Electronic Supplementary Material (ESI) for Chemical Science.

Supplementary Information

Discovery and biosynthesis of bosamycin from *Streptomyces* sp. 120454

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Figure S120-121. ^{1}H NMR (400 MHz) and ^{13}C (100 MHz) spectrum of compound **J** in DMSO-d_6.

References

Experimental Procedures

Heterologous expression of cosmid pHG06015 in *Streptomyces albus* J1074.

Cosmid library for strain 120454 was constructed according to the protocol.[2] The cosmid pHG06015 that covers partial *bsm* gene cluster was obtained through PCR screening from 2000 clones using primers listed in Table 3. The cosmid pHG06015 was transferred into the *E. coli*/ET12567 (pUZ8002) strain, and then introduced into *Streptomyces albus* J1074 by intergrative conjugation. The recombinant strain was grown on MS 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, pH 7.0) for 1 days at 30 °C and 250 rpm. Subsequently, 15 mL seed cultures were inoculated into 2 L baffled flasks containing 300 mL of the fermentation medium (dextrin 40 g, tomato paste 7.5 g, NZ Amine 2.5 g, primary yeast 5 g in 1 L distilled water, pH 7.0), and incubated for 7 days at 160 rpm and 30 °C. Finally, the fermentation broth was filtered and absorbed with XAD-16 resin. The resin was washed with water and eluted with acetone.

Co-expression of *bsmF* , *bsmG* and *bsmH* in *Streptomyces albus* J1074 /pHG06015 strain.

A DNA fragment containing three genes of *bsmF*, *bsmG* and *bsmH* was amplified from genomic DNA and subcloned into *E. coli–Streptomyces* expression shuttle vector pUWL201PWT plasmid to afford pHG06016 plasmid. The plasmid pHG06016 was transformed into *E. coli* ET12567/pUZ8002 and then introduced into *S. albus*/pHG06015 strain by conjugation. Clones harboring pHG06016 plasmid were selected by thiostrepton resistance and verified by diagnostic PCR. The resulting recombinant strain was then fermented for 7 days at 30 °C. The crude extract was analyzed by LC-MS and HPLC.

Isolation and purification of bosamycins from *S.* sp. 120454 wild-type and recombinant strains.

For isolation of compounds, a large scale fermentation (20 L) for wild-type strain was carried out using the same medium as mention above. The resin was harvested after seven days' cultivation, and extracted with methanol for three times. The combined methanol phases were evaporated to dryness, and the resulting extract was subjected to silica-gel column, and eluted with the mixture of methylene dichloride and methanol (100:1 to 1:1). Fractions were combined according to HPLC analysis, and further separated by Sephadex LH-20 chromatography. Fractions containing the target compounds were finally purified by semi-preparative HPLC. Compounds **1**-**6** were purified from *S.* sp. 120454 wild-type strain. Compound **13** were purified from HG06012 strain. Compound **14** were purified from HG06013 strain.

Physical data for bosamycins.

Compound **1**: white amorphous solid; $[\alpha]_D^{25}$ -26.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 218 (3.84), 228 (3.83), 278 (3.31); NMR data see Table S4; HRESIMS *m/z* 876.4166 $[M+H]^+$ (calcd for $C_{40}H_{58}N_7O_{15}$, 876.3913).

Compound **2**: white amorphous solid; $[\alpha]_D^{25}$ +30.8 (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε) 212 (3.61), 226 (3.57), 279 (3.08); NMR data see Table S5; HRESIMS *m/z* 1082.4697 $[M+H]^+$ (calcd for $C_{50}H_{68}N_9O_{18}$, 1082.4677).

Compound **3**: white amorphous solid; $[\alpha]_D^{25}$ -20.0 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.23), 228 (4.27), 281 (3.58); NMR data see Table S6; HRESIMS *m/z* 1126.4572 $[M+H]^+$ (calcd for $C_{51}H_{68}N_9O_{20}$, 1126.4575).

Compound **4**: white amorphous solid; $[\alpha]_D^{25}$ +8.7 (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.01), 229 (3.12), 280 (3.49); NMR data see Table S7; HRESIMS *m/z* 1081.4725 $[M+H]^+$ (calcd for $C_{51}H_{69}N_8O_{18}$, 1081.4724).

Compound **5**: white amorphous solid; $[\alpha]_D^{25}$ -6.7 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 218 (3.88), 229 (3.98), 278 (3.48); NMR data see Table S8; HRESIMS *m/z* 1140.4743 $[M+H]^+$ (calcd for $C_{52}H_{70}N_9O_{20}$, 1140.4732).

Compound **6**: white amorphous solid; $[\alpha]_D^{25}$ +8.7 (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.07), 227 (4.08), 280 (3.64); NMR data see Table S9; HRESIMS *m/z* 1095.4879 $[M+H]^+$ (calcd for $C_{52}H_{71}N_8O_{18}$, 1095.4881).

Compound **13**: white amorphous solid; $[\alpha]_D^{25}$ +8.0 (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ε) 218 (3.86), 229 (3.97), 281 (3.40); NMR data see Table S10; HRESIMS *m/z* 1009.4564 $[M+H]^+$ (calcd for $C_{48}H_{65}N_8O_{16}$, 1009.4513).

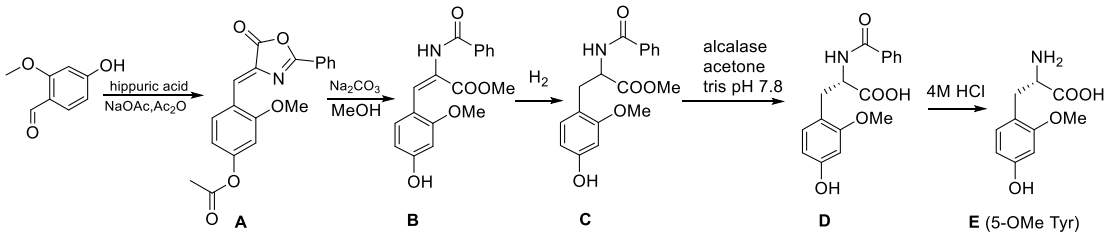
Compound **14**: white amorphous solid; $[\alpha]_D^{25}$ +5.3 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 218 (3.65), 227 (3.67), 278 (3.26); NMR data see Table S11; HRESIMS *m/z* 1039.4631 $[M+H]^+$ (calcd for $C_{49}H_{67}N_8O_{17}$, 1039.4619).

Preparation and analysis of Marfey's derivatives.

The absolute configuration of the bosamycins was determined by advanced Marfey's method.[3] Briefly, Compound **3** (0.5 mg) was dissolved in 6 N HCl (1 mL) and hydrolyzed at 110 °C for 12 h. After cooling, the solution was evaporated to dryness and dissolved in H_2O (100 µL). To this mixture was added a 1% (w/v) solution (200 µL) of 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (L-FDAA) and1-fluoro-2,4-dinitrophenyl-5-D-alanine amide (D-FDAA) in acetone respectively. After adding $NaHCO_3$ solution (1 M, 50 µL), the reaction mixture was heated at 45 °C for 1 h and then acidified with 2 N HCl (25 µL). The standards L-Leu, L-OMe Tyr, L-Ser, *erythro* L-OHAsp,*threo* L-OHAsp and L-Tyr were derivatized in a similar manner. Derivatized hydrolysate (20 µL) and standard amino acids (20 µL) were subjected to LC-MS analysis.

Because compound **3** has two tyrosines that have different configurations, whereas compound **1** only have one tyrosine that can be assigned unambiguously by Marfey's method. Thus, **1** was hydrolyzed and derivatized in a similar manner. Results can be found in Figure S2.

Chemical synthesis of 5-OMe Tyr.[4-7]



Compound **A**: 4-Hydroxy-2-methoxybenzaldehyd (1 g, 6.6 mmol), hippuric acid (1.2 g, 6.7 mmol), sodium acetate (0.56 g,7 mmol) and acetic anhydride (1.5 ml, 1.6 g, 16 mmol) were added to a 100-ml round-bottom flask, heated in an oil bath at 100 ℃ for 2 h. The resulting solid mixture was cooled to room temperature before H_2O (10 ml) was added. The mixture was filtrated, washed with aqueous Na_2CO_3 and dried under vacuum. The desired compound **A** was then obtained after recrystallization from acetone/H_2O (2:1) as a yellow solid; yield: 1.2 g (53%). ^1H NMR data see Table S13. HRESIMS *m/z*: 338.0992 $[M+H]^+$ (calcd. for $[C_{19}H_{15}NO_5H]^+$ 338.0878).

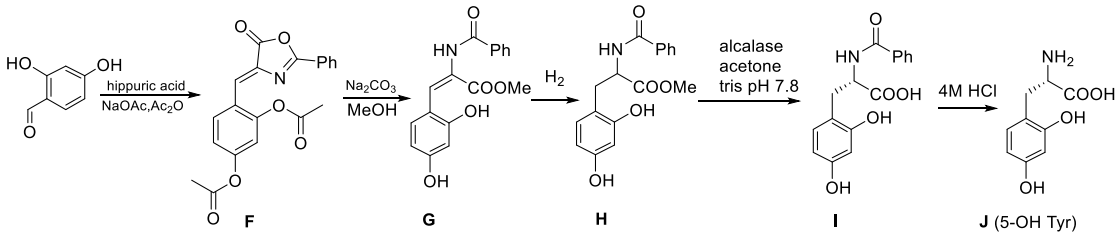
Compound **C**: To a 100-ml round bottom flask was added compound **A** (1 g, 3.38 mmol), 1:1 mixture of CH_2Cl_2 and MeOH (12 ml) and Na_2CO_3 (283 mg, 1.41 mmol). The mixture was stirred at room temperature for 12 hours, then filtered and concentrated to afford compound **B** (850mg, 85%). ^1H NMR data see Table S13. HRESIMS *m/z*: 350.1016 $[M+Na]^+$ (calcd. for $[C_{18}H_{17}NO_5Na]^+$ 350.1107). Compound **B** was dissolved in a solution of Pd/C (70 mg) in MeOH (15 mL) and acetic acid (2.5 mL). The reaction suspension was then hydrogenated (50 bar H_2) for 24 h. The mixture was filtered and concentrated to give compound **C** as a white crystalline solid (723 mg, 72%). ^1H NMR data see Table S13. HRESIMS *m/z*: 330.0549 $[M+H]^+$ (calcd. for $[C_{18}H_{19}NO_5H]^+$ 330.1297).

Compound **D**: Compound **C** (500 mg, 1.5 mmol) was dissolved in DMSO (1.5 mL) and diluted with acetone (12 mL) then diluted with Tris buffer (80 mM) pH 7.8. The mixture was warmed to 37 °C, and Alcalase 1ml (3 mL, > 2.4 U / mL, Sigma) from *Bacillus licheniformis* was added to the reaction system. The reaction was periodically adjusted to pH 7.8 by the addition of 1 M NaOH until conversion was stopped by HPLC (2 days). The volatiles were removed

in vacuo. The organic phase was acidified with HCl to pH = 2, and then extracted with EtOAc. The organic extract was concentrated, and the resulting solid was recrystallized by diluting in MeOH and EtOAc (~1:1) to give compound **D** as a white needle (323 mg, 67%). ^{1}H NMR data see Table S13. HRESIMS *m/z*: 338.1012 $[M+Na]^+$ (calcd. for $[C_{17}H_{17}NO_5Na]^+$ 338.1140).

Compound **E** (5-OMe Tyr): Compound **D** (200 mg, 0.63 mmol) was hydrolyzed with 4N HCl. The mixture was cooled, washed with EtOAc, and concentrated to give a crude brown compound **E** (75 mg, 56%). ^{1}H NMR data see Table S13. HRESIMS *m/z*: 212.0940 $[M+H]^+$ (calcd. for $[C_{10}H_{12}NO_4H]^+$ 212.0878).

Chemical synthesis of 5-OH Tyr.[4-7]



5-OH Tyr (30 mg) was prepared using the method as mentioned in 5-OMe Tyr. NMR data see Table S13, Figures S120 and S121. HRESIMS *m/z*: 198.0873 $[M+H]^+$ (calcd. for $[C_{19}H_{15}NO_5H]^+$ 198.0688).

Chemical complementation of 5-OMe Tyr or 5-OH Tyr to Δ*bsm*F, Δ*bsm*G and Δ*bsm*H mutant.

*Δbsm*F, *Δbsm*G and *Δbsm*H mutant was individually cultured in a 50 ml flasks containing 20 mL B medium. After 24 hours cultivation, 5-OMe Tyr (1.3 mg) or 5-OH Tyr (1.3 mg) dissolved in DMSO was supplemented into fermentation broth and cultured for another 6 days. After extraction by XAD-16, the eluted organic solution was then analyzed by LC-MS**.** The LC-MS analysis was performed using a 18 min solvent gradient from 10 % to 90 % methanol in water supplied with 0.1 TFA at a flow rate of 0.4 mL/min.

Chemical complementation of L-erythro-β-OH-Asp into Δ*bsmC* mutant.

Δ*bsm*C mutant was individually cultured in a 50 ml flasks containing 20 mL B medium. After 24 hours cultivation, L-erythro-β-OH-Asp (1.2 mg) dissolved in DMSO was supplemented into fermentation broth and cultured for another 6 days. After extraction by XAD-16, the eluted organic solution was then analyzed by LC-MS**.** The LC-MS analysis was performed using a 18 min solvent gradient from 10 % to 90 % methanol in water supplied with 0.1 TFA at a flow rate of 0.4 mL/min.

Protein expression and purification.

DNA fragments containing target genes including BsmA (A_1), BsmB (A_4-T_4), BsmD (C_6-A_6-T_6), BsmF (A_0-T_0) and BsmH were individually amplified from genomic DNA of *S*. sp. 120454 with primers listed in Table S3. The purified PCR product of BsmA (A_1), BsmB (A_4-T_4) and BsmF (A_0-T_0) were ligated with linearized pET28a (linearized by NdeI and HindIII) to afford pHG06017, pHG06018 and pHG06020, DNA fragments containing BsmD (C_6-A_6-T_6) and BsmH were ligated with linearized pET22b (treated with NdeI and HindIII) to afford pHG06019 and pHG06021. The obtained plasmids were transformed into *E. coli* BL21(DE3), respectively. A single colony was picked to inoculate a 4 mL LB starter culture grown overnight at 37 °C, 200 rpm. The following day, 0.4 L LB media supplemented with kanamycin or ampicillin was inoculated with the starter culture and incubated at 37 °C, 200 rpm until the OD600 reached 0.6. The culture was cooled to 4 °C and induced with 0.125 mM IPTG. Cultures were incubated at 16 °C, 200 rpm for 18 h. Cell pellets were resuspended in lysis buffer (100 mM Tris, pH 8.0, 15 mM imidazole, 300 mM

NaCl, 10 % glycerol) and sonicated on ice. After centrifugation at 15000 rpm for 30 min, the supernatant was filtered and loaded onto a 5 mL Histrap HP column (GE lifesciences). Fractions containing the proteins were pooled and desalted by a PD10 column (GE Healthcare) with 100 mM Tris-HCl buffer (pH 7.5) and 10% glycerol and stored at -80°C.

Adenylation activities of A domain.

The A domain specificity assays were conducted in a 50 μL reaction volume containing 100 mM Tris-HCl, pH 7.5, 20 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 1 hour, an equal volume of the Master Reaction Mix (Sigma-Aldrich Pyrophosphate Assay Kit MAK 168) were added to each of the sample, and incubated for another 30 minutes. Then, the fluorescence intensity (λex=316 / λem=456 nm) was measured by a microplate reader (TECAN infinite M200PRO).

***In vitro* assay of BsmH.**

Enzymatic reaction was performed in 100 mM phosphate buffer (pH 7.2) containing 10 μM BsmH, 0.5 mM 5-OH Tyr, 1 mM SAM. After incubation at 30 °C for 2 h, 50 μL acetonitrile were added to quench the reaction. Then the mixture was centrifuged at 14,000 g for 10 min and the supernatant was analyzed by analytic HPLC using a 20 min solvent gradient from 5% to 20% acetonitrile in water supplied with 0.1 TFA at a flow rate of 0.5 mL/min.

Biological activity assay of SHP2.

The catalytic activity of SHP2 was monitored using the surrogate substrate DiFMUP in a prompt fluorescence assay format.[8] The phosphatase reactions were performed at room temperature in 96-well black polystyrene plate, flat bottom, low flange, nonbinding surface (Corning, cat. no. 3575) using a final reaction volume of 100 μl and the following assay buffer conditions: 60 mM HEPES, pH 7.2, 75 mM NaCl, 75 mM KCl, 1 mM EDTA, 0.05% P-20, 5 mM DTT. 1 nM of $SHP2^{WT}$(residues 1-525) was co-incubated with of 1 μM of bisphosphorylated IRS1 peptide (sequence:H2N-LN(pY)IDLDLV(dPEG8)LST(pY)ASINFQK-amide) and 30 μM of tested compounds. Under the same buffer conditions, the phosphatase assays of $SHP2^{E76K}$ (0.5 nM) or $SHP2^{PTP}$ (1 nM) was incubated with Compound **5** at various concentrations. After 30-60 min incubation at 25 °C, the surrogate substrate DiFMUP (Invitrogen, cat. no. D6567, 100 μM) was added to the reaction and incubated at 25 °C for 30 min. The reaction was then quenched by the addition of 20 μl of a 160 μM solution of bpV (Phen) (Enzo Life Sciences cat. no. ALX-270-204). The fluorescence signal was monitored using a microplate reader (TECAN, M200PRO) using excitation and emission wavelengths of 340 and 450 nm, respectively.

Compounds **1**-**6**, **13**-**14** were screened at 30 μM against Src homology 2-containing protein tyrosine phosphatase 2 (SHP2), which is a major phosphatase involved in growth factor and cytokine-mediated signaling. Studies have shown that SHP2 allosteric inhibitors have shown remarkable anti-tumor benefits.[8-11] In our initial single-concentration assays, only compound **5** had the inhibitory effect on the SHP2 (Figure S3, A). To further evaluate the acting mechanisms of compound **5** we used three SHP2 proteins to test sensitivities of **5**: (1) wild-type (WT) SHP2 (residues 1–525); (2) $SHP2^{E76K}$ mutant with a partially open conformation in SHP2; (3) SHP2 PTP domain with a completely open conformation. Compound **5** was shown to inhibit SHP2 enzyme activity in a dose-dependent manner, and the IC_{50} value of $SHP2^{WT}$, $SHP2^{E76K}$, $SHP2^{PTP}$ were 24.25, 45.56, and 89.98 μM, respectively (Figure S3, B and C), suggesting **5** could be a novel allosteric inhibitor of SHP2.

Table S1. Bacterial plasmidsand strains.

Plasmid/Strain	Relevant characteristics	Source
Plasmid		
pKC1139	*E.coli-Streptomyces* shuttle plasmid used for gene disruption, temperature sensitive	12
PJTU2554	Cosmid vector for genomic library construction	13
pSET152-kasOp*	pSET152 derived plasmid containing the promoter *kas*Op*	14
pUWL201PWT	*E. coli-Streptomyces* expression shuttle vector harboring *oriT* (cloned into the *PstI* site)	15
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 C-terminal His-tag, ampicillin resistance	Novagen
pHG06001	pKC1139 derived plasmid for disruption of *bsmA-C*$_1$	This study
pHG06002	pKC1139 derived plasmid for disruption of *bsmC*	This study
pHG06003	pKC1139 derived plasmid for disruption of *bsmD*	This study
pHG06004	pKC1139 derived plasmid for disruption of *bsmF*	This study
pHG06005	pKC1139 derived plasmid for disruption of *bsmG*	This study
pHG06006	pKC1139 derived plasmid for disruption of *bsmH*	This study
pHG06007	pKC1139 derived plasmid for disruption of *orf(-1)*	This study
pHG06008	pKC1139 derived plasmid for disruption of *bsmI*	This study
pHG06009	pSET152-kasOp* derived plasmid for complementation of *bsmC*	This study
pHG06010	pSET152-kasOp* derived plasmid for complementation of *bsmF*	This study
pHG06011	pSET152-kasOp* derived plasmid for complementation of *bsmG*	This study
pHG06012	pSET152-kasOp* derived plasmid for complementation of *bsmF*-T281A	This study
pHG06013	pSET152-kasOp* derived plasmid for complementation of *bsmF*-F380A	This study
pHG06014	pSET152-kasOp* derived plasmid for complementation of *bsmF*-C387A	This study
pHG06015	Cosmid which contains *bsm* biosynthetic gene cluster	This study
pHG06016	pUWL201PWT derived plasmid harboring *bsmF*, *bsmG*, *bsmH*	This study
pHG06017	pET28a derived plasmid for expressing N-terminal His-tag BsmA (A_1)	This study
pHG06018	pET28a derived plasmid for expressing N-terminal His-tag BsmB (A_4-T_4)	This study
pHG06019	pET22b derived plasmid for expressing C-terminal His-tag BsmD (C_6-A_6-T_6)	This study
pHG06020	pET28a derived plasmid for expressing N-terminal His-tag BsmF (A_0-T_0)	This study
pHG06021	pET22b derived plasmid for expressing C-terminal His-tag BsmH	This study
***E. coli* strains**		
DH5α	General cloning host	16
BL21 (DE3)	Heterologous host for protein expression	NEB

ET12567 (pUZ8002)	Methylation-deficient host used for *E. coli-Streptomyces* intergeneric conjugation	3
Strains		
S. albus J1074	Model actinomycete used for gene heterologous expression	17
120454	Wild type strain for bosamycins production	This study
HG06001	Δ*bsmA-C*$_1$, in-frame deletion mutant strain in WT, bosamycin D producing	This study
HG06002	Δ*bsmC*, in-frame deletion mutant strain in WT, bosamycins non-producing	This study
HG06003	Δ*bsmD*, in-frame deletion mutant strain in WT, bosamycins non-producing	This study
HG06004	Δ*bsmF*, in-frame deletion mutant strain in WT, bosamycins non-producing	This study
HG06005	Δ*bsmG*, in-frame deletion mutant strain in WT, bosamycins non-producing	This study
HG06006	Δ*bsmH*, in-frame deletion mutant strain in WT, bosamycins non-producing	This study
HG06007	Δ*orf(-1)*, in-frame deletion mutant strain in WT, bosamycins producing	This study
HG06008	Δ*bsmI*, in-frame deletion mutant strain in WT, bosamycin D producing	This study
HG06009	complementation of *ΔbsmC* mutant by *bsmC*, bosamycins producing	This study
HG06010	complementation of *ΔbsmF* mutant by *bsmF*, bosamycins producing	This study
HG06011	complementation of *ΔbsmG* mutant by *bsmG* , bosamycins producing	This study
HG06012	*Streptomyces albus* J1074 integrated with plasmid pJTU2554	This study
HG06013	*Streptomyces albus* J1074 integrated with plasmid pHG06015 which contains *bsm* biosynthetic gene cluster	This study
HG06014	HG06013 containing plasmid pHG06016	This study

Table S2. Oligonucleotide primers used in this study.

Oligonucleotide	Sequence[a]	Enzyme sites
a. for amplification of homologous arms from genomic DNA for gene disruption (5'-3')		
orf(-1)-up-F	**AACGACGGCCAGTGCC<u>AAGCTT</u>**GCTTCAAGAGCACGTCGGATAC	*Hin*dIII
orf(-1)-up-R	ATCCCGGCACTCACCAACGACCGCACAGCGTCGAAG	
orf(-1)-down-F	CTTCGACGCTGTGCGGTCGTTGGTGAGTGCCGGGAT	
orf(-1)-down-R	**AGCTATGACATGATTAC<u>GAATTC</u>**CTGCTCACCATCCACCATCT	*Eco*RI
bsmI-up-F	**AACGACGGCCAGTGCC<u>AAGCTT</u>**GTCGTCCACTTCCAGCAATAG	*Hin*dIII
bsmI-up-R	GGCATGCAGCTGATCACCCCACTGGCCGACGAGATC	
bsmI-down -F	GATCTCGTCGGCCAGTGGGGTGATCAGCTGCATGCC	
bsmI-down-R	**AGCTATGACATGATTAC<u>GAATTC</u>**GCACAAGACACCCAGACAAC	*Eco*RI
bsmA-C$_1$-up-F	**AACGACGGCCAGTGCC<u>AAGCTT</u>**GGACCATCAGGCACGACATAAC	*Hin*dIII
bsmA-C$_1$-up-R	CAATGGCCTTTGACCGCACCGCTGCACATCACGGTG	
bsmA-C$_1$-down-F	CACCGTGATGTGCAGCGGTGCGGTCAAAGGCCATTG	
bsmA-C$_1$- down -R	**AGCTATGACATGATTAC<u>GAATTC</u>**GAACCAGACGATCAGCAAGAAC	*Eco*RI
bsmC -up-F	**AACGACGGCCAGTGCC<u>AAGCTT</u>**TGTTGGCGGTGCTCAAG	*Hin*dIII
bsmC -up-R	TTGTCGATCAGCAGCAGATGTACTCGGTCGAGGTGTAGAT	
bsmC -down-F	ATCTACACCTCGACCGAGTACATCTGCTGCTGATCGACAA	
bsmC - down -R	**AGCTATGACATGATTAC<u>GAATTC</u>**ATGTGCCGTCCTGTTCC	*Eco*RI
bsmD -up-F	**AACGACGGCCAGTGCC<u>AAGCTT</u>**AAGACCCTCACCACCCTCTT	
bsmD -up-R	**TTGCTCGGCCTGGACACGCAGTTCCGGGTAGCGCGC**	
bsmD -down-F	**GCGCGCTACCCGGAACTGCGTGTCCAGGCCGAGCAA**	
bsmD - down -R	**AGCTATGACATGATTAC<u>GAATTC</u>**CGATACGGAAACCACGCAACT	
bsmF -up-F	**AACGACGGCCAGTGCC<u>AAGCTT</u>**GTATCGAACTGGGAGAAGTG	*Hin*dIII
bsmF -up-R	CATGGCCAGCGAGTCACCTTTCTCCTTGGGTGTCAT	
bsmF- down -F	ATGACACCCAAGGAGAAAGGTGACTCGCTGGCCATG	
bsmF - down -R	**AGCTATGACATGATTAC<u>GAATTC</u>**CCCTTCCTCGCTACTTCTTGTG	*Eco*RI
bsmG -up-F	**AACGACGGCCAGTGCC<u>AAGCTT</u>**TGCTGGCAGTTGGTCTTC	*Hin*dIII
bsmG -up-R	ATCTCTCGTACCAATTGGGCCTGTATCGCTGTGTCGTTCA	
bsmG - down -F	TGAACGACACAGCGATACAGGCCCAATTGGTACGAGAGAT	
bsmG - down -R	**AGCTATGACATGATTAC<u>GAATTC</u>**CCACTCCATACGATGACCTTAC	*Eco*RI
bsmH -up-F	**AACGACGGCCAGTGCC<u>AAGCTT</u>**GATCAGTGCCGCGATCAT	*Hin*dIII
bsmH-up-R	CTACTTCTTGTGCCCGATGAGGTGGAAATGAGTCCGTAGAC	
bsmH - down -F	GTCTACGGACTCATTTCCACCTCATCGGGCACAAGAAGTAG	
bsmH - down -R	**AGCTATGACATGATTAC<u>GAATTC</u>**CCGTGGAAGTAGCCGAAG	*Eco*RI
b. for screening of the correct mutants (5'-3')		
orf(-1)- Diag-F	CAGCGCGATCATGCTATCT	
orf(-1)- Diag-R	ACAGGTGGCGTTCAACTATT	
bsmI- Diag-F	AGTCTGTCGTGGCCATTCTA	
bsmI- Diag-R	CACAGGTGGCGTTCAACTATT	
bsmA-C$_1$ - Diag-F	GACCTGCTTTGCGAGTTTAC	
bsmA-C$_1$ - Diag-R	CGGAGAACCGATTACCTCTATG	

bsmC - Diag-F	CACAGGGAACAGAGGAGAATG	
bsmC - Diag-R	GCAAGGTCGAACGAGTACC	
bsmD - Diag-F	TCCCGAACCCACTGATGA	
bsmD - Diag-R	CAACTCACCGTACGAGAACAC	
bsmF - Diag-F	TGCGCTACATCGAAGAGAAC	
bsmF - Diag-R	CGAAGAGTTCGACGAGCAG	
bsmG - Diag-F	AGCTGGAGACCGACTTCTT	
bsmG - Diag-R	GTGTCCGGCTTGCCTTC	
bsmH - Diag-F	CTTCGACCCGATGACGTTC	
bsmH - Diag-R	GATGCCACATCCGGACAG	
c. for genes complementation (5'-3')		
152- *bsmC* -F	**TGCTGCATGCATACGTACTAGT**CTCAAGCGCCCGGAAAGG	*Spe*I
152- *bsmC* -R	**CTATGACATGATTACGAATTC**TCACCCGGACATGGCGAC	*Eco*RI
152- *bsmF* -F	**TGCTGCATGCATACGTACTAGT**CCGTTCGAGGCGTACCGC	*Spe*I
152- *bsmF* -R	**CTATGACATGATTACGAATTC**TCACTGTGCCTCTCGCCC	*Eco*RI
152- *bsmG* -F	**TGCTGCATGCATACGTACTAGT**GGTGACTCGCTGGCCATG	*Spe*I
152- *bsmG* -R	**CTATGACATGATTACGAATTC**TCATGGACGTTGCTCCTC	*Eco*RI
152-bsmF-T281A-F	CTGACGCACCTCGTCTCCACC	
152-bsmF-T281A-R	GCGCTGGTAACCCCCTGCGACGAG	
152-bsmF-F380A-F	GGTTCCGGTCCGCACTACTGC	
152-bsmF-F380A-R	GCGCCCGAGGTGGCGGCCGTC	
152-bsmF-C387A-F	CTGGGCGCCCCGTTCGCC	
152-bsmF- C387A-R	GCGGTAGTGCGGACCGGAACCGAA	
201- *bsmFGH*-F	**AAAGAGGAGAAATTACATATG**ATGACACCCAAGGAGAAA	*Nde*I
201-*bsmFGH*-R	**CAGGAATTCGATATCAAGCTT**AACTCAGCGGCGTGATA	*Hin*dIII
d. for protein expression		
BsmA (A_1)-28a-F	**GTGCCGCGCGGCAGCCATATG**CTGTTCGAGGCGCGGGTT	*Nde*I
BsmA (A_1)-28a-R	**CTCGAGTGCGGCCGCAAGCTT**TCAGCGTAGTTTGACCTGGTC	*Hin*dIII
BsmB (A_4-T_4)-28a-F	**GTGCCGCGCGGCAGCCATATG**CTGGCGAGCCTCCTCGAT	*Nde*I
BsmB (A_4-T_4)-28a-R	**CTCGAGTGCGGCCGCAAGCTT**TCACGACAGCGGCATCCGCTC	*Hin*dIII
BsmD (C_6-A_6-T_6)-22b-F	**AAGAAGGAGATATACATATG**GTGCCGTTGTCGTTT	*Nde*I
BsmD(C_6-A_6-T_6)-22b-R	**CTCGAGTGCGGCCGCAAGCTT**GGGCACCTCGCCCACTCC	*Hin*dIII
BsmF (A_0-T_0)-28a-F	**GTGCCGCGCGGCAGCCATATG**GCCGAGTGGAACGACACC	*Nde*I
BsmF (A_0-T_0)-28a-R	**CTCGAGTGCGGCCGCAAGCTT**TCACTGTGCCTCTCGCCC	*Hin*dIII
BsmH-22b-F	**AAGAAGGAGATATACATATG**ATGTCCCGCGCACTTGAG	*Nde*I
BsmH-22b-R	**CTCGAGTGCGGCCGCAAGCTT**CTTCTTGTGCCCGATGAC	*Hin*dIII

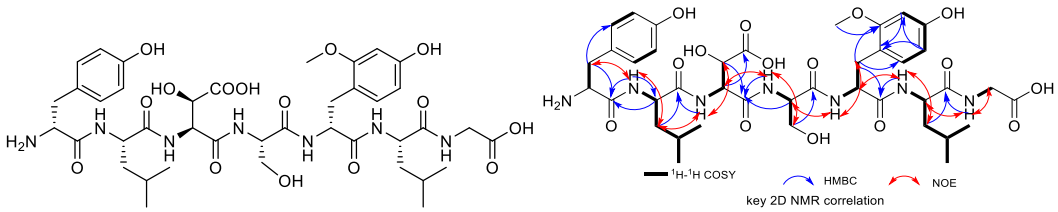
[a] Letters highlighted in bold are sequences used for ligation independent cloning and the enzyme sites are indicated by underline

Table S3. Substrate specificity predictions for the adenylation domains of the NRPSs encoded in the *bsm* gene cluster.

Module	Substrate recognition sequence	Corresponding amino acid	Predicted amino acid
M1	DASTIAAVCK	Tyr	Tyr
M2	DASTIAAVCK	Tyr	Tyr
M3	DAWMVGAVCK	Leu	Phe
M4	DLTKLGVVNK	Asp	Asn
M5	DVWHFSLVDK	Ser	Ser
M6	DASTIGAVCK	OMe-Tyr	Tyr
M7	DAWMVGAVCK	Leu	Phe
M8	DILQLGVIWK	Gly	Gly
M0	DGSIAALVWK	Tyr	Tyr

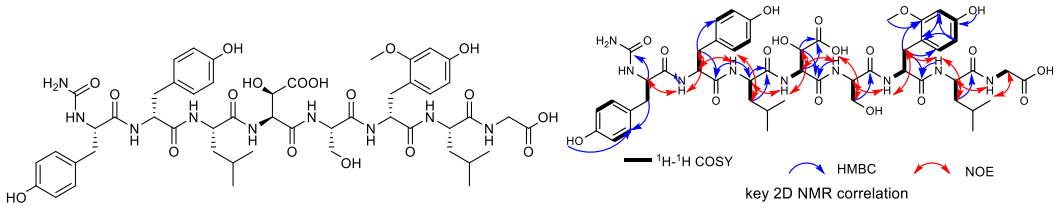
The prediction of the substrate specificity was based on NRPS Predictor2.[18]

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



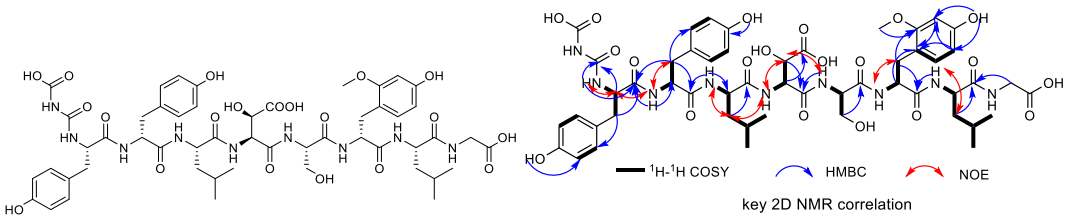
No	δ_C (type)	δ_H, multi. (*J* in Hz)	No	δ_C (type)	δ_H, multi. (*J* in Hz)
D-Tyr	169.44, C		D-OCH_3-Tyr	171.49, C	
	54.67, CH	3.92, d (7.4)		54.28, CH	4.30, dd (14.6, 7.1)
	36.85, CH_2	2.86, d (7.2)		31.57, CH_2	2.92, dd (13.5, 6.5)
	125.39, C				2.72, dd (13.3, 8.5)
	130.69, CH	7.00, d (8.1)		115.96, C	
	115.65, CH	6.68,d (8.0)		157.74, C	
	156.96, C			55.55, CH_3	3.70, s
	115.65, CH	6.68, d (8.0)		99.06, CH	6.31, s
	130.69, CH	7.00, d (8.1)		158.49, C	
L-Leu	171.47, C			131.51, CH	6.83, d (8.1)
	51.27, CH	4.28, m		106.87, CH	6.18, d (8.0)
	39.41, CH_2	1.39, 1.26, m		NH	8.12, d (6.5)
	23.99, CH	1.02, m	L-Leu	172.32, C	
	23.63, CH_3	0.75, d (6.5)		51.55, CH	4.14, dd (15.8,7.8)
	23.7, CH3	0.77, d (6.5)		40.59, CH_2	1.41, m
	NH	8.67, d (7.4)		24.15, CH	1.26, m
L- *erythro* -OHAsp	169.72, C			21.49, CH_3	0.68, d (6.4)
	57.63, CH	4.29, m		21.69, CH_3	0.70, d (6.4)
	72.86, CH	3.94, d (4.1)		NH	7.88, d (8.2)
	174.47, C		Gly	171.86, C	
	NH	8.04, d (5.7)		41.68, CH_2	3.70, d (5.4)
L-Ser	170.33, C			NH	8.21, t (5.4)
	55.91, CH	4.12, m			
	61.92, CH_2	3.60, m			
	NH	7.78, d (7.2)			

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



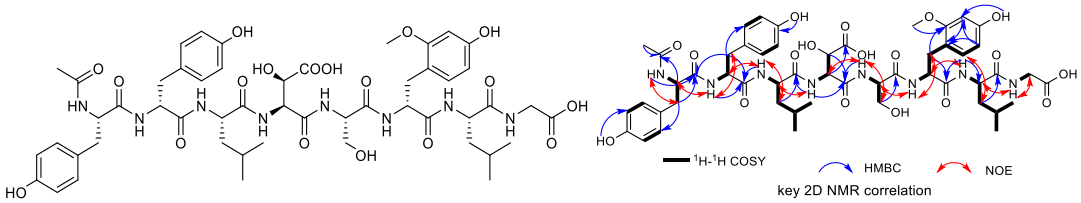
No	δ_C (type)	δ_H, multi. (*J* in Hz)	No	δ_C (type)	δ_H, multi. (*J* in Hz)
L-Tyr	172.41, C		L-Ser	170.29, C	
	54.82, CH	4.25, m		55.76, CH	4.18, q (6.0)
	38.04, CH_2	2.57, dd (13.8, 4.0),		62.06, CH_2	3.56, dd (10.6, 5.3),
		2.35, dd (13.9, 8.6)			3.48, dd (10.1, 3.6)
	128.32, C			NH	7.79, d (7.4)
	130.62, CH	6.70, d (8.1)	D-OCH_3-Tyr	171.31, C	
	115.16, CH	6.54, d (8.4)		54.2, CH	4.33, dd (14.7, 7.4)
	156.1, C			31.87, CH_2	2.82, dd (13.6, 4.3)
	115.16, CH	6.54, d (8.4)			2.71, dd (13.3, 7.4)
	130.62, CH	6.70, d (8.1)		115.53, C	
	158.74, C			157.84, C	
D-Tyr	171.62, C			55.53, CH_3	3.70, s
	54.92, CH	4.46, dd (14.2, 8.7)		99.07, CH	6.30, d (1.9)
	37.67, CH_2	2.86, 2.59, m		158.52, C	
	128.17, C			131.44, CH	6.79, d (8.1)
	130.69, CH	7.06, d (8.3)		106.88, CH	6.17, dd (8.1, 2.2)
	115.23, CH	6.62, d (8.4)		NH	7.93, br s
	156.31, C		L-Leu	172.88, C	
	115.23, CH	6.62, d (8.4)		51.31, CH	4.11, dd (15.4, 8.1)
	130.69, CH	7.06, d (8.3)		40.73, CH_2	1.35, m
	NH	8.24, d (8.6)		24.08, CH	1.17, m
L-Leu	172.55, C			21.69, CH_3	0.68, d (6.5)
	51.27, CH	4.42, m		21.74, CH_3	0.77, d (6.5)
	41.35, CH_2	1.46, 1.41, m		NH	7.88, d (8.2)
	24.45, CH	1.42, m	Gly	171.53, C	
	23.62, CH_3	0.76, d (5.7)		41.13, CH_2	3.71,s
	23.71, CH_3	0.80, d (5.7)		NH	8.11, br s
	NH	8.19, d (9.0)			
L- *erythro* -OHAsp	169.09, C				
	55.53, CH	4.70, t (5.4)			
	71.46, CH	4.08, d (5.3)			
	173.27, C				
	NH	8.29, br s			

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



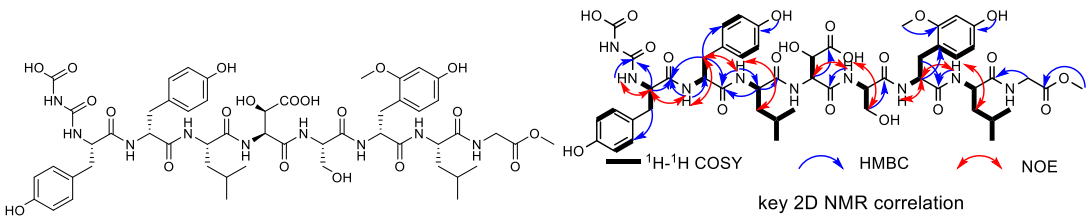
No	δ_C δ_N (type)	δ_H, multi. (*J* in Hz)	No	δ_C δ_N (type)	δ_H, multi. (*J* in Hz)
L-Tyr	169.84, C		L- *erythro* -OHAsp	168.65, C	
	53.85, CH	4.47, m		55.34, CH	4.71, dd (7.6, 5.8)
	36.59, CH_2	2.69, 2.58, m		71.85, CH	4.06, d (3.7)
	127.11, C			171.14, C	
	130.11, CH	6.69, d (8.3)		NH	8.353, br s
	114.82, CH	6.53, d (8.4)	L-Ser	169.74, C	
	155.92, C			55.24, CH	4.16, m
	114.82. CH	6.53, d (8.4)		61.56, CH_2	3.56, dd (10.4, 5.0),
	130.11, CH	6.69, d (8.3)			3.49, dd (10.4, 4.0)
	114.93, NH	8.20, d (8.6)		115.34, NH	7.76, d (6.6)
	158.84, C		D-OCH_3-Tyr	170.89, C	
	158.07, C			54.20, CH	4.33, dd (14.9, 7.5)
D-Tyr	170.85, C			31.37, CH_2	2.71, dd (13.0, 7.7),
	54.92, CH	4.54, m			2.89, dd (13.4, 4.5)
	37.68, CH_2	2.88, dd (13.4, 4.5),		115.23, C	
		2.63, m		156.31, C	
	128.05, C			55.09, CH_3	3.70, s
	130.23, CH	7.07, d (8.4)		98.62, CH	6.30, d (2.0)
	114.79, CH	6.63, d (8.4)		157.35, C	
	155.79, C			131.04, CH	6.80, d (8.1)
	114.79, CH	6.63, d (8.4)		106.43, CH	6.18, dd (8.1, 2.0)
	130.23, CH	7.07, d (8.4)		120.75, NH	7.96, br s
	117.76, NH	8.40, d (8.3)	L-Leu	172.43, C	
L-Leu	172.07, C			50.97, CH	4.12, dd (15.7, 7.9)
	50.67, CH	4.44, m		40.27, CH_2	1.36, m
	41.16, CH_2	1.43, m		23.68, CH	1.23, m
	24.09, CH	1.43, m		21.29, CH_3	0.69, d (6.4)
	23.17, CH_3	0.80, d (6.6)		21.22, CH_3	0.78, d (6.4)
	23.27, CH_3	0.76, d (6.6)		120.37, NH	7.89, d (8.3)
	119.15, NH	8.25, d (8.4)	Gly	172.84, C	
				40.85, CH_2	3.71, s

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



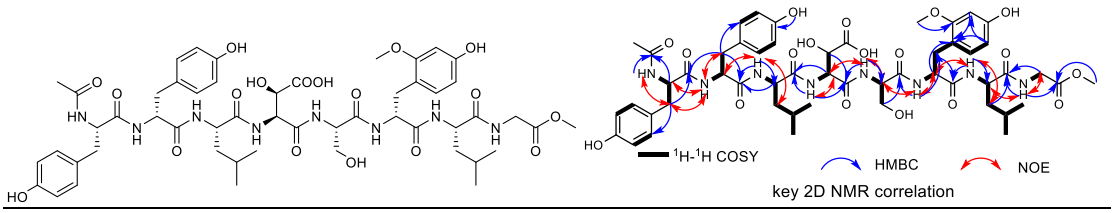
No	δ_C (type)	δ_H, multi. (*J* in Hz)	No	δ_C (type)	δ_H, multi. (*J* in Hz)
L-Tyr	170.89, C		L- *erythro* –OHAsp	168.66, C	
	54.73, CH	4.47, m		55.62, CH	4.72, m
	37.27, CH_2	2.37, 2.58, m		71.40, CH	4.00, m
	128.45, C			171.34, C	
	130.5, CH	6.88, d (7.8)		NH	8.322, d (7.6)
	115.34, CH	6.61, d (8.1)	L-Ser	169.82, C	
	155.8, C			55.69, CH	4.19, m
	115.34, CH	6.61, d (7.8)		62.03, CH_2	3.50. 3.56, m
	130.5, CH	6.88, d (8.1)		NH	7.80, d (7.1)
	NH	8.27, d (8.2)	D-OCH_3-Tyr	171.11, C	
	169.26, C			54.21, CH	4.31, m
	22.83, CH_3	1.721, s		31.93, CH_2	2.71, 2.82, m
D-Tyr	169.73, C			115.57, C	
	54.68, CH	4.54, dd (13.4, 8.3)		157.42, C	
	37.95, CH_2	2.85, 2.60, m		55.51, CH_3	3.71, s
	128.02, C			99.13, CH	6.31, s
	130.67, CH	7.01, d (7.9)		158.07, C	
	115.33, CH	6.59, d (7.8)		131.42, CH	6.79, d (8.0)
	156.28			106.88, CH	6.18, d (8.1)
	115.33, CH	6.59, d (7.8)		NH	7.93, br s
	130.67, CH	7.01, d (7.9)	L-Leu	172.4, C	
	NH	8.40, d (7.9)		51.33, CH	4.11, dd (13.4,6.6)
L-Leu	172.15, C			40.78, CH_2	1.35, m
	51.11, CH	4.41, m		24.12, CH	1.17, m
	41.63, CH_2	1.43, m		21.74, CH_3	0.69, d (6.1)
	24.58, CH	1.42, m		21.57, CH_3	0.76, d (6.1)
	23.65, CH_3	0.77, d (6.4)		NH	7.89, d (8.0)
	23.74, CH_3	0.80, d (6.4)	Gly	172.73, C	
	NH	8.19, d (7.8)		41.15, CH_2	3.71, s
				NH	8.12, br s

