

# Genome mining and metabolic profiling uncover polycyclic tetramate macrolactams from *Streptomyces koyangensis* SCSIO 5802

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**Table S1.** Strains and plasmids used and constructed in this study.

Strains or plasmids	Description	Reference or source
<b>Strains</b>		
<i>E. coli</i> DH5 $\alpha$	Host for general cloning	
<i>E. coli</i> BW25113	Host strain for PCR-targeting	[1]
<i>E. coli</i> ET12567/pUZ8002	Host strain for conjugation	[2]
<i>S. koyangensis</i> SCSIO 5802	Wild-type producer of abyssomycins	This study
<i>S. koyangensis</i> SCSIO 5802A	Abyssomycins-free strain originated from <i>S. koyangensis</i> SCSIO 5802	This study
<i>S. koyangensis</i> SCSIO 5802AC	Abyssomycins/candididins-free strain originated from <i>S. koyangensis</i> SCSIO 5802	This study
<i>S. koyangensis</i> SCSIO 5802ACM	Abyssomycins/candididins/PTM-free strain originated from <i>S. koyangensis</i> SCSIO 5802	This study
<b>Plasmids</b>		
pIJ790	Cml <sup>r</sup> , including $\lambda$ -RED ( <i>gam</i> , <i>bet</i> , <i>exo</i> ) for PCR-targeting	[3]
pIJ773	Apr <sup>r</sup> , source of <i>acc(3)IV</i> and <i>oriT</i> fragment	[3]
pUZ8002	Kan <sup>r</sup> , including <i>tra</i> for conjugation	[4]
7-8F	Amp <sup>r</sup> , Kan <sup>r</sup> , harboring <i>can</i> gene	This study
6-8A	Amp <sup>r</sup> , Kan <sup>r</sup> , harboring <i>skoB</i> gene	This study

**Table S2.** Primers used in this study.

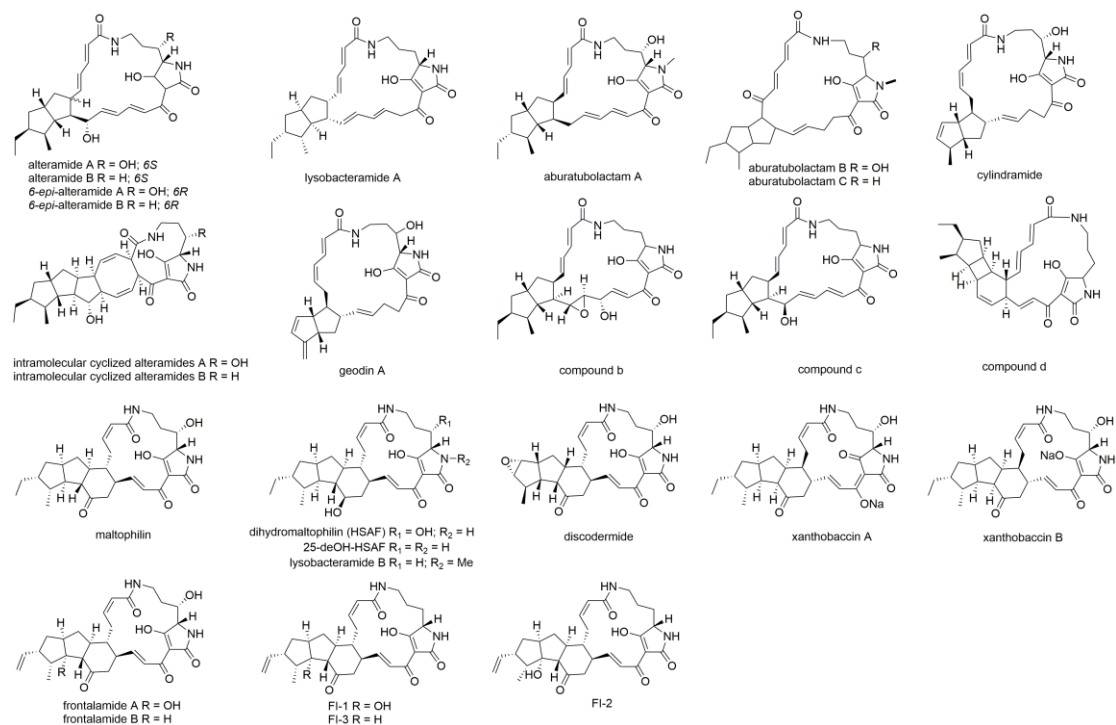
<b>Primer</b>	<b>Sequence (5'-3')</b>
<b>For PCR screening of genomic library</b>	
sko-orf5567-up-scr-F	GGGCAAGGTCGTCGTCA
sko-orf5567-up-scr-R	CCGTCCGAACCGTAGTTGA
sko-orf5573-mid-scr-F	CGGCACGATGATGACGATG
sko-orf5573-mid-scr-R	GCCAGTGCGGTCTTGATGAG
sko-orf5576-down-scr-F	AGCCGGGTCTACGAGAAGG
sko-orf5576-down-scr-R	TCCAGGAAGGTGGTGAGGG
<b>For construction of gene disruption mutant</b>	
canD-delF	GCGCGCGTCGAGGTGGGCGAGCAGCCGGGCGG
	CCTGGGCACTAGTATTCCGGGGATCCGTCGACC
canD-delR	GAGGTGCCCCCGCTCCTGCGCGGCCTGGTCCGC
	ACCCGCACTAGTTGTAGGCTGGAGCTGCTTC
skoA-delF	TTGGCTGACGCGATCAACGACGCCCCGACGGGC
	AAGGTAATTCCGGGGATCCGTCGACC
skoA-delR	CCAGACCAGACGCCGGTCCTCGGGCTCCAGCCG
	CCGTCCTGTAGGCTGGAGCTGCTTC
canD-testF	GCCAGCGAGTGGGTGACG
canD-testR	CGGCGAACTGTCCGAGCG
skoA-testF	CATCCCCTCCACCGCGCGAAG
skoA-testR	GACCGGTTGCTCTCCAGG

**Table S3.**  $^1\text{H}$  (700 MHz) and  $^{13}\text{C}$  NMR (175 MHz) NMR Data for 10-*epi*-HSAF (**1**) and koyanamide A (**2**).

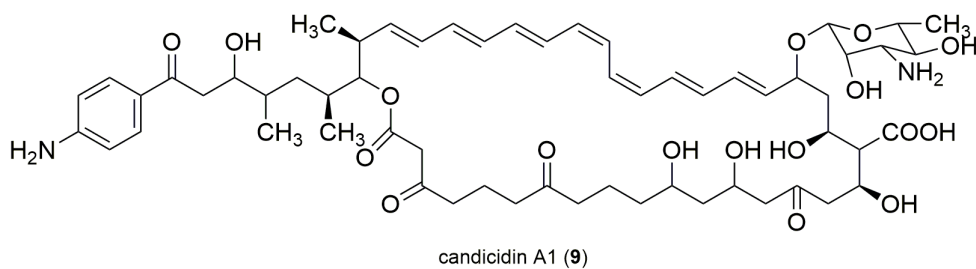
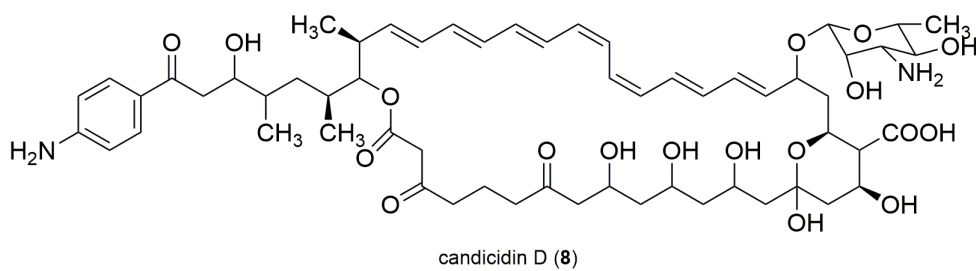
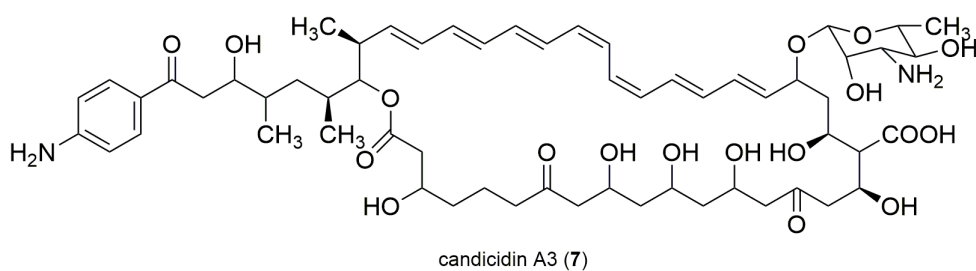
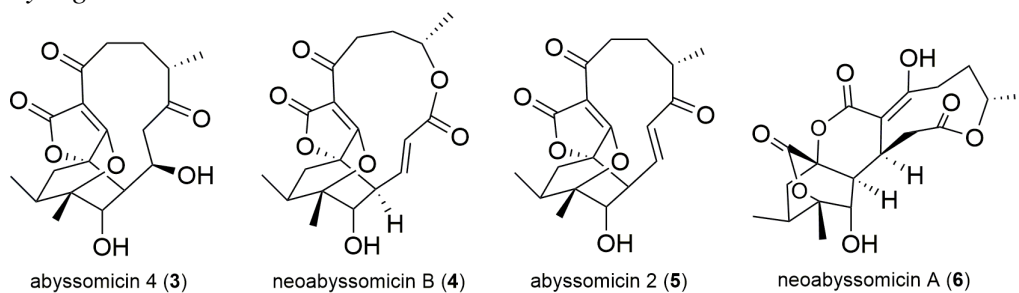
position	10- <i>epi</i> -HSAF ( <b>1</b> ) <sup>a</sup>		koyanamide A ( <b>2</b> ) <sup>b</sup>	
	$\delta_{\text{C}}$ , type	$\delta_{\text{H}}$ , mult ( <i>J</i> in Hz)	$\delta_{\text{C}}$ , type	$\delta_{\text{H}}$ , mult ( <i>J</i> in Hz)
1	165.5, C		180.7, C	
2	124.2, CH	5.74, m	46.6, CH	3.91, m
3	138.8, CH	5.91, m	125.9, CH	5.46, t (9.1)
4	27.8, CH <sub>2</sub>	1.87-1.92, m; 3.44-3.45, m	138.3, CH	5.56, d (9.8)
5	43.6, CH	1.25-1.28, m	46.4, CH	2.92-2.95, m
6	47.2, CH	1.61-1.65, m	58.4, CH	1.89-1.92, m
7	37.3, CH <sub>2</sub>	0.82-0.88, m; 1.98-2.06, m	36.5, CH <sub>2</sub>	0.99-1.00, 1.96-1.98, m
8	41.4, CH	2.34, m	50.5, CH	2.79-2.81, m
9	40.5, CH <sub>2</sub>	0.82-0.88, m; 1.98-2.06, m	41.6, CH <sub>2</sub>	0.94-0.95, 2.10, m
10	53.5, CH	1.29-1.36, m	54.9, CH	1.38-1.39, m
11	46.5, CH	1.23-1.29, m	47.8, CH	1.25-1.28, m
12	58.0, CH	1.73-1.75, m	54.2, CH	1.67-1.72, m
13	59.2, CH	1.09-1.13, m	65.4, CH	1.83-1.86, m
14	72.7, CH	3.21-3.24, m	82.1, CH	3.67-3.68, m, 1H
15	42.2, CH <sub>2</sub>	1.20-1.31, m; 1.73-1.75, m	59.6, CH	3.17-3.21, m
16	44.8, CH	1.98-2.06, m	138.2, CH	5.75, t (8.8)
17	148.8, CH	6.52, m	126.1, CH	5.67, t (8.7)
18	119.9, CH	6.79, m	47, CH	3.92, m
19	171.5, C		207.7, C	
20	100.5, C		— <sup>c</sup> , C	
21	177.1, C		176.7 (Z), 174.7 (E), C	
22		8.88, s		— <sup>c</sup>
23	68.4, CH	3.85, s	69.8, 69.9, CH	3.94-3.97, m
24	192.0, C		207.7, C	
25	69.9, CH	3.80, m	71.2 (Z), 72.5 (E), CH	3.74-3.76, m
26	31.3, CH <sub>2</sub>	1.17, m; 1.36-1.41, m	30.8, 31.3, CH <sub>2</sub>	1.36, 1.29, m
27	36.3, CH <sub>2</sub>	2.57, m; 3.21-3.24, m	37.2, CH <sub>2</sub>	3.64-3.69, m, 2H
28		7.97, t (5.5)		— <sup>c</sup>
29	25.9, CH <sub>2</sub>	1.01-1.02, m; 1.53-1.57, m	27.2, CH <sub>2</sub>	1.1, 1.62-1.64, m
30	12.6, CH <sub>3</sub>	0.85, m	13.0, CH <sub>3</sub>	0.91, d (7.5)
31	18.4, CH <sub>3</sub>	1.06, d (6.3)	18.4, CH <sub>3</sub>	1.05, d (6.5)

