Supporting Information

Ulleungamides A and B, Modified *α,β*-Dehydropipecolic Acid-Containing Cyclic Depsipeptides from *Streptomyces* sp. KCB13F003

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Experimental Section

1-1 General experimental procedures

ODS (75 μm, Cosmosil) was used for vacuum liquid chromatography with LP grade solvents (SK chemicals). Analytic C_{18} (4.6 × 150 mm, 5 μm, YMC), semi-preparative C_{18} (10 × 250 mm, 10 μm, Optimapak), and preparative C_{18} (20 × 250 mm, 10 μm, GROM-SIL) columns were used for HPLC on a YL9100 HPLC system (Younglin) equipped with an YL9160 PDA detector (Younglin) using HPLC grade solvents (Burdick & Jackson). UV spectra were obtained on a Shimadzu UV-1601 spectrophotometer. Specific rotations were measured on a JASCO P-1020 polarimeter using a 100 mm glass microcell. Circular dichroism (CD) spectra were measured on Jasco J-715 spectropolarimeter at 298K using a quartz cuvette of 0.1 cm path length. The NMR spectra were recorded on a Bruker Biospin Advance II 900 NMR spectrometer (900 MHz for ^{1}H and 225 MHz for ^{13}C), Bruker AVANCE HD 800 NMR spectrometer (800 MHz for ^{1}H and 200 MHz for ^{13}C), and Bruker AVANCE HD 700 NMR spectrometer (700 MHz for ^{1}H and 175 MHz for ^{13}C) at Korea Basic Science Institute (KBSI) in Ochang, Korea. NMR spectra were recorded in DMSO-d_6 and chemical shifts were referenced to residual solvent signal. High resolution electrospray ionization mass spectrometry (HRESIMS) data were acquired on a Q-TOF mass spectrometer (SYNAPT G2, Waters) at KBSI in Ochang, Korea. A liquid chromatography-mass spectrometry (LC-MS) was performed using an LTQ XL linear ion trap (Thermo Scientific, USA) equipped with an electrospray ionization (ESI) source that was coupled to a rapid separation LC (RSLC; ultimate 3000, Thermo Scientific) system (ESI-LC-MS) using a HSS T3 column (Waters, UK) (2.1 × 150 mm; 2.5 um particle size) with a linear gradient of the binary solvent system consisting of solvent A (water with 0.1% formic acid) and solvent B (acetonitrile) at a flow rate of 0.3 mL/min. A linear gradient was initiated with 5% B and linearly increased to 100% at 0–15 min. The ESI (negative ion) parameters were the source voltage (+5 KV), entrance capillary voltage (+18 V), entrance capillary temperature (275 ℃), and tube lens voltage (+120 V). The scan range was fixed from *m/z* 50 to 1500. The data-dependent mass spectrometry experiments were controlled using the menu driven software provide with the Xcalibur system (version 2.2 SP1.48; Thermo Scientific).

1-2 Microbial source

Soil samples were collected at 5–10 cm depth in Ulleung Island and air-dried. Actinobacteria were isolated by dilution plating method on HV agar medium (1.0 g humic acid, 0.5 g Na_2HPO_4, 1.7 g KCl, 0.05 g $MgSO_4 \cdot 7H_2O$, 0.01 g $FeSO_4 \cdot 7H_2O$, 1 g $CaCl_2$, and 12 g agar per 1 L distilled water, pH 7.2) supplemented with cycloheximide (100 μg/mL). Strain KCB13F003 was recognized after two weeks of incubation at 28 ℃ and maintained on SY agar medium (10 g starch, 1 g yeast extract, 1 g tryptone, 17 g agar per 1 L distilled water). The strain exhibited the highest 16S rRNA gene sequence similarities to *Streptomyces chattanoogensis* NBRC 12754 (99.4%), *Streptomyces lydicus* ATCC 25470 (99.4%), and *Streptomyces staurosporininus* BK179 (99.4%). Therefore, the strain KCB13F003 was identified and named as *Streptomyces* sp. KCB13F003.

1-3 Cultivation

Streptomyces sp. KCB13F003 maintained on SY agar medium was inoculated into 500 mL baffled Erlenmeyer flask containing 100 mL of GLY medium (15.8 mL glycerol, 10 g lactose, 5 g malt extract, 5 g yeast extract, and 1 g $CaCO_3$ per 1 L distilled water). The cultures were grown at 28 ℃ for 3 days on a rotary shaker operating at 125 rpm. 3 mL of seed medium was inoculated to each 250 mL of the same production medium in 1 L baffed Erlenmeyer flasks (30 × 250 mL). Fermentation was carried out at 28 ℃ for 5 days with agitation at 115 rpm.

1-4 Extraction and isolation

The fermentation broth (7.5 L) was centrifuged and the supernatant was adsorbed onto Amberlite XAD-7. The resin was collected and transferred to an open column, and eluted with MeOH. The eluate was concentrated *in vacuo* to yield white crude extract. The cell pellet was extracted with acetone and concentrated *in vacuo*. The combined crude extracts from the supernatant and cell pellet were suspended in H_2O and partitioned with EtOAc using a separation funnel to yield yellow oily extract. The extract (10.0 g) was fractionated by reversed-phase C_{18} flash column chromatography using a stepwise gradient of MeOH-H_2O (from 20:80, 40:60, 60:40, 80:20 to 100:0; 1 L for each step). The 80% MeOH fraction was subjected to preparative HPLC (GROM-SIL, C_{18}, 20 × 250 mm, 10 μm, 6.5 mL/min) using a gradient elution of 45–80% CH_3CN/H_2O in 35 min to yield 4 subfractions. Further purification of the first subfraction by semi-preparative HPLC (Optimapak, C_{18}, 10 × 250 mm, 10 μm, 3.0 mL/min) with a gradient solvent system of 40–52% CH_3CN/H_2O over 35 min provided ulleungamide B (**2**) (48.0 mg, t_R = 19.6 min). The second subfraction was purified by semi-preparative HPLC (Optimapak, C_{18}, 10 × 250 mm, 10 μm, 3.0 mL/min) using a gradient elution of 40–70% CH_3CN/H_2O to afford ulleungamide A (**1**) (24.0 mg, t_R = 21.2 min).

Ulleungamide A (**1**): white powder; $[\alpha]^{21}_D$ +88.4 (*c* 0.05, MeOH); UV(MeOH) λ_{max} (log ε) 204 (3.6), 210 (3.3); for NMR data, see Table S1; HRESIMS *m/z* 986.4861 $[M + H]^+$ (calcd for $C_{51}H_{68}N_7O_{13}$, 986.4875).
Ulleungamide B (**2**): white powder; $[\alpha]^{25}_D$ +69.6 (*c* 0.05, MeOH); UV(MeOH) λ_{max} (log ε) 204 (3.6); 210 (3.2); for NMR data, see Table S2; HRESIMS *m/z* 1024.4642 $[M + Na]^+$ (calcd for $C_{51}H_{67}N_7O_{14}$, 1024.4644).

1-5 Methyl esterification

Solution of compound **1** (2.7 mg) in MeOH (0.5 mL) were treated with 100 μL of trimethylsilyldiazomethane (2 M in diethyl ether) at room temperature for 3 h. The product formation was confirmed by LC/MS (ESIMS *m/z* 1000 $[M + H]^+$), and the reaction mixture was purified by semi-preparative HPLC (Optimapak, C_{18}, 10 × 250 mm, 10 μm, 3.0 mL/min) eluting with a gradient solvent system of 40–80% CH_3CN/H_2O over 35 min to afford methylated product **1b** (1.9 mg, t_R = 21.2 min).

1-6 Determination of the absolute configuration at C-5 and C-26 by modified Mosher's method

Compound **1b** (1.9 mg) was divided into two 20 mL vials, and each was dissolved in 1.0 mL of anhydrous pyridine. To each solution of **1b** was added a slight excess of dimethylaminopyridine (DMAP). Reaction mixtures were stirred for 5 min and treated

with 40 μL of (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) and 40 μL of (*S*)-MTPA-Cl, respectively. The reaction was continued for 24 h at room temperature. Following confirmation of successful product formation by LC/MS (ESIMS *m*/*z* 1432 $[M + H]^+$), the reaction was quenched by addition of 50 μL of H_2O. The crude product mixtures were purified by semi-preparative HPLC (Optimapak, C_{18}, 10 × 250 mm, 10 μm, 3.0 mL/min) using a gradient solvent system of 70–100% CH_3CN/H_2O over 20 min to yield bis-*S*-MTPA ester (**1c**) (0.6 mg, t_R = 19.4 min) and bis-*R*-MTPA ester (**1d**) (0.6 mg, t_R = 18.9 min).

1-7 Determination of the absolute configuration at C-9, C-15, C-32, and C-36 by advanced Marfey's analysis

Compound **1** (0.9 mg) was hydrolyzed in 0.5 mL of 6 N HCl at 100 ℃ for 1 h. Afterwards, the hydrolysate was evaporated *in vacuo* and divided into two portions. To a hydrolysate of each were added 100 μL of 1 N $NaHCO_3$. Either 100 μL of *N*-α-(5-fluoro-2,4-dinitrophenyl)-L-leucinamide (L-FDLA) or D-FDLA (1% w/v in acetone) was added to each hydrolysate and the mixtures were heated at 40 ℃ for 1 h. 20 μL of 2 N HCl was added to neutralize the mixtures and a 20 μL aliquot of each reaction mixture was dissolved in 20 μL of CH_3CN. The resulting mixture was analyzed by LC-MS as described in general experimental procedures. The L- and D-FDLA derivatives for compound **2** were also obtained using an identical method.

Retention times (t_R, min) of the L- and D-FDLA derivatives for compound **1** were as follows: Pip 13.35, 12.94; Phe 14.29, 13.13; γ-OH-Pip 11.53, 11.25; Thr 10.82, 11.84; *N*-Me-Phe 13.64, 13.43.
Retention times (t_R, min) of the L- and D-FDLA derivatives for compound **2** were as follows: Pip 13.37, 12.97; Phe 14.31, 13.15; γ-OH-Pip 11.57, 11.28; Thr 10.86, 11.88; *N*-Me-Phe 13.65, 13.44.
Elution orders of the L- and D-FDLA derivatives of authentic L-amino acids: L-Pip (D→L), L-Phe (L→D), L-Thr (L→D), L-*allo*-Thr (L→D), *N*-Me-L-Phe (L→D).
Retention times (t_R, min) of the L- and D-FDLA derivatives for (2*S*, 4*R*)-4-hydroxypipecolic acid (Manchester Organics): 11.52, 11.24

1-8 Determination of the absolute configuration at C-33 by 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) derivatization

To the hydrolysate of **1** (0.3 mg) solution in 200 μL of H_2O were added 200 μL of Et_3N (6% w/v in acetone) and 200 μL of GITC (1% w/v in acetone). After stirring at room temperature for 20 min, the reaction mixture was diluted with 100 μL of 5% acetic acid, and a 5 μL of aliquot was analyzed by LC/MS as described in general experimental procedures (t_R = 9.59, ESIMS *m*/*z* 509 $[M + H]^+$). The GITC derivatives of standard amino acids, L-Thr and L-*allo*-The, were obtained and analyzed by LC/MS in the same manner (t_R = 9.59 and 9.48 min, respectively).

1-9 Determination of the absolute configuration at C-47 by phenylglycine methyl ester (PGME) derivatization

Compound **1** (2.6 mg) was divided into two 4 mL vials, and each was dissolved in 1.0 mL of tetrahydrofuran (THF). Each vial was treated with 7.6 mg of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and 6 mg of *S*- or *R*-PGME. The reaction was continued at room temperature for 15 h, and the product formation was confirmed by LC/MS (ESIMS *m*/*z* 1133 $[M + H]^+$). The reaction mixtures were evaporated *in vacuo*, and the (*S*)-PGME amide (**1e**) (0.9 mg, t_R = 19.4 min) and *R*-PGME (**1f**) (0.8 mg, t_R = 20.7 min) amide were obtained by semi-preparative HPLC (Optimapak, C_{18}, 10 × 250 mm, 10 μm, 3.0 mL/min) using a gradient solvent system of 50–65% CH_3CN/H_2O over 30 min. The *S*- and *R*-PGME amides of compound **2** were also obtained using the same method (t_R = 14.4 min and 15.7, respectively).

1-10 Determination of the absolute configuration of 4,5-diol moiety by Snatzke's method

1 mL of stock solution of dimolybdenum tetraacetate $[Mo_2(OAc)_4]$ in DMSO (0.7 mg/mL) was added to 0.5 mg of compound **2** and the CD spectrum was recorded immediately after mixing. The CD spectrum was recorded every 10 min until a stationary spectrum was reached (40 min after mixing). The inherent CD from **2** was subtracted to give the induced CD of the complex. The observed signs of the diagnostic bands at 322 (band IV) and 282 nm (band V) were correlated to the absolute configuration at 4,5-diol moiety. The same procedure was also used for **1** and the CD spectrum was recorded until 32 min after mixing.

1-11 Cell proliferation assay

The sensitivity of the normal (NRK, MRC-5, and 267B1) and cancer cell lines (HeLa, MCF-7, and PC-3) to **1** and **2** was evaluated with the methylthiazole tetrazolium (MTT) assay.[1] NRK, HeLa, MCF-7, and PC-3 cells were cultured in DMEM (HyClone) medium at 37 ℃ under a 5% CO_2 atmosphere. MRC-5 and 267B1 were cultured in RPMI 1640 (HyClone) media at 37 ℃ under a 5% CO_2 atmosphere. Each medium was supplemented with 100 units penicillin, 100 μg/mL streptomycin, and 10% fetal bovine serum (HyClone). Cells (5×10^3 cells/mL) were seeded into a 96-well plate and then treated with various concentrations of **1** and **2** dissolved in DMSO (maximum concentrations were 50 μM). After 48 h incubation, 10 μL of MTT solution (AMRESCO) (5 mg/mL in PBS) was added to each well and incubated for 2 h. Then, the supernatant were removed, and the precipitates were dissolved in 100 μL DMSO. The absorbance at 570 nm was measured in a microplate reader.

1-12 Antimicrobial assay

The following microorganisms were used in the assay; *Staphylococcus aureus* KCTC 1916, *Salmonella typhimurium* KCTC 1926, *Bacillus subtilis* KCTC 1022, *Escherichia coli* KCTC 1039, *Pseudomonas aeruginosa* KCTC 1750, *Klebsiella pneumoniae* KCTC 2246, *Enterococcus faecalis* KCTC 3206, *Candida albicans* KCTC 7678, *Penicillium griseofulvum* KCTC 6435, and *Alternaria brassicicola* ATCC 96836. Each strain was precultured for 24 h in the following media; nutrient broth (3 g beef extract, and 5 g peptone per 1 L distilled water, adjusted to pH 6.8 before sterilization) for *S. typhimurium*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *E. faecalis*, LB broth (10 g tryptone, 5 g yeast extract, and 5 g NaCl per 1 L distilled water) for *S. aureus*, and potato dextrose broth (Difco) for *C. albicans*, *P. griseofulvum*, and *A. brassicicola*. The precultured broth (1% (v/v) of agar medium) was inoculated into the corresponding 0.7% agar medium which was autoclaved and cooled to 45 ℃. 5 mL of the resulting suspension was poured into the 90 mm Petri dish containing 15 mL of prepoured LB agar and distributed evenly on the plate. After the agar overlay was solidified, 6 mm filter paper disks containing 100 μg, 50 μg, and 25 μg of **1** and **2** dissolved in DMSO were placed, and

the plates were incubated at 28 ℃ (*B. subtilis*, *C. albicans*, *P. griseofulvum*, and *A. brassicicola*) or 37 ℃ (*S. aureus*, *S. typhimurium*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *E. faecalis*) for 24 h. The growth inhibitory effects were determined by measuring the diameter of inhibition zone. Only compound **1** indicated inhibition zones against *S. aureus* and *S. typhimurium*. Kanamycin, chloramphenicol, and tetracycline served as control.

Diameter of inhibition zone in mm at 100, 50, 25 μg/disk.

organism	ulleungamide A (**1**)	kanamycin	chloramphenicol	tetracycline
Staphylococcus aureus	19, 16.5, 14	19, 17.5, 15	21, 18.5, 16	22, 21, 20
Salmonella typhimurium	13, 9, 0	0, 0, 0	19, 17, 14	25, 22, 21

Refererence

(1) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55–63.

Table S1. NMR spectral data of **1** in DMSO-d_6

	δ_C, type	δ_H, mult (J in Hz)	COSY[b]	HMBC	ROESY[b]
5-hydroxy-6-methyl-2,3-dehydropipecolic acid					
1	162.3, C [162.4]				
2	129.4, C [129.5]				
3	120.2, CH [119.7]	5.98, brt (7.5) [5.85, brt (7.5)]	4a, 4b	1, 2, 4, 5	4a, 4b
4a	27.3, CH_2 [27.3]	2.28, ovl[a] [2.28, ovl[a]]	3, 4b, 5	2, 3, 5	3, 4b, 5, 7
4b		2.01, ovl[a] [2.01, ovl[a]]	3, 4a	2, 3, 5, 6	3, 4a, 5
5	65.2, CH [65.2]	3.75, s [3.75, s]	4a, 5-OH, 6	3	4a, 4b, 6, 7
6	53.6, CH [53.6]	3.93, m [3.93, m]	5, 7	2, 4, 5, 7, 8	5, 7, 9, 10a, 10b
7	14.5, CH_3	0.97, ovl[a]	6	5, 6	4a, 5, 6, 9, 10a
5-OH		4.79, ovl[a]	5		
Pipecolic acid					
8	170.6, C [170.6]				
9	50.2, CH	5.56, ovl[a]	10a, 10b	8, 10, 11, 13, 14	6, 7, 10b, 11a, 13b
10a	26.7, CH_2 [26.8]	1.94, m	9, 10b, 11a, 11b		6, 7, 10b, 11b,
10b		1.59, ovl[a]	9, 10a, 11a	9, 12	9, 10a, 11a
11a	19.6, CH_2	1.53, ovl[a]	10a, 10b, 11b, 12a, 12b	10	9, 10b, 11b, 12a, 13b
11b		1.31, m	10a, 11a, 12a, 12b	12	10a, 11a, 12b
12a	24.7, CH_2	1.52, ovl[a]	11a, 11b, 12b, 13a, 13b	10	11a, 12b, 13b
12b		1.24, m	11a, 11b, 12a, 13a, 13b	13	11b, 12a, 13a
13a	42.7, CH_2	3.89, brd (13.3)	12a, 12b, 13b	9, 11, 12, 14	12b, 13b, 15
13b		3.10, brt (12.5)	12a, 12b, 13a		9, 11a, 12a, 13a, 15
Phenylalanine					
14	170.0, C				
15	48.4, CH [48.3]	5.15, dd (16.2, 8.4)	15-NH, 16a, 16b	14, 16, 17, 23	13a, 13b
16a	37.6, CH_2	3.00, ovl[a]	15, 16b	15, 18, 22	
16b		2.82, dd (13.6, 8.5)	15, 16a	14, 15, 18, 22	
17	137.6, C				
18	129.3, CH	7.20, ovl[a]		16, 20, 22	
19	128.0, CH	7.23, ovl[a]		17, 21	
20	126.2, CH	7.16, ovl[a]		18, 22	
21	128.0, CH	7.23, ovl[a]		17, 19	
22	129.3, CH	7.20, ovl[a]		16, 18, 20	
15-NH		8.90, m [8.89, m]	15	15, 23	24, 25b
4-hydroxypipecolic acid					
23	170.8, C				
24	52.4, CH [52.4]	4.29, m	25a, 25b	23, 25, 26, 28, 29	15-NH, 25a, 25b, 30a
25a	33.2, CH_2 [33.3]	1.82, ovl[a]	24, 25b, 26		24, 25b, 26
25b		1.41, m	24, 25a, 26	23, 24, 26, 27	15-NH, 24, 25a, 28b
26	61.9, CH	3.65, brs	25a, 25b, 27a, 26-OH	24, 28	25a, 27a, 27b
27a	31.3, CH_2 [31.3]	1.59, ovl[a]	26, 27b, 28a, 28b	28	26, 27b, 28a
27b		1.45, m	27a, 28a, 28b		26, 27a, 28a, 28b
28a	34.1, CH_2 [34.2]	4.17, m	27a, 27b, 28b	24, 26, 27, 29	24, 27a, 27b, 28b
28b		3.19, ovl[a]	27a, 27b, 28a	26, 27, 29	25b, 28a
26-OH		4.43, brs	26		
Glycine					
29	168.0, C				
30a	40.5, CH_2 [40.4]	4.54, m [4.54, m]	30b, 30-NH	29	24, 30b
30b		3.48, m [3.48, m]	30a, 30-NH	29	13b, 30a, 30-NH,
30-NH		8.04, m	30a, 30b	31	30b, 32, 33
Threonine					

31	167.4, C				
32	54.6, CH [54.6]	4.77, ovl[a] [4.85, dd (9.1, 2.9)]	33, 32-NH	31, 35	30-NH, 33, 34, 32-NH
33	71.4, CH [71.3]	5.48, m [5.53, m]	32, 34	1, 34	30-NH, 32, 34
34	15.9, CH_3 [15.8]	0.97, ovl[a] [0.94, d (6.1)]	33	32, 33	32, 33
32-NH		7.69, d (8.8) [8.15, d (8.4)]	32	35	32, 36, 44
***N*-methylphenylalanine**					
35	170.9, C				
36	56.7, CH [59.4]	5.57, ovl[a] [5.11, t (7.3)]	37a, 37b	35, 37, 38, 44, 45	32-NH, 37a, 39, 43
37a	34.7, CH_2 [35.6]	3.20, dd (14.4, 5.4) [3.32, ovl[a]]	36, 37b	35, 36, 38, 39, 43	36, 37b, 39, 43, 44
37b		2.90, dd (13.8, 11.0) [3.00, ovl[a]]	36, 37a	35, 36, 39, 43	37a, 39, 43
38	137.8, C [137.5]				
39	128.8, CH [128.9]	7.26, ovl[a] [7.34, d (7.4)]		37, 41, 43	
40	128.0, CH [128.3]	7.23, ovl[a] [7.28, m]		38, 42	
41	126.1, CH [126.4]	7.16, ovl[a] [7.20, ovl[a]]		39, 43	
42	128.0, CH [128.3]	7.23, ovl[a] [7.28, m]		38, 40	
43	128.8, CH [128.9]	7.26, ovl[a] [7.34, d (7.4)]		37, 39, 41	
44	31.4, CH_3 [28.9]	2.94, s [2.76, s]		36, 45	32-NH, 36, 46b
2-isopropylsuccinic acid					
45	172.0, C [171.7]				
46a	31.8, CH_2 [31.9]	2.52, ovl[a] [2.60, m]	46b		
46b		2.10, brd (13.9) [2.42, m]	46a, 47		44, 46a
47	46.8, CH [47.1]	2.48, ovl[a] [2.52, m]	46b, 48	45, 51	49, 50
48	29.2, CH [29.5]	1.73, m [1.85, m]	47, 49, 50	46, 47, 49, 50, 51	49, 50
49	19.7, CH_3 [20.0]	0.78, d (6.4) [0.88, ovl[a]]	48	47, 48, 50	47, 48
50	19.7, CH_3 [19.8]	0.76, d (6.4) [0.89, ovl[a]]	48	47, 48, 49	47, 48
51	175.6, C [175.6]				
51-OH		11.87, brs			

[a]Overlapped with other signals

[b]Correlations between signals in the region of 7.15–7.30 ppm cannot be determined due to highly overlapped cross-peaks in COSY and ROESY spectra.

