Supplementary Materials

Article

Flavonol–glycoside and Rare Triterpenoid Derivatives Isolated from Leaves of *Combretum glutinosum* Perr. Ex Dc. with *In Vitro* Cytotoxic Activity

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Abstract: Combretaceae plants are used traditionally by many cultures especially Sudanese patients for the treatment of diverse ailments such as anti-inflammatory, antimicrobial, antitumor, and antioxidant disorders. From these plants, the genus *Combretum* are traditional medicinal plants. Thus, they are formed from non-polar or polar extracts of many isolated phytochemicals. Of these necessities, the use of *Combretum* extracts for their medicinal properties can be proofed the earliest of myths and traditions used to document those plants' ability to treat diseases. *Combretum glutinosum* Perr. Ex Dc. is a common shrub native to the Africa continent, especially Sudan. Currently, there is no published data about its cytotoxic activity. Additionally, there are few chemical and biological reports of *C. glutinosum*. Therefore, the current study aimed to isolate the chemical bioactive compounds **(1–6)** from ethyl acetate (EtOAc) extract of *C. glutinosum*. A new flavonoid compound, namely glutosinumoside **(4)** was afforded, and five known compounds; three oleanane-glycosides **(1-3)** and two phenolic acids **(5,6)**. The structures of the six compounds were determined by spectroscopic analysis, including one- and two-dimensional (1D– & 2D–) NMR, mass spectrometry, and chromatographic analysis. Moreover, *in vitro* cytotoxic evaluation of the successive extracts and the bio-actives EtOAc fractions of *C. glutinosum* against MCF7 (breast), HT29 (colon), HepG2 (liver), and MRC5 (normal lung) cell lines was performed. The isolated compounds showed comparable cytotoxic activities with the crude EtOH extract and doxorubicin against the tested cell lines. Compounds **(1)** and **(6)** exhibited the highest cytotoxicity against MCF7 (1.37±0.21 and 1.48±0.34 μg/mL, respectively) and

HepG2 (3.30±0.02 and 2.10±0.22 μg/mL, respectively) in the MTT assay. In addition, compounds **(1)** and **(3)** showed significant upregulation of cancer's two important hallmarks (caspase 3 and bax genes) by inducing apoptosis and perturbing the MCF7 cell cycle.

Kerwords: *Combretum glutinosum*; cytotoxicity; flavonol glycoside; triterpenoid saponin; phenolic compounds

Table S1. HR–ESI–MS (– mode) spectral data of compound **(1)**.

Chemical Formula	**$C_{42}H_{67}O_{13}$**	
HR–ESI–MS (–)	Molecular weight $C_{42}H_{67}O_{13}$, *m*/*z* (rel. int. %)	*m*/*z*= 843.4384 [M+HCOOH, 31%, calcd. 843.4697], 795.3777 [M–H^+, 10%, calcd. 759.4531], 727.3915 [M–H^+– 4OH^-, 100%, calcd. 727.4421], 744.3812 [M–H^+– 3OH^-, 85%, calcd. 744.4449], and 717.3630 [M –3CH_3 – 2HO^-, 75%, calcd. 717.3850]

Table S2. HR–ESI–MS (+/– mode) spectral data of compound **(4)**.

Chemical Formula	**$C_{38}H_{50}O_{23}$**	
HR–ESI–MS (+)	Molecular weight $C_{38}H_{50}O_{23}$, *m*/*z* (rel. int. %)	*m*/*z* = 575.2101 [M – 7OH^- –$C_9H_8O_4^{2-}$, 100%, calcd. 575.2422].
HR–ESI–MS (–)		*m*/*z* = 759.2730 [M–2H – 3OH^- – 2CH_3O^-, 2%, calced. 759.2136]; 587.1901 [M– 2H –6OH^- – CH_3O^- –$C_8H_8O_3^{2-}$, 2%, calcd. 587.1765]; 517.1487 [M–H – 2 glucose units – CH_3, 5%, calcd. 517.1346]; 371.15604 [M– 3glucose parts, 1%, calcd. 371.1131]; 265.1478 [M– 3sugar moieties – CH_3 –3CH_3O^-, 100%, calcd. 265.0344].

Table S3. NMR Spectroscopic Data for the Compounds **(2,3)** in Deuterated Methanol[1] and Pyridine[2].

Position	Type	Compound (2)[1]		Compound (3)[2]		Oleanolic acid [a, a`]	
		[*]δ_H (*J* in *Hz*)	[**]δ_C	[*]δ_H (*J* in *Hz*)	[**]δ_C	δ_H (*J* in *Hz*) [a, a`]	δ_C [a, a`]
1	CH_2			2.01, *m*, 1.91, *brd*	33.8	1.57, 1.02	39.0
2	CH_2	0.91, *m*,	29.2	2.01, *br.t*, 0.99, *br.s*	29.8	1.82	28.1
3	CH	3.24, *d* (3.5)	82.9	3.54, *d* (4)	81.7	3.44, *dd*	78.2
4	C		36.5		36.3		39.4
5	CH	3.77, *m*	57.7	1.84, *br.d* (11.5)	48.8	0.88, *d*	55.9
6	CH_2			1.65, *m*, 1.44, *br.t*	19.5	1.58, 1.39	18.8
7	CH_2	1.45, *m*, 1.28, *m*	34.6	1.67, *m*, 1.43, *br.t*	33.6	1.53, 1.36	33.4
8	C		43.1		41.0		39.8
9	CH	1.90, *m*	48.7	2.01, *br.t*	49.2	1.71, *tr*	48.2
10	C		39.7		44.1		37.4
11	CH_2	1.94, *m*, 1.25, *brs*	25.5	2.06, *br.s*	25.0	1.96	23.8
12	CH	5.30, *br.s*	125.2	5.47, *s*	124.6	5.49, *s*	122.6
13	C		144.2		145.1		144.8
14	C		43.1		42.8		42.2
15	CH_2	1.26, *br.s*	31.0	2.01, *br.t*, 2.34, *t*	29.8	2.19, 1.22	28.4
16	CH_2			2.78, *br.t*, 2.07, *m*	28.7	2.12, 1.96	23.8
17	C		49.6		47.8		46.7
18	CH	3.01, *m*	45.5	3.50, *br.s*	45.3	3.30, *dd*	42.1
19	CH_2	1.88, *m*, 0.86, *d* (6.4)	48.2	2.27, *m*, 1.34, *t*	48.3	1.83, 1.32	46.6
20	C		29.4		39.0		31.0
21	CH_2	1.45, *m*, 1.28, *m*	34.6	1.67, *m*, 1.43, *br.t*	33.6	1.46, 1.23	34.3
22	CH_2	1.73, *m*, 1.62, *m*	33.7	2.01, *br.t*	29.6	2.04, 1.82	33.2
23	CH_3	0.91, *s*	25.6	0.95, *s*	25.6	1.24, *s*	28.8
24	CH_3	0.97, *s*	29.9	1.18, *s*	18.4	1.02, *s*	16.5
25	CH_3	0.95, *s*	17.9	1.10, *s*	18.1	0.93, *s*	15.6
26	CH_3	0.69, *s*	18.2	1.06, *s*	15.0	1.04, *s*	17.5
27	CH_3	1.26, *s*	25.5	1.52, *s*	25.5	1.30, *s*	26.2
28	C		179.0		178.1		172.0
29	CH_3	0.91, *s*	25.6	1.12, *s*	29.5	0.97, *s*	33.4
30	CH_3	1.26, *s*	25.5	0.95, *s*	25.3	1.02, *s*	23.8
				β–D–Glucose [b / (C–28) c, c`]			
1`		5.33, *d* (8.3)	96.3	6.37, *d* (8.0)	96.6	6.31, *d* (8.0)	96.0 [b]
						5.37, *d* (8.1)	95.7 [c, c`]
2`		3.28, *m*	74.5	4.22, *m*	74.9	4.23	74.2 [b]
						3.33, *m*	73.9 [c, c`]
3`		3.34, *m*	78.8	4.30, *m*	79.7	4.30	79.1 [b]
						3.35, *d* (8.5)	78.2 [c, c`]
4`		3.77, *br.s*	70.9	4.37, *m*	71.5	4.34	71.3 [b]
						3.39, *m*	71.1 [c, c`]
5`		3.33, *m*	78.8	4.02, *d* (8.5)	80.1	4.04	79.5 [b]
						3.42, *m*	78.5 [c, c`]
6`		3.78, *m*; 3.65, *dd*	62.8	4.43, *m*	62.8	4.43, 4.48, *d* (11.8)	62.4 [b]
						3.80; 3.71 *m*	62.5 [c, c`]
		β–Galactose [d]					
1``		5.33, *d* (8.3)	96.3			6.31, *d* (8.0)	96.0 [b]

					4.7	103.3 [d]
2``	3.27, *m*	74.5			4.23	74.2 [b]
					4.92	71.3 [d]
3``	3.31, *br.s*	71.8			4.30	79.1 [b]
					5.13	71.9 [d]
4``	3.31, *br.s*	71.2			4.34	71.3 [b]
					5.12	68.6 [d]
5``	3.28, *m*	74.5			4.04	79.5 [b]
					3.73	75.1 [d]
6``	3.78, *m;* 3.65, *dd*	62.8			4.43, 4.48, *d* (11.8)	62.4 [b]
					4.33, 4.06	62.3 [d]
			α–Arabinose [b / e]			
1``			6.06, *d* (5.5)	96.6	4.16	97.6 [b]
					4.75 (*d*; 6.9)	107.8 [e]
2``			4.37, *m*	71.5	3.43	72.9 [b]
					4.44	73.1 [e]
3``			4.22, *m*	74.9	3.57	73.5 [b]
					4.16	74.8 [e]
4``			4.23, *m*	69.6	3.85	69.6 [b]
					4.31	69.8 [e]
5``			4.18, *m*, 3.70, *d* (10.0)	67.1	3.82, 3.57	67.2 [b]
					3.81 (*d*; 10.6), 4.31	67.0 [e]

[*] Measured at 500 MHz, [**] Measured at 125 MHz, [a, a`] [1,2], [b] [3], [c, c`] [4,5], [d] [6], [e] [7], Assignments were based on the HMBC, HSQC, COSY and DEPT experiments.

Table S4. NMR Spectroscopic Data for the Compounds **(5,6)** in Deuterated Methanol.

position	**Compound (5)**		**Vanillic acid** [a]		**Compound (6)**		**Syringic acid** [b]	
(C)	[*]δ_H (*J* in *Hz*)	[**]δ_C	[*]δ_H (*J* in *Hz*)	[**]δ_C	[*]δ_H (*J* in *Hz*)	[**]δ_C	[*]δ_H (*J* in *Hz*)	[**]δ_C
1				122.0		149.3		150.4
2	7.50 (1H, *s*)	116.2	7.45 (1H, *s*)	115.4	7.27 (1H, *s*)	108.7	7.32 (1H, *s*)	115.4
3		149.1		147.6		123.0		120.9
4		152.8		150.5		142.0		145.1
5	6.76 (1H, *d, J* = 8.0 Hz)	114.3	6.85 (1H, *d, J* = 7.8 Hz	113.1		123.0		120.9
6	7.48(1H, *br.d, J* = 9.0 Hz)	125.7	7.44 (1H, *s*)	123.9	7.27 (1H, *s*)	108.7	7.32 (1H, *s*)	115.4
7		169.2		167.6		171.0		165.8
O–CH_3	3.85 (3H, *s*)	56.8	3.81 (3H, *s*)	55.95	3.87(6H, *s*)	57.2	3.87 (6H, *s*)	60.1

[*] Measured at 500 MHz, [**] Measured at 125 MHz, [a] [8], [b] [9].

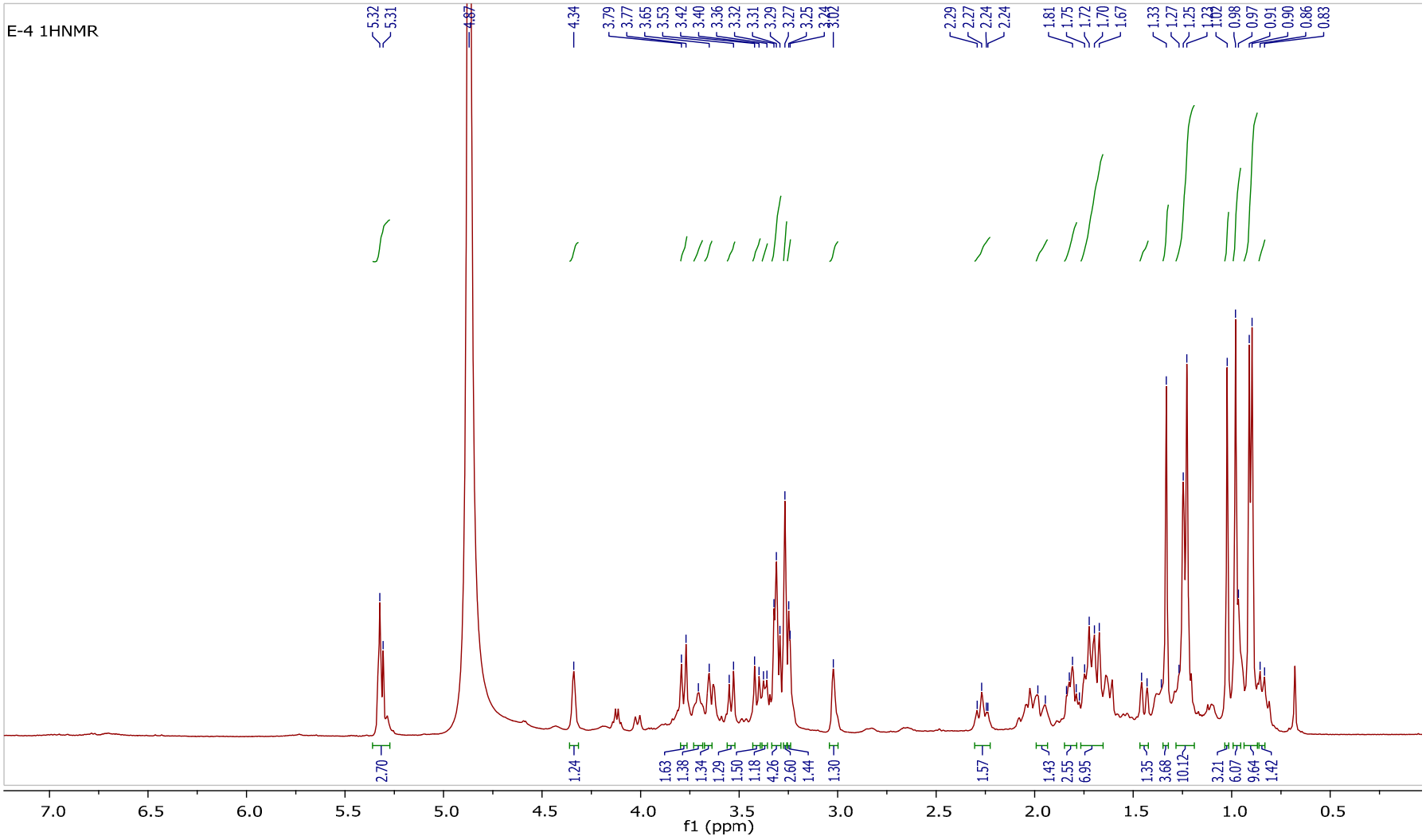


Figure S1. ^{1}H–NMR spectrum of compound **(1)** in CD_3OD.

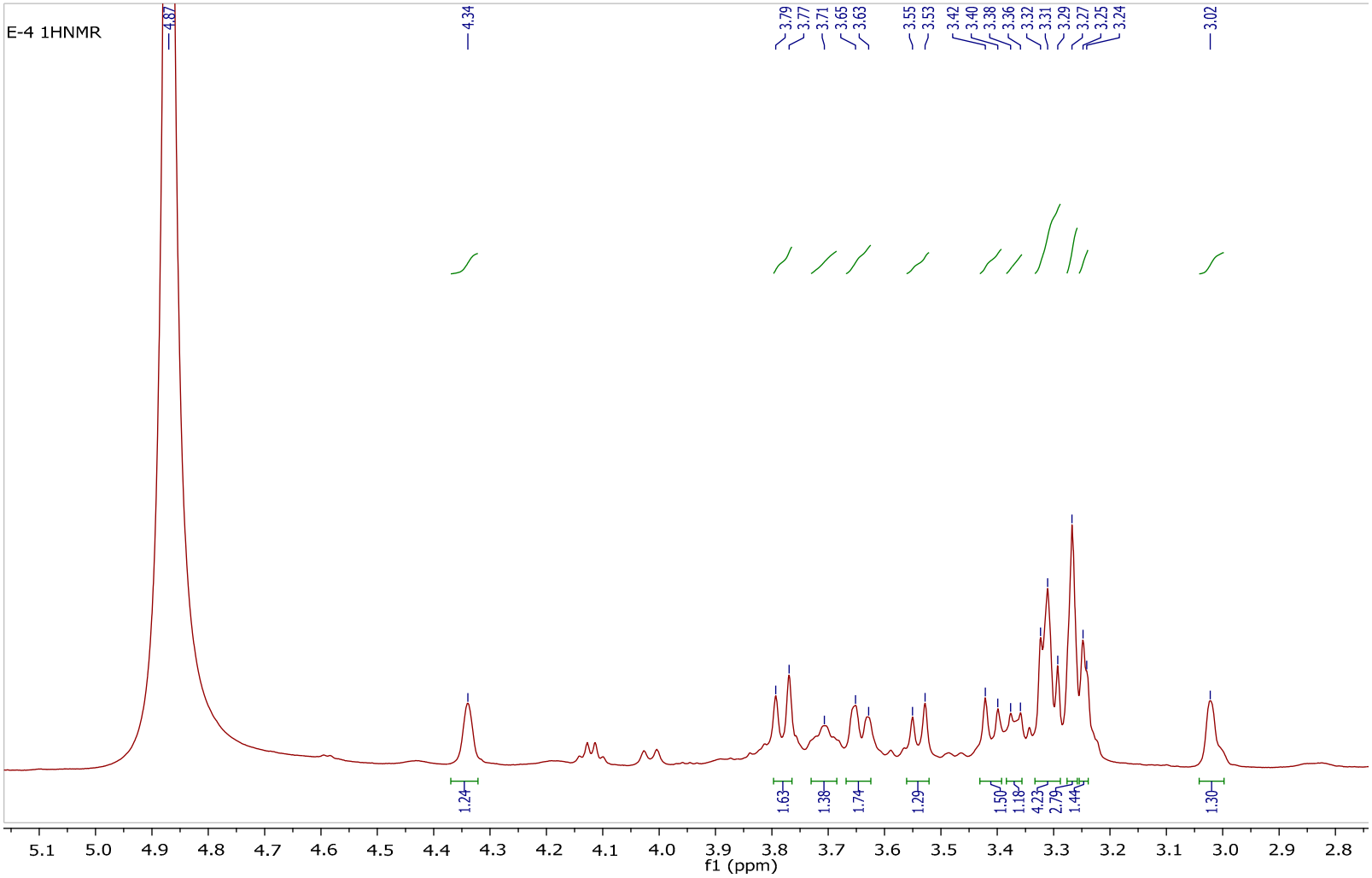


Figure S2. ^{1}H–NMR spectrum (expansion from 2.8–5.1 ppm) of compound **(1)** in CD_3OD.

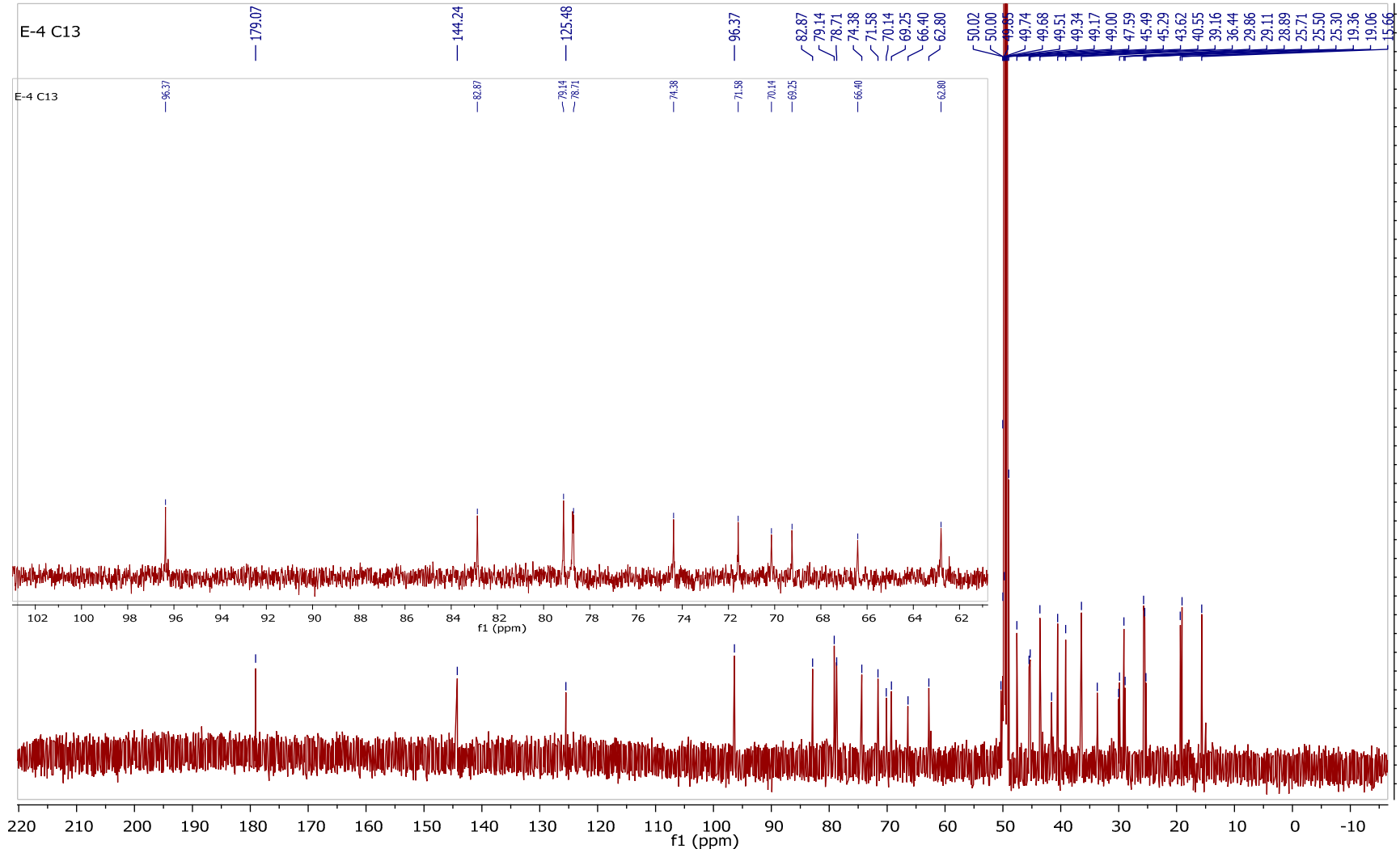


Figure S3. ^{13}C–NMR spectrum of compound **(1)** in CD_3OD.

