Supporting Information

The effect of MR1 ligand glyco-analogues on mucosal-associated invariant T (MAIT) cell activation

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Chemistry Experimental

General experimental: All reactions were performed open to air. Reactions were monitored by TLC analysis on Macherey-Nagel silica gel coated plastic sheets (0.20 mm with fluorescent indicator UV_{254}) via detection by UV absorption (254 nm) where relevant and dipping in 10% H_2SO_4 in EtOH followed by charring or dipping in a solution of $KMnO_4$ (0.05 M), K_2CO_3 (0.4 M), and NaOH (0.06%) in water. Column chromatography was performed using Pure Science silica gel (40–63 μm) and Agilent Bondesil C18 (40 μm). All solvents were removed by evaporation under reduced pressure. High resolution mass spectra (HRMS) were recorded on an Agilent 6530 Q-TOF mass spectrometer utilising a JetStream™ electro-spray ionisation (ESI) source in positive or negative mode. Optical rotations were recorded on an Autopol II (Rudolph Research Analytical) at 589 nm (sodium D line). Infrared (IR) spectra were recorded as thin films using either a Bruker Platinum-ATR spectrometer. Nuclear magnetic resonance spectra were obtained at 20 °C in DMSO-d_6 or D_2O using a Varian INOVA operating at 500 MHz. Chemical shifts are given in ppm (δ) relative to the solvent residual peak. NMR peak assignments were made using COSY, HSQC, and HMBC 2D experiments.

General procedure for the synthesis of glycitylamines: The sugar (1 mmol) was dissolved in a saturated solution of NH_4OAc in EtOH (20 mL/mmol), and $NaCNBH_3$ (188 mg, 3 equiv.) and 30% aq. NH_3 (8 mL/mmol) were added. The mixture was stirred at reflux for 24 h, cooled to room temperature, and concentrated *in vacuo*. The resulting residue was dissolved in H_2O, loaded on to a Dowex H^+ ion-exchange resin and washed several times with H_2O to remove excess salt. The amine product was then eluted with 30% aq. NH_3 and the eluent was concentrated. The residue was then subjected to silica gel flash column chromatography (DCM/EtOH/MeOH/NH_3 (aq), 15/2/2/1 → 5/2/2/1, v/v/v/v) to give the corresponding glycitylamine.

1-Amino-1-deoxy-D-ribitol (7a). The general procedure for the synthesis of glycitylamines was carried out on D-ribose to yield 1-amino-1-deoxy-D-ribitol **7a** (124 mg, 0.82 mmol, 82%). R_f = 0.01 (DCM/EtOH/ MeOH/NH_3 (aq) 5/2/2/1 v/v/v/v); $[\alpha]_D^{21}$ = - 6.0 (*c* = 1.0, H_2O); IR (film) 3240, 2935, 2495, 1456, 1041 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 3.95 (ddd, *J* = 3.1, 5.3, 8.8 Hz, 1H), 3.75 (dd, *J* = 3.0, 11.5 Hz, 1H), 3.70 (dd, *J* = 3.0, 6.6 Hz, 1H), 3.66 (dd, *J* = 5.2, 6.6 Hz, 1H), 3.60 (dd, *J* = 6.2, 11.6 Hz, 1H), 3.16 (dd, *J* = 3.4, 13.2 Hz, 1H), 2.97 (dd, *J* = 9.2, 13.2

Hz, 1H); ^{13}C NMR (125 MHz, D_2O) δ 72.7, 71.7, 67.8, 62.5, 40.9; HRMS (ESI): calcd. for $[C_5H_{14}NO_4]^+$: 152.0917, obsd. 152.0923.

1-Amino-1-deoxy-L-ribitol (7b). The general procedure for the synthesis of glycitylamines was carried out on L-ribose to yield 1-amino-1-deoxy-L-ribitol **7b** (121 mg, 0.80 mmol, 80%). R_f = 0.01 (DCM/EtOH/ MeOH/NH_3 (aq) 5/2/2/1 v/v/v/v); $[\alpha]_D^{21}$ = + 6.0 (*c* = 1.0, H_2O); IR (film) 3286, 2953, 2493, 1457, 1071 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 3.95 (ddd, *J* = 3.1, 5.3, 8.8 Hz, 1H), 3.75 (dd, *J* = 3.0, 11.5 Hz, 1H), 3.70 (dd, *J* = 3.0, 6.6 Hz, 1H), 3.66 (dd, *J* = 5.2, 6.6 Hz, 1H), 3.60 (dd, *J* = 6.2, 11.6 Hz, 1H), 3.16 (dd, *J* = 3.4, 13.2 Hz, 1H), 2.97 (dd, *J* = 9.2, 13.2 Hz, 1H); ^{13}C NMR (125 MHz, D_2O) δ 72.7, 71.7, 67.8, 62.5, 40.9; HRMS (ESI): calcd. for $[C_5H_{14}NO_4]^+$: 152.0917, obsd. 152.0918.

1-Amino-1,2-dideoxy-D-ribitol (7c). The general procedure for the synthesis of glycitylamines was carried out on 2-deoxy-D-ribose to yield 1-amino-1,2-dideoxy-D-ribitol **7c** (99 mg, 0.73 mmol, 73%). R_f = 0.02 (DCM/EtOH/ MeOH/NH_3 (aq) 5/2/2/1 v/v/v/v); $[\alpha]_D^{21}$ = - 4.0 (*c* = 1.0, H_2O); IR (film) 3316, 3037, 2932, 2507, 1463, 1030 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 3.78-3.66 (m, 2H), 3.65-3.53 (m, 2H), 3.22-3.06 (m, 2H), 2.04-1.92 (m, 1H), 1.84-1.71 (m, 1H); ^{13}C NMR (125 MHz, D_2O) δ 74.3, 69.6, 62.3, 37.2, 29.3; HRMS (ESI): calcd. for $[C_5H_{14}NO_3]^+$: 136.0974, obsd. 136.0972.

1-Amino-1-deoxy-D-arabinitol (7d). The general procedure for the synthesis of glycitylamines was carried out on D-arabinose to yield 1-amino-1-deoxy-D-arabinitol **7d** (119 mg, 0.79 mmol, 79%). R_f = 0.01 (DCM/EtOH/ MeOH/NH_3 (aq) 5/2/2/1 v/v/v/v); $[\alpha]_D^{21}$ = + 13.0 (*c* = 1.0, H_2O); IR (film) 3362, 3247, 2997, 2419, 1457, 1065 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 4.19-4.14 (m, 1H), 3.84, (dd, *J* = 2.8, 11.9 Hz, 1H), 3.77-3.71 (m, 1H), 3.67 (dd, *J* = 6.0, 11.6 Hz, 1H), 3.54 (dd, *J* = 1.9, 8.9 Hz, 1H), 3.18 (d, *J* = 6.9 Hz, 2H); ^{13}C NMR (125 MHz, D_2O) δ 71.0, 70.3, 66.2, 62.6, 42.4; HRMS (ESI): calcd. for $[C_5H_{14}NO_4]^+$: 152.0917, obsd. 152.0922.

1-Amino-1-deoxy-D-glucitol (7e). The general procedure for the synthesis of glycitylamines was carried out on D-glucose to yield 1-amino-1-deoxy-D-glucitol **7e** (140 mg, 0.77 mmol, 77%). R_f = 0.01 (DCM/EtOH/ MeOH/NH_3 (aq) 5/2/2/1 v/v/v/v); $[\alpha]_D^{21}$ = - 6.0 (*c* = 1.0, H_2O); IR (film) 3322, 2931, 2472, 1618, 1407, 1078, 1021 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 4.00-3.95 (m, 1H), 3.79-3.74 (m, 2H), 3.73-3.68 (m, 1H), 3.63-3.57 (m, 2H), 3.16 (dd, *J* = 3.3, 13.0 Hz, 1H), 3.01 (dd, *J* = 8.6, 13.0 Hz, 1H); ^{13}C NMR (125 MHz, D_2O) δ 70.8, 70.7, 70.5, 69.2, 62.6, 41.7; HRMS (ESI): calcd. for $[C_6H_{16}NO_5]^+$: 182.1023, obsd. 182.1035.

1-Amino-1-deoxy-D-xylitol (7f). The general procedure for the synthesis of glycitylamines was carried out on D-xylose to yield 1-Amino-1-deoxy-D-xylitol **7f** (122 mg, 0.81 mmol, 81%). R_f = 0.01 (DCM/EtOH/ MeOH/NH_3 (aq) 5/2/2/1 v/v/v/v); $[\alpha]_D^{23}$ = - 6.5 (*c* = 11, D_2O); IR (film) 3250, 2929, 1566, 1458, 1069, 1028 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 3.98-3.90 (m, 1H), 3.76-3.69 (m, 1H), 3.64 (dd, *J* = 11.8, 4.3 Hz, 1H), 3.61-3.51 (m, 2H), 3.19-3.11 (m, 1H), 3.10-3.02 (m, 1H); ^{13}C NMR (125 MHz, D_2O) δ 71.6, 71.5, 67.7, 62.3, 41.9; HRMS (ESI): calcd. for $[C_5H_{14}NO_4]^+$: 152.0917, obsd. 152.0919.

1-Amino-1-deoxy-L-lyxitol (7g). The general procedure for the synthesis of glycitylamines was carried out on L-lyxose to yield 1-Amino-1-deoxy-L-lyxitol **7g** (115 mg, 0.76 mmol, 76%). R_f = 0.01 (DCM/EtOH/ MeOH/NH_3 (aq) 5/2/2/1 v/v/v/v); $[\alpha]_D^{23}$ = +1.1 (*c* = 0.5, D_2O); IR (film) 3237, 2927, 1565, 1459, 1067, 1027 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 3.68 (ddd, *J* = 9.1, 7.9, 3.4 Hz, 1H), 3.64 (ddd, *J* = 7.6, 5.8, 2.1 Hz, 1H), 3.46-3.34 (m, 2H), 3.31 (dd, *J* = 8.0, 2.0 Hz, 1H), 3.11 (dd, *J* = 13.2, 3.4 Hz, 1H), 2.79 (dd, *J* = 13.2, 9.2 Hz, 1H); ^{13}C NMR (125 MHz, D_2O) δ 71.8, 69.8, 67.1, 62.7, 42.2; HRMS (ESI): calcd. for $[C_5H_{14}NO_4]^+$: 152.0917, obsd. 152.0926.

1-Amino-1-deoxy-D-allitol (7h). The general procedure for the synthesis of glycitylamines was carried out on D-allose to yield 1-Amino-1-deoxy-D-allitol **7h** (145 mg, 0.80 mmol, 80%). R_f = 0.01 (DCM/EtOH/ MeOH/NH_3 (aq) 5/2/2/1 v/v/v/v); $[\alpha]_D^{23}$ = - 2.9 (*c* = 5, D_2O); IR (film) 3283, 2939, 1617, 1400, 1030 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 4.01 (ddd, *J* = 8.7, 5.0, 3.3 Hz, 1H), 3.79-3.71 (m, 2H), 3.68 (dd, *J* = 11.9, 3.1 Hz, 1H), 3.64 (t, *J* = 6.2 Hz, 1H), 3.54 (dd, *J* = 11.9, 7.0 Hz, 1H), 3.18 (dd, *J* = 13.2, 3.2 Hz, 1H), 2.99 (dd, *J* = 13.2, 9.1 Hz, 1H); ^{13}C NMR (125 MHz, D_2O) δ 72.9, 72.1, 71.7, 67.6, 62.2, 41.0; HRMS (ESI): calcd. for $[C_6H_{16}NO_5]^+$: 182.1023, obsd. 182.1078.

6-Chloro-5-nitrouracil (9). 6-Chlorouracil (**8**) (2.00 g, 13.65 mmol) was dissolved in conc. H_2SO_4 (6 mL) and the mixture stirred at 0 °C. Fuming nitric acid (2 mL) was added dropwise with continuous stirring, at which point the solution turned yellow. The solution was stirred for a further 30 min at 0 °C, then poured carefully on to ice chips with vigorous stirring. The aqueous solution was extracted three times with EtOAc. The organic layers were combined and washed twice with H_2O, dried with $MgSO_4$, filtered and concentrated *in vacuo* to give uracil **9** (1.72 g, 8.98 mmol, 66%) as a light yellow solid. R_f = 0.4 (PE/EA, 1/1, v/v); IR (film)

3022, 2843, 1670, 1419, 1345, 1038 cm^{-1}; ^{13}C NMR (125 MHz, DMSO-d_6) δ 160.3, 150.8, 113.5; HRMS (ESI): calcd. for $[C_4HClN_3O_4]^-$: 189.9661, obsd. 189.9664.

General procedure for the synthesis of 5-nitro-6-glycitylaminouracils: To a solution of 6-chloro-5-nitrouracil (500 mg, 2.61 mmol) in EtOH (5 mL/mmol) was added a solution of glycitylamine (3.92 mmol, 1.5 equiv.) in H_2O (5 mL/mmol) and the resulting bright yellow solution maintained at pH 8 via the addition of 1 M NaOH. The solution was stirred at r.t. for 24 hours, after which time the EtOH was removed under reduced pressure. The resulting solution was loaded on to a Zerolit-FFIP $HCOO^-$ ion-exchange resin, washed with water to remove excess salt and then eluted with 1 M HCOOH. The eluent was concentrated *in vacuo* and the residue purified using isocratic (H_2O) C18 reverse-phase chromatography to afford the product as a light yellow amorphous solid.

5-Nitro-6-((D-ribityl)amino)uracil (10a). The general procedure for the synthesis of 5-nitro-6-glycitylaminouracils was carried out on **7a** to yield 5-nitro-6-((D-ribityl)amino)uracil **10a** (591 mg, 1.93 mmol, 74%). R_f = 0.6 (DCM/EtOH/ MeOH/NH_3 (aq) 5/2/2/1 v/v/v/v); $[\alpha]_D^{20}$ = + 16.0 (*c* = 0.1, DMSO); IR (film) 3410, 3178, 1696, 1682, 1405, 1035 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 4.15-4.08 (m, 1H), 3.88-3.62 (m, 6H); ^{13}C NMR (125 MHz, D_2O) δ 157.1, 152.6, 147.6, 107.8, 70.2, 70.0, 67.1, 60.2, 42.6; HRMS (ESI): calcd. for $[C_9H_{13}N_4O_8]^-$: 305.0739, obsd. 305.0773.

5-Nitro-6-((L-ribityl)amino)uracil (10b). The general procedure for the synthesis of 5-nitro-6-glycitylaminouracils was carried out on **7b** to yield 5-nitro-6-((L-ribityl)amino)uracil **10b** (605 mg, 1.98 mmol, 76%). R_f = 0.6 (DCM/EtOH/ MeOH/NH_3 (aq) 5/2/2/1 v/v/v/v); $[\alpha]_D^{20}$ = - 7.0 (*c* = 0.1, DMSO); IR (film) 3416, 3178, 2530, 1677, 1609, 1403, 1068 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 4.15-4.08 (m, 1H), 3.88-3.62 (m, 6H); ^{13}C NMR (125 MHz, D_2O) δ 157.1, 152.6, 147.6, 107.8, 70.2, 70.0, 67.1, 60.2, 42.6; HRMS (ESI): calcd. for $[C_9H_{13}N_4O_8]^-$: 305.0739, obsd. 305.0765.

5-Nitro-6-((2′-deoxy-D-ribityl)amino)uracil (10c). The general procedure for the synthesis of 5-nitro-6-glycitylaminouracils was carried out on **7c** to yield 6-((2′-deoxy-D-ribityl)amino)-5-nitrouracil **10c** (515 mg, 1.77 mmol, 68%). R_f = 0.7 (DCM/EtOH/ MeOH/NH_3 (aq) 5/2/2/1 v/v/v/v); $[\alpha]_D^{20}$ = + 19.0 (*c* = 0.1, DMSO); IR (film) 3436, 3181, 2974, 1706, 1671, 1628, 1408, 1220 1040 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 3.77-3.45 (m, 6H), 2.10-1.97 (m, 1H), 1.84-1.72

(m, 1H); ^{13}C NMR (125 MHz, D_2O) δ 162.0, 160.0, 157.2, 111.1, 74.8, 70.0, 62.8, 38.7, 31.4; HRMS (ESI): calcd. for $[C_9H_{13}N_4O_7]^-$: 289.0790, obsd. 289.0787.

5-Nitro-6-((D-arabinityl)amino)uracil (10d). The general procedure for the synthesis of 5-nitro-6-glycitylaminouracils was carried out on **7d** to yield 6-(D-arabinityl)amino-5-nitrouracil **10d** (573 mg, 1.87 mmol, 72%). R_f = 0.6 (DCM/EtOH/ MeOH/NH_3 (aq) 5/2/2/1 v/v/v/v); $[\alpha]_D^{20}$ = - 2.0 (*c* = 0.1, DMSO); IR (film) 3302, 3013, 2826, 1696, 1620, 1213, 1048 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 4.27-4.22 (m, 1H), 3.85 (dd, *J* = 2.8, 11.8 Hz, 1H), 3.79-3.71 (m, 3H), 3.67 (dd, *J* = 6.0, 11.9 Hz, 1H), 3.59 (dd, *J* = 1.8, 8.9 Hz, 1H); ^{13}C NMR (125 MHz, D_2O) δ 159.3, 154.4, 149.3, 110.0, 71.2, 70.7, 67.6, 62.8, 46.2; HRMS (ESI): calcd. for $[C_9H_{13}N_4O_8]^-$: 305.0739, obsd. 305.0739.

5-Nitro-6-((D-glucityl)amino)uracil (10e). The general procedure for the synthesis of 5-nitro-6-glycitylaminouracils was carried out on **7e** to yield 5-nitro-6-((D-glucityl)amino)uracil **10e** (658 mg, 1.96 mmol, 75%). R_f = 0.6 (DCM/EtOH/ MeOH/NH_3 (aq) 5/2/2/1 v/v/v/v); $[\alpha]_D^{20}$ = + 11.0 (*c* = 0.1, DMSO); IR (film) 3273, 2938, 2433, 1671, 1626, 1421, 1038 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 4.09-4.03 (m, 1H), 3.81 (dd, *J* = 2.7, 5.0 Hz, 1H), 3.74 (dd, *J* = 2.7, 11.8 Hz, 1H), 3.73-3.67 (m, 2H), 3.63 (dd, *J* = 2.6, 8.0 Hz, 1H), 3.61-3.55 (m, 2H,); ^{13}C NMR (125 MHz, D_2O) δ 159.2, 154.3, 149.0, 110.2, 71.4, 71.2, 70.6, 70.5, 62.9, 46.0; HRMS (ESI): calcd. for $[C_{10}H_{15}N_4O_9]^-$: 335.0845, obsd. 335.0896.

5-Nitro-6-((D-xylityl)amino)uracil (10f). The general procedure for the synthesis of 5-nitro-6-glycitylaminouracils was carried out on **7f** to yield 5-nitro-6-((D-xylityl)amino)uracil **10f** (615 mg, 2.00 mmol, 77%). R_f = 0.6 (DCM/EtOH/ MeOH/NH_3 (aq) 5/2/2/1 v/v/v/v); $[\alpha]_D^{23}$ = + 6.2 (*c* = 0.4, DMSO); IR (film) 3359, 2892, 1680, 1617, 1419, 1067 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 3.99 (dt, *J* = 8.1, 4.4 Hz, 1H), 3.86 (dt, *J* = 6.7, 4.5 Hz, 1H), 3.77 (dd, *J* = 13.9, 4.7 Hz, 1H), 3.72 (dd, *J* = 11.8, 4.1 Hz, 1H), 3.68-3.60 (m, 2H), 3.55 (dd, *J* = 13.9, 7.7 Hz, 1H); ^{13}C NMR (125 MHz, D_2O) δ 162.0, 160.6, 157.9, 111.2, 72.1, 71.5, 70.1, 62.6, 44.0, 43.9; HRMS (ESI): calcd. For $[C_9H_{13}N_4O_8]^-$: 305.0739, obsd. 305.0786.

5-Nitro-6-((L-lyxityl)amino)uracil (10g). The general procedure for the synthesis of 5-nitro-6-glycitylaminouracils was carried out on **7g** to yield 5-nitro-6-((L-lyxityl)amino)uracil **10g** (585 mg, 1.91 mmol, 73%). R_f = 0.6 (DCM/EtOH/ MeOH/NH_3 (aq) 5/2/2/1 v/v/v/v); $[\alpha]_D^{23}$ = + 16.5 (*c* = 0.4, DMSO); IR (film) 3388, 3195, 2971, 1685, 1626, 1424, 1023 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 3.98-3.90 (m, 2H), 3.83 (dd, *J* = 14.3, 3.3 Hz, 1H), 3.73 (dd, *J* = 14.4, 6.0 Hz, 1H), 3.67 (d, *J* = 6.3 Hz, 2H), 3.51 (dd, *J* = 8.7, 2.1 Hz, 1H); ^{13}C NMR (125 MHz, D_2O) δ

162.2, 160.9, 157.9, 111.3, 71.1, 70.2, 69.3, 63.1, 44.0; HRMS (ESI): calcd. For $[C_9H_{13}N_4O_8]^-$: 305.0739, obsd. 305.0748.

5-Nitro-6-((D-allityl)amino)uracil (10h). The general procedure for the synthesis of 5-nitro-6-glycitylaminouracils was carried out on **7h** to yield 5-nitro-6-((D-allityl)amino)uracil **10h** (606 mg, 1.80 mmol, 69%). R_f = 0.6 (DCM/EtOH/ MeOH/NH_3 (aq) 5/2/2/1 v/v/v/v); $[\alpha]_D^{23}$ = + 7.8 (*c* = 1, DMSO); IR (film) 3300, 3006, 1649, 1537, 1405, 1043 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 4.23-4.14 (m, 1H), 3.96-3.75 (m, 6H), 3.71 (dd, *J* = 14.4, 7.2 Hz, 1H), 3.65 (dd, *J* = 12.0, 6.9 Hz, 1H); ^{13}C NMR (125 MHz, D_2O) δ 159.2, 154.3, 149.1, 109.7, 72.5, 72.3, 72.1, 69.2, 62.3, 45.0; HRMS (ESI): calcd. For $[C_{10}H_{15}N_4O_9]^-$: 335.0845, obsd. 335.0847.

*Synthesis of lumazines **2** and **11**:* To a solution of **10a** (50 mg, 0.15 mmol) in 10% AcOH/H_2O (v/v) (20 mL/mmol), was added Fe powder (42 mg, 0.75 mmol) and the mixture was stirred at 90 °C for 2 h, after which time TLC analysis (DCM/EtOH/MeOH/NH_3 (aq), 5/2/2/1, v/v/v/v) indicated complete consumption of the starting material and formation of the diaminouracil intermediate. The mixture was cooled to r.t. and butane-2,3-dione (40 µL, 0.45 mmol) was added. The mixture was then heated to 80 °C for 3 h, after which time all the intermediate had reacted as indicated by TLC. The reaction was cooled to r.t. and concentrated under reduced pressure. The resulting residue was purified via silica gel flash column chromatography (MeOH/DCM 1/19 → 1/5, v/v/v/v), followed by isocratic (H_2O) C18 reverse-phase chromatography to afford **2** (19 mg, 0.06 mmol, 39%) and **11** (10 mg, 0.03 mmol, 26%) as yellow oils.

D-Ribityl lumazine 6,7-diMe (2). R_f = 0.5 (MeOH/DCM 1/5 v/v); $[\alpha]_D^{20}$ = + 11.0 (*c* = 0.1, H_2O); IR (film) 3236, 1632, 1529, 1038 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 4.98 (d, *J* = 13.0 Hz, 1H), 4.80-4.68 (m, 1H), 4.43-4.34 (m, 1H), 3.91 (ddd, *J* = 3.3, 7.5, 14.0 Hz, 1H), 3.88-3.81 (m, 2H), 3.69 (dd, *J* = 6.9, 11.9 Hz, 1H), 2.87 (s, 3H), 2.65 (s, 3H); ^{13}C NMR (125 MHz, D_2O) δ 162.9, 157.8, 152.3, 150.4, 145.1, 130.7, 73.3, 71.9, 69.0, 62.5, 51.3, 21.4, 18.2; HRMS (ESI): calcd. for $[C_{13}H_{19}N_4O_6]^+$: 327.1299, obsd. 327.1246. Characterisation data matched those reported in literature.[1-2]

Reduced D-ribityl lumazine 6,7-diMe (11). This compound was isolated as a mixture of diastereomers. This compound was kept under an Ar atmosphere at all times, as it rapidly oxidises in air to form lumazine **2**. R_f = 0.4 (MeOH/DCM 1/5 v/v); ^1H NMR (500 MHz, D_2O) δ 4.27-4.17 (m, 1H), 4.07-3.98 (m, 1H), 3.87-3.52 (m, 5H), 2.08 (s, 3H), 1.21 (d, *J* = 6.5 Hz,

3H); HRMS (ESI): calcd. for $[C_{13}H_{21}N_4O_6]^+$: 329.1456, obsd. 329.1438. Characterisation data matched those reported in literature.[1]

General procedure for the synthesis of 6-Me-7-OH lumazines: To a solution of 5-nitro-6-glycitylaminouracil in 10% AcOH/H_2O (v/v) (20 mL/mmol), was added Fe powder (5 equiv.) and the mixture stirred at 90 °C for 2 h, after which time TLC analysis (DCM/EtOH/MeOH/NH_3 (aq), 5/2/2/1, v/v/v/v) indicated complete consumption of the starting material and formation of the diaminouracil intermediate. The mixture was cooled to room temp. and sodium pyruvate (20 equiv.) was added. The mixture was the heated to 80 °C for 3 hours, after which all the intermediate had reacted as indicated by TLC. The reaction was cooled to room temp. and concentrated under reduced pressure. The resulting residue was purified further by using silica gel flash chromatography (DCM/EtOH/MeOH/NH_3 (aq), 15/2/2/1 → 5/2/2/1, v/v/v/v), followed by isocratic (H_2O) C18 reversed-phase chromatography to afford the lumazine product.

D-Ribityl lumazine 6-Me-7-OH (3a). The general procedure for the synthesis of 6-Me-7-OH lumazines was carried out on **10a** (50 mg, 0.15 mmol) to yield **3a** (19 mg, 0.06 mmol, 40%). R_f = 0.6 (DCM/EtOH/ MeOH/NH_3 (aq) 5/2/2/1 v/v/v/v); $[\alpha]_D^{20}$ = + 9.0 (*c* = 0.1, H_2O); IR (film) 3233, 1659, 1630, 1528, 1050 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 4.53 (dd, *J* = 8.2, 13.7 Hz, 1H), 4.36 (dd, *J* = 2.7, 13.7 Hz, 1H), 4.18-4.12 (m, 1H), 3.86 (ddd, *J* = 2.7, 7.1, 13.1 Hz, 1H), 3.80 (dd, *J* = 3.1, 11.9 Hz, 1H), 3.70 (t, *J* = 5.6 Hz, 1H), 3.65 (dd, *J* = 7.1, 12.1 Hz, 1H), 2.31 (s, 3H); ^{13}C NMR (125 MHz, D_2O) δ 164.1, 159.2, 158.4, 152.3, 146.7, 110.4, 73.2, 72.0, 69.9, 62.4, 43.3, 19.1; HRMS (ESI): calcd. for $[C_{12}H_{17}N_4O_7]^+$: 329.1092, obsd. 329.1095.

L-Ribityl lumazine 6-Me-7-OH (3b). The general procedure for the synthesis of 6-Me-7-OH lumazines was carried out on **10b** (50 mg, 0.15 mmol) to yield **3b** (17 mg, 0.05 mmol, 35%). R_f = 0.6 (DCM/EtOH/ MeOH/NH_3 (aq) 5/2/2/1 v/v/v/v)); $[\alpha]_D^{20}$ = - 2.0 (*c* = 0.1, H_2O); IR (film) 3234, 1629, 1597, 1527, 1051 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 4.53 (dd, *J* = 8.2, 13.7 Hz, 1H), 4.36 (dd, *J* = 2.7, 13.7 Hz, 1H), 4.18-4.12 (m, 1H), 3.86 (ddd, *J* = 2.7, 7.1, 13.1 Hz, 1H), 3.80 (dd, *J* = 3.1, 11.9 Hz, 1H), 3.70 (t, *J* = 5.6 Hz, 1H), 3.65 (dd, *J* = 7.1, 12.1 Hz, 1H), 2.31 (s, 3H); ^{13}C NMR (125 MHz, D_2O) δ 164.1, 159.2, 158.4, 152.3, 146.7, 110.4, 73.2, 72.0, 69.9, 62.4, 43.3, 19.1; HRMS (ESI): calcd. for $[C_{12}H_{17}N_4O_7]^+$: 329.1092, obsd. 329.1099.

2-Deoxy-D-ribityl lumazine 6-Me-7-OH (3c). The general procedure for the synthesis of 6-Me-7-OH lumazines was carried out on **10c** (50 mg, 0.17 mmol) to yield **3c** (14 mg, 0.04 mmol,

27%). R_f = 0.7 (DCM/EtOH/ MeOH/NH_3 (aq) 5/2/2/1 v/v/v/v)); $[\alpha]_D^{20}$ = + 2.0 (*c* = 0.1, H_2O); IR (film) 3232, 1625, 1526, 1063 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 4.34 (dd, *J* = 6.2, 7.8 Hz, 2H), 3.71 (dd, *J* = 2.7, 11.5 Hz, 1H), 3.63-3.51 (m, 3H), 2.33 (s, 3H), 2.07-1.97 (m, 1H), 1.83-1.72 (m, 1H); ^{13}C NMR (125 MHz, D_2O) δ 164.1, 158.9, 158.6, 152.0, 146.7, 110.6, 74.3, 69.1, 62.5, 38.6, 29.7, 19.1; HRMS (ESI): calcd. for $[C_{12}H_{15}N_4O_6]^-$: 311.0997, obsd. 311.0943.

D-Arabinityl lumazine 6-Me-7-OH (3d). The general procedure for the synthesis of 6-Me-7-OH lumazines was carried out on **10d** (100 mg, 0.30 mmol) to yield **3d** (36 mg, 0.11 mmol, 37%). R_f = 0.6 (DCM/EtOH/ MeOH/NH_3 (aq) 5/2/2/1 v/v/v/v)); $[\alpha]_D^{20}$ = - 3.0 (*c* = 0.1, H_2O); IR (film) 3127, 1599, 1527, 1050 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 4.46 (dd, *J* =7.4, 13.4 Hz, 1H), 4.33 (dd, *J* = 6.7, 13.4 Hz, 1H), 4.24 (t, *J* = 6.7 Hz, 1H), 3.82 (dd, *J* = 2.7, 12.0 Hz, 1H), 3.78-3.72 (m, 1H), 3.61 (dd, *J* = 6.0, 12.0 Hz, 1H), 3.44 (d, *J* = 9.1 Hz, 1H), 2.33 (s, 3H); ^{13}C NMR (125 MHz, D_2O) δ 164.1, 159.1, 158.6, 152.3, 146.9, 110.5, 70.4, 70.1, 67.0, 63.0, 43.7, 18.9; HRMS (ESI): calcd. for $[C_{12}H_{15}N_4O_7]^-$: 327.0946, obsd. 327.0939.

D-Glucityl lumazine 6-Me-7-OH (3e). The general procedure for the synthesis of 6-Me-7-OH lumazines was carried out on **10e** (100 mg, 0.28 mmol) to yield **3e** (35 mg, 0.10 mmol, 35%). R_f = 0.5 (DCM/EtOH/ MeOH/NH_3 (aq) 5/2/2/1 v/v/v/v)); $[\alpha]_D^{20}$ = + 11.0 (*c* = 0.1, H_2O); IR (film) 3245, 1630, 1527, 1057 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 4.44 (dd, *J* = 9.2, 13.4, 1H), 4.28 (dd, *J* = 5.7, 13.8 Hz, 1H), 4.18-4.11 (m, 1H), 3.82-3.70 (m, 4H), 3.62 (dd, *J* = 5.7, 12.3 Hz, 1H), 2.31 (s, 3H); ^{13}C NMR (125 MHz, D_2O) δ 164.0, 159.0, 158.5, 152.2, 146.7, 110.4, 71.6, 70.8, 70.0, 69.8, 62.5, 43.8, 19.1; HRMS (ESI): calcd. for $[C_{13}H_{19}N_4O_8]^+$: 359.1197, obsd. 359.1186.

General procedure for the synthesis of 5-amino-6-glycitylaminouracils: To a solution of 5-nitro-6-glycitylaminouracil in H_2O (12.5 mL/mmol) were added 2 drops of 2M KOH followed by $Na_2S_2O_4$ (4 equiv.) and the resulting solution was stirred at r.t. for 2 h until HRMS analysis showed the complete consumption of starting material. The reaction mixture was directly subjected to C18 reversed-phase column chromatography to afford fractions containing the product, which were acidified with 1M HCl and lyophilised to give the pure product as a pink amorphous solid.

5-Amino-6-((D-ribityl)amino)uracil (12a). The general procedure for the synthesis of 5-amino-6-glycitylaminouracils was carried out on **10a** (50 mg, 0.16 mmol) to yield 5-nitro-6-

((D-ribityl)amino)uracil **12a** (31 mg, 71%). $[\alpha]_D^{25}$ = + 10.2 (*c* = 1.0, H_2O); IR (film) 3208, 2948, 1706, 1624, 1183, 1047 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 4.01-3.93 (m, 1H), 3.88-3.75 (m, 2H), 3.72 (t, *J* = 6.2 Hz, 1H), 3.69-3.58 (m, 2H), 3.51 (dd, *J* = 14.8, 7.3 Hz, 1H); ^{13}C NMR (125 MHz, D_2O) δ 160.9, 150.8, 150.3, 82.6, 72.2, 72.1, 70.4, 62.3, 44.6; HRMS (ESI): calcd. for $[C_9H_{15}N_4O_6]^-$: 275.0997, obsd. 275.0998.

5-Amino-6-((L-ribityl)amino)uracil (12b). The general procedure for the synthesis of 5-amino-6-glycitylaminouracils was carried out on **10b** (50 mg, 0.16 mmol) to yield 5-nitro-6-((L-ribityl)amino)uracil **12b** (30 mg, 67%). $[\alpha]_D^{25}$ = - 9.1 (*c* = 1.0, H_2O); IR (film) 3269, 2940, 1705, 1636, 1179, 1055 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 4.01-3.94 (m, 1H), 3.85-3.74 (m, 2H), 3.70 (t, *J* = 6.1 Hz, 1H), 3.68-3.59 (m, 2H), 3.53 (dd, *J* = 14.7, 7.6 Hz, 1H); ^{13}C NMR (125 MHz, D_2O) δ 160.9, 150.8, 150.4, 82.6, 72.2, 72.0, 70.3, 62.3, 44.5; HRMS (ESI): calcd. for $[C_9H_{15}N_4O_6]^-$: 275.0997, obsd. 275.0976.

5-Amino-6-((2′-deoxy-D-ribityl)amino)uracil (12c). The general procedure for the synthesis of 5-amino-6-glycitylaminouracils was carried out on **10c** (50 mg, 0.17 mmol) to yield 5-nitro-6-((2′-deoxy-D-ribityl)amino)uracil **12c** (26 mg, 58%). $[\alpha]_D^{25}$ = + 12.2 (*c* = 1.0, H_2O); IR (film) 3279, 2930, 1718, 1644, 1190, 1056 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 3.76-3.62 (m, 2H), 3.63-3.50 (m, 2H), 3.49-3.44 (m, 2H), 2.01-1.89 (m, 1H), 1.75-1.63 (m, 1H); ^{13}C NMR (125 MHz, D_2O) δ 160.8, 150.9, 149.9, 74.4, 68.7, 62.3, 38.9, 31.0; HRMS (ESI): calcd. for $[C_9H_{15}N_4O_5]^-$: 259.1048, obsd. 259.1052.

5-Amino-6-((D-arabinityl)amino)uracil (12d). The general procedure for the synthesis of 5-amino-6-glycitylaminouracils was carried out on **10d** (50 mg, 0.16 mmol) to yield 5-nitro-6-((D-arabinityl)amino)uracil **12d** (28 mg, 63%). $[\alpha]_D^{25}$ = - 12.0 (*c* = 0.1, H_2O); IR (film) 3203, 2941, 1703, 1635, 1175, 1068 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 4.05 (td, *J* = 6.4, 1.9 Hz, 1H), 3.83 (dd, *J* = 11.9, 3.0 Hz, 1H), 3.78-3.71 (m, 1H), 3.68-3.61 (m, 1H), 3.57-3.47 (m, 3H); ^{13}C NMR (125 MHz, D_2O) δ 160.8, 150.8, 150.1, 82.5, 70.7, 70.5, 68.5, 62.7, 45.4; HRMS (ESI): calcd. for $[C_9H_{15}N_4O_6]^-$: 275.0997, obsd. 275.0989.