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



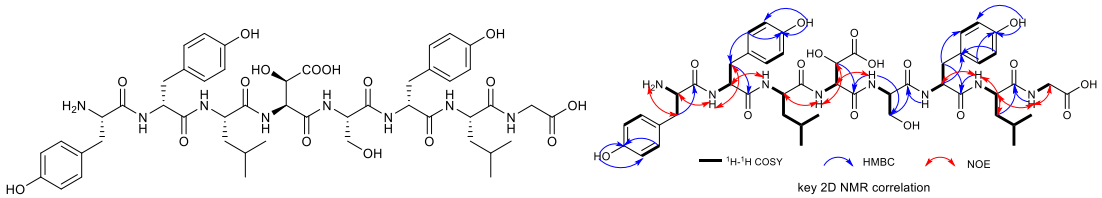
No	δ_C (type)	δ_H, multi. (*J* in Hz)	No	δC (type)	δ_H, multi. (*J* in Hz)
L-Tyr	170.24, C		L- *erythro* -OHAsp	169.24, C	
	54.43, CH	4.48, m		56.49, CH	4.63, m
	37.67, CH_2	2.72, 2.62, m		72.98, CH	3.98, d (3.8)
	127.52, C			170.04, C	
	130.56, CH	6.71, d (8.3)	L-Ser	170.39, C	
	115.26, CH	6.53, d (8.4)		55.66, CH	4.11, m
	156.37, C			62.01, CH_2	3.60, 3.52, m
	115.26, CH	6.53, d (8.4)		NH	7.67, d (6.9)
	130.56, CH	6.71, d (8.3)		54.94, CH	4.52, m
	NH	8.25, d (9.1)	D-OCH_3-Tyr	171.51, C	
	159.31, C			54.18, CH	4.34, dd (14.9, 7.8)
	157.67, C			31.64, CH_2	2.70, m
D-Tyr	171.30, C				2.98, dd (13.3, 4.7)
	54.94, CH	4.52, m		115.23, C	
	37.97, CH_2	2.89, 2.66, m		156.39, C	
	128.18, C			55.54, CH_3	3.70, s
	130.56, CH	7.06, d (8.4)		99.05, CH	6.30, d (1.9)
	115.25, CH	6.63, d (8.4)		158.49, C	
	156.27, C			131.59, CH	6.82, d (8.2)
	115.25, CH	6.63, d (8.4)		106.88, CH	6.18, dd (8.1, 1.9)
	130.56, CH	7.06, d (8.4)		NH	8.15, br s
	NH	8.40, d (8.3)	L-Leu	173.25, C	
L-Leu	172.35, C			51.68, CH	4.16, dd (14.6, 9.1)
	51.25, CH	4.43, m		41.11, CH_2	1.44, m
	40.68, CH_2	1.44, m		24.27, CH	1.314 m
	24.46, CH	1.44, m		21.79, CH_3	0.73, d (6.4)
	23.75, CH_3	0.82, d (5.4)		21.77, CH_3	0.79, d (6.4)
	23.52, CH_3	0.80, d (5.4)		NH	7.96, d (8.3)
	NH	8.22, d (9.1)	Gly	52.06, CH_3	3.58, s
				170.58, C	
				41.36, CH_2	3.81, dd (7.6, 6.6)

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



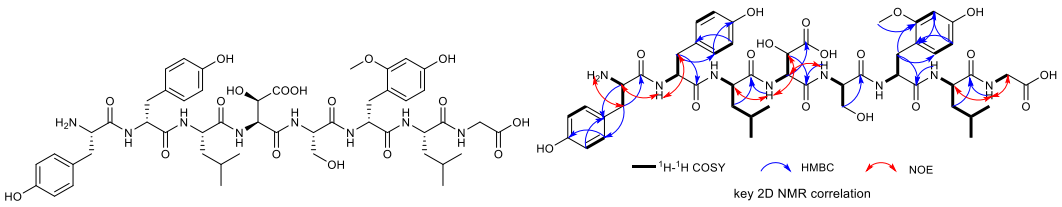
No	δ_C (type)	δ_H, multi. (*J* in Hz)	No	δ_C (type)	δ_H, multi. (*J* in Hz)
L-Tyr	171.82, C		L- *erythro* -OHAsp	169.04, C	
	54.8, CH	4.38, m		55.45, CH	4.73, dd (8.77, 5.9)
	37.35, CH_2	2.38, dd (13.7, 9.6)		71.38, CH	4.10, d (5.8)
		2.55, dd (13.2, 9.4)		173.08, C	
	128.45, C			NH	8.32, d (6.5)
	130.53, CH	6.88, d (8.4)	L-Ser	170.37, C	
	115.32, CH	6.61, d (8.3)		55.69, CH	4.20, dd (12.4, 5.0)
	156.15, C			62.06, CH_2	3.50,3.55, dd (11.0, 5.3)
	115.34, CH	6.61, d (8.3)		NH	7.77, d (7.5)
	130.51, CH	6.88, d (8.3)	D-OCH_3-Tyr	171.4, C	
	NH	7.91, d (7.9)		54.24, CH	4.30, dd (14.7, 7.4)
	169.79, C			31.82, CH_2	2.71, dd (13.3, 7.1),
	22.86, CH_3	1.73, s			2.82, dd (13.6, 5.1)
D-Tyr	171.65, C			115.57, C	
	54.91, CH	4.46, d (14.3, 8.5)		157.86, C	
	37.71, CH_2	2.83, 2.61, m		55.54, CH_3	3.70, s
	128.02, C			99.03, CH	6.31, d (6.3)
	130.67, CH	7.01, d (8.5)		158.52, C	
	115.33 CH	6.59, d (8.3)		131.41, CH	6.77, d (8.2)
	156.28, C			106.88, CH	6.18, dd (8.1, 2.0)
	115.33, CH	6.59, d (8.5)		NH	7.96, d (6.7)
	130.67, CH	7.01, d (8.5)	L-Leu	173.11, C	
	NH	8.29, d (7.8)		51.23, CH	4.11, d (5.8)
L-Leu	172.64, C			40.57, CH_2	1.34, m
	51.29, CH	4.37, m		24.04, CH	1.15, m
	41.17, CH_2	1.46, m		21.74, CH_3	0.68, d (6.5)
	24.41, CH	1.42, m		21.58, CH_3	0.75, d (6.5)
	23.57, CH_3	0.77, d (5.6)		NH	7.93, d (5.5)
	23.68, CH_3	0.79, d (5.6)	Gly	52.26, CH_3	3.60, s
	NH	8.19, d (8.2)		170.57, C	
				41.05, CH_2	3.80, d (5.9)
				NH	8.16, d (6.0)

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



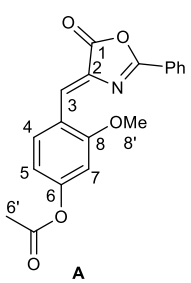
No	δ_C (type)	δ_H, multi. (*J* in Hz)	No	δ_C (type)	δ_H, multi. (*J* in Hz)
L-Tyr	170.64, C		L-Ser	169.40, C	
	54.12, CH	4.70, s		55.10, CH	4.22, m
	36.19, CH_2	2.57, m		61.70, CH_2	3.50, dd (10.3, 5.3),
		2.79, m			3.54, dd (10.3, 5.3)
	127.15, C			NH	7.74, d (7.6)
	130.56, CH	6.79, d (8.7)	D-Tyr	170.54, C	
	114.81, CH	6.61, d (8.7)		54.80, CH	4.41, m
	156.55, C			37.95, CH_2	2.69, m
	114.81, CH	6.61, d (8.7)			2.79, m
	130.56, CH	6.79, d (8.7)		127.26, C	
	NH	8.78, d (8.6)		130.28, CH	7.03, d (8.4)
D-Tyr	167.73, C			114.81, CH	6.61, d (8.4)
	54.80, CH	4.40, m		156.06, C-OH	9.18,s
	37.05, CH_2	2.69, 2.78, m		114.81, CH	6.61, d (8.4)
	124.60, C			130.28, CH	7.03, d (8.4)
	130.16, CH	6.96, d (8.4)		NH	7.89, br s
	114.81, CH	6.61, d (8.4)	L-Leu	172.39, C	
	155.84,C-OH	9.16, s		50.75, CH	4.21, dd (15.4, 8.1)
	114.81, CH	6.61, d (8.4)		40.63, CH_2	1.35, m
	130.16, CH	6.96, d (8.4)		23.77, CH	1.27, m
	NH	8.47, d (8.2)		21.27, CH_3	0.72, d (6.3)
L-Leu	172.34, C			21.11, CH_3	0.75, d (6.3)
	50.79, CH	4.38, m		NH	8.13, d (8.3)
	40.67, CH_2	1.44, m	Gly	171.08, C	
	24.09, CH	1.44, m		40.62, CH_2	3.71, t (5.9)
	23.17, CH_3	0.82, d (6.2)		NH	8.19, t (5.9)
	23.28, CH_3	0.79, d (6.2)			
	NH	7.94, d (7.5)			
L- *erythro* -OHAsp	168.60, C				
	54.10, CH	4.68, m			
	71.00, CH	4.07, d (5.7)			
	172.66, C				
	NH	8.33, d (7.4)			

Table S11. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data of compound **14** in DMSO-d_6.

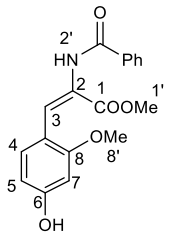


No	δ_C (type)	δ_H, multi. (*J* in Hz)	No	δ_C (type)	δ_H, multi. (*J* in Hz)
L-Tyr	171.26, C		L-Ser	170.08, C	
	55.00, CH	4.71, m		55.50, CH	4.21, m
	38.46, CH_2	2.58, m		61.90, CH_2	3.60, dd (10.9, 5.0),
		2.81, m			3.64, dd (11.0, 5.4)
	127.60, C			NH	7.78, d (7.7)
	130.74, CH	7.03, d (8.4)	D-OCH_3-Tyr	171.04, C	
	115.44, CH	6.62, d (8.4)		54.09, CH	4.35, dd (14.7, 7.4)
	156.50, C			32.05, CH_2	2.79, m
	115.44, CH	6.62, d (8.4)			2.71, m
	130.74, CH	7.03, d (8.4)		115.44, C	
	NH	8.78, d (8.7)		158.53, C	
D-Tyr	168.16, C			55.55, CH_3	3.70, s
	54.03, CH	4.40, m		99.08, CH	6.31, d (1.3)
	36.64, CH_2	2.77, 2.80, m		157.87, C-OH	9.20, s
	125.08, C			131.41, CH	6.79, dd (8.1, 3.9)
	131.03, CH	6.79, d (8.1)		106.88, CH	6.18, d (8.0)
	115.44, CH	6.62, d (8.3)		NH	7.88, br s
	157.87,C-OH	9.17, s	L-Leu	172.77, C	
	115.44, CH	6.62, d (8.3)		51.26, CH	4.13, m
	131.03, CH	6.79, d (8.3)		40.81, CH_2	1.34, m
	NH	8.47, d (8.2)		24.05, CH	1.17, m
L-Leu	172.64, C			21.77, CH_3	0.69, d (6.5)
	51.18, CH	4.40, m		21.54, CH_3	0.79, d (6.5)
	41.40, CH_2	1.44, m		NH	7.90, d (7.5)
	24.55, CH	1.44, m	Gly	173.07, C	
	23.62, CH_3	0.76, d (5.7)		41.08, CH_2	3.71, s
	23.72, CH_3	0.83, d (5.7)		NH	8.10, d (5.8)
	NH	7.90, d (9.0)			
L- *erythro* -OHAsp	169.06, C				
	55.00, CH	4.71, m			
	71.44, CH	4.07, d (5.8)			
	172.89, C				
	NH	8.37, d (8.7)			

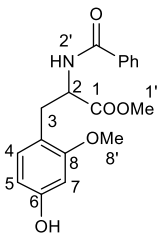
Table S12. ^{1}H NMR (400 MHz) data of **A**-**E** in DMSO-d_6.



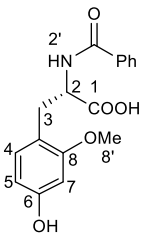
A



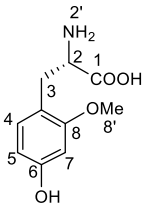
B



C



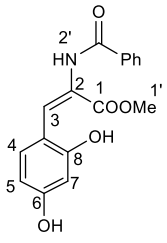
D



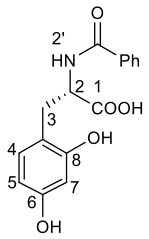
E

No	**A**	**B**	**C**	**D**	**E**
	δ_H (multi, *J*, Hz)	δ_H (multi, *J*, Hz)	δ_H (multi, *J*, Hz)	δ_H (multi, *J*, Hz)	δ_H (multi, *J*, Hz)
1’		3.67 (s)	3.60 (s)		
2			4.58 (dd, 13.6, 9.0)	4.55 (m)	3.96 (t, 5.5)
2'-NH			8.66 (d, 7.6)	8.49 (d, 8.0)	8.12 (s)
3	7.51 (s)	7.70 (s)	2.86 (dd, 13.5, 9.7)	2.82 (dd,13.5, 10.5)	2.86 (dd, 14.0, 7.1)
			3.10 (dd, 13.5, 5.5)	3.16 (dd, 13.5, 4.4)	3.30 (dd, 14.0, 6.4)
4	8.81 (d, 8.6)	7.48 (d, 7.7)	6.97 (d, 8.1)	7.00 (d, 8.0)	6.91 (d, 8.1)
5	6.94 (dd, 8.6,1.8)	6.20 (d, 8.6)	6.97 (d, 8.1)	6.23 (dd, 8.1, 1.5)	6.29 (dd, 8.1, 2.2)
6-OH		9.75 (brs)	9.35 (brs)	9.24 (brs)	9.47 (brs)
6’	2.31 (s)				
7	7.00 (d, 1.8 **)**	6.32 (s)	6.37 (d, 1.5)	6.36 (d, 1.5)	6.40 (d, 2.1 **)**
8’	3.91 (s)	3.75 (s)	3.74 (s)	3.73 (s)	3.71 (s)
Ph	8.12 (d, 7.4)	7.95 (d, 7.5)	7.79 (d, 7.4)	7.77 (d, 7.5)	
	8.12 (d, 7.4)	7.95 (d, 7.5)	7.79 (d, 7.4)	7.77 (d, 7.5)	
	7.73 (m)	7.56 (t, 7.1)	7.53 (t, 7.2)	7.53 (t, 7.2)	
	7.64 (t, 7.6)	7.48 (t, 7.6)	7.46 (t,7.1)	7.45 (t, 7.5)	
	7.64 (t, 7.6)	7.48 (t, 7.6)	7.46 (t,7.1)	7.45 (t, 7.5)	

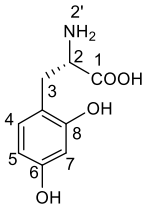
Table S13. ^{1}H NMR (400 MHz) data of **G**, **I** and **J** in DMSO-d_6.



G



I



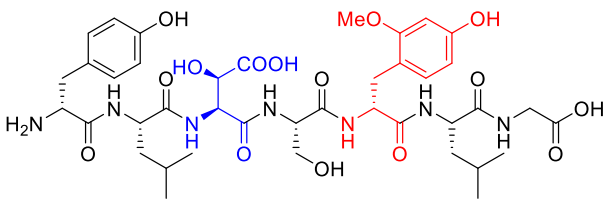
J

No	**G**	**I**	**J**
	δ_H (multi, *J*, Hz)	δ_H (multi, *J*, Hz)	δ_H (multi, *J*, Hz)
1’	3.69 (s)		
2		4.55 (m)	3.95 (t, 5.5)
2'-NH		8.49 (brd, 7.7)	8.28 ((brd, 7.7)
3	7.70 (s)	2.80 (dd,13.6, 10.3)	2.85 (dd, 13.8, 7.4)
		3.08 (dd, 13.6, 4.3)	2.98 (dd, 13.8, 6.6)
4	7.52 (d, 8.6)	6.92 (d, 8.2)	6.83 (d, 8.2)
5	6.19 (dd, 8.6, 2.1)	6.09 (dd, 8.1, 2.5)	6.16 (dd, 8.1, 2.4)
6-OH	9.75 (s)	9.03 (brs)	
7	6.37 (d, 2.1)	6.27 (d, 2.3)	6.39 (d, 2.3 **)**
8-OH	10.10 (brs)	9.47 (brs)	9.66 (brs)
Ph	7.96 (d, 7.4)	7.77 (d, 7.5)	
	7.96 (d, 7.4)	7.77 (d, 7.5)	
	7.58 (t, 7.4)	7.52 (t, 7.5)	
	7.48 (t, 7.4)	7.45 (t, 7.5)	
	7.48 (t, 7.4)	7.45 (t, 7.5)	

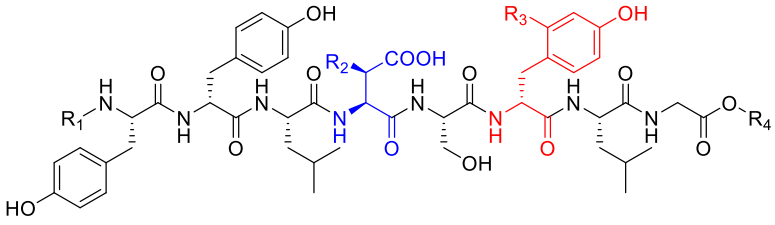
Table S14. Conserved sequence regions in the alignment comparisons of BsmF and other known P450s (numbering indicated for BsmF).[19,20]

P450	B-B2 loop N-term		B-B2 loop C-term						F-helix				G-helix					I-helix			β-1 sheet		
	87	90	107	108	109	111	114	115	194	195	196	197	216	217	218	219	222	270	271	277	324	325	326
OxyD	G	I	S	G	G	M	V	S	H	A	F	G	A	H	T	E	V	N	C	G	A	M	H
ClоI	G	L	A	S	G	M	V	T	H	A	W	S	A	K	N	E	L	N	C	G	S	L	H
NovI	G	L	A	S	G	M	V	T	H	A	W	S	A	K	N	E	L	N	C	G	S	L	H
SimD1	G	L	A	S	R	M	L	T	H	A	L	S	A	K	N	E	L	N	C	G	S	L	H
Sky32	G	L	A	A	G	M	V	T	S	A	L	S	A	R	N	E	L	N	C	G	A	M	H
Consensus*	G	L	A	(1)	G	M	V	T	H	A	(2)	S	A	(3)	N	E	(4)	**N**	C	**G**	(5)	LM	H
BsmF-P450	A	G	M	G	S	Q	F	N	S	Y	E	R	L	L	D	K	A	N	A	G	S	Q	Y

* Identity residues shown in bold and underlined, mismatching residues or similar residues indicated in normal font. Exceptions are: (1) Small residue (S, G, A),(2) large hydrophobic (W, F, L), (3) positively charged residue (K, H, R), (4) majority hydrophobic (L, V; also S and G), (5) majority small (S, A; also V). Protein accession number: OxyD (3MGX_A); ClоI (AAN65225); NovI (Q9L9F9); SimD1 (AAK06805); Sky32 (4L0F_A).



1 bosamycin A



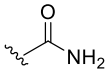
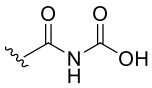
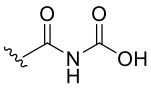
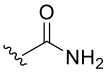
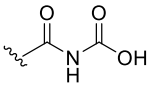
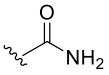
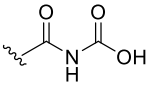
		R_1	R_2	R_3	R_4
2	bosamycin B		-OH	-OMe	H
3	bosamycin C		-OH	-OMe	H
4	bosamycin D		-OH	-OMe	H
5	bosamycin E		-OH	-OMe	-Me
6	bosamycin F		-OH	-OMe	-Me
7	bosamycin G		-OH	H	H
8	bosamycin H		-OH	H	H
9	bosamycin I		-OH	H	H
10	bosamycin J		H	-OMe	H
11	bosamycin K		H	-OMe	H
12	bosamycin L		H	-OMe	-Me
13	bosamycin M	H	-OH	H	H
14	bosamycin N	H	-OH	-OMe	H

Figure S1. Structures of bosamycins.

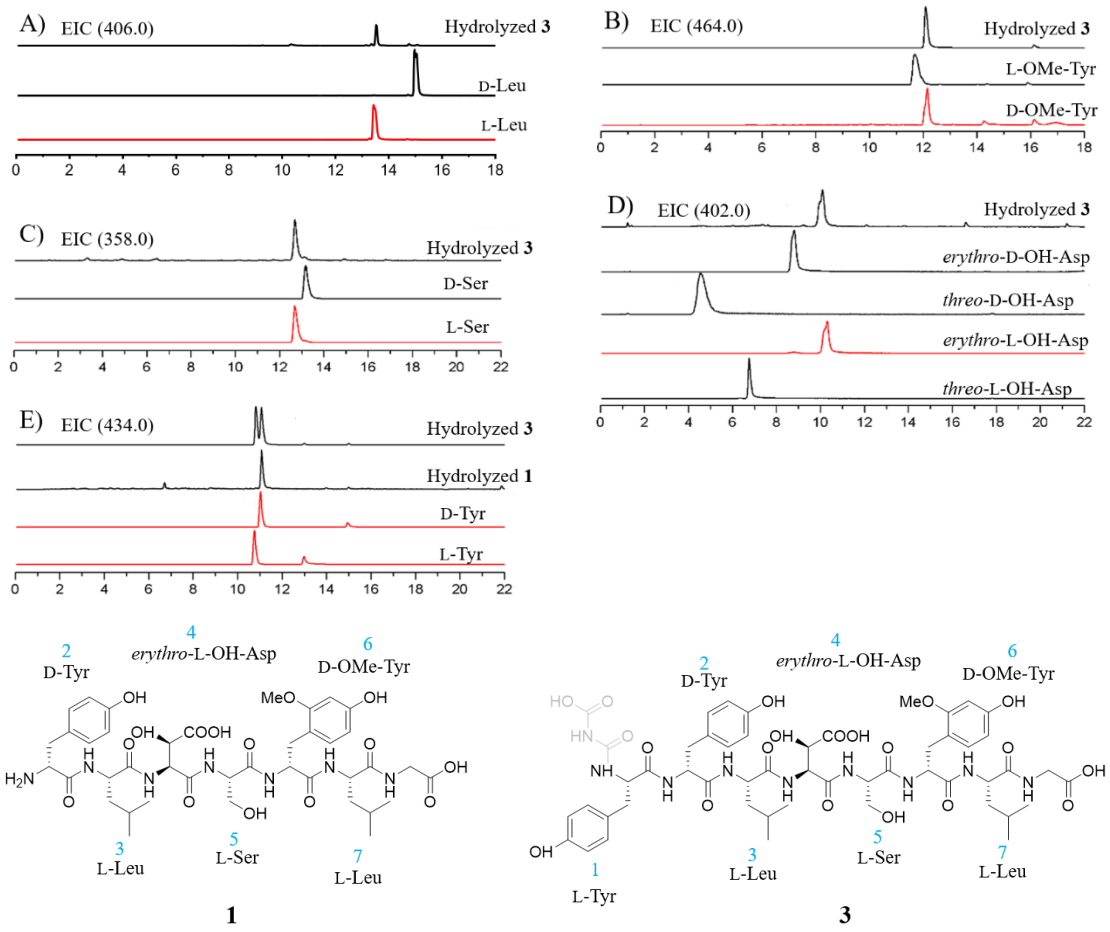
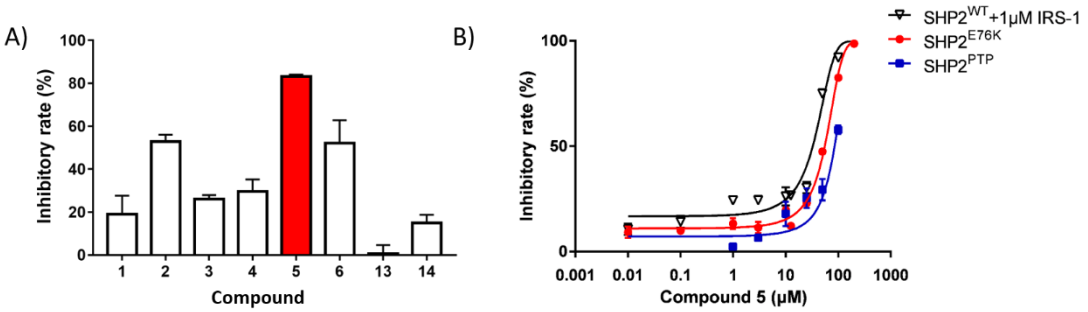


Figure S2. LC-MS analysis of L-FDAA and D-FDAA derivatives of the amino acid residues in **1** and **3**. Panel A indicates 3rd and 7th Leu in **3** is L-type; Panel B indicates 6th OMe-Tyr in **3** is D-type, Panel C indicates 5th Ser in **3** is L-type; Panel D indicates 4th OH-Asp in **3** is *erythro*-L-OH-Asp; Panel E indicates 2nd Tyr in **1** and **3** is D-type, 1st Tyr in **3** is L-type. The deduced D-type configurations in 2nd, and 6th amino acid residues are consistent with the presence of E domains in their corresponding modules (Scheme 1).



C)

Enzyme	IC_{50} (μM)
$SHP2^{WT}$	24.25
$SHP2^{E76K}$	45.56
$SHP2^{PTP}$	89.98

Figure S3. Identification of compound **5** as a novel inhibitor of SHP2. A) Primary screen the biological activity of compounds on SHP2 enzyme activity were examined. SHP2 was screened in the presence of 1 μM 2P-IRS-1 and 30 μM of each compound. B) Phosphatase activities of $SHP2^{WT}$, $SHP2^{E76K}$, $SHP2^{PTP}$ were assessed in the presence of compound **5** at various concentrations. C) The IC_{50} value of **5** against $SHP2^{WT}$, $SHP2^{E76K}$, $SHP2^{PTP}$.

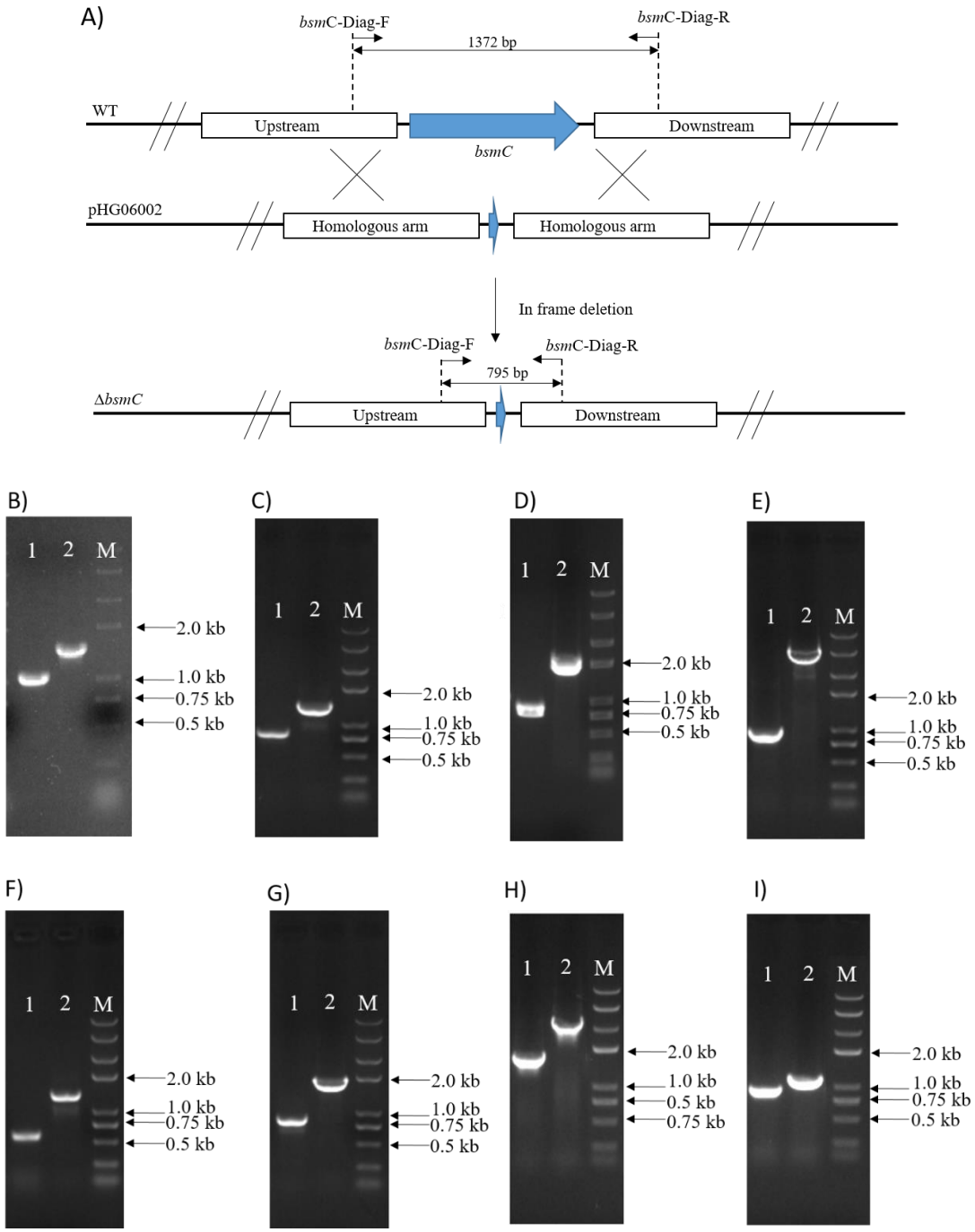


Figure S4. Generation of the *S.* sp. 120454 mutant strains. A) Gene disruption with homologous recombination strategies. B) The *S.* sp. 120454 HG06001 mutant (Δ*bsmA*-C_1). Lane 1, mutant strain; Lane 2, *S.* sp. 120454 WT; C) The *S.* sp. 120454 HG06002 mutant (Δ*bsm*C). Lane 1, mutant strain; Lane 2, *S.* sp. 120454 WT; D) The *S.* sp. 120454 HG06003 mutant (Δ*bsm*D). Lane 1, mutant strain; Lane 2, *S.* sp. 120454 WT; E) The *S.* sp. 120454 HG06004 mutant (Δ*bsm*F). Lane 1, mutant strain; Lane 2, *S.* sp. 120454 WT; F) The *S.* sp. 120454 HG06005 mutant (Δ*bsm*G). Lane 1, mutant strain; Lane 2, *S.* sp. 120454 WT; G) The *S.* sp. 120454 HG06006 mutant (Δ*bsm*H). Lane 1, mutant strain; Lane 2, *S.* sp. 120454 WT; H) The *S.* sp. 120454 HG06007 mutant (Δ*orf(-1)*). Lane 1, mutant strain; Lane 2, *S.* sp. 120454 WT; I) The *S.* sp. 120454 HG06008 mutant (Δ*bsmI*). Lane 1, mutant strain; Lane 2, *S.* sp. 120454 WT; Lane M, *Trans*2K® Plus II DNA marker.

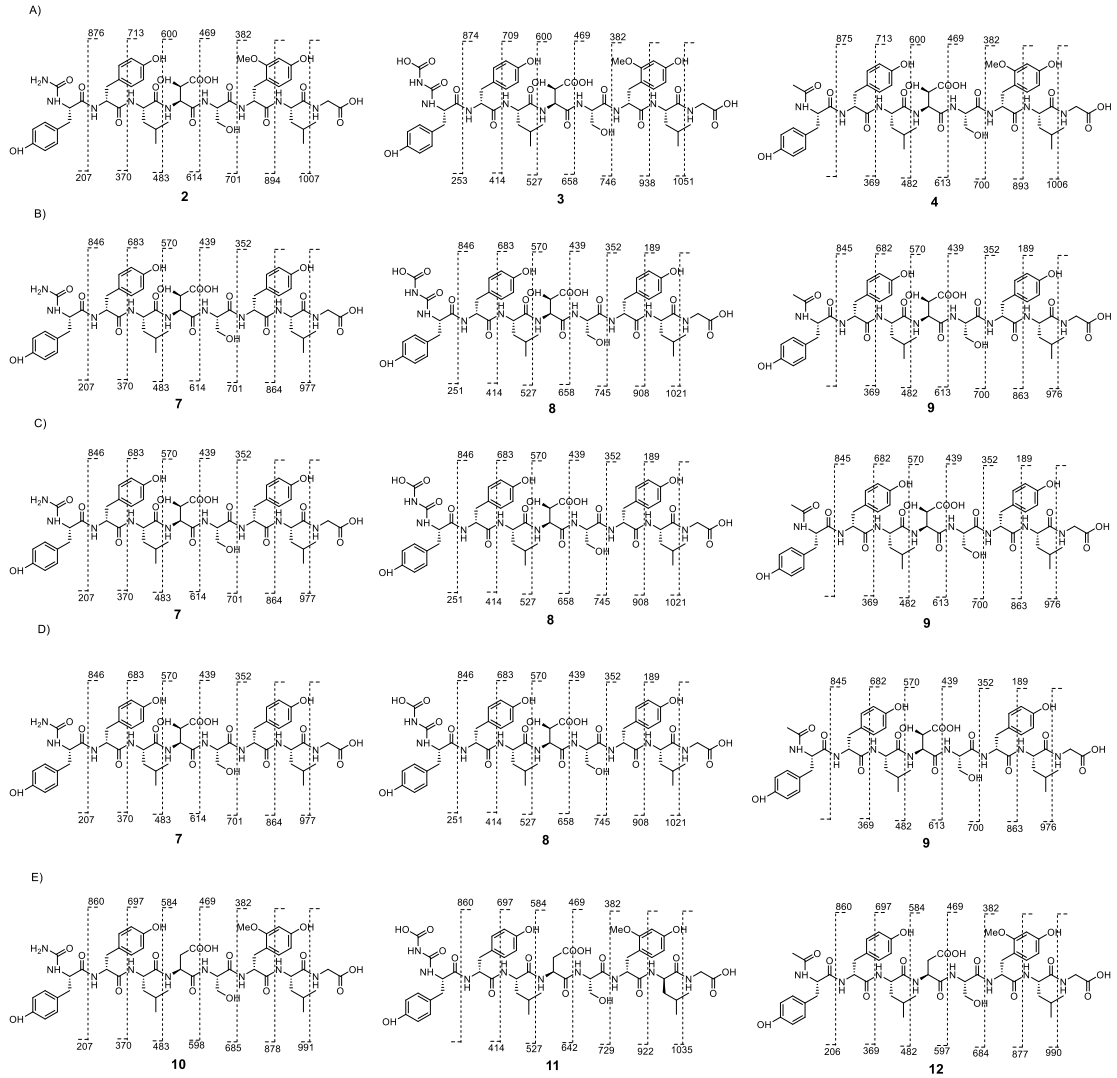


Figure S5. MS/MS analysis of metabolite extracts from wild type and mutant strains. A) *S.* sp. 120454 WT. B) The *S.* sp. 120454 HG06004 mutant (Δ*bsm*F). C) The *S.* sp. 120454 HG06005 mutant (Δ*bsm*G). D) The *S.* sp. 120454 HG06005 mutant (Δ*bsm*H). E) The *S.* sp. 120454 HG06002 mutant (Δ*bsm*C).

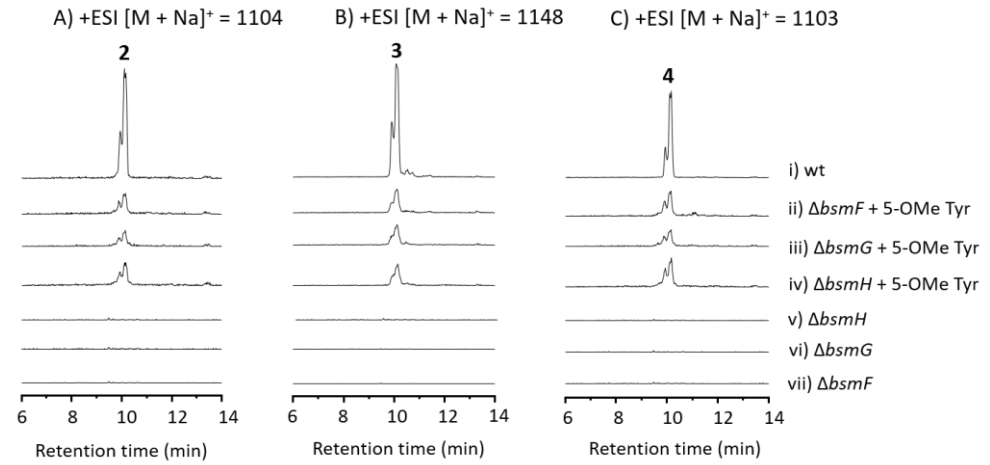


Figure S6. Chemical complementation of 5-OMe Tyr into mutants. LC-MS analyses of A) compound **2**, B) compound **3** and C) compound **4** for different mutant strains. i) wt; ii) Δ*bsmF* mutant fed with 5-OMe Tyr; iii) Δ*bsmG* mutant fed with 5-OMe Tyr; iv) Δ*bsmH* mutant fed with 5-OMe Tyr; v) Δ*bsmH* mutant; vi) Δ*bsmG* mutant; vii) Δ*bsmF* mutant.

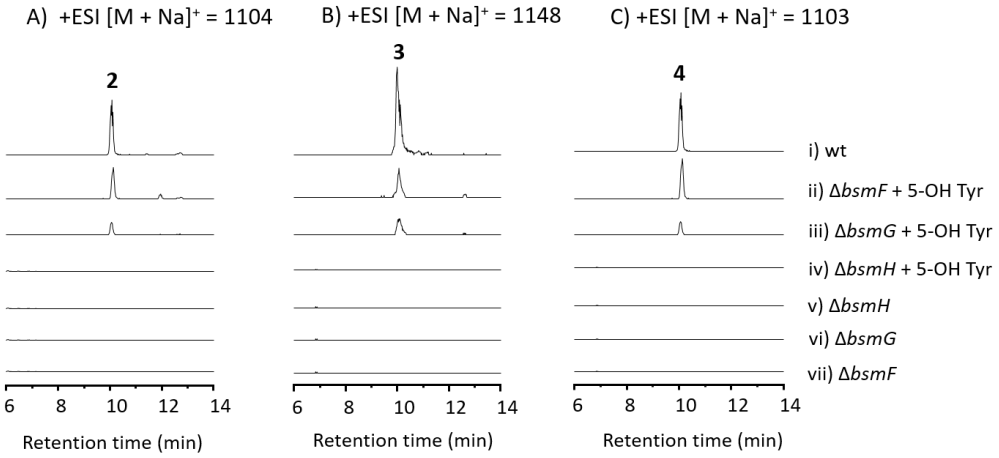


Figure S7. Chemical complementation of 5-OH Tyr into mutants. LC-MS analyses of A) compound **2**, B) compound **3** and C) compound **4** for different mutant strains. i) wt; ii) Δ*bsmF* mutant fed with 5-OH Tyr; iii) Δ*bsmG* mutant fed with 5-OH Tyr; iv) Δ*bsmH* mutant fed with 5-OH Tyr; v) Δ*bsmH* mutant; vi) Δ*bsmG* mutant; vii) Δ*bsmF* mutant.

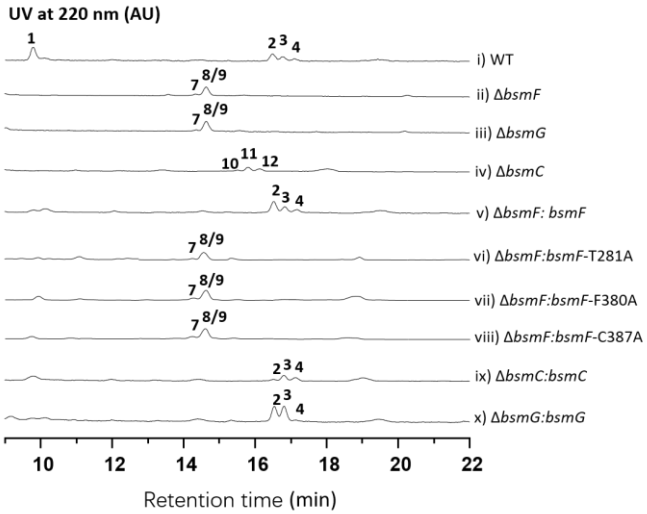


Figure S8. HPLC analysis of metabolite extracts from mutant strains and gene complementation strains. i) wild type; ii) Δ*bsmF* mutant strain; iii) Δ*bsmG* mutant strain; iv) Δ*bsmC* mutant strain; v) complementation of Δ*bsmF* mutant by *bsmF*; vi) complementation of Δ*bsmF*-T281A mutant by *bsmF*; vii) complementation of Δ*bsmF*-F281A mutant by *bsmF*. viii) complementation of Δ*bsmF*-C281A mutant by *bsmF*; ix) complementation of Δ*bsmC* mutant by *bsmC*; x) complementation of Δ*bsmG* mutant by *bsmG*. **8** and **9** have indentical retention time.

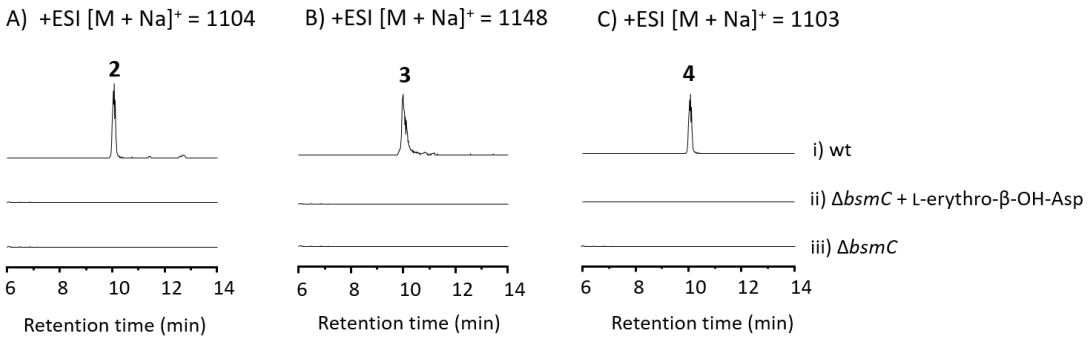


Figure S9. Chemical complementation fed L-erythro-β-OH-Asp into Δ*bsmC* mutant. LC-MS analyses of A) compound **2**, B) compound **3** and C) compound **4** for different strains. i) wt; ii) Δ*bsmC* mutant fed L-erythro-β-OH-Asp; iii) Δ*bsmC* mutant.

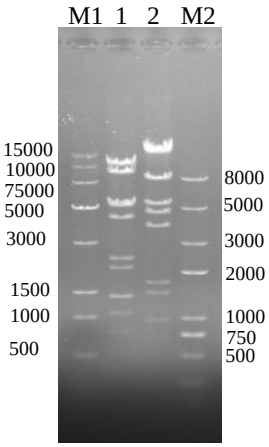


Figure S10. Verification of cosmid pHG06015. A) Physical map of pJTU2554 harboring the *bsm* gene cluster. B) agarose gel electrophoresis of pHG06015 cosmid. Legend: M1, 15K marker; 1, pHG06015 digested by BamHI; 2, pHG06011 digested by KpnI; M2, 8K marker

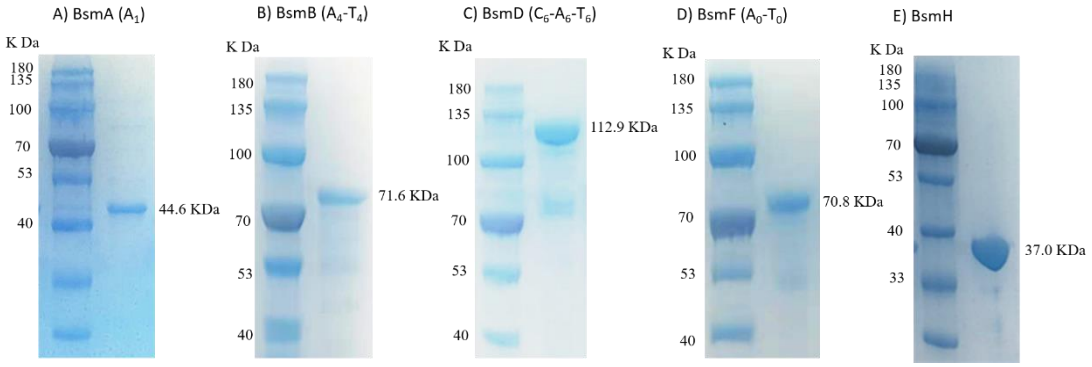


Figure S11. SDS-PAGE analysis of proteins. A) BsmA (A_1) (calculated molecule weight: 44.6 KDa); B) BsmB (A_4-T_4) (calculated molecule weight: 71.6 KDa); C) BsmD (C_6-A_6-T_6) (calculated molecule weight: 112.9 KDa); D) BsmF (A_0-T_0) (calculated molecule weight: 70.8 KDa). E) BsmH (calculated molecule weight: 37.0 KDa).

