

SUPPORTING INFORMATION

Beyond Basicity: Discovery of Nonbasic DENV Protease Inhibitors with Potent Activity in Cell Culture

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Expression and Purification of the Viral Proteases

The DENV (serotype 2) and WNV NS2B–NS3 protease constructs were described before.¹⁻² The cofactor (NS2B) is covalently connected to the protease domain (NS3) by a glycine-serine linker (GGGGSGGGG) in both viral enzymes. The proteases were expressed and purified according to protocol established by Steuer (2001), with slight modifications.³ DENV-2 and WNV NS2B-NS3 genes were synthesized by a commercial supplier (Geneart), and inserted into an expression vector, pET28a(+) (Novagen, Germany), which was used to transform *E. coli* BL 21 λ (DE3) cells. The cells were grown overnight in 25 mL LB-Kan (50 µg/mL kanamycin) medium (140 rpm, 37 °C). From this preculture, large scale expression was carried out in two shaking flasks (2 L), each containing 500 mL LB-Kan (50 µg/mL kanamycin) until an OD₆₀₀ of 0.6 was reached. The expression was initiated by adding IPTG to a final concentration of 1 mM, followed by incubation for 4 – 5 h at 30 °C, with a shaking speed of 120 rpm. After incubation period, the cells were harvested by centrifugation (Sorvall RC 6 Plus; rotor: SLA 1500, 4500 rpm) for 10 min. The resulting pellet was frozen in liquid nitrogen and stored at -80 °C. The pellet was resuspended in lysis buffer (25 mM Tris pH 7.9, 0.5 M NaCl, 5 mM imidazole, 5% glycerol), the cells were disrupted by passing through a cell disruptor (Constant Cell Disruption Systems, Daventry, UK, pressure 1.7 kbar) and finally the solution was centrifuged (Sorvall RC 6 Plus; rotor: SS-34, 18000 rpm, 38720 g) at 4 °C for 40 min. DNase I (Sigma-Aldrich) was added to the supernatant and purification was carried out by Ni²⁺ affinity chromatography using NTA-agarose column (Chelating Sepharose TM, Fast Flow, GE Healthcare). The sample was loaded and washed with 500 mL lysis buffer, and then the target protein was eluted with the elution buffer (25 mM Tris pH 7.9, 0.5 M NaCl, 250 mM imidazole, 5% glycerol). Fractions were collected and checked by SDS-page. The identity of the eluted target protein was confirmed by MALDI-TOF MS and HR-ESI. For the latter, the protein was initially desalting on a SephadexG-25 column in NH₄HCO₃ buffer (50 mM). The fractions containing the target protein were desalting and concentrated with a centrifugal concentrator (Vivaspin®, Sartorius: 10 kDa MWCO; Hettieh Rotina 420R; rotor: 4723, 4500 rpm, 4000 g). An exchange to the storage buffer (100 mM Tris pH 7.9, 50 mM NaCl) was done by 5 washings at 4 °C to remove imidazole. Protein aliquots (15 µl) in a glycerol/storage buffer (1:1) mixture were frozen in liquid nitrogen for storage at -80 °C. Before carrying out an assay, the aliquot concentration was determined by measuring the absorbance of a solution in assay

buffer at 280 nm. The calculated extinction coefficients (ExPASyProtParam tool) were found to be 41940 L·mol⁻¹·cm⁻¹ for DENV protease and 55920 L·mol⁻¹·cm⁻¹ for WNV protease.

Inhibitory Activity of Compounds against Isolated Viral Proteases

DENV and WNV Protease Relative Inhibition Assay

The DENV and WNV protease relative inhibition assays were performed as described before.⁴⁻⁵ Continuous enzymatic assays were performed in black 96-well V-bottom plates (Greiner Bio-One, Germany) using a BMG Labtech Fluostar OPTIMA Microtiter fluorescence plate reader at an excitation wavelength of 320 nm and an emission wavelength of 405 nm or an excitation wavelength of 330 nm and an emission wavelength of 430 nm. Stock solutions of the inhibitors (10 mM in DMSO) were diluted to a final concentration of 50 µM in triplicates, and preincubated for 15 min with the DENV protease (100 nM) or WNV protease (150 nM) in the assay buffer (50 mM Tris-HCl pH 9, ethylene glycol (10% v/v), and 0.0016% Brij 58). The reaction was then initiated by the addition of the FRET substrate (final concentration 50 µM) to obtain a final assay volume of 100 µL per well. The enzymatic activity was monitored for 15 min and determined as a slope of relative fluorescence units per second (RFU/s) for each concentration. Compounds **MB-8**⁶, **MB-53**⁷ and aprotinin⁸ were used as control inhibitors. Percentage inhibition was calculated relative to a positive control (without the inhibitor), as a mean of the triplicates and respective standard deviation.

Table S1. Inhibitory Activity of Compounds against isolated DENV-2 and WNV proteases

No.	DENV ^a	WNV ^b	No.	DENV ^a	WNV ^b
	%	%		%	%
1	n.i.	20±4			
2	n.i.	n.i.	46	n.i.	n.i.
3	n.i.	15±2	47	13±4	n.i.
4	n.i.	n.i.	48	13±2	n.i.
5	n.i.	n.i.	49	n.i.	n.i.
6	n.i.	n.i.	50	19±8	13±3
7	n.i.	n.i.	51	n.i.	12±7
8	12±4	16±4	52	n.i.	n.i.
9	n.i.	20±5	53	n.i.	31±4
10	n.i.	n.i.	54	11±3	10±2
11	n.i.	n.i.	55	n.i.	n.i.
12	19±4	10±2	56	n.i.*	n.i.*
13	17±4	26±3	57	n.i.	n.i.
14	n.i.	22±2	58	29±1	66±4
15	n.i.	17±2	59	20±9	n.i.
16	n.i.	27±2	60	20±3	20±8
17	n.i.	n.i.	61	18±2	28±4
18	13±7	22±1	62	n.i.	n.i.
19	19±4	34±3	63	12±2	33±3
20	n.i.	22±3	64	12±8	n.i.
21	n.i.	15±4	65	n.i.	24±3
22	23±5	34±5	66	n.i.	13±2
23	28±4	55±3	67	19±3	47±2
24	46±1	20±6	68	n.i.	24±2
25	n.i.	n.i.	69	16±2	32±4
26	n.i.	21±1	70	11±6	n.i.
27	n.i.	n.i.	71	n.i.	23±5
28	16±8	n.i.	72	n.i.	21±6
29	n.i.	17±3	73	n.i.	19±2
30	n.i.	n.i.	74	20±2	31±7
31	n.i.	n.i.	75	22±3	42±2
32	15±4	24±6	76	14±6	66±1
33	38±4	24±4	77	n.i.	n.i.
34	20±7	25±2	78	n.i.	n.i.
35	n.i.	n.i.	79	n.i.	33±5
36	11±7	39±0	80	n.i.	n.i.
37	27±3	29±1	81	10±4	25±7
38	37±0	11±5	82	n.i.	19±4
39	51±3	25±2	83	12±4	15±9
40	n.i.	n.i.	84	20±5	45±0
41	n.i.	13±6	85	18±0*	28±0*
42	n.i.	16±7	86	n.i.	n.i.

43	25 ± 4	19 ± 5	87	19 ± 8	20 ± 5
44	31 ± 4	65 ± 1	88	n.i.	n.i.
45	n.i.	30 ± 1			
MB-8⁶	67 ± 6	18 ± 4	MB-53⁷	99 ± 1 $IC_{50} = 0.47 \pm 0.04$	89 ± 6 $IC_{50} = 4.0 \pm 0.4$
aprotinin^{8 c}	100 ± 1 $IC_{50} = 0.021 \pm 0.01$	103 ± 3 $IC_{50} = 0.028 \pm 0.03$			

^aInhibition of DENV serotype 2 NS2B-NS3 (inhibitor 50 μM, substrate 50 μM). ^bInhibition of WNV NS2B-NS3 (inhibitor 50 μM, substrate 50 μM). ^cMeasurements with aprotinin were performed at a concentration of 30 nM for DENV-2 and WNV proteases. *measured with HPLC. If inhibition ≤ 10% = no inhibition (n.i.). All measurements were carried out in triplicate.

DENV and WNV Protease Relative Inhibition Assay with detergent CHAPS

The DENV and WNV protease relative inhibition assays were performed as described before.⁴⁻⁵ Continuous enzymatic assays were performed in black 96-well V-bottom plates (Greiner Bio-One, Germany) using a BMG Labtech Fluostar OPTIMA Microtiter fluorescence plate reader at an excitation wavelength of 330 nm and an emission wavelength of 430 nm. Stock solutions of the inhibitors (10 mM in DMSO) were diluted to a final concentration of 50 μM in triplicates, and preincubated for 15 min with the DENV protease (100 nM) or WNV protease (150 nM) in the assay buffer (50 mM Tris-HCl pH 9, ethylene glycol (10% v/v), and 1 mM CHAPS). The reaction was then initiated by the addition of the FRET substrate (final concentration 50 μM) to obtain a final assay volume of 100 μL per well. The enzymatic activity was monitored for 15 min and determined as a slope of relative fluorescence units per second (RFU/s) for each concentration. Compounds **NK-189⁹** and **MB-53⁷** were used as control inhibitors. Percentage inhibition was calculated relative to a positive control (without the inhibitor), as a mean of the triplicates and respective standard deviation.

Table S2. Inhibitory Activity of Compounds against Isolated DENV-2 and WNV Proteases

Cpd.	DENV ^a		WNV ^b	
	%	IC ₅₀ [μM]	%	IC ₅₀ [μM]
1	19 ± 2	n.d.	16 ± 2	n.d.
2	20 ± 5	n.d.	17 ± 3	n.d.
3	n.i.	n.d.	n.i.	n.d.
4	n.i.	n.d.	13 ± 1	n.d.
5	28 ± 5	n.d.	14 ± 1	n.d.
6	34 ± 5	n.d.	12 ± 1	n.d.
7	13 ± 2	n.d.	13 ± 1	n.d.
8	n.i.	n.d.	n.i.	n.d.
9	59 ± 6	n.d.	24 ± 2	n.d.
10	12 ± 7	n.d.	21 ± 2	n.d.
11	14 ± 3	n.d.	13 ± 1	n.d.
12	100 ± 4	29 ± 2	44 ± 3	n.d.
13	20 ± 6	n.d.	27 ± 6	n.d.
14	50 ± 7	n.d.	44 ± 3	n.d.
15	70 ± 3	36 ± 2	53 ± 0	n.d.
16	75 ± 9	n.d.	20 ± 2	n.d.
17	n.i.	n.d.	19 ± 8	n.d.
18	57 ± 2	n.d.	19 ± 2	n.d.
19	85 ± 2	12 ± 1	71 ± 4	n.d.
20	45 ± 3	n.d.	28 ± 7	n.d.
21	43 ± 4	n.d.	19 ± 6	n.d.
22	43 ± 5	n.d.	51 ± 2	n.d.
23	98 ± 2	11 ± 1	71 ± 3	n.d.
24	84 ± 3	n.d.	29 ± 2	n.d.
25	11 ± 4	n.d.	n.i.	n.d.
26	53 ± 4	n.d.	23 ± 5	n.d.
27	n.i.	n.d.	n.i.	n.d.
28	n.i.	n.d.	n.i.	n.d.
29	15 ± 4	n.d.	22 ± 2	n.d.
30	n.i.	n.d.	n.i.	n.d.
31	66 ± 2	n.d.	20 ± 1	n.d.
32	64 ± 5	n.d.	33 ± 6	n.d.
33	77 ± 3	n.d.	66 ± 3	n.d.
34	101 ± 8	18 ± 2	58 ± 7	n.d.
35	89 ± 8	10 ± 1	55 ± 4	n.d.
36	88 ± 9	8.1 ± 0.4	59 ± 1	n.d.
37	90 ± 5	7.3 ± 0.3	91 ± 1	7.8 ± 0.9
38	95 ± 3	6.9 ± 0.7	68 ± 1	n.d.
39	93 ± 5	13 ± 0	53 ± 4	n.d.
40	n.i.	n.d.	21 ± 2	n.d.
41	30 ± 4	n.d.	23 ± 4	n.d.
42	43 ± 5	n.d.	13 ± 0	n.d.

Cpd.	DENV ^a		WNV ^b	
	%	IC ₅₀ [μM]	%	IC ₅₀ [μM]
43	90 ± 3	7.8 ± 0.5	32 ± 2	n.d.
44	70 ± 2	n.d.	86 ± 2	8.6 ± 0.9
45	81 ± 1	n.d.	76 ± 2	6.7 ± 0.8
46	92 ± 1	19 ± 1	22 ± 2	n.d.
47	15 ± 4	n.d.	19 ± 1	n.d.
48	80 ± 4	n.d.	32 ± 7	n.d.
49	n.i.	n.d.	10 ± 1	n.d.
50	16 ± 7	n.d.	18 ± 2	n.d.
51	52 ± 8	n.d.	37 ± 2	n.d.
52	66 ± 5	n.d.	46 ± 2	n.d.
53	62 ± 6	n.d.	41 ± 1	n.d.
54	70 ± 7	n.d.	30 ± 2	n.d.
55	n.d.	n.d.	n.d.	n.d.
56	n.d.	n.d.	n.d.	n.d.
57	98 ± 2	8.3 ± 0.8	66 ± 5	n.d.
58	98 ± 3	9.2 ± 0.7	88 ± 2	4.2 ± 0.3
59	92 ± 9	6.1 ± 0.3	68 ± 2	n.d.
60	90 ± 3	17 ± 2	41 ± 4	n.d.
61	80 ± 2	n.d.	33 ± 2	n.d.
62	84 ± 3	n.d.	52 ± 1	n.d.
63	89 ± 1	9.7 ± 1.1	47 ± 3	n.d.
64	87 ± 1	14 ± 1	21 ± 1	n.d.
65	77 ± 1	n.d.	47 ± 4	n.d.
66	79 ± 2	n.d.	49 ± 1	n.d.
67	95 ± 2	5.6 ± 0.1	63 ± 3	n.d.
68	90 ± 2	8.8 ± 0.4	60 ± 2	n.d.
69	93 ± 3	8.2 ± 0.9	82 ± 1	9.5 ± 0.6
70	92 ± 1	10 ± 0	83 ± 0	21 ± 2
71	92 ± 6	8.0 ± 0.6	57 ± 1	n.d.
72	55 ± 4	n.d.	30 ± 1	n.d.
73	53 ± 4	n.d.	30 ± 4	n.d.
74	90 ± 2	9.5 ± 1.0	67 ± 4	n.d.
75	91 ± 2	6.6 ± 0.7	86 ± 1	4.5 ± 0.1
76	87 ± 0	n.d.	90 ± 2	3.1 ± 0.5
77	97 ± 3	3.7 ± 0.2	69 ± 1	n.d.
78	102 ± 5	8.3 ± 0.4	46 ± 1	n.d.
79	75 ± 2	n.d.	50 ± 2	n.d.
80	56 ± 7	n.d.	14 ± 1	n.d.
81	35 ± 5	n.d.	17 ± 0	n.d.
82	18 ± 6	n.d.	n.i.	n.d.
83	76 ± 3	n.d.	64 ± 1	n.d.
84	83 ± 3	8.3 ± 0.9	80 ± 1	6.9 ± 0.5
85	n.d.	n.d.	n.d.	n.d.
86	98 ± 2	5.6 ± 0.5	71 ± 4	16 ± 0

Cpd.	DENV ^a		WNV ^b	
	%	IC ₅₀ [μM]	%	IC ₅₀ [μM]
87	17 ± 3	n.d.	40 ± 2	n.d.
88	67 ± 2	33 ± 2	55 ± 0	n.d.
NK-189	97 ± 2	11 ± 1	79 ± 1	n.d.
MB-53	100 ± 2	0.43 ± 0.04	89 ± 1	4.0 ± 0.8

^aInhibition of DENV serotype 2 NS2B-NS3 (inhibitor 50 μM, substrate 50 μM). ^bInhibition of WNV NS2B-NS3 (inhibitor 50 μM, substrate 50 μM). If inhibition ≤ 10% = no inhibition (n.i.). n.d. = not determined. All measurements were carried out in triplicate.

Characterization of Compound 56 and 85

Compounds **56** and **85** were found to exhibit fluorescence. For compound **56** excitation and emission wavelength were 340 nm and 425 nm, respectively. For compound **85** excitation and emission wavelength were 310 nm and 405 nm, respectively.

HPLC-based DENV and WNV Protease Assays with Compound 56 and 85

After performing the fluorimetric assay, the enzymatic reaction was stopped by adding 10 μL of TFA (4%) to each well, and the plate was centrifuged. The HPLC analysis was carried out on a Jasco HPLC system equipped with a RP-18 column Phenomenex Luna C₁₈(2) (5 μm, 150 × 3 mm) and a FP-2020 plus fluorescence detector (excitation: 320 nm; emission: 405 nm). The conditions were used for DENV assay as previously described.⁴⁻⁵ Accordingly, flow rate: 1.0 mL/min; eluent A: water (0.1% TFA); eluent B: acetonitrile (0.1% TFA); and gradient: 10% B (0 min), 10% B (1 min), 95% B (9.5 min), 95% B (9.6 min), 10% B (12.6 min), and 10% B (15 min). Different conditions were used for the WNV assay: flow rate: 1.2 mL/min; eluent A: water (0.1% TFA); eluent B: acetonitrile (0.1% TFA); and gradient: 10% B (0 min), 20% B (1 min), 95% B (5 min), 95% B (6 min), 10% B (6.1 min), and 10% B (8 min).⁷ Percentage inhibition was calculated relative to a positive control (without the inhibitor). All experiments were performed in triplicate and the final value was obtained as the average.

Enzyme Kinetic Studies

To determine the dissociation constant of the enzyme-inhibitor complex (K_i), IC₅₀ values of the inhibitor at different substrate concentrations (50, 100, 200, and 300 μM) were measured using the previous parameters. Cheng-Prusoff's¹⁰⁻¹¹ method was used for K_i calculations. Hence, the K_i value was obtained as the intercept at the y-axis from a linear plot of IC₅₀ values as a function of the substrate concentration. Linear regressions were performed using Prism 6.01 (Graphpad Software, Inc.).

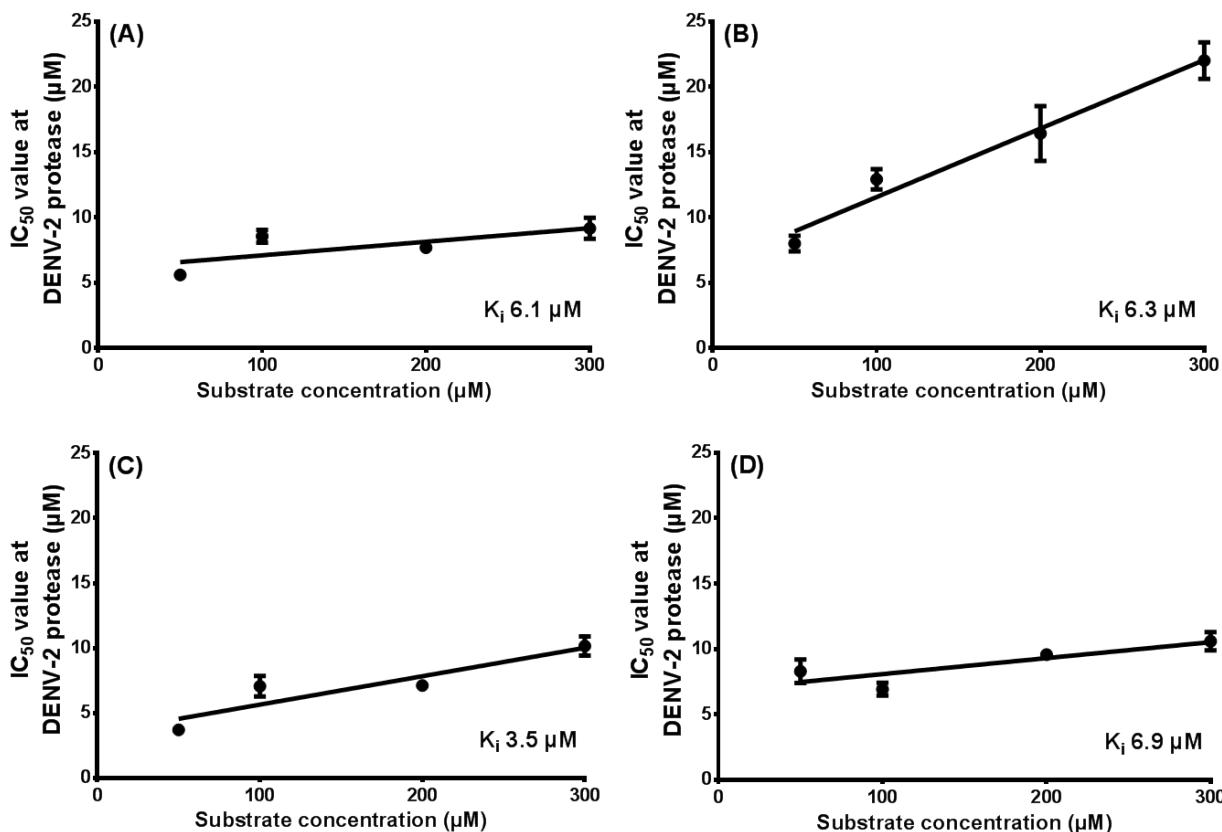


Figure S1: Kinetic studies of inhibitors at isolated DENV-2 protease. (A) Cheng-Prusoff plot of compound **67**. (B) Cheng-Prusoff plot of compound **71**. (C) Cheng-Prusoff plot of compound **77**. (D) Cheng-Prusoff plot of compound **84**. All plots indicate a competitive inhibition mechanism.

DENV-2 Michaelis-Menten kinetics with compound **67** and **84**

Michaelis-Menten kinetics were performed in black 96-well V-bottom plates (Greiner Bio-One, Germany) using a BMG Labtech Fluostar OPTIMA Microtiter fluorescence plate reader at an excitation wavelength of 330 nm and an emission wavelength of 430 nm. Stock solutions of the inhibitors (10 mM in DMSO) were diluted to a final concentration of 2 μ M, 10 μ M or 15 μ M in triplicates in the assay buffer (50 mM Tris-HCl pH 9, ethylene glycol (10% v/v), and 1 mM CHAPS). K_m and V_{max} determinations were performed as previously described for the DENV protease.⁴ Fluorescence inner filter effects were corrected as described in literature.¹² Correction factors were taken from literature.⁴ A linear calibration curve for 2-aminobenzoic acid was created to convert the measured relative fluorescence units into substrate cleavage per minute [μ M/s] (figure S2). Linear regressions were performed using Prism 6.01 (Graphpad Software, Inc.).

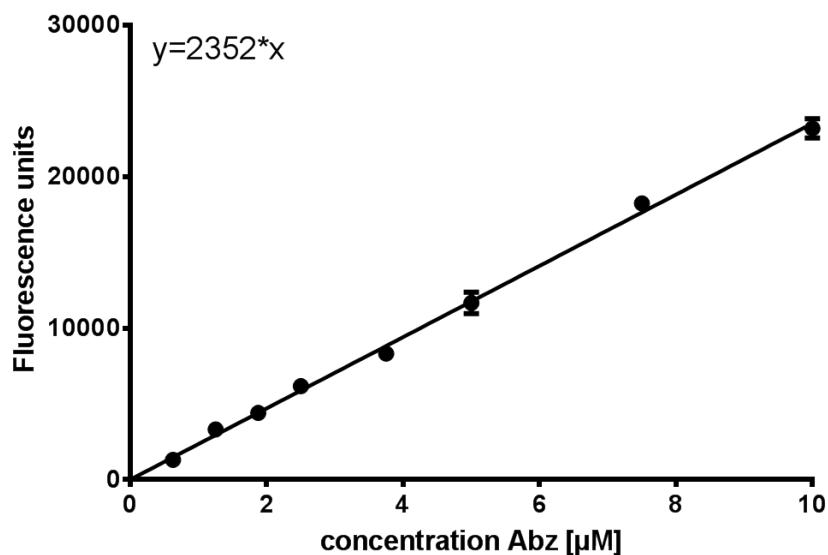


Figure S2: Linear calibration curve for 2-aminobenzoic acid (Abz). Correlation coefficient (R^2) = 0.998.

K_m , V_{max} and K_i calculations for compound **67** and **84** were performed using Prism 6.01 (Graphpad Software, Inc.) by using nonlinear regression and the competitive enzyme inhibition equation.

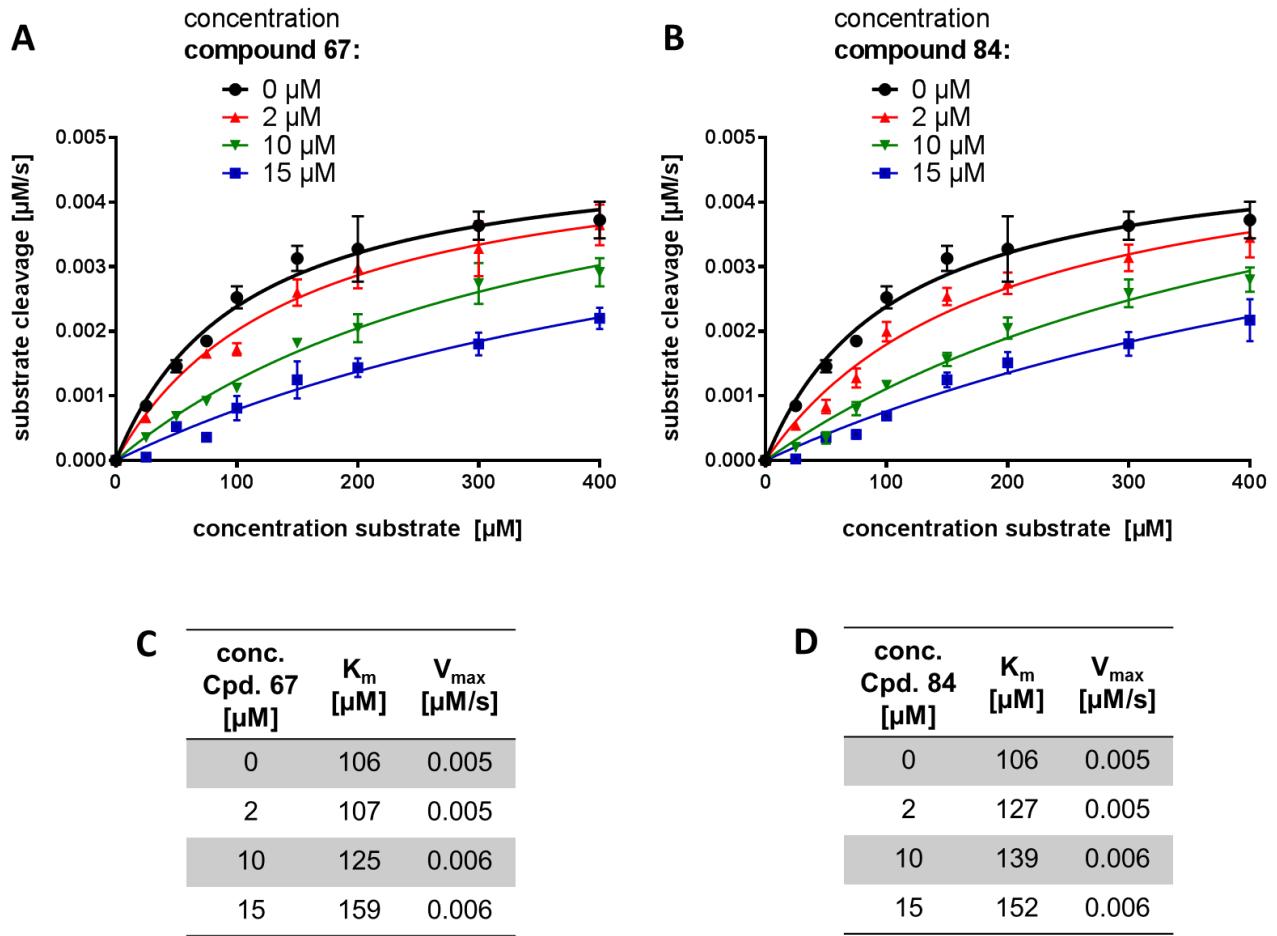


Figure S3: Michaelis-Menten kinetics of DENV-2 NS2-NB3 in the presence of compound **67** and **84**. K_i values calculated for compounds **67** and **84** were 5.2 μM and 4.1 μM , respectively. (A) Michaelis-Menten plot with different concentrations of compound **67**. (B) Michaelis-Menten plot with different concentrations of compound **84**. (C) K_m and V_{max} values at different concentrations of compound **67**. (D) K_m and V_{max} values at different concentrations of compound **84**.

Tryptophan Fluorescence Quenching Assay

The tryptophan fluorescence quenching assay was performed as described by Bodenreider *et al.*¹³ DENV protease (0.2 μ M) was titrated with different concentrations (0, 1.25, 2.5, 5, 10, 20, 30, 40, 50 μ M) of inhibitor in buffer (50 mM Tris-HCl pH 9.0) and the samples were incubated for 1 h at room temperature. For the fluorescence displacement experiment, the protease and NATA controls were incubated with the inhibitor (50 μ M) and aprotinin (10 μ M) under the same conditions. Fluorescence of the enzymes (0.20 μ M DENV) in the presence of aprotinin (10 μ M), without the inhibitor, was also determined and used to correct the results of aprotinin displacement; in order to exclude aprotinin intrinsic fluorescence or inner filter effects. Fluorescence emission at 340 nm was monitored on a Tecan Safire II instrument (excitation at 280 nm). All experiments were performed in triplicates and the values were obtained in relative fluorescence units (RFU). Tryptophan quenching was plotted as a curve of the mean and standard deviation of the values against the respective concentrations of the inhibitor in comparison to the fluorescence displacement experiment using Prism 6.01 (Graphpad Software, Inc.).

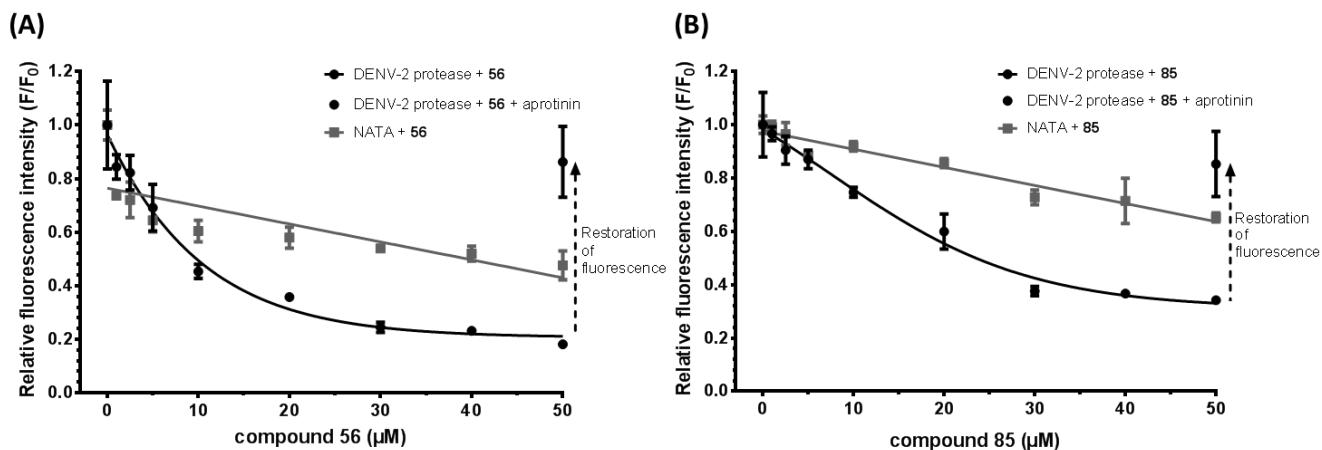


Figure S4. The addition of Compound **56** (A) and **85** (B) to DENV-2 protease led to a concentration-dependent quenching of the fluorescence of the tryptophan residues in the substrate binding region. The fluorescence is partially restored when aprotinin is added. NATA = *N*-Acetyl-L-tryptophanamide.

Inhibitory Activity of Compounds against Thrombin and Trypsin

Thrombin Assay

Thrombin was purchased from Sigma-Aldrich (Germany). The thrombin assay was performed as reported.⁵ Continuous fluorimetric assay was done in black 96 well V-bottom plates (Greiner Bio-One, Germany), using a BMG Labtech Fluostar OPTIMA microtiter fluorescence plate reader. Excitation wavelength of 355 nm and an emission wavelength of 460 nm were used. The inhibitors (final concentration 25 µM, from 10 mM stock solutions in DMSO) were preincubated with thrombin (10 nM) in the assay buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.05% Tween 20) for 15 min. Enzymatic cleavage was initiated by the addition of the Boc-Val-Pro-Arg-AMC substrate (Bachem, Germany) at a final concentration of 50 µM. The activity of thrombin was monitored for 15 min and determined as a slope of relative fluorescence units per second (RFU/s). Camostat mesylate was used as inhibition control. All experiments were performed in triplicates and percentage inhibition was calculated as the mean and respective standard deviation of the values. Values were obtained in relation to a positive control.

Trypsin Assay

Trypsin was purchased from Sigma-Aldrich (Germany). The inhibition of trypsin was determined as described before.¹⁴ Continuous fluorimetric assay was done in black 96 well V-bottom plates (Greiner Bio-One, Germany), using a BMG Labtech Fluostar OPTIMA microtiter fluorescence plate reader. Excitation wavelength of 355 nm and an emission wavelength of 460 nm were used. The inhibitors (final concentration 50 µM, from 10 mM stock solutions in DMSO) were preincubated with trypsin (1 nM) in the assay buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.05% Tween 20) for 15 min. Enzymatic cleavage was initiated by the addition of the Boc-Val-Pro-Arg-AMC substrate (Bachem, Germany) at a final concentration of 50 µM. The activity of thrombin was monitored for 15 min and determined as a slope of relative fluorescence units per second (RFU/s). Camostat mesylate was used as positive control. All experiments were performed in triplicates and percentage inhibition was calculated as the mean and respective standard deviation of the values. Values were obtained in relation to a positive control (without inhibitor).

Table S3. Inhibitory Activity of Compounds against Thrombin and Trypsin

No.	thrombin ^a	trypsin ^b	No.	thrombin ^a	trypsin ^b
	%	%		%	%
1	10 ± 5	n.i.			
2	n.i.	n.i.	46	n.i.	n.i.
3	n.i.	n.i.	47	n.i.	n.i.
4	n.i.	n.i.	48	n.i.	n.i.
5	n.i.	n.i.	49	n.i.	39 ± 3
6	n.i.	n.i.	50	n.i.	n.i.
7	n.i.	n.i.	51	n.i.	n.i.
8	n.i.	12 ± 3	52	n.i.	n.i.
9	12 ± 2	10 ± 2	53	n.i.	n.i.
10	n.i.	11 ± 2	54	n.i.	n.i.
11	n.i.	11 ± 3	55	n.i.	n.i.
12	11 ± 4	n.i.	56	n.i.	n.i.
13	n.i.	n.i.	57	n.i.	n.i.
14	n.i.	n.i.	58	n.i.	n.i.
15	n.i.	35 ± 4	59	n.i.	n.i.
16	n.i.	n.i.	60	n.i.	11 ± 5
17	n.i.	n.i.	61	n.i.	n.i.
18	n.i.	n.i.	62	n.i.	27 ± 8
19	n.i.	22 ± 2	63	n.i.	12 ± 4
20	n.i.	n.i.	64	n.i.	n.i.
21	12 ± 6	n.i.	65	n.i.	n.i.
22	n.i.	11 ± 3	66	n.i.	n.i.
23	16 ± 3	n.i.	67	n.i.	n.i.
24	16 ± 6	n.i.	68	n.i.	n.i.
25	n.i.	n.i.	69	n.i.	26 ± 10
26	n.i.	n.i.	70	n.i.	n.i.
27	n.i.	n.i.	71	n.i.	16 ± 1
28	13 ± 0	11 ± 2	72	n.i.	n.i.
29	n.i.	n.i.	73	n.i.	n.i.
30	n.i.	n.i.	74	n.i.	n.i.
31	17 ± 6	n.i.	75	n.i.	n.i.
32	n.i.	n.i.	76	n.i.	n.i.
33	12 ± 8	n.i.	77	n.i.	n.i.
34	10 ± 0	12 ± 7	78	n.i.	n.i.
35	n.i.	10 ± 1	79	n.i.	n.i.
36	n.i.	n.i.	80	n.i.	n.i.
37	n.i.	n.i.	81	n.i.	n.i.
38	n.i.	n.i.	82	n.i.	n.i.
39	n.i.	n.i.	83	n.i.	13 ± 3
40	11 ± 5	37 ± 6	84	n.i.	n.i.
41	n.i.	n.i.	85	n.i.	18 ± 9
42	n.i.	n.i.	86	n.i.	17 ± 1

43	11 ± 4	15 ± 2	87	n.i.	n.i.
44	n.i.	n.i.	88	n.i.	16 ± 9
45	n.i.	31 ± 4	camostat	99 ± 4	101 ± 2

^aInhibition of thrombin (inhibitor 25 µM, substrate 50 µM). ^bInhibition of trypsin (inhibitor 50 µM, substrate 50 µM). If inhibition ≤ 10% = no inhibition (n.i.). All measurements were carried out in triplicate.

Residual Plots for DENV-2 Titer Reduction Curves

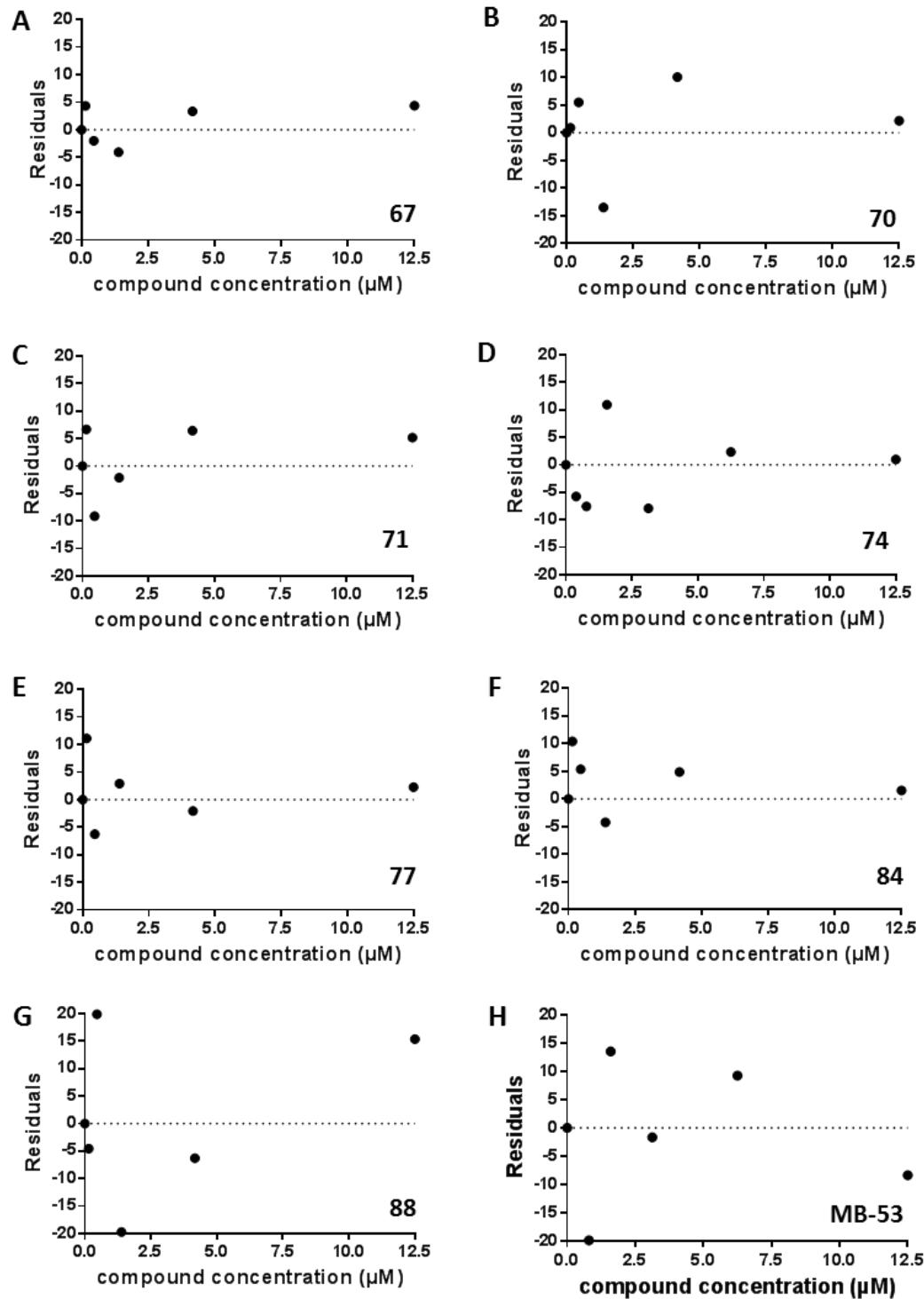


Figure S5: Residual plots for DENV-2 titer reduction curves.

Parallel Artificial Membrane Permeability Assay (PAMPA)

The permeability was evaluated as reported, using a pre-coated PAMPA plate system (BD Gentest, BD Bioscience, Germany).¹⁴ Phosphate buffered saline (PBS) pH 7.4 (Sigma-Aldrich, Germany) was used for all experiments. Concentration determinations were carried out on a Jasco HPLC system with a UV-detector and an RP-18 column (ReproSil-Pur-ODS-3, Dr. Maisch GmbH, Germany, 5 µm, 50 mm × 2 mm). The conditions for the method used were eluent A, water (0.1% TFA); eluent B, acetonitrile (0.1% TFA); and gradient, 1% B (0.2 min), 100% B (3.5 min), 100% B (4.5 min), 1% B (4.6 min), and 1% B (5 min). UV-detection was performed at 254 nm. Six-point calibration curves (10, 25, 50, 100, 150, and 200 µM) were generated for all analyzed compounds and references (caffeine, carbamazepine and phenytoin) with correlation coefficients (R^2) being at least 0.9. The PAMPA plate was warmed to room temperature for 1 h. In the donor plate, 300 µL of the 200 µM compound solutions in PBS were dispensed in triplicate, and in the acceptor plate, 200 µL of PBS buffer were added to all wells. The plates were combined, and the system was incubated at 25 °C for 5 h. After incubation, 100 µL samples from each of the donor and acceptor wells were transferred to 96 well U-bottom polypropylene plates (Greiner Bio-One, Germany) and quantitatively analyzed by UV absorbance on HPLC. The concentrations were determined from calibration curves generated beforehand. Permeability (Pe) and mass retention (R) were calculated as described in the literature.¹⁵⁻¹⁶

Metabolic Stability against Liver Microsomes

Metabolic stability measurement was performed as described before.^{7, 14, 17} Pooled liver microsomes from male Sprague-Dawley were purchased from Sigma-Aldrich (Germany). To determine the metabolic stability, liver microsomal proteins (0.2 mg/mL) were supplemented with NADPH (5 mM) in Dulbecco's Phosphate Buffered Saline (DPBS (Sigma-Aldrich, Germany)) and preincubated at 37 °C for 15 min. Additionally, test compounds (100 µM) were added and incubated at 37 °C for 30 min. Aliquots were removed at various time points (0, 5, 10, 20, 30, 60 min). The reaction was terminated by the addition of acetonitrile, and the samples were cooled with ice for 15 min before centrifugation (3000g at 4 °C for 20 min). The supernatants were used for further analysis. The loss of parent compound was monitored by HPLC on a Jasco HPLC system with UV detector and RP-18 column (ReproSil-Pur-ODS, Dr. Maisch GmbH, Germany, 3 µm, 50 mm × 2 mm) using the method: eluent A, water (+ 0.1% TFA); eluent B. acetonitrile (+ 0.1% TFA); flow rate, 1 mL/min; gradient, 1% B (0.2 min), 100% B (3.5 min), 100% B (4.5 min), 1% B (4.6 min), 1% B (5 min). The metabolic stability was determined by dividing the peak areas of the unaltered parent compound in the metabolized sample by the peak areas of the parent compound in the reference sample. The activity of the microsomal preparations was verified by using a positive control (testosterone).

Stability against Pancreatic Enzymes

Stability against α -chymotrypsin and trypsin was performed as described before.¹⁷⁻¹⁸ α -Chymotrypsin (from bovine pancreas, ≥ 40 units/mg protein) and trypsin (from bovine pancreas, Type V-S, $\geq 8,500$ BAEE units/mg protein) were purchased from Sigma-Aldrich (Germany). In brief, a solution of the respective enzyme (10 μ M) was preincubated at 37 °C in a phosphate buffer (pH 7.4) for 15 min. Compounds and **MB-53** as positive control were added (final concentration 0.1 mg/mL) and incubated at 37 °C for up to 120 min. At six time points, aliquots were taken (0, 5, 15, 30, 60, 120 min) and quenched with twice the amount of acetonitrile. The samples were cooled on ice before centrifugation (3000g at 4 °C for 20 min). The supernatants were used for further analysis. The loss of parent compound was monitored by HPLC on a Jasco HPLC system with UV detector and RP-18 column (ReproSil-Pur-ODS, Dr. Maisch GmbH, Germany, 3 μ m, 50 mm \times 2 mm) using the method: eluent A, water (+ 0.1% TFA); eluent B. acetonitrile (+ 0.1% TFA); flow rate, 1 mL/min; gradient, 1% B (0.2 min), 100% B (3.5 min), 100% B (4.5 min), 1% B (4.6 min), 1% B (5 min). The stability was determined by dividing the peak areas of the unaltered parent compound in the metabolized sample by the peak areas of the parent compound in the reference sample.

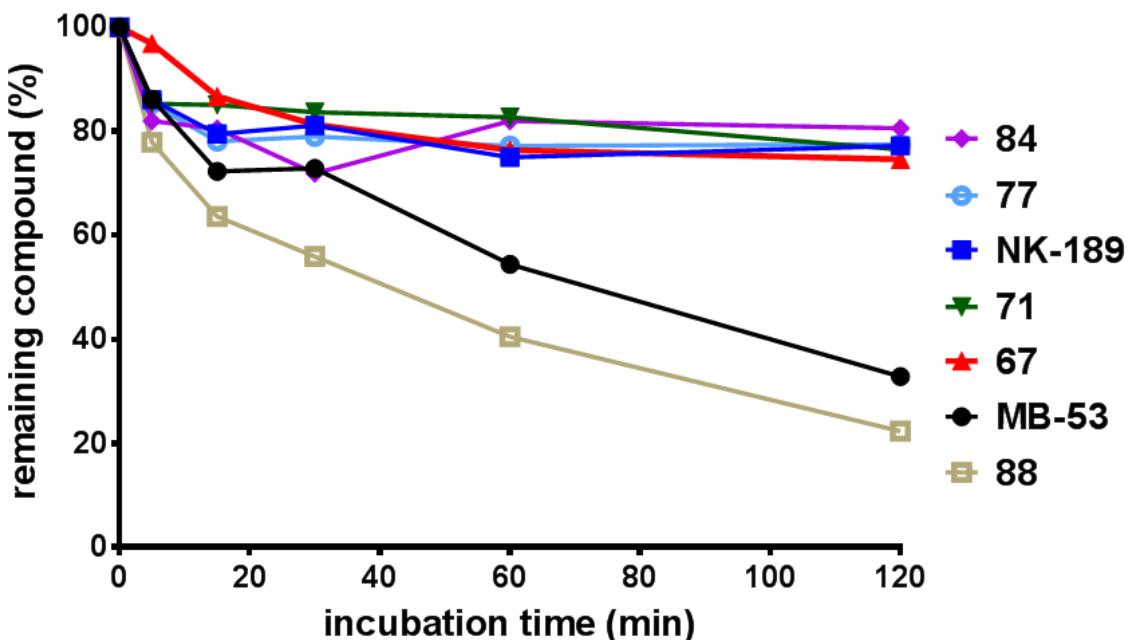


Figure S6: Degradation of compounds and reference substance **MB-53** in the presence of bovine trypsin.

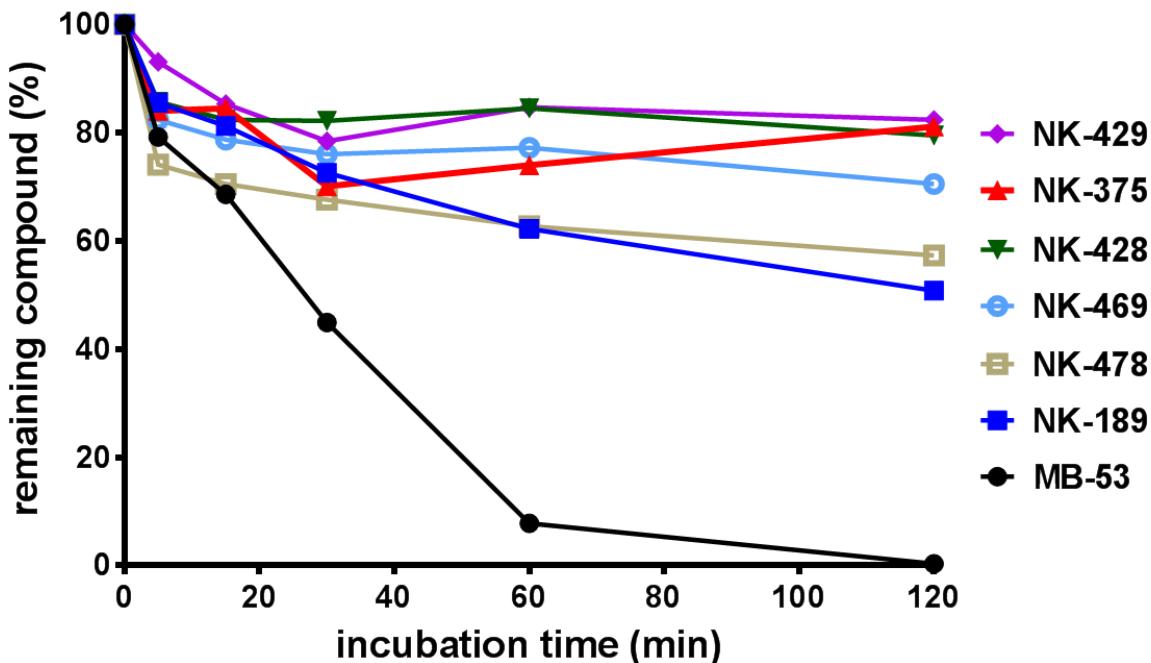


Figure S7: Degradation of compounds and reference substance **MB-53** in the presence of bovine α -chymotrypsin.