<sup>a</sup>Measured in DMSO-*d*<sub>6</sub>. <sup>b</sup>Measured in CD<sub>3</sub>OD. <sup>c</sup>Not observed.

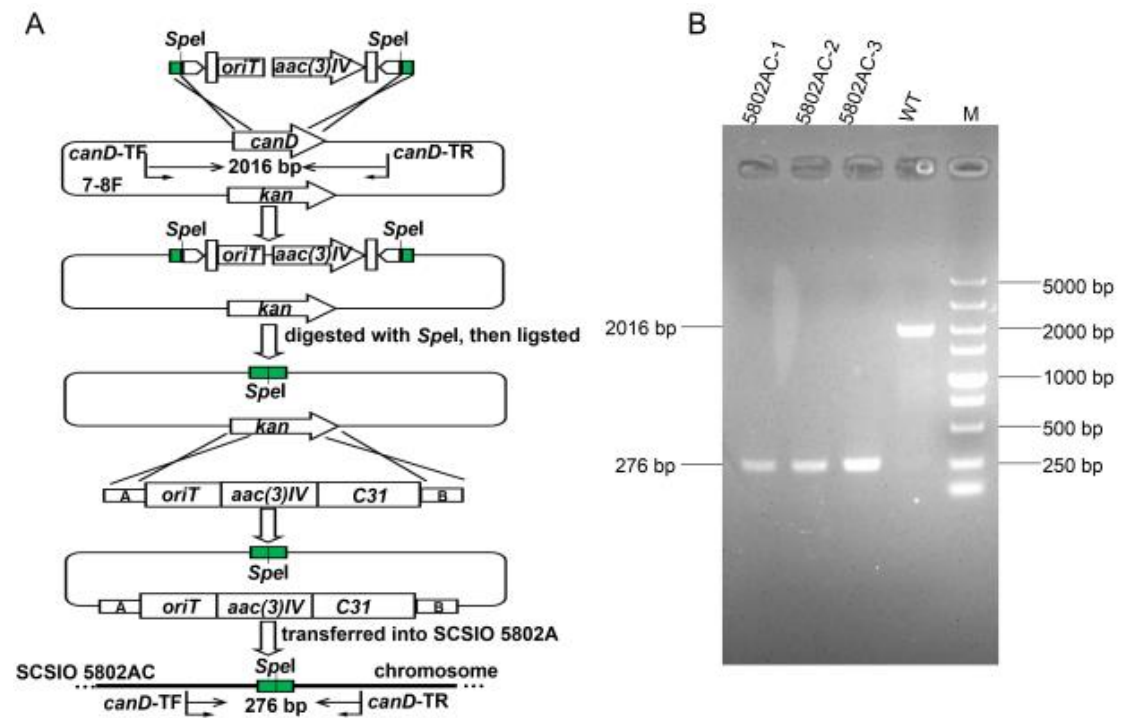
**Figure S1.** Structures of PTMs with a subset of 5/5 bicyclic ring system and 5/5/6 tricyclic ring system.



