catalysts

Supplementary Materials: Production of New Isoflavone Glucosides from Glycosylation of 8-Hydroxydaidzein by Glycosyltransferase from *Bacillus subtilis* ATCC 6633

Chien-Min Chiang [1], Tzi-Yuan Wang [2], Szu-Yi Yang [3], Jiumn-Yih Wu [4,*] and Te-Sheng Chang [3,*]

Table S1. NMR spectroscopic data for compound (**1**)/(**2**) (in DMSO-d_6; 700MHz).

Compound	8OHDe [a]		8OHDe-7-*O*-β-glucoside Compound (**1**)		8OHDe-8-*O*-β-glcoside Compound (**2**)		
Position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	HMBC
Isoflavone moiety							
2	152.66	8.34, s	153.7	8.40, s	153.0	8.32, s	
3	122.67		123.6		123.3		H-2, H-2′(6′)
4	175.13		175.7		175.0		H-2, H-5
5	115.63	7.46, d (8.6)	115.2	7.52, d (8.9)	121.5	7.76, d (8.8)	
6	114.13	6.95, d (8.6)	114.1	7.30, d (8.9)	115.5	7.02, d (8.8)	H-5,
7	149.91	10.32 OH [d]	149.0		154.6		H-5, H-6 [b]
8	132.86	9.44 OH [d]	136.0		131.9		H-6, [c]
8a	146.69		146.3		150.6		H-2, H-5
4a	117.43		120.1		117.4		H-6,
1′	122.90		122.9		122.6		H-3′(5′)
2′(6′)	130.13	7.39, d (8.6)	130.6	7.40, d (8.1)	130.2	7.38, d (7.9)	H-2′(6′), H-3′(5′)
3(5′)	114.91	6.81, d (8.6)	115.4	6.82, d (8.1)	115.1	6.81, d (7.9)	H-3′(5′)
4′	157.11	-	157.6	-	157.3	-	H-2′(6′), H-3′(5′)
		9.53 OH [d]		9.63 OH [d]		9.63 OH [d]	
Glucose moiety							
1″			102.2	4.91, d (7.6)	104.5	4.93, d (7.7)	H-2″
2″			73.7	3.38, m	74.1	3.36, m	H-1″, H-3″
3″			76.1	3.32, m	76.4	3.27, m	H-1″, H-2″, H-4″
				5.20 OH [d]		5.21 OH [d]	
4″			70.1	3.21, m	69.8	3.18, m	H-3″, H-5″, H-6″
				5.14 OH [d]		5.07 OH [d]	
5″			77.7	3.41, m	77.4	3.18, m	H-1″, H-4″, H-6″
6″			61.1	3.73, m	60.9	3.63, m	H-4″, H-5″
				3.50, m		3.47, m	
				4.70 OH [d]		4.50 OH [d]	

(a) Goto, H.; Terao, Y.; Akai, S. Synthesis of various kinds of isoflavones, isoflavanes, and biphenyl-ketones and their 1,1-diphenyl-2-picrylhydrazyl radical-scavenging activities. *Chem. Pharm. Bull.* **2009**, *57*, 346-360.
(b) A correlation between Glc-H-1″ and C-7 observed in HMBC spectrum of compound (**1**).
(c) A correlation between Glc-H-1″ and C-8 observed in HMBC spectrum of compound (**2**).
(d) OH indicates the hydroxy proton.

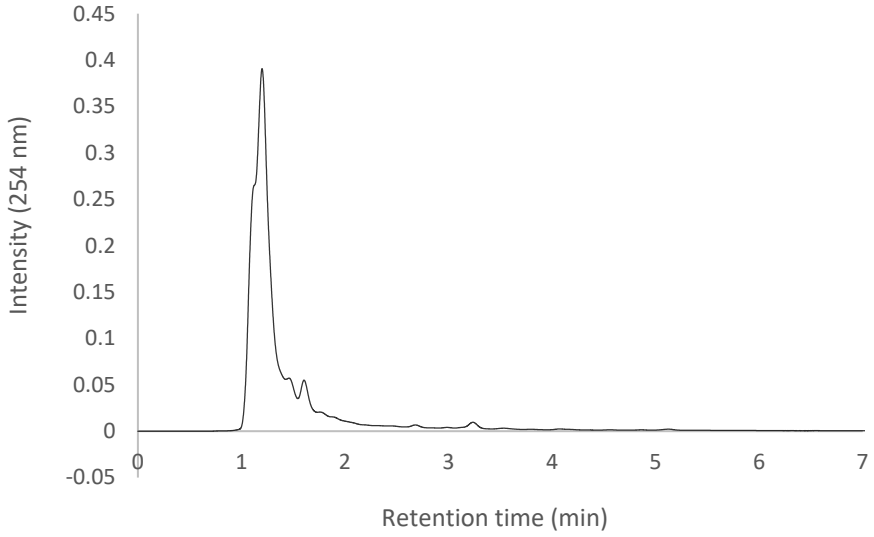


Figure S1. UPLC analysis of the 24 h fermentation broth by *B. subtilis* ATCC 6633 without adding of 8-OHDe.

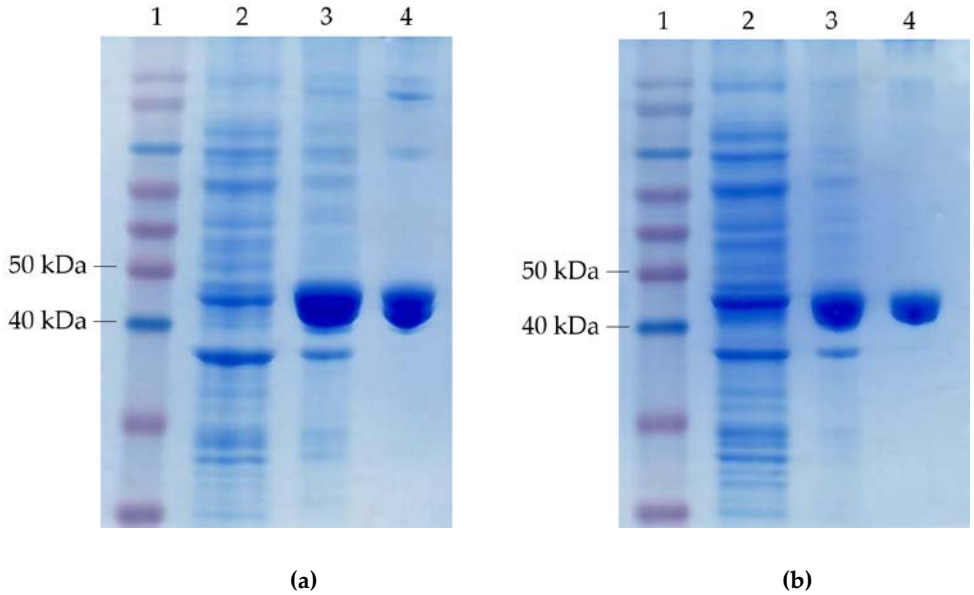
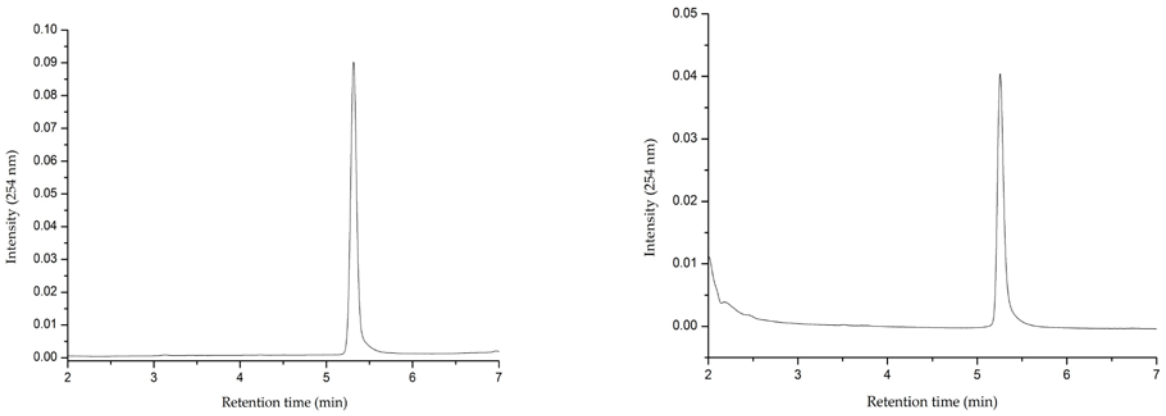


Figure S2. SDS-PAGE analysis of expressed and purified proteins from recombinant *E. coli* harboring pETDuet-BsGT292 (**a**) and pETDuet-BsGT292 (**b**). Lane 1: molecular marker; lane 2: total protein before induction; lane 3: total protein after 20 h induction; lane 4: purified protein.