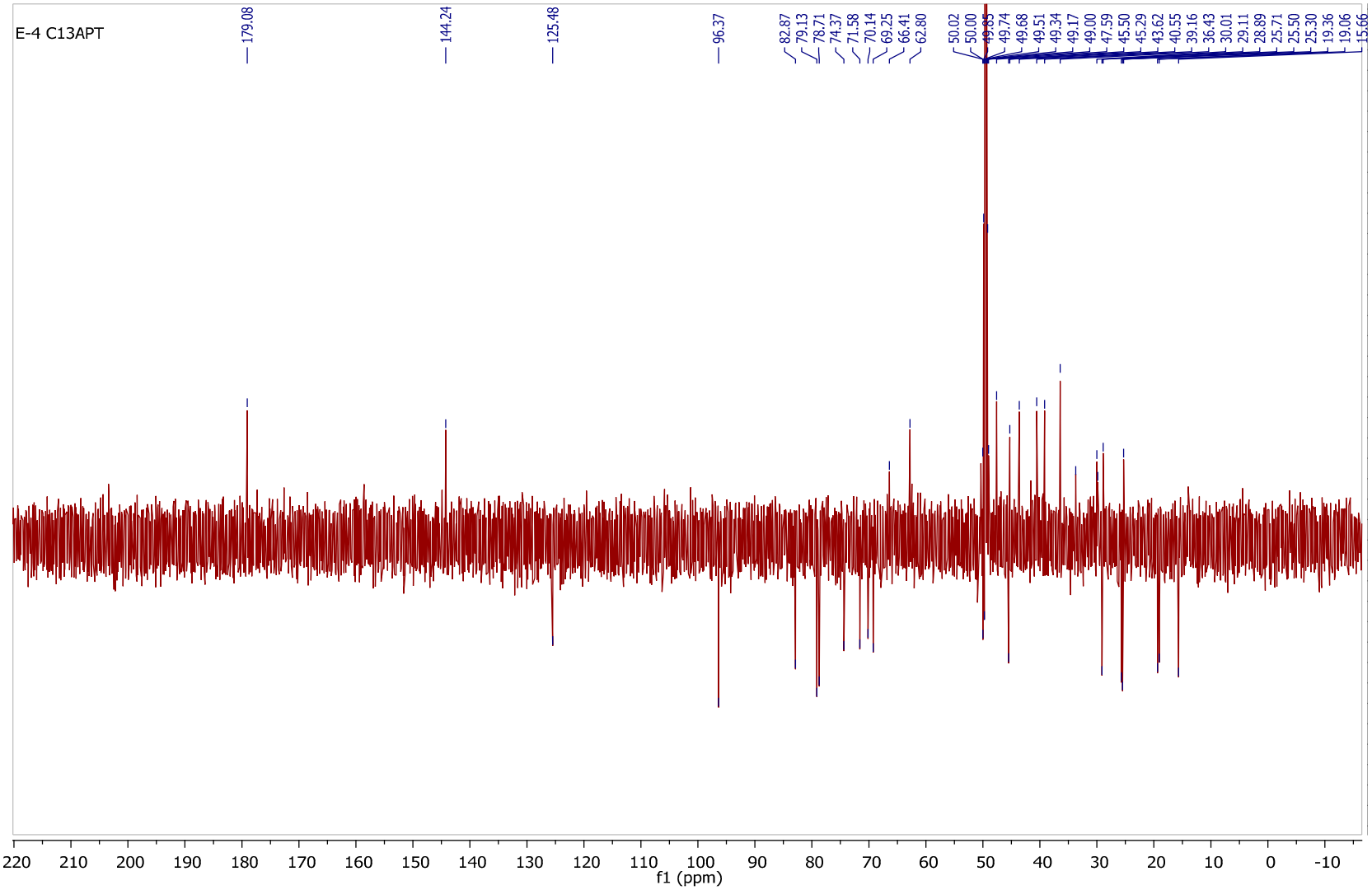
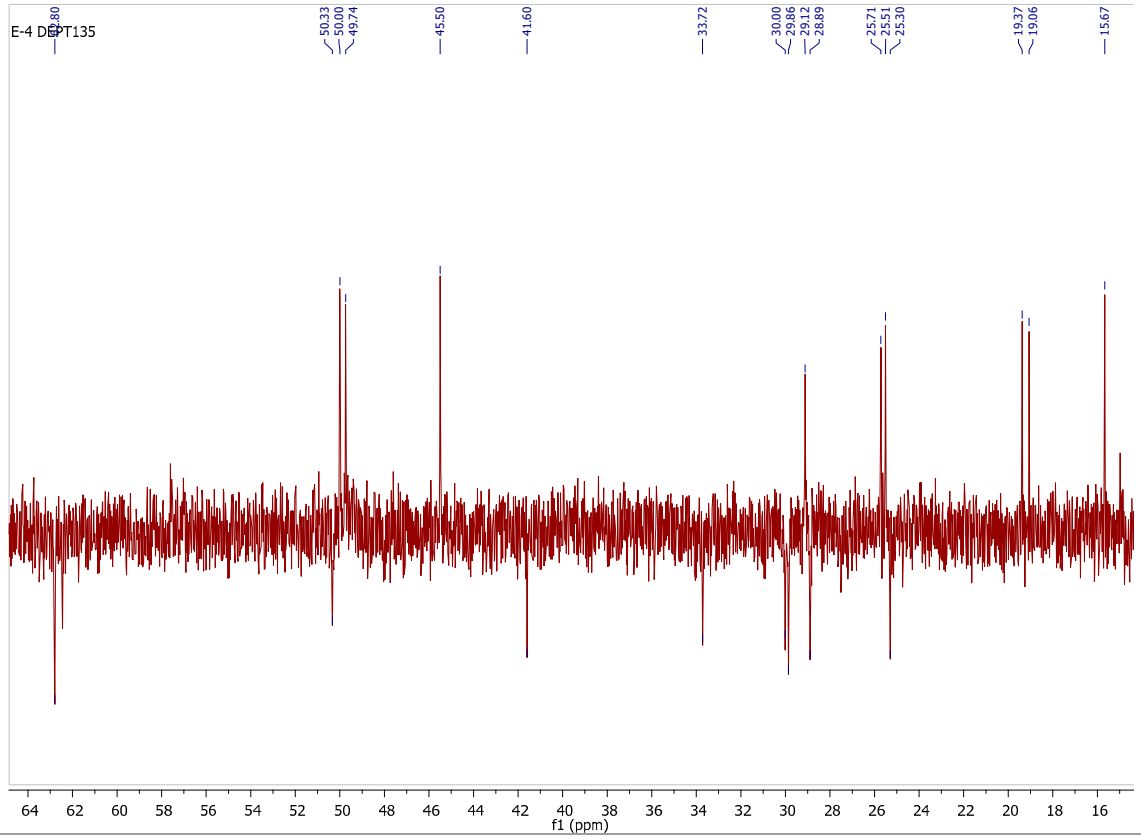


Figure S4. APT –NMR spectrum of compound **(1)** in CD_3OD.

(A)



(B)

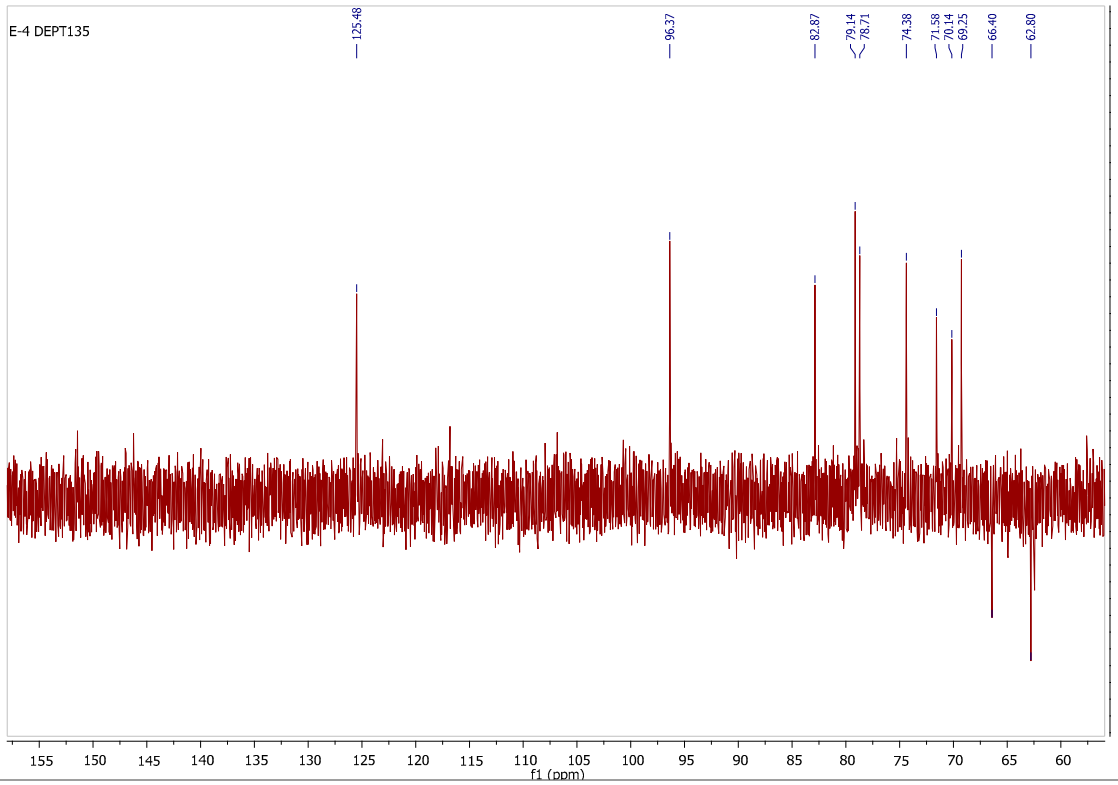


Figure S5. DEPT –NMR spectrum of compound **(1)** in CD_3OD; **(A):** (expansion range 16–64 ppm) **(B):** (expansion range 60–155 ppm).

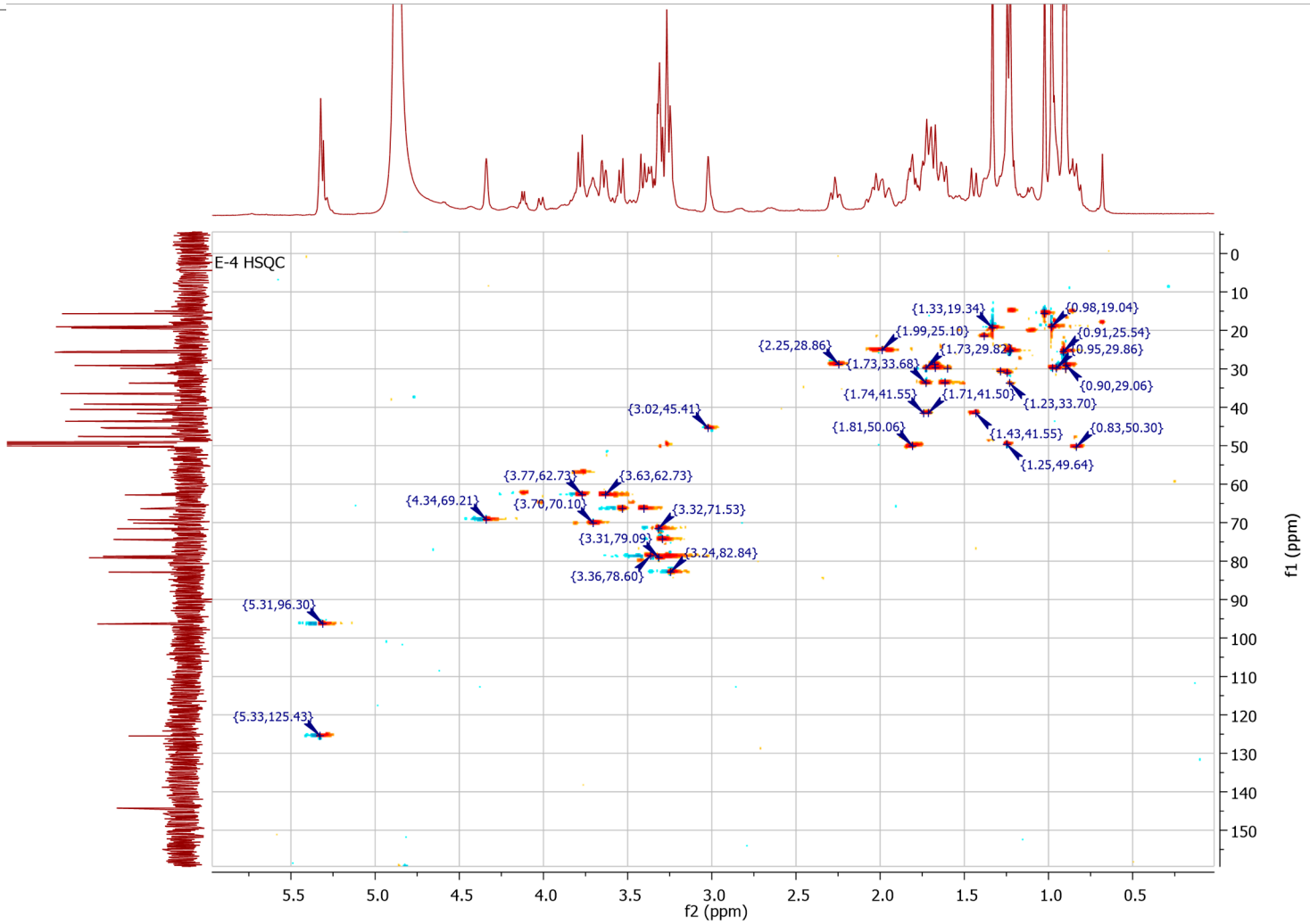


Figure S6. HSQC spectrum of compound **(1)** in CD_3OD.

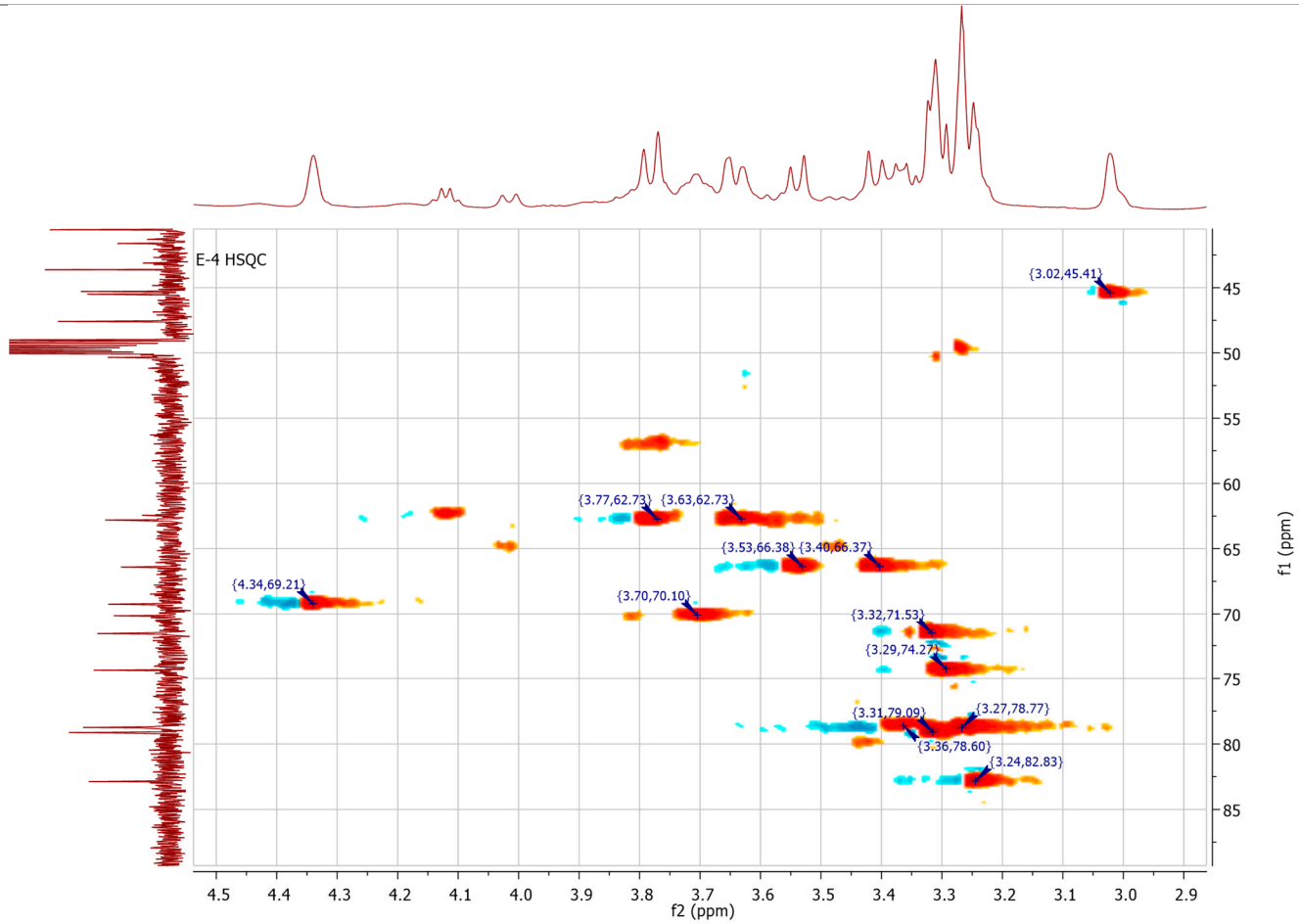


Figure S7. HSQC spectrum (expansion range 2.9–4.5 ppm) of compound **(1)** in CD_3OD.

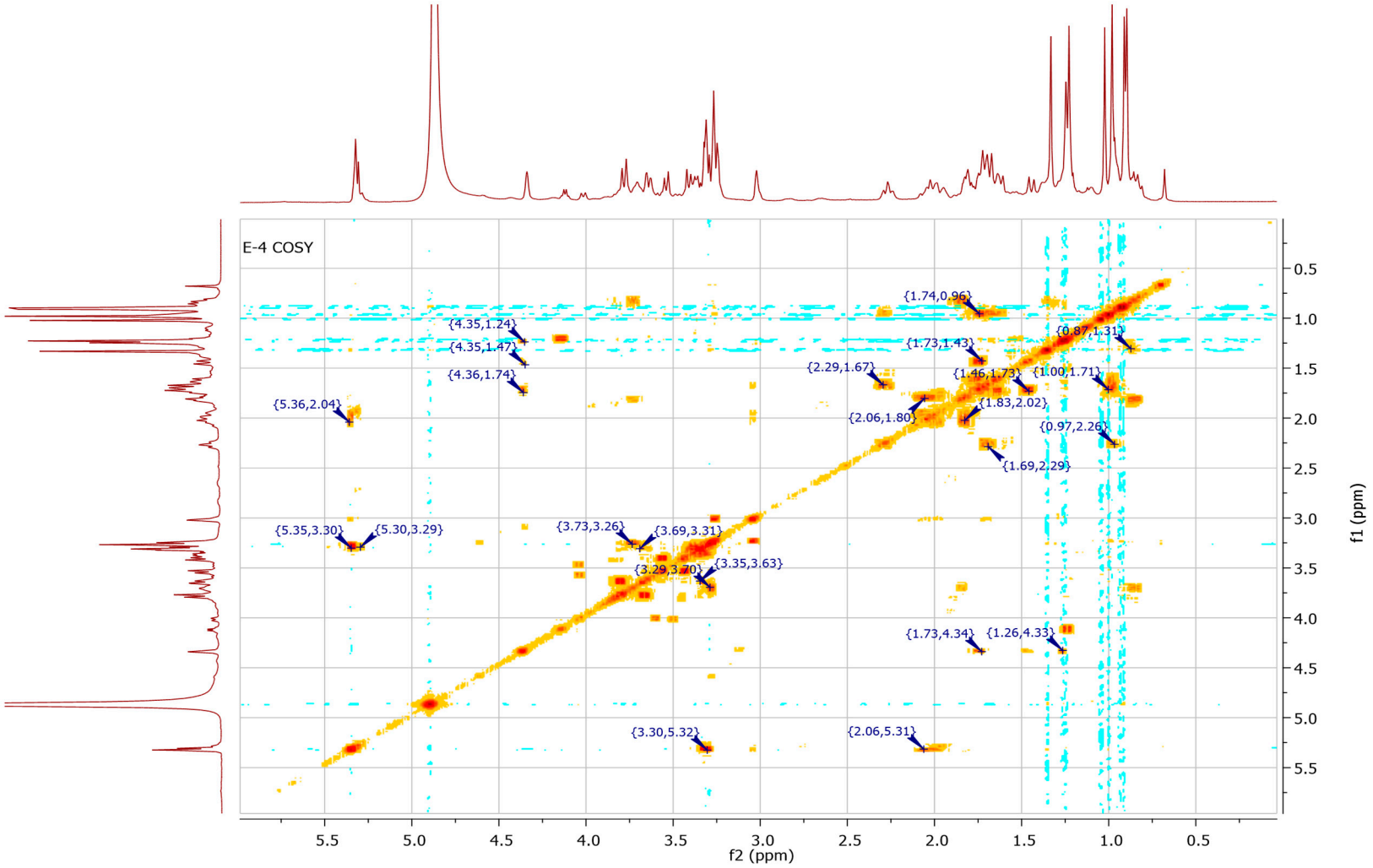


Figure S8. COSY spectrum of compound **(1)** in CD_3OD.

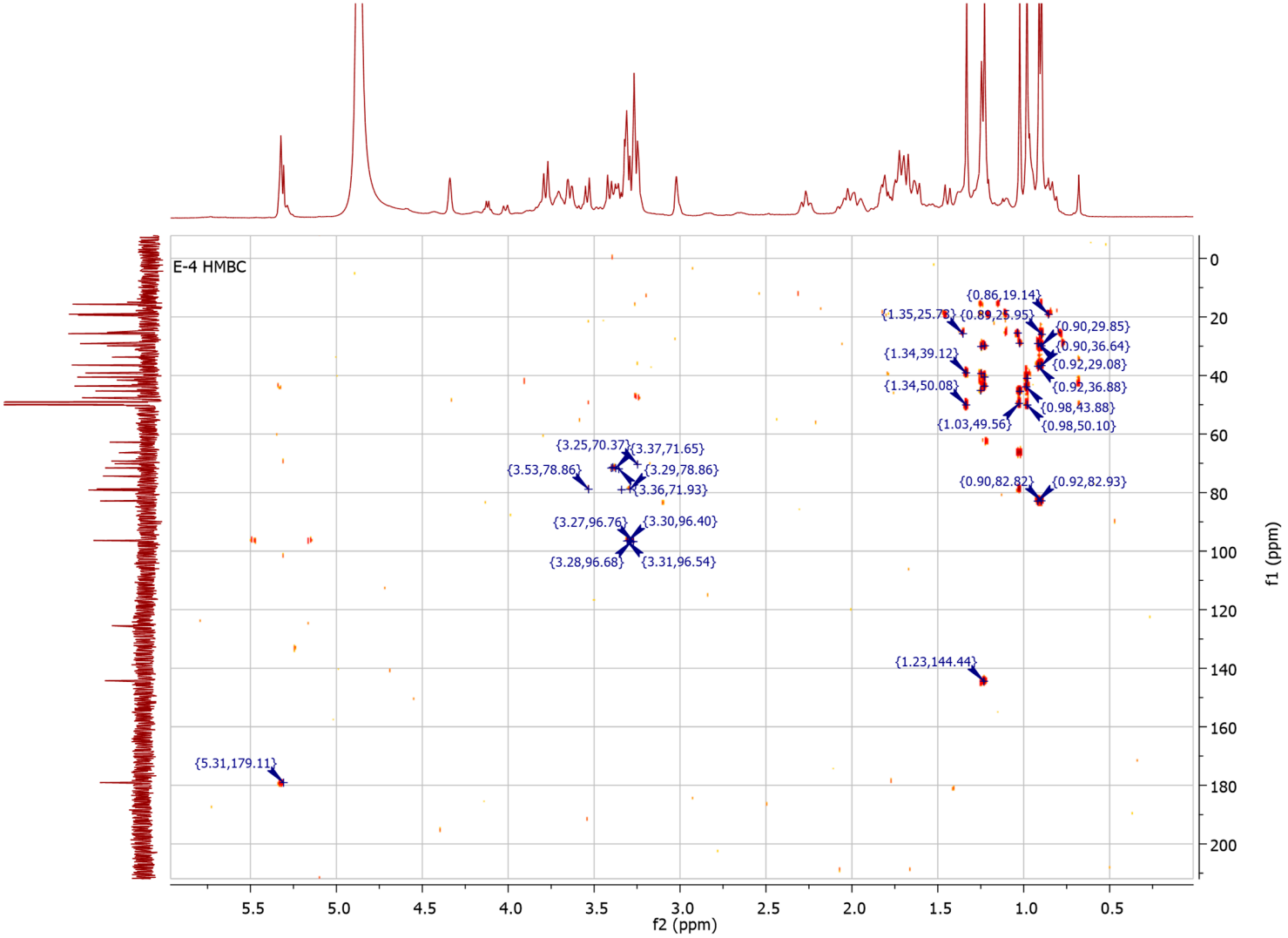


Figure S9. HMBC spectrum of compound **(1)** in CD_3OD.

