SUPPORTING INFORMATION

Discovery of Potent, Reversible and Competitive Cruzain Inhibitors with Trypanocidal Activity: A Structure-Based Drug Design Approach

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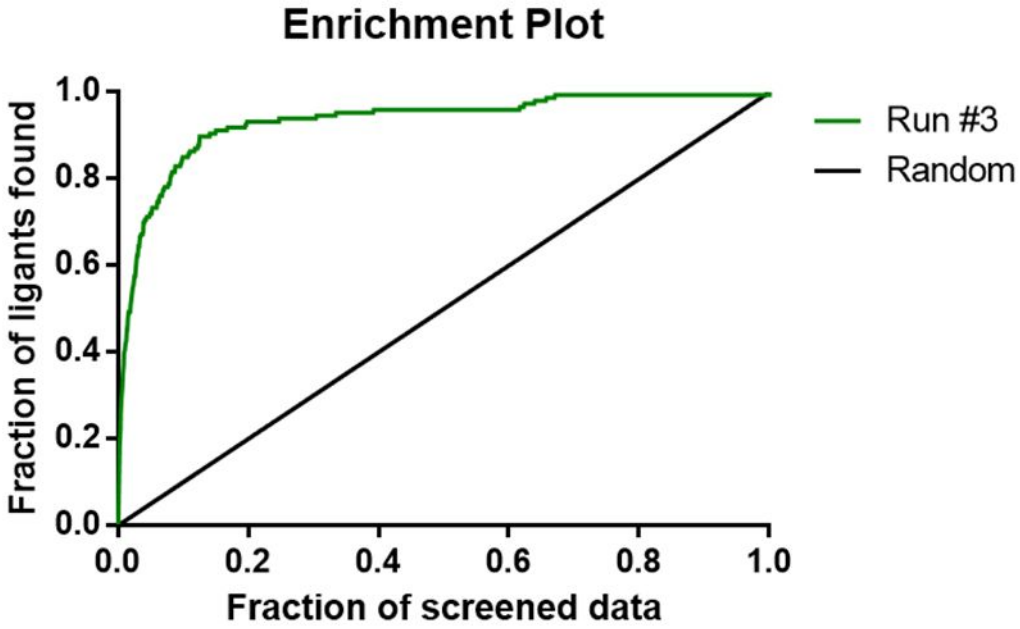
Supporting Figures



	Run #3	Random
Baseline	0.0	0.0
Total Area	0.9313	0.4990
Total Peak Area	0.9313	0.4990
Number of Peaks	1.000	1.000
Peak 1		
First X=	0.000997	0.000997
Last X=	1.000	1.000
Peak X=	1.000	1.000
Peak Y=	0.9966	0.9985
Area=	0.9313	0.4990
%Area=	100.0	100.0

Figure S1. Enrichment plot with an AUC of 0.9313 obtained using DOCK 3.5.54.

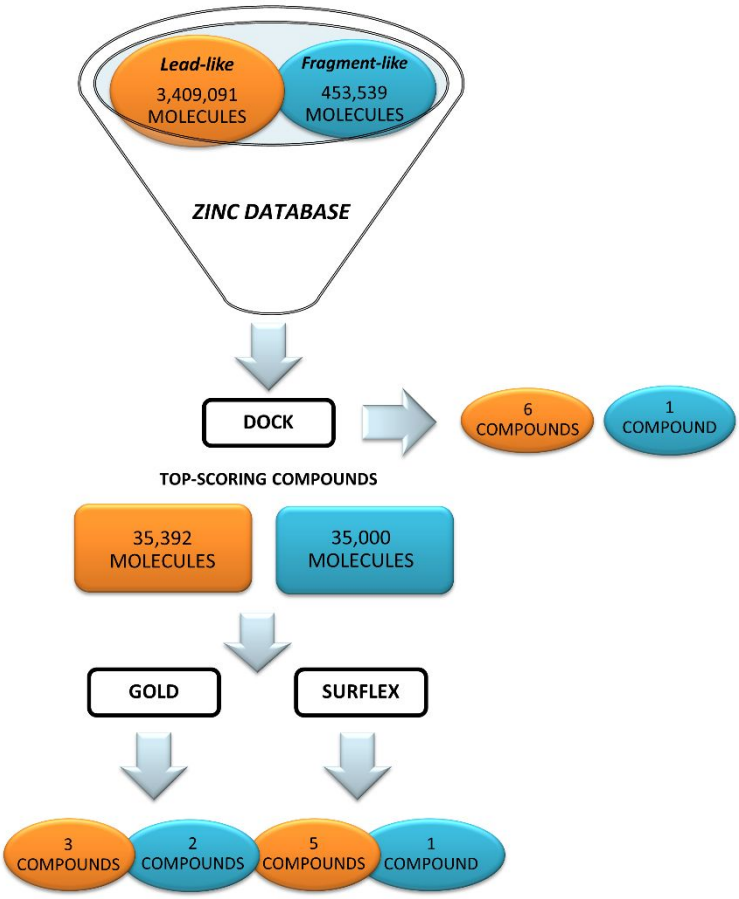


Figure S2. Virtual screening strategy used in the selection of compounds for experimental profiling toward cruzain.

Supporting Table

Table S1. *In vivo* acute toxicity (maximum tolerated dose - MTD)[a]

Dose (mg/kg)	Compound	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
300	1	P	P	P	P	P	-	-
	45	P	P	P	P	P	-	-
150	1	P	P	-	-	-	-	-
	45	P	P	P	-	-	-	-
100	1	-	-	-	-	-	-	-
	45	-	-	-	-	-	-	-
75	1	-	-	-	-	-	-	-
	45	-	-	-	-	-	-	-
Vehicle		-	-	-	-	-	-	-

[a](P) piloerection. Non-infected Swiss female mice (2 animals per group) treated i.p. with four different doses of each compound (75, 100, 150, and 300 mg/Kg). Each mouse received a single dose of the testing compounds. Vehicle solution: 0.9% NaCl + 10% DMSO.

Supporting Experimental Procedures

Synthesis

Unless noted, all reactions were performed under an atmosphere of argon with dry solvents and magnetic stirring. Dichloromethane (DCM) and triethylamine (Et_3N) were distilled from CaH_2. Tetrahydrofuran (THF) was distilled from sodium/benzophenone. Dimethyl formamide (DMF) was purchased from Aldrich (anhydrous) and used without further purification. Yields refer to homogeneous materials obtained after purification of reaction products by flash column chromatography using silica gel (200-400 mesh) or recrystallization. Analytical thin-layer chromatography was performed on silica-gel 60 and GF (5-40 µm thickness) plates, and visualization was accomplished using UV light, basic potassium permanganate staining or ninhydride solution followed by heating. Melting points were measured with a Buchi M-565 equipament and are uncorrected. ^1H and proton-decoupled ^{13}C NMR spectra were acquired in $CDCl_3$, CD_3OD or d_6-DMSO at 250 MHz (^1H) and 62.5 MHz (^{13}C) (Bruker DPX250), at 400 MHz (^1H) and 100 MHz (^{13}C) (Bruker Avance 400), at 500 MHz (^1H) and 125 MHz (^{13}C) (Varian Inova 500), or at 600 MHz (^1H) and 150 MHz (^{13}C) (Bruker Avance 600). Chemical shifts (δ) are reported in ppm using residual undeuterated solvent as an internal standard ($CDCl_3$ at 7.26 ppm, CD_3OD at 3.31 ppm, d_6-DMSO at 2.50 ppm, and TMS at 0.00 ppm for ^1H NMR spectra and $CDCl_3$ at 77.16 ppm, CD_3OD at 49.0 ppm, d_6-DMSO at 39.52 ppm for ^{13}C NMR spectra). Multiplicity data are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, br s = broad singlet, dd = doublet of doublets, dt = doublet of triplets, ddd = doublet of doublet of doublets, tt = triplet of triplets, app d = apparent doublet, app t = apparent triplet, m = multiplet, and br m = broad multiplet. The multiplicity is followed by the coupling constant(s) in Hz and integration. High resolution mass spectrometry (HRMS) were measured using electrospray ionization (ESI) (waters xevo Q-tof, thermo LTQ-FT ultra, or thermos Q exactive) or using electron ionization (EI) (GCT premier waters).

Method A: Etherification reaction

To a solution of the corresponding phenol (1.5 equiv.) in DMF (in a minimum concentration of 0.1 $mol.L^{-1}$) was added K_2CO_3 (2 equiv.) and the alkyl-tosylate **42** (1 equiv.). The reaction was stirred at 60° C and monitored by TLC. After completion, a NaOH solution (1 $mol.L^{-1}$) was added at room temperature and the resulting mixture was extracted with Et_2O (3 times). The organic layer was washed with brine, dried over $MgSO_4$ and concentrated to give the desired ether product.

Method B: Boc deprotection reaction

To a solution of the corresponding carbamate (1 equiv.) in DCM (in a minimum concentration of 0.1 $mol.L^{-1}$) was added HCl (4 $mol.L^{-1}$ in dioxane, 3 equiv.) at room temperature. The reaction was monitored by TLC. After completion, the solvent was removed under reduced pressure generating the desired ammonium hydrochloride.

Method C: Acylation reaction with CDI

To a solution of the corresponding ammonium hydrochloride (1 equiv.) in DMF (in a concentration of 0.5-1 $mol.L^{-1}$) was added CDI (1.5 equiv.) at room temperature. The reaction was monitored by TLC. After completion, distilled water was added (10 times the volume of DMF used) at 0°C. When precipitation occurred (compounds **1**, **28**, **29**, **30**, **31**, **33**, **34**, **36**, **37**, **38**, **39**, **45**, **46** and **50**), the solid was filtered and washed with distilled water, generating the desired acyl-imidazole. When the product did not precipitate, an extraction was performed with Et_2O (3 times). The organic layer was washed with brine, dried over $MgSO_4$, and concentrated, generating the desired imidazole derivatives (compounds **56**, **61** and **60**). After extraction, the compounds **32**, **35**, **53**, **60**, and **68-71** were purified by flash column chromatography (hexane / EtOAc gradient of polarity) generating the desired acyl-imidazole.

Method D: Esterification reaction

To a solution of the corresponding carboxylic acid (1.1 equiv.) and alcohol (1 equiv.) in DCM (in a minimum concentration of 0.1 $mol.L^{-1}$) were added EDC (1.2 equiv.) and DMAP (10 mol%) at 0°C. The reaction was stirred at room temperature and monitored by TLC. After completion, the solution was washed with distilled water and brine. The organic layer was dried over $MgSO_4$ and

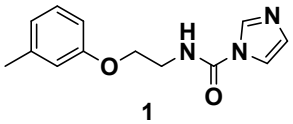
concentrated. The crude product was purified by flash column chromatography (hexane / EtOAc gradient of polarity) generating the desired ester.

Method E: Amidation reaction

To a solution of the corresponding carboxylic acid (1.1 equiv.) and amine (1.0 equiv.) in DMF or DCM (in a minimum concentration of 0.1 mol.L^{-1}) were added EDC (1.2 equiv.) and HOBt (1.2 equiv.) at 0°C. For examples in which the ammonium hydrochloride salt was used, 2 equiv. of Et_3N was added. The reaction was stirred at room temperature and monitored by TLC. After completion, an extraction was performed in DCM or Et_2O and distilled water, and the organic layer was washed with brine. The organic layer was dried over $MgSO_4$ and concentrated. The crude product was purified by flash column chromatography (hexane / EtOAc gradient of polarity) generating the desired amide.

Method F: Carbonyl substitution reaction.

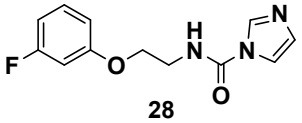
To a solution of amine (1.5 equiv.) in DMF (0.1 mol.L^{-1}) was added NaH (60% in mineral oil; for the compounds **85** and **86**) followed by addition of **1** (1 equiv.) and the mixture was stirred for 12 - 14 h at room temperature (compounds **85 – 88 and 92**) or 100°C (compounds **89 – 91**). The reaction mixture was poured on water (5 mL) and extracted with Et_2O (2 × 15 mL). The combined organic layer was dried over $MgSO_4$, and concentrated. Purification by flash column chromatography (hexane / EtOAc gradient of polarity) gave the title compound.



1

Method A, followed by B and C.

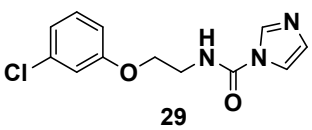
White solid; M.P.: 89-91°C; **^{1}H NMR (600 MHz, $CDCl_3$)** δ 8.19 (s, 1H), 7.42 (t, *J* = 1.3 Hz, 1H), 7.19 (t, *J* = 7.8 Hz, 1H), 7.11 (s, 1H), 6.96 (s, 1H), 6.82 (d, *J* = 7.5 Hz, 1H), 6.75 – 6.69 (m, 2H), 4.19 – 4.15 (m, 2H), 3.84 (dd, *J* = 10.4, 5.4 Hz, 2H), 2.34 (s, 3H). **^{13}C NMR (151 MHz, $CDCl_3$)** δ 158.34, 149.24, 139.94, 136.08, 130.54, 129.54, 122.46, 116.19, 115.41, 111.44, 66.18, 40.77, 21.63. HRMS calcd. for $[M+H]^+$: 246.12370; observed: 246.12292.



28

Method A, followed by B and C.

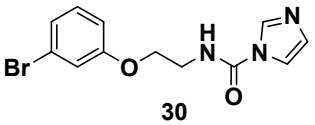
White solid; M.P.: 124-127°C; **^{1}H NMR (500 MHz, $CDCl_3$)** δ 8.22 (s, 1H), 7.46 (s, 1H), 7.28 – 7.18 (m, 2H), 7.11 (s, 1H), 6.74 – 6.67 (m, 2H), 6.62 (dt, *J* = 10.7, 2.3 Hz, 1H), 4.18 (t, *J* = 5.0 Hz, 2H), 3.86 (dd, *J* = 10.5, 5.3 Hz, 2H). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 163.70 (d, *J* = 245.6 Hz), 159.70 (d, *J* = 10.9 Hz), 149.28, 136.08, 130.65 (d, *J* = 10.0 Hz), 130.43, 116.33, 110.17 (d, *J* = 2.7 Hz), 108.50 (d, *J* = 21.4 Hz), 102.50 (d, *J* = 25.0 Hz), 66.59, 40.59. HRMS calcd. for $[M+H]^+$: 250.09918; observed: 250.09793.



29

Method A, followed by B and C.

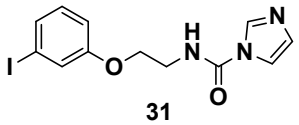
White solid; M.P.: 106-109°C; **^{1}H NMR (500 MHz, $CDCl_3$)** δ 8.25 (s, 1H), 7.84 (t, *J* = 5.3 Hz, 1H), 7.51 (s, 1H), 7.20 (t, *J* = 8.2 Hz, 1H), 7.08 (s, 1H), 6.96 (dd, *J* = 8.0, 1.0 Hz, 1H), 6.87 (t, *J* = 2.1 Hz, 1H), 6.77 (dd, *J* = 8.2, 2.1 Hz, 1H), 4.16 (t, *J* = 5.1 Hz, 2H), 3.83 (dd, *J* = 10.4, 5.3 Hz, 2H). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 158.98, 149.26, 135.99, 135.03, 130.43, 129.96, 121.58, 116.51, 114.99, 112.75, 77.33, 77.07, 76.82, 66.41, 40.50. HRMS calcd. for $[M+H]^+$: 266.06963; observed: 266.06849.



30

Method A, followed by B and C.

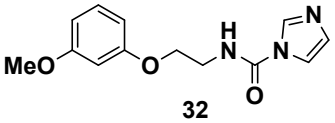
White solid; M.P.: 127-131°C; **^{1}H NMR (500 MHz, d_6-DMSO)** δ 8.77 (t, *J* = 4.7 Hz, 1H), 8.27 (s, 1H), 7.71 (s, 1H), 7.26 (t, *J* = 8.1 Hz, 1H), 7.20 (s, 1H), 7.15 (d, *J* = 7.9 Hz, 1H), 7.05 (s, 1H), 7.00 (dd, *J* = 8.3, 1.8 Hz, 1H), 4.18 (t, *J* = 5.5 Hz, 2H), 3.64 (dd, *J* = 5.4, 10.4 Hz, 2H). **^{13}C NMR (151 MHz, d_6-DMSO)** δ 159.79, 149.46, 136.46, 131.73, 130.08, 124.18, 122.51, 117.89, 117.07, 114.57, 66.67, 40.12. HRMS calcd. for $[M+H]^+$: 310.01911; observed: 310.01769.



31

Method A, followed by B and C.

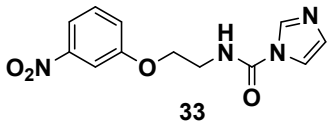
White solid; M.P.: 126-130°C; **^{1}H NMR (600 MHz, d_6-DMSO)** δ 8.73 (s, 1H), 8.25 (s, 1H), 7.68 (s, 1H), 7.38 – 7.26 (m, 2H), 7.12 – 6.94 (m, 3H), 4.14 (t, *J* = 5.4 Hz, 2H), 3.61 (dd, *J* = 9.9, 4.7 Hz, 2H). **^{13}C NMR (126 MHz, d_6-DMSO)** δ 159.02, 149.00, 135.96, 131.36, 129.65, 129.60, 123.10, 116.57, 114.46, 95.04, 66.04. HRMS calcd. for $[M+H]^+$: 358.00524; observed: 358.00371.



32

Method A, followed by B and C.

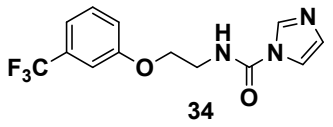
White solid; M.P.: 112-117°C; **^{1}H NMR (600 MHz, $CDCl_3$)** δ 8.22 (s, 1H), 7.52 (t, *J* = 5.2 Hz, 1H), 7.48 (s, 1H), 7.19 (t, *J* = 8.2 Hz, 1H), 7.08 (s, 1H), 6.55 (dd, *J* = 8.2, 2.4 Hz, 1H), 6.49 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.45 (t, *J* = 2.3 Hz, 1H), 4.15 (t, *J* = 5.1 Hz, 2H), 3.82 (dd, *J* = 10.4, 5.4 Hz, 2H), 3.79 (s, 3H). **^{13}C NMR (151 MHz, $CDCl_3$)** δ 160.93, 159.49, 149.23, 136.02, 130.12, 130.10, 116.38, 106.84, 106.58, 101.10, 66.17, 55.32, 40.60. HRMS calcd. for $[M+H]^+$: 262.11862; observed: 262.11798.



33

Method A, followed by B and C.

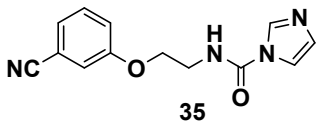
White solid; M.P.:133-136°C; **^{1}H NMR (600 MHz, d_6-DMSO)** δ 8.77 (s, 1H), 8.25 (s, 1H), 7.82 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.74 (t, *J* = 2.3 Hz, 1H), 7.68 (t, *J* = 1.2 Hz, 1H), 7.58 (t, *J* = 8.2 Hz, 1H), 7.45 (dd, *J* = 8.2, 2.3 Hz, 1H), 7.02 (s, 1H), 4.28 (t, *J* = 5.5 Hz, 2H), 3.67 (q, *J* = 5.3 Hz, 2H).**^{13}C NMR (151 MHz, d_6-DMSO)** δ 158.84, 149.02, 148.74, 135.96, 130.75, 129.60, 121.97, 116.56, 115.72, 108.84, 66.69, 39.57. HRMS calcd. for $[M+H]^+$: 277.09368; observed: 277.09266.



34

Method A, followed by B and C.

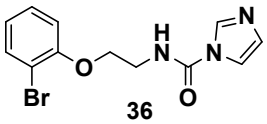
White solid; M.P.: 104-105°C; **^{1}H NMR (500 MHz, $CDCl_3$)** δ 8.17 (s, 1H), 7.43 – 7.37 (m, 2H), 7.24 (d, *J* = 7.7 Hz, 1H), 7.12 (s, 1H), 7.09 (s, 1H), 7.06 (d, *J* = 8.3 Hz, 1H), 6.79 (s, 1H), 4.21 (t, *J* = 5.0 Hz, 2H), 3.86 (dd, *J* = 10.5, 5.3 Hz, 2H). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 158.46, 149.25, 136.09, 132.24 (q, *J* = 32.4 Hz), 130.72, 130.38, 125.02, 122.85, 118.34 (q, *J* = 3.6 Hz), 117.01 (q, *J* = 222.7 Hz), 111.54 (q, *J* = 3.9 Hz), 66.65, 40.61. HRMS calcd. for $[M+H]^+$: 300.09544; observed: 300.10468.



35

Method A, followed by B and C.

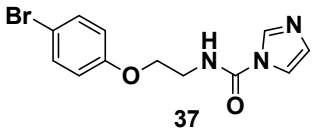
White solid; M.P.: 135-137°C; **^{1}H NMR (500 MHz, d_6-DMSO)** δ 8.76 (t, *J* = 5.1 Hz, 1H), 8.26 (s, 1H), 7.70 (s, 1H), 7.54 – 7.46 (m, 2H), 7.43 (d, *J* = 7.5 Hz, 1H), 7.34 (dd, *J* = 8.4, 2.4 Hz, 1H), 7.05 (s, 1H), 4.23 (t, *J* = 5.5 Hz, 2H), 3.66 (q, *J* = 5.4 Hz, 2H**); ^{13}C NMR (126 MHz, d_6-DMSO)** δ 158.93, 149.51, 136.45, 131.39, 130.11, 125.26, 120.95, 119.08, 117.91, 117.06, 112.77, 66.84.



36

Method A, followed by B and C.

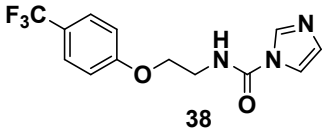
White solid; M.P.: 132-135°C; **^{1}H NMR (250 MHz, $CDCl_3$)** δ 8.16 (s, 1H), 7.56 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.37 (s, 1H), 7.34 – 7.25 (m, 2H), 7.12 (s, 1H), 6.99 – 6.83 (m, 2H), 6.40 (s, 1H), 4.24 (t, *J* = 5.0 Hz, 2H), 3.90 (dd, *J* = 10.3, 5.3 Hz, 2H). **^{13}C NMR (63 MHz, $CDCl_3$)** δ 154.49, 149.13, 136.08, 133.39, 130.26, 128.77, 122.91, 116.12, 114.04, 112.39, 67.61, 40.25. HRMS calcd. for $[M+H]^+$: 310.01857; observed: 310.01815.



37

Method A, followed by B and C.

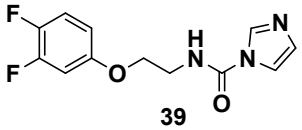
White solid; M.P.: 110-113°C; **^{1}H NMR (500 MHz, $CDCl_3$)** δ 8.27 (s, 1H), 8.16 (t, *J* = 5.5 Hz, 1H), 7.53 (t, *J* = 1.2 Hz, 1H), 7.36 (d, *J* = 9.0 Hz, 2H), 7.06 (s, 1H), 6.74 (d, *J* = 9.0 Hz, 2H), 4.13 (t, *J* = 5.1 Hz, 2H), 3.81 (dd, *J* = 10.5, 5.3 Hz, 2H). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 157.41, 149.30, 136.04, 132.43, 129.78, 116.65, 116.19, 113.54, 66.40, 40.55. HRMS calcd. for $[M+H]^+$: 310.01857; observed: 310.01816.



38

Method A, followed by B and C.

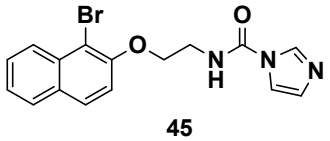
White solid; M.P.: 94-95°C; **^{1}H NMR (500 MHz, $CDCl_3$)** δ 8.18 (s, 1H), 7.55 (d, *J* = 8.6 Hz, 2H), 7.40 (s, 1H), 7.09 (s, 1H), 6.95 (d, *J* = 8.6 Hz, 2H), 6.87 (s, 1H), 4.21 (t, *J* = 5.0 Hz, 2H), 3.86 (dd, *J* = 10.5, 5.3 Hz, 2H). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 160.74, 149.25, 136.08, 130.67, 127.25 (q, *J* = 3.7Hz), 125.46 – 123.30 (m), 116.18, 114.55, 66.56, 40.56. HRMS calcd. for $[M+H]^+$: 300.09544; observed: 300.09969.



39

Method A, followed by B and C.

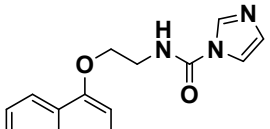
White solid; M.P.:141-142°C; **^{1}H NMR (400 MHz, d_6-DMSO)** δ 8.76 (t, *J* = 5.3 Hz, 1H), 8.26 (s, 1H), 7.69 (t, *J* = 1.3 Hz, 1H), 7.04 (s, 1H), 6.83 – 6.71 (m, 3H), 4.18 (t, *J* = 5.5 Hz, 2H), 3.63 (dd, *J* = 5.5, 10.4 Hz, 2H). **^{13}C NMR (101 MHz, d_6-DMSO)** δ 163.07 (dd, *J* = 243.9 and 16.4 Hz), 160.45 (t, *J* = 14.2 Hz), 149.50, 136.44, 130.10, 117.04, 99.49 – 96.53 (m), 67.21. HRMS calcd. for $[M+H]^+$: 268.08921; observed: 268.08883.



45

Method A, followed by B and C.

White solid; M.P.: 136-140°C; ^{1}H **NMR (500 MHz, $CDCl_3$)** δ 8.20 (t, *J* = 4.2 Hz, 2H), 7.82 (dd, *J* = 11.5, 8.6 Hz, 2H), 7.60 (t, *J* = 7.4 Hz, 1H), 7.45 (t, *J* = 7.4 Hz, 1H), 7.41 (s, 1H), 7.26 (d, *J* = 14.2 Hz, 1H), 7.11 (s, 1H), 6.77 (s, 1H), 4.37 (t, *J* = 5.0 Hz, 2H), 3.92 (dd, *J* = 10.3, 5.3 Hz, 2H). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 152.31, 149.08, 136.13, 132.91, 130.64, 130.44, 129.48, 128.20,

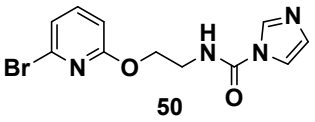


46

128.13, 126.19, 125.09, 115.99, 115.47, 110.15, 68.91, 40.46. HRMS calcd. for $[M+H]^+$: 360.03476; observed: 360.03323.

Method A, followed by B and C.

White solid; M.P.: 132-136°C; **^{1}H NMR (500 MHz, $CDCl_3$)** δ 8.16 (d, *J* = 8.6 Hz, 2H), 7.81 (d, *J* = 8.1 Hz, 1H), 7.58 (t, *J* = 5.3 Hz, 1H), 7.51 – 7.42 (m, 4H), 7.38 (t, *J* = 7.9 Hz, 1H), 7.02 (s, 1H), 6.81 (d, *J* = 7.6 Hz, 1H), 4.33 (t, *J* = 5.0 Hz, 2H), 3.94 (dd, *J* = 10.4, 5.3 Hz, 2H). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 153.91, 149.26, 135.88, 134.51, 129.99, 127.69, 126.61, 125.83, 125.45, 125.36, 121.49, 121.04, 116.43, 105.02, 66.44, 40.70. HRMS calcd. for $[M+H]^+$: 282.12425; observed: 282.12294.

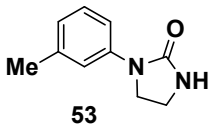


50

To a solution of alcohol **48** (0.1 g, 0.62 mmol, 1.1 equiv.) in anhydrous THF (5 mL), was added NaH (60% in mineral oil; 27 mg, 0.67 mmol, 1.2 equiv.) at 0°C and stirred for 20 min. Then, 2,6-dibromopyridine was added at 0°C. After 29 h at room temperature, distilled water (10 ml) was added and the solution was extracted with EtOAc (3 x 15 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product was purified by flash column chromatography (hexane / EtOAc gradient of polarity) generating the pyridyl ether derivative.

Then, Method B and C were used to prepare **50**.

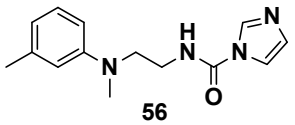
White solid; M.P.: 141-144°C; **^{1}H NMR (500 MHz, d_6-DMSO)** δ 8.72 (t, *J* = 4.4 Hz, 1H), 8.23 (s, 1H), 7.69 – 7.62 (m, 2H), 7.22 (d, *J* = 7.5 Hz, 1H), 7.02 (s, 1H), 6.87 (d, *J* = 8.2 Hz, 1H), 4.40 (t, *J* = 5.5 Hz, 2H), 3.63 (dd, *J* = 5.4, 10.0 Hz, 2H). **^{13}C NMR (126 MHz, d_6-DMSO)** δ 162.74, 149.00, 142.02, 137.65, 135.97, 129.60, 120.73, 116.58, 109.84, 64.46. HRMS calcd. for $[M+H]^+$: 311.01381; observed: 311.01342.



53

Method A, followed by B and C.

White solid; **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.39 (s, 1H), 7.31 (d, *J* = 7.8 Hz, 1H), 7.22 (t, *J* = 7.8 Hz, 1H), 6.88 (d, *J* = 7.8 Hz, 1H), 5.03 (s, 1H), 3.93 (dd, *J* = 8.9, 7.8 Hz, 2H), 3.56 (t, *J* = 7.8 Hz, 2H), 2.36 (s, 3H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 159.95, 140.09, 138.81, 128.79, 123.83, 118.90, 115.24, 45.61, 37.68, 21.82. HRMS calcd. for $[M+H]^+$: 177.10224; observed: 177.10184.



56

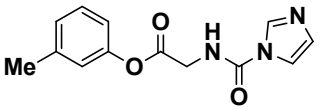
To a solution of *m*-toluidine (4.7 mmol, 0.5 mL) in methanol (10 mL), were added paraformaldehyde (3 equiv.; 0.42 g) and sodium methoxide (5 equiv.; 5 ml of a 25% solution (w/v) in methanol). The reaction was stirred at 65° C for 1 h. Then, $NaBH_4$ (3 equiv.; 0.53 g) was added at room temperature. The reaction was stirred at 65° C for 1.5 h. The reaction was concentrated

under reduced pressure, distilled water (10 mL) was added and the solution was extracted with EtOAc (3 x 15 mL). The organic layer was dried over Mg_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by flash column chromatography (hexane / EtOAc gradient of polarity) generating the methylamine in 93% yield.

To a solution of methyl amine (0.61 mmol, 74 mg) in THF (3 mL), was added NaH (1 eq.; 24.4 mg) at 0° C. After 30 min, alkyl-tosylate **42** (1 equiv.; 0.19 g) was added at 0° C. After 4 h at room temperature, distilled water (10 ml) was added and the solution was extracted with EtOAc (3 x 15 mL). The organic layer was dried over Mg_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by flash column chromatography (hexane / DCM gradient of polarity) generating the tertiary amine in 25% yield (48% of *m*-toluidine was recovered).

Then, the method B was performed, followed by C.

White solid; M.P.: 99-103°C; **^{1}H NMR (500 MHz, $CDCl_3$)** δ 7.95 (s, 1H), 7.25 (t, *J* = 1.4 Hz, 1H), 7.18 – 7.12 (m, 1H), 7.04 – 7.00 (m, 1H), 6.77 (s, 1H), 6.66 – 6.57 (m, 3H), 3.68 – 3.60 (m, 4H), 2.97 (s, 3H), 2.30 (s, 3H). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 149.55, 149.05, 139.33, 135.71, 130.15, 129.38, 118.46, 116.08, 113.61, 110.01, 51.60, 38.82, 38.52, 21.85. HRMS calcd. for $[M+H]^+$: 259.15534; observed: 259.15476.

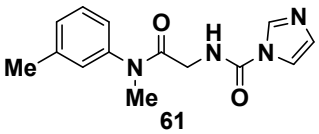


60

Method D, followed by B and C.

The carboxilic acid **57** was prepared as reported in the literature.[1]

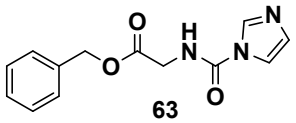
White solid; M.P.: 84-89°C; **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.12 (s, 1H), 7.78 (t, *J* = 4.2 Hz, 1H), 7.40 (s, 1H), 7.34 (t, *J* = 7.6 Hz, 1H), 7.21 (d, *J* = 7.7 Hz, 1H), 7.07 – 6.99 (m, 3H), 3.88 (d, *J* = 4.7 Hz, 2H), 3.29 (s, 3H), 2.39 (s, 3H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 168.09, 148.92, 141.51, 140.56, 136.15, 130.32, 130.09, 129.69, 127.60, 124.00, 115.94, 42.80, 37.70, 21.27. HRMS calcd. for $[M+H]^+$: 260.10352; observed: 260.10246.



61

Method E, followed by B and C.

White solid; M.P.: 84-89°C; **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.12 (s, 1H), 7.78 (t, *J* = 4.2 Hz, 1H), 7.40 (s, 1H), 7.34 (t, *J* = 7.6 Hz, 1H), 7.21 (d, *J* = 7.7 Hz, 1H), 7.07 – 6.99 (m, 3H), 3.88 (d, *J* = 4.7 Hz, 2H), 3.29 (s, 3H), 2.39 (s, 3H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 168.09, 148.92, 141.51, 140.56, 136.15, 130.32, 130.09, 129.69, 127.60, 124.00, 115.94, 42.80, 37.70, 21.27. HRMS calcd. for $[M+H]^+$: 273.13515; observed: 273.13410.

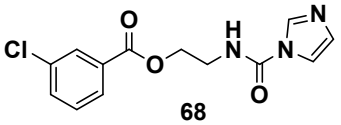


63

The benzylic ester **62** was prepared as reported in the literature.[2]

Then, the method C was performed.

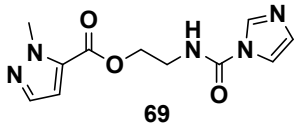
White solid; M.P.: 118-121°C; **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.20 (s, 1H), 7.60 (t, *J* = 5.1 Hz, 1H), 7.44 (t, *J* = 1.3 Hz, 1H), 7.40 – 7.34 (m), 7.05 (s, 1H), 5.24 (s, 2H), 4.21 (d, *J* = 5.4 Hz, 2H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 169.32, 149.13, 136.03, 134.87, 130.22, 128.74, 128.47, 116.35, 67.68, 42.36. HRMS calcd. for $[M+H]^+$: 260.10297; observed: 260.10729.



68

Method D, followed by B and C.

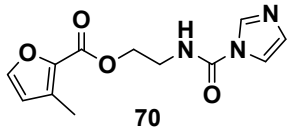
Colorless oil; **^{1}H NMR (500 MHz, $CDCl_3$)** δ 8.17 (s, 1H), 8.04 (t, *J* = 1.8 Hz, 1H), 7.95 (d, *J* = 7.8 Hz, 1H), 7.60 (ddd, *J* = 8.0, 2.1, 1.0 Hz, 1H), 7.47 – 7.41 (m, 1H), 7.39 (dd, *J* = 3.8, 2.4 Hz, 1H), 7.13 (d, *J* = 4.3 Hz, 1H), 6.79 (s, 1H), 4.65 – 4.60 (m, 2H), 3.86 (dd, *J* = 10.3, 5.2 Hz, 2H). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 166.35, 148.98, 135.90, 134.79, 133.65, 131.03, 130.72, 129.93, 129.84, 127.91, 115.85, 63.88, 41.04.



69

Method D, followed by B and C.

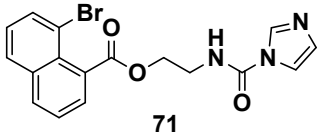
White solid; M.P.: 105-106°C; **^{1}H NMR (500 MHz, $CDCl_3$)** δ 8.13 (s, 1H), 7.45 (d, *J* = 1.9 Hz, 1H), 7.39 (s, 1H), 7.20 (s, 1H), 7.06 (s, 1H), 6.80 (d, *J* = 1.9 Hz, 1H), 4.52 (t, *J* = 5.1 Hz, 2H), 4.14 (s, 3H), 3.80 (dd, *J* = 10.4, 5.3 Hz, 2H). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 160.36, 149.16, 138.06, 135.98, 131.63, 130.53, 116.24, 111.78, 63.51, 40.74, 39.73. HRMS calcd. for $[M+H]^+$: 264.10912; observed: 264.10896.



70

Method D, followed by B and C.