Detectable chemical shifts for ^{1}H and ^{13}C of the minor conformer are presented in brackets.

Table S2. NMR spectral data of **2** in DMSO-d_6

	δ_C, type	δ_H, mult (*J* in Hz)	COSY[b]	HMBC	ROESY[b]
4,5-dihydroxy-6-methyl-2,3-dehydropipecolic acid					
1	162.3, C [162.4]				
2	130.4, C [130.4]				
3	121.4, CH [121.1]	5.73, brs [5.63, brs]	4	1, 2, 5	4
4	62.9, CH [62.7]	4.16, ovl[a] [4.17, ovl[a]]	3, 5	2, 3	3, 5, 7
5	66.9, CH [66.9]	3.60, brs [3.60, brs]	4, 6, 5-OH	3	4, 6, 7
6	56.0, CH [56.0]	4.08, m	5, 7	2, 4, 5, 7, 8	5, 7, 9, 10a
7	14.5, CH_3 [14.5]	1.09, ovl[a] [1.09, ovl[a]]	6	5, 6	4, 5, 6, 9, 10a
4-OH					
5-OH		4.82, ovl[a]	5		
Pipecolic acid					
8	170.9, C				
9	50.2, CH	5.54, ovl[a]	10a, 10b	8, 10, 11, 13, 14	6, 7, 10b
10a	26.8, CH_2 [26.9]	1.89, m	9, 11a		6, 7, 10b
10b		1.57, m	9, 11b	8, 11	9, 10a
11a	19.6, CH_2	1.54, ovl[a]	10a, 11b		11b, 13b
11b		1.28, ovl[a]	10b, 11a		11a
12a	24.7, CH_2	1.50, ovl[a]	12b, 13a, 13b		12b, 13b
12b		1.24, ovl[a]	12a, 13b, 13a		12a, 13a
13a	42.6, CH_2	3.91, m	12a, 12b, 13b	9, 11, 12	12b, 13b, 15
13b		3.08, brt (12.3)	12a, 12b, 13a		11a, 12a, 13a, 15
Phenylalanine					
14	169.8, C				
15	48.4, CH [48.4]	5.13, ovl[a]	16a, 16b, 15-NH	14, 16, 17	13a, 13b, 16a, 16b, 18, 22, 15-NH
16a	37.6, CH_2 [37.5]	3.00, m	15, 16b	14, 15, 17, 18, 22	15, 16b, 18, 22, 15-NH
16b		2.82, dd (13.6, 8.6)	15, 16a	14, 15, 17, 18, 22	15, 16a, 18, 22, 15-NH
17	137.6, C				
18	129.3, CH	7.20, ovl[a]		16, 20, 22	15, 16a, 16b, 15-NH
19	128.0, CH	7.23, ovl[a]		17, 21	20
20	126.2, CH	7.16, ovl[a]		18, 22	19, 21
21	128.0, CH	7.23, ovl[a]		17, 19	20
22	129.3, CH	7.20, ovl[a]		16, 18, 20	15, 16a, 16b, 15-NH
15-NH		8.95, ovl[a]	15	15, 23	25b, 16a, 16b, 30a, 30b, 24, 15, 18, 22
4-hydroxypipecolic acid					
23	170.8, C				
24	52.5, CH	4.27, m	25a, 25b	23, 25, 26, 28, 29	15-NH, 25a, 30a
25a	33.3, CH_2 [33.3]	1.81, m	24, 25b		24, 25b, 26
25b		1.39, m	24, 25a, 26	23, 24, 26, 27	15-NH, 25a
26	61.9, CH [62.0]	3.65, m	25b, 27b, 26-OH		25a, 27a, 27b, 28b
27a	31.3, CH_2 [31.3]	1.62, m	27b, 28a, 28b	25, 28	26, 27b, 28a
27b		1.43, m	26, 27a, 28b	26	26, 27a, 28b
28a	34.3, CH_2 [34.4]	4.17, ovl[a]	27a, 28b	24, 26, 27, 29	27a, 28b
28b		3.16, ovl[a]	27a, 27b, 28a	27, 29	26, 27b, 28a
26-OH		4.43, d (6.1)	26		
Glycine					
29	168.1, C [168.1]				
30a	40.4, CH_2 [40.2]	4.56, m	30b, 30-NH	29, 31	15-NH, 24, 30b
30b		3.43, ovl[a] [3.42, ovl[a]]	30a, 30-NH	29, 31	15-NH, 30a, 30-NH
30-NH		8.05, brs	30a, 30b		30b, 32, 33
Threonine					

31	167.3, C				
32	54.5, CH [54.4]	4.77, dd (9.0 2.8) [4.89, dd (9.2, 3.3)]	33, 32-NH	31, 35	30-NH, 33, 34, 32-NH, 36
33	71.7, CH [71.4]	5.49, m [5.54, ovl[a]]	32, 34	1	30-NH, 32, 34, 32-NH
34	15.7, CH_3 [15.6]	0.97, d (6.1) [0.90, ovl[a]]	33	32, 33	32, 33
32-NH		7.79, brs [8.36, d (9.1)]	32	35	32, 33, 36
***N*-methylphenylalanine**					
35	170.9, C				
36	56.8, CH [59.3]	5.53, ovl[a] [5.13, ovl[a]]	37a, 37b	35, 37, 44, 45	32, 32-NH, 37a, 39, 43, 44
37a	34.7, CH_2 [35.8]	3.22, dd (14.1, 5.7) [3.31, ovl[a]]	36, 37b	36, 38, 39, 43	36, 37b, 39, 43
37b		2.89, dd (14.1, 10.5) [2.96, ovl[a]]	36, 37a	36, 38, 39, 43	37a, 39, 43
38	137.8, C [137.4]				
39	128.8, CH [128.9]	7.26, ovl[a]		37, 41, 43	
40	128.0, CH [128.3]	7.23, ovl[a]		38. 42	
41	126.1, CH [126.4]	7.16, ovl[a]		39, 43	
42	128.0, CH [128.3]	7.23, ovl[a]		38, 40	
43	128.8, CH [128.9]	7.26, ovl[a]		37, 39, 41	
44	31.5, CH_3 [28.9]	2.92, s [2.76, s]		36, 45	36, 46a, 46b, 47
2-isopropylsuccinic acid					
45	171.9, C [171.7]				
46a	31.9, CH_2	2.53, ovl[a] [2.62, dd (16.4, 10.3)]	46b, 47 46a, 47	45, 47, 51 45, 47, 51	44, 46b 44, 46a, 47
46b		2.09, dd (16.1, 3.5) [2.41, m]			
47	46.8, CH [47.1]	2.46, ovl[a] [2.54, ovl[a]]	46a, 46b, 48	46, 48, 49, 50, 51	44, 46b, 48, 49
48	29.3, CH [29.5]	1.73, m [1.86, m]	47, 49, 50	46, 47, 49, 50, 51	47, 49, 50
49	19.7, CH_3 [20.1]	0.78, d (6.9) [0.90, ovl[a]]	48	47, 48, 50	47, 48, 50
50	19.7, CH_3 [19.7]	0.76, d (6.9) [0.90, ovl[a]]	48	47, 48, 49	48, 49
51	175.6, C [175.6]				
51-OH		11.85, brs			

[a]Overlapped with other signals

[b]Correlations between signals in the region of 7.15–7.30 cannot be determined due to highly overlapped cross-peaks in COSY and ROESY spectra.

Detectable chemical shifts for ^{1}H and ^{13}C of the minor conformer are presented in brackets.

Figure S1. ^{1}H NMR spectrum of **1** in DMSO-d_6 (900 MHz)

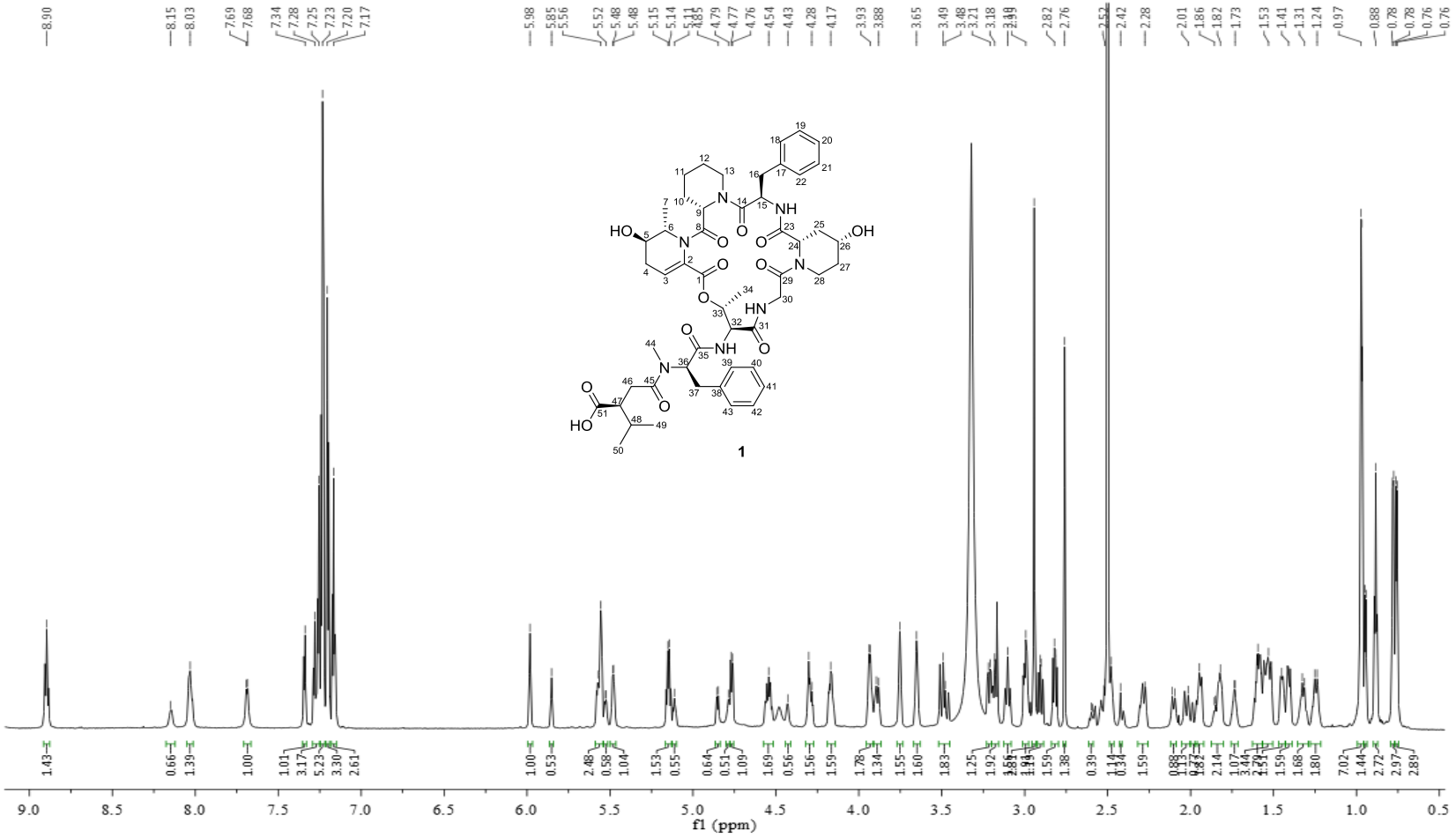


Figure S2. ^{1}H NMR spectrum of **1** in DMSO-d_6 (800 MHz)

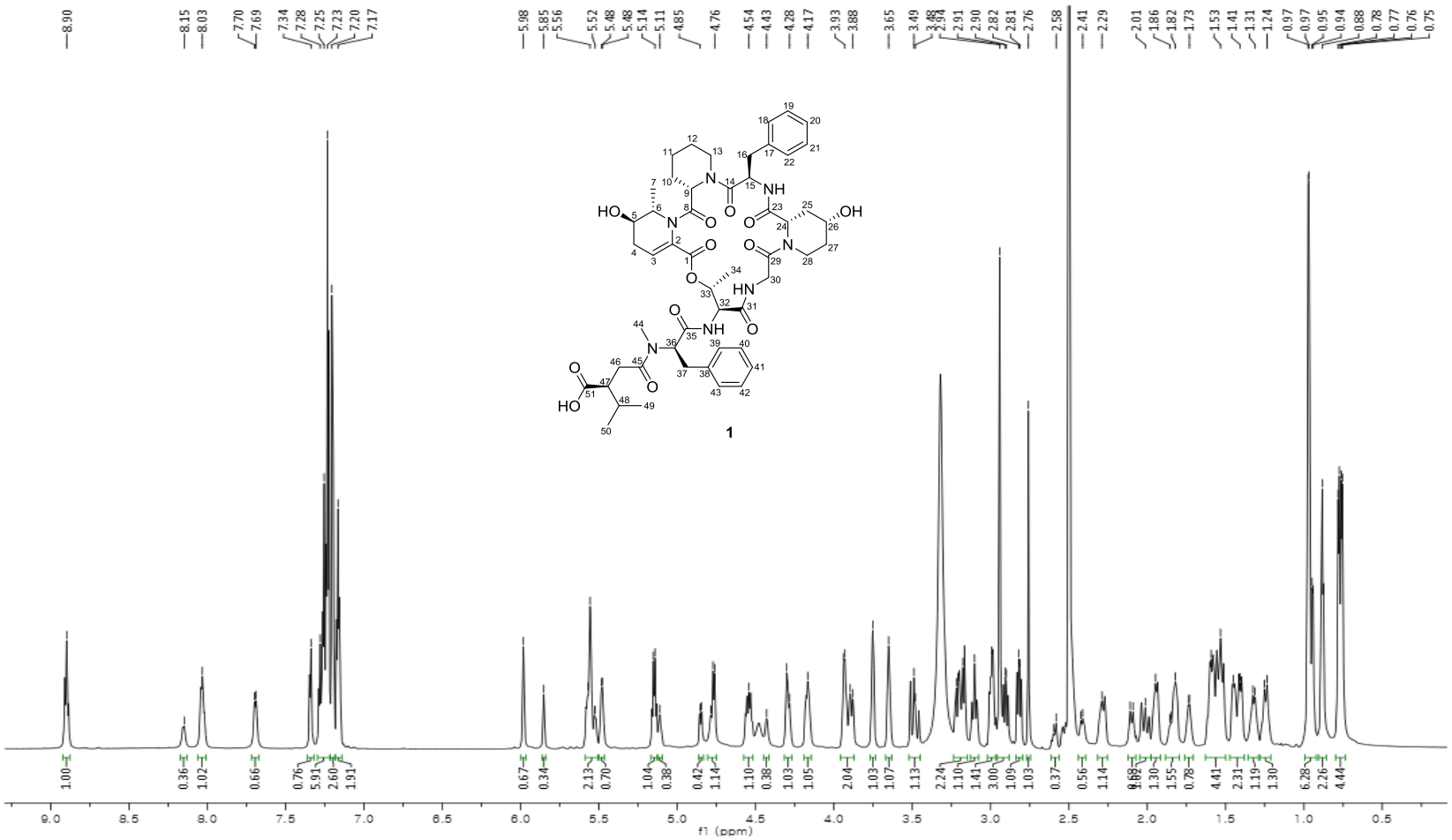


Figure S3. ^{1}H NMR spectrum of **1** in DMSO-d_6 (800 MHz) expanded in the region of 7.5–13.0 ppm

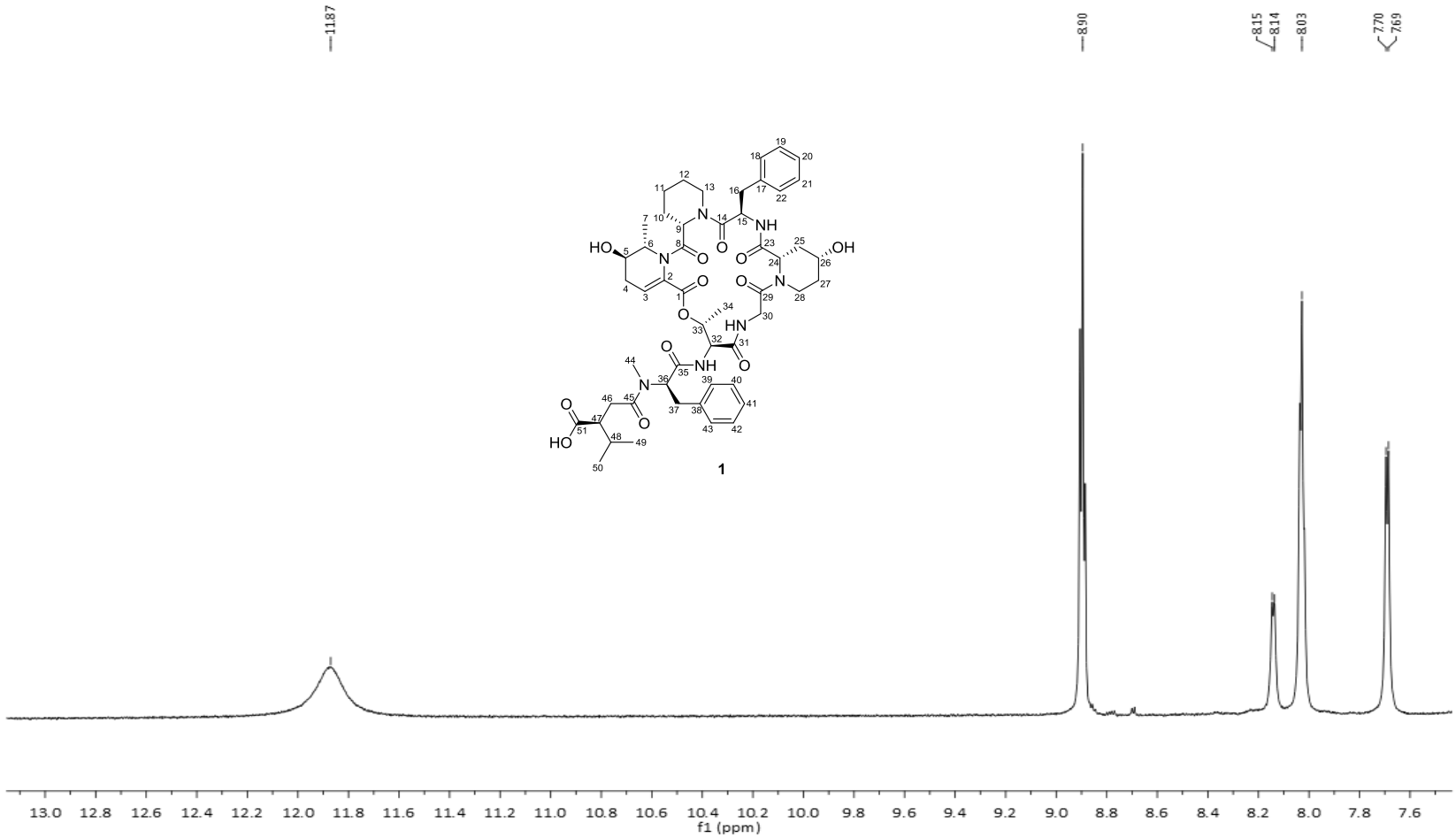


Figure S4. ^{13}C NMR spectrum of **1** in DMSO-d_6 (200 MHz)

