

SUPPLEMENTARY MATERIAL

Three new bioactive diterpenoids from the roots of *Croton crassifolius*

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ABSTRACT

Phytochemical investigation of *Croton crassifolius* led to the isolation of two new halimane diterpenoids (**1** and **2**), a new *nor*-clerodane diterpenoid (**3**), along with three known analogues (**4–6**). Their structures including absolute configurations were elucidated by spectroscopic analysis, single-crystal X-ray diffraction, and CD analysis. All isolates were evaluated for their inhibitory effects on the nitric oxide (NO) production induced by lipopolysaccharide (LPS) in RAW264.7 macrophage cells, and compound **1** exhibited moderate inhibition of NO production with an IC₅₀ value of 25.8 ± 0.9 μM.

Keywords: *Croton crassifolius*; halimane diterpenoids; clerodane diterpenoids; NO production; single-crystal X-ray diffraction

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Table S1. ^1H (500 MHz) and ^{13}C (125 MHz) NMR data for **1–3** (δ in ppm) in CDCl_3 .

No.	1		2		3	
	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}
1	a 2.50, m b 2.16, m	24.7	a 2.34, m b 2.19, m	24.7	a 2.20, m b 2.14, m	25.0
2	a 2.22, m b 1.97, m	24.5	a 2.17, m b 1.95, m	23.3	a 2.11, m b 1.80, m	23.7
3	4.77, dd (12.1, 3.8)	72.6	5.19, dd (10.8, 3.9)	71.6	a 2.31, m b 2.12, m	19.1
4		57.1		52.8		125.3
5		135.9		131.8		166.7
6	4.26, m	74.3	4.18, m	63.6	4.98, br d (13.7)	77.0
7	α 1.93, m β 1.86, m	34.0	α 1.99, m β 1.61, m	35.9	α 1.24, m β 2.52, m	34.8
8	1.80, m	36.2	2.02, m	33.9	2.26	38.3
9		52.3		52.9		48.3
10		130.6		136.4	3.13, br d (12.3)	38.4
11	a 2.77, dd (14.0, 8.7) b 2.27, dd (14.0, 8.7)	40.0	a 2.86, dd (14.0, 8.8) b 2.35, dd (14.0, 5.8)	41.3	a 3.23, d (17.8) b 3.08, d (17.8)	48.1
12	5.48, t (8.7)	72.4	5.48, dd (8.8, 5.8)	72.0		191.1
13		124.7		125.8		127.1
14	6.42, br s	108.0	6.38, br s	108.0	6.71, br s	108.5
15	7.46, br s	144.2	7.45, br s	144.2	7.44, br s	144.4
16	7.48, br s	139.6	7.44, br s	139.0	8.01, br s	146.9
17	1.10, d (6.8)	16.1	1.02, d (6.9)	15.4	1.03, d (6.8)	19.3
18		170.9		172.2		173.4
19	α 3.62, d (9.0) β 4.62, d (9.0)	75.2	a 4.65, d (12.0) b 4.28, d (12.0)	62.0		
20		176.6		176.2		173.3
OMe-18	3.77, s	52.7	3.71, s	52.3		
OMe-20					3.67	51.5
OAc-3	2.04, s	20.9	2.03, s	20.7		
		170.1		170.0		
OAc-19			2.06, s	21.0		
				170.8		

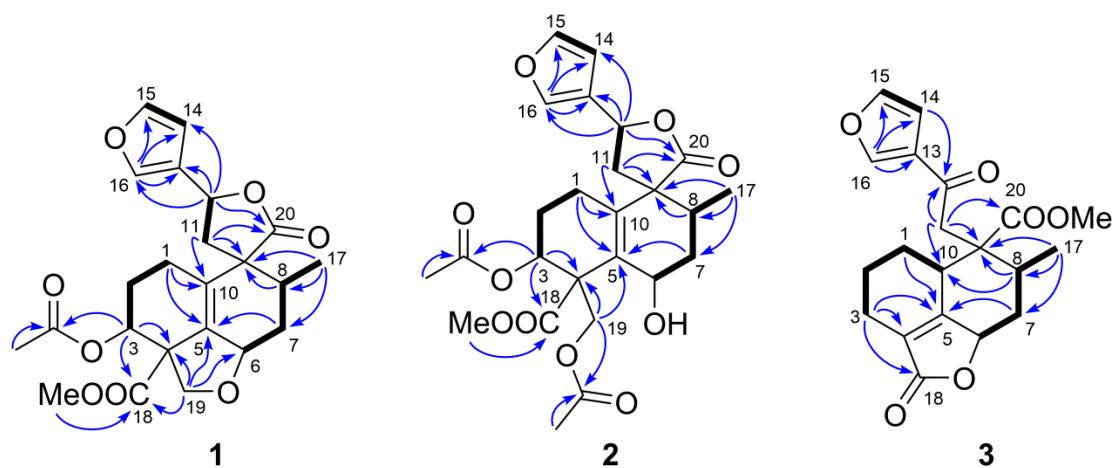


Figure S1. Key ^1H - ^1H COSY (—) and HMBC (—) correlations of 1–3.

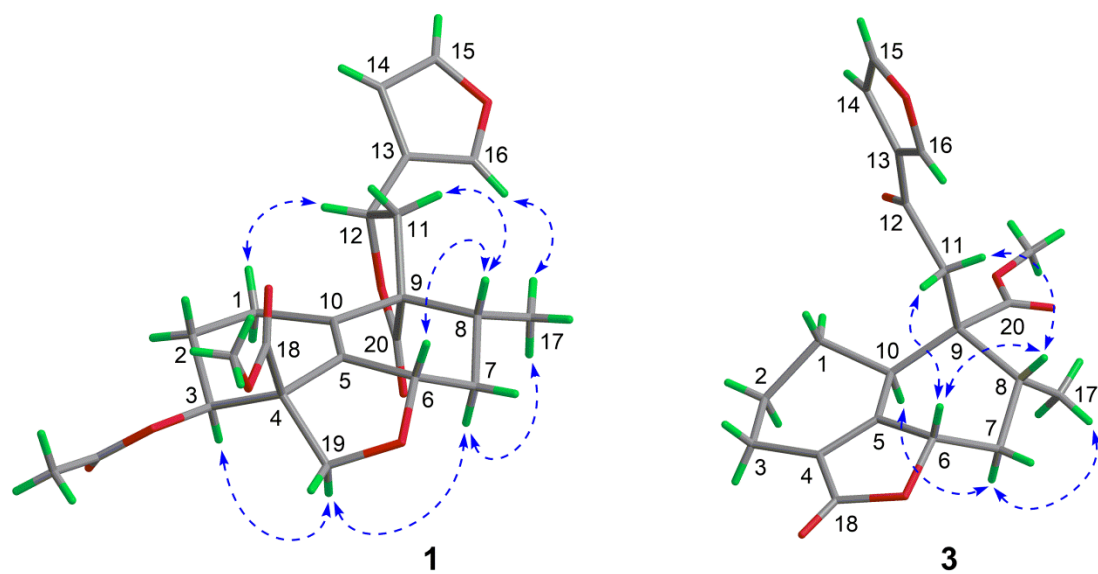


Figure S2. Key NOE (↔) correlations of 1 and 3.

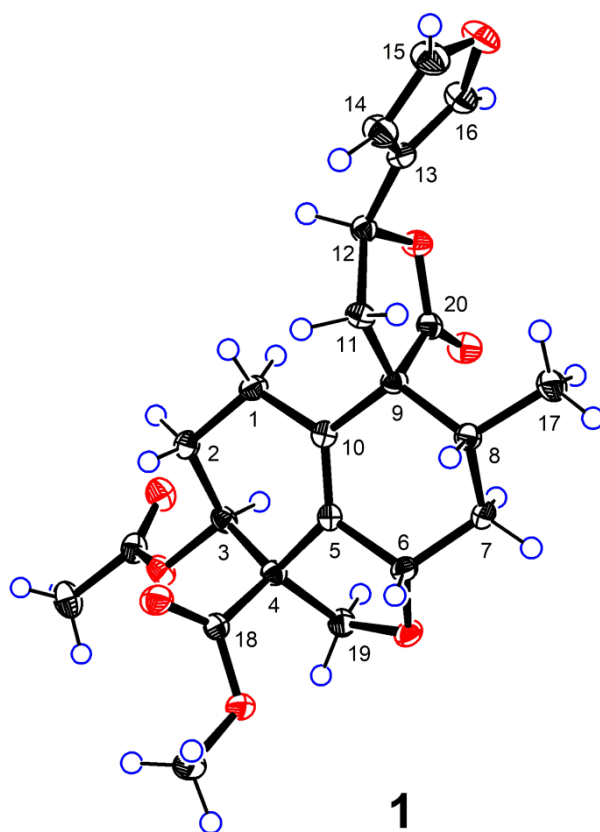


Figure S3. Single-crystal X-ray structure of **1**.

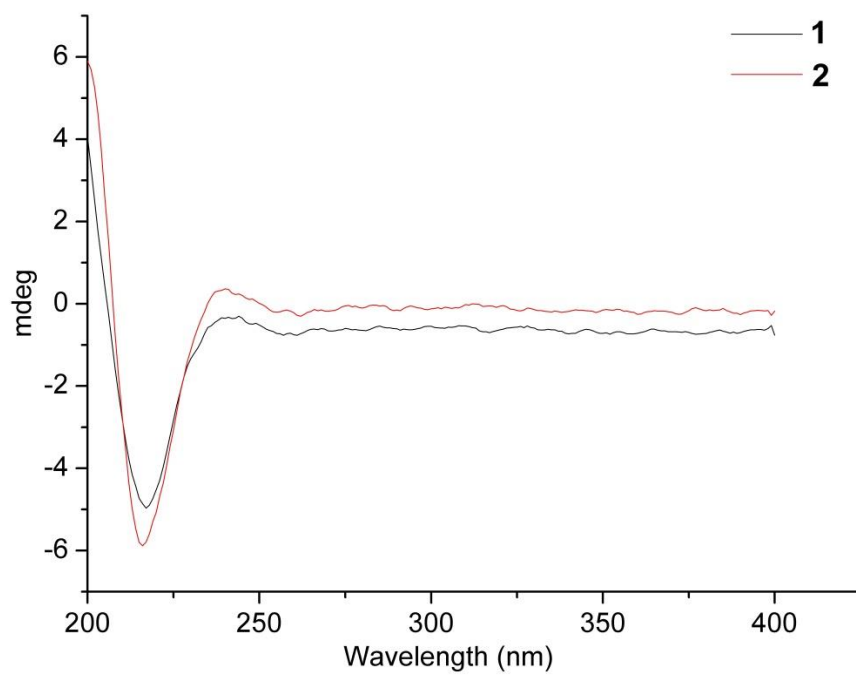


Figure S4. CD spectra of **1** and **2** (in MeCN).

Figure S5. ^1H NMR spectrum of **1** in CDCl_3 .