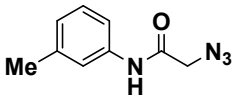
White solid; M.P.: 110-112°C; **^{1}H NMR (500 MHz, $CDCl_3$)** δ 8.19 (s, 1H), 7.77 (s, 1H), 7.43 (d, *J* = 6.6 Hz, 2H), 7.02 (s, 1H), 6.34 (s, 1H), 4.51 (t, *J* = 5.2 Hz, 2H), 3.77 (dd, *J* = 10.4, 5.2 Hz, 2H), 2.31 (s, 3H). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 160.05, 149.24, 145.57, 139.61, 136.06, 132.65, 130.07, 116.45, 115.52, 62.97, 40.76, 11.76. HRMS calcd. for $[M+H]^+$: 264.09788; observed: 264.09782.



71

Method D, followed by B and C.

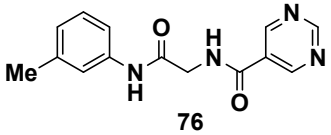
Colorlles oil; **^{1}H NMR (600 MHz, $CDCl_3$)** δ 8.11 (s, 1H), 7.97 (d, *J* = 8.1 Hz, 1H), 7.89 (d, *J* = 8.0 Hz, 1H), 7.84 (d, *J* = 7.4 Hz, 1H), 7.66 (d, *J* = 7.0 Hz, 1H), 7.52 (t, *J* = 7.6 Hz, 1H), 7.39 (t, *J* = 7.8 Hz, 1H), 7.33 (s, 1H), 7.07 (s, 1H), 6.52 (s, 1H), 4.64 – 4.56 (m, 2H), 3.88 – 3.78 (m, 2H). **^{13}C NMR (151 MHz, $CDCl_3$)** δ 170.79, 149.03, 136.02, 135.74, 133.51, 132.00, 131.29, 130.79, 129.14, 129.09, 128.53, 127.23, 125.60, 119.27, 115.99, 77.37, 77.16, 76.95, 64.66, 40.37, 0.14. HRMS calcd. for $[M+H]^+$: 388.02913; observed: 388.02957.



74

To a solution of *m*-toluidine (3.0 g, 28.0 mmol, 1.0 equiv.) in a two-phase mixture of EtOAc (27 mL) and saturated $NaHCO_3$ (27 mL) at 0°C was added dropwise bromoacetyl bromide (2.93 mL, 33.6 mmol, 1.2 equiv.). The reaction was warmed to 25°C and stirred for 1 h. The reaction was diluted with EtOAc (20 mL) and the organic layer was separated. The organic layer was washed with saturated aqueous solution of $NaHCO_3$ (20 mL), 10% aqueous citric acid solution (w/v, 60 mL), and brine (30 mL). The organic layer was then dried over $MgSO_4$ and concentrated to give the desired amide as an off-white solid that was used without further purification.

Sodium azide (1.45 g, 22.3 mmol, 1.2 equiv.) was added to a solution of 2-bromo-*N*-*m*-tolylacetamide (4.26 g, 18.6 mmol, 1.0 equiv.) in DMF (37 mL) and the suspension was stirred for 18 h at room temperature. The reaction mixture was poured on water (100 mL) and EtOAc (50 mL), the organic layer was separated, and the aqueous layer was extracted with EtOAc (2 × 50 mL). The combined organic layer was washed with water (30 mL), brine (50 mL), dried over $MgSO_4$, and concentrated. Purification by flash column chromatography (4:1 hexane/EtOAc) gave the azide **74**.

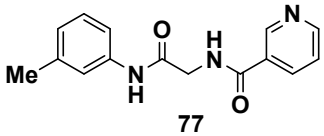


76

A solution of 2-azido-*N*-*m*-tolylacetamide (1.50 g, 7.81 mmol, 1.0 equiv.) in methanol (30 mL) was added to a suspension of 5% Pd/C (90 mg) in methanol (5 mL) that was activated by bubbling H_2 (balloon) through the suspension for 10 min. The reaction was stirred under an atmosphere of hydrogen for 2 h, and then filtered through a pad of celite. The reaction mixture was concentrated to give the title compound (1.28 g, 99%) as a yellow oil, which was used in the next step without further purification.

To a solution of 2-amino-*N*-*m*-tolylacetamide (0.200 g, 1.21 mmol, 1.0 equiv.) in DCM (6 mL) and DMF (1 mL) were added pyrimidine-5-carboxylic acid (0.150 g, 1.21 mmol, 1.0 equiv.) and EDC (0.255 g, 1.33 mmol, 1.1 equiv.) and the mixture was stirred for 18 h at room temperature. The reaction mixture was poured on water (20 mL) and extracted with EtOAc (2 × 30 mL) and DCM (30 mL). The combined organic layer was washed with brine (20 mL), dried over $MgSO_4$, and concentrated. The yellow solid was triturated with EtOAc to give the title compound (140 mg, 43%) as a white solid.

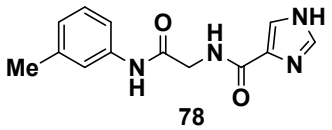
White solid; **^{1}H NMR (400 MHz, d_6-DMSO)** δ 9.99 (s, 1H), 9.34 (s, 1H), 9.27 (t, *J* = 5.6 Hz, 1H), 9.22 (s, 2H), 7.43 (s, 1H), 7.39 (d, *J* = 8.3 Hz, 1H), 7.19 (t, *J* = 7.8 Hz, 1H), 6.87 (d, *J* = 7.5 Hz, 1H), 4.11 (d, *J* = 5.8 Hz, 2H), 2.27 (s, 3H). **^{13}C NMR (101 MHz, d_6-DMSO)** δ 167.65, 164.04, 160.64, 156.47, 139.19, 138.40, 129.06, 127.90, 124.56, 120.33, 116.99, 43.67, 21.64. HRMS calcd. for $[M+H]^+$: 271.11895; observed: 271.11881.



77

To a solution of 2-amino-*N*-m-tolylacetamide (0.225 g, 1.37 mmol, 1.0 equiv.) in DMF (6 mL) was added nicotinic acid (0.168 g, 1.37 mmol, 1.0 equiv.) and EDC (0.289 g, 1.51 mmol, 1.1 equiv.) and the mixture was stirred for 18 h. The reaction mixture was poured on water (20 mL) and extracted with CH_2Cl_2 (2 × 20 mL) and EtOAc (3 × 20 mL). The combined organic layer was washed with brine (20 mL), dried over $MgSO_4$, and concentrated. The yellow solid was triturated with CH_2Cl_2/Et_2O (1:1, v/v, 2 mL) to give the title compound (45 mg, 12%) as a white solid.

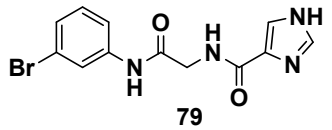
White solid; **^{1}H NMR (400 MHz, d_6-DMSO)** δ 10.01 (s, 1H), 9.09 (s, 1H), 8.75 (d, *J* = 4.0 Hz, 1H), 8.26 (d, *J* = 7.9 Hz, 1H), 7.55 (dd, *J* = 7.7, 4.9 Hz, 1H), 7.49 – 7.37 (m, 2H), 7.20 (t, *J* = 7.8 Hz, 1H), 6.89 (d, *J* = 7.3 Hz, 1H), 4.11 (d, *J* = 5.8 Hz, 2H), 2.29 (s, 3H). **^{13}C NMR (101 MHz, d_6-DMSO)** δ 167.95, 165.77, 152.50, 149.01, 139.29, 138.39, 135.55, 129.96, 129.05, 124.48, 123.96, 120.25, 116.91, 43.71, 21.65·HRMS calcd. for $[M+H]^+$: 270.12370; observed: 270.12822.



78

To a solution of 2-amino-*N*-*m*-tolylacetamide (0.250 g, 1.52 mmol, 1.0 equiv.) in DCM (20 mL) and DMF (1 mL) were added imidazole-4-carboxylic acid (0.170 g, 1.52 mmol, 1.0 equiv.) and EDC (0.320 g, 1.67 mmol, 1.1 equiv.) and the mixture was stirred for 18 h at room temperature. The reaction mixture was poured on water (20 mL) and extracted with CH_2Cl_2 (2 × 30 mL). The combined organic layer was washed with water (10 mL), brine (20 mL), dried over $MgSO_4$, and concentrated to give the title compound (50 mg, 13%) as a white solid.

White solid; **^{1}H NMR (400 MHz, d_6-DMSO)** δ 12.71 (s, 1H), 10.18 (s, 1H), 8.12 (s, 1H), 7.76 (s, 1H), 7.67 (s, 1H), 7.44 (d, *J* = 14.4 Hz, 2H), 7.19 (t, *J* = 7.4 Hz, 1H), 6.87 (d, *J* = 6.5 Hz, 1H), 4.08 (d, *J* = 4.4 Hz, 2H), 2.28 (s, 3H). **^{13}C NMR (101 MHz, d_6-DMSO)** δ 168.27, 139.41, 138.32, 136.12, 129.02, 124.33, 120.10, 119.52, 116.77, 42.96, 21.67. HRMS calcd. for $[M+Na]^+$: 281.10562; observed: 281.10090.



79

To a solution of 3-bromoaniline (2.0 g, 11.62 mmol, 1.0 equiv.) in a two-phase mixture of EtOAc (20 mL) and saturated aqueous $NaHCO_3$ (20 mL) at 0°C was added dropwise bromoacetyl bromide (1.3 mL, 15.11 mmol, 1.3 equiv.). The reaction was warmed to 25°C and stirred for 3 h. The organic layer was separated and the aqueous layer was extracted with EtOAc (2 × 30 mL). The combined organic layer was washed with 10% aqueous citric acid solution (w/v, 20 mL), dried over $MgSO_4$, and concentrated to give the desired amide (3.4 g, 99%) that was used in the next step without further purification.

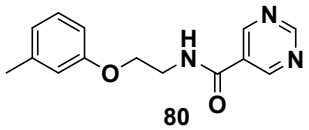
Sodium azide (0.88 g, 13.5 mmol, 1.2 equiv.) was added to a solution of 2-bromo-*N*-(3-bromophenyl)acetamide (3.30 g, 11.2 mmol, 1.0 equiv.) in DMF (20 mL) and the suspension was stirred for 48 h. The reaction mixture was poured on water (30 mL) and EtOAc (20 mL), the organic layer was separated and the aqueous layer was extracted with EtOAc (2 × 20 mL). The combined organic layer was washed with water (20 mL), brine (20 mL), dried over $MgSO_4$, and concentrated to give the desired azide (2.43 g, 81%) that was used in the next step without further purification.

A solution of 2-azido-*N*-(3-bromophenyl)acetamide (1.50 g, 5.64 mmol, 1.0 equiv.) in methanol (30 mL) was added to a suspension of 5% Pd/C (100 mg) in methanol (5 mL) that was activated by bubbling H_2 (balloon) through the suspension for 10 min. The reaction was stirred under an atmosphere of hydrogen for 1 h, and then filtered through a pad of celite. The reaction mixture

was concentrated to give the desired amine (1.33 g, 99%) that was used in the next step without further purification.

To a solution of 2-amino-*N*-m-bromophenylacetamide (0.500 g, 2.08 mmol, 1.0 equiv.) in DCM (20 mL) and DMF (1 mL) were added imidazole-4-carboxylic acid (0.233 g, 2.08 mmol, 1.0 equiv.) and EDC (0.438 g, 2.29 mmol, 1.1 equiv.) and the mixture was stirred for 18 h at room temperature. The reaction mixture was poured on brine (20 mL) and extracted with DCM (2 × 20 mL) and EtOAc (3 × 20 mL). The combined organic layer was dried over $MgSO_4$, and concentrated to a yellow solid. The yellow solid was triturated with DCM to give the title compound (20 mg, 3%) as a white solid.

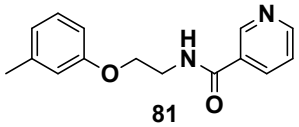
White solid; **^{1}H NMR of conformers mixture (500 MHz, d_6-DMSO)** δ 12.52 (s, 1H), 10.23 (s, 0.77H), 10.04 (s, 0.36H), 8.12 (s, 1H), 7.95 (s, 0.7 H), 7.77 (s, 1H), 7.66 (s, 1H), 7.60 (d, *J* = 7.9 Hz, 0.6H), 7.52 (d, *J* = 8.0 Hz, 0.6H), 7.24-7.34 (m, 2H), 7.06 (t, *J* = 7.4 Hz, 0.36H), 4.06 (d, *J* = 5.8 Hz, 2H). **^{13}C NMR of conformers mixture (d_6-DMSO, 126 MHz)** δ 168.79, 168.31, 163.06, 141.05, 139.41, 136.33, 136.13, 131.29, 129.25, 126.29, 123.68, 122.04, 121.88, 119.63, 119.53, 118.30, 42.97, 42.89. HRMS calcd. for $[M+H]^+$: 323.01381; observed: 323.01343.



80

Method E.

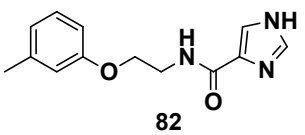
White solid; M.P.: 70-73°C; **^{1}H NMR (400 MHz, $CDCl_3$)** δ 9.34 (s, 1H), 9.13 (s, 2H), 7.19 (t, *J* = 7.8 Hz, 1H), 6.82 (d, *J* = 7.5 Hz, 2H), 6.76 – 6.68 (m, 2H), 4.18 (t, *J* = 5.4 Hz, 2H), 3.91 (dd, *J* = 10.3, 5.4 Hz, 2H), 2.34 (s, 3H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 163.65, 160.62, 158.25, 155.65, 139.83, 129.43, 127.80, 122.34, 115.31, 111.31, 39.76, 21.51. HRMS calcd. for $[M+H]^+$: 258.12370; observed: 258.12360.



81

To a solution of 2-amino-*N*-*m*-tolylether (0.288 g, 1.53 mmol, 1.0 equiv.) in DCM (6 mL) were added nicotinic acid (0.188 g, 1.53 mmol, 1.0 equiv.), EDC (0.323 g, 1.69 mmol, 1.1 equiv.), and Et_3N (0.21 mL, 1.53 mmol, 1.0 equiv.) and the mixture was stirred for 18 h at room temperature. The reaction mixture was poured on brine (10 mL) and extracted with EtOAc (3 × 25 mL). The combined organic layer was dried over $MgSO_4$, and concentrated to a brown oil. Purification by flash column chromatography (20:1 $CHCl_3$/MeOH) and (EtOAc) gave the title compound (76 mg, 19%) as a light yellow oil.

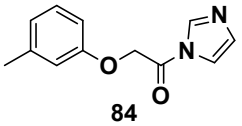
Light yellow oil; **^{1}H NMR (500 MHz, $CDCl_3$)** δ 9.02 (d, *J* = 1.9 Hz, 1H), 8.76 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.18 – 8.12 (m, 1H), 7.42 (dd, *J* = 7.9, 4.9 Hz, 1H), 7.21 (t, *J* = 7.8 Hz, 1H), 6.83 (d, *J* = 7.5 Hz, 1H), 6.76 (dd, *J* = 11.9, 3.7 Hz, 3H), 4.19 (t, *J* = 5.0 Hz, 2H), 3.92 (dd, J = 10.4, 5.4 Hz, 2H), 2.36 (s, 3H). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 165.77, 158.35, 152.35, 147.95, 139.77, 135.19, 130.10, 129.41, 123.55, 122.24, 115.36, 111.36, 66.49, 39.68, 21.52. HRMS calcd. for $[M+H]^+$: 257.12845; observed: 257.12886.



82

Method E.

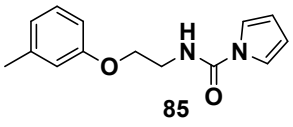
Light yellow oil; **^{1}H NMR (400 MHz, $CDCl_3$)** δ 11.60 (s, 1H), 7.69 (s, 1H), 7.60 (d, *J* = 8.6 Hz, 2H), 7.17 (t, *J* = 7.8 Hz, 1H), 6.83 – 6.68 (m, 3H), 4.13 (t, *J* = 5.3 Hz, 2H), 3.85 (dd, *J* = 11.0, 5.5 Hz, 2H), 2.33 (s, 3H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 163.57, 158.50, 139.60, 135.88, 135.51, 129.26, 121.96, 119.31, 115.42, 111.36, 66.56, 38.69, 21.49. HRMS calcd. for $[M+H]^+$: 246.12370; observed: 246.12345.



84

To a solution of carboxylic acid **83** (0.105 g, 0.63 mmol, 1.0 equiv.) in CH_3CN (5 mL) was added CDI (0.205 g, 1.26 mmol, 2.0 equiv.) and the mixture was stirred for 8 h at room temperature. The CH_3CN was removed by evaporation and the crude was purified by flash column chromatography (9.5:0.5 DCM/MeOH) to give the title compound.

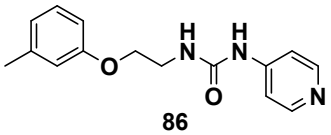
Light yellow oil; **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.46 (s, 2H), 7.16 – 7.01 (m, 2H), 6.77 (d, *J* = 7.5 Hz, 1H), 6.71 – 6.61 (m, 2H), 4.56 (s, 2H), 2.28 (s, 3H). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 174.78, 158.21, 139.66, 134.24, 129.29, 122.34, 119.23, 115.68, 111.76, 67.19, 21.34. HRMS calcd. for $[M+Na]^+$: 239.07910; observed: 239.08047.



85

Method F.

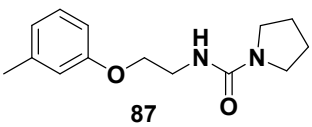
Light yellow oil; **^{1}H NMR (500 MHz, $CDCl_3$)** δ 7.25 – 7.18 (m, 3H), 6.84 (d, *J* = 7.5 Hz, 1H), 6.79 – 6.73 (m, 2H), 6.31 (t, *J* = 2.3 Hz, 1H), 2H), 6.03 (s, 1H), 4.17 (t, *J* = 5.0 Hz, 2H), 3.85 (dd, *J* = 10.3, 5.4 Hz, 2H), 2.37 (s, 3H). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 158.45, 151.16, 139.90, 129.52, 122.38, 118.54, 115.47, 112.14, 111.49, 66.58, 40.60, 21.64. HRMS calcd. for $[M+Na]^+$: 245.12811; observed: 245.12845.



86

Method F.

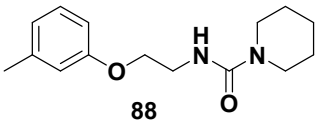
Light yellow oil; **^{1}H NMR (500 MHz, $CDCl_3$)** δ 8.98 (s, 1H), 8.23 (d, *J* = 5.4 Hz, 2H), 7.10 (dd, *J* = 8.3, 7.6 Hz, 1H), 6.73 (d, *J* = 7.5 Hz, 1H), 6.60 (d, *J* = 7.4 Hz, 2H), 6.37 (s, 1H), 4.00 (t, *J* = 5.0 Hz, 2H), 3.63 (dd, *J* = 10.1, 5.1 Hz, 2H), 2.26 (s, 3H). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 158.52, 155.29, 149.11, 148.32, 139.77, 129.43, 122.17, 115.34, 112.95, 111.47, 67.08, 39.72, 21.60. HRMS calcd. for $[M+Na]^+$: 272.13935; observed: 272.13931.



87

Method F.

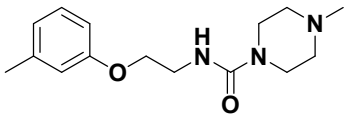
White solid; M.P.: 52,8 - 53,7 °C; **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.18 (t, *J* = 7.8 Hz, 1H), 6.80 (d, *J* = 7.4 Hz, 1H), 6.74 (dd, *J* = 11.0, 2.0 Hz, 2H), 4.70 (s, 1H), 4.06 (t, *J* = 5.0 Hz, 2H), 3.66 (dd, *J* = 10.5, 5.4 Hz, 2H), 3.35 (t, *J* = 6.6 Hz, 4H), 2.35 (s, 3H), 1.94 – 1.88 (m, 4H); ^{13}C **NMR (101 MHz, $CDCl_3$)** δ 158.77, 156.70, 139.60, 129.28, 121.80, 115.35, 111.41, 67.53, 45.52, 40.20, 25.54, 21.50; HRMS calcd. for $[M+H]^+$: 249.15975; observed: 249.15970.



88

Method F.

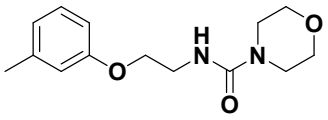
White solid; M.P.: 44,8 - 46,0 °C; **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.19 (t, *J* = 7.8 Hz, 1H), 6.80 (d, *J* = 7.5 Hz, 1H), 6.77 – 6.71 (m, 2H), 4.92 (s, 1H), 4.07 (t, *J* = 5.0 Hz, 2H), 3.65 (dd, *J* = 10.3, 5.4 Hz, 2H), 3.40 – 3.29 (m, 4H), 2.35 (s, 3H), 1.65 – 1.52 (m, 6H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 158.75, 157.60, 139.62, 129.29, 121.83, 115.36, 111.43, 67.45, 44.87, 40.52, 25.58, 24.39, 21.51; HRMS calcd. for $[M+H]^+$: 263.17540; observed: 263. 17542.



89

Method F.

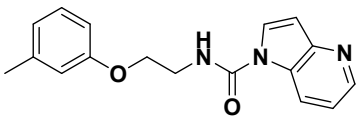
White solid; M.P.: 117,5 - 118,5 °C; **^{1}H NMR (500 MHz, $CDCl_3$)** δ 7.19 (t, *J* = 7.8 Hz, 1H), 6.81 (d, *J* = 7.6 Hz, 1H), 6.78 – 6.70 (m, 2H), 4.98 (d, *J* = 4.8 Hz, 1H), 4.07 (t, *J* = 5.0 Hz, 2H), 3.66 (dd, *J* = 10.3, 5.3 Hz, 2H), 3.46 – 3.38 (m, 4H), 2.45 – 2.38 (m, 4H), 2.35 (s, 3H), 2.32 (s, 3H); **^{13}C NMR (126 MHz, $CDCl_3$)** δ 158.68, 157.56, 139.65, 129.32, 121.90, 115.34, 111.39, 67.29, 54.66, 46.15, 43.70, 40.52, 21.55; HRMS calcd. for $[M+H]^+$: 278.18630; observed: 278.18633.



90

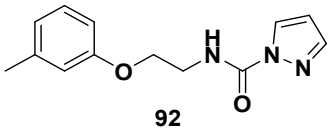
Method F.

Yellow oil; **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.19 (t, *J* = 7.8 Hz, 1H), 6.81 (d, *J* = 7.5 Hz, 1H), 6.77 – 6.70 (m, 2H), 4.95 (s, 1H), 4.07 (t, *J* = 5.0 Hz, 2H), 3.73 – 3.64 (m, 6H), 3.40 – 3.34 (m, 4H), 2.35 (s, 3H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 158.63, 157.67, 139.68, 129.33, 121.96, 115.33, 111.38, 67.22, 66.47, 43.96, 40.50, 21.51; HRMS calcd. for $[M+H]^+$: 265.15467; observed: 265.15460.



91

White solid; M.P.: 121,1 - 123,5 °C; **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.55 (d, *J* = 3.9 Hz, 1H), 8.46 (d, *J* = 8.4 Hz, 1H), 7.70 (d, *J* = 3.7 Hz, 1H), 7.25 (dd, *J* = 8.4, 4.7 Hz, 1H), 7.20 (t, *J* = 7.7 Hz, 1H), 6.87 - 6.80 (m, 2H), 6.74 - 6.76 (m, 2H), 6.27 (s, 1H), 4.22 (t, *J* = 5.0 Hz, 2H), 3.91 (dd, *J* = 10.3, 5.3 Hz, 2H), 2.35 (s, 3H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ 158.27 (s), 151.51 (s), 148.11 (s), 145.46 (s), 139.83 (s), 129.43 (s), 128.93 (s), 126.69 (s), 122.35 (s), 121.97 (s), 118.90 (s), 115.32 (s), 111.36 (s), 108.31 (s), 66.38 (s), 40.55 (s), 21.51 (s); HRMS calcd. for $[M+H]^+$: 296.13935; observed: 296.13935.

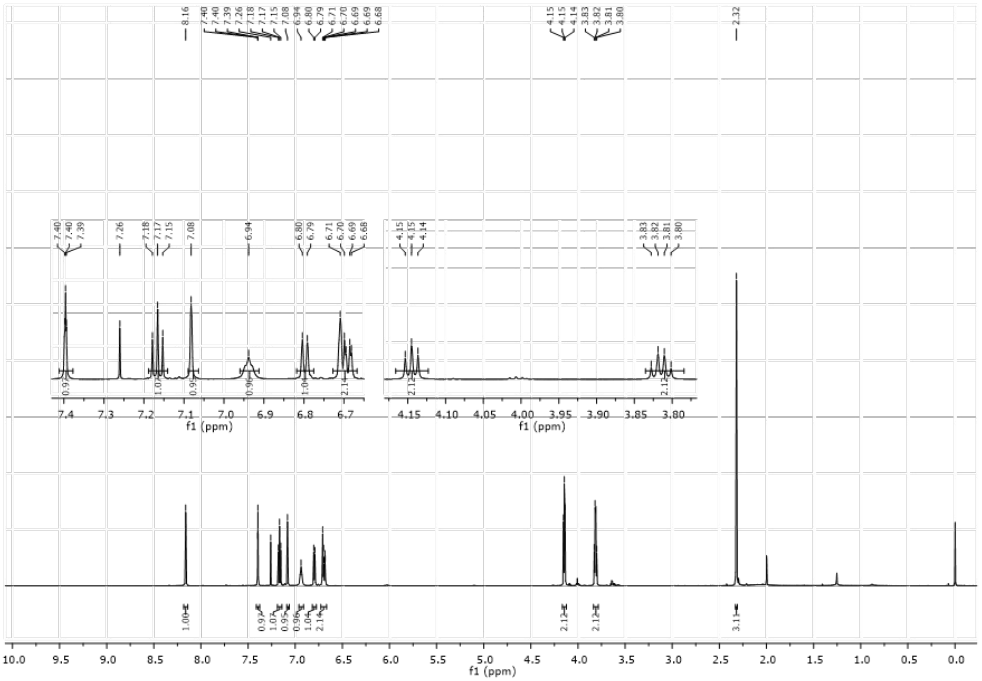


92

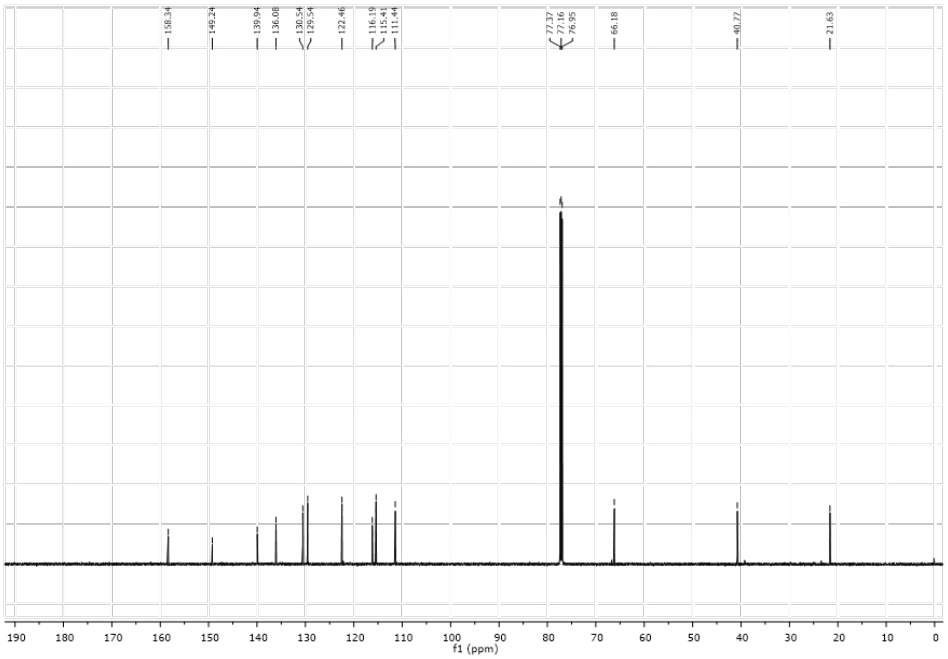
Method F.

Yellow solid; M.P.: 50,8 - 51,8 °C; **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.25 (d, *J* = 2.7 Hz, 1H), 7.64 (d, *J* = 1.0 Hz, 1H), 7.60 (s, 1H), 7.19 (t, *J* = 7.8 Hz, 1H), 6.84 - 6.72 (m, 3H), 6.42 (dd, *J* = 2.7, 1.6 Hz, 1H), 4.16 (t, *J* = 5.2 Hz, 2H), 3.86 (dd, *J* = 10.9, 5.5 Hz, 2H), 2.35 (s, 3H); ^{13}C NMR **(100 MHz, $CDCl_3$)** δ 158.42, 149.92, 142.28, 139.65, 129.29, 128.63, 122.09, 115.44, 111.38, 108.43, 66.37, 39.93, 21.50.; HRMS calcd. for $[M + Na]^+$: 268.1057; observed: 268.1035.

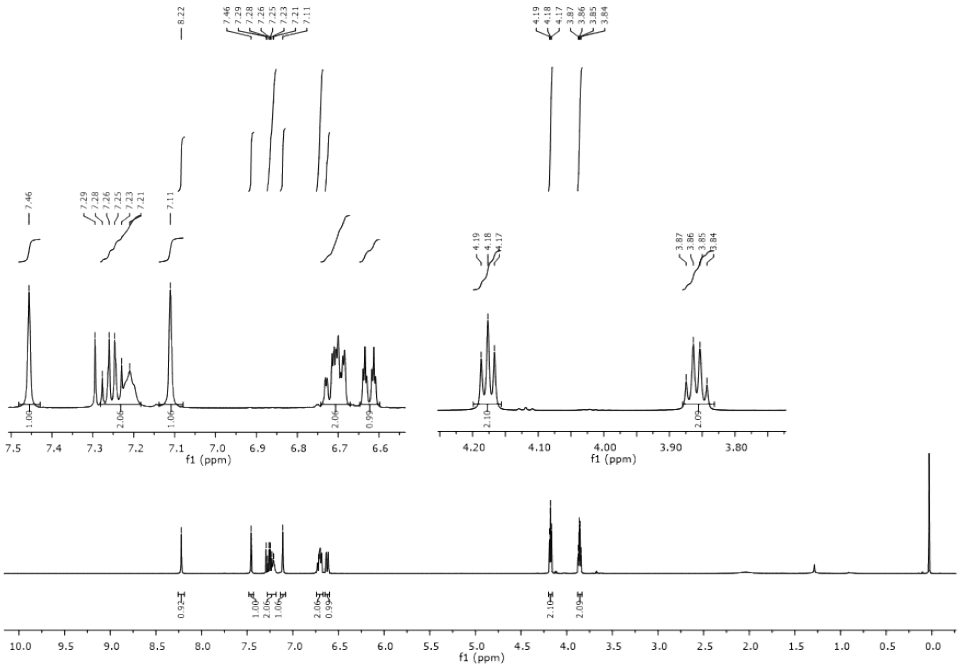
NMR Spectra



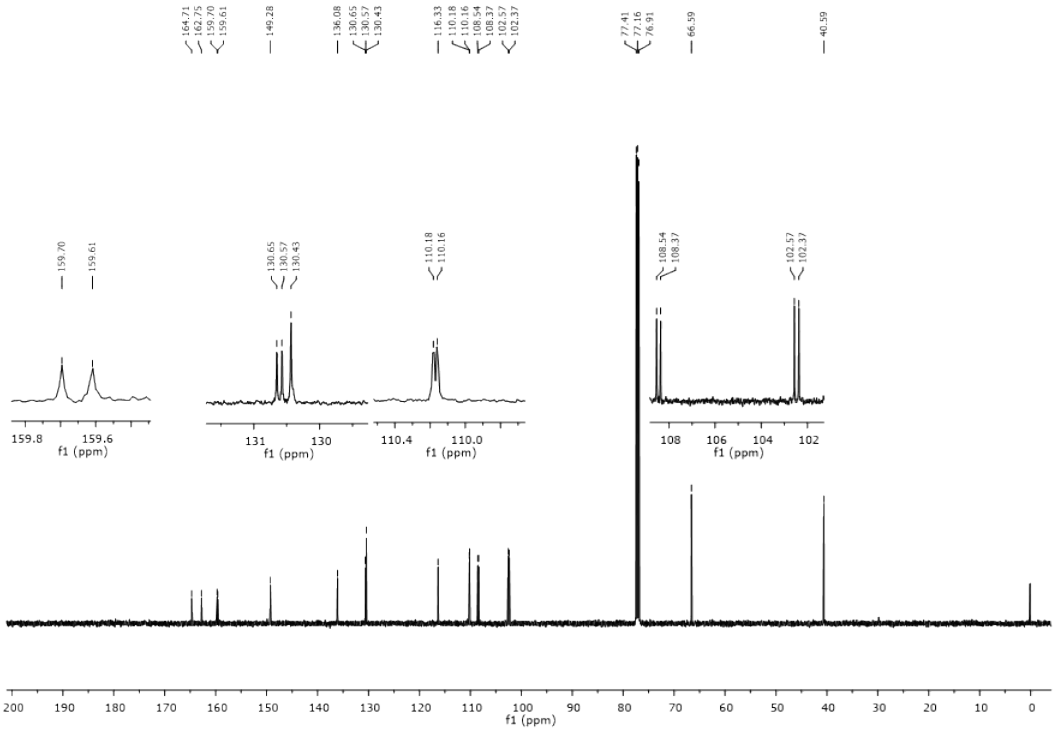
^{1}H NMR of 1 (600 MHz, $CDCl_3$)



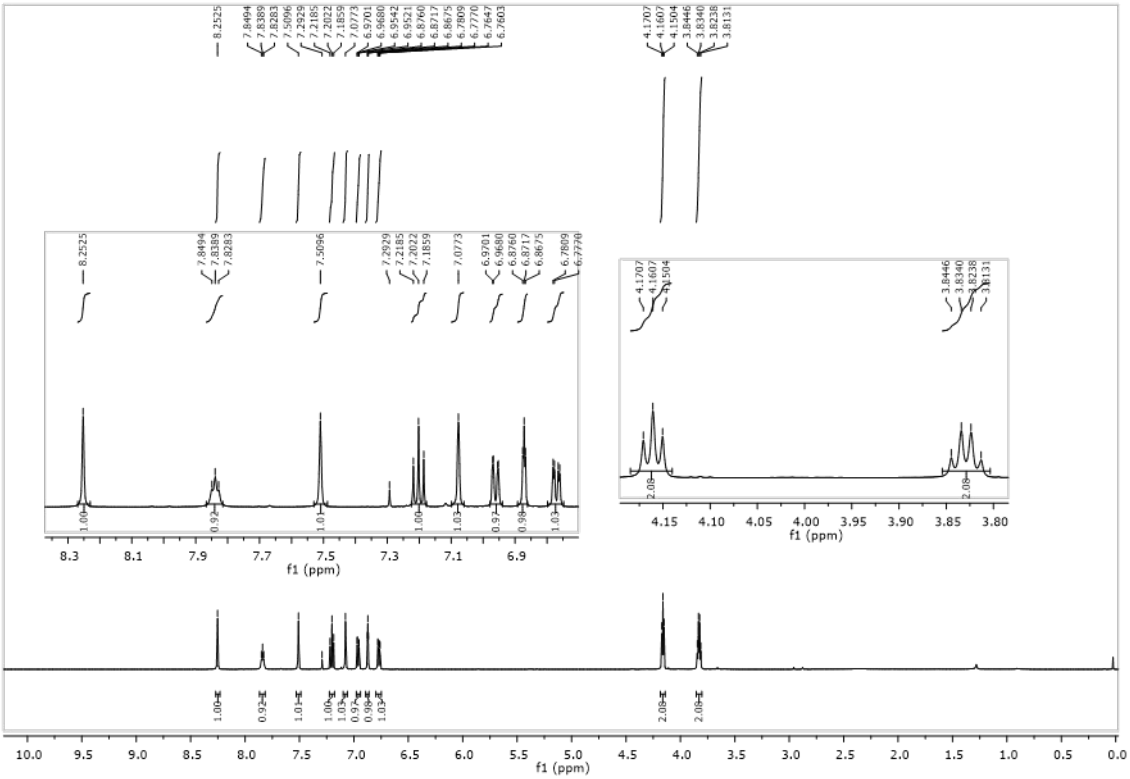
^{13}C NMR of 1 (151 MHz, $CDCl_3$)



