Supporting Information

The development of a PET Radiotracer for Imaging Alpha Synuclein Aggregates in Parkinson's Disease

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Synthesis of **[^{3}H]M503-1619**. A mixture of desmethyl **M503-1619** (0.6 mg and cesium carbonate (3 mg) was prepared in dimethyl sulfoxide (100 μL). [^{3}H]Methyl iodide (27 mCi) in dimethyl sulfoxide (300 μL) was then added, and the reaction was stirred at ambient temperature overnight. The reaction mixture was dissolved in 1 mL of HPLC eluent and 100 μL of 2% trifluoroacetic acid (TFA) before purification by high-performance liquid chromatography (HPLC). Chromatographic separation was performed on a KROMASIL C18 column (7 μm, 250 × 10 mm) using a mobile phase of 27% acetonitrile in 0.1% TFA at a flow rate of 2.0 mL/min, with UV detection at 254 nm. Fractions eluting at 12 minutes were collected, combined, and diluted to 20 mL with water. The resulting solution was passed through a Sep-Pak® Plus Short C18 cartridge (pre-washed sequentially with ethanol and water), followed by a final wash with water. The purified product was eluted from the Sep-Pak cartridge using 10 mL of ethanol. The final purified product had an activity of 10 mCi, a concentration of 1.0 mCi/mL, and a molar activity of 82 Ci/mmol.

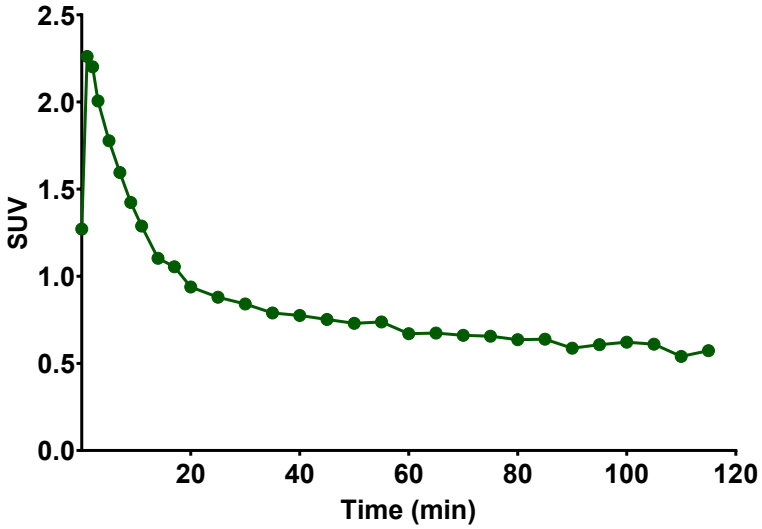


Figure S1. Whole brain uptake (SUV) of [^{11}C]**M503-1619** in cynomolgus monkey brain.

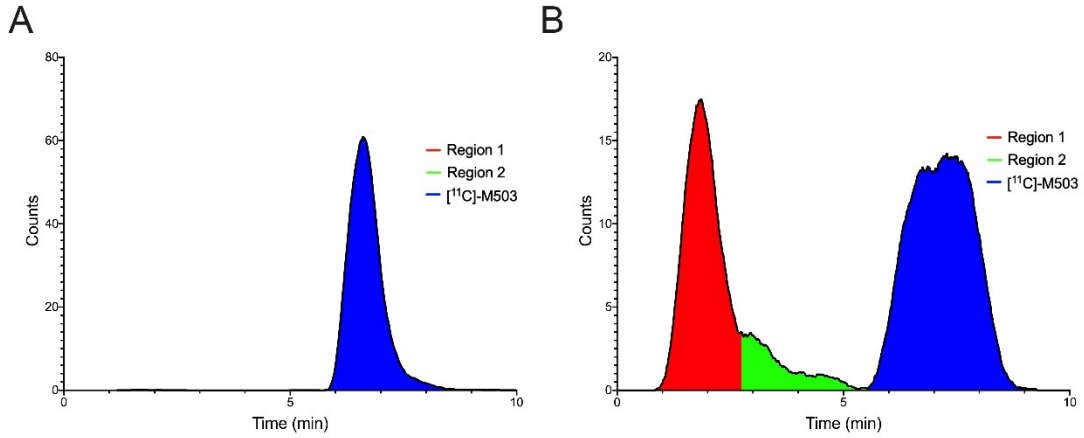


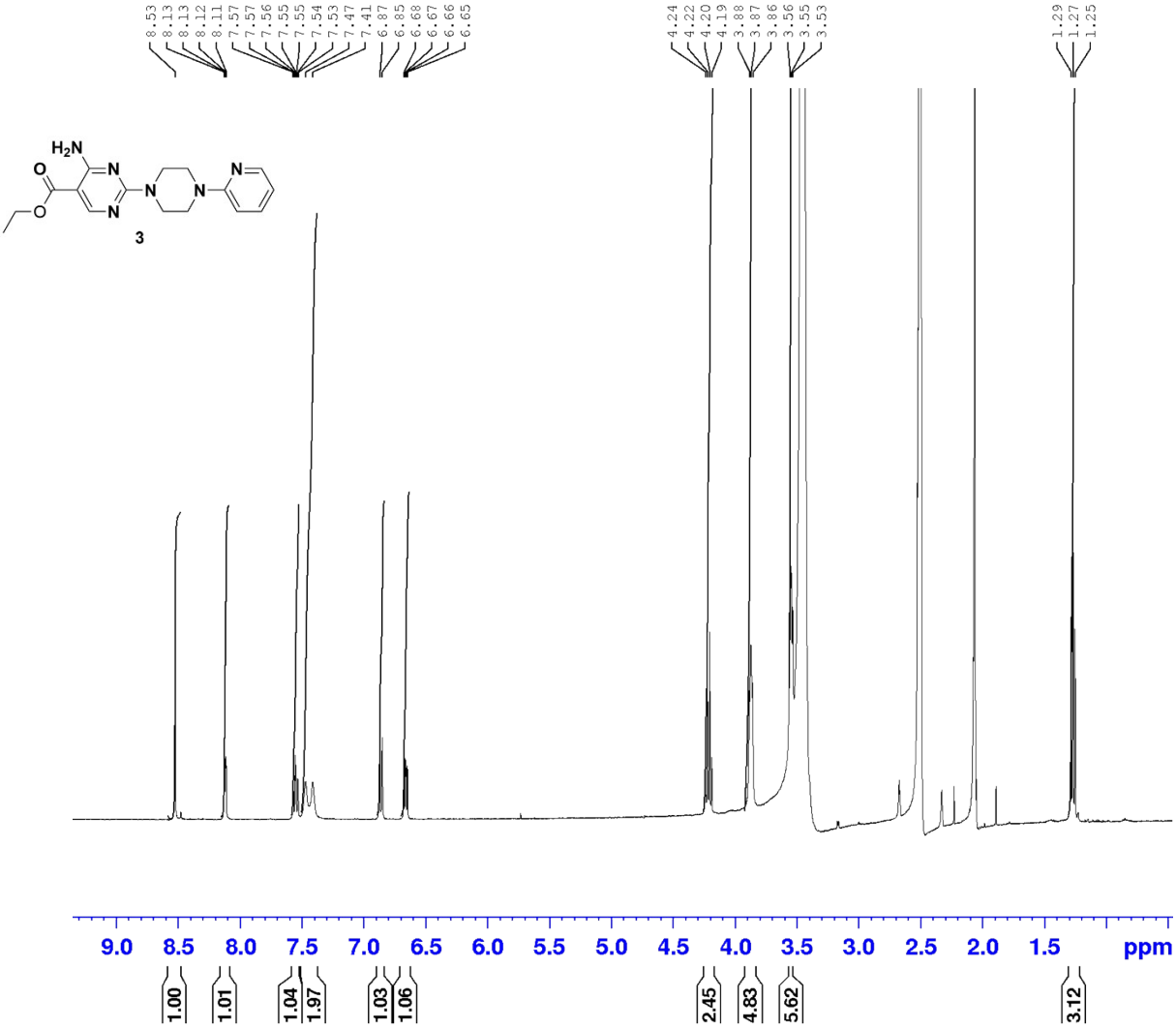
Figure S2. Example HPLC radiochromatograms of **(A)** [^{11}C]**M503-1619** dose and **(B)** plasma sampled at 7.5 minutes post-injection.. The dose was measured after remaining at room temperature for the duration of imaging. Parent compound is shown in blue, radiometabolites are shown in red (region 1) and green (region 2).

Table S1. Metabolite data for [^{11}C]**M503-1619** in a rhesus monkey

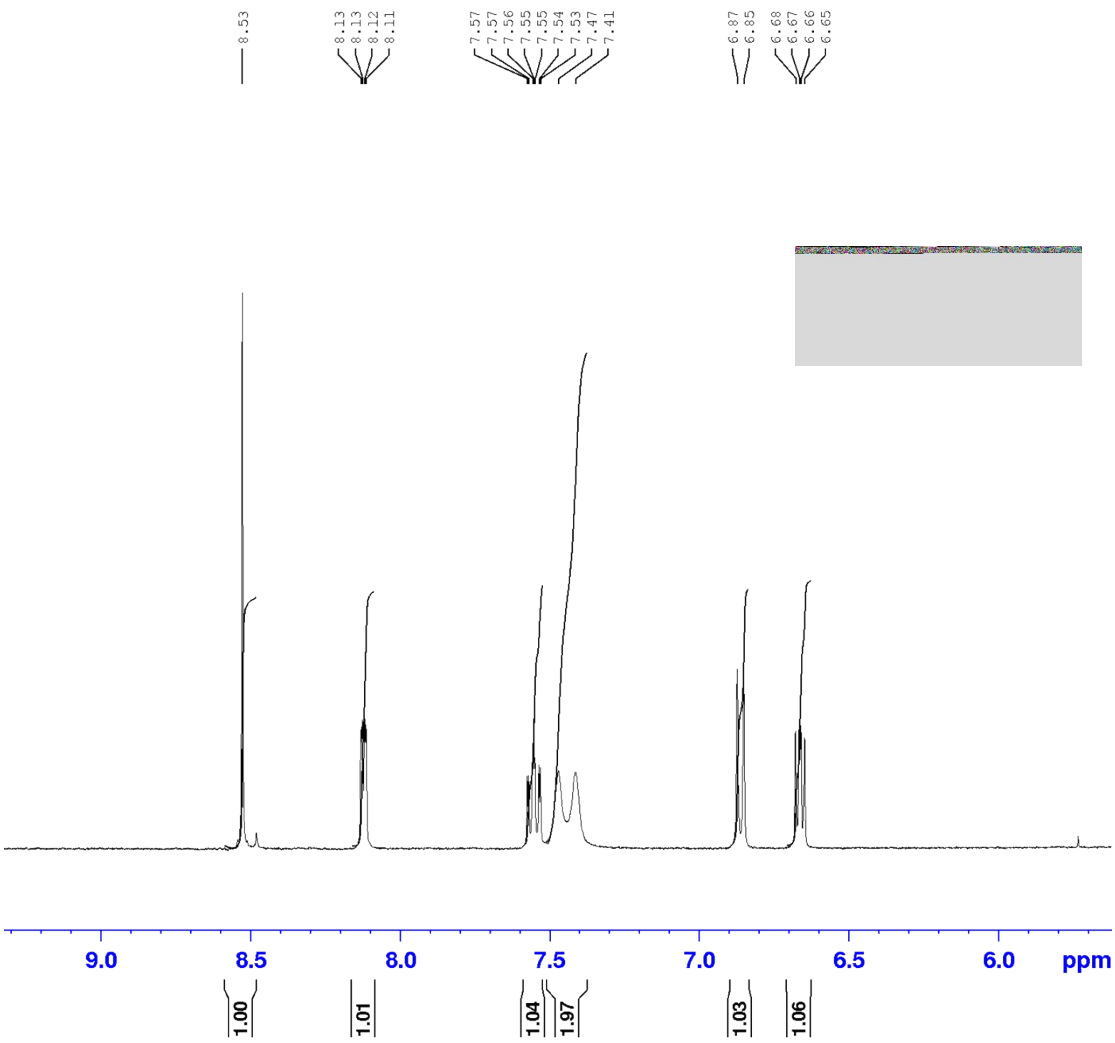
Time (min)	Parent (%) (R_T = 7.5 min)	Metabolite 1 (%) (R_T = 2 min)	Metabolite 2 (%) (R_T = 3 min)
7.5	57	35	8
17	24	67	9
30	12	76	12

^{1}H and ^{13}C NMR data

Ethyl 4-amino-2-(4-(pyridin-2-yl)piperazin-1-yl)pyrimidine-5-carboxylate (**3**)



^{1}H-NMR 400 MHz (DMSO) δ 8.53 (s, 1H), 8.12 (dd, 1H, J = 4.9 Hz, J = 1.4 Hz), 7.57-7.53 (m, 1H), 7.47 (s, 1H), 7.41 (s, 1H), 6.86 (d, 1H, J = 8.6 Hz),), 6.66 (q, 1H, J = 3.9 Hz), 4.21 (q, 2H, J=7.1 Hz), 3.87 (t, 4H, J = 5.1 Hz), 3.55 (t, 4H, J = 5.1 Hz), 1.27 (t, 3H, J = 7.1 Hz).