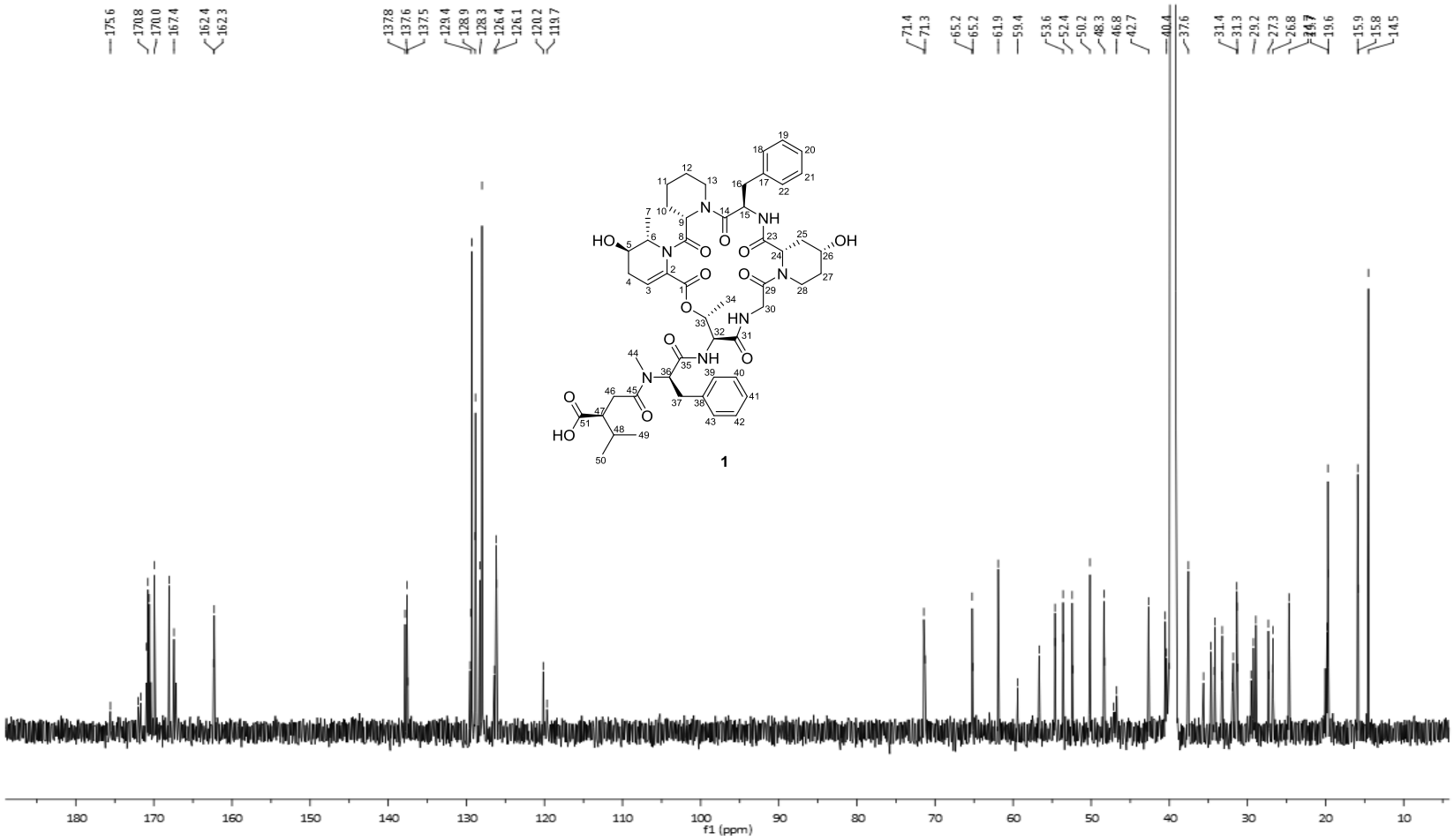


Figure S5. COSY spectrum of **1** in DMSO-d_6 (800 MHz)

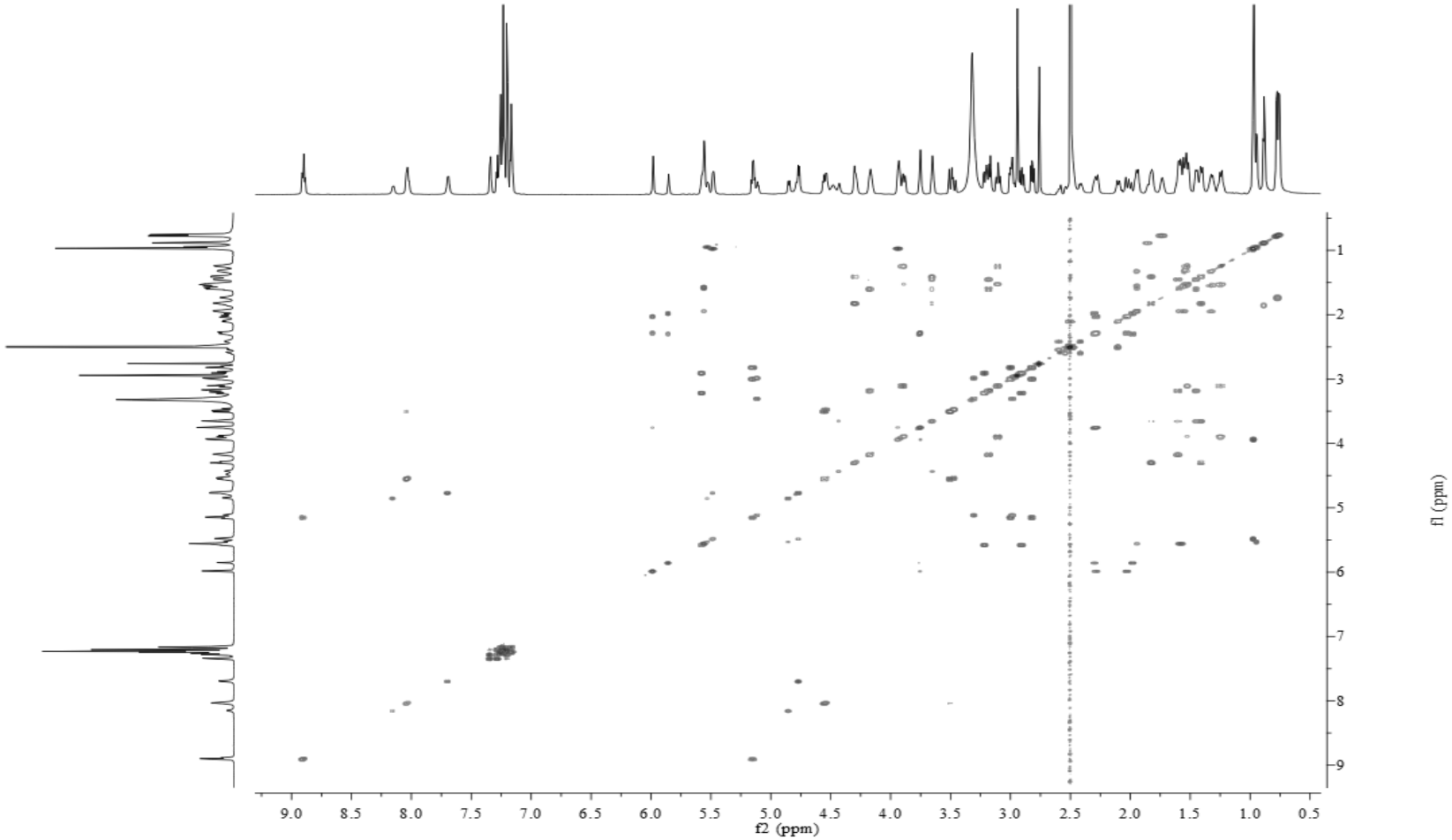


Figure S6. DQF-COSY spectrum of **1** in DMSO-d_6 (900 MHz)

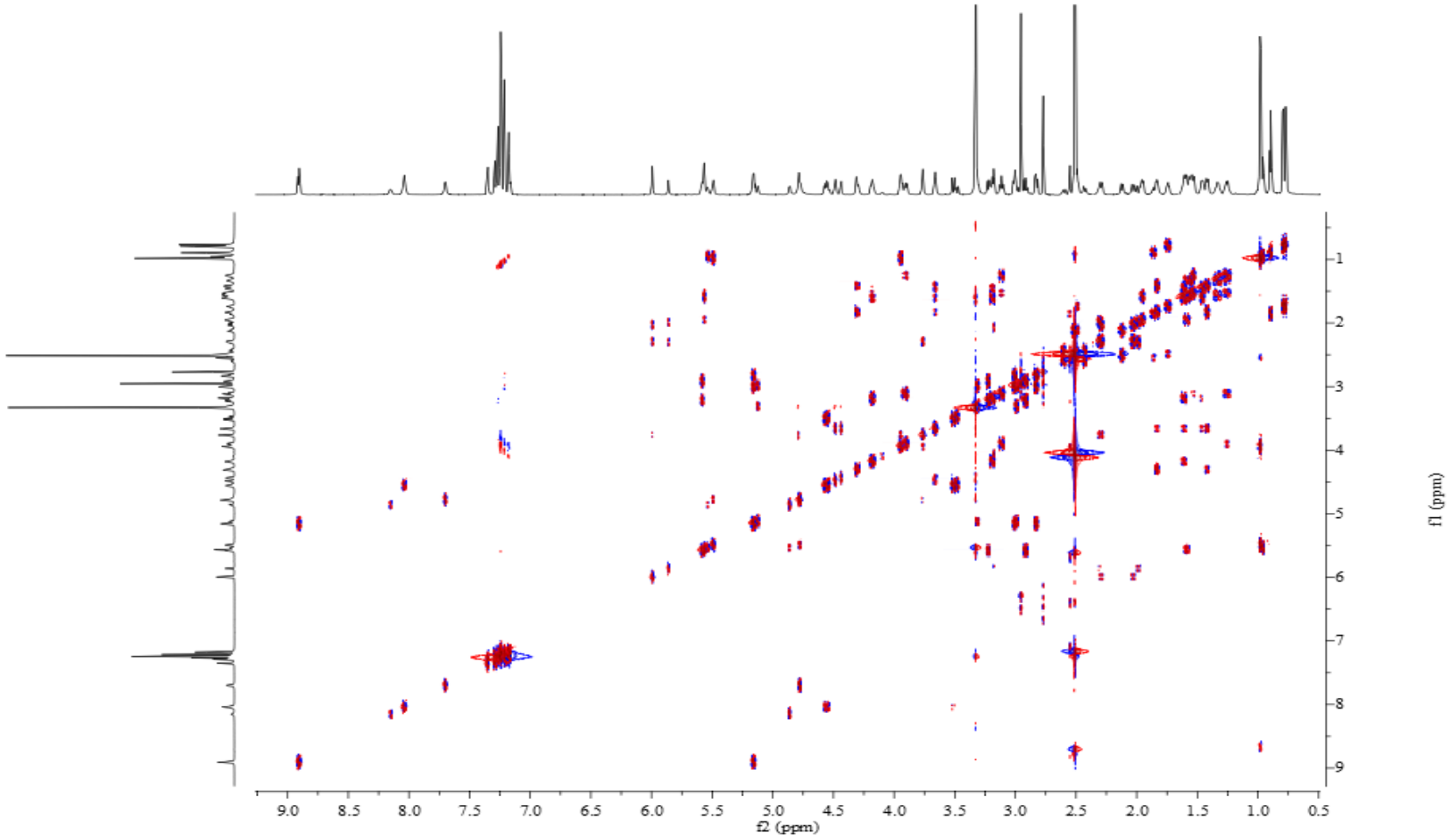


Figure S7. HSQC-DEPT spectrum of **1** in DMSO-d_6 (800 MHz)

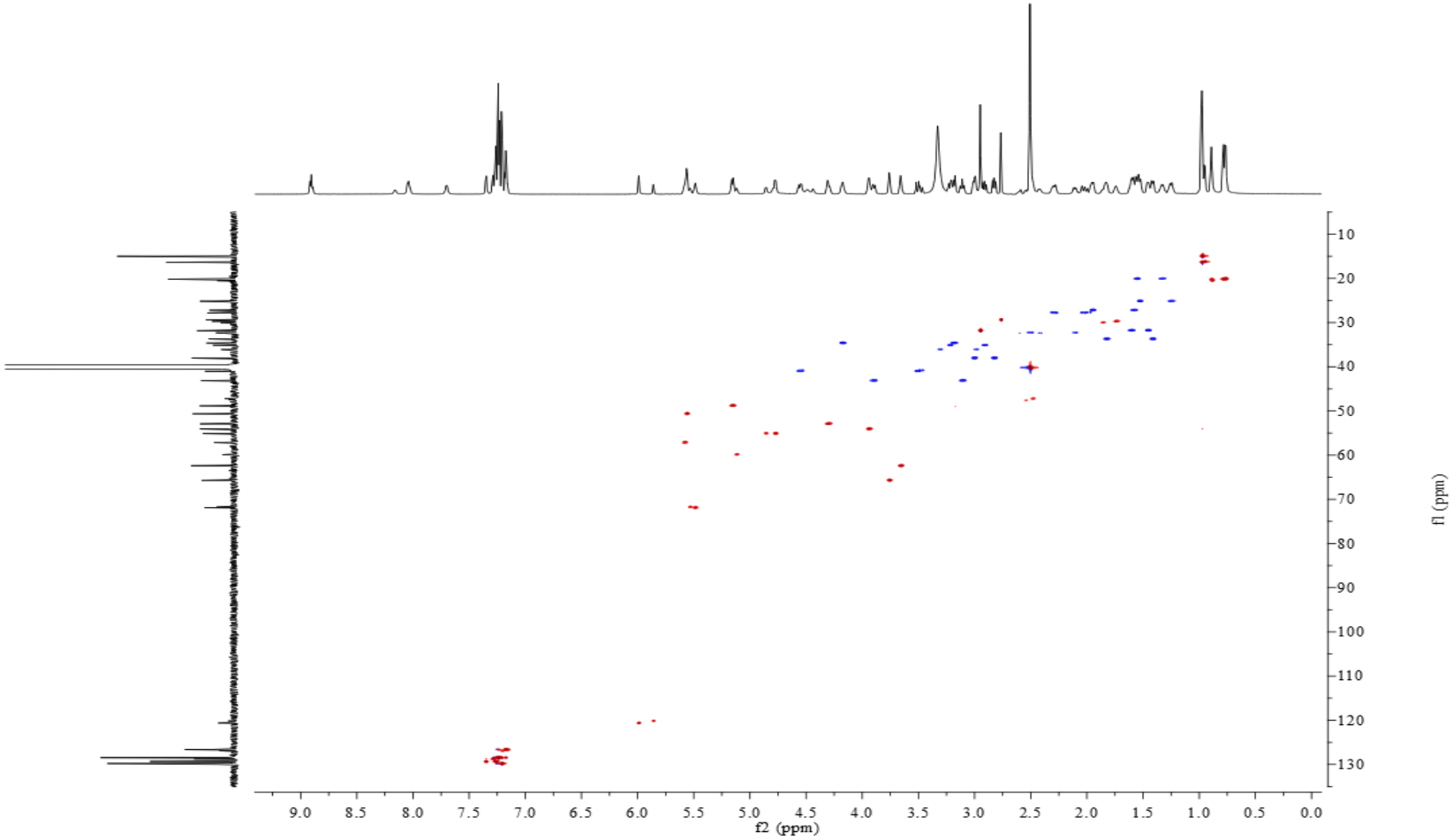


Figure S8. TOCSY spectrum of **1** in DMSO-d_6 (900 MHz)

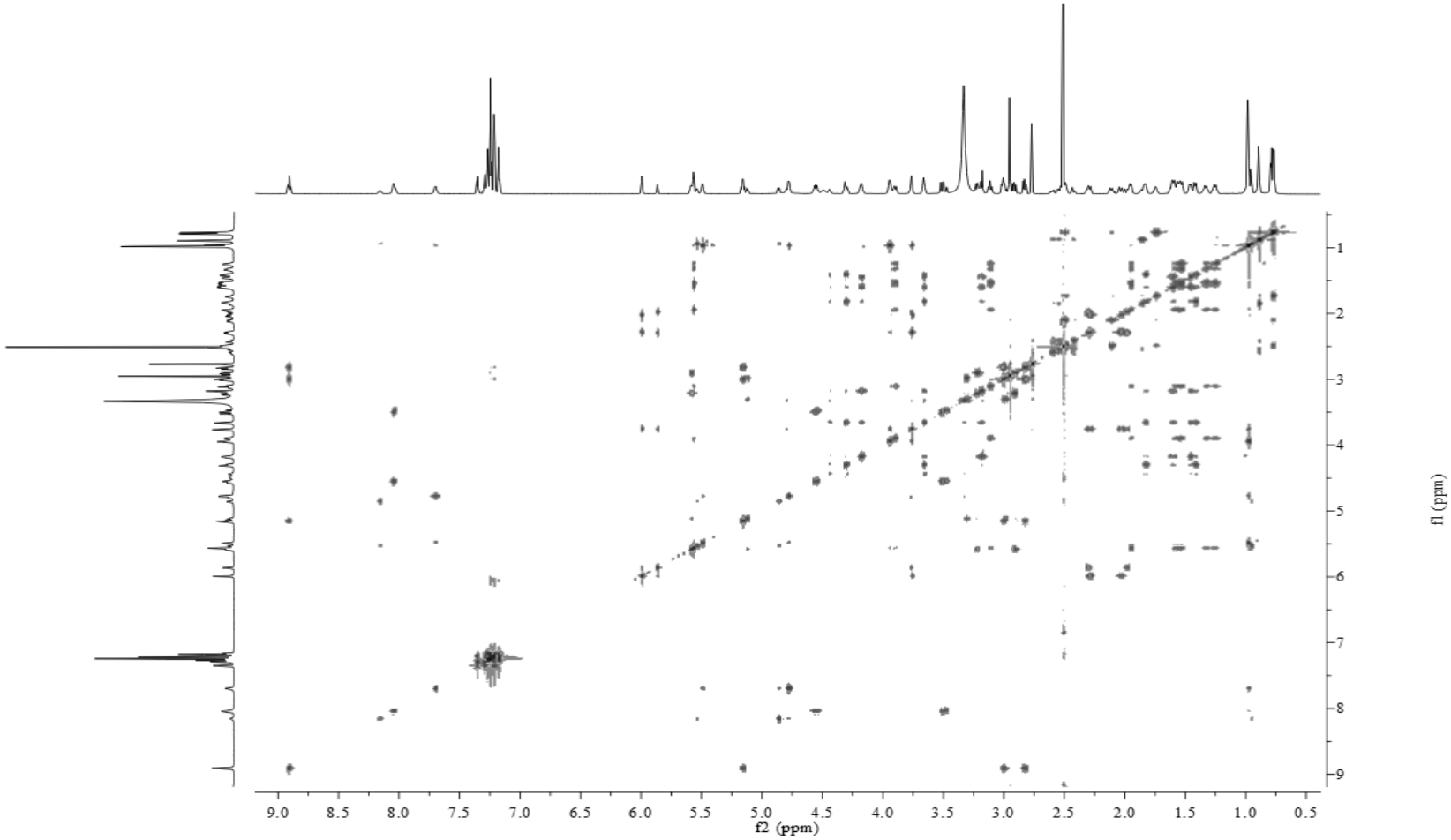


Figure S9. HSQC-TOCSY spectrum of **1** in DMSO-d_6 (800 MHz)