FRET Substrates Synthesis

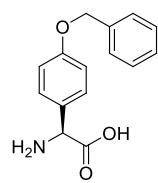
DENV and WNV protease FRET substrates peptides, with the respective sequences 2-Abz-Nle-Lys-Arg-Arg-Ser-(3-NO₂)-Tyr-NH₂ ($K_m = 106 \mu\text{M}$) and 2-Abz-Gly-Lys-Lys-Arg-Gly-(3-NO₃)-Tyr-Ala-Lys-NH₂ ($K_m = 36 \mu\text{M}$), were synthesized according to standard Fmoc SPPS procedure, as described for the dengue FRET substrate.⁴ Purity was determined by RP-HPLC using the same method as for the inhibitors.

Synthesis and Analytical Data of Precursors

General Procedure for the Synthesis of (4-Benzylxyloxy)phenylglycine

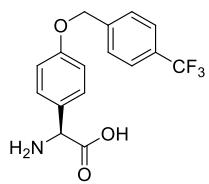
Benzylxyloxyamino acids were synthesized as previously described.⁷ A solution of 4-hydroxyphenylglycine (1 equiv) in 1 M NaOH (1 equiv) and a solution of CuSO₄·5 H₂O (0.67 equiv) in water (10 mL) were warmed to 50 °C under stirring. Both solutions were combined, and the reaction mixture was stirred for 30 min at 50 °C. After cooling on an ice-water bath, the blue precipitate of the amino acid Cu-complex separated immediately. The precipitate was isolated, washed with water and dried. The Cu-complex was dissolved in a mixture of MeOH (25 mL) and 1 M NaOH (1 equiv). The corresponding benzyl bromide (1.1 equiv) was added, and the mixture was stirred at room temperature overnight. The insoluble Cu-complex of the resulting ether was collected by filtration, washed with MeOH, and then with water to remove excess of the unreacted starting material. Finally, 1 M HCl (2 equiv) was added to release the product from the Cu-complex. The precipitated product was washed with water and dried under reduced pressure. The crude product was used for subsequent synthetic steps without further purification.

(4-Benzylxyloxy)-L-phenylglycine (115)



Compound **115** was obtained according to the general procedure from (4-hydroxy)-L-phenylglycine (2009 mg, 12.0 mmol) and benzyl bromide (1.568 mL, 13.2 mmol) as a beige solid (2105 mg, 60% yield). ¹H NMR (300 MHz, DMSO-*d*₆/NaOD) δ 7.49–7.36 (m, 5H), 7.29 (d, *J* = 8.7 Hz, 2H), 6.97 (d, *J* = 8.7 Hz, 2H), 5.09 (s, 2H), 4.16 (s, 1H). ¹³C NMR (75 MHz, DMSO-*d*₆/NaOD) δ 178.8, 155.5, 135.3, 133.9, 127.4, 127.2, 126.8, 126.3, 113.8, 68.9, 59.6. HRMS (ESI): *m/z* [M–H][−] calcd for C₁₅H₁₄NO₃: 256.0979, found: 256.0968.

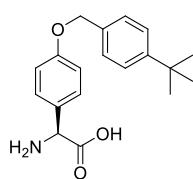
[4-(4-Trifluoromethyl)benzylxyloxy]-L-phenylglycine (116)



Compound **116** was obtained according to the general procedure from (4-hydroxy)-L-phenylglycine (994 mg, 5.9 mmol) and 4-(trifluoromethyl)benzyl bromide (1610 mg, 6.7 mmol) as a beige solid (652 mg, 33% yield). ¹H NMR (300 MHz, DMSO-*d*₆/NaOD) δ 7.73 (d, *J* = 8.1 Hz, 2H), 7.64 (d, *J* = 8.1 Hz,

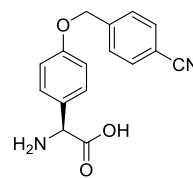
2H), 7.26 (d, J = 8.6 Hz, 2H), 6.84 (d, J = 8.1 Hz, 2H), 5.16 (s, 2H), 3.94 (s, 2H), 3.59 (s, 2H). HRMS (ESI): m/z [M–H][–] calcd for C₁₆H₁₃F₃NO₃: 324.0853, found: 324.0750.

[4-(4-*tert*-Butyl)benzyloxy]-L-phenylglycine (117)



Compound **117** was obtained according to the general procedure from (4-hydroxy)-L-phenylglycine (995 mg, 5.9 mmol) and 4-*tert*-butylbenzyl bromide (1.213 mL, 6.7 mmol) as a beige solid (1090 mg, 58% yield). ¹H NMR (300 MHz, DMSO-*d*₆/NaOD) δ 7.38 (d, J = 8.3 Hz, 2H), 7.33 (d, J = 8.3 Hz, 2H), 7.24 (d, J = 8.7 Hz, 2H), 6.81 (d, J = 8.6 Hz, 2H), 5.00 (s, 2H), 3.91 (s, 1H), 3.55 (s, 3H), 1.26 (s, 9H). ¹³C NMR (APT, 75 MHz, DMSO-*d*₆/NaOD) δ 176.3, 156.4, 150.2, 138.2, 134.5, 127.7, 127.5, 125.2, 113.9, 69.0, 60.4, 34.5, 31.3. HRMS (ESI): m/z [M–H][–] calcd for C₁₉H₂₂NO₃: 312.1605, found: 312.1599.

[4-(4-Cyano)benzyloxy]-L-phenylglycine (118)



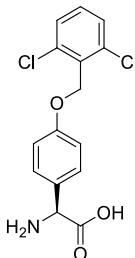
Compound **118** was obtained according to the general procedure from (4-hydroxy)-L-phenylglycine (993 mg, 5.9 mmol) and 4-cyanobenzyl bromide (1234 mg, 6.3 mmol) as a beige solid (857 mg, 51% yield). ¹H NMR (300 MHz, DMSO-*d*₆/NaOD) δ 7.83 (d, J = 8.3 Hz, 2H), 7.61 (d, J = 8.3 Hz, 2H), 7.27 (d, J = 8.7 Hz, 2H), 6.83 (d, J = 8.6 Hz, 2H), 5.14 (s, 2H), 3.95 (s, 1H), 3.52 (s, 2H). ¹³C NMR (APT, 75 MHz, DMSO-*d*₆/NaOD) δ 176.5, 156.2, 143.3, 139.0, 132.2, 128.1, 127.9, 127.7, 127.0, 119.0, 117.7, 114.0, 110.4, 70.1, 60.1. HRMS (ESI): m/z [M–H][–] calcd for C₁₆H₁₃N₂O₃: 281.0932, found: 281.0943.

[4-(3-Methoxy)benzyloxy]-L-phenylglycine (119)



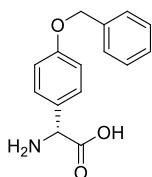
Compound **119** was obtained according to the general procedure from (4-hydroxy)-L-phenylglycine (993 mg, 5.9 mmol) and 3-methoxybenzyl bromide (0.909 mL, 6.5 mmol) as a light brown solid (779 mg, 46% yield). ¹H NMR (300 MHz, DMSO-*d*₆/NaOD) δ 7.34–7.20 (m, 2H), 6.98 (d, J = 6.8 Hz, 2H), 6.91–6.79 (m, 4H), 5.02 (s, 2H), 3.91 (s, 1H), 3.72 (s, 3H), 3.51 (s, 2H). HRMS (ESI): m/z [M–H][–] calcd for C₁₆H₁₆NO₄: 286.1085, found: 286.1085.

[4-(2,6-Dichlorobenzyl)oxy]-L-phenylglycine (120)



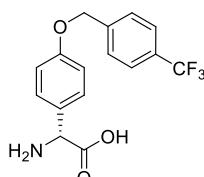
Compound **120** was obtained according to the general procedure from (4-hydroxy)-L-phenylglycine (993 mg, 5.9 mmol) and 2,6-dichlorobenzyl bromide (1632 mg, 6.8 mmol) as a beige solid (996 mg, 51% yield). ¹H NMR (300 MHz, DMSO-*d*₆/NaOD) δ 7.54 (d, *J* = 8.7 Hz, 2H), 7.48–7.38 (m, 1H), 7.29 (d, *J* = 8.6 Hz, 2H), 6.88 (d, *J* = 8.6 Hz, 2H), 5.17 (s, 2H), 3.95 (s, 1H), 3.49 (s, 2H). ¹³C NMR (APT, 75 MHz, DMSO-*d*₆/NaOD) δ 176.6, 156.6, 139.2, 136.0, 132.1, 131.4, 128.8, 127.7, 113.5, 65.0, 60.2. HRMS (ESI): *m/z* [M–H][–] calcd for C₁₅H₁₂Cl₂NO₃: 324.0200, found: 324.0190.

(4-Benzyl)oxy-D-phenylglycine (121)



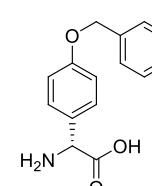
Compound **121** was obtained according to the general procedure from (4-hydroxy)-D-phenylglycine (2402 mg, 14.4 mmol) and benzyl bromide (1.9 mL, 15.8 mmol) as a beige solid (2045 mg, 55% yield). ¹H NMR (300 MHz, DMSO-*d*₆/NaOD) δ 7.45–7.34 (m, 5H), 7.25 (d, *J* = 8.7 Hz, 2H), 6.83 (d, *J* = 8.7 Hz, 2H), 5.04 (s, 2H), 3.92 (s, 1H). ¹³C NMR (75 MHz, DMSO-*d*₆/NaOD) δ 176.6, 156.6, 138.8, 137.6, 128.6, 128.0, 127.9, 127.7, 114.0, 69.3, 60.3. HRMS (ESI): *m/z* [M–H][–] calcd for C₁₅H₁₄NO₃: 256.0979, found: 256.0989.

[4-(4-Trifluoromethyl)benzyloxy]-D-phenylglycine (122)



Compound **122** was obtained according to the general procedure from (4-hydroxy)-D-phenylglycine (601 mg, 3.6 mmol) and 4-(trifluoromethyl)benzyl bromide (0.67 mL, 4.3 mmol) as a beige solid (527 mg, 45% yield). ¹H NMR (300 MHz, DMSO-*d*₆/NaOD) δ 7.74 (d, *J* = 8.1 Hz, 2H), 7.66–7.21 (m, 4H), 6.84 (d, *J* = 8.6 Hz, 2H), 3.98 (s, 1H), 3.55 (s, 2H). HRMS (ESI): *m/z* [M–H][–] calcd for C₁₅H₁₂Cl₂NO₃: 324.0853, found: 324.0859.

[4-(3,4-Dichlorobenzyl)oxy]-D-phenylglycine (123)



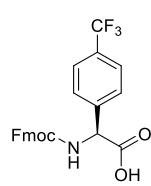
Compound **123** was obtained according to the general procedure from (4-hydroxy)-D-phenylglycine (499 mg, 3.0 mmol) and 3,4-dichlorobenzyl bromide (0.547 mL, 3.3 mmol) as a beige solid (596 mg, 61% yield). ¹H NMR (300 MHz, DMSO-*d*₆/NaOD) δ 7.69 (d, *J* = 7.7 Hz, 1H), 7.65 (d, *J* = 8.2 Hz,

1H), 7.41 (dd, $J = 8.3, 1.9$ Hz, 1 H), 7.29 (d, $J = 8.6$ Hz, 2 H), 6.86 (d, $J = 8.6$ Hz, 2H), 5.06 (d, $J = 6.1$ Hz, 1H), 3.94 (s, 1H). ^{13}C NMR (APT, 75 MHz, DMSO-*d*₆/NaOD) δ 175.5, 154.2, 136.6, 131.2, 130.9, 129.5, 128.4, 128.1, 127.7, 114.3, 70.4, 60.8. HRMS (ESI): *m/z* [M-H]⁻ calcd for C₁₅H₁₂Cl₂NO₃: 324.0200, found: 324.0197.

General Procedure for the Synthesis of N_α-Fmoc-protected Amino Acids.

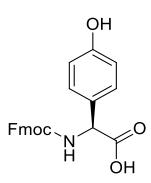
Benzylxoyamino acids were Fmoc-protected with a previously published method.⁷ A solution of the amino acid or its hydrochloride salt (1 equiv) and DIPEA (2 – 3 equiv) in 30 mL of ACN/H₂O (1:1) was stirred at room temperature for 20 min, then Fmoc-OSu (0.9–0.95 equiv) was added. After 30 min, most of the solids have dissolved. The reaction progress was monitored for the disappearance of Fmoc-OSu by TLC (solvent system: ethyl acetate). When the reaction was completed (1.5 – 2 h), the solution was acidified to pH 1 – 2 using 1 N HCl, 10 mL of water were added, and the mixture was allowed to stir for an additional hour. Finally, the resulting precipitate was collected by filtration, washed with water, dried under reduced pressure and used as crude product without further purification for solid phase peptide synthesis.

N_α-Fmoc-(4-trifluoromethyl)-L-phenylglycine (124)



Following the general procedure, (4-trifluoromethyl)-L-phenylglycine (211 mg, 1.0 mmol), DIPEA (0.450 mL, 2.6 mmol) and Fmoc-OSu (339 mg, 1.0 mmol) were reacted together to give **124** as a white solid (436 mg, 94% yield). ^1H NMR (300 MHz, CDCl₃) δ 8.00 (s, 1H), 7.80–7.66 (m, 2H), 7.60–7.47 (m, 4H), 7.43–7.28 (m, 4H), 7.22–7.08 (m, 2H), 4.96 (d, $J = 5.2$ Hz, 1H), 4.44 (s, 2H), 4.07–3.98 (m, 1H). HRMS (ESI): *m/z* [M-H]⁻ calcd for C₂₄H₁₇F₃NO₄: 440.1115, found: 440.1141.

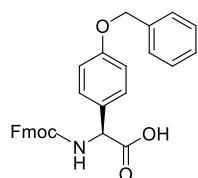
N_α-Fmoc-(4-hydroxy)-L-phenylglycine (125)



Following the general procedure, 4-(hydroxyl)-L-phenylglycine (1503 mg, 9.5 mmol), DIPEA (3.830 mL, 22.4 mmol) and Fmoc-OSu (2871 mg, 8.5 mmol) were reacted together to give **125** as a white solid (2214 mg, 63% yield). ^1H NMR (300 MHz, CD₃CN/D₂O) δ 7.83 (d, $J = 7.5$ Hz, 2H), 7.67 (d, $J = 7.2$ Hz, 2H), 7.42 (t, $J = 7.4$ Hz, 2H), 7.37–7.28 (m, 4H), 7.23 (d, $J = 8.1$ Hz, 2H), 6.82 (d, $J = 8.3$ Hz, 2H), 5.13 (d, $J = 6.8$ Hz, 1H), 4.33 (d, $J = 7.0$ Hz, 2H), 4.22 (t, $J = 6.9$ Hz, 1H). ^{13}C NMR (75 MHz, CD₃CN /D₂O) δ

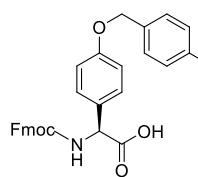
172.6, 158.2, 156.6, 145.2, 142.1, 130.0, 128.7, 128.1, 126.5, 126.2, 121.0, 116.5, 67.5, 58.4, 48.0. HRMS (ESI): m/z [M–H][–] calcd for C₂₃H₁₈NO₅: 388.1190, found: 388.1191.

N_α-Fmoc-(4-benzyloxy)-L-phenylglycine (126)



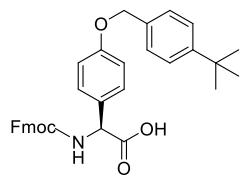
Following the general procedure, **115** (2867 mg, 9.8 mmol), DIPEA (4.166 mL, 24.4 mmol) and Fmoc-OSu (3130 mg, 9.3 mmol) were reacted together to give **126** as a beige or brown solid (4590 mg, 98% yield). ¹H NMR (300 MHz, CDCl₃) δ 7.75 (d, J = 7.7 Hz, 3H), 7.56 (d, J = 7.0 Hz, 2H), 7.44–7.28 (m, 11H), 6.97 (d, J = 7.4 Hz, 2H), 5.92–5.76 (m, 1H), 5.40–5.24 (m, 1H), 5.04 (s, 2H), 4.39 (d, J = 6.4 Hz, 2H), 4.24–4.16 (m, 1H). HRMS (ESI): m/z [M–H][–] calcd for C₃₀H₂₄NO₅: 478.1660, found: 478.1662.

N_α-Fmoc-[4-(4-trifluoromethyl)benzyloxy]-L-phenylglycine (127)



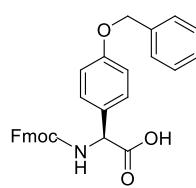
Following the general procedure, **116** (652 mg, 2.0 mmol), DIPEA (0.856 mL, 5.0 mmol) and Fmoc-OSu (644 mg, 1.9 mmol) were reacted together to give **127** as a beige solid (975 mg, 89% yield). ¹H NMR (300 MHz, CDCl₃) δ 7.77 (t, J = 7.4 Hz, 2H), 7.64 (d, J = 8.1 Hz, 2H), 7.59–7.50 (m, 3H), 7.44–7.28 (m, 5H), 7.20 (s, 1H), 6.99–6.88 (m, 2H), 5.77 (d, J = 6.4 Hz, 1H), 5.33 (d, J = 6.7 Hz, 1H), 5.11 (s, 2H), 4.39 (d, J = 5.9 Hz, 2H), 4.24–4.15 (m, 1H). HRMS (ESI): m/z [M–H][–] calcd for C₃₁H₂₃F₃NO₅: 546.1534, found: 546.1554.

N_α-Fmoc-[4-(4-tert-butyl)benzyloxy]-L-phenylglycine (128)



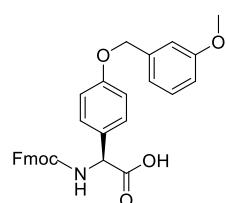
Following the general procedure, **117** (1090 mg, 3.5 mmol), DIPEA (1.485 mL, 8.7 mmol) and Fmoc-OSu (1117 mg, 3.3 mmol) were reacted together to give **128** as a beige solid (1446 mg, 77% yield). ¹H NMR (300 MHz, CDCl₃) δ 7.77 (t, J = 7.6 Hz, 2H), 7.65–7.54 (m, 1H), 7.44–7.39 (m, 2H), 7.41–7.27 (m, 8H), 7.21 (s, 1H), 6.96 (d, J = 7.9 Hz, 2H), 5.80 (d, J = 6.7 Hz, 1H), 5.33 (d, J = 6.7 Hz, 1H), 5.01 (s, 2H), 4.39 (d, J = 6.4 Hz, 2H), 4.24–4.16 (m, 1H), 1.32 (s, 9H). HRMS (ESI): m/z [M–H][–] calcd for C₃₄H₃₂NO₅: 534.2286, found: 534.2314.

N_α-Fmoc-[4-(4-cyanomethyl)benzyloxy]-L-phenylglycine (129)



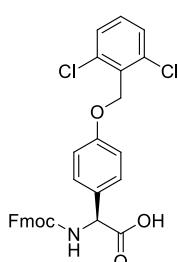
Following the general procedure, **118** (857 mg, 3.0 mmol), DIPEA (1.296 mL, 7.6 mmol) and Fmoc-OSu (974 mmol, 2.9 mmol) were reacted together to give **129** as a brown oil (1212 mg, 79% yield). ¹H NMR (300 MHz, CDCl₃) δ 7.76 (t, *J* = 7.3 Hz, 3H), 7.67 (d, *J* = 8.3 Hz, 2H), 7.61–7.54 (m, 1H), 7.52 (d, *J* = 8.3 Hz, 2H), 7.42–7.28 (m, 6H), 6.96–6.87 (m, 2H), 5.83 (d, *J* = 6.7 Hz, 1H), 5.33 (d, *J* = 6.5 Hz, 1H), 5.10 (s, 2H), 4.43–4.35 (m, 2H), 4.23–4.16 (m, 1H). HRMS (ESI): *m/z* [M–H][–] calcd for C₃₁H₂₃N₂O₅: 503.1612, found: 503.1629.

N_α-Fmoc-[4-(3-methoxy)benzyloxy]-L-phenylglycine (130)



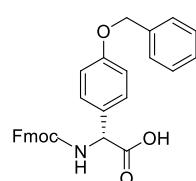
Following the general procedure, **119** (779 mg, 2.7 mmol), DIPEA (1.157 mL, 6.8 mmol) and Fmoc-OSu (872 mg, 2.6 mmol) were reacted together to give **130** as a beige solid (982 mg, 71% yield). ¹H NMR (300 MHz, CDCl₃) δ 7.81–7.72 (m, 2H), 7.66–7.55 (m, 1H), 7.44–7.27 (m, 8H), 7.02–6.92 (m, 4H), 6.86 (dd, *J* = 8.0, 2.2 Hz, 1H), 5.80 (d, *J* = 6.6 Hz, 1H), 5.32 (d, *J* = 6.7 Hz, 1H), 5.03 (s, 2H), 4.39 (d, *J* = 6.6 Hz, 2H), 4.25–4.16 (m, 1H), 3.81 (s, 3H). HRMS (ESI): *m/z* [M–H][–] calcd for C₃₁H₂₆NO₆: 508.1766, found: 508.1788.

N_α-Fmoc-[4-(2,6-dichloro)benzyloxy]-L-phenylglycine (131)



Following the general procedure, **120** (996 mg, 3.1 mmol), DIPEA (1.303 mL, 7.6 mmol) and Fmoc-OSu (976 mg, 2.9 mmol) were reacted together to give **131** as a beige solid (1361 mg, 81% yield). ¹H NMR (300 MHz, CDCl₃) δ 7.80–7.69 (m, 2H), 7.65–7.55 (m, 1H), 7.44–7.29 (m, 9H), 7.24–7.20 (m, 1H), 7.06–6.98 (m, 2H), 5.77 (d, *J* = 6.6 Hz, 1H), 5.36 (d, *J* = 6.7 Hz, 1H), 5.27 (s, 2H), 4.40 (d, *J* = 6.8 Hz, 2H), 4.25–4.17 (m, 1H). HRMS (ESI): *m/z* [M–H][–] calcd for C₃₀H₂₂Cl₂NO₅: 546.0881, found: 546.0886.

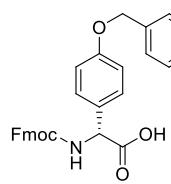
N_α-Fmoc-(4-benzyloxy)-D-phenylglycine (132)



Following the general procedure, **121** (521 mg, 2.0 mmol), DIPEA (0.864 mL, 5.1 mmol) and Fmoc-OSu (650 mg, 1.9 mmol) were reacted together to give **132** as a beige solid (916 mg, 94% yield). ¹H NMR (300 MHz, CDCl₃) δ 7.79–7.68

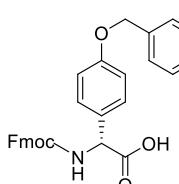
(m, 3H), 7.63–7.52 (m, 3H), 7.45–7.31 (m, 9H), 7.00–6.89 (m, 2H), 5.79 (d, J = 6.3 Hz, 1H), 5.34 (d, J = 6.4 Hz, 1H), 5.05 (s, 2H), 4.40 (d, J = 6.4 Hz, 2H), 4.25–4.09 (m, 1H). ^{13}C NMR (75 MHz, DMSO-*d*₆) δ 172.4, 158.1, 156.6, 156.2, 146.3, 141.4, 128.8, 128.2, 127.9, 127.6, 127.2, 120.1, 115.5, 70.2, 57.6, 47.3 HRMS (ESI): *m/z* [M–H][–] calcd for C₃₀H₂₄NO₅: 478.1660, found: 478.1663.

N_α-Fmoc-[4-(4-trifluoromethyl)benzyloxy]-D-phenylglycine (133)



Following the general procedure, **122** (430 mg, 1.3 mmol), DIPEA (0.564 mL, 3.3 mmol) and Fmoc-OSu (424 mg, 1.3 mmol) were reacted together to give **133** as a beige solid (604 mg, 83% yield). ^1H NMR (300 MHz, CDCl₃) δ 7.74 (t, J = 8.2 Hz, 2H), 7.64 (d, J = 8.1 Hz, 2H), 7.61–7.09 (m, 10H), 7.01–6.84 (m, 2H), 5.36 (d, J = 6.6 Hz, 1H), 5.11 (s, 2H), 4.39 (d, J = 6.2 Hz, 2H), 4.25–4.13 (m, 1H). HRMS (ESI): *m/z* [M–H][–] calcd for C₃₁H₂₃F₃NO₅: 546.1534, found: 546.1528.

N_α-Fmoc-[4-(3,4-dichloro)benzyloxy]-D-phenylglycine (134)

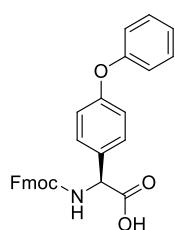


Following the general procedure, **123** (161 mg, 0.5 mmol), DIPEA (0.211 mL, 1.2 mmol) and Fmoc-OSu (156 mg, 0.5 mmol) were reacted together to give **134** as a brown solid (241 mg, 87% yield). ^1H NMR (300 MHz, CDCl₃) δ 7.78 (t, J = 7.7 Hz, 2H), 7.63–7.54 (m, 2H), 7.48 (d, J = 8.2 Hz, 1H), 7.43–7.31 (m, 5H), 7.26 (s, 1H), 7.19 (s, 2H), 6.96 (dd, J = 11.5, 8.6 Hz, 2H), 5.75 (d, J = 6.7 Hz, 1H), 5.39 (d, J = 6.7 Hz, 1H), 5.03 (s, 2H), 4.50–4.38 (m, 2H), 4.28–4.18 (m, 1H). HRMS (ESI): *m/z* [M–H][–] calcd for C₃₀H₂₂Cl₂NO₅: 546.0881, found: 546.0872.

General Procedure for the Synthesis of (4-Phenoxy)phenylglycine

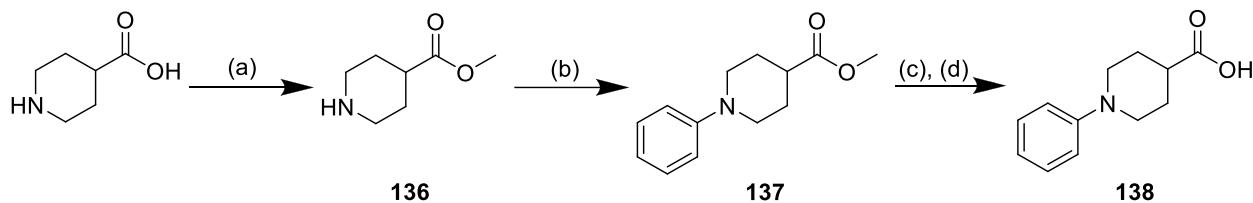
(4-Phenoxy)phenylglycine derivatives were synthesized according to a procedure modified from literature.¹⁹ 4 Å molecular sieves were given to a mixture of *N*_α-Fmoc-(4-hydroxy)-phenylglycine (1 equiv), the respective arylboronic acid (2 equiv) and Cu(OAc)₂ (2 equiv). 10 mL DCM was added and the resulting colored suspension was treated with 4 equivalents pyridine. After stirring the reaction mixture at ambient atmosphere overnight, all solvents were removed under reduced pressure. The resulting residue was suspended in ethyl acetate, filtered and the filtrate was washed 3 times with 1 N HCl. The organic layer was dried over anhydrous MgSO₄, filtered and concentrated *in vacuo*. The crude mixture was purified by flash chromatography if necessary or used for subsequent synthetic steps without further purification.

N_α-Fmoc-(4-phenoxy)-L-phenylglycine (135)



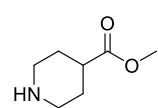
Following the general procedure, **125** (397 mg, 1.0 mmol), benzeneboronic acid (258 mg, 2.1 mmol), Cu(OAc)₂ (381 mg, 2.1 mmol) and pyridine (0.332 mL, 4.1 mmol) were reacted together to give **135** as pale yellow solid (229 mg, 48% yield). ¹H NMR (300 MHz, acetone-*d*₆) δ 11.10 (s, 1H), 7.93–7.80 (m, 2H), 7.51 (d, *J* = 8.0 Hz, 2H), 7.46–7.25 (m, 10H), 7.02 (t, *J* = 8.0 Hz, 1H), 6.85 (d, *J* = 8.2 Hz, 2H), 5.38 (d, *J* = 7.5 Hz, 1H), 4.44–4.30 (m, 2H), 4.24 (t, *J* = 6.9 Hz, 1H). ¹³C NMR (APT, 75 MHz, acetone-*d*₆) δ 172.8, 162.6, 156.3, 155.0, 146.6, 142.0, 136.8, 134.9, 129.3, 128.5, 128.3, 128.0, 126.2, 120.8, 119.4, 116.3, 67.4, 58.4, 48.0. HRMS (ESI): *m/z* [M–H][−] calcd for C₂₉H₂₂NO₅: 464.1503, found: 464.1501.

Scheme S1. Synthesis of 1-Phenylpiperidine-4-carboxylic acid



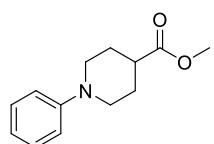
(a) SOCl₂, MeOH, 0 °C → reflux overnight, (b) phenylboronic acid pinacol ester, Cu(OAc)₂, Et₃N, 3 Å sieves, DCM, (c) LiOH, THF/H₂O, (d) HCl.

Methyl piperidine-4-carboxylate (136)



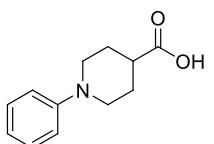
To a solution of piperidine-4-carboxylic acid (1503 mg, 11.6 mmol) in dry MeOH (15 mL) was added SOCl₂ (2.531 mL, 34.8 mmol) dropwise at 0 °C. The mixture was heated to reflux and was stirred overnight. The solution was concentrated *in vacuo* to afford a beige solid (1648 mg, 99% yield). ¹H NMR (300 MHz, DMSO-*d*₆) δ 3.62 (s, 3H), 3.25–3.12 (m, 2H), 2.96–2.80 (m, 2H), 2.74–2.62 (m, 1H), 2.02–1.90 (m, 2H), 1.85–1.68 (m, 2H). ¹³C NMR (APT, 75 MHz, DMSO-*d*₆) δ 173.6, 51.7, 42.0, 37.6, 24.4. HRMS (ESI): *m/z* [M+H]⁺ calcd for C₇H₁₄NO₂: 144.1019, found: 144.1028.

Methyl 1-phenylpiperidine-4-carboxylate (137)



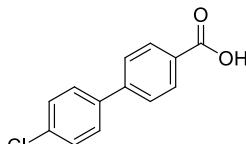
Compound **137** was synthesized according to a procedure modified from literature.²⁰ 3 Å molecular sieves were given to a mixture of **136** (204 mg, 1.4 mmol), phenylboronic acid pinacol ester (572 mg, 2.8 mmol) and Cu(OAc)₂ (508 mg, 2.8 mmol). 10 mL ACN was added under nitrogen atmosphere and the resulting colored suspension was treated with 4 equivalents Et₃N (0.774 ml, 5.6 mmol). After stirring the reaction mixture at 80 °C overnight, the suspension was dried under reduced pressure. The resulting residue was suspended in H₂O and ethyl acetate. The suspension was filtered and the filtrate was washed 3 times with H₂O and 0.1 N NaOH. The organic layer was dried over anhydrous MgSO₄ and concentrated to afford a yellow oil (94 mg, 31% yield). ¹H NMR (300 MHz, acetone-*d*₆) δ 7.17–7.09 (m, 2H), 6.82 (d, *J* = 7.6 Hz, 2H), 6.71 (t, *J* = 7.3 Hz, 1H), 3.65 (s, 3H), 3.64–3.60 (m, 2H), 2.78 (td, *J* = 12.2, 2.9 Hz, 2H), 2.57–2.45 (m, 1H), 2.01–1.92 (m, 2H), 1.82–1.72 (m, 2H). ¹³C NMR (APT, 75 MHz, CDCl₃) δ 175.8, 150.9, 129.4, 120.1, 116.4, 52.0, 49.8, 40.9, 28.5. HRMS (ESI): *m/z* [M+H]⁺ calcd for C₁₃H₁₈NO₂: 220.1332, found: 220.1322.

1-Phenylpiperidine-4-carboxylic acid (138)



137 (94 mg, 0.3 mmol) was dissolved in a solution of LiOH (29 mg, 1.1 mmol) in THF/H₂O (2:1, 10 mL). The solution was stirred for 4 h. Most of the volume was removed *in vacuo* and the pH was acidified with 2 N HCl. The product was extracted with ethyl acetate, washed with several portions of brine and dried under reduced pressure to afford a pale yellow solid (88 mg, 85% yield). ¹H NMR (300 MHz, acetone-*d*₆) δ 7.38–7.23 (m, 3H), 7.19 (s, *J* = 8.7 Hz, 2H), 3.85–3.33 (m, 4H), 2.61–2.46 (m, 3H), 1.74–1.54 (m, 2H). HRMS (ESI): *m/z* [M+H]⁺ calcd for C₁₂H₁₅NO₂: 206.1176, found: 206.1174.

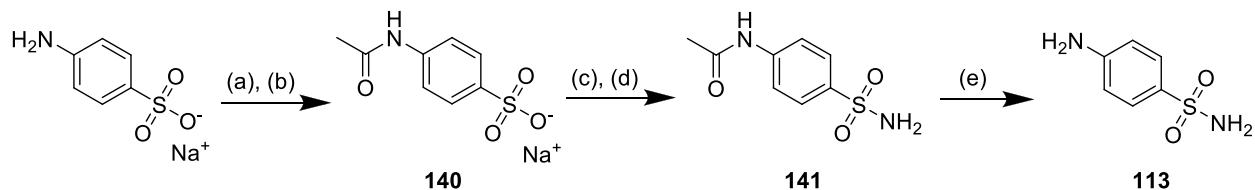
4'-Chlorobiphenyl-4-carboxylic acid (139)



Methyl 4'-chlorobiphenyl-4-carboxylate (445 mg, 1.8 mmol) was dissolved in a mixture of THF/H₂O 4:1 (20 mL). LiOH (108 mg, 4.5 mmol) was added under ice-bath cooling and the solution was stirred overnight. The pH of the mixture was adjusted to 1 with concentrated HCl. The resulting precipitate was collected by filtration and washed with 1 N HCl and water. The white solid (419 mg, 100% yield) was dried under reduced pressure and used without further purification. ¹H NMR (300 MHz,

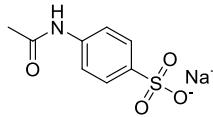
DMSO-*d*₆) δ 13.00 (br. s, 1H), 8.02 (d, *J* = 7.7 Hz, 2H), 7.87–7.70 (m, 4H), 7.55 (d, *J* = 7.9 Hz, 2H). HRMS (ESI): *m/z* [M–H][−] calcd for C₁₃H₈ClO₂: 231.0218, found: 231.0217.

Scheme S2. Synthesis of 4-Aminobenzenesulfonamide (Sulfanilamide)



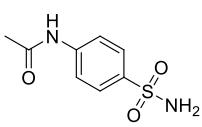
(a) Pyridine, acetic anhydride; (b) NaOH, 0 °C → rt, EtOH; (c) thionyl chloride, DMF, -4 °C; (d) NH₄OH, THF; (e) HCl, H₂O/EtOH, reflux, 1 h → rt.

Sodium 4-acetamidobenzenesulfonate (140)

 **140** was synthesized according to a previously published method.²¹ Sodium 4-aminobenzenesulfonate (2014 mg, 9.4 mmol) was dissolved in pyridine (15 mL) under nitrogen atmosphere. Acetic anhydride (2.864 mL, 14.2 mmol) was added dropwise and the solution was stirred for 2 hours. The solvent was removed under reduced pressure and further co-evaporated with several portions of ethanol. After adding ethanol to the residue, the resulting suspension was cooled to 0 °C, filtered and washed with cold ethanol and Et₂O. The obtained solid was dried *in vacuo* to yield a pale red solid (pyridinium 4-acetamidobenzenesulfonate, 2379 mg, 86% yield).

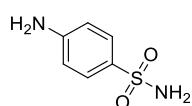
This salt (2010 mg, 6.8 mmol) was added to an aqueous 1 N NaOH solution at 0 °C. After warming the mixture to room temperature, ethanol was added and the mixture was concentrated *in vacuo* by about 75%. An additional portion of ethanol was added and the mixture was concentrated to dryness under reduced pressure. The solid was filtered, washed with ethanol, Et₂O and dried to dryness to afford a white solid (1492 mg, 92% yield). ¹H NMR (300 MHz, DMSO-*d*₆) δ 7.39–7.01 (m, 4H), 6.43 (d, *J* = 8.5 Hz, 1H), 1.70 (s, 3H). HRMS (ESI): *m/z* [M–H][−] calcd for C₈H₈NO₄S: 214.0180, found: 214.0175.

N-(4-Sulfamoylphenyl)acetamide (141)



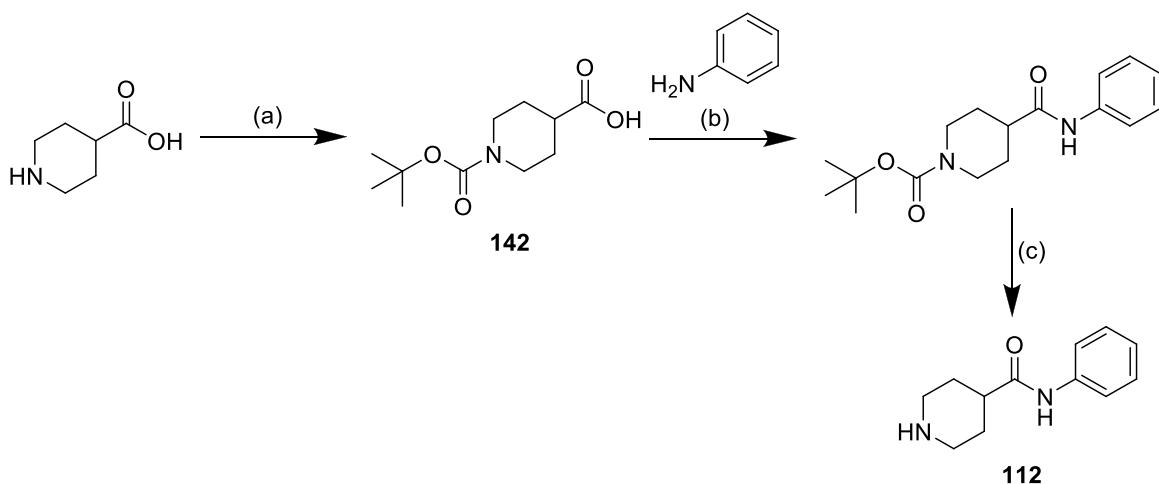
141 was synthesized according to a previously published method.²¹ **140** (987 mg, 4.2 mmol) was dissolved in DMF (10 mL) under nitrogen atmosphere and cooled with an ice-brine bath. Thionyl chloride (0.906 mL, 12.5 mmol) was added dropwise and the mixture was stirred for 2 hours. The mixture was poured into a beaker with water and ice. The resulting yellow solid was collected by filtration, washed with water and dried *in vacuo*. The solid was added portionwise to a stirred solution of THF (10 mL) and concentrated NH₄OH solution (8 mL). The mixture was stirred overnight and concentrated under reduced pressure. Cold water was added and the solid was filtered and washed with Et₂O. After drying under reduced pressure **141** was obtained as a grey solid (454 mg, 51% yield). ¹H NMR (300 MHz, DMSO-*d*₆) δ 7.47–7.24 (m, 3H), 7.13–6.93 (s, 2H), 6.54–6.37 (m, 2H), 1.62 (s, 3H). HRMS (ESI): *m/z* [M–H][−] calcd for C₈H₉N₂O₃S: 213.0339, found: 213.0359.

4-Aminobenzenesulfonamide (113)



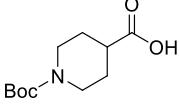
113 was synthesized according to a previously published method.²¹ **141** (402 mg, 1.9 mmol) was added to a mixture of ethanol (10 mL) and water (2 mL). After addition of concentrated HCl (10 mL), the solution was brought to reflux for 1 hour. The mixture was cooled to room temperature, quenched with saturated aqueous NaHCO₃ and extracted 3 times with ethyl acetate. The combined organic layers were washed with water and brine, dried over anhydrous MgSO₄, filtered and concentrated *in vacuo* to afford an off-white solid (221 mg, 68% yield). ¹H NMR (300 MHz, DMSO-*d*₆) δ 7.45 (d, *J* = 8.6 Hz, 2H), 6.89 (s, 2H), 6.59 (d, *J* = 8.7 Hz, 2H), 5.79 (s, 2H). ¹³C NMR (APT, 75 MHz, DMSO-*d*₆) δ 152.3, 130.4, 127.9, 112.9. HRMS (ESI): *m/z* [M+H]⁺ calcd for C₆H₉N₂O₂S: 195.0199, found: 195.0200.

Scheme S3. Synthesis of *N*-Phenylpiperidine-4-carboxamide

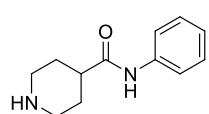


(a) Boc₂O, NaOH, THF, 0 °C -> rt; (b) HATU, TMP, DCM/DMF, 0 °C -> rt; (c) TFA, DCM.

1-(*tert*-Butoxycarbonyl)piperidine-4-carboxylic acid (142)

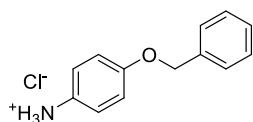
 **142** was synthesized according to a previously published method.²² To a solution of piperidine-4-carboxylic acid (589 mg, 4.6 mmol) in THF was added 1 N NaOH (15 mL) at 0 °C. To this di-*tert*-butyl dicarbonate (2.098 mL, 9.8 mmol) was added dropwise with vigorous stirring. The solution was warmed to room temperature and stirred overnight. The resulting mixture was concentrated to half of the volume and acidified with 1 N HCl to a pH of 5. A precipitate was formed which was filtered and washed with water to yield a white solid (787 mg, 75% yield). ¹H NMR (300 MHz, CDCl₃) δ 4.10–3.92 (m, 2H), 2.91–2.77 (m, 2H), 2.55–2.41 (m, 1H), 1.96–1.81 (m, 2H), 1.72–1.55 (m, 2H), 1.45 (s, 9H). HRMS (ESI): *m/z* [M-H]⁻ calcd for C₁₁H₁₈NO₄: 228.1241, found: 228.1254.

N-Phenylpiperidine-4-carboxamide (112)



142 (155 mg, 0.7 mmol), HATU (299 mg, 0.8 mmol) and TMP (0.156 mL, 1.2 mmol) were dissolved in DCM/DMF (1:1). Aniline (0.072 mL, 0.8 mmol) was added dropwise at 0 °C. The solution was warmed to room temperature and was allowed to stir overnight. After that the solvents were removed *in vacuo* and 2 mL of TFA/DCM (1:1) were added. The mixture was stirred for 1.5 hours and the solvents were evaporated under reduced pressure. The resulting residue was dissolved in ethyl acetate and washed 2 times with 1 N NaOH and water. The organic layer was dried with anhydrous MgSO₄ and the solvent was removed *in vacuo* to yield a yellow oil (96 mg, 70% yield). ¹H NMR (300 MHz, DMSO-*d*₆) δ 7.63–7.56 (m, 3H), 7.50–7.45 (m, 2H), 7.33–7.24 (m, 2H), 7.09–7.00 (m, 1H), 3.40–3.29 (m, 2H), 3.00–2.85 (m, 2H), 2.02–1.88 (m, 5H). HRMS (ESI): *m/z* [M+H]⁺ calcd for C₁₂H₁₇N₂O: 205.1335, found: 205.1339.

4-(Benzyl)aniline hydrochloride (111)

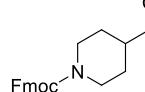


Compound **111** was synthesized according to a previously published method.²³ To a stirred solution of 4-aminophenol (1021 mg, 9.4 mmol) in DMF was added KOtBu (1160 mg, 10.3 mmol) at 0 °C. After the dropwise addition of benzyl bromide (1.222 mL, 10.3 mmol) the suspension was allowed to stir at room temperature for 4 hours. The solution was cooled again to 0 °C and a further 1.1 equiv of KOtBu (1153 mg, 10.3 mmol) prior to the dropwise addition of benzyl bromide (1.222 mL, 10.3 mmol) were added. This mixture was stirred overnight before it was quenched with H₂O. The solution was extracted 3 times with ethyl acetate and the combined organic phases were washed with H₂O and brine. The solution was dried over MgSO₄ and concentrated under reduced pressure. HCl in 1,4-dioxane (4 M) was added and the resulting brown precipitate was filtered, washed several times with diethyl ether and dried *in vacuo* (HCl salt, 1391 mg, 63% yield). ¹H NMR (300 MHz, DMSO-*d*₆) δ 10.15 (s, 3H), 7.48–7.33 (m, 5H), 7.43–7.28 (m, 2H), 7.14–7.07 (m, 2H), 5.12 (s, 2H). ¹³C NMR (APT, 75 MHz, DMSO-*d*₆) δ 158.1, 137.1, 128.9, 128.4, 128.2, 125.1, 124.7, 116.2, 70.0. HRMS (ESI): *m/z* [M+H]⁺ calcd for C₁₃H₁₄NO: 200.1070, found: 200.1068.

General Procedure for the Protection of Amines with Fmoc-OSu (procedure A)

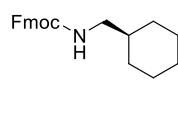
Sodium bicarbonate (2 equiv) and the amine were dissolved in water. If the amine was not soluble, ACN was added dropwise until all solids were dissolved. The solution was cooled with an ice-water bath and a solution of Fmoc-OSu (1.2 equiv) in ACN was added dropwise. After 20 minutes the mixture was allowed to warm to room temperature and was stirred overnight. The solvent was then removed *in vacuo* and the resulting suspension was extracted 3 times with ethyl acetate. The organic layer was back extracted twice with saturated sodium bicarbonate solution. The combined aqueous layers were acidified to a pH of 1 – 3 with a 1 N HCl solution. The resulting suspension was extracted 3 times with ethyl acetate and the combined organic layers were washed with 1 N HCl and water. The organic layer was dried over anhydrous MgSO₄ and the solvent was removed *in vacuo*. The protected amine was purified by flash chromatography if necessary.

1-(((9H-Fluoren-9-yl)methoxy)carbonyl)piperidine-4-carboxylic acid (143)



According to the general procedure for the protection of amines (*procedure A*), piperidine-4-carboxylic acid (408 mg, 3.2 mmol) was reacted with sodium bicarbonate (533 mg, 6.3 mmol) and Fmoc-OSu (1281 mg, 3.8 mmol) resulting in a white solid (1099 mg, 99% yield). ¹H NMR (300 MHz, CDCl₃) δ 7.77 (d, *J* = 7.4 Hz, 2H), 7.58 (d, *J* = 7.5 Hz, 2H), 7.40 (t, *J* = 7.2 Hz, 2H), 7.32 (td, *J* = 7.4, 1.1 Hz, 2H), 4.45 (d, *J* = 6.5 Hz, 2H), 4.25 (t, *J* = 6.7 Hz, 1H), 4.14–3.87 (m, 2H), 3.03–2.87 (m, 2H), 2.58–2.46 (m, 1H), 2.00–1.83 (m, 2H), 1.72–1.54 (m, 2H). HRMS (ESI): *m/z* [M–H][–] calcd for C₂₁H₂₀NO₄: 350.1398, found: 350.1402.

(1*r*,4*r*)-4-(((9H-Fluoren-9-yl)methoxy)carbonylamino)methyl)cyclohexanecarboxylic acid (144)



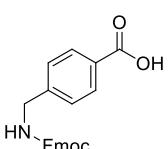
According to the general procedure for the protection of amines (*procedure A*), Tranexamic acid (512 mg, 3.3 mmol) was reacted with sodium bicarbonate (544 mg, 6.5 mmol) and Fmoc-OSu (1178 mg, 3.9 mmol) resulting in a white solid (1174 mg, 95% yield). ¹H NMR (300 MHz, CDCl₃) δ 7.77 (d, *J* = 7.4 Hz, 2H), 7.59 (d, *J* = 7.5 Hz, 2H), 7.40 (t, *J* = 7.2 Hz, 2H), 7.32 (t, *J* = 7.5 Hz, 2H), 4.44 (d, *J* = 6.7 Hz, 2H), 4.21 (t, *J* = 6.5 Hz, 1H), 3.05 (t, *J* = 6.6 Hz, 2H), 2.12–2.05 (m, 1H), 1.82 (d, *J* = 13.1 Hz, 2H), 1.54–1.33 (m, 4H), 1.29–1.22 (m, 2H), 1.05–0.87 (m, 2H). HRMS (ESI): *m/z* [M–H][–] calcd for C₂₃H₂₄NO₄: 378.1711, found: 378.1701.