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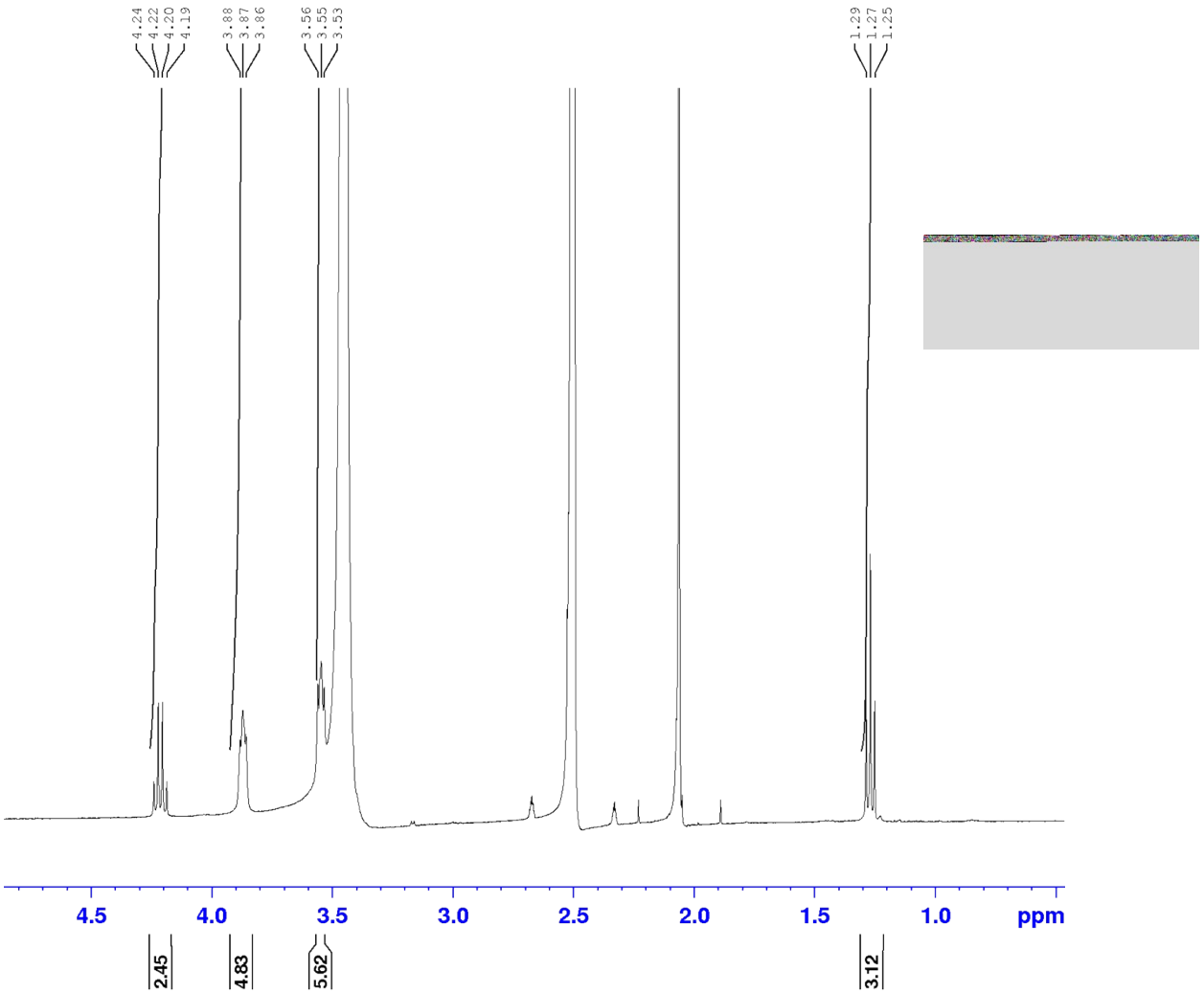

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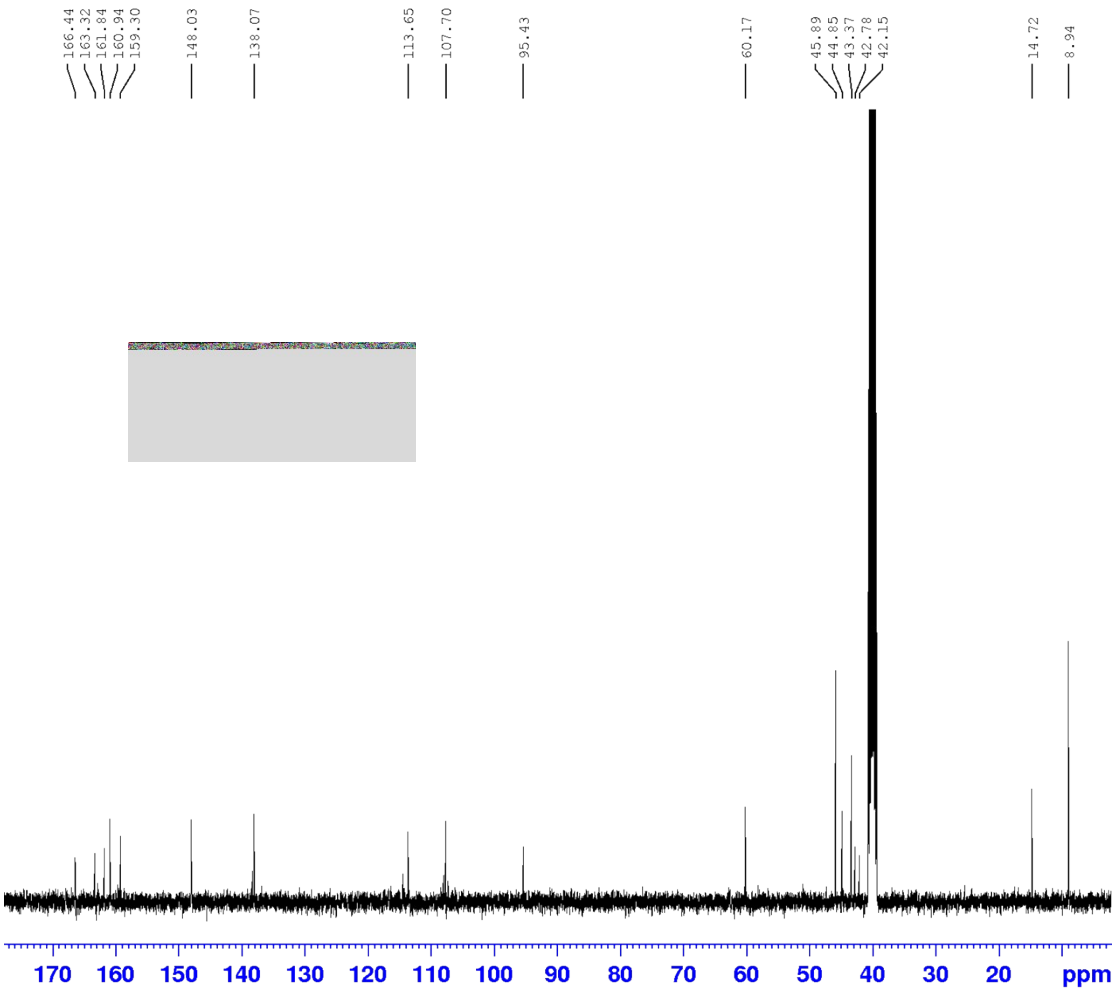
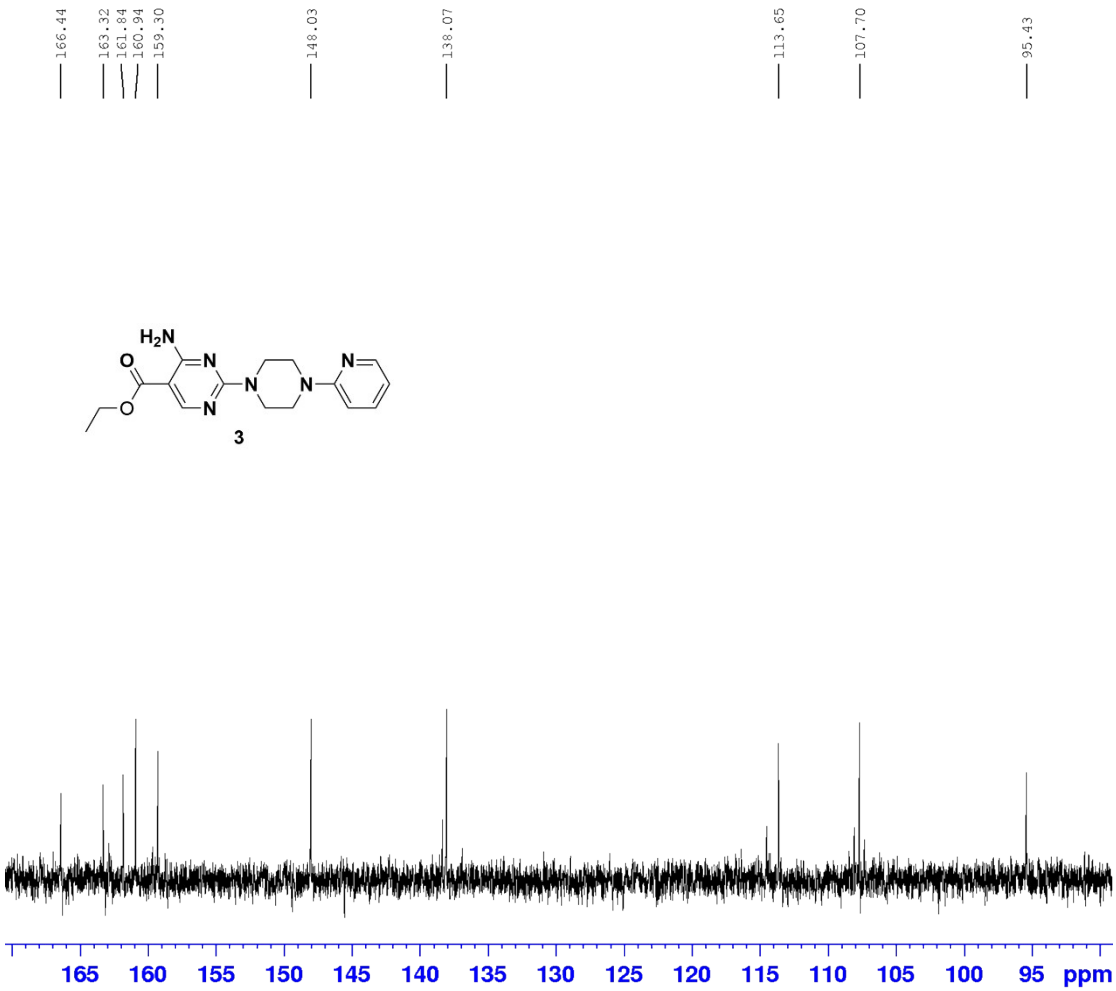
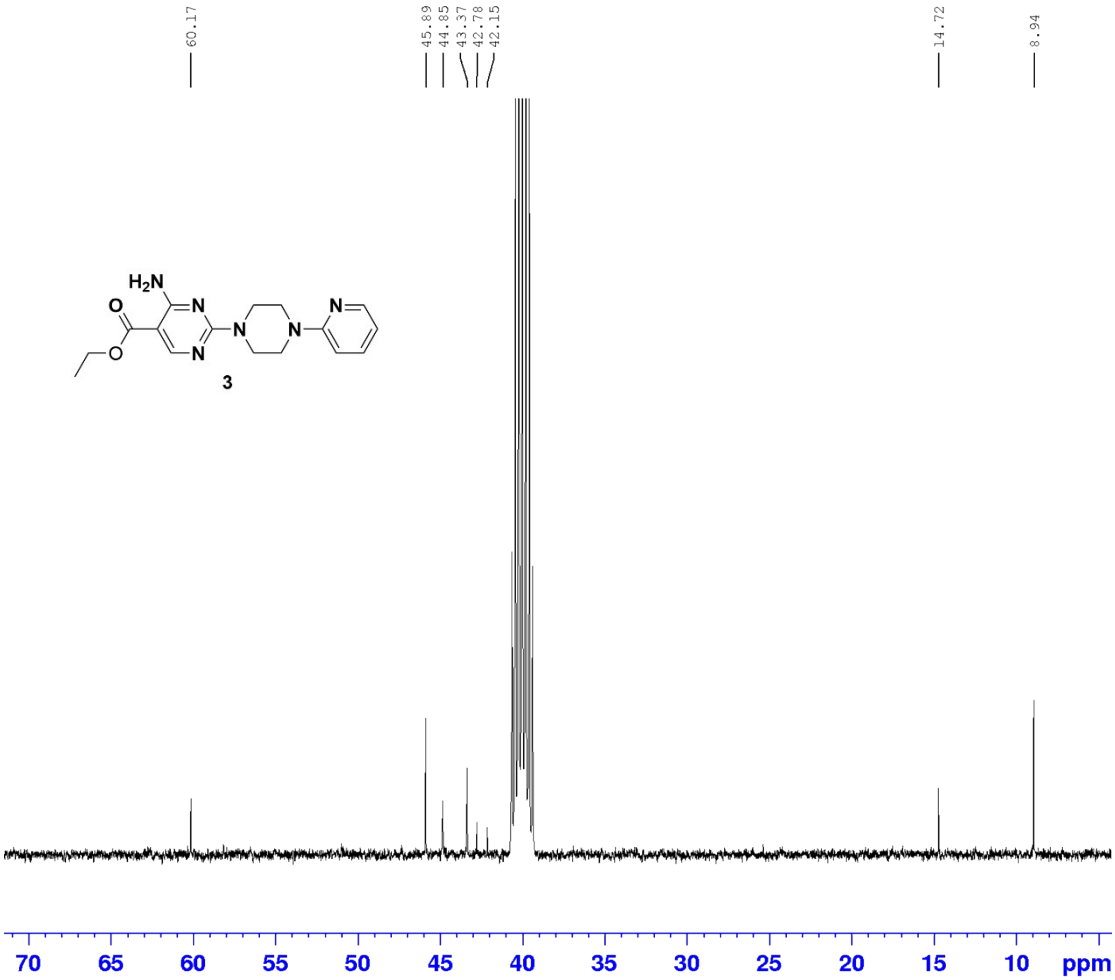


