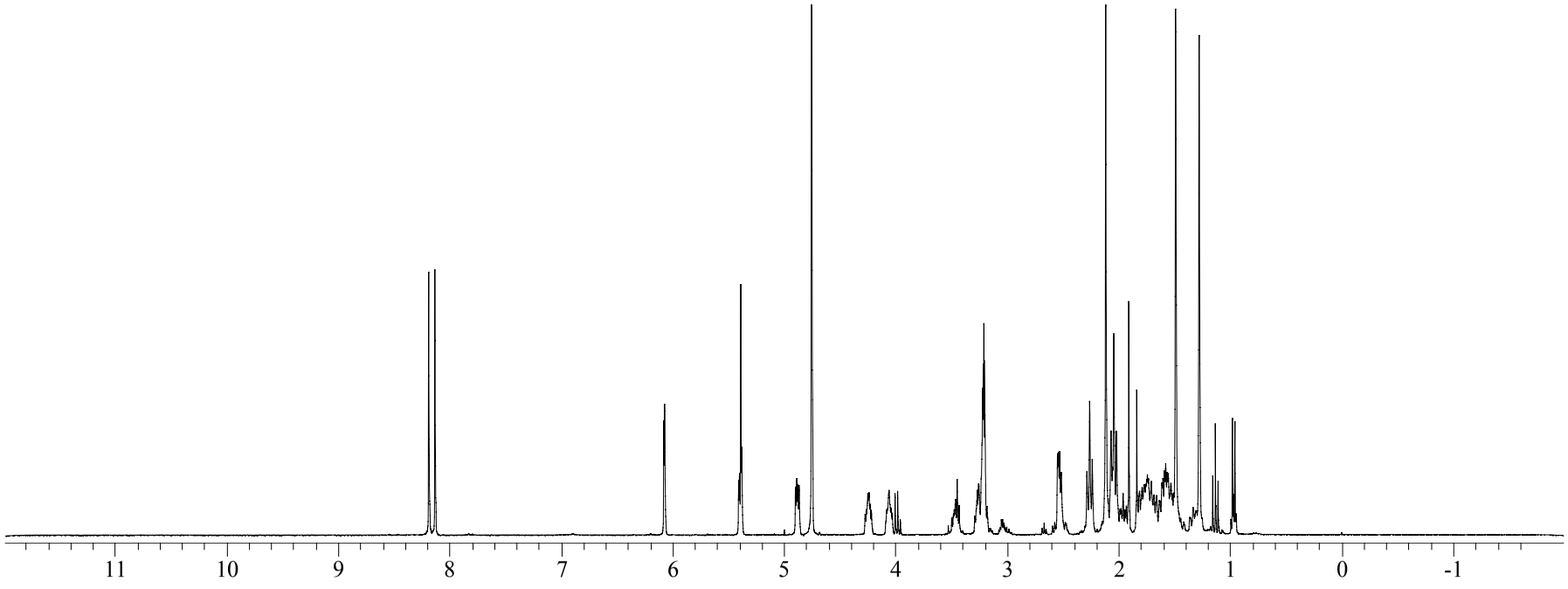


75 MHz ^{1}H NMR spectrum (MeOD) of S8

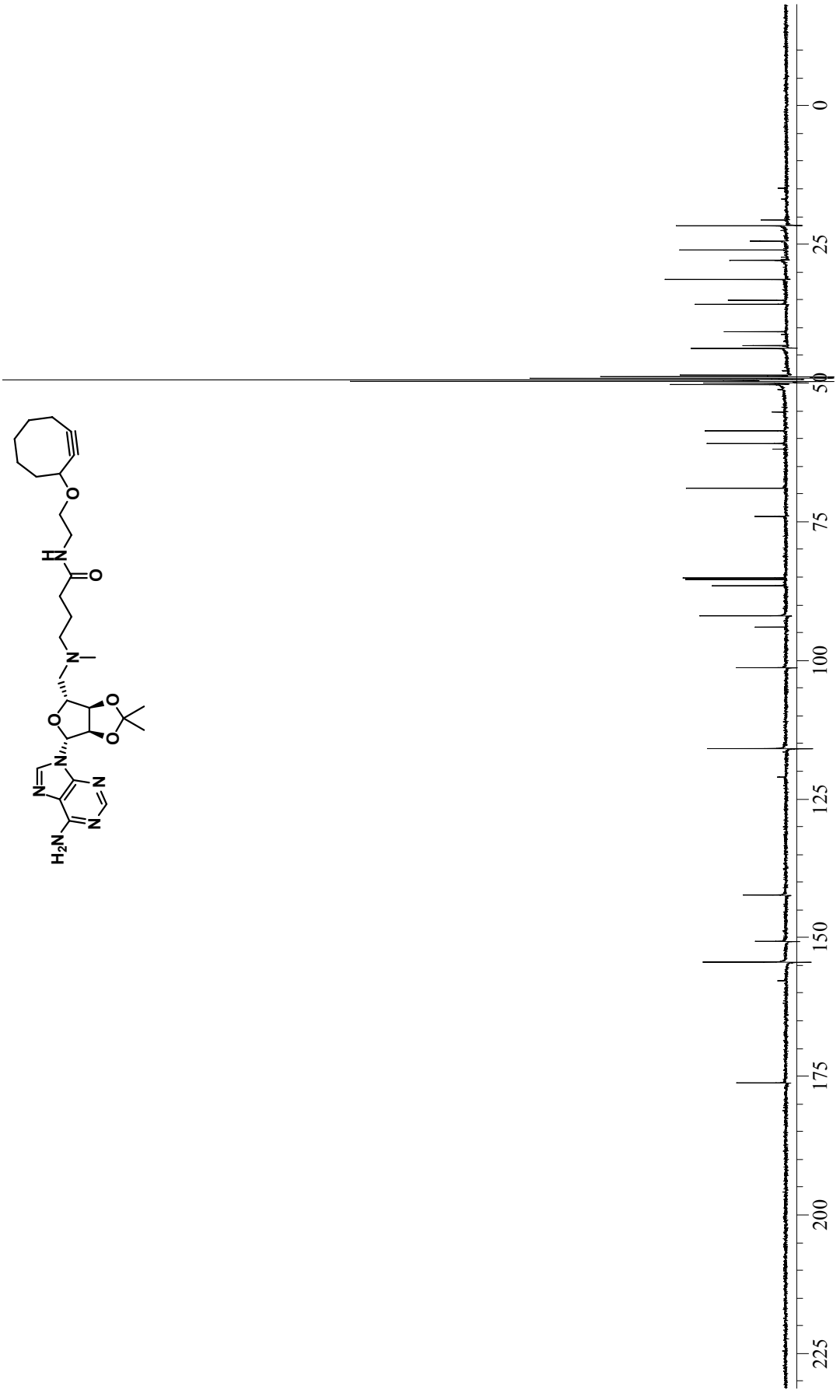


500 MHz ^{1}H NMR spectrum (MeOD) of S9

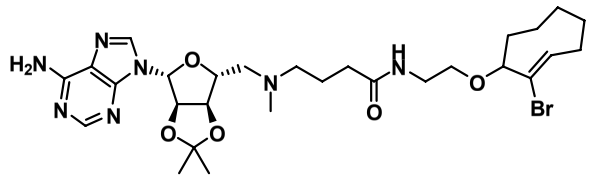


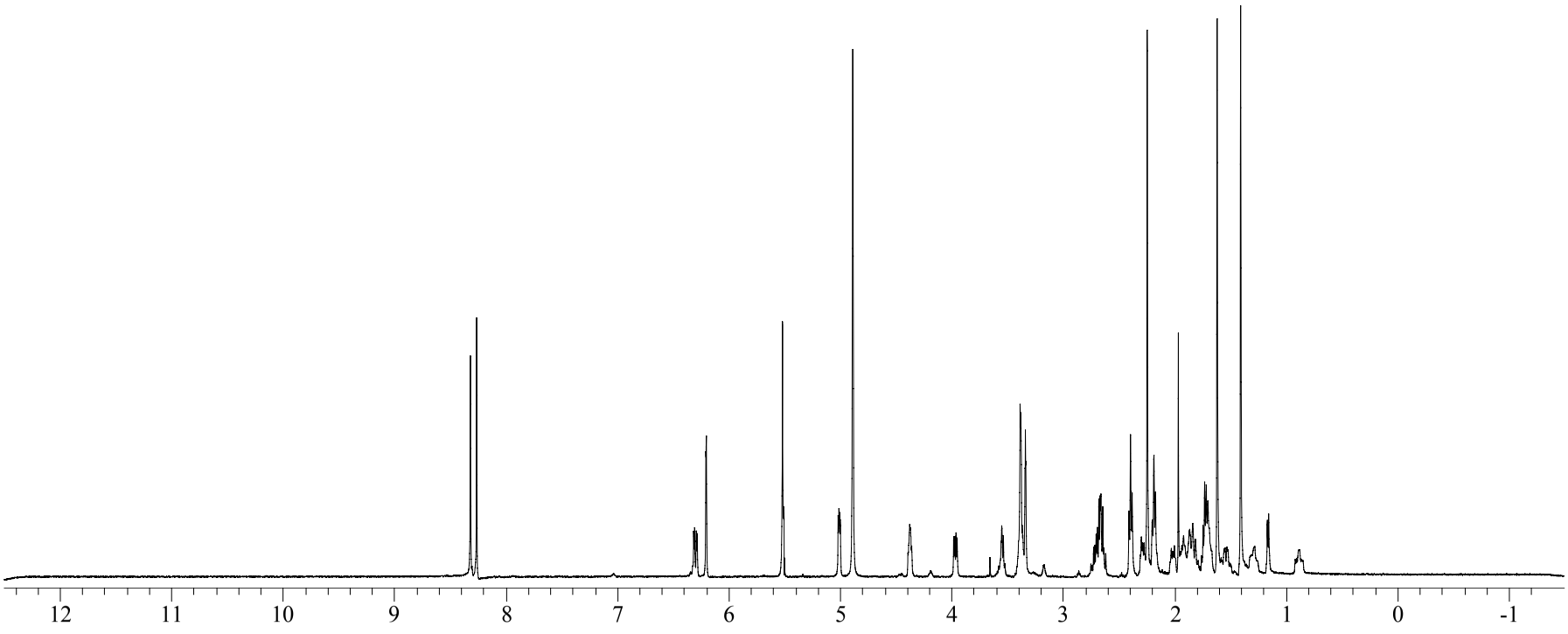