(a) **(b)**

Figure S3. Biotransformation of 8-OHDe by the purified BsGT110 (**a**) and BsGT296 (**b**). Two micrograms of the purified BsGT292 (**a**) or BsGT296 (**b**) was incubated with 0.4 mM uridine diphosphate (UDP)-glucose and 0.02 mg/mL of 8-OHDe in the presence of 50 mM Tris at pH 8.0 and 10 mM of $MgCl_2$ at 40 °C for 30 min. After the reaction, the mixtures were analyzed with ultra-performance liquid chromatography (UPLC). The UPLC operation conditions are described in Materials and Methods.

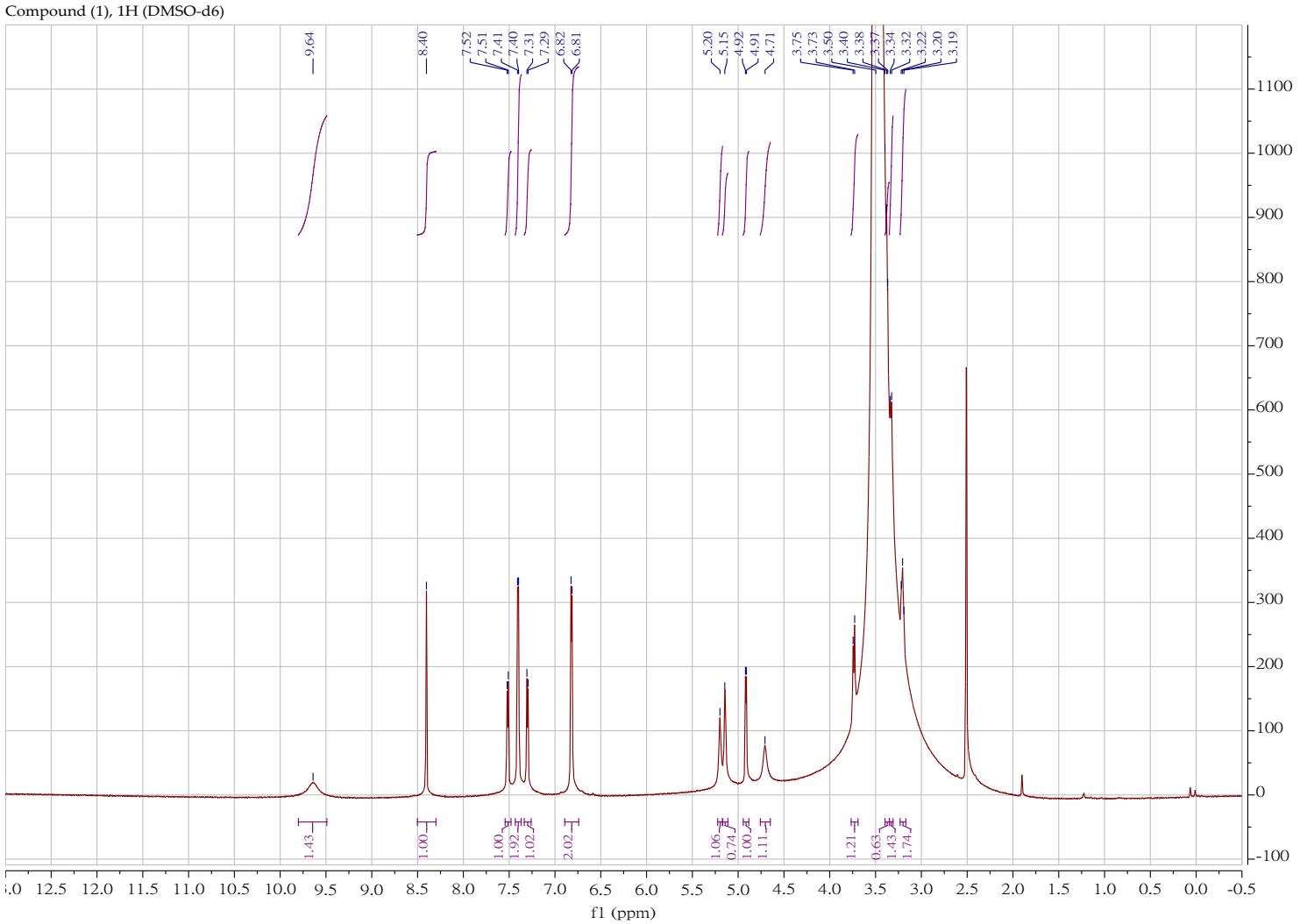


Figure S4. The 1H-NMR (700 MHz, DMSO-*d*6) spectrum of compound (**1**).

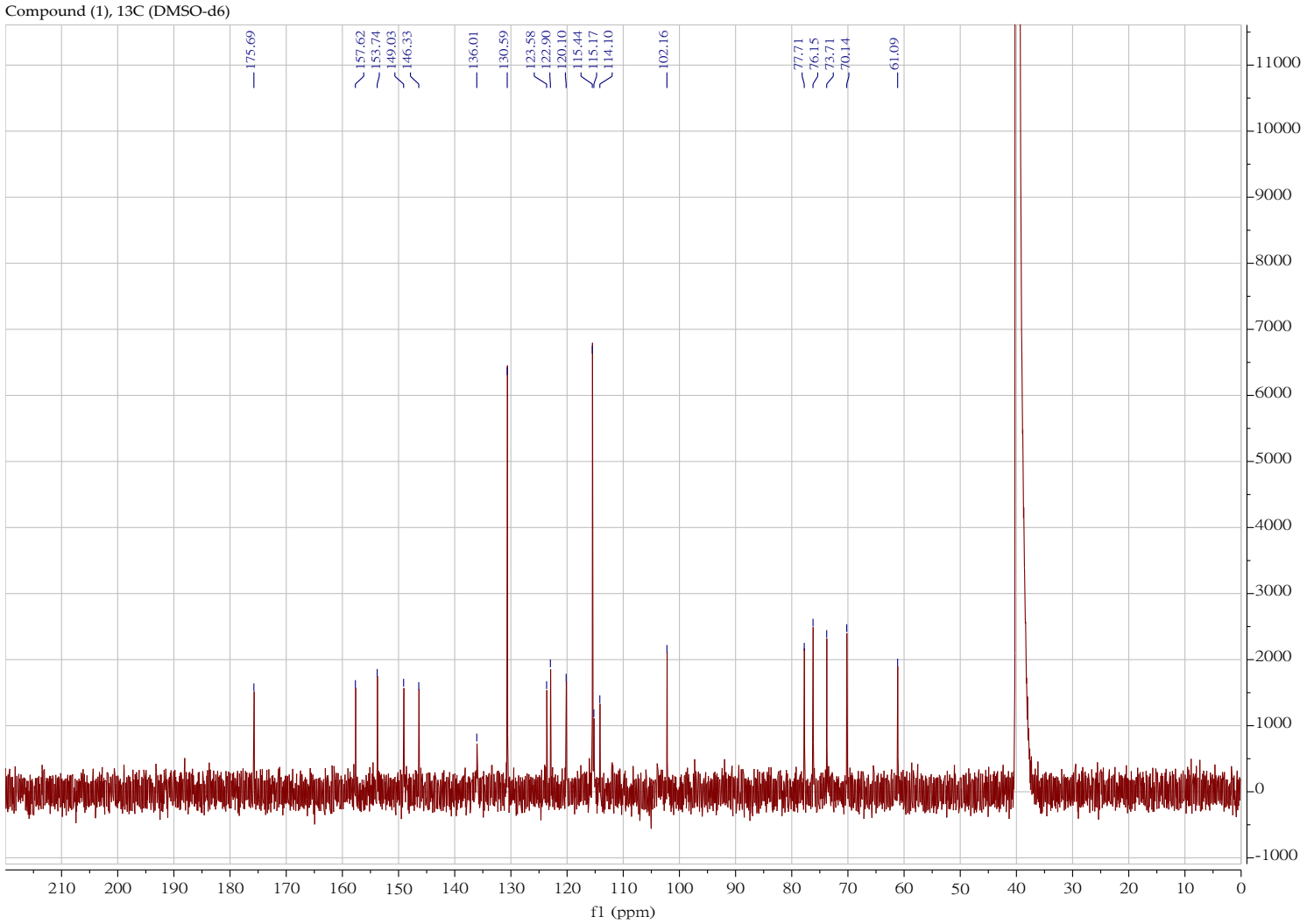


Figure S5. The 13C-NMR (176 MHz, DMSO-*d*6) spectrum of compound (**1**).