^{13}C-NMR 100 MHz (DMSO) δ 166.4, 163.3, 161.8, 160.9, 159.3, 148.0, 138.1, 113.7, 107.7, 95.4, 60.2, 45.9, 44.9, 43.4, 42.8, 42.2, 14.7, 8.9.

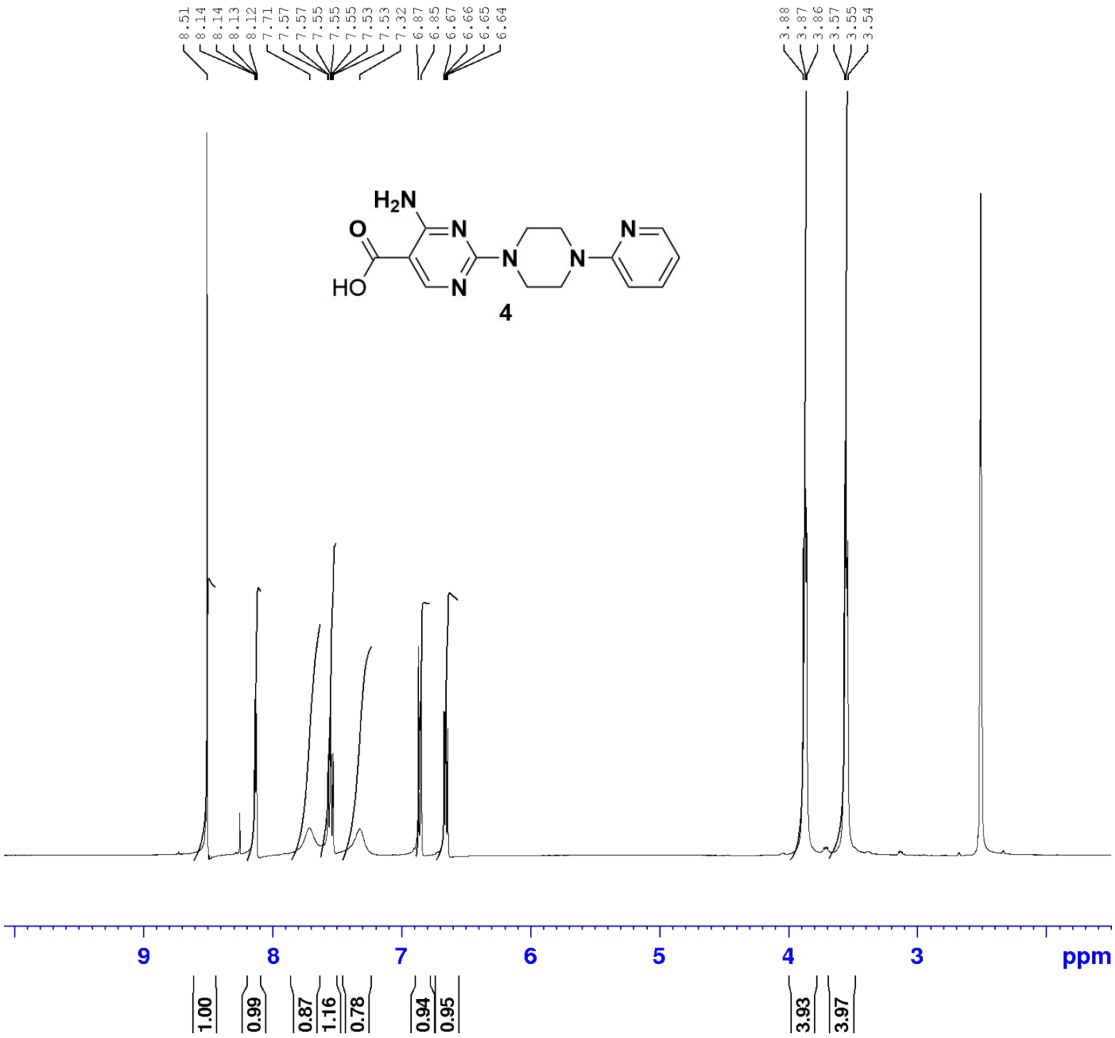


^{13}C -NMR 100 MHz (DMSO) δ 166.4, 163.3, 161.8, 160.9, 159.3, 148.0, 138.1, 113.7, 107.7, 95.4, 60.2, 45.9, 44.9, 43.4, 42.8, 42.2, 14.7, 8.9.

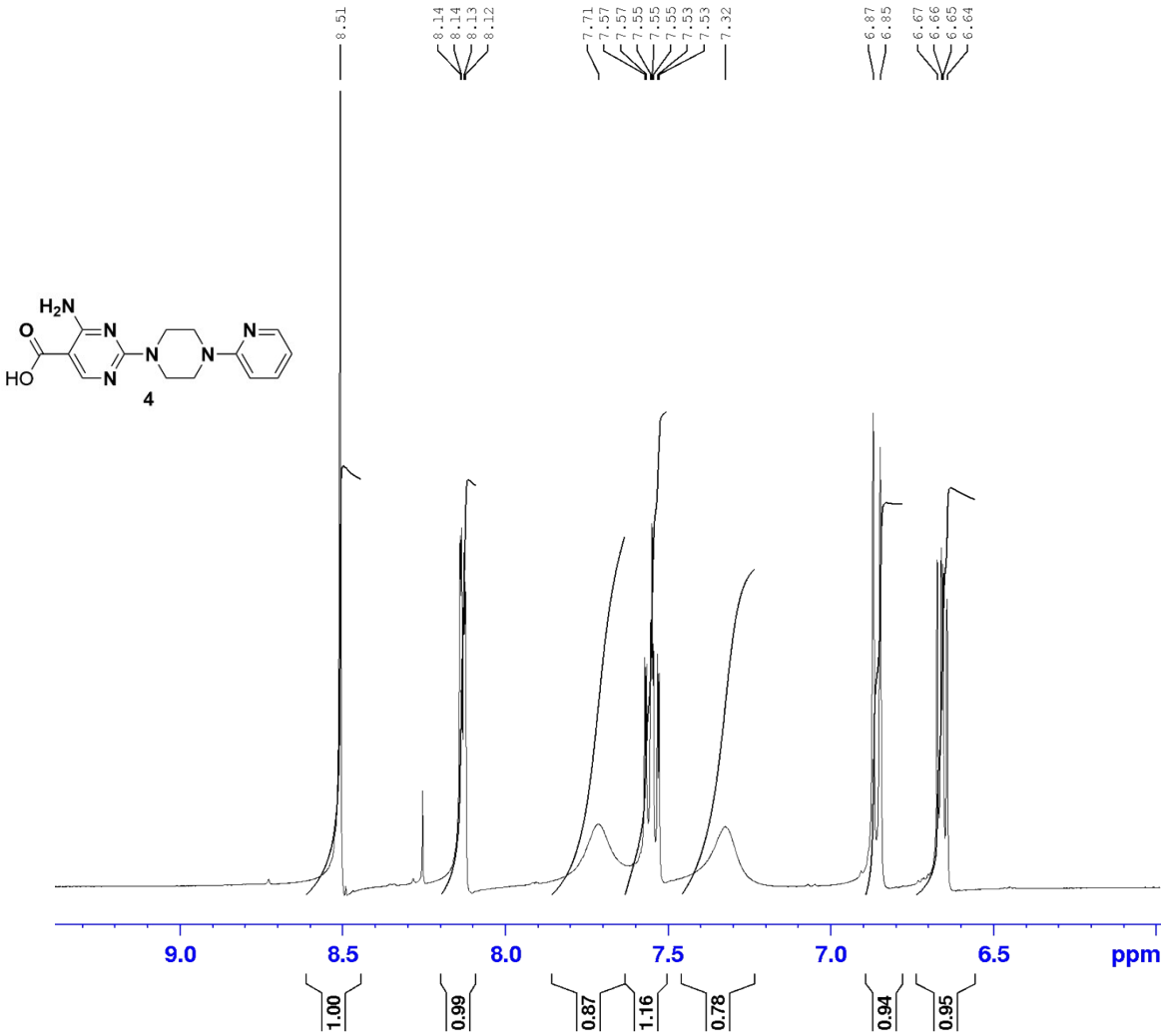


^{13}C -NMR 100 MHz (DMSO) δ 166.4, 163.3, 161.8, 160.9, 159.3, 148.0, 138.1, 113.7, 107.7, 95.4, 60.2, 45.9, 44.9, 43.4, 42.8, 42.2, 14.7, 8.9.

