

## Supplementary Note

### Supplementary Note 1

#### Supplementary Note 1.1

UPLC-MS detection of *N*-acetylcysteamine (SNAC) thioesters P2

#### Supplementary Note 1.2

Structure characterization data of compounds **12a** and **12b** P3

#### Supplementary Note 1.3

Structure characterization data of compound **13** P6

#### Supplementary Note 1.4

Structure characterization data of compound **14** P13

### Supplementary Note 2

Structure characterization data of compound **10** P22

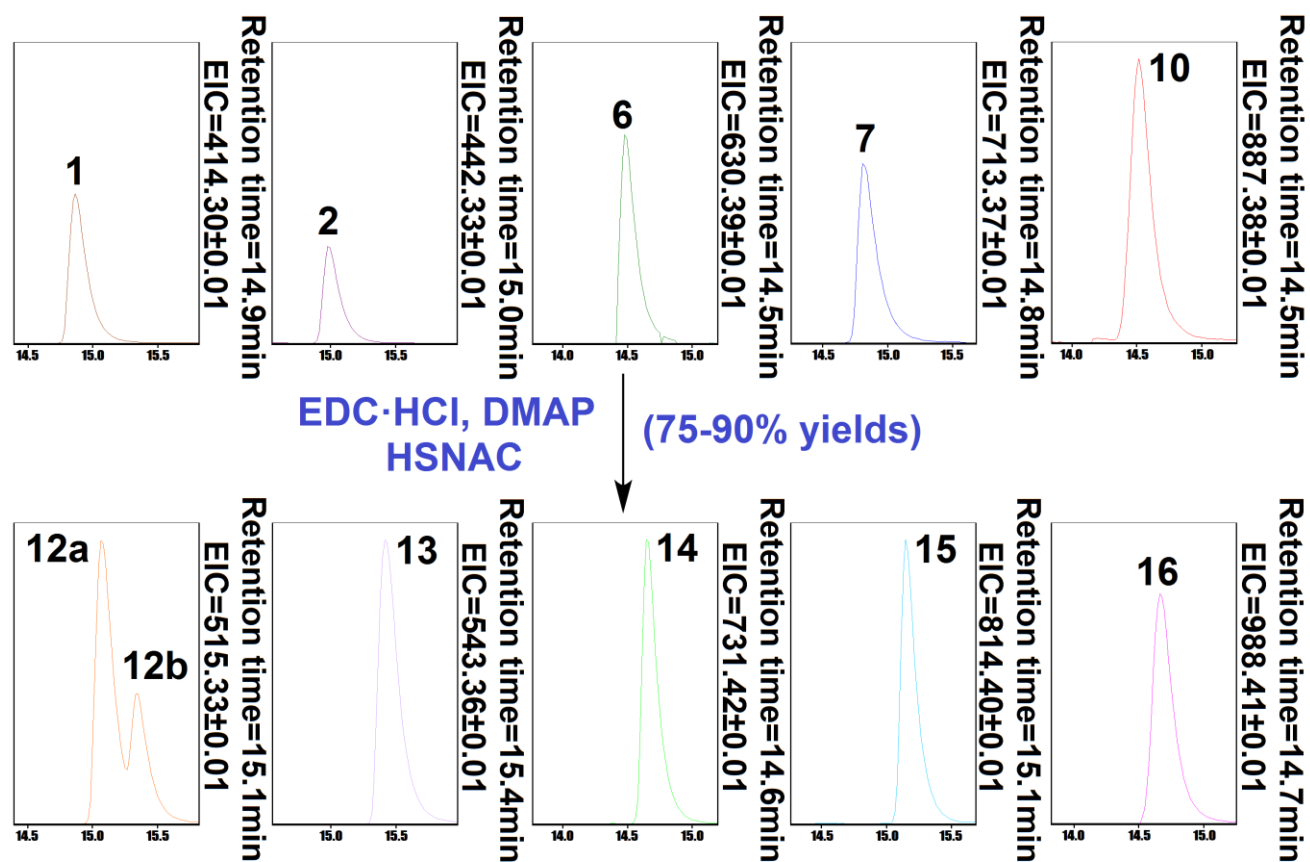
### Supplementary Note 3

Structure characterization data of compound **6** P39

## Supplementary Note 1

### Supplementary Note 1.1

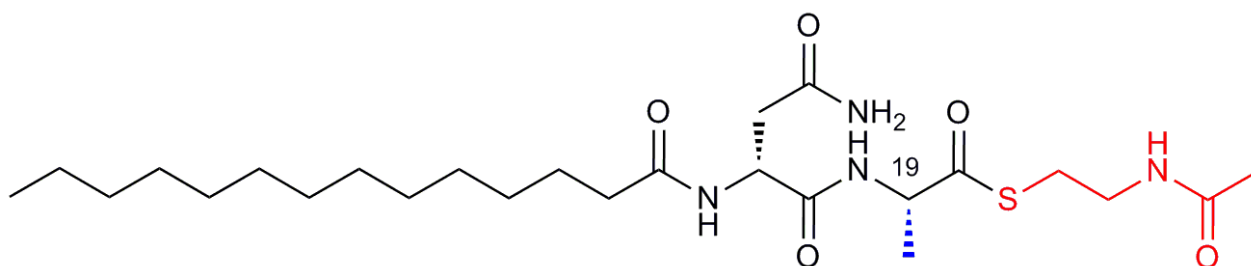
UPLC-MS detection of *N*-acetylcysteamine (SNAC) thioesters



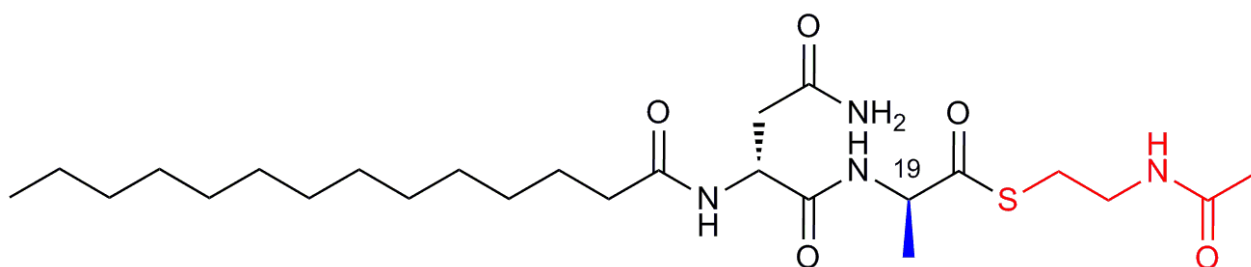
UPLC-MS extracted ion chromatograms of compounds **1**, **2**, **6**, **7**, **10** and their corresponding *N*-acetylcysteamine (SNAC) thioesters, **12–16**, synthesized via EDC/DMAP coupling reactions.

## Supplementary Note 1.2

Structure characterization data of compounds **12a** and **12b**

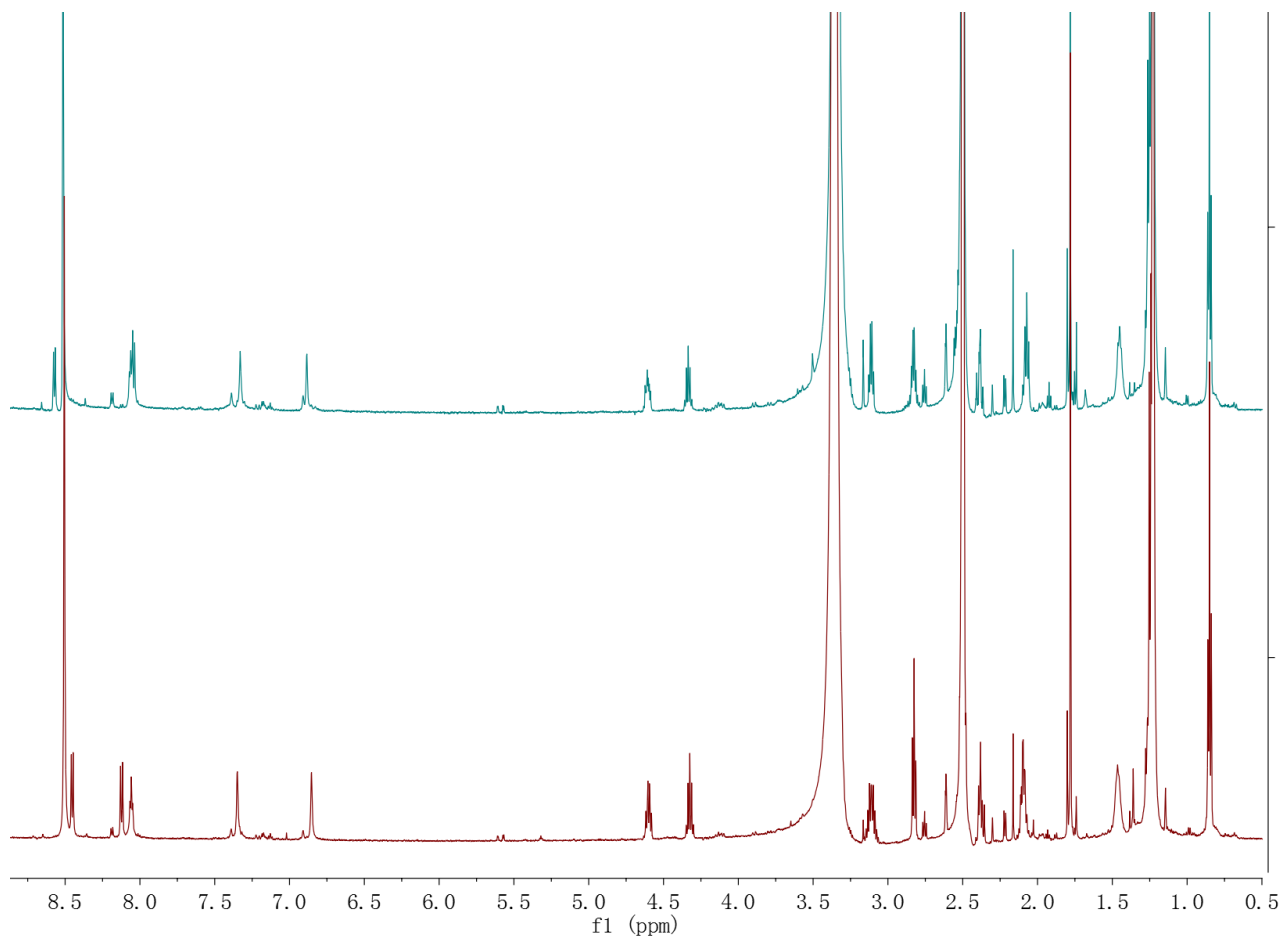


**12a**

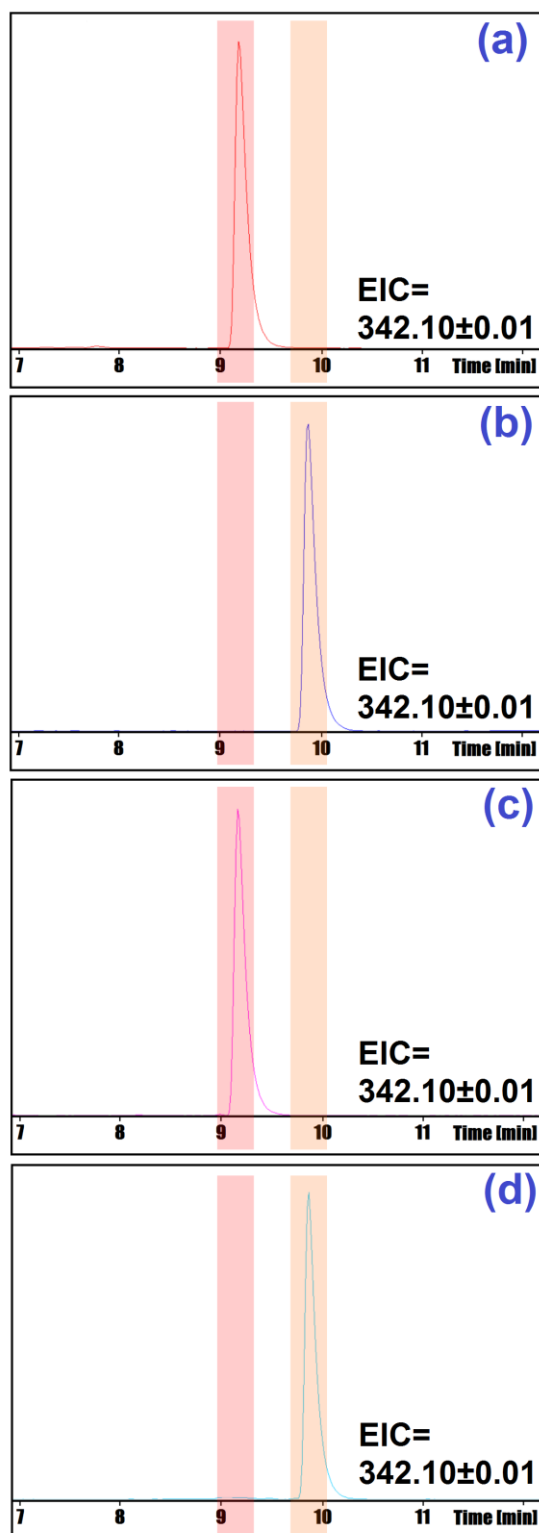


**12b**

The chemical structures of two *N*-acetylcysteamine (SNAC) thioester diastereomers (**12a** and **12b**) of compound **1** (precolibactin-413).



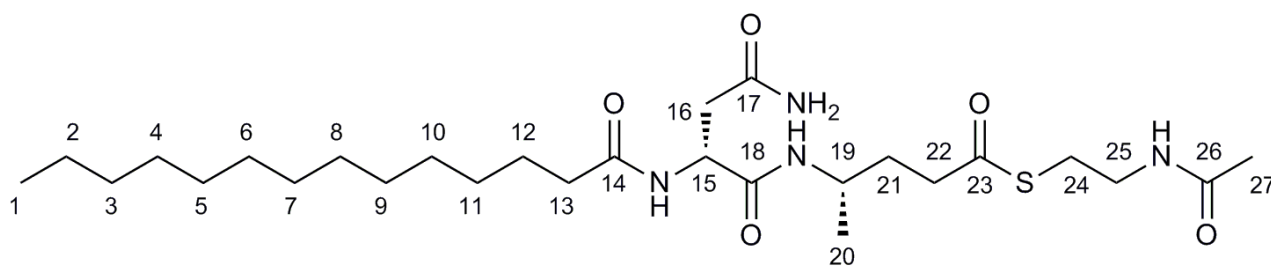
Overlay of the  $^1\text{H}$  NMR spectra of **12a** and **12b**. The spectra were recorded in  $\text{DMSO-}d_6$  at 600 MHz. (Maroon = **12a**; Teal = **12b**).



Marfey's analysis to determine the alanine configurations in **12a** and **12b**. The retention times of DNPA (2,4-dinitrophenyl-5-L-alaninamide)-standard L-Ala and DNPA-standard D-Ala were 9.1 min (a) and 9.9 min (b), respectively. Based on the retention times of DNPA-**12a** Ala [9.1 min, (c)] and DNPA-**12b** Ala [9.9 min (d)], the configurations of alanine in **12a** and **12b** were determined as L and D, respectively.

## Supplementary Note 1.3

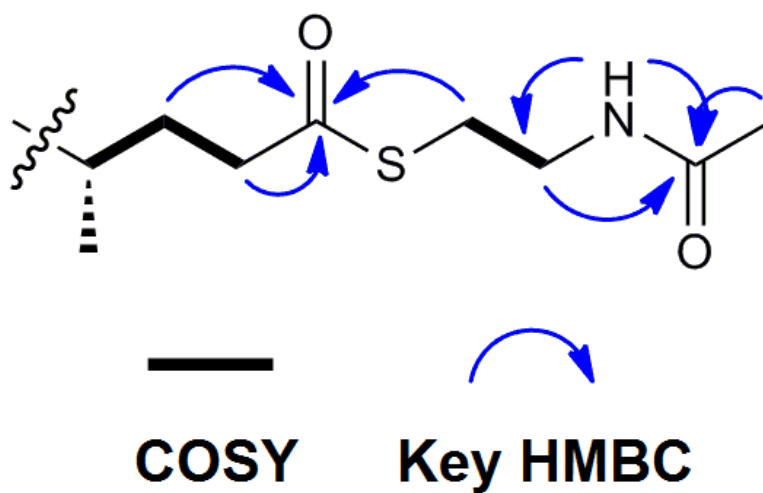
### Structure characterization data of compound **13**



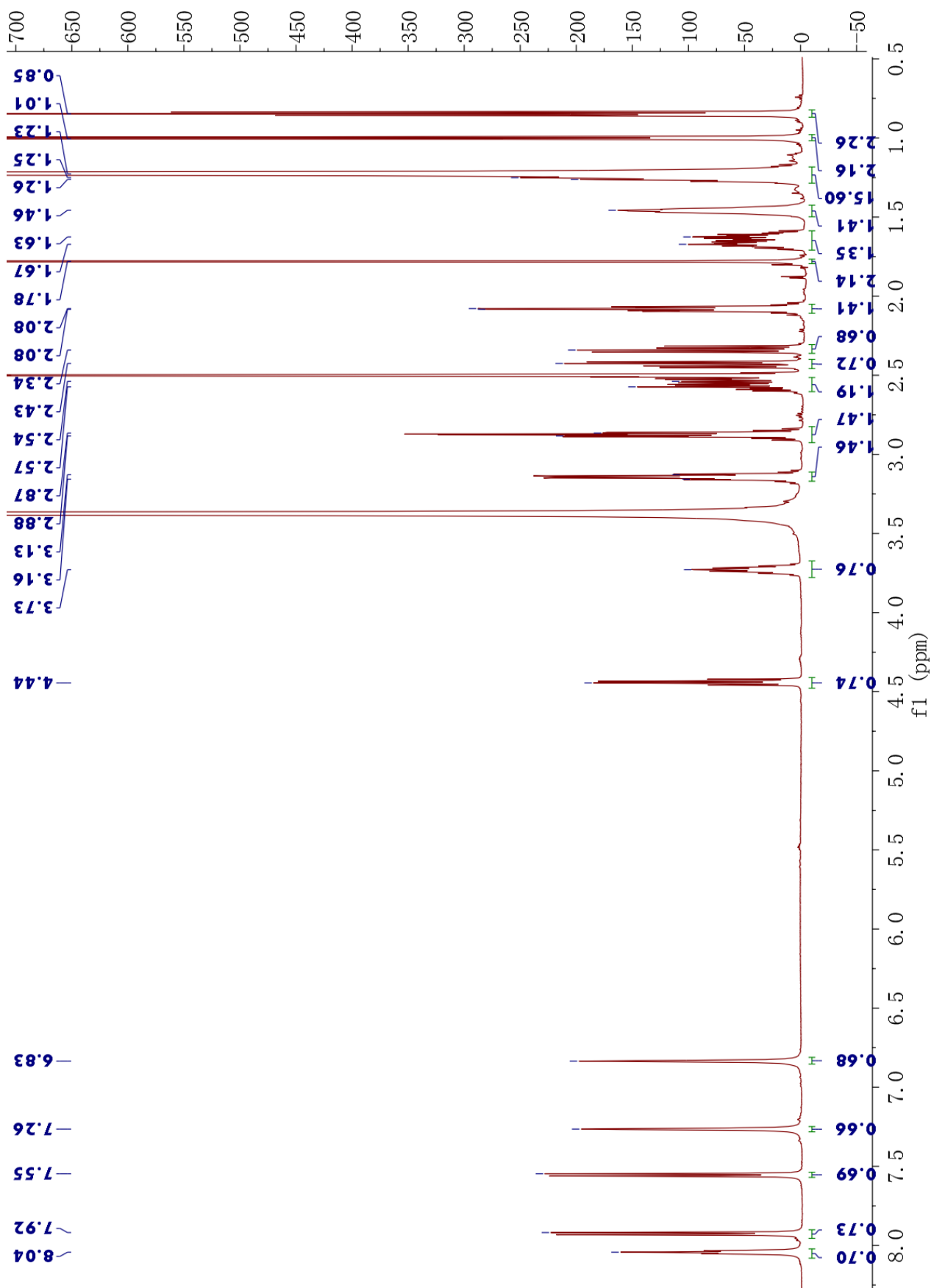
NMR spectroscopic data for **13** in DMSO-*d*<sub>6</sub>.