3-((9*H*-Fluoren-9-yl)methoxy)carbonylamino)benzoic acid (145)



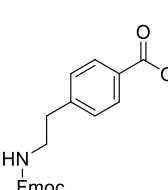
According to the general procedure for the protection of amines (*procedure A*), 3-aminobenzoic acid (1496 mg, 10.9 mmol) was reacted with sodium bicarbonate (1838 mg, 21.9 mmol) and Fmoc-OSu (4795 mg, 14.2 mmol) resulting in a white solid (3192 mg, 81% yield). ^1H NMR (300 MHz, DMSO-*d*₆) δ 12.89 (s, 1H), 9.91 (s, 1H), 8.13 (s, 1H), 7.91 (d, *J* = 7.4 Hz, 2H), 7.76 (d, *J* = 7.3 Hz, 2H), 7.68 (t, *J* = 6.9 Hz, 1H), 7.58 (d, *J* = 7.8 Hz, 1H), 7.49–7.30 (m, 5H), 4.50 (d, *J* = 6.7 Hz, 2H), 4.32 (t, *J* = 6.6 Hz, 1H). ^{13}C NMR (APT, 75 MHz, DMSO-*d*₆) δ 167.2, 153.4, 143.7, 140.8, 139.3, 131.4, 129.0, 127.7, 127.1, 126.8, 125.1, 123.3, 120.2, 119.0, 65.7, 46.6. HRMS (ESI): *m/z* [M–H][–] calcd for C₂₂H₁₆NO₄: 358.1085, found: 358.1084.

4-(((9*H*-Fluoren-9-yl)methoxy)carbonylamino)methyl)benzoic acid (146)



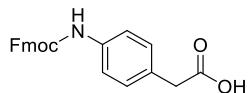
According to the general procedure for the protection of amines (*procedure A*), 4-(aminomethyl)benzoic acid (2502 mg, 16.5 mmol) was reacted with sodium bicarbonate (2782 mg, 33.1 mmol) and Fmoc-OSu (6699 mg, 19.8 mmol) resulting in a white solid (5700 mg, 92% yield). ^1H NMR (300 MHz, DMSO-*d*₆) δ 12.81 (br. s, 1H), 7.96–7.82 (m, 4H), 7.70 (d, *J* = 7.3 Hz, 2H), 7.47–7.28 (m, 6H), 7.26–7.11 (m, 1H), 4.38 (d, *J* = 6.5 Hz, 2H), 4.41–4.34 (m, 3H). HRMS (ESI): *m/z* [M–H][–] calcd for C₂₃H₁₈NO₄: 372.1241, found: 372.1264.

4-(2-((9*H*-Fluoren-9-yl)methoxy)carbonylamino)ethyl)benzoic acid (147)



According to the general procedure for the protection of amines (*procedure A*), 4-(2-aminoethyl)benzoic acid (622 mg, 3.1 mmol) was reacted with sodium bicarbonate (519 mg, 6.2 mmol) and Fmoc-OSu (1259 mg, 3.7 mmol) resulting in a white solid (1195 mg, 94% yield). ^1H NMR (300 MHz, DMSO-*d*₆) δ 12.75 (br. s, 1H), 7.91–7.81 (m, 4H), 7.65 (d, *J* = 7.4 Hz, 2H), 7.45–7.27 (m, 6H), 7.10–7.00 (m, 1H), 4.29 (d, *J* = 6.6 Hz, 2H), 4.23–4.15 (m, 1H), 3.28–3.20 (m, 2H), 2.83–2.71 (m, 2H). HRMS (ESI): *m/z* [M–H][–] calcd for C₂₄H₂₀NO₄: 386.1398, found: 386.1400.

2-(4-((9*H*-Fluoren-9-yl)methoxy)carbonylamino)phenyl)acetic acid (148)

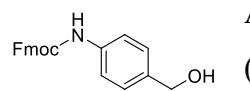


According to the general procedure for the protection of amines (*procedure A*), 2-(4-aminophenyl)acetic acid (497 mg, 3.3 mmol) was reacted with sodium bicarbonate (550 mg, 6.6 mmol) and Fmoc-OSu (1332 mg, 3.9 mmol) resulting in a white solid (1153 mg, 94% yield). ^1H NMR (300 MHz, DMSO-*d*₆) δ 12.23 (br. s, 1H), 9.65 (s, 1H), 7.92 (d, *J* = 7.3 Hz, 2H), 7.76 (d, *J* = 7.5 Hz, 2H), 7.49–7.29 (m, 6H), 7.15 (d, *J* = 8.0 Hz, 2H), 6.77 (s, 1H), 4.48 (d, *J* = 6.5 Hz, 2H), 4.31 (t, *J* = 6.5 Hz, 2 H), 3.48 (s, 2H). ^{13}C NMR (APT, 75 MHz, DMSO-*d*₆) δ 173.3, 153.9, 144.2, 141.2, 138.0, 130.1, 129.5, 128.1, 127.6, 125.6, 120.6, 118.7, 66.0, 47.1, 40.5. HRMS (ESI): *m/z* [M–H][–] calcd for C₂₃H₁₈NO₄: 372.1241, found: 372.1274.

General Procedure for the Protection of Amines with Fmoc-OSu (*procedure B*)

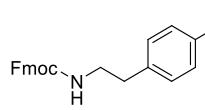
Sodium bicarbonate (2 equiv) and the amine were dissolved in H₂O/ACN (1:1, v/v). The solution was cooled with an ice-water bath and a solution of Fmoc-OSu (1.0 equiv) in ACN was added dropwise. After 20 minutes this mixture was allowed to warm to room temperature and was stirred overnight. The solvent was removed *in vacuo* and the resulting suspension was acidified to a pH of 1 – 3 with a 1 N HCl. This mixture was extracted 3 times with ethyl acetate or DCM and the combined organic layers were washed 2 times with 1 N HCl and water. The organic layer was dried over anhydrous MgSO₄ and the solvent was removed *in vacuo*. The protected amine was purified by flash chromatography if necessary.

(9*H*-Fluoren-9-yl)methyl 4-(hydroxymethyl)phenylcarbamate (149)



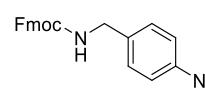
According to the general procedure for the protection of amines (*procedure B*), (4-aminophenyl)methanol (511 mg, 4.1 mmol) was reacted with sodium bicarbonate (695 mg, 8.3 mmol) and Fmoc-OSu (1403 mg, 4.1 mmol) resulting in a pale yellow solid (1421 mg, 99% yield). ^1H NMR (300 MHz, CDCl₃) δ 7.79 (d, *J* = 7.4 Hz, 2H), 7.62 (d, *J* = 7.8 Hz, 2H), 7.42 (t, *J* = 7.2 Hz, 2H), 7.37–7.28 (m, 6H), 6.67 (br. s, 1H), 4.65 (s, 2H), 4.56 (d, *J* = 6.6 Hz, 2H), 4.28 (t, *J* = 6.5 Hz, 1H), 1.58 (s, 1H). HRMS (ESI): *m/z* [M+H]⁺ calcd for C₂₂H₂₀NO₃: 368.1257, found: 368.1255.

(9H-Fluoren-9-yl)methyl 4-hydroxyphenethylcarbamate (150)



According to the general procedure for the protection of amines (*procedure B*), tyramine hydrochloride (398 mg, 2.3 mmol) was reacted with sodium bicarbonate (385 mg, 4.6 mmol) and Fmoc-OSu (773 mg, 2.3 mmol) resulting in a white solid (687 mg, 83% yield). ^1H NMR (300 MHz, DMSO-*d*₆) δ 7.92–7.81 (m, 2H), 7.67 (d, *J* = 7.3 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.32 (t, *J* = 7.3 Hz, 2H), 6.97 (d, *J* = 8.1 Hz, 2H), 6.84–6.71 (m, 1H), 6.66 (d, *J* = 8.2 Hz, 2H), 4.29 (d, *J* = 6.6 Hz, 2H), 4.25–4.15 (m, 1H), 3.19–3.08 (m, 2H), 2.59 (t, *J* = 7.4 Hz, 2H). HRMS (ESI): *m/z* [M+H]⁺ calcd for C₂₃H₂₂NO₃: 382.1414, found: 382.1408.

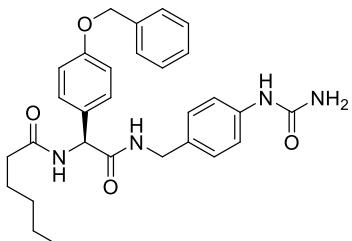
(9H-Fluoren-9-yl)methyl 4-aminobenzylcarbamate (151)



151 was synthesized according to a previously published method.²⁴ In short triethylamine (0.970 mL, 7.0 mmol) and DMF (1 mL) were added to a solution of 4-(aminomethyl)aniline (853 mg, 7.0 mmol) in ACN under nitrogen atmosphere. Fmoc-OSu (2356 mg, 7.0 mmol) was added dropwise as a solution in ACN. After 1 hour the resulting suspension was concentrated *in vacuo* and extracted with ethyl acetate. The resulting solution was washed with water and brine, dried over anhydrous MgSO₄ and concentrated. The resulting residue was purified by flash chromatography on silica gel to yield a white solid (2100 mg, 88% yield). ^1H NMR (300 MHz, acetone-*d*₆) δ 7.85 (d, *J* = 7.6 Hz, 2H), 7.70 (d, *J* = 7.3 Hz, 2H), 7.40 (t, *J* = 7.2 Hz, 2H), 7.36–7.26 (m, 2H), 7.01 (d, *J* = 8.2 Hz, 2H), 6.72 (br. s, 1H), 6.61 (d, *J* = 8.4 Hz, 2H), 4.52 (s, 2H), 4.34 (d, *J* = 7.2 Hz, 2H), 4.24 (d, *J* = 6.9 Hz, 1H), 4.18 (d, *J* = 6.1 Hz, 2H). HRMS (ESI): *m/z* [M+H]⁺ calcd for C₂₂H₂₁N₂O₂: 345.1598, found: 345.1596.

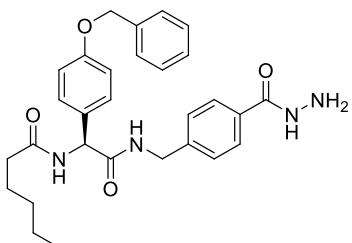
Synthesis and Analytical Data of Inhibitors

(*S*)-*N*-(1-(4-(Benzylxy)phenyl)-2-oxo-2-(4-ureidobenzylamino)ethyl)hexanamide (**22**).



Synthesis of compound **22** was described before.⁹ To a solution of acetic acid (1 mL) in water (0.250 mL) was added compound **101** (14 mg, 0.02 mmol). To this mixture a solution of sodium cyanate (3 mg, 0.05 mmol) in water was added. A grey precipitate was formed and the suspension was stirred overnight. The mixture was cooled with an ice-water bath and filtered. The collected solid was washed several times with cold water and then dried *in vacuo*. The solid was further purified with reversed phase liquid chromatography and freeze-dried in H₂O/ACN to afford a white powder (10 mg, 83% yield). ¹H NMR (300 MHz, acetone-*d*₆) δ 8.04 (br. s, 1H), 7.71 (t, *J* = 6.1 Hz, 1H), 7.55–7.43 (m, 3H), 7.44–7.29 (m, 7H), 7.06 (d, *J* = 8.4 Hz, 2H), 6.95 (d, *J* = 8.7 Hz, 2H), 5.52–5.46 (m, 1H), 5.40 (br. s, 2H), 5.11 (s, 2H), 4.30 (d, *J* = 5.9 Hz, 2H), 2.25 (t, *J* = 7.5 Hz, 2H), 1.64–1.51 (m, 2H), 1.35–1.21 (m, 4H), 0.86 (t, *J* = 6.8 Hz, 3H). HRMS (ESI): *m/z* [M+H]⁺ calcd for C₂₉H₃₅N₄O₄: 503.2653, found: 503.2649.

(*S*)-*N*-(1-(4-(Benzylxy)phenyl)-2-(4-(hydrazinecarbonyl)benzylamino)-2-oxoethyl)hexanamide (**23**).

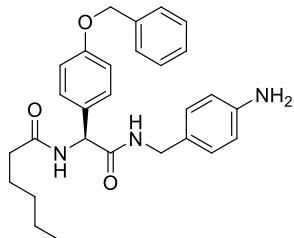


Synthesis of compound **23** was described before.⁹ **25** (28 mg, 0.06 mmol) was dissolved in one drop of dry THF under nitrogen atmosphere. To this solution carbonyldiimidazole (14 mg, 0.09 mmol) in dry THF (0.5 mL) was added. The mixture was stirred for 2 hours at room temperature and hydrazine monohydrate (7 mg, 21 mmol) in dry THF (0.5 mL) was added. The mixture was further stirred for 5 hours and the solvent was removed under reduced pressure. The resulting residue was dissolved in ethyl acetate and was washed 2 times with 0.05 N NaOH and water. The

organic phase was concentrated *in vacuo*, filtered over anhydrous MgSO₄ and was further purified with reversed phase liquid chromatography. The purified product was re-dissolved in H₂O/ACN and freeze-dried to yield a white powder (11 mg, 38% yield). ¹H NMR (300 MHz, acetone-*d*₆) δ 7.93 (t, *J* = 5.9 Hz, 1H), 7.75 (d, *J* = 6.9 Hz, 2H), 7.54 (d, *J* = 7.9 Hz, 1H), 7.47 (d, *J* = 7.1 Hz, 2H), 7.43–7.22 (m, 7H), 6.98 (d, *J* = 8.6 Hz, 2H), 5.50 (d, *J* = 7.4 Hz, 1H), 5.12 (s, 2H), 4.45 (d, *J* = 6.1 Hz, 2H), 2.26 (t, *J* = 7.4 Hz, 2H), 1.64–1.51 (m, 2H), 1.33–1.22 (m, 4H), 0.86 (t, *J* = 6.9 Hz, 3H). HRMS (ESI): *m/z* [M+H]⁺ calcd for C₂₉H₃₅N₄O₄: 503.2653, found: 503.2656.

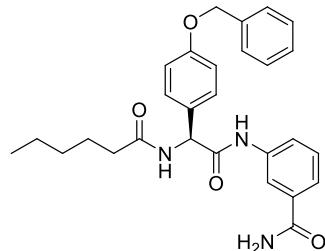
The inhibitors / intermediates listed below were obtained with solid-phase synthesis according to the Fmoc protocol as described in the main part. Synthesis of compound **101** on solid support was described before.⁹

(*S*)-*N*-(2-(4-Aminobenzylamino)-1-(4-(benzyloxy)phenyl)-2-oxoethyl)hexanamide (**101**)



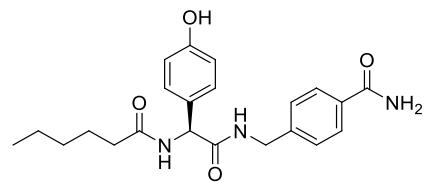
¹H NMR (300 MHz, acetone-*d*₆) δ 7.98 (t, *J* = 5.3 Hz, 1H), 7.74–4.63 (m, 1H), 7.47 (d, *J* = 7.3 Hz, 2H), 7.43–7.27 (m, 7H), 7.11 (d, *J* = 8.2 Hz, 2H), 6.96 (d, *J* = 8.3 Hz, 2H), 5.51 (d, *J* = 5.2 Hz, 1H), 5.11 (s, 2H), 4.42 (s, 2H), 2.26 (t, *J* = 7.4 Hz, 2H), 1.64–1.51 (m, 2H), 1.36–1.21 (m, 4H), 0.85 (t, *J* = 6.5 Hz, 3H). HRMS (ESI): *m/z* [M+H]⁺ calcd for C₂₈H₃₄N₃O₃: 460.2595, found: 460.2607.

(*S*)-3-(2-(4-(Benzylamino)phenyl)-2-hexanamidoacetamido)benzamide (**15**)



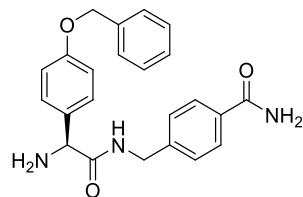
HRMS (ESI): *m/z* [M+H]⁺ calcd for C₂₈H₃₂N₃O₄: 474.2384, found: 474.2374.

(S)-4-((2-Hexanamido-2-(4-hydroxyphenyl)acetamido)methyl)benzamide (29)



HRMS (ESI): *m/z* [M+H]⁺ calcd for C₂₂H₂₈N₃O₄: 398.2074, found: 398.2077.

(S)-4-((2-Amino-2-(4-(benzyloxy)phenyl)acetamido)methyl)benzamide (40)



HRMS (ESI): *m/z* [M+H]⁺ calcd for C₂₃H₂₄N₃O₃: 390.1812, found: 390.1813.

HPLC Purity of Inhibitors

Purity of inhibitors was determined by HPLC on a Jasco HPLC system with a Jasco UV-2070 Plus Intelligent UV/VIS Detector on an RP-18 column (ReproSil-Pur-ODS-3, Dr. Maisch GmbH, Germany, 5 µm, 50 mm × 2 mm) using the following method: eluent A: water (0.1% TFA); eluent B: acetonitrile (0.1% TFA); injection volume: 10 µL; flow rate: 1 mL/min; and gradient: 1% B (0.2 min), 100% B (7 min), 100% B (8 min), 1% B (8.1 min), and 1% B (10 min). Chromatograms recorded at 254 nm were used for purity assessment.

Table S4. RP-HPLC data of compounds

Compound	HPLC t_R (min)	HPLC purity (254 nm)
1	4.72	90%
2	4.70	> 95%
3	5.11	> 95%
4	5.00	> 95%
5	5.05	> 95%
6	4.67	> 95%
7	5.02	> 95%
8	5.27	> 95%
9	4.38	> 95%
10	4.48	> 95%
11	4.19	> 95%
12	4.70	> 95%
13	4.18	89%
14	4.55	> 95%
15	4.35	> 95%
16	4.27	> 95%
17	4.29	92%
18	4.20	> 95%
19	4.09	> 95%
20	4.37	> 95%
21	3.93	> 95%
22	4.34	> 95%
23	4.29	> 95%
24	4.21	> 95%

Compound	HPLC t_R (min)	HPLC purity (254 nm)
25	4.32	> 95%
26	4.51	> 95%
27	3.64	> 95%
28	3.92	89%
29	3.29	92%
30	3.77	> 95%
31	4.24	> 95%
32	4.74	> 95%
33	4.54	> 95%
34	4.67	> 95%
35	4.10	> 95%
36	4.38	> 95%
37	4.55	> 95%
38	4.67	> 95%
39	4.04	> 95%
40	3.26	> 95%
41	4.07	> 95%
42	4.20	> 95%
43	4.59	> 95%
44	4.59	95%
45	4.77	91%
46	4.07	> 95%
47	3.78	> 95%
48	3.91	> 95%
49	3.47	> 95%
50	3.40	> 95%
51	4.18	> 95%
52	4.48	> 95%
53	3.76	> 95%
54	3.99	94%
55	4.69	> 95%
56	4.83	> 95%
57	4.71	> 95%
58	4.67	94%
59	4.61	> 95%

Compound	HPLC t_R (min)	HPLC purity (254 nm)
60	4.63	> 95%
61	4.75	93%
62	4.30	> 95%
63	3.66	94%
64	4.84	> 95%
65	4.47	> 95%
66	4.59	> 95%
67	4.53	> 95%
68	4.44	> 95%
69	4.38	> 95%
70	4.34	95%
71	4.89	> 95%
72	4.67	> 95%
73	4.68	> 95%
74	4.54	> 95%
75	4.44	> 95%
76	4.11	88%
77	4.29	> 95%
78	4.24	> 95%
79	3.62	> 95%
80	3.64	> 95%
81	4.80	> 95%
82	5.02	> 95%
83	4.47	> 95%
84	4.89	> 95%
85	4.16	> 95%
86	3.82	> 95%
87	3.38	> 95%
88	3.88	> 95%

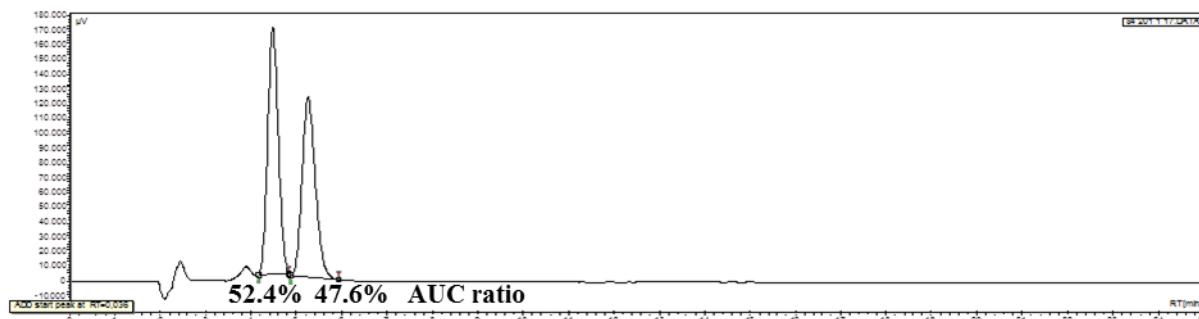
Enantiomeric Purity of Inhibitors

The enantiomeric purity HPLC analysis was carried out on a Jasco HPLC system equipped with a ReproSil Chiral-NR column ($8 \mu\text{m}$, $150 \times 4.6 \text{ mm}$) and a Jasco UV-2070 Plus Intelligent UV/VIS Detector. The following method was used: eluent A: water (0.1% TFA); eluent B: methanol (0.1% TFA); injection volume: $20 \mu\text{L}$; flow rate: 1 mL/min ; isocratic elution with 15% A and 85% B for 25 minutes. Chromatograms recorded at 254 nm were used for assessment.

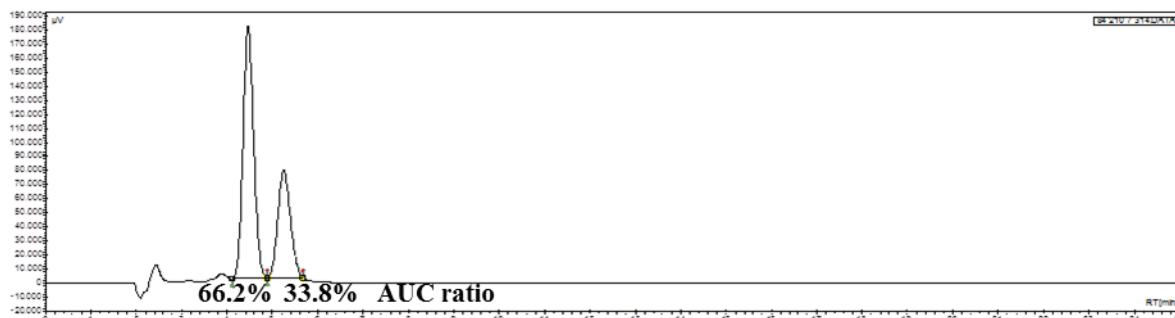
Exemplary Separation of Enantiomeric Compounds

The method for enantiomeric separation was developed and validated by using different mixtures of L-configured compound **18** and D-configured compound **31**. Furthermore a 1:2 mixture of L-configured compound **32** and D-configured compound **33** was separated.

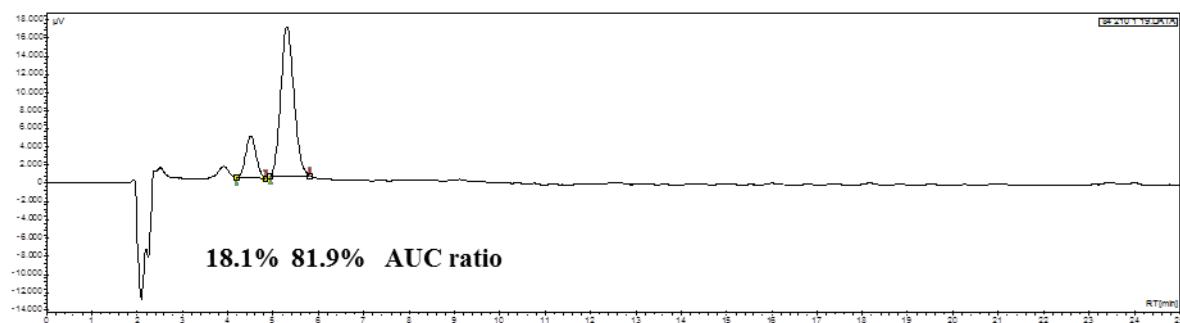
Compound **18** / **31** 1:1 mixture



Compound **18** / **31** 2:1 mixture



Compound 18 / 31 2:8 mixture



Compound 32 / 33 1:2 mixture

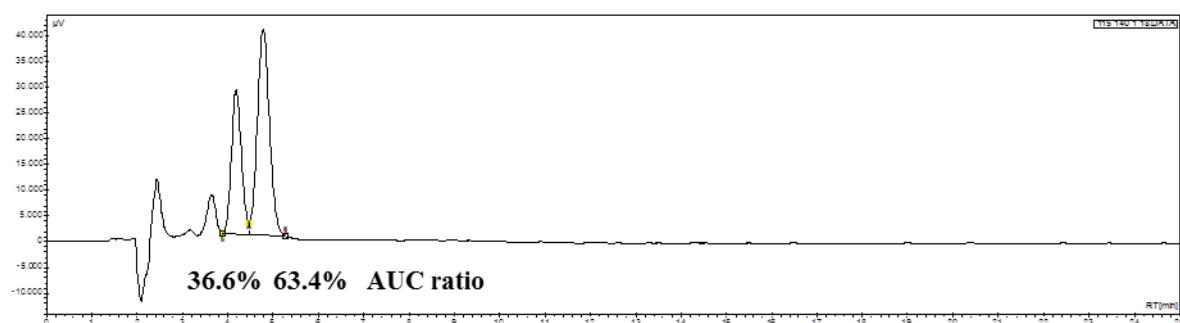
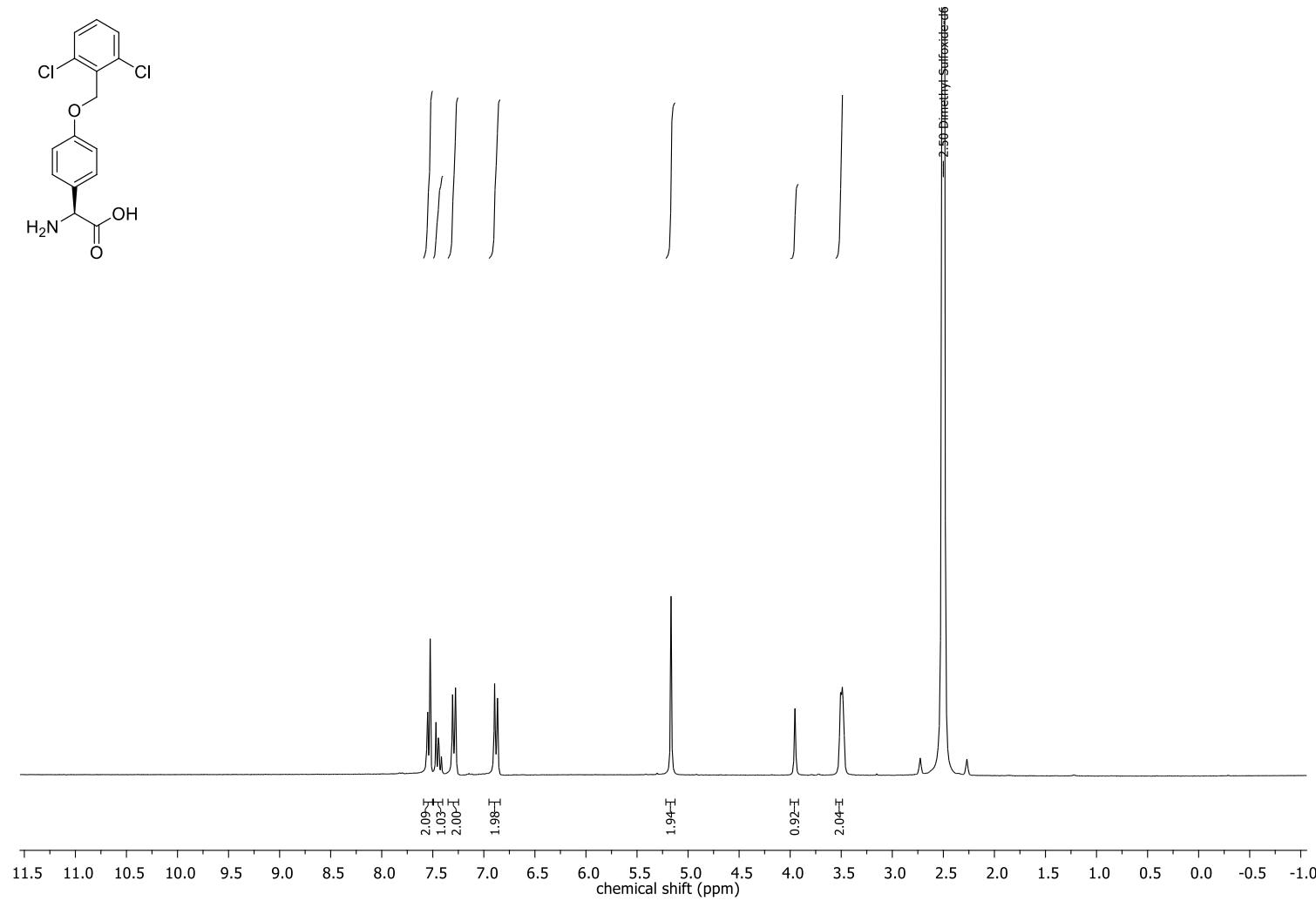


Table S5. Enantiomeric Purity of Selected Compounds

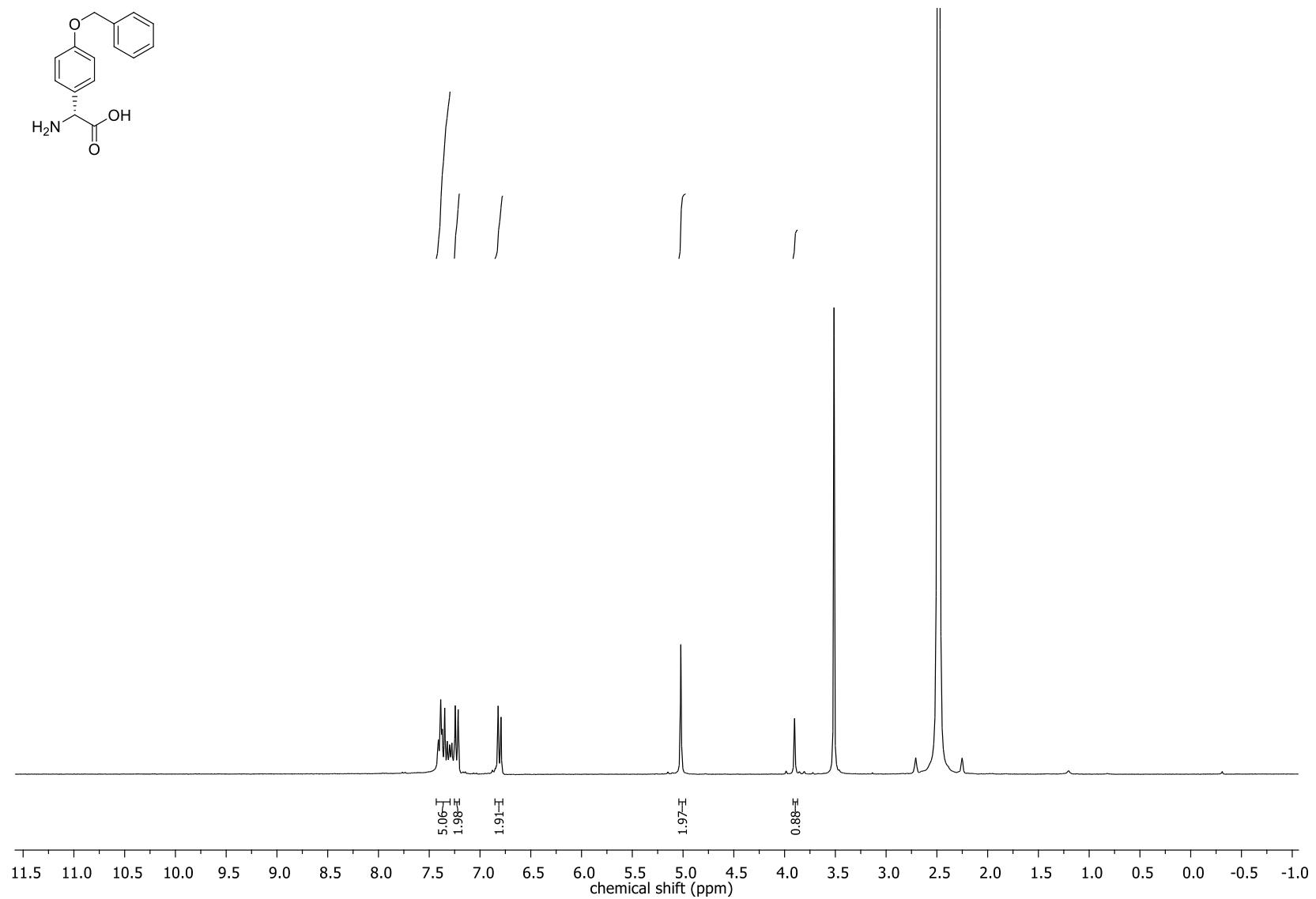
Compound	L-Enantiomer [%]	D-Enantiomer [%]
18	98.9	1.1
31	2.1	97.9
32	95.2	4.8
33	3.2	96.8
57	99.5	0.5
67	97.0	3.0
71	96.6	3.4
84	98.2	1.8
88	98.1	1.9

Selected ^1H and ^{13}C NMR Spectra

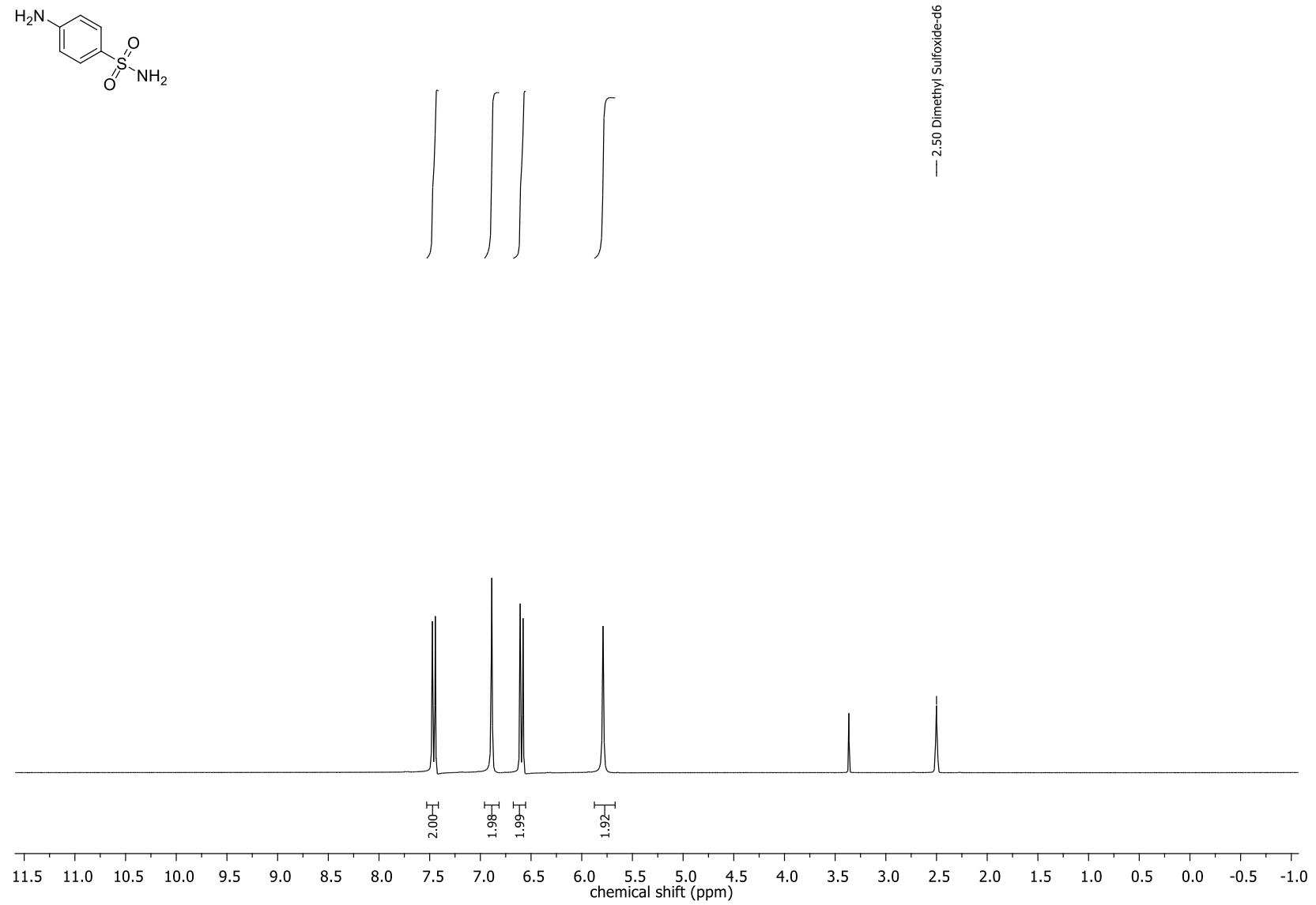
Compound **120**, ^1H NMR (300 MHz, DMSO-d₆/NaOD)



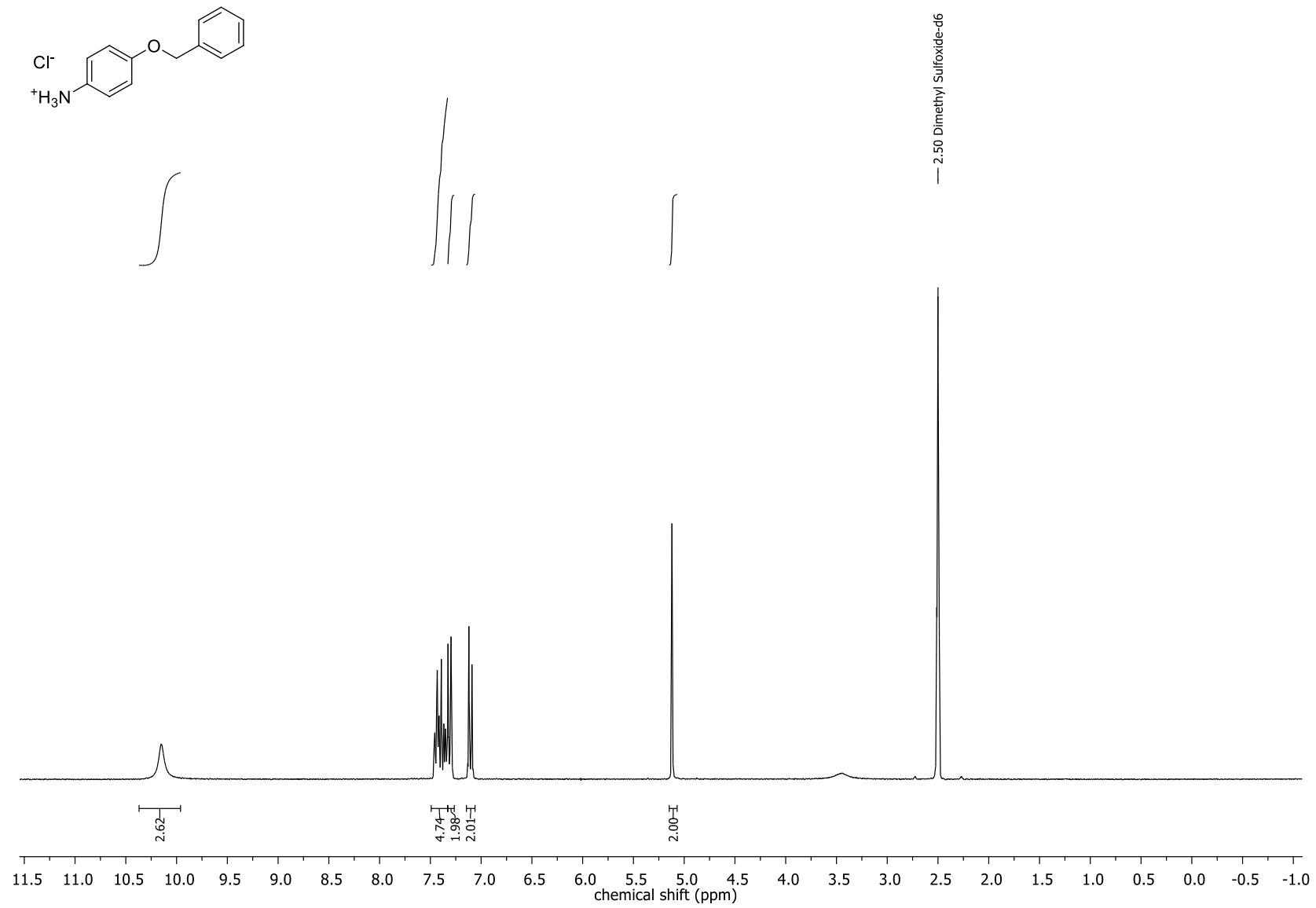
Compound **121**, ^1H NMR (300 MHz, DMSO-d₆/NaOD)



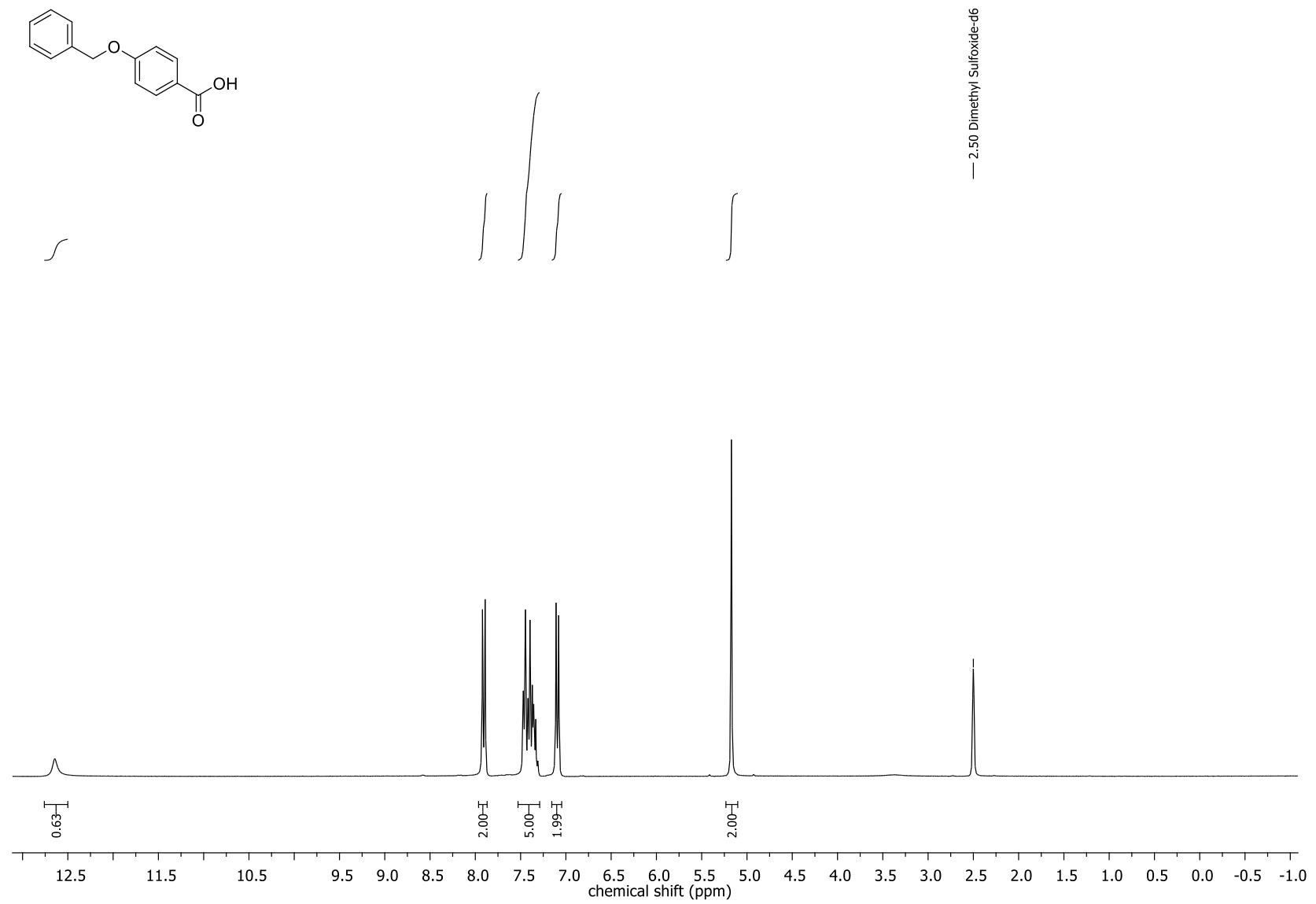
Compound **113**, ^1H NMR (300 MHz, DMSO- d_6)



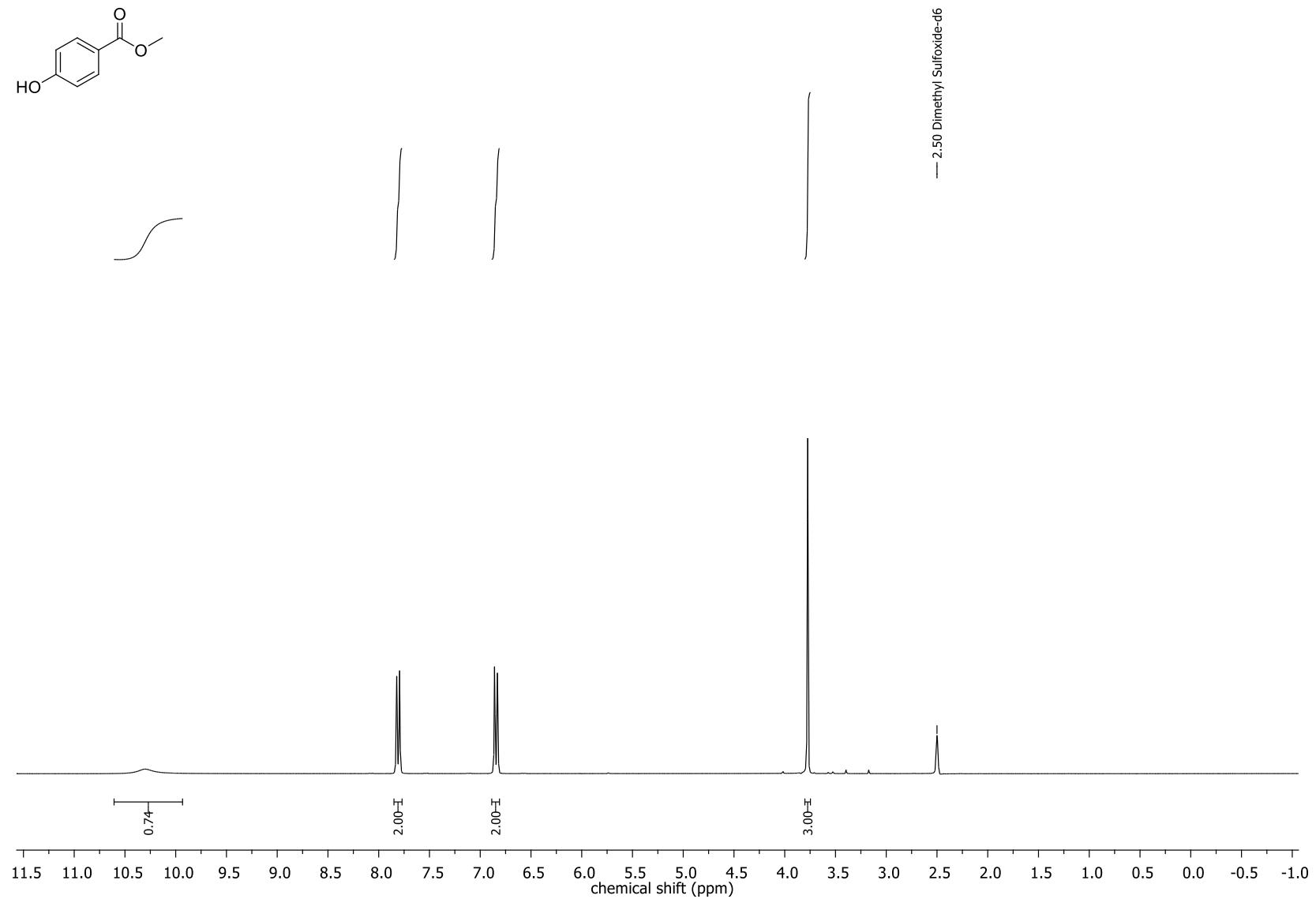
Compound **111**, ^1H NMR (300 MHz, DMSO- d_6)