5-Amino-6-((D-glucityl)amino)uracil (12e). The general procedure for the synthesis of 5-amino-6-glycitylaminouracils was carried out on **10e** (50 mg, 0.15 mmol) to yield 5-nitro-6-((D-glucityl)amino)uracil **12e** (31 mg, 68%). $[\alpha]_D^{25}$ = + 5.6 (*c* = 0.1, H_2O); IR (film) 3231, 2960, 1708, 1642, 1167, 1053 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 4.10-4.00 (m, 1H), 3.93-3.76 (m, 3H), 3.76-3.61 (m, 3H), 3.56 (ddd, *J* = 14.7, 7.7, 2.4 Hz, 1H); ^{13}C NMR (125 MHz, D_2O) δ

161.0, 150.9, 150.4, 83.0, 71.3, 71.0, 70.9, 69.9, 62.7, 45.1; HRMS (ESI): calcd. for $[C_{10}H_{17}N_4O_7]^-$: 305.1103, obsd. 305.1108.

5-Amino-6-((D-xylityl)amino)uracil (12f). The general procedure for the synthesis of 5-amino-6-glycitylaminouracils was carried out on **10f** (50 mg, 0.16 mmol) to yield 5-nitro-6-((D-xylityl)amino)uracil **12f** (28 mg, 63%). $[\alpha]_D^{25}$ = + 9.6 (*c* = 1.0, H_2O); IR (film) 3299, 2941, 1703, 1629, 1178, 1056 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 3.98 (dt, *J* = 7.9, 4.0 Hz, 1H), 3.83 (dt, *J* = 6.5, 4.5 Hz, 1H), 3.72 (dd, *J* = 11.8, 4.3 Hz, 1H), 3.69-3.49 (m, 5H); ^{13}C NMR (125 MHz, D_2O) δ 161.0, 150.8, 150.8, 150.3, 82.6, 71.8, 71.1, 70.2, 62.4, 45.2; HRMS (ESI): calcd. for $[C_9H_{15}N_4O_6]^-$: 275.0997, obsd. 275.0990.

5-Amino-6-((L-lyxityl)amino)uracil (12g). The general procedure for the synthesis of 5-amino-6-glycitylaminouracils was carried out on **10g** (50 mg, 0.16 mmol) to yield 5-nitro-6-((L-lyxityl)amino)uracil **12g** (27 mg, 60%). $[\alpha]_D^{25}$ = + 14.5 (*c* = 0.1, H_2O); IR (film) 3189, 3002, 1701, 1624, 1182, 1050 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 4.08-3.96 (m, 2H), 3.83-3.71 (m, 3H), 3.70-3.58 (m, 2H); ^{13}C NMR (125 MHz, D_2O) δ 161.9, 151.4, 150.6, 92.8, 71.0, 70.1, 69.8, 63.2, 45.0; HRMS (ESI): calcd. for $[C_9H_{15}N_4O_6]^-$: 275.0997, obsd. 275.0995.

5-Amino-6-((D-allityl)amino)uracil (12h). The general procedure for the synthesis of 5-amino-6-glycitylaminouracils was carried out on **10h** (50 mg, 0.15 mmol) to yield 5-nitro-6-((D-allityl)amino)uracil **12h** (28 mg, 61%). $[\alpha]_D^{25}$ = + 21.1 (*c* = 0.1, H_2O); IR (film) 3211, 2937, 1705, 1643, 1176, 1048 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 4.06 (ddd, *J* = 8.0, 5.7, 2.7 Hz, 1H), 3.89 (td, *J* = 6.6, 3.2 Hz, 1H), 3.84 (t, *J* = 5.8 Hz, 1H), 3.82-3.76 (m, 2H), 3.69-3.62 (m, 2H), 3.54 (dd, *J* = 14.8, 7.6 Hz, 1H); ^{13}C NMR (125 MHz, D_2O) δ 160.9, 150.9, 150.4, 82.6, 72.4, 72.2, 72.2, 70.3, 62.3, 44.7; HRMS (ESI): calcd. for $[C_{10}H_{17}N_4O_7]^-$: 305.1103, obsd. 305.1105.

Computational Procedures

Molecular docking simulations were carried out using GOLD (v 5.7.1). Before docking, RL-6-Me-7-OH and 5-OP-RU were re-docked into the MR1-MAIT crystal structures 4L4V and 4PJ7 from PDB, respectively, to reproduce the binding pose from the crystal structure and to identify scoring functions best able to predict the crystal pose (data not shown). Docked ligand poses were scored with Chemscore in GOLD. Molecular graphics and analyses were performed using the UCSF Chimera package.[3] Chimera is developed by the Resource for Biocomputing,

Visualization, and Informatics at the University of California, San Francisco (supported by NIGMS Grant P41-GM103311).[4]

Biology Experimental

General experimental: Ac-6-FP and methylglyoxal were purchased from Schircks Laboratory and Sigma, respectively. 5OPRU and its analogues were freshly prepared before each experiment by combining 10 mM of 5-A-RU or analogues with 40 mM of methylglyoxal. For all experiments, cells were analysed using an ACEA Biosciences NovoCyte flow cytometer. The data were analysed using FlowJo software version 10. One-way ANOVA was used for all statistical analysis (Prism 8.1.1).

Cell lines: The MAIT cell line 6C2 was generated by retroviral gene transduction of MAIT αβTCRs into the murine thymoma TG40, as described previously.[5] MAIT αβTCR sequences were kindly provided by Prof. Olivier Lantz (Institut Curie, France). A murine MR1-overexpressing NIH.cl9 cell line was established by retroviral gene transduction of murine MR1 to the previously described NiH3T3 fibroblast cell line.[6] For tetramer generation, soluble biotinylated mouse MR1β_2m-complexes were produced as previously descirbed.[7] Biotinylated MR1 (1 mg/mL, 0.02 mM, 10 μL) was then tetramerised after adding a molar ratio of 1:5 streptavidin-APC (1 mg/mL, 3 nM, 12.5 μL) to MR1.

MR1 tetramer assay: The compounds (5 mM) were mixed with APC-conjugated mouse MR1 tetramers at a concentration of 10% (v/v) (>10,000 fold excess) and incubated overnight at 4 °C. 6C2 cells and TG40 cells (10^5 cells/mL) were then stained with the tetramer complex for 45 mins at room temperature before staining with PE-conjugated anti-mouse CD3 (Biolegend) and 7AAD (Biolegend), followed by flow cytometric analysis.

MAIT cell activation and inhibition assay: NIH.cl9 cells (10^5 cells/mL) were cultured in RPMI-1640 (without folic acid and supplemented with 10% fetal calf serum) and stimulated with the indicated concentration of ligands at 37 °C/5% CO_2 for 1 hr, after which time 6C2 cells (5 x 10^5 cells/mL) were added to the wells and incubated at 37 °C/5% CO_2 for 24 hours. For inhibition studies, NIH.cl9 cells (10^5 cells/mL) were stimulated with the indicated concentration of ligands at 37 °C/5% CO_2 for 1 hr, after which time 5-OP-RU (10 μM) and 6C2 cells (5 x 10^5 cells/mL) were added to the wells and incubated at 37 °C/5% CO_2 for 24

hours. Cells were then stained with PE-conjugated anti-mouse CD137 (Biolegend), APC-conjugated anti-mouse TCRβ (Biolegend) and 7AAD, followed by flow cytometric analysis.

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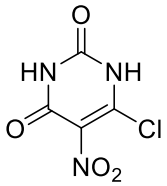
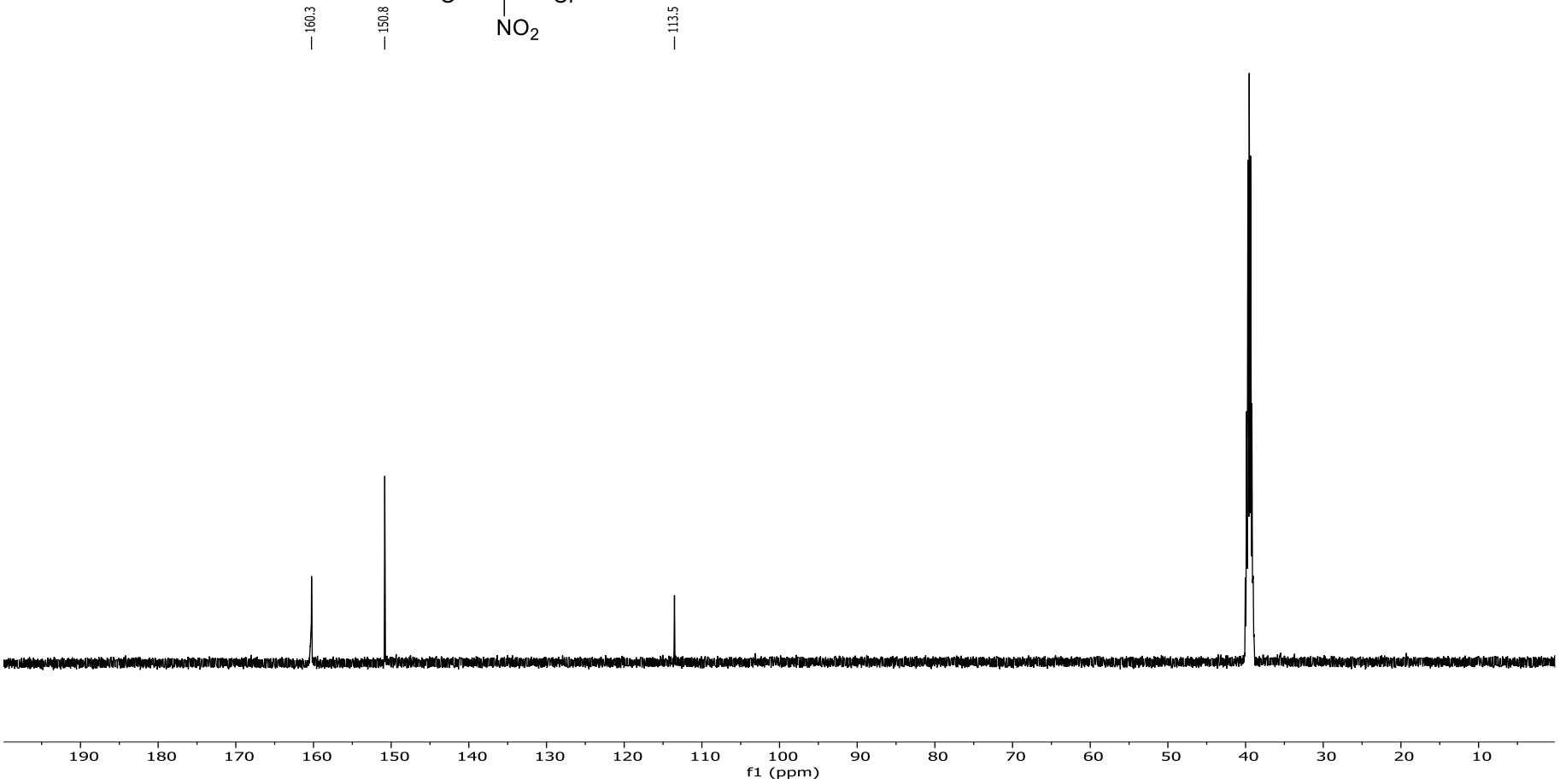
NMR Spectra

5-nitro-6-chlorouracil (9)

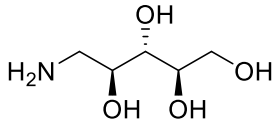
^{13}C NMR (DMSO-d_6, 125 MHz)

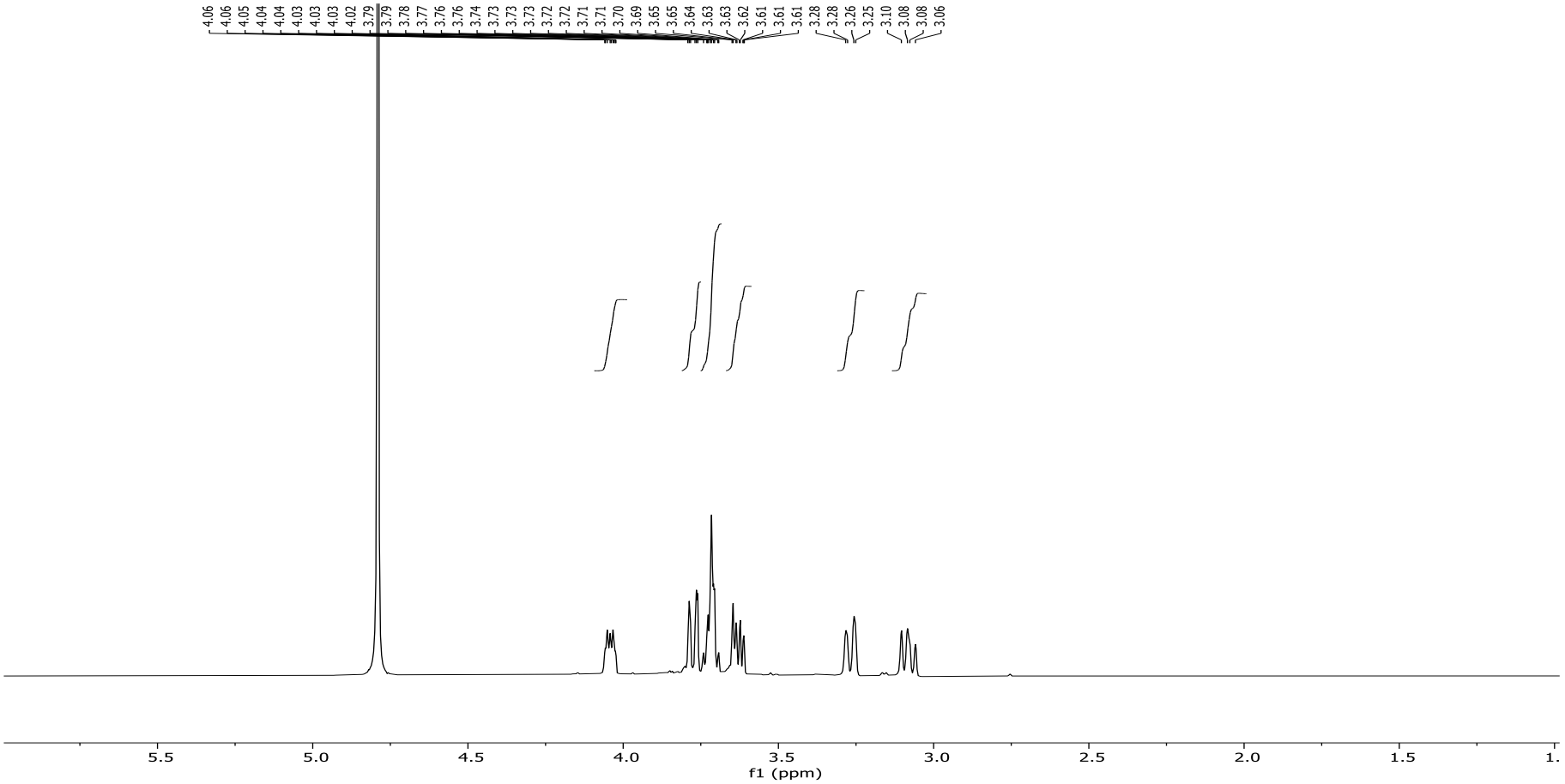




1-Amino-1-deoxy-D-ribitol (7a)

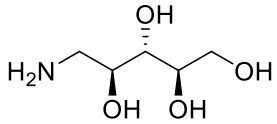
^{1}H NMR (D_2O, 500 MHz)

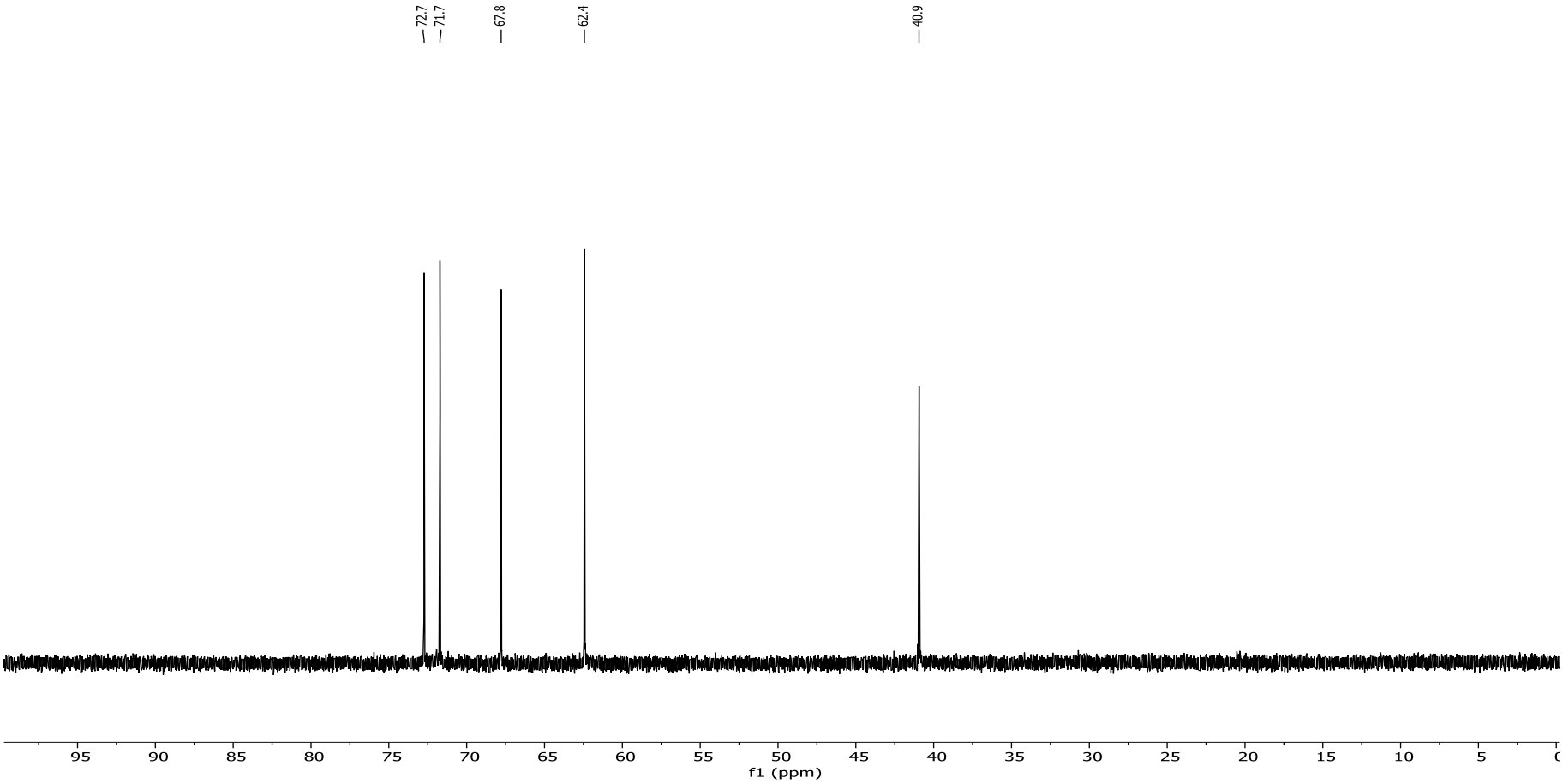




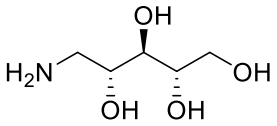
1-Amino-1-deoxy-D-ribitol (7a)

^{1}H NMR (D_2O, 500 MHz)

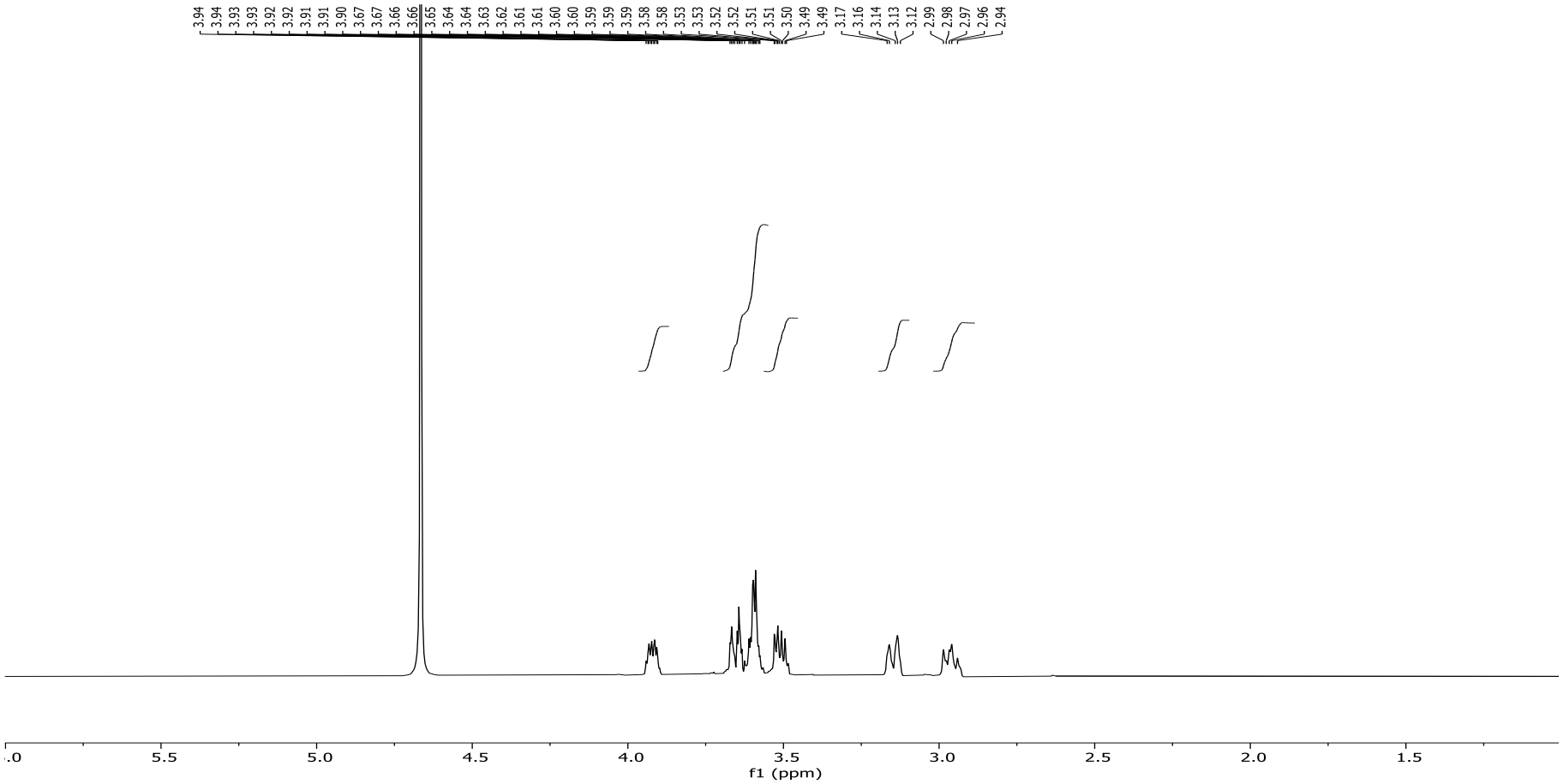




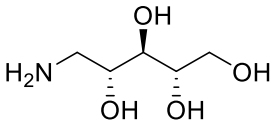
1-Amino-1-deoxy-L-ribitol (7b)



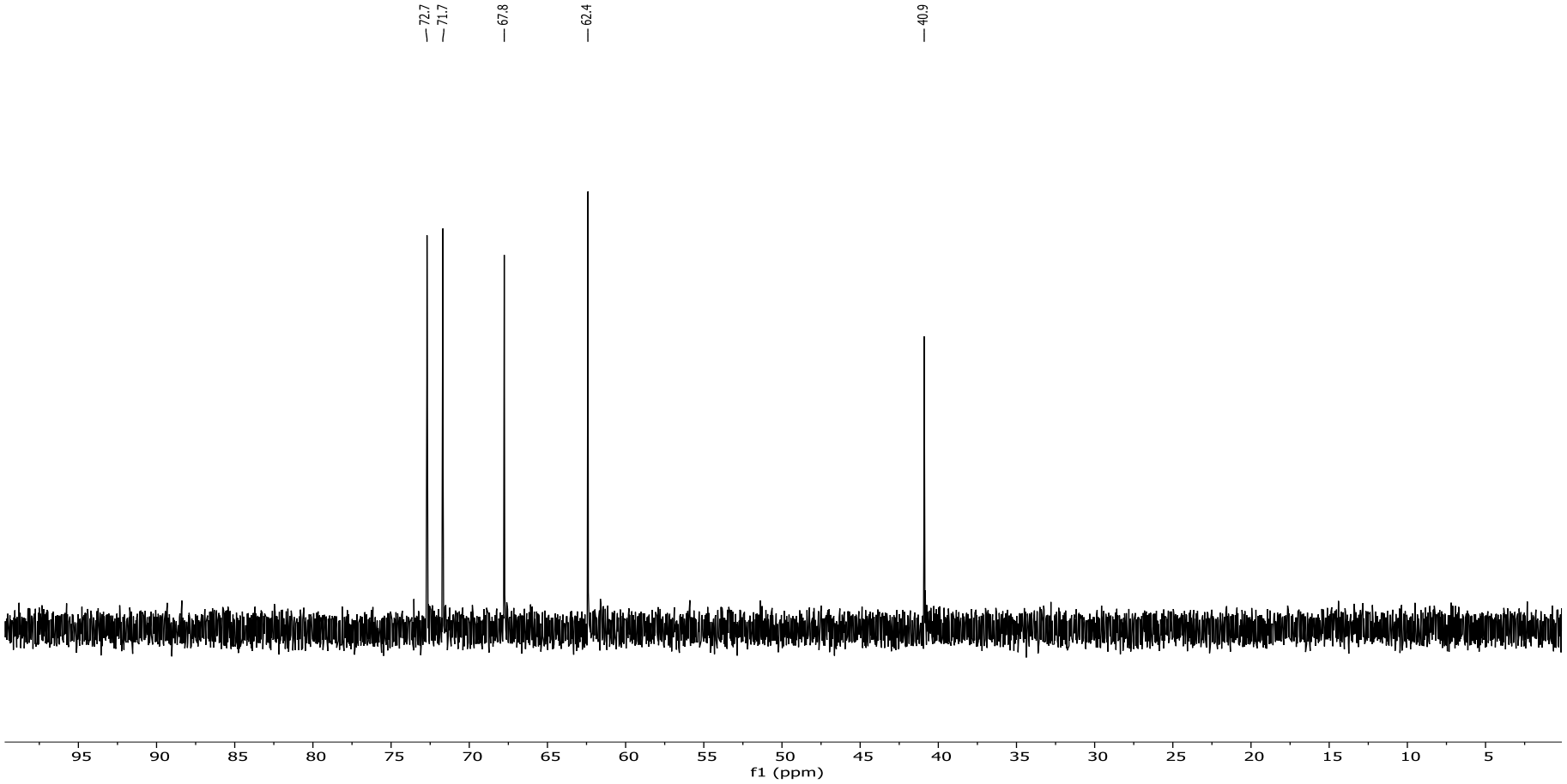
^{1}H NMR (D_2O, 500 MHz)



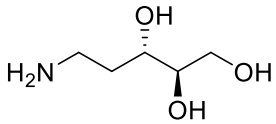
1-Amino-1-deoxy-L-ribitol (7b)



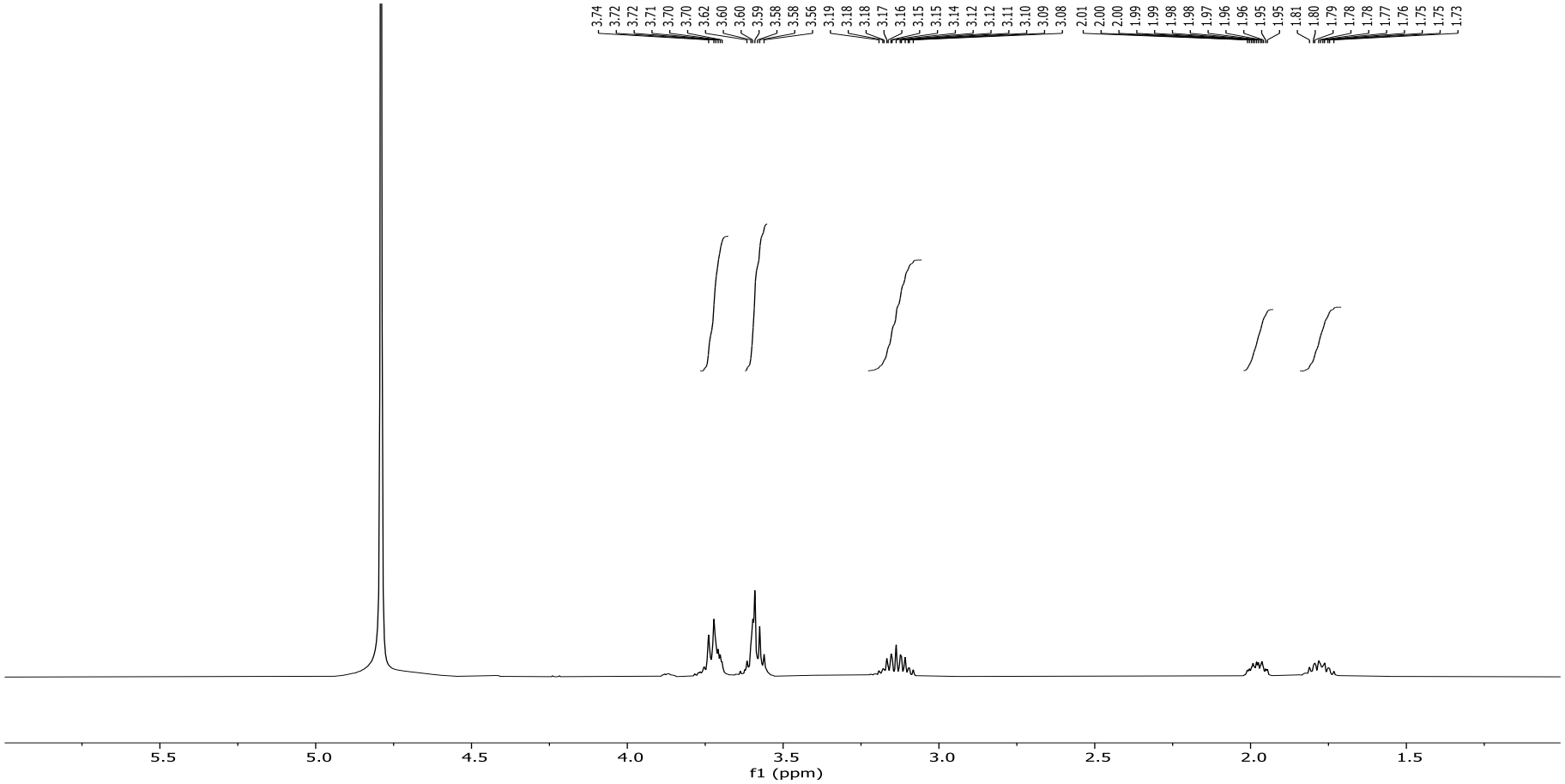
^{13}C NMR (D_2O, 125 MHz)



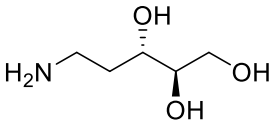
1-Amino-1,2-dideoxy-D-ribitol (7c)



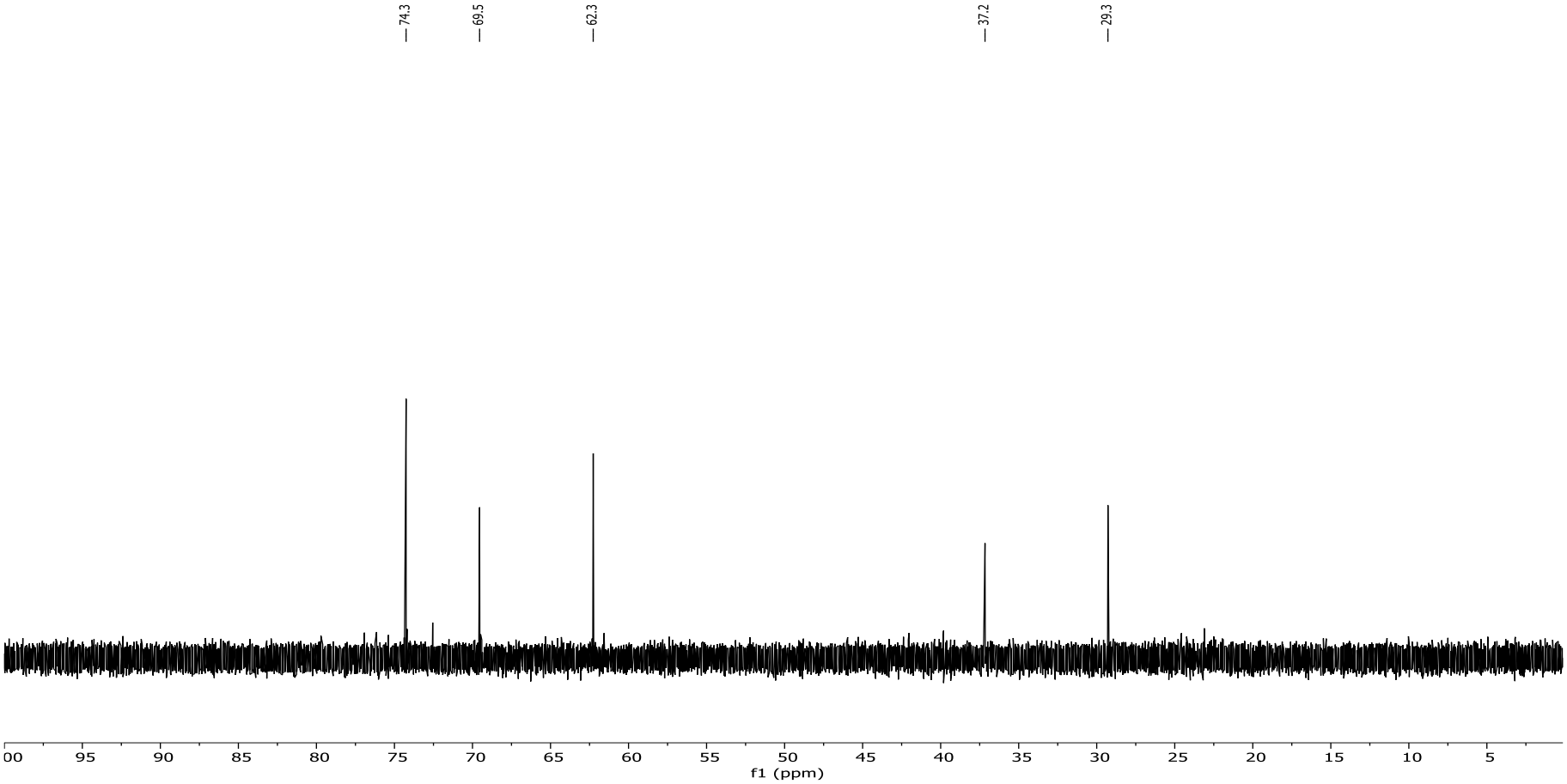
^{1}H NMR (D_2O, 500 MHz)



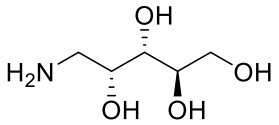
1-Amino-1,2-dideoxy-D-ribitol (7c)