**Figure S2.** Structures of abyssomicins/neoabyssomicins and candididins isolated from *S. koyangensis* SCSIO 5802.

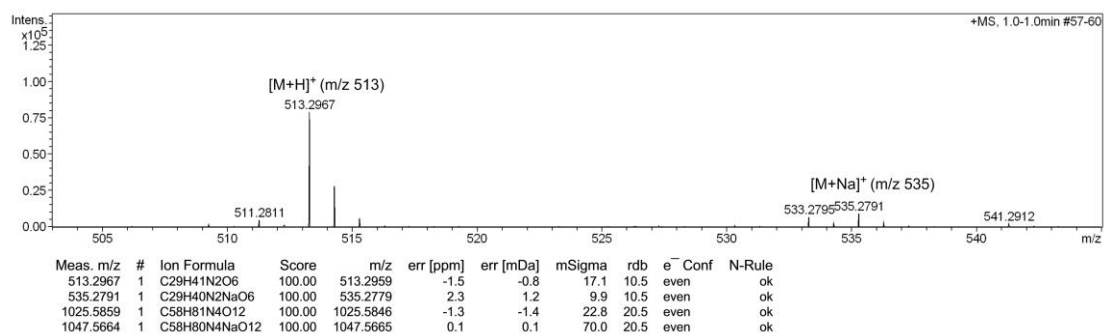


**Figure S3.** *canD* disruption in *S. koyangensis* SCSIO 5802A via PCR-targeting.

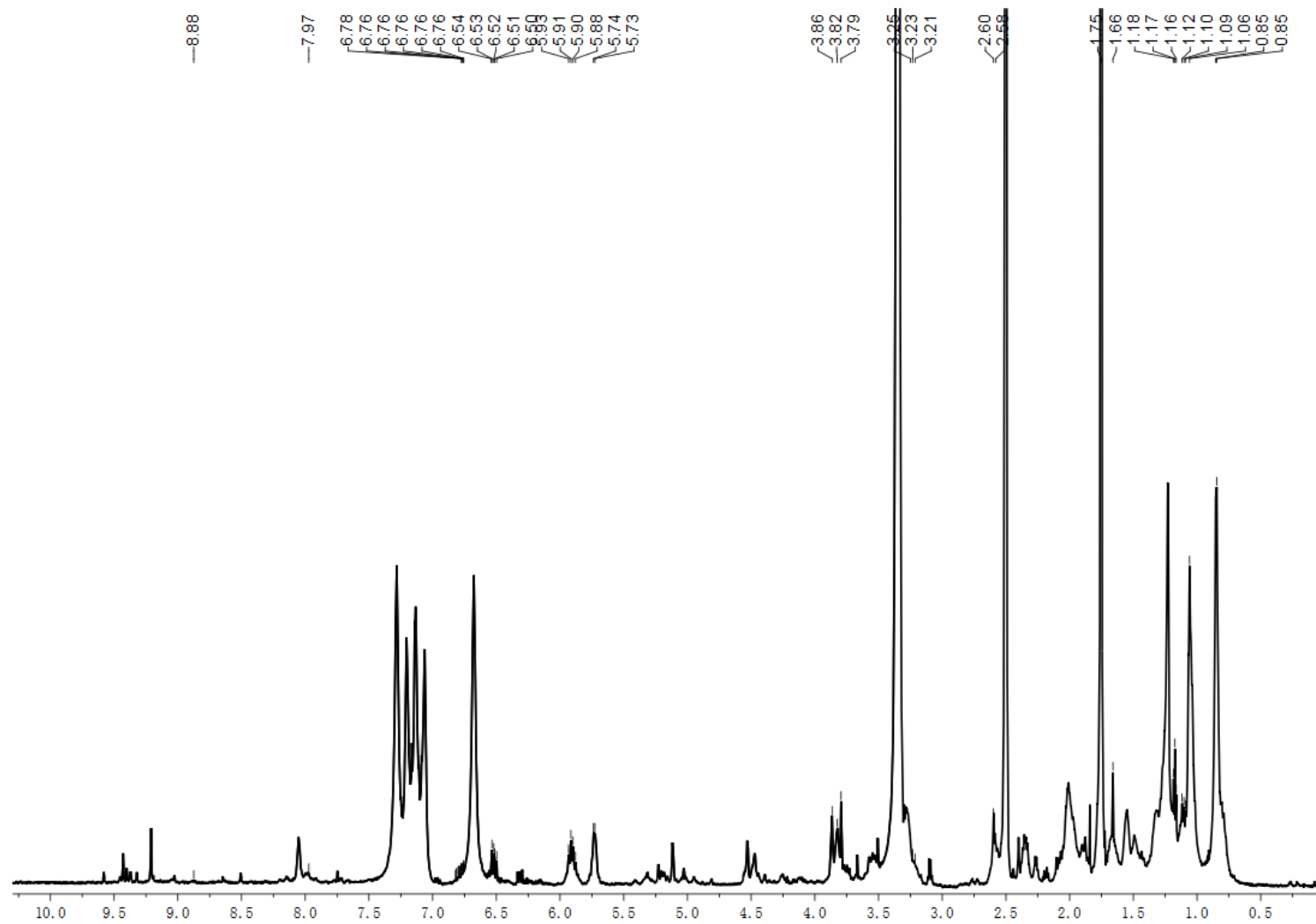




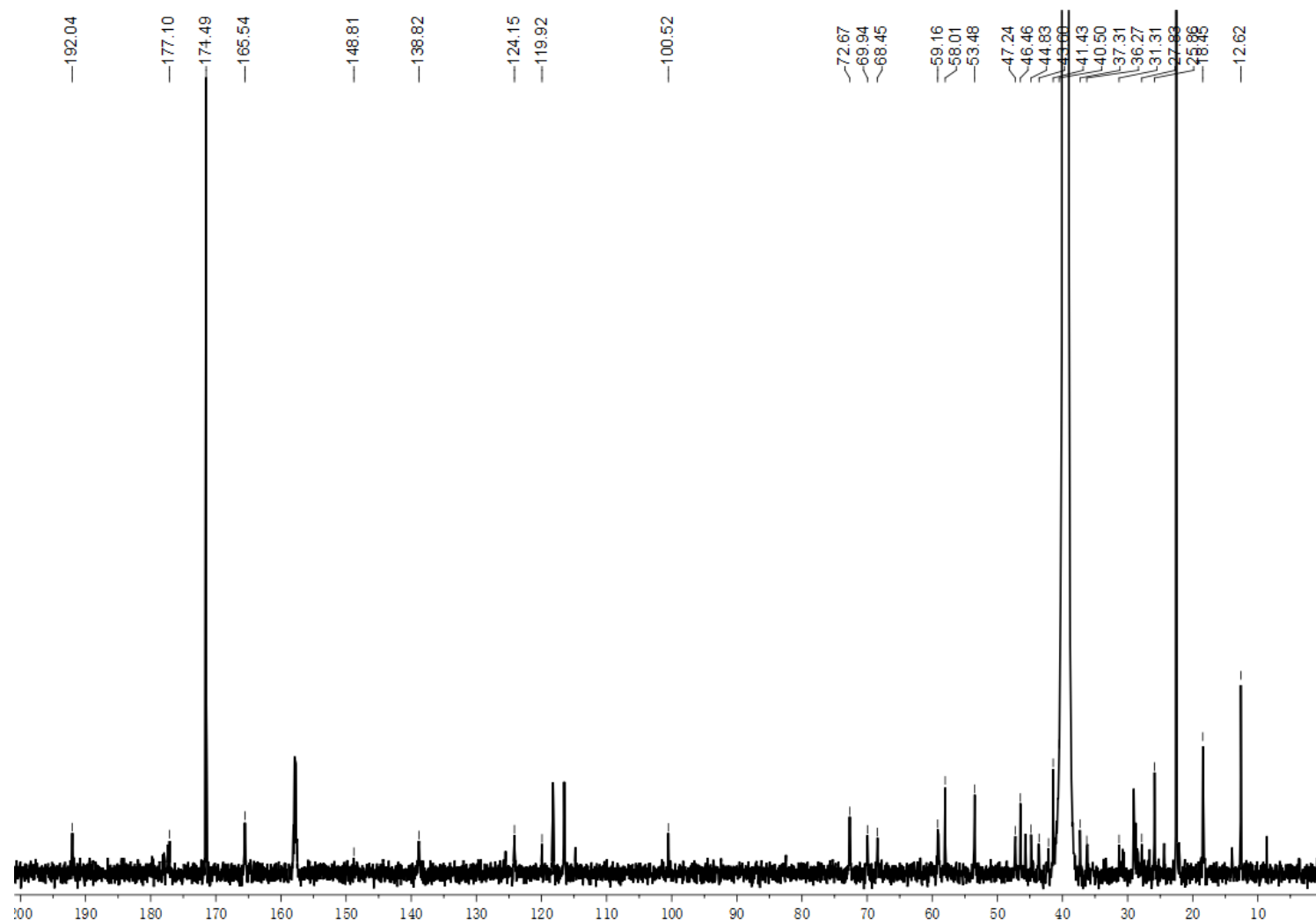
**Figure S4.** HRESIMS spectrum of compound 1.



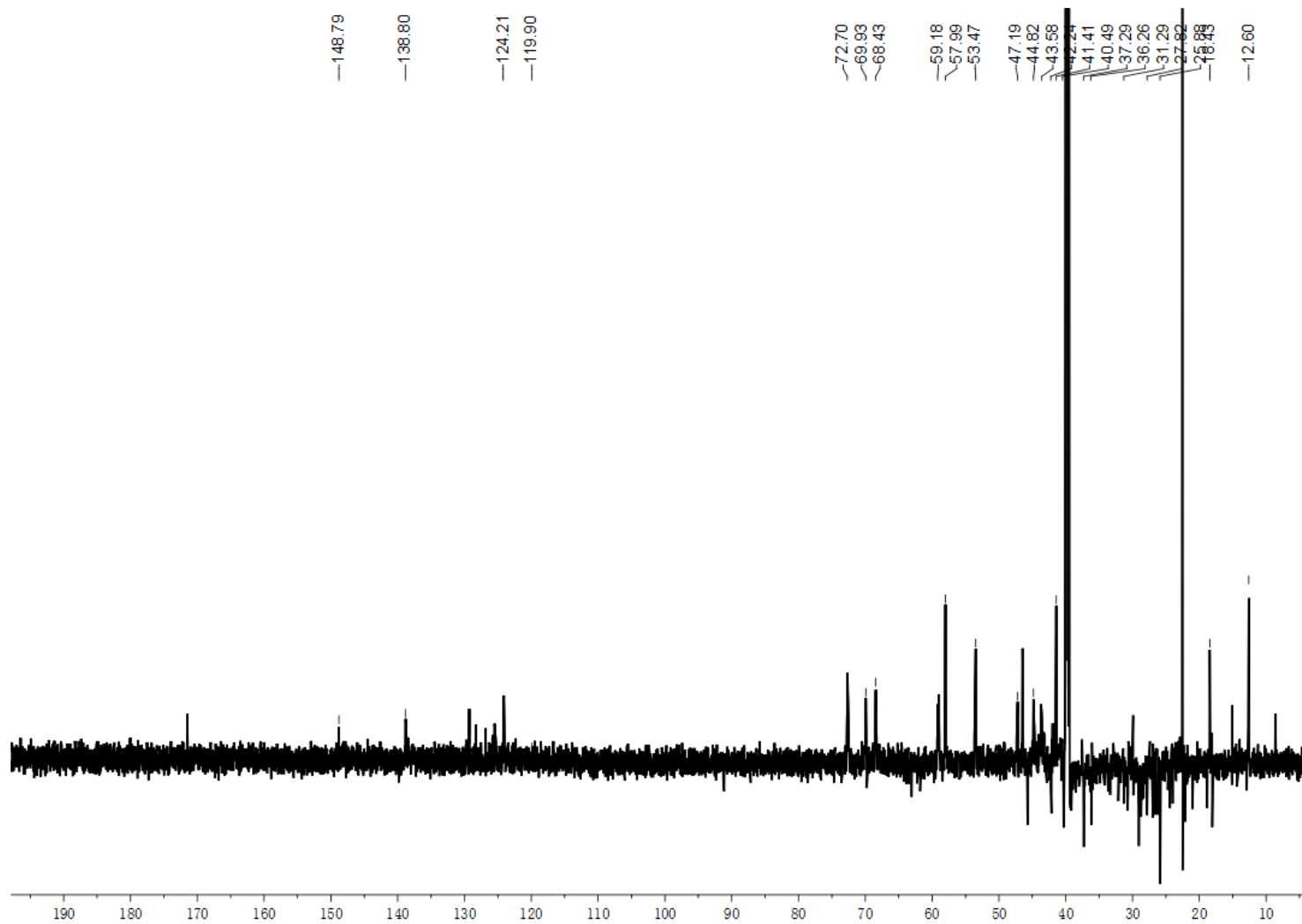
**Figure S5.**  $^1\text{H}$  NMR spectrum of **1** in  $\text{DMSO-}d_6$ .



