Supplementary Information

Identification of 3-hydroxy-1,2-dimethylpyridine-4(1*H*)-thione as a Metal-Binding Motif for the Inhibition of Botulinum Neurotoxin A

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1. Biological Evaluation

1.1 Interference Compound Filter

All final compounds were examined using publicly accessible PAINS filters:
ZINC patterns search (http://zinc15.docking.org/patterns/home/)
Shoichet's aggregation advisor (https://advisor.bkslab.org/).[1]
No PAINS compounds were identified.

1.2 SNAPtide FRET Assay

BoNT/A1 LC (truncated, 1–425) was kindly provided by the Johnson lab at University of Wisconsin. SNAPtide FRET substrate #523 was purchased from List Labs. The SNAPtide assay was performed as described previously.[2, 3] BoNT/A1 LC (1–425) was incubated with compound for 30 minutes at 25 °C in HEPES (40 mM, pH 7.4) containing TRITON X-100 (0.01%) in CoStar 96-well black opaque-bottom plates. Substrate was added after incubation and the rate of fluorescence (λ_{ex}= 490 nm, λ_{em}= 523 nm) immediately read on a SpectraMax i3x (Molecular Devices) plate reader for a duration of 20 minutes. Final concentrations: BoNT/A1 LC (10 nM), SNAPtide substrate #523 (3.8 µM). Measurements were performed in technical and experimental triplicate. Data were analyzed using GraphPad Prism 8 and IC_{50} values obtained using the four-parameter dose-response inhibition fit.

1.3 Metal Binding Fragment Screen

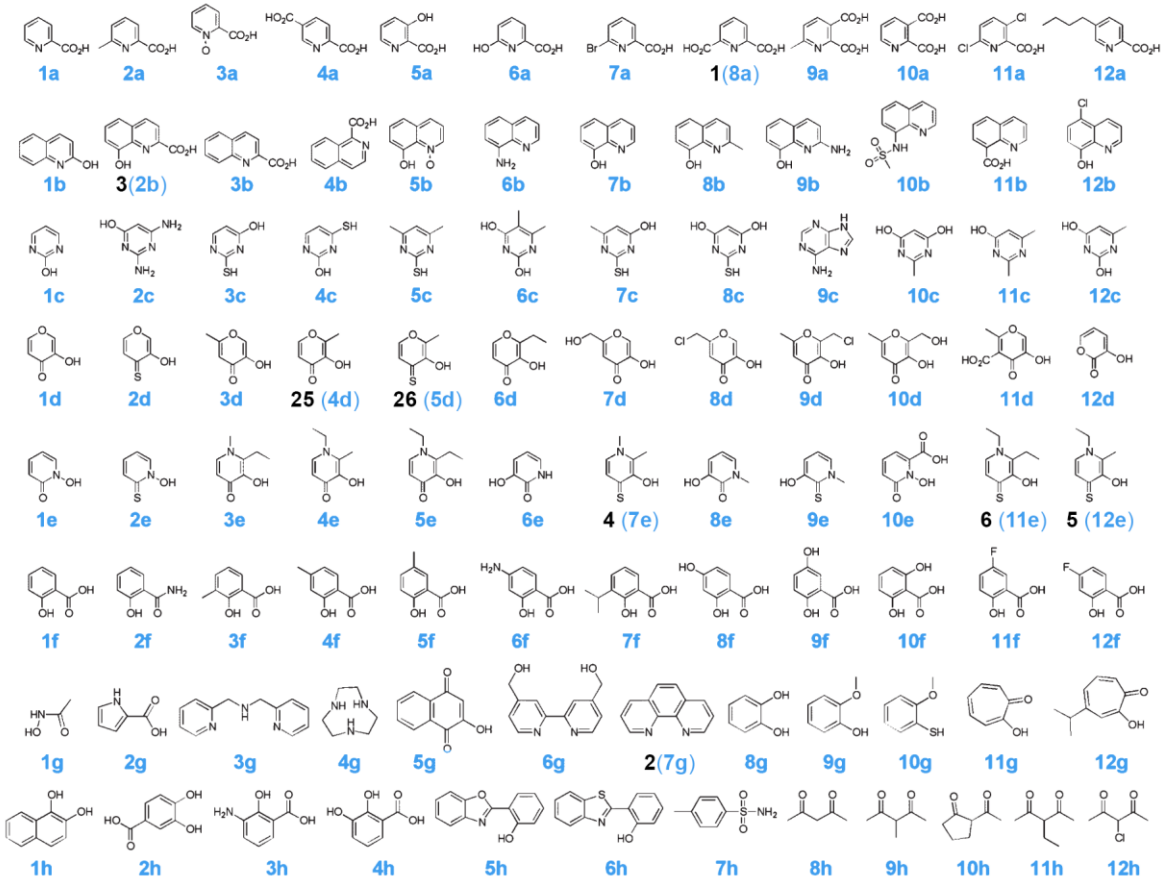


Figure S1: Compounds contained in CFL-1.1. Blue text reflects original library numbering. Black text corresponds to manuscript numbering.[4]

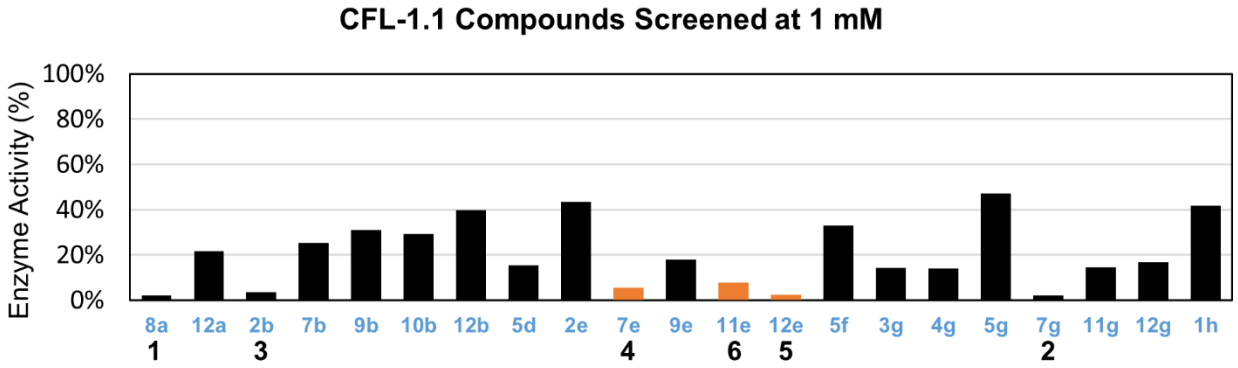


Figure S2: CFL-1 compounds with greater than 50% inhibition of BoNT/A1 LC, screened at 1 mM. Compounds indicated by orange bars possess the 3,4-HOPTO scaffold. Blue numbers reflect the original library (see above), black numbers correspond to manuscript numbering.

1.4 SNAPtide Competition Assay

The SNAPtide FRET assay was performed as described in **Section 1.2** and as previously described[2, 3], with the following modifications: 5 µL of each compound was added to 20 µL of BoNT/A1 LC and the plate was incubated at room temperature for 30 min followed by addition of 20 µL of substrate for a total assay volume of 50 µL. Data were analyzed using GraphPad Prism 8 with the custom equation in **Figure 2B**. The extra sum-of-squares test was used to compare the mutually exclusive binding case ($\alpha = 0$) and the non-mutually exclusive binding (α unconstrained).

Table S1. Results of the extra sum-of-squares F test to compare fits.

Comparison of Fits	
Null hypothesis	$\alpha = 0$ (mutually exclusive binding)
Alternative hypothesis	α unconstrained (non-mutually exclusive binding)
P value	0.5069
Conclusion (alpha = 0.05)	Do not reject null hypothesis
Preferred model	$\alpha = 0$
F (DFn, DFd)	0.4489 (1, 38)

1.5 Cell-Based Assay

The cell-based BoNT/A1 activity assay was performed as previously described.[5] The hiPSC derived GABA neurons and culture medium were purchased from Cellular Dynamics International (Madison, WI), and cultured in PLO-matrigel coated 96-well TPP plates (Midsci) for 12 days prior to the assay. For the inhibition assay, 200 LD_{50} Units of BoNT/A1 (150 kDa, specific activity 1.7 x 10^8 U/mg, produced from *C. botulinum* strain Hall A hyper as previously described[6]) was added to the cells in 50 µL stimulation medium (modified neurobasal medium containing 2.2 mM $CaCl_2$ and 56 mM KCl (Life Technologies), supplemented with B27 and glutaMAX). Cells were incubated at 37 °C in a humidified 5% CO_2 atmosphere for 7.5 min. The toxin was removed, and cells washed twice using 300 µL of culture medium. Inhibitor concentrations were added 30 minutes post toxin addition with a final DMSO concentration of 1–2%, ran in triplicate. Positive control (+/0) was toxin without inhibitor in culture media, and negative control (-/0) was culture media, both containing 1% DMSO. Cells were incubated for 7 h post toxin

addition at 37 °C, 5% CO_2 to allow for SNAP-25 cleavage. Culture medium was aspirated and cells lysed in 50 µL of 1× LDS lysis buffer (Life Technologies), and samples analyzed by Western blot using a monoclonal anti-SNAP-25 antibody (Synaptic Systems, Germany) as described previously.[7] Bands were visualized using Phosphaglo chemiluminescent reagent (KPL) on a Azure C600 imaging system equipped with a CCD camera.

1.6 MMP Inhibition Assay

The matrix metalloproteinase inhibitor profiling kit was purchased from Enzo Life Sci. (product #BML-AK308-0001) and the assay performed according to the product manual's specification. Briefly, the assay was conducted in CoStar 96-well black opaque-bottom plates, in the recommended assay buffer (50 mM HEPES, 10 mM $CaCl_2$, 0.05% Brij-35). MMPs were diluted to the recommended concentration and incubated with inhibitor at 37 °C. Substrate was added after incubation and the rate of fluorescence (λ_{ex}= 545 nm, λ_{em}= 576 nm) immediately read on a SpectraMax i3x (Molecular Devices) plate reader for a duration of 10 minutes. Measurements were performed in technical and experimental triplicate. Data were analyzed using GraphPad Prism 8 and IC_{50} values obtained using the four-parameter dose-response inhibition fit.

Table S2. MMP Inhibition values of **40**.

Enzyme	**IC_{50} (µM)**
MMP-1	14.2 ± 1.6
MMP-2	5.9 ± 2.0
MMP-3	5.1 ± 1.3
MMP-7	15.5 ± 3.0
MMP-8	3.6 ± 1.0
MMP-9	1.5 ± 0.1
MMP-13	3.6 ± 1.1
MMP-19	5.5 ± 2.3
BoNT/A LC	1.8 ± 0.1

1.7 Western Blots

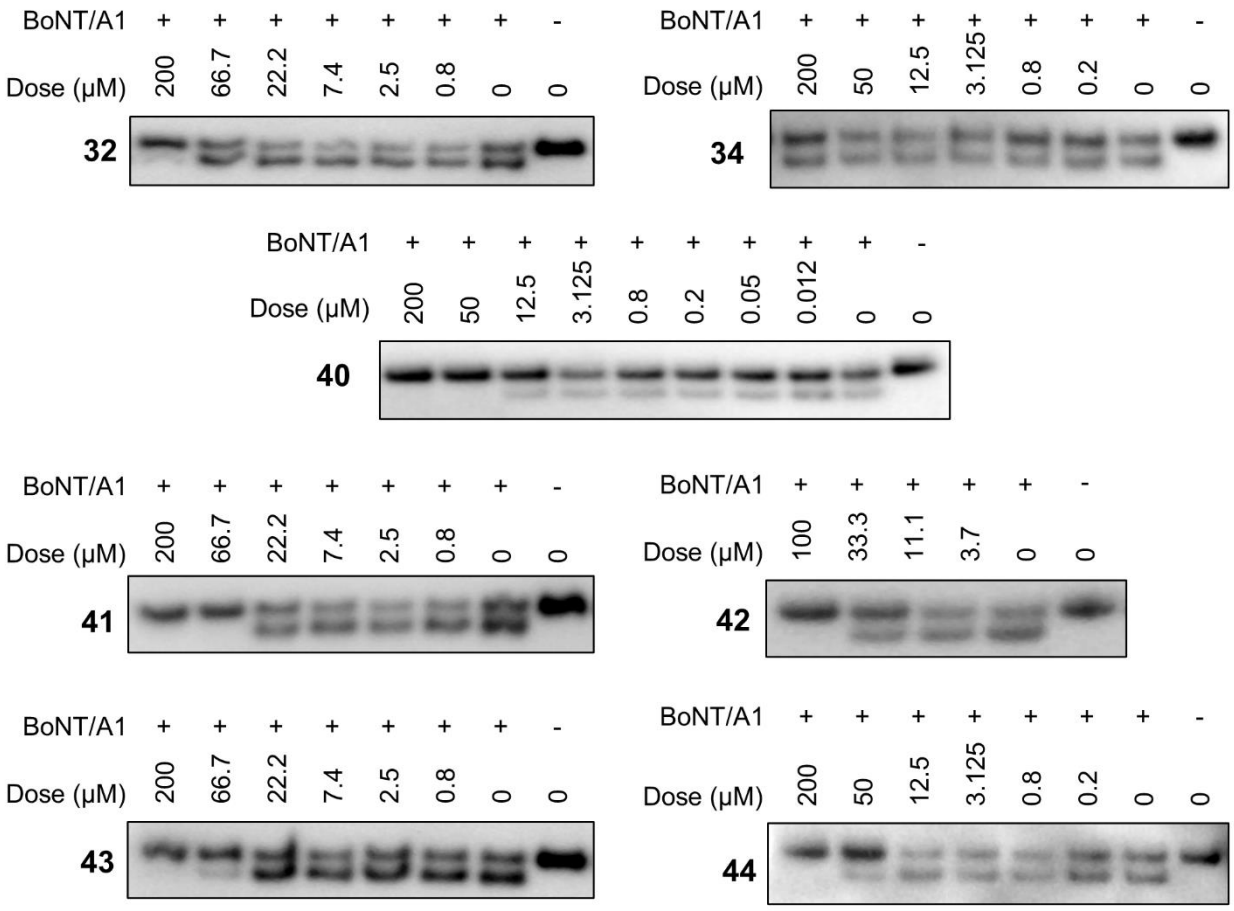


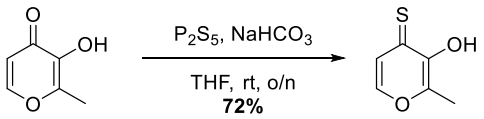
Figure S3: Representative images of cell-assay Western blots.

2. Chemistry

2.1 General Procedures and Instrumentation

Reactions were carried out under atmospheric conditions and all reagents obtained from commercial sources and used without further purification. Microwave reactions were carried out using a Biotage® Initiator. All reactions were monitored for completion using liquid chromatography-mass spectrometry (LC-MS). LC-MS analysis was performed on an Agilent 1260 Infinity II instrument coupled to a single quadrupole InfinityLab LC/MSD instrument running a gradient of increasing MeCN/water (5–95%) containing 0.1% formic acid, at a flow rate of 0.5 mL min^{-1} on a Zorbax 300SB-C8 column. Compound purity was determined using HPLC-MS and found to be ≥95%. Normal-phase (NP) and reverse-phase (RP) automated column chromatography (ACC) were performed on a CombiFlash Rf+ Lumen using RediSep Rf silica cartridges (15–30 g) columns for NP, or RediSep Rf Gold C_{18} HP 15.5 g cartridge for RP. ^{1}H NMR were recorded at 600 MHz on a Bruker AVIII HD 600 NMR spectrometer equipped with a 5 mm CPQCl CryoProbe. ^{13}C NMR were recorded at 151 MHz on a Bruker AVIII 600 NMR spectrometer equipped with a 5 mm CPDCH CryoProbe. Chemical shifts are reported in ppm, with reference to the residual solvent peak. Multiplicities are reported with coupling constants and are given to the nearest 0.1 Hz. High resolution mass spectrometry (HRMS) was carried out using an Agilent 1260 Infinity II instrument coupled to an Agilent 6230 TOF-MS spectrometer using electro spray ionisation (ES+/-), giving masses correct to four decimal places.

2.2 Synthesis and Characterization of Thiomaltol (26) and 3,4-HOPTO derivatives 21, 27-44



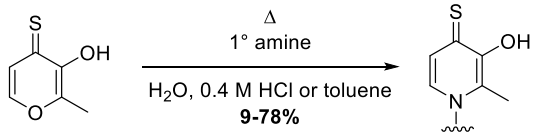
25 **26**

(**26**) Thiomaltol

Thiomaltol was synthesized according to a literature procedure outlined by Flematti *et al*.[8] Maltol (1.26 g, 10.0 mmol, 1.0 equiv.) was dissolved in THF (30 mL) and a solution of P_2S_5 (3.34 g, 15.0 mmol, 1.5 equiv.) dissolved in THF (10 mL) was added and stirred for 5 min. $NaHCO_3$ (5.00 g, 60.0 mmol, 6.0 equiv.) was added and the reaction stirred at r.t overnight. The reaction mixture was poured into water (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layers were dried over Na_2SO_4 and reduced *in vacuo* to give the titled compound (1.03 g, 7.24 mmol, 72%) as yellow crystalline needles. Spectral data agree with published data.

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.76 (br.s, 1H), 7.58 (d, *J* = 5.0 Hz, 1H), 7.31 (d, *J* = 5.0 Hz, 1H), 2.43 (s, 3H); **^{13}C NMR (151 MHz, $CDCl_3$)**: δ 186.0, 150.7, 147.1, 145.8, 124.3, 15.2; **MS (ES^+)**: *m/z* calcd for $[C_6H_7O_2S]^+$: 143.0, found: 143.0 – compound ionizes poorly.

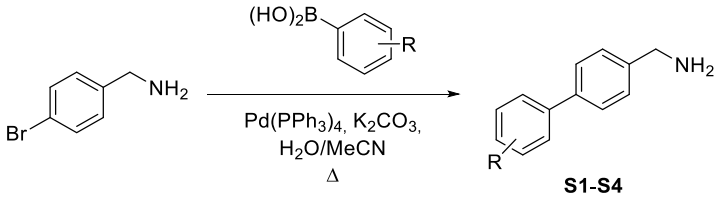
Method A



26 **21, 27-44**

Protocol adapted from syntheses outlined by Agrawal *et al* and Garner *et al*.[4, 9] Thiomaltol (1.0 equiv.) and the appropriate amine (1.2–2.0 equiv.) were suspended in either water (1–3 mL), 0.4 M HCl (1–3 mL), or toluene (0.5 mL) and the mixture refluxed for 16 h, or heated by microwave at 150 °C for 15 min. See individual compounds for purification.

Method B



S1-S4

4-Bromobenzylamine (1.0 equiv.), the appropriate boronic acid (1.1 equiv.) were dissolved in a well-stirred mixture of 2 M aqueous K_2CO_3 and MeCN (3:10) and the reaction heated to 90 °C. $Pd(PPh_3)_4$ (0.05 mmol, 0.05 equiv.) was added and the reaction heated overnight. See individual compounds for purification.

(**21**) 3-Hydroxy-2-methyl-1-(4-methylphenethyl)pyridine-4(1*H*)-thione
Synthesized by Method A (microwave) using thiomaltol (150 mg, 1.06 mmol, 1.0 equiv.), 4-methylphenethylamine (243 mg, 1.79 mmol, 1.7 equiv.) and 0.4 M HCl (1 mL).). The reaction mixture was poured into EtOAc (20 mL) and the organic layer separated. The aqueous layer was extracted with EtOAc (2 × 20 mL) and the combined organic layers dried over Na2SO4 and reduced in vacuo to give the crude product. The crude product was purified by NP ACC (50–100% EtOAc–hexane, followed by 10% MeOH flush) to afford the titled compound (130 mg, 0.50 mmol, 47%) was collected as an off-white amorphous solid.
^{1}H NMR (600 MHz, $CDCl_3$): δ 8.76 (br.s, 1H), 7.35 (d, *J* = 6.7 Hz, 1H), 7.10 (d, *J* = 7.7 Hz, 2H), 6.91 (d, *J* = 7.9 Hz, 2H), 6.83 (d, *J* = 6.7 Hz, 1H), 4.19 (t, *J* 7.0 Hz, 2H), 3.01 (t, *J* 7.0 Hz, 2H), 2.42 (s, 3H), 2.32 (s, 3H); **^{13}C NMR (151 MHz, $CDCl_3$)**: δ 171.0, 154.1, 137.5, 132.6, 131.1, 130.0, 128.7, 127.0, 126.1, 56.9, 36.7, 21.2, 12.7; **HPLC**, t_R 6.26 min (>98%, UV_{280}); **HRMS (ES$^+$)**: *m/z* calcd for $[C_{15}H_{18}NOS]^+$: 260.1104, found: 260.1094.

(**27**) 3-Hydroxy-2-methyl-1-(4-methylbenzyl)pyridine-4(1*H*)-thione
Synthesized by Method A (microwave) using thiomaltol (220 mg, 1.55 mmol, 1.0 equiv.), 4-methylbenzylamine (335 µL, 2.64 mmol, 1.7 equiv.) and 0.4 M HCl (1 mL). The mixture was cooled to r.t and then extracted with EtOAC, dried over Na_2SO_4 and reduced *in vacuo* to reveal the crude product. The crude product was triturated in hexane:EtOAc (1:1) and the titled compound (180 mg, 0.73 mmol, 47%) collected as a yellow amorphous solid.
^{1}H NMR (600 MHz, $CDCl_3$): δ 8.76 (br.s, 1H), 7.51 (d, *J* = 6.7 Hz, 1H), 7.21–7.16 (m, 3H), 6.92 (d, *J* = 8.0 Hz, 2H), 5.17 (s, 2H), 2.39 (s, 3H), 2.35 (s, 3H); **^{13}C NMR (151 MHz, $CDCl_3$)**: δ 171.6, 154.4, 139.0, 131.7, 130.8, 130.3, 127.9, 126.32, 126.30, 58.7, 21.2, 13.0; **HPLC**, t_R 6.11 min (>98%, UV_{280}); **HRMS (ES$^+$)**: *m/z* calcd for $[C_{14}H_{16}NOS]^+$: 246.0947, found: 246.0956.

(**28**) 1-(4-Chlorobenzyl)-3-hydroxy-2-methylpyridine-4(1*H*)-thione
Synthesized by Method A (thermal) using thiomaltol (142 mg, 1.00 mmol, 1.0 equiv), 4-chlorobenzylamine (243 µL, 2.00 mmol, 2.0 equiv.) and 0.4 M HCl (2 mL). The reaction mixture was diluted with 1M HCl and the corresponding precipitate filtered. The titled compound (76 mg, 0.29 mmol, 29%) was collected as a beige amorphous solid.
^{1}H NMR (600 MHz, $CDCl_3$): δ 8.77 (br.s, 1H), 7.52 (d, *J* = 6.7 Hz, 1H), 7.39–7.34 (m, 2H), 7.18 (d, *J* = 6.7 Hz, 1H), 6.98–6.95 (m, 2H), 5.18 (s, 2H), 2.37 (s, 3H); **^{13}C NMR (151 MHz, $CDCl_3$)**: δ 172.4, 154.5, 135.1, 132.5, 131.6, 129.9, 127.6, 127.4, 126.4, 58.2, 13.0; **HPLC**, t_R 6.17 min (>98%, UV_{280}); **HRMS (ES$^+$)**: *m/z* calcd for $[C_{13}H_{13}ClNOS]^+$: 266.0401, found: 266.0414.