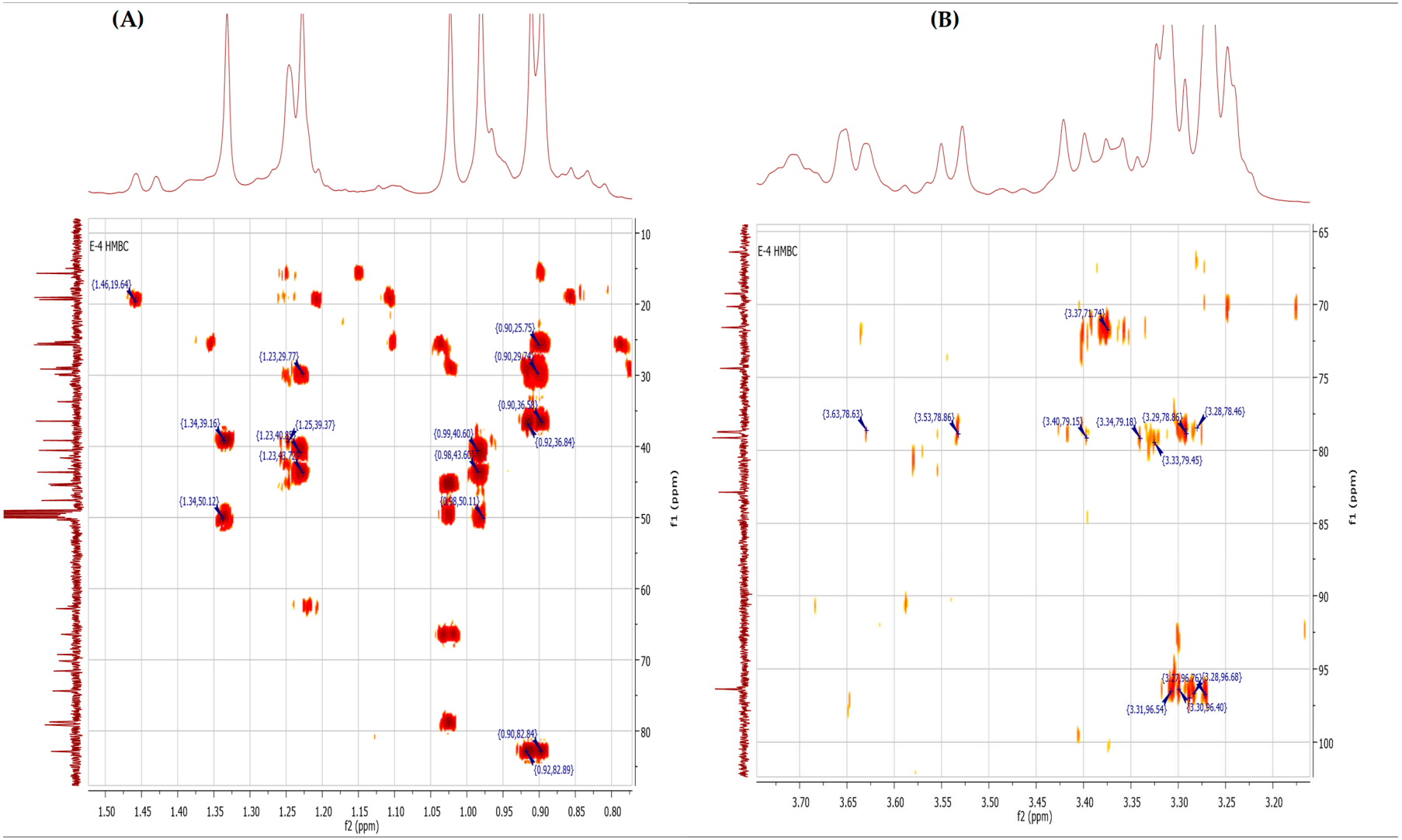


Figure S10. HMBC spectrum of compound **(1)** in CD_3OD; **(A):** (expansion range 0.80–1.50 ppm) **(B):** (expansion range 3.20–3.70 ppm

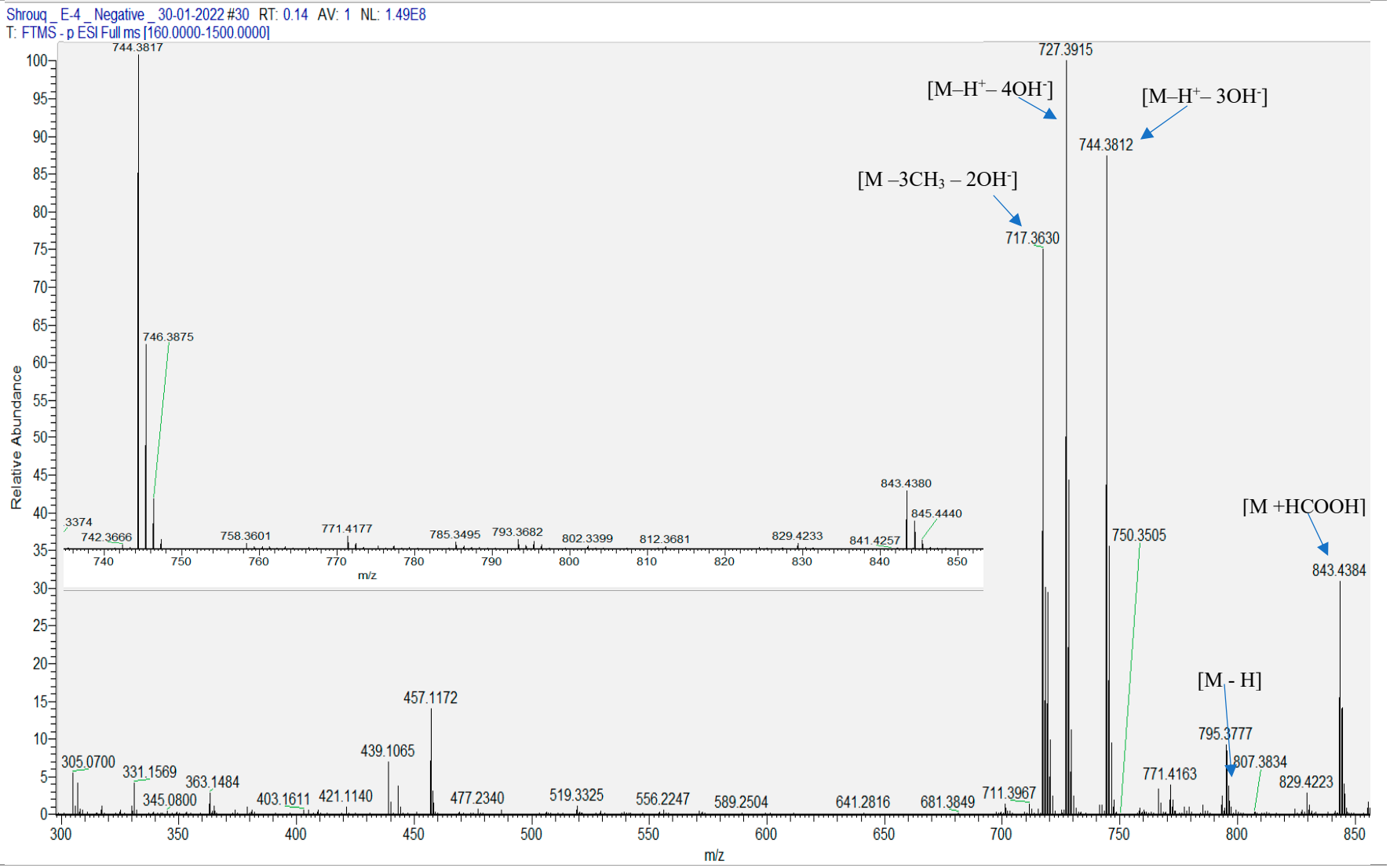


Figure S11. HR–ESI–MS spectrum (– mode) of compound **(1)** in CD_3OD.

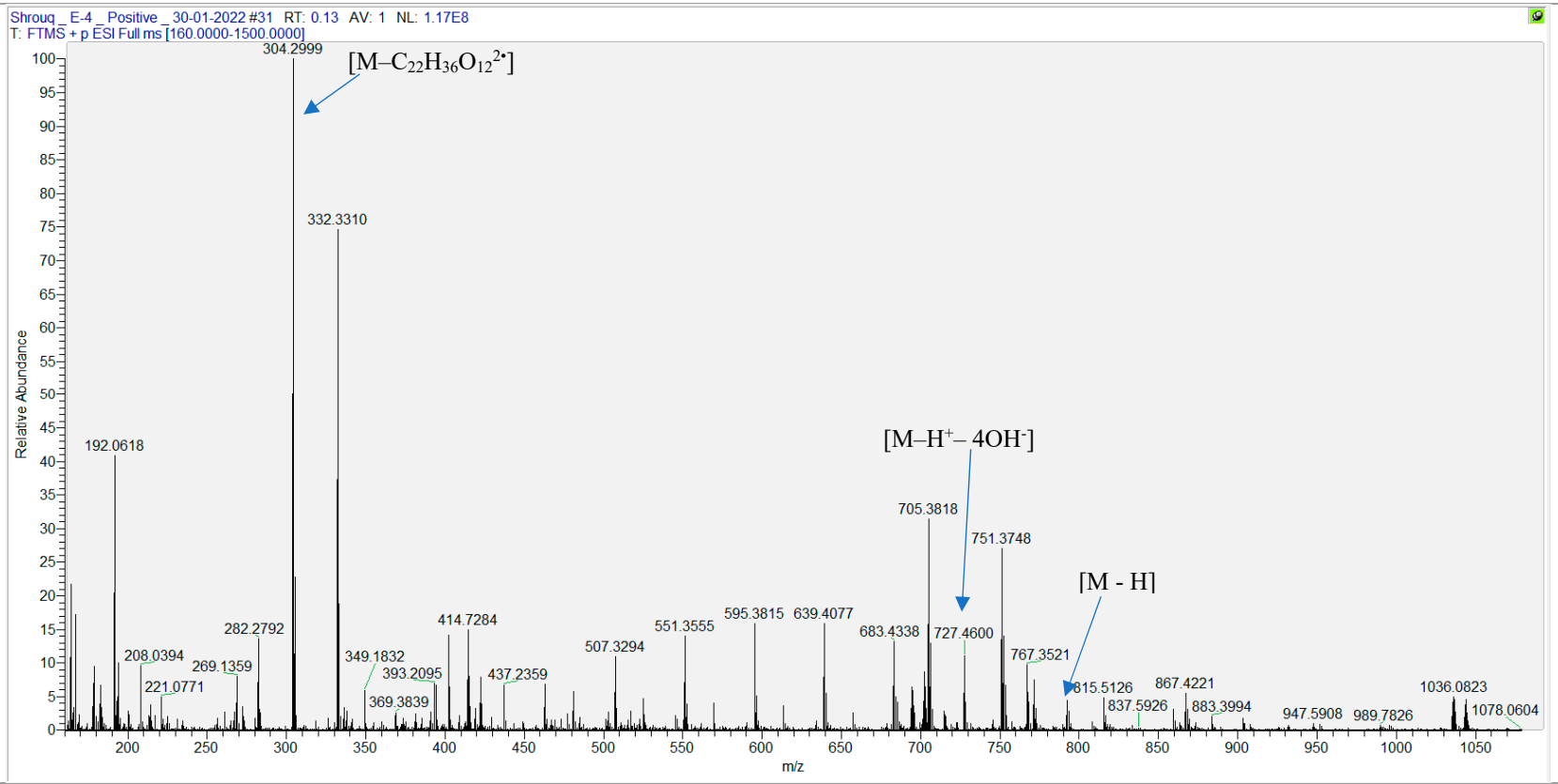


Figure S12. HR–ESI–MS spectrum (+ mode) of compound **(1)** in CD_3OD.

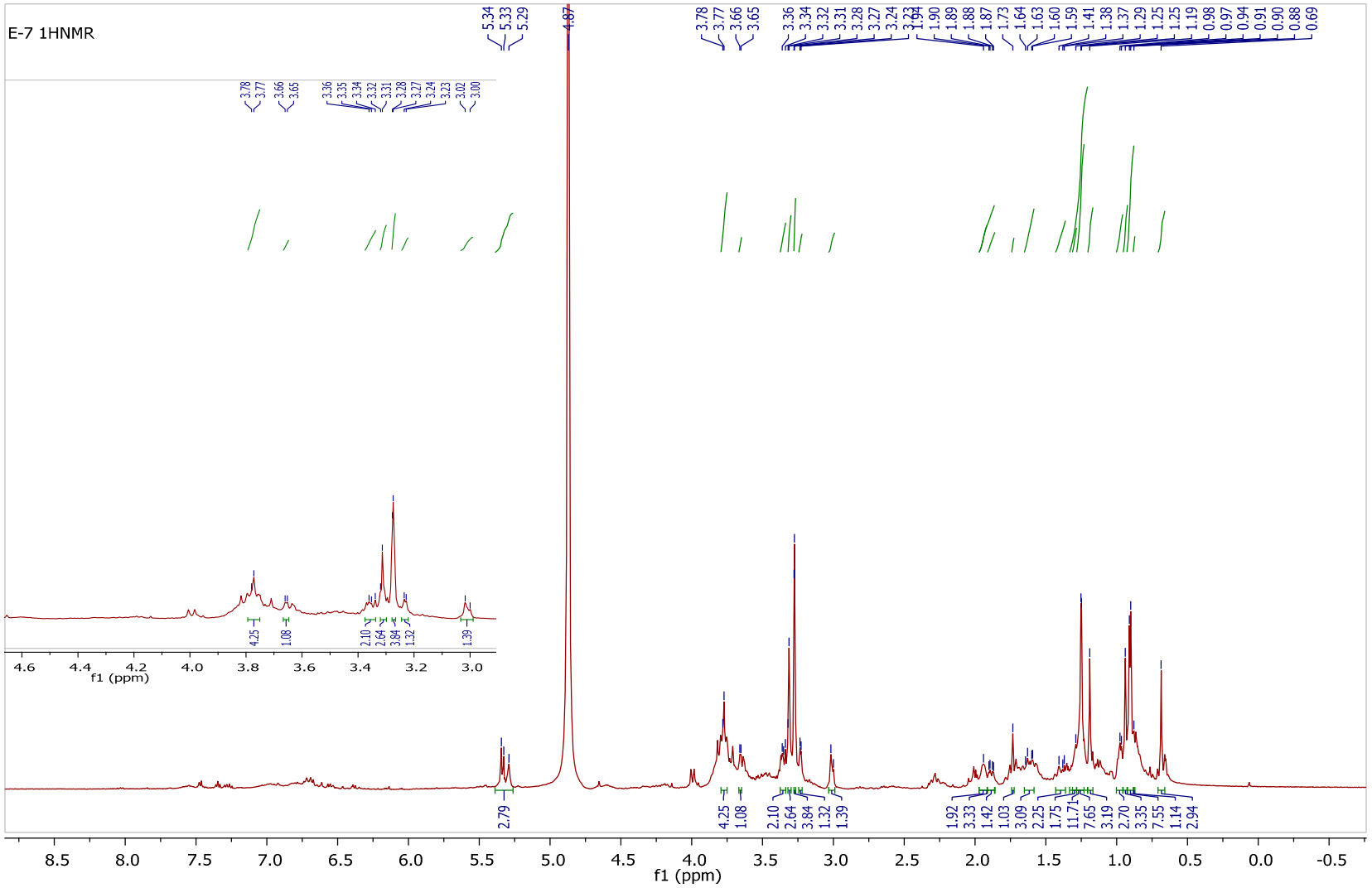


Figure S13. ^{1}H–NMR spectrum of compound **(2)** in CD_3OD.

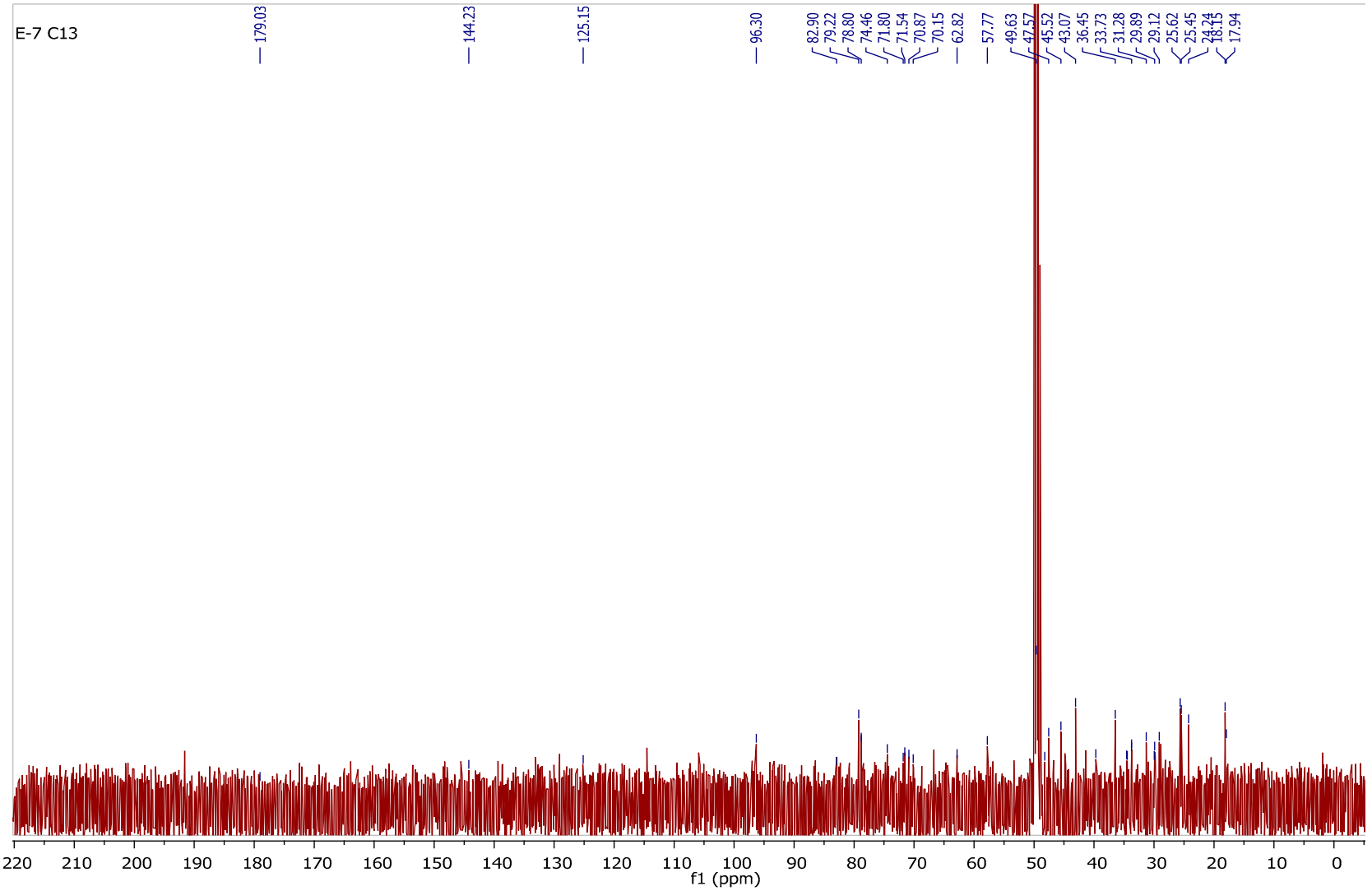


Figure S14. ^{13}C–NMR spectrum of compound **(2)** in CD_3OD.

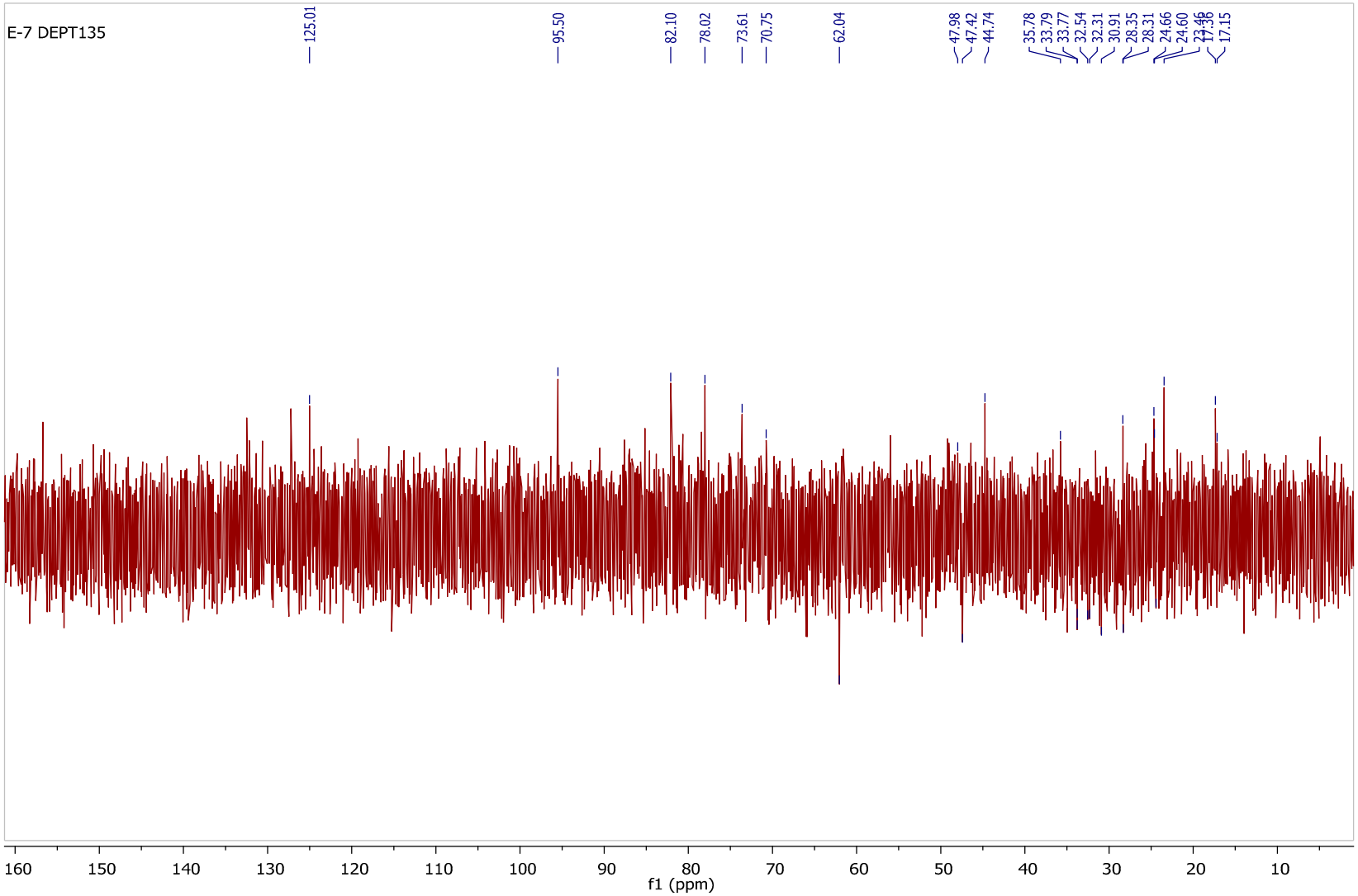


Figure S15. DEPT–NMR spectrum of compound **(2)** in CD_3OD.

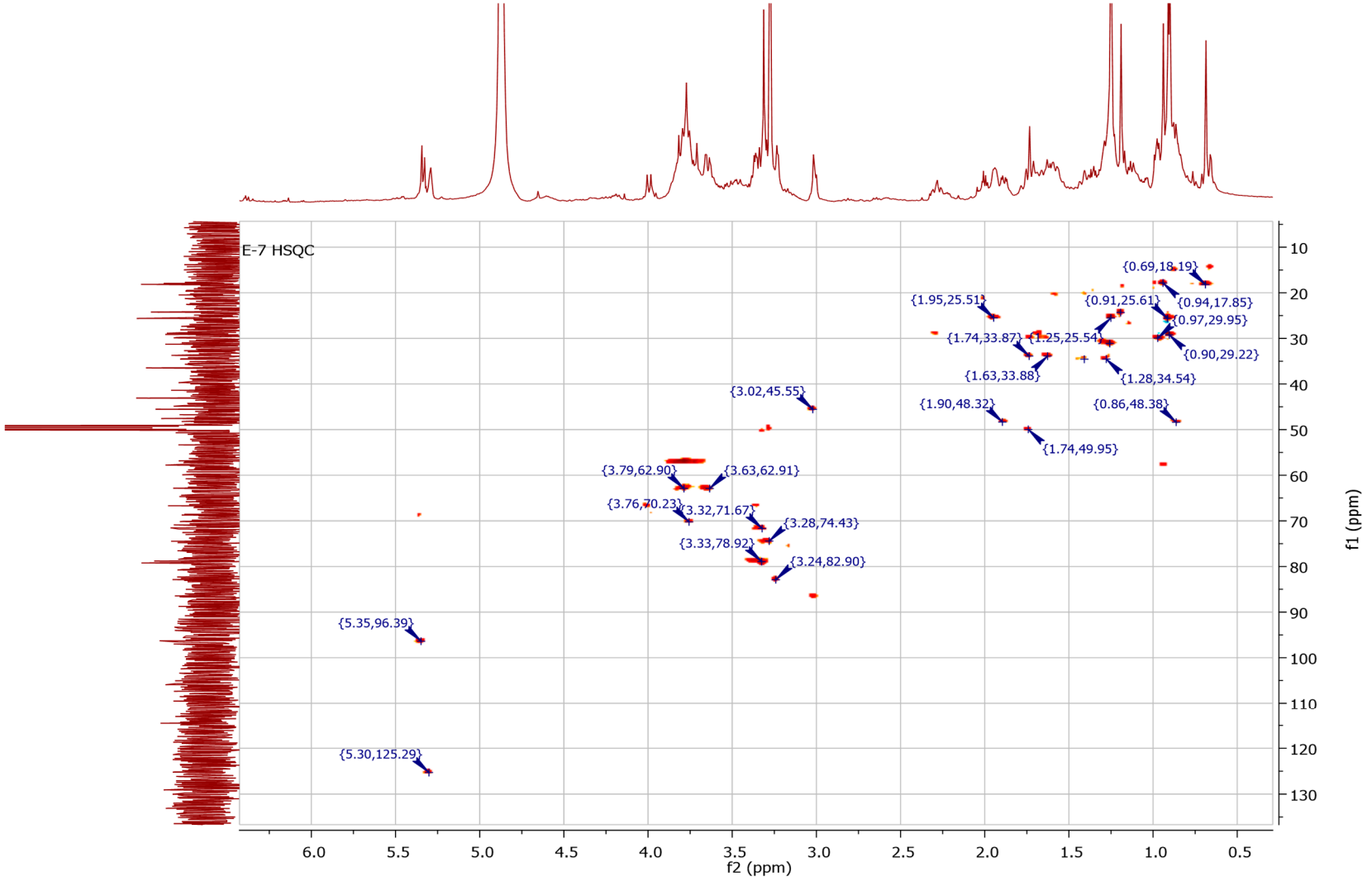


Figure S16. HSQC–NMR spectrum of compound **(2)** in CD_3OD.