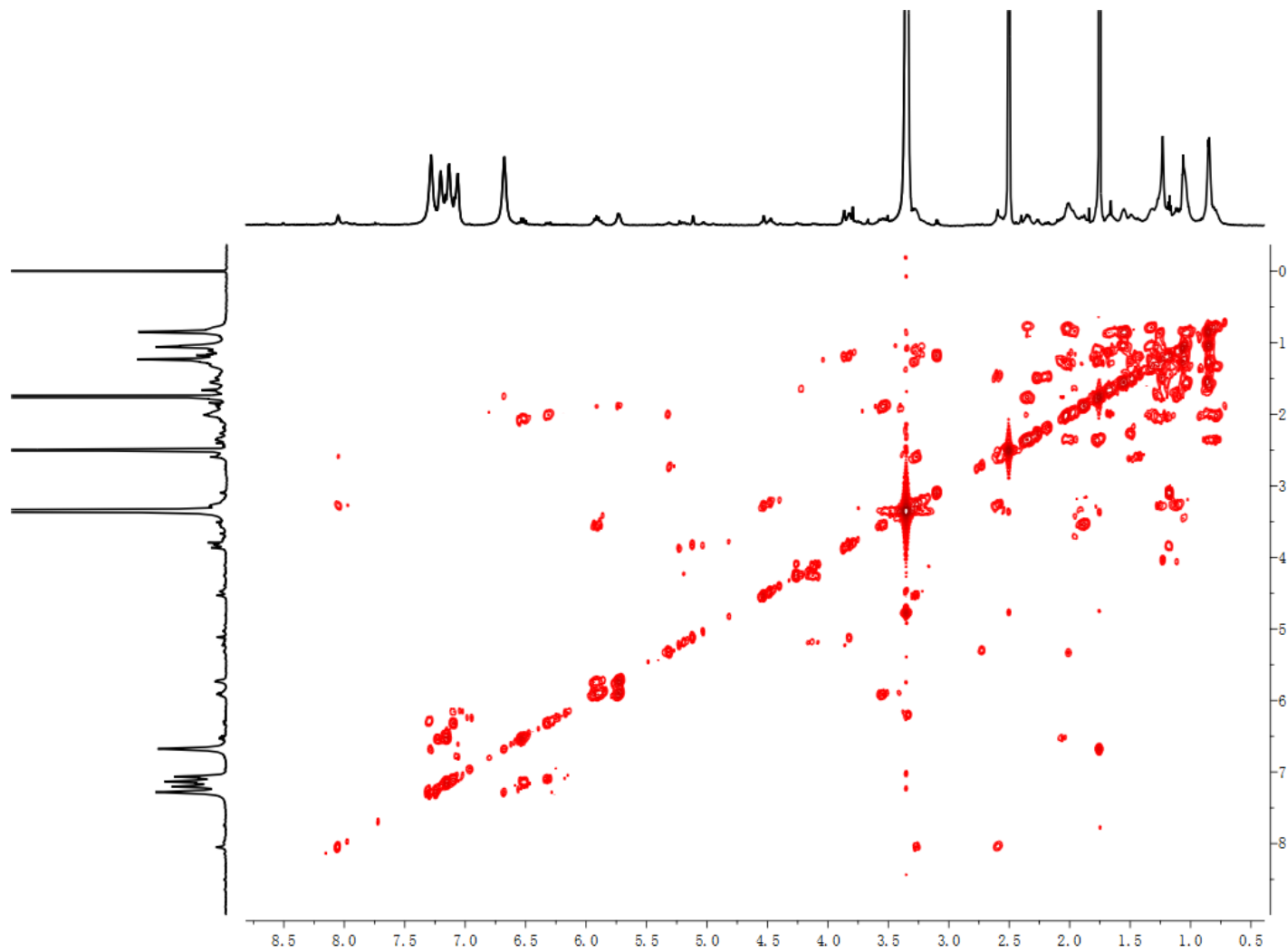
**Figure S6.**  $^{13}\text{C}$  NMR spectrum of **1** in  $\text{DMSO-}d_6$ .



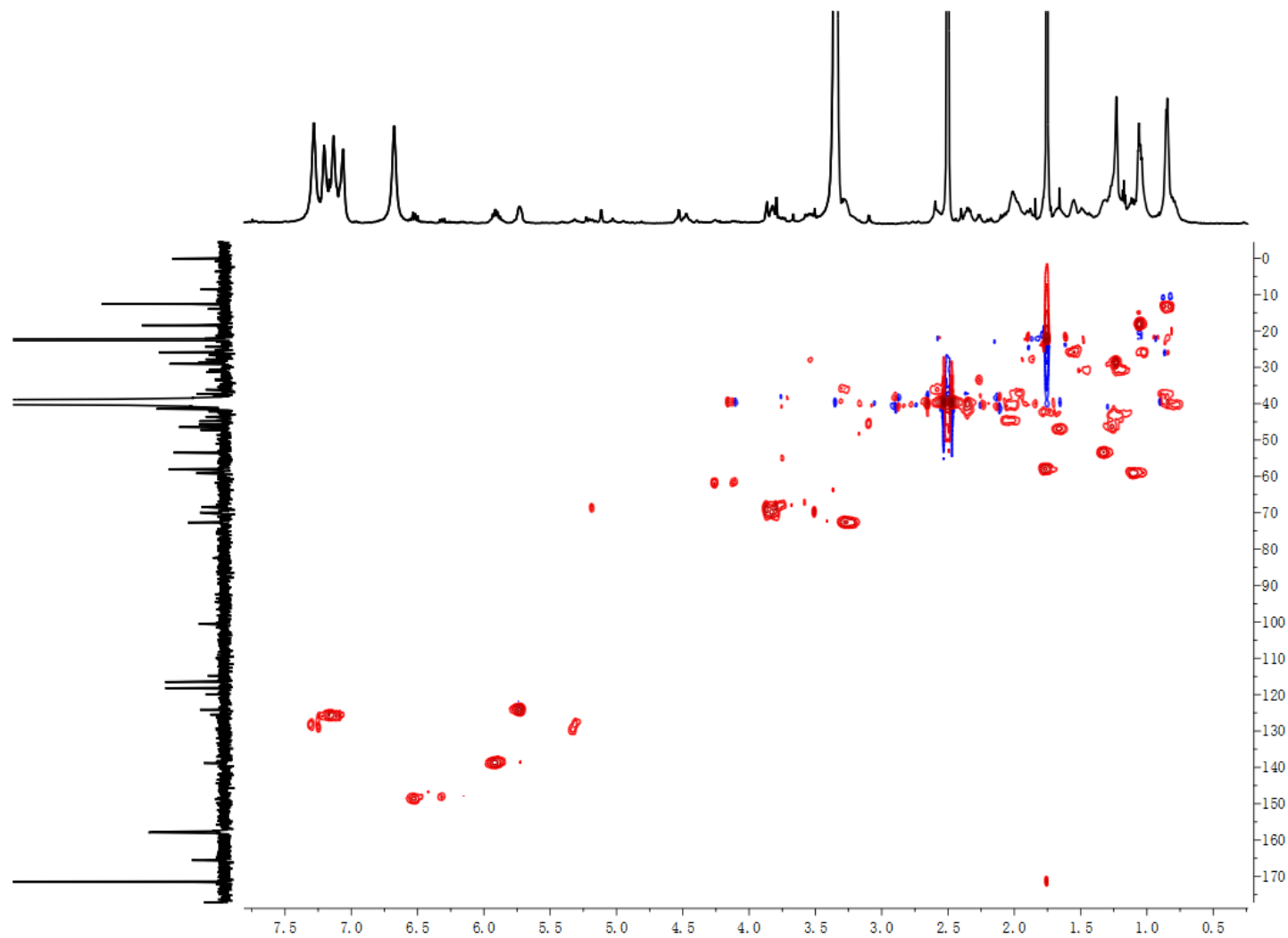
**Figure S7.**  $^{13}\text{C}$  DEPT spectrum of **1** in DMSO-*d*<sub>6</sub>.



