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Supplementary Information

Comparative Genome Mining and Heterologous Expression of an Orphan NRPS Gene Cluster Directs the Production of Ashimides

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Experimental Procedures

General experimental procedures.

All NMR spectra were collected on a Bruker Avance 600 at 600 MHz for ^{1}H and 150 MHz for ^{13}C nuclei. HRESIMS data were performed on an Agilent 6530 TOF LC/MS mass spectrometer. X-ray single crystal diffraction data were collected on a Bruker APEX-II CCD diffractometer. UV absorbance was measured on a Nanodrop 2000c spectrometer with a 10 mm cuvette. CD spectra were collected on a Jasco J-710 circular dichroism spectropolarimeter. The semipreparative RP-HPLC was performed on an Agilent 1200 HPLC with an Agilent Eclipse XDB-C18 column (5 μ, 250×9.4 mm). Sephadex LH-20 was purchased from GE Biotechnology. All chemicals used in the study were of analytical grade.

Strain isolation and identification.

Strain NA03103 was isolated from root sample collected in a mangrove forest of Hainan province, China. 16S rDNA gene was amplified with general primers [27F and 1492R] by using standard DNA extraction and PCR protocols. A BLAST (http://blast.ncbi.nlm.nih.gov) search of the partial 16S rRNA sequenced revealed 100% identity match with a *Streptomyces* sp. BCG23 strain (gene accession number KJ018714.1).

Construction of cosmid library.

The cosmid library of *S.* sp. NA03103 was constructed using an established protocol.[1] For the generation of a cosmid library, high quality chromosomal DNA (>150 kb) was isolated by salting-out procedure and partially digested with *Mbo*I, dephosphorylated with Alkaline Phosphatase (Thermo), and size-fractionated in low-melting temperature agarose by using pulsed-field gel electrophoresis (PFGE). The chromosome DNA fragments aroune 40 kb were recovered from the low-melting agarose gel that was digested by b-agarase (New England Biolabs). The recovered DNA was ligated with linealized cosmid vector pJTU2554 in a 1:1 molar ratio. After an overnight ligation at 16 °C, the mixture was packaged using MaxPlax^{TM} Lambda Packaging Extracts and transfected into *E. coli* EPI300 cell. The apramycin resistant colonies were picked individually and inoculated in LB (100 μL) that was supplied with apramycin in each well of 96-well plates. These plates were grown at 37 °C for 20 h, and then 50% glycerol (100 μL) was added into each well for storage at -80°C. Finally, The cosmid library of *Streptomyces* sp. NA3103 consisted of 5, 376 colonies with an average insert size of 40 kb.

Heterologous Expression and HPLC detection.

The cosmid pHG4001 was was transferred into the *E. coli*/ET12567 (pUZ8002) strain, and further introduced into *S. lividans* SBT18 by conjugation.[2] The recombiant strain was grown on ISP4 agar medium supplemented with 50 μg/mL of apramycin for sporulation. The seed culture was prepared by inoculating fresh spores into 250-mL baffled flasks containing 50 mL of TSB medium (17.0 g tryptone, 3.0 g soytone, 2.5 g glucose, 5.0 g sodium chloride, 2.5 g Na_2HPO_4 in 1 L water, pH7.0) for 2 days at 28 °C and 250 rpm. Subsequently, 20 mL seed cultures were inoculated into 1 L baffled flasks containing 400 mL of the fermentation medium (10 g soluble starch, 4 g yeast extract, 2 g peptone, 1 g $CaCO_3$, 40 mg $Fe_2(SO_4)_3$, 0.1 g KBr, 27 g sea salt in 1 L water), and incubated for 10 days at 160 rpm and 28 °C. Finally, the fermentation broth was filtered and absorbed with XAD-16 resin. The resin was washed with water and eluted with methanol. The afforded methanol extract was directly used for HPLC analysis. Each sample (10 μL) was injected into the column and first eluted with a linear gradient of 10% methanol to 90% methanol in water for 13 min, and finally with 100% methanol for 3 min at a flow rate of 0.5 mL/min.

Fermentation and extraction.

A fresh spore solution of *S.lividans* SBT18/pHG4001 was inoculated into 1-L flasks containing 200 mL of TSB medium. After growing at 28 °C and 160 rpm for 2 days, 20 mL of seed culture were transferred to 50 flasks each containing 200 mL of fermentation medium (10 g soluble starch, 4 g yeast extract, 2 g peptone, 1 g $CaCO_3$, 40 mg $Fe_2(SO_4)_3$, 0.1 g KBr, 27 g sea salt in 1 L water, pH 7.0) and incubated at 28 °C on rotary shakers (160 rpm) for 10 days. Then, XAD-16 resin (600 g) was added to the fermentation broth. The resin and cell mass were filtered and washed with water, and eluted with methanol (2 L). The methanol extracts were concentrated under reduced pressure to afford 8.3 g crude extract.

The procedure for large scale fermentation (5 L) and extraction of the mutant Δ*asmI* strain is similar to that described in *S.lividans* SBT18/pHG4001 strain, which led to afford 0.9 g crude extract.

Isolation and structure elucidation of metabolites from *S.lividans* SBT18/ pHG4001 and mutants.

The 8.3 g crude extract was loaded on a silica-gel (200 g) column, and eluted with CH_2Cl_3/MeOH (100:0, 100:2, 100:4, 100:8, 100:16, 100:32, 50:50) to give 7 fractions (A1-A7). Fractions A2 and A3 were furterh separated by a Sephadex LH-20 chromatography and fractionated using MeOH/H_2O (1:1) to give subfractions A2.1−A2.6 and A3.1−A3.7, respectively. Subfractions A2.2 were purified by semi-preparative HPLC eluted with 30% MeOH in H_2O at a flow rate of 2 ml/ min to afford **1** (20 mg) and **2** (25 mg). Subfractions A2.3 were separated using semipreparative HPLC elucted with 40% MeCN in H_2O to afford **3** (2.1 mg), **6** (1.8 mg), and **7** (0.8 mg). Subfractions A3.3 was purified using semipreparative HPLC elucted with MeCN-H_2O (gradient eluction in 25 min from 15% MeCN to 30% MeCN with a flow rate 2.0 mL/min) to afford **4** (1.1 mg), **5** (2.1 mg), and **8** (1.7 mg). Compound **9** (1.6 mg) was purifed from subfraction A3.4 using semipreparative HPLC eluted with 60% MeOH at a flow rate of 2 mL/min.

The 0.9 g crude extract obtained from Δ*asmI* strain was loaded on a silica-gel (30 g), and eluted with CH_2Cl_3/MeOH (100:0, 100:2, 100:4, 100:8, 100:16, 100:32, 50:50) to give 6 fractions (B1-B6). The fraction B2 was further fractionated by a Sephadex LH-20 chromatography and fractionated using MeOH to give subfractions B2.1−B2.5. Subfraction B2.2 were separated using semipreparative HPLC elucted with a gradient MeCN in H_2O (from 15% MeCN to 50% MeCN in 30 min with a flow rate 2.0 mL/min) to afford **17** (5.1 mg), **18** (6.6 mg), and **19** (4.0 mg).

Compound **1**: pale yellow crystal; NMR data see Table S4; HRESIMS *m/z* 465.1260 $[M+H]^+$ (calcd for $C_{19}H_{20}N_4O_{10}$, 465.1252); UV (MeOH): λ_{max} (log ε) =253 (3.16), 310 nm (2.81).

Compound **2**: yellowish oil; NMR data see Table S5; HRESIMS *m/z* 483.0919 $[M+H]^+$ (calcd for $C_{19}H_{19}ClN_4O_9$, 483.0913); UV (MeOH): λ_{max} (log ε) =253 (2.97), 310 nm (2.62).

Compound **3**: yellowish oil; NMR data see Table S6; HRESIMS *m/z* 453.0812 $[M+H]^+$ (calcd for $C_{18}H_{17}ClN_4O_8$, 453.0808); UV (MeOH): λ_{max} (log ε) =253 (3.06), 314 nm (2.72).

Compound **4**: yellowish oil; NMR data see Table S7; HRESIMS *m/z* 471.0909 $[M+H]^+$ (calcd for $C_{18}H_{19}ClN_4O_9$, 471.0913); UV (MeOH): λ_{max} (log ε) =253 (3.12), 314 nm (2.89).

Compound **5**: yellowish oil; NMR data see Table S8; HRESIMS *m/z* 501.1024 $[M+H]^+$ (calcd for $C_{19}H_{21}ClN_4O_{10}$, 501.1019); UV (MeOH): λ_{max} (log ε) =253 (3.22), 310 nm (3.20).

Compound **6**: yellowish oil; NMR data see Table S9; HRESIMS *m/z* 350.0948 $[M+H]^+$ (calcd for $C_{15}H_{15}N_3O_7$, 350.0943); UV (MeOH): λ_{max} (log ε) =253 (2.24), 310 nm (3.06).

Compound **7**: yellowish oil; NMR data see Table S10; HRESIMS *m/z* 425.0863 $[M+H]^+$ (calcd for $C_{17}H_{17}ClN_4O_7$, 425.0858); UV (MeOH): λ_{max} (log ε) =253 (2.89), 320 nm (2.98).

Compound **8**: yellowish oil; NMR data see Table S11; HRESIMS *m/z* 240.0507 $[M+H]^+$ (calcd for $C_{10}H_9NO_6$, 240.0503); UV (MeOH): λ_{max} (log ε) =253 (2.71), 310 nm (3.16).

Compound **9**: yellowish oil; NMR data see Table S12; HRESIMS *m/z* 254.0653 $[M+H]^+$ (calcd for $C_{11}H_{11}NO_6$, 254.0659); UV (MeOH): λ_{max} (log ε) =253 (2.91), 310 nm (3.07).

Compound **10**: yellowish oil; NMR data see Table S13; HRESIMS *m/z* 206.0453 $[M+H]^+$ (calcd for $C_{10}H_7NO_4$, 206.0448); UV (MeOH): λ_{max} (log ε) =288 (2.76), 360 nm (3.17).

Compound **17**: white powder; NMR data see Table S17; HRESIMS *m/z* 431.1204 $[M+H]^+$ (calcd for $C_{19}H_{18}N_4O_8$, 431.1197); UV (MeOH): λ_{max} (log ε) =288 (2.88), 360 nm (2.92).

Compound **18**: white powder; NMR data see Table S18; HRESIMS *m/z* 449.0853 $[M+H]^+$ (calcd for $C_{19}H_{17}ClN_4O_7$, 449.0859); UV (MeOH): λ_{max} (log ε)=288 (2.97), 360 nm (3.06).

Compound **19**: white powder; NMR data see Table S19; HRESIMS *m/z* 453.0959 $[M+H]^+$ (calcd for $C_{19}H_{19}ClN_4O_8$, 467.0964); UV (MeOH): λ_{max} (log ε) =288 (2.79), 360 nm (3.05).

Crystal data for ashimide A (1)

$C_{19}H_{20}N_4O_{10} + 2H_2O$ (M =500.42 g/mol), monoclinic, space group P 21 21 21 (no. 19), a = 6.9557(8) Å, b = 7.4897(8) Å, c = 41.571(5) Å, β = 90°, V = 2165.7(4) $Å^3$, Z = 4, T = 153 K, μ(Cu Kα) = 1.120 mm^{-1}, Dcalc = 1.535 g/cm^3, F(000) = 1048; 10157 reflections measured (6° ≤ Θ ≤ 68.4°), 3968 unique (R_{int} = 0.0468, R_{sigma} = 0.0412) which were used in all calculations. The final R_1 was 0.0412 (I > 2σ(I)) and wR_2 was 0.0923 (all data); The crystallographic data have been deposited in the Cambridge Crystallographic Data Centre as CCDC1861590.

Construction of in-frame deletion in cosmid pHG4001.

The gene inactivation was performed by two temperature-sensitive plasmid pKOV-Kan and pDF25 to generate the in-frame deletion mutants as previously described.[3] Briefly, the up- and downstream homologous arms were amplified with primers upF/R and downF/R listed in Table S2 using genomic DNA as template. The purified PCR products were ligated to *Bam*HI linearized pKOV-Kan to generate mutation carring plasmids (pHG4002−pHG4015). The individual plasmid (pHG4002−pHG4015) carring mutation were transformed into *E. Coli* DH10B competent cells contating pDF25 (chloramphenicol resistant) and grown on the LB argar medium supplemented with Chl, Kan and Apr at 30 °C over night. Several colonies were picked and streaked onto LB agar medium containing all three antibiotic as mentioned above and cultured at 43 °C over night for selection of single cross-over clones. The genotypes of these candidate clones were confirmed by PCR (primers listed in Table S2). The correct single cross over cosmids were isolated, re-transformed into pDF25 competent cells and grown at 30 °C with Chl, Kan, and Apr for double cross-over homologous recombination. The transformants were subjected to liquid LB supplemented with Apr and allowed to resolve at 43 °C for 24 h to elimate the pDF25 and pKOV-Kan. The cell suspension was diluted at different concentrations and spread on LB agar medium plate supplemented with Apr and sucrose, and incubated at 30 °C for 24 h. Colonies were picked and re-streaked on LB agar medium plate supplemented with Apr and sucrose, and allowed to grow at 30 °C overnight. Colonies that grew on medium with Apr and sucrose were analyzed by PCR (primers listed in Table S2). The genotype of mutated cosmid were confirmed by PCR and DNA sequencing (Table S2 and Figure S3). The afforded mutated cosmid were transformed into *S. lividans* SBT18 through conjugation as mentionbed above. The resulting mutant strains were used for further fermentation.

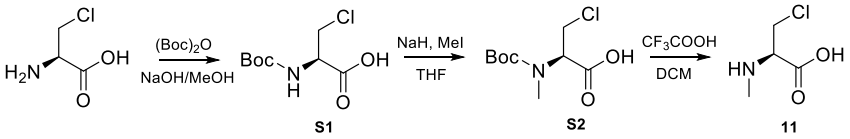
Stable isotope labeling experiment

L-alanine (^{15}N) , glycine-^{15}N, L-serine-3-^{13}C, L-alanine-2,3,3,3-D_4 were purchased from Sigma-aldrich. L-alanine-^{15}N (400 mg total) was added to 5 × 400 ml cultures, yielding 0.2 mg ashimide B (**2**) for N-H HMBC analysis. Glycine-^{15}N (100 mg total), L-serine-3-^{13}C (100 mg total) and L-alanine-2,3,3,3-D_4 (100 mg total) were added to 400 ml cultures individually and the cultures were harvested after 10 days' fermentaion. The metabolic extracts were used directly for LC-MS analysis.

Adenylation activities of A domain[4]

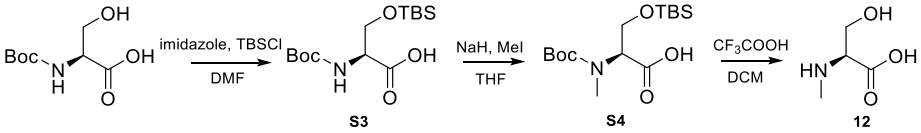
To determine the adenylation activity of A domains against different substrate, we carried out a coupled continuous assay for inorganic pyrophosphate using a unique fluorogenic pyrophosphate sensor in which the presence of pyrophosphate results in the production of a fluorescent product (λ_{ex}=316 /λ_{em}=456 nm) proportional to the pyrophosphate present (Sigma-Aldrich Pyrophosphate Assay Kit MAK 168).The A domain specificity assays were conducted in a 50 μL reaction volume containing 50 mM Tris-HCl, pH 7.5, 60 μM NRPS protein, 12.5 mM $MgCl_2$, 2.0 mM TCEP, 2 mM amino acid, 4 mM ATP. After reaction at room temperature for 30 min,[5,6] an equal volume of the Master Reaction Mix were added to each of the sample, and the reaction was incubated for 20 minutes at room temperature. Then , the fluorescence intensity (λ_{ex}=316/λ_{em}=456 nm) was measured by a microplate reader (TECAN infinite M200PRO).

Chemical synthesis of compound 11.



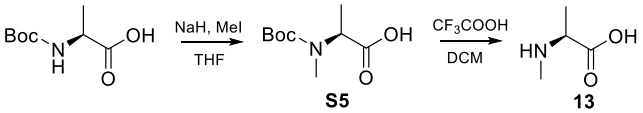
To a stirring solution of L-3-chloroalanine (1g, 8.1 mmol) in 20 mL of 1 M NaOH/MeOH (3:2) solution was added Boc_2O (3.5 g, 16.2 mmol, 2 eq.) at room temperature for 12 h.[7] The reaction mixture was cooled to 0 °C, acidified to pH 6.0, and extracted with EtOAC (50 mL × 3). The combined organic solvent was washed with brine, dried over Na_2SO_4, and evaporated to dryness to afford **S1** (1.2 g), which was directly used for next reaction without purification. Then a suspension of NaH in THF at 0 °C was slowly added the solution of 400 mg **S1** (1.8 mmol) and CH_3I (0.6 g, 4.0 mmol, 2.2 eq.) sequentially. The reaction mixture was warm to room temperature and stirred for 8 h. The reaction mixture was then diluted with diethyl ether (40 mL) and quenched with water (15 mL). The layers were separated, and the aqueous layer was extracted with diethyl ether (2 × 30 mL), acidified with 10% citric acid solution, and extracted with ethyl acetate (3 × 60 mL). The combined organic layers were washed by saturated brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo to yield an colourless oil. The crude material was purified via column chromatography using 30% EtOAc in petroleum ether, to afford **S2** (273 mg, 65%). **S2** (273 mg, 1.2 mmol) was dissolved in a solution of DCM (4 mL) and TFA (3 mL), and stirred at room tempreture for 2 h, then concentrated in vacuo to yield almost pure amino acid **11** as a trifluoroacetic acid salt (301 mg, 93%).[8] ^{1}H NMR data see Table S14; HRESIMS *m/z* 138.0312 [M + H]+, (calcd for $[C_4H_9ClNO_2]^+$ *m/z*, 138.0316).

Chemical synthesis of compound 12.



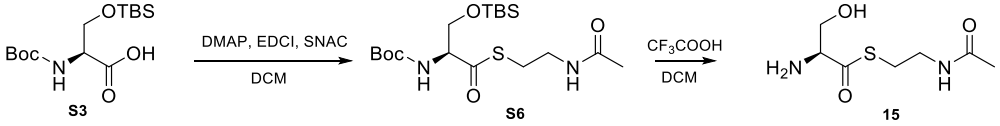
To a solution of N-(tert-butoxycarbonyl)-L-serine (1 g, 4.9 mmol) in anhydrous DMF (24 ml), 15 mmol imidazole (1 g, 14.7 mmol, 3 eq) was added at 0°C, followed by addition of TBSCl (1 g, 6.7 mmol, 1.4 eq). The mixture was warmed to room temperature slowly and stirred for 10 h. Upon total consumption of the substrate, the reaction mixture was poured into a mixture of ice cooled 1N HCl (5 ml) and ether (20 ml) to hydrolyze the silyl ester. The organic layer was separated and the aqueous layer was extracted with ether (20 mL) twice. The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo to yield the desired product **S3** (1.32g, 85%) withour purification.[9] The almost pure compound **12** (275 mg) was prepared using the method as mentioned in compound **11**. ^{1}H NMR data see Table S14; HRESIMS *m/z* 120.0651 [M + H]+, (calcd for $[C_4H_{10}NO_3]^+$ *m/z*, 120.0655).

Chemical synthesis of compound 13.



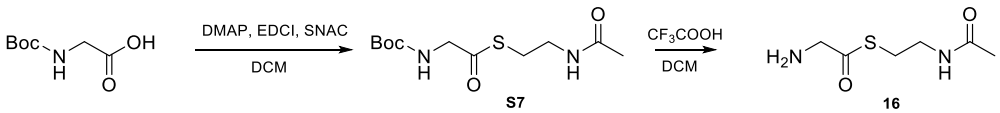
Compound **13** (245 mg) was prepared using the method as mentioned in compound **11**. ^{1}H NMR data see Table S14; HRESIMS *m/z* 104.0709 $[M + H]^+$, (calcd for $[C_4H_{10}NO_2]^+$ *m/z*, 104.0706).

Chemical synthesis of compound 15.[10]



To a solution of **S3** (50 mg, 0.16 mmol) in DCM (5 ml), EDCI (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride, 33 mg; 1.1 eq), DMAP (4-Dimethylaminopyridine, 3.8 mg, 0.03 mmol, 0.2 eq) and SNAC (N-Acetylcysteamine, 21 mg, 0.18 mmol, 1.1 eq) were added at room temperature. The reaction was stirred for 12 h, and comcentrated under reduced pressure. The product **S6** (51.3 mg) was purified by semi-preparative HPLC using 30 % MeCN in H_2O at a flow rate 2 mL/min to afford **S6** (56 mg). Deprotection of the Boc and TBS groups using 30% TFA in DCM at room temperature for 30 min from **S6** directly obtain compound **15** (21 mg) as an almost pure product. ^{1}H and ^{13}C NMR data see Table S15; HRESIMS *m/z* 207.2671 $[M + H]^+$, (calcd for $[C_7H_{15}N_2O_3S]^+$ *m/z*, 207.0798).

Chemical synthesis of compound 16.



Compound **16** (89 mg) was prepared using the same method as mentioned in **15**. ^{1}H and ^{13}C NMR data see Table S16; HRESIMS *m/z* 177.0690 $[M + H]^+$, (calcd for $[C_6H_{13}N_2O_2S]^+$ *m/z*, 177.0692).

Chemical complementation of compound 10 into *ΔasmH* mutant, *ΔasmJ* mutant and *ΔasmR* mutant.

ΔasmH mutant, *ΔasmJ* mutant and *ΔasmR* mutant were cultured in a 20 ml scale at 28°C in fermentation medium individually. After 24 hours cultivation, compound **10** (1.3 mg, 2 μmol) dissolved in DMSO was supplemented into fermentation broth and cultured for another 12 hours. After extraction by XAD-16 resin, the eluted methanol solution was then analyzed by LC-MS.

Chemical complementation of compounds 11-14 into *ΔasmO* mutant and *ΔasmP* mutant.

ΔasmO mutant and *ΔasmP* mutant strains were cultured in a 20 ml scale at 28°C in fermentation medium, respectively. After 24 hours cultivation, compounds **11–14** (each 5 mg) dissolved in H_2O was individually supplemented into the fermentation broth and cultivated for another 12 hours. After extraction by XAD-16 resin, the eluted methanol solution was then analyzed by LC-MS.

Chemical complementation of compounds 17–18 into *ΔasmS* mutant.

ΔasmS mutant strain was cultured in a 20 mL scale at 28°C in fermentation medium. After 24 hours cultivation, compounds **11**−**13** (each 1.5 mg) dissolved in DMSO were supplemented into fermentation broth individually and cultured for another 12 hours. The metabolic extract was then analyzed by LC-MS.

Protein Expression and Purification.

DNA fragments containing target genes including *asmD*, *asmI*, ferredoxin gene (ctg1_441) and ferredoxin reductase gene (ctg1_4885) were amplified from genomic DNA of *S.* sp. NA03103 with primers listed in Table S2. The purified PCR products were ligated with linearized pET28a (linearized by NdeI and HindIII) to afford pHG4016-pHG4019, respectively. And DNA fragments containing target genes including *asmC*, *asmM*, *asmN* and *asmS* were ligated with linearized pET22b (treated with *Nde*I and HindIII) to afford pHG4020−pHG4023, respectively. The obtained pHG4016-pHG4023 were further introduced into *E. coli* BL21(DE3), respectively. The transformants were cultivated in 400 mL LB medium supplemented with 50 μg/mL kanamycin (pHG4016−pHG4019) or 50 μg/mL Ampicillin (pHG4020-pHG4023) at 37 °C (220 rpm) until OD_{600} value reached around 0.6. The culture was cooled to 4 °C and induced with 0.1 mM IPTG, and continued to cultivate at 16 °C (220 rpm) for 18 h. After centrifugation at 4000 rpm for 10 min, cells were resuspended in lysis buffer (100 mM Tris, pH 8.0, 15 mM imidazole, 300 mM NaCl, 10 % glycerol) and lysed on ice by sonication. After centrifugation at 15000 rpm for 30 min, the supernatant was filtered and purified by ÄKTA FPLC system equipped with a 5 mL Histrap HP column (GE lifesciences).The proteins were pooled and desalted by a PD10 column (GE Healthcare) with 100 mM phosphate buffer (pH 7.0) and 10% glycerol and stored at -80°C.

***In vitro* assay of AsmD.**

The AsmD reaction solution (100 μL) contained 5 μM AsmD, 1mM compound **20**, 1 mM THF, 10 μM PLP, and 100 mM phosphate buffer (pH 7.5). Reaction was incubated at 30 °C for 1 h, and terminated by adding 100 μL acetonitrile. Then the mixture was centrifuged at 15,000 g for 30 min and the supernatant was analyzed by HPLC. The HPLC analysis was performed using a 18 min solvent gradient from 5% (0–10 min) and 5% to 100% (10–18 min) methanol in water supplied with 0.1 TFA at a flow rate of 0.5 mL/min.

***In vitro* assay of AsmI.**

The AsmI-catalyzed reaction was carried out in a 100 μL reaction system containing 100 mM phosphate buffer (pH 7.0), 100 μM substrate, 5 mM NADPH, 2μM FDR, 2μM FDX and 30 μM AsmI. After incubation at 30°C for 1 h, 1.5 h, 2 h, and 3 h, the reaction was quenched by adding 100 μL methanol. The reaction mixture was then centrifuged at 15, 000 g for 10 min and the supernatant was analyzed by HPLC or LC-MS. The HPLC analysis was performed using a 18 min solvent gradient from 10% to 90% (0–15 min) and 100% (15–18min) methanol in water supplied with 0.1 TFA at a flow rate of 0.5 mL/min with a UV detection at 310 nm. The LC-MS analysis was performed using a 18 min solvent gradient from 10% to 90% (0–13 min) and 100% (13–15 min) methanol in water supplied with 0.1 TFA at a flow rate of 0.5 mL/min.

Cytotoxicity assay.

The in vitro cytotoxicity against the tested cell lines was determined using the MTT method. MCF-7 cells was seeded in 96-well plates at density of 1.5×10^4 cells per well and different concentrations compound **2** was added into the medium after 8 h. 4 mg/mL of MTT (20 μl per well) was added to the medium at the end of culture and incubated at 37°C for 4 h. After 4 h incubation, the formazan production was dissolved by DMSO and readied absorbance at 570 nm.

Apoptosis analysis.

The apoptosis induction of compound **2** in MCF-7 was measured by FITC-labeled annexin-V/PI apoptosis detection kit according to manufacturer's instruction. Briefly, various concentrations of compound **2** was added to the medium of MCF-7 cells (1×10^5 cells per wells in 6-well plates) and cultured for 48 h. After harvested washed with PBS, MCF-7 cells were stained with FITC-labeled annexin-V and PI diluted in binding buffer. 15 minutes later, the cells were analyzed using flow cytometry (FACScalibur, USA)

Table S1. Bacterial plasmids and strains.

Plasmid/Strain	Relevant characteristics	Source
Plasmid		
pJTU2554	Cosmid vector	Ref 11
pKOV -Kan	plasmid used for gene disruption, temperature sensitive	Ref 12
pDF25	plasmid used for gene disruption, temperature sensitive	Ref 13
pET28a(+)	Protein expression vector used in *E.coli*, encoding N-terminal His-tag, kanamycin resistance	Novagen
pET22b(+)	Protein expression vector used in *E.coli*, encoding N-terminal His-tag, Ampicillin resistance	Novagen
pHG4001	pJTU2554 harboring the intact *asm* gene cluster	This study
pHG4002	pKOV derived plasmid for disruption of *asmA*	This study
pHG4003	pKOV derived plasmid for disruption of *asmB*	This study
pHG4004	pKOV derived plasmid for disruption of *asmC*	This study
pHG4005	pKOV derived plasmid for disruption of *asmD*	This study
pHG4006	pKOV derived plasmid for disruption of *asmH*	This study
pHG4007	pKOV derived plasmid for disruption of *asmI*	This study
pHG4008	pKOV derived plasmid for disruption of *asmJ*	This study
pHG4009	pKOV derived plasmid for disruption of *asmK*	This study
pHG4010	pKOV derived plasmid for disruption of *asmM*	
pHG4011	pKOV derived plasmid for disruption of *asmN*	
pHG4012	pKOV derived plasmid for disruption of *asmO*	This study
pHG4013	pKOV derived plasmid for disruption of *asmP*	This study
pHG4014	pKOV derived plasmid for disruption of *asmR*	This study
pHG4015	pKOV derived plasmid for disruption of *asmS*	This study
pHG4016	pET28a(+) derived plasmid for expressing N-terminal His-tag AsmD	This study
pHG4017	pET28a(+) derived plasmid for expressing N-terminal His-tag AsmI	This study
pHG4018	pET28a(+) derived plasmid for expressing N-terminal His-tag Ctg1_4885	This study
pHG4019	pET28a(+) derived plasmid for expressing N-terminal His-tag Ctg1_441	This study
pHG4020	pET22b(+) derived plasmid for expressing N-terminal His-tag AsmC	This study
pHG4021	pET22b(+) derived plasmid for expressing N-terminal His-tag AsmM	This study
pHG4022	pET22b(+) derived plasmid for expressing N-terminal His-tag AsmN	This study
pHG4023	pET22b(+) derived plasmid for expressing N-terminal His-tag AsmS	This study
***E. coli* strains**		
DH5α	General cloning host	Ref 14
DH10B	General cloning host	
BL21 (DE3)	Heterologous host for protein expression	NEB
ET12567 (pUZ8002)	Methylation-deficient host used for *E. coli-Streptomyces* intergeneric conjugation	Ref 15
***Streptomyces* strains**		
S. lividans SBT18	Model actinomycete used for gene heterologous expression.	Ref 16
NA03103	Wild type strain for ashimides production	This study
HG04001	Heterologous expression of cosmid 31E5 in *S. lividans* SBT18	This study
HG04002	*ΔasmA*, in-frame deletion mutant strain	This study
HG04003	*ΔasmB*, in-frame deletion mutant strain	This study
HG04004	*ΔasmC*, in-frame deletion mutant strain	This study
HG04005	*ΔasmD*, in-frame deletion mutant strain	This study
HG04006	*ΔasmH*, in-frame deletion mutant strain	This study
HG04007	*ΔasmI*, in-frame deletion mutant strain	This study
HG04008	*ΔasmI*, in-frame deletion mutant strain	This study
HG04009	*ΔasmJ*, in-frame deletion mutant strain	This study
HG04010	*ΔasmK*, in-frame deletion mutant strain	This study
HG04011	*ΔasmO*, in-frame deletion mutant strain	This study
HG04012	*ΔasmM*, in-frame deletion mutant strain	This study
HG04013	*ΔasmN*, in-frame deletion mutant strain	This study
HG04014	*ΔasmP*, in-frame deletion mutant strain	This study
HG04015	*ΔasmR*, in-frame deletion mutant strain	This study
HG04016	*ΔasmS*, in-frame deletion mutant strain	This study

Table S2. Primers used in this study.

Name	Sequence
screen-up-F	GGACAACCTGCTCACCAA
screen-up-R	GGCGTTCTCGATGTGCT
screen-down-F	TGTCCTTCGGGTCGTAGAA
screen-down-R	GAGCTGGGCAAGGAGATG
asmA-up-F	CTGGCGGCCGTTACTAGTGGATCCTGGTGAGGACGGAGTTGA
asmA-up-R	ATGTACGCCGTCAGCACGCCGCTCATACTGCGCCAC
asmA-down-F	TTCACACCGGAAACCCTTTACGTGATCCGGCAGGCG
asmA-down-R	TCTCCGGTCGACTCTAGAGGATCCGAGGCAAACGCCTGGTC
asmB-up-F	CTGGCGGCCGTTACTAGTGGATCCGCTCGGGATGTCGAAGAAG
asmB-up-R	CGTTCTCGATGTGCTCGGGGGTACCAGCCGTAGTACGG
asmB-down-F	GGGTTCGGTTACGTGATCCCTTCTACCGCGTCGACAAG
asmB-down-R	TCTCCGGTCGACTCTAGAGGATCCATGGGAGGAGGAGCGGTT
asmC-up-F	CTGGCGGCCGTTACTAGTGGATCCTGAGCCGCTGATCCAGA
asmC-up-R	TGGGAGGTCACGGTCAAGAGGCGAGCTTCTTCCAC
asmC-down-F	GTGGAAGAAGCTCGCCTCTTGACCGTGACCTCCCA
asmC-down-R	TCTCCGGTCGACTCTAGAGGATCCGCGTCGAGGAAGATGATGAA
asmD-up-F	CTGGCGGCCGTTACTAGTGGATCCCGGAGGTCAGGATGACGTA
asmD-up-R	GATGACGTGGACTGGGAAACGATCGGGACCAACACACTC
asmD-down-F	GAGTGTGTTGGTCCCGATCGTTTCCCAGTCCACGTCATC
asmD-down-R	TCTCCGGTCGACTCTAGAGGATCCGCCGATTCCAGGGTCTTG
asmH-up-F	CTGGCGGCCGTTACTAGTGGATCCCTCATCGGTAAGGTCTCACATC
asmH-up-R	CTGGACGAGAGGAAGCCGGACCCCTTCATGAGCCTT
asmH-down-F	AAGGCTCATGAAGGGGTCCGGCTTCCTCTCGTCCAG
asmH-down-R	TCTCCGGTCGACTCTAGAGGATCCCTGAACAACGTCCTGCTCAAC
asmI-up-F	CTGGCGGCCGTTACTAGTGGATCCAGGCGGAGAAGGTGTTGTAGA
asmI-up-R	TCGGAACCCTCCGCACCACCGCCGGACTCAGTCCTG
asmI-down-F	CAGGACGTAGTCCGGCGGTGGTGCGGAGGGTTCCGA
asmI-down-R	TCTCCGGTCGACTCTAGAGGATCCCGGGTTCAGCCGTGGTTATG
asmJ-up-F	CTGGCGGCCGTTACTAGTGGATCCAGGTTCTCGGGCATGAAGA
asmJ-up-R	ACGTACACCACGCAGCCCGAGAGCACCGGGAACTCA
asmJ-down-F	TGAGTTCCCGGTGCTCTCGGGCTGCGTGGTGTACGT
asmJ-down-R	TCTCCGGTCGACTCTAGAGGATCCAGCATGAACTGGCGGTTCTC
asmK-up-F	CTGGCGGCCGTTACTAGTGGATCCGATGACGGTGTCCTCCTTG
asmK-up-R	TGGAAAGCAAGGTCGGCACTCCACCCACTTCTGTCT
asmK-down-F	AGACAGAAGTGGGTGGAGTGCCGACCTTGCTTTCCA
asmK-down-R	TCTCCGGTCGACTCTAGAGGATCCACCGACGACCAGGTCAA
asmM-up-F	AACGACGGCCAGTGCCAAGCTTTCATCCGGCCCGCGTCC
asmM-up-R	CCCGTGAGCCGCTGCGTGTGGTCCCGCATGACGGG
asmM-down-F	CCCGTCATGCGGGACCACACGCAGCGGCTCACGGG
asmM-down-R	AGCTATGACATGATTACGAATTCTGCGCGAGCGTGGACC
asmN-up-F	AACGACGGCCAGTGCCAAGCTTTCGACGGTCCGGTAGTCGAG
asmN-up-R	TTCCAGGCCGGGCTGGCCAGCCCGGCCTGGAAG
asmN-down-F	GTGTCGGCGAGTGCCTGCCAGCCCGGCCTGGAAG
asmN-down-R	AGCTATGACATGATTACGAATTCGTCGGGTGTGGTCACGGC
asmO-up-F	CTGGCGGCCGTTACTAGTGGATCCCCGACATGACCGGCAACT
asmO-up-R	TCCTGCGGTCGGGTGTGGCATGGCTCCACCTCGCAC
asmO-down-F	GTGCGAGGTGGAGCCATGCCACACCCGACCACAGGA
asmO-down-R	TCTCCGGTCGACTCTAGAGGATCCCACCCGACCACAGGA
asmP-up-F	CTGGCGGCCGTTACTAGTGGATCCGTCTCGCGCCAGTAGGCCA
asmP-up-R	TTTTCGCGGGAAGGGGCCGCCGCTCCTCCTGTGGTCG
asmP-down-F	CGACCACAGGAGGAGCGGCGGCCCCTTCCCGCGAAAA
asmP-down-R	TCTCCGGTCGACTCTAGAGGATCCGGATCAGCGGCTTCACGCC
asmR-up-F	CTGGCGGCCGTTACTAGTGGATCCGACGATGCTGTCCTTCTACAAC
asmR-up-R	GGCCTTCCAGCTGATCAGGCAAGGAACGTAAGGCCC
asmR-down-F	GGGCCTTACGTTCCTTGCCTGATCAGCTGGAAGGCC
asmR-down-R	TCTCCGGTCGACTCTAGAGGATCCGACGTTGAGCACCTGGAA
asmS-up-F	CTGGCGGCCGTTACTAGTGGATCCCGGCAGTCGTCAGCCTAAAT
asmS-up-R	AGCGGTACTGCATCAACTCGTGCTCTCCGGGTTCAC
asmS-down-F	GTGAACCCGGAGAGCACGAGTTGATGCAGTACCGCT
asmS-down-R	TCTCCGGTCGACTCTAGAGGATCCCACTACGTGGAGGAGTTCTT
asmA-PO-F	GGCTCGACCATTCTGTTGAT
asmA-PO-R	GCTCCAGCATCGTGTTGA

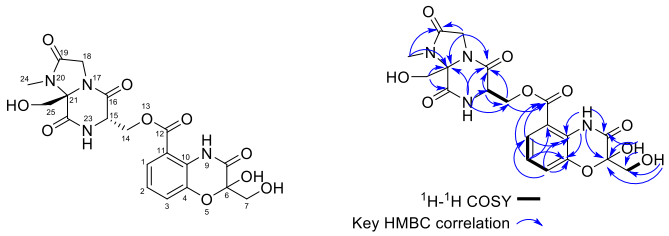
asmA-NE-F	TATGAGCGGGTGGAGAAAGTA
asmA-NE-R	GGATCACGTAACCGAACCC
asmB-PO-F	GTATGAGCGGGTGGAGAAAG
asmB-PO-R	CAGCGTGACCGTGAACA
asmB-NE-F	TCGGACGAGGACCTCAC
asmB-NE-R	TTGTCGACGCGGTAGAAAC
asmC-PO-F	CCCGAGCACATCGAGAAC
asmC-PO-R	TCAACCGGAACGTCGTG
asmC-NE-F	GGACTCCACGCACATCTC
asmC-NE-R	ACGTTCGGCCACATGAA
asmD-PO-F	TTGACCGTGACCTCCCA
asmD-PO-R	GCAGATAGCCGTTCACCAC
asmD-NE-F	GAGGACCATGTGGGTGTC
asmD-NE-R	ACTGGGAAACCGATGCC
asmH-PO-F	CCAGTTCACCGGTCACG
asmH-PO-R	CCACCTGTACAAGCCCTTC
asmH-NE-F	TCGTCGAGTCCATCGTCA
asmH-NE-R	CGGGTCGAACTTCGTCATC
asmI-PO-F	GCGGAACAGGGTCAACTC
asmI-PO-R	ACAACCACCTGCTGAACAA
asmI-NE-F	GTGCCGAAGGGCTTGTA
AsmI-NE-R	CTACAACGACGAGGAGAACTG
asmJ-PO-F	CACGTTCGCTTCCCTTTCT
asmJ-PO-R	ACTCCACCCACTTCTGTCT
asmJ-NE-F	CCTTCTCGTTGCCGAACA
asmJ-NE-R	CCTGAACGGCTCCGAAC
asmK-PO-F	CCTTCTCGTTGCCGAACA
asmK-PO-R	GATCAGACTGCCGTAGATGC
asmK-NE-F	ACAGAAGTGGGTGGAGTG
asmK-NE-R	TGGGAAACGCCGAGATG
asmM-PO-F	CCGGAACTCGTCGCCTTC
asmM-PO-R	CTCCTCGGCCACATCCGG
asmN-PO-F	GCCGCCCACCGGTCA
asmN-PO-R	CCACGGTGGGAGGCCCC
asmO-PO-F	CGGTCATCGACTCCAACAG
asmO-PO-R	GGCACTTGTCGAGGAAGAA
asmO-NE-F	TATGCCGTGCATCCGAAG
asmO-NE-R	GCGGTAGAACGGCATCAT
asmP-PO-F	TCACCGGACTCACCTACC
asmP-PO-R	GTACTGTCGTCGGTCATGAAG
asmP-NE-F	CAAACCGGTGCTGATGATCTA
asmP-NE-R	ACCGAGTACCGGGTGAA
asmR-PO-F	CCAGCAGCCTGACTTGTAAT
asmR-PO-R	GGACGGCTTCTCGTGTTC
asmR-NE-F	GACGCGCAGGTCTACTAC
asmR-NE-R	GATGCACATCGCCTCCT
asmS-PO-F	GCCCTGATCAGCTGGAAG
asmS-PO-R	AAGACCGAGGGCGTCTA
asmS-NE-F	CCAGGTGCTCAACGTCTAC
asmS-NE-R	CATGTACGCGGGCAGAA
asmI-pET28a-F	GGTGCCGCGCGGCAGCCATATGAACGAGTCGGAACCC
asmI- pET 28a-R	GCTCGAGTGCGGCCGCAAGCTTTCAGGTCCGCCGCCGGAC
4885- pET 28a-F	GGTGCCGCGCGGCAGCCATATGGTGGTCGACGCGGATCAG
4885- pET 28a-R	GCTCGAGTGCGGCCGCAAGCTTCTATGCGACGAGGCTTTC
441- pET 28a-F	GGTGCCGCGCGGCAGCCATATGCGGATCGGCATCGAC
441- pET 28a-R	GCTCGAGTGCGGCCGCAAGCTTTCAGCCCACCGGCTCCGA
asmD-pET28a-F	GGTGCCGCGCGGCAGCCATATGGTGGACTGGGAAACCGAT
asmD-pET28a-R	GCTCGAGTGCGGCCGCAAGCTTTCACGGCTGCATCACCTC
asmC-pET22b-F	AAGAAGGAGATATACATATGGTGATCCAGCAGTCCGCG
asmC-pET22b-R	CTCGAGTGCGGCCGCAAGCTTCGACGCGGAGTCGTCCGC
asmM-pET22b-F	AAGAAGGAGATATACATATG AGCCTGGACCGGTGGGG
asmM-pET22b-R	CTCGAGTGCGGCCGCAAGCTT GCGTTCGCCGGGCACCCG
asmN-pET22b-F	AAGAAGGAGATATACATATG GTGACACACGTCGACGAT
asmN-pET22b-R	CTCGAGTGCGGCCGCAAGCTTTCGGGCCCGTCCTTCCCG
asmS-pET22b-F	AAGAAGGAGATATACATATG GTGAACCCGGAGAGCACG
asmS-pET22b-R	CTCGAGTGCGGCCGCAAGCTT ACTCGATCTCCTTGAGGC

Table S3. Deduced functions of ORFs in the *asm* gene cluster[a]

ORF	Amino acids[b]	Blastp homologue	Identity/coverage [%]	Protein ID[c]
AsmA	291	response regulator	95/100	EHN75269.1
AsmB	482	sensor histidine kinase	93/98	KPC71983.1
AsmC	1076	non-ribosomal peptide synthetase		
AsmD	450	FmoH alanine/alpha-methylserine hydroxymethyltransferase	53/87	BAP16692.1
AsmE	488	transmembrane transporter	44/96	AAM77999.1
AsmF	180	hypothetical protein		
AsmG	398	putative 3-deoxy-D-arabinose-heptulosonic-7-phosphate synthase	49/97	ACN39016.1
AsmH	648	phenazine biosynthesis protein phzE	52/94	SDS26303.1
AsmI	476	BM3 cytochrome P450	39/91	3PSX_A
AsmJ	239	SgcG oxidoreductase	48/87	AAL06666.1
AsmK	413	LtmK cytochrome P450	41/88	ABB88538.1
AsmL	273	hypothetical protein		
AsmM	1089	non-ribosomal peptide synthetase		
AsmN	1331	non-ribosomal peptide synthetase		
AsmO	394	CmdE halogenase	25/77	Q0VZ69.1
AsmP	222	CtvB O-methyl transferase	32/92	Q0C9L6.1
AsmQ	258	transcriptional regulator	34/95	BAL56037.1
AsmR	461	SgcD5 amide bond formation	48/92	ANY94435.1
AsmS	806	non-ribosomal peptide synthase		

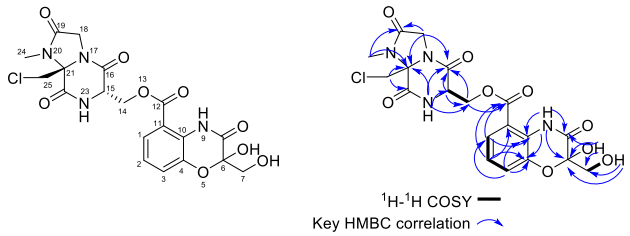
[a] The sequence has been deposited in GenBank with the accession number MH752201.[b] Numbers are in amino acids. [c] Given in numbers are NCBI accession numbers.

Table S4. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data of **1** in DMSO-d_6.



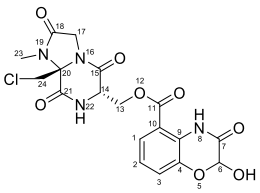
No.	δ_C	δ_H (mult, *J*, Hz)
1	124.5, CH	7.58 (d, 8.4, 1.8)
2	122.8, CH	7.08 (td, 8.4, 1.8)
3	122.9, CH	7.30 (d, 8.4)
4	142.4, C	
6	97.2, C	
6-OH		7.67 (d, 14.1)
7	63.5, CH_2	3.96 (dd, 11.5, 4.8) 3.58 (m)
7-OH		5.22 (t, 4.8)
8	163.4, C	
9		NH 10.04 (d, 3.4)
10	128.7, C	
11	113.9, C	
12	166.4, C	
13		
14	63.3, CH_2	4.65 (m) 4.61 (m)
15	53.1, CH	4.77 (q, 3.0)
16	162.8, C	
17		
18	47.4, CH_2	4.14 (t, 14.4) 3.82 (m)
19	168.7, C	
20		
21	79.9, C	
22	166.9, C	
23		NH 9.00 (d, 11.7)
24	27.7, CH_3	3.04 (s)
25	61.4, CH_2	3.91 (m) 3.78 (m)
25-OH		5.75 (t, 5.4)

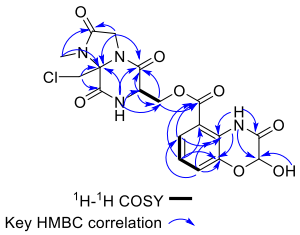
Table S5. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data of **2** in DMSO-d_6.



No.	δ_C	δ_H (mult, *J*, Hz)
1	124.6, CH	7.62 (dd, 8.0, 3.2)
2	122.9, CH	7.08 (t, 8.0)
3	122.9, CH	7.31 (d, 8.0)
4	142.4, C	
6	97.2, C	
7	63.5, CH_2	3.96 (dd, 11.5, 3.9) 3.59 (m)
8	163.4, C	
9		NH 10.06 (d, 3.4)
10	128.7, C	
11	113.8, C	
12	166.4, C	
13		
14	63.0, CH_2	4.64 (m) 4.62 (m)
15	52.8, CH	4.97 (m)
16	163.4, C	
17		
18	48.0, CH_2	3.89 (d, 15.9) 4.20 (dd, 15.9, 11.5)
19	168.5, C	
20		
21	78.8, C	
22	165.5, C	
23		NH 9.22 (d, 11.7)
24	27.5, CH_3	3.06 (s)
25	47.3, CH_3	4.48 (d, 12.6) 4.42 (dd, 12.6, 2.6)

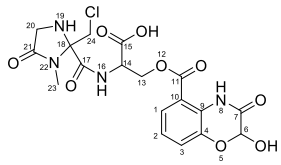
Table S6. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data of **3** in DMSO-d_6.

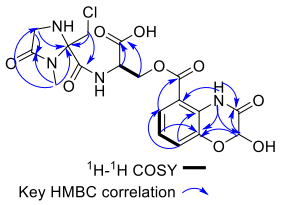




No.	δ_C	δ_H (mult, *J*, Hz)
1	125.5, CH	7.79 (d, 7.8, 1.8)
2	122.8, CH	7.11 (t, 7.8)
3	123.5, CH	7.37 (d, 7.8)
4	141.5, C	
6	90.2, C	
6-OH		5.63 (s)
7	162.5, CH_2	
8		NH 10.15 (d, 12.8)
9	128.7, C	
10	114.0, C	
11	166.1, C	
12		
13	61.9, CH_2	4.72 (m) 4.56 (m)
14	51.1, CH	4.76 (m)
15	164.7, C	
16		
17	48.7, CH_2	4.54 (m) 4.49 (m)
18	167.1, C	
19		
20	79.9, C	
21	167.2, C	
22		
23	25.1, CH_3	2.75 (s)
24	43.8, CH_2	4.60 (m) 4.40(m)

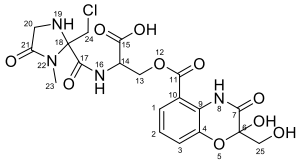
Table S7. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data of **4** in DMSO-d_6.

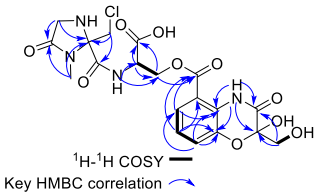




No.	δ_C	δ_H (mult, *J*, Hz)
1	125.0, CH	7.78 (dd, 7.8, 1.2)
2	122.5, CH	7.11 (t, 7.8)
3	123.0, CH	7.37 (d, 7.8)
4	141.0, C	
6	89.8, C	5.63 (s)
7	162.0, CH_2	
8		NH 10.12 (d, 7.2)
9	128.4, C	
10	113.6, C	
11	165.7, C	
12		
13	61.4, CH_2	4.72 (m) 4.50 (m)
14	50.6, CH	4.68 (m)
15	166.8, C	
16		NH 8.70 (brs)
17	164.3, C	4.49 (m)
18	81.5, C	
19		
20	48.2, CH_2	
21	166.7, C	
22		
23	24.6, CH_3	2.75 (s)
24	43.4, CH_2	4.60 (m) 4.41(m)

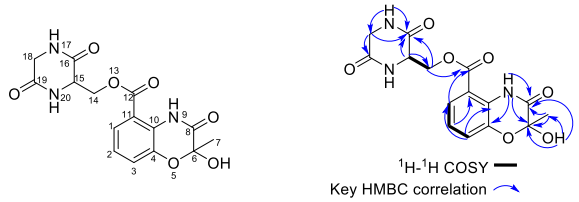
Table S8. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data of **5** in DMSO-d_6.





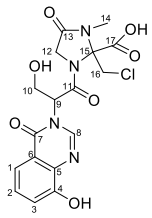
No.	δ_C	δ_H (mult, *J*, Hz)
1	124.5, CH	7.76 (dd, 7.8, 1.2)
2	122.3, CH	7.09 (t, 7.8)
3	122.6, CH	7.33 (d, 7.8)
4	141.8, C	
6	96.8, C	
7	162.9, CH_2	
8		NH 10.13 (d, 7.2)
9	128.6, C	
10	112.8, C	
11	165.9, C	
12		
13	61.4, CH_2	4.73 (m) 4.60 (m)
14	50.6, CH	4.77 (brs)
15	166.8, C	
16		NH 8.73 (brs)
17	164.2, C	
18	81.5, C	
19		NH 8.73 (brs)
20	48.1, CH_2	4.41 (m) 4.62 (m)
21	166.9, C	
22		
23	24.6, CH_3	2.75 (s)
24	43.3, CH_2	4.49 (m)
25	63.0, CH_2	3.97 (dd, 11.5, 3.4) 3.61 (dd, 11.5, 2.0)

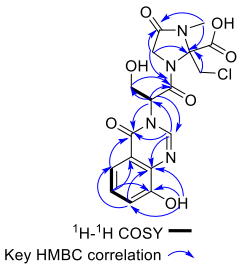
Table S9. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data of **6** in DMSO-d_6.



No.	δ_C	δ_H (mult, *J*, Hz)
1	124.7, CH	7.96 (dd, 7.8, 1.2)
2	122.3, CH	7.09 (t, 7.8)
3	122.8, CH	7.32 (d, 7.8)
4	141.8, C	
6	95.2, C	
7	22.7, CH_3	1.68 (s)
8	163.5, C	
9		NH 10.01 (d, 7.2)
10	129.3, C	
11	113.2, C	
12	165.8, C	
13		
14	63.3, CH_2	4.68 (m) 4.64 (m)
15	51.4, CH	4.36 (m)
16	166.1, C	
17		NH 8.95 (brs)
18	40.9, CH_2	3.91 (m) 3.90 (m)
19	170.6, C	
20		NH 8.46 (brs)

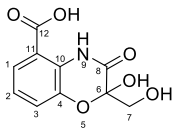
Table S10. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data of **7** in DMSO-d_6.

