Supporting Information

The Discovery of Cyclic Lipopeptide Olenamidonins in a Deepsea-Derived *Streptomyces* Strain by Knocking out a DtxR Family Regulator

Qiannan Sun[1], Dongqi Yu[1], Xueqing Zhang[1], Fei Xiao[1]*, Wenli Li[1,2,3]*

***** Author to whom correspondence should be addressed

Fei Xiao Email: xiaofei3450@ouc.edu.cn

Wenli Li Email: liwenli@ouc.edu.cn

[1]Key Laboratory of Marine Drugs, Ministry of Education of China, School of Medicine and Pharmacy, Ocean University of China, Qingdao, China

[2]Laboratory for Marine Drugs and Bioproducts, Qingdao National Laboratory for Marine Science and Technology, Qingdao, China

[3]College of Chemistry & Pharmacy, Northwest A&F University, Yangling, China

Table of contents

Table S1. A 1490 nt 16S rRNA sequence of *S. olivaceus* SCSIO 1071.

***S. olivaceus* SCSIO 1071**	**Sequence**
16S rRNA	AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTG CTTAACACATGCAAGTCGAACGATGAACCACTTCGGTGGG GATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTG CCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAAT ACCGGATATTGATCTTCACGGGCATCTGTGAGGTTCGAAA GCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTT GTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAG CCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGA CACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAAT ATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCG TGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGC AGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGC GCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG GGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCT CGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGG CTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAG TTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAA ATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCG GATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGT GGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCC GTAAACGGTGGGCACTAGGTGTGGGCAACATTCCACGTT GTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGG GAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGG GGGCCCGCACAAGCGGCGGAGCATGTGGCTTAATTCGAC GCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGG AAACGGCCAGAGATGGTCGCCCCCTTGTGGTCGGTGTAC AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATG TTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGT GTTGCCAGCAAGCCCCTTCGGGGGTGTTGGGGACTCAC GGGAGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGA CGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCAC ACGTGCTACAATGGCCGGTACAATGAGCTGCGATACCGCA AGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGAT TGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTA GTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCG GGCCTTGTACACACCGCCCGTCACGTCACGAAAGTCGGT AACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGG AGCTGTCGAAGGTGGGACTGGCGATTGGGACGAAGTCG TAACAAGGTAGCC

Table S2. *dtxR*$_{so}$ sequence of *S. olivaceus* SCSIO 1071.

***S. olivaceus* SCSIO 1071**	**Sequence**
dtxR$_{so}$	ATGTCCGGACTGATCGACACCACGGAGATGTACCTCCGC ACCATCCTGGAGCTGGAGGAGGAAGGTGTGGTCCCGATG CGCGCCCGGATCGCCGAGCGGCTCGACCAGAGCGGCCC GACGGTCAGTCAGACGGTGGCGCGCATGGAGCGCGACG GTCTGGTGTCCGTGGCGGCCGACCGGCACCTGGAGCTG ACCGAGGAGGGCAGGCGGCTGGCGACGCGCGTGATGC GCAAGCACCGGCTCGCGGAGTGCCTGCTCGTCGACGTG ATCGGCCTGGAGTGGGAGCAGGTGCACGCCGAGGCGTG CCGCTGGGAGCACGTGATGAGCGAGGCCGTCGAGCGCC GCGTGCTCGAGCTGCTGCGCCACCCGACCGAGTCGCCG TACGGGAACCCGATTCCGGGCCTGGAGGAGCTGGGGGA GACGGACATCGCCGACCCGTTCCTGGACGAGGGCATGG TCTCGCTCGCCGACCTCGACCCGGGCATGGAGGGCAAG ACGGTCGTCGTGCGGCGGATCGGCGAGCCGATCCAGAC GGACGCGCAGCTGATGTACACGCTGCGCCGGGCGGGTG TGCAGCCCGGTTCGGTGGTGAGCGTGACCGAGTCGGCG GGCGGCGTGCTGGTGGGCAGTGGCGGCGAGGCGGCCG AGCTGGAGTCGGACACCGCCTCCCACGTGTTCGTGGCC AAGCGCTGA

Table S3. Bacteria and plasmids used in this study.

Strains or plasmids	**Description**	**Reference or source**
Strains		
E. coli DH5α	Host strain for general cloning	Stratagene
E. coli ET12567/pUZ8002	Host strain for conjugation	[1]
E. coli BW25113/pIJ790	Host strain for PCR-targeting	[2]
Streptomyces olivaceus SCSIO 1071	Wild-type strain	This study
Plasmids		
pIJ773	Apr^R, source of *acc(3)IV-oriT* cassette	[1]
pIJ790	Cm^R, λ RED recombination plasmid	[1]
pWLI1001	Genomic library cosmid harboring the *dtxR*$_{so}$ gene	This study
pWLI1002	pWLI1001 derivative where *dtxR*$_{so}$ was replaced with a*cc(3)IV-oriT* cassette	This study

Table S4. The primer pairs used in this study[a].

Primers	**Sequence (5'→3')**
Knockout	
$\Delta dtxR_{so}$-MF	ATGTCCGGACTGATCGACACCACGGAGATGTACCTCCGCATTCCG GGGATCCGTCGACC
$\Delta dtxR_{so}$-MR	TCAGCGCTTGGCCACGAACACGTGGGAGGCGGTGTCCGATGTAGG CTGGAGCTGCTTC
Confirmation of the mutants	
$\Delta dtxR_{so}$-CF	GGGCACGGTTGTCGGTGGTA
$\Delta dtxR_{so}$-CR	GCCAGGAGCCCCTTCGTCAT

[a]Underlined letters represent nucleotides homologous to the DNA regions internal to target genes.

Table S5. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data of **4** and **5** in DMSO-*d6*.

	4		**5**	
Position	δ_C, type	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)
1-N				
2	44.6, CH_2	4.17, d (14.3) 3.31, m	44.6, CH_2	4.17, d (14.3) 3.31, m
3	168.8, C		168.8, C	
4-NH		7.21, m		7.21, m
5	53.9, CH	4.49, td (8.8, 6.4)	53.9, CH	4.49, td (8.8, 6.4)
6	171.1, C		171.1, C	
7-NH		8.71, d (7.7)		8.71, d (7.7)
8	52.2, CH	4.36, q (7.3)	52.2, CH	4.36, q (7.3)
9	171.8, C		171.8, C	
10-NH		6.98, t (5.9)		6.98, t (5.9)
11	38.9, CH_2	3.50, dd (13.7, 6.2) 3.24, dd (10.4, 5.0)	38.9, CH_2	3.50, dd (13.7, 6.2) 3.24, dd (10.4, 5.0)
12	58.9, CH	3.77, d (8.4)	58.9, CH	3.77, d (8.4)
13-NH		2.99, d (9.4)		2.99, d (9.4)
14	76.3, C		76.3, C	
15	172.8, C		172.8, C	
16	36.3, CH_2	3.07, dd (13.9, 8.7) 2.78, dd (13.9, 6.2)	36.3, CH_2	3.07, dd (13.9, 8.7) 2.78, dd (13.9, 6.2)
17	137.3, C		137.3, C	
18/22	128.8, CH	7.18, m	128.8, CH	7.18, m
19/21	128.3, CH	7.24, m	128.3, CH	7.24, m
20	126.3, CH	7.17, m	126.3, CH	7.17, m
23	27.4, CH_3	1.26, m	27.4, CH_3	1.26, m
24	25.9, CH_3	1.18, s	25.9, CH_3	1.18, s
25	37.7, CH_2	3.31, m	37.7, CH_2	3.31, m
26-NH		7.77, t (5.7)		7.77, t (5.7)
27	166.6, C		166.6, C	
28	103.5, CH	5.55, d (14.0)	103.5, CH	5.55, d (14.0)
29	133.8, CH	7.59, dd (13.9, 11.1)	133.8, CH	7.59, dd (13.9, 11.1)
30-NH		10.35, d (11.0)		10.35, d (11.0)
1′	171.5, C		171.5, C	
2′	35.4, CH_2	2.24, t (7.3)	35.3, CH_2	2.24, t (7.3)
3′	24.7, CH_2	1.54, m	24.7, CH_2	1.54, m
4′	28.4, CH_2	1.25, m	28.6, CH_2	1.25, m
5′	28.5, CH_2	1.24, m	29.0, CH_2	1.24, m
6′	31.1, CH_2	1.24, m	26.7, CH_2	1.26, m
7′	22.0, CH_2	1.26,	38.4, CH_2	1.13, m
8′	13.9, CH_3	0.89, t (7.0)	27.4, CH	1.49, m
9′			22.5, CH_3	0.84, d
10′			22.5, CH_3	0.84, d

[a]Signals were overlapped with other signals.

Table S6. The proposed functions of proteins encoded by the *ole* biosynthetic gene cluster in *S. olivaceus* SCSIO 1071.

Protein	Size (aa)	Proposed function	Homolog	Identity/ Similarity (%)
-1	518	Carboxylesterase	Carboxylesterase (MDA4890073.1), *Streptomyces* sp. MS2A	84/89
OleA	438	Histidine kinase	SceX (ANH11419.1), *Streptomyces* sp. SD85	45/58
OleB	727	Transporter	Transporter (WP_103817670.1), *Streptomyces* sp. Ru62	50/68
OleC	68	MbtH family protein	Tsk13 (UMM61374.1), *Streptomyces tasikensis* P46	59/74
OleD	317	ABC transporter	Atr29 (QBG38790.1), *Streptomyces atratus* SCSIO ZH16	58/73
OleE	245	4'-phosphopantetheinyl transferase	AsuC1(AIL50185.1), *Streptomyces aureus*	36/48
OleF	275	SARP family transcriptional regulator	SARP family transcriptional regulator (BAX64243.1), *Streptomyces* sp. MSC090213JE08	45/60
OleG	344	ornithine cyclodeaminase	ornithine cyclodeaminase (WP_130881053.1), *Streptomyces syringium*	58/73
OleH	309	3-oxoacyl-ACP synthase	3-oxoacyl-ACP synthase (KUN30549.1), *Streptomyces corchorusii*	53/70
OleI	480	ATP-grasp protein	ATP-grasp protein (QCW80778.1), *Streptomyces* sp. S6	61/70
OleJ	427	Decarboxylase	EtmH (QSI97707.1), *Streptomyces achromogenes* subsp. *streptozoticus*	57/69
OleK	92	Acyl carrier protein	EtmI (QSI97708.1), *Streptomyces achromogenes* subsp. *streptozoticus*	43/67
OleL	296	Hypothetical protein	Hypothetical protein (OEV19204.1), *Streptomyces nanshensis*	36/46
OleM	234	Acyl-CoA dehydrogenase	EtmK (QSI97710.1), *Streptomyces achromogenes* subsp. *streptozoticus*	34/46
OleN	387	Acyl-CoA dehydrogenase	acyl-CoA dehydrogenase (WP_311120300.1), *Streptomyces durocortorensis*	57/71
OleO	214	Transcriptional regulator	Transcriptional regulator (WP_150259904.1), *Streptomyces venezuelae*	75/87
OleP	278	2,3-diaminopropionate biosynthesis protein	2,3-diaminopropionate biosynthesis protein (WP_274911273.1), *Streptomyces* sp. WZ-12	68/77
OleQ	2183	Non-ribosomal peptide synthetase	Atr21 (QBG38782.1), *Streptomyces atratus*	38/50
OleR	2416	Non-ribosomal peptide synthetase	Atr22 (QBG38783.1), *Streptomyces atratus*	48/59
OleS	273	ABC transporter	Atr30 (QBG38791.1), *Streptomyces atratus*	42/61
OleT	187	Hypothetical protein	Hypothetical protein (GGW99313.1), *Streptomyces malachitofuscus*	83/87
OleU	195	TetR family transcriptional regulator	TetR family transcriptional regulator (NEC45849.1), *Streptomyces* sp. SID8016	82/88
OleV	168	DinB family protein	DinB family protein (WP_186776788.1), *Streptomyces salinarius*	80/89
OleW	208	SAM-dependent methyltransferase	SAM-dependent methyltransferase (MDG9694720.1), *Streptomyces* sp. DH17	87/93
OleX	194	XRE family transcriptional regulator	XRE family transcriptional regulator (AUO16412.1), *Streptomyces* sp. IMB7-145	62/72
OleY	418	DUF4190 domain-containing protein	DUF4190 domain-containing protein (MZE69516.1), *Streptomyces* sp. SID5789	80/87
OleZ	250	GntR family transcriptional regulator	GntR family transcriptional regulator (MCF2125887.1), *Streptomyces* sp. STD 3.1	96/98
+1	61	SPOR domain-containing protein	SPOR domain-containing protein (WP_055418037.1), *Streptomyces pactum*	88/91
+2	144	Hydrolase	Hydrolase (ADM46379.1), *Streptomyces* sp. CS	66/73

Note: The *ole* BGC is almost identical to the *aut* BGC (GenBank ID: WP_194276129.1-WP_194276149).