(**29**) 1-(4-Bromobenzyl)-3-hydroxy-2-methylpyridine-4(1*H*)-thione

Synthesized by Method A (microwave) using thiomaltol (150 mg, 1.06 mmol, 1.0 equiv.), 4-bromobenzylamine (267 µL, 2.11 mmol, 2.0 equiv.) and water (1 mL). The crude product was purified in the same manner as outlined for compound **27**. The titled compound (130 mg, 0.42 mmol, 40%) was collected as a brown amorphous solid.
^{1}H NMR (600 MHz, $CDCl_3$): δ 8.77 (br.s, 1H), 7.54–7.50 (m, 3H), 7.17 (d, *J* = 6.7 Hz, 1H), 6.91 (d, *J* = 8.4 Hz, 2H), 5.16 (s, 2H), 2.37 (s, 3H); **^{13}C NMR (151 MHz, $CDCl_3$)**: δ 172.4, 154.5, 133.0, 132.9, 131.6, 127.8, 127.4, 126.4, 123.1, 58.2, 13.0; **HPLC**, t_R 6.27 min (>98%, UV_{280}); **HRMS (ES^+)**: *m/z* calcd for $[C_{13}H_{13}BrNOS]^+$: 309.9896 (^{79}Br) and 311.9876 (^{81}Br), found: 309.9883 and 311.9879.

(**30**) 3-Hydroxy-1-(4-methoxybenzyl)-2-methylpyridine-4(1*H*)-thione
Synthesized by Method A (thermal) using thiomaltol (142 mg, 1.00 mmol, 1.0 equiv.), 4-methoxybenzylamine (261 µL, 2.00 mmol, 2.0 equiv.) and 0.4 M HCl (2 mL). The reaction mixture was diluted with 1 M HCl (8 mL) and extracted with EtOAc (3 × 15 mL), dried over Na_2SO_4 and reduced *in vacuo* to give the crude product. The crude product was purified by RP ACC (0–100% MeCN–H_2O in 0.1% formic acid) and the titled compound (85 mg, 0.33 mmol, 33%) collected as a brown solid after lyophilization.
^{1}H NMR (600 MHz, $CDCl_3$): δ 8.76 (br.s, 1H), 7.50 (d, *J* = 6.7 Hz, 1H), 7.17 (d, *J* = 6.7 Hz, 1H), 7.00–6.96 (m, 2H), 6.92–6.88 (m, 2H), 5.14 (s, 2H), 3.80 (s, 3H), 2.41 (s, 3H); **^{13}C NMR (151 MHz, $CDCl_3$)**: δ 171.6, 160.1, 154.4, 131.5, 128.0, 127.8, 126.3, 125.6, 115.0, 58.4, 55.5, 13.0; **HPLC**, t_R 5.84 min (>95%, UV_{280}); **HRMS (ES^+)**: *m/z* calcd for $[C_{14}H_{16}NO_2S]^+$: 262.0896, found: 262.0907.

(**31**) *3*-Hydroxy-1-(4-hydroxybenzyl)-2-methylpyridine-4(1*H*)-thione
Synthesized by Method A (microwave) using thiomaltol (160 mg, 1.13 mmol, 1.0 equiv.), 4-hydroxybenzylamine (277 mg, 2.25 mmol, 2.0 equiv.) and water (1 mL). The crude product was purified in the same manner as outlined for compound **21**. The titled compound (50 mg, 0.20 mmol, 18%) was obtained as a brown amorphous solid.
^{1}H NMR (600 MHz, DMSO-d_6): δ 9.39 (s, 1H), 9.03 (br.s, 1H), 7.83 (br.s, 1H), 7.23–7.19 (m, 2H), 7.08 (d, *J* = 5.2 Hz, 1H), 6.72–6.69 (m, 2H), 4.09 (s, 2H), 2.34 (s, 3H); **^{13}C NMR (151 MHz, DMSO-d_6)**: δ 156.6, 147.2, 144.9, 139.8, 135.9, 130.1, 126.3, 118.5, 115.2, 33.7, 19.4; **HPLC**, t_R 4.22 min (>95%, UV_{280}); **HRMS (ES^+)**: *m/z* calcd for $[C_{13}H_{14}NO_2S]^+$: 248.0740, found: 248.0736.

(**32**) 3-Hydroxy-2-methyl-1-(4-nitrobenzyl)pyridine-4(1*H*)-thione
Synthesized by Method A (microwave) using thiomaltol (150 mg, 1.06 mmol, 1.0 equiv.), 4-nitrobenzylamine hydrochloride (398 mg, 2.11 mmol, 2.0 equiv.) and water (2 mL). The reaction mixture was diluted with 1 M HCl (10 mL) and the resulting precipitate filtered and triturated with DCM. The titled compound (75 mg, 0.27 mmol, 26%) was collected as an off-white amorphous solid.
^{1}H NMR (600 MHz, DMSO-d_6): δ 8.76 (br.s, 1H), 8.27–8.24 (m, 2H), 7.86 (d, *J* = 6.7 Hz, 1H), 7.43 (d, *J* = 6.7 Hz, 1H), 7.39–7.36 (m, 2H), 5.64 (br.s, 2H), 2.28 (s, 3H); **^{13}C NMR (151 MHz, DMSO-d_6)**: δ 170.3, 152.7, 147.1, 143.1, 133.6, 128.1, 127.6, 125.1, 124.2, 56.9, 12.5; **HPLC**, t_R 5.84 min (>97%, UV_{254}); **HRMS (ES^+)**: *m/z* calcd for $[C_{13}H_{13}N_2O_3S]^+$: 277.0641, found: 277.0632.

(**33**) 1-(2,4-Dichlorobenzyl)-3-hydroxy-2-methylpyridine-4(1*H*)-thione
Synthesized by Method A (microwave) using thiomaltol (280 mg, 1.97 mmol, 1.0 equiv.), 2,4-dichlorobenzylamine (530 µL, 3.94 mmol, 2.0 equiv.) and 0.4 M HCl (2 mL). The reaction mixture was diluted with EtOAc (10 mL) and sat. $NaHCO_3$ (5 mL). The resulting yellow solid was filtered and crystallized from $CHCl_3$ using hexane as the anti-solvent. The titled compound (180 mg, 0.60 mmol, 30%) was collected as yellow microneedles.
^{1}H NMR (600 MHz, DMSO-d_6): δ 8.77 (s, 1H), 7.78 (d, *J* = 2.1 Hz, 1H), 7.73 (d, *J* = 6.7 Hz, 1H), 7.44 (dd, *J* 8.4 and 2.1 Hz, 1H), 7.41 (d, *J* 6.7 Hz, 1H), 6.71 (d, *J* = 8.4 Hz, 1H), 5.50 (s, 2H), 2.28 (s, 3H); **^{13}C NMR (151 MHz,**

DMSO-d_6): δ 170.4, 152.7, 133.6, 133.4, 132.4, 132.2, 129.3, 128.7, 128.34, 128.27, 125.2, 54.9, 12.3; **HPLC**, t_R 6.54 min (>98%, UV_{280}); **HRMS (ES$^+$)**: *m/z* calcd for $[C_{13}H_{12}Cl_2NOS]^+$: 300.0011, found: 300.0015.

(**34**) 1-(2,4-Dichlorophenethyl)-3-hydroxy-2-methylpyridine-4(1*H*)-thione
Synthesized by Method A (microwave) using thiomaltol (150 mg, 1.06 mmol, 1.0 equiv.), 2,4-dichlorophenethylamine (341 mg, 1.79 mmol, 1.7 equiv.) and water (1 mL). The crude product was purified in the same manner as outlined for compound **33**. The titled compound (260 mg, 0.83 mmol, 78%) was collected as brown microcrystals.
^{1}H NMR (600 MHz, $CDCl_3$): δ 8.76 (br.s, 1H), 7.44 (d, *J* 2.0 Hz, 1H), 7.38 (d, *J* 6.5 Hz, 1H), 7.18 (dd, *J* 8.1 and 2.0 Hz, 1H), 6.93 (d, *J* 8.2 Hz, 1H), 6.88 (d, *J* 6.6 Hz, 1H), 4.23 (t, *J* 7.1 Hz, 2H), 3.16 (t, *J* 6.9 Hz, 2H), 2.49 (s, 3H); **^{13}C NMR (151 MHz, $CDCl_3$)**: δ 171.7, 154.2, 134.77, 134.76, 132.03, 131.96, 130.9, 130.0, 128.1, 126.8, 126.3, 54.3, 34.6, 12.7; **HPLC**, t_R 6.54 min (>98%, UV_{280}); **HRMS (ES$^+$)**: *m/z* calcd for $[C_{14}H_{14}Cl_2NOS]^+$: 314.0168, found: 314.0176.

(**35**) 1-(4-Chlorophenethyl)-3-hydroxy-2-methylpyridine-4(1*H*)-thione
Synthesized by Method A (microwave) using thiomaltol (150 mg, 1.06 mmol, 1.0 equiv.), 2-(4-chlorophenyl)ethylamine (200 µL, 1.43 mmol, 1.3 equiv.) and water (1 mL). The crude product was purified in the same manner as outlined for compound **33**. The titled compound (184 mg, 0.66 mmol, 62%) was collected as off-white plates.
^{1}H NMR (600 MHz, $CDCl_3$): δ 8.74 (br.s, 1H), 7.35 (d, *J* = 6.5 Hz, 1H), 7.28 (d, *J* = 8.2 Hz, 2H), 6.96 (d, *J* = 8.3 Hz, 2H), 6.82 (d, *J* = 6.6 Hz, 1H), 4.20 (t, *J* 6.9 Hz, 2H), 3.03 (t, *J* 6.7 Hz, 2H), 2.43 (s, 3H); **^{13}C NMR (151 MHz, $CDCl_3$)**: δ 171.2, 154.1, 134.1, 133.8, 131.0, 130.2, 129.5, 126.8, 126.1, 56.5, 36.4, 12.7; **HPLC**, t_R 6.28 min (>98%, UV_{280}); **HRMS (ES$^+$)**: *m/z* calcd for $[C_{14}H_{15}ClNOS]^+$: 280.0557, found: 280.0547.

(**36**) 1-(2,4-Dimethylphenethyl)-3-hydroxy-2-methylpyridine-4(1*H*)-thione
Synthesized by Method A (microwave) using thiomaltol (150 mg, 1.06 mmol, 1.0 equiv.), 2,4-dimethylphenethylamine (268 mg, 1.79 mmol, 1.7 equiv.) and water (1 mL). The crude product was purified in the same manner as outlined for compound **27**. The titled compound (127 mg, 0.46 mmol, 44%) was collected as yellow amorphous solid.
^{1}H NMR (600 MHz, $CDCl_3$): δ 8.75 (br.s, 1H), 7.36 (d, *J* = 6.6 Hz, 1H), 6.99 (s, 1H), 6.93 (d, *J* = 7.6 Hz, 1H), 6.82 (d, *J* = 6.7 Hz, 1H), 6.79 (d, *J* 7.7 Hz, 1H), 4.16 (t, *J* = 7.1 Hz, 2H), 3.03 (t, *J* = 7.0 Hz, 2H), 2.43 (s, 3H), 2.29 (s, 3H), 2.19 (s, 3H); **^{13}C NMR (151 MHz, $CDCl_3$)**: δ 171.0, 154.1, 137.6, 135.8, 131.8, 131.0, 130.9, 129.6, 127.6, 127.0, 126.2, 55.8, 34.0, 21.2, 19.3, 12.6; **HPLC**, t_R 6.48 min (>98%, UV_{280}); **HRMS (ES$^+$)**: *m/z* calcd for $[C_{16}H_{20}NOS]^+$: 274.1260, found: 274.1273.

(**37**) 1-(3,4-Dimethylphenethyl)-3-hydroxy-2-methylpyridine-4(1*H*)-thione
Synthesized by Method A (microwave) using thiomaltol (150 mg, 1.06 mmol, 1.0 equiv.), 2,4-dimethylphenethylamine (268 mg, 1.79 mmol, 1.7 equiv.) and water (1 mL). The crude product was purified in the same manner as outlined for compound **27**. The titled compound (127 mg, 0.46 mmol, 44%) was collected as a yellow amorphous solid.
^{1}H NMR (600 MHz, $CDCl_3$): δ 8.76 (br.s, 1H), 7.36 (d, *J* = 6.6 Hz, 1H), 7.05 (d, *J* = 7.6 Hz, 1H), 6.86 (d, *J* = 6.7 Hz, 1H), 6.81 (s, 1H), 6.75 (d, *J* = 7.6 Hz, 1H), 4.18 (t, *J* = 7.1 Hz, 2H), 2.97 (t, *J* = 7.1 Hz, 2H), 2.44 (s, 3H), 2.23 (s, 3H), 2.22 (s, 3H); **^{13}C NMR (151 MHz, $CDCl_3$)**: δ 170.0, 154.1, 137.6, 136.1, 133.0, 131.1, 130.4, 130.0, 127.0, 126.13, 126.06, 56.9, 36.7, 19.9, 19.5, 12.7; **HPLC**, t_R 6.48 min (>98%, UV_{280}); **HRMS (ES$^+$)**: *m/z* calcd for $[C_{16}H_{20}NOS]^+$: 274.1260, found: 274.1264.

(**38**) 1-(((3r,5r,7r)-Adamantan-1-yl)methyl)-3-hydroxy-2-methylpyridine-4(1*H*)-thione
Synthesized by Method A (microwave) using thiomaltol (150 mg, 1.06 mmol, 1.0 equiv.), amantadine hydrochloride (230 mg, 1.23 mmol, 1.2 equiv.) and 0.5 M aqueous K_2CO_3 (1 mL). The crude product was purified in the same manner as outlined for compound **21**. The titled compound (91 mg, 0.31 mmol, 30%) was collected as an off-white semi-solid.
^{1}H NMR (600 MHz, $CDCl_3$): δ 8.74 (br.s, 1H), 7.46 (d, *J* = 6.7 Hz, 1H), 7.04 (d, *J* = 6.7 Hz, 1H), 3.74 (s, 2H), 2.50 (s, 3H), 2.03 (br.s, 3H), 1.72 (br.d, *J* = 12.3 Hz, 3H), 1.59 (br.d, *J* = 11.7 Hz, 3H), 1.53 (br.d, *J* = 2.1 Hz, 6H); **^{13}C NMR (151 MHz, $CDCl_3$)**: δ 170.3, 154.0, 132.4, 128.5, 125.3, 65.9, 40.7, 36.4, 36.0, 28.1, 14.3; **HPLC**, t_R 6.88 min (>98%, UV_{280}); **HRMS (ES^+)**: *m/z* calcd for $[C_{17}H_{24}NOS]^+$: 290.1573, found: 290.1574.

(**39**) 1-(2-((3r,5r,7r)-Adamantan-1-yl)ethyl)-3-hydroxy-2-methylpyridine-4(1*H*)-thione
Synthesized by Method A (microwave) using thiomaltol (150 mg, 1.06 mmol, 1.0 equiv.), 2-(1-adamantyl)ethanamine hydrochloride (387 mg, 1.79 mmol, 1.7 equiv.), 0.5 M aqueous K_2CO_3 (1 mL). The crude product was purified in the same manner as outlined for compound **2s1**. The titled compound (138 mg, 0.45 mmol, 43%) was collected as off-white amorphous solid.**^{1}H NMR (600 MHz, $CDCl_3$)**: δ 8.75 (br.s, 1H), 7.48 (d, *J* = 5.2 Hz, 1H), 7.10 (d, *J* = 6.3 Hz, 1H), 4.01–3.96 (m, 2H), 2.48 (s, 3H), 2.02 (br.s, 3H), 1.75 (br.d, *J* = 12.6 Hz, 3H), 1.64 (br.d, *J* = 12.0 Hz, 3H), 1.57 (br.s, 6H), 1.53–1.49 (m, 2H); **^{13}C NMR (151 MHz, $CDCl_3$)**: δ 170.1, 154.2, 130.8, 127.2, 126.6, 51.2, 45.3, 42.2, 36.8, 32.3, 28.4, 12.7; **HPLC**, t_R 7.26 min (>98%, UV_{280}); **HRMS (ES^+)**: *m/z* calcd for $[C_{18}H_{26}NOS]^+$: 304.1730, found: 304.1744.

(**40**) 1-(2-([1,1'-Biphenyl]-4-yl)ethyl)-3-hydroxy-2-methylpyridine-4(1*H*)-thione
Synthesized by Method A (microwave) using thiomaltol (100 mg, 0.70 mmol, 1.0 equiv.), 2-(4-biphenyl)ethylamine (166 mg, 0.84 mmol, 1.2 equiv.) and water (3 mL). The reaction mixture was diluted with 1 M HCl (3 mL) and extracted with DCM (3 × 20 mL), dried over Na_2SO_4 and reduced *in vacuo* to give the crude product. The crude product was purified by RP ACC (50–75% hold MeCN–H_2O in 0.1% formic acid). Appropriate fractions were pooled and extracted with DCM, dried over Na_2SO_4 and reduced *in vacuo* to afford the titled compound (98 mg, 0.30 mmol, 44%) as an off-white amorphous solid. **^{1}H NMR (600 MHz, $CDCl_3$)**: δ 8.80 (br.s, 1H), 7.58–7.53 (m, 4H), 7.46–7.43 (m, 2H), 7.39 (d, *J* = 6.7 Hz, 1H), 7.38–7.34 (m, 1H), 7.13–7.10 (m, 2H), 6.89 (d, *J* = 6.7 Hz, 1H), 4.25 (t, *J* = 7.1 Hz, 2H), 3.10 (t, *J* = 7.0 Hz, 2H), 2.47 (s, 3H); **^{13}C NMR (151 MHz, $CDCl_3$)**: δ 171.4, 154.3, 140.8, 140.3, 134.7, 131.0, 129.3, 129.0, 128.0, 127.7, 127.1, 126.9, 126.2, 56.7, 36.8, 12.7; **HPLC**, t_R 6.80 min (>95%, UV_{254}); **HRMS (ES^+)**: *m/z* calcd for $[C_{20}H_{20}NOS]^+$: 322.1260, found: 322.1272.

(**S1**) (4'-fluoro-[1,1'-biphenyl]-4-yl)methanamine
Synthesized by Method B using 4-bromobenzylamine (316 µL, 2.50 mmol, 1.0 equiv.), 4-fluorophenylboronic acid (384 mg, 2.75 mmol, 1.1 equiv.), $Pd(PPh_3)_4$ (145 mg, 0.13 mmol, 0.05 equiv.), 2 M aqueous K_2CO_3 (3 mL) and MeCN (10 mL). The reaction mixture was filtered through celite and concentrated. The crude residue was diluted with EtOAc and washed successively with water (2 × 20 mL) and sat. $NaHCO_3$ (20 mL). The organic phase was then extracted with 1 M HCl (2 × 20 mL), and the aqueous phase basified by addition of 15% NaOH. The aqueous phase was extracted with EtOAc (2 × 20 mL), and the organic phase dried over Na_2SO_4, and concentrated *in vacuo* to yield the titled compound (456 mg, 2.50 mmol, 91%) as a pale yellow amorphous solid. **^{1}H NMR (600 MHz, DMSO-*d*$_6$)** δ 8.43 (br.s, 1H), 7.75–7.60 (m, 4H), 7.51 (d, *J* = 7.7 Hz, 2H), 7.28 (t, *J* = 8.6 Hz, 2H), 3.97 (s, 2H); **^{13}C NMR (151 MHz, DMSO-*d*$_6$)** δ 161.9 (d, *J* = 244.4 Hz), 138.5, 136.2 (d, *J* = 3.4 Hz), 129.0, 128.6 (d, *J* = 8.2 Hz), 126.6, 115.7 (d, *J* = 21.2 Hz), 42.5, one Ar-C not observed; **LC-MS**: *m/z* calcd for $[C_{13}H_{13}FN]^+$: 202.1, found: 185.0 – mass adduct minus NH_2.

(**41**) 1-((4'-fluoro-[1,1'-biphenyl]-4-yl)methyl)-3-hydroxy-2-methylpyridine-4(1*H*)-thione

Synthesized by Method A (thermal) using thiomaltol (100 mg, 0.70 mmol, 1.0 equiv.), **S1** (283 mg, 1.41 mmol, 2.0 equiv.), and 0.4 M HCl (3 mL). The reaction mixture was extracted in DCM (3 × 10 mL), dried over Na_2SO_4, and concentrated *in vacuo*. Crude product was purified by RP ACC (10–100% MeCN–H_2O in 0.1% formic acid). The titled compound (64 mg, 0.28 mmol, 28%) was collected as pale-yellow crystals. **^{1}H NMR (600 MHz, $CDCl_3$)**: δ 8.78 (br.s, 1H), 7.57–7.49 (m, 5H), 7.23 (d, *J* = 6.6 Hz, 1H), 7.13 (t, *J* = 8.6 Hz, 2H), 7.09 (d, *J* = 7.9 Hz, 2H), 5.25 (s, 2H), 2.42 (s, 3H); **^{13}C NMR (151 MHz, $CDCl_3$)**: δ 172.0, 162.9 (d, *J* = 247.6 Hz), 154.4, 141.1, 136.1 (d, *J* = 3.3 Hz), 132.9, 131.7, 128.8 (d, *J* = 8.2 Hz), 128.1, 127.8, 126.8, 126.4, 116.0 (d, *J* = 21.4 Hz), 58.6, 13.0; **HPLC**, t_R 6.69 min (>95%, UV_{254}); **HRMS (ES$^+$)**: *m/z* calcd for $[C_{19}H_{17}FNOS]^+$: 326.1009, found: 326.1023.

(**S2**) 4-(4-Chlorophenyl)benzylamine
Synthesized by Method B using 4-bromobenzylamine (126 µL, 1.00 mmol, 1.0 equiv.), 4-chlorophenylboronic acid (172 mg, 1.10 mmol, 1.1 equiv.), $Pd(PPh_3)_4$ (58 mg, 0.05 mmol, 0.05 equiv.), 2 M aqueous K_2CO_3 (3 mL) and MeCN (10 mL). The crude product was purified in the same manner as outlined for compound **S1**. The titled compound (110 mg, 0.51 mmol, 51%) was collected as a white solid*. **^{1}H NMR (600 MHz, DMSO-*d*$_6$)**: δ 7.69–7.66 (m, 2H), 7.61–7.59 (m, 2H), 7.51–7.48 (m, 2H), 7.42 (d, *J* = 7.9 Hz, 2H), 3.75 (s, 2H); **^{13}C NMR (151 MHz, DMSO-*d*$_6$)**: δ 143.8, 139.0, 136.8, 132.0, 128.8, 128.3, 127.7, 126.4, 45.2; **LC-MS**: *m/z* calcd for $[C_{13}H_{13}ClN]^+$: 218.1, found: 201.3 – mass adduct minus NH_2.
*~20% impurity arising from boronic acid, but compound used without further purification.