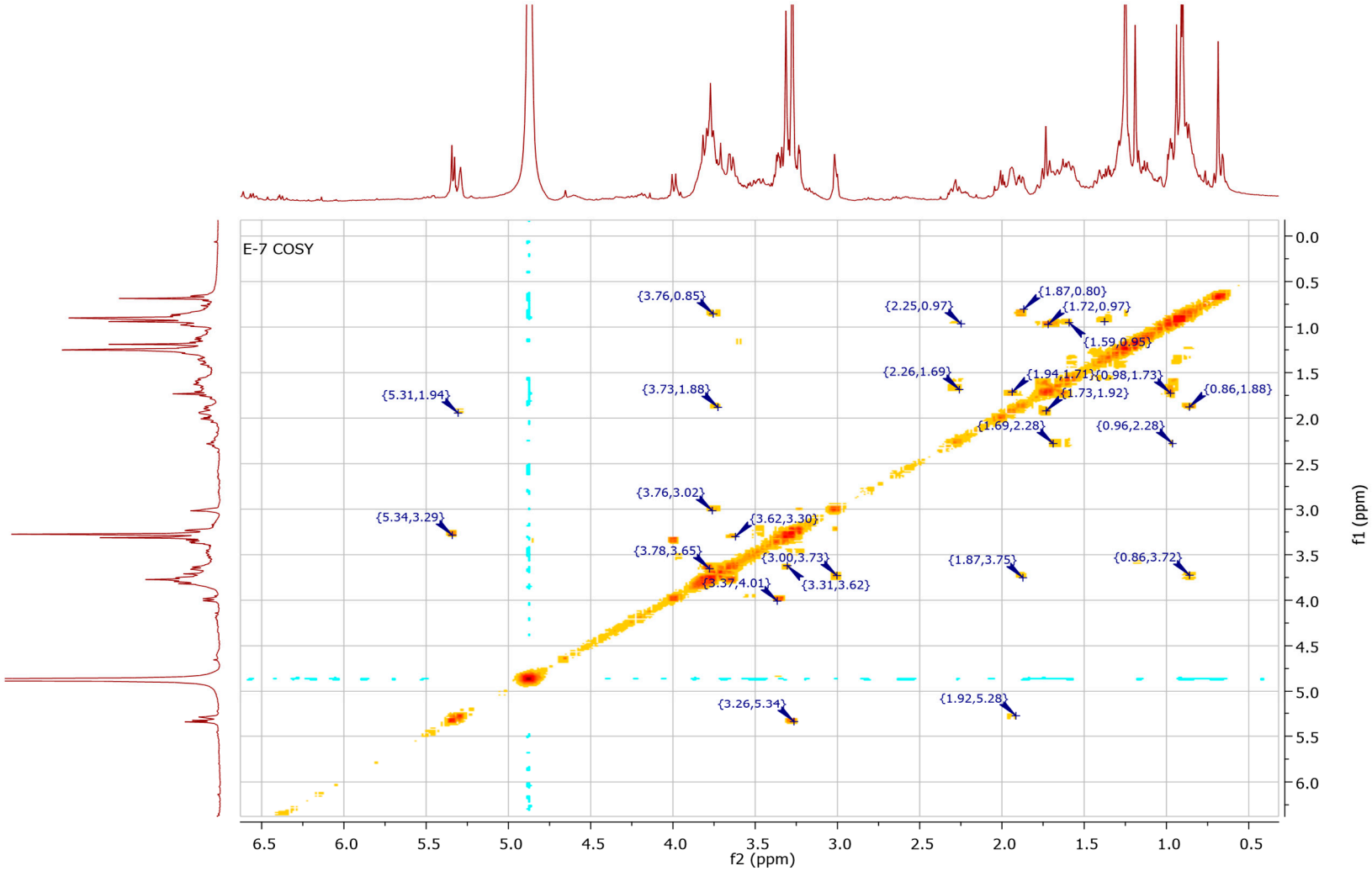


Figure S17. COSY spectrum of compound **(2)** in CD_3OD.

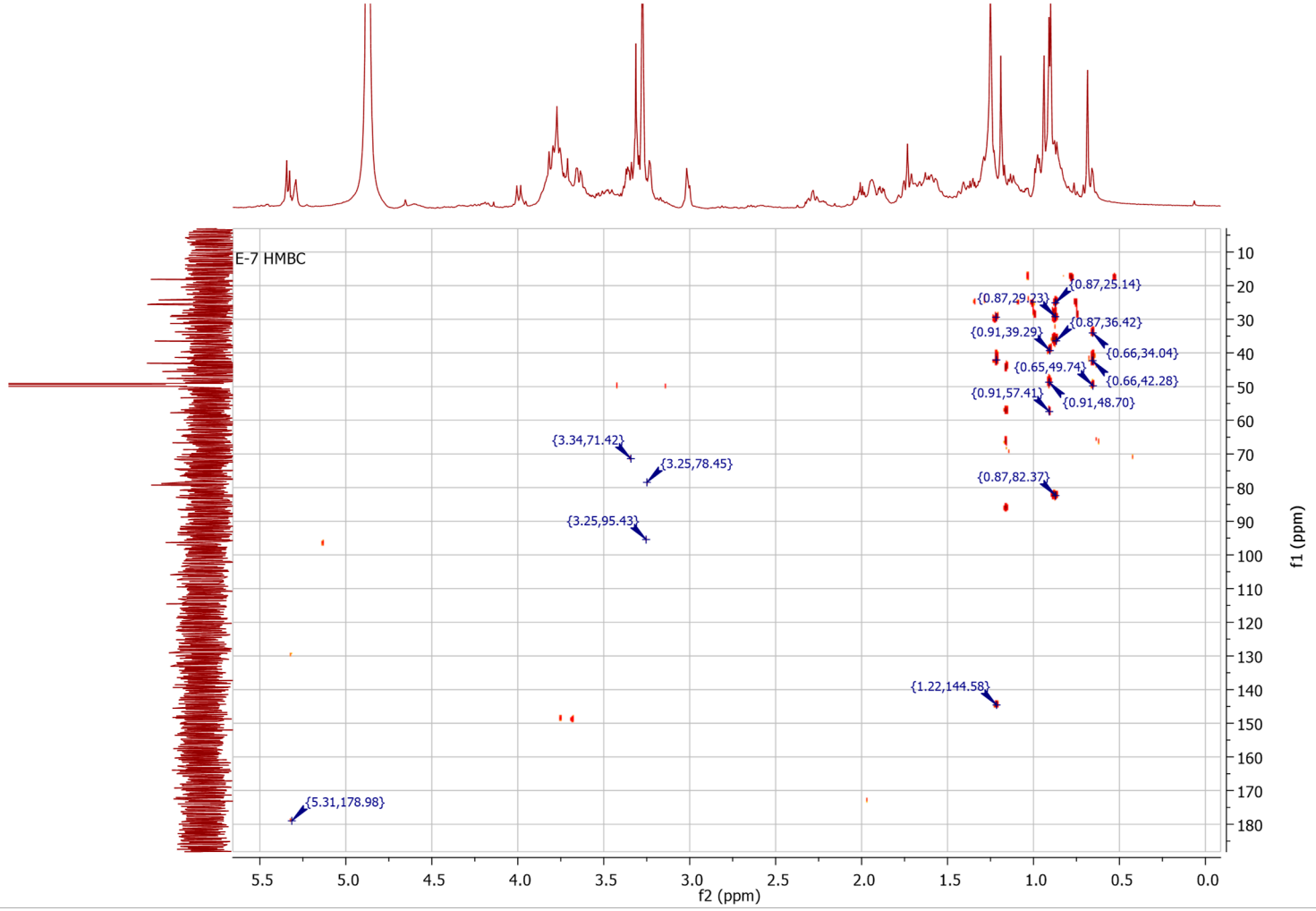


Figure S18. HMBC spectrum of compound **(2)** in CD_3OD.

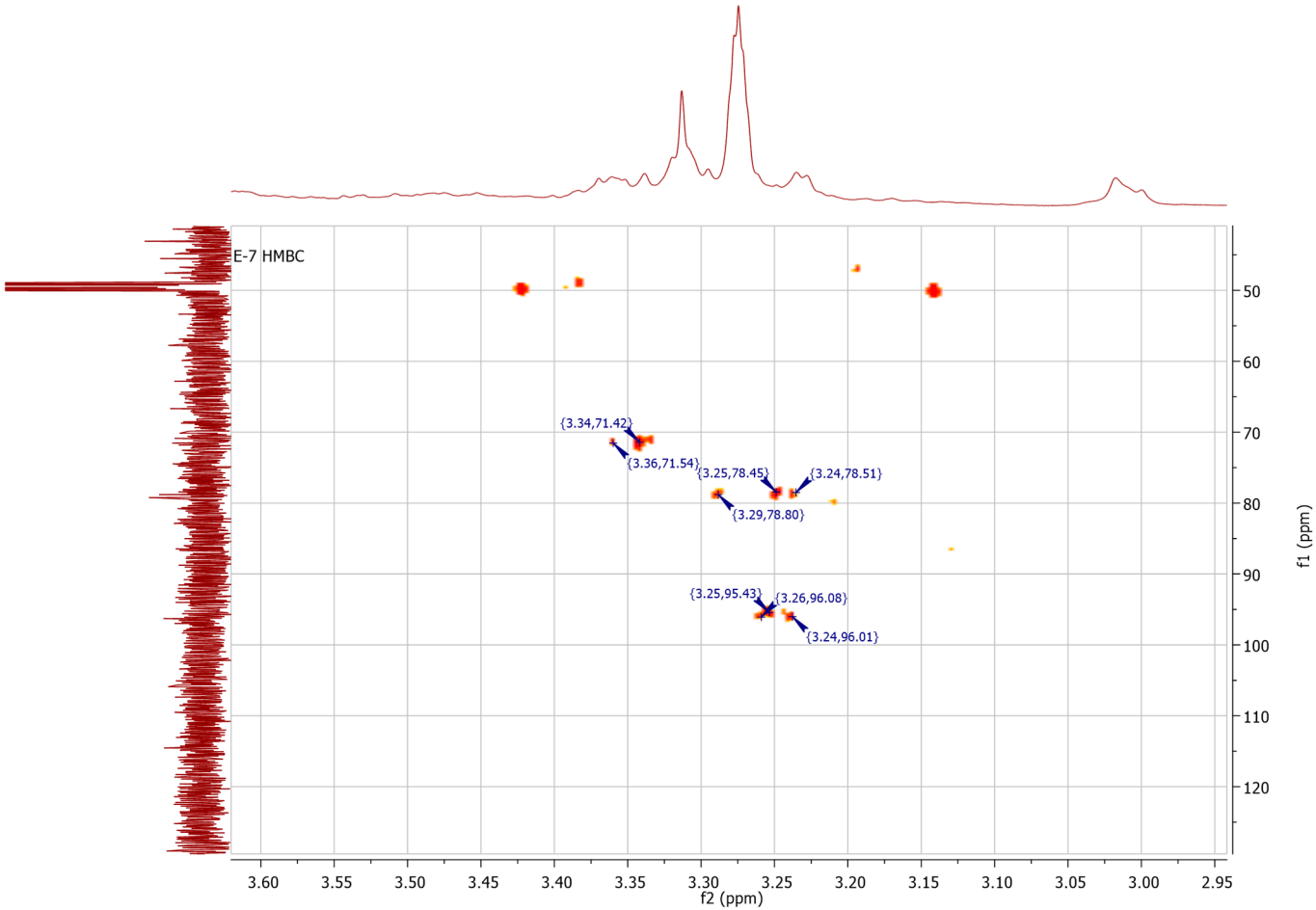


Figure S19. HMBC spectrum (expansion range 2.95–3.60 ppm) of compound **(2)** in CD_3OD.

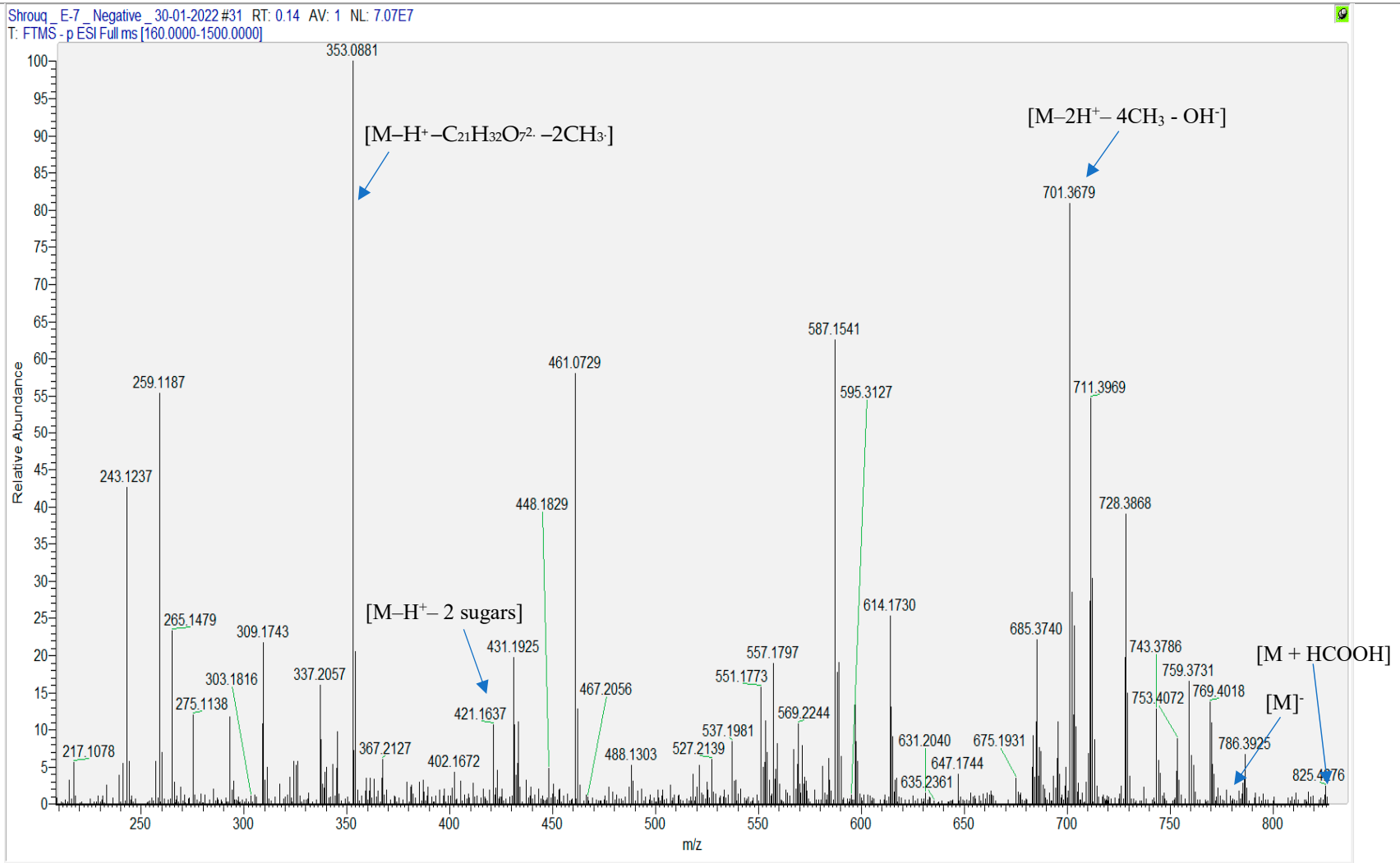


Figure S20. HR–ESI–MS spectrum (– mode) of compound **(2)** in CD_3OD.

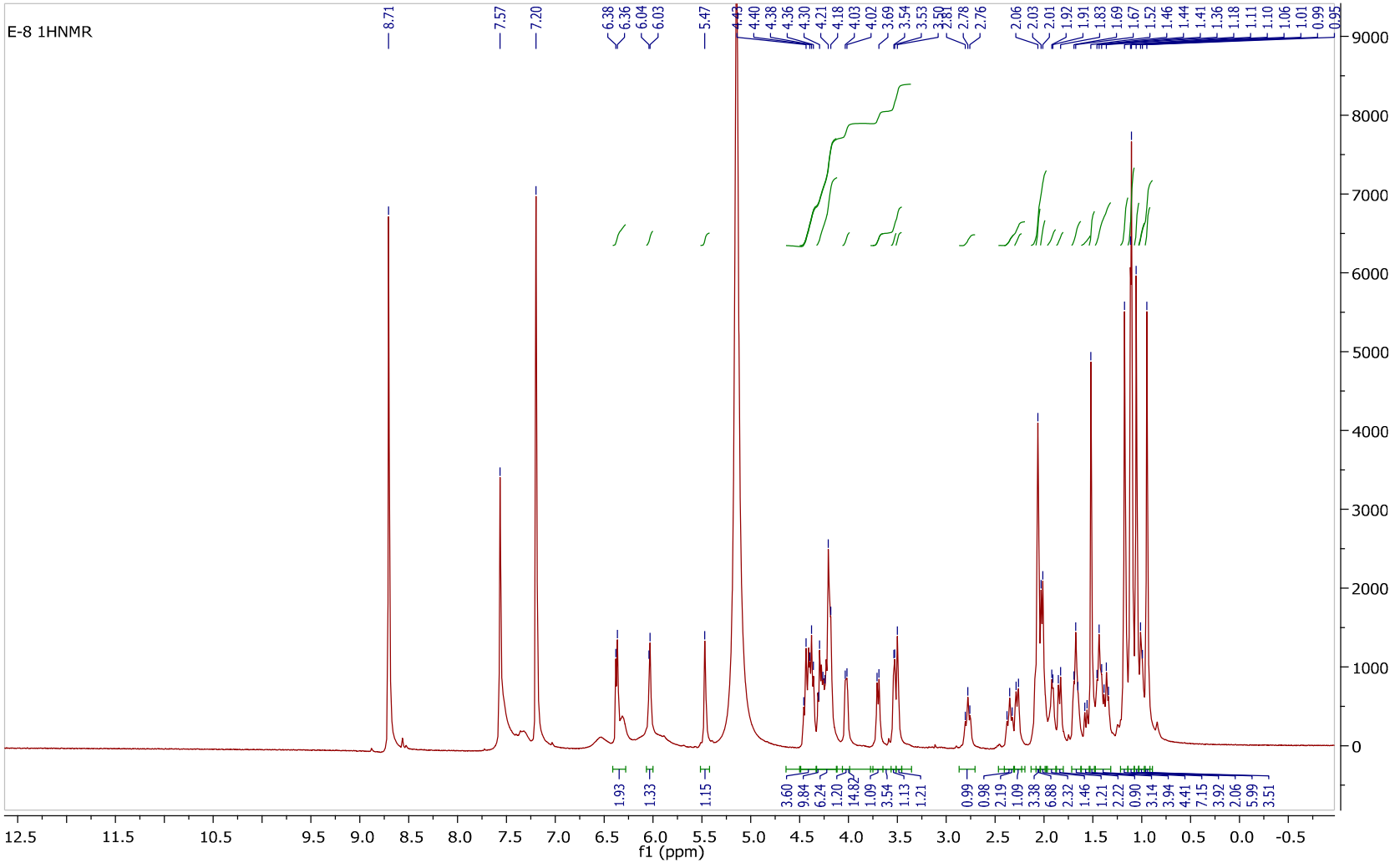


Figure S21. ^{1}H–NMR spectrum of compound **(3)** in pyridine–d_6.

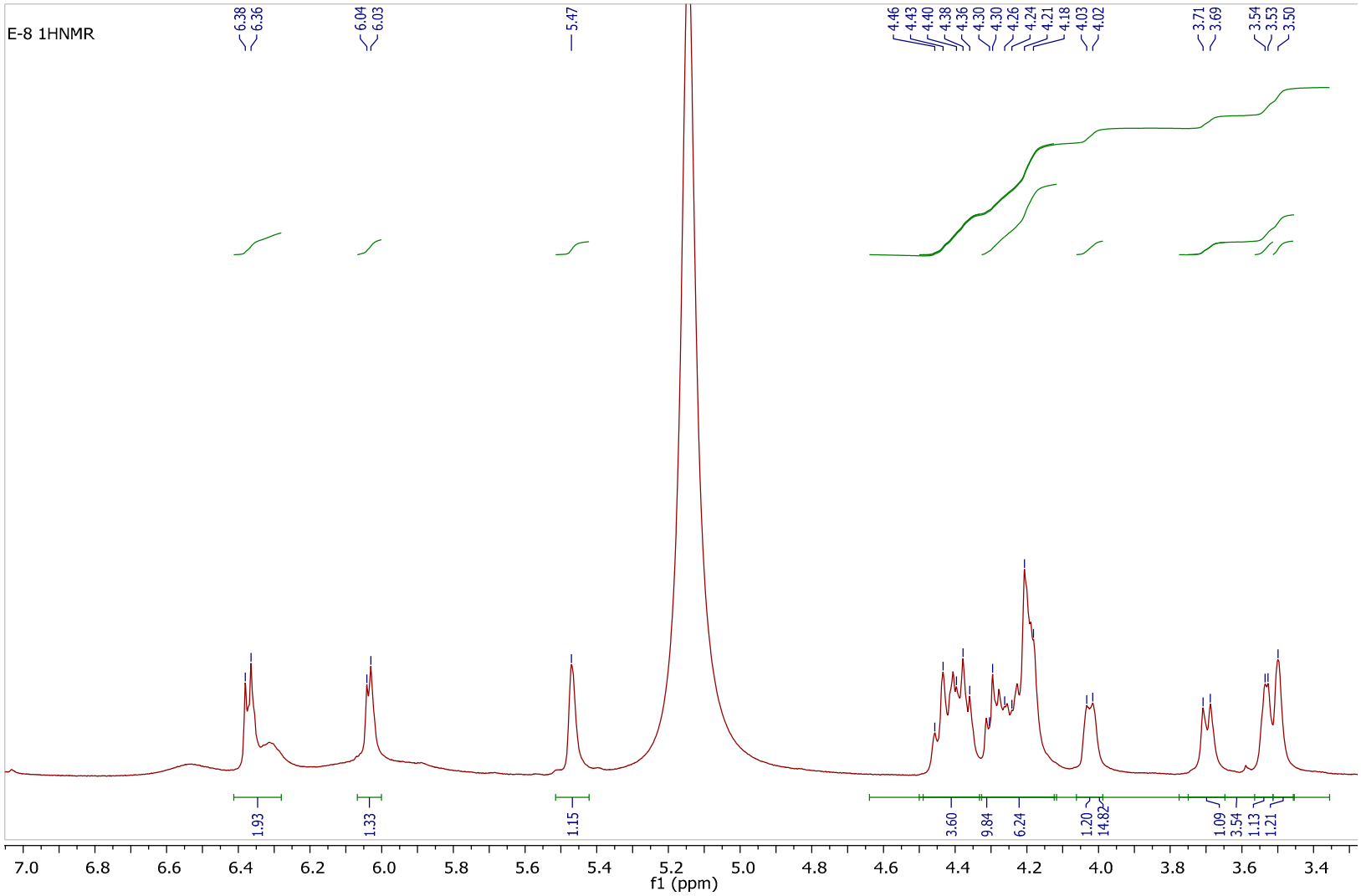


Figure S22. ^{1}H–NMR spectrum (expansion range 3.4–7.0 ppm) of compound **(3)** in pyridine–d_6.