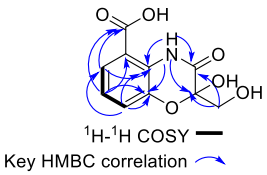




No.	δ_C	δ_H (mult, *J*, Hz)
1	116.0, CH	7.56 (dd, 7.8, 1.2)
2	128.0, CH	7.37 (t, 7.8)
3	119.1, CH	7.23 (d, 7.8)
4	153.0, C	4-OH 9.94 (s)
5	136.2, C	
6	121.9, C	
7	160.0, C	
8	144.3, C	8.28 (s)
9	57.6, C	5.52 (dd, 8.4, 5.4)
10	58.3, C	
11	166.0, C	
12	48.5, CH_2	3.90 (m) 4.61 (m)
13	166.7, C	4.77 (brs)
14	24.5, CH_3	2.69 (s)
15	81.2, C	
16	43.2, CH_2	4.33 (m)
17	167.1, C	

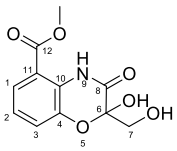
Table S11. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data of **8** in DMSO-d_6.

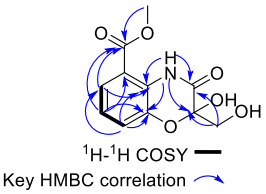




No.	δ_C	δ_H (mult, *J*, Hz)
1	124.5, CH	7.61 (d, 7.8)
2	122.9, CH	7.06 (t, 7.8)
3	122.3, CH	7.26 (d, 7.8)
4	142.3, C	
6	97.2, C	
7	63.5, CH_2	3.58 (d, 11.4) 3.96 (d, 11.4)
8	163.3, C	
9		NH 10.55(s)
10	129.1, C	
11	114.6, C	
12	169.3, C	

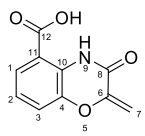
Table S12. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data of **9** in DMSO-d_6.

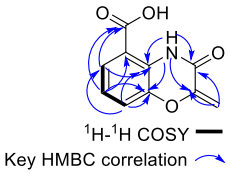




No.	δ_C	δ_H (mult, *J*, Hz)
1	123.6, CH	7.60 (d, 7.8)
2	122.5, CH	7.08 (t, 7.8)
3	122.2, CH	7.30 (d, 7.8)
4	142.5, C	
6	96.8, C	
7	63.0, CH_2	3.58 (d, 11.4) 3.96 (d, 11.4)
8	162.9, C	
9		NH 10.25(s)
10	128.3, C	
11	113.7, C	
12	166.9, C	
12-OCH_3	52.6, CH_3	3.89 (s)

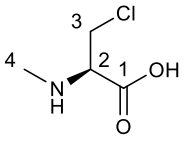
Table S13. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data of **10** in DMSO-d_6.



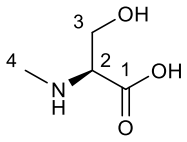


No.	δ_C	δ_H (mult, *J*, Hz)
1	125.3, CH	7.63 (d, 7.8)
2	122.5, CH	7.08 (t, 7.8)
3	122.0, CH	7.31 (d, 7.8)
4	141.5, C	
6	148.0, C	
7	98.9, CH_2	5.47 (brs) 5.14 (brs)
8	155.6, C	
9		NH 10.97 (s)
10	127.8, C	
11	115.5, C	
12	169.2, C	

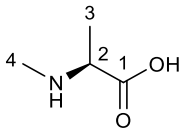
Table S14. ^{1}H NMR (400 MHz) data of **11**–**13** in D_2O.



11



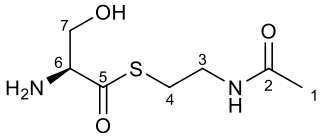
12



13

No.	**11**	**12**	**13**
	δ_H (mult, *J*, Hz)	δ_H (mult, *J*, Hz)	δ_H (mult, *J*, Hz)
2	3.68 (dd, 7.2, 6.8)	3.80 (m)	3.47 (q, 4.8)
3	4.48 (brs)	3.83 (dd, 5.2, 4.0);	1.46 (d, 4.8)
	3.80 (brs)	3.73 (dd, 5.2, 4.0)	
4	2.75 (s)	3.23 (s)	2.65 (s)

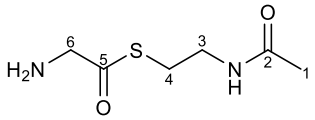
Table S15. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data of **15** in D_2O.



15

No.	δ_C	δ_H (mult, *J*, Hz)
1	23.0, CH_3	1.91 (s)
2	174.1, C	
3	42.2, CH_2	3.26 (t, 4.4)
4	38.1, CH_2	2.56 (t, 4.4)
5	195.6, C	
6	48.8, CH	3.24 (m)
7	60.6, CH_2	4.37 (m) 3.90 (m)

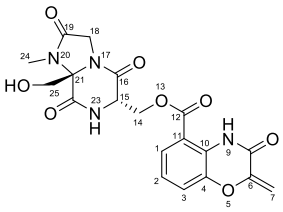
Table S16. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) data of **16** in D_2O.

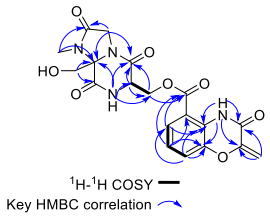


16

No.	δ_C	δ_H (mult, *J*, Hz)
1	21.4, CH_3	1.73 (s)
2	174.3, C	
3	38.2, CH_2	3.16 (t, 6.4)
4	27.9, CH_2	2.92 (t, 6.4)
5	194.3, C	
6	46.4, CH_2	3.24 (s)

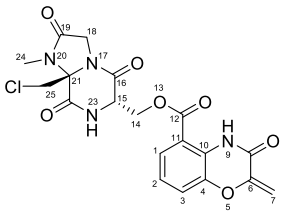
Table S17. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data of **17** in DMSO-d_6.

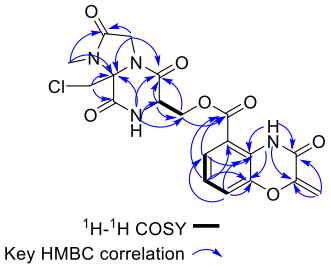




No.	δ_C	δ_H (mult, *J*, Hz)
1	125.8, CH	7.79 (dd, 8.0, 3.2)
2	122.8, CH	7.13 (t, 8.0)
3	120.9, CH	7.40 (d, 8.0)
4	141.5, C	
6	147.8, C	
7	99.4, CH_2	5.50 (d, 1.8) 5.18 (d, 1.8)
8	155.5, C	
9		NH 10.31 (s)
10	127.8, C	
11	113.6, C	
12	163.9, C	
13		
14	61.8, CH_2	4.67 (m) 4.60 (m)
15	51.1, CH	4.65 (m)
16	167.4, C	
17		
18	48.2, CH_2	4.49 (d, 15.9) 4.26 (d, 15.9)
19	167.3, C	
20		
21	83.0, C	
22	166.1, C	
23		
24	25.2, CH_3	2.71 (s)
25	70.2, CH_2	3.48 (m) 3.54(m)

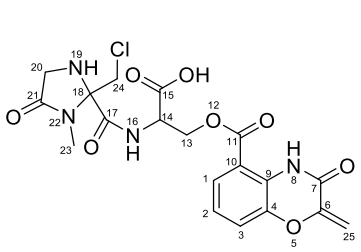
Table S18. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data of **18** in DMSO-d_6.

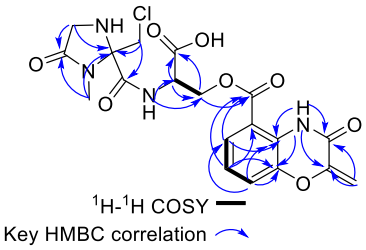




No.	δ_C	δ_H (mult, *J*, Hz)
1	123.3, CH	7.49 (dd, 8.0, 3.2)
2	123.4, CH	7.13 (t, 8.0)
3	119.4, CH	7.31 (d, 8.0)
4	141.5, C	
6	147.9, C	
7	99.1, CH_2	5.45 (d, 1.8) 5.15 (d, 1.8)
8	155.3, C	
9		NH 11.36 (s)
10	126.4, C	
11	110.9, C	
12	164.3, C	
13		
14	67.9, CH_2	4.69 (m) 4.62 (m)
15	67.4, CH	5.42 (m)
16	167.8, C	
17		
18	49.1, CH_2	4.43 (d, 15.9) 4.69 (d, 15.9)
19	167.6, C	
20		
21	81.6, C	
22	167.3, C	
23		
24	24.9, CH_3	2.76 (s)
25	43.4, CH_2	4.37 (m)

Table S19. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data of **19** in DMSO-d_6.





No.	δ_C	δ_H (mult, *J*, Hz)
1	123.4, CH	7.48 (dd, 7.8, 1.2)
2	123.2, CH	7.12 (t, 7.8)
3	119.5, CH	7.31 (d, 7.8)
4	141.5, C	
6	147.9, C	
7	155.3, CH	
8		NH 11.36 (d, 7.2)
9	128.8, C	
10	111.0, C	
11	164.3, C	
12		
13	67.84, CH_2	4.69 (m) 4.61 (m)
14	67.2, CH	5.41 (brs)
15	167.6, C	
16		
17	167.3, C	
18	81.5, C	
19		
20	48.9, CH_2	4.443 (m) 4.69 (m)
21	167.8, C	
22		
23	24.9, CH_3	2.76 (s)
24	43.4, CH_2	4.37 (m)
25	99.1, CH_2	5.45 (d, 1.8) 5.15 (d, 1.8)

Measured in DMSO-d_6.

Table S20. Substrate specificity predictions for the adenylation domains of the NRPSs encoded in the *asm* Gene Cluster.[17]

A domain	Siganture residues	Predicted substrate
AsmC	D L Y N V A S V F R	cysteine
AsmM	D I Y H L G L I T-	glycine
AsmN	D M F N V G L I P R	cysteine
AsmS	G M V L R A V V P D	glycine/alanine/valine

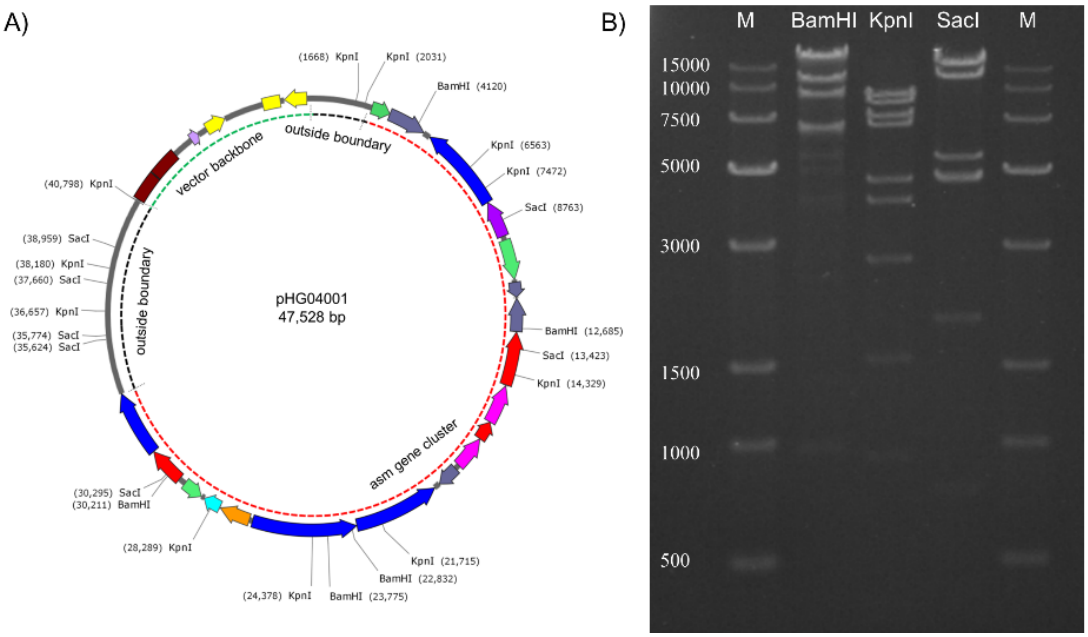


Figure S1. Verification of cosmid pHG4001. A) Physical map of pJTU2554 harboring the intact *asm* gene cluster. B) agarose gel electrophoresis of pHG4001 cosmid. M: 15K marker.

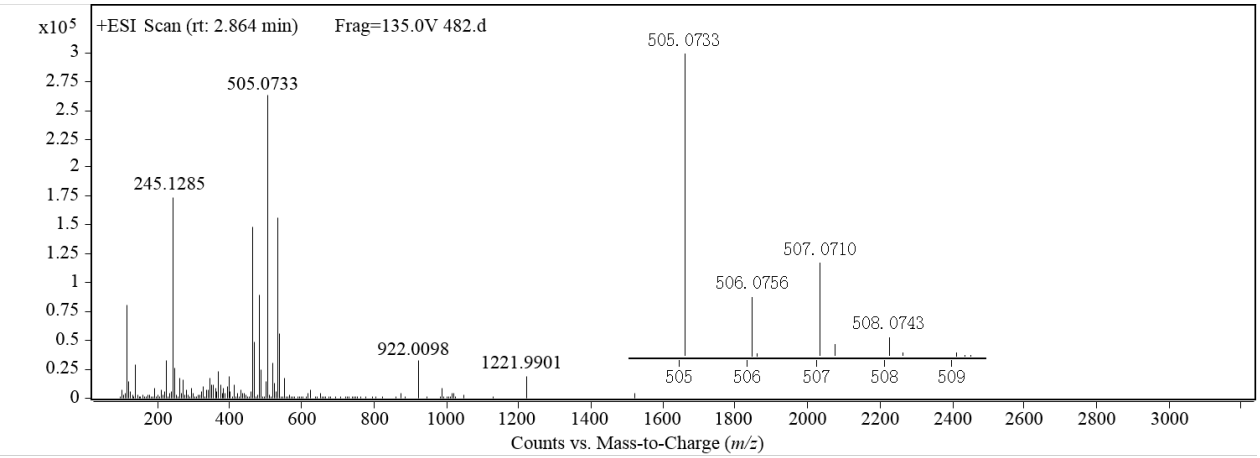


Figure S2. HR-ESIMS spectrum of **2**.

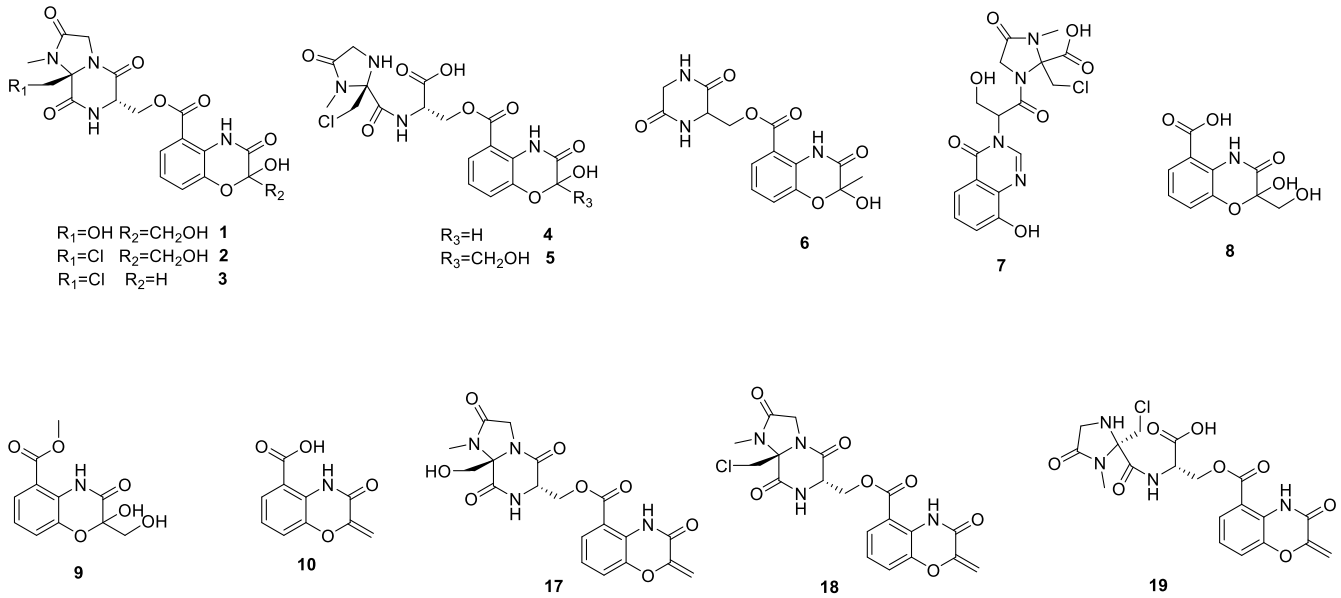


Figure S3. Stuctures of compounds **1**–**10** and **17**–**19**.

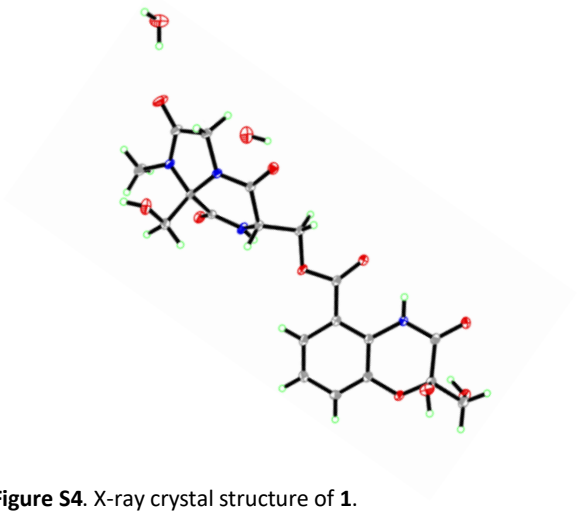


Figure S4. X-ray crystal structure of **1**.

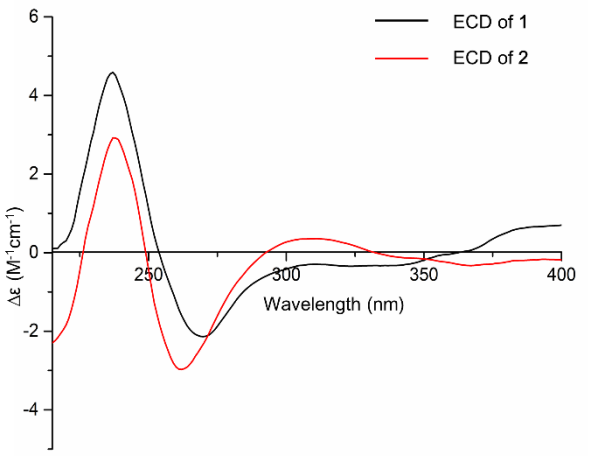


Figure S5. Comparision of CD spectra for **1** and **2**.

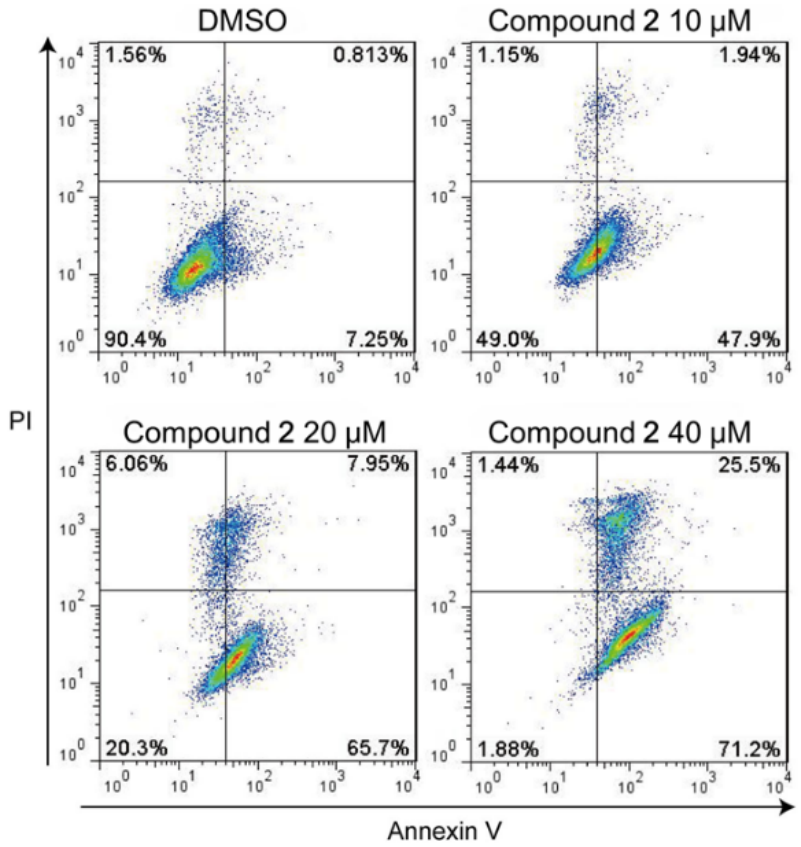


Figure S6. Apoptosis of MCF-7 cell was detected through flow cytometry after treated with different concentration of compound **2** contrasted with DMSO. The percente of apoptotic cells including Annexin-V^+ PI^- early apoptotic cells in lower-right quadrant and Annexin-V^+ PI^+ late apoptotic cells in upper-right quadrant was increased along with compound concentration.

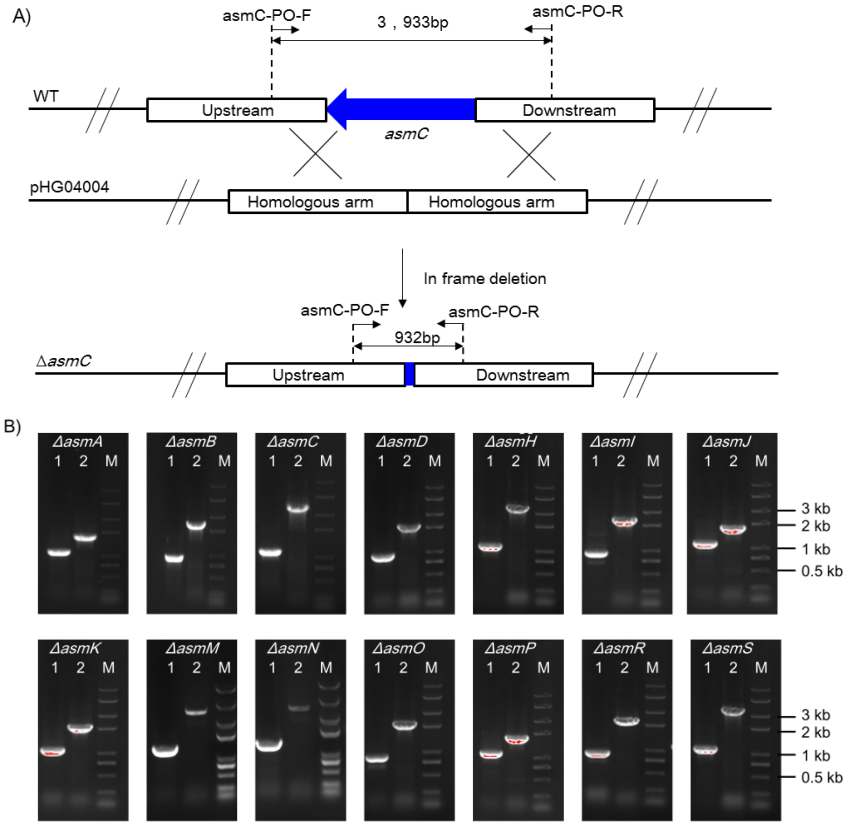


Figure S7. Construction of in-frame deletion in cosmid pHG4001. A) Gene disruption with homologous recombination strategies. B) PCR verification of *asm* mutants: Lane1, amplified with PO-F/R and mutants; Lane 2, amplified with PO-F/R and pHG4001.

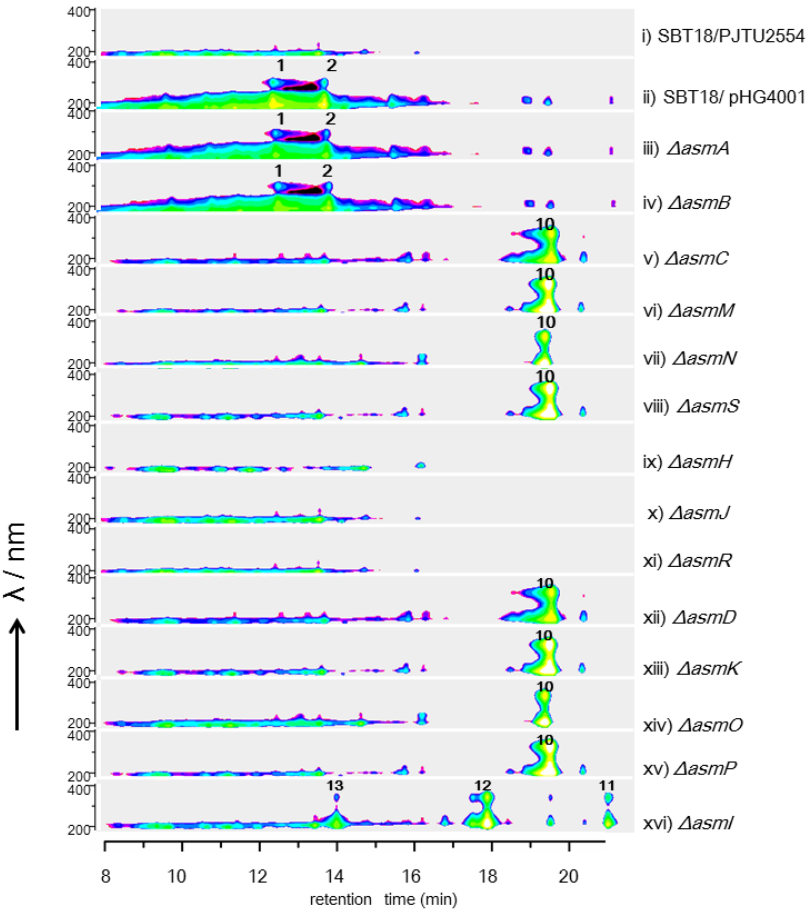


Figure S8. HPLC contour plot of metabolic extracts. The metabolic extracts from mutant strains were analyzed through HPLC equipped with DAD (Diode Array Detector), which can cover the UV absorption from 200 nm to 400 nm.

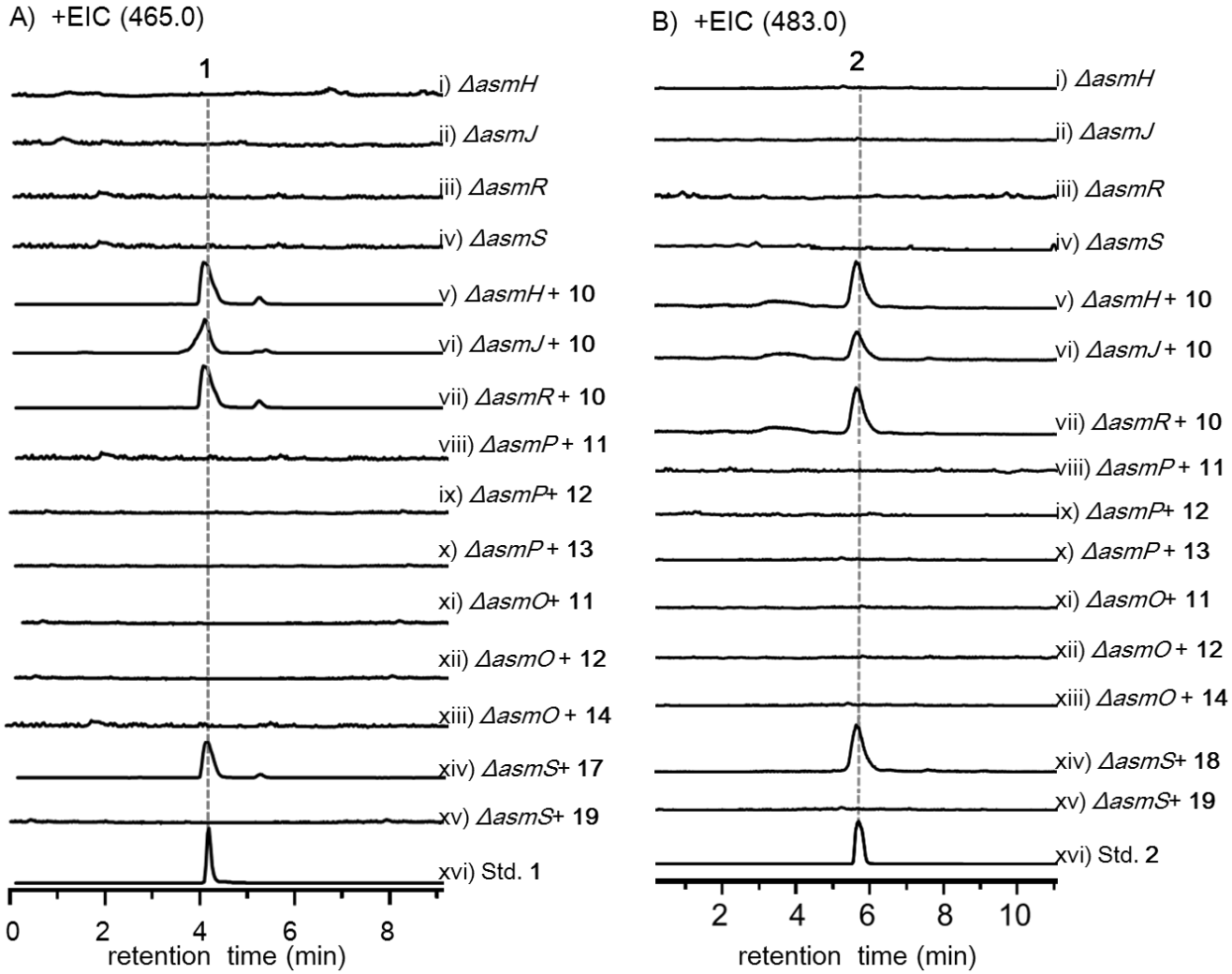


Figure S9. Chemical complementation of compounds into mutants. LC-MS analyses of A) compound **1** and B) compound **2** for diffient mutant strains. i) *ΔasmH* mutant; ii) *ΔasmJ* mutant; iii) *ΔasmR* mutant; iv) *ΔasmR* mutant; v) *ΔasmH* mutant fed with **10**; vi) *ΔasmJ* mutant fed with **10**; vii) *ΔasmR* mutant fed with **10**; viii) *ΔasmS* mutant fed with **17**; ix) *ΔasmS* mutant fed with **19**; x) *ΔasmO* mutant fed with **11**; xi) *ΔasmP* mutant fed with **11**; xii) *ΔasmO* mutant fed with **12**; xiii) *ΔasmP* mutant fed with **13**; xiv) *ΔasmO* mutant fed with **13**; xv) *ΔasmP* mutant fed with **14**; A, xvi) standard of **1**. B, xvi) standard of **2**.

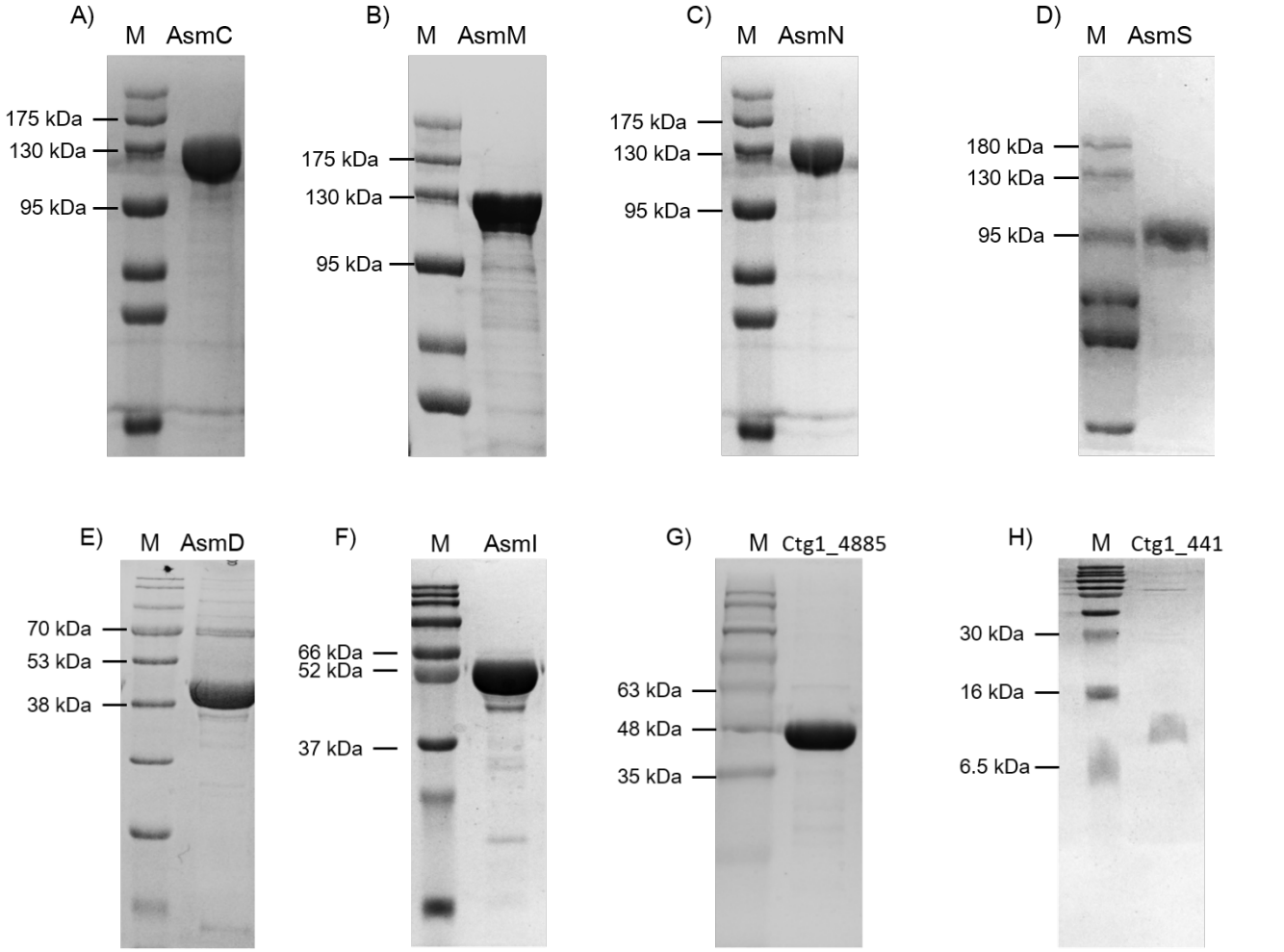


Figure S10. SDS-PAGE analysis of proteins. A) AsmC (calculated molecule weight: 118.6 KDa); B) AsmM (calculated molecule weight: 115.8 KDa); C) AsmN (calculated molecule weight 144.4 KDa); D) AsmS (calculated molecule weight: 87.2 KDa); E) AsmD (calculated molecule weight 49.2 KDa); F) AsmI (calculated molecule weight 55.2 KDa); G) Ctg1_4885 (ferredoxin reductase from *S.* sp. NA03103, calculated molecule weight: 47.3 KDa); H) Ctg1_441 (ferredoxin from *S.* sp. NA03103, calculated molecule weight: 8.9 KDa).

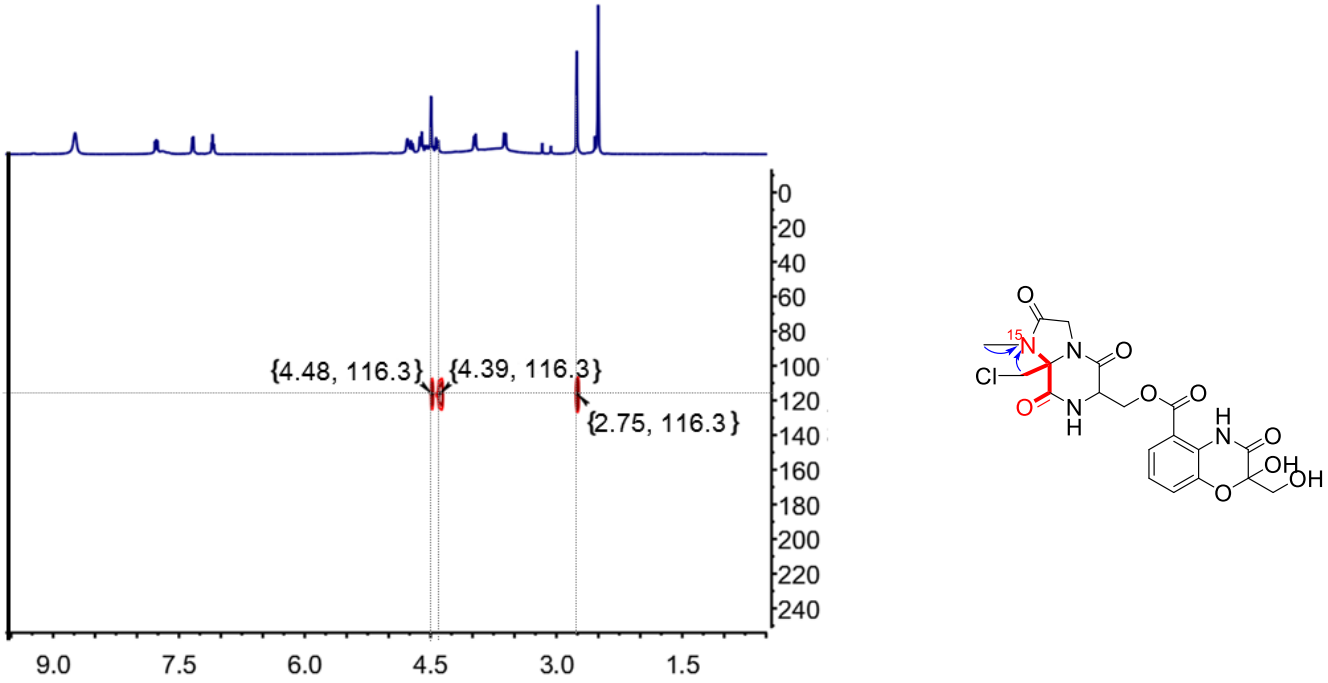


Figure S11. ^{1}H-^{15}N HMBC correlations of **2** feeding with ^{15}N-alanine in DMSO-d_6.

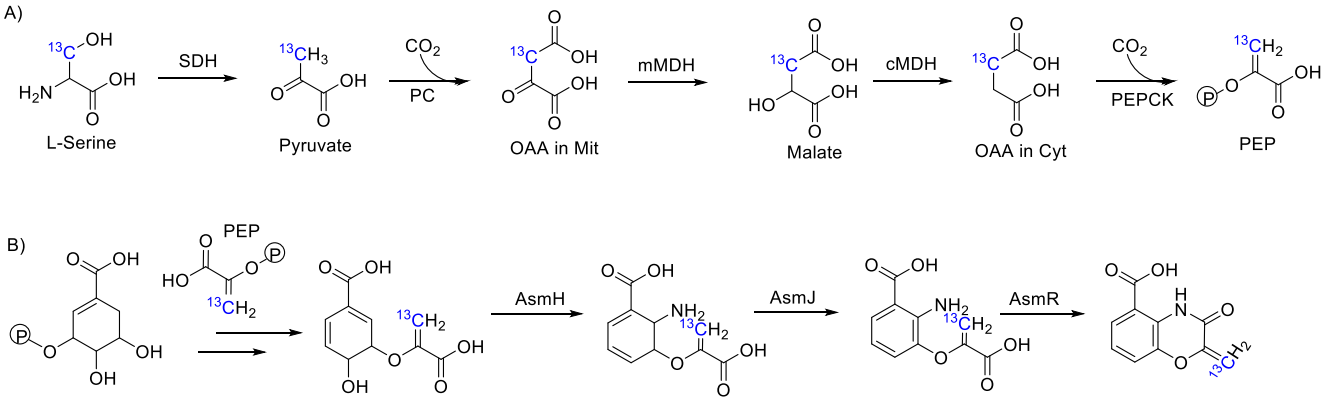


Figure S12. A) Pathway of L-serine to phosphoenolpyruvic acid (PEP);[18] B) Pathway of chorismic acid to compound **10.**[19]
SDH: serine dehydratase, mMDH and cMDH: mitochondrial and cytosolic malate dehydrogenase, respectively, PEP: phosphoenolpyruvate, PEPCK: phosphoenolpyruvate carboxykinase, OAA: oxaloacetate, Mit: mitochondria.[20]

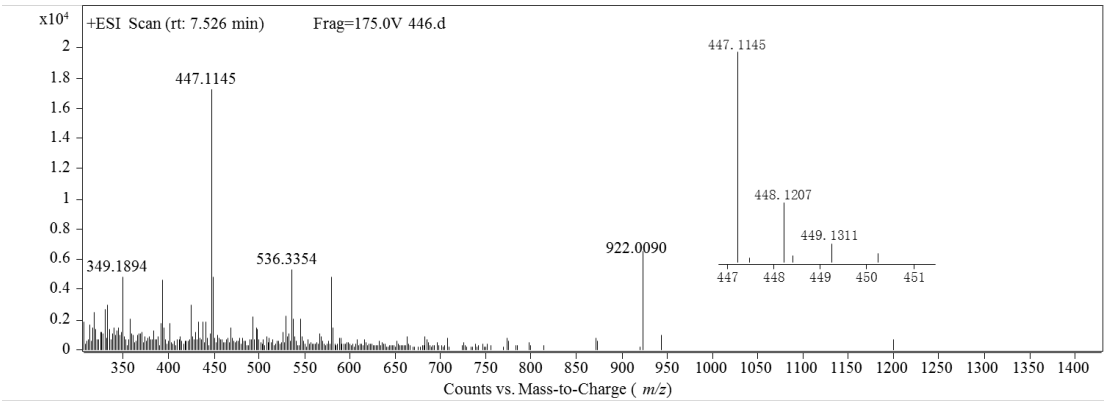


Figure S13. HRESIMS spectrum of **20**.

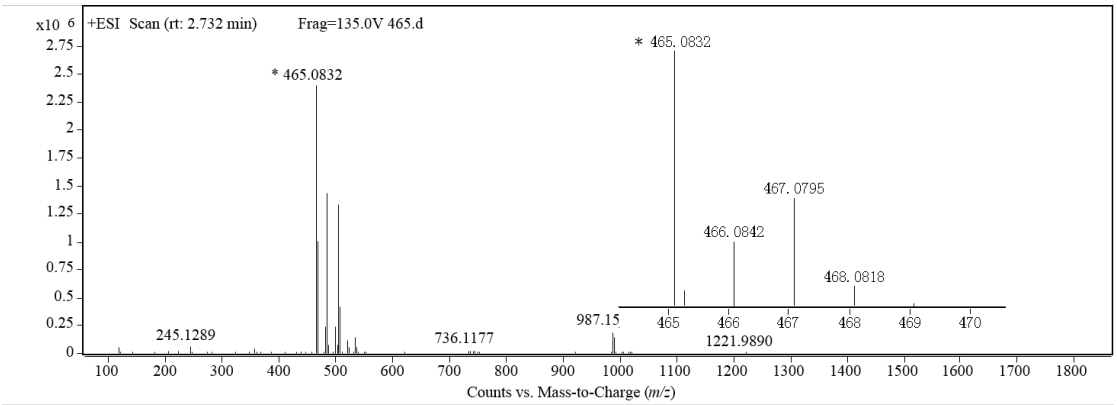


Figure S14. HRESIMS spectrum of **21**.

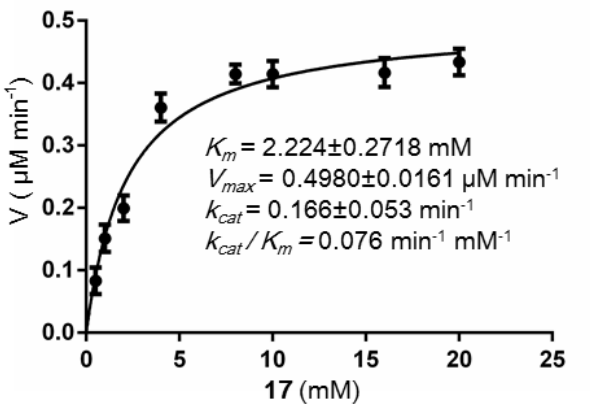


Figure S15. Pseudo-first order kinetic studies of AsmI toward the formation of **1** from **17**. Under steady-state conditions, plots of initial velocity of formation of **1** versus the concentration of **17** (0.5mM, 1 mM, 2 mM, 4 mM, 8mM, 10mM, 16 mM and 20mM) displayed Michaelis-Menten kinetics.

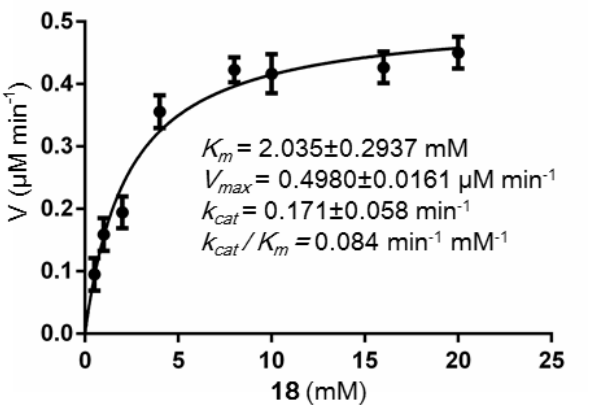


Figure S16. Pseudo-first order kinetic studies of AsmI toward the formation of **2** from **18**. Under steady-state conditions, plots of initial velocity of formation of **2** versus the concentration of **18** (0.5mM, 1 mM, 2 mM, 4 mM, 8mM, 10mM, 16 mM and 20mM) displayed Michaelis-Menten kinetics.

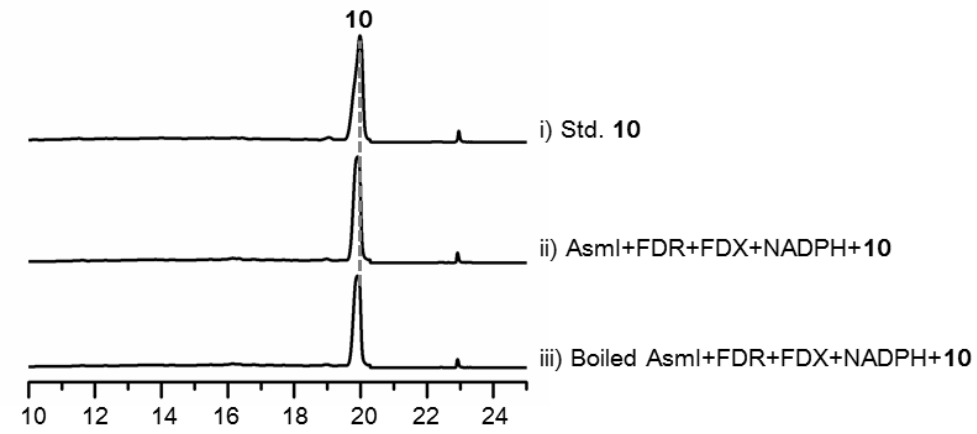


Figure S17. *In vitro* assays of AsmI using **10** as substrate.

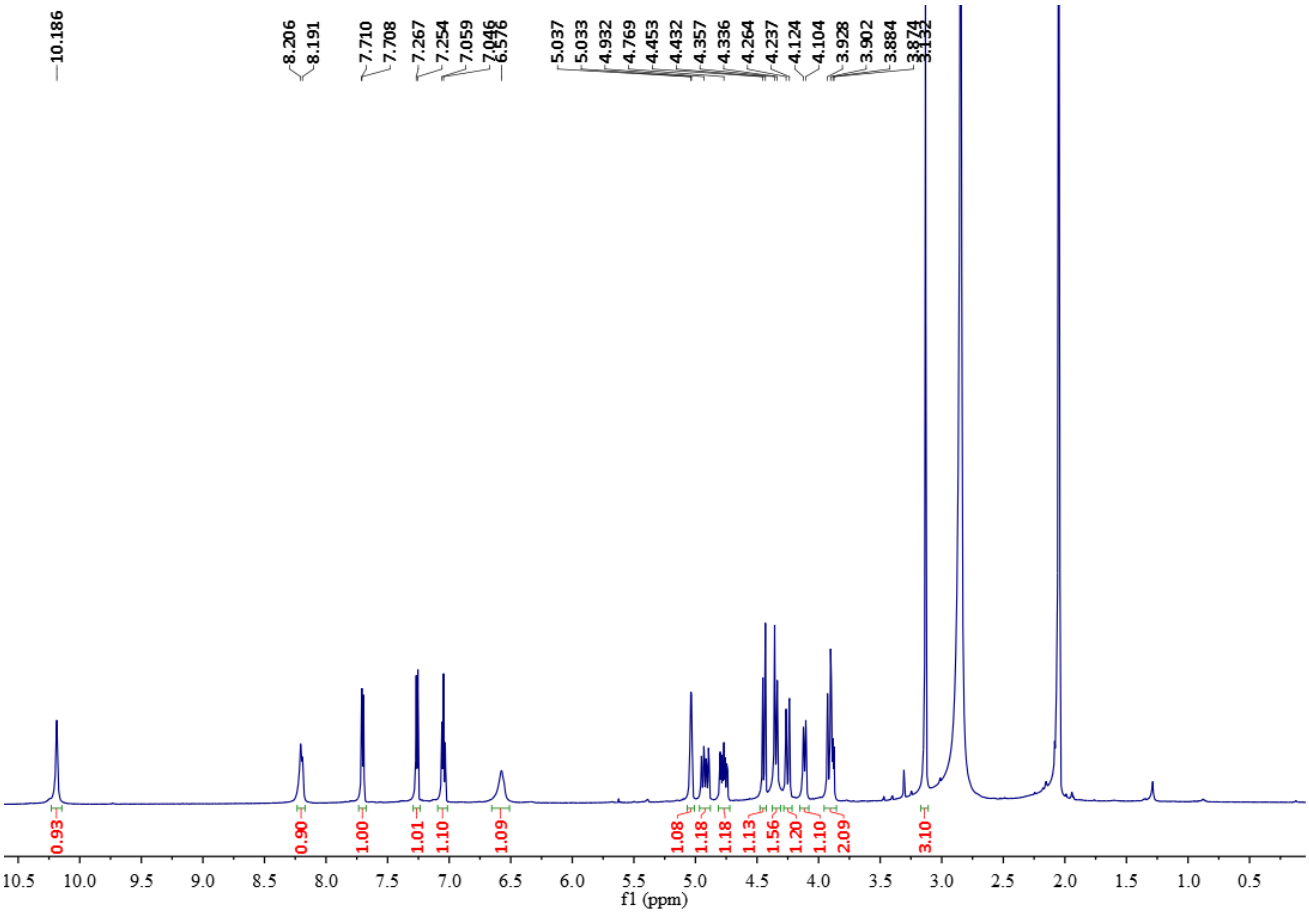


Figure S18. ^{1}H NMR spectrum of ashimide A (**1**) in DMSO-d_6 at 600 MHz.

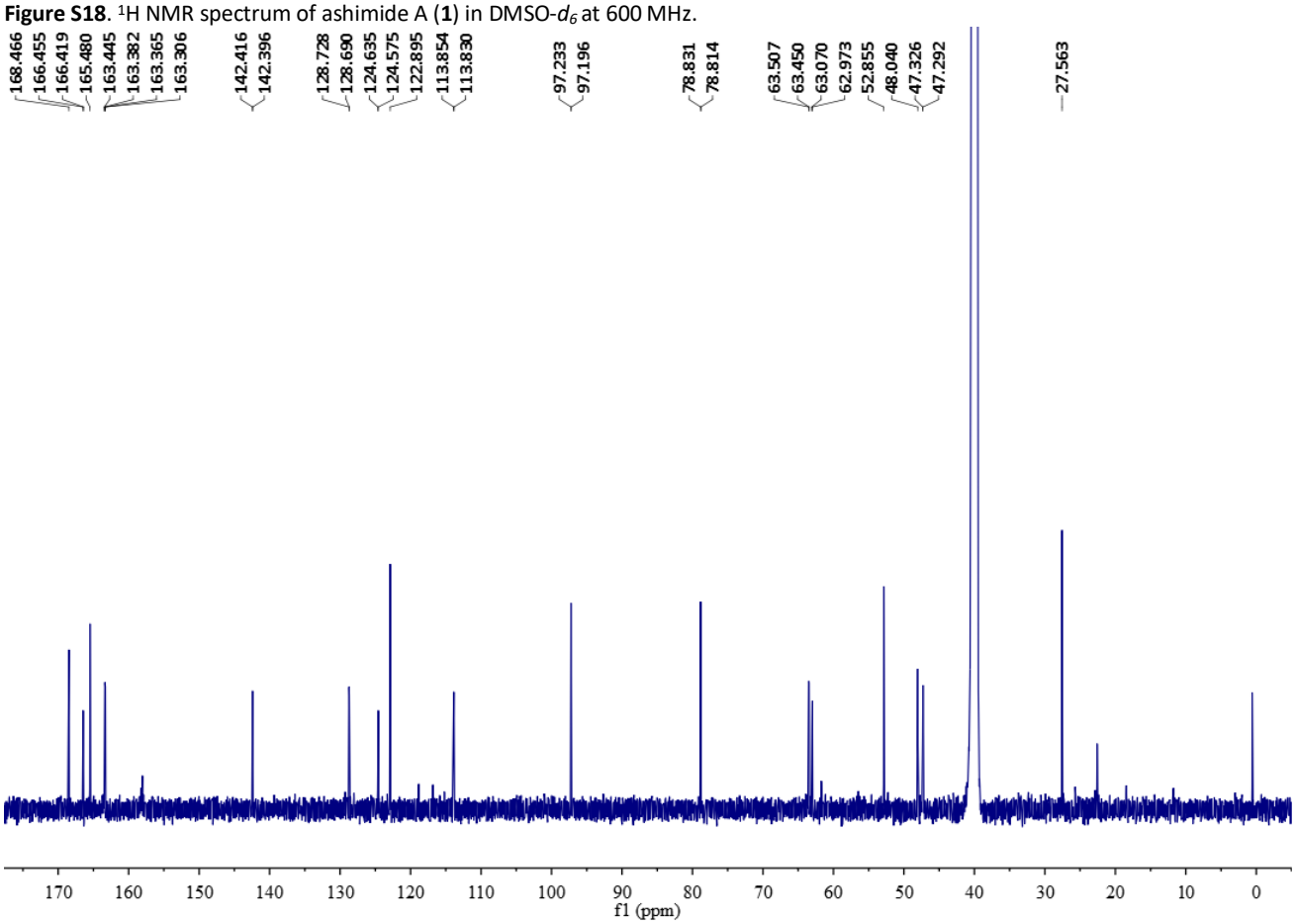


Figure S19. ^{13}C NMR spectrum of ashimide A(**1**) in DMSO-d_6 at 150MHz.