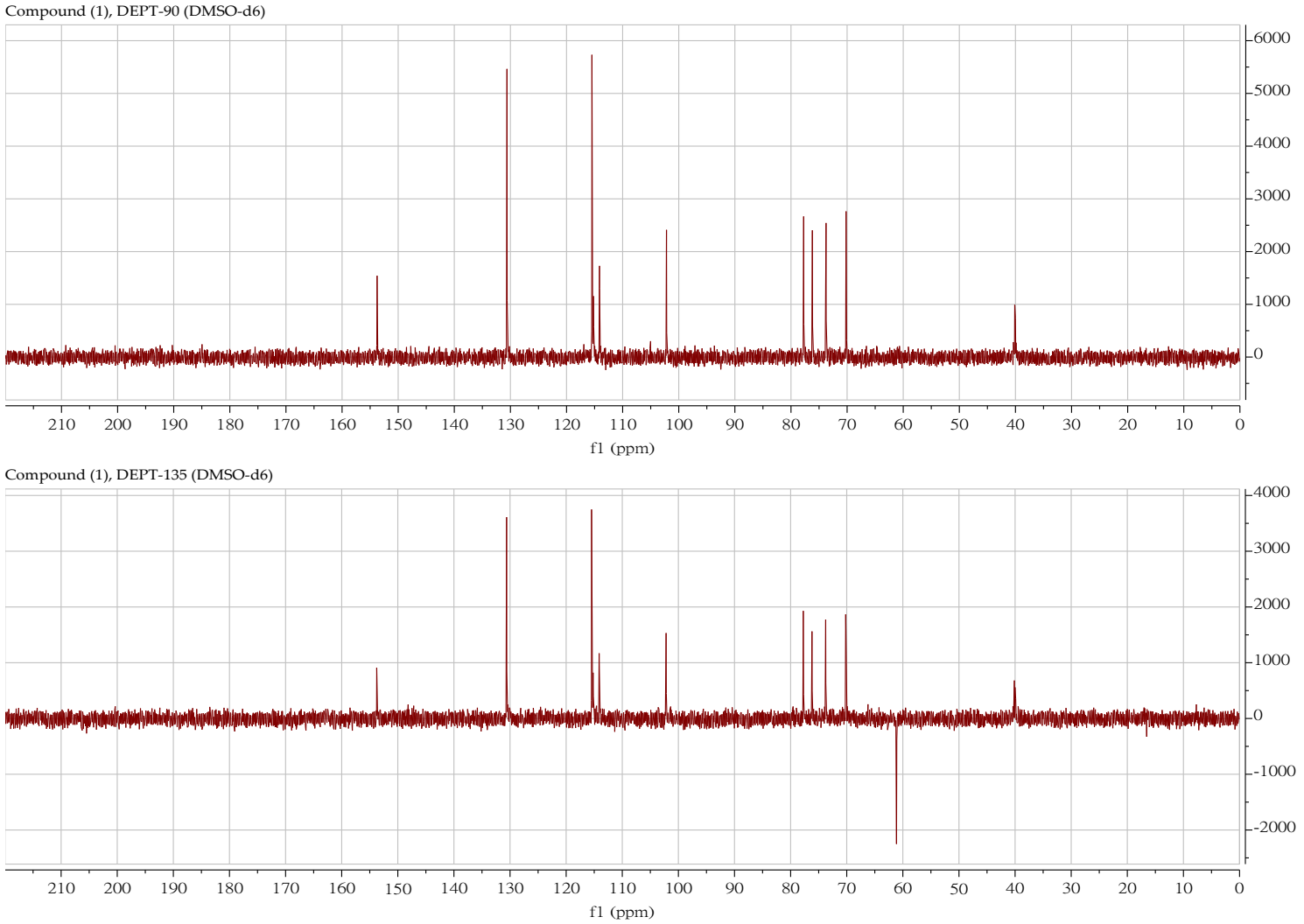


Figure S6. The DEPT-90 and DEPT-135 (176 MHz, DMSO-*d*6) spectra of compound (**1**).

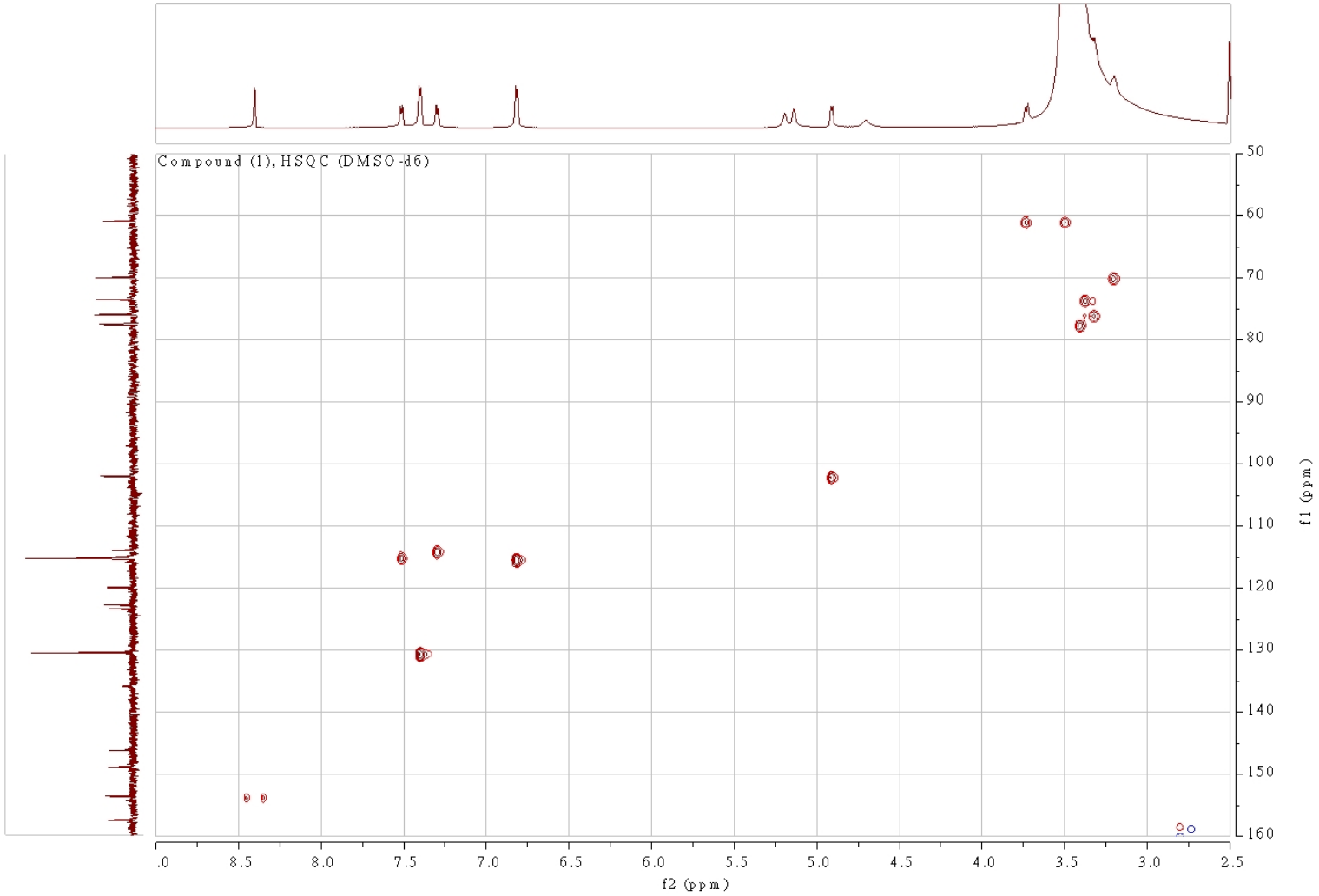


Figure S7. The HSQC (700 MHz, DMSO-*d*6) spectrum of compound (**1**).