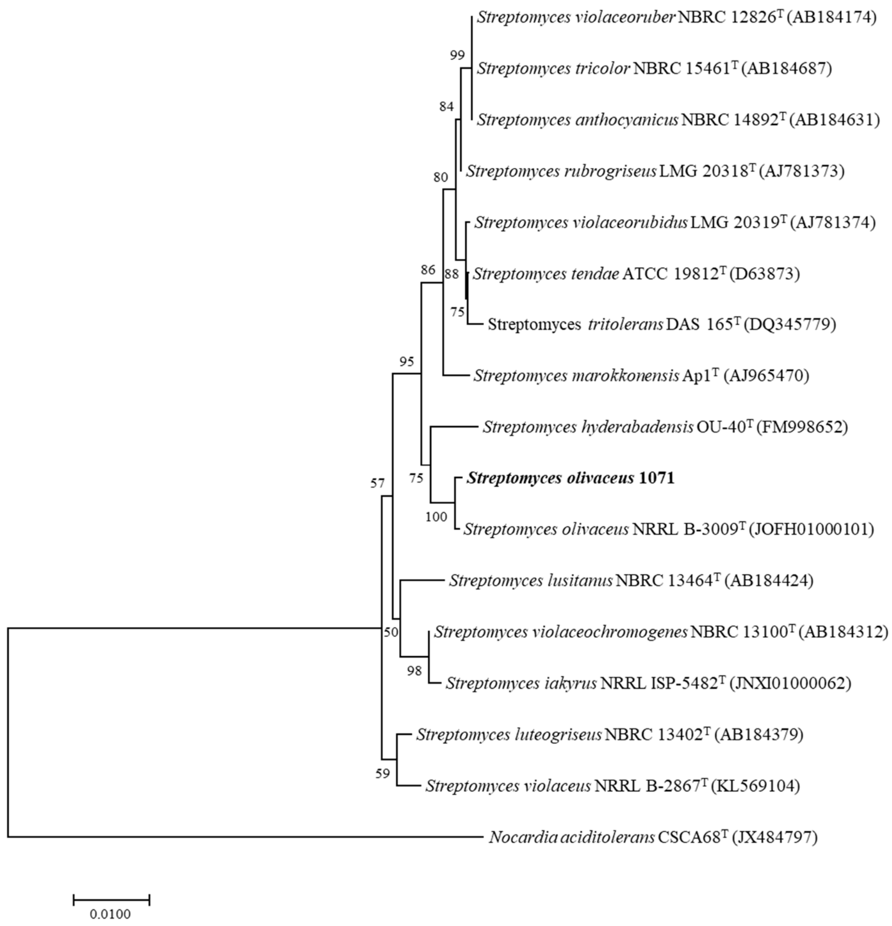

Figure S1. A neighbor-joining phylogenetic tree of the *S. olivaceus* SCSIO 1071 based on 16S rDNA sequences.

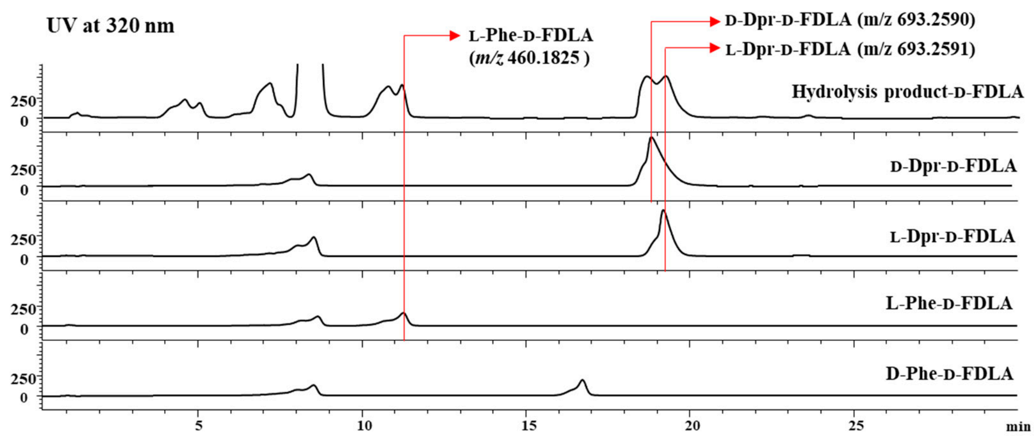

Figure S2. HPLC chromatogram data of D-FDAA derivatives of amino acid units in olenamidonin A (**1**).

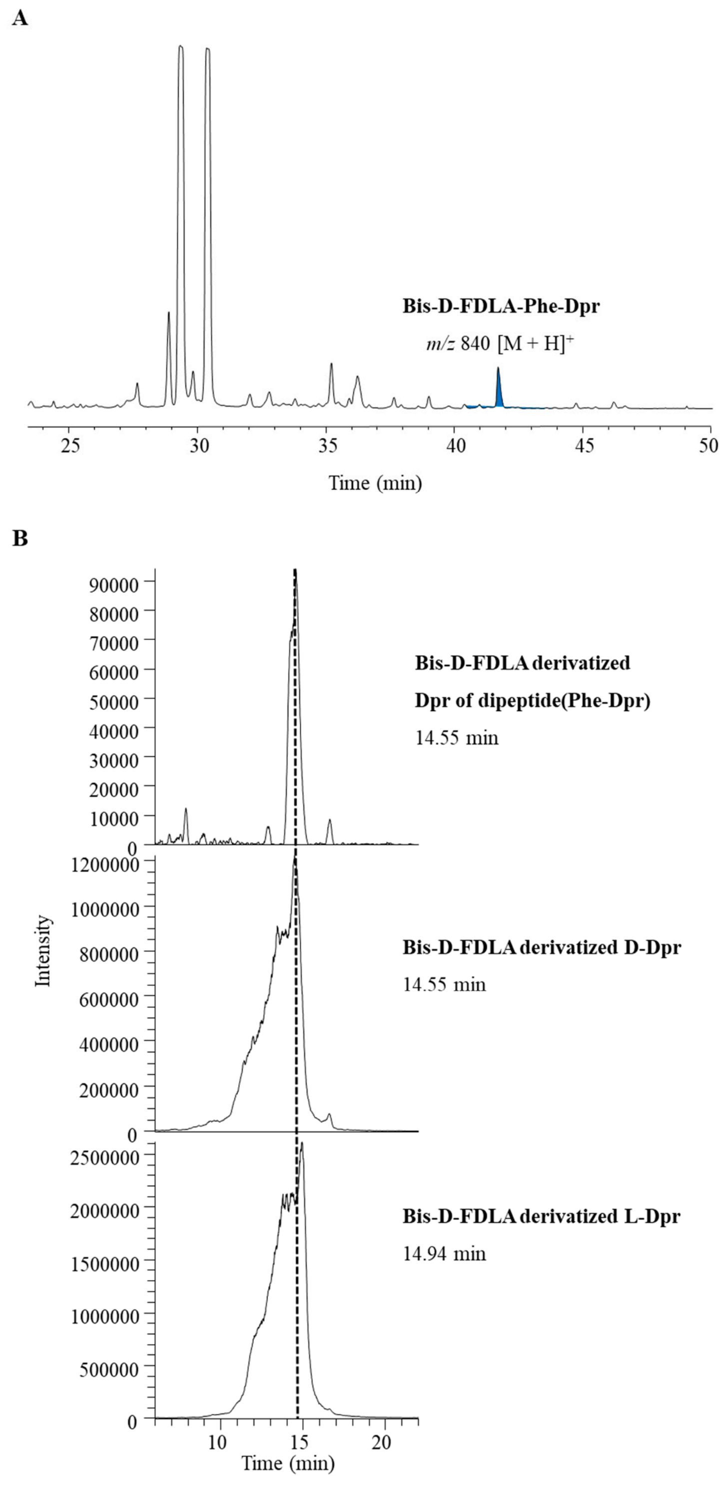

Figure S3. An advanced Marfey's analysis of the D-FDLA-derivatized Phe-Dpr dipeptide. (A) An HPLC chromatogram of the partial hydrolysate of **1** derivatized with D-FDLA. The peak for bis-D-FDLA-Phe-Dpr is colored in blue. (B) Extracted ion chromatograms at *m/z* 693 $[M+H]^+$ of the D-FDLA-derivatized Dpr of the dipeptide and D-FDLA-derivatized authentic D- and L-Dpr.

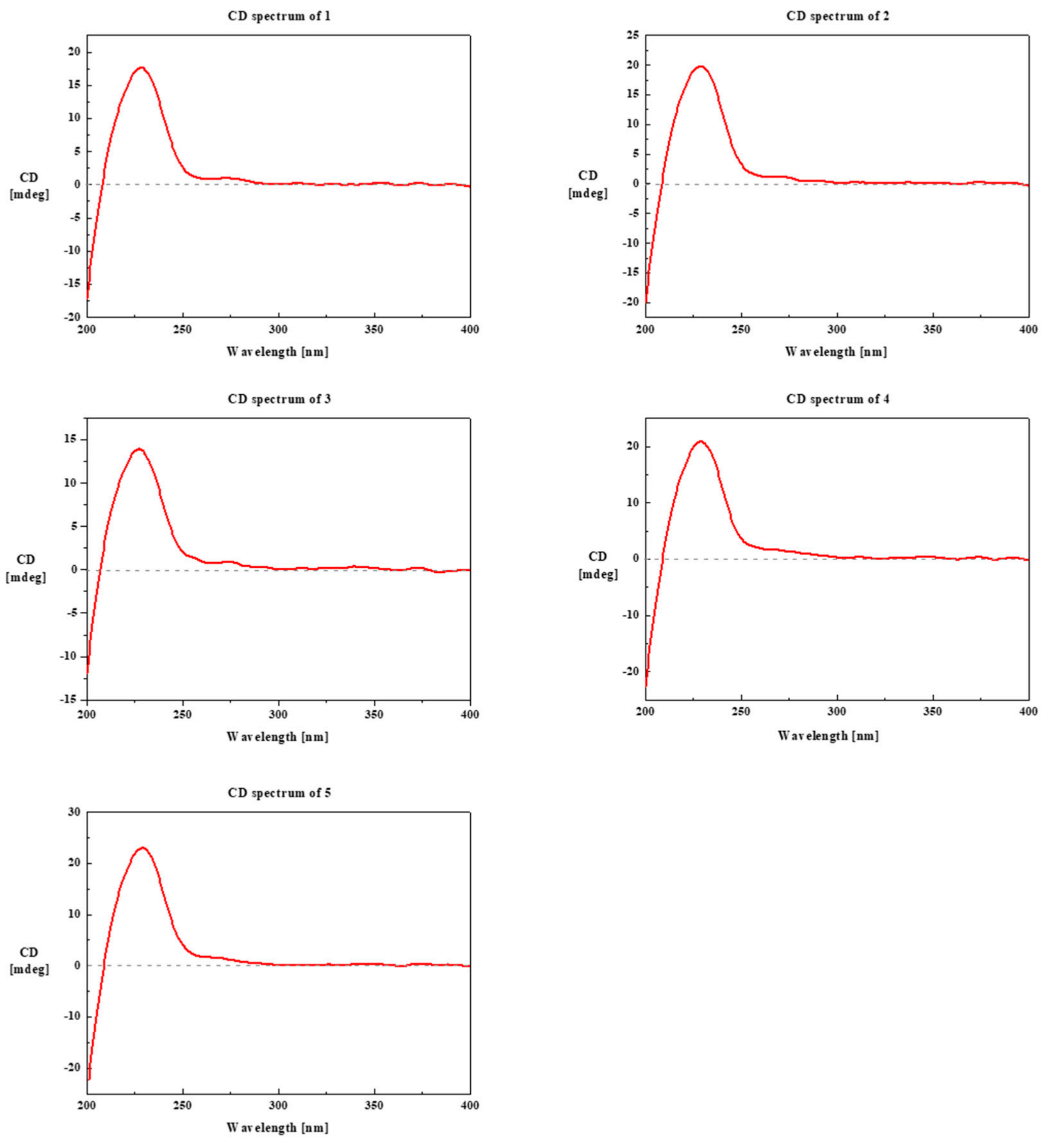

Figure S4. ECD spectra of **1-5** in MeOH**.**

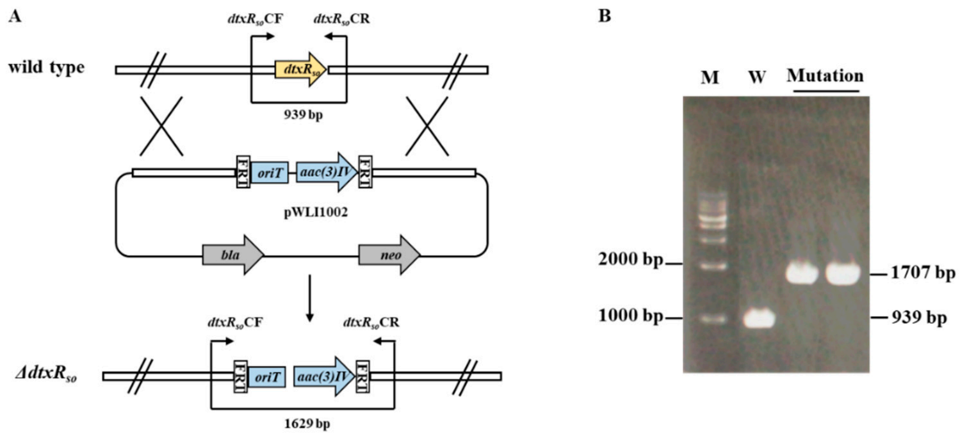

Figure S5. Inactivation of *dtxR$_{so}$*. (A) Construction of *dtxR$_{so}$* inactivation mutant. (B) PCR confirmation of double-crossover mutant. M: 1 kb DNA marker; W: *S. olivaceus* SCSIO 1071 wild-type strain; M: *$dtxR_{so}$* gene inactivation mutant.

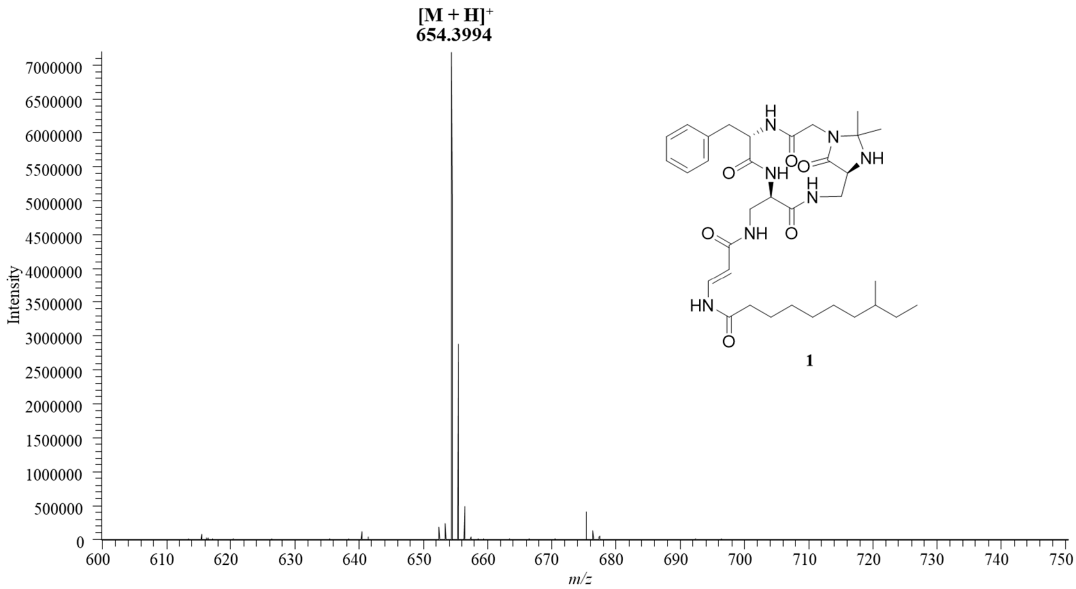

Figure S6. HRESIMS spectrum of **1**.