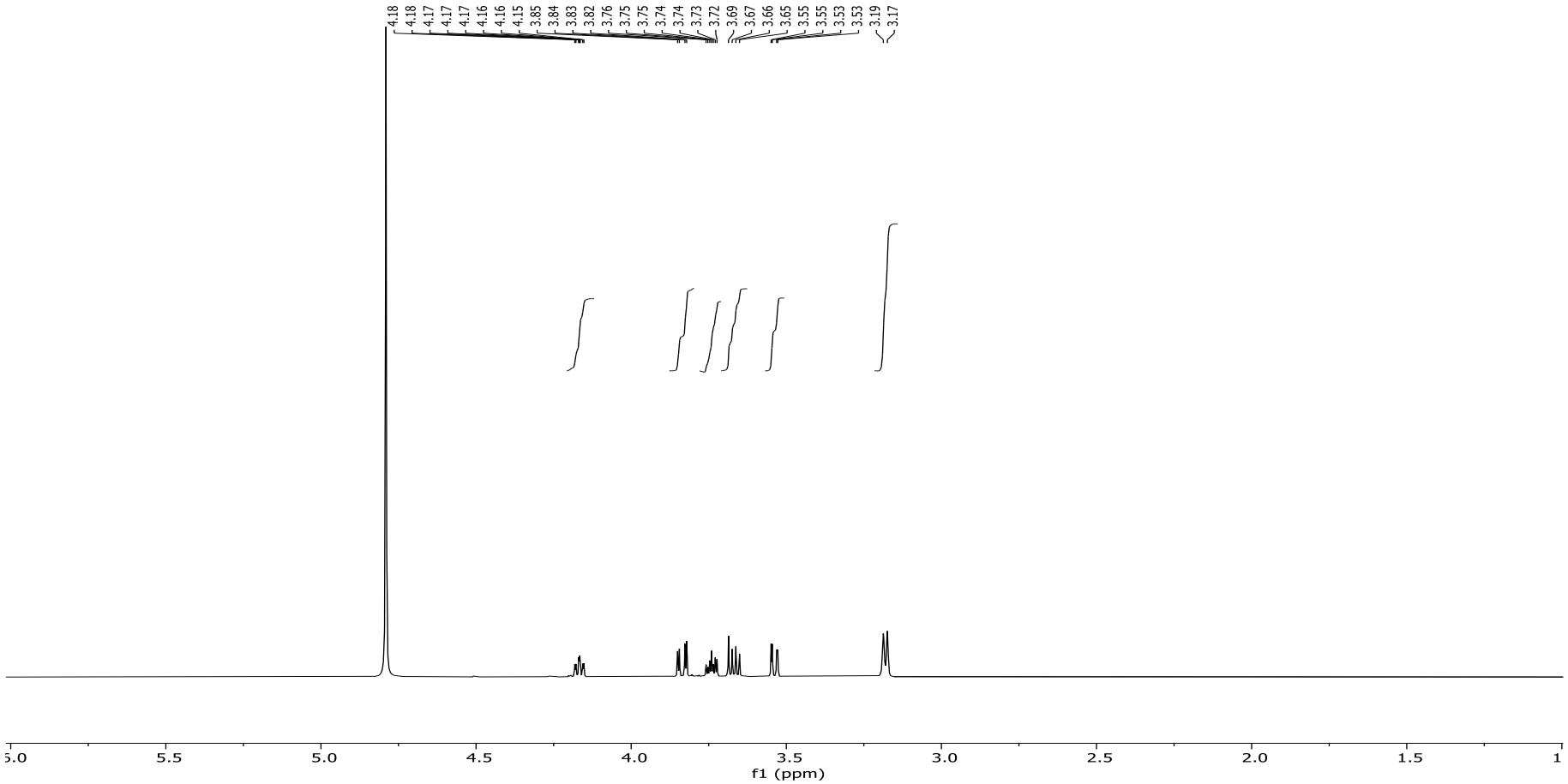
^{13}C NMR (D_2O, 125 MHz)



1-Amino-1-deoxy-D-arabinitol (7d)

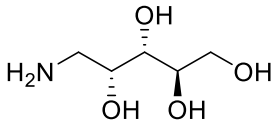


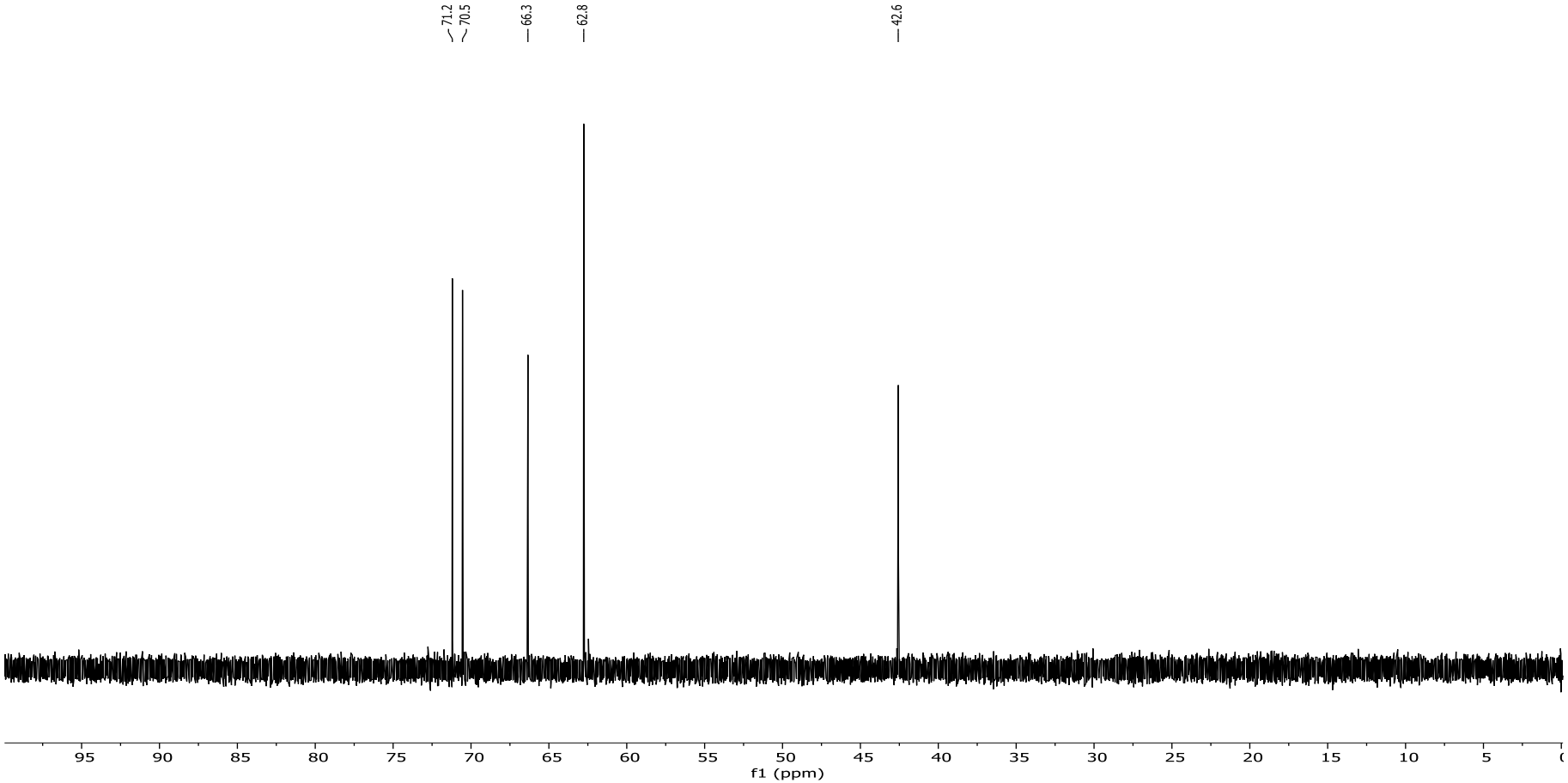
^{1}H NMR (D_2O, 500 MHz)



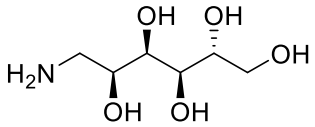
1-Amino-1-deoxy-D-arabinitol (7d)

^{1}H NMR (D_2O, 500 MHz)

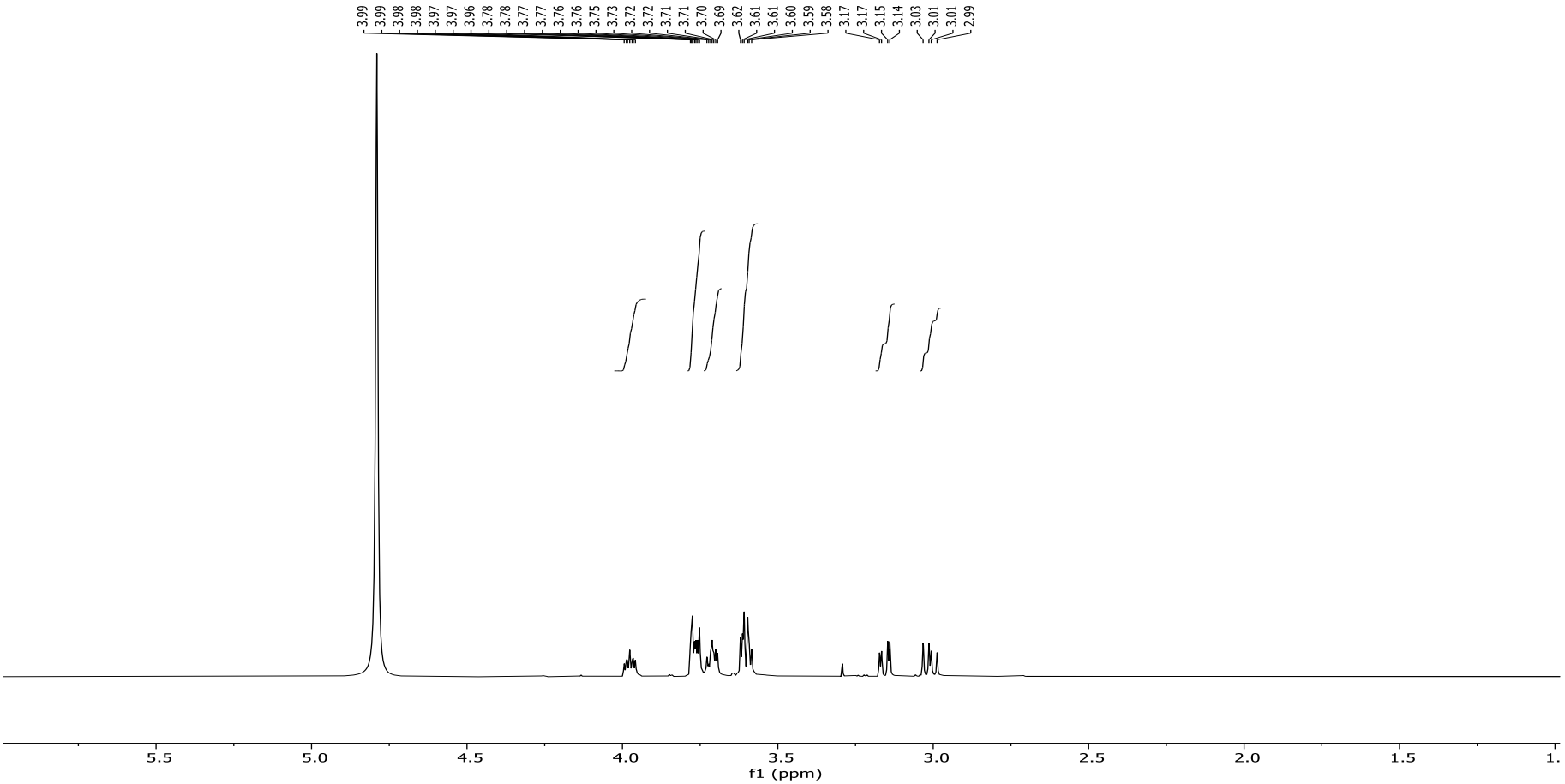




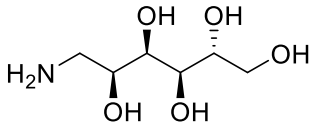
1-Amino-1-deoxy-D-glucitol (7e)



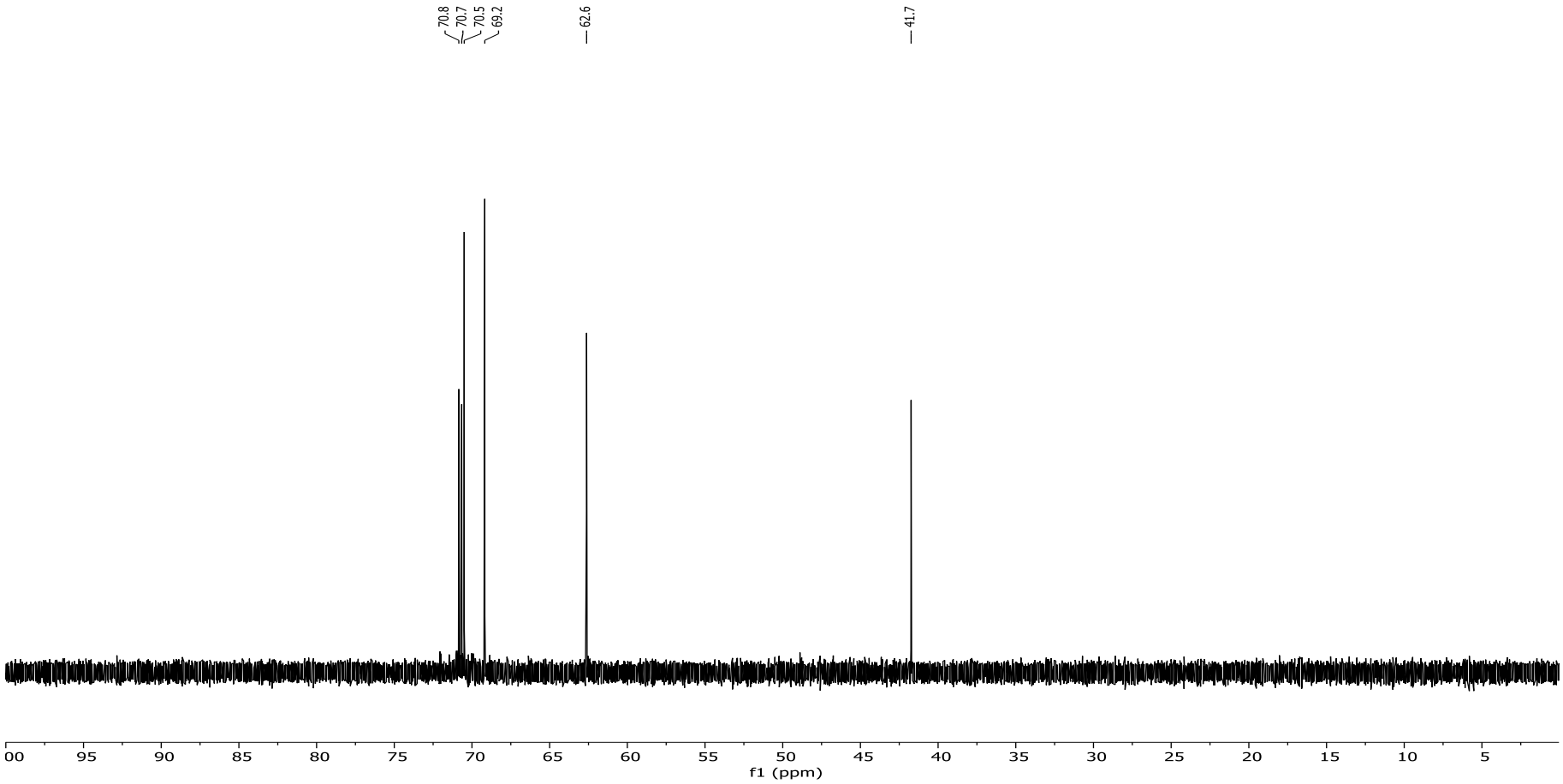
^{13}C NMR (D_2O, 125 MHz)



1-Amino-1-deoxy-D-glucitol (7e)

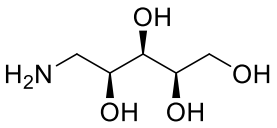


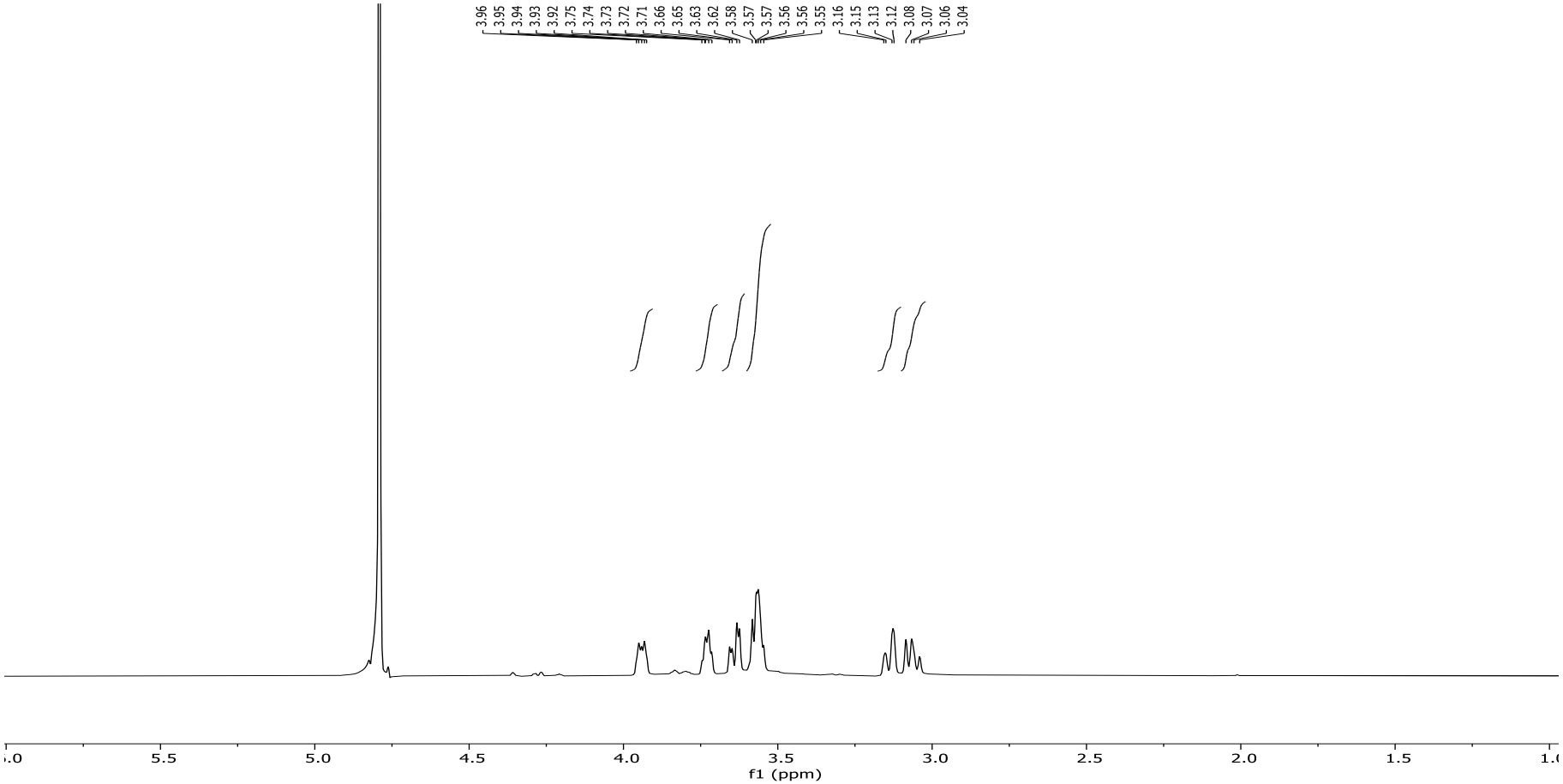
^{13}C NMR (D_2O, 125 MHz)



1-Amino-1-deoxy-D-xylitol (7f)

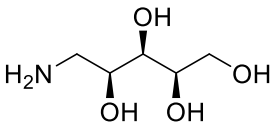
^{1}H NMR (D_2O, 500 MHz)

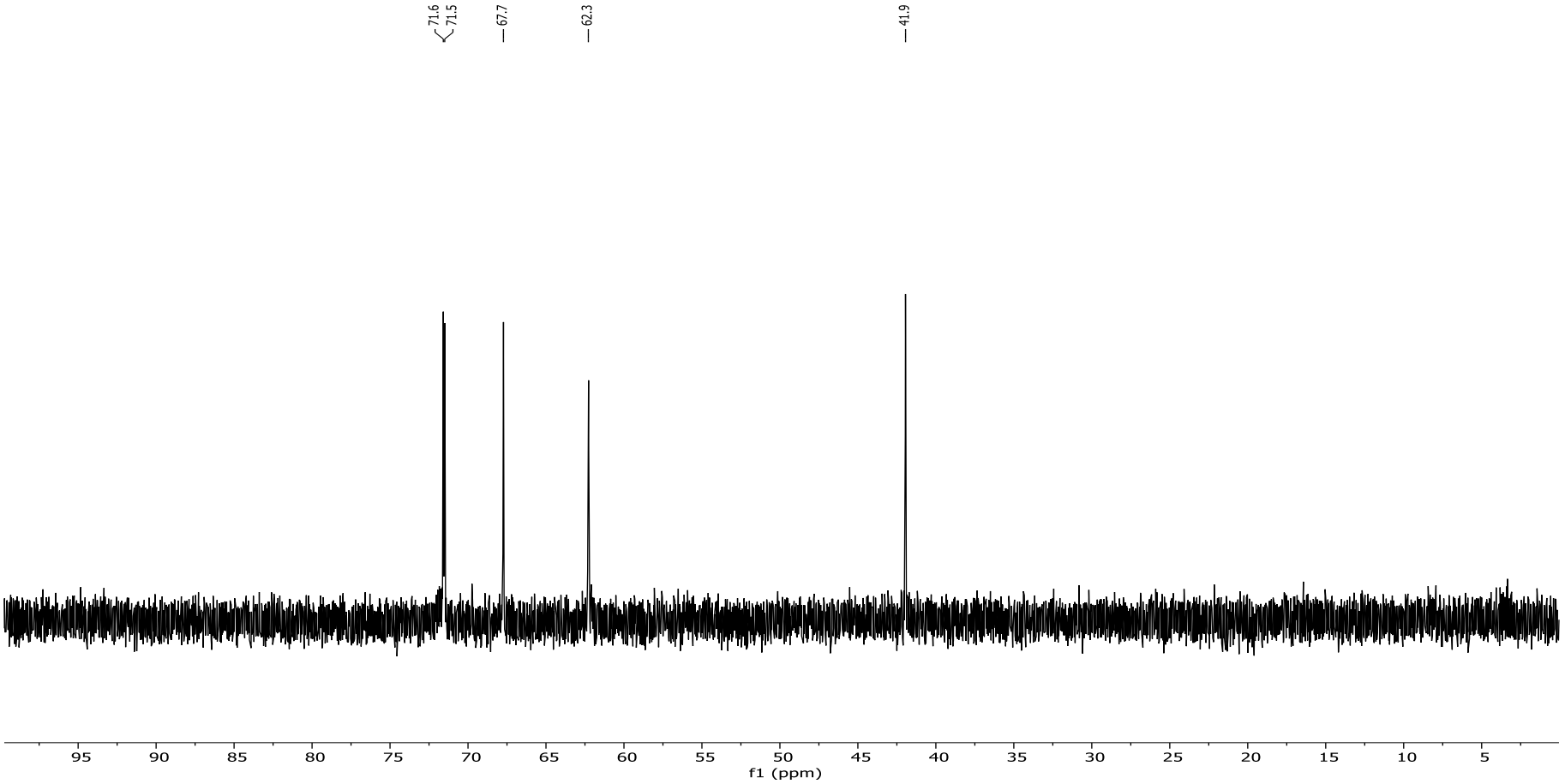




1-Amino-1-deoxy-D-xylitol (7f)

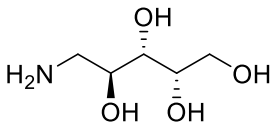
^{13}C NMR (D_2O, 125 MHz)

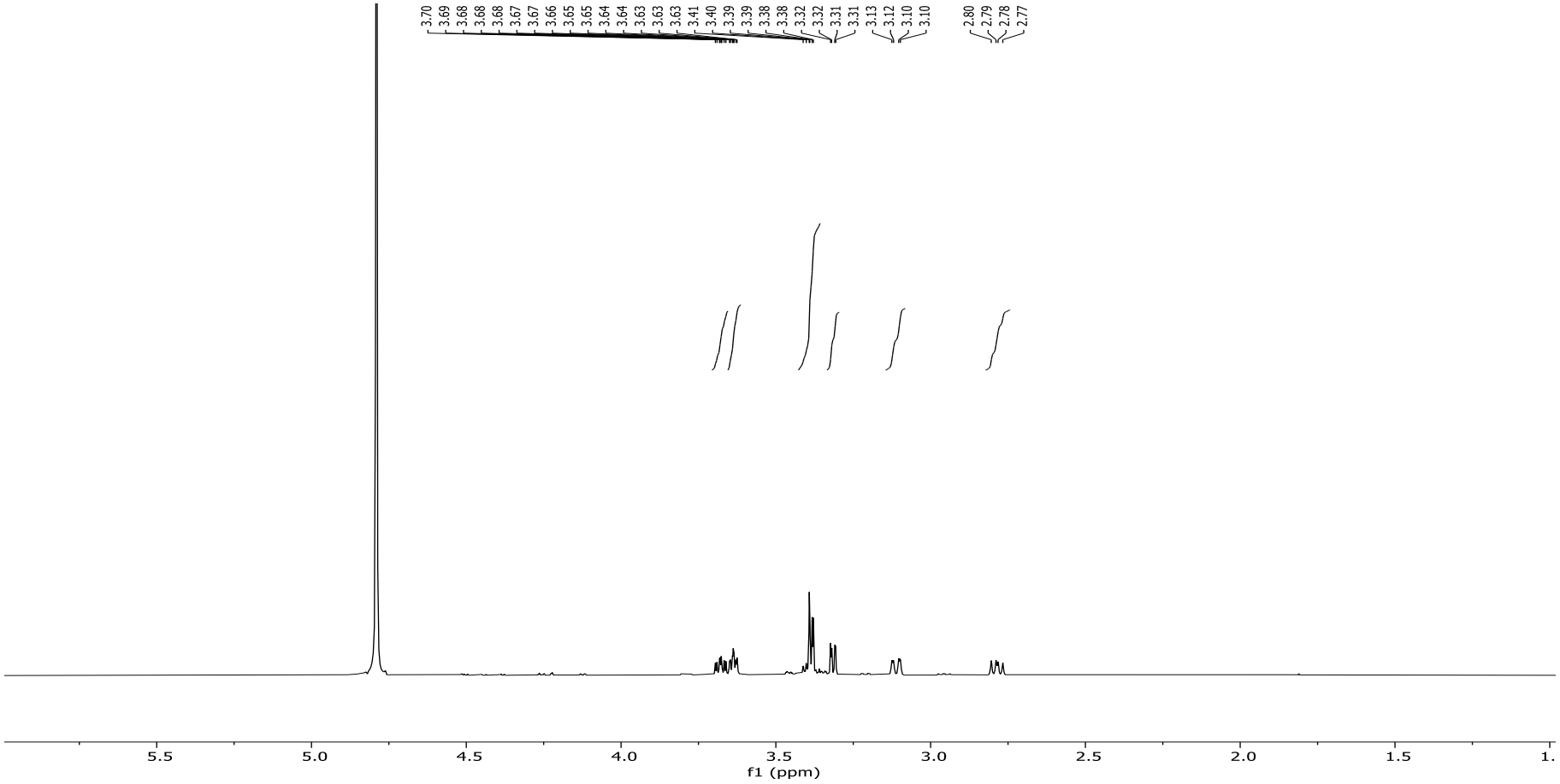




1-Amino-1-deoxy-L-lyxitol (7g)

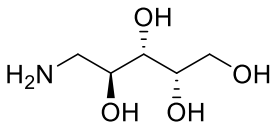
^{1}H NMR (D_2O, 500 MHz)

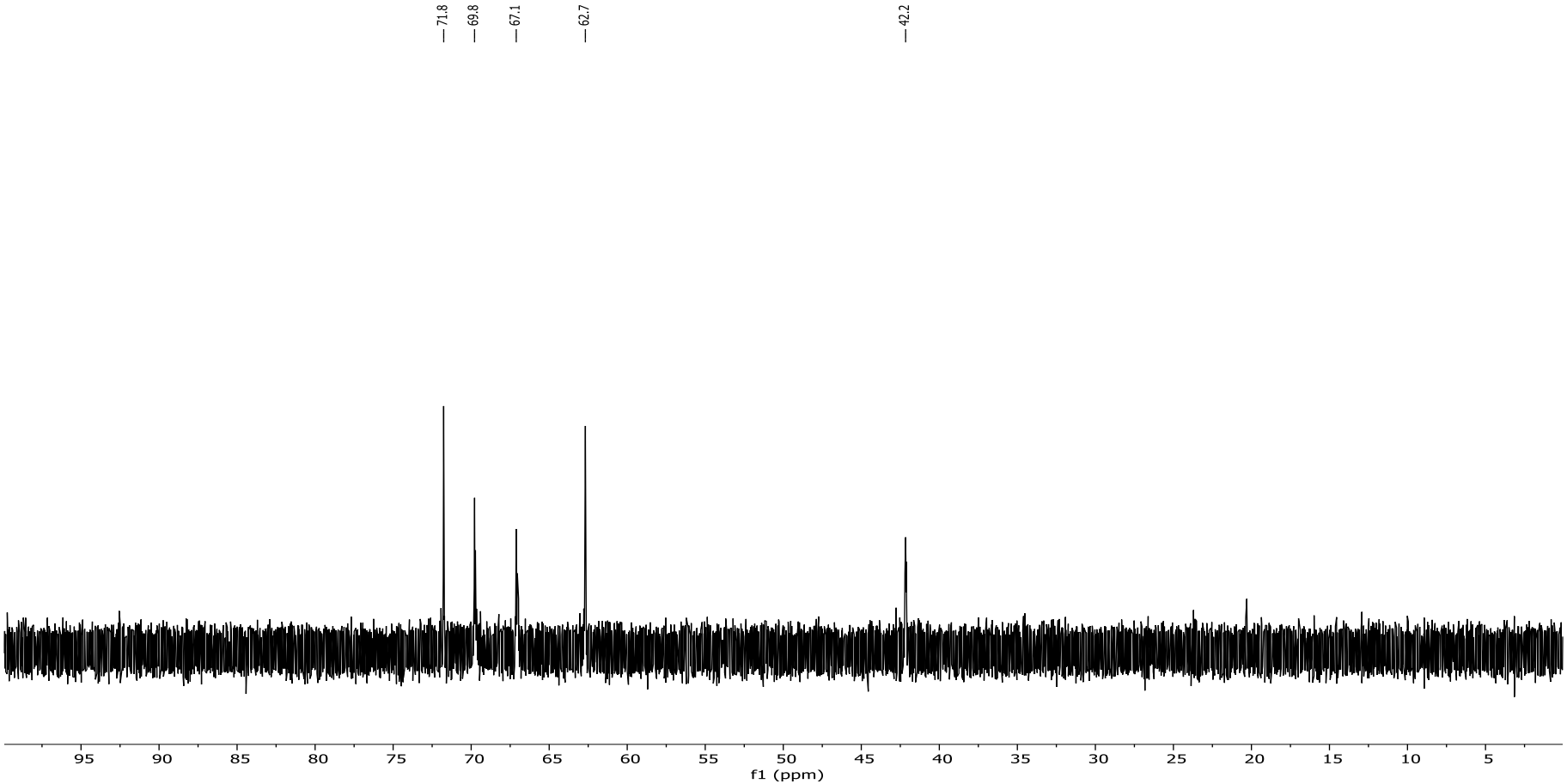




1-Amino-1-deoxy-L-lyxitol (7g)

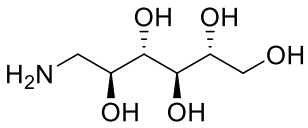
^{13}C NMR (D_2O, 125 MHz)

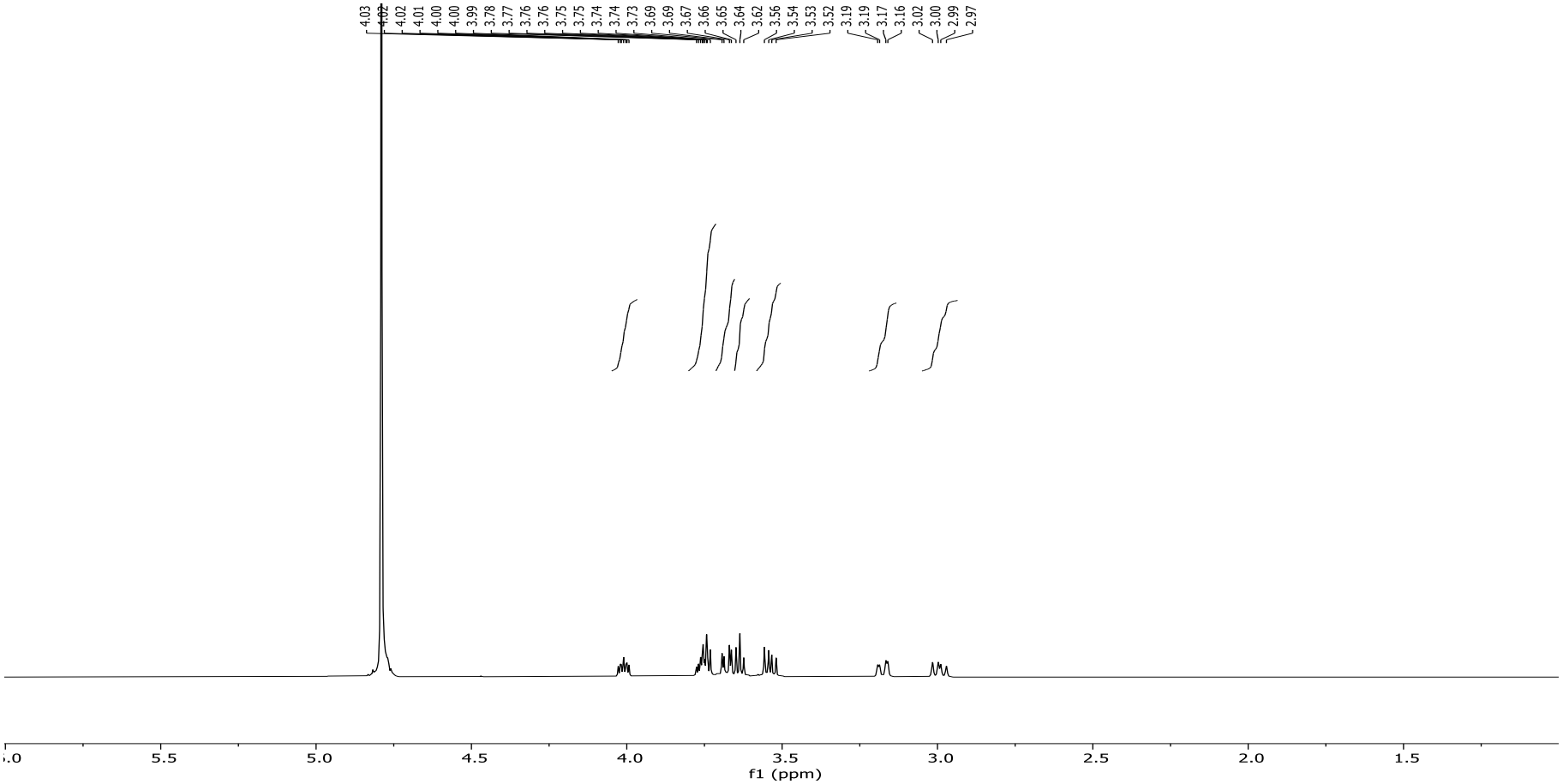




1-Amino-1-deoxy-D-allitol (7h)

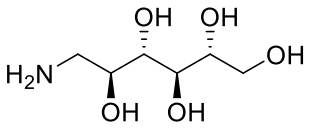
^{1}H NMR (D_2O, 500 MHz)

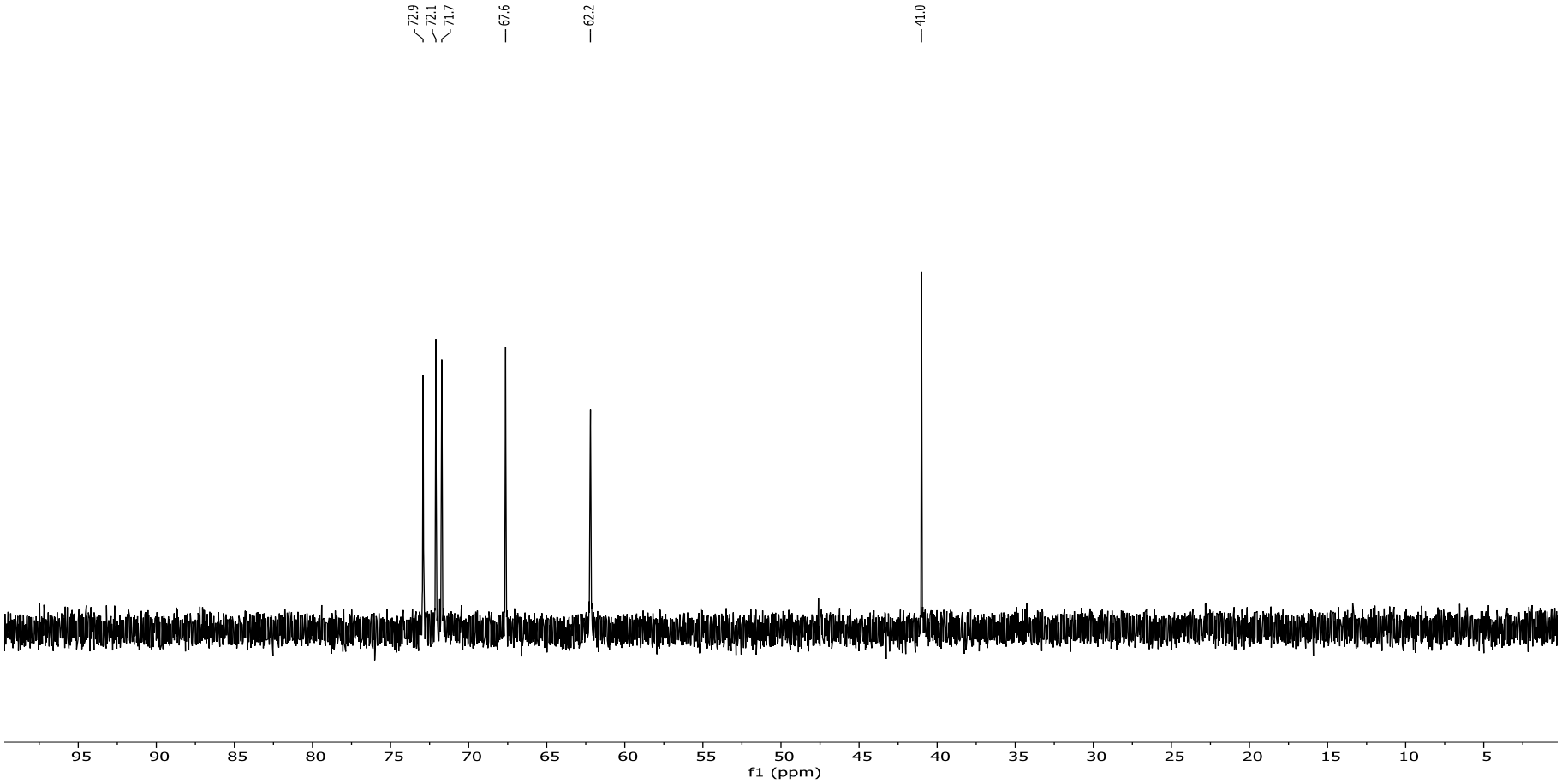




1-Amino-1-deoxy-D-allitol (7h)