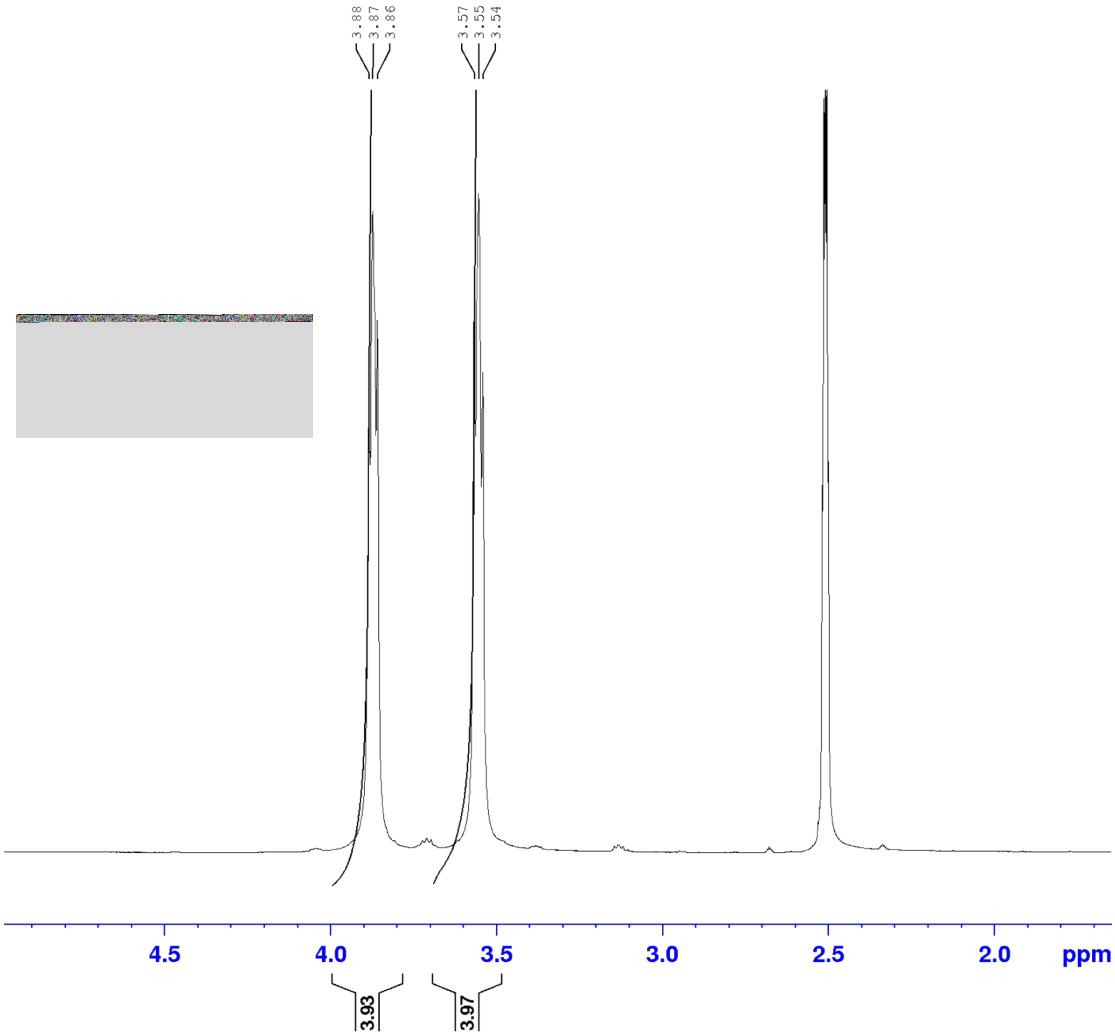
4-amino-2-(4-(pyridin-2-yl)piperazin-1-yl)pyrimidine-5-carboxylic acid (**4**)



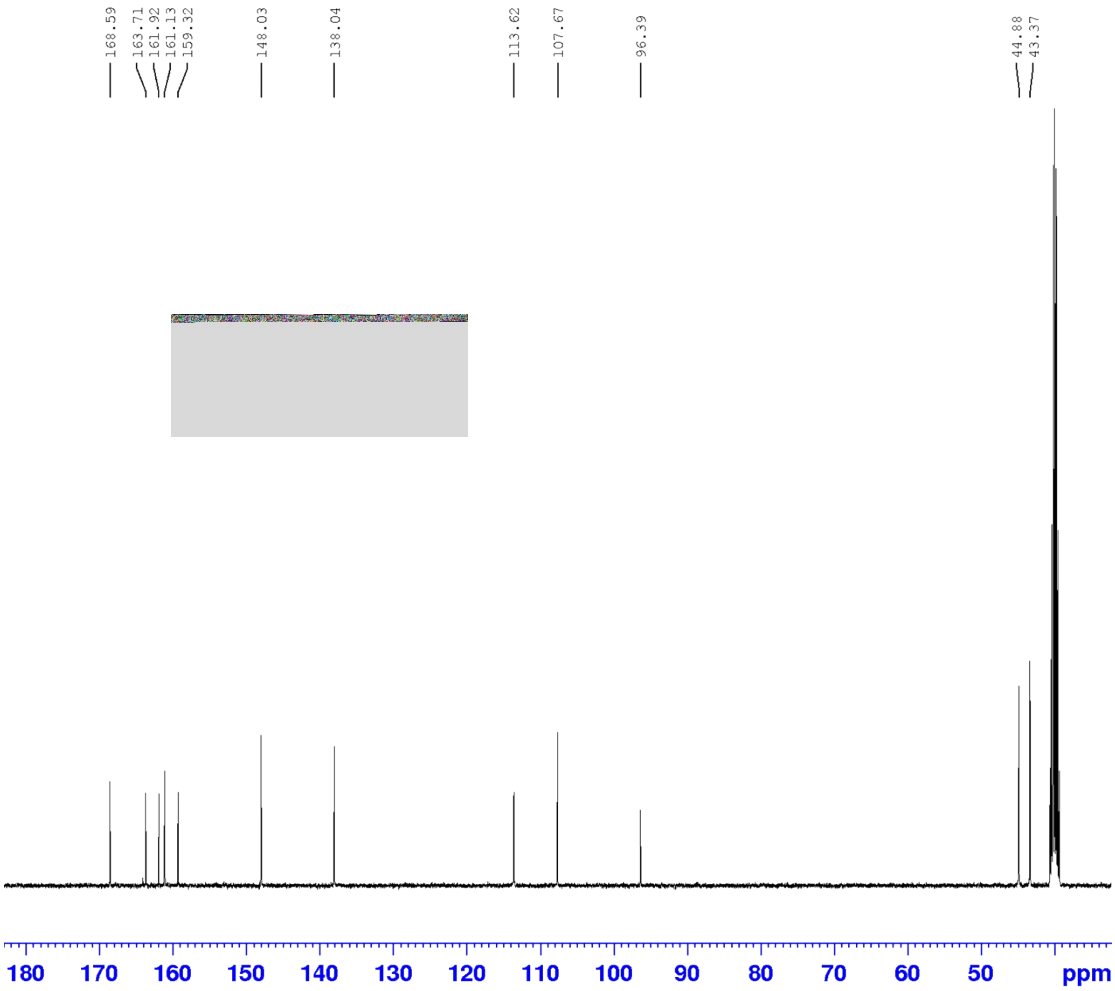
^{1}H-NMR 400 MHz (DMSO) δ 8.51 (s, 1H), 8.13 (dd, 1H, J = 4.8 Hz, J = 1.4 Hz), 7.71 (br s, 1H), 7.56-7.52 (m, 1H), 7.32 (br s, 1H), 6.86 (d, 1H, J = 8.6 Hz), 6.66 (q, 1H, J = 4.0 Hz), 3.87 (t, 4H, J = 5.1 Hz), 3.55 (t, 4H, J = 5.1 Hz).



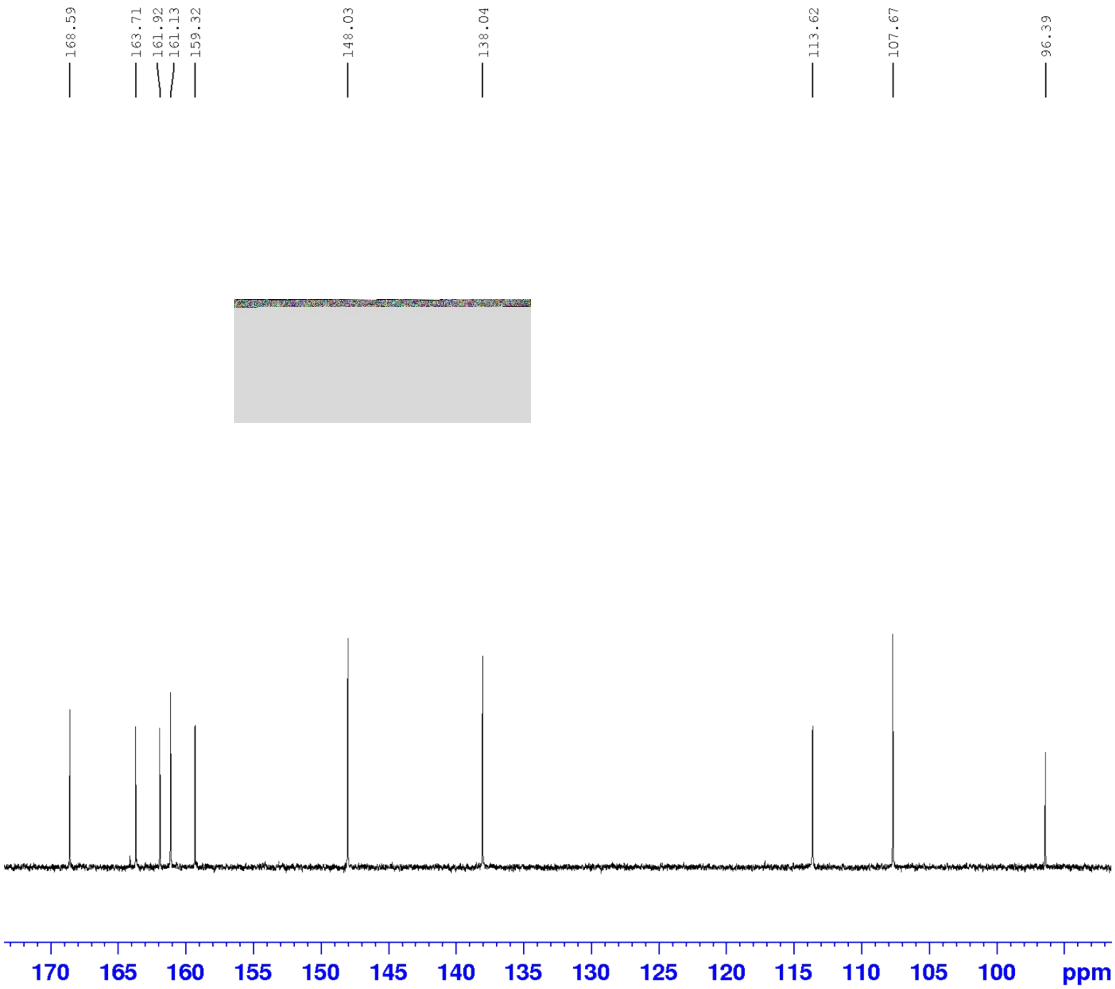
^{1}H -NMR 400 MHz (DMSO) δ 8.51 (s, 1H), 8.13 (dd, 1H, J = 4.8 Hz, J = 1.4 Hz), 7.71 (br s, 1H), 7.56-7.52 (m, 1H), 7.32 (br s, 1H), 6.86 (d, 1H, J = 8.6 Hz), 6.66 (q, 1H, J = 4.0 Hz), 3.87 (t, 4H, J = 5.1 Hz), 3.55 (t, 4H, J = 5.1 Hz).