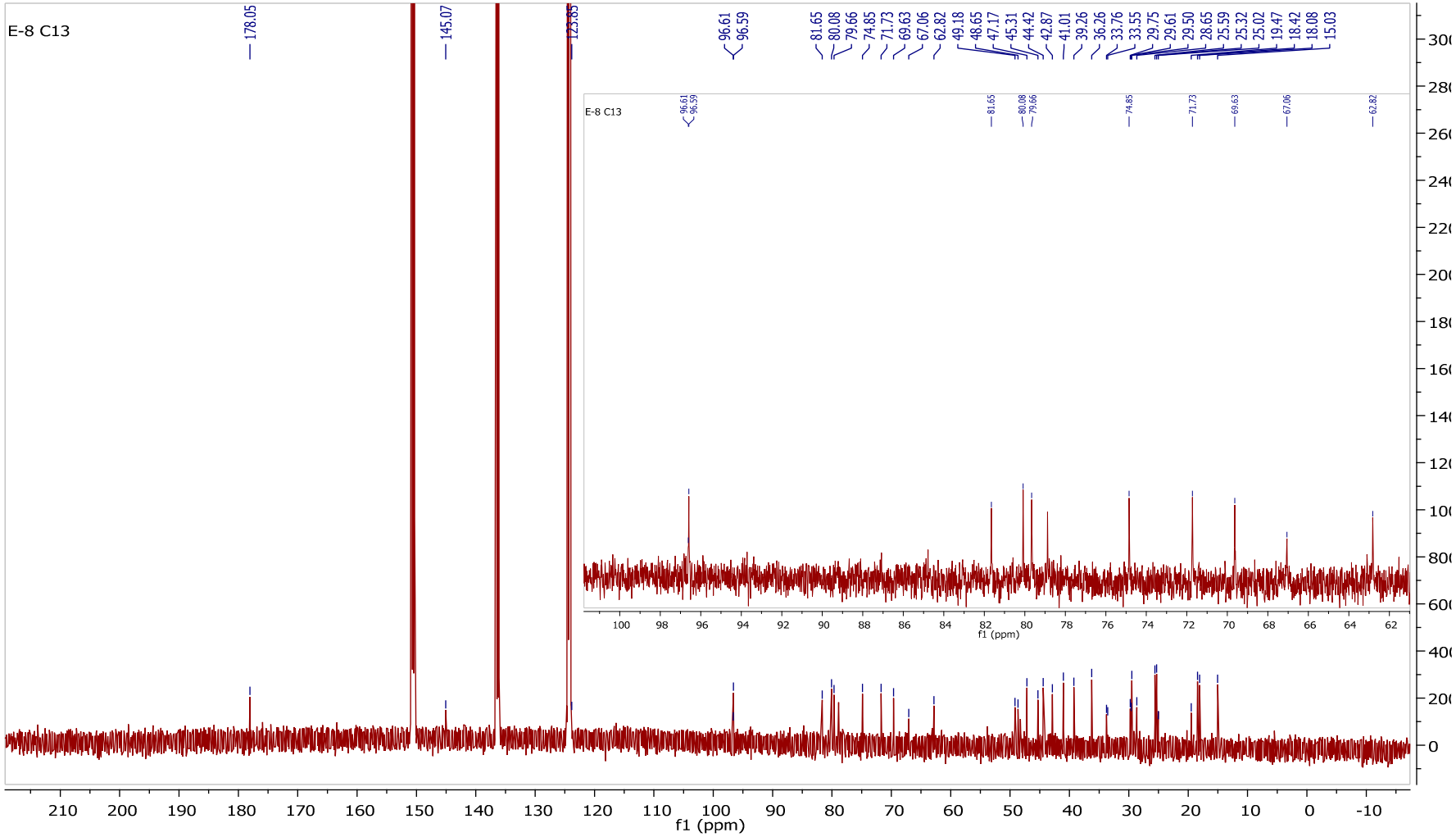


Figure S23. ^{13}C–NMR spectrum of compound **(3)** in pyridine–d_6.

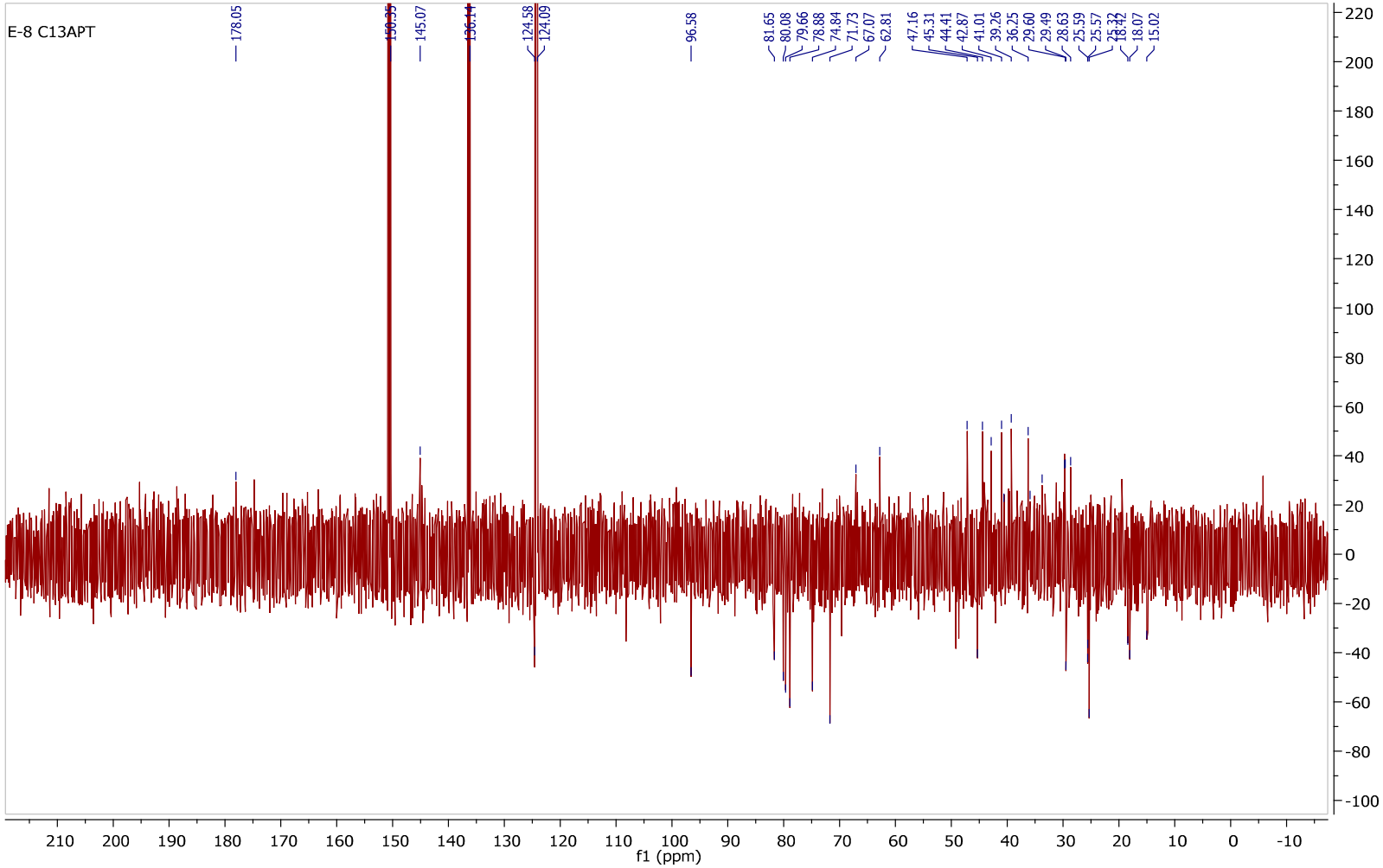


Figure S24. APT –NMR spectrum of compound **(3)** in pyridine–d_6.

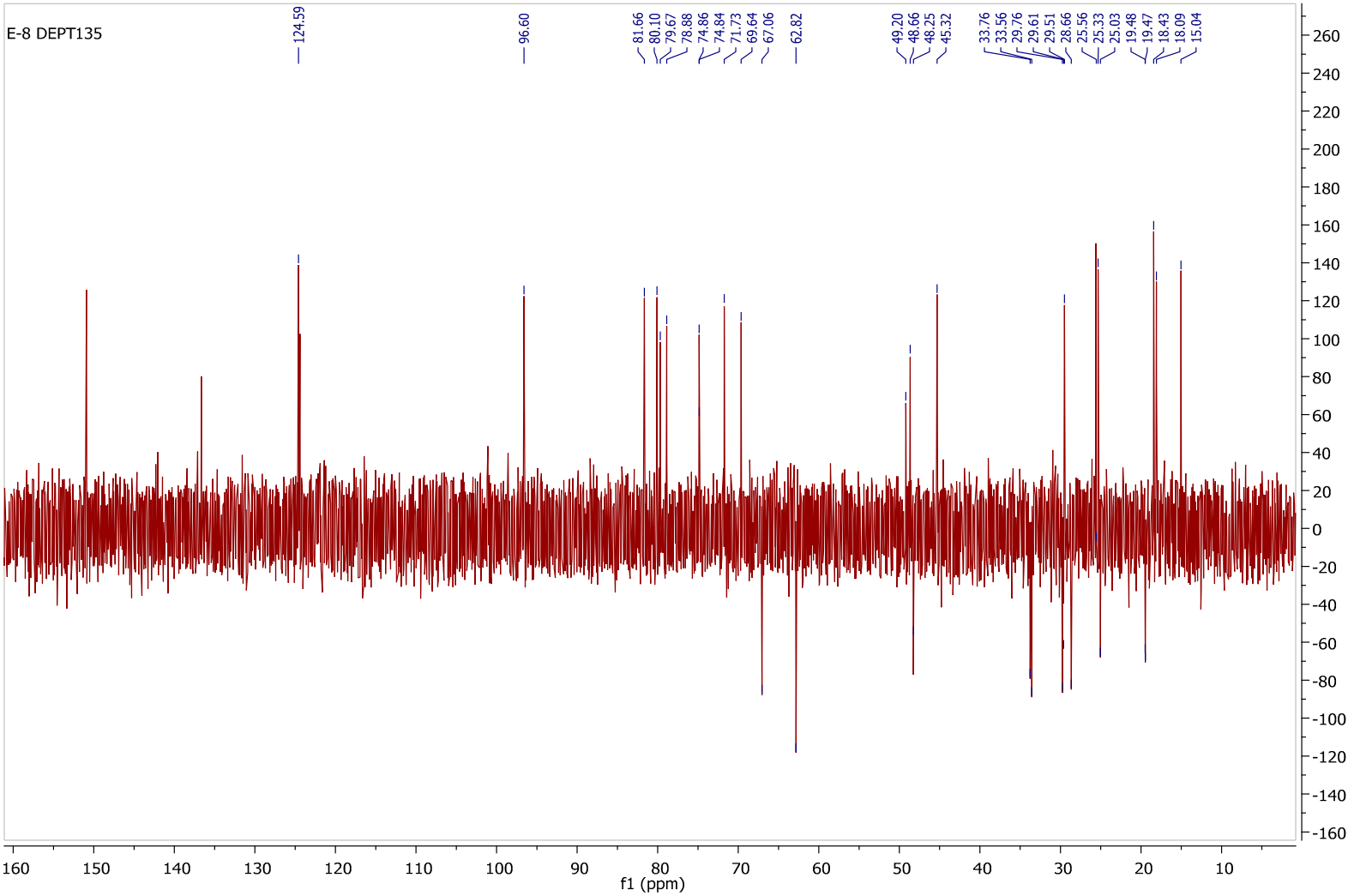


Figure S25. DEPT–NMR spectrum of compound **(3)** in pyridine–d_6.

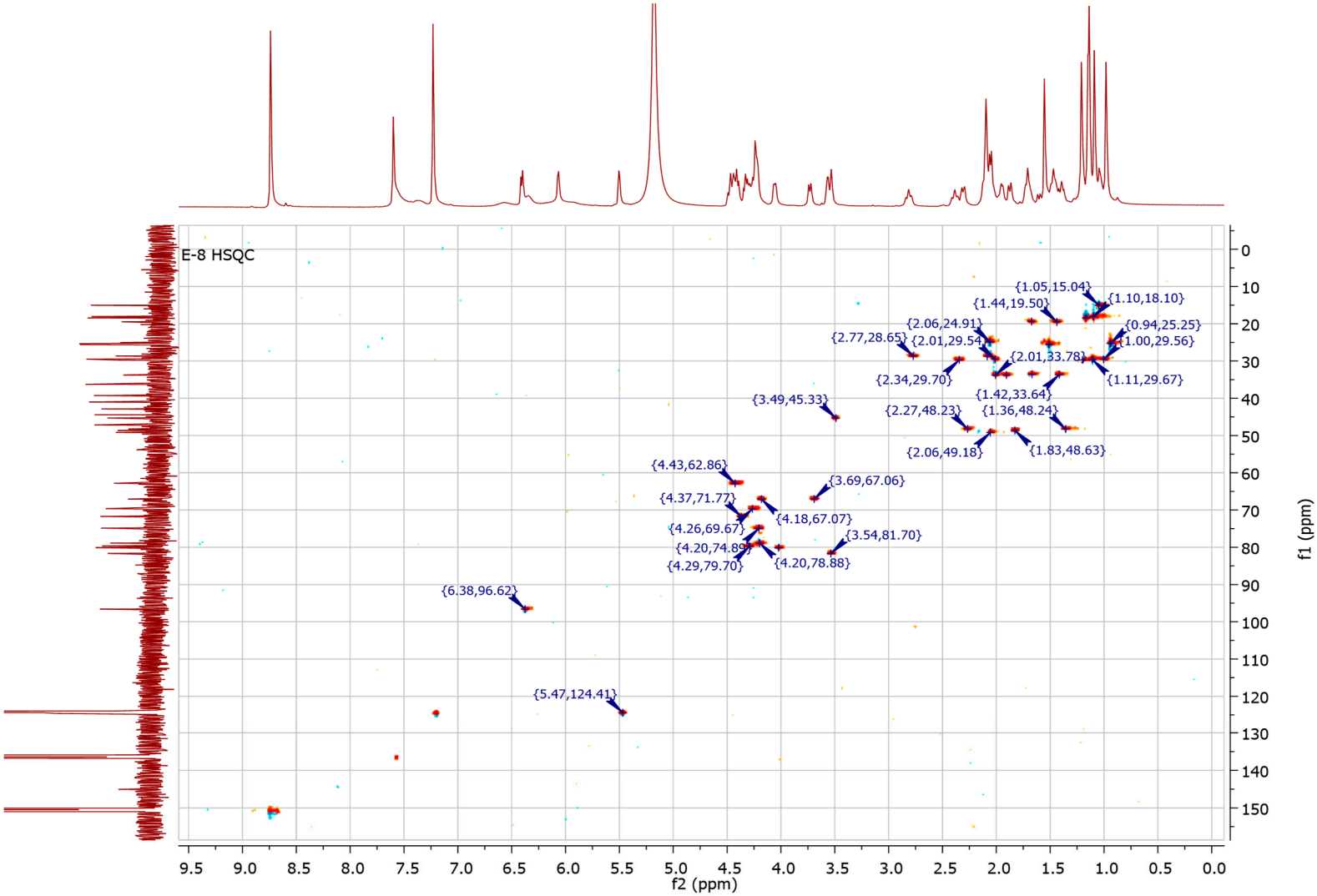


Figure S26. HSQC–NMR spectrum of compound **(3)** in pyridine–d_6.

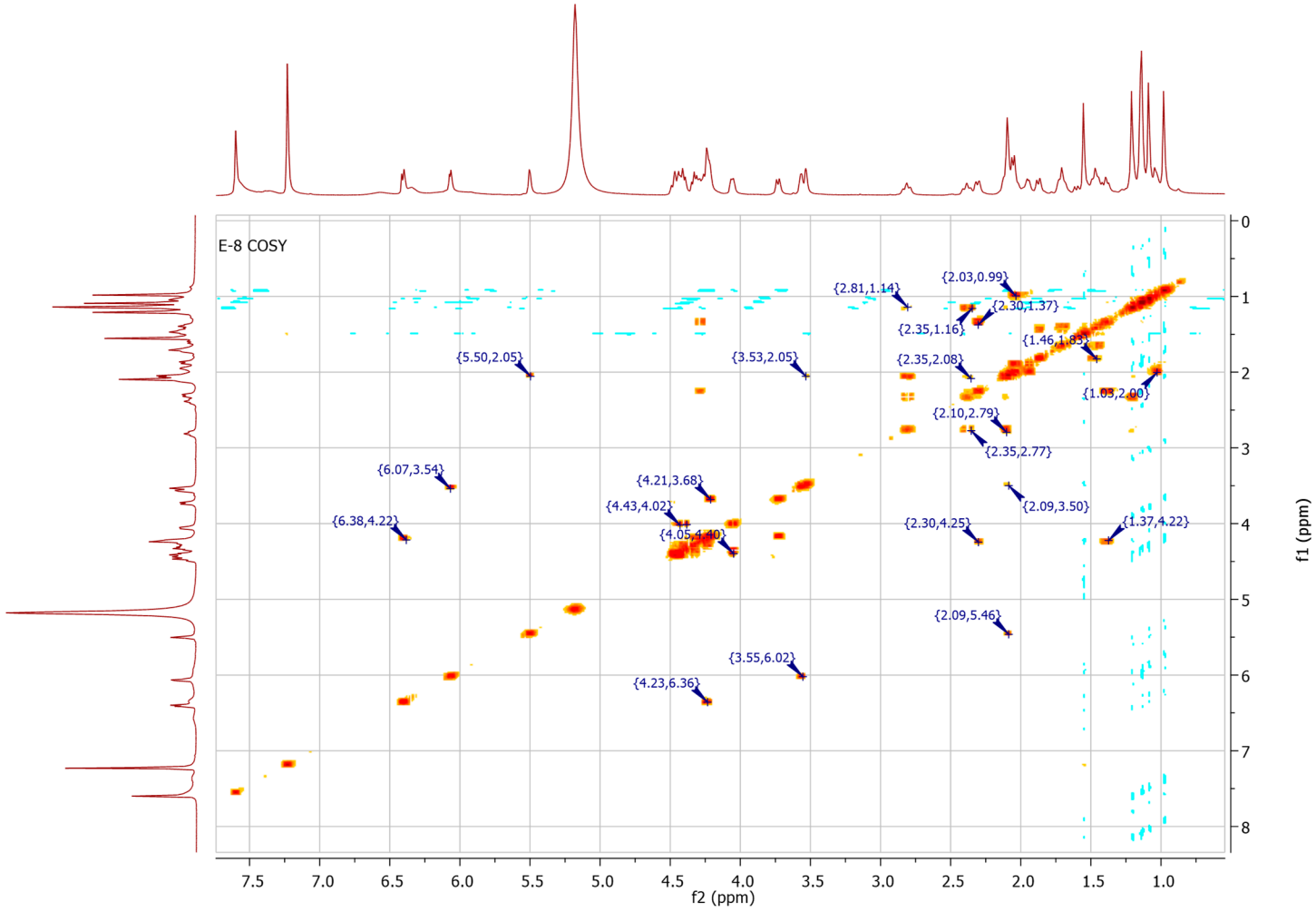


Figure S27. COSY spectrum of compound **(3)** in pyridine–d_6.

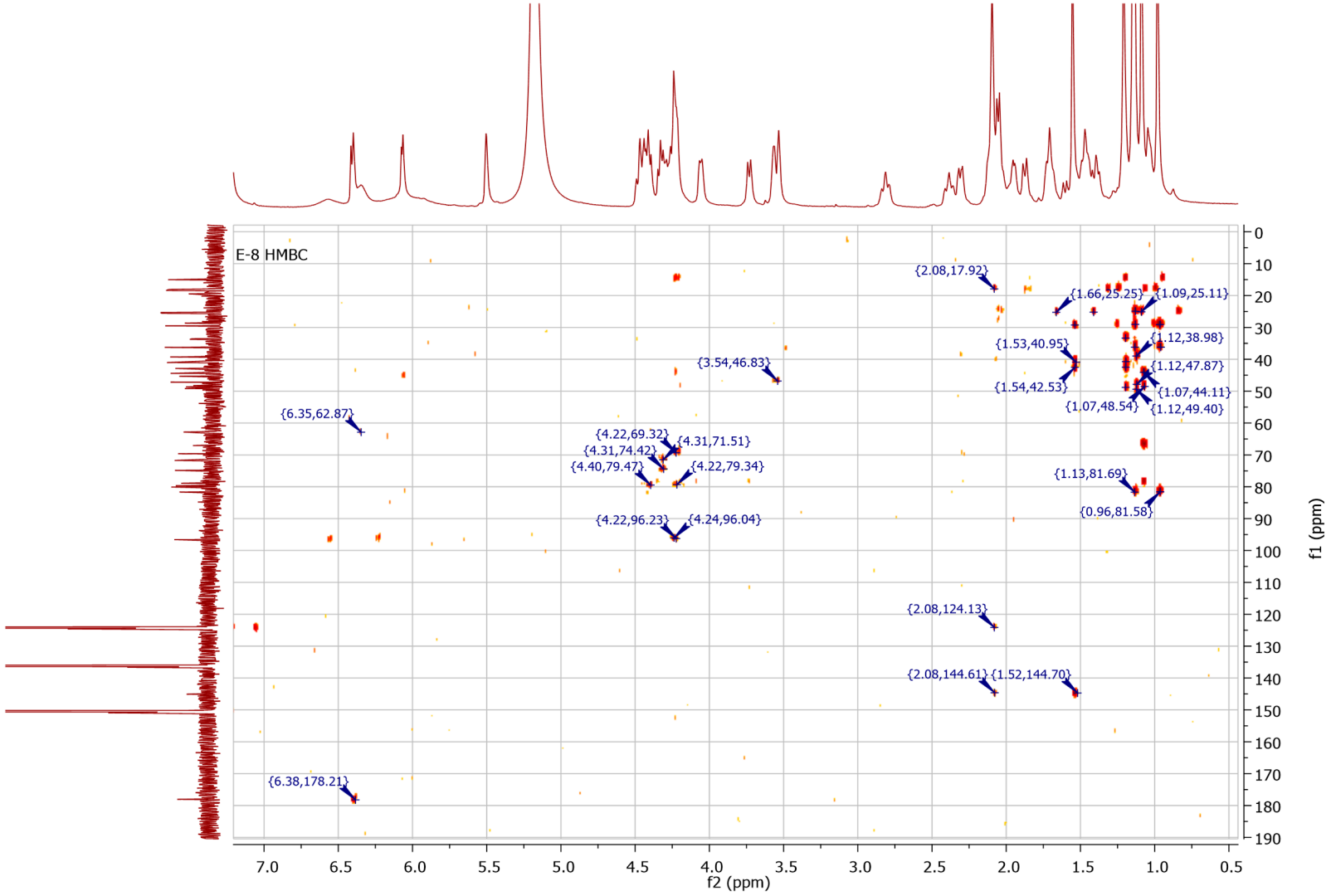


Figure S28. HMBC spectrum of compound **(3)** in pyridine–d_6.