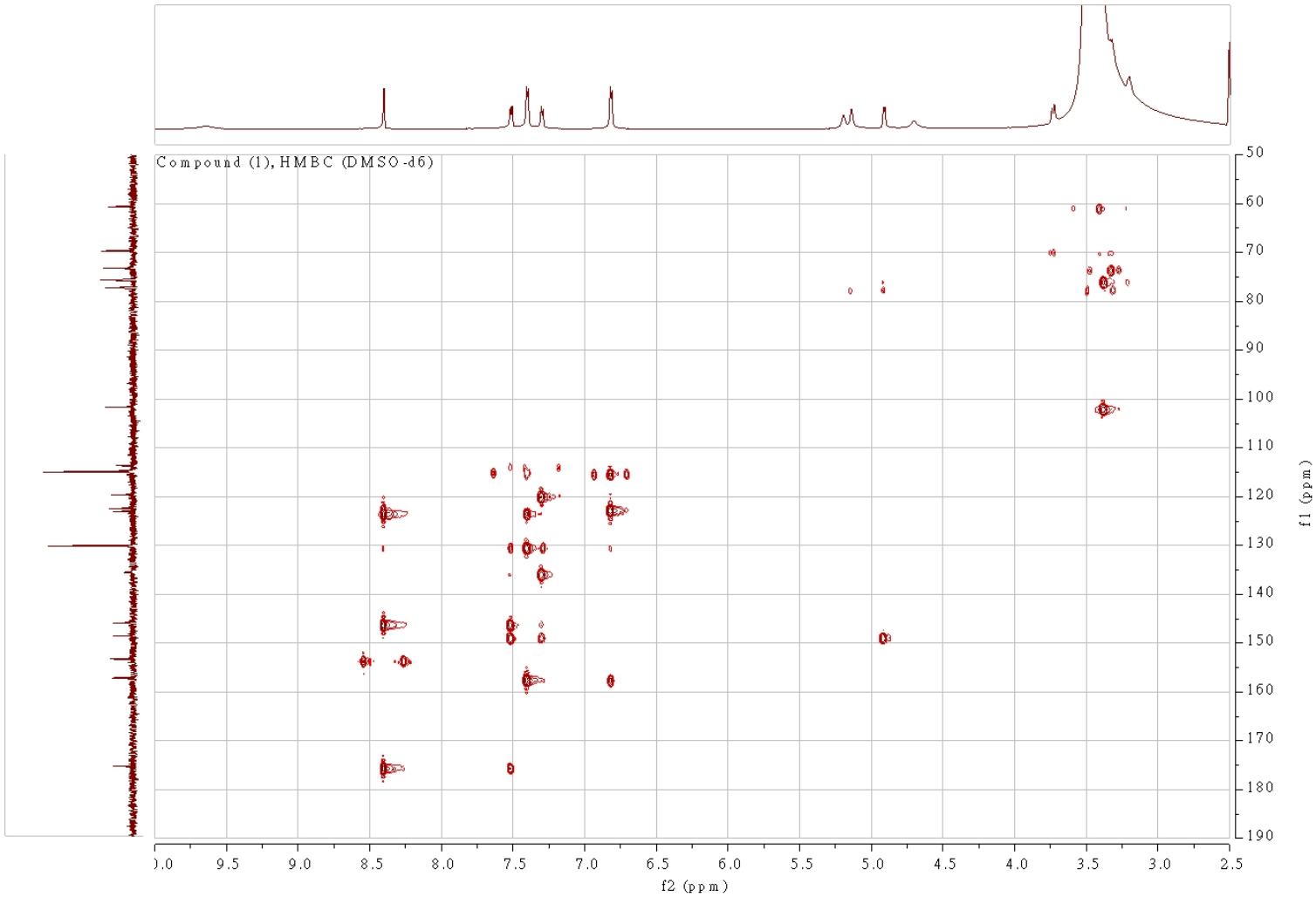


Figure S8. The HMBC (700 MHz, DMSO-*d*6) spectrum of compound (**1**).

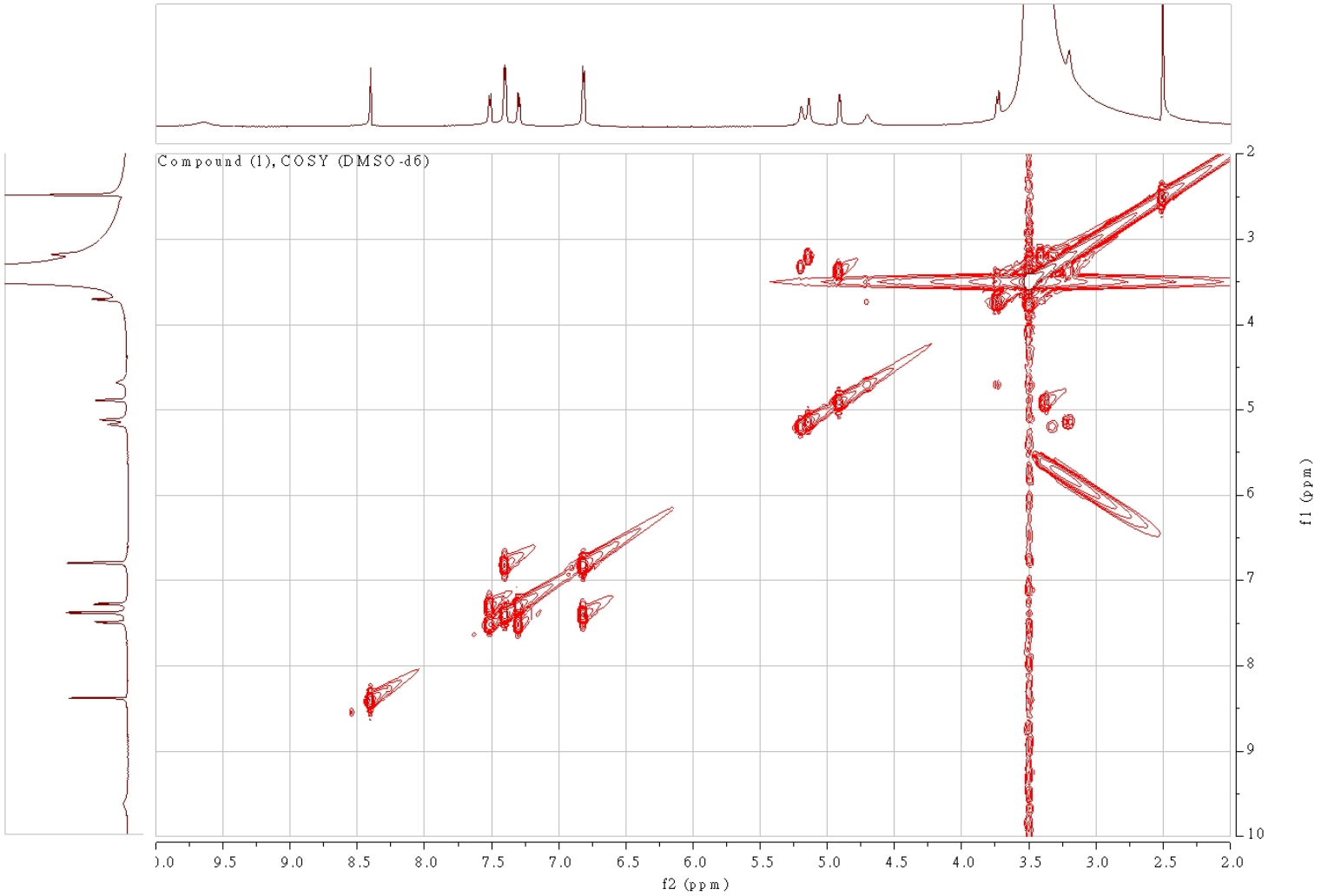


Figure S9. The H-H COSY (700 MHz, DMSO-*d*6) spectrum of compound (**1**).