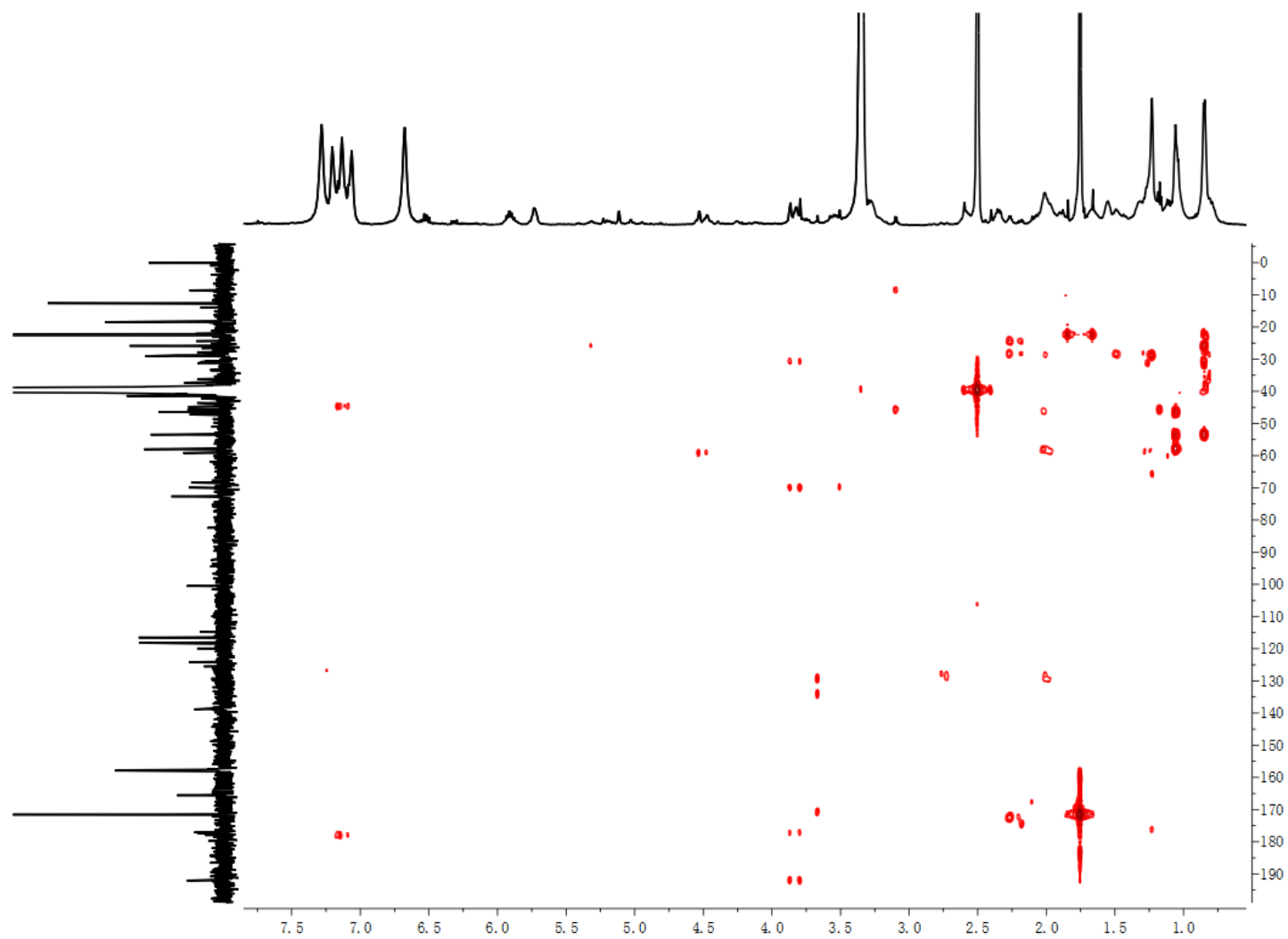
**Figure S8.**  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of **1** in  $\text{DMSO-}d_6$ .



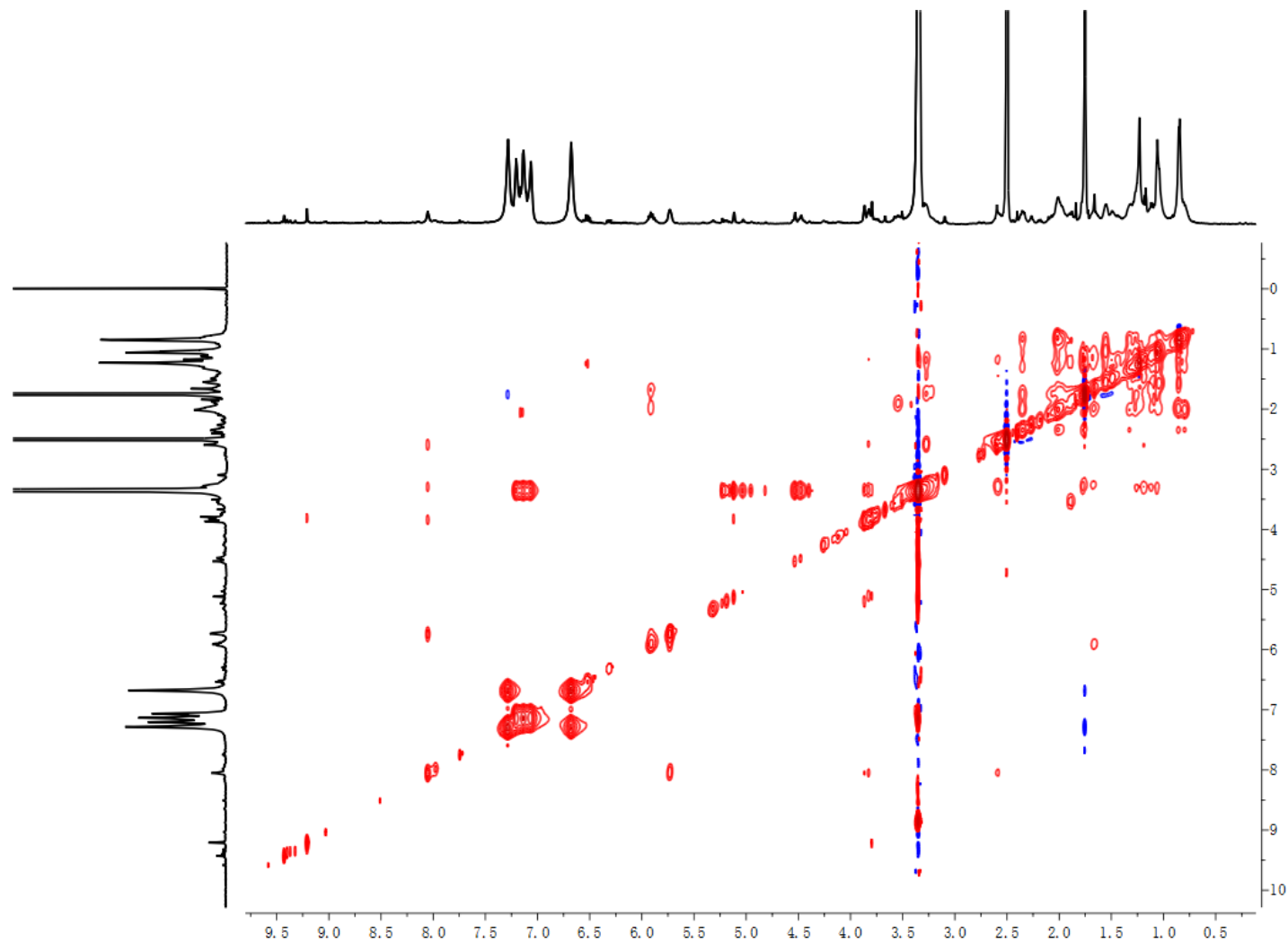
**Figure S9.** HSQC spectrum of **1** in DMSO-*d*<sub>6</sub>.