300 MHz ^{1}H NMR spectrum (MeOD) of S9

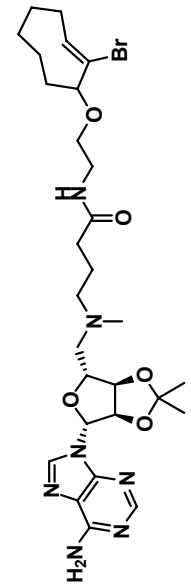


500 MHz ^{1}H NMR spectrum (MeOD) of 13

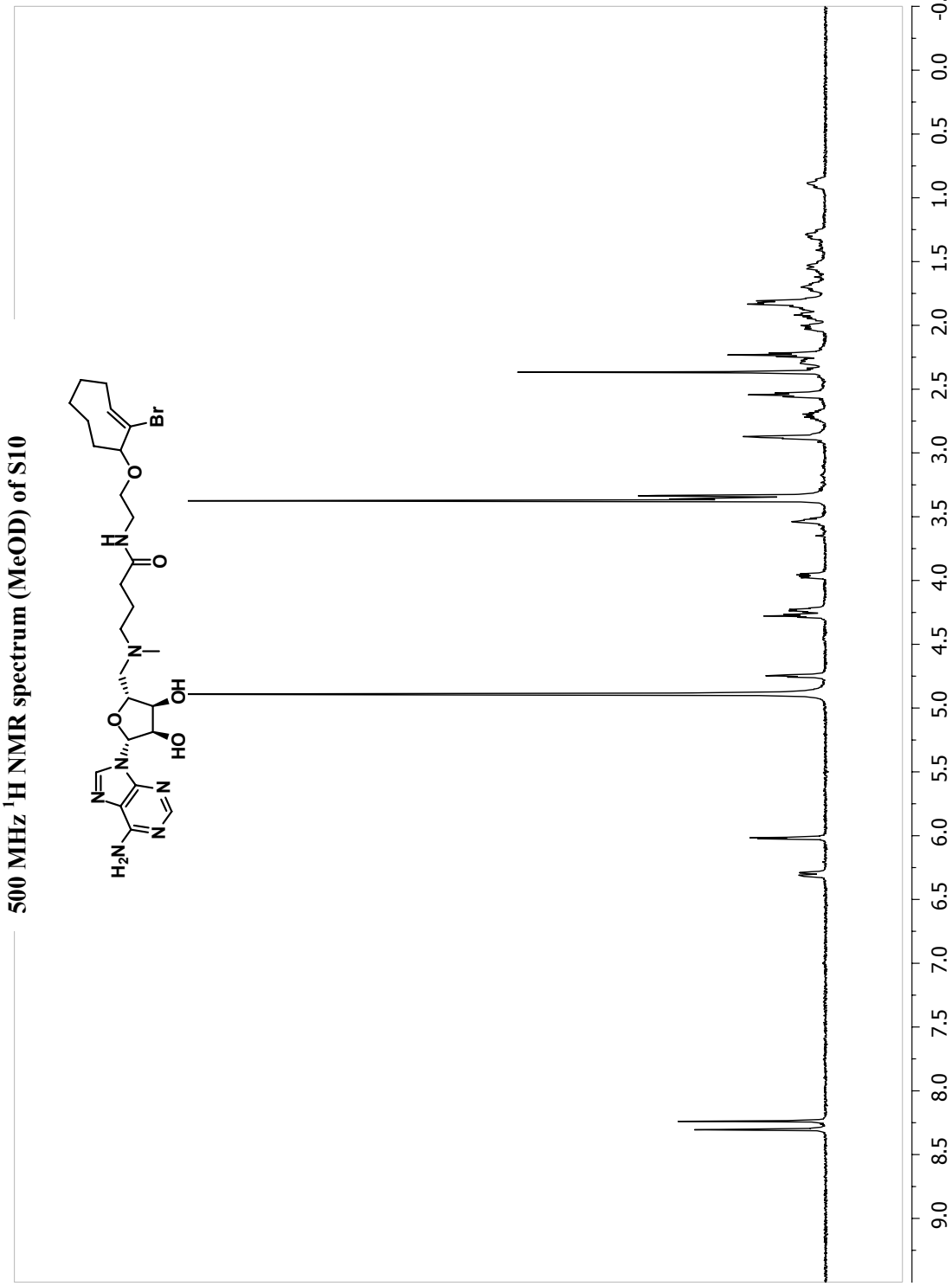




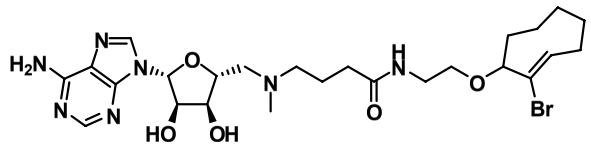