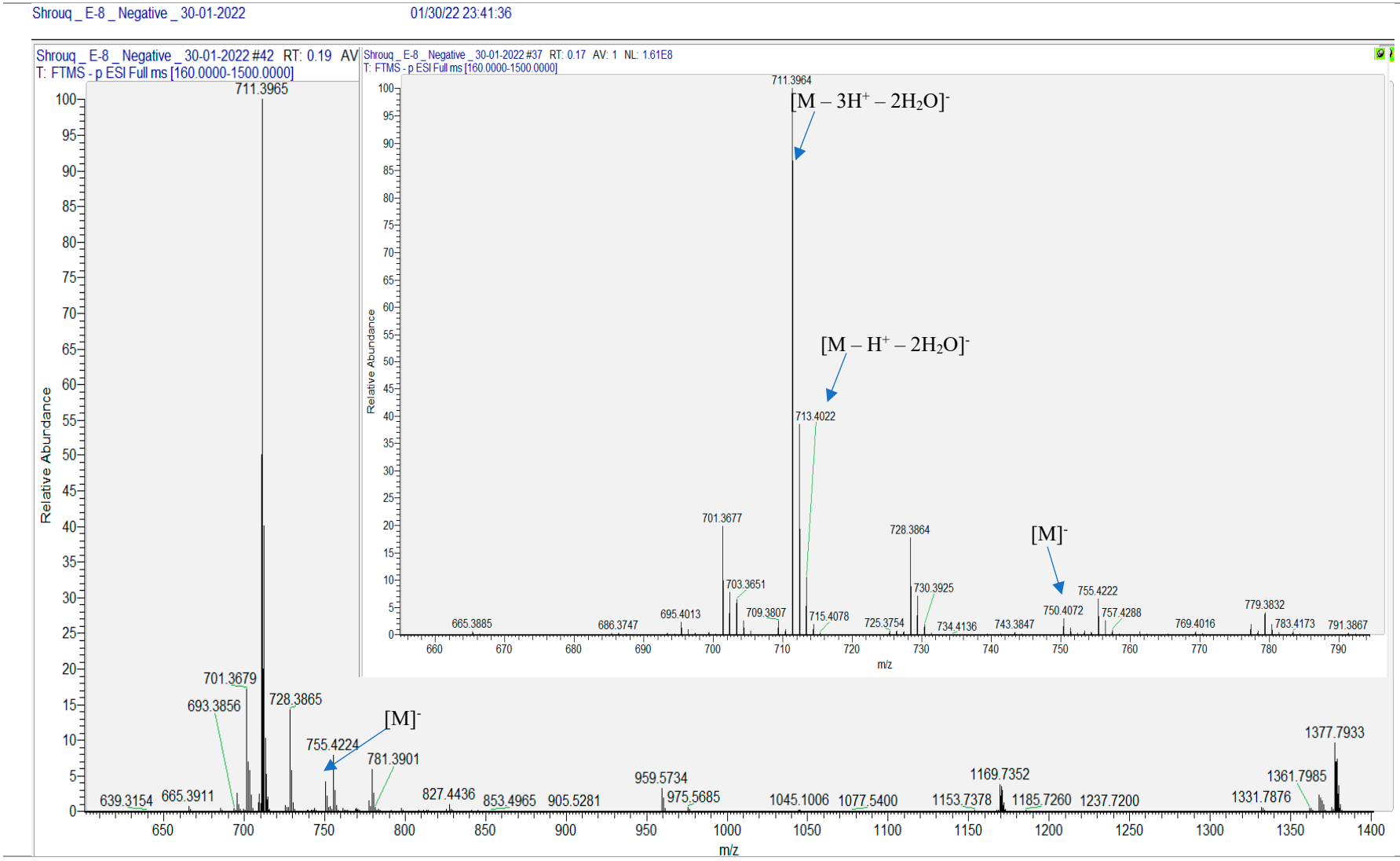


Figure S29. HR–ESI–MS spectrum (– mode) of compound **(3)** in pyridine–d_6.

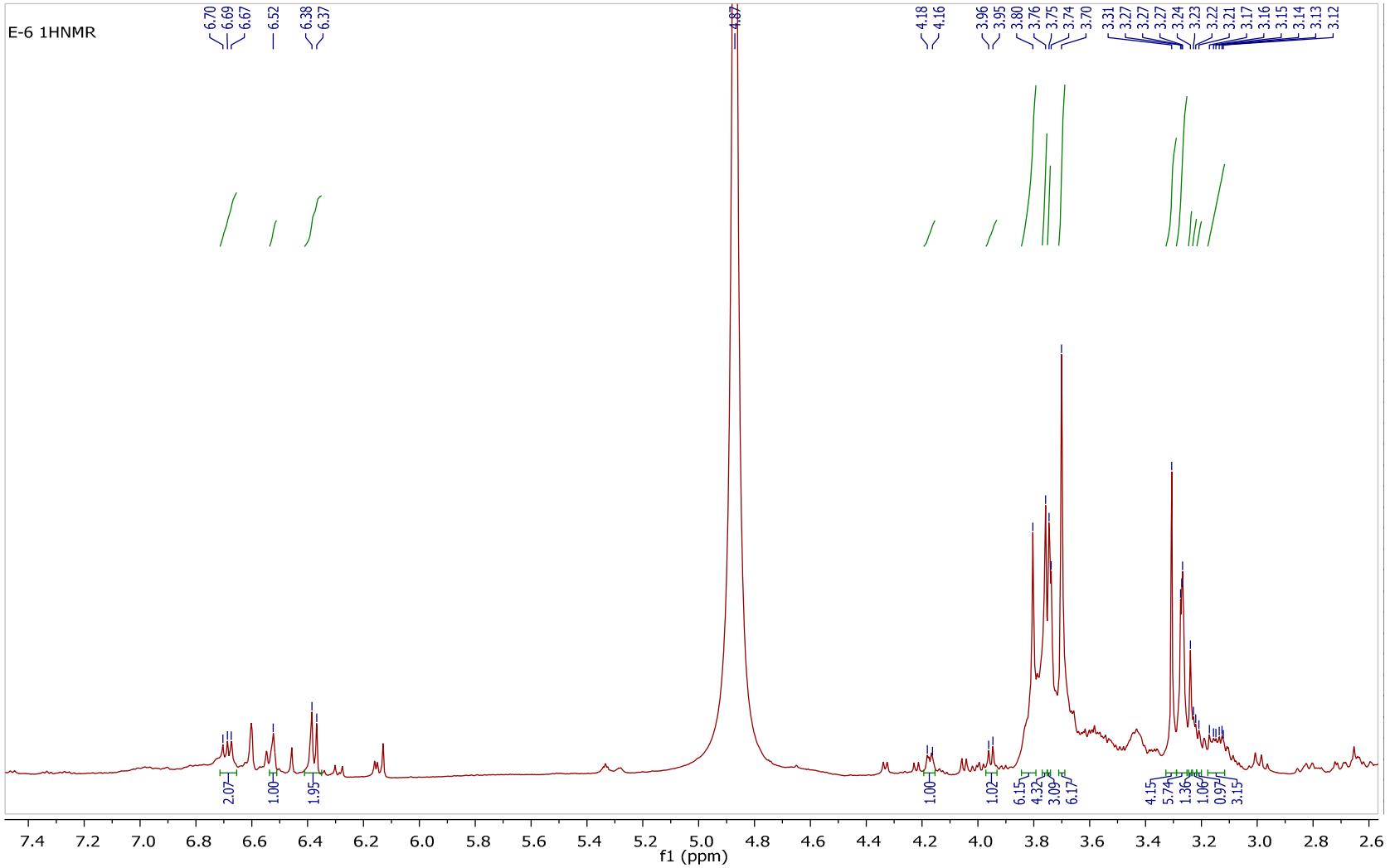


Figure S30. ^{1}H–NMR spectrum of compound **(4)** in CD_3OD.

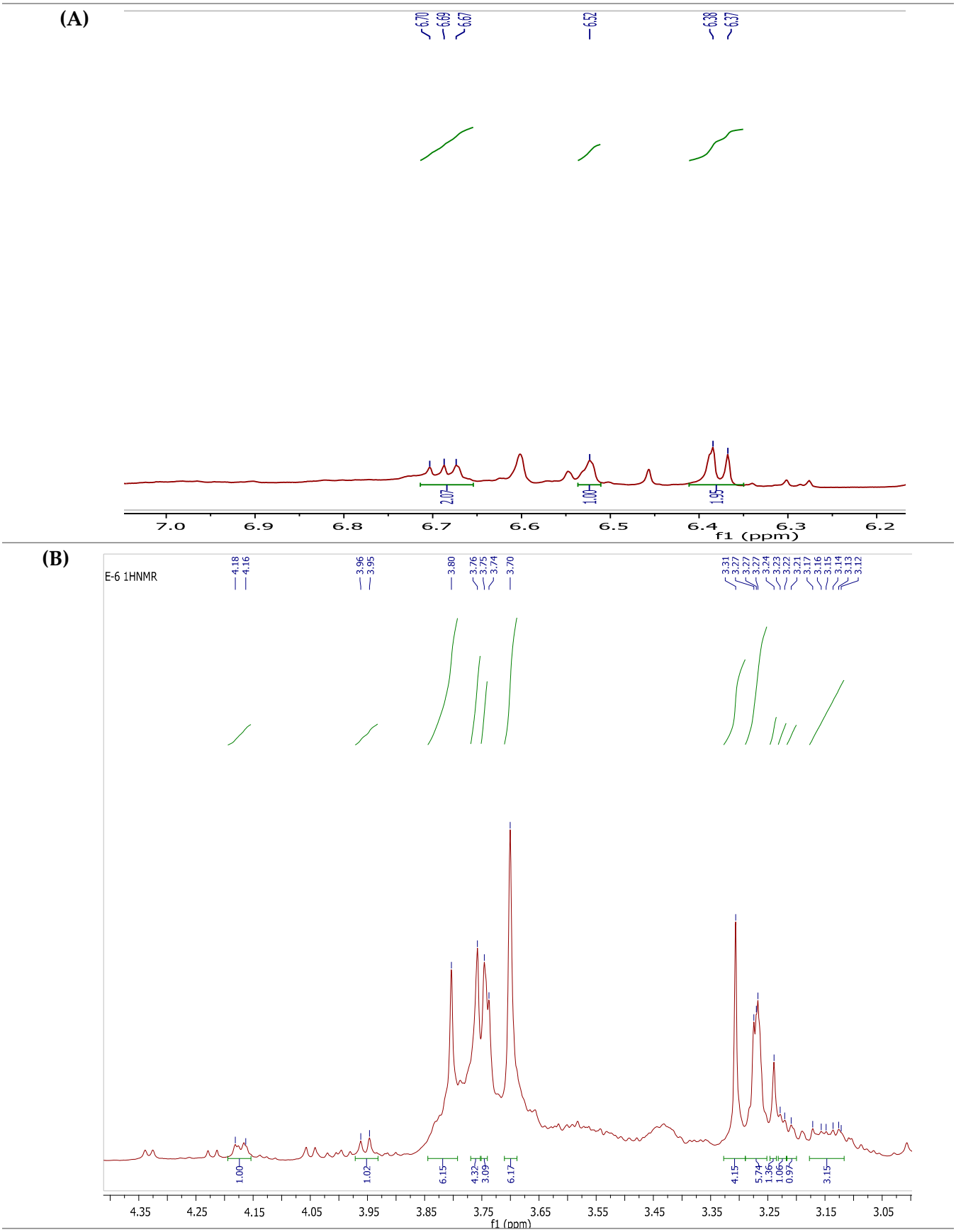


Figure S31. ^{1}H–NMR spectrum of compound **(4)** in CD_3OD; **(A):** (expansion range 6.2–7.0 ppm), **(B):** (expansion range 3.0–4.35 ppm).

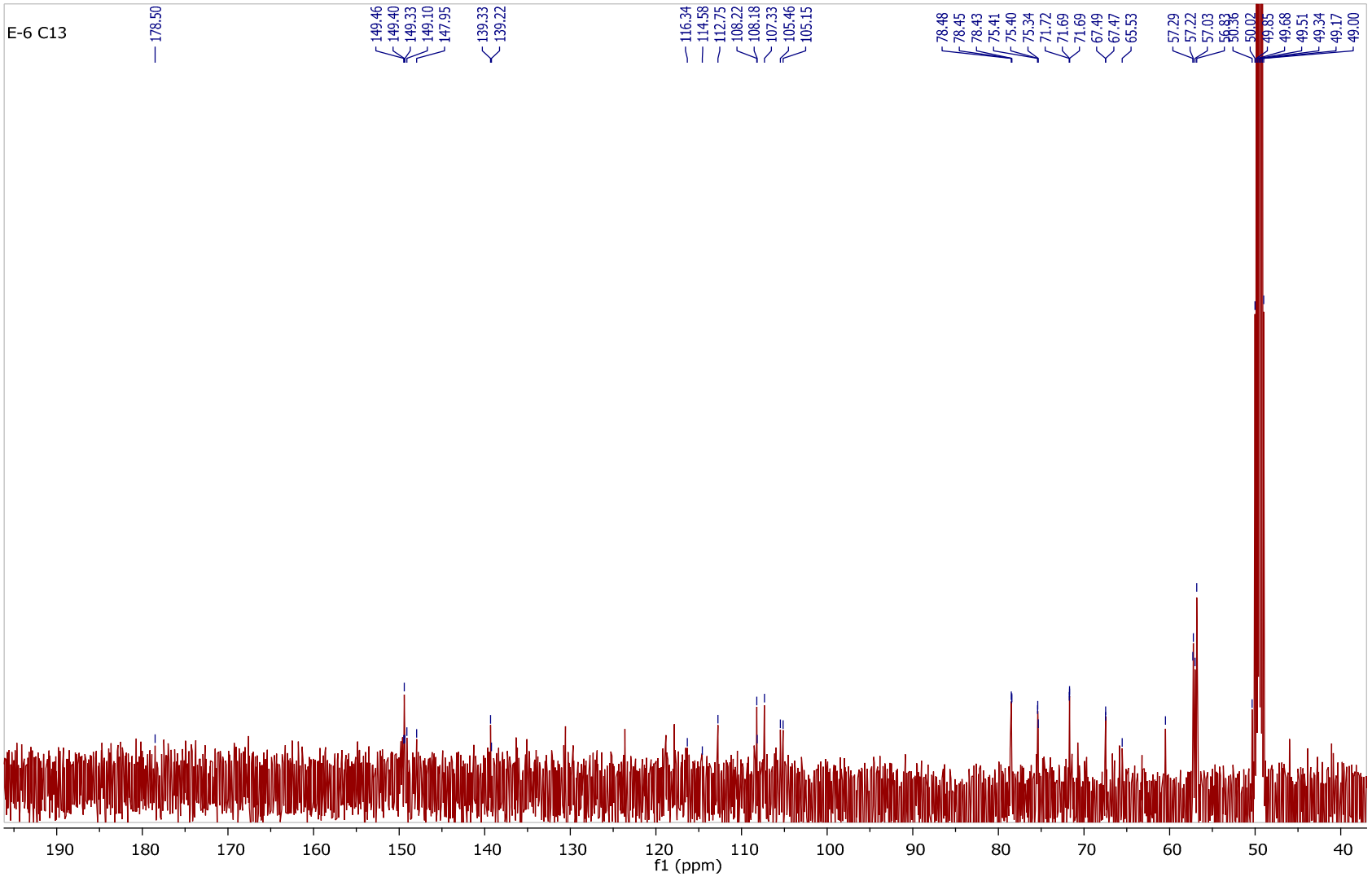


Figure S32. ^{13}C–NMR spectrum (expansion 40–190 ppm) of compound **(4)** in CD_3OD.

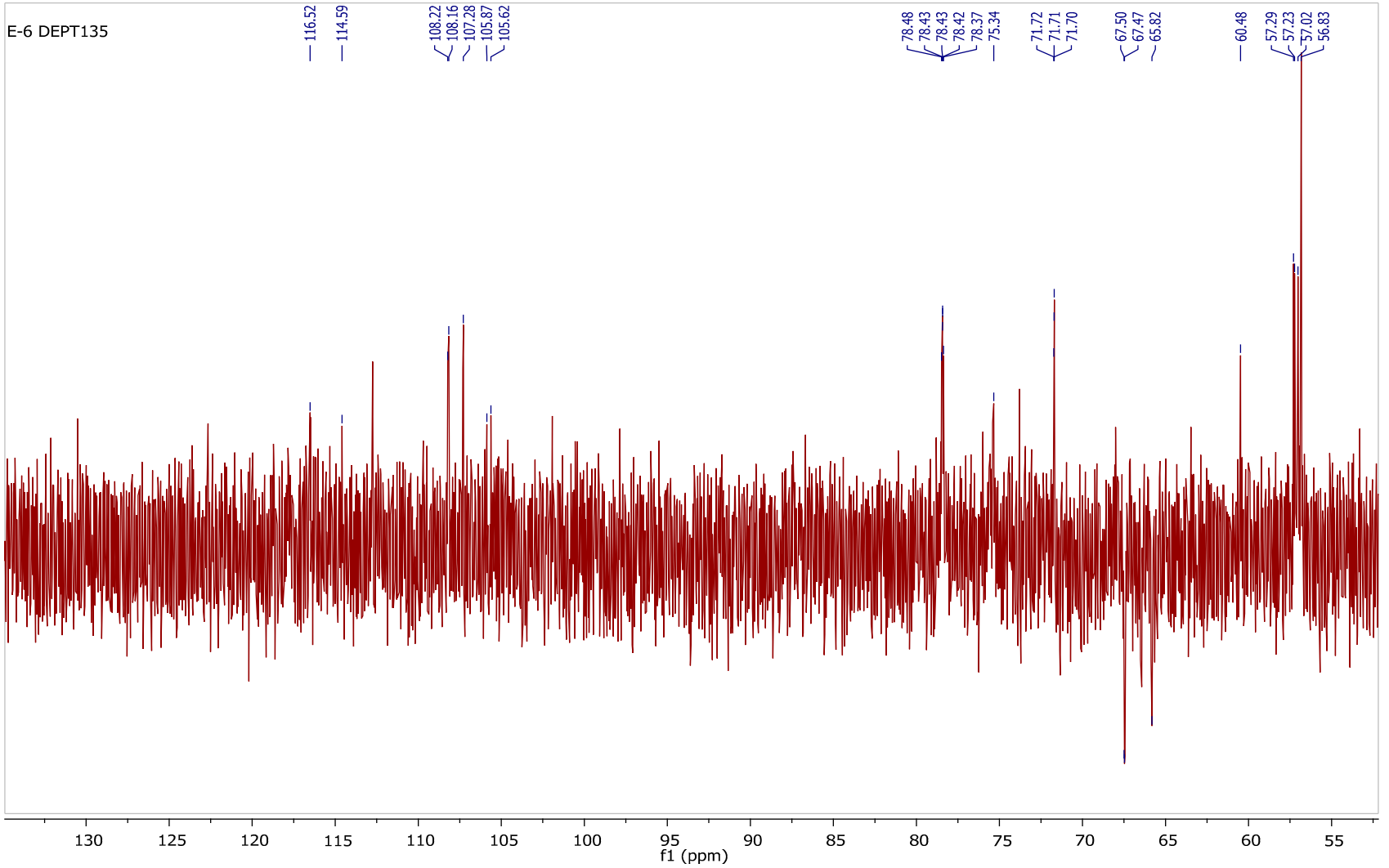


Figure S33. DEPT –NMR spectrum (expansion 55–130 ppm) of compound **(4)** in CD_3OD.

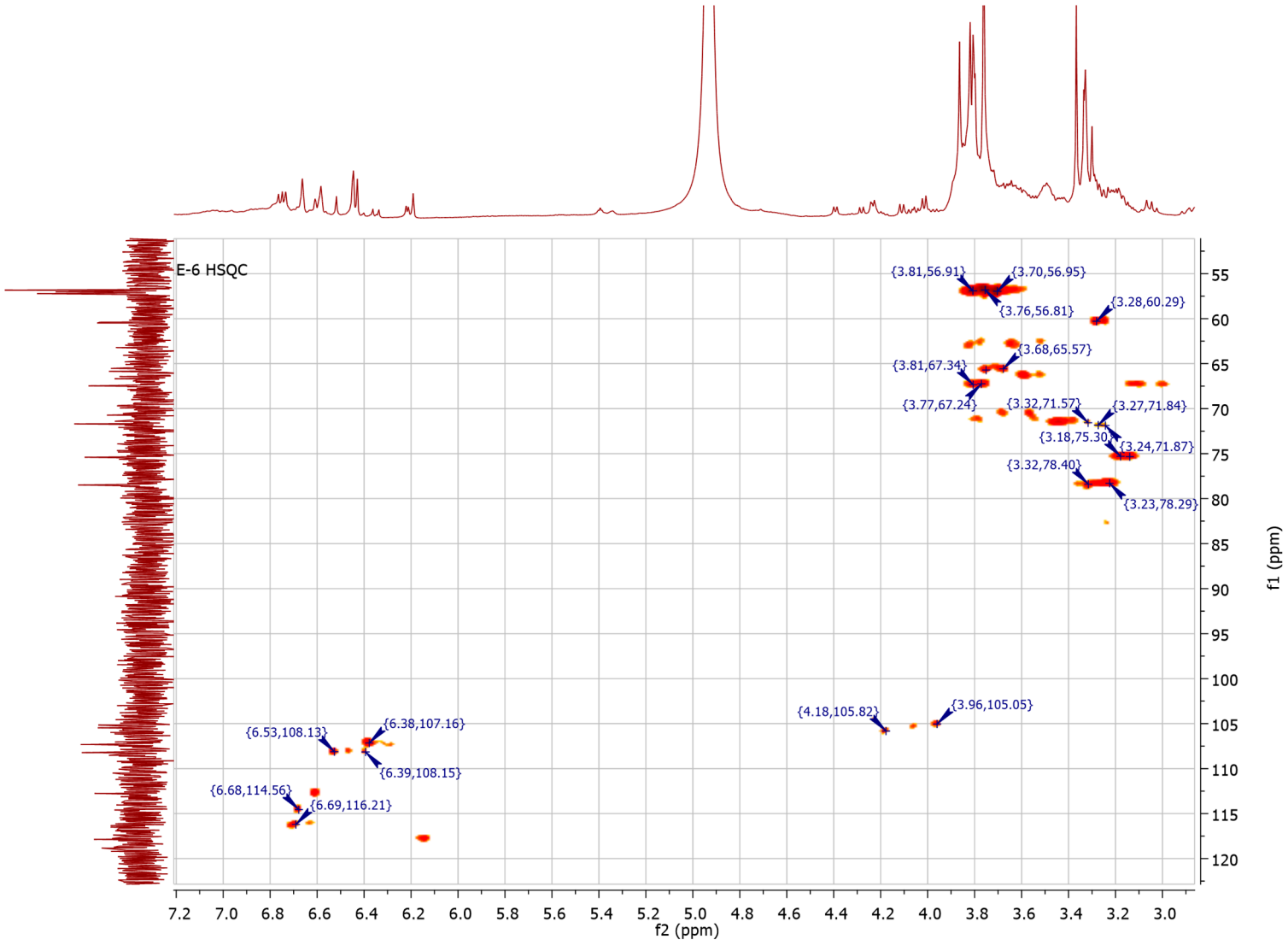


Figure S34. HSQC–NMR spectrum (expansion 3.0–7.2 ppm) of compound **(4)** in CD_3OD.

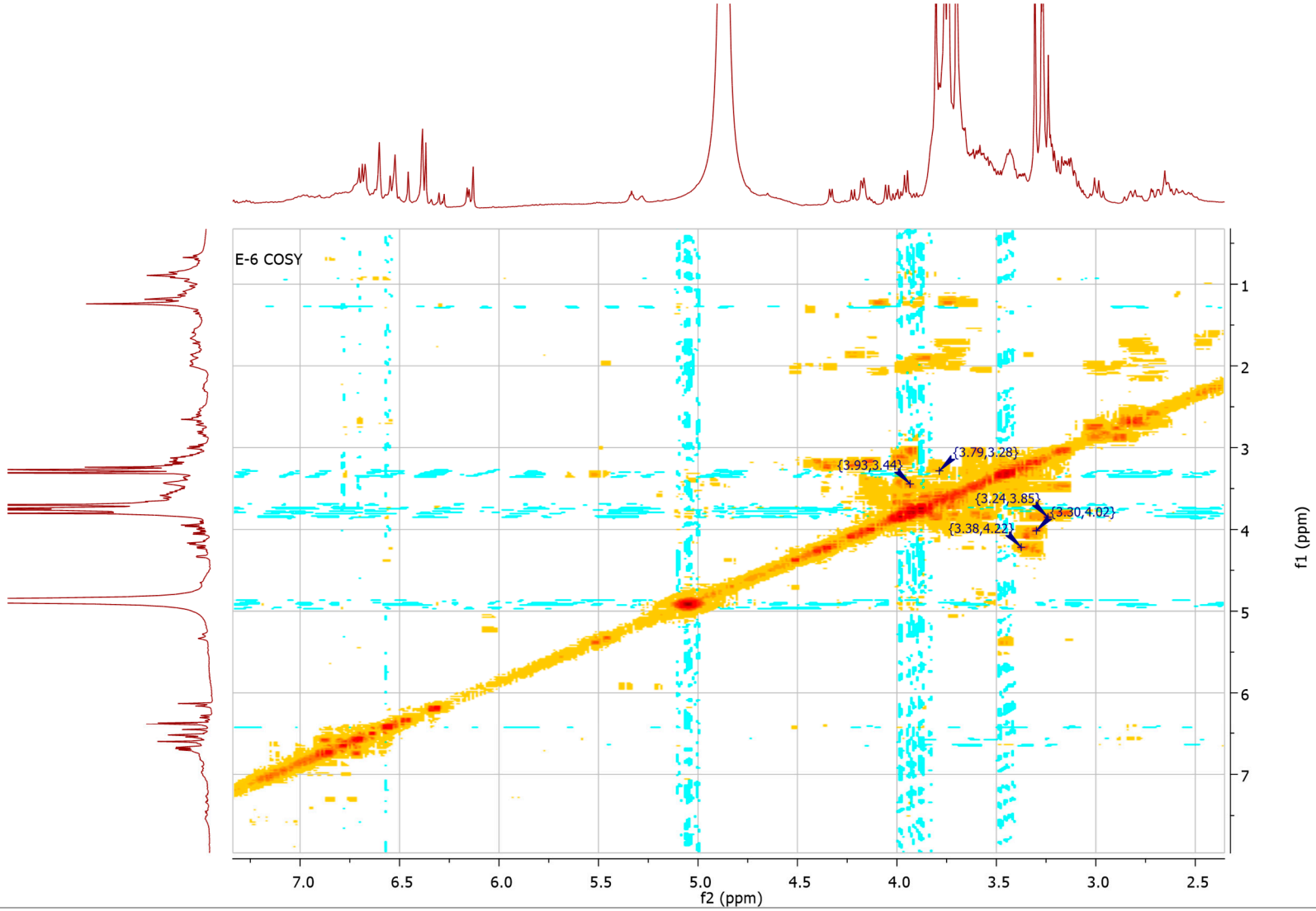


Figure S35. COSY spectrum (expansion 2.5–7.0 ppm) of compound **(4)** in CD_3OD.

