SUPPORTING INFORMATION

ABHD17 regulation of plasma membrane palmitoylation and N-Ras-dependent cancer growth

Jarrett R. Remsberg^, Radu M. Suciu^, Noemi A. Zambetti^, Thomas W. Hanigan, Ari J. Firestone, Anagha Inguva, Amy Long, Nhi Ngo, Kenneth M. Lum, Cassandra L. Henry, Stewart K. Richardson, Marina Predovic, Ben Huang, Melissa M. Dix, Amy R. Howell[5], Micah J. Niphakis*, Kevin Shannon*, and Benjamin F. Cravatt*

Table of Contents

Supplementary Tables

Supplementary Table 1. Small molecule screening data

Category	Parameter	Description
Assay	Type of assay	Gel-based ABPP
	Target	mABHD17B
	Primary measurement	mABHD17B fluorescent gel-band intensity
	Key reagents	FP-Rh: (2-[3-(dimethylamino)-6-dimethyliminio-xanthen-9-yl]-5-[5-[10-[ethoxy(fluoro)phosphoryl]decoxycarbonylamino]pentylcarbamoyl]benzoate)
	Assay protocol	Inhibitor activity against mABHD17B, was determined by competitive gel-based ABPP in the particulate fraction of mouse brain homogenates using FP-Rh competition. Brain proteomes (50 ug, 1.0 mg/mL) were first treated with each test compound or DMSO for 30 min at 37 ºC and subsequently treated with FP-Rh (1.0 μM) for an additional 30 min at room temperature. The assays were then quenched with 4X SDS-PAGE loading buffer and FP-Rh-labeled enzymes were resolved by SDS-PAGE (10% acrylamide) and their relative activities measured by in-gel fluorescence imaging using a Bio-Rad ChemiDoc™ XRS imager. Quantification of enzyme activities was performed by densitometric analysis using ImageJ software (NIH).
	Additional comments	-
Library	Library size	~5000
	Library composition	Structurally diverse collection of serine hydrolase-directed electrophiles
	Source	Proprietary screening library designed and synthesized at Abide Therapeutics (now owned by Lundbeck)
	Additional comments	-
Screen	Format	96-well plate
	Concentration(s) tested	1.0 and 10 μM
	Plate controls	>2 DMSO control lanes / gel (up to 28 lanes / gel)
	Reagent/ compound dispensing system	Manual
	Detection instrument and software	Bio-Rad ChemiDoc™ XRS imager
	Assay validation/QC	-
	Correction factors	-
	Normalization	-
	Additional comments	Compounds were screened on a weekly basis as they were synthesized over ~18 months
Post-HTS analysis	Hit criteria	>50% inhibition of FP-Rh labeling at 10 μM
	Hit rate	~1%
	Additional assay(s)	Validation against hABHD17B using methods described in Supplementary information
	Confirmation of hit purity and structure	LC/MS and 1H NMR
	Additional comments	-

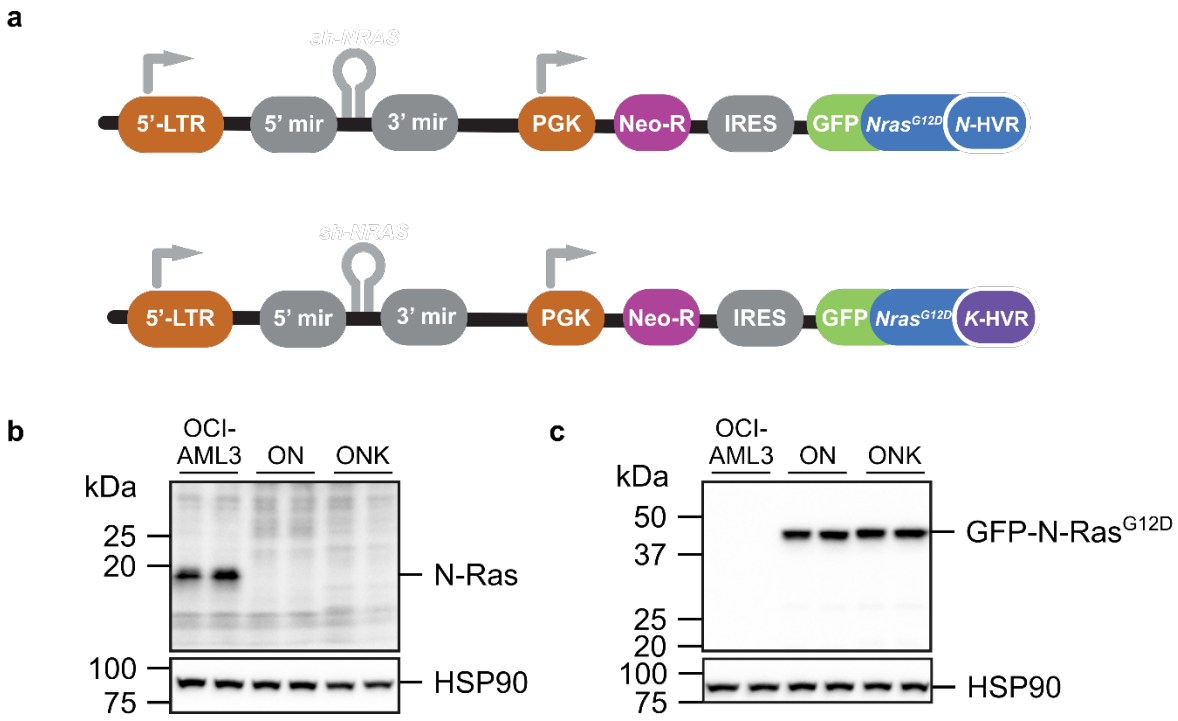
Supplementary Table 2. Properties of ABHD17 inhibitors. Potency values for inhibiting human ABHD17B and LYPLA1 were measured by gel-ABPP in lysates of HEK293T cells stably expressing recombinant human ABHD17B following a 30 min inhibitor preincubation. Inhibition of endogenously expressed LYPLA1 was also measured in these experiments in parallel. Data represent average values and 95% confidence intervals from three independent experiments fit with a normalized response curve. Standard deviation values for IC50s determined independently for each each experimental replicate are also provided. Intrinsic clearance was measured in human liver microsomes (HLM) (n = 1).

Compound	ABHD17B IC_{50} (μM)	LYPLA1 IC_{50} (μM)	HLM (μL/min/mg)
5	0.89 (0.71-1.1, s.d 0.07)	0.05 (0.04-0.06, s.d. 0.01)	-
6	0.92 (0.73 – 1.2, s.d. 0.05)	0.43 (0.35-0.53, s.d. 0.12)	-
7	0.25 (0.20-0.32, s.d. 0.07)	11 (7.6-16, s.d. 3.4)	-
8	0.14 (0.11-0.16, s.d. 0.03)	>10	480
4 (ABD957)	0.21 (0.16-0.28, s.d. 0.04)	>10	4.8

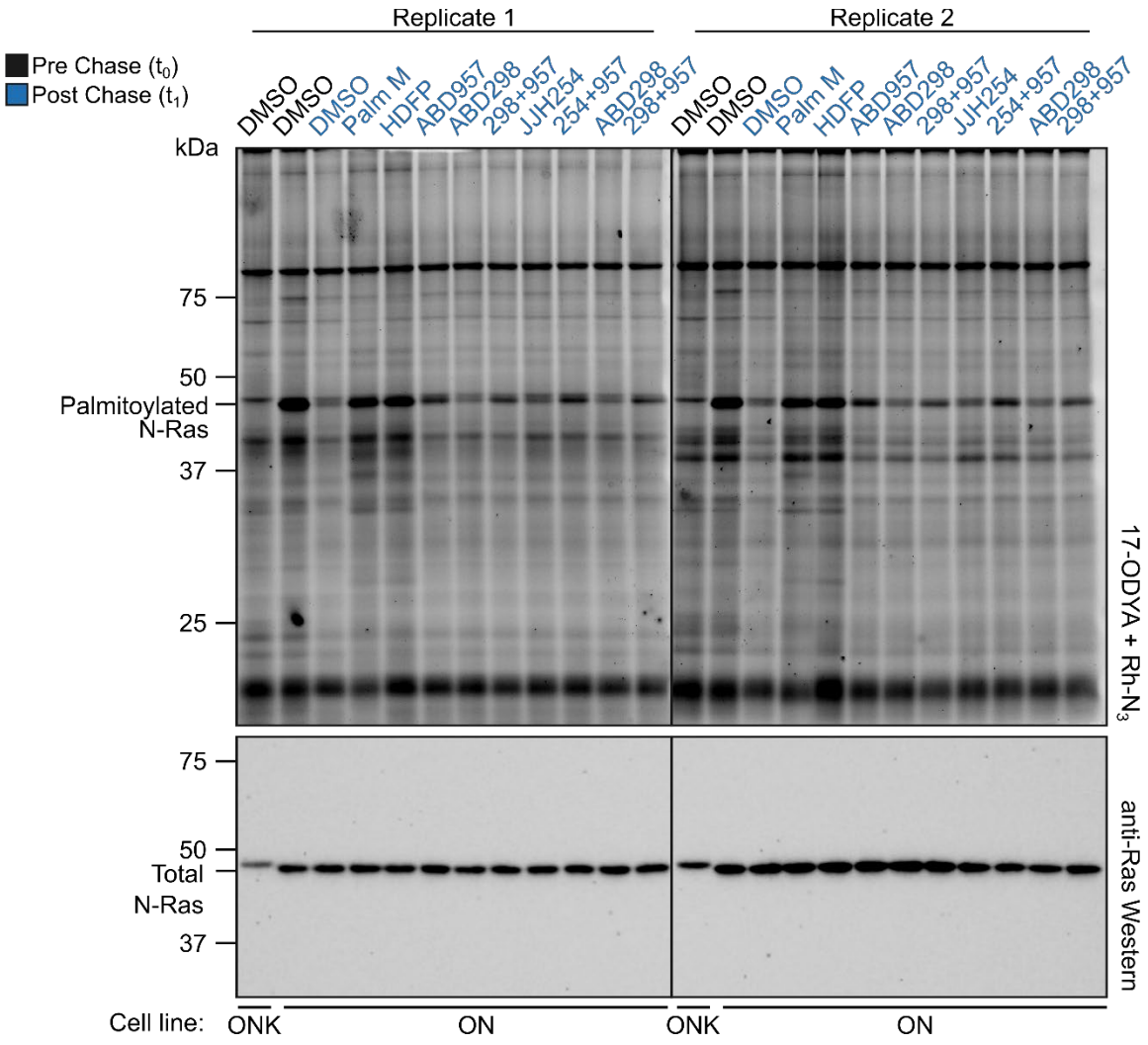
Supplementary Table 3. Peptide sequences and targeting parameters used for parallel reaction monitoring.

Protein (human)	Peptide Sequence	m/z	z	t start (min)	t stop (min)	Collision Energy (%)	AGC Target	Max Injection Time (ms)
ABHD17A	ELDTIEVFPTK	646.3426	2	29.05	33.05	30	600000	500
ABHD17A	FISQELPSQR	602.8197	2	19.63	23.63	30	600000	500
ABHD17B	WTLHLSER	521.2774	2	19.7	23.7	30	600000	500
ABHD17B	ADWQYSSR	506.7278	2	16.29	20.29	30	600000	500
ABHD17C	ADWQYSQR	527.2411	2	16.22	20.22	30	600000	500
ABHD17C	VAFPDTR	403.2138	2	17.34	21.34	30	600000	500
LYPLA1	ASFPQGPIGGANR	636.3282	2	20.09	24.09	30	400000	200
LYPLA1	TLVNPANVTFK	602.3402	2	24.17	28.17	30	400000	200
LYPLA2	IPVTLNMK	458.2702	2	21.73	25.73	30	400000	200
LYPLA2	AFPQAANGSAK	531.2724	2	12.46	16.46	30	400000	200
PCCA	IAWDDEETR	567.7567	2	19.88	23.88	30	400000	200
PCCA	LSSQEAASSFGDDR	735.3288	2	17.96	21.96	30	400000	200
PCCA	SFGLPSIGR	467.2613	2	26.2	30.2	30	400000	200
PCCA	LSQYQEPLHLPGVR	546.2984	3	23.31	27.31	30	400000	200
PCCA	FLSDVYPDGFK	644.3164	2	28.58	32.58	30	400000	200
PCCA	VTEDTSSVLR	553.788	2	16.64	20.64	30	400000	200
MCCC1	QEGIIFIGPPPSAIR	797.9512	2	32.07	36.07	30	400000	200
MCCC1	IIEEAPAPGIK	569.3293	2	19.69	23.69	30	400000	200
MCCC1	IPLSQEEITLQGHAFEA R	680.3567	3	25.25	29.25	30	400000	200
MCCC1	EGSIEIDIPVPK	648.8559	2	30.38	34.38	30	400000	200