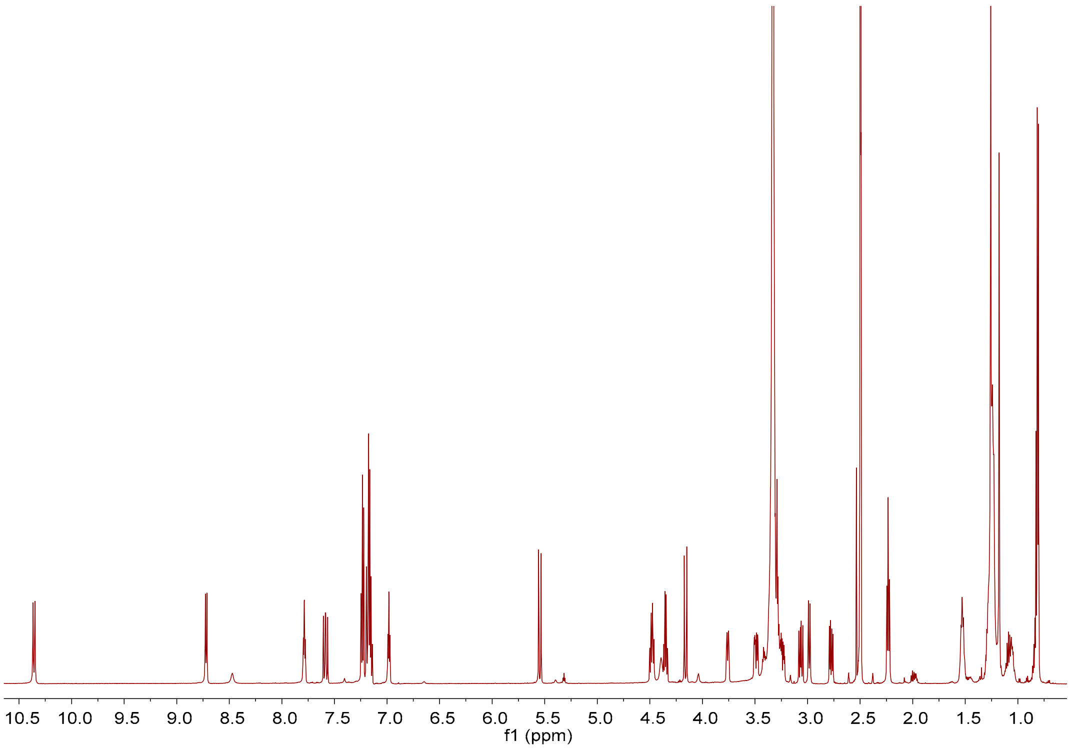

Figure S7. ^{1}H NMR spectrum of **1** in DMSO-d_6.

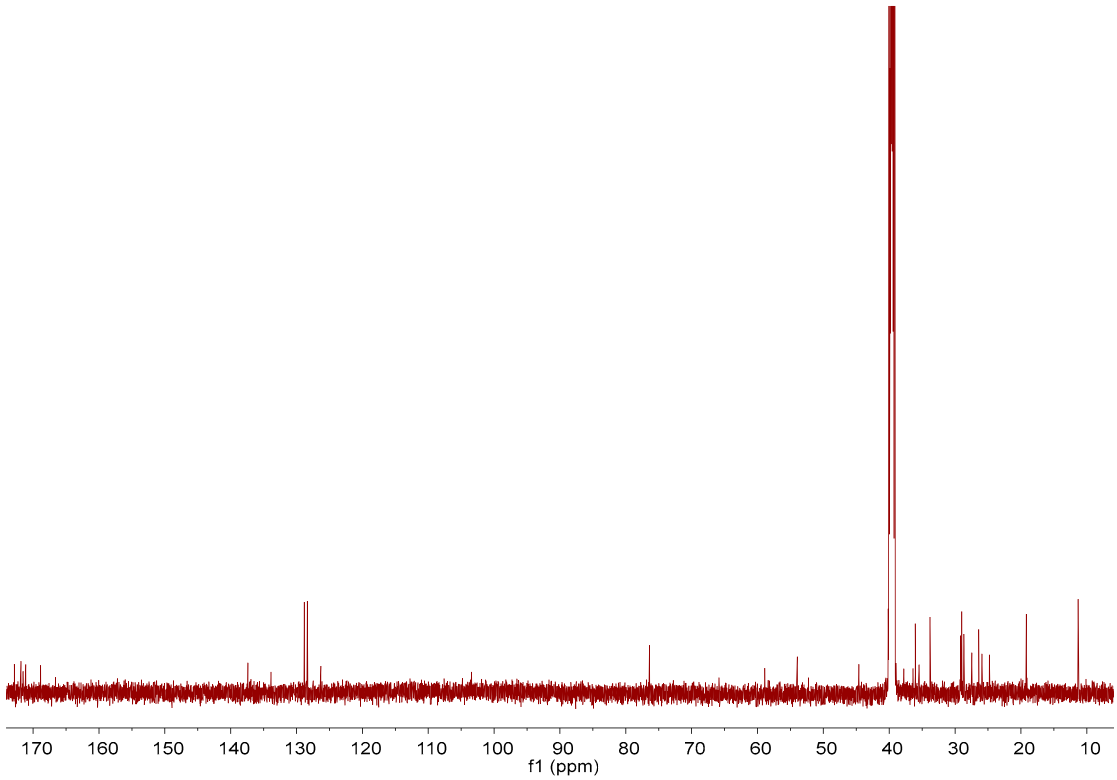

Figure S8. ^{13}C NMR spectrum of **1** in DMSO-*d*₆.

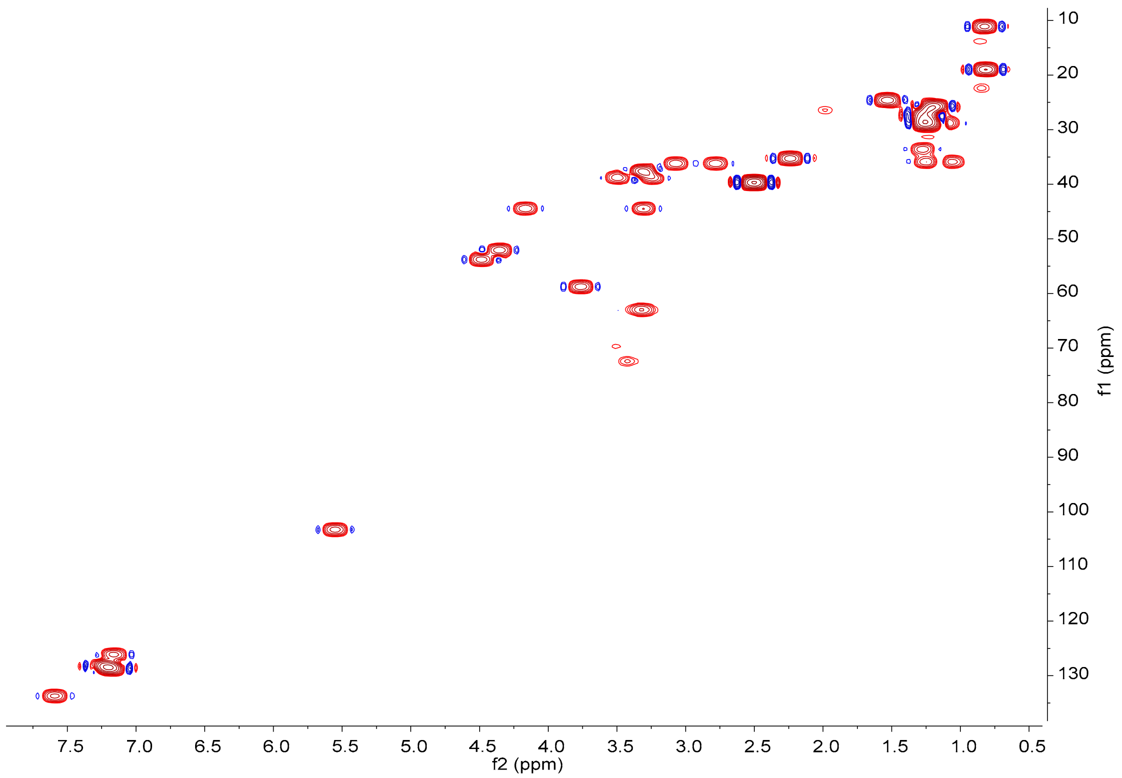

Figure S9. HSQC spectrum of **1** in DMSO-d_6.

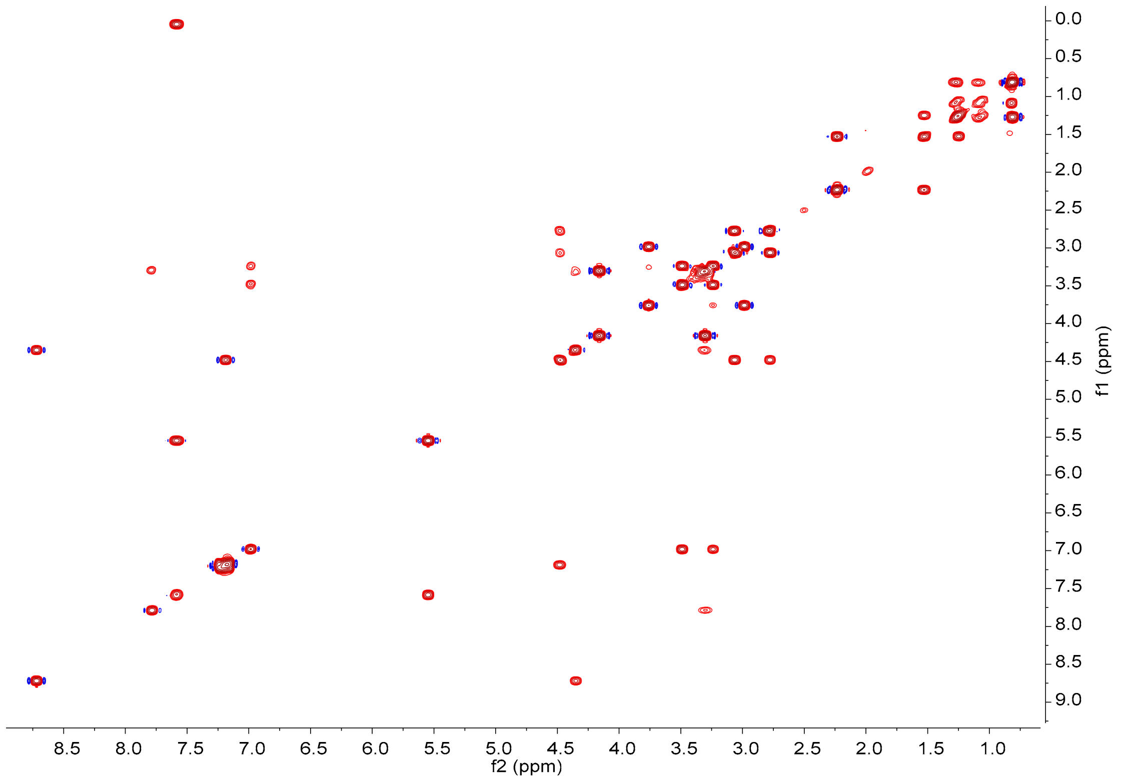

Figure S10. ^{1}H-^{1}H COSY spectrum of **1** in DMSO-*d*$_6$.

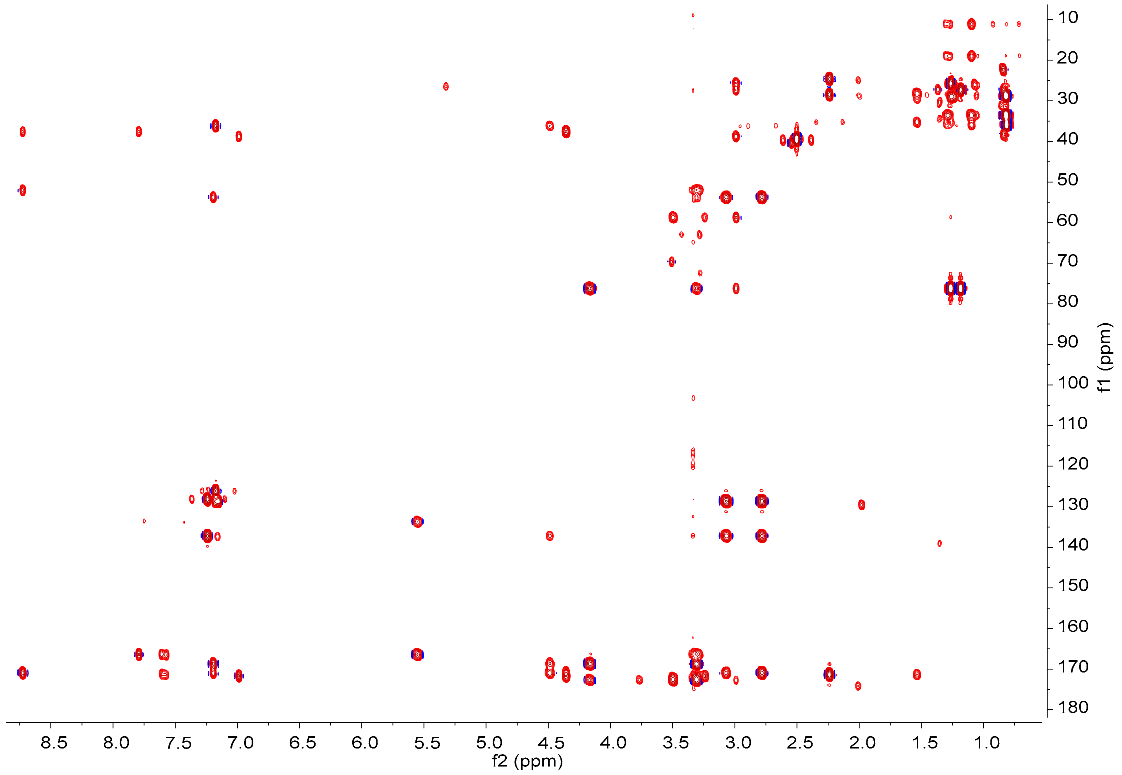

Figure S11. HMBC spectrum of **1** in DMSO-*d*$_6$.