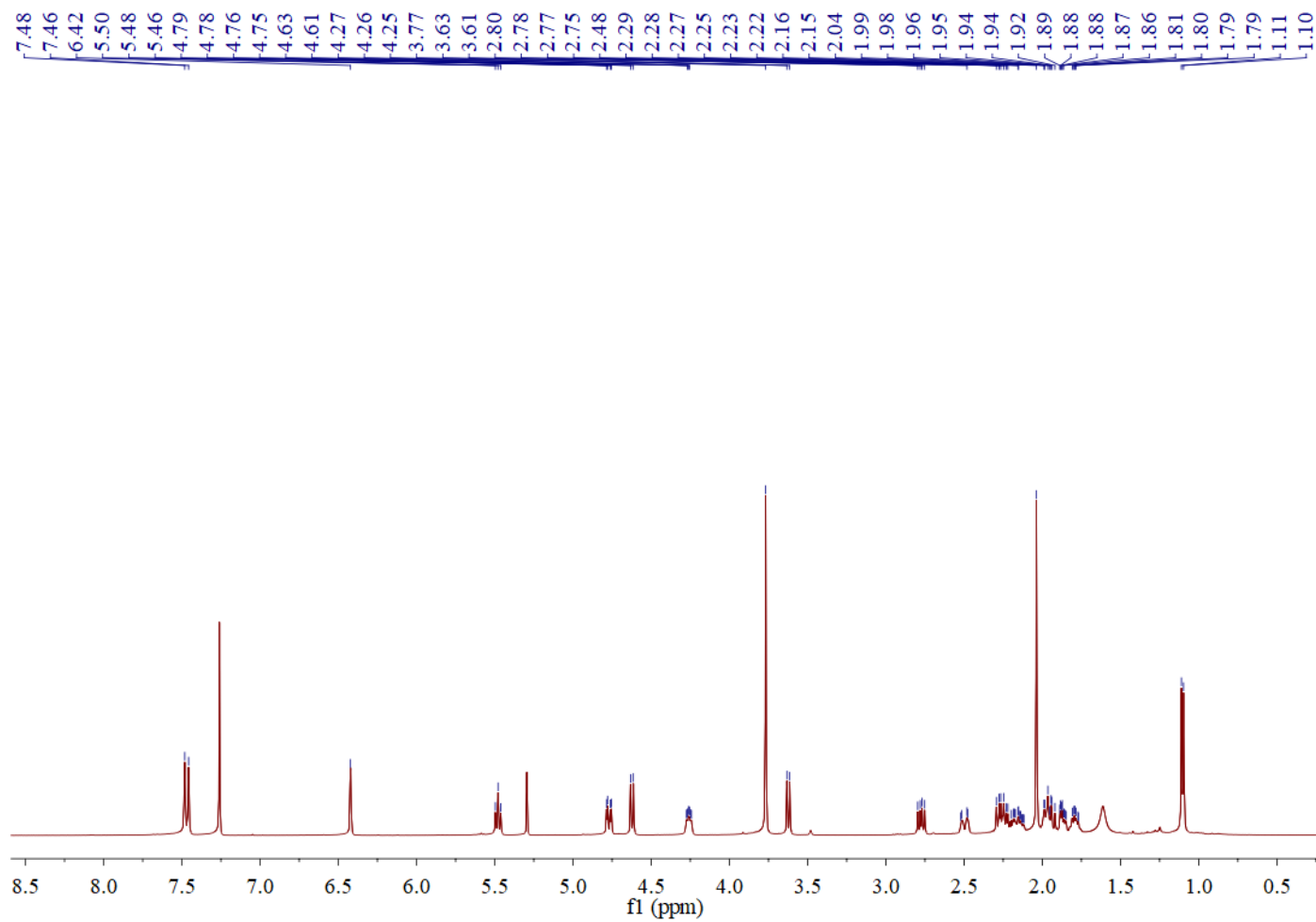


Figure S6. ^{13}C and DEPT 135 NMR spectra of **1** in CDCl_3 .

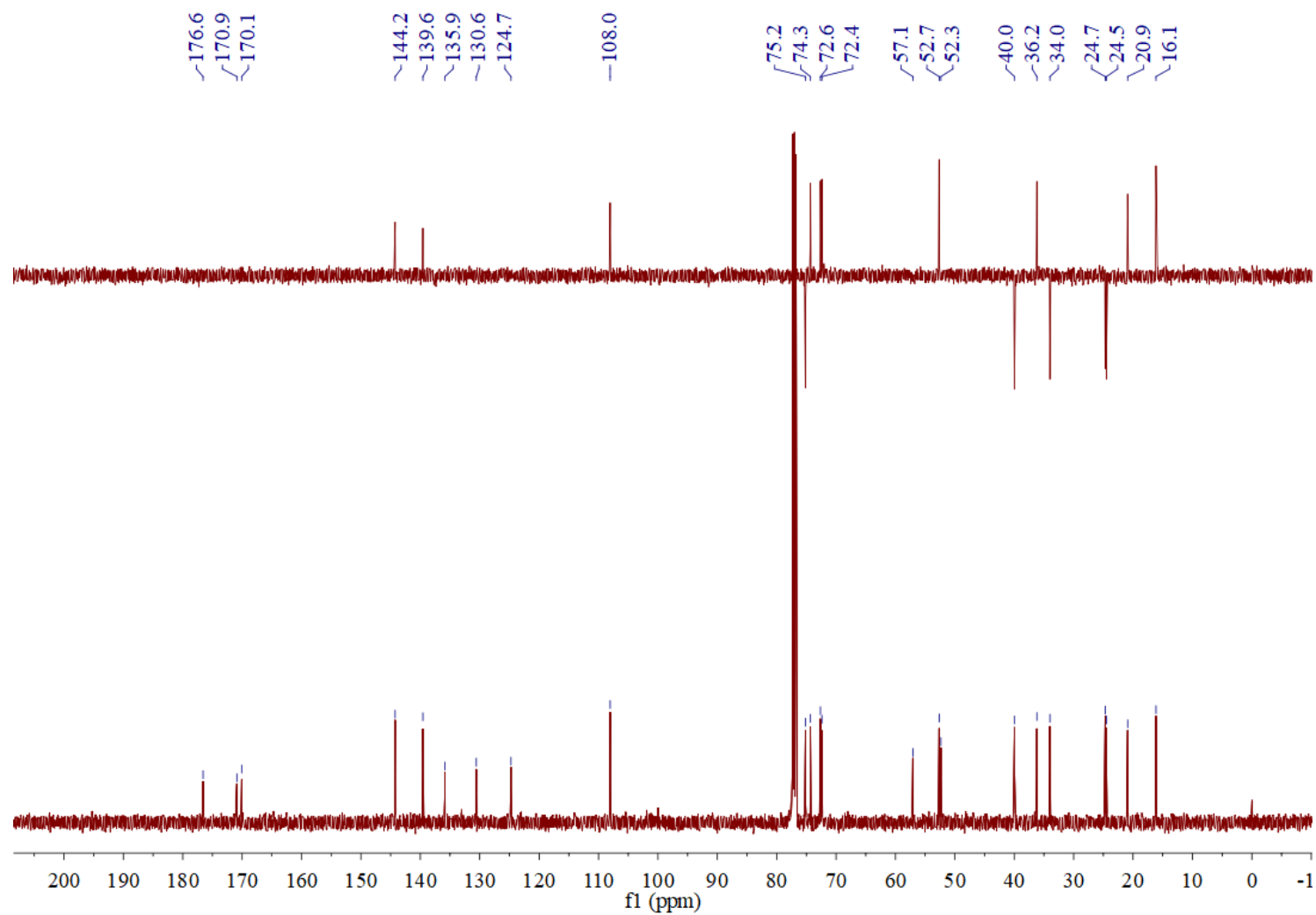


Figure S7. HSQC spectrum of **1** in CDCl₃.

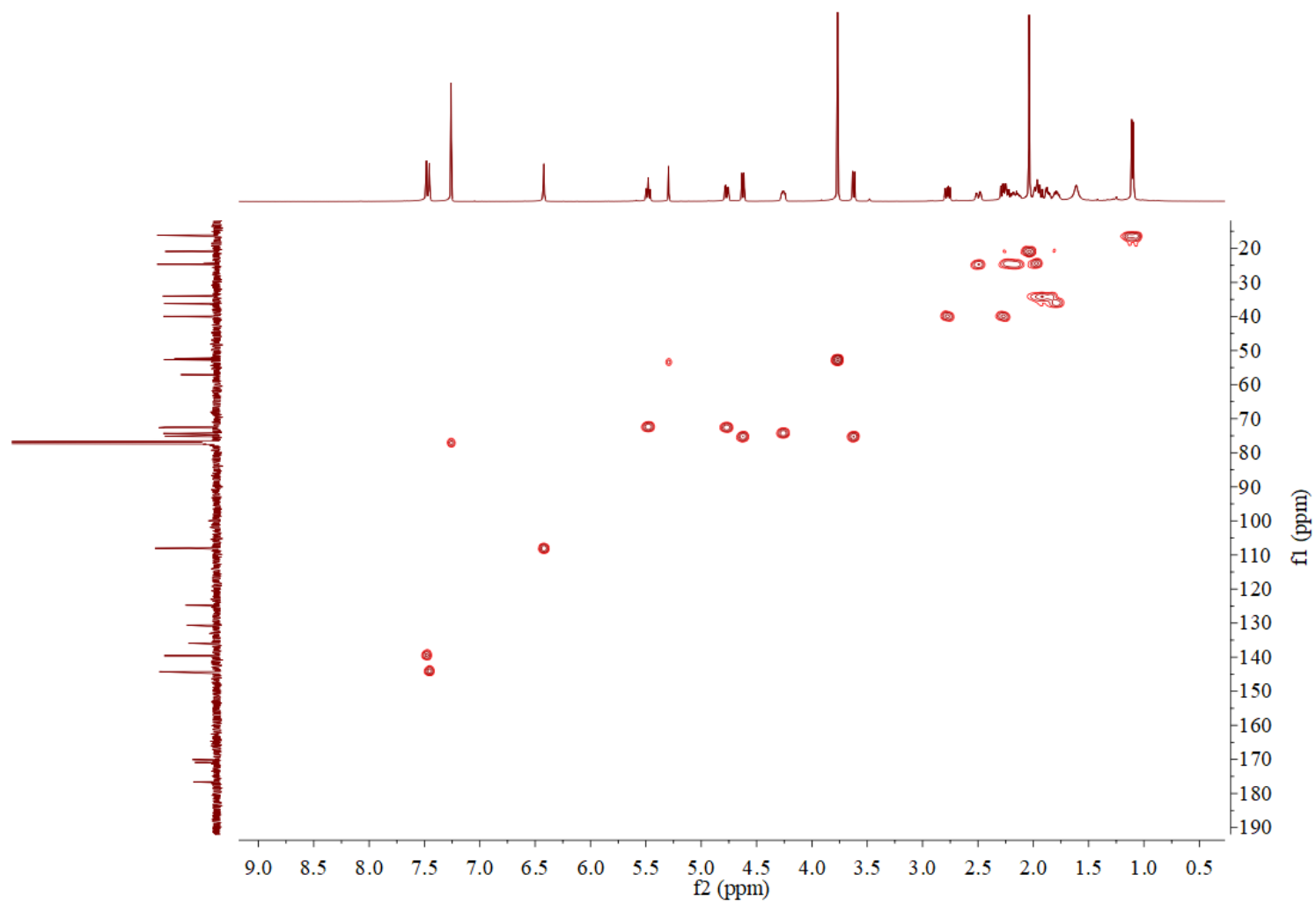


Figure S8. HMBC spectrum of **1** in CDCl₃.

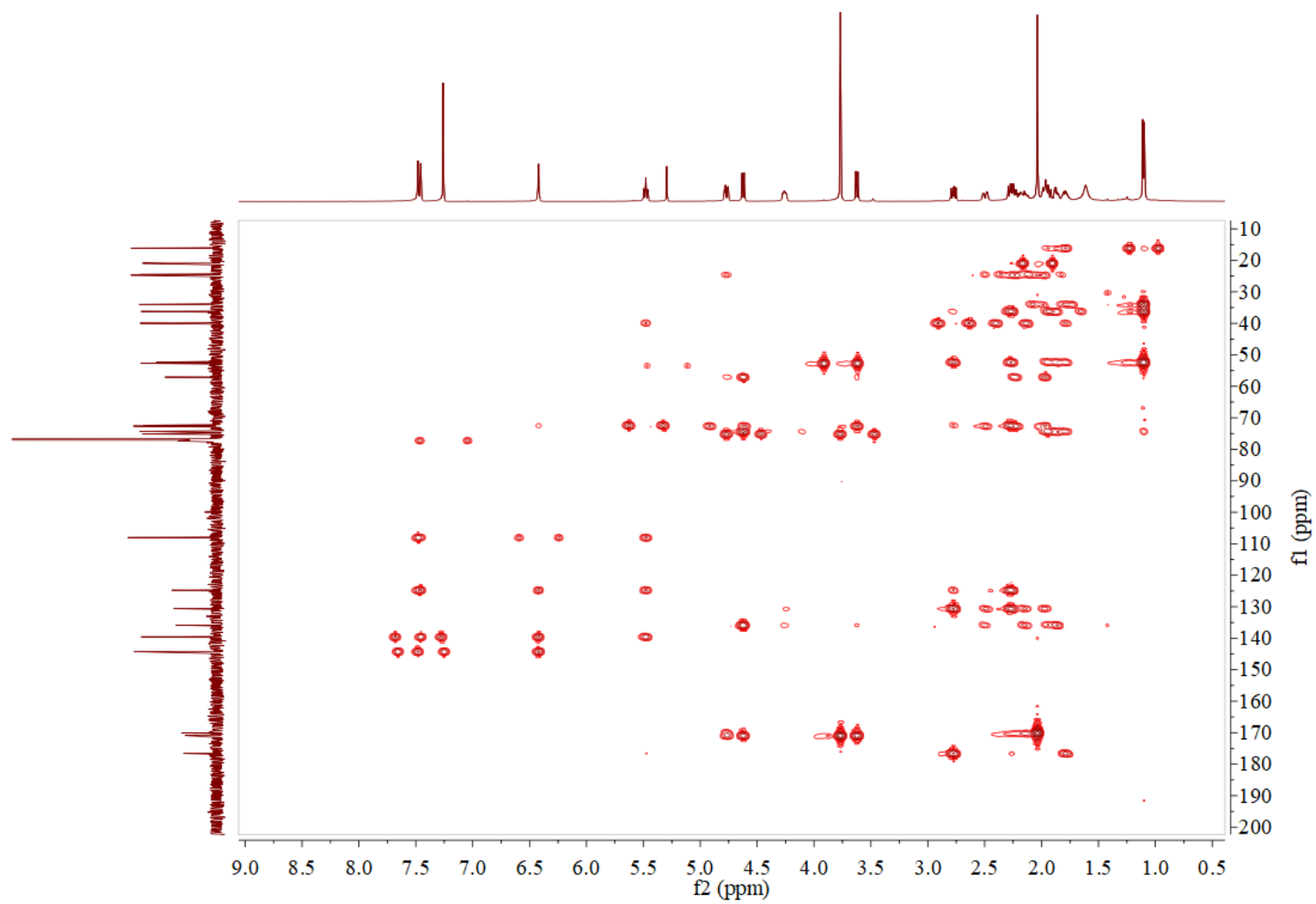


Figure S9. ^1H – ^1H COSY spectrum of **1** in CDCl_3 .