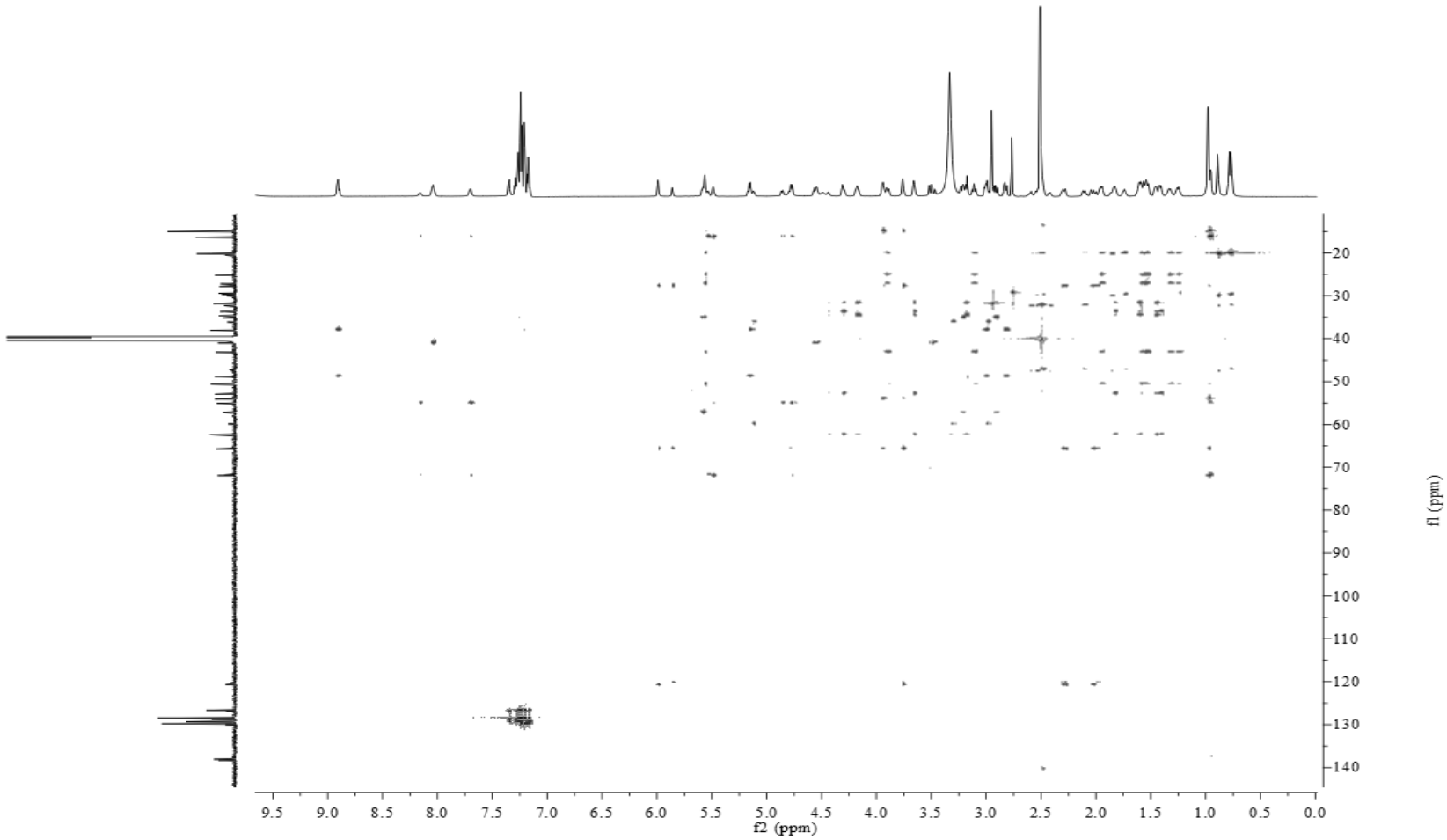


Figure S10. HMBC spectrum of **1** in DMSO-d_6 (800 MHz)

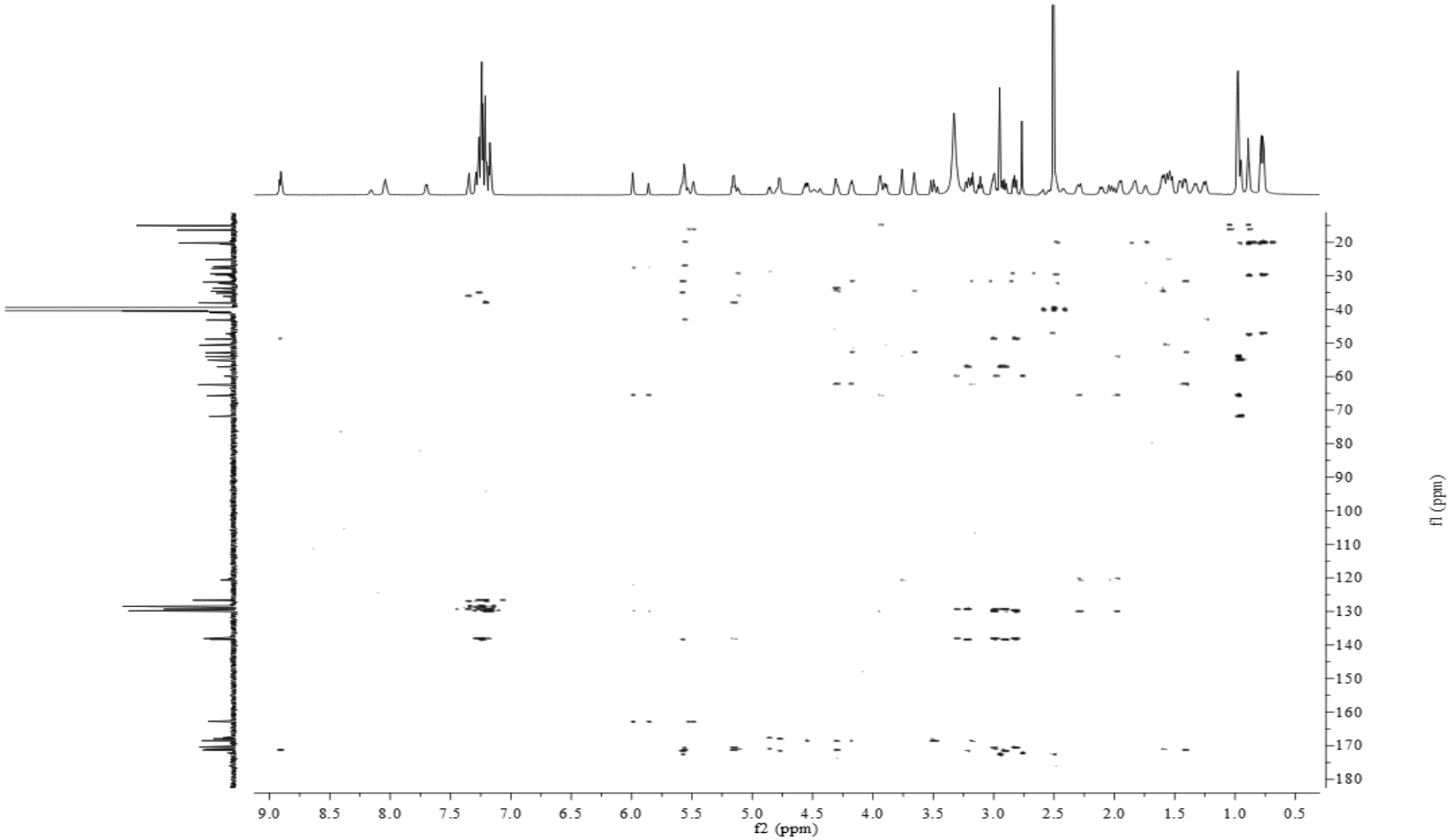


Figure S11. HMBC spectrum of **1** in DMSO-d_6 (900 MHz) (1)

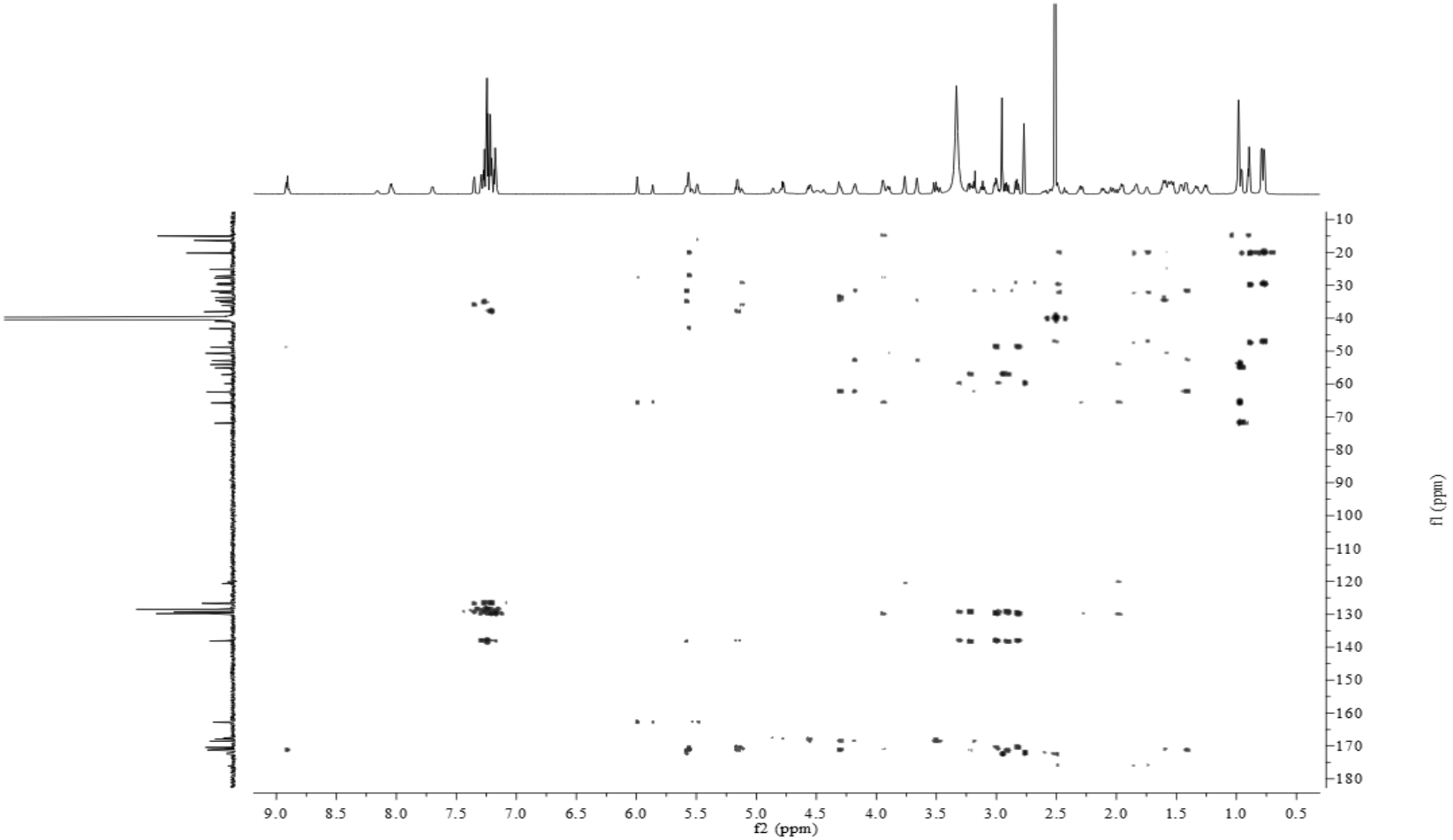


Figure S12. HMBC spectrum of **1** in DMSO-d_6 (900 MHz) (2)

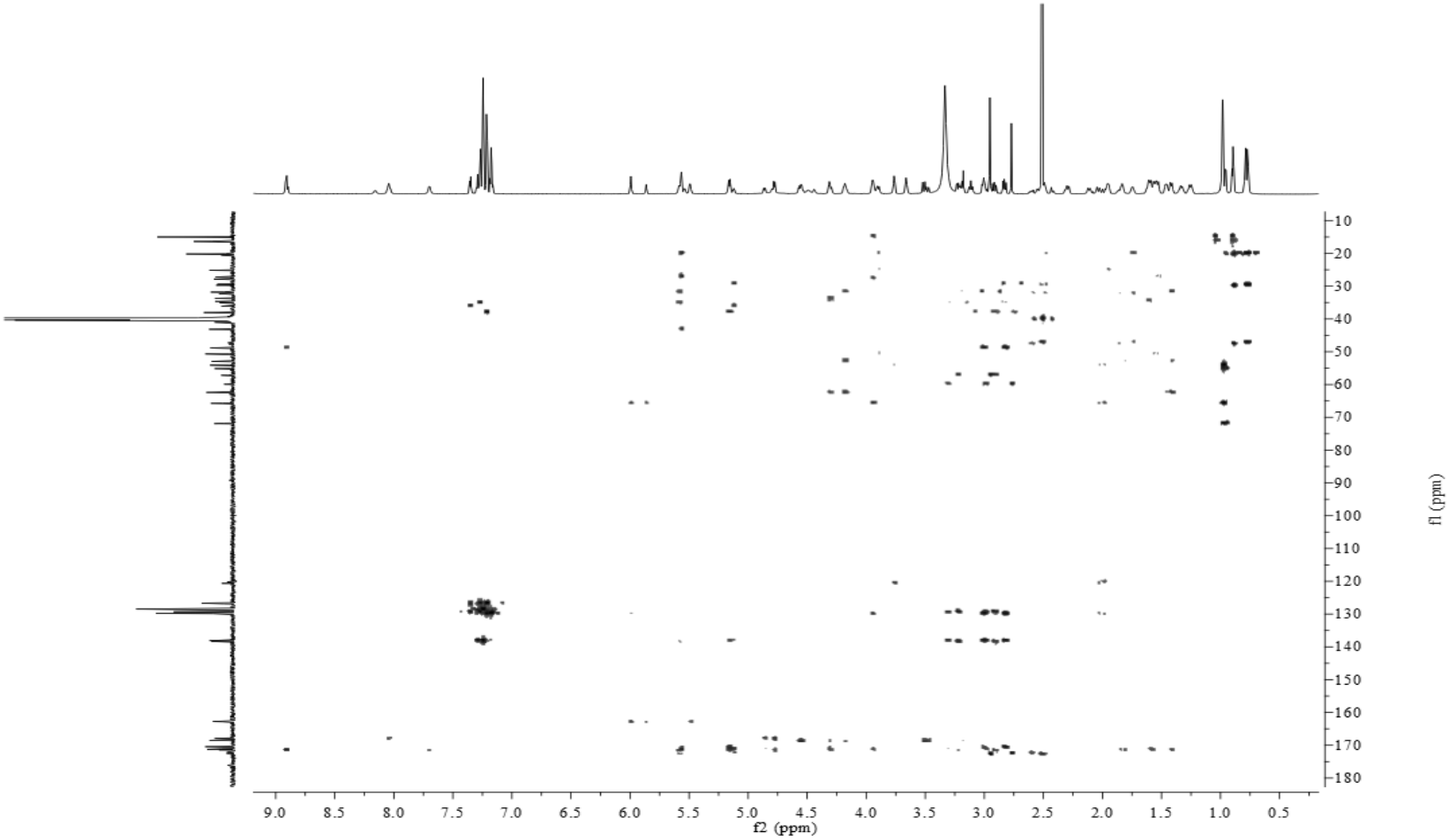


Figure S13. ROESY spectrum of **1** in DMSO-d_6 (800 MHz)

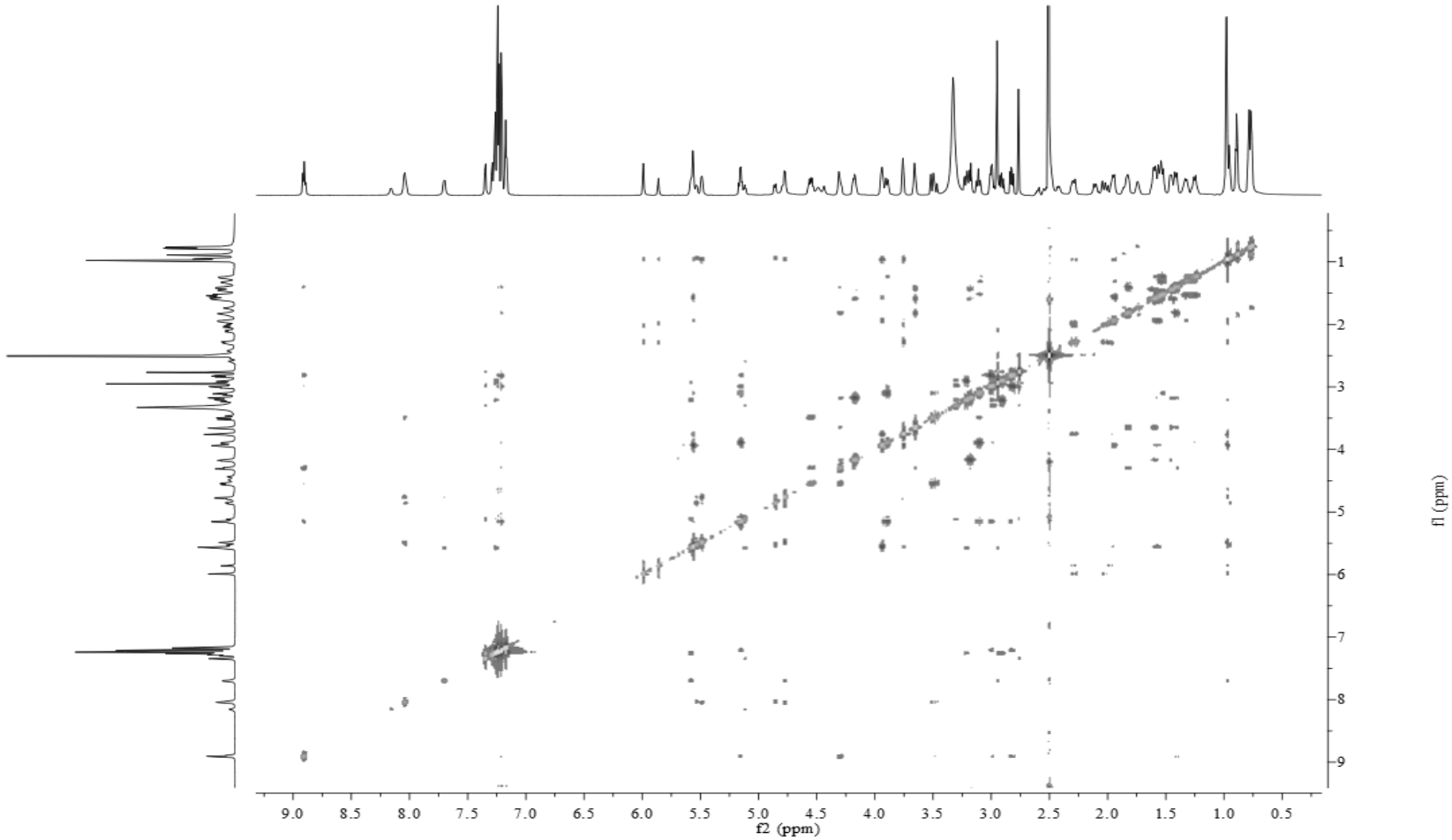


Figure S14. ^{1}H NMR spectrum of **2** in DMSO-d_6 (900 MHz)