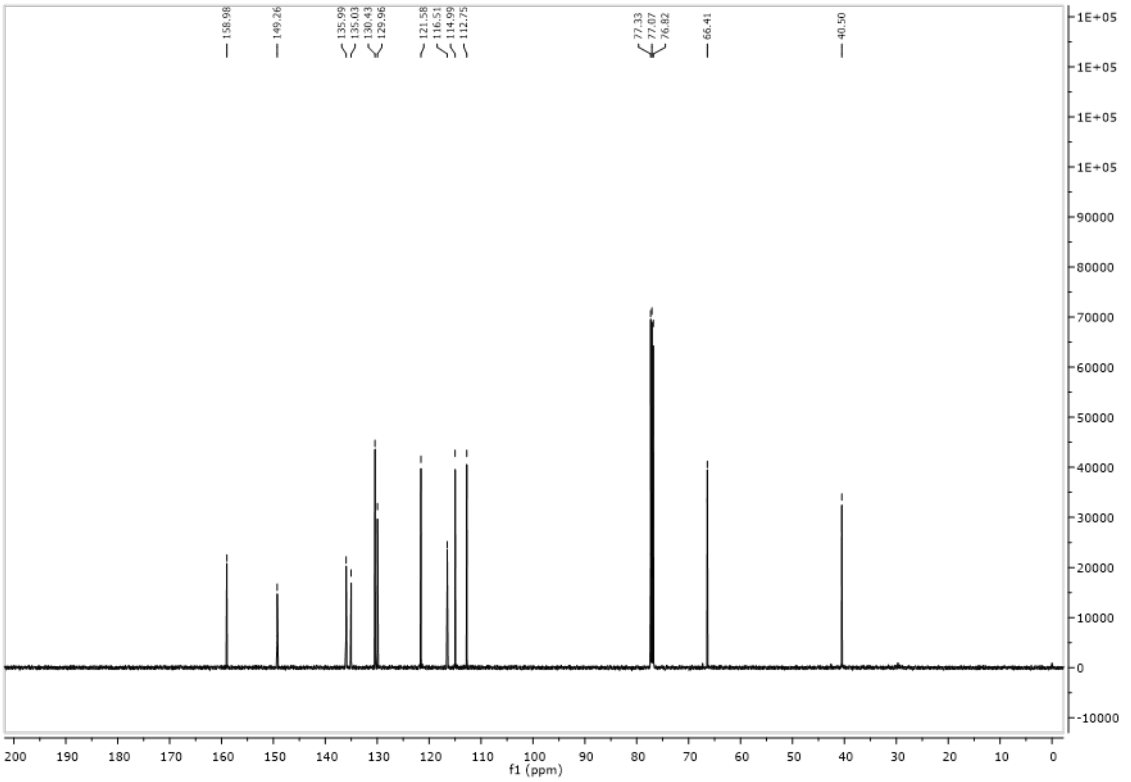
^{1}H NMR of 28 (500 MHz, CDCl3)



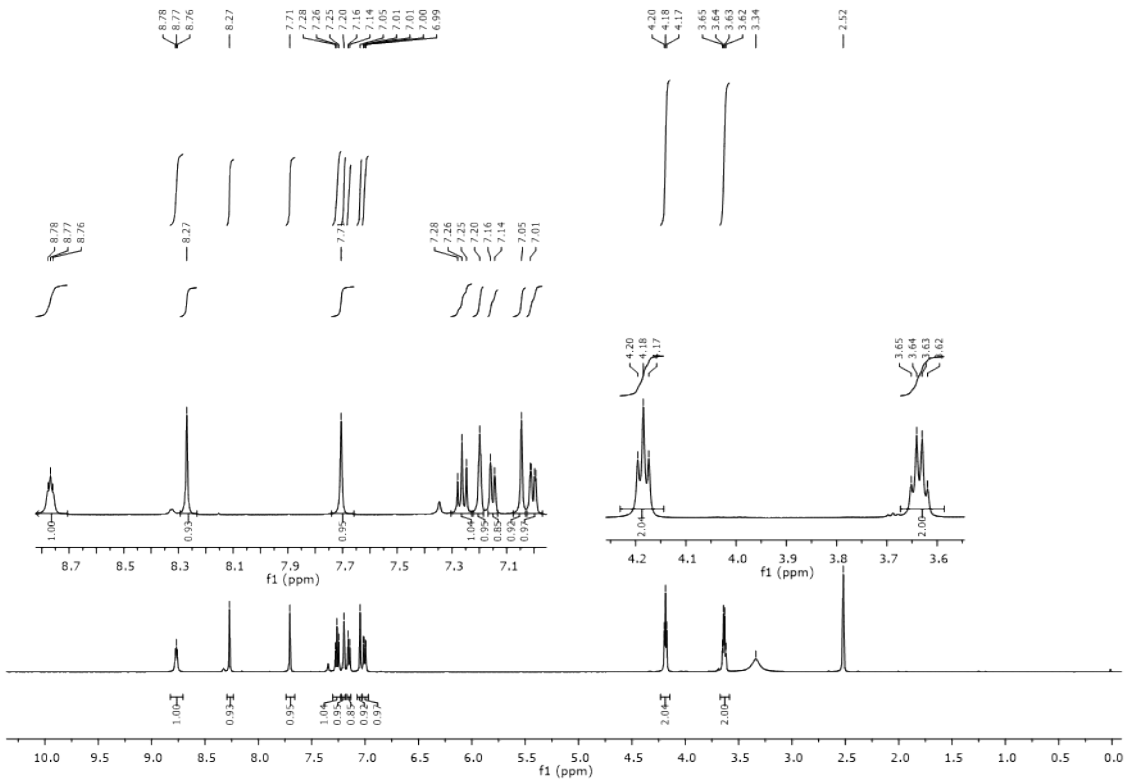
^{13}C NMR of 28 (126 MHz, CDCl3)



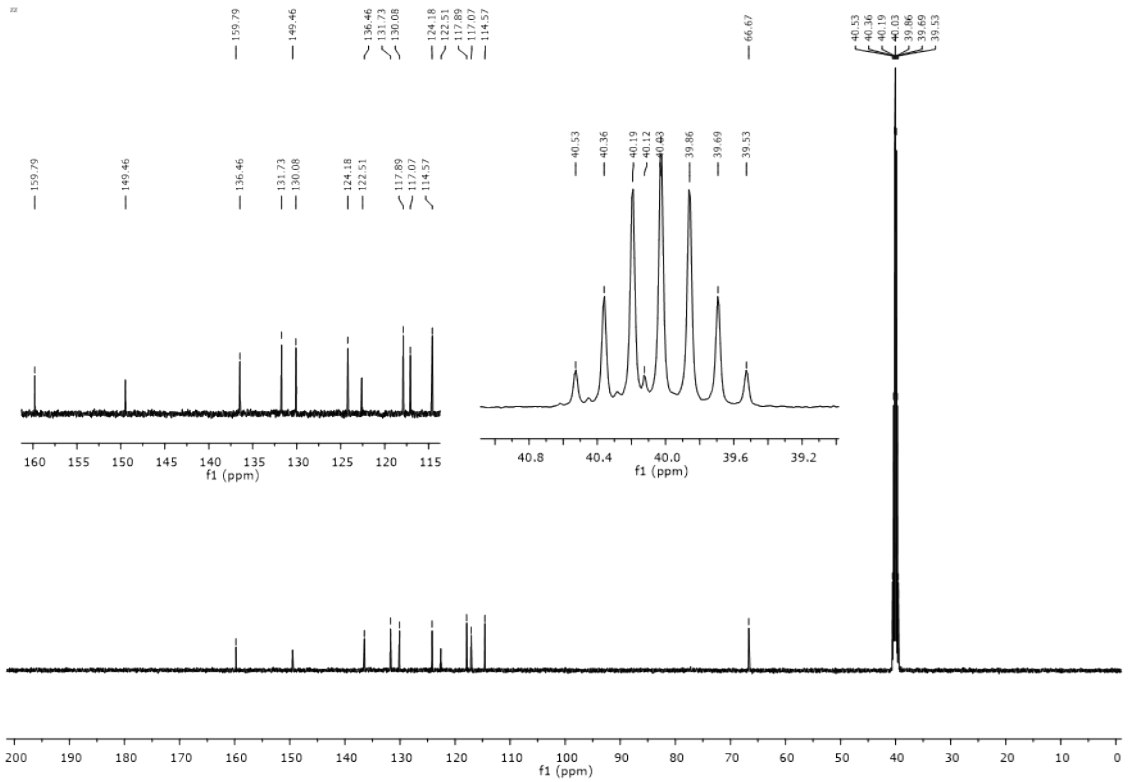
^{1}H NMR of 29 (500 MHz, $CDCl_3$)



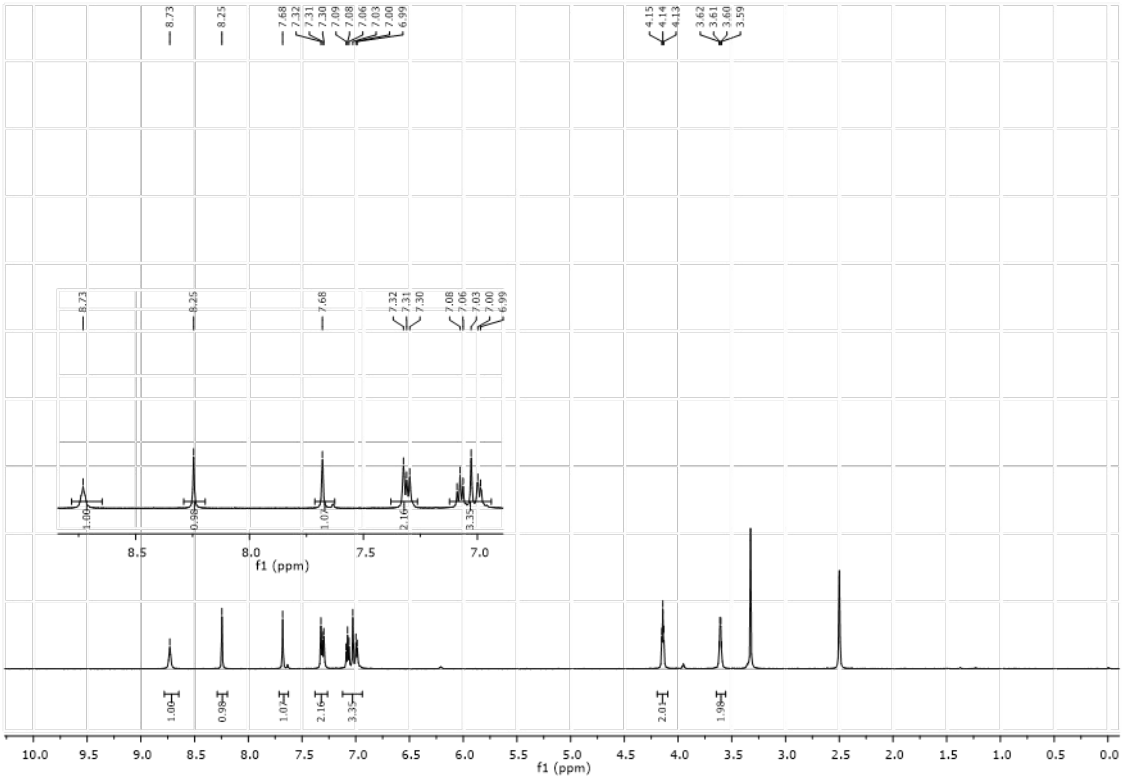
^{13}C NMR of 29 (126 MHz, $CDCl_3$)



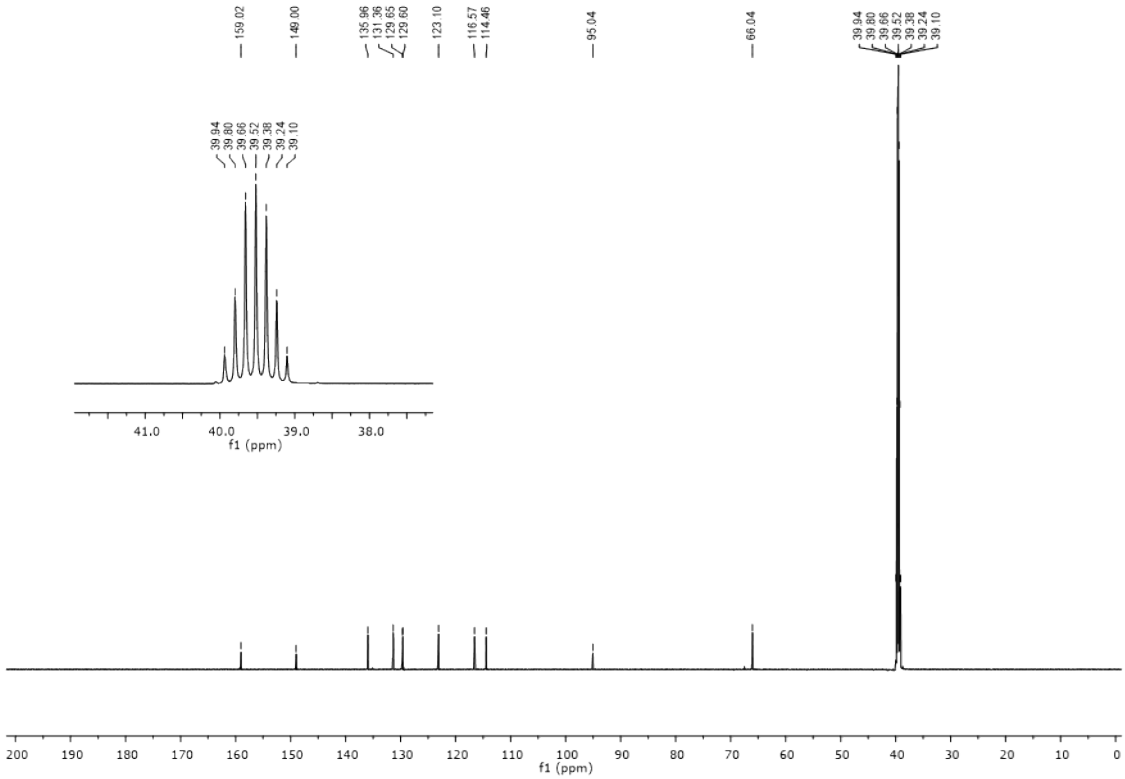
^{1}H NMR of 30 (500 MHz, DMSO)



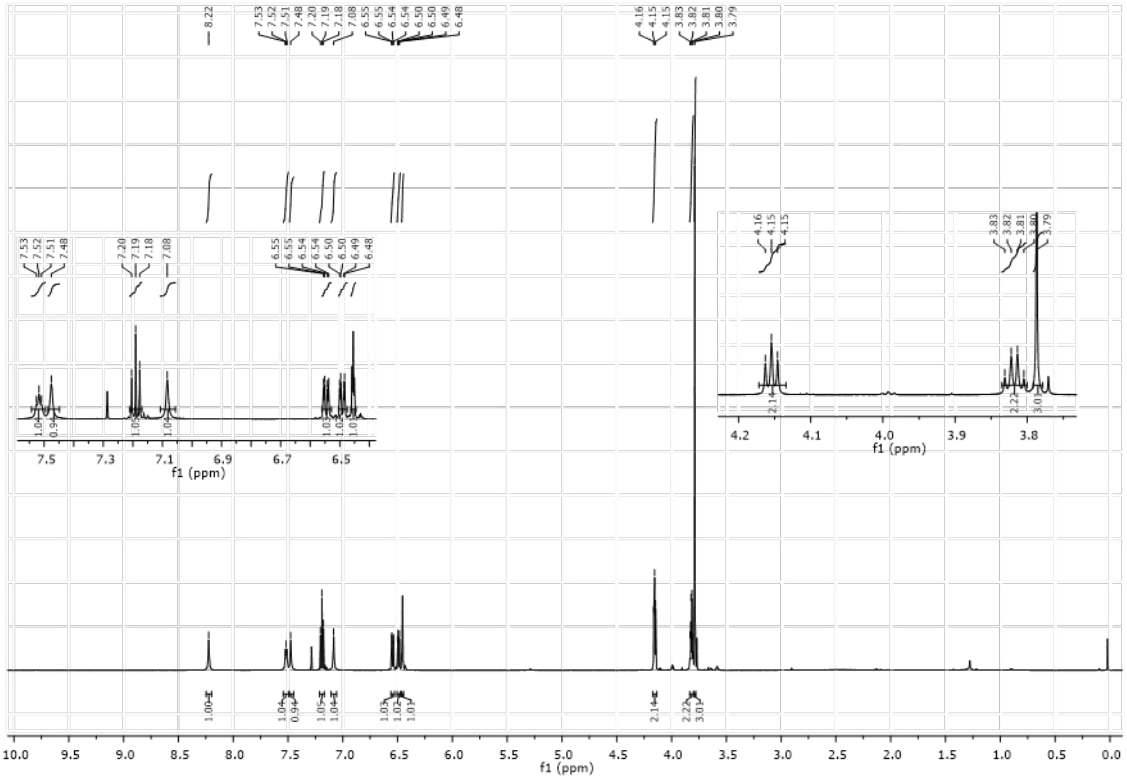
^{13}C NMR of 30 (126 MHz, DMSO)



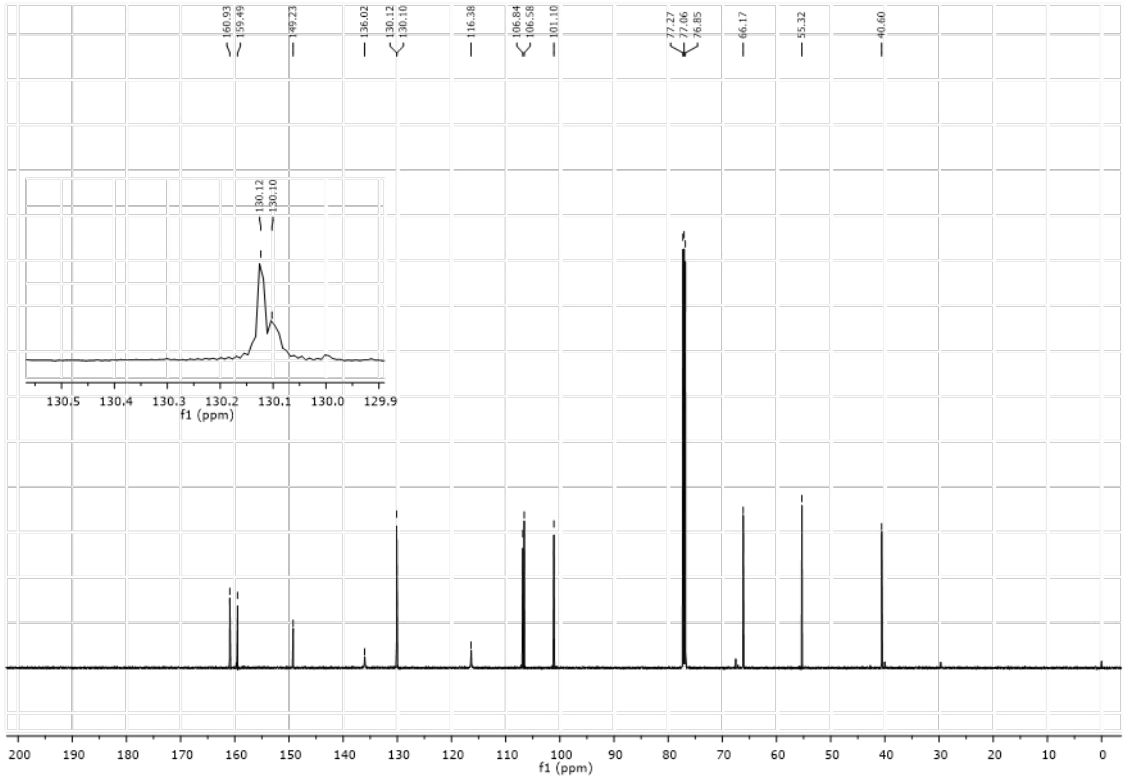
^{1}H NMR of 31 (600 MHz, DMSO-d_6)



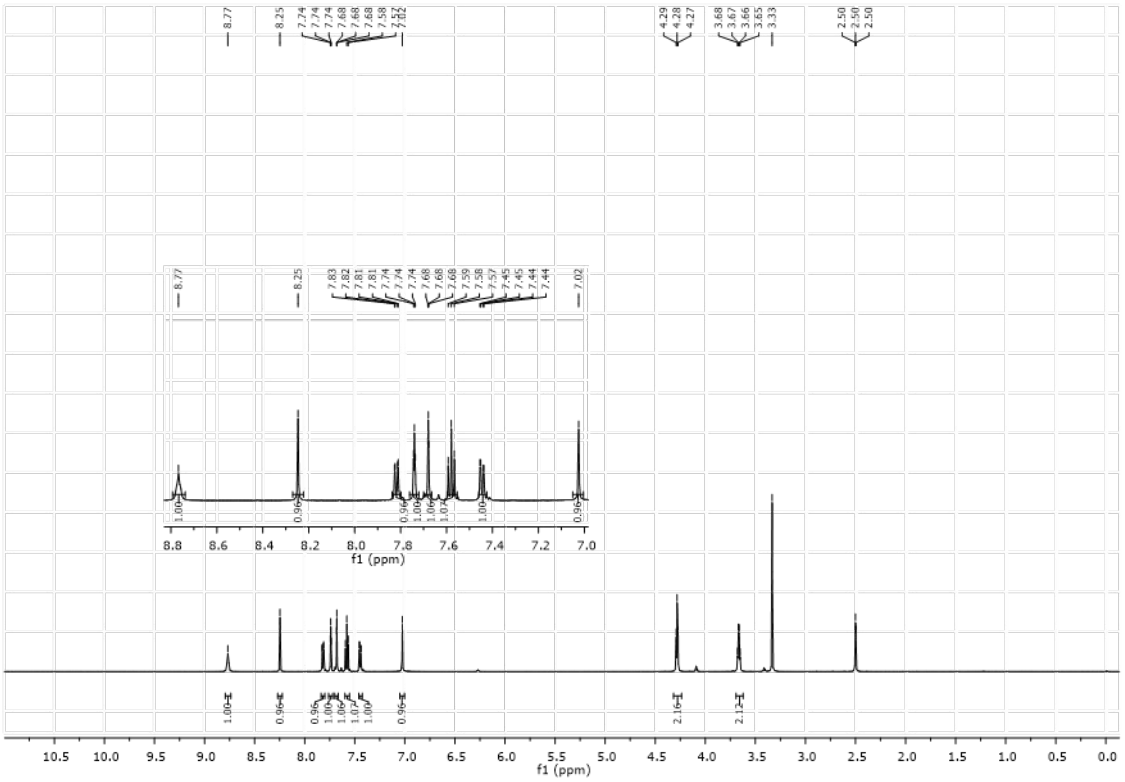
^{13}C NMR of 31 (126 MHz, DMSO)



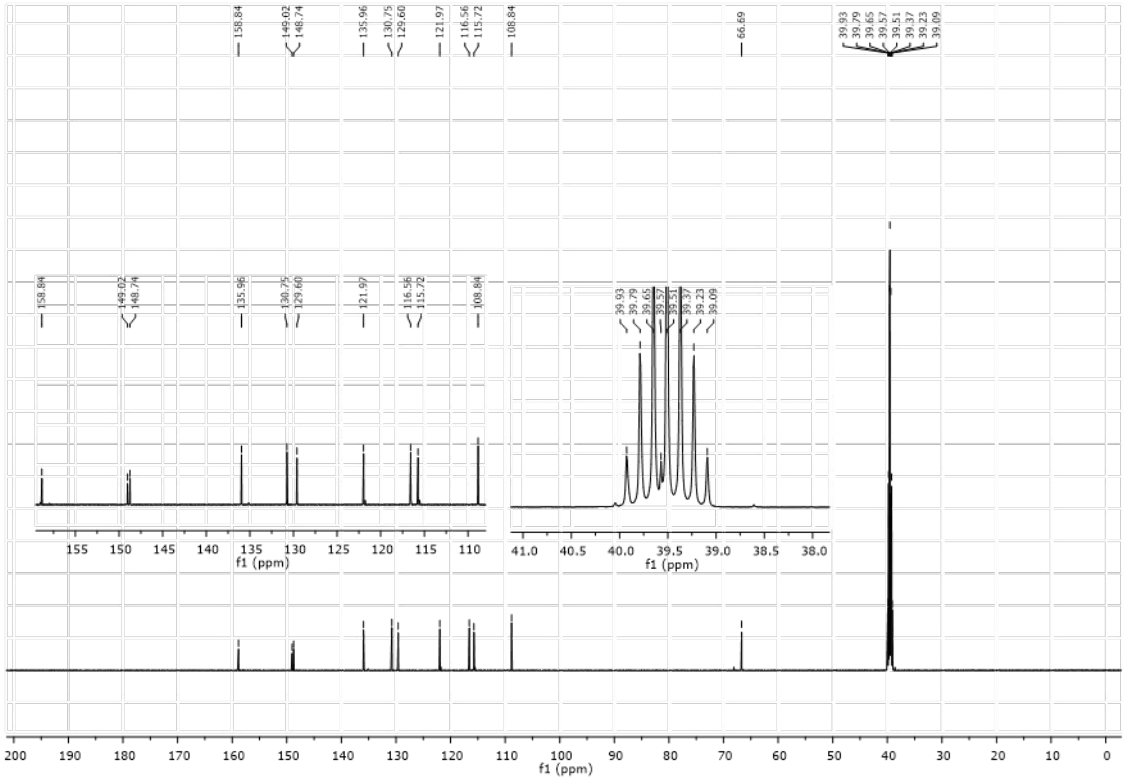
^{1}H NMR of 32 (600 MHz, $CDCl_3$)



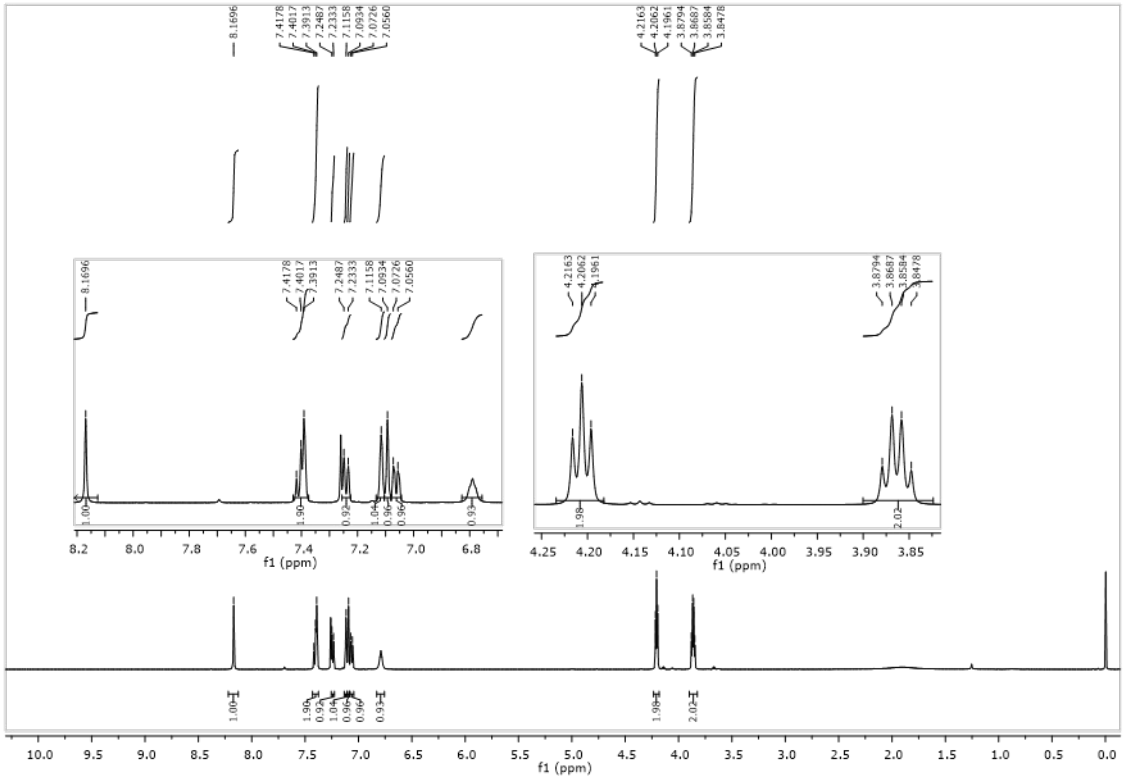
^{13}C NMR of 32 (151 MHz, $CDCl_3$)



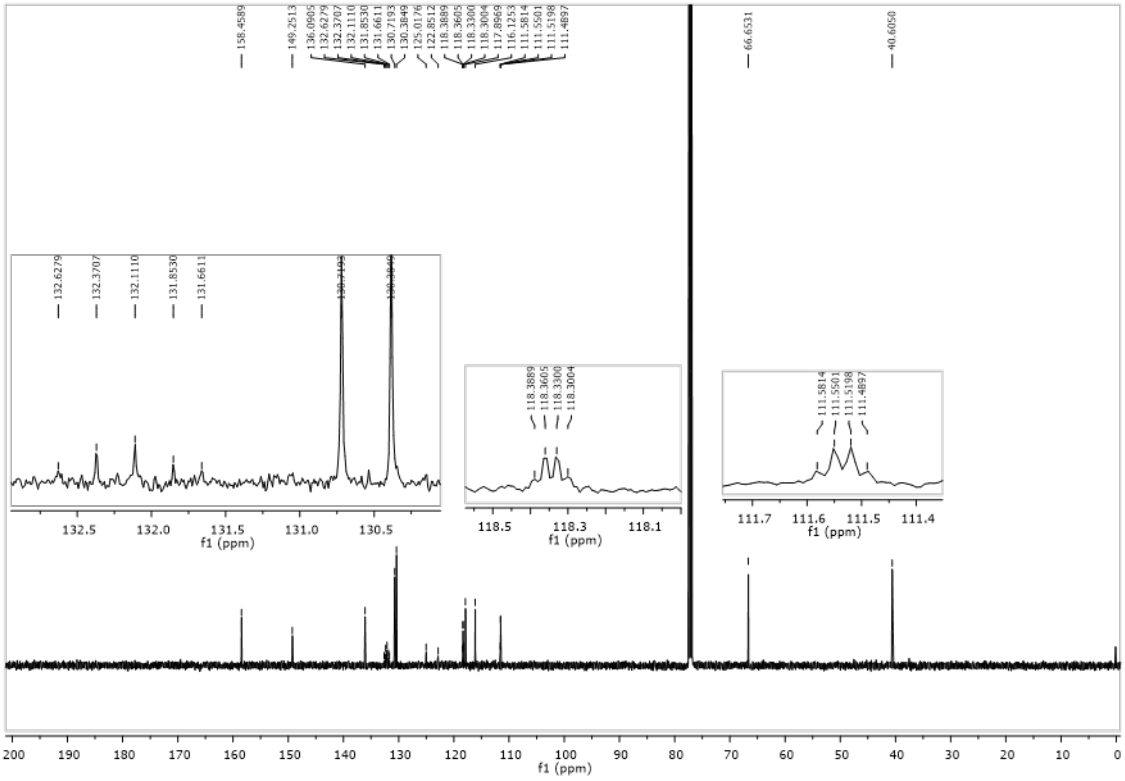
^{1}H NMR of 33 (600 MHz, DMSO)



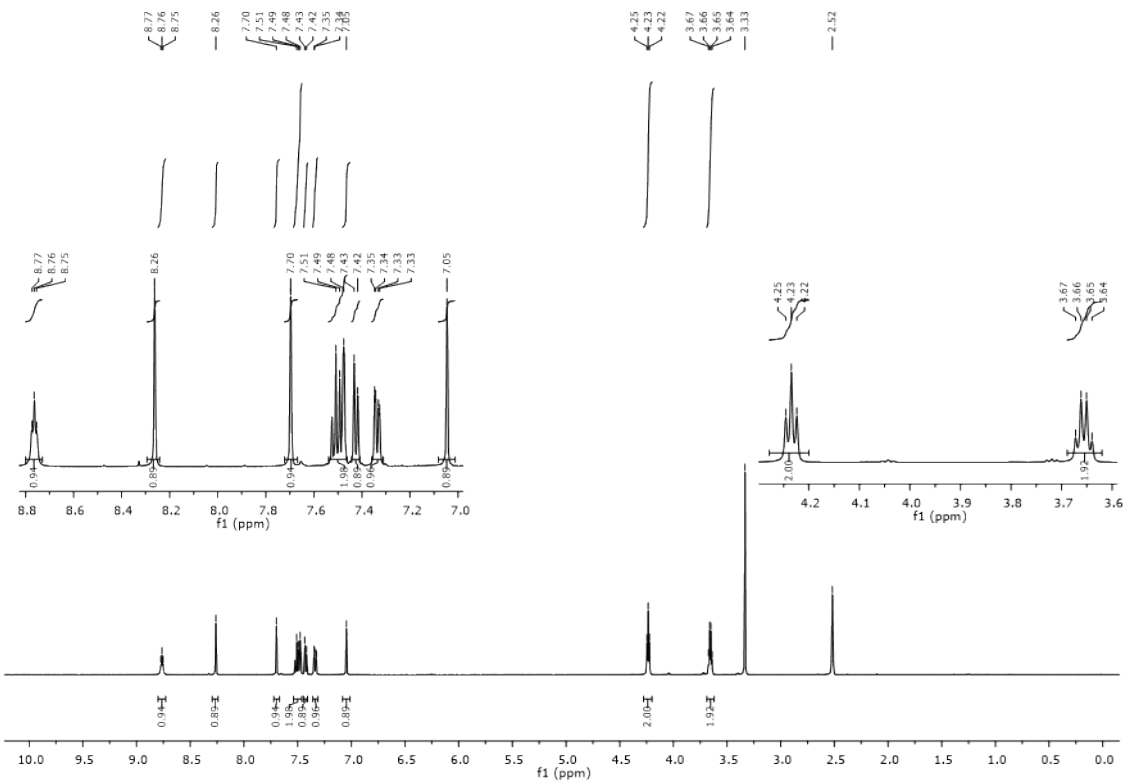
^{13}C NMR of 33 (151 MHz, DMSO)



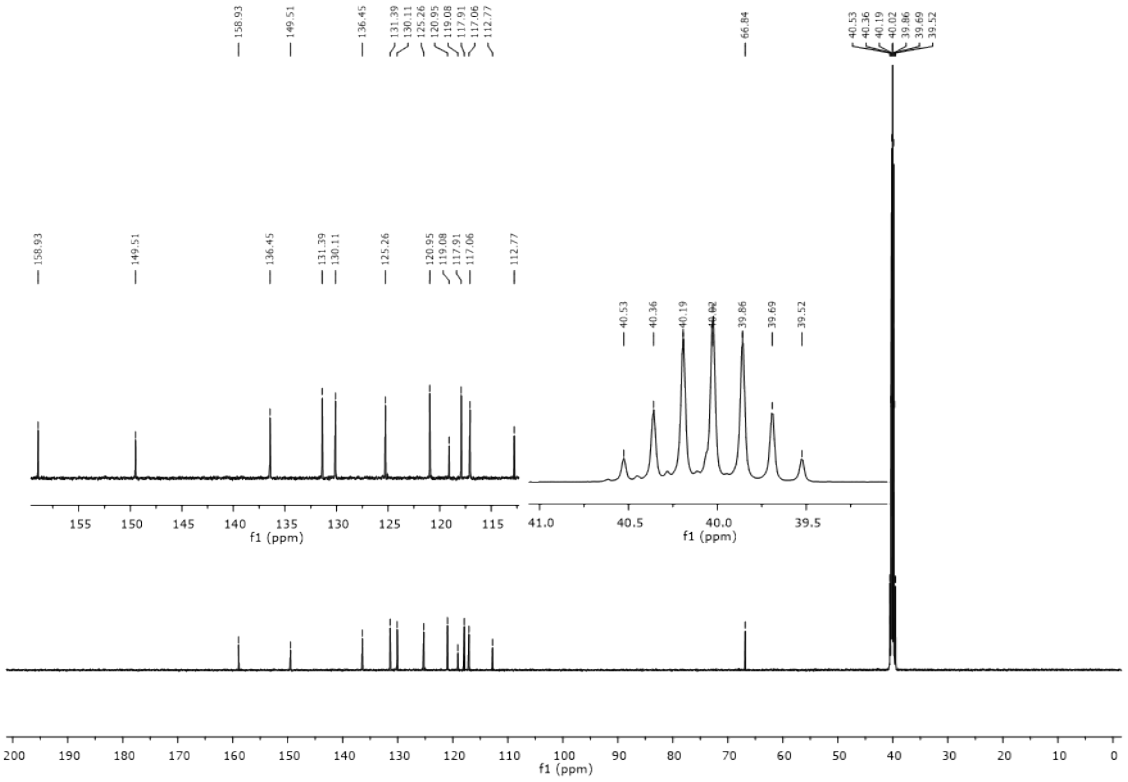
^{1}H NMR of 34 (500 MHz, $CDCl_3$)