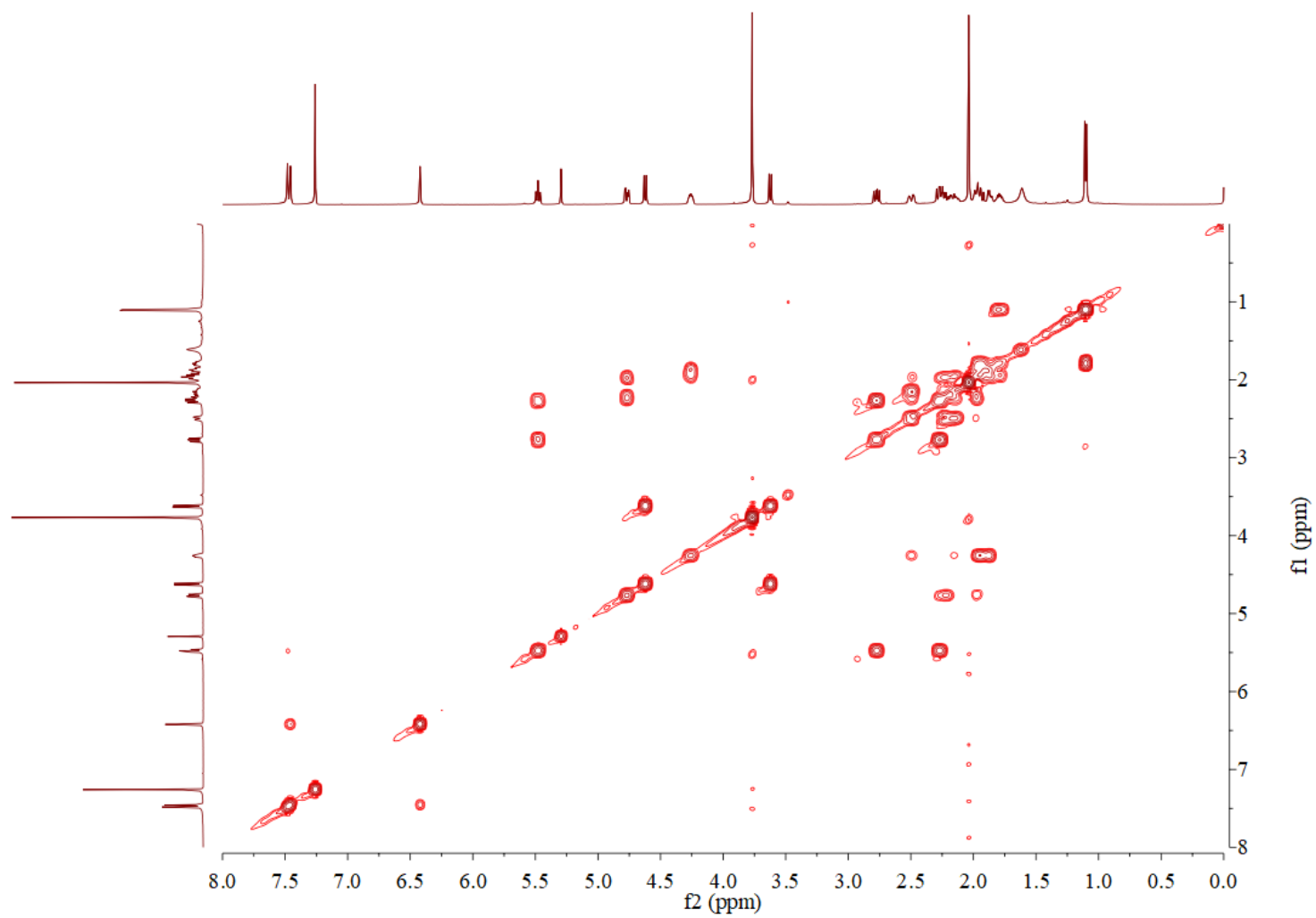


Figure S10. NOESY spectrum of **1** in CDCl₃.

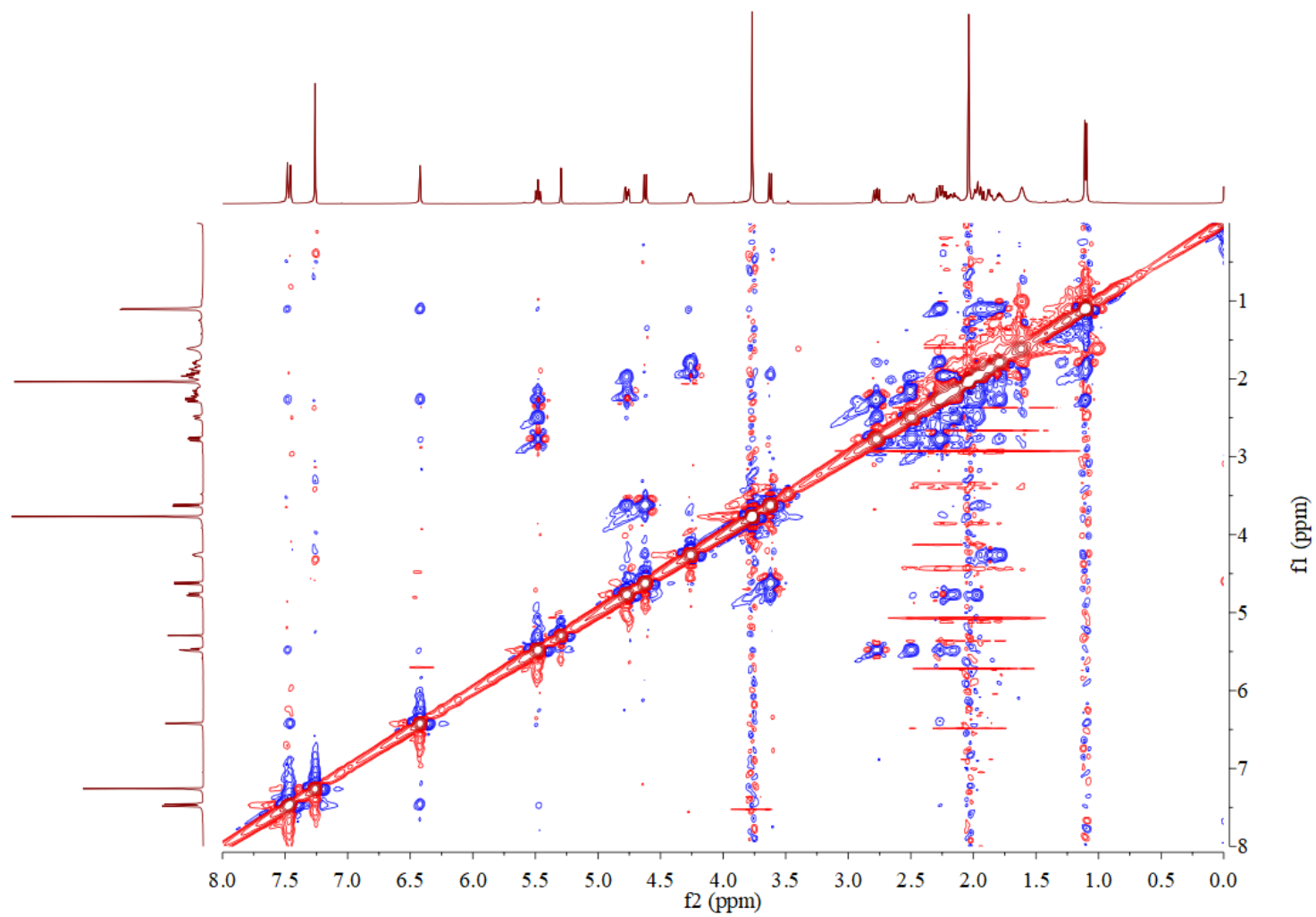


Figure S11. HRESIMS spectrum of **1**.