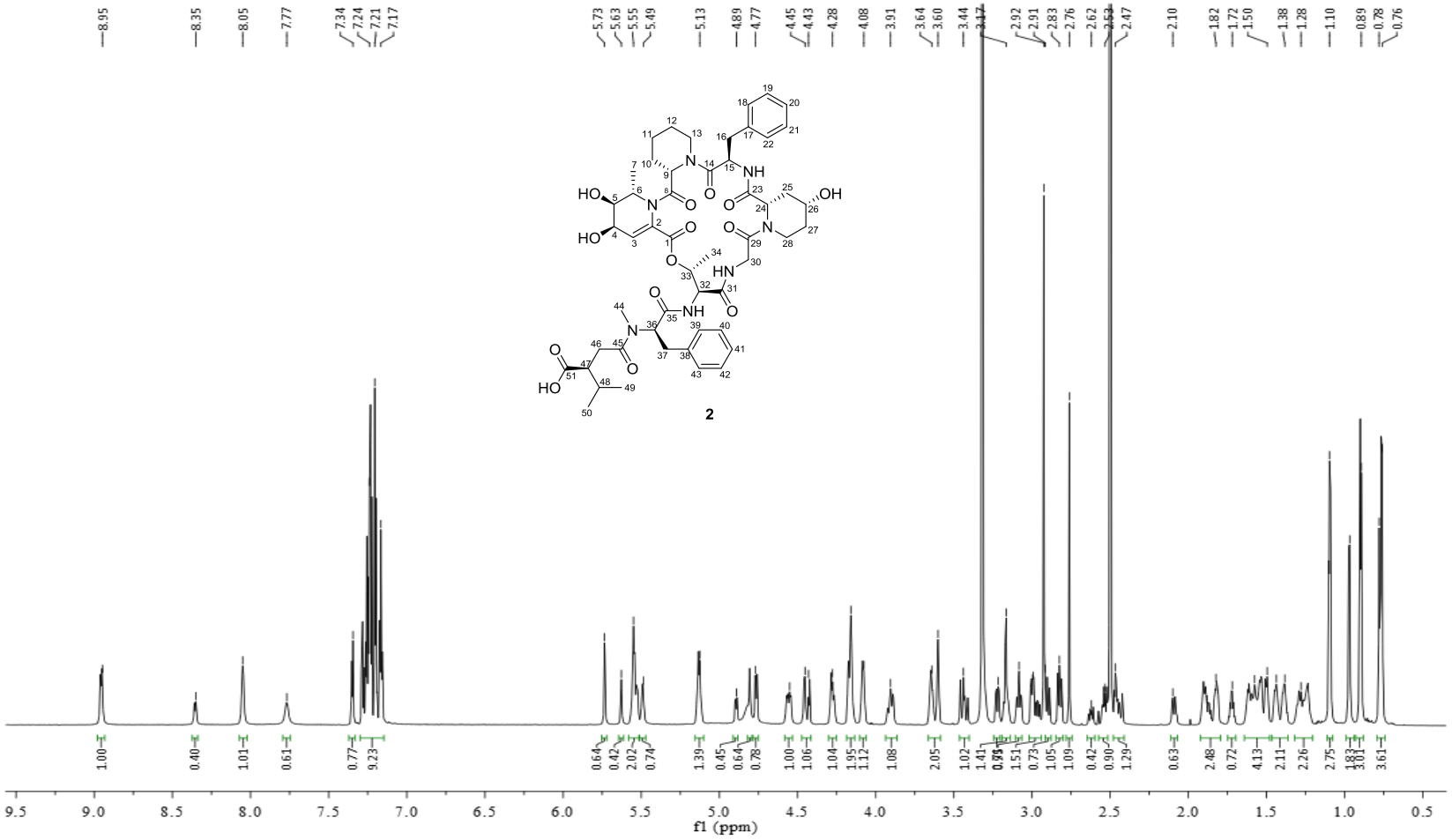


Figure S15. ^{1}H NMR spectrum of **2** in DMSO-d_6 (900 MHz) expanded in the region of 7.5–13.0 ppm

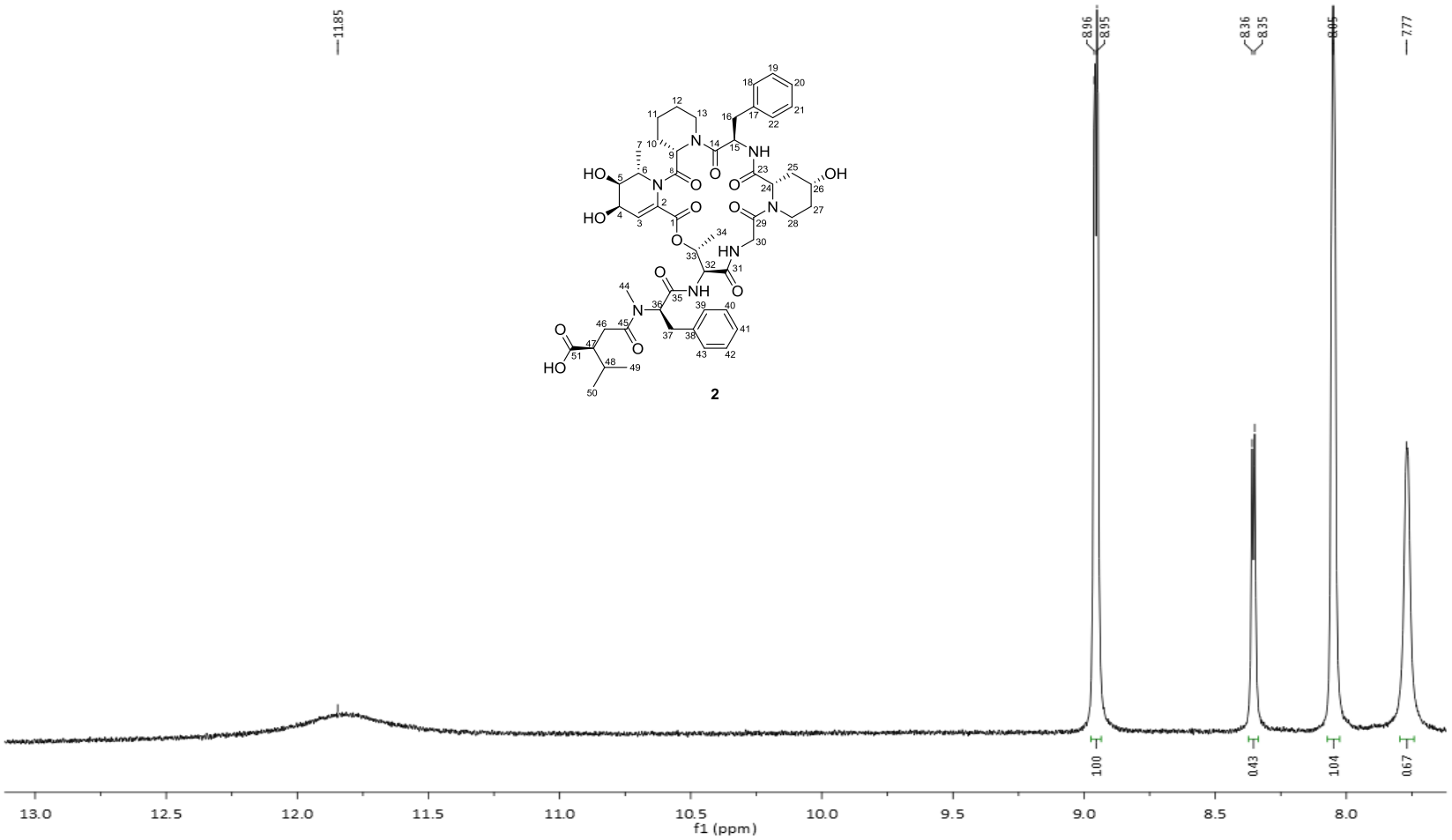


Figure S16. ^{13}C NMR spectrum of **2** in DMSO-d_6 (225 MHz)

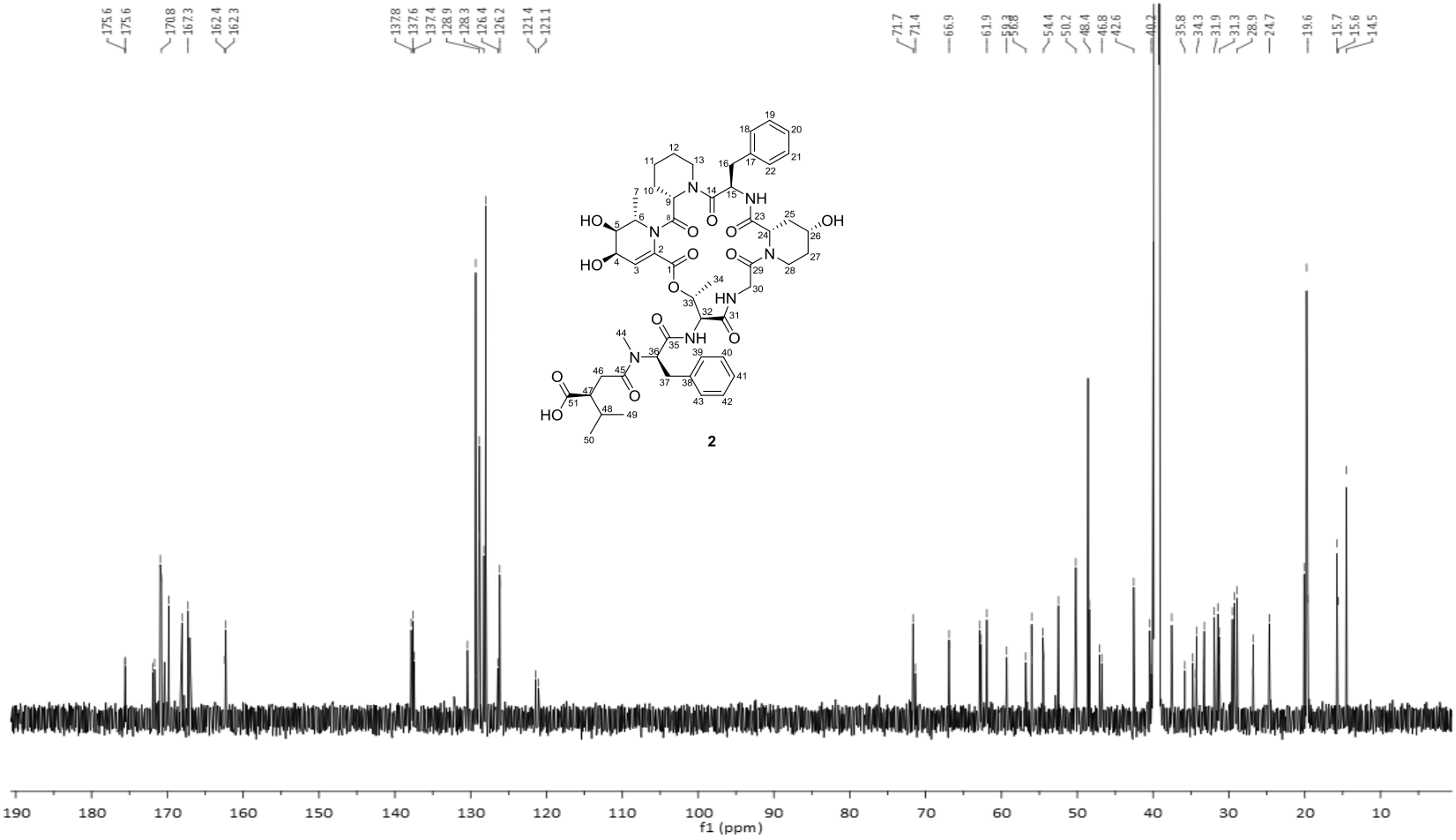


Figure S17. COSY spectrum of **2** in DMSO-d_6 (900 MHz)

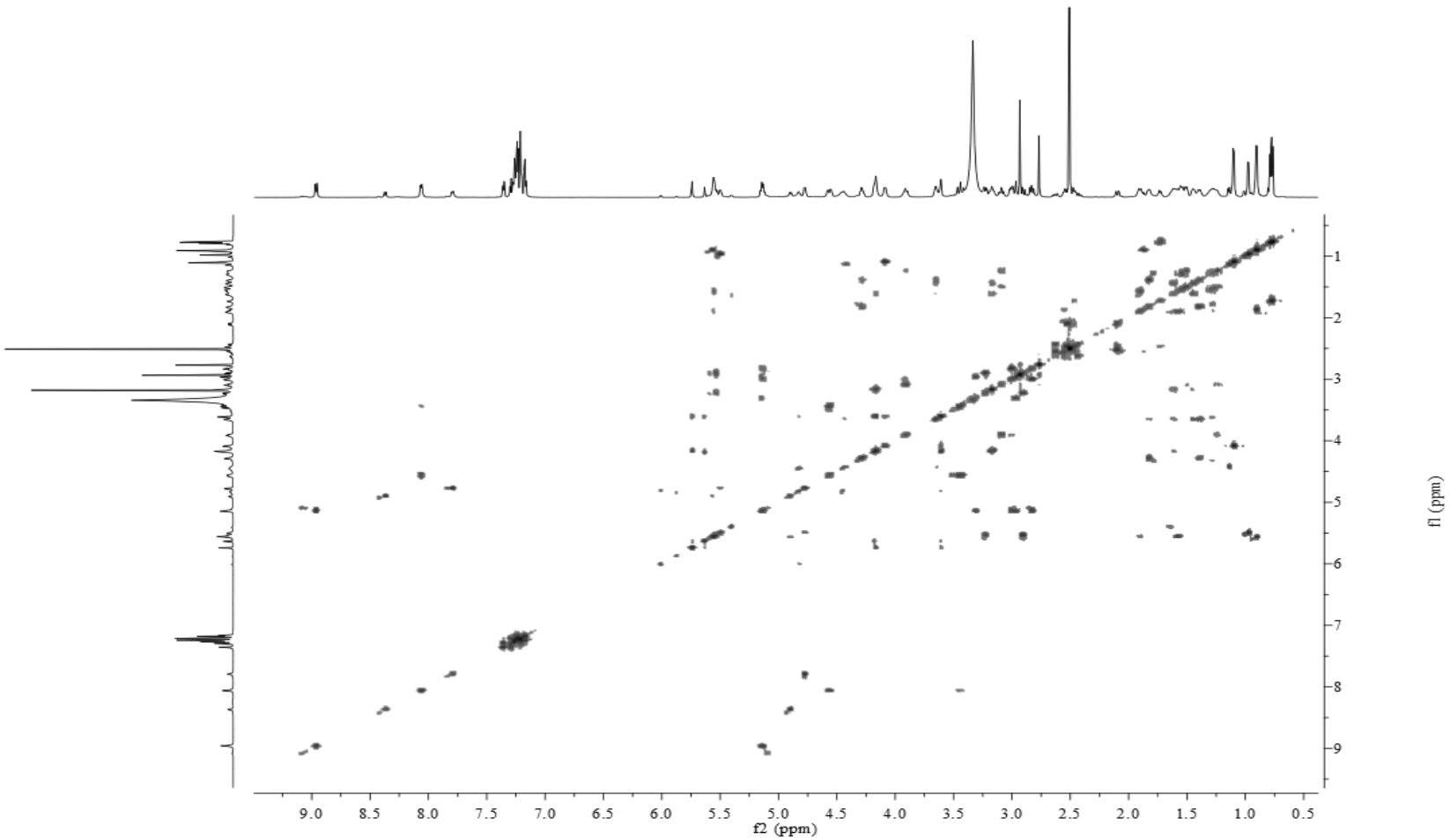

9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f2 (ppm)
1
2
3
4
5
6
7
8
9
f1 (ppm)

Figure S18. DQF-COSY spectrum of **2** in DMSO-d_6 (900 MHz)

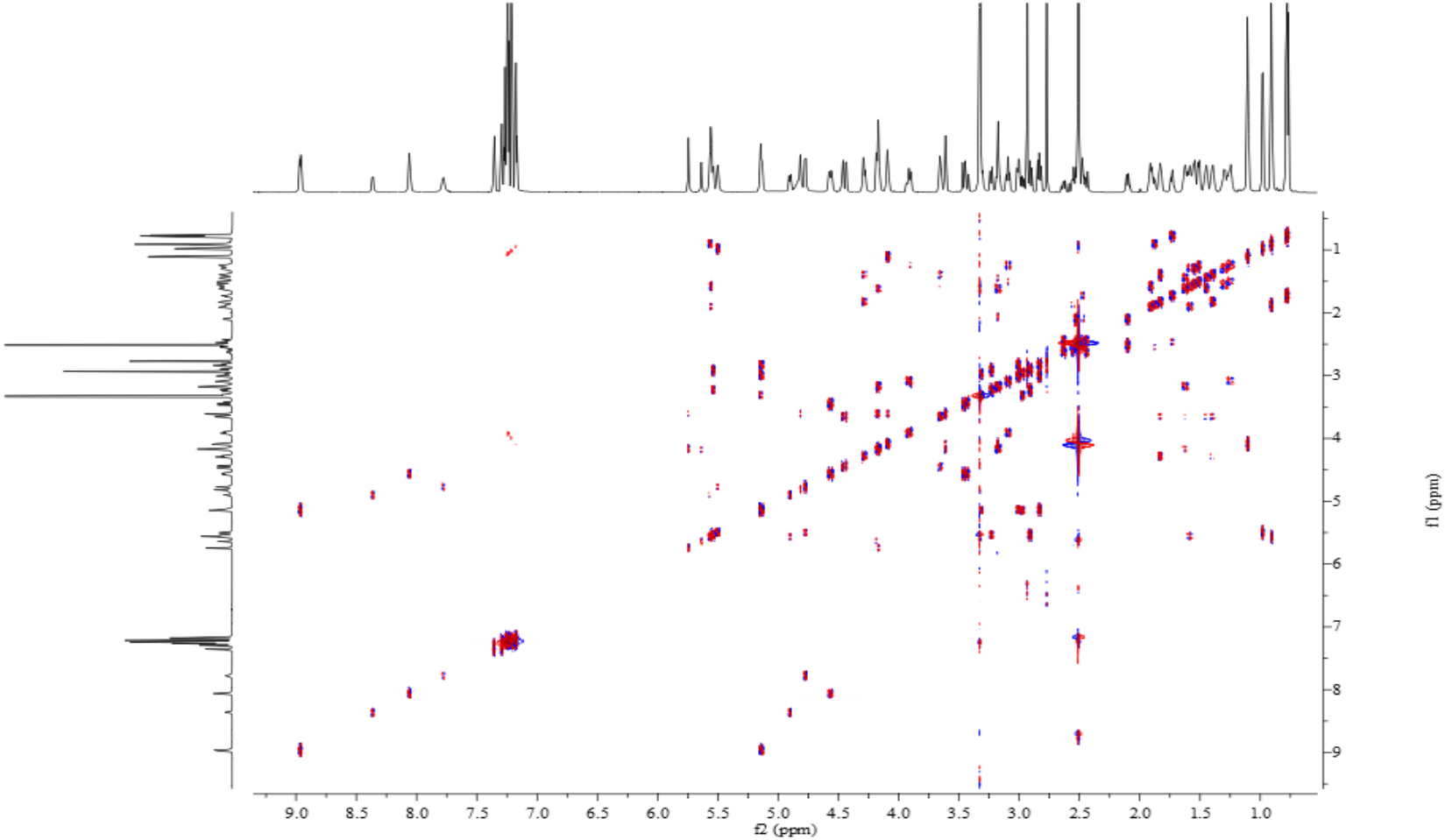


Figure S19. HSQC-DEPT spectrum of **2** in DMSO-d_6 (900 MHz)

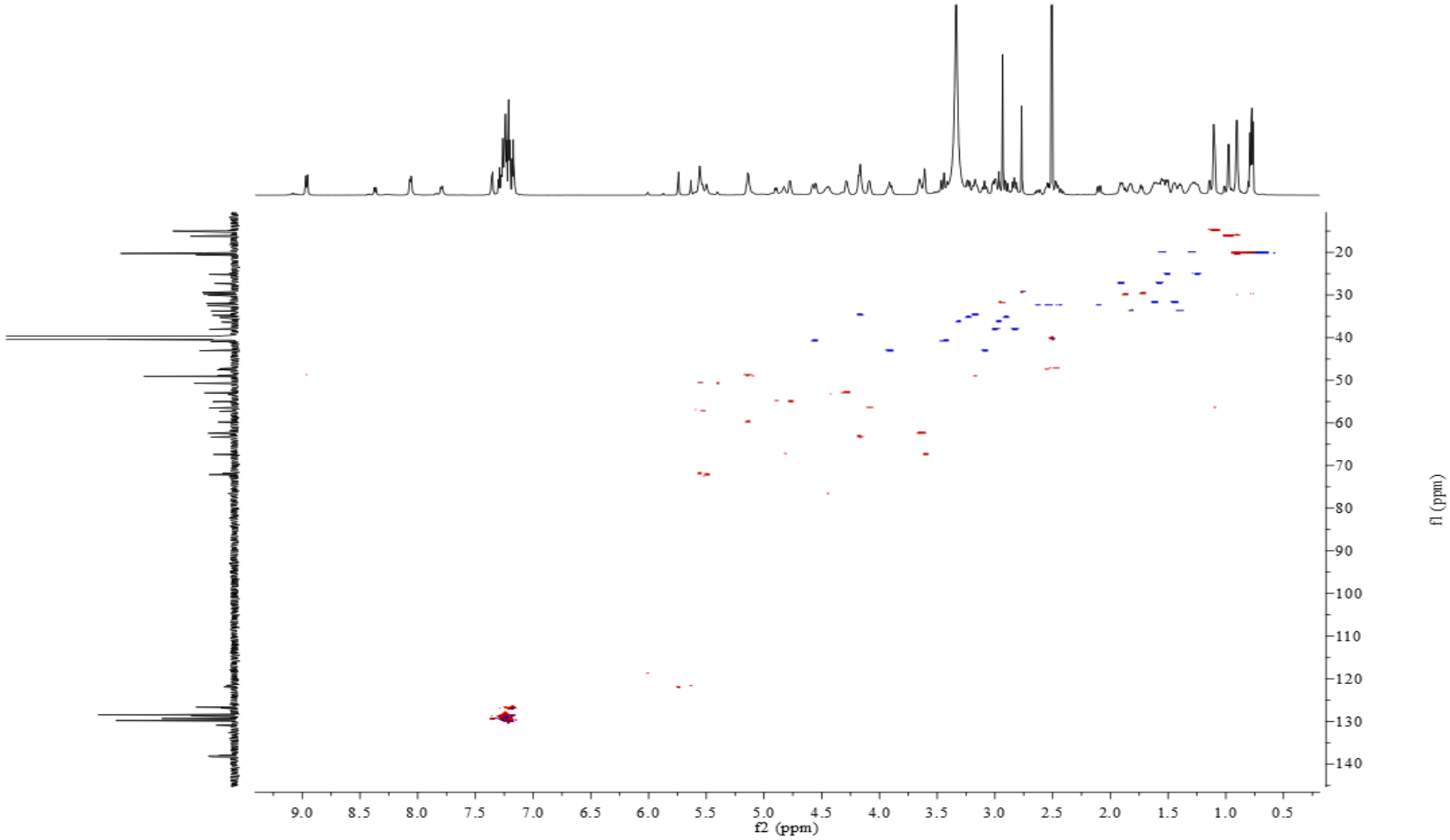


Figure S20. TOCSY spectrum of **2** in DMSO-d_6 (900 MHz)

