SUPPLEMENTARY MATERIALS

Furofuranoid-type lignans and related phenolics from *Anisacanthus virgularis* (Salisb.) Nees with promising anticholinesterase and anti-ageing properties: A study supported by molecular modelling

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# Both authors contributed equally to this work

List of content

List of abbreviation:

ACh: acetylcholine

AChE: acetyl cholinesterase

AD: Alzheimer's disease

ARG: arginine

CC: column chromatography

CNS: central nervous system

DCM: dichloromethane

DMSO: dimethyl sulphoxide

DTNB: 5,5-dithiobis-2-nitrobenzoic acid

EtOAc: ethyl acetate

GLY: glycine

HFB4: normal melanocytes cell line

LYS: lysine

MeOH: methanol

NMDA: N-methyl-D-aspartate

OD: optical density

PHE: phenylalanine

RPE: retinal pigment epithelial

SABC: streptavidin conjugated to Horseradish peroxidase

SER: serine

TERT: telomerase reverse transcriptase

TMB: 3,3′ ,5,5′ - tetramethylbenzidine

VLC: vacuum liquid chromatography

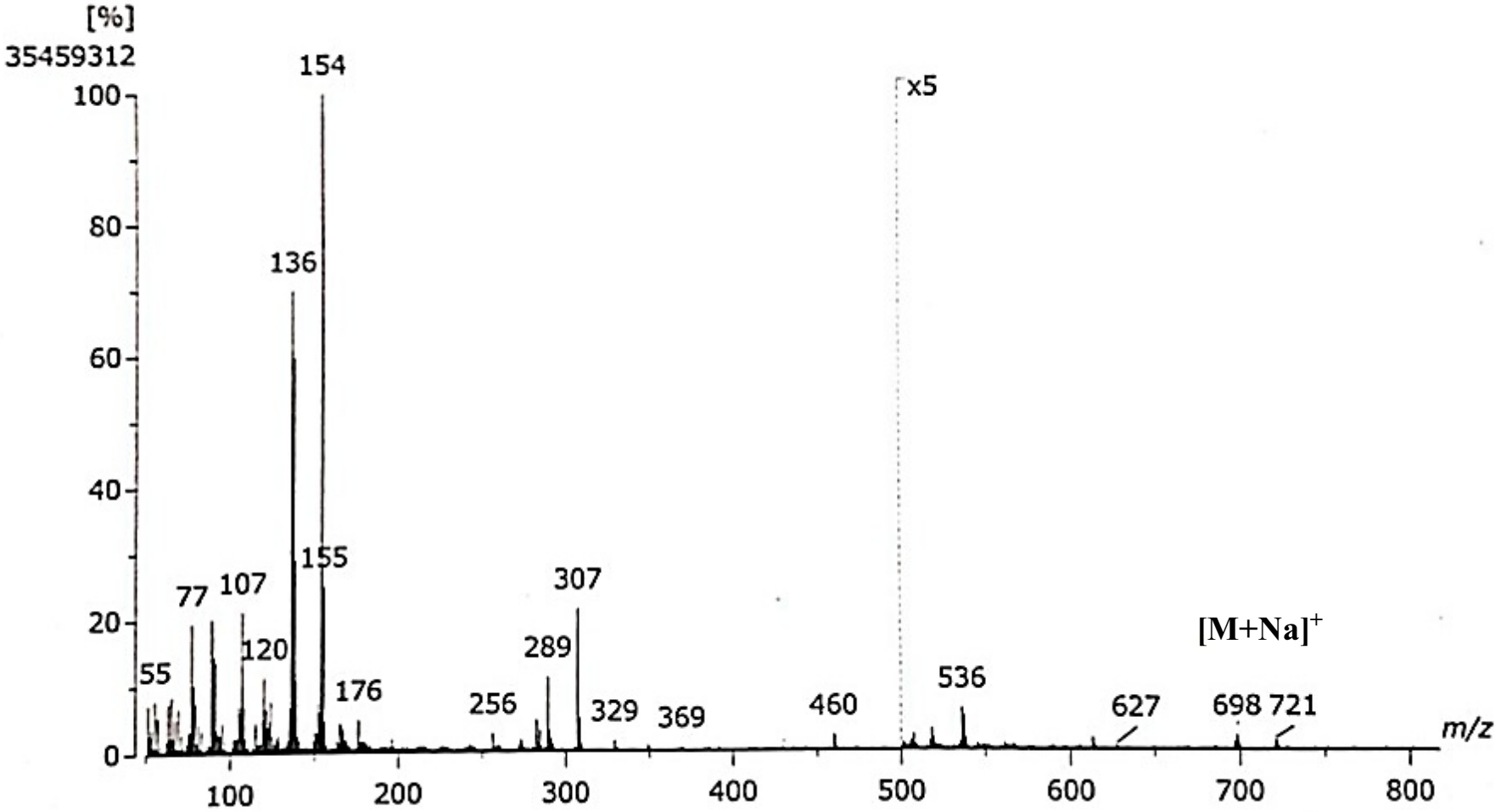


Figure S1. Positive FAB-MS spectrum of compound 1

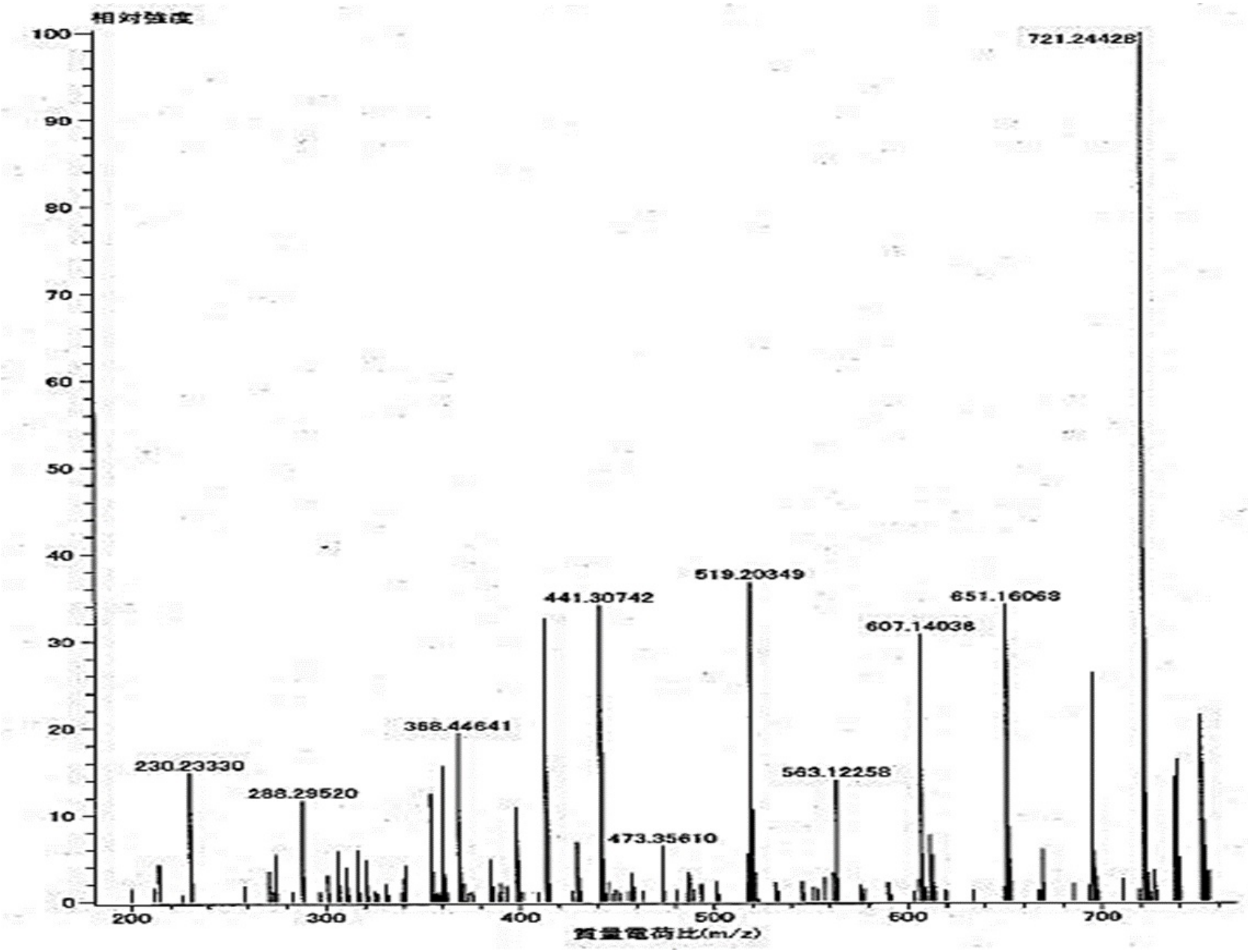


Figure S2. Positive HRESIMS spectrum of compound 1

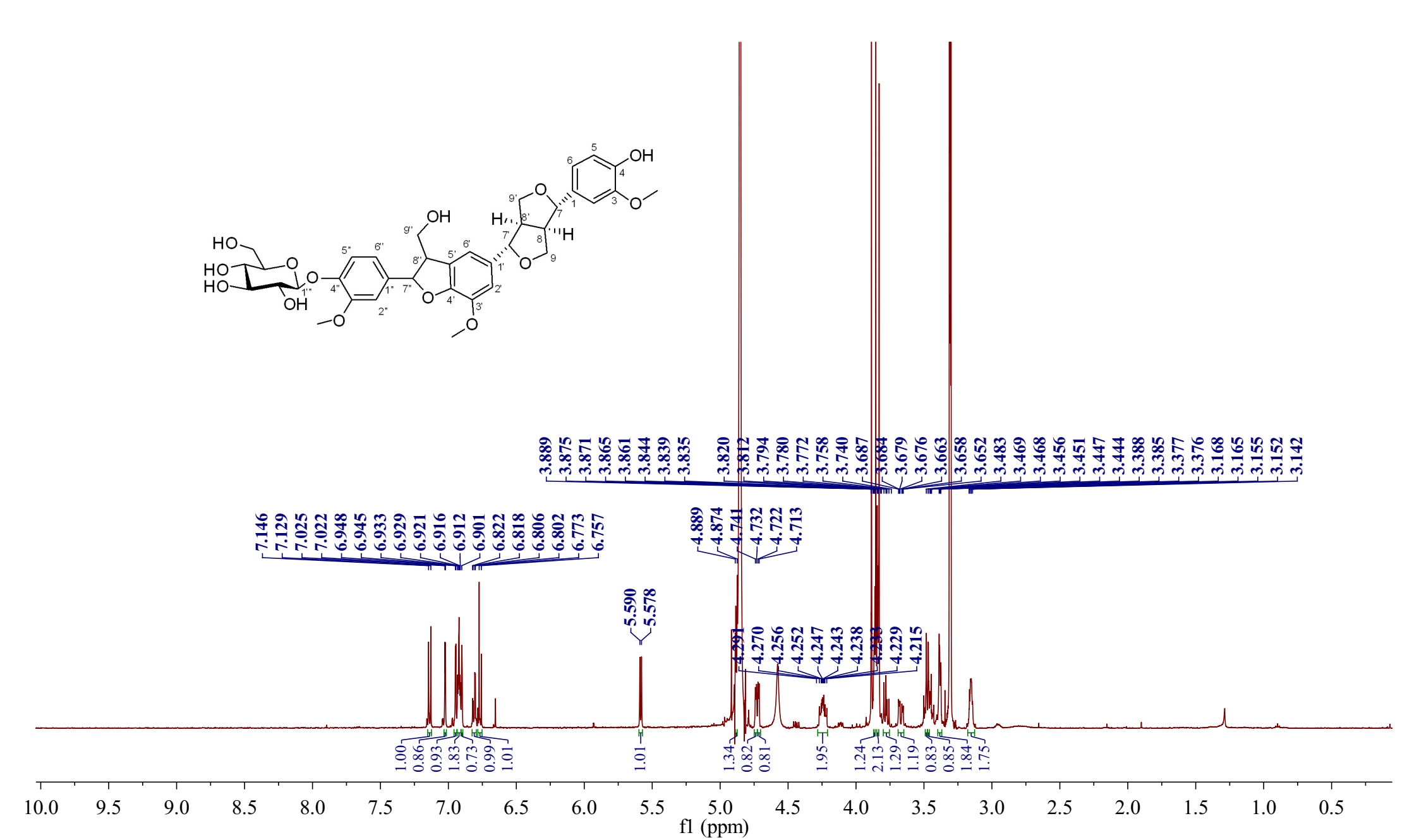


Figure S3. ^{1}H NMR spectrum of compound 1 (CD_3OD, 500 MHz)