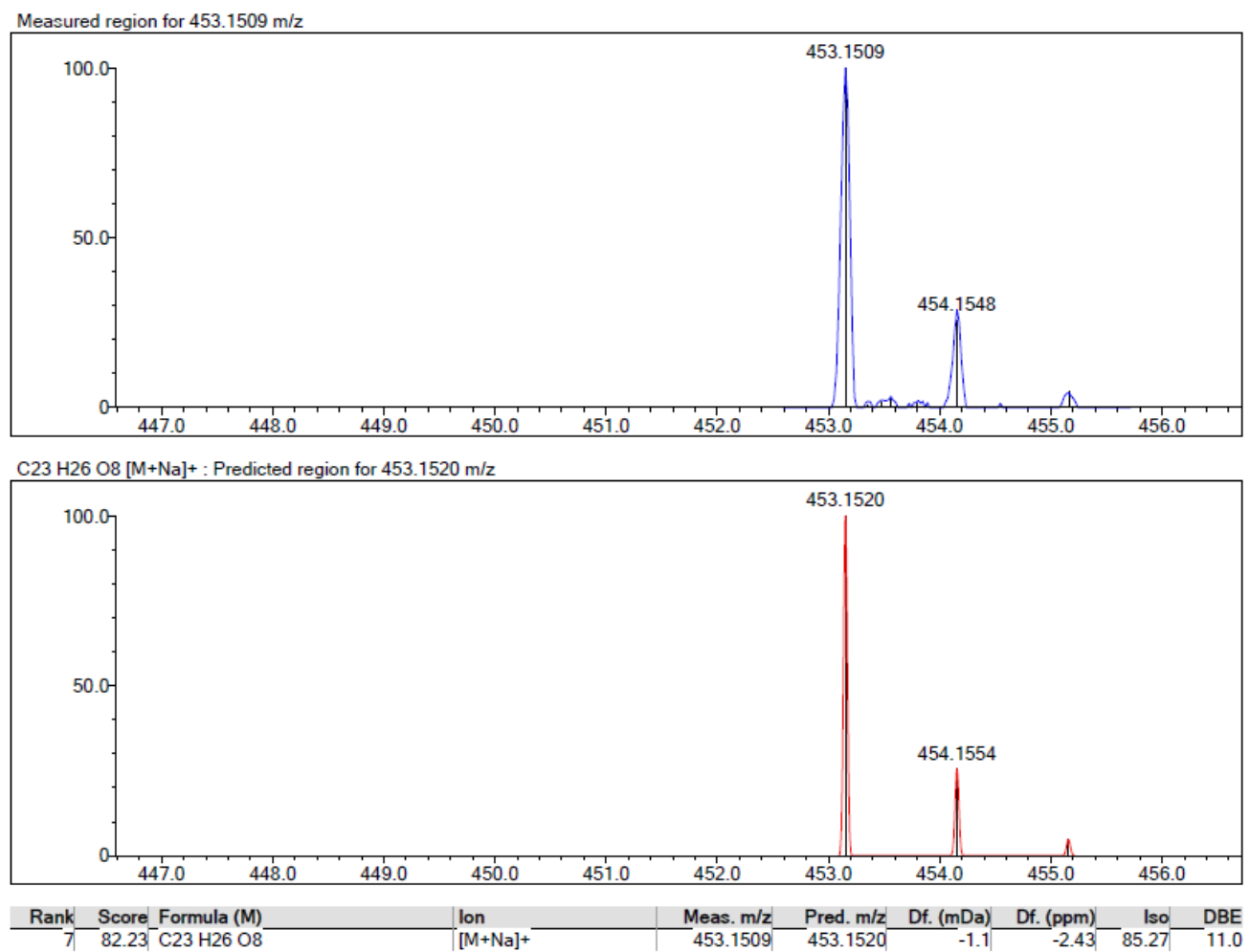
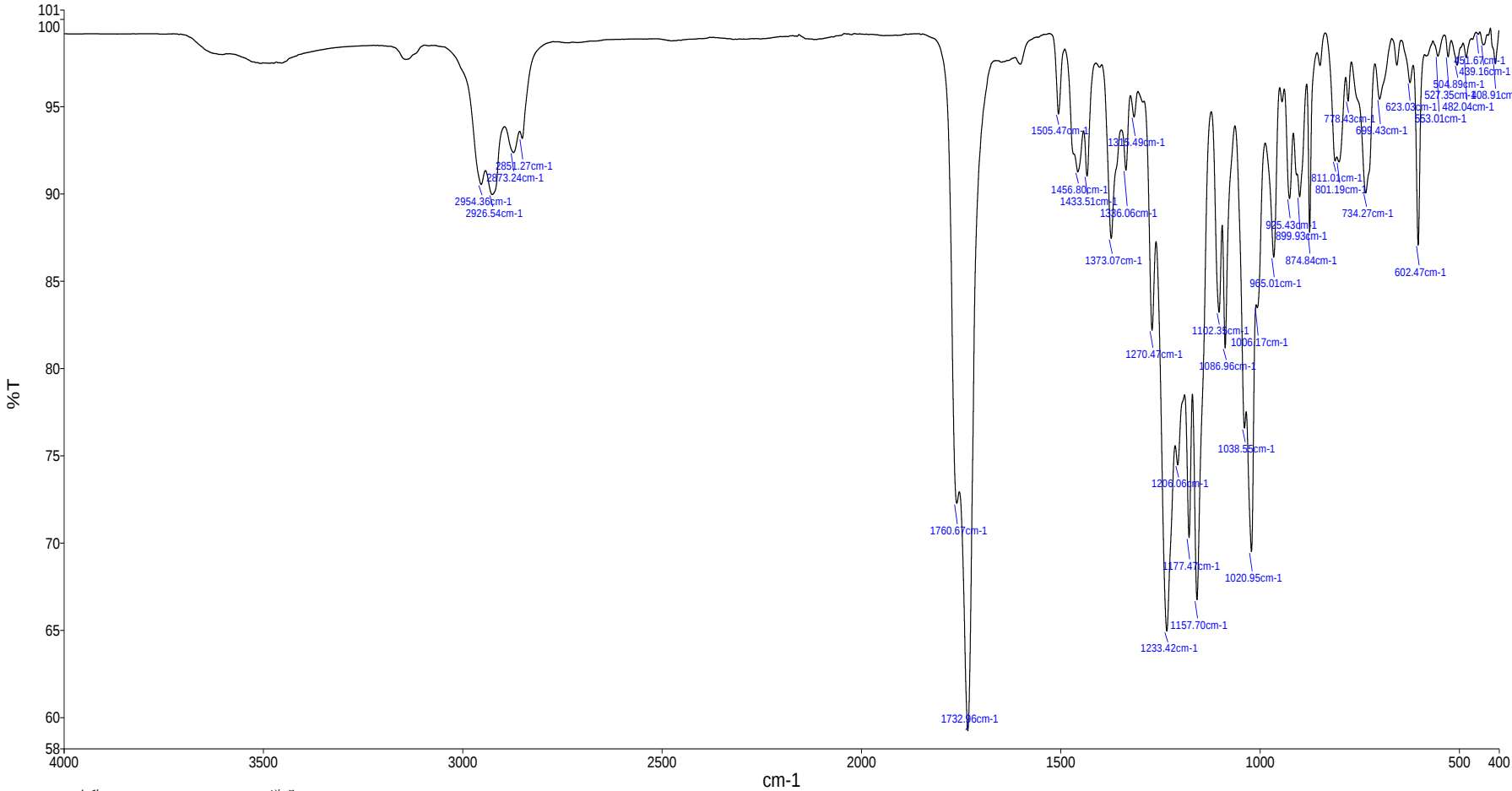


Figure S12. IR (KBr disc) spectra of 1.



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Figure S13. ^1H NMR spectrum of **2** in CDCl_3 .

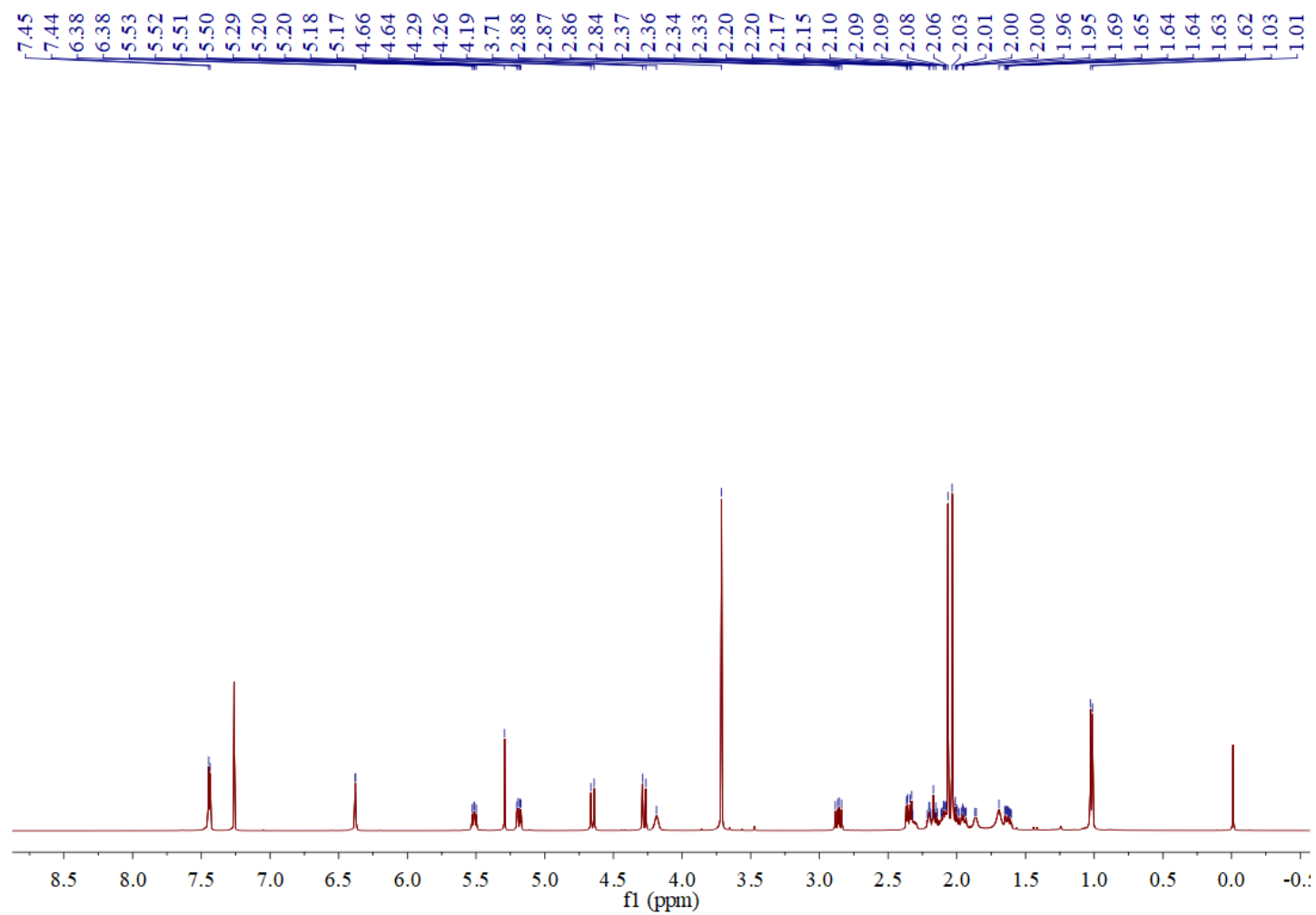


Figure S14. ^{13}C and DEPT 135 NMR spectra of **2** in CDCl_3 .

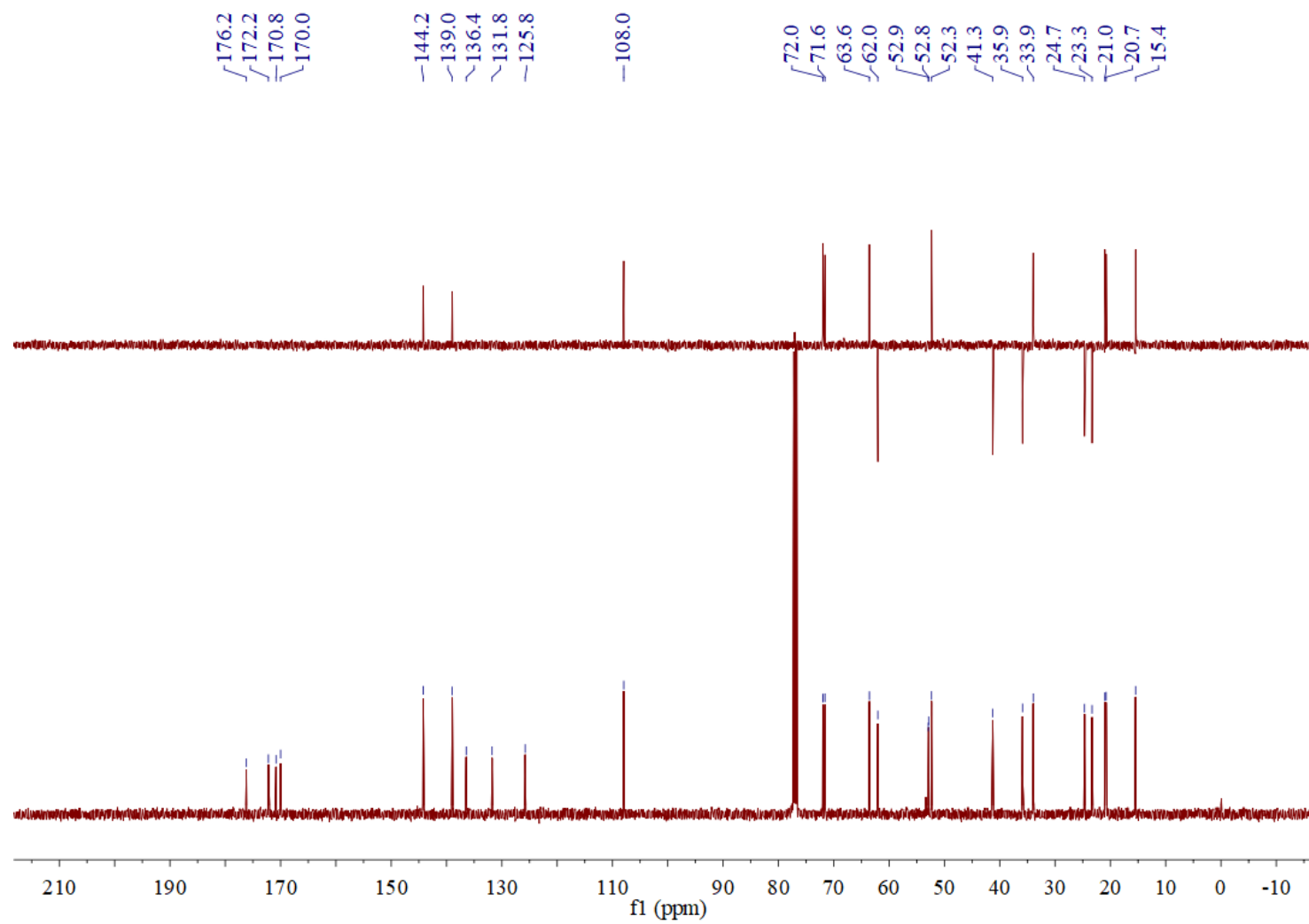


Figure S15. HSQC spectrum of **2** in CDCl₃.