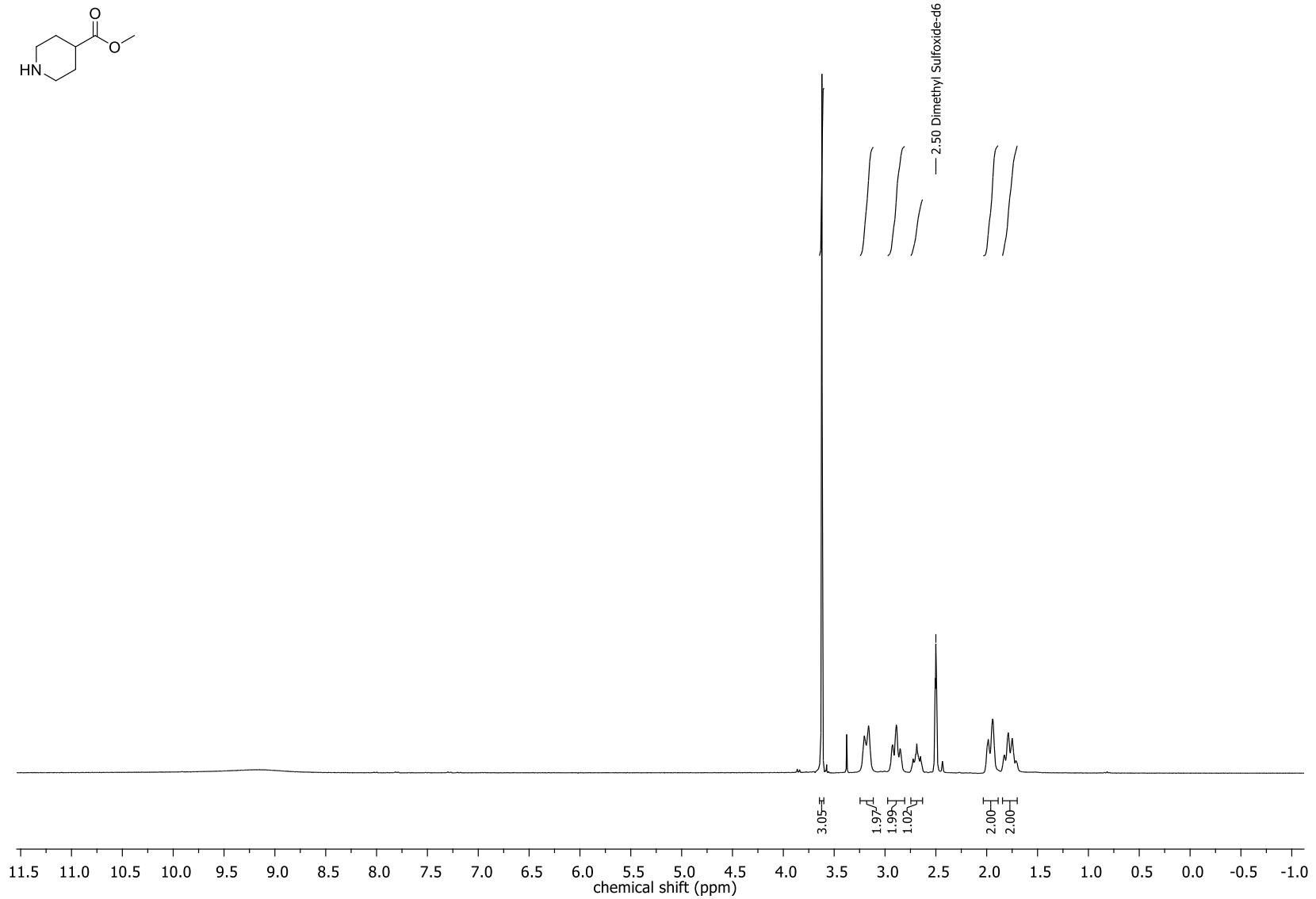
Compound **89**, ^1H NMR (300 MHz, DMSO- d_6)



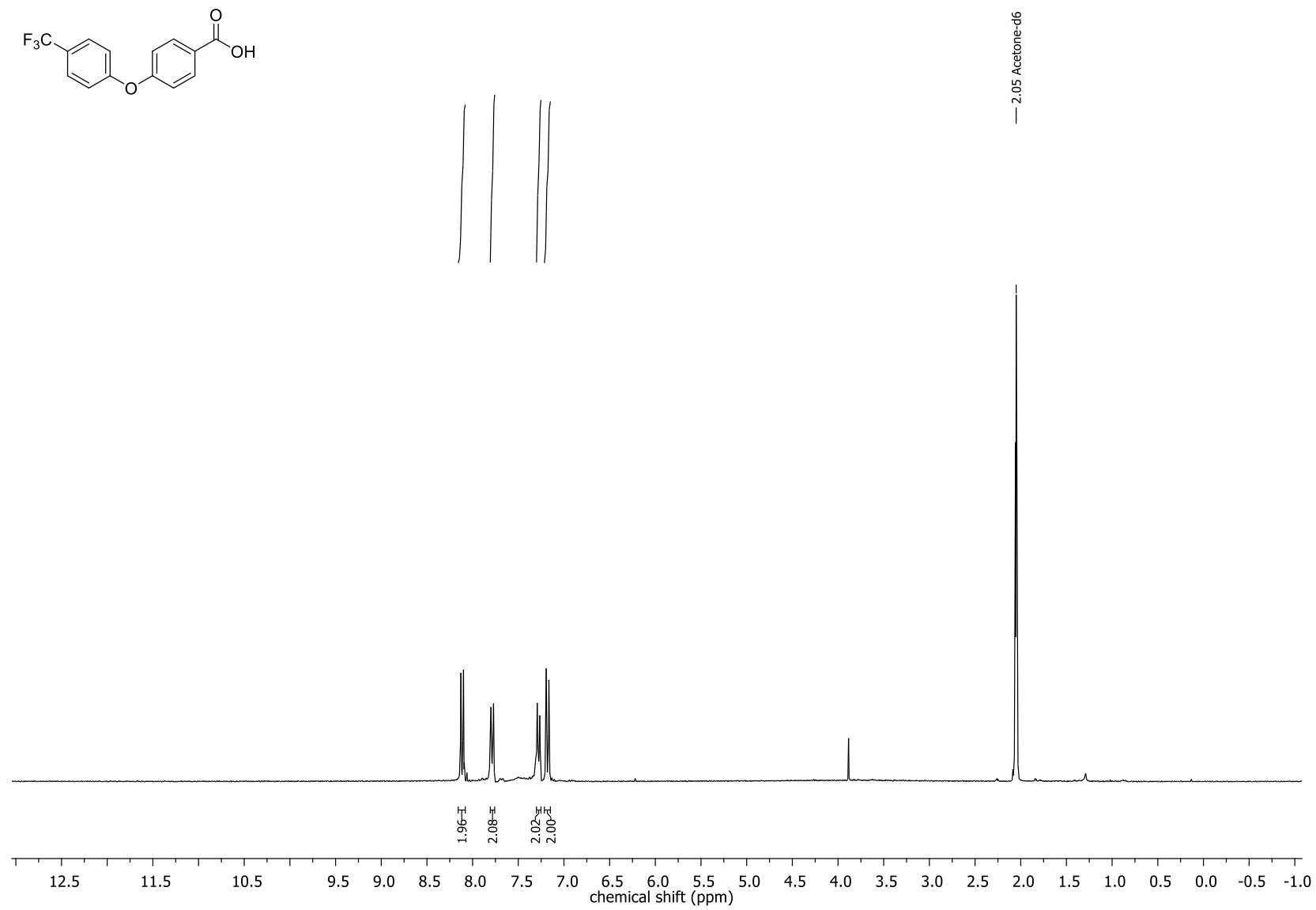
Compound **91**, ^1H NMR (300 MHz, DMSO- d_6)



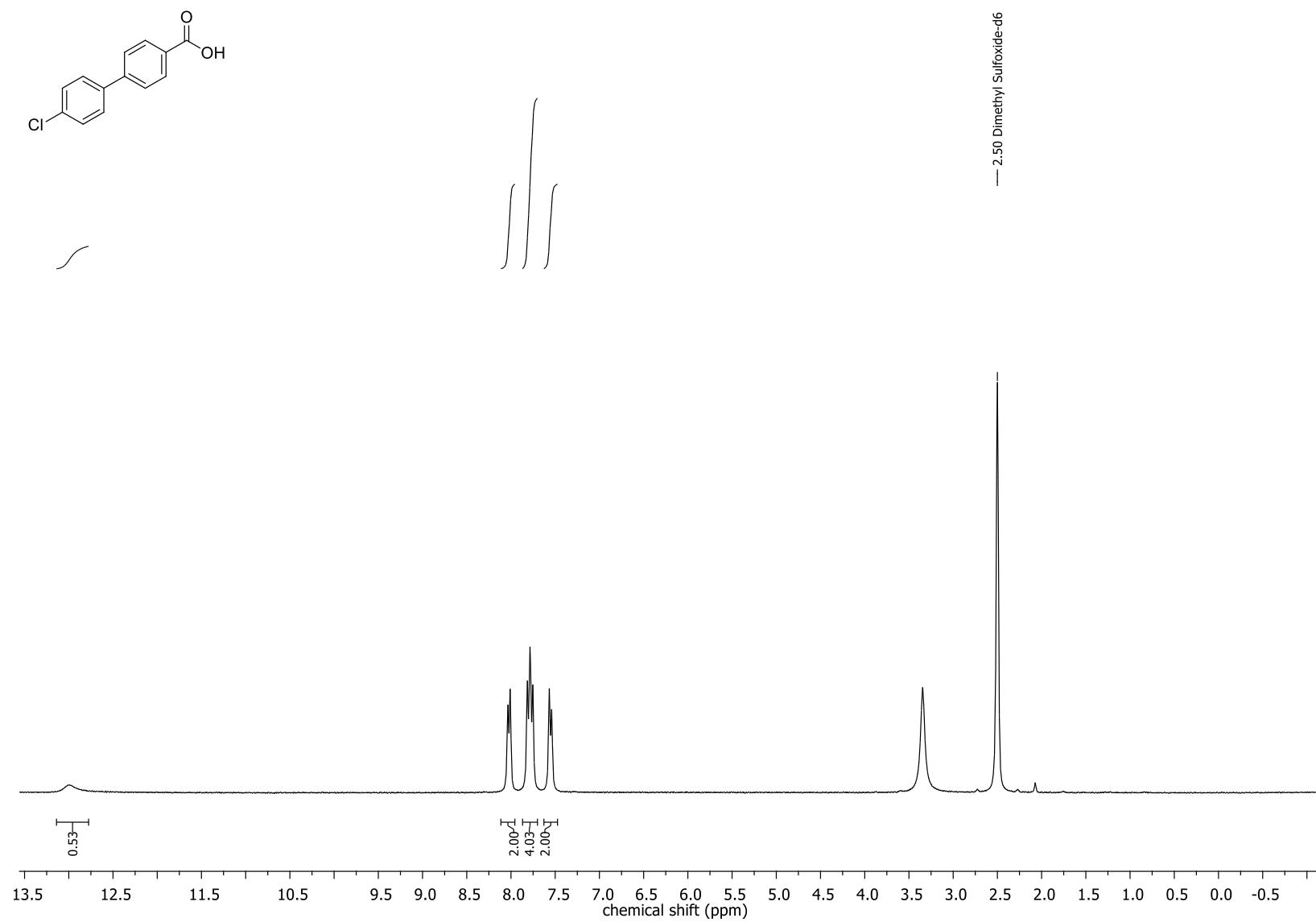
Compound **136**, ^1H NMR (300 MHz, DMSO- d_6)



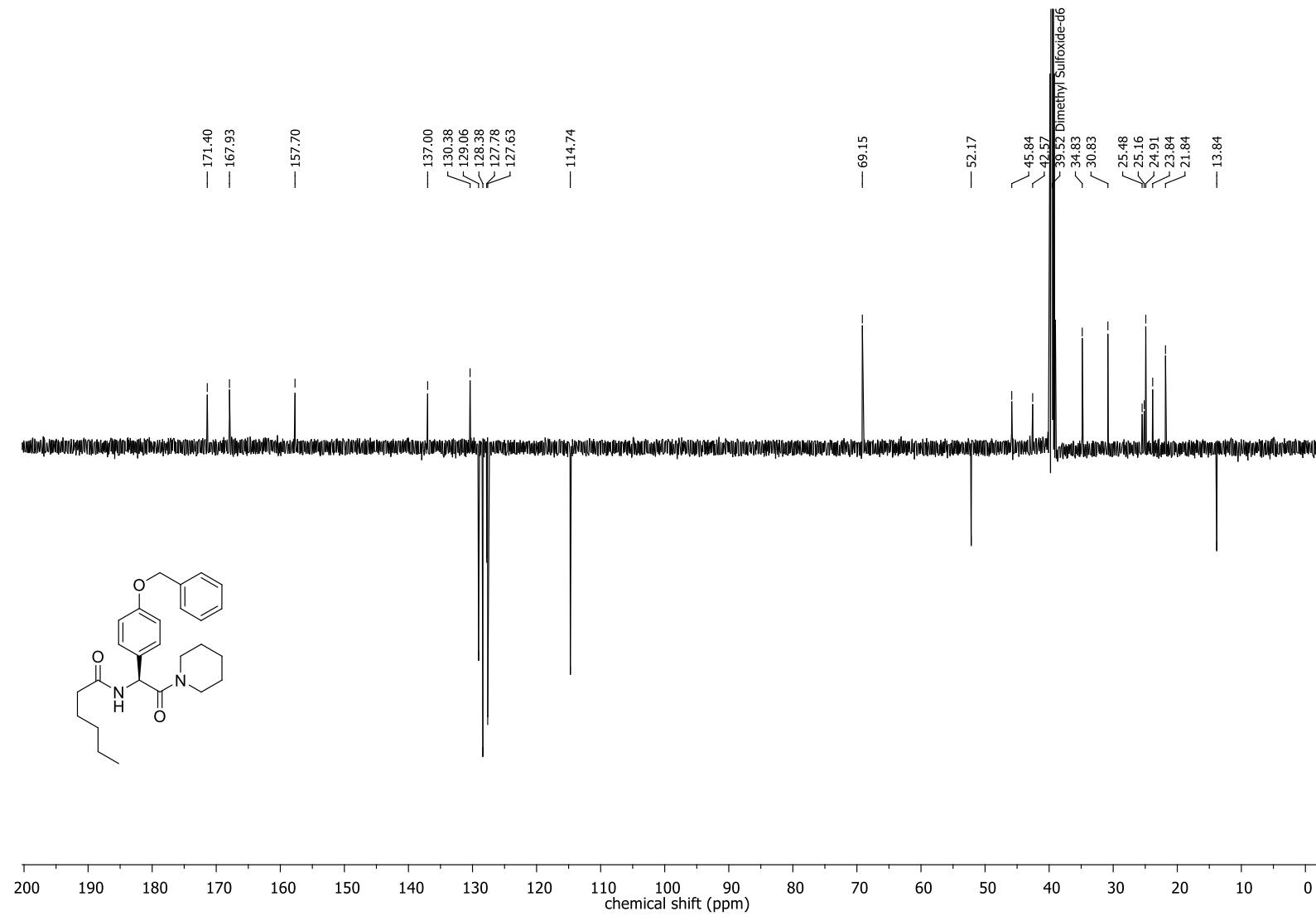
Compound 95, ^1H NMR (300 MHz, acetone- d_6)



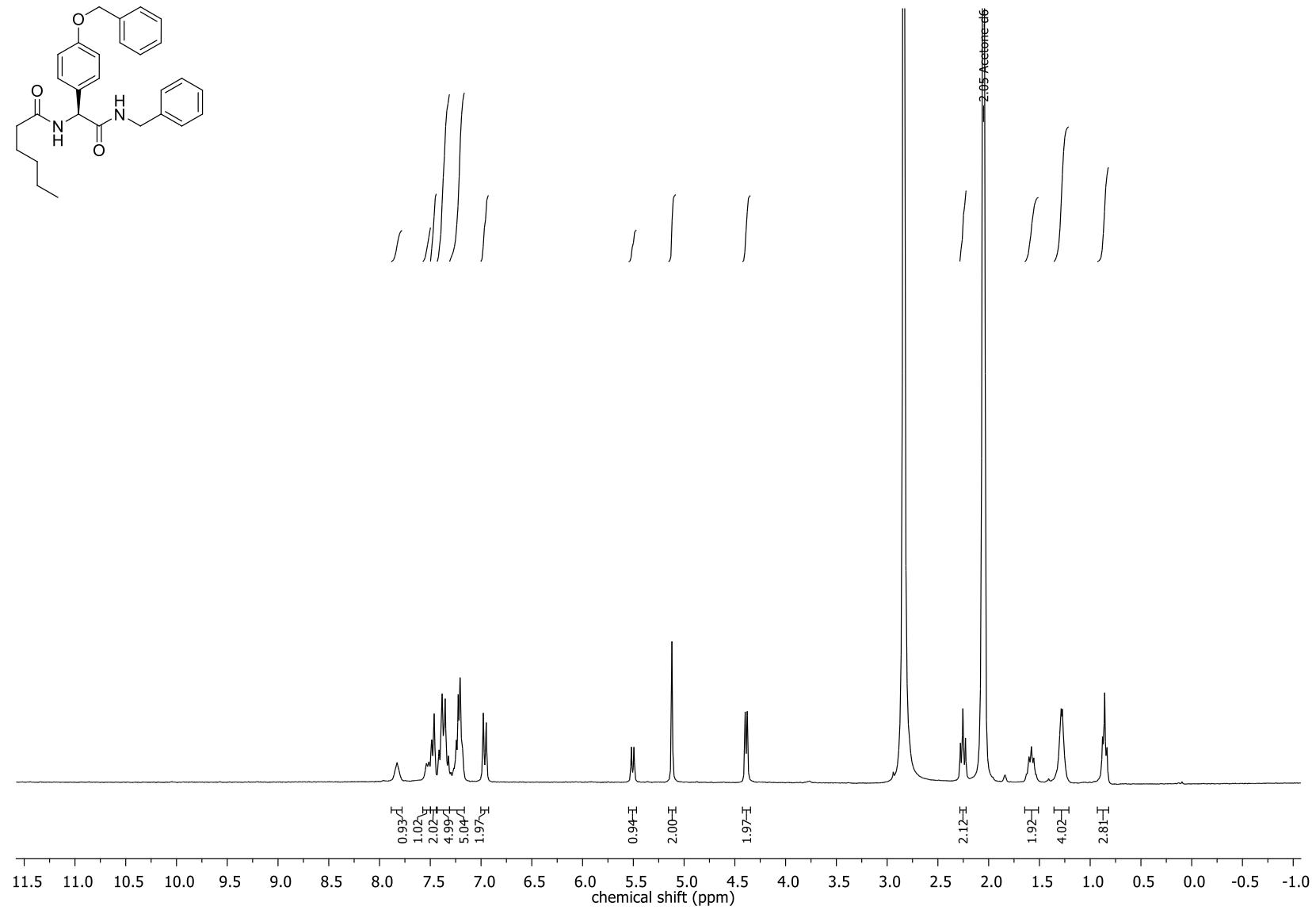
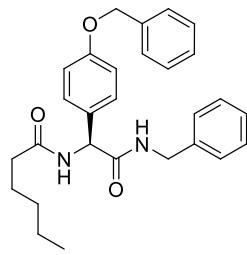
Compound **139**, ^1H NMR (300 MHz, DMSO- d_6)



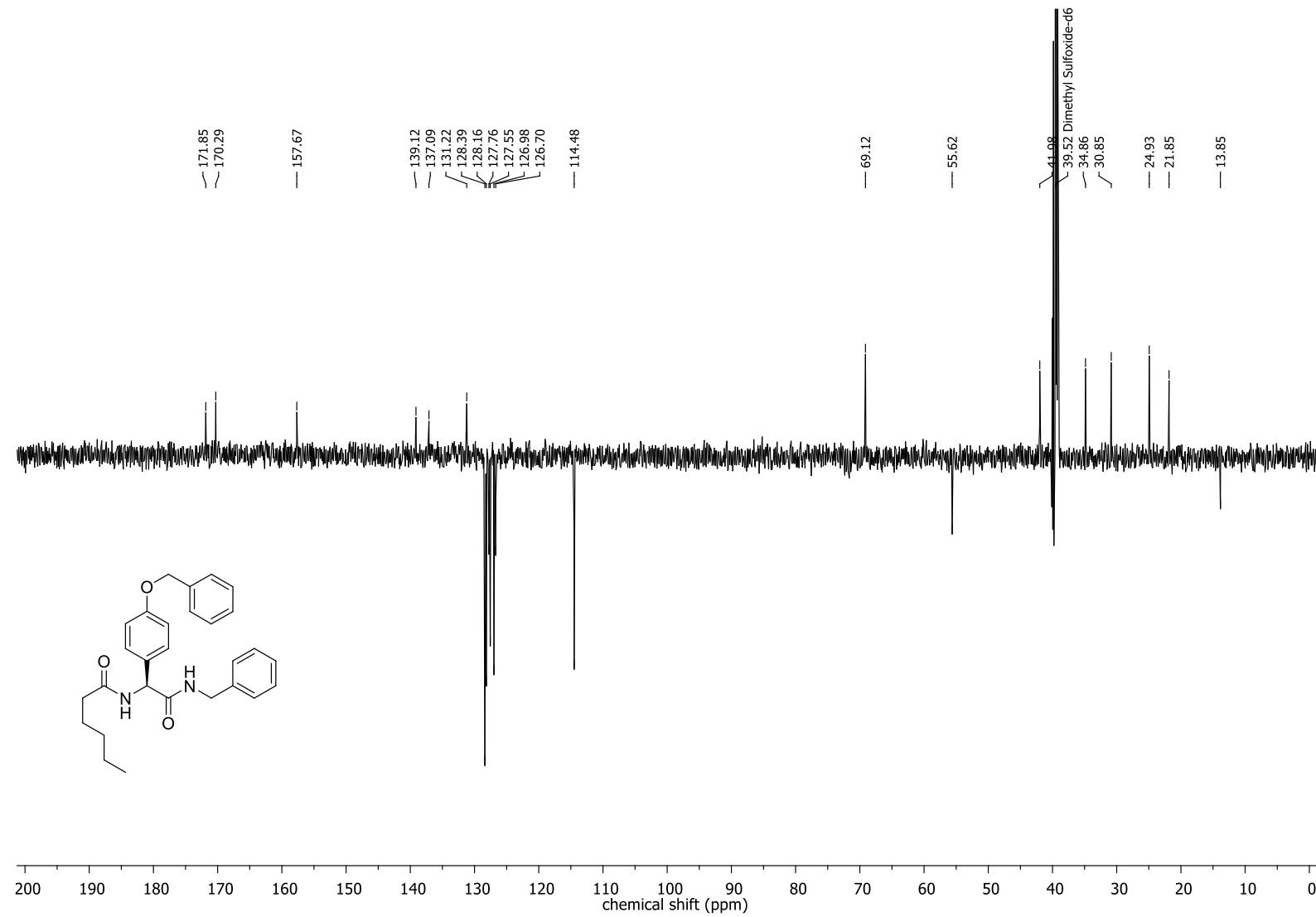
Compound 1, ^{13}C NMR (APT, 126 MHz, $\text{DMSO}-d_6$)



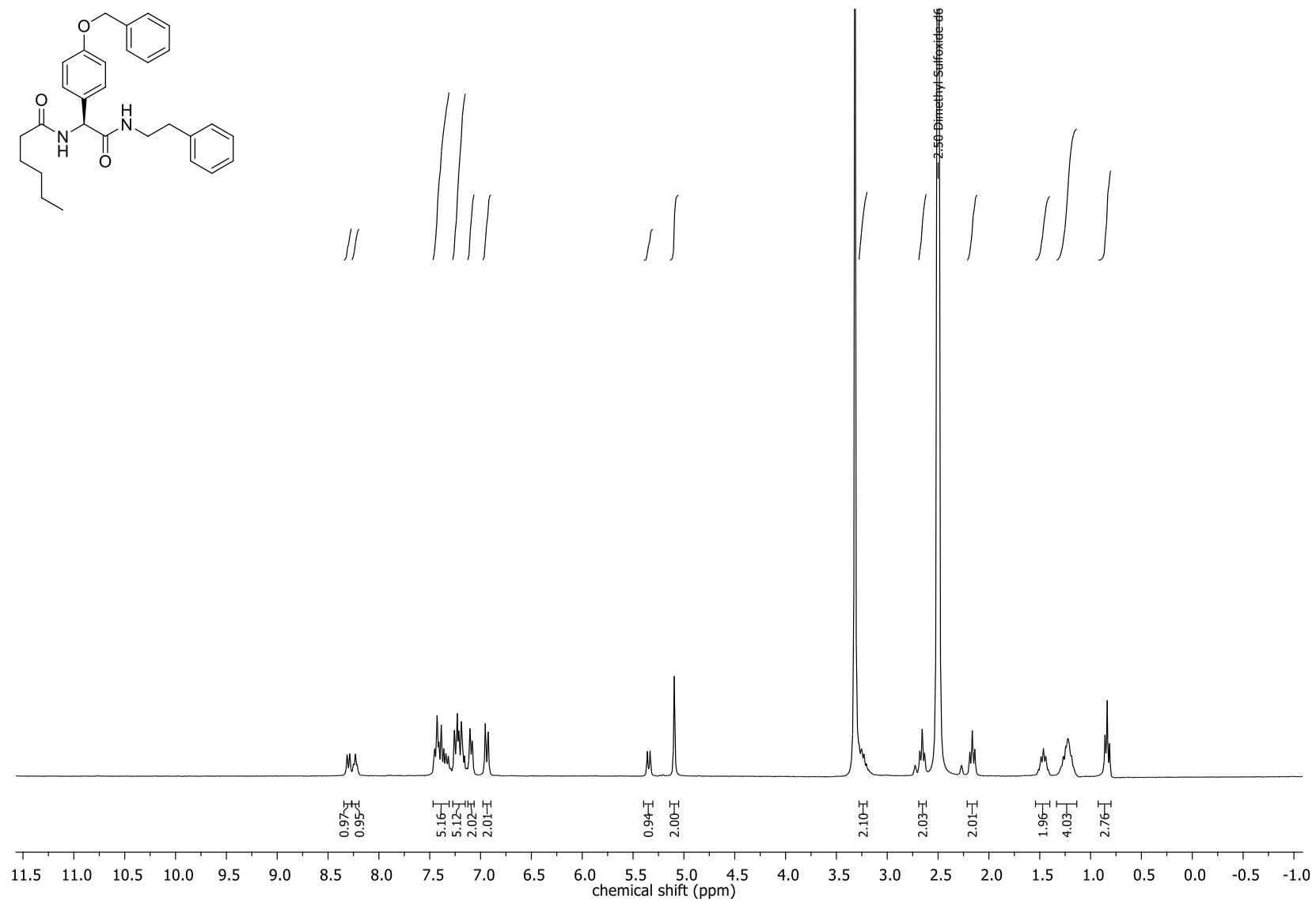
Compound 2, ^1H NMR (300 MHz, acetone- d_6)



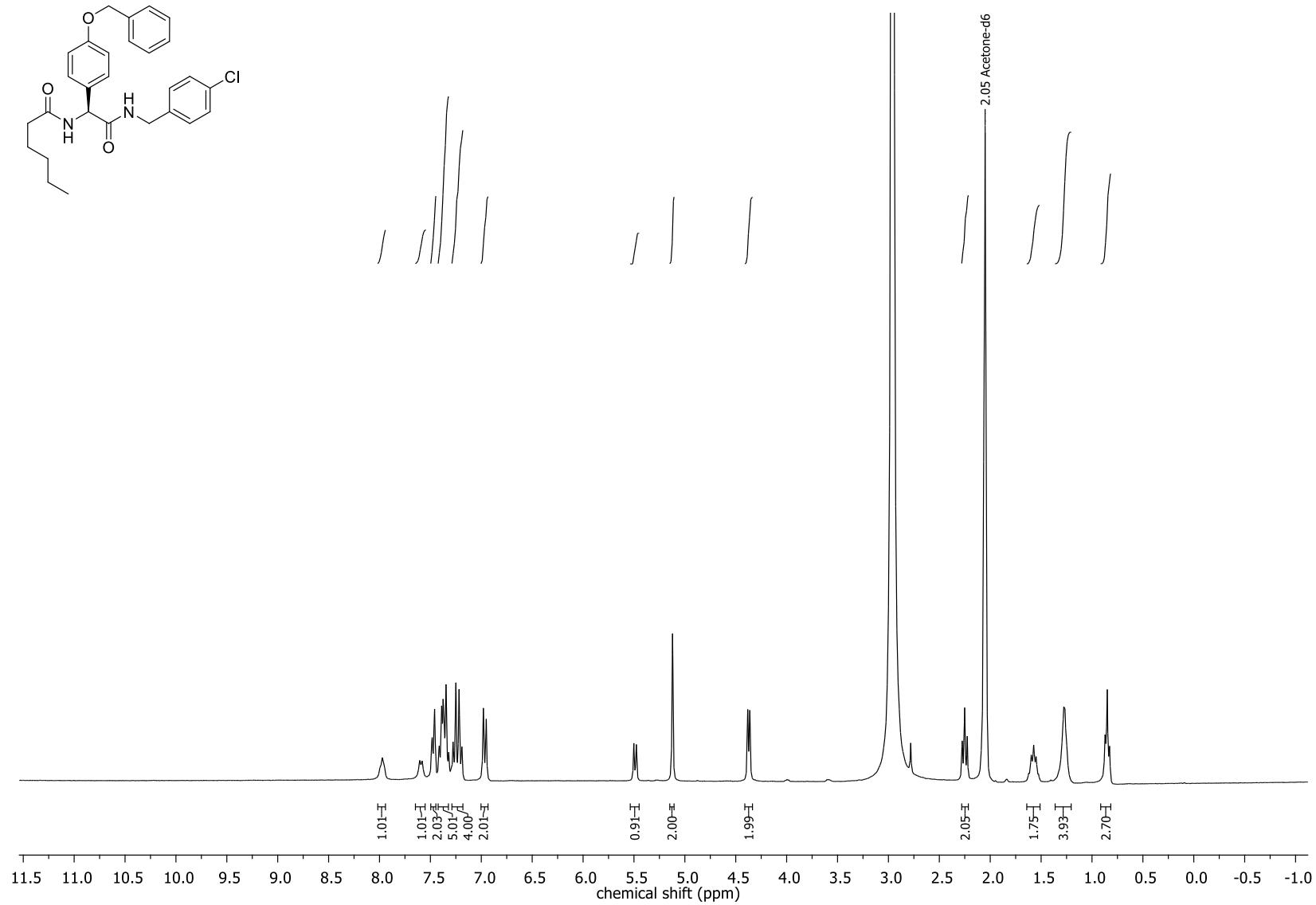
Compound 2, ^{13}C NMR (APT, 126 MHz, $\text{DMSO}-d_6$)



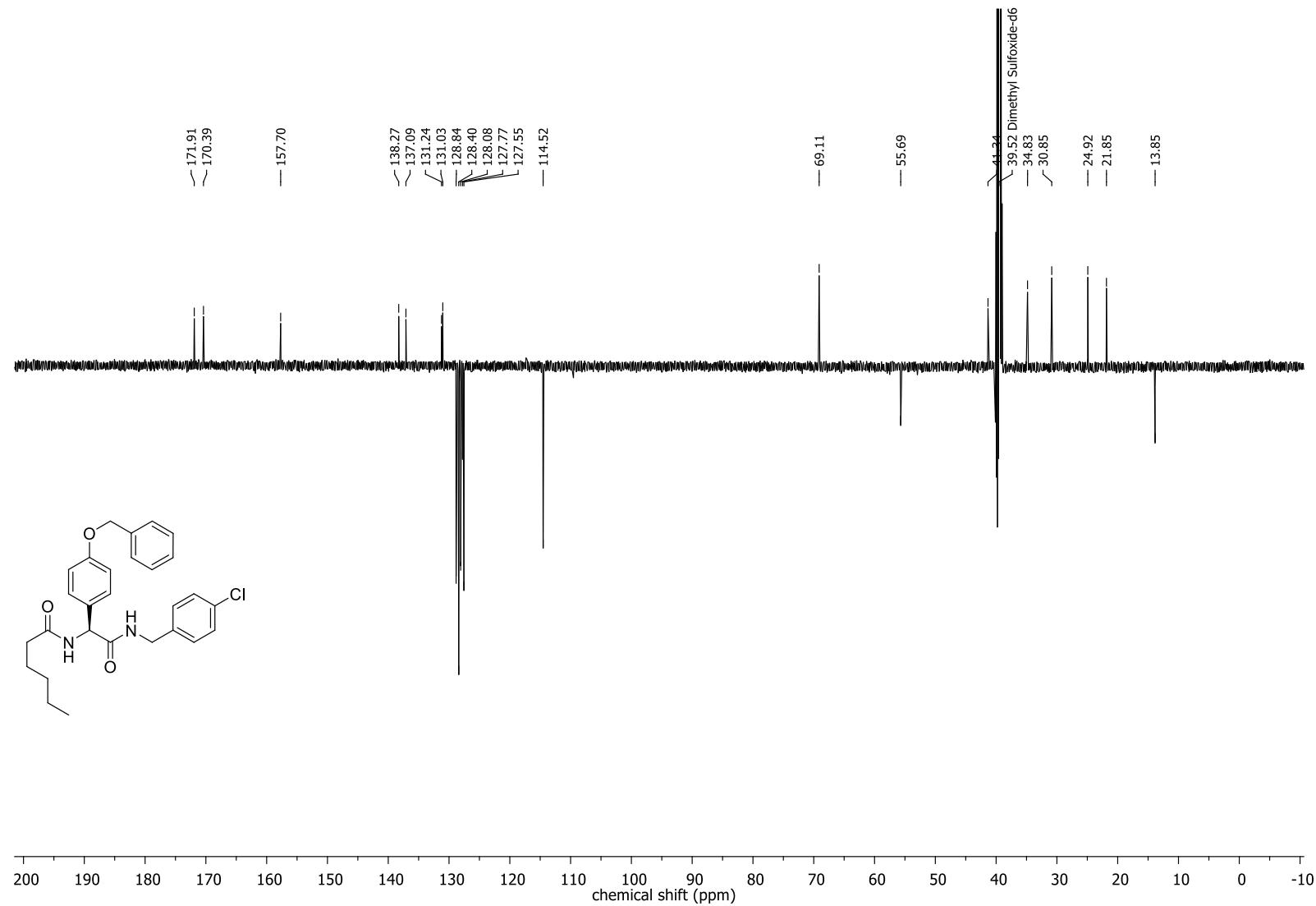
Compound 3, ^1H NMR (300 MHz, DMSO- d_6)



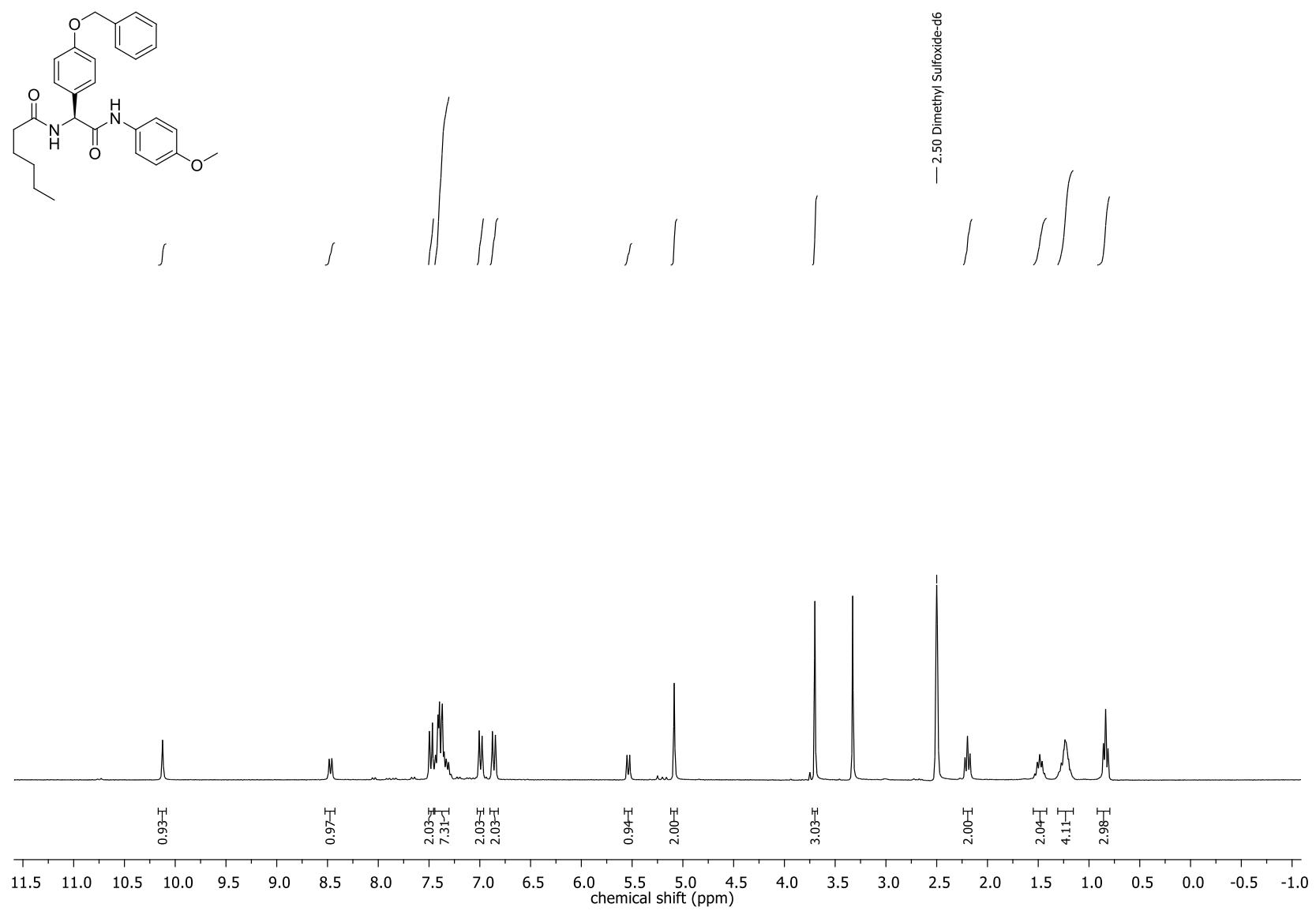
Compound 4, ^1H NMR (300 MHz, acetone- d_6)



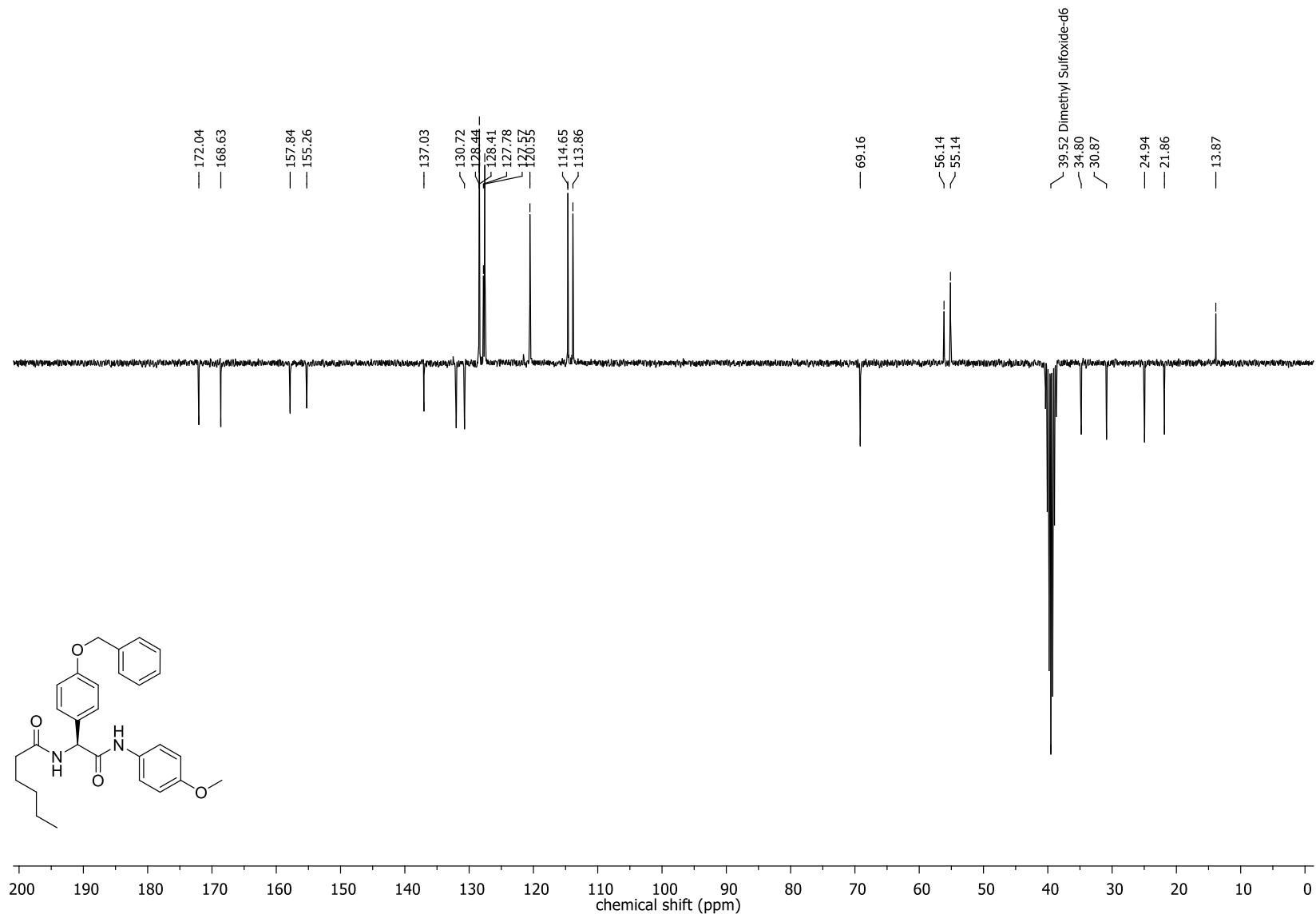
Compound 4, ^{13}C NMR (APT, 126 MHz, $\text{DMSO}-d_6$)



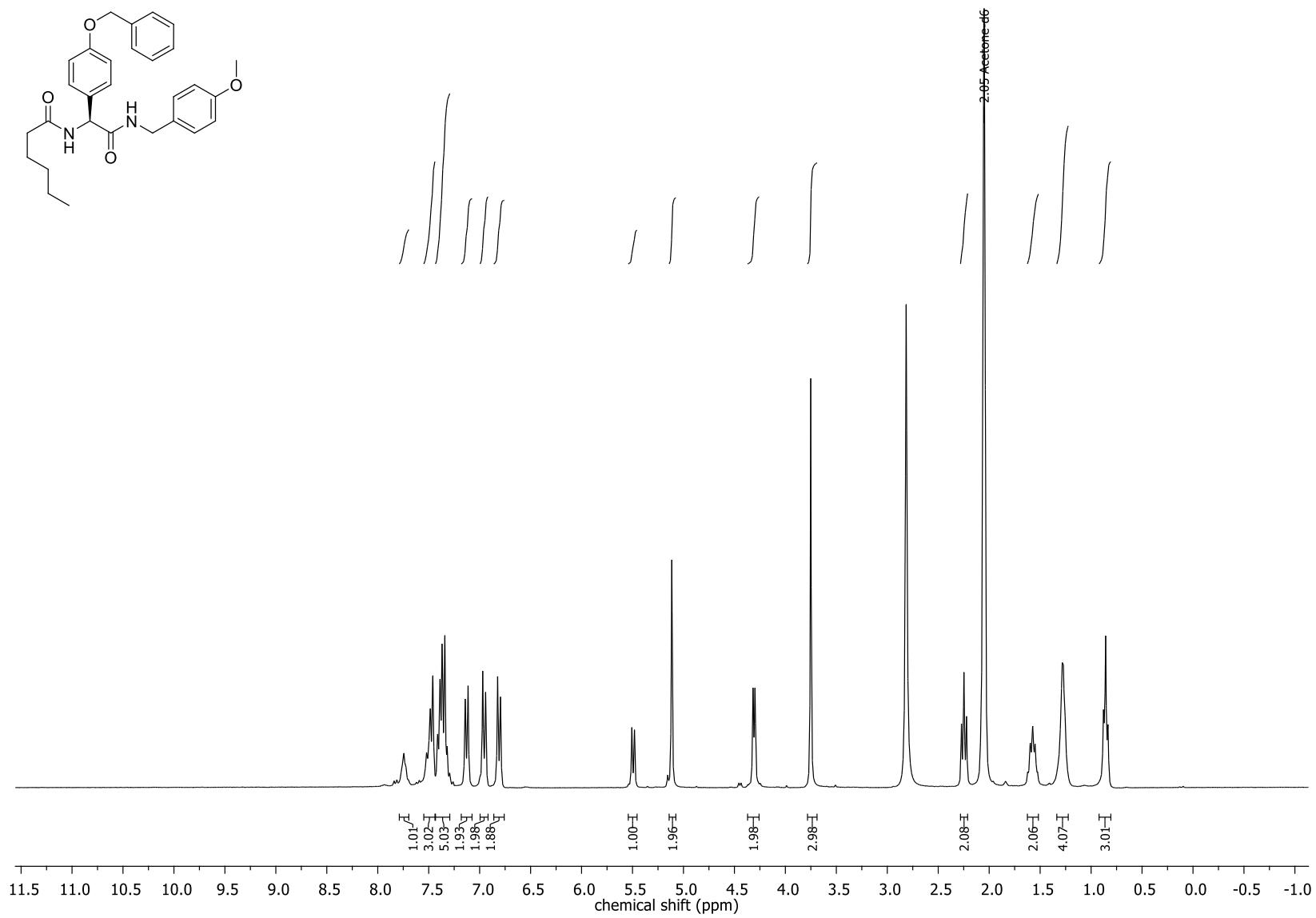
Compound 5, ^1H NMR (300 MHz, DMSO- d_6)



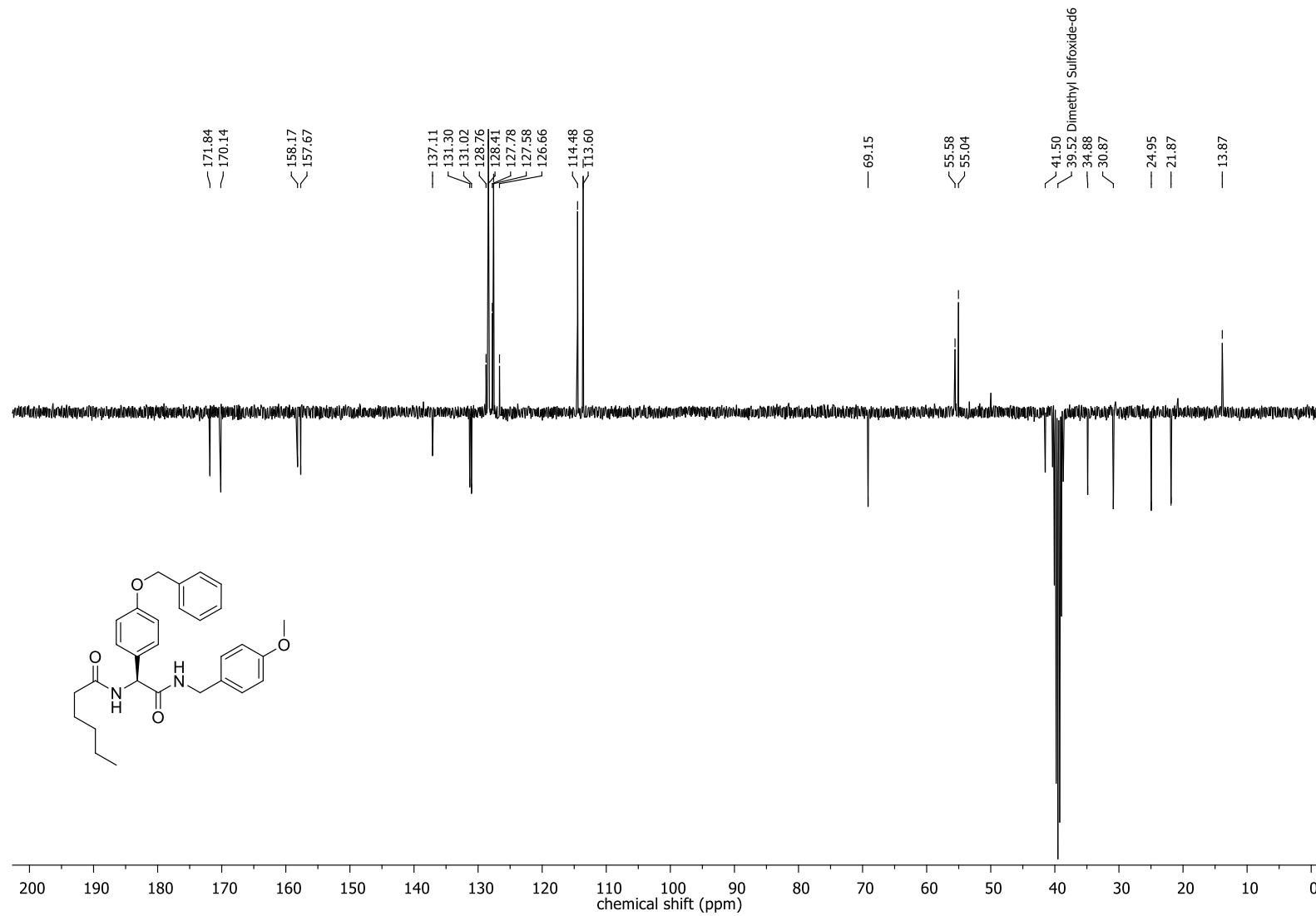
Compound 5, ^{13}C NMR (APT, 75 MHz, DMSO- d_6)