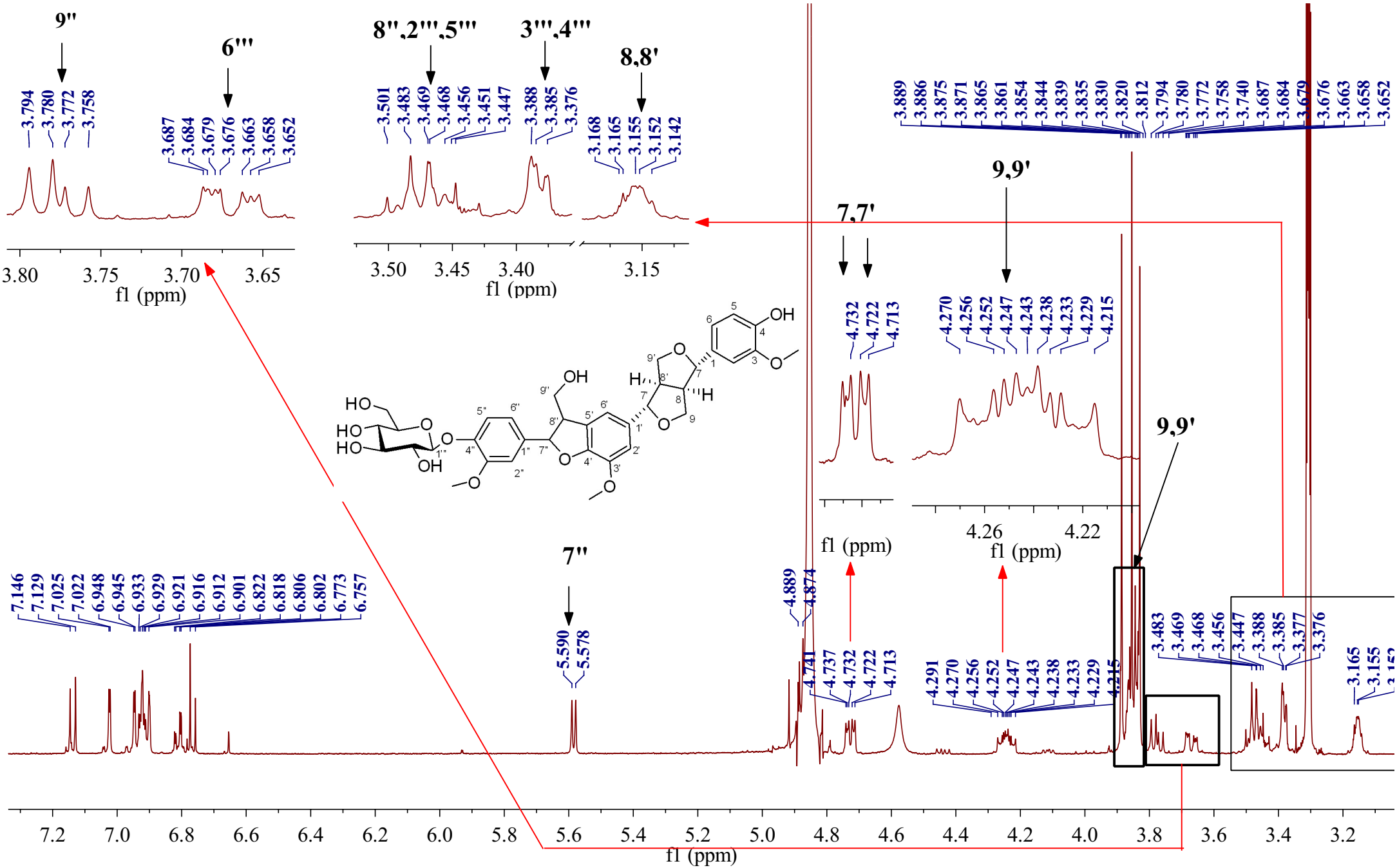


Figure S4. Expanded ^{1}H NMR spectrum of compound 1 (CD_3OD, 500 MHz)

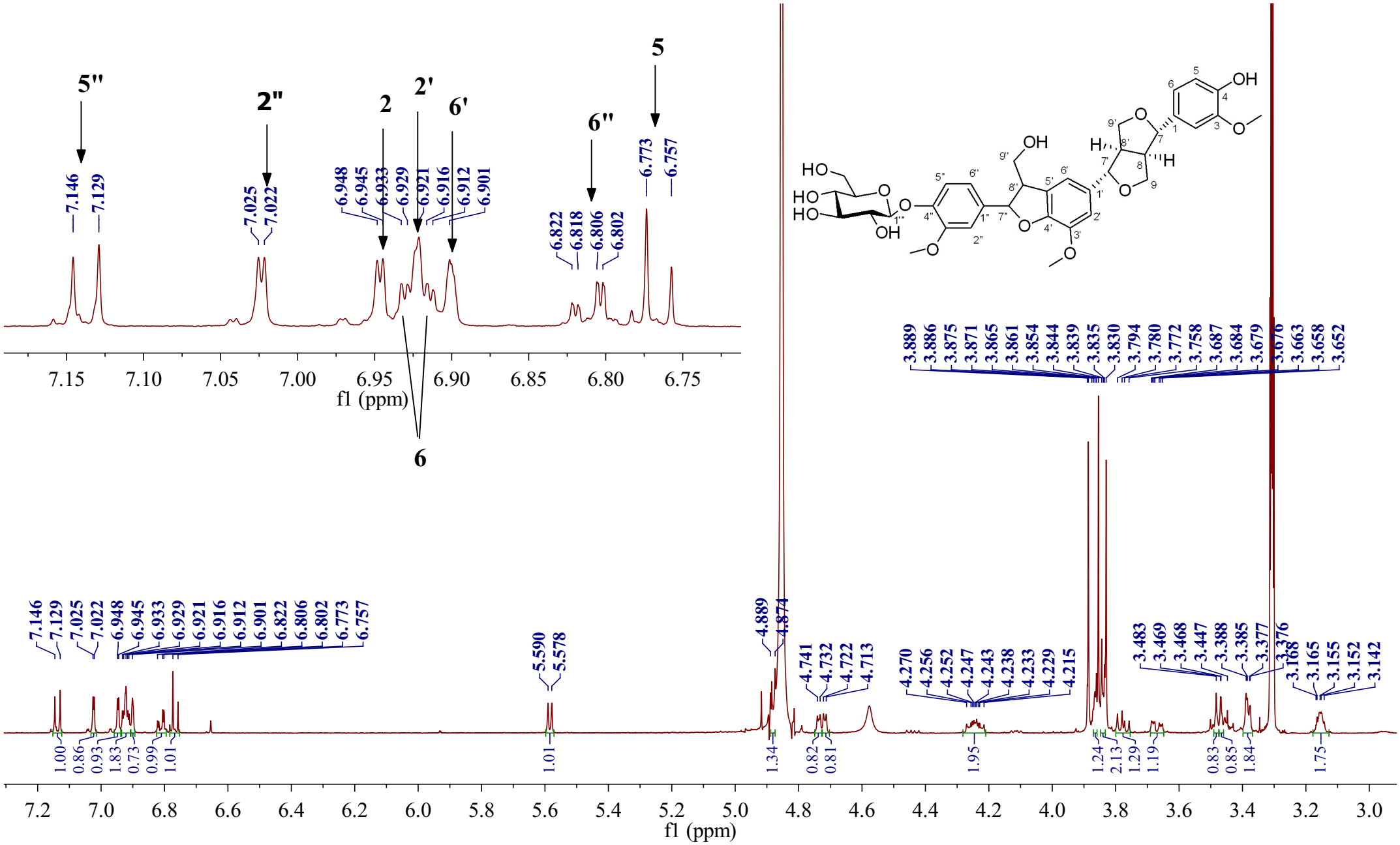


Figure S5. Expanded ^{1}H NMR spectrum of compound 1 (CD_3OD, 500 MHz)

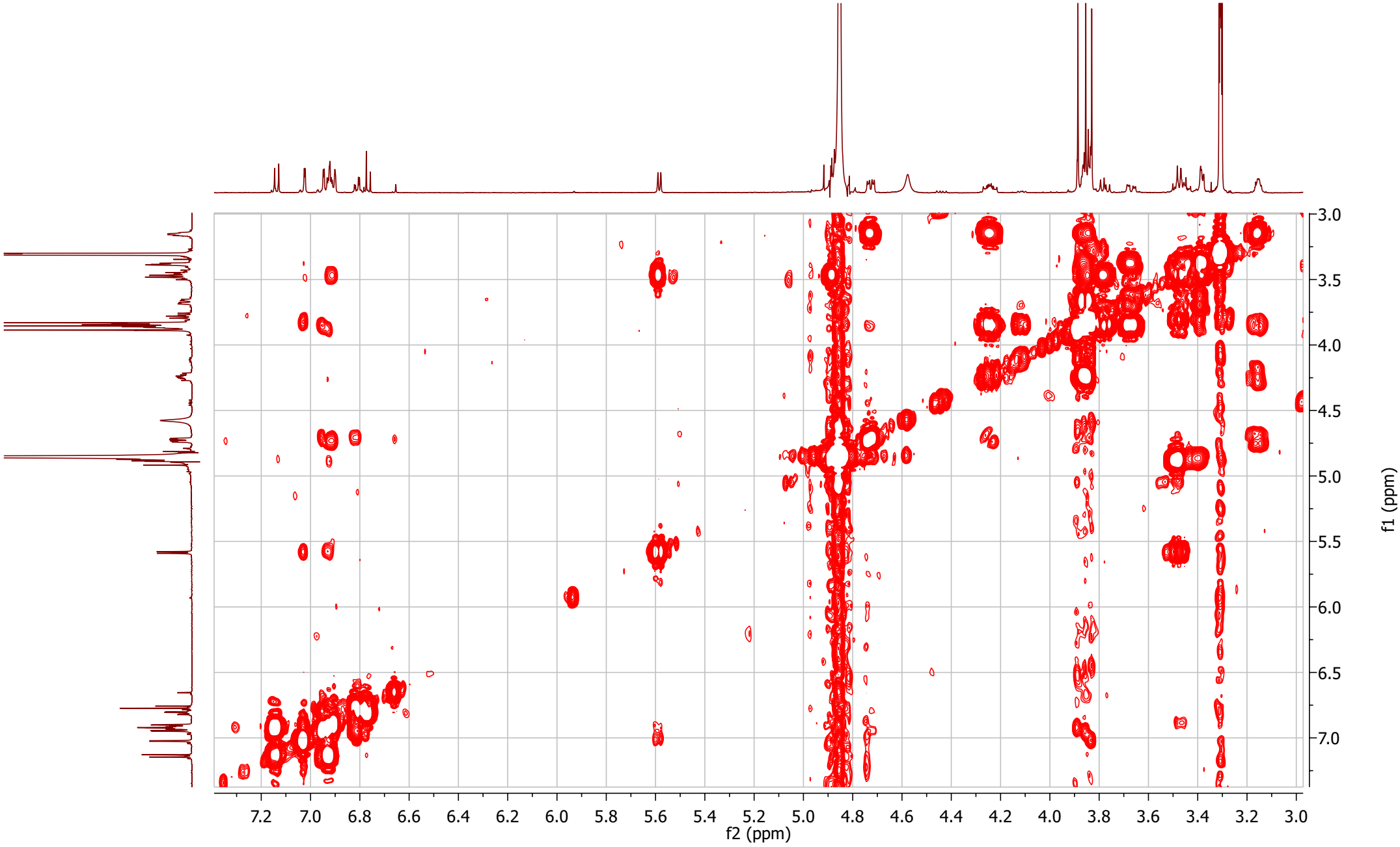


Figure S6. ^{1}H-^{1}H COSY spectrum of compound 1 (CD_3OD, 500 MHz)