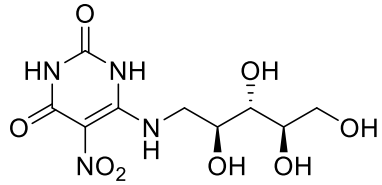
^{13}C NMR (D_2O, 125 MHz)

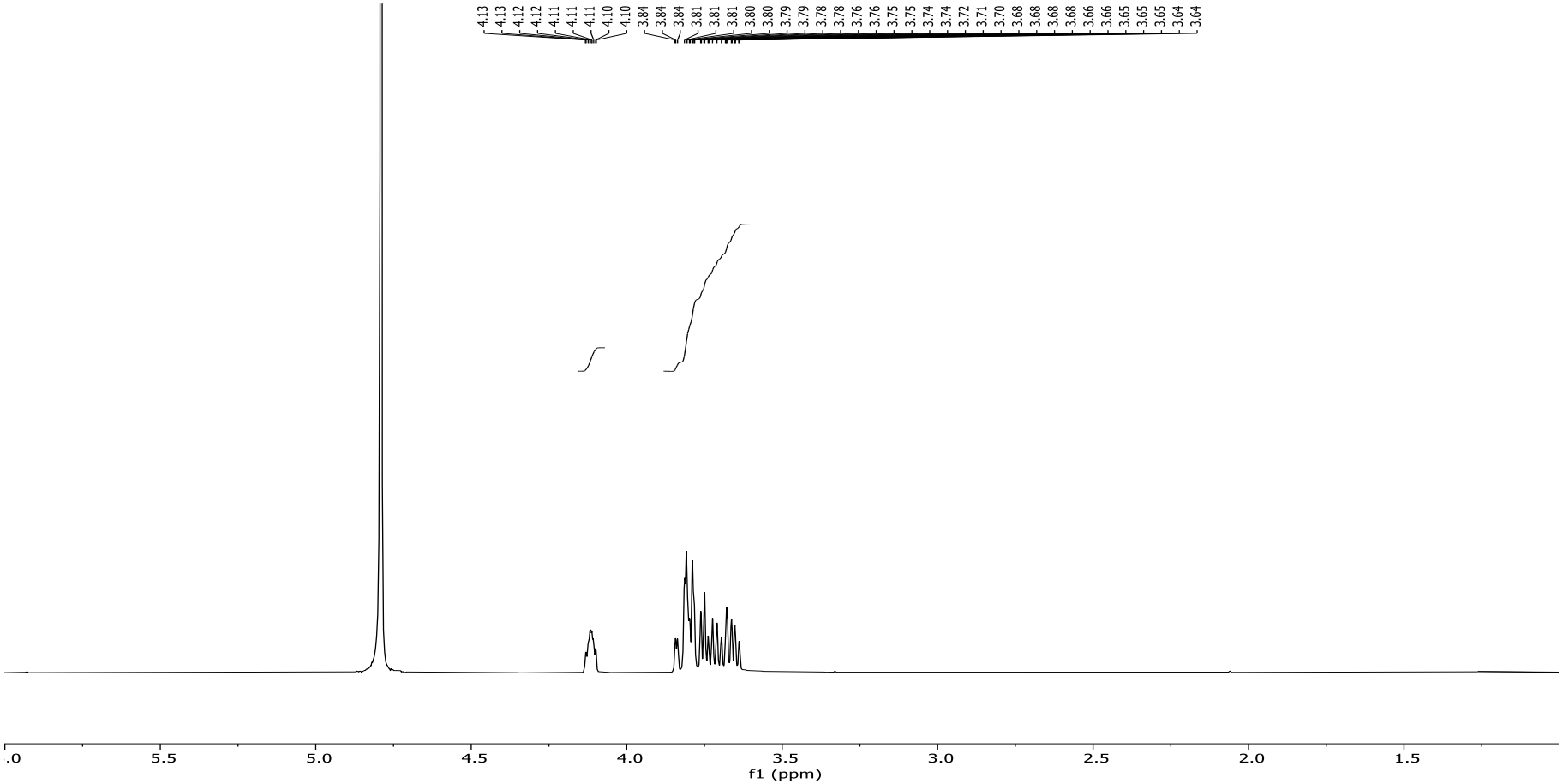




5-Nitro-6-((D-ribityl)amino)uracil (10a)

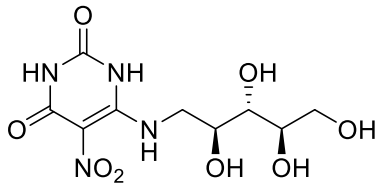
^{1}H NMR (D_2O, 500 MHz)

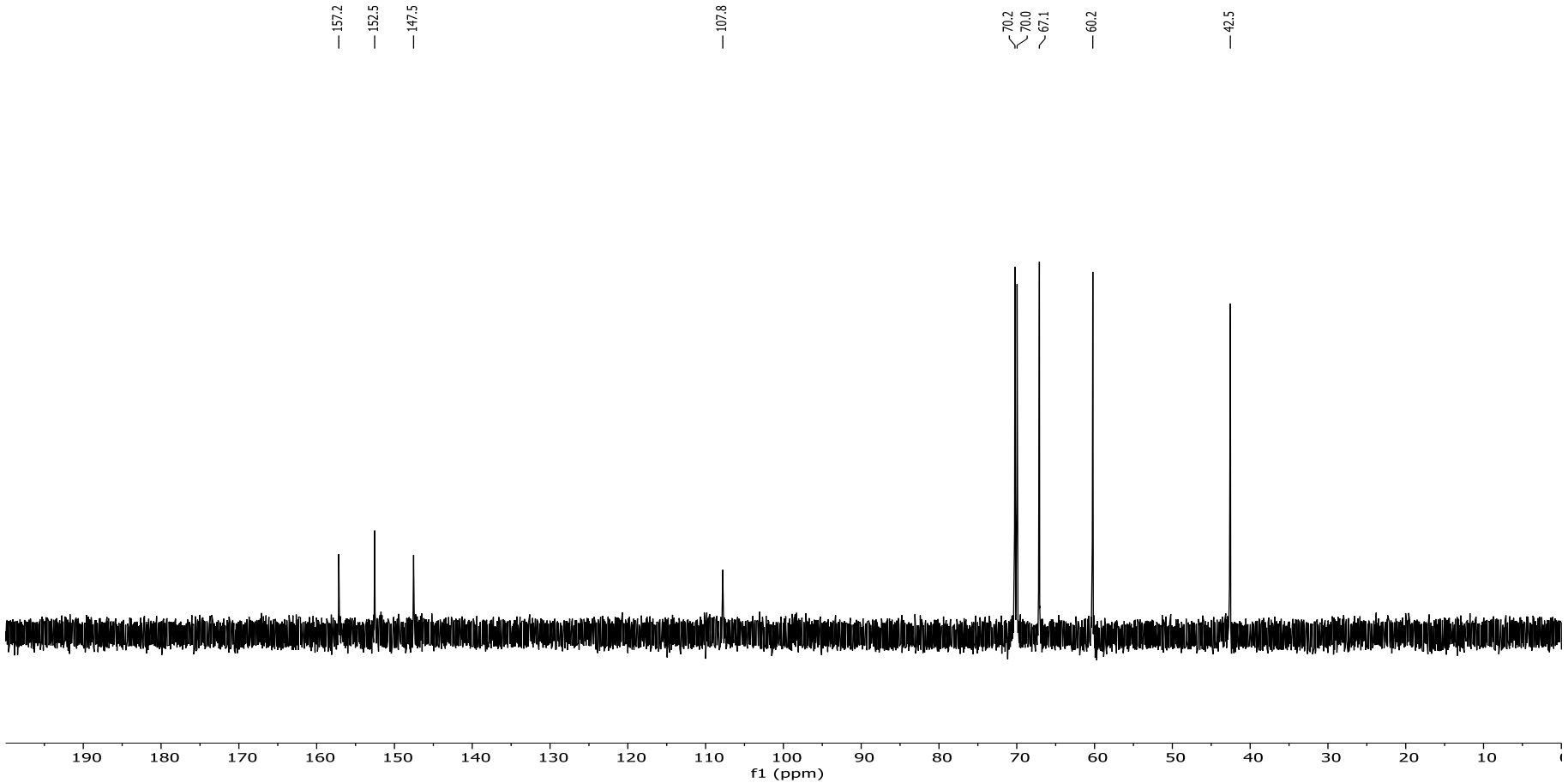




5-Nitro-6-((D-ribityl)amino)uracil (10a)

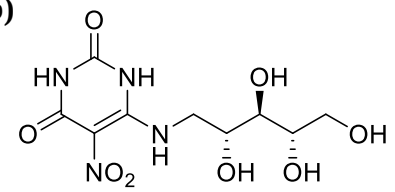
^{13}C NMR (D_2O, 125 MHz)

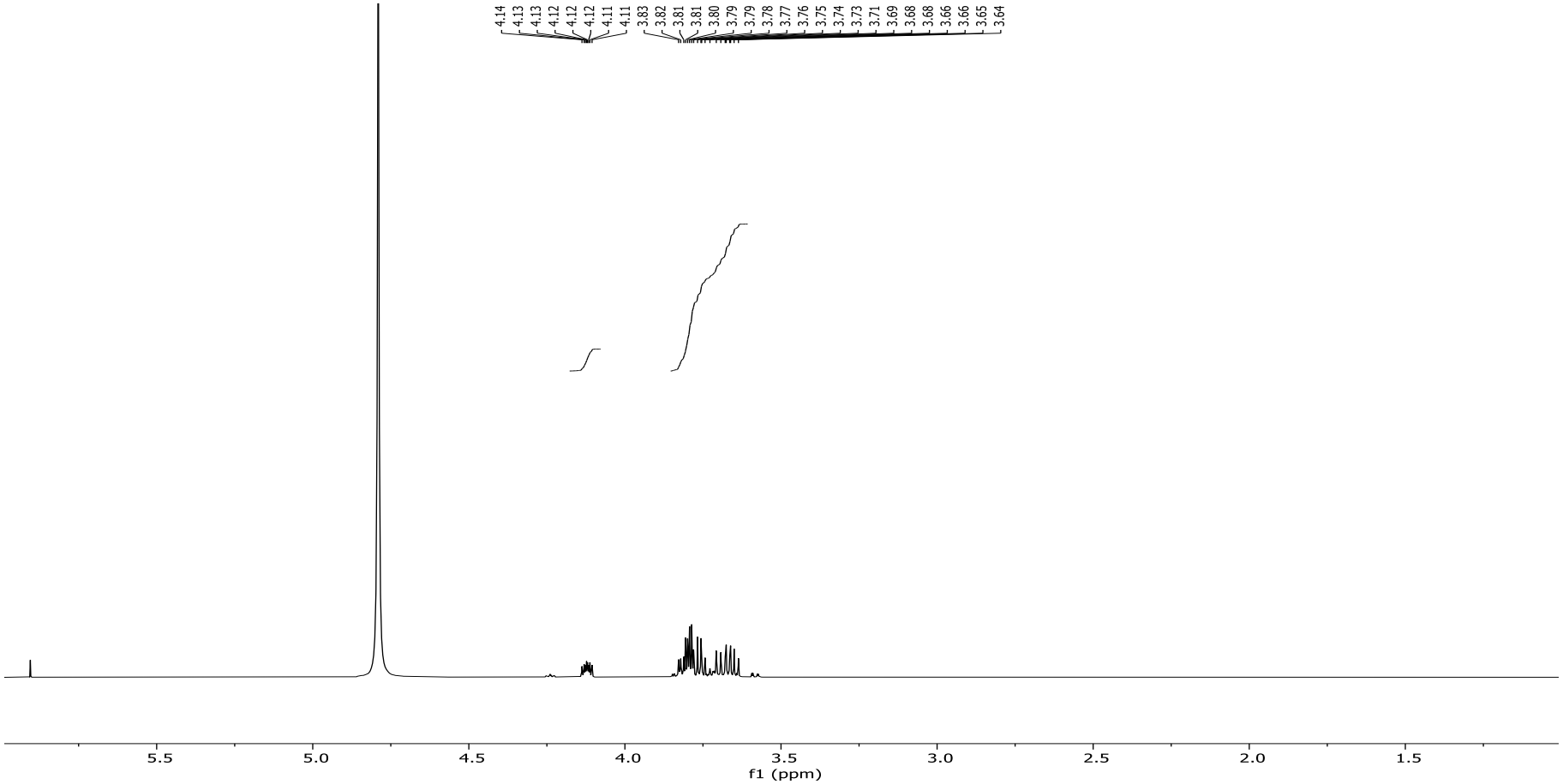




5-Nitro-6-((L-ribityl)amino)uracil (10b)

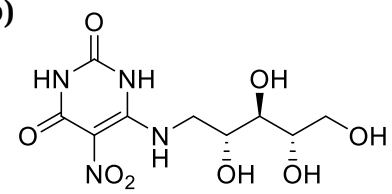
^{1}H NMR (D_2O, 500 MHz)





5-Nitro-6-((L-ribityl)amino)uracil (10b)

^{13}C NMR (D_2O, 125 MHz)



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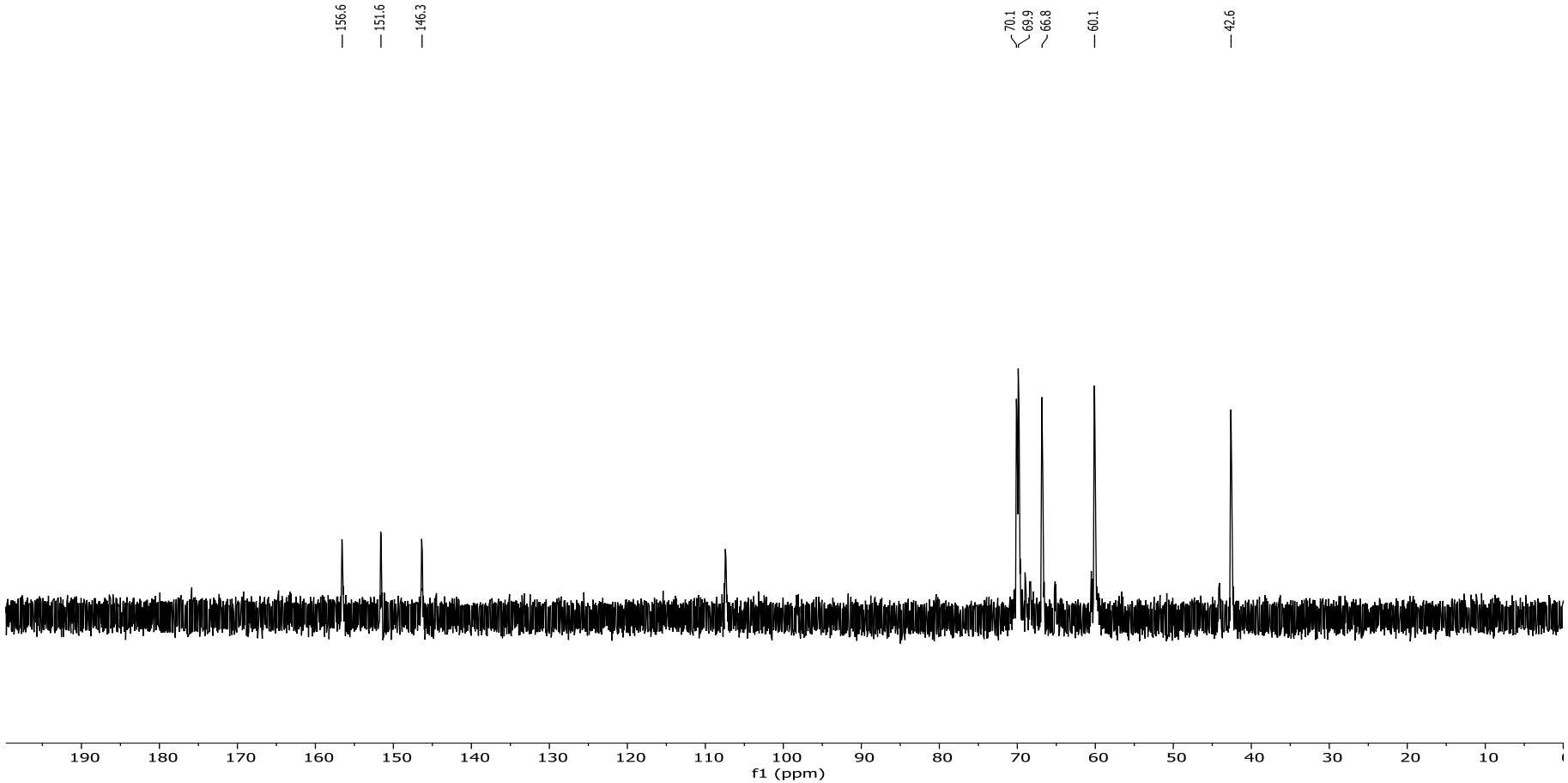
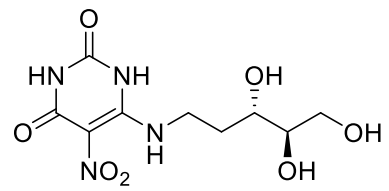
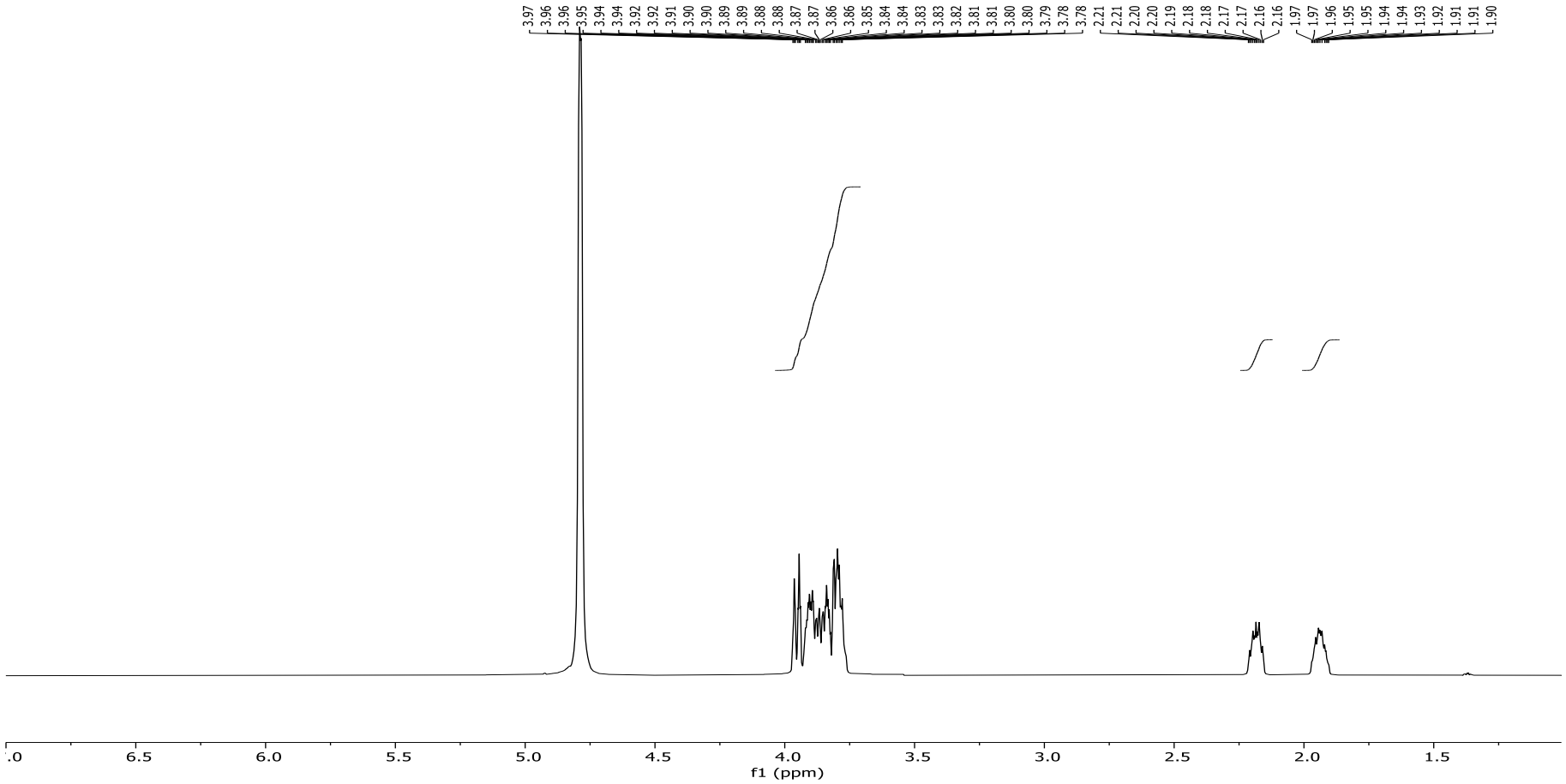


5-Nitro-6-((2′-deoxy-D-ribityl)amino)uracil (10c)

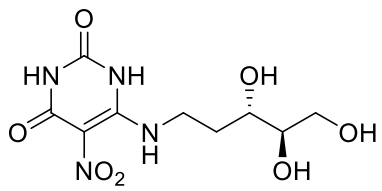
^{1}H NMR (D_2O, 500 MHz)

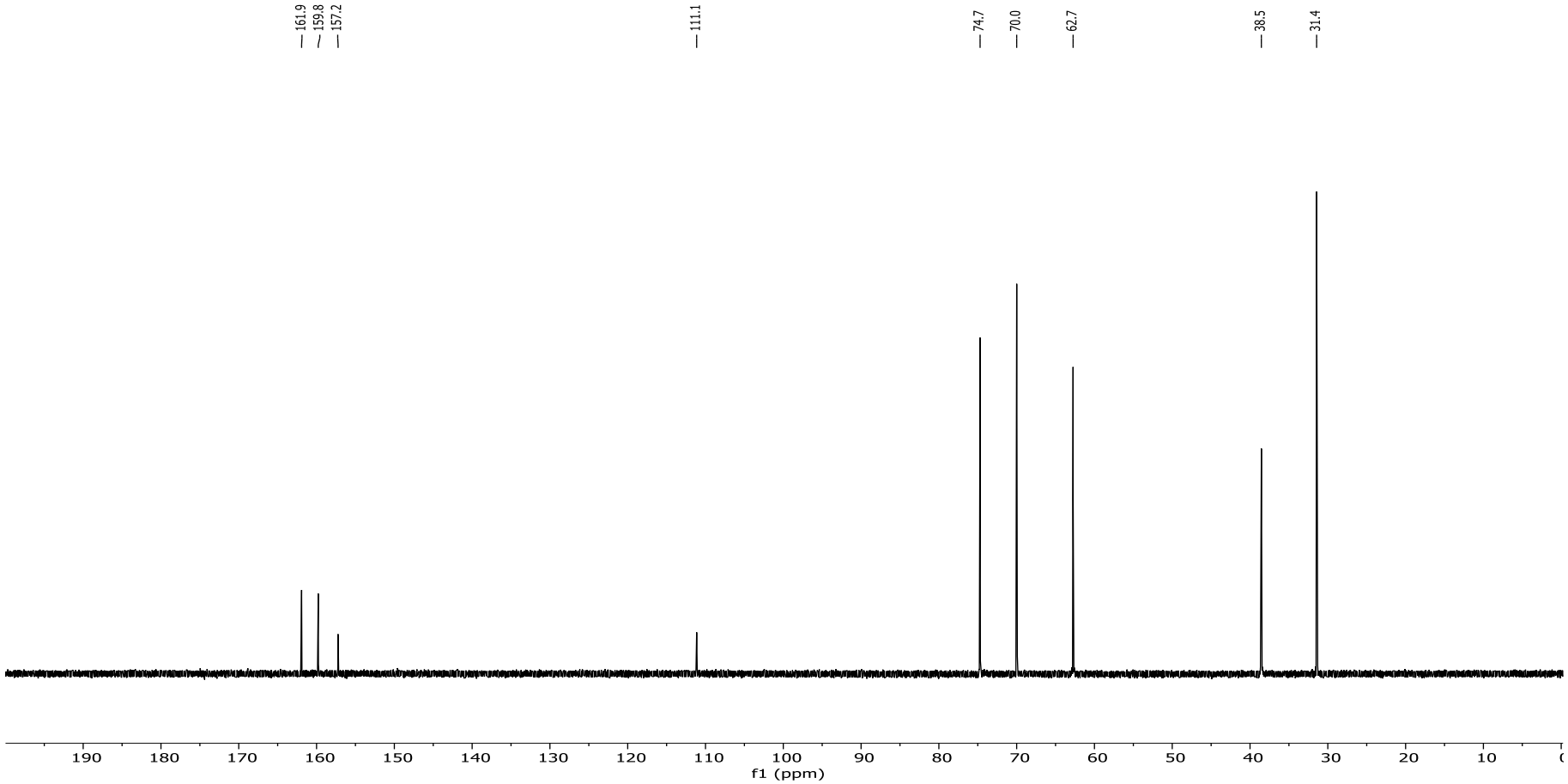




5-Nitro-6-((2′-deoxy-D-ribityl)amino)uracil (10c)

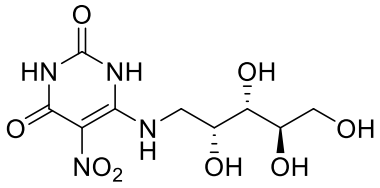
^{13}C NMR (D_2O, 125 MHz)

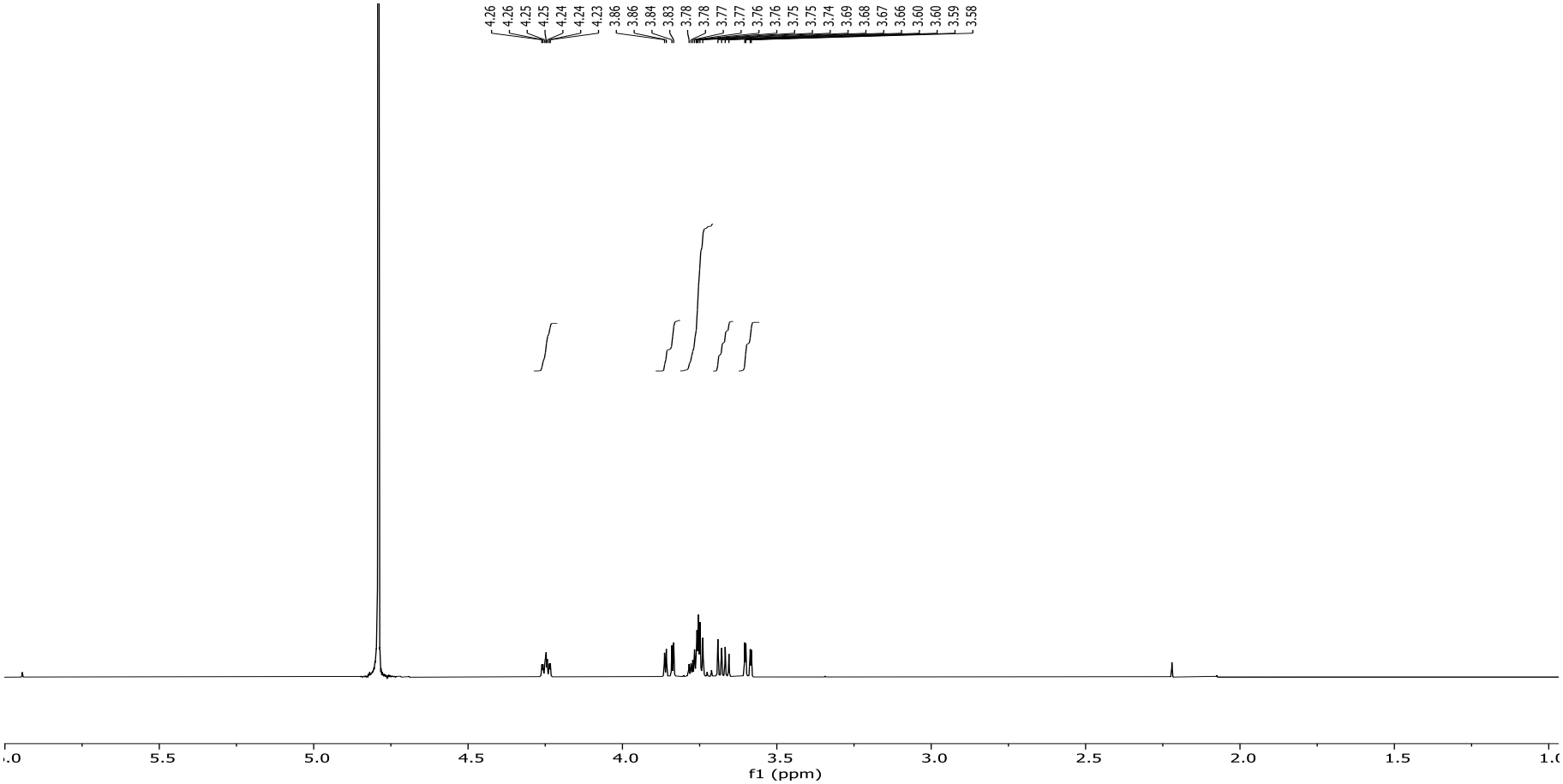




5-Nitro-6-((D-arabinityl)amino)uracil (10d)

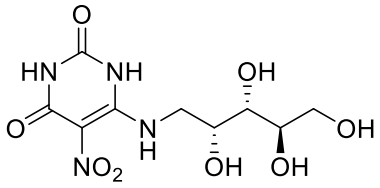
^{1}H NMR (D_2O, 500 MHz)

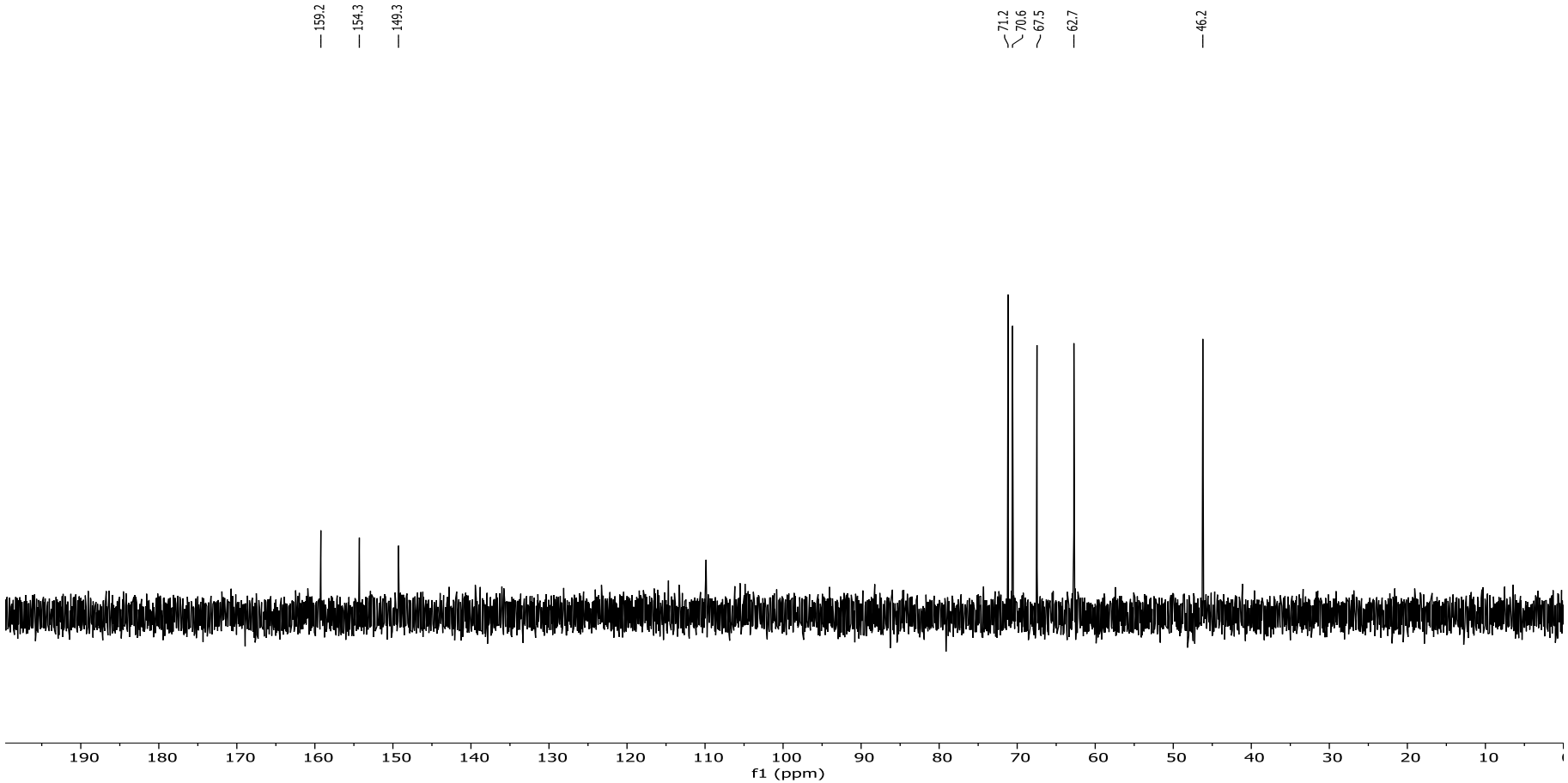




5-Nitro-6-((D-arabinityl)amino)uracil (10d)

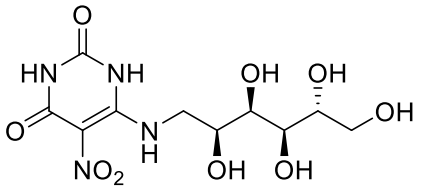
^{13}C NMR (D_2O, 125 MHz)

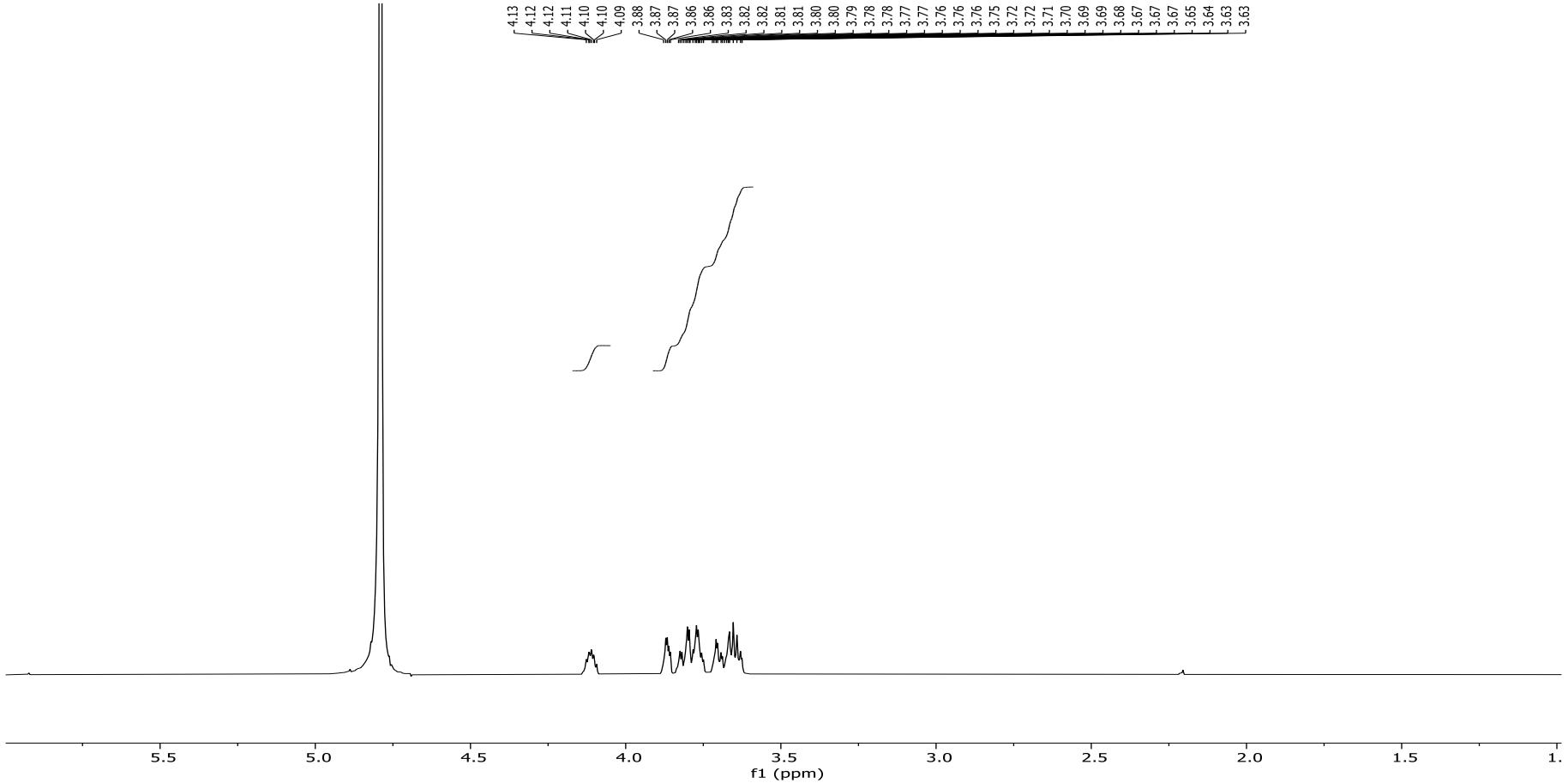




5-Nitro-6-((D-glucityl)amino)uracil (10e)

