Supporting Information

Agonistic or antagonistic mucosal-associated invariant T (MAIT) cell activity is determined by the 6-alkylamino substituent on uracil MR1 ligands

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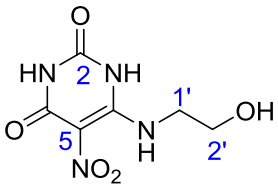
Chemistry Experimental

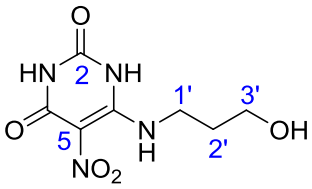
General experimental: All reactions were performed open to air. 6-chlorouracil (Carbosynth), ethanolamine (Sigma), 3-amino-1-propanol (Riedel-de Haën), 5-amino-1-pentanol (Sigma), 40 wt % methylamine in H_2O (Riedel-de Haën), propylamine (Sigma), pentylamine (Alfa Aesar), decylamine (Sigma), NaOH (Pure Science), Na_2S_2O4 (Sigma), KOH (Pure Science), EtOH (Fisher Scientific), $(CH_3)_2CO$ (Fisher Scientific), DMSO (Roth), DMSO-d_6 (Apollo) and D_2O (Apollo) were used as received. 5-OP-RU (**5a**) was prepared as described previously.[1] Reactions were monitored by high resolution mass spectrometry (HRMS) recorded on an Agilent 6530 Q-TOF mass spectrometer utilising a JetStream™ electro-spray ionisation (ESI) source in positive or negative mode. Column chromatography was performed using Bondesil C18 (40 μm). All solvents were removed by evaporation under reduced pressure. Infrared (IR) spectra were recorded as thin films using a Bruker Platinum-ATR spectrometer. Nuclear magnetic resonance (NMR) spectra were obtained at 20 °C in DMSO-d_6 or D_2O using a JEOL NMR spectrometer 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.

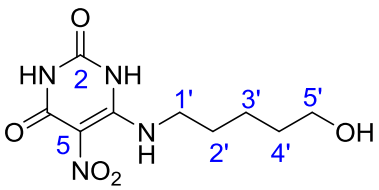
General procedure for the synthesis of 5-nitro-6-aminouracils: To a solution of 6-chloro-5-nitrouracil (100 mg, 0.53 mmol, 1.5 equiv.) in 1:1 EtOH:H_2O (10 mL/mmol) was added the amine (0.35 mmol, 1 equiv.) and the resulting yellow solution was maintained at pH 8 via the addition of 1 M NaOH. The solution was stirred at r.t. for 24 hours, after which time the solvents were removed under reduced pressure. The residue was suspended in $(CH_3)_2CO$ and filtered to give the desired product as a light yellow amorphous solid.



5-Nitro-6-((2-hydroxyethyl)amino)uracil (7b). The general procedure for the synthesis of 5-nitro-6-aminouracils was carried out using ethanolamine (**6b**) to yield 5-Nitro-6-((2-hydroxyethyl)amino)uracil **7b** (61 mg, 0.28 mmol, 79%). IR (film) 3381, 3035, 2845, 1723, 1618, 1369, 1049 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 11.05 (s, 1H, NH), 10.19 (t, $J_{NH,\,1'}$ = 5.4 Hz, 1H, NH), 9.74 (s, 1H, NH), 3.69-3.48 (m, 4H, CH_2-1′, CH_2-2′); ^{13}C NMR (125 MHz, DMSO-d_6) δ 156.5 (C-2), 153.9 (C-6), 148.1 (C-4), 109.1 (C-5), 59.1 (C-2′), 44.9 (C-1′); HRMS *m/z* calcd for $[C_6H_7N_4O_5]^-$ 215.0422, obsd 215.0414.

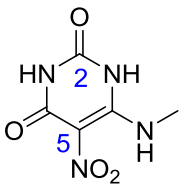


5-Nitro-6-((3-hydroxypropyl)amino)uracil (7c). The general procedure for the synthesis of 5-nitro-6-aminouracils was carried out using 3-amino-1-propanol (**6c**) to yield 5-Nitro-6-((3-hydroxypropyl)amino)uracil **7c** (67 mg, 0.29 mmol, 83%). IR (film) 3373, 3140, 2874, 1656, 1423, 1065 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 11.01 (s, 1H, NH), 10.24 (t, $J_{NH,\,1'}$ = 5.8 Hz, 1H, NH), 3.50 (q, $J_{1',NH} = J_{1',2'}$ = 6.5 Hz, 2H, CH_2-1′), 3.46 (t, $J_{3',2'}$ = 6.2 Hz, 2H, CH_2-3′), 1.73 (p, $J_{2',1'} = J_{2',3'}$ = 6.1 Hz, 2H, CH_2-2′).; ^{13}C NMR (125 MHz, DMSO-d_6) δ 156.5 (C-2), 153.2 (C-6), 148.0 (C-4), 109.0 (C-5), 58.3 (C-3′), 40.4 (C-1′), 30.9 (C-2′); HRMS *m/z* calcd for $[C_7H_9N_4O_5]^-$ 229.0578, obsd 229.0579.

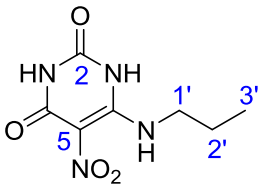


5-Nitro-6-((5-hydroxypentyl)amino)uracil (7d). The general procedure for the synthesis of 5-nitro-6-aminouracils was carried out using 5-amino-1-pentanol (**6d**) to yield 5-Nitro-6-((5-hydroxypentyl)amino)uracil **7d** (70 mg, 0.27 mmol, 78%). IR (film) 3283, 2931, 2857, 1642, 1448, 1036 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 11.02 (s, 1H, NH), 10.12 (t, $J_{NH,\,1'}$ = 6.1 Hz, 1H, NH), 9.76 (s, 1H, NH), 3.65-3.39 (m, 4H, CH_2-1′, CH_2-

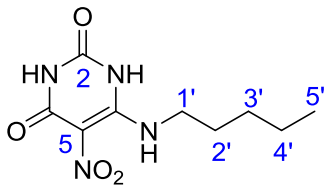
5′), 1.56 (p, $J_{2',1'} = J_{2',3'}$ = 7.5 Hz, 2H, CH_2-2′), 1.47-1.37 (m, 2H, CH_2-4′), 1.39-1.20 (m, 2H, CH_2-3′); ^{13}C NMR (125 MHz, DMSO-d_6) δ 156.4 (C-2), 153.1 (C-6), 148.0 (C-4), 109.0 (C-5), 60.5 (C-5′), 42.0 (C-1′), 32.0 (C-4′), 28.3 (C-2′), 22.6 (C-3′); HRMS *m/z* calcd for $[C_9H_{13}N_4O_5]^-$ 257.0891, obsd 257.0889.



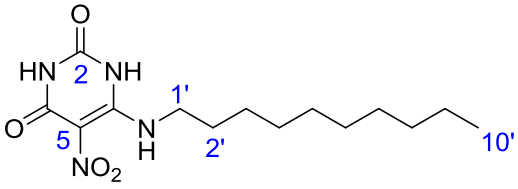
5-Nitro-6-(methylamino)uracil (7e). The general procedure for the synthesis of 5-nitro-6-aminouracils was carried out using methylamine (**6e**) to yield 5-Nitro-6-(methylamino)uracil **7e** (56 mg, 0.30 mmol, 86%). IR (film) 3342, 2977, 2760, 1650, 1623, 1405, 1036 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.31-9.24 (m, 1H, NH), 9.23-9.18 (m, 1H, NH), 2.83 (d, $J_{Me,NH}$ = 4.6 Hz, 3H, Me); ^{13}C NMR (125 MHz, DMSO-d_6) δ 160.4 (C-2), 159.9 (C-6), 155.6 (C-4), 110.2 (C-5), 27.8 (C-Me); HRMS *m/z* calcd for $[C_5H_5N_4O_4]^-$ 185.0316, obsd 185.0321.



5-Nitro-6-(propylamino)uracil (7f). The general procedure for the synthesis of 5-nitro-6-aminouracils was carried out using propylamine (**6f**) to yield 5-Nitro-6-(propylamino)uracil **7f** (62 mg, 0.29 mmol, 84%). IR (film) 3310, 2957, 2876, 1637, 1426, 1056 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.49 (s, 1H, NH), 9.38 (t, $J_{NH,1'}$ = 5.5 Hz, 1H, NH), 3.34-3.25 (m, 2H, CH_2-1′), 1.53 (h, $J_{2,1} = J_{2,3}$ = 7.4 Hz, 2H, CH_2-2′), 0.89 (t, $J_{3,2}$ = 7.4 Hz, 3H, CH_3-3′); ^{13}C NMR (125 MHz, DMSO-d_6) δ 160.4 (C-2), 159.8 (C-6), 155.8 (C-4), 110.2 (C-5), 42.0 (C-1′), 22.1 (C-2′), 11.5 (C-3′); HRMS *m/z* calcd for $[C_7H_9N_4O_4]^-$ 213.0629, obsd 213.0623.



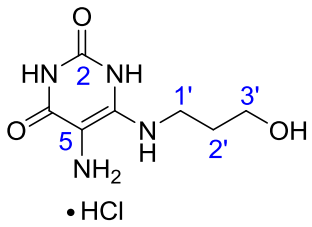
5-Nitro-6-(pentylamino)uracil (7g). The general procedure for the synthesis of 5-nitro-6-aminouracils was carried out using pentylamine (**6g**) to yield 5-Nitro-6-(pentylamino)uracil **7g** (70 mg, 0.29 mmol, 82%). IR (film) 3318, 2931, 2868, 1634, 1426, 1060 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.03 (s, 1H, NH), 10.12 (t, $J_{NH,1'}$ = 6.1 Hz, 1H, NH), 3.48 (q, $J_{1',NH} = J_{1',3'}$ = 6.8 Hz, 2H, CH_2-1′), 1.56 (p, $J_{2',1'} = J_{2',3'}$ = 7.4 Hz, 2H, CH_2-2′), 1.38-1.18 (m, 4H, CH_2-3′, CH_2-4′), 0.88 (t, $J_{5',4'}$ = 6.9 Hz, 3H, CH_3-5′).; ^{13}C NMR (125 MHz, DMSO-d_6) δ 156.4 (C-2), 153.1 (C-6), 148.0 (C-4), 109.0 (C-5), 42.0 (C-1′), 28.2 (C-3′), 28.1 (C-4′), 21.8 (C-2′), 13.9 (C-5′); HRMS *m/z* calcd for $[C_9H_{13}N_4O_4]^-$ 241.0942, obsd 241.0947.



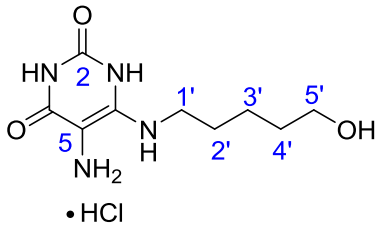
5-Nitro-6-(decylamino)uracil (7h). The general procedure for the synthesis of 5-nitro-6-aminouracils was carried out using decylamine (**6h**) to yield 5-Nitro-6-(decylamino)uracil **7h** (91 mg,

0.29 mmol, 83%). IR (film) 3179, 2918, 2850, 1637, 1398, 1048 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.35 (t, $J_{NH,1'}$ = 5.4 Hz, 1H, NH), 9.31 (s, 1H, NH), 3.37-3.30 (m, 2H, CH_2-1′), 1.57-1.44 (m, 2H, CH_2-2′), 1.35-1.14 (m, 14H, CH_2-3′-CH_2-9′), 0.85 (t, $J_{10',9'}$ = 7.7 Hz, 3H, CH_3-10′); ^{13}C NMR (125 MHz, DMSO-d_6) δ 160.0 (C-2), 159.8 (C-6), 155.6 (C-4), 110.1 (C-5), 40.5 (C-1′), 31.3, 29.0, 29.0, 28.8, 28.7, 26.6, 22.1 (C-2′-C-9′), 14.0 (C-10′); HRMS *m/z* calcd for $[C_{14}H_{23}N_4O_4]^-$ 311.1725, obsd 311.1731.

General procedure for the synthesis of 5-amino-6-hydroxyalkylaminouracils ***8c*** *and* ***8d****:* To a solution of 5-nitro-6-hydroxylalkylaminouracil in H_2O (12.5 mL/mmol) was 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 isocratic (H_2O) 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.

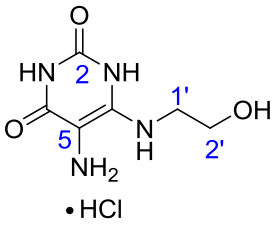


5-Amino-6-((3-hydroxypropyl)amino)uracil (8c). The general procedure for the synthesis of 5-amino-6-hydroxyalkylaminouracils was carried out on **7c** (50 mg, 0.22 mmol) to yield **8c** (39 mg, 75%). IR (film) 2938, 1689, 1618, 1208, 1054 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 3.67 (t, $J_{3',2'}$ = 6.1 Hz, 2H, CH_2-3′), 3.43 (t, $J_{1',2'}$ = 6.9 Hz, 2H, CH_2-1′), 1.85 (p, $J_{2',1'} = J_{2',3'}$ = 6.6 Hz, 2H, CH_2-2′); ^{13}C NMR (125 MHz, D_2O) δ 160.9, 151.0 (C-2, C-4), 150.1 (C-6), 82.1 (C-5), 58.4 (C-3′), 39.0 (C-1′), 30.6 (C-2′); HRMS *m/z* calcd for $[C_7H_{11}N_4O_3]^-$ 199.0837, obsd 199.0842.

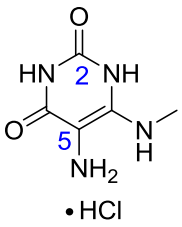


5-Amino-6-((5-hydroxypentyl)amino)uracil (8d). The general procedure for the synthesis of 5-amino-6-hydroxyalkylaminouracils was carried out on **7d** (50 mg, 0.19 mmol) to yield **8d** (36 mg, 72%). IR (film) 2933, 1632, 1172, 1051 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 3.63 (t, $J_{5',4'}$ = 6.4 Hz, 2H, CH_2-5′), 3.39 (t, $J_{1',2'}$ = 7.0 Hz, 2H, CH_2-1′), 1.70 (p, $J_{2',1'} = J_{2',3'}$ = 7.3 Hz, 2H, CH_2-2′), 1.60 (p, $J_{4',3'} = J_{4',5'}$ = 6.9 Hz, 2H, CH_2-4′), 1.50-1.40 (m, 1H, 2H, CH_2-3′); ^{13}C NMR (125 MHz, D_2O) δ 161.2, 152.4 (C-2, C-4), 149.0 (C-6), 81.8 (C-5), 62.0 (C-5′), 42.1 (C-1′), 31.5 (C-4′), 27.9 (C-2′), 21.2 (C-3′); HRMS *m/z* calcd for $[C_9H_{15}N_4O_3]^-$ 227.1150, obsd 227.1147.

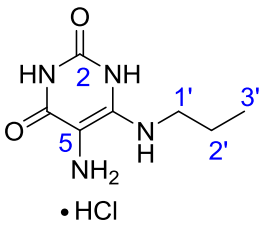
*General procedure for the synthesis of 5-amino-6-alkylaminouracils **8b**, **8e-h**:* To a solution of 5-nitro-6-alkylaminouracil in DMSO:EtOH:H_2O 1:1:1 (12.5 mL/mmol) was added 2 drops of 2M KOH followed by $Na_2S_2O_4$ (4 equiv.) and the resulting solution was stirred at r.t. for 2 h until HRMS analysis showed the complete consumption of starting material. The reaction mixture was acidified with 1M HCl, dried under an atmosphere of argon to remove EtOH and lyophilised. The residue was then subjected to C18 reversed-phase column chromatography (100:0 H_2O → 100: MeOH) to afford fractions containing the product, which were lyophilised to give the pure product as a pink amorphous solid.



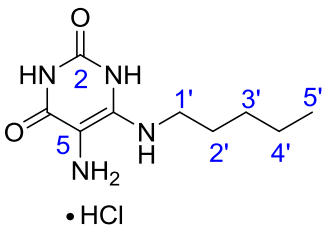
5-Amino-6-((2-hydroxyethyl)amino)uracil (8b). The general procedure for the synthesis of 5-amino-6-alkylaminouracils was carried out on **7b** (50 mg, 0.23 mmol) to yield **8b** (35 mg, 68%). IR (film) 2930, 1716, 1637, 1172, 1048 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 3.91 (t, $J_{2',1'}$ = 5.7 Hz, 2H, CH_2-2′), 3.67 (t, $J_{1',2'}$ = 5.6 Hz, 2H, CH_2-1′); ^{13}C NMR (125 MHz, D_2O) δ 161.1, 151.2 (C-2, C-4), 150.5 (C-6), 82.8 (C-5), 60.4 (C-2′), 44.7 (C-1′); HRMS *m/z* calcd for $[C_6H_9N_4O_3]^-$ 185.0680, obsd 185.0673.