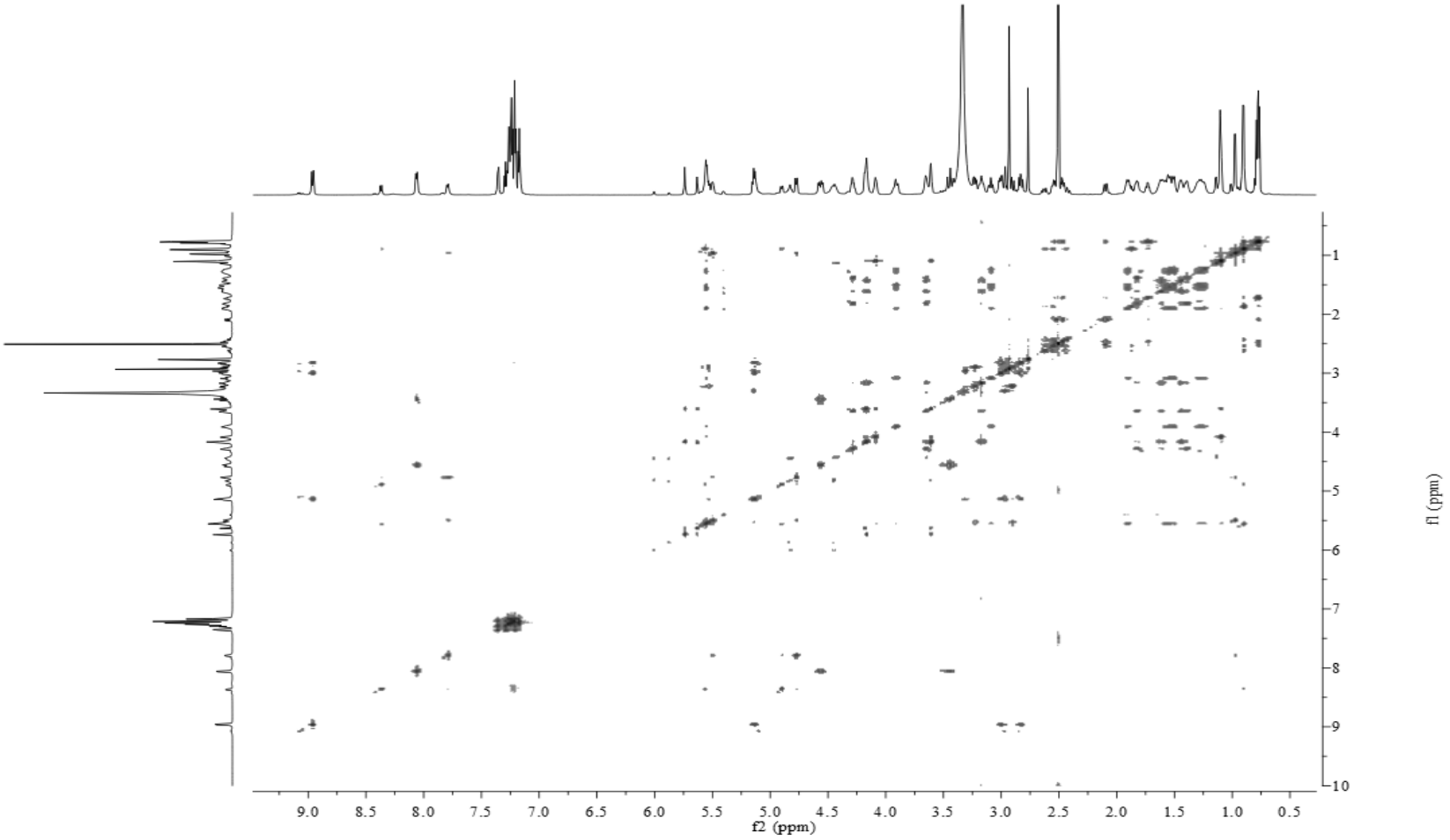


Figure S21. HMBC spectrum of **2** in DMSO-d_6 (900 MHz)

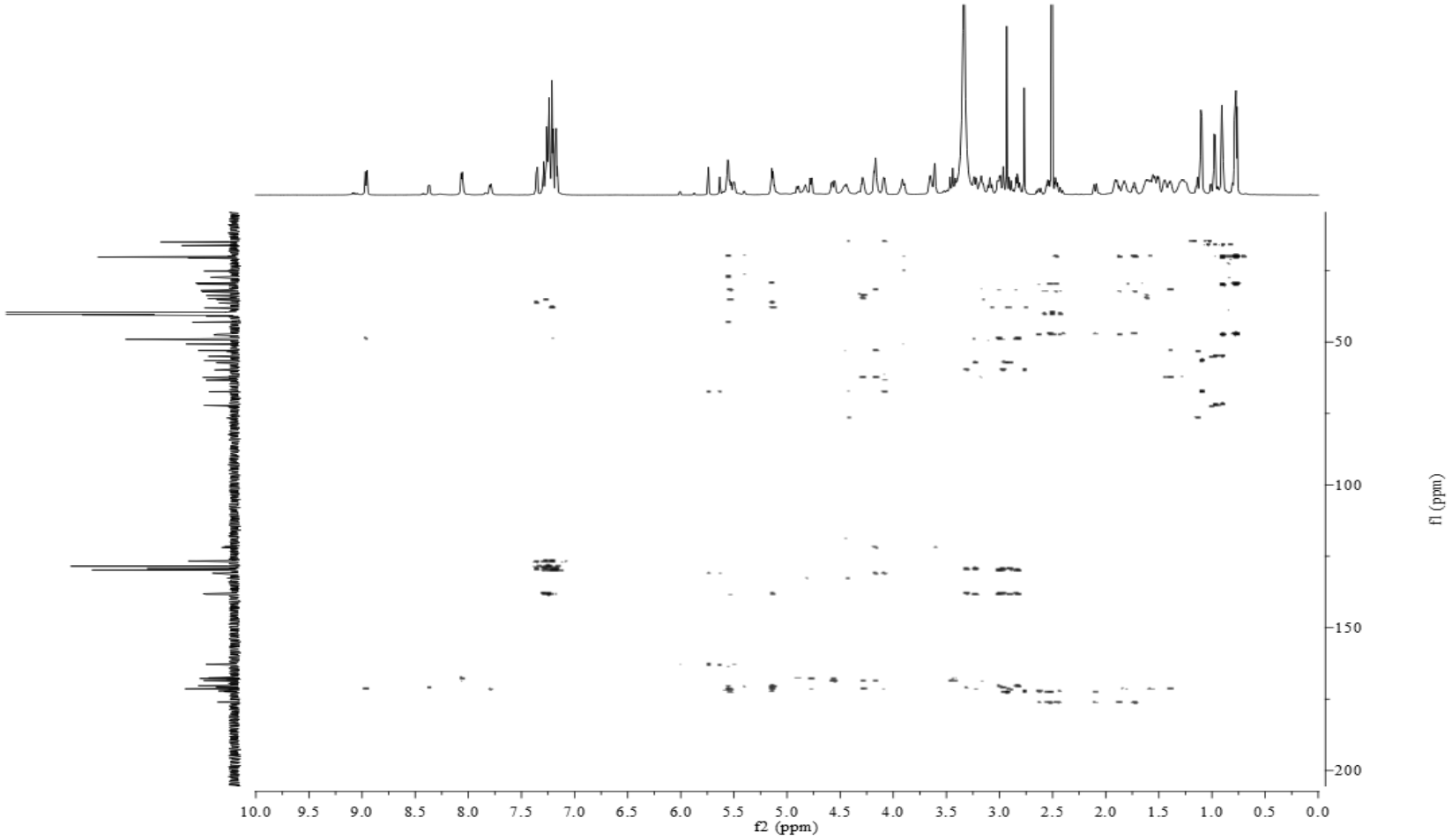


Figure S22. ROESY spectrum of **2** in DMSO-d_6 (900 MHz)

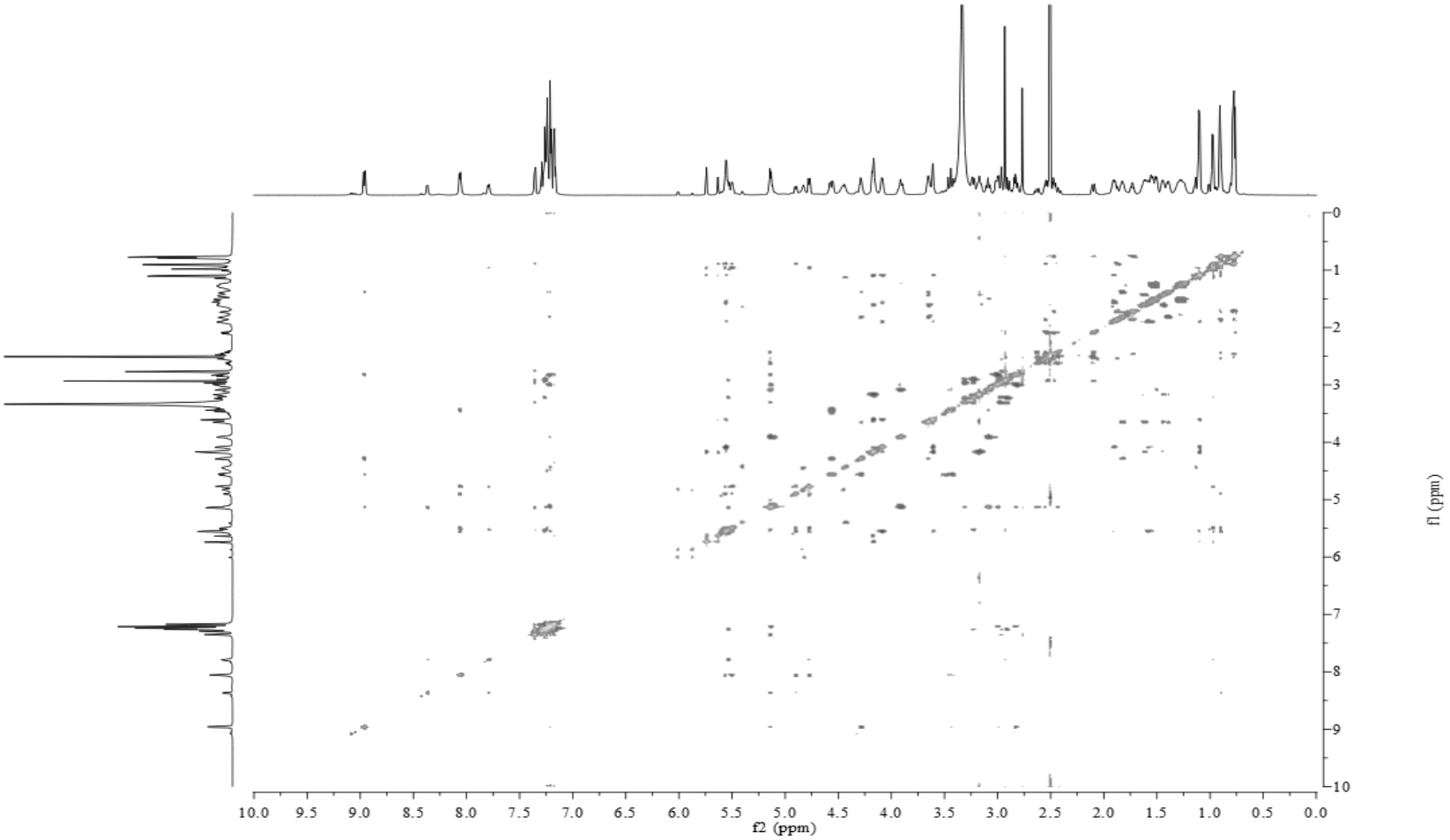


Figure S23. ^{1}H NMR spectrum of bis-*S*-MTPA ester **1c** in DMSO-d_6 (900 MHz)

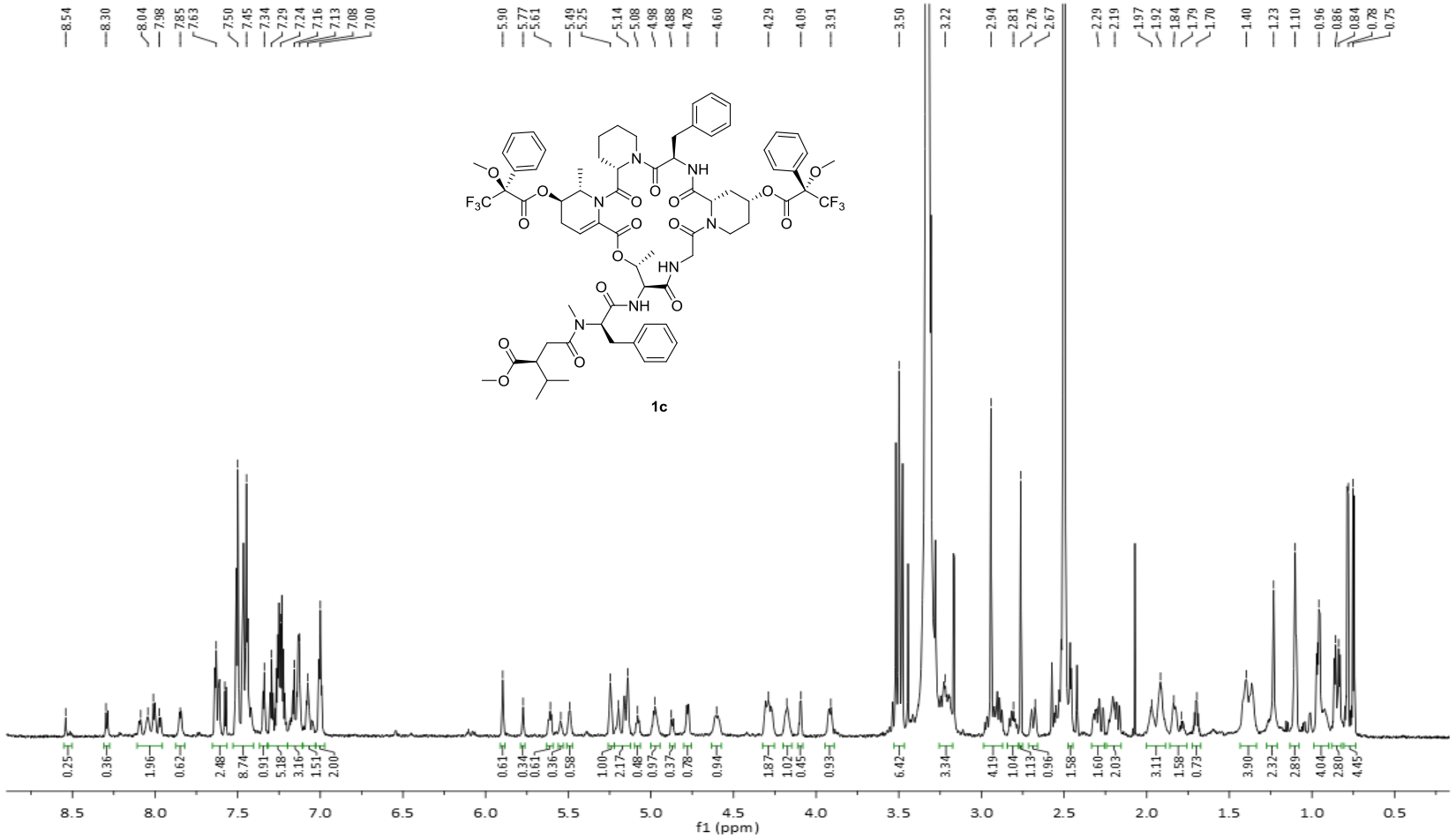


Figure S24. HSQC-DEPT spectrum of bis-*S*-MTPA ester **1c** in DMSO-d_6 (900 MHz)

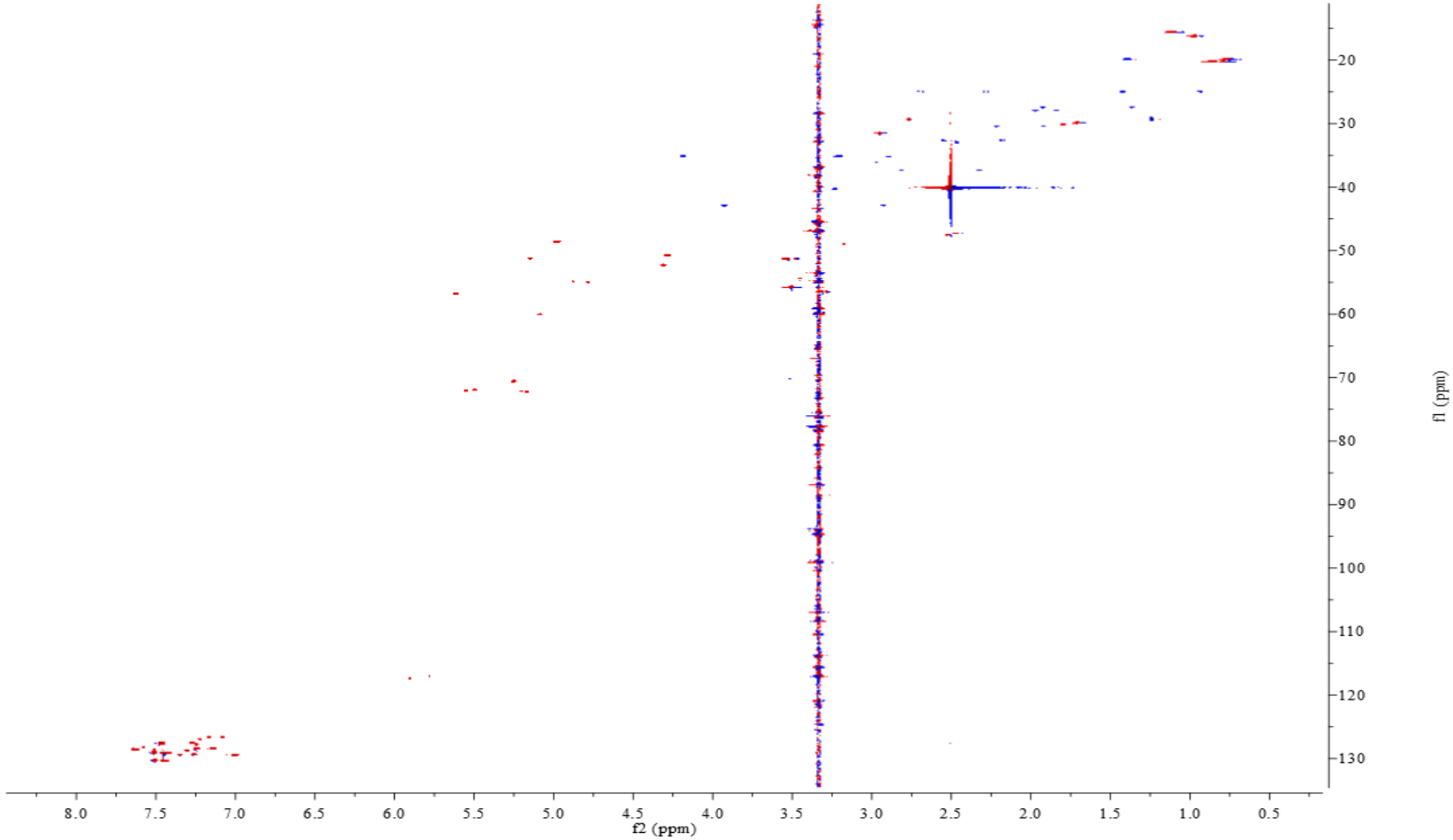


Figure S25. ROESY spectrum of bis-*S*-MTPA ester **1c** in DMSO-d_6 (900 MHz)