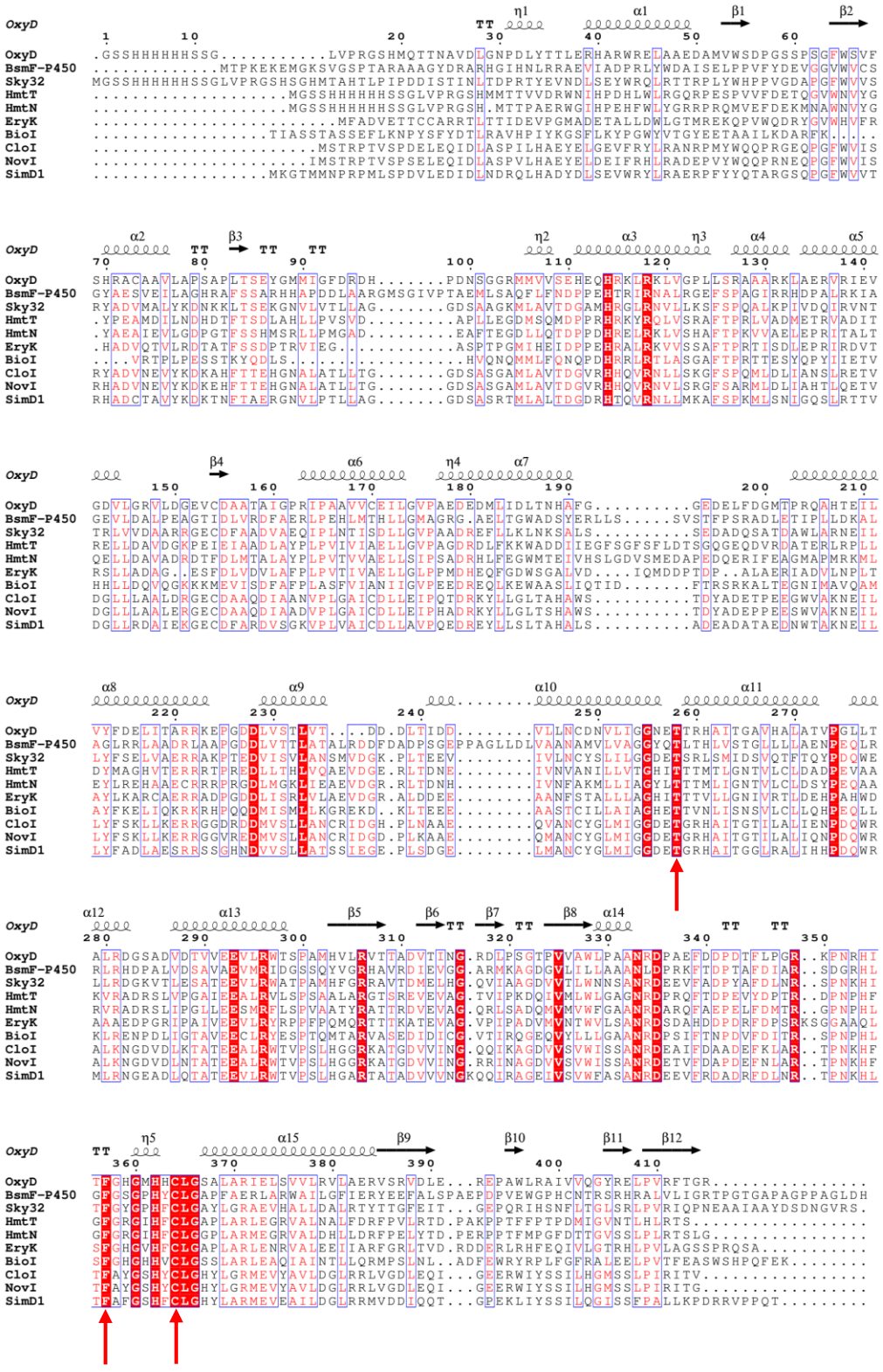
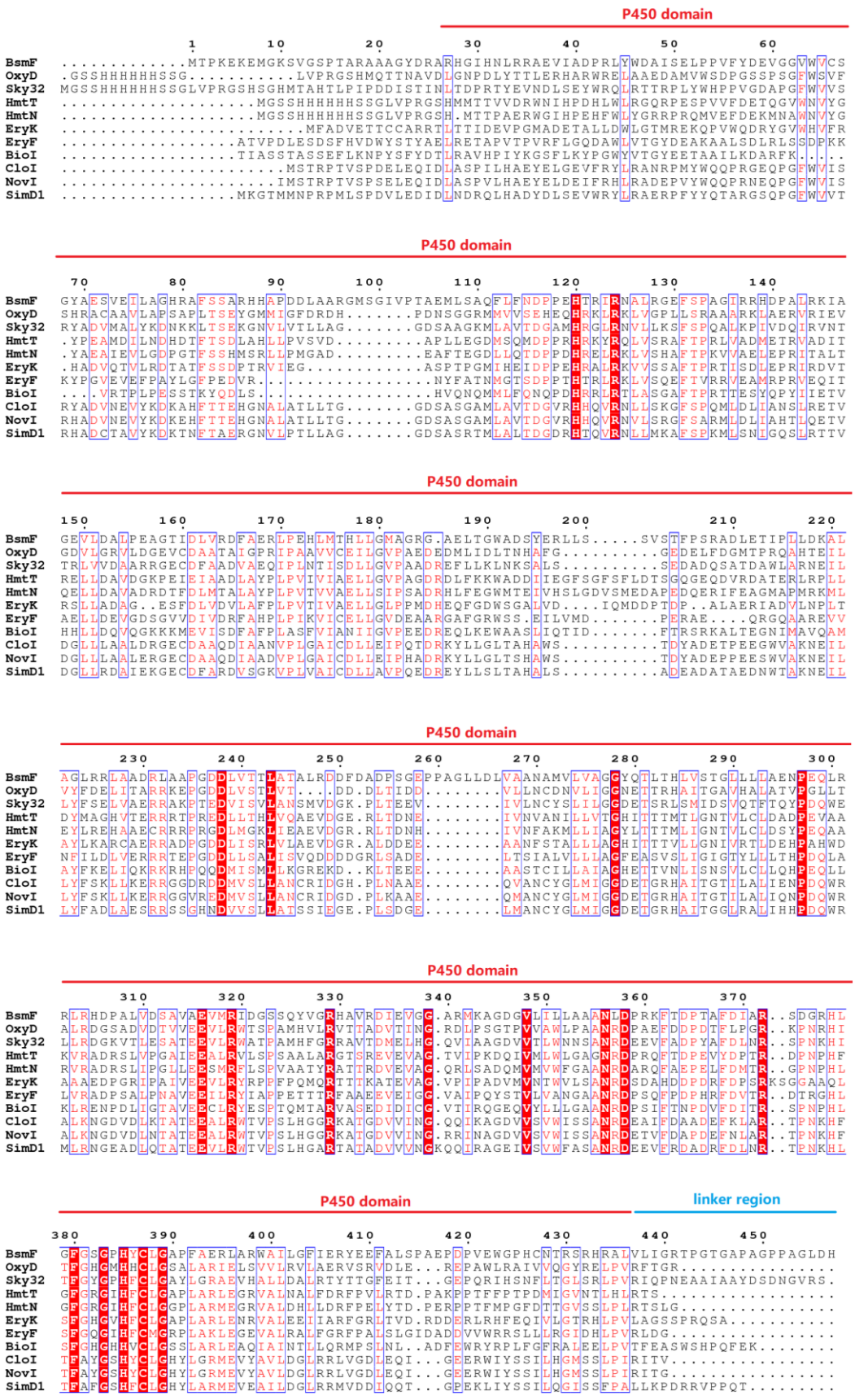


Figure S12. Sequence alignment of BsmF-P450 with other P450 proteins. The Glu/Thr residues are important in interactions with and proton transfer to iron-oxo intermediates in the P450 catalytic cycle. The heme-binding motif conserved Cys residues that act as the proximal ligand to the heme iron, the conserved phenylalanines as a regulator of heme iron potential.[21,22] Protein accession number: OxyD (3MGX_A); Sky32 (4L0F_A); HmtT (CBZ42154); HmtN (5XW2_A); EryK (P48635.3); CloI (AAN65225); NovI (Q9L9F9); SimD1 (AAK06805); BioI (AGG62423.1). The multiple alignment was generated by ClustalW servers and rendered with ESPript 3.0.[23]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P450 domain
P450 domain
P450 domain
P450 domain
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OxyD
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HmtT
HmtN
EryK
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BioI
CloI
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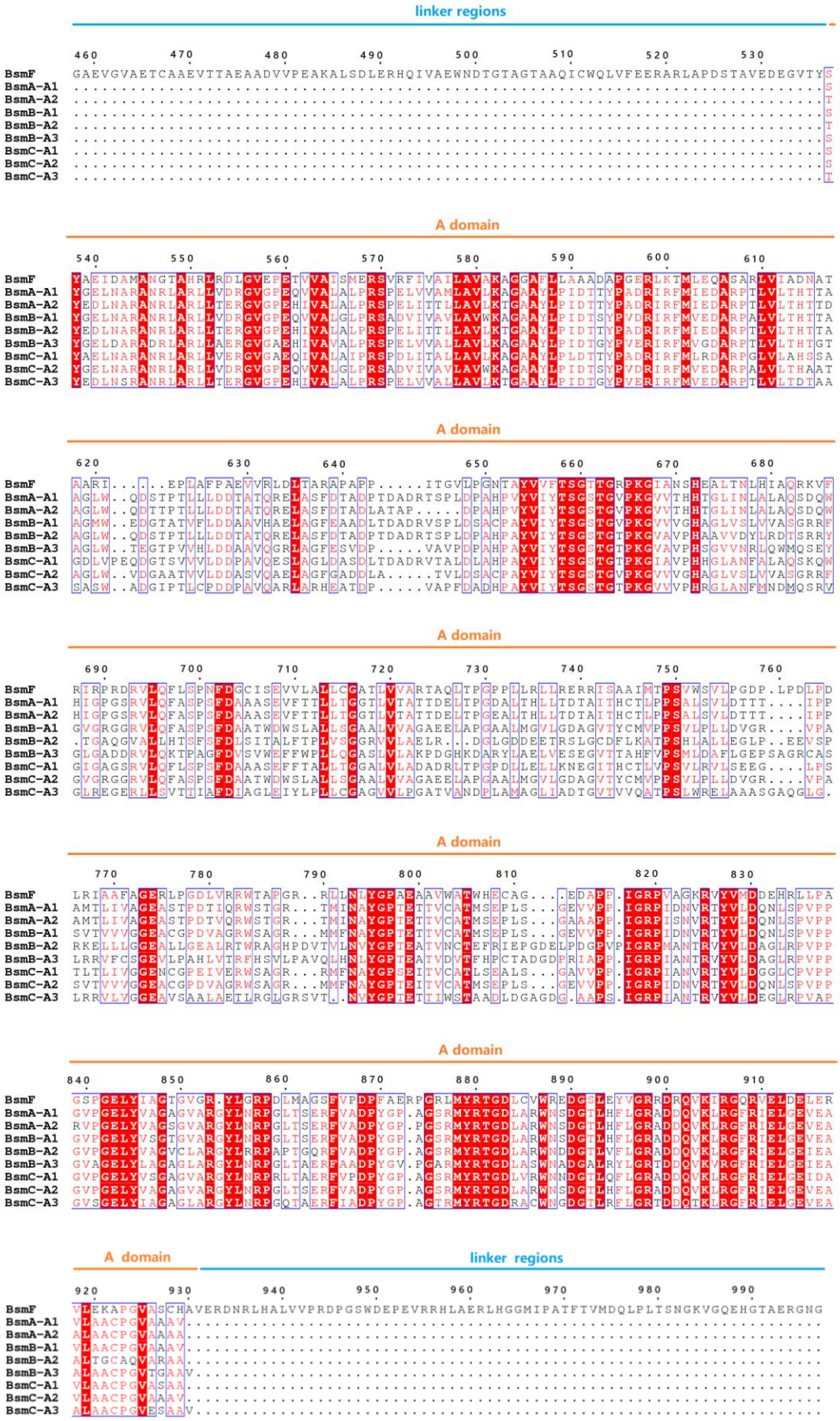
SimD1


linker regions
A domain
A domain
A domain
A domain
A domain
A domain
linker regions
BsmF
BsmA-A1
BsmA-A2
BsmB-A1
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BsmC-A1
BsmC-A2
BsmC-A3

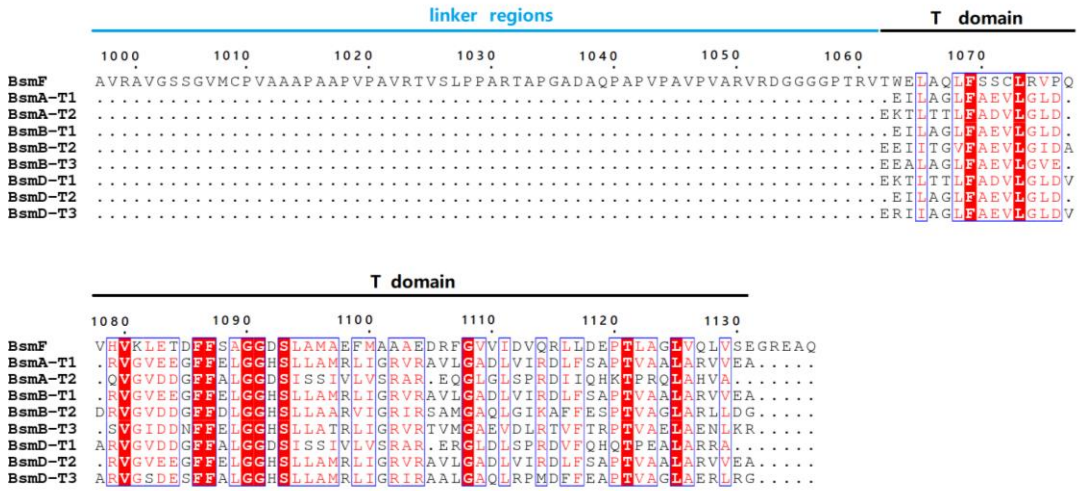

Figure S13. Domain analyze of BsmF. P450 domain (27-436 aa); A domain (537-930 aa); T domain (1062-1131 aa); linker regions (437-536 aa and 931-1061 aa).

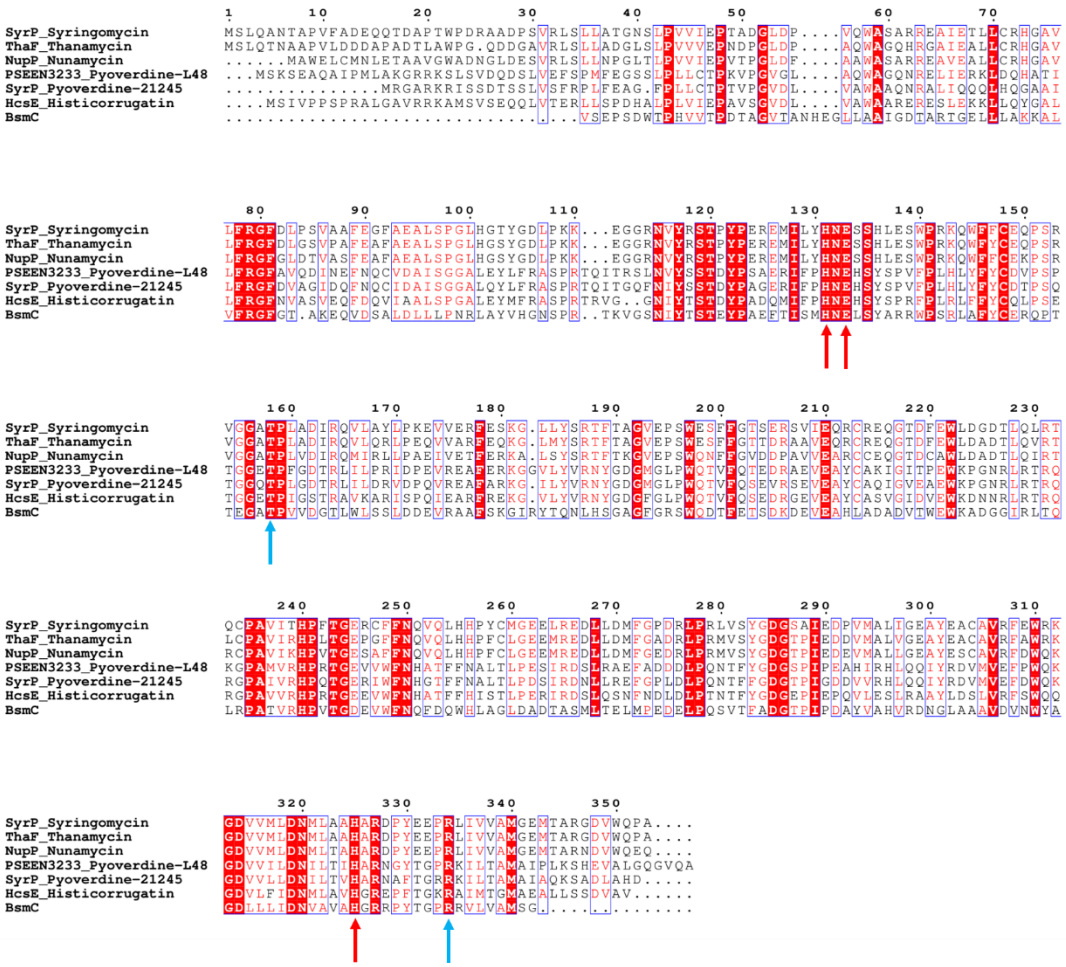

Figure S14. Sequence alignment of BsmC with other β-hydroxylases. Fe(II) and α-ketoglutarate binding residues are marked with red arrows and blue arrows.[24] Protein accession number: SyrP_Syringomycin (AKF46133.1); ThaF_Thanamycin (ALG65284.1); NupP_Nunamycin (KPN90375.1); PSEEN3233_Pyoverdine-L48 (WP_011534378.1); SyrP_Pyoverdine-21245 (AJW67533.1); HcsE_Histicorrugatin (WP_053122094.1). The multiple alignment was generated by ClustalW servers and rendered with ESPript 3.0.[23]

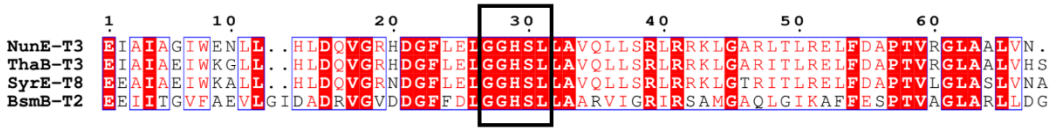

Figure S15. Sequence alignment of BsmB-T_2 with other T domains. Strictly conserved GGHSL motif in the thiolation domain are marked with black box.[24] Protein accession number: NupE-T_3 (KPN90369.1); SyrE-T_8 (AAY37647.1); ThaB-T_3 (AED90003.1). The multiple alignment was generated by ClustalW servers and rendered with ESPript 3.0.[23]

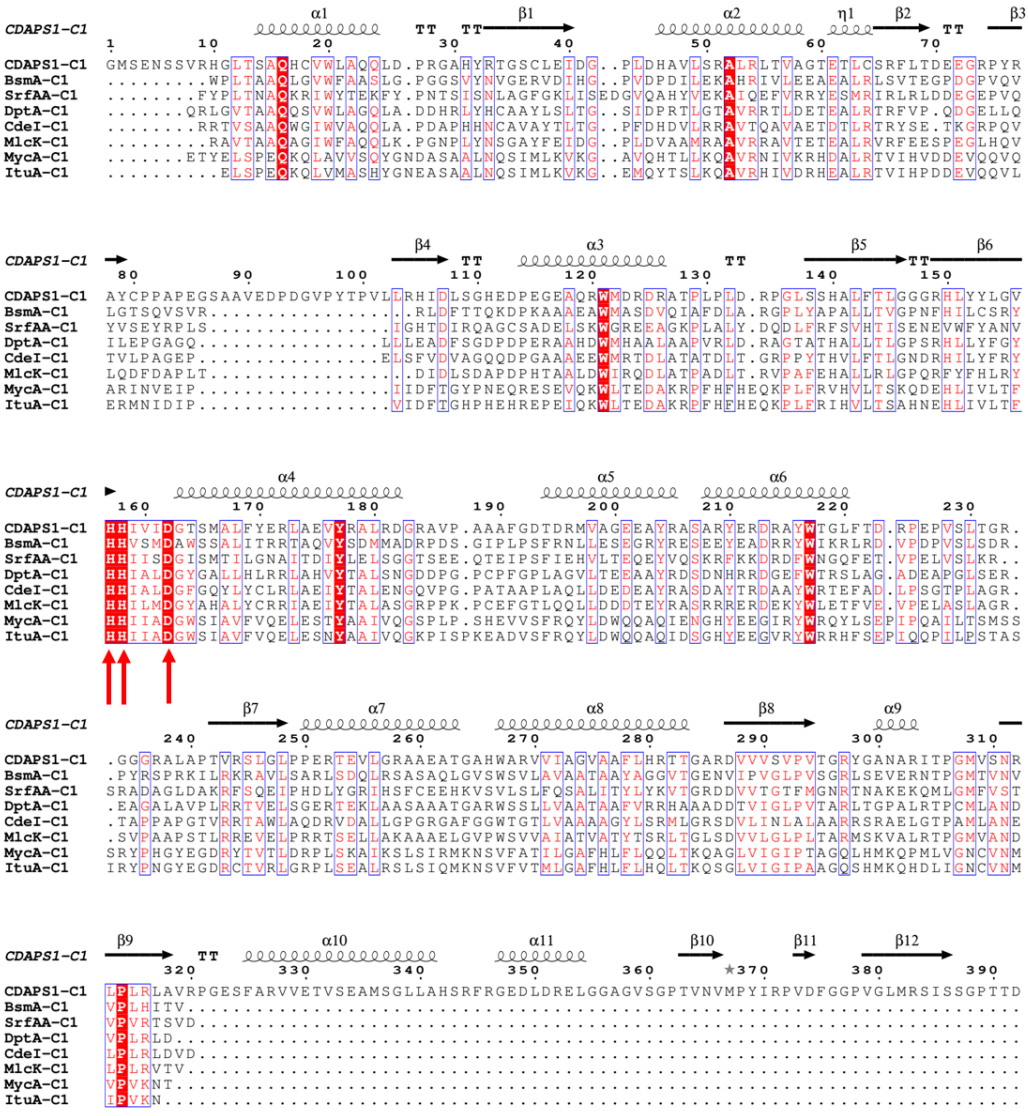

Figure S16. Sequence alignment of BsmA-C1 with other C domains. The conserved histidine and aspartate residues are marked by red arrows.[25] Protein accession number: CDAPS1-C1 (CAB38517); SrfAA-C1 (CAE02630); DptA-C1 (AHX36919); CdeI-C1 (QBC75021); MlcK-C1 (ARU08073); MycA-C1 (Q9R9J1); ItuA-C1 (BAB69698). The multiple alignment was generated by ClustalW servers and rendered with ESPript 3.0.[23]

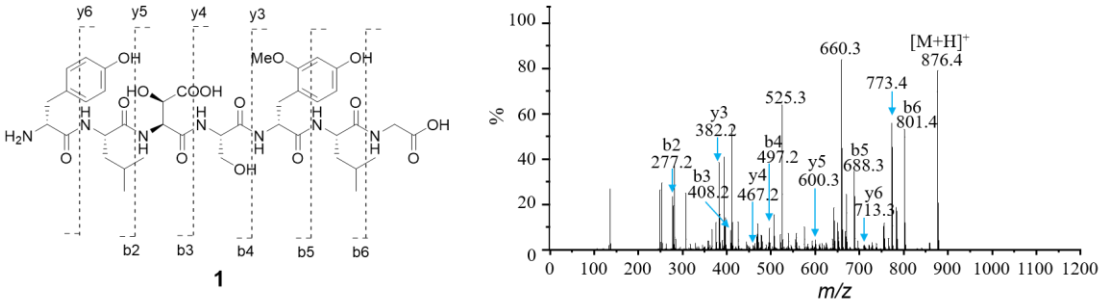

Figure S17. MS/MS analysis of **1**.

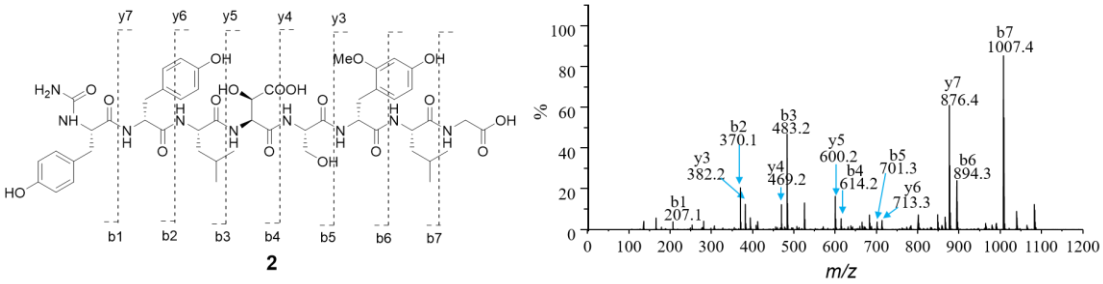

Figure S18. MS/MS analysis of **2**.

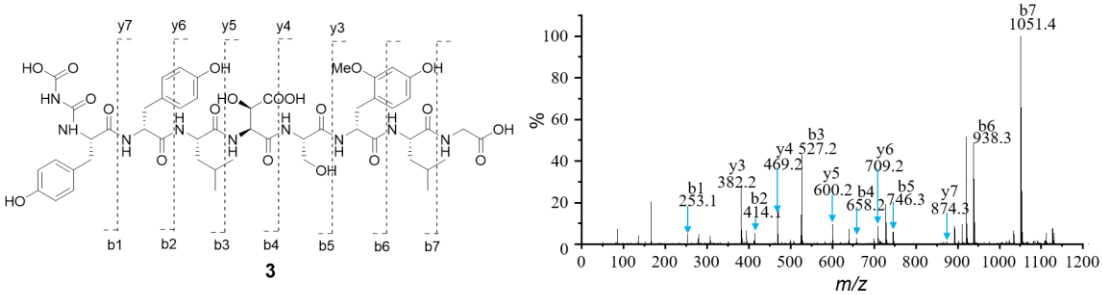

Figure S19. MS/MS analysis of **3**.

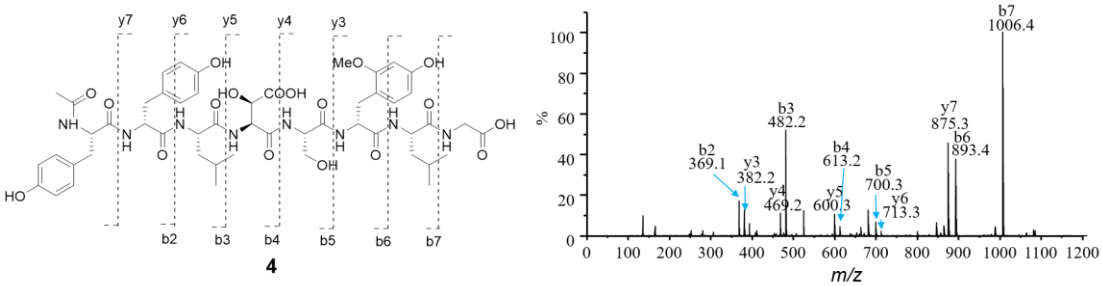

Figure S20. MS/MS analysis of **4**.

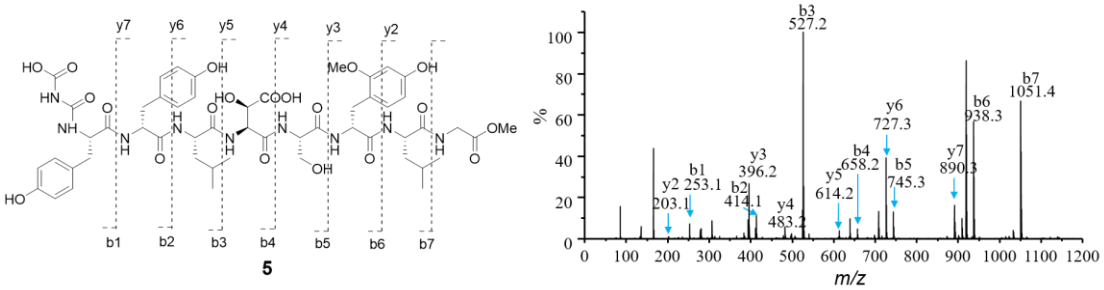

Figure S21. MS/MS analysis of **5**

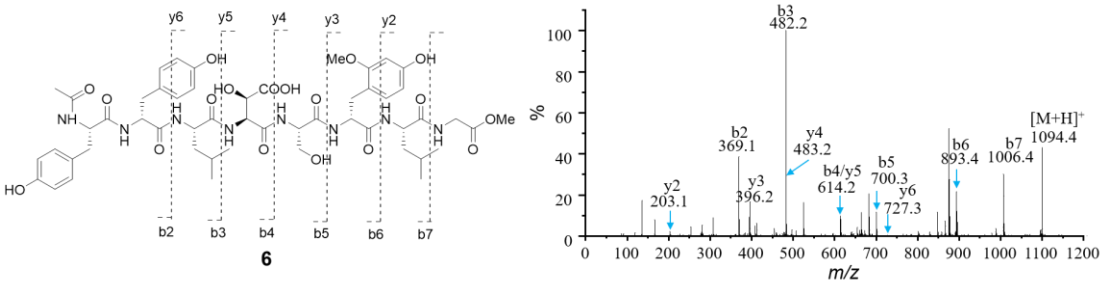

Figure S22. MS/MS analysis of **6**.

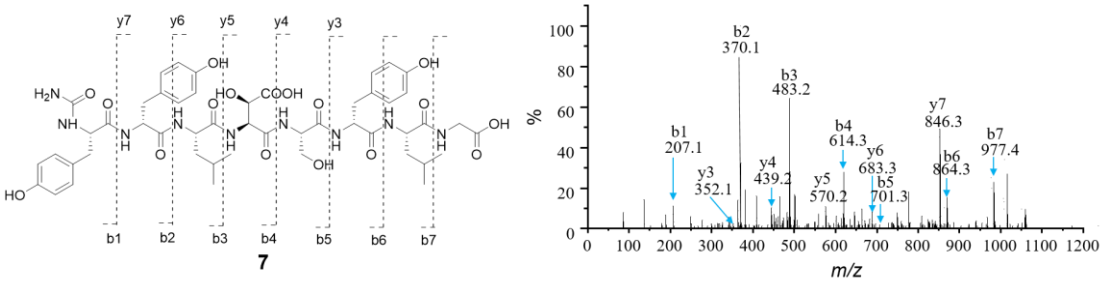

Figure S23. MS/MS analysis of **7** in Δ*bsmF* mutant strain.

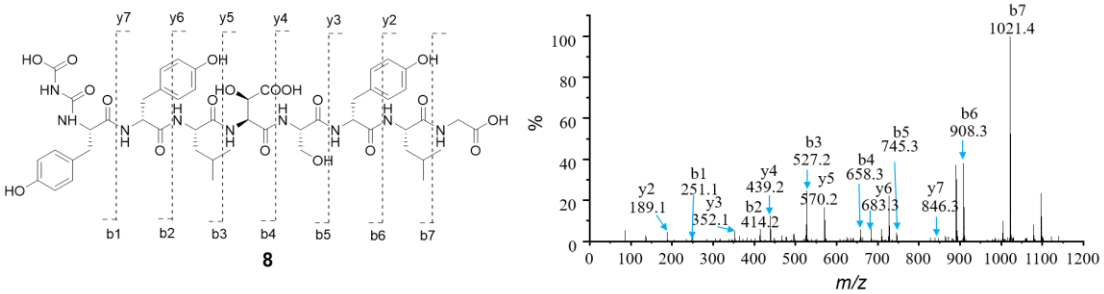

Figure S24. MS/MS analysis of **8** in Δ*bsmF* mutant strain.

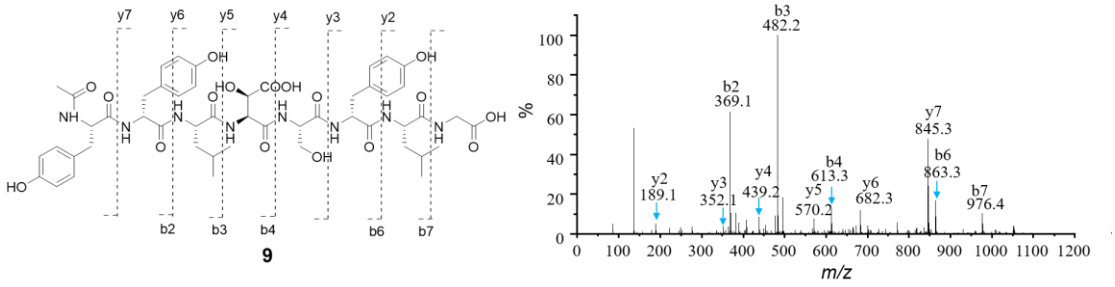

Figure S25. MS/MS analysis of **9** in Δ*bsmF* mutant strain.

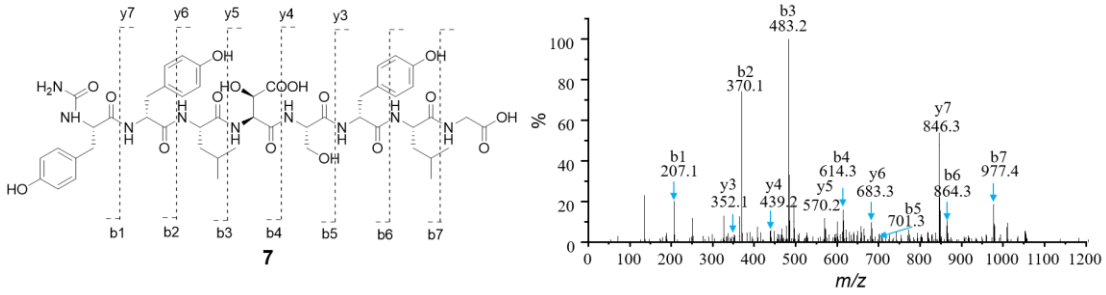

Figure S26. MS/MS analysis of **7** in Δ*bsmG* mutant strain.

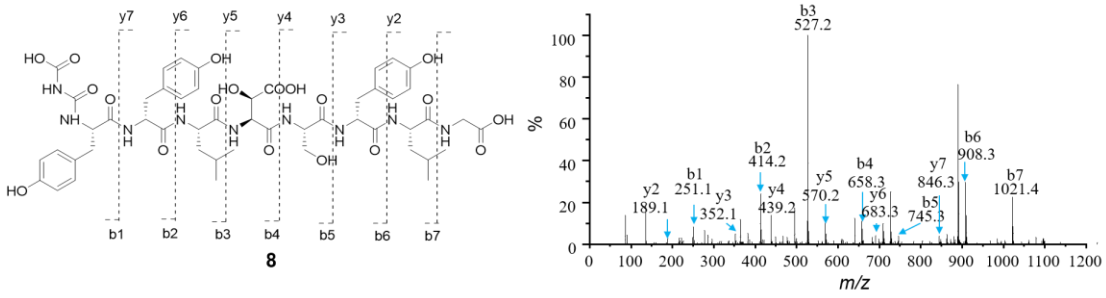

Figure S27. MS/MS analysis of **8** in Δ*bsmG* mutant strain.

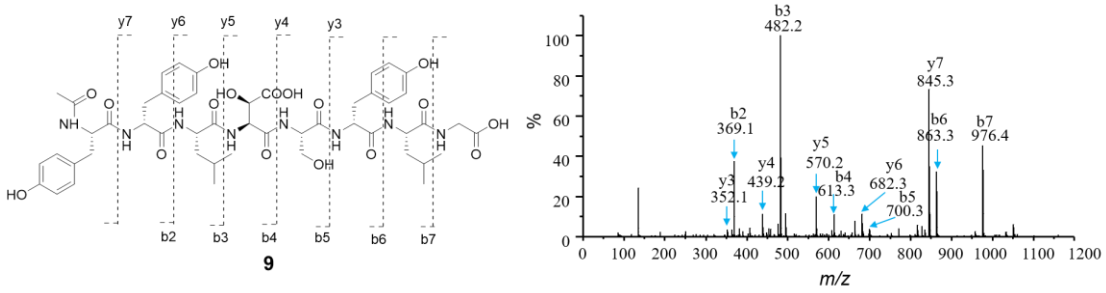

Figure S28. MS/MS analysis of **9** in Δ*bsmG* mutant strain.

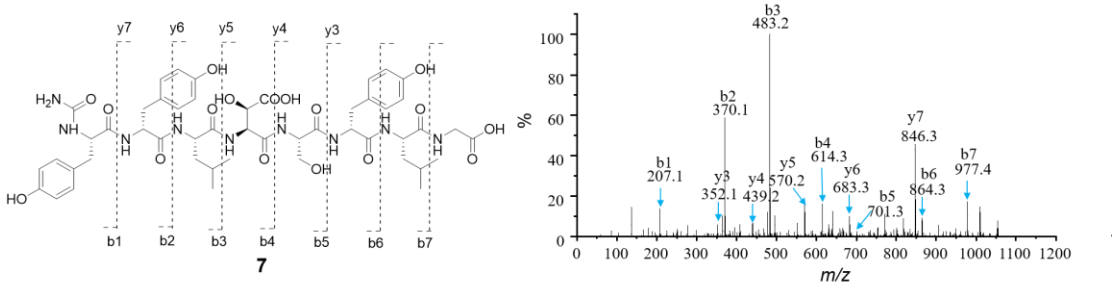

Figure S29. MS/MS analysis of **7** in Δ*bsmH* mutant strain.

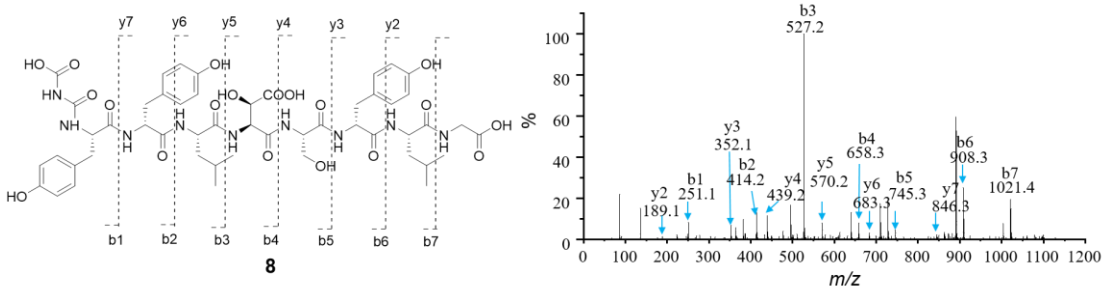

Figure S30. MS/MS analysis of **8** in Δ*bsmH* mutant strain.

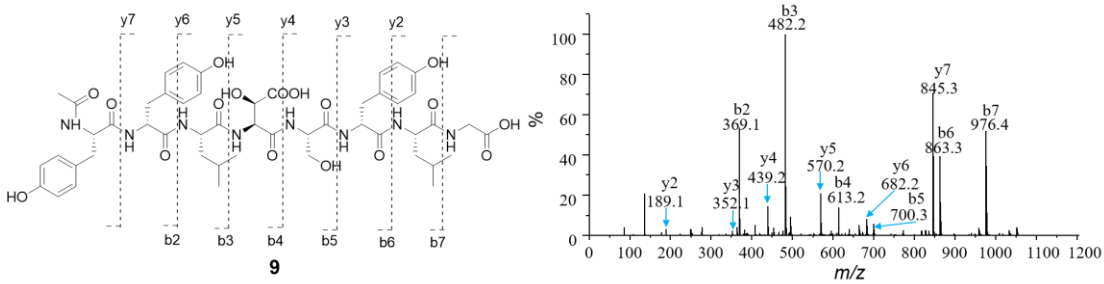

Figure S31. MS/MS analysis of **9** in Δ*bsmG* mutant strain.

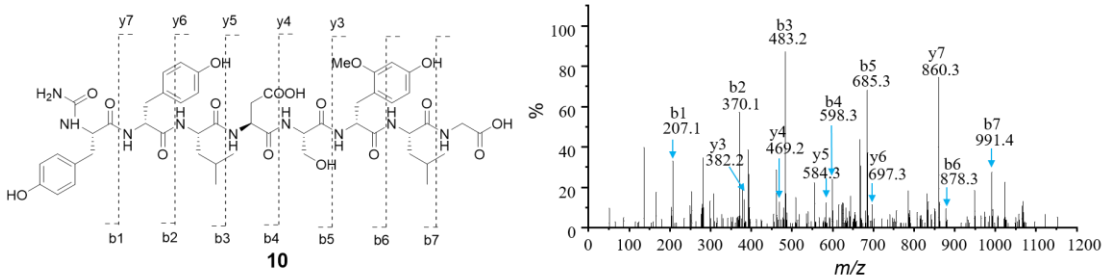

Figure S32. MS/MS analysis of **10** in Δ*bsmC* mutant strain.

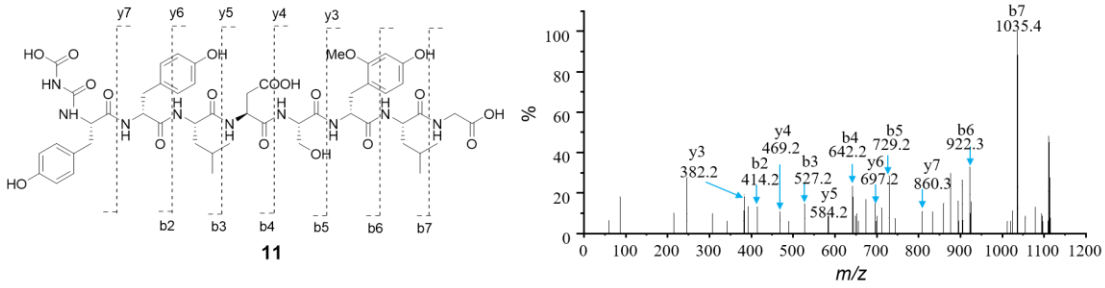

Figure S33. MS/MS analysis of **11** in Δ*bsmC* mutant strain.

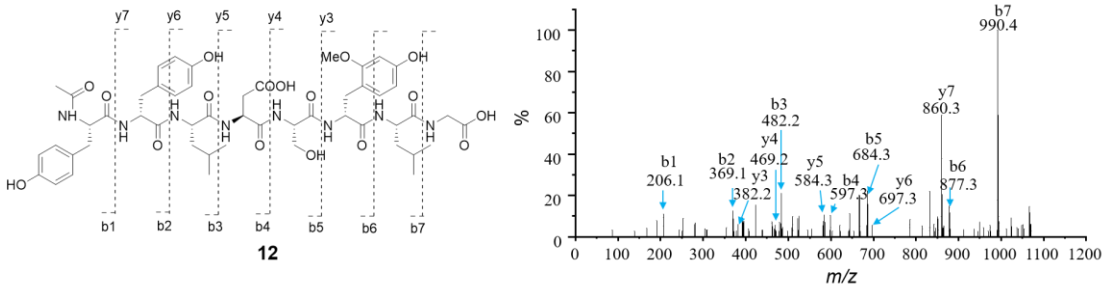

Figure S34. MS/MS analysis of **12** in Δ*bsmC* mutant strain.

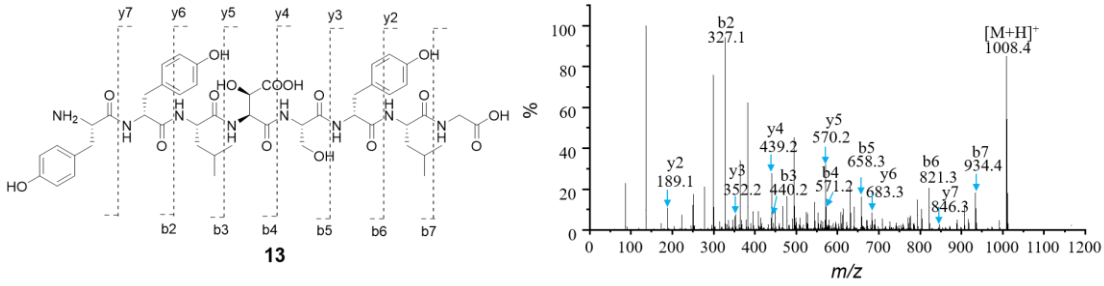

Figure S35. MS/MS analysis of **13.**

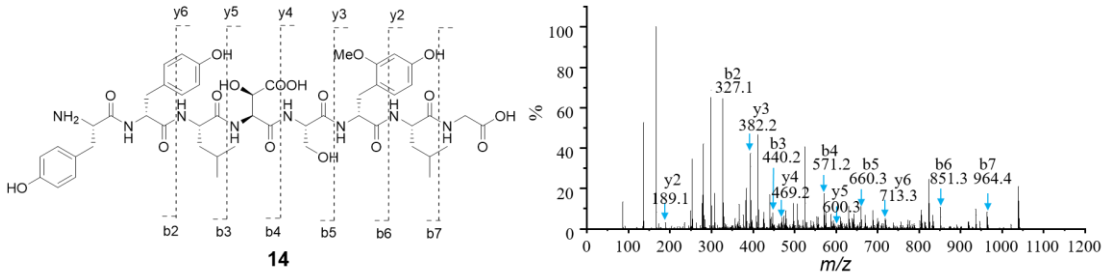

Figure S36. MS/MS analysis of **14**.

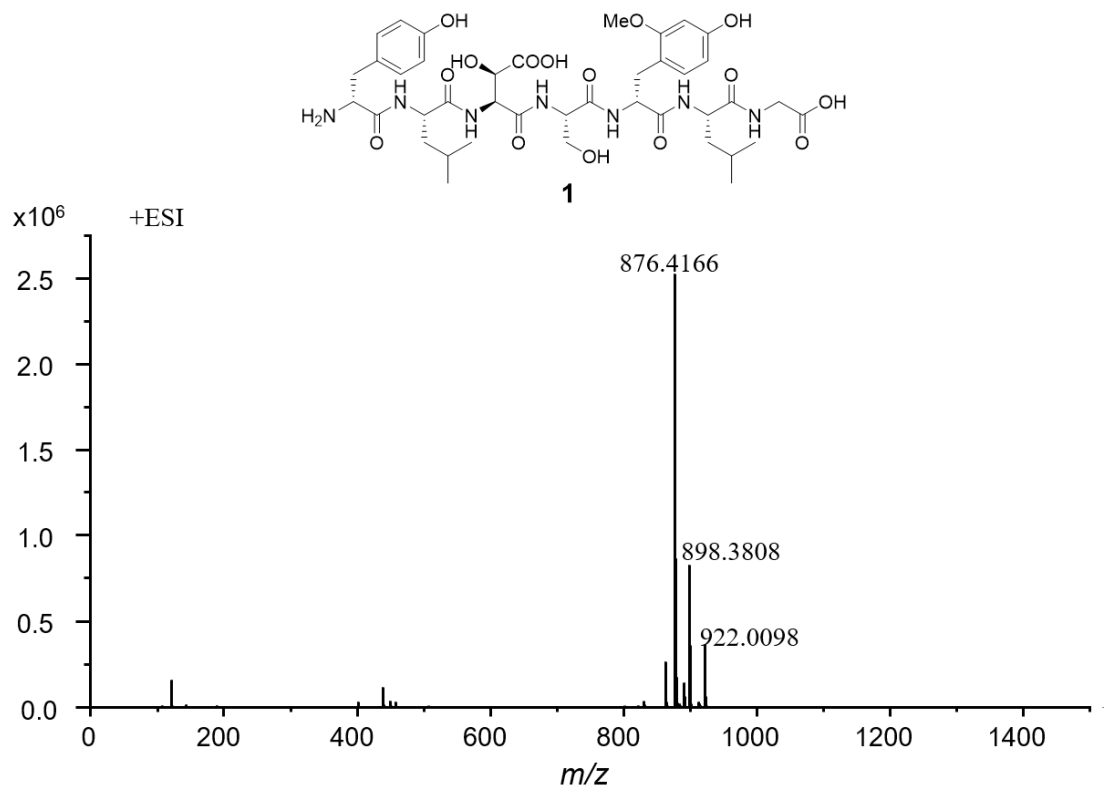

Figure S37. HRESIMS spectrum of **1**.