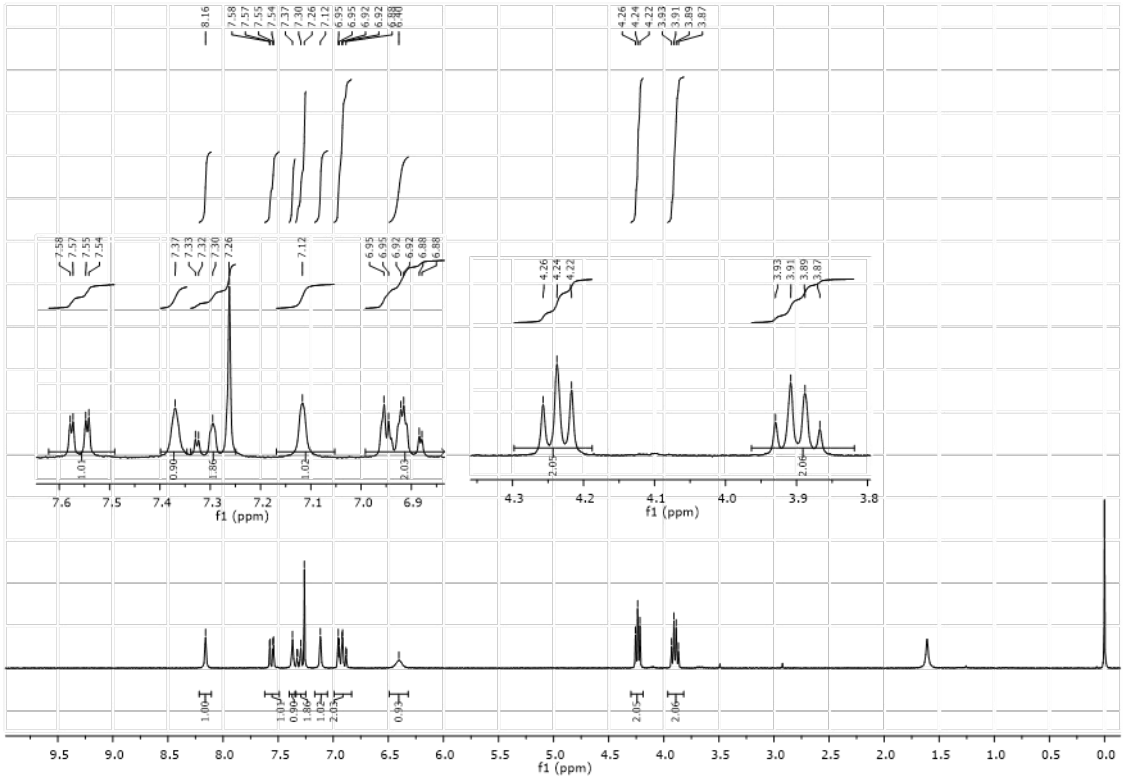
^{13}C NMR of 34 (126 MHz, $CDCl_3$)



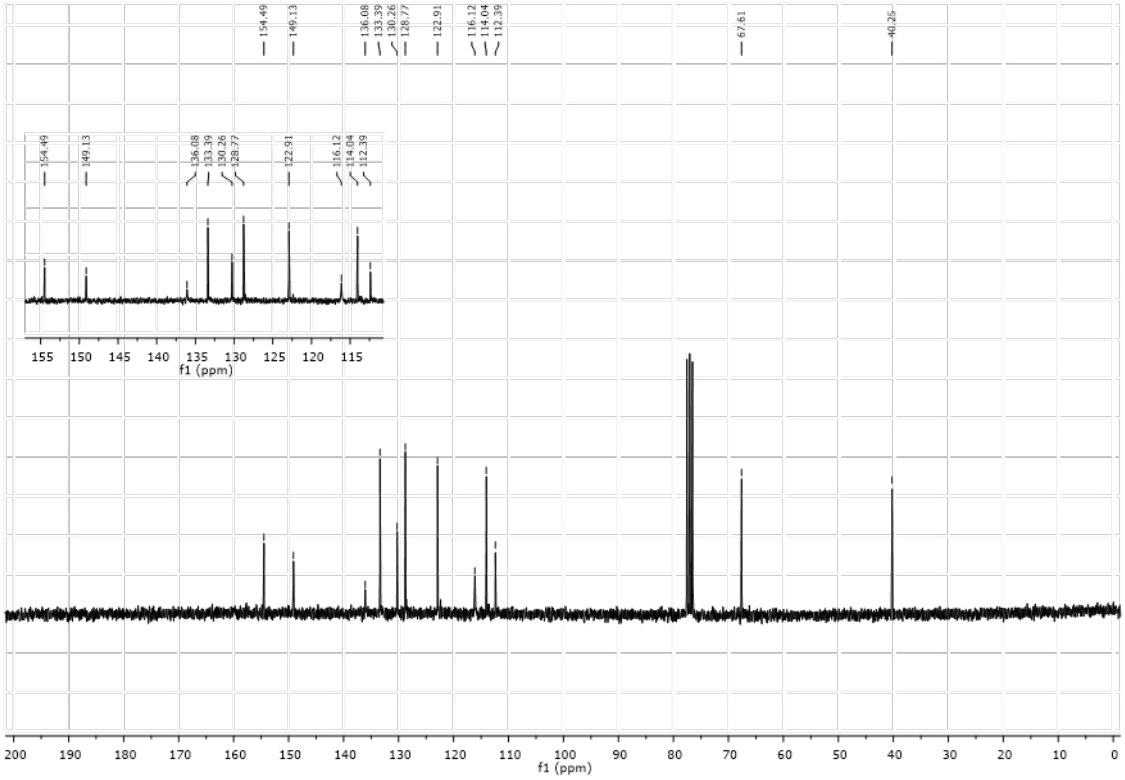
^{1}H NMR of 35 (500 MHz, DMSO)



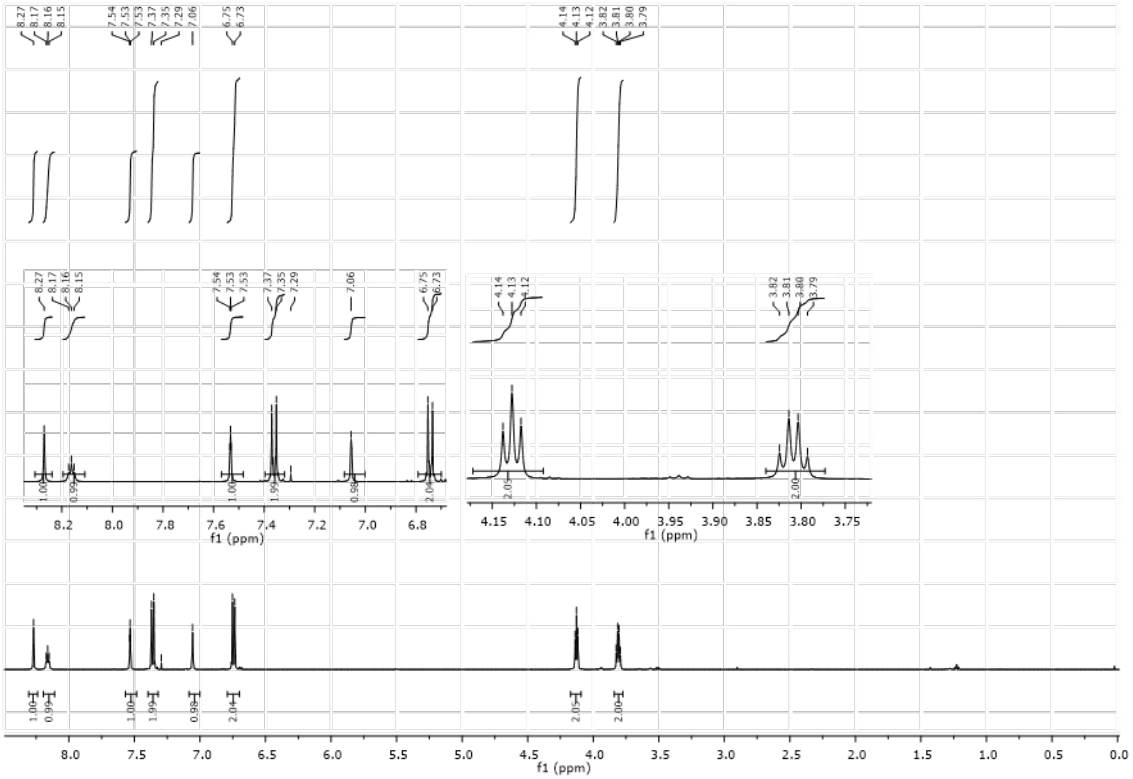
^{13}C NMR of 35 (126 MHz, DMSO)



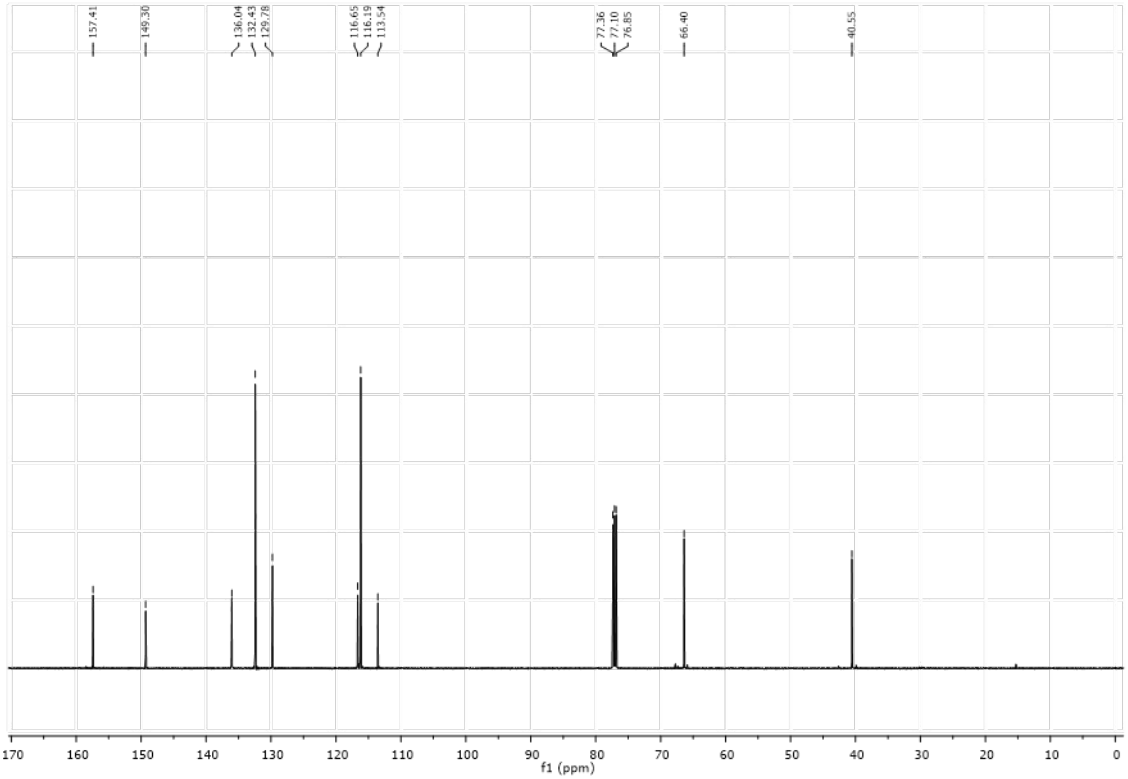
^{1}H NMR of 36 (250 MHz, $CDCl_3$)



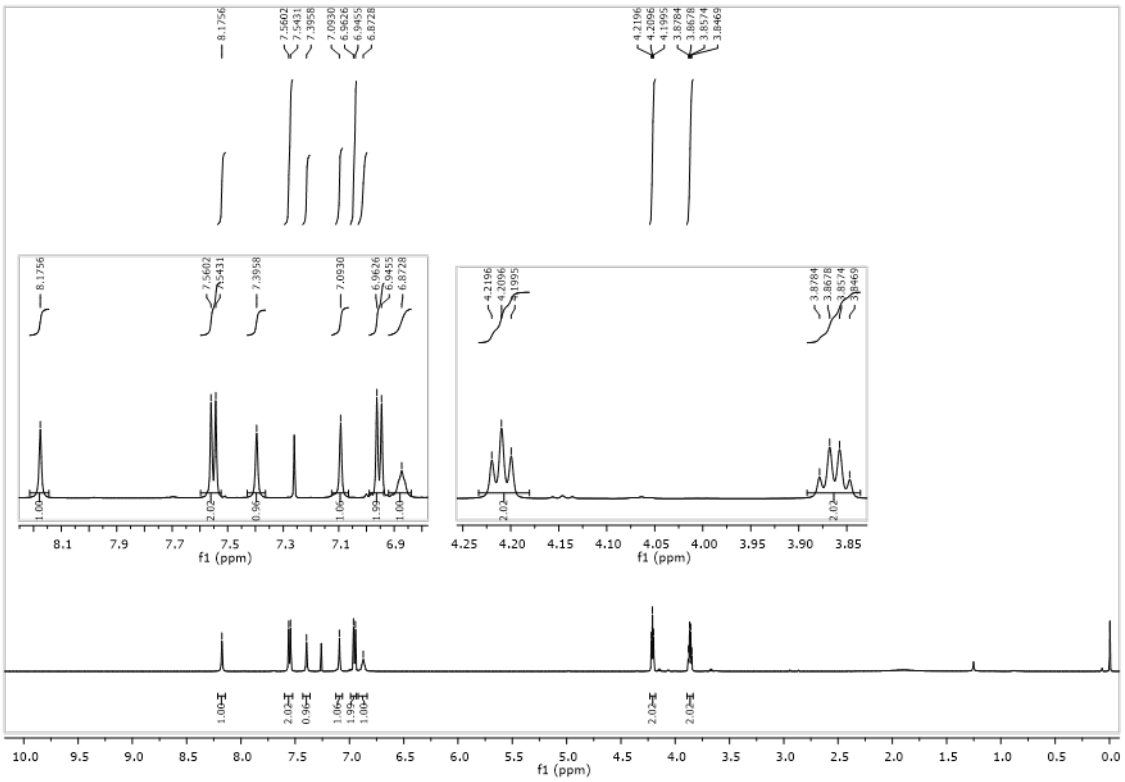
^{13}C NMR of 36 (63 MHz, $CDCl_3$)



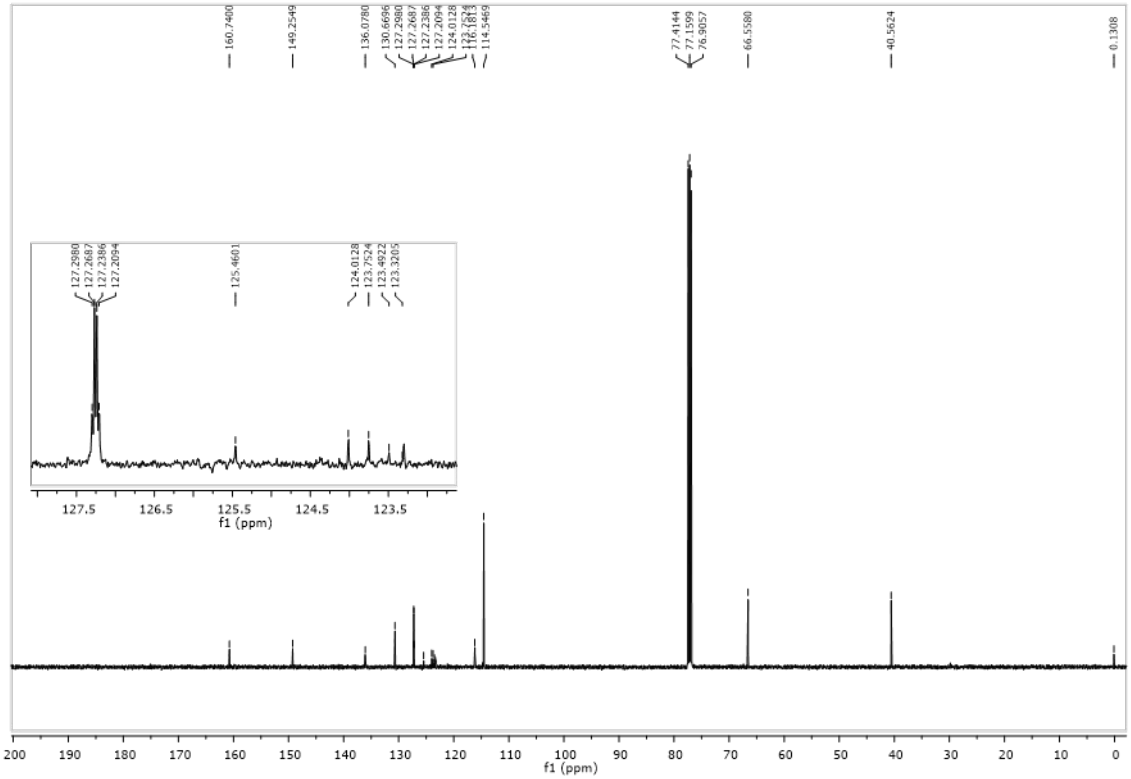
^{1}H NMR of 37 (500 MHz, $CDCl_3$)



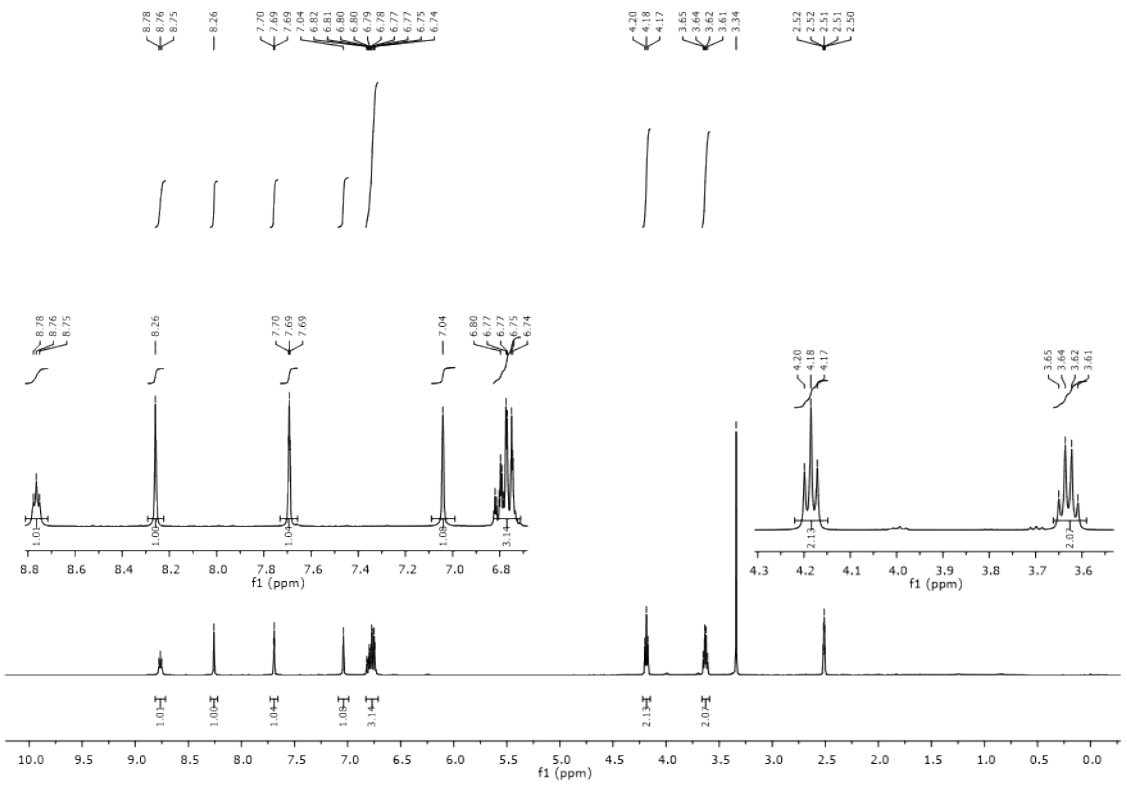
^{13}C NMR of 37 (126 MHz, $CDCl_3$)



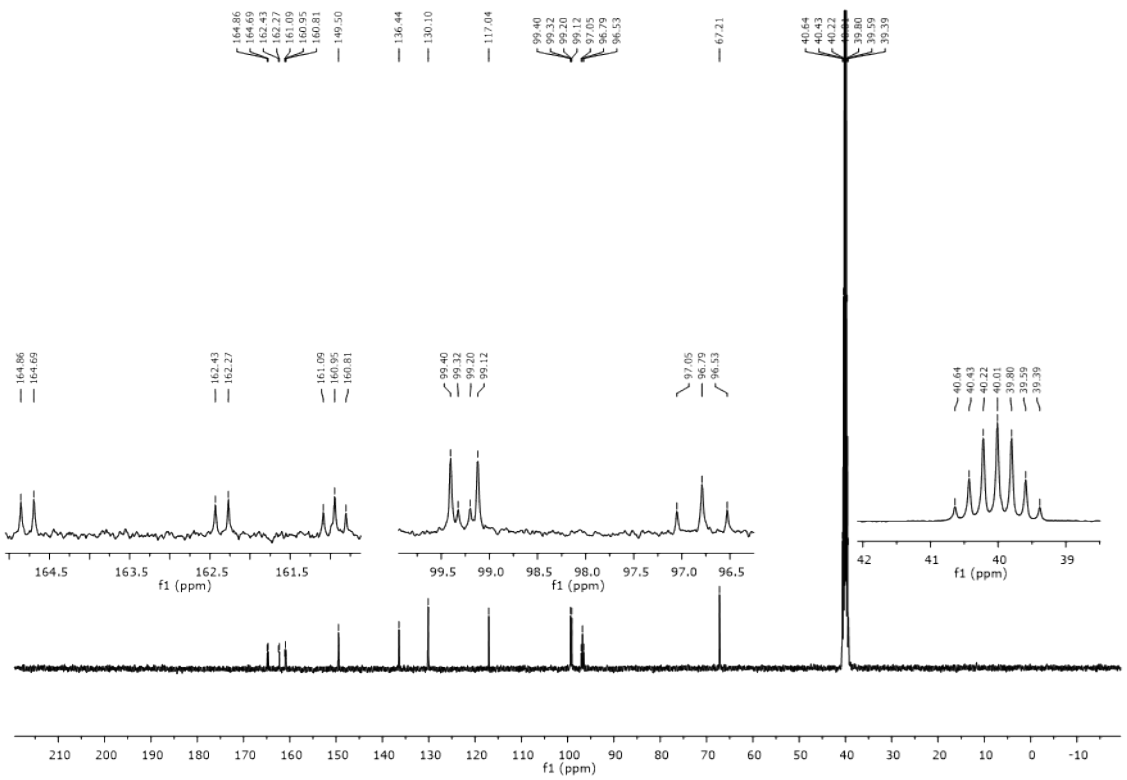
^{1}H NMR of 38 (500 MHz, $CDCl_3$)



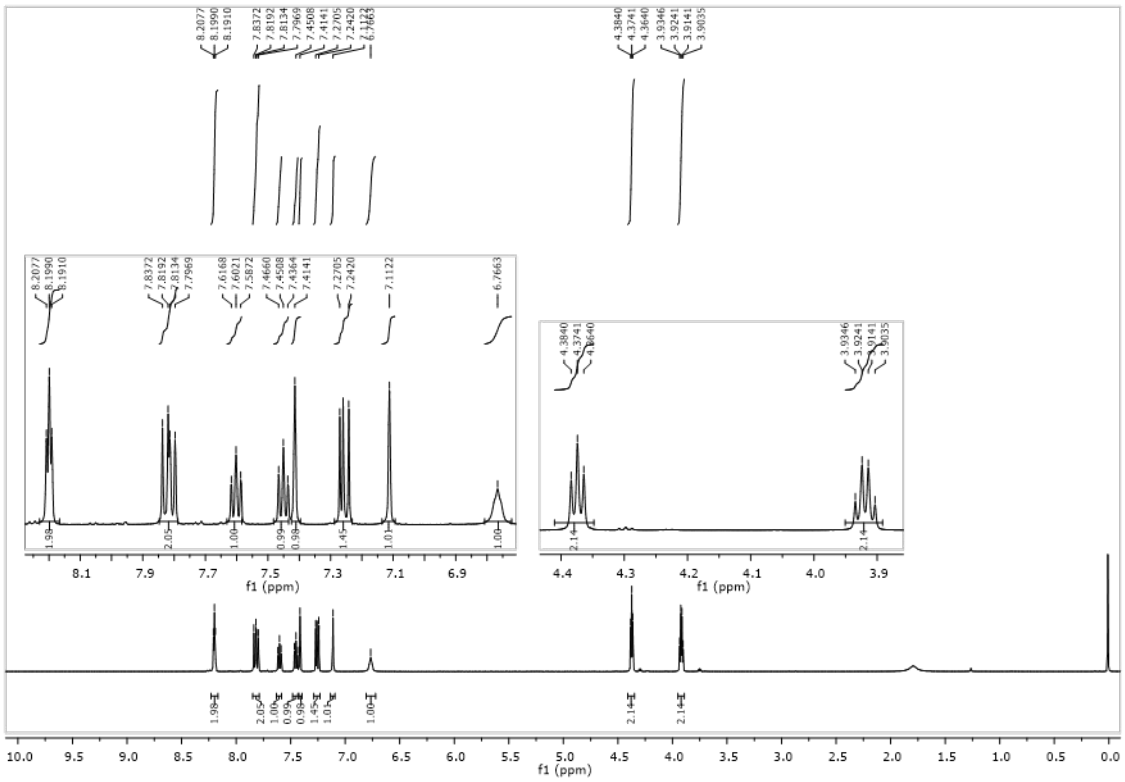
^{13}C NMR of 38 (126 MHz, $CDCl_3$)



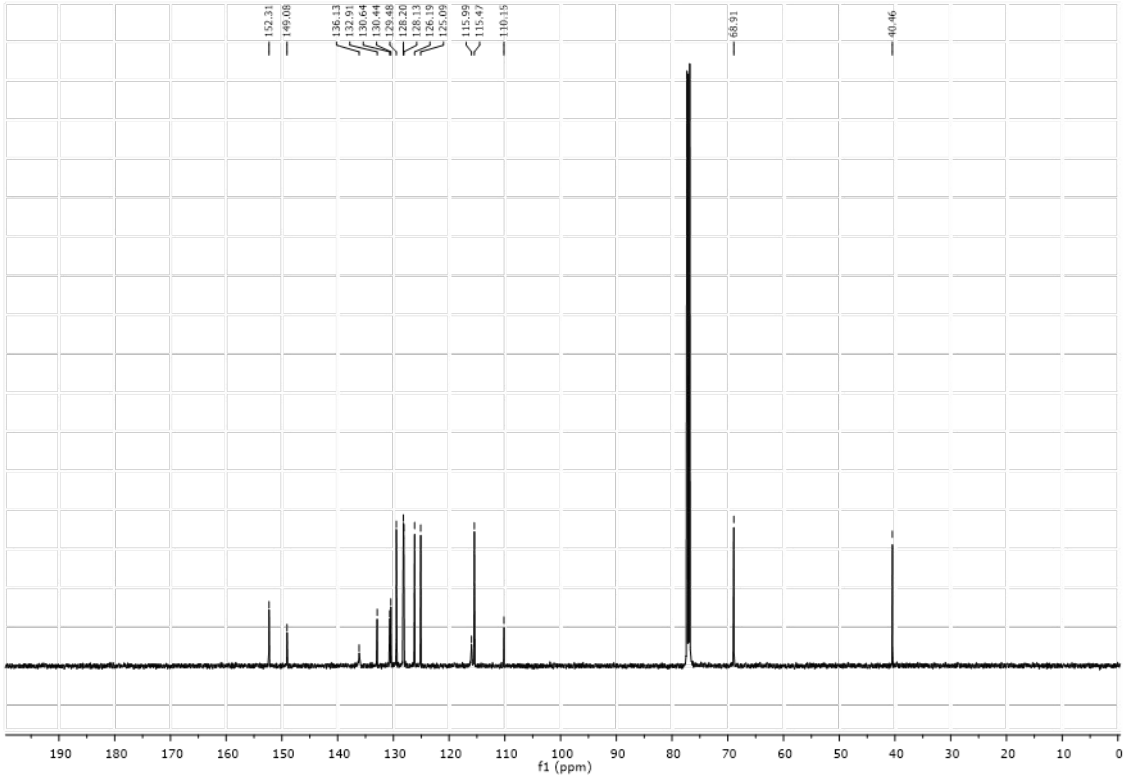
^{1}H NMR of 39 (400 MHz, DMSO)



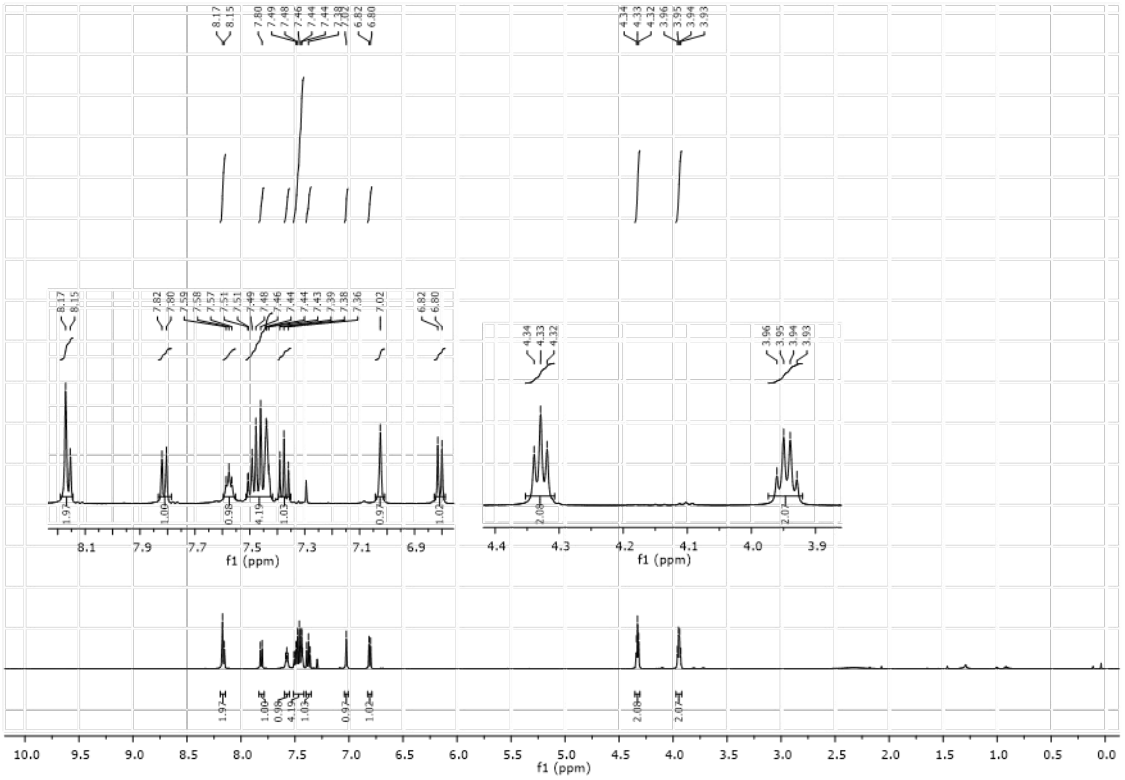
^{13}C NMR of 39 (101 MHz, DMSO)



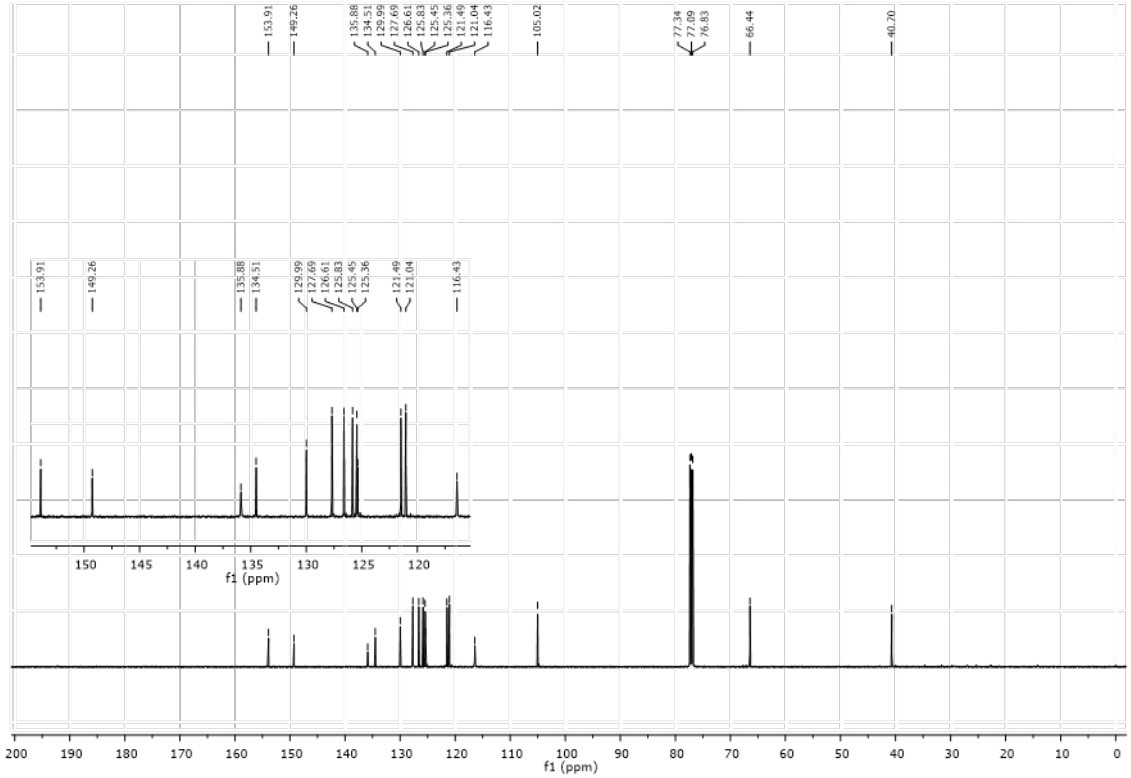
^{1}H NMR of 45 (500 MHz, $CDCl_3$)



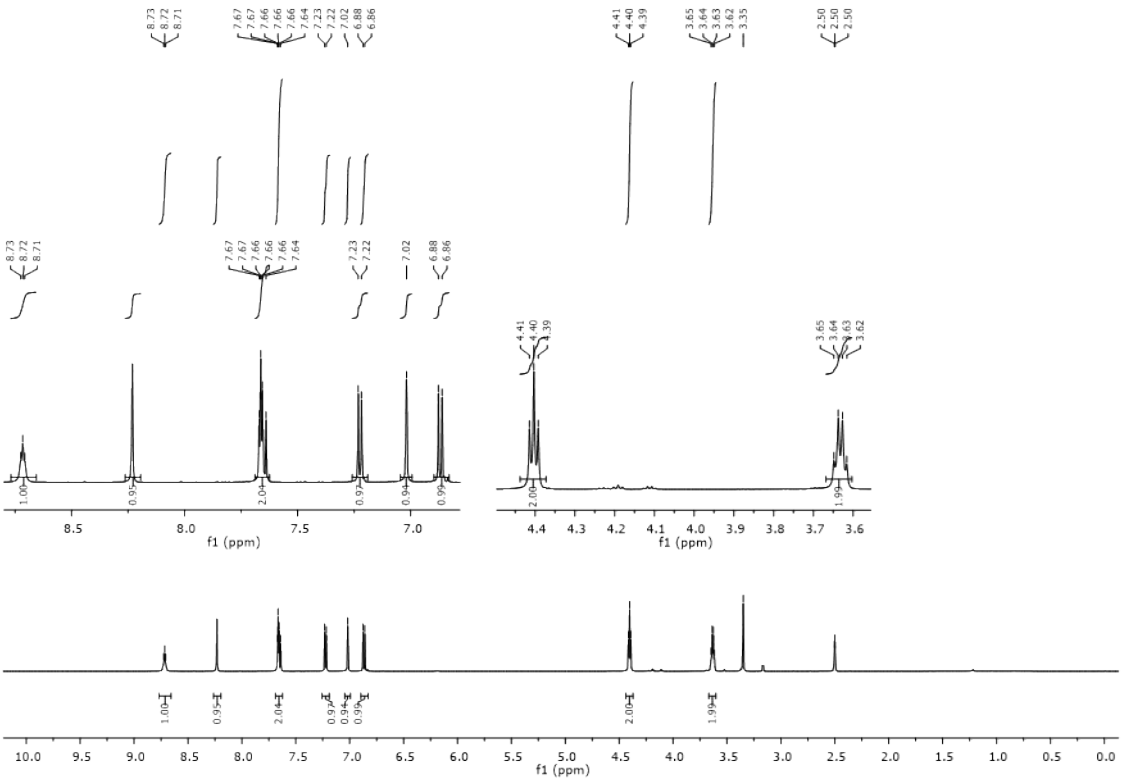
^{13}C NMR of 45 (126 MHz, $CDCl_3$)