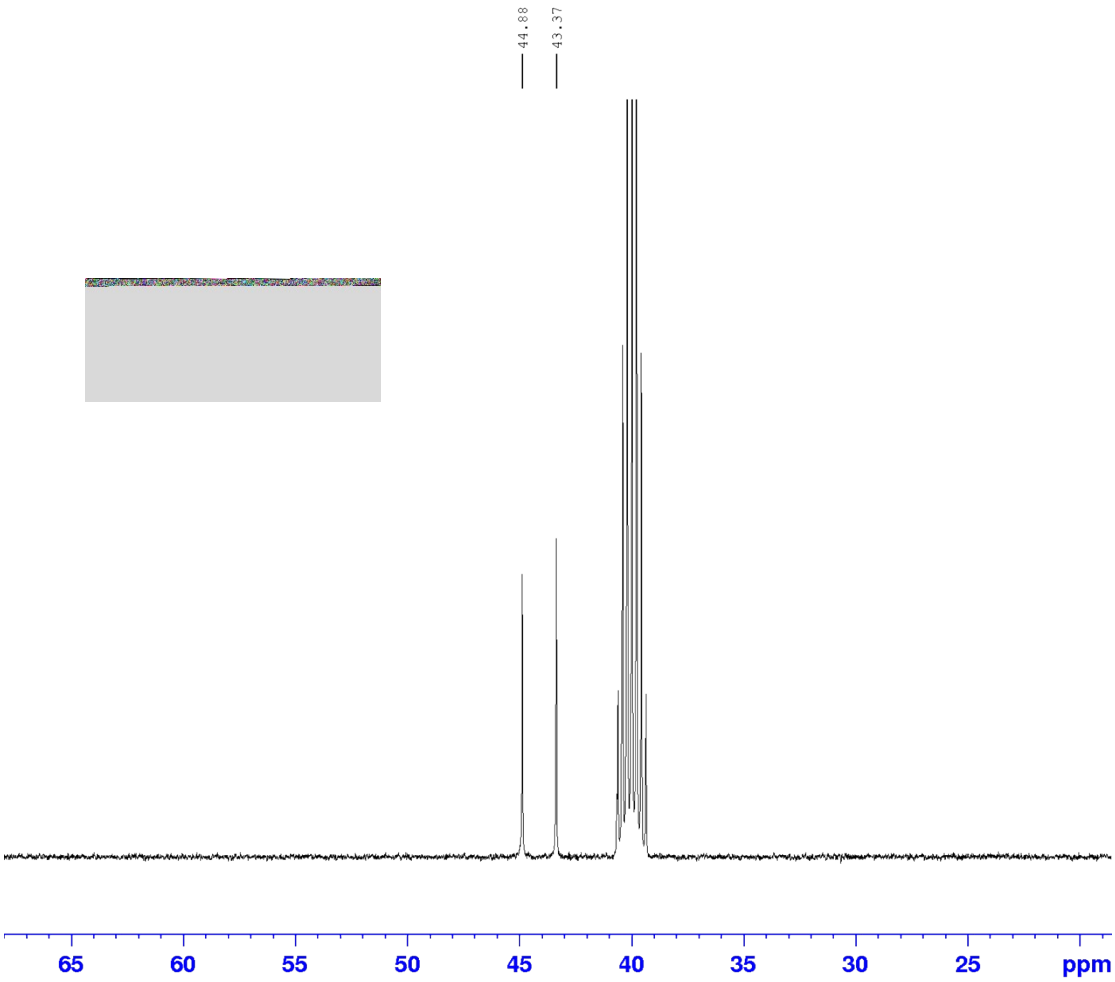
^{1}H -NMR 400 MHz (DMSO) δ 8.51 (s, 1H), 8.13 (dd, 1H, J = 4.8 Hz, J = 1.4 Hz), 7.71 (br s, 1H), 7.56-7.52 (m, 1H), 7.32 (br s, 1H), 6.86 (d, 1H, J = 8.6 Hz), 6.66 (q, 1H, J = 4.0 Hz), 3.87 (t, 4H, J = 5.1 Hz), 3.55 (t, 4H, J = 5.1 Hz).



^{13}C-NMR 100 MHz (DMSO) δ 168.6, 163.7, 161.9, 161.1, 159.3, 148.0, 138.0, 113.6, 107.7, 96.4, 44.9, 43.4.

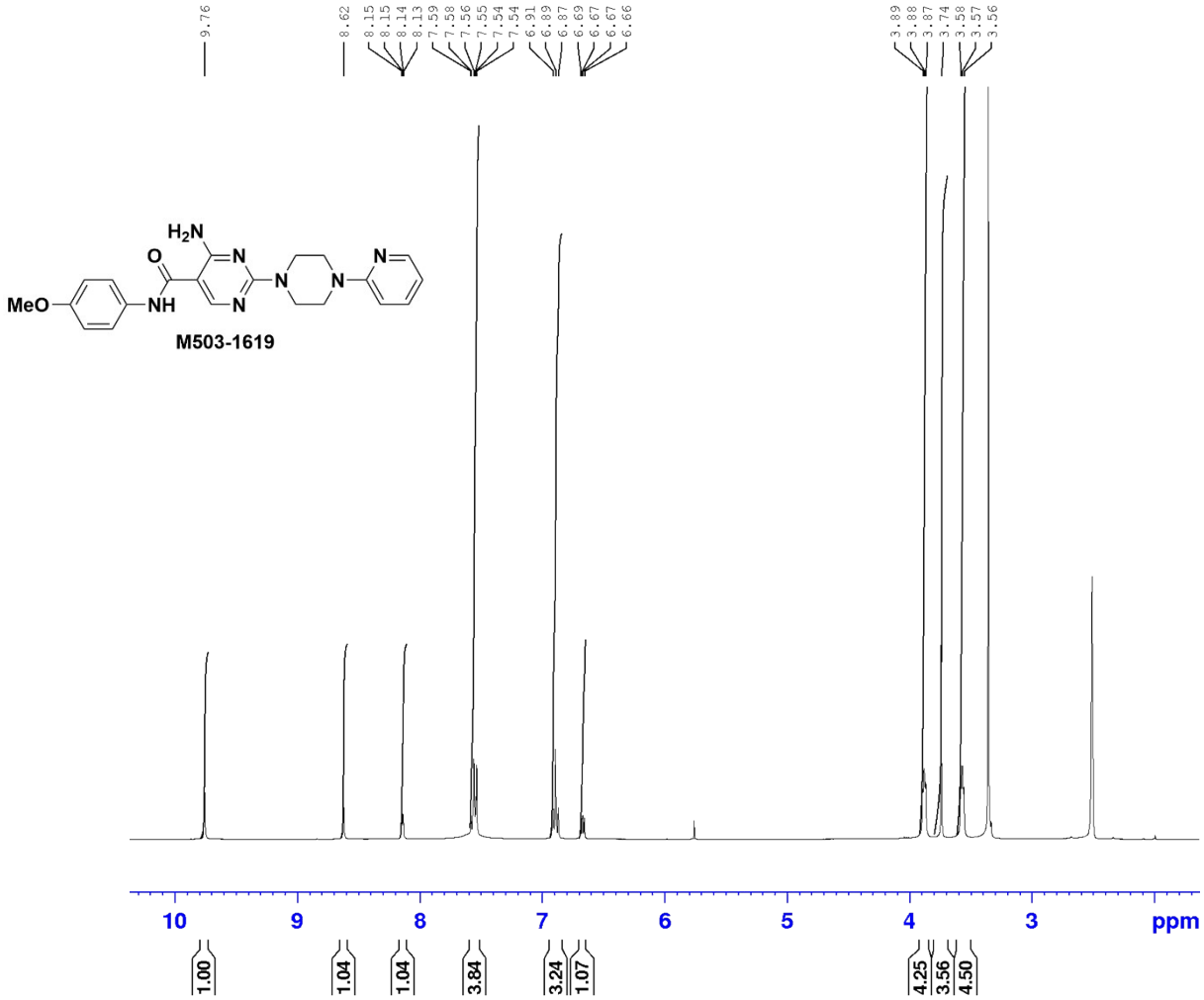


^{13}C -NMR 100 MHz (DMSO) δ 168.6, 163.7, 161.9, 161.1, 159.3, 148.0, 138.0, 113.6, 107.7, 96.4, 44.9, 43.4.

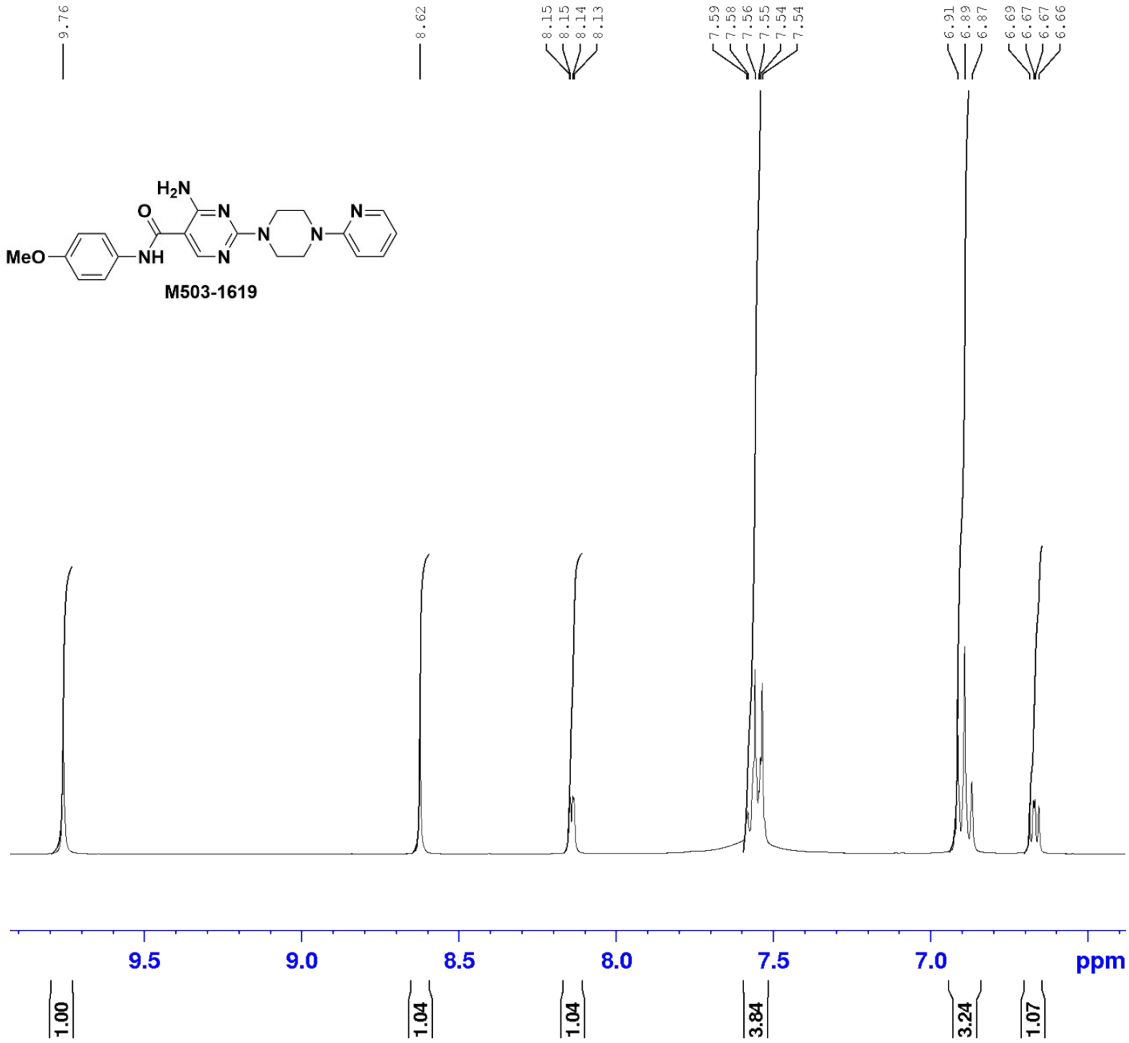


^{13}C-NMR 100 MHz (DMSO) d 168.6, 163.7, 161.9, 161.1, 159.3, 148.0, 138.0, 113.6, 107.7, 96.4, 44.9, 43.4.

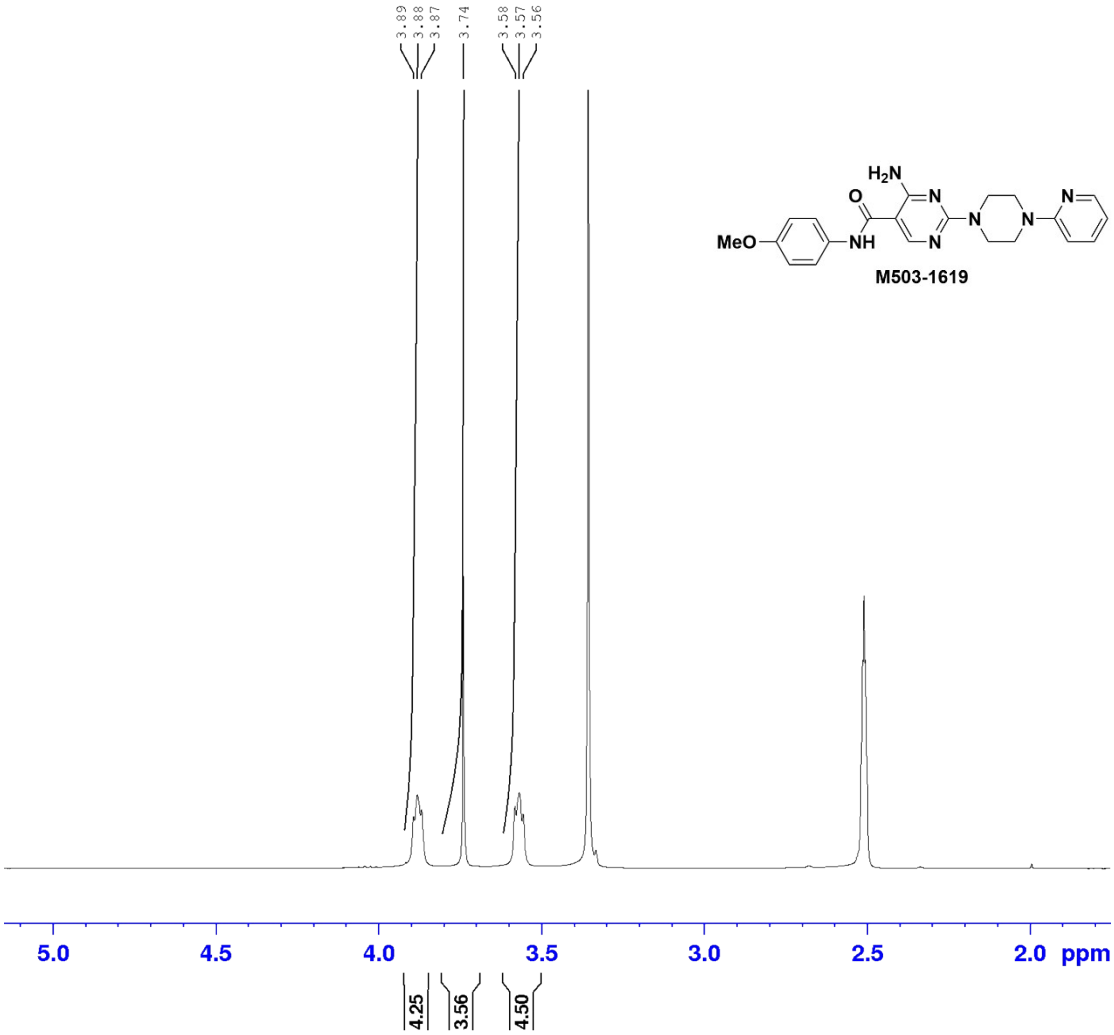
4-amino-*N*-(4-methoxyphenyl)-2-(4-(pyridin-2-yl)piperazin-1-yl)pyrimidine-5-carboxamide (**M5030-1619**)