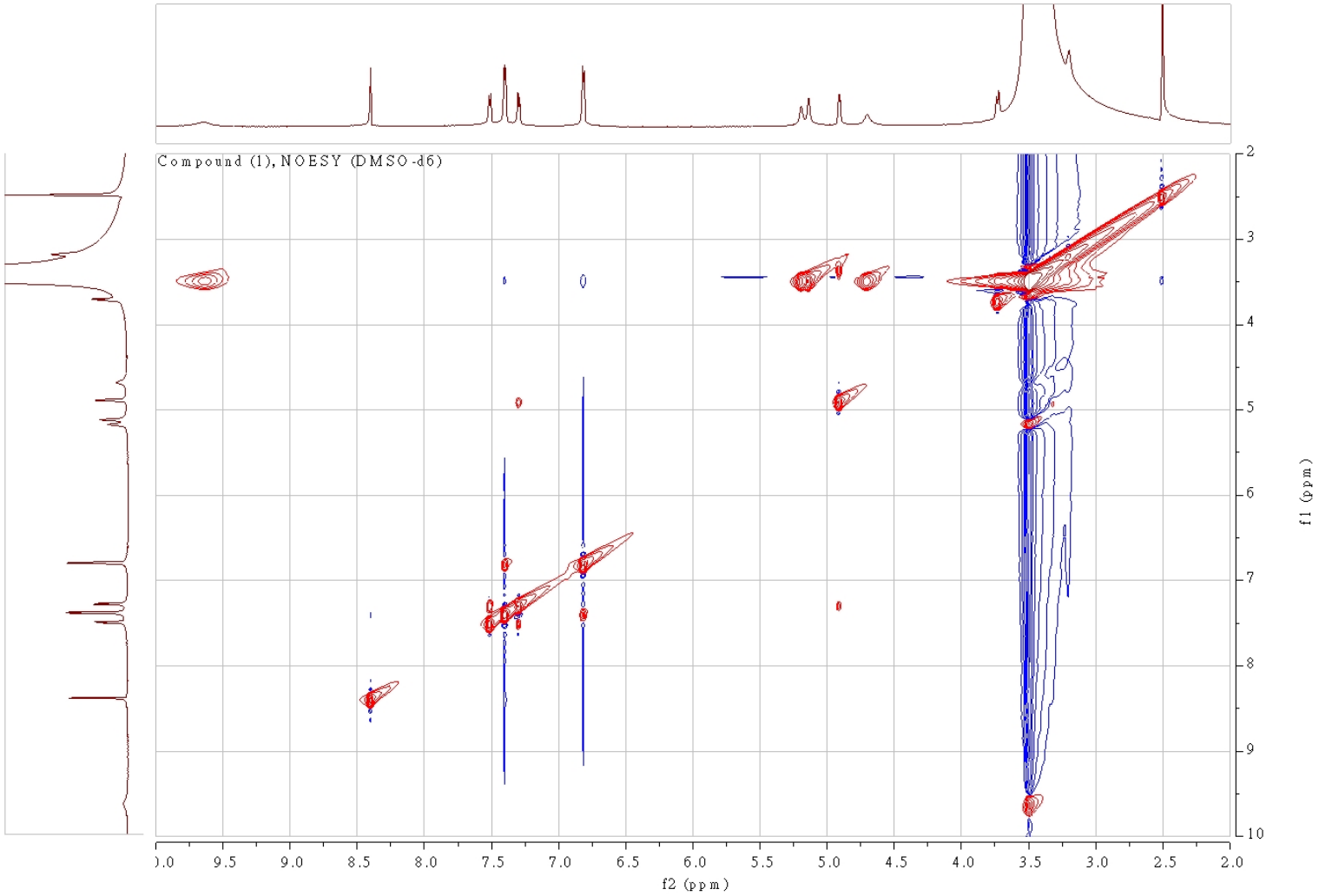


Figure S10. The H-H NOESY (700 MHz, DMSO-*d*6) spectrum of compound (**1**).

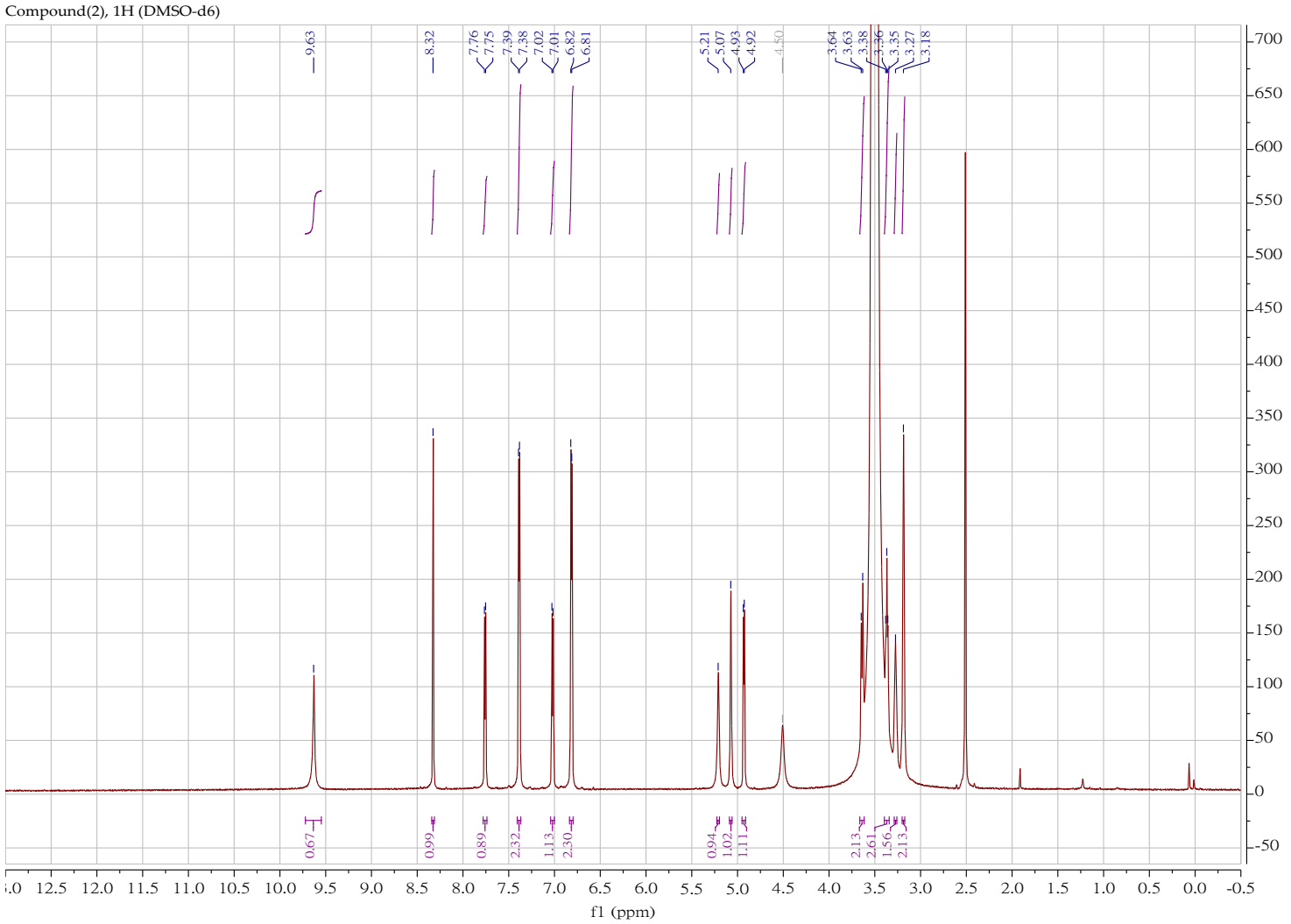


Figure S11. The 1H-NMR (700 MHz, DMSO-*d*6) spectrum of compound (**2**).