300 MHz ^{1}H NMR spectrum (MeOD) of 13

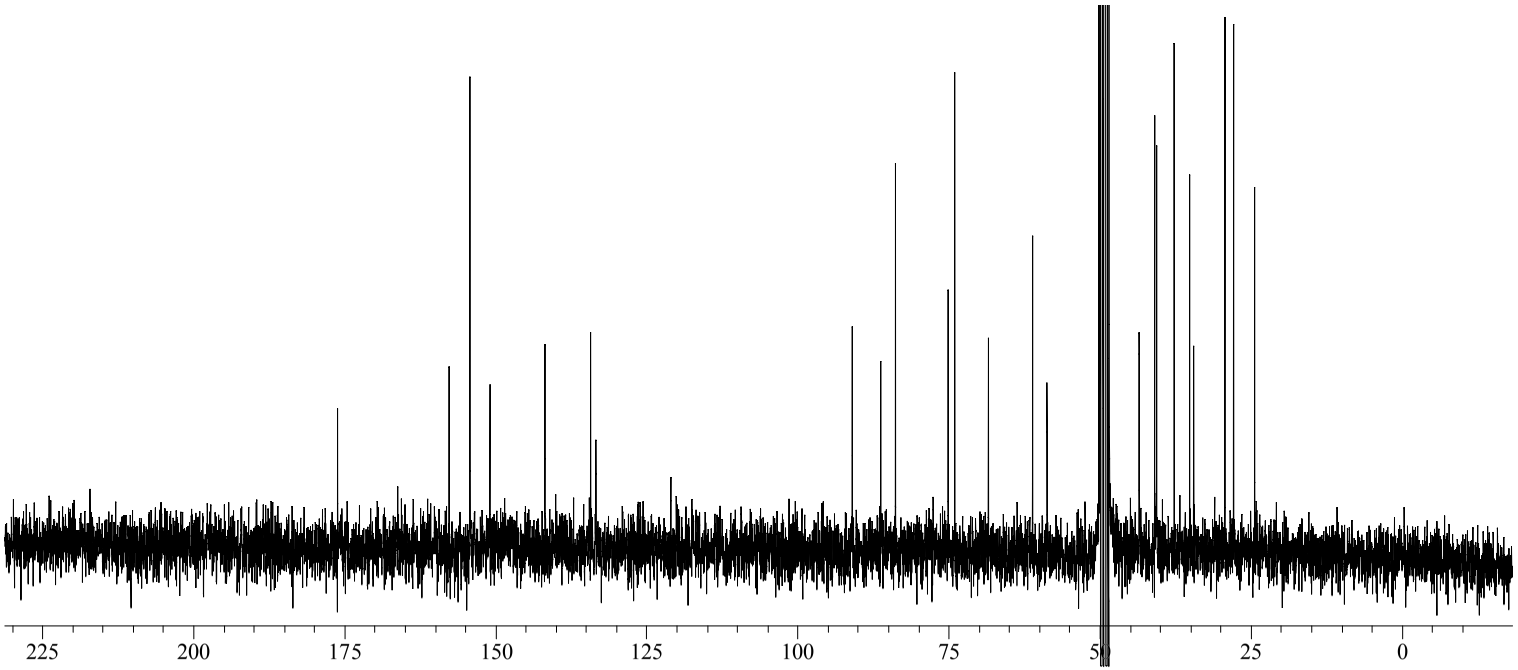


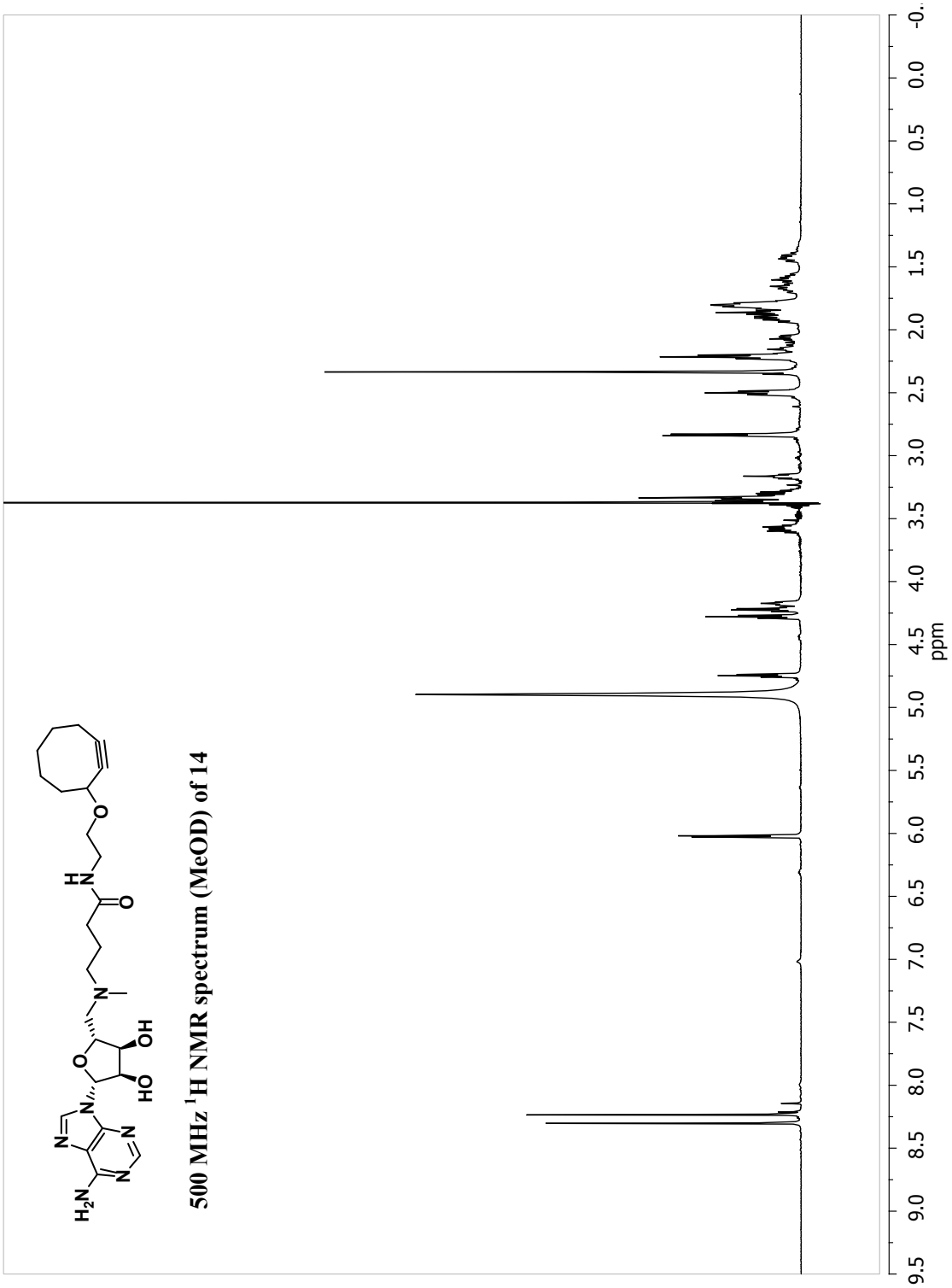
500 MHz ^{1}H NMR spectrum (MeOD) of S10



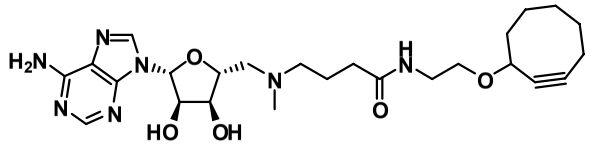
300 MHz ^{1}H NMR spectrum (MeOD) of S10