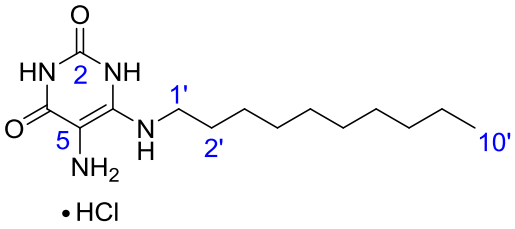
5-Amino-6-(methylamino)uracil (8e). The general procedure for the synthesis of 5-amino-6-alkylaminouracils was carried out on **7e** (50 mg, 0.27 mmol) to yield **8e** (35 mg, 67%). IR (film) 2986, 1641, 1180, 1061 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 2.98 (s, 3H, Me); ^{13}C NMR (125 MHz, D_2O) δ 160.7, 151.0 (C-2, C-4), 150.7 (C-6), 81.8 (C-5), 28.2 (C-Me); HRMS *m/z* calcd for $[C_5H_7N_4O_2]^-$ 155.0574, obsd 155.0567.



5-Amino-6-(propylamino)uracil (8f). The general procedure for the synthesis of 5-amino-6-alkylaminouracils was carried out on **7f** (50 mg, 0.23 mmol) to yield **8f** (39 mg, 77%). IR (film) 2923, 1638, 1196, 1043 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 3.31 (t, $J_{1',2'}$ = 7.1 Hz, 2H, CH_2-1′), 1.64 (h, $J_{2',1'}$ = $J_{2',3'}$ = 7.4 Hz, 2H, CH_2-2′), 0.94 (t, $J_{3',2'}$ = 7.4 Hz, 3H, CH_3-3′); ^{13}C NMR (125 MHz, D_2O) δ 160.9, 151.1 (C-2, C-4), 150.0 (C-6), 81.9 (C-5), 43.9 (C-1′), 21.8 (C-2′), 10.3 (C-3′); HRMS *m/z* calcd for $[C_7H_{11}N_4O_2]^-$ 183.0887, obsd 183.0893.



5-Amino-6-(pentylamino)uracil (8g). The general procedure for the synthesis of 5-amino-6-alkylaminouracils was carried out on **7g** (50 mg, 0.21 mmol) to yield **8g** (39 mg, 75%). IR (film) 2977, 1726, 1189, 1032 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 3.34 (t, $J_{1',2'}$ = 7.1 Hz, 2H, CH_2-1′), 1.68-1.59 (m, 2H, CH_2-2′), 1.39-1.27 (m, 4H, CH_2-3′, CH_2-4′), 0.91-0.84 (m, 3H, CH_3-5′); ^{13}C NMR (125 MHz, D_2O) δ 160.8, 151.0 (C-2, C-4), 150.0 (C-6), 81.8 (C-5), 42.2 (C-1′), 28.0 (C-3′), 27.9 (C-2′), 21.6 (C-4′), 13.1 (C-5′); HRMS *m/z* calcd for $[C_9H_{15}N_4O_2]^-$ 211.1200, obsd 211.1196.



5-Amino-6-(decylamino)uracil (8h). The general procedure for the synthesis of 5-amino-6-alkylaminouracils was carried out on **7h** (50 mg, 0.16 mmol) to yield **8h** (36 mg, 71%). IR (film) 2918, 1635, 1170, 1046 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.91 (s, 1H, NH), 7.91 (t, $J_{NH,1'}$ = 5.5 Hz, 1H, NH), 3.25 (q, $J_{1',NH}$ = $J_{1',2'}$ = 6.7 Hz, 2H, CH_2-1′), 1.50 (p, $J_{2',1'}$ = $J_{2',3'}$ = 7.2 Hz, 2H, CH_2-2′), 1.36-1.11 (m, 14H, CH_2-3′-CH_2-9′), 0.83 (t, $J_{10',9'}$ = 6.8 Hz, 3H, CH_3-10); ^{13}C NMR (125 MHz, DMSO-d_6) δ 159.9, 149.9 (C-2, C-4), 149.1 (C-6), 81.8 (C-5), 41.8 (C-1′), 31.4, 29.1, 29.1, 28.9, 28.8, 28.8, 26.1, 22.2 (C-2′-C-9′), 14.1 (C-10′); HRMS *m/z* calcd for $[C_{14}H_{25}N_4O_2]^-$ 281.1983, obsd 281.1994.

Computational Procedures

Molecular docking simulations were carried out using GOLD (v 5.7.1). Before docking, 5-OP-RU was re-docked into the MR1-MAIT crystal structure 4PJ7 from PDB 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.[2] Chimera is developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIGMS Grant P41-GM103311).[3]

Biology Experimental

General experimental: Ac-6-FP and methylglyoxal were purchased from Schircks Laboratory and Sigma, respectively. 5-OP-RU (**5a**) and its analogues **5b-h** were freshly prepared before each experiment by combining 10 mM of 5-A-RU or analogues with 40 mM of methylglyoxal.[1] 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.[1, 4] 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.[5] For tetramer generation, soluble biotinylated mouse MR1β_2m-complexes were produced as previously descirbed.[1, 6] Biotinylated MR1 was then tetramerised after adding a molar ratio of 1:5 streptavidin-APC:MR1.

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 hour, 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 hour, 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 (Biolegend), followed by flow cytometric analysis.

MR1 tetramer assay: The compounds (5 mM) were mixed with APC-conjugated mouse MR1 tetramer at a concentration of 10% (v/v) and incubated overnight at 4 °C. 6C2 cells and TG40 cells (5 x 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, followed by flow cytometric analysis.

MR1 surface expression 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 4 hours, after which time the cells were stained with PE-conjugated anti-mouse MR1 (Biolegend) and 7AAD, followed by flow cytometric analysis.

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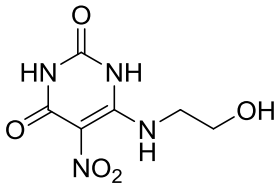
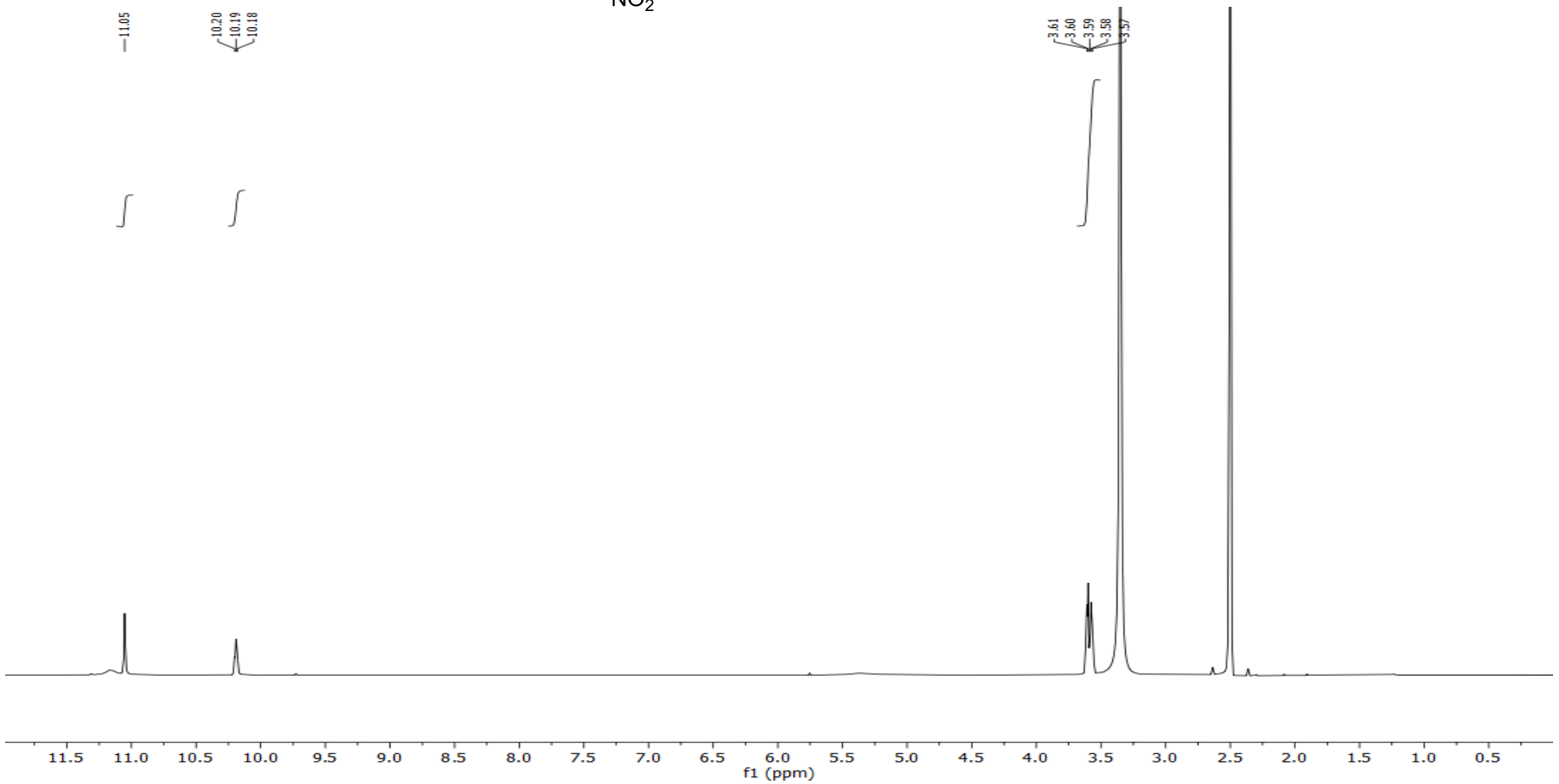
NMR Spectra

5-Nitro-6-((2-hydroxyethyl)amino)uracil (**7b**)

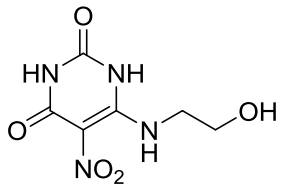


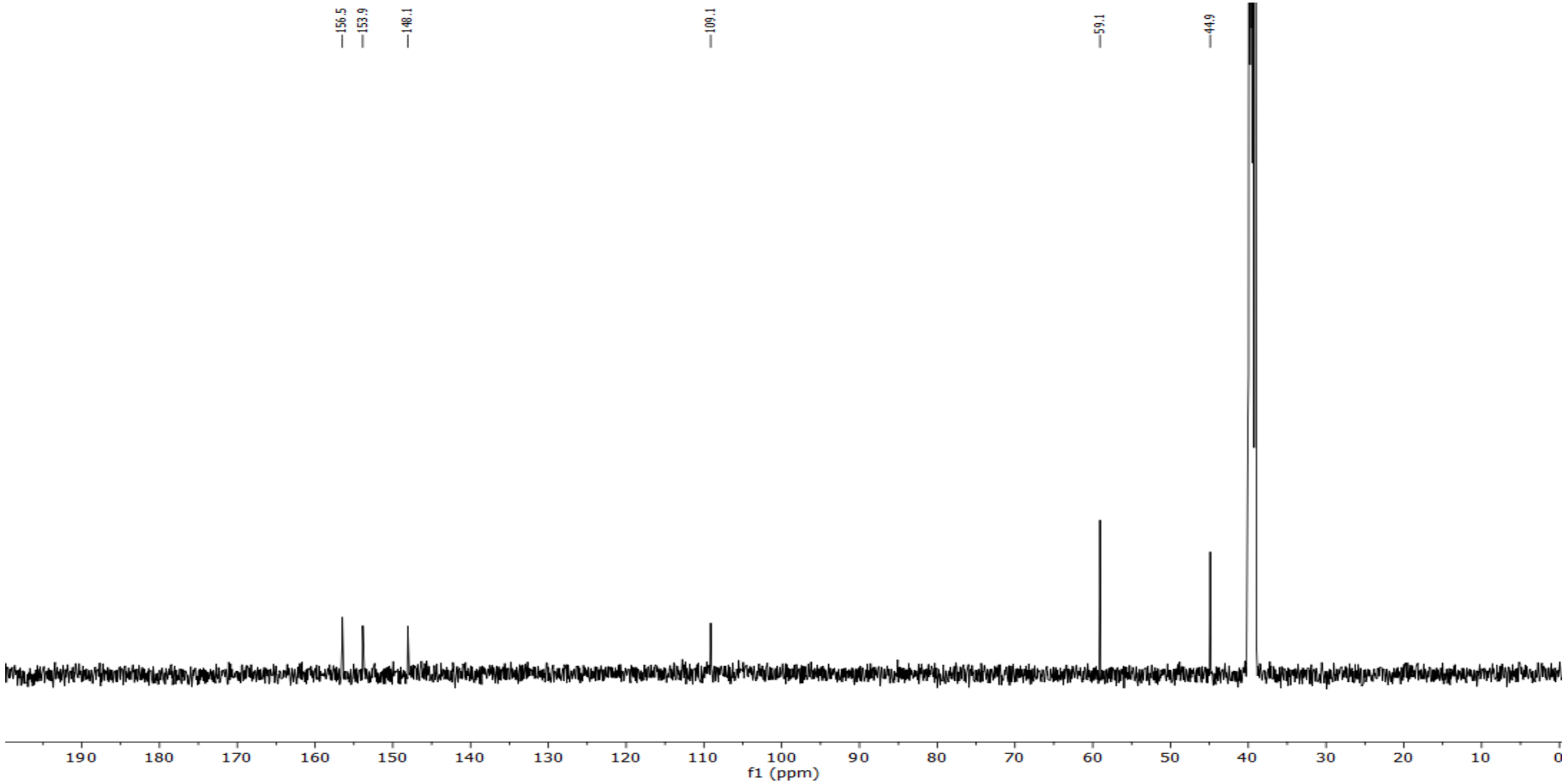
^{1}H NMR (DMSO-d_6, 500 MHz)



5-Nitro-6-((2-hydroxyethyl)amino)uracil (**7b**)

^{13}C NMR (DMSO-d$_6$, 125 MHz)





5-Nitro-6-((3-hydroxypropyl)amino)uracil (**7c**)

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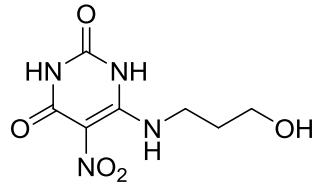