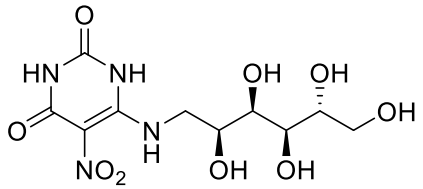
^{1}H NMR (D_2O, 500 MHz)

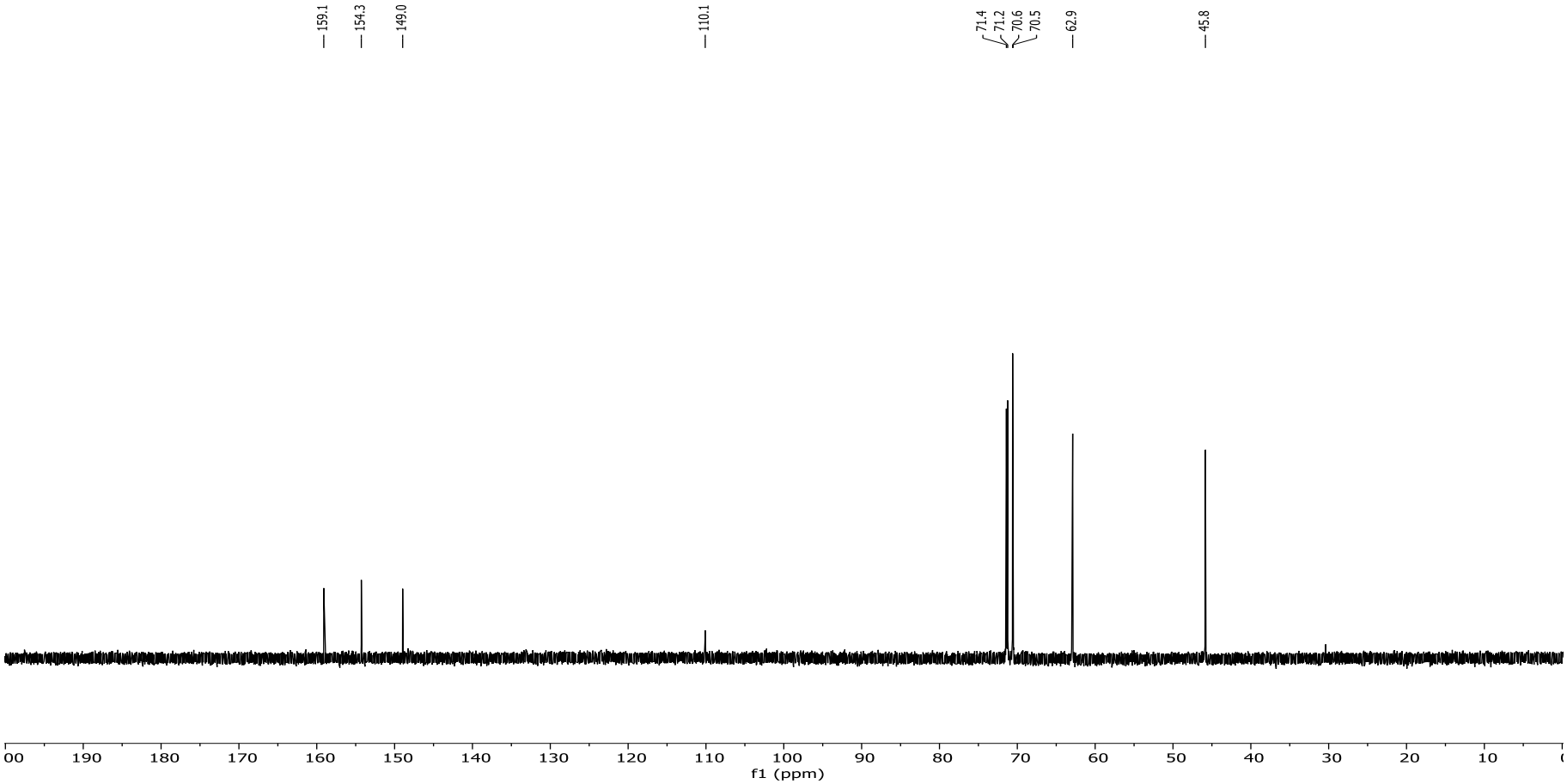




5-Nitro-6-((D-glucityl)amino)uracil (10e)

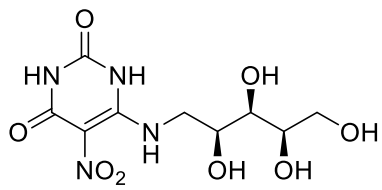
^{13}C NMR (D_2O, 125 MHz)

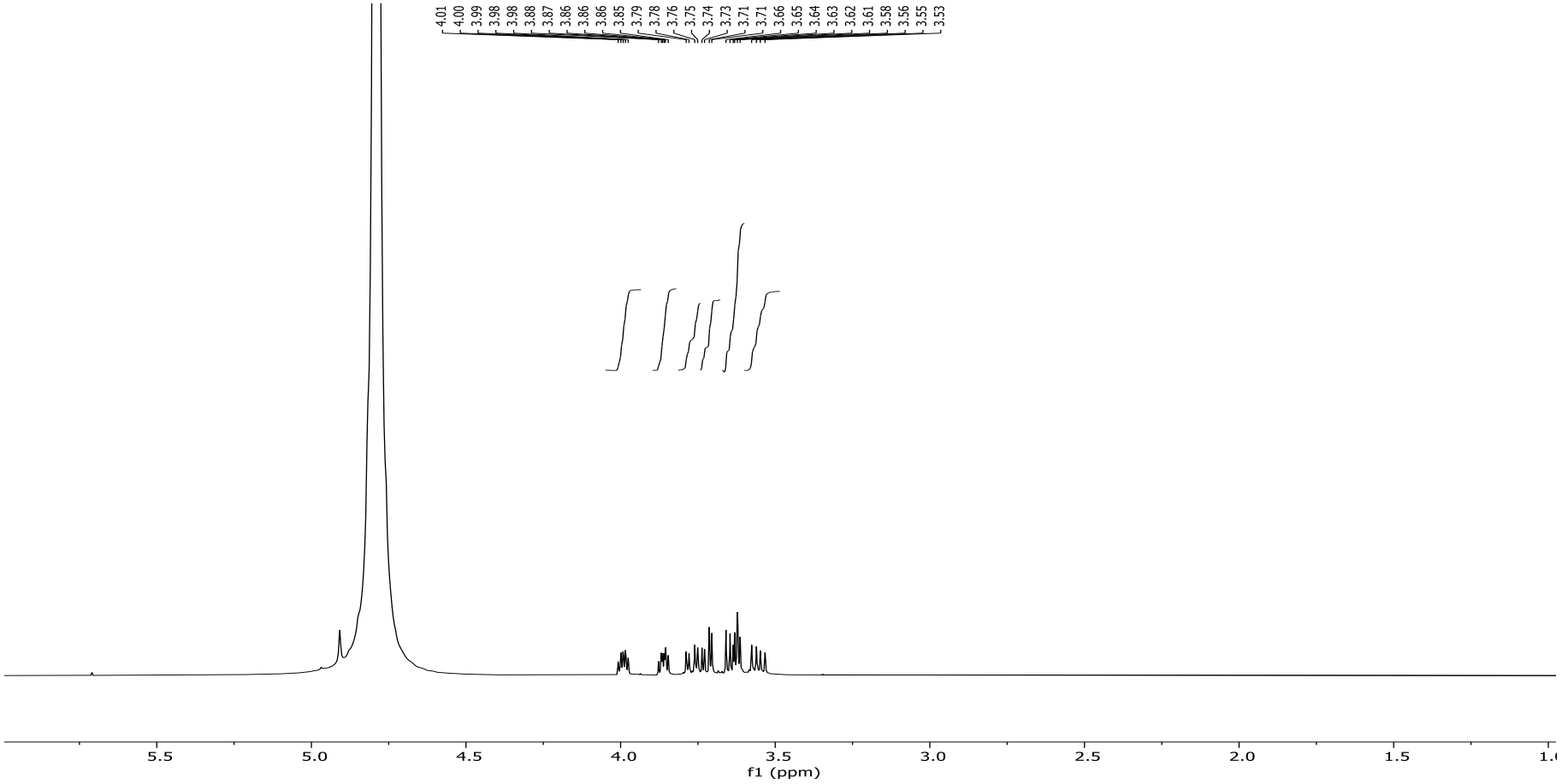




5-Nitro-6-((D-xylityl)amino)uracil (10f)

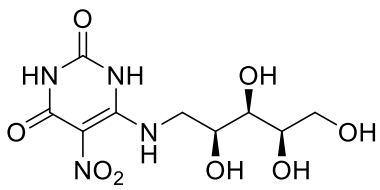
^{1}H NMR (D_2O, 500 MHz)

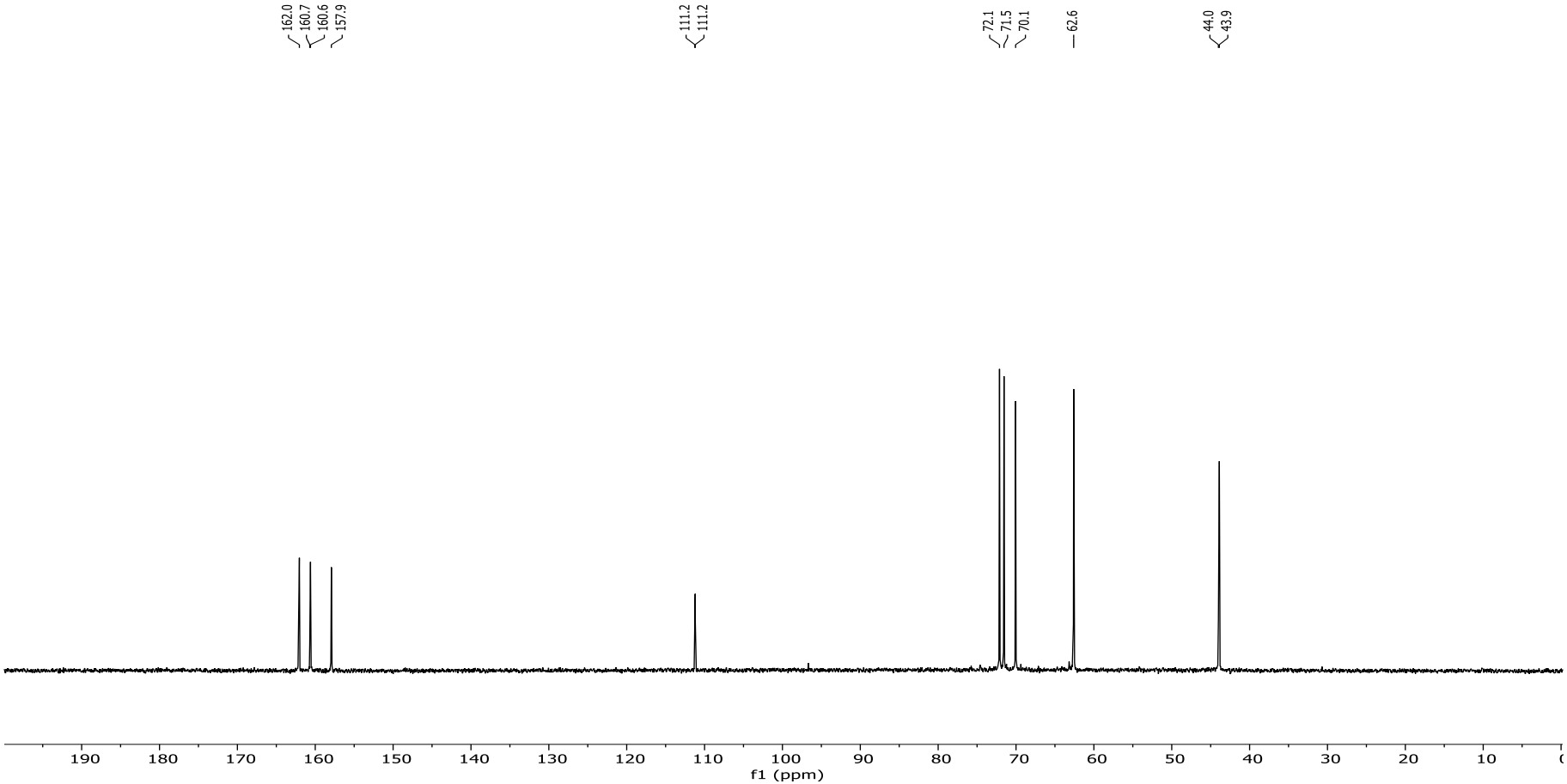




5-Nitro-6-((D-xylityl)amino)uracil (10f)

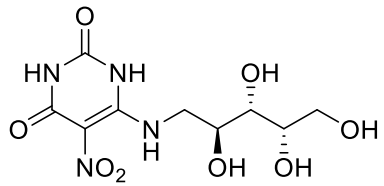
^{13}C NMR (D_2O, 125 MHz)

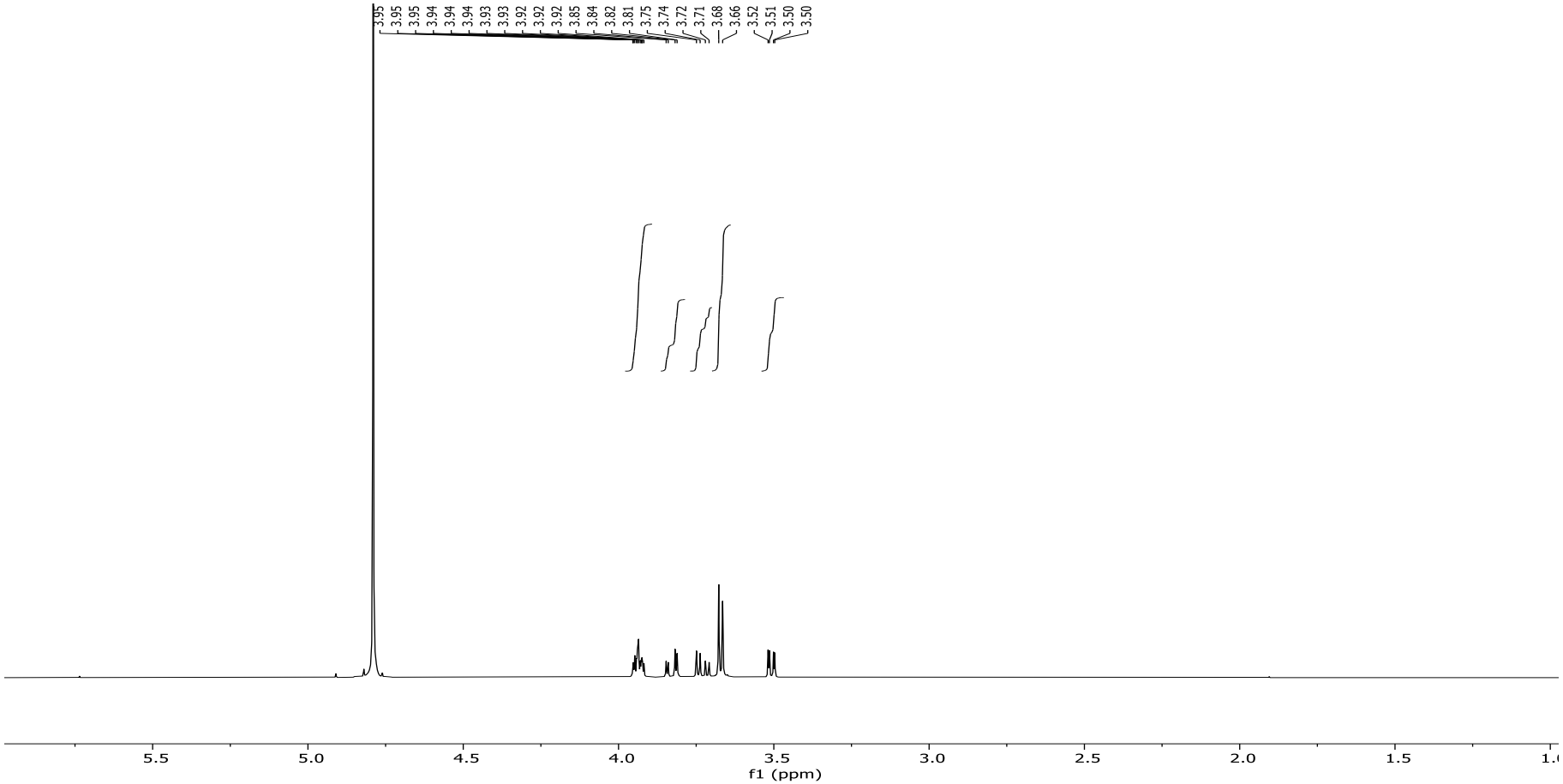




5-Nitro-6-((L-lyxityl)amino)uracil (10g)

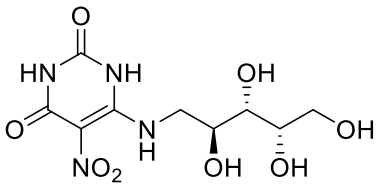
^{1}H NMR (D_2O, 500 MHz)

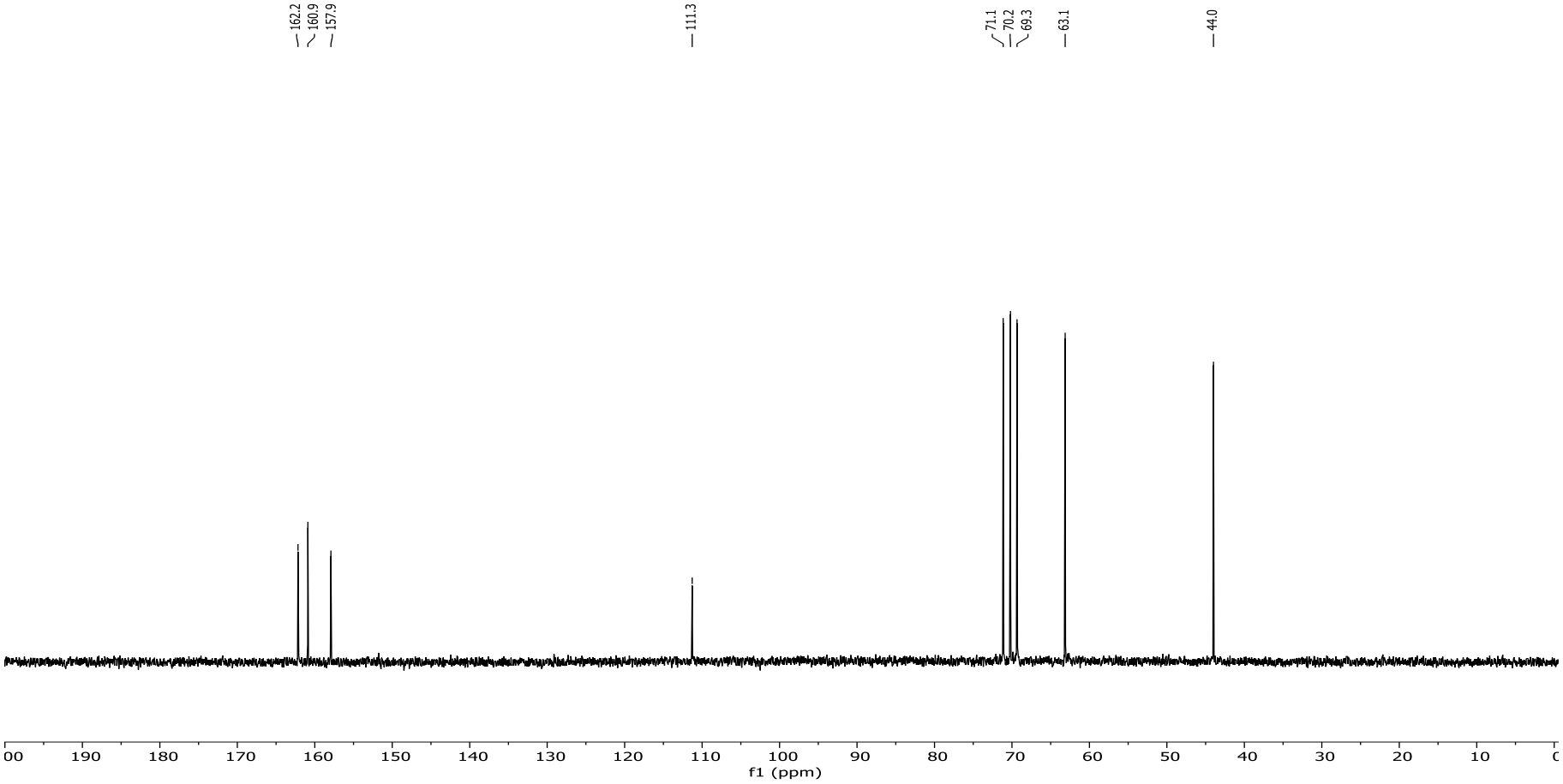




5-Nitro-6-((L-lyxityl)amino)uracil (10g)

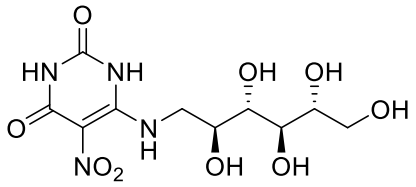
^{13}C NMR (D_2O, 125 MHz)

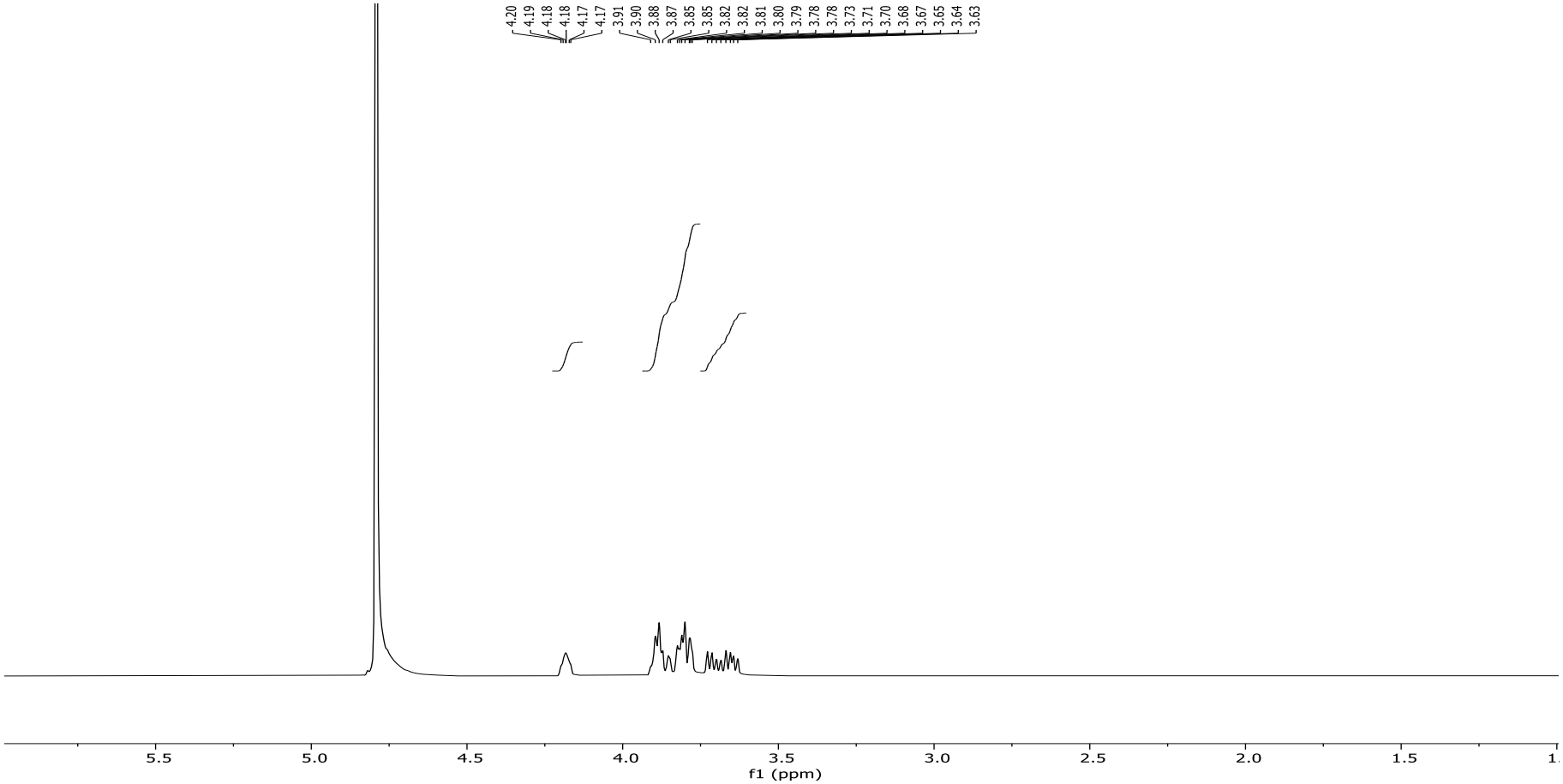




5-Nitro-6-((D-allityl)amino)uracil (10h)

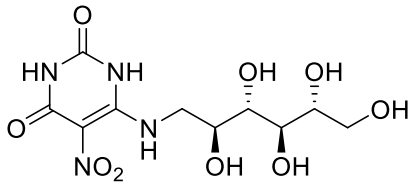
^{1}H NMR (D_2O, 500 MHz)

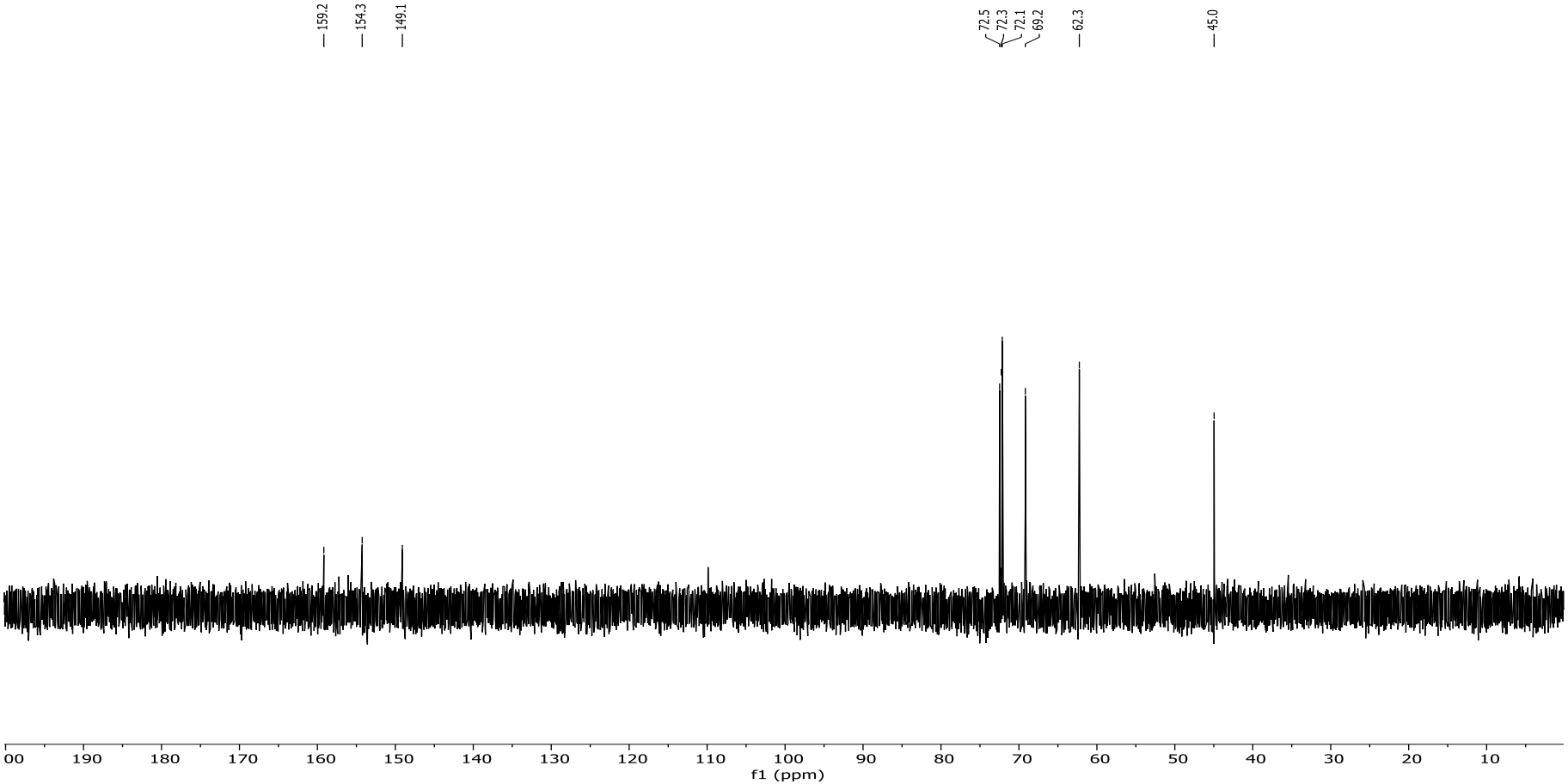




5-Nitro-6-((D-allityl)amino)uracil (10h)

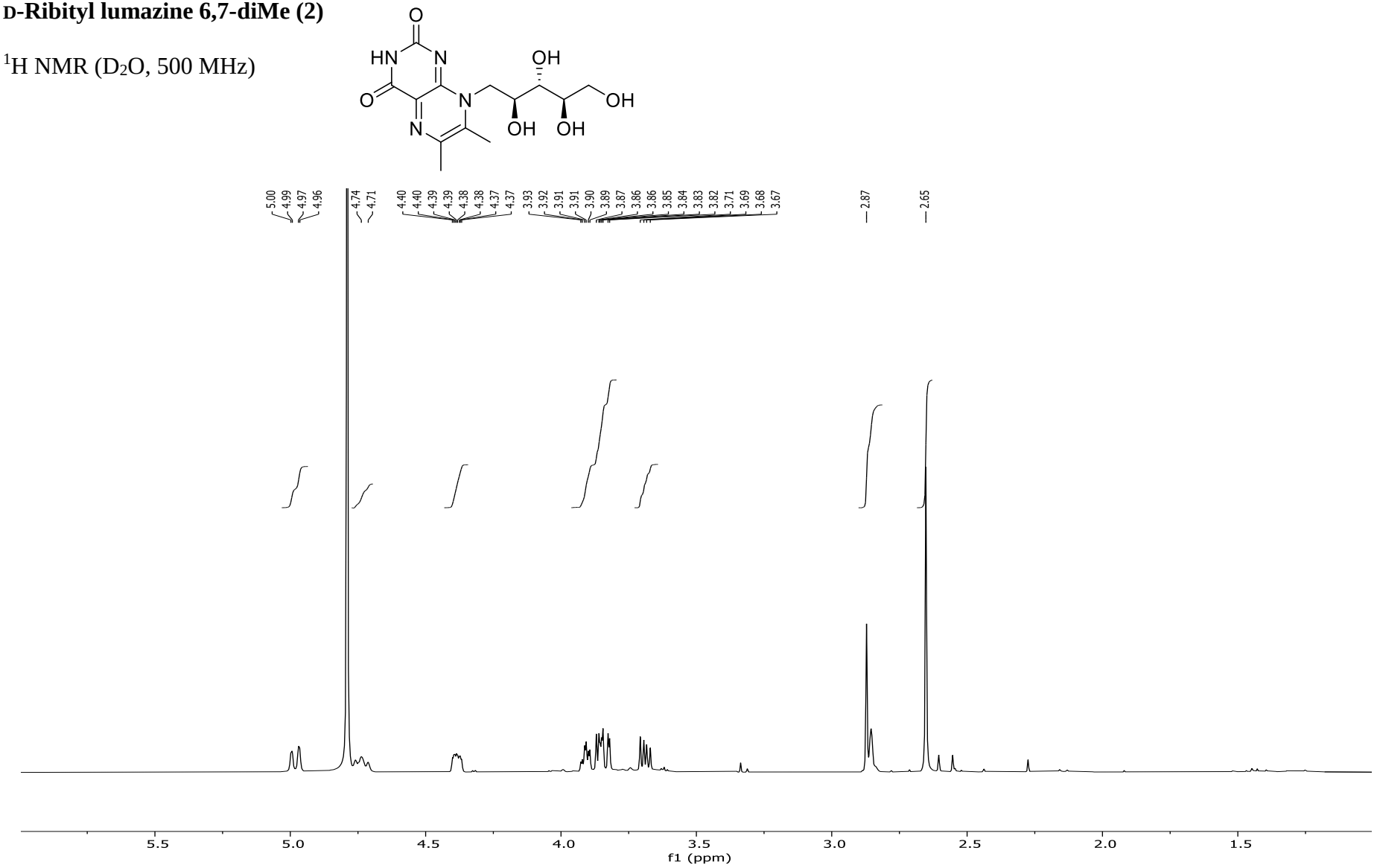
^{13}C NMR (D_2O, 125 MHz)





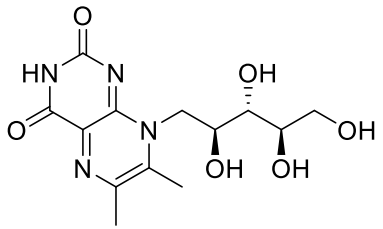
D-Ribityl lumazine 6,7-diMe (2)