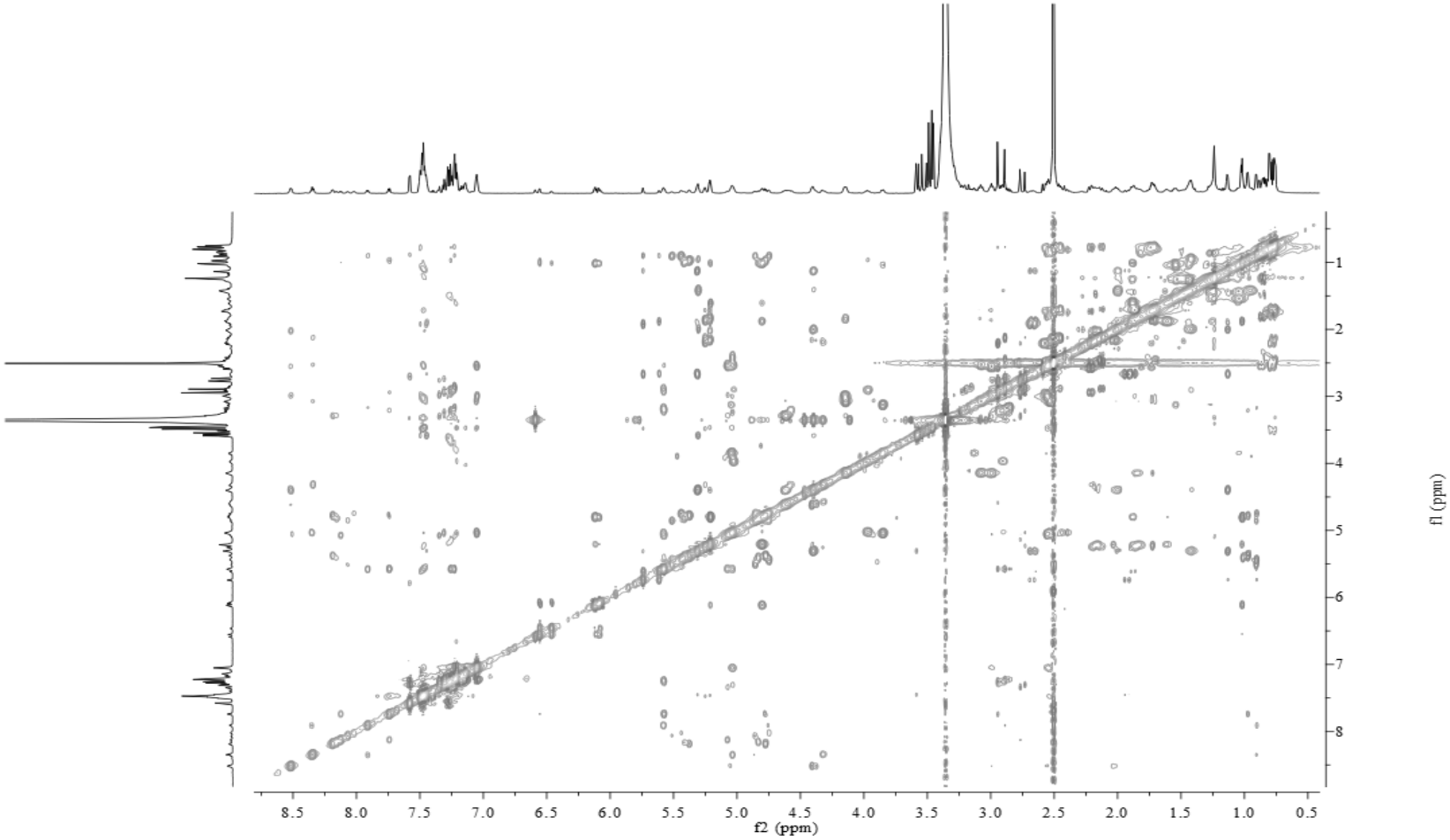


Figure S26. ^{1}H NMR spectrum of bis-*R*-MTPA ester **1d** in DMSO-d_6 (900 MHz)

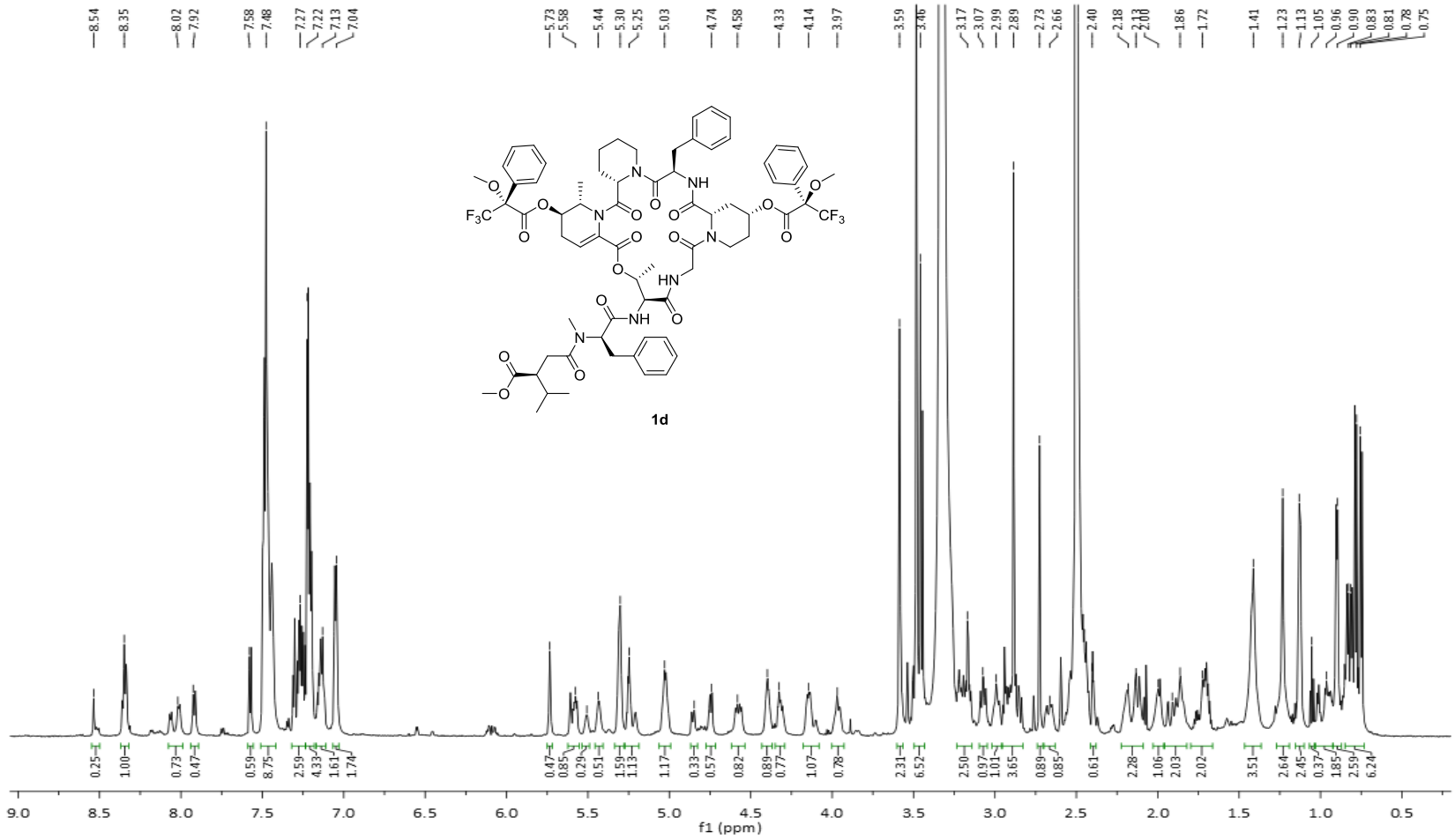


Figure S27. HSQC-DEPT spectrum of bis-*R*-MTPA ester **1d** in DMSO-d_6 (900 MHz)

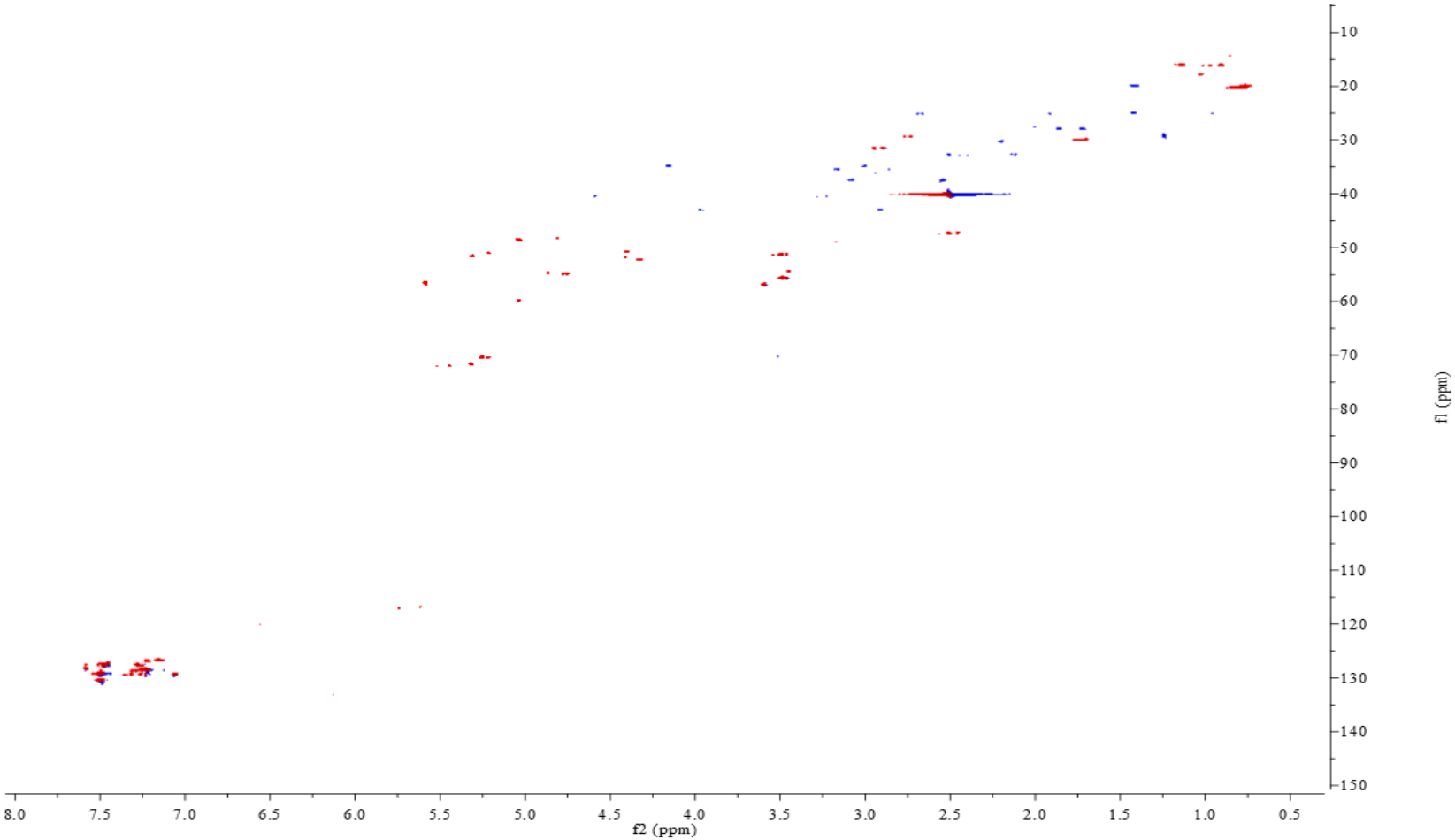


Figure S28. ^{1}H NMR spectrum of *S*-PGME amide **1e** in DMSO-d_6 (800 MHz)

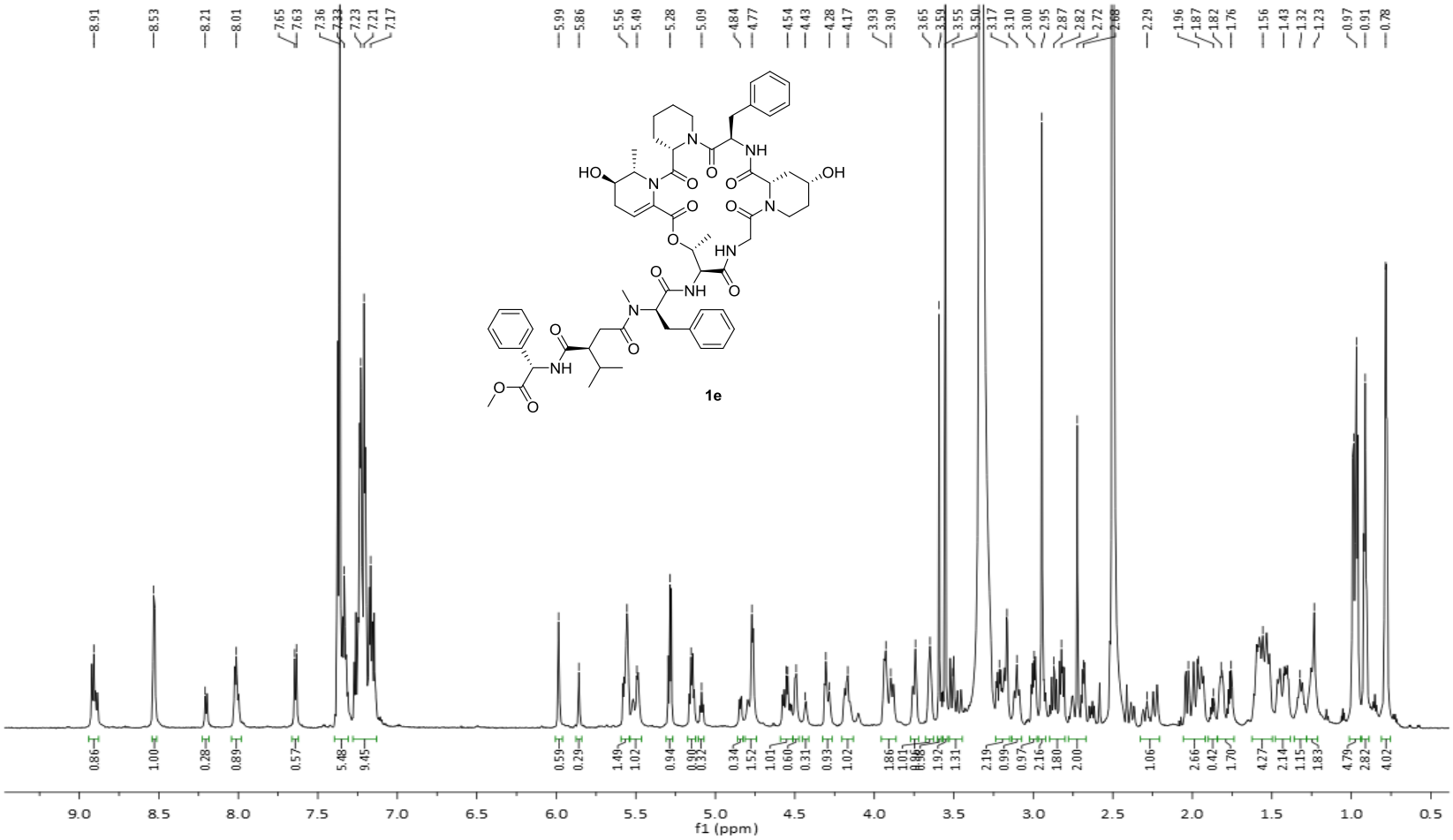


Figure S29. HSQC-DEPT spectrum of *S*-PGME amide **1e** in DMSO-d_6 (800 MHz)

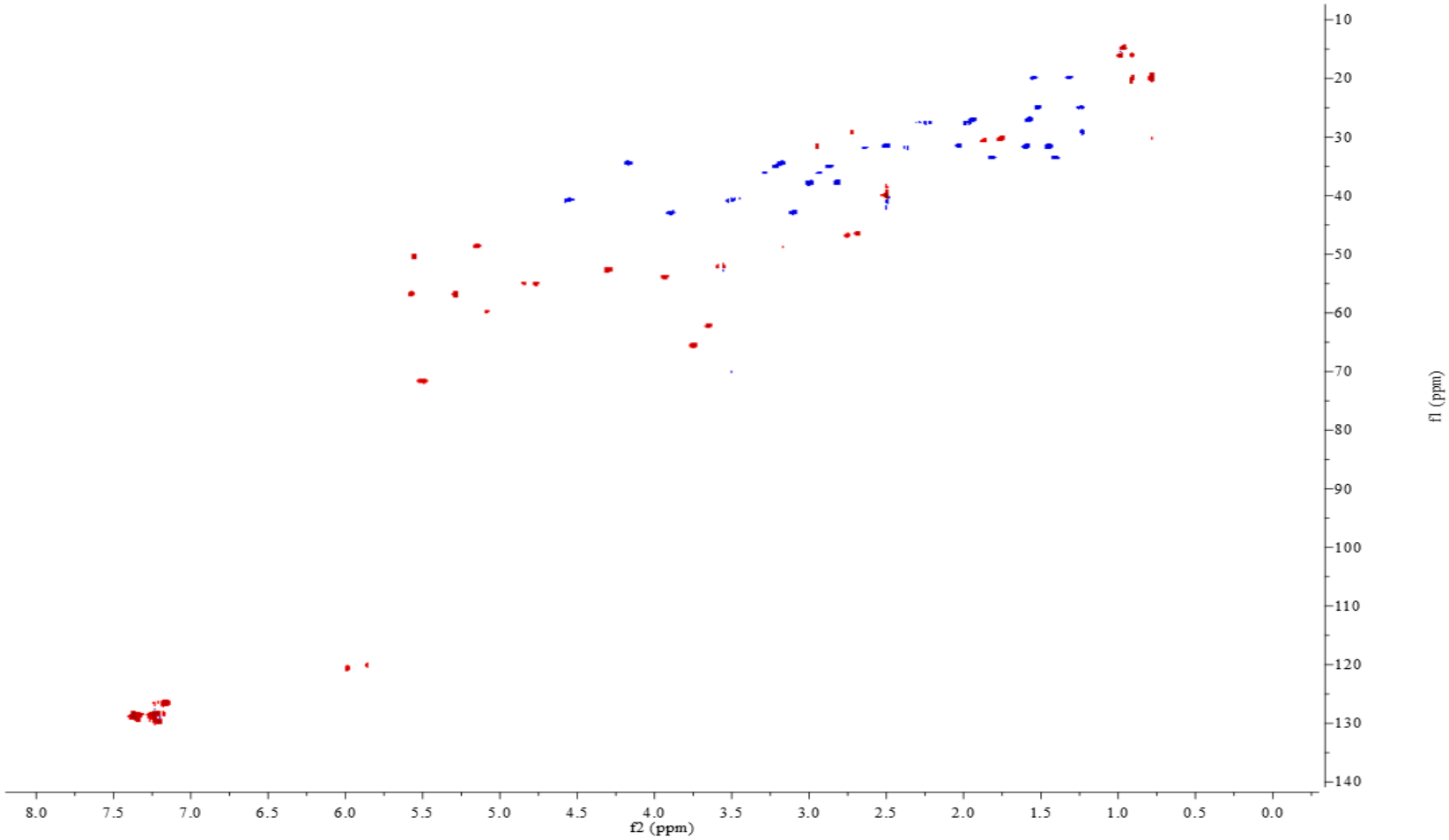


Figure S30. ^{1}H NMR spectrum of *R*-PGME amide **1f** in DMSO-d_6 (800 MHz)