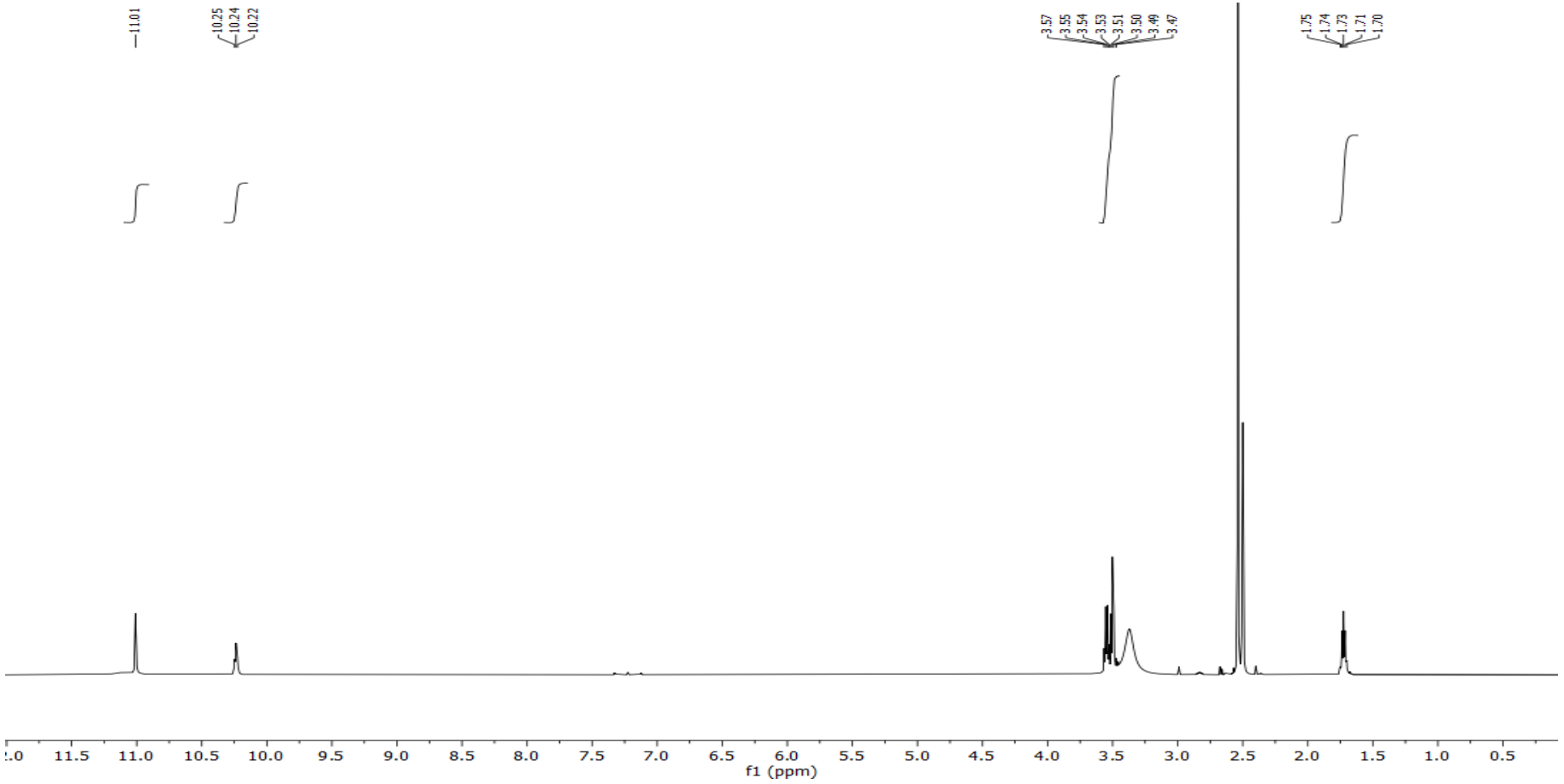

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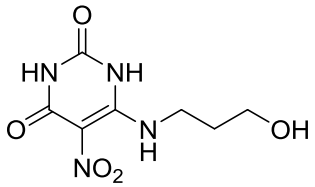
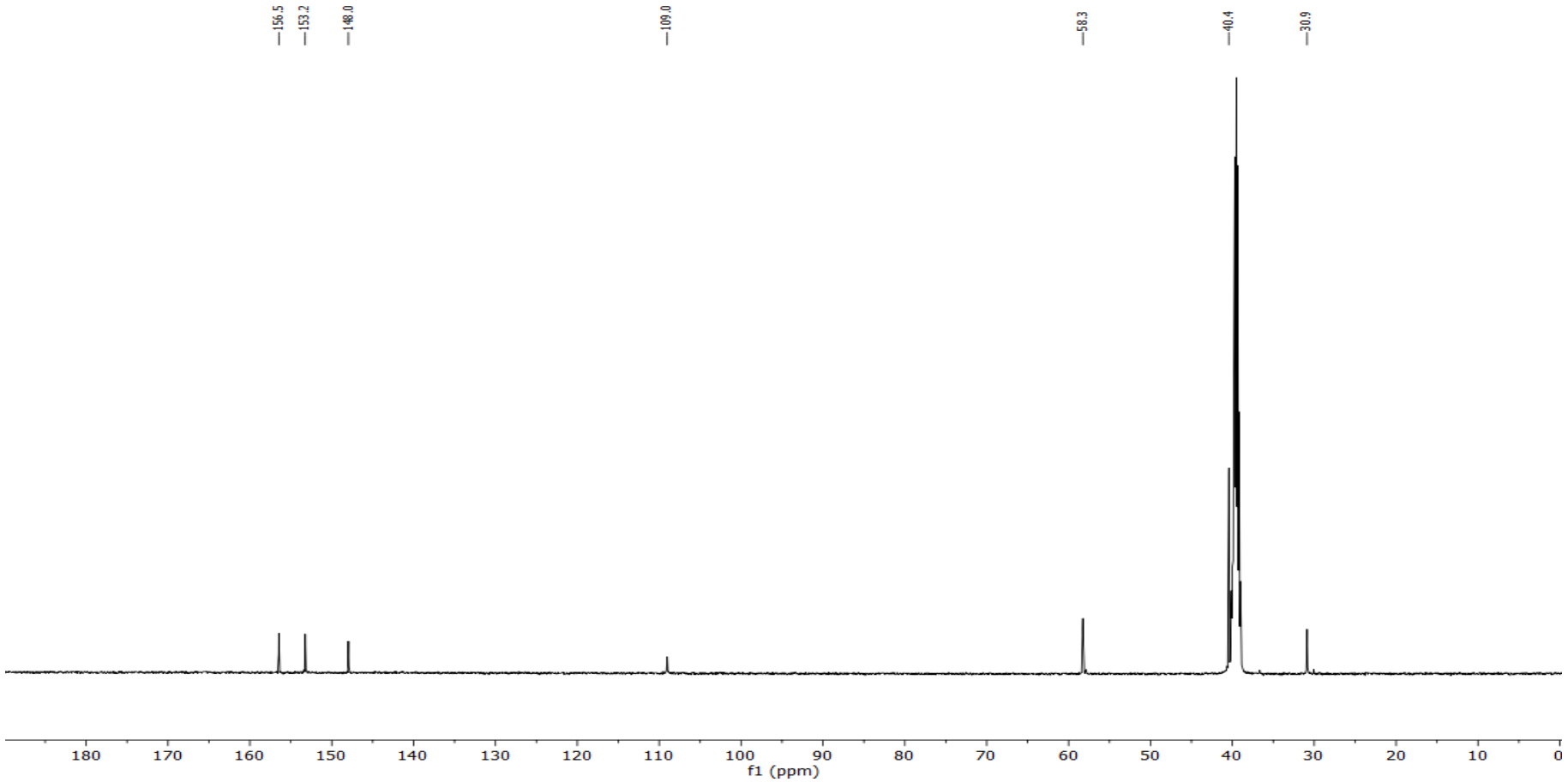
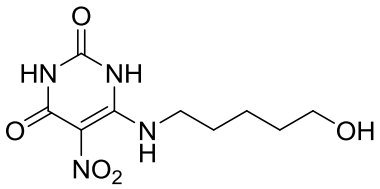
^{13}C NMR (DMSO-d_6, 125 MHz)

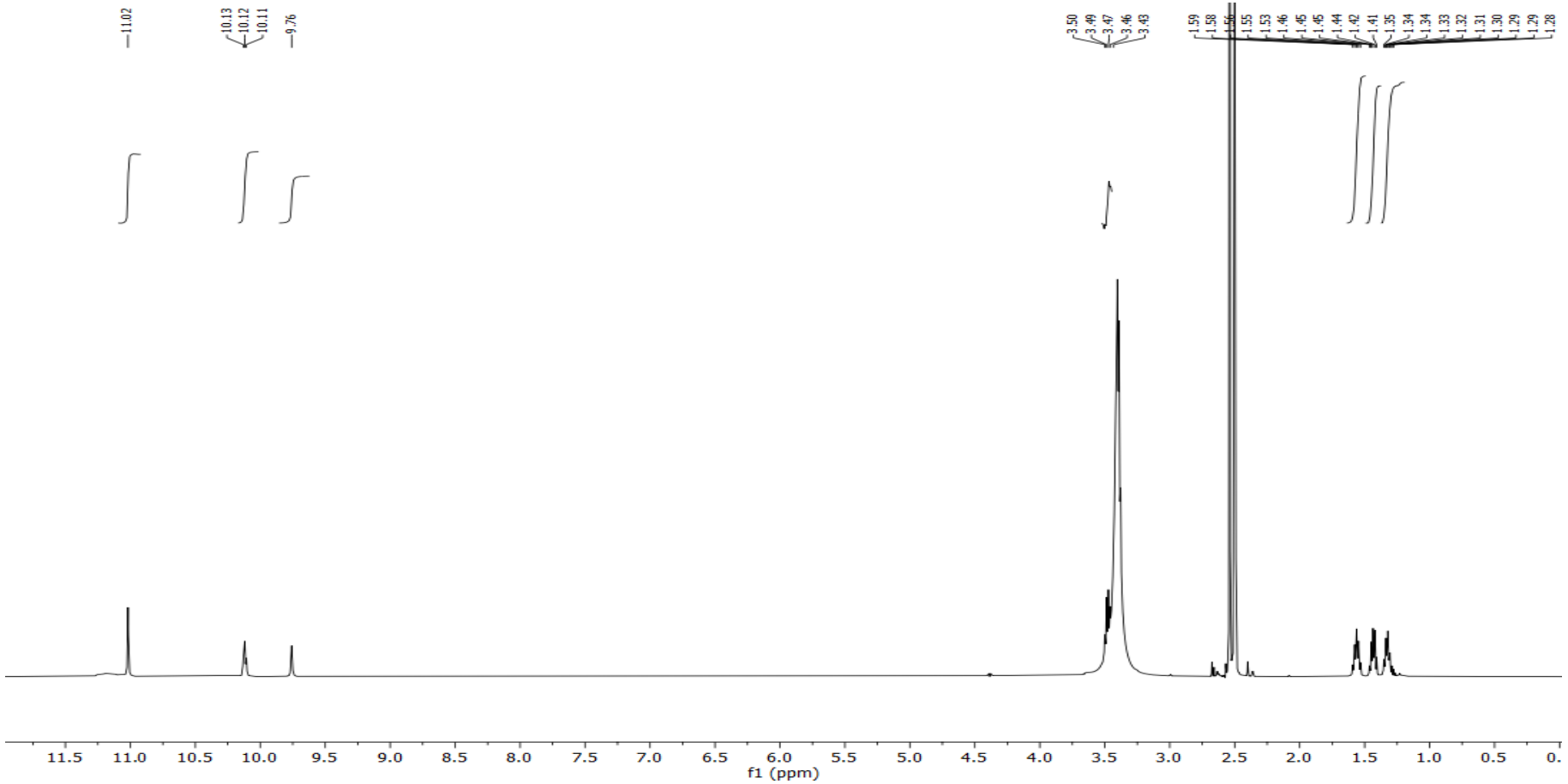




5-Nitro-6-((5-hydroxypentyl)amino)uracil (**7d**)

^{1}H NMR (DMSO-d_6, 500 MHz)





5-Nitro-6-((5-hydroxypentyl)amino)uracil (**7d**)

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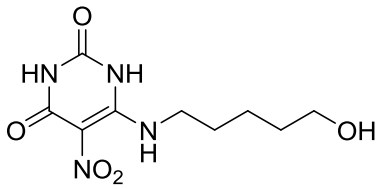
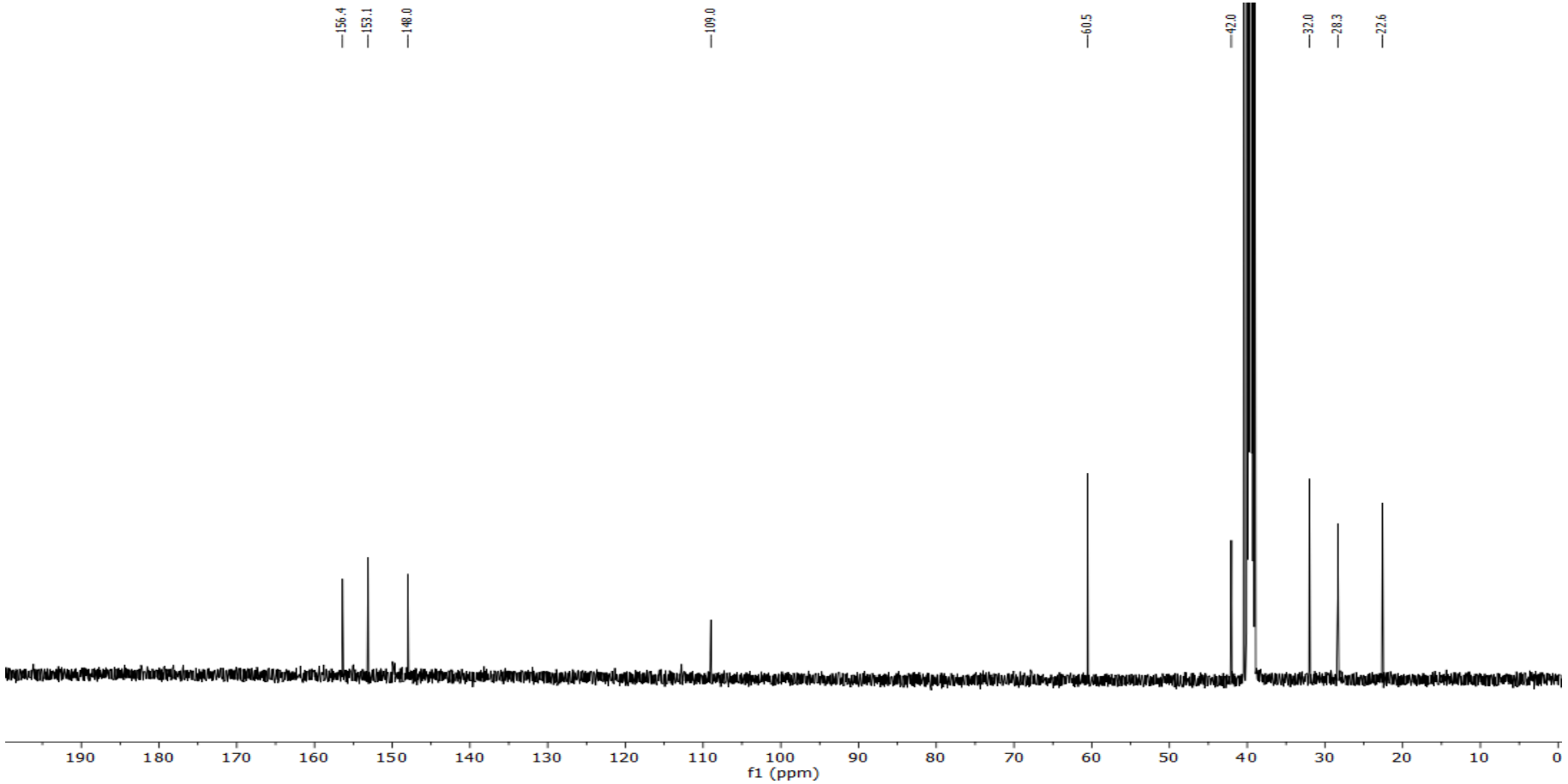
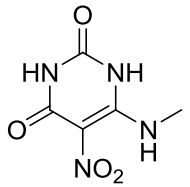
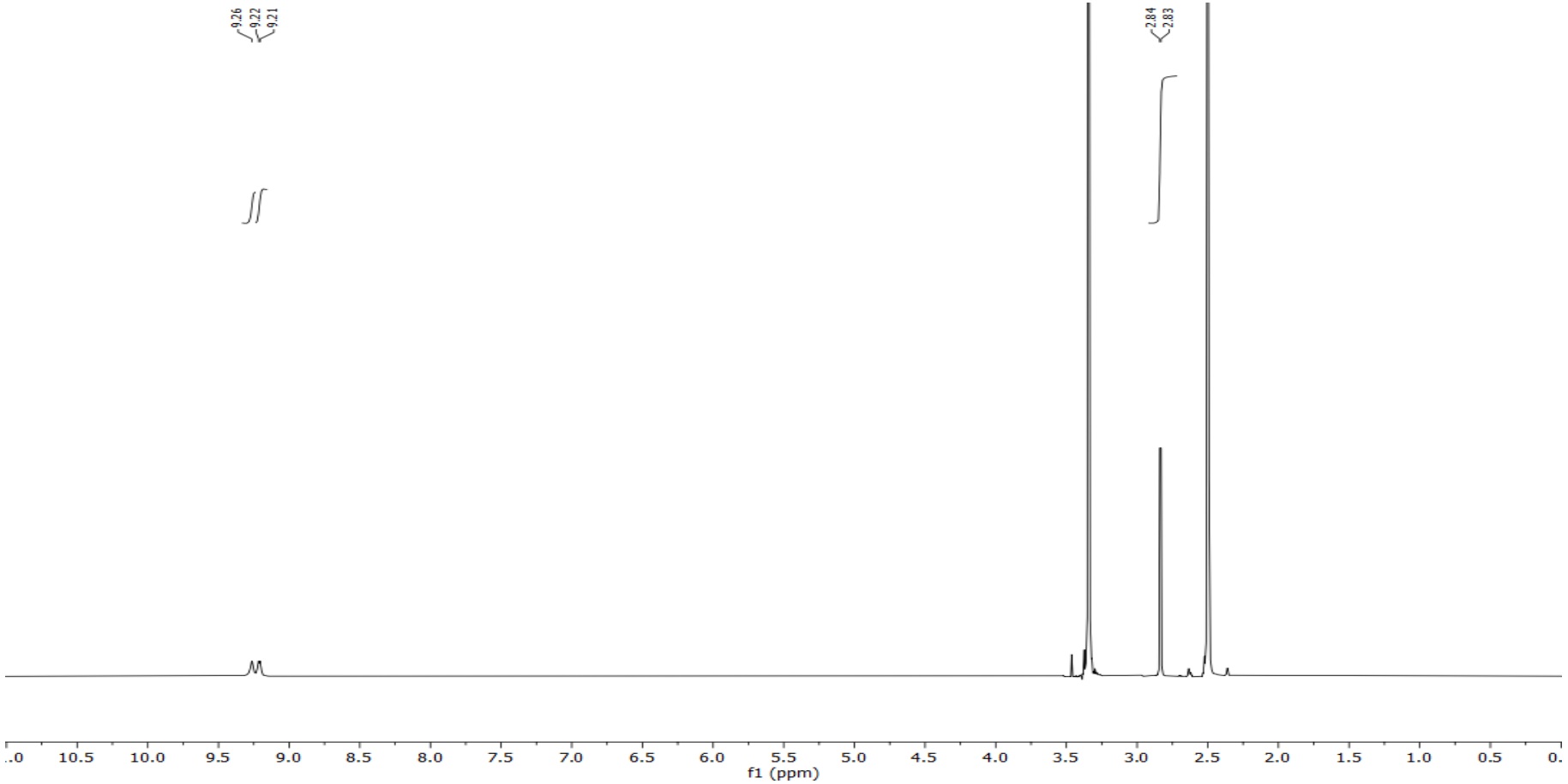