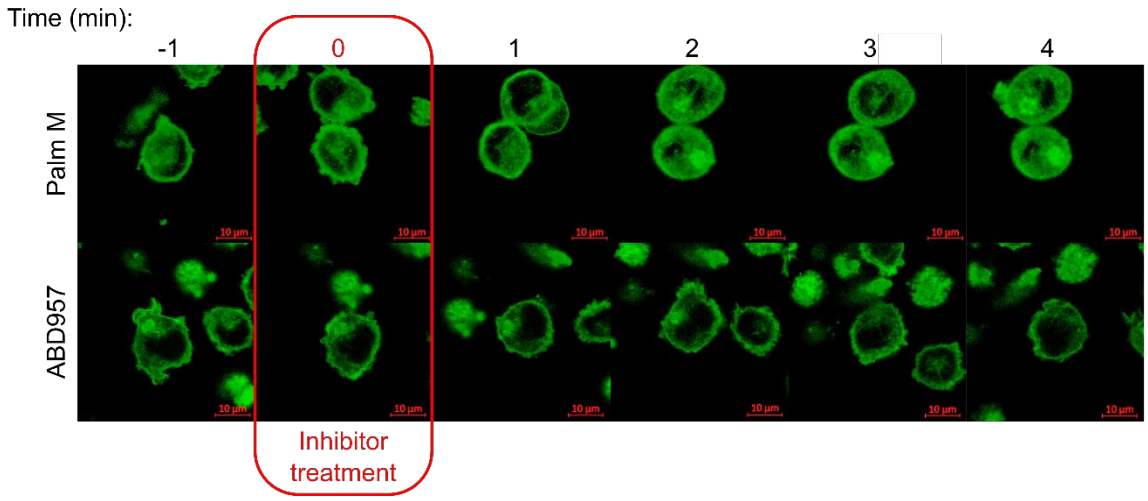
Supplementary Figures



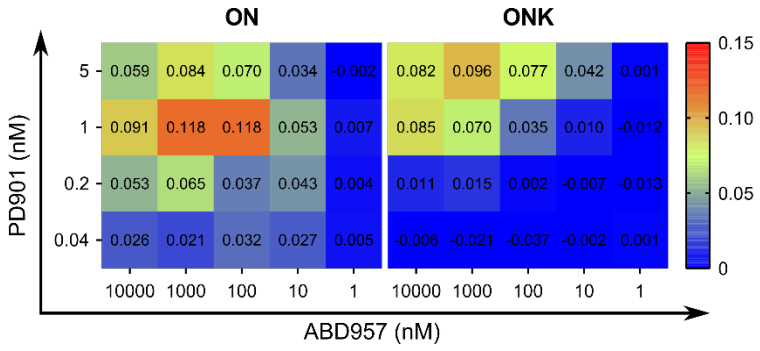
Supplementary Figure 1. Establishment of OCI-AML3 sublines expressing GFP-N-Ras^{G12D} (ON cells) or GFP-N-Ras$^{G12D\text{-}KRAS\text{-}HVR}$ (ONK cells). a, Schematic of viral construct containing an shRNA for N-Ras and cDNAs for GFP-N-Ras^{G12D} or GFP-N-Ras$^{G12D\text{-}KRAS\text{-}HVR}$. **b,** Western blot confirming endogenous N-Ras knockdown in stably infected ON and ONK cells using N-Ras specific antibody[1]. **c,** Western blot analysis with an anti-Ras (G12D mutant) antibody confirming expression of GFP-N-Ras^{G12D} or GFP-N-Ras$^{G12D\text{-}KRAS\text{-}HVR}$ in ON and ONK cells, respectively. For Western blots, two biological replicates are shown for each group.



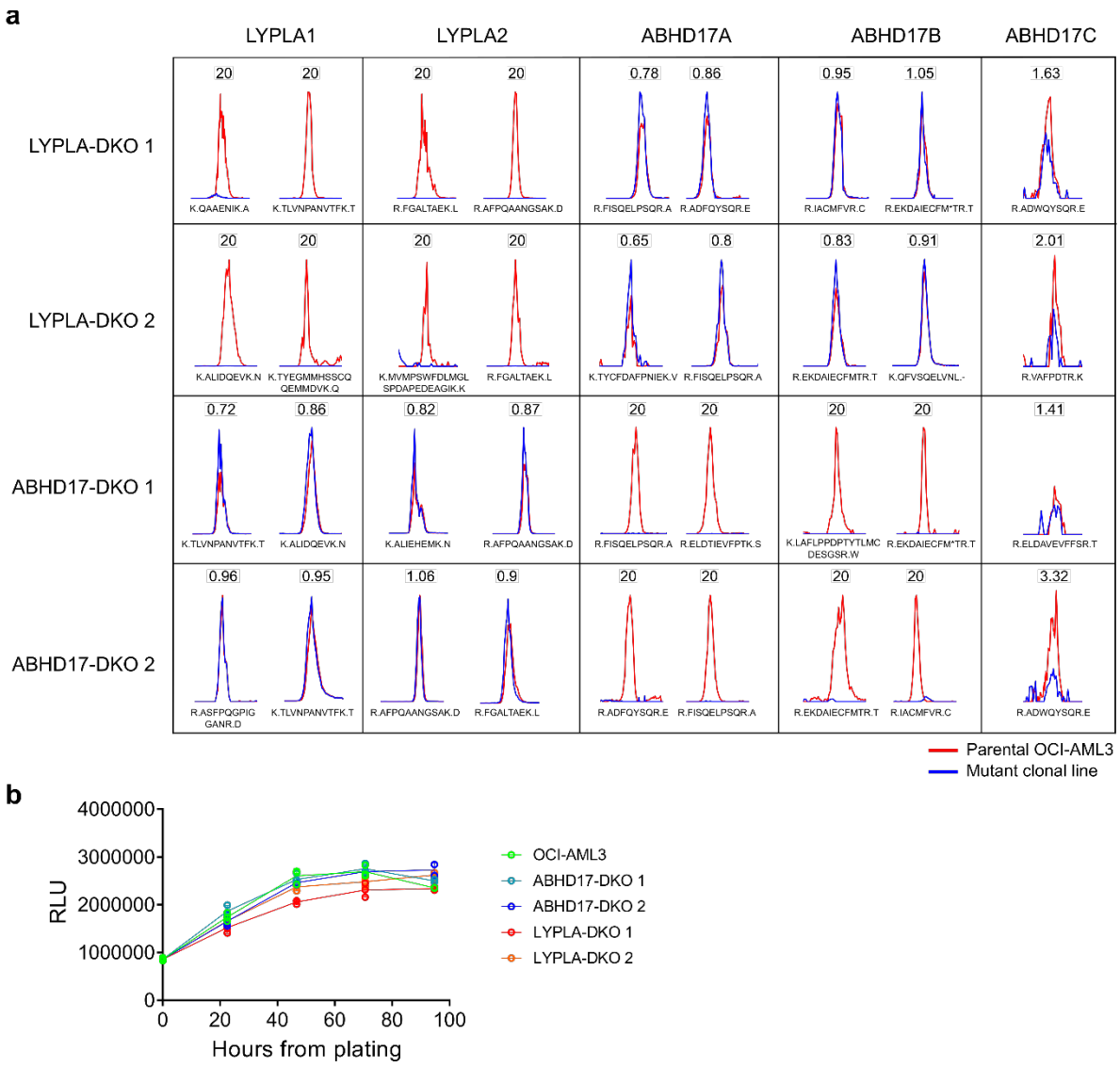
Supplementary Figure 2. Effects of inhibitor treatment on global palmitoylation as assessed by SDS-PAGE and in-gel fluorescence scanning. Representative SDS-PAGE analysis of protein modification by 17-ODYA comparing effects of treatment with Palm M (10 μM), HDFP (20 μM), ABD957 (500 nM), JJH254 (1 μM), and ABD298 (500 nM) in ON cells. Palmitoylation visualized by rhodamine attached via CuAAC to the alkyne of 17-ODYA (top gel). Total N-Ras quantity in each sample was visualized by Western blotting with a Ras antibody. Gels shows are from two independent experiments.



Supplementary Figure 3. Effect of inhibitor treatment on GFP-N-Ras localization in ON cells. Time course images taken once every minute following administration of DMSO, Palm M (10 μM), or ABD957 (500 nM). Compound was infused into the medium at t = 0 (boxed in red). Green channel shows GFP-N-Ras. Note that only cells within the focal plane of the image collections were evaluated for N-Ras localization. Additional cells that were out of the focal plane are also seen in certain fields (identified by typically smaller sizes with entire cell showing green color). Representative images taken from three separate biological replicate time course experiments. Scale bar, 10 μM.



Supplementary Figure 4. Bliss independence score quantifying synergy of ABD957 and PD901 in **Figure 5c,d**. A positive Bliss score indicates that the observed combined response is greater than expected and indicative of synergy.



Supplementary Figure 5. Proteomic validation of targeted enzyme disruption in LYPLA1/2 and ABHD17A/B KO lines generated by CRISPR-Cas9 gene editing. a, MS1 chromatograms for representative unique peptides from the indicated serine hydrolases quantified by MS-ABPP in parental OCI-AML3 vs. clonal KO cell lines. Note that a single unique peptide was detected for ABHD17C, albeit at low and variable signals, across all cell lines. **b,** Estimated growth rates of parental OCI-AML3 cells and clonal KO cell lines as determined by luminescence measurement of ATP content over time. Data represent average values ± s.d. (biological replicates; n = 2 for 94.75 hour time point, n = 3 for all others).

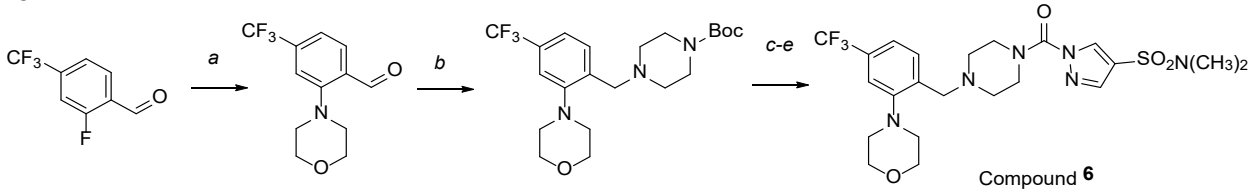
Supplementary Videos

Supplementary Video 1. Effect of inhibitor treatment on GFP-N-Ras localization in ON cells. Representative time-lapse videos from ON cells before and after treatment with Palm M (10 μM) (**a**), ABD957 (500 nM) (**b**), or DMSO control (**c**). Green channel shows GFP-N-Ras signal. Frames were acquired every 90s over 680 x 680 pixels for 10 frames prior to compound infusion and a total of 35 frames is shown, with each frame requiring 13.37 seconds to scan. Relative acquisition time is shown in red text in the upper left. Frames where compound infusion begins are captioned in the upper left in red text with the name of compound used for the experiment. Data shown are a representative of three biological replicates. One medial z-plane over a 400 x 400-pixel area is shown for clarity. Scale bar, 10 μm.

Supplementary Note – Chemistry Methods

All commercially available chemicals were obtained from Aldrich, Acros, Fisher, Fluka, Maybridge, or the like and were used without further purification. Palm M and HDFP were synthesized as generally described[2,3]. Anhydrous solvents and oven-dried glassware were used for synthetic transformations sensitive to moisture and/or oxygen. All reactions are typically carried out under an inert nitrogen atmosphere using oven-baked glassware unless otherwise noted. Flash chromatography is performed using 230–400 mesh silica gel 60 using Isco Combiflash instruments. NMR spectra were generated on either Bruker 300 or Bruker 400 instruments. Chemical shifts are typically recorded in ppm relative to tetramethylsilane (TMS) with multiplicities given as s (singlet), bs (broad singlet), d (doublet), t (triplet), dt (double of triplets), q (quadruplet), qd (quadruplet of doublets), hept (heptuplet), and m (multiplet). Chemical purities were >95% for all final compounds as assessed by LC/MS analysis with detection at 210 and 254 nm.

N,N-dimethyl-1-(4-(2-morpholino-4-(trifluoromethyl)benzyl)piperazine-1-carbonyl)-1*H*-pyrazole-4-sulfonamide (6).



*Step A: 2-morpholino-4-(trifluoromethyl)benzaldehyde (**11**)*
A 100-mL round-bottom flask was charged with 2-fluoro-4-(trifluoromethyl)benzaldehyde (400 mg, 2.08 mmol, 1.00 equiv) in DMSO (10 mL). Morpholine (271 mg, 3.11 mmol, 1.50 equiv), potassium carbonate (861 mg, 6.18 mmol, 3.00 equiv) was added under nitrogen. The resulting solution was stirred overnight at 80 °C. The reaction progress was monitored by LCMS and upon completion the reaction was quenched with water (10 mL). The resulting solution was extracted with EtOAc (3 x 10 mL) and the organic layers were combined, washed with brine (2 x 10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with EtOAc/petroleum ether (1/5) to yield 223 mg (41% yield) of **11** as yellow oil.

1H NMR (400 MHz, CDCl3): δ 10.35 (s, 1H), 7.93 (d, J = 8.0 Hz, 1H), 7.41 (d, J = 8.0 Hz, 1H), 7.35 (s, 1H), 3.99 - 3.90 (m, 4H), 3.16 (m, J = 5.7 Hz, 3.5 Hz, 4H); LCMS (ESI, m/z): 260 $[M+H]^+$.

*Step B: tert-butyl 4-(2-morpholino-4-(trifluoromethyl)benzyl)piperazine-1-carboxylate (**12**)*
A 100-mL round-bottom flask was charged with **11** (223 mg, 0.860 mmol, 1.00 equiv) in 1,2-dichloroethane (10 mL), *tert*-butyl piperazine-1-carboxylate (240 mg, 1.29 mmol, 1.50 equiv), triethylamine (260 mg, 2.57 mmol, 3.00 equiv) was added. The resulting solution was stirred for 30 min at room temperature. Sodium triacetoxyborohydride (547 mg, 2.58 mmol, 3.00 equiv) was added. The resulting solution was stirred overnight at room temperature. The reaction progress was monitored by LCMS and upon completion the reaction was then quenched with water (10 mL). The resulting solution was extracted with DCM (3 x 10 mL) and the organic layers were combined, washed with brine (2 x 10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with MeOH/DCM (1/20) to yield 300 mg (81% yield) of **12** as yellow oil.