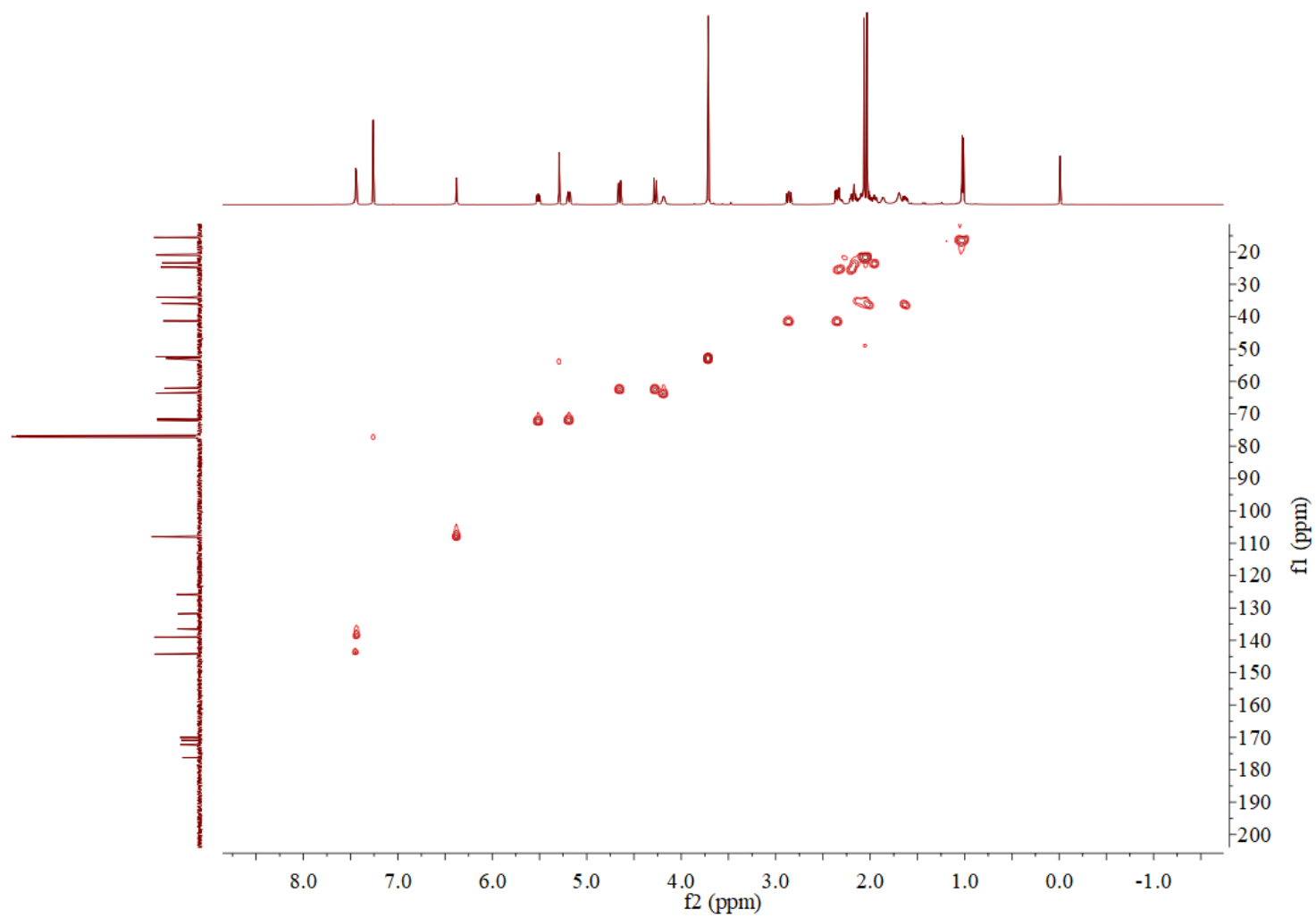


Figure S16. HMBC spectrum of **2** in CDCl₃.

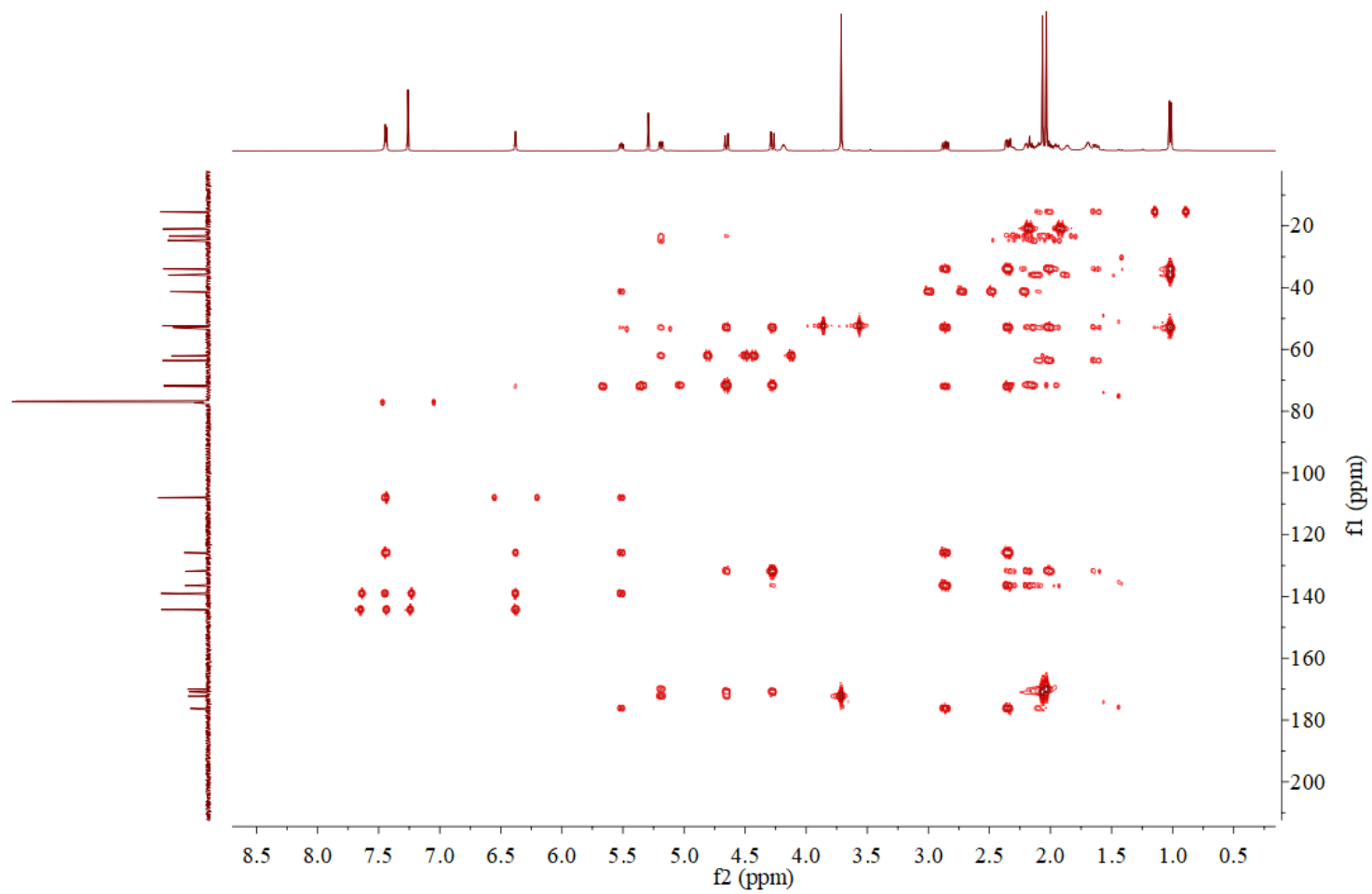


Figure S17. ^1H – ^1H COSY spectrum of **2** in CDCl_3 .

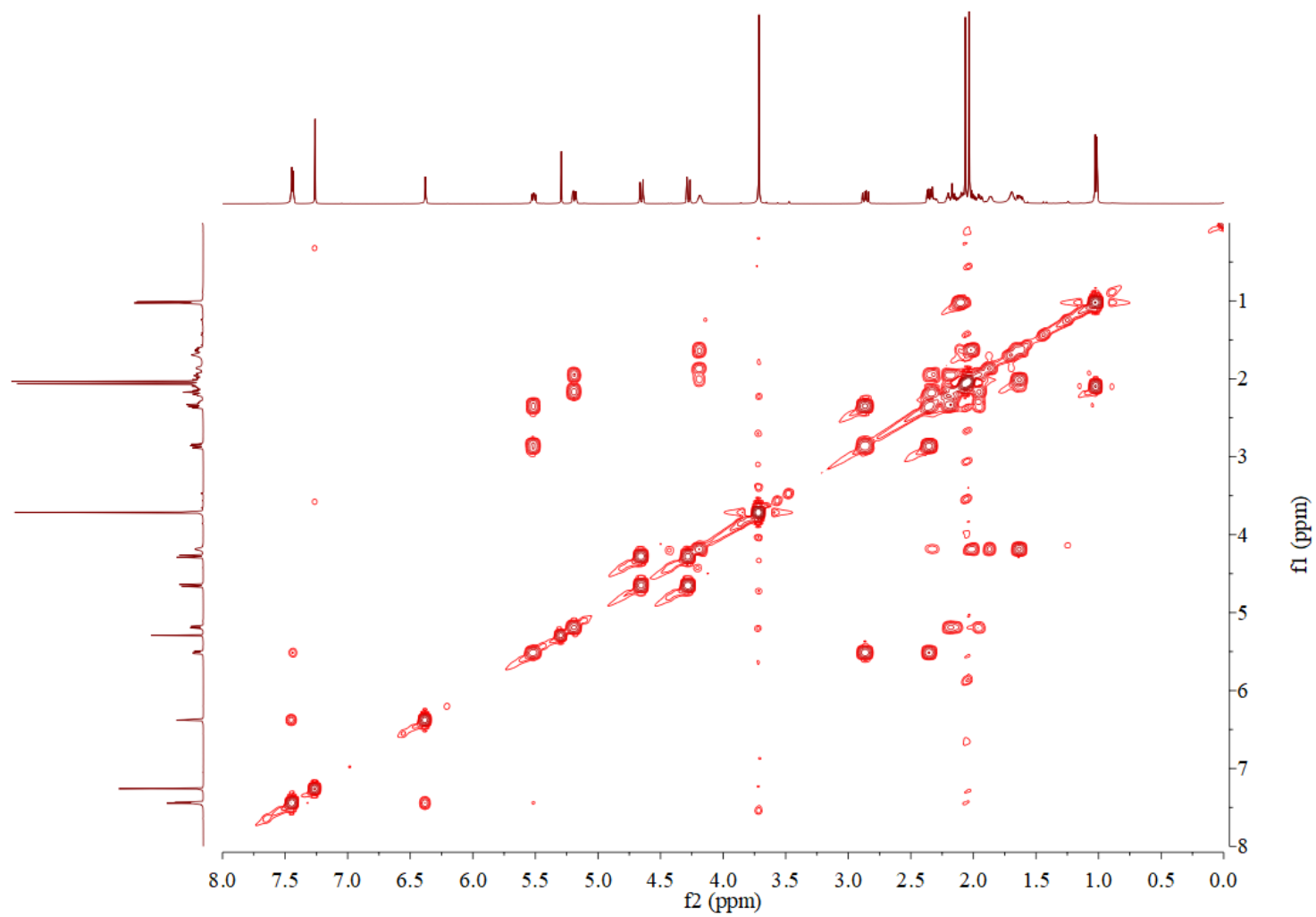


Figure S18. NOESY spectrum of **2** in CDCl₃.