C	$\delta_C$	$\delta_H$ (J in Hz)	COSY	HMBC ( $^1H$ to $^{13}C$ )
1	14.1	0.85 t (6.6)	2	2, 3
2	22.6	1.25 m	1	1, 3
3	31.4	1.23-1.25 m		
4-10	28.8, 29.0, 29.1, 29.2, 29.2, 29.2	1.23-1.25 m		
11	28.8	1.23-1.25 m		
12	25.4	1.46 m	13a, 13b	11, 13, 14
13	35.4	a 2.08 m b 2.08 m	12, 13b 12, 13a	11, 12, 14 11, 12, 14
14	172.3	- NH, 7.92 d (8.4)	15	14, 15, 16
15	50.0	4.44 m	14NH, 16a, 16b	16, 18
16	37.4	a 2.34 dd (8.4, 15.6) b 2.43 dd (6.0, 15.0)	15, 16b 15, 16a	15, 17 15, 17
17	171.5	- a NH, 6.83 s b NH, 7.26 s	17bNH 17aNH	16, 17 17
18	170.6	- NH, 7.55 d (8.4)	19	18, 19, 20, 21
19	43.8	3.73 m	18NH, 20, 21	18, 20, 21, 22
20	20.5	1.01 d (6.6)	19	19, 21
21	31.6	a 1.63 m b 1.67 m	19, 21b, 22a, 22b 19, 21a, 22a, 22b	19, 20, 22, 23 19, 20, 22, 23
22	40.5	a 2.54 m b 2.57 m	21a, 21b, 22b 21a, 21b, 22a	19, 21, 23 19, 21, 23
23	198.5	-		

24	28.1	a 2.87 m b 2.88 m	24b, 25a, 25b 24a, 25a, 25b	23, 25 23, 25
25	38.4	a 3.13 m  b 3.16 m	24a, 24b, 25b, 25NH 24a, 24b, 25a, 25NH	24, 26 24, 26
		NH, 8.04 t (5.4)	25a, 25b	25, 26
26	169.4	-		
27	22.2	1.78 s		24, 25, 26

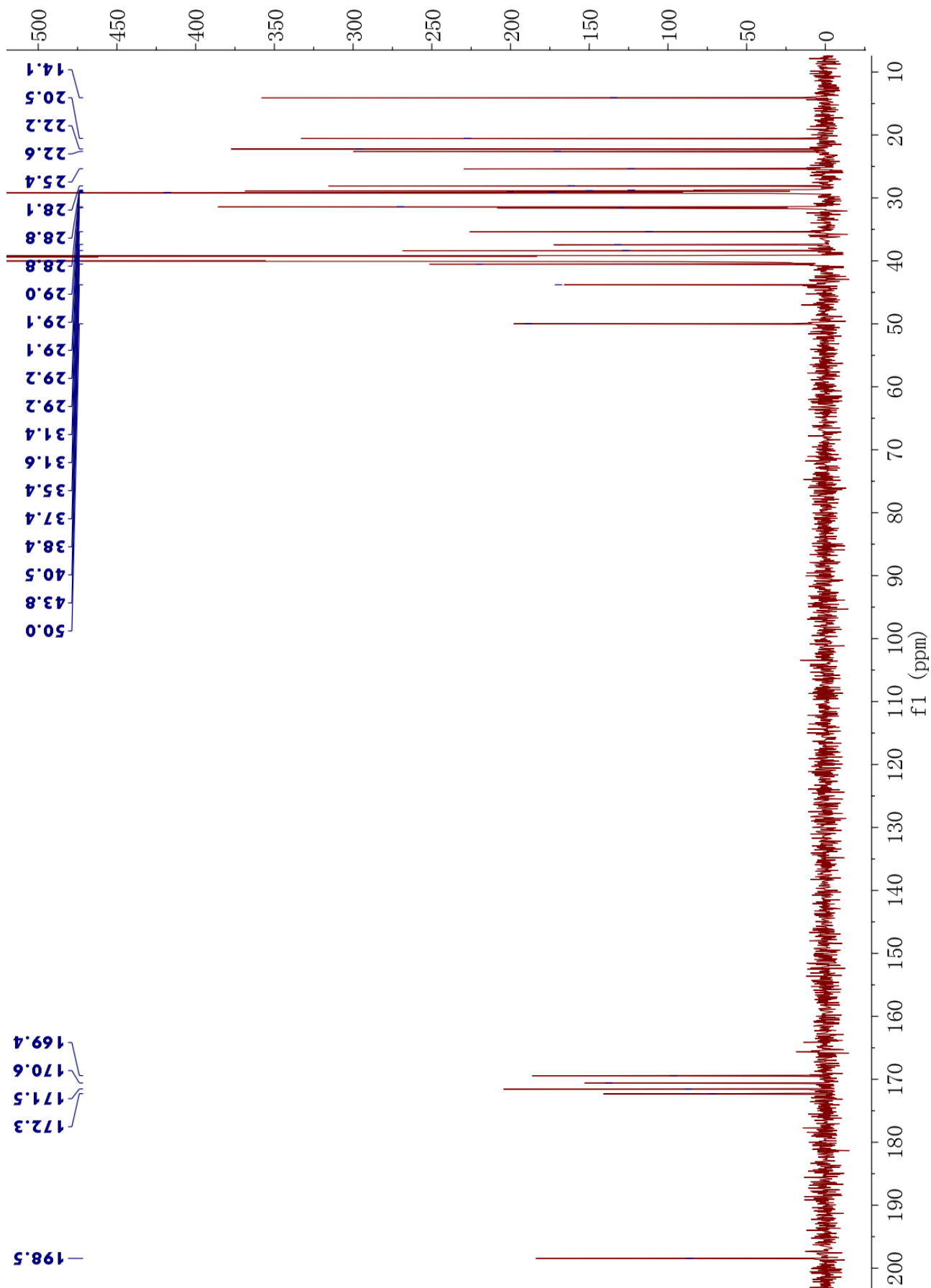


The NMR-based key correlations for the structural assignment of **13**.

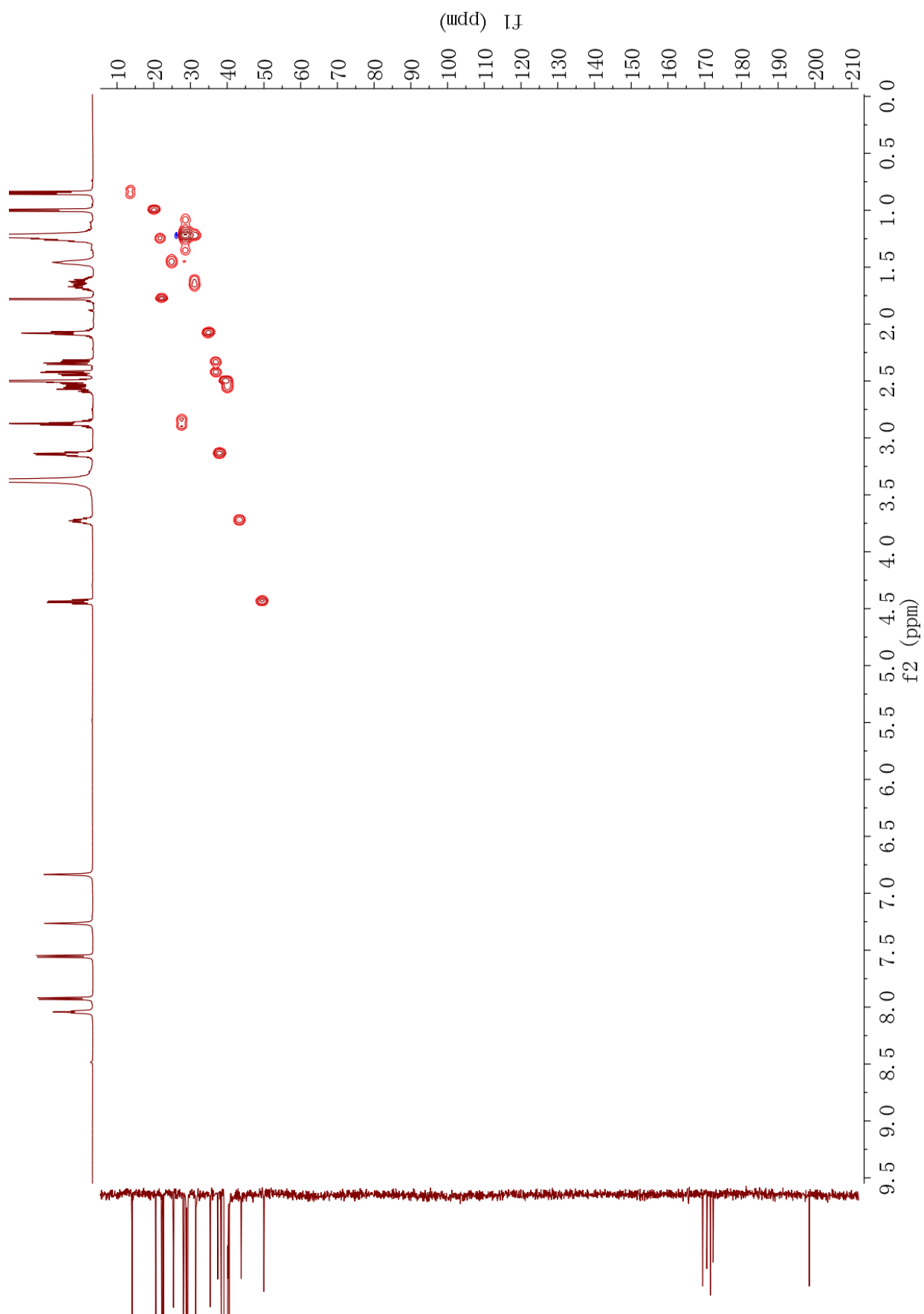


$^1\text{H}$  NMR spectrum of **13** (recorded in  $\text{DMSO}-d_6$  at 600 MHz).

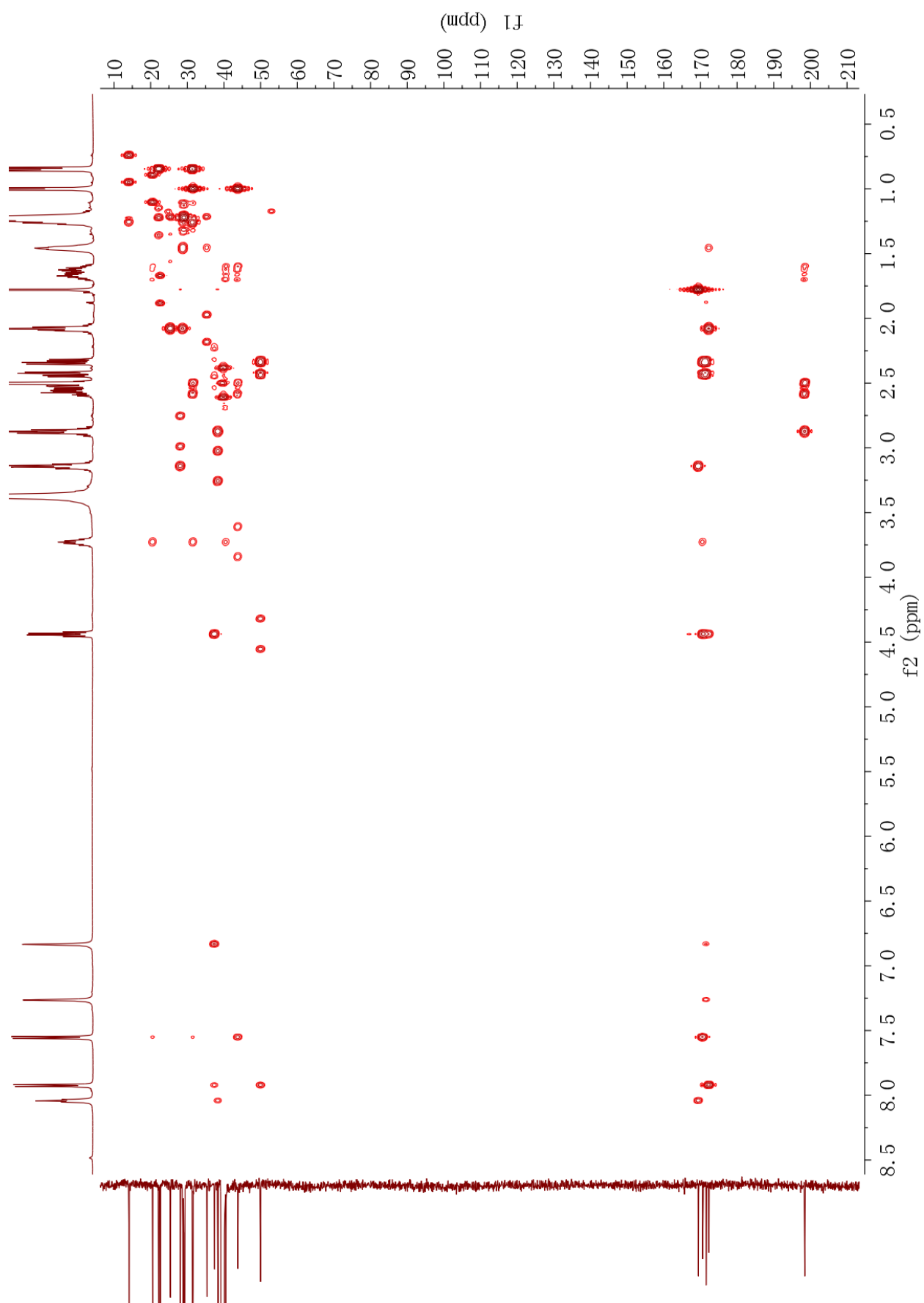




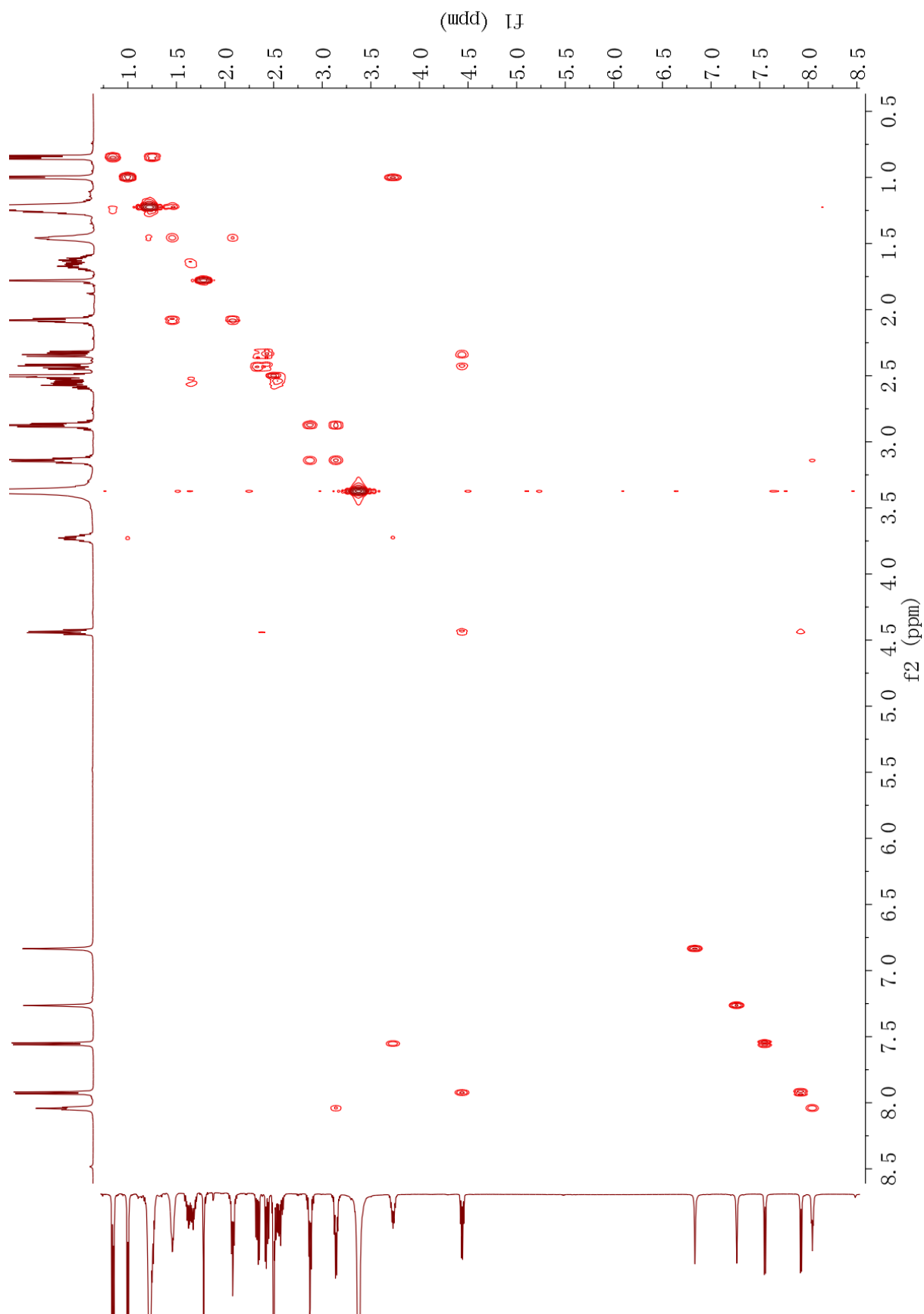
<sup>13</sup>C NMR spectrum of **13** (recorded in DMSO-*d*<sub>6</sub> at 150 MHz).



$^1\text{H}$ - $^{13}\text{C}$  HSQC spectrum of **13** (recorded in  $\text{DMSO-}d_6$  at 600 MHz).



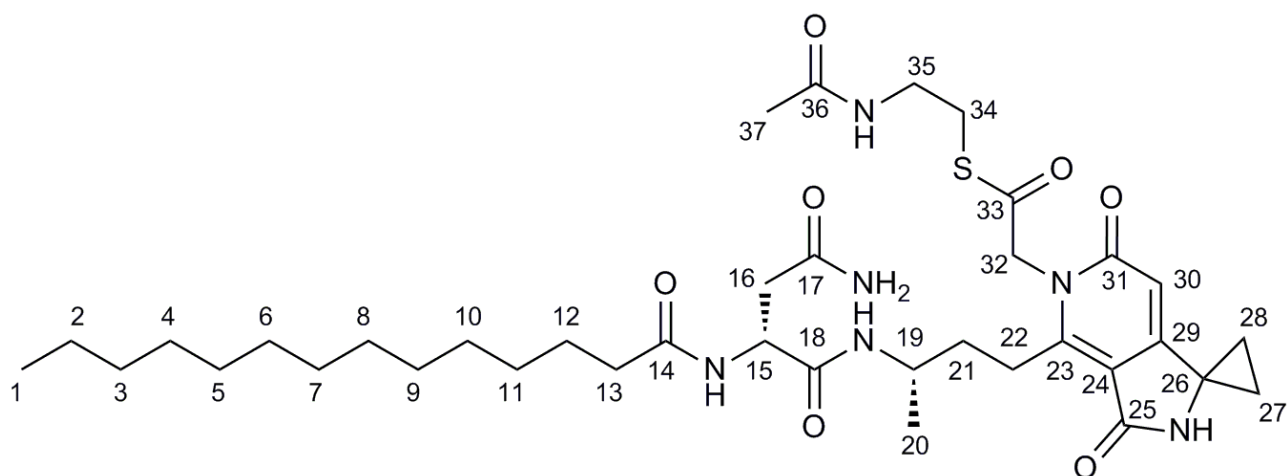
$^1\text{H}$ - $^{13}\text{C}$  HMBC spectrum of **13** (recorded in  $\text{DMSO}-d_6$  at 600 MHz).



$^1\text{H}$ - $^1\text{H}$  COSY spectrum of **13** (recorded in  $\text{DMSO-}d_6$  at 600 MHz).