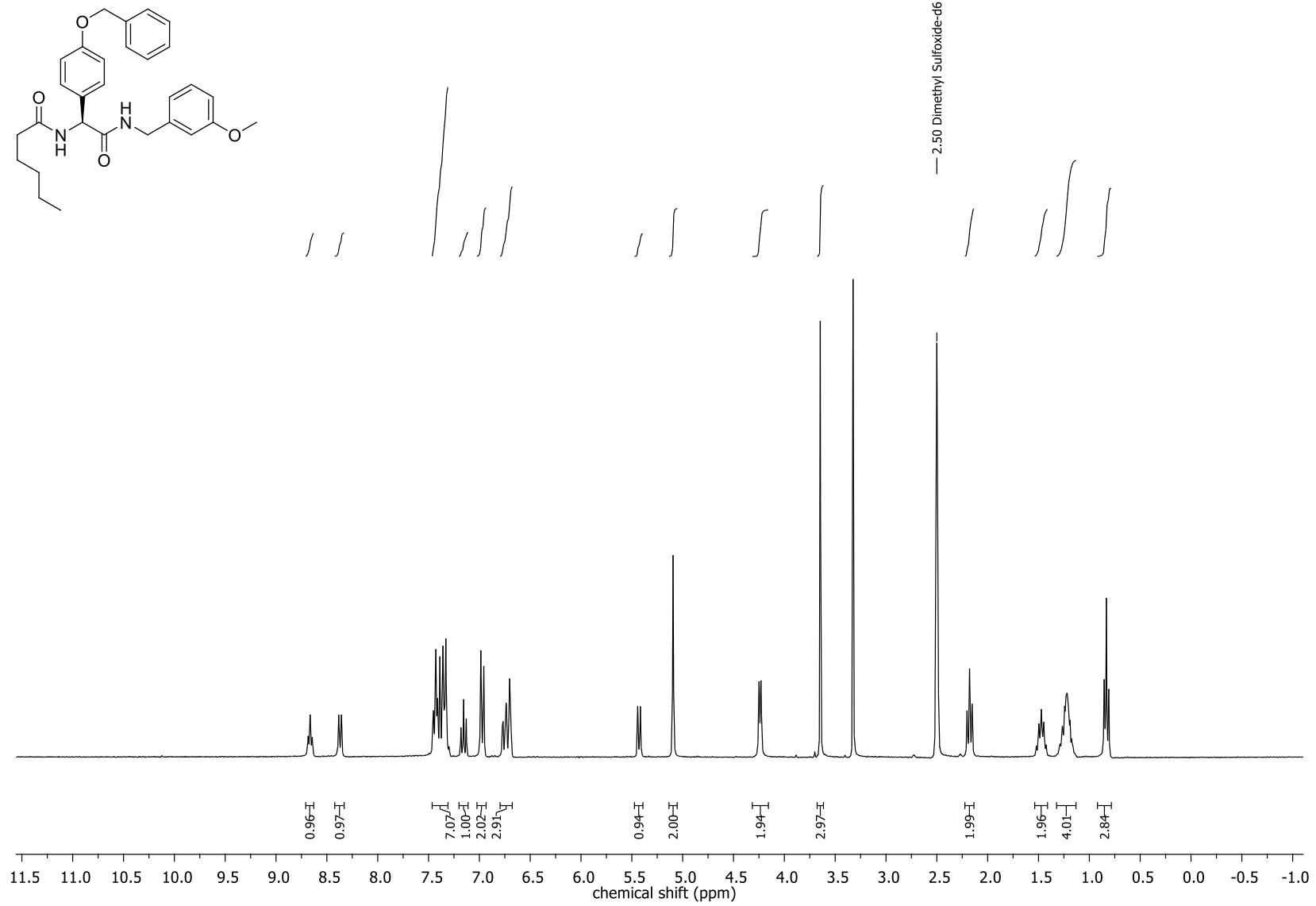
Compound 6, ^1H NMR (300 MHz, acetone- d_6)



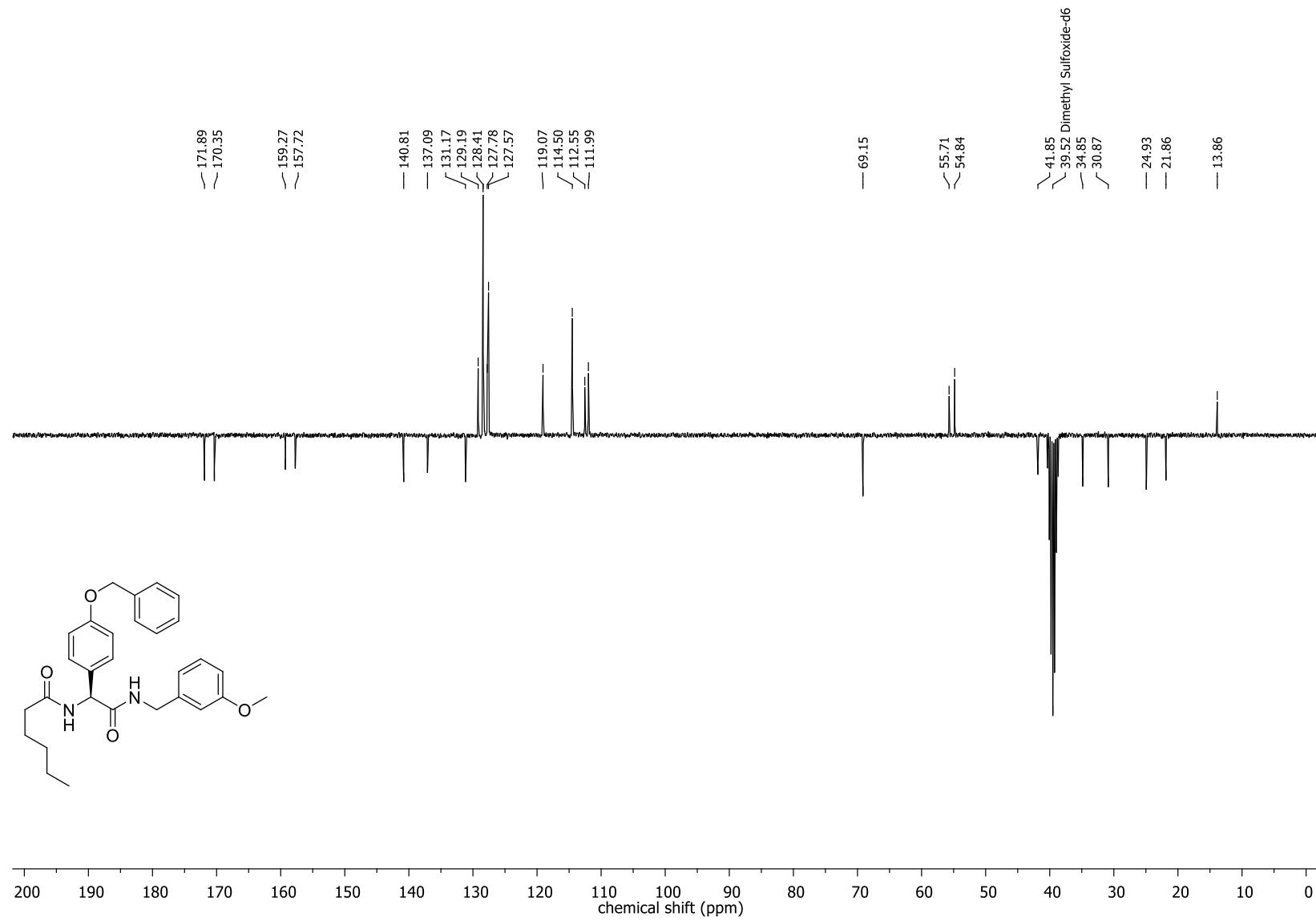
Compound 6, ^{13}C NMR (APT, 75 MHz, DMSO- d_6)



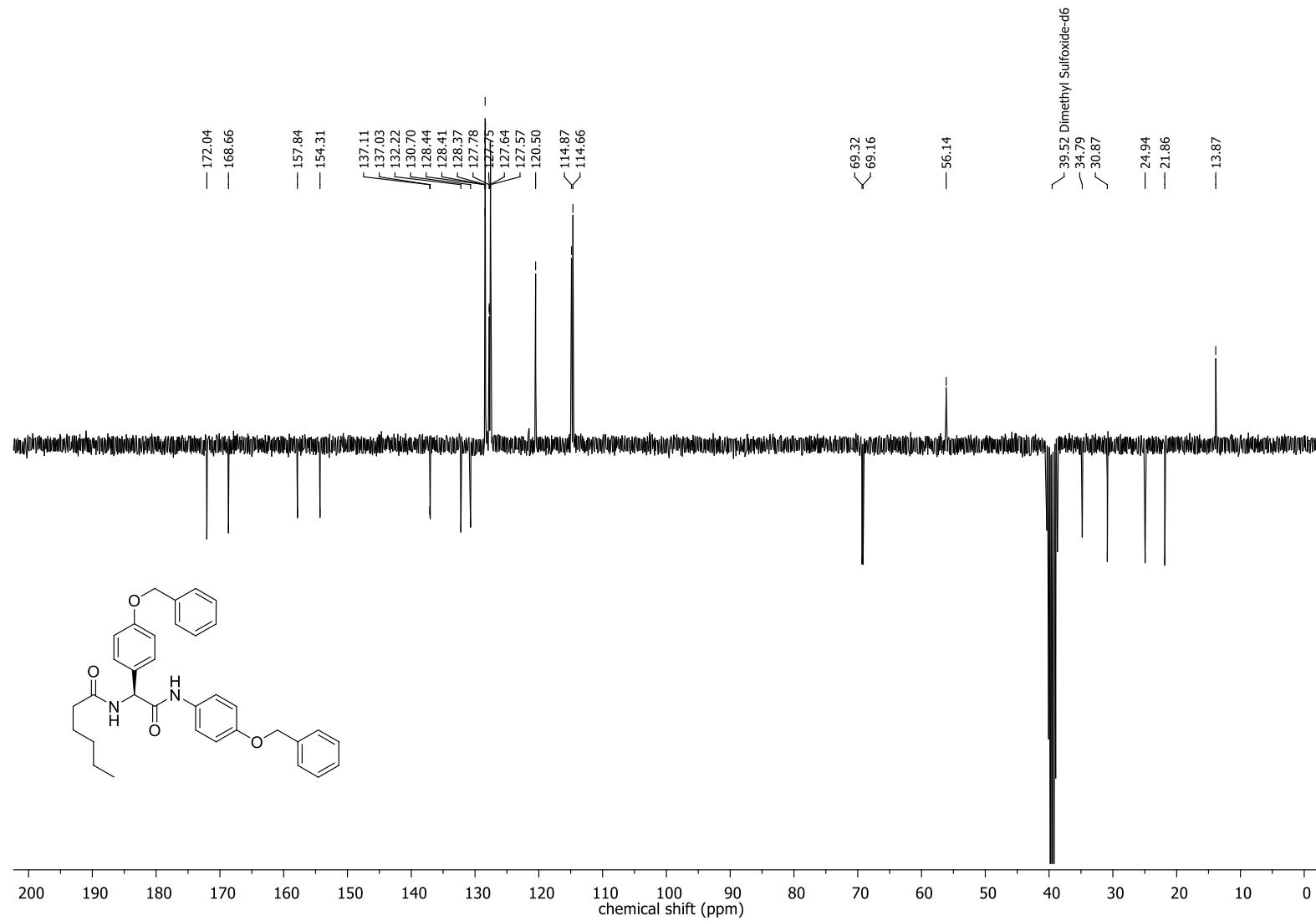
Compound 7, ^1H NMR (300 MHz, DMSO- d_6)



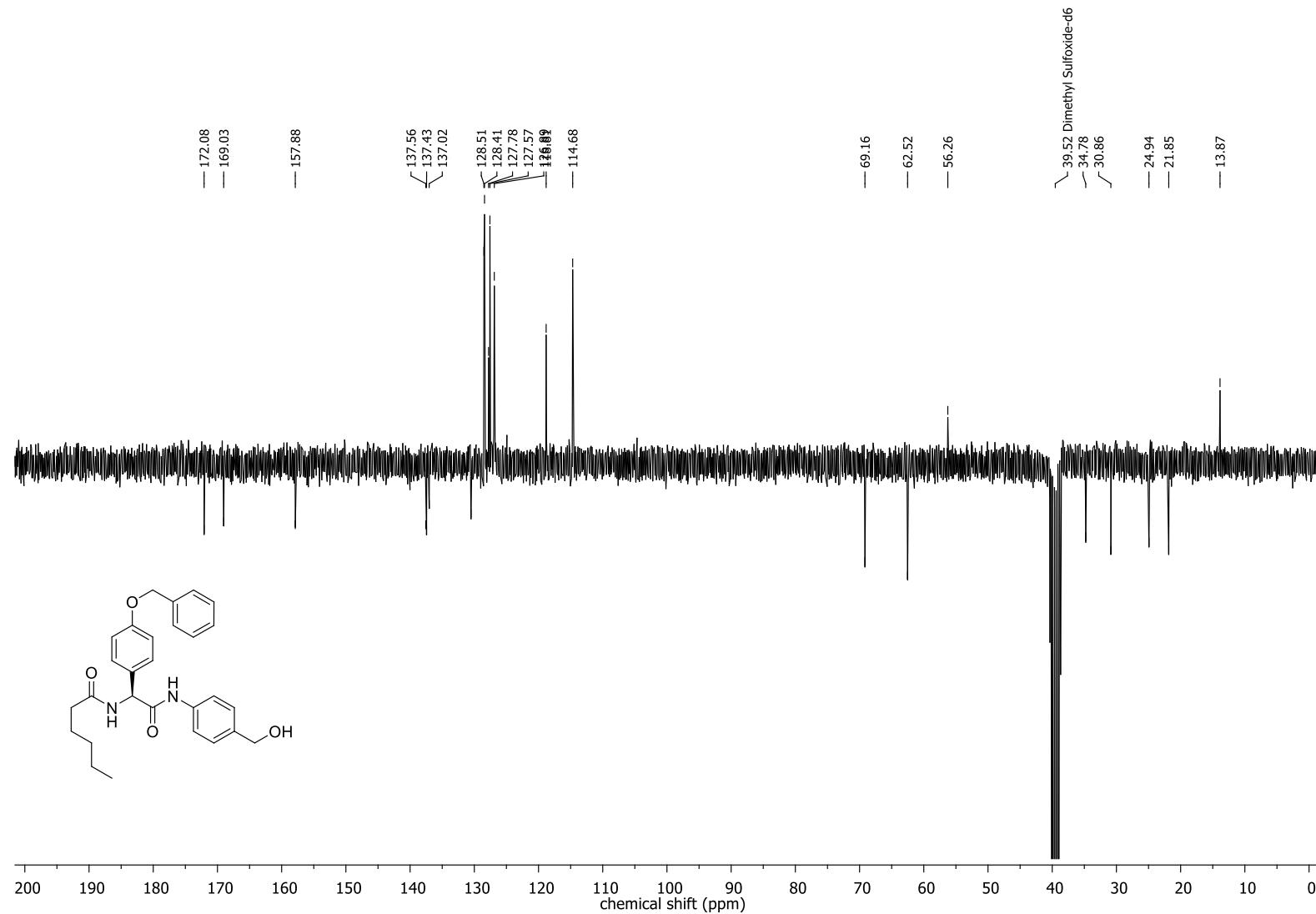
Compound 7, ^{13}C NMR (APT, 75 MHz, DMSO- d_6)



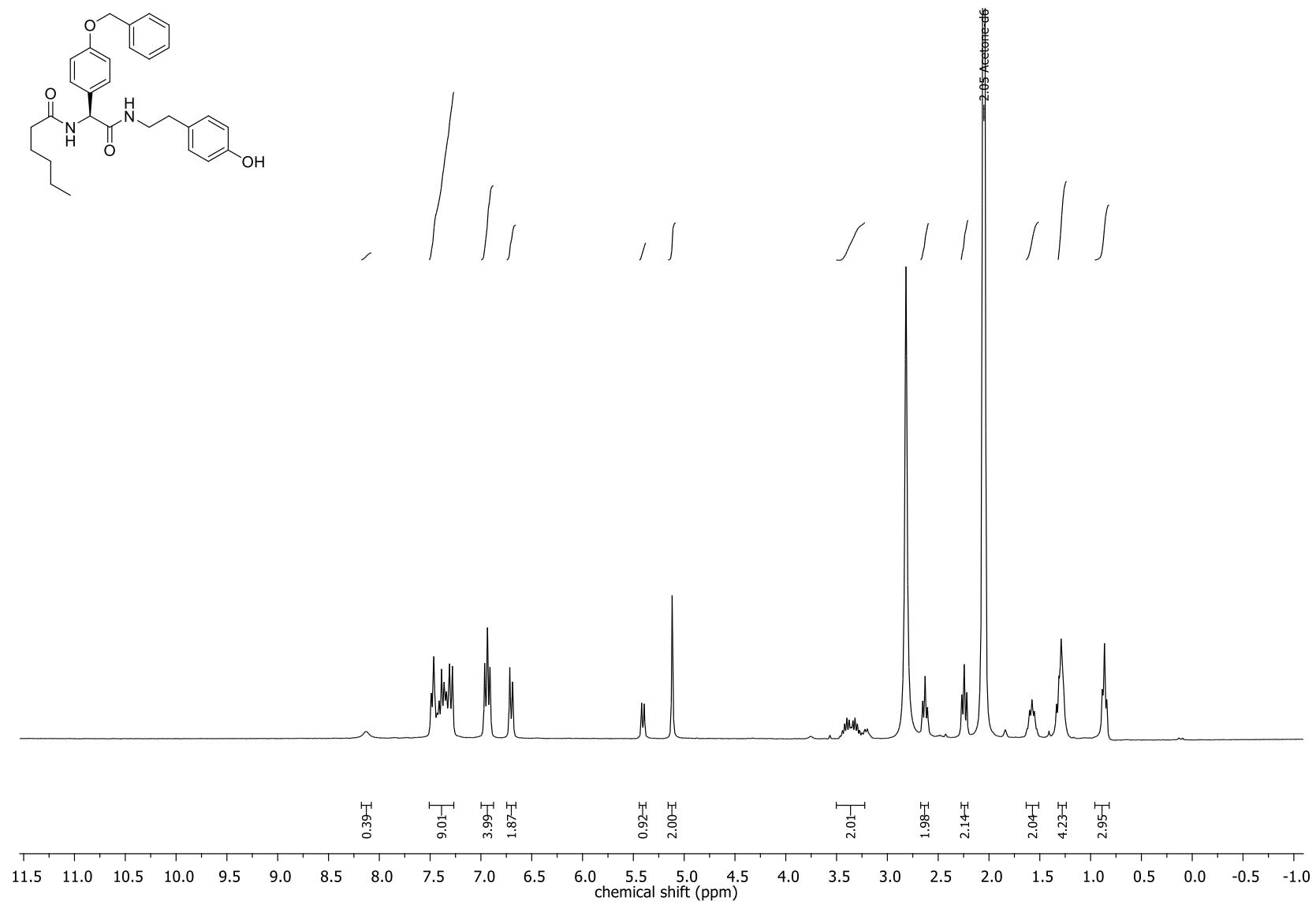
Compound 8, ^{13}C NMR (APT, 75 MHz, DMSO- d_6)



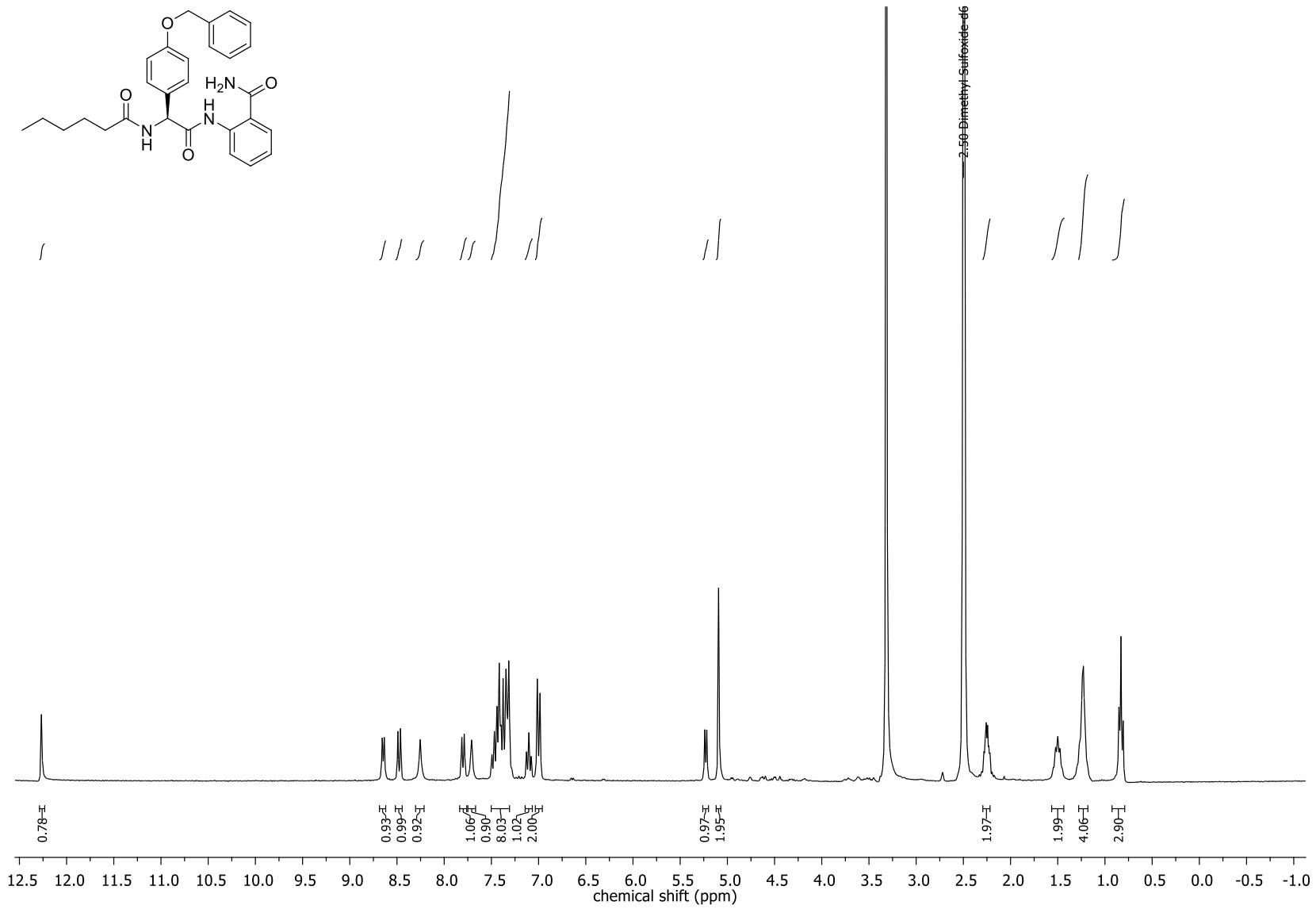
Compound 9, ^{13}C NMR (APT, 75 MHz, DMSO- d_6)



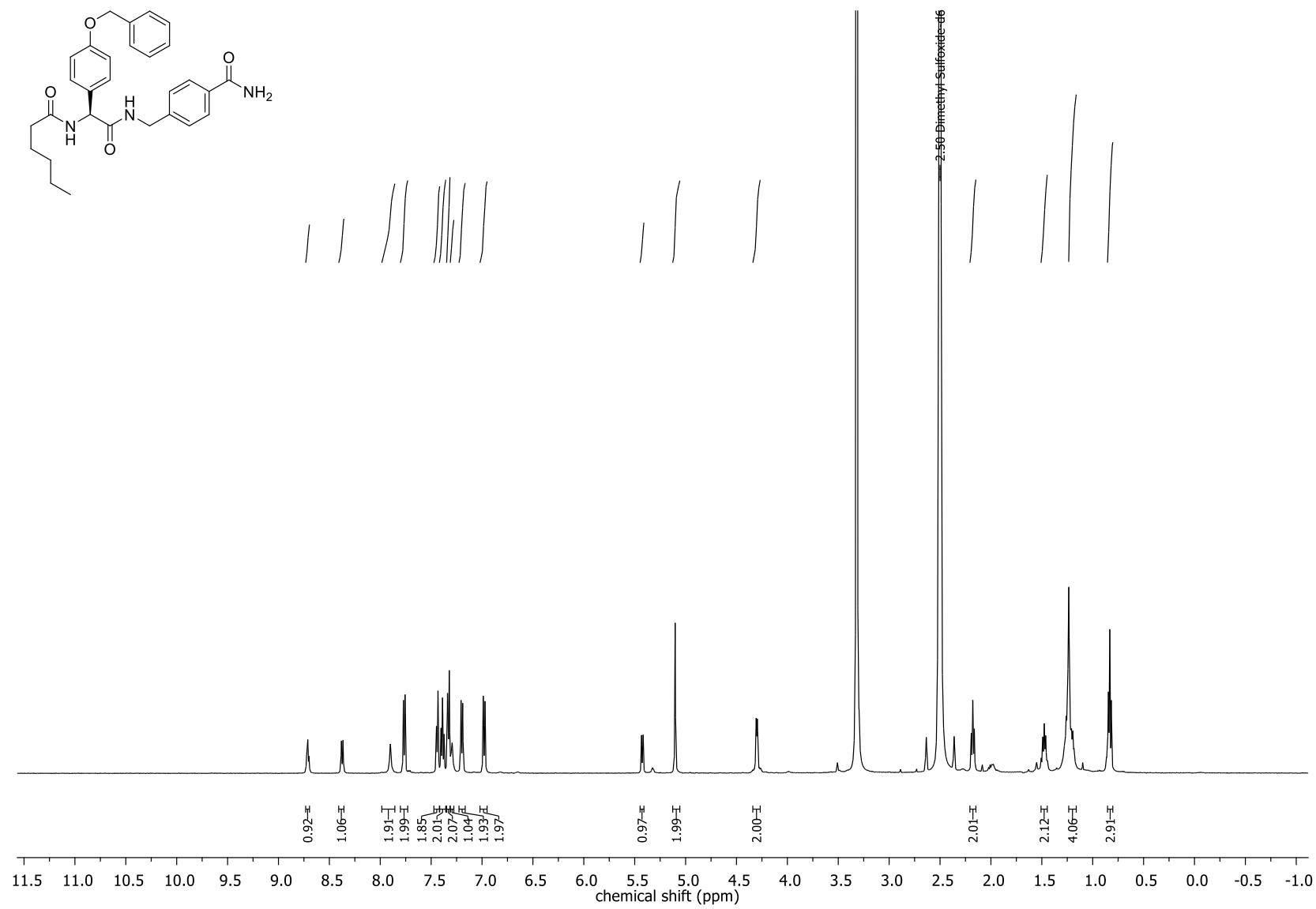
Compound **10**, ^1H NMR (300 MHz, acetone- d_6)



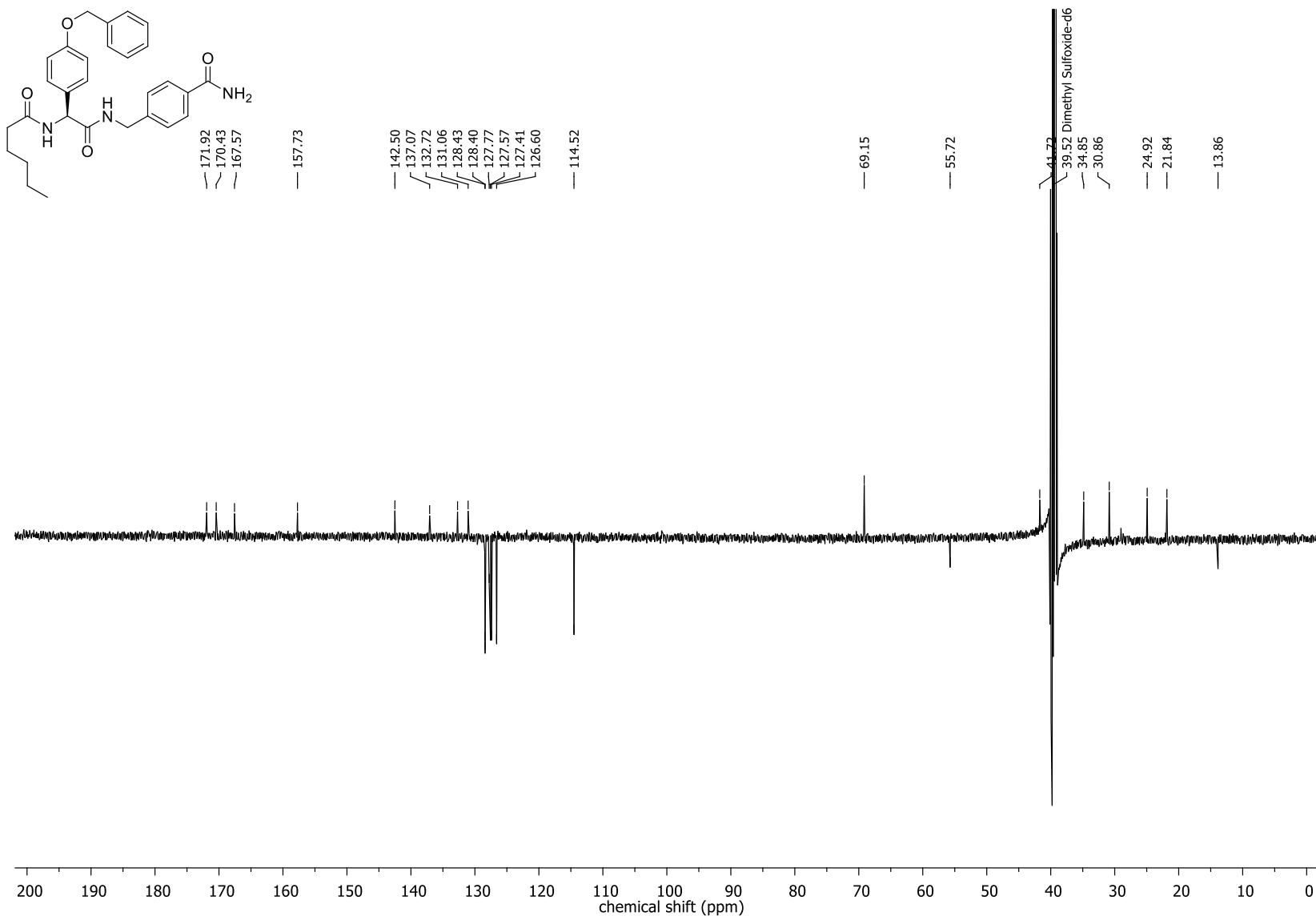
Compound 16, ^1H NMR (300 MHz, DMSO- d_6)



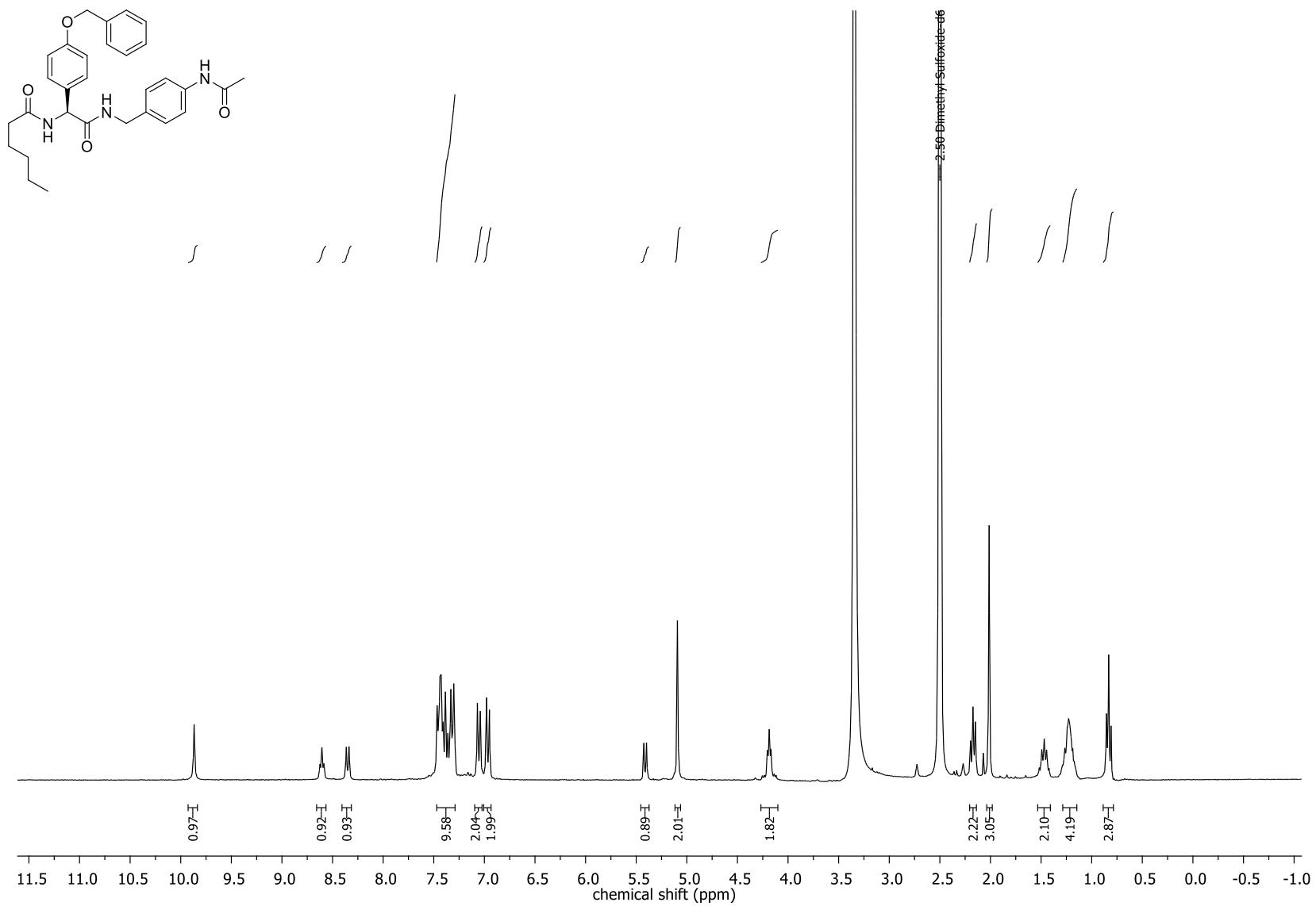
Compound **18**, ^1H NMR (500 MHz, $\text{DMSO}-d_6$)



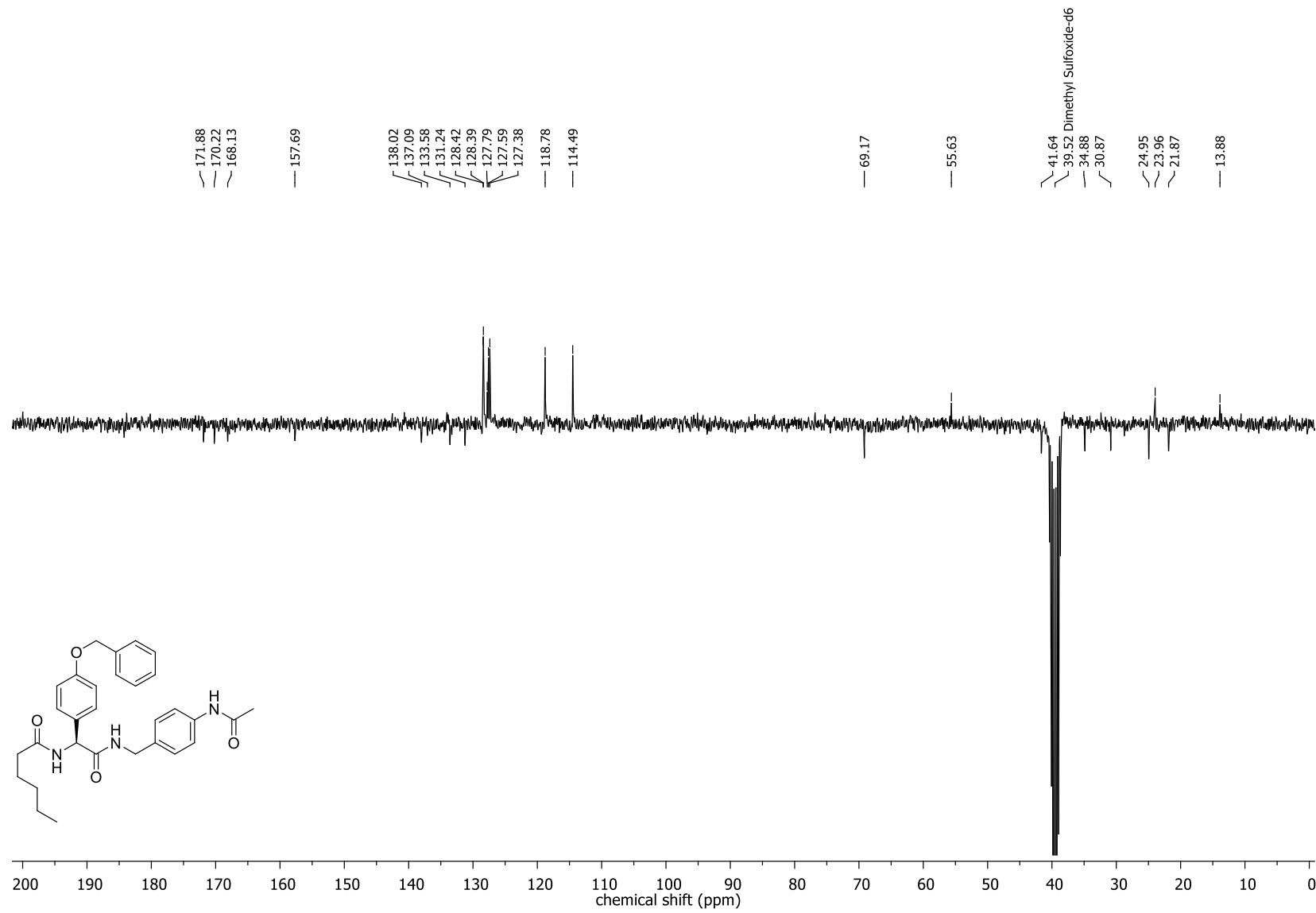
Compound **18**, ^1H NMR (126 MHz, DMSO-*d*₆)



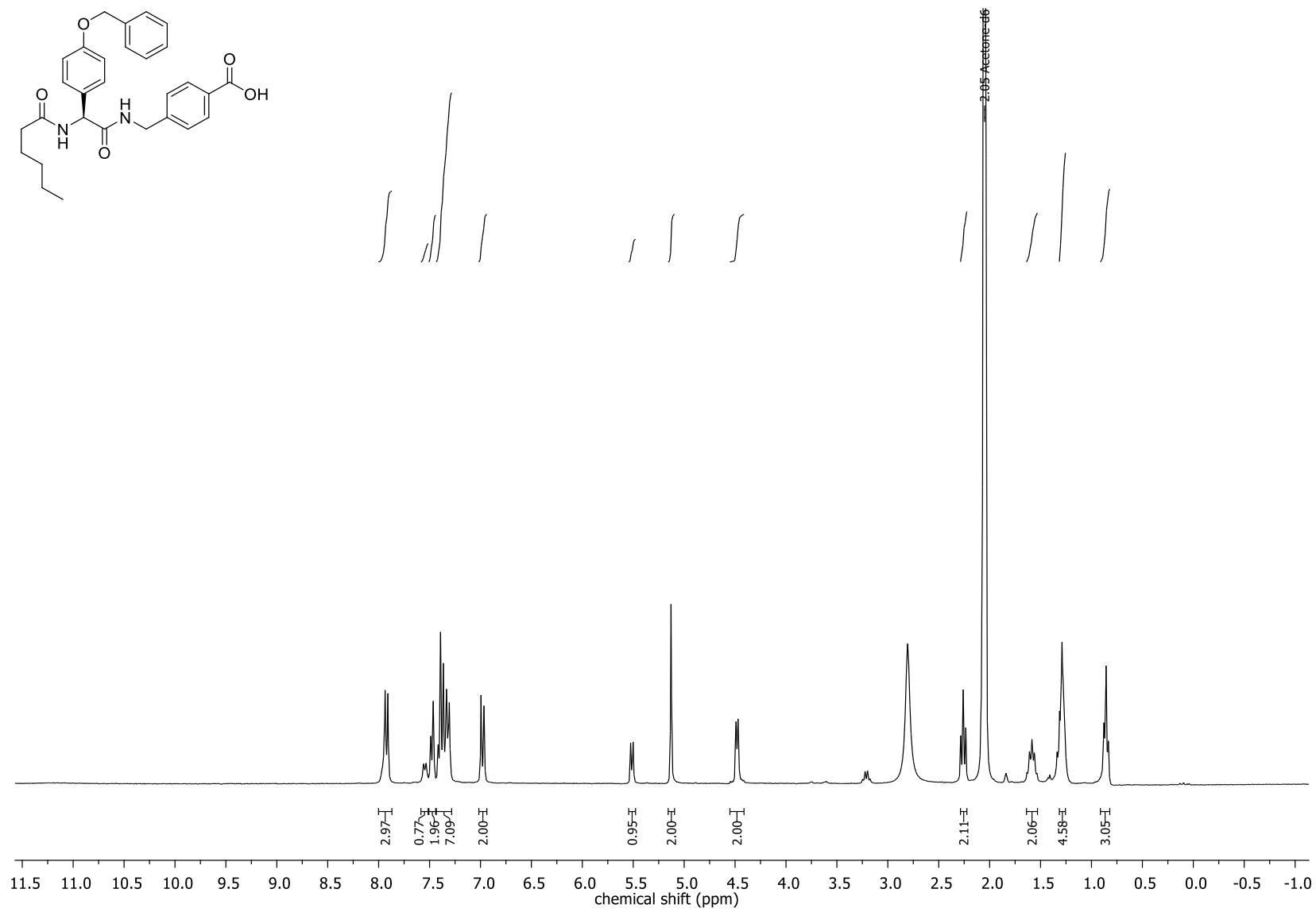
Compound **21**, ^1H NMR (300 MHz, DMSO- d_6)



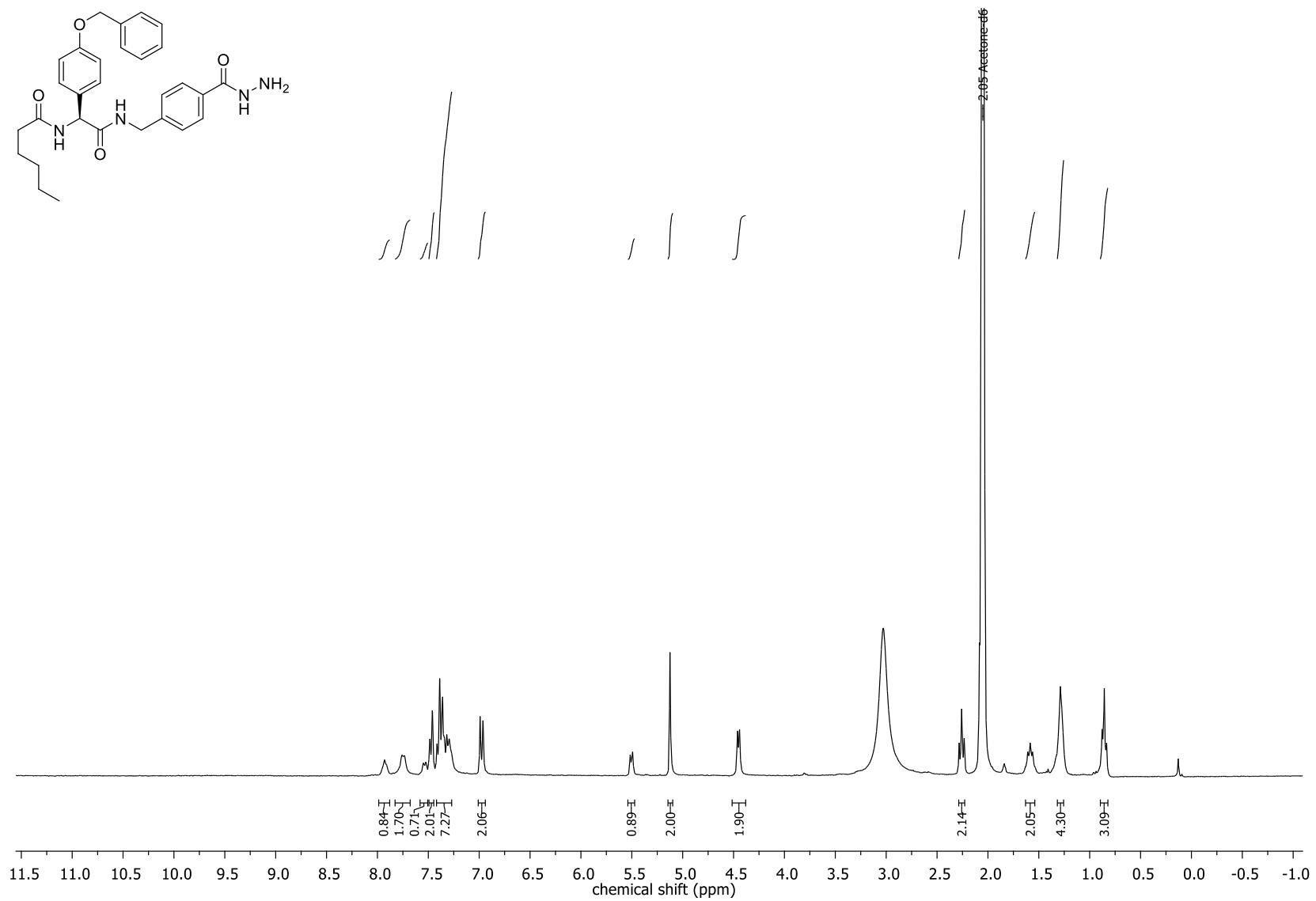
Compound **21**, ^{13}C NMR (APT, 75 MHz, $\text{DMSO}-d_6$)



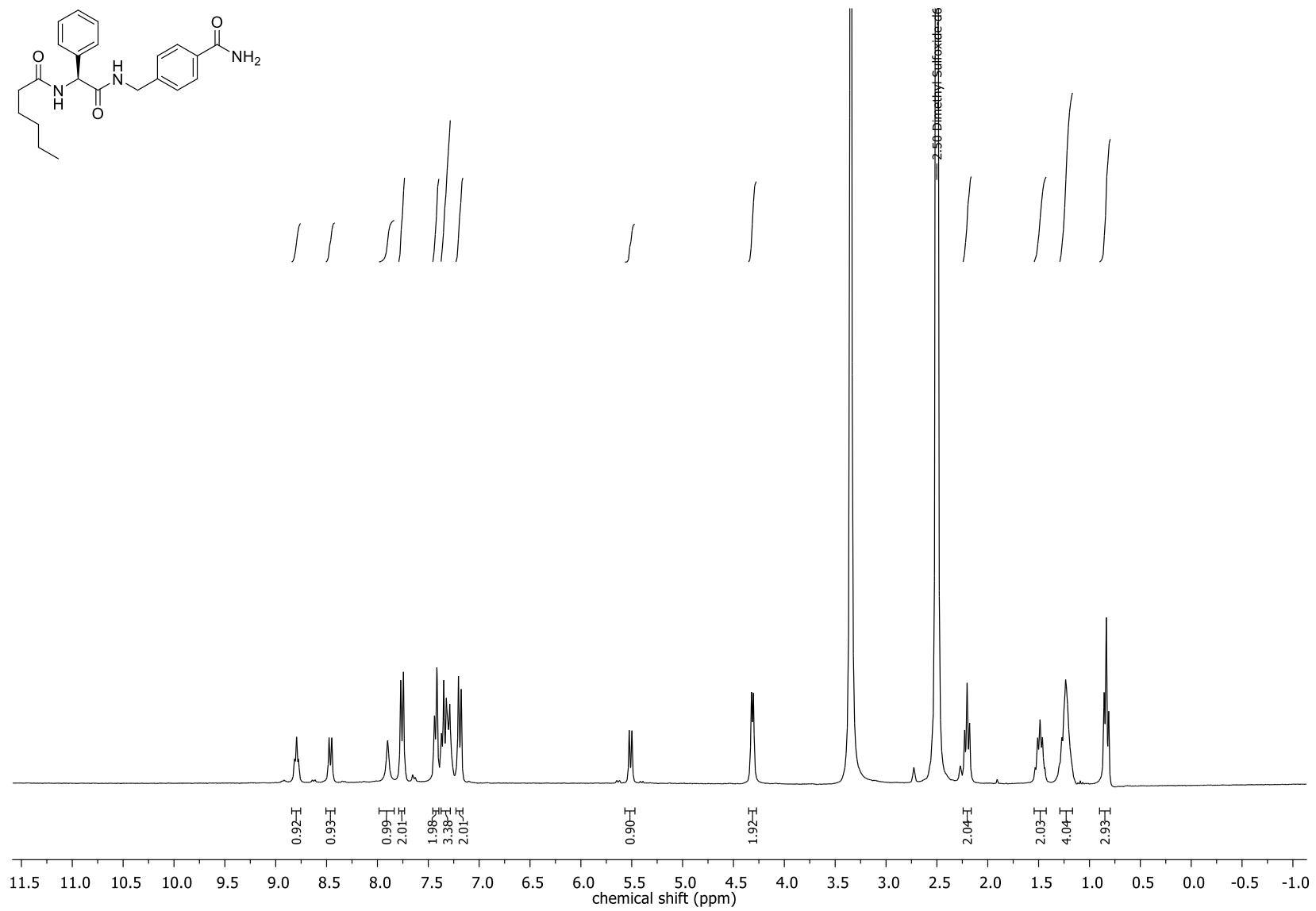
Compound **25**, ^1H NMR (300 MHz, acetone- d_6)