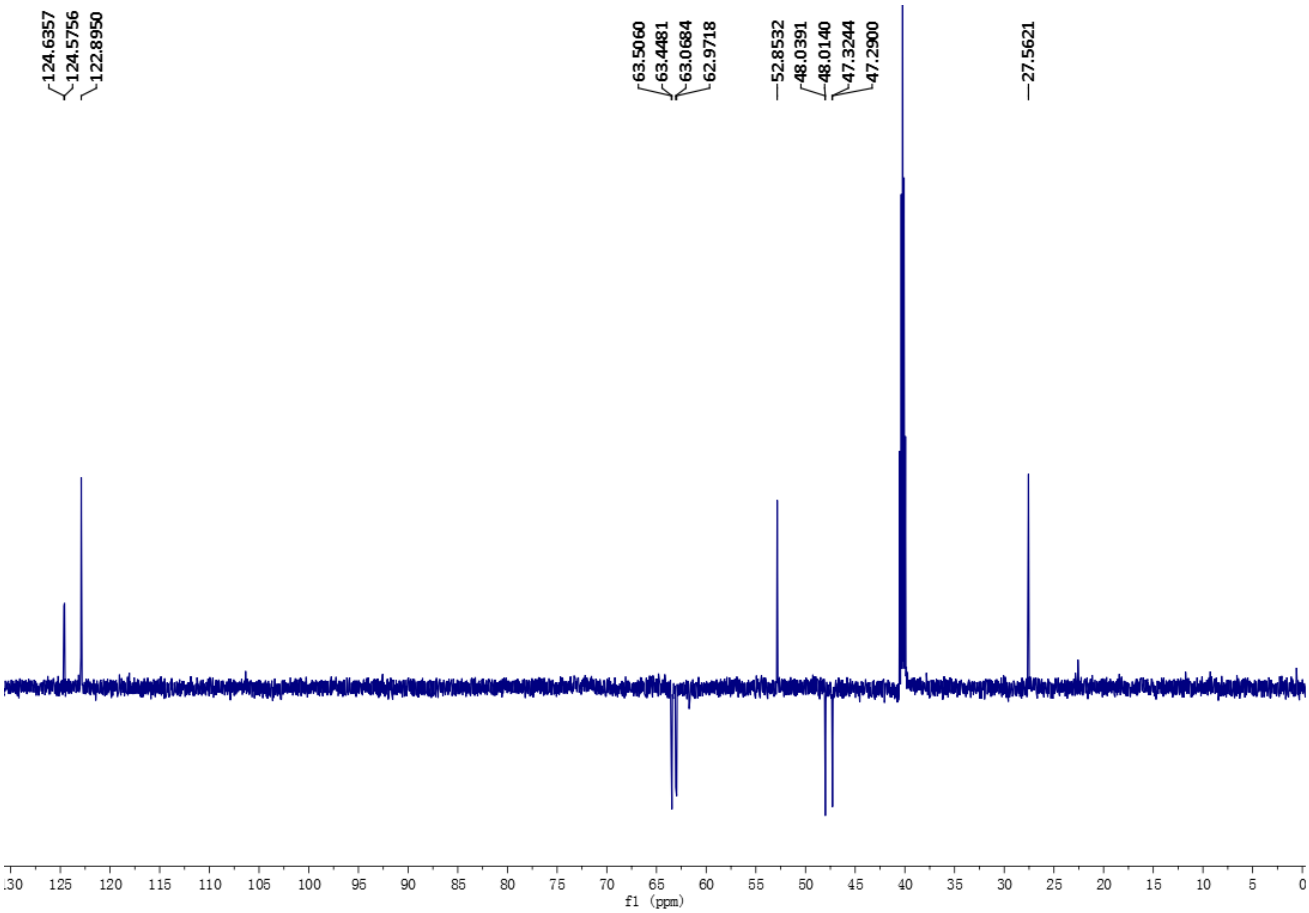


Figure S20. DEPT 135 spectrum of ashimide A(**1**) in DMSO-d_6.

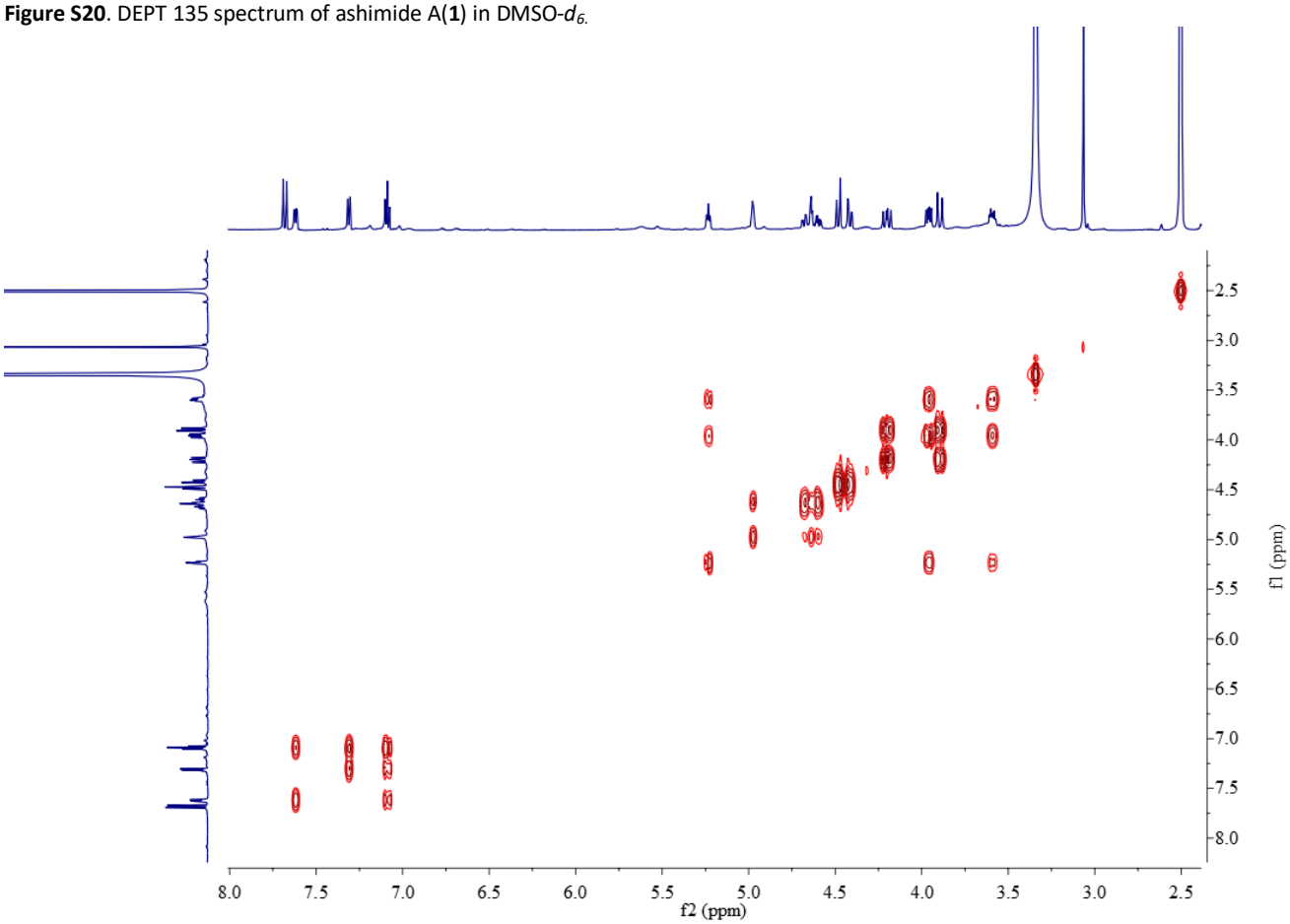


Figure S21. ^{1}H-^{1}H COSY NMR spectrum of ashimide A(**1**) in DMSO-d_6.

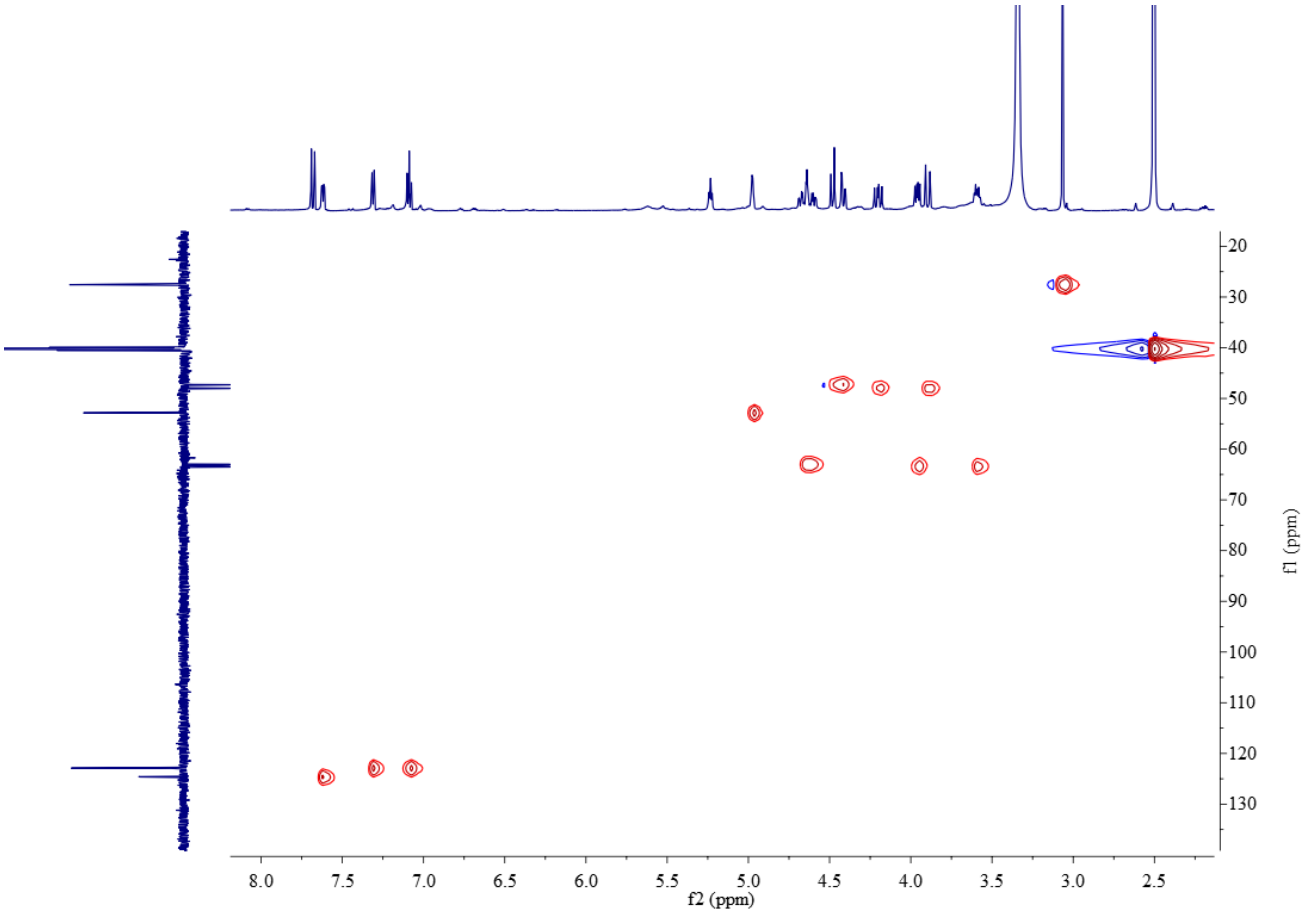


Figure S22. HSQC NMR spectrum of ashimide A(**1**) in DMSO-d_6.

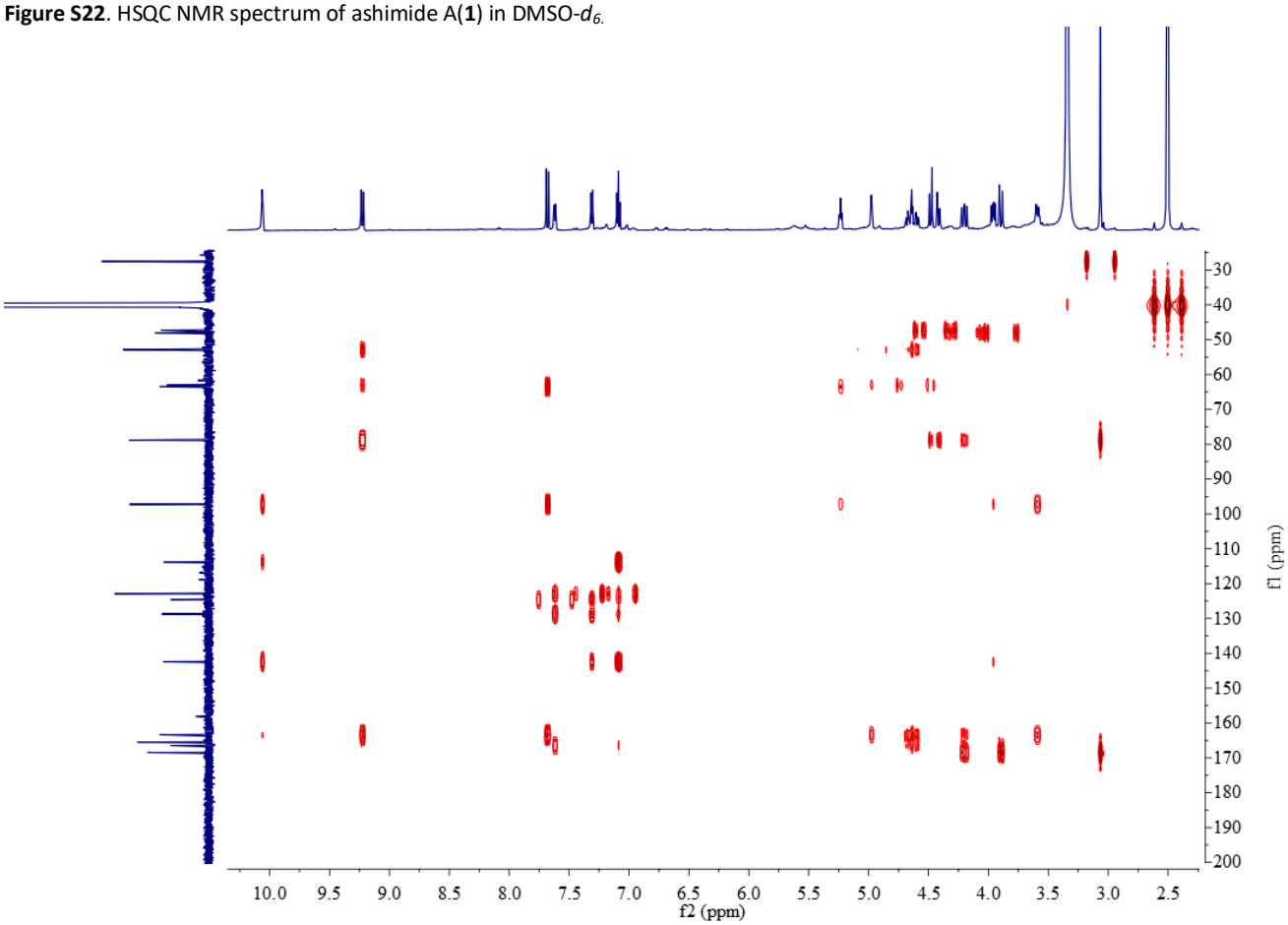


Figure S23. HMBC NMR spectrum of ashimide A(**1**) in DMSO-d_6.

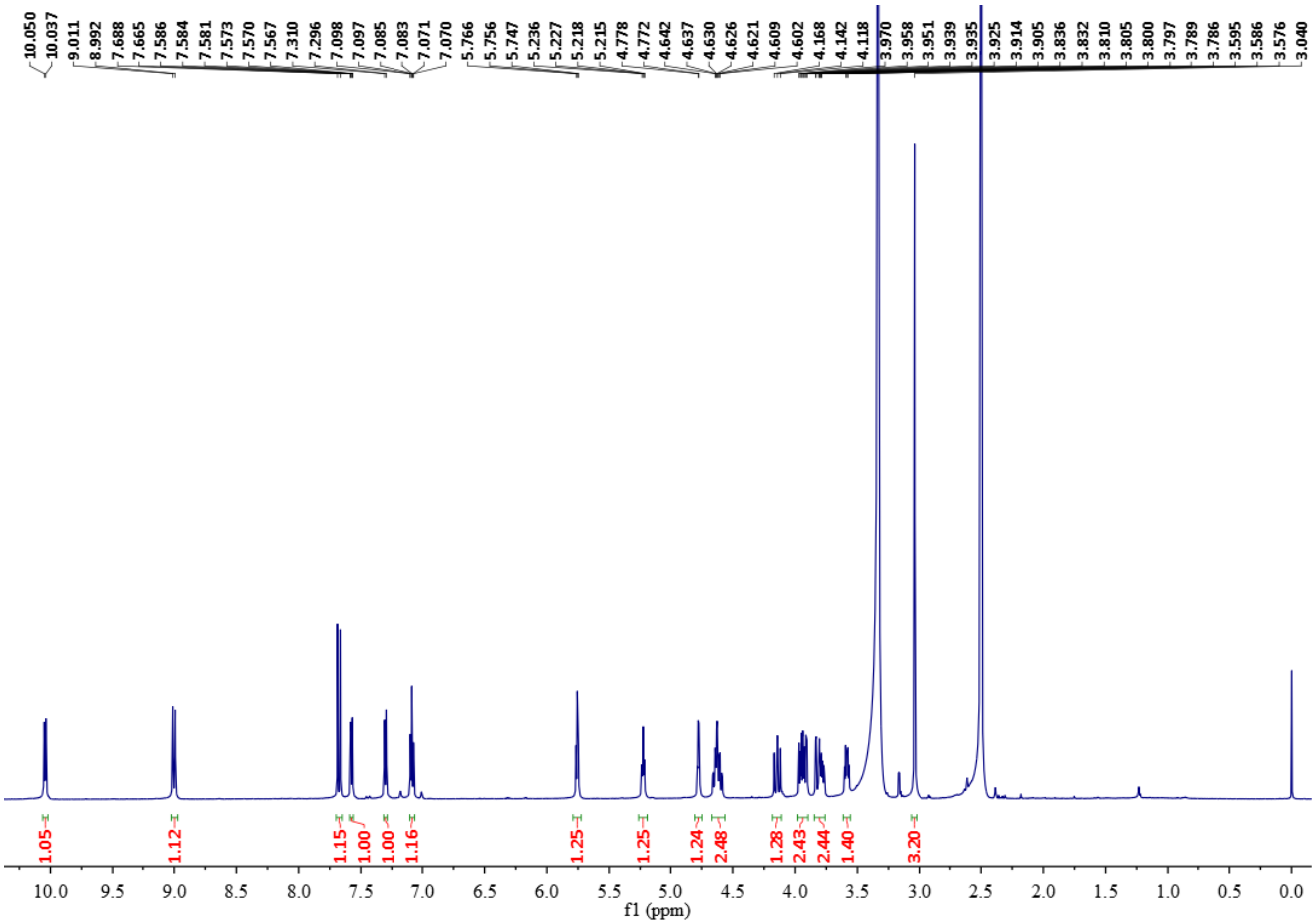


Figure S24. ^{1}H NMR spectrum of ashimide B (**2**) in DMSO-d_6 at 600 MHz.

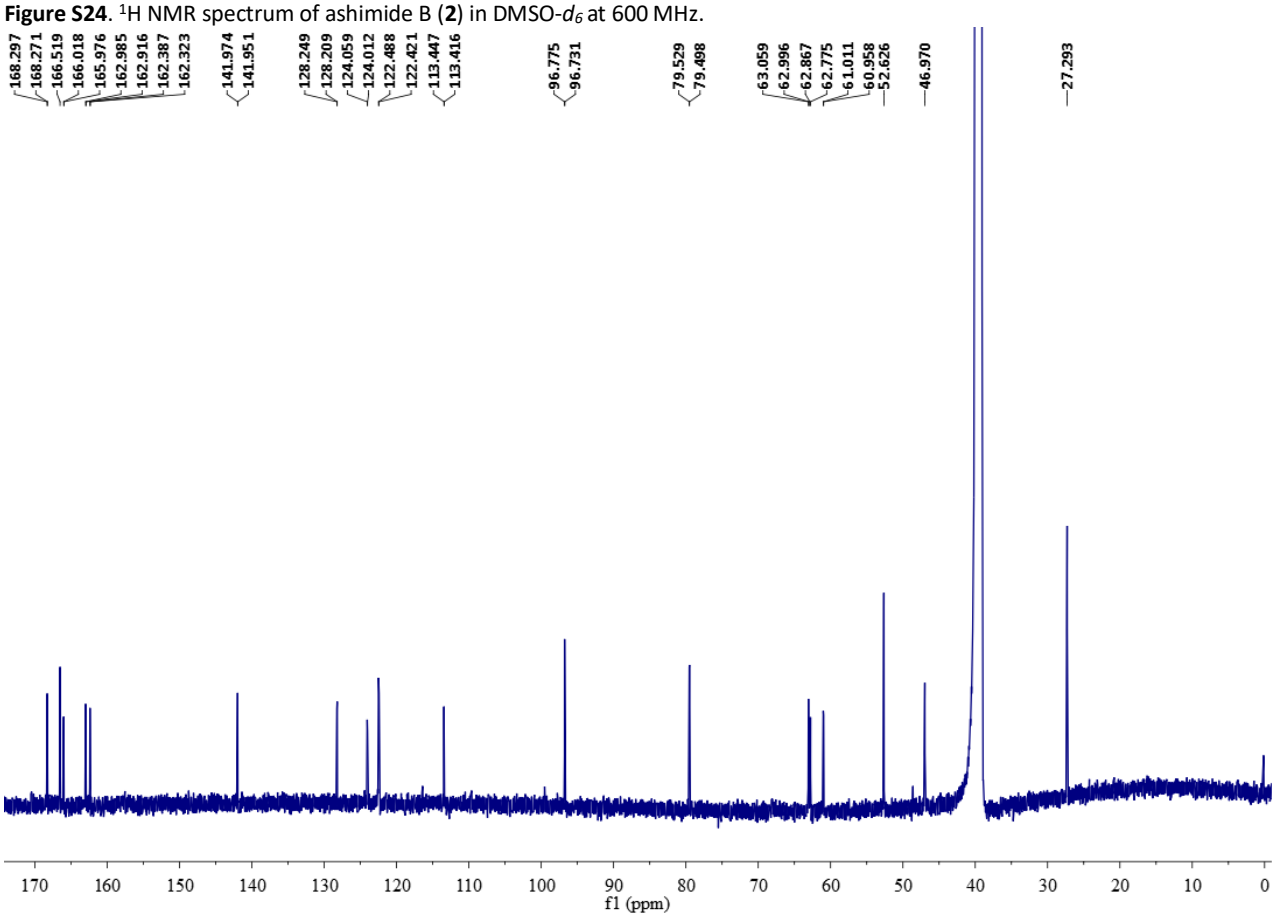


Figure S25. ^{13}C NMR spectrum of ashimide B (**2**) in DMSO-d_6 at 150MHz.

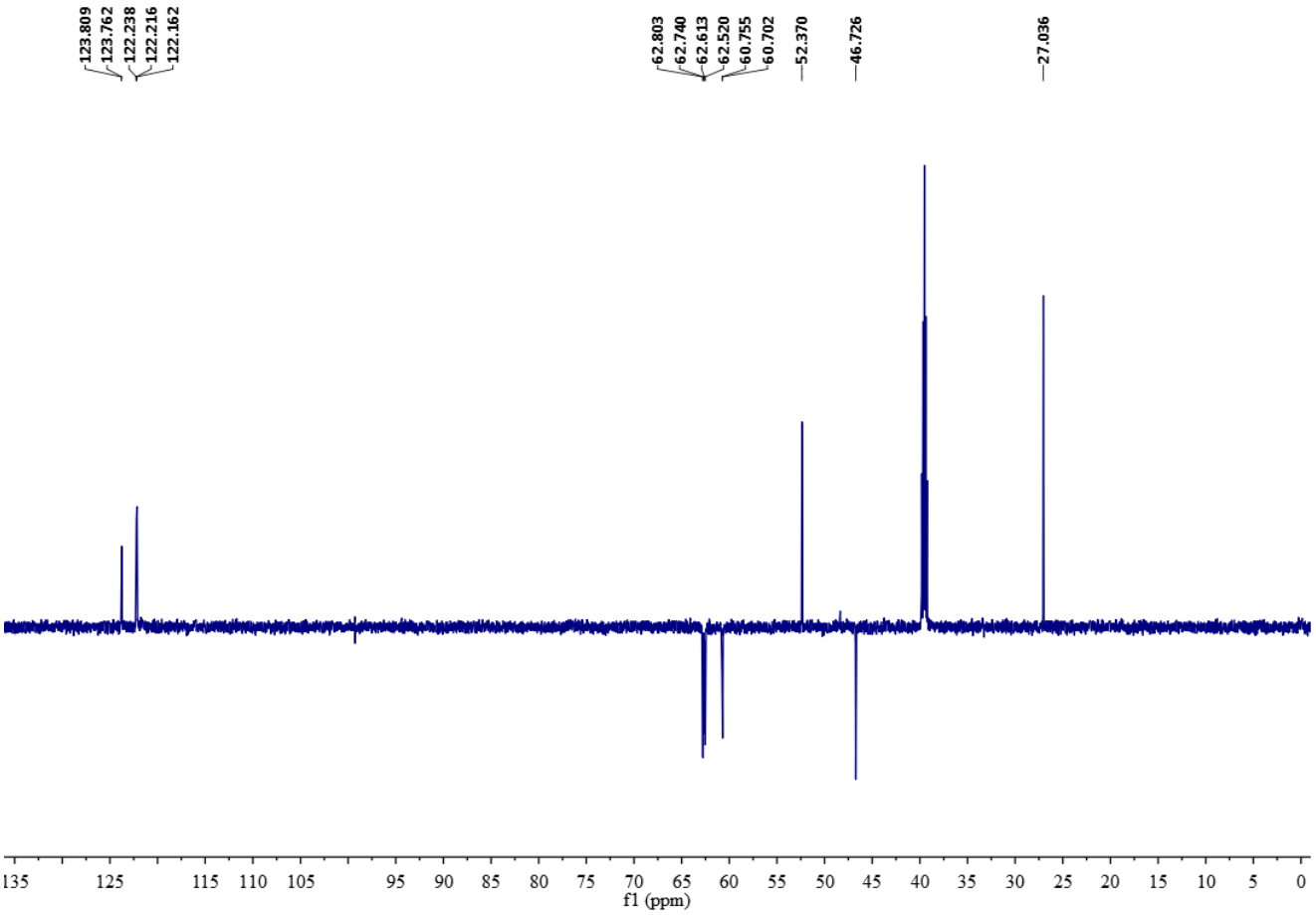


Figure S26. DEPT 135 spectrum of ashimide B (**2**) in DMSO-d_6.

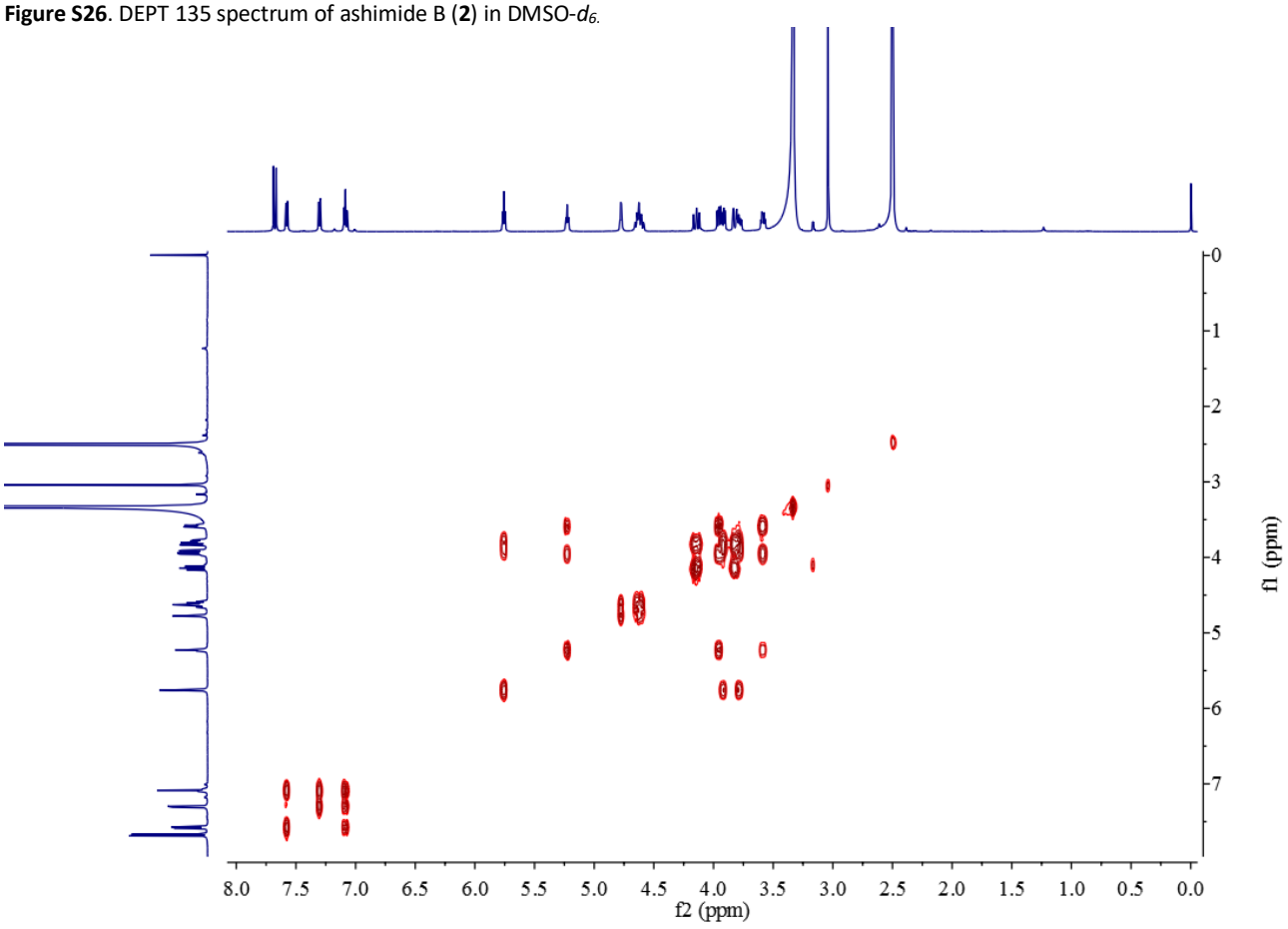


Figure S27. ^{1}H-^{1}H COSY NMR spectrum of ashimide B (**2**) in DMSO-d_6.

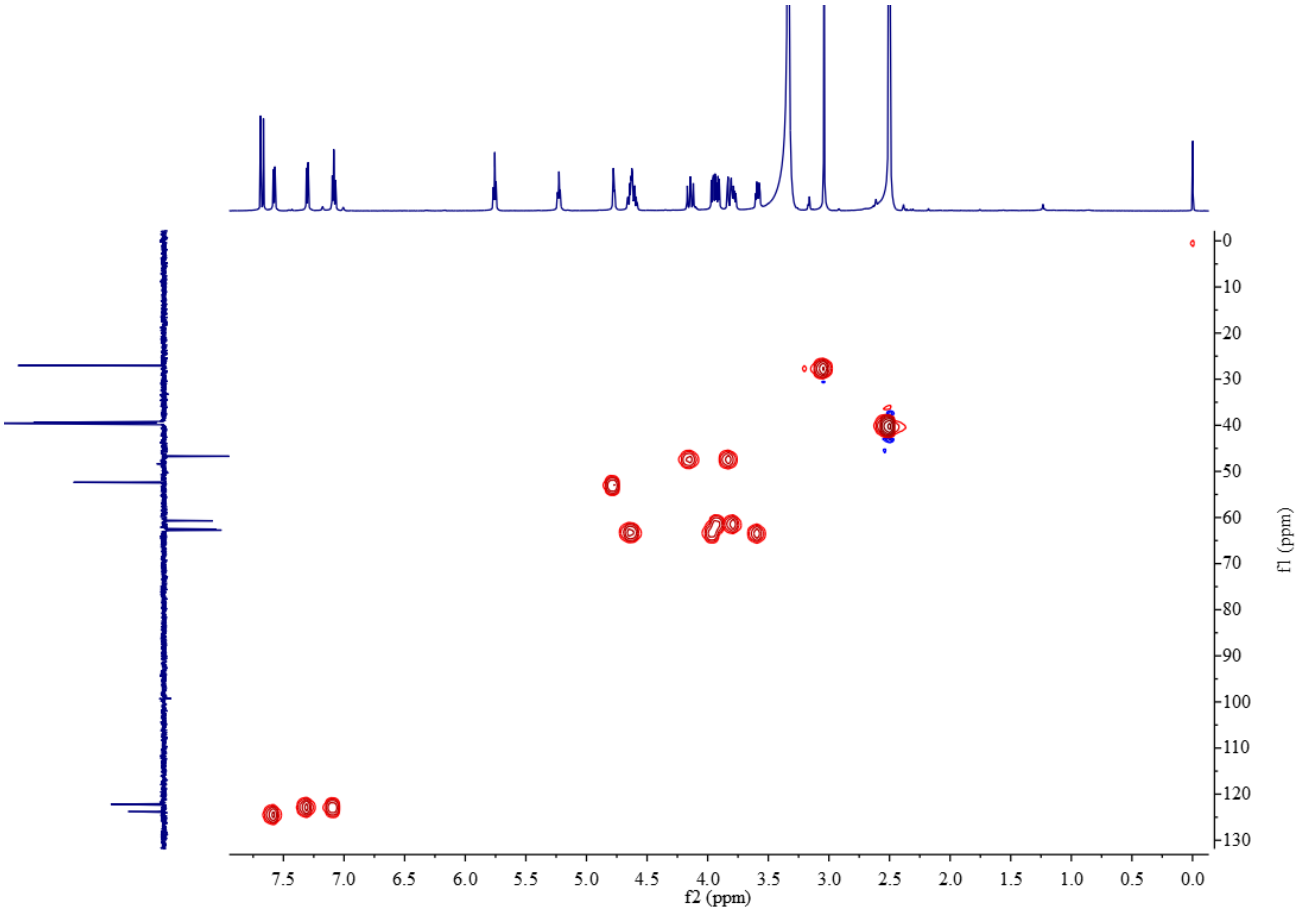


Figure S28. HSQC NMR spectrum of ashimide B (**2**) in DMSO-d_6.

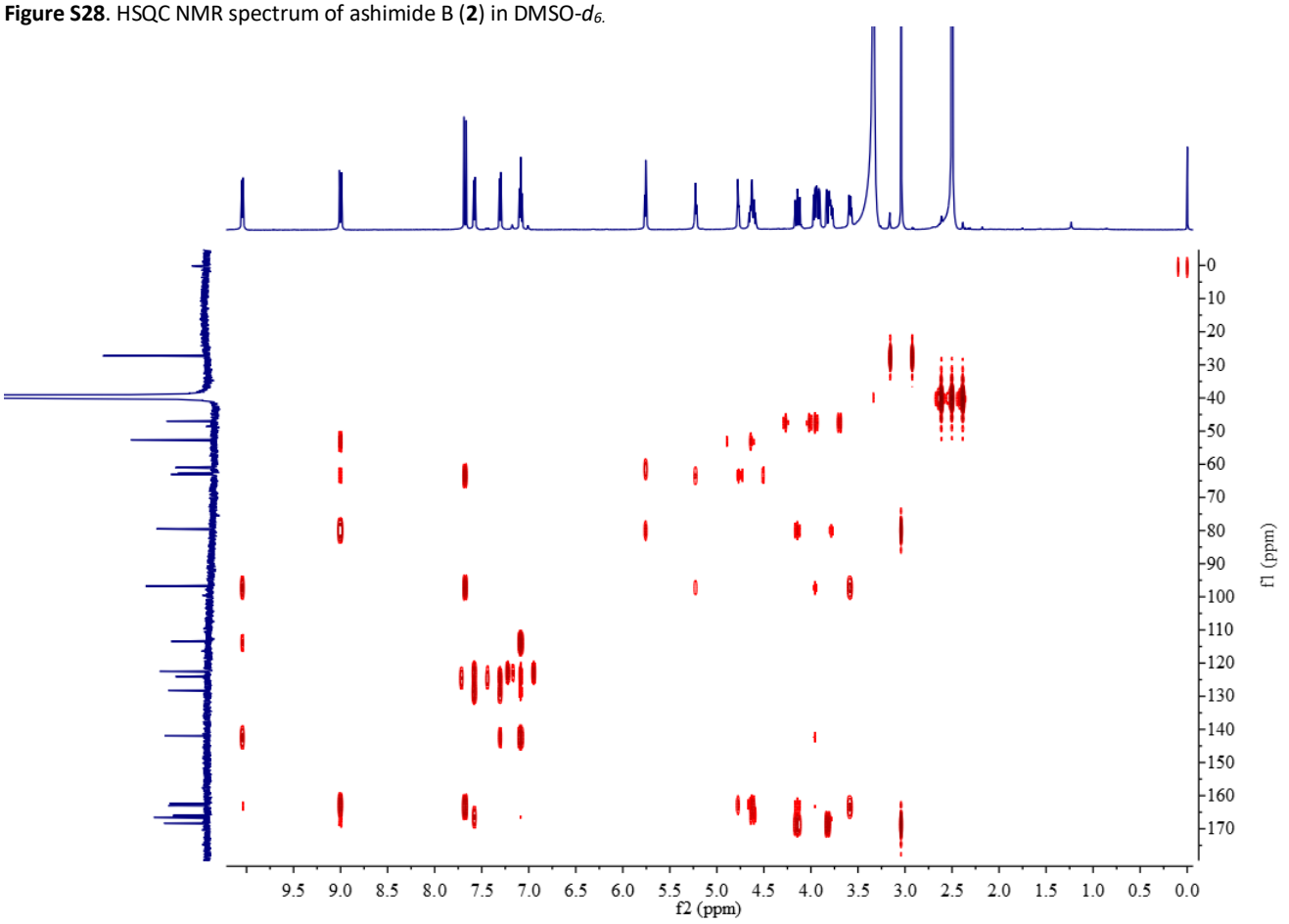


Figure S29. HMBC NMR spectrum of ashimide B (**2**) in DMSO-d_6.

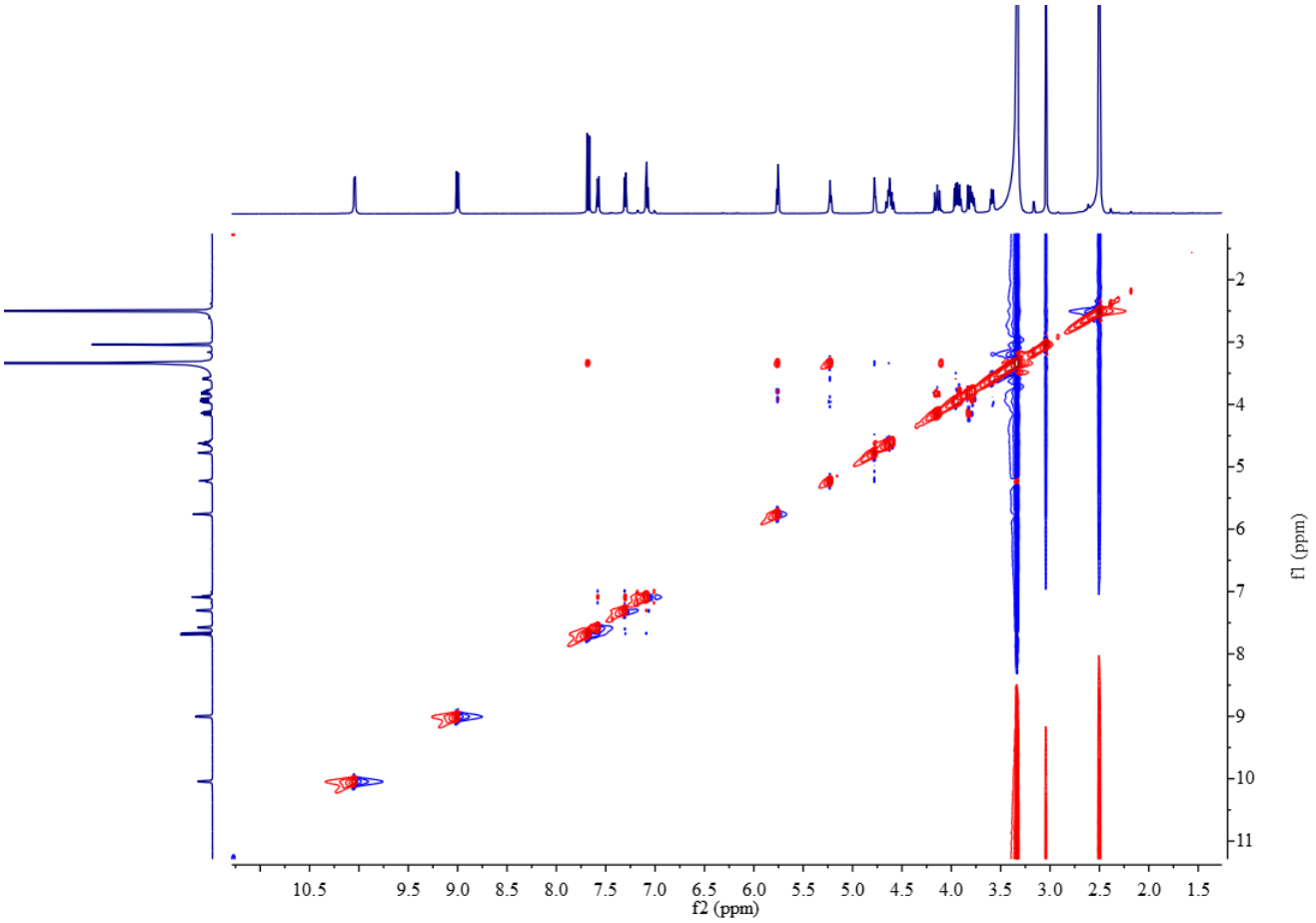


Figure S30. NOESY spectrum of ashimide B (**2**) in DMSO-d_6.

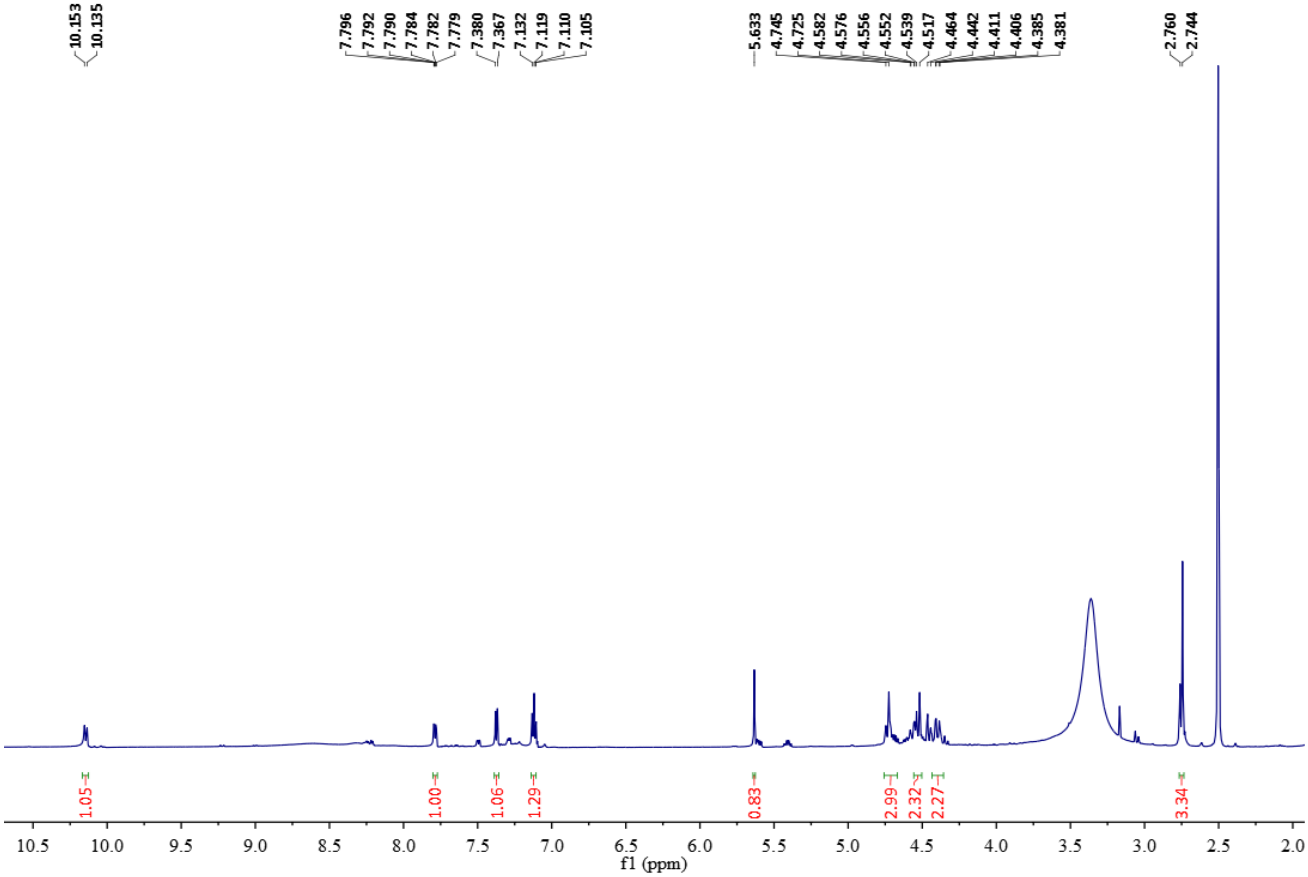


Figure S31. ^{1}H NMR spectrum of **3** in DMSO-d_6 at 600 MHz.

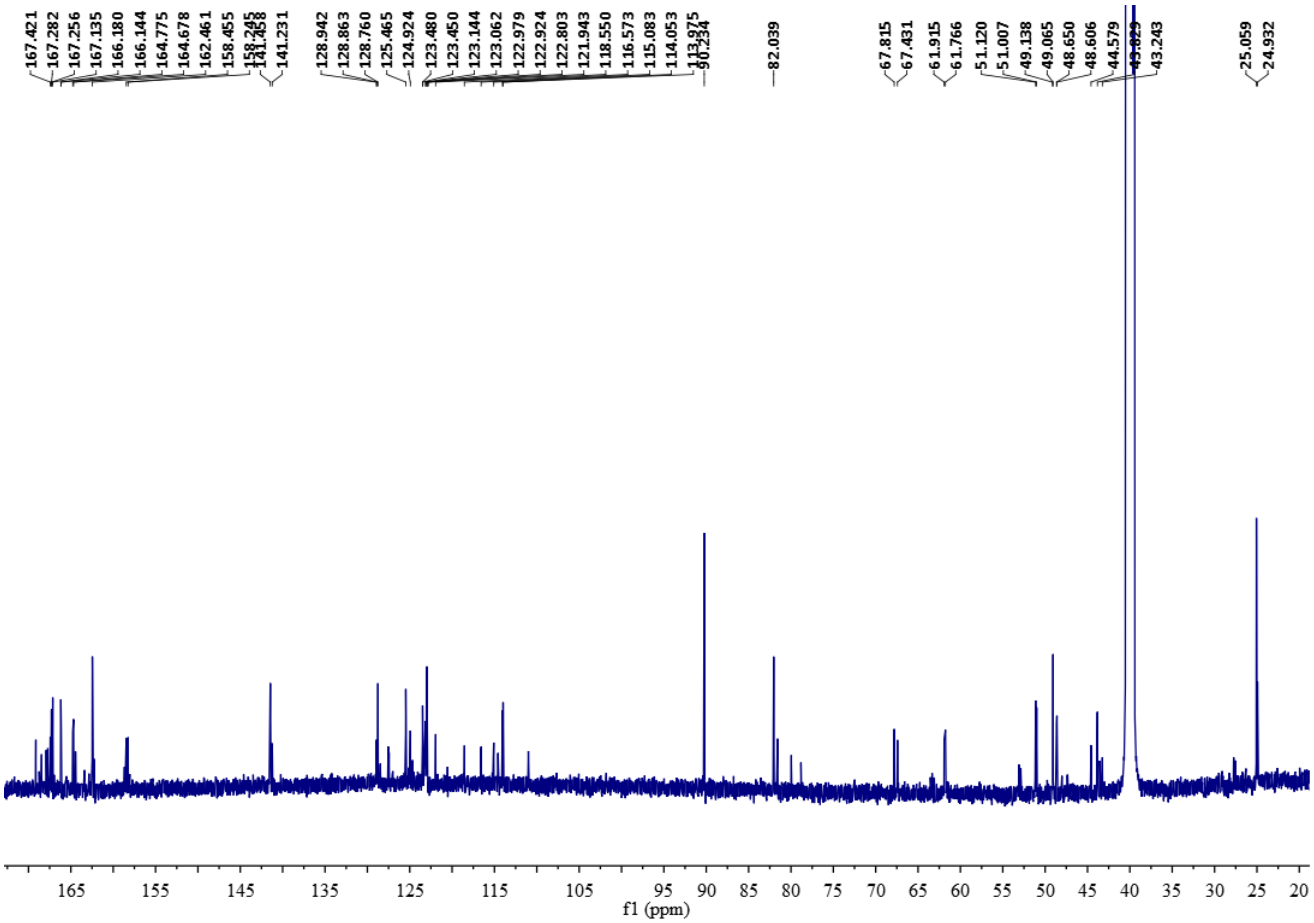


Figure S32. ^{13}C NMR spectrum of **3** in DMSO-d_6 at 150MHz.