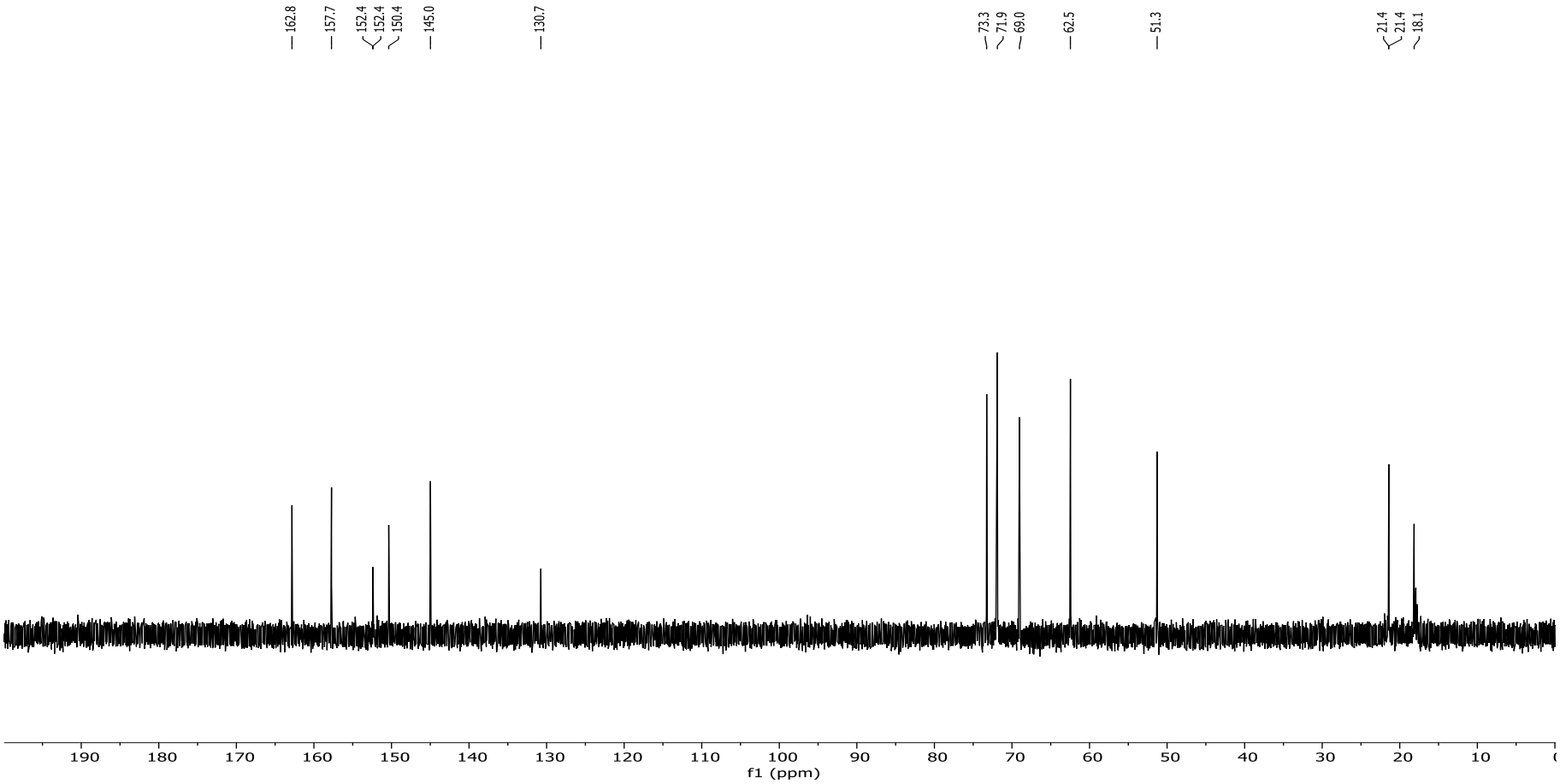
^{1}H NMR (D_2O, 500 MHz)



D-Ribityl lumazine 6,7-diMe (2)

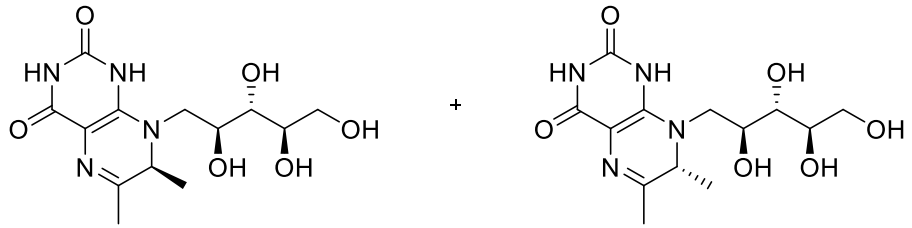
^{13}C NMR (D_2O, 125 MHz)

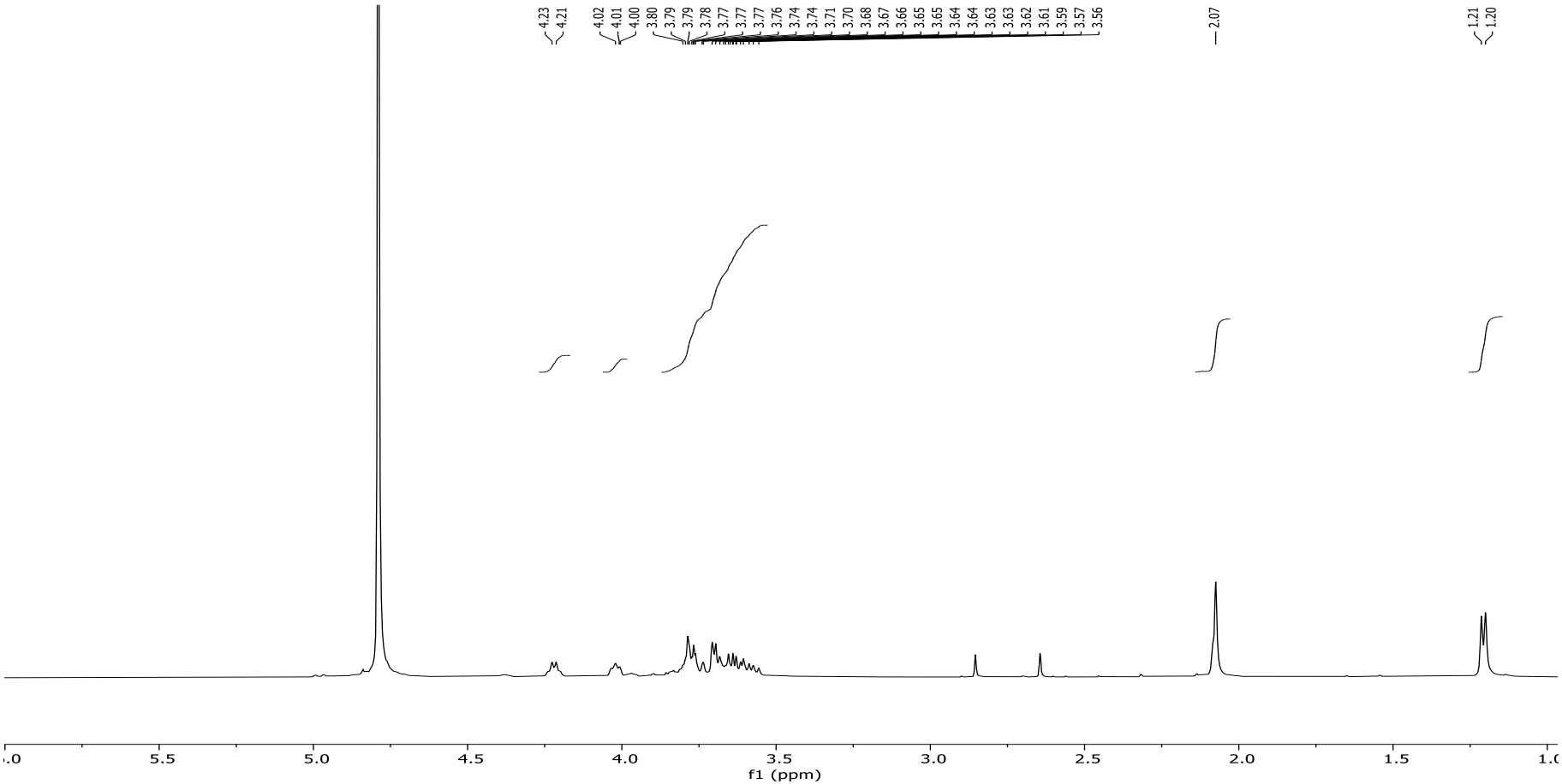




Reduced D-ribityl lumazine 6,7-diMe (11)

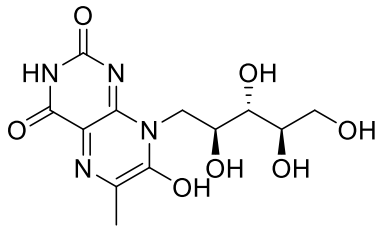
^{1}H NMR (D_2O, 500 MHz)

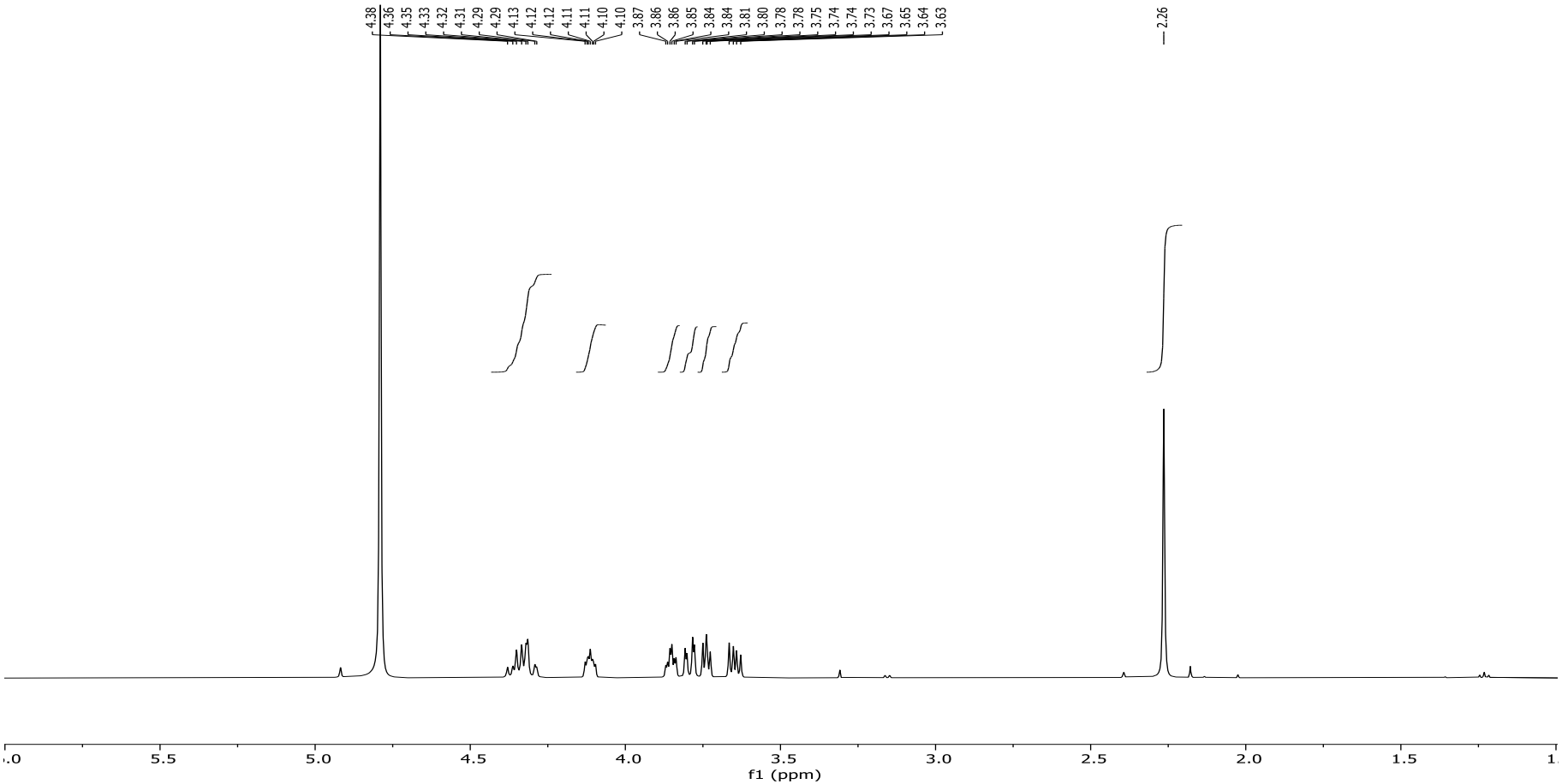




D-Ribityl lumazine 6-Me-7-OH (3a)

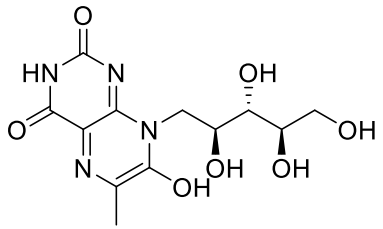
^{1}H NMR (D_2O, 500 MHz)

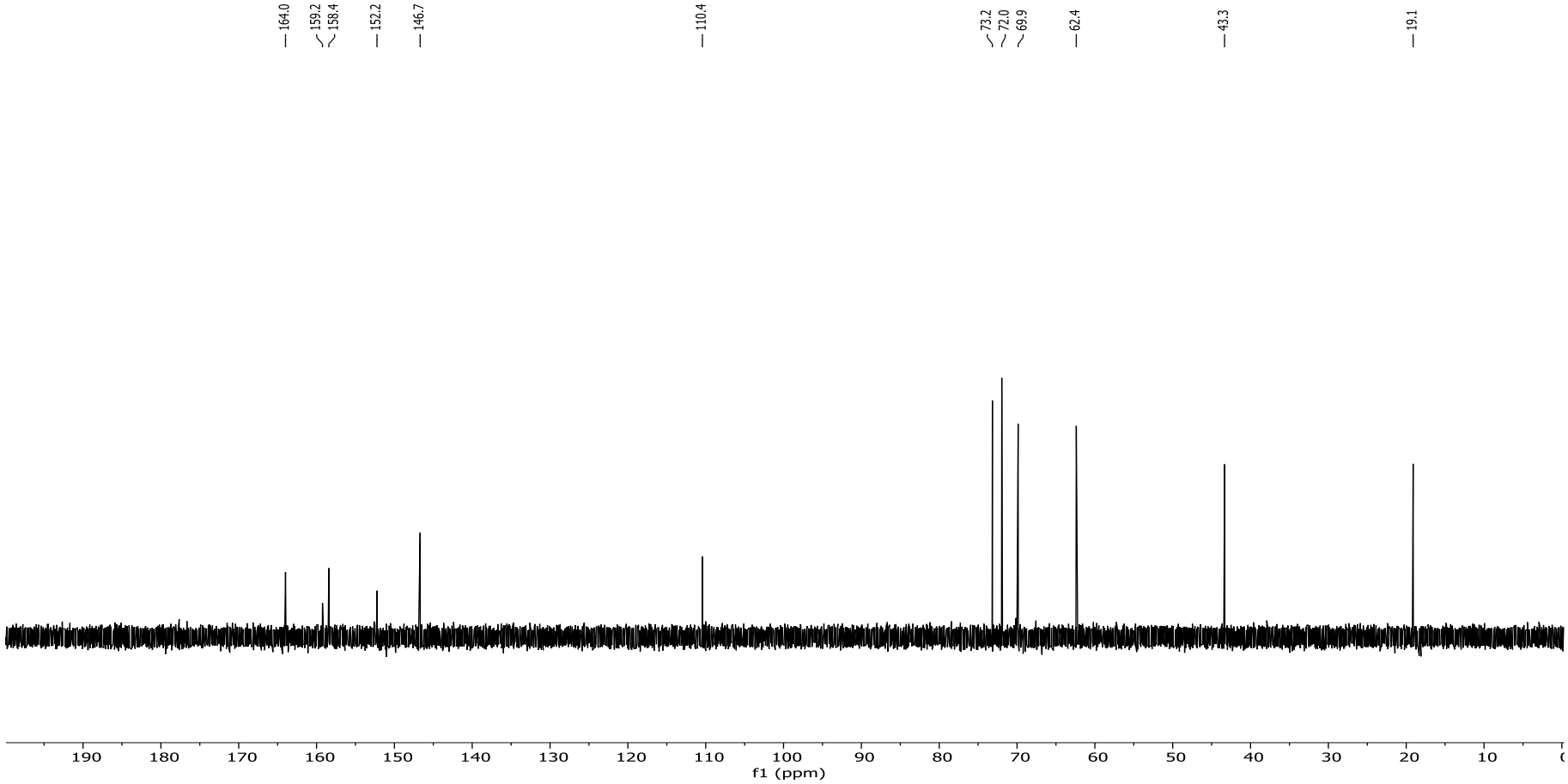




D-Ribityl lumazine 6-Me-7-OH (3a)

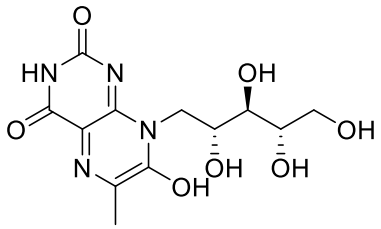
^{13}C NMR (D_2O, 125 MHz)

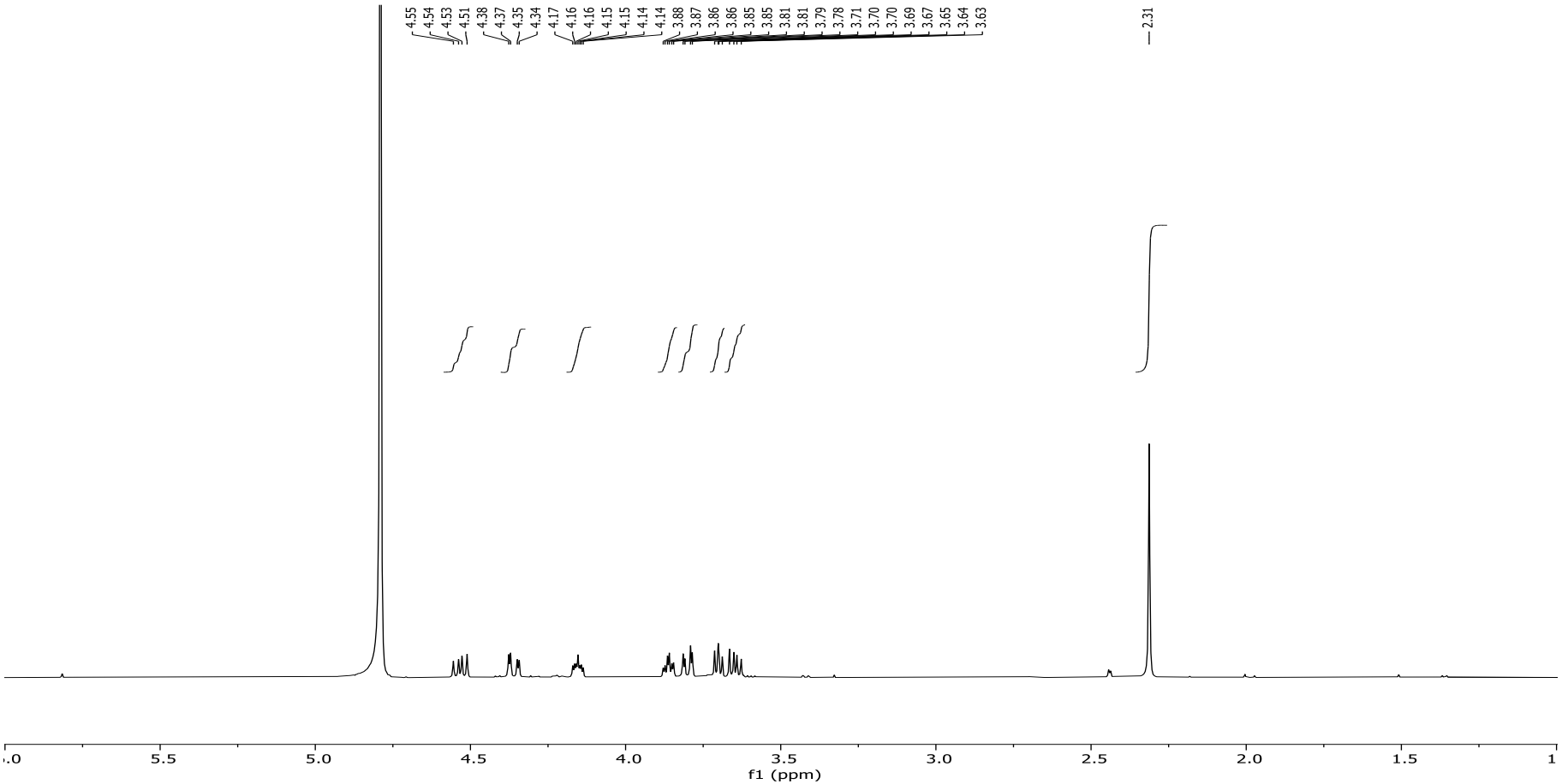




L-Ribityl lumazine 6-Me-7-OH (3b)

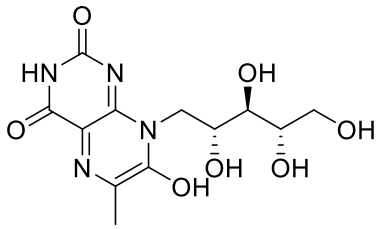
^{1}H NMR (D_2O, 500 MHz)

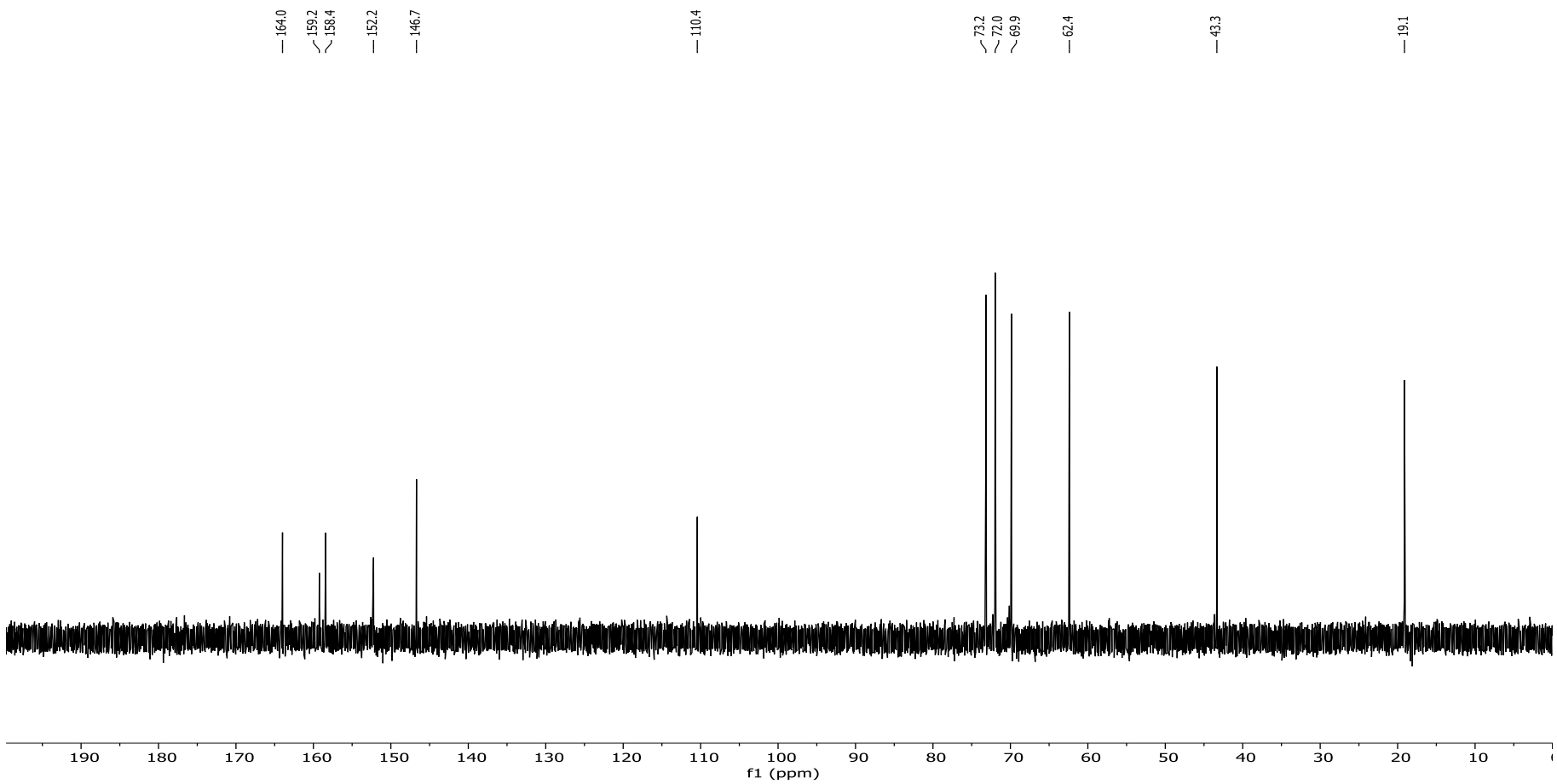




L-Ribityl lumazine 6-Me-7-OH (3b)

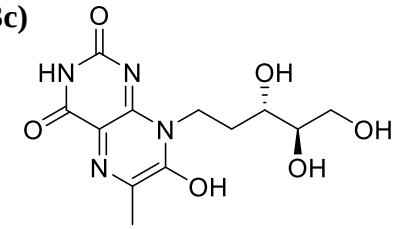
^{13}C NMR (D_2O, 125 MHz)

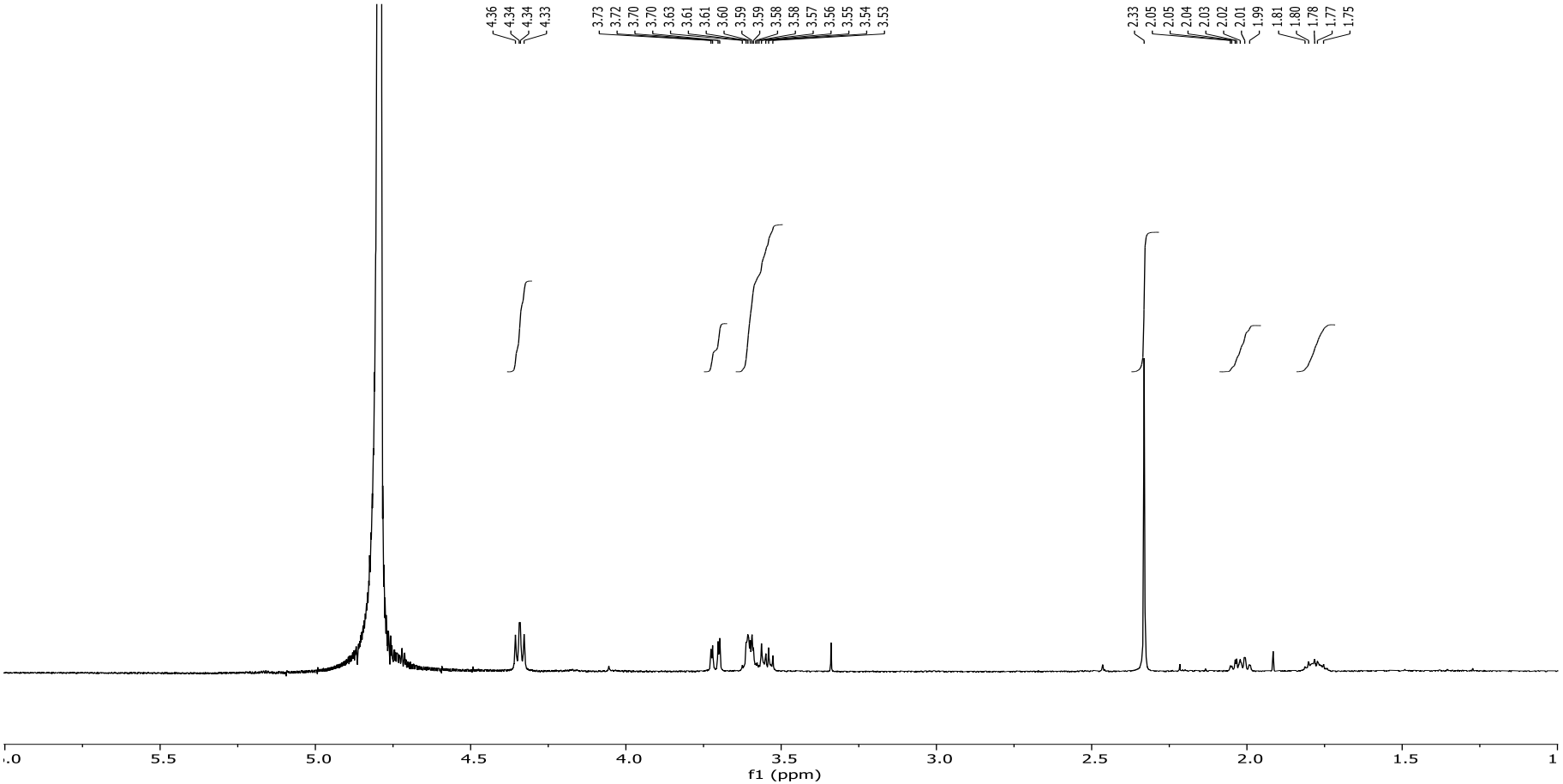




2-Deoxy-D-ribityl lumazine 6-Me-7-OH (3c)

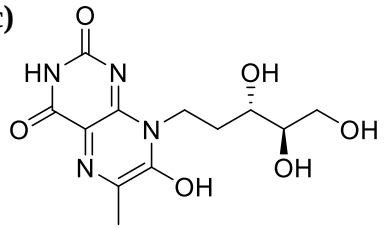
^{1}H NMR (D_2O, 500 MHz)

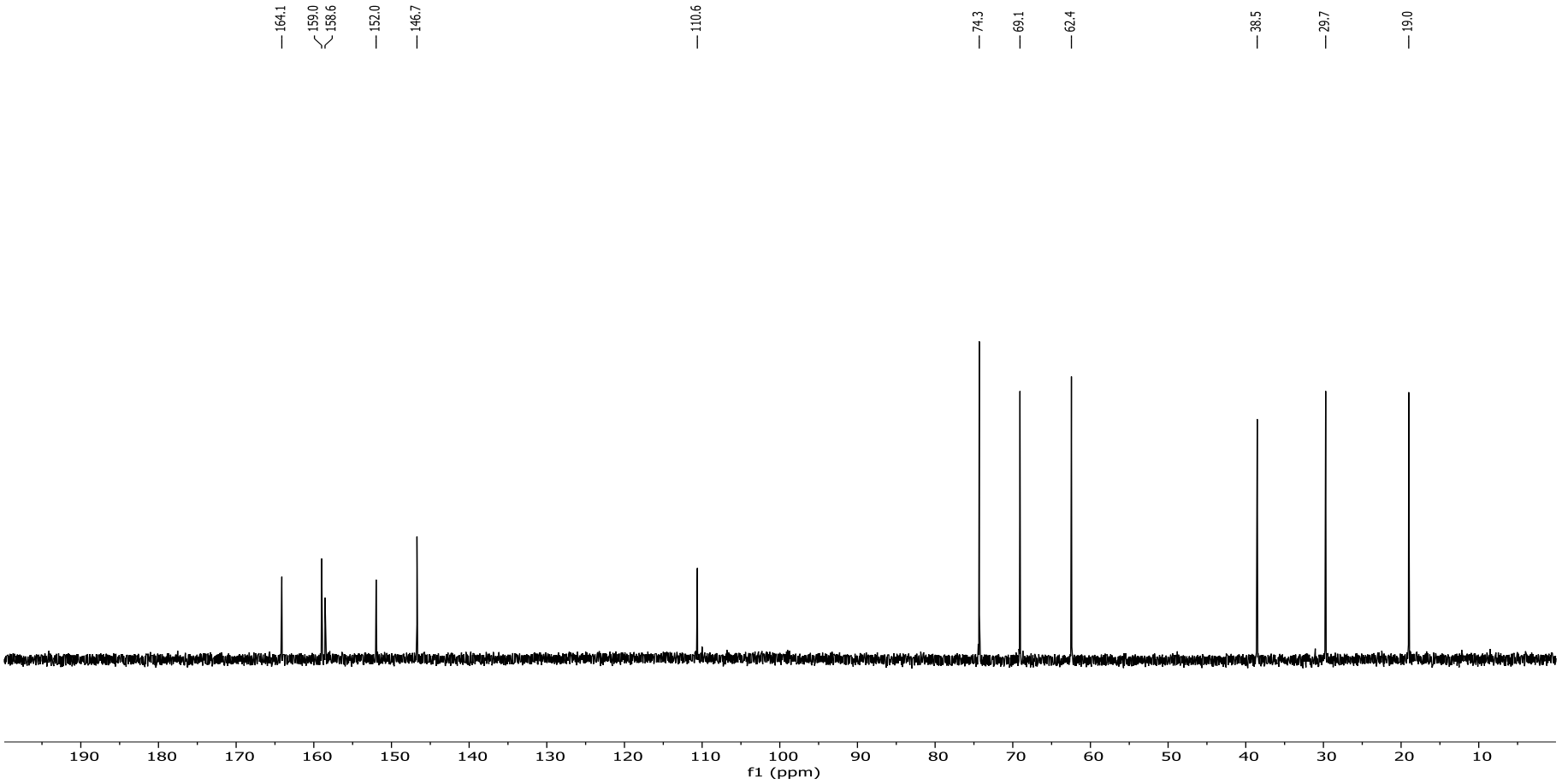




2-Deoxy-D-ribityl lumazine 6-Me-7-OH (3c)

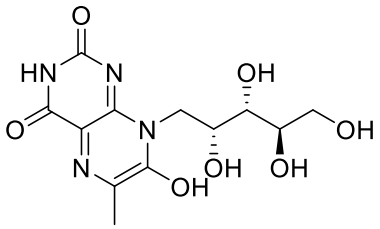
^{13}C NMR (D_2O, 125 MHz)

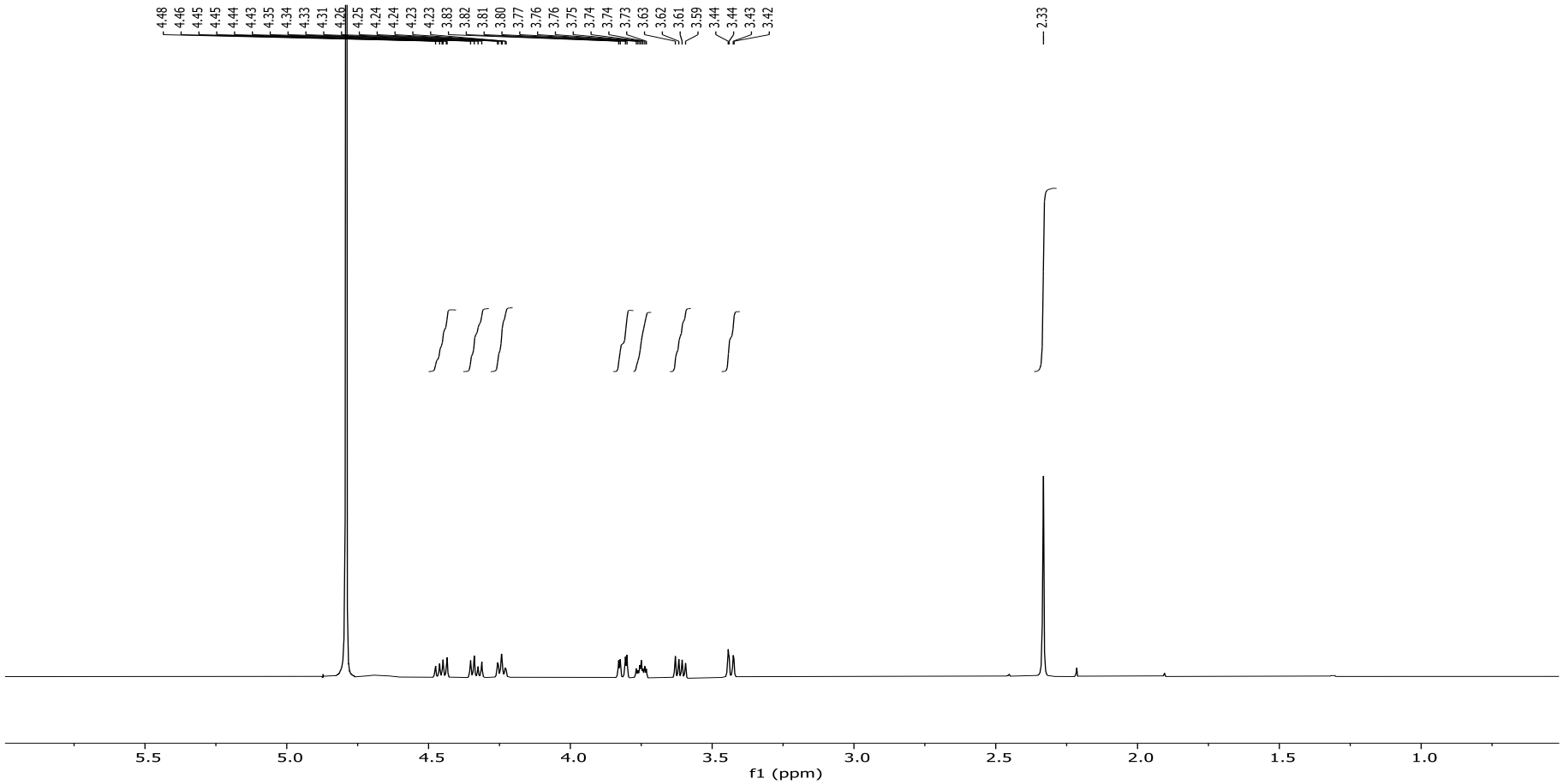




D-Arabinityl lumazine 6-Me-7-OH (3d)

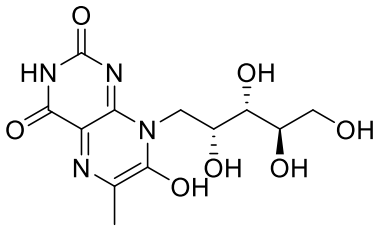
^{1}H NMR (D_2O, 500 MHz)