## Supplementary Note 1.4

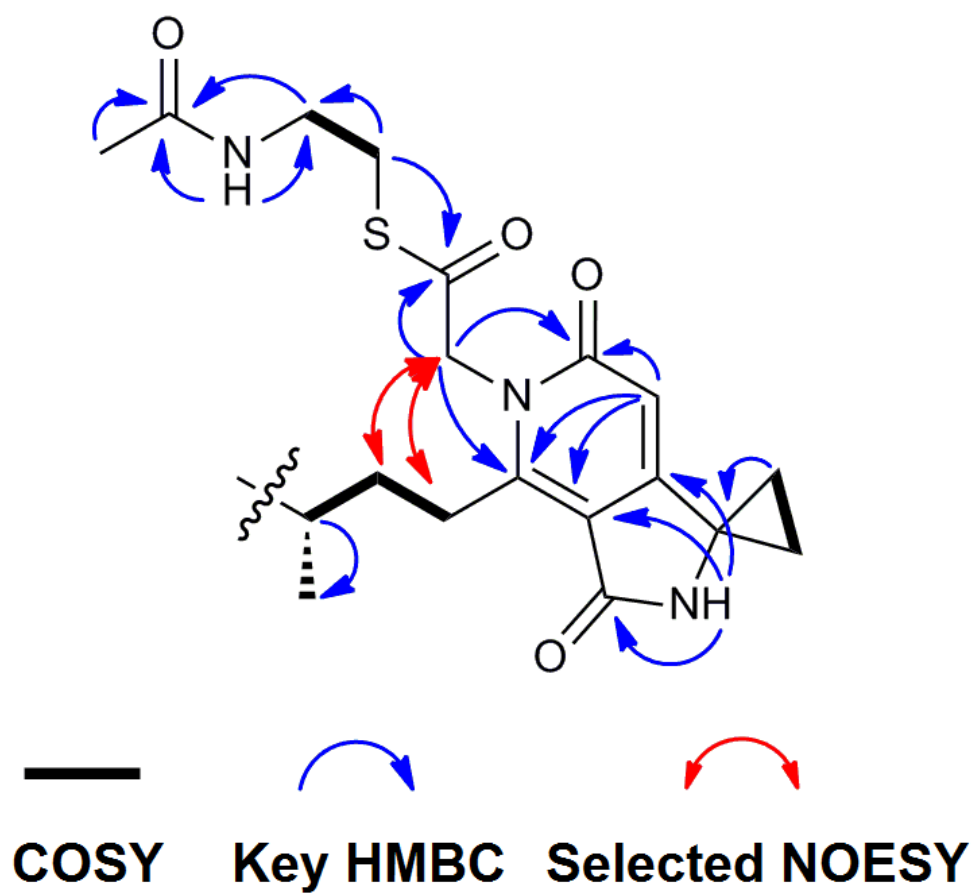
### Structure characterization data of compound **14**



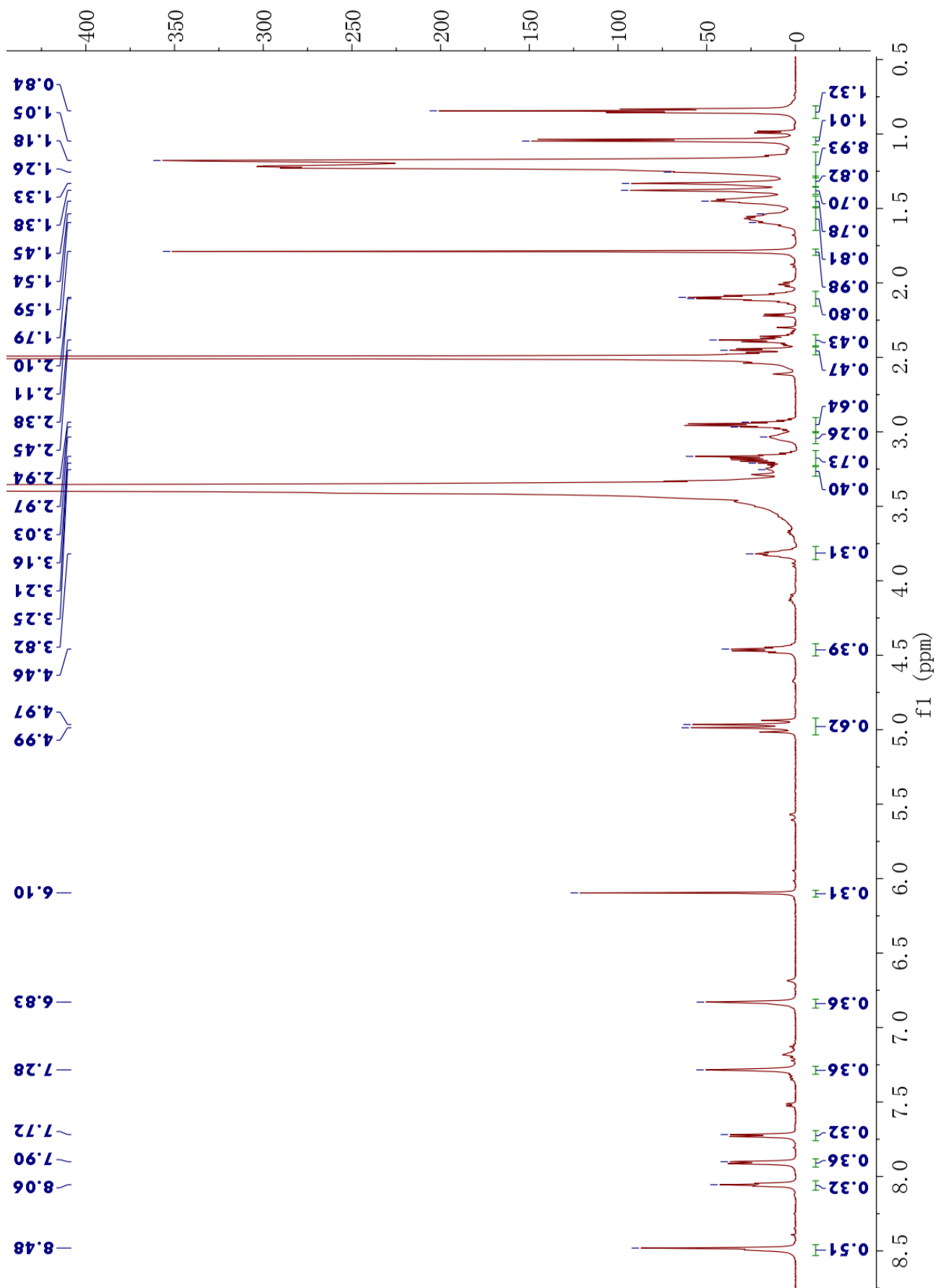
NMR spectroscopic data for **14** in DMSO-*d*<sub>6</sub>.

C	$\delta_C$	$\delta_H$ (J in Hz)	COSY	HMBC ( $^1H$ to $^{13}C$ )
1	14.1	0.84 t (6.6)	2	2, 3
2	22.2	1.26 m	1	1, 3
3	31.4	1.18-1.26 m		
4-10	28.7, 29.0, 29.1, 29.1, 29.2, 29.2, 29.2	1.18-1.26 m		
11	28.8	1.18-1.26 m	12	
12	25.3	1.45 m	11, 13a, 13b	
13	35.3	a 2.10 m b 2.11 m	12, 13b 12, 13a	11, 12, 14 11, 12, 14
14	172.4	- NH, 7.90 d (7.2)	15	14, 15, 16
15	50.2	4.46 m	14NH, 16a, 16b	16, 18
16	37.5	a 2.38 m b 2.45 m	15, 16b 15, 16a	15, 17 15, 17
17	171.6	- a NH, 6.83 s b NH, 7.28 s	17bNH 17aNH	16, 17 17
18	170.7	- NH, 7.72 d (7.8)	19	18, 19, 20, 21
19	44.7	3.82 m	18NH, 20, 21a, 21b	18, 20, 21, 22
20	20.4	1.05 d (6.6)	19	19, 21
21	35.2	a 1.54 m	19, 21b, 22a,	19, 20, 22

			22b	
		b 1.59 m	19, 21a, 22a,	19, 20, 22
			22b	
22	24.1	a 3.03 m	21a, 21b, 22b	
		b 3.25 m	21a, 21b, 22a	
23	153.2	-		
24	109.5	-		
25	166.9	-		
		NH, 8.48 s		23, 24, 25, 26, 29
26	40.1	-		
27	15.3	a 1.33 m	27b, 28a, 28b	26, 28, 29
		b 1.38 m	27a, 28a, 28b	26, 28, 29
28	15.3	a 1.33 m	27a, 27b, 28b	26, 27, 29
		b 1.38 m	27a, 27b, 28a	26, 27, 29
29	159.9	-		
30	103.1	6.10 s		23, 24, 26, 31
31	161.9	-		
32	52.9	a 4.97 d (16.8)		23, 31, 33
		b 4.99 d (16.8)		23, 31, 33
33	194.6	-		
34	28.2	a 2.94 m	34b, 35a, 35b	33, 35
		b 2.97 m	34a, 35a, 35b	33, 35
35	38.1	a 3.16 m	34a, 34b,	34, 36
			35b, 35NH	
		b 3.21 m	34a, 34b,	34, 36
			35a, 35NH	
		NH, 8.06 t (5.4)	35a, 35b	35, 36
36	169.6	-		
37	22.6	1.79 s		36

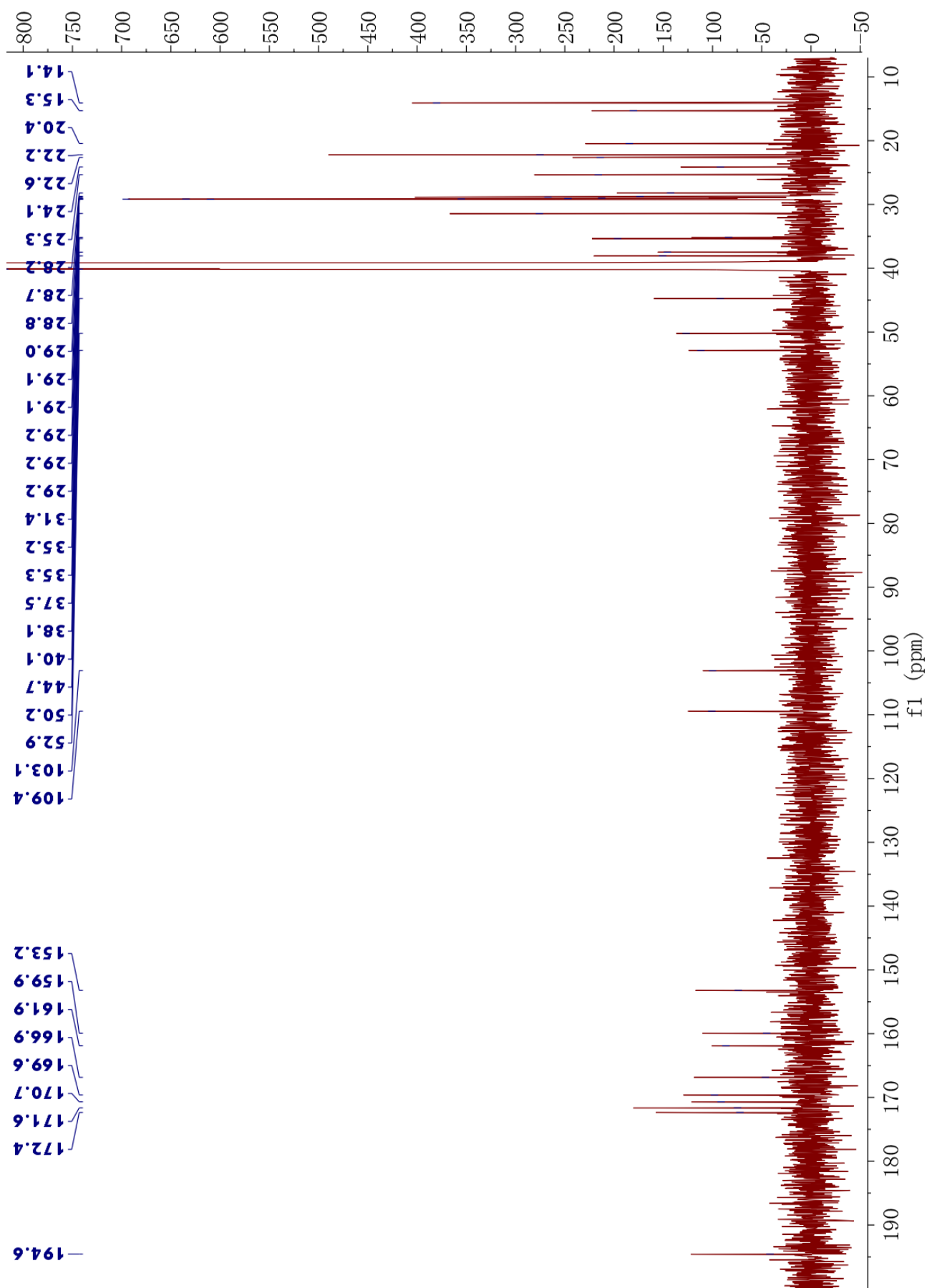


The NMR-based key correlations for the structural assignment of **14**.

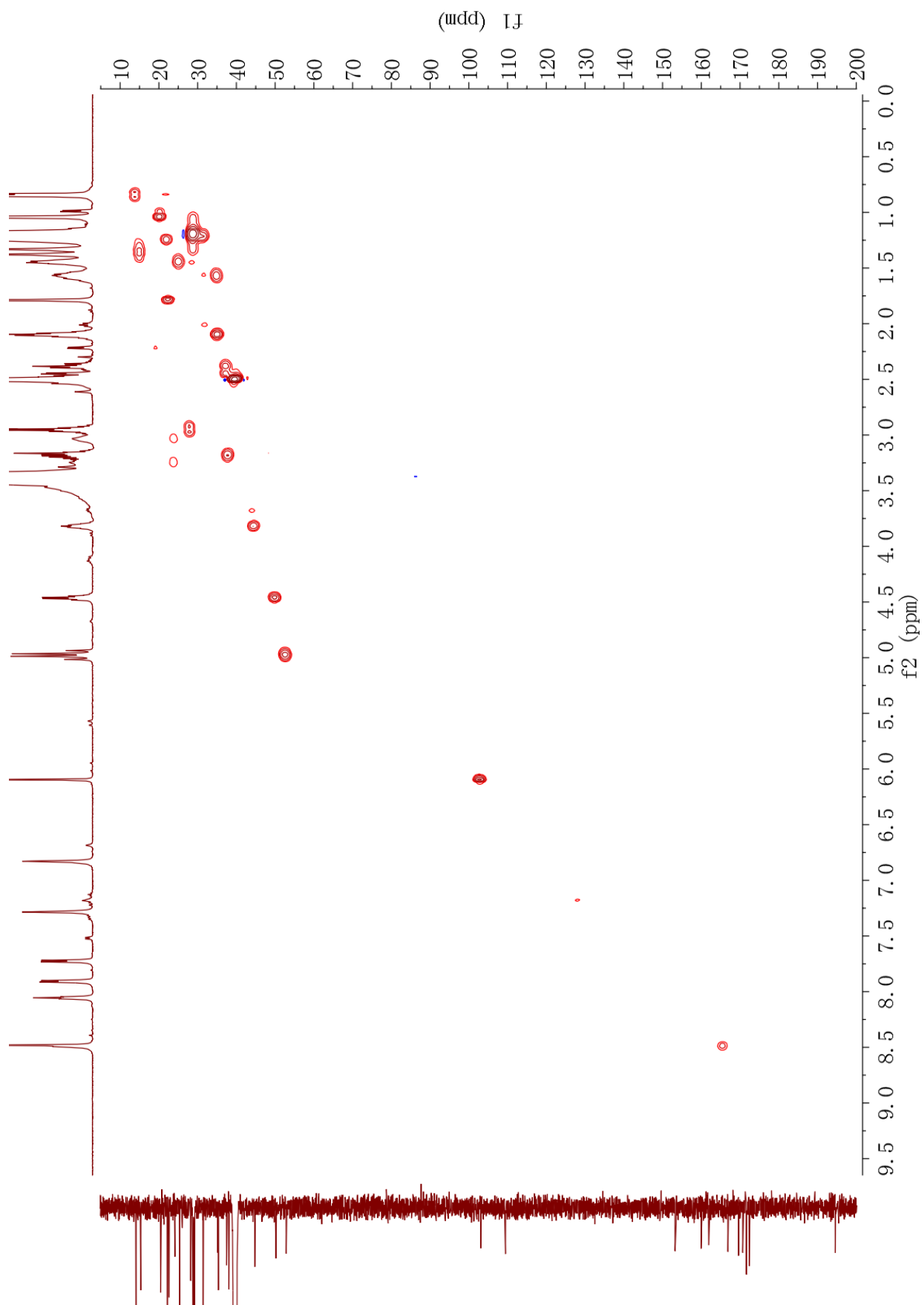


$^1\text{H}$  NMR spectrum of **14** (recorded in  $\text{DMSO-}d_6$  at 600 MHz).

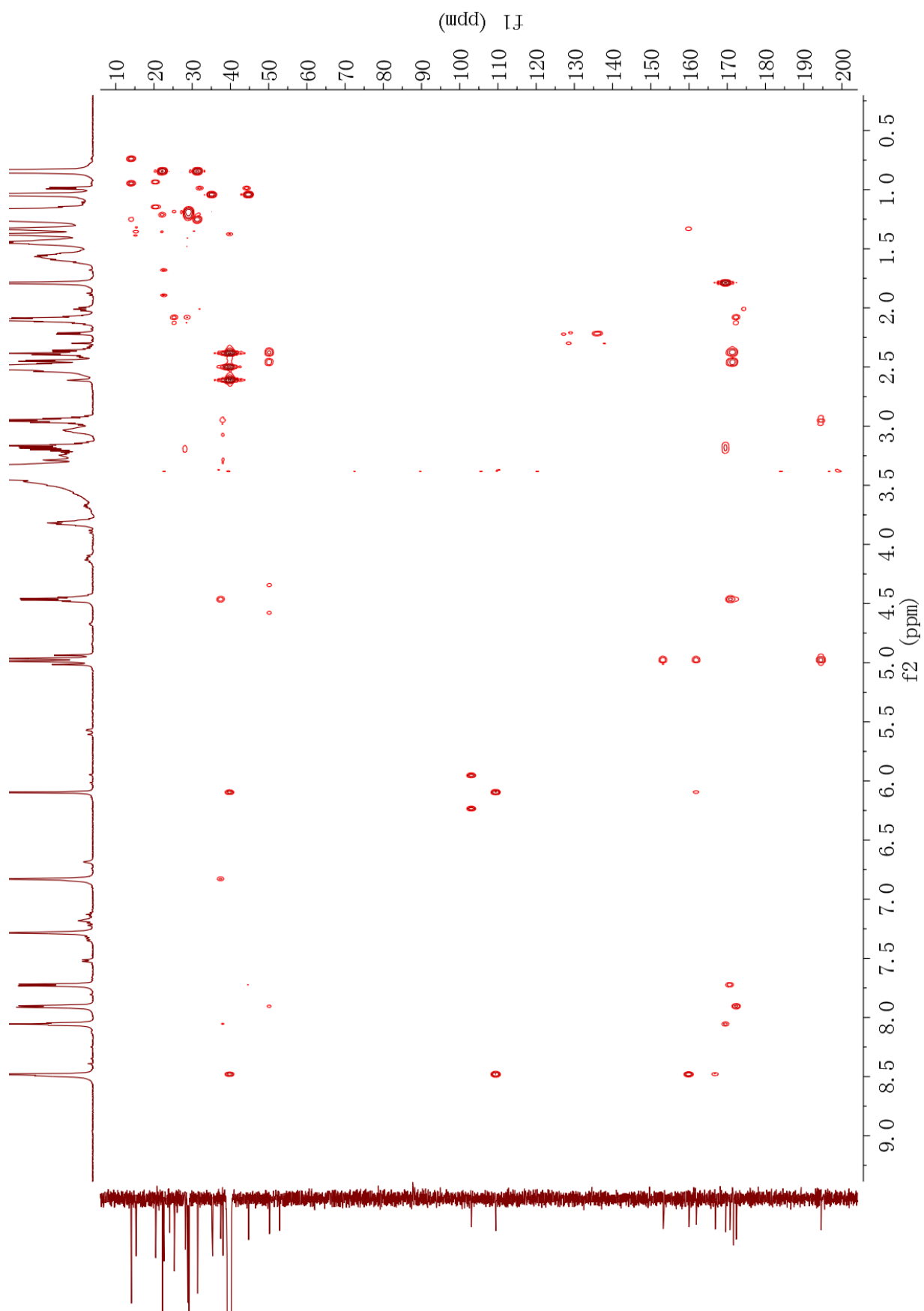




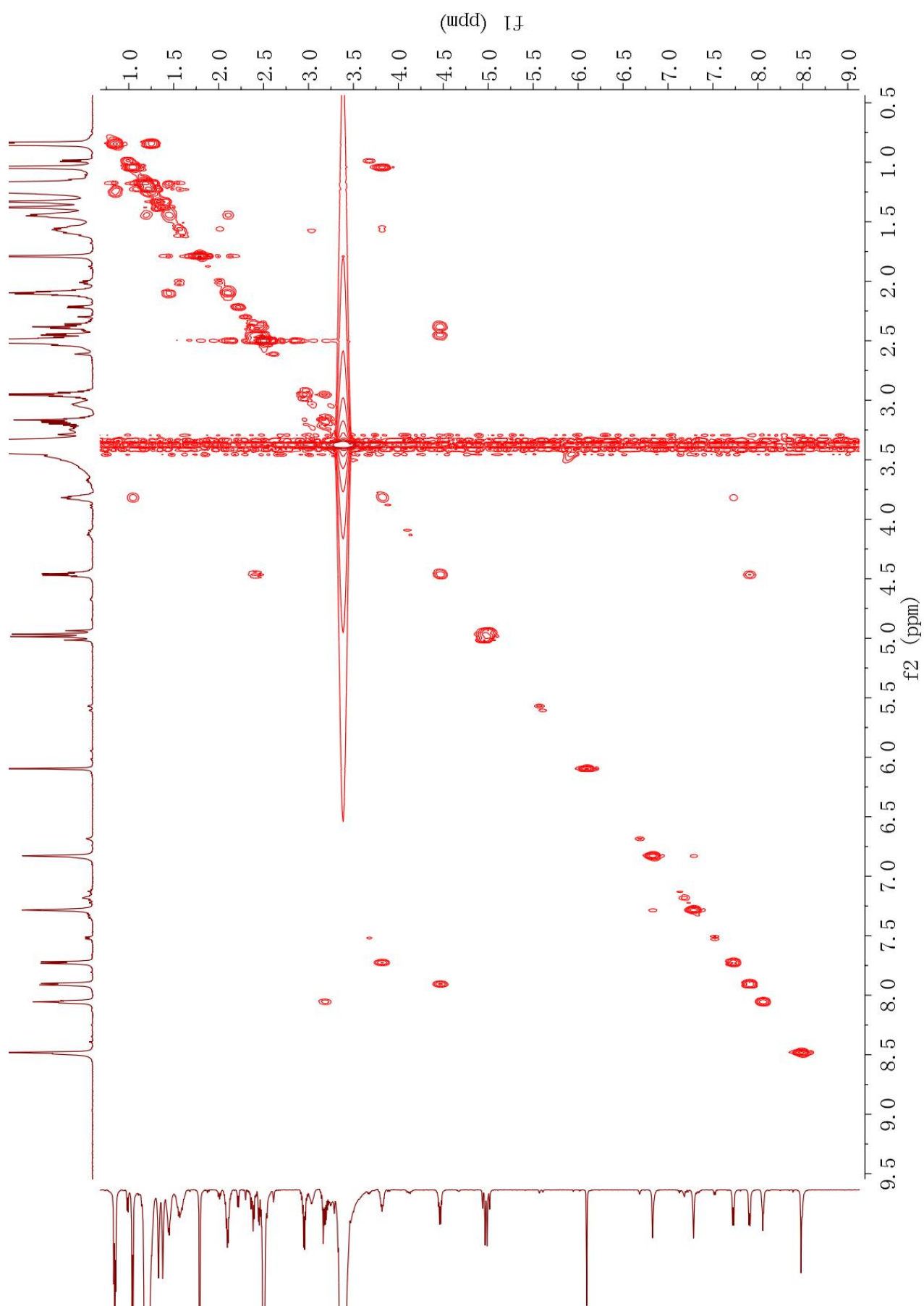
$^{13}\text{C}$  NMR spectrum of **14** (recorded in  $\text{DMSO-}d_6$  at 150 MHz).