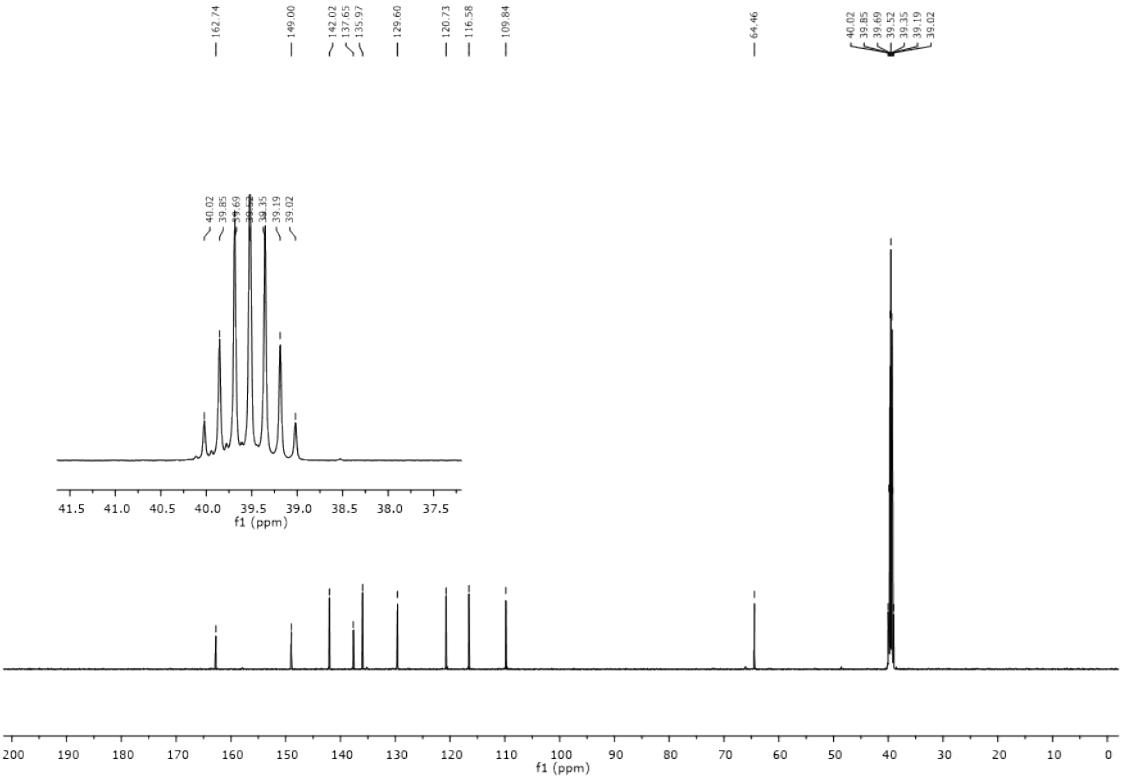
^{1}H NMR of 46 (500 MHz, $CDCl_3$)



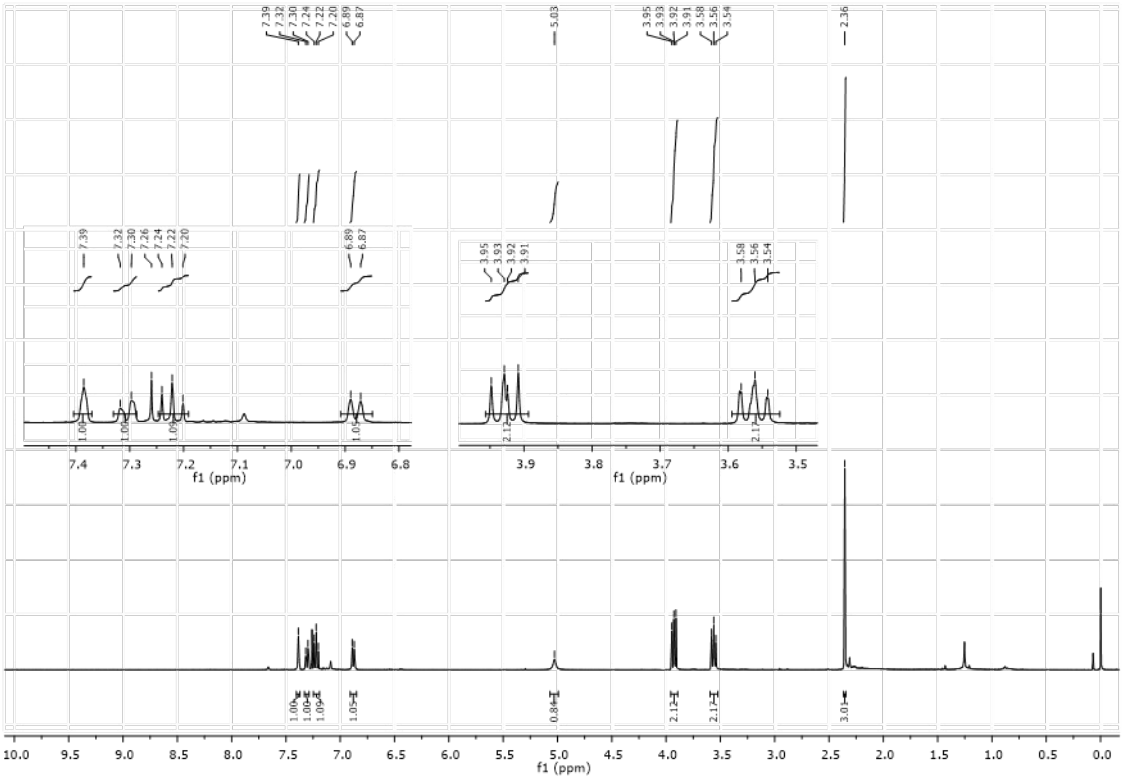
^{13}C NMR of 46 (126 MHz, $CDCl_3$)



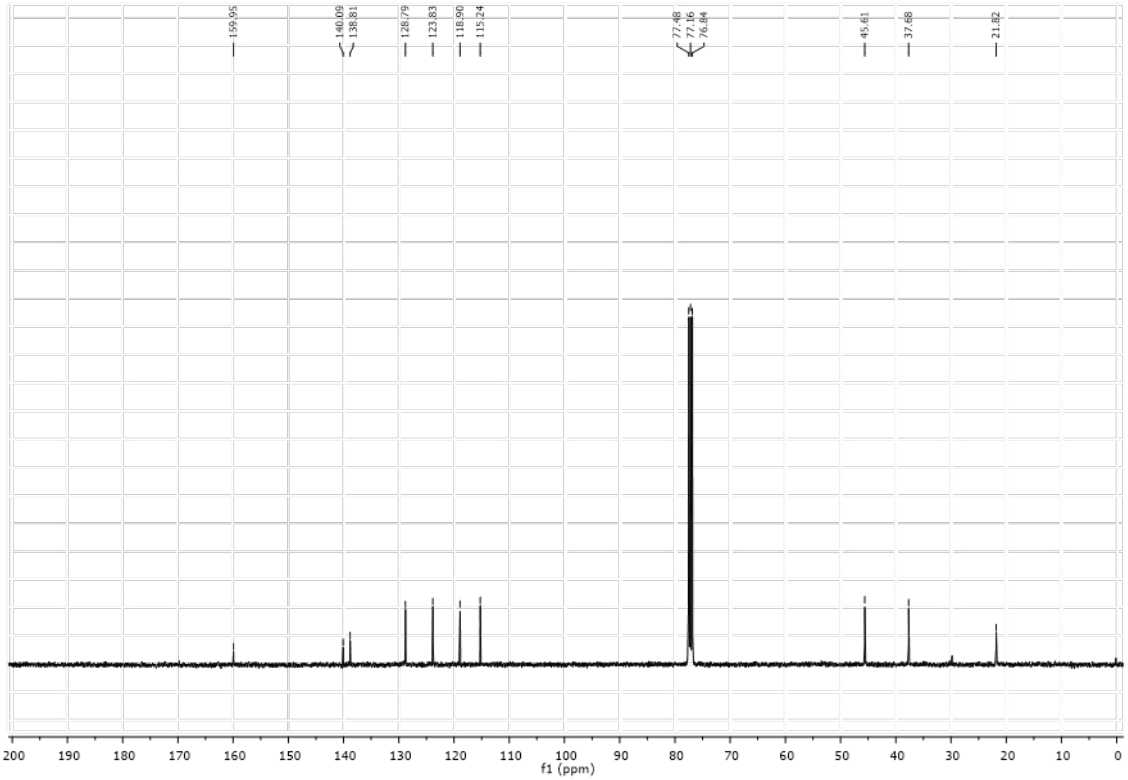
^{1}H NMR of 50 (500 MHz, DMSO)



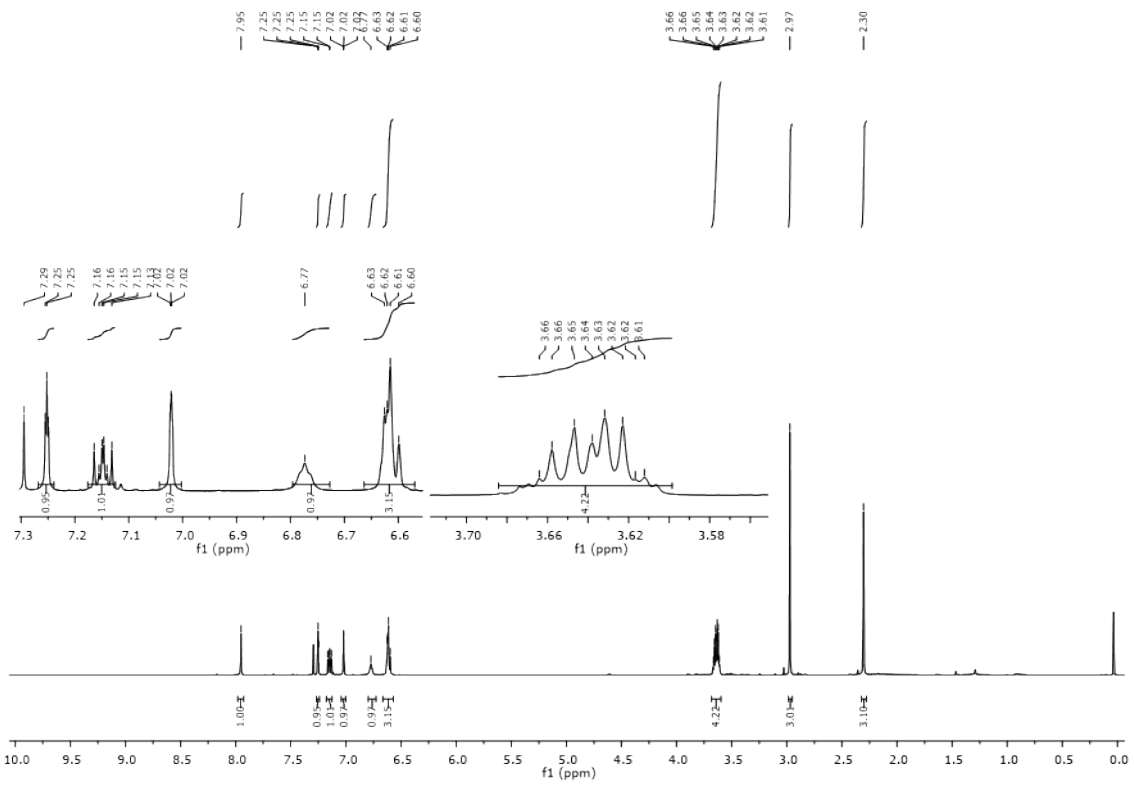
^{13}C NMR of 50 (126 MHz, DMSO)



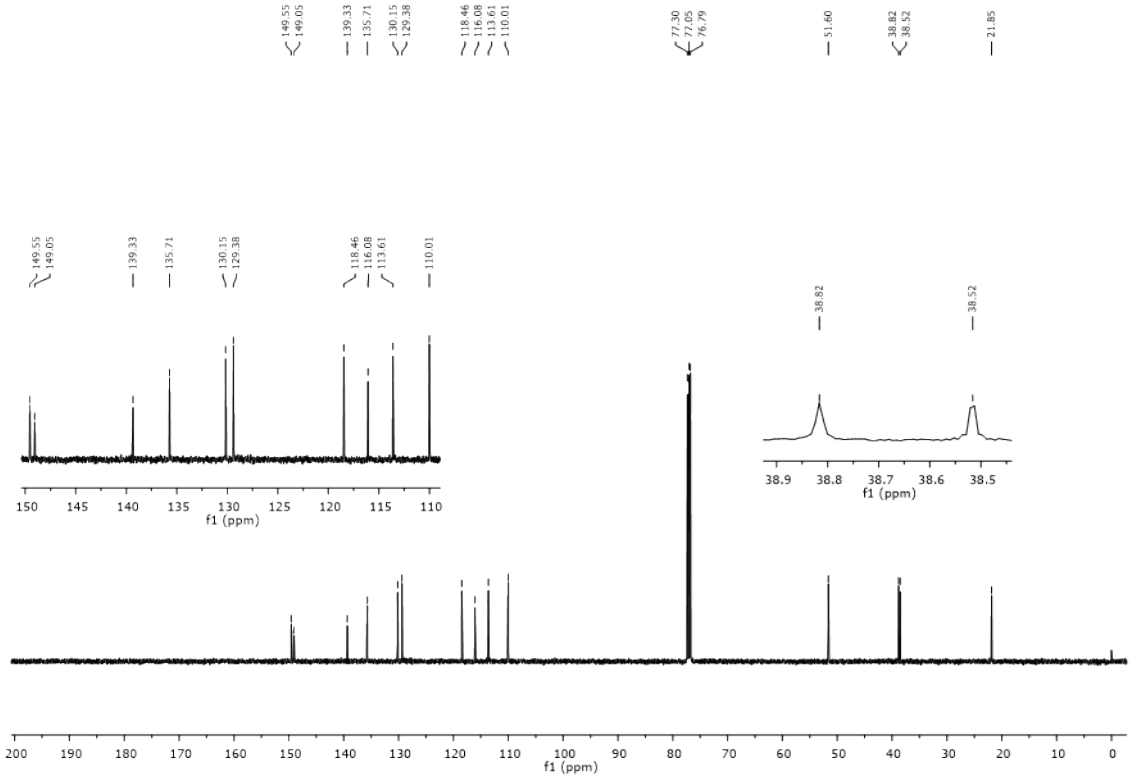
^{1}H NMR of 53 (400 MHz, $CDCl_3$)



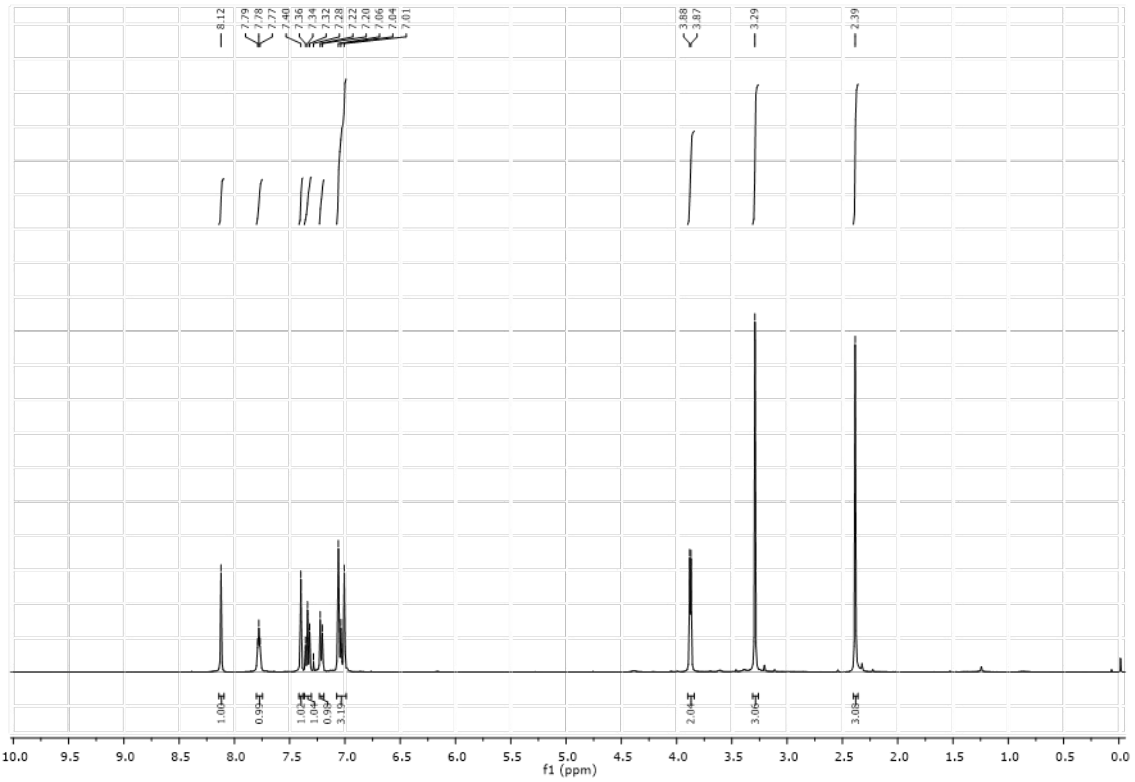
^{13}C NMR of 53 (101 MHz, $CDCl_3$)



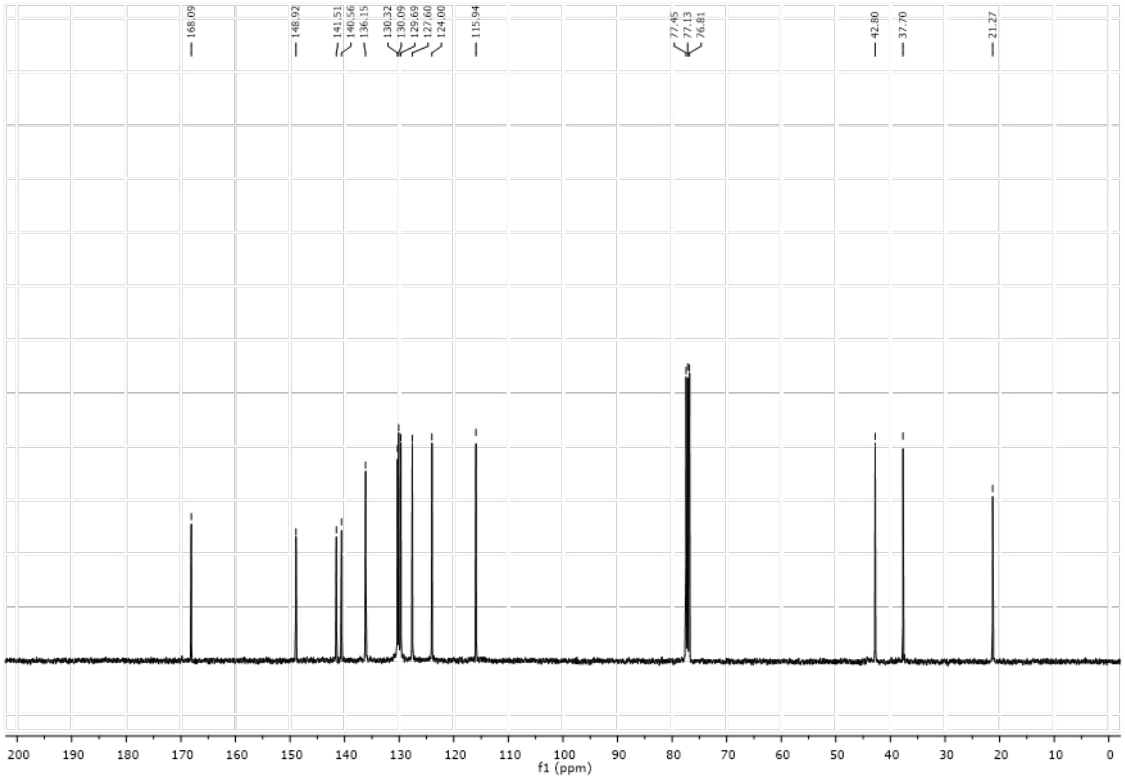
^{1}H NMR of 56 (500 MHz, $CDCl_3$)



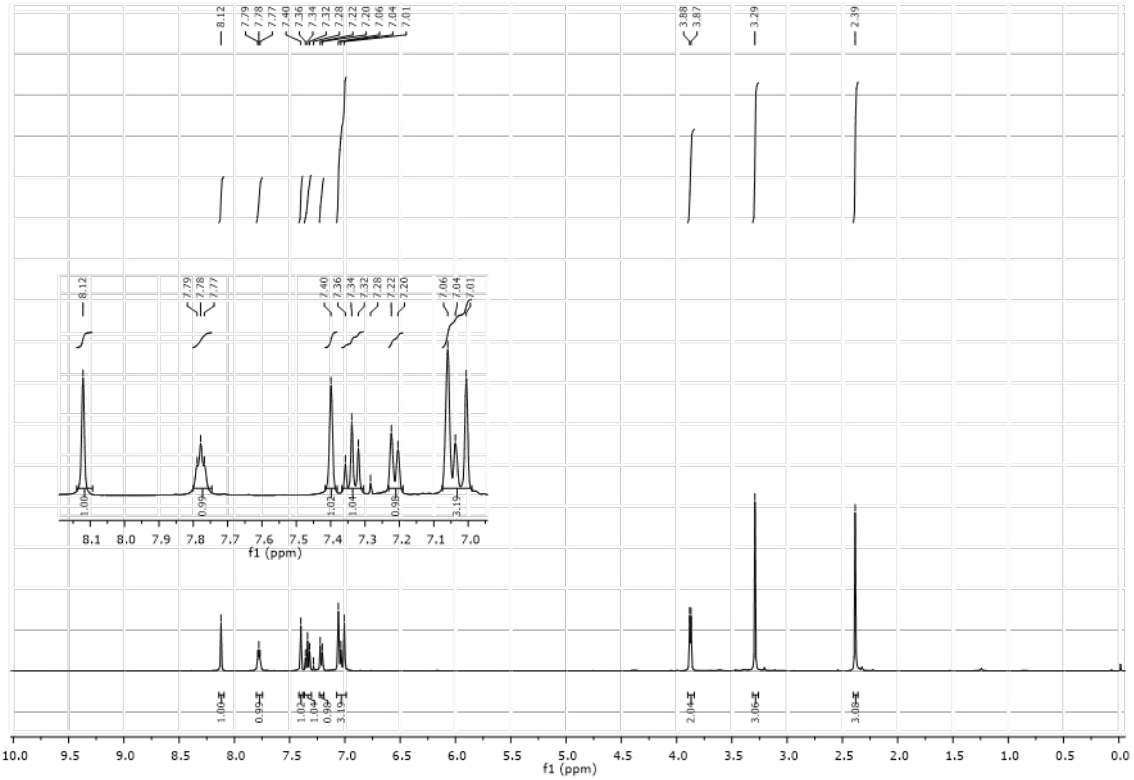
^{13}C NMR of 56 (126 MHz, CDCl3)



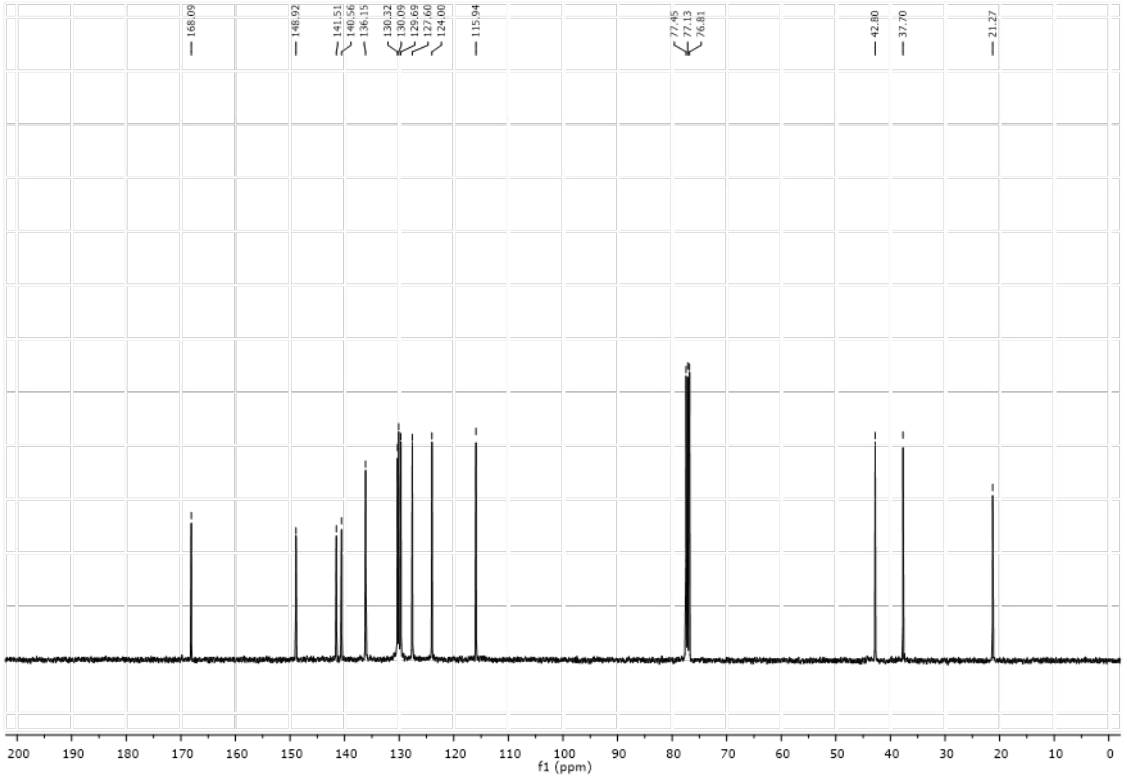
^{1}H NMR of 60 (400 MHz, $CDCl_3$)



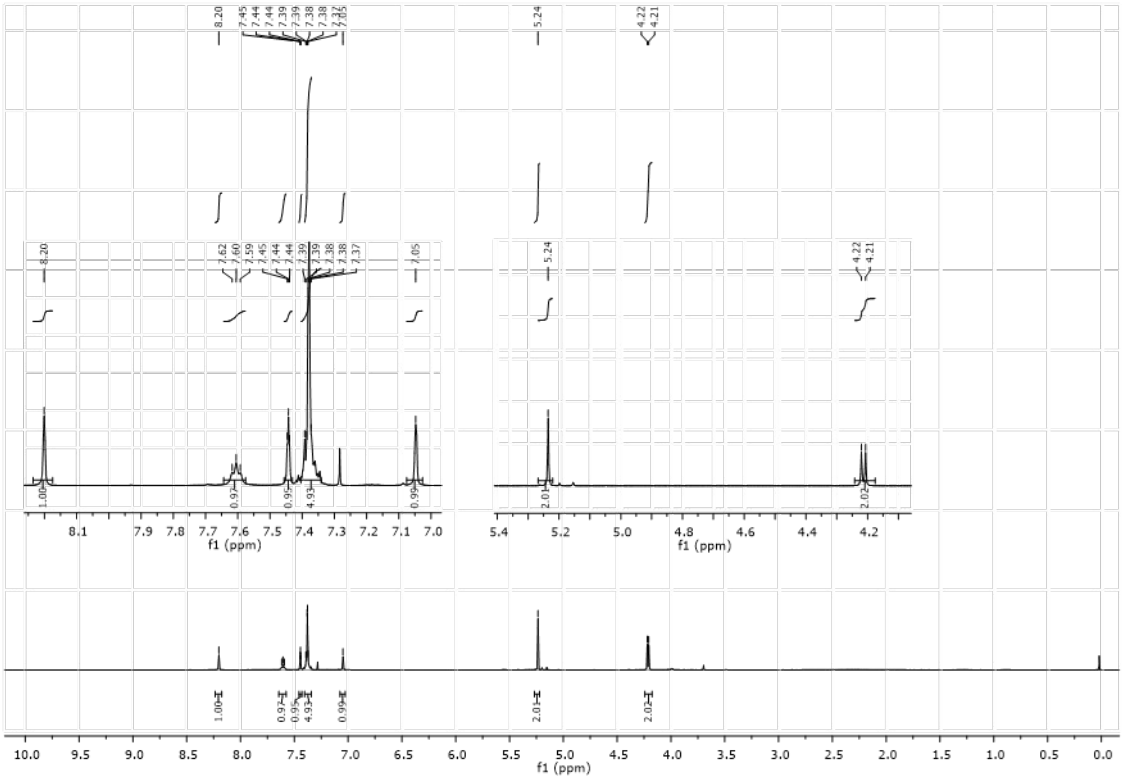
^{13}C NMR of 60 (101 MHz, CDCl3)



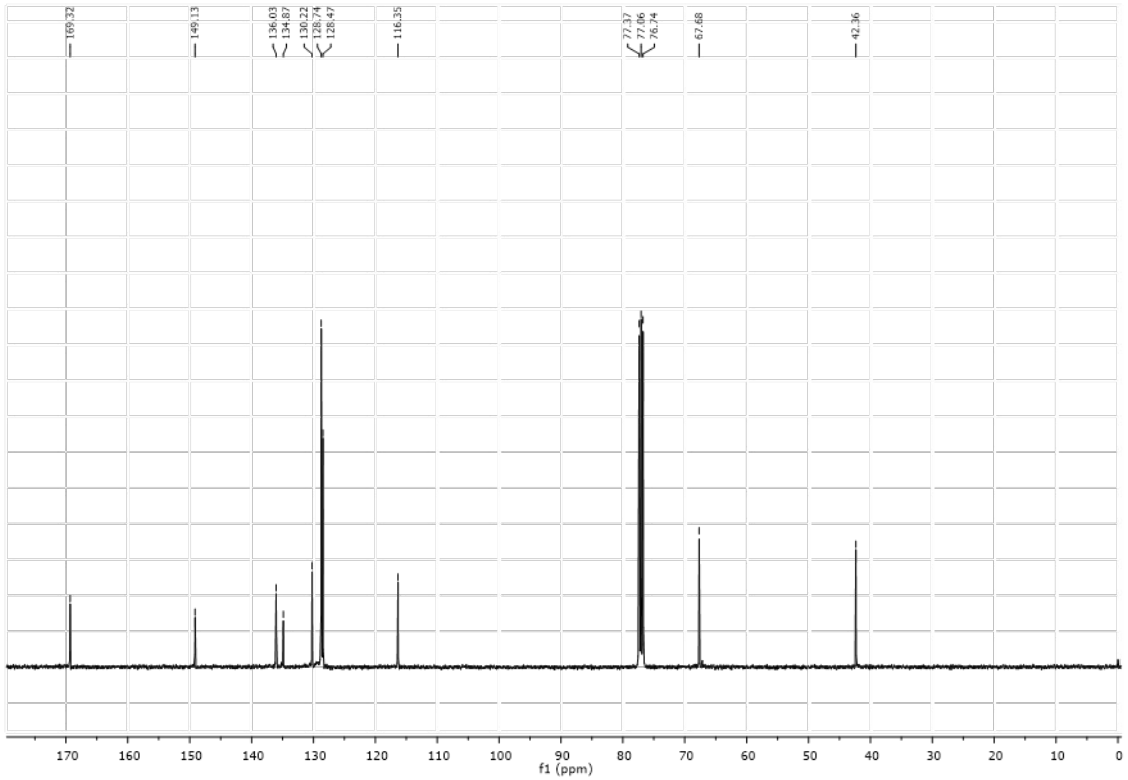
^{1}H NMR of 61 (400 MHz, $CDCl_3$)



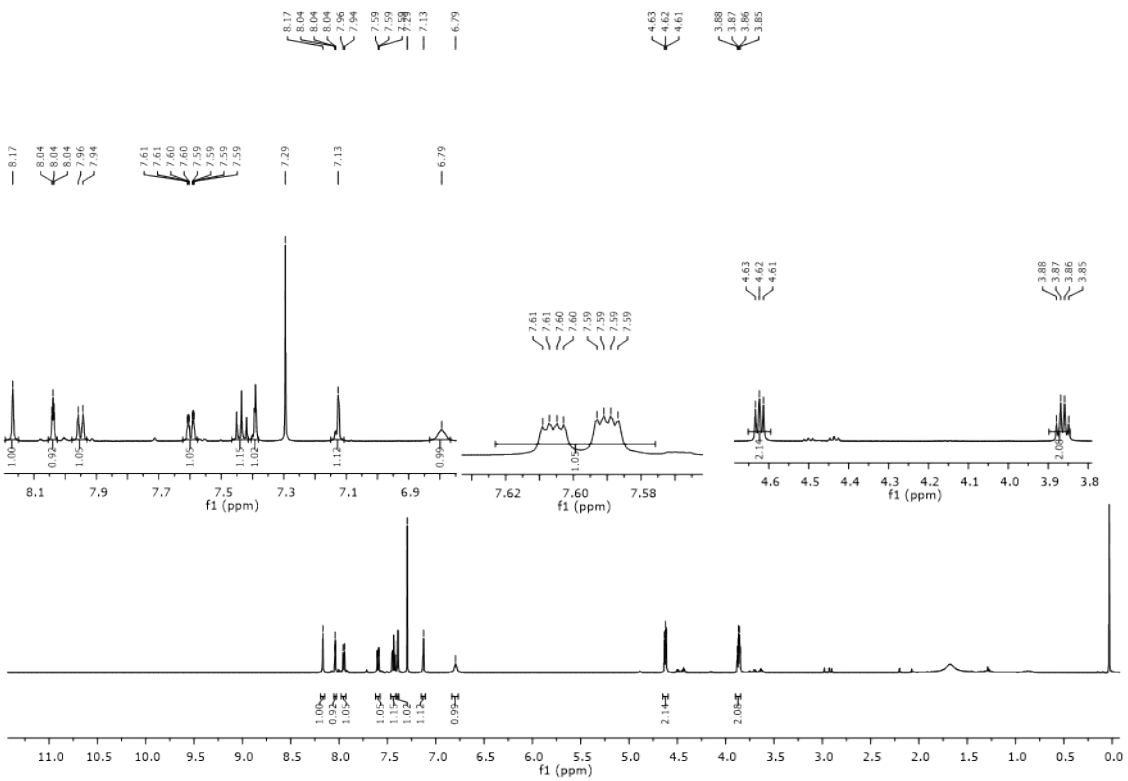
^{13}C NMR of 61 (101 MHz, CDCl3)



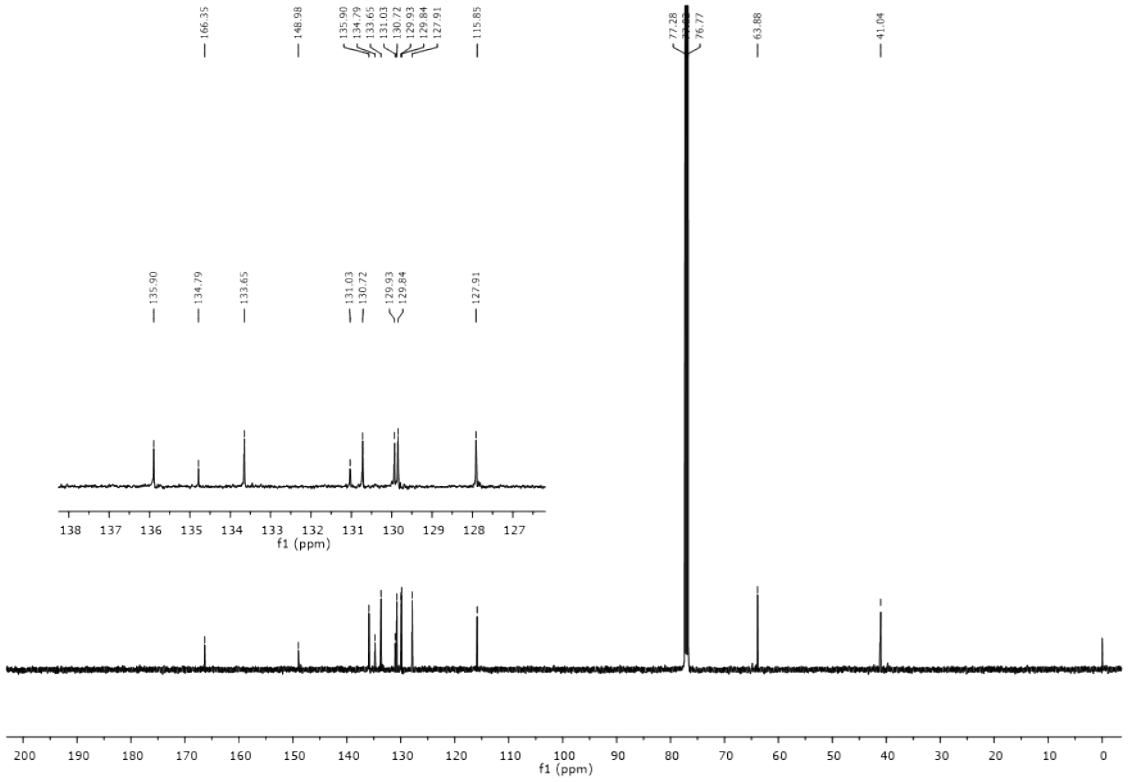
^{1}H NMR of 63 (400 MHz, $CDCl_3$)