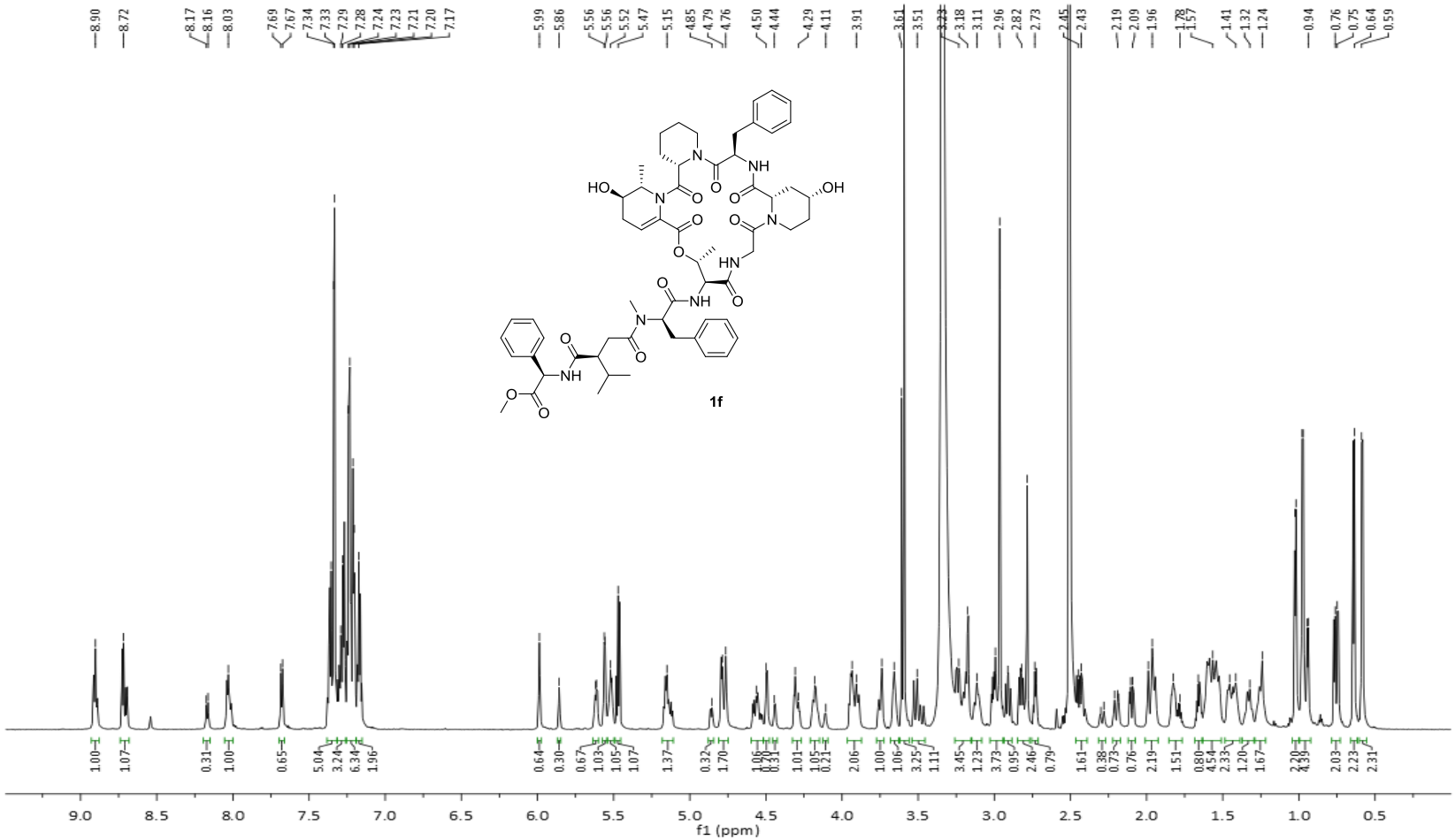


Figure S31. HSQC-DEPT spectrum of *R*-PGME amide **1f** in DMSO-d_6 (800 MHz)

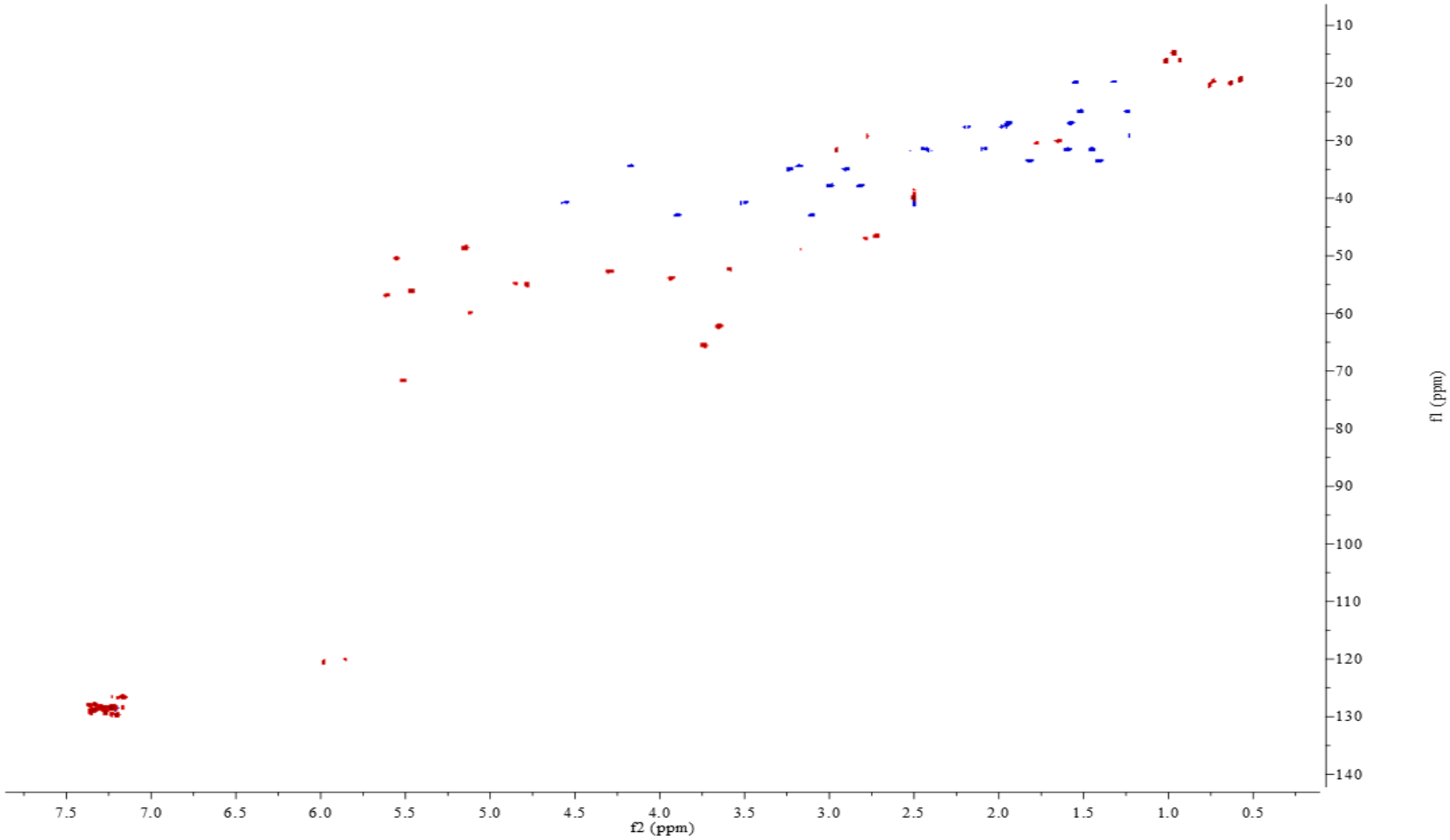

10
20
30
40
50
60
70
80
90
100
110
120
130
140
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f2 (ppm)

Figure S32. ^{1}H NMR spectrum of *S*-PGME amide of **2** in DMSO-d_6 (700 MHz)

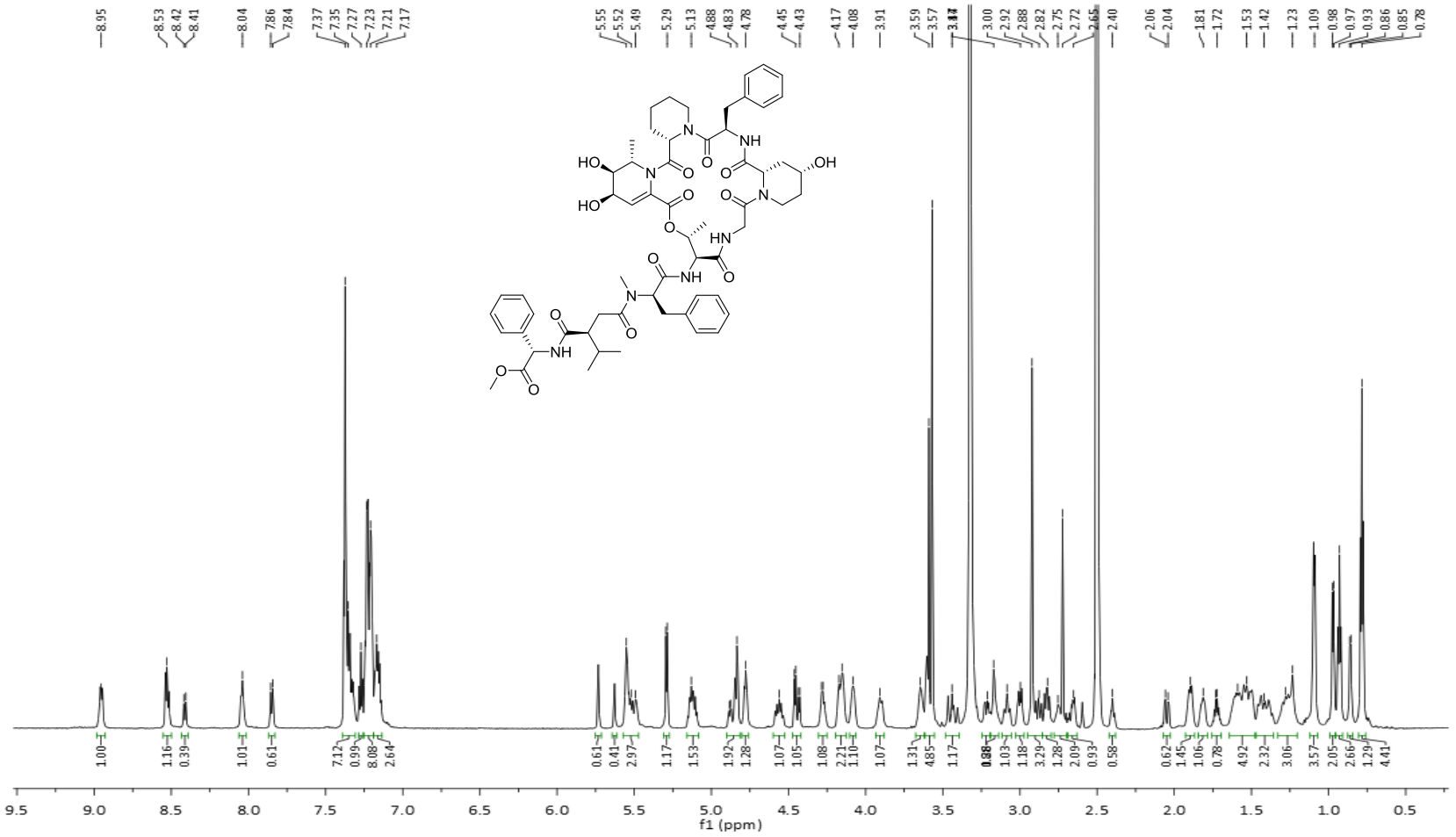


Figure S33. ^{1}H NMR spectrum of *R*-PGME amide of **2** in DMSO-d_6 (700 MHz)

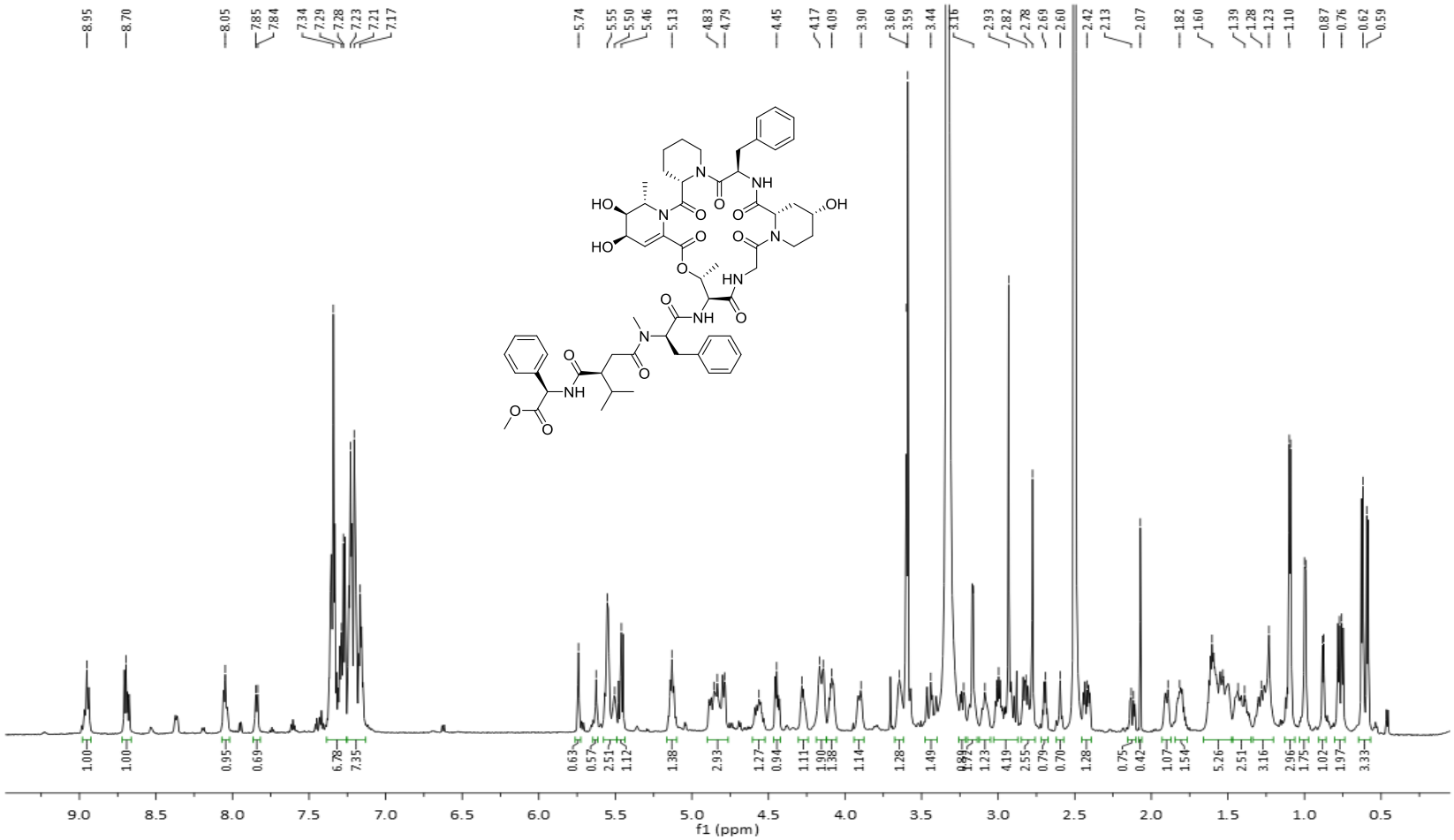


Figure S34. $\Delta\delta_{S-R}$ values around C-47 obtained for *S*- and *R*-PGME amides of **2**

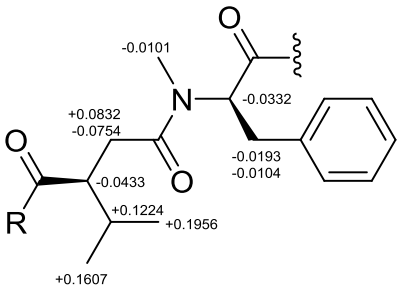


Figure S35. Time evolution of ICD spectra of **1** in solution of dimolybdenum tetraacetate in DMSO

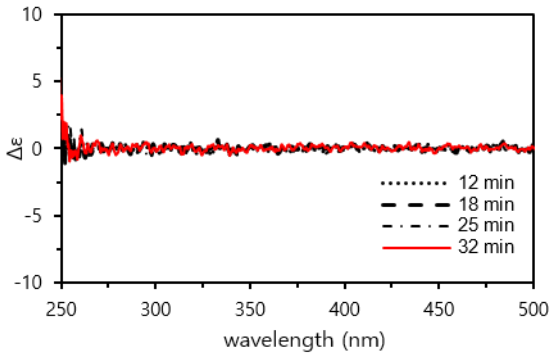
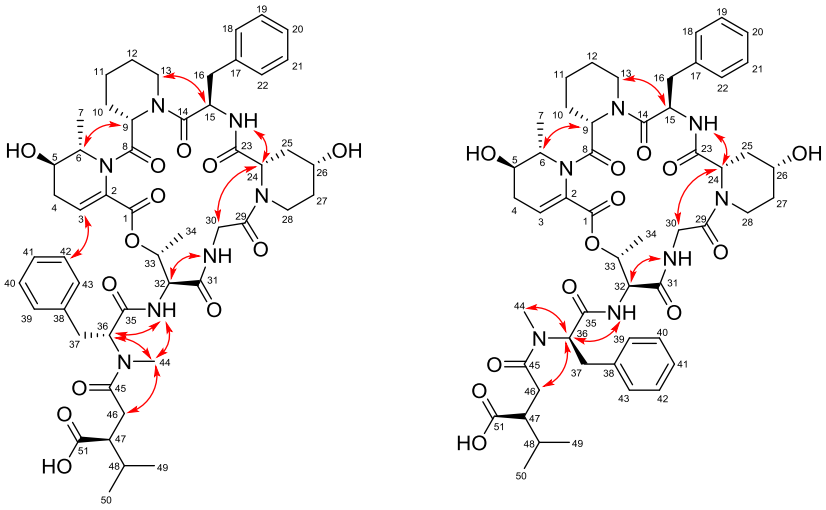


Figure S36. Key ROESY correlations of major and minor conformers of **1**



major conformer of **1** minor conformer of **1**

Figure S37. HRESIMS data of **1**

Elemental Composition Report

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Minimum:				-1.5			
Maximum:		5.0	50.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Formula
986.4861	986.4875	-1.4	-1.4	21.5	210.4	0.704	C51 H68 N7 O13

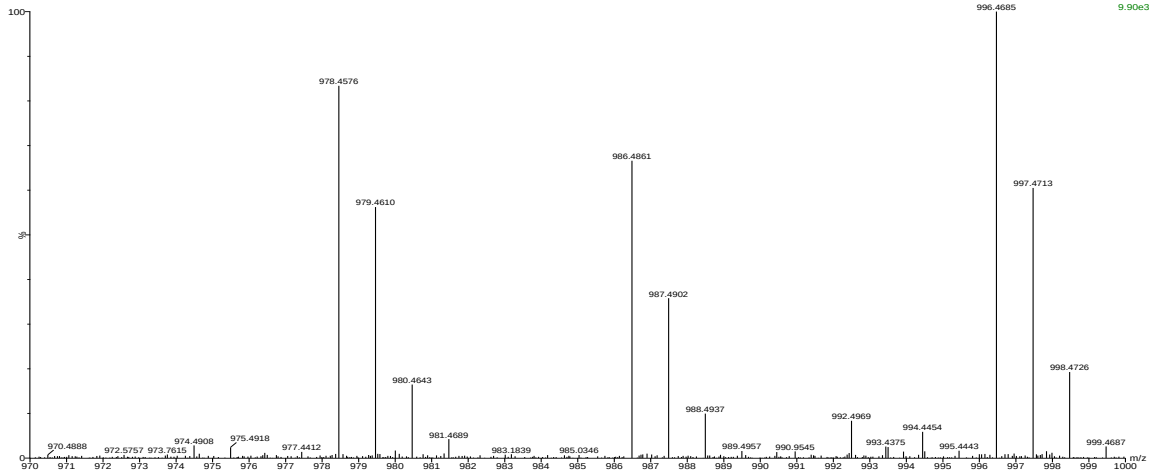


Figure S38. HRESIMS data of **2**

Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Minimum:				-1.5		
Maximum:		5.0	5.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
1024.4642	1024.4644	-0.2	-0.2	21.5	431.9	C51 H67 N7 O14 Na

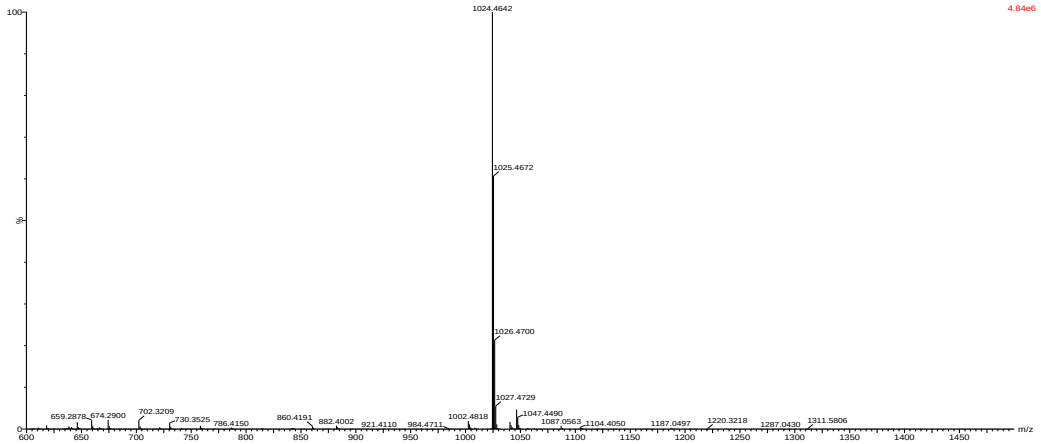


Figure S39. CD spectrum of **1** in MeOH

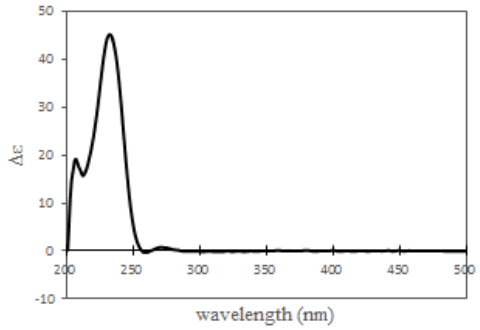


Figure S40. CD spectrum of **2** in MeOH