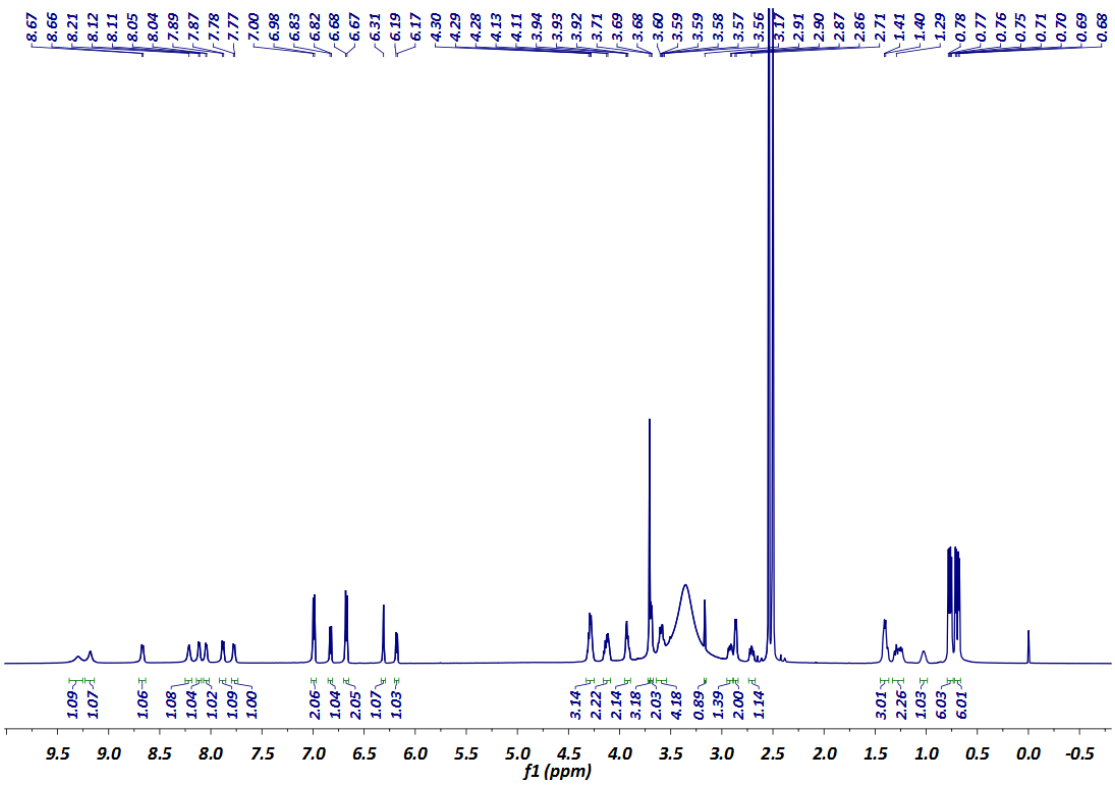

Figure S38. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of compound **1**.

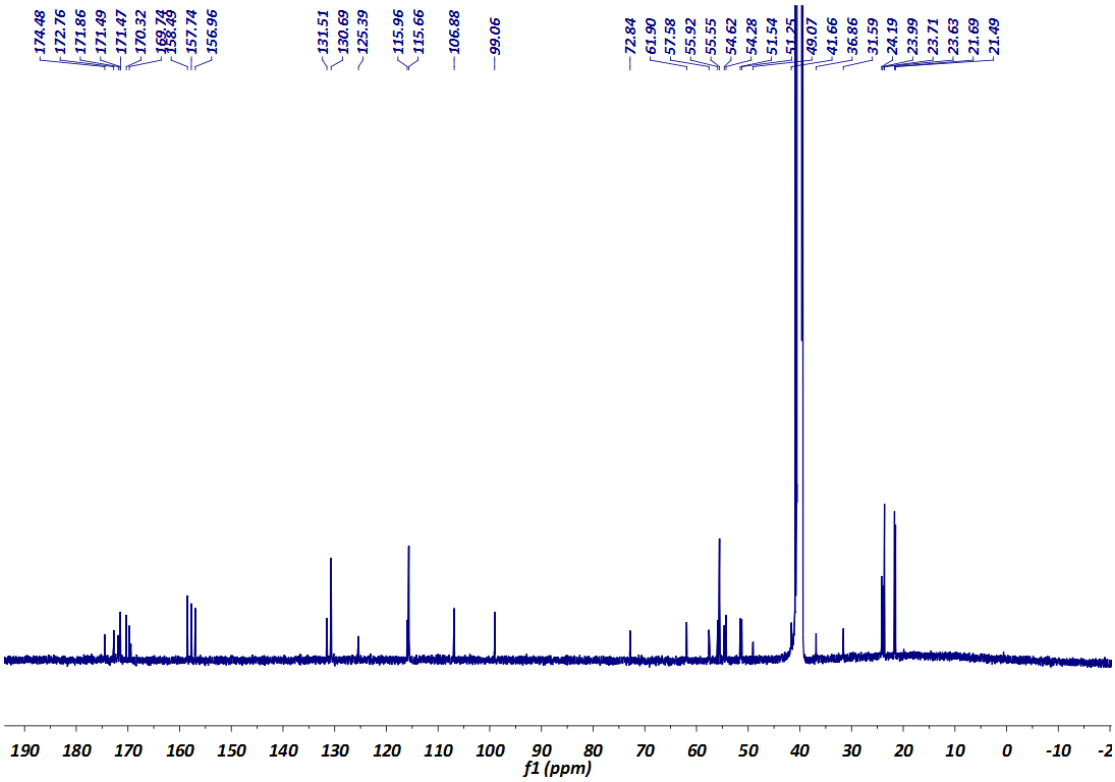

Figure S39. ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of compound **1**.

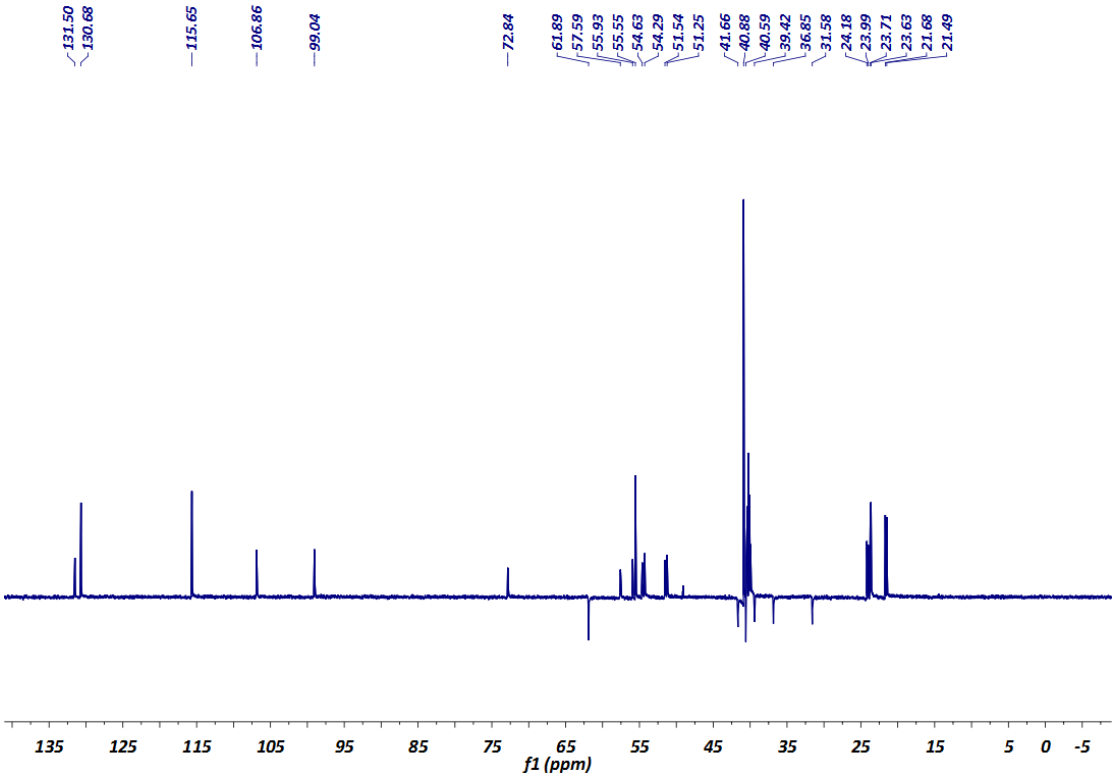

Figure S40. DEPT NMR (150 MHz, DMSO-d_6) spectrum of compound **1**.

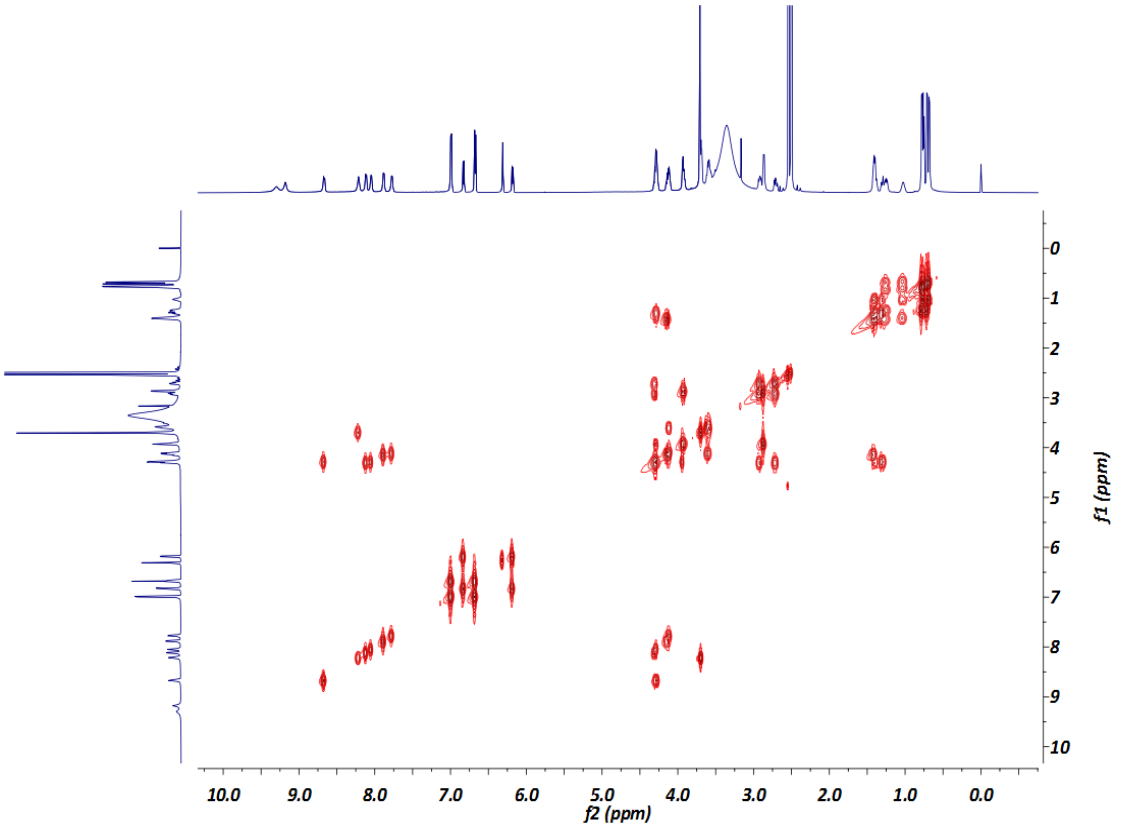

Figure S41. $^{1}H–^{1}H$ COSY NMR (600 MHz, DMSO-d_6) spectrum of compound **1**.

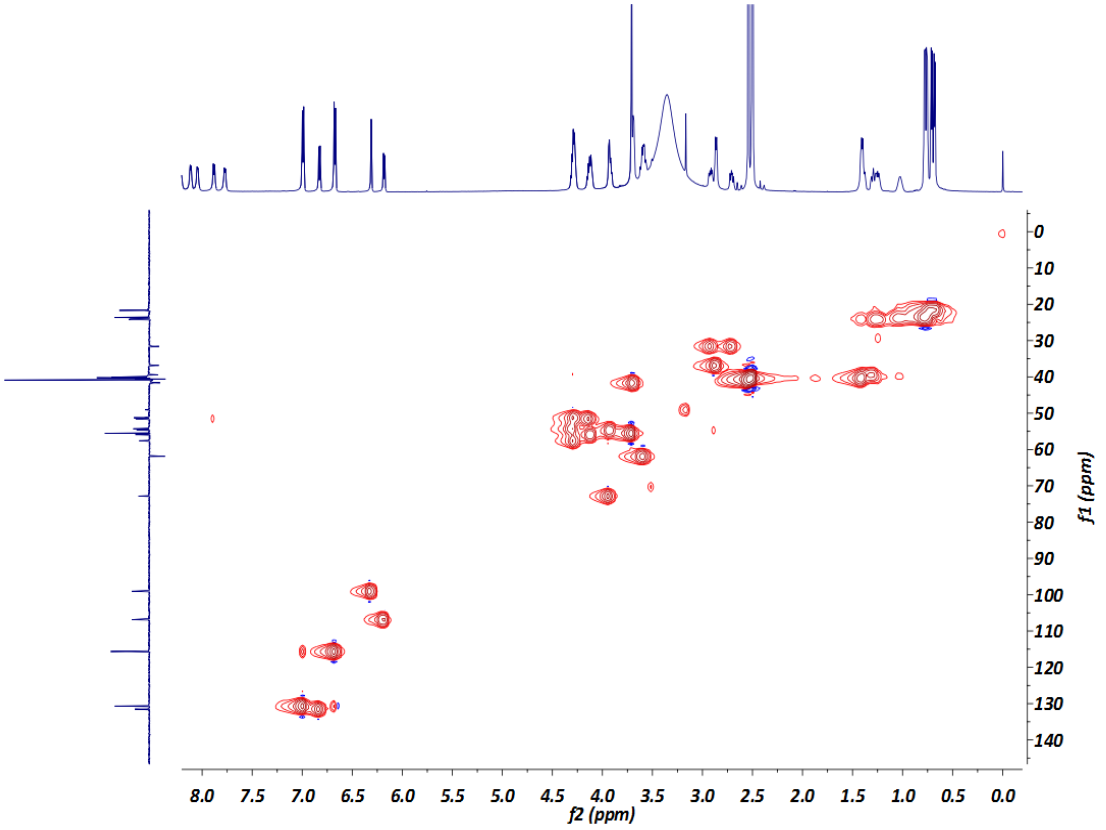

Figure S42. HSQC NMR (600 MHz, DMSO-d_6) spectrum of compound **1**.

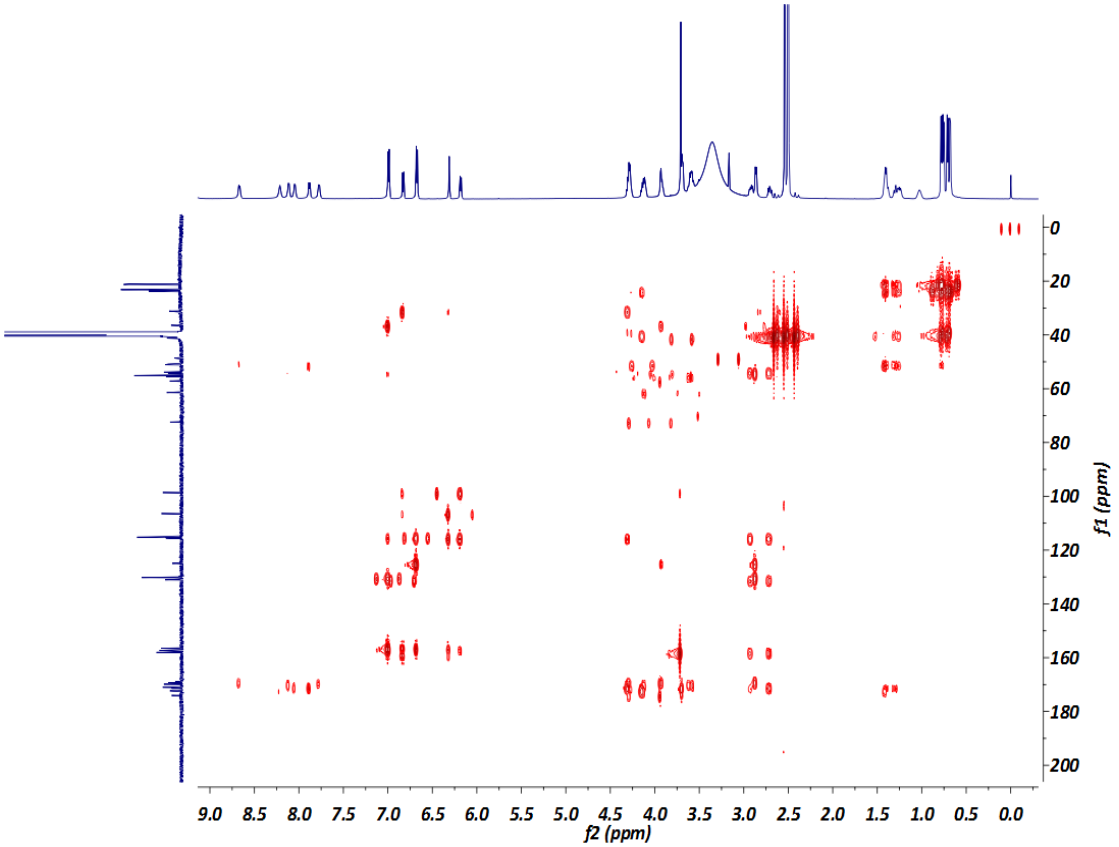

Figure S43. HMBC NMR (600 MHz, DMSO-d_6) spectrum of compound **1**.

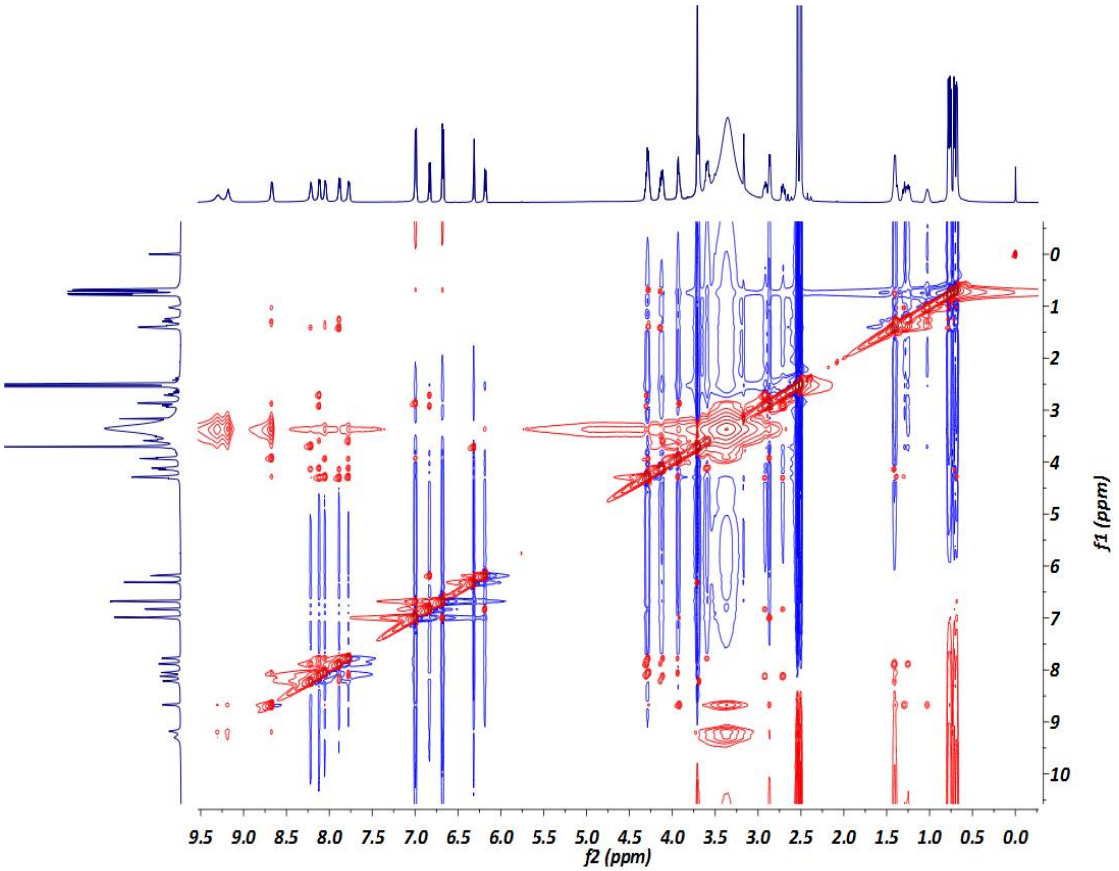

Figure S44. NOESY NMR (600 MHz, DMSO-d_6) spectrum of compound **1**.

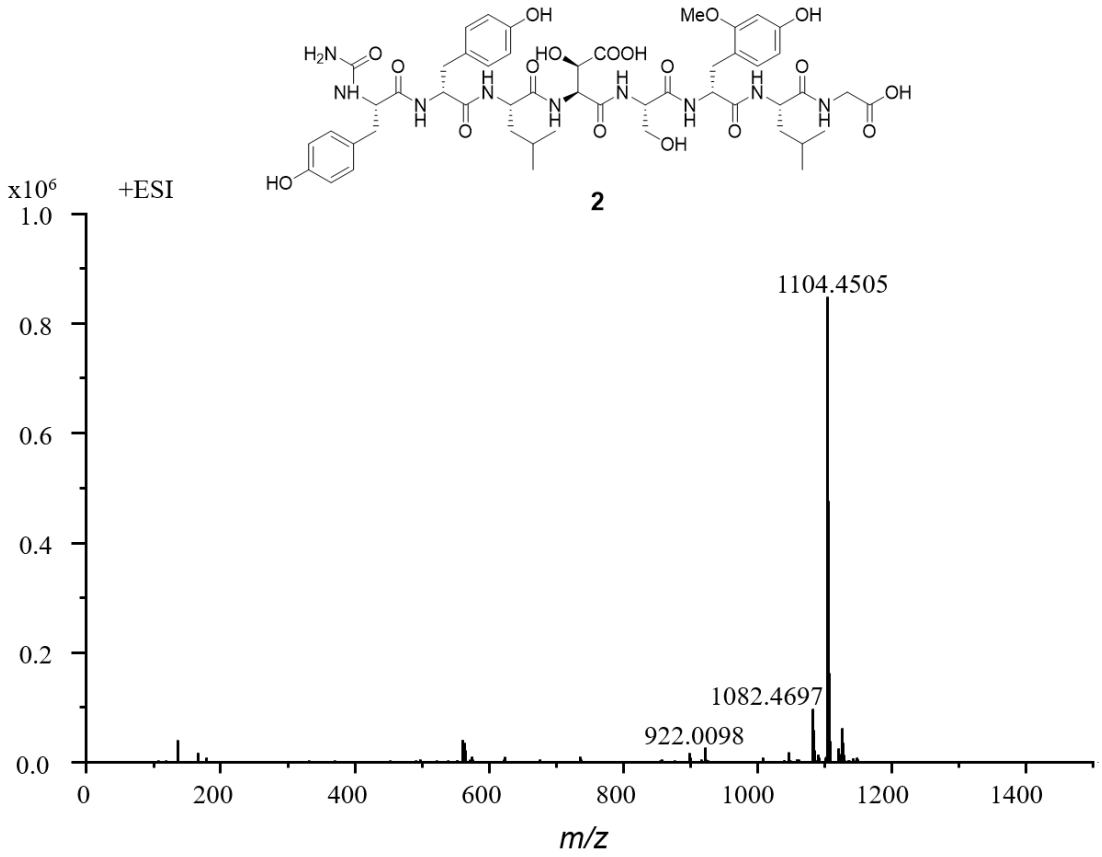

Figure S45. HRESIMS spectrum of **2**.

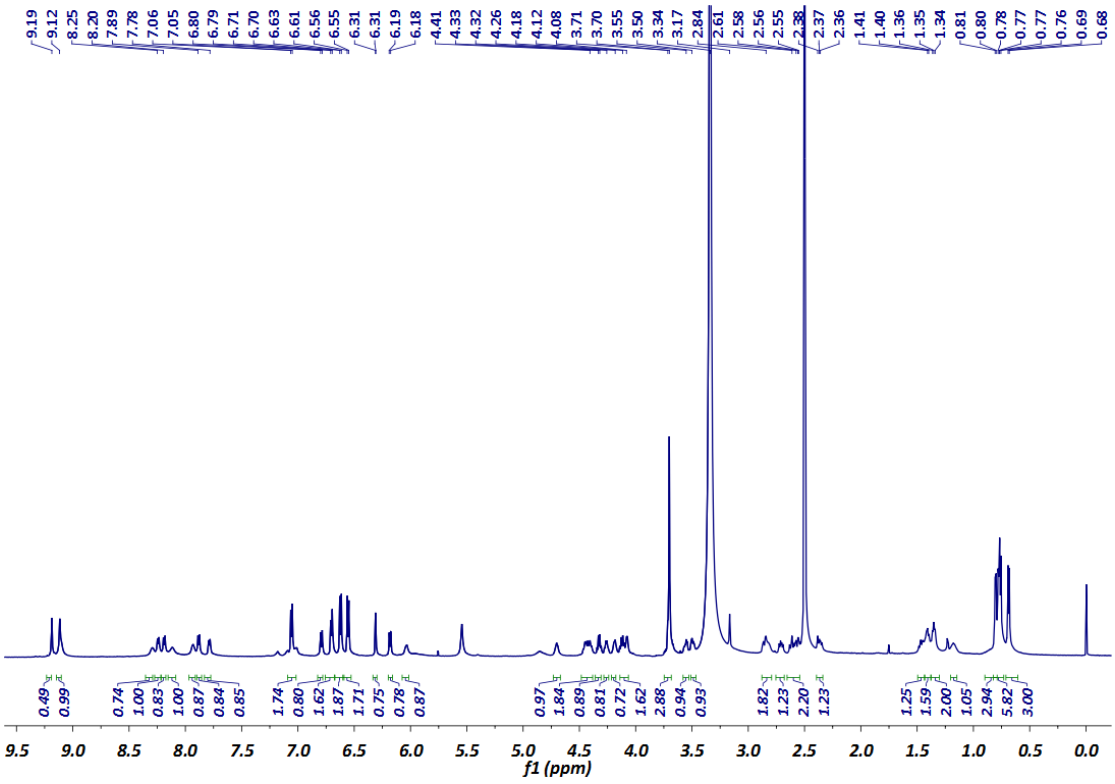

Figure S46. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of compound **2**.

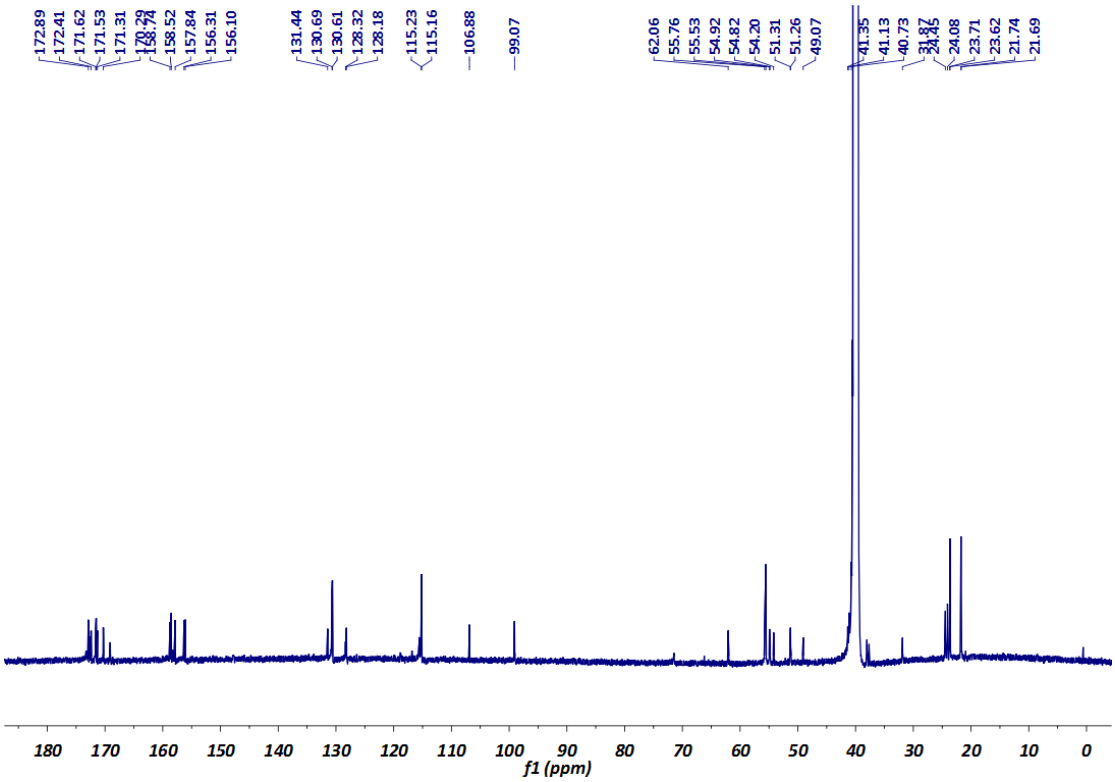

Figure S47. ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of compound **2**.

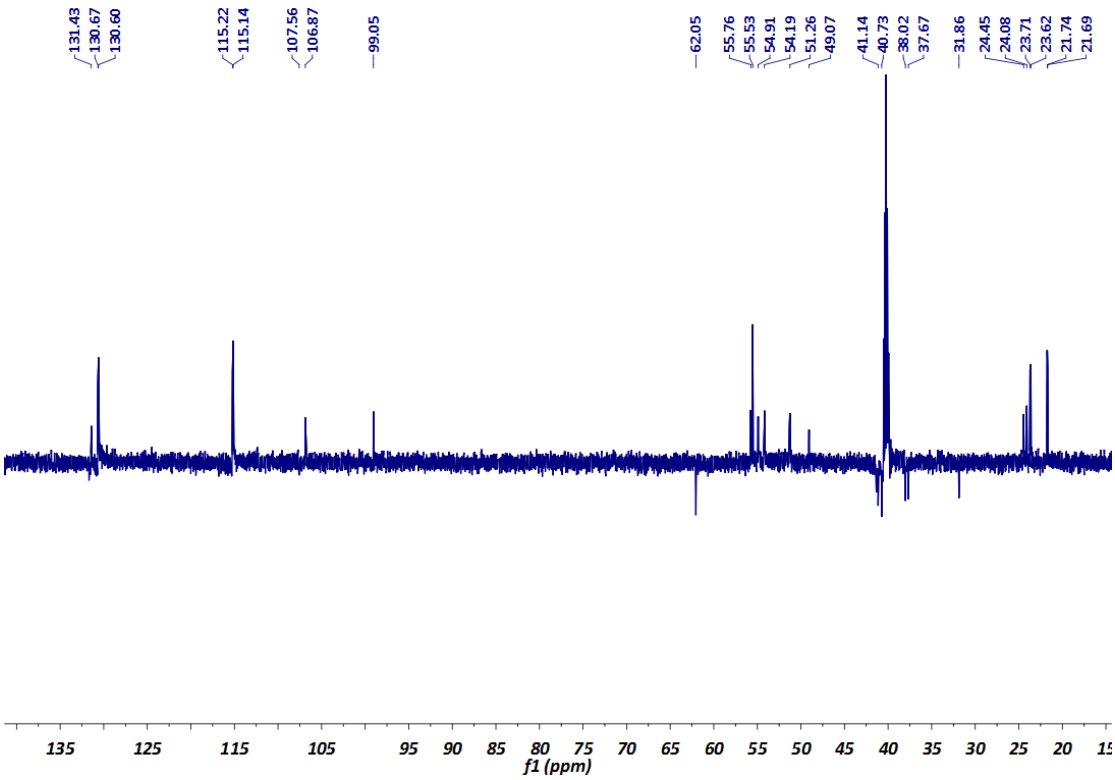

Figure S48. DEPT NMR (150 MHz, DMSO-d_6) spectrum of compound **2**.

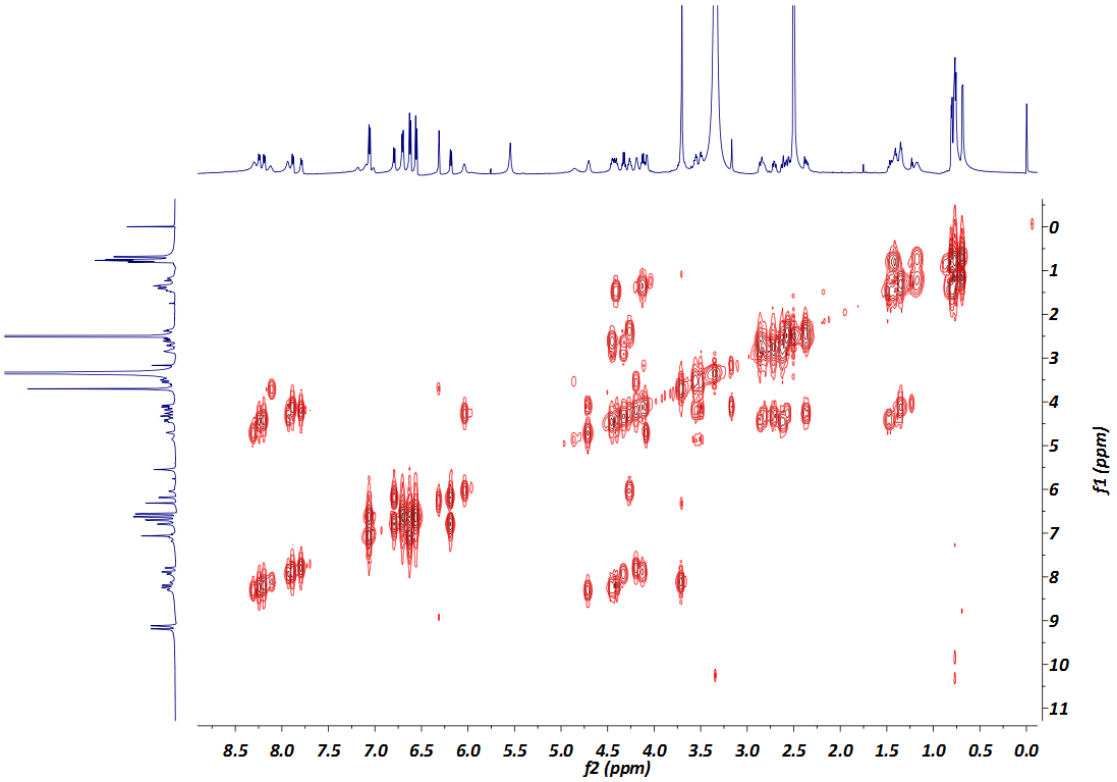

Figure S49. $^{1}H–^{1}H$ COSY NMR (600 MHz, DMSO-d_6) spectrum of compound **2**.

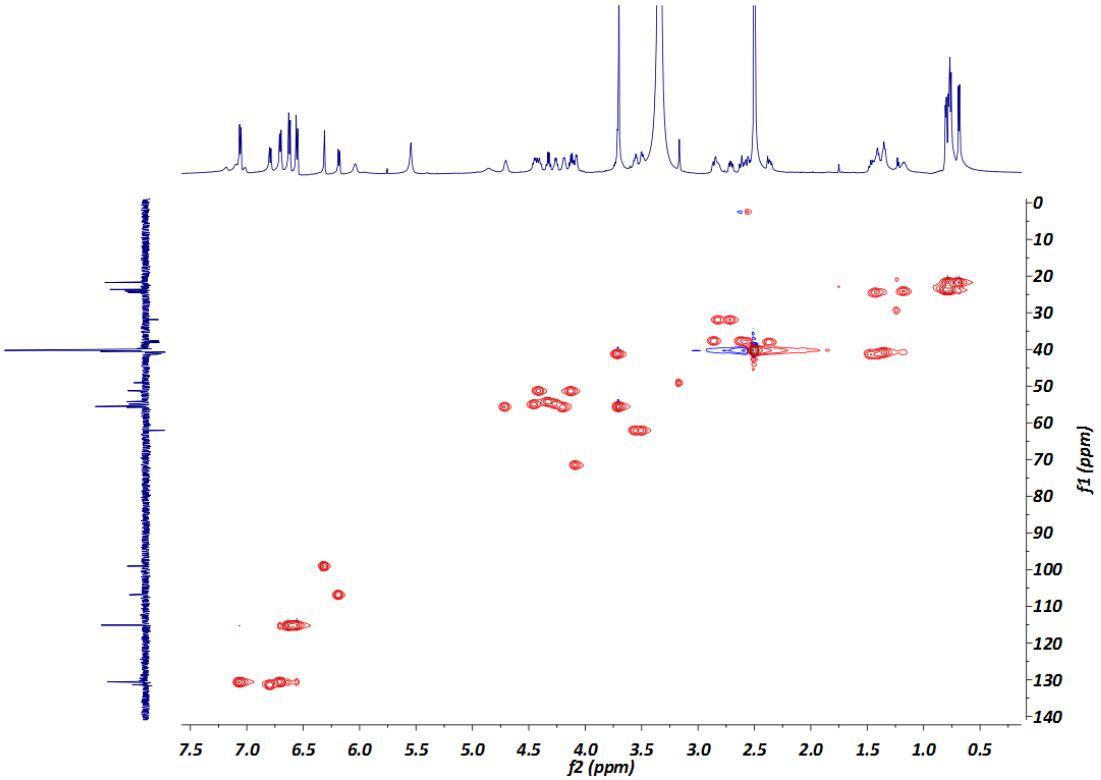

Figure S50. HSQC NMR (600 MHz, DMSO-d_6) spectrum of compound **2**.

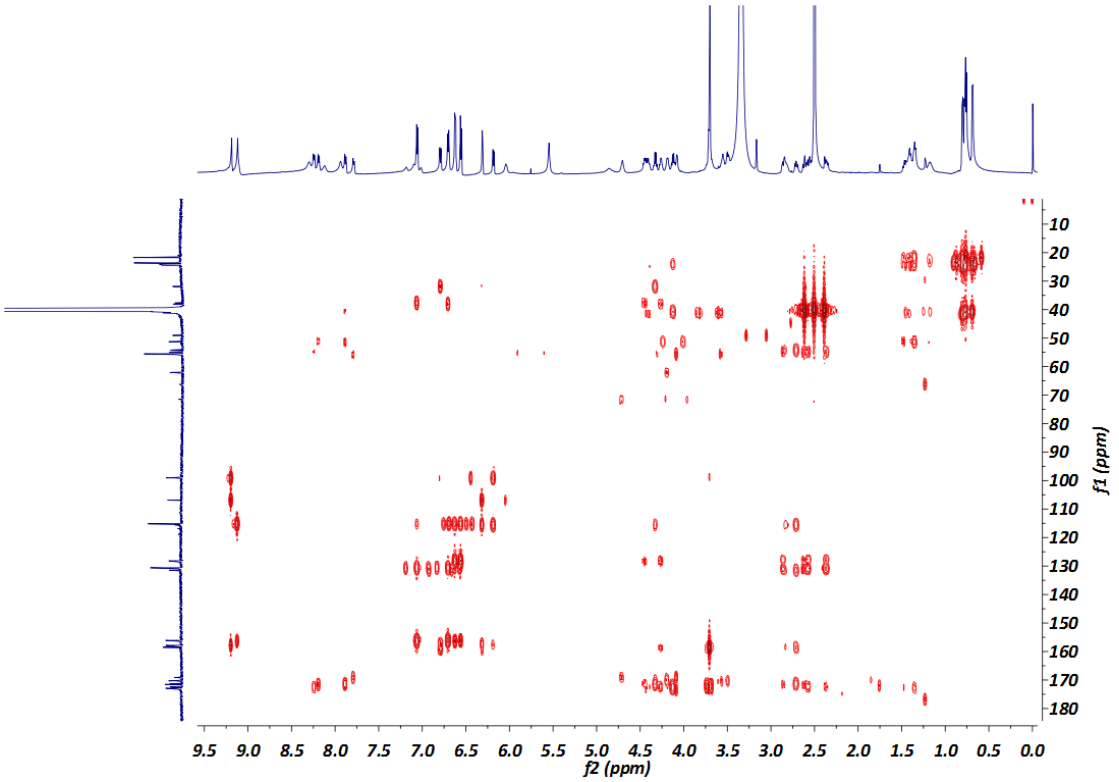

Figure S51. HMBC NMR (600 MHz, DMSO-d_6) spectrum of compound **2**.

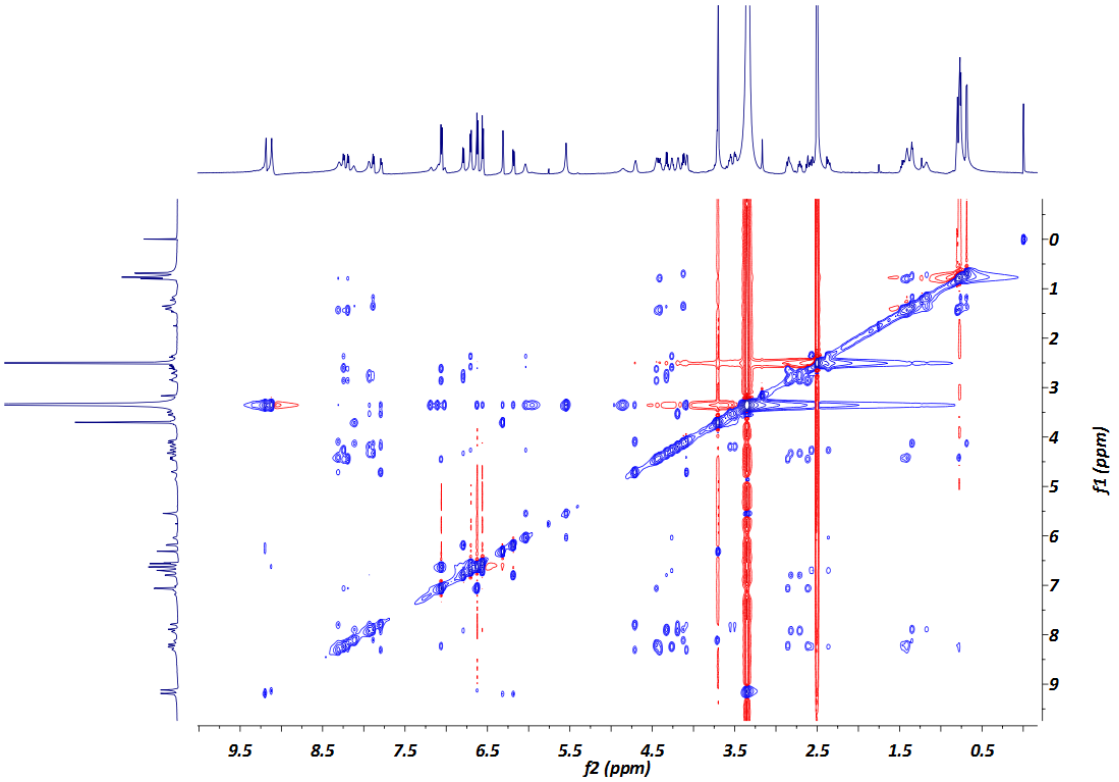

Figure S52. NOESY NMR (600 MHz, DMSO-d_6) spectrum of compound **2**.

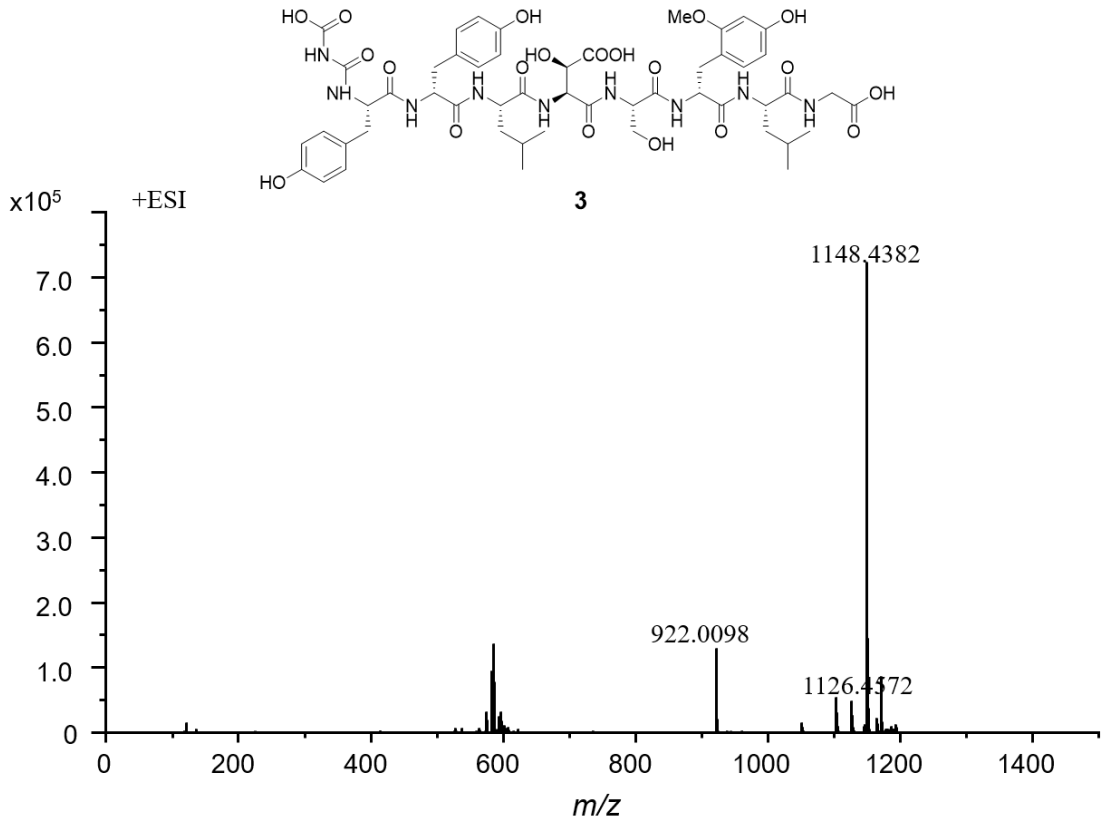

Figure S53. HRESIMS spectrum of **3**.

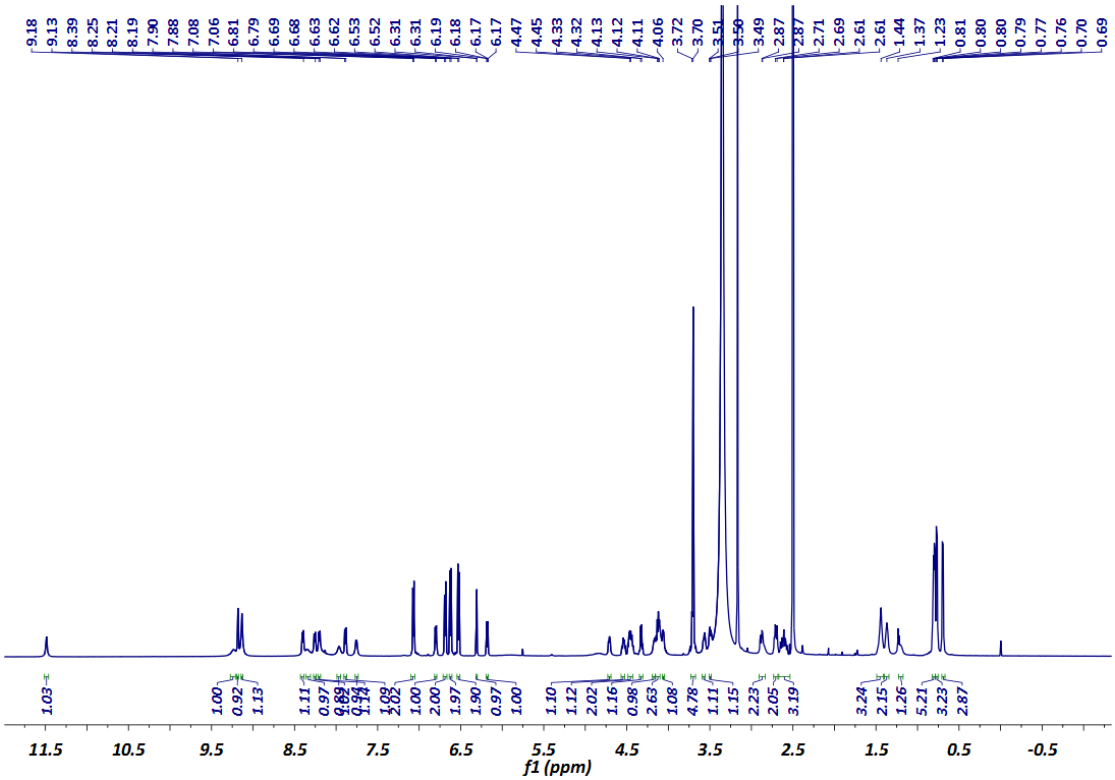

Figure S54. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of compound **3**.