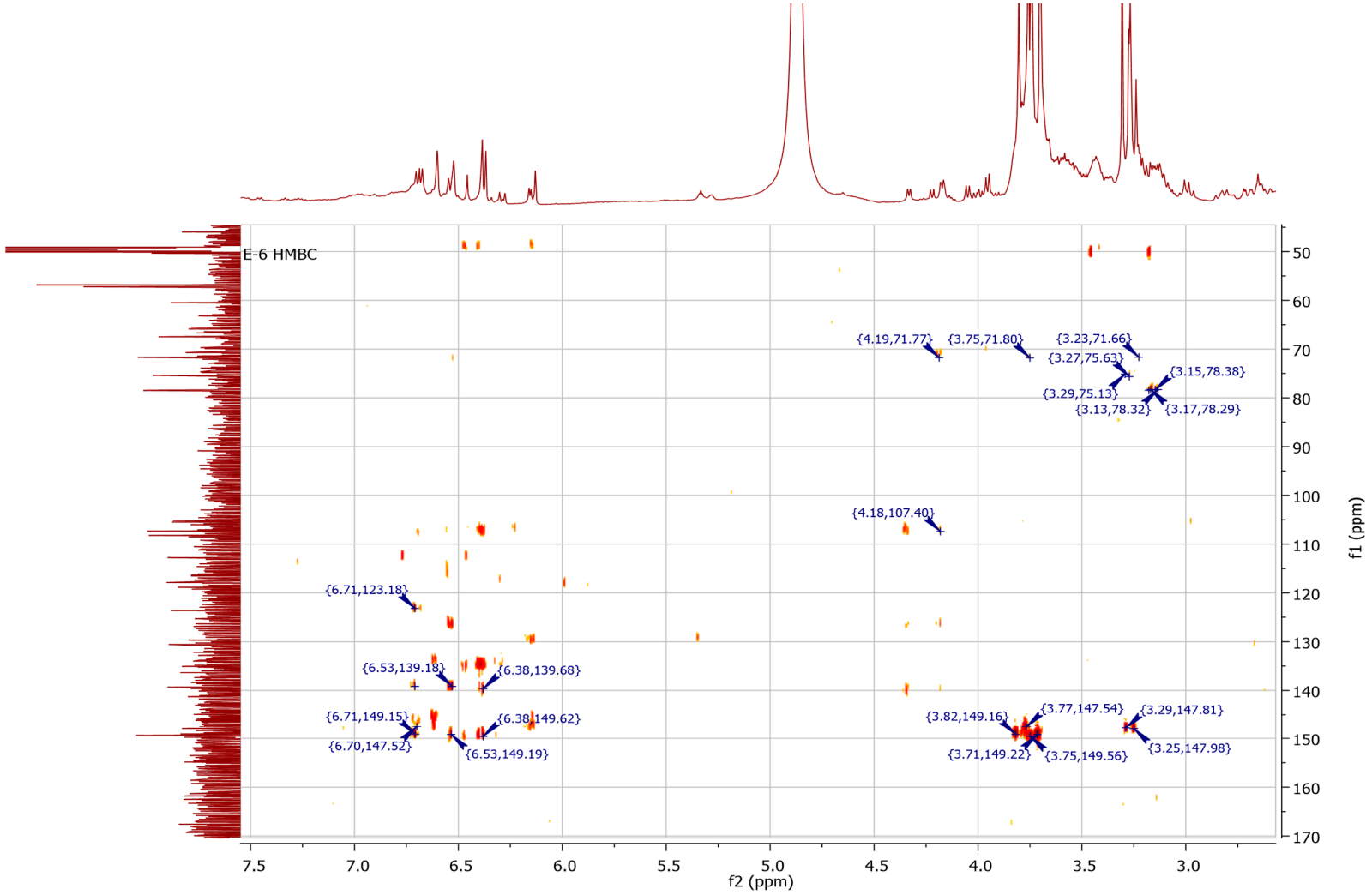


Figure S36. HMBC spectrum (expansion 3.2–7.5 ppm) of compound **(4)** in CD_3OD.

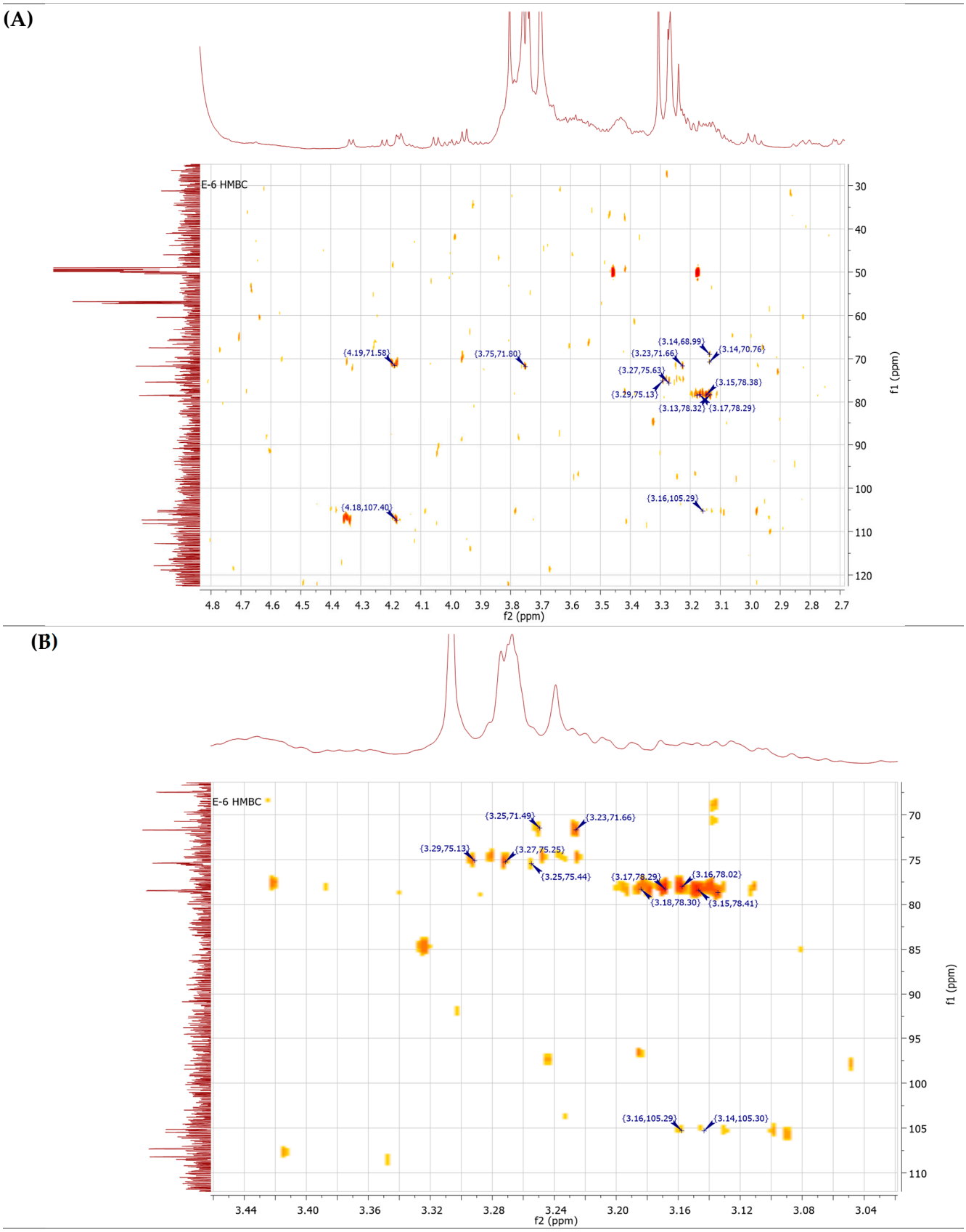


Figure S37. HMBC spectrum of compound **(4)** in CD_3OD; **(A):** (expansion 2.7–4.8 ppm), and **(B):** (expansion 3.04–3.44 ppm).

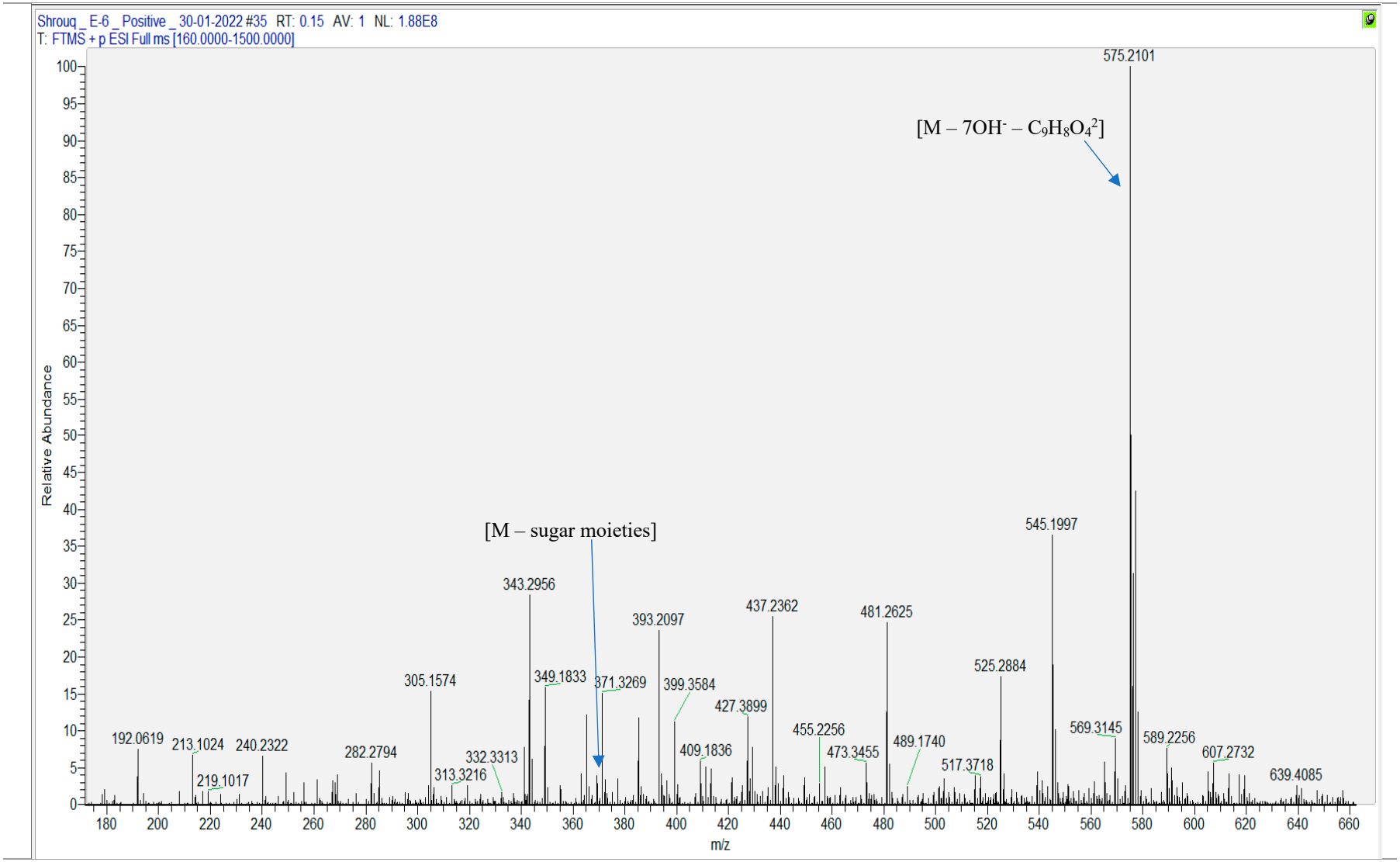


Figure S38. HR–ESI–MS spectrum (+ mode) of compound **(4)** in CD_3OD.

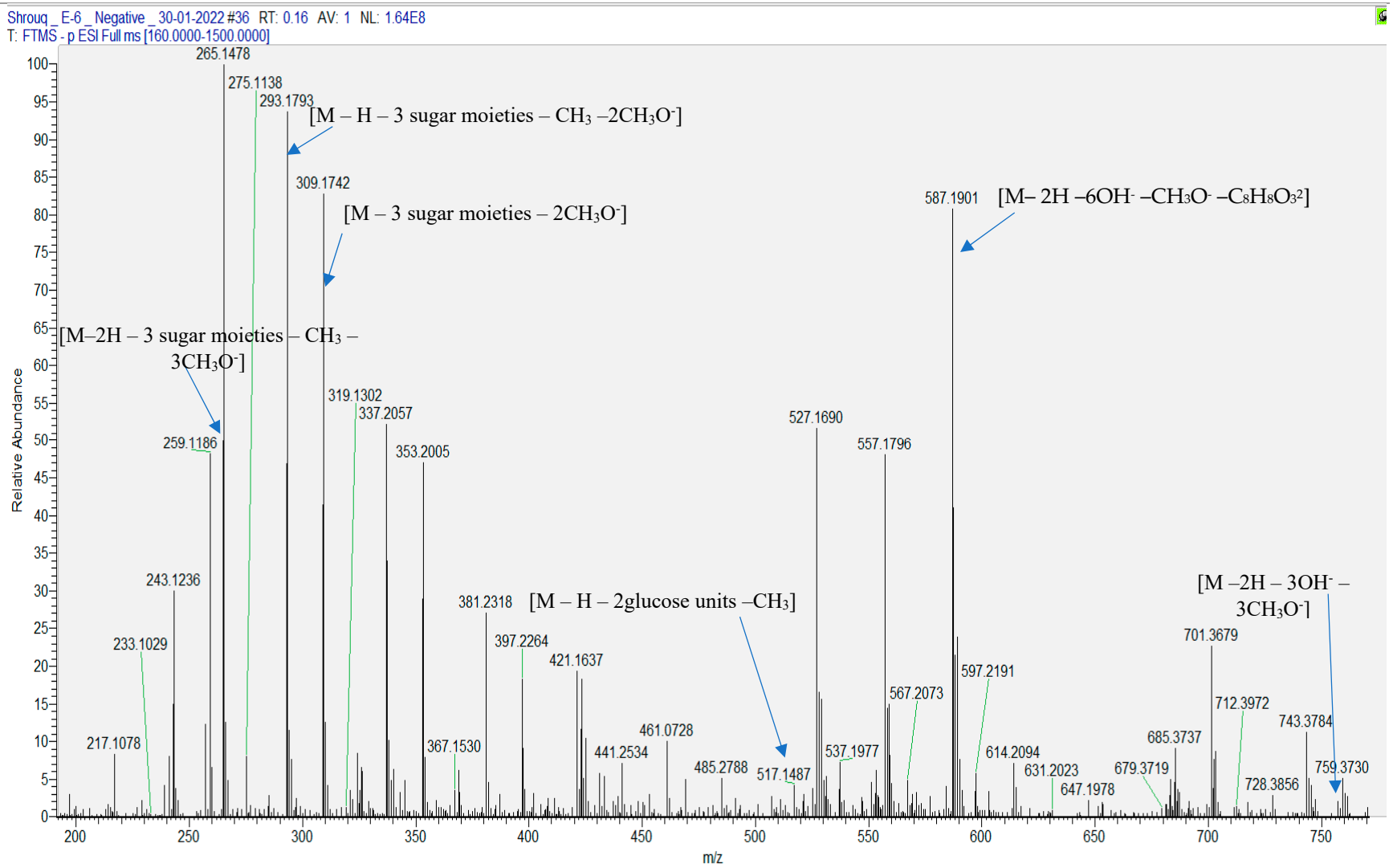


Figure S39. HR–ESI–MS spectrum (– mode) of compound **(4)** in CD_3OD.

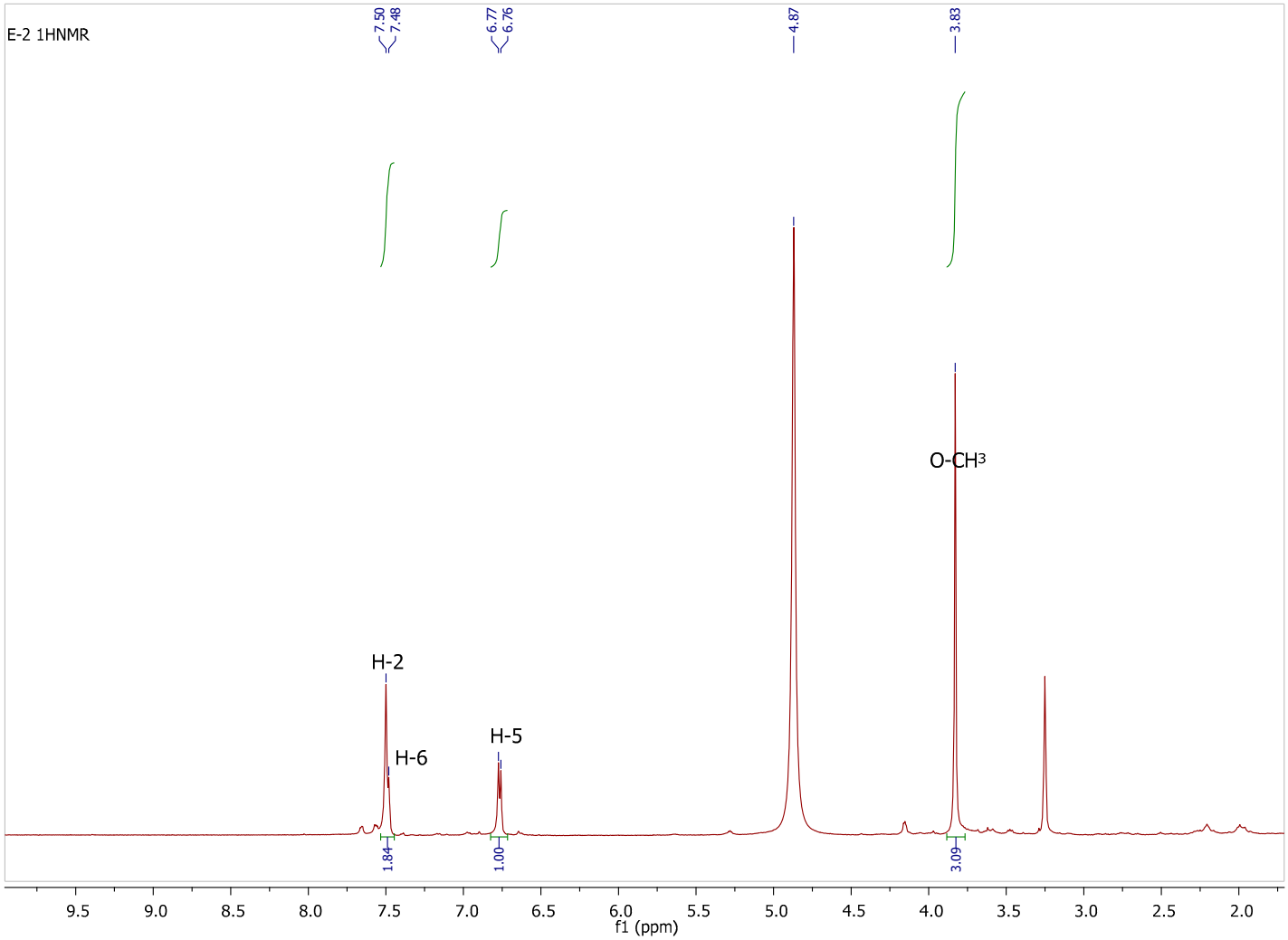


Figure S40. ^{1}H–NMR spectrum of compound **(5)** in CD_3OD.

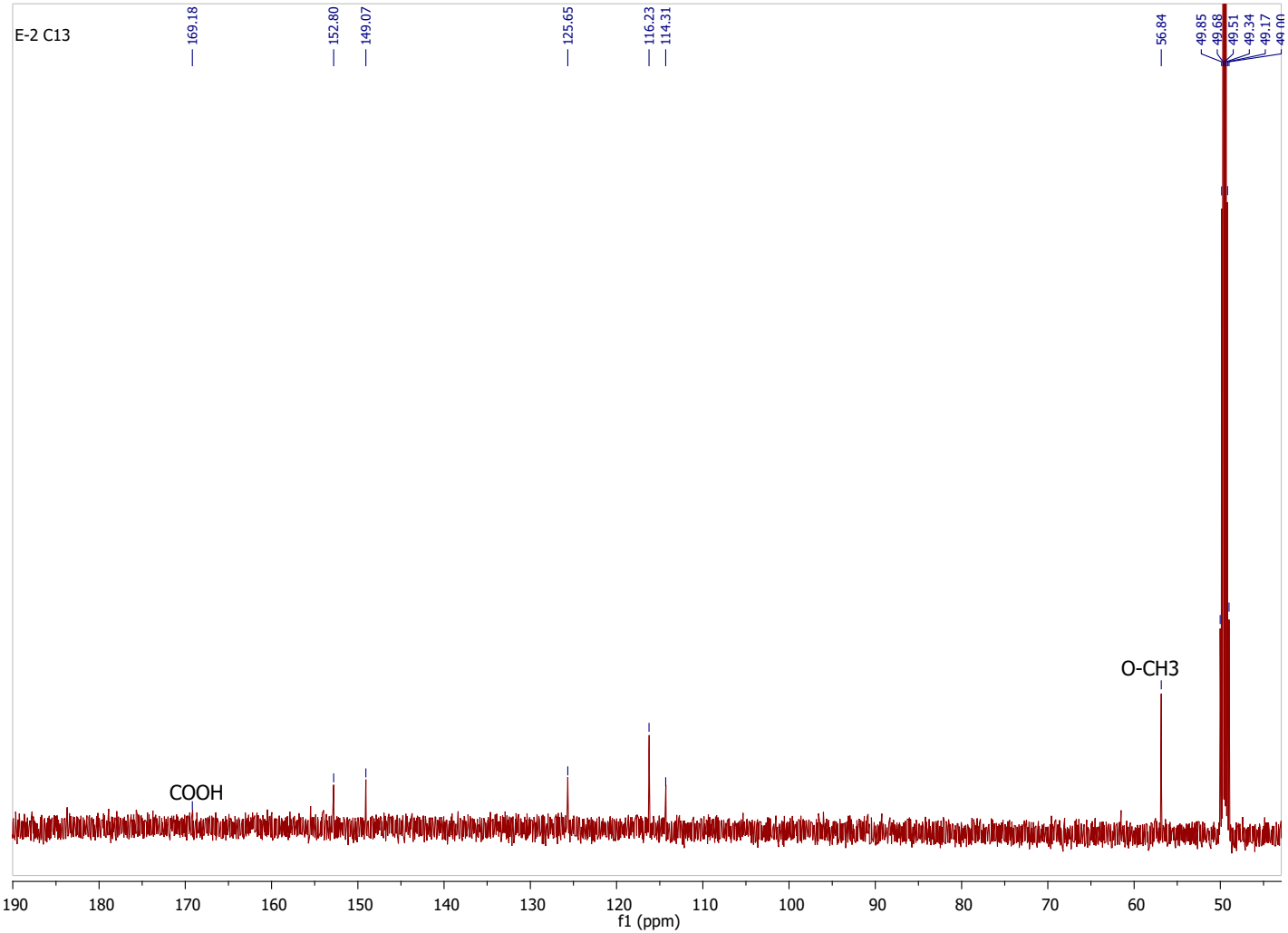


Figure S41. ^{13}C–NMR spectrum of compound **(5)** in CD_3OD.