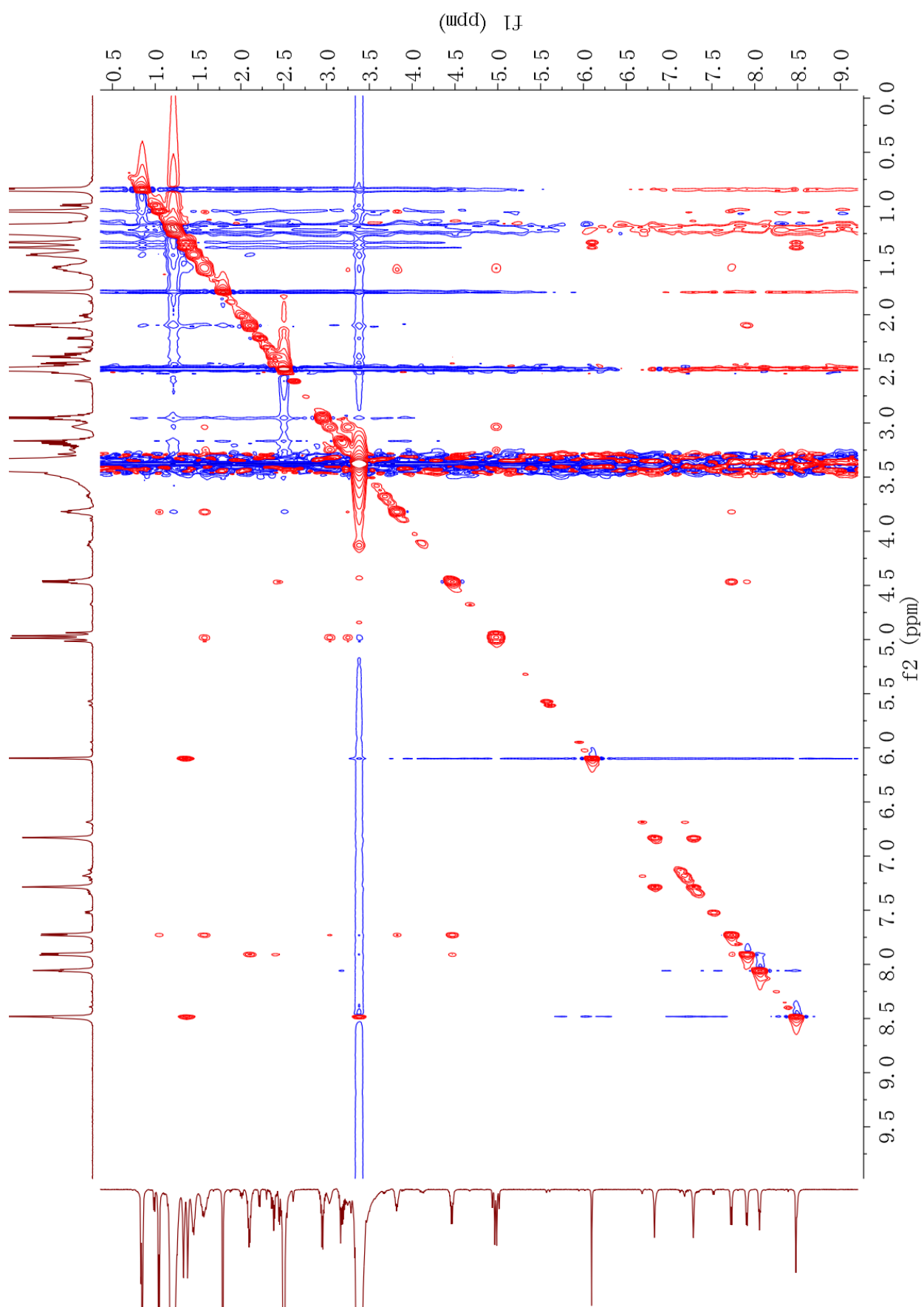
$^1\text{H}$ - $^{13}\text{C}$  HSQC spectrum of **14** (recorded in  $\text{DMSO}-d_6$  at 600 MHz).



$^1\text{H}$ - $^{13}\text{C}$  HMBC spectrum of **14** (recorded in  $\text{DMSO-}d_6$  at 600 MHz).



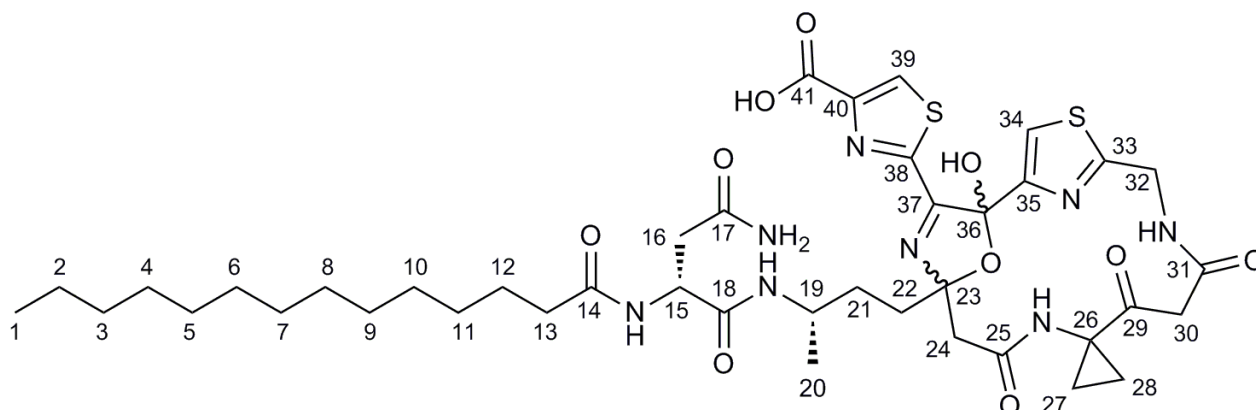
$^1\text{H}$ - $^1\text{H}$  COSY spectrum of **14** (recorded in DMSO- $d_6$  at 600 MHz).



$^1\text{H}$ - $^1\text{H}$  NOESY spectrum of **14** (recorded in  $\text{DMSO}-d_6$  at 600 MHz).

## Supplementary Note 2

### Structure characterization data of compound **10**

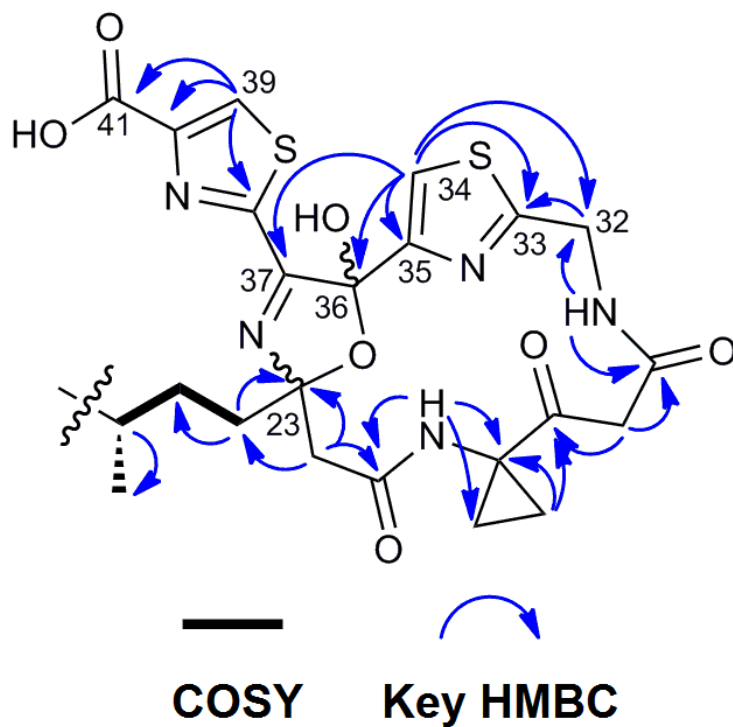


NMR spectroscopic data for **10** (**10a** and **10b** two stereoisomers) in DMSO-*d*<sub>6</sub>.

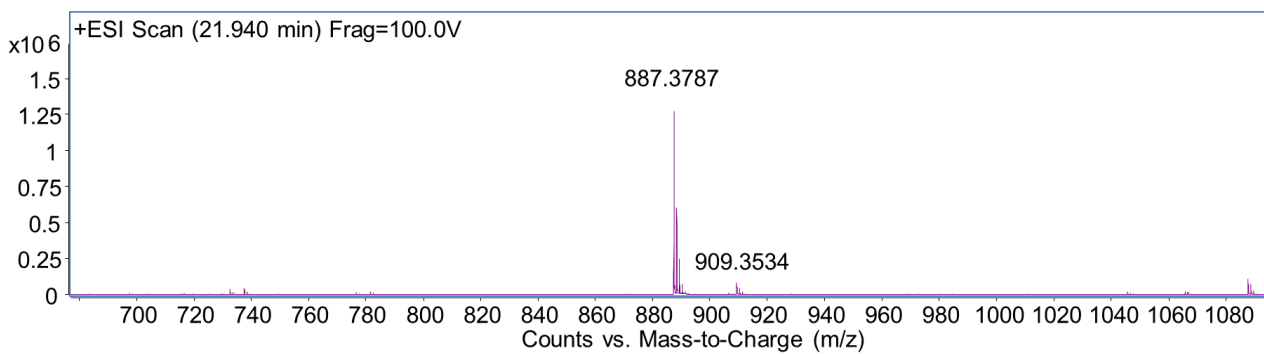
C	$\delta_C$	$\delta_H$ (J in Hz)	COSY	HMBC ( <sup>1</sup> H to <sup>13</sup> C)
1	14.1	0.84 t (6.8)	2	2, 3
2	22.3	1.25 m	1	1
3	31.5	1.14-1.27 m		
4-10	28.8, 29.0, 29.0, 29.0, 29.1, 29.1, 29.2	1.14-1.27 m		
11	28.8	1.14-1.27 m		
12	25.4	1.41 m	13	11, 13, 14
13	35.4	2.05 m	12	11, 12, 14
14	172.5 <b>10a</b> / 172.4 <b>10b</b>	-		
		NH, 8.03 <b>10a</b> d (7.7)/ NH, 8.01 <b>10b</b> d (7.7)	15	14, 15, 16
15	50.2	4.46 m	14NH, 16a, 16b	16, 18
16	37.7 <b>10a</b> / 37.8 <b>10b</b>	a 2.35 dd (8.5, 15.3) b 2.41 dd (4.3, 15.3)	15, 16b	15, 17
17	171.6	-	15, 16a	15, 17
		a NH, 6.84 s b NH, 7.31 s	17NHb 17NHa	16, 17 17
18	170.6 <b>10a</b> / 170.5 <b>10b</b>	-		
		NH, 7.58 <b>10a</b> d (7.7)/ NH, 7.66 <b>10b</b> d (7.7)	19	18, 19, 20, 21

19	44.7	3.70 m	18NH, 20, 21a, 21b	18, 20, 21, 22
20	20.4	0.99 d (6.0)	19, 21b	19, 21
21	30.3 <b>10a</b> / 30.4 <b>10b</b>	a 1.44 m  b 1.57 m	19, 21b, 22a, 22b	19, 22
22	34.9 <b>10a</b> / 35.0 <b>10b</b>	a 1.69 <b>10a</b> m/ a 1.79 <b>10b</b> m b 1.79 <b>10a</b> m/ b 1.89 <b>10b</b> m	21a, 21b, 22a, 22b	19, 21, 23
23	107.8	-		
24	45.8 <b>10a</b> / 46.1 <b>10b</b>	a 2.60 m  b 3.31 d (12.8)	24b  24a	22, 23, 25
25	170.1 <b>10a</b> / 170.2 <b>10b</b>	-  NH, 7.86 br s		25, 26, 27, 28
26	39.4	-		
27	22.1	a 0.87 m b 1.10 m	27b, 28a, 28b	26
28	22.1	a 0.87 m b 1.10 m	27a, 28a, 28b	29
29	205.4	-		
30	48.4	a 3.10 d (14.5) b 3.71 m	30b 30a	26, 29, 31
31	166.9	-  NH, 9.06 s	32a, 32b	31, 32
32	40.6	a 4.27 d (14.5) b 4.78 dd (6.8, 17.0)	31NH, 32b 31NH, 32a	33
33	168.1	-		
34	120.1	8.03 s		32, 33, 35, 36, 37
35	154.1	-		
36	107.6	-		
37	160.4	-		
38	156.3	-		
39	127.6	8.17 s		38, 40, 41
40	156.1	-		
41	163.9	-		

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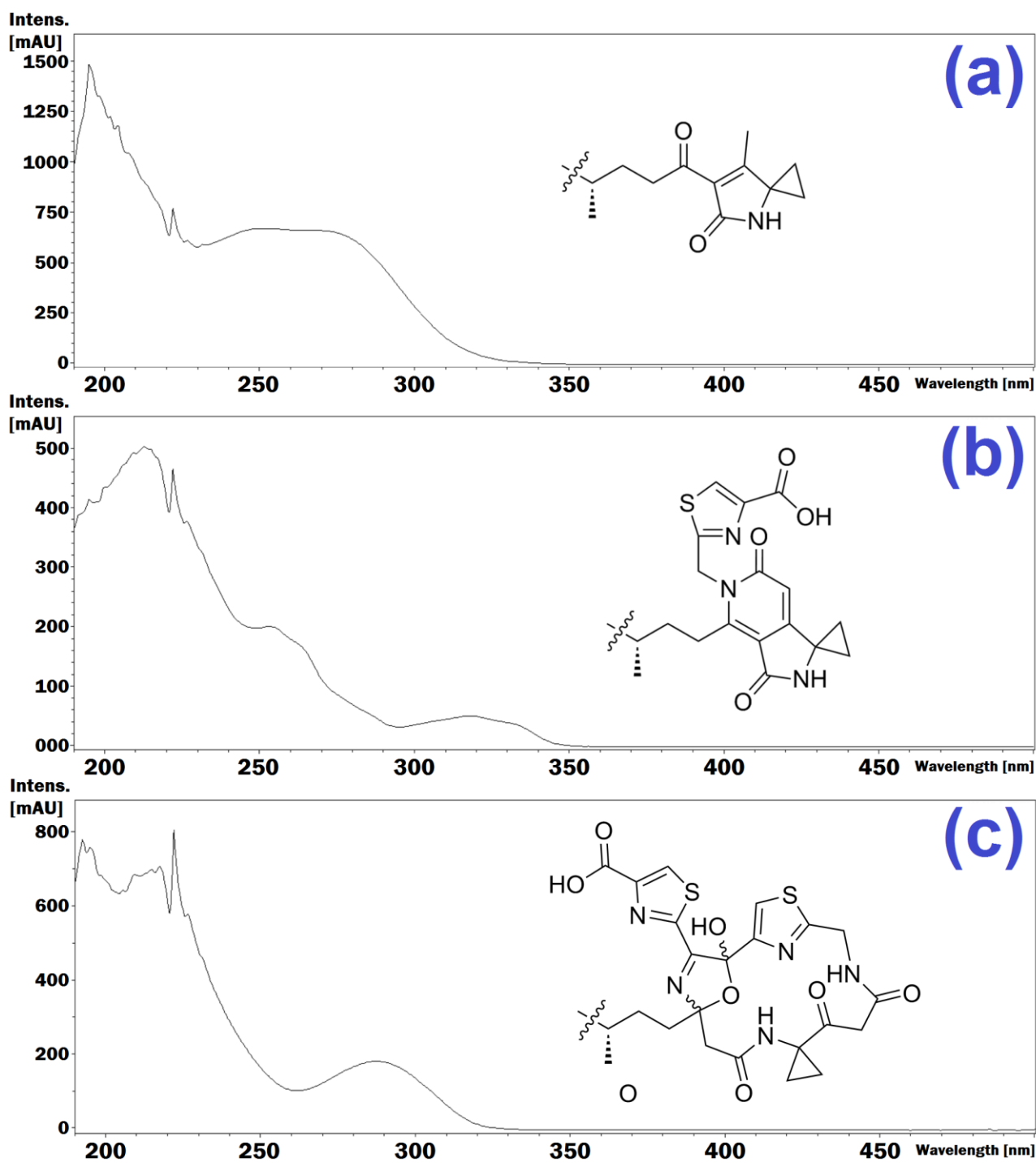


NMR-based key correlations for the structural assignment of **10**.