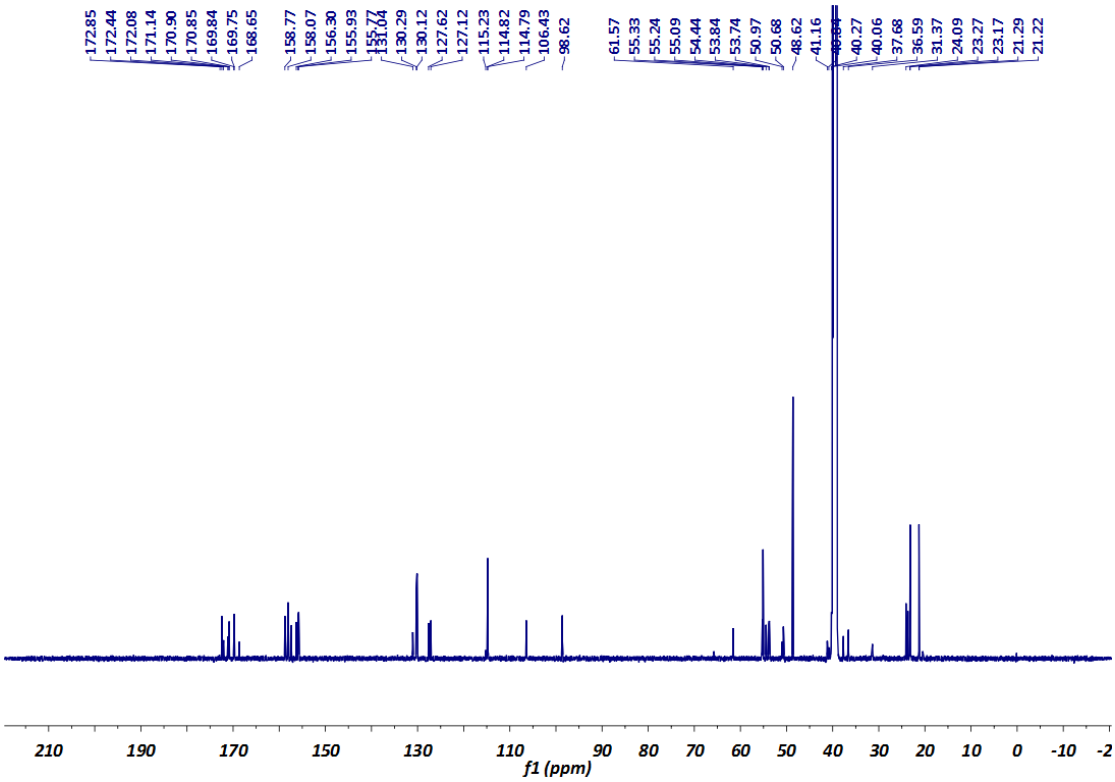

Figure S55.[13]C NMR (150 MHz, DMSO-d_6) spectrum of compound **3**.

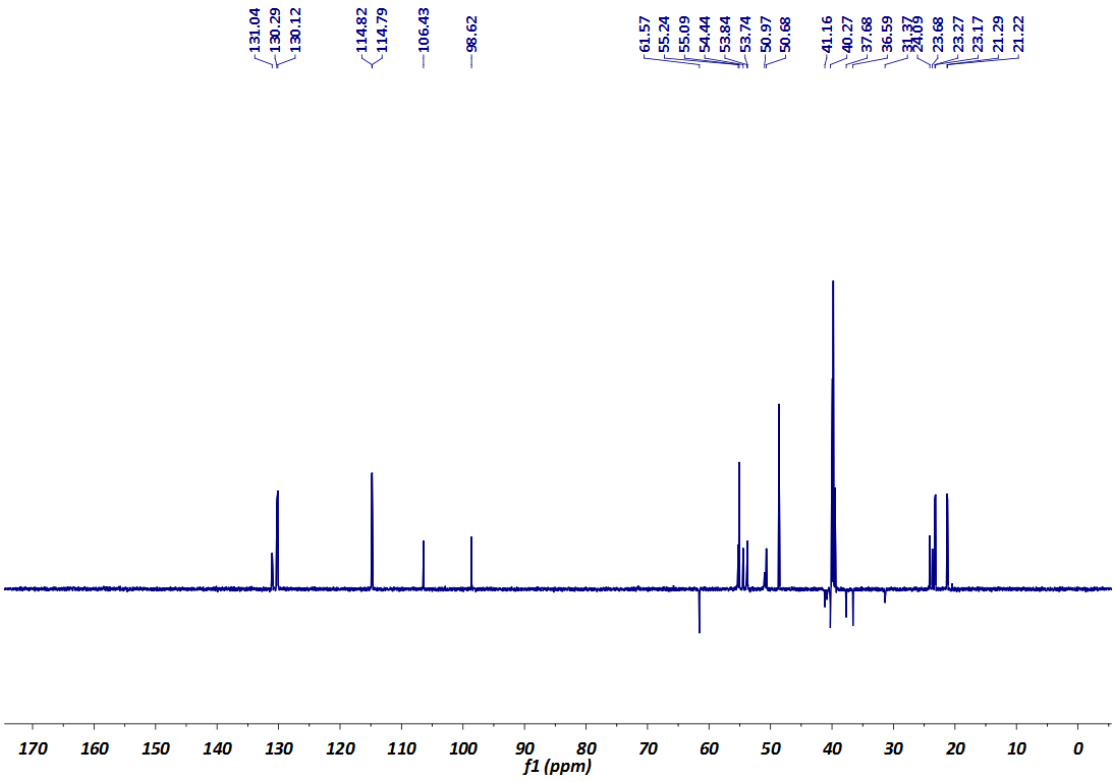

Figure S56. DEPT NMR (150 MHz, DMSO-d_6) spectrum of compound **3**.

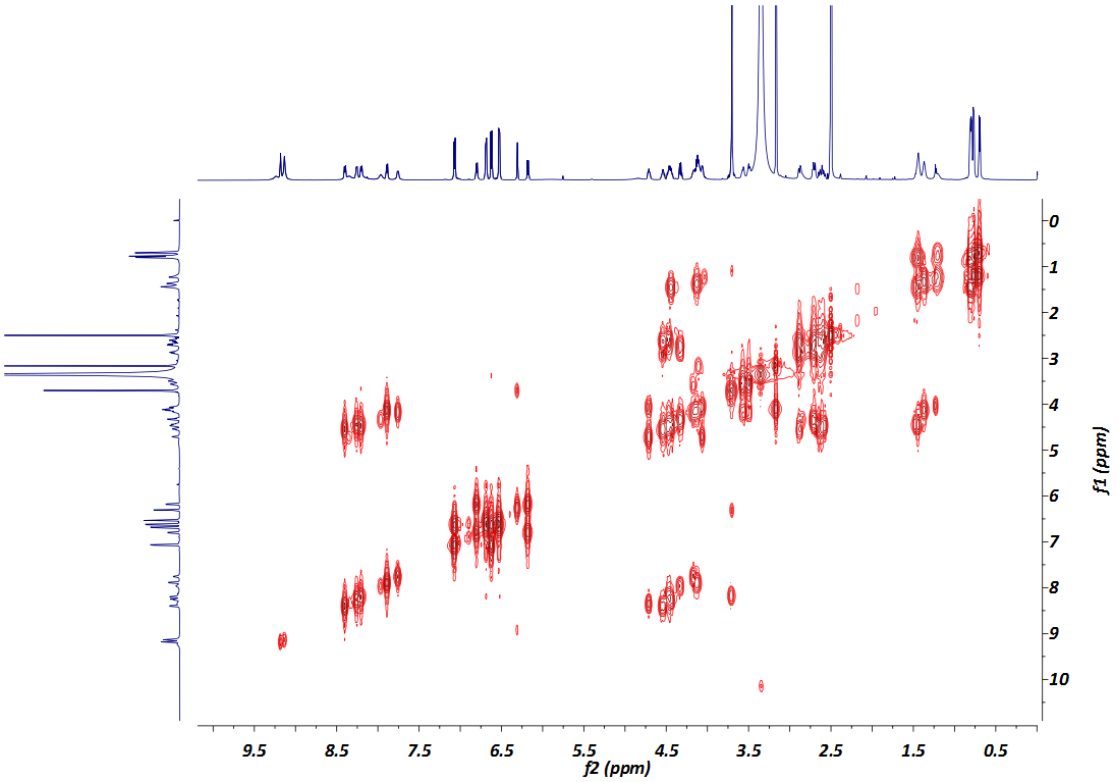

Figure S57. $^{1}H–^{1}H$ COSY NMR (600 MHz, DMSO-d_6) spectrum of compound **3**.

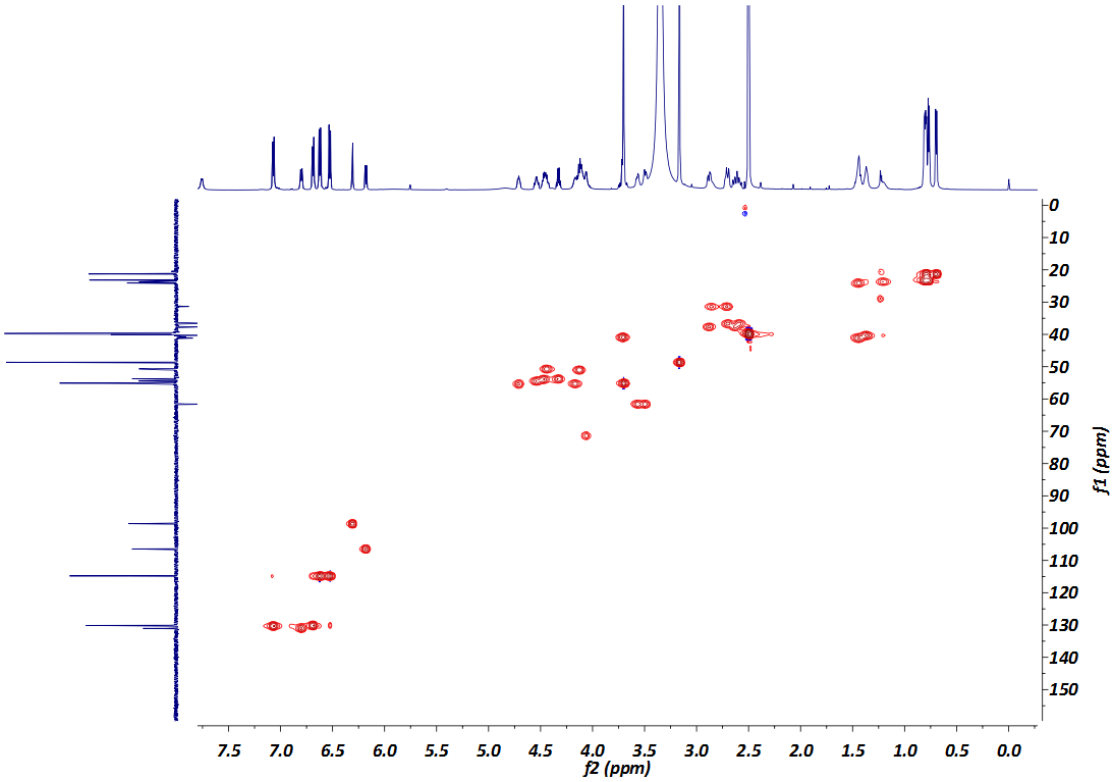

Figure S58. HSQC NMR (600 MHz, DMSO-d_6) spectrum of compound **3**.

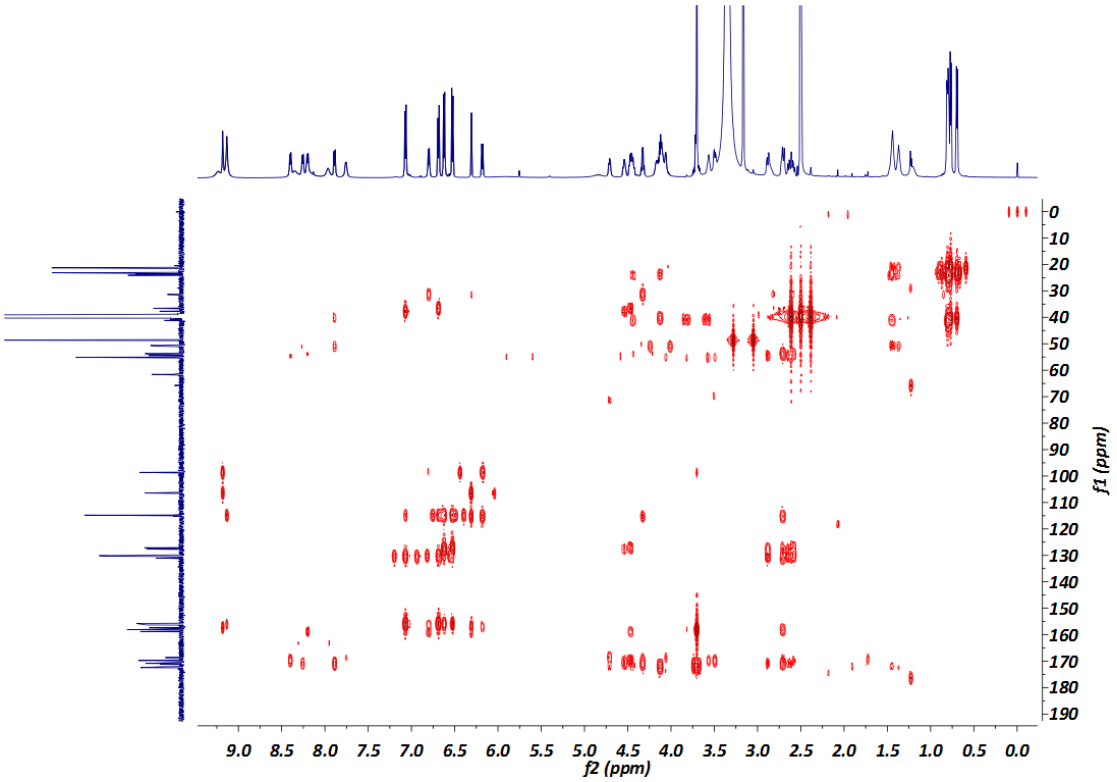

Figure S59. HMBC NMR (600 MHz, DMSO-d_6) spectrum of compound **3**.

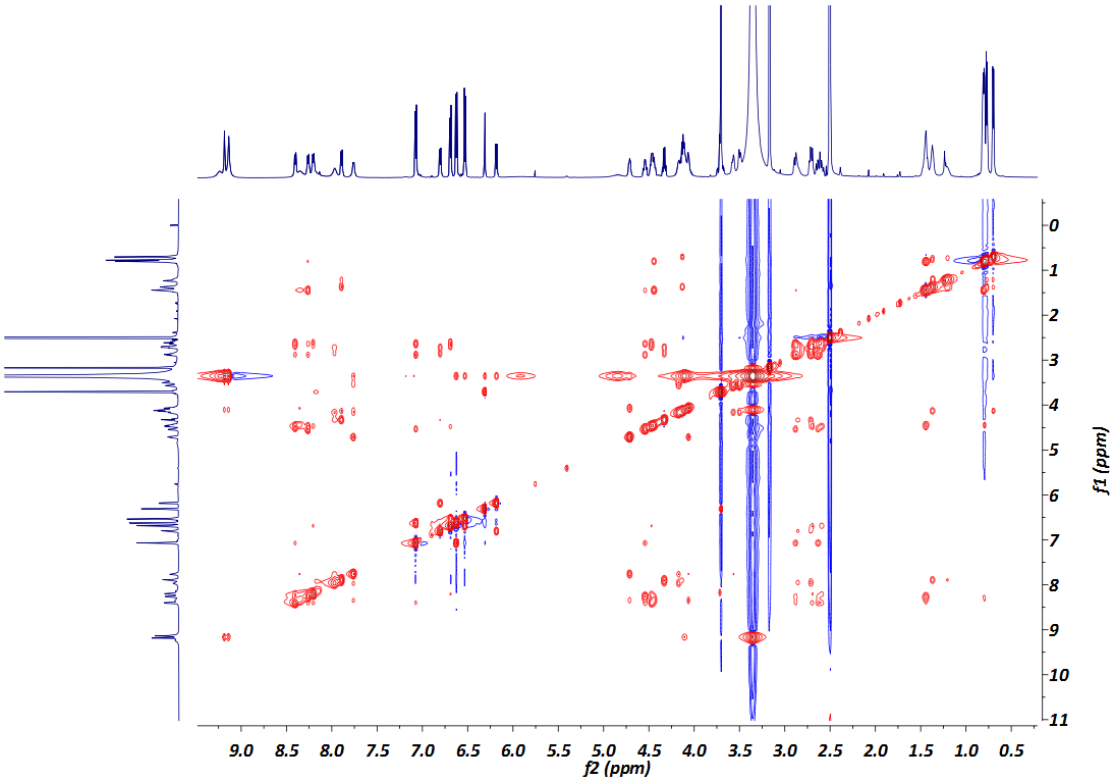

Figure S60. NOESY NMR (600 MHz, DMSO-d_6) spectrum of compound **3**.

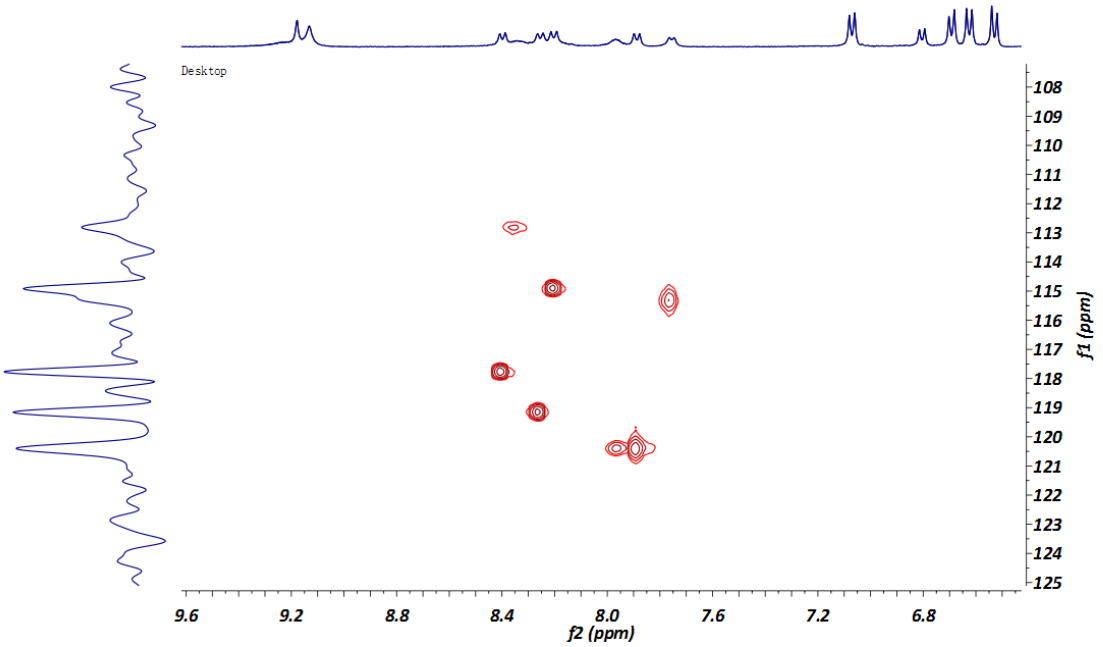

Figure S61. N-H HSQC NMR (600 MHz, DMSO-d_6) spectrum of compound **3**.

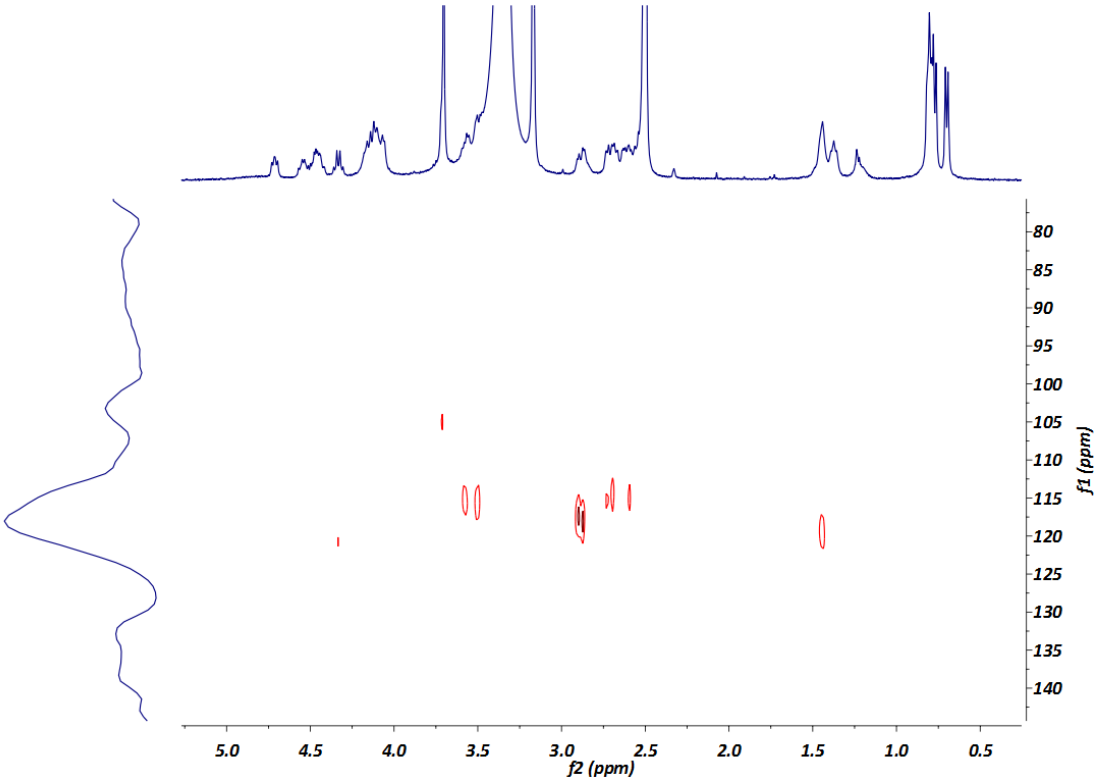

Figure S62. N-H HMBC NMR (600 MHz, DMSO-d_6) spectrum of compound **3**.

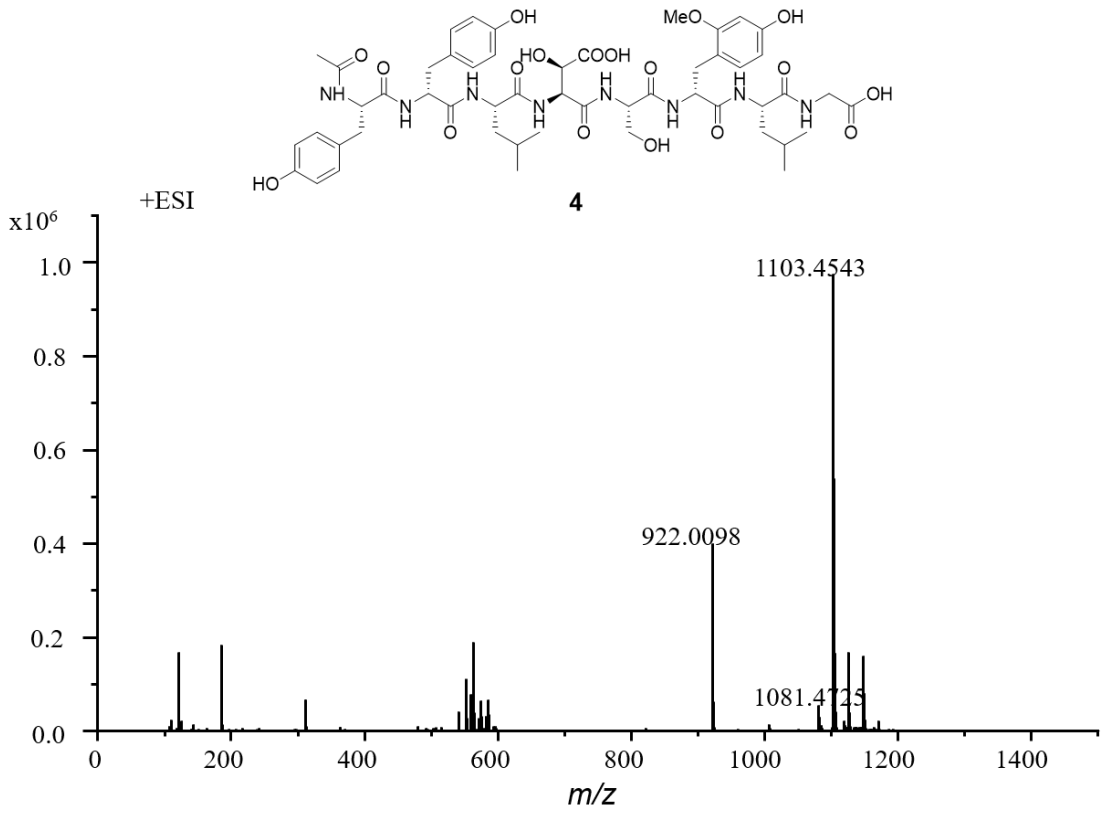

Figure S63. HRESIMS spectrum of **4**.

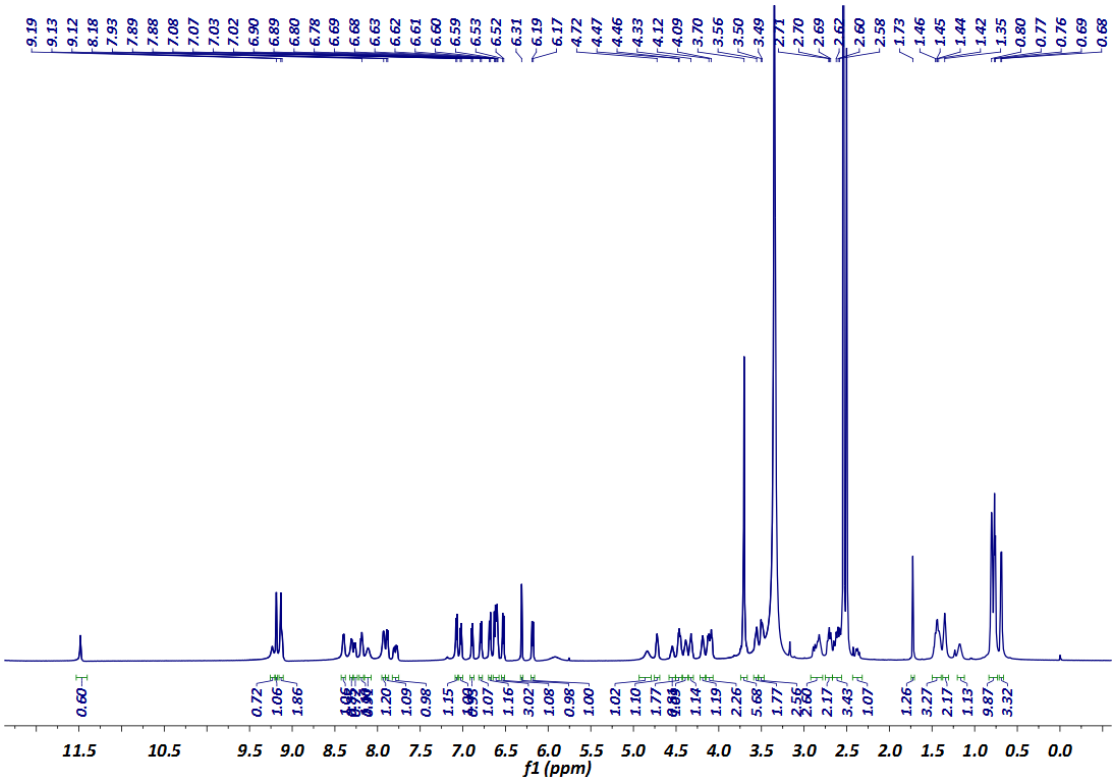

Figure S64. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of compound **4**.

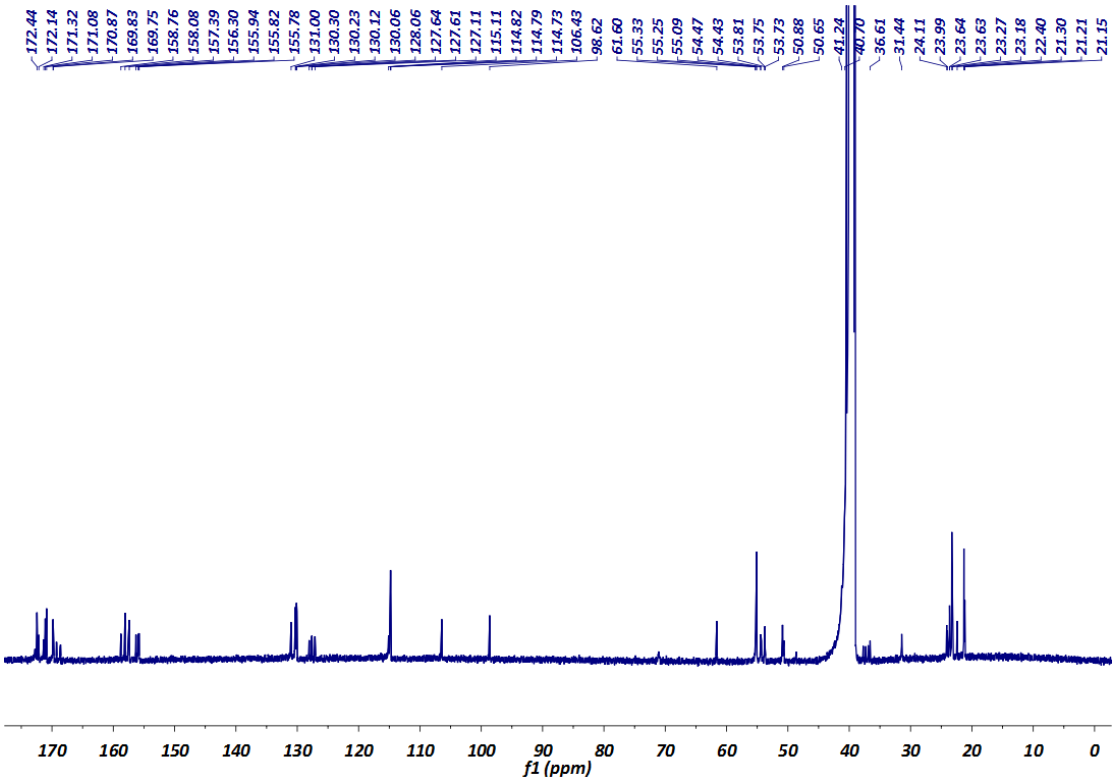

Figure S65. ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of compound **4**.

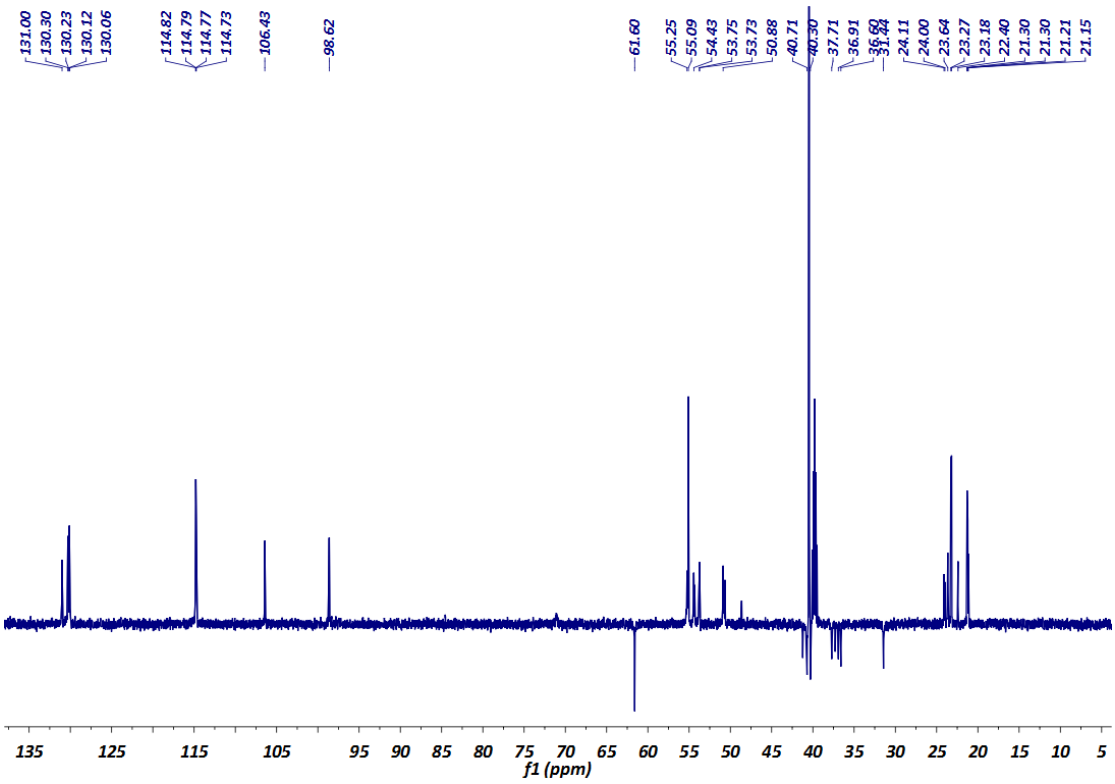

Figure S66. DEPT NMR (150 MHz, DMSO-d_6) spectrum of compound **4**.

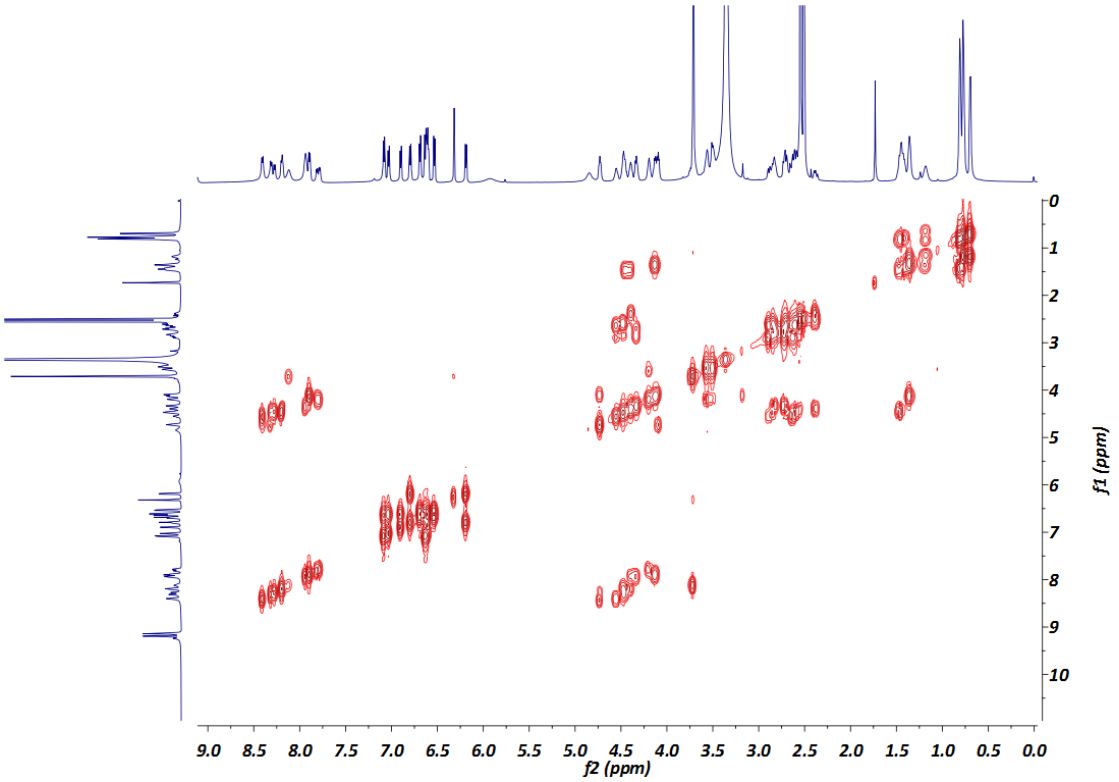

Figure S67. $^{1}H–^{1}H$ COSY NMR (600 MHz, DMSO-d_6) spectrum of compound **4**.

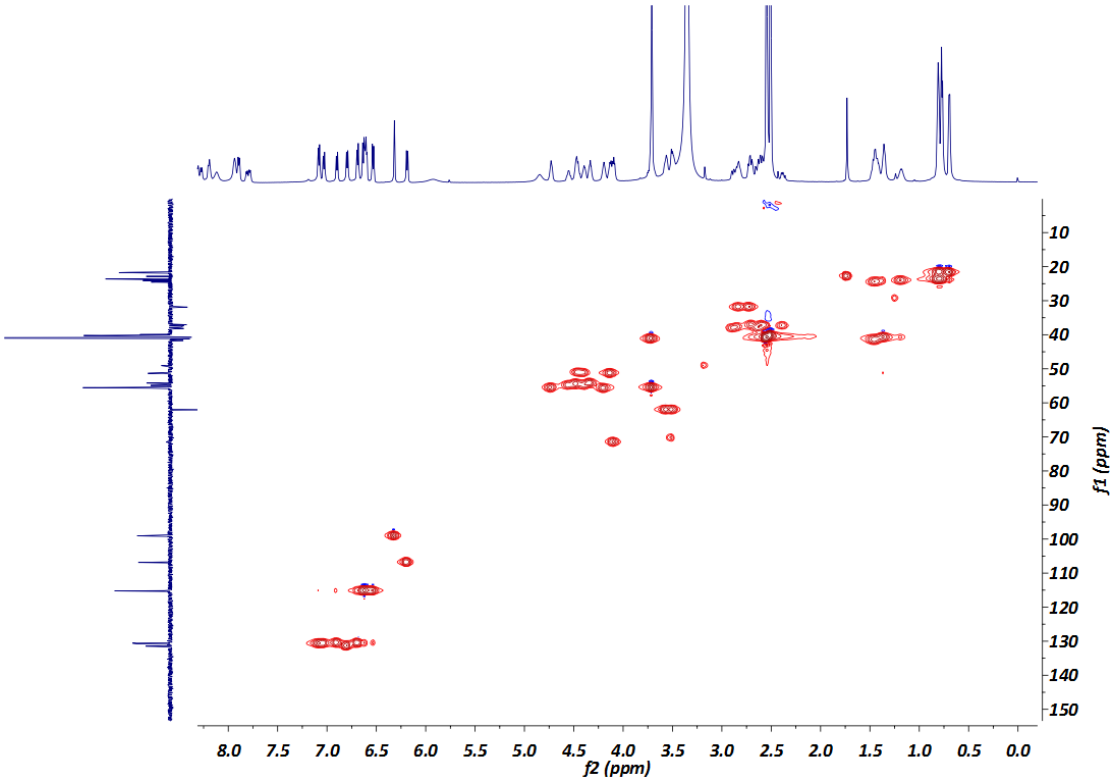

Figure S68. HSQC NMR (600 MHz, DMSO-d_6) spectrum of compound **4**.

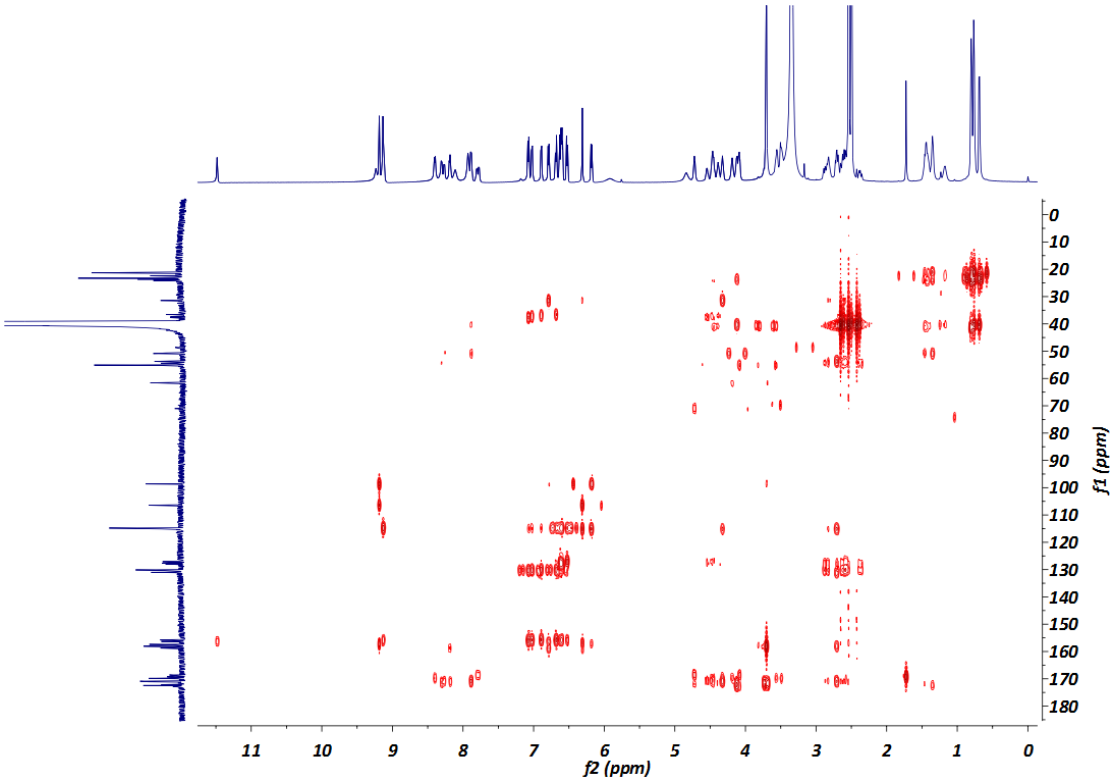

Figure S69. HMBC NMR (600 MHz, DMSO-d_6) spectrum of compound **4**.

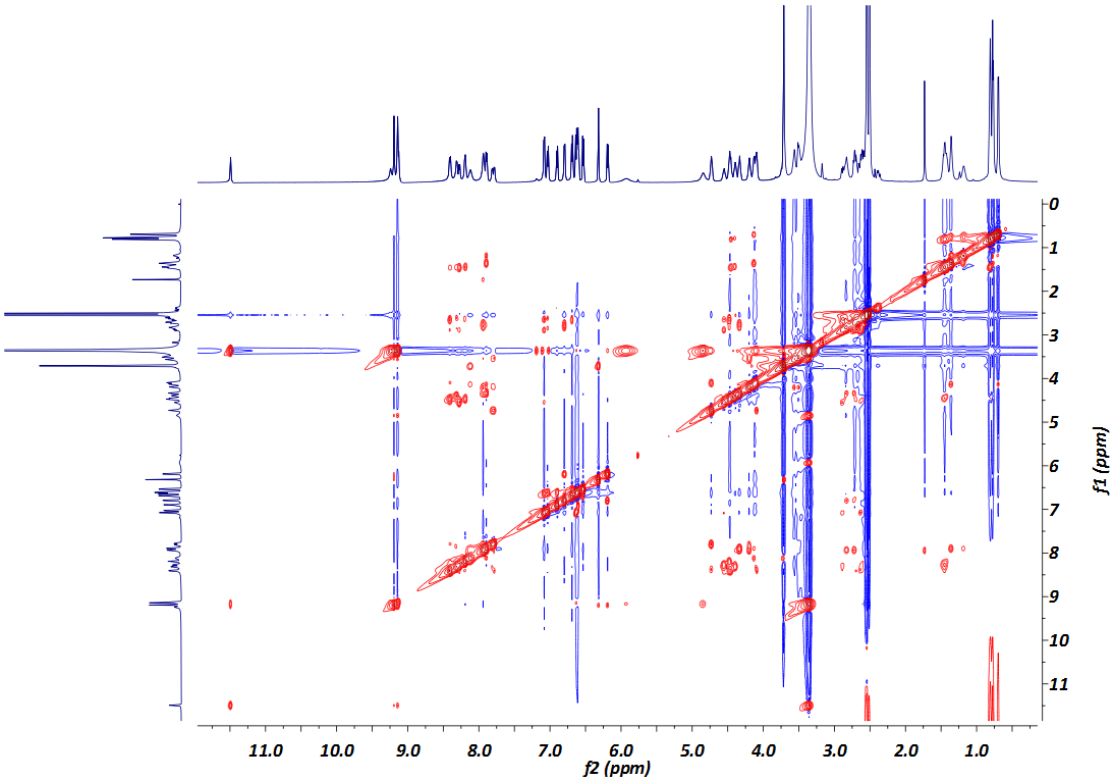

Figure S70. NOESY NMR (600 MHz, DMSO-d_6) spectrum of compound **4**.