1H NMR (400 MHz, CDCl3): δ 7.61 (d, *J* = 7.1 Hz, 1H), 7.39 - 7.29 (m, 2H), 3.94 - 3.80 (m, 4H), 3.61 (s, 2H), 3.52 - 3.34 (m, 4H), 3.11 - 2.91 (m, 4H), 2.60 - 2.27 (m, 4H), 1.47 (s, 9H); LCMS (ESI, *m/z*): 430 $[M+H]^+$.

*Step C: 4-[2-(piperazin-1-ylmethyl)-5-(trifluoromethyl)phenyl]morpholine (**13**)*
A 100-mL round-bottom flask was charged with **12** (300 mg, 0.700 mmol, 1.00 equiv) in DCM (10 mL), trifluoroacetic acid (2.50 mL). The resulting solution was stirred for 5 h at room temperature. The reaction progress was monitored by LCMS and upon completion the resulting mixture was concentrated under reduced pressure to yield 230 mg (100% yield) of **13** as yellow oil and was carried to the next step without further purification. LCMS (ESI, *m/z*): 330 $[M+H]^+$

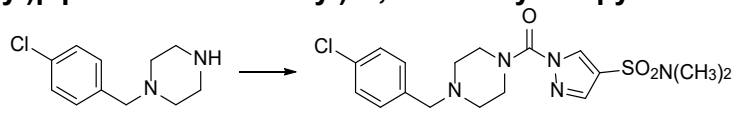
*Step D: 4-[[2-(morpholin-4-yl)-4-(trifluoromethyl)phenyl]methyl]piperazine-1-carbonyl chloride (**14**)*
A 40-mL round-bottom flask was charged with triphosgene (104 mg, 0.350 mmol, 0.50 equiv) in DCM (10 mL), **13** (230 mg, 0.700 mmol, 1.00 equiv), N-ethyl-N-isopropylpropan-2-amine (181 mg, 1.40 mmol, 2.00 equiv) was added. The resulting solution was stirred for 3 h at 0 °C. The reaction was then quenched with water (10 mL). The resulting solution was extracted with DCM (3 x 10 mL) and the organic layers were combined, washed with brine (2 x 10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to yield 300 mg (crude) of **14** as yellow oil and was carried to the next step without further purification. LCMS (ESI, *m/z*): 392 $[M+H]^+$.

*Step E: N,N-dimethyl-1-(4-(2-morpholino-4-(trifluoromethyl)benzyl)piperazine-1-carbonyl)-1H-pyrazole-4-sulfonamide (**6**)*

A 40-mL round-bottom flask was charged with **14** (300 mg, 0.770 mmol, 1.00 equiv) in THF (10 mL), *N,N*-dimethyl-1H-pyrazole-4-sulfonamide (161 mg, 0.920 mmol, 1.20 equiv), *N,N*-diisopropylethylamine (297 mg, 2.30 mmol, 3.00 equiv), 4-dimethylaminopyridine (9.36 mg, 0.0800 mmol, 0.10 equiv) was added. The resulting solution was stirred overnight at 60 °C. The reaction progress was monitored by LCMS and upon completion the reaction was then quenched with water (10 mL). The mixture was extracted with DCM (3 x 10 mL) and the organic layers were combined, washed with brine (2 x 10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product (710 mg) was purified by preparative HPLC using the following gradient conditions: 30% CH_3CN/80% Phase A increasing to 80% CH_3CN over 10 min, then to 100% CH_3CN over 0.1 min, holding at 100% CH_3CN for 1.9 min, then reducing to 30% CH_3CN over 0.1 min, and holding at 30% for 1.9 min, on a Waters 2767-5 Chromatograph. Column: Xbridge Prep C18, 19*150mm 5μm; Mobile phase: Phase A: aqueous NH_4HCO_3 (0.05%); Phase B: CH_3CN; Detector, UV220 & 254nm. Purification resulted in 118.0 mg (29% yield) of **6** as a white solid.

1H NMR (300 MHz, CDCl3): δ 8.47 (s, 1H), 7.82 (s, 1H), 7.61 - 7.66 (m, 1H), 7.31 - 7.40 (m, 2H), 3.77 - 3.92 (m, 8H), 3.67 (bs, 2H), 2.87 - 3.02 (m, 4H), 2.74 (s, 6H), 2.63 (bs, 4H); 13C NMR (101 MHz, DMSO-*d*6): δ 152.36, 149.20, 139.96, 136.85, 134.12, 131.10, 128.50 (q, *J* = 31.5 Hz), 124.23 (q, *J* = 272.3 Hz), 119.68 (q, *J* = 3.5 Hz), 117.88, 116.07 (q, *J* = 3.6 Hz), 66.50, 56.25, 52.46, 52.33, 37.46; HRMS (m/z): [M+H]+ calcd. for C22H29F3N6O4S, 531.1996; found 531.2003.

1-(4-(4-chlorobenzyl)piperazine-1-carbonyl)-*N,N*-dimethyl-1H-pyrazole-4-sulfonamide (5).

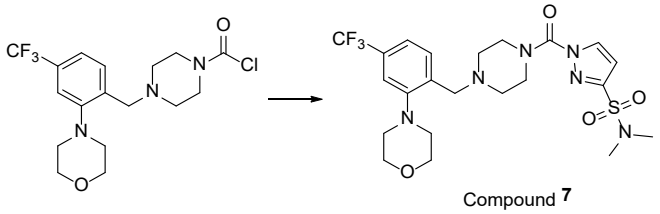


Compound **5**

To a stirred mixture solution of 1-(4-chlorobenzyl)piperazine (450 mg, 2.13 mmol, 1.00 equiv) and *N,N*-diisopropylethylamine (1.10 g, 8.52 mmol, 4.00 equiv) in DCM (5.00 mL) was added triphosgene (238 mg, 0.852mmol, 0.400 equiv) dropwise at 0 °C. After 1 h, a mixture of *N,N*-dimethyl-1H-pyrazole-4-sulfonamide (370 mg, 2.13 mmol, 1.00 equiv), DMAP (260 mg, 2.13 mmol, 1.00 equiv) and *N,N*-diisopropylethylamine (275 mg, 2.13 mmol, 1.00 equiv) in DCM (5.00 mL) was added dropwise to the above reaction solution at 0 °C. The reaction mixture was stirred overnight at 45 °C before quenching with water (10 mL). The resulting solution was extracted with DCM and the combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by Prep-HPLC with the following gradient conditions: Column: XBridge Shield RP18 OBD Column, 30×150mm 5um; Mobile Phase A: Water (10 mmol/L NH_4HCO_3 + 0.1% NH_4OH), Mobile Phase B: ACN; Flow rate: 60 mL/min; Gradient: 36 B to 63 B in 8 min; 220 nm; RT1: 7.7 min to provide 122.3 mg (14% yield) of **5** as a white solid.

1H NMR (300 MHz, CDCl3): δ 8.46 (s, 1H), 7.82 (s, 1H), 7.26 - 7.33 (m, 4H), 3.86 (bs, 4H), 3.53 (s, 2 H), 2.74 (s, 6H), 2.56 (s, 4H); 13C NMR (101 MHz, DMSO-*d*6): δ 149.18, 139.95, 136.69, 134.13, 131.62, 130.72, 128.20, 117.86, 60.69, 52.02, 37.45; HRMS (m/z): [M+H]+ calcd. for C17H22ClN5O3S, 412.1205; found 412.1208.

***N,N*-dimethyl-1-(4-(2-morpholino-4-(trifluoromethyl)benzyl)piperazine-1-carbonyl)-1H-pyrazole-3-sulfonamide (7)**



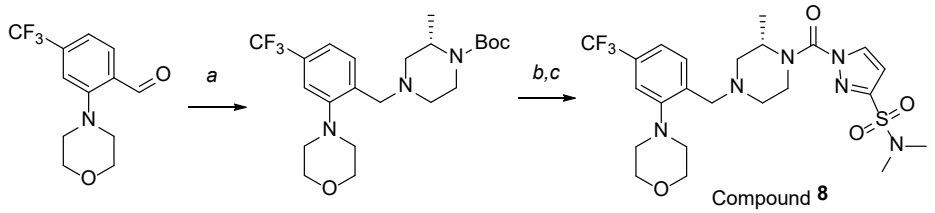
Compound **7**

A 50-mL round-bottom flask was charged with *N,N*-dimethyl-1H-pyrazole-3-sulfonamide (107 mg, 0.610 mmol, 1.10 equiv), THF (10 mL), 4-dimethylaminopyridine (20.4 mg, 0.170 mmol, 0.30 equiv), *N,N*-diisopropylethylamine (144 mg, 1.12 mmol, 2.00 equiv), **14** (218 mg, 0.560 mmol, 1.00 equiv). The resulting solution was stirred overnight at 60 °C and quenched with water (10 mL). The resulting mixture was extracted with DCM (3 x 15 mL) and the organic layers were combined, washed with brine (1 x 50 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product (450 mg) was purified by preparative HPLC using the following gradient conditions: 20% CH_3CN/80% Phase A increasing to 80% CH_3CN over 10 min, then to 100% CH_3CN over 0.1 min, holding at 100% CH_3CN for 1.9 min, then reducing to 20% CH_3CN over 0.1 min, and holding at 20% for 1.9 min, on a Waters 2767-5 Chromatograph. Column: Xbridge Prep C18, 19*150mm 5um; Mobile phase: Phase A: aqueous NH_4HCO_3 (0.05%); Phase B: CH_3CN; Detector, UV220 & 254nm. Purification resulted in 75.7 mg (26% yield) of **7** as a yellow solid.

1H NMR (300 MHz, CDCl3): δ 8.18 (d, *J* = 2.7 Hz, 1H), 7.62 - 7.60 (m, 1H), 7.36 - 7.32 (m, 2H), 6.74 (d, *J* = 2.7 Hz, 1H), 3.87 - 3.84 (m, 8H), 3.65 (bs, 2H), 2.98 - 2.96 (m, 4H), 2.86 (s, 6H), 2.60 (bs, 4H); 13C NMR (101 MHz, DMSO-*d*6): δ 152.35, 149.37, 148.79, 136.89, 134.18,

131.07, 128.49 (q, *J* = 31.4 Hz), 124.23 (q, *J* = 272.3 Hz), 119.71 (q, *J* = 3.7 Hz), 116.08 (d, *J* = 3.4 Hz), 108.12, 66.50, 56.20, 52.45, 52.29, 37.61; HRMS (m/z): [M+H]+ calcd. for C22H29F3N6O4S, 531.1996; found 531.1986.

(*S*)-*N,N*-dimethyl-1-(2-methyl-4-(2-morpholino-4-(trifluoromethyl)benzyl)piperazine-1-carbonyl)-1H-pyrazole-3-sulfonamide (8)



*Step A: tert-butyl (S)-2-methyl-4-(2-morpholino-4-(trifluoromethyl)benzyl)piperazine-1-carboxylate (**15**)*

A 50-mL round-bottom flask was charged with **11** (300 mg, 1.16 mmol, 1.00 equiv), *tert*-butyl (2S)-2-methylpiperazine-1-carboxylate (278 mg, 1.39 mmol, 1.20 equiv), DCM (10 mL). The mixture was stirred for 2 h at room temperature. Sodium triacetoxyborohydride (982 mg, 4.63 mmol, 4.00 equiv) was added. The resulting solution was stirred overnight at room temperature and then quenched with water (50 mL). The resulting mixture was extracted with DCM (3 x 50 mL) and the organic layers were combined, washed with brine (1 x 100 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with MeOH/DCM (1/20) to provide 470 mg (92% yield) of **15** as yellow oil.

1H NMR (400MHz, CDCl3): δ 7.60 (d, *J* = 7.9 Hz, 1H), 7.36 - 7.29 (m, 2H), 4.22 (s, 1H), 3.99 - 3.74 (m, 5H), 3.61 (d, *J* = 13.3 Hz, 1H), 3.48 (d, *J* = 13.3 Hz, 1H), 3.20 - 3.02 (m, 3H), 3.01 - 2.92 (m, 2H), 2.87 - 2.72 (m, 1H), 2.69 - 2.54 (m, 1H), 2.25 (dd, *J* = 11.2 Hz, 3.9 Hz, 1H), 2.14 - 2.02 (m, 1H), 1.47 (s, 9H), 1.21 (d, *J* = 6.7 Hz, 3H); LCMS (ESI, *m/z*): 444 $[M+H]^+$.

*Step B: (S)-4-(2-((3-methylpiperazin-1-yl)methyl)-5-(trifluoromethyl)phenyl)morpholine (**16**)*

A 250-mL round-bottom flask was charged with **15** (470 mg, 1.06 mmol, 1.00 equiv), trifluoroacetic acid (5 mL), DCM (15 mL). The resulting solution was stirred overnight at room temperature and concentrated under reduced pressure. The crude product was dissolved in 1M NaOH solution (10 mL) and extracted with DCM (3 x 20 mL). The organic layers were combined, washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to provide 340 mg (93% yield) of **16** as brown oil and was carried to the next step without further purification. LCMS (ESI, *m/z*): 344 $[M+H]^+$.