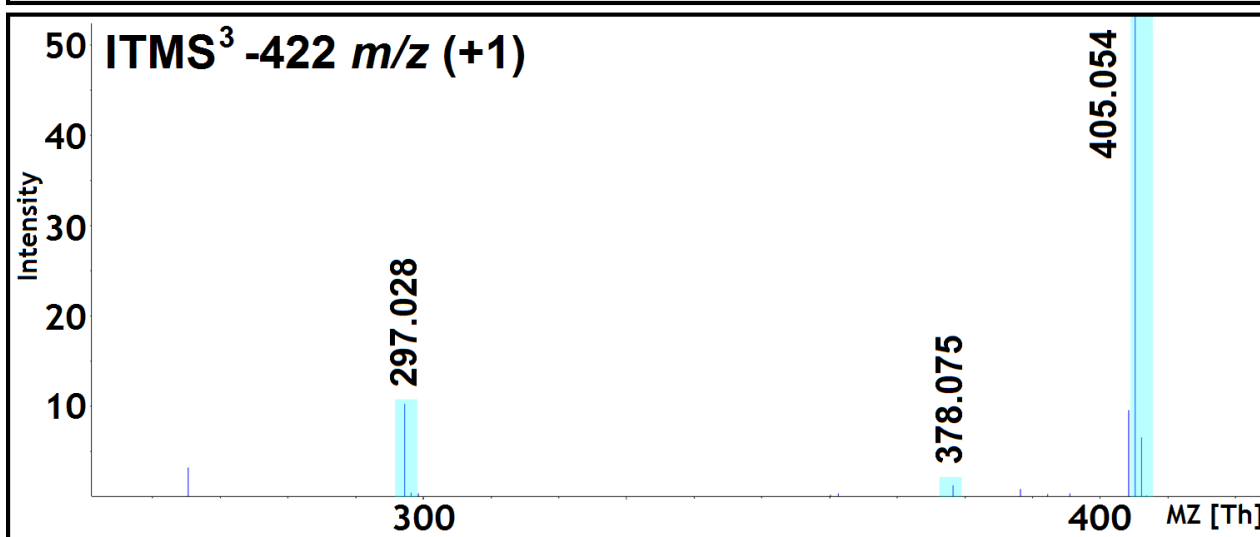
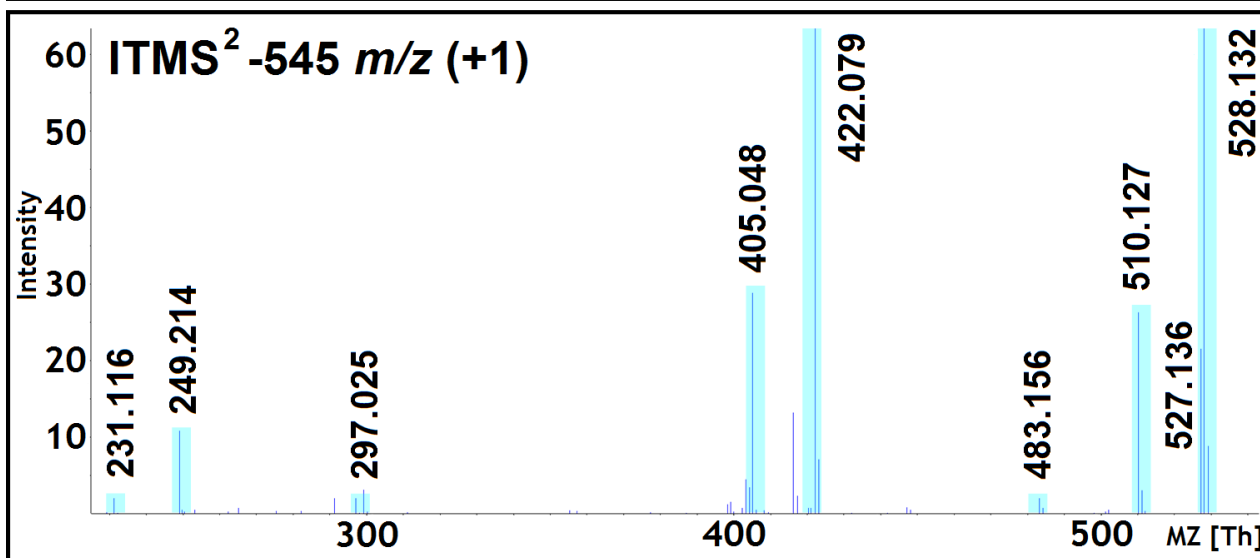
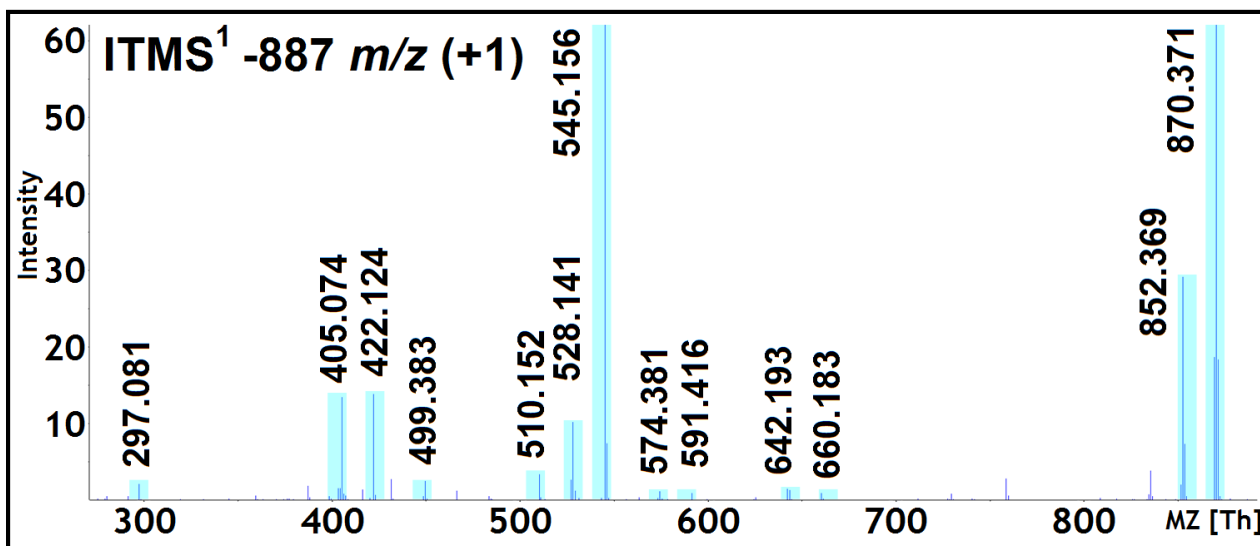


HRESIMS of **10**.

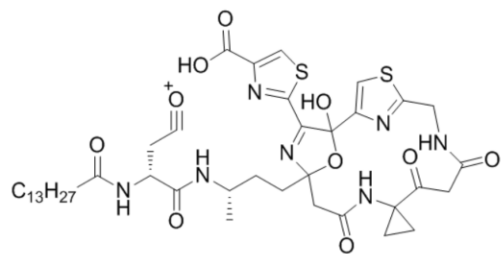




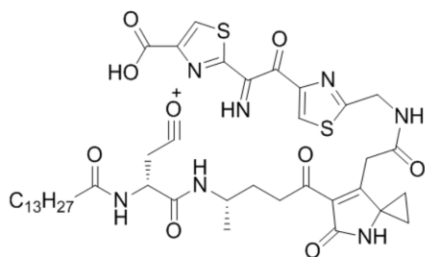
UV spectrum of **10** (c) in comparison with those of **5** (a) and **7** (b).



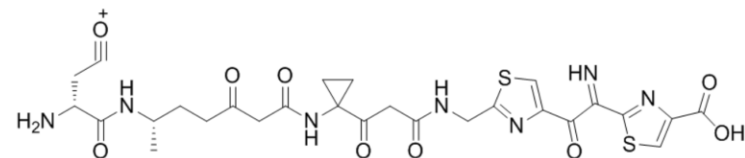
MS<sup>n</sup> fragmentation pattern of **10**.



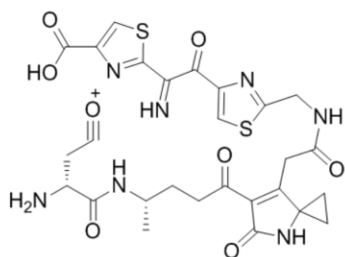
**Frag. 1**



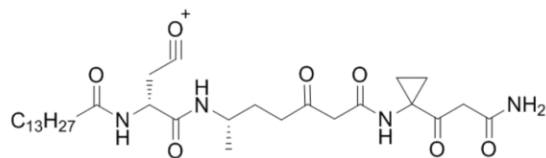
**Frag. 2**



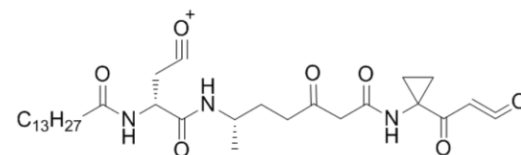
**Frag. 3**



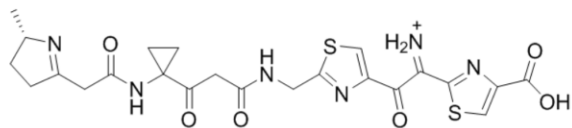
**Frag. 4**



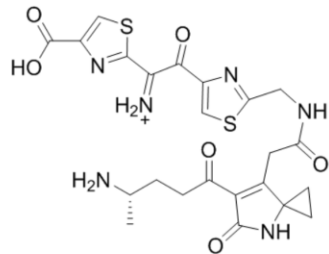
**Frag. 5**



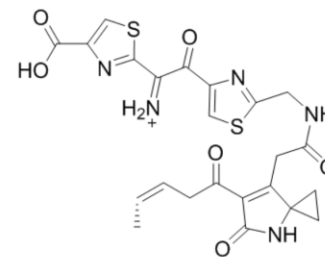
**Frag. 6**



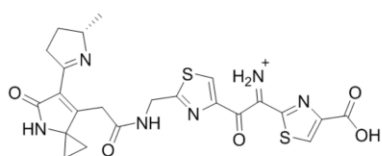
**Frag. 7**



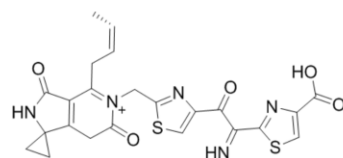
**Frag. 7**



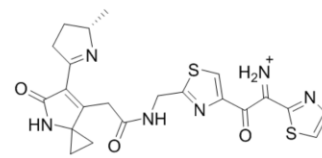
**Frag. 8**



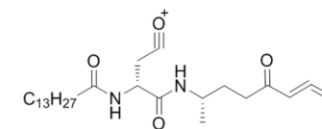
**Frag. 9**



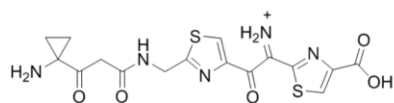
**Frag. 10**



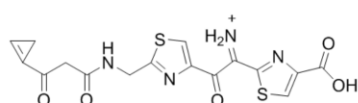
**Frag. 11**



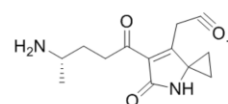
**Frag. 12**



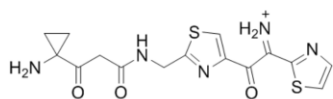
**Frag. 13**



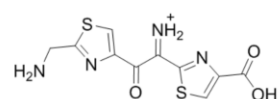
**Frag. 14**



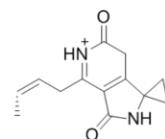
**Frag. 17**



**Frag. 15**



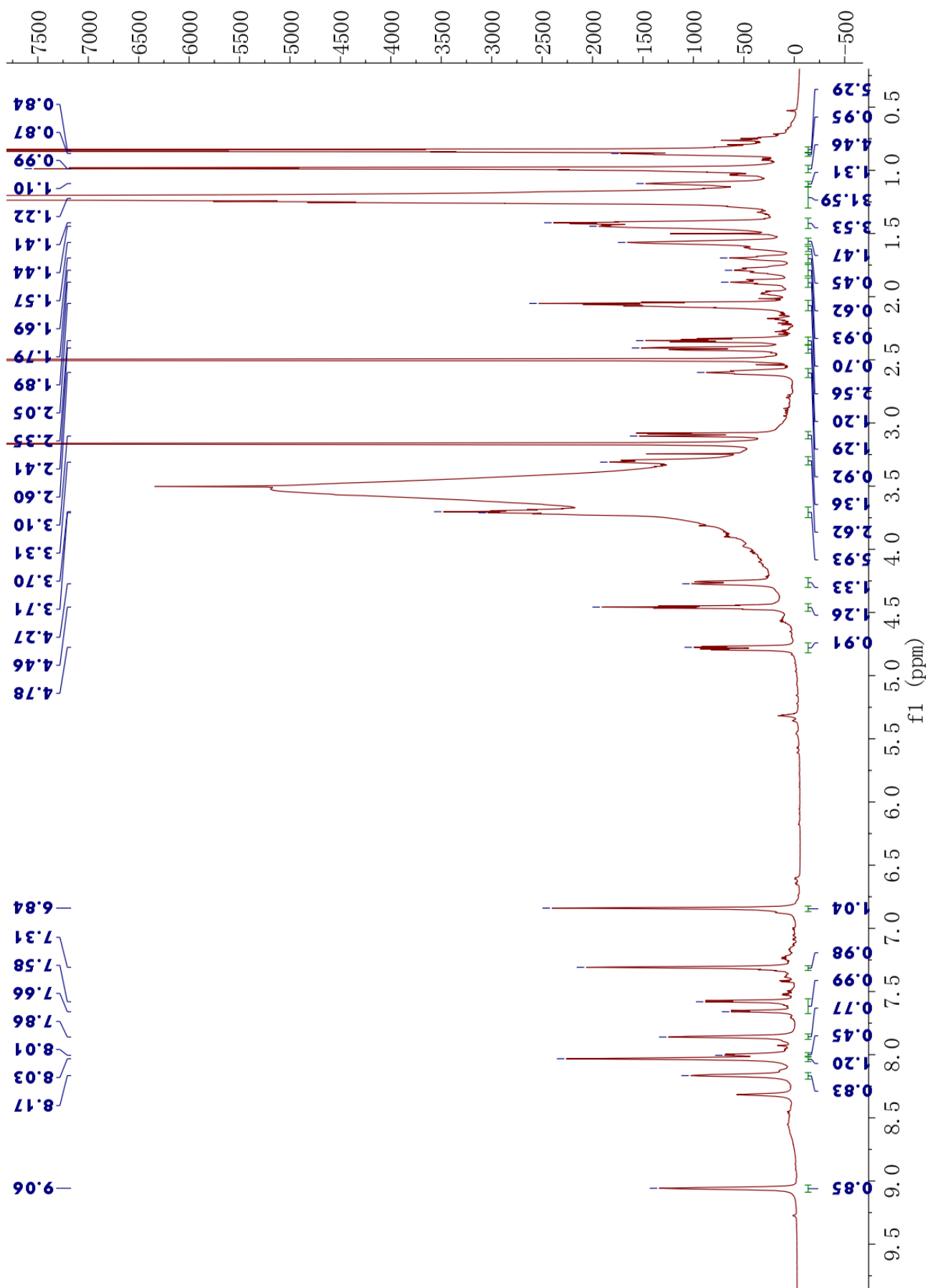
**Frag. 16**



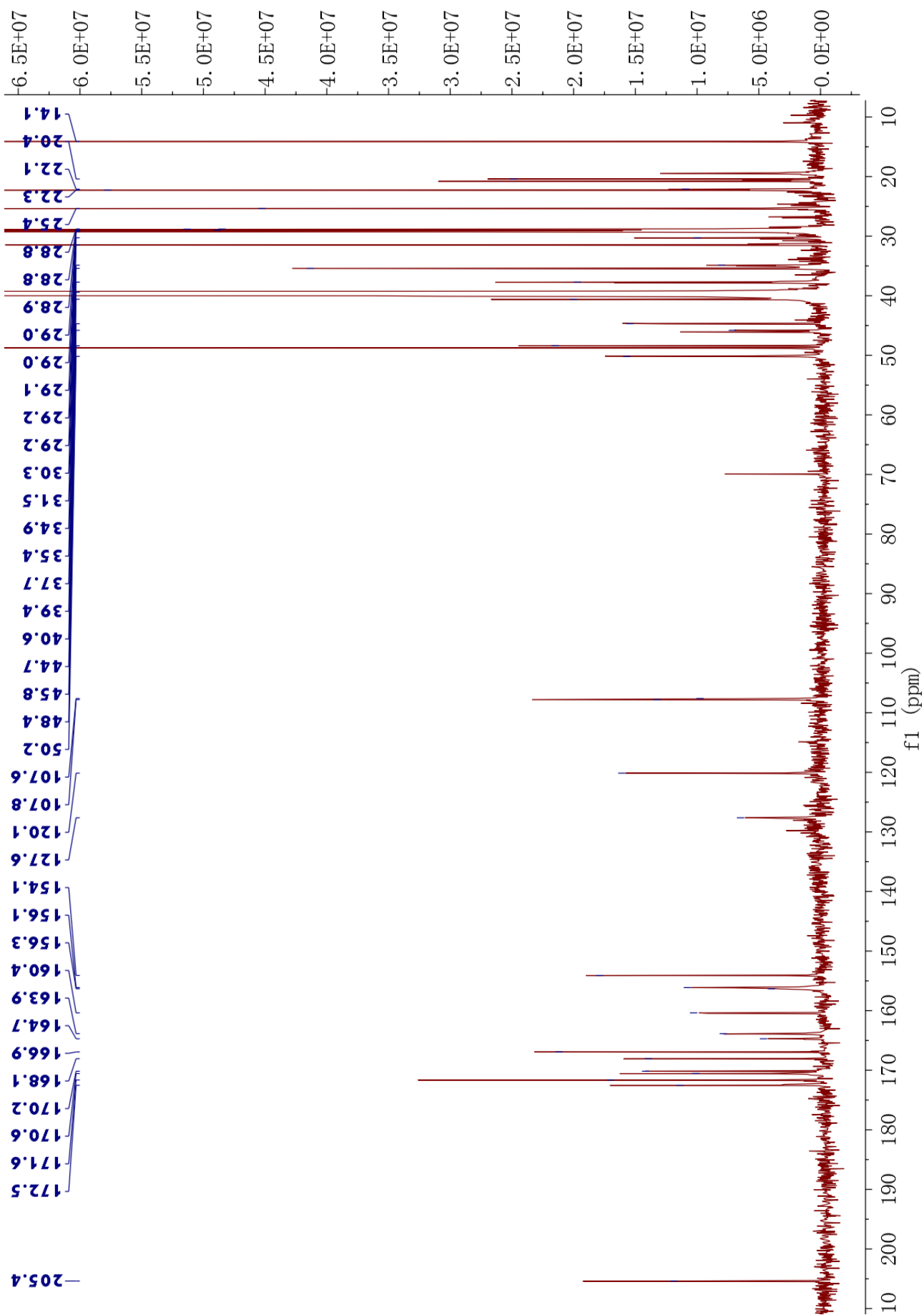
**Frag. 18**

Fragmentation	MS <sup>n</sup>	Obs. mass	Calc. mass	Error [Da]
1	MS <sup>2</sup>	870.371	870.353	0.018
2	MS <sup>2</sup>	852.369	852.342	0.027
3	MS <sup>2</sup>	660.183	660.155	0.028
4	MS <sup>2</sup>	642.193	642.144	0.049
5	MS <sup>2</sup>	591.416	591.376	0.040
6	MS <sup>2</sup>	574.381	574.349	0.032
7	MS <sup>2</sup>	545.156	545.128	0.028
8	MS <sup>2</sup> /MS <sup>3</sup>	528.141	528.101	0.040
9	MS <sup>3</sup>	527.136	527.117	0.019
10	MS <sup>2</sup> /MS <sup>3</sup>	510.152	510.091	0.061
11	MS <sup>3</sup>	483.156	483.127	0.029
12	MS <sup>2</sup>	449.383	449.302	0.081
13	MS <sup>2</sup> /MS <sup>3</sup>	422.124	422.059	0.065
14	MS <sup>2</sup> /MS <sup>3</sup> /MS <sup>4</sup>	405.074	405.033	0.041
15	MS <sup>4</sup>	378.075	378.070	0.006
16	MS <sup>2</sup> /MS <sup>3</sup> /MS <sup>4</sup>	297.081	297.012	0.069
17	MS <sup>3</sup>	249.214	249.124	0.090
18	MS <sup>3</sup>	231.116	231.113	0.003

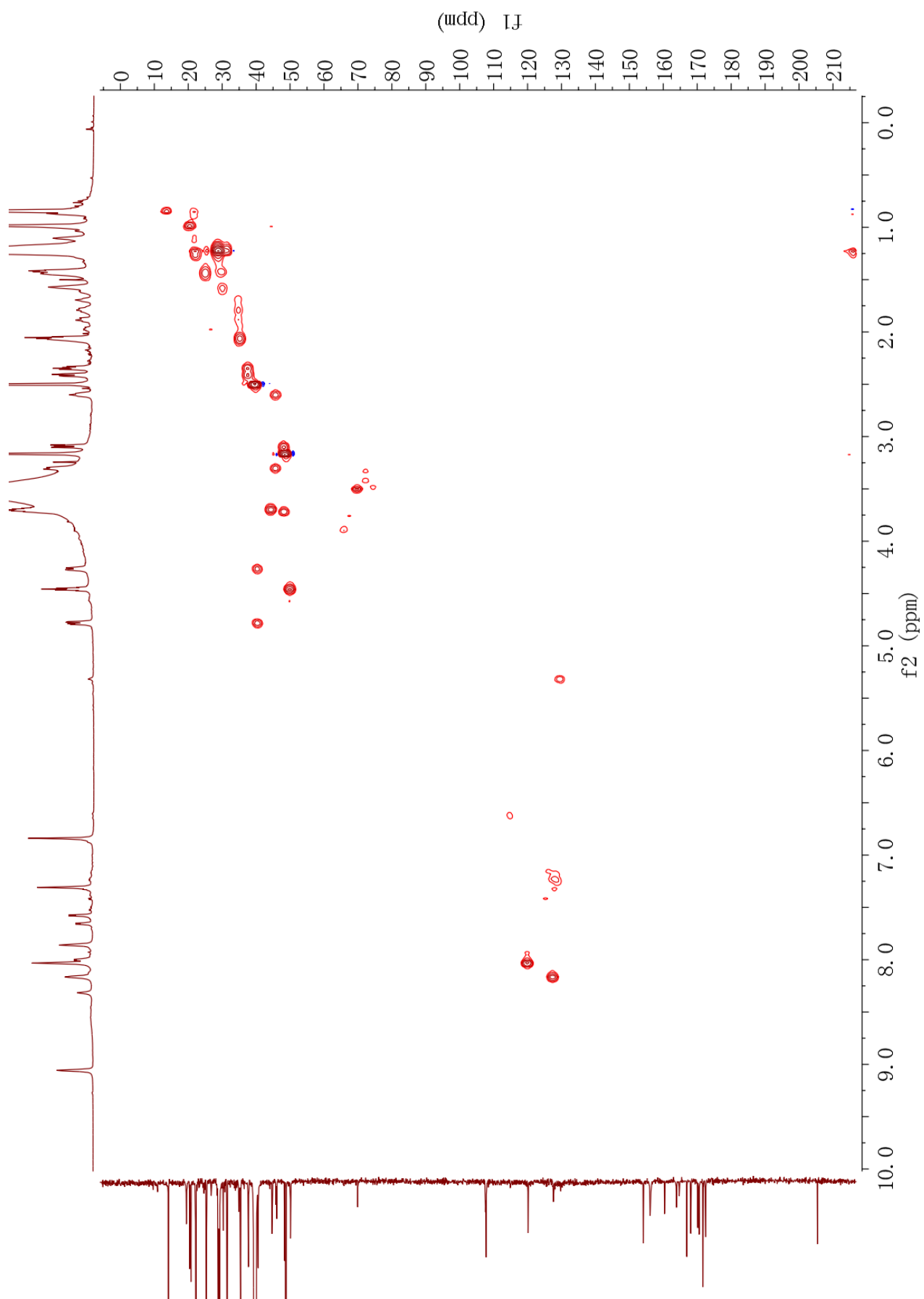
The major fragmentation species from MS<sup>n</sup> measurement of **10**. Fragmentation was acquired with collision energy of 28 V. Obs. = observed; Calc. = calculated.