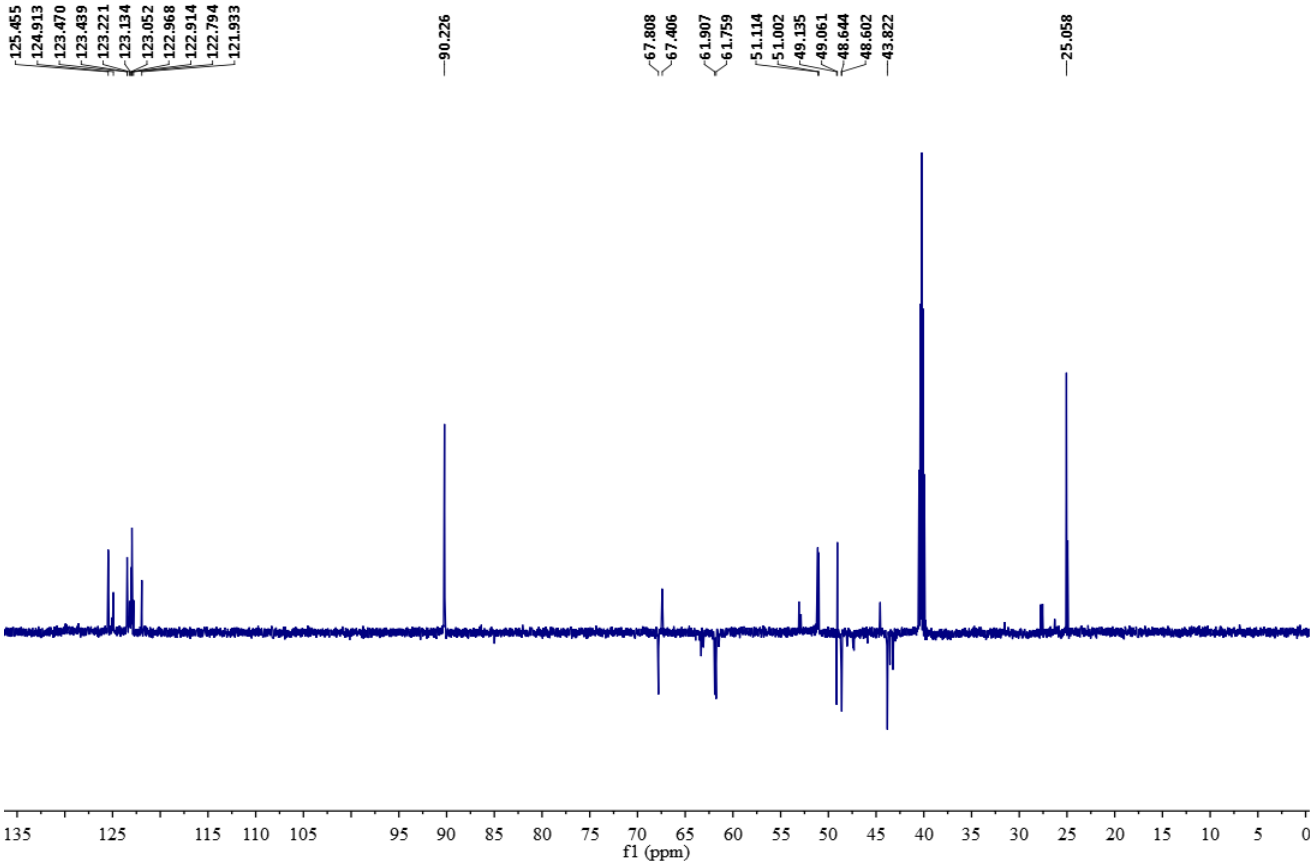


Figure S33. DEPT 135 spectrum of **3** in DMSO-d_6.

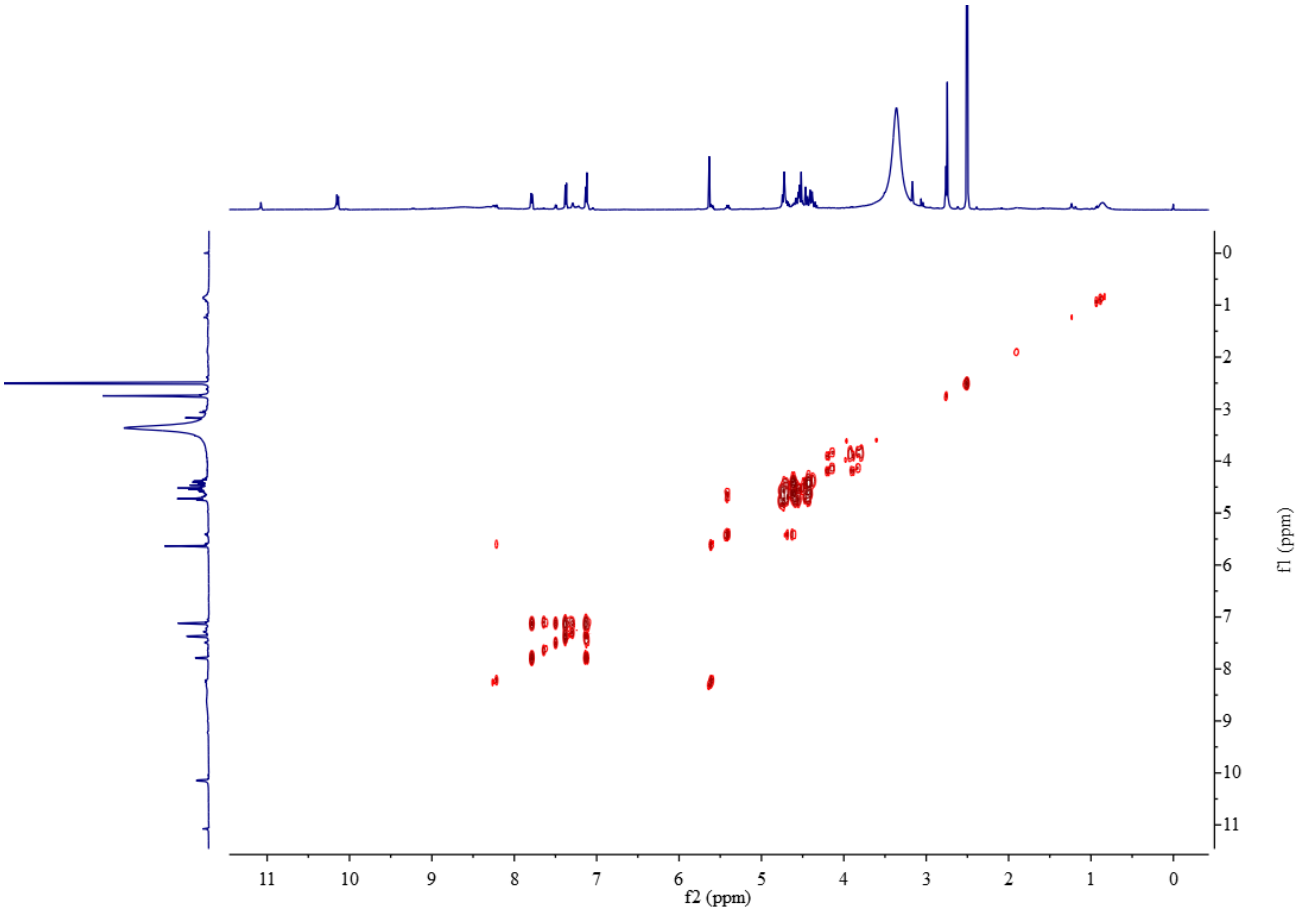


Figure S34.^{1}H-^{1}H COSY NMR spectrum of **3** in DMSO-d_6.

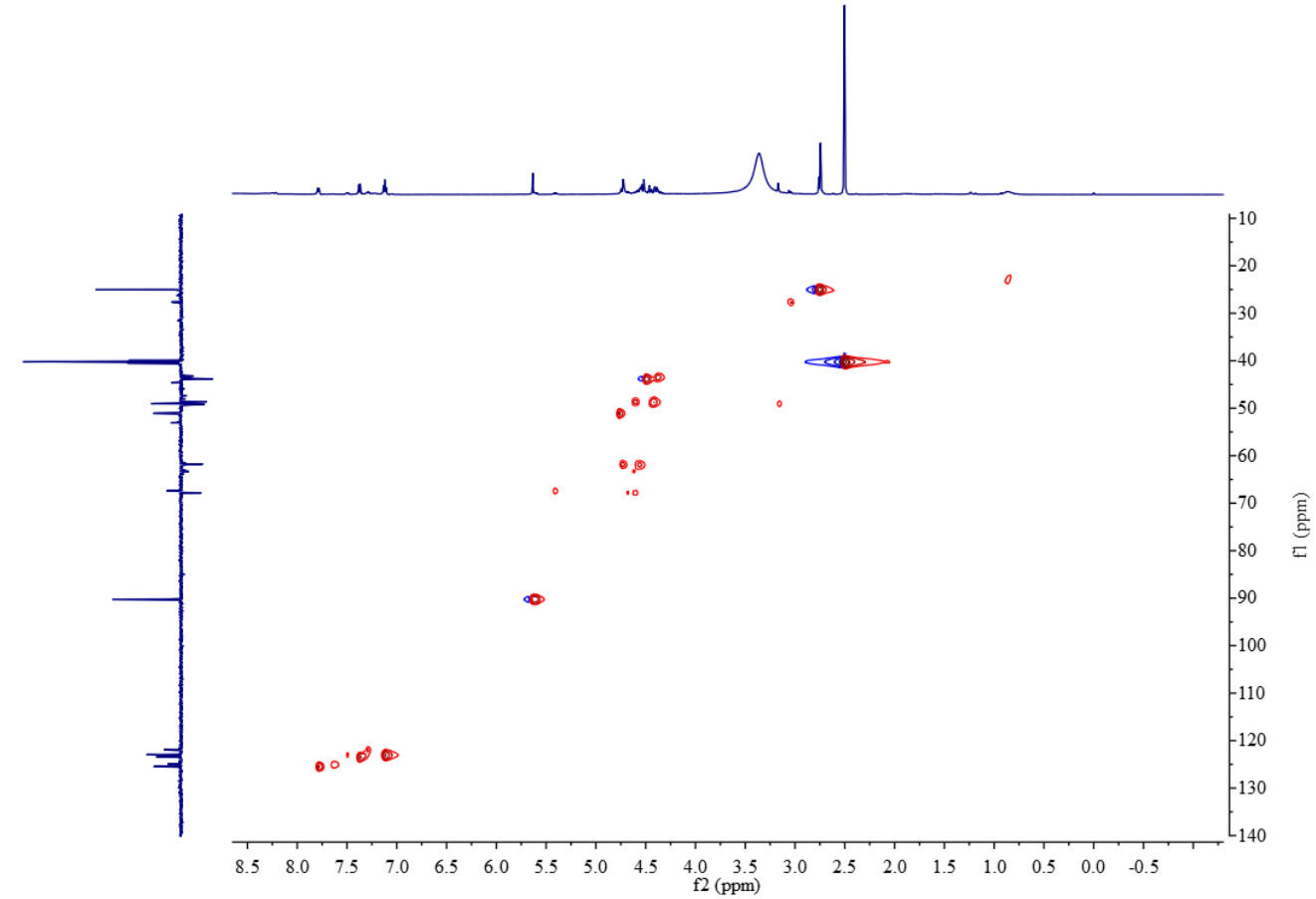


Figure S35. HSQC NMR spectrum of **3** in DMSO-d_6.

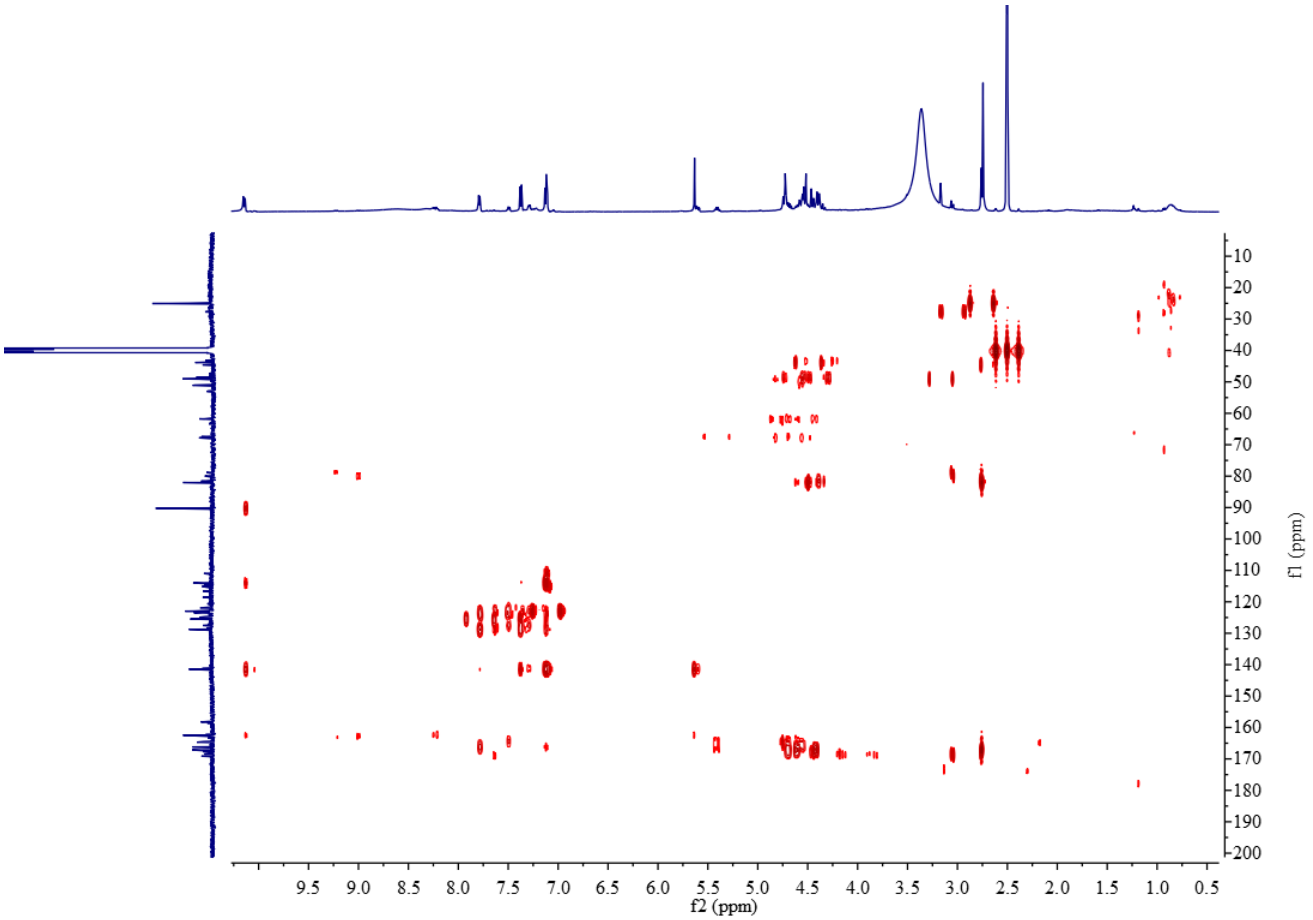


Figure S36. HMBC NMR spectrum of **3** in DMSO-d_6.

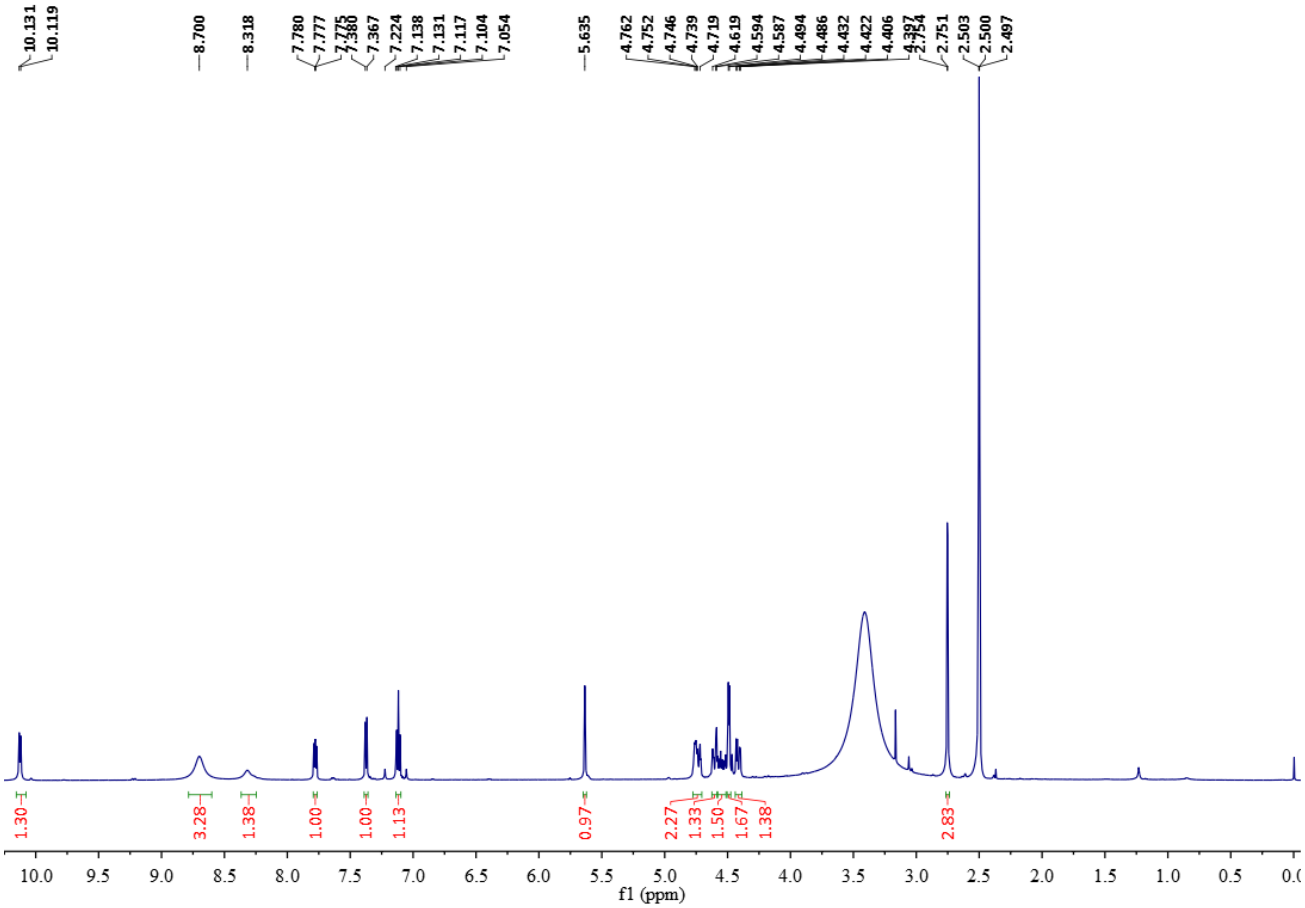


Figure S37. ^{1}H NMR spectrum of **4** in DMSO-d_6 at 600 MHz.

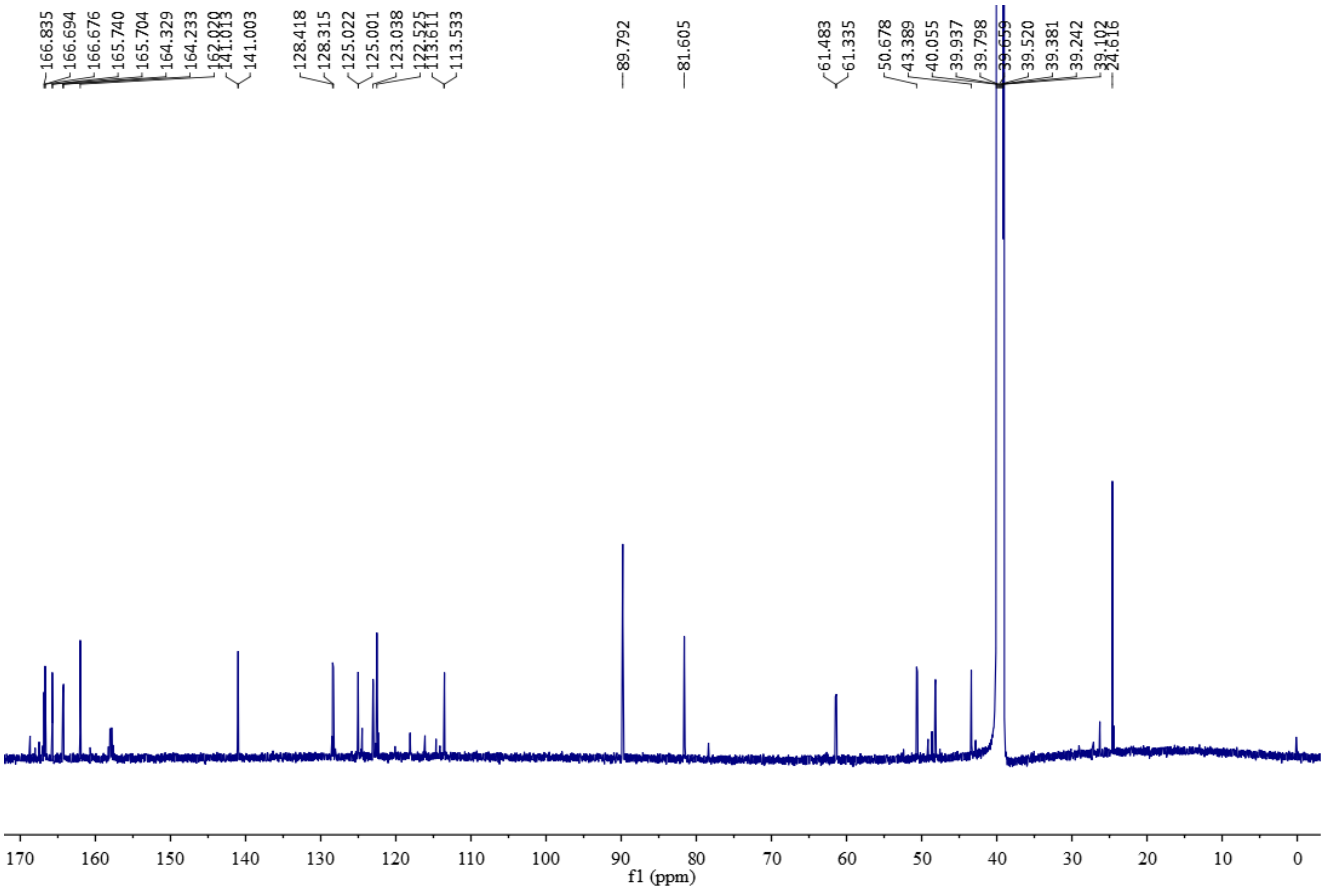


Figure S38. ^{13}C NMR spectrum of **4** in DMSO-d_6 at 150MHz.

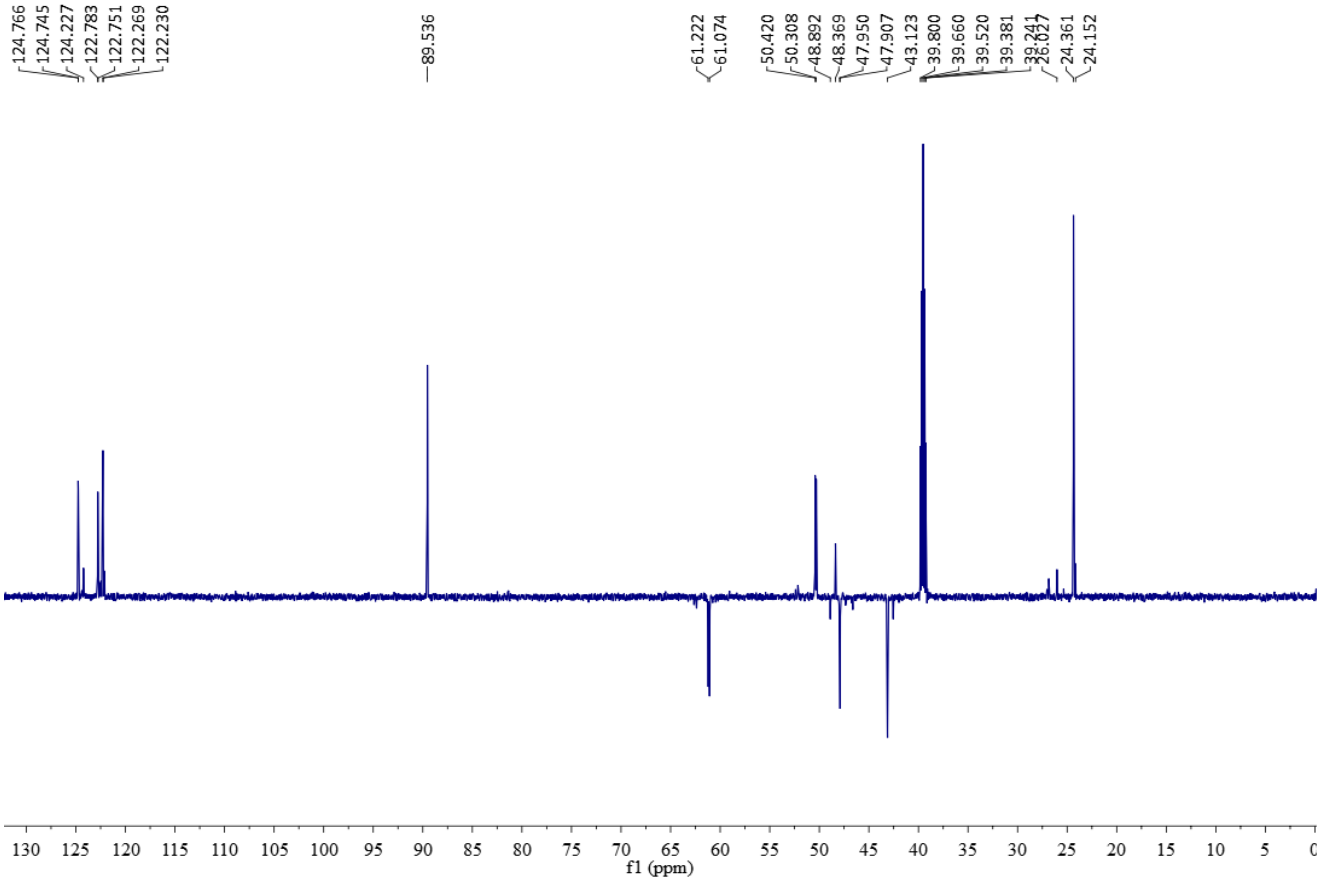


Figure S39. DEPT 135 spectrum of **4** in DMSO-d_6.

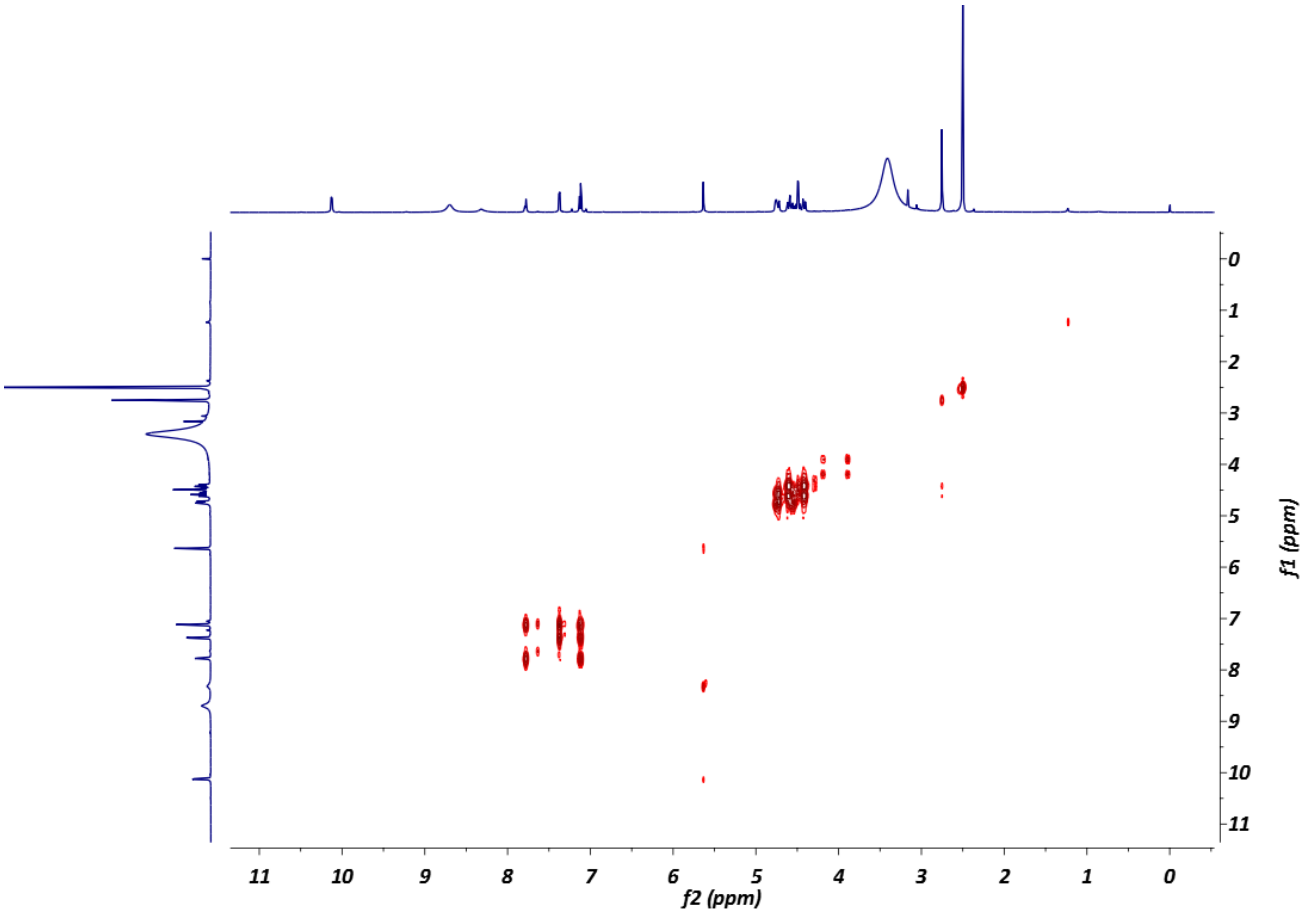


Figure S40. ^{1}H-^{1}H COSY NMR spectrum of **4** in DMSO-d_6.

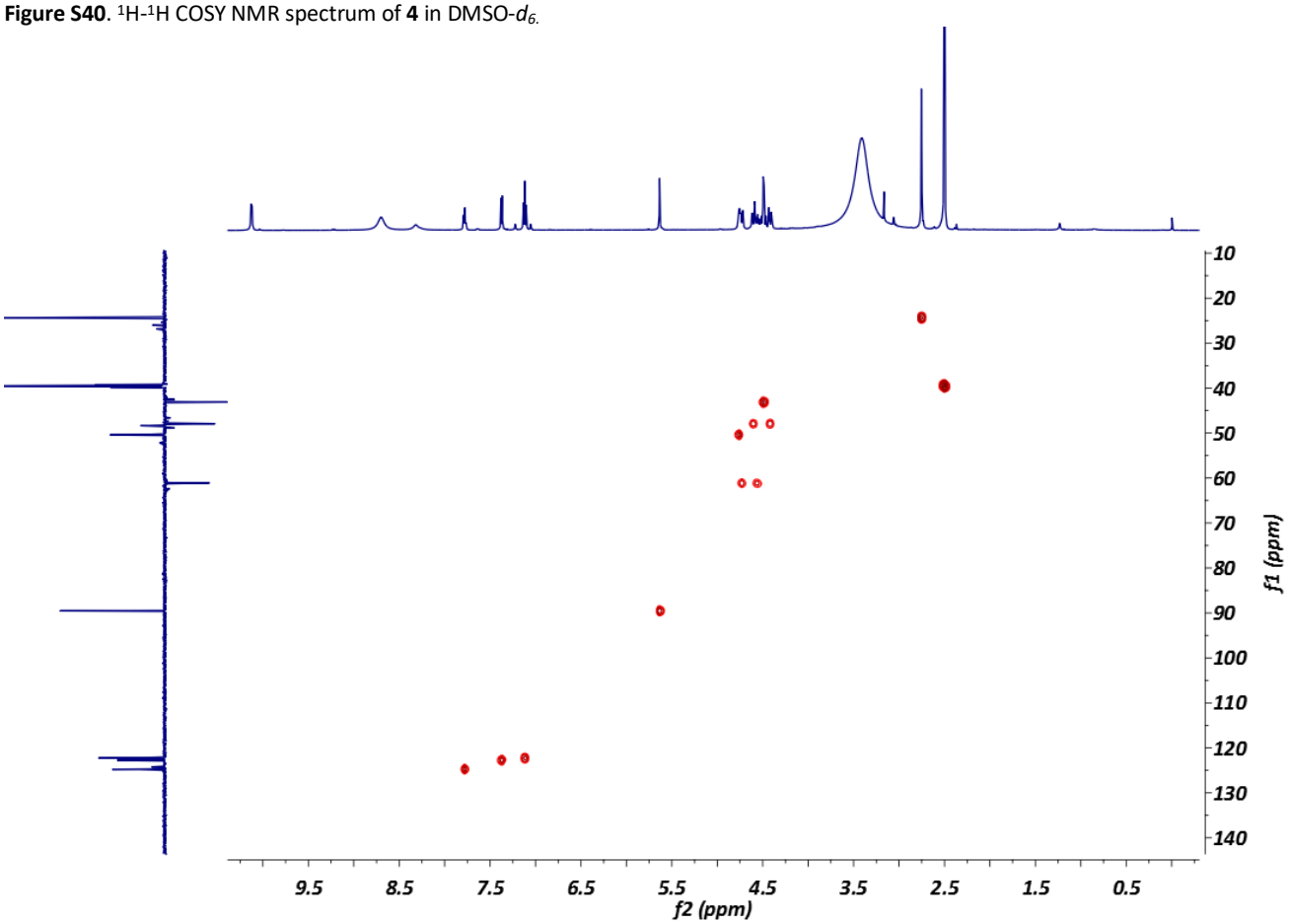


Figure S41. HSQC NMR spectrum of **4** in DMSO-d_6.

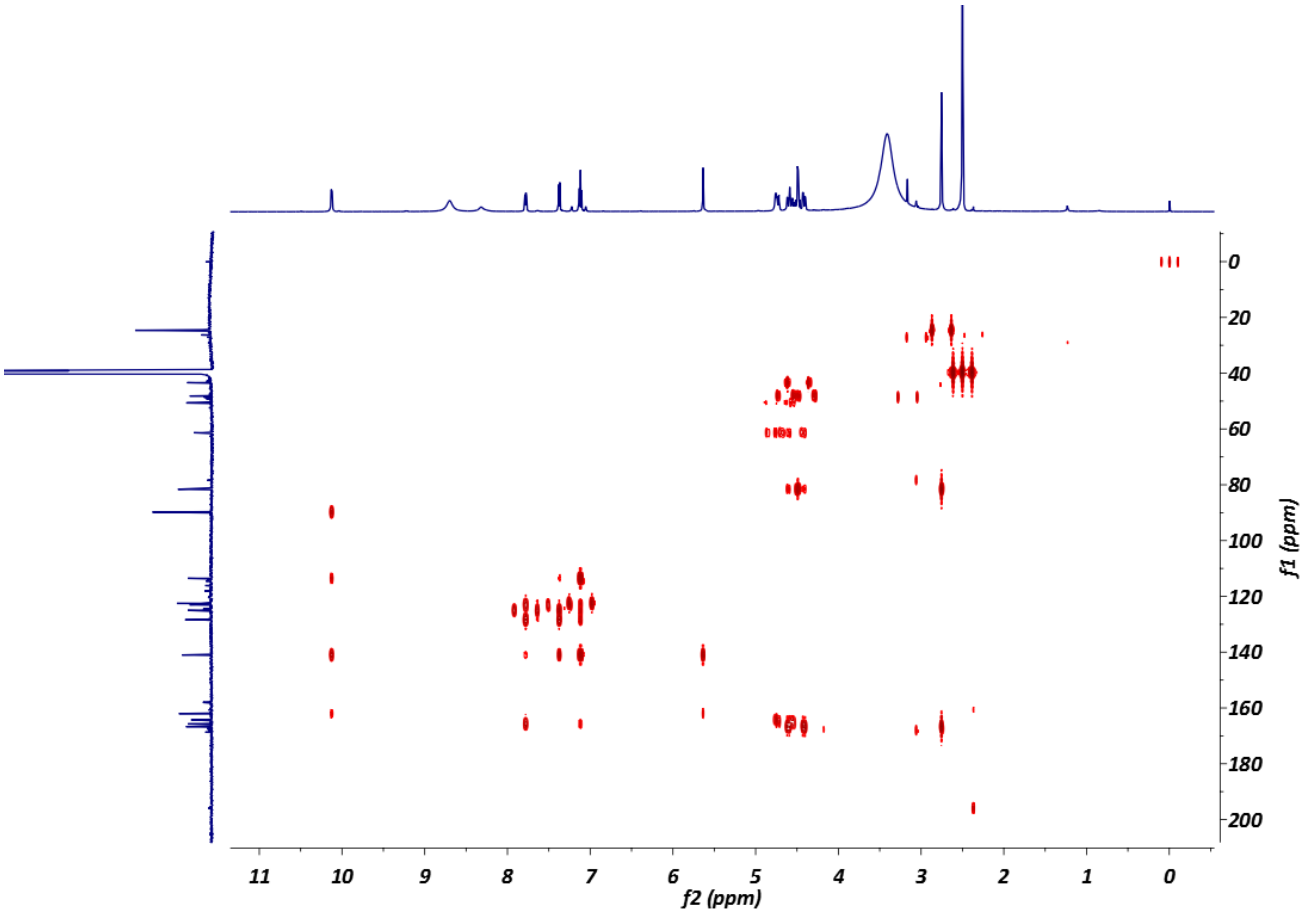


Figure S42. HMBC NMR spectrum of **4** in DMSO-d_6.

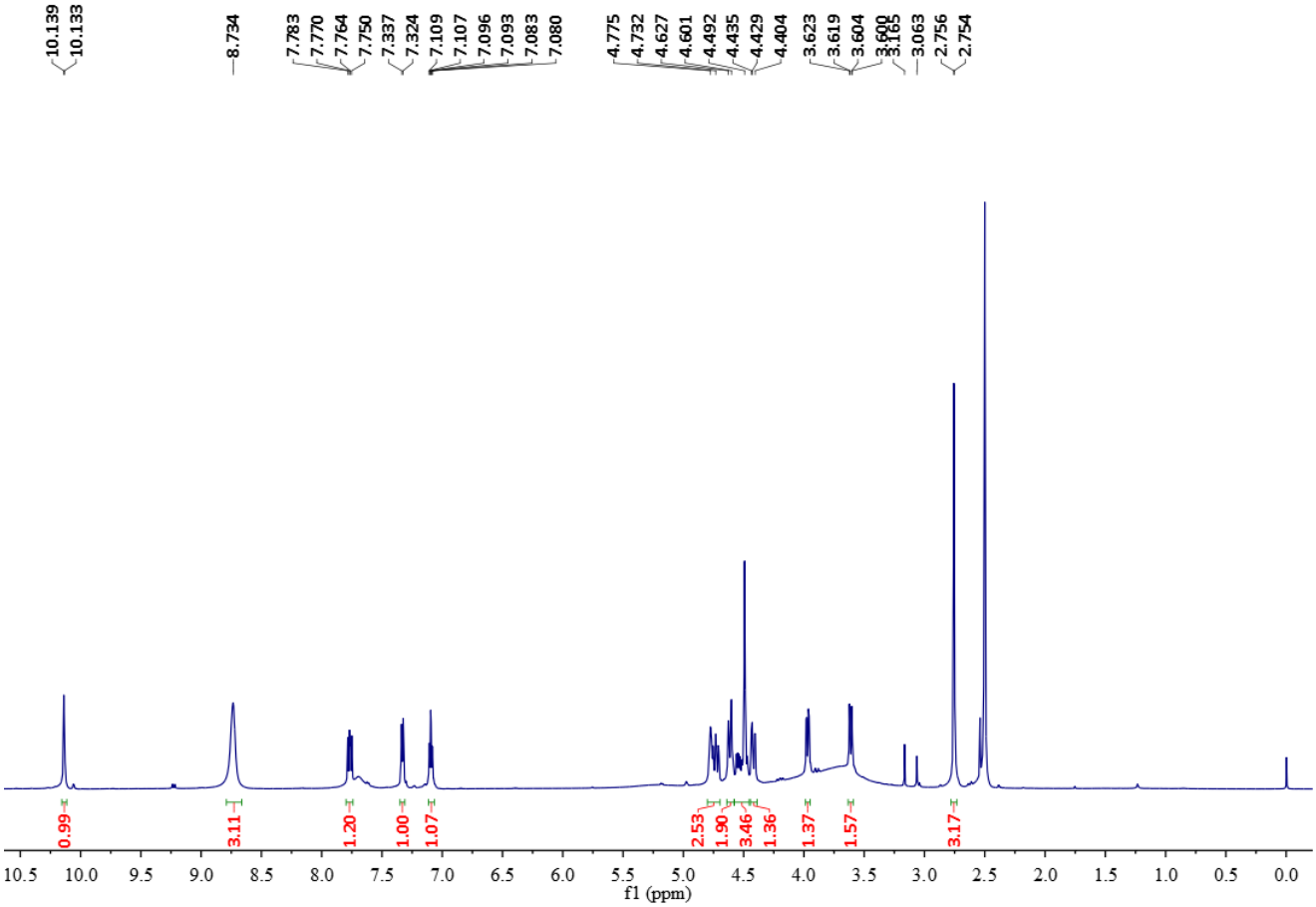


Figure S43. ^{1}H NMR spectrum of **5** in DMSO-d_6 at 600 MHz.

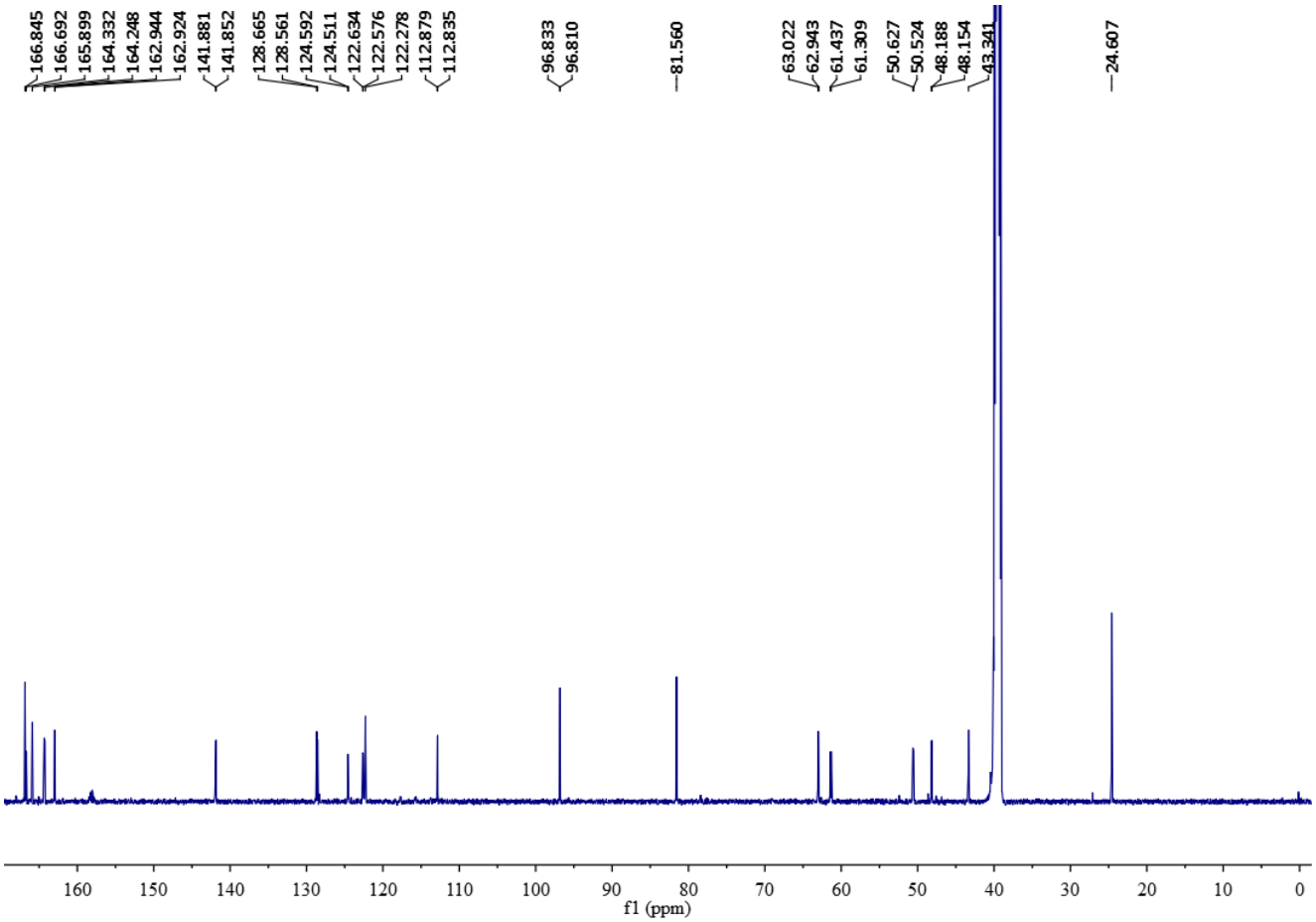


Figure S44. ^{13}C NMR spectrum of **5** in DMSO-d_6 at 150MHz.

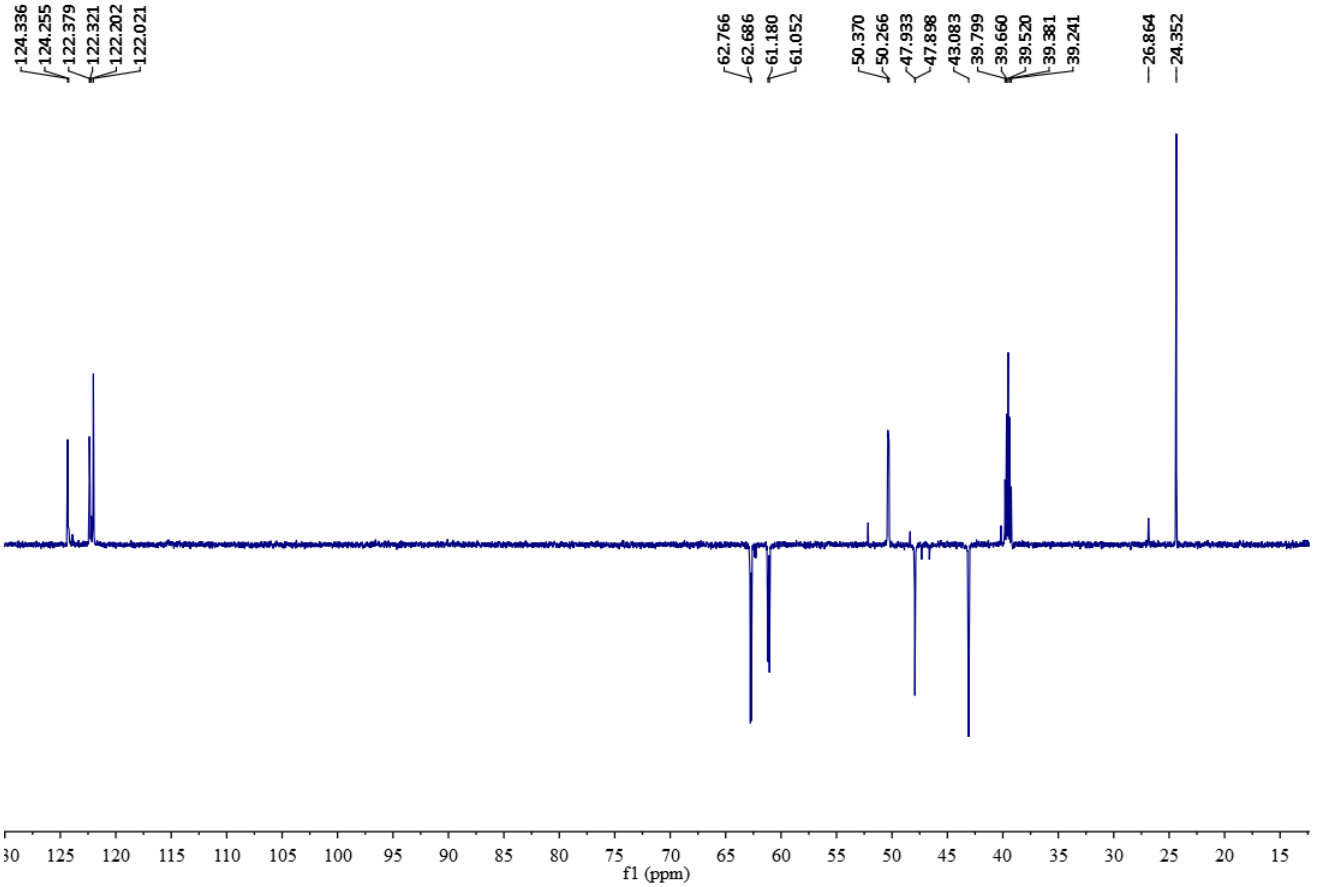


Figure S45. DEPT 135 spectrum of **5** in DMSO-d_6.

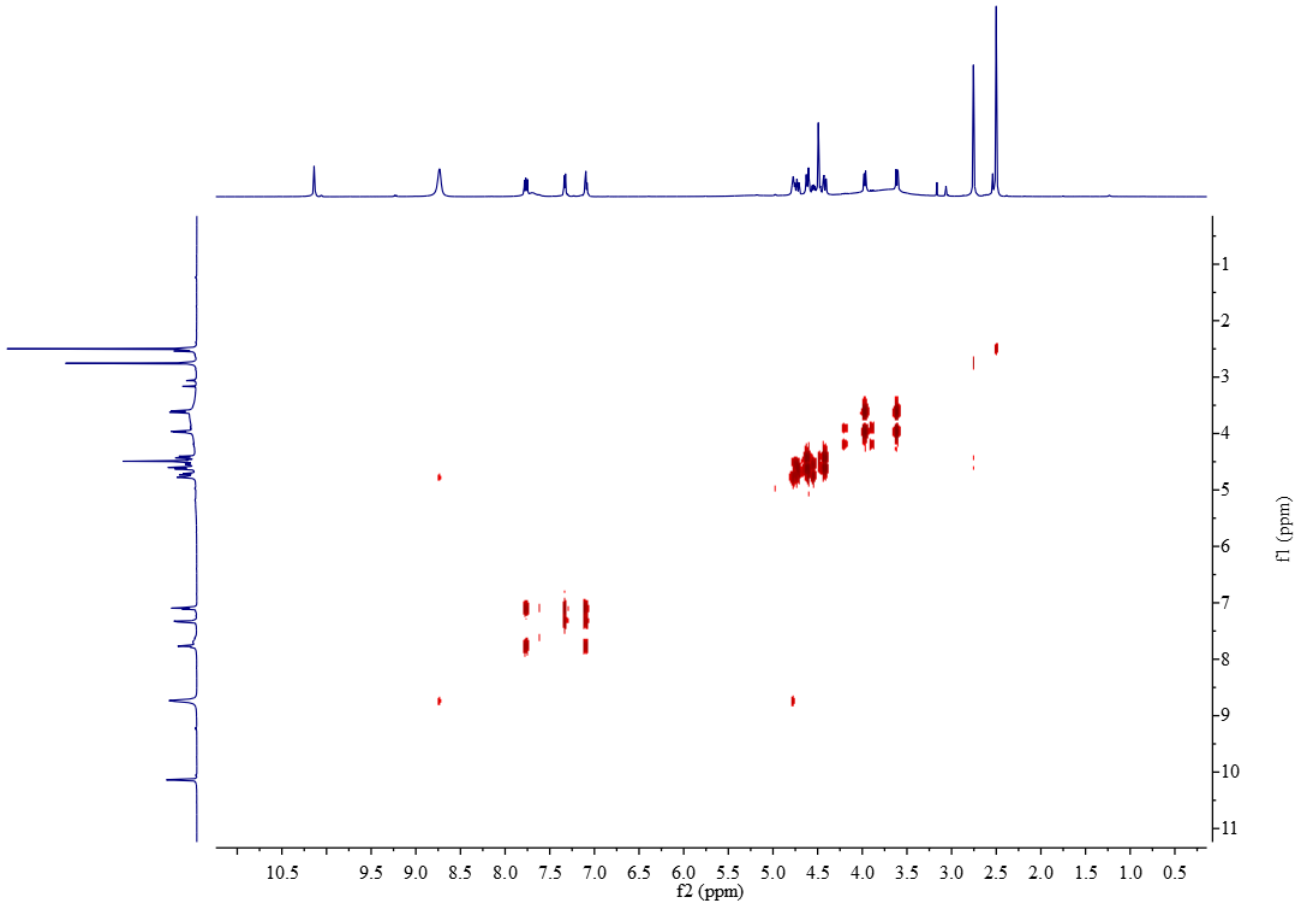


Figure S46. ^{1}H-^{1}H COSY NMR spectrum of **5** in DMSO-d_6.