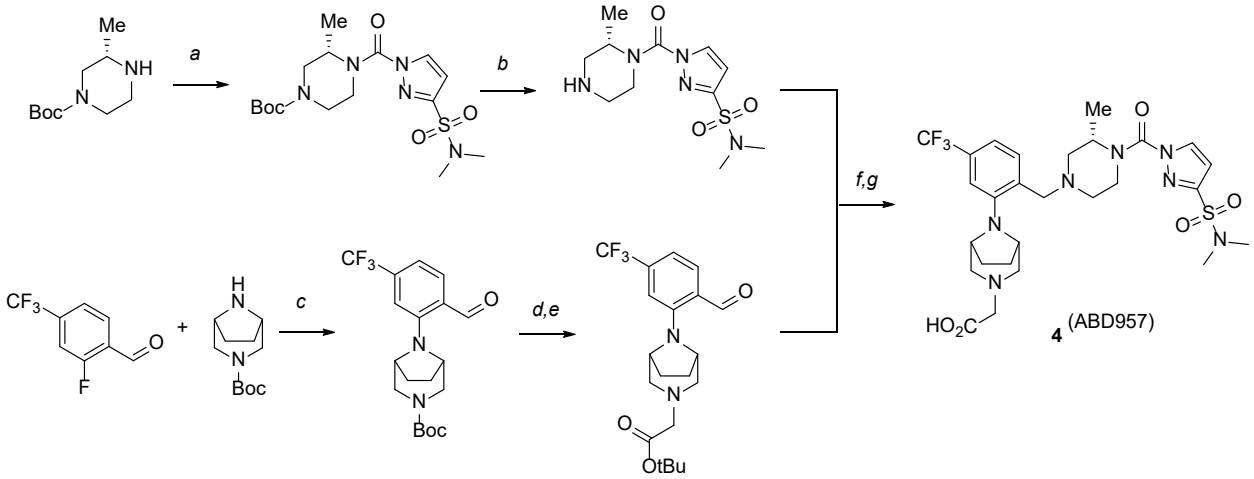
*Step C: (S)-N,N-dimethyl-1-(2-methyl-4-(2-morpholino-4-(trifluoromethyl)benzyl)piperazine-1-carbonyl)-1H-pyrazole-3-sulfonamide (**8**)*

A 50-mL round-bottom flask was charged with triphosgene (103 mg, 0.350 mmol, 0.70 equiv), DCM (5 mL). *N,N*-Dimethyl-1H-pyrazole-3-sulfonamide (175 mg, 1.00 mmol, 2.00 equiv) was added at 0 °C. *N,N*-Diisopropylethylamine (256 mg, 1.98 mmol, 4.00 equiv) was added at 0 °C. The mixture was stirred for 2 h at room temperature. **16** (170 mg, 0.500 mmol, 1.00 equiv) was added. The resulting solution was stirred overnight at room temperature and then quenched with water (50 mL). The resulting mixture was extracted with DCM (3 x 50 mL) and the organic layers were combined, washed with brine (1 x 100 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product (400 mg) was purified by preparative HPLC using the following gradient conditions: 20% CH_3CN/80% Phase A increasing to 80%

CH_3CN over 10 min, then to 100% CH_3CN over 0.1 min, holding at 100% CH_3CN for 1.9 min, then reducing to 20% CH_3CN over 0.1 min, and holding at 20% for 1.9 min, on a Waters 2767-5 Chromatograph. Column: Xbridge Prep C18, 19*150mm 5μm; Mobile phase: Phase A: aqueous NH_4HCO_3 (0.05%); Phase B: CH_3CN; Detector, UV220 & 254nm. Purification resulted in 98.7 mg (37% yield) of **8** as a white solid.

1H NMR (400 MHz, CDCl3): δ 8.17 (d, *J* = 2.7 Hz, 1H), 7.61 (bs, 1H), 7.39 – 7.28 (m, 2H), 6.74 (d, *J* = 2.7 Hz, 1H), 4.67 (bs, 1H), 4.38 – 4.28 (m, 1H), 3.93 – 3.77 (m, 4H), 3.73 – 3.48 (m, 2H), 3.47 – 3.39 (m, 1H), 3.08 – 2.90 (m, 5H), 2.85 (s, 6H), 2.76 – 2.63 (m, 1H), 2.48 – 2.18 (m, 2H), 1.46 (d, *J* = 6.2 Hz, 3H); 13C NMR (101 MHz, DMSO-*d*6): δ 152.41, 149.37, 148.74, 136.73, 134.18, 131.19, 128.54 (q, *J* = 31.3 Hz), 124.23 (q, *J* = 272.3 Hz), 119.55 (q, *J* = 3.9 Hz), 115.96 (q, *J* = 3.9 Hz), 108.14, 66.51, 57.29, 56.41, 52.45, 52.40, 37.60, 16.09; HRMS (m/z): [M+H]+ calcd. for C23H31F3N6O4S, 545.2152; found 545.2158.

2-((1R,5S)-8-(2-(((S)-4-(3-(*N,N*-dimethylsulfamoyl)-1H-pyrazole-1-carbonyl)-3-methylpiperazin-1-yl)methyl)-5-(trifluoromethyl)phenyl)-3,8-diazabicyclo[3.2.1]octan-3-yl)acetic acid (4, ABD957*)*



*Step A: tert-butyl (S)-4-(3-(N,N-dimethylsulfamoyl)-1H-pyrazole-1-carbonyl)-3-methylpiperazine-1-carboxylate (**17**)*

A 40-mL vial was charged with *tert*-butyl (3S)-3-methylpiperazine-1-carboxylate (600 mg, 3.00 mmol, 1.00 equiv), DCM (10 mL), triphosgene (446 mg, 1.50 mmol, 0.50 equiv). *N,N*-Diisopropylethylamine (1.16 g, 8.99 mmol, 3.00 equiv) was added dropwise at 0 °C. The resulting solution was stirred for 2 h at room temperature and quenched by water (10 mL). The mixture was extracted with DCM (3 x 10 mL) and the organic layers were combined, washed with brine (3 x 10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to provide 600 mg (crude) of *tert*-butyl (*S*)-4-(chlorocarbonyl)-3-methylpiperazine-1-carboxylate (**18**) as yellow oil which was carried to the next step without further purification.

A 40-mL vial was charged with **18** (600 mg, 2.28 mmol, 1.00 equiv), THF (10 mL), *N,N*-dimethyl-1H-pyrazole-3-sulfonamide (401 mg, 2.29 mmol, 1.00 equiv), *N,N*-diisopropylethylamine (886 mg, 6.86 mmol, 3.00 equiv) and 4-dimethylaminopyridine (56.0 mg, 0.460 mmol, 0.20 equiv). The resulting solution was stirred overnight at 60 °C and quenched by water (10 mL). The mixture was extracted with EtOAc (3 x 10 mL) and the organic layers were combined, washed with brine (3 x 10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The

residue was chromatographed on a silica gel column with EtOAc/petroleum ether (1/1) to provide 900 mg (98% yield) of **17** as a yellow oil.

1H NMR (400 MHz, CD3OD): *δ* 8.31 (d, *J* = 2.7 Hz, 1H), 6.84 (d, *J* = 2.7 Hz, 1H), 4.65 - 4.49 (m, 1H), 4.19 (d, 1H), 4.07 (d, *J* = 13.4 Hz, 1H), 3.97 - 3.85 (m, 1H), 3.49 - 3.37 (m, 1H), 3.30 - 3.00 (m, 2H), 2.86 (s, 6H), 1.50 (s, 9H), 1.38 (d, *J* = 6.8 Hz, 3H); LCMS (ESI, m/z): 402 $[M+H]^+$.

*Step B: (S)-N,N-dimethyl-1-(2-methylpiperazine-1-carbonyl)-1H-pyrazole-3-sulfonamide (**19**)*

A 40-mL vial was charged with **17** (900 mg, 2.24 mmol, 1.00 equiv), DCM (10 mL), trifluoroacetic acid (4 mL). The resulting solution was stirred for 2 h at room temperature and concentrated under reduced pressure to provide 660 mg (crude) of **19** as a yellow oil which was carried to the next step without further purification. LCMS (ESI, *m/z*): 302 $[M+H]^+$.

*Step C: tert-butyl 8-(2-formyl-5-(trifluoromethyl)phenyl)-3,8-diazabicyclo[3.2.1]octane-3-carboxylate (**20**)*

A 40mL vial was charged with dimethyl sulfoxide (10 mL), 2-fluoro-4-(trifluoromethyl)benzaldehyde (576 mg, 3.00 mmol, 1.00 equiv), *tert*-butyl 3,8-diazabicyclo[3.2.1]octane-3-carboxylate (623 mg, 2.93 mmol, 1.00 equiv), potassium carbonate (1.24 g, 8.97 mmol, 3.00 equiv) under nitrogen. The resulting solution was stirred overnight at 120 °C and quenched by water (10 ml). The mixture was extracted with EtOAc (3 x 10 mL) and the organic layers were combined, washed with brine (1 x 10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with EtOAc/petroleum ether (1/3) to provide 450 mg (39% yield) of **20** as a yellow oil.

1H NMR (400 MHz, CDCl3): *δ* 10.33 (s, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.28 (s, 1H), 7.19 (d, *J* = 1.5 Hz, 1H), 4.11 - 3.68 (m, 4H), 3.49 - 3.13 (m, 2H), 2.03 - 1.71 (m, 4H), 1.50 (s, 9H); LCMS (ESI, *m/z*): 385 $[M+H]^+$.

*Step D: 2-(3,8-diazabicyclo[3.2.1]octan-8-yl)-4-(trifluoromethyl)benzaldehyde (**21**)*

A 40-mL vial was charged with **20** (150 mg, 0.390 mmol, 1.00 equiv), DCM (10 mL), trifluoroacetic acid (2 mL). The resulting solution was stirred for 2 h at room temperature and concentrated under reduced pressure. The pH value of the solution was adjusted to 9 with sodium hydroxide solution (1 mol/L aq). The mixture was extracted with DCM (3 x 30 mL) and the organic layers were combined, washed with brine (2 x 20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to yield 100 mg (crude) of **21** as a yellow oil. LCMS (ESI, *m/z*): 285 $[M+H]^+$.

*Step E: tert-butyl 2-(8-(2-formyl-5-(trifluoromethyl)phenyl)-3,8-diazabicyclo[3.2.1]octan-3-yl)acetate (**22**)*

A 40-mL vial was charged with **21** (100 mg, 0.350 mmol, 1.00 equiv), acetone (10 mL), *tert*-butyl 2-bromoacetate (137 mg, 0.700 mmol, 2.00 equiv), potassium carbonate (146 mg, 1.06 mmol, 3.00 equiv). The resulting solution was stirred overnight at 80 °C and quenched with water (10 ml). The mixture was extracted with EtOAc (3 x 10 mL) and the organic layers were combined, washed with brine (1 x 10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with EtOAc/petroleum ether (1/3) to provide 125 mg (86% yield) of **22** as yellow oil.

1H NMR (400 MHz, CDCl3): *δ* 10.33 (s, 1H), 7.88 (d, *J* = 8.0 Hz, 1H), 7.26 - 7.16 (m, 2H), 3.95 - 3.80 (m, 2H), 3.21 (s, 2H), 2.86 (s, 4H), 2.08 (s, 2H), 1.90 (s, 2H), 1.49 (s, 9H); LCMS (ESI, m/z): 399 $[M+H]^+$.

*Step F: tert-butyl 2-(8-(2-(((S)-4-(3-(N,N-dimethylsulfamoyl)-1H-pyrazole-1-carbonyl)-3-methylpiperazin-1-yl)methyl)-5-(trifluoromethyl)phenyl)-3,8-diazabicyclo[3.2.1]octan-3-yl)acetate (**23**)*

A 40-mL vial was charged with 1,2-dichloroethane (10 mL), **19** (125 mg, 0.410 mmol, 1.00 equiv), **22** (165 mg, 0.410 mmol, 1.00 equiv), triethylamine (126 mg, 1.25 mmol, 3.00 equiv). The resulting solution was stirred for 1 h at room temperature, then sodium triacetoxyborohydride (264 mg, 1.25 mmol, 3.00 equiv) was added. The resulting solution was stirred overnight at room temperature and then quenched with water (10 mL). The resulting mixture was extracted with DCM (3 x 10 mL) and the organic layers were combined, washed with brine (1 x 10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with EtOAc/petroleum ether (1/1) to provide 100 mg (35% yield) of **23** as a yellow oil.

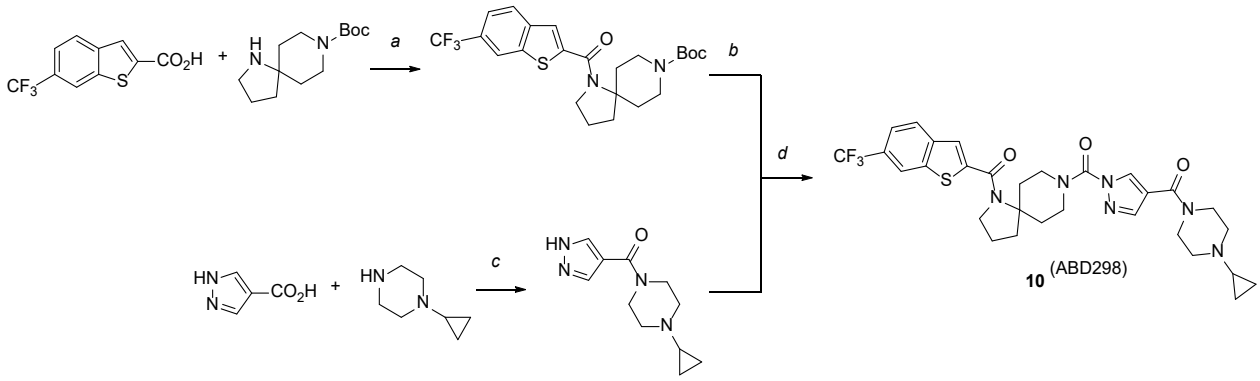
1H NMR (400 MHz, CDCl3): *δ* 8.20 (d, *J* = 2.7 Hz, 1H), 7.57 (d, *J* = 7.9 Hz, 1H), 7.24 - 7.18 (m, 1H), 7.13 - 7.08 (m, 1H), 6.77 (d, *J* = 2.7 Hz, 1H), 4.70 (s, 1H), 4.35 (d, *J* = 13.5 Hz, 1H), 3.90 (s, 1H), 3.80 (s, 1H), 3.66 (d, *J* = 13.4 Hz, 1H), 3.57 (d, *J* = 13.4 Hz, 1H), 3.50 - 3.38 (m, 1H), 3.19 (s, 2H), 2.94 (d, *J* = 11.2 Hz, 1H), 2.89 (s, 6H), 2.84 - 2.69 (m, 5H), 2.44 - 2.35 (m, 1H), 2.34 - 2.24 (m, 1H), 2.11 - 2.01 (m, 2H), 1.95 - 1.84 (m, 2H), 1.52 - 1.42 (m, 12H); LCMS (ESI, m/z): 684 $[M+H]^+$.