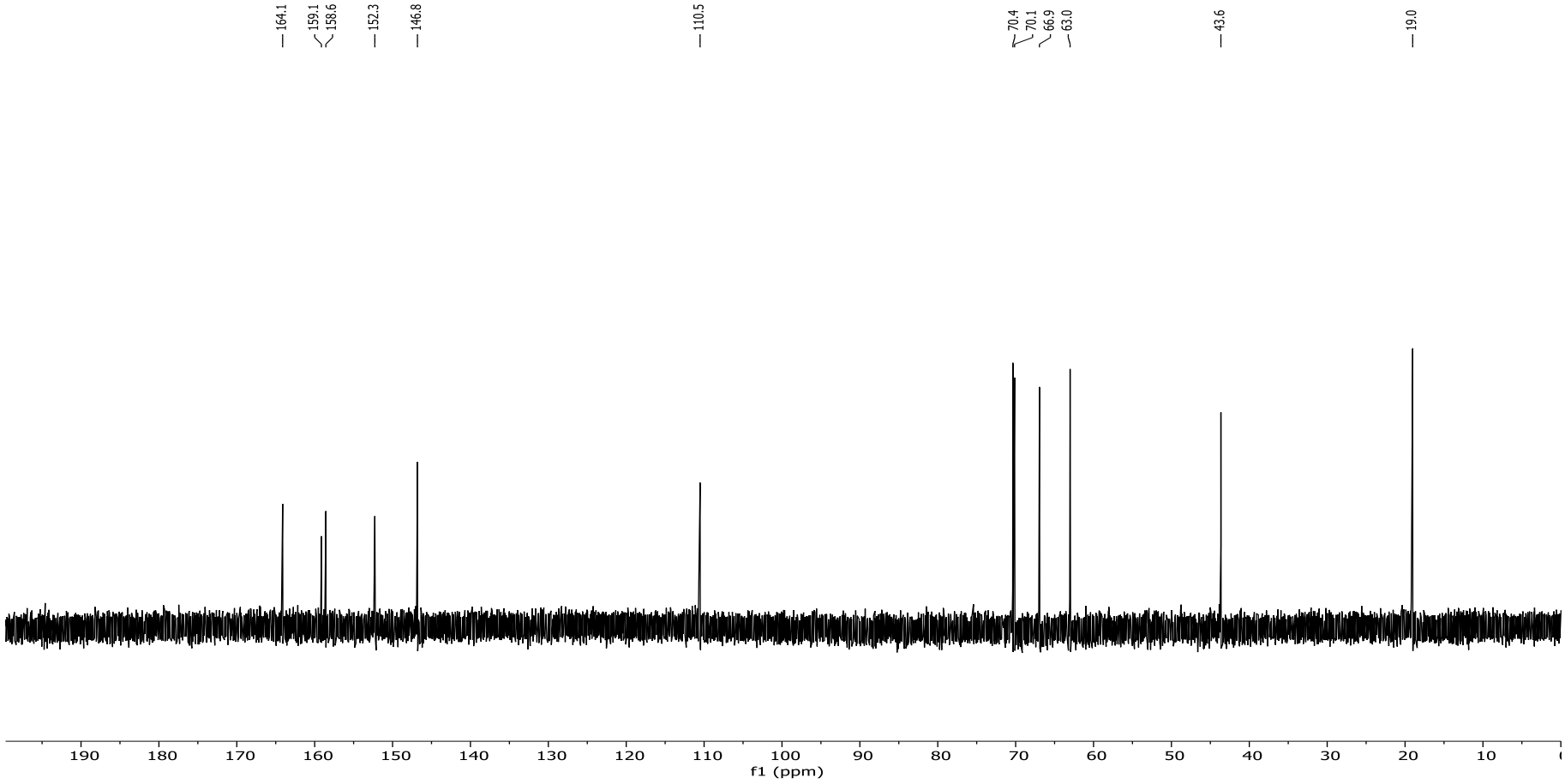




D-Arabinityl lumazine 6-Me-7-OH (3d)

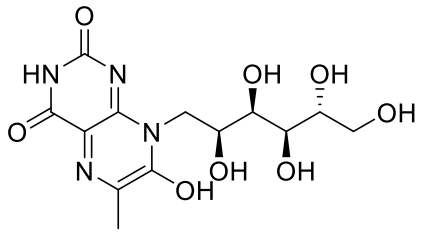
^{13}C NMR (D_2O, 125 MHz)

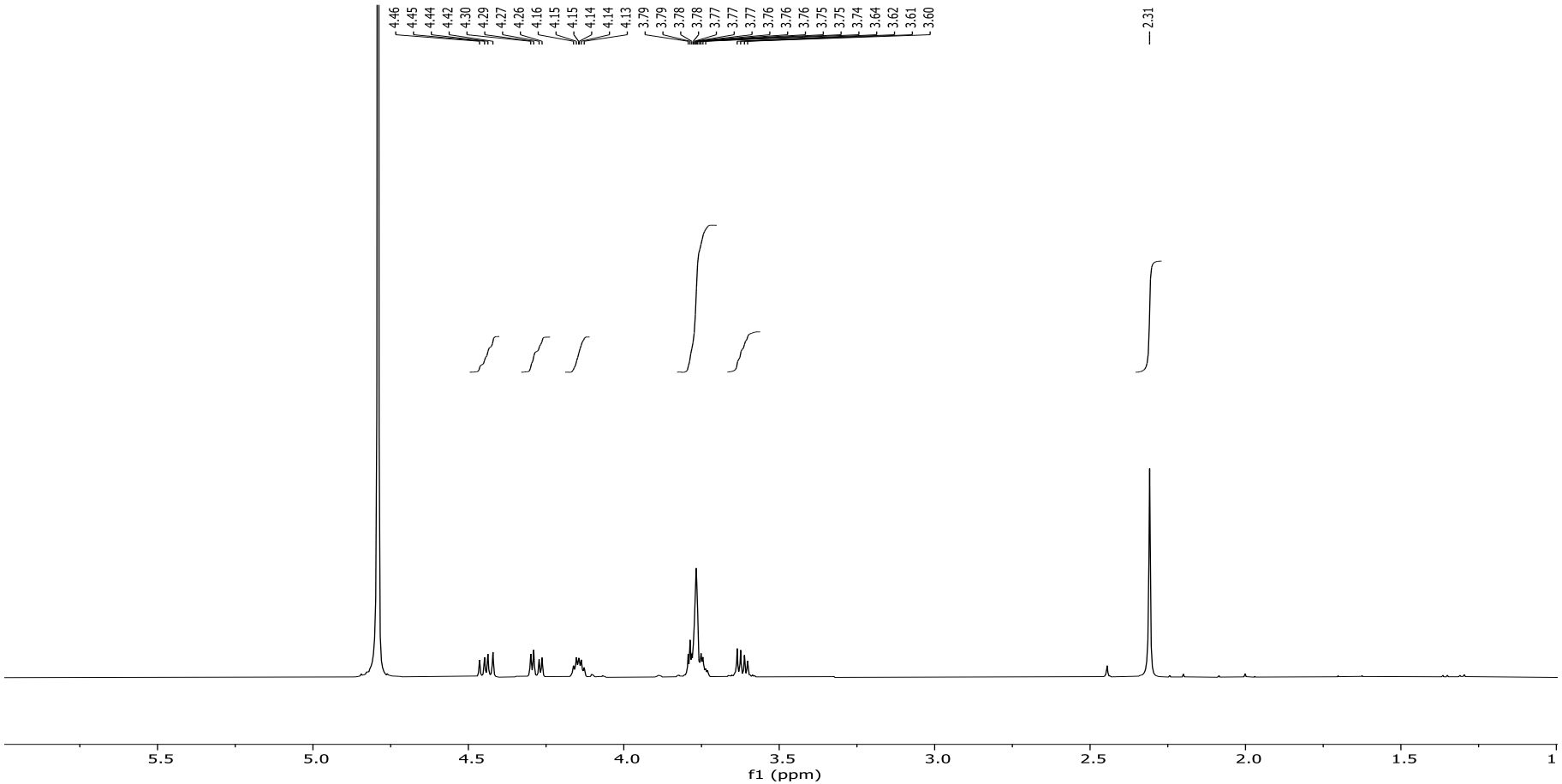




D-Glucityl lumazine 6-Me-7-OH (3e)

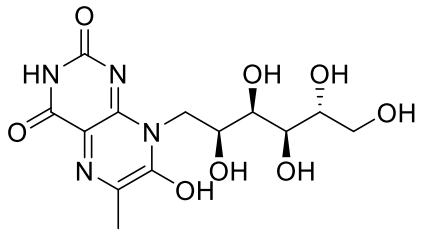
^{1}H NMR (D_2O, 500 MHz)

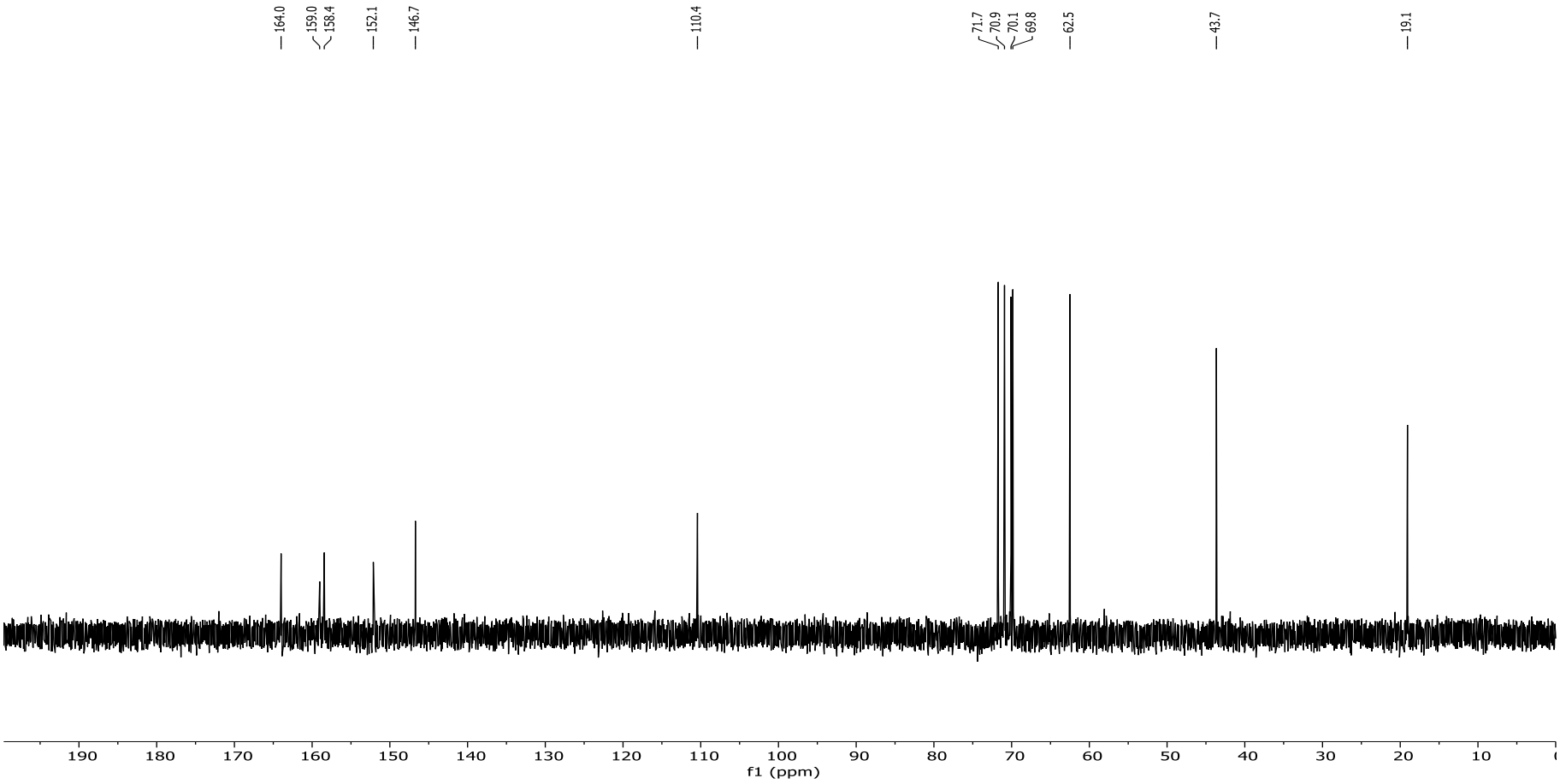




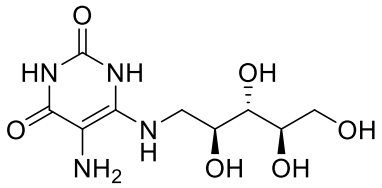
D-Glucityl lumazine 6-Me-7-OH (3e)

^{13}C NMR (D_2O, 125 MHz)

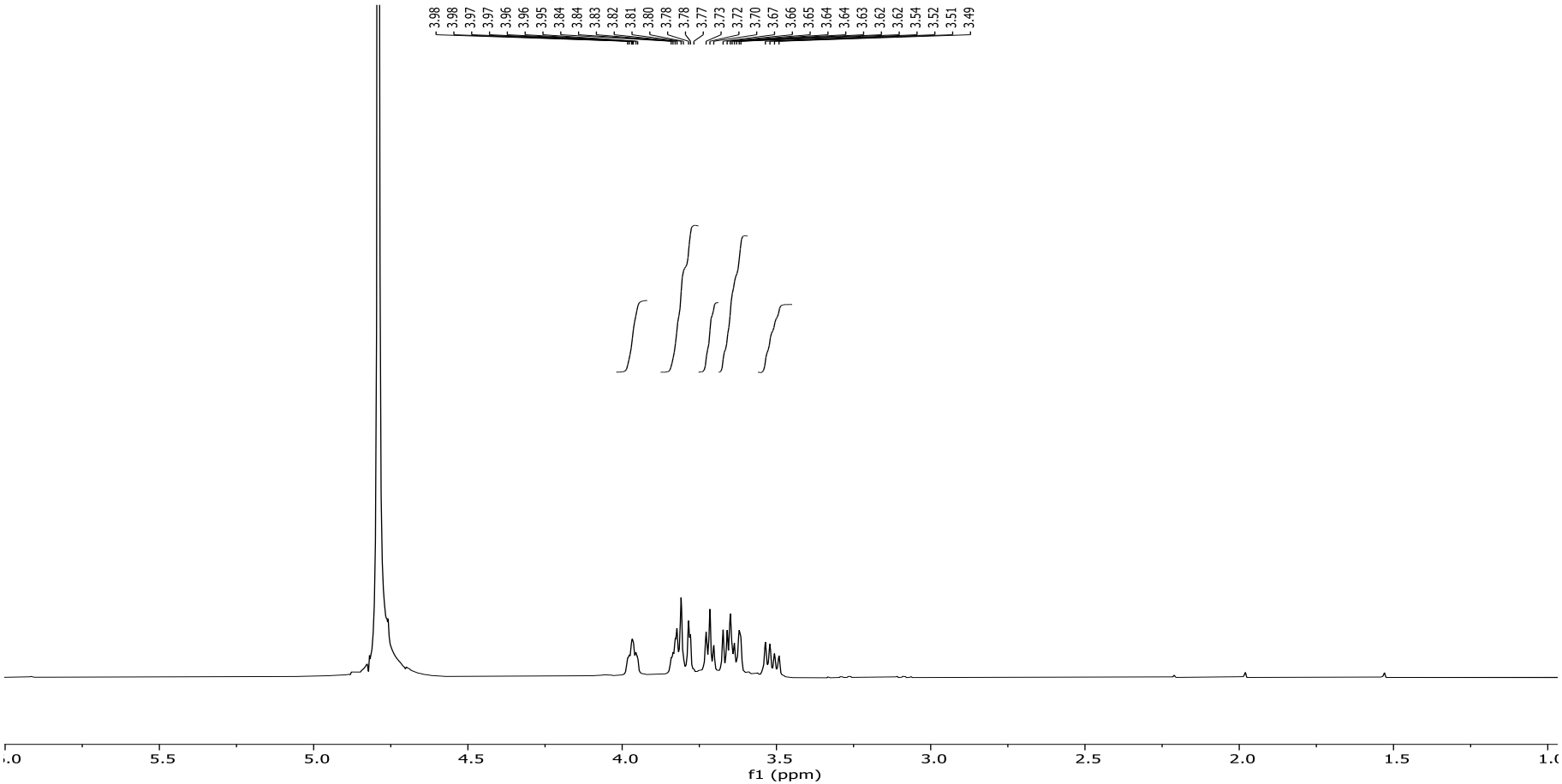




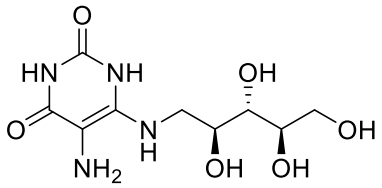
5-Amino-6-((D-ribityl)amino)uracil (12a)



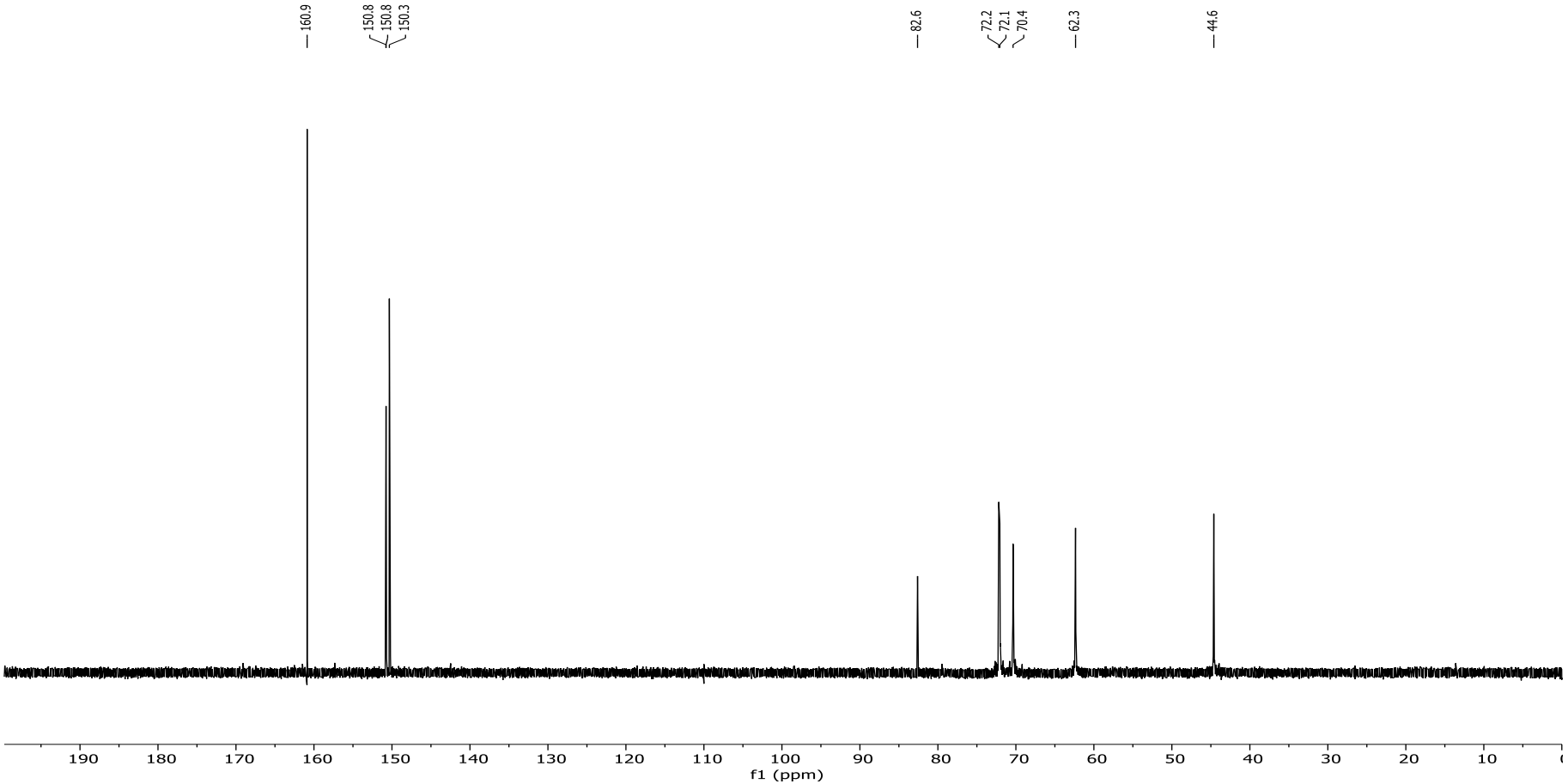
^{1}H NMR (D_2O, 500 MHz)



5-Amino-6-((D-ribityl)amino)uracil (12a)

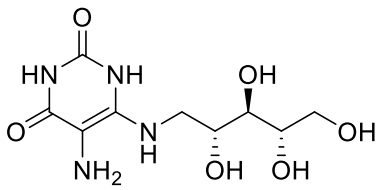


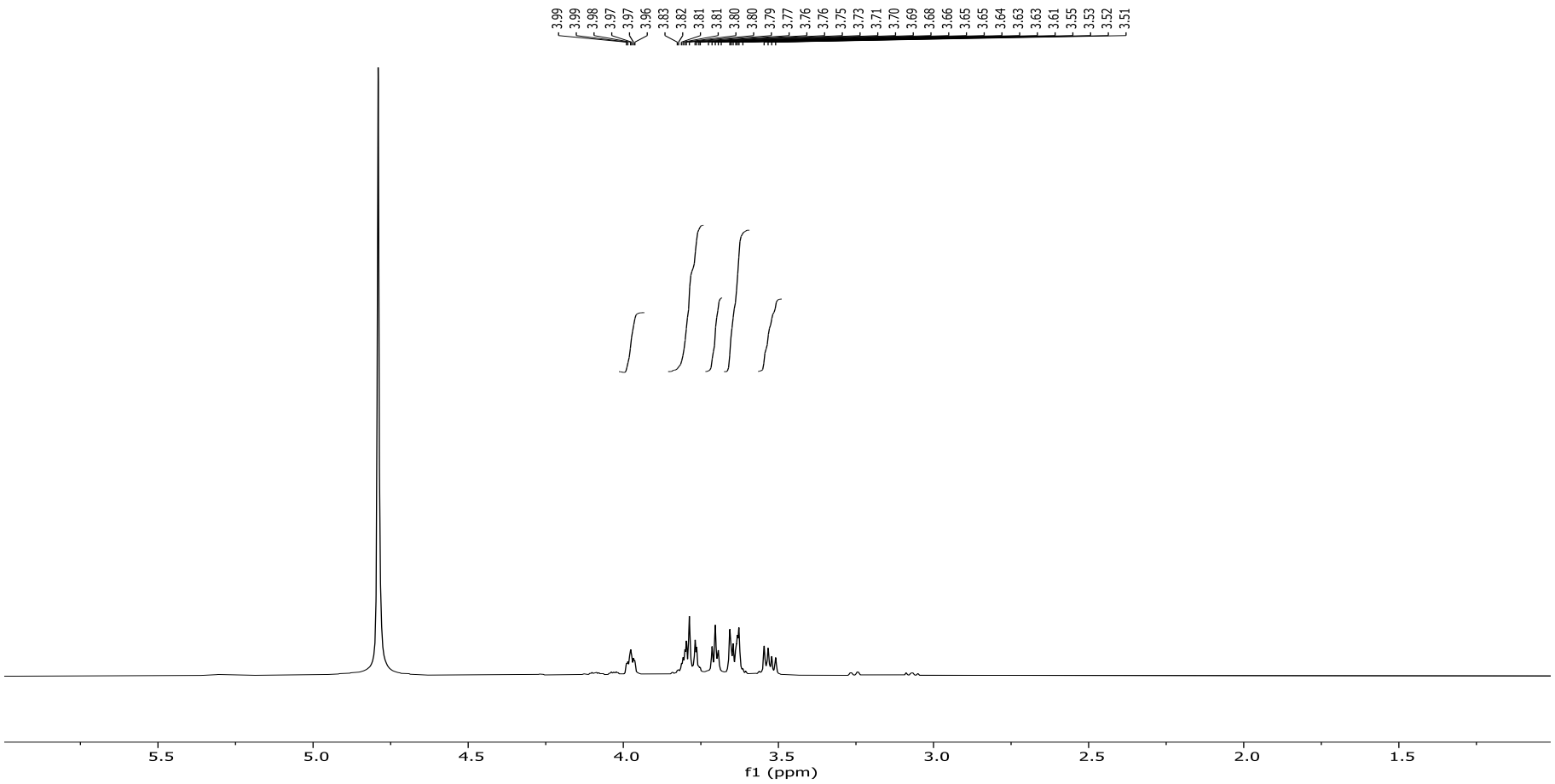
^{13}C NMR (D_2O, 125 MHz)



5-Amino-6-((L-ribityl)amino)uracil (12a)

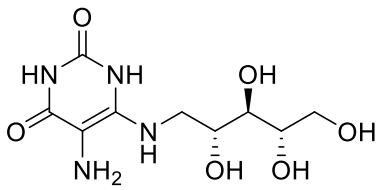
^{1}H NMR (D_2O, 500 MHz)

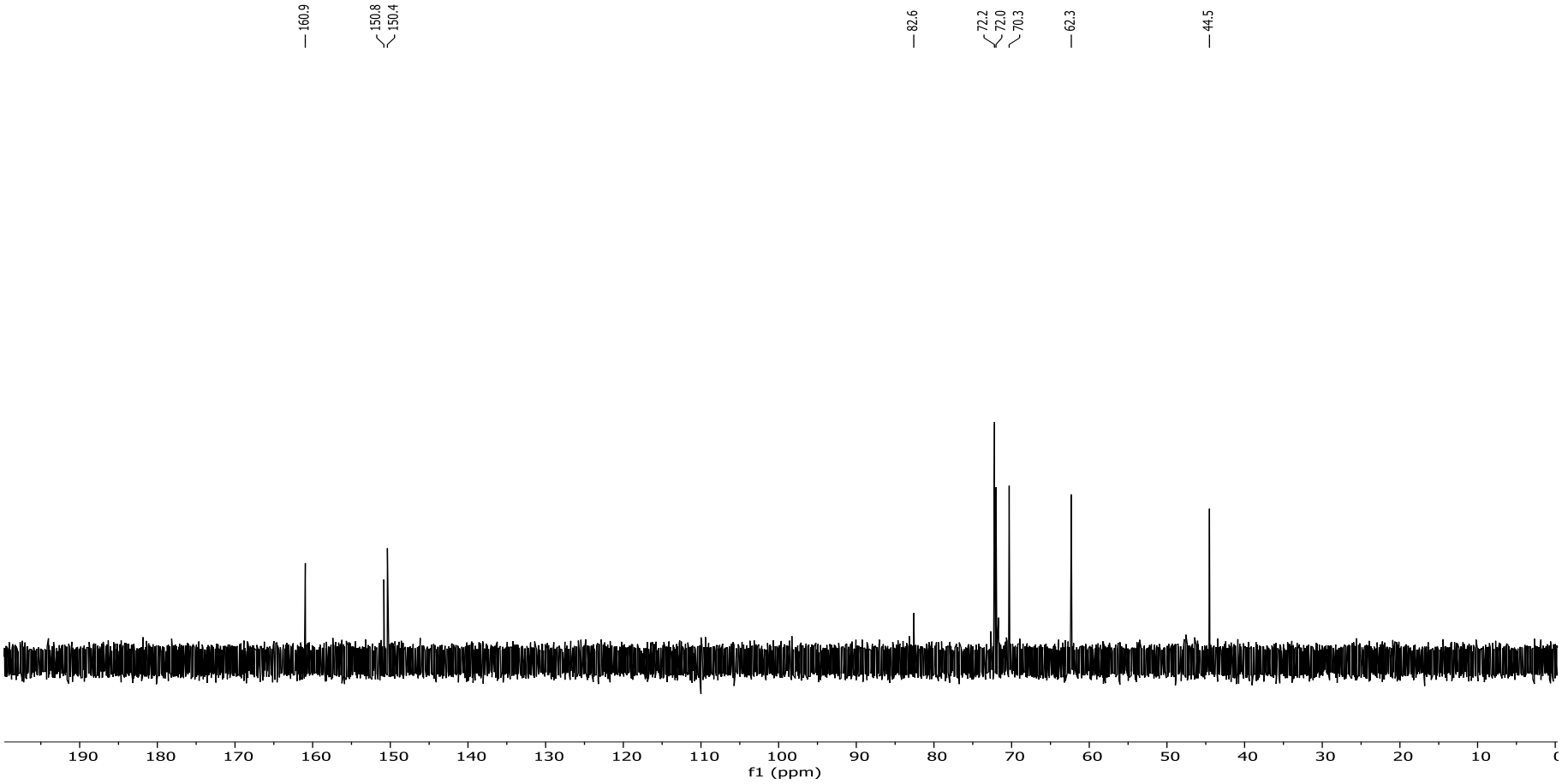




5-Amino-6-((L-ribityl)amino)uracil (12a)

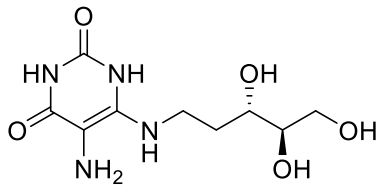
^{13}C NMR (D_2O, 125 MHz)

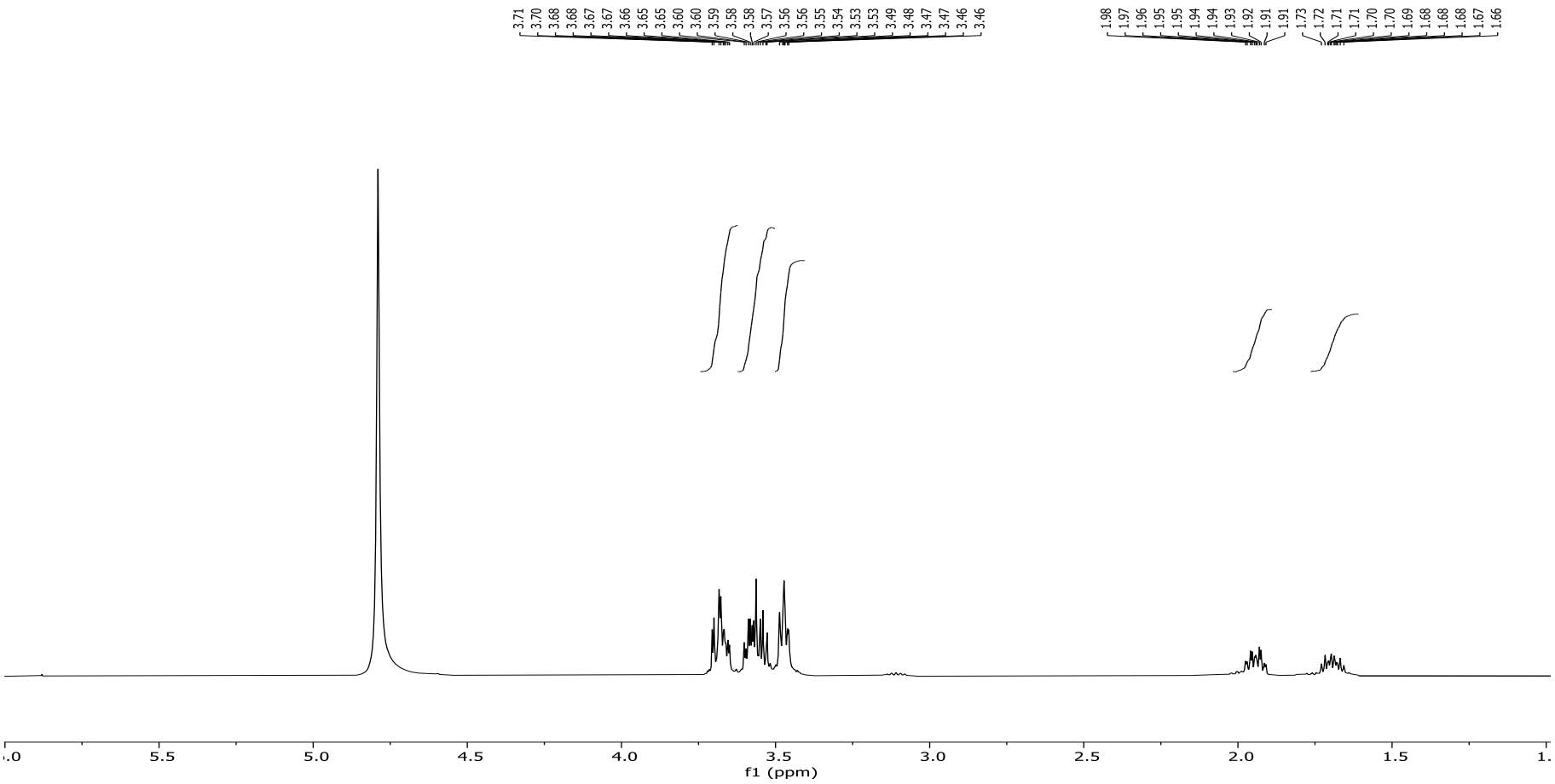




5-Amino-6-((2′-deoxy-D-ribityl)amino)uracil (12c)

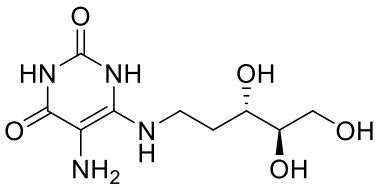
^{1}H NMR (D_2O, 500 MHz)

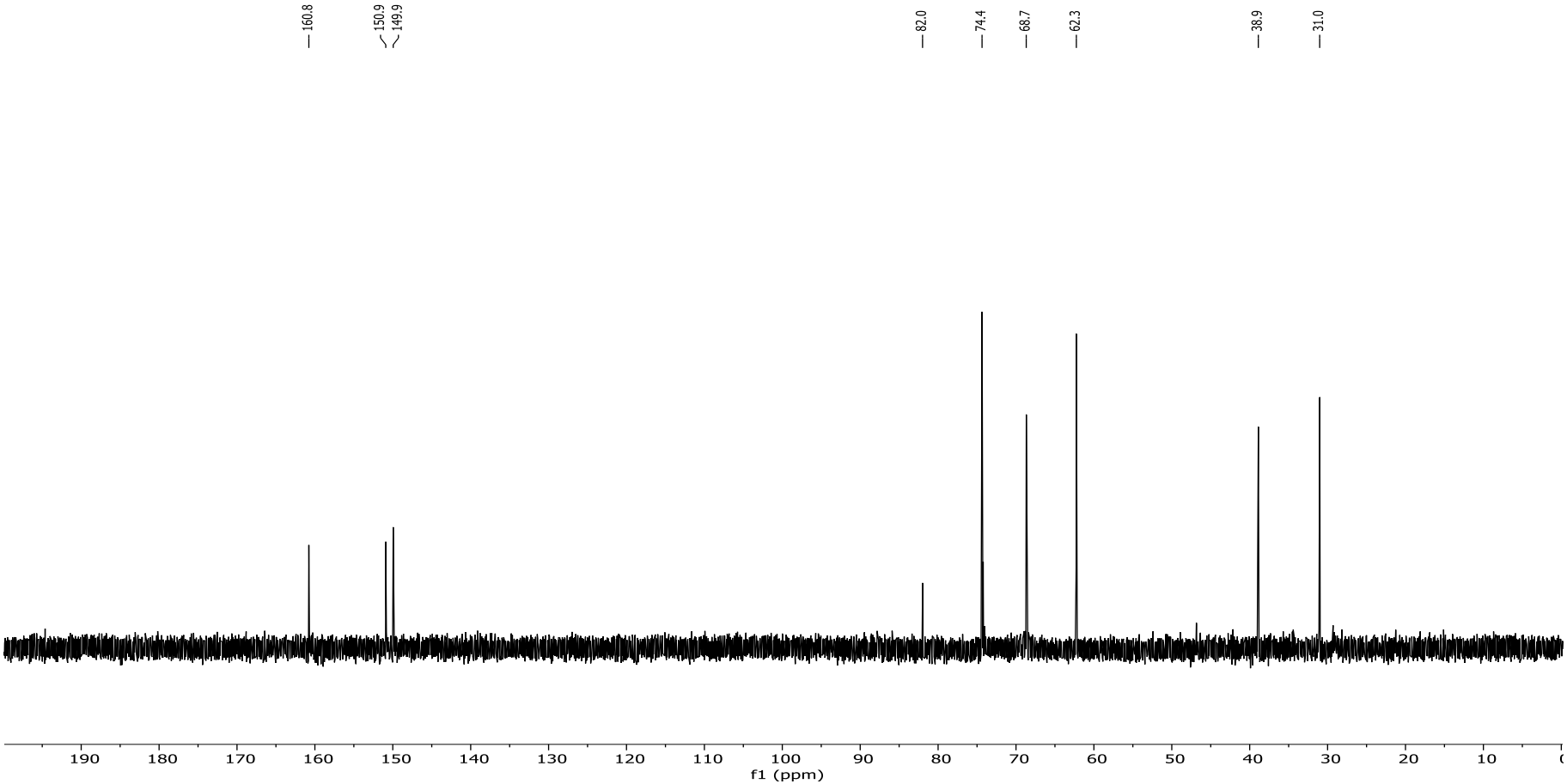




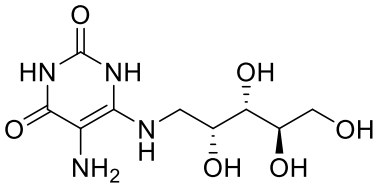
5-Amino-6-((2′-deoxy-D-ribityl)amino)uracil (12c)