**Figure S10.** HSBC spectrum of **1** in DMSO-*d*<sub>6</sub>.

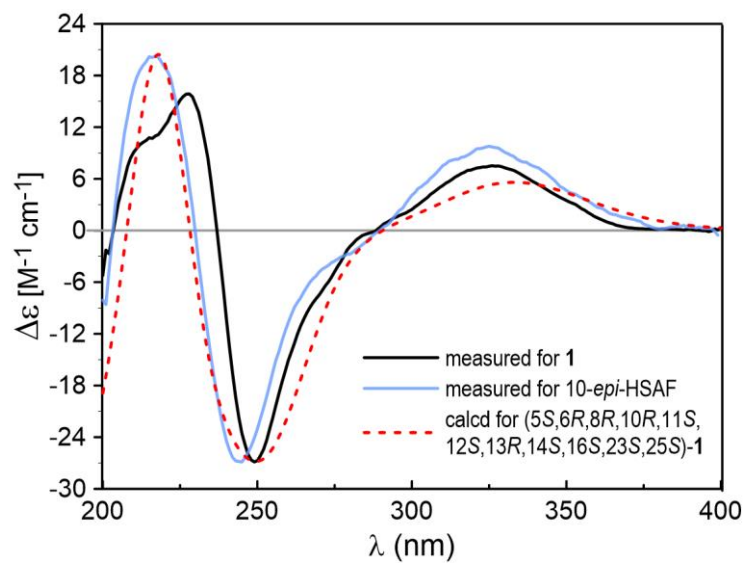


**Figure S11.** NOESY spectrum of **1** in DMSO-*d*<sub>6</sub>.

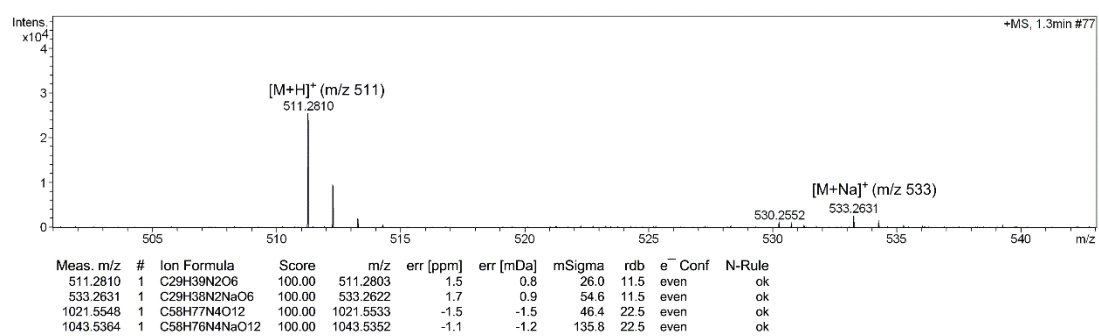




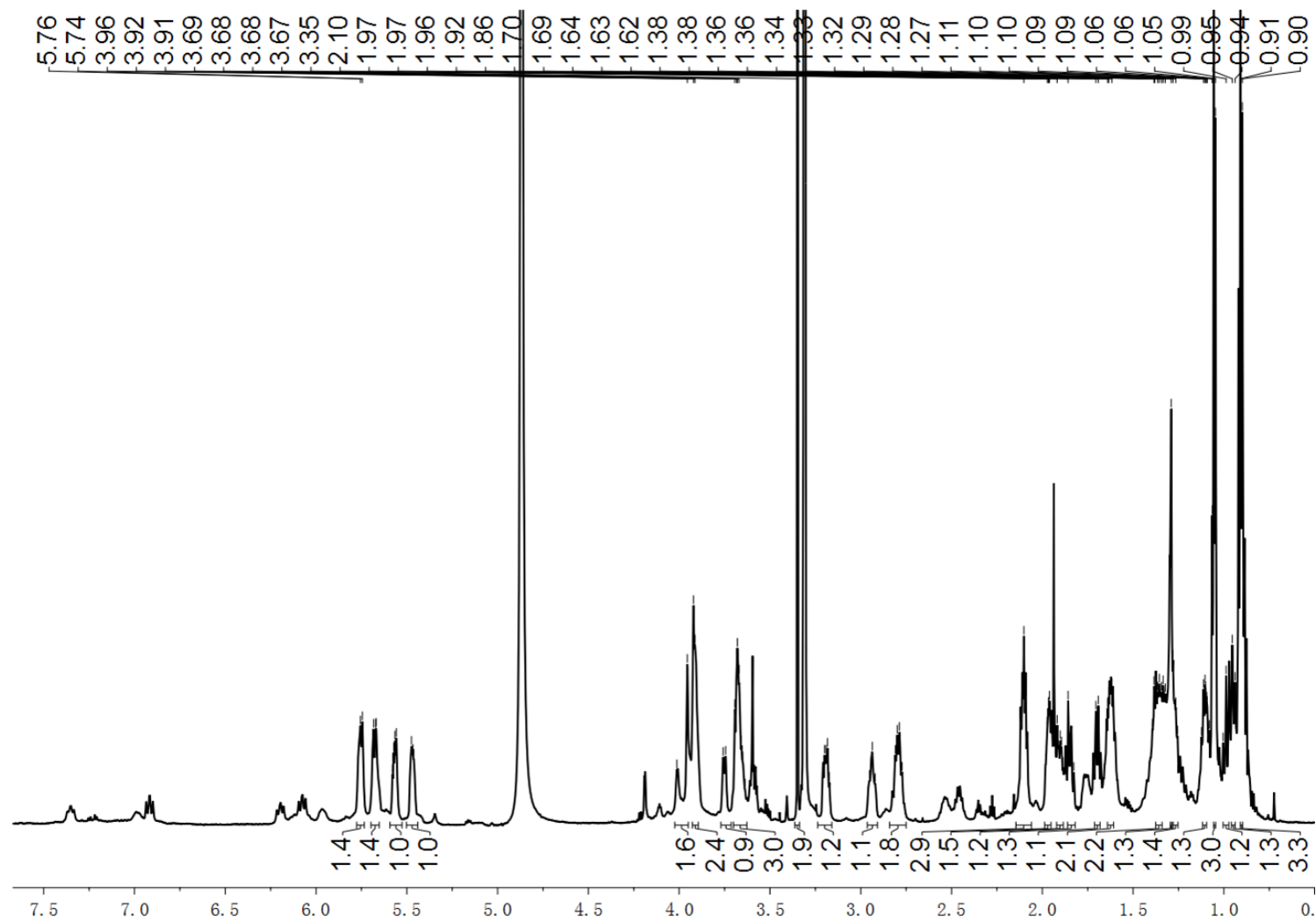
**Figure S12.** Comparison among the ECD spectra measured for compound **1** and 10-*epi*-HSAF and calculated for (5*S*,6*R*,8*R*,10*R*,11*S*,12*S*,13*R*,14*S*,16*S*,23*S*,25*R*)-**1**.



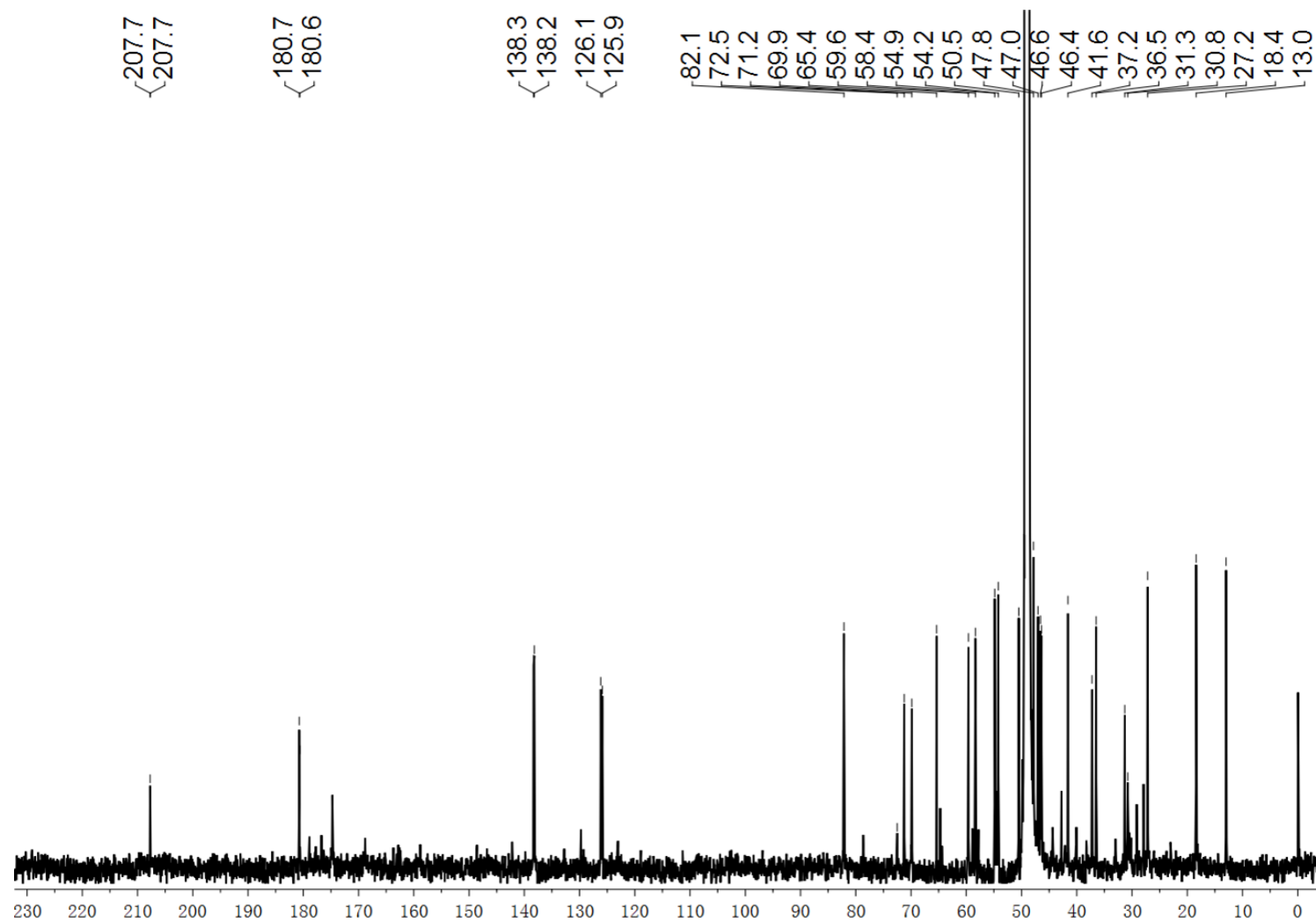
**Figure S13.** HRESIMS spectrum of **2**.



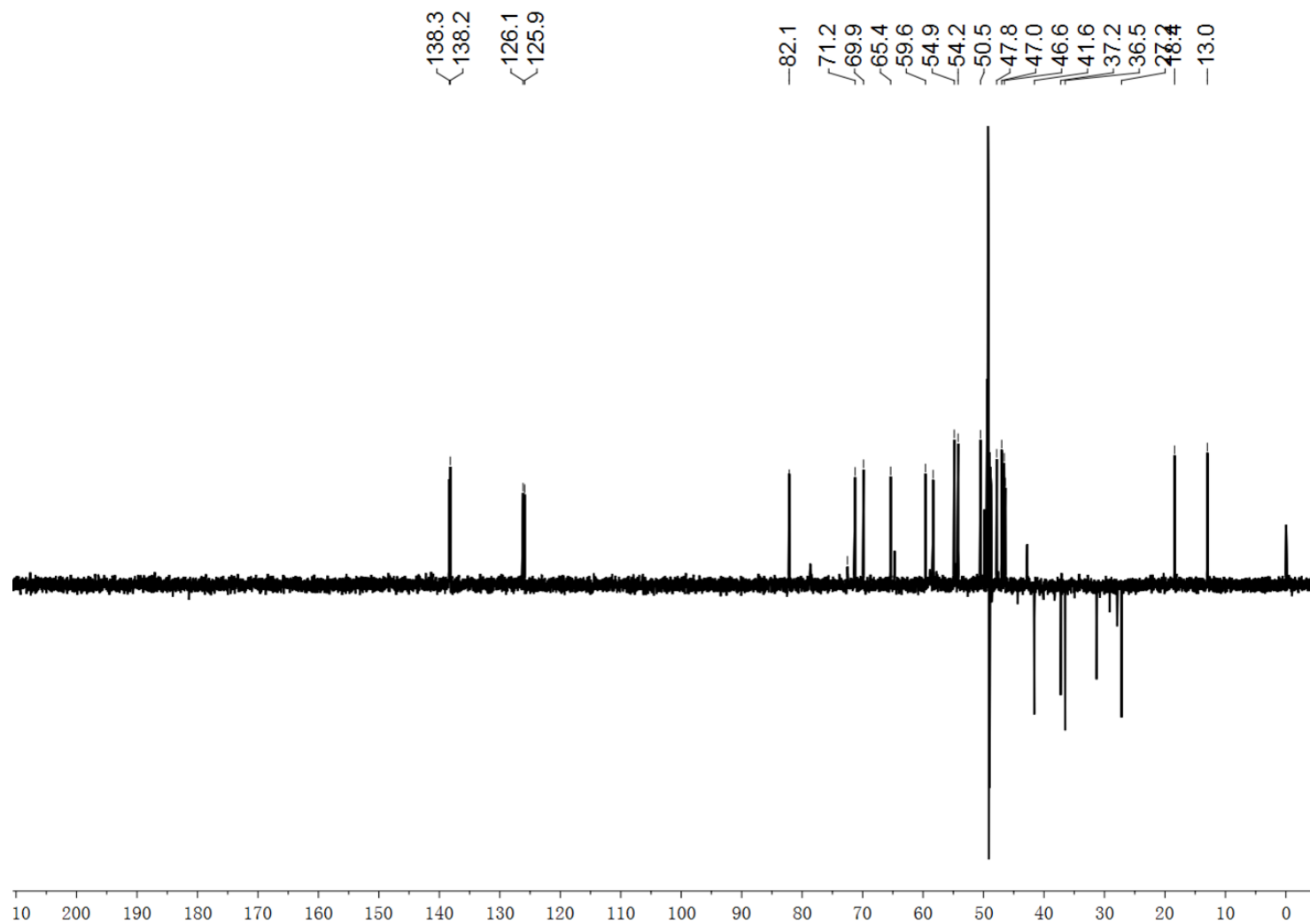
**Figure S14.**  $^1\text{H}$  NMR spectrum of **2** in  $\text{MeOH-}d_4$ .