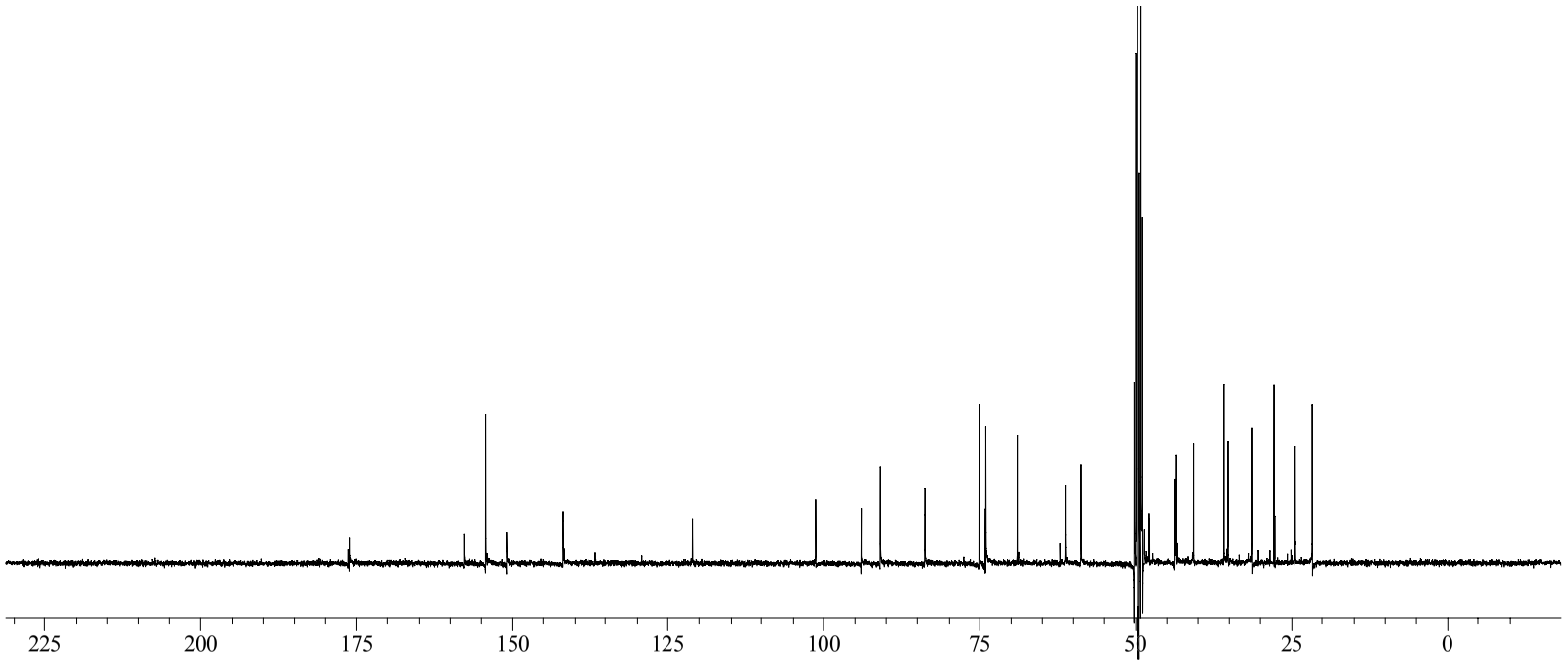




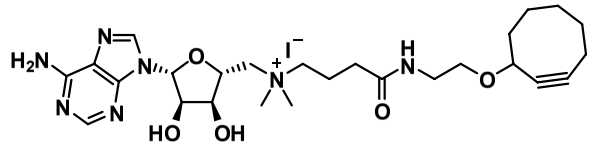

500 MHz ^{1}H NMR spectrum (MeOD) of 14
ppm
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

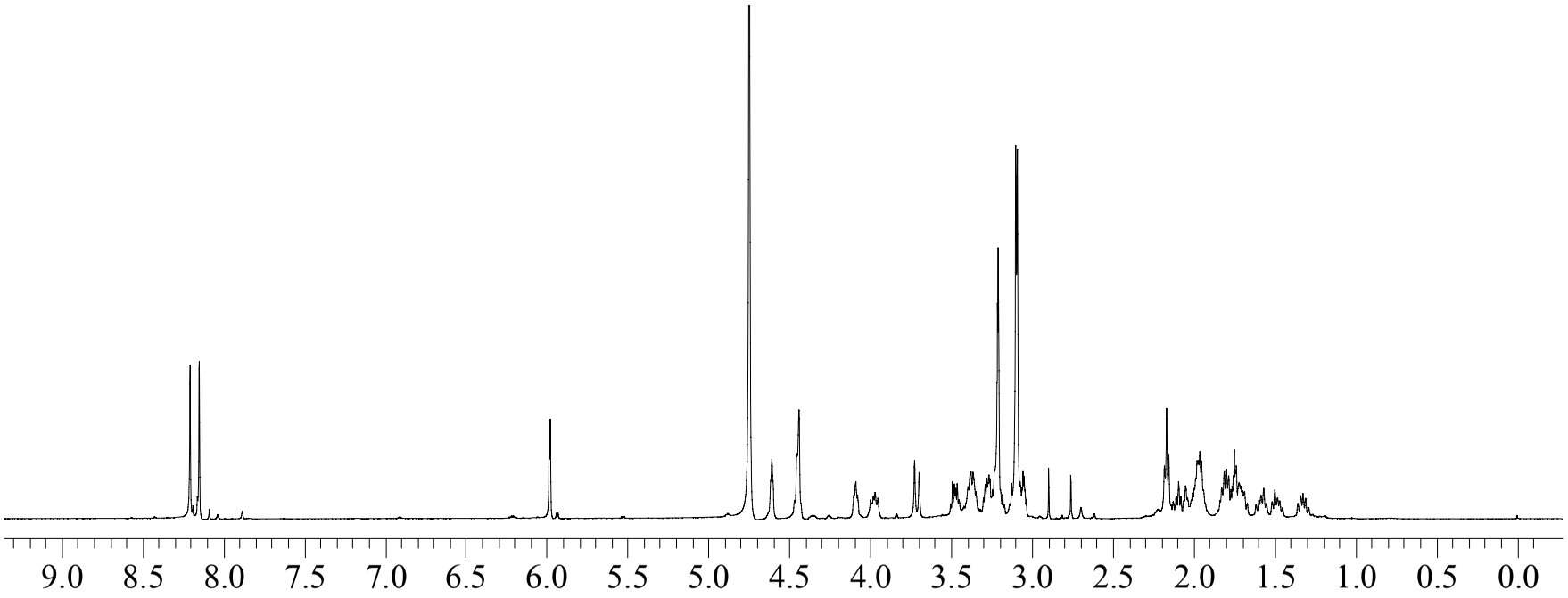
300 MHz ^{1}H NMR spectrum (MeOD) of 14



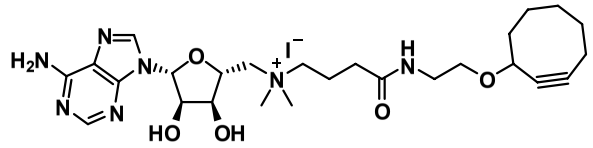


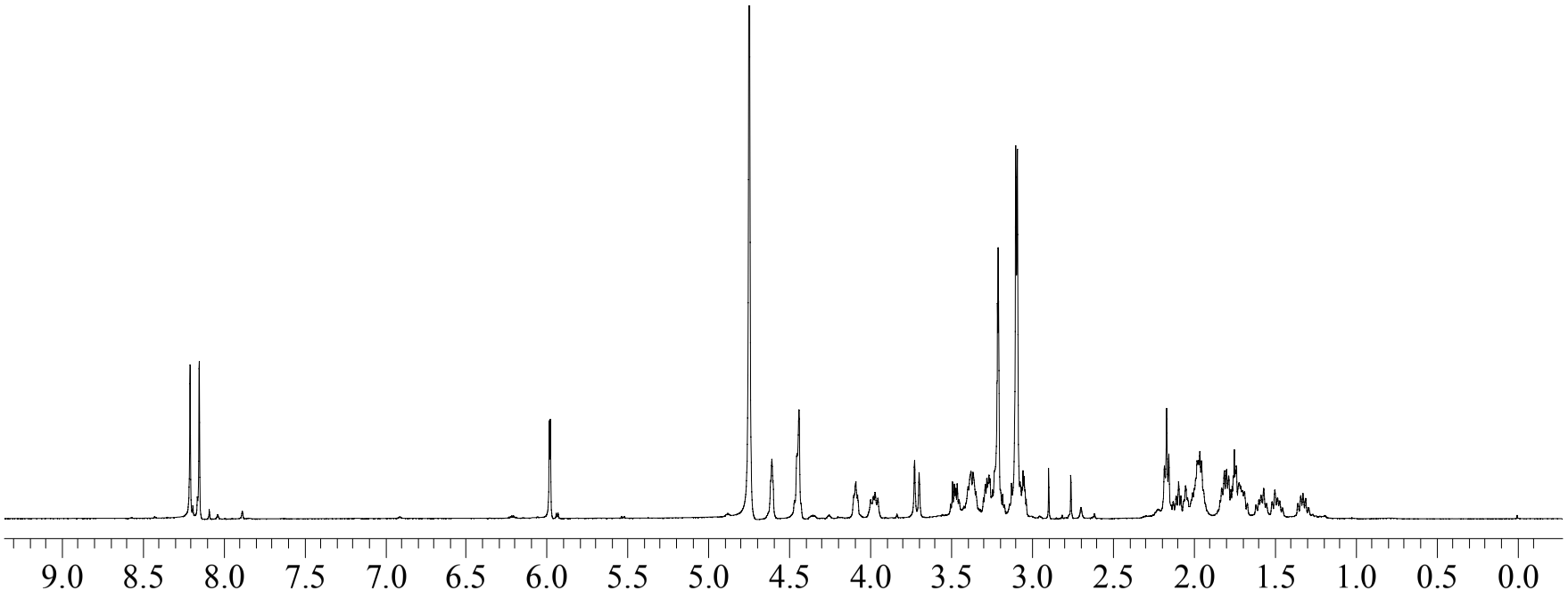
500 MHz ^{1}H NMR spectrum (MeOD) of 16





500 MHz ^{1}H NMR spectrum (MeOD) of 14





Materials and methods

E. coli strain and cloning vector

The BL21(DE3)-Gold (Stratagene) strain of *E. coli* was used for the expression of MetJ. The strain has been developed to provide high transformation efficiency and high levels of expression of genes cloned into expression vectors containing the T7 promoter. The strain contains a mutated gene that encodes a degraded RNAase E enzyme preventing degradation of mRNA. The strain is also deficient in the Lon protease, which can degrade recombinant proteins. In addition the strain is also deficient in the outer membrane protease, OmpT.