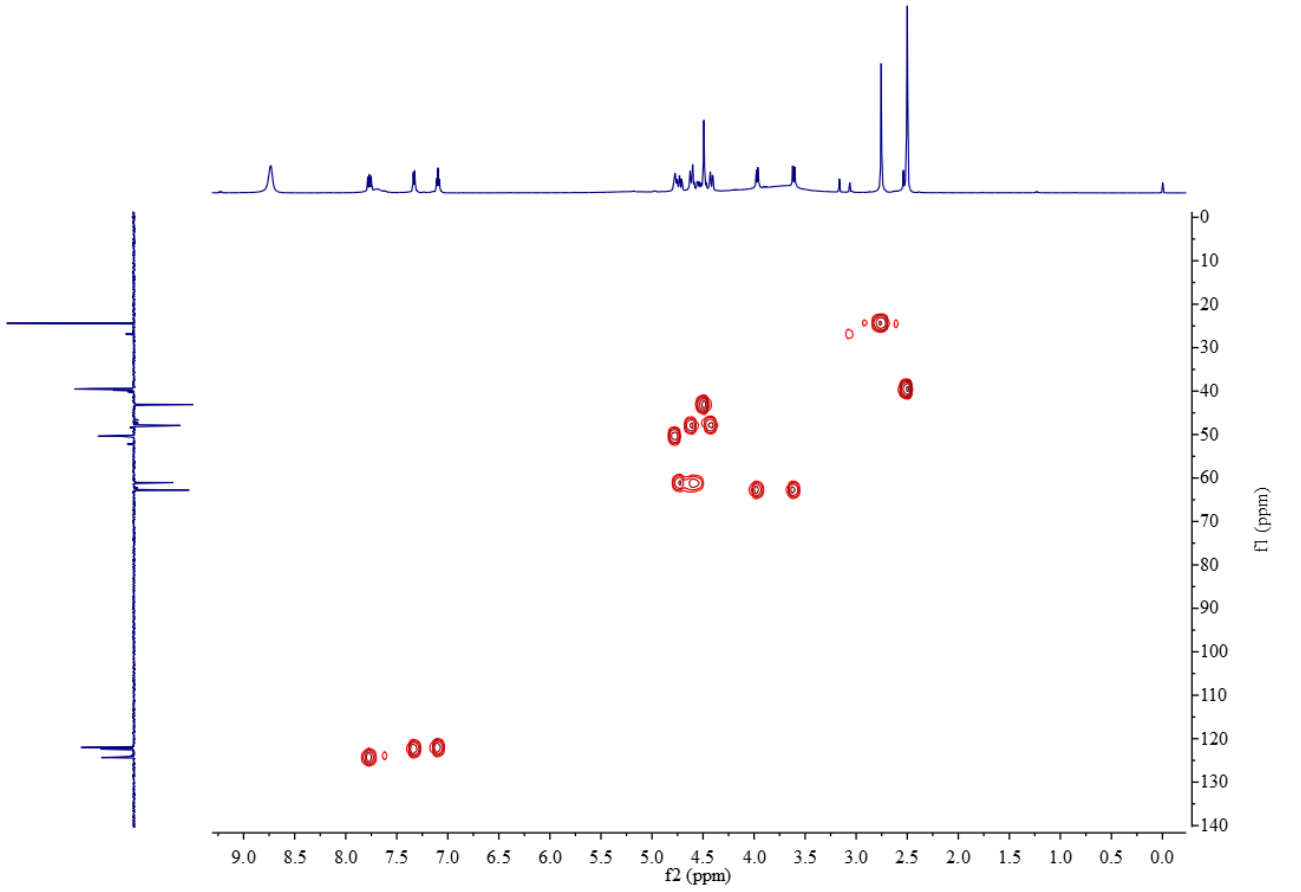


Figure S47. HSQC NMR spectrum of **5** in DMSO-d_6.

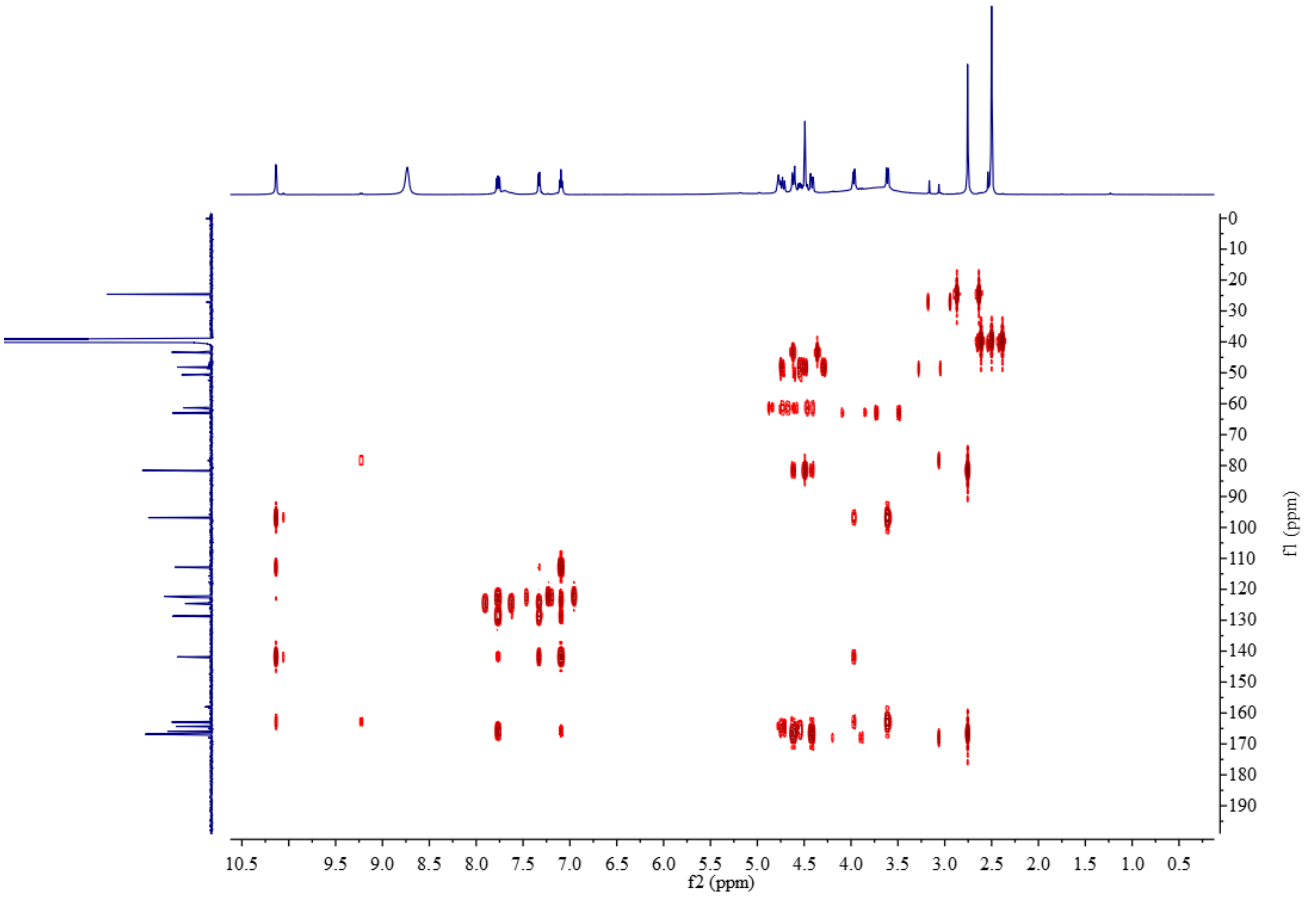


Figure S48. HMBC NMR spectrum of **5** in DMSO-d_6.

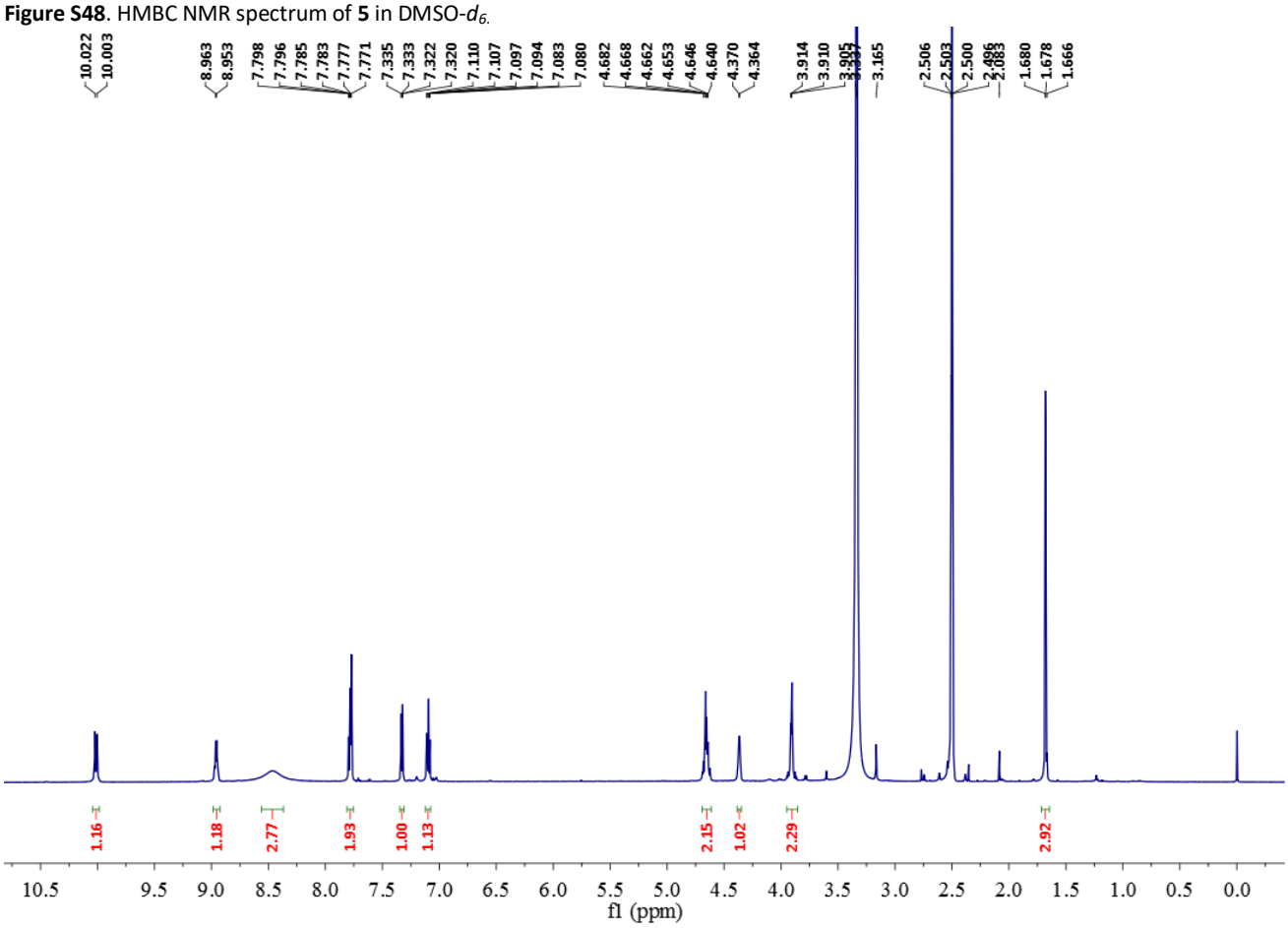


Figure S49. ^{1}H NMR spectrum of **6** in DMSO-d_6 at 600 MHz.

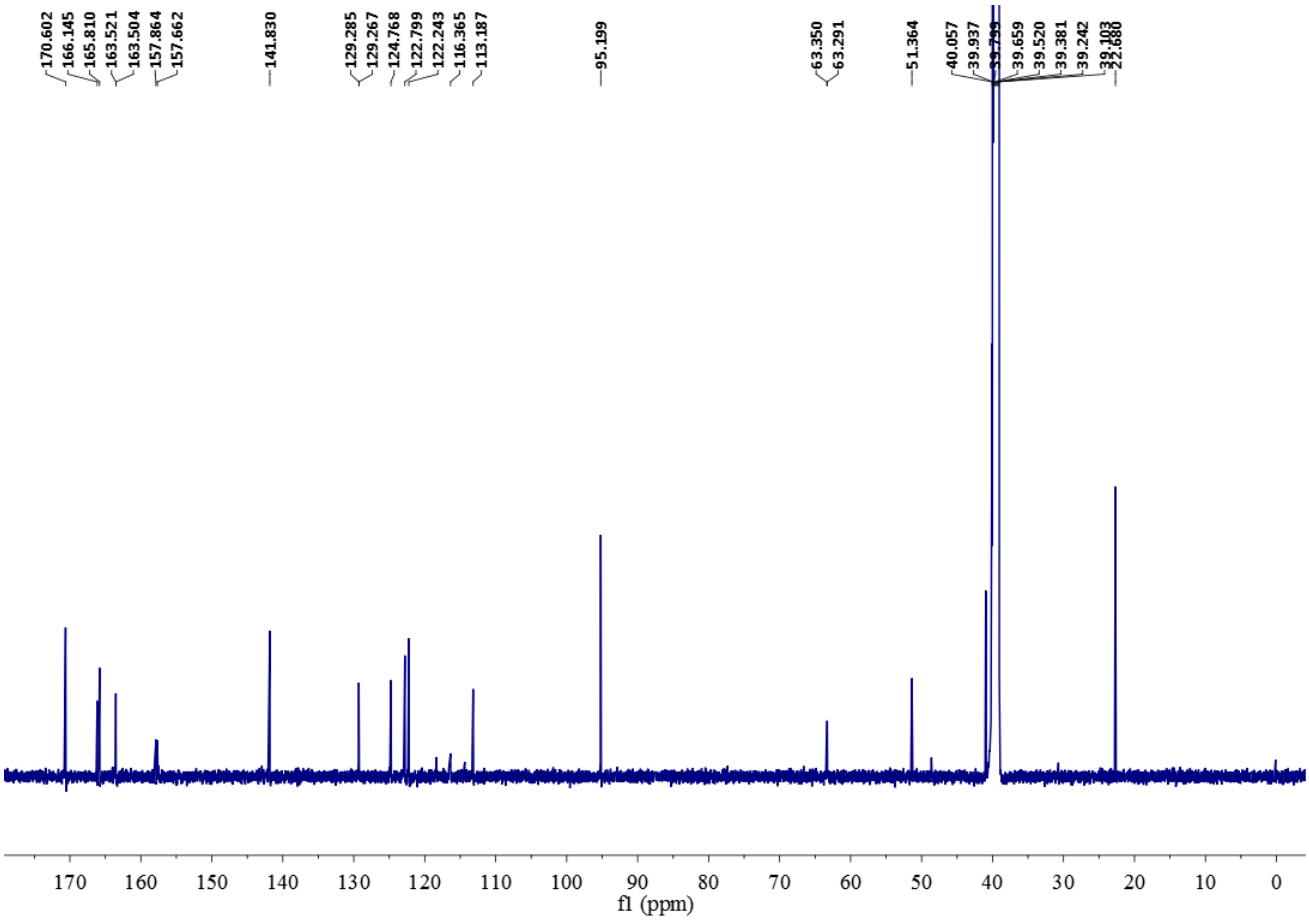


Figure S50. ^{13}C NMR spectrum of **6** in DMSO-d_6 at 150MHz.

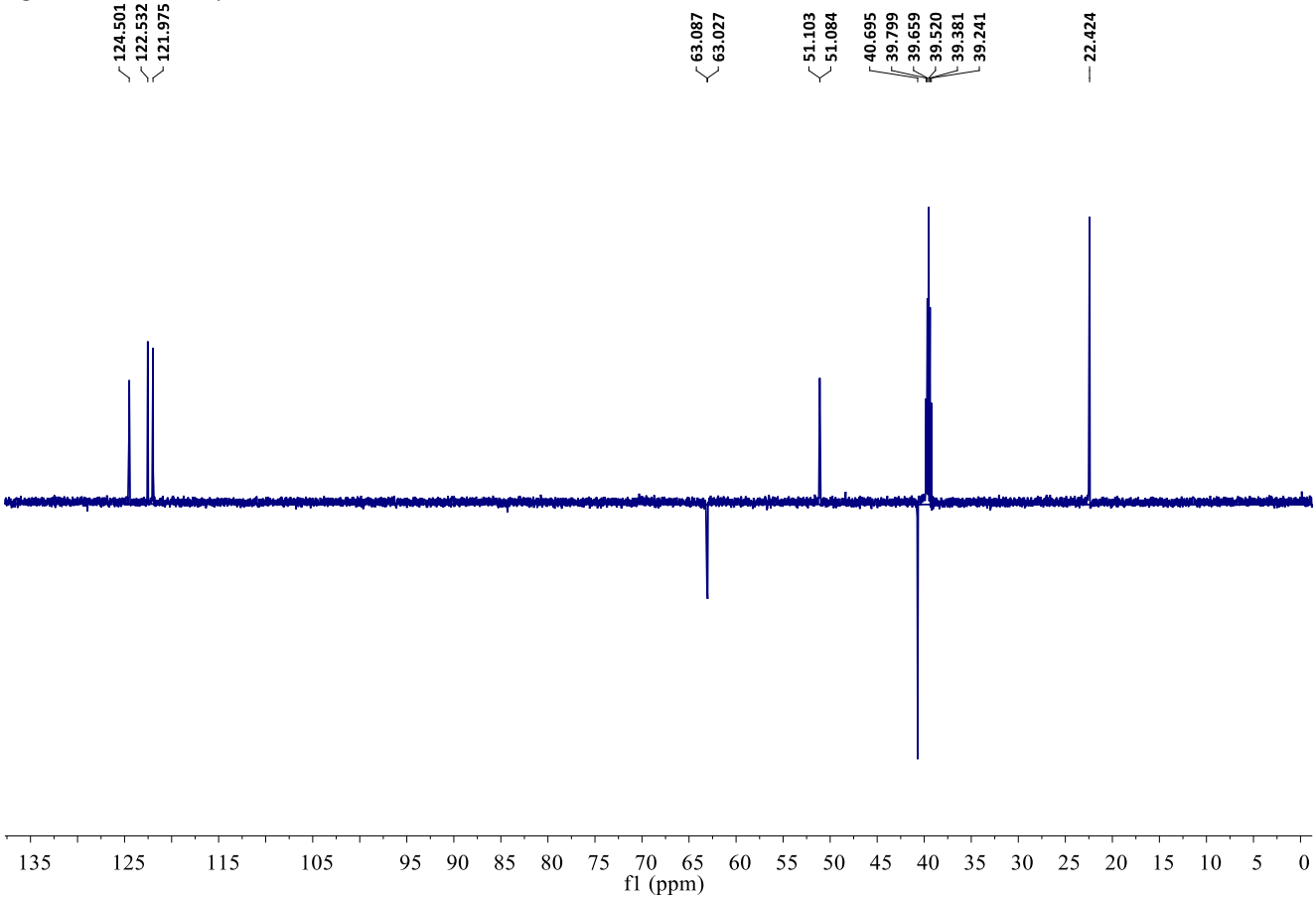


Figure S51. DEPT 135 spectrum of **6** in DMSO-d_6.

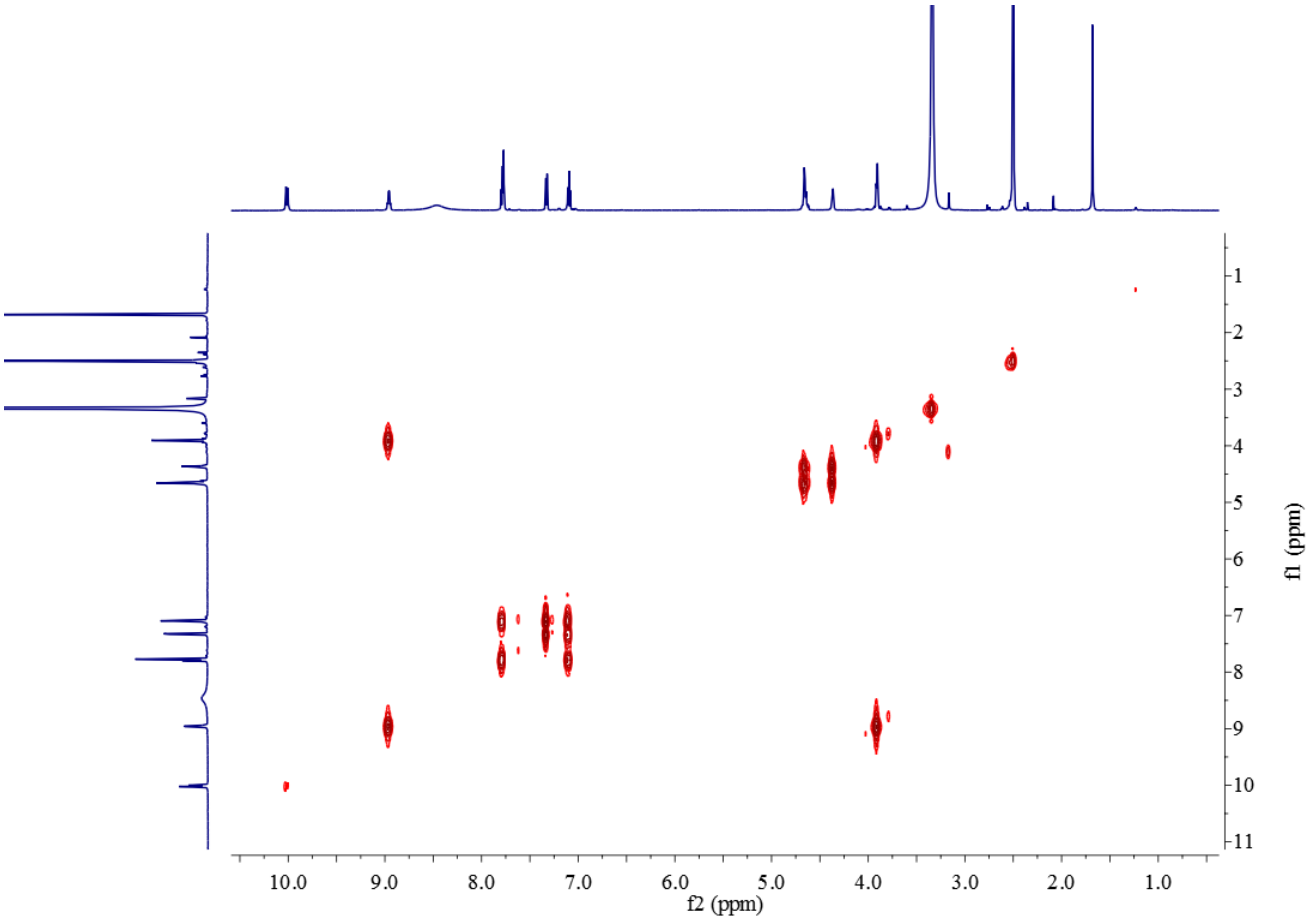


Figure S52. ^{1}H-^{1}H COSY NMR spectrum of **6** in DMSO-d_6.

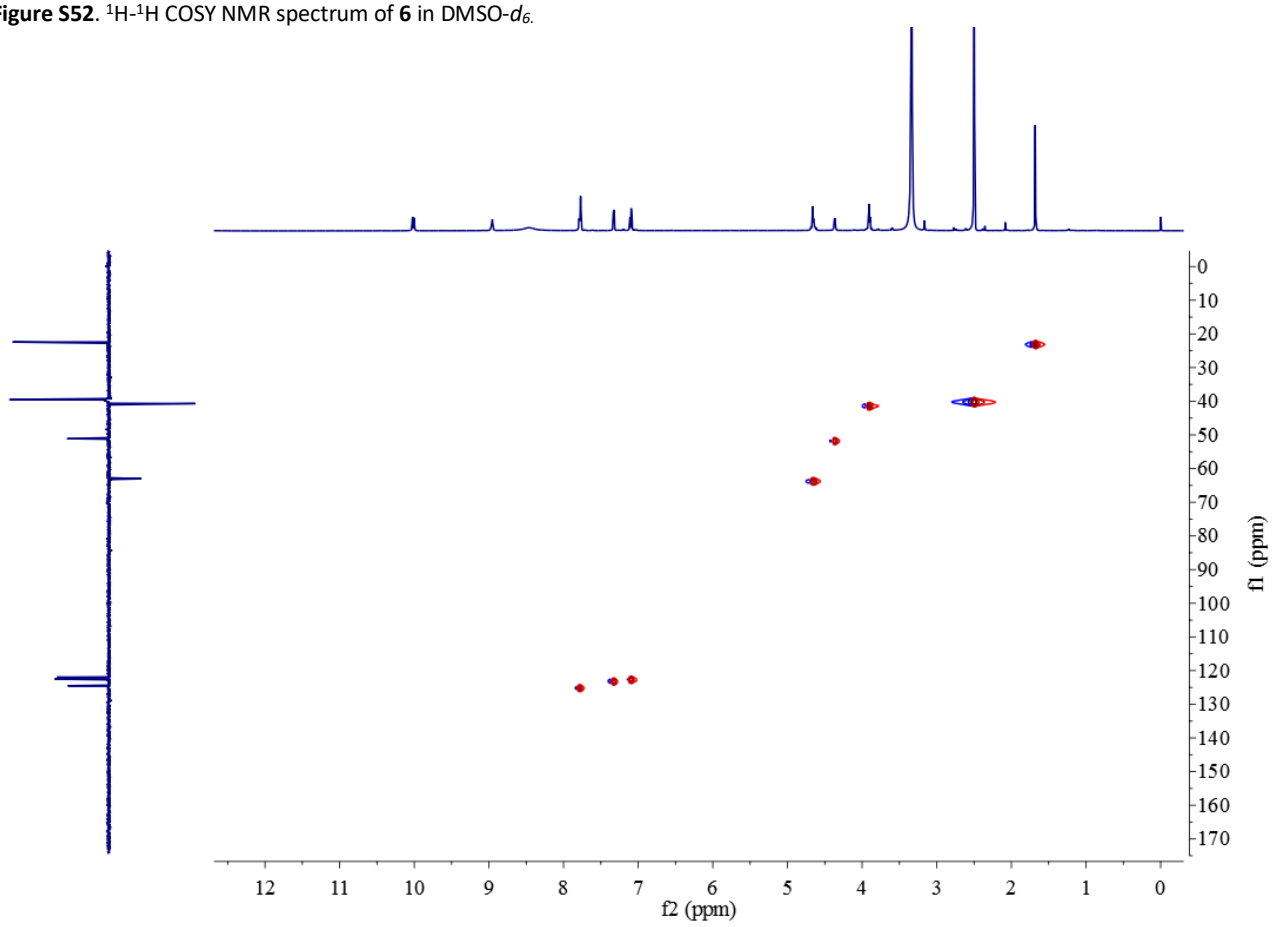


Figure S53. HSQC NMR spectrum of **6** in DMSO-d_6.

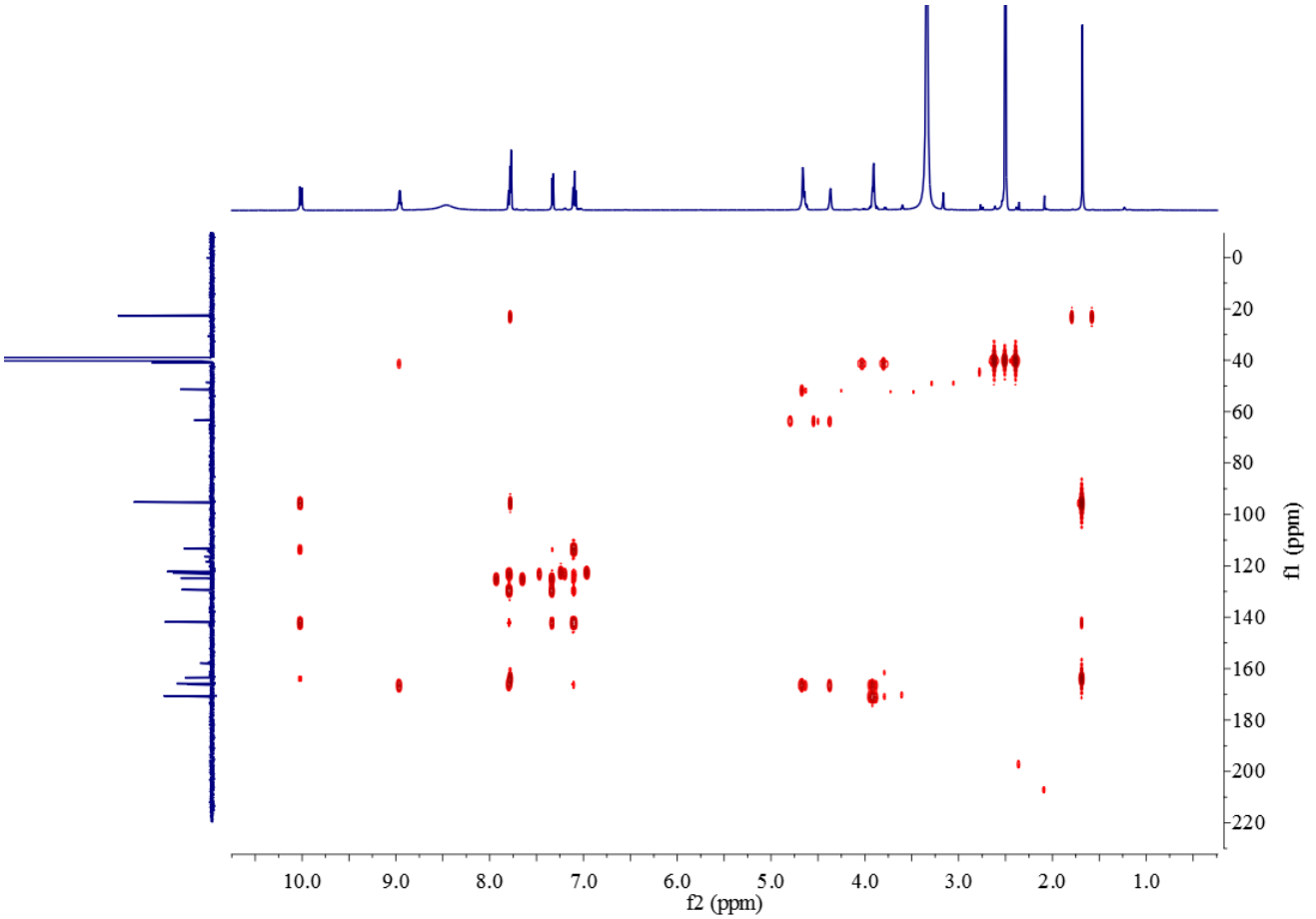


Figure S54. HMBC NMR spectrum of **6** in DMSO-d_6.

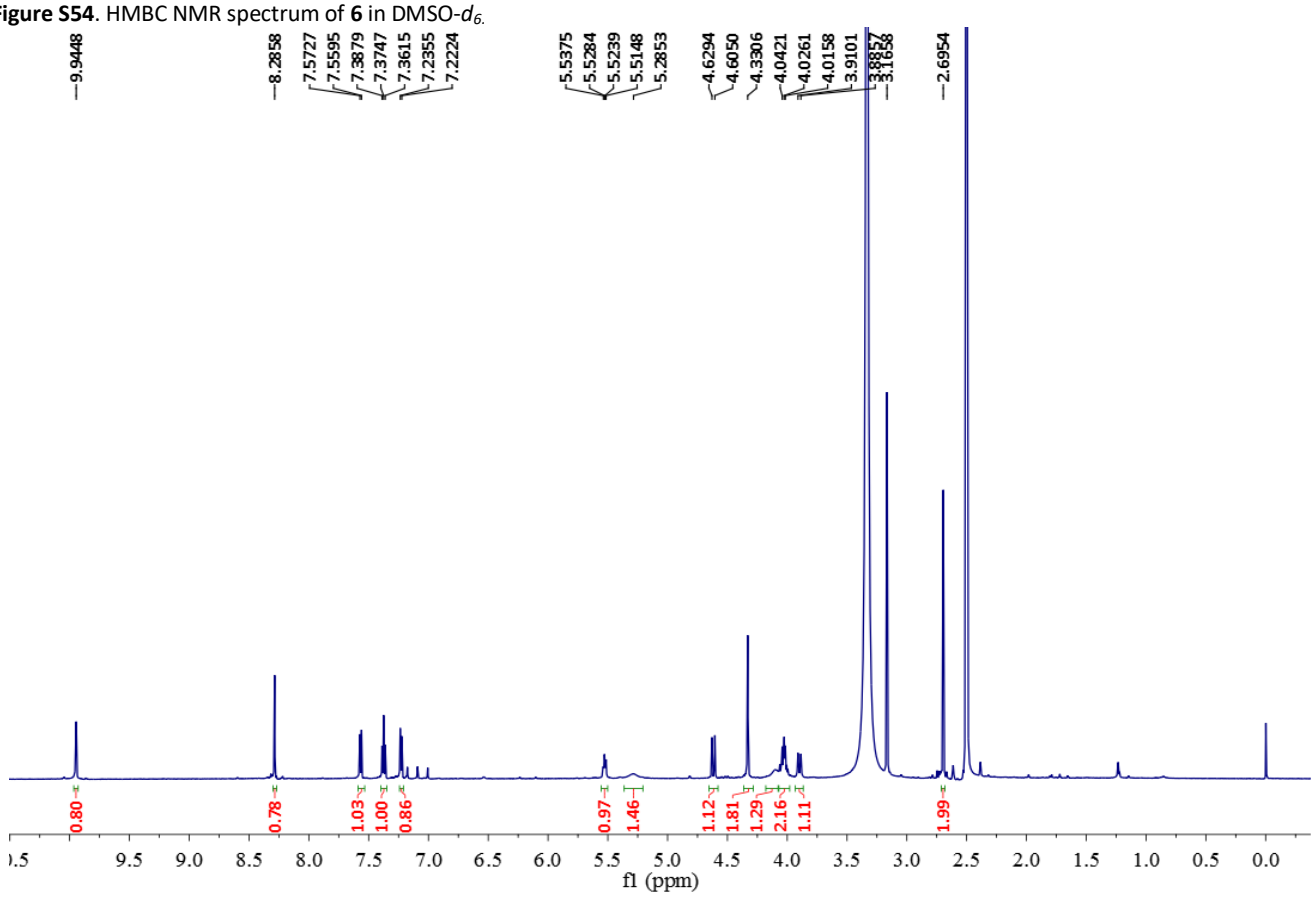


Figure S55. ^{1}H NMR spectrum of **7** in DMSO-d_6 at 600 MHz.

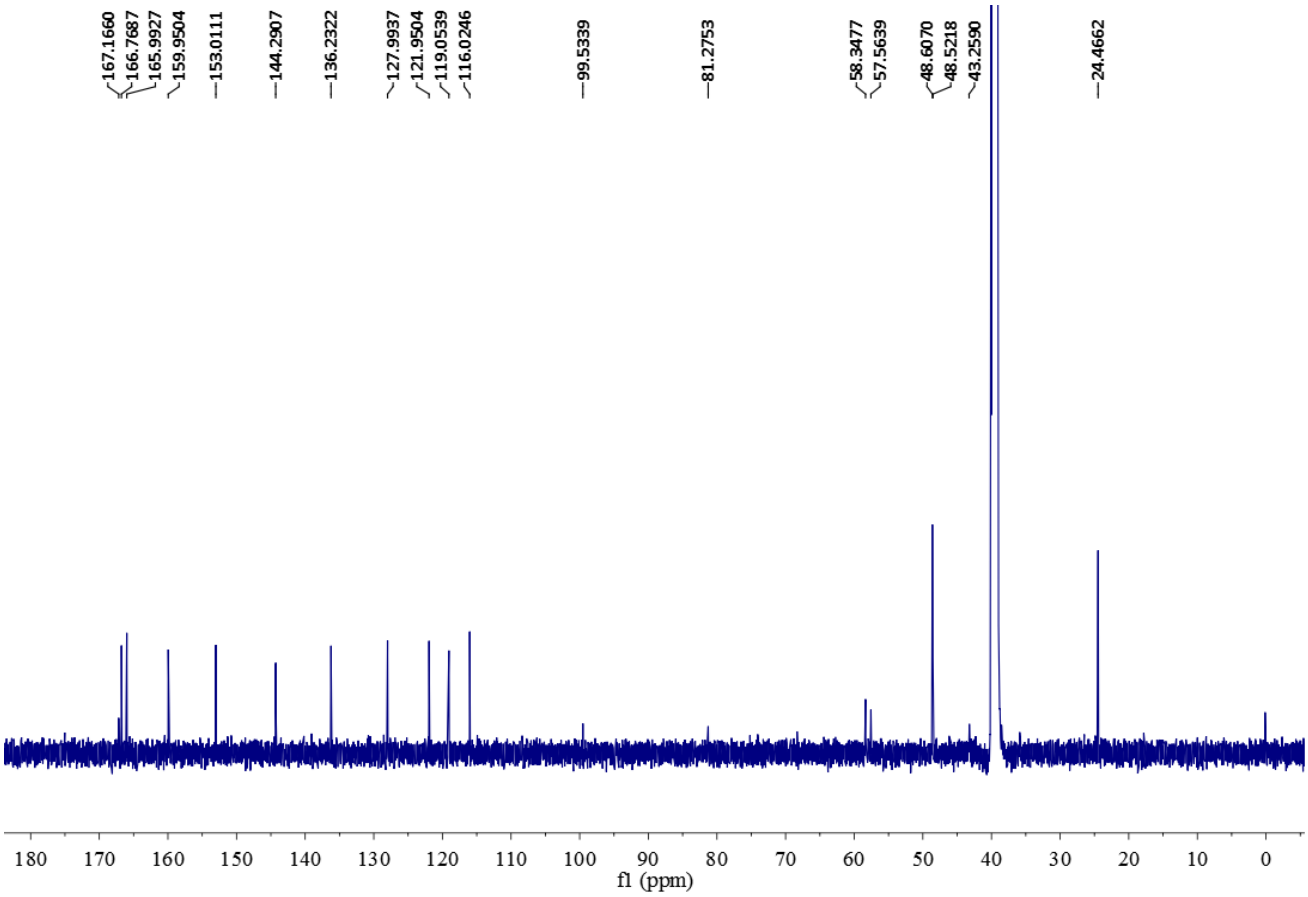


Figure S56. ^{13}C NMR spectrum of **7** in DMSO-d_6 at 150MHz.

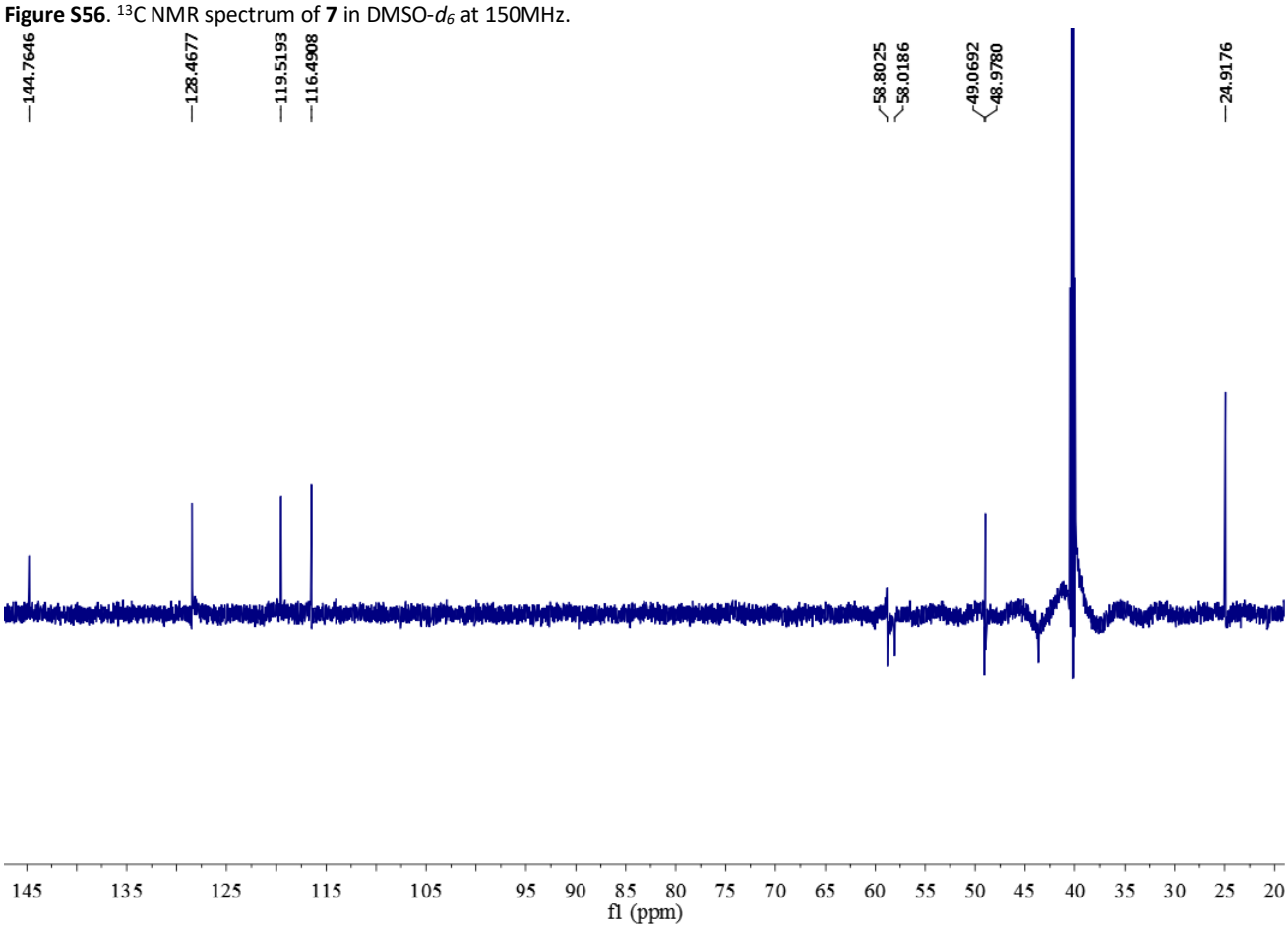


Figure S57. DEPT 135 spectrum of **7** in DMSO-d_6.

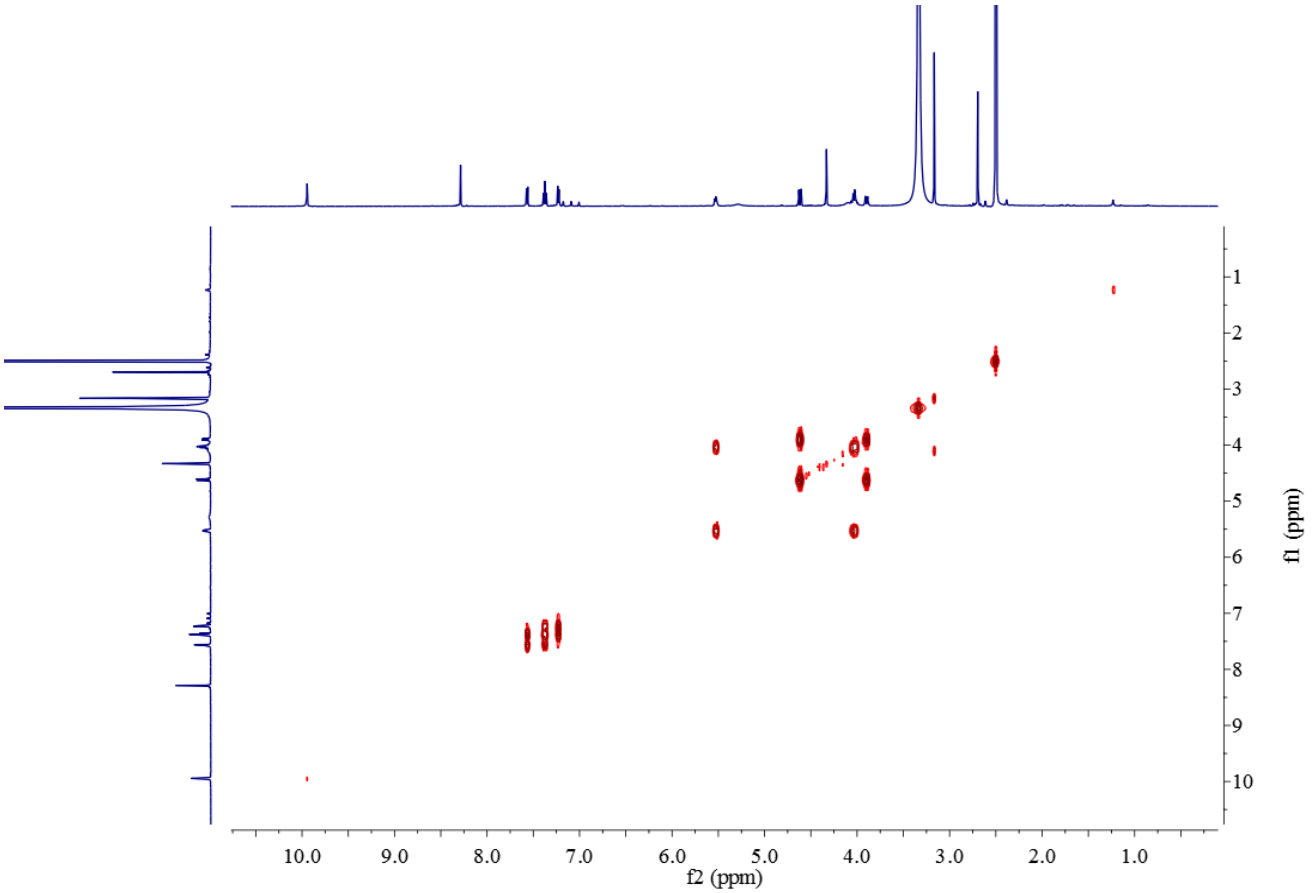


Figure S58. ^{1}H-^{1}H COSY NMR spectrum of **7** in DMSO-d_6.

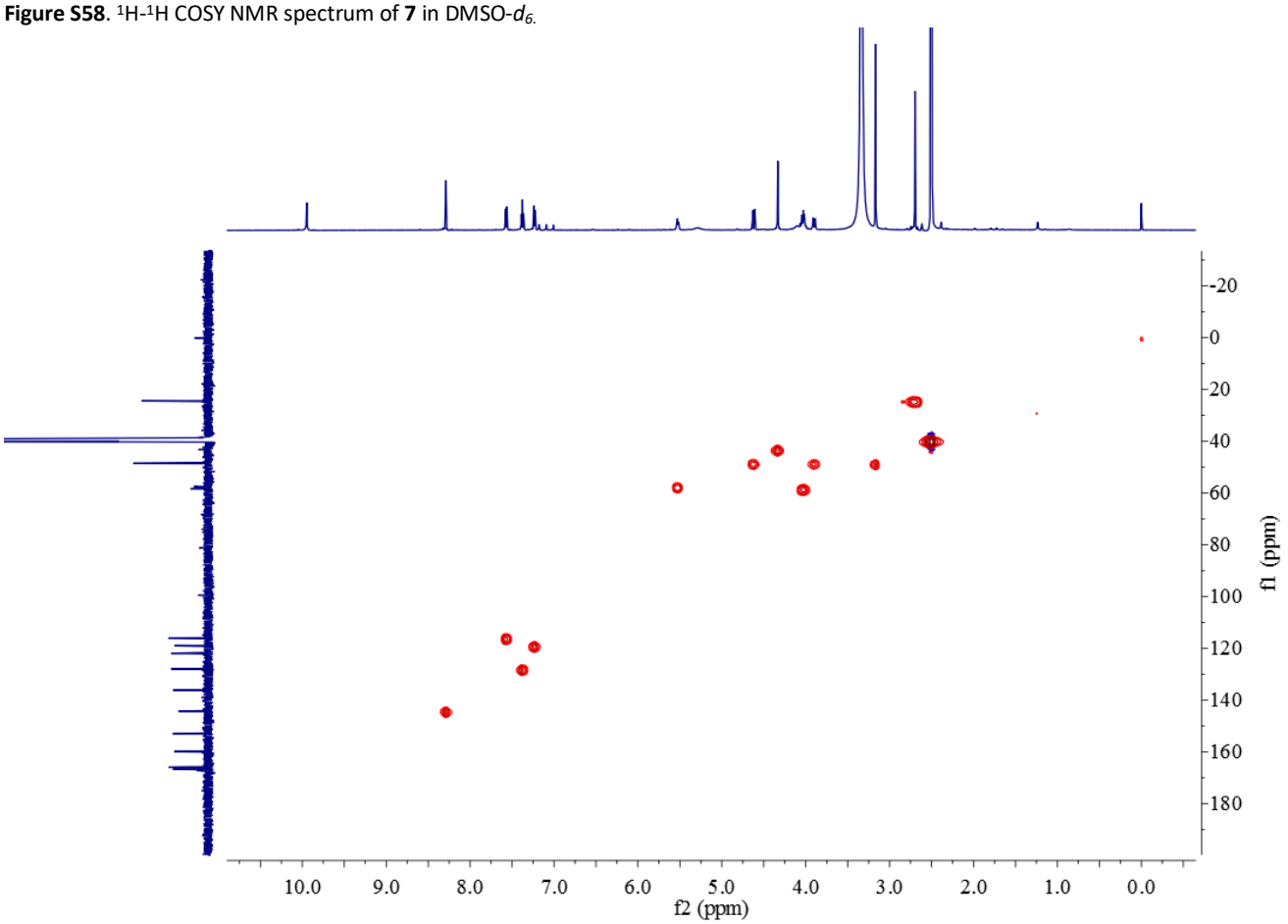


Figure S59. HSQC NMR spectrum of **7** in DMSO-d_6.

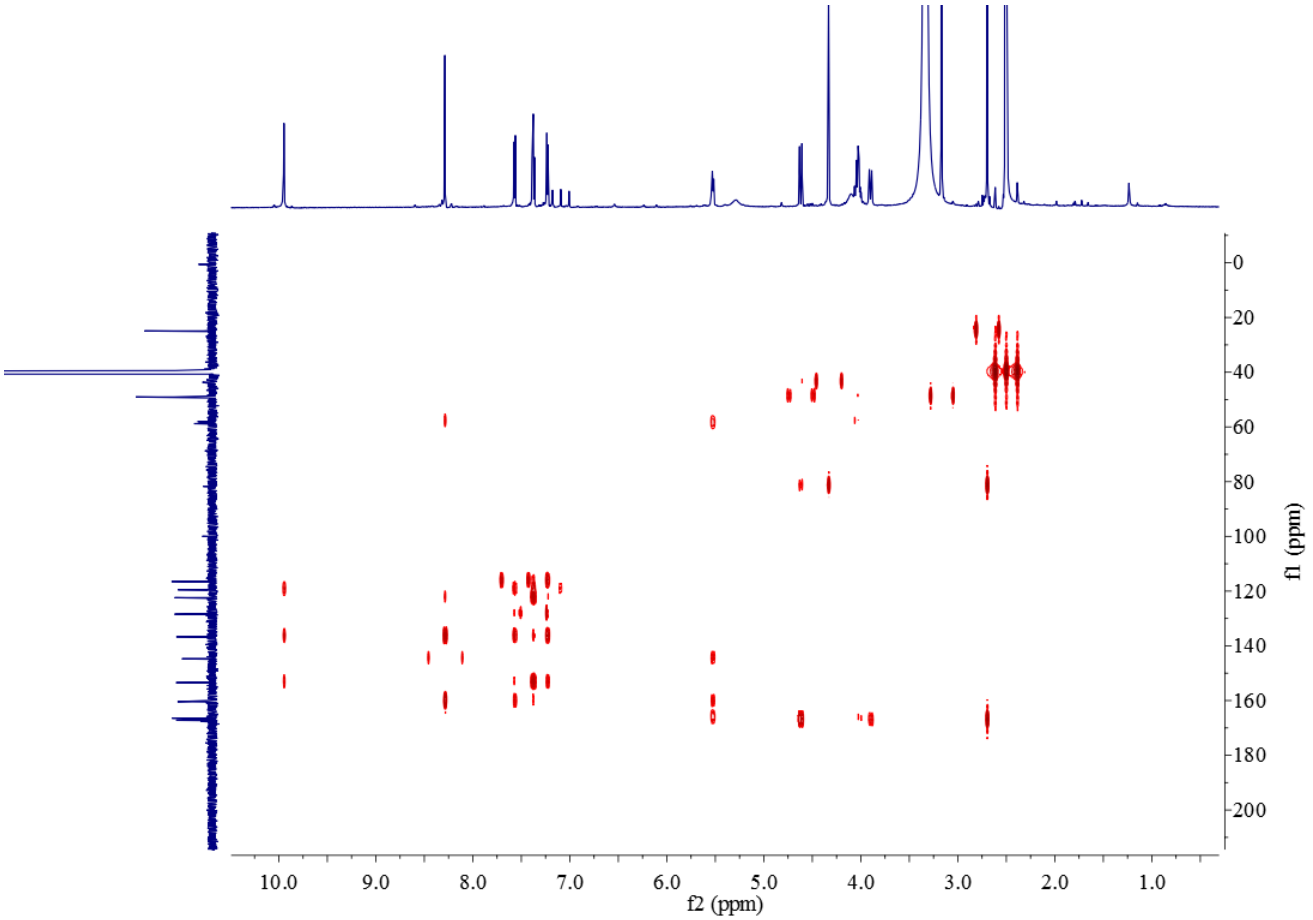


Figure S60. HMBC NMR spectrum of **7** in DMSO-d_6.