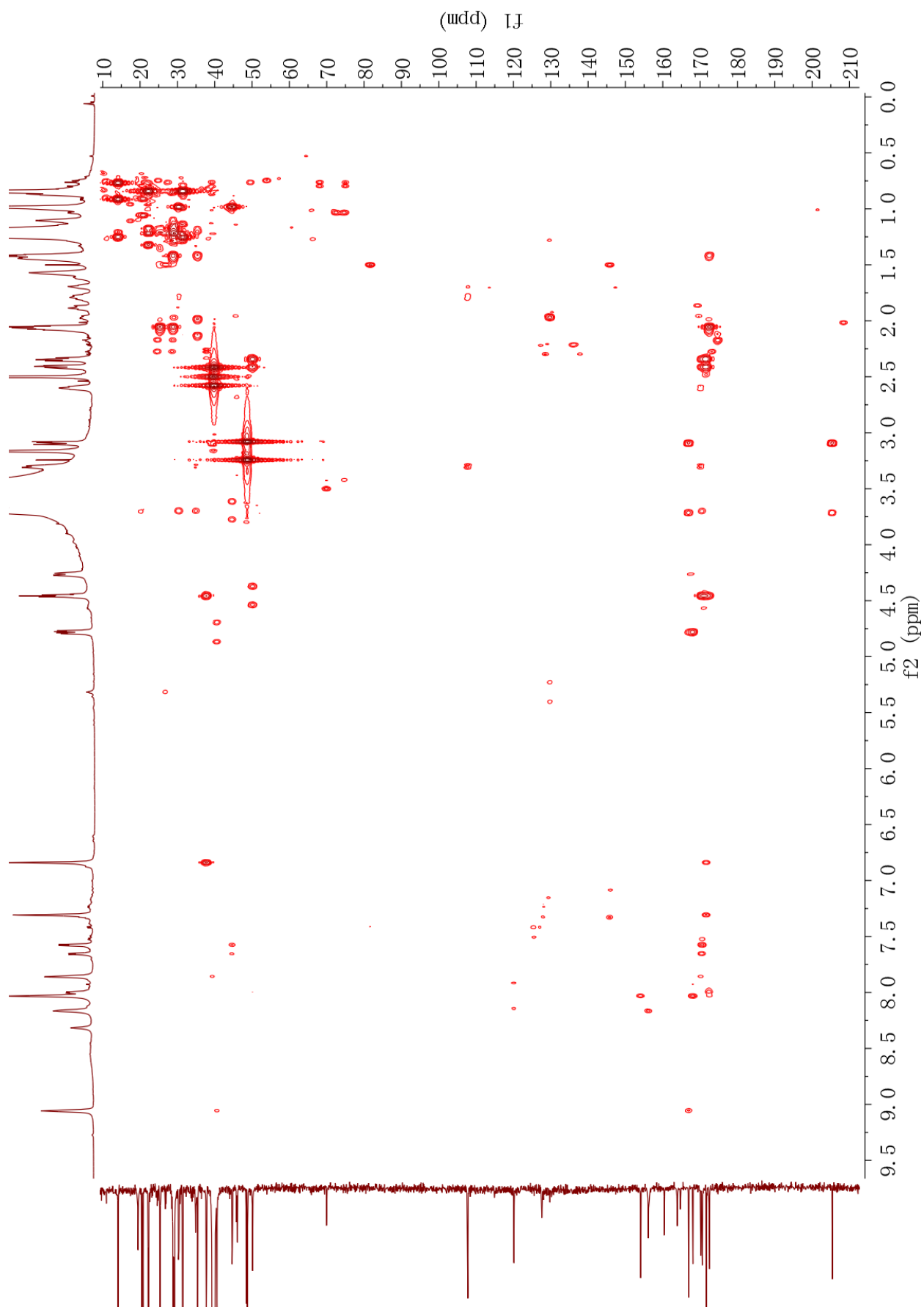
$^1\text{H}$  NMR spectrum of **10** (recorded in  $\text{DMSO-}d_6$  at 850 MHz).



$^{13}\text{C}$  NMR spectrum of **10** (recorded in  $\text{DMSO-}d_6$  at 212.5 MHz).

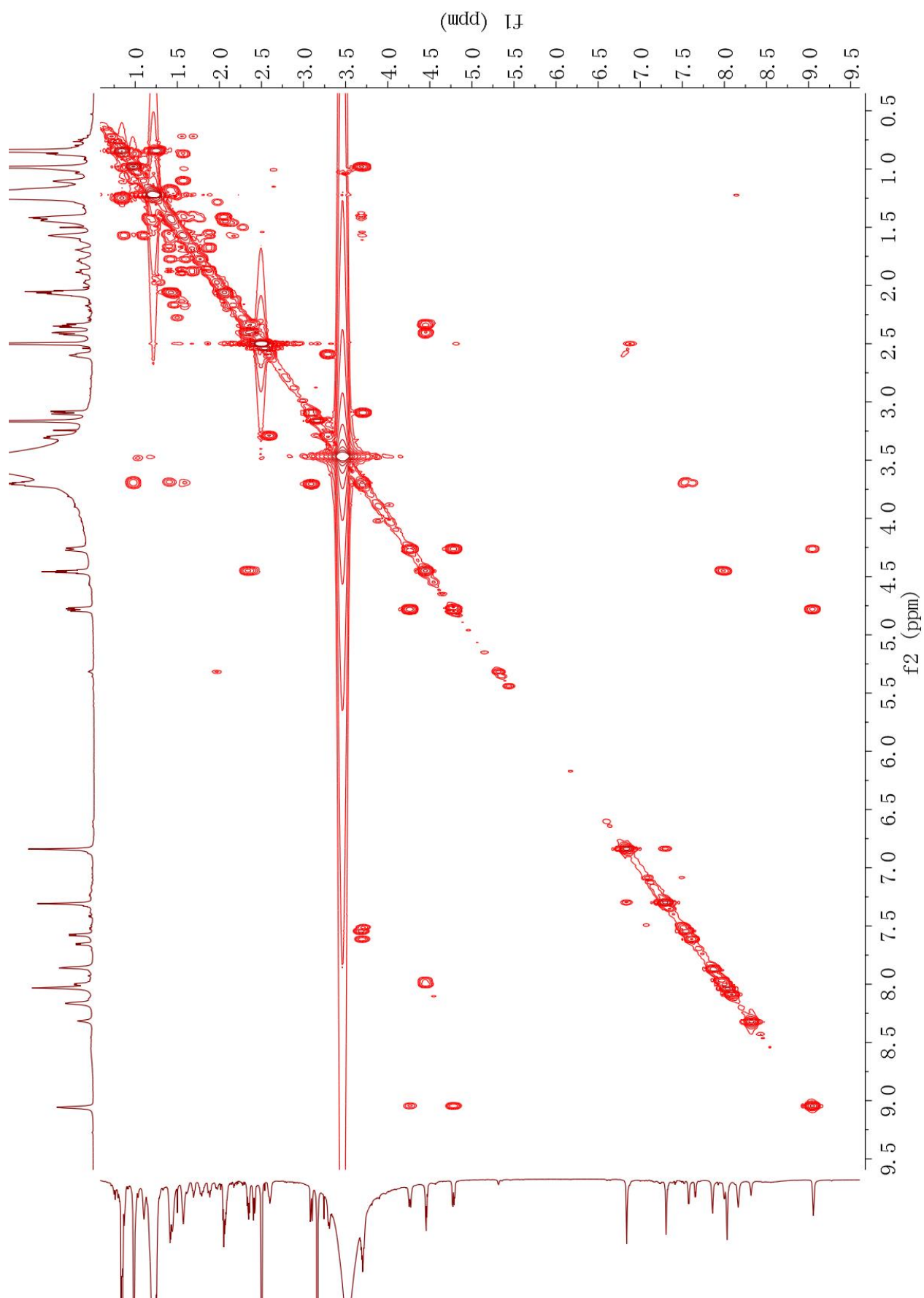


$^1\text{H}$ - $^{13}\text{C}$  HSQC spectrum of **10** (recorded in  $\text{DMSO-}d_6$  at 850 MHz).

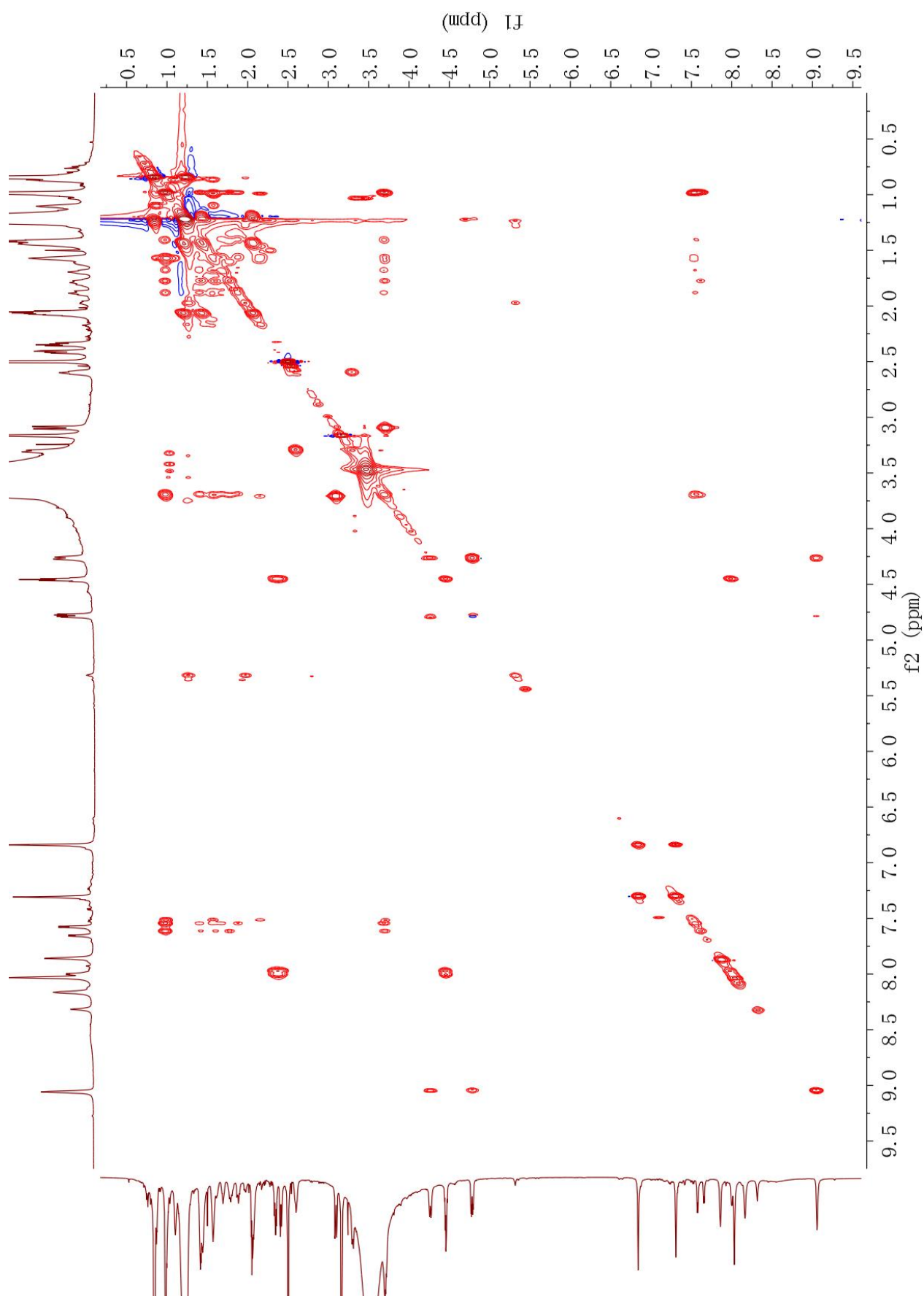


$^1\text{H}$ - $^{13}\text{C}$  HMBC spectrum of **10** (recorded in  $\text{DMSO-}d_6$  at 850 MHz).

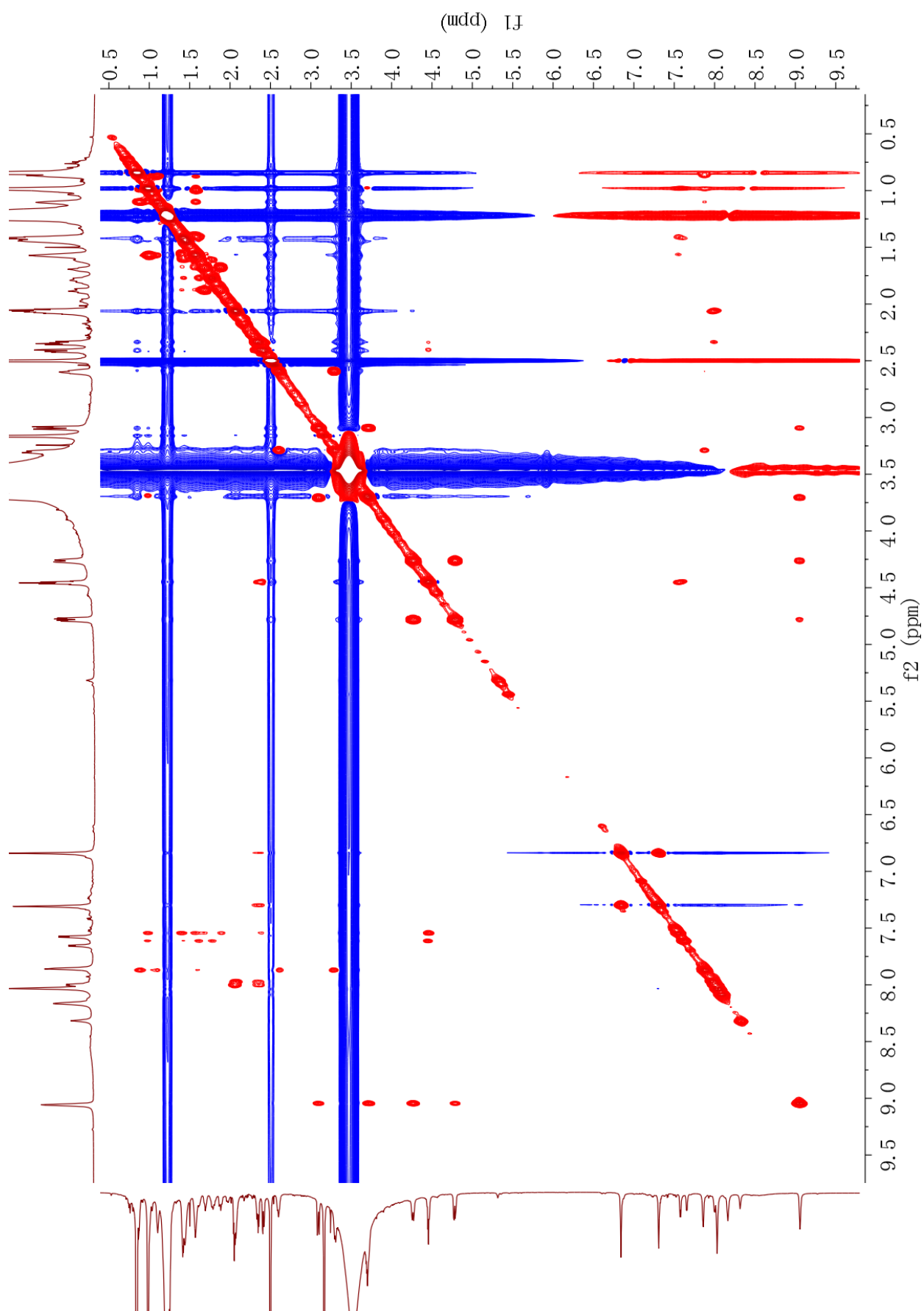




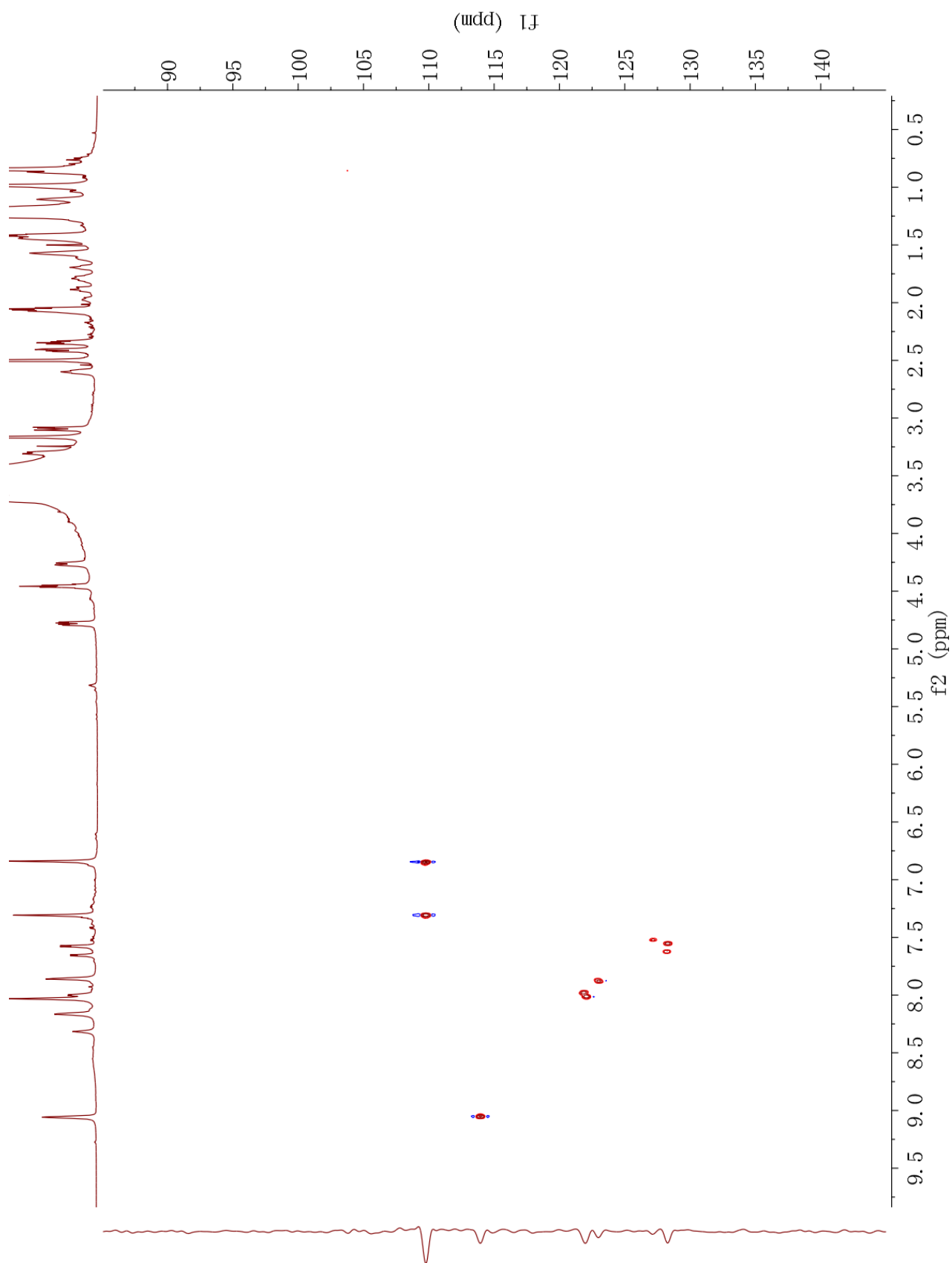
$^1\text{H}$ - $^1\text{H}$  COSY spectrum of **10** (recorded in  $\text{DMSO-}d_6$  at 850 MHz).