^{13}C NMR (D_2O, 125 MHz)

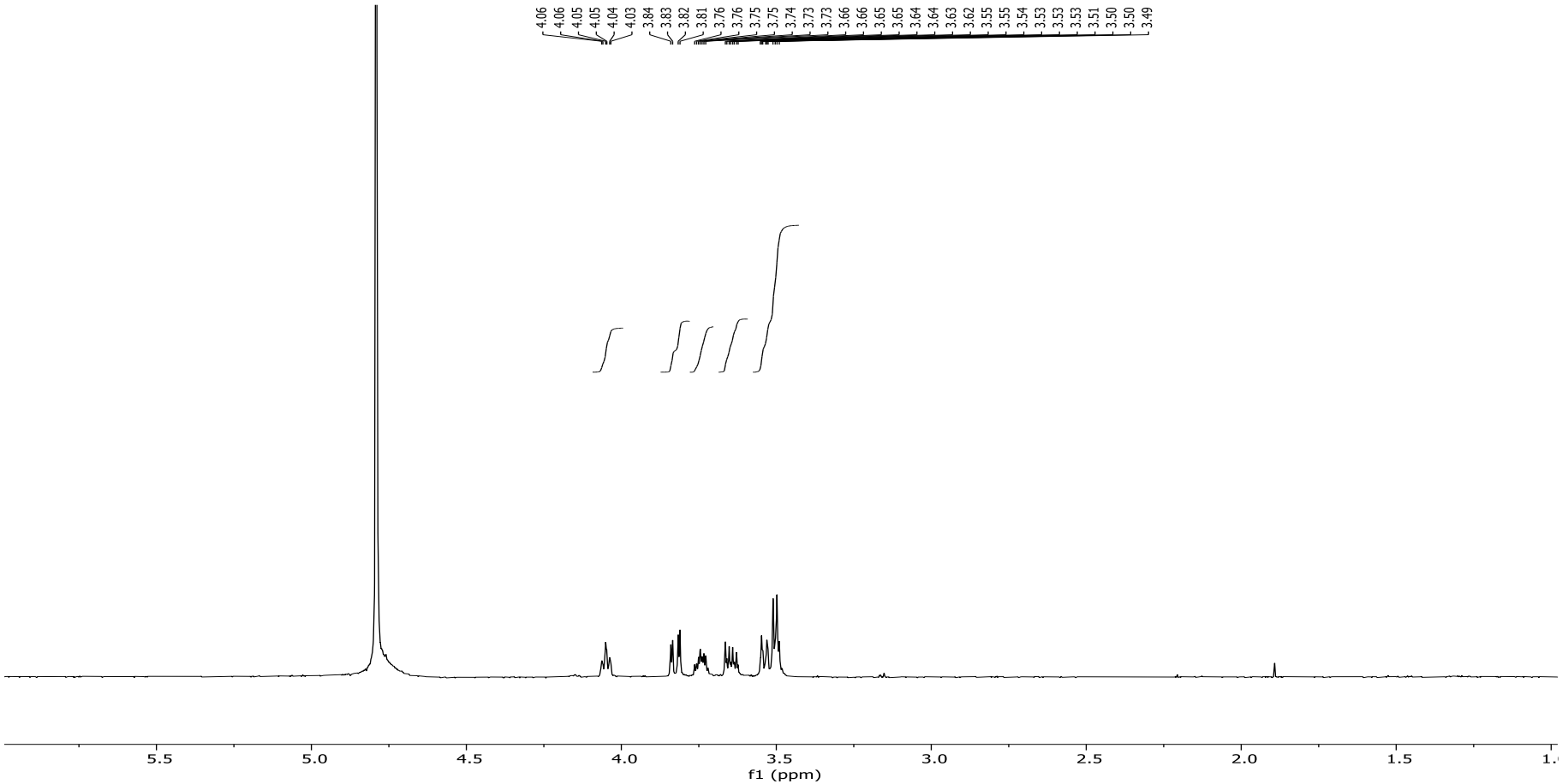




5-Amino-6-((D-arabinityl)amino)uracil (12d)

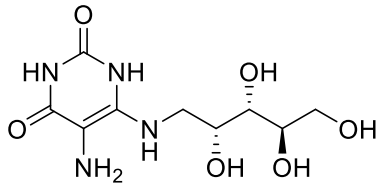


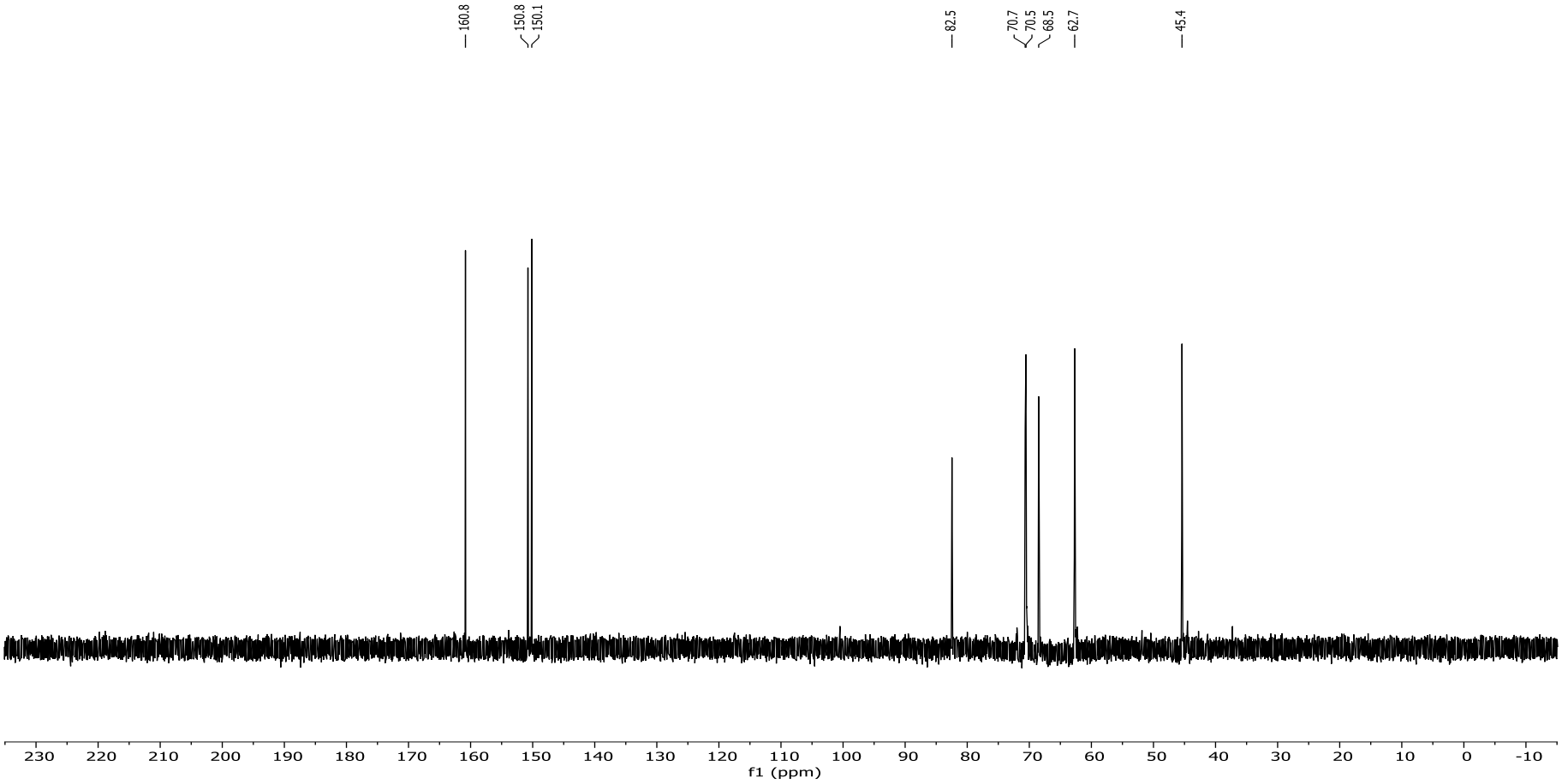
^{1}H NMR (D_2O, 500 MHz)



5-Amino-6-((D-arabinityl)amino)uracil (12d)

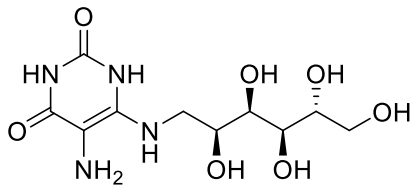
^{13}C NMR (D_2O, 125 MHz)

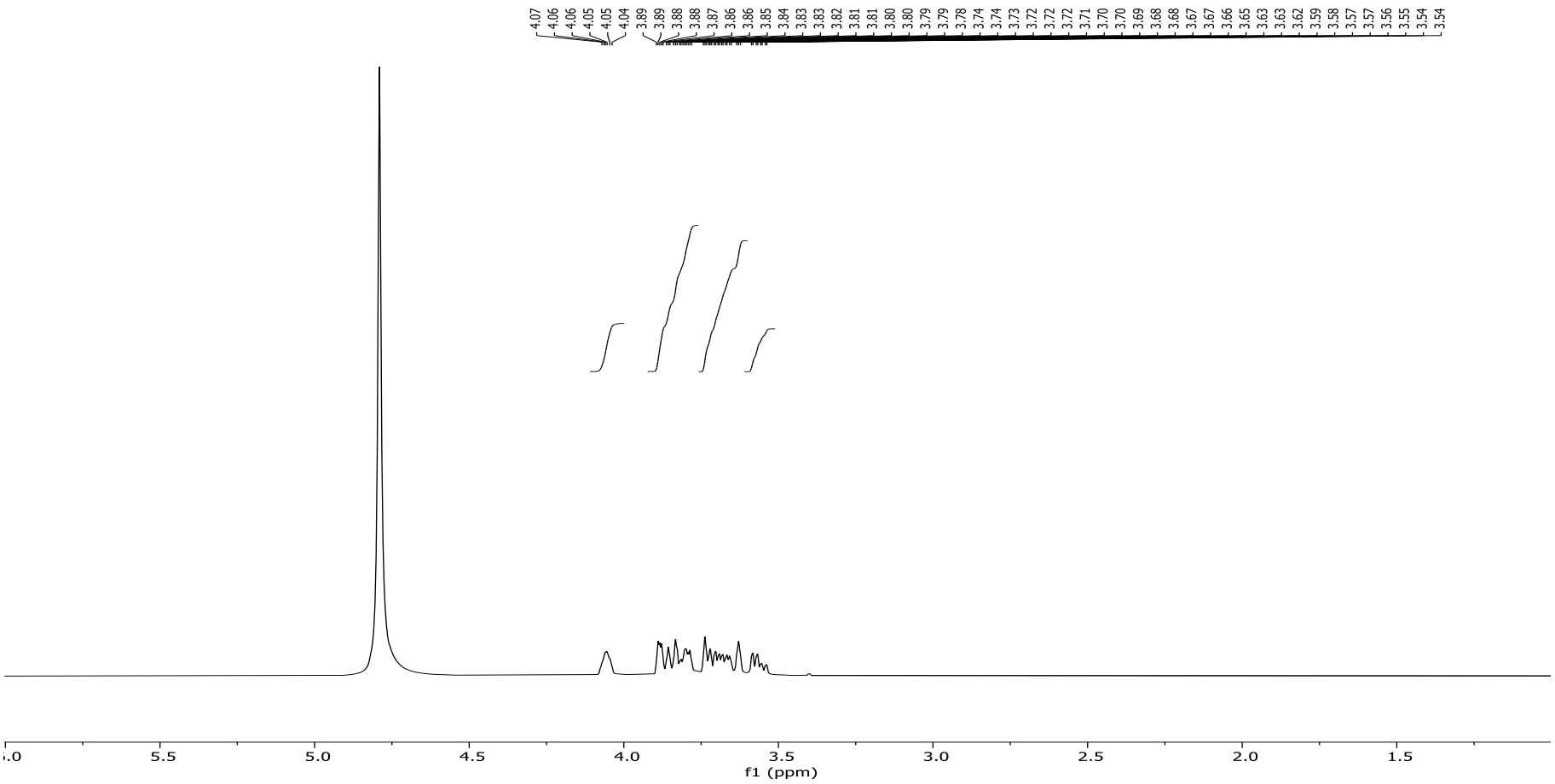




5-Amino-6-((D-glucityl)amino)uracil (12e)

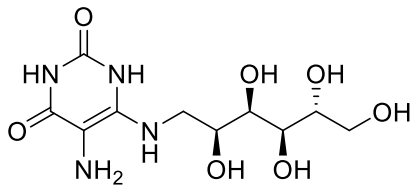
^{1}H NMR (D_2O, 500 MHz)

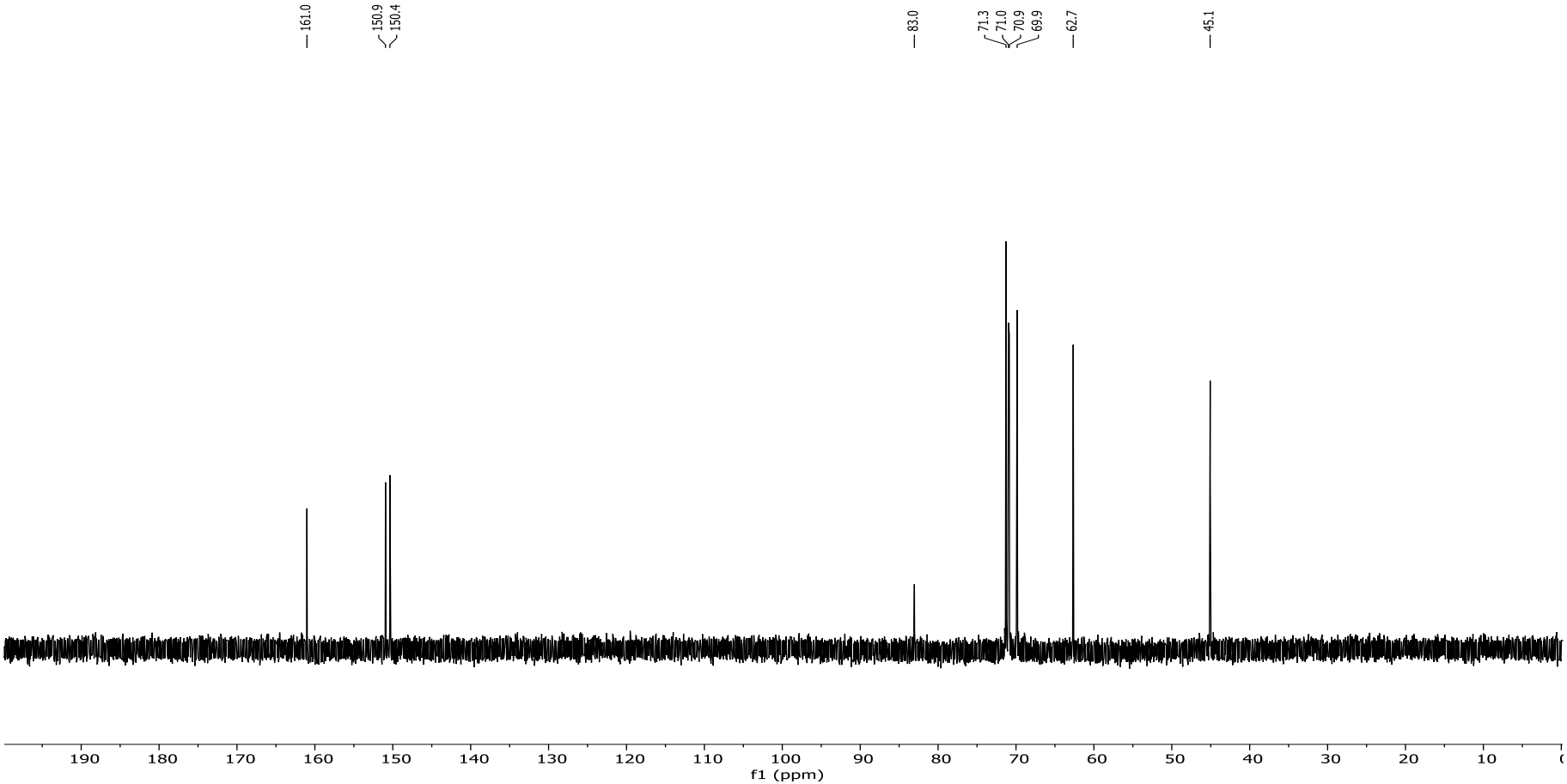




5-Amino-6-((D-glucityl)amino)uracil (12e)

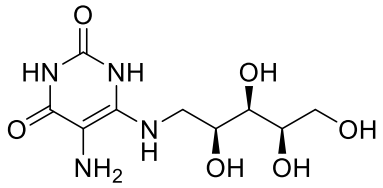
^{13}C NMR (D_2O, 125 MHz)

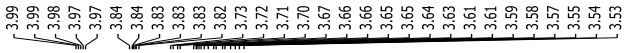


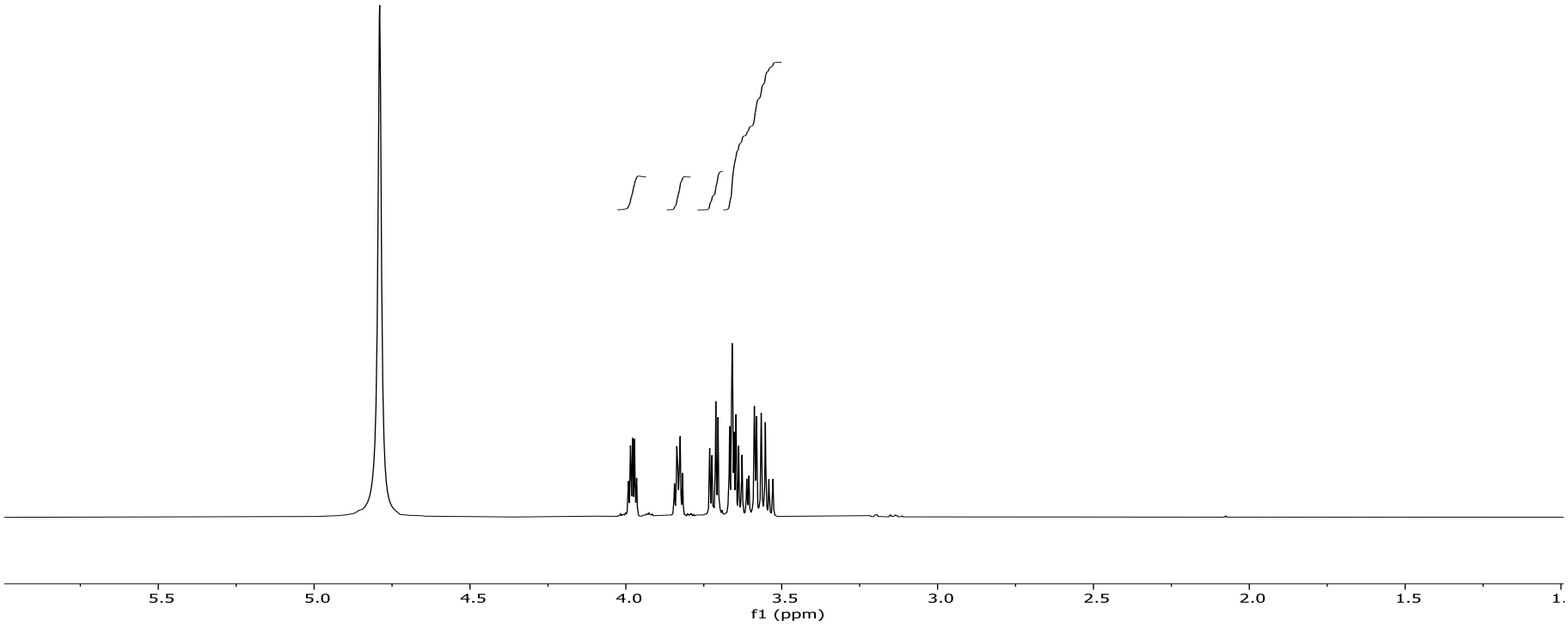


5-Amino-6-((D-xylityl)amino)uracil (12f)

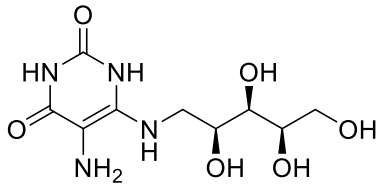
^{1}H NMR (D_2O, 500 MHz)



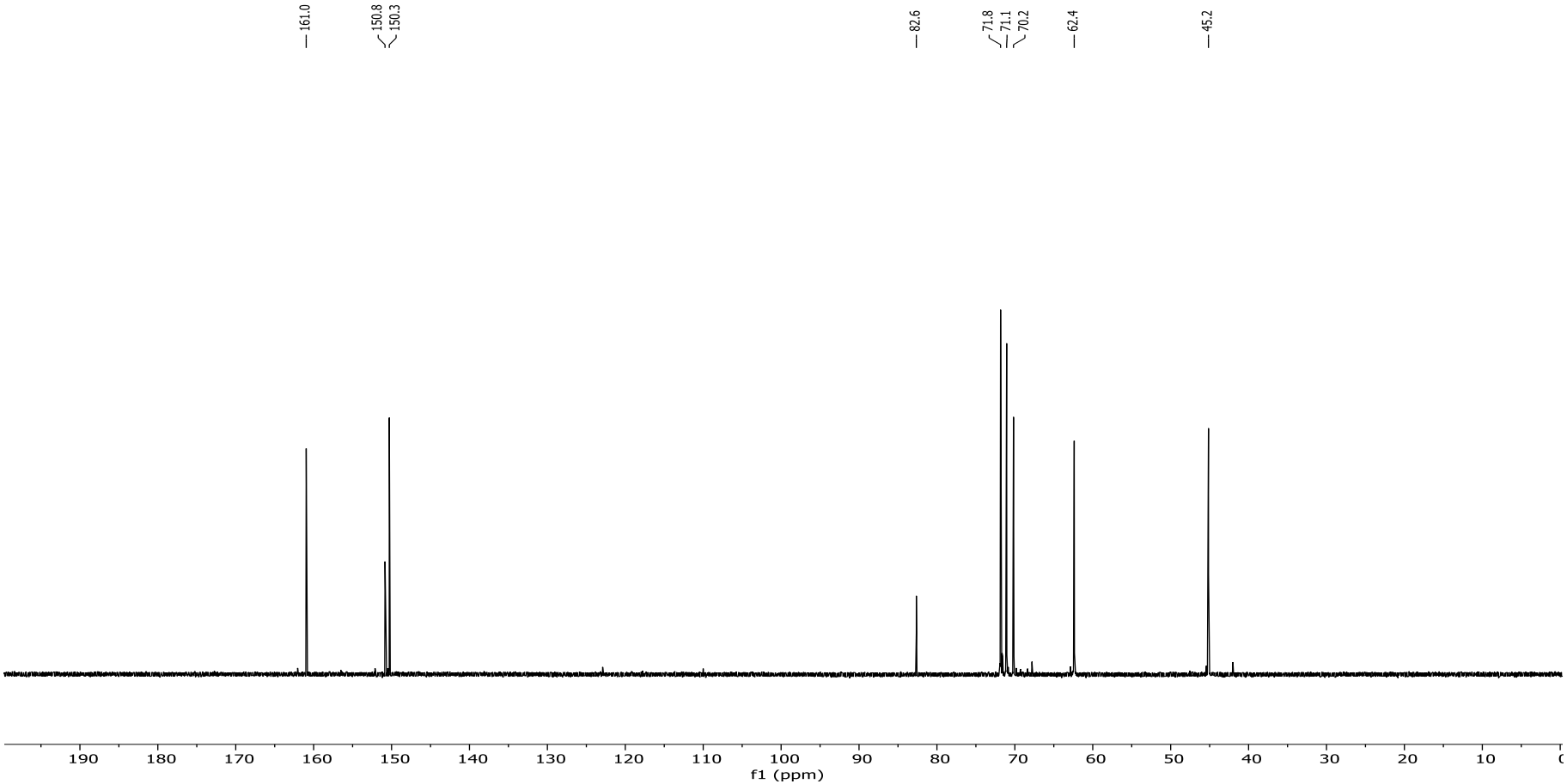




5-Amino-6-((D-xylityl)amino)uracil (12f)

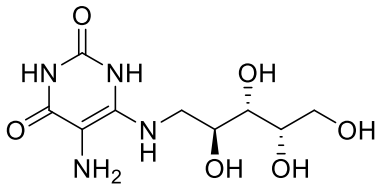


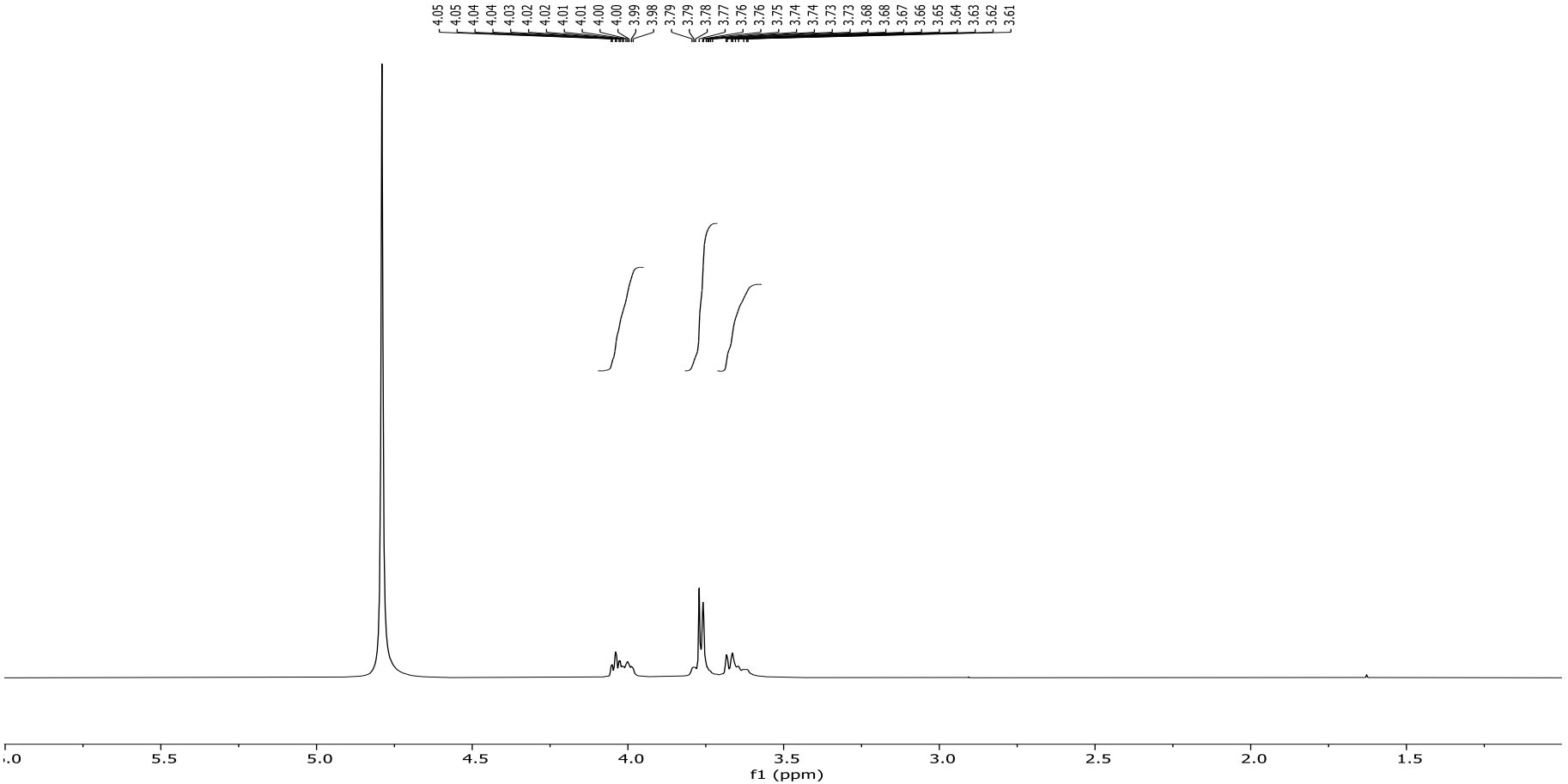
^{13}C NMR (D_2O, 125 MHz)



5-Amino-6-((L-lyxityl)amino)uracil (12g)

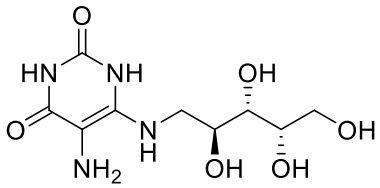
^{1}H NMR (D_2O, 500 MHz)

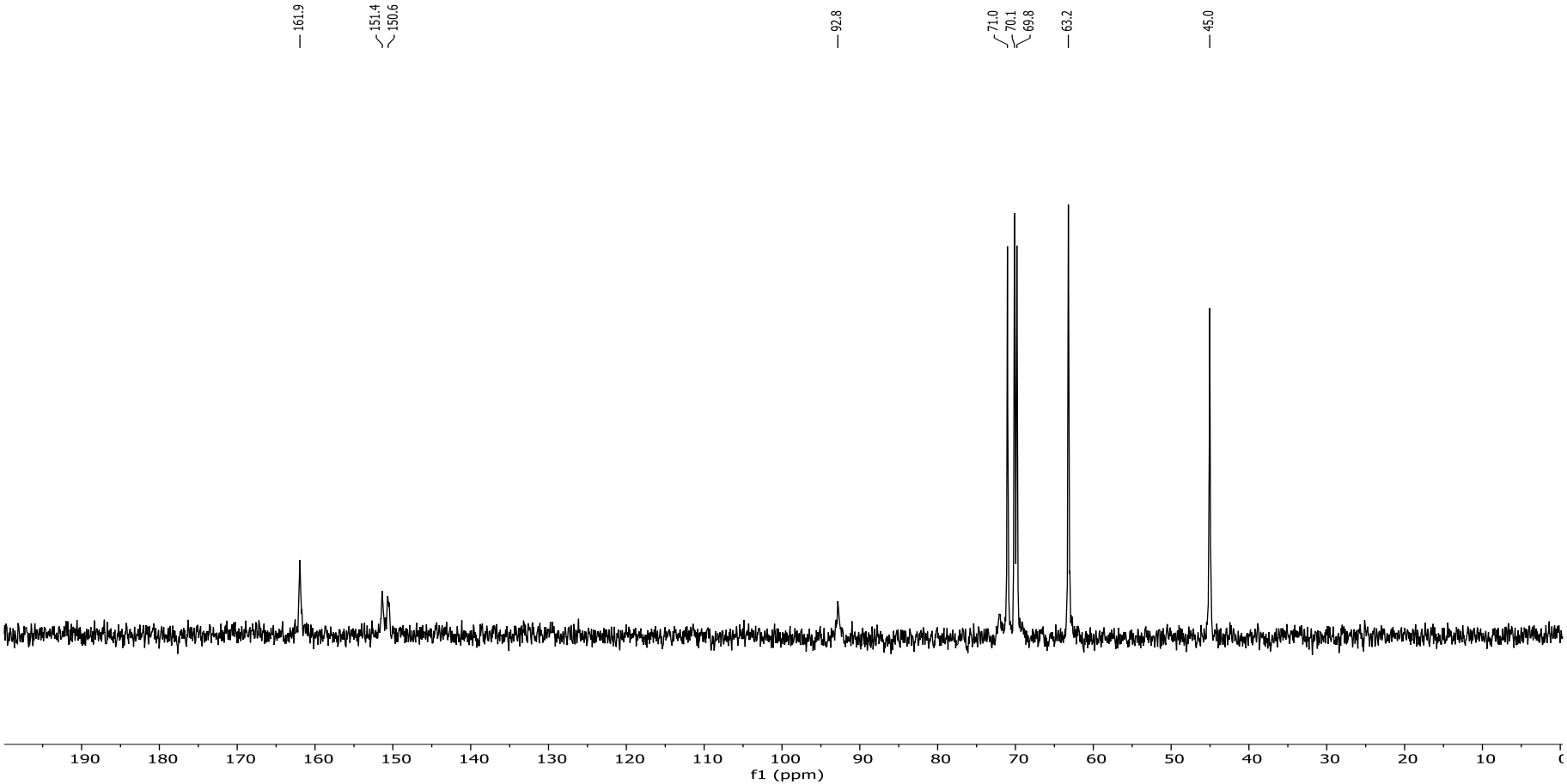




5-Amino-6-((L-lyxityl)amino)uracil (12g)

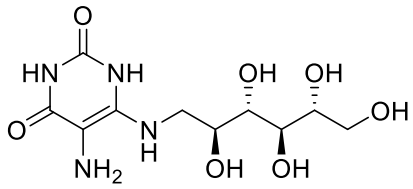
^{13}C NMR (D_2O, 125 MHz)

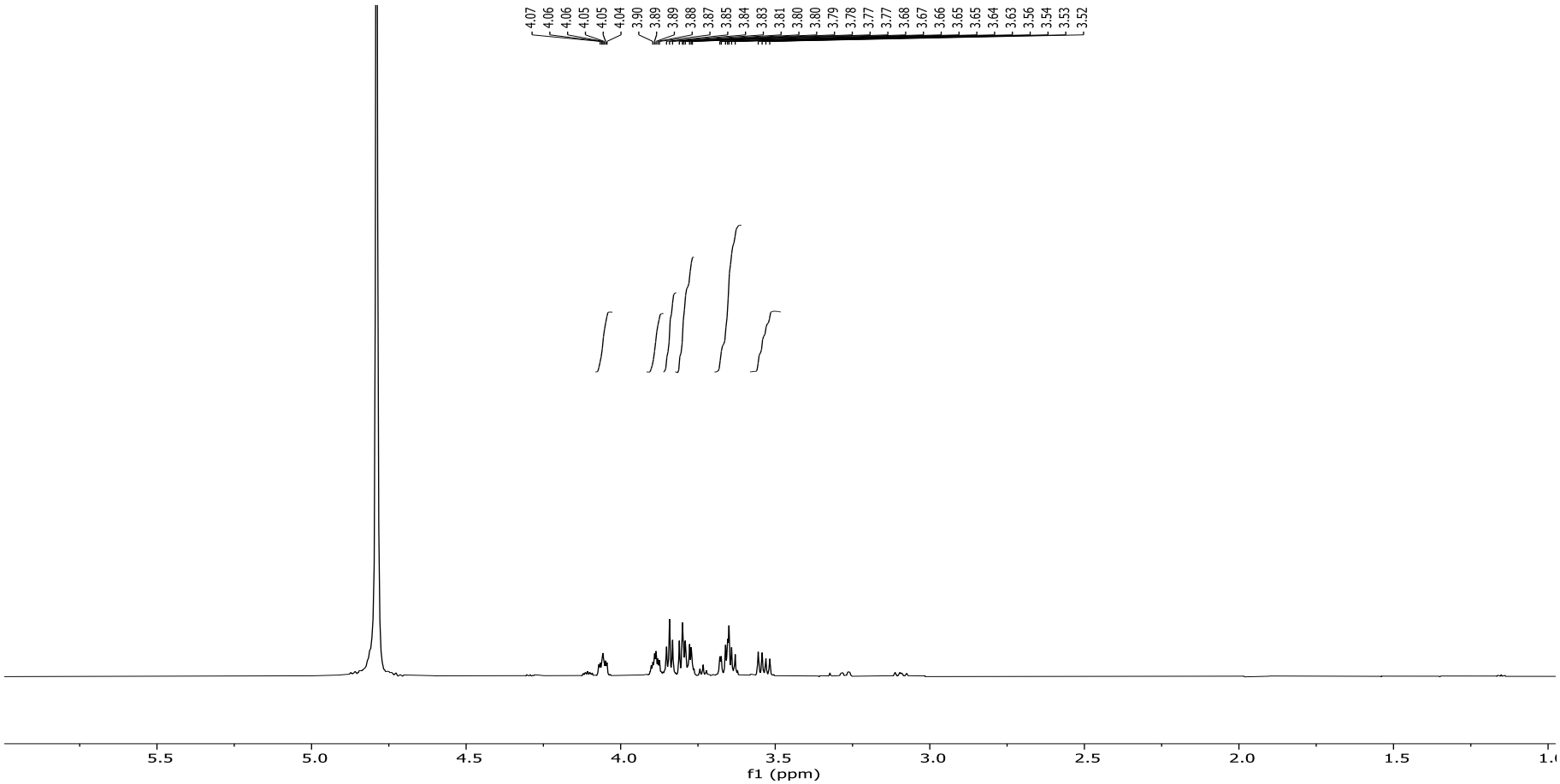




5-Amino-6-((D-allityl)amino)uracil (12h)

^{1}H NMR (D_2O, 500 MHz)





5-Amino-6-((D-allityl)amino)uracil (12h)

^{13}C NMR (D_2O, 125 MHz)