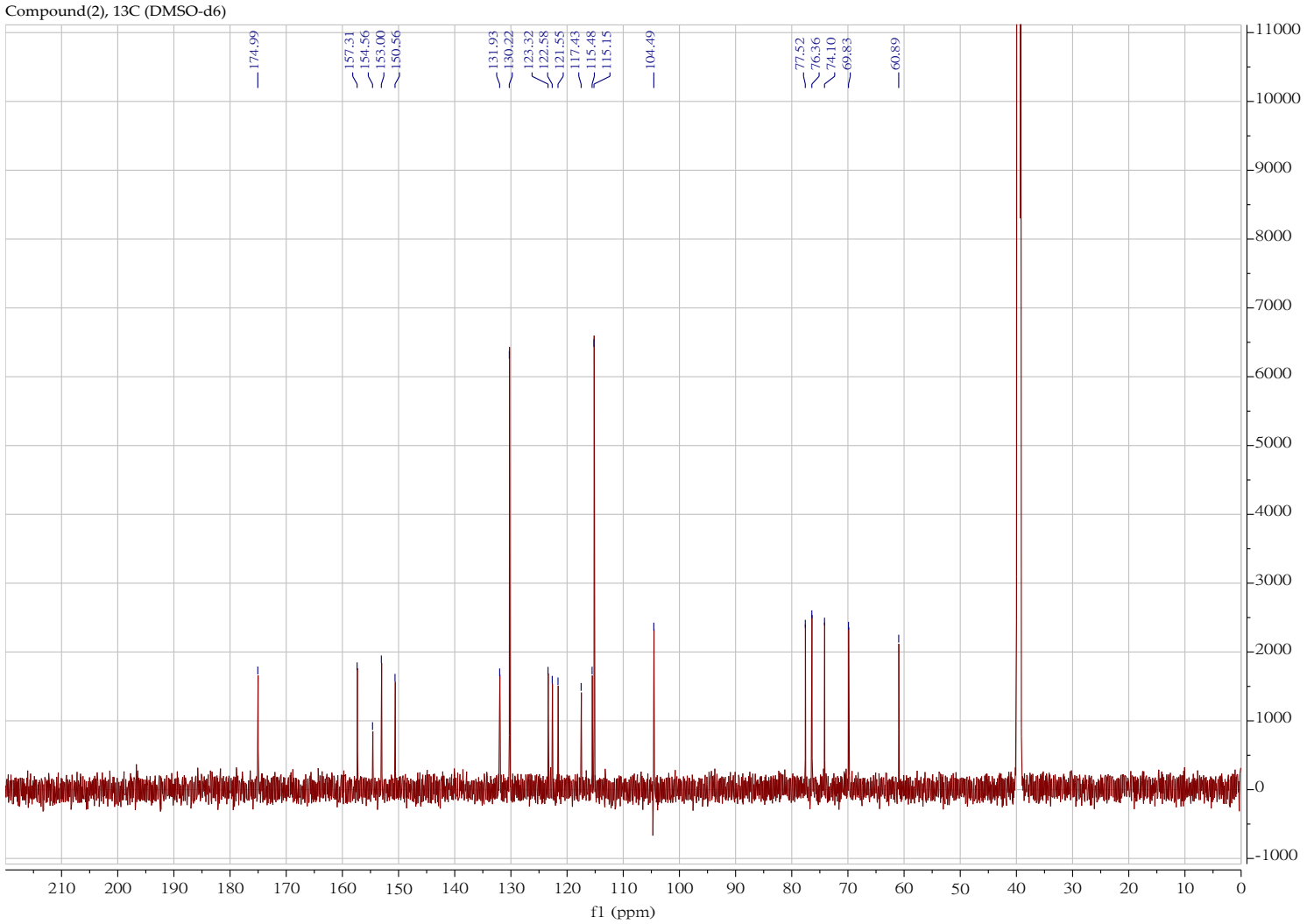


Figure S12. The 13C-NMR (176 MHz, DMSO-*d*6) spectrum of compound (**2**).

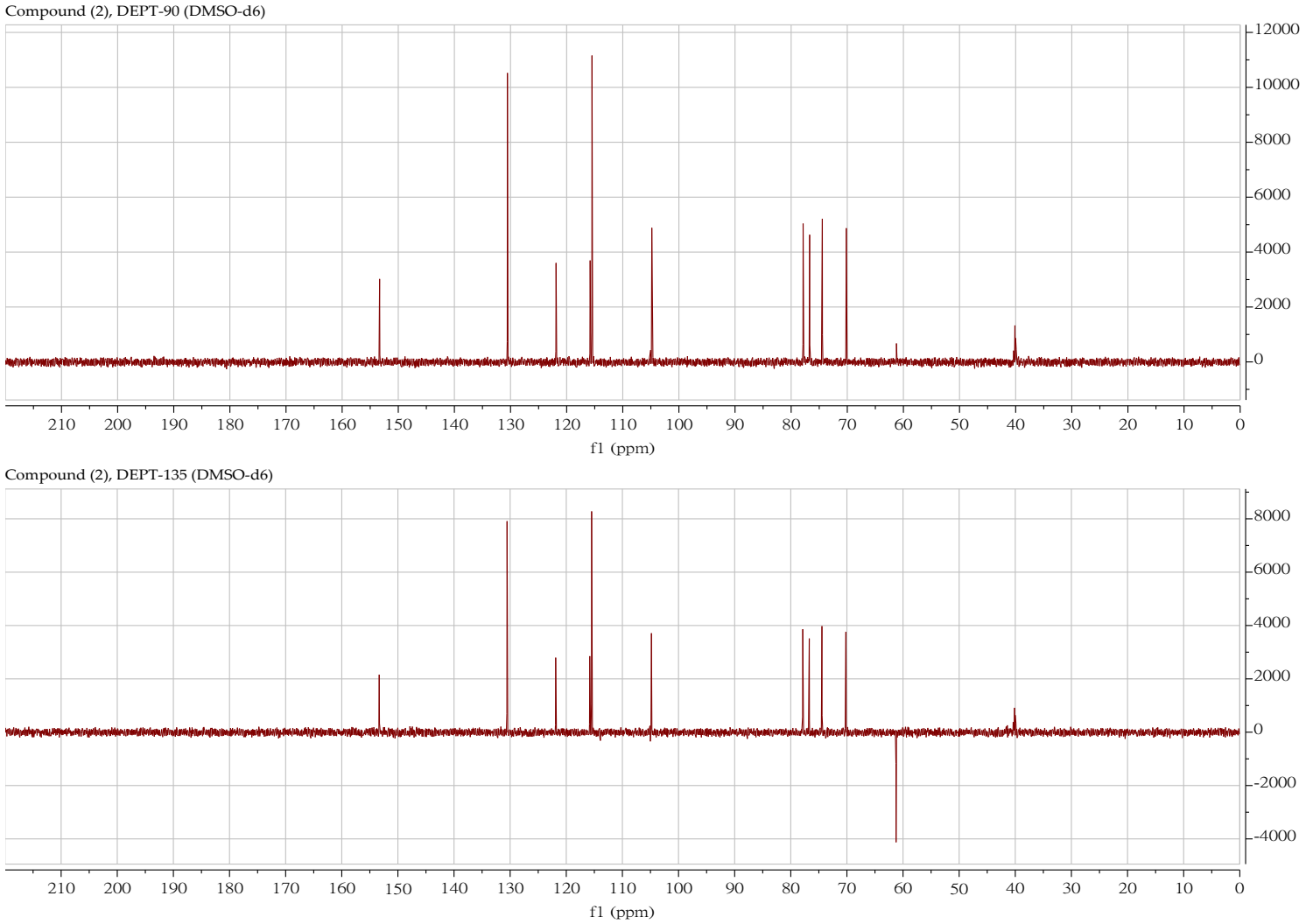


Figure S13. The DEPT-90 and DEPT-135 (176 MHz, DMSO-*d*6) spectra of compound (**2**).