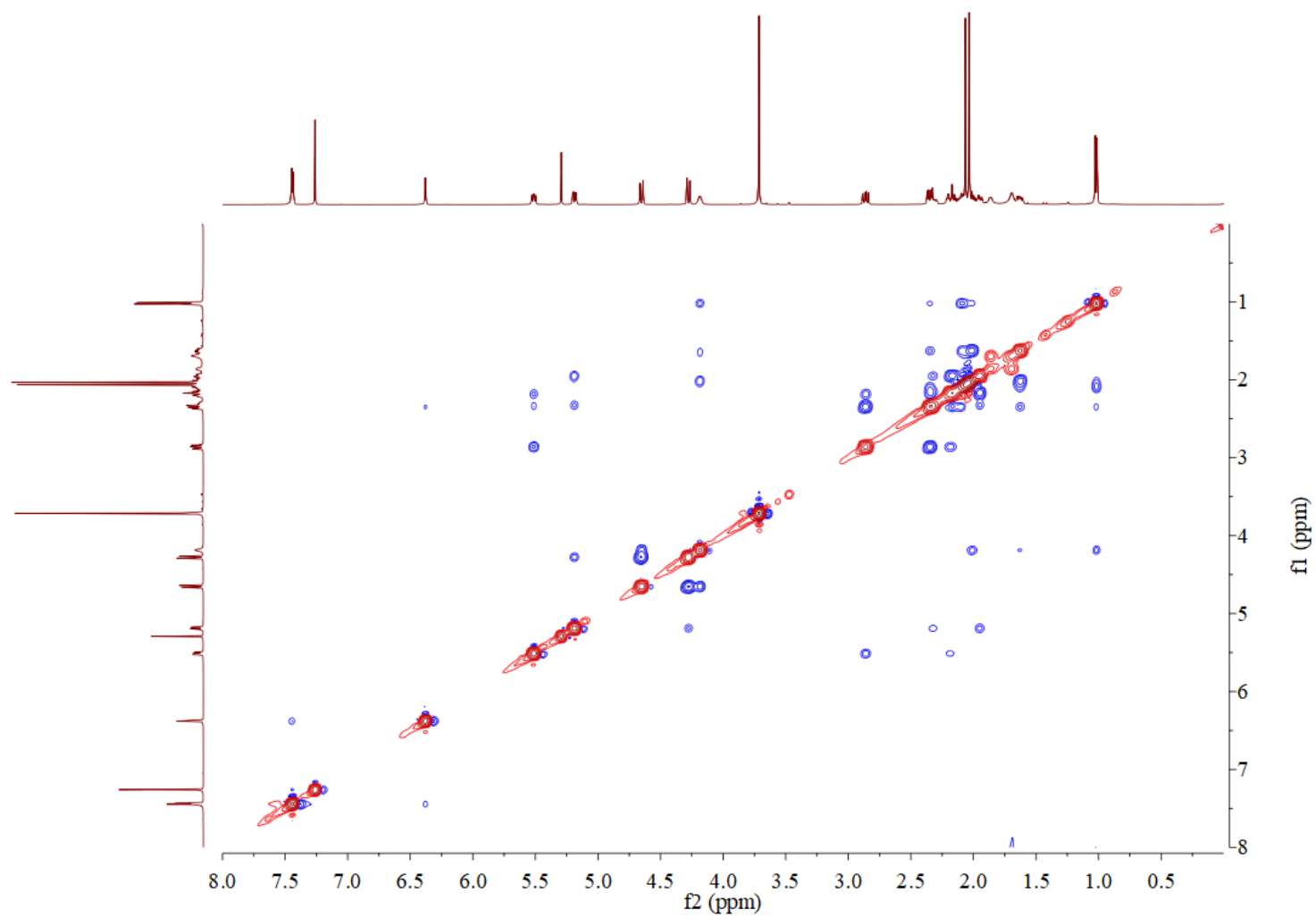


Figure S19. HRESIMS spectrum of **2**.

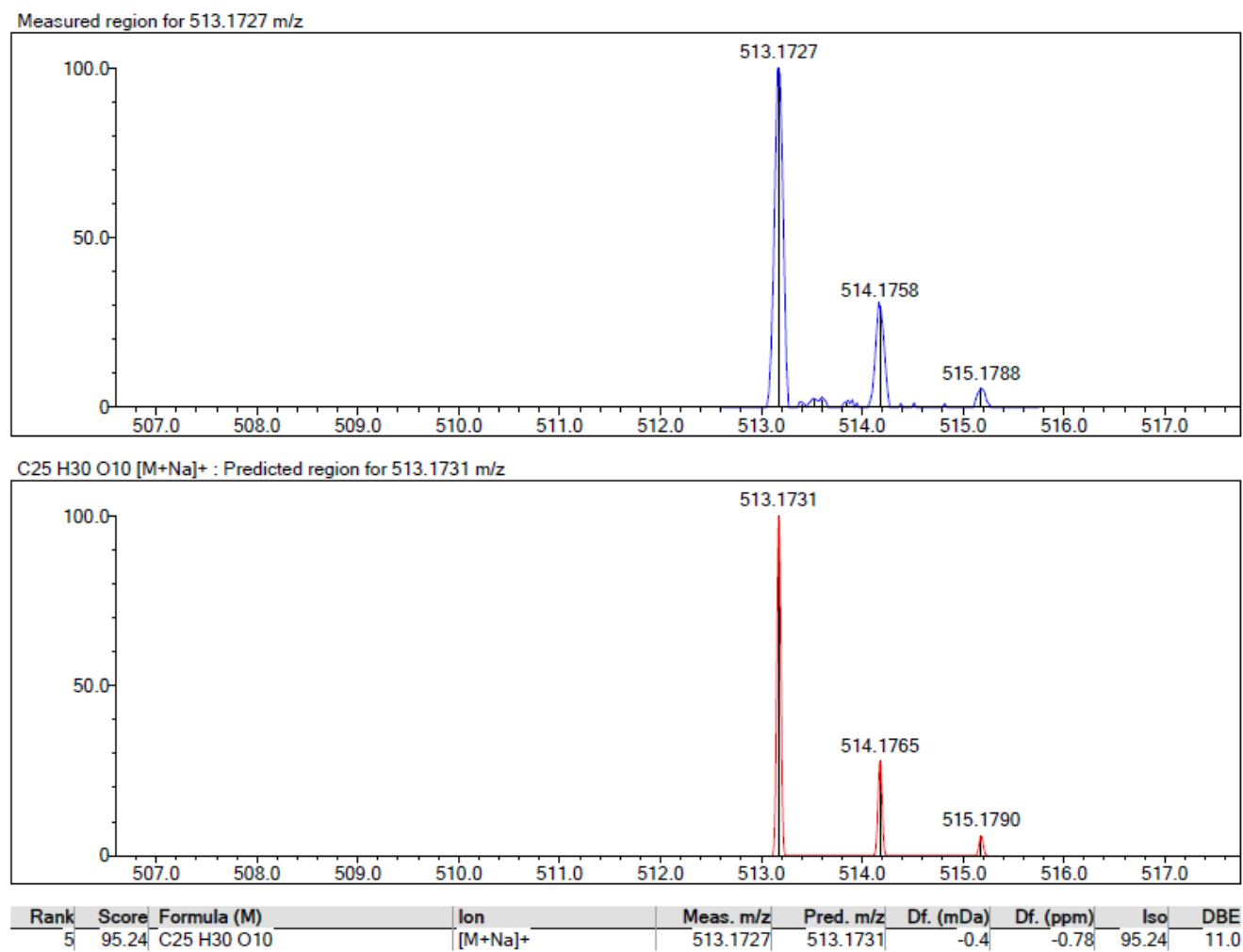
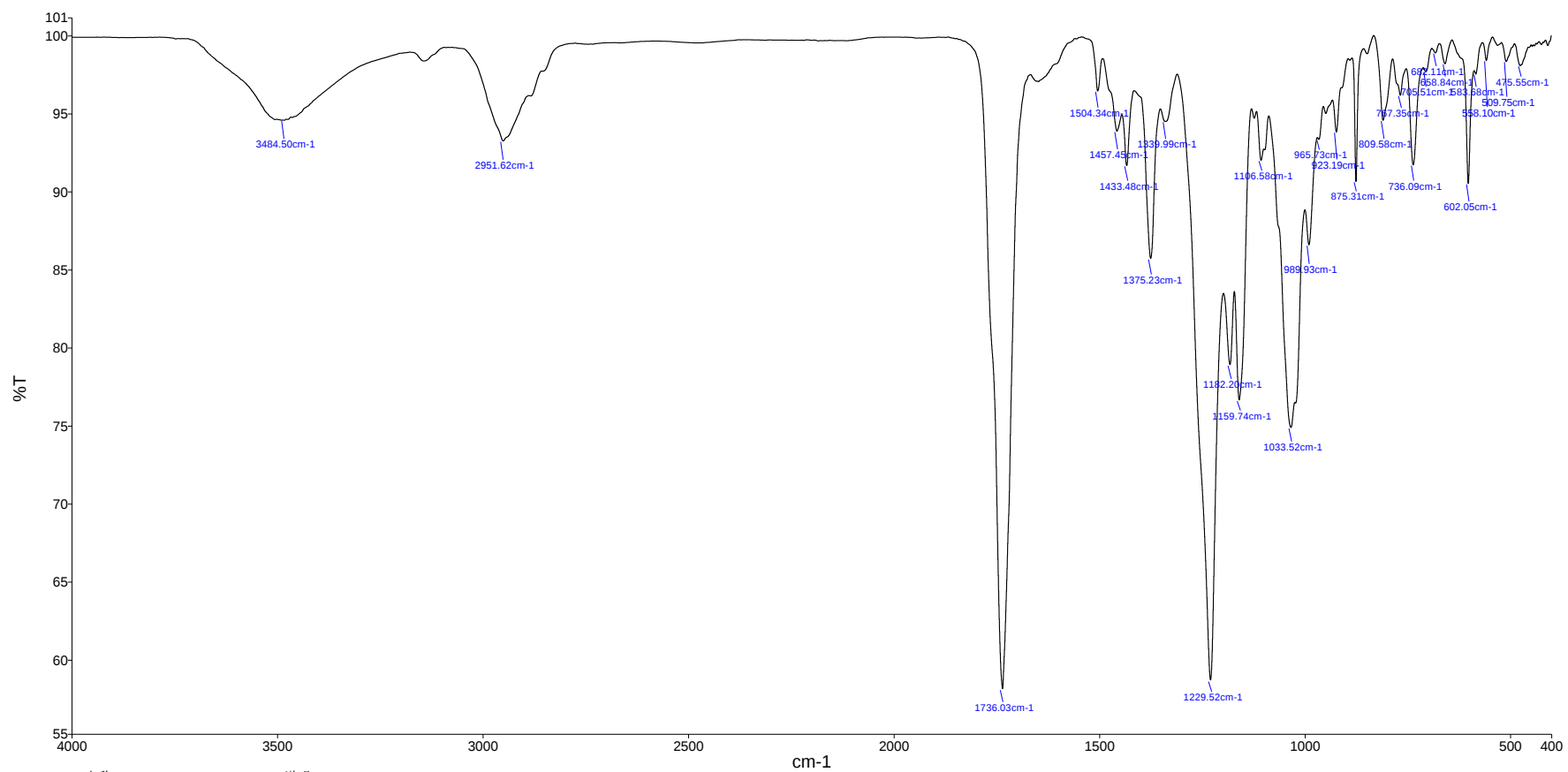


Figure S20. IR (KBr disc) spectra of **2**.



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Figure S21. ^1H NMR spectrum of **3** in CDCl_3 .

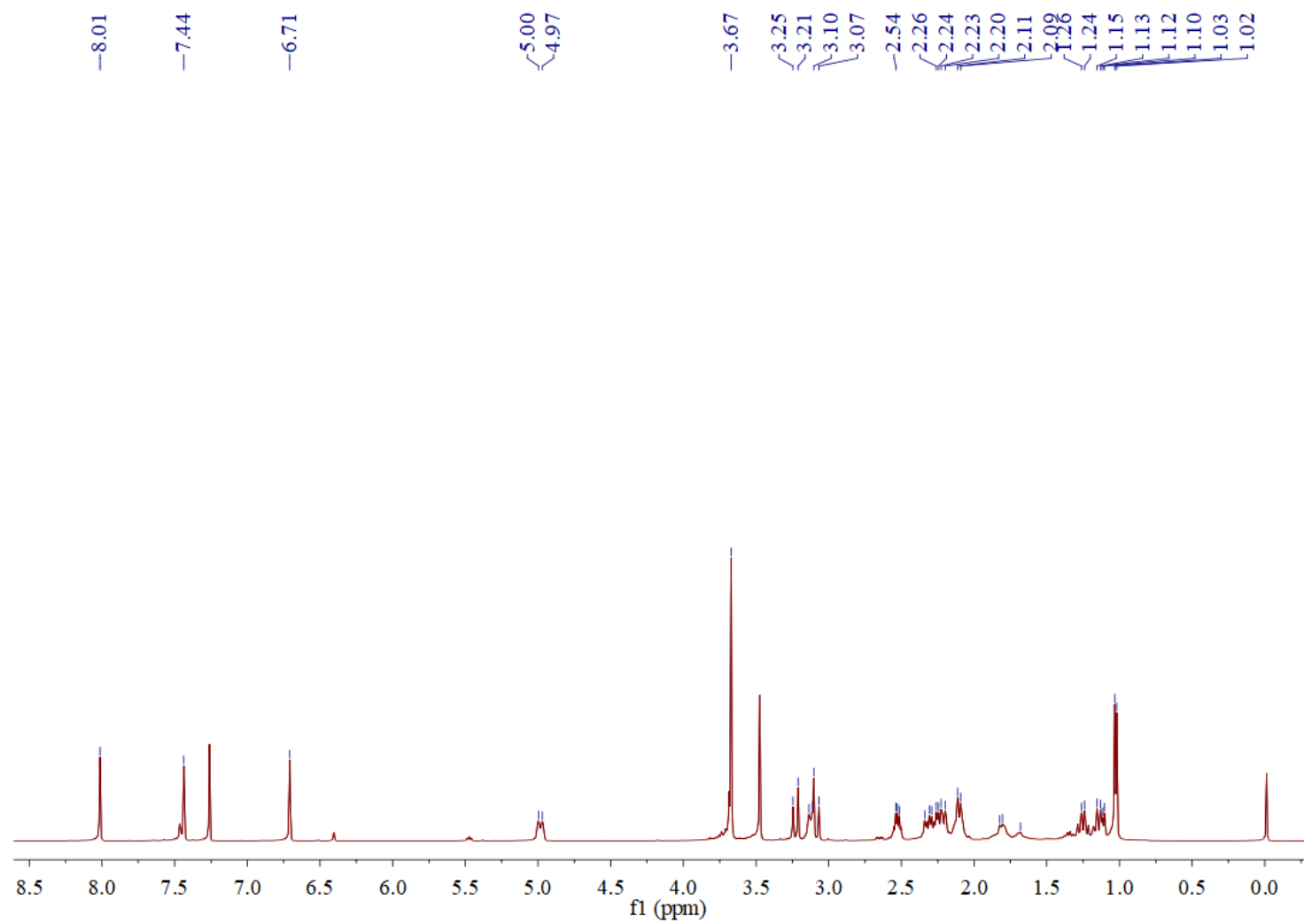


Figure S22. ^{13}C and DEPT 135 NMR spectra of **3** in CDCl_3 .