(**42**) 1-((4'-Chloro-[1,1'-biphenyl]-4-yl)methyl)-3-hydroxy-2-methylpyridine-4(1*H*)-thione
Synthesized by Method A (thermal) using thiomaltol (213 mg, 1.50 mmol, 1.0 equiv.), **S2** (718 mg, 3.30 mmol, 2.2 equiv.) and 0.4 M HCl (2 mL). The reaction mixture was reduced *in vacuo* and the crude product was purified by RP ACC (10–100% MeCN–H_2O in 0.1% formic acid). The titled compound (47 mg, 0.14 mmol, 9%) was collected as a yellow amorphous solid. **^{1}H NMR (600 MHz, DMSO-*d*$_6$)**: δ 8.76 (br.s, 1H), 7.87 (d, *J* = 6.7 Hz, 1H), 7.72–7.66 (m, 4H), 7.53–7.49 (m, 2H), 7.42 (d, *J* = 6.6 Hz, 1H), 7.23 (d, *J* = 8.2 Hz, 2H), 5.51 (s, 2H), 2.35 (s, 3H); **^{13}C NMR (151 MHz, DMSO-*d*$_6$)**: δ 169.7, 152.7, 138.5, 138.2, 135.0, 133.5, 132.6, 128.9, 128.4, 128.3, 127.3, 127.2, 124.9, 57.3, 12.6; **HPLC**, t_R 7.02 min (>95%, UV_{254}); **HRMS (ES$^+$)**: *m/z* calcd for $[C_{19}H_{17}ClNOS]^+$: 342.0714, found: 342.0705.

(**S3**) (3',4'-Difluoro-[1,1'-biphenyl]-4-yl)methanaminium formate
Synthesized by Method B using 4-bromobenzylamine (126 µL, 1.00 mmol, 1.0 equiv.), 3,4-difluorophenylboronic acid (176 mg, 1.10 mmol, 1.1 equiv.), $Pd(PPh_3)_4$ (58 mg, 0.05 mmol, 0.05 equiv.), 2 M $K_2CO_{3(aq)}$ (3 mL) and MeCN (10 mL). The reaction mixture was poured into EtOAc (30 mL) and water (30 mL) added. The organic layer was separated and washed with water (20 mL), brine (20 mL), dried (Na_2SO_4) and reduced *in vacuo* to give the crude product. The crude product was purified by RP ACC (10–100% MeCN–H_2O in 0.1% formic acid) and the titled compound (190 mg, 0.87 mmol, 87%) collected as fluffy white solid after lyophilization. **^{1}H NMR (600 MHz, DMSO-*d*$_6$)**: δ 8.36 (s, 1H), 7.80 (ddd, *J* = 12.3, 7.8 and 2.2 Hz, 1H), 7.74–7.70 (m, 2H), 7.58–7.49 (m, 4H), 3.99 (s, 2H); **^{13}C NMR (151 MHz, $CDCl_3$)**: δ 165.1, 149.8 (dd, *J* 245.3 and 12.8 Hz), 149.1 (dd, *J* 246.5 and 12.7 Hz), 137.4, 137.2 (dd, *J* 6.1 and 3.7 Hz), 136.1, 129.2, 126.8, 123.4 (dd, *J* 6.2 and 3.2 Hz), 117.9 (d, *J* 17.0 Hz), 115.7 (d, *J* 17.6 Hz), 42.4; **LC-MS**: *m/z* calcd for $[C_{13}H_{12}F_2N]^+$: 220.1, found: 203.4 – mass adduct minus NH_2.

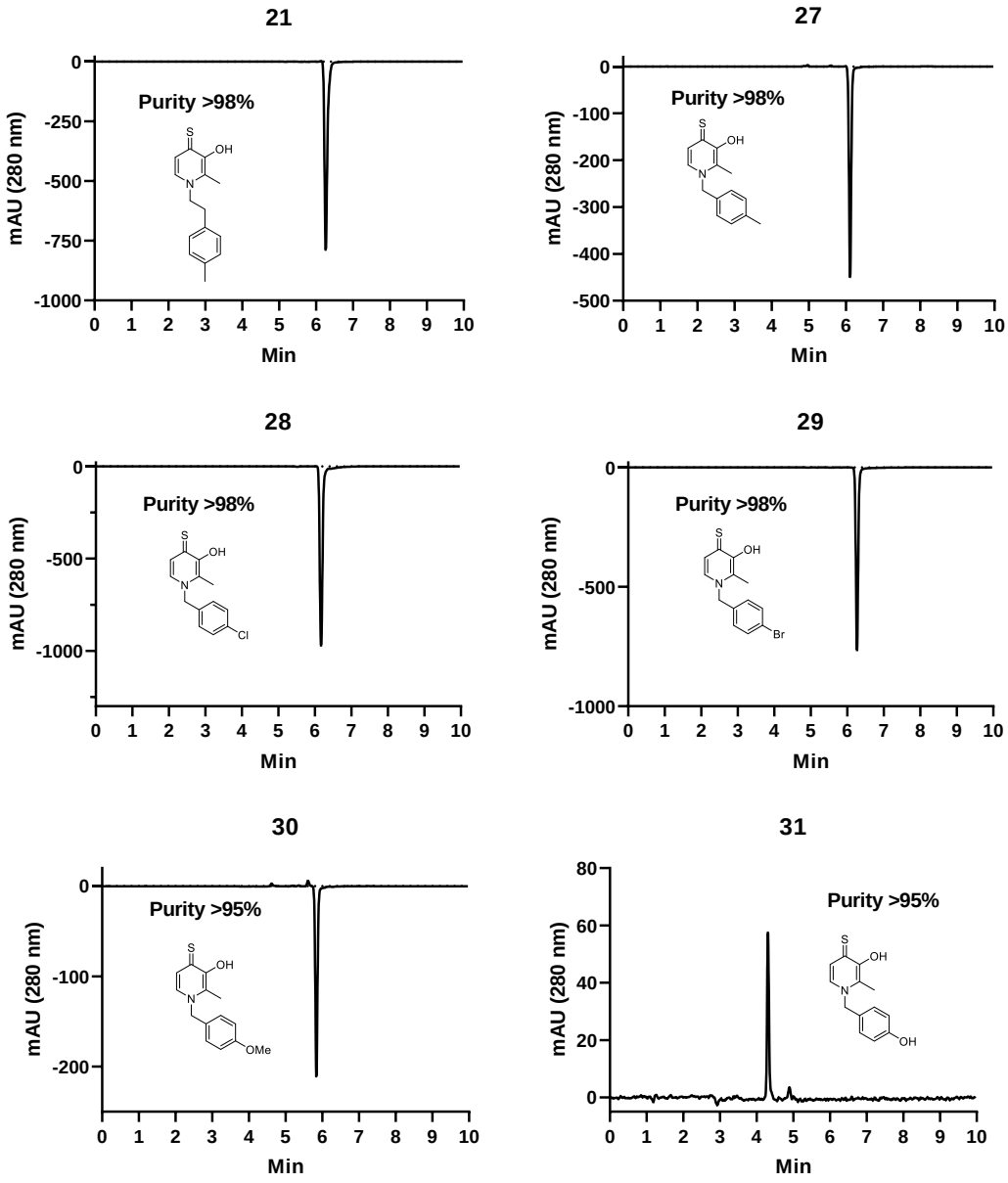
(**43**) 1-((3',4'-Difluoro-[1,1'-biphenyl]-4-yl)methyl)-3-hydroxy-2-methylpyridine-4(1*H*)-thione
Synthesized by Method A (thermal) using thiomaltol (81 mg, 0.57 mmol, 1.0 equiv.), **S3** (190 mg, 0.87 mmol, 1.5 equiv.) and toluene (0.5 mL). The reaction mixture was reduced *in vacuo* and the crude product purified by RP ACC (10–100% MeCN–H_2O in 0.1% formic acid). The titled compound (35 mg, 0.10 mmol, 18%) was collected as a yellow amorphous solid. **^{1}H NMR (600 MHz, DMSO-d$_6$)**: δ 8.76 (br.s, 1H), 7.88 (d, *J* = 6.7 Hz, 1H), 7.79–

7.74 (m, 1H), 7.72 (d, *J* 8.3 Hz, 2H), 7.54–7.49 (m, 2H), 7.42 (d, *J* = 6.6 Hz, 1H), 7.23 (d, *J* = 8.0 Hz, 2H), 5.51 (s, 2H), 2.34 (s, 3H); **^{13}C NMR (151 MHz, $CDCl_3$)**: δ 172.1, 154.5, 150.6 (dd, *J* 248.7 and 12.7 Hz), 150.4 (dd, *J* 249.8 and 12.6 Hz), 140.0, 137.1 (t, *J* 4.9 Hz), 133.5, 131.7, 128.1, 127.7, 126.9, 126.4, 123.2 (dd, *J* 6.3 and 3.5 Hz), 118.0 (d, *J* 17.2 Hz), 116.2 (d, *J* 17.8 Hz), 58.5, 13.1; **HPLC**, t_R 6.87 min (>95%, UV_{254}); **HRMS (ES^+)**: *m/z* calcd for $[C_{19}H_{16}F_2NOS]^+$: 344.0915, found: 344.0930.

(**S4**) (4-(Benzo[d][1,3]dioxol-5-yl)phenyl)methanamine
Synthesized by Method B using 4-bromobenzylamine (634 μL, 5.00 mmol, 1.0 equiv.), 1,3-benzodioxol-5-ylboronic acid (912 mg, 5.50 mmol, 1.1 equiv.), $Pd(PPh_3)_4$ (288 mg, 0.25 mmol, 0.05 equiv.), 2 M aqueous K_2CO_3 (7.5 mL) and MeCN (25 mL). The crude product was purified in the same manner as outlined for compound **S1**. The titled compound (810 mg, 3.55 mmol, 71%) was collected as a pale-yellow amorphous solid*. **^{1}H NMR (600 MHz, $CDCl_3$)**: δ 7.50–7.47 (m, 2H), 7.35 (d, *J* = 8.1 Hz, 2H), 7.07–7.04 (m, 2H), 6.88 (d, *J* = 7.9 Hz, 1H), 5.99 (s, 2H), 3.90 (s, 2H); **^{13}C NMR (151 MHz, $CDCl_3$)**: δ 148.2, 147.1, 142.2, 139.6, 135.5, 127.6, 127.2, 120.6, 108.7, 107.7, 101.3, 46.3; **LC-MS**: *m/z* calcd for $[C_{14}H_{14}NO_2]^+$: 228.1, found: 211.1 – mass adduct minus NH_2.
*~10% impurity arising from boronic acid, compound used without further purification.

(**44**) 1-(4-(Benzo[d][1,3]dioxol-5-yl)benzyl)-3-hydroxy-2-methylpyridine-4(1*H*)-thione
Synthesized by Method A (thermal) using thiomaltol (213 mg, 1.50 mmol, 1.0 equiv.), **S4** (681 mg, 3.00 mmol, 2.0 equiv.) and 0.4 M HCl (3 mL). The crude product was purified in the same manner as outlined for compound **30**. The titled compound (110 mg, 0.31 mmol, 21%) was collected as a pale-yellow amorphous solid. **^{1}H NMR (600 MHz, DMSO-d_6):** δ 8.76 (s, 1H), 7.87 (d, *J* = 6.7 Hz, 1H), 7.65–7.62 (m, 2H), 7.42 (d, *J* = 6.6 Hz, 1H), 7.24 (d, *J* = 1.9 Hz, 1H), 7.20–7.16 (d, *J* = 8.2 Hz, 2H), 7.13 (dd, *J* = 8.1 and 1.9 Hz, 1H), 6.99 (d, *J* = 8.1 Hz, 1H), 6.05 (s, 2H), 5.48 (s, 2H), 2.35 (s, 3H); **^{13}C NMR (151 MHz, DMSO-d_6):** δ 169.2, 152.2, 147.6, 146.6, 139.2, 133.7, 133.2, 133.1, 127.9, 126.6, 169.64, 126.61, 124.5, 119.9, 108.3, 106.6, 100.7, 56.9, 12.1; **HPLC**, t_R 6.67 min (>95%, UV_{214}); **HRMS (ES^+):** *m/z* calcd for $[C_{20}H_{18}NO_3S]^+$: 352.1002, found: 352.1017.

2.3 HPLC and NMR Spectra



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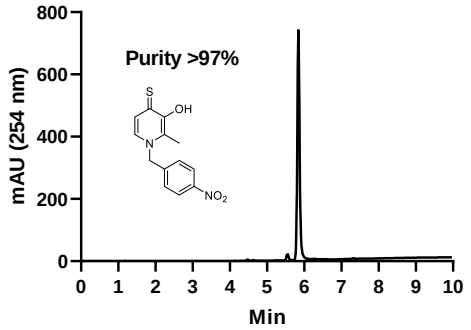

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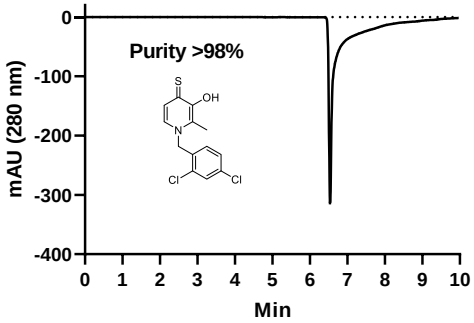

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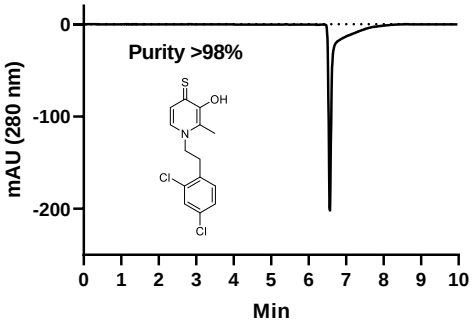

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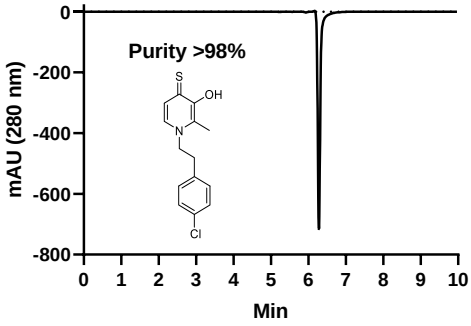

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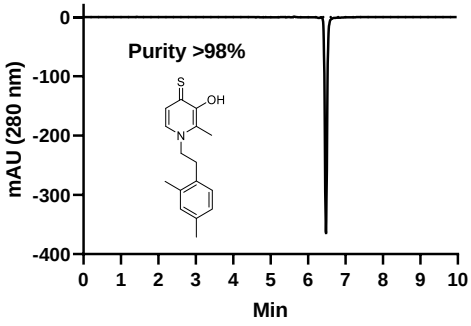

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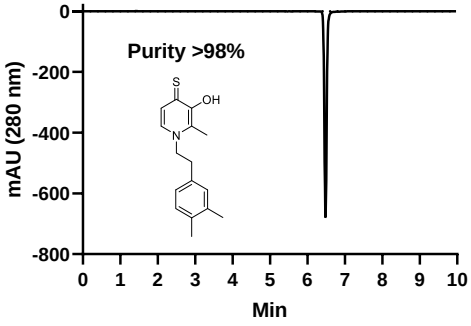

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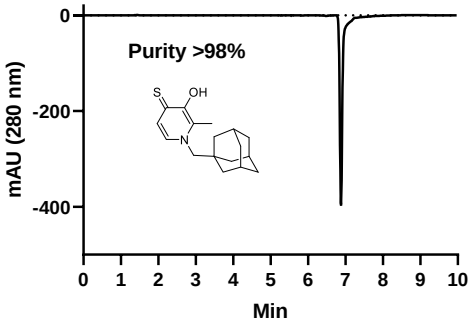

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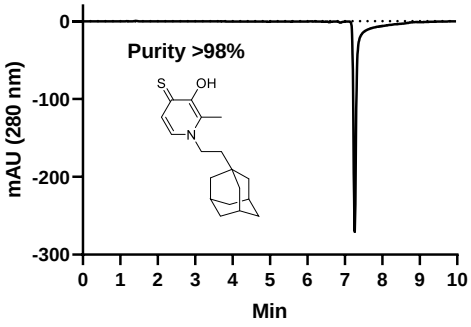

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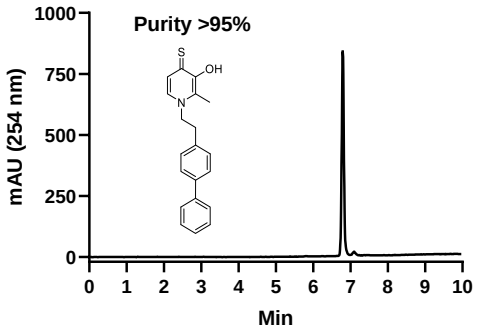
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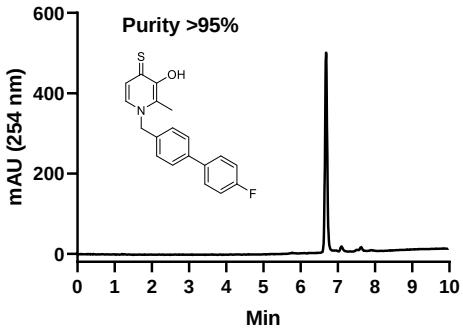
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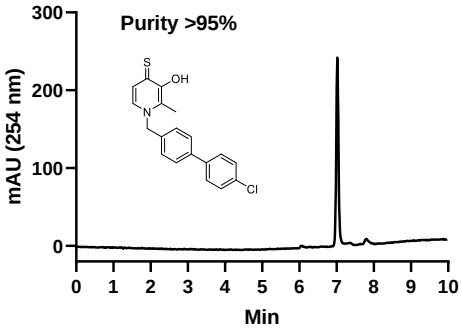
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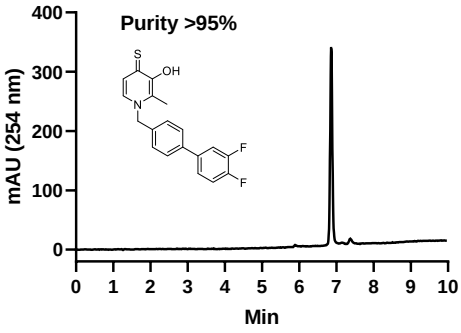
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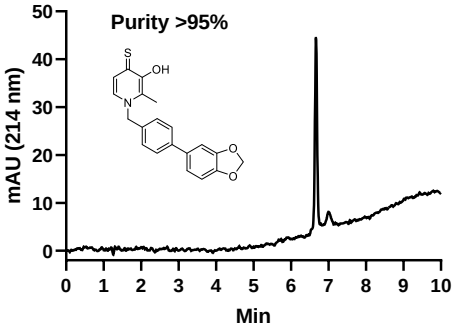
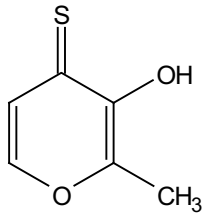
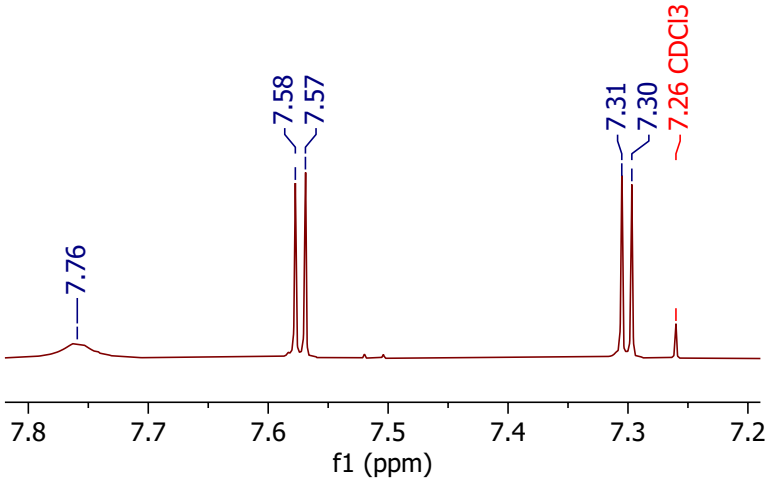
44

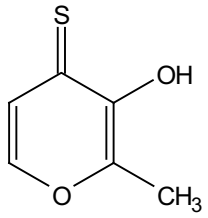

Purity >95%
mAU (214 nm)
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10
Min
S
OH
N
O
O

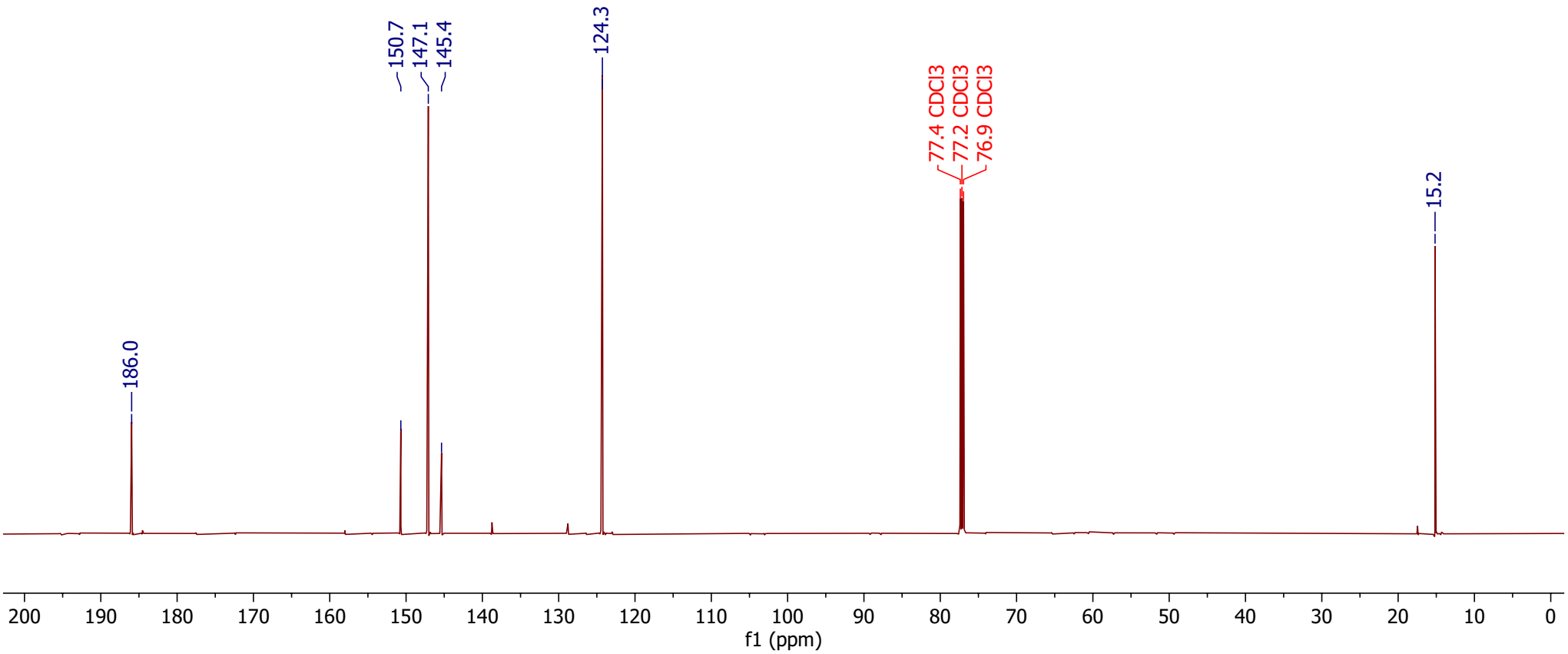
Compound 26
^{1}H NMR (600 MHz, CDCl3)