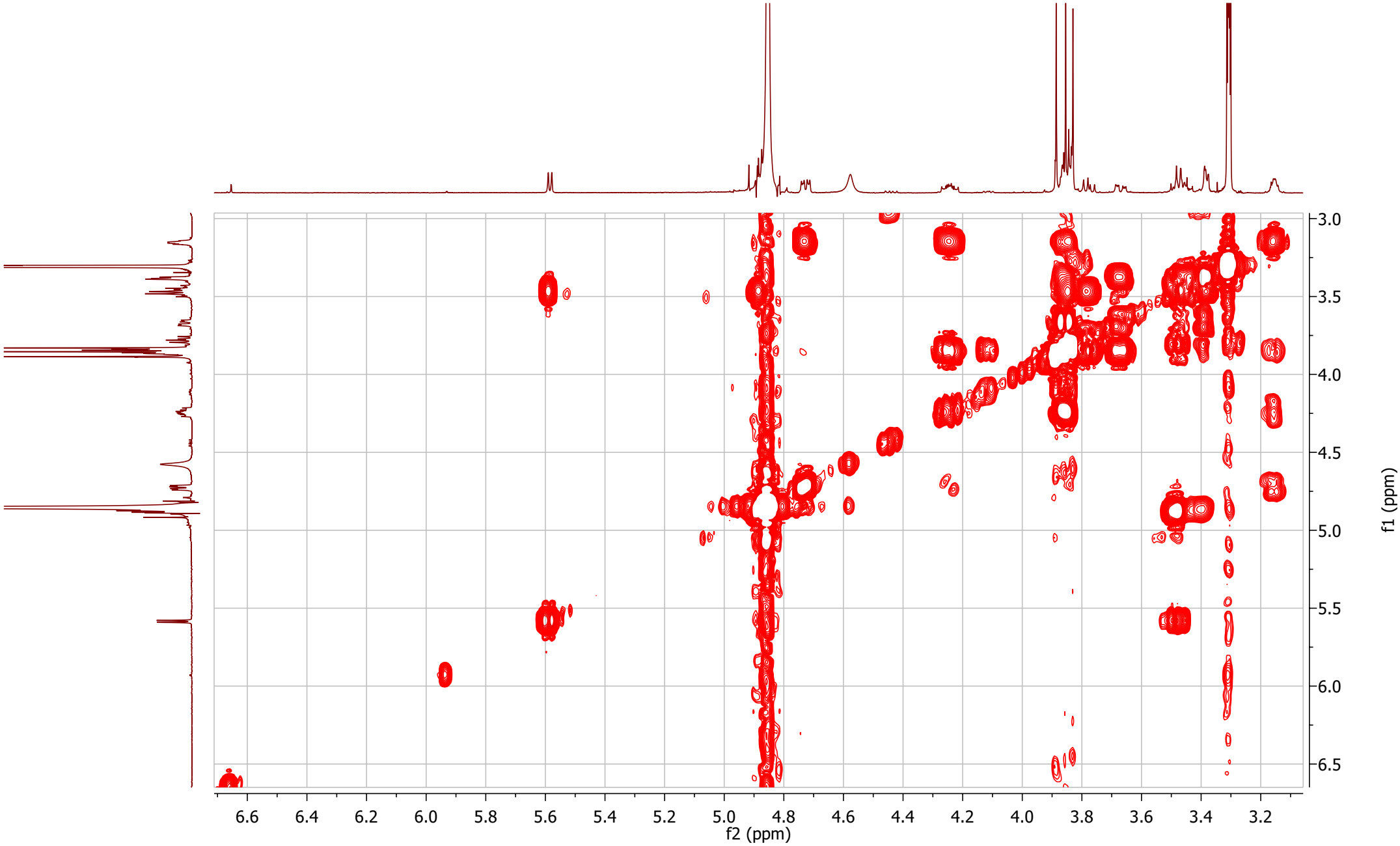


Figure S7. Expanded ^{1}H-^{1}H COSY spectrum of compound 1 (CD_3OD, 500 MHz)

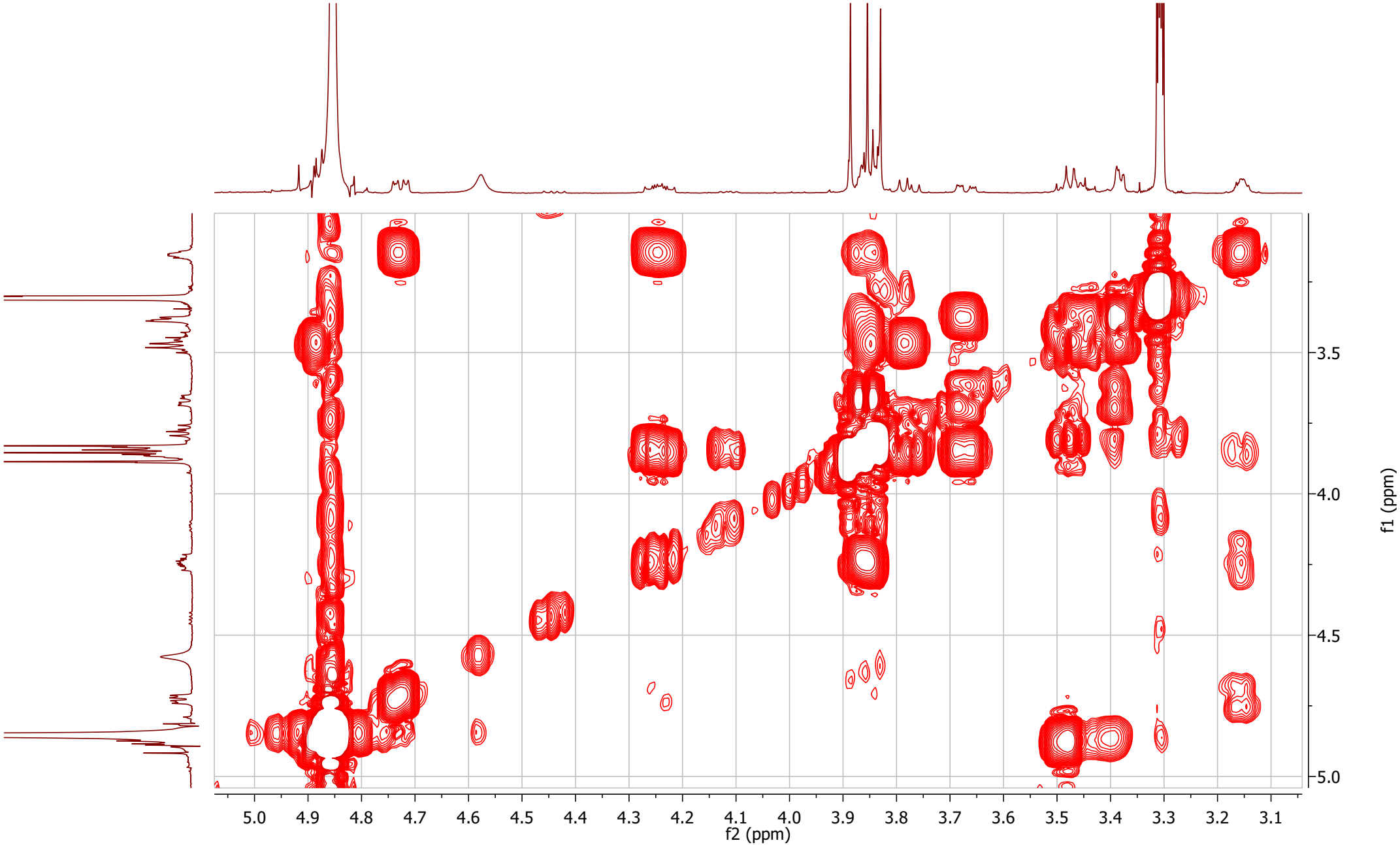


Figure S8. Expanded ^{1}H-^{1}H COSY spectrum of compound 1 (CD_3OD, 500 MHz)

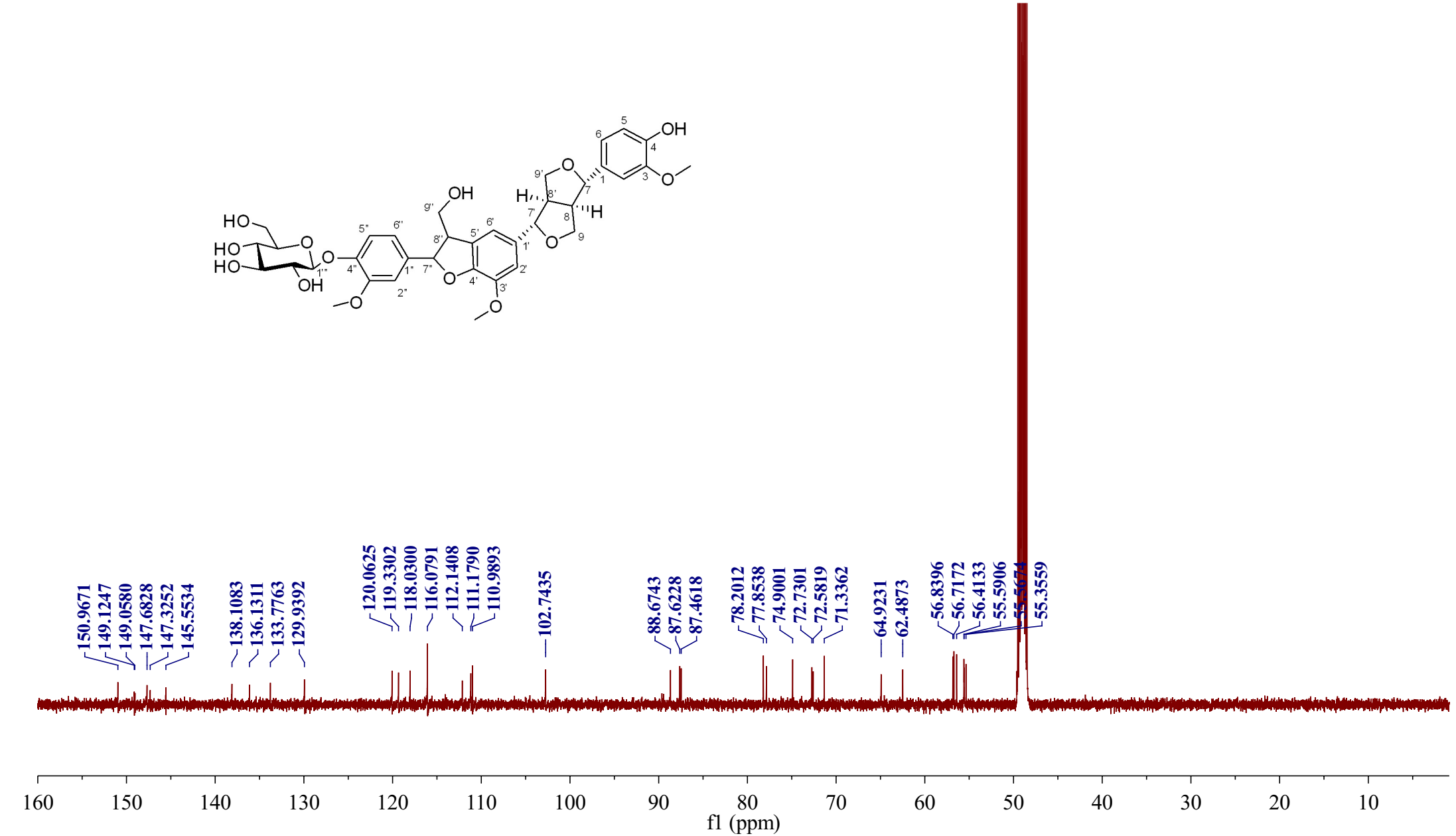


Figure S9. ^{13}CNMR spectrum of compound 1 (CD_3OD, 125 MHz)

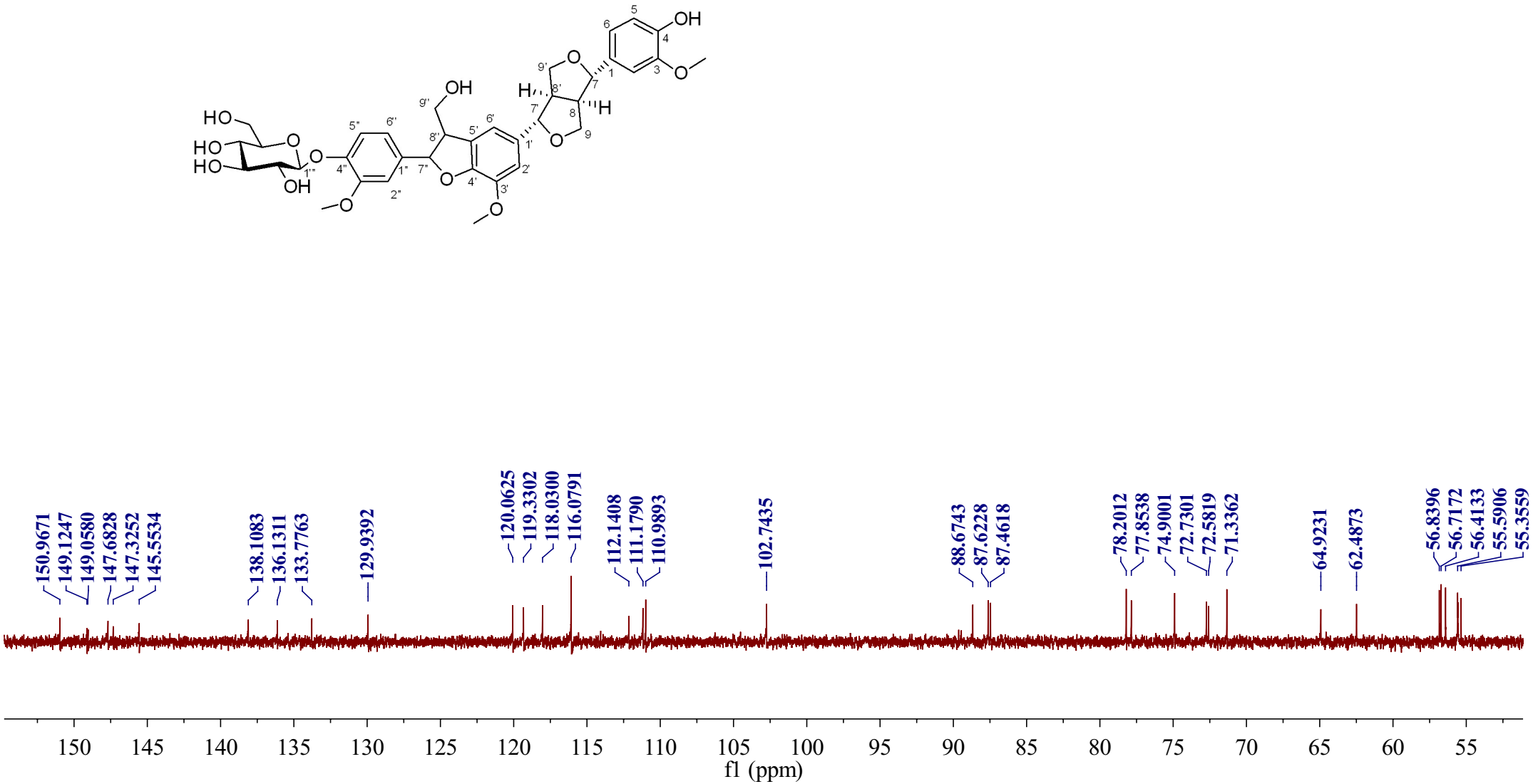


Figure S10. Expanded ^{13}CNMR spectrum of compound 1 (CD_3OD, 125 MHz)