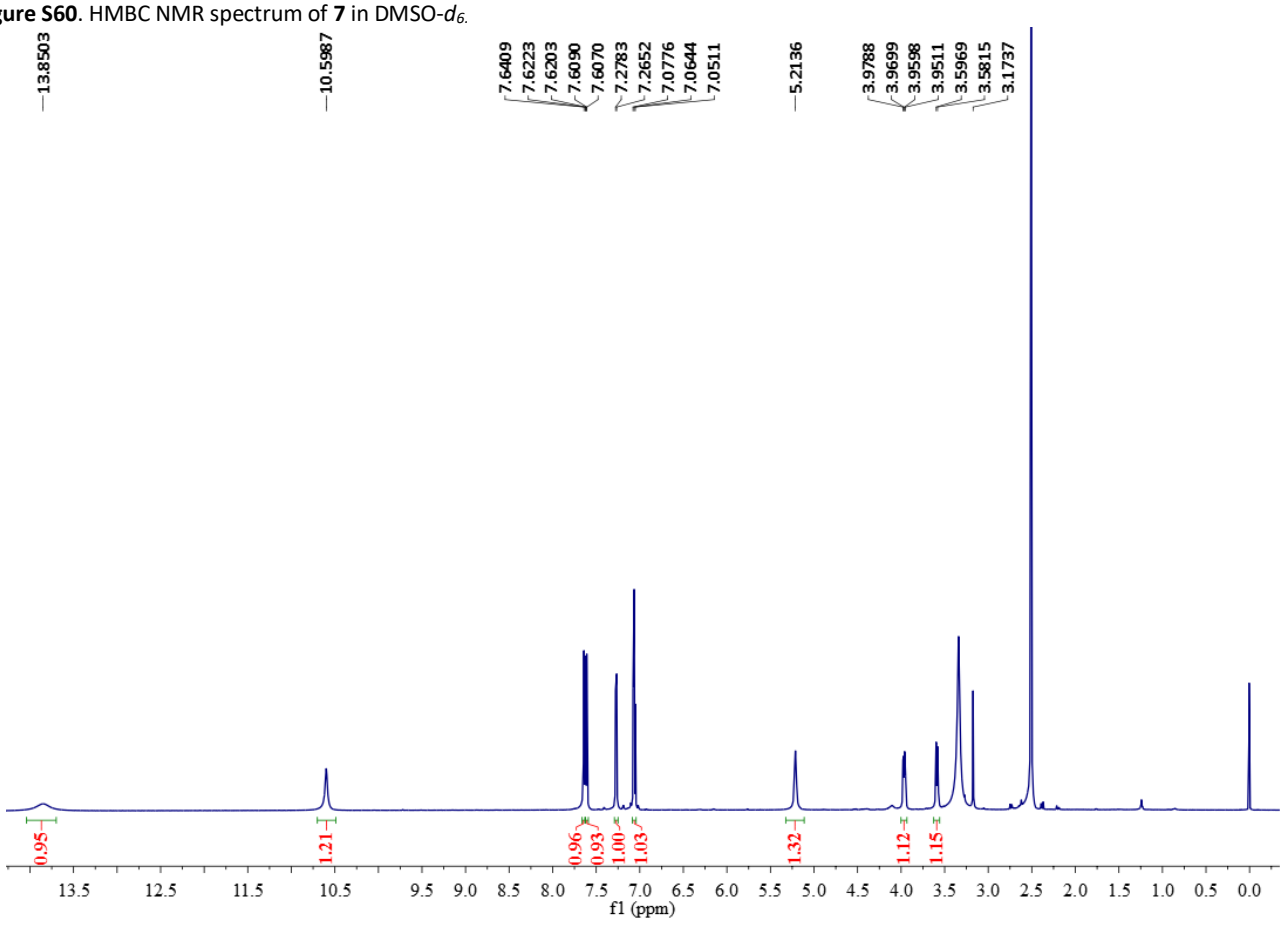


Figure S61. ^{1}H NMR spectrum of **8** in DMSO-d_6 at 600 MHz.

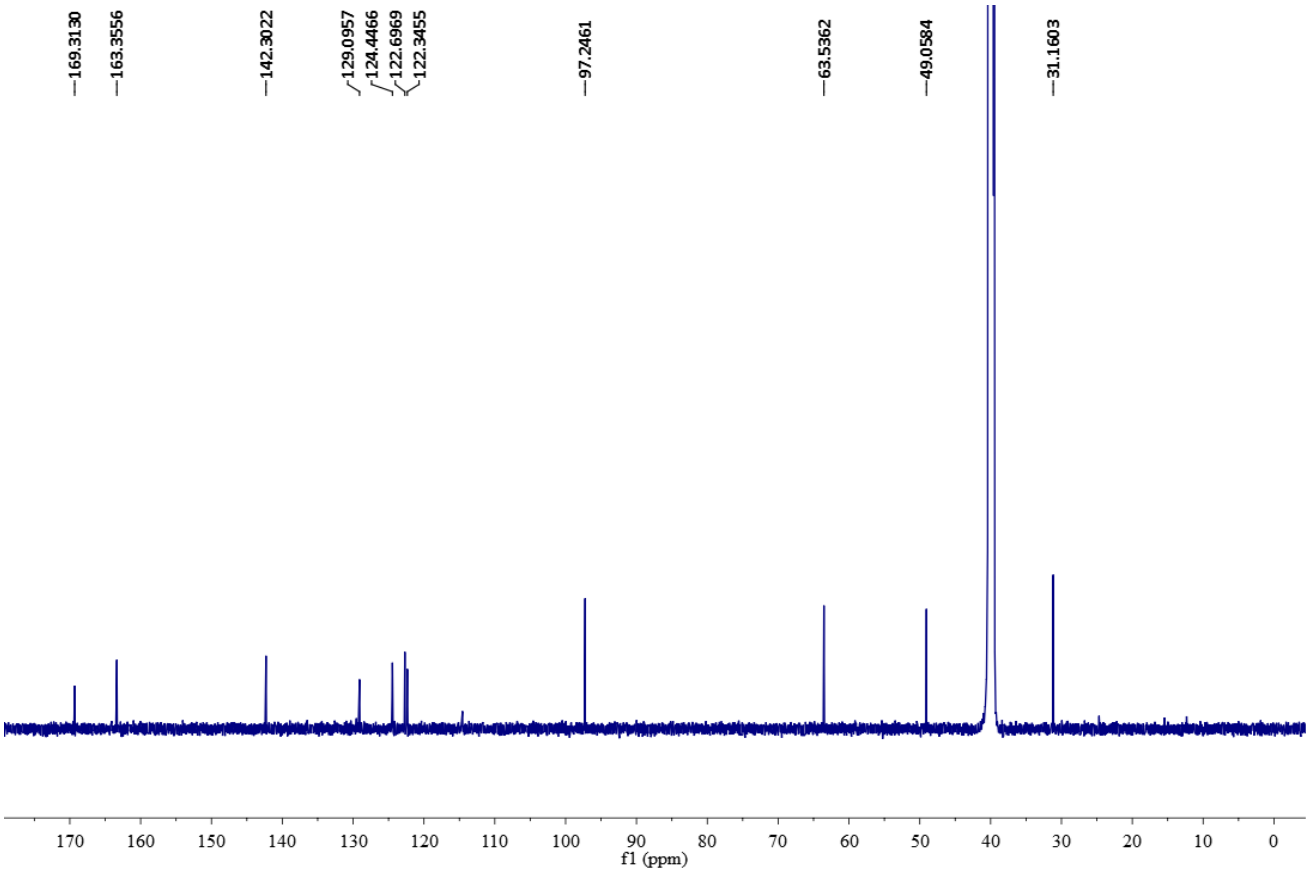


Figure S62. ^{13}C NMR spectrum of **8** in DMSO-d_6 at 150MHz.

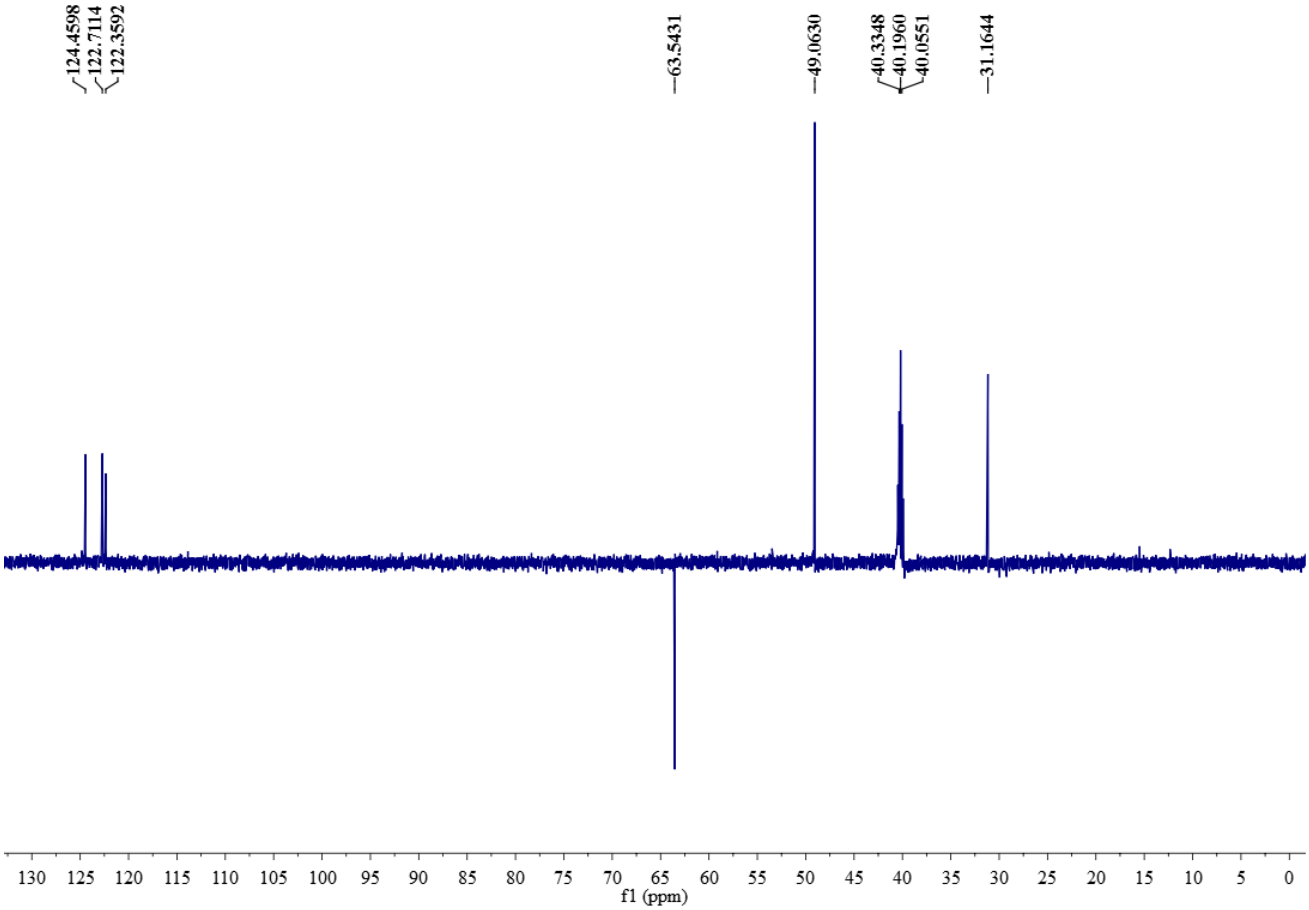


Figure S63. DEPT 135 spectrum of **8** in DMSO-d_6.

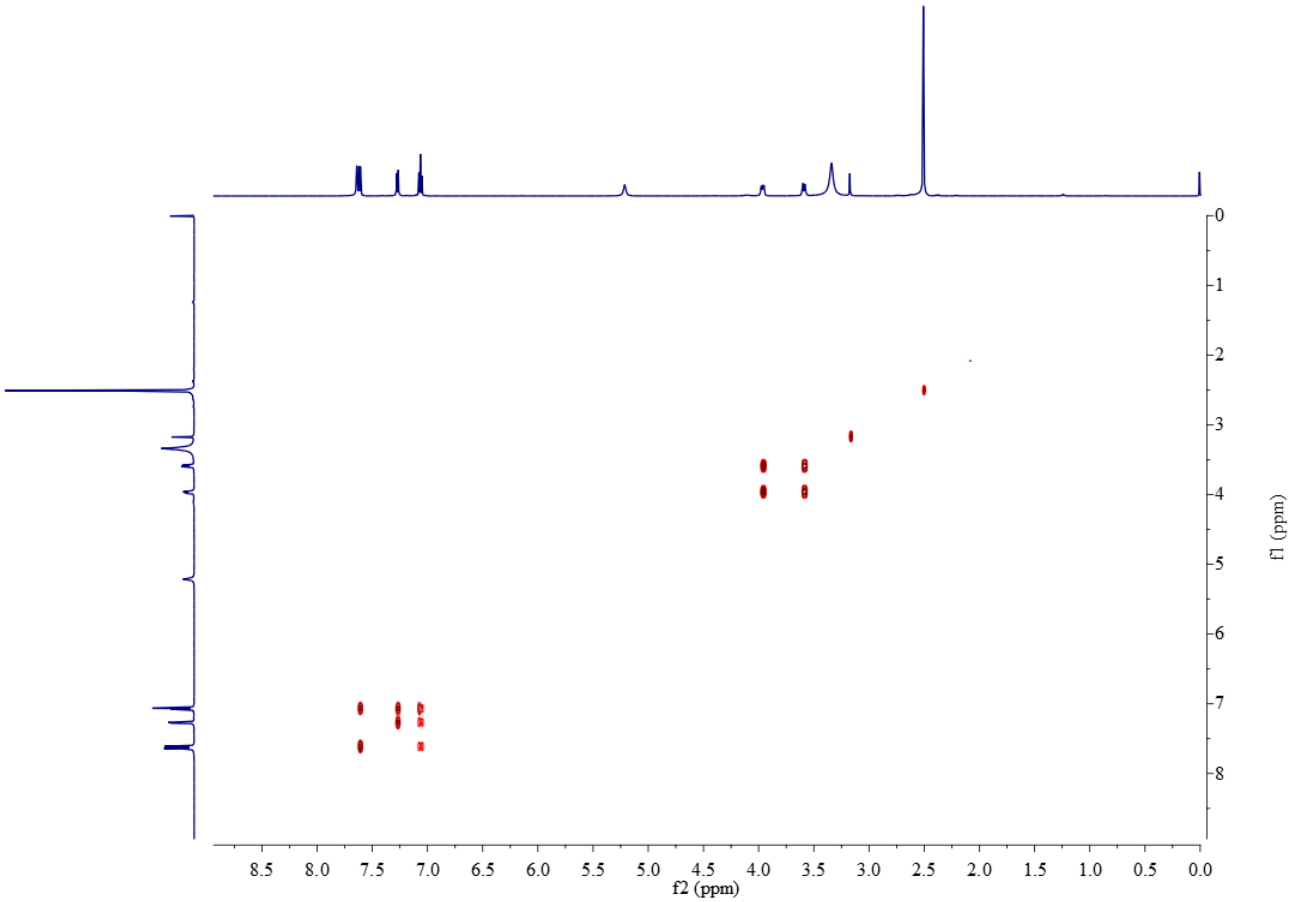


Figure S64. ^{1}H-^{1}H COSY NMR spectrum of **8** in DMSO-d_6.

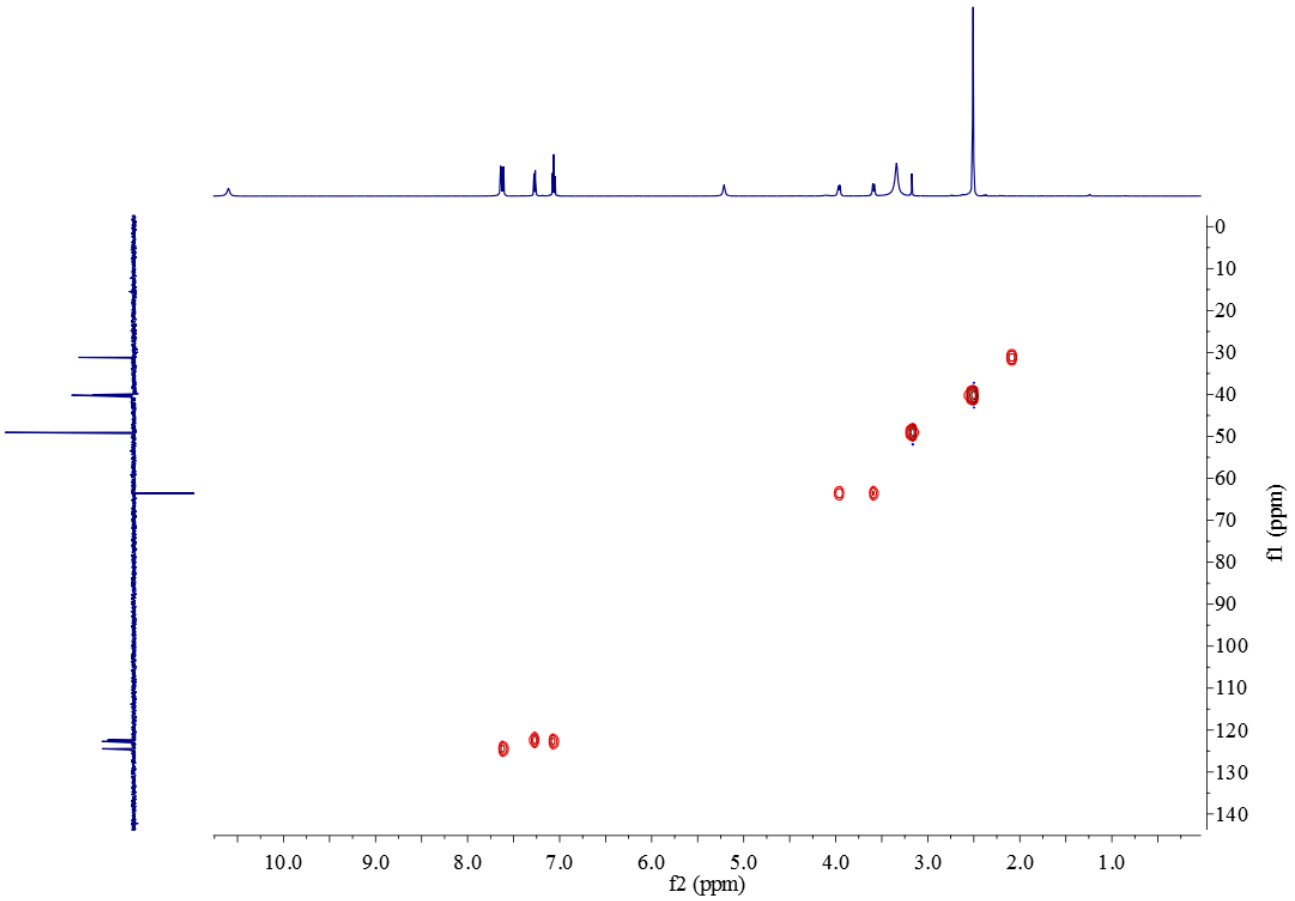


Figure S65. HSQC NMR spectrum of **8** in DMSO-d_6.

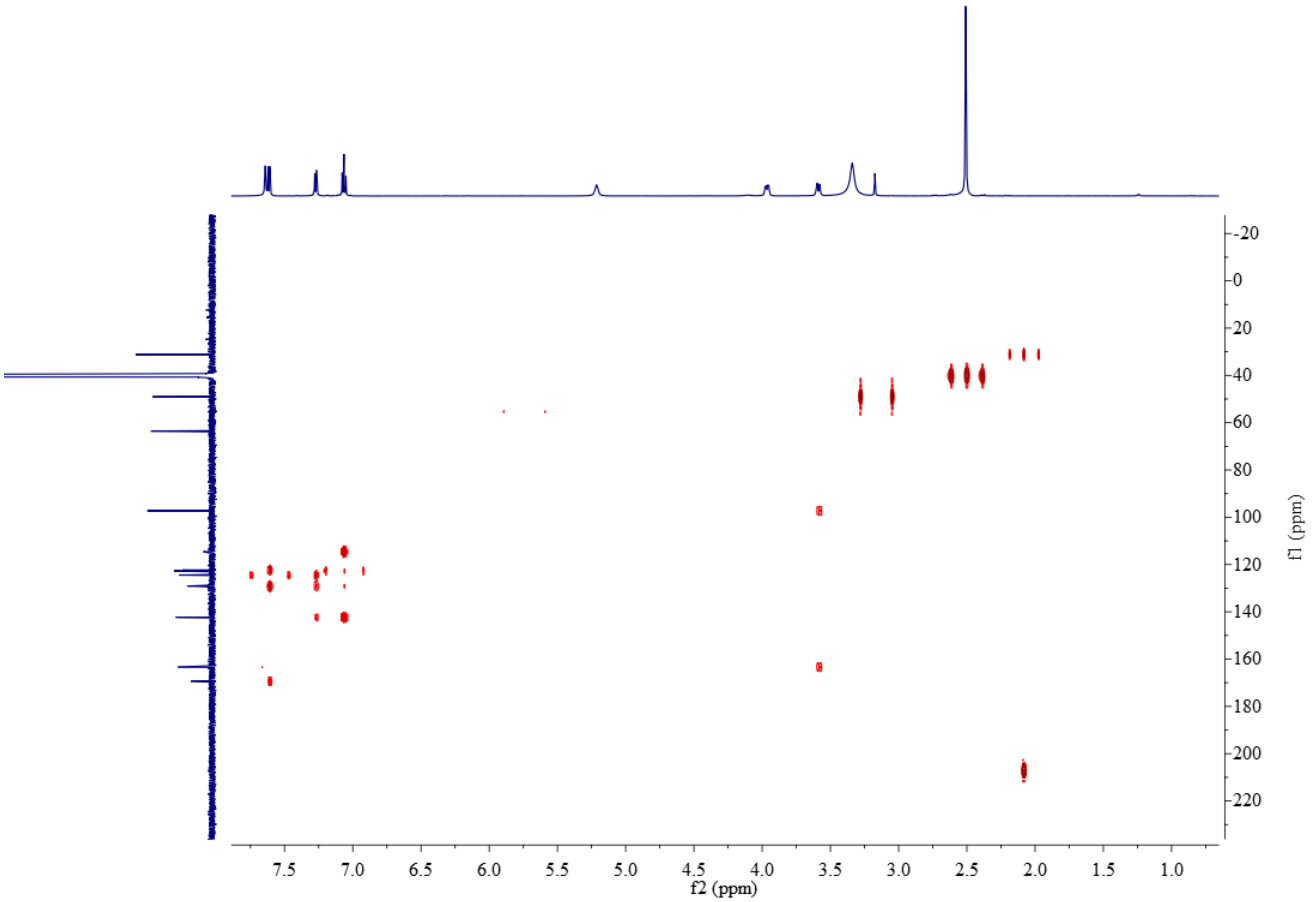


Figure S66. HMBC NMR spectrum of **8** in DMSO-d_6.

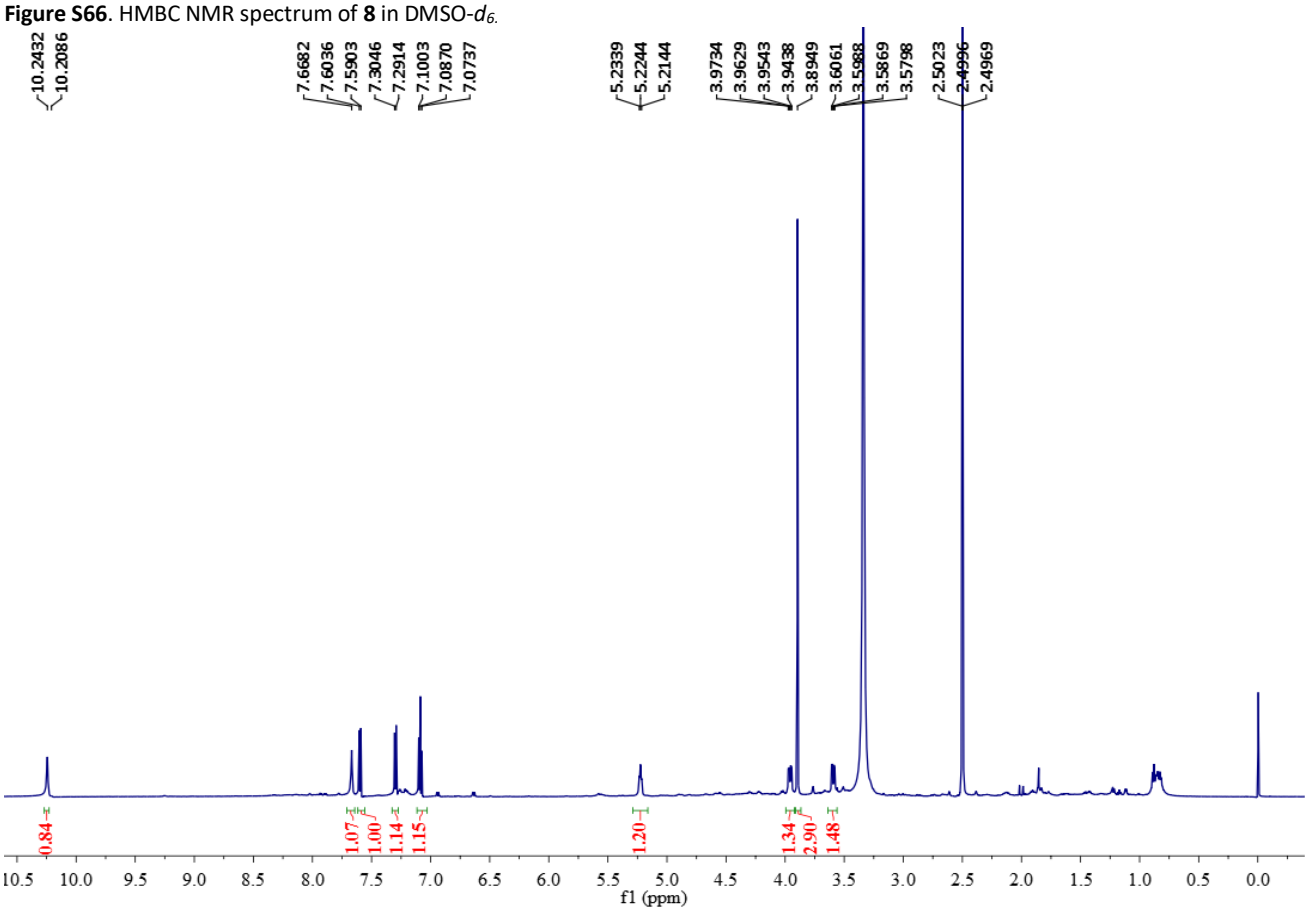


Figure S67. ^{1}H NMR spectrum of **9** in DMSO-d_6 at 600 MHz.

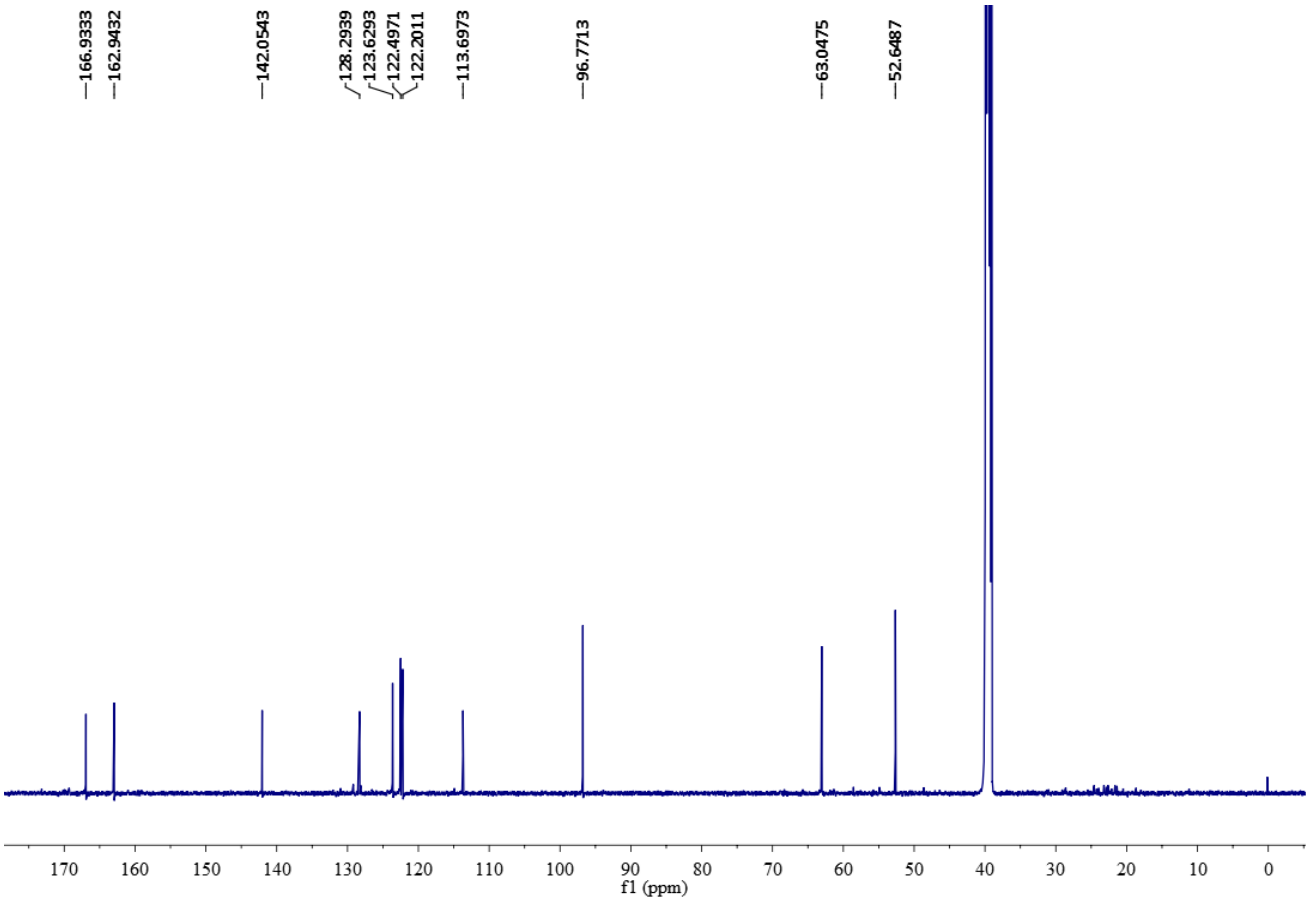


Figure S68. ^{13}C NMR spectrum of **9** in DMSO-d_6 at 150MHz.

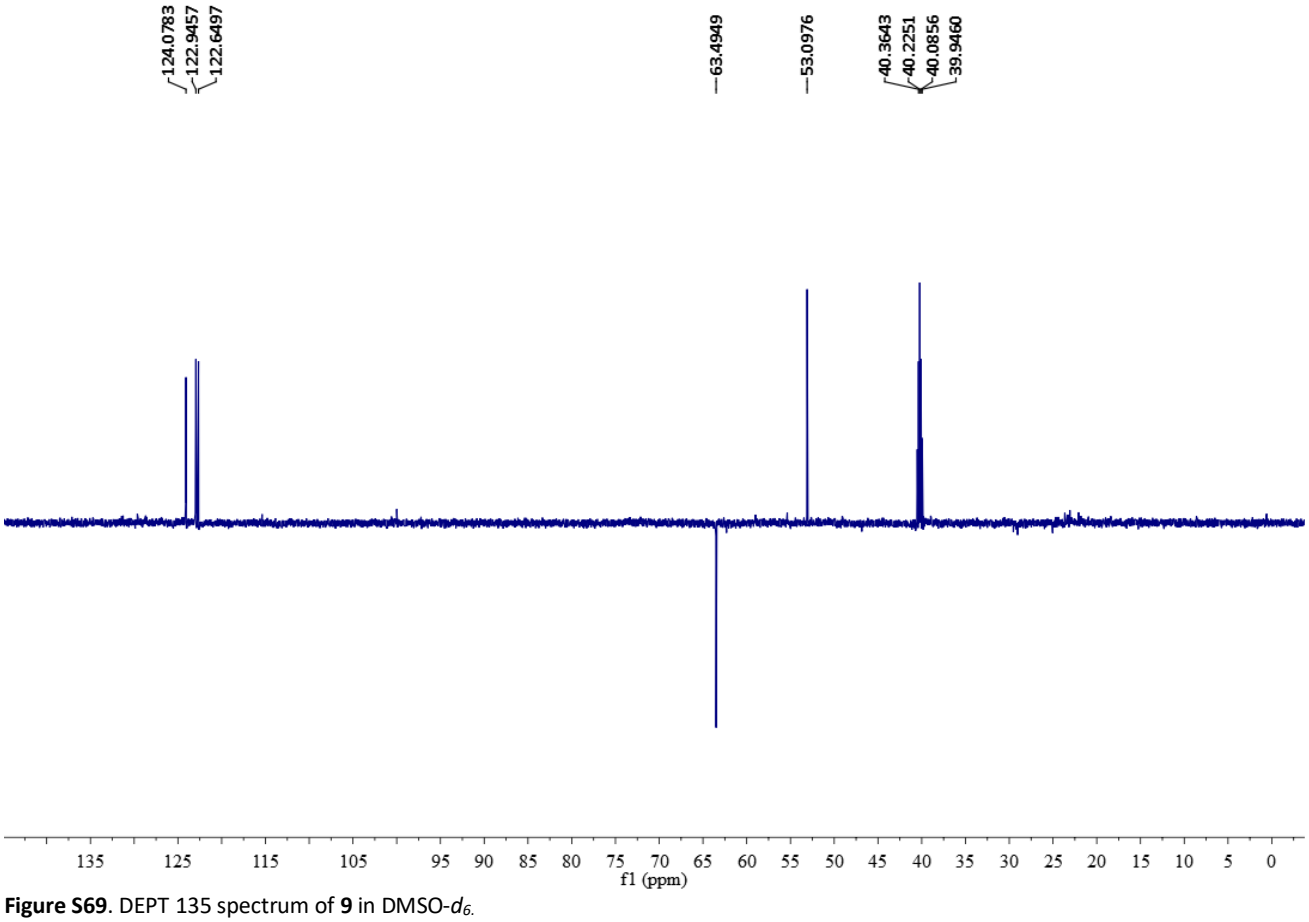


Figure S69. DEPT 135 spectrum of **9** in DMSO-d_6.

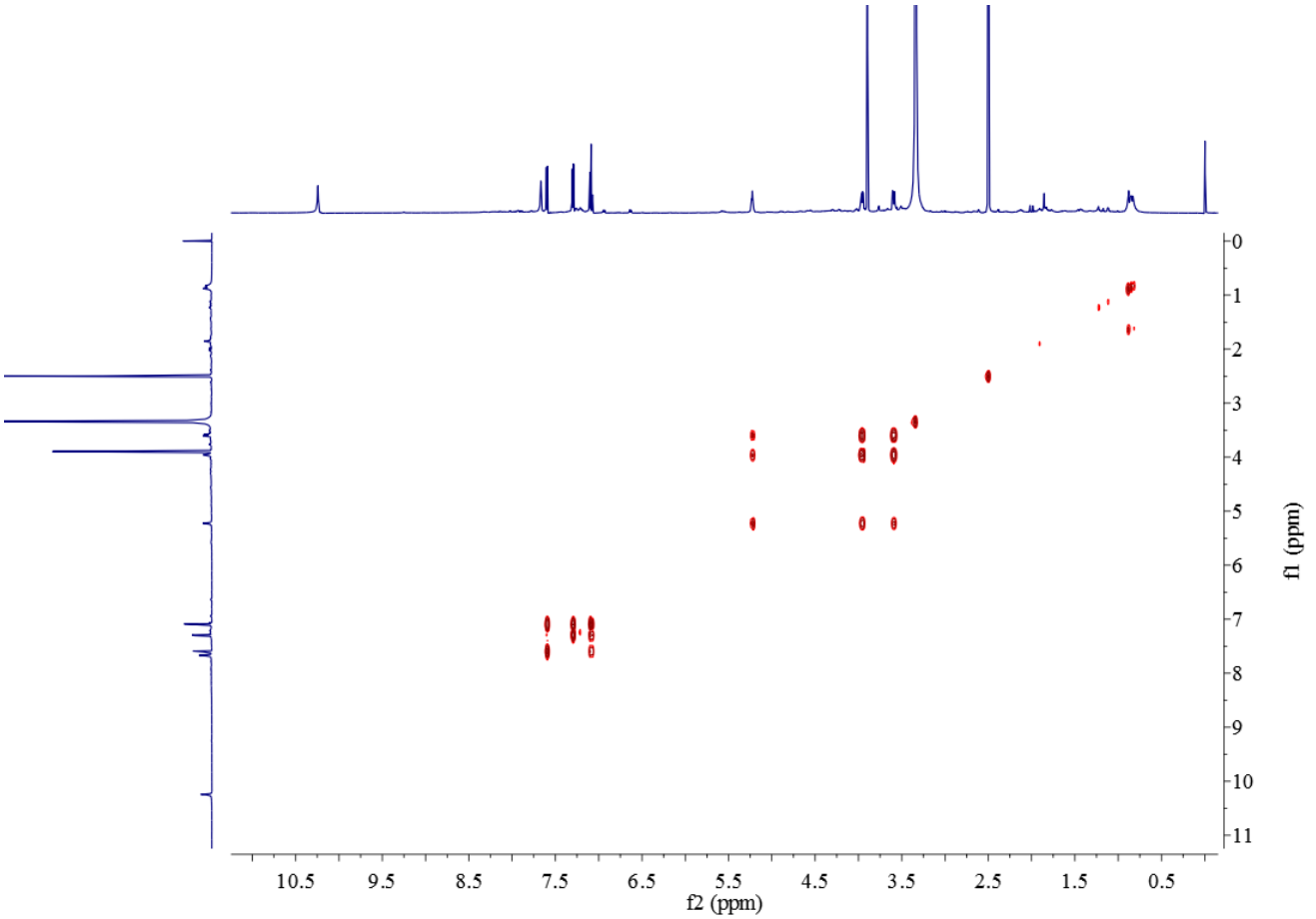


Figure S70. ^{1}H-^{1}H COSY NMR spectrum of **9** in DMSO-d_6.

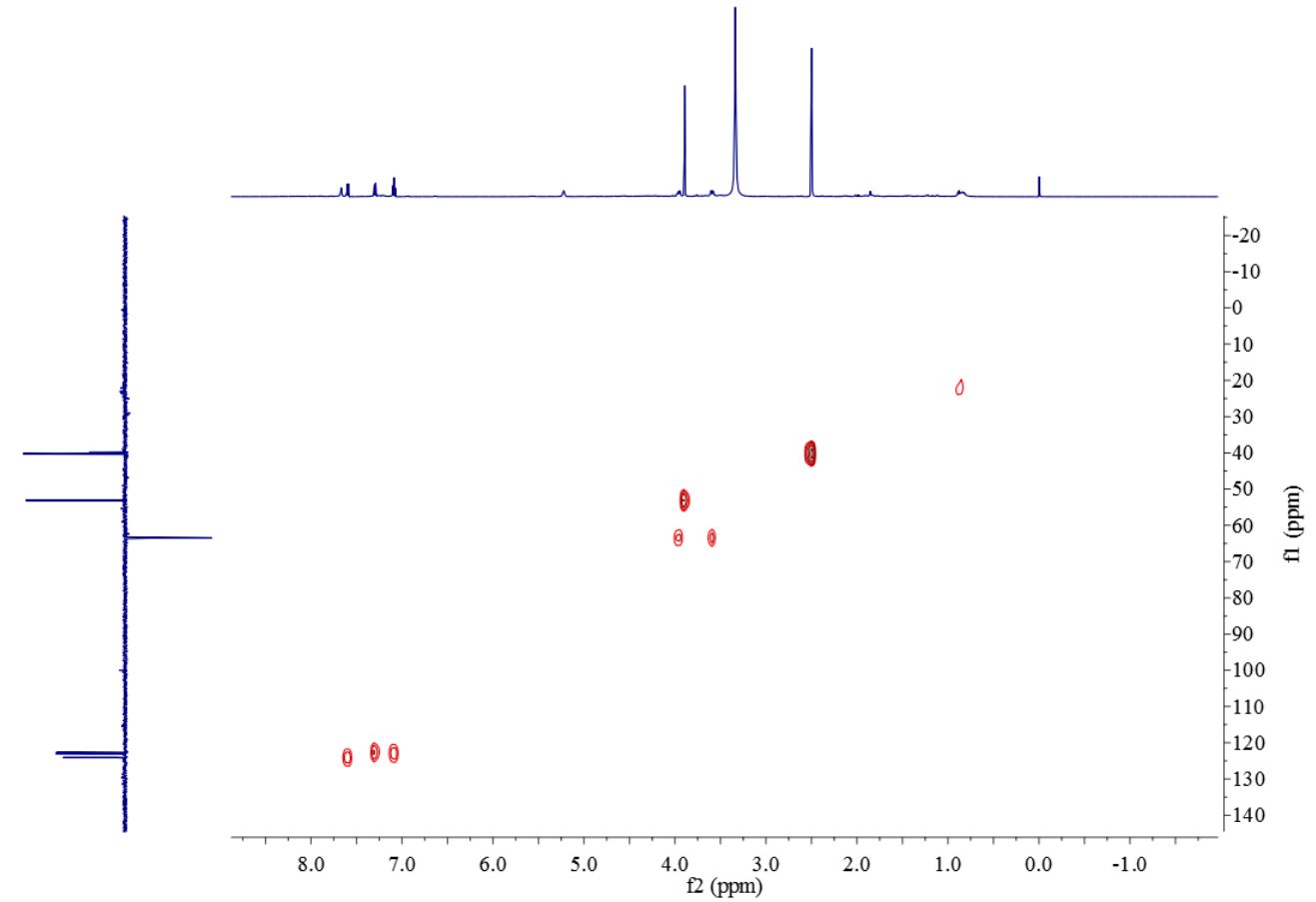


Figure S71. HSQC NMR spectrum of **9** in DMSO-d_6.

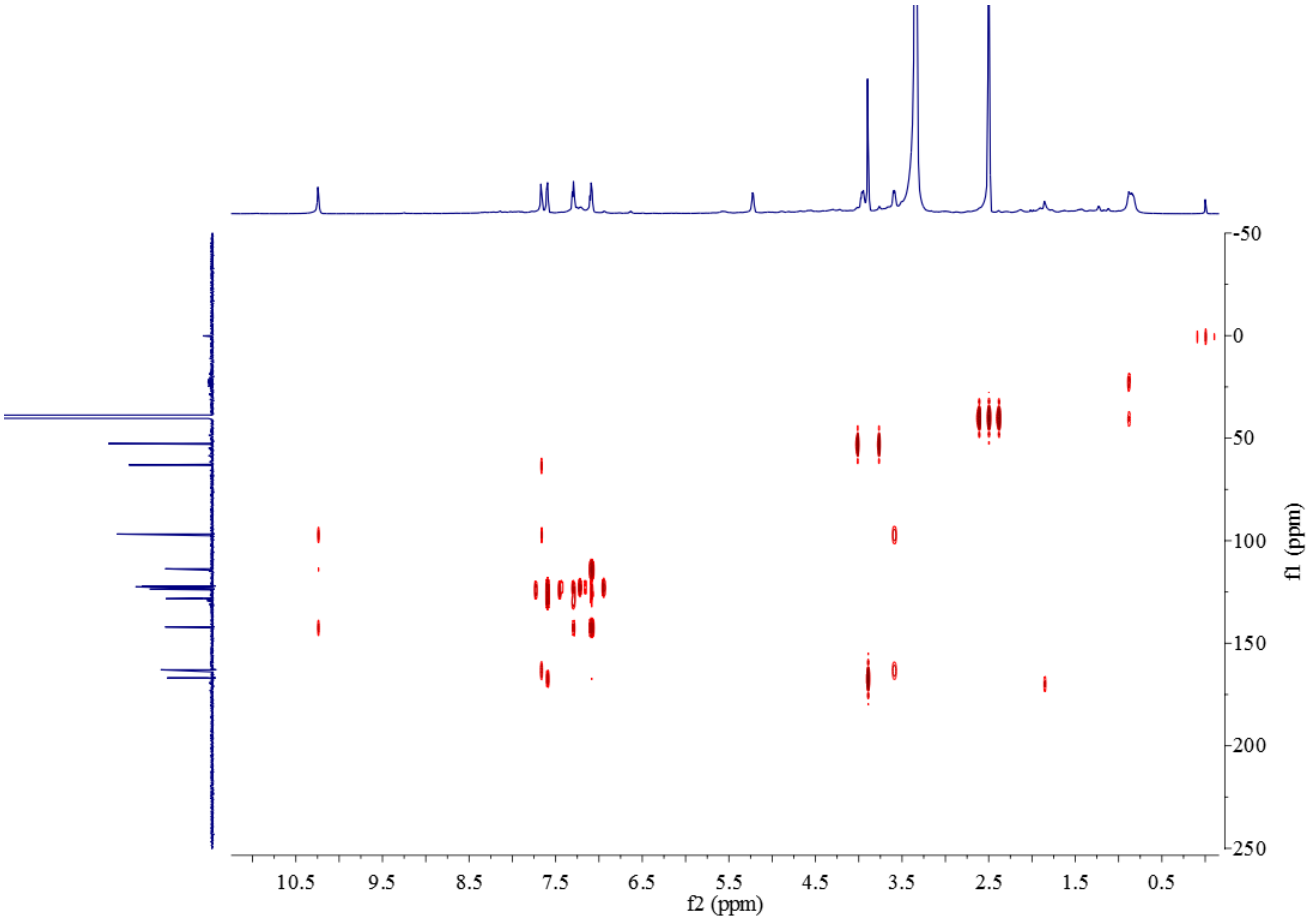


Figure S72. HMBC NMR spectrum of **9** in DMSO-d_6.

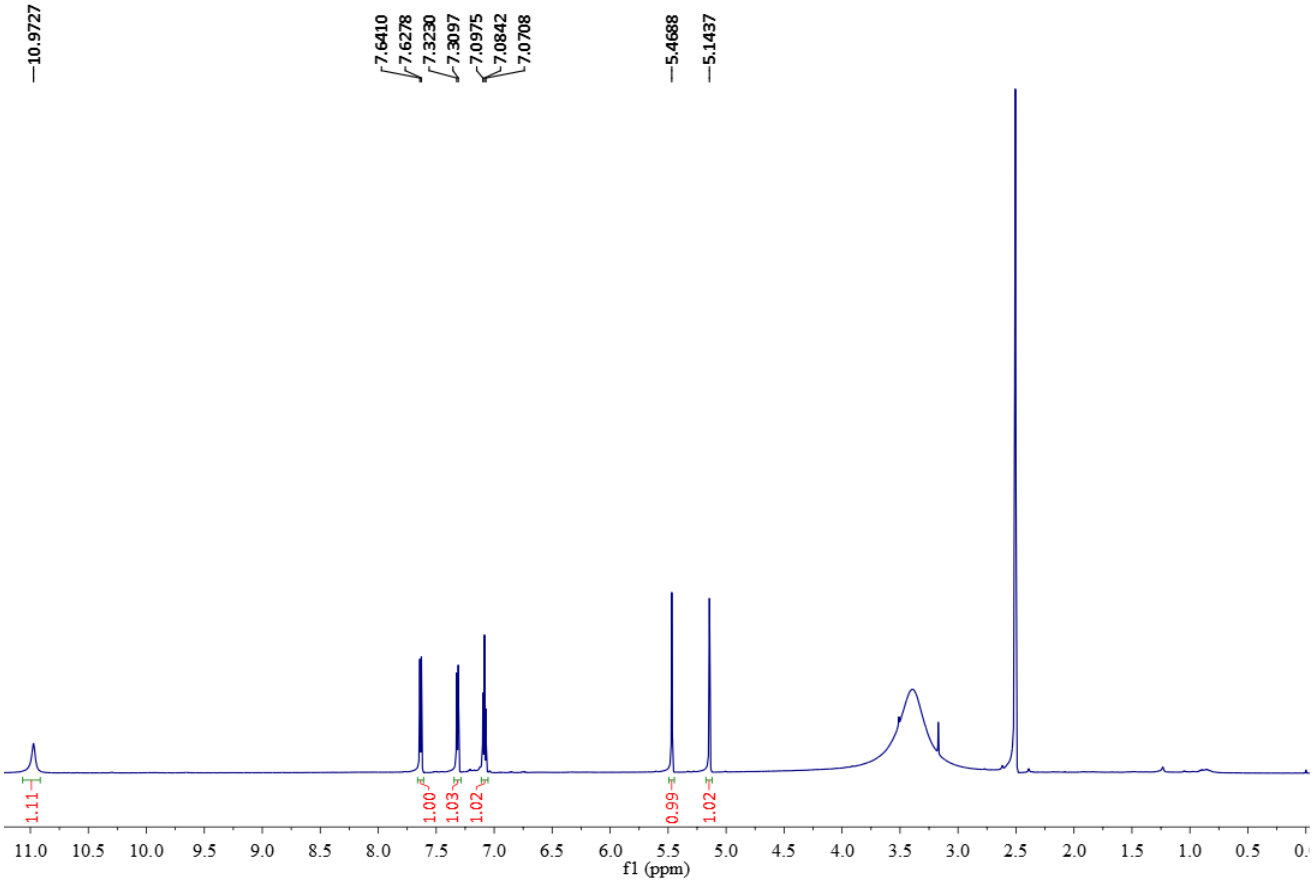


Figure S73.[1]H NMR spectrum of **10** in DMSO-d_6 at 600 MHz.

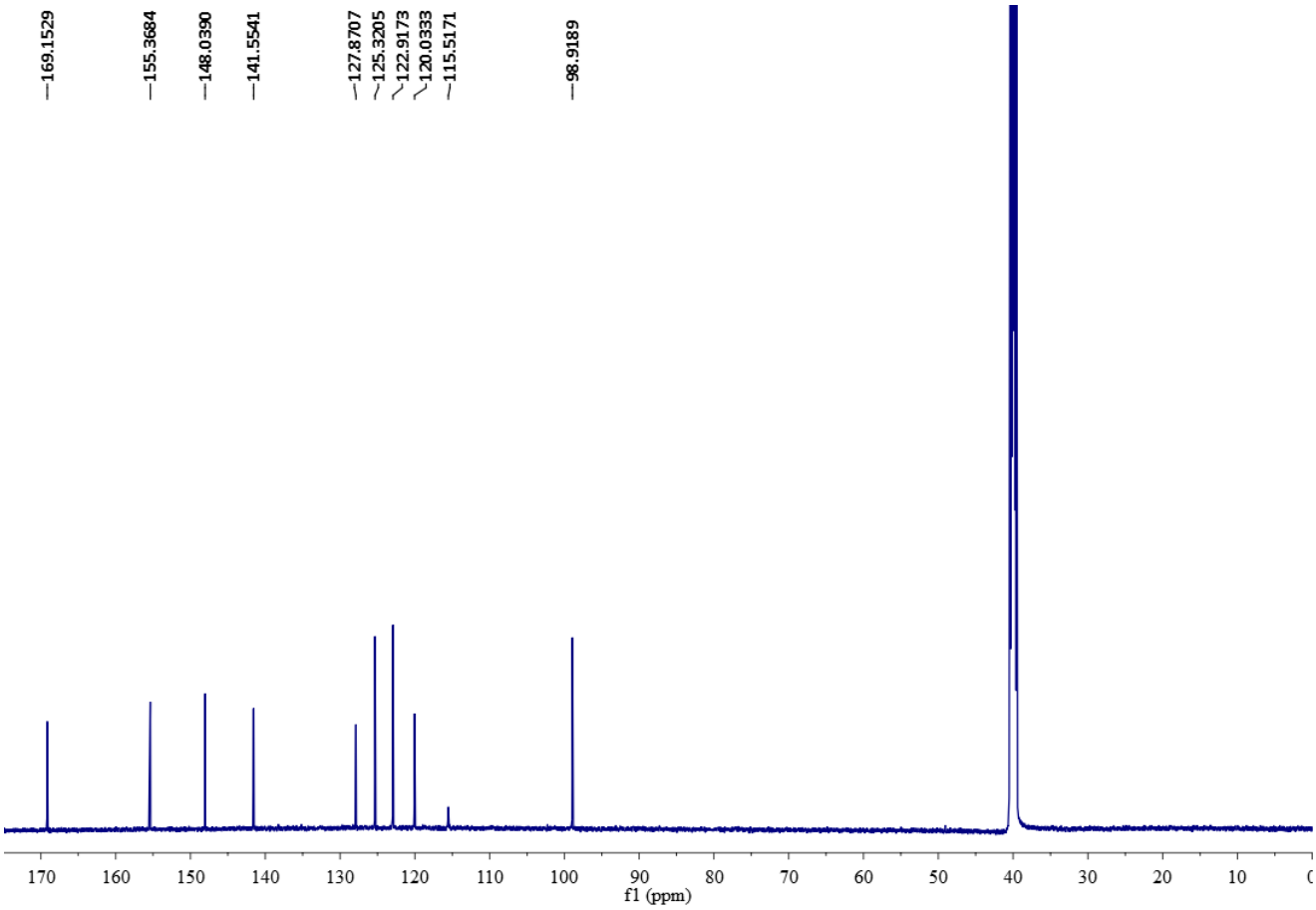


Figure S74.[13]C NMR spectrum of **10** in DMSO-d_6 at 150MHz.