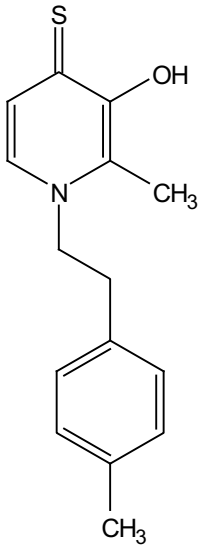


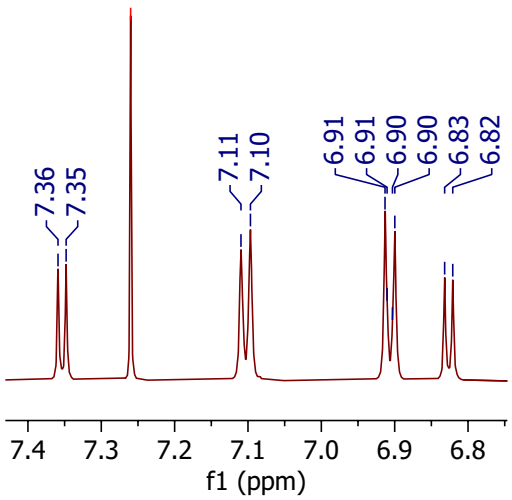
Compound 26
^{13}C NMR (151 MHz, CDCl3)

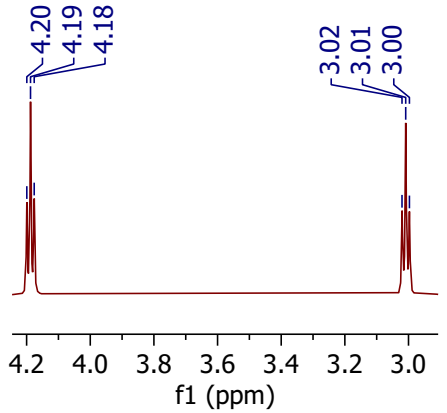


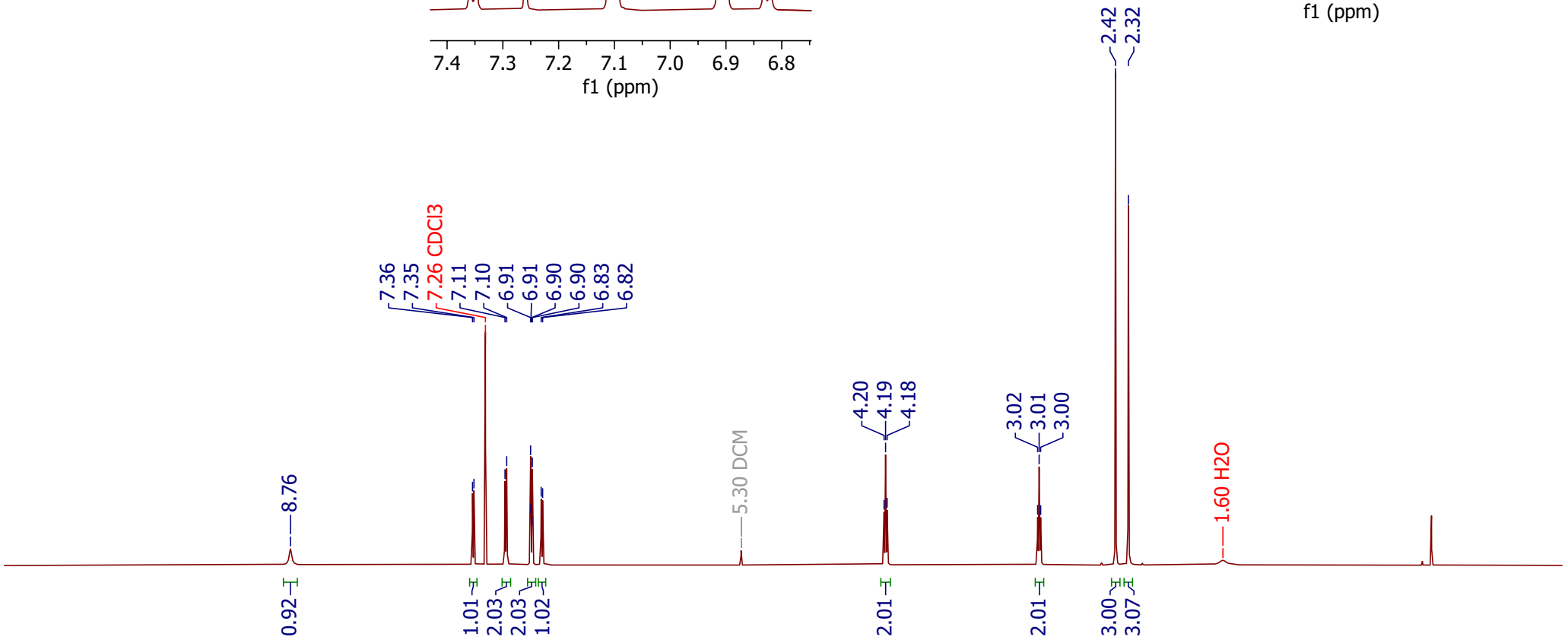


Compound 21
^{1}H NMR (600 MHz, CDCl3)

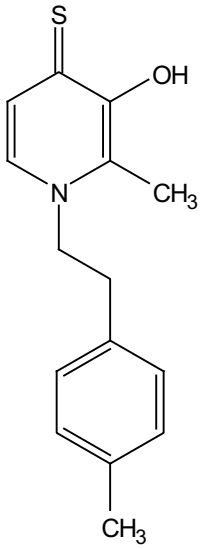




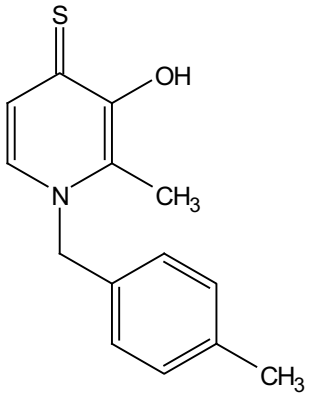


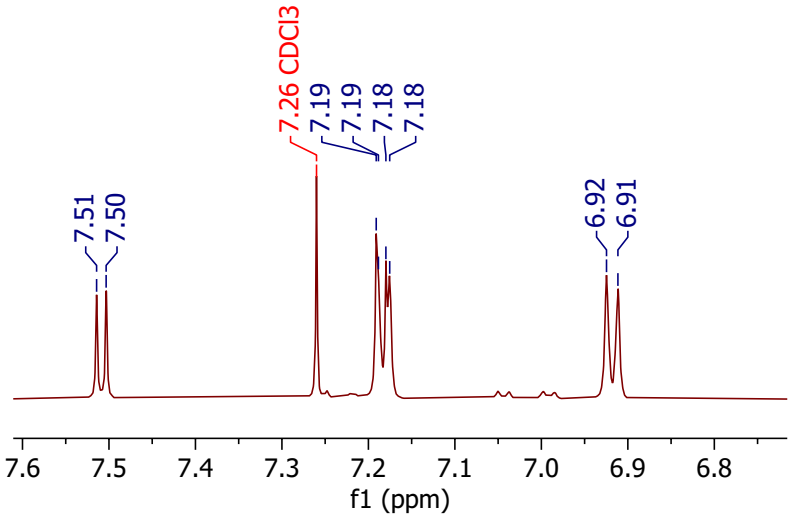


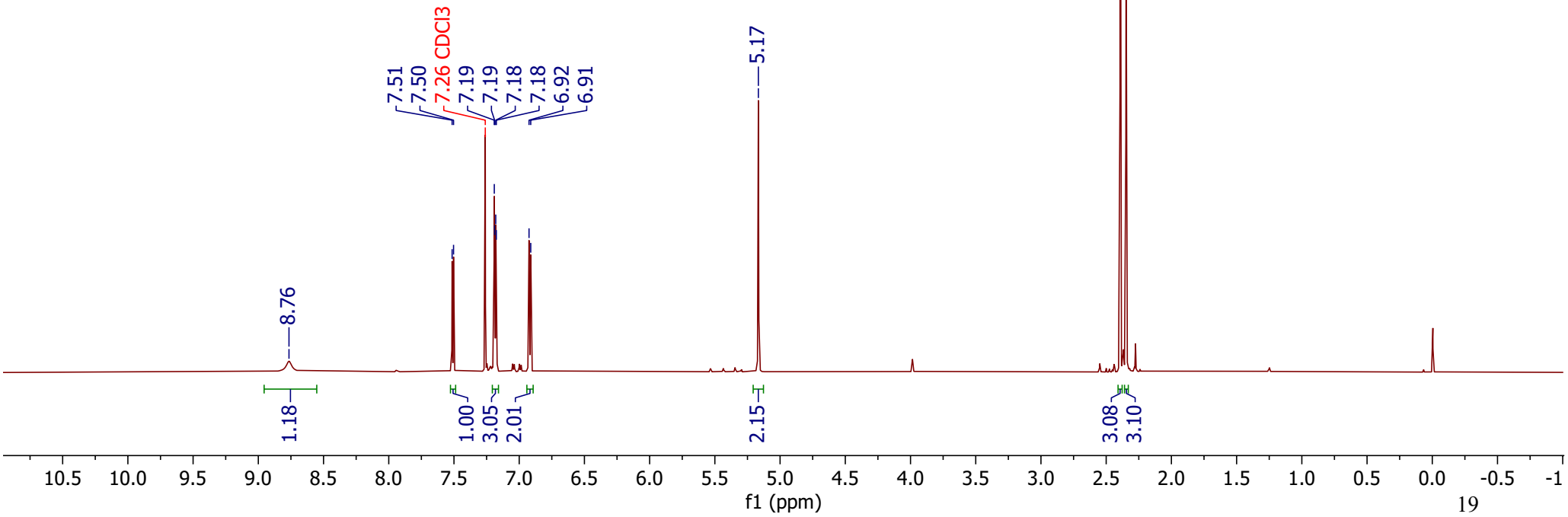
Compound 21
^{13}C NMR (151 MHz, CDCl3)



Compound 27
^{1}H NMR (600 MHz, CDCl3)

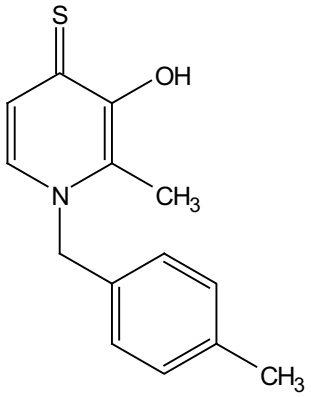




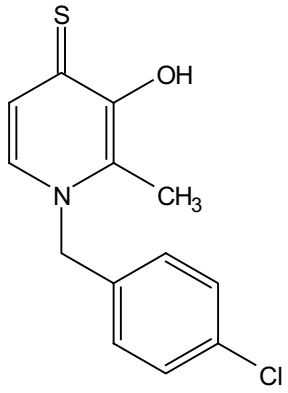


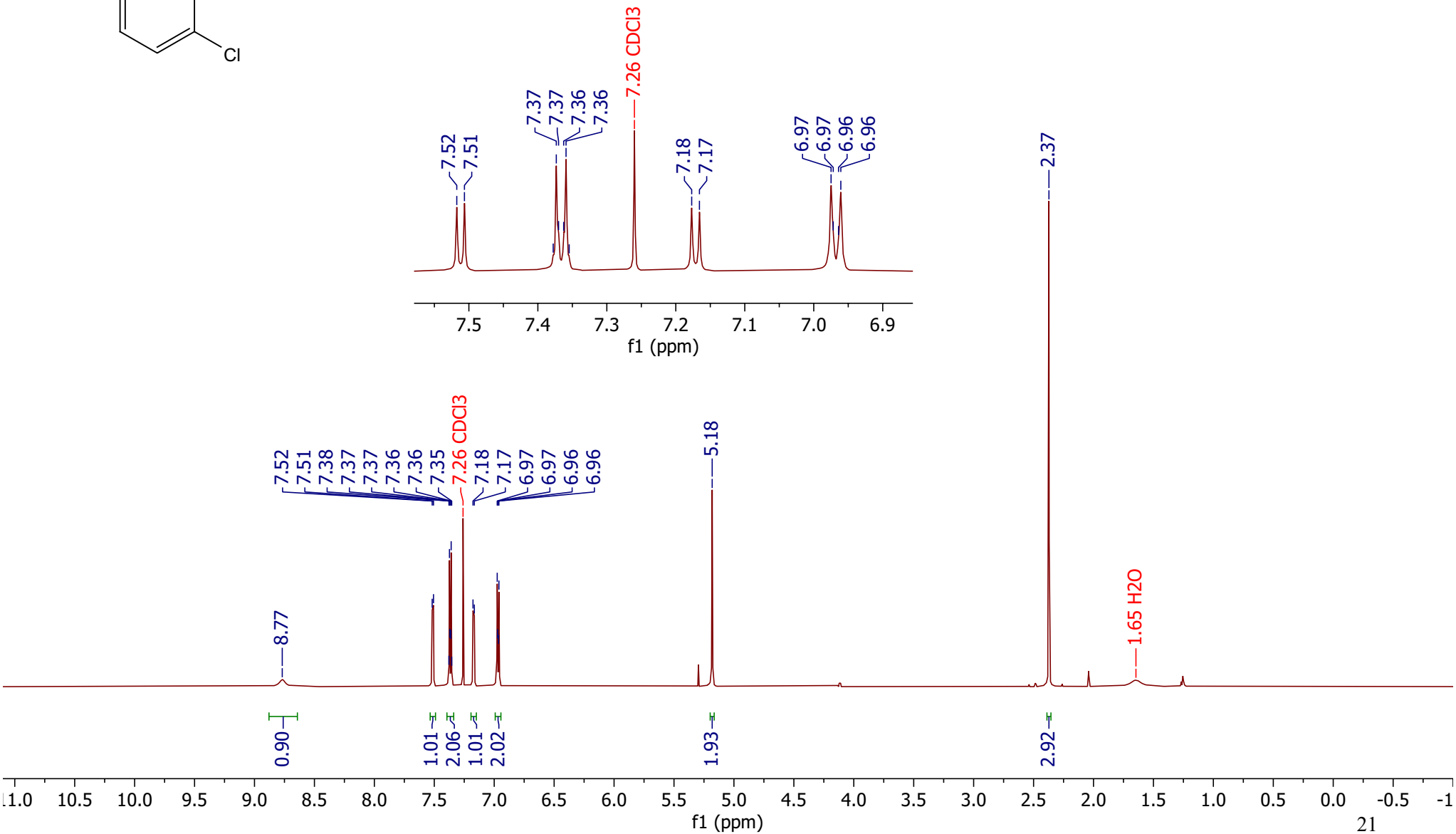
Compound 27

^{13}C NMR (151 MHz, CDCl3)

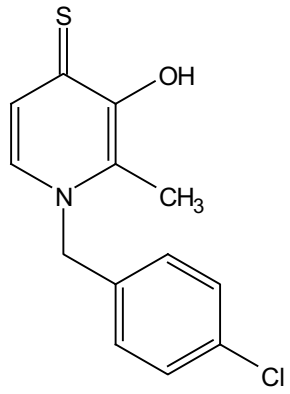


Compound 28
H NMR (600 MHz, CDCl3)

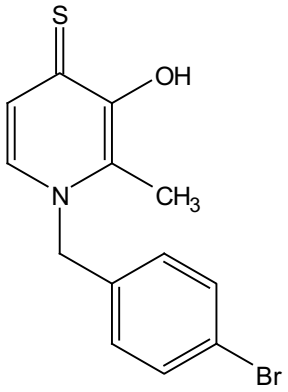


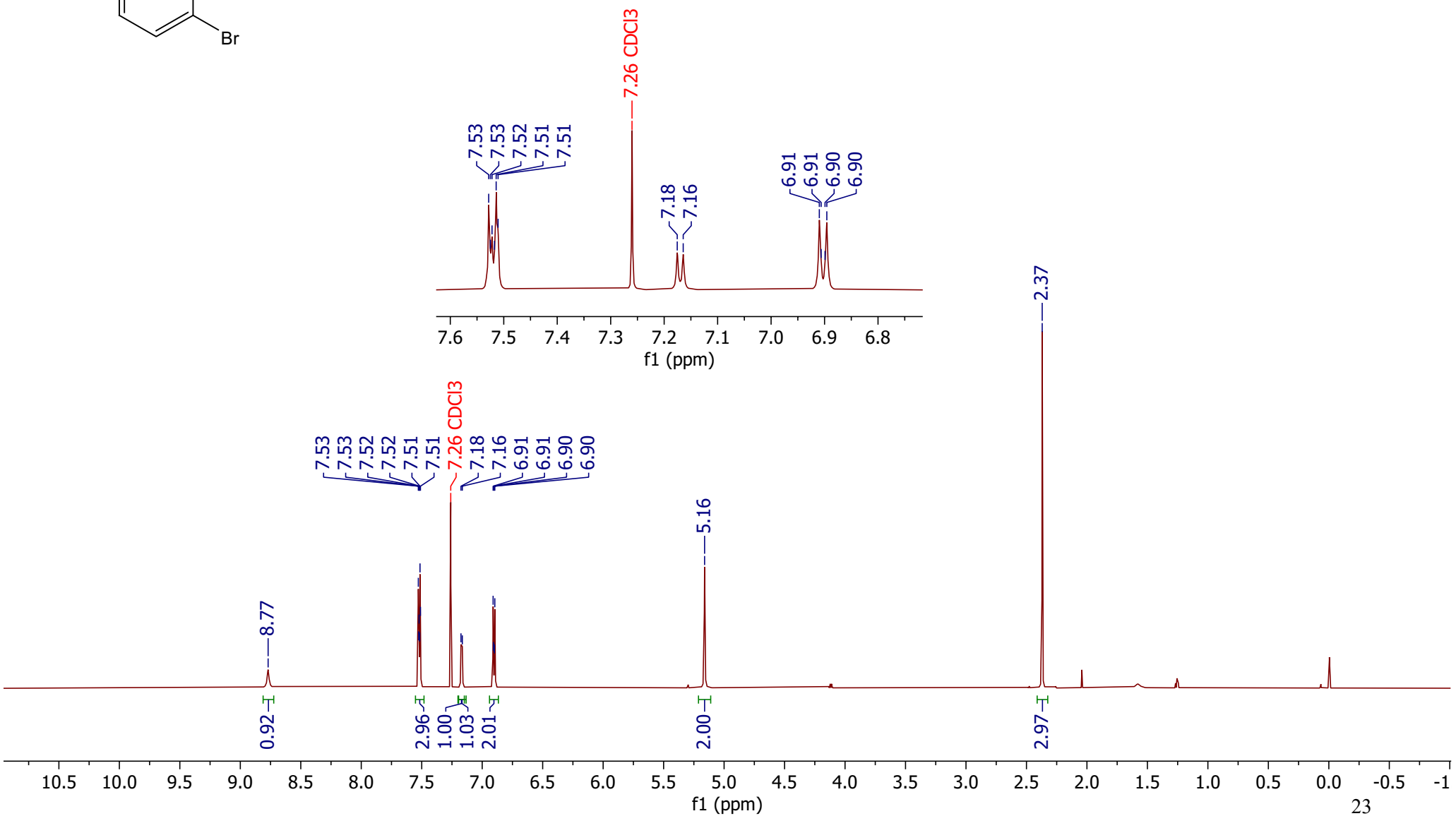


Compound 28
^{13}C NMR (151 MHz, CDCl3)

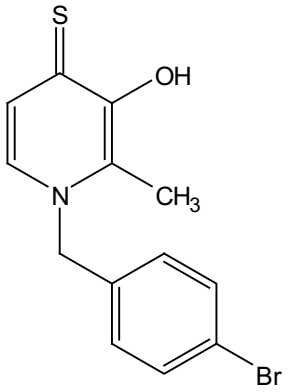


Compound 29
^{1}H NMR (600 MHz, CDCl3)

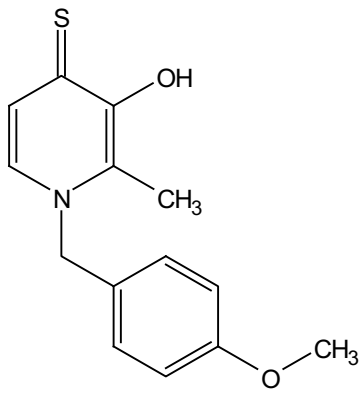


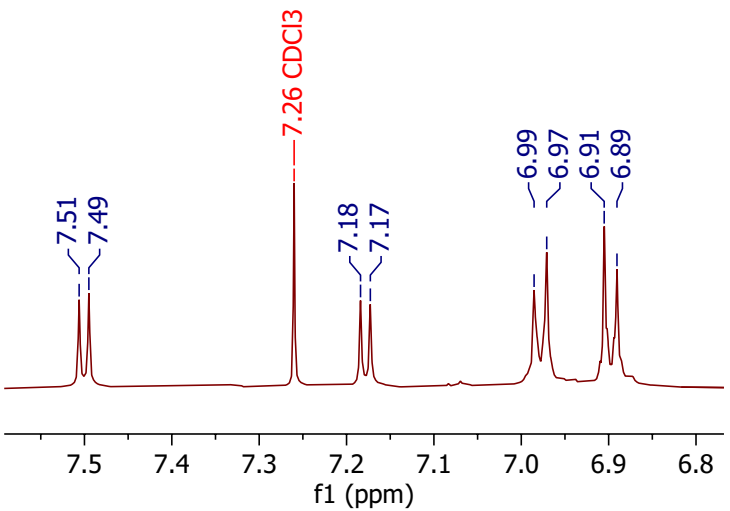


Compound 29
^{13}C NMR (151 MHz, CDCl3)



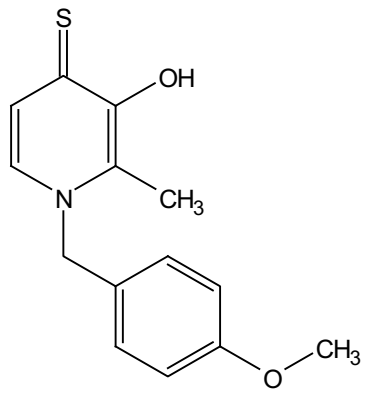
Compound 30
H NMR (600 MHz, CDCl3)