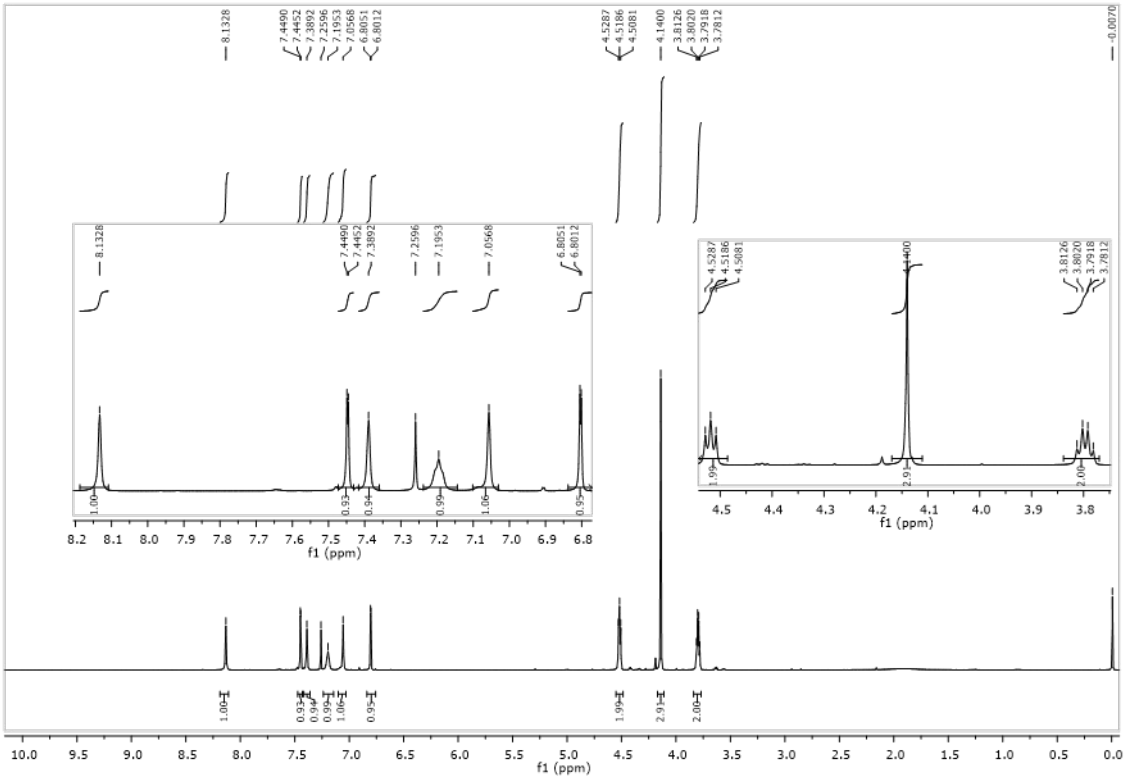
^{13}C NMR of 63 (101 MHz, $CDCl_3$)



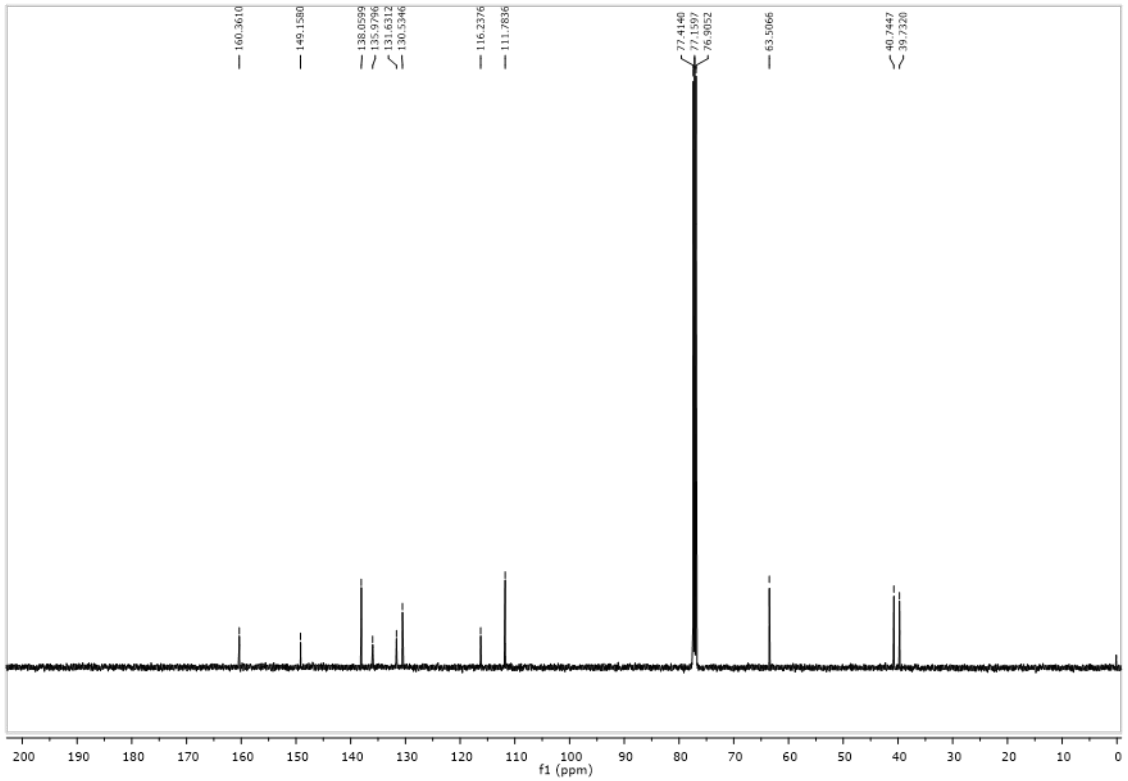
^{1}H NMR of 68 (500 MHz, $CDCl_3$)



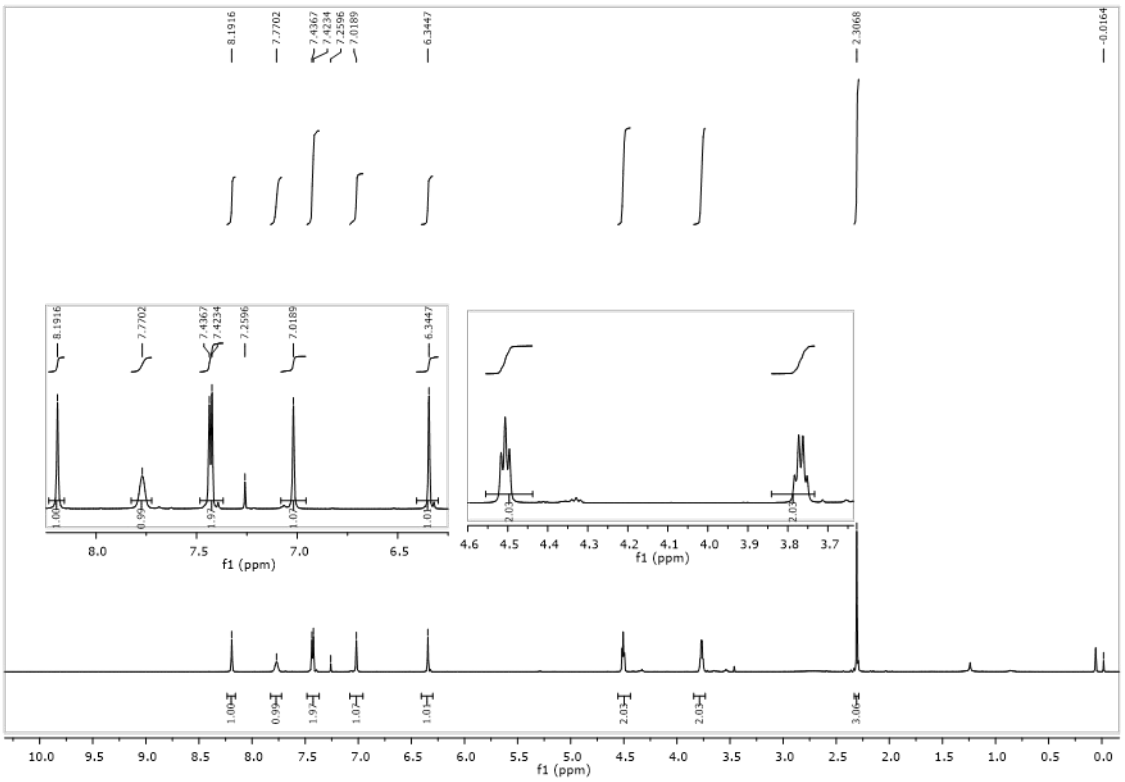
^{13}C NMR of 68 (126 MHz, $CDCl_3$)



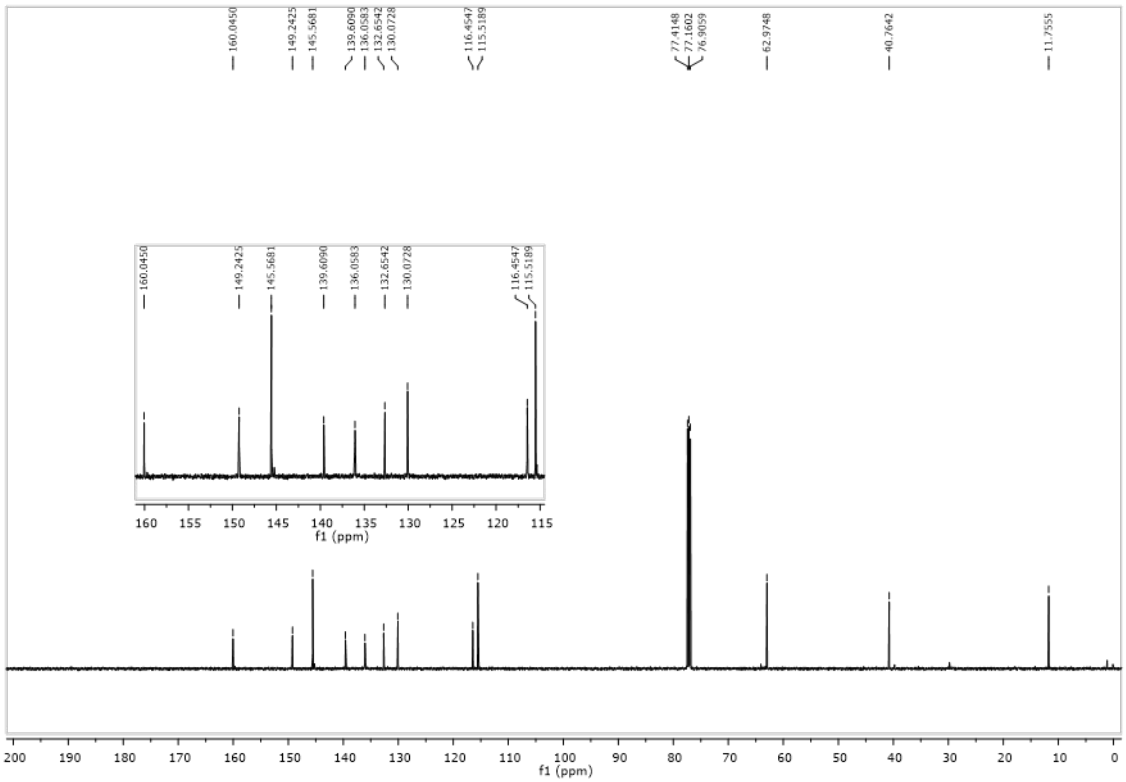
^{1}H NMR of 69 (500 MHz, $CDCl_3$)



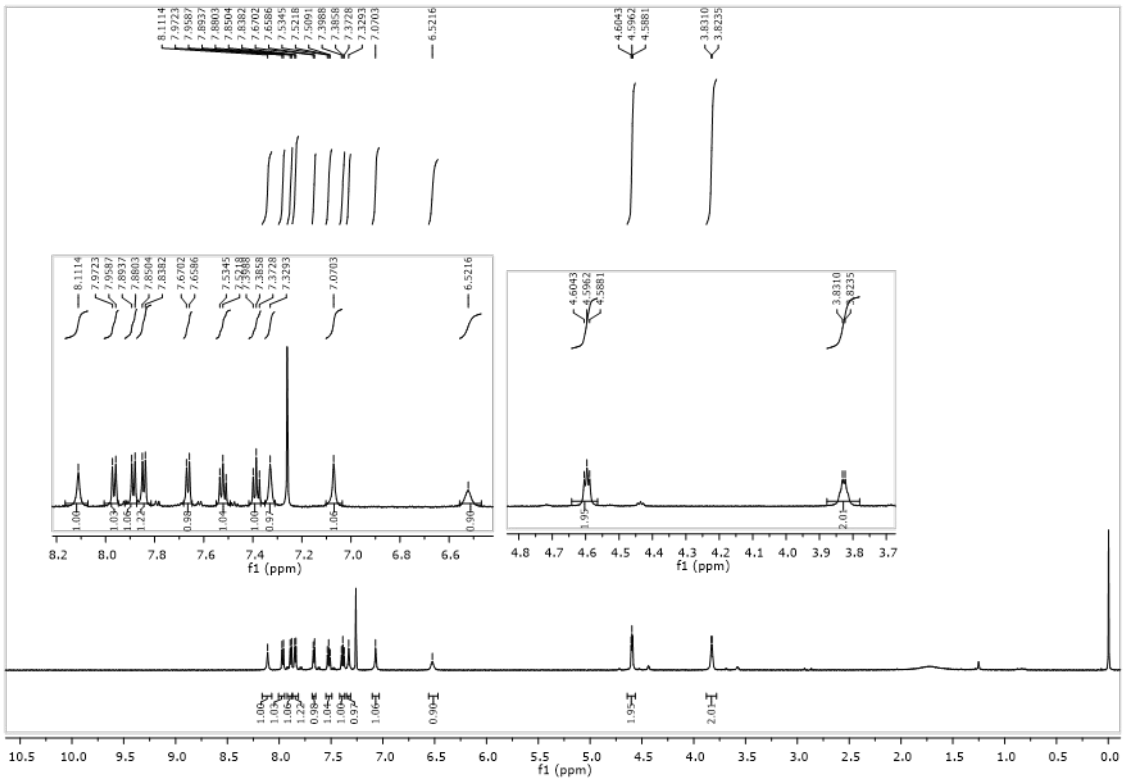
^{13}C NMR of 69 (126 MHz, $CDCl_3$)



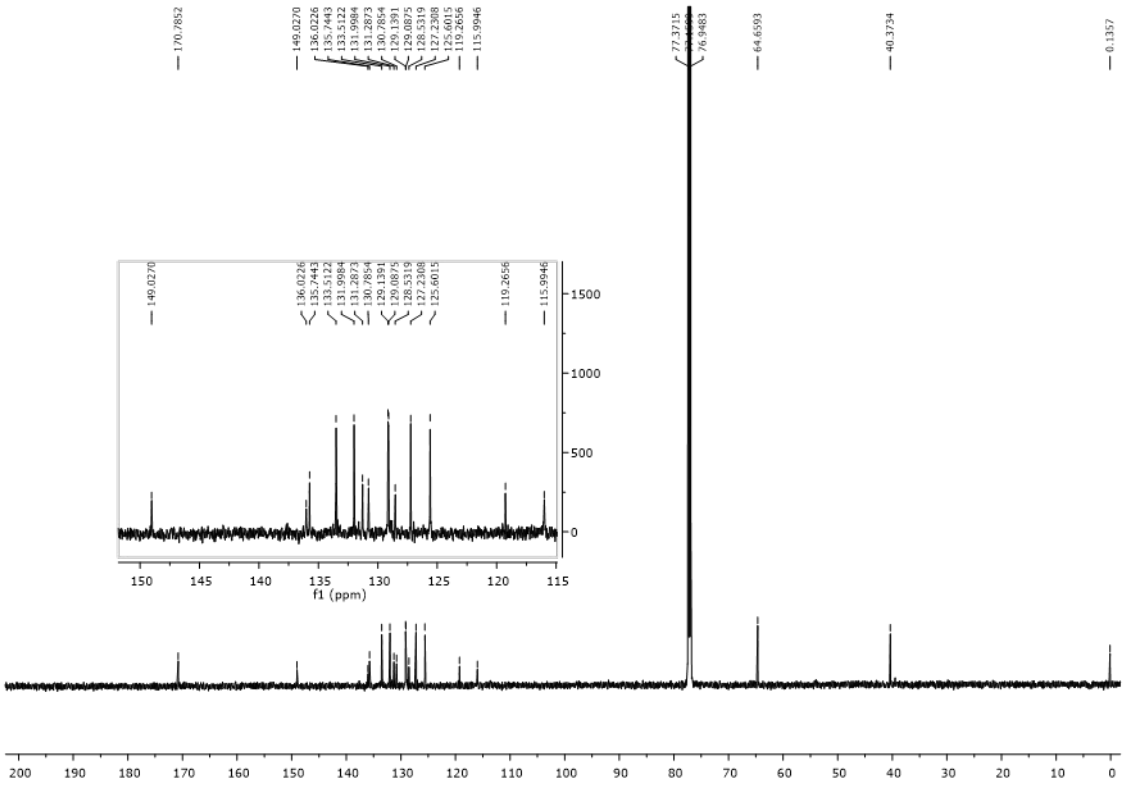
^{1}H NMR of 70 (500 MHz, $CDCl_3$)



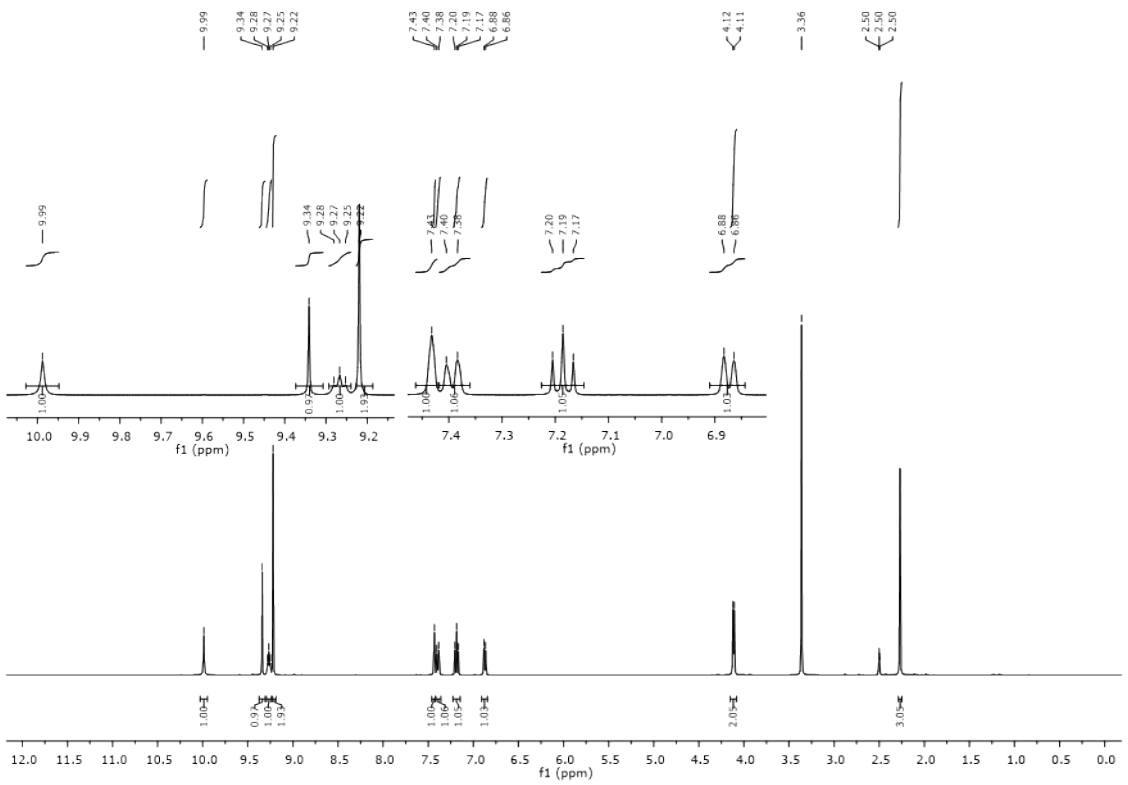
^{13}C NMR of 70 (126 MHz, $CDCl_3$)



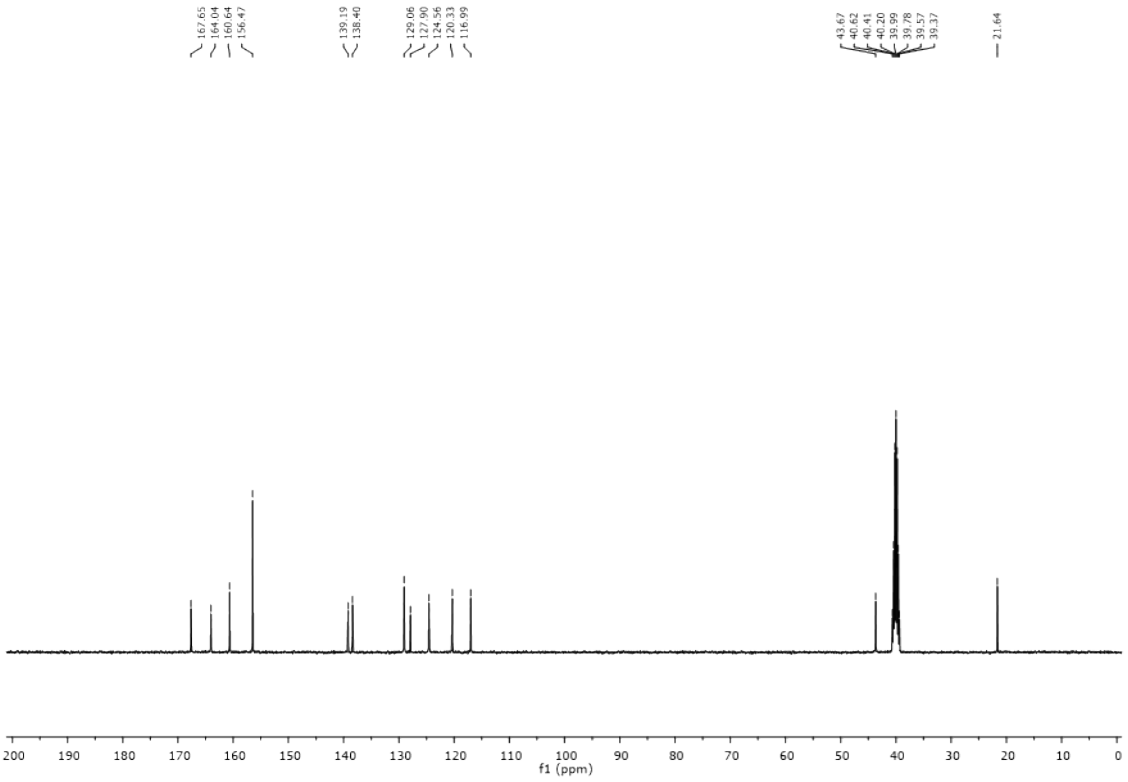
^{1}H NMR of 71 (600 MHz, $CDCl_3$)



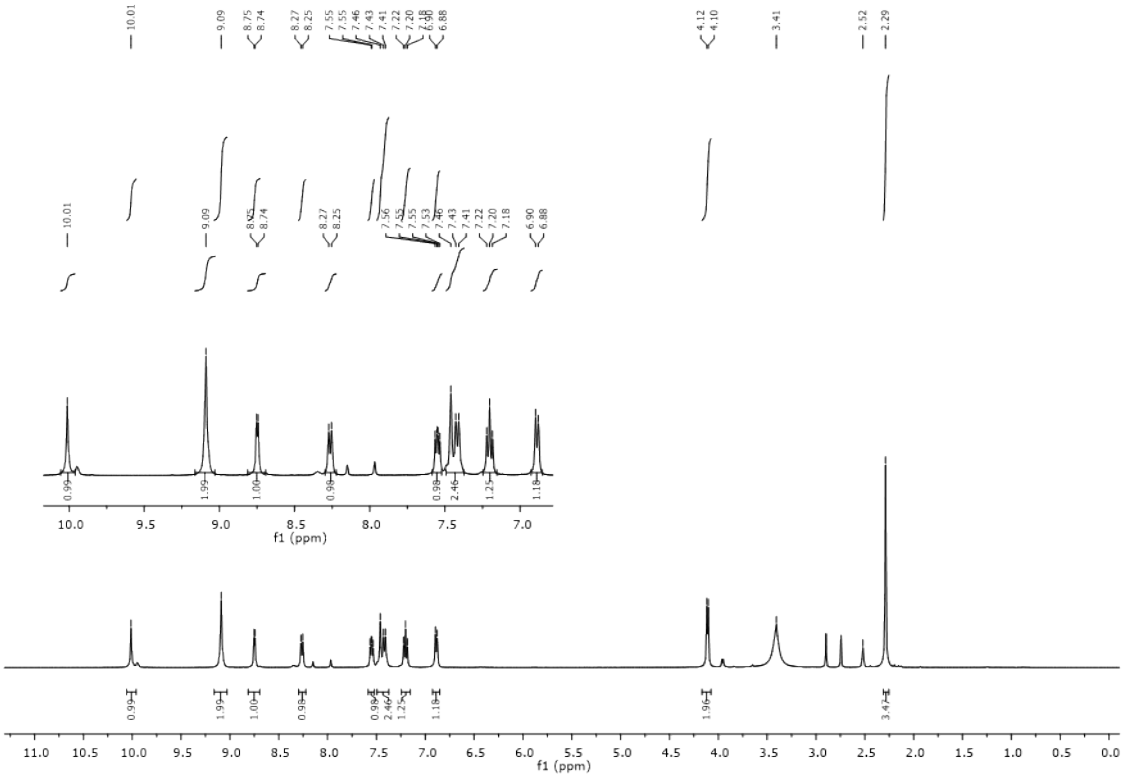
^{13}C NMR of 71 (151 MHz, $CDCl_3$)



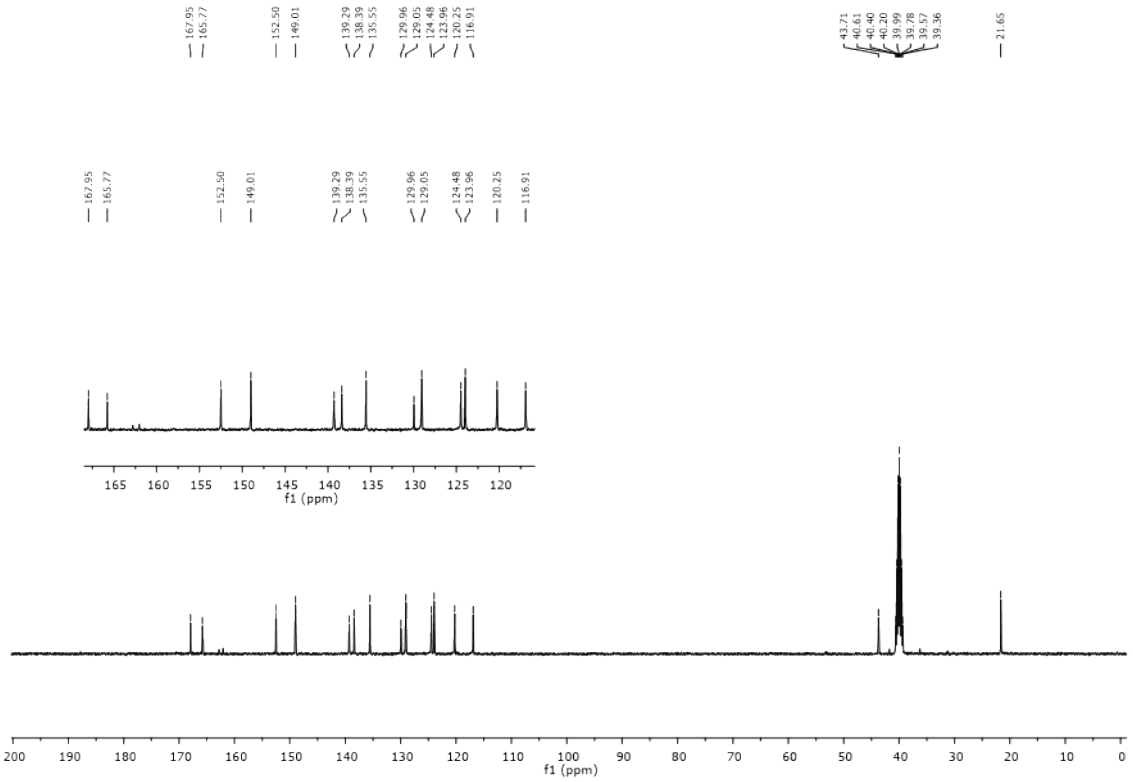
^{1}H NMR of 76 (400 MHz, DMSO-d_6)



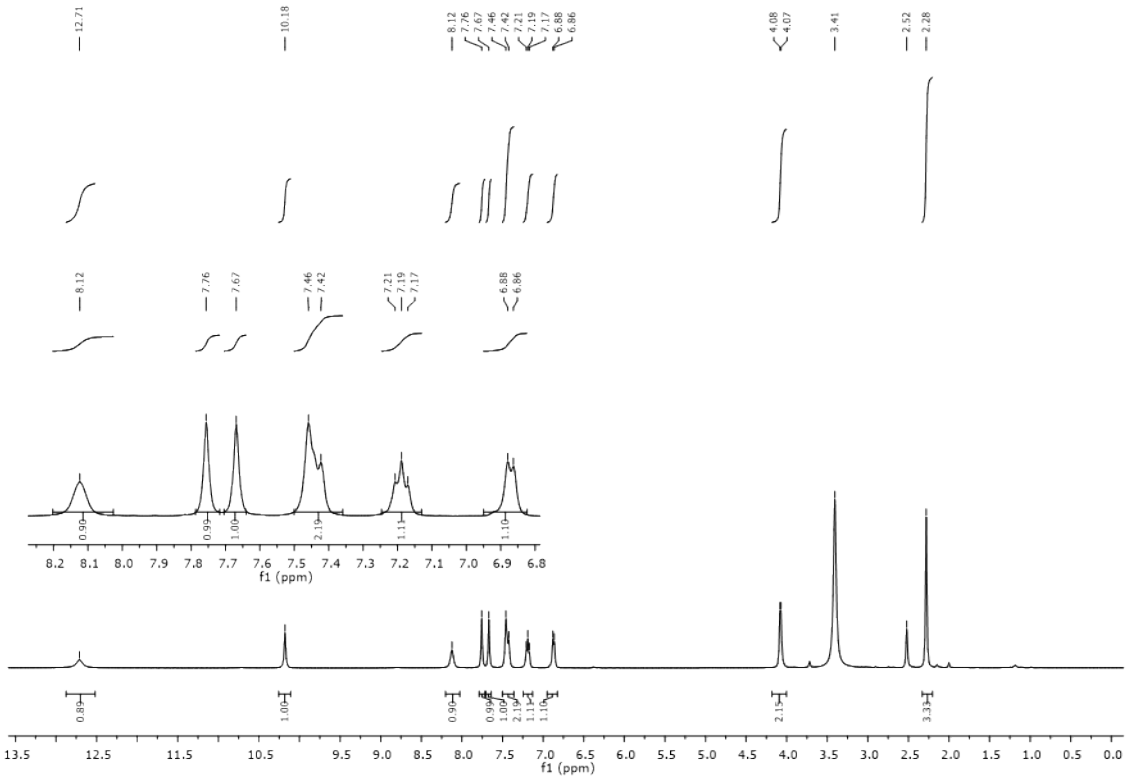
^{13}C NMR of 76 (100 MHz, DMSO-d_6)



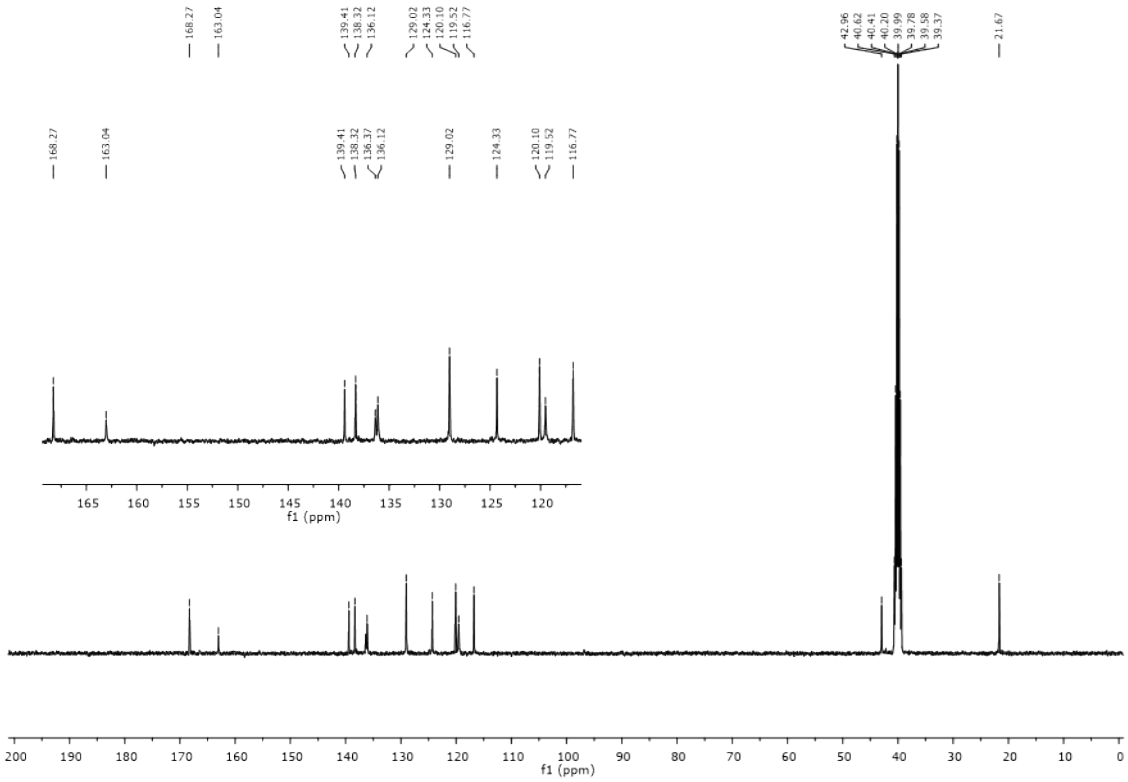
^{1}H NMR of 77 (400 MHz, DMSO-d_6)



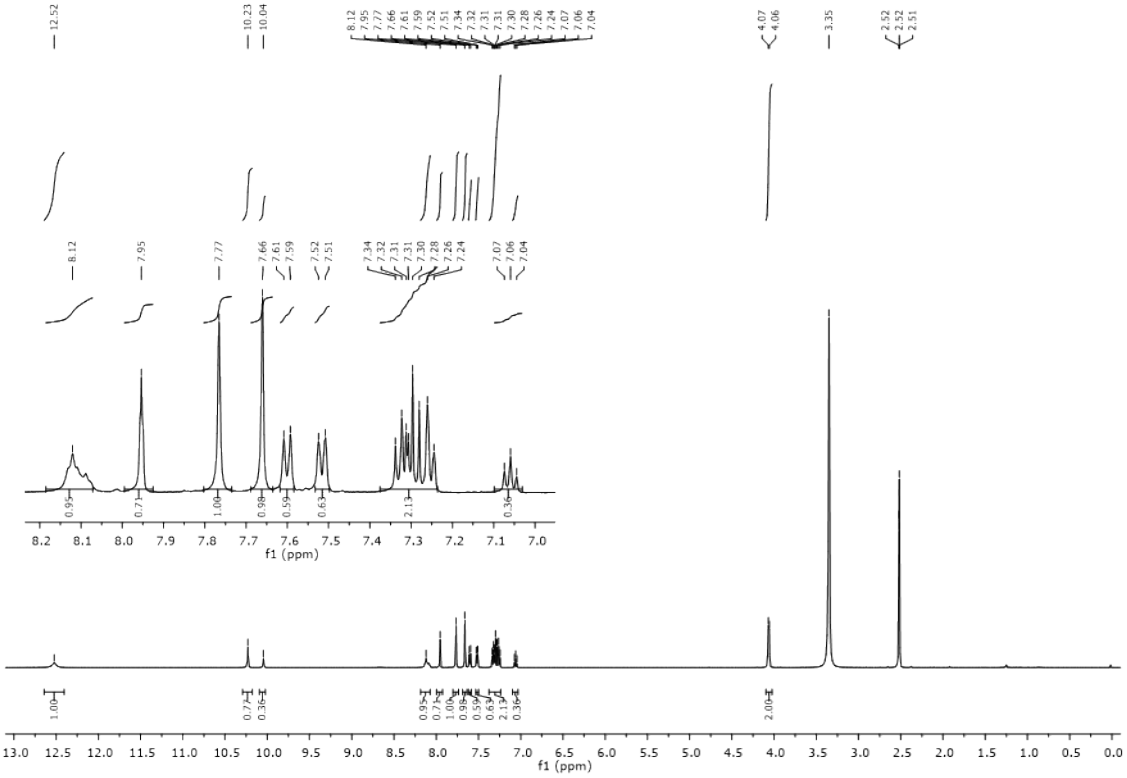
^{13}C NMR of 77 (100 MHz, DMSO-d_6)