5-Nitro-6-(methylamino)uracil (**7e**)

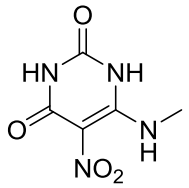
^{1}H NMR (DMSO-d_6, 500 MHz)

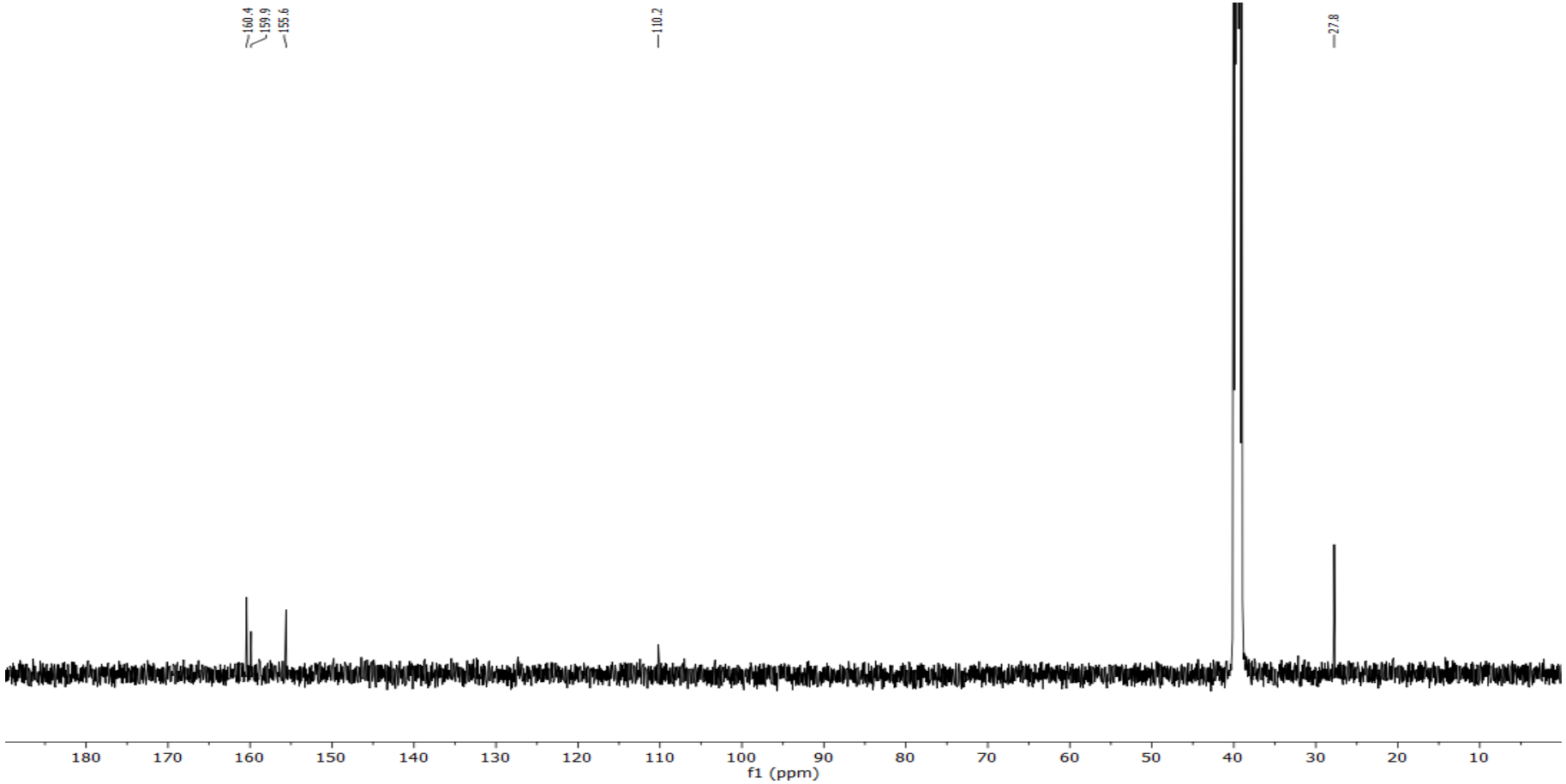




5-Nitro-6-(methylamino)uracil (**7e**)

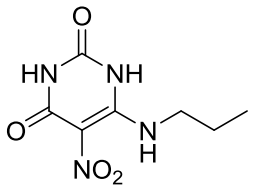
^{13}C NMR (DMSO-d$_6$, 125 MHz)

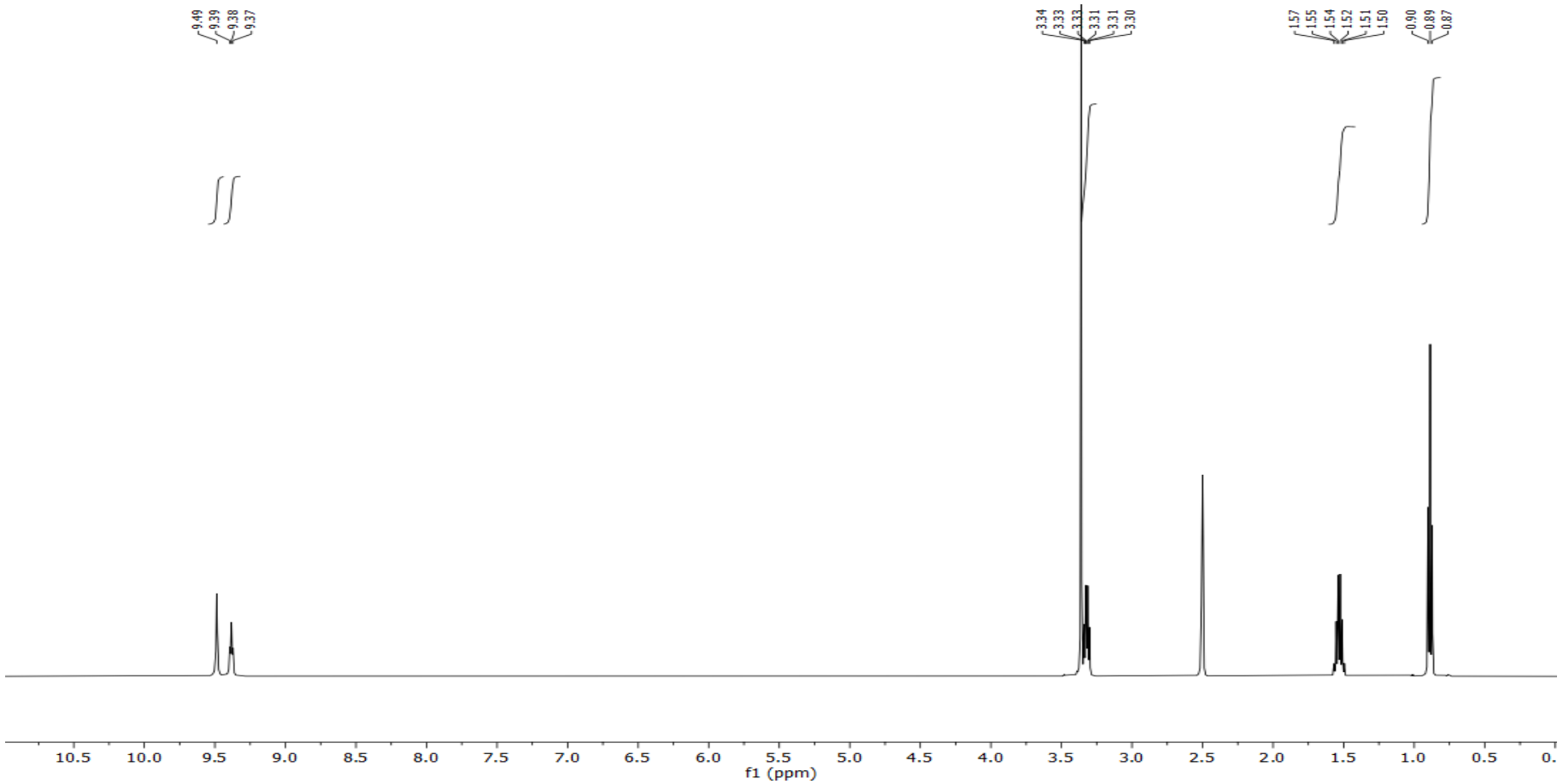




5-Nitro-6-(propylamino)uracil (**7f**)

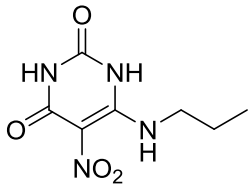
^{1}H NMR (DMSO-d_6, 500 MHz)

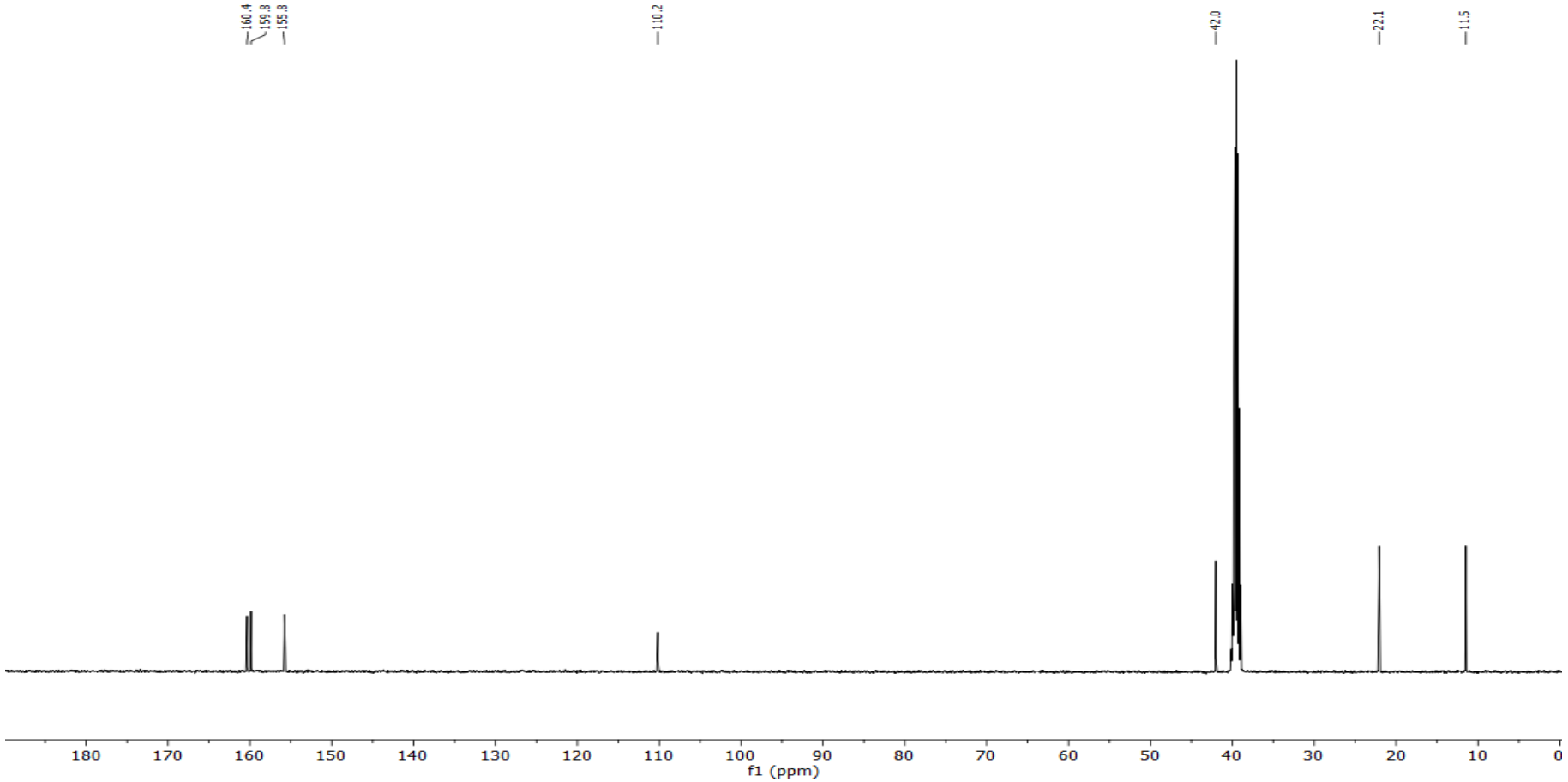




5-Nitro-6-(propylamino)uracil (**7f**)

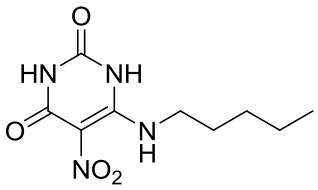
^{13}C NMR (DMSO-d$_6$, 125 MHz)

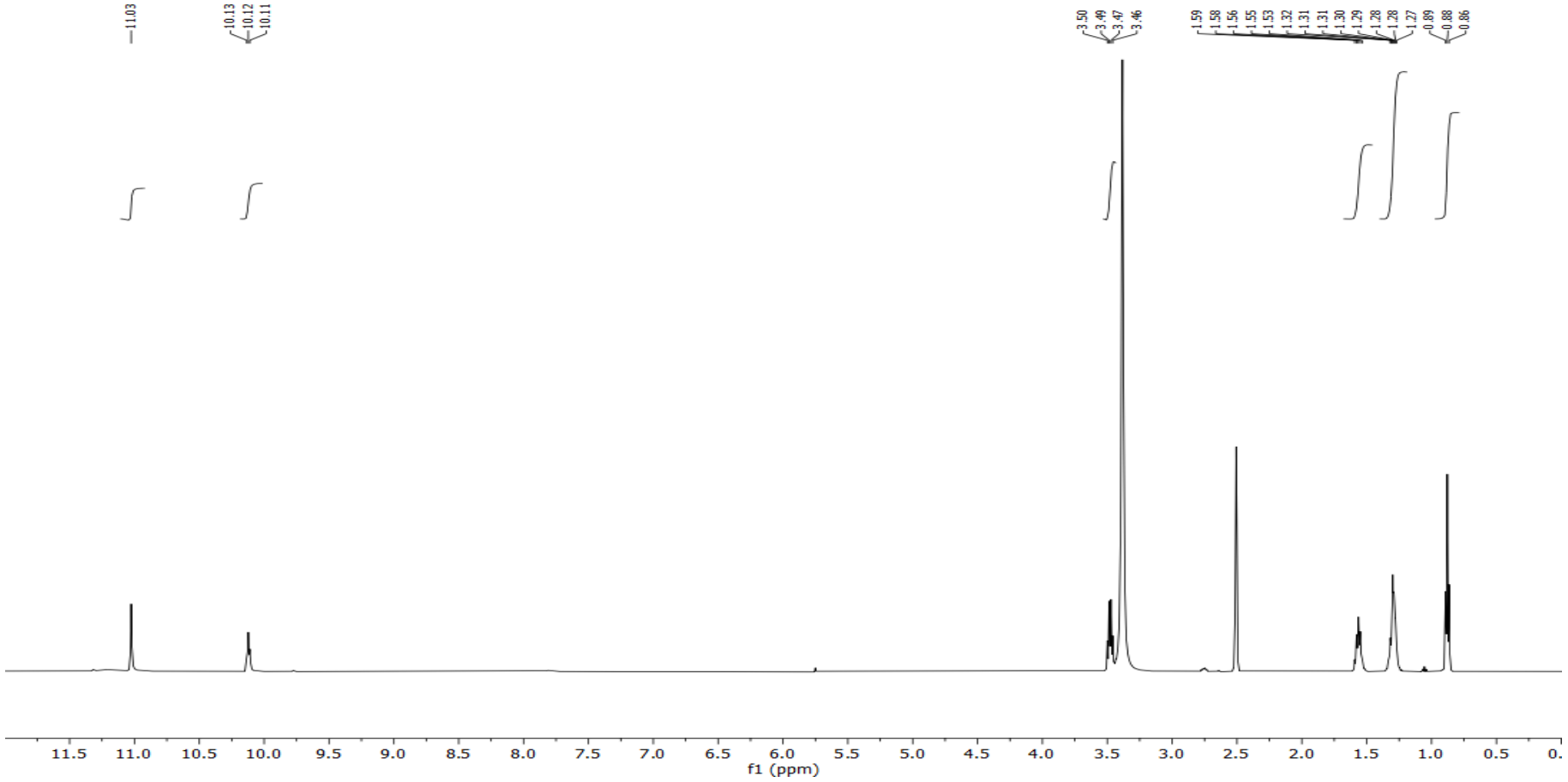




5-Nitro-6-(pentylamino)uracil (**7g**)