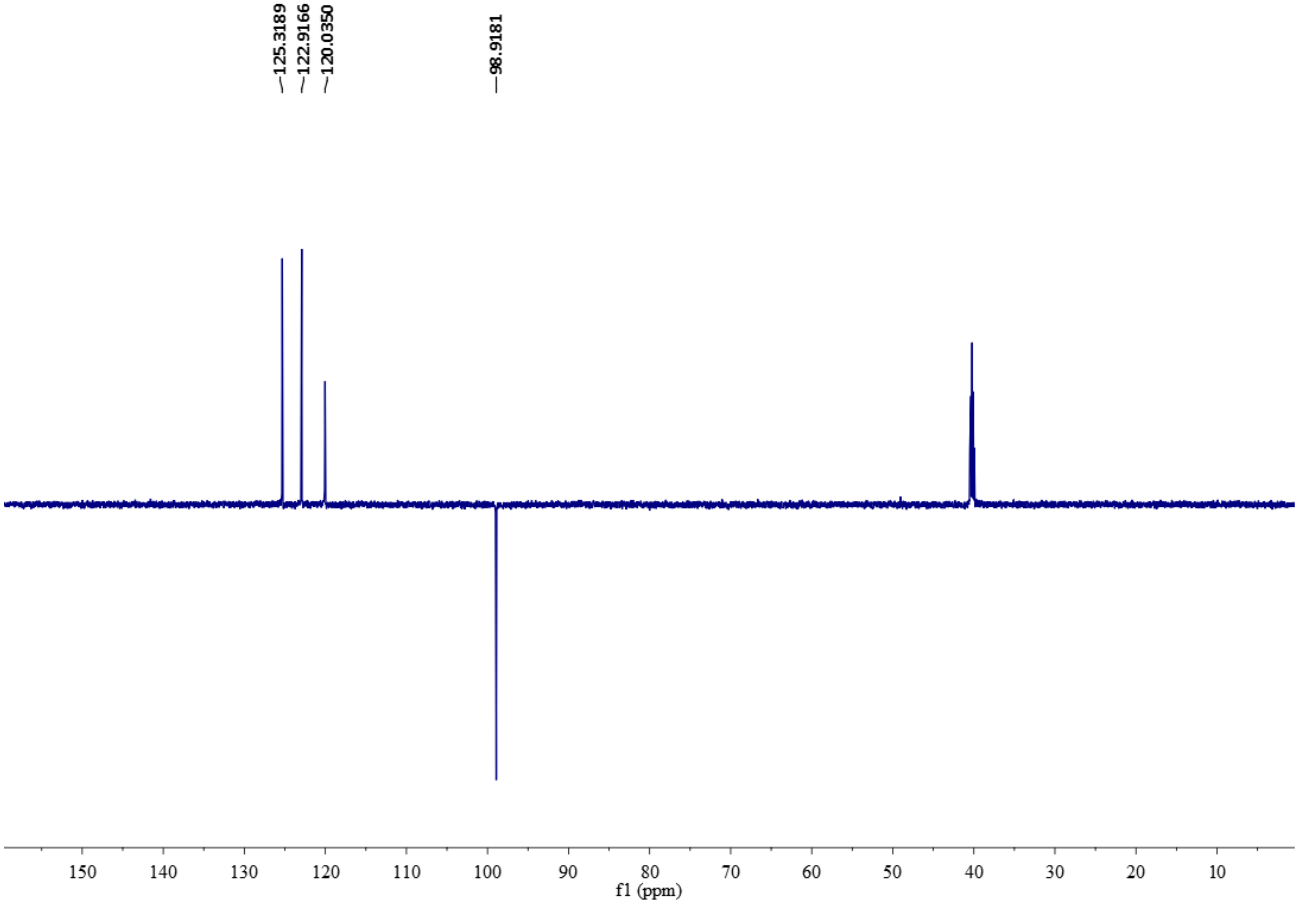


Figure S75. DEPT 135 spectrum of **10** in DMSO-d_6.

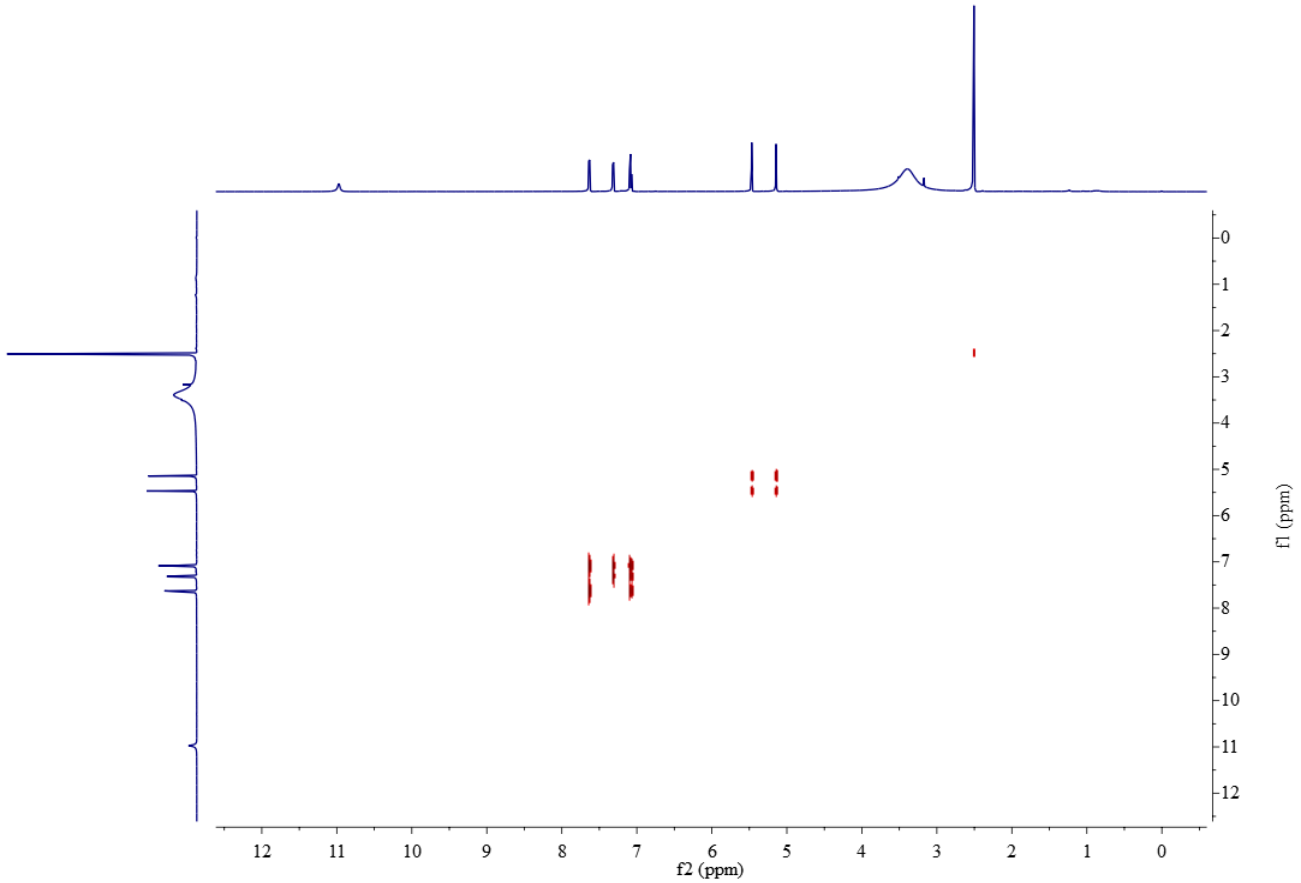


Figure S76. ^{1}H-^{1}H COSY NMR spectrum of **10** in DMSO-d_6.

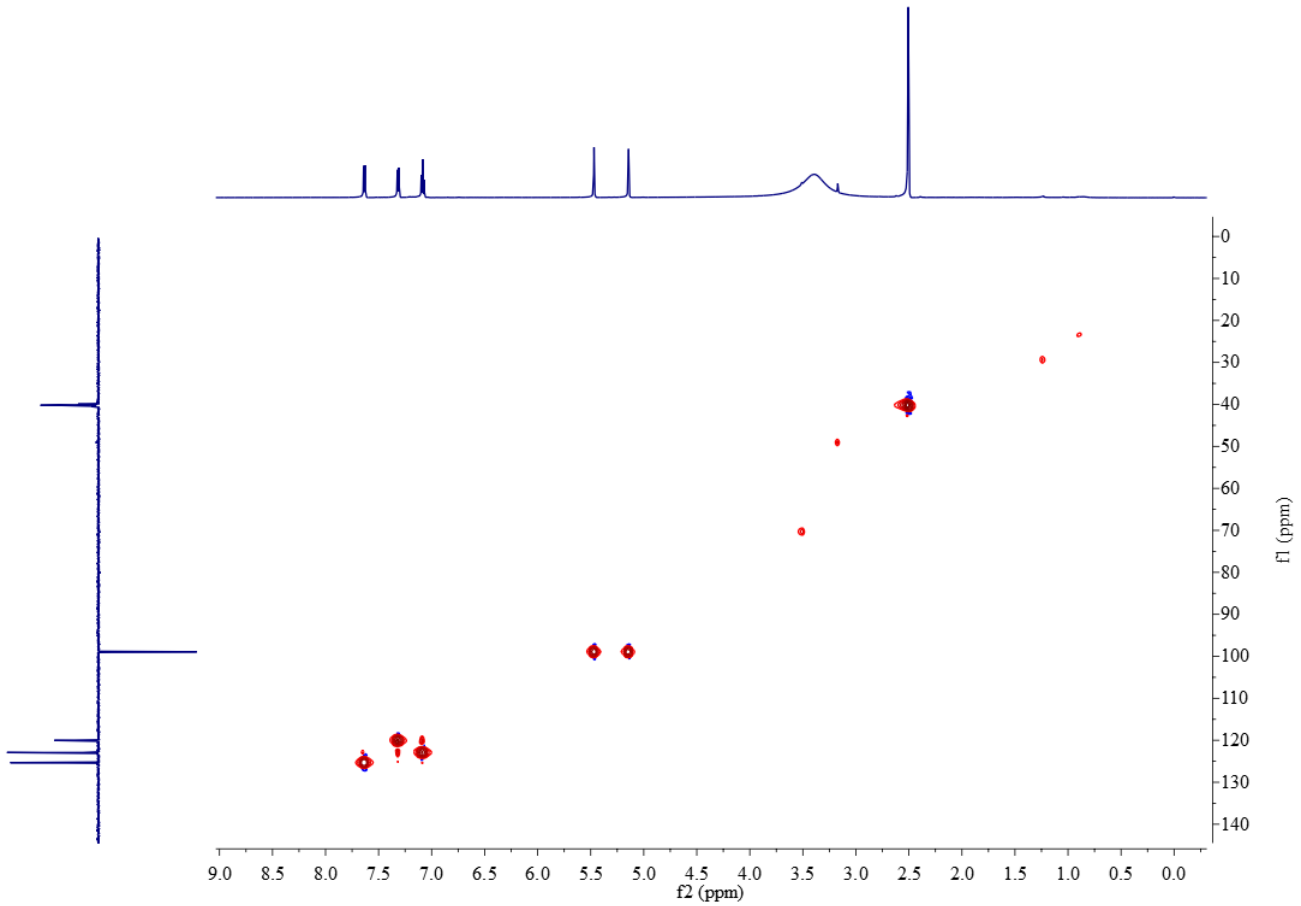


Figure S77. HSQC NMR spectrum of **10** in DMSO-d_6.

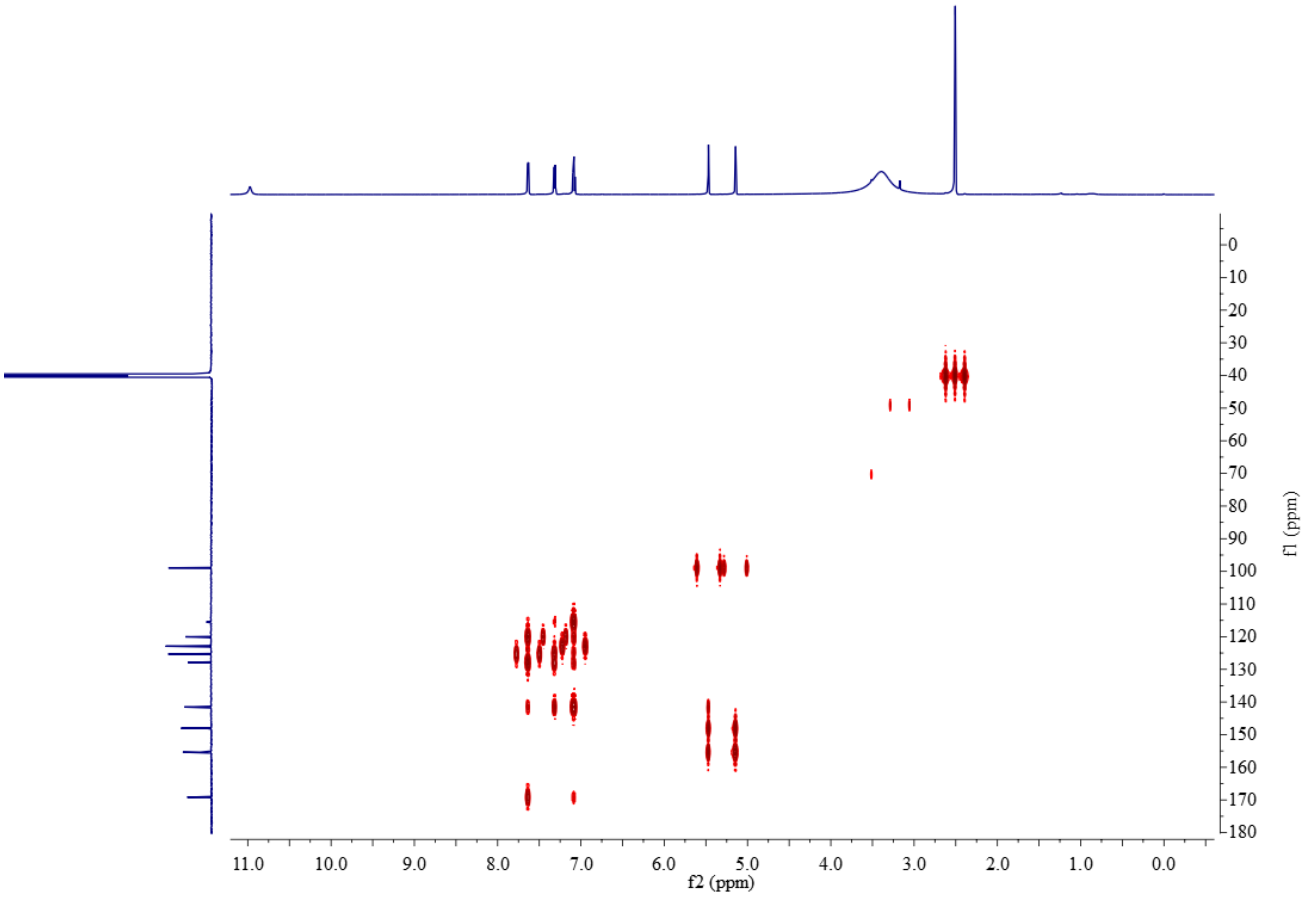


Figure S78. HMBC NMR spectrum of **10** in DMSO-d_6.

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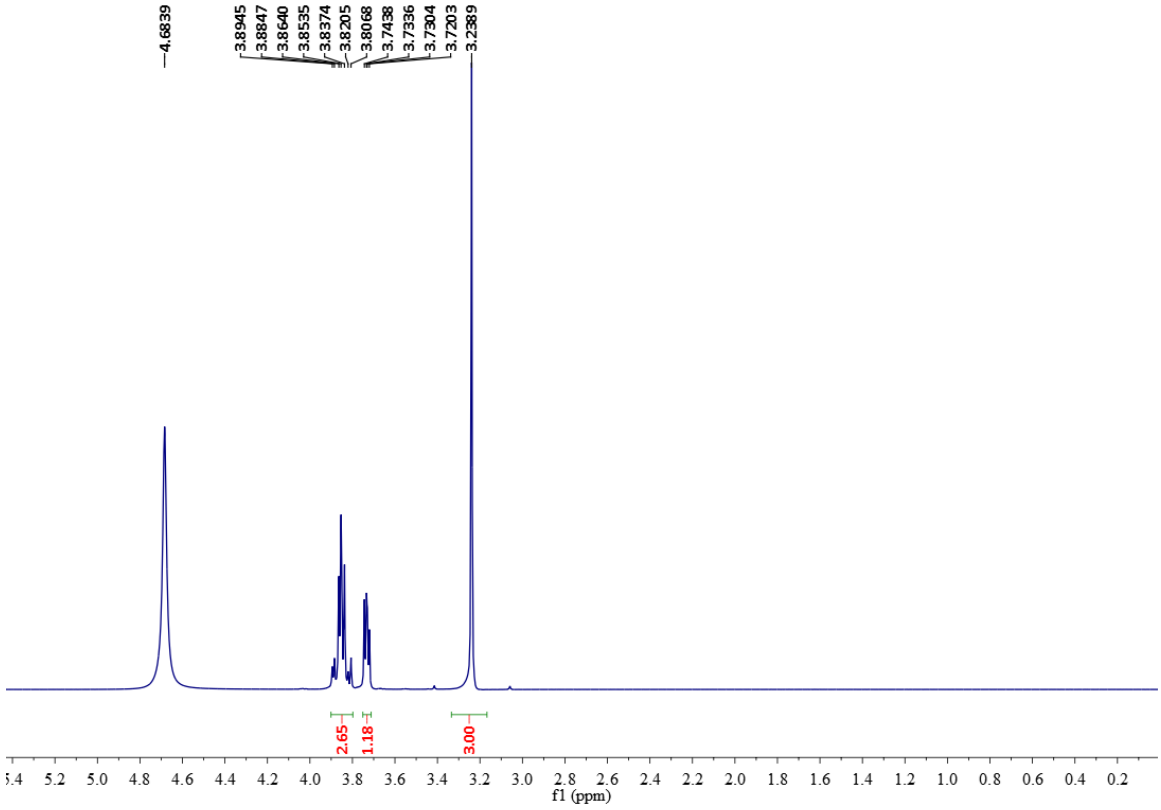

Figure S79. ^{1}H NMR spectrum of **12** in D_2O at 400 MHz.

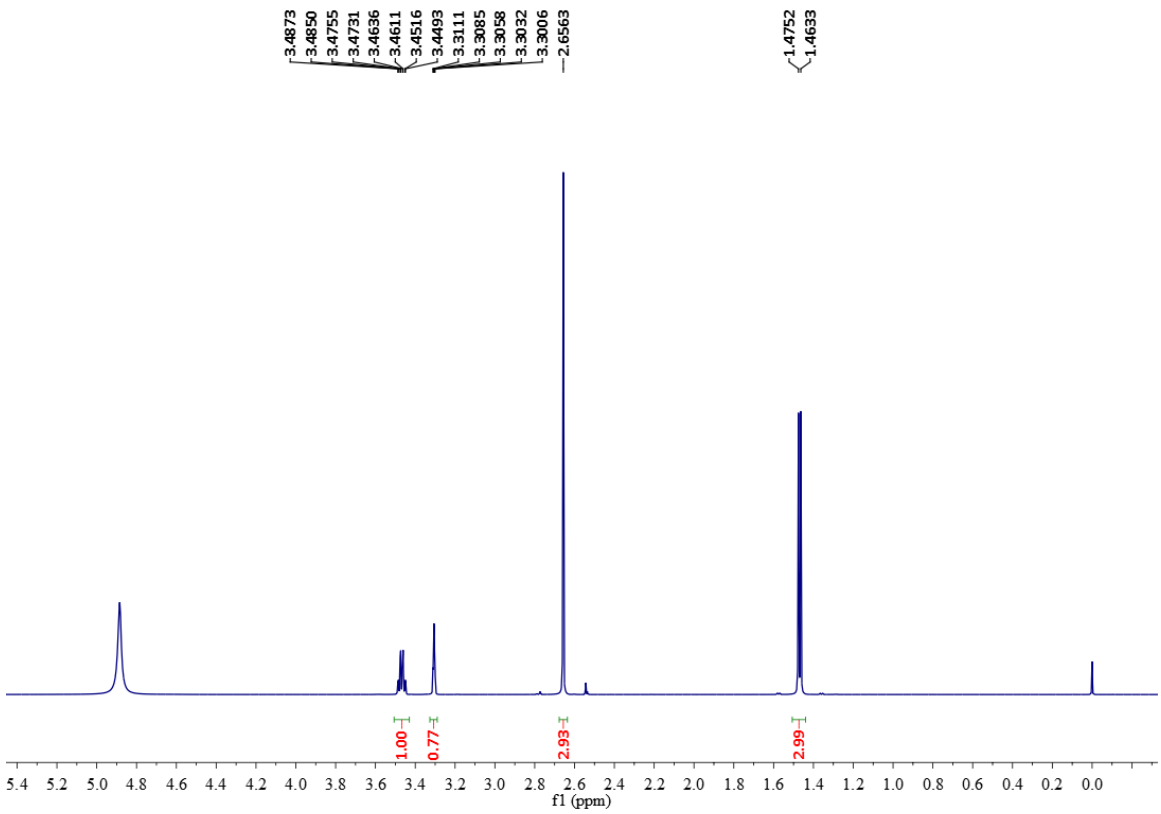

Figure S80. ^{1}H NMR spectrum of **13** in D_2O at 400 MHz.

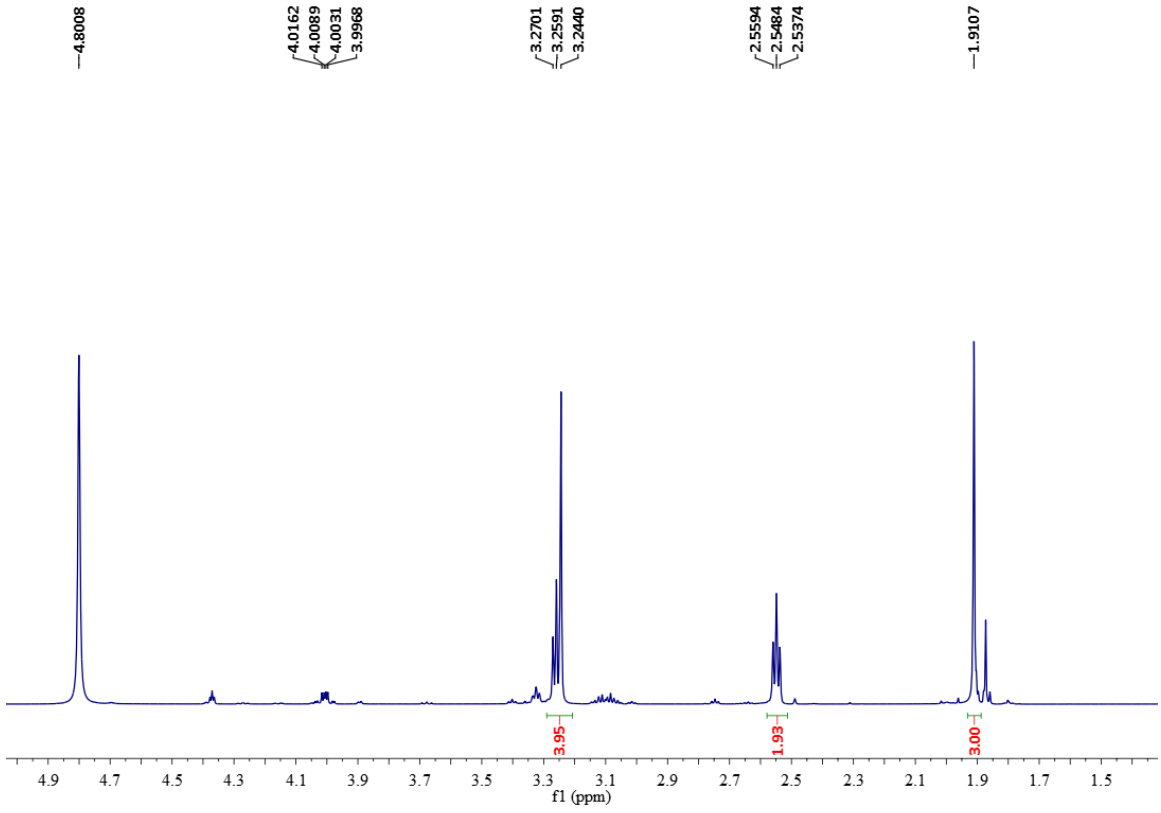

Figure S81. ^{1}H NMR spectrum of **15** in D_2O at 600 MHz.

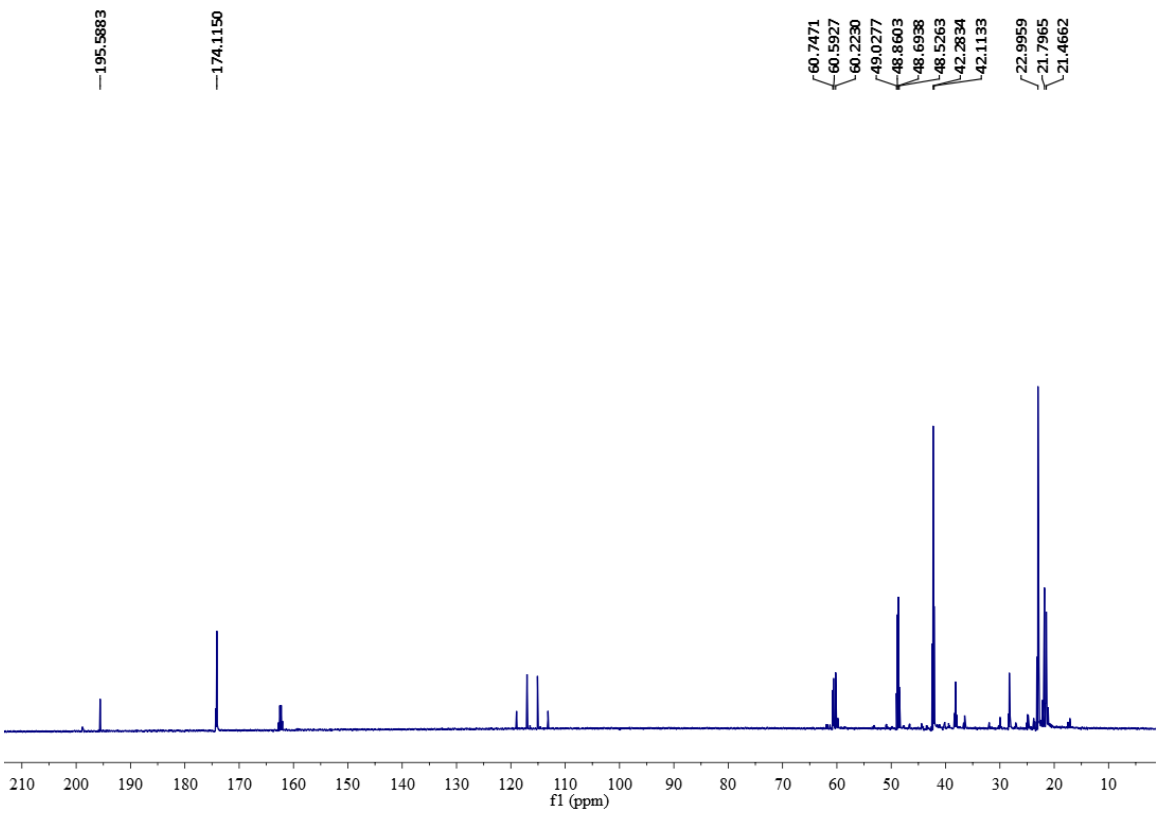

Figure S82. ^{13}C NMR spectrum of **15** in D_2Oat 150MHz.

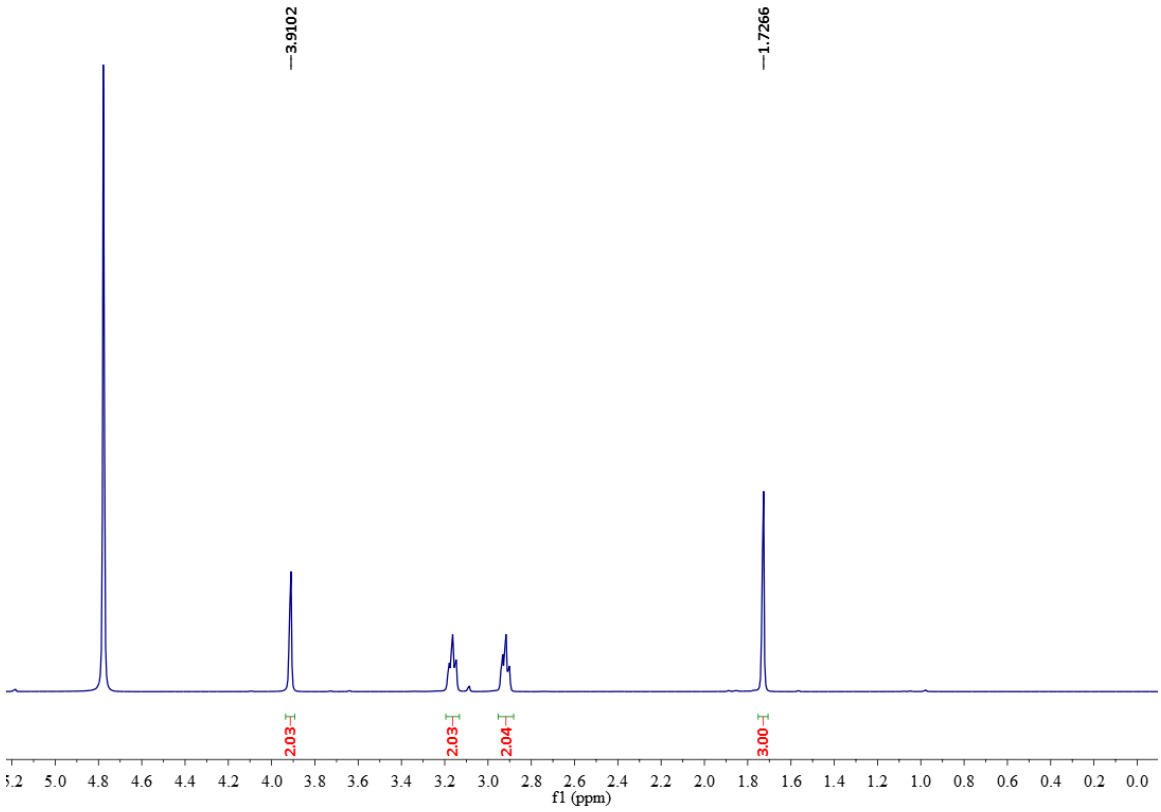

Figure S83. ^{1}H NMR spectrum of **16** in D_2O at 400 MHz.

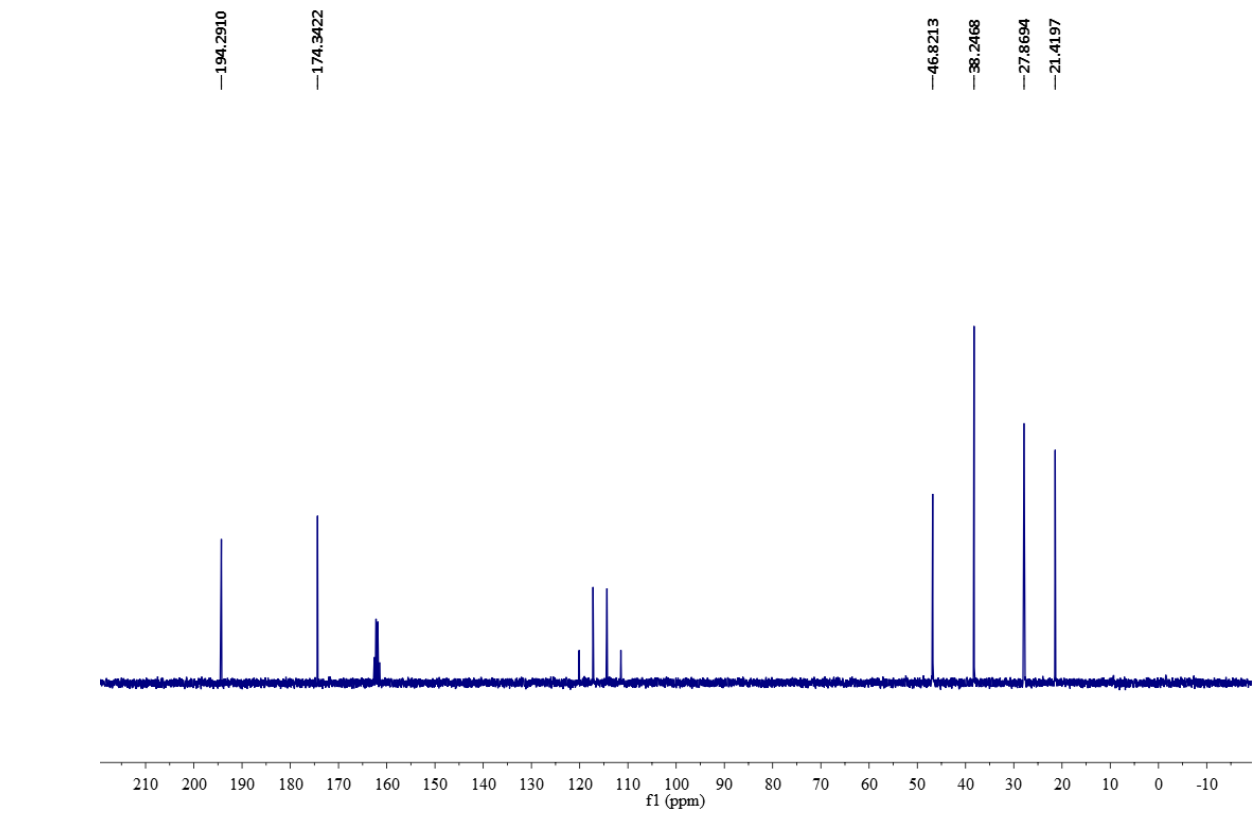

Figure S84. ^{13}C NMR spectrum of **16** in D_2Oat 100MHz.

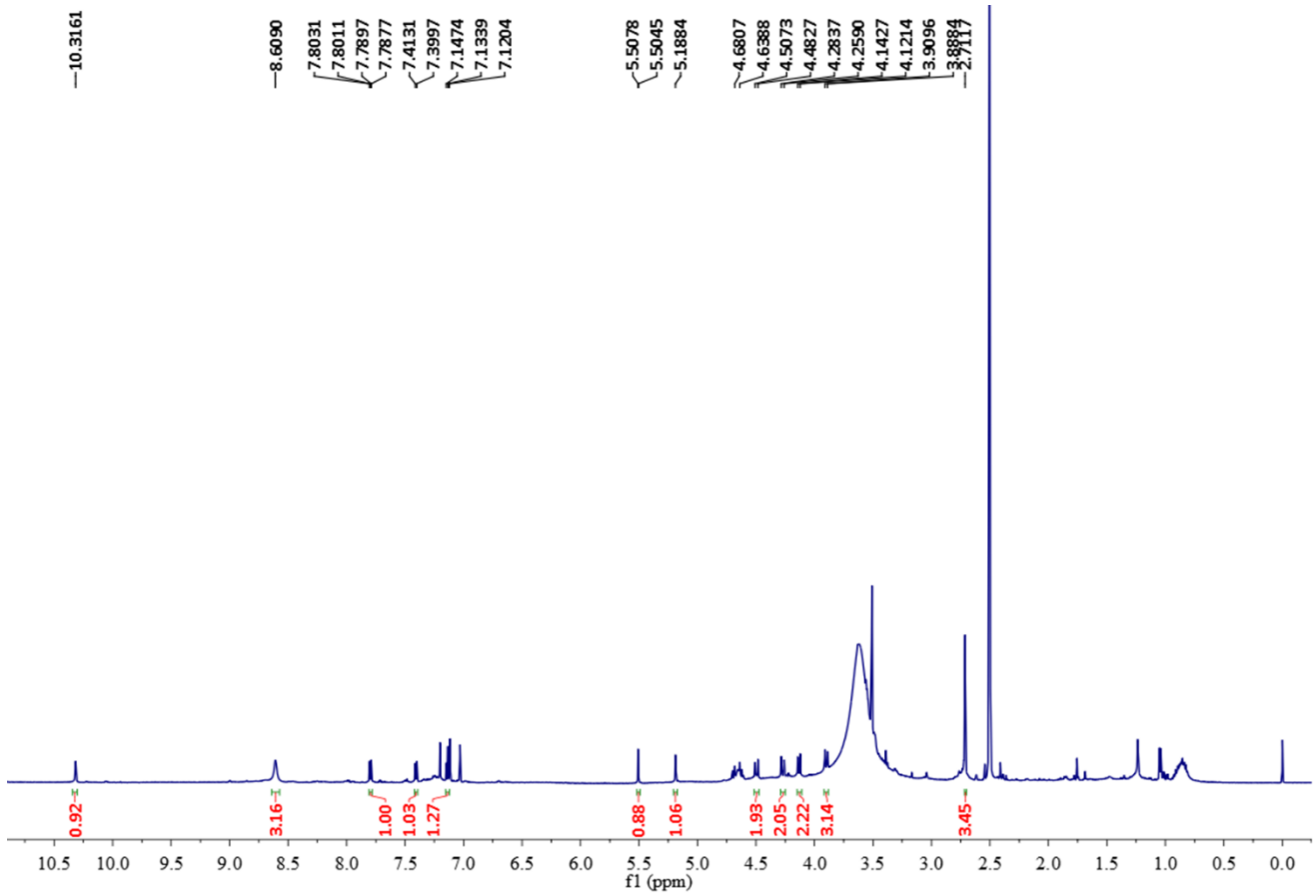

Figure S85. ^{1}H NMR spectrum of **17** in DMSO-d_6 at 600 MHz.

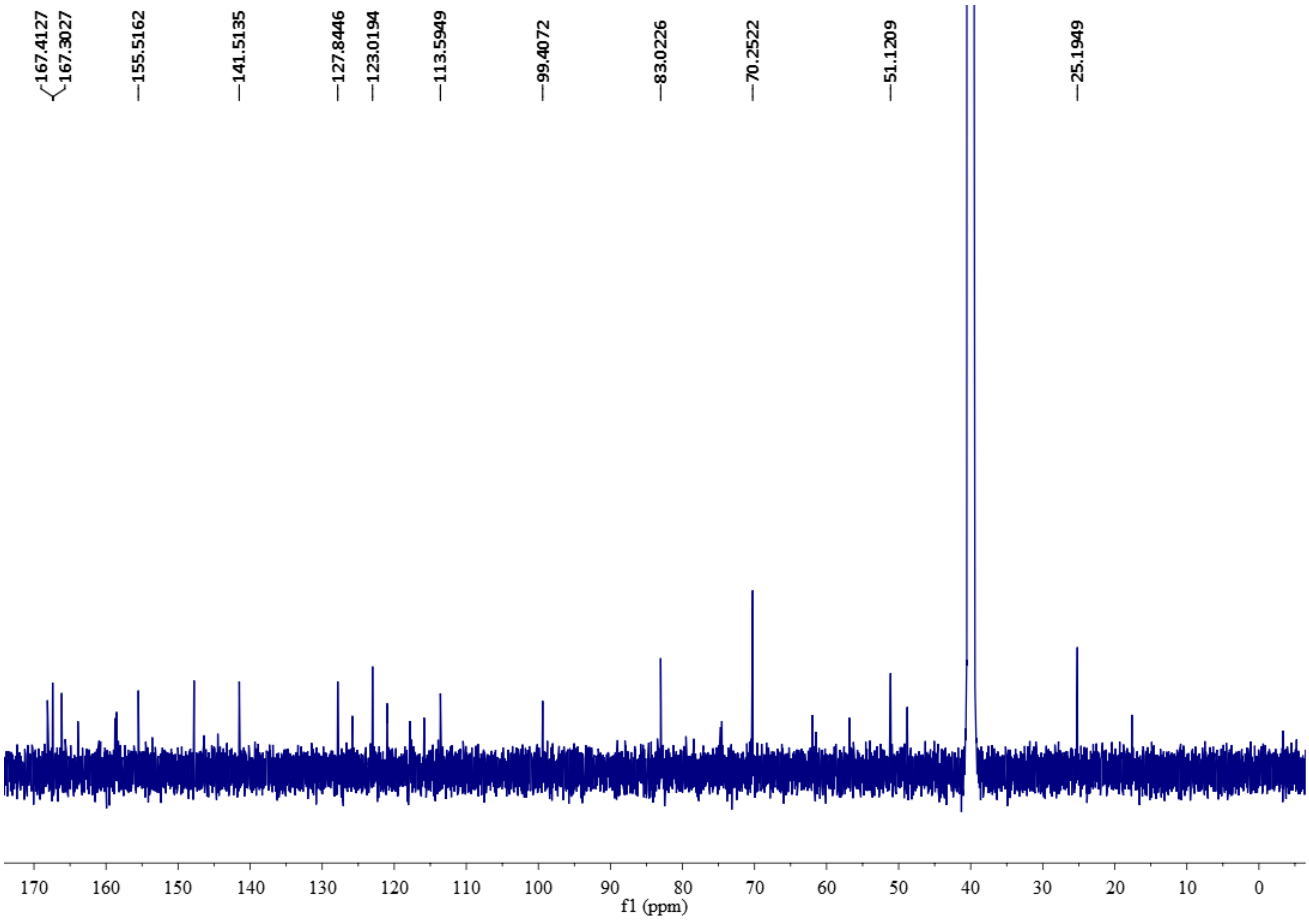

Figure S86. ^{13}C NMR spectrum of **17** in DMSO-d_6 at 150MHz.

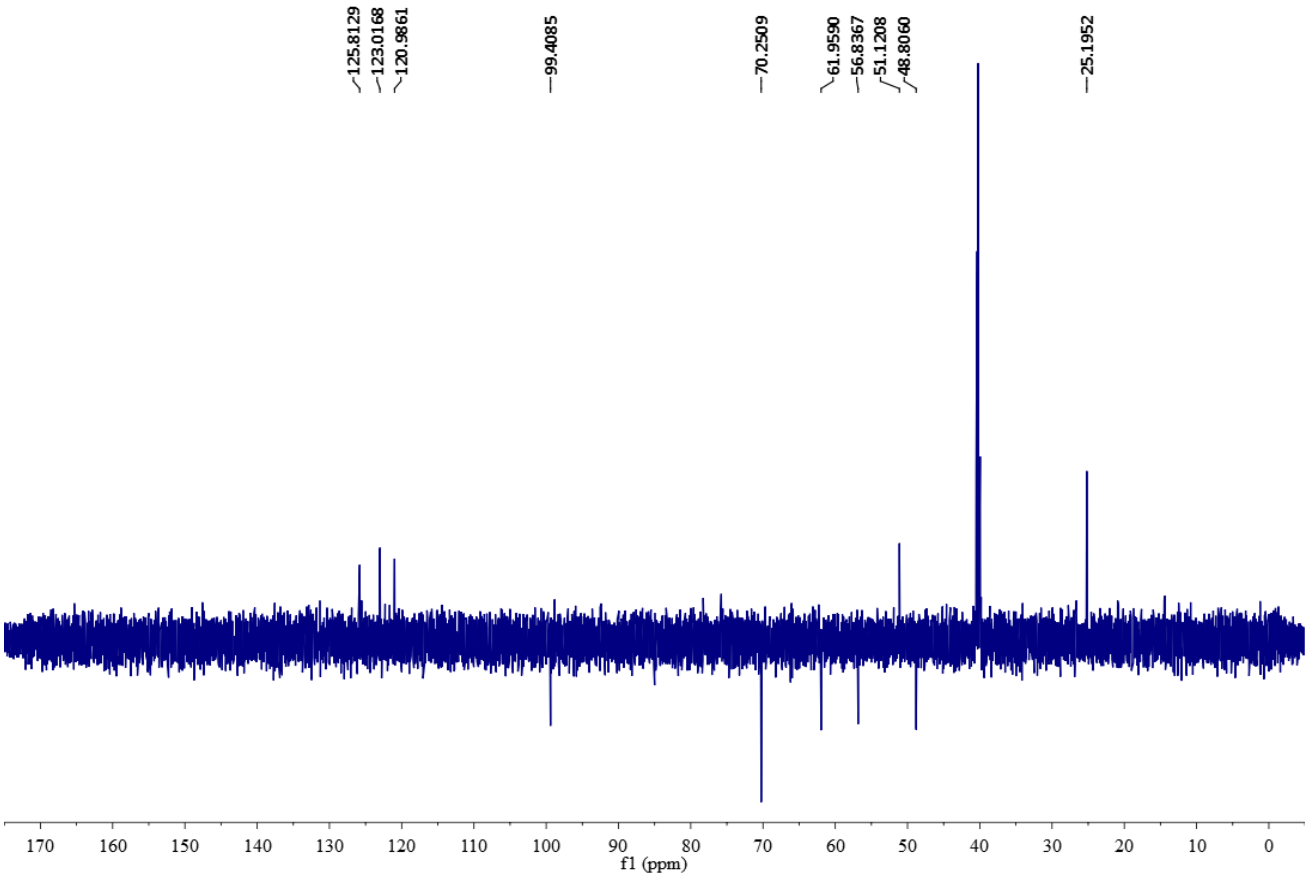

Figure S87. DEPT 135 spectrum of **17** in DMSO-d_6.

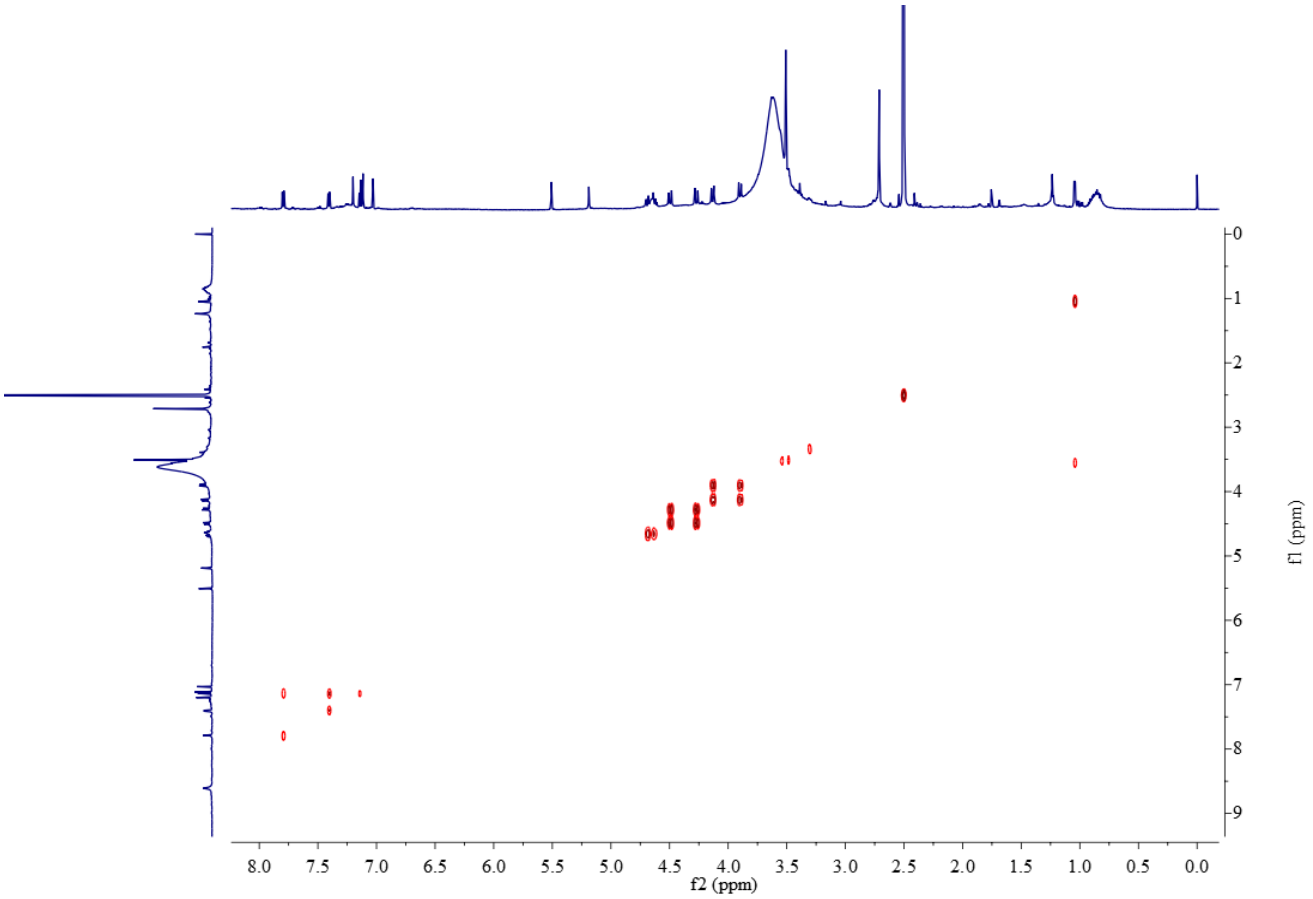

Figure S88. ^{1}H-^{1}H COSY NMR spectrum of **17** in DMSO-d_6.

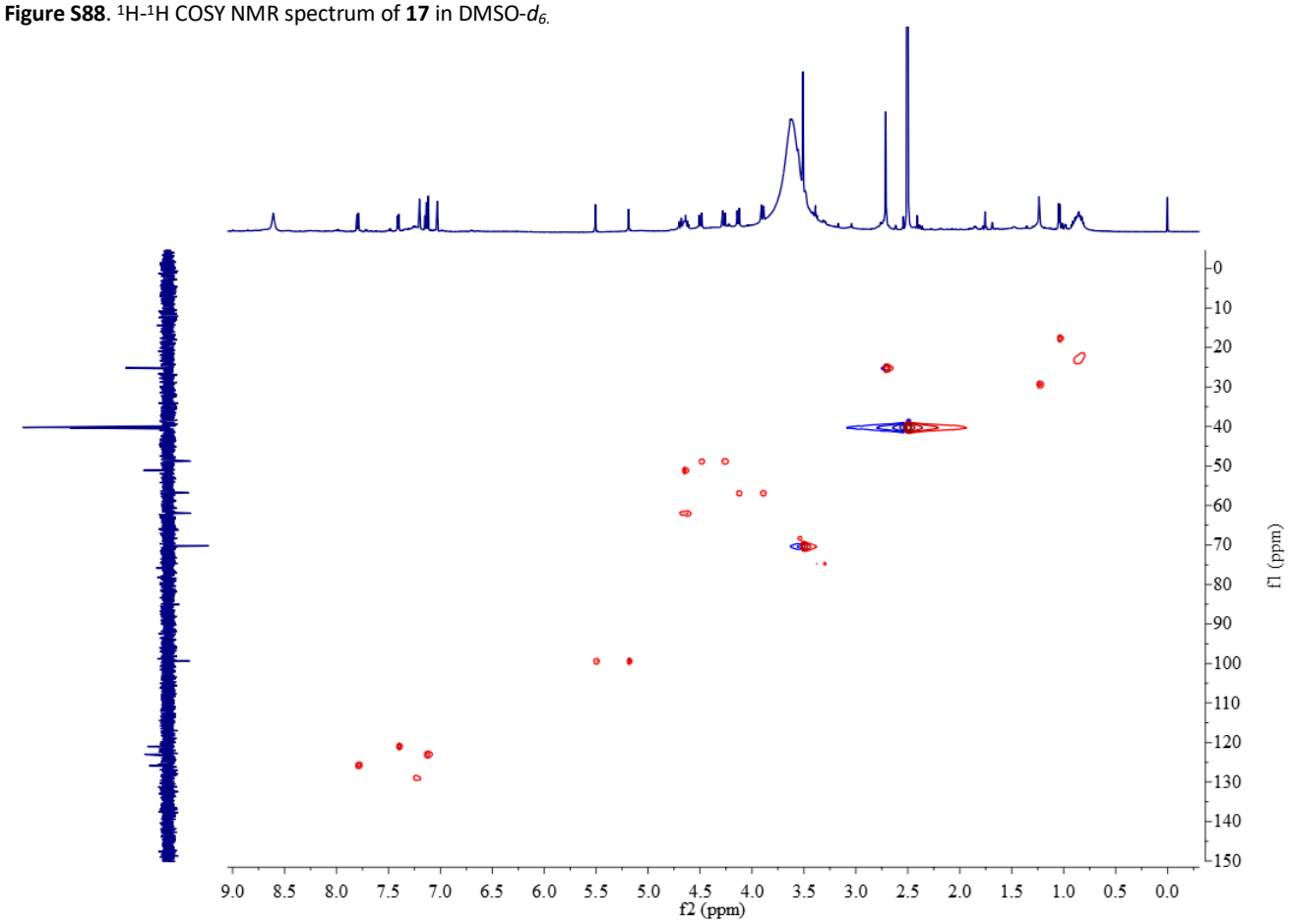

Figure S89. HSQC NMR spectrum of **17** in DMSO-d_6.