*Step G: 2-(8-(2-(((S)-4-(3-(N,N-dimethylsulfamoyl)-1H-pyrazole-1-carbonyl)-3-methylpiperazin-1-yl)methyl)-5-(trifluoromethyl)phenyl)-3,8-diazabicyclo[3.2.1]octan-3-yl)acetic acid (**4**, ABD957)*

A 40-mL vial was charged with **23** (100 mg, 0.150 mmol, 1.00 equiv), DCM (10 mL), trifluoroacetic acid (4 mL). The resulting solution was stirred for 2 h at room temperature and concentrated under reduced pressure. The crude product (100 mg) was purified by preparative HPLC using the following gradient conditions: 38% CH_3CN/62% Phase A increasing to 60% CH_3CN over 7 min, then to 100% CH_3CN over 0.1 min, holding at 100% CH_3CN for 1.9 min, then reducing to 38% CH_3CN over 0.1 min, and holding at 38% for 1.9 min, on a Waters 2767-5 Chromatograph. Column: Xbridge Prep C_{18}, 19*150mm 5um; Mobile phase: Phase A: aqueous NH_4HCO_3 (0.05%); Phase B: CH_3CN; Detector, UV220 & 254nm. Purification resulted in 20.4 mg (22% yield) of **4** (ABD957) as a white solid.

1H NMR (300 MHz, CD3OD): δ 8.29 (d, *J* = 2.7 Hz, 1H), 7.66 (d, *J* = 8.0 Hz, 1H), 7.31 (d, *J* = 7.9 Hz, 1H), 7.18 (s, 1H), 6.82 (d, *J* = 2.7 Hz, 1H), 4.56 (bs, 1H), 4.30 (d, *J* = 17.2 Hz, 2H), 4.20 (d, *J* = 13.8 Hz, 1H), 3.75 – 3.23 (m, 9H), 2.96 (d, *J* = 10.4 Hz, 1H), 2.83 (s, 6H), 2.81 (d, *J* = 10.4 Hz, 1H), 2.43 (dd, *J* = 11.6, 3.7 Hz, 1H), 2.38 – 2.26 (m, 1H), 2.15 (s, 4H), 1.45 (d, *J* = 6.8 Hz, 3H); 13C NMR (101 MHz, DMSO-*d*6): δ 171.56, 150.33, 149.38, 148.73, 134.18, 133.86, 131.64, 128.23 (q, *J* = 30.7 Hz), 124.32 (q, *J* = 272.1 Hz), 117.11 (q, *J* = 3.4 Hz), 113.13 (q, *J* = 3.3 Hz), 108.14, 59.53, 59.34, 58.32, 58.26, 57.75, 57.61, 57.33, 52.55, 37.62, 26.93, 16.09; HRMS (m/z): [M+H]+ calcd. for C27H36F3N7O5S, 628.2523; found 628.2534.

8-[4-(4-cyclopropylpiperazine-1-carbonyl)pyrazole-1-carbonyl]-1-[6-(trifluoromethyl)-1-benzothiophene-2-carbonyl]-1,8-diazaspiro[4.5]decane (10, ABD298)



*Step A: tert-butyl 1-(6-(trifluoromethyl)benzo[b]thiophene-2-carbonyl)-1,8-diazaspiro[4.5]decane-8-carboxylate (**24**)*

A mixture of 6-(trifluoromethyl)-1-benzothiophene-2-carboxylic acid (1.00 g, 4.06 mmol, 1.00 equiv), *tert*-butyl 1,8-diazaspiro[4.5]decane-8-carboxylate (0.98 g, 0.004 mmol, 1.00 equiv), EDCI (1.56 g, 0.008 mmol, 2.00 equiv), HOBt (0.66 g, 0.005 mmol, 1.20 equiv) and *N,N*-diisopropylethylamine (1.57 g, 0.012 mmol, 3.00 equiv) in DCM (20 mL) was stirred overnight at room temperature under nitrogen atmosphere. The reaction was quenched with water (20 mL) at room temperature. The resulting mixture was extracted with DCM (3 x 20 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by reverse flash chromatography with the following conditions: column, C18 silica gel; mobile phase, ACN in water, 10% to 50% gradient in 10 min; detector, UV 254/220 nm. This resulted in **24** (1.40 g, 73%) as an off-white solid.

1H NMR (400 MHz, $CDCl_3$): δ 8.16 (d, J = 1.7 Hz, 1H), 7.92 (d, J = 8.4 Hz, 1H), 7.66 – 7.60 (m, 2H), 4.19 (d, J = 42.6 Hz, 2H), 3.89 (t, J = 6.6 Hz, 2H), 3.10 (s, 2H), 2.83 (s, 2H), 2.09 (s, 2H), 2.02 – 1.95 (m, J = 6.7 Hz, 2H), 1.49 (s, 9H), 1.43 (s, 2H); LCMS (ESI, *m/z*): 469 $[M+H]^+$.

Step B: *(1,8-diazaspiro[4.5]decan-1-yl)(6-(trifluoromethyl)benzo[b]thiophen-2-yl)methanone hydrochloride (**25**)*

To a stirred mixture of **24** (1.40 g, 2.99 mmol, 1.00 equiv) in DCM (10 mL), was added HCl(*g*) in 1,4-dioxane (5 mL) dropwise at 0 °C under nitrogen atmosphere. The resulting mixture was stirred overnight at room temperature. The resulting mixture was concentrated under reduced pressure. This resulted in **25** (1.40 g, crude) as an off-white solid. LCMS (ESI, *m/z*): 369 $[M+H]^+$.

Step C: *(4-cyclopropylpiperazin-1-yl)(1H-pyrazol-4-yl)methanone (**26**)*

A mixture of 1-cyclopropylpiperazine (1.50 g, 11.8 mmol, 1.00 equiv), 1*H*-pyrazole-4-carboxylic acid (1.33 g, 11.8 mmol, 1.00 equiv), EDCI (4.56 g, 23.7 mmol, 2.00 equiv), HOBt (1.93 g, 14.2 mmol, 1.20 equiv) and *N,N*-diisopropylethylamine (4.61 g, 35.6 mmol, 3.00 equiv) in DCM (20 mL) was stirred overnight at room temperature under nitrogen atmosphere. The reaction was quenched with water (20mL) at room temperature. The resulting mixture was extracted with DCM (3 x 20mL). The combined organic layers were washed with brine (20mL), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by reverse flash chromatography with the following conditions: column, C18 silica gel; mobile phase, ACN in water, 10% to 50% gradient in 10 min; detector, UV 254/220 nm. This resulted in **26** (1.0 g, 38%) as an off-white solid.

1H NMR (400 MHz, $CDCl_3$): *δ* 11.53 (s, 1H), 7.81 (d, *J* = 1.8 Hz, 2H), 3.72 (s, 4H), 2.67 (s, 4H), 1.74 – 1.65 (m, 1H), 0.57 – 0.38 (m, 4H); LCMS (ESI, *m/z*): 221 $[M+H]^+$.

Step D: *(4-cyclopropylpiperazin-1-yl)(1-(1-(6-(trifluoromethyl)benzo[b]thiophene-2-carbonyl)-1,8-diazaspiro[4.5]decane-8-carbonyl)-1H-pyrazol-4-yl)methanone (**10**, ABD298)*

A stirred mixture of **25** (700 mg, 1.90 mmol, 1.00 equiv), *N,N*-diisopropylethylamine (736 mg, 5.70 mmol, 3.00 equiv) and triphosgene (225 mg, 0.760 mmol, 0.40 equiv) in DCM (20 mL) was stirred for 2 h at room temperature under nitrogen atmosphere prior to addition a mixture of **26** (460 mg, 2.09 mmol, 1.10 equiv) and DMAP (69.6 mg, 0.570 mmol, 0.30 equiv) in DCM (10 mL) at 0°C under nitrogen atmosphere. The resulting mixture was stirred overnight at room temperature before quenching with water (30 mL) at room temperature. The resulting mixture was extracted with DCM (3 x 30 mL). The combined organic layers were washed with brine (30 mL), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The crude product was purified by Prep-HPLC with the following conditions to afford **10** (ABD298) (250 mg, 26%) as a white solid.

Prep-HPLC conditions: Column: XBridge Shield RP18 OBD Column, 30*150mm,5um; Mobile Phase A:Water (10 mmol/L NH_4HCO_3), Mobile Phase B:ACN; Flow rate:60 mL/min; Gradient:35% B to 65% B in 7 min; 254/220 nm; RT1:8.02 min.

1H NMR (400 MHz, $CDCl_3$): δ 8.31 (s, 1H), 8.17 (s, 1H), 7.92 (d, *J* = 8.4 Hz, 1H), 7.85 (s, 1H), 7.69 – 7.59 (m, 2H), 4.68 (s, 2H), 3.93 (t, *J* = 6.6 Hz, 2H), 3.70 (s, 4H), 3.35 – 3.15 (m, 4H), 2.67 (s, 4H), 2.18 (t, *J* = 6.8 Hz, 2H), 2.08 – 1.97 (m, 2H), 1.68 (s, 1H), 1.58 – 1.55 (m, 2H), 0.55 – 0.43 (m, 4H); 13C NMR (101 MHz, CDCl3): δ 162.71, 161.76, 150,24, 144.50, 141.90, 141.33, 139.96, 132.17, 128.10 (q, *J* = 32.6 Hz), 125.36, 124.6, 124.36 (q, *J* = 272.3 Hz), 121.49 (q, *J* = 3.5 Hz), 120.02 (q, *J* = 4.4 Hz), 118.15, 65.97, 53.50, 51.49, 44.87, 38.43, 35.91, 32.50, 23.49, 6.04; HRMS (m/z): [M+H]+ calcd. for C30H33F3N6O3S, 615.2360; found 615.2365.

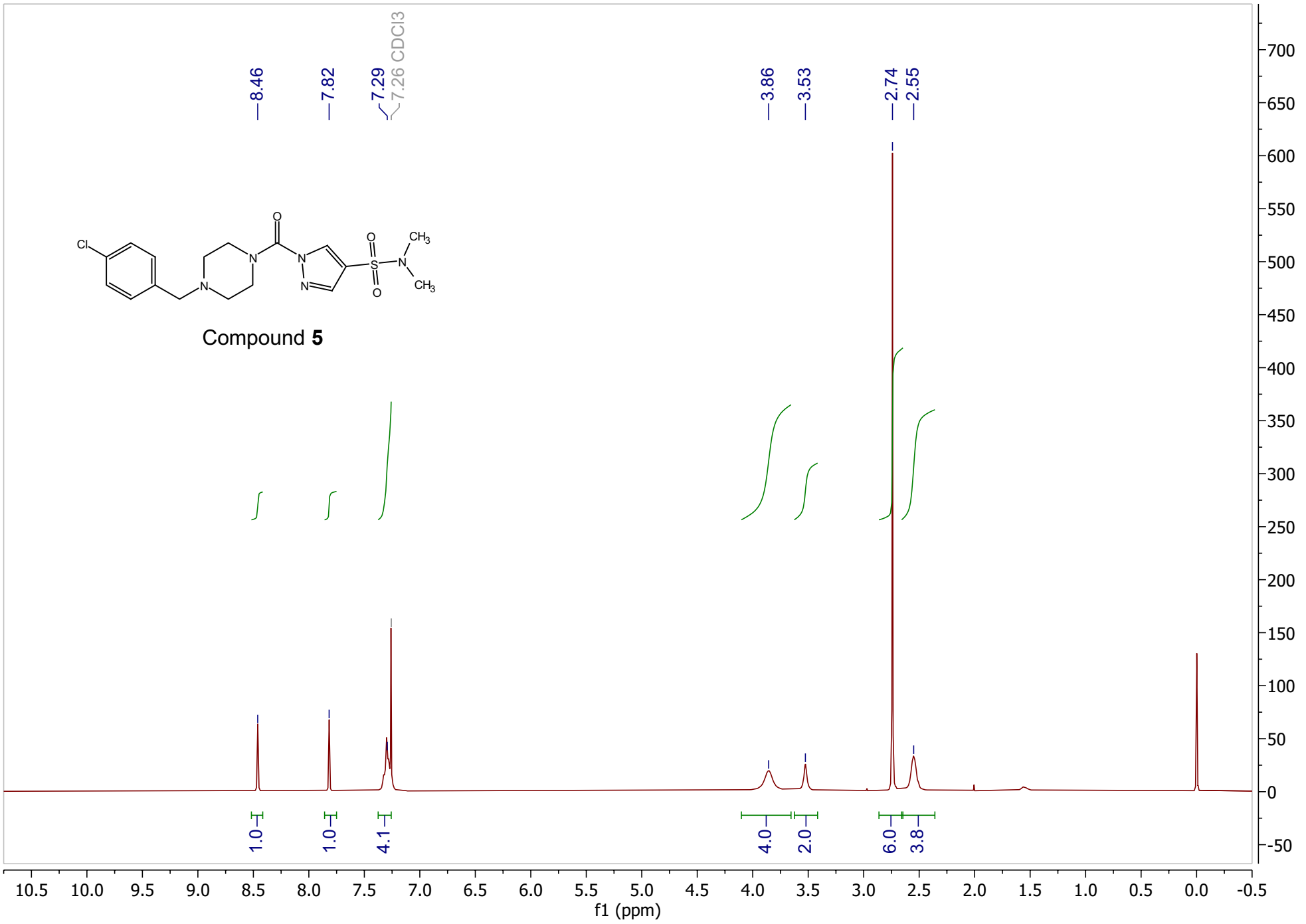
References

1. Waters, A.M. et al. Evaluation of the selectivity and sensitivity of isoform- and mutation-specific RAS antibodies. *Sci Signal* **10**(2017).
2. Hedberg, C. et al. Development of highly potent inhibitors of the Ras-targeting human acyl protein thioesterases based on substrate similarity design. *Angew Chem Int Ed Engl* **50**, 9832-7 (2011).
3. Martin, B.R., Wang, C., Adibekian, A., Tully, S.E. & Cravatt, B.F. Global profiling of dynamic protein palmitoylation. *Nat Methods* **9**, 84-9 (2011).