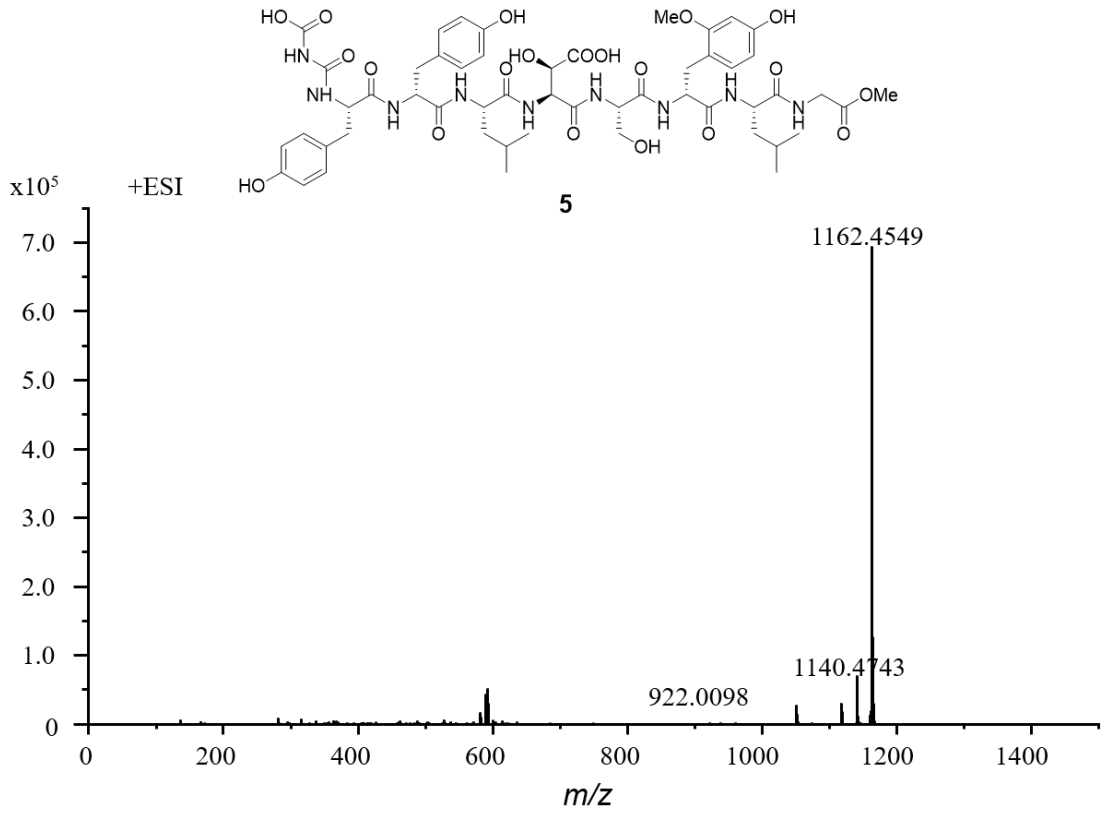

Figure S71. HRESIMS spectrum of **5**

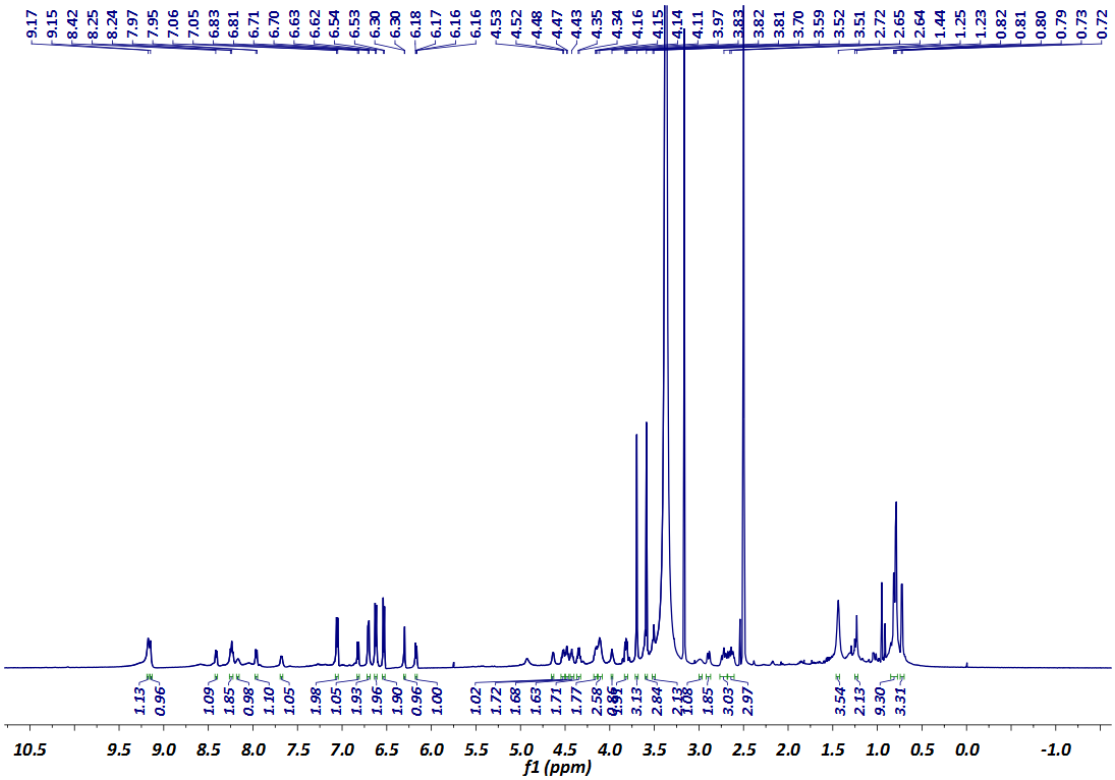

Figure S72. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of compound **5**.

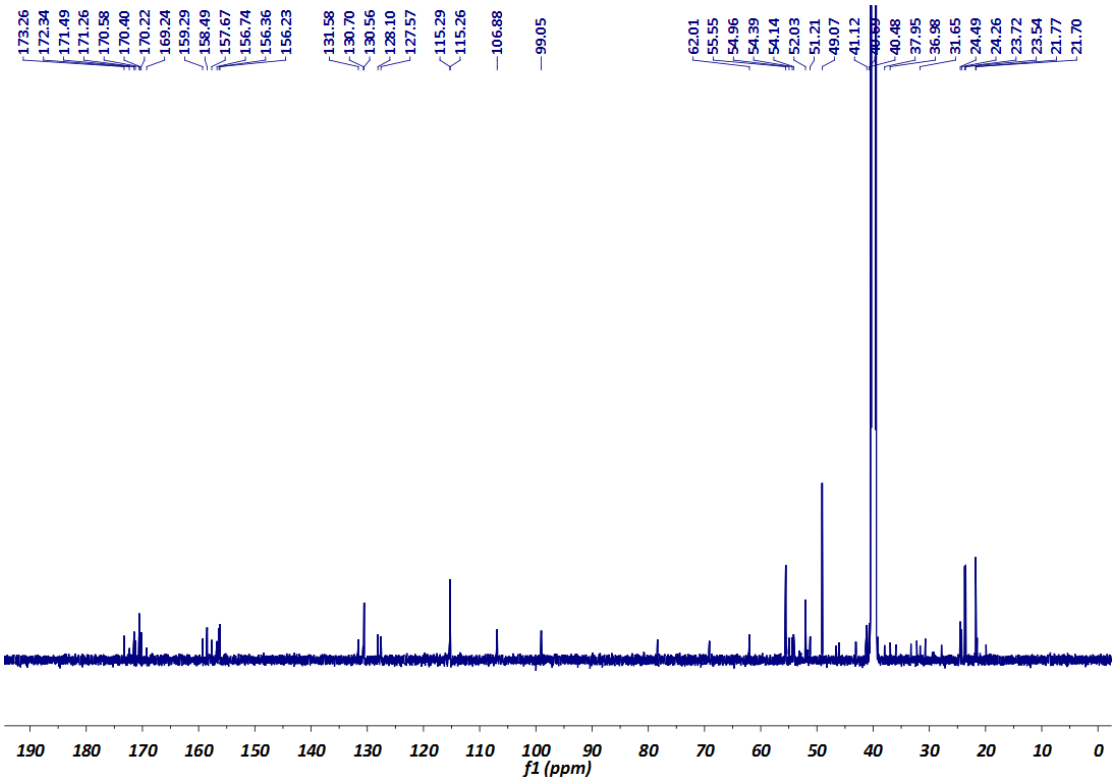

Figure S73. ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of compound **5**.

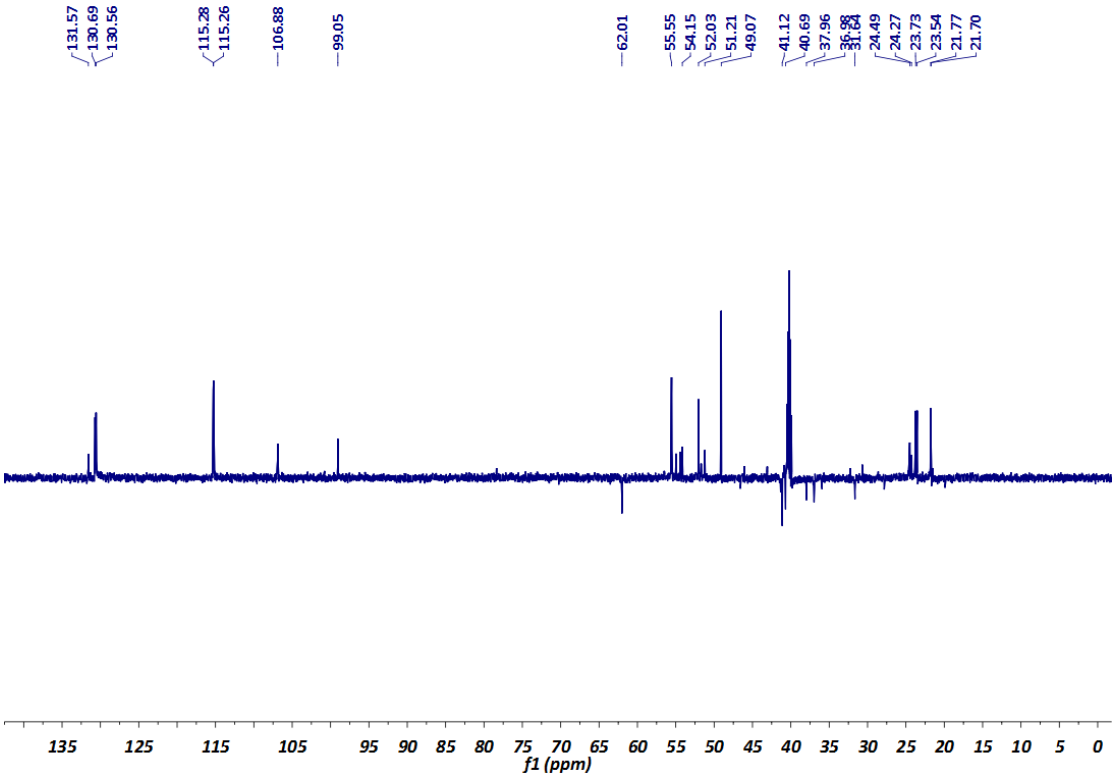

Figure S74. DEPT NMR (150 MHz, DMSO-d_6) spectrum of compound **5**.

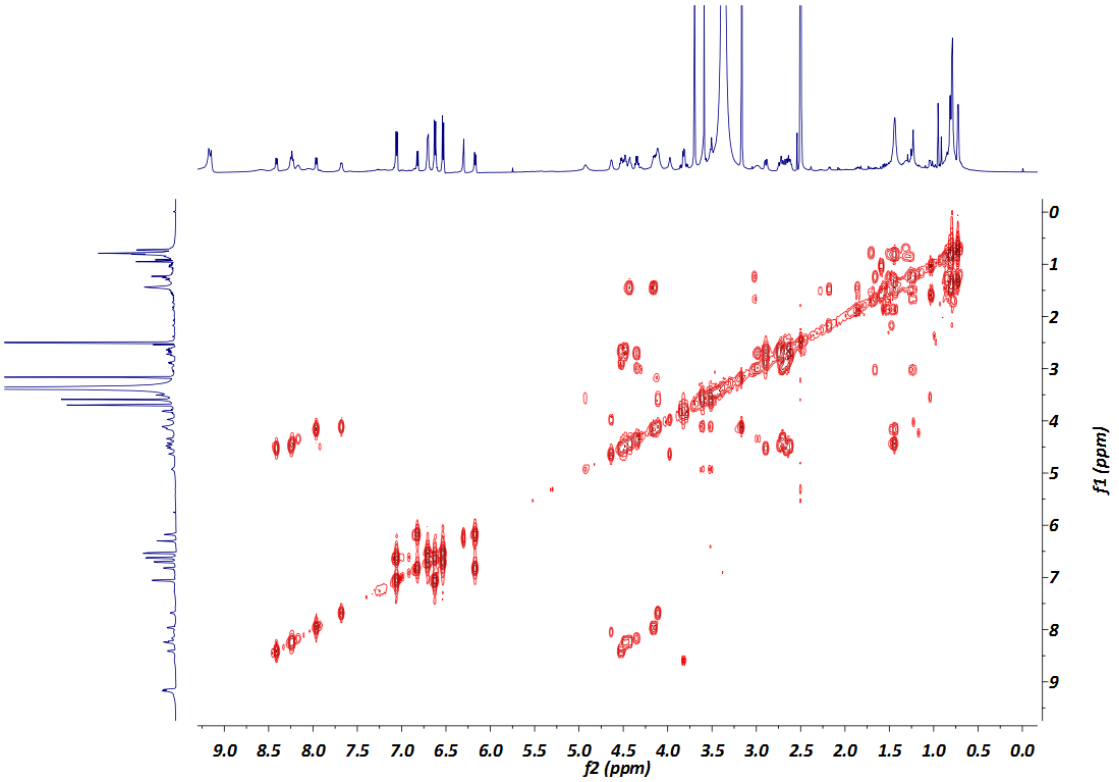

Figure S75. $^{1}H–^{1}H$ COSY NMR (600 MHz, DMSO-d_6) spectrum of compound **5**.

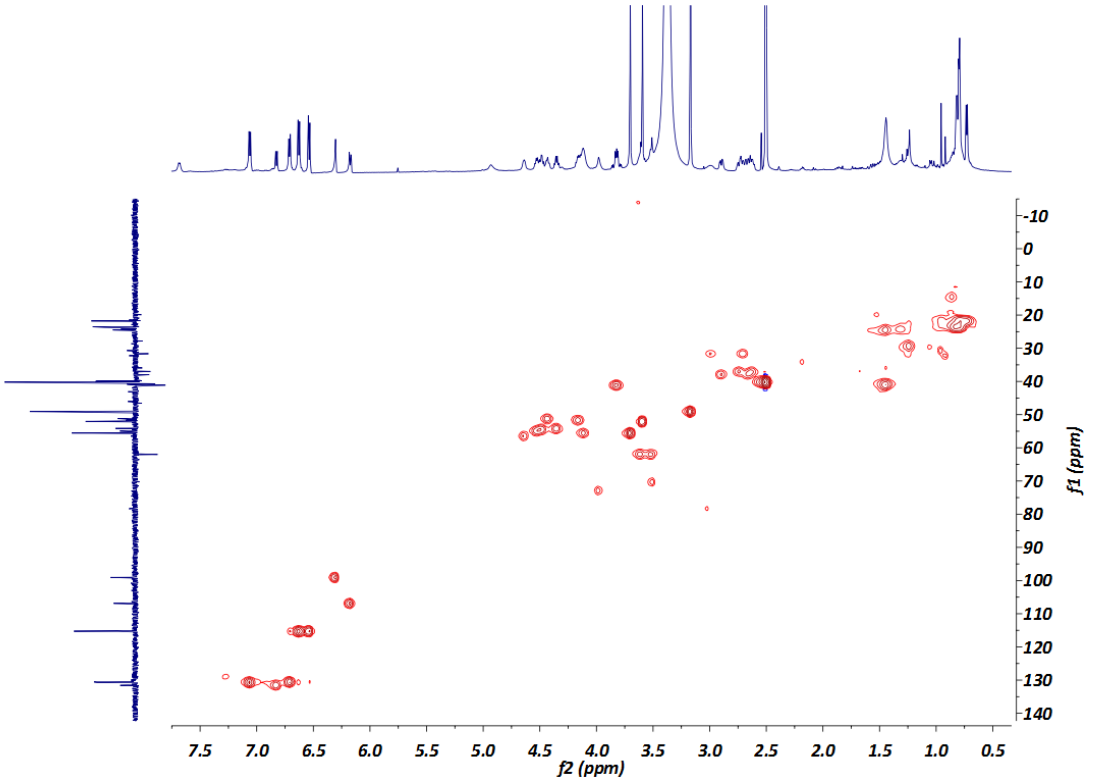

Figure S76. HSQC NMR (600 MHz, DMSO-d_6) spectrum of compound **5**.

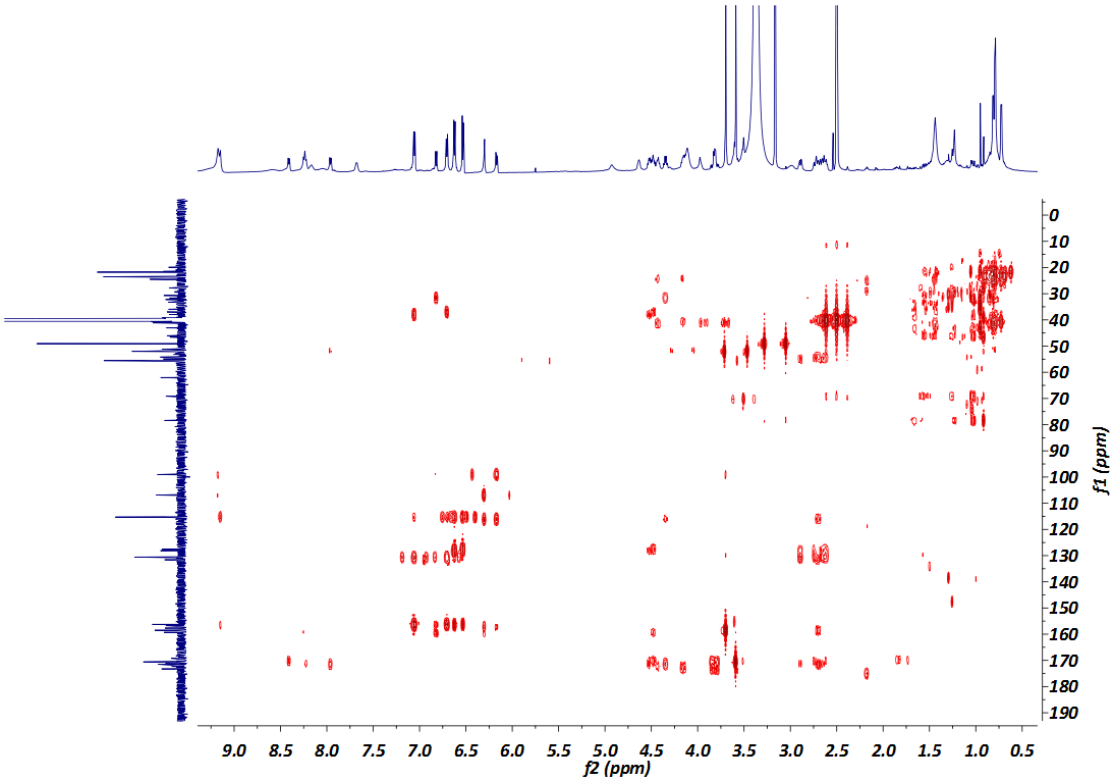

Figure S77. HMBC NMR (600 MHz, DMSO-d_6) spectrum of compound **5**.

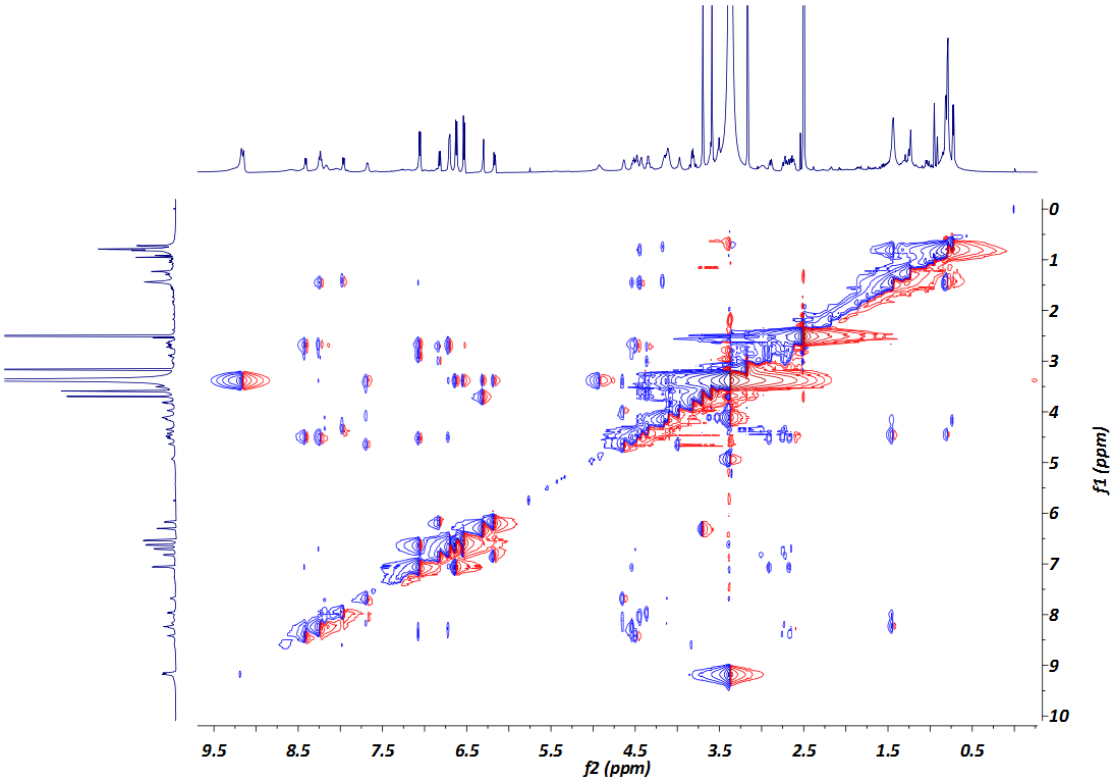

Figure S78. NOESY NMR (600 MHz, DMSO-d_6) spectrum of compound **5**.

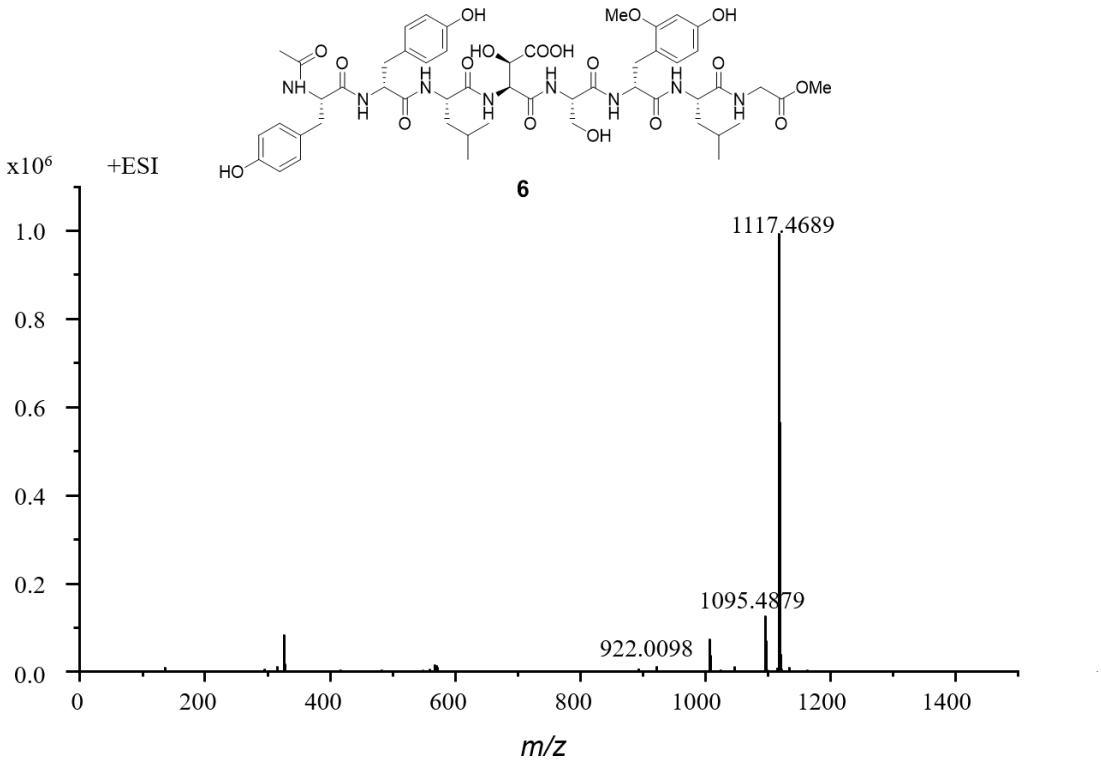

Figure S79. HRESIMS spectrum of **6**.

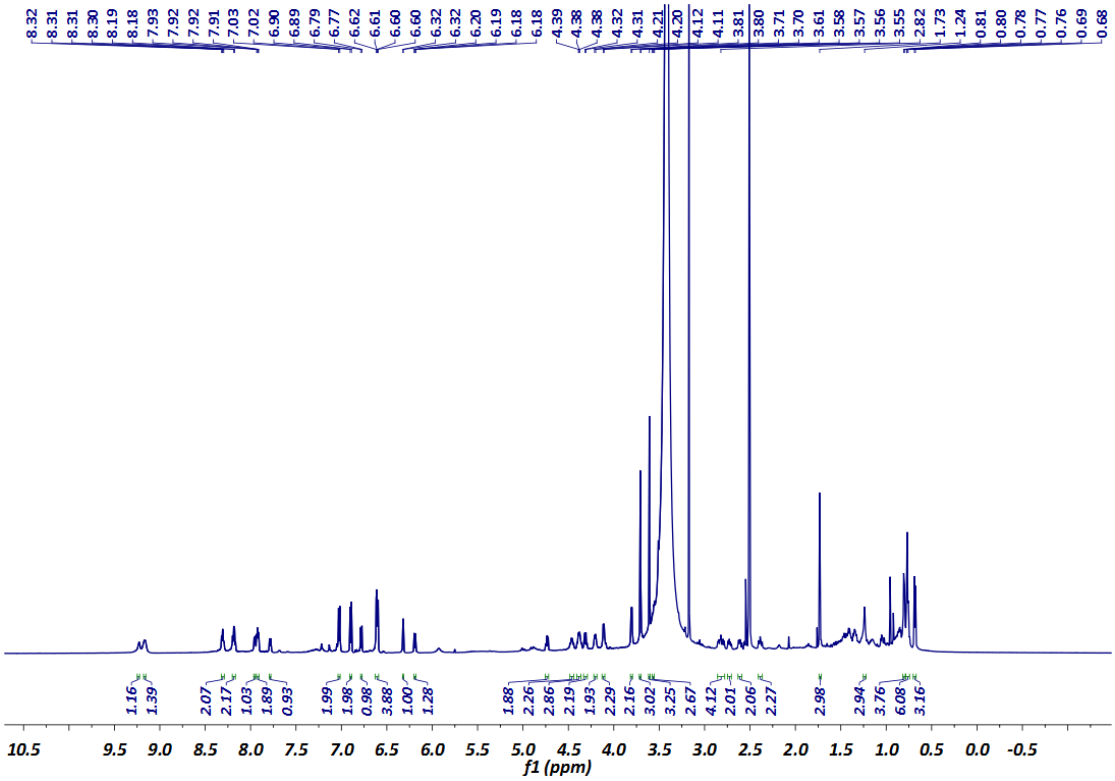

Figure S80. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of compound **6**.

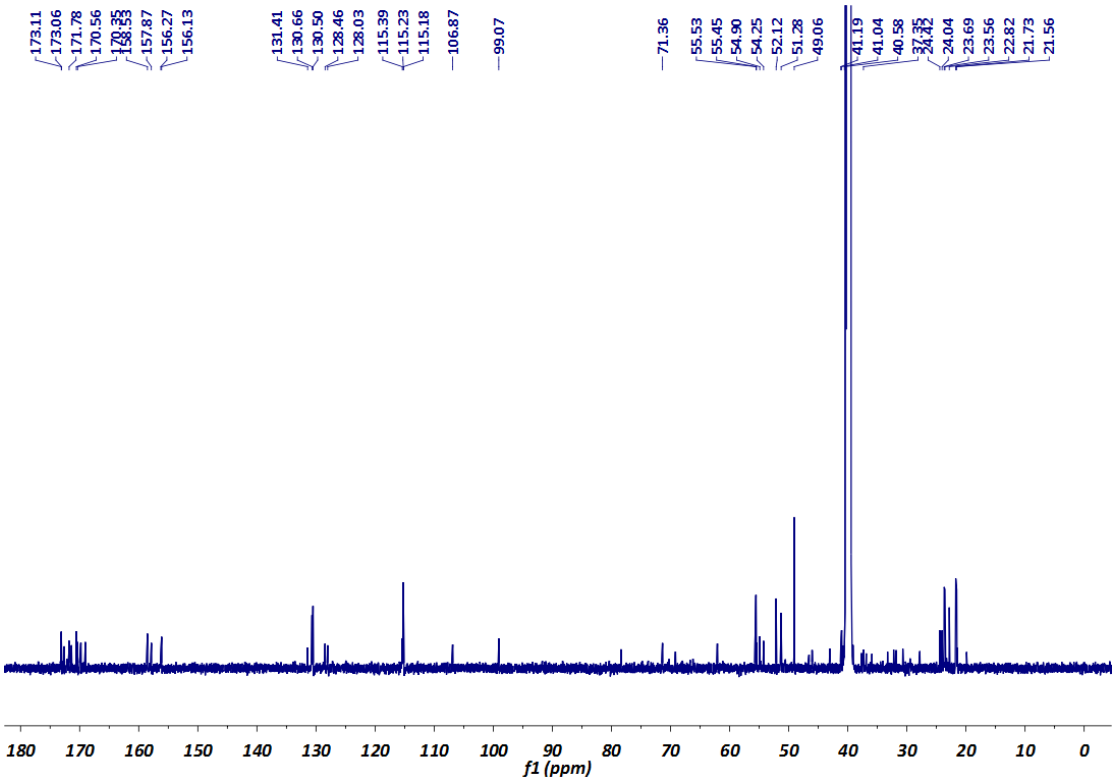

Figure S81. ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of compound **6**.

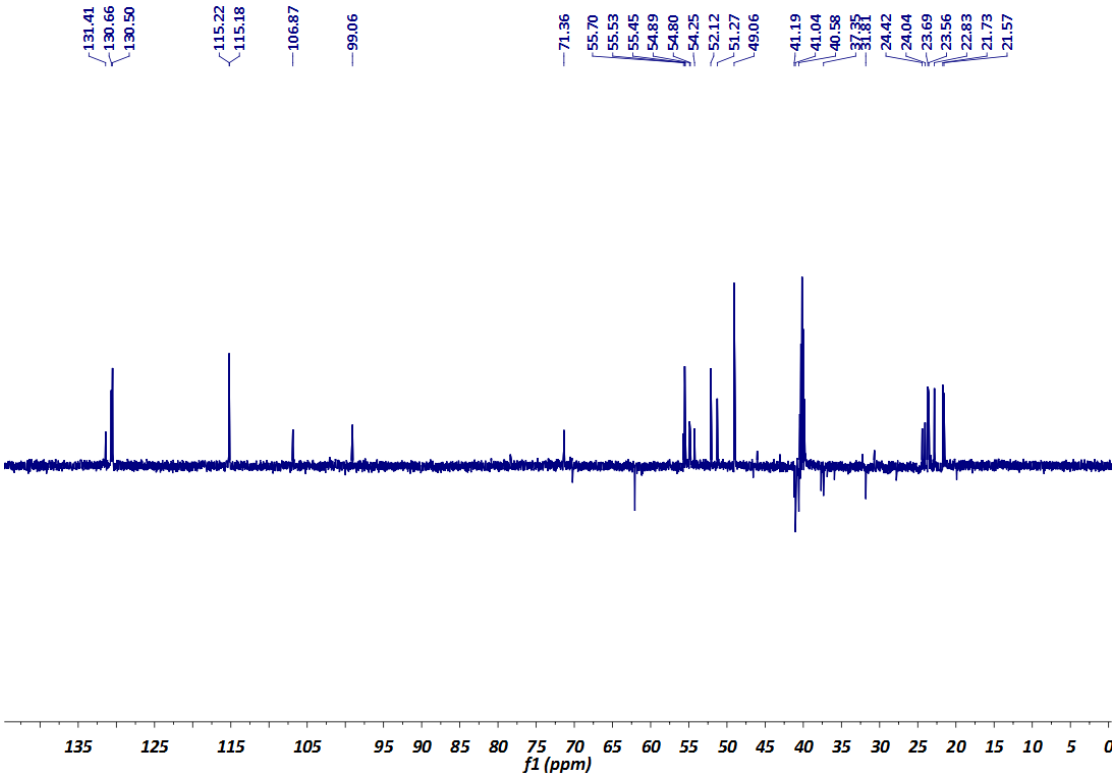

Figure S82. DEPT NMR (150 MHz, DMSO-d_6) spectrum of compound **6**.

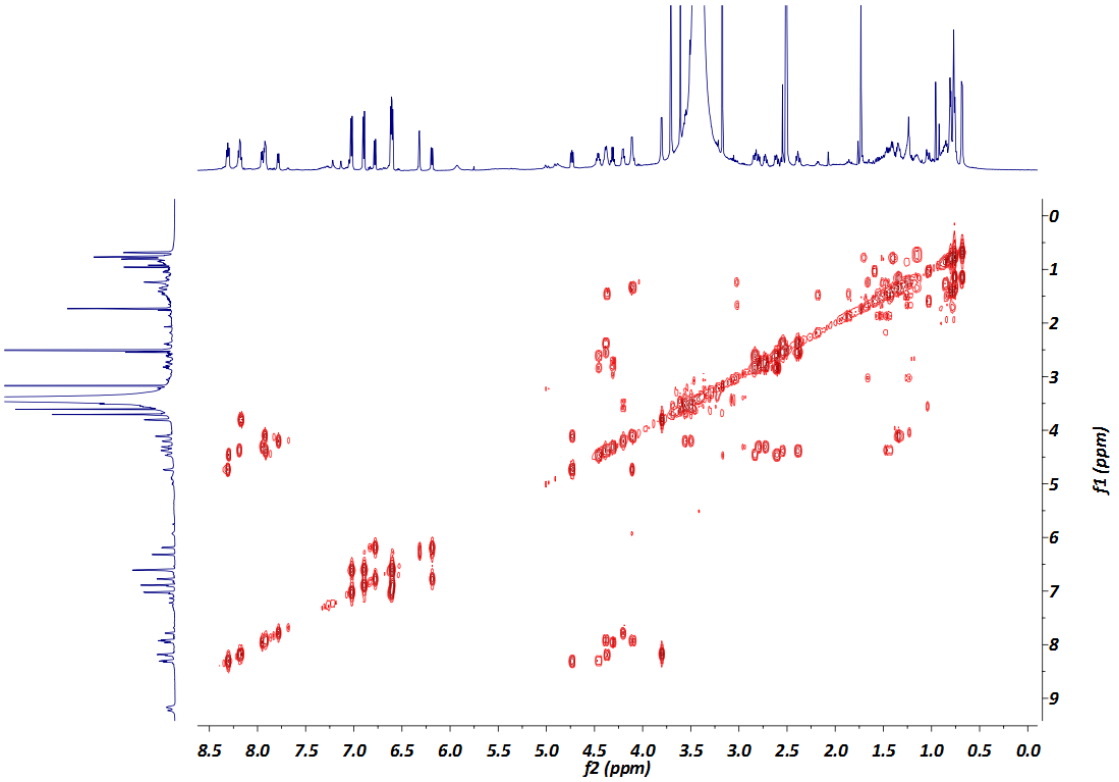

Figure S83. $^{1}H–^{1}H$ COSY NMR (600 MHz, DMSO-d_6) spectrum of compound **6**.

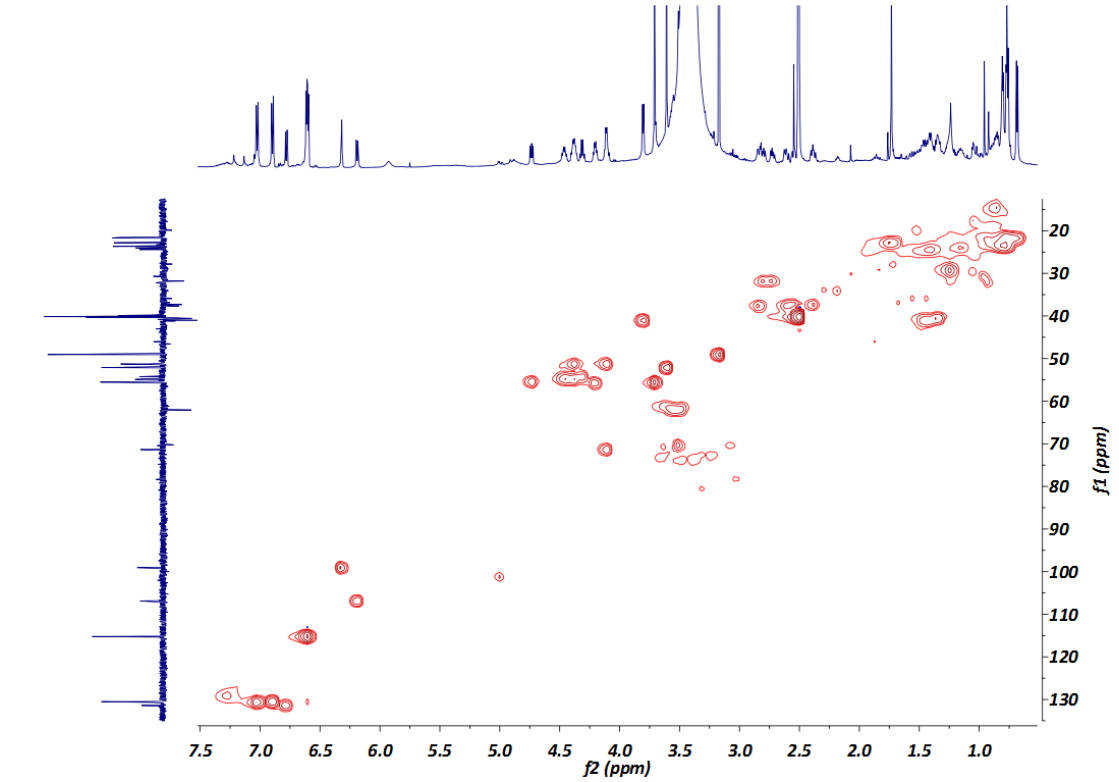

Figure S84. HSQC NMR (600 MHz, DMSO-d_6) spectrum of compound **6**.

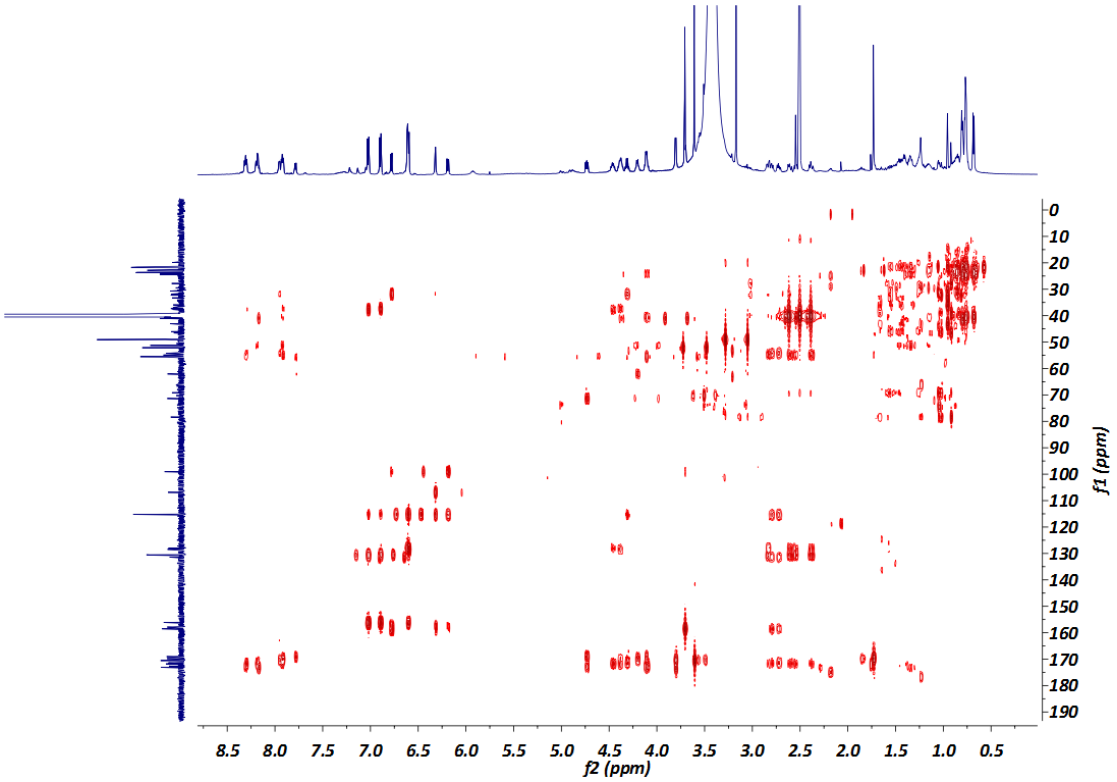

Figure S85. HMBC NMR (600 MHz, DMSO-d_6) spectrum of compound **6**.

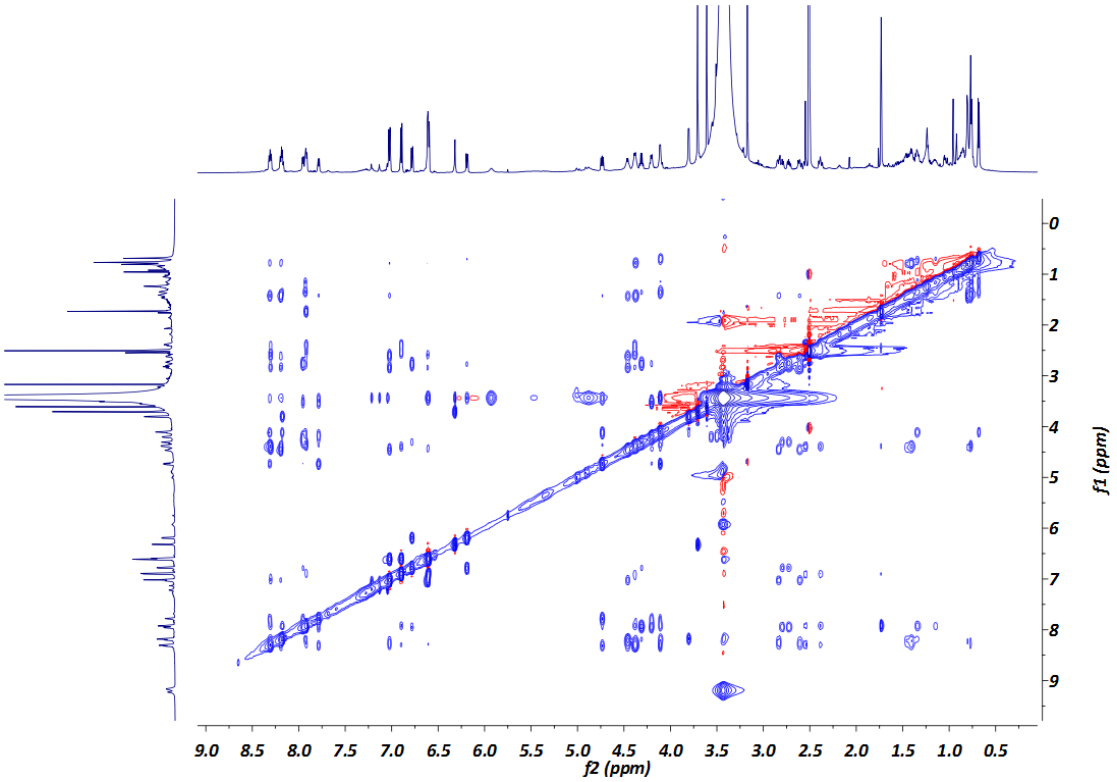

Figure S86. NOESY NMR (600 MHz, DMSO-d_6) spectrum of compound **6**.

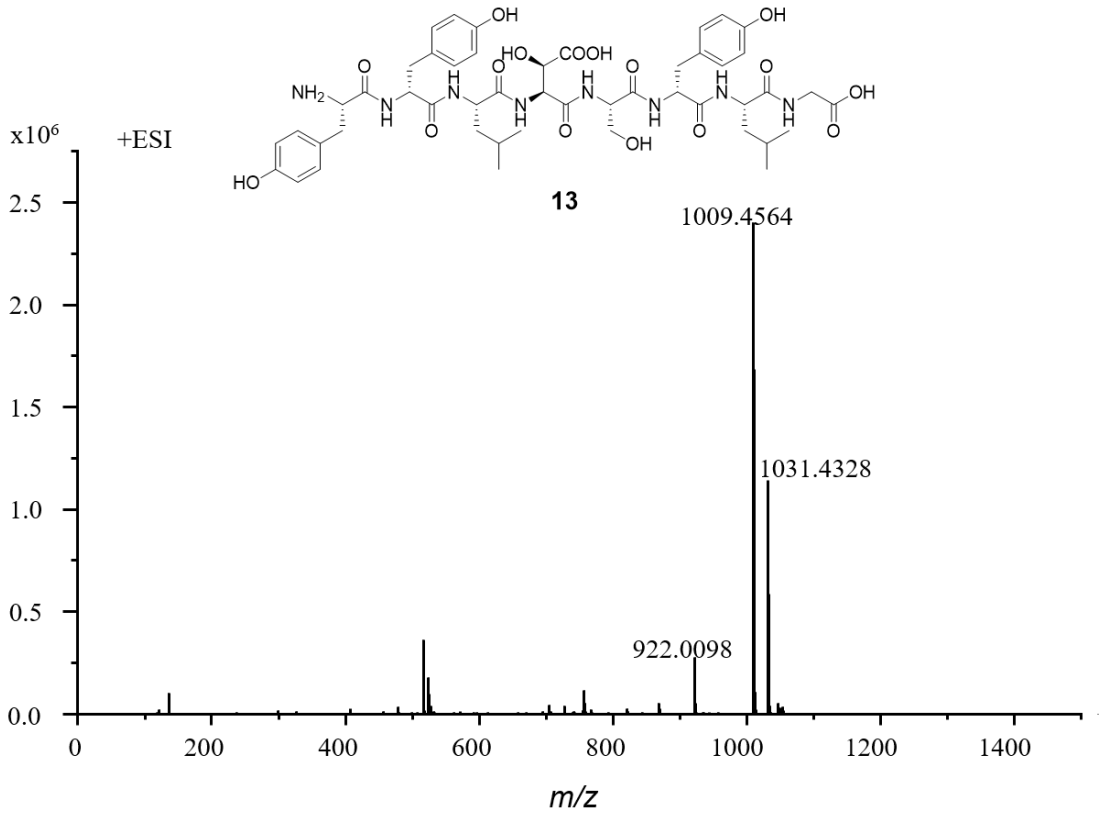

Figure S87. HRESIMS spectrum of **13**.