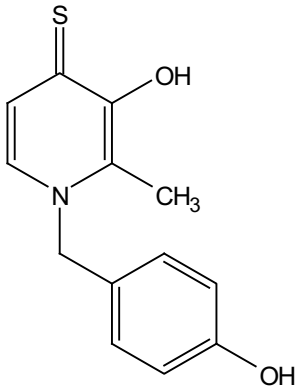


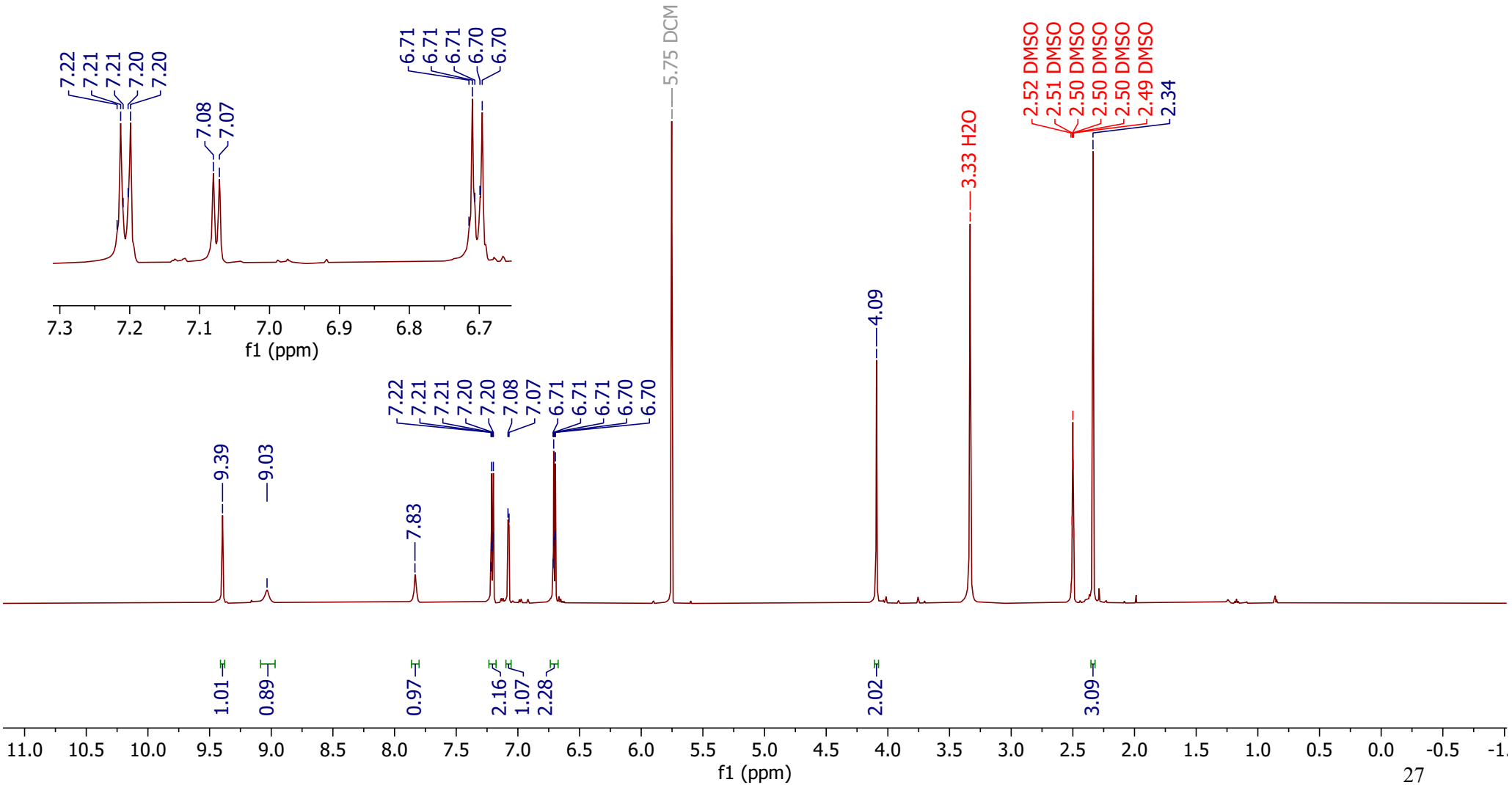
Compound 30

^{13}C NMR (151 MHz, CDCl3)



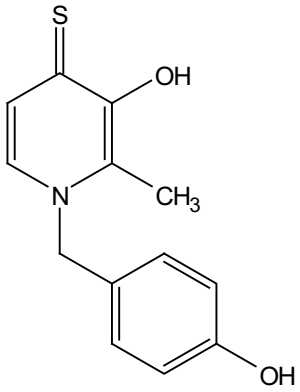
Compound 31
^{1}H NMR (600 MHz, DMSO-d6)



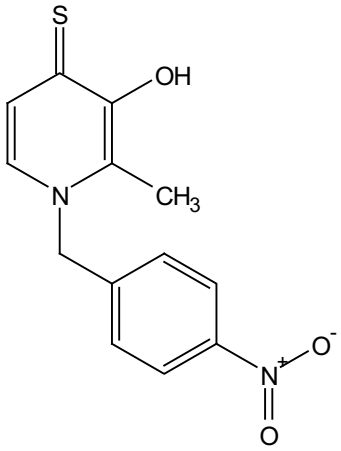


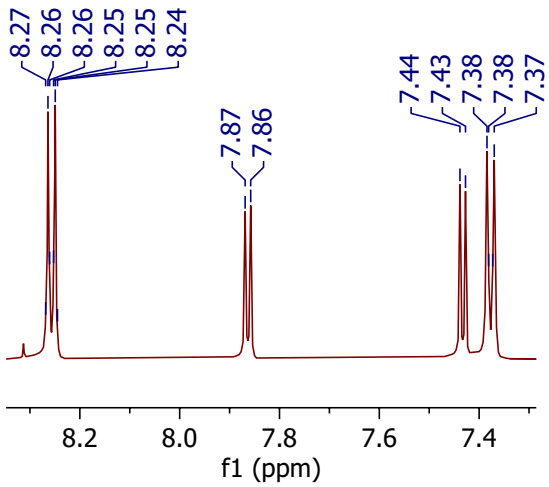
Compound 31

^{13}C NMR (151 MHz, DMSO-d6)



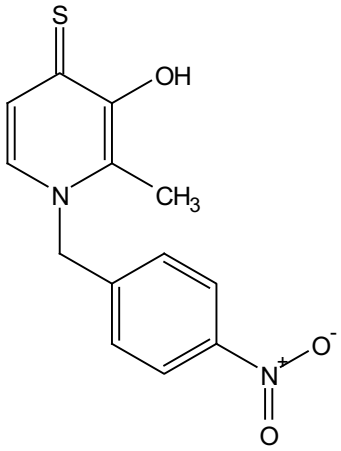
Compound 32
^{1}H NMR (600 MHz, DMSO-d6)



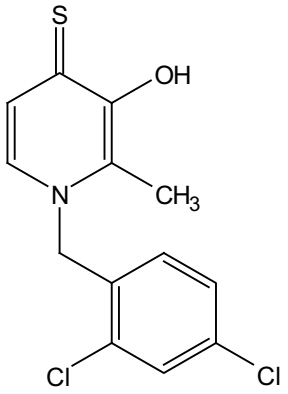


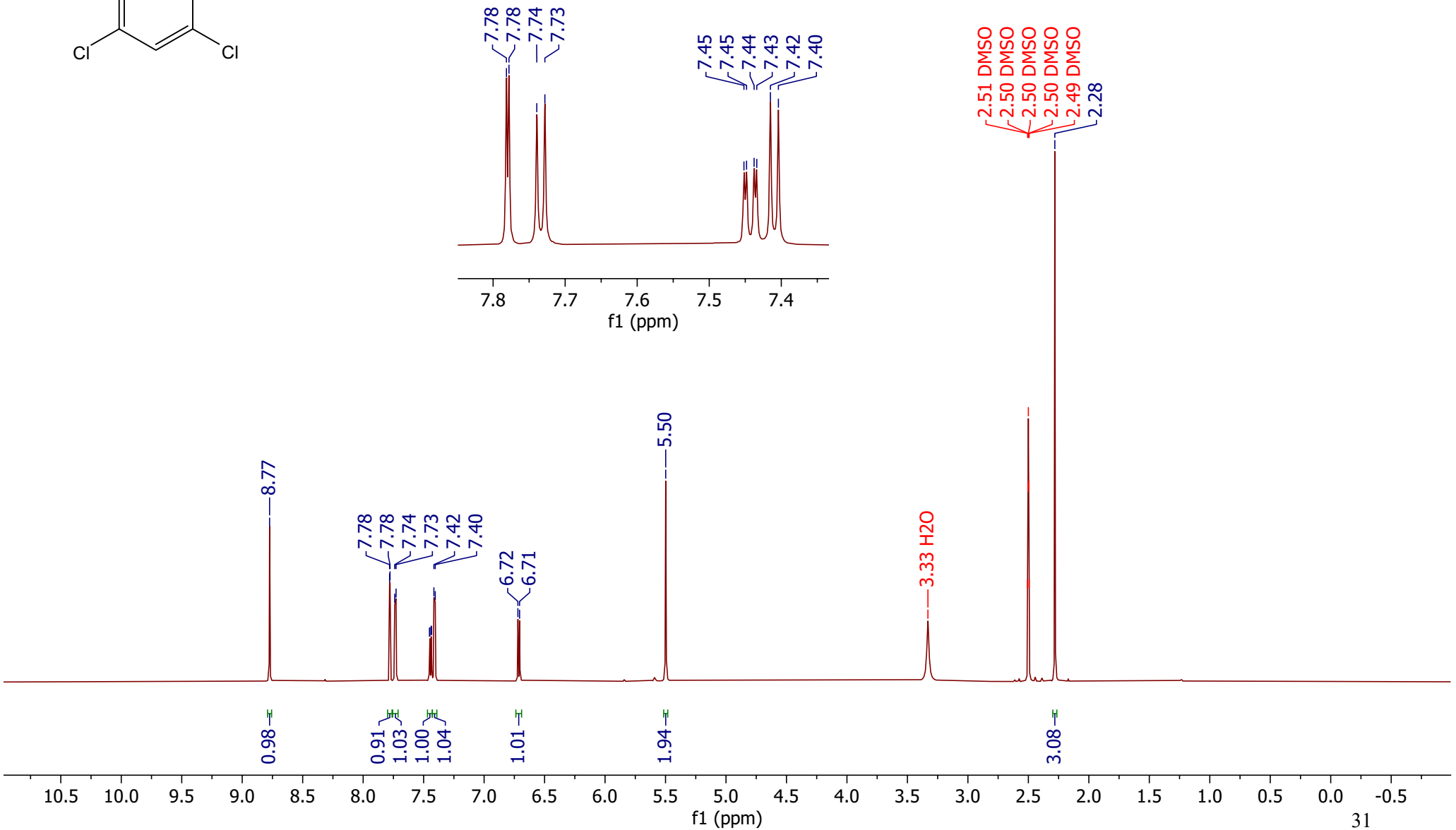
Compound 32

^{13}C NMR (151 MHz, DMSO-d6)



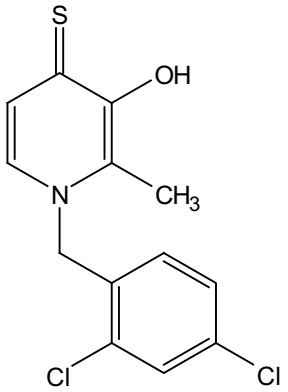
Compound 33
^{1}H NMR (600 MHz, DMSO-d6)



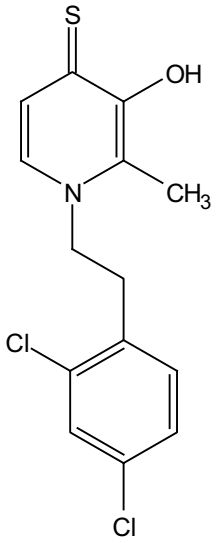


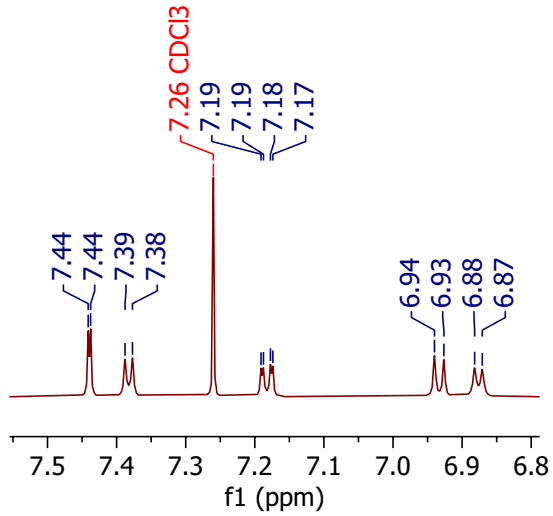
Compound 33

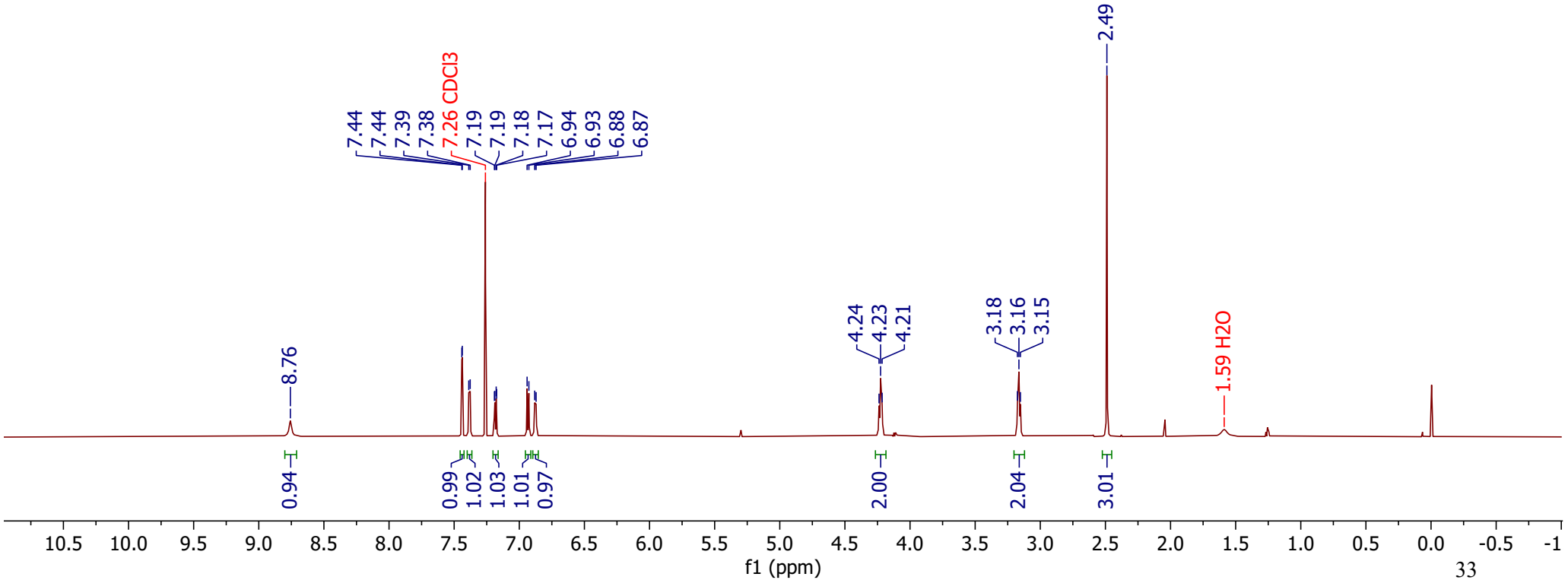
^{13}C NMR (151 MHz, DMSO-d6)



Compound 34
^{1}H NMR (600 MHz, CDCl3)

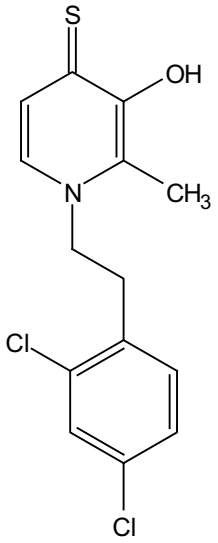




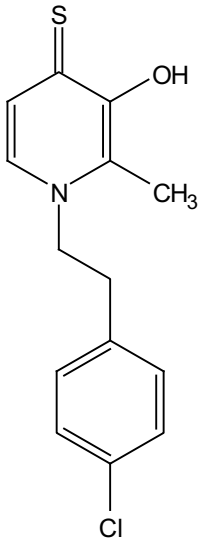


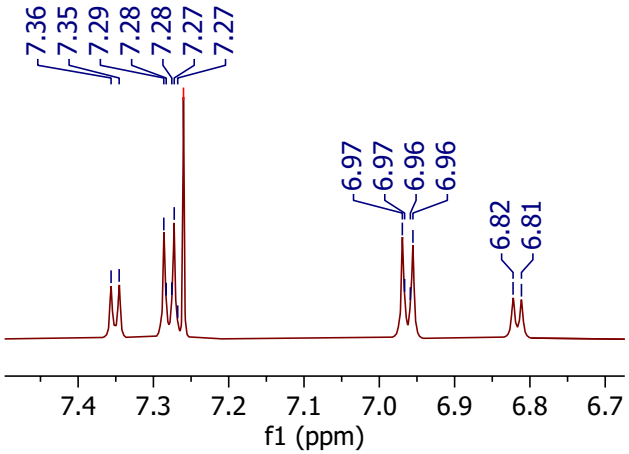
Compound 34

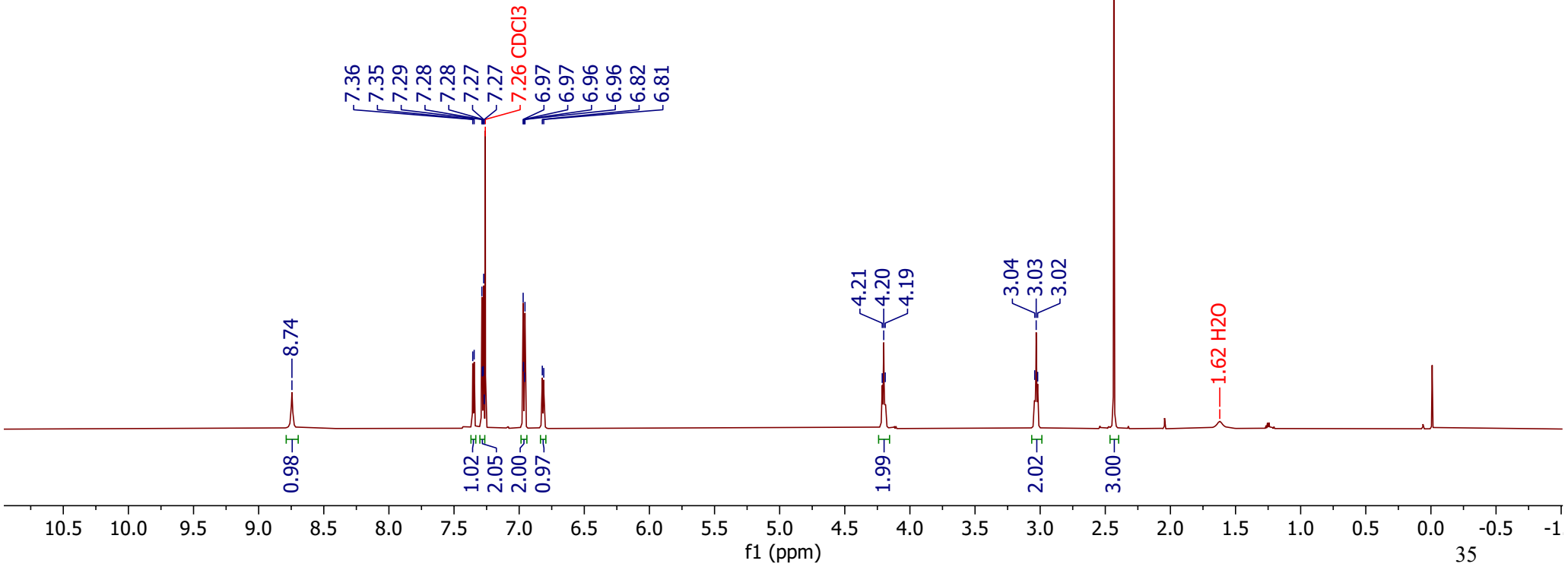
^{13}C NMR (151 MHz, CDCl3)



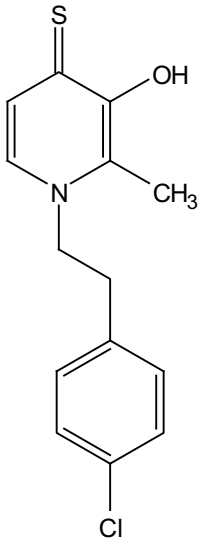
Compound 35
^{1}H NMR (600 MHz, CDCl3)



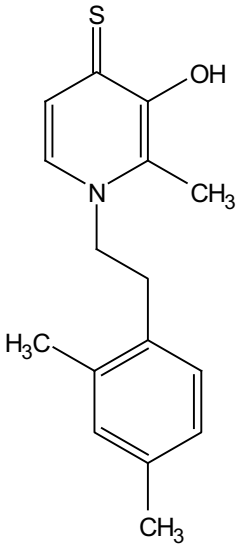


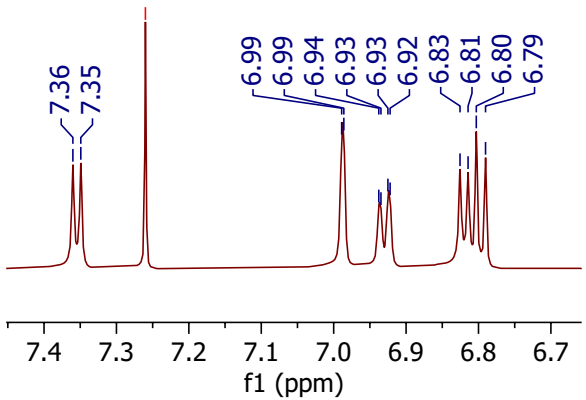


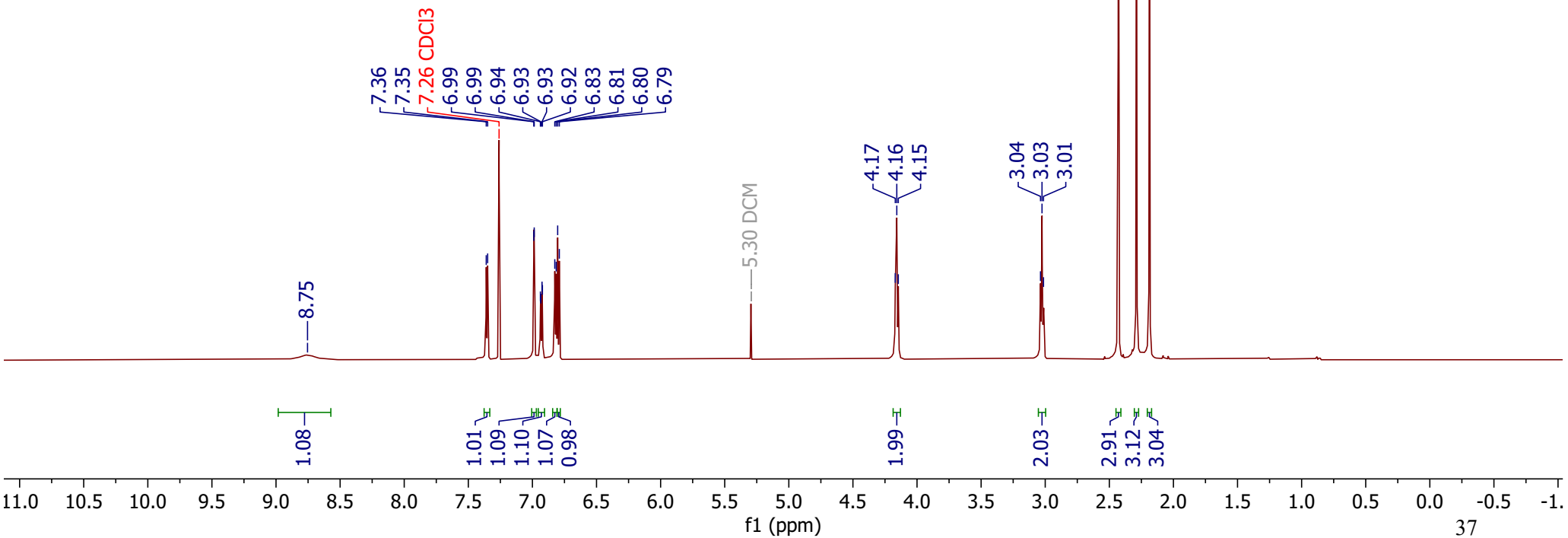
Compound 35
^{13}C NMR (151 MHz, CDCl3)



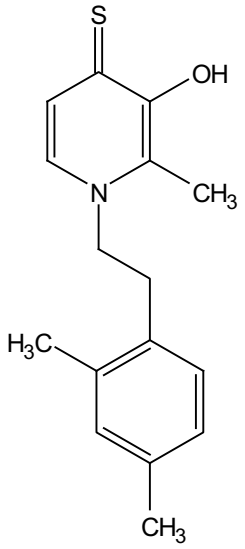
Compound 36
^{1}H NMR (600 MHz, CDCl3)