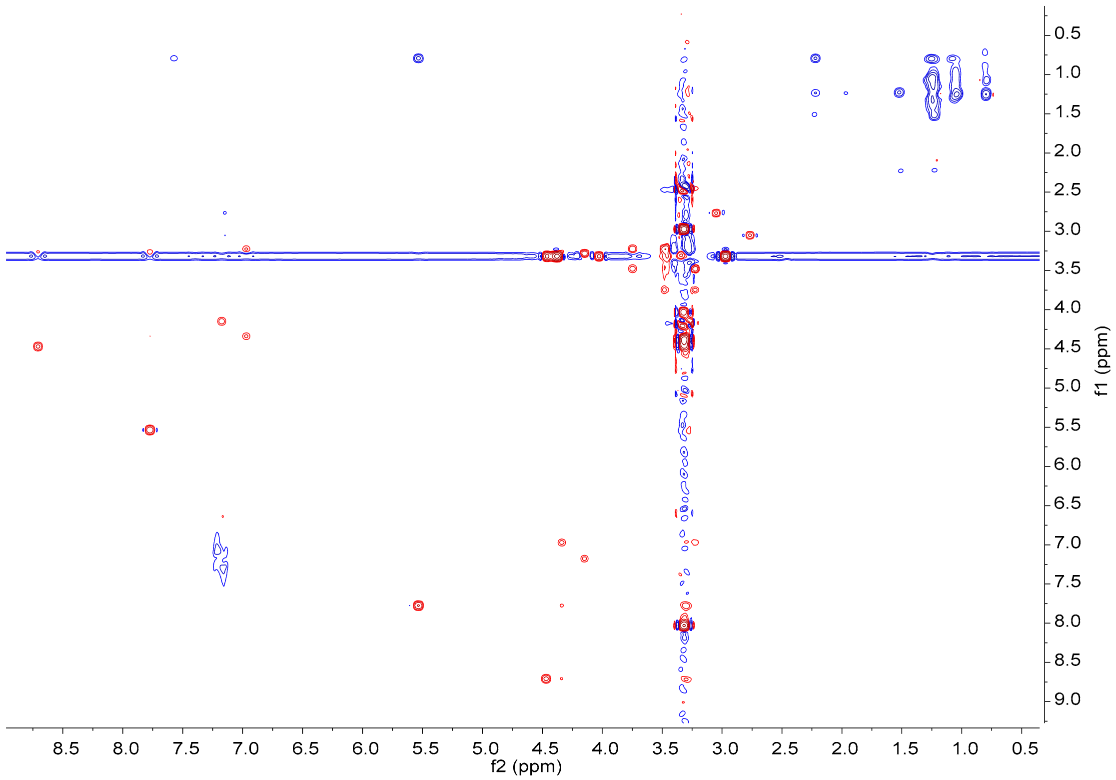

Figure S12. NOESY spectrum of **1** in DMSO-d_6.

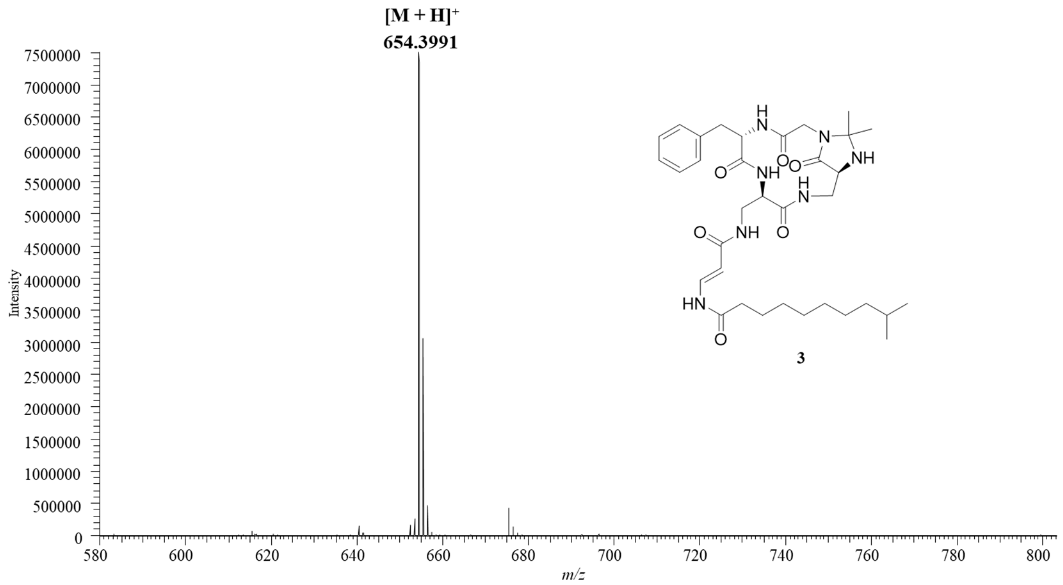

Figure S13. HRESIMS spectrum of **2**.

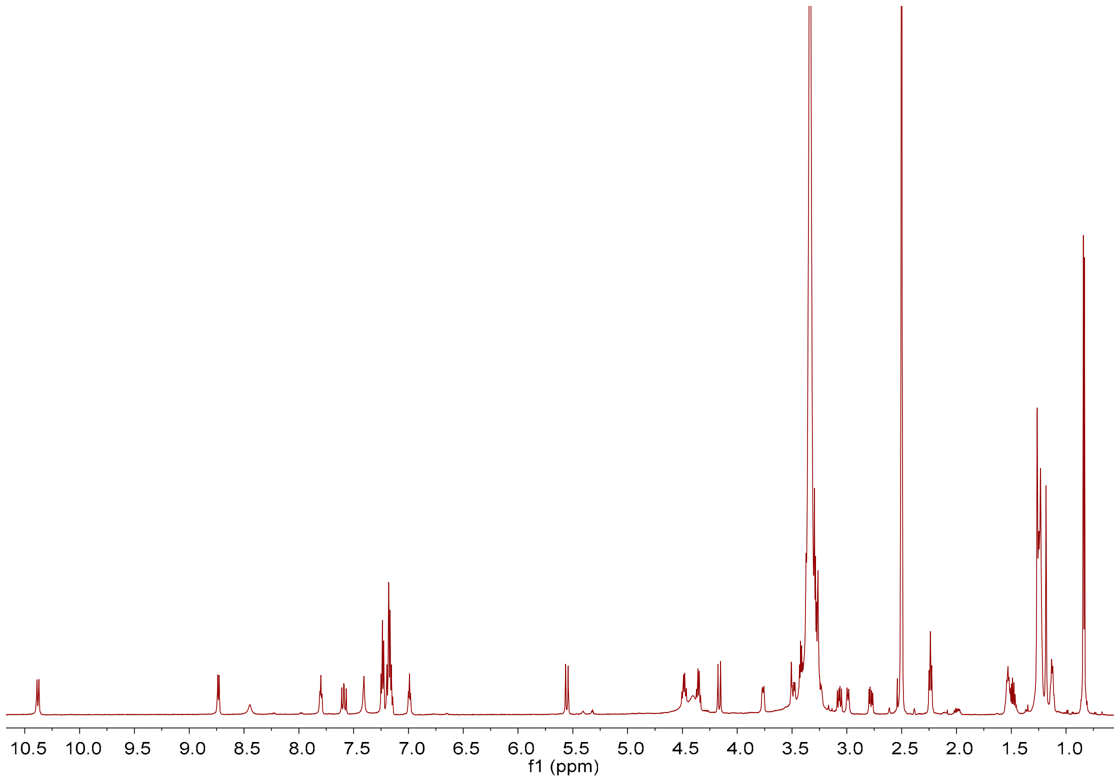

Figure S14. ^{1}H NMR spectrum of **2** in DMSO-d_6.

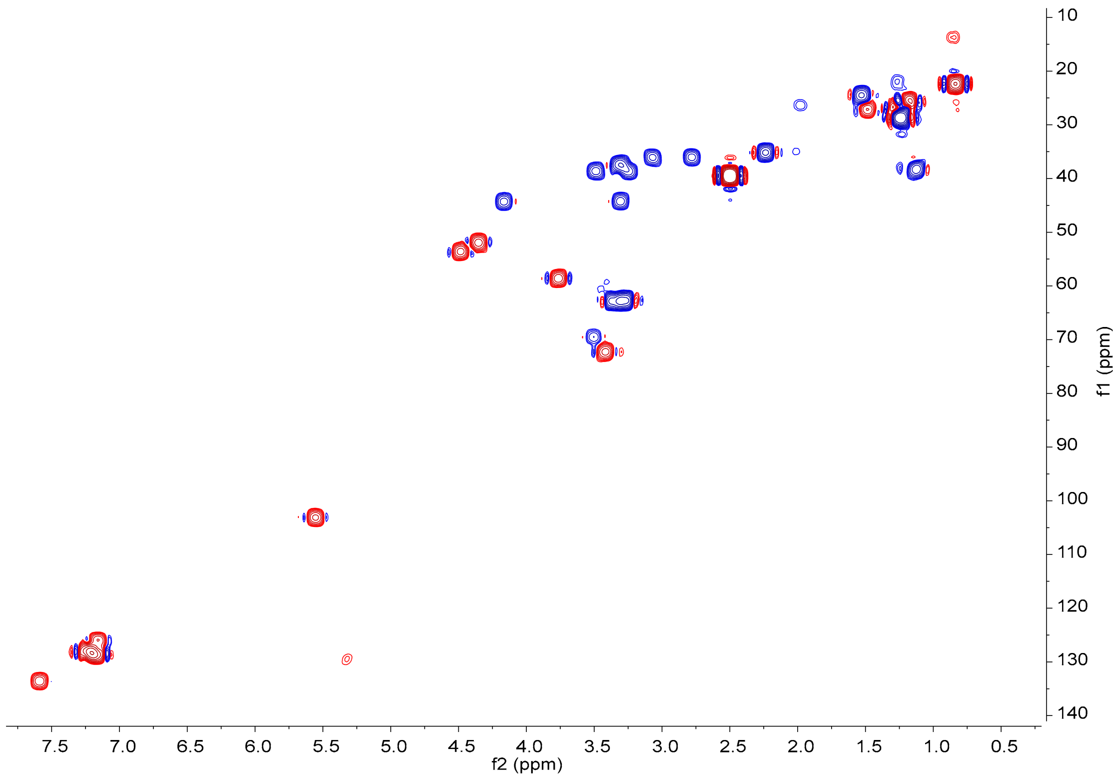

Figure S15. HSQC spectrum of **2** in DMSO-d_6.

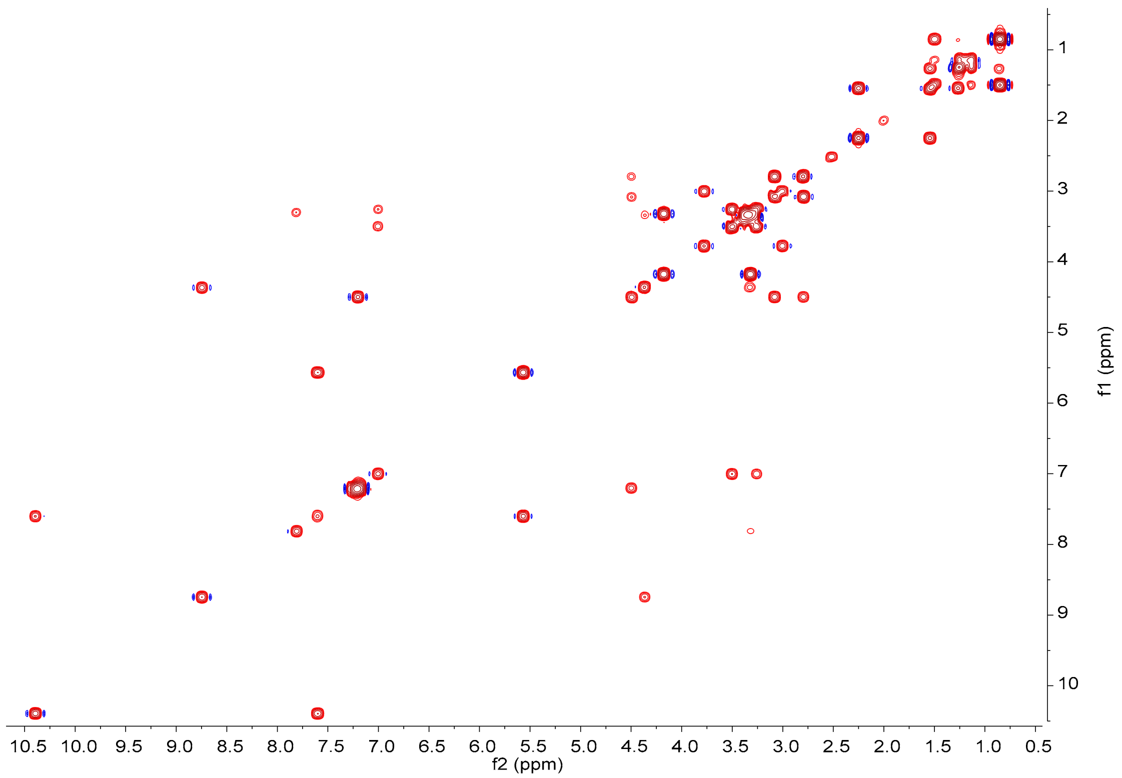

Figure S16. ^{1}H-^{1}H COSY spectrum of **2** in DMSO-d_6.