NMR Spectra

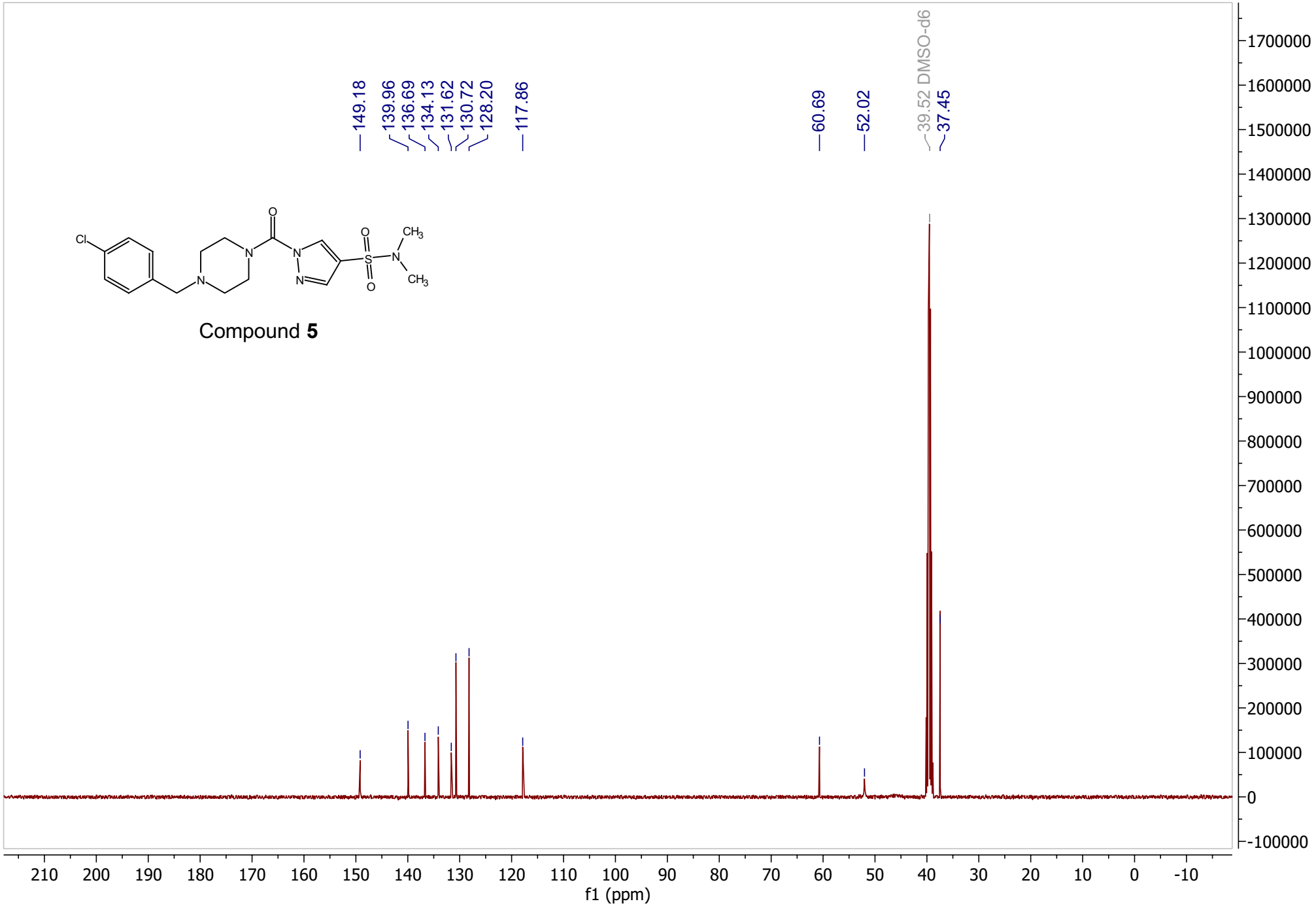
(next pages)

1H NMR (CDCl3)

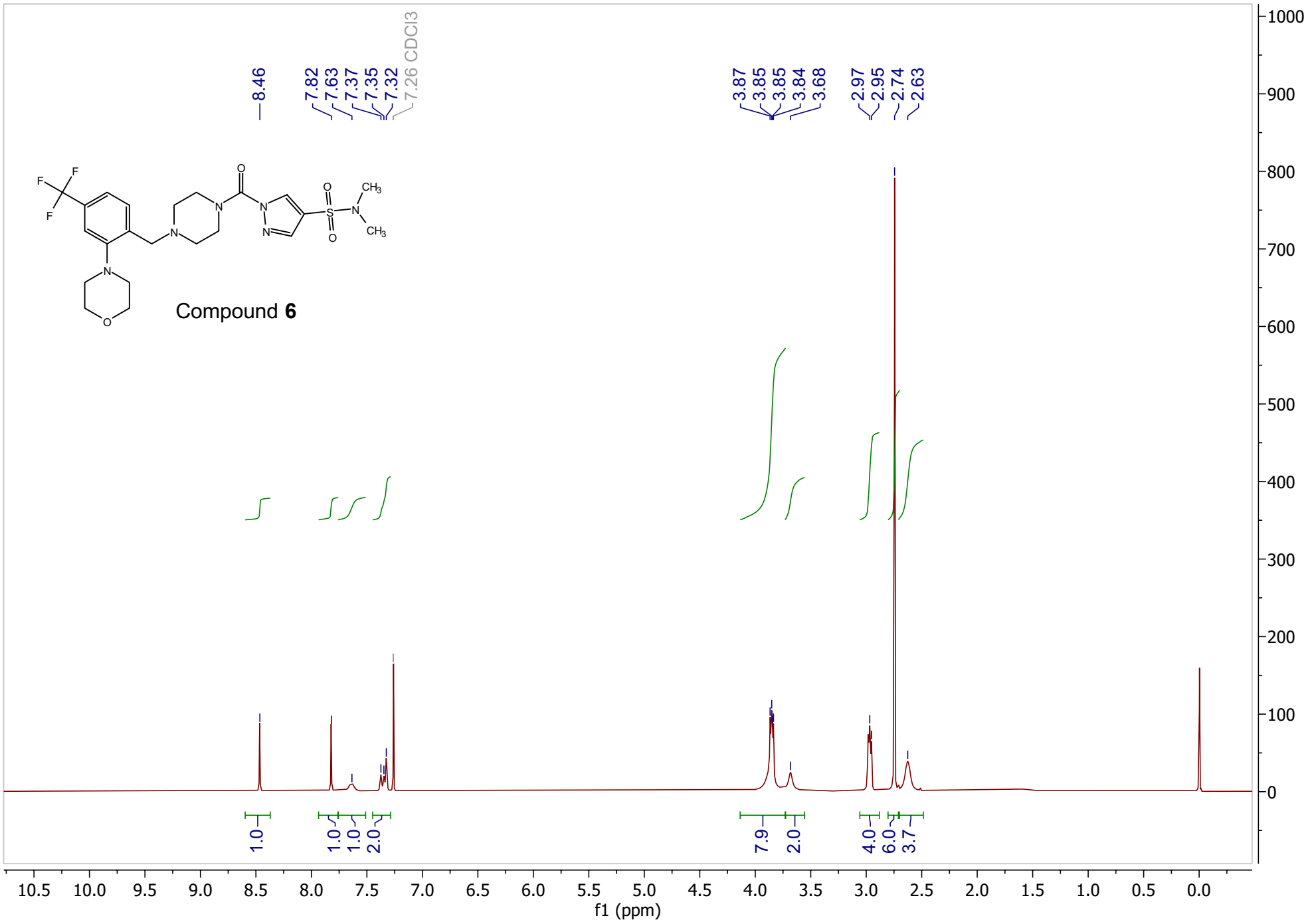


Compound **5**

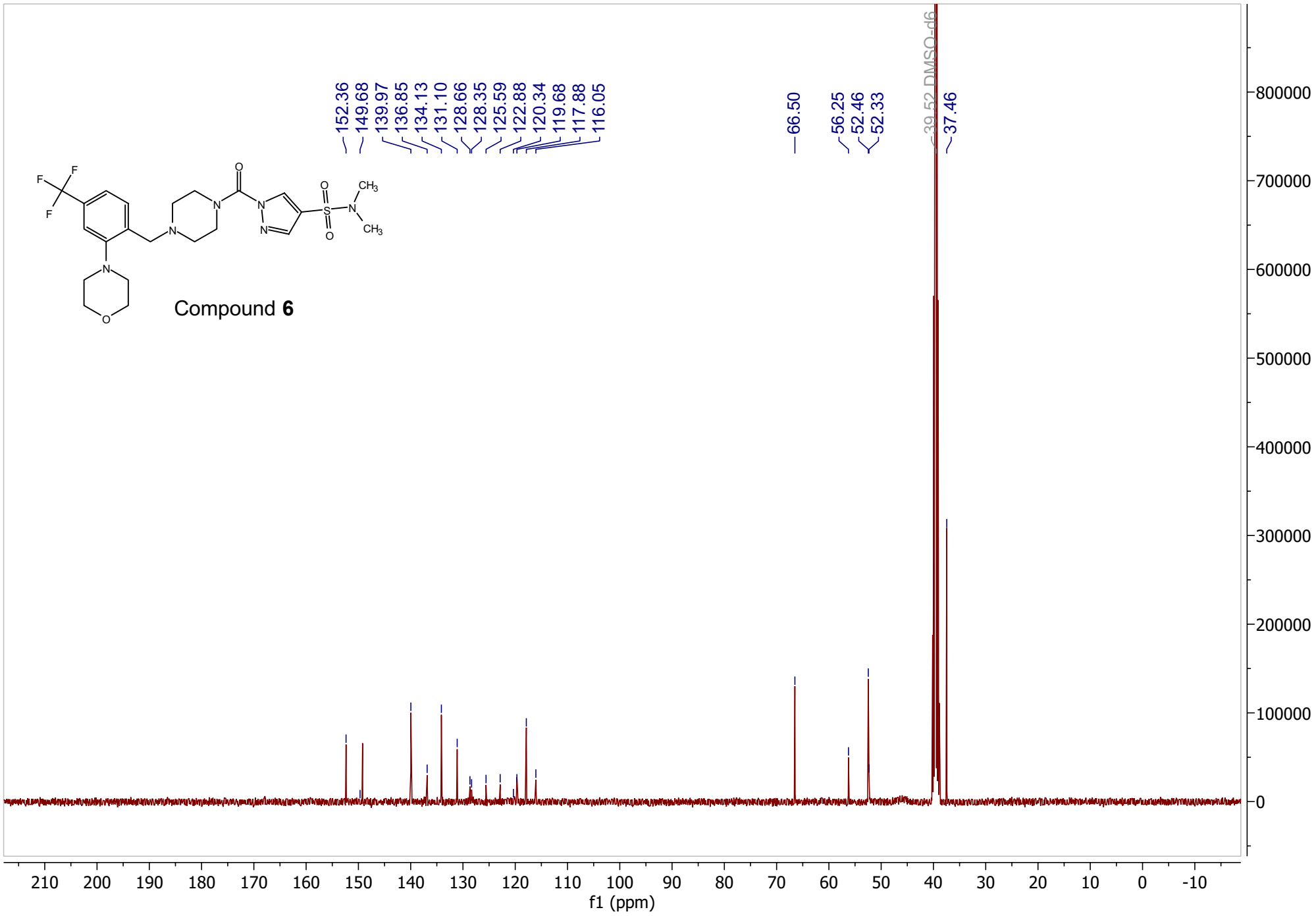
13C NMR (DMSO-d6)