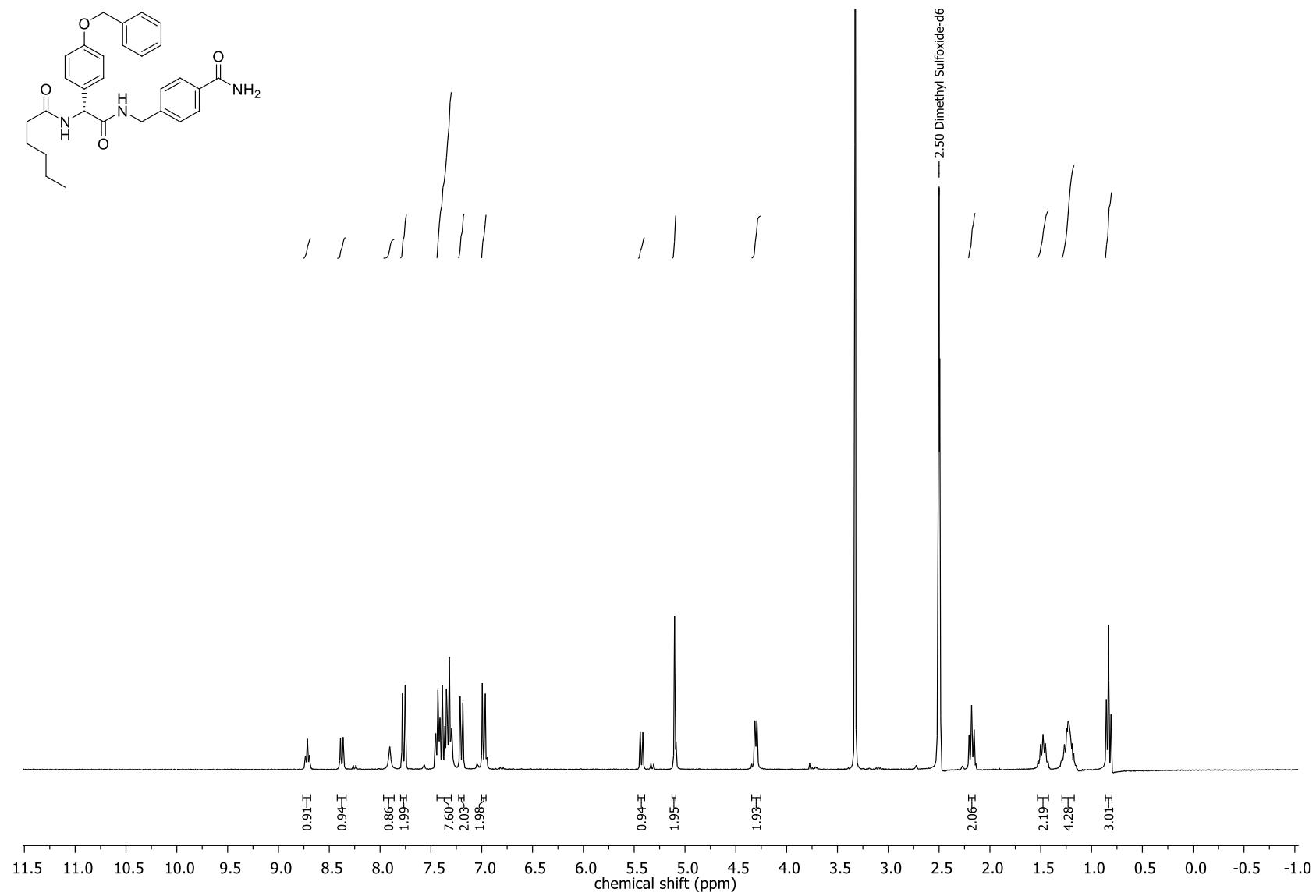
Compound **23**, ^1H NMR (300 MHz, acetone- d_6)



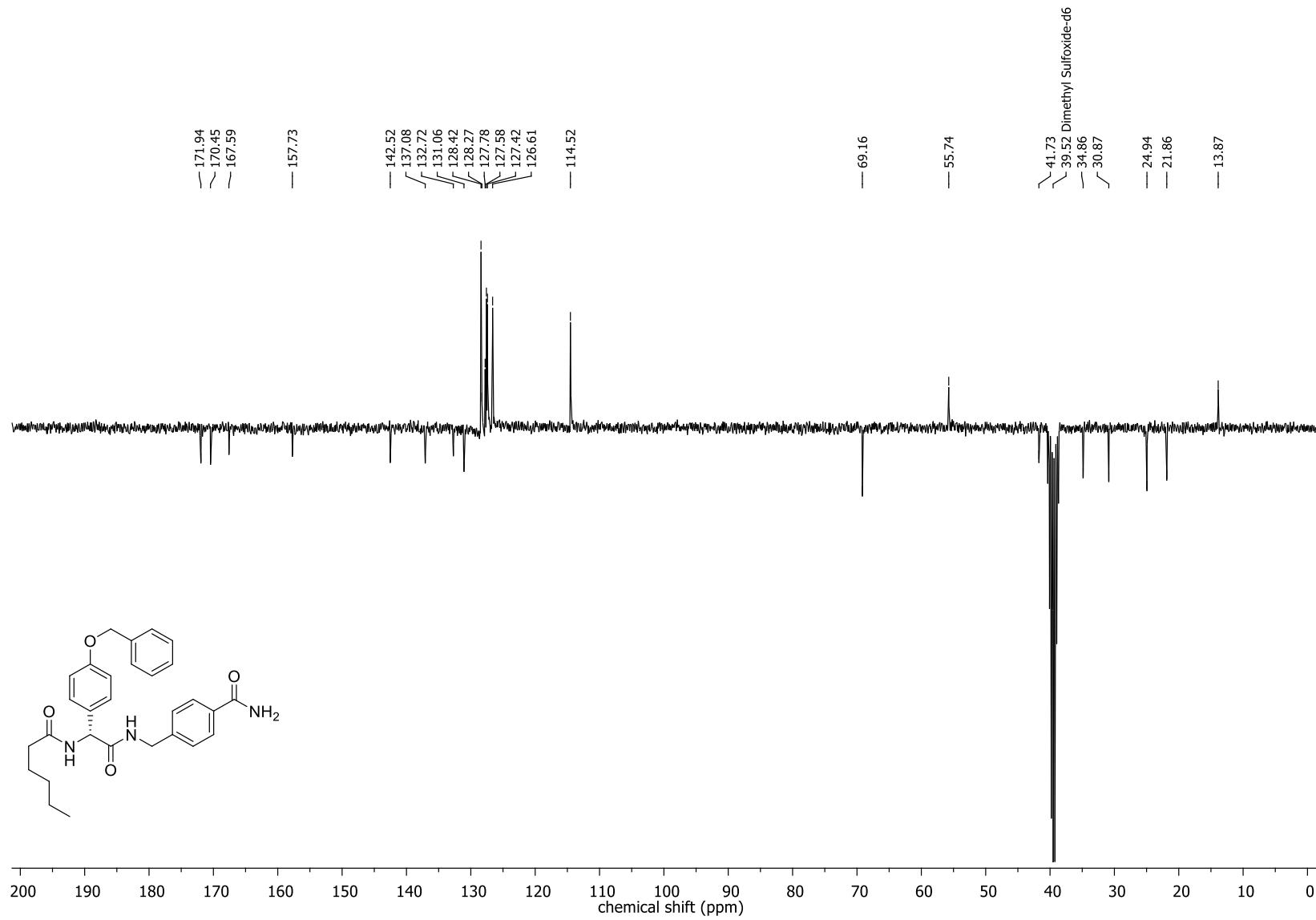
Compound 27, ^1H NMR (300 MHz, $\text{DMSO}-d_6$)



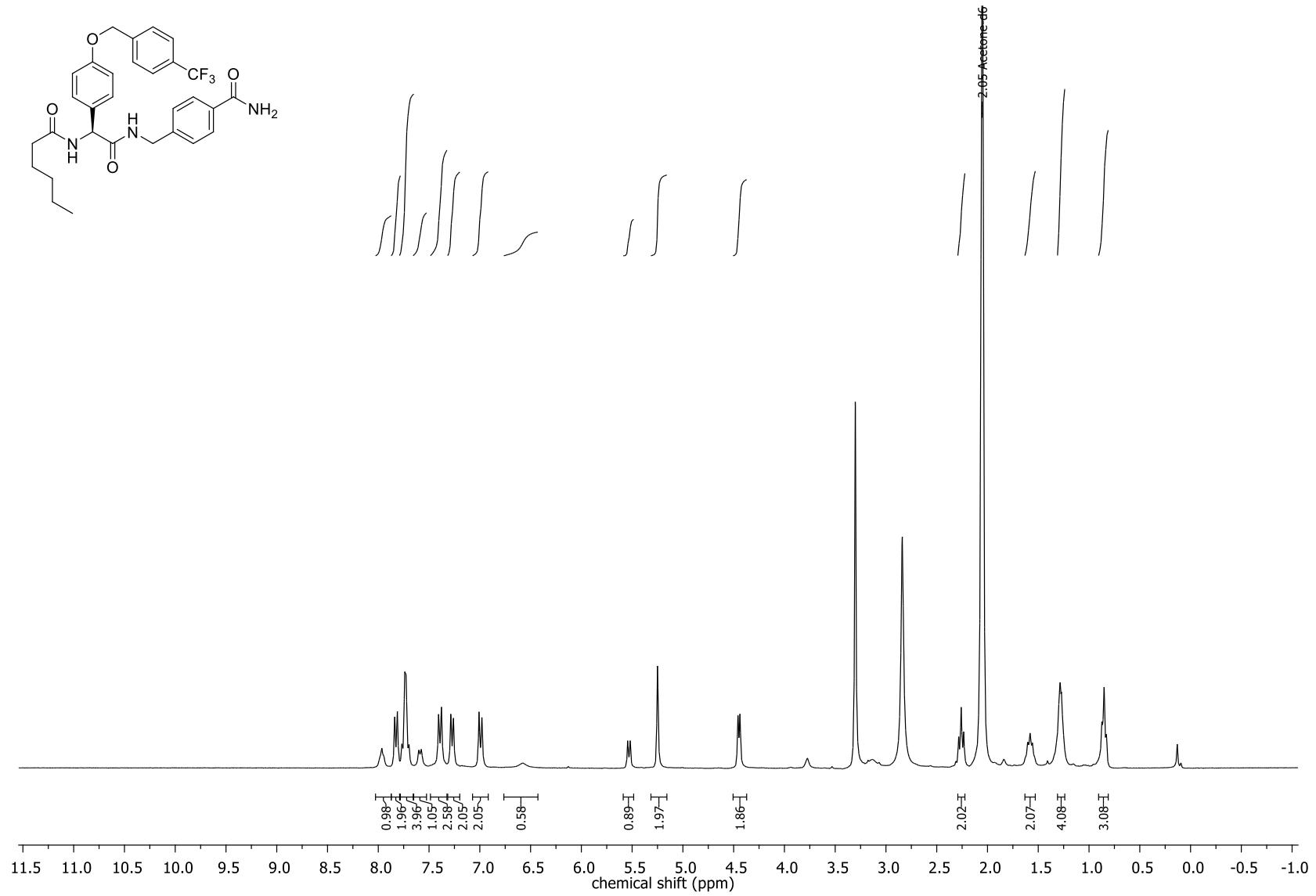
Compound 31, ^1H NMR (300 MHz, DMSO- d_6)



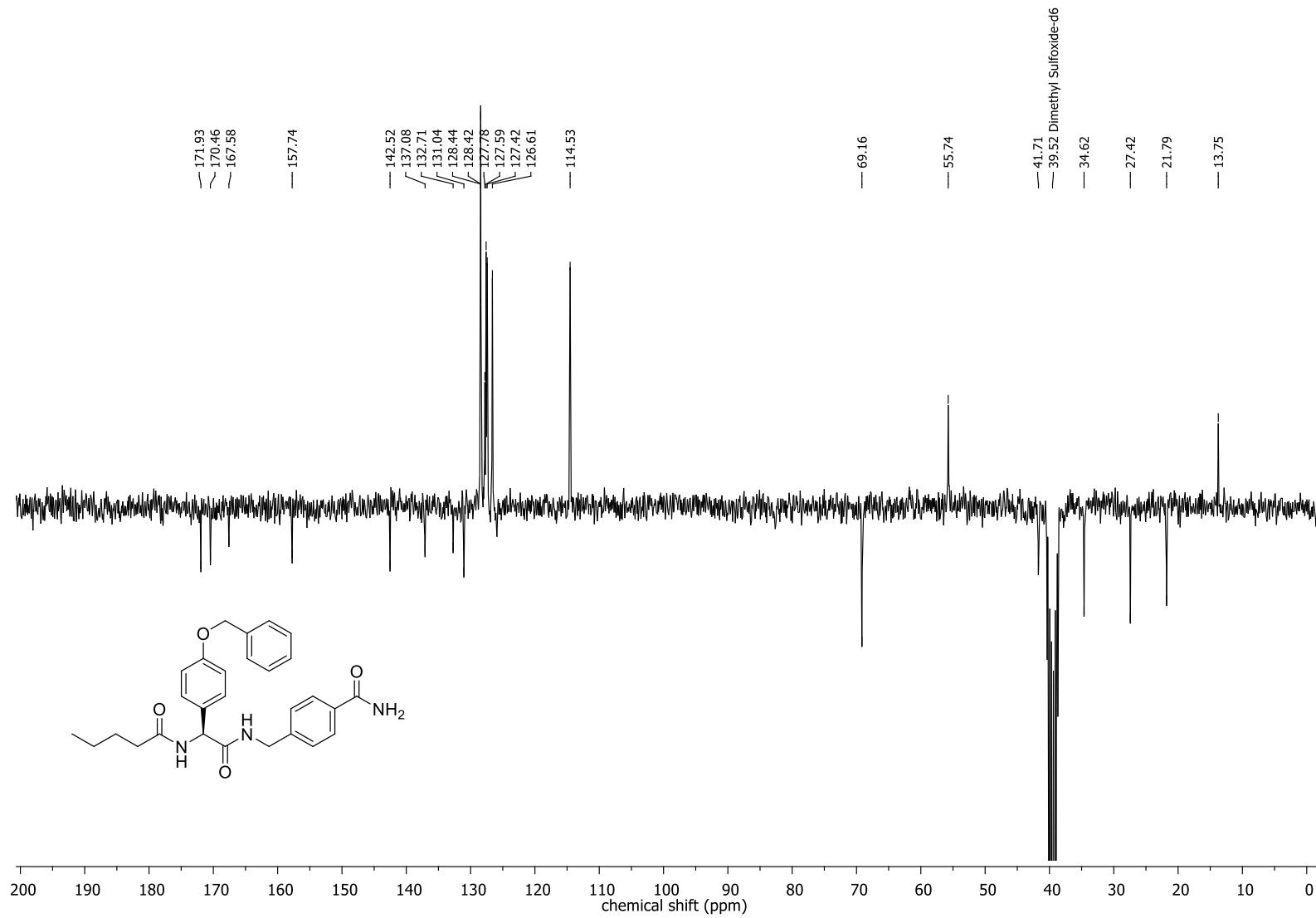
Compound **31**, ^{13}C NMR (APT, 75 MHz, $\text{DMSO}-d_6$)



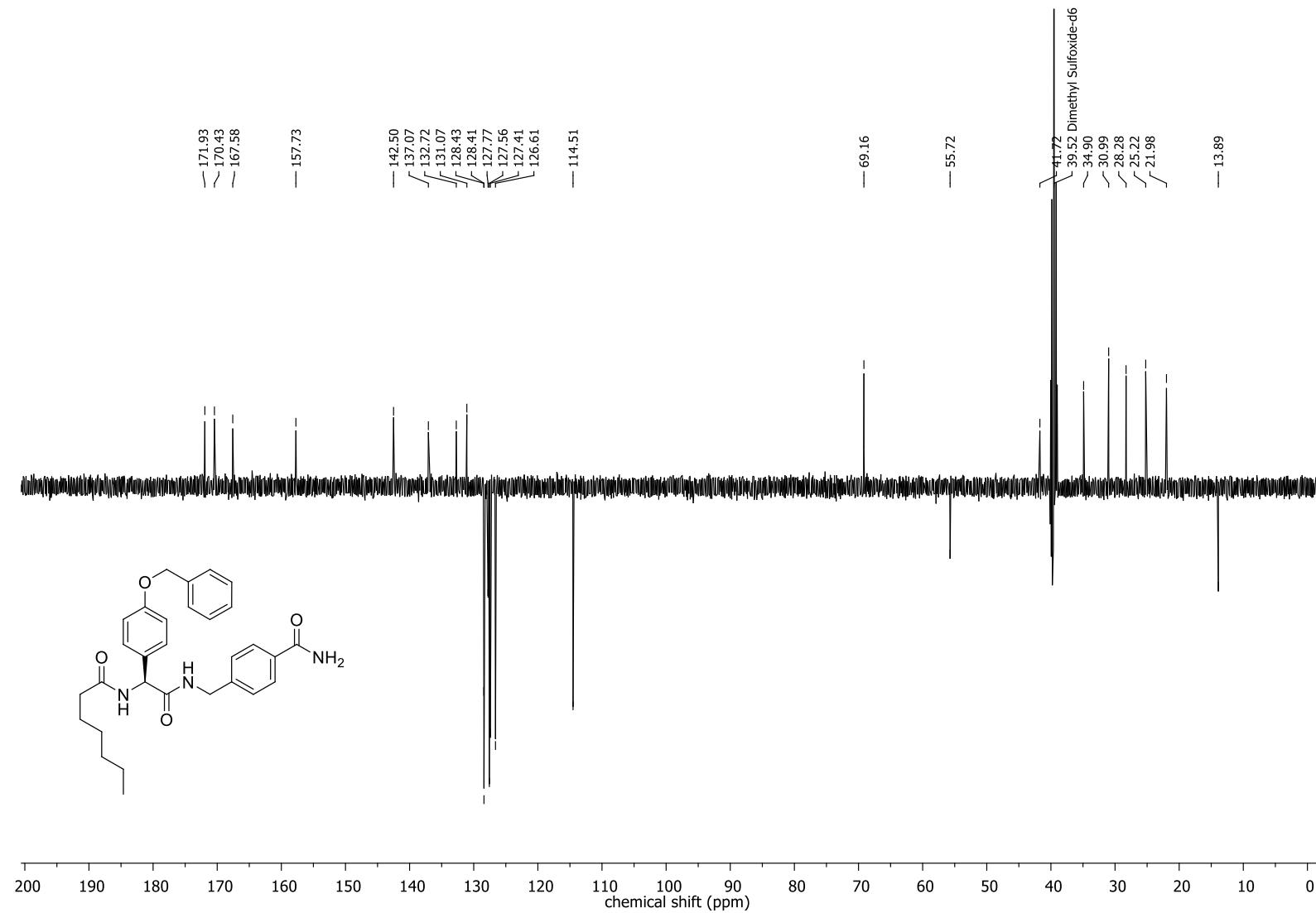
Compound **32**, ^1H NMR (300 MHz, acetone- d_6)



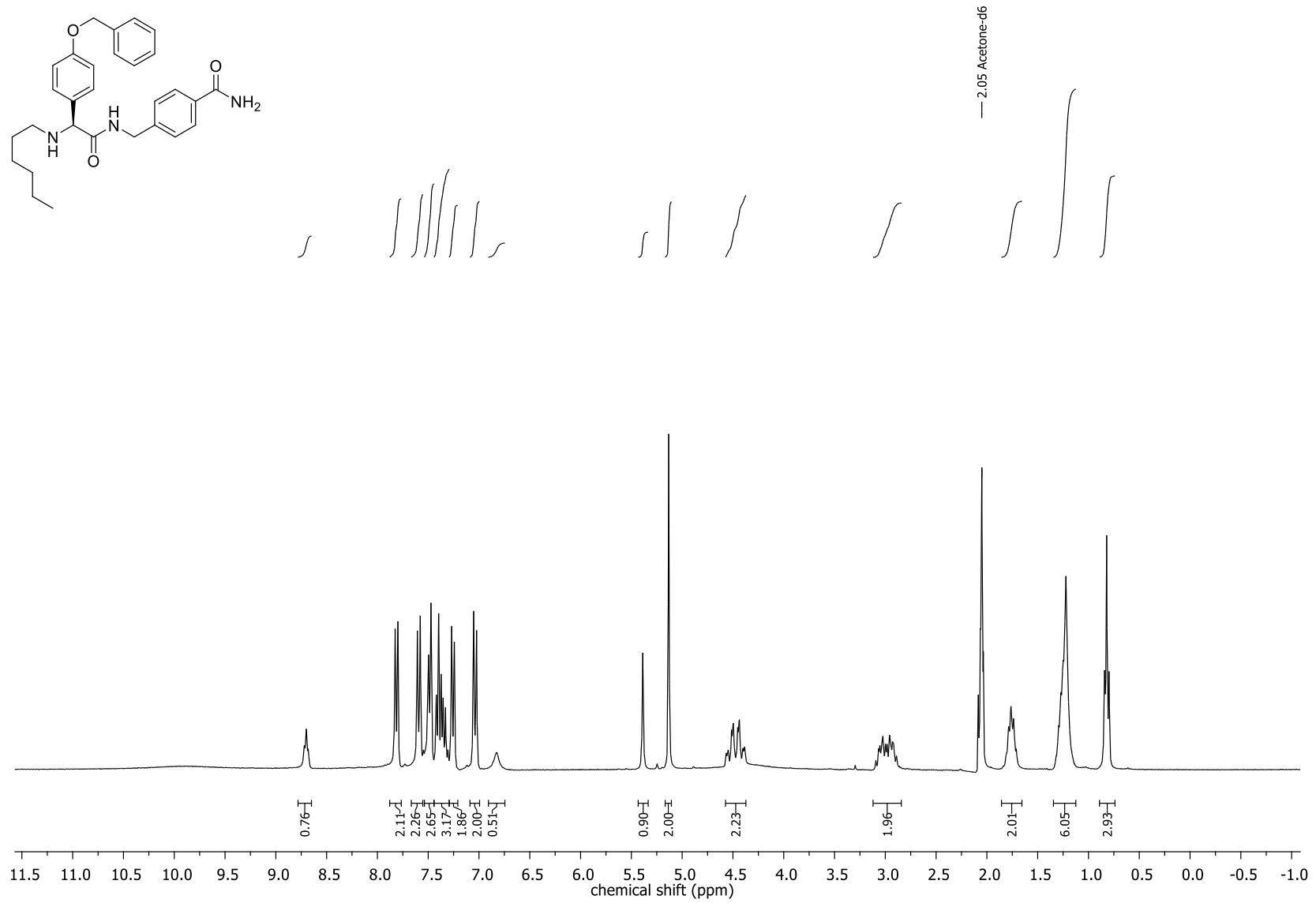
Compound 42, ^{13}C NMR (APT, 75 MHz, $\text{DMSO}-d_6$)



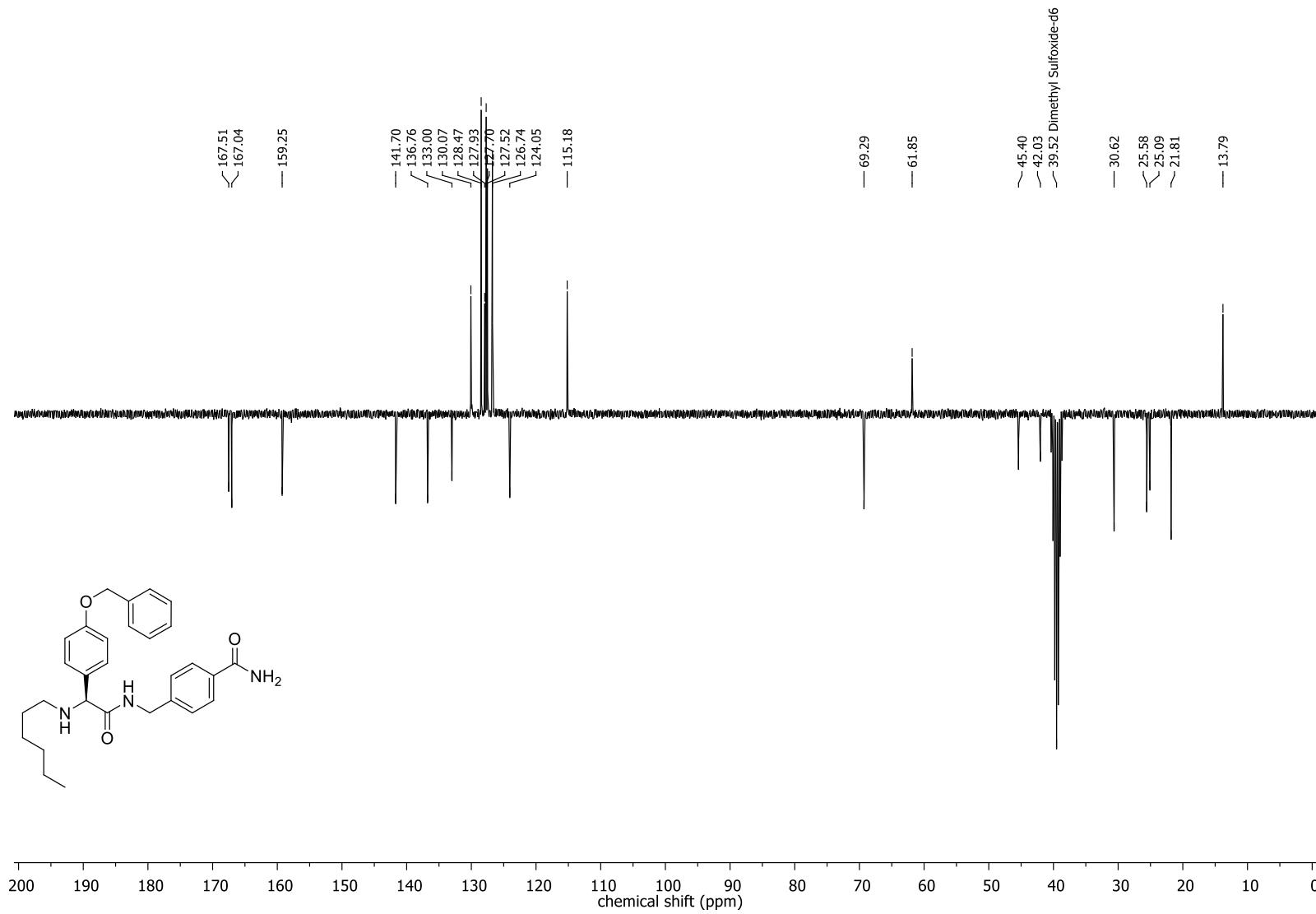
Compound 43, ^{13}C NMR (APT, 126 MHz, $\text{DMSO}-d_6$)



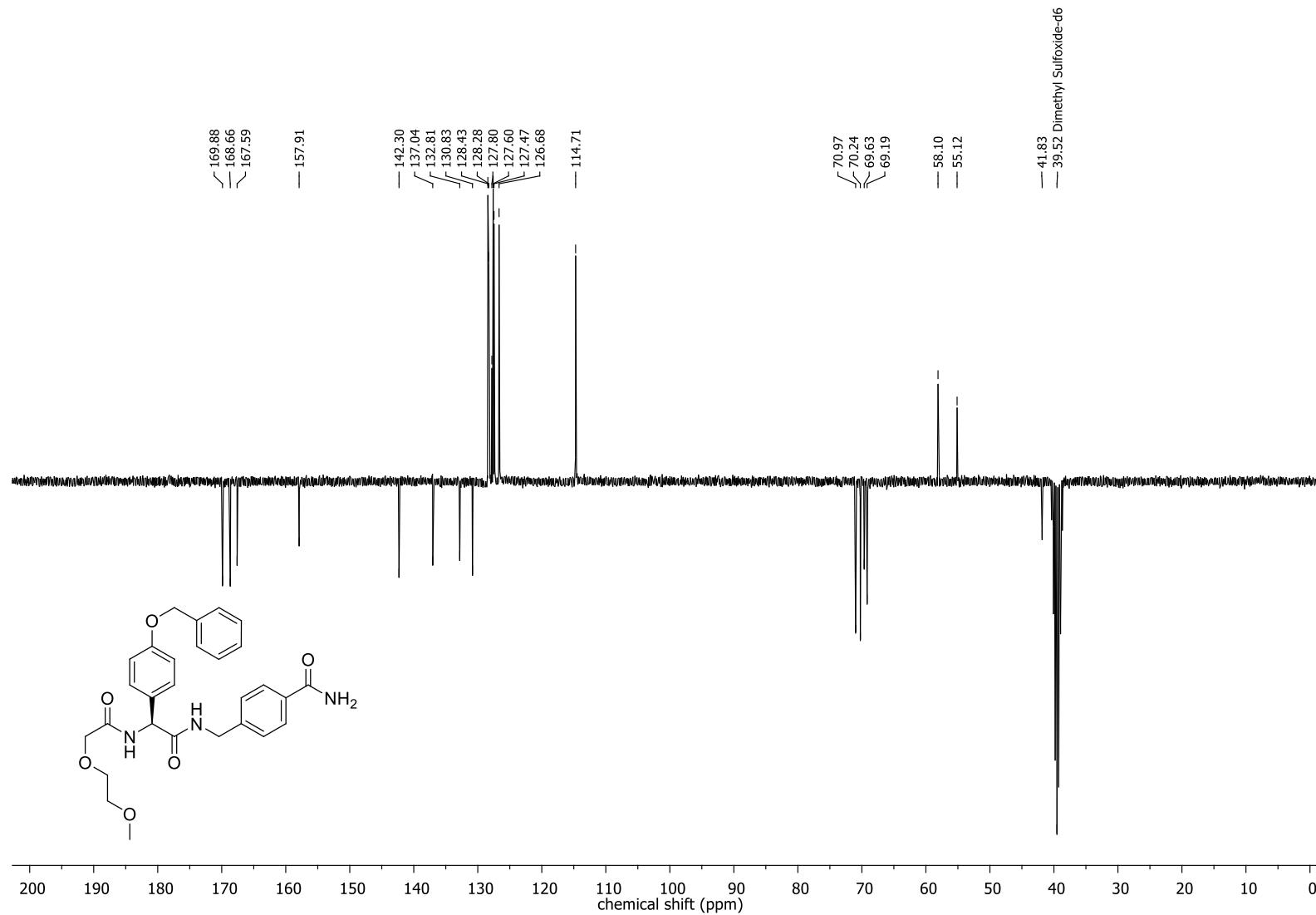
Compound 46, ^1H NMR (300 MHz, acetone- d_6)



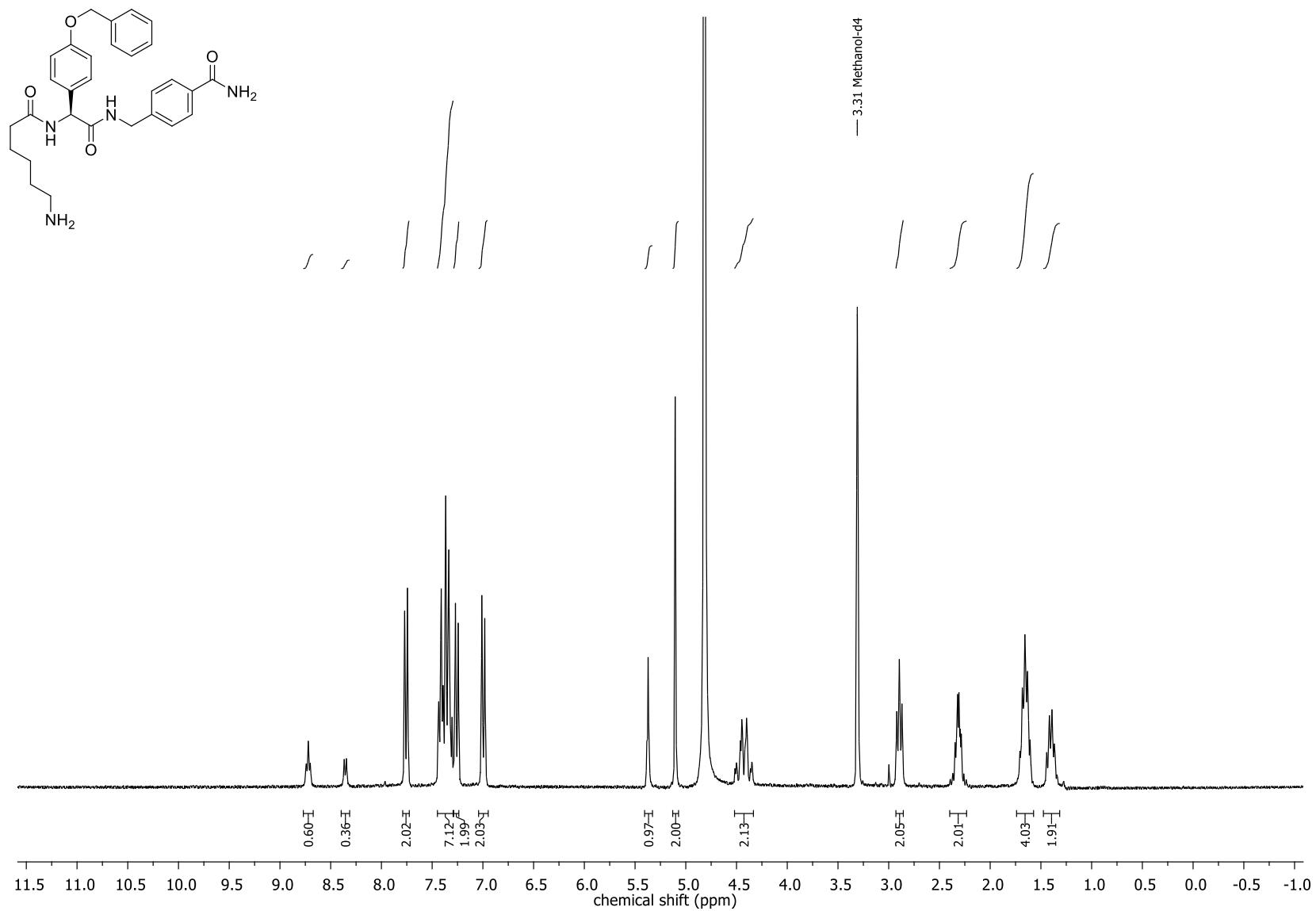
Compound 46, ^{13}C NMR (APT, 75 MHz, $\text{DMSO}-d_6$)



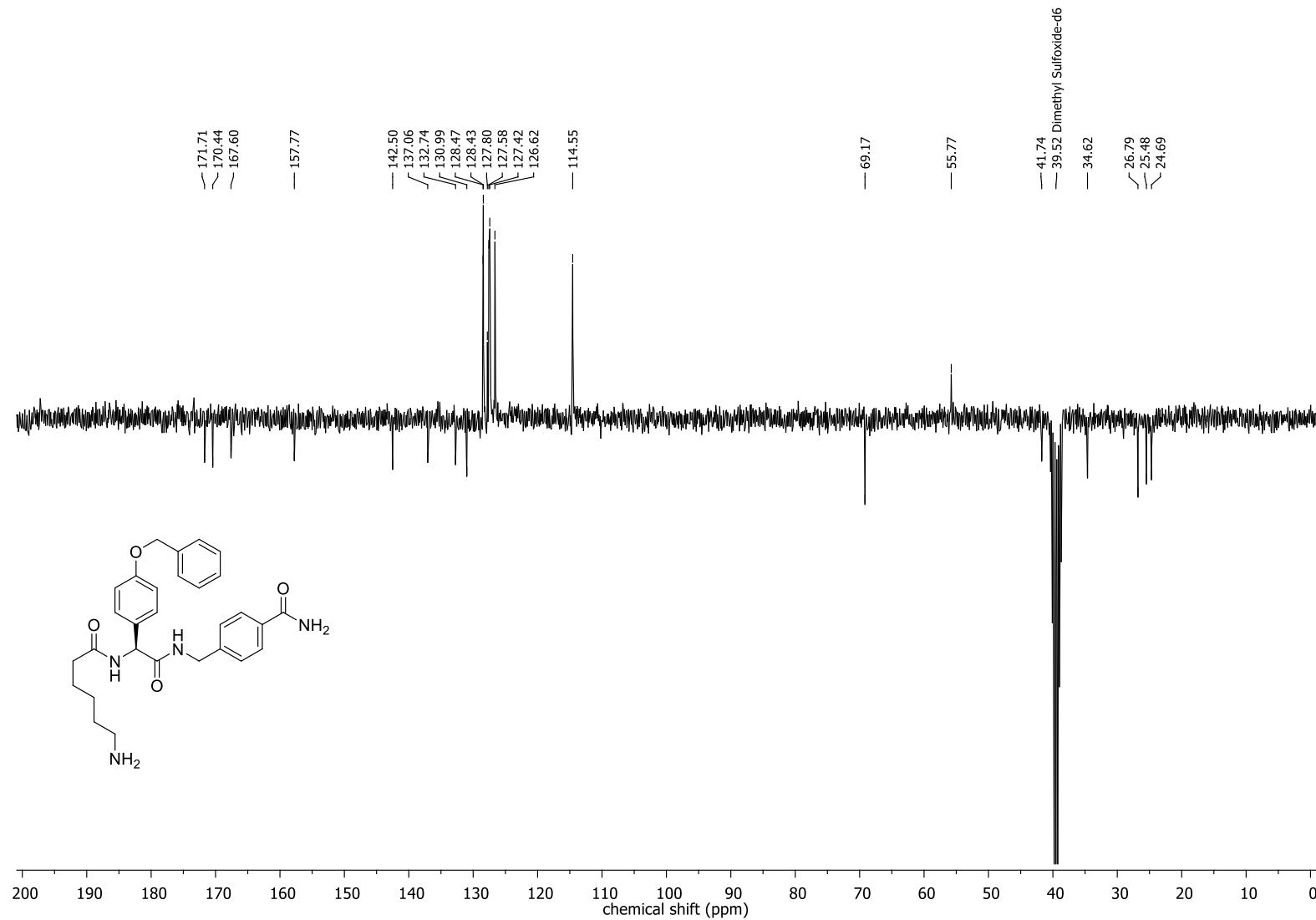
Compound 47, ^{13}C NMR (APT, 75 MHz, $\text{DMSO}-d_6$)



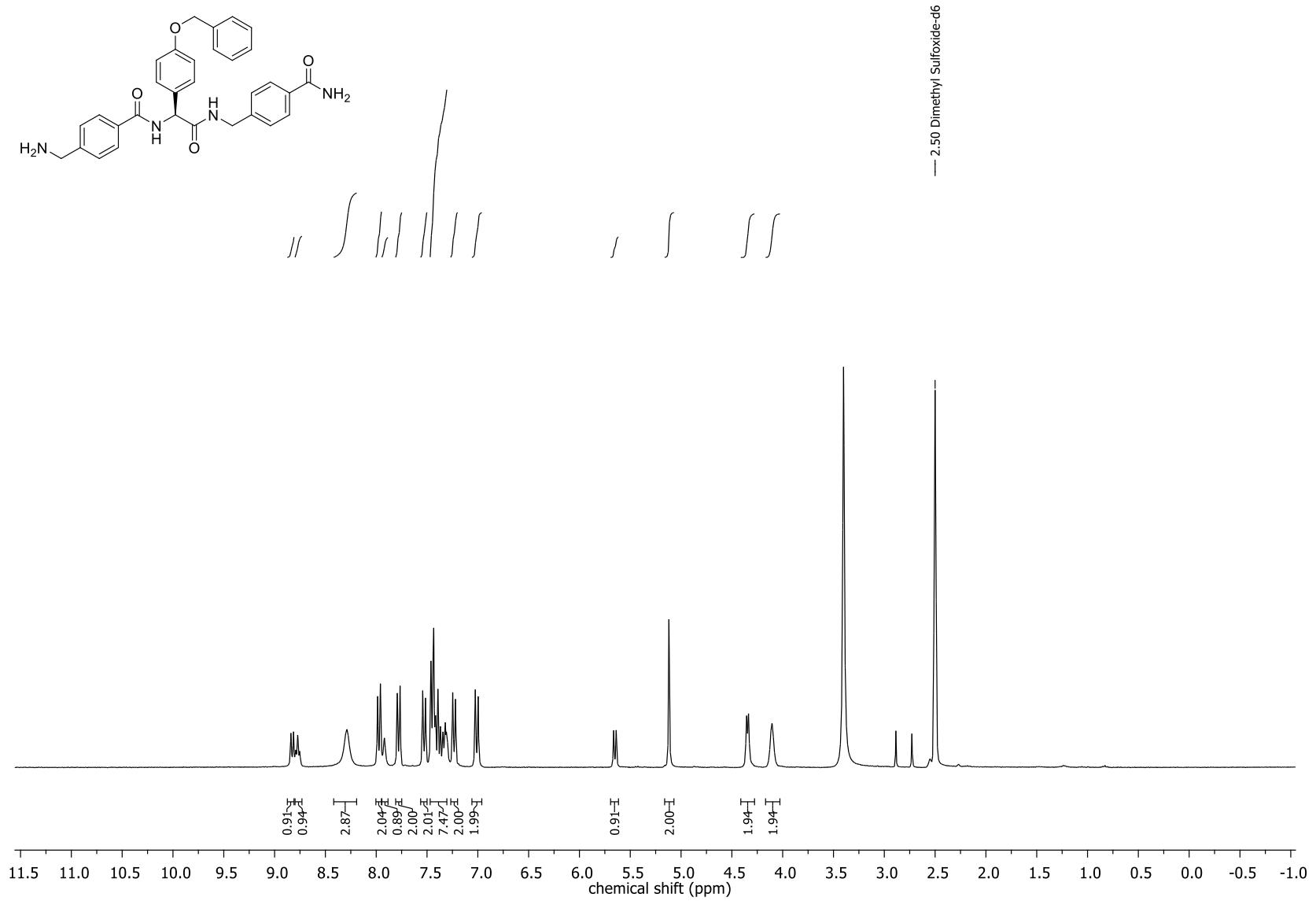
Compound 49, ^1H NMR (300 MHz, CD_3OD)



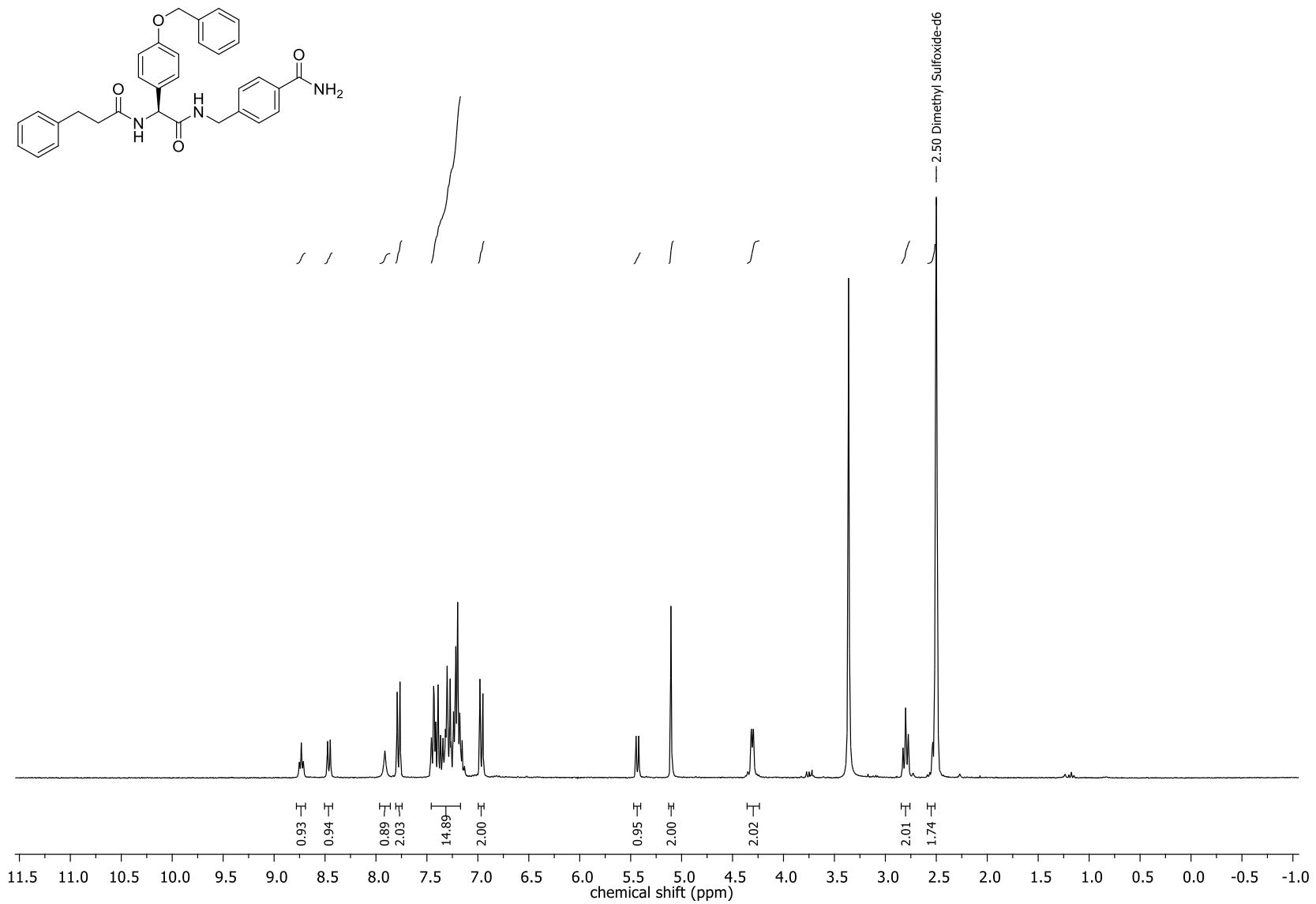
Compound **49**, ^{13}C NMR (APT, 75 MHz, $\text{DMSO}-d_6$)



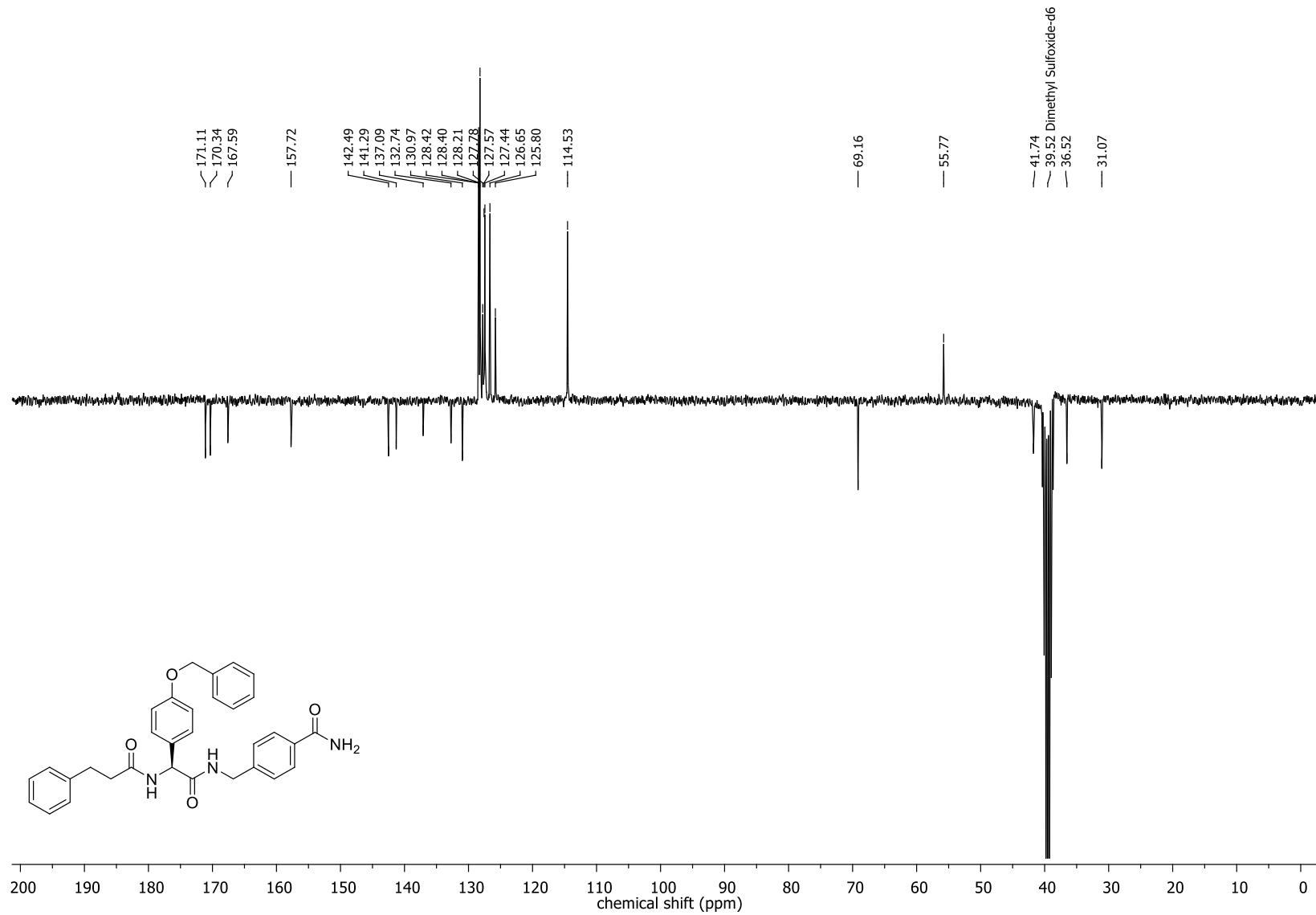
Compound **50**, ^1H NMR (300 MHz, DMSO- d_6)