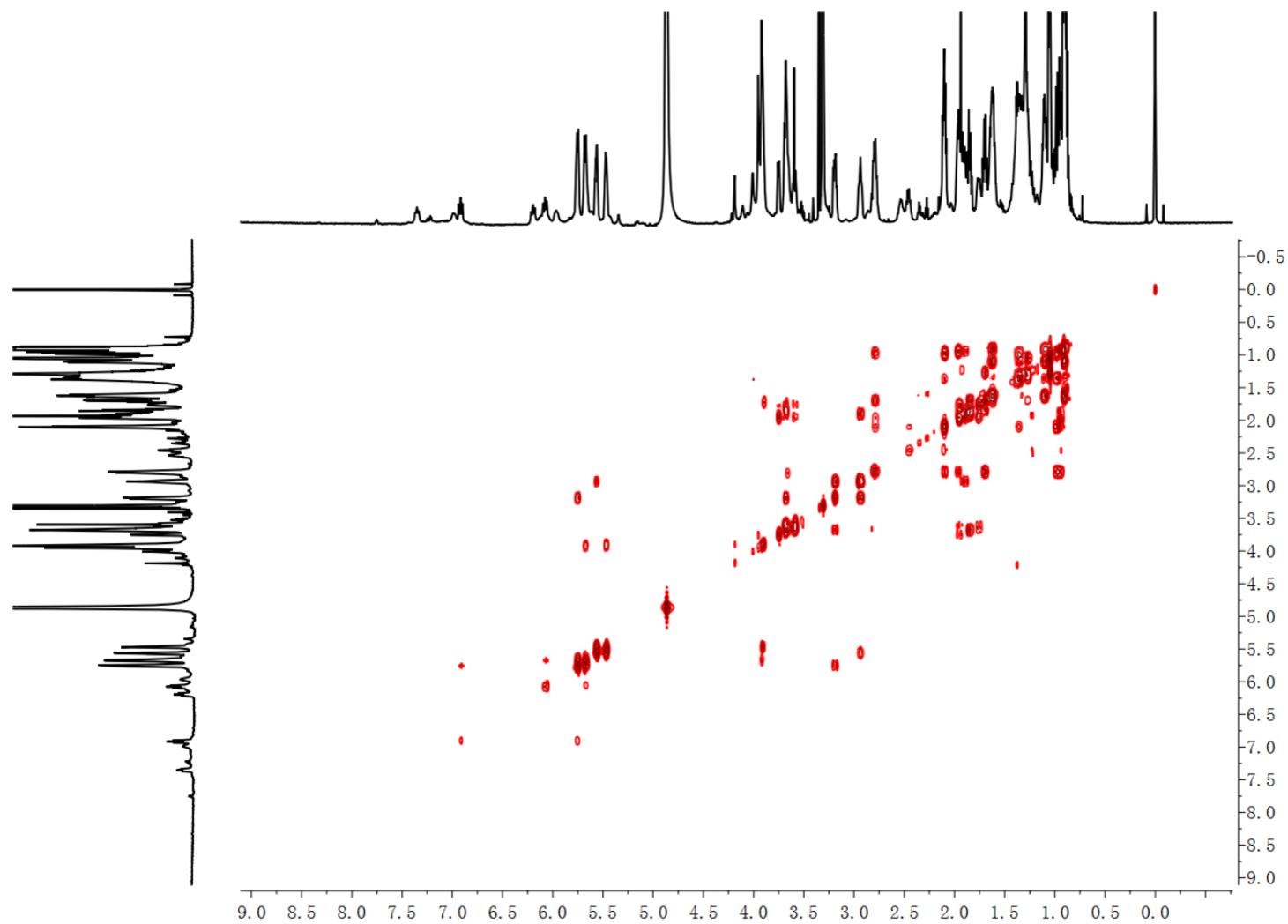
**Figure S15.**  $^{13}\text{C}$  NMR spectrum of **2** in  $\text{MeOH-}d_4$ .



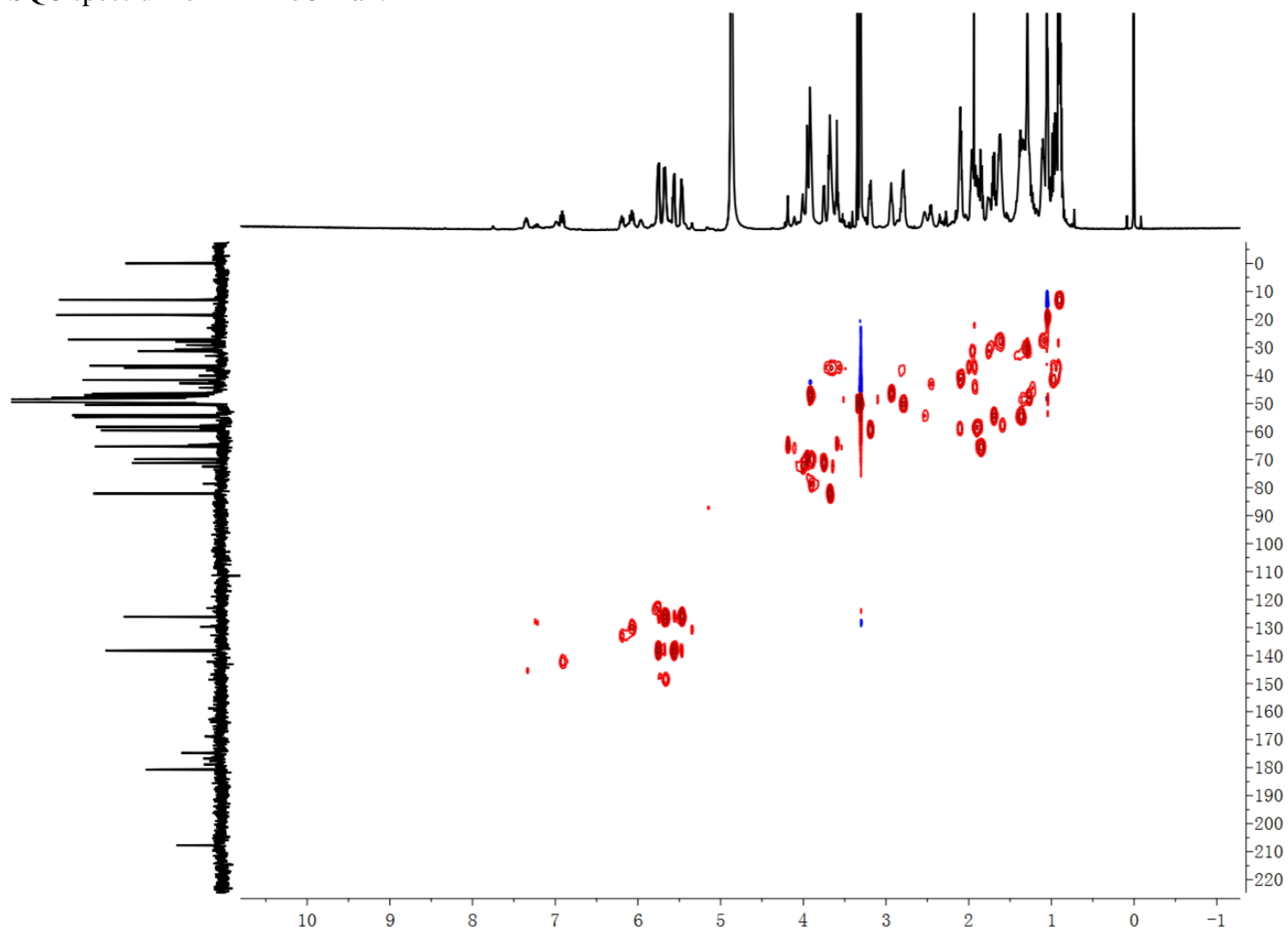
**Figure S16.**  $^{13}\text{C}$  DEPT spectrum of **2** in  $\text{MeOH-}d_4$ .