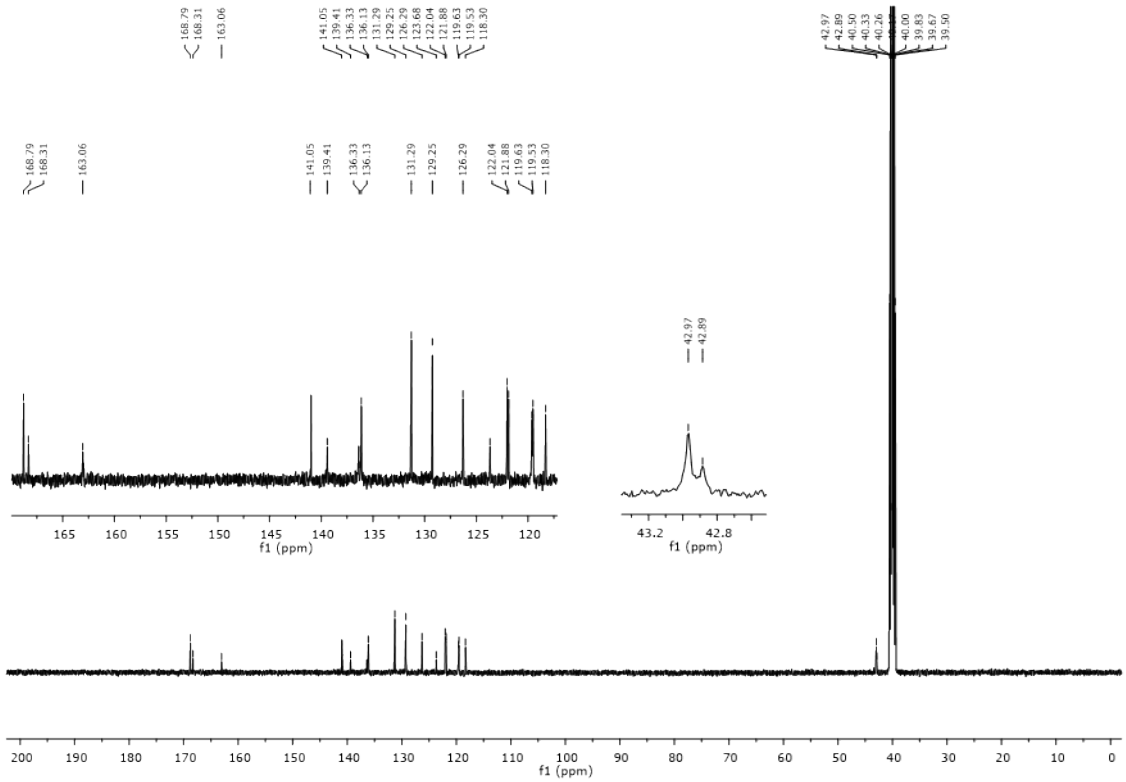
^{1}H NMR of 78 (400 MHz, DMSO-d_6)



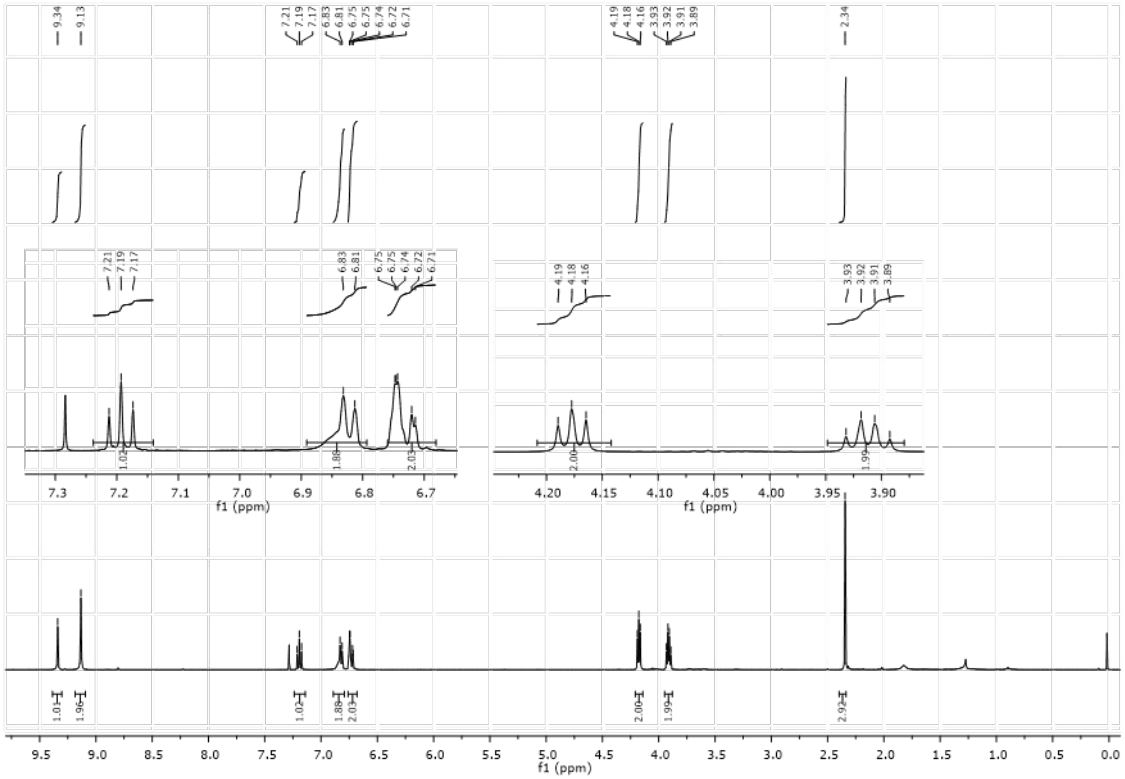
^{13}C NMR of 78 (100 MHz, DMSO-d_6)



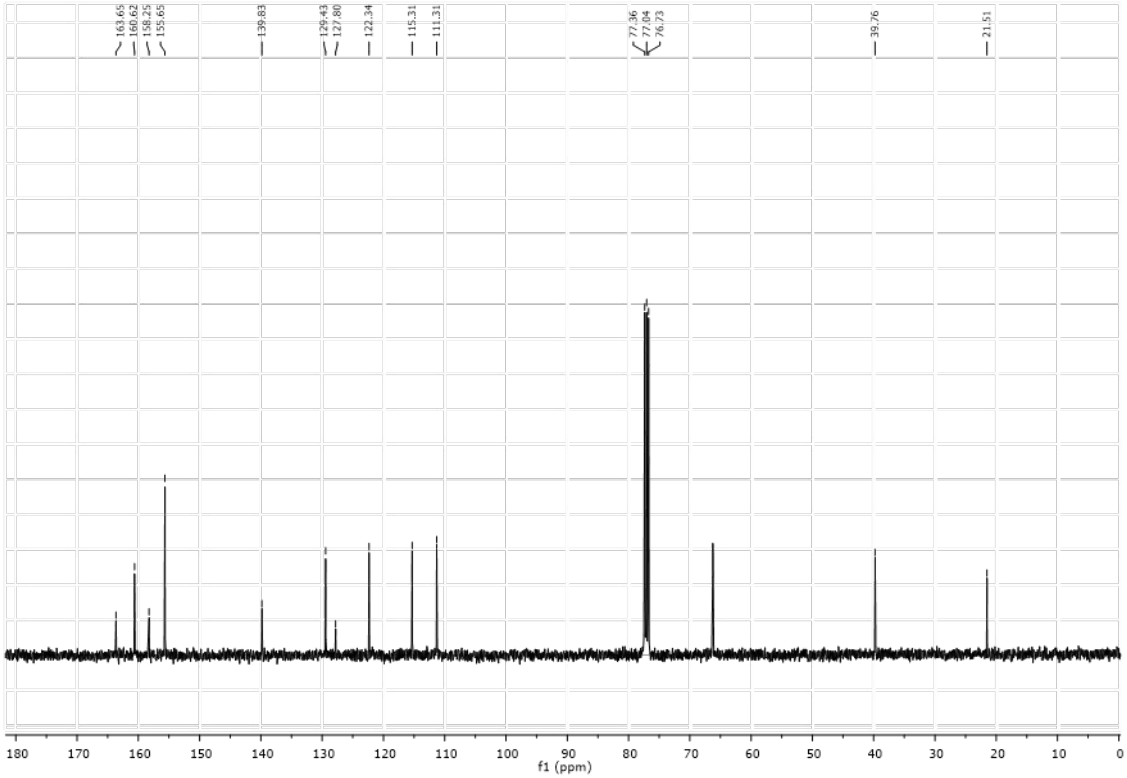
^{1}H NMR of 79 (500 MHz, DMSO-d_6)



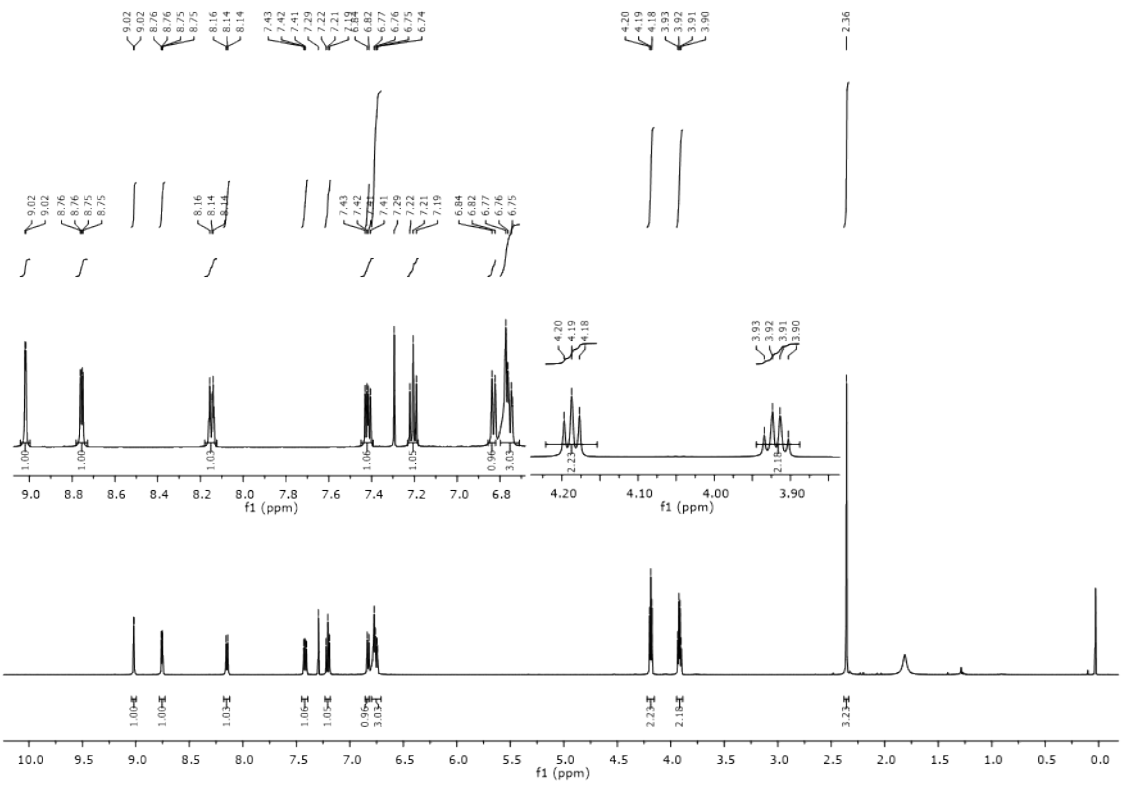
^{13}C NMR of 79 (125 MHz, DMSO-d_6)



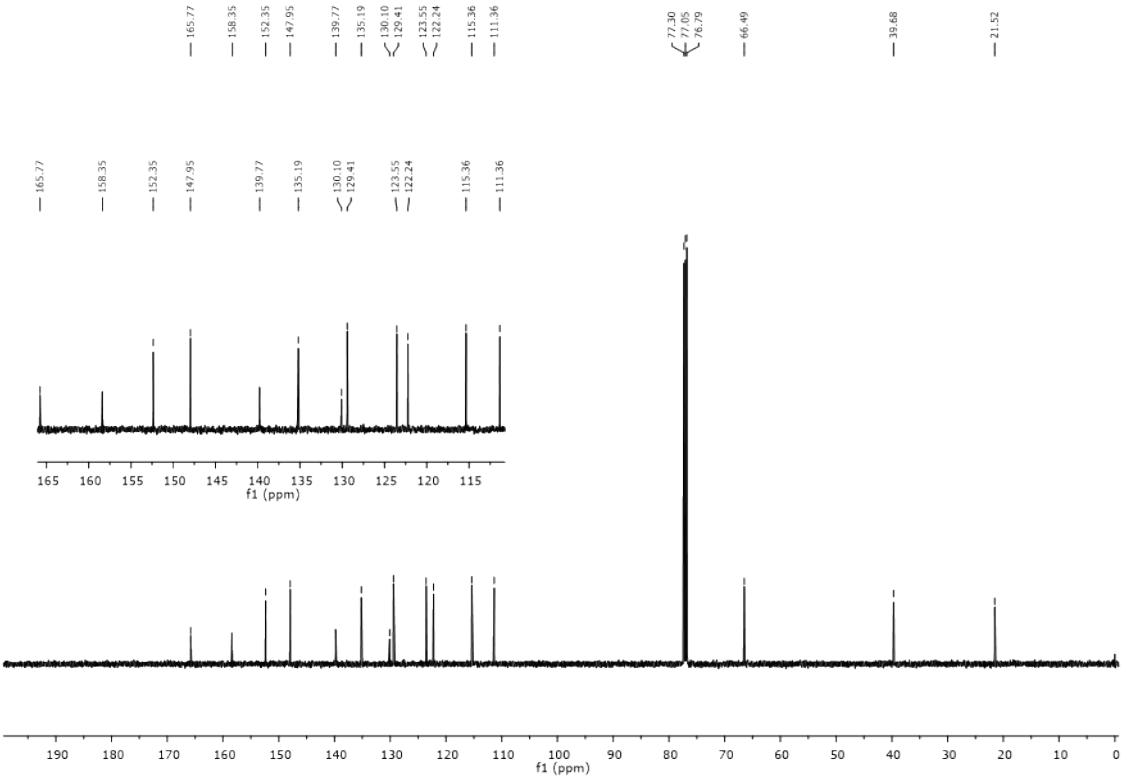
^{1}H NMR of 80 (400 MHz, $CDCl_3$)



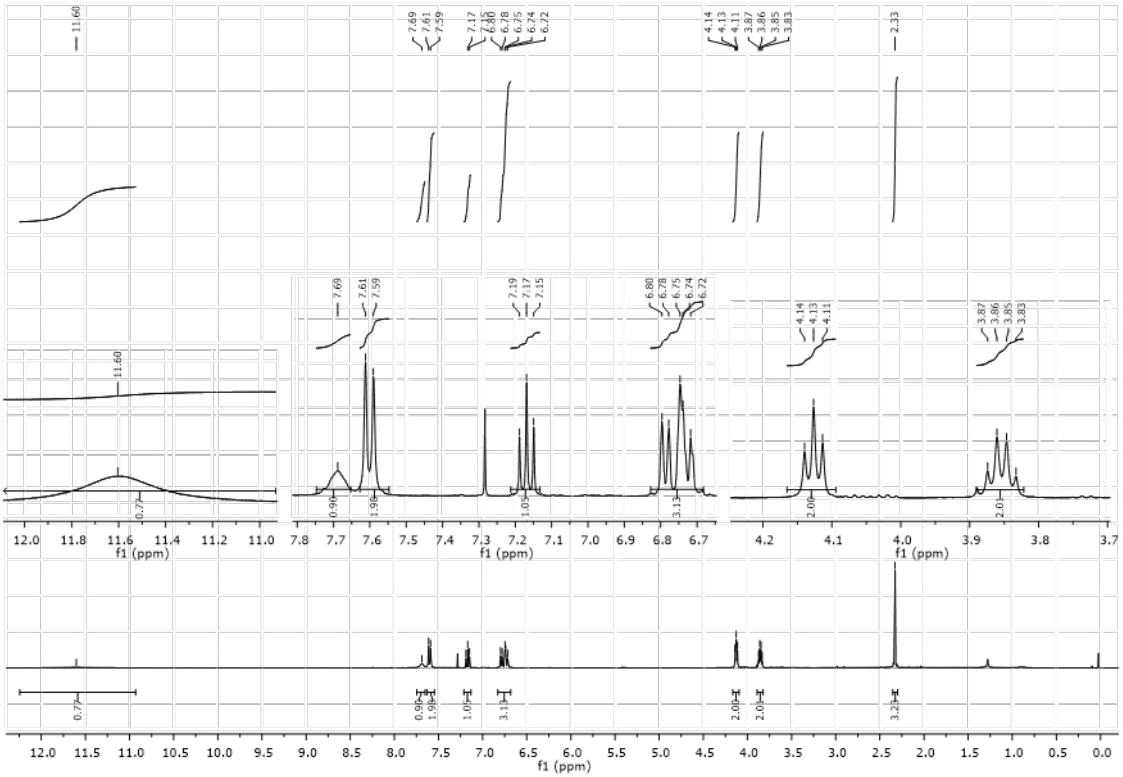
^{13}C NMR of 80 (101 MHz, $CDCl_3$)



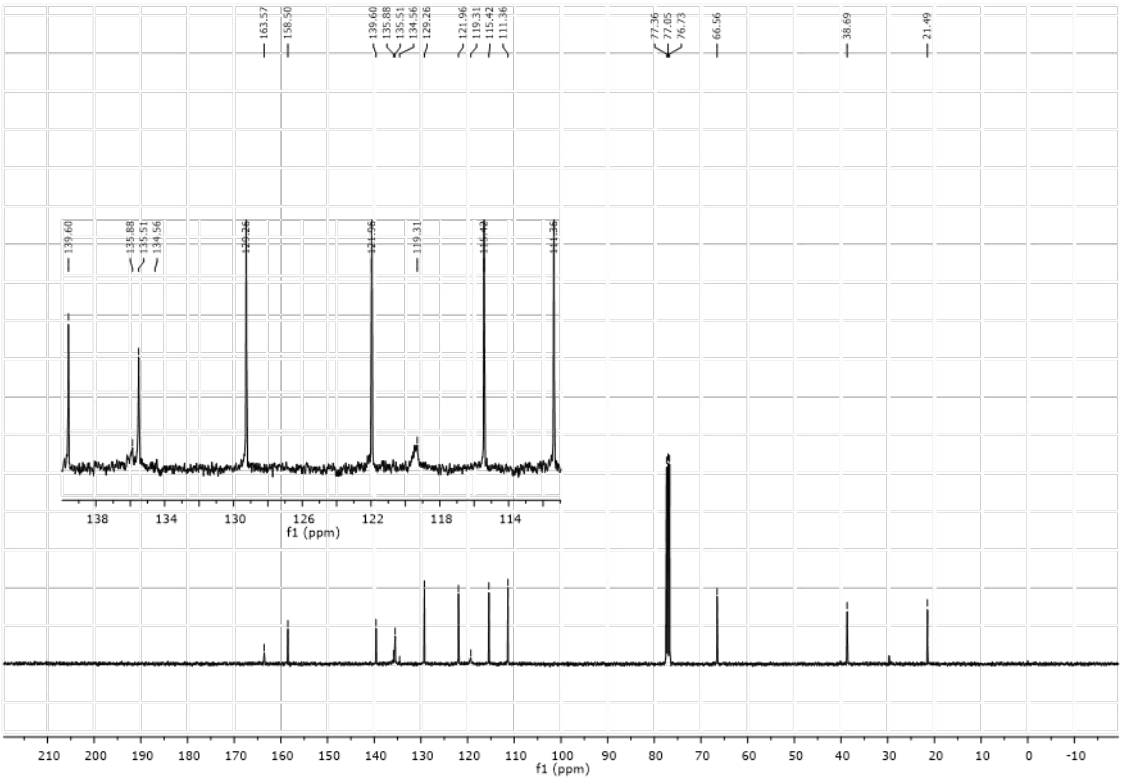
^{1}H NMR of 81 (500 MHz, $CDCl_3$)



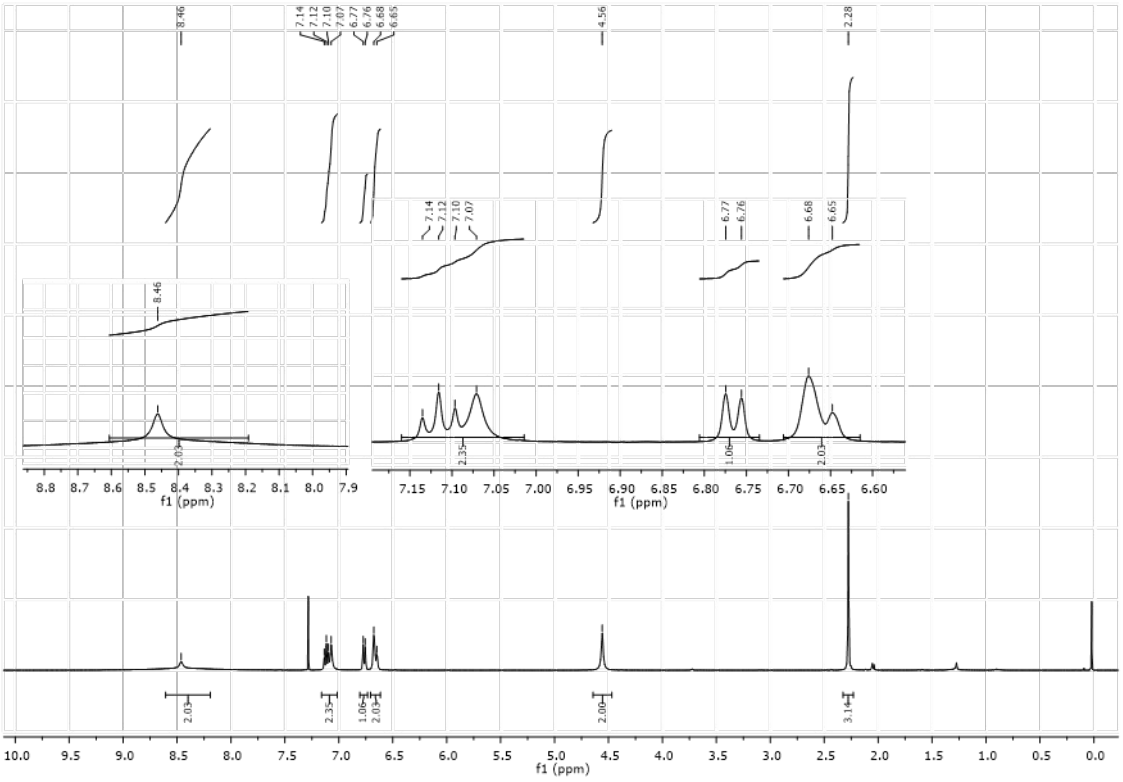
^{13}C NMR of 81 (125 MHz, $CDCl_3$)



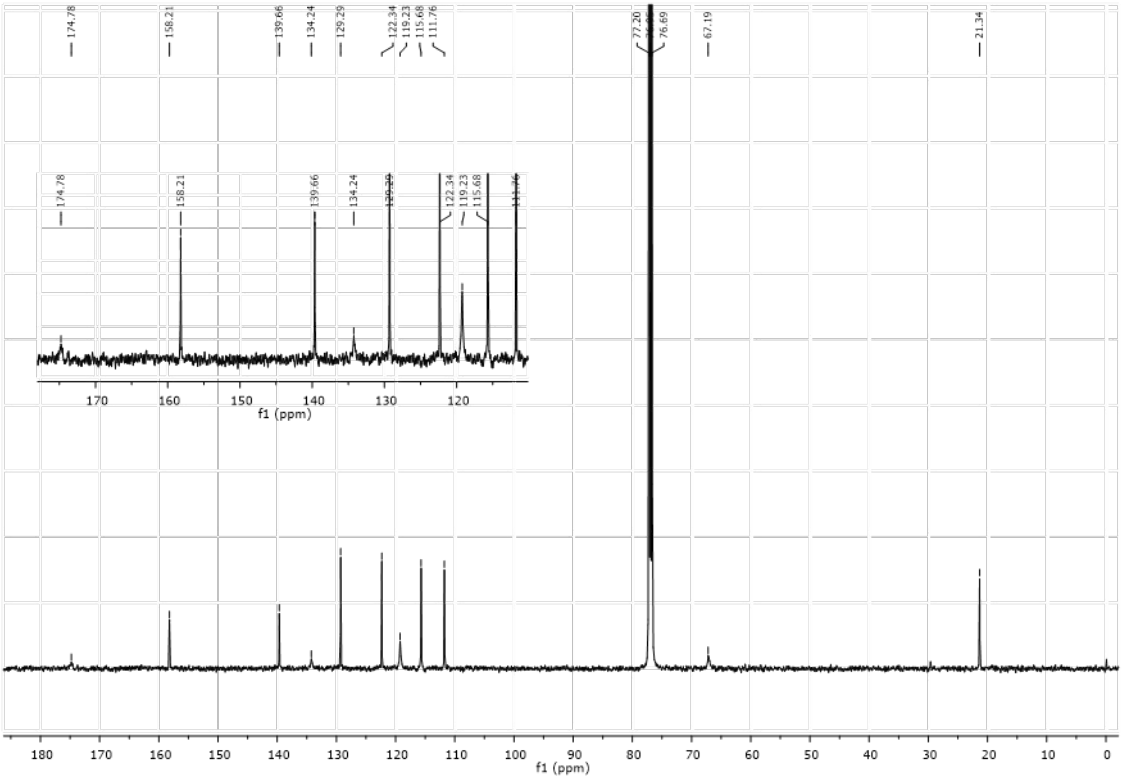
^{1}H NMR of 82 (400 MHz, $CDCl_3$)



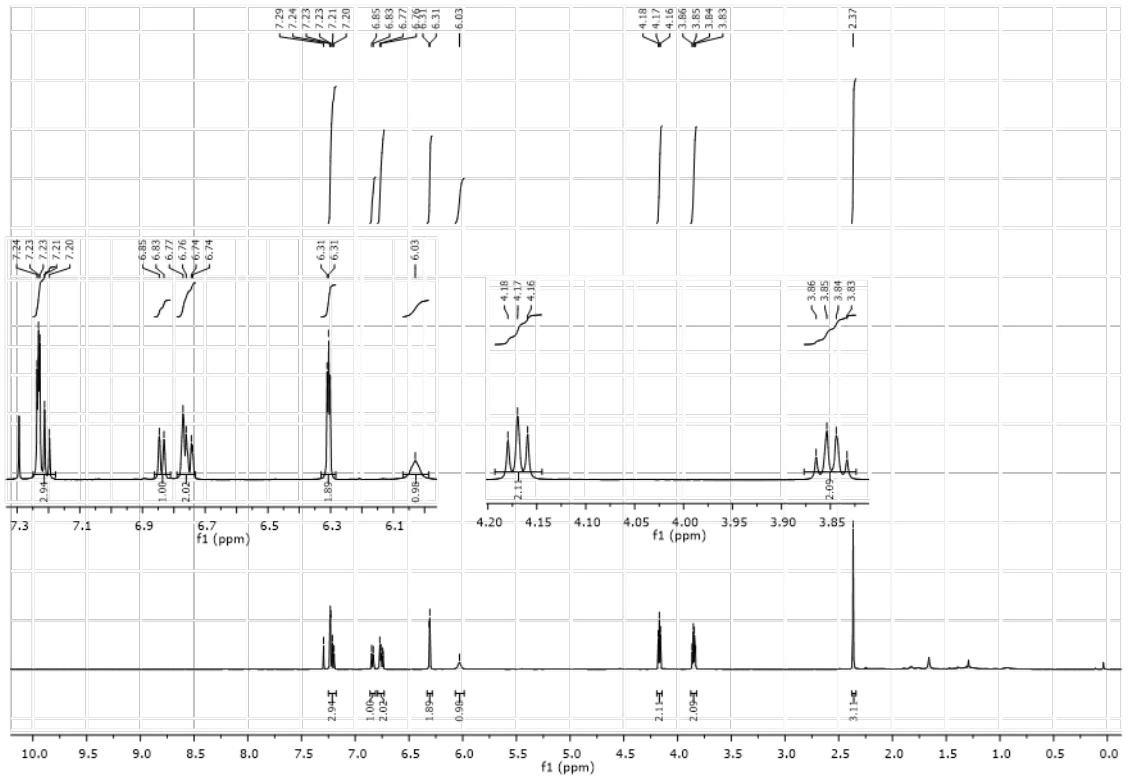
^{13}C NMR of 82 (101 MHz, $CDCl_3$)



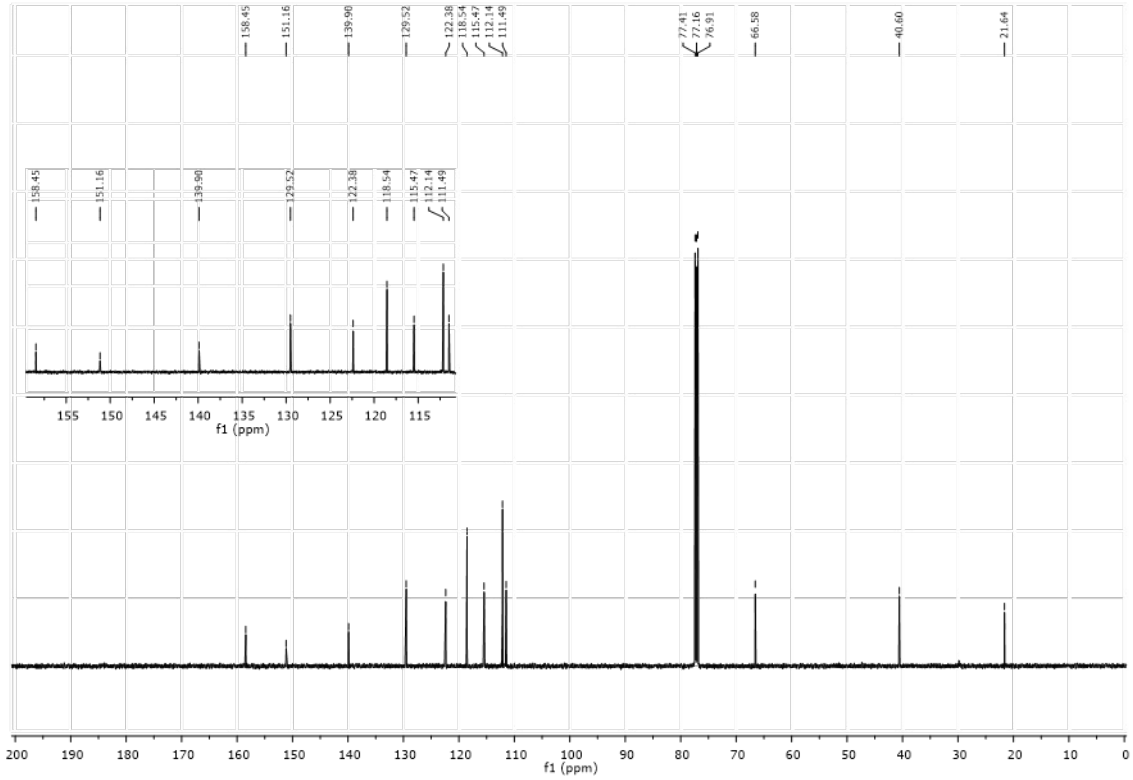
^{1}H NMR of 84 (400 MHz, $CDCl_3$)



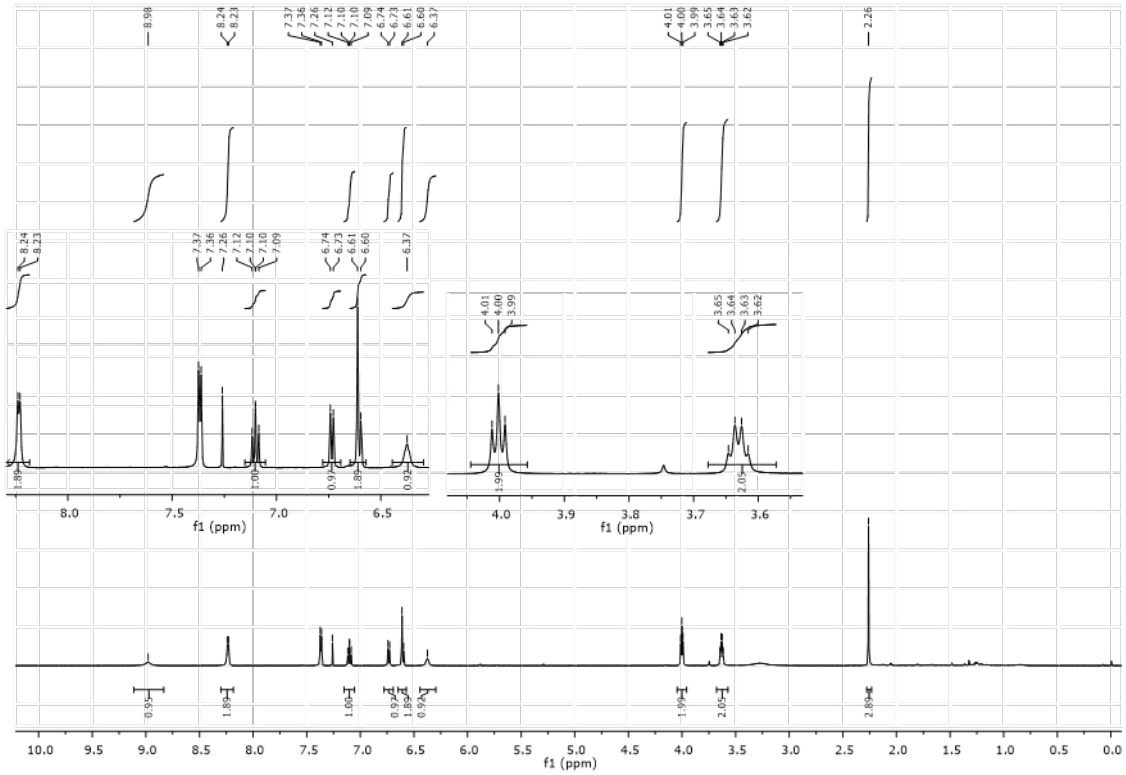
^{13}C NMR of 84 (101 MHz, $CDCl_3$)