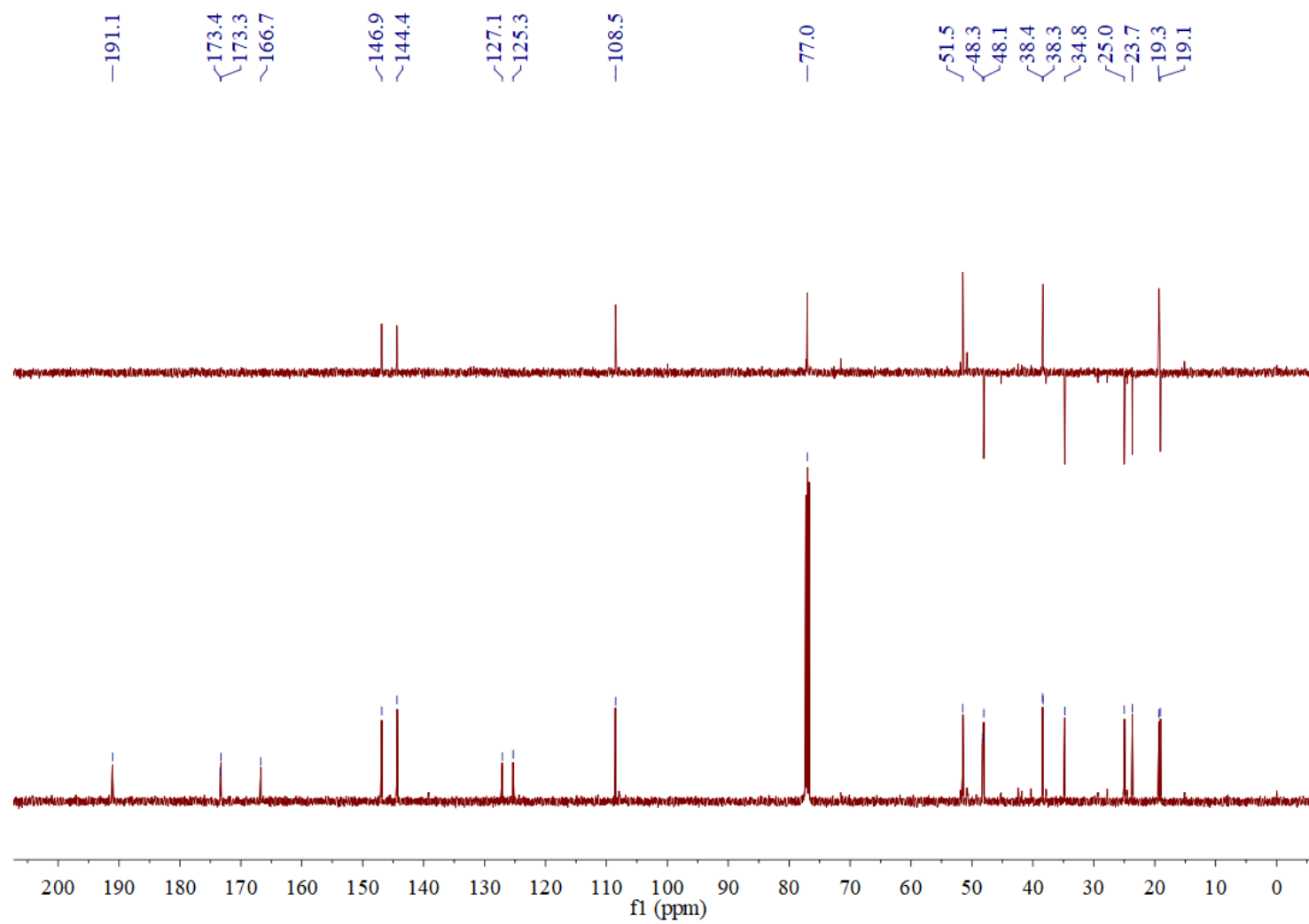


Figure S23. HSQC spectrum of **3** in CDCl₃.

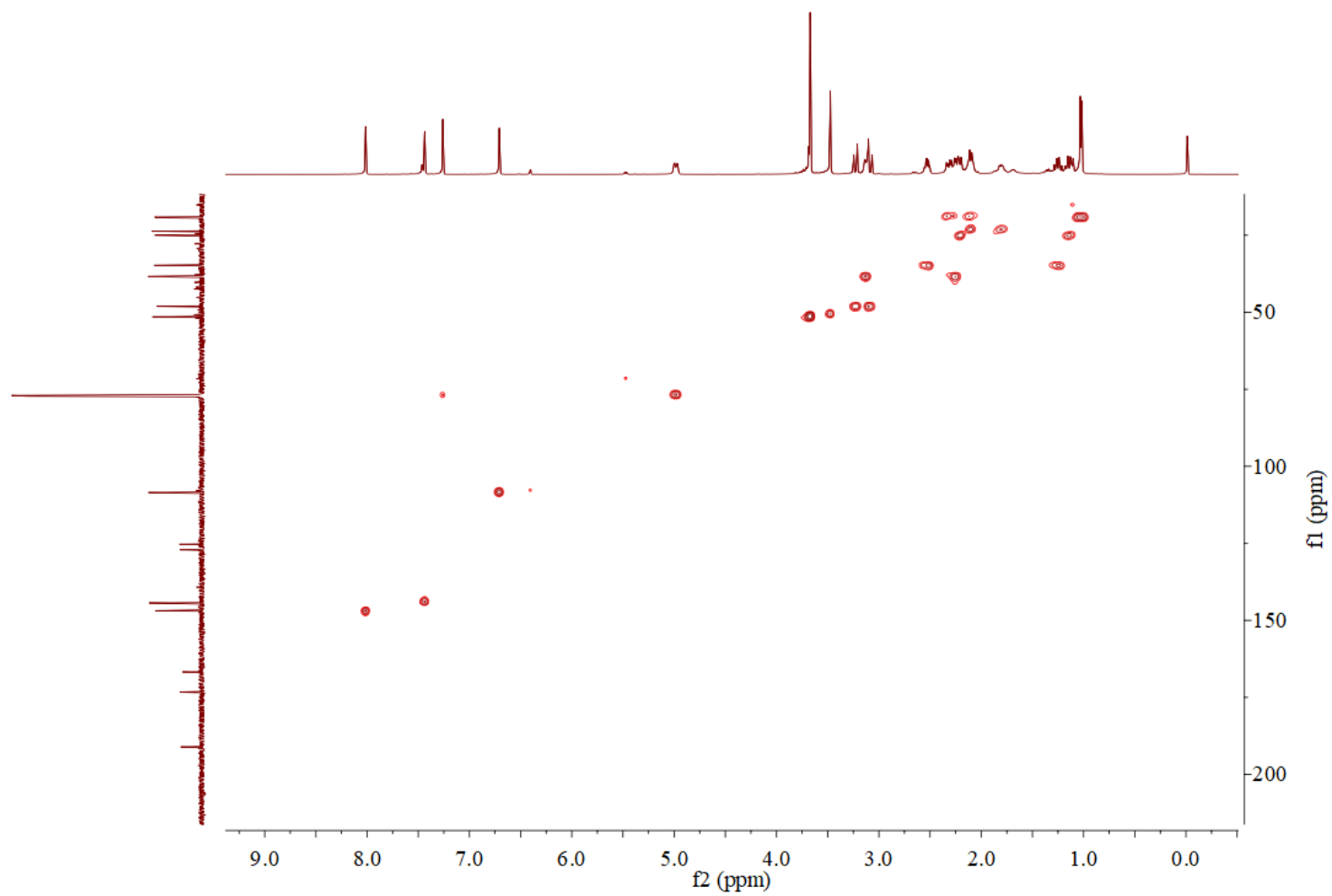


Figure S24. HMBC spectrum of **3** in CDCl₃.

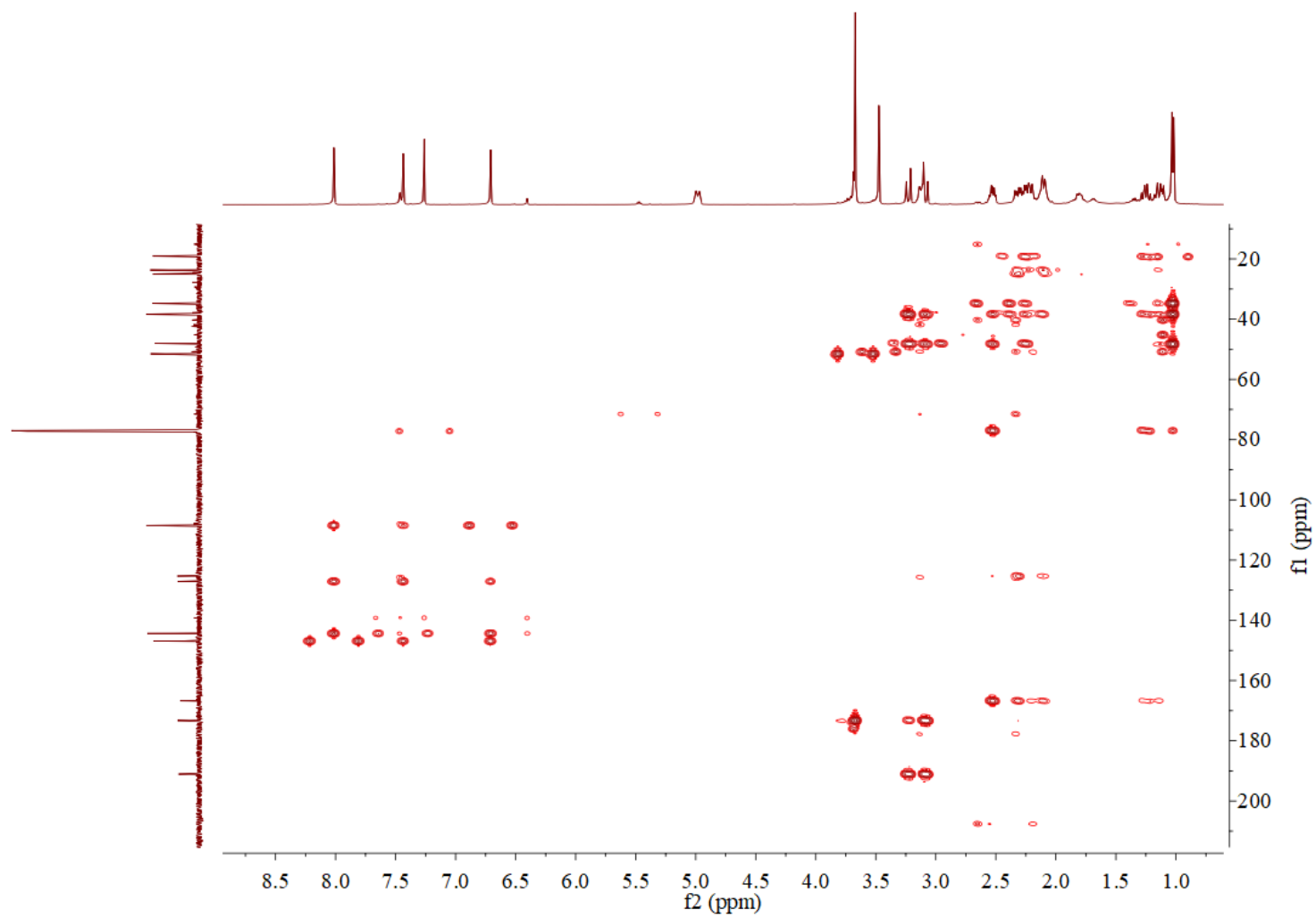


Figure S25. ^1H - ^1H COSY spectrum of **3** in CDCl_3 .