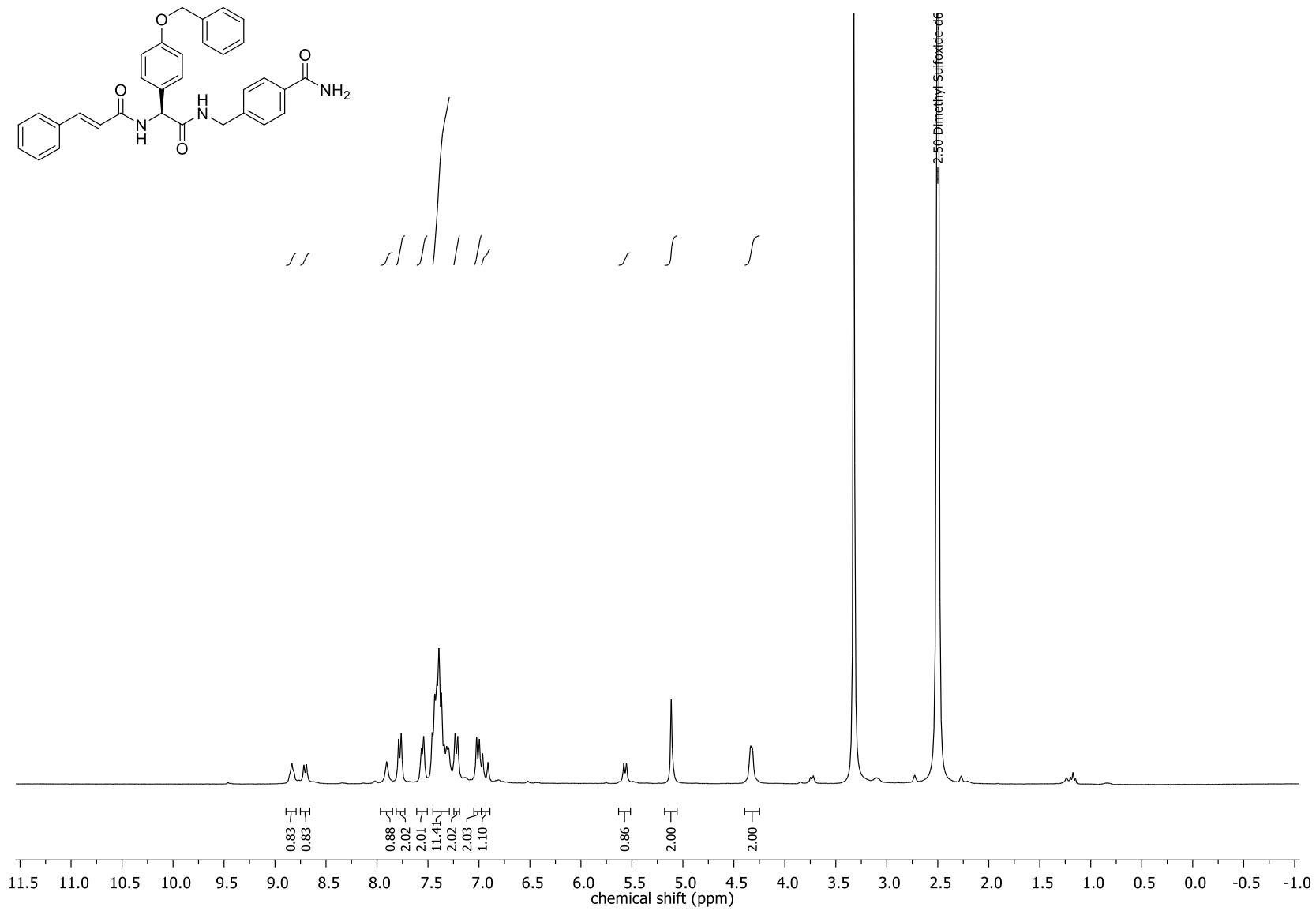
Compound **51**, ^1H NMR (300 MHz, DMSO- d_6)



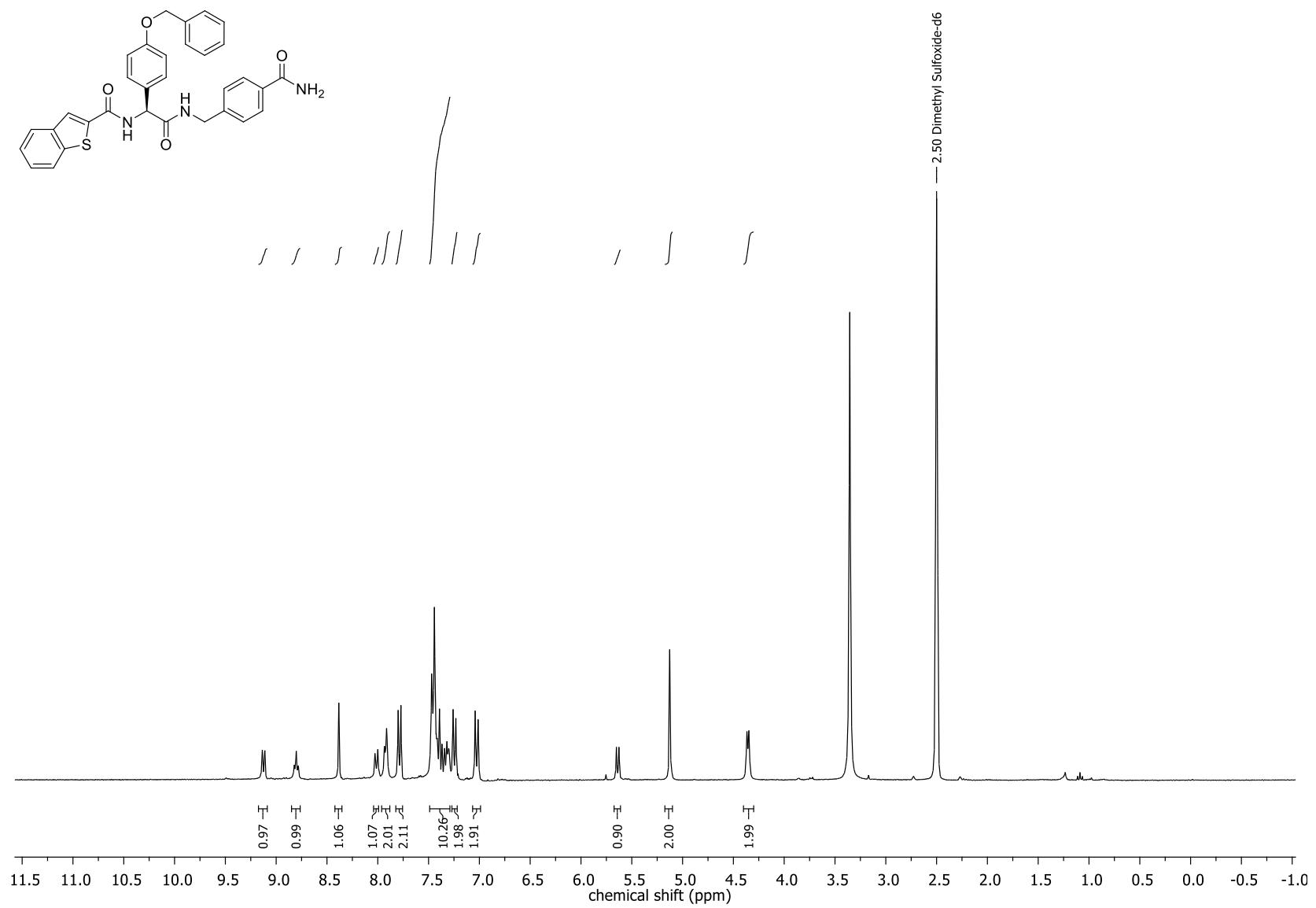
Compound **51**, ^{13}C NMR (APT, 75 MHz, $\text{DMSO}-d_6$)



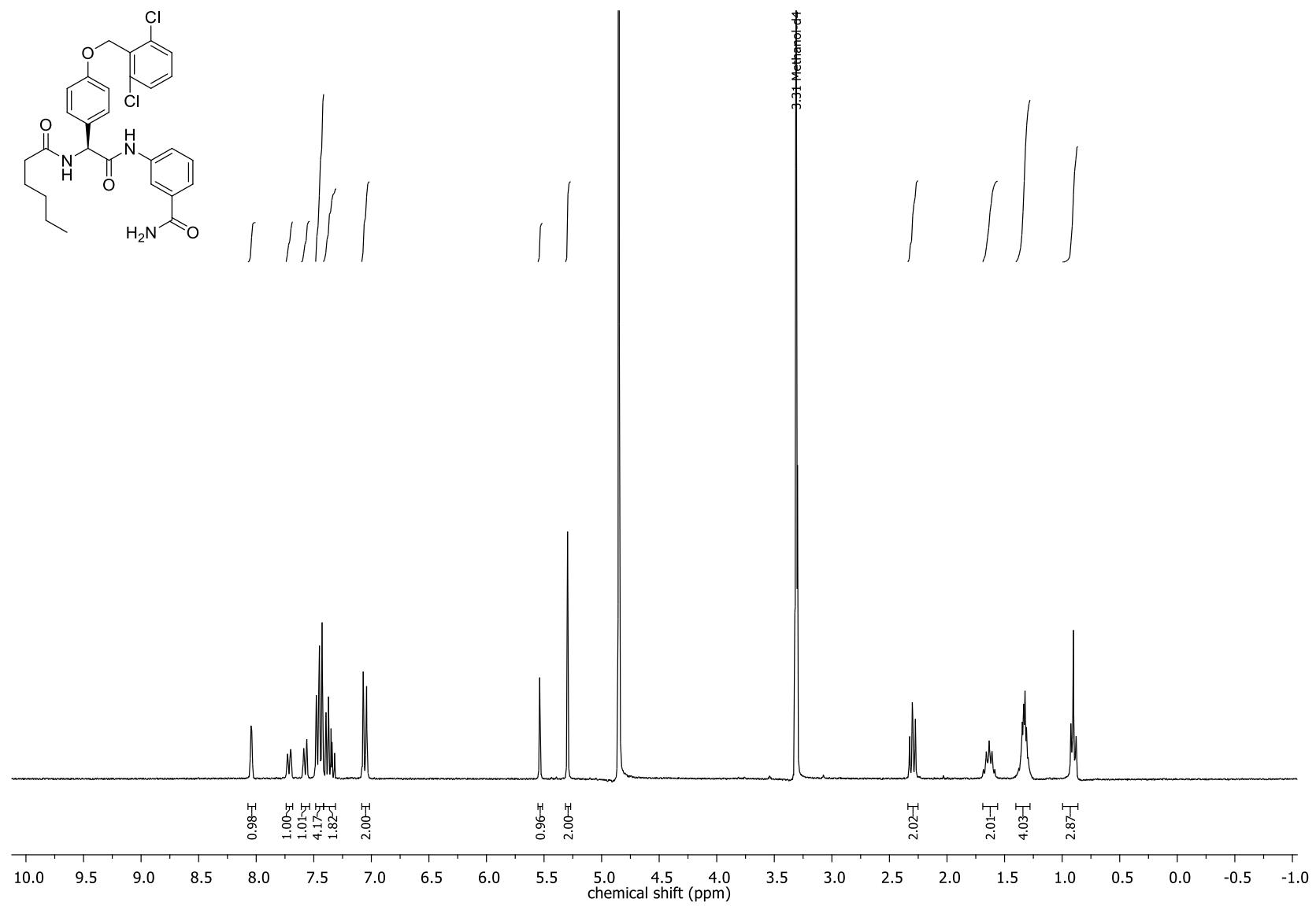
Compound **52**, ^1H NMR (300 MHz, DMSO- d_6)



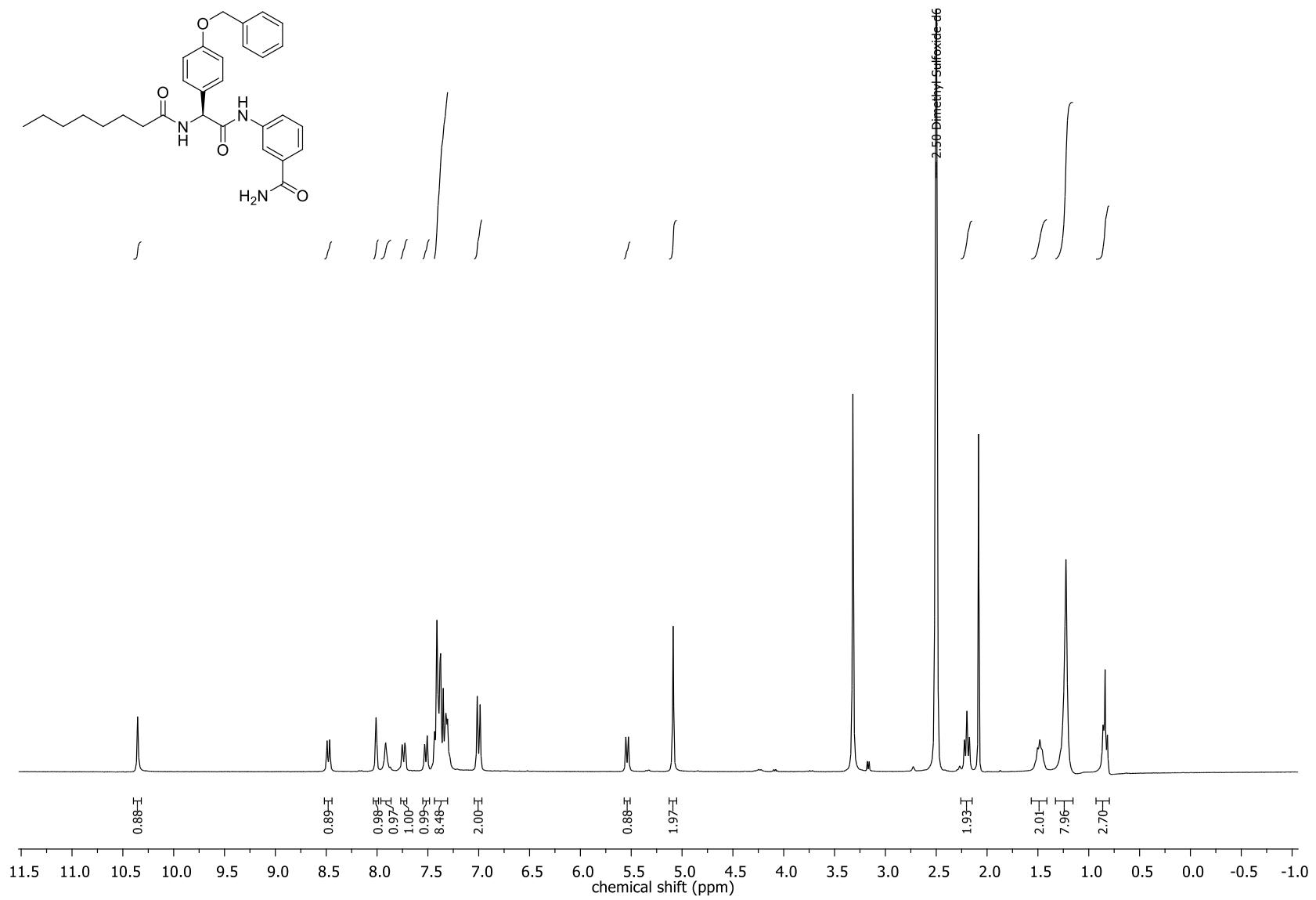
Compound **55**, ^1H NMR (300 MHz, DMSO- d_6)



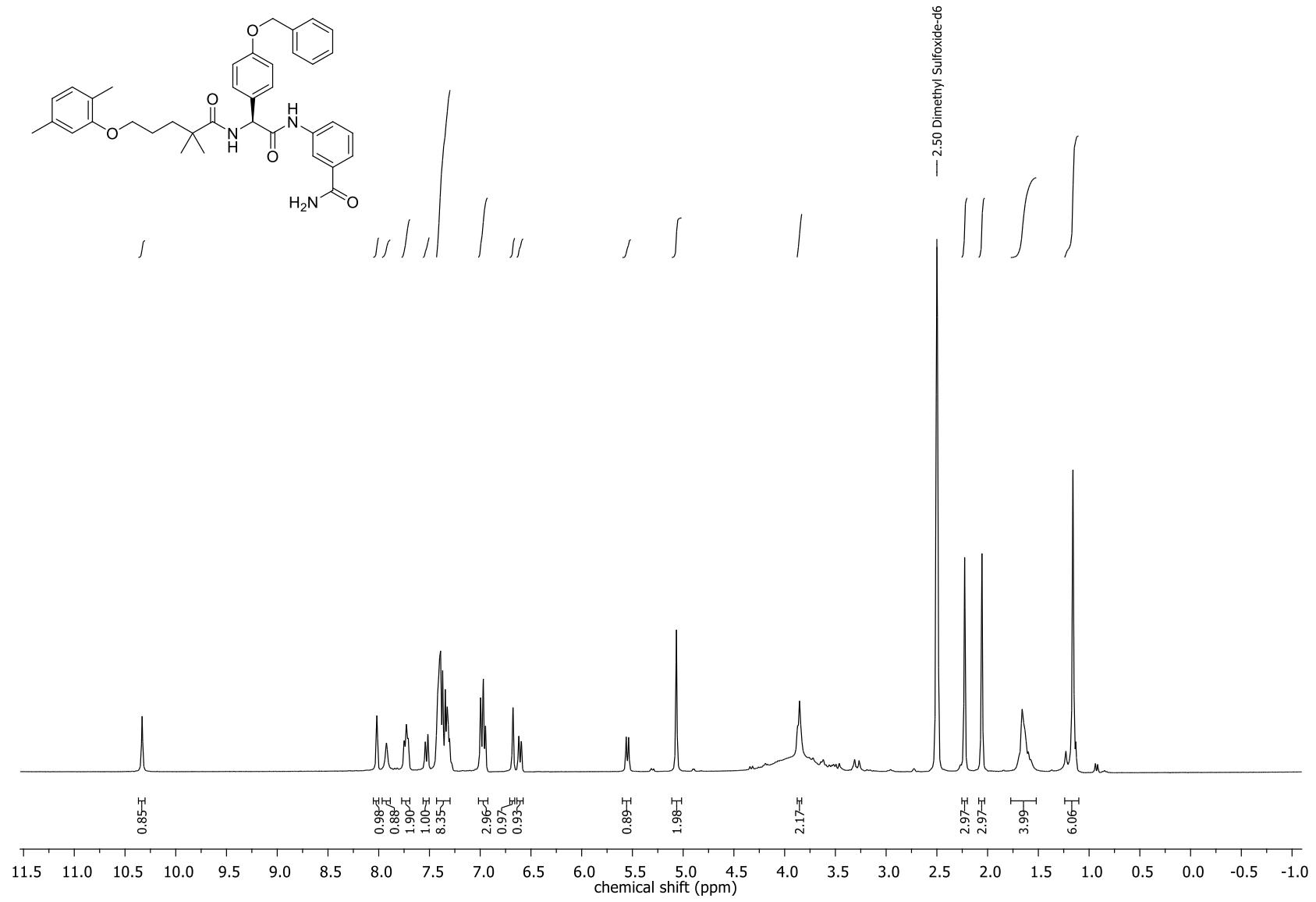
Compound **60**, ^1H NMR (300 MHz, CD_3OD)



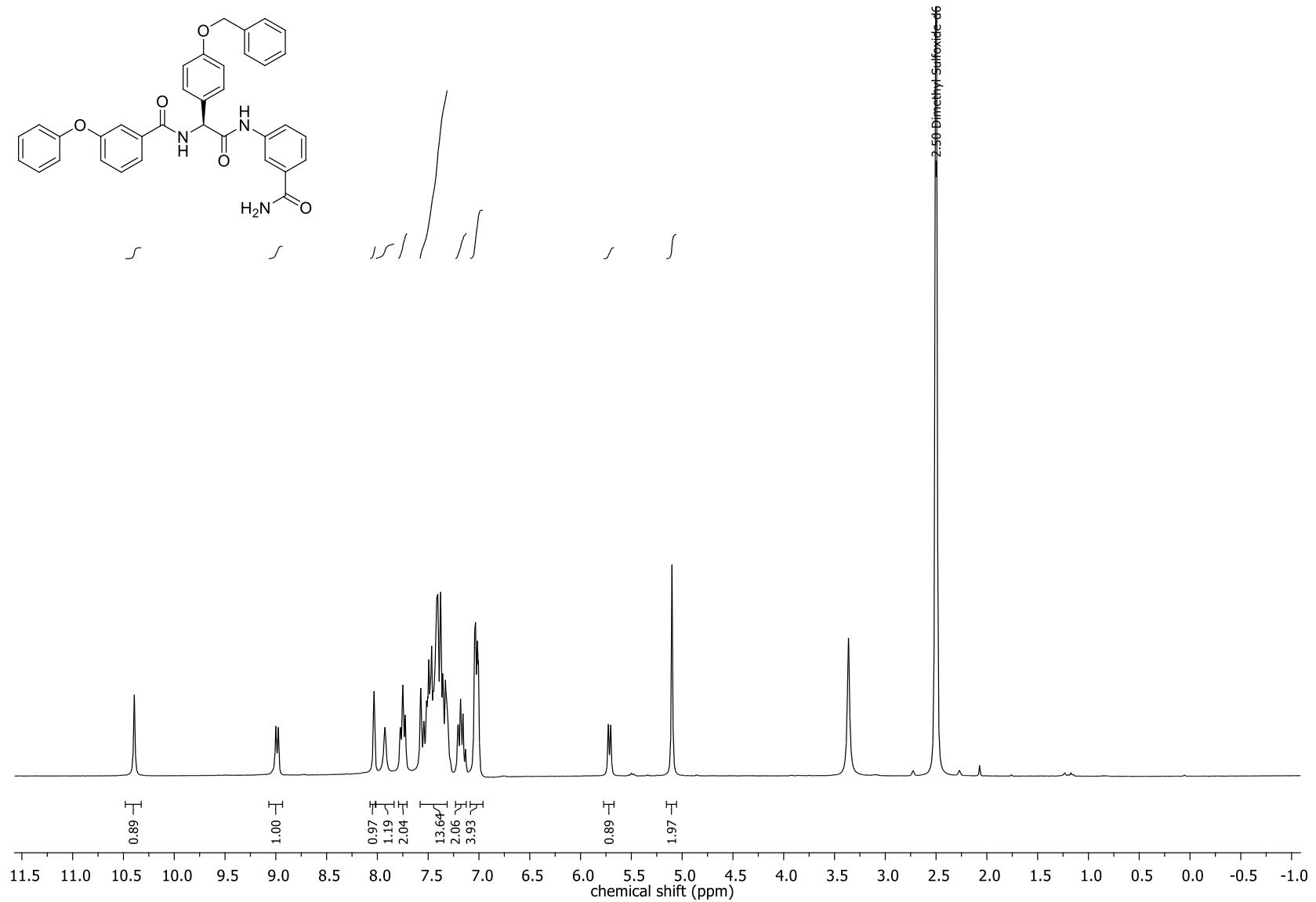
Compound **61**, ^1H NMR (300 MHz, DMSO- d_6)



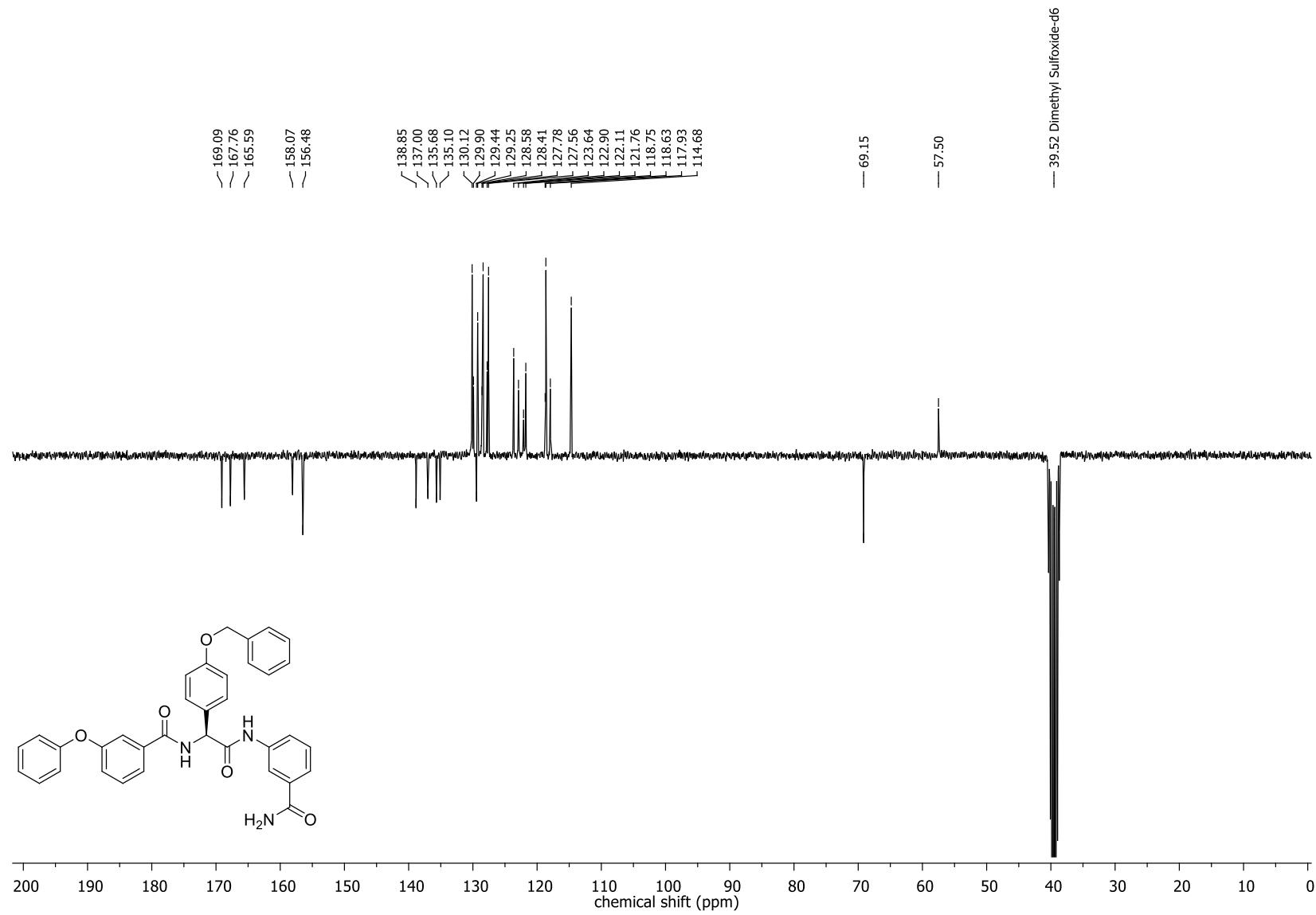
Compound **82**, ^1H NMR (300 MHz, DMSO- d_6)



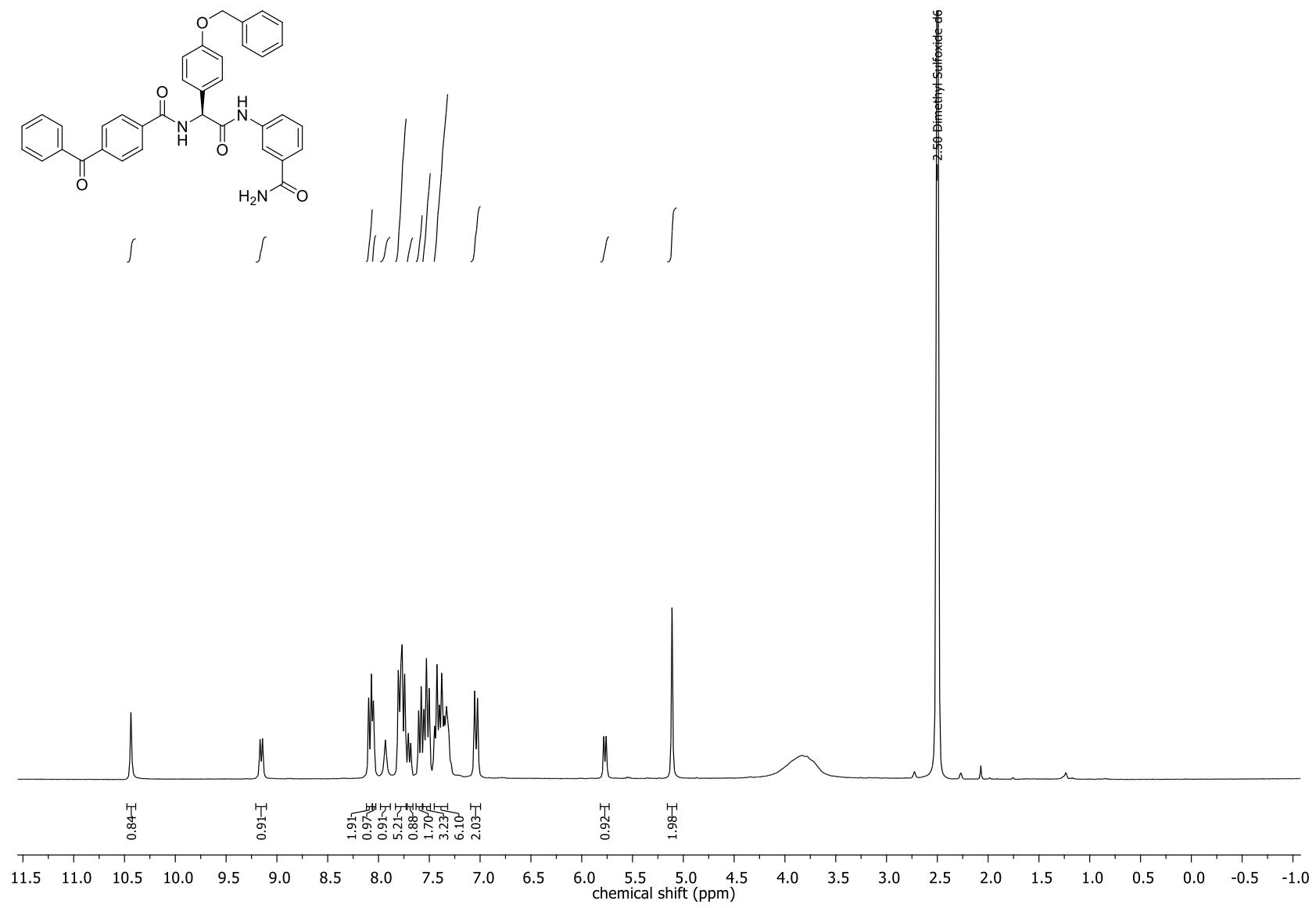
Compound **68**, ^1H NMR (300 MHz, $\text{DMSO}-d_6$)



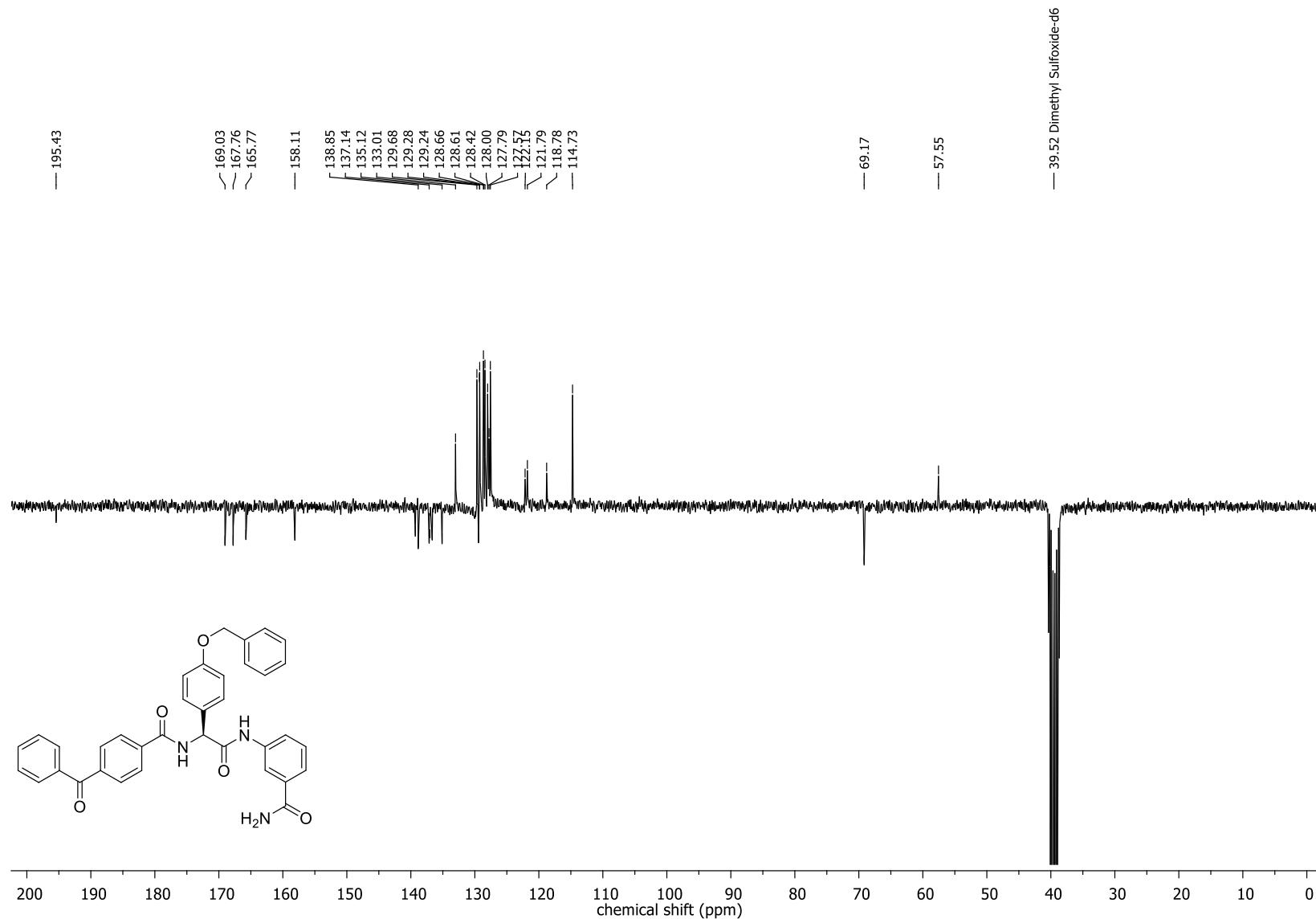
Compound **68**, ^{13}C NMR (APT, 75 MHz, $\text{DMSO}-d_6$)



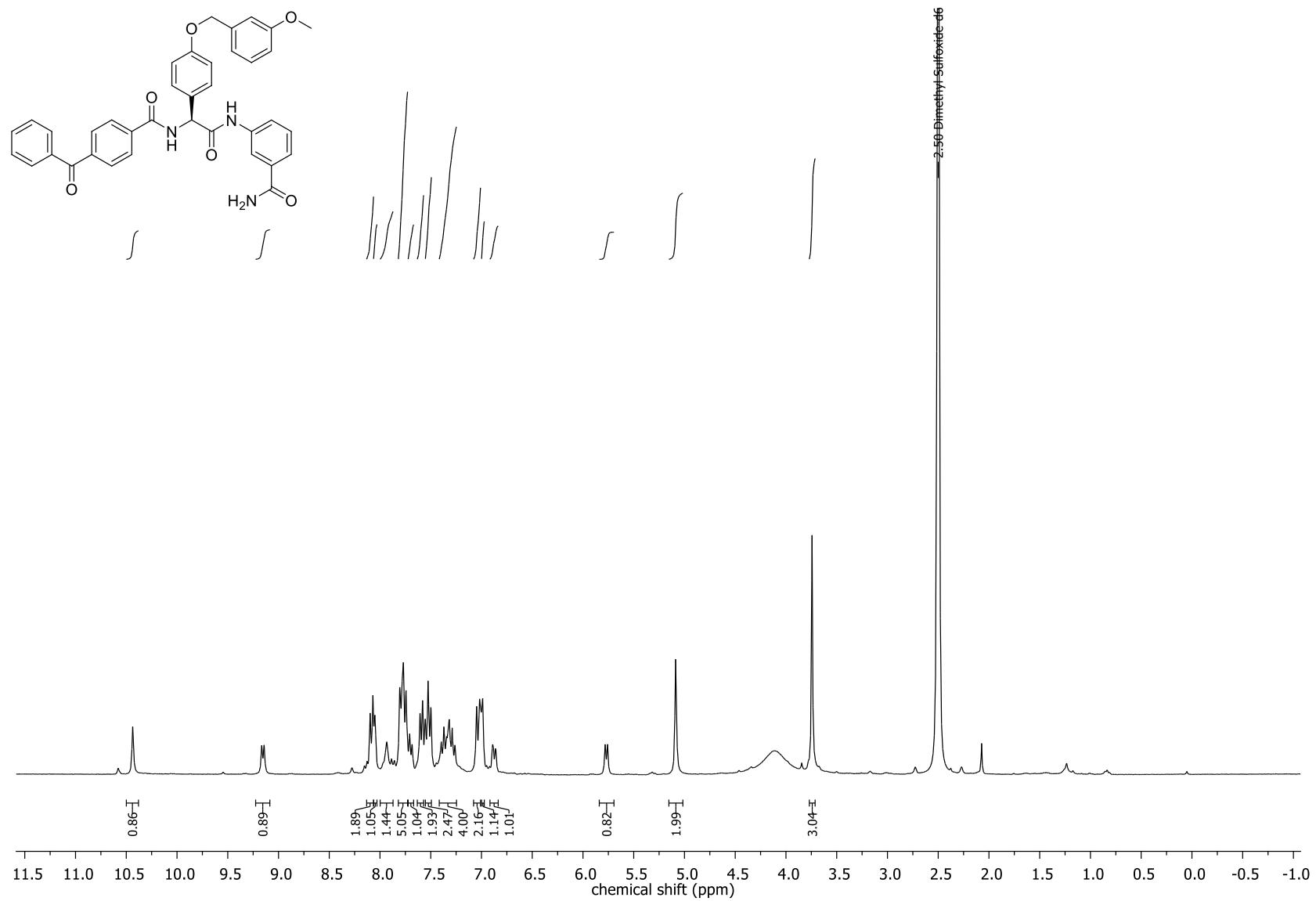
Compound 77, ^1H NMR (300 MHz, $\text{DMSO}-d_6$)



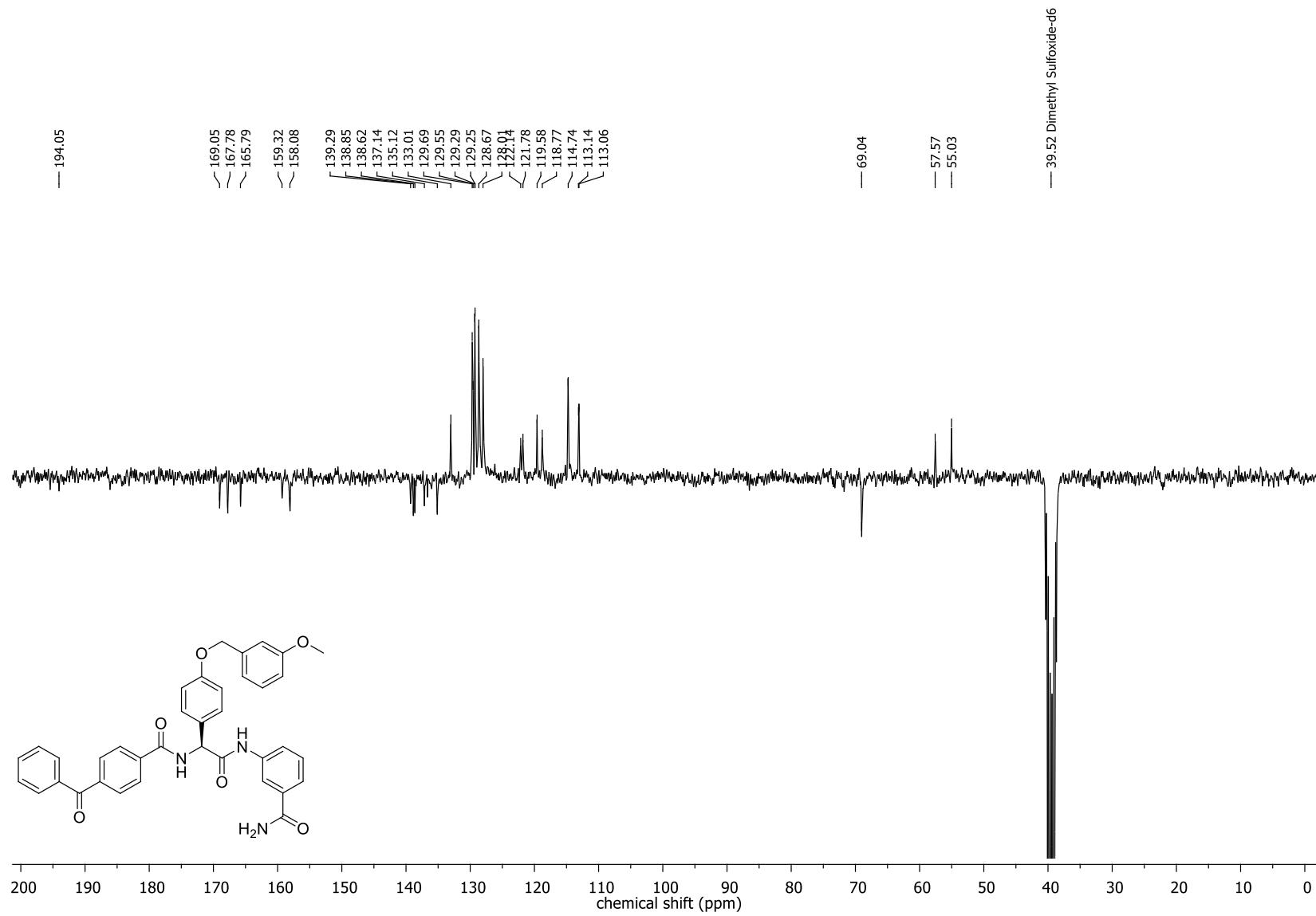
Compound 77, ^{13}C NMR (APT, 75 MHz, $\text{DMSO}-d_6$)



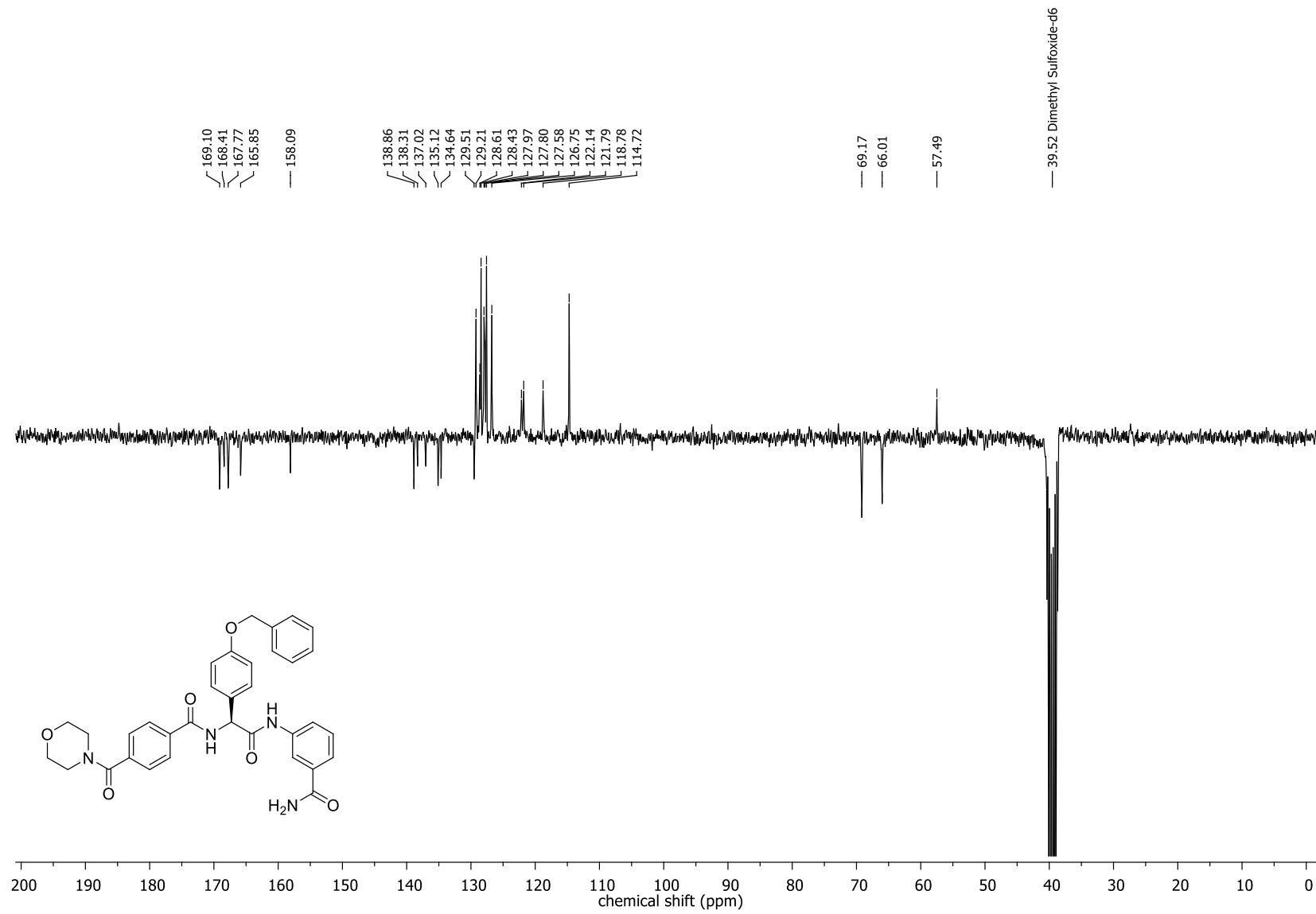
Compound **78**, ^1H NMR (300 MHz, $\text{DMSO}-d_6$)



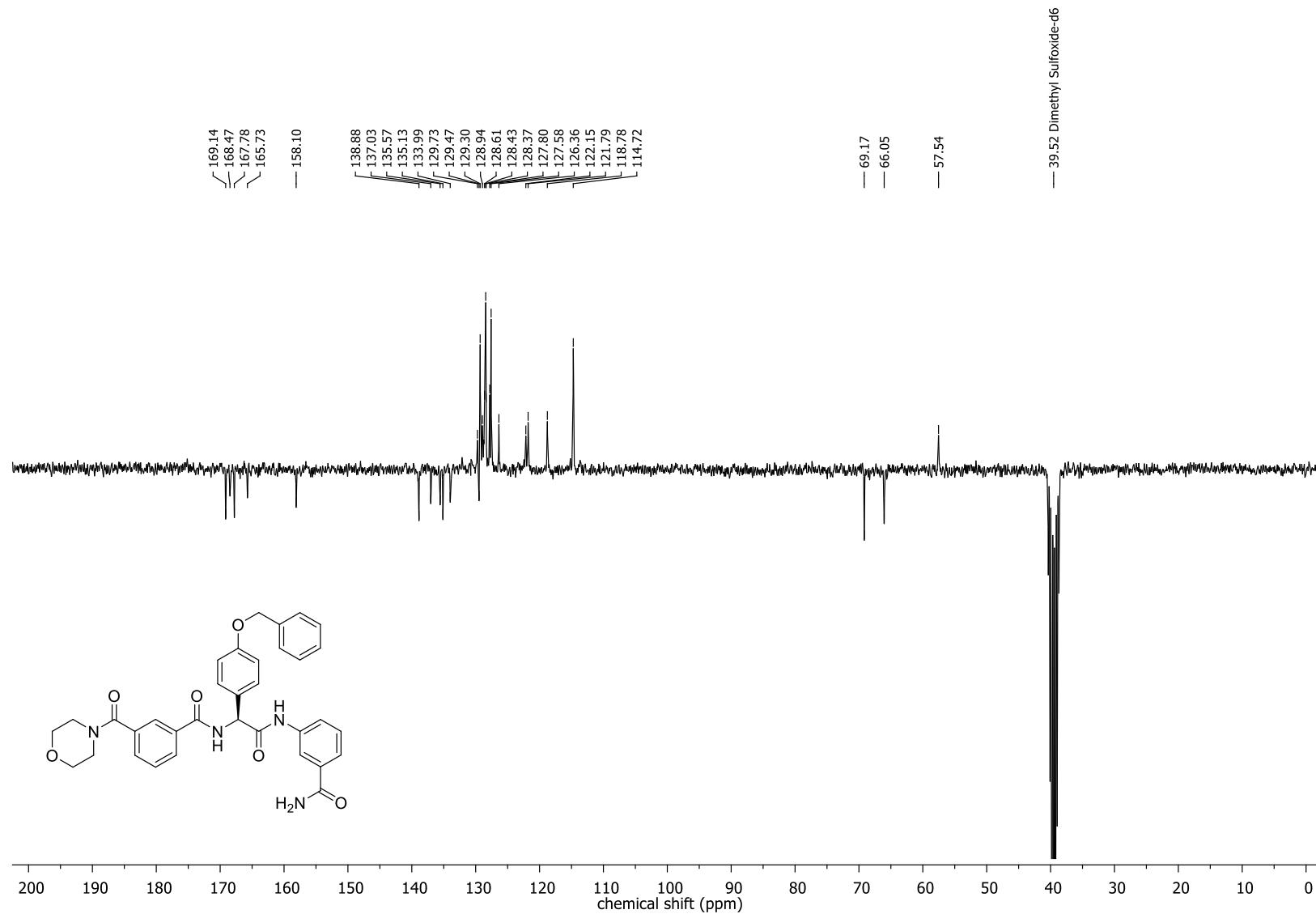
Compound **78**, ^{13}C NMR (APT, 75 MHz, $\text{DMSO}-d_6$)