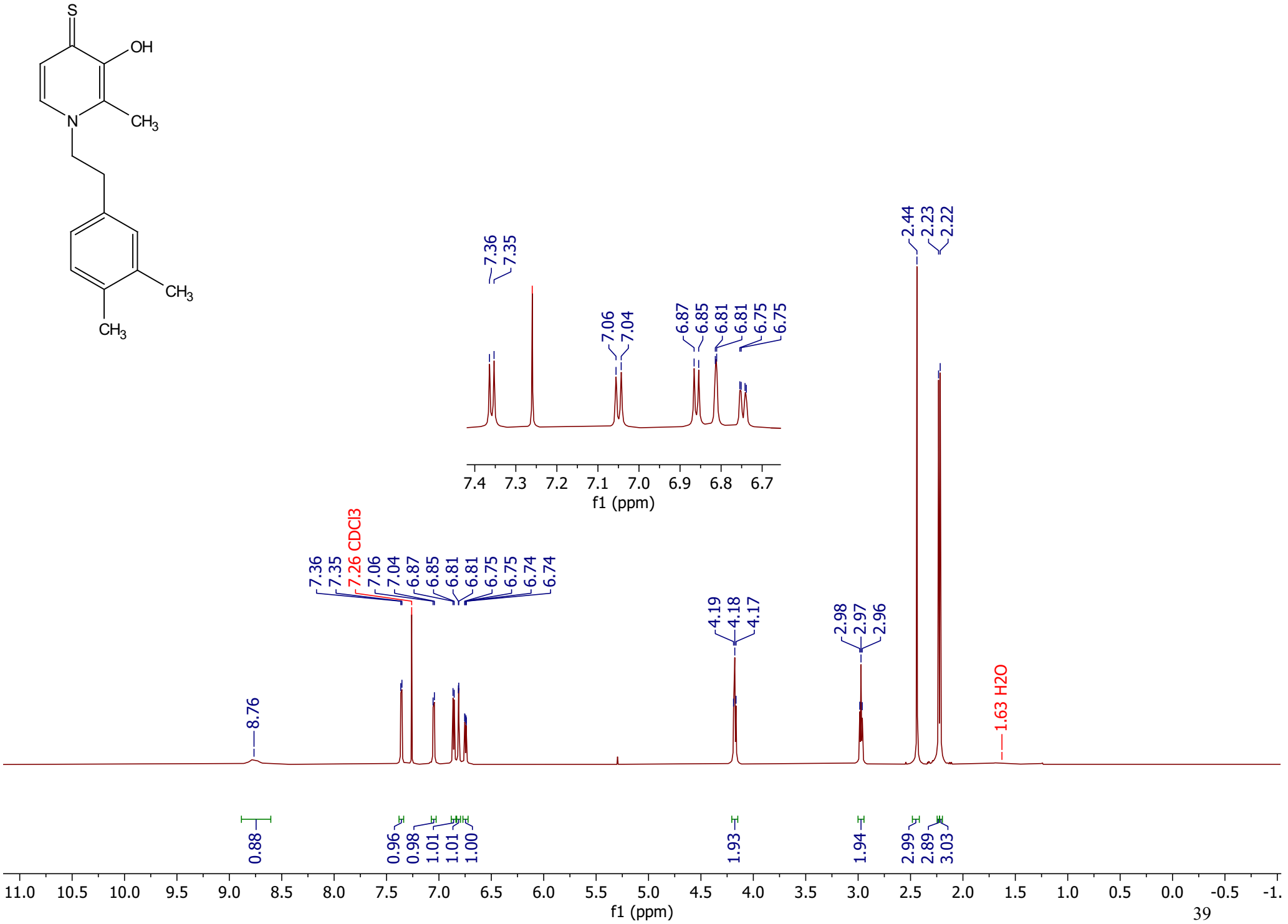




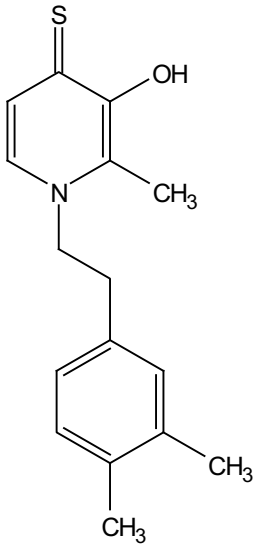
Compound 36
^{13}C NMR (151 MHz, CDCl3)



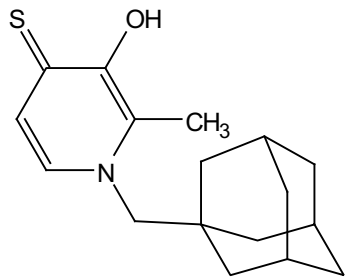
Compound 37
^{1}H NMR (600 MHz, CDCl3)

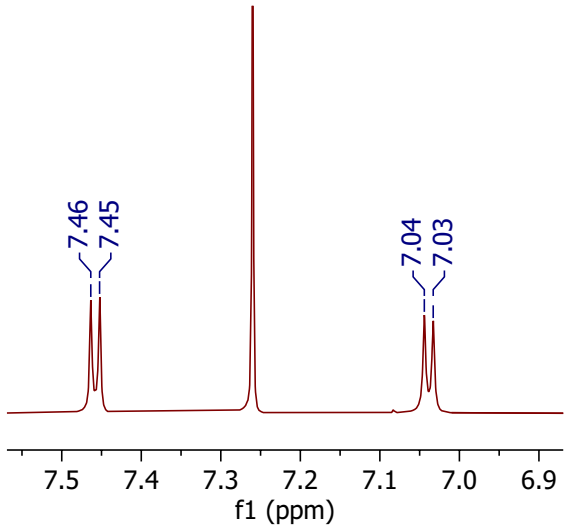


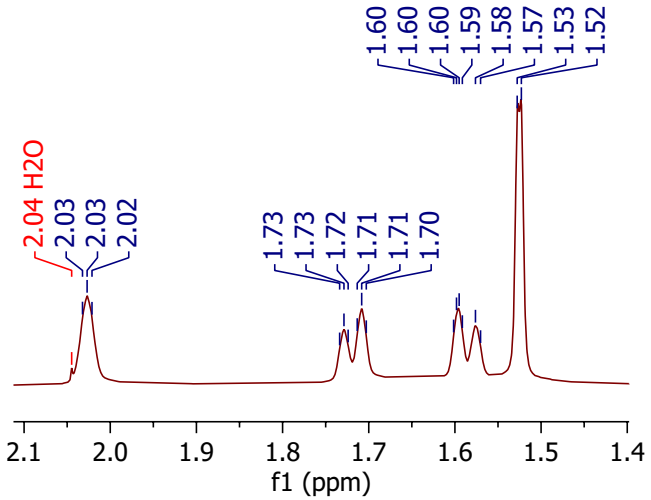
Compound 37
^{13}C NMR (151 MHz, CDCl3)

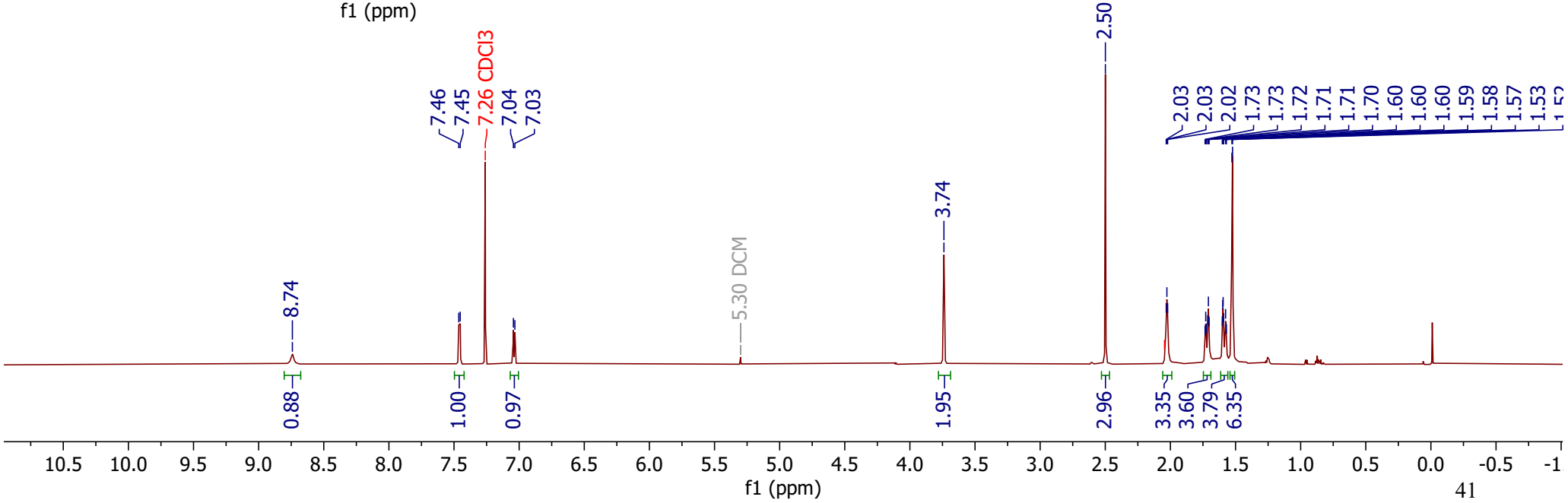


Compound 38
^{1}H NMR (600 MHz, CDCl3)

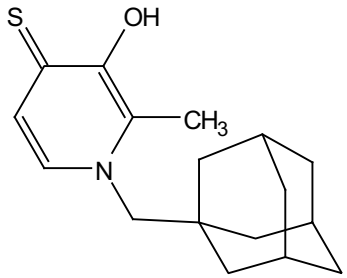




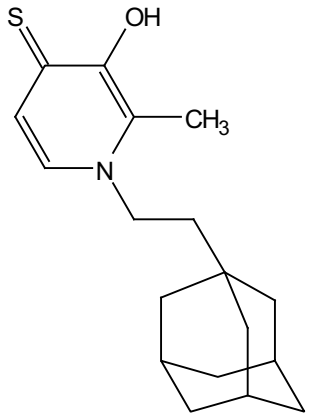


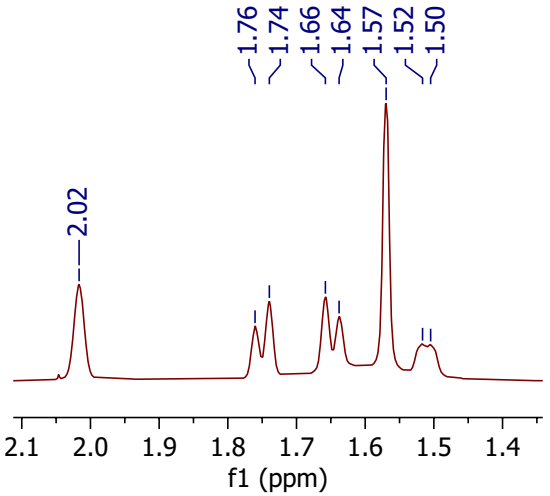


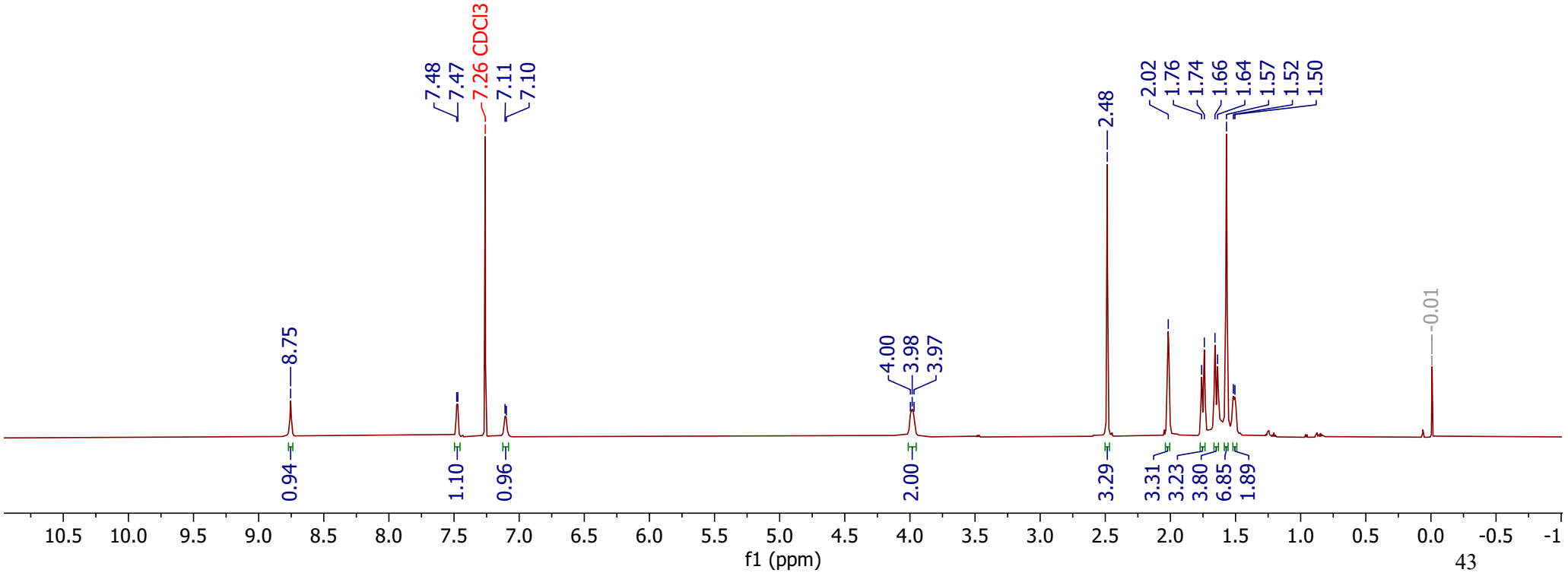
Compound 38
^{13}C NMR (151 MHz, CDCl3)



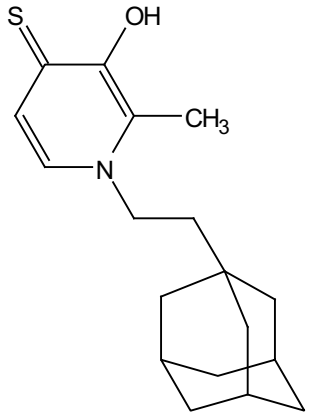
Compound 39
^{1}H NMR (600 MHz, CDCl3)

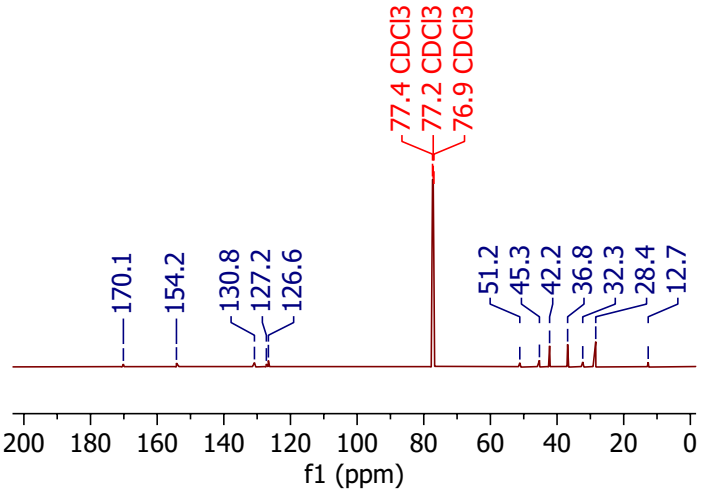




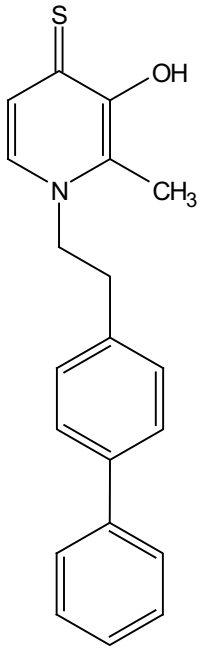


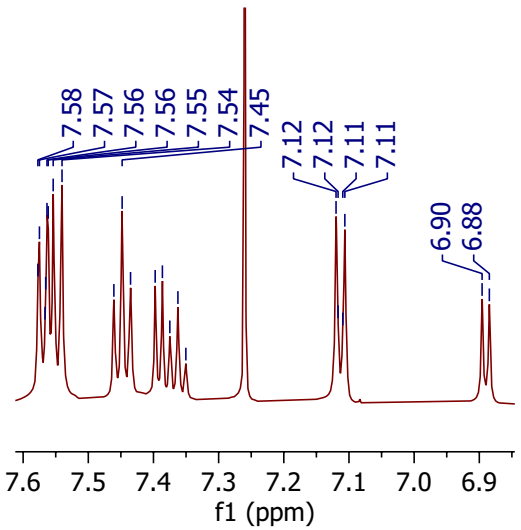
Compound 39
^{13}C NMR (151 MHz, CDCl3)

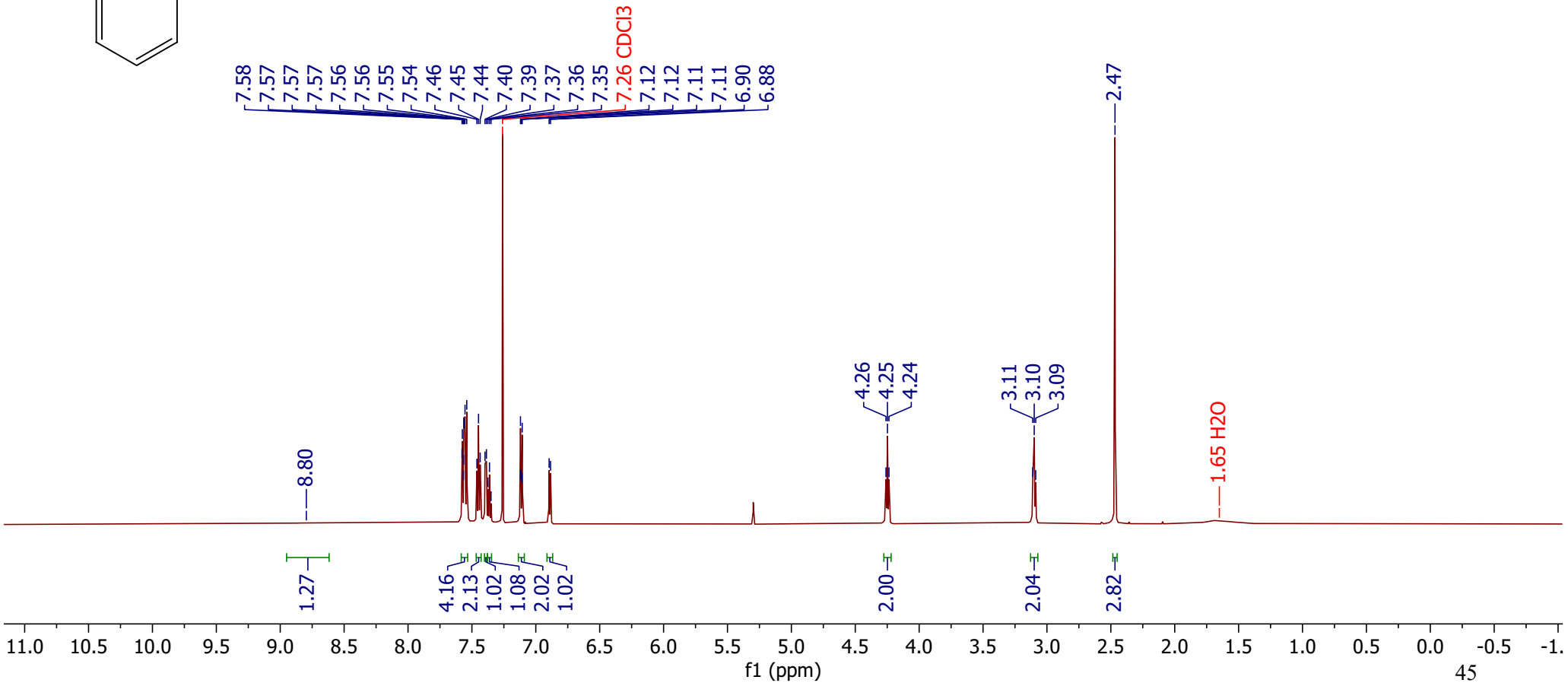




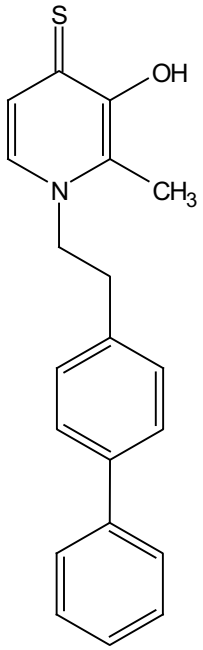
Compound 40
^{1}H NMR (600 MHz, CDCl3)



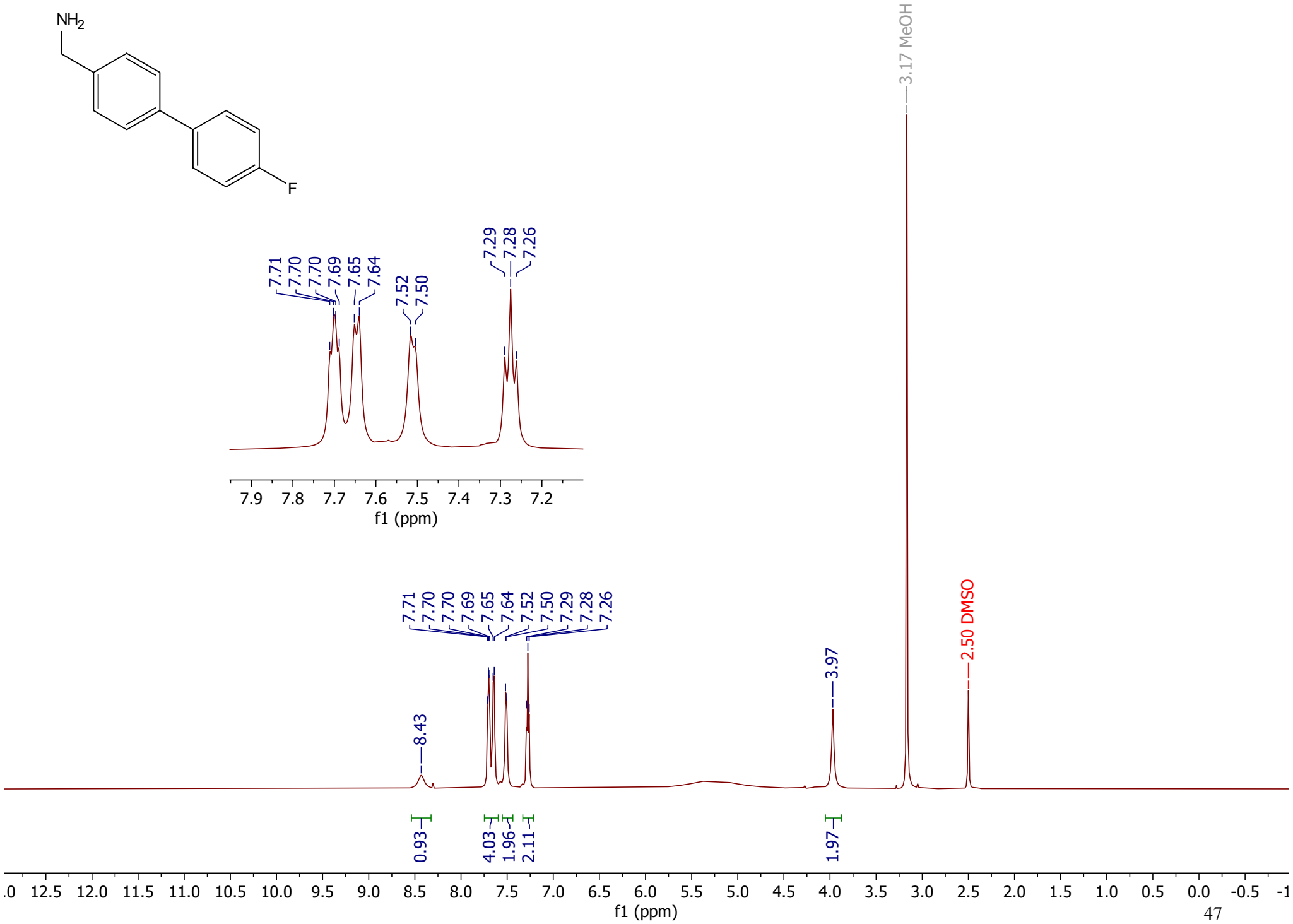




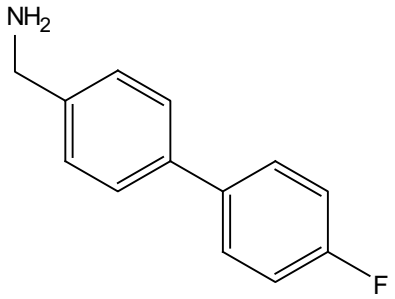
Compound 40
^{13}C NMR (151 MHz, CDCl3)