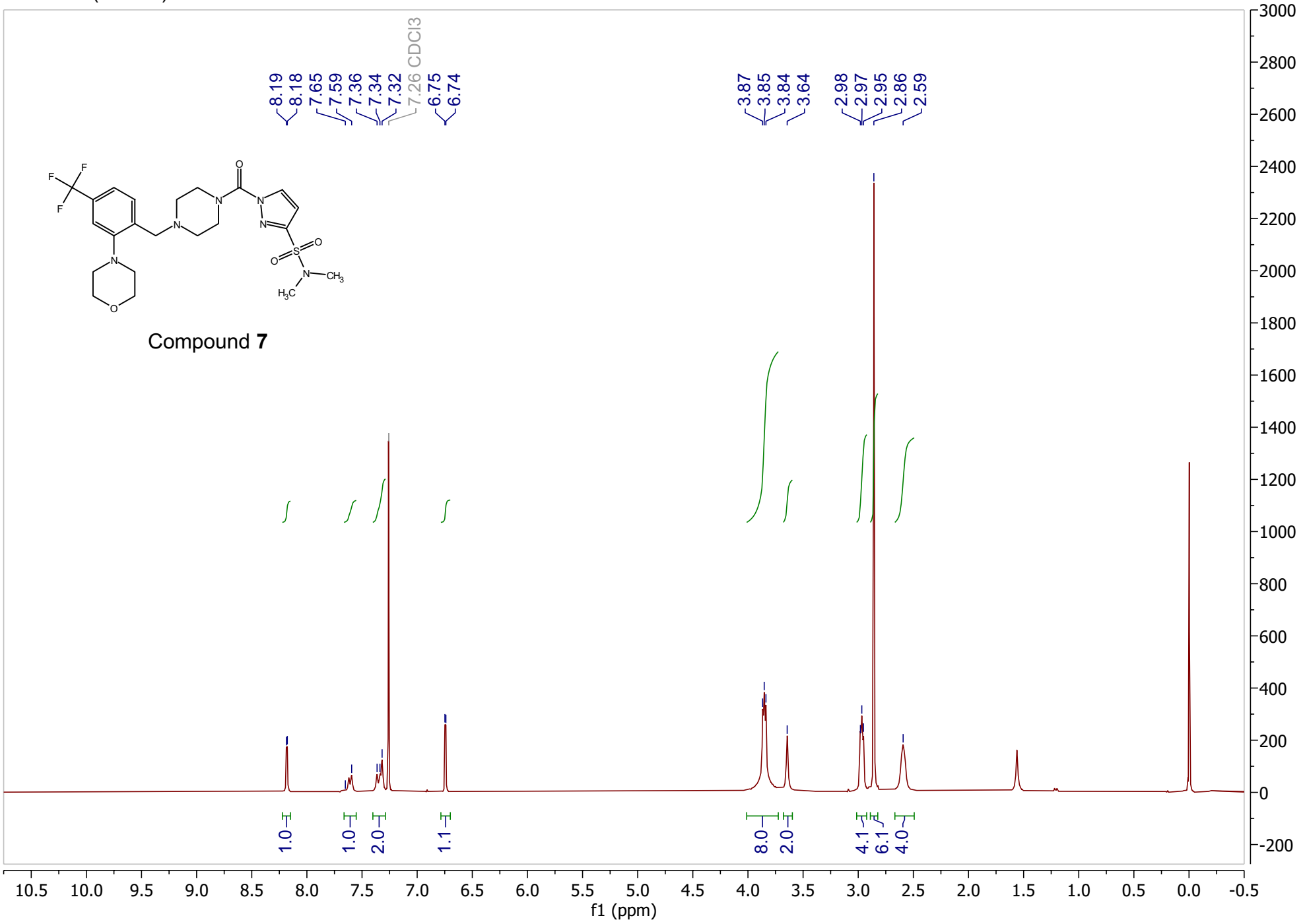
1H NMR (CDCl3)


8.46
7.82
7.63
7.37
7.35
7.32
7.26 CDCl3
3.87
3.85
3.85
3.84
3.68
2.97
2.95
2.74
2.63
Compound 6
1.0
1.0
1.0
2.0
7.9
2.0
4.0
6.0
3.7
f1 (ppm)

13C NMR (DMSO-d6)

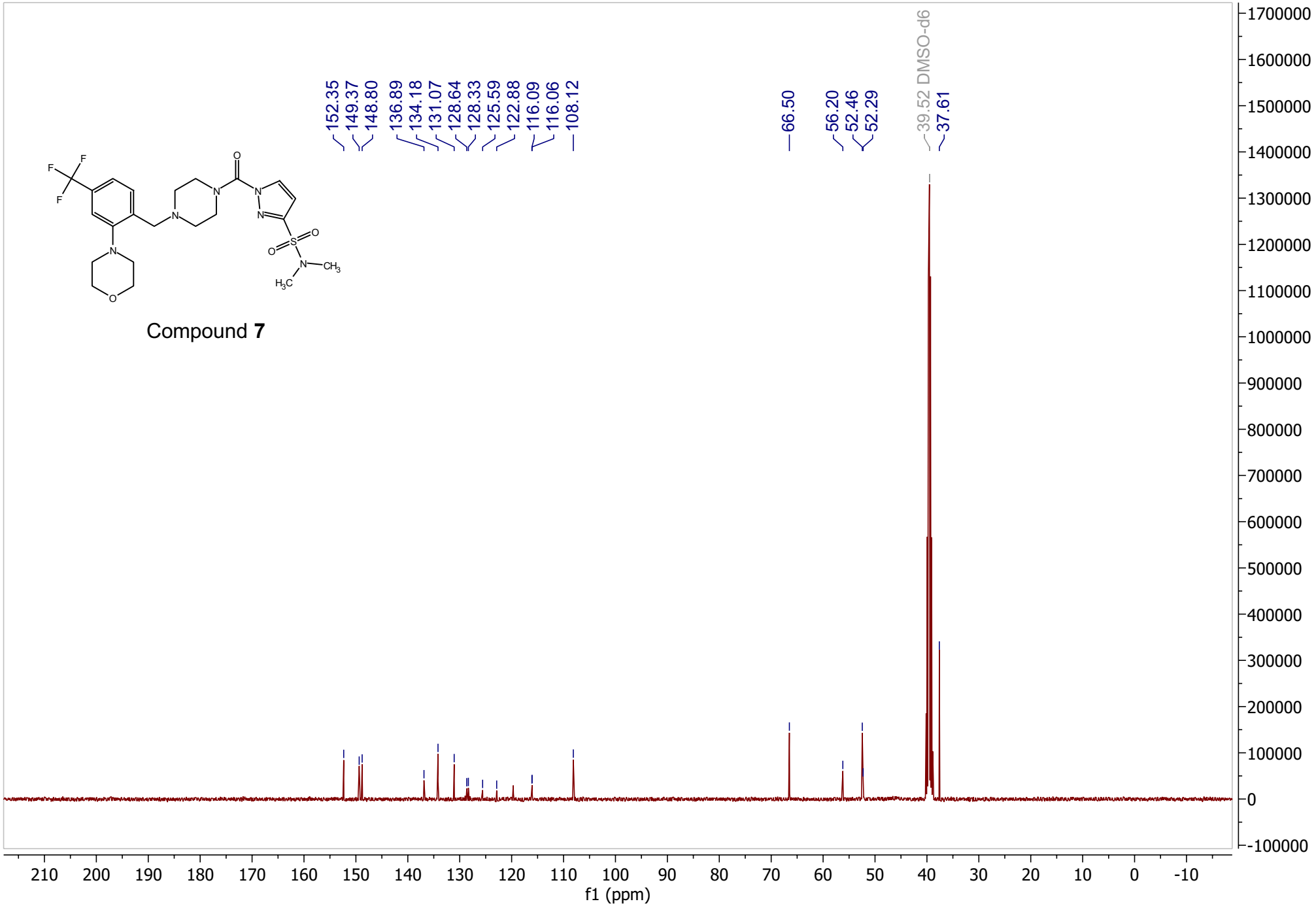


1H NMR (CDCl3)

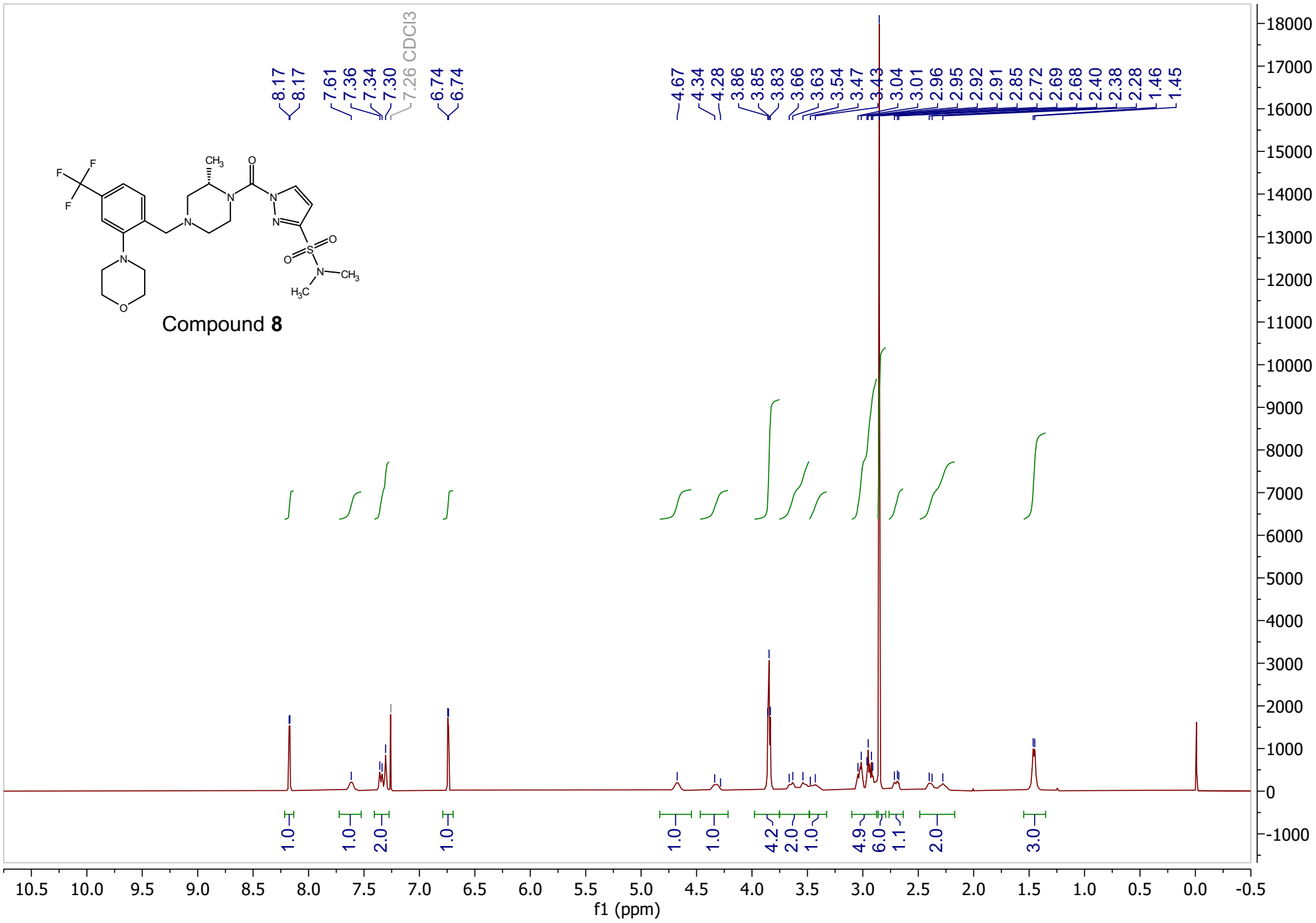


Compound **7**

13C NMR (DMSO-d6)

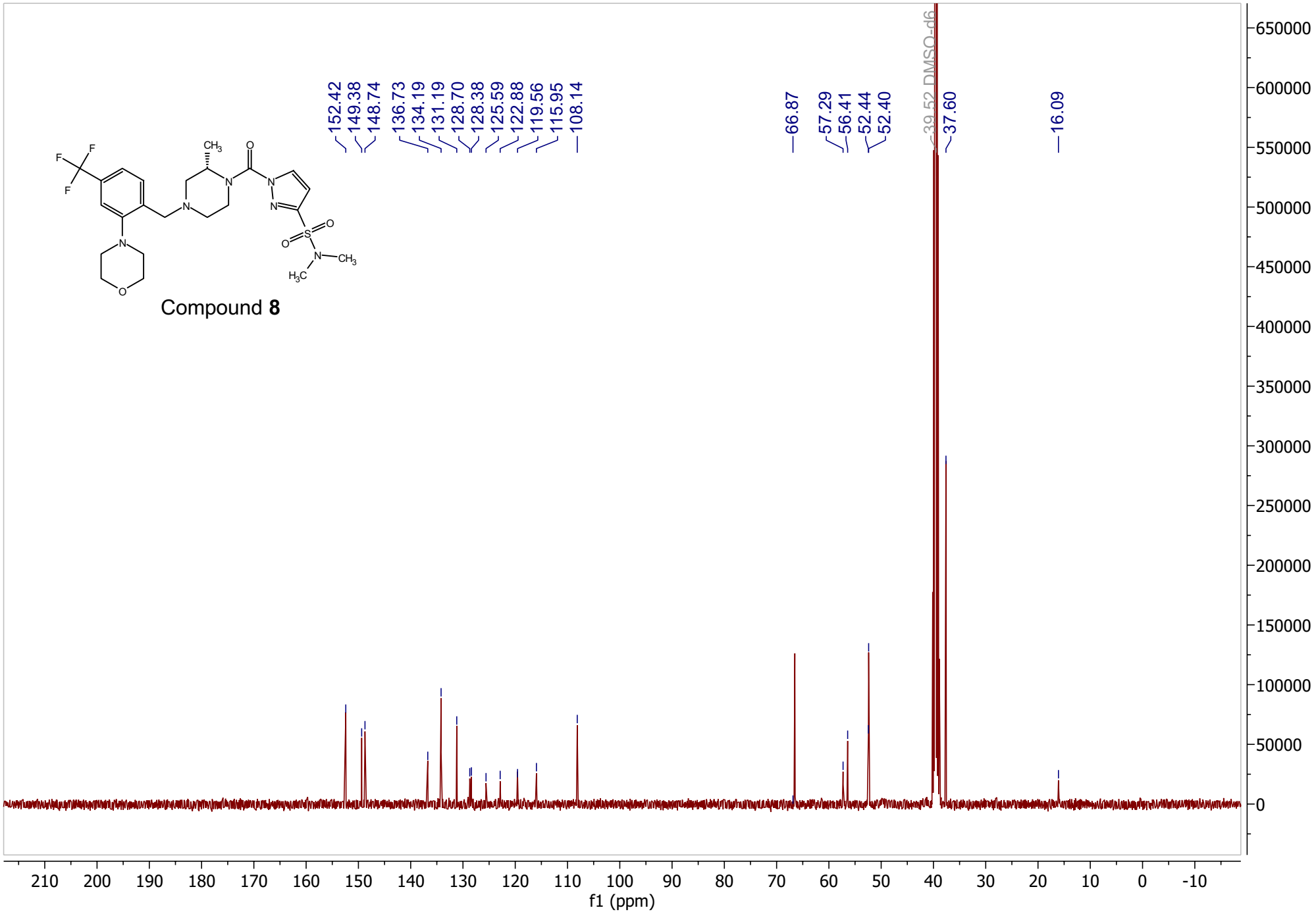


1H NMR (CDCl3)