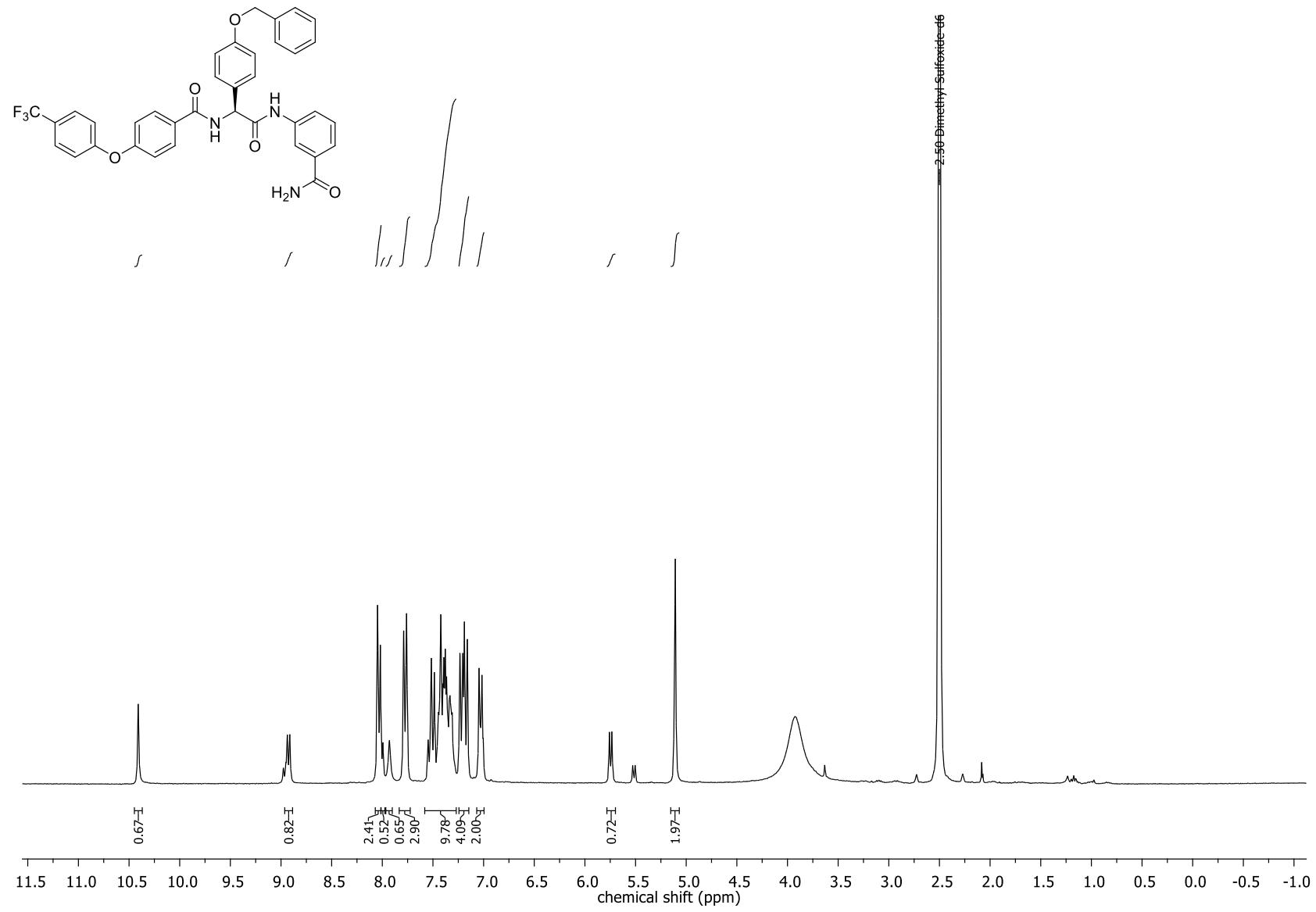
Compound **79**, ^{13}C NMR (APT, 75 MHz, $\text{DMSO}-d_6$)



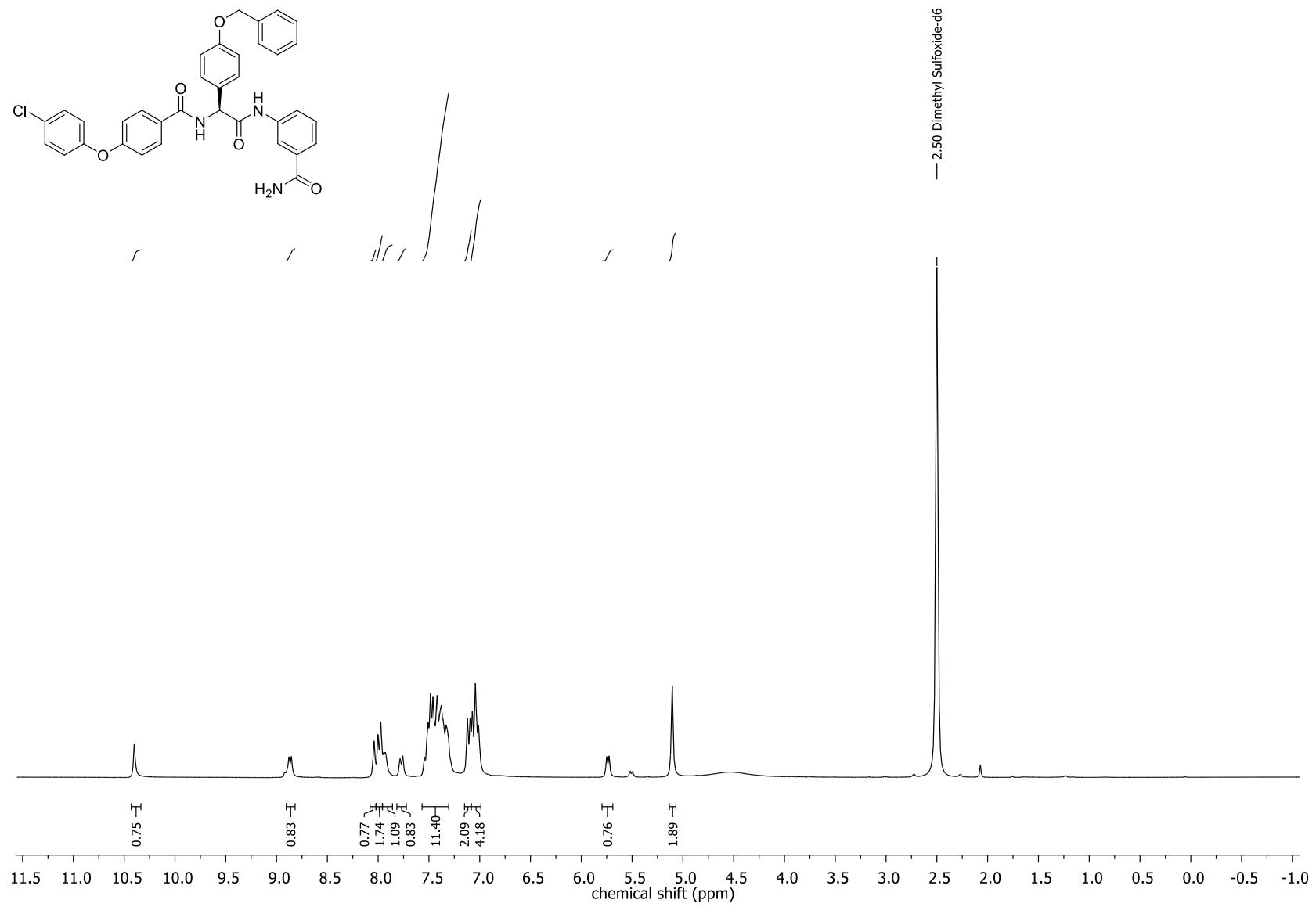
Compound 80, ^{13}C NMR (APT, 75 MHz, DMSO- d_6)



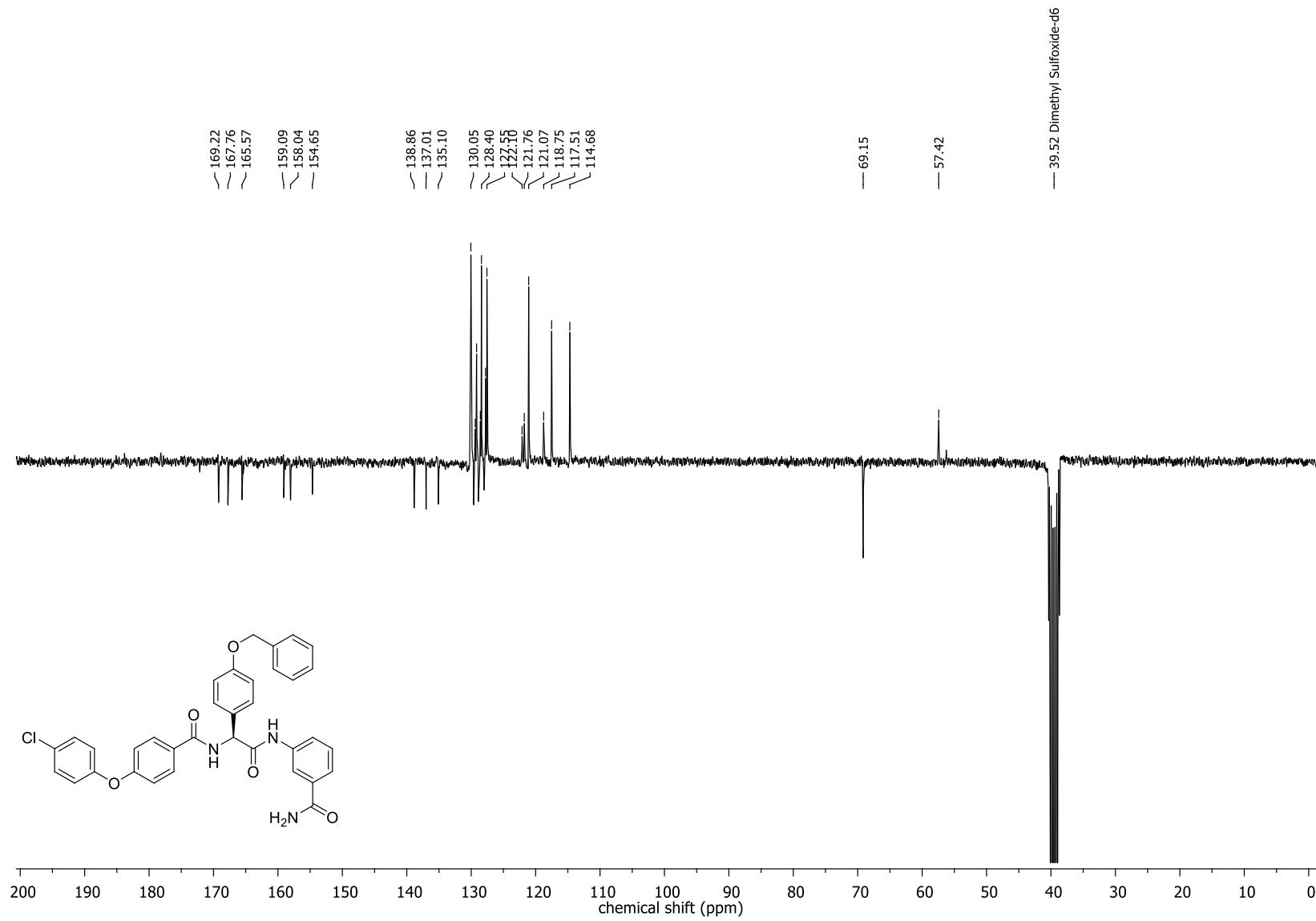
Compound **71**, ^1H NMR (300 MHz, $\text{DMSO}-d_6$)



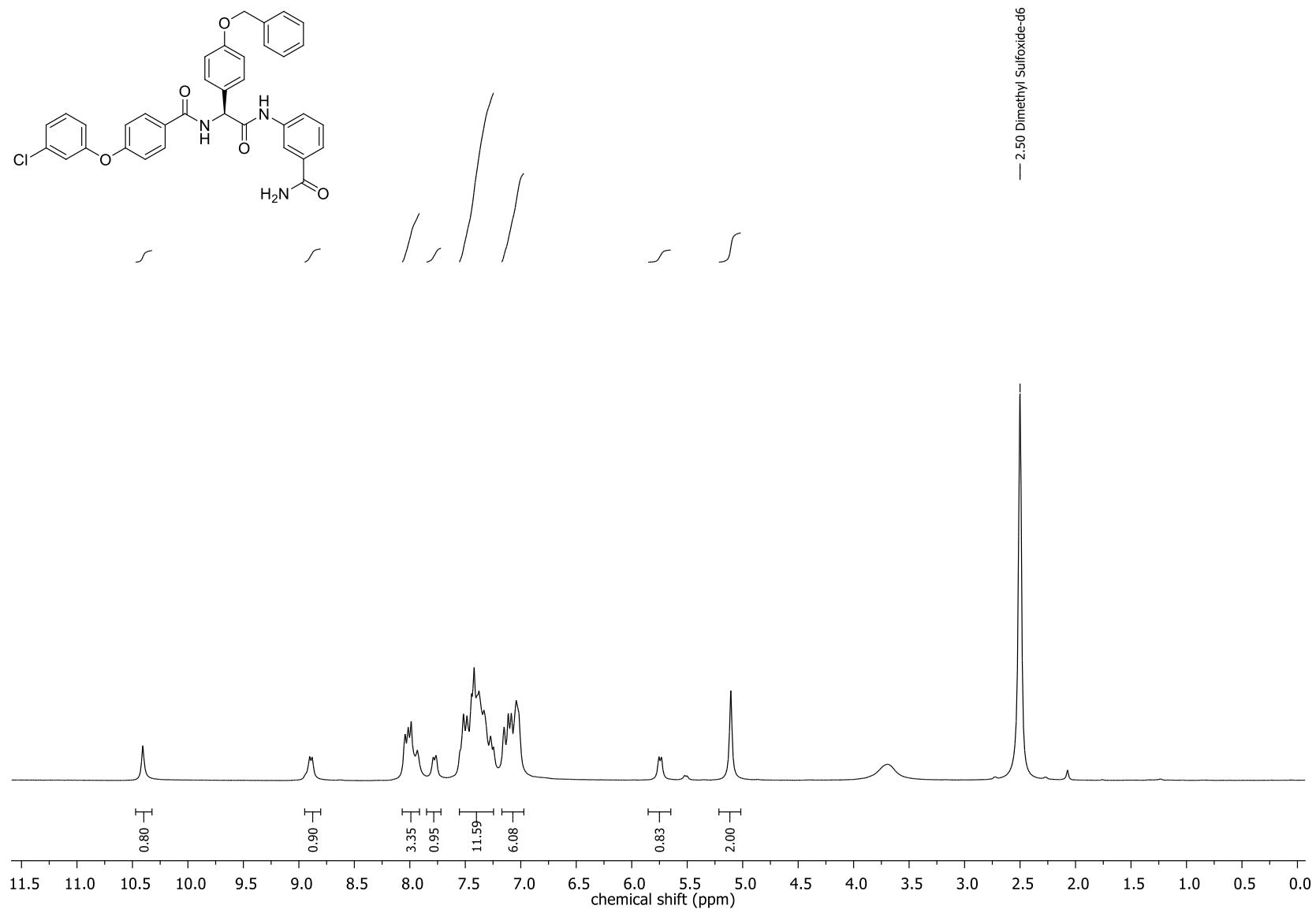
Compound **72**, ^1H NMR (300 MHz, DMSO- d_6)



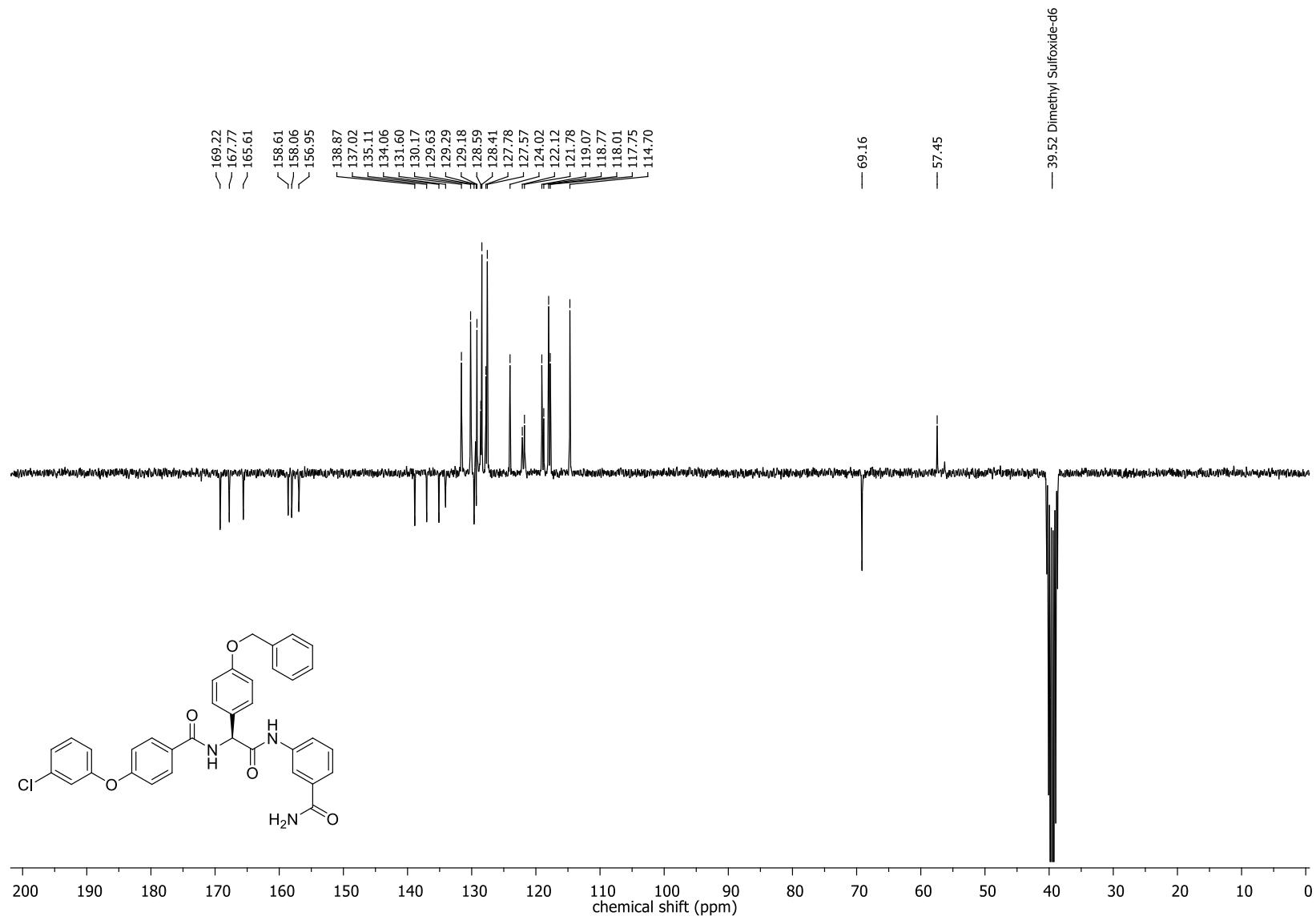
Compound **72**, ^{13}C NMR (APT, 75 MHz, $\text{DMSO}-d_6$)



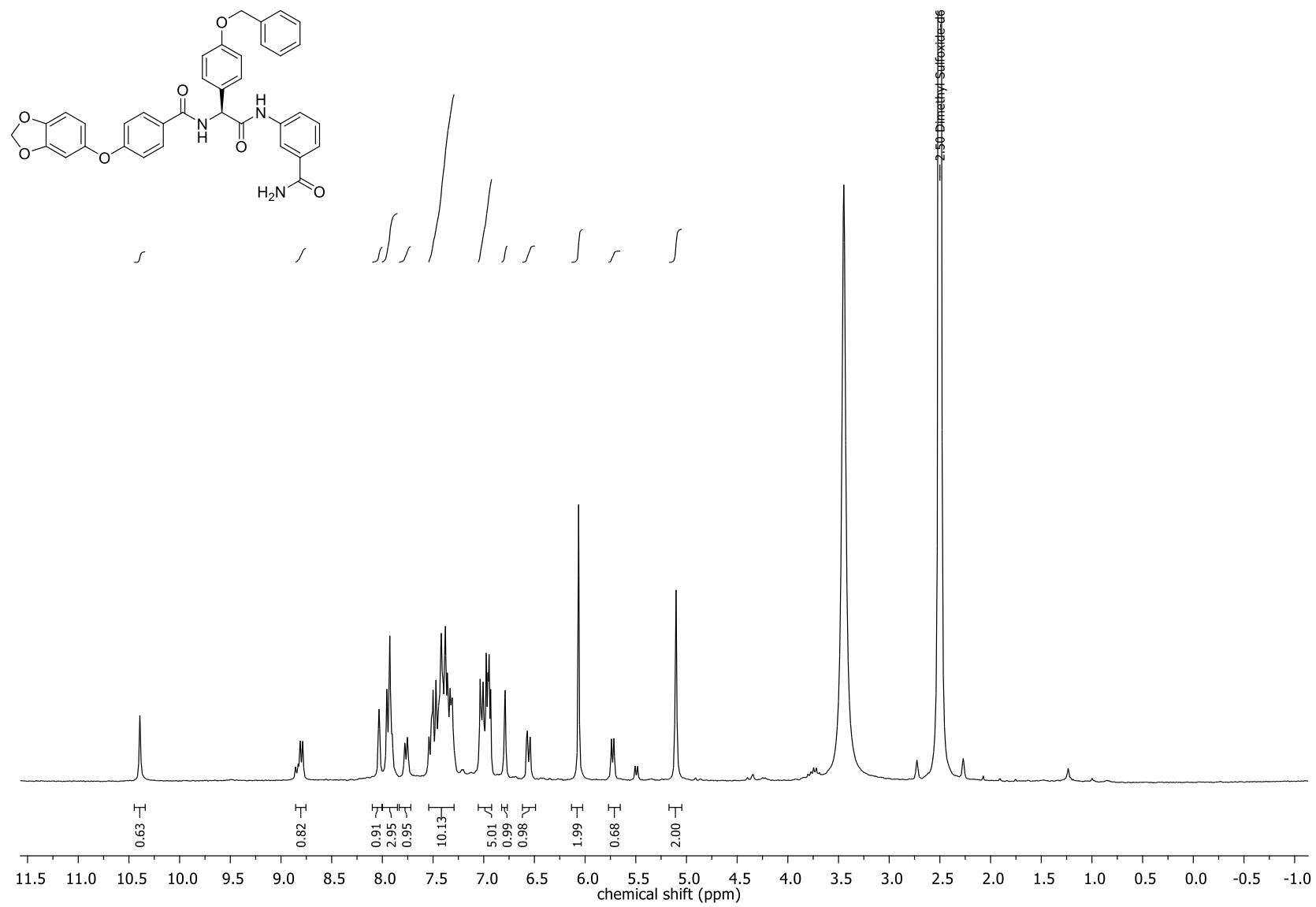
Compound **73**, ^1H NMR (300 MHz, DMSO- d_6)



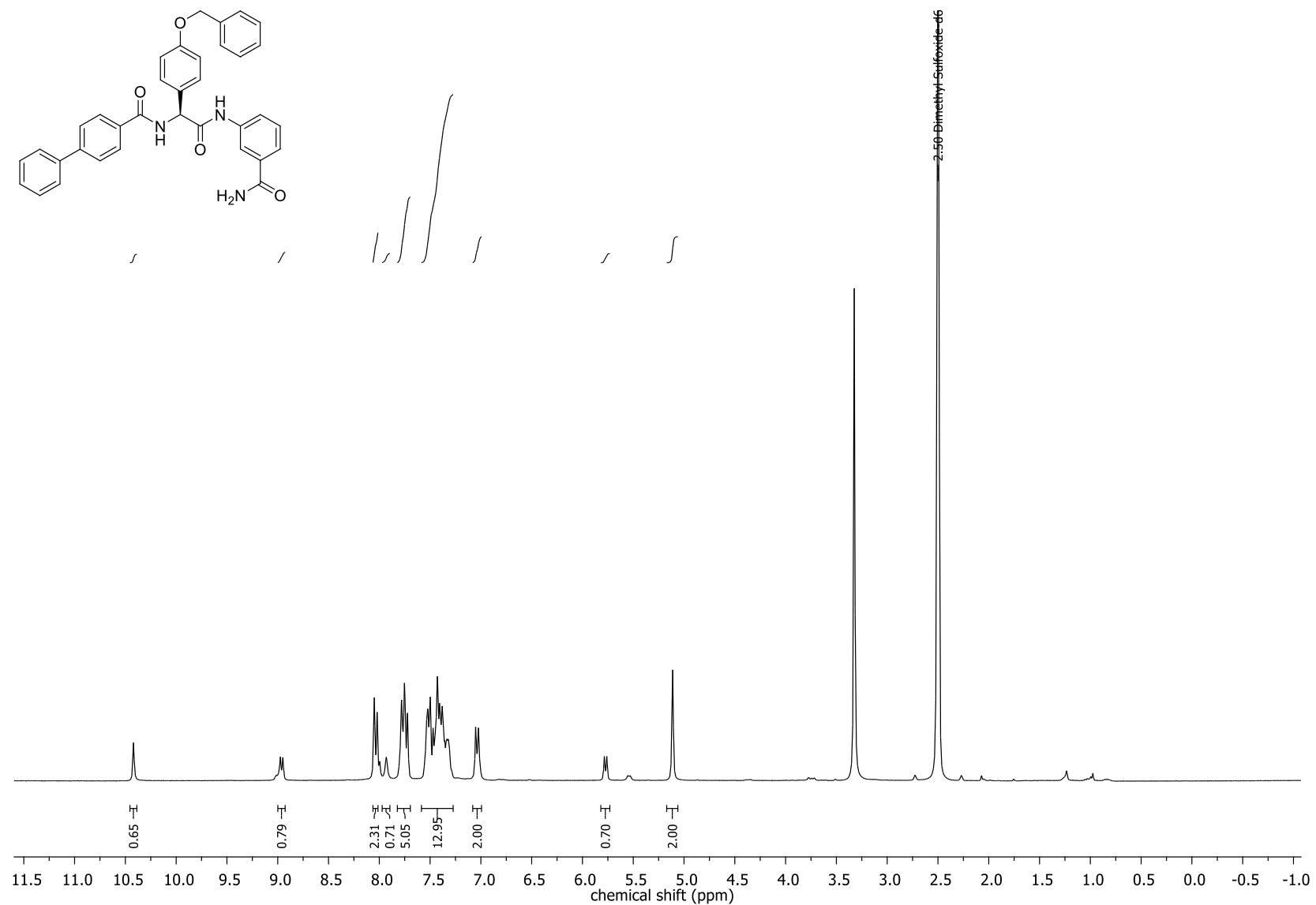
Compound **73**, ^{13}C NMR (APT, 75 MHz, $\text{DMSO}-d_6$)



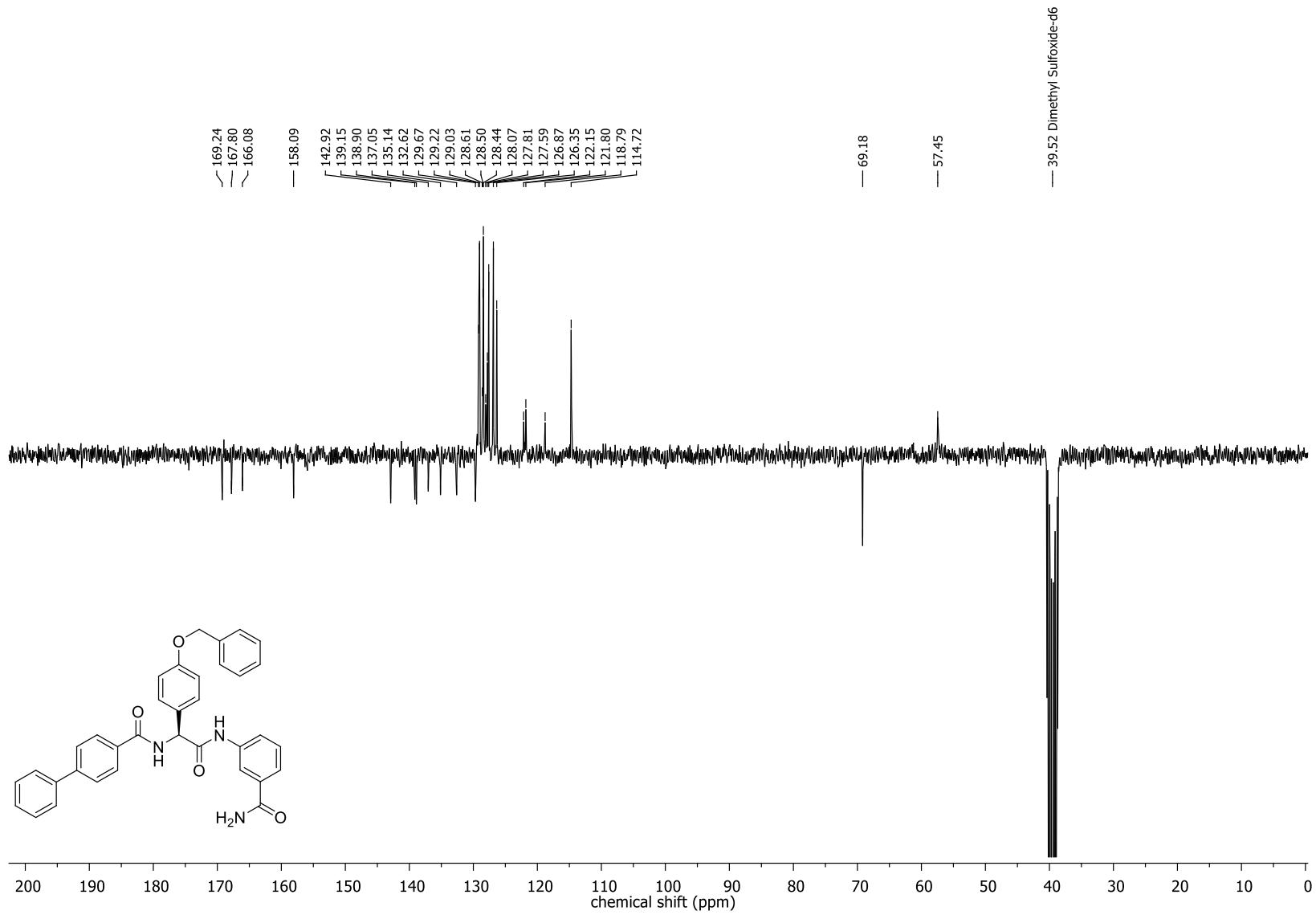
Compound 74, ^1H NMR (300 MHz, $\text{DMSO}-d_6$)



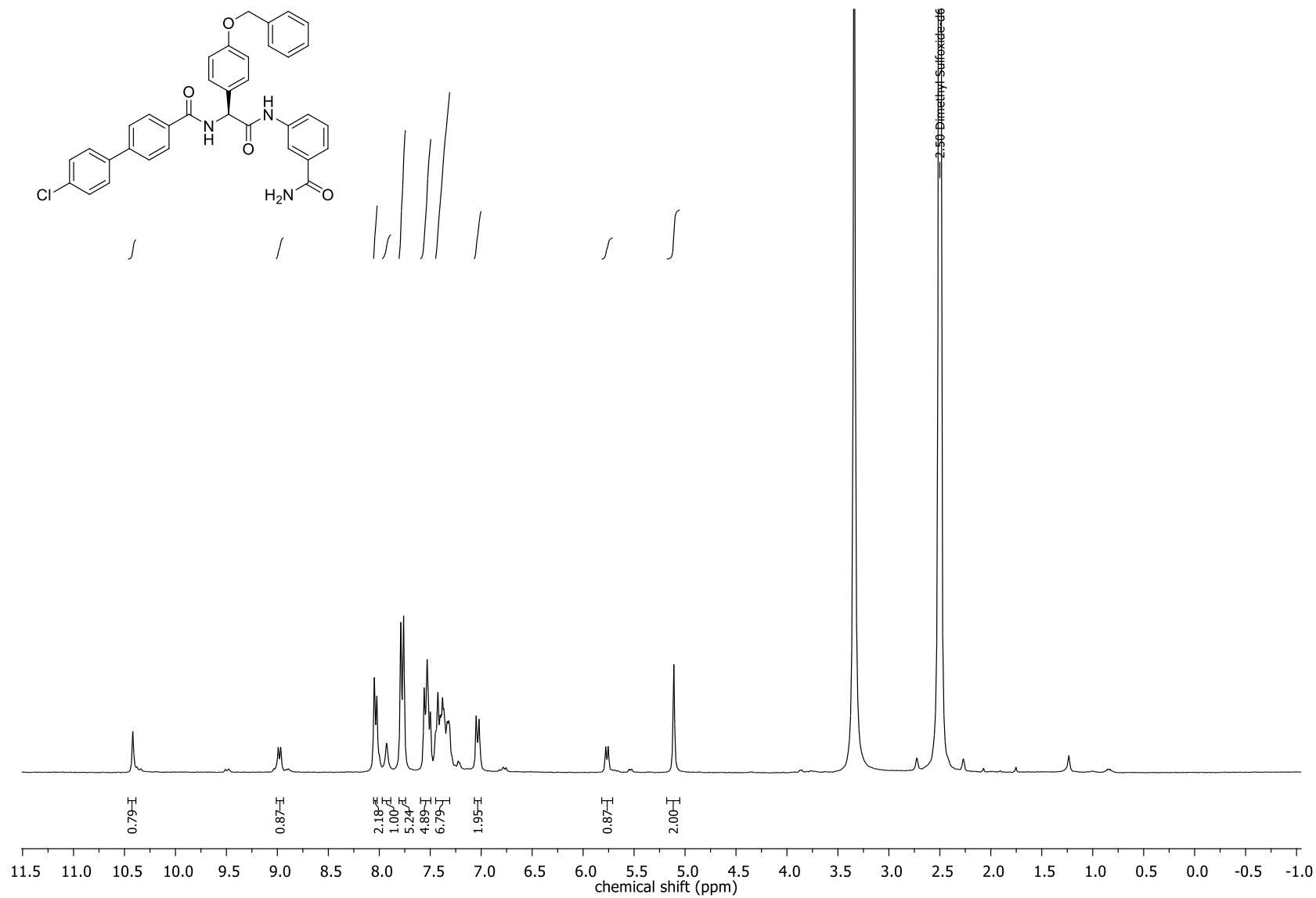
Compound **83**, ^1H NMR (300 MHz, DMSO- d_6)



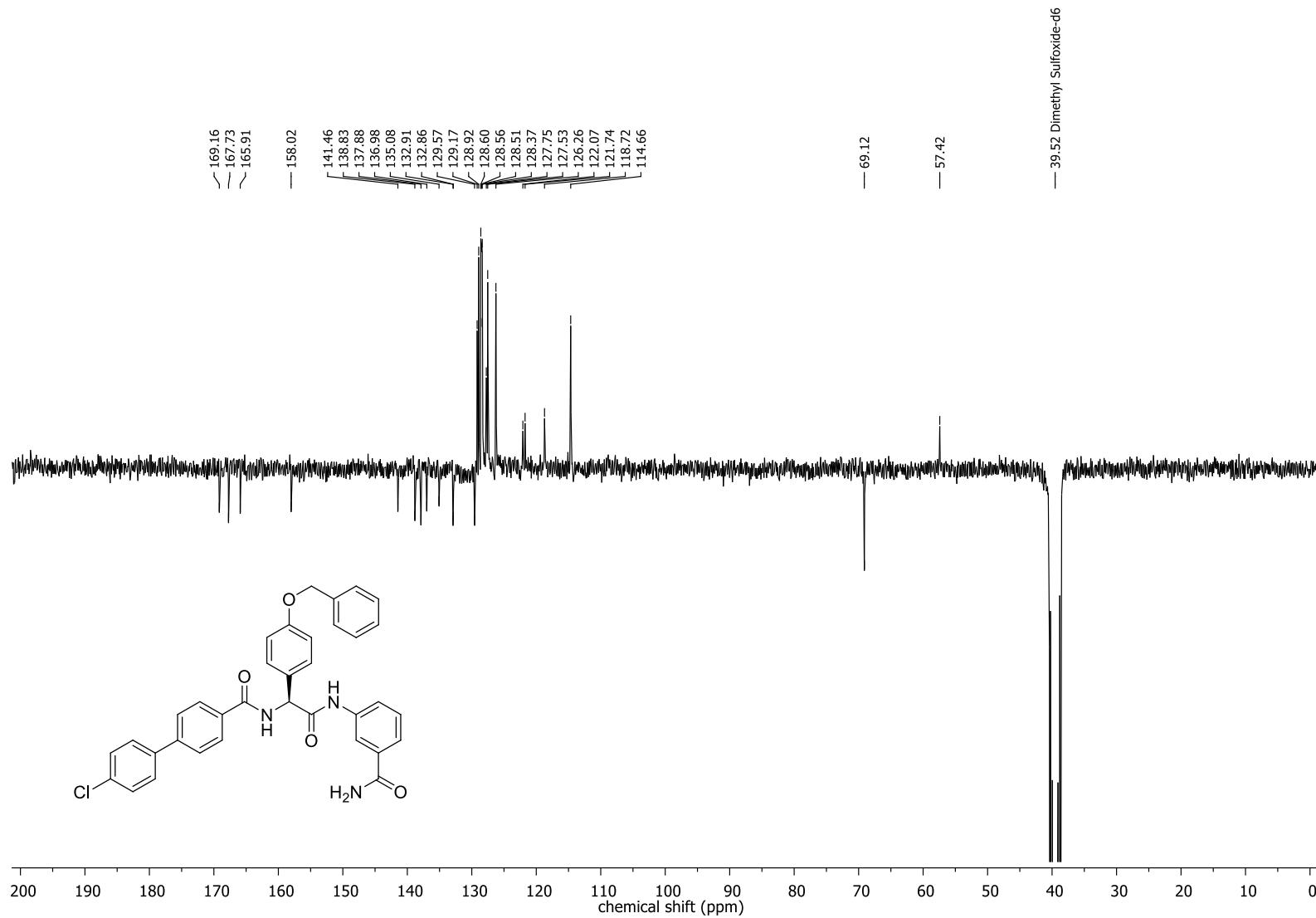
Compound 83, ^{13}C NMR (APT, 75 MHz, $\text{DMSO}-d_6$)



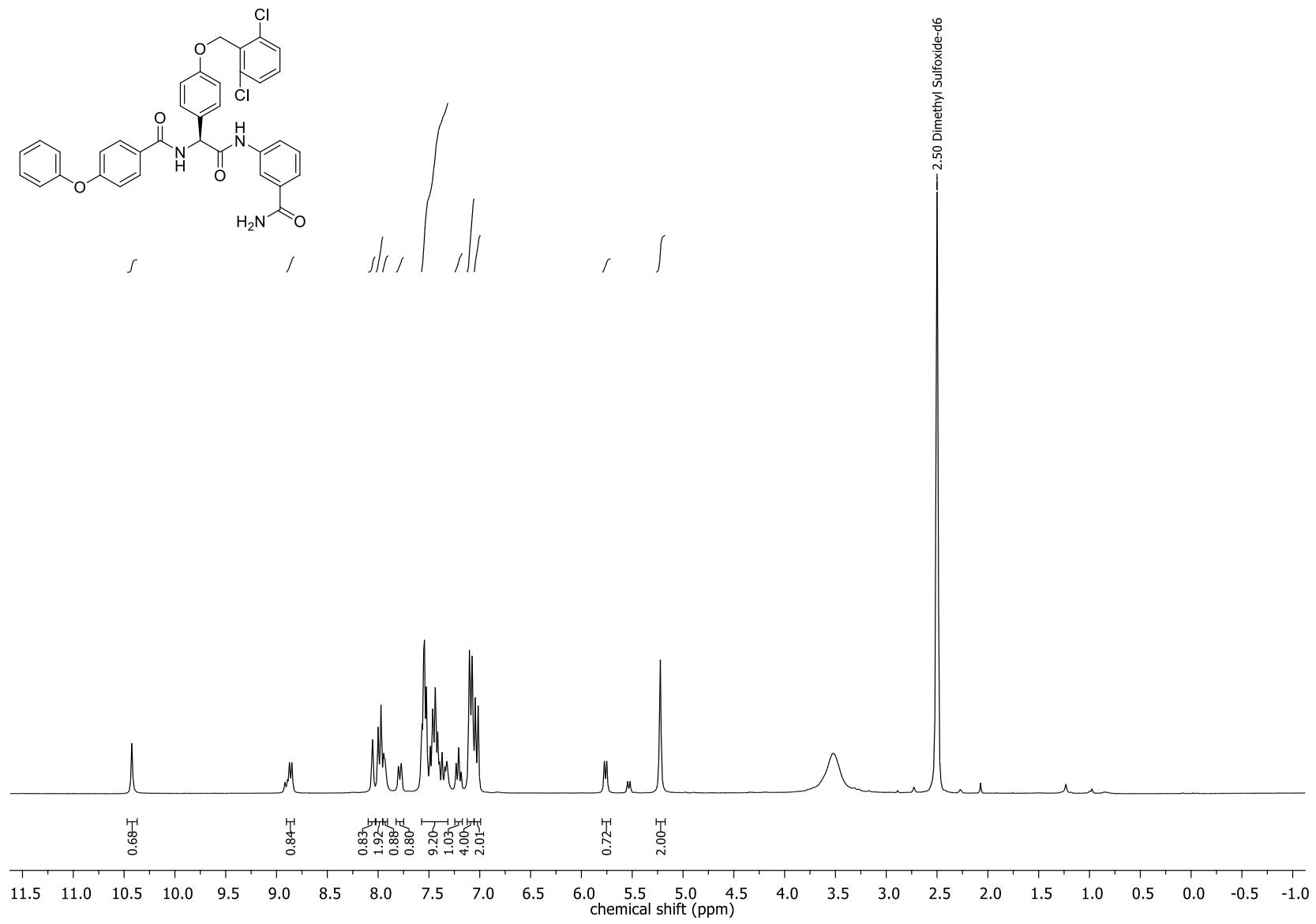
Compound **84**, ^1H NMR (300 MHz, $\text{DMSO}-d_6$)



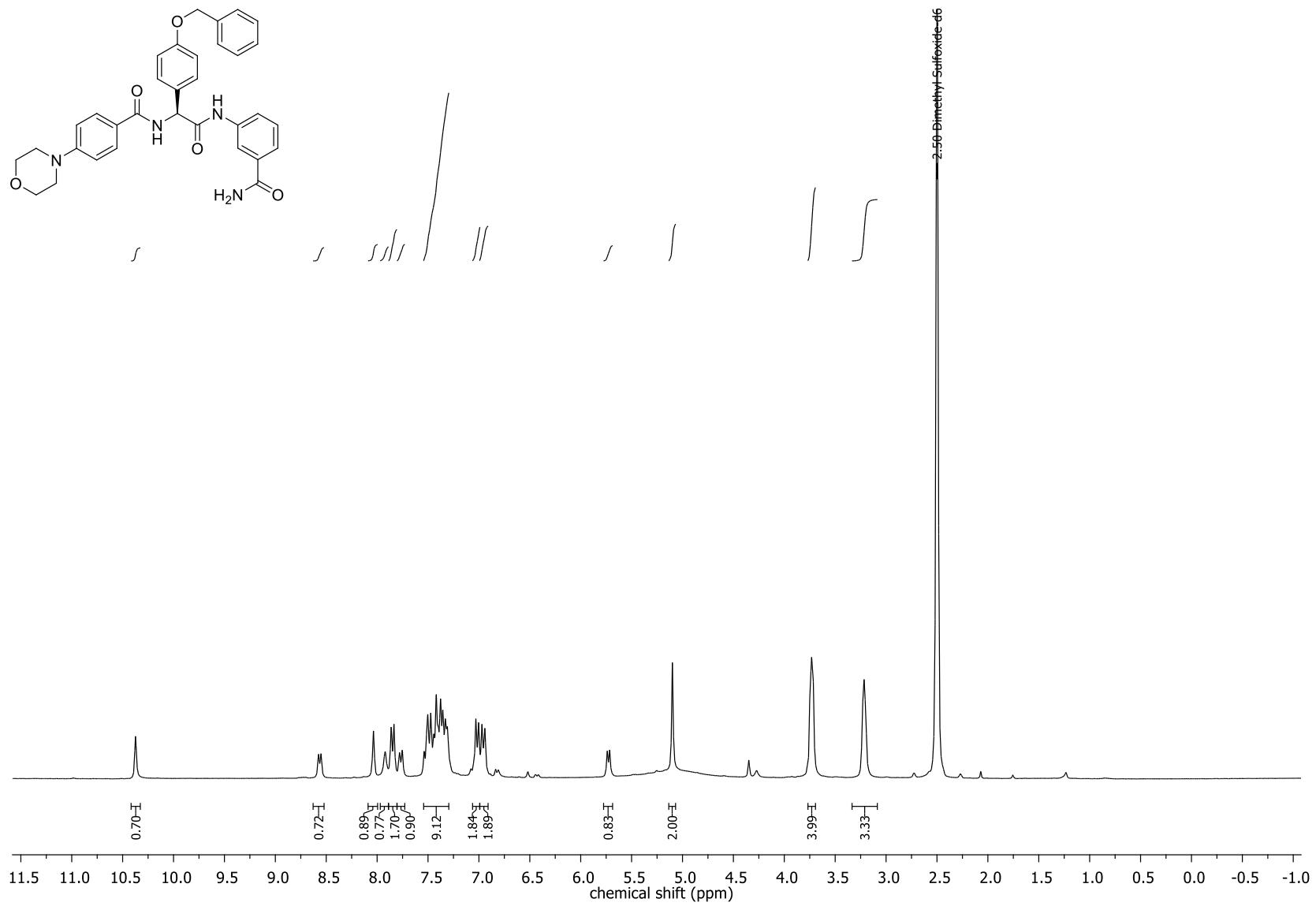
Compound **84**, ^{13}C NMR (APT, 75 MHz, $\text{DMSO}-d_6$)



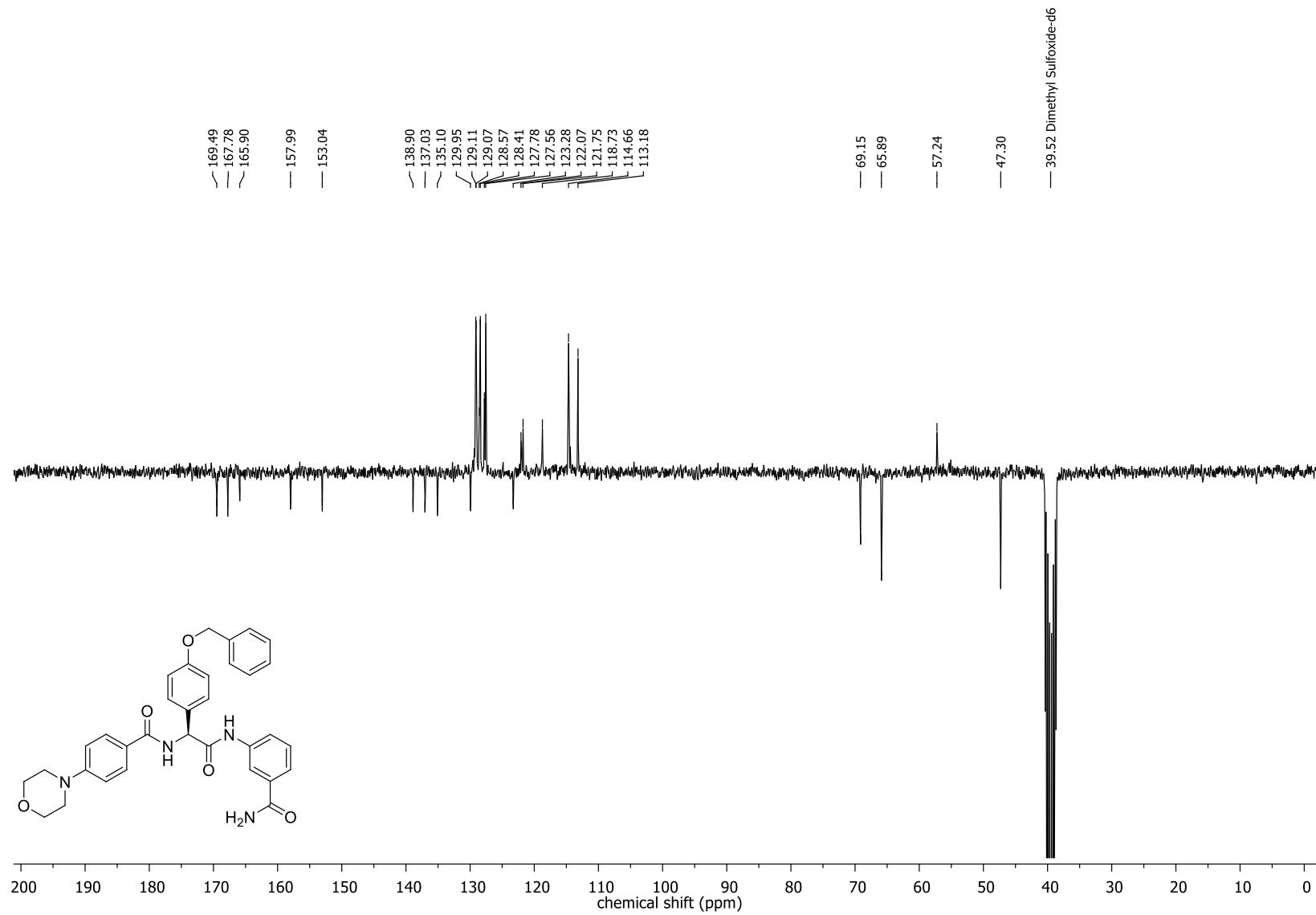
Compound **64**, ^1H NMR (300 MHz, DMSO- d_6)



Compound **88**, ^1H NMR (300 MHz, DMSO- d_6)



Compound **88**, ^{13}C NMR (APT, 75 MHz, $\text{DMSO}-d_6$)



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