^{1}H-NMR (DMSO) δ 9.76 (s, 1H), 8.62 (s, 1H), 8.15 – 8.13 (m, 1H), 7.58 – 7.54 (m, 4H), 6.91 – 6.87 (m, 3H), 6.69 – 6.66 (m, 1H), 3.88 (t, 4H, J = 5.0 Hz), 3.74 (m, 3H), 3.57 (t, 4H, J = 5.0 Hz)



^{1}H-NMR (DMSO) δ 9.76 (s, 1H), 8.62 (s, 1H), 8.15 – 8.13 (m, 1H), 7.58 – 7.54 (m, 4H), 6.91 – 6.87 (m, 3H), 6.69 – 6.66 (m, 1H), 3.88 (t, 4H, J = 5.0 Hz), 3.74 (m, 3H), 3.57 (t, 4H, J = 5.0 Hz)



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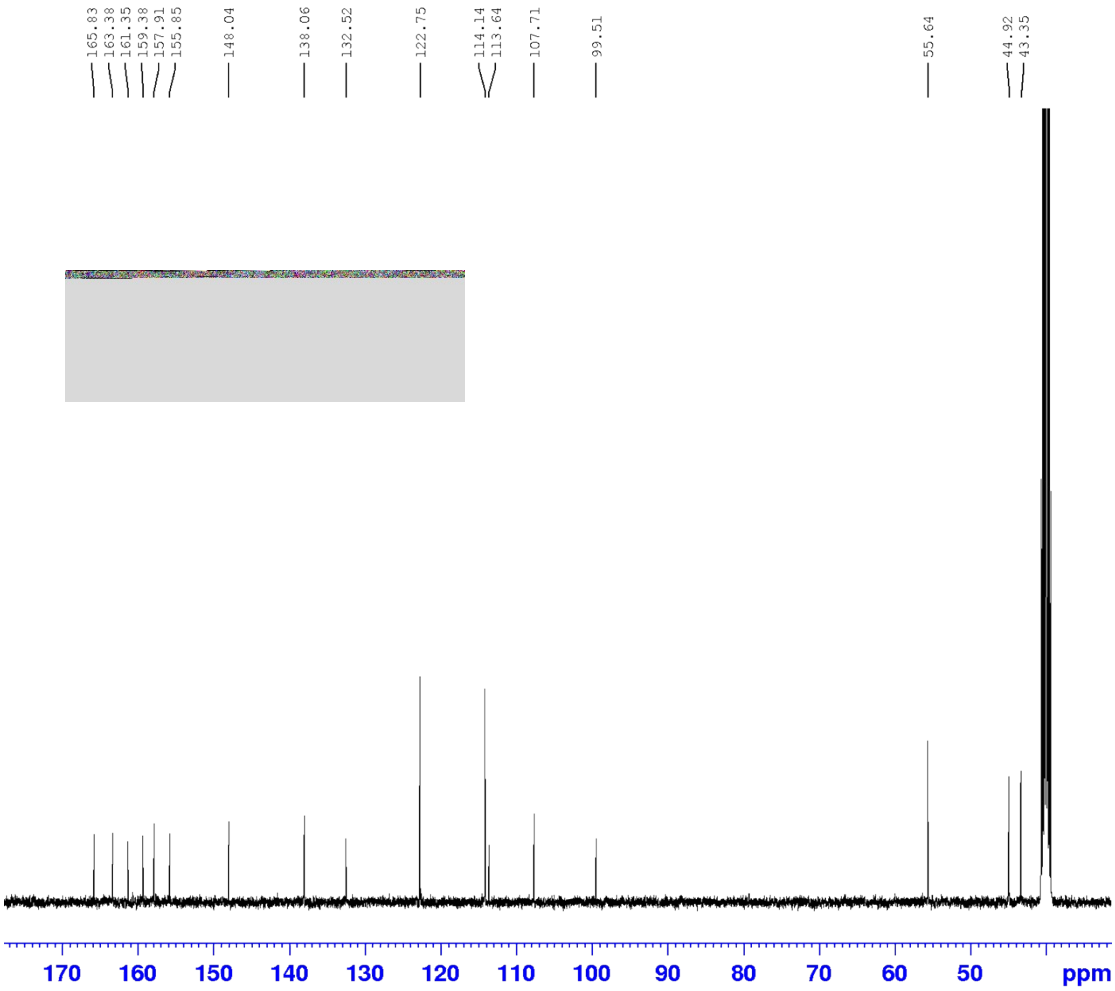
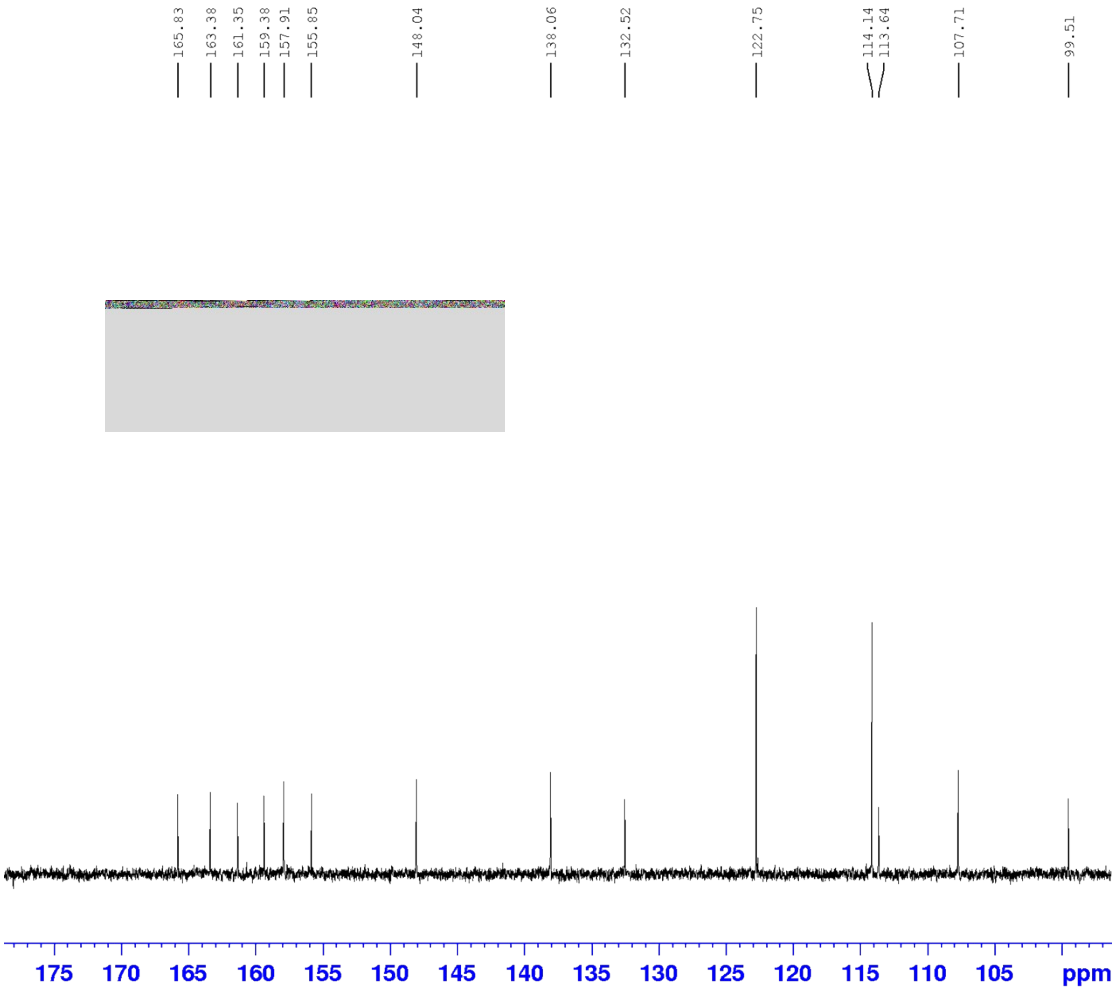
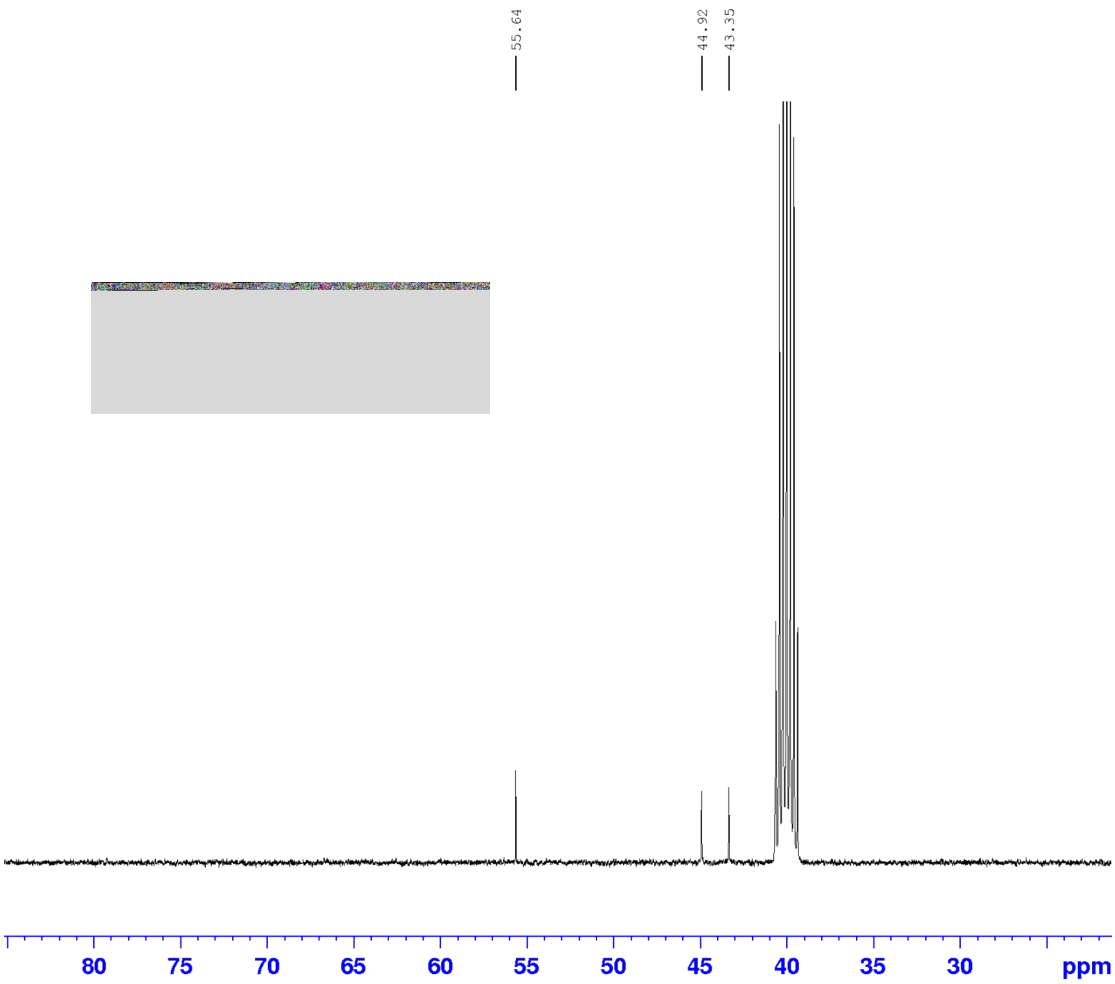


^{13}C-NMR 100 MHz (DMSO) δ 165.8, 163.4, 161.4, 159.4, 157.9, 155.9, 148.0, 138.1,132.5, 122.7, 114.1,113.6, 107.7, 99.5, 55.6, 44.9, 43.4.