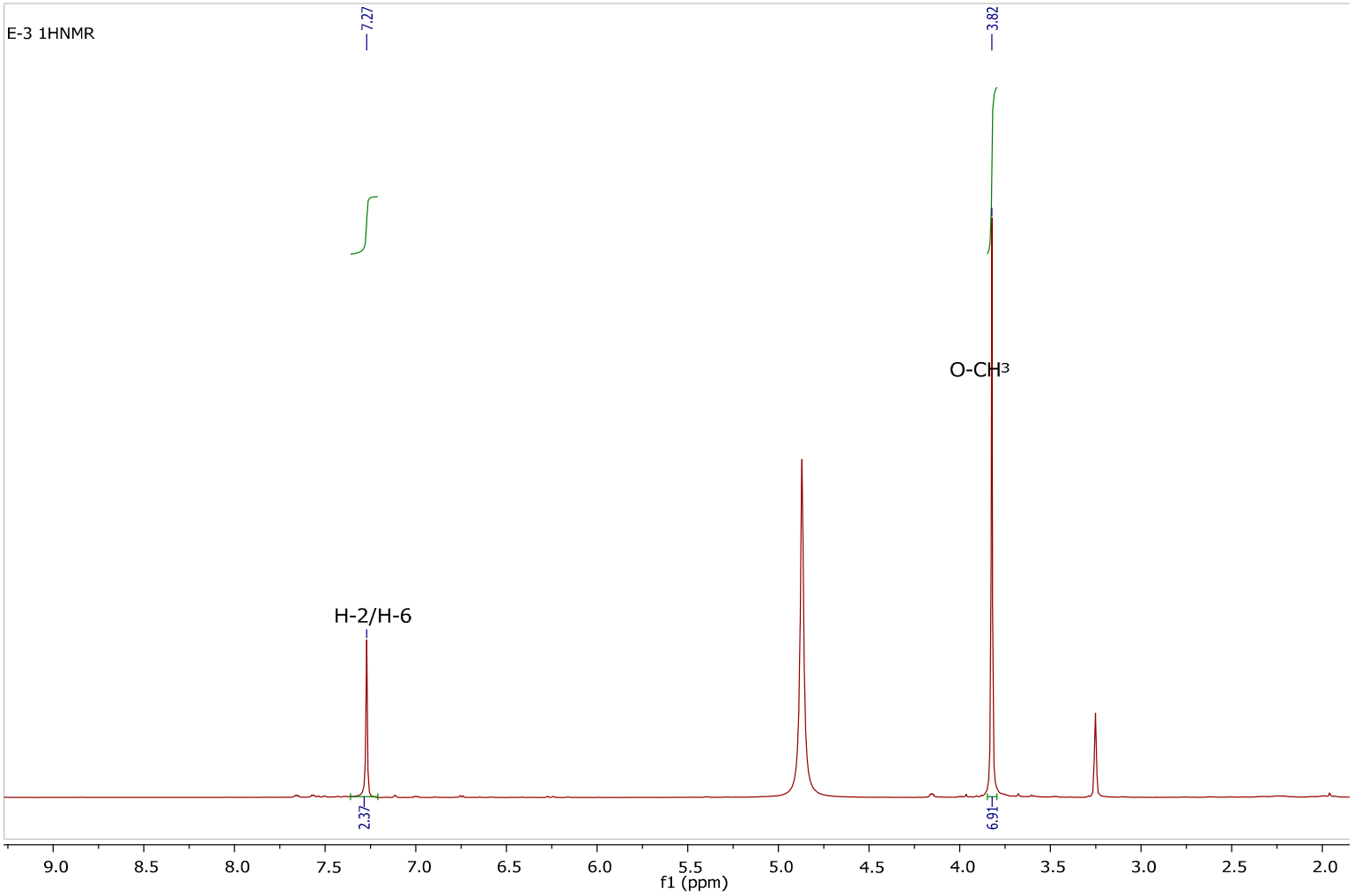


Figure S42. ^{1}H–NMR spectrum of compound **(6)** in CD_3OD.

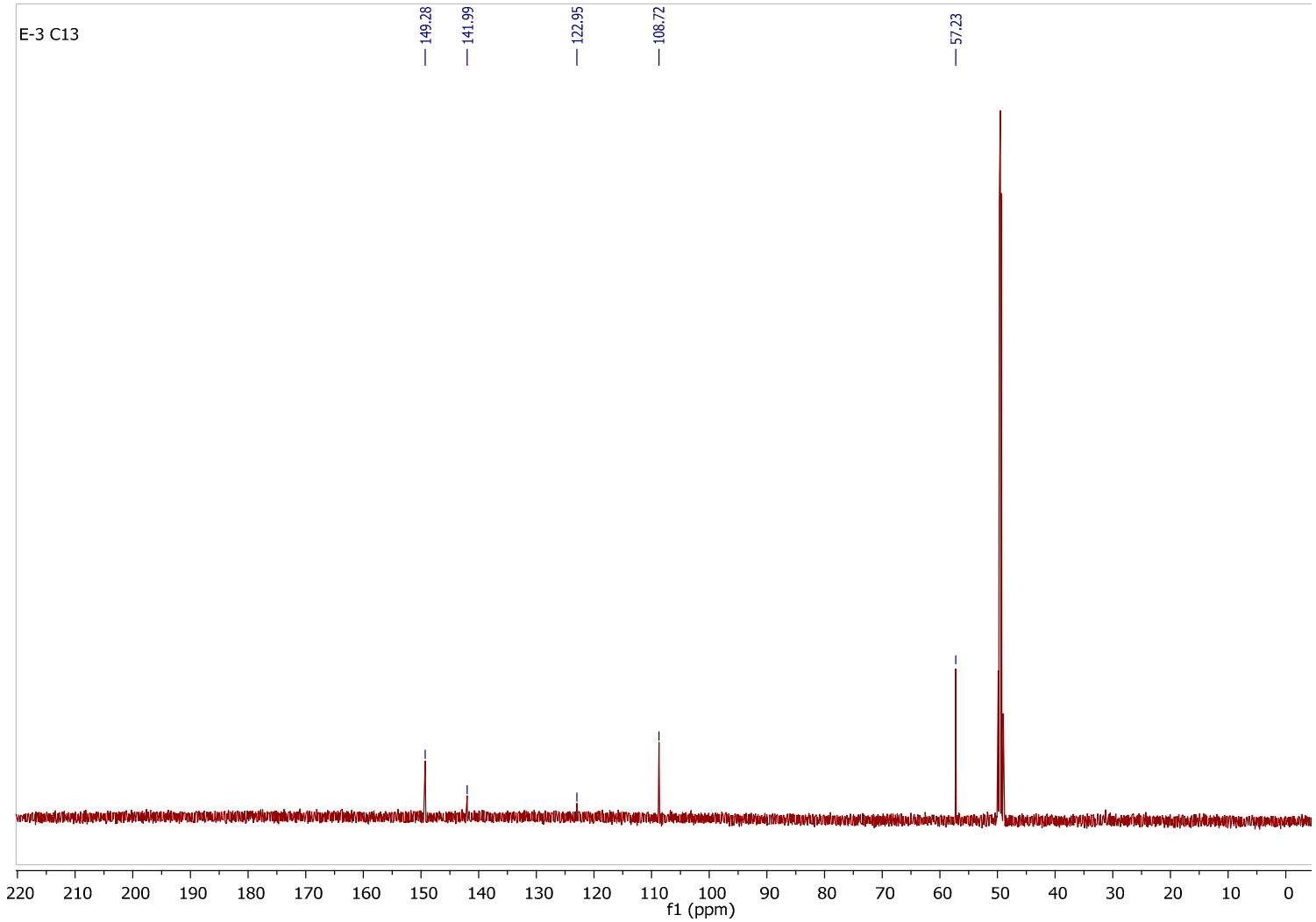


Figure S43. ^{13}C–NMR spectrum of compound **(6)** in CD_3OD.

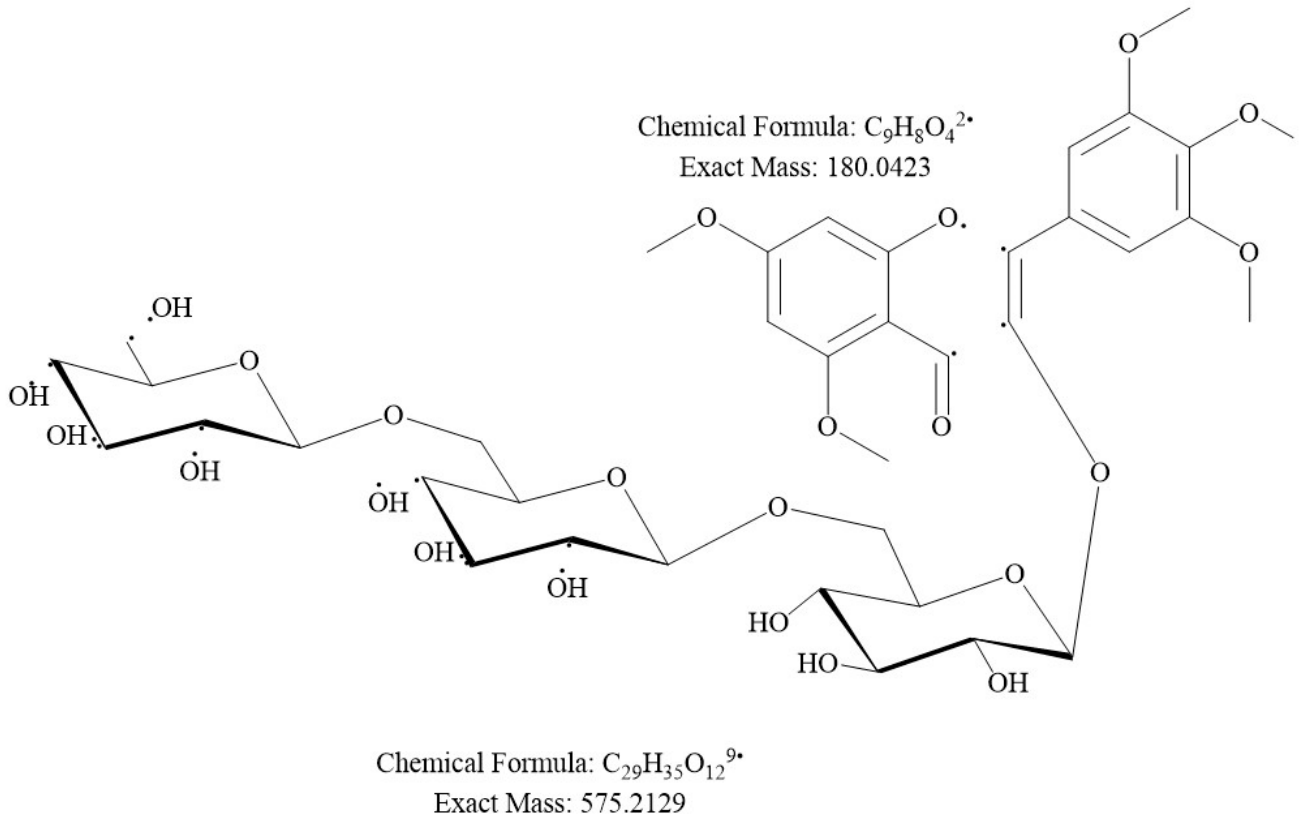


Figure S44. HR–ESI–MS Fragmentation (+ mode) of Compound **(4)**.

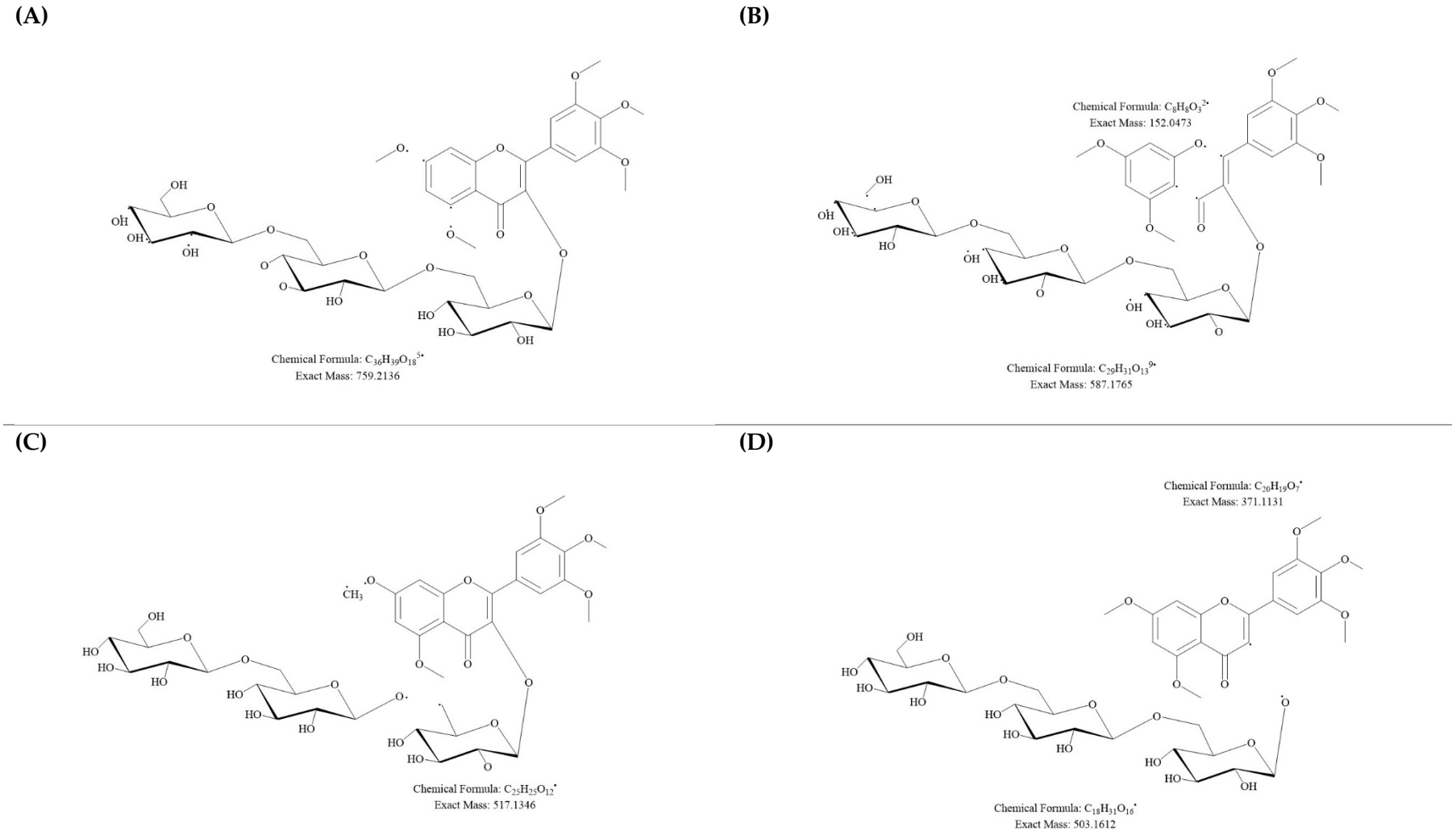
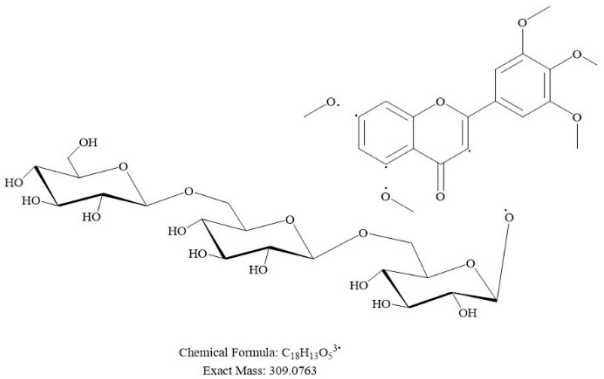


Figure S45. HR–ESI–MS (– mode) of Compound **(4)**; **(A):** Fragmentation-1, **(B):** Fragmentation-2, **(C):** Fragmentation-3, and **(D):** Fragmentation-4.

(E)



(F)

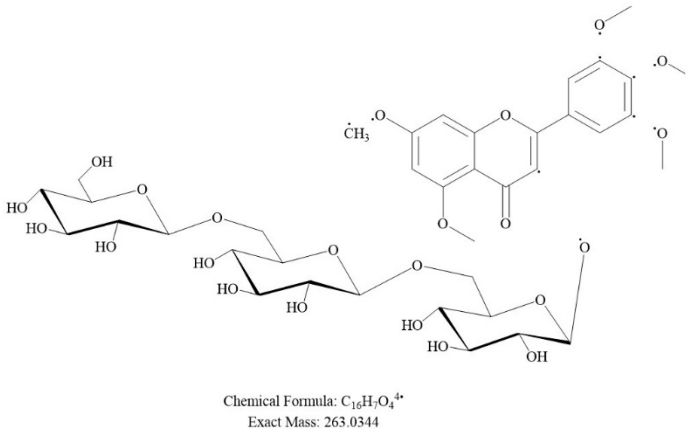
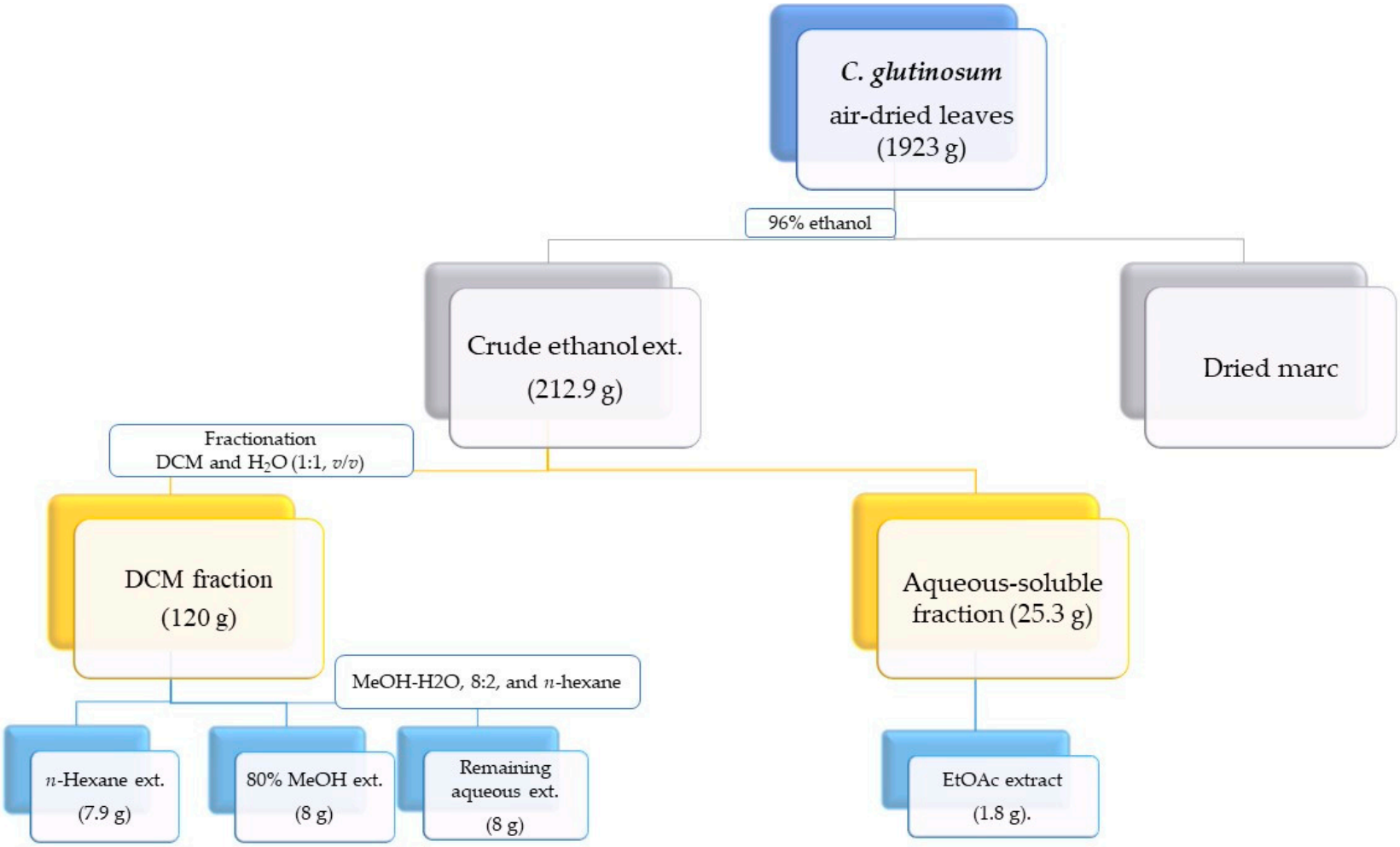
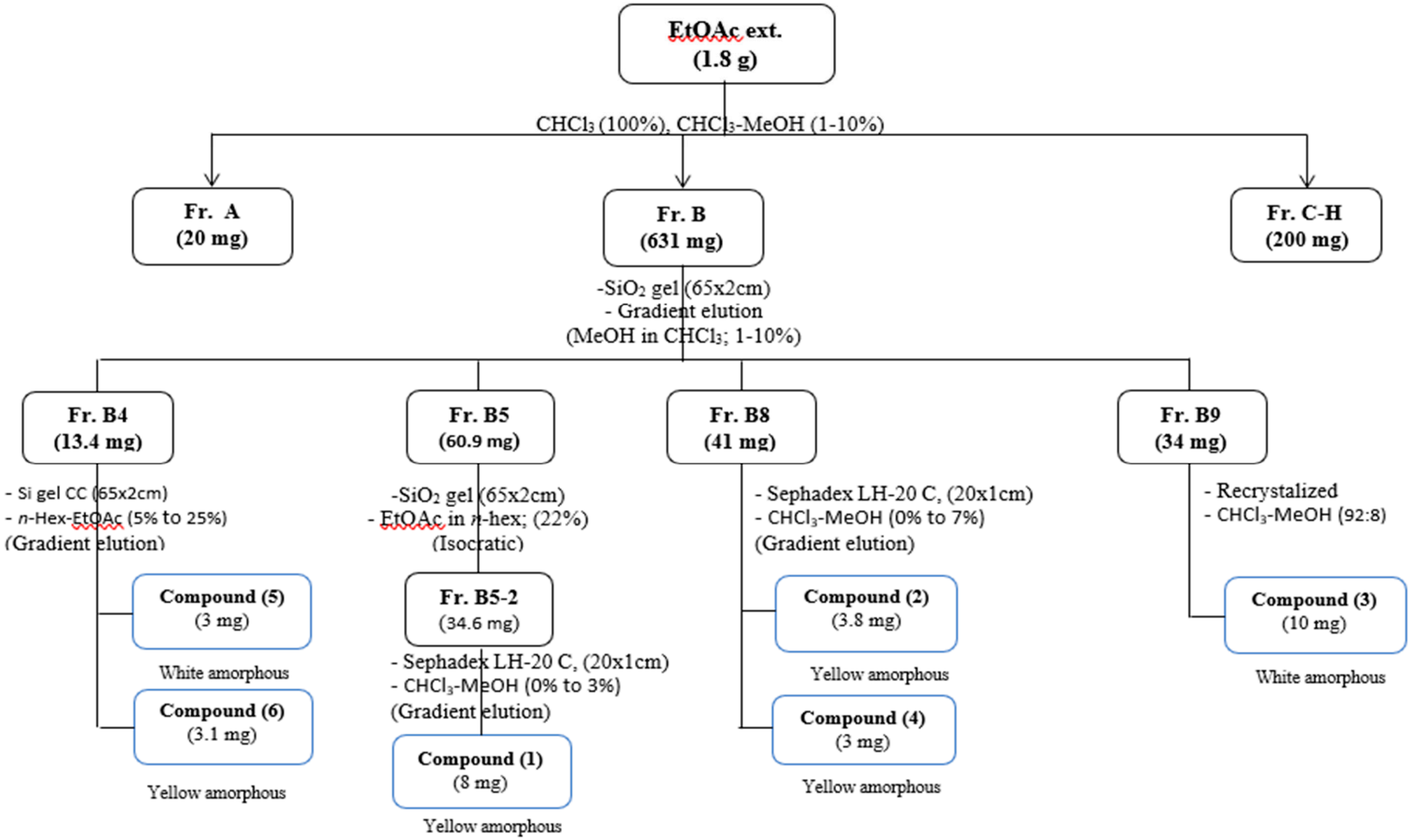


Figure S45 (cont.'). HR–ESI–MS (– mode) of Compound **(4)**; **(E):** Fragmentation-5, and **(F):** Fragmentation-6.



Flowchart 1: Extraction and fractionation procedure of the *C. glutinosum* leaves.



Flowchart 2: Fractionation and purification of the ethyl acetate extract of the *C. glutinosum* leaves.

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