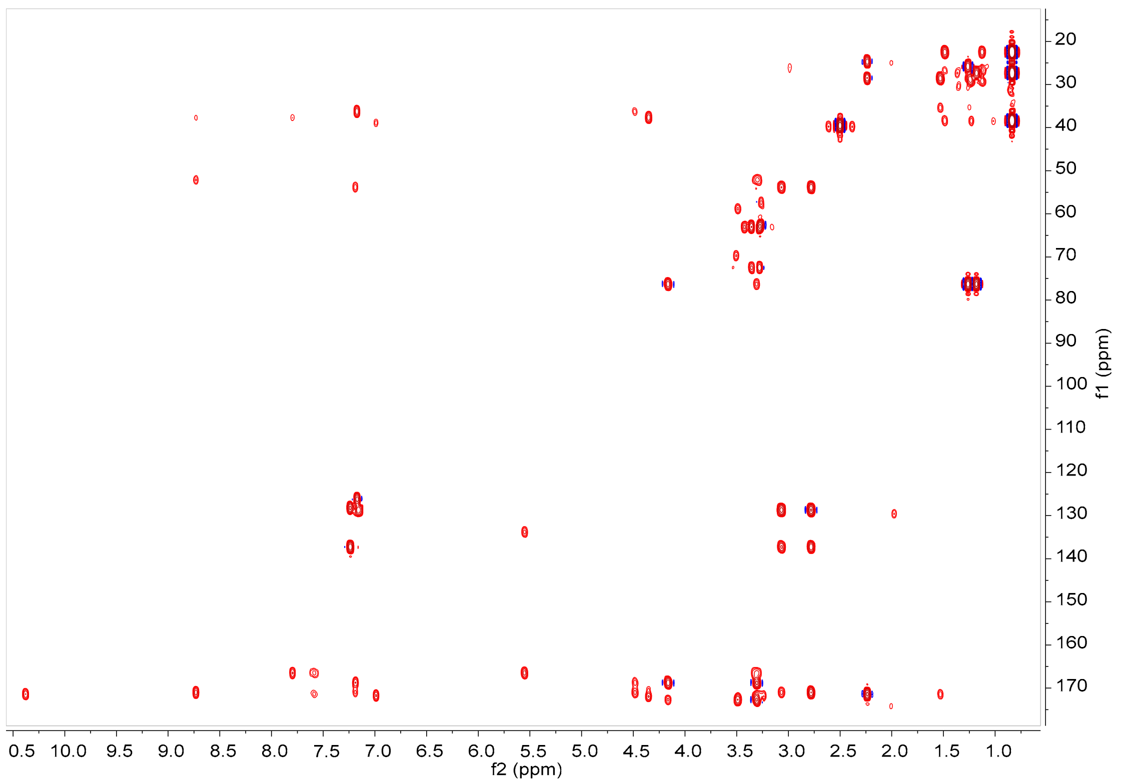

Figure S17. HMBC spectrum of **2** in DMSO-d_6.

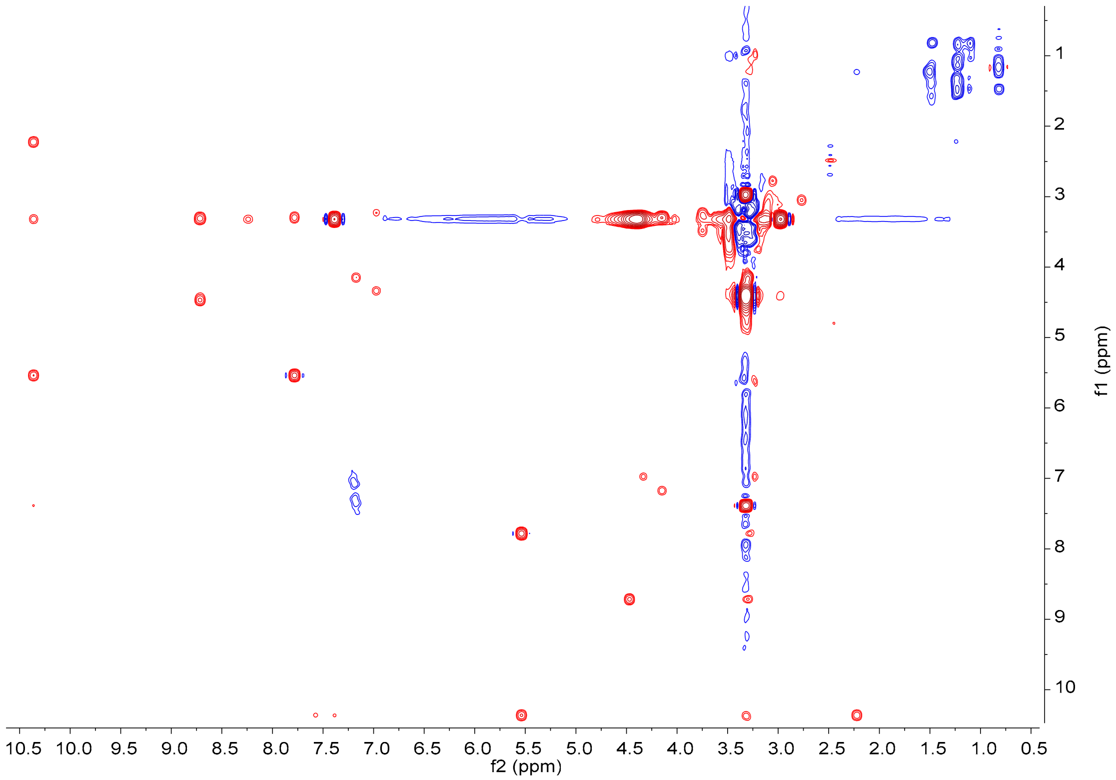

Figure S18. NOESY spectrum of **2** in DMSO-d_6.

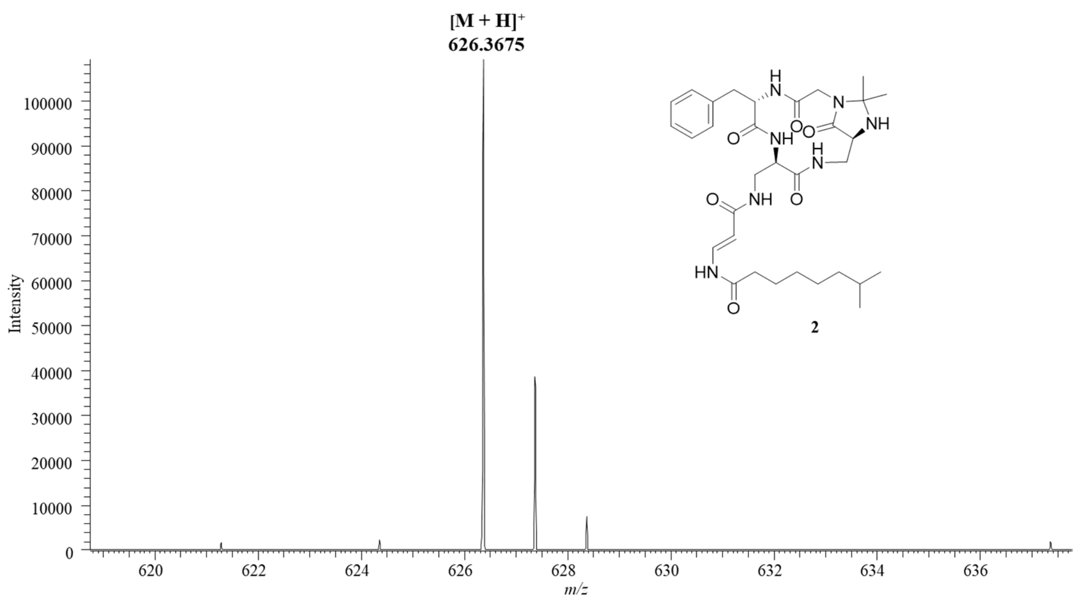

Figure S19. HRESIMS spectrum of **3**.

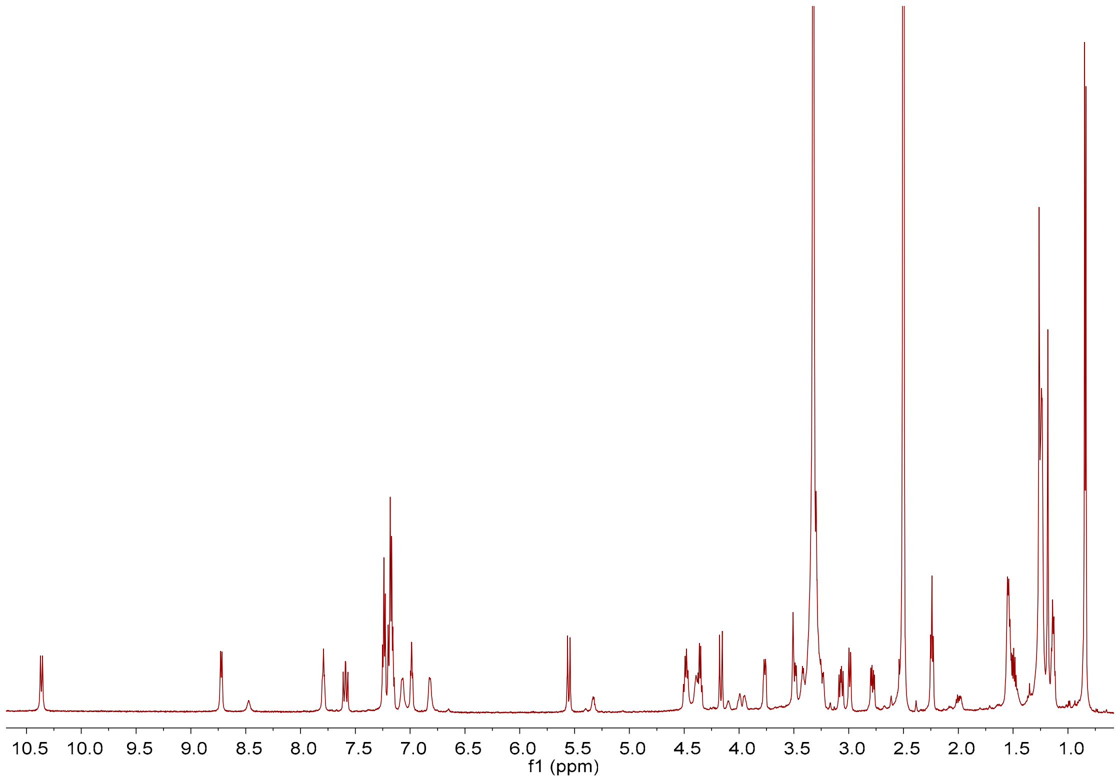

Figure S20. ^{1}H NMR spectrum of **3** in DMSO-d_6.

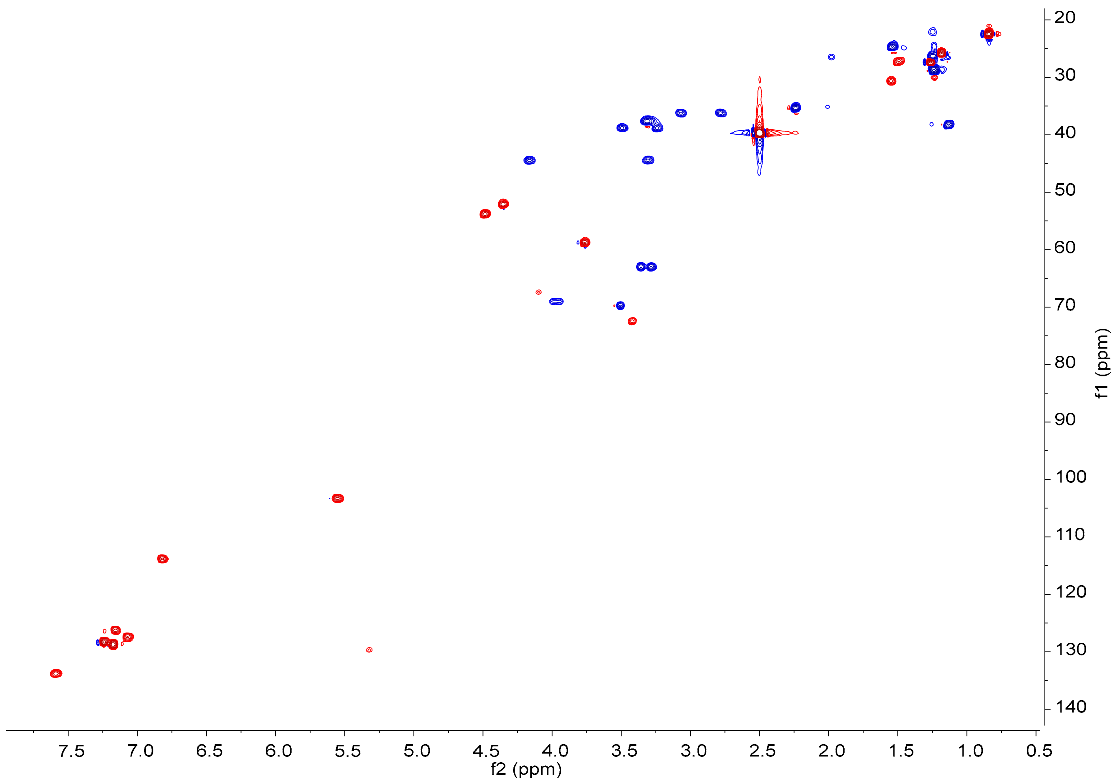

Figure S21. HSQC spectrum of **3** in DMSO-d_6.