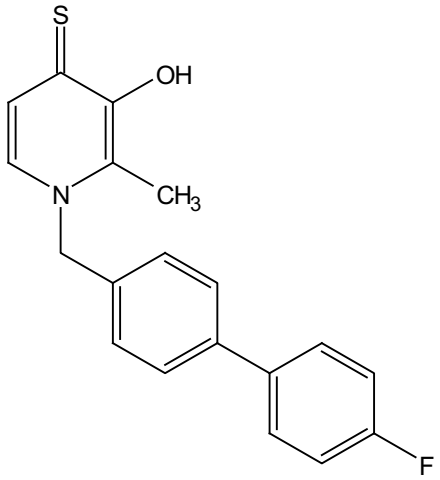
Compound S1
H NMR (600 MHz, DMSO-d6)

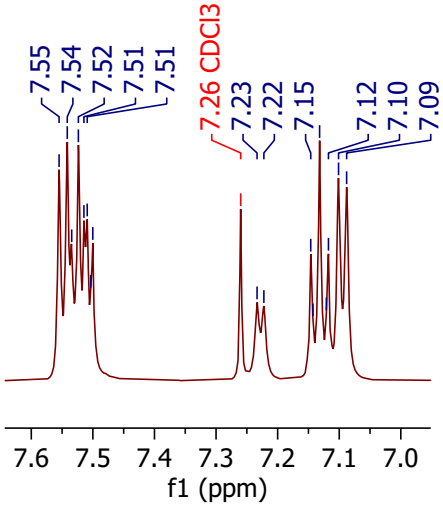


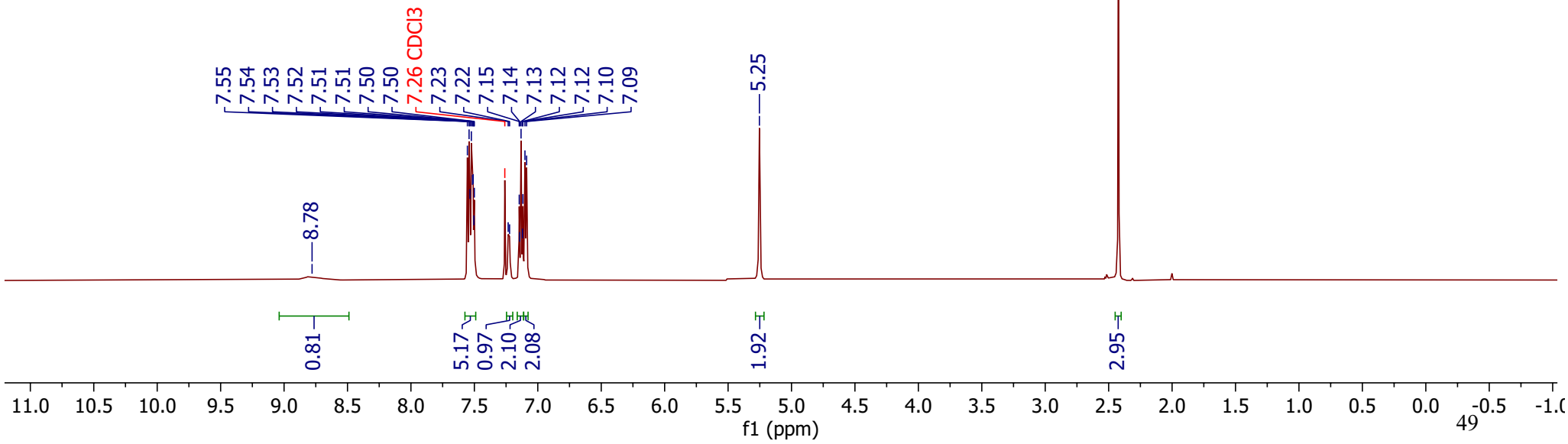
Compound S1
^{13}C NMR (151 MHz, DMSO-d6)



Compound 41
^{1}H NMR (600 MHz, CDCl3)

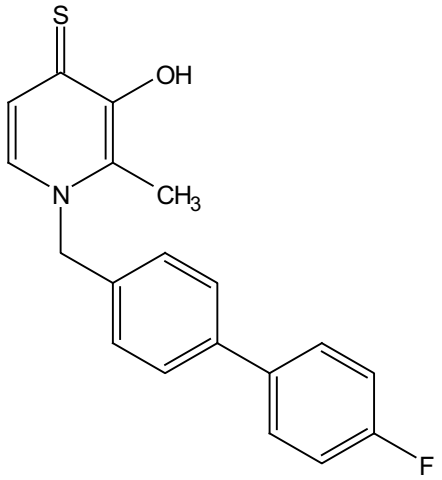




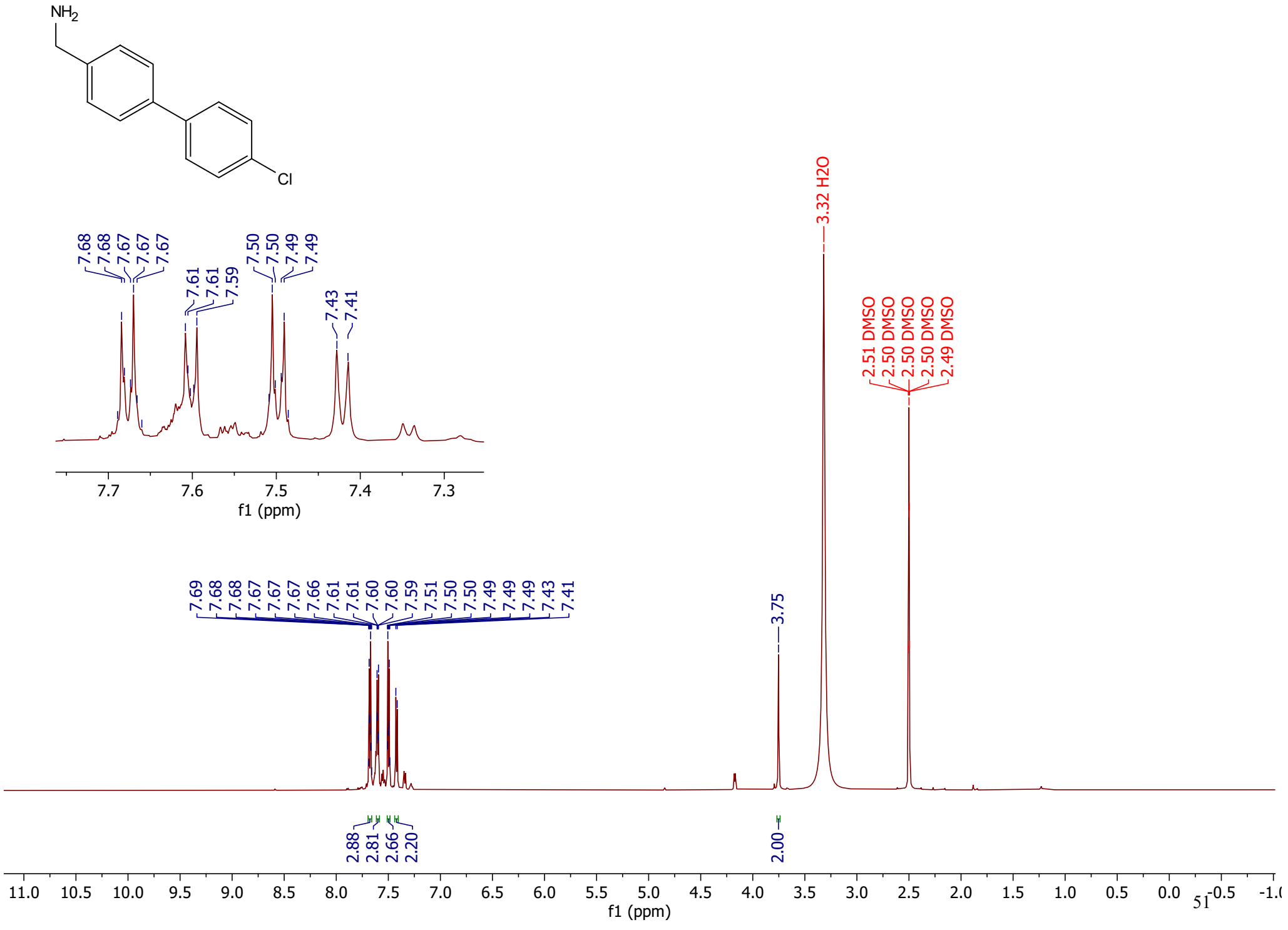


Compound 41

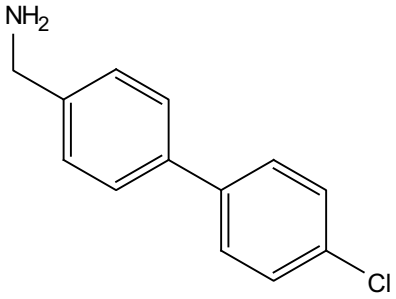
^{13}C NMR (151 MHz, CDCl3)

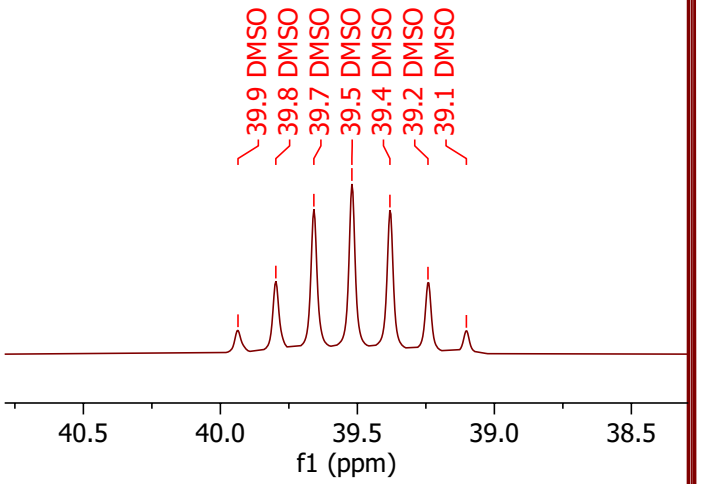


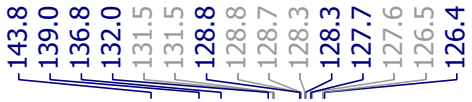
Compound S2
^{1}H NMR (600 MHz, DMSO-d6)



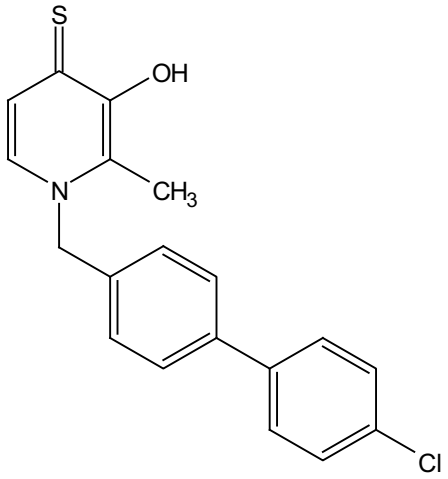
Compound S2
^{13}C NMR (151 MHz, DMSO-d6)

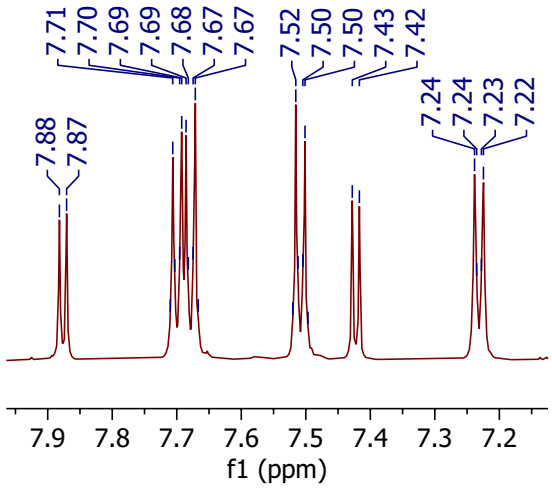


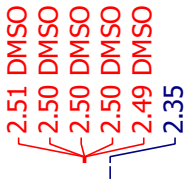


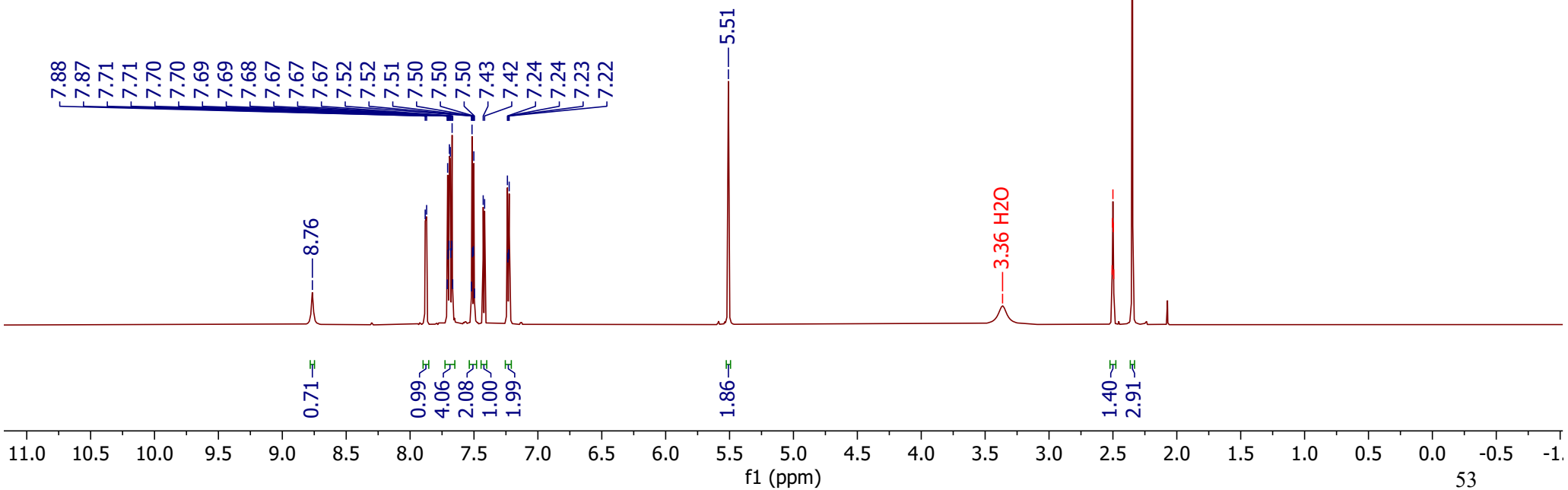


Compound 42
^{1}H NMR (600 MHz, DMSO-d6)

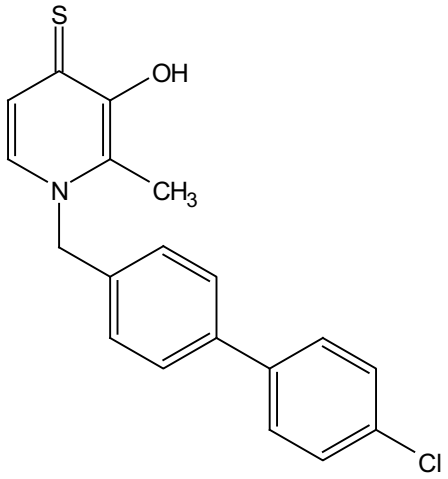




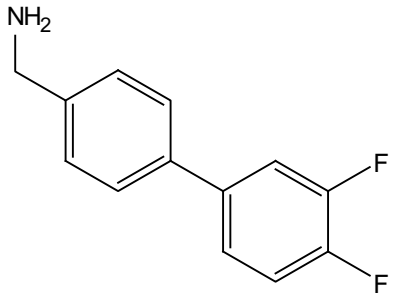


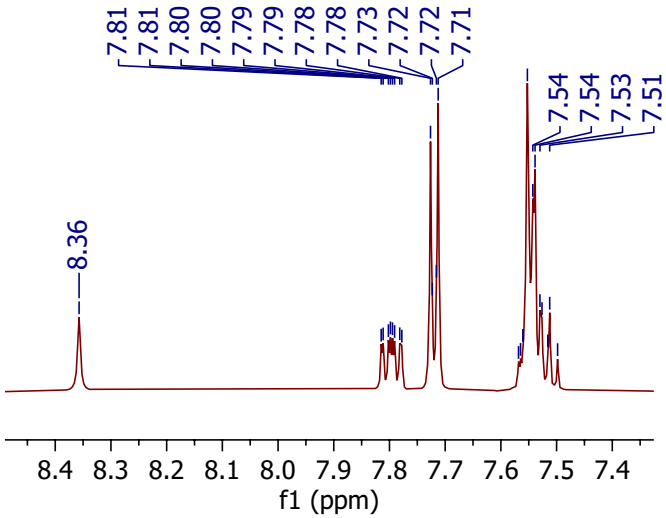


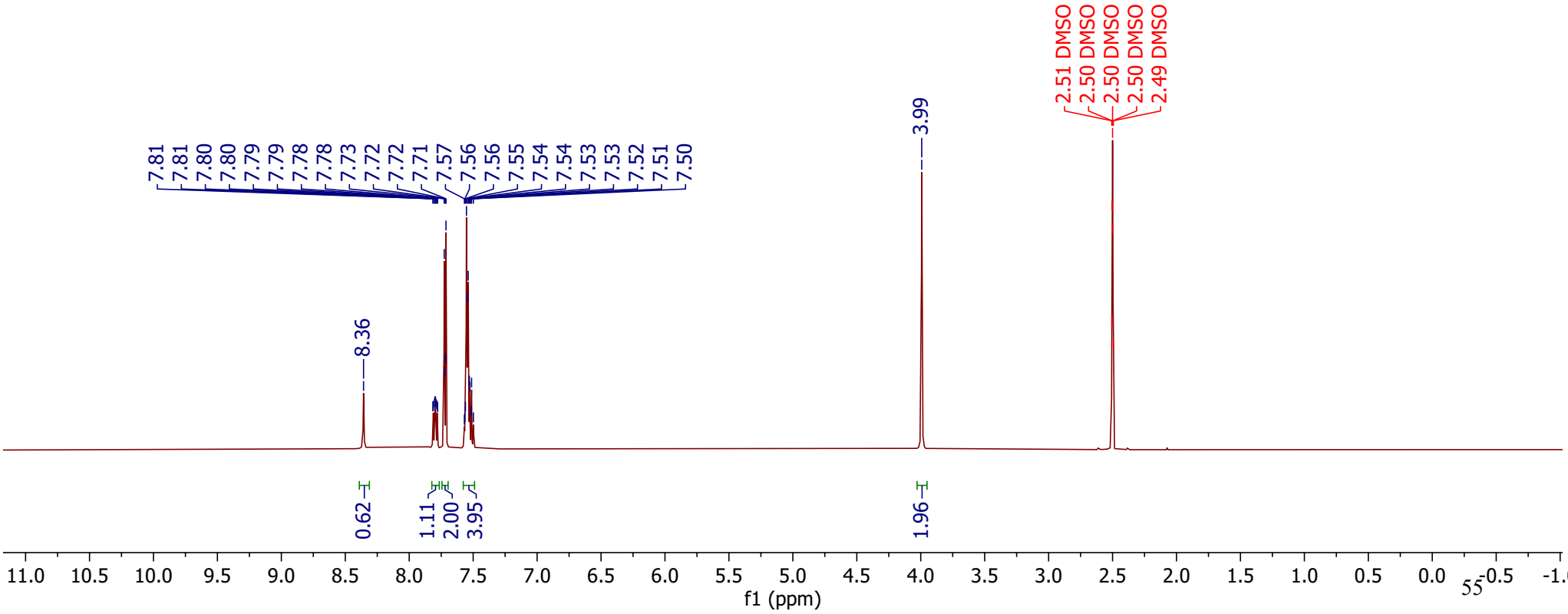
Compound 42
^{13}C NMR (151 MHz, DMSO-d6)



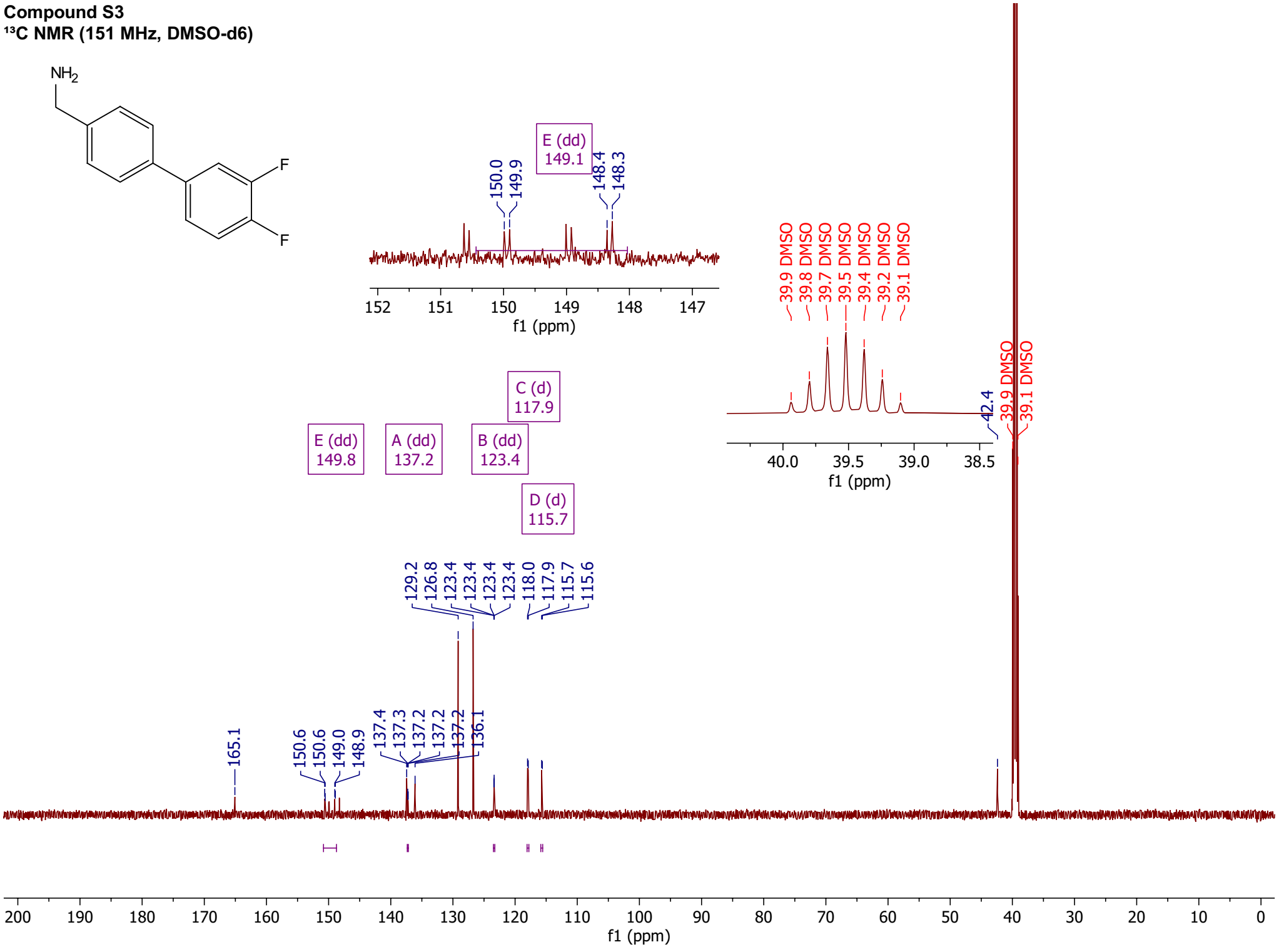
Compound S3
^{1}H NMR (600 MHz, DMSO-d6)