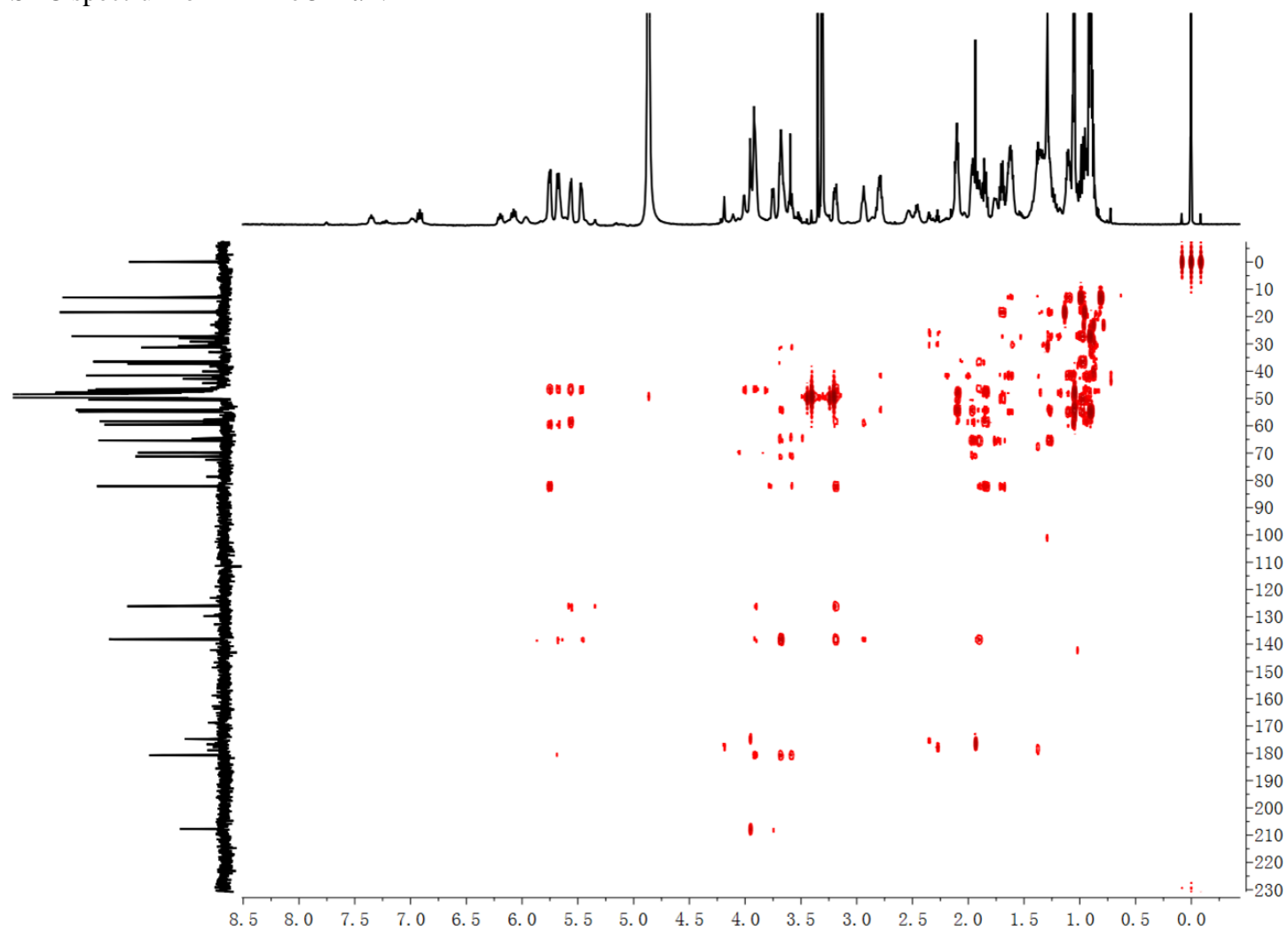
**Figure S17.**  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of **2** in  $\text{MeOH-}d_4$ .



**Figure S18.** HSQC spectrum of **2** in MeOH-*d*<sub>4</sub>.

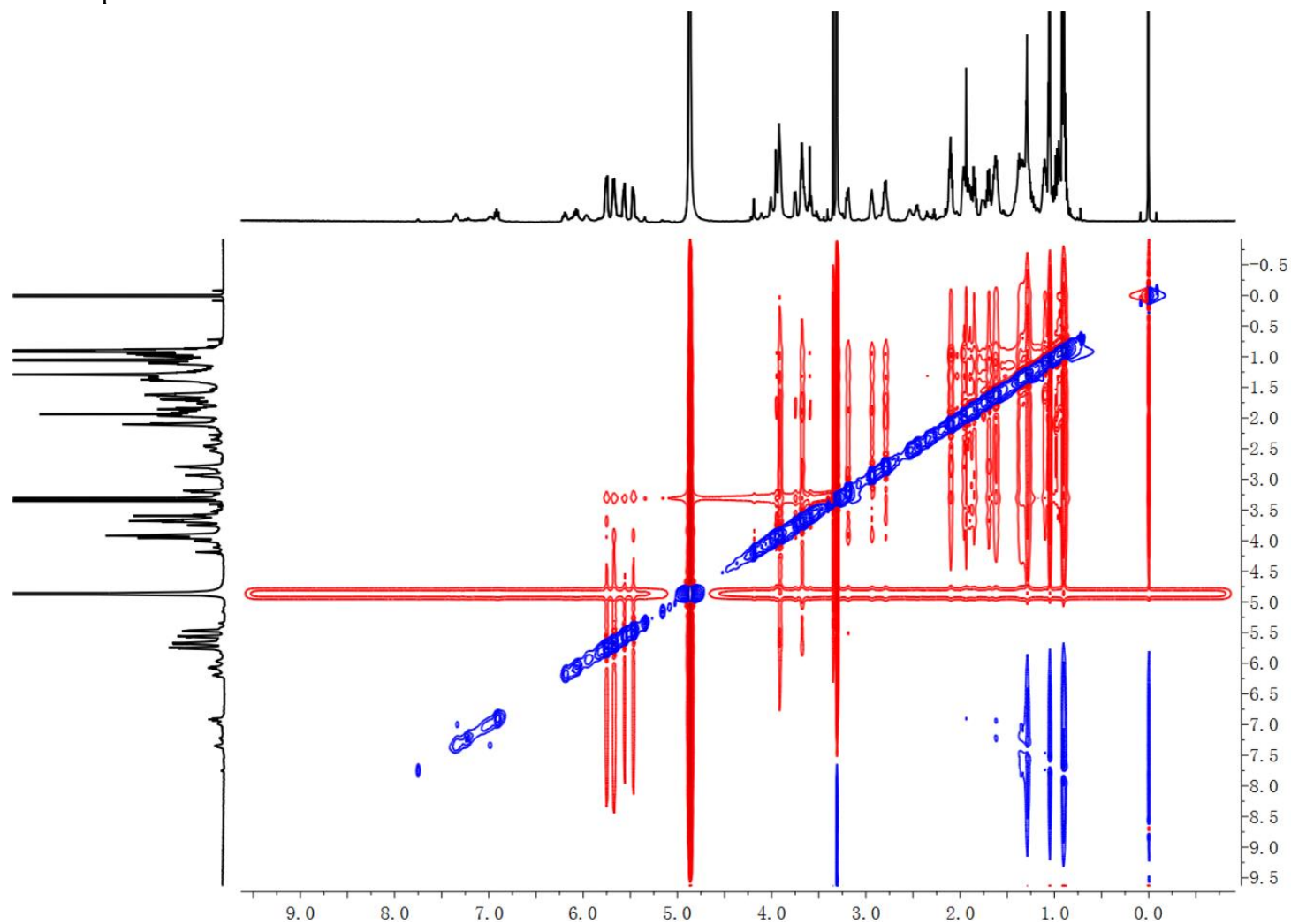


**Figure S19.** HSBC spectrum of **2** in MeOH-*d*<sub>4</sub>.

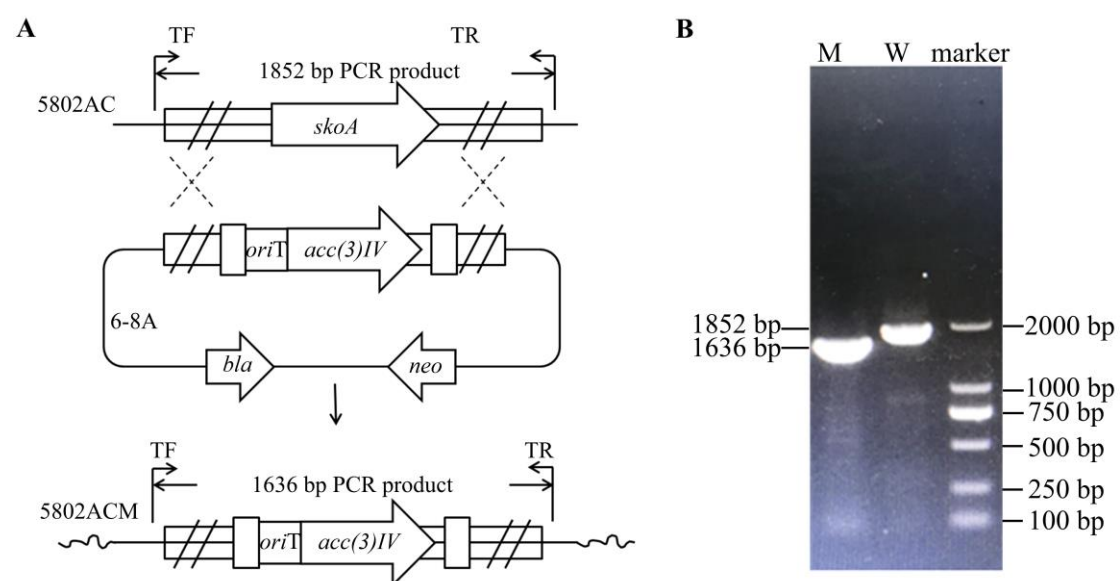




**Figure S20.** NOESY spectrum of **2** in MeOH-*d*<sub>4</sub>.



**Figure S21.** *skoA* disruption in *S. koyangensis* SCSIO 5802AC via PCR-targeting.



### **Supplemental References.**

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- [2] Datsenko, K. A.; Wanner, B. L. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6640-6645.
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- [4] Paget, M. S. B., Chamberlin, L.; Atrih, A.; Foster, S. J.; Buttner, M. J. *J. Bacteriol.* **1999**, *181*, 204-211.