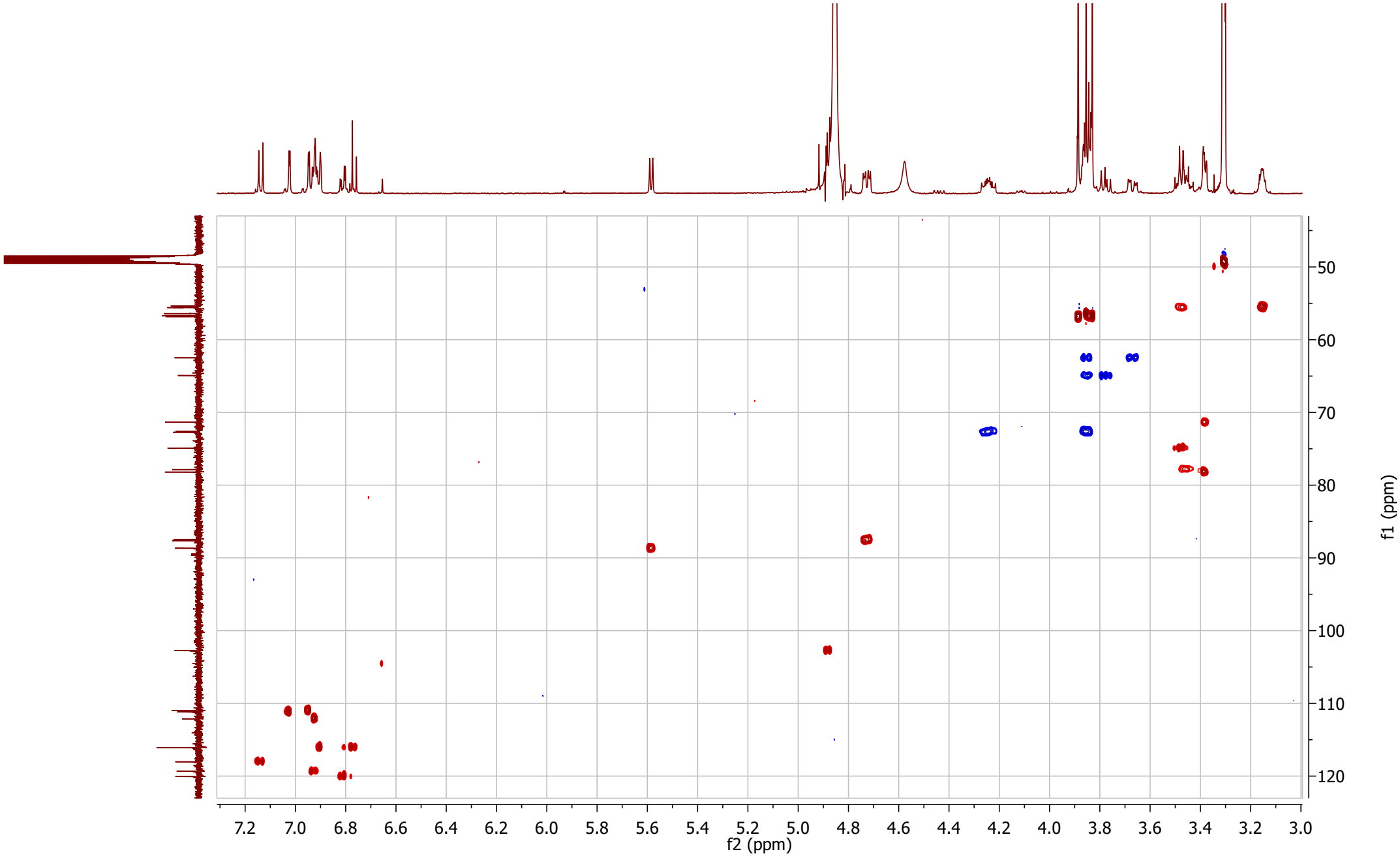


Figure S11. HSQC spectrum of compound 1

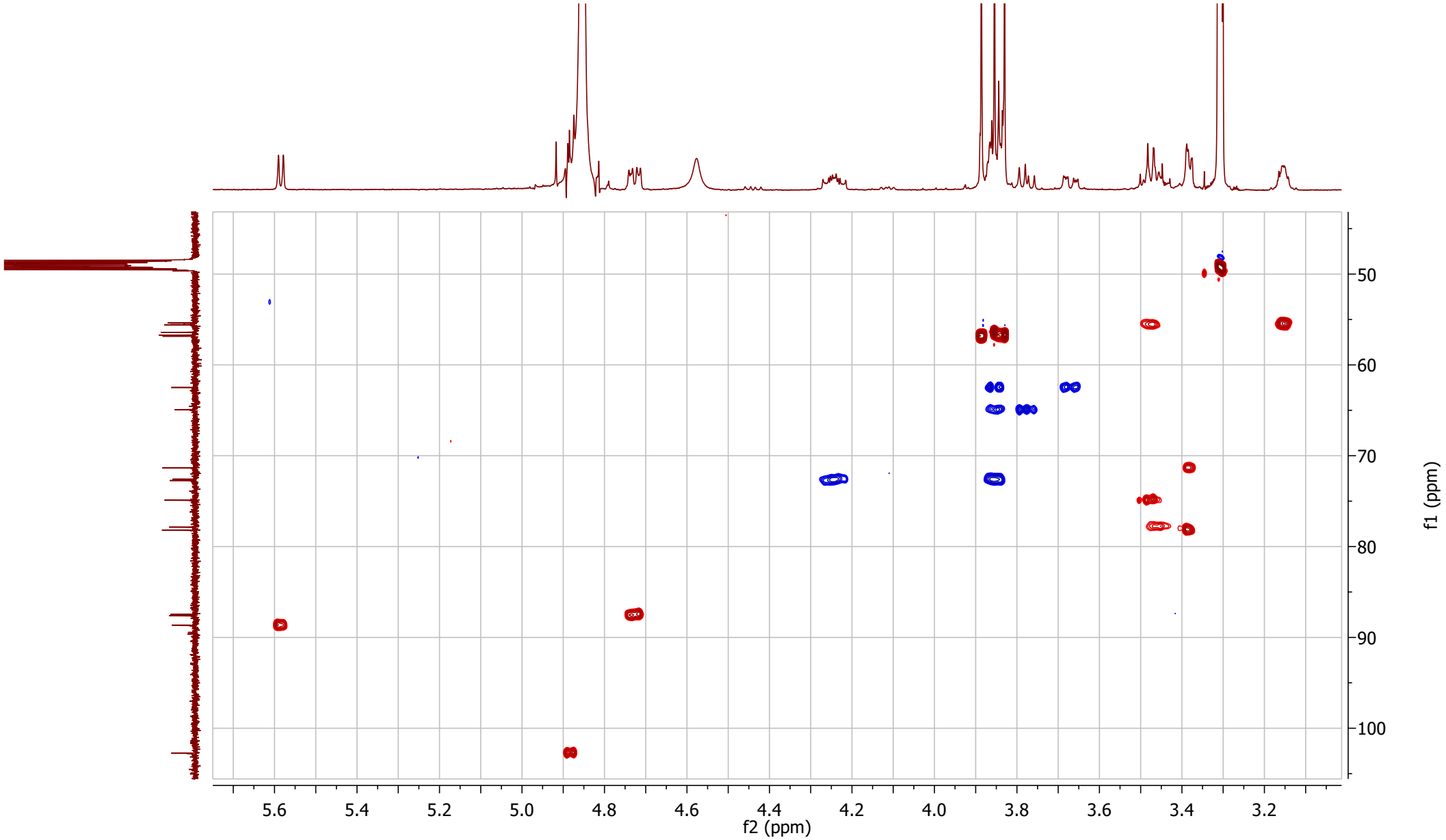


Figure S12. Expanded HSQC spectrum of compound 1

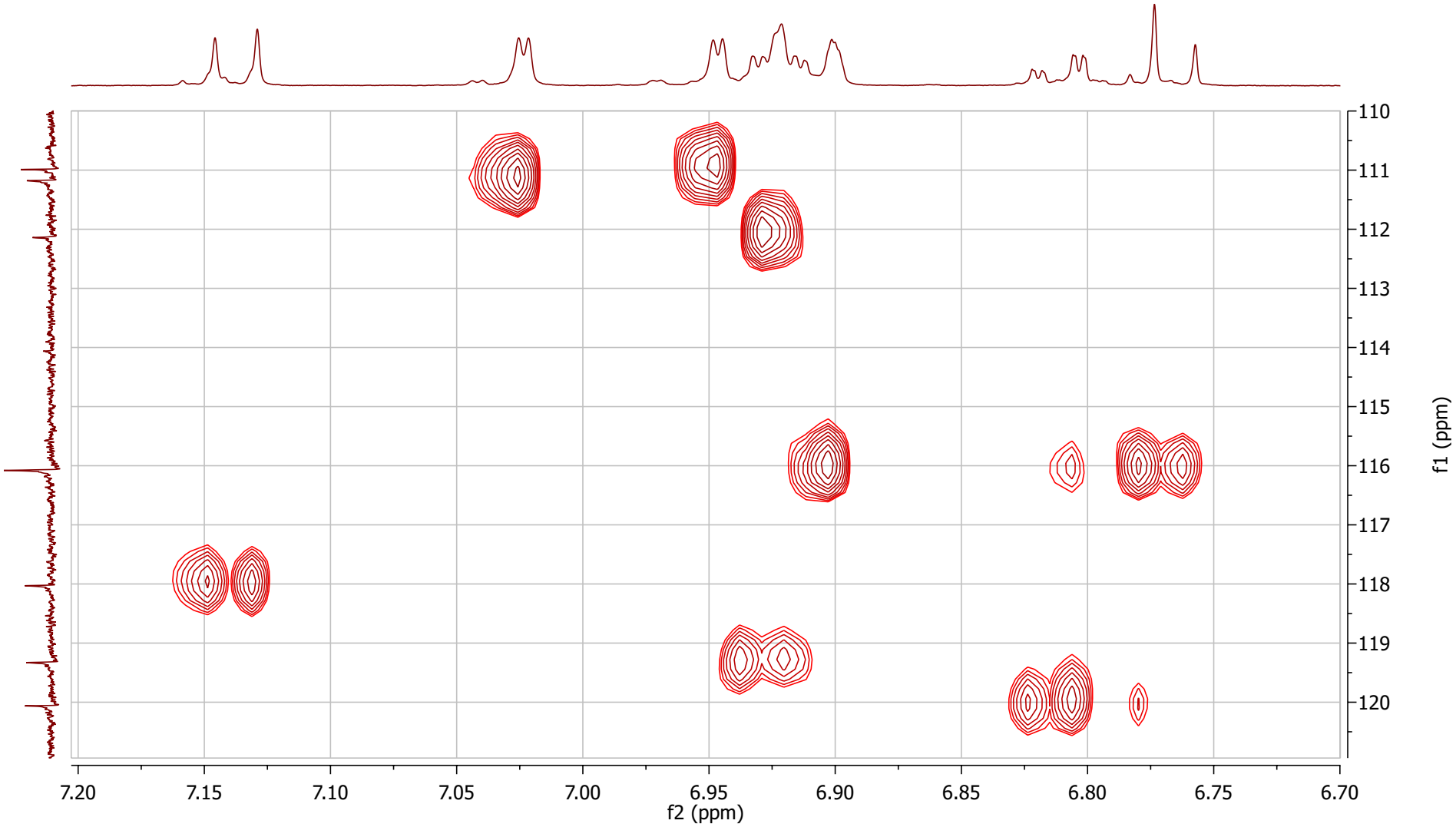


Figure S13. Expanded HSQC spectrum of compound 1