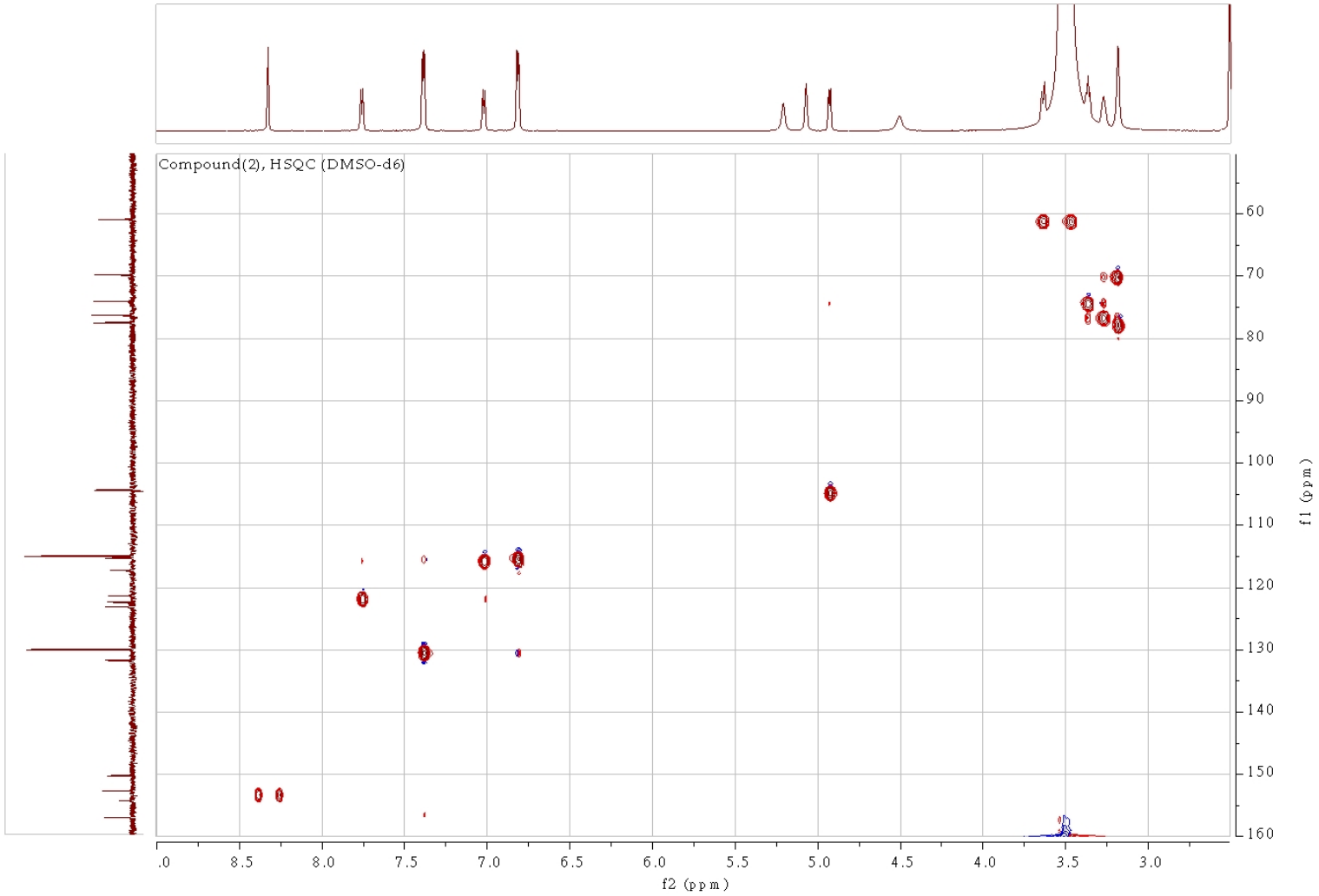


Figure S14. The HSQC (700 MHz, DMSO-*d*6) spectrum of compound (**2**).

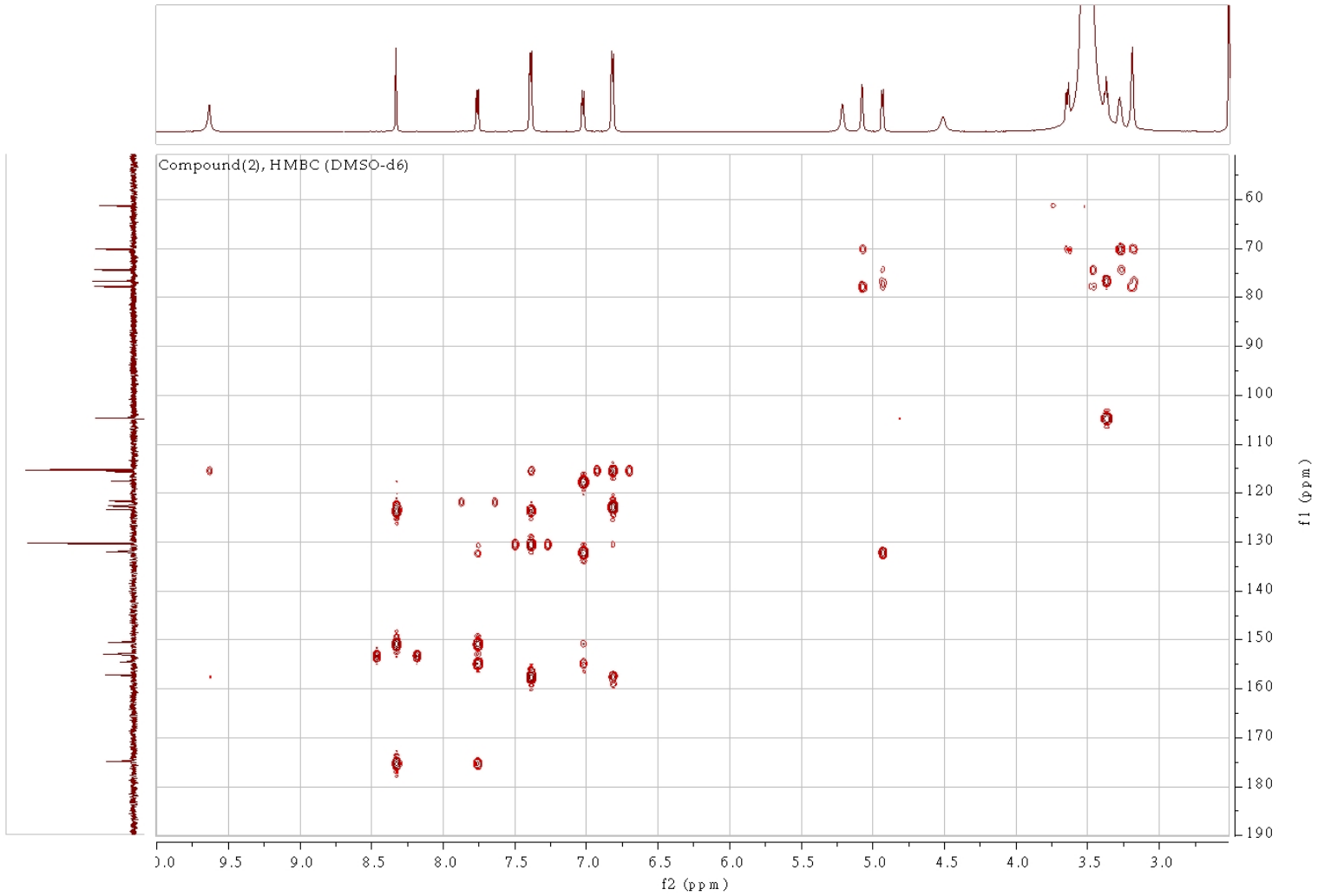


Figure S15. The HMBC (700 MHz, DMSO-*d*6) spectrum of compound (**2**).