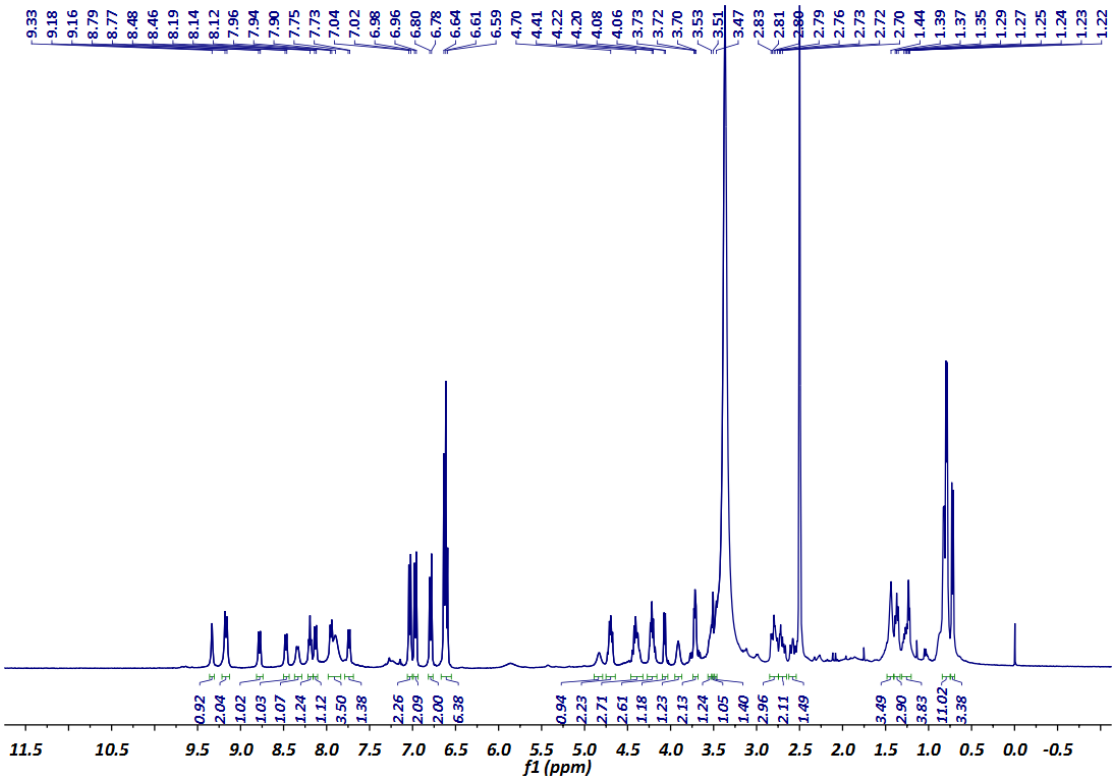

Figure S88. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of compound **13**.

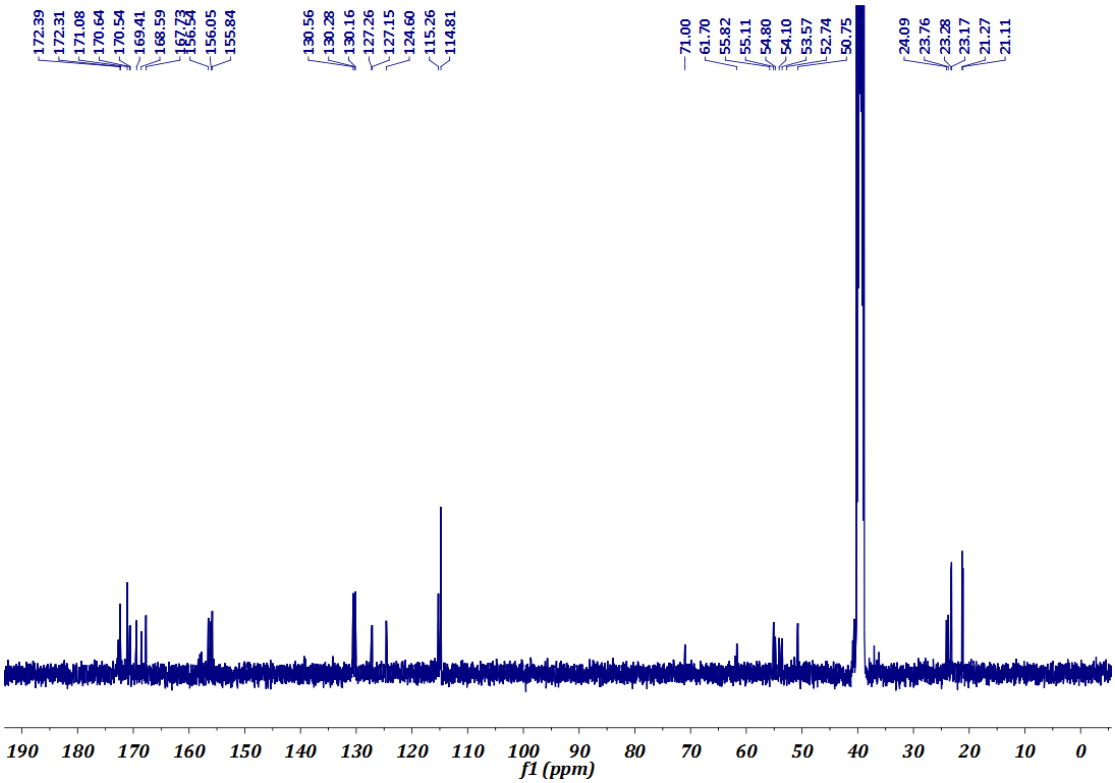

Figure S89. ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of compound **13**.

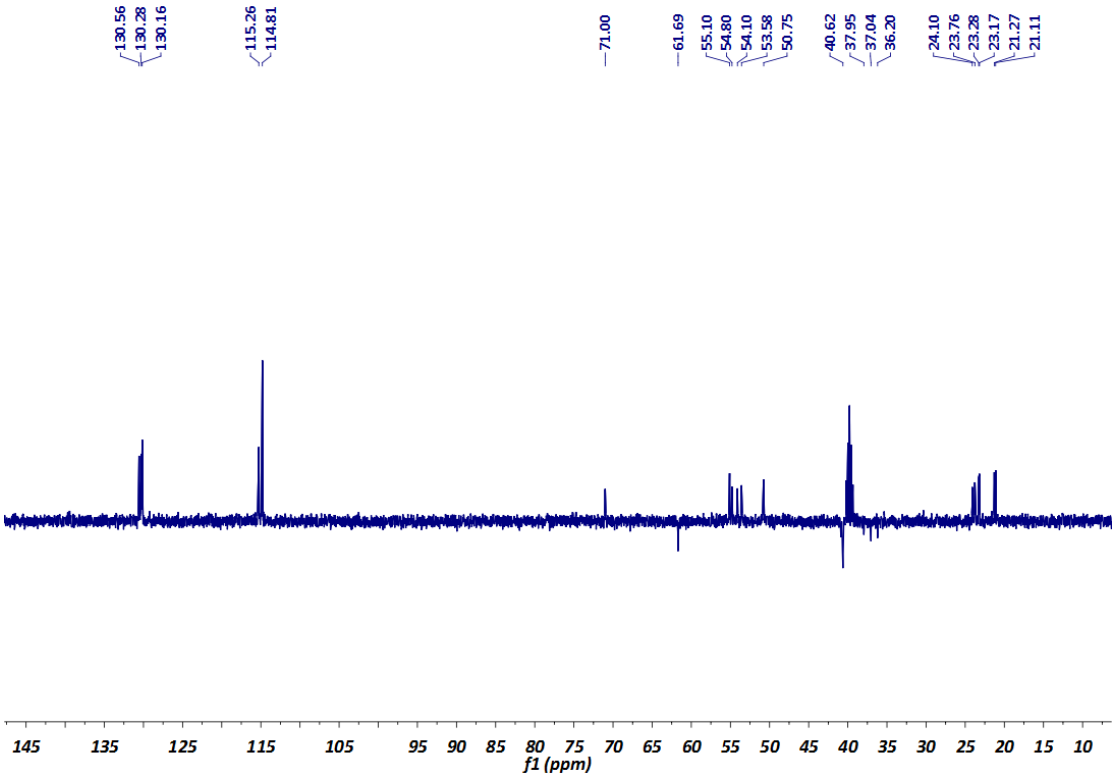

Figure S90. DEPT NMR (150 MHz, DMSO-d_6) spectrum of compound **13**.

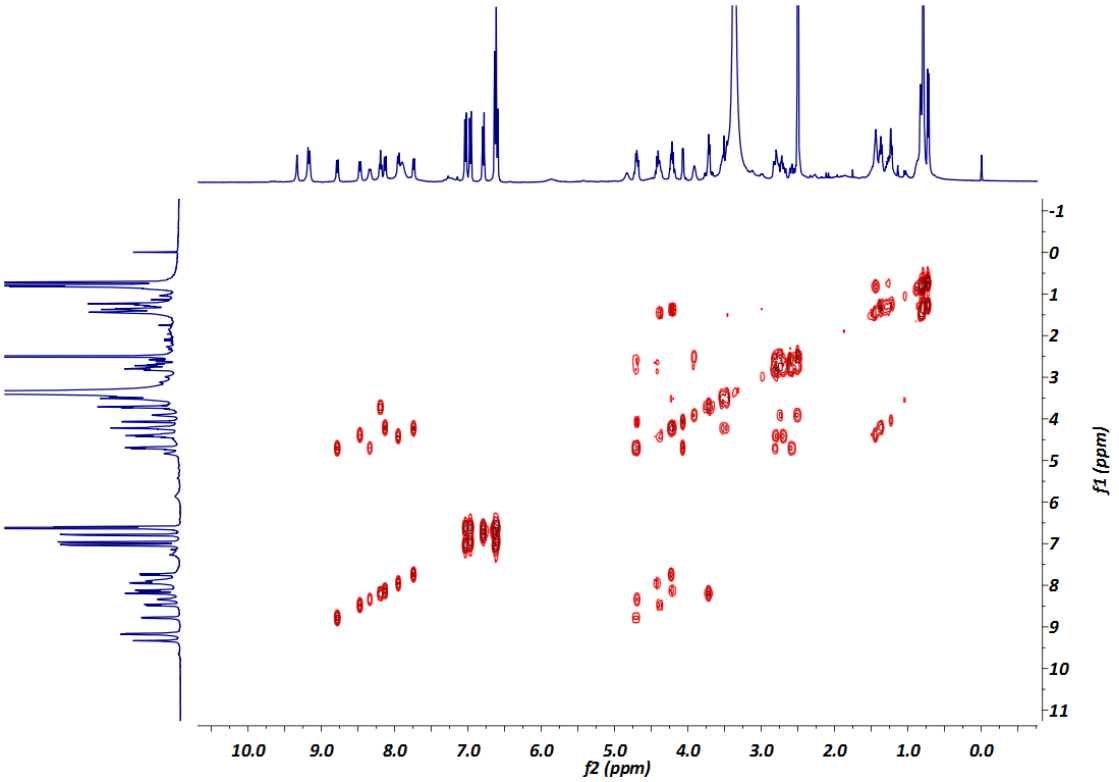

Figure S91. $^{1}H–^{1}H$ COSY NMR (600 MHz, DMSO-d_6) spectrum of compound **13**.

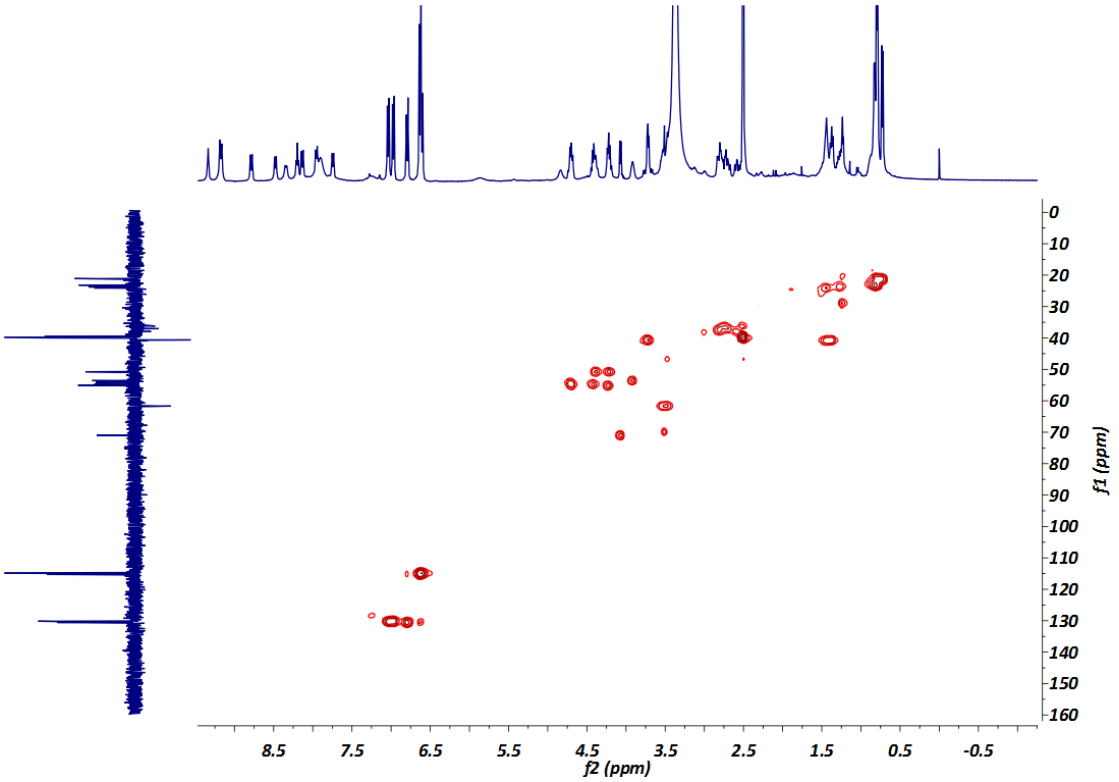

Figure S92. HSQC NMR (600 MHz, DMSO-d_6) spectrum of compound **13**.

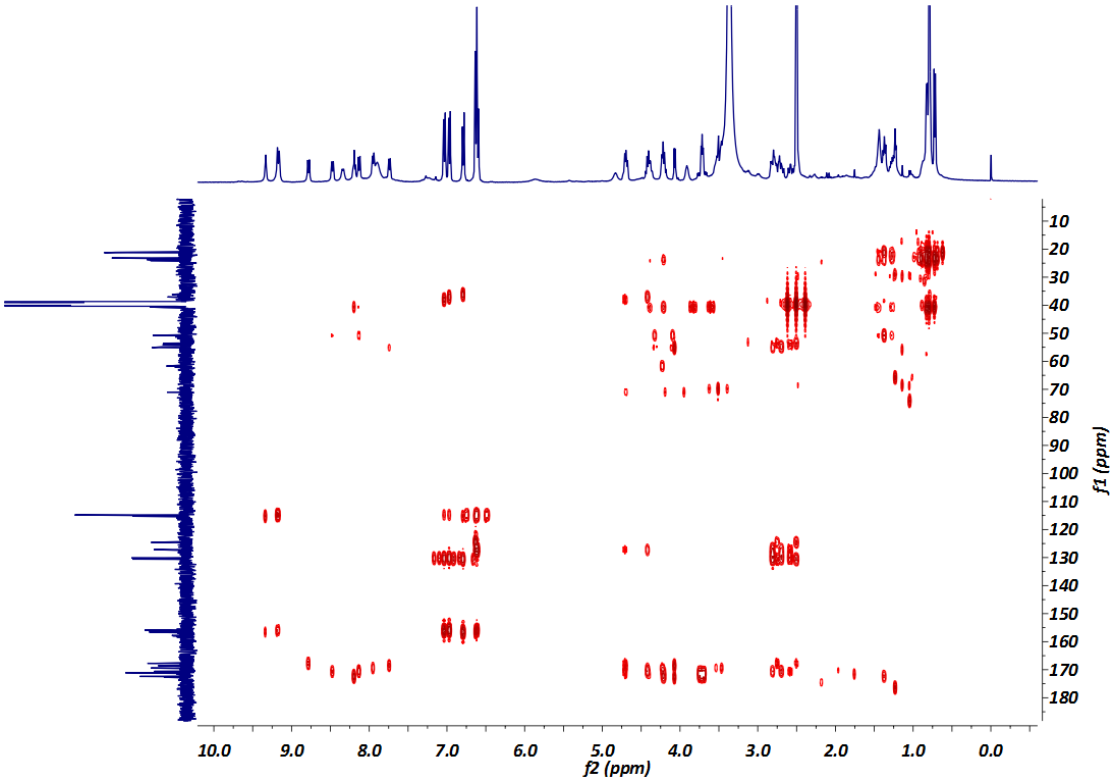

Figure S93. HMBC NMR (600 MHz, DMSO-d_6) spectrum of compound **13**.

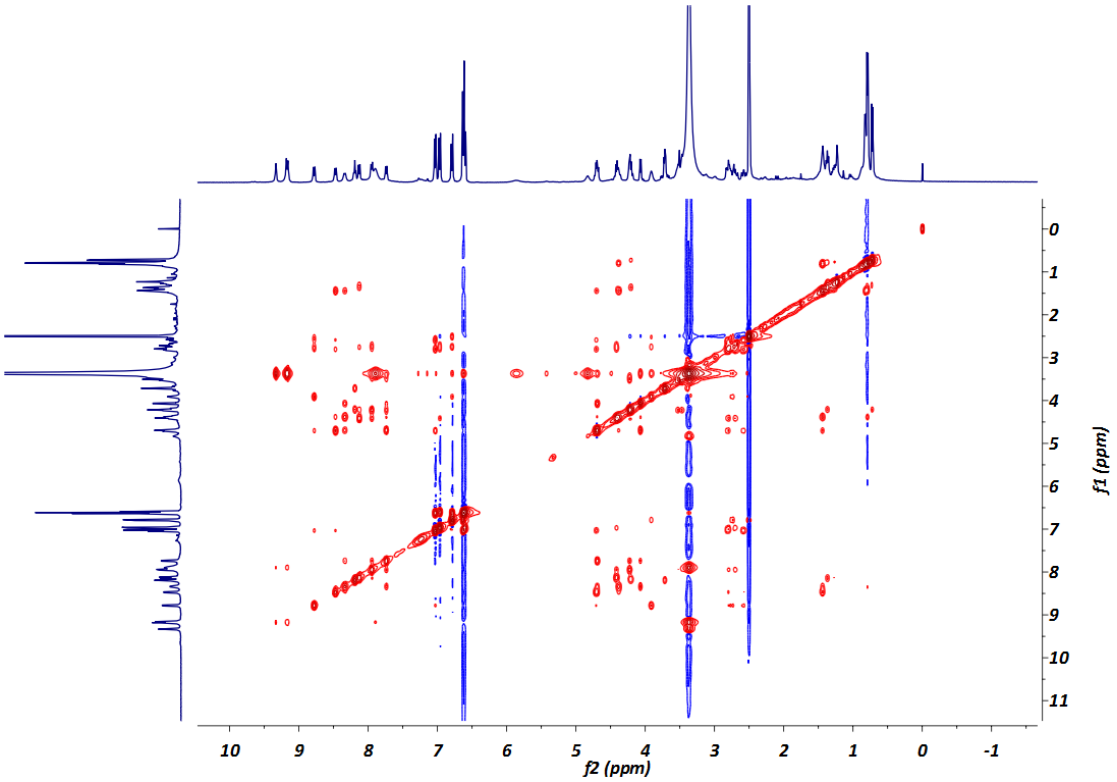

Figure S94. NOESY NMR (600 MHz, DMSO-d_6) spectrum of compound **13**.

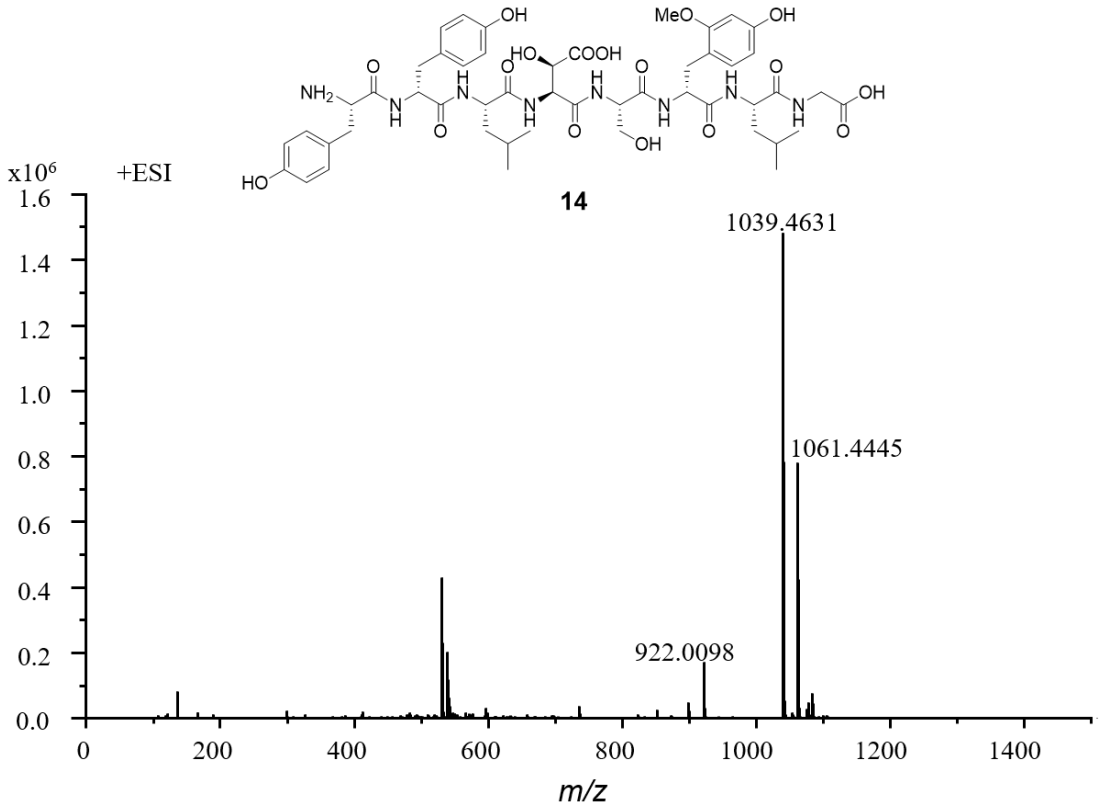

Figure S95. HRESIMS spectrum of **14**.

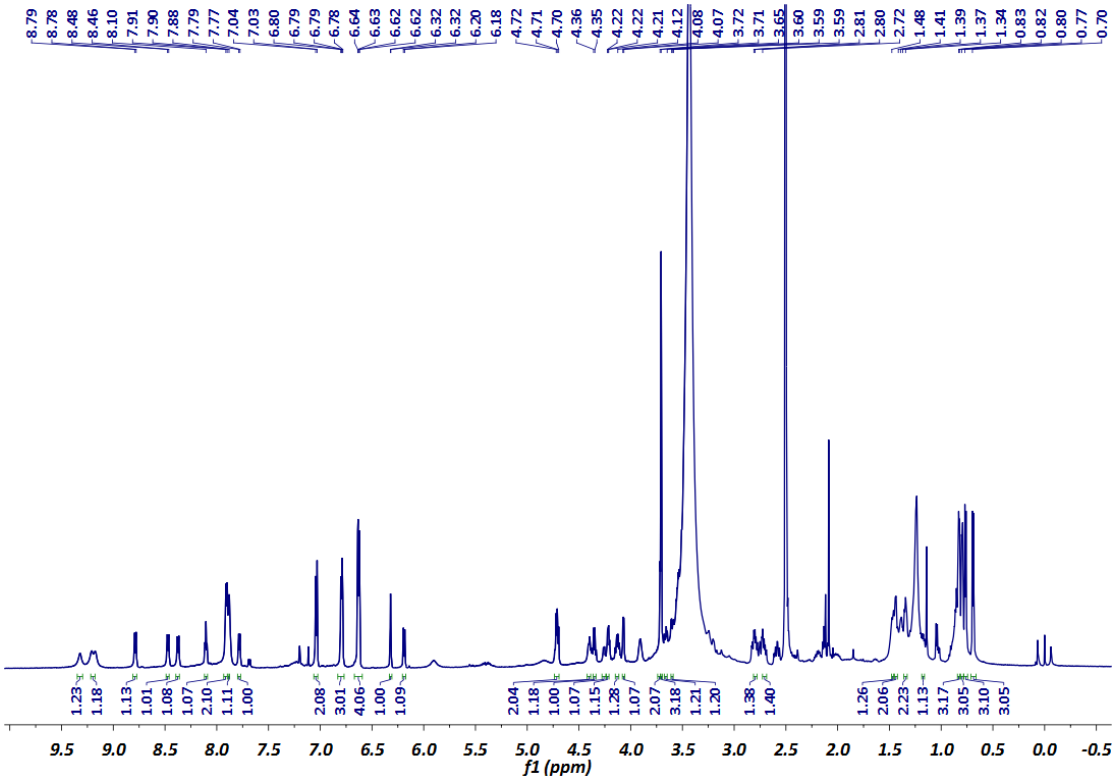

Figure S96. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of compound **14**.

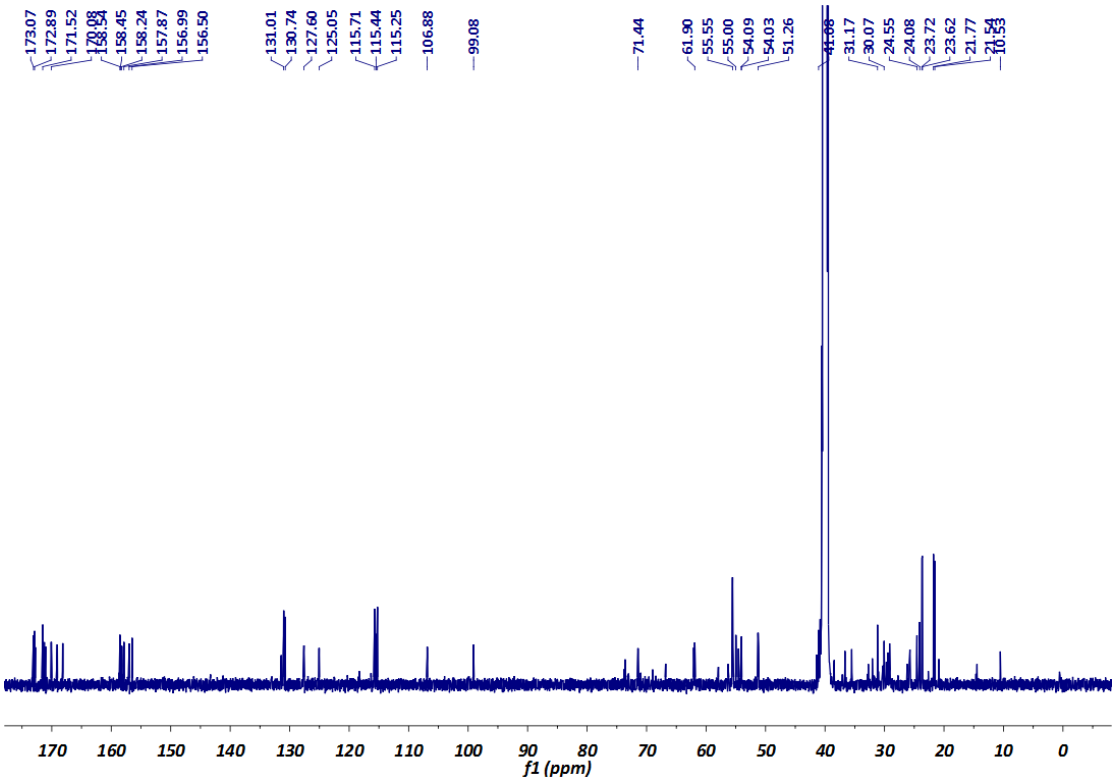

Figure S97. ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of compound **14**.

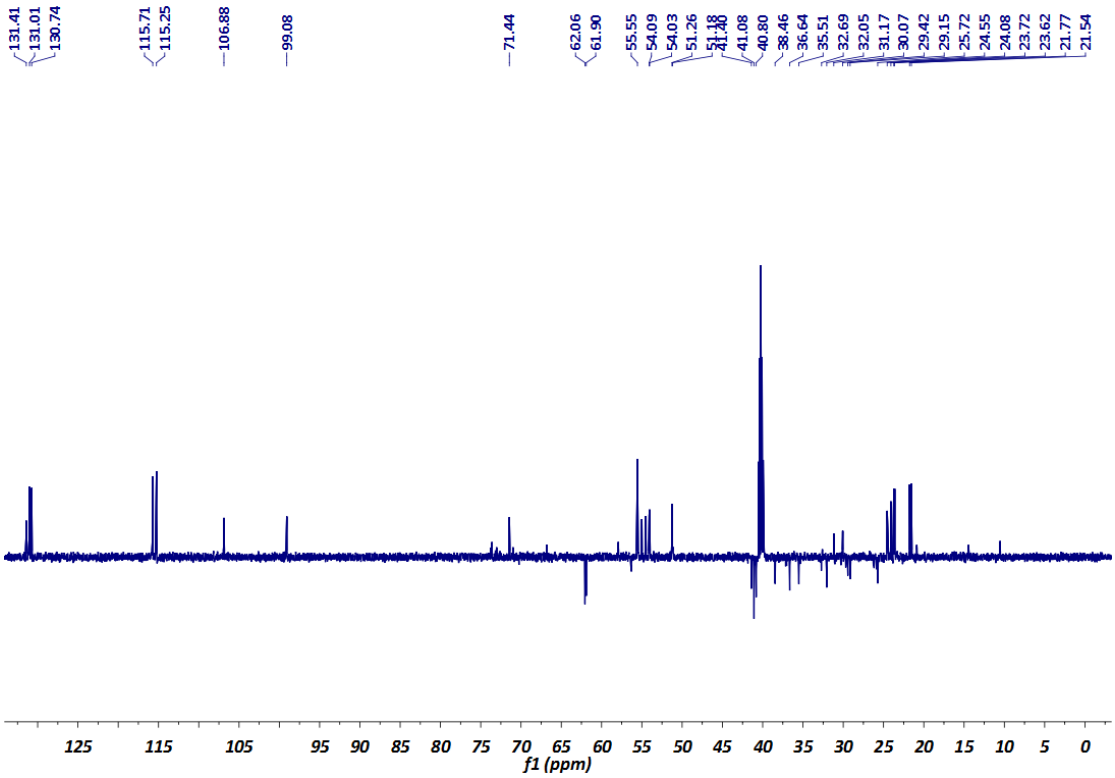

Figure S98. DEPT NMR (150 MHz, DMSO-d_6) spectrum of compound **14**.

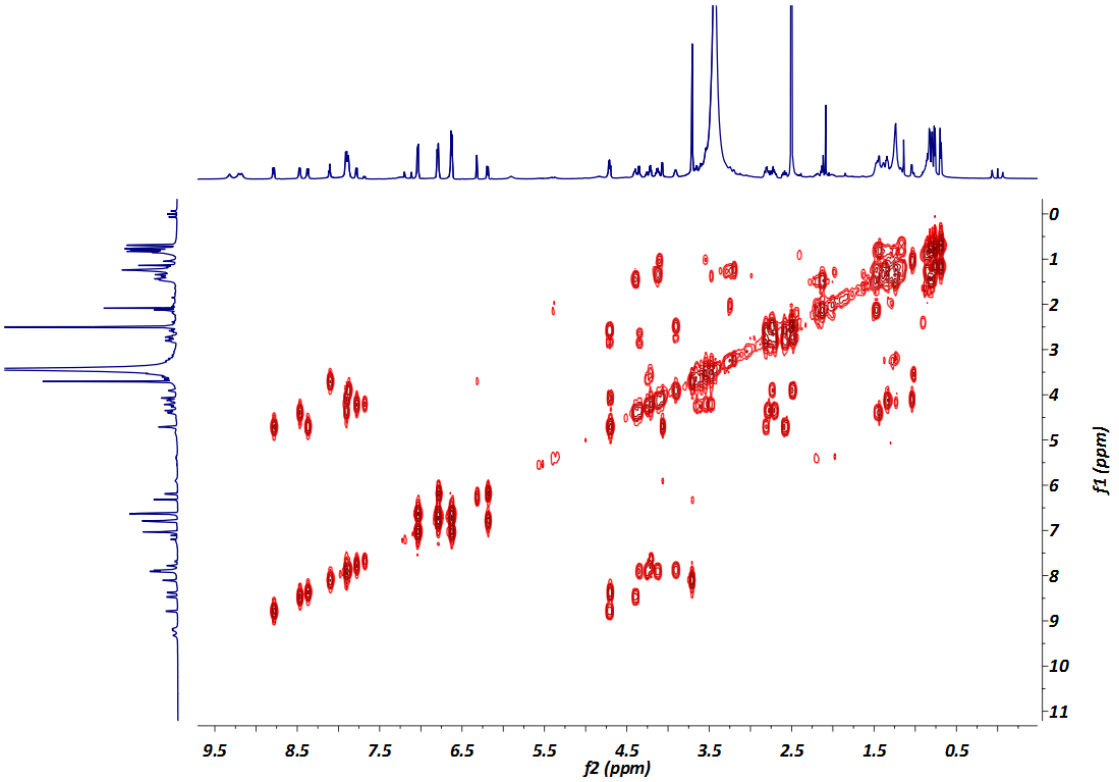

Figure S99. ^{1}H–^{1}H COSY NMR (600 MHz, DMSO-d_6) spectrum of compound **14**.

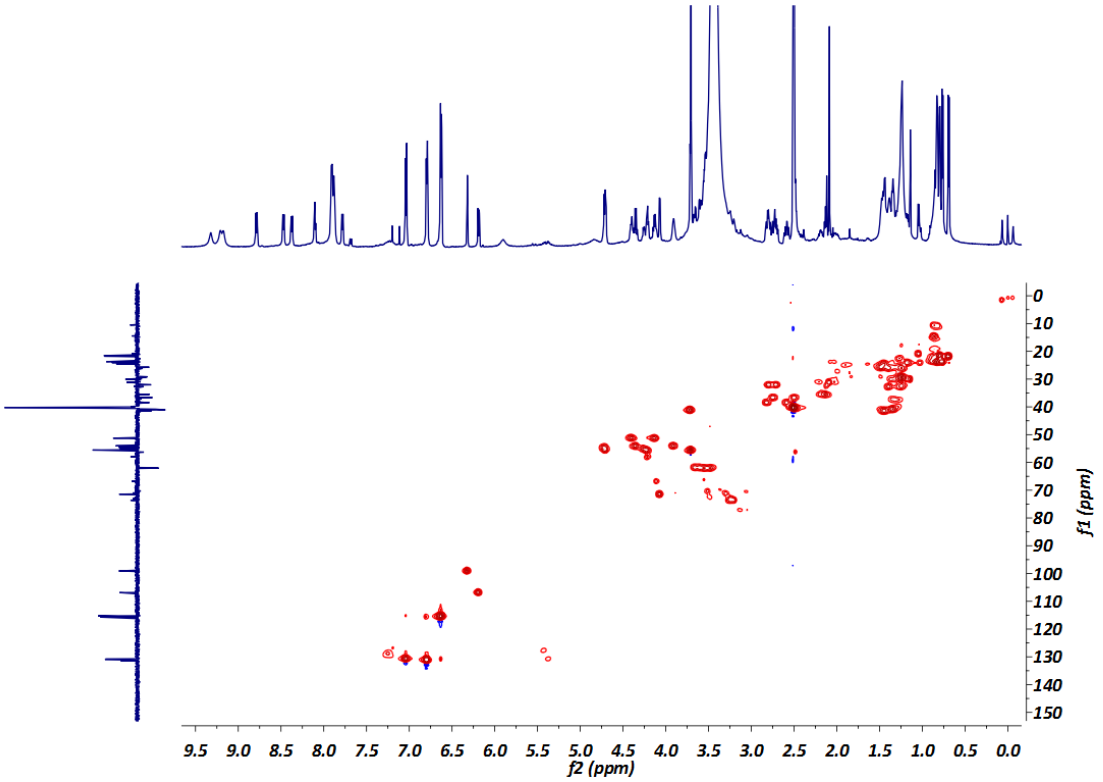

Figure S100. HSQC NMR (600 MHz, DMSO-d_6) spectrum of compound **14**.

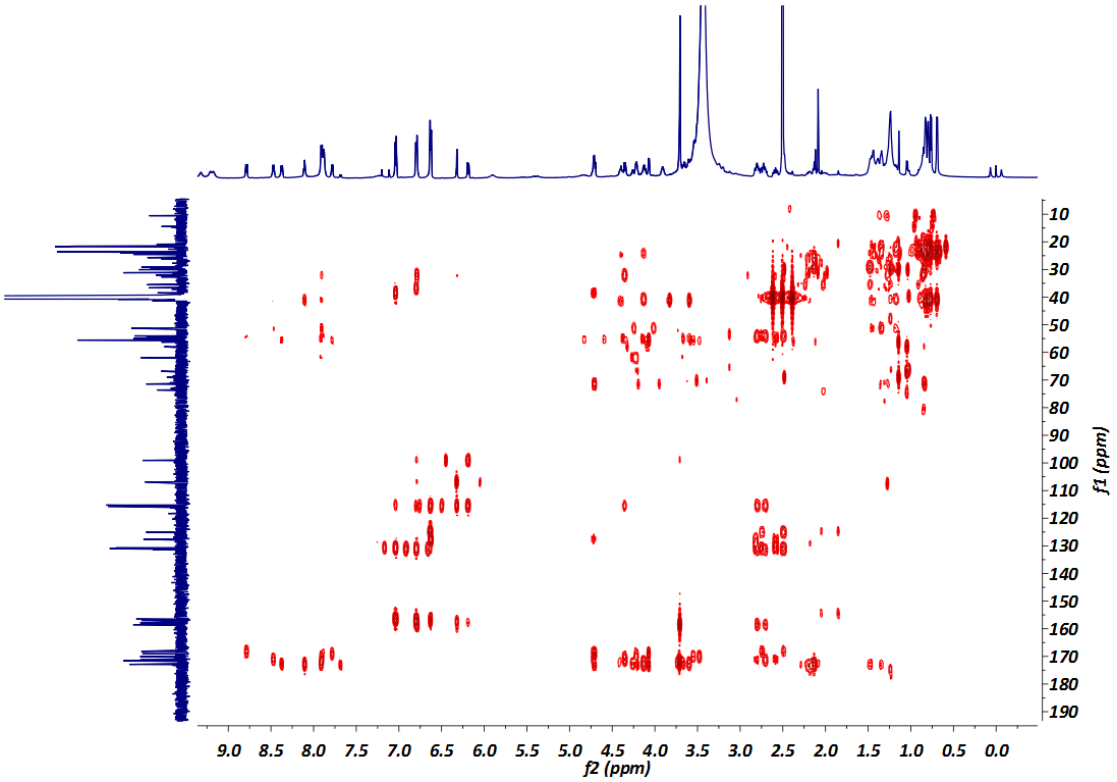

Figure S101. HMBC NMR (600 MHz, DMSO-d_6) spectrum of compound **14**.

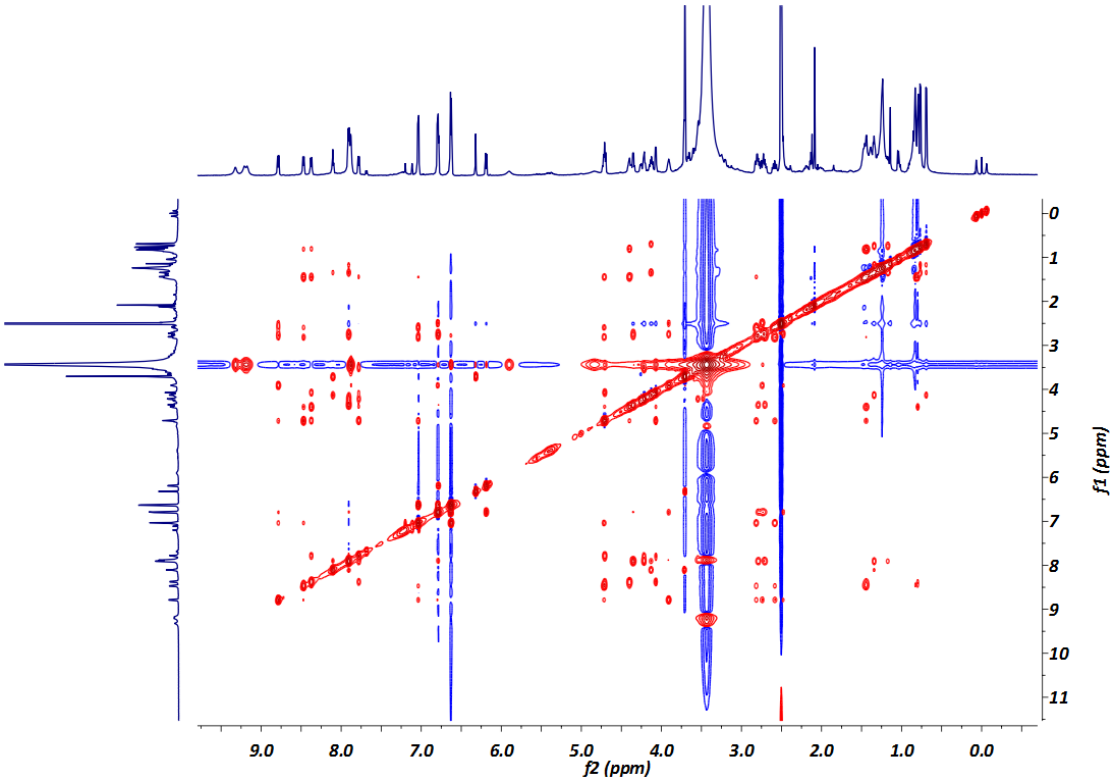

Figure S102. NOESY NMR (600 MHz, DMSO-d_6) spectrum of compound **14**.

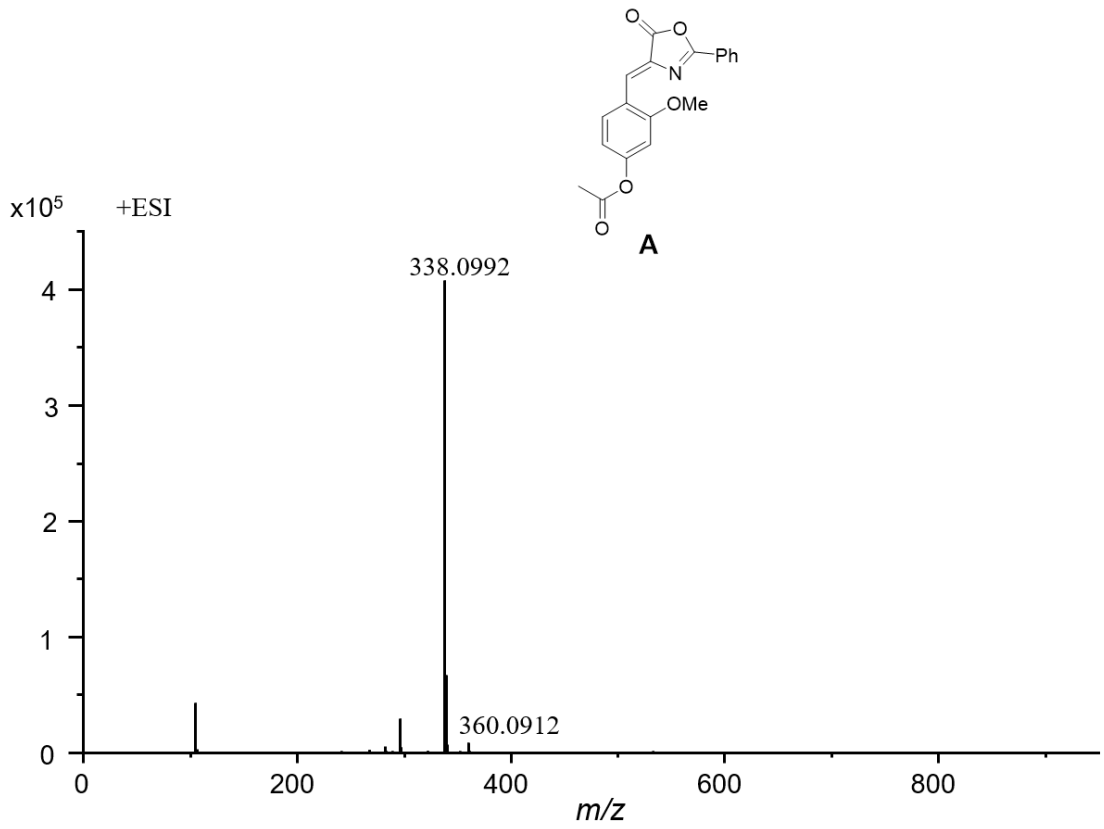

Figure S103. HRESIMS spectrum of **A**.

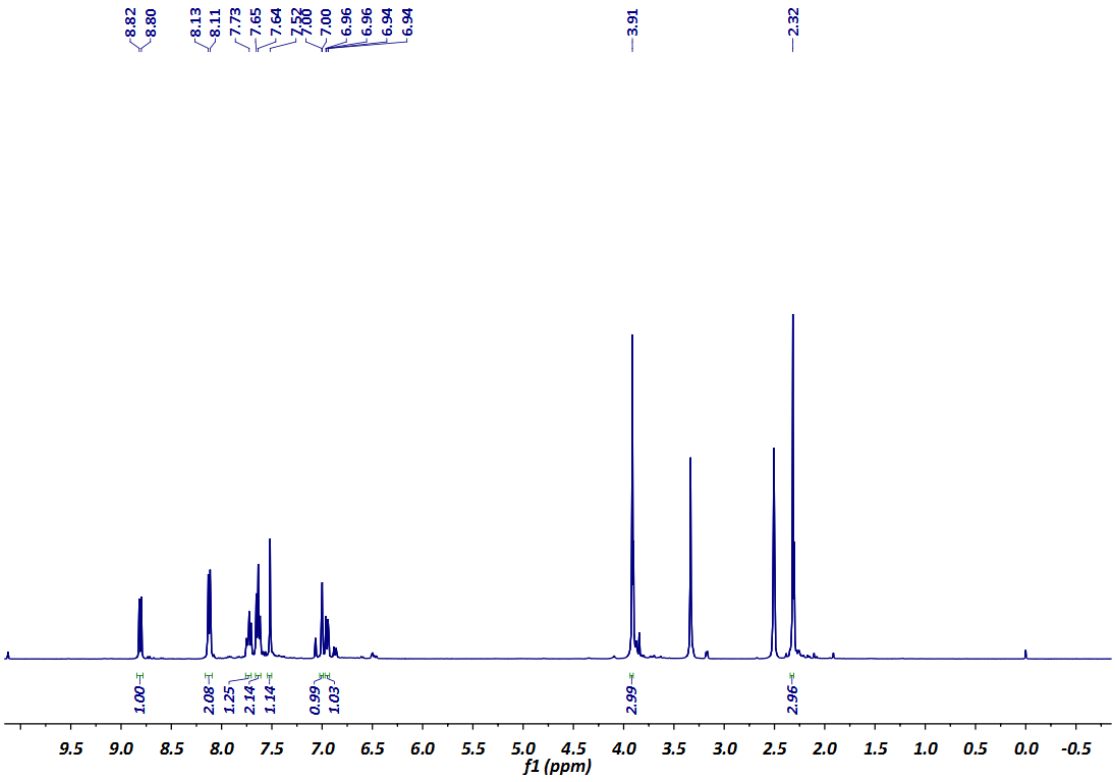

Figure S104. ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **A**.