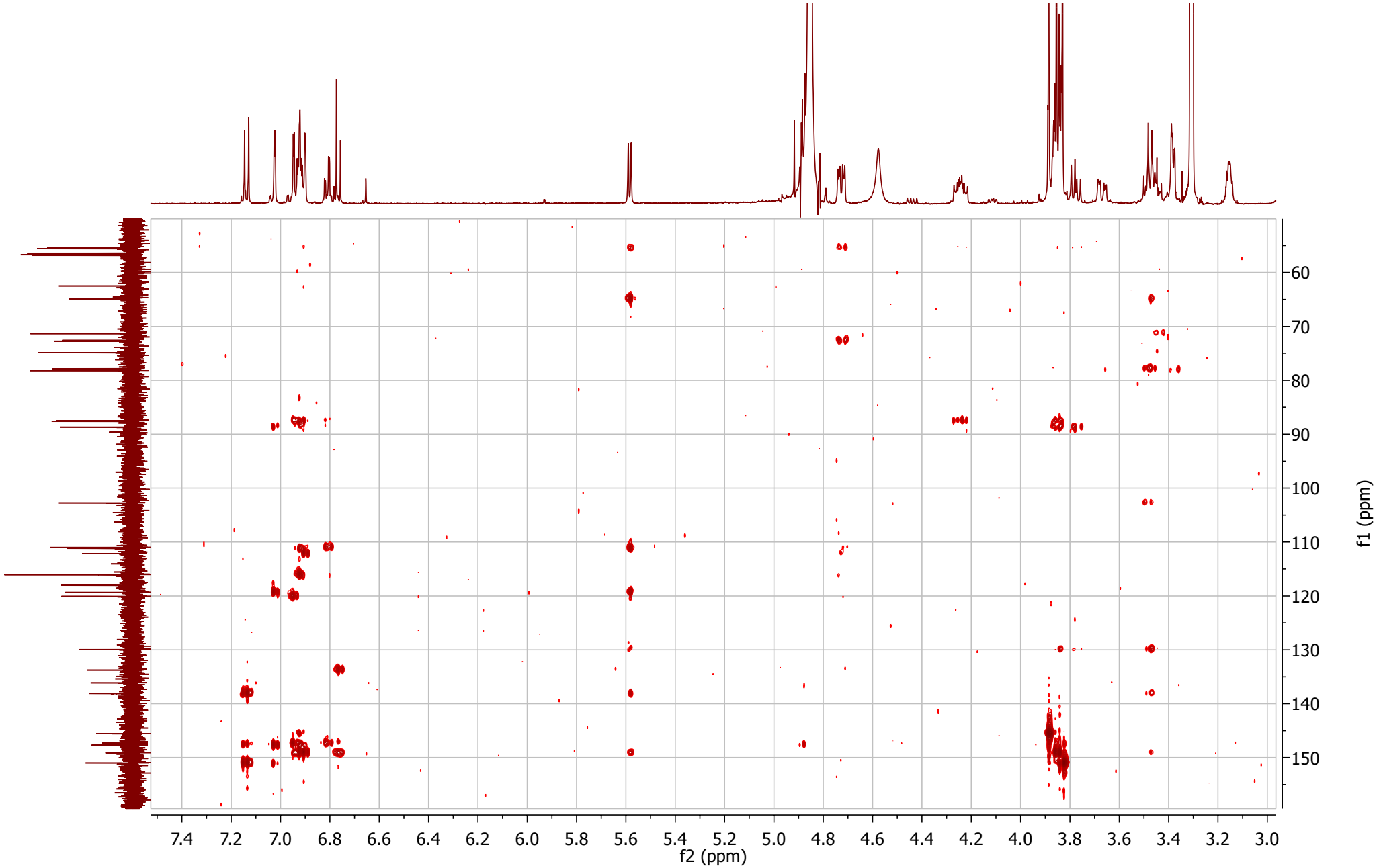


Figure S14. HMBC spectrum of compound 1

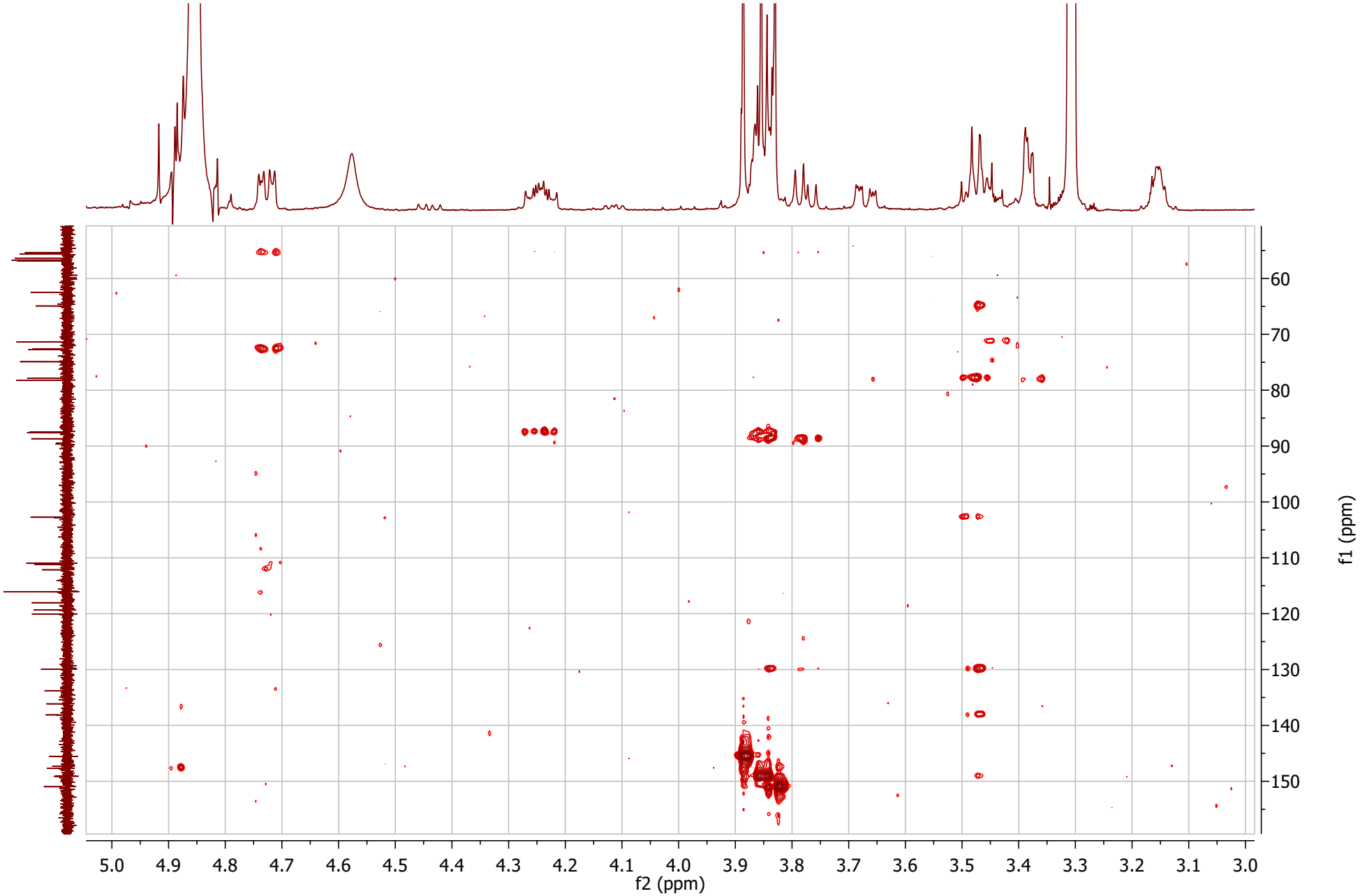


Figure S15. Expanded HMBC spectrum of compound 1

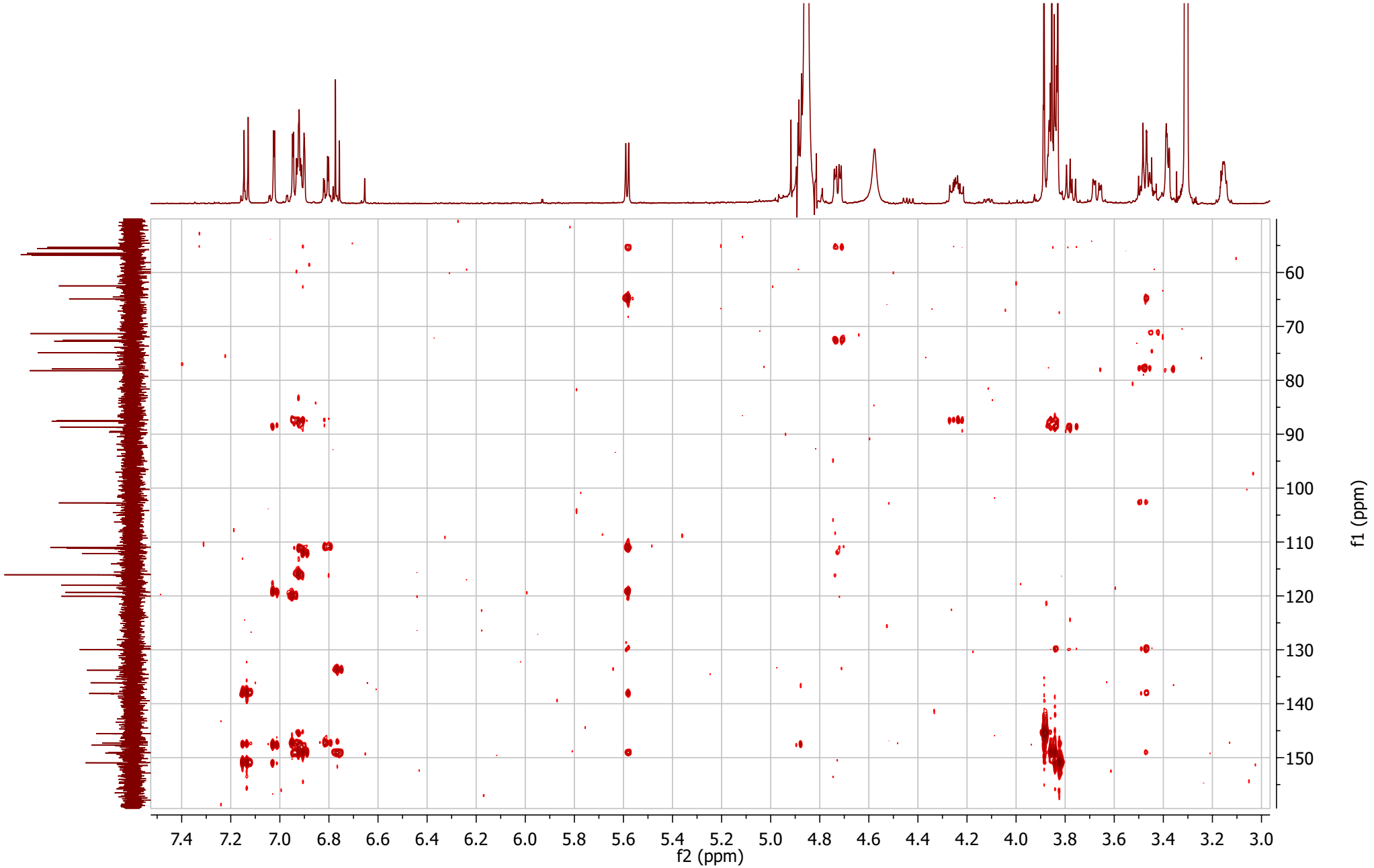


Figure S16. Expanded HMBC spectrum of compound 1