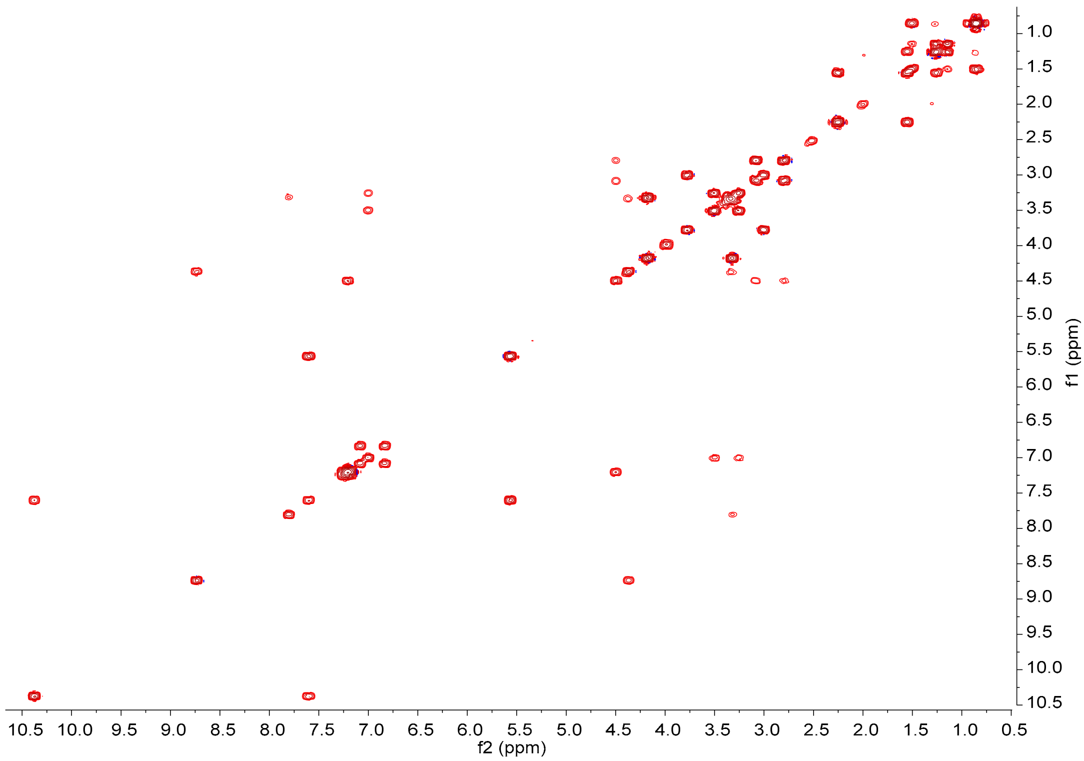

Figure S22. ^{1}H-^{1}H COSY spectrum of **3** in DMSO-d_6.

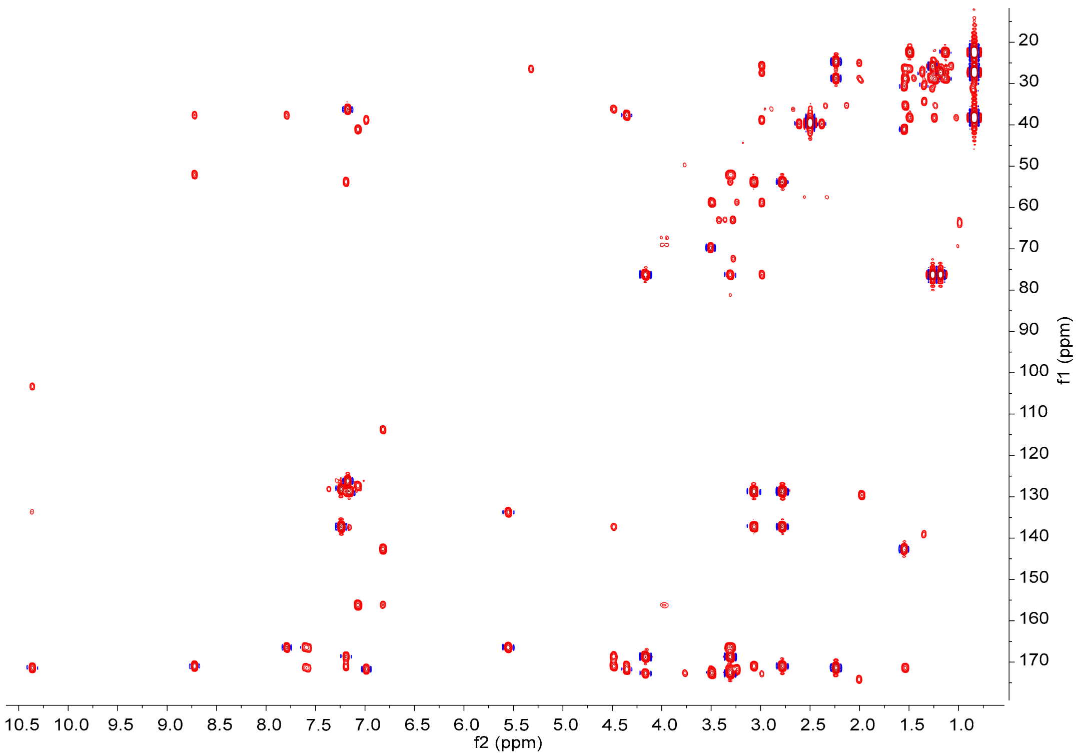

Figure S23. HMBC spectrum of **3** in DMSO-d_6.

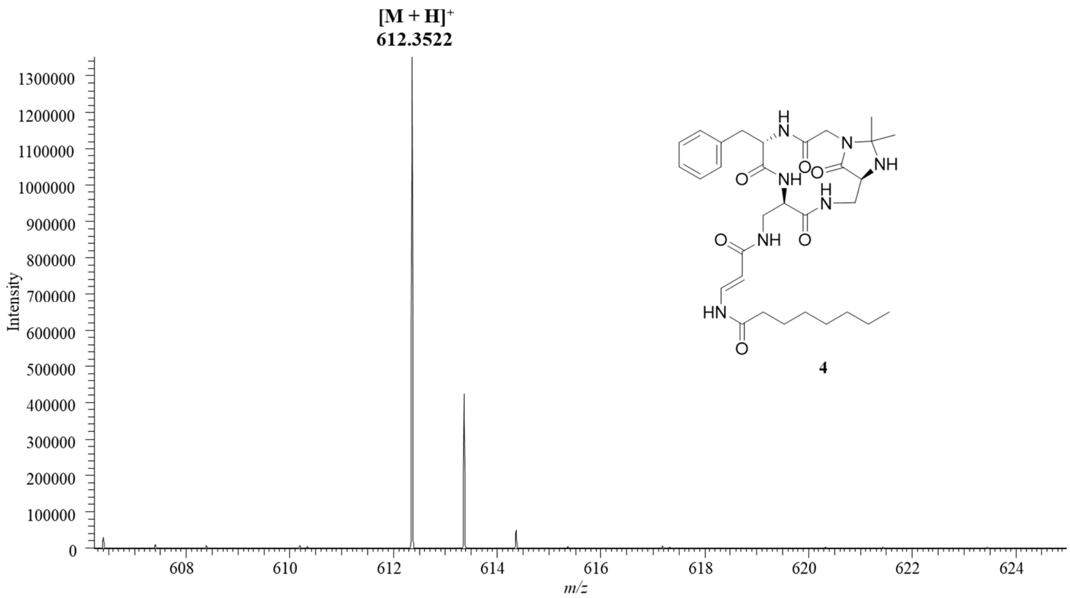

Figure S24. HRESIMS spectrum of **4**.

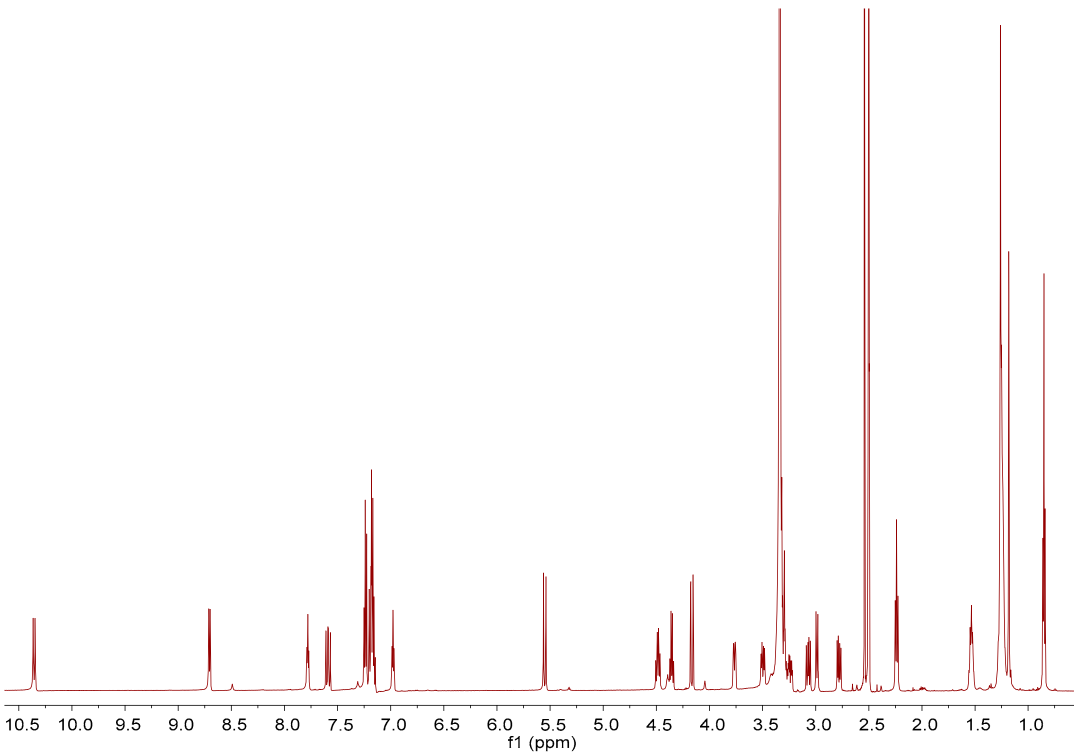

Figure S25. ^{1}H NMR spectrum of **4** in DMSO-d_6.

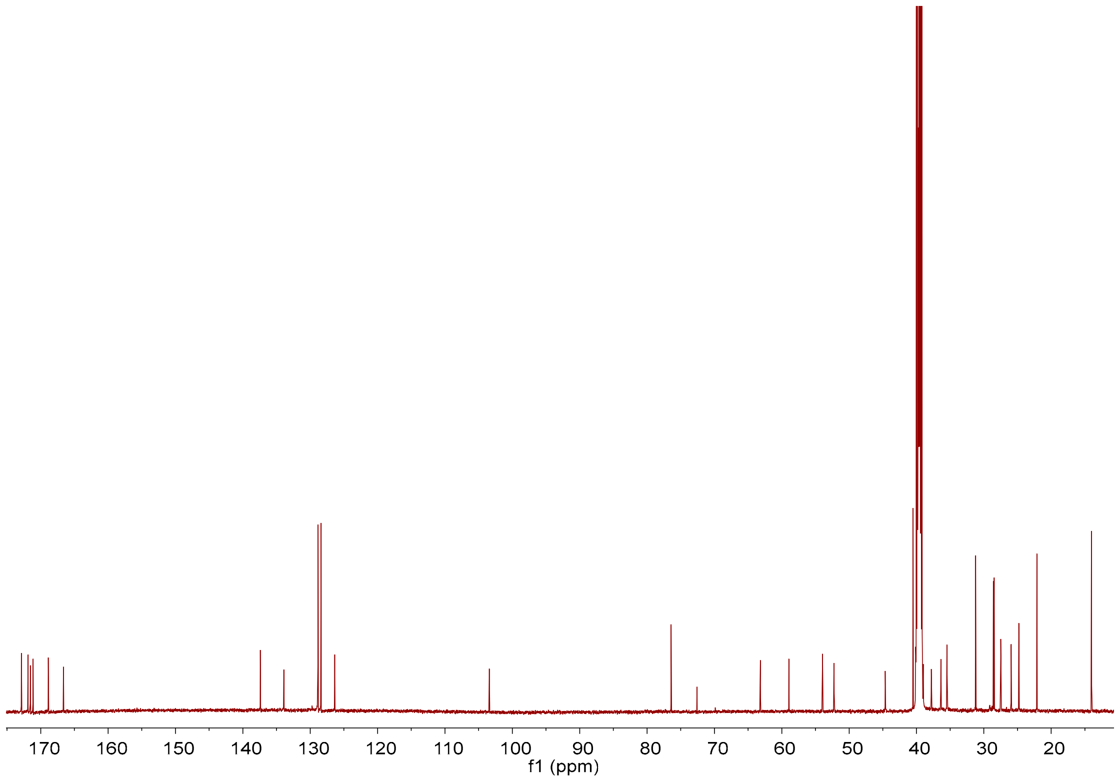

Figure S26. ^{13}C NMR spectrum of **4** in DMSO-*d*₆.

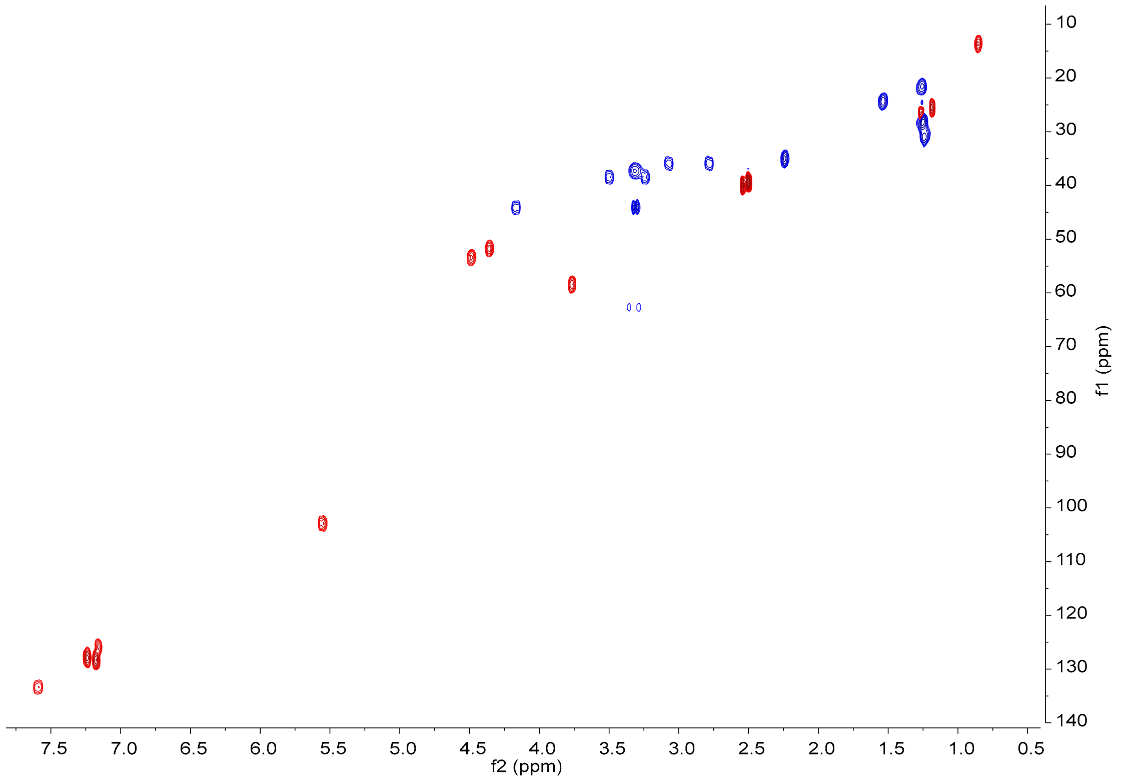

Figure S27. HSQC spectrum of **4** in DMSO-*d*₆.