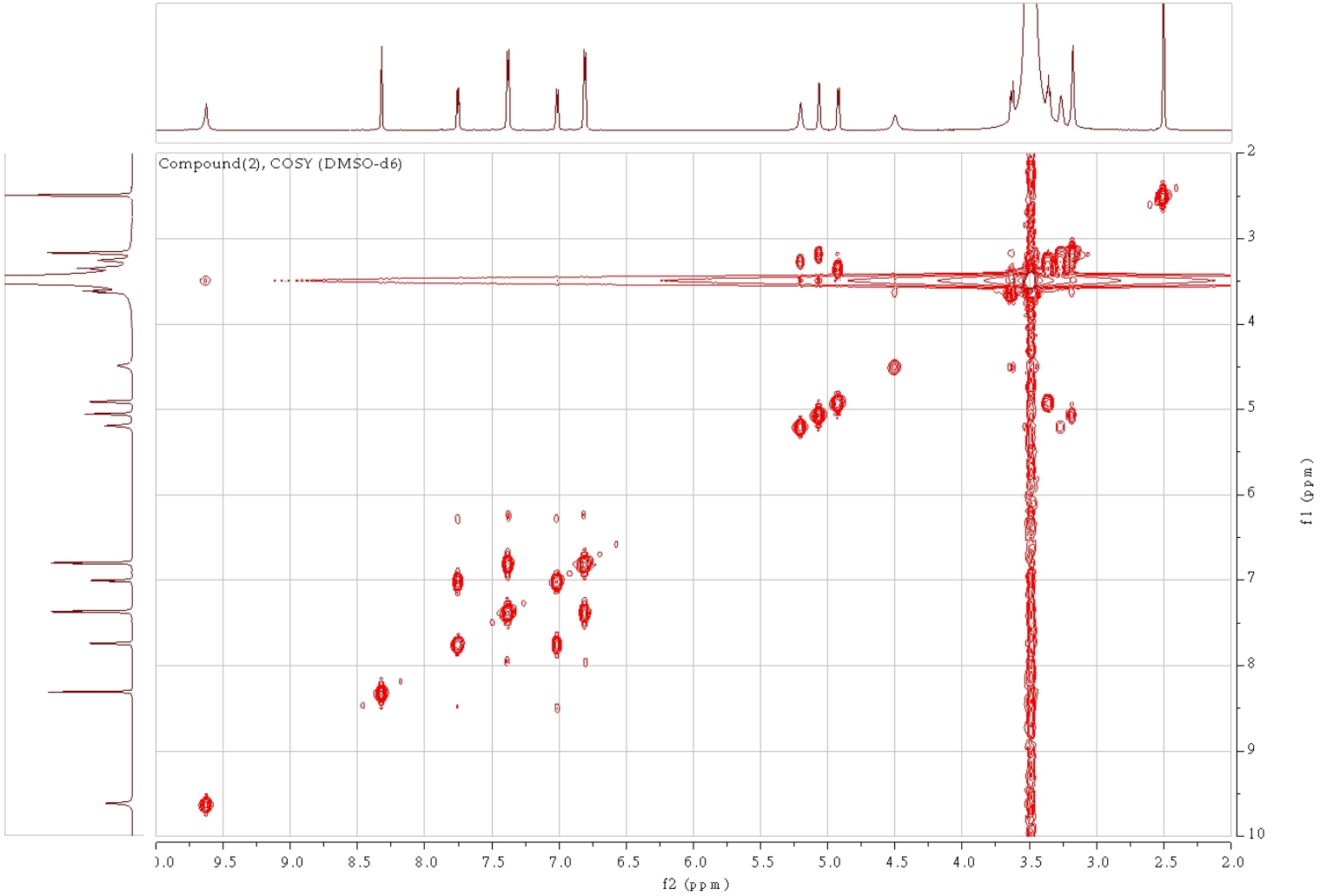


Figure S16. The H-H COSY (700 MHz, DMSO-*d*6) spectrum of compound (**2**).

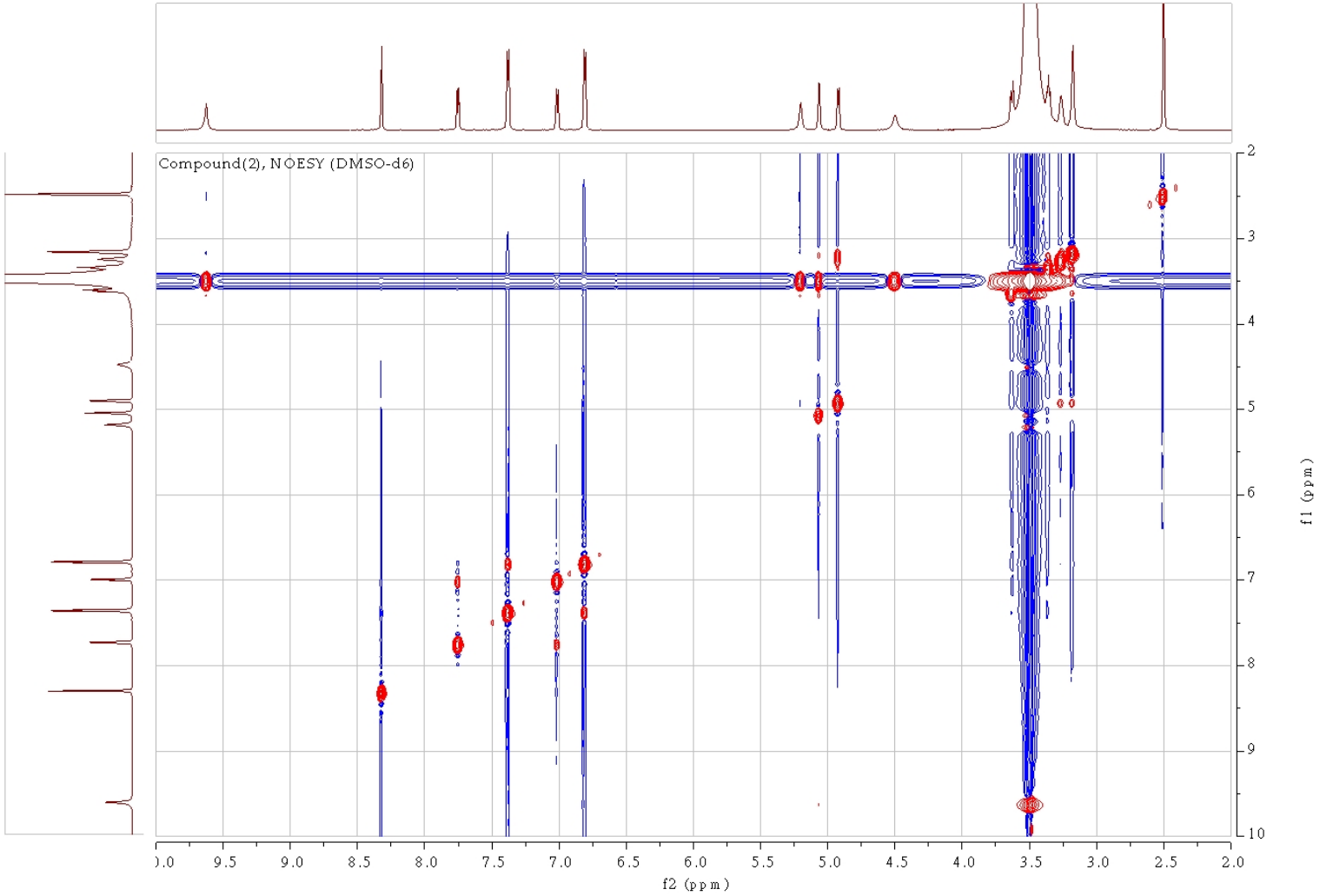


Figure S17. The H-H NOESY (700 MHz, DMSO-*d*6) spectrum of compound (**2**).