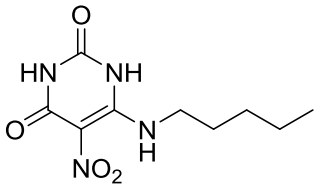
^{1}H NMR (DMSO-d$_6$, 500 MHz)

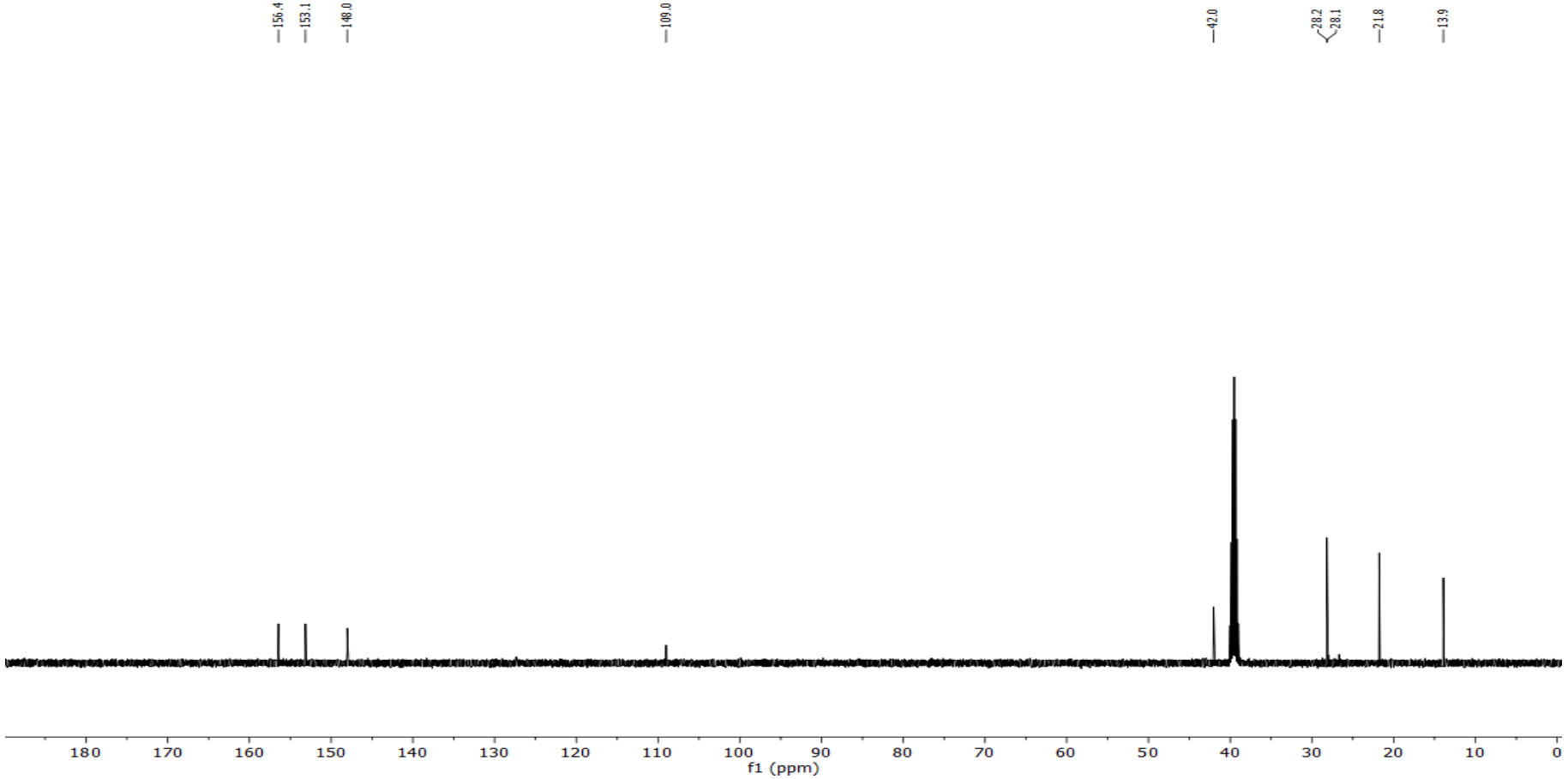




5-Nitro-6-(pentylamino)uracil (**7g**)

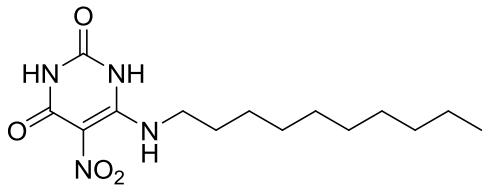
^{13}C NMR (DMSO-d$_6$, 125 MHz)

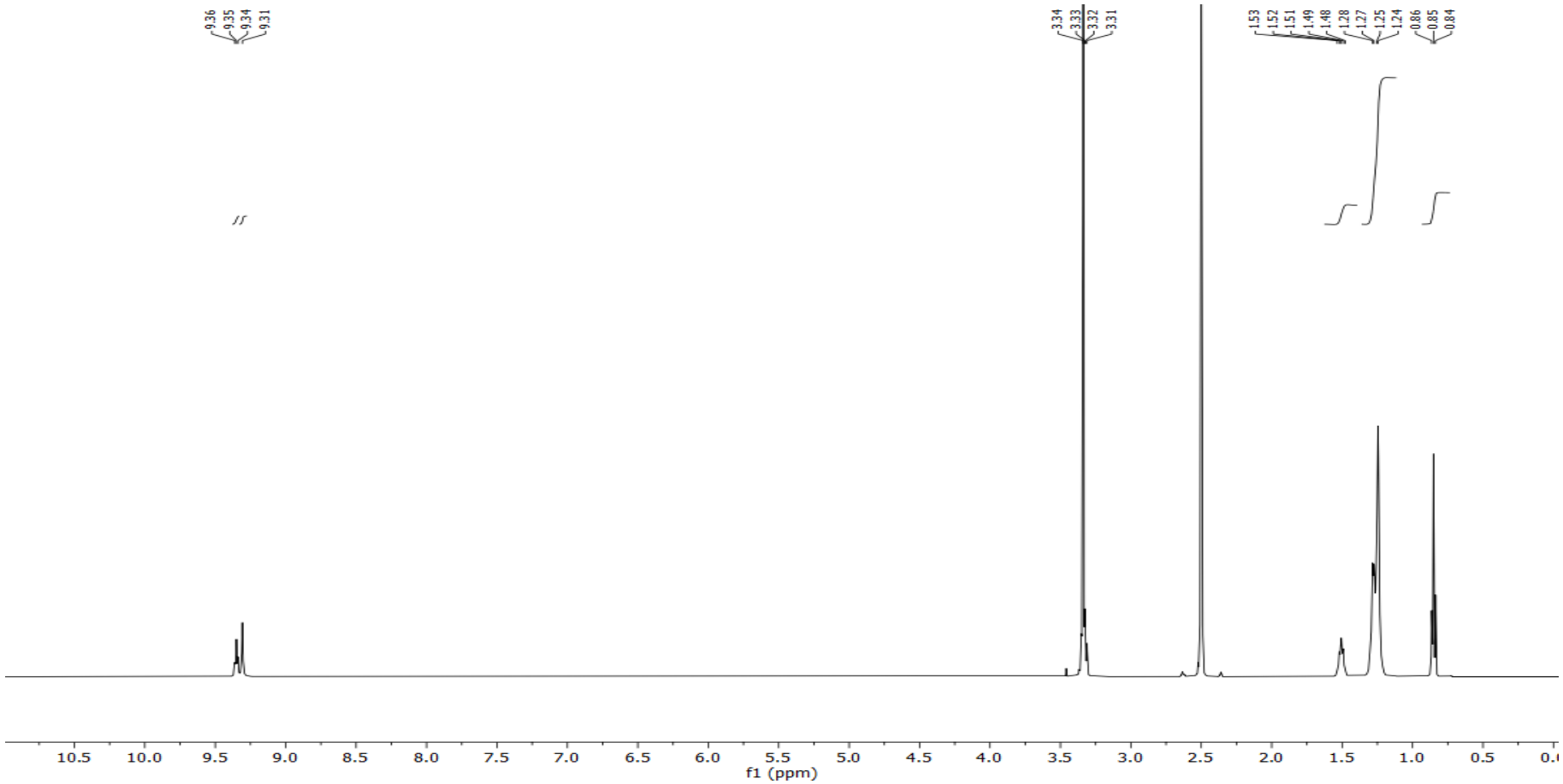




5-Nitro-6-(decylamino)uracil (**7h**)

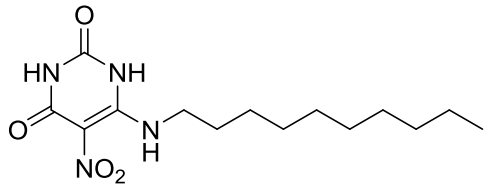
^{1}H NMR (DMSO-d_6, 500 MHz)

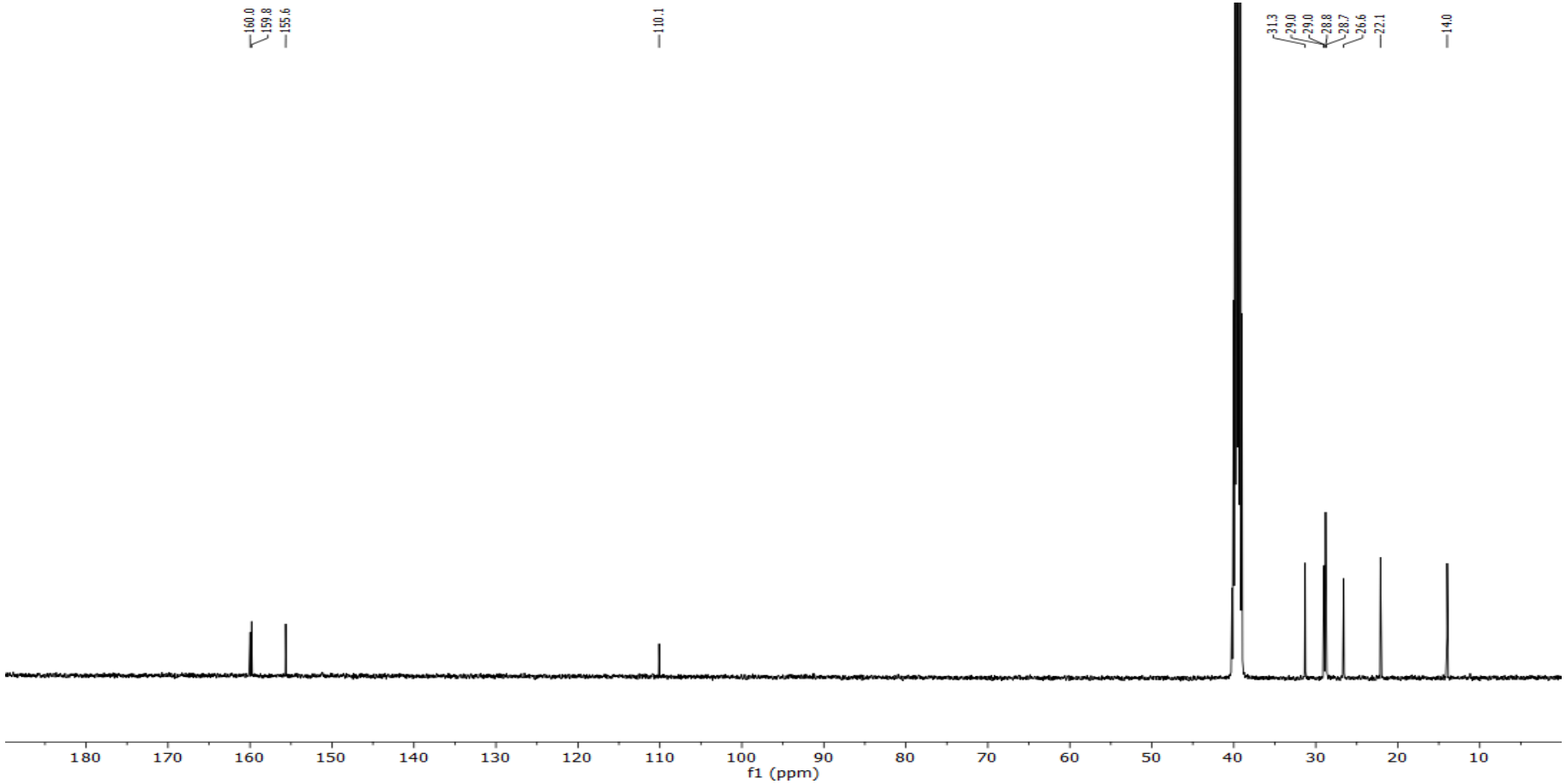




5-Nitro-6-(decylamino)uracil (**7h**)

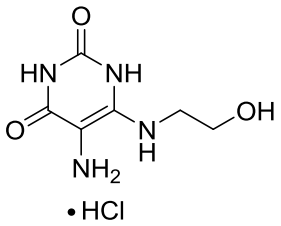
^{13}C NMR (DMSO-d$_6$, 125 MHz)

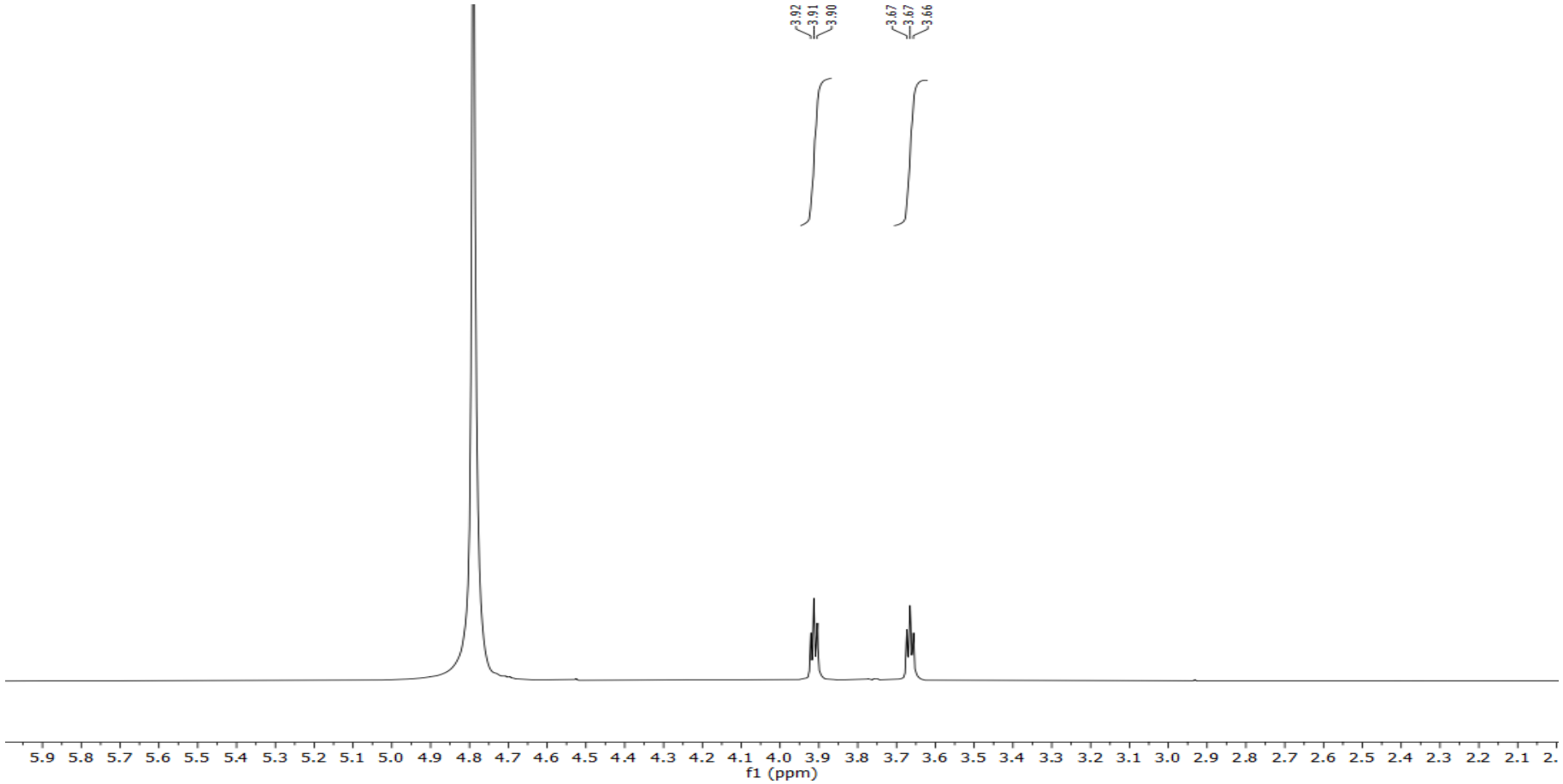




5-Amino-6-((2-hydroxyethyl)amino)uracil (**8b**)