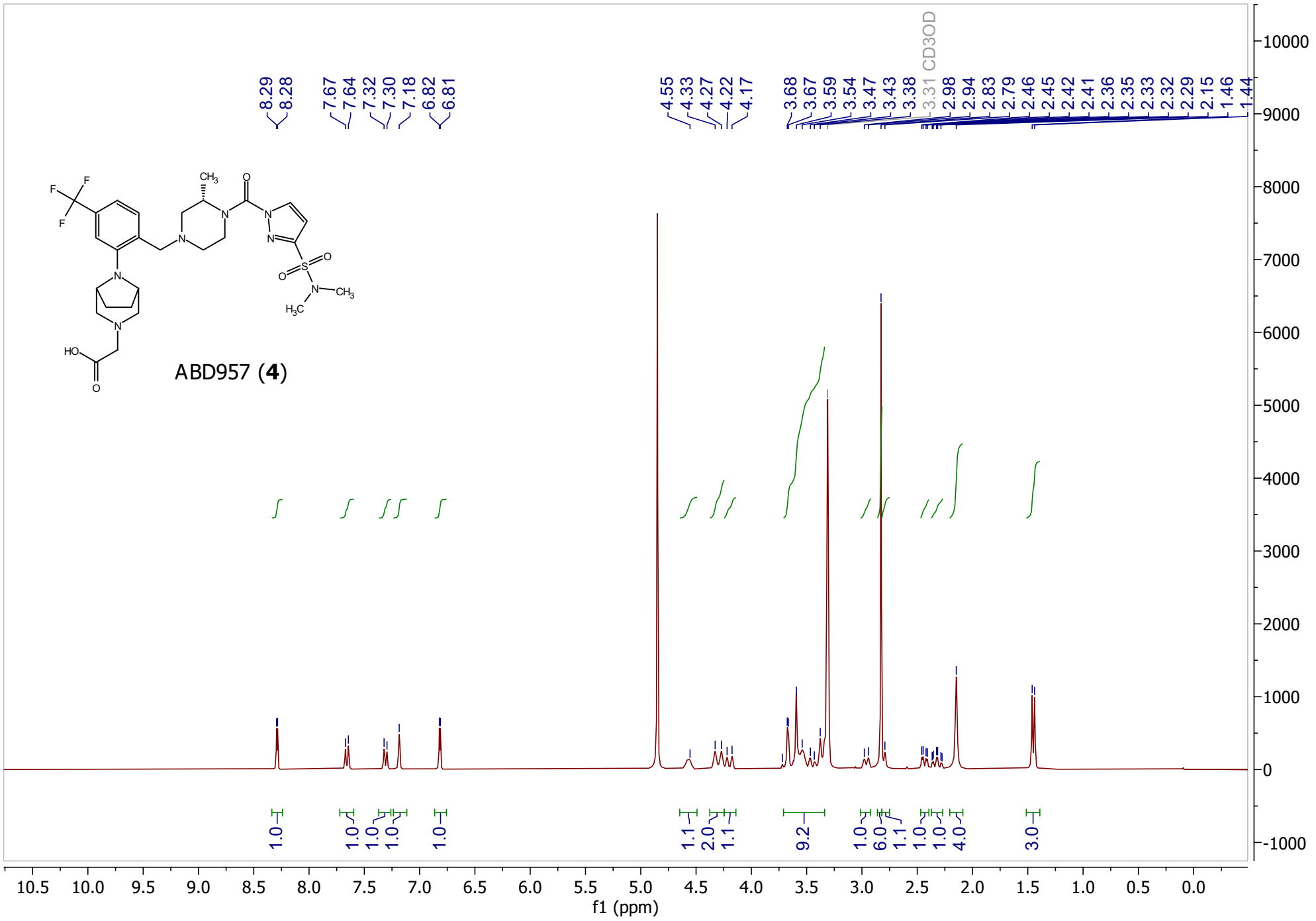
Compound **8**

13C NMR (DMSO-d6)

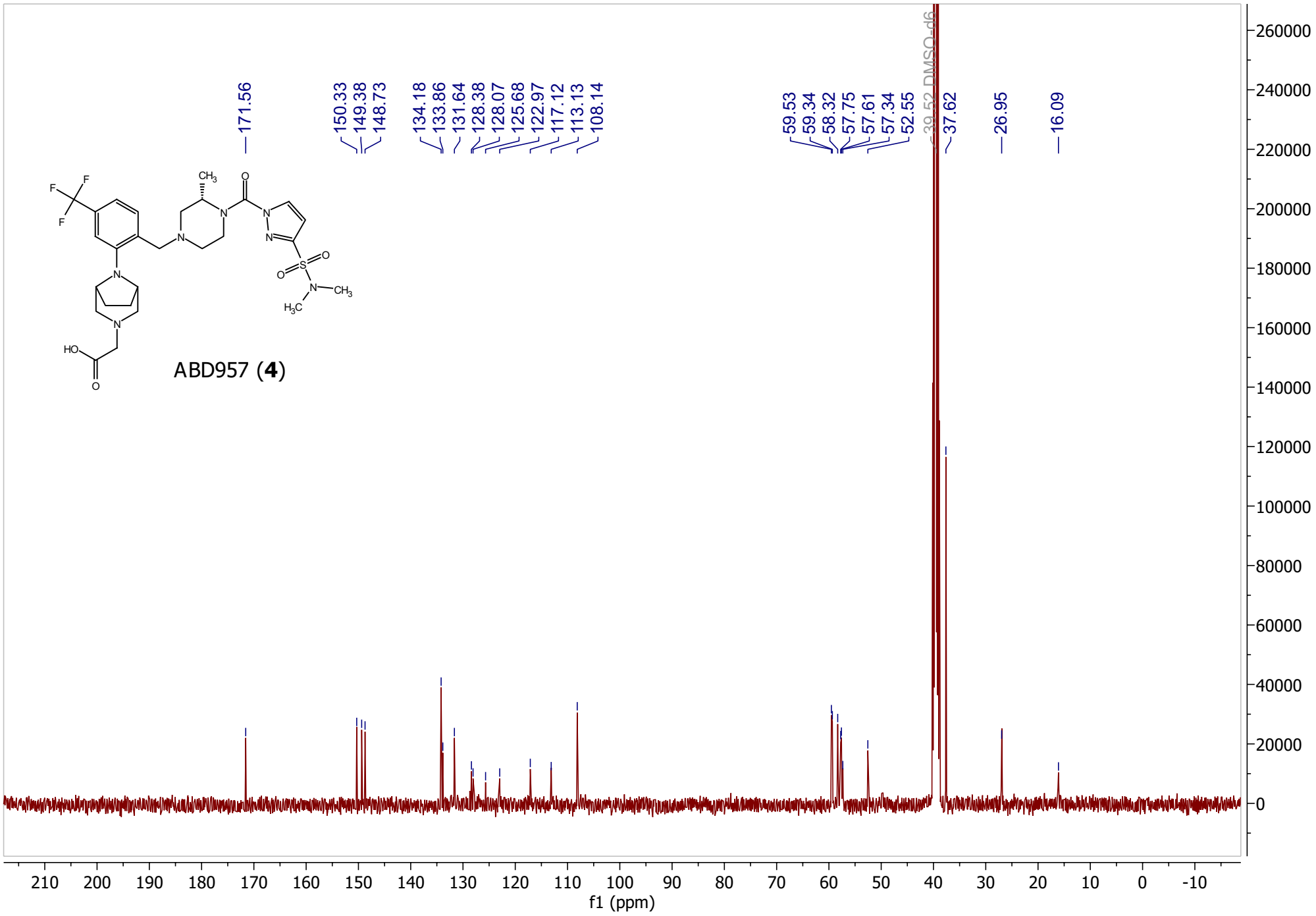


Compound **8**

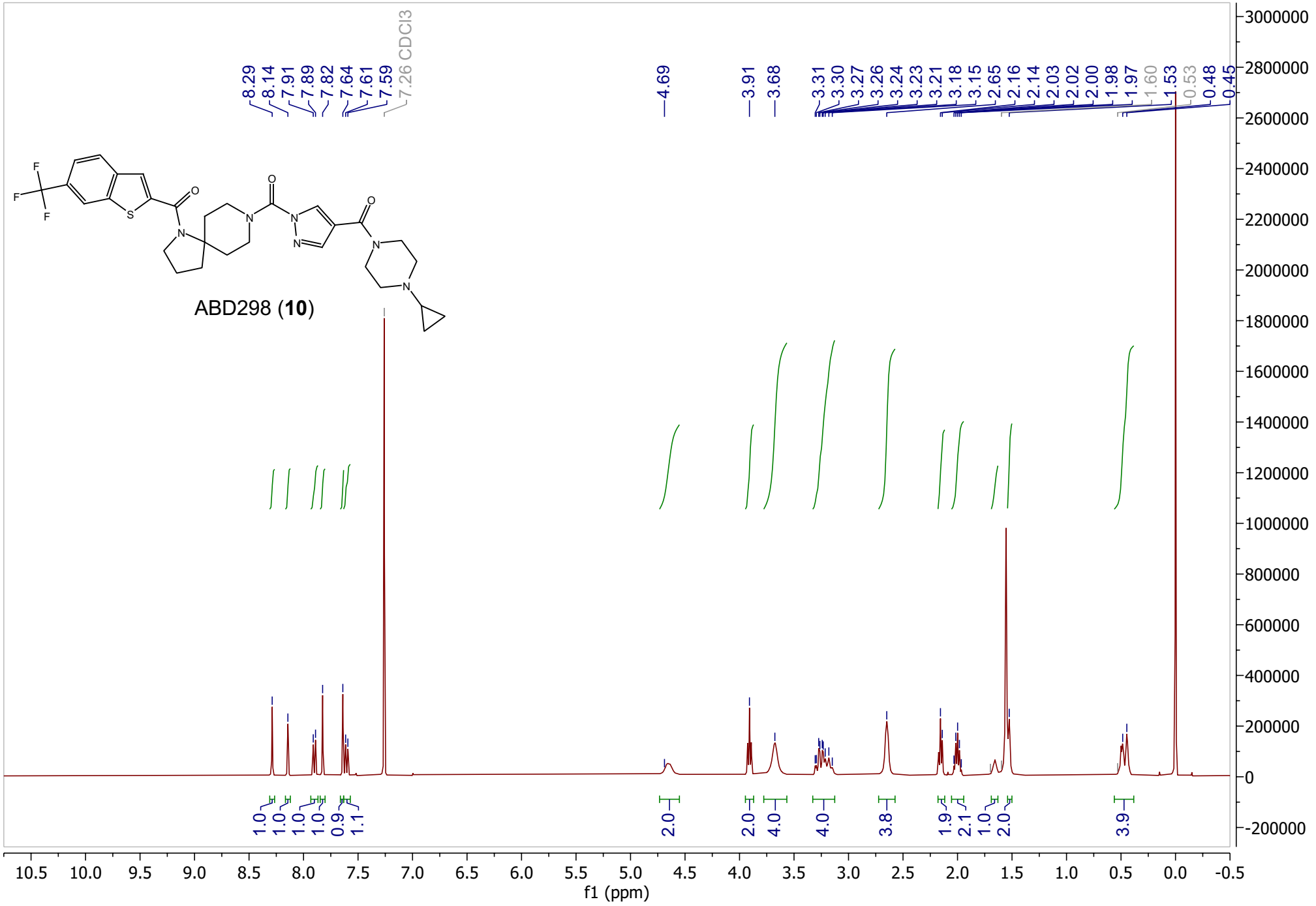
1H NMR (CD3OD)


8.29
8.28
7.67
7.64
7.32
7.30
7.18
6.82
6.81
4.55
4.33
4.27
4.22
4.17
3.68
3.67
3.59
3.54
3.47
3.43
3.38
3.31 CD3OD
2.98
2.94
2.83
2.79
2.46
2.45
2.42
2.41
2.36
2.35
2.33
2.32
2.29
2.15
1.46
1.44
ABD957 (4)
1.0
1.0
1.0
1.0
1.0
1.1
2.0
1.1
9.2
1.0
6.0
1.1
1.0
1.0
4.0
3.0
f1 (ppm)

13C NMR (DMSO-d6)



1H NMR (CDCl3)


8.29
8.14
7.91
7.89
7.82
7.64
7.61
7.59
7.26 CDCl3
4.69
3.91
3.68
3.31
3.30
3.27
3.26
3.24
3.23
3.21
3.18
3.15
2.65
2.16
2.14
2.03
2.02
2.00
1.98
1.97
1.60
1.53
0.53
0.48
0.45
ABD298 (10)
1.0
1.0
1.0
1.0
0.9
1.1
2.0
2.0
4.0
4.0
3.8
1.9
2.1
1.0
2.0
3.9
f1 (ppm)

13C NMR (CDCl3)