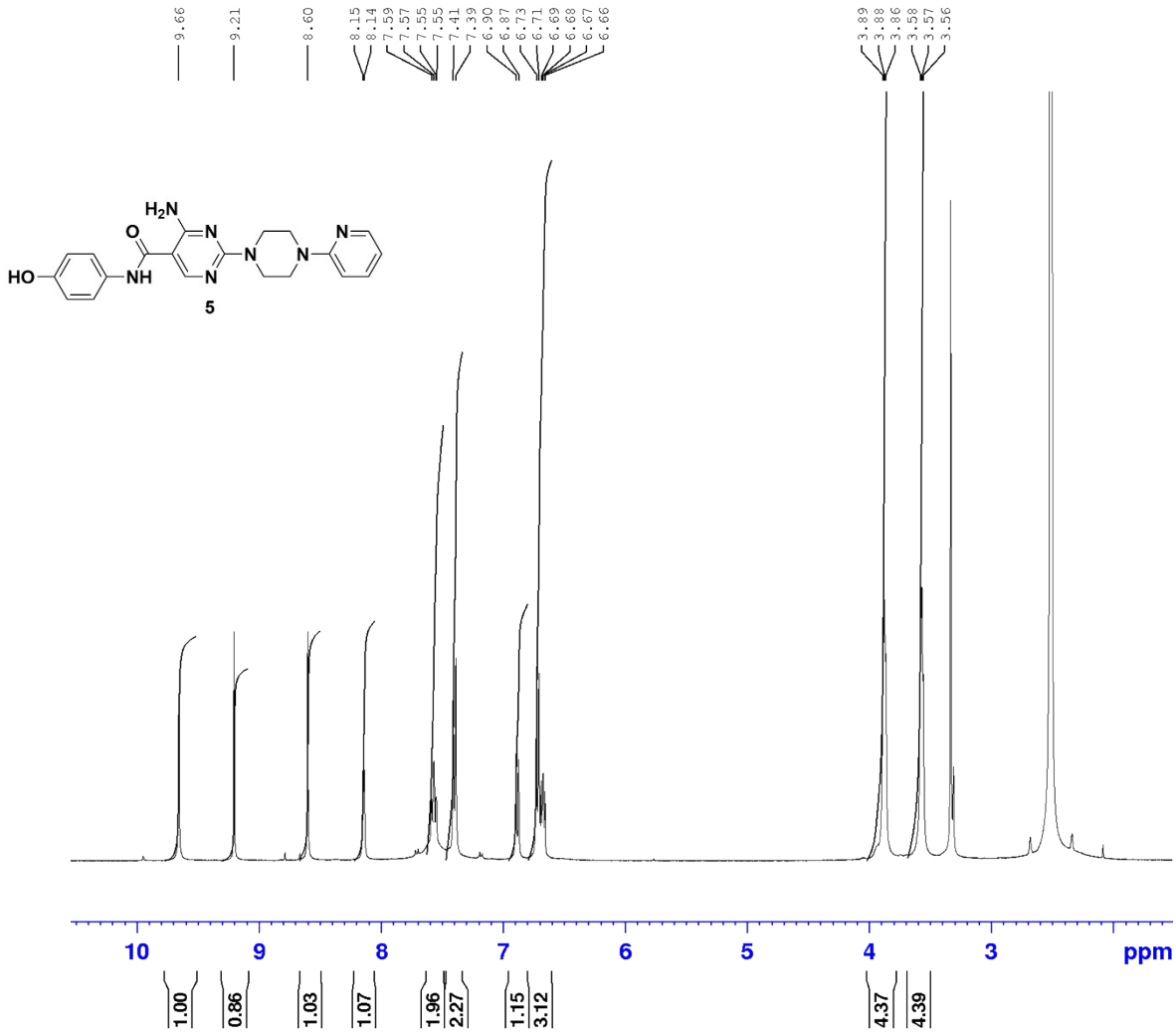


^{13}C -NMR 100 MHz (DMSO) δ 165.8, 163.4, 161.4, 159.4, 157.9, 155.9, 148.0, 138.1,132.5, 122.7, 114.1,113.6, 107.7, 99.5, 55.6, 44.9, 43.4.

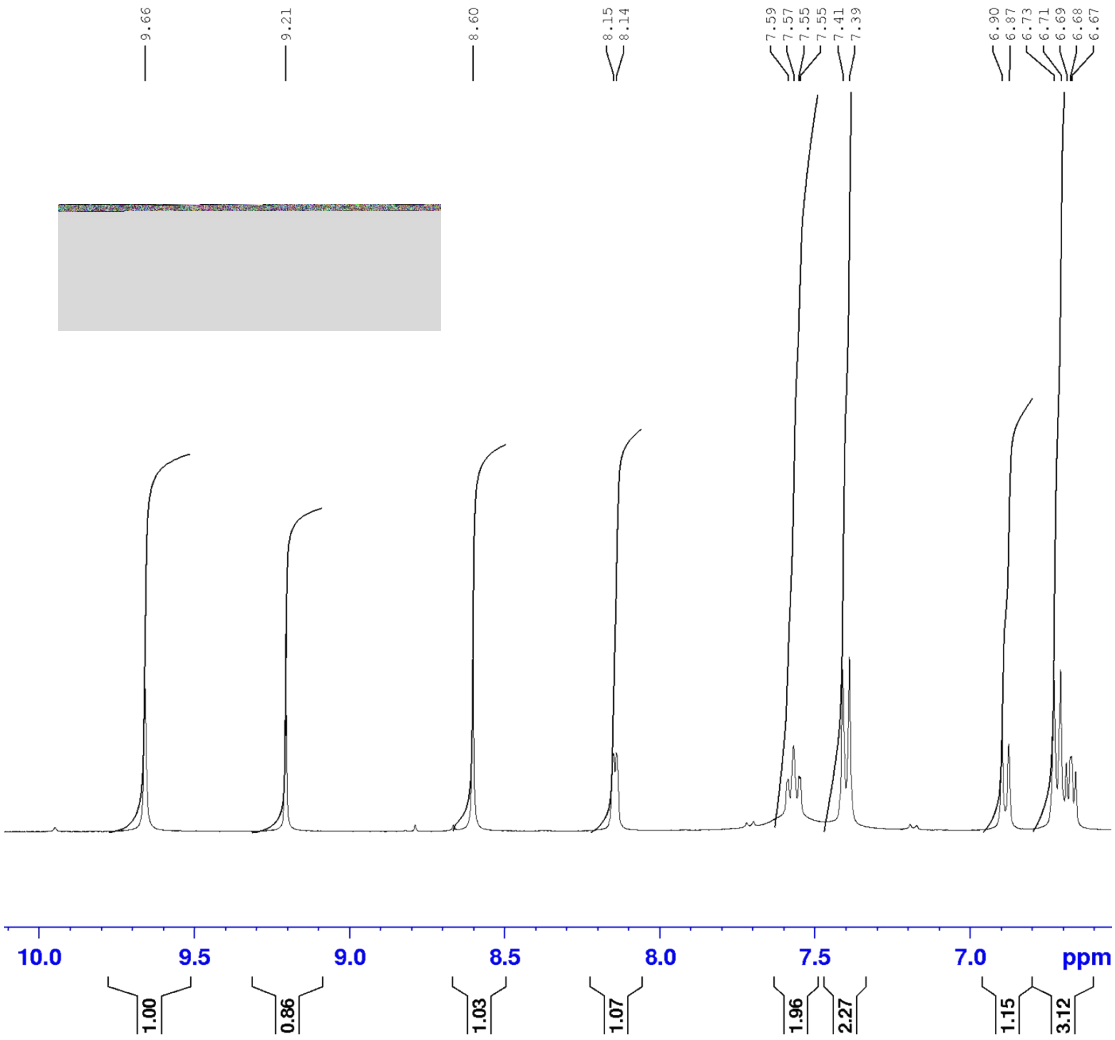


^{13}C -NMR 100 MHz (DMSO) δ 165.8, 163.4, 161.4, 159.4, 157.9, 155.9, 148.0, 138.1,132.5, 122.7, 114.1,113.6, 107.7, 99.5, 55.6, 44.9, 43.4.

4-Amino-*N*-(4-hydroxyphenyl)-2-(4-(pyridin-2-yl)piperazin-1-yl)pyrimidine-5-carboxamide (**5**)



^{1}H-NMR 400 MHz (DMSO) δ 9.66 (s, 1H), 9.21 (s, 1H), 8.61 (s, 1H), 8.14 (d, 1H, J = 3.6 Hz), 7.57 (t, 2H, J = 5.2 Hz), 7.40 (d, 2H, J = 8.7 Hz), 6.89 (d, 1H, J = 8.6 Hz), 6.73-6.66 (m, 3H), 3.88 (t, 4H, J = 4.7 Hz), 3.57 (t, 4H, J = 4.7 Hz).



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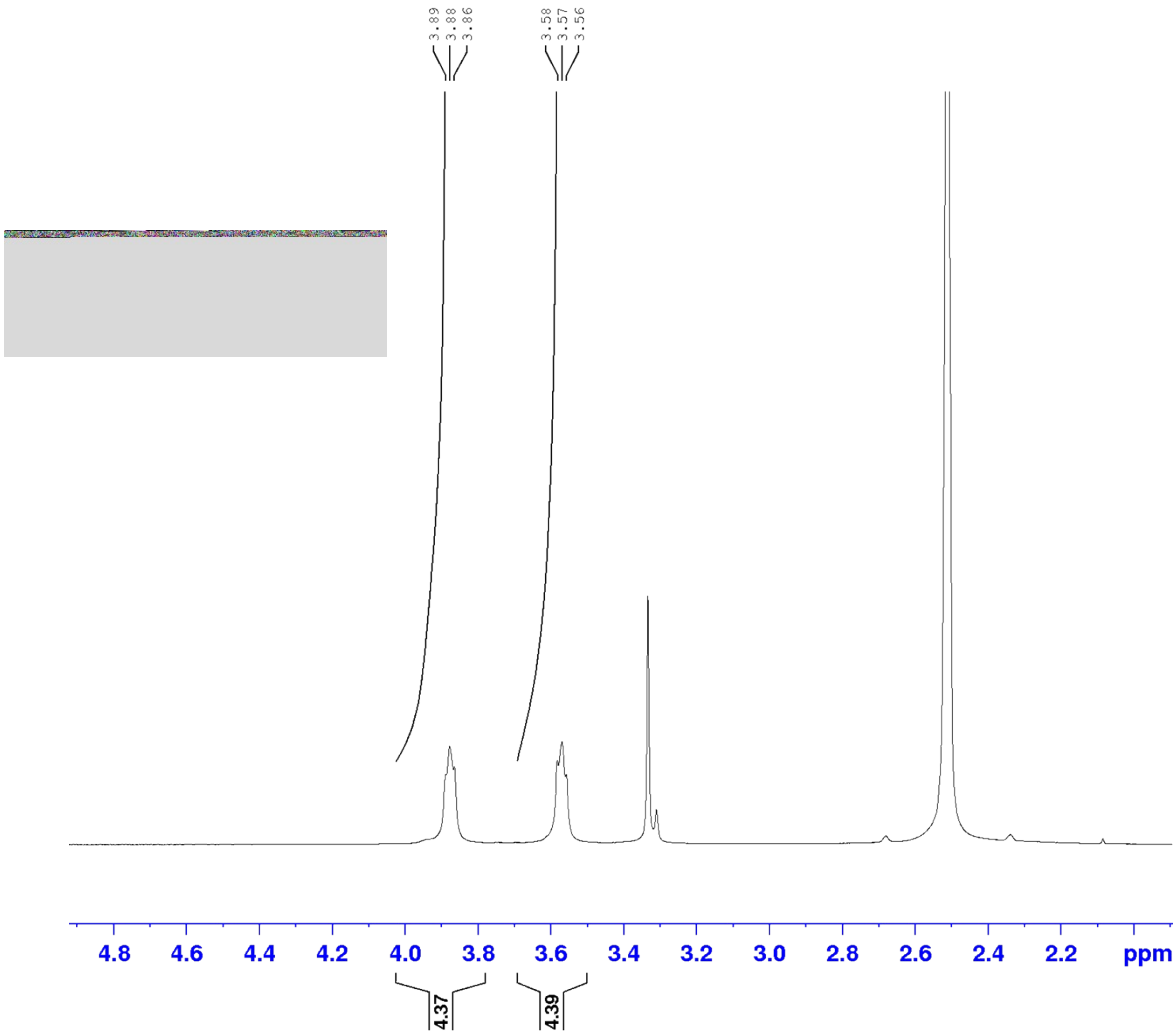