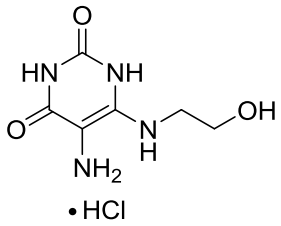
^{1}H NMR (D_2O, 500 MHz)

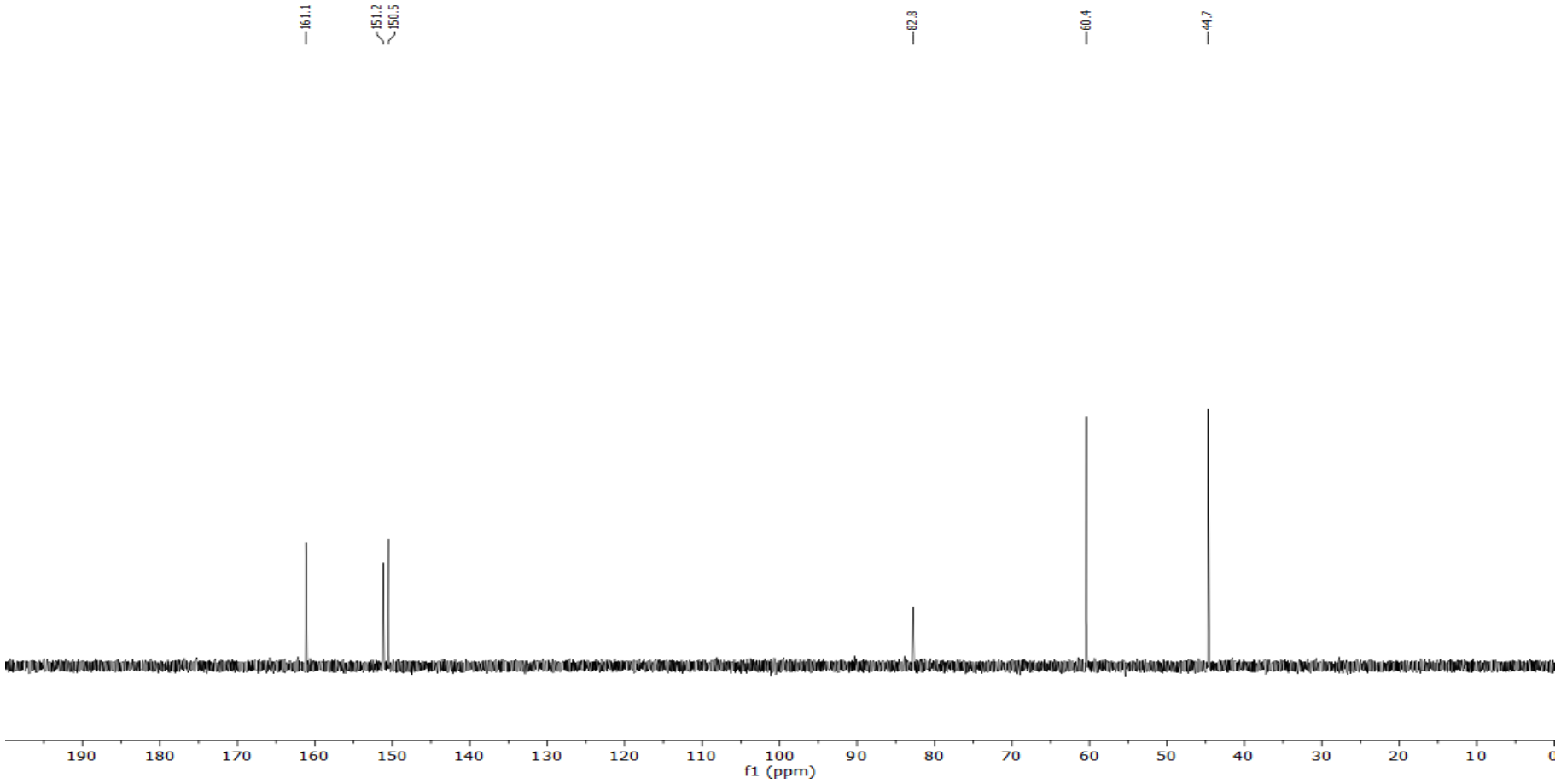




5-Amino-6-((2-hydroxyethyl)amino)uracil (**8b**)

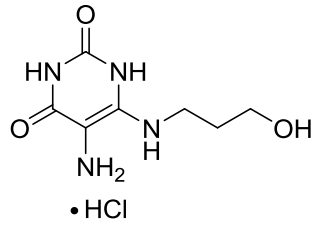
^{13}C NMR (D_2O, 125 MHz)

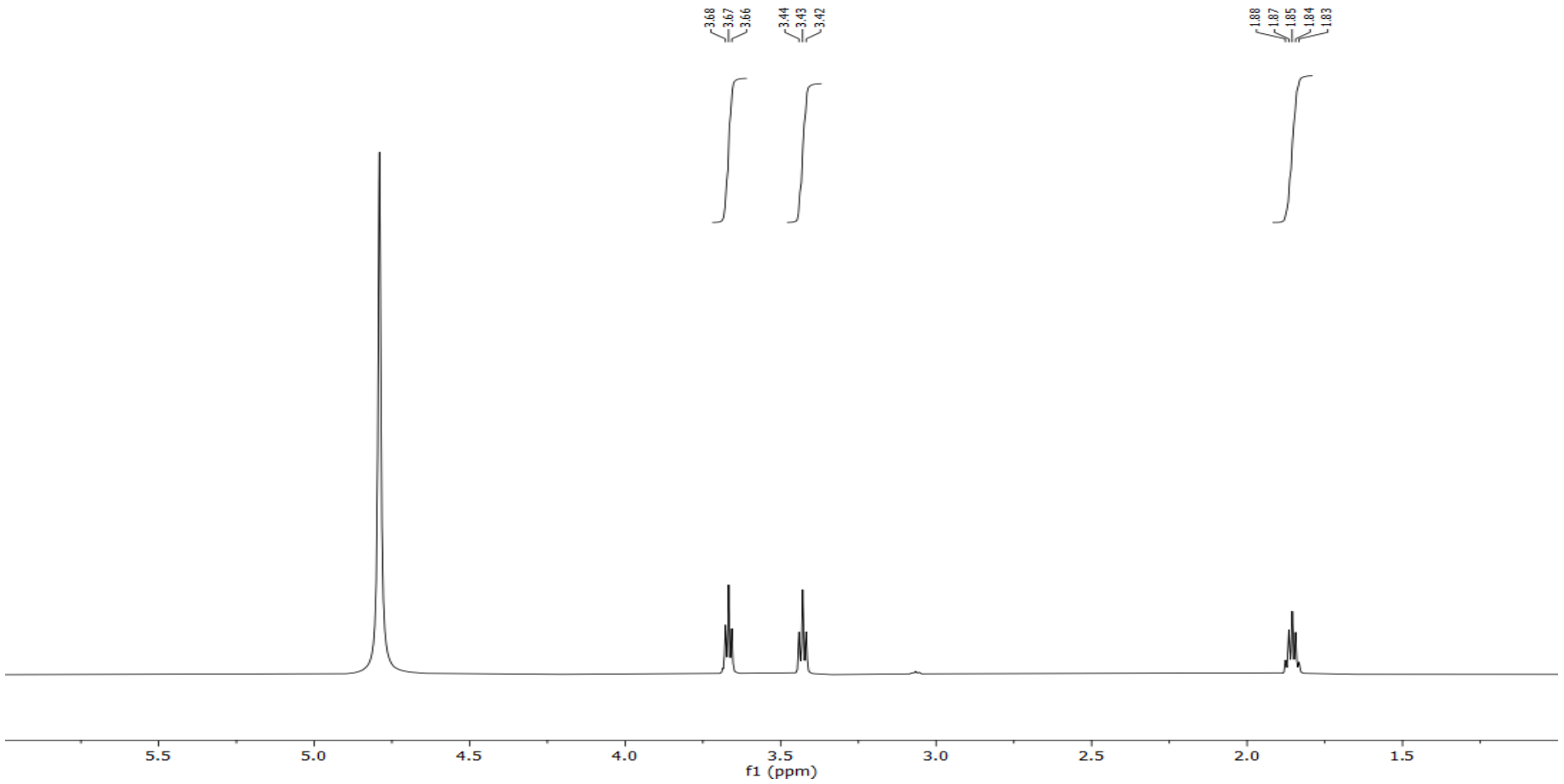




5-Amino-6-((3-hydroxypropyl)amino)uracil (**8c**)

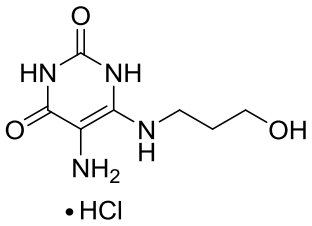
^{1}H NMR (D_2O, 500 MHz)

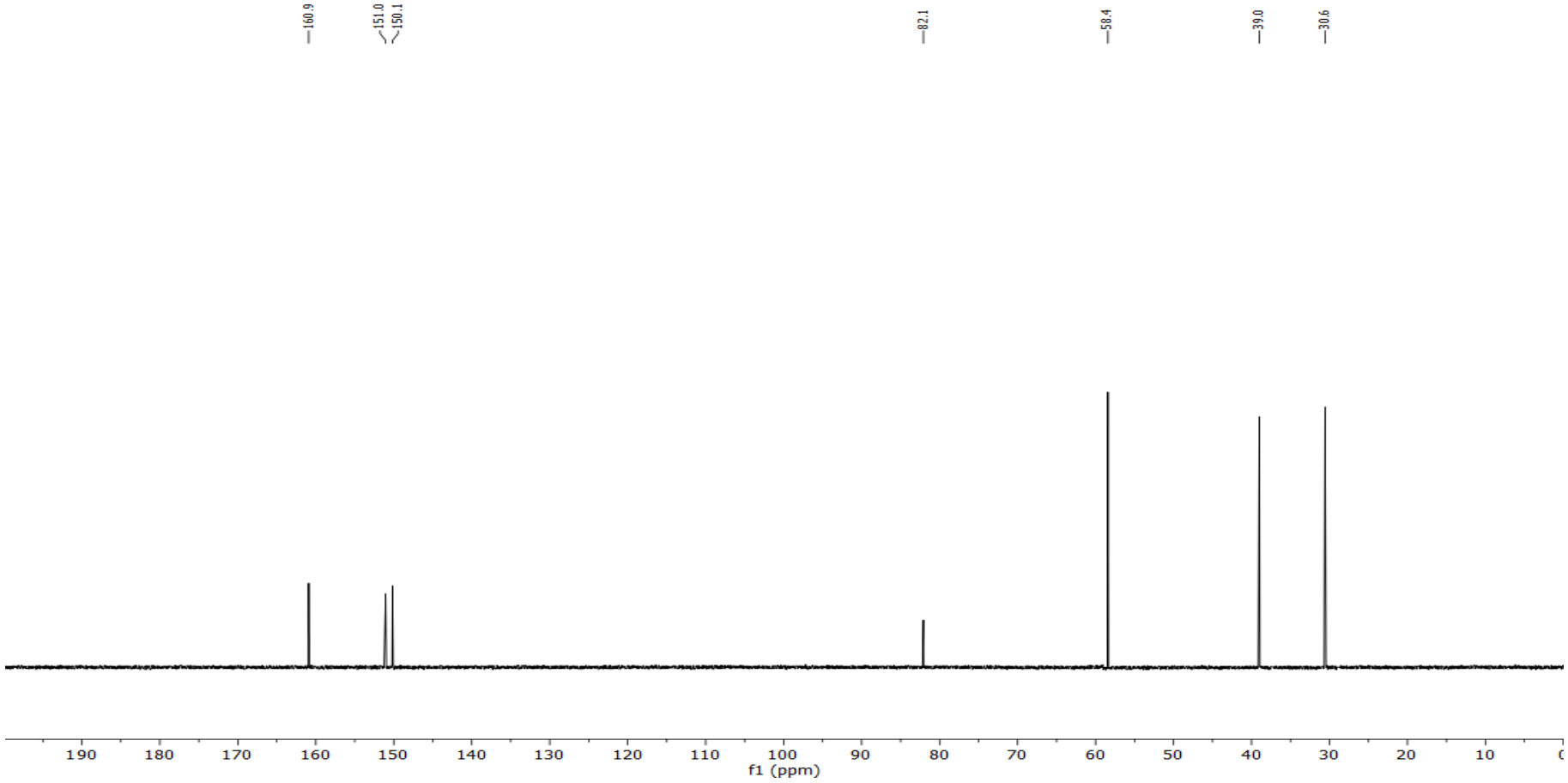




5-Amino-6-((3-hydroxypropyl)amino)uracil (**8c**)

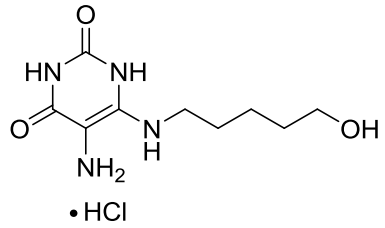
^{13}C NMR (D_2O, 125 MHz)

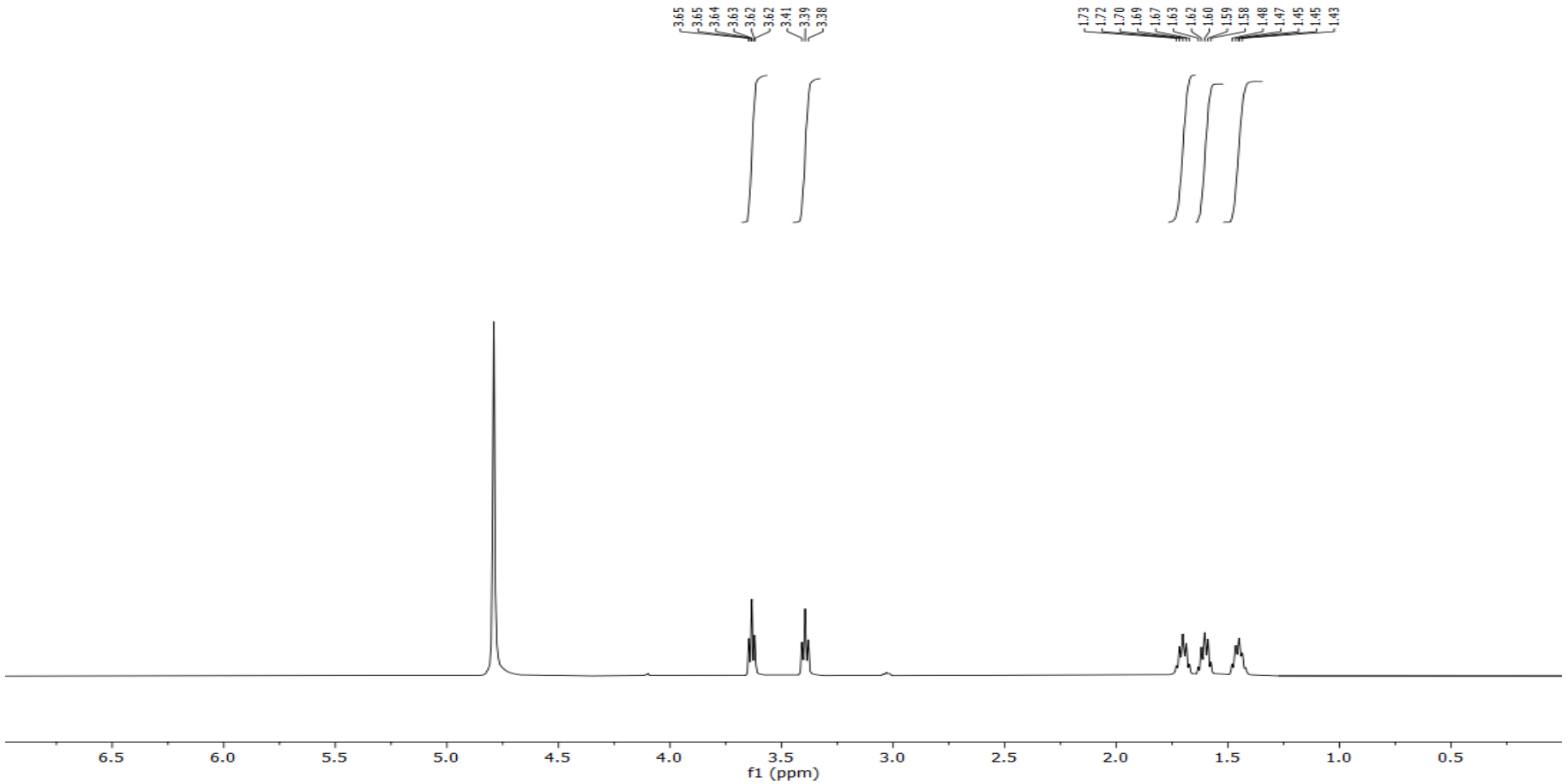




5-Amino-6-((5-hydroxypentyl)amino)uracil (**8d**)