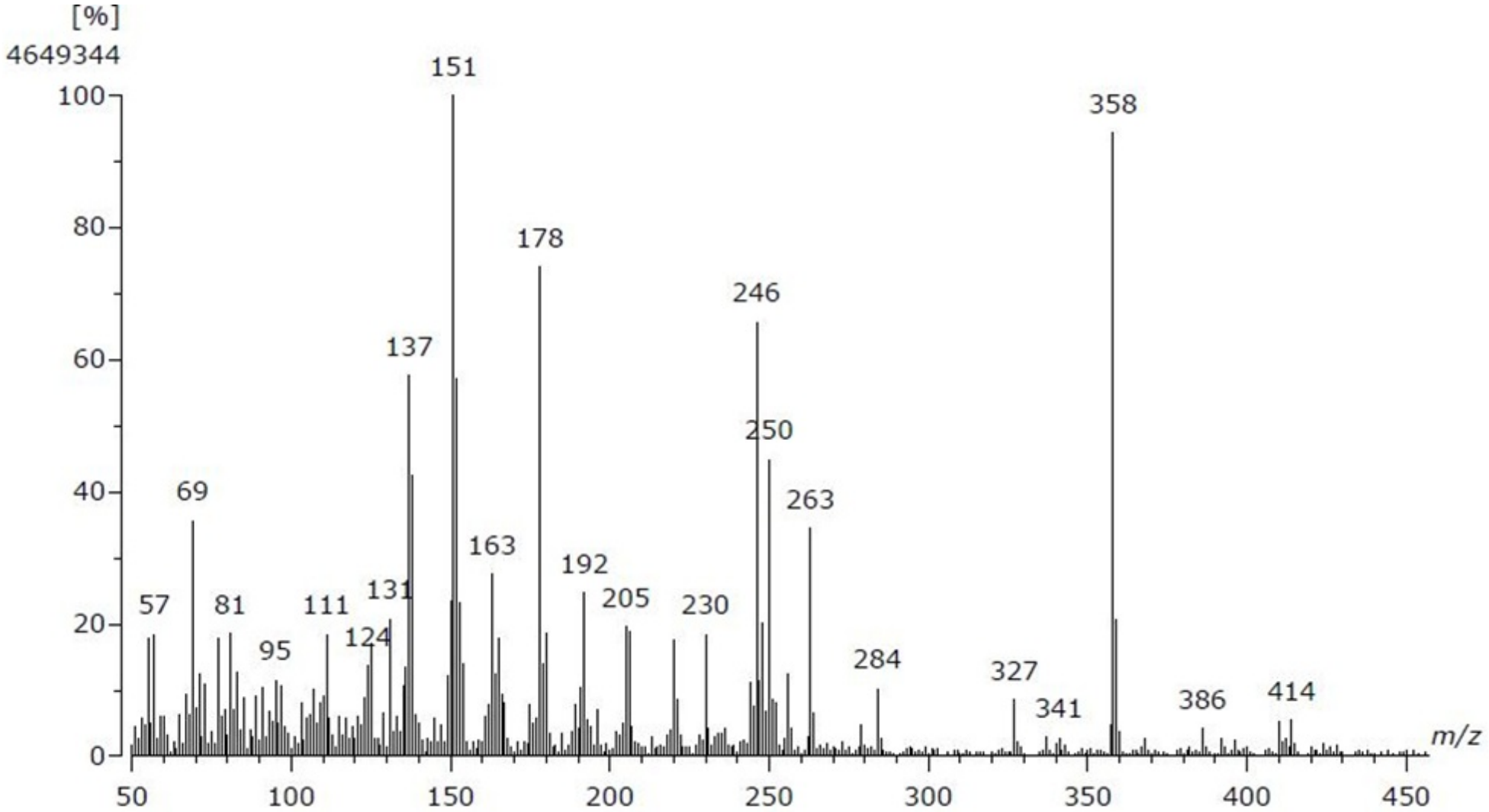


Figure S17. EI-MS spectrum of compound 2

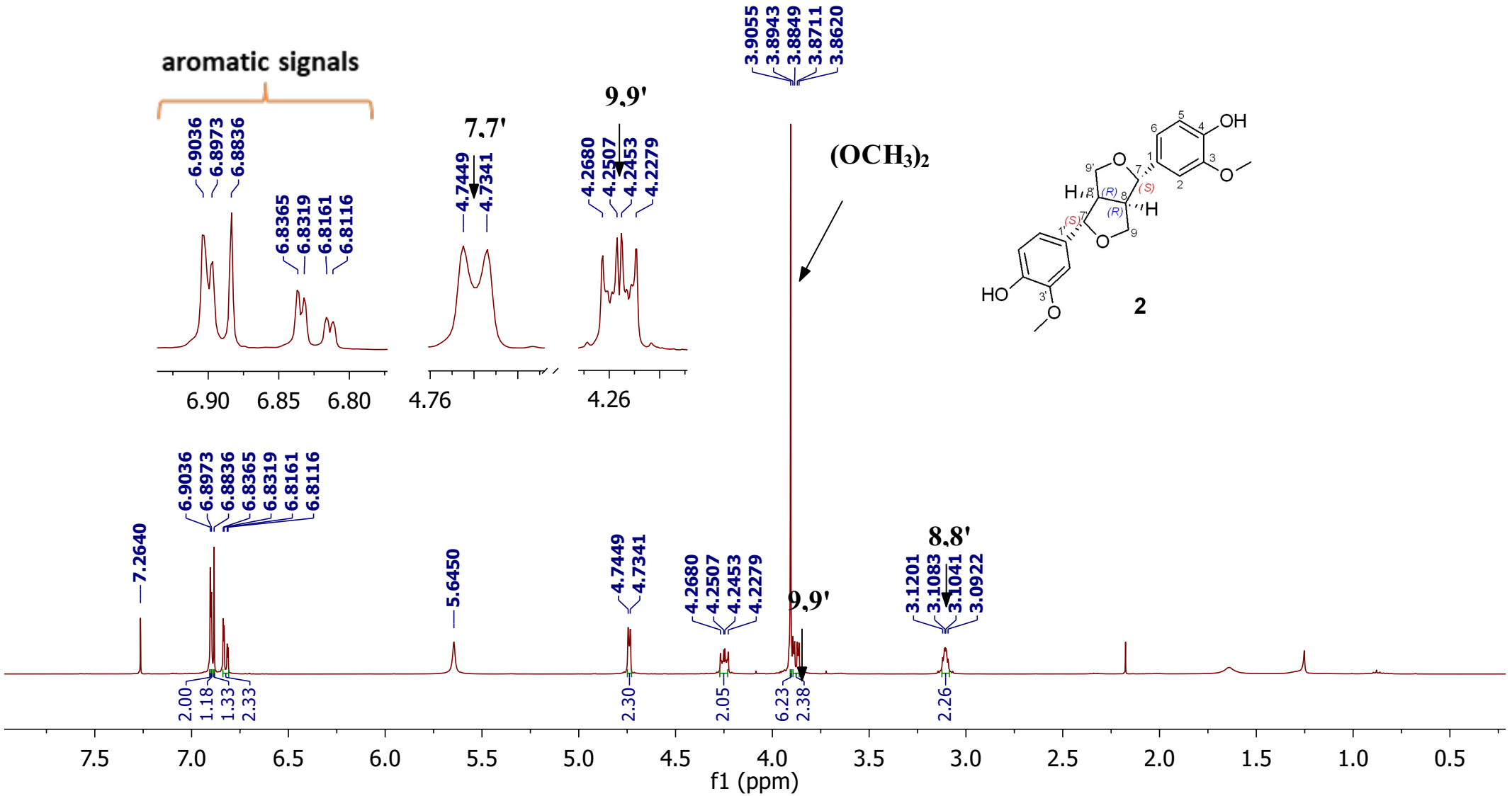


Figure S18. ^{1}HNMR spectrum of compound 2 (CD_3OD, 400 MHz)

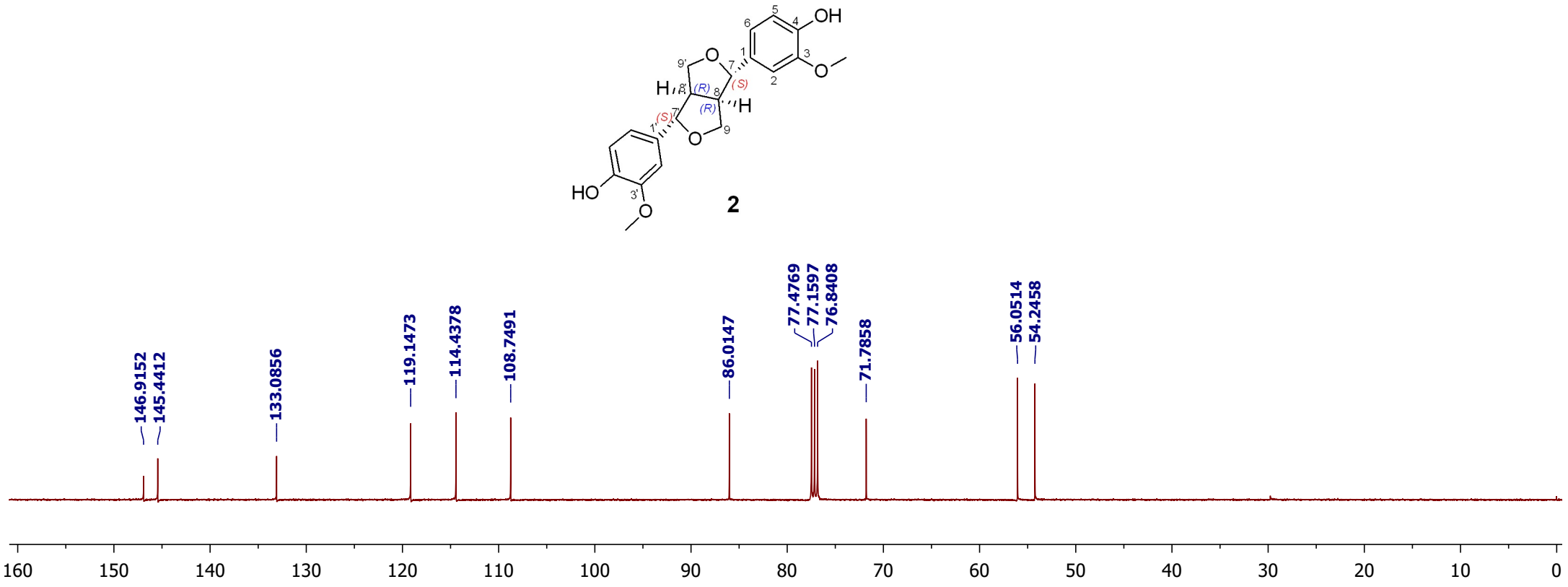


Figure S19. ^{13}CNMR spectrum of compound 2 (CD_3OD, 100 MHz)