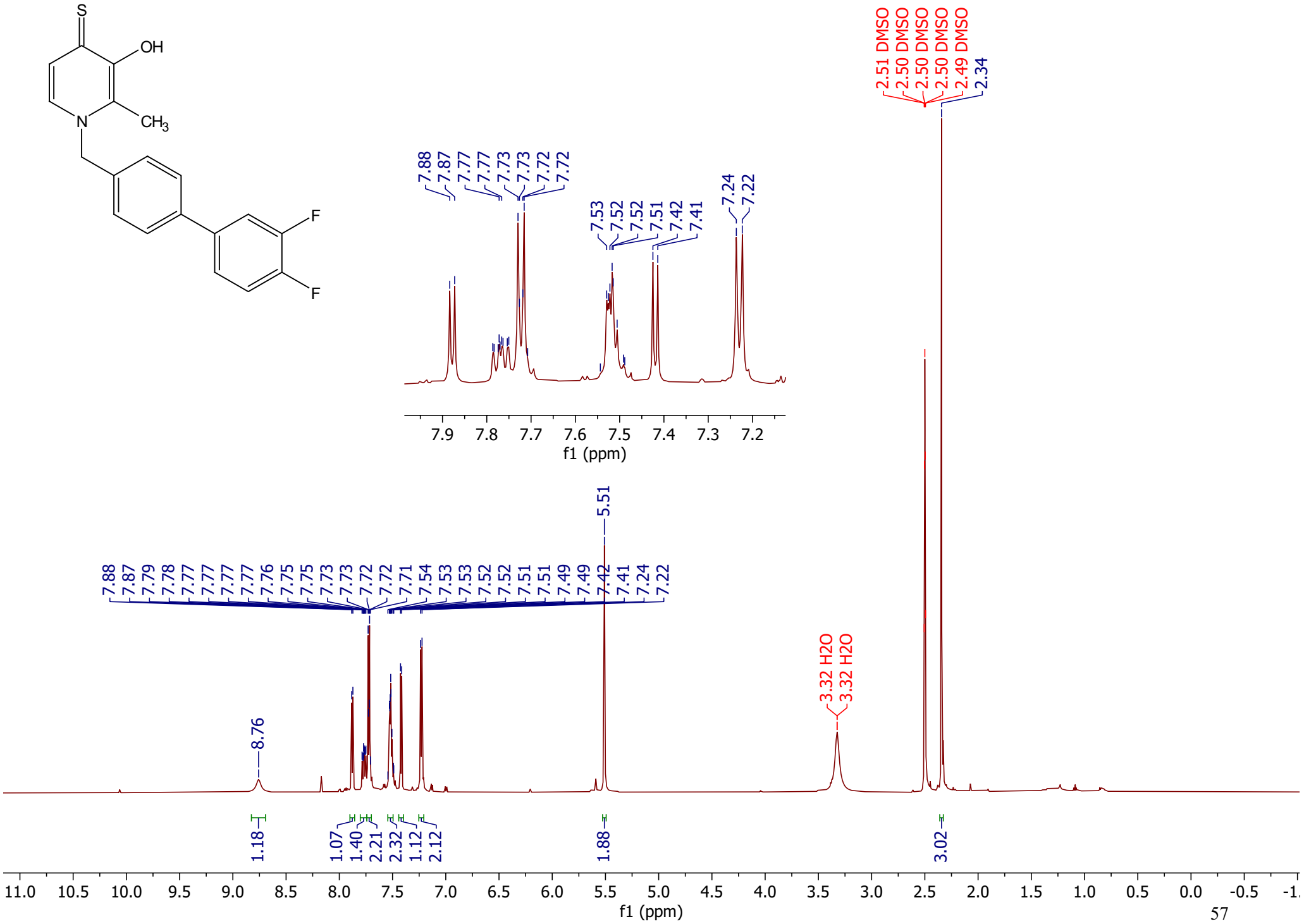




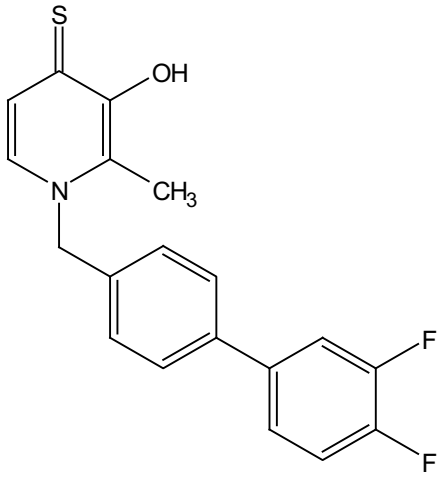
Compound S3
^{13}C NMR (151 MHz, DMSO-d6)

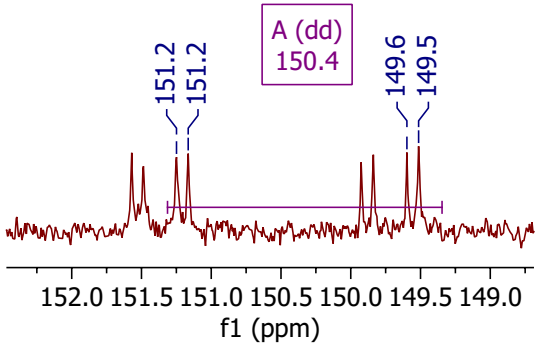


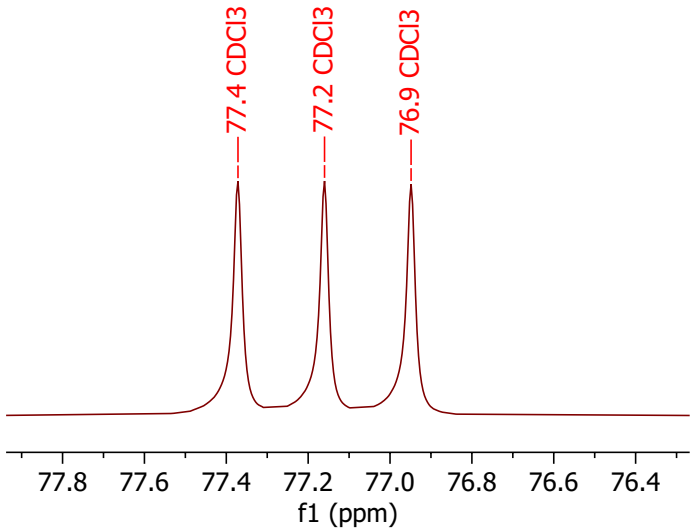
Compound 43
^{1}H NMR (600 MHz, DMSO-d6)

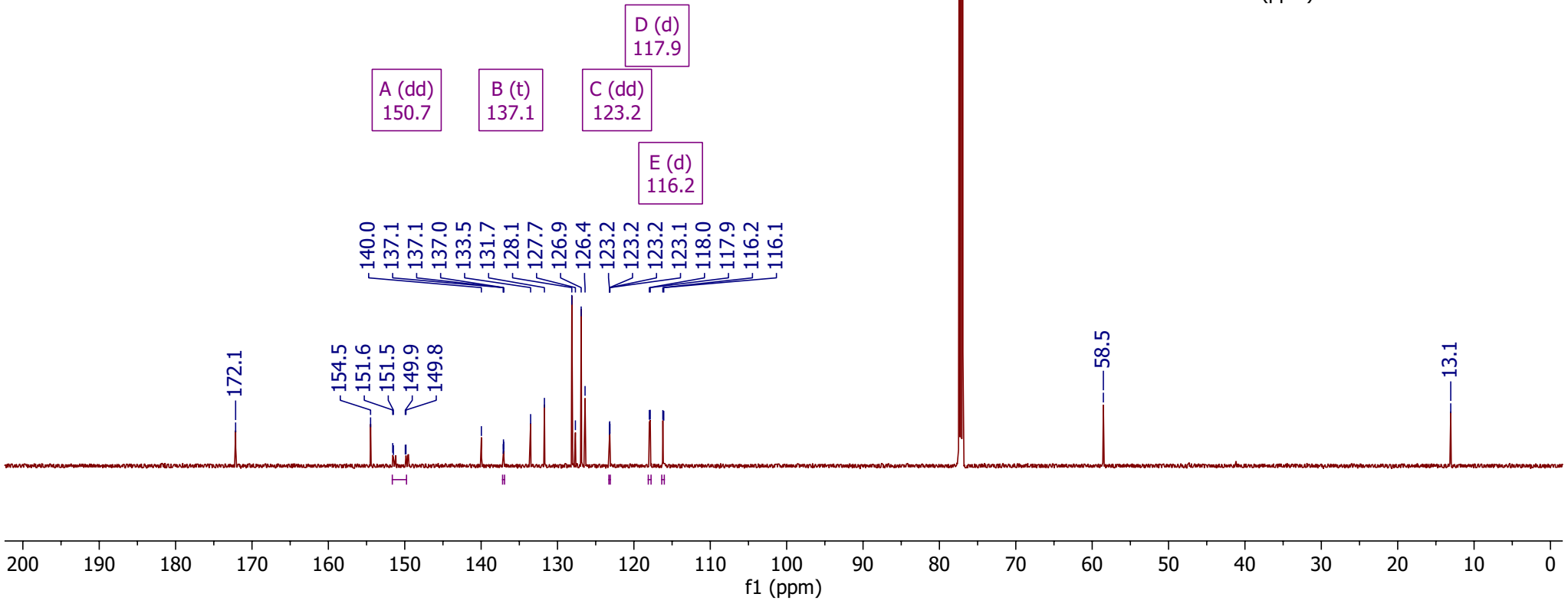


Compound 43
^{13}C NMR (151 MHz, CDCl3)

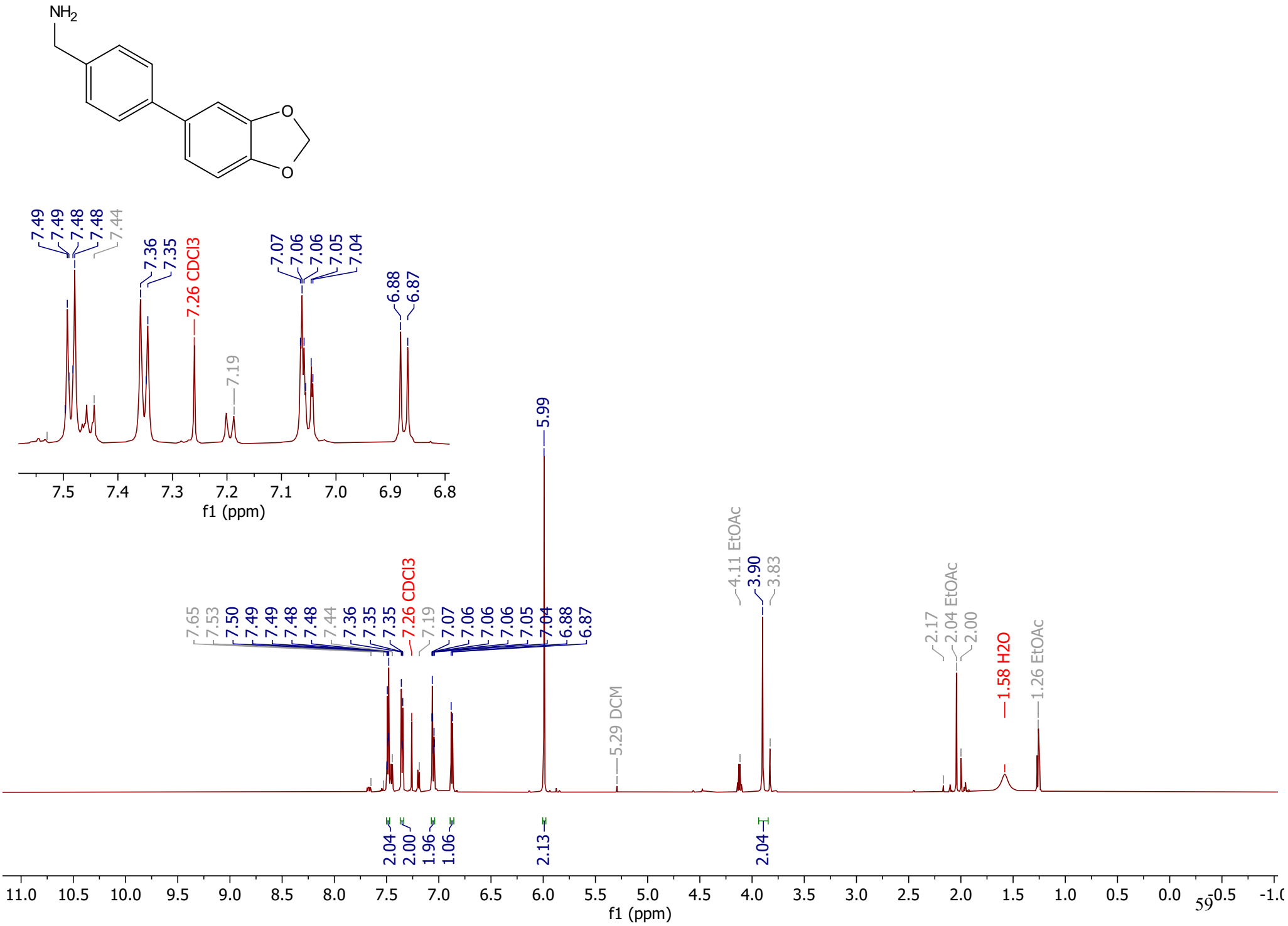




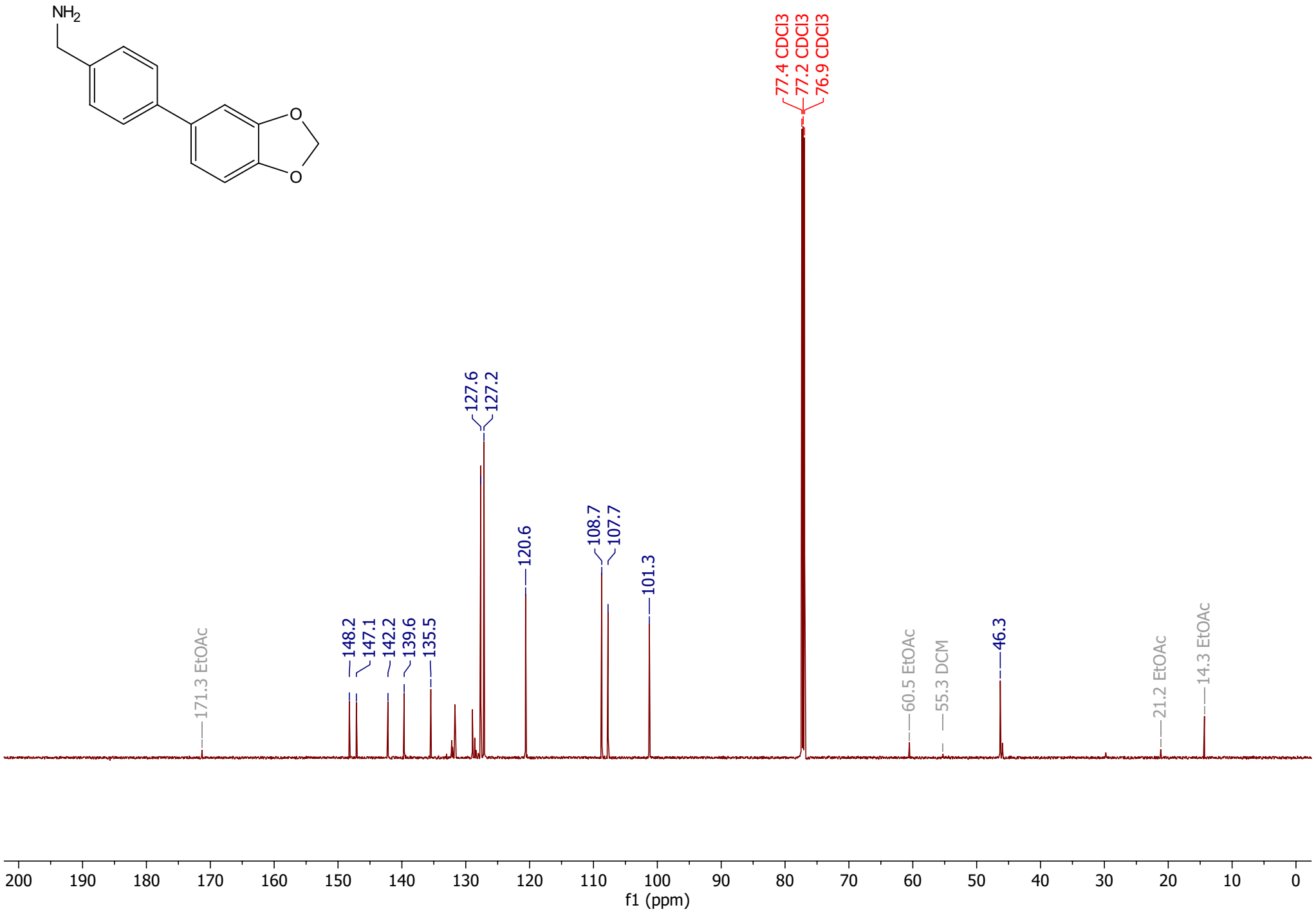




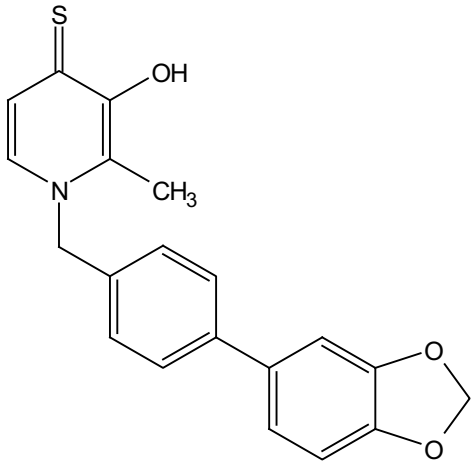
Compound S4
^{1}H NMR (600 MHz, CDCl3)

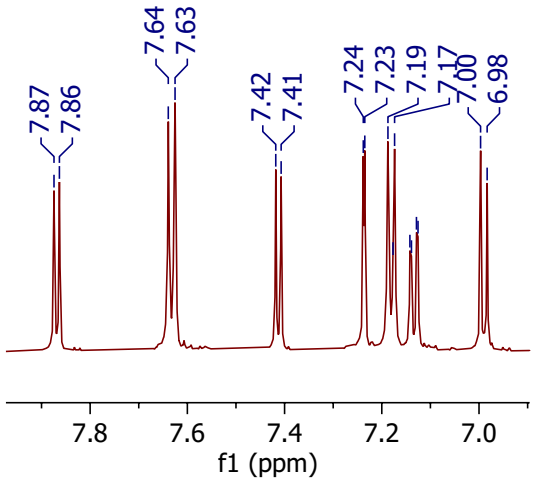


Compound S4
^{13}C NMR (151 MHz, CDCl3)

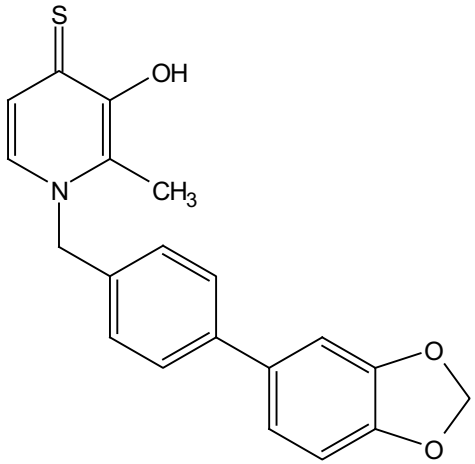


Compound 44
^{1}H NMR (600 MHz, DMSO-d6)





Compound 44
^{13}C NMR (151 MHz, DMSO-d6)



3. References

1. J. J. Irwin, D. Duan, H. Torosyan, A. K. Doak, K. T. Ziebart, T. Sterling, G. Tumanian and B. K. Shoichet, *J Med Chem*, 2015, **58**, 7076-7087.
2. P. T. Bremer, M. Adler, C. H. Phung, A. K. Singh and K. D. Janda, *J Med Chem*, 2017, **60**, 338-348.
3. L. Lin, M. E. Olson, T. Sugane, L. D. Turner, M. A. Tararina, A. L. Nielsen, E. K. Kurbanov, S. Pellett, E. A. Johnson, S. M. Cohen, K. N. Allen and K. D. Janda, *J Med Chem*, 2020, **63**, 11100-11120.
4. A. Agrawal, S. L. Johnson, J. A. Jacobsen, M. T. Miller, L.-H. Chen, M. Pellecchia and S. M. Cohen, *ChemMedChem*, 2010, **5**, 195-199.
5. S. Pellett, W. H. Tepp, C. M. Clancy, G. E. Borodic and E. A. Johnson, *Febs Lett*, 2007, **581**, 4803-4808.
6. C. J. Malizio, M. C. Goodnough and E. A. Johnson, in *Bacterial Toxins: Methods and Protocols*, ed. O. Holst, Humana Press, Totowa, NJ, 2000, DOI: 10.1385/1-59259-052-7:27, pp. 27-39.
7. S. Pellett, W. H. Tepp, C. M. Clancy, G. E. Borodic and E. A. Johnson, *FEBS Lett*, 2007, **581**, 4803-4808.
8. G. R. Flematti, E. D. Goddard-Borger, D. J. Merritt, E. L. Ghisalberti, K. W. Dixon and R. D. Trengove, *J Agr Food Chem*, 2007, **55**, 2189-2194.
9. A. L. Garner, A. K. Struss, J. L. Fullagar, A. Agrawal, A. Y. Moreno, S. M. Cohen and K. D. Janda, *ACS Med Chem Lett*, 2012, **3**, 668-672.