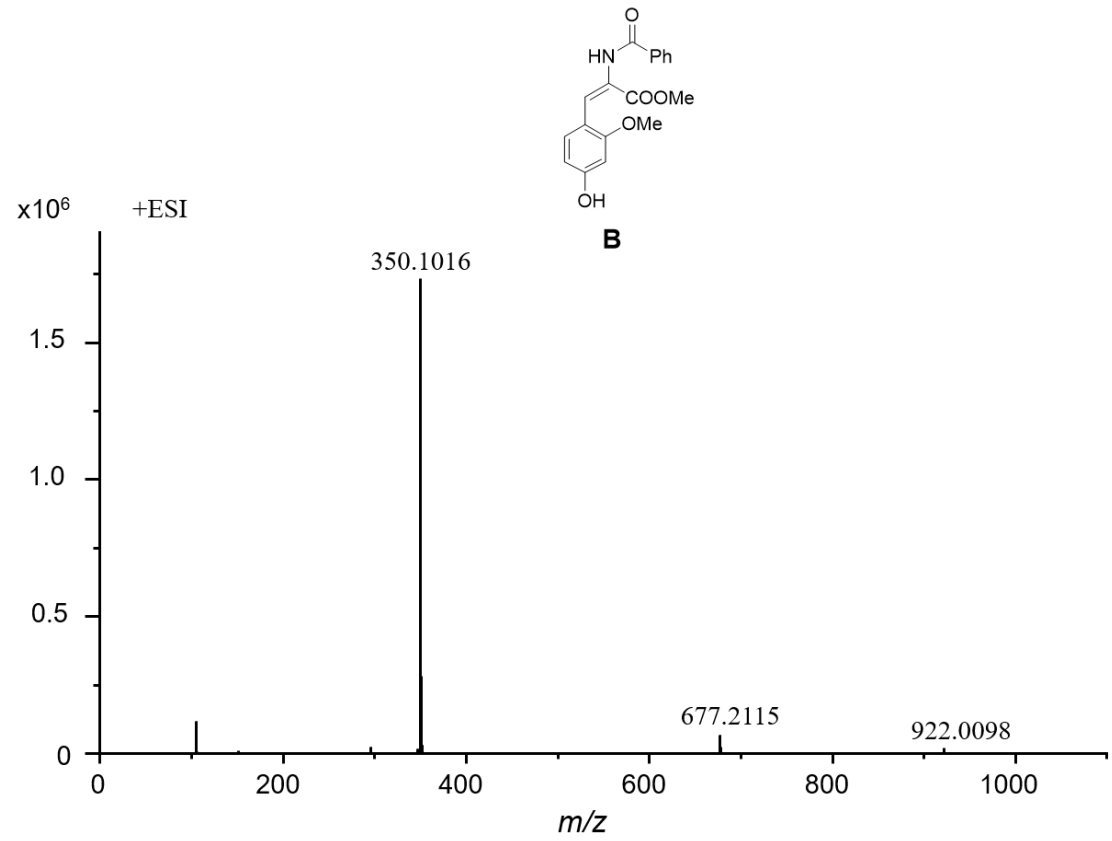

Figure S105. HRESIMS spectrum of **B**.

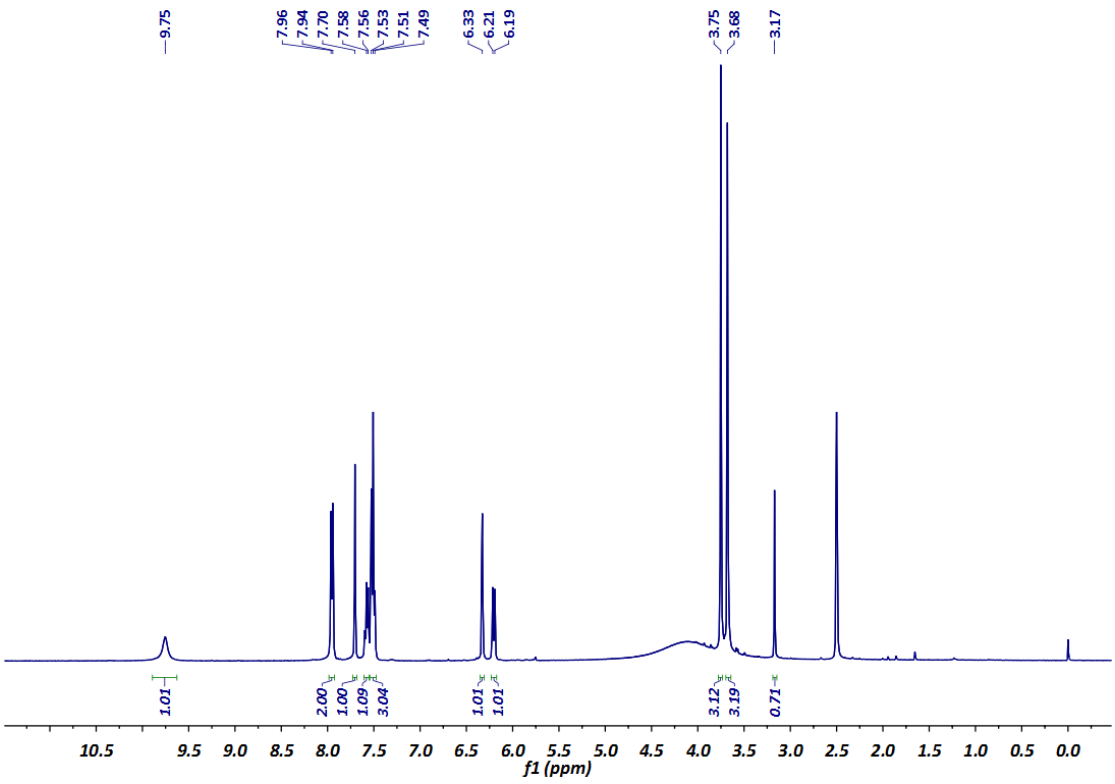

Figure S106. ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **B**.

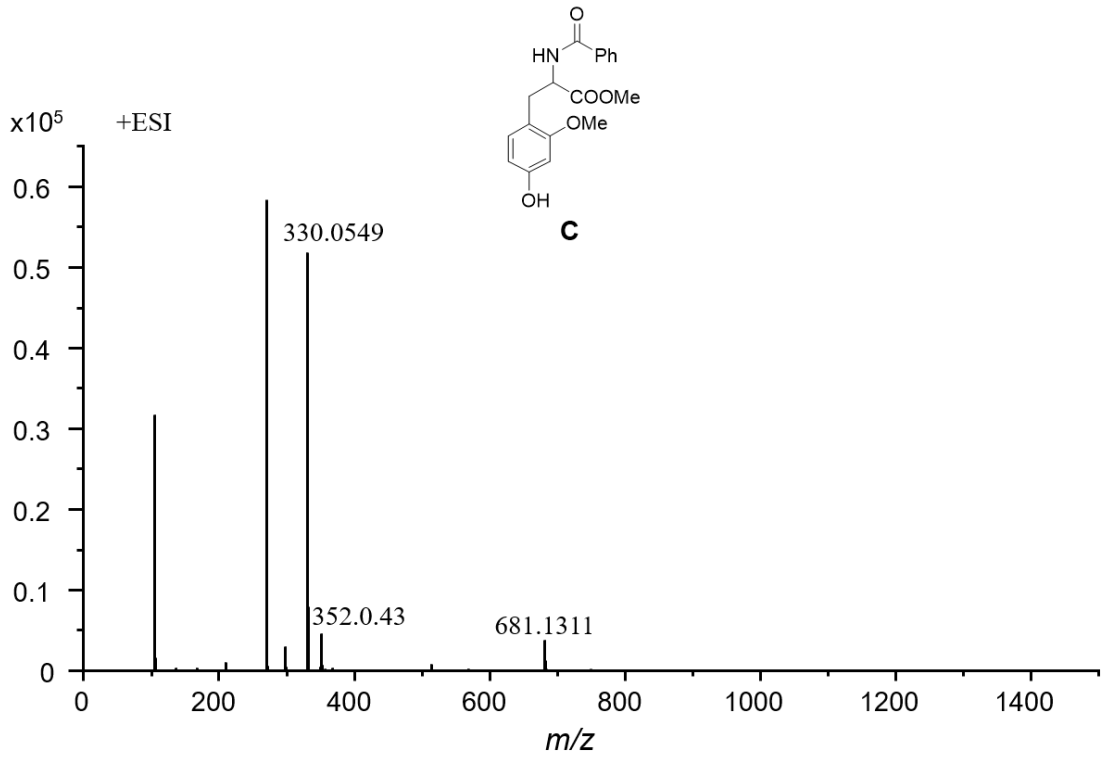

Figure S107. HRESIMS spectrum of **C**.

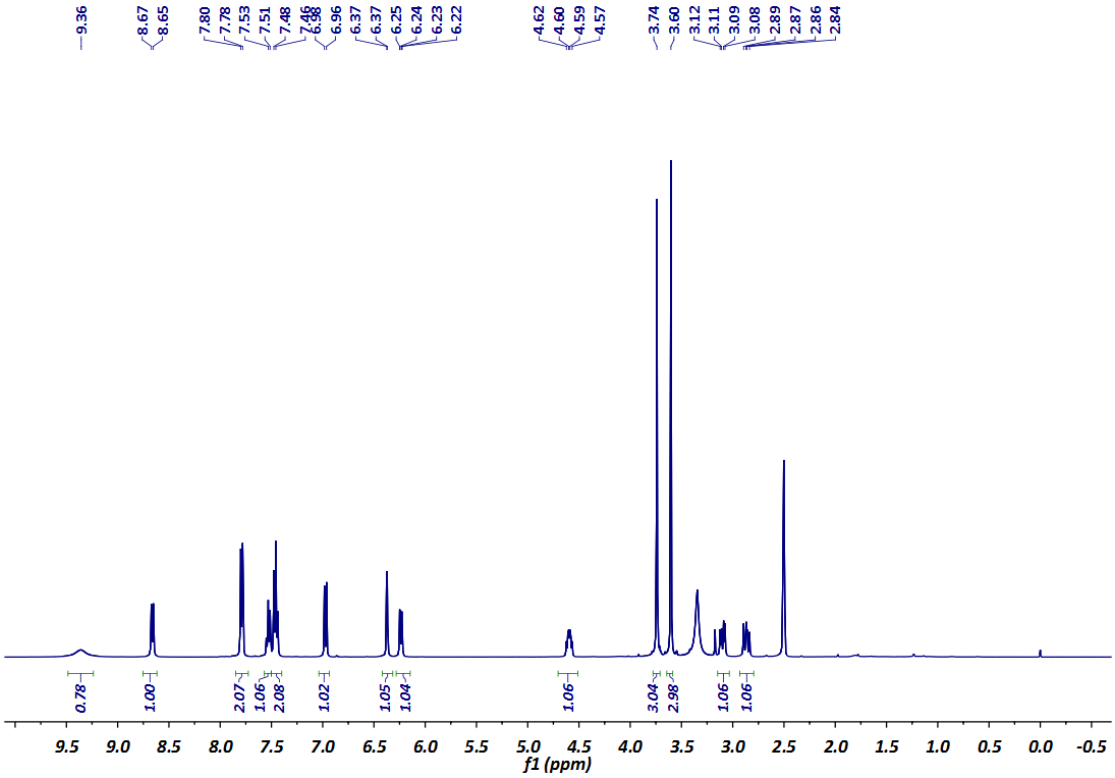

Figure S108. ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **C**.

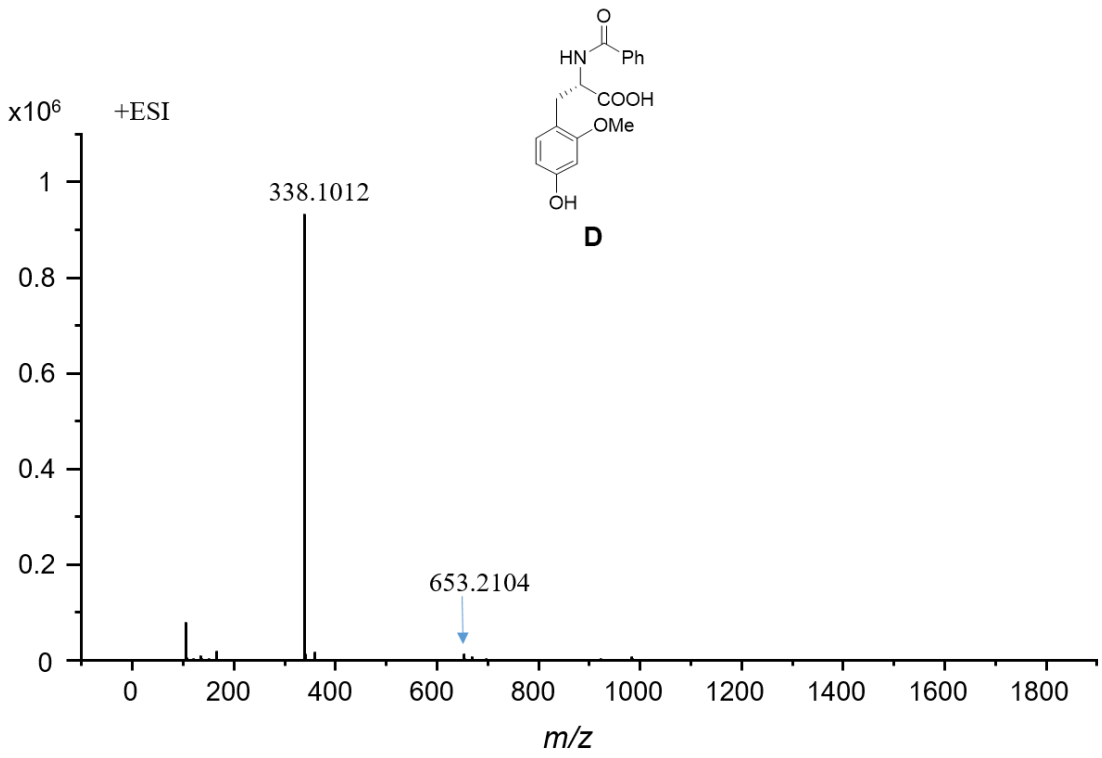

Figure S109. HRESIMS spectrum of **D**.

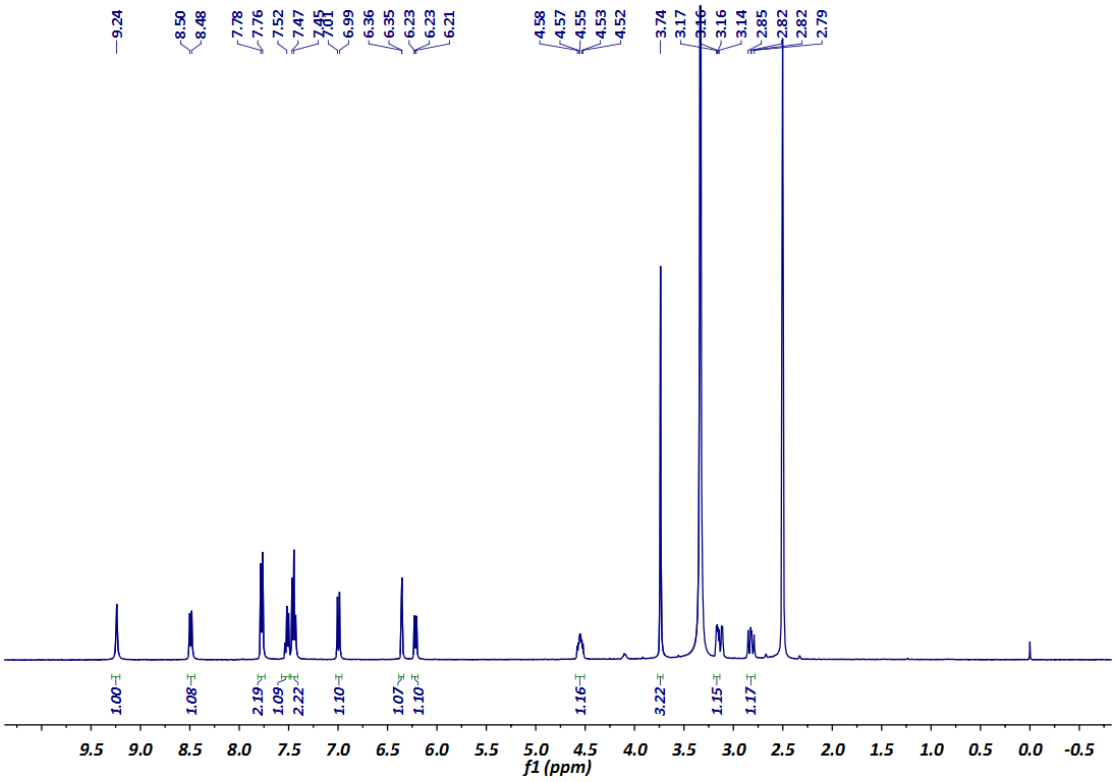

Figure S110. ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **D**.

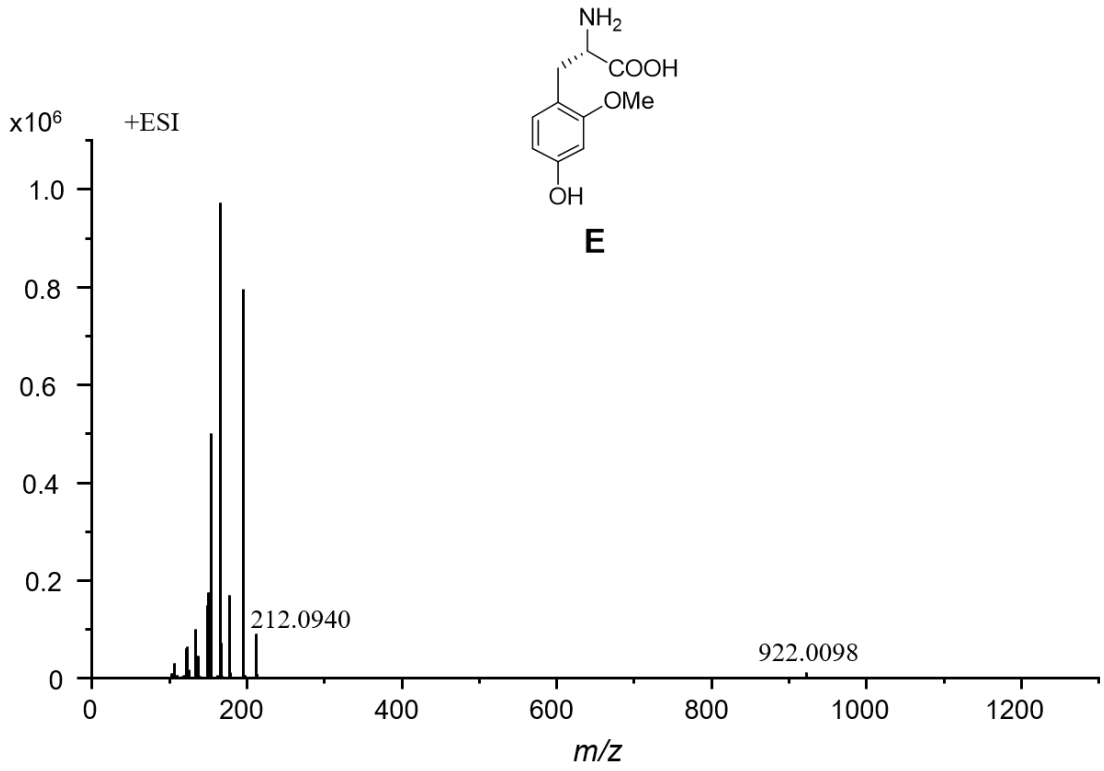

Figure S111. HRESIMS spectrum of **E**.

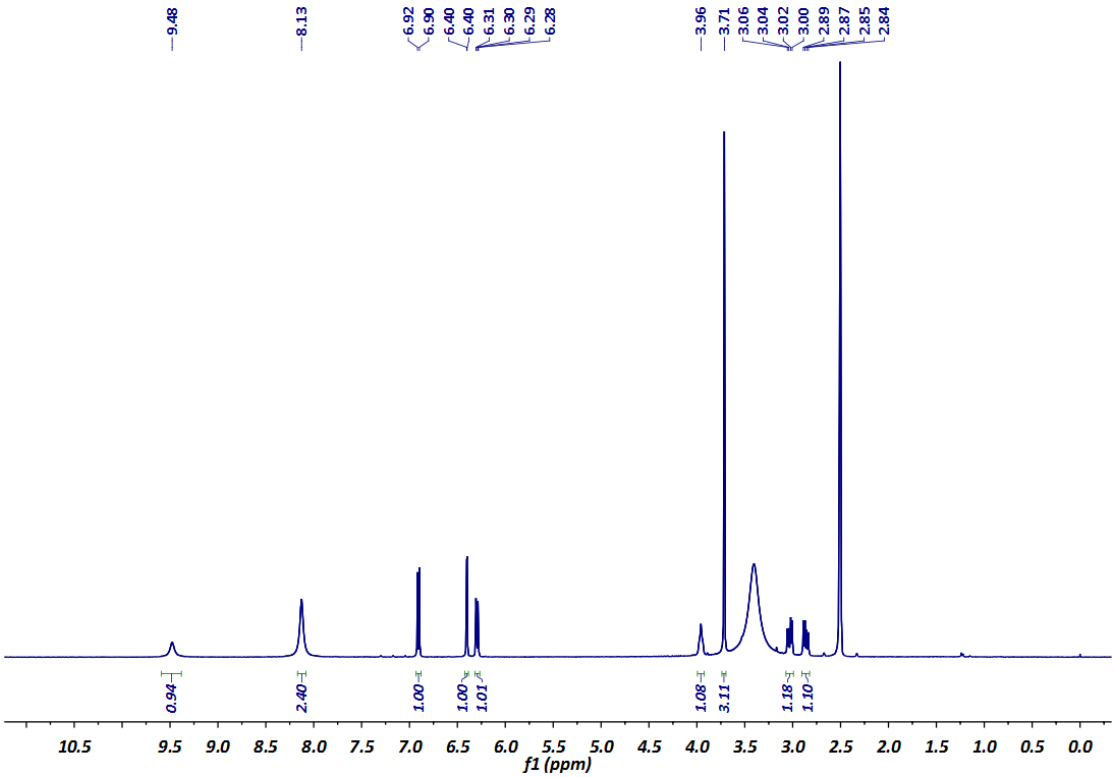

Figure S112. ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **E**.

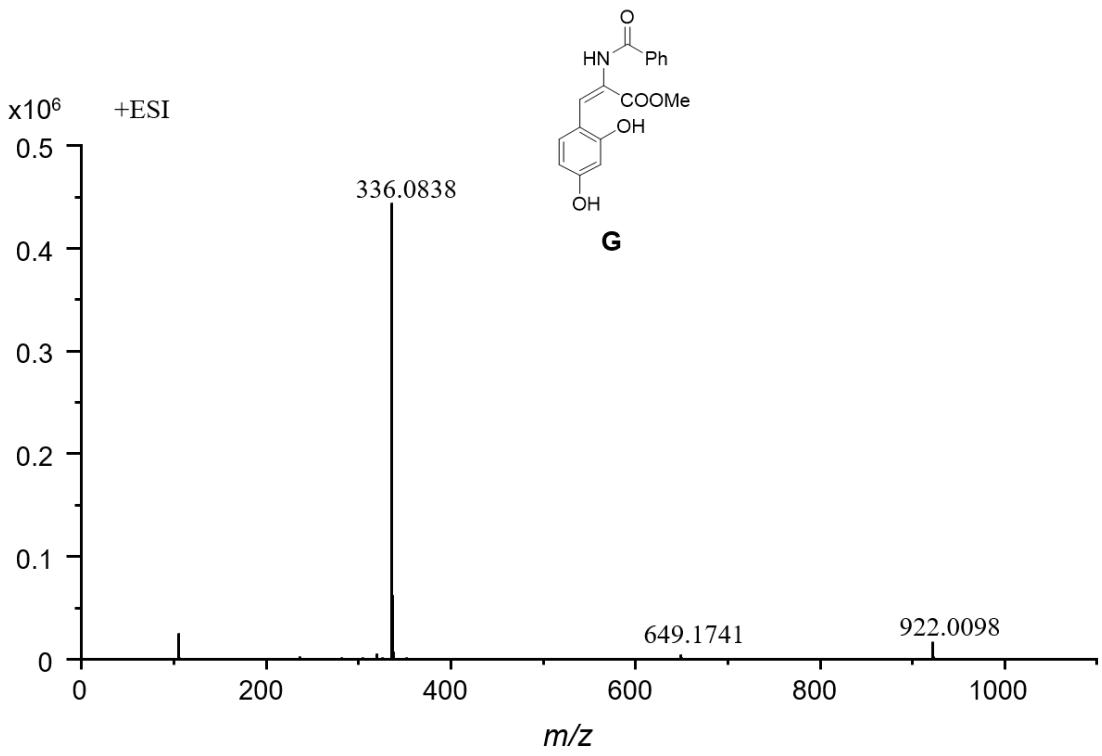

Figure S113. HRESIMS spectrum of **G**.

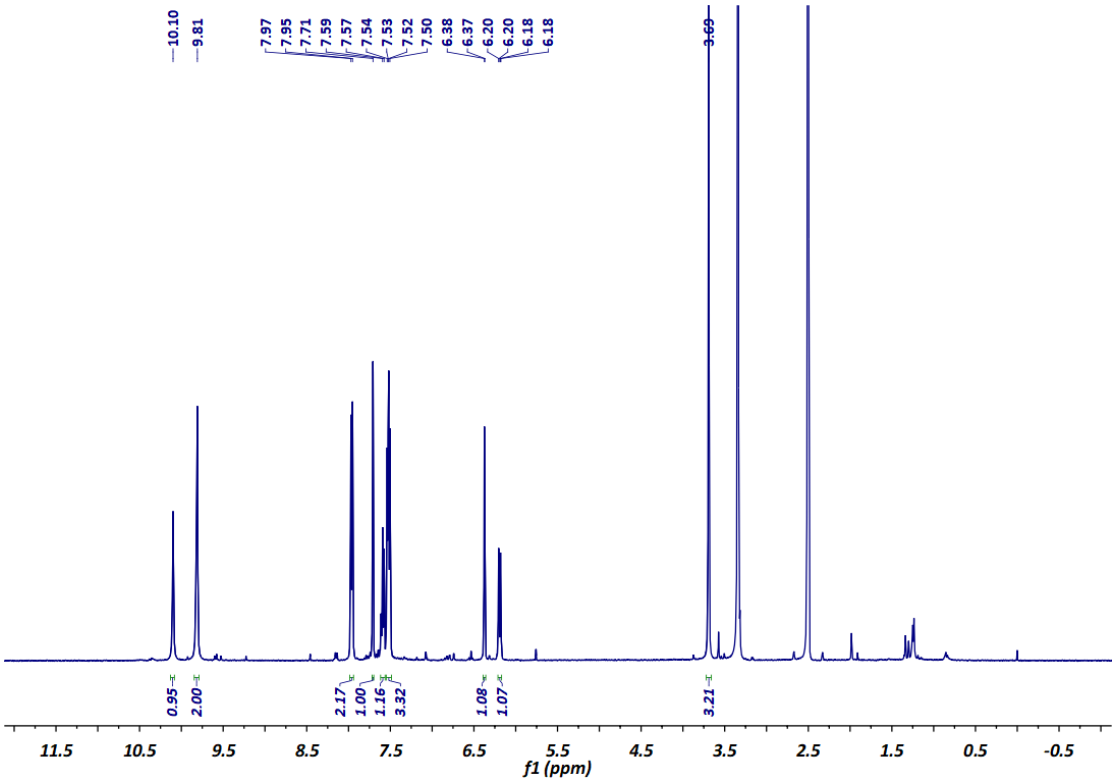

Figure S114. ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **G**.

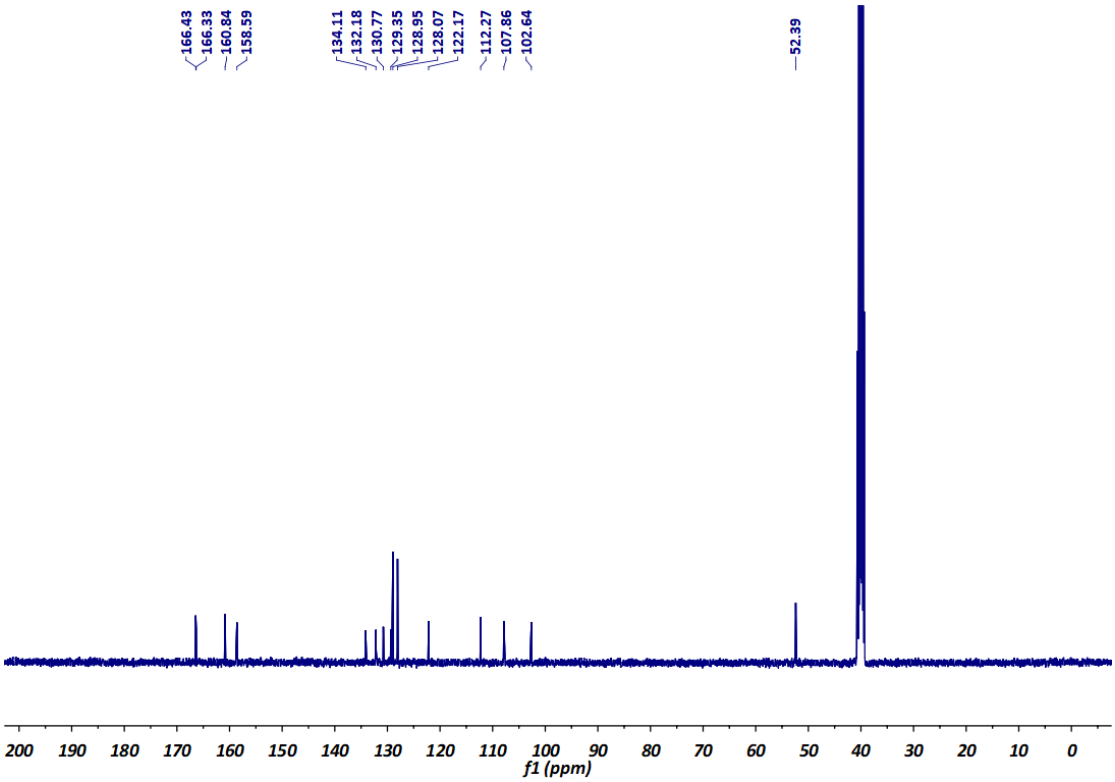

Figure S115. ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **G**.

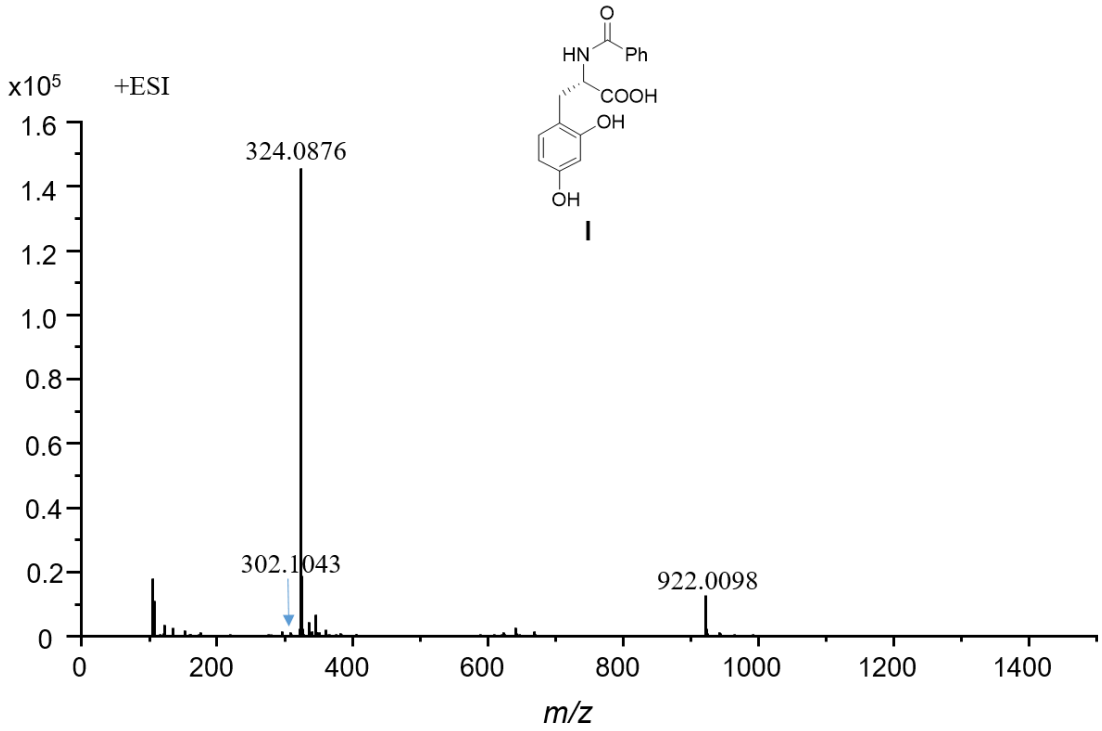

Figure S116. HRESIMS spectrum of **I**.

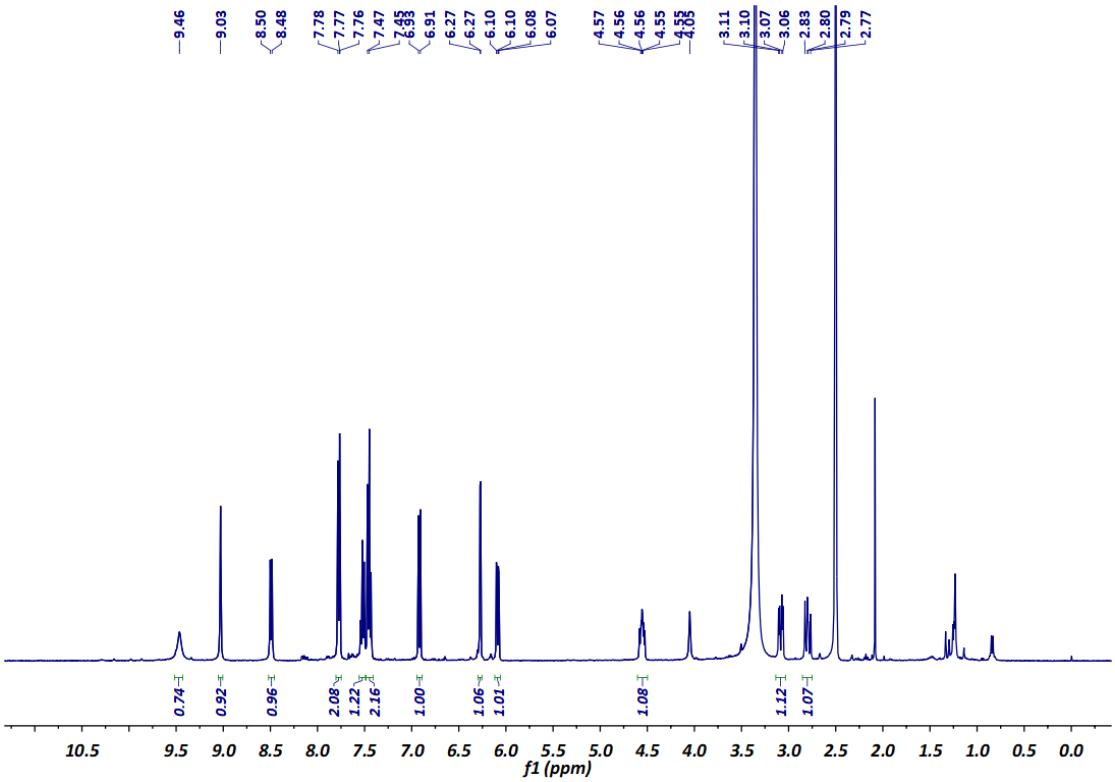

Figure S117. ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **I**.

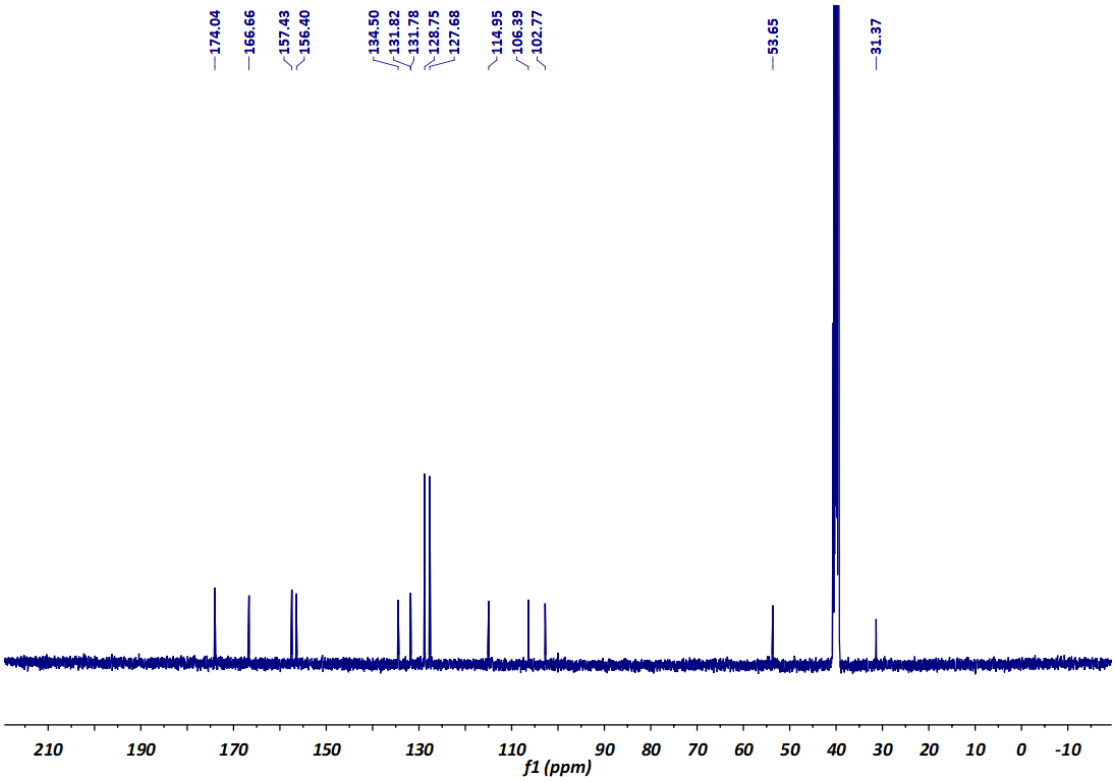

Figure S118. ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **I**.

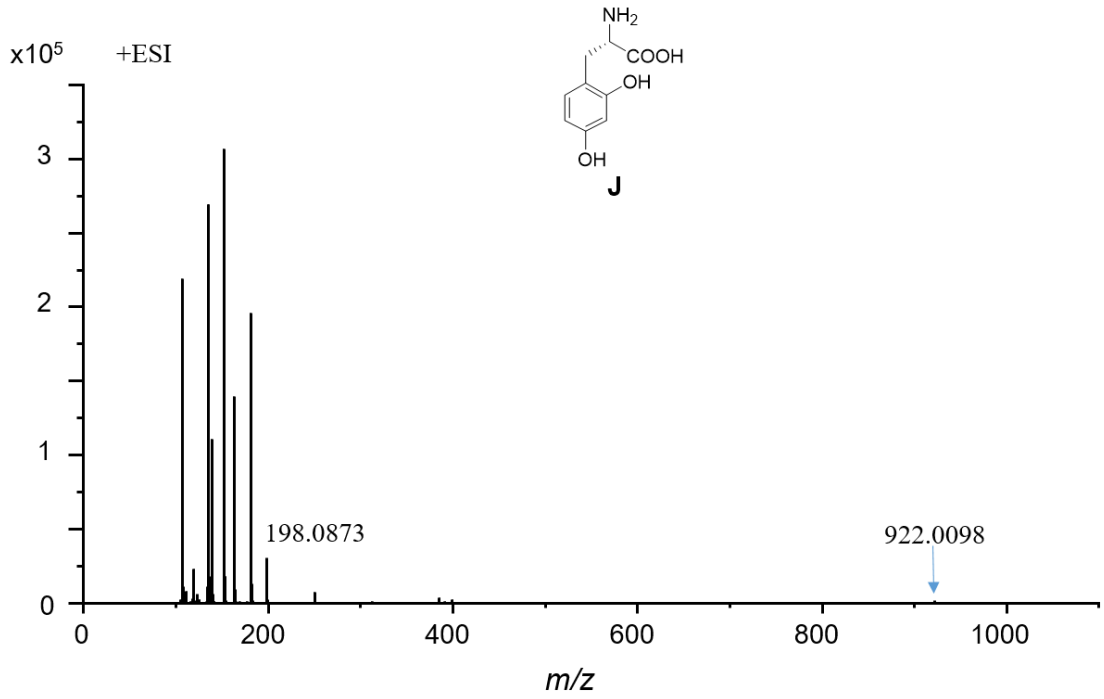

Figure S119. HRESIMS spectrum of **J**.

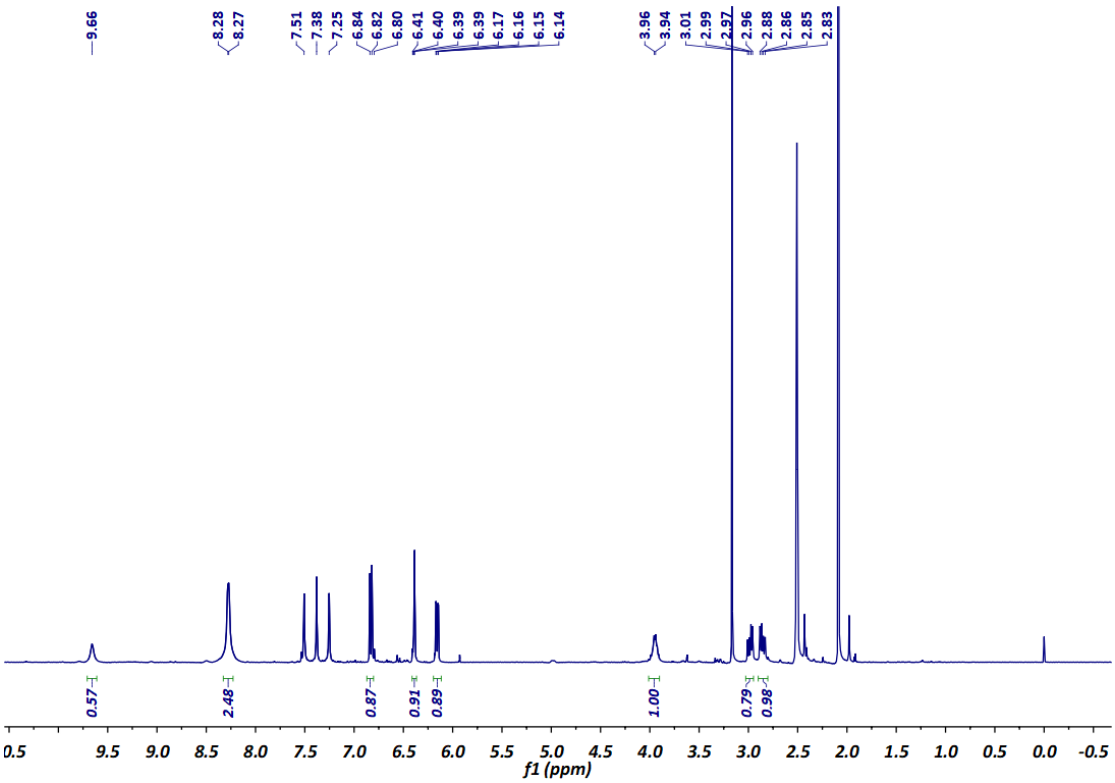

Figure S120. ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **J**.

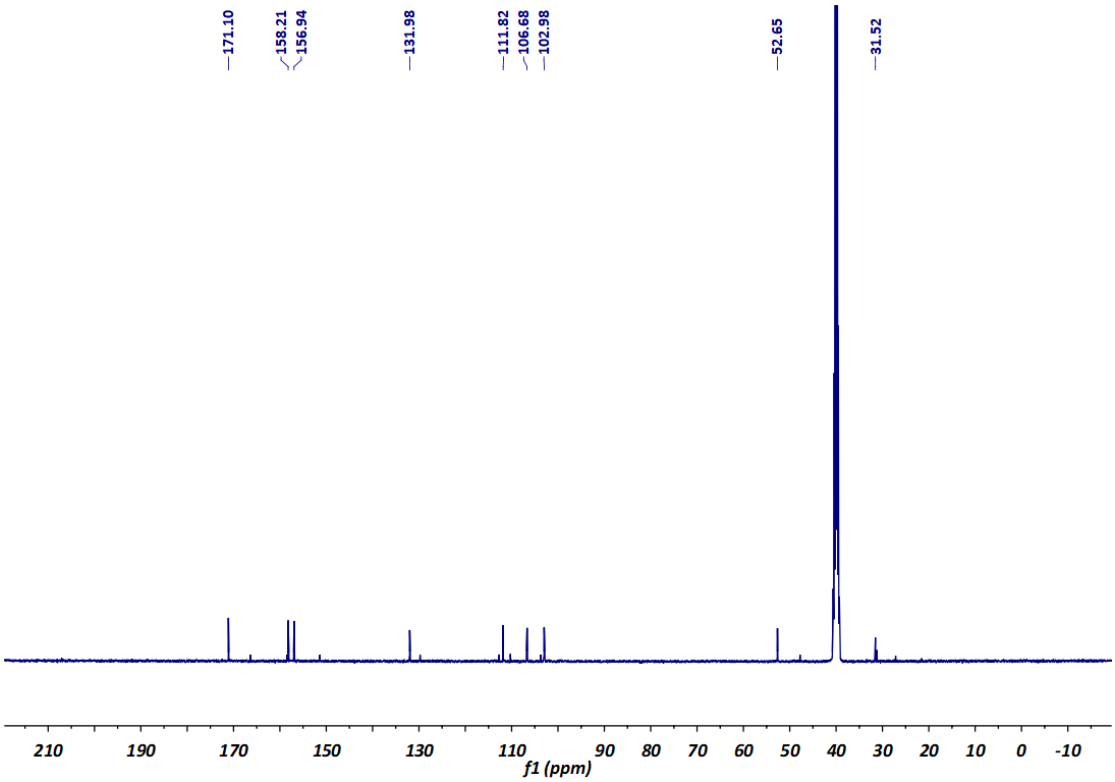

Figure S121. ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **J**.

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