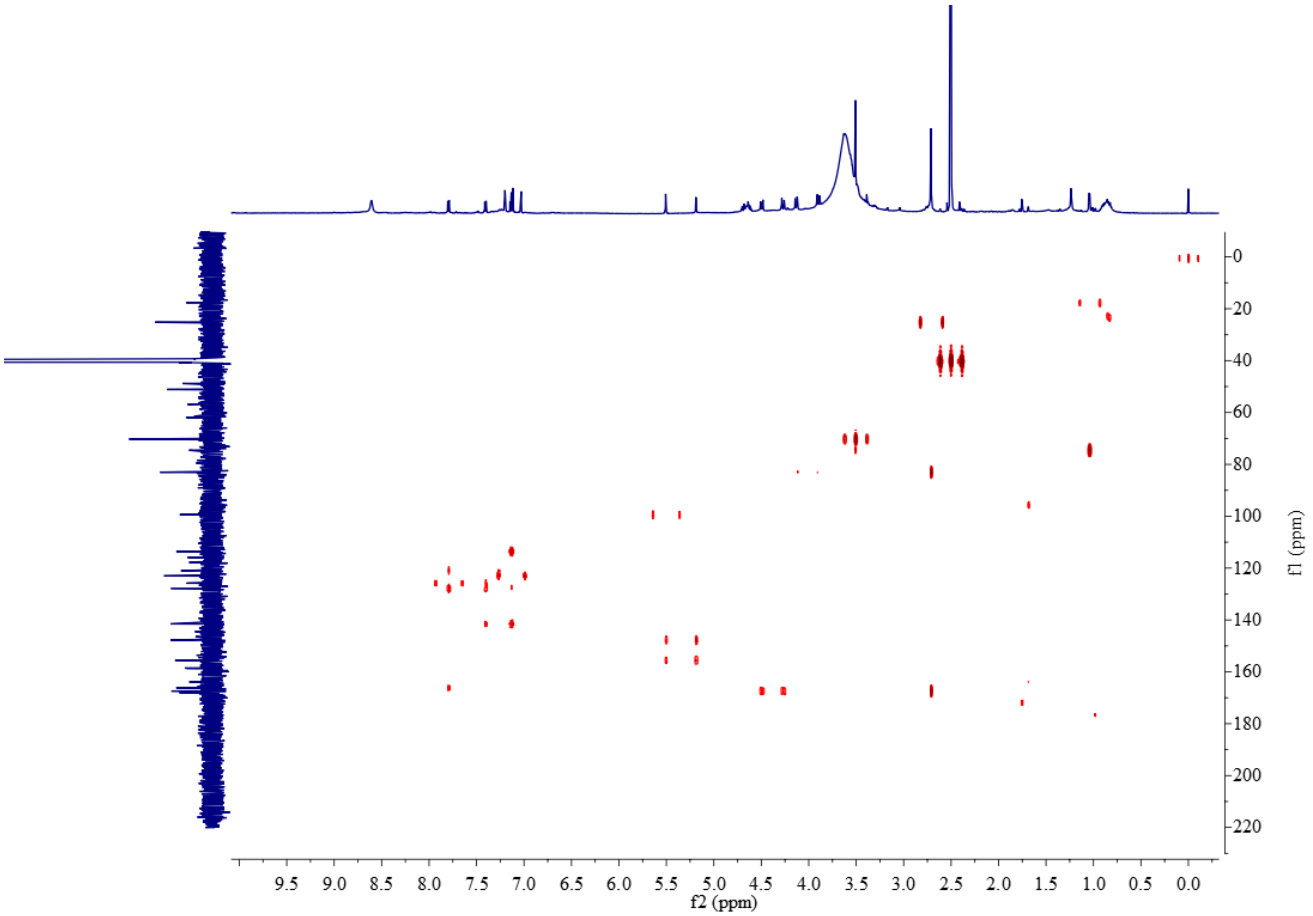

Figure S90. HMBC NMR spectrum of **17** in DMSO-d_6.

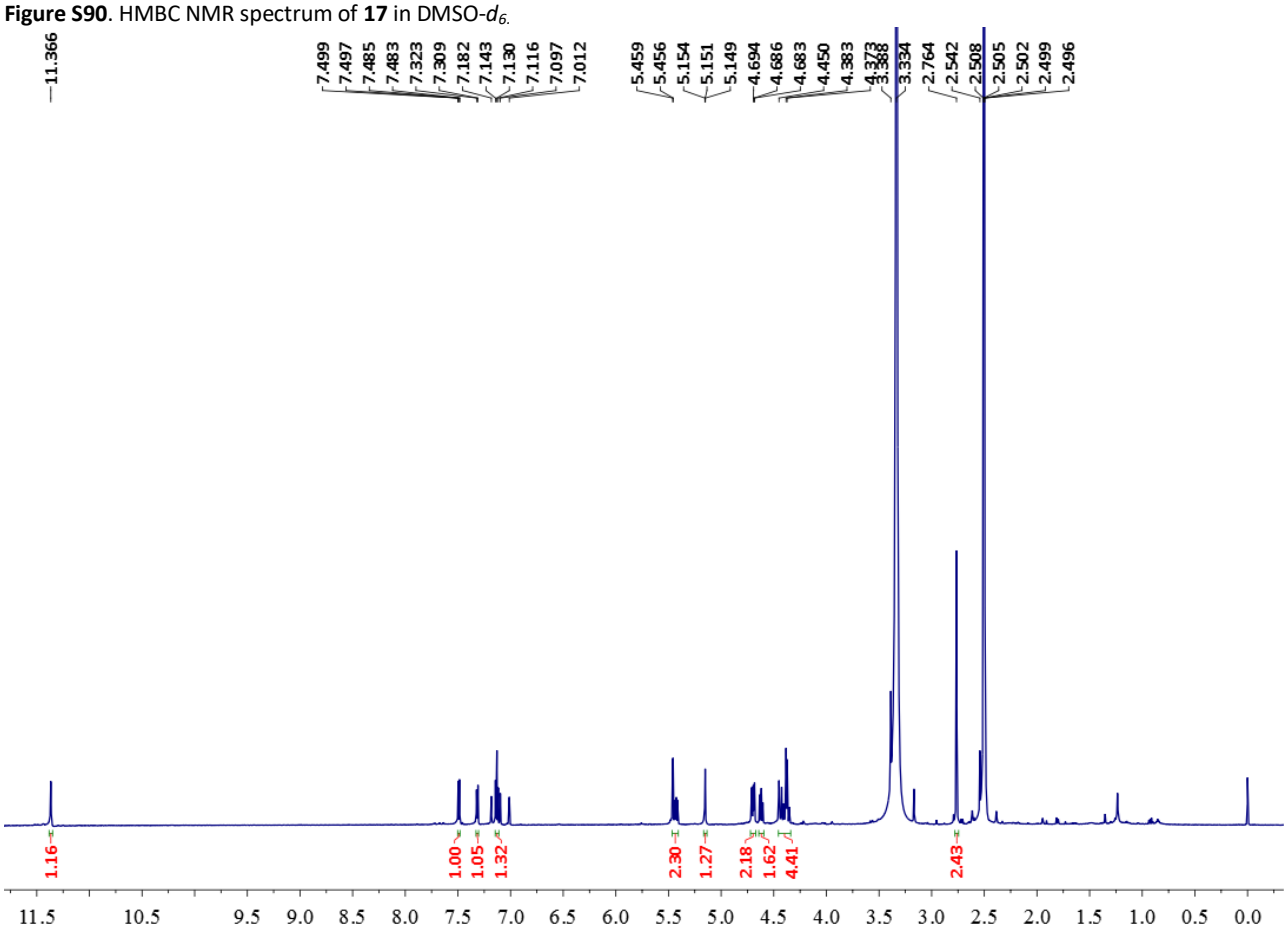

Figure S91. ^{1}H NMR spectrum of **18** in DMSO-d_6 at 600 MHz.

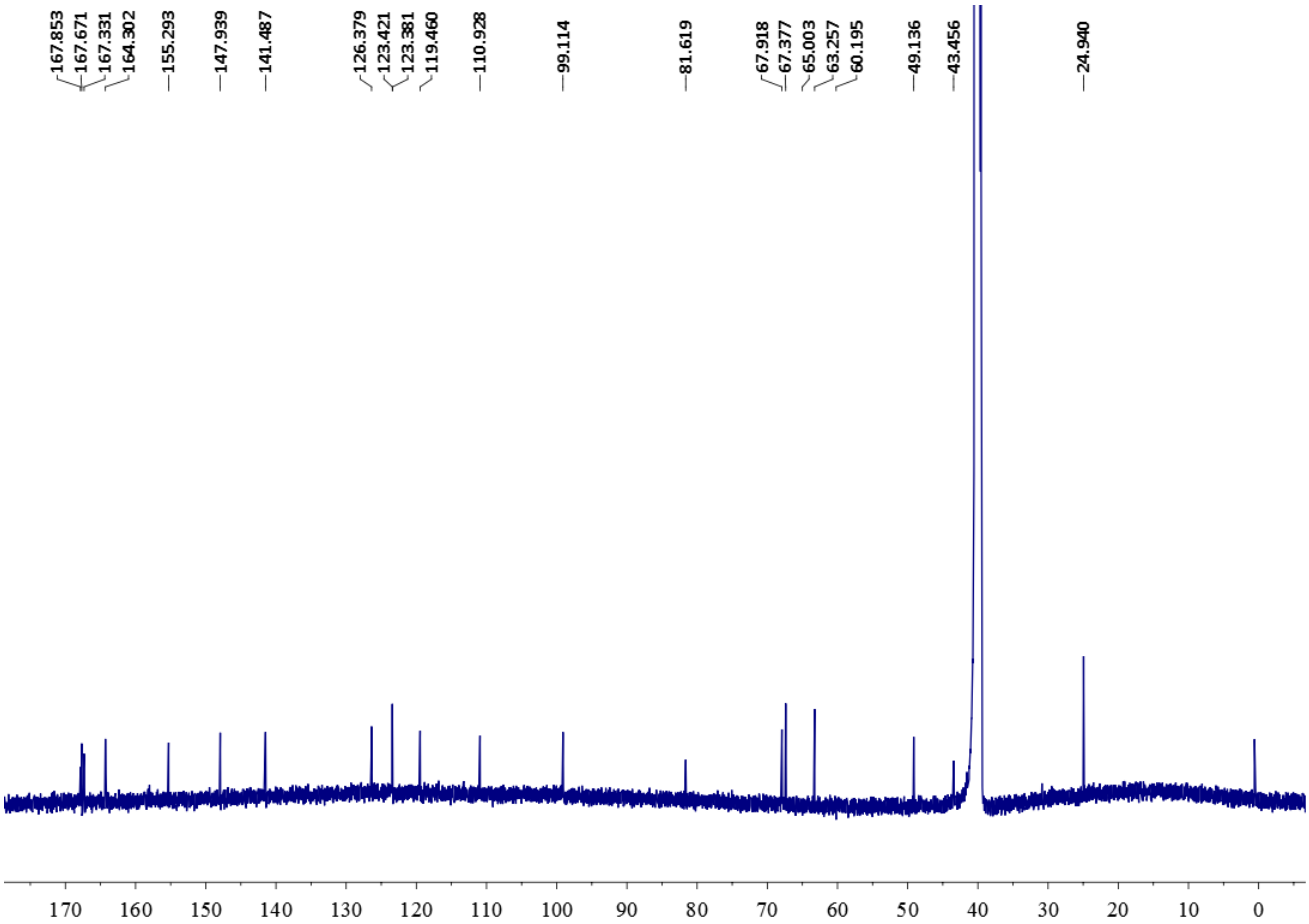

Figure S92. ^{13}C NMR spectrum of **18** in DMSO-d_6 at 150MHz.

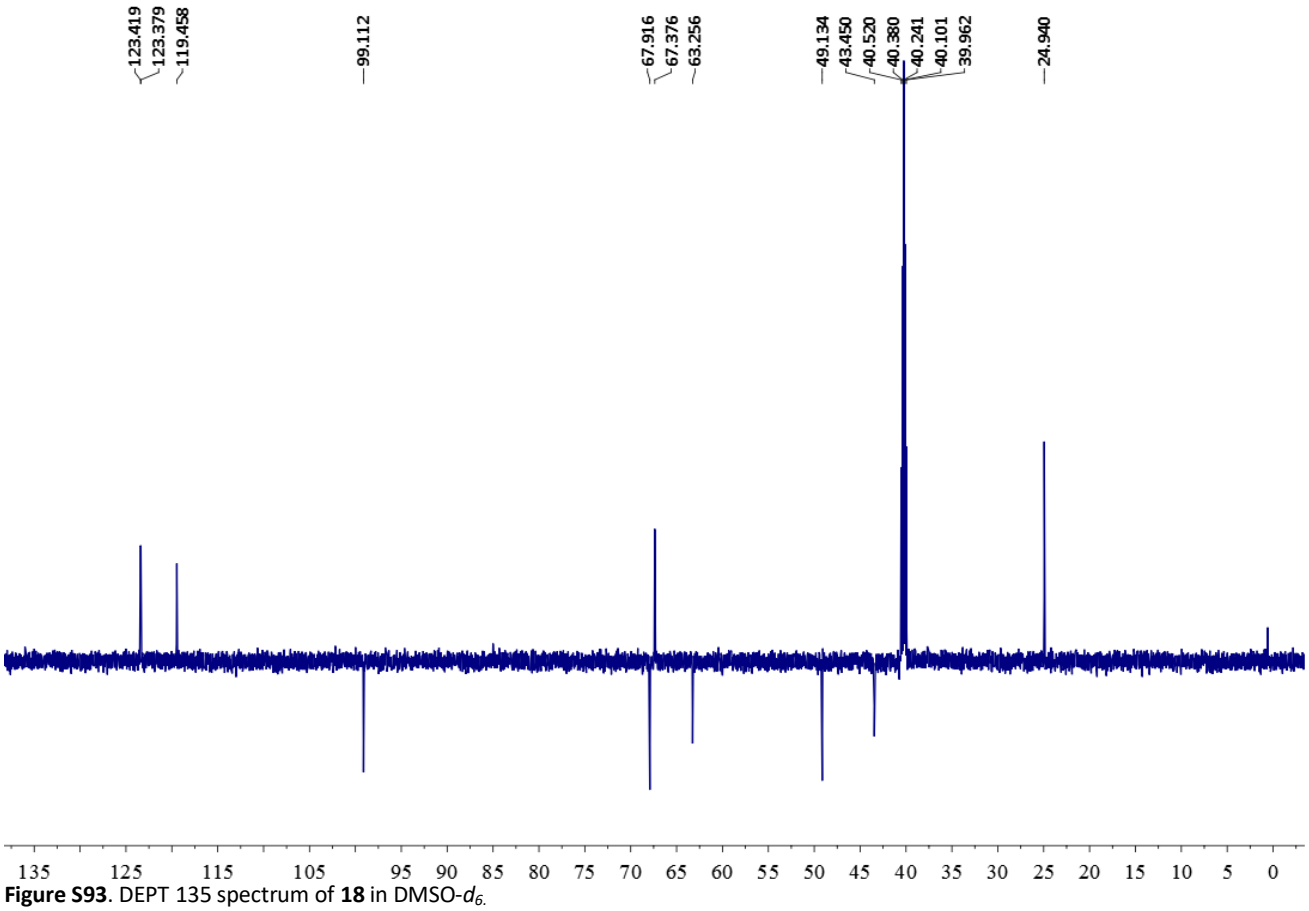

Figure S93. DEPT 135 spectrum of **18** in DMSO-d_6.

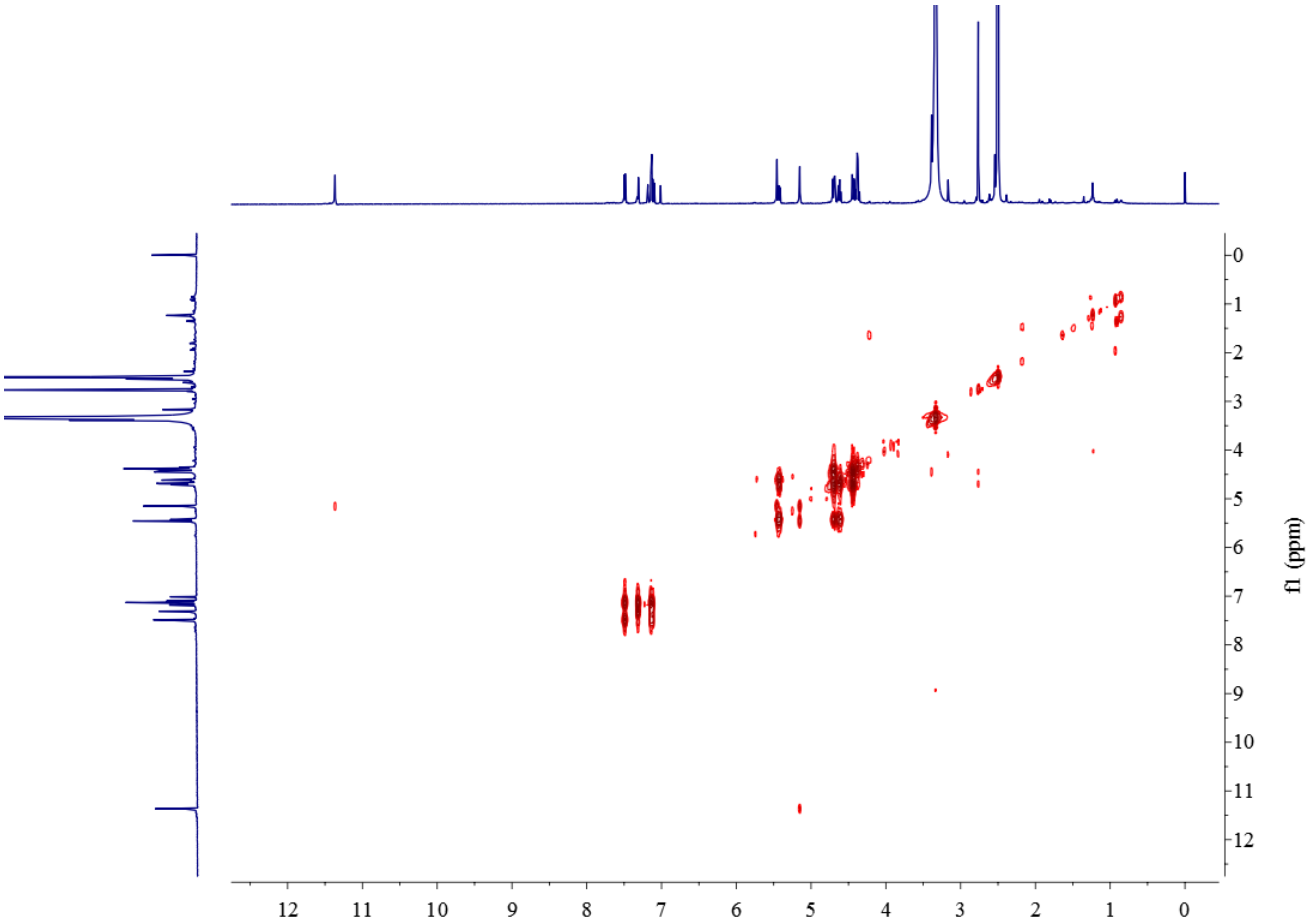

Figure S94. ^{1}H-^{1}H COSY NMR spectrum of **18** in DMSO-d_6.

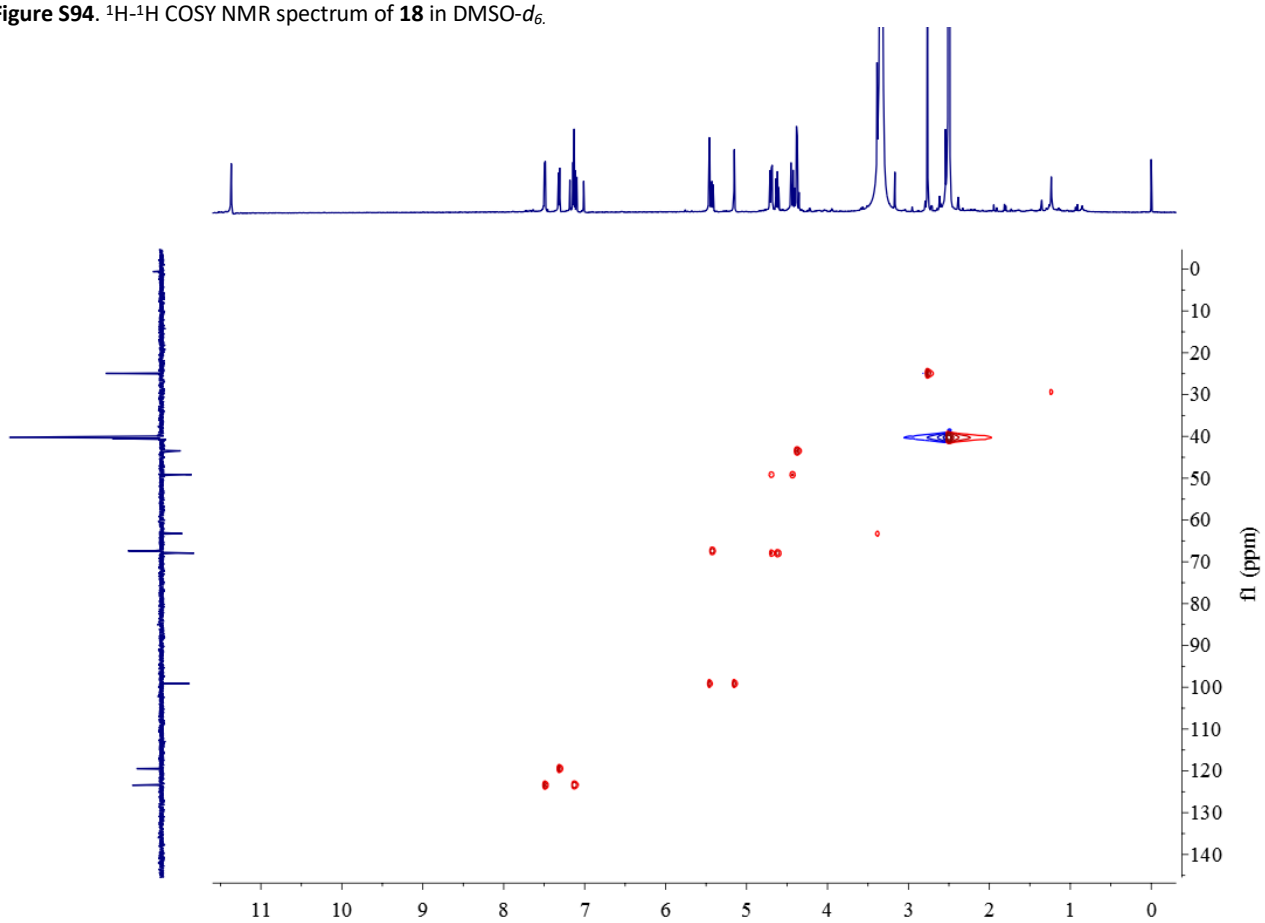

Figure S95. HSQC NMR spectrum of **18** in DMSO-d_6.

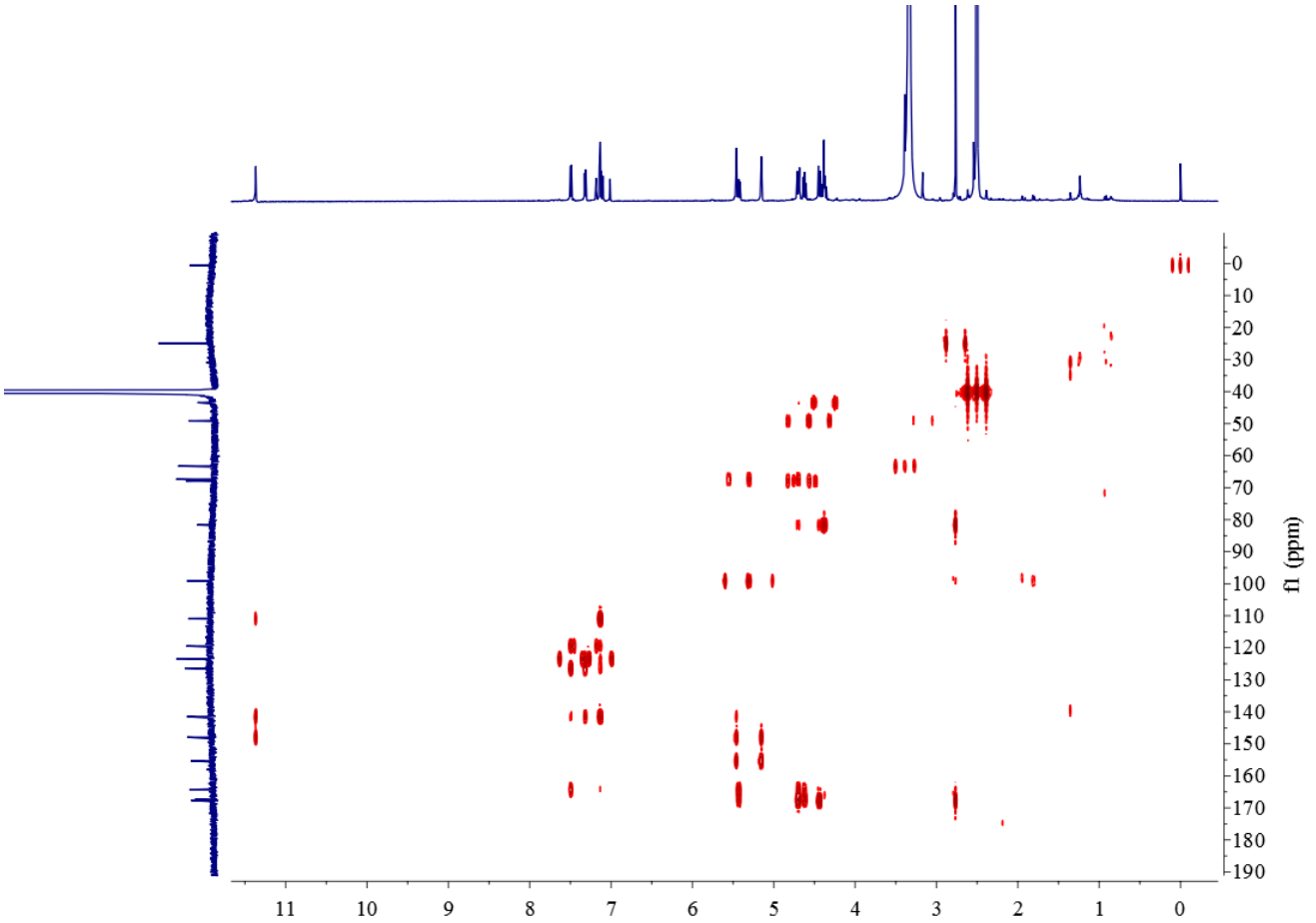

Figure S96. HMBC NMR spectrum of **18** in DMSO-d_6.

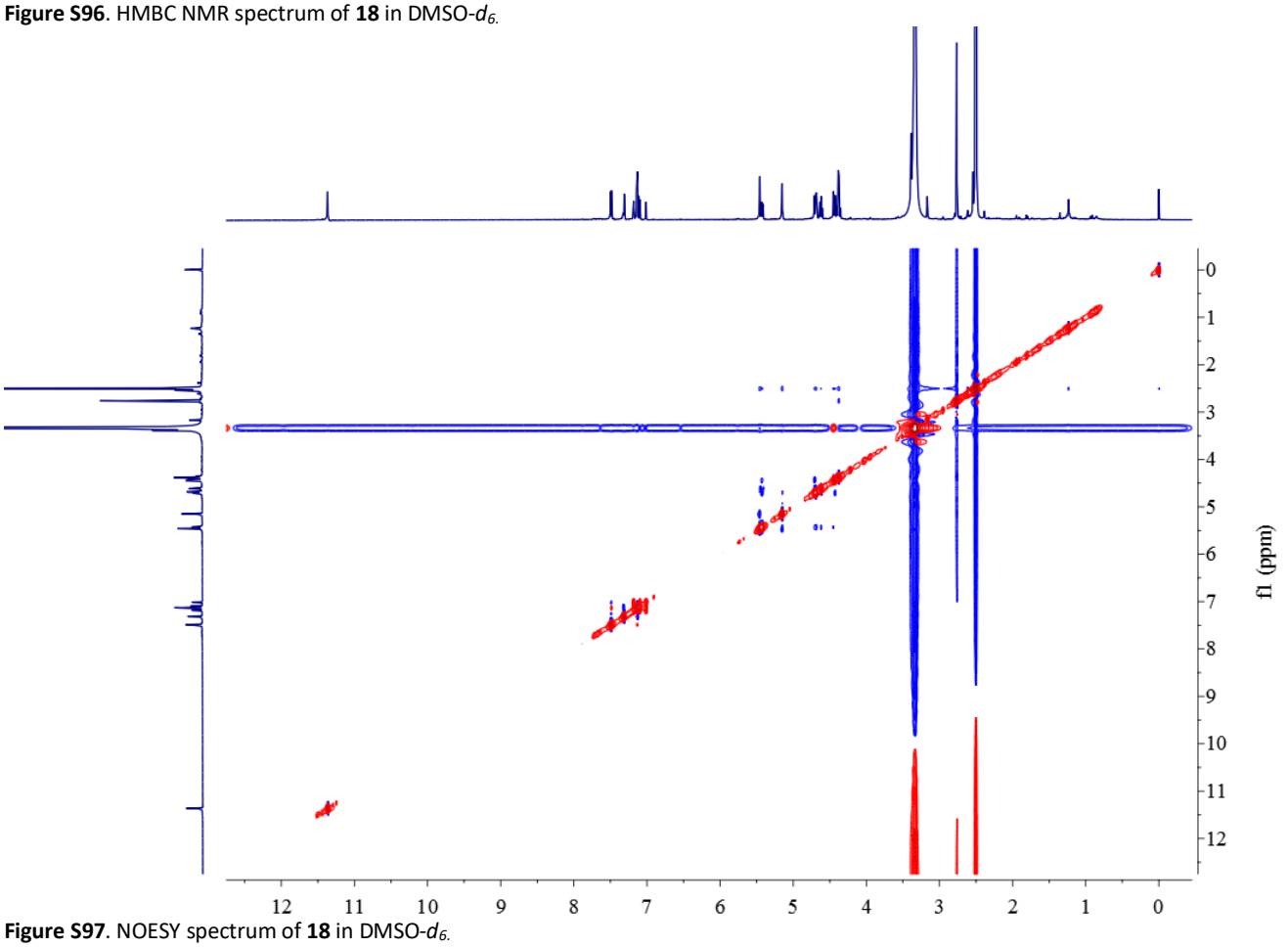

Figure S97. NOESY spectrum of **18** in DMSO-d_6.

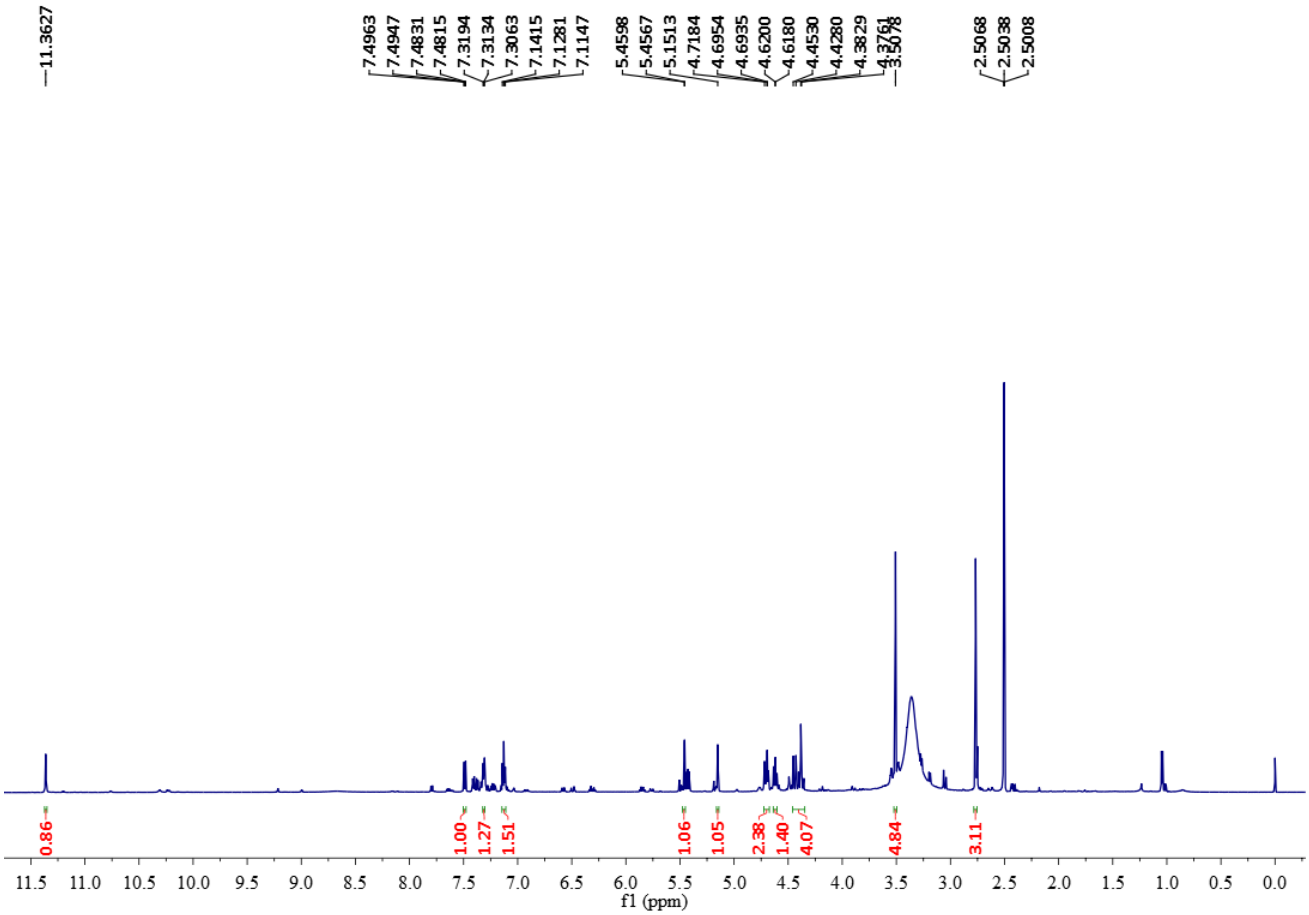

Figure S98. ^{1}H NMR spectrum of **19** in DMSO-d_6 at 600 MHz.

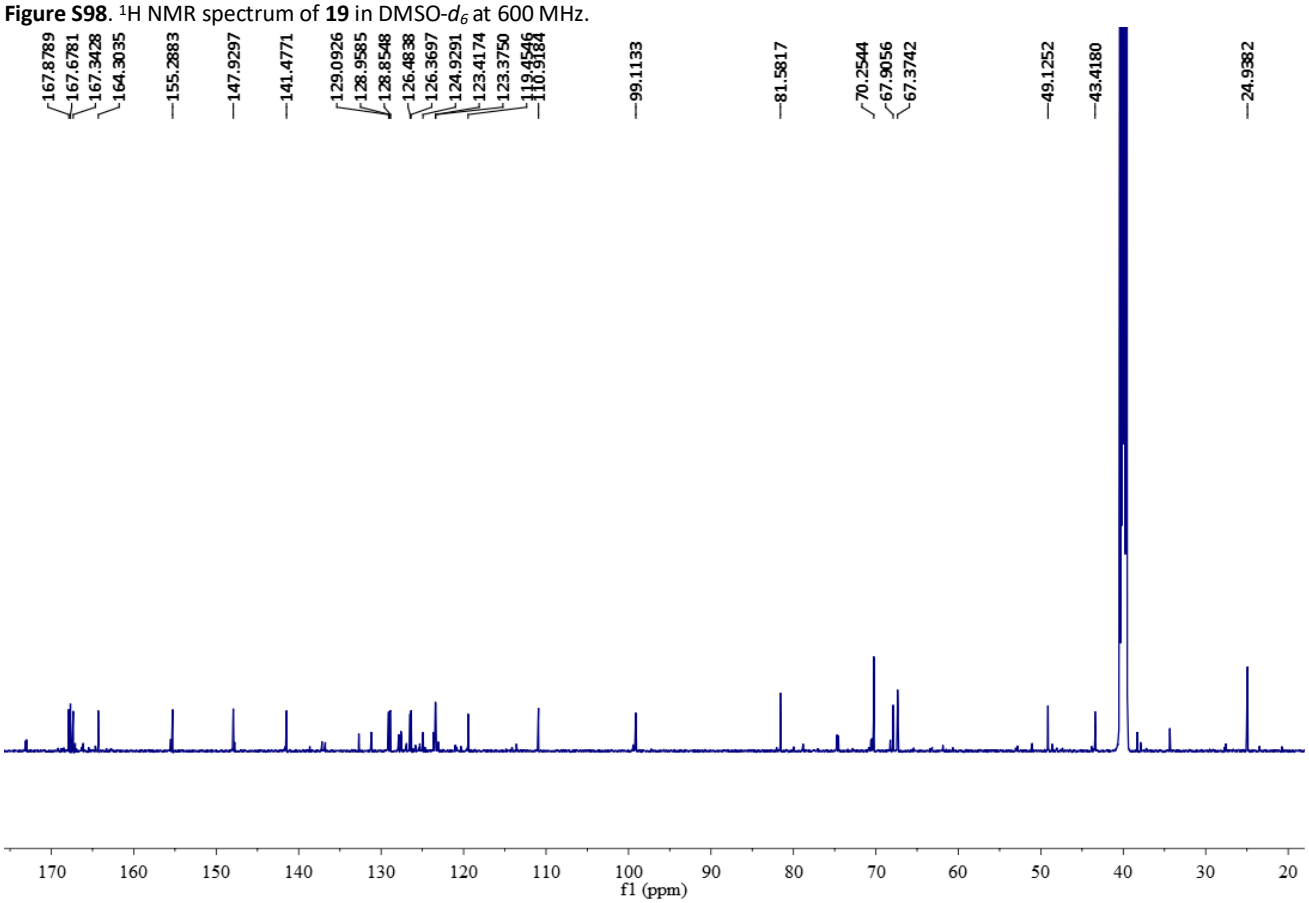

Figure S99. ^{13}C NMR spectrum of **19** in DMSO-d_6 at 150MHz.

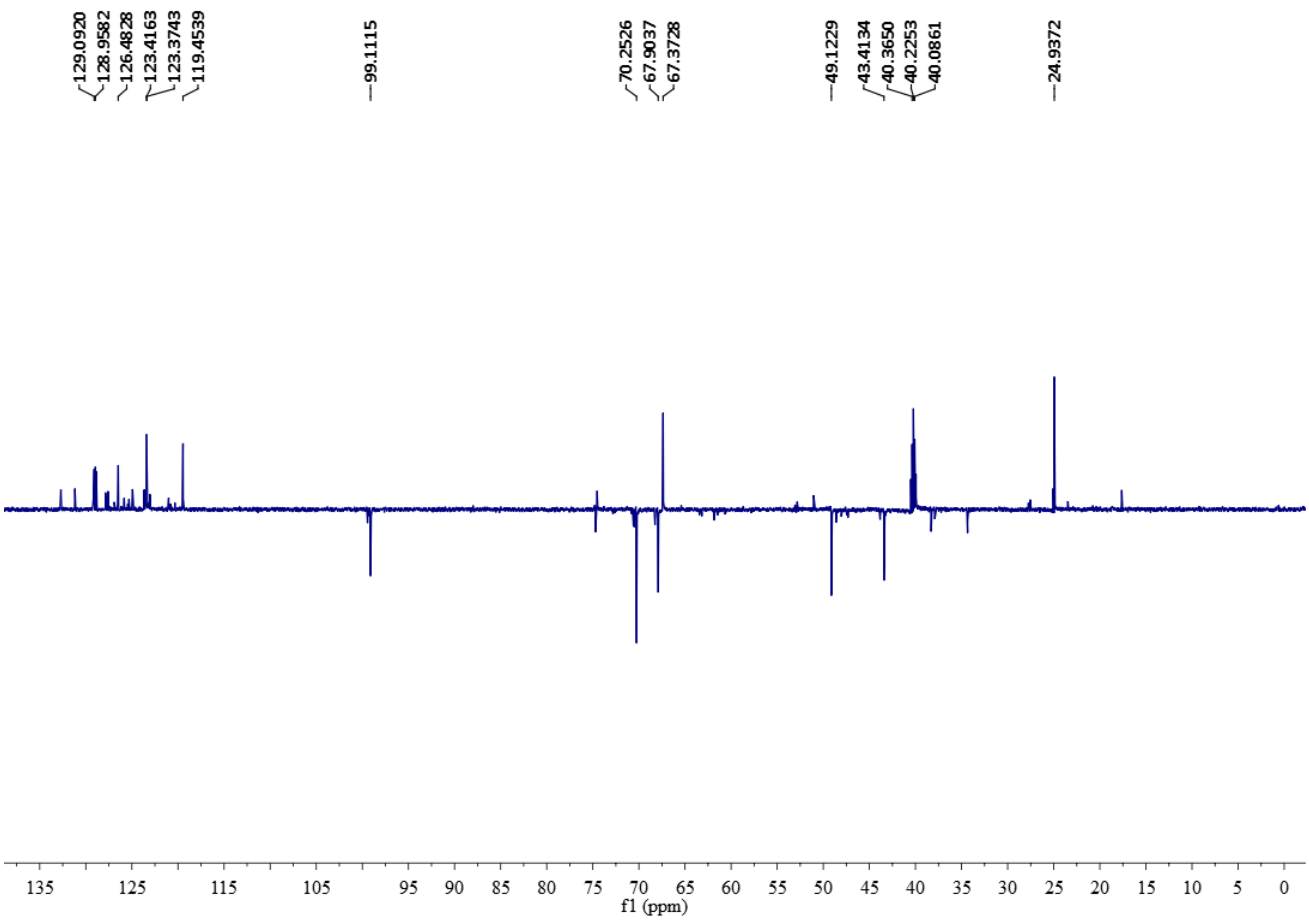

Figure S100. DEPT 135 spectrum of **19** in DMSO-d_6.

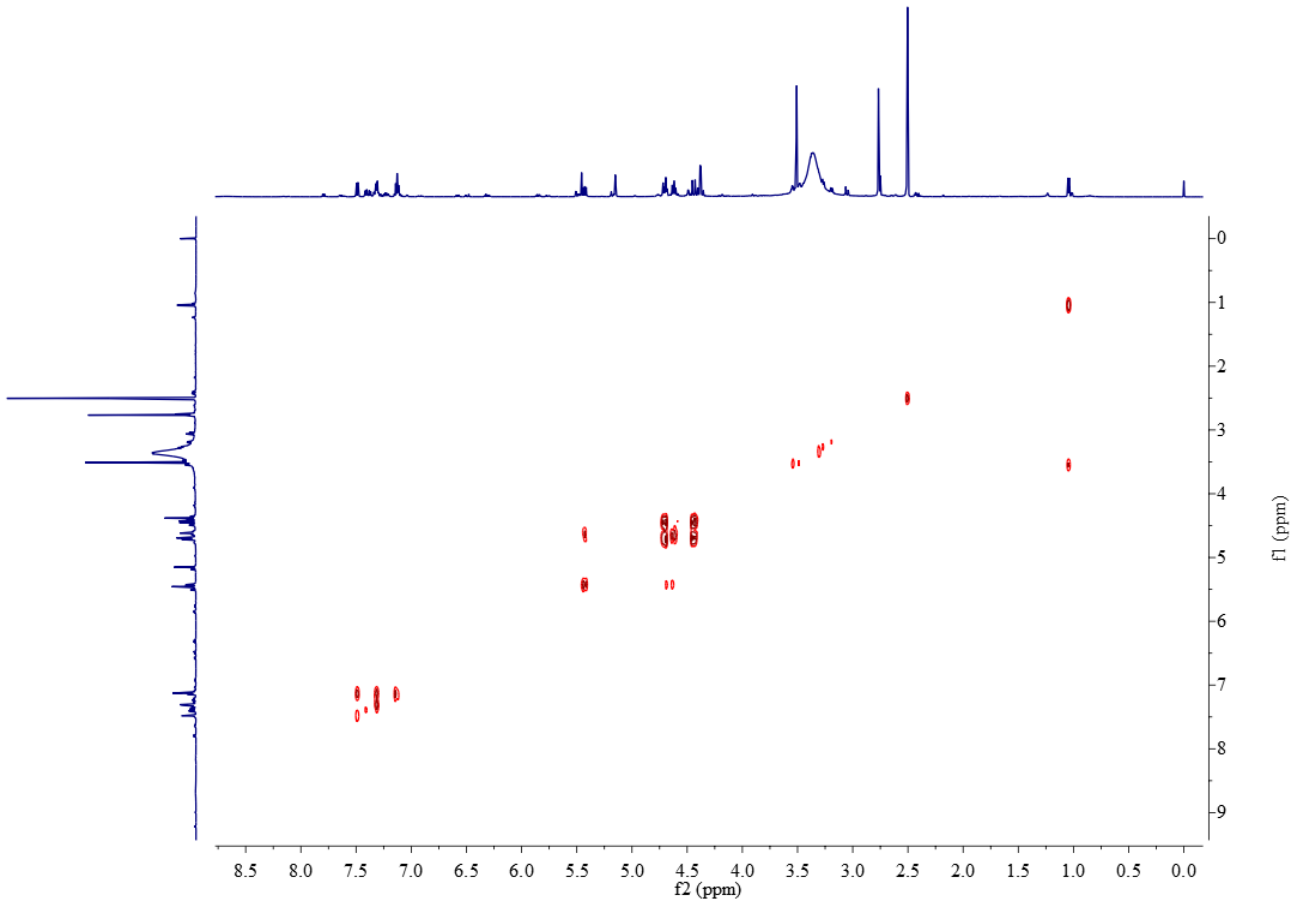

Figure S101. ^{1}H-^{1}H COSY NMR spectrum of **19** in DMSO-d_6.

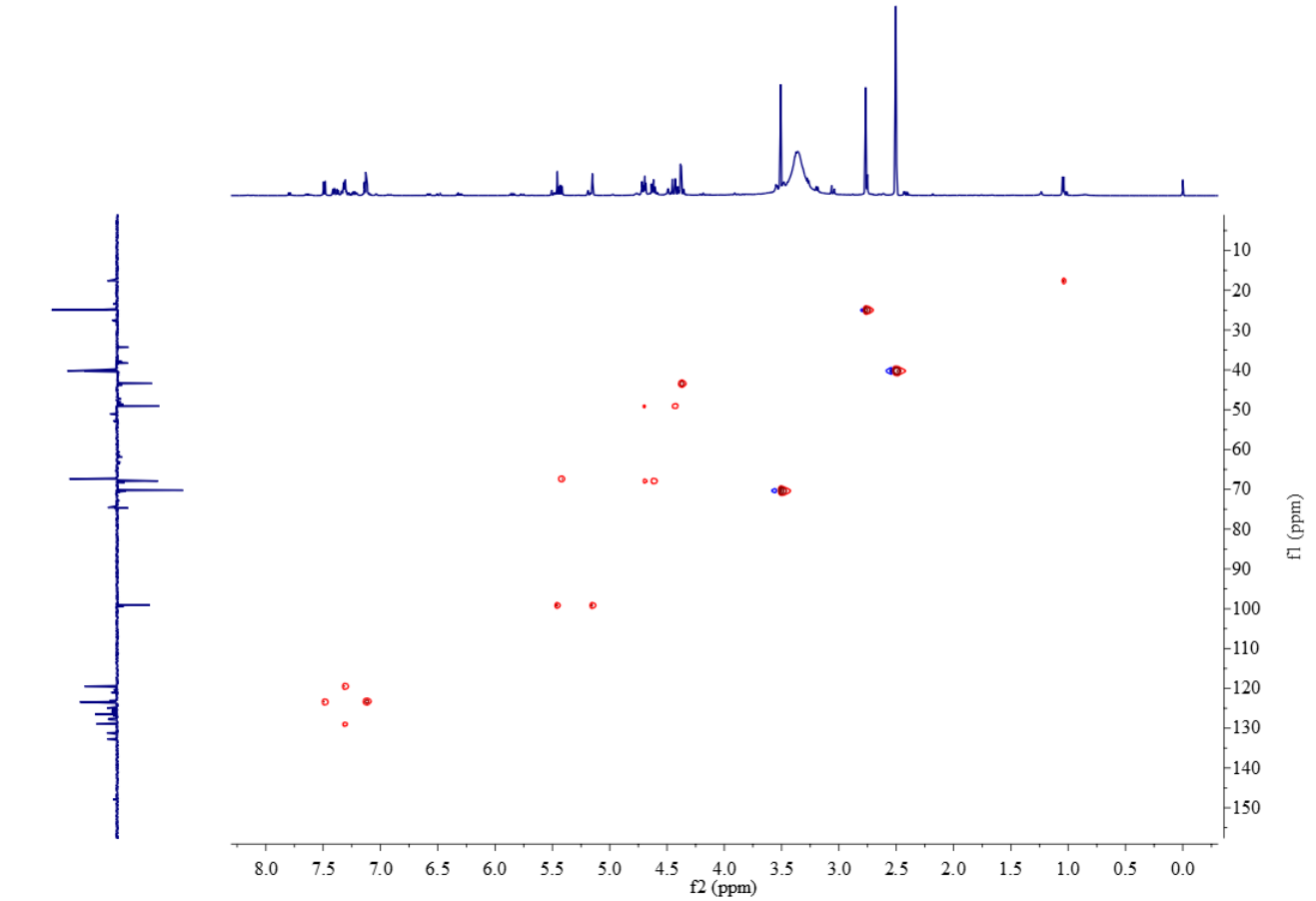

Figure S102. HSQC NMR spectrum of **19** in DMSO-d6.

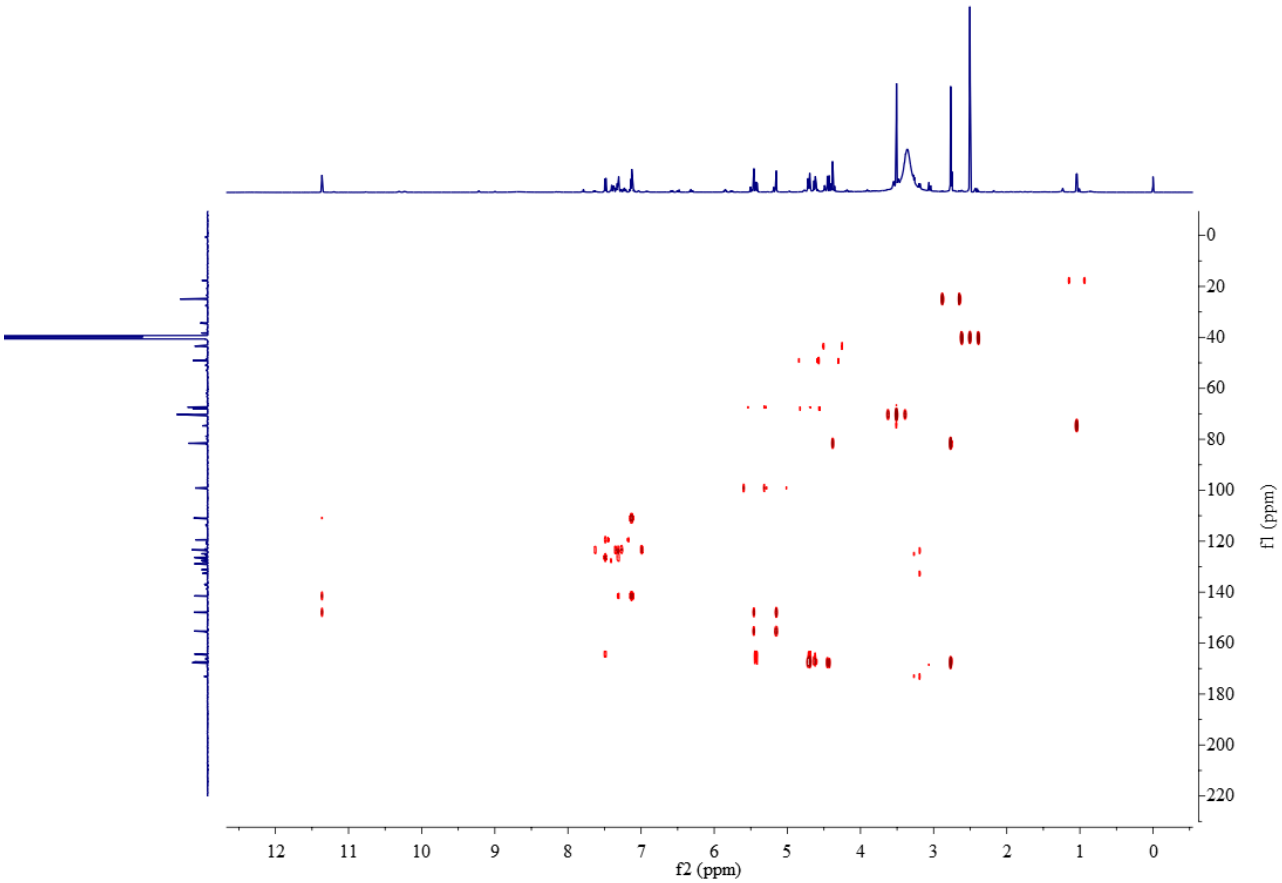

Figure S103. HMBC NMR spectrum of **19** in DMSO-d_6.

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