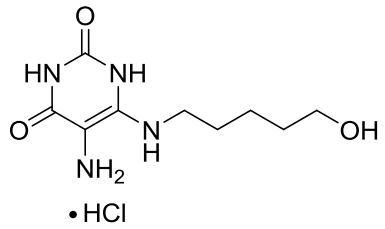
^{1}H NMR (D_2O, 500 MHz)

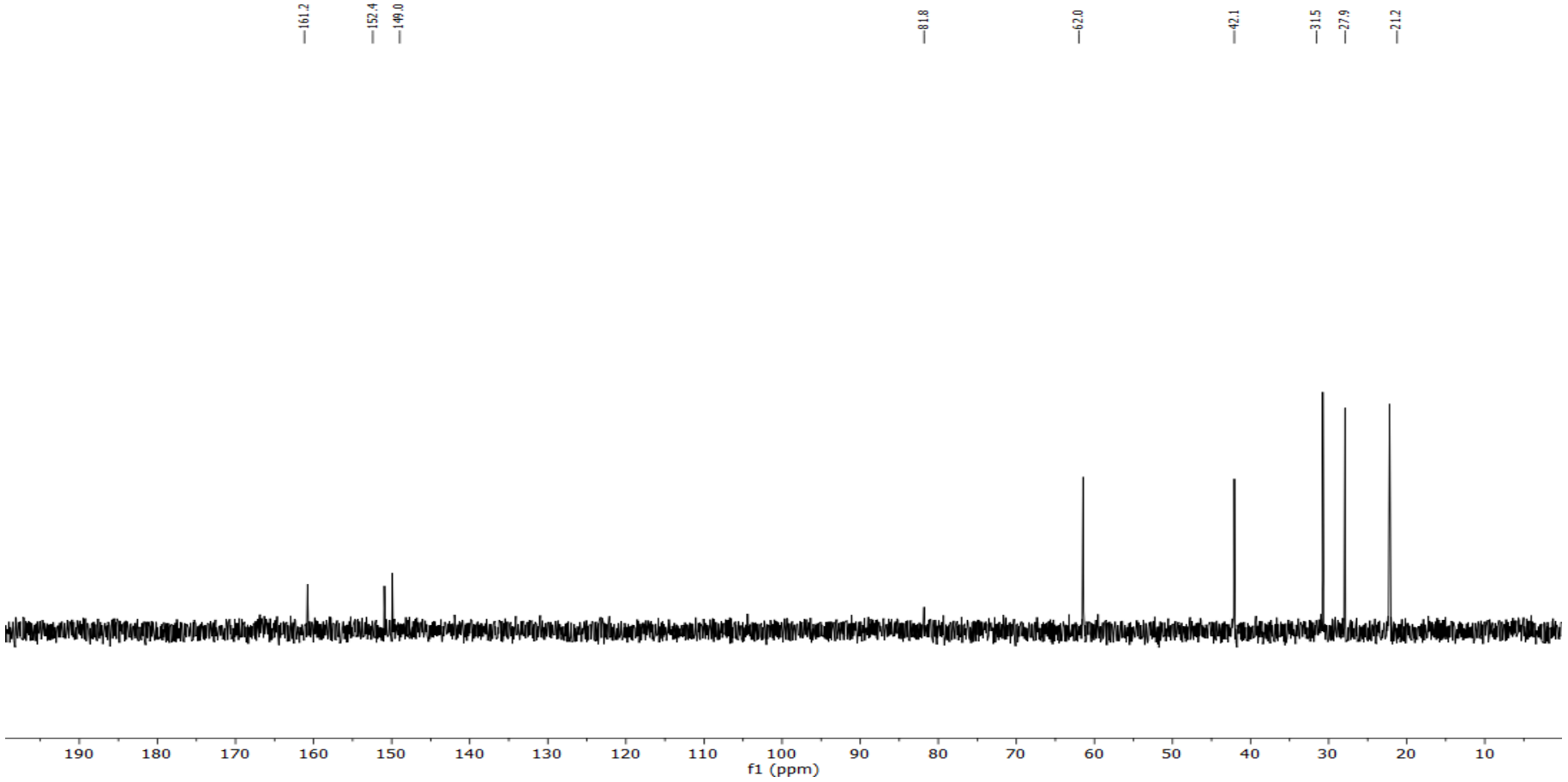




5-Amino-6-((5-hydroxypentyl)amino)uracil (**8d**)

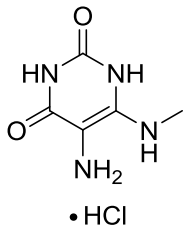
^{13}C NMR (D_2O, 125 MHz)

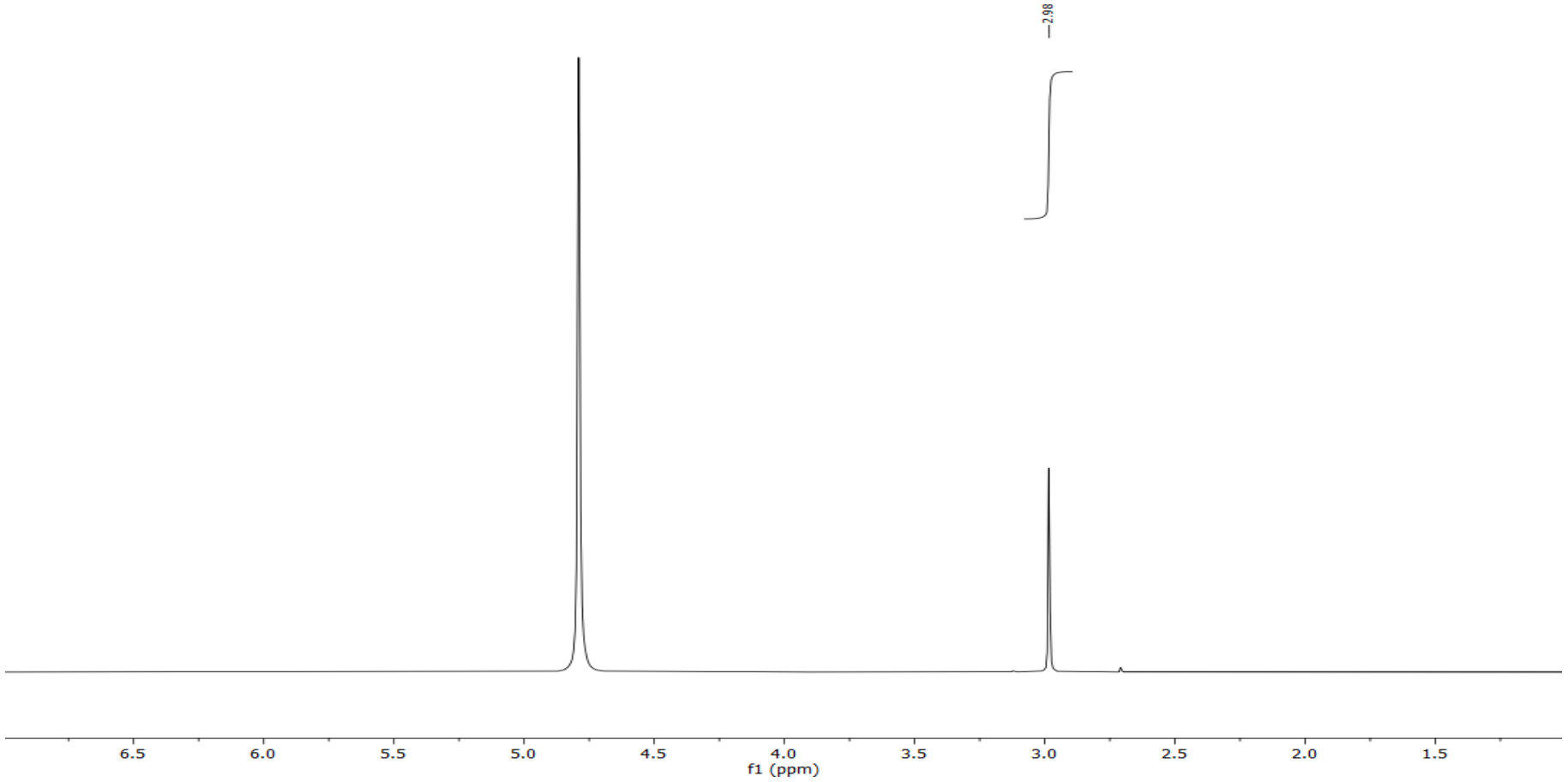




5-Amino-6-(methylamino)uracil (**8e**)

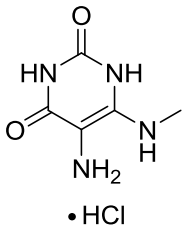
^{1}H NMR (D_2O, 500 MHz)

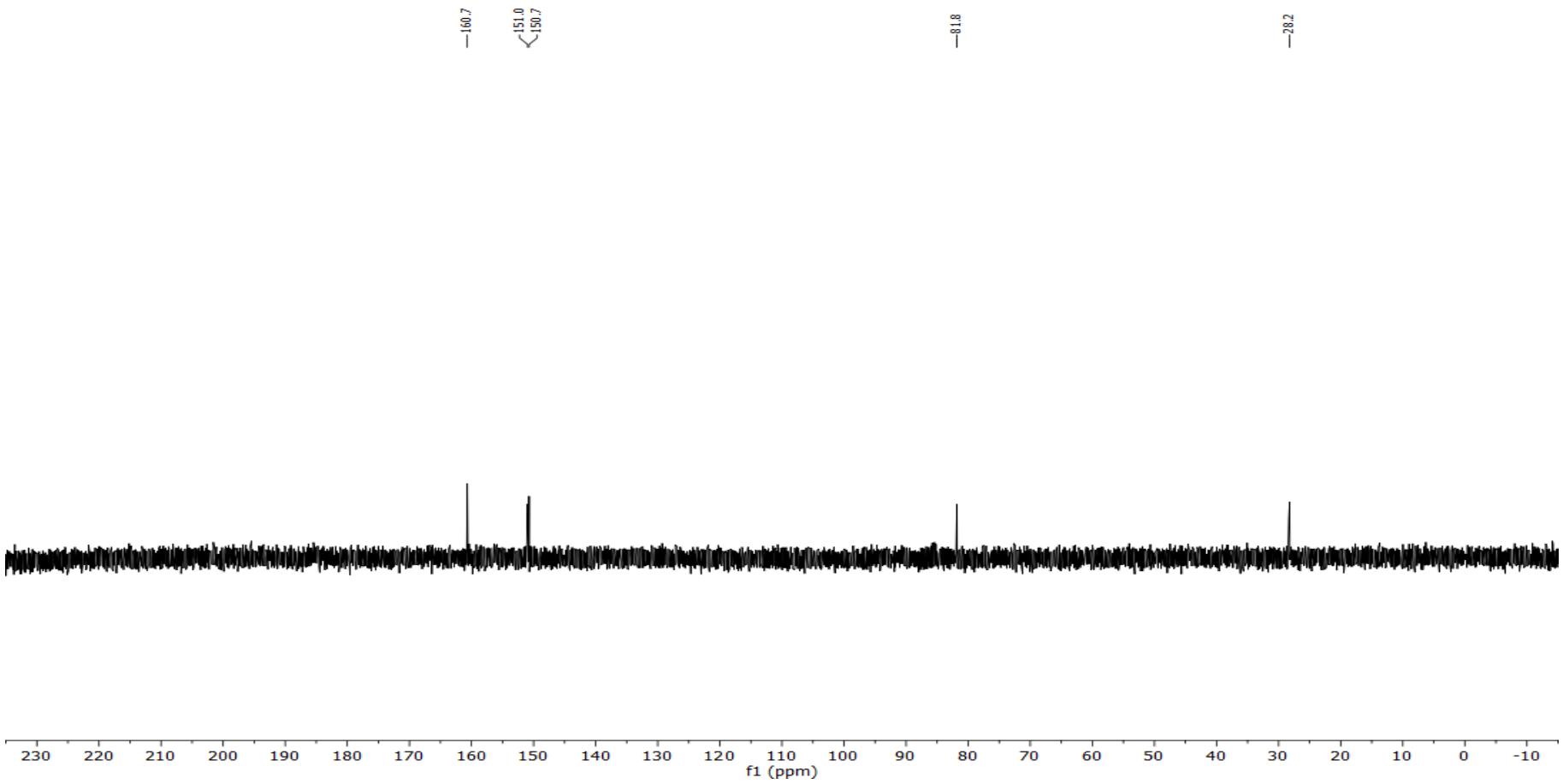




5-Amino-6-(methylamino)uracil (**8e**)

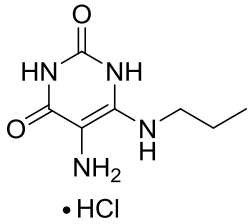
^{13}C NMR (D_2O, 125 MHz)

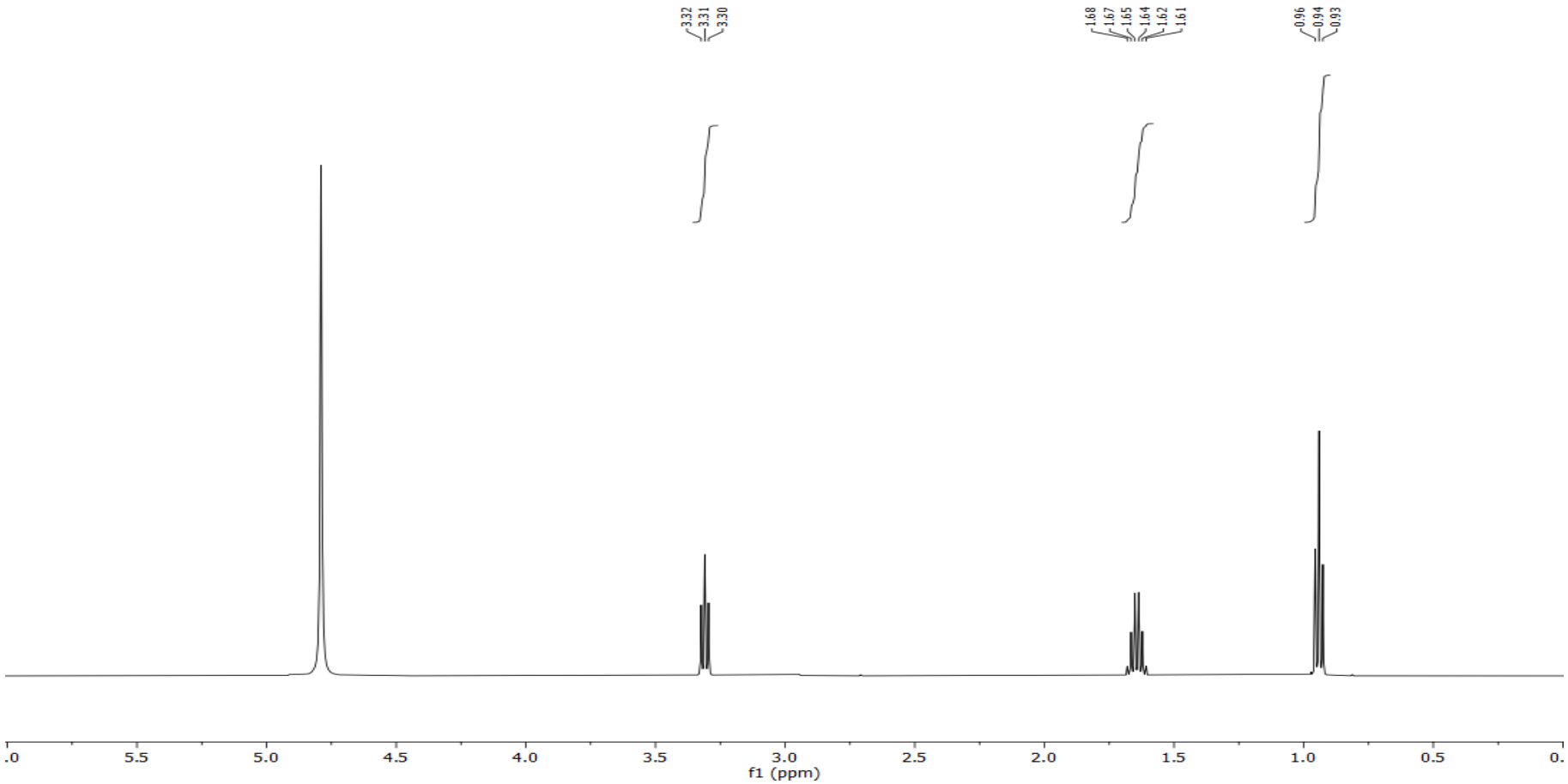




5-Amino-6-(propylamino)uracil (**8f**)

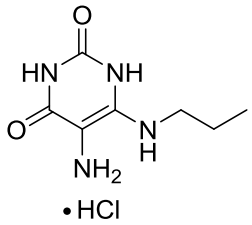
^{1}H NMR (D_2O, 500 MHz)