The pET22b(+) vector (Novagen) was used for the cloning and expression of MetJ. The gene was cloned into the pET plasmid under the control of the strong bacteriophage T7 transcription and translation signals. The expression host (BL21(DE3)-Star) contained a chromosomal copy of the T7 RNA polymerase gene under *lacUV5* control. Expression was induced by addition of isopropyl β-*D*-1-thiogalactopyranoside (IPTG) to the bacterial culture. The pET22b(+) vector carries an N-terminal *pelB* signal sequence for potential periplasmic location and resistance to ampicillin.[6]

Transformation protocol

BL21(DE3)-Star competent cells (Stratagene) were thawed on ice. The plasmid DNA (pET22b(+) vector containing the wild-type MetJ gene (pET22b(+) *metJ*) (2 µl) was added to a pre-chilled, sterilised ependorf tube. Competent cells (100 µl) were added to the DNA and the mixture incubated on ice for 30 min. The sample was then heat-shocked at 42 ºC for 20 seconds and incubated on ice for 2 min. 2YT media (0.9 ml, pre-heated to 42 ºC) was added to the competent cells, which were then incubated at 37 ºC with shaking (225-250 rpm) for one hour. The cells were concentrated by centrifugation (200 × g) for five min. The supernatant (900 µl) was removed with a pipette and the cells resuspended with shaking. The transformation was plated onto a single LB Agar plate with ampicillin 100 µg/ml. The plate was incubated at 37 ºC for 18h.

Table of buffers and media required for the expression and purification of MetJ

Buffer or media	Formulation
2YT media	Tryptone: 4.8 g Yeast Extract: 3.0 g NaCl: 1.5 g H_2O 300 ml
LB media	Tryptone: 3.0 g Yeast Extract: 1.5 g NaCl: 3.0 g H_2O: 300 ml
LB with agar	Tryptone: 3.0 g Yeast Extract: 1.5 g NaCl: 3.0 g Agar: 3.0 g H_2O: 300 ml
Sonication buffer	100 mM potassium phosphate pH 7.6 1 mM EDTA 10 mM β-mercaptoethanol 1 broad spectrum protease inhibitor per 50 ml
Q Sepharose buffer A	50 mM Tris.HCl 75 mM KCl 1mM EDTA
Q Sepharose buffer B	50 mM Tris.HCl 500 mM KCl 1mM EDTA
SP Sepharose buffer A	25 mM potassium phosphate pH 7.6 1 mM EDTA
SP Sepharose buffer B	400 mM potassium phosphate pH 7.6 1 mM EDTA

Expression and purification of MetJ

Single colonies of pET22b(+) *metJ* transformed BL21(DE3)-Star cells plated on LB Agar with ampicillin 100 μg/ml were each used to inoculate one of six 5 ml cultures of LB with

ampicillin 100 µg/ml. The cells were grown for 18 h at 37 ºC with shaking (225-250 rpm). Four of the overnight cultures were each used to inoculate 500 ml LB with ampicillin 100 µg/ml. The cells were grown until OD_{580} = 1.0 and a sample (100 µl) was taken and mixed with loading buffer (100 µl). Expression was induced by addition of IPTG to a final concentration of 1 mM. Further ampicillin, to a final concentration of 100 µg/ml, was added at this stage. The cells were grown until OD_{580} = 1.5 and a sample (100 µl) was taken and mixed with loading buffer (100 µl). The cells were harvested by centrifugation (2500 rpm, 4 ºC, 20 min) and stored at −80 ºC.

The pellet was defrosted on ice and resuspended in 80 ml of sonication buffer. The cells were lysed by sonication on ice. The cell debris was removed by centrifugation (15000 rpm, 4 ºC, 15 min). The supernatant was decanted and centrifuged (15000 rpm, 4 ºC, 30 min). The remaining sample was treated with DNaseI (Invitrogen, 1 mg DNaseI/100 g cells, 3mM $MgCl_2$) for 90 min at ambient temperature. The sample was made up to 20% ammonium sulfate, left on ice for 20 min and centrifuged (10000 rpm, 4 ºC, 15 min). The supernatant was decanted, made up to 70% ammonium sulfate, left on ice for 20 min and centrifuged (10000 rpm, 4 ºC, 15 min). The pellet was resuspended in Q Sepharose buffer A (with 1 broad-spectrum protease inhibitor tablet per 1000 ml) and dialysed extensively against the same buffer (with 1 broad-spectrum protease inhibitor tablet per 1000 ml). Purification of the protein was achieved by passage of the cell extract through two ion exchange columns: Q Sepharose (gradient of 50 mM Tris.HCl pH 7.6; 1 mM EDTA;75-500 mM KCl). The fractions containing MetJ were identified using SDS-PAGE. The combined MetJ-containing fractions were dialysed extensively against SP Sepharose buffer A (with 1 broad-spectrum protease inhibitor tablet per 1000 ml). Final purification was achieved by passage of the partially purified protein through a SP Sepharose (gradient of 25-400 potassium phosphate pH 7.6; 1 mM EDTA) The fractions containing MetJ were identified using SDS-PAGE (**Figure S1**). The purified protein was stored as a 70% ammonium sulfate precipitate at 4 ºC.

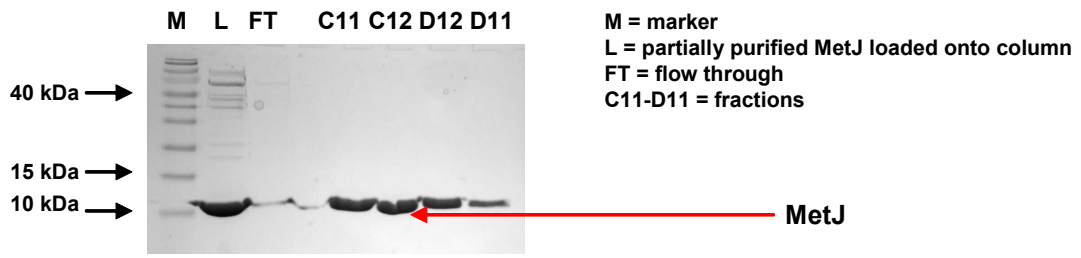


Figure S1: The MetJ-containing fractions were identified by SDS-PAGE.

Protein concentration was determined by spectrophotometry using $E_{280} = 15340\ M^{-1}cm^{-1}$ for the MetJ monomer.[7] The molecular weight of the MetJ monomer was confirmed by mass spectrometry (**Figure S2**).