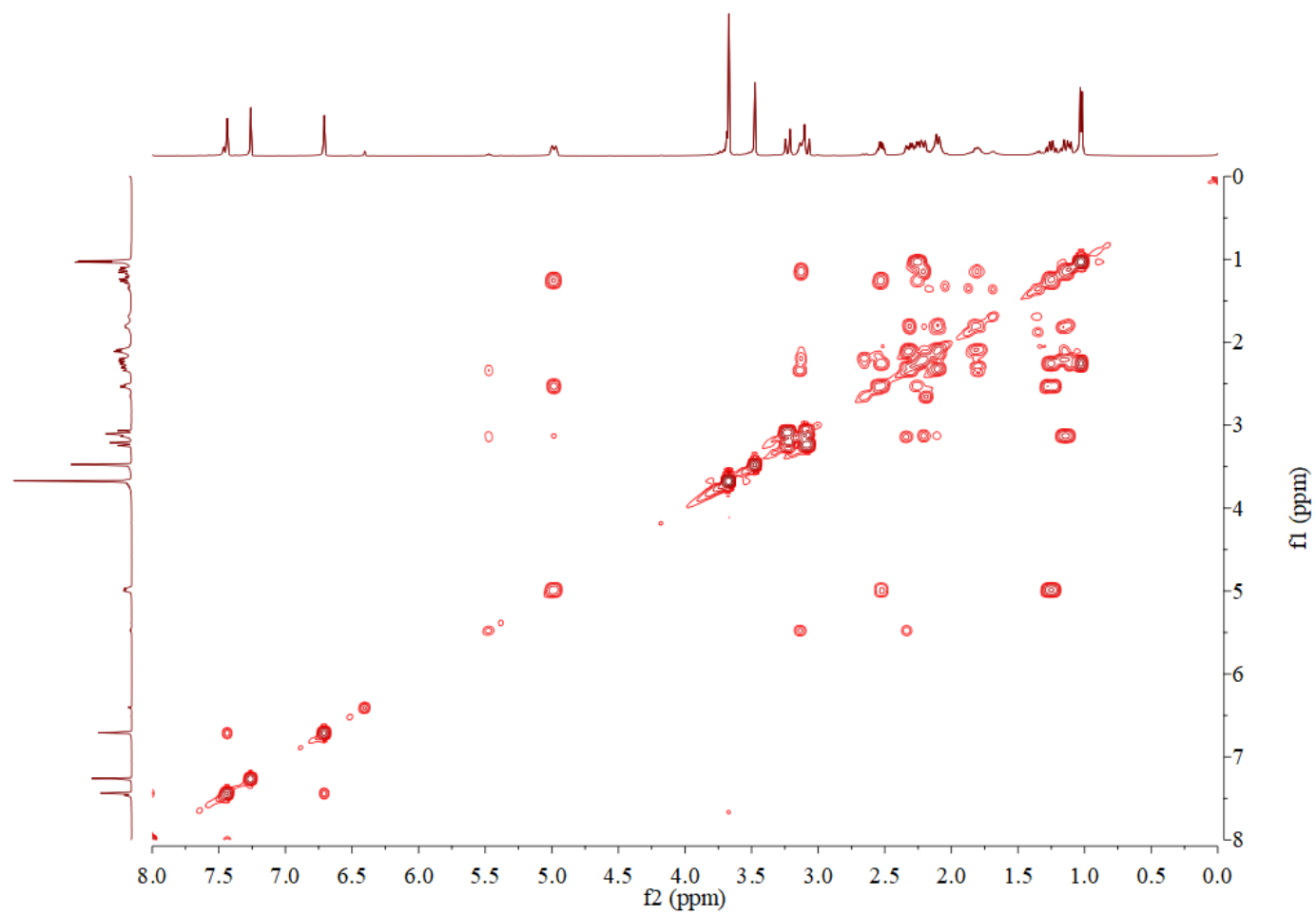


Figure S26. NOESY spectrum of **3** in CDCl₃.

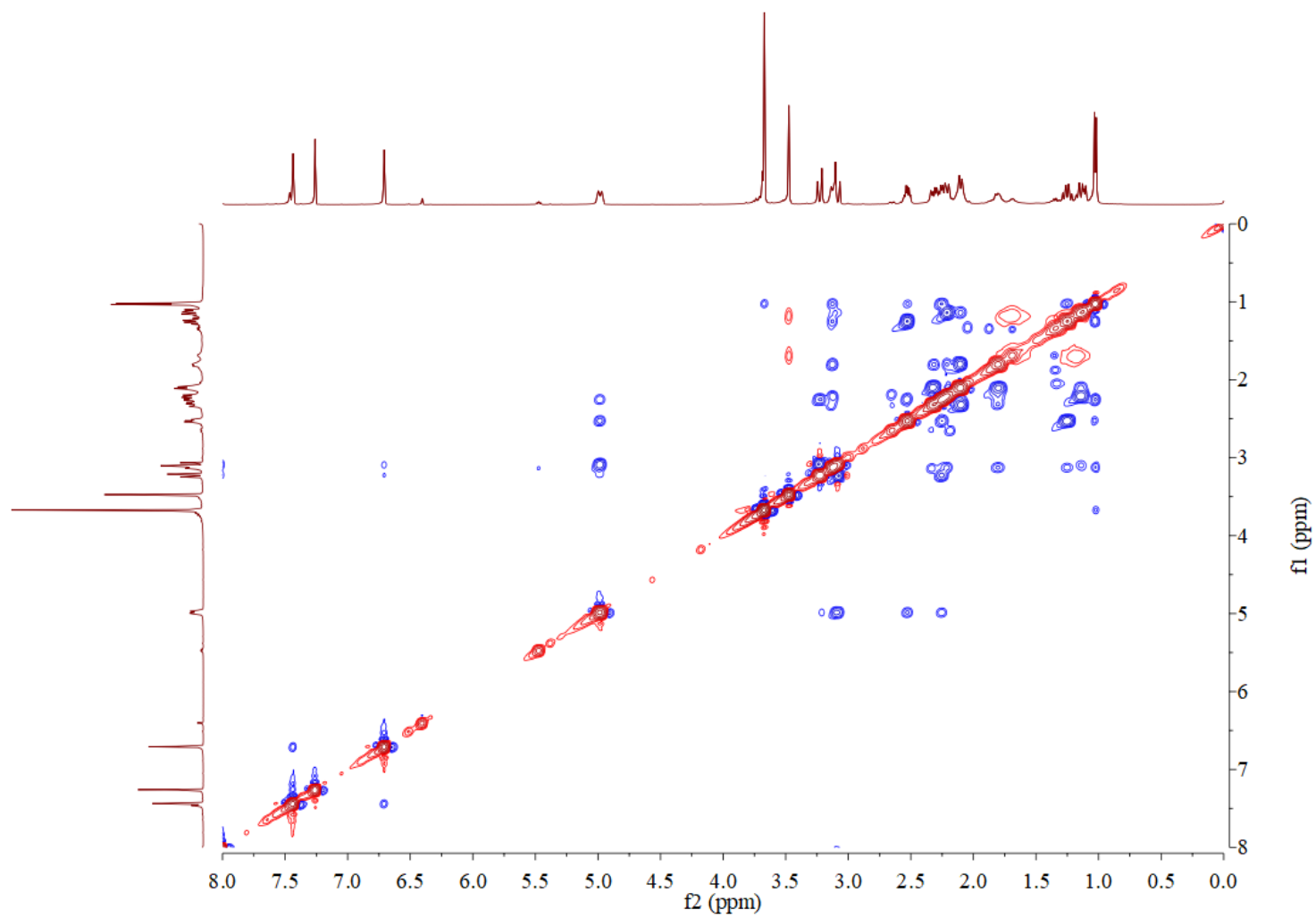


Figure S27. HRESIMS spectrum of **3**.

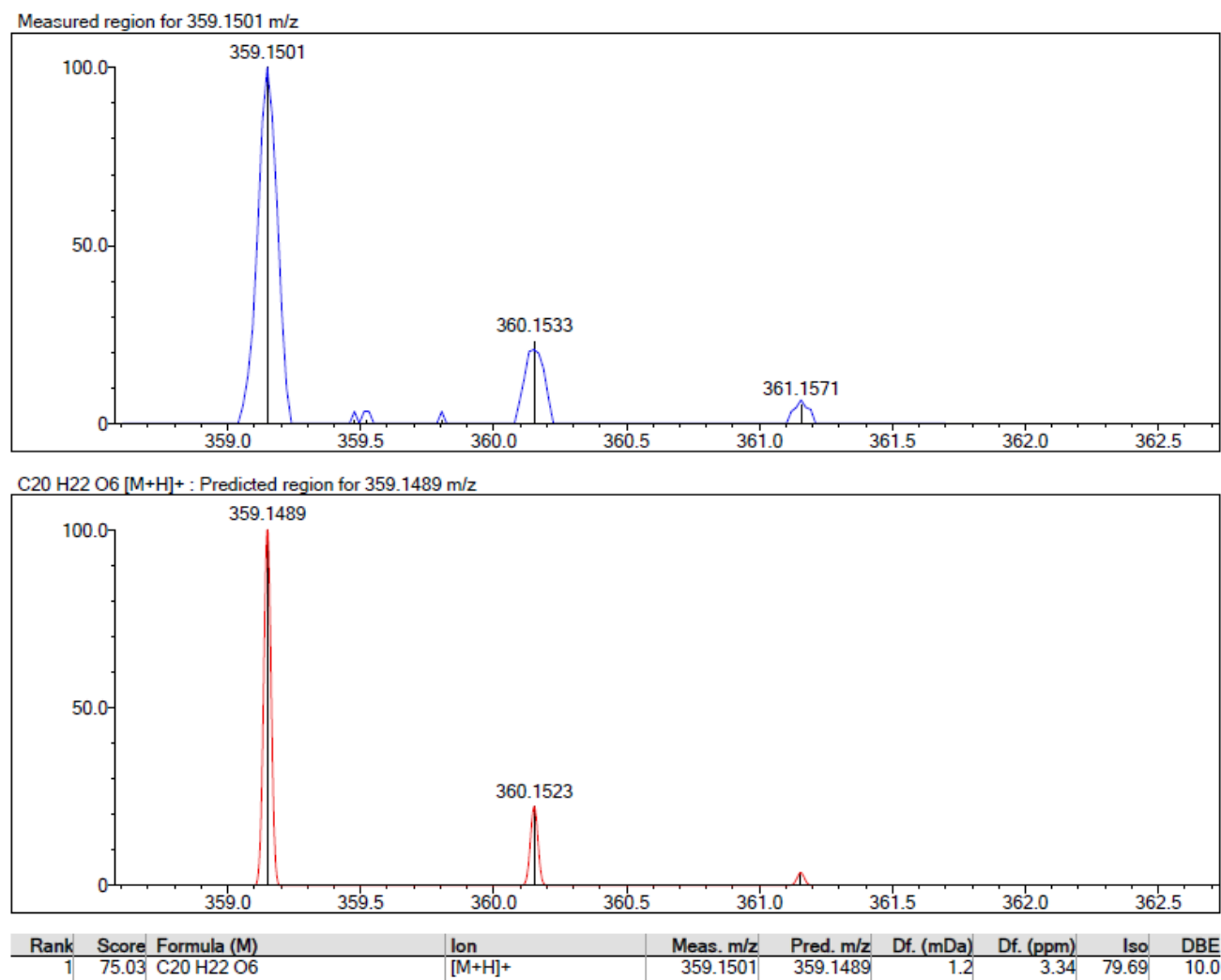


Figure S28. IR (KBr disc) spectra of **3**.