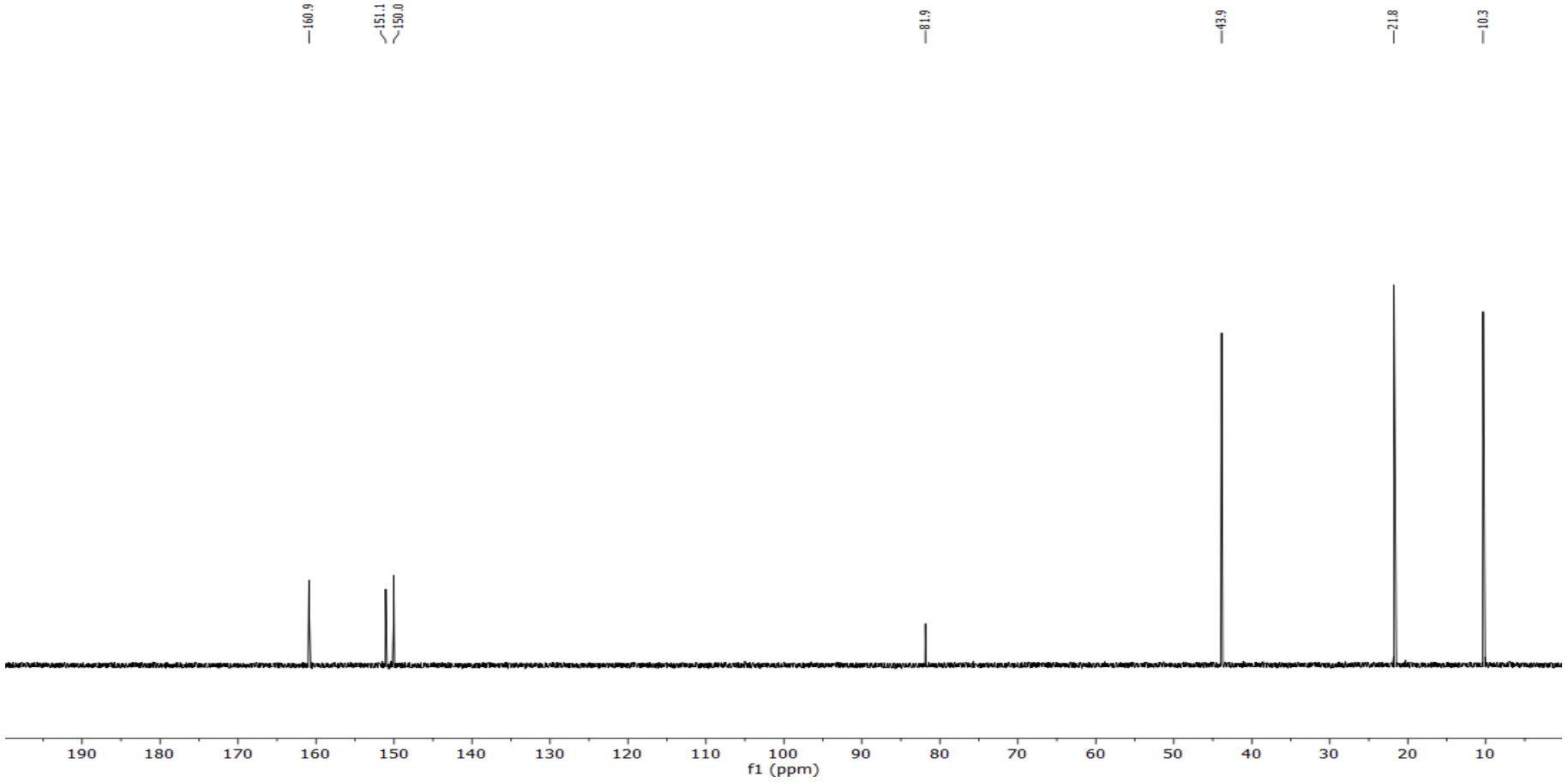




5-Amino-6-(propylamino)uracil (**8f**)

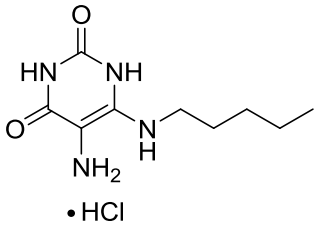
^{13}C NMR (D_2O, 125 MHz)

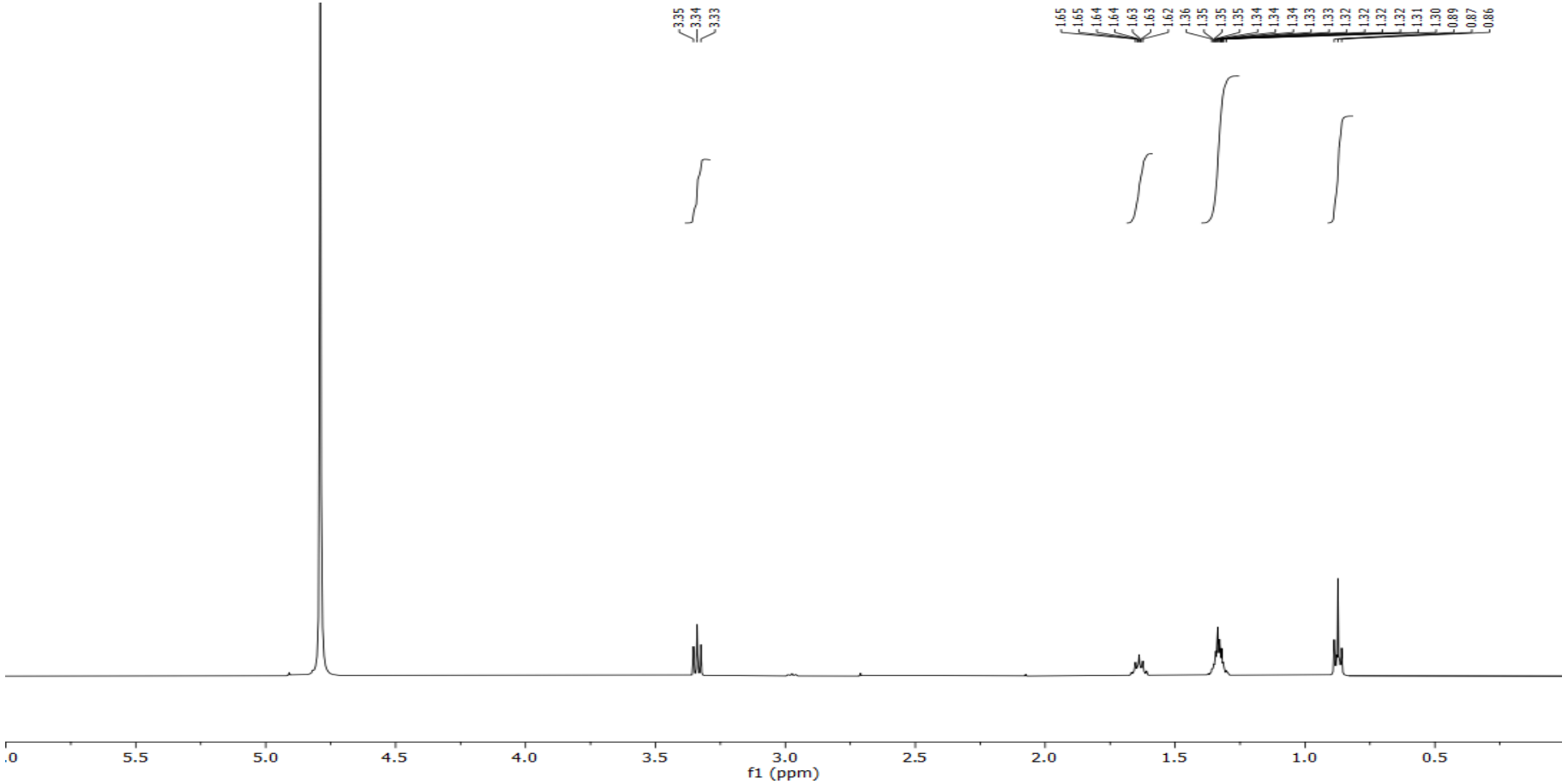




5-Amino-6-(pentylamino)uracil (**8g**)

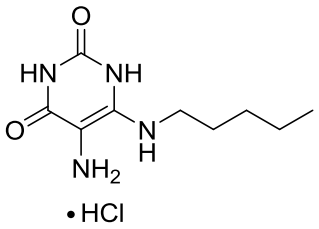
^{1}H NMR (D_2O, 500 MHz)

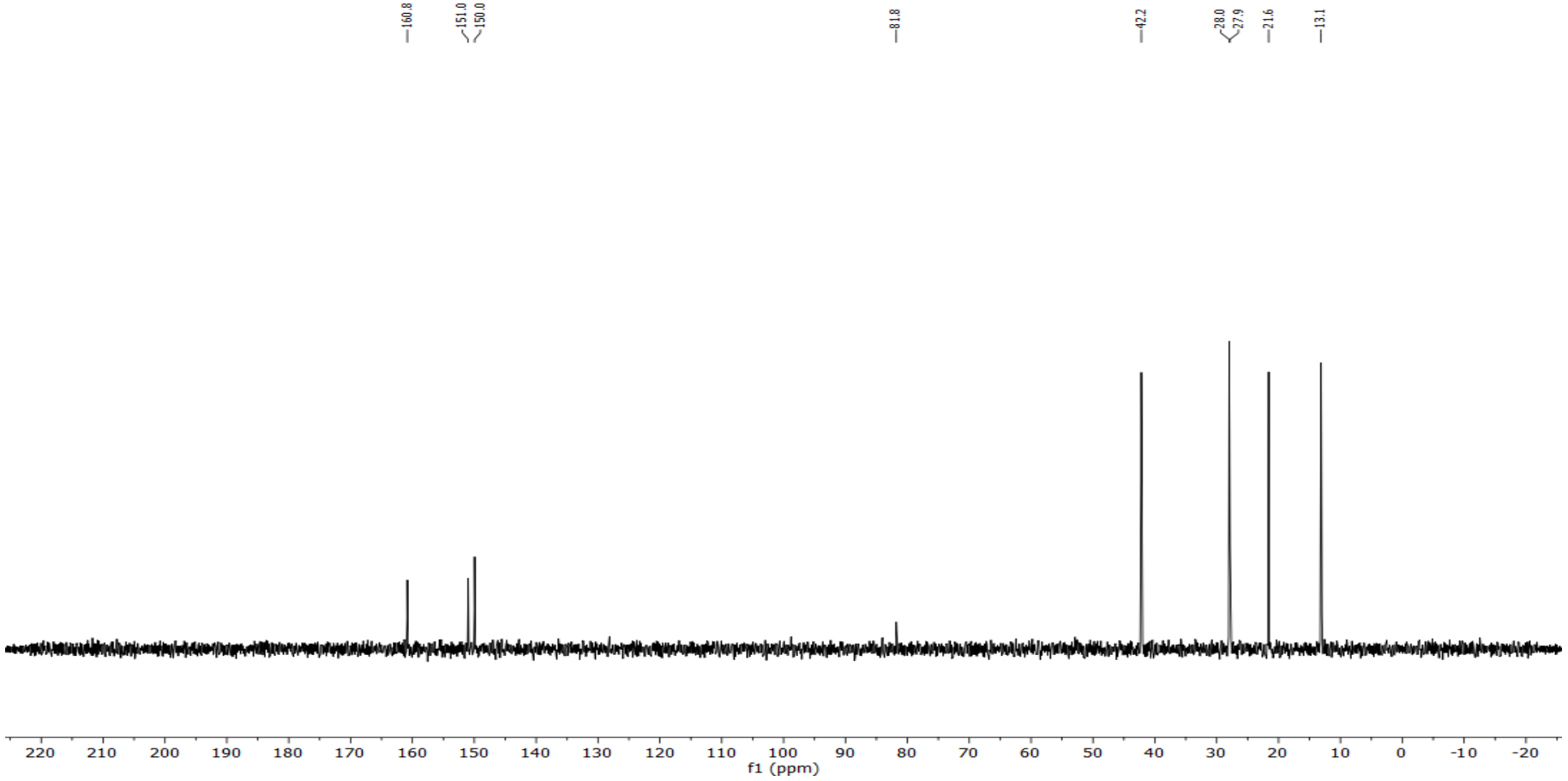




5-Amino-6-(pentylamino)uracil (**8g**)

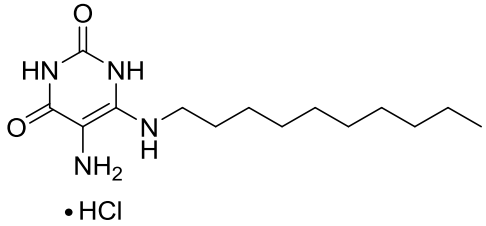
^{13}C NMR (D_2O, 125 MHz)

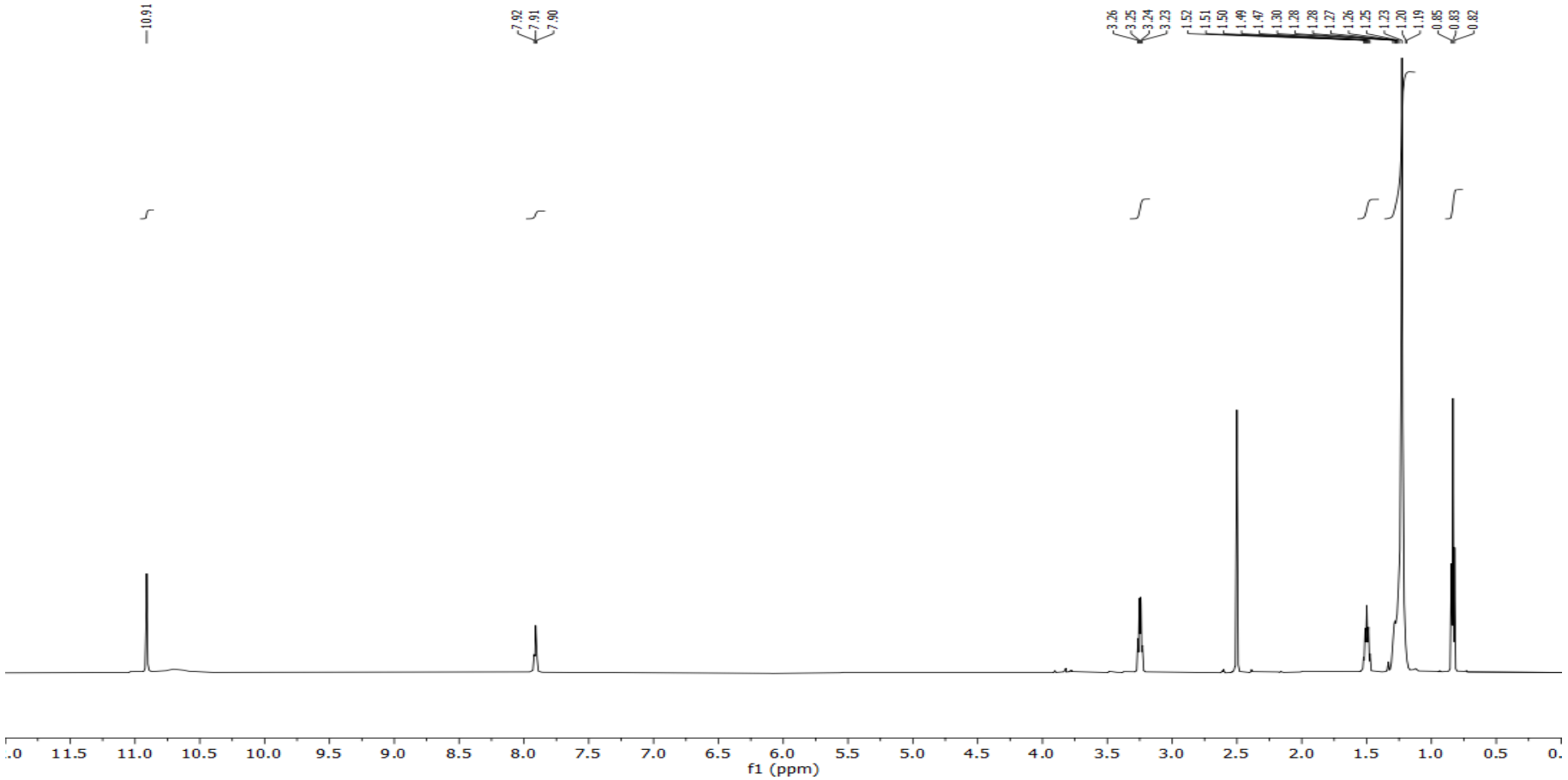




5-Amino-6-(decylamino)uracil (**8h**)

^{1}H NMR (DMSO-d_6, 500 MHz)





5-Amino-6-(decylamino)uracil (**8h**)

^{13}C NMR (DMSO-d$_6$, 125 MHz)