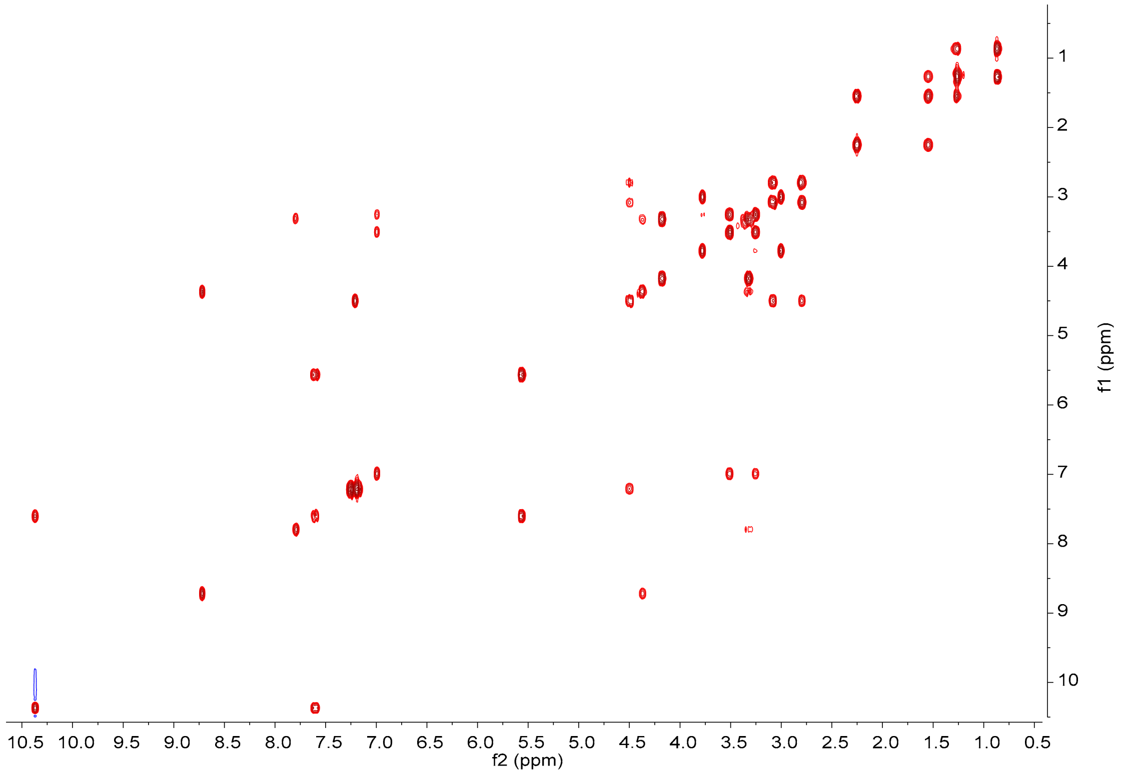

Figure S28. ^{1}H-^{1}H COSY spectrum of **4** in DMSO-d_6.

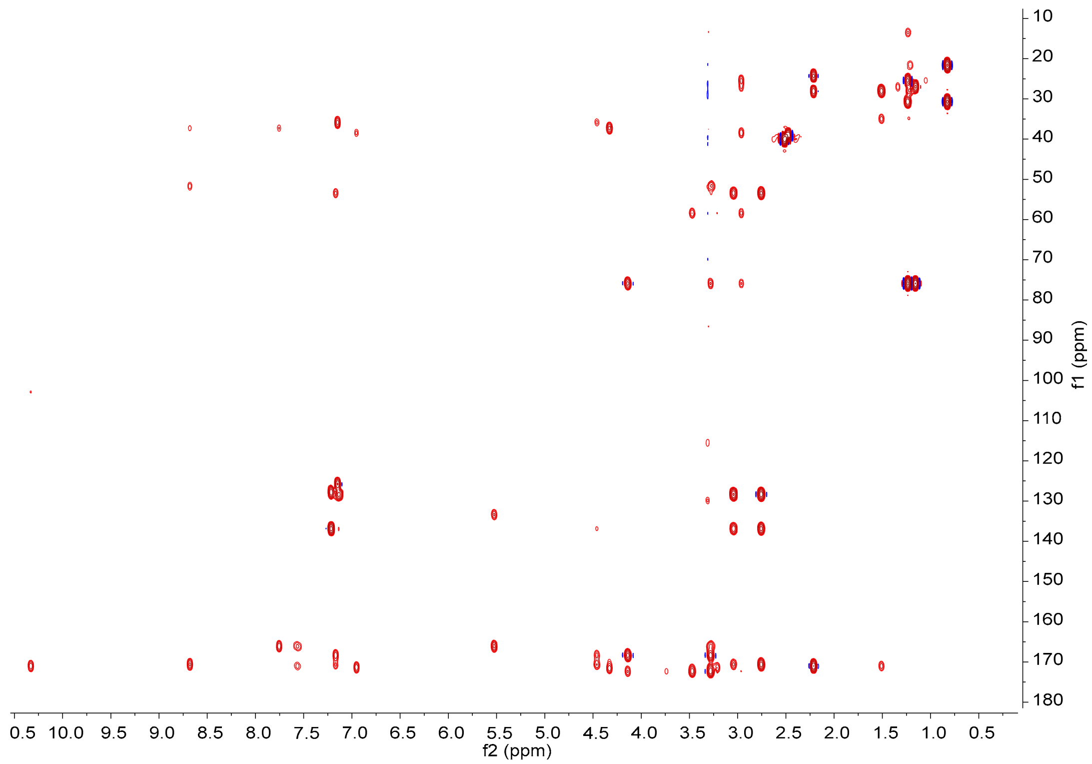

Figure S29. HMBC spectrum of **4** in DMSO-d_6.

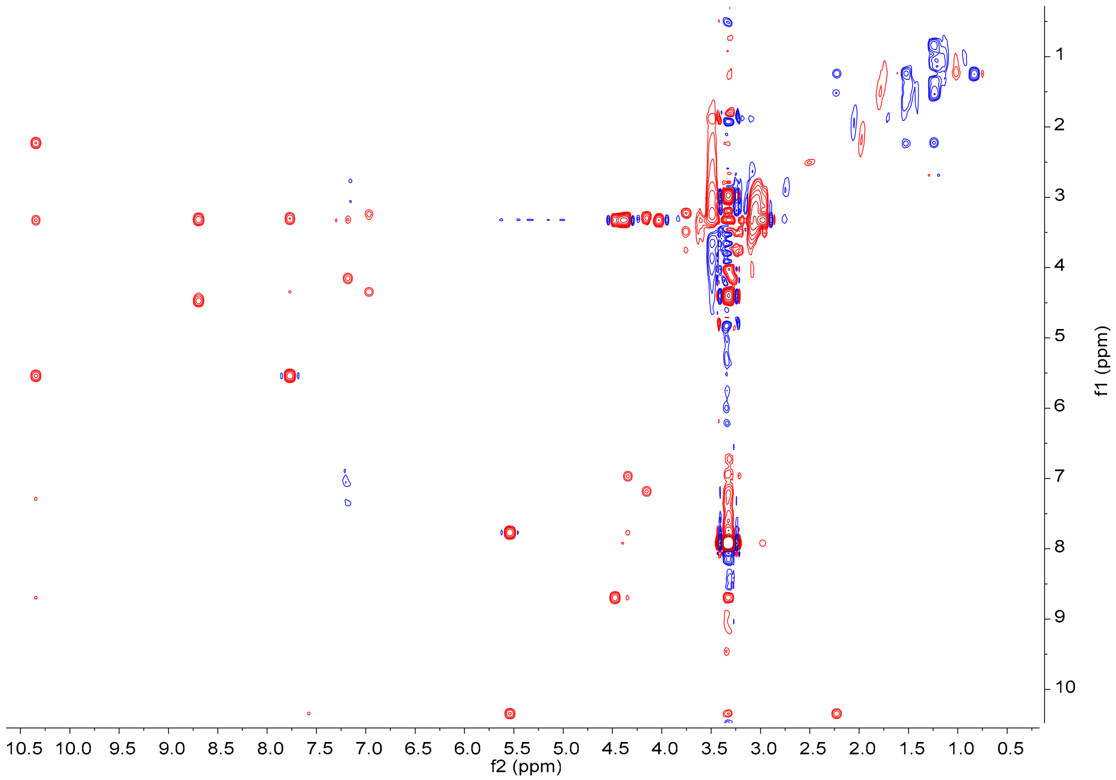

Figure S30. NOESY spectrum of **4** in DMSO-d_6.

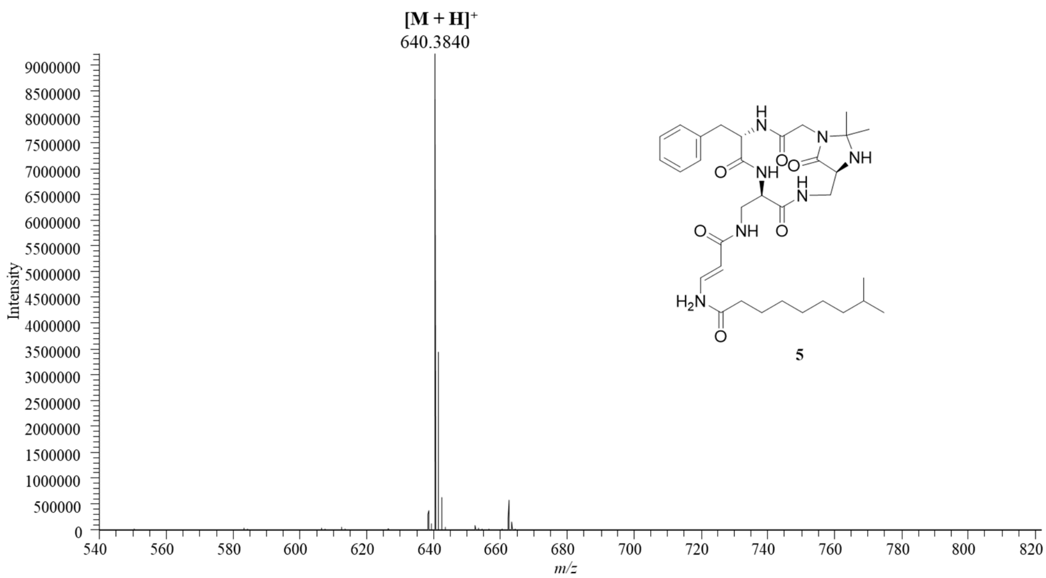

Figure S31. HR-ESIMS spectrum of **5**.

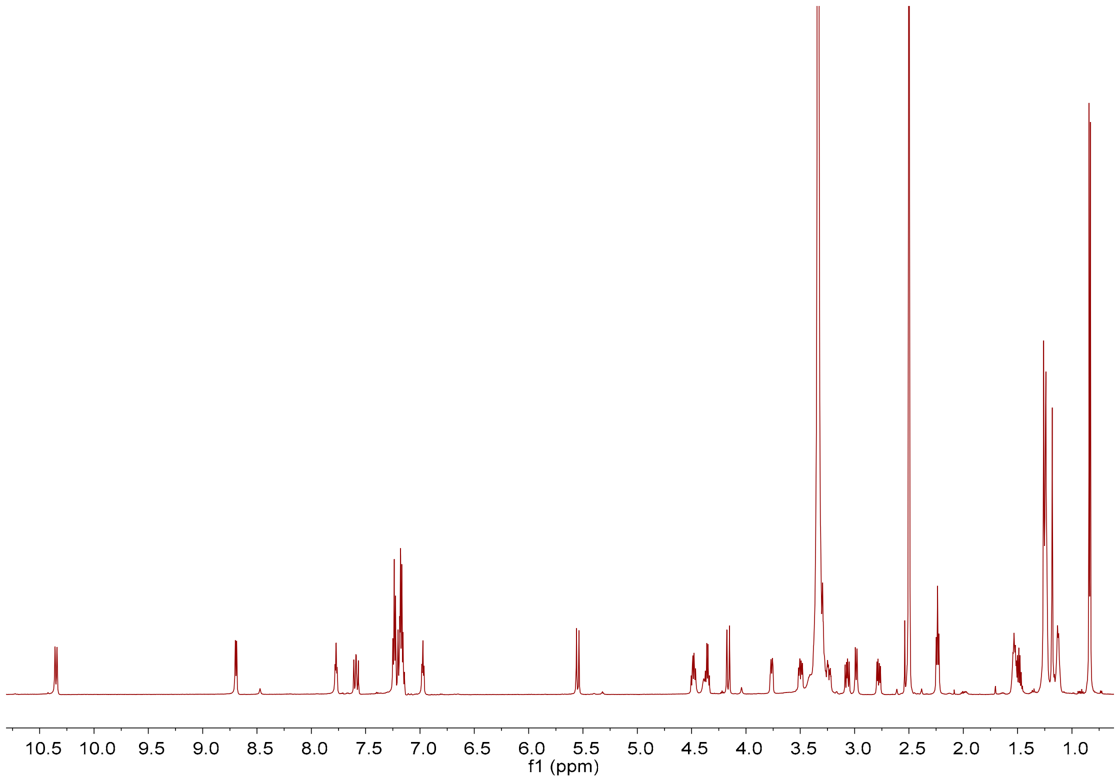

Figure S32. ^{1}H NMR spectrum of **5** in DMSO-*d6*.