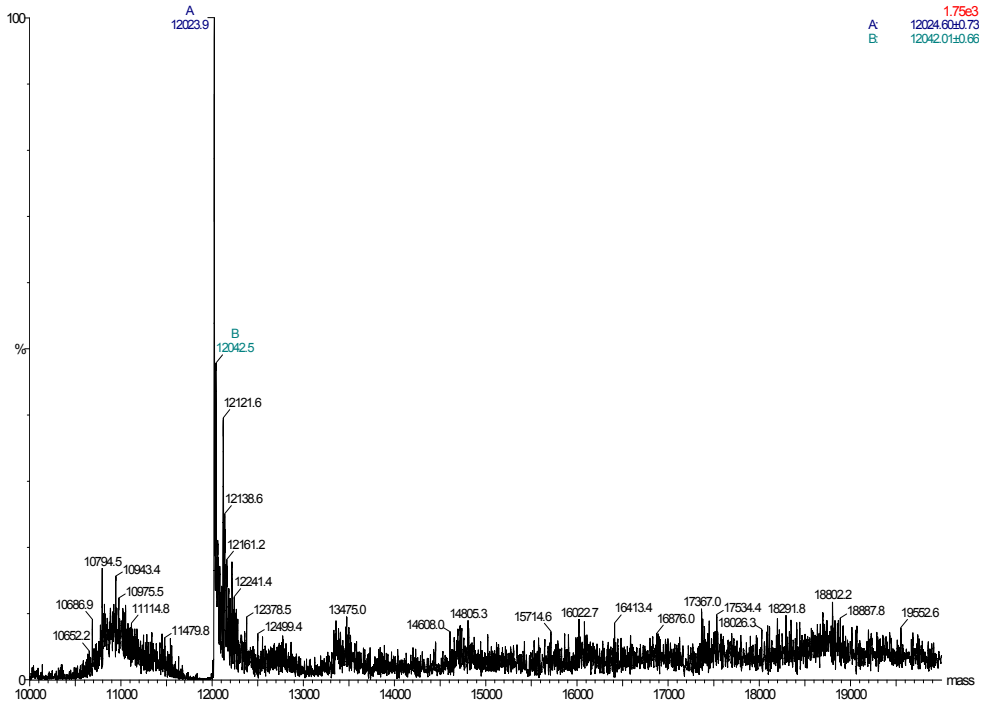


Figure S2: Mass spectrum of the MetJ monomer. The spectrum was acquired on a Waters Micromass LCT premier orthogonal acceleration time of flight spectrometer fitted with an Advion Nanomate automated nano-electrospray ionisation injector. The data was processed using the MaxEnt algorithm which uses the maximum entropy method to produce true molecular mass spectra from multiply-charged electrospray spectra.

Fluorescence anisotropy experiments

Analysis of novel SAM analogues binding to MetJ in the presence of operator DNA was carried out using fluorescence anisotropy. Fluorescence anisotropy experiments were performed on a Spex Fluorolog Tau spectrofluorometer (HORIBA Jobin Yvon) controlled by Datamax software. The excitation and emission wavelengths were set to 488 nm and 517 nm respectively. Slit widths were set to 5 nm and all experiments were carried out at 19 °C. The detector integration time was 5 seconds and three readings were taken of each sample to obtain an average anisotropy. Increase in fluorescence anisotropy was observed upon titration of MetJ into a solution of F-MetC (10 nM) with and without ligand (2 μM) in TK buffer pH 7.6 (50 mM Trizma hydrochloride pH 7.6, 100 mM KCl) with a total starting volume of 200 μl. During titrations, the reaction volume did not increase by more than 10%. The final DMSO concentration was 2% or less. After each addition, the sample was mixed with a pipette and left to equilibrate for five minutes before the fluorescence anisotropy readings

were acquired. The intensity of linear and perpendicular light, compared to the excitation light is measured experimentally and the anisotropy was calculated using the following equation:

$$r = \frac{(F_{\parallel} - F_{\perp})}{(F_{\parallel} + 2F_{\perp})}$$

Where r = fluorescence anisotropy

$F_{\parallel}$ = fluorescence intensity parallel to the excitation plane

$F_{\perp}$ = fluorescence intensity perpendicular to the excitation plane

The data was fitted to a sigmoidal growth logistic model using the following equation:

$$\mathbf{y = A2 + \left(\frac{(A1 - A2)}{(1 + (x/x0)} \right)^{p}}$$

where A1 refers to the initial value, A2 refers to the final value, x0 refers to the centre and p is a value relating to the slope of the transition. Errors refer the standard deviation of three titration repeats. An arbitrary weighting function with the following equation was employed:

$$\mathbf{w = \left(\frac{1}{st.\,dev.\,of\,y} \right)^{2}}$$

The fitting was carried out using OriginPro 7.5 software.

F-*MetC* was obtained as two complementary single stranded oligonucleotides from MWG:

5' TAG ACA TCC AGA CGT ATA 3' (with 5'-fluorescein)

5' TAT ACG TCT GGA TGT CTA 3'

The fluorescein-labelled anti-Met-box DNA was also obtained as two complementary strands from MWG:

5’ TCC GGC AGG CCG GCA GGA 3' (with 5'-fluorescein)

5’ TCC TGC CGG CCT GCC GGA 3’

A 2:1 ratio of unlabelled–labelled oligonucleotides were heated to 80 °C in a water-bath. The water-bath was switched off and the mixture was allowed to cool to room temperature.