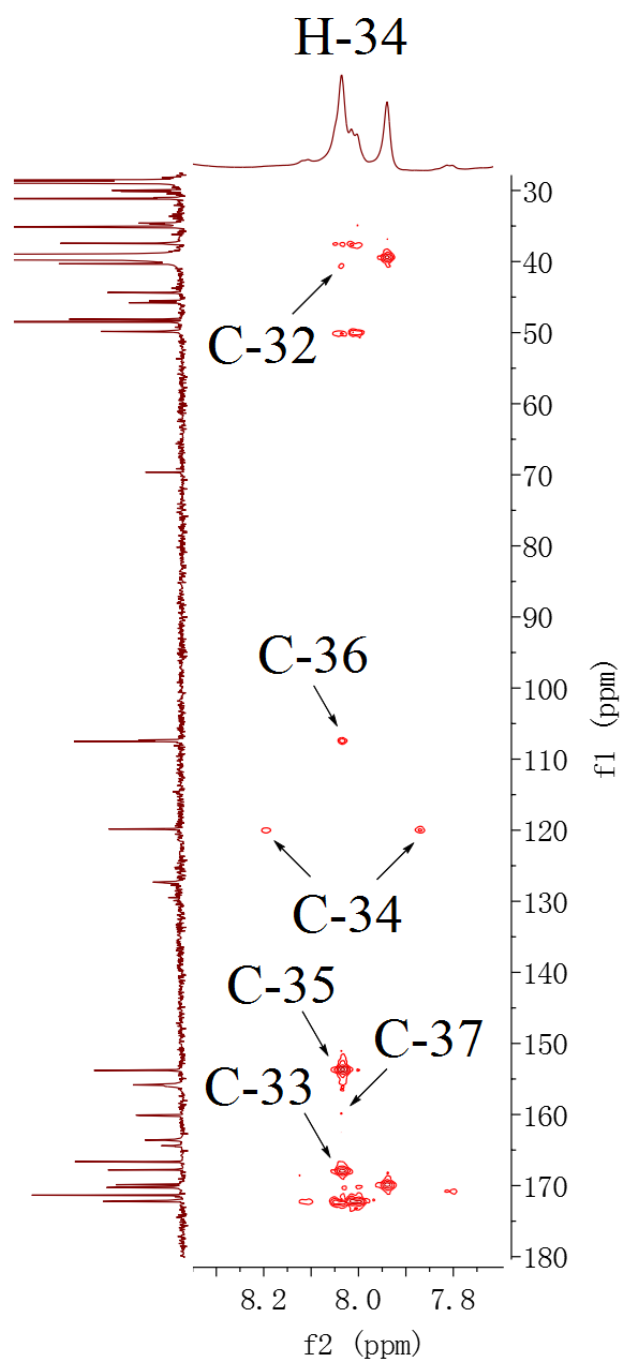
$^1\text{H}$ - $^1\text{H}$  TOCSY spectrum of **10** (recorded in  $\text{DMSO}-d_6$  at 850 MHz).



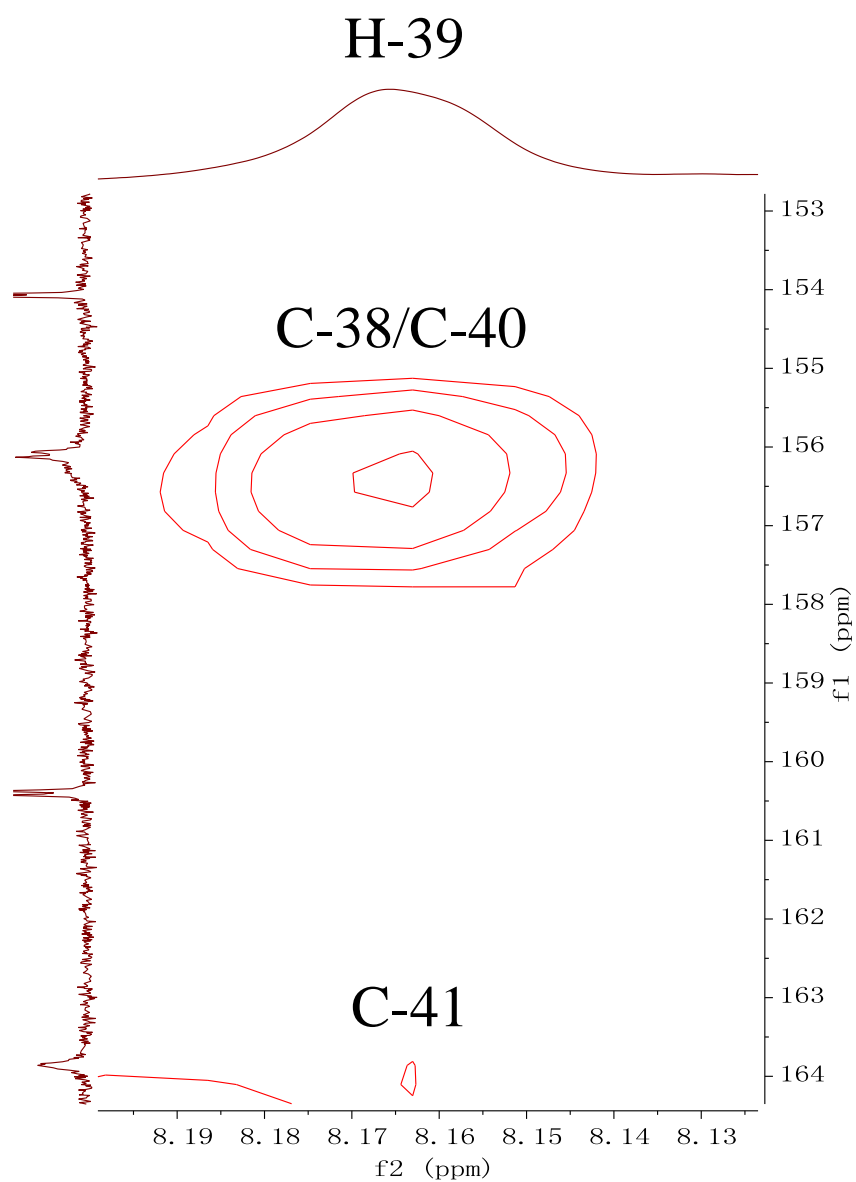
$^1\text{H}$ - $^1\text{H}$  NOESY spectrum of **10** (recorded in  $\text{DMSO-}d_6$  at 850 MHz).



$^1\text{H}$ - $^{15}\text{N}$  HSQC spectrum of **10** (recorded in  $\text{DMSO-}d_6$  at 850 MHz).



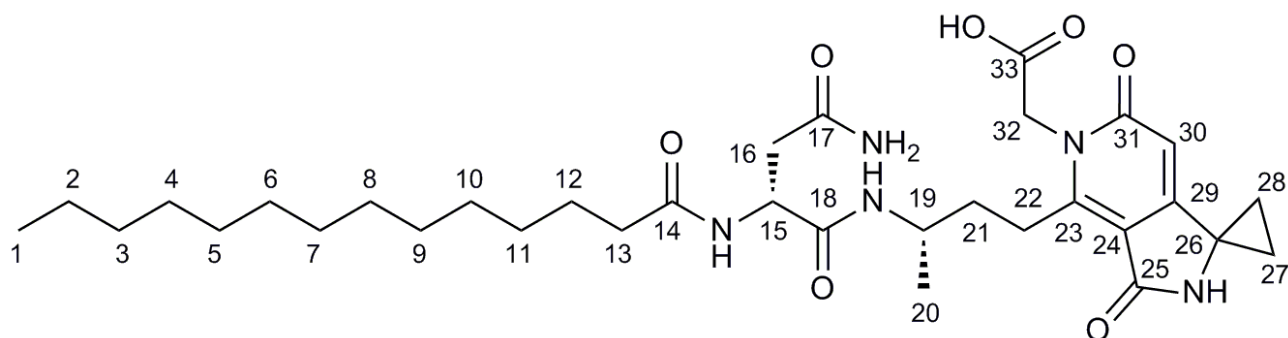
Expanded  $^1\text{H}$ - $^{13}\text{C}$  HMBC spectrum of **10** showing the key correlations of H-34 to C-32, C-33, C-35, C-36 and C-37 (recorded in  $\text{DMSO-}d_6$  at 600 MHz with a 1.7 mm cryoprobe).



Expanded  $^1\text{H}$ - $^{13}\text{C}$  HMBC spectrum of **10** showing the key correlations of H-39 to C-38, C-40 and C-41 (recorded in  $\text{DMSO-}d_6$  at 850 MHz).

## Supplementary Note 3

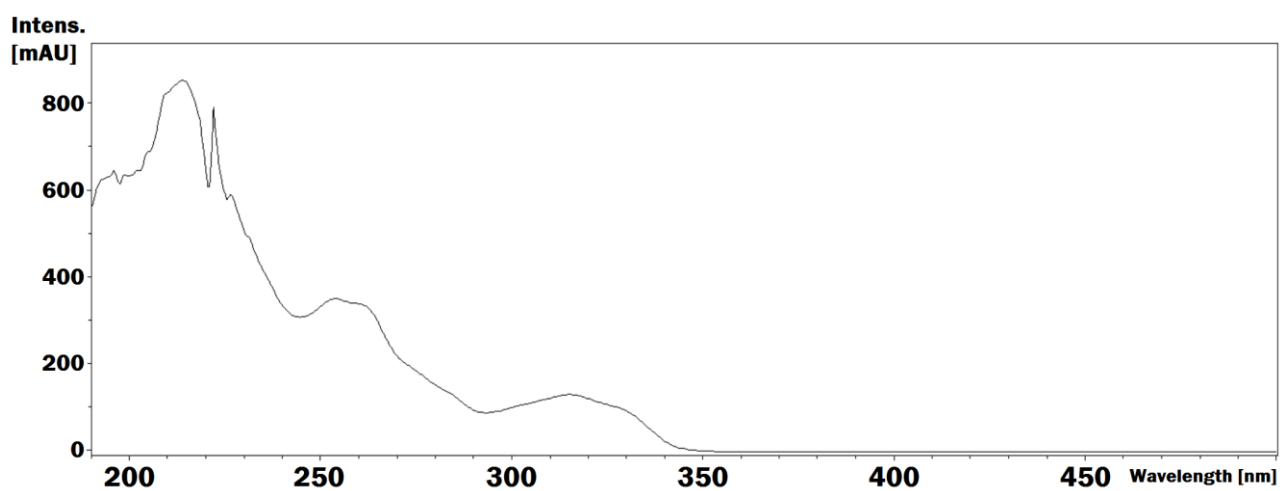
### Structure characterization data of compound **6**



NMR spectroscopic data for **6** in DMSO-*d*<sub>6</sub>.

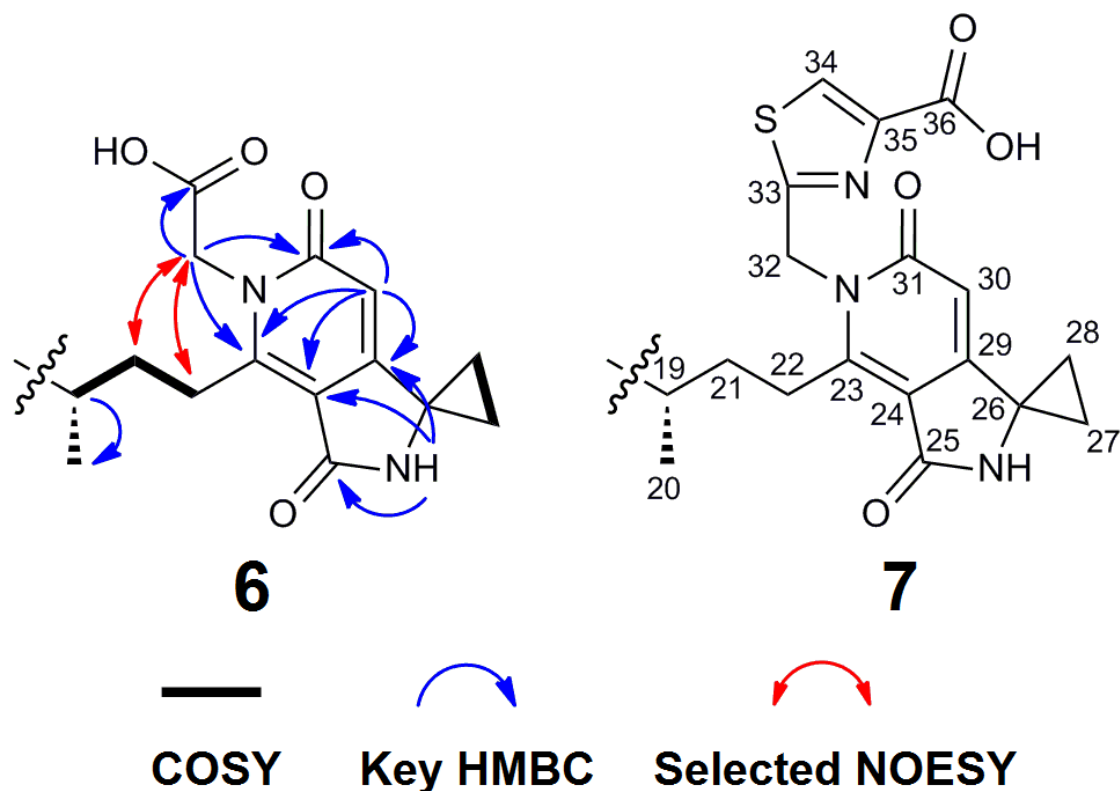
C	$\delta_C$	$\delta_H$ (J in Hz)	COSY	HMBC ( $^1H$ to $^{13}C$ )
1	14.1	0.84 t (7.2)	2	2, 3
2	22.3	1.26 m	1	1, 3
3	31.5	1.19-1.24 m		
4-10	28.9, 29.1, 29.2, 29.2, 29.2, 29.2	1.19-1.24 m		
11	28.8	1.19-1.24 m	12	
12	25.3	1.46 m	11, 13a, 13b	11, 13, 14
13	35.5	a 2.11 m b 2.17 m	12, 13b 12, 13a	11, 12, 14 11, 12, 14
14	172.6	- NH, 8.13 br s	15	14
15	50.2	4.50 m	14NH, 16a, 16b	16, 18
16	37.7	a 2.38 m b 2.47 m	15, 16b 15, 16a	15, 17 15, 17
17	171.8	- a NH, 6.80 s b NH, 7.37 s	17bNH 17aNH	16, 17 17
18	170.9	- NH, 7.62 d (7.8)	19	15, 18, 19, 20, 21
19	44.7	3.82 m	18NH, 20, 21a, 21b	18, 20, 21, 22
20	20.6	1.03 d (6.6)	19	19, 21, 22
21	35.0	a 1.56 m  b 1.64 m	19, 21b, 22a, 22b 19, 21a, 22a,	19, 20, 22 19, 20, 22

22	24.3	a 2.92 m b 3.26 m	21a, 21b, 22b 21a, 21b, 22a
23	153.3	-	
24	108.9	-	
25	167.3	-	
		NH, 8.37 s	23, 24, 25, 26, 29, 30
26	40.1	-	
27	15.2	a 1.30 m b 1.36 m	27b, 28a, 28b 26, 28, 29 27a, 28a, 28b 26, 28, 29
28	15.2	a 1.30 m b 1.36 m	27a, 27b, 28b 26, 27, 29 27a, 27b, 28a 26, 27, 29
29	159.3	-	
30	102.8	6.03 s	23, 24, 26, 29, 31
31	162.2	-	
32	46.0	a 4.56 d (18.0) b 4.67 d (16.8)	23, 31, 33 23, 31, 33
33	169.7	-	



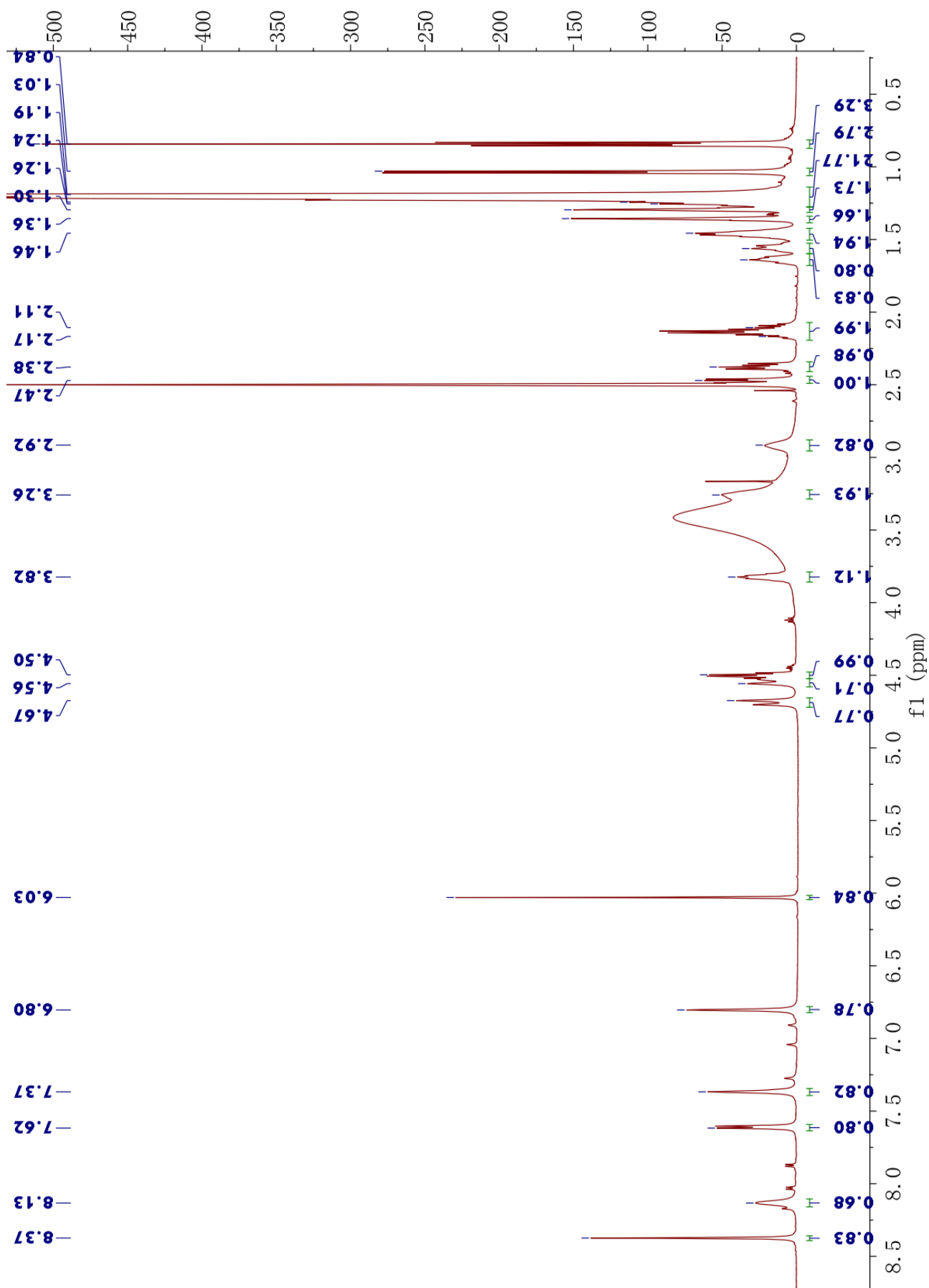
UV spectrum of **6**.