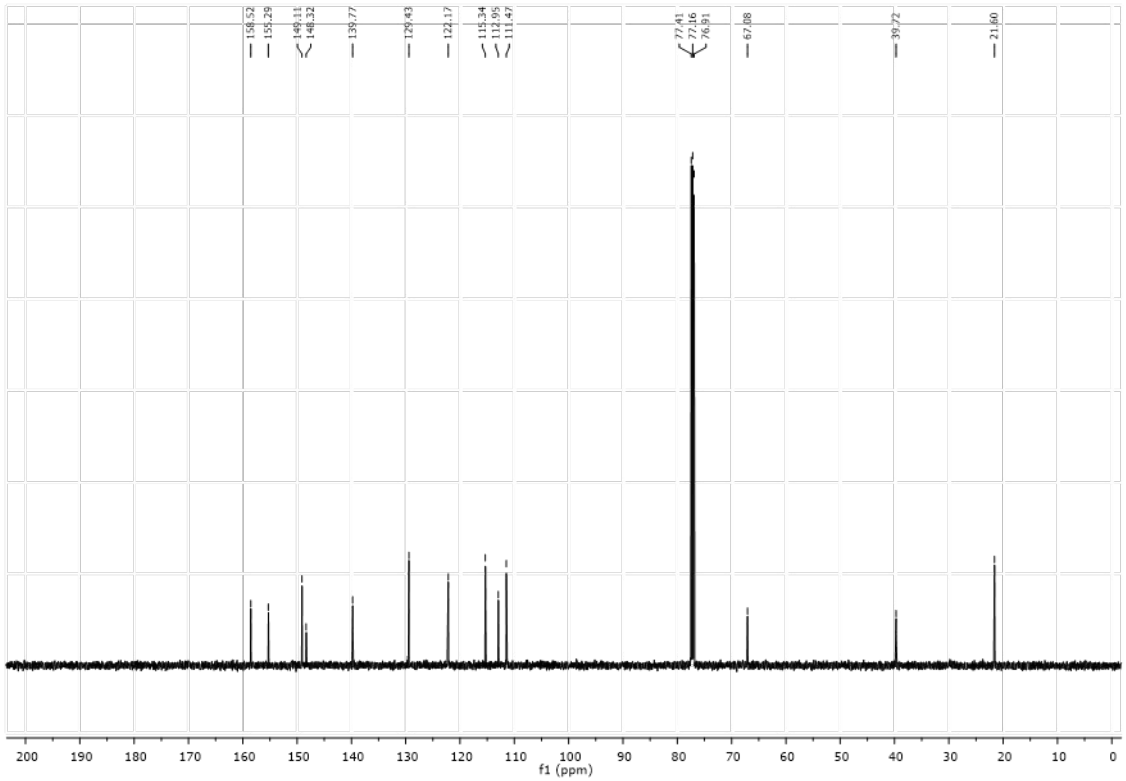
^{1}H NMR of 85 (500 MHz, $CDCl_3$)



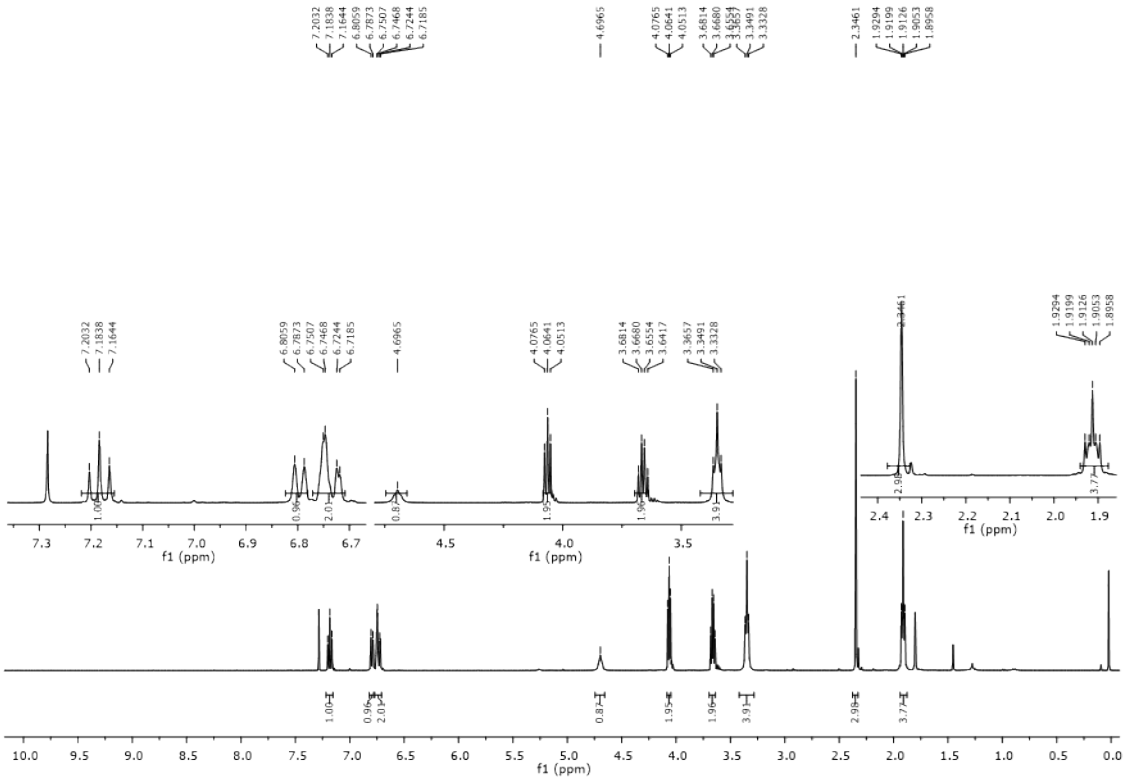
^{13}C NMR of 85 (126 MHz, $CDCl_3$)



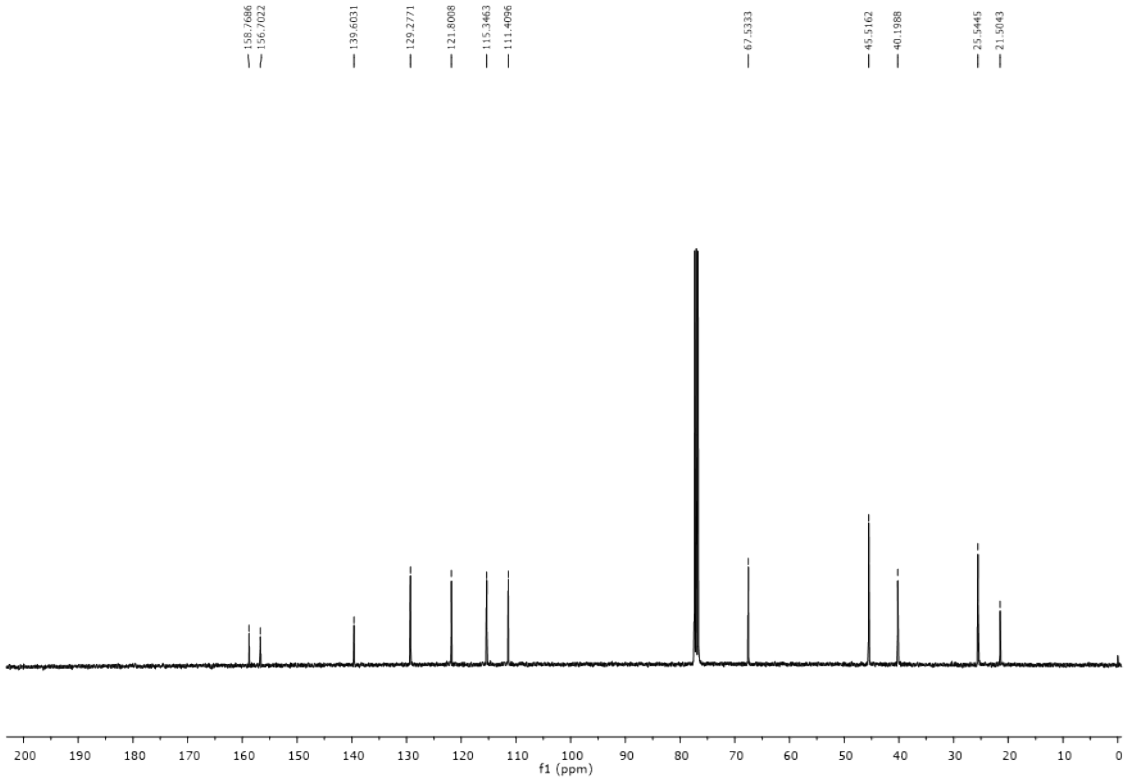
^{1}H NMR of 86 (500 MHz, $CDCl_3$)



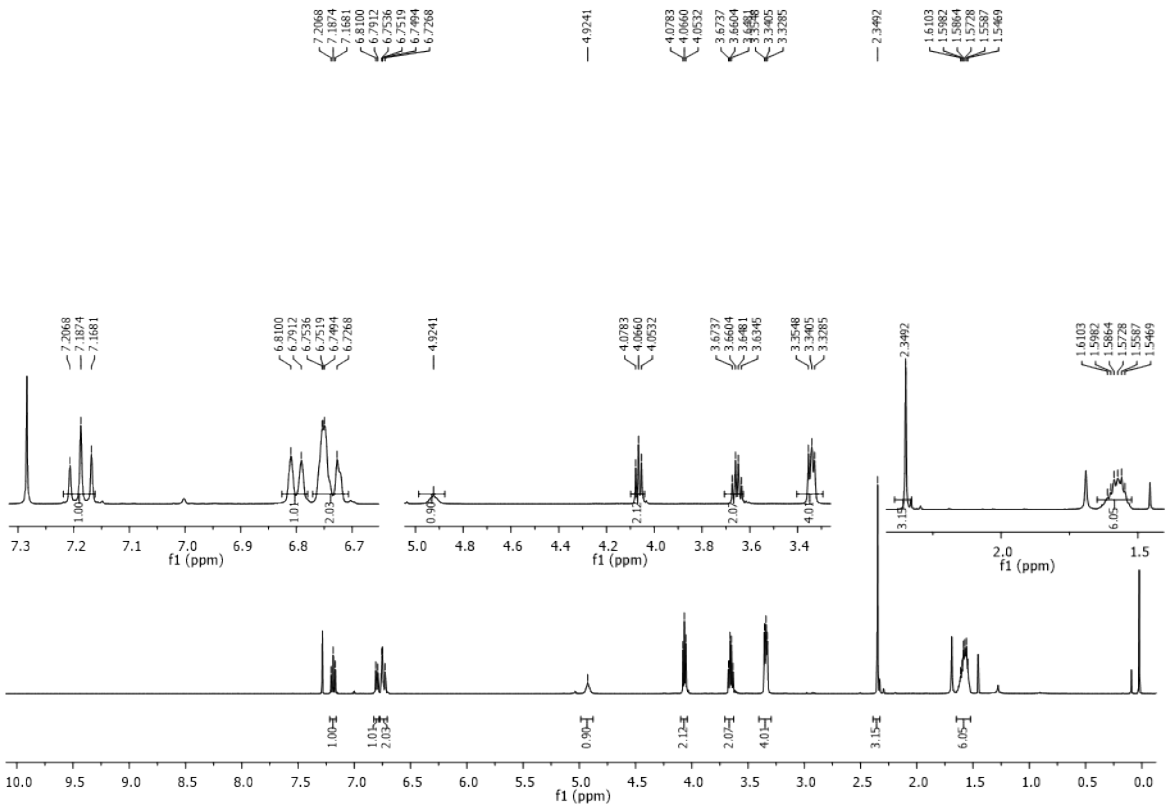
^{13}C NMR of 86 (126 MHz, $CDCl_3$)



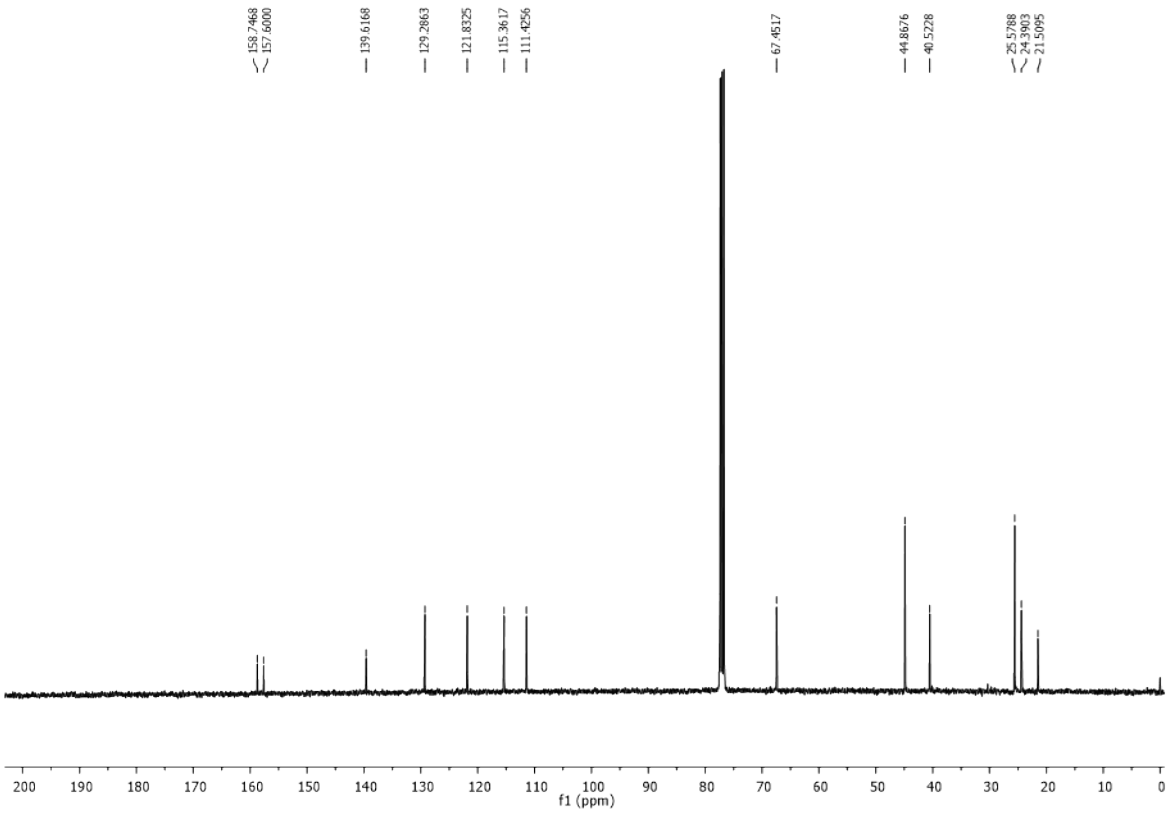
^{1}H NMR of 87 (400 MHz, $CDCl_3$)



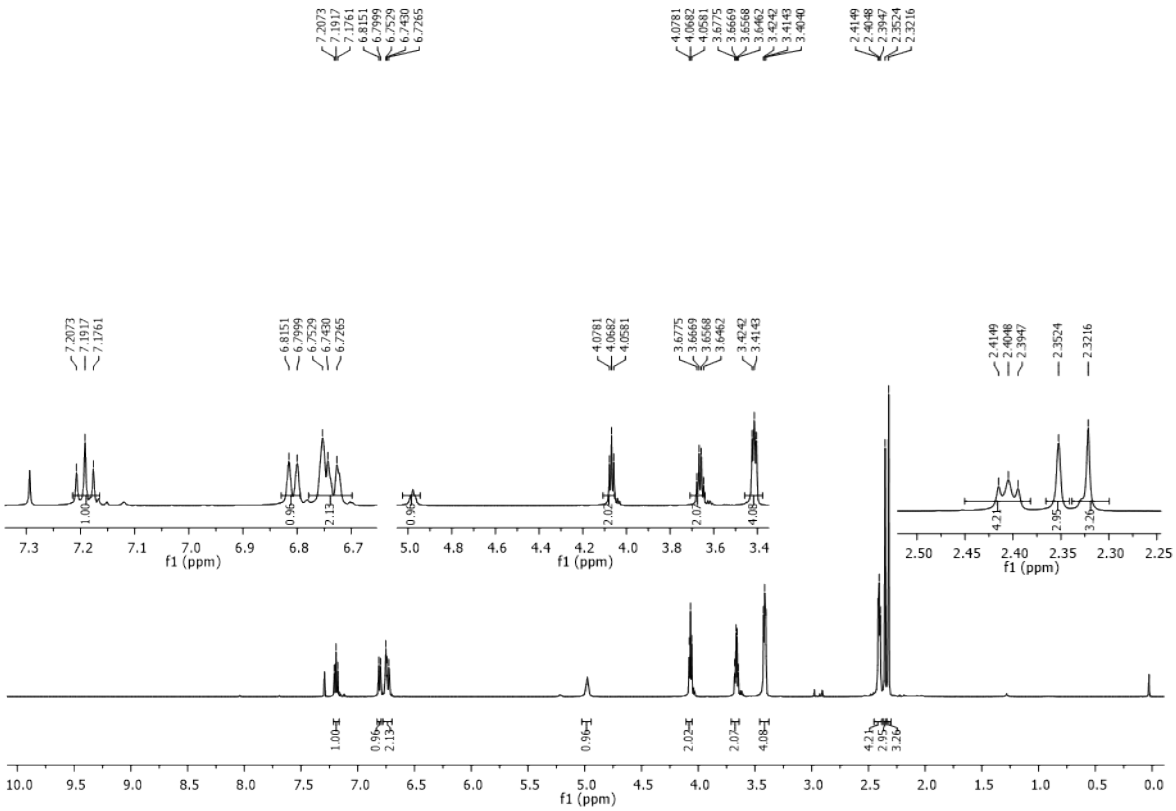
^{13}C NMR of 87 (101 MHz, $CDCl_3$)



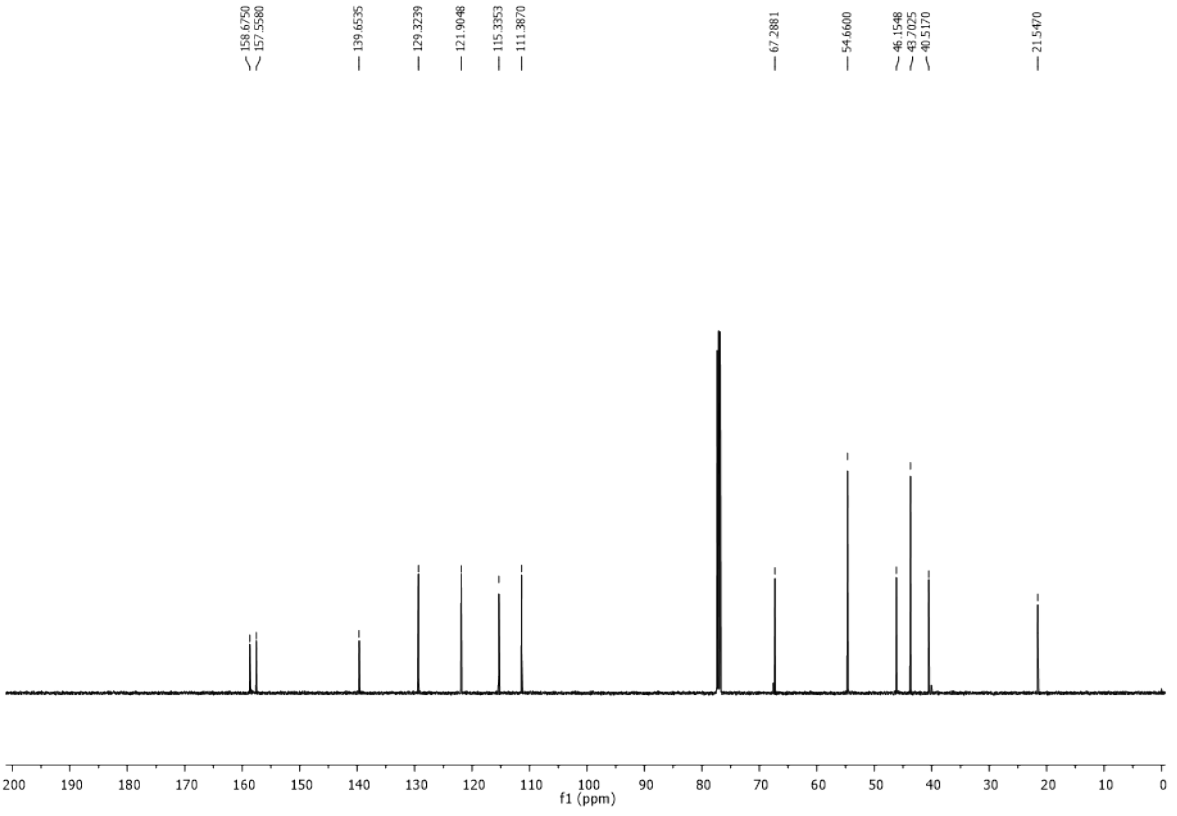
^{1}H NMR of 88 (400 MHz, $CDCl_3$)



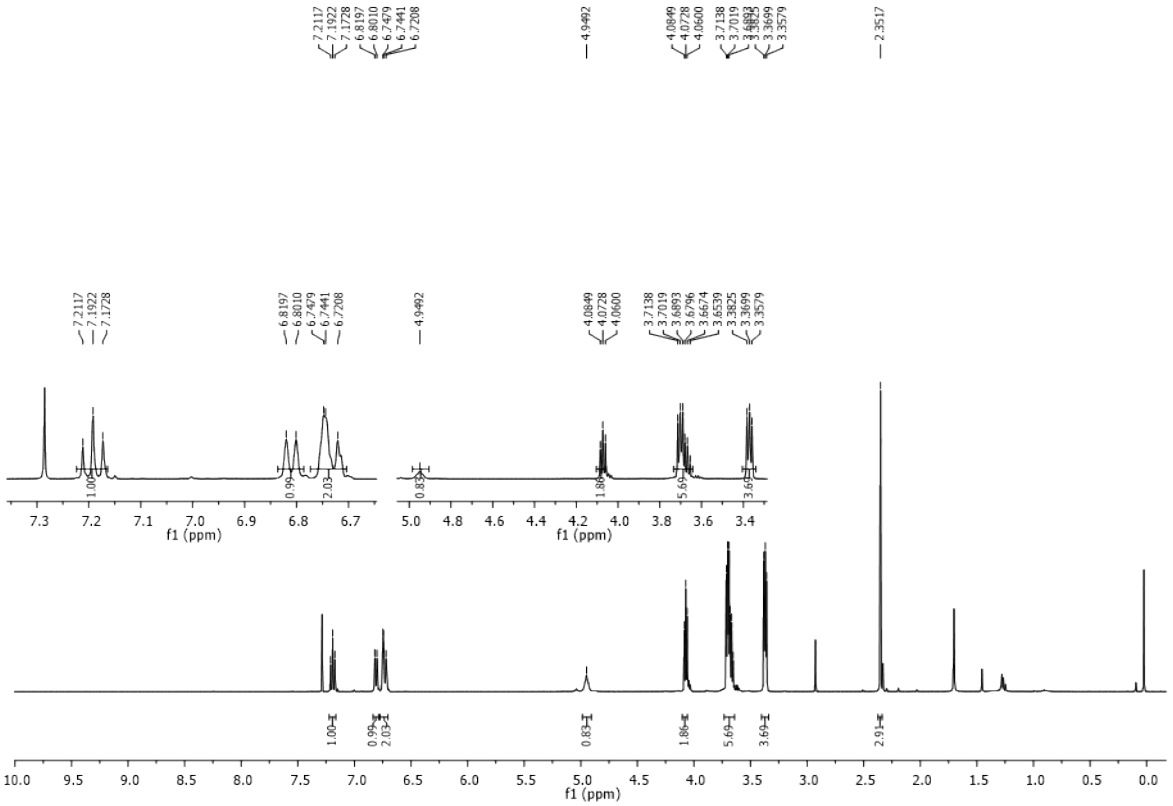
^{13}C NMR of 88 (101 MHz, $CDCl_3$)



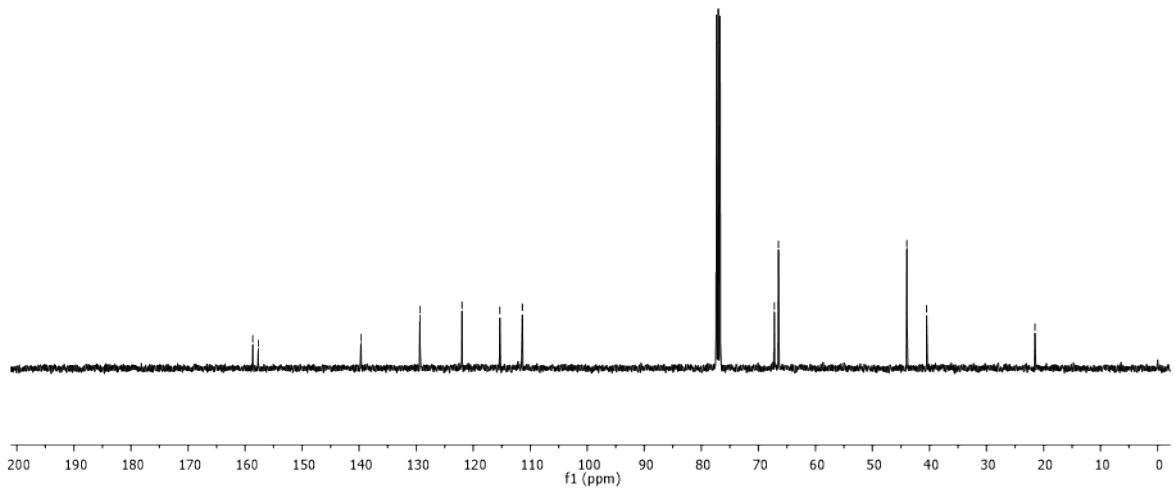
^{1}H NMR of 89 (500 MHz, $CDCl_3$)



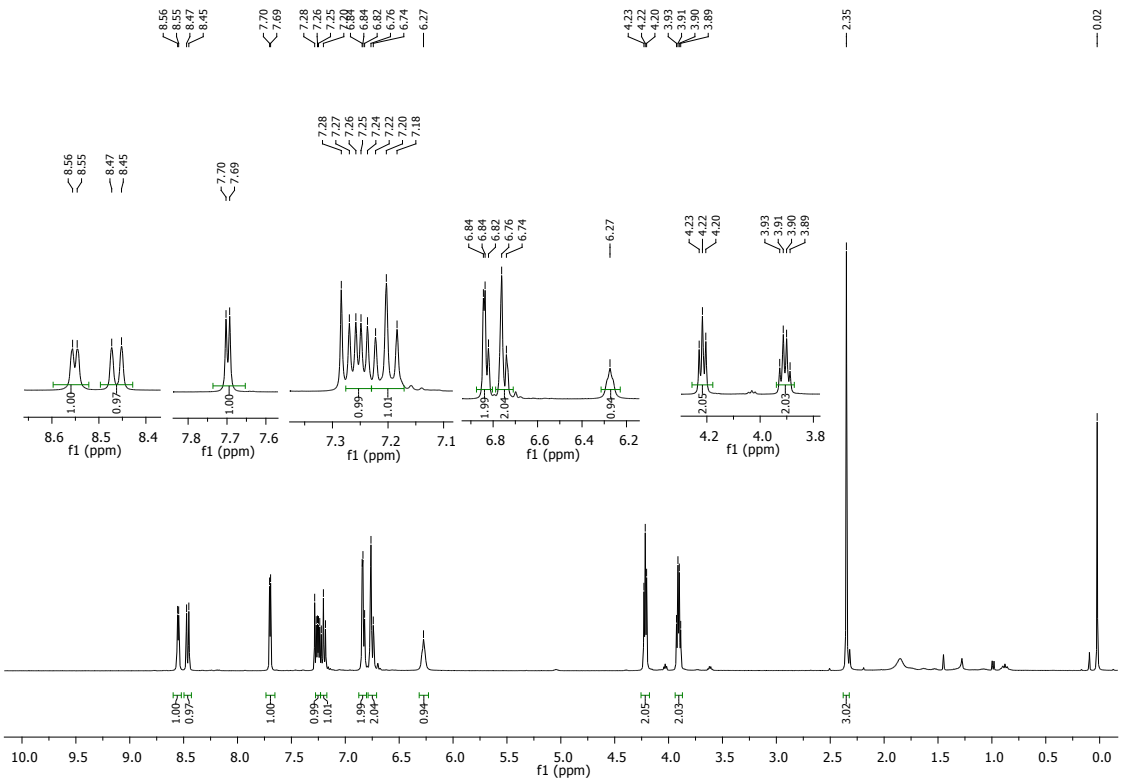
^{13}C NMR of 89 (126 MHz, $CDCl_3$)



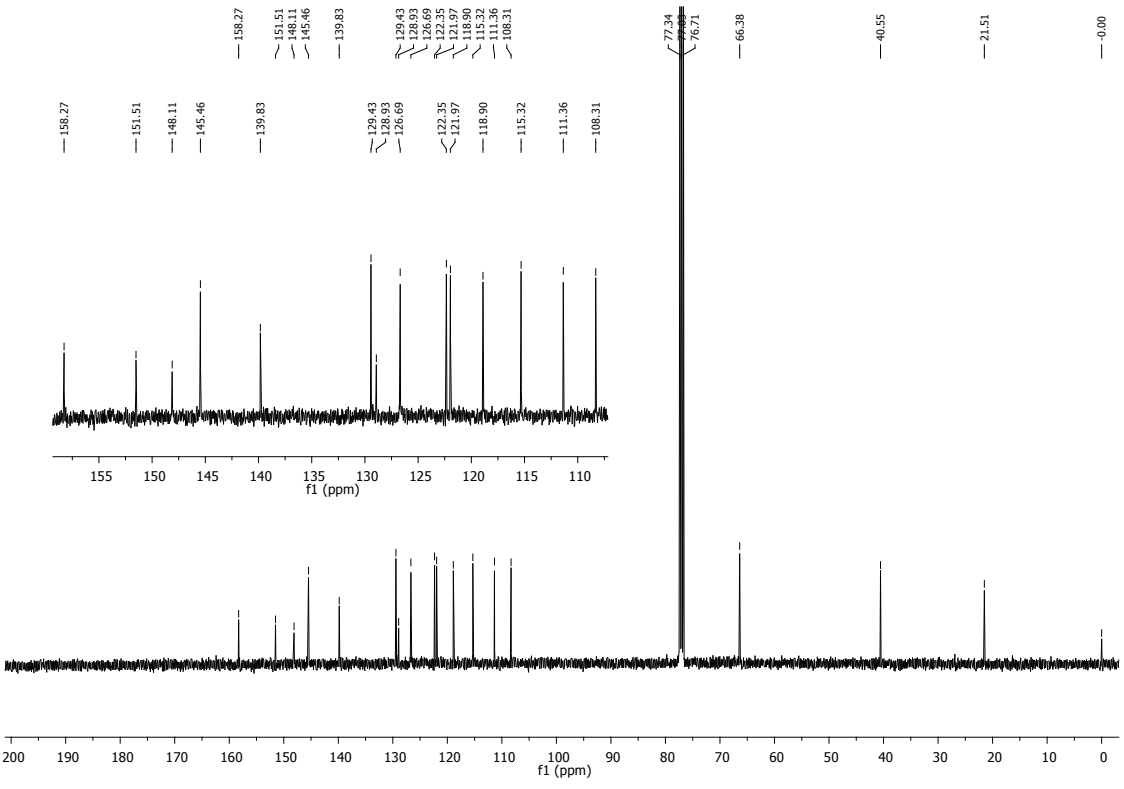
^{1}H NMR of 90 (400 MHz, $CDCl_3$)



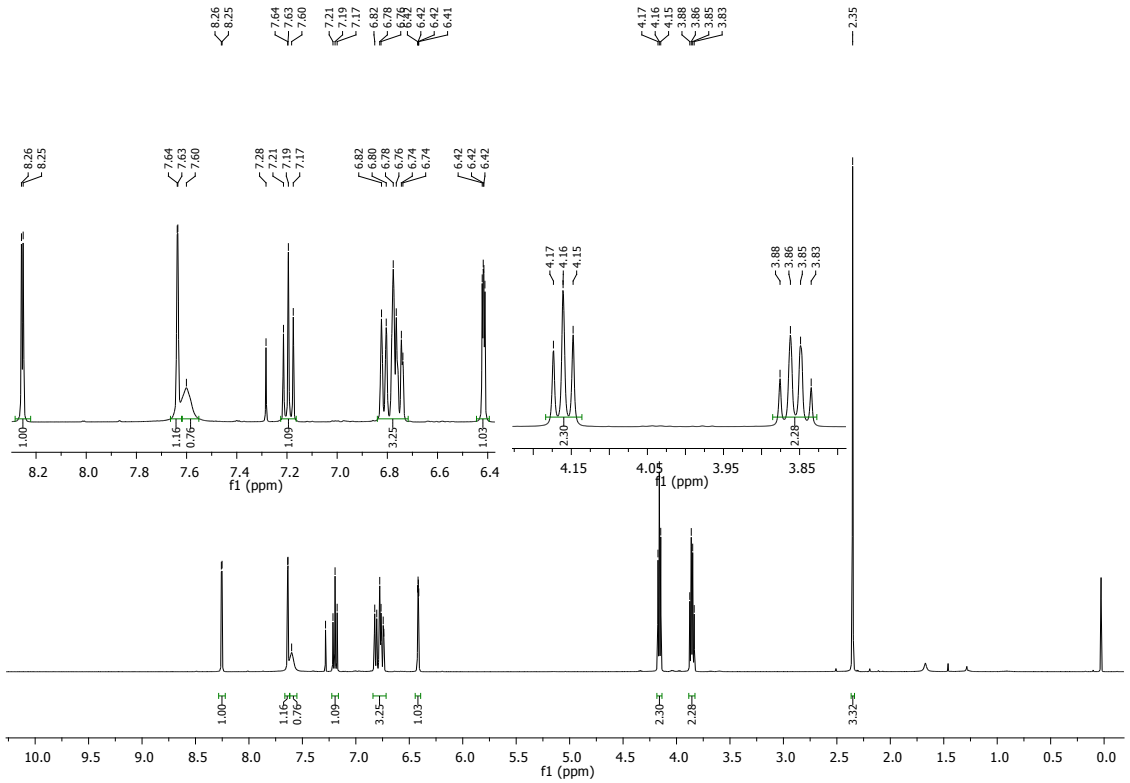
^{13}C NMR of 90 (101 MHz)



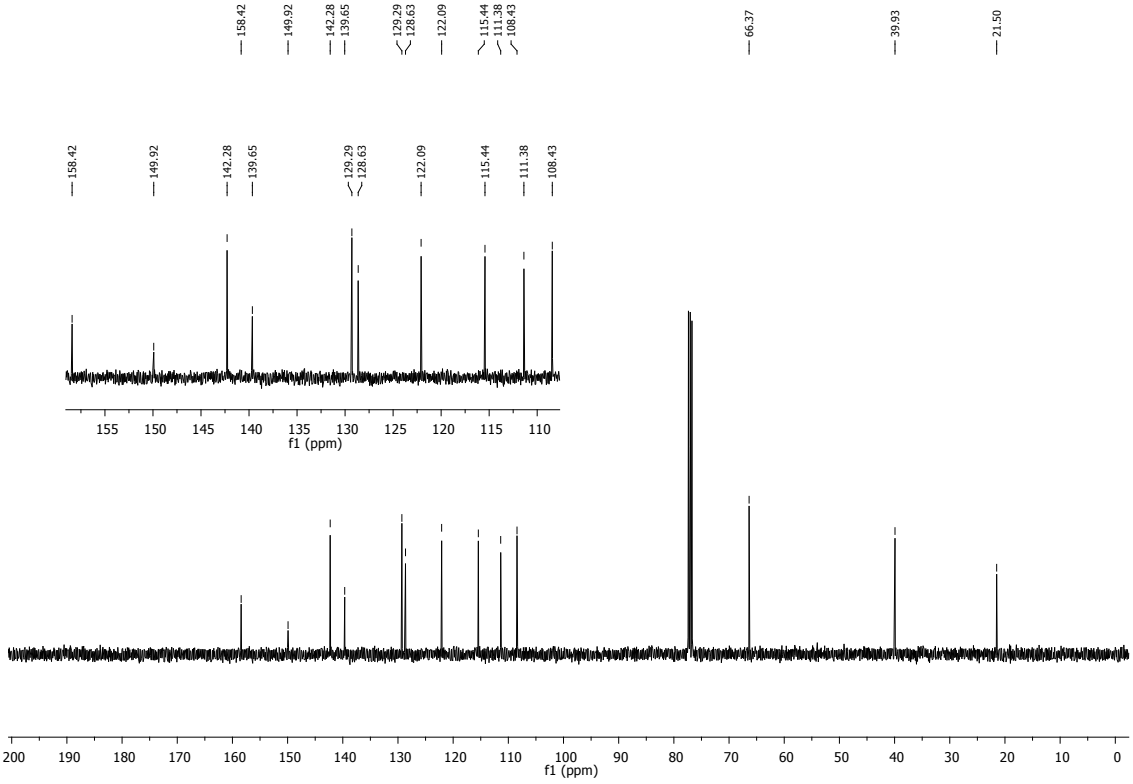
^{1}H NMR of 91 (400 MHz, $CDCl_3$)



^{13}C NMR of 91 (101 MHz)



^{1}H NMR of 92 (400 MHz, $CDCl_3$)



^{13}C NMR of 92 (101 MHz)

Supporting References

1. Johansson, H., Boesgaard, M.W., Norskov-Lauritsen, L., Larsen, I., Kuhne, S., Gloriam, D.E., Bräuner-Osborne, H., Pedersen, D.S. (2015). Selective Allosteric Antagonists for the G Protein-Coupled Receptor GPRC6A Based on the 2-Phenylindole Privileged Structure Scaffold. J. Med. Chem. *58*, 8938–8951.

2. P. Martin, M. Mueller, D. Spielvogel, D. Flubacher, A. Boudier, US 20080242857A1; Oct 2, 2008.