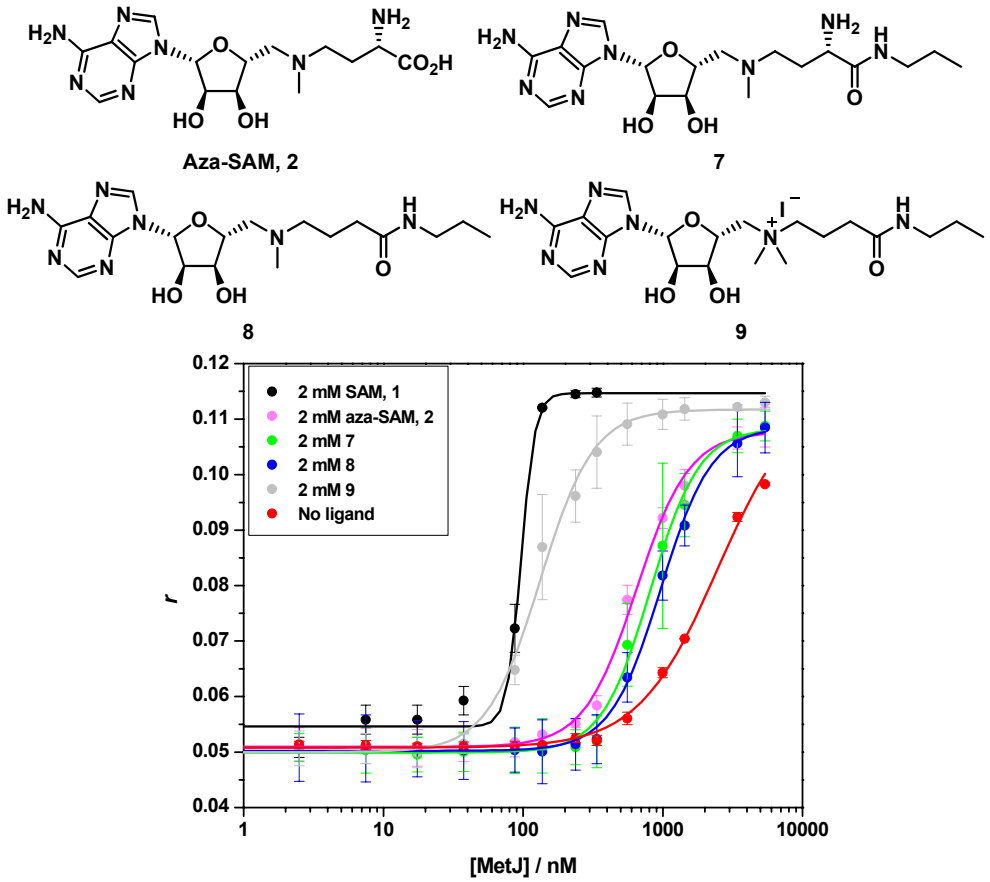


Figure S3: Increase in fluorescence anisotropy observed upon titration of MetJ into a solution of F-*MetC* (10 nM) with ligand (2 mM) in TK buffer pH 7.6: SAM 1 (black), aza-SAM 2 (magenta), 7 (green), 8 (blue), 9 (light grey), no ligand (red). Excitation was at 488 nm and emission was measured at 517 nm. Results shown are an average of three repeats. Data is normalised to a starting anisotropy value of 0.051. The data was fitted to a sigmoidal growth logistic model using the OriginPro 7.5 software as previously described.

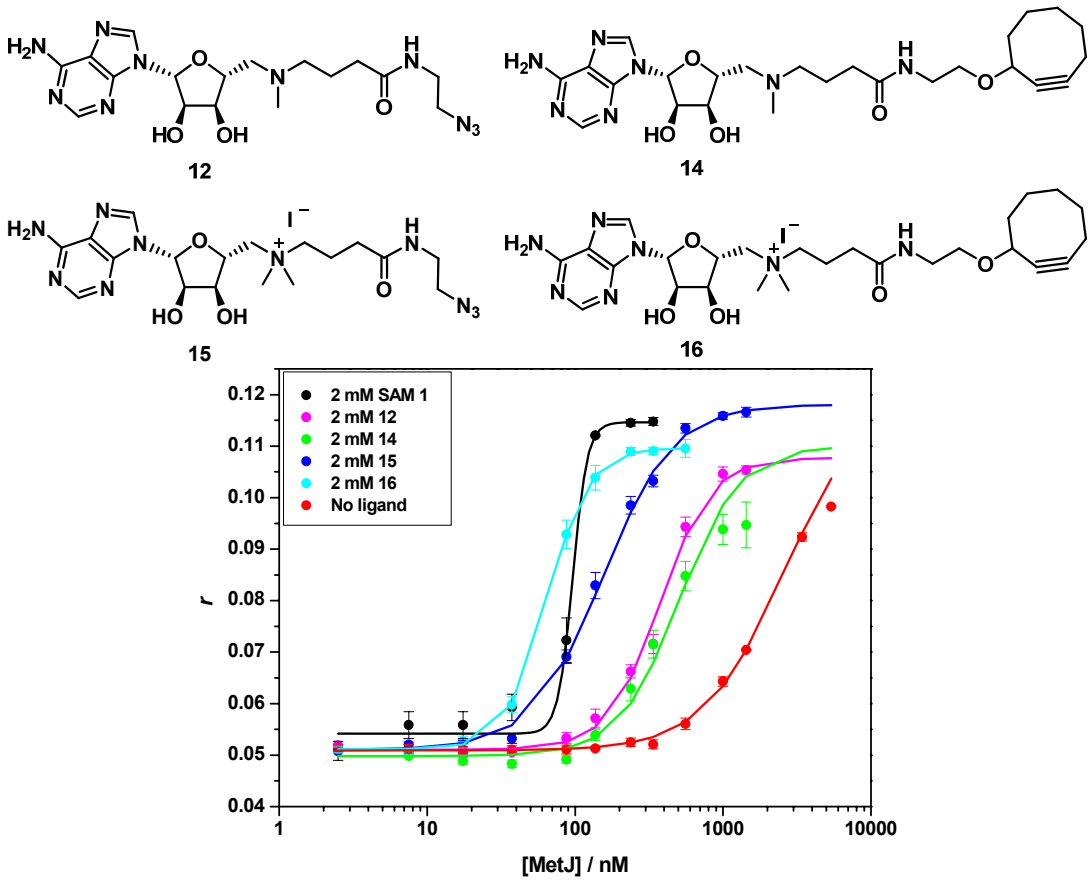


Figure S4: Increase in fluorescence anisotropy observed upon titration of MetJ into a solution of F-*MetC* (10 nM) with ligands (2 mM) in TK buffer pH 7.6. SAM 1 (black), 12 (magenta), 14 (green), 15 (blue), 16 (cyan) and no ligand (red). Excitation was at 488 nm and emission was measured at 517 nm. Results shown are an average of three repeats. Data is normalised to a starting anisotropy value of 0.051. The data was fitted to a sigmoidal growth logistic model using the OriginPro 7.5 software as previously described.

References

1. W. C. Still, M. Kahn and A. Mitra, *J. Org. Chem.*, 1978, **43**, 2923-2925.
2. M. J. Thompson, A. Mekhalfia, D. P. Hornby and G. M. Blackburn, *J. Org. Chem.*, 1999, **64**, 7467-7473.
3. C. J. Marasco, Jr., D. L. Kramer, J. Miller, C. W. Porter, C. J. Bacchi, D. Rattendi, L. Kucera, N. Iyer, R. Bernacki, P. Pera and J. R. Sufrin, *J. Med. Chem*, 2002, **45**, 5112-5122.
4. G. A. Olah, R. Karpeles and S. C. Narang, *Synthesis*, 1982, 963-965.
5. L. Skattebol and S. Solomon, *Organic Syntheses*, Wiley and Sons: New York, 1973.
6. F. W. Studier and B. A. Moffatt, *J. Mol. Biol.*, 1986, **189**, 113-130.
7. C. M. Johnson, A. Cooper and P. G. Stockley, *Biochemistry*, 1992, **31**, 9717-9724.