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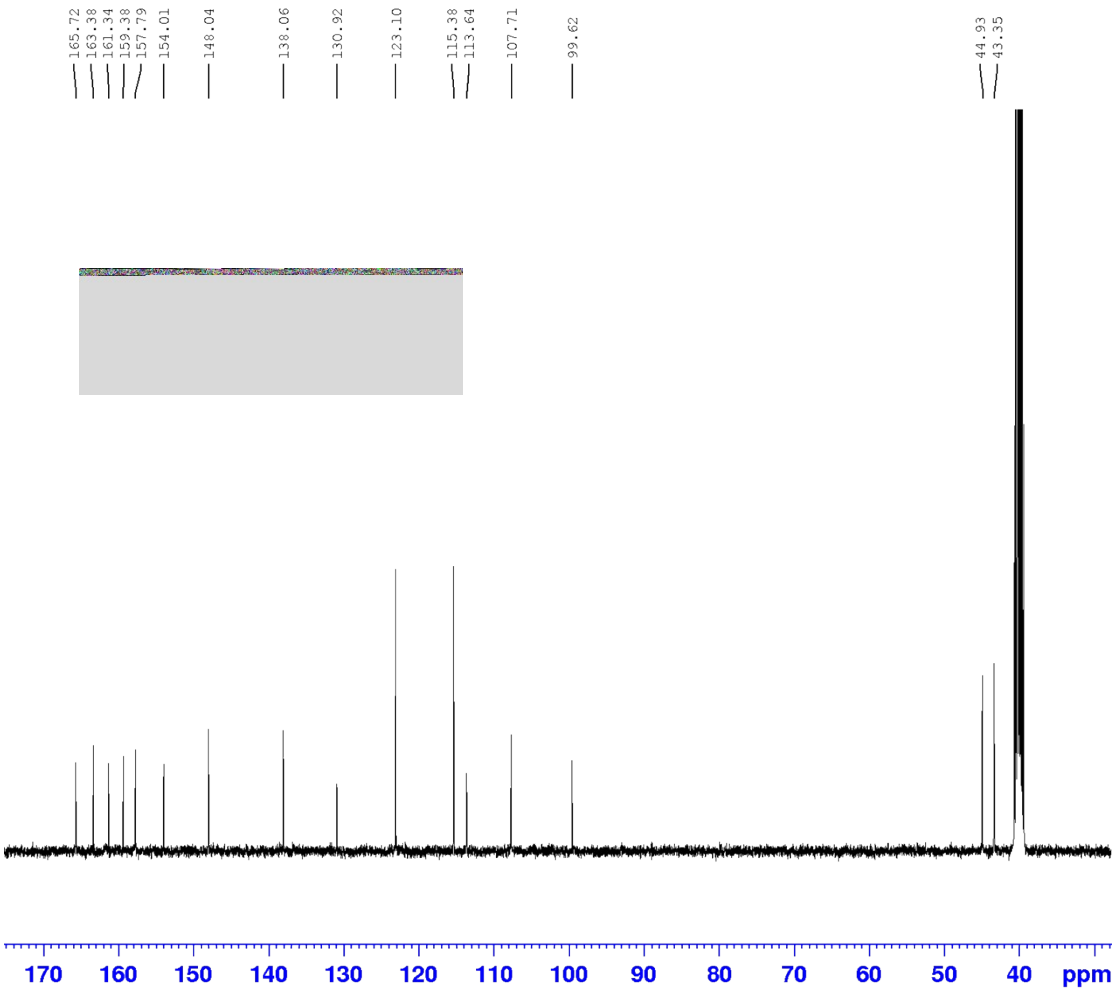
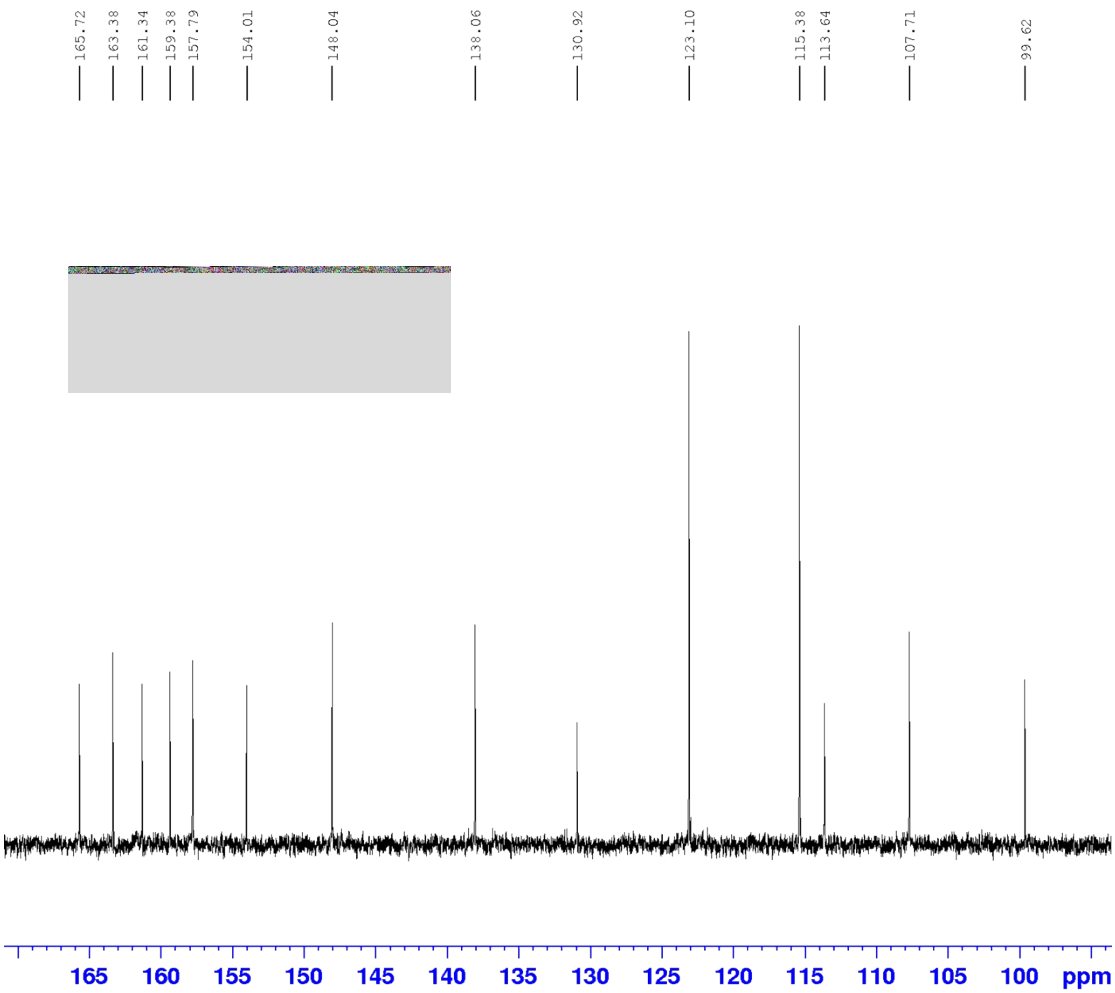
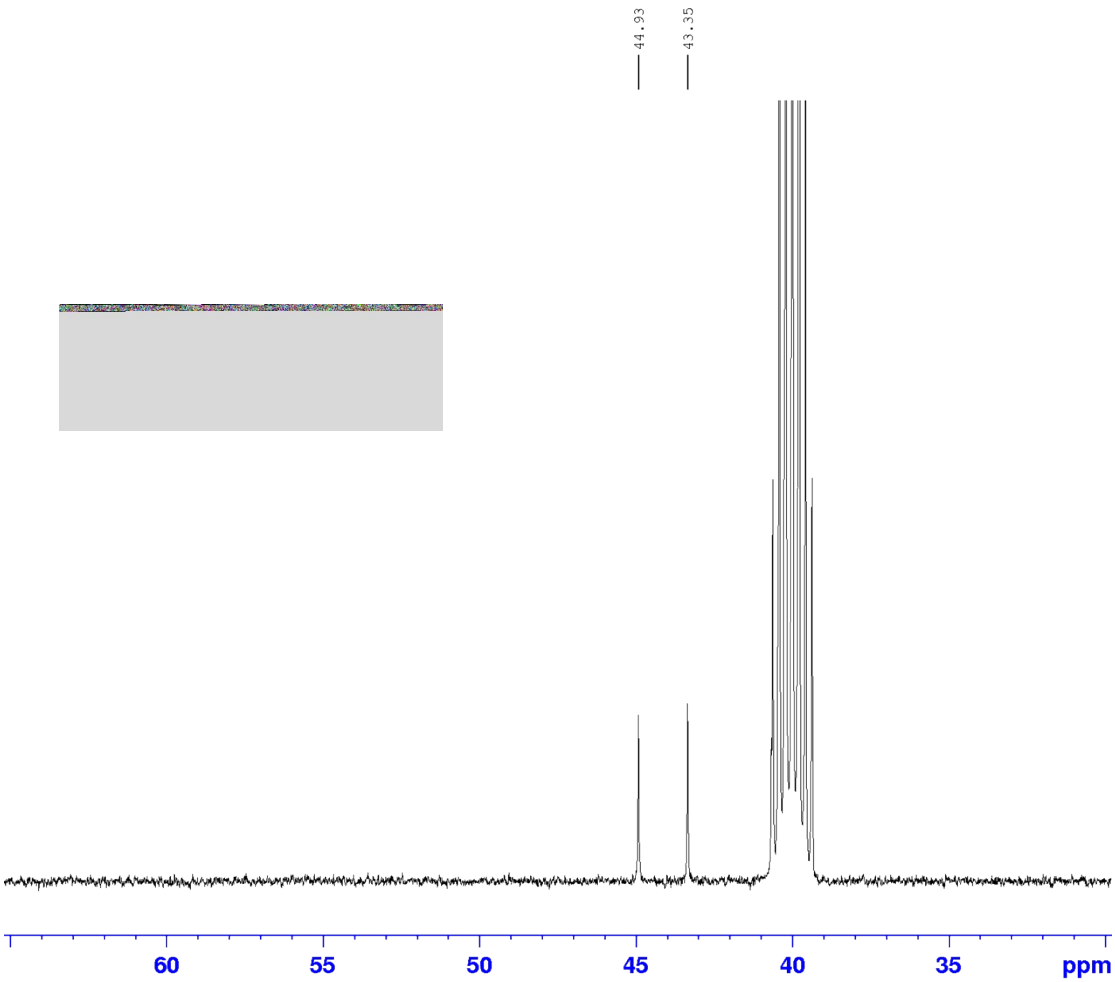


^{13}C-NMR 100 MHz (DMSO) δ 165.7, 163.4, 161.3, 159.4, 157.8, 154.0, 148.0, 138.0, 130.9, 123.1, 115.4, 113.6, 107.7, 99.6, 44.9, 43.4.



^{13}C-NMR 100 MHz (DMSO) δ 165.7, 163.4, 161.3, 159.4, 157.8, 154.0, 148.0, 138.0, 130.9, 123.1, 115.4, 113.6, 107.7, 99.6, 44.9, 43.4.



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