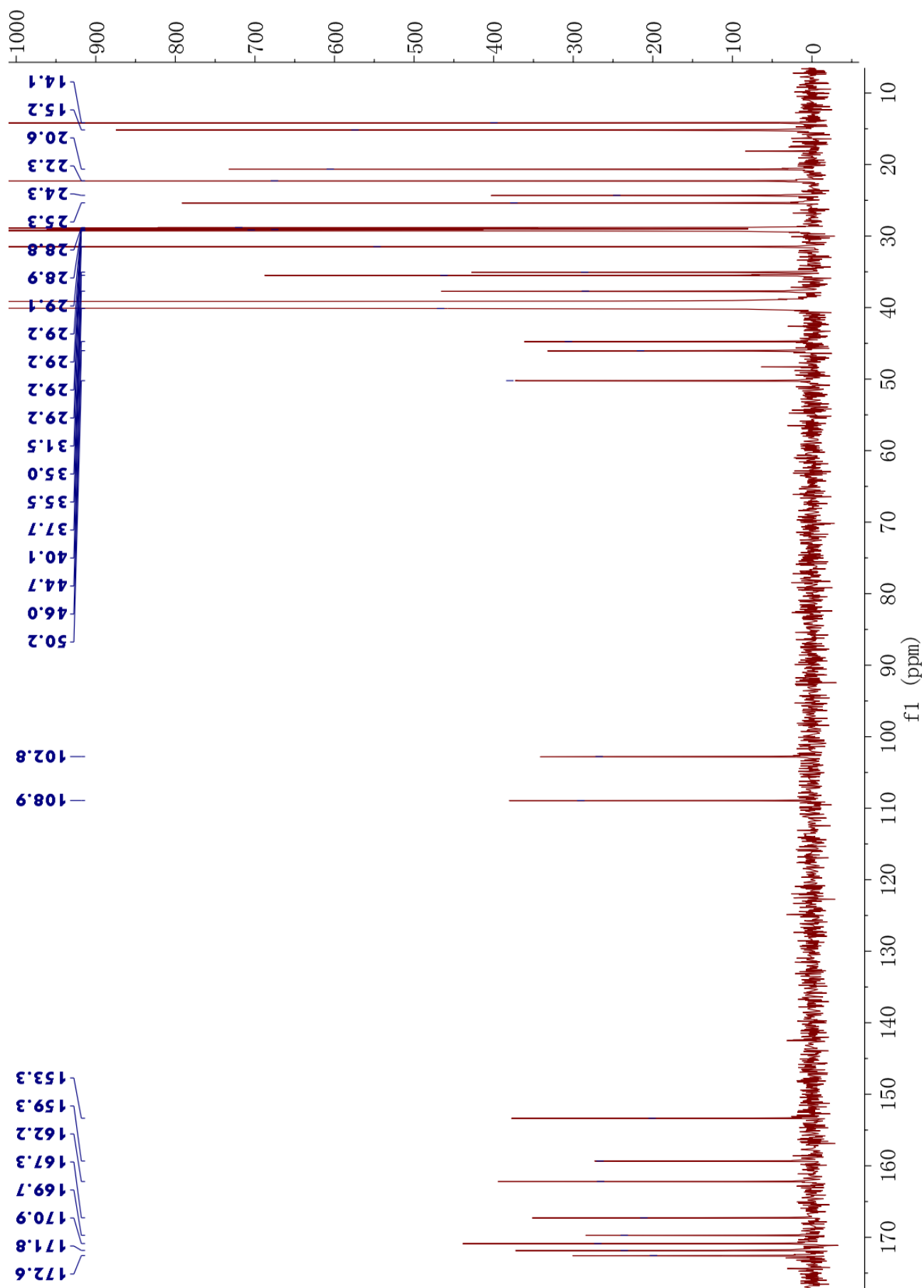


NMR-based key correlations for the structural assignment of **6** as compared to the structure of known **7** (precolibactin-712).

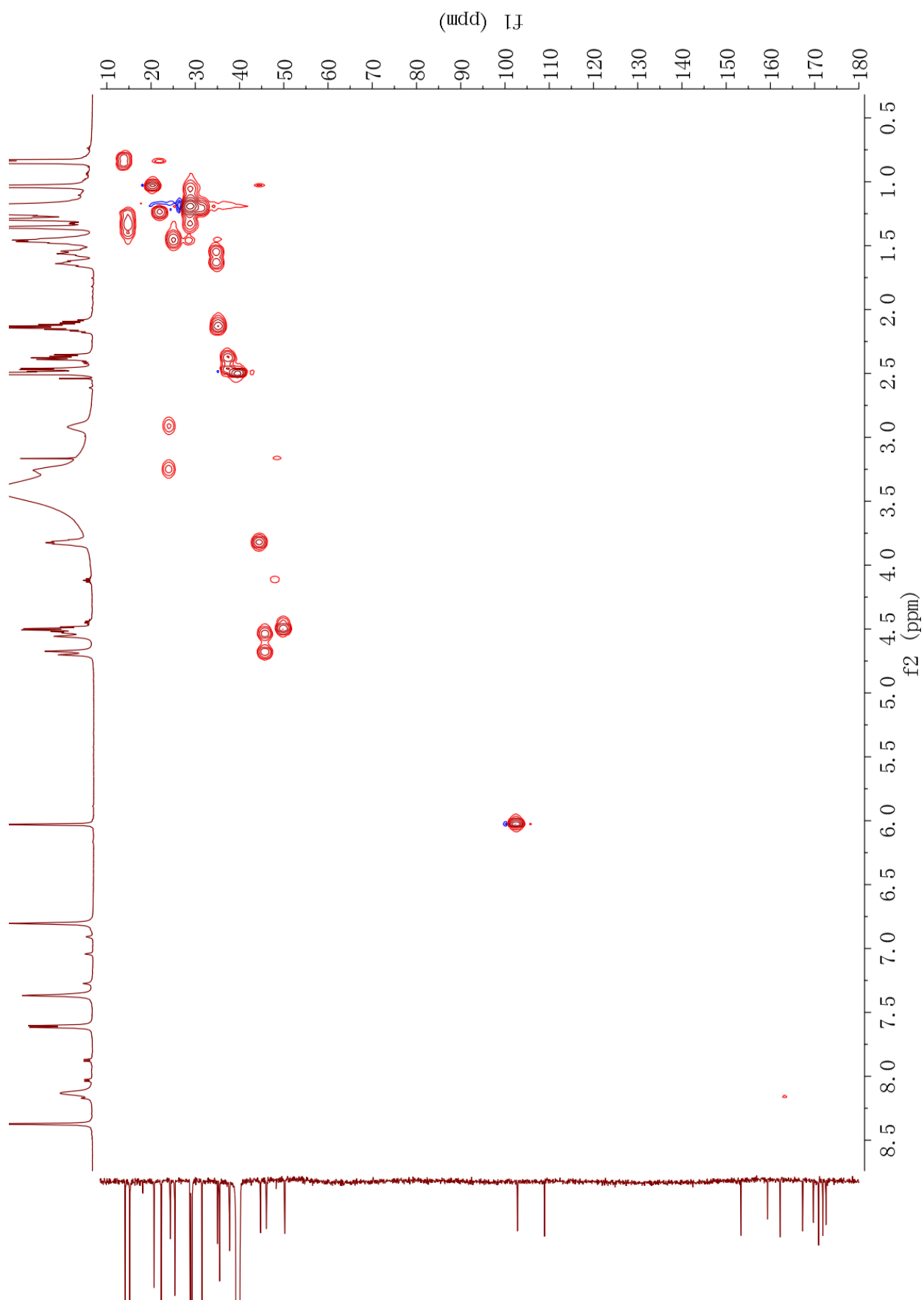
**Note for structure elucidation of 6:** Compound **6** was obtained as white and amorphous powder. The molecular formula was determined as  $C_{33}H_{51}N_5O_7$  based on the HRESIMS analysis ( $m/z$  630.3842  $[M + H]^+$ , calcd 630.3867). A comparison of the 1D and 2D NMR spectra of **6** and **7** indicated that **6** contains the same *N*-myristoyl-D-asparagine residue and 1*H*-pyrrolo[3,4-*c*]pyridine-3,6(2*H*,5*H*)-dione unit as in **7**. However, the thiazole proton resonance at  $\delta_H$  8.09 (H-34), and the carbon signals at  $\delta_C$  164.2 (C-33),  $\delta_C$  126.9 (C-34) and  $\delta_C$  151.7 (C-35) in **7** were not observed in **6**, suggesting an absence of the thiazole ring in **6**. Instead, C-32 ( $\delta_C$  46.0) in **6** was observed to connect the 1*H*-pyrrolo[3,4-*c*]pyridine-3,6(2*H*,5*H*)-dione unit and the terminal carboxyl group, as evidenced by the HMBC correlations from the methylene protons at  $\delta_H$  4.56 (H-32) to the carbon signals at C-23 ( $\delta_C$  153.3), C-31 ( $\delta_C$  162.2) and C-33 ( $\delta_C$  169.7). As an intermediate from the *clb* pathway, **6** can be envisioned to be released from the first NRPS module of ClbJ, which would fill the gap between previously isolated precolibactin-546 (**5**) and precolibactin-712 (**7**).



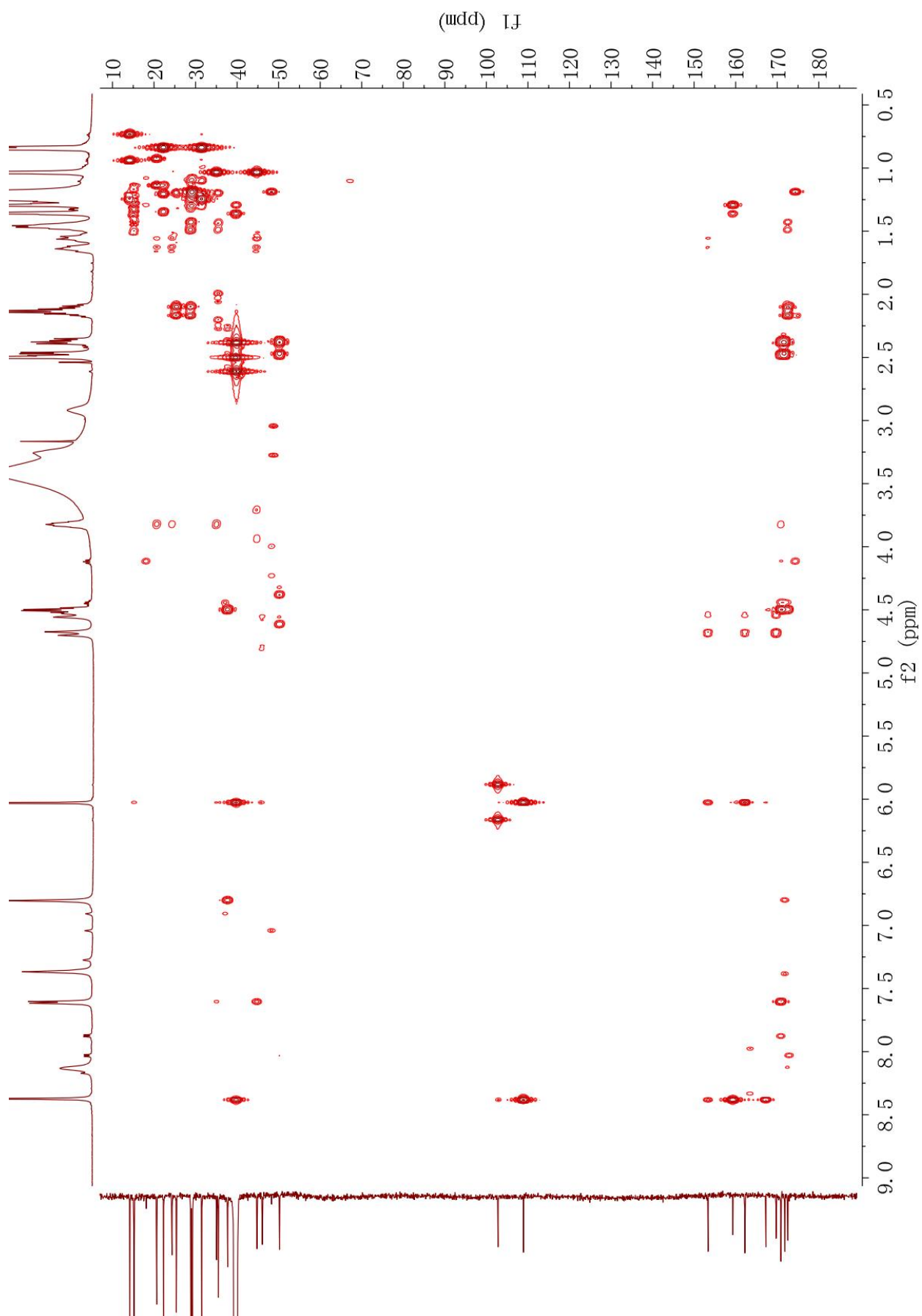
$^1\text{H}$  NMR spectrum of **9** (recorded in  $\text{DMSO-}d_6$  at 600 MHz).



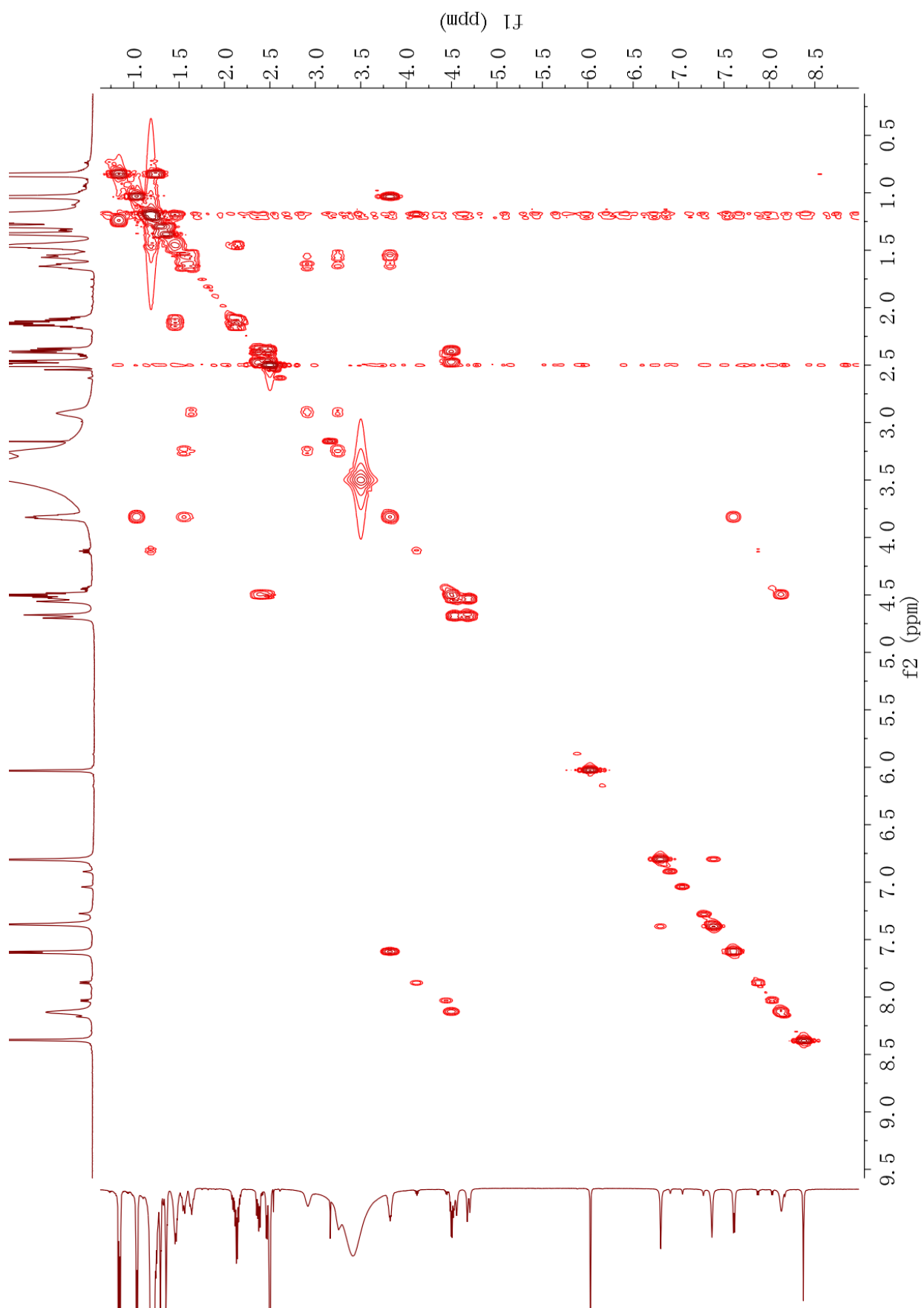
<sup>13</sup>C NMR spectrum of **9** (recorded in DMSO-*d*<sub>6</sub> at 150 MHz).

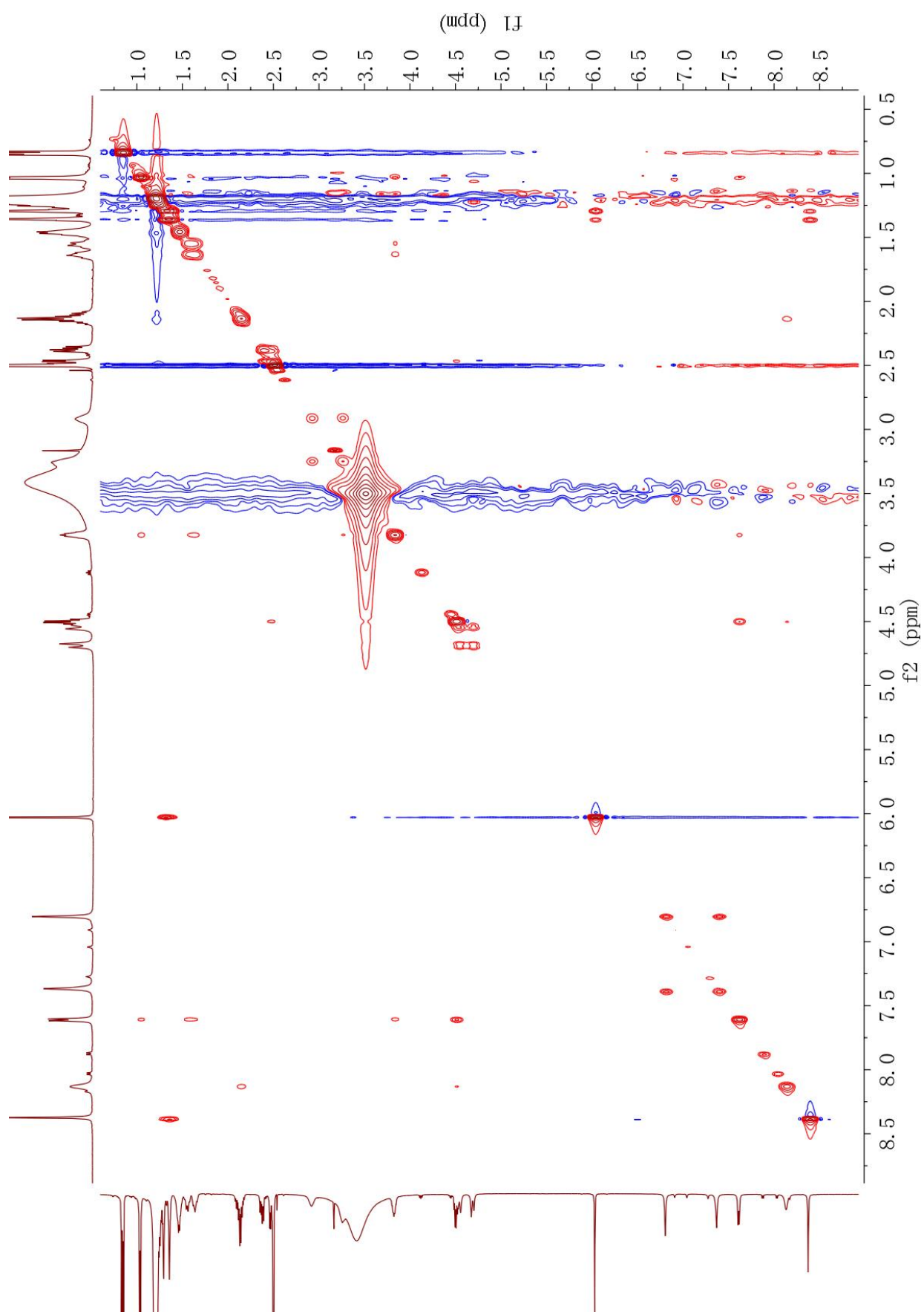


$^1\text{H}$ - $^{13}\text{C}$  HSQC spectrum of **6** (recorded in  $\text{DMSO}-d_6$  at 600 MHz).



$^1\text{H}$ - $^{13}\text{C}$  HMBC spectrum of **6** (recorded in  $\text{DMSO}-d_6$  at 600 MHz).





$^1\text{H}$ - $^1\text{H}$  NOESY spectrum of **6** (recorded in  $\text{DMSO}-d_6$  at 600 MHz).