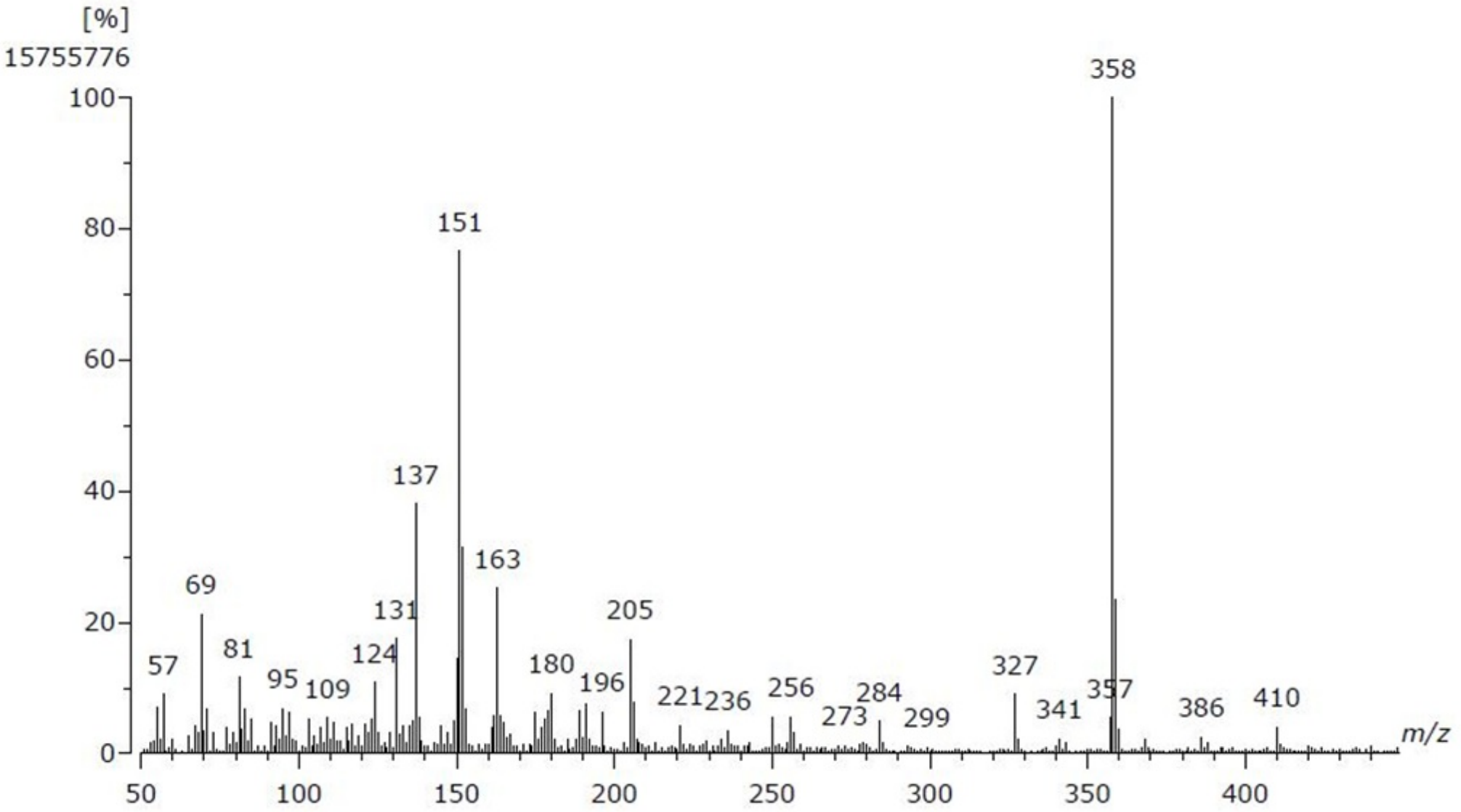


Figure S20. EI-MS spectrum of compound 3

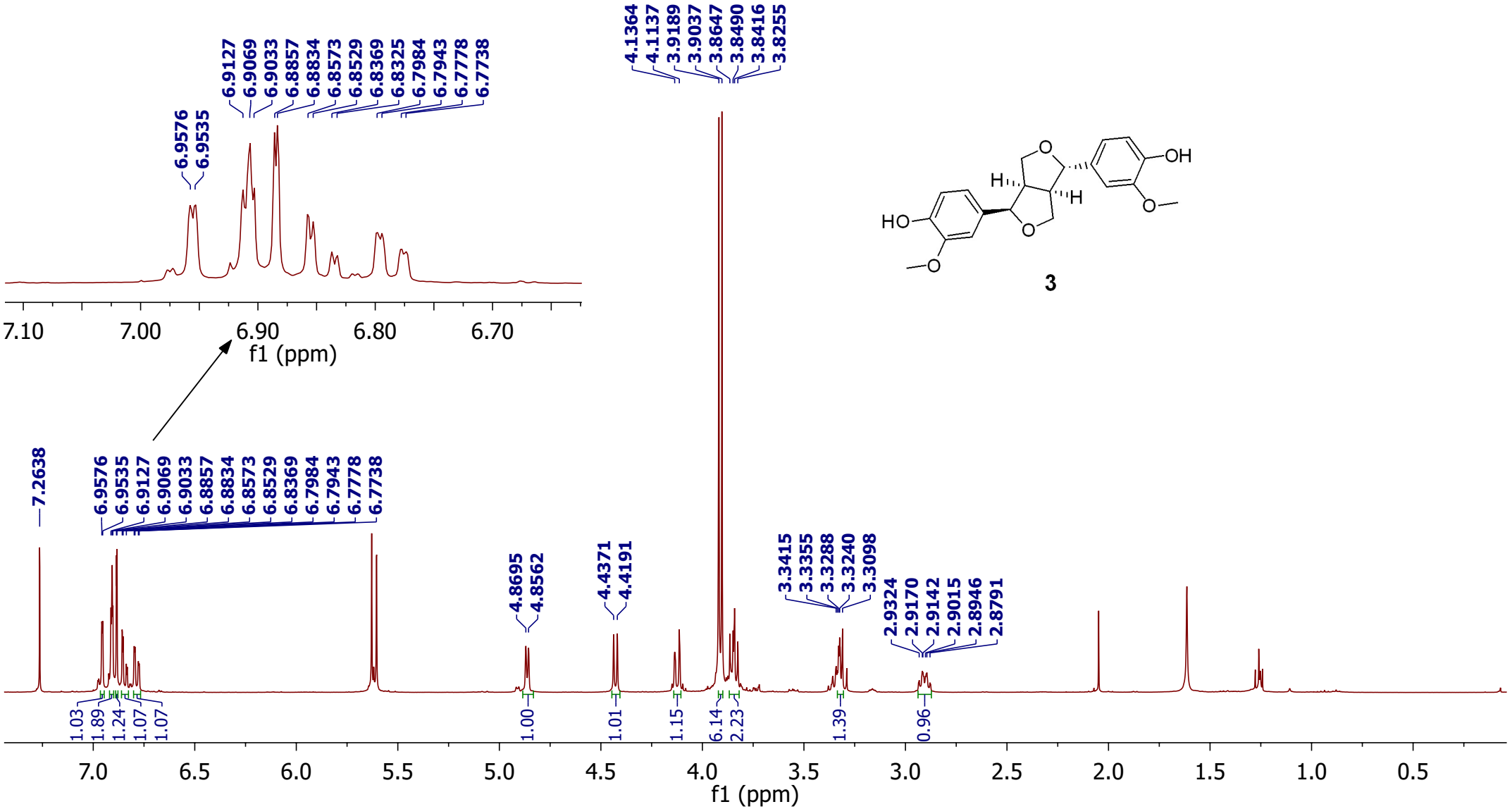
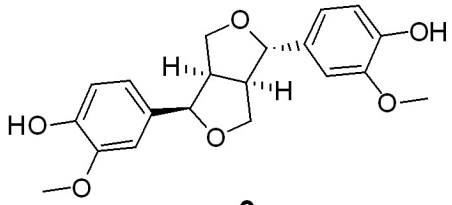


Figure S21. ^{1}HNMR spectrum of compound 3 ($CDCL_3$, 100 MHz)



3

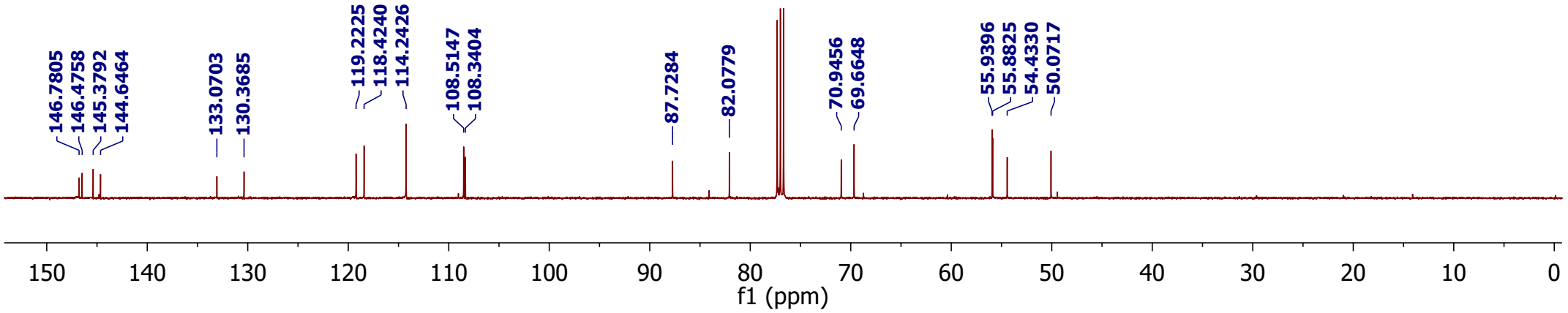


Figure S22. ^{13}CNMR spectrum of compound 3 ($CDCL_3$, 100 MHz)

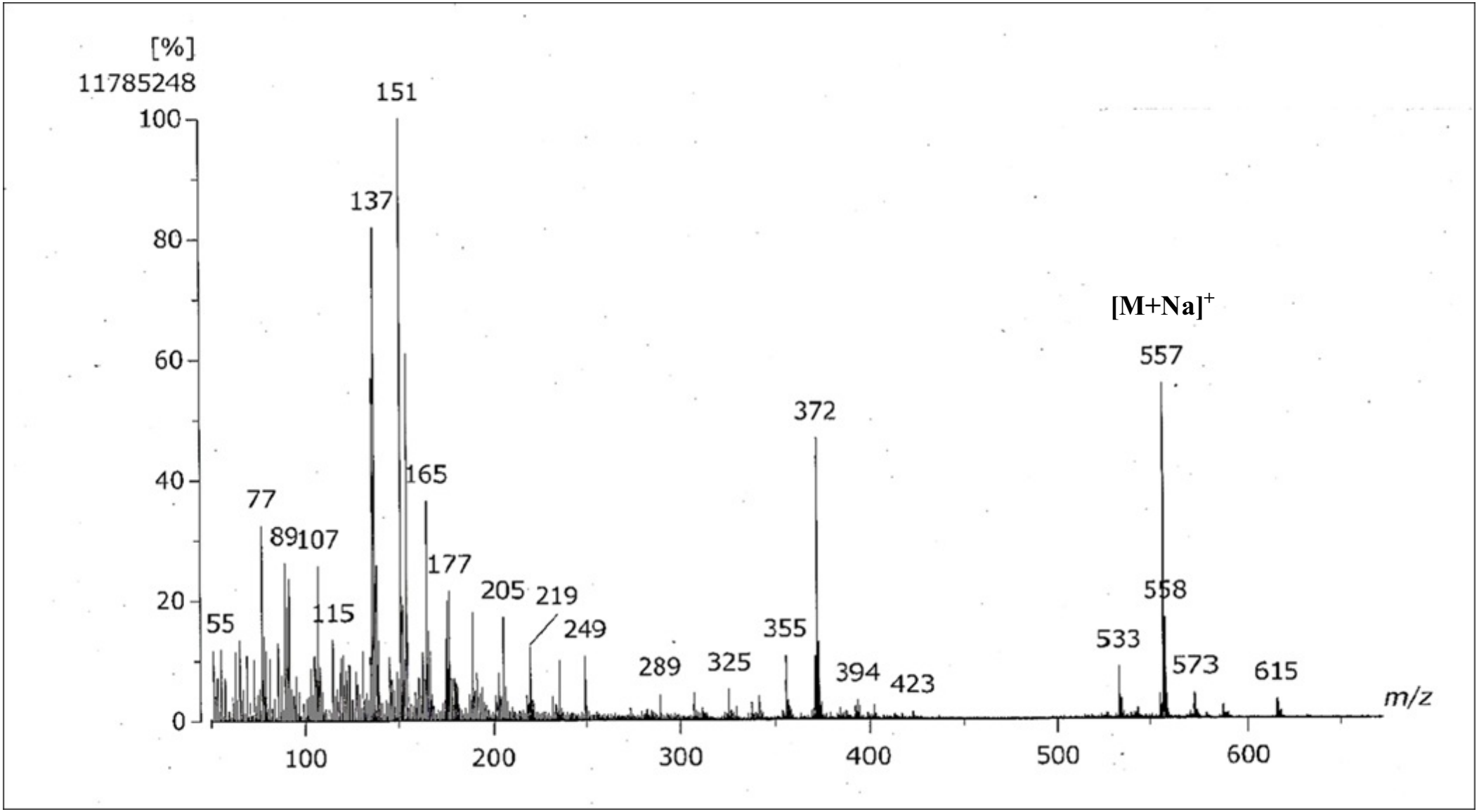


Figure S23. Positive FAB-MS spectrum of compound 4

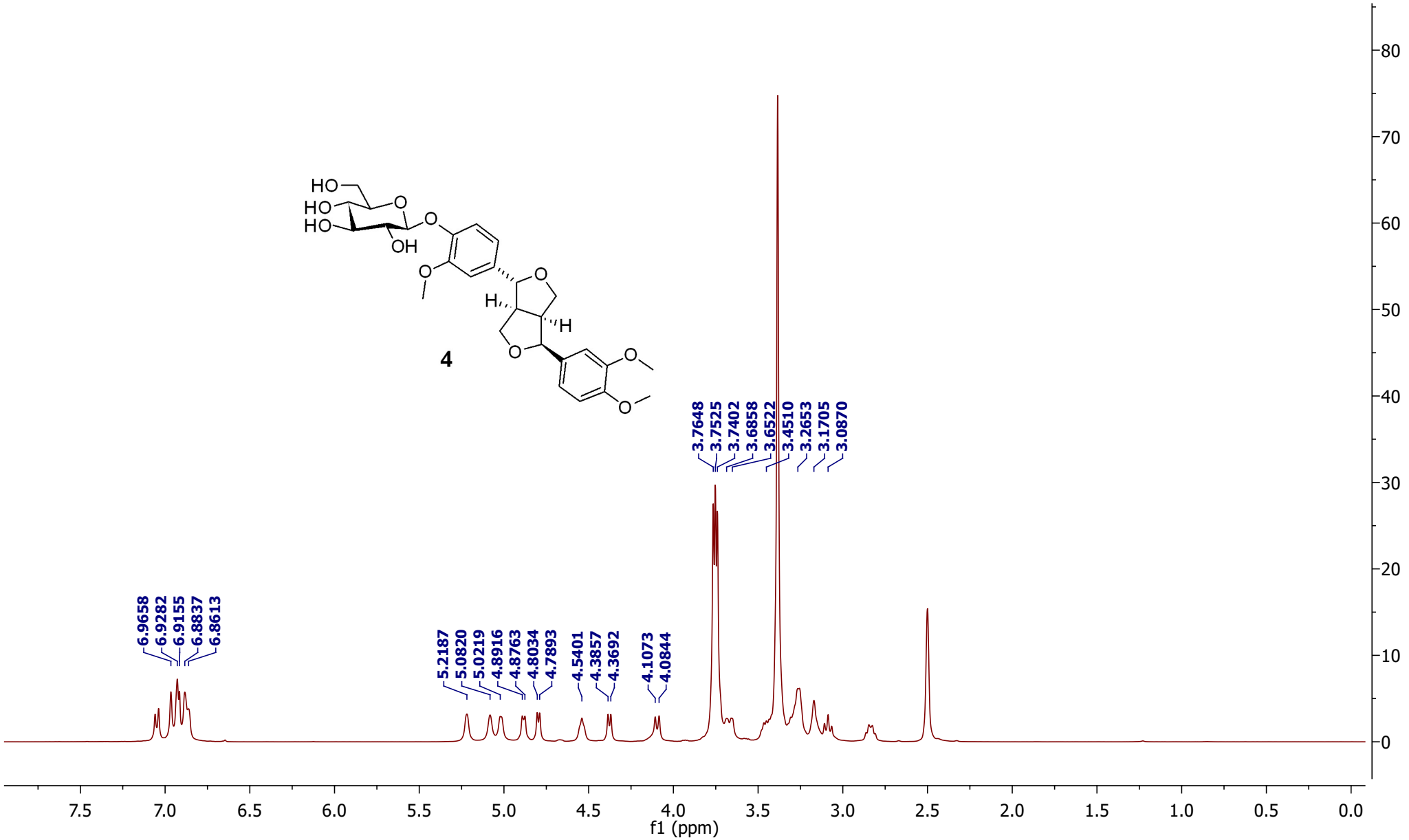


Figure S24. ^{1}HNMR spectrum of compound 4 (DMSO-d_6, 400 MHz)