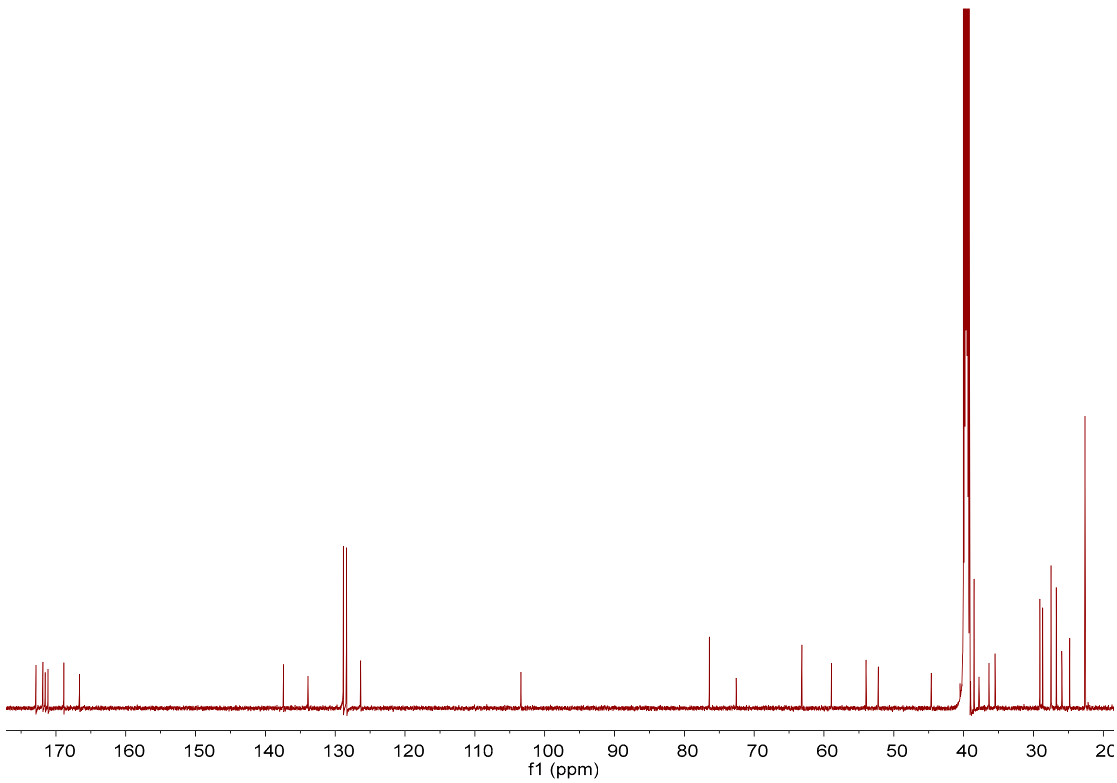

Figure S33. ^{13}C NMR spectrum of **5** in DMSO-*d6*.

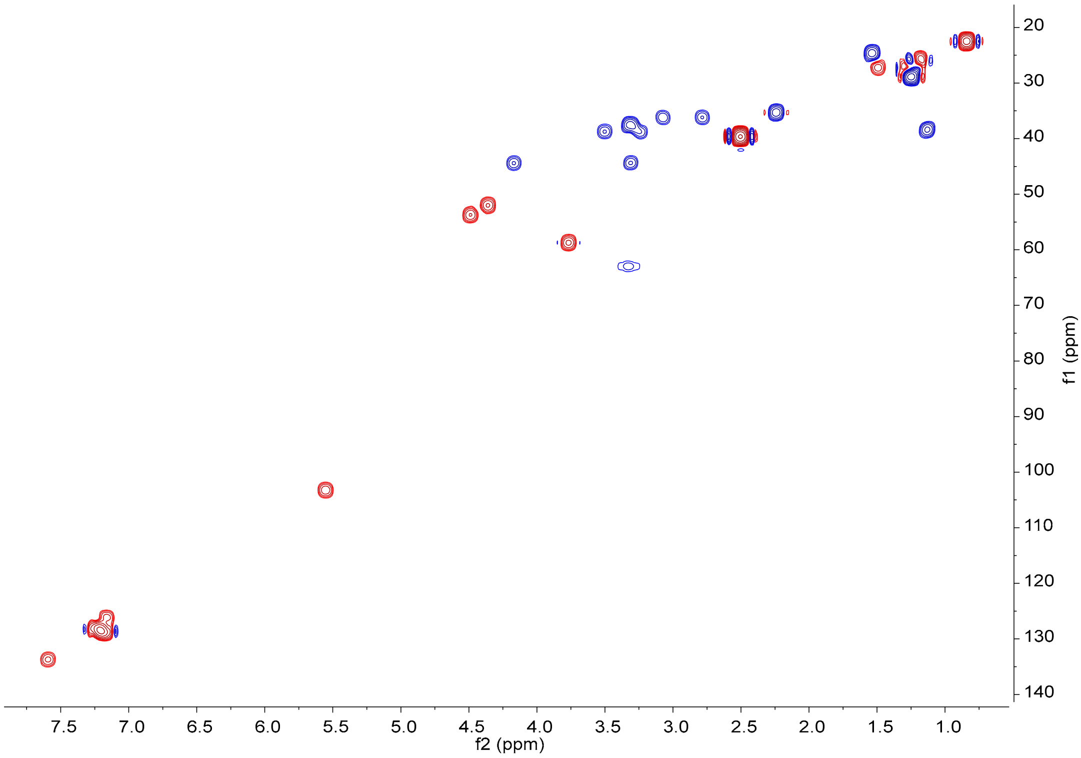

Figure S34. HSQC spectrum of **5** in DMSO-d_6.

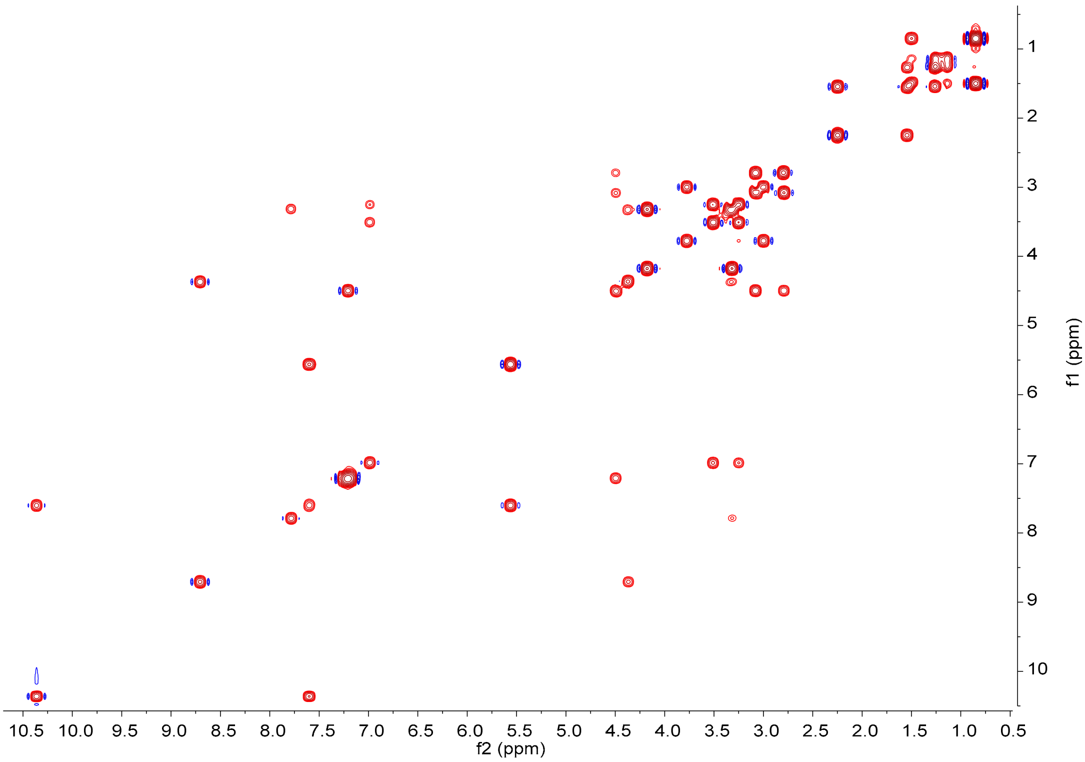

Figure S35. ^1H-^1H COSY spectrum of **5** in DMSO-d_6.

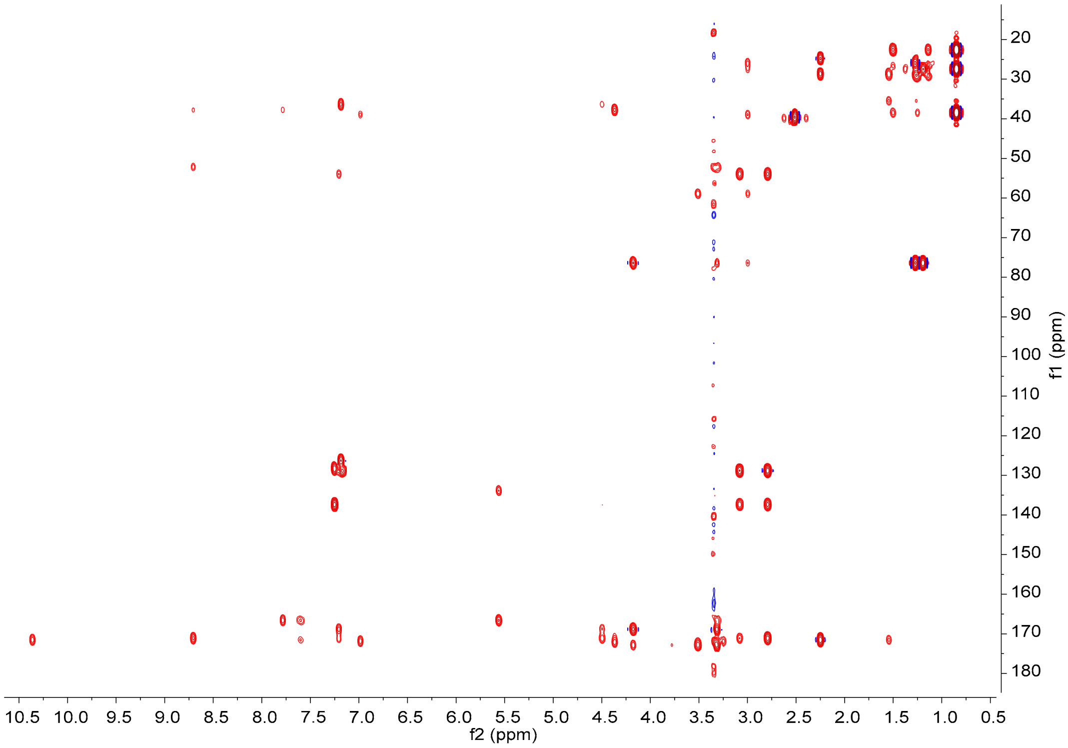

Figure S36. HMBC spectrum of **5** in DMSO-*d*$_6$.

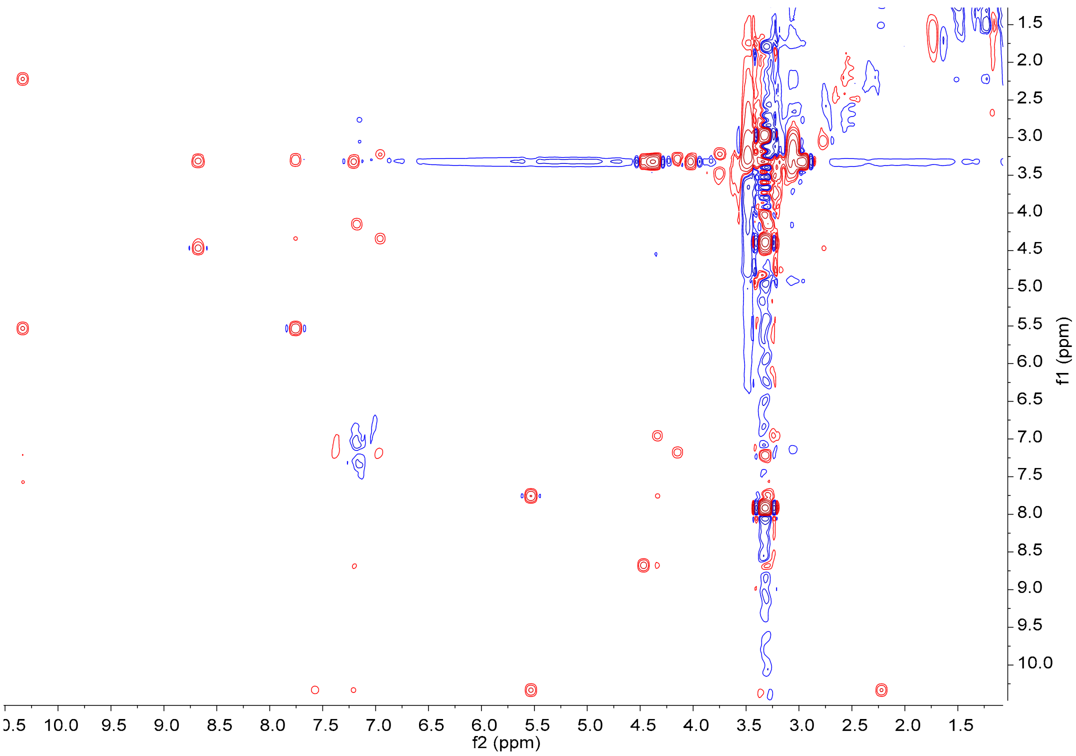

Figure S37. NOESY spectrum of **5** in DMSO-*d*$_6$.

References

[1] Gust B, Challis G L, Fowler K, Kieser T, Chater K F. PCR-targeted Streptomyces gene replacement identifies a protein domain needed for biosynthesis of the sesquiterpene soil odor geosmin[J]. *Proc Natl Acad Sci U S A*, 2003, 100(4): 1541-1546.

[2] Macneil D J, Occi J L, Gewain K M, Macneil T, Gibbons P H, Ruby C L, Danis S J. Complex organization of the *Streptomyces avermitilis* genes encoding the avermectin polyketide synthase[J]. *Gene*, 1992, 115(1-2): 119-125.