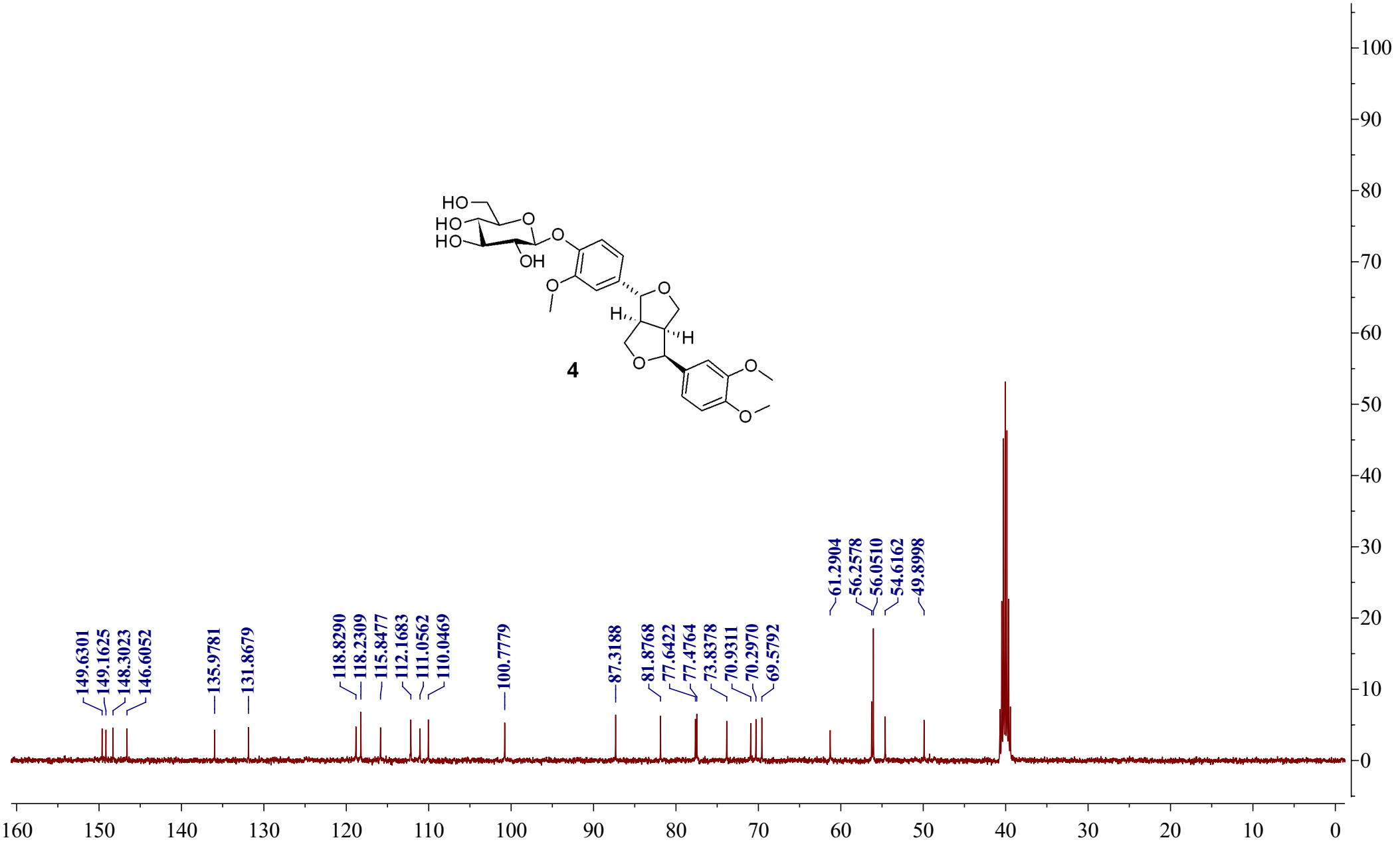


Figure S25. ^{13}CNMR spectrum of compound 4 (DMSO-d_6, 100 MHz)

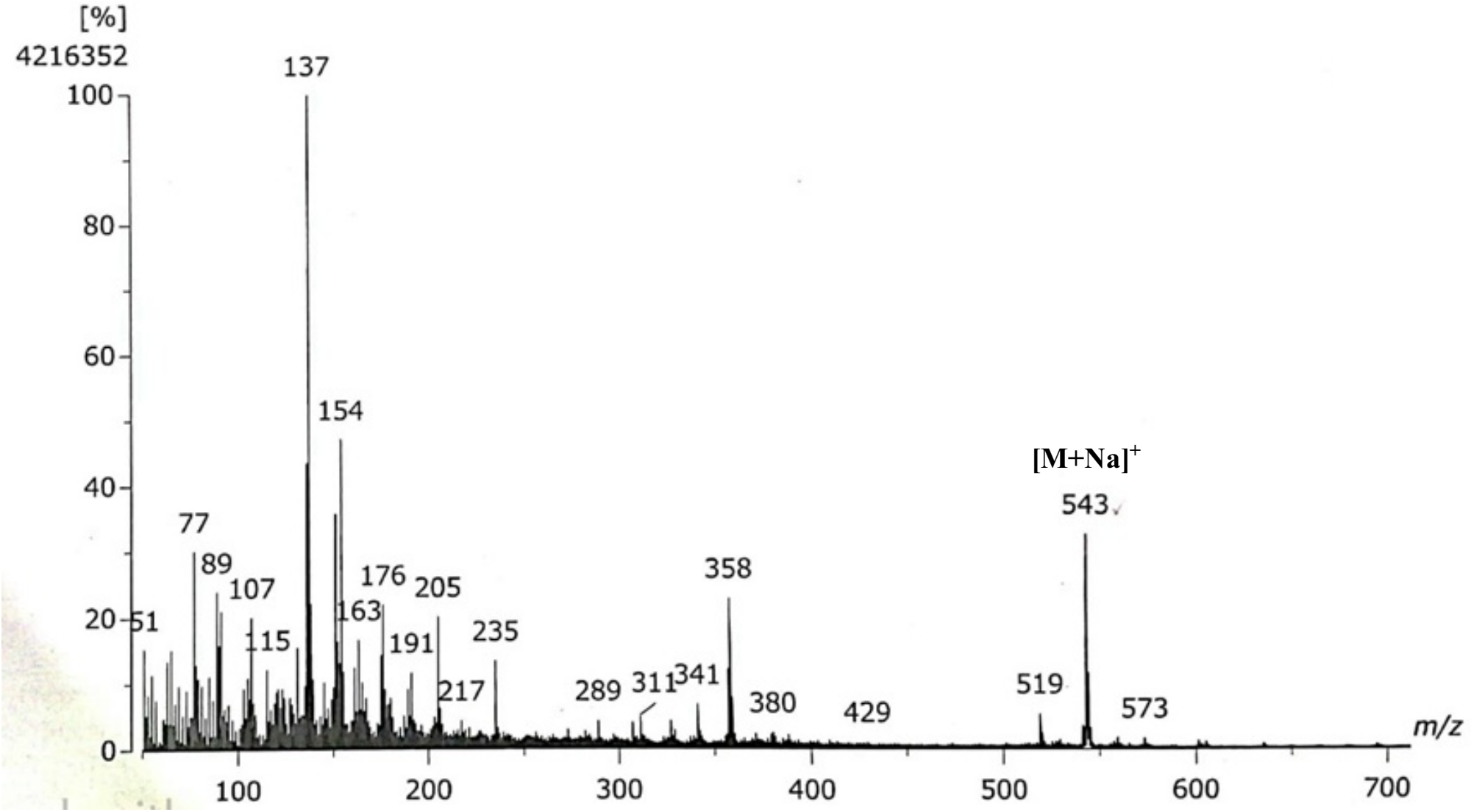


Figure S26. Positive FAB-MS spectrum of compound 5

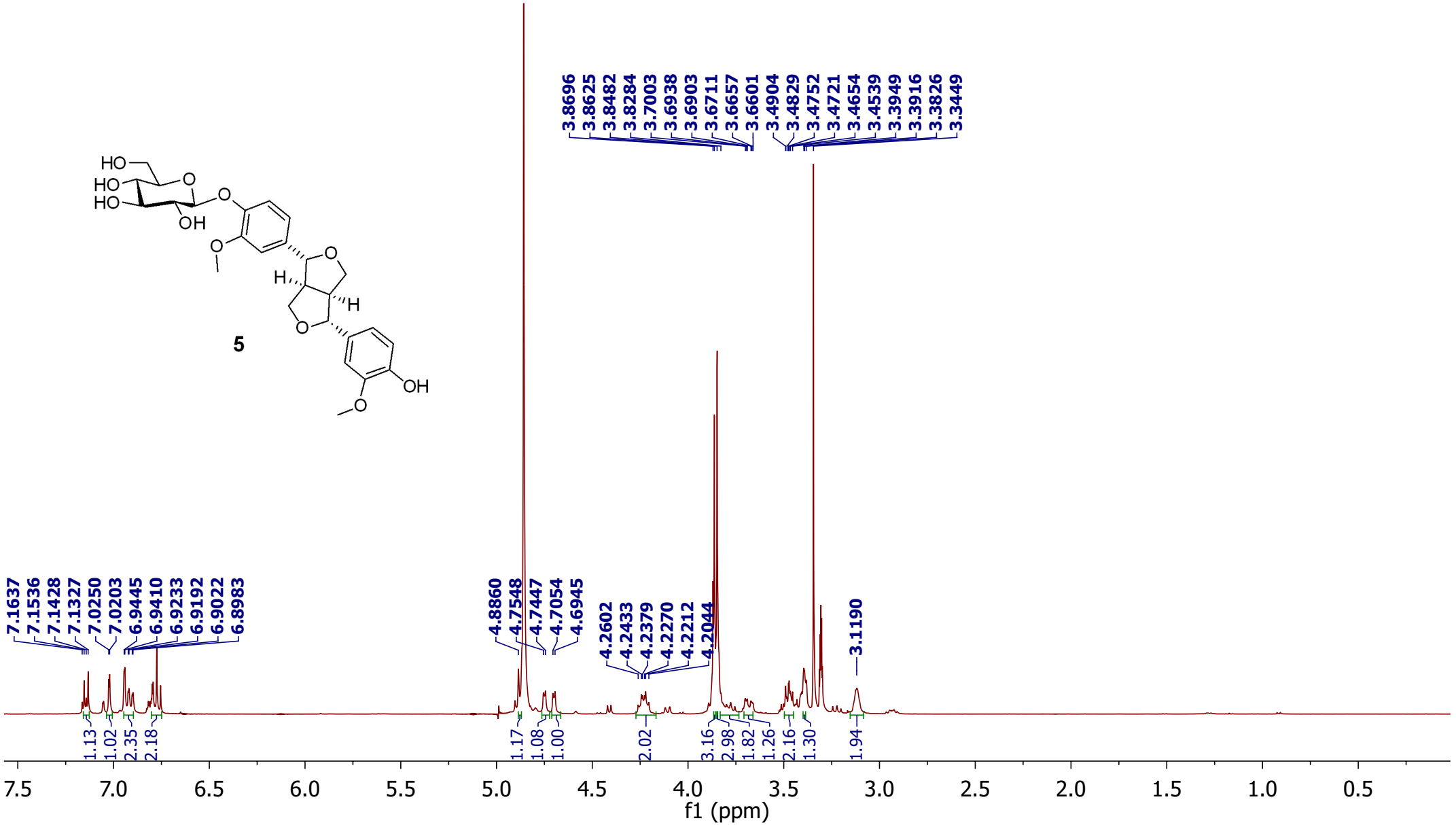


Figure S27. ^{1}HNMR spectrum of compound 5 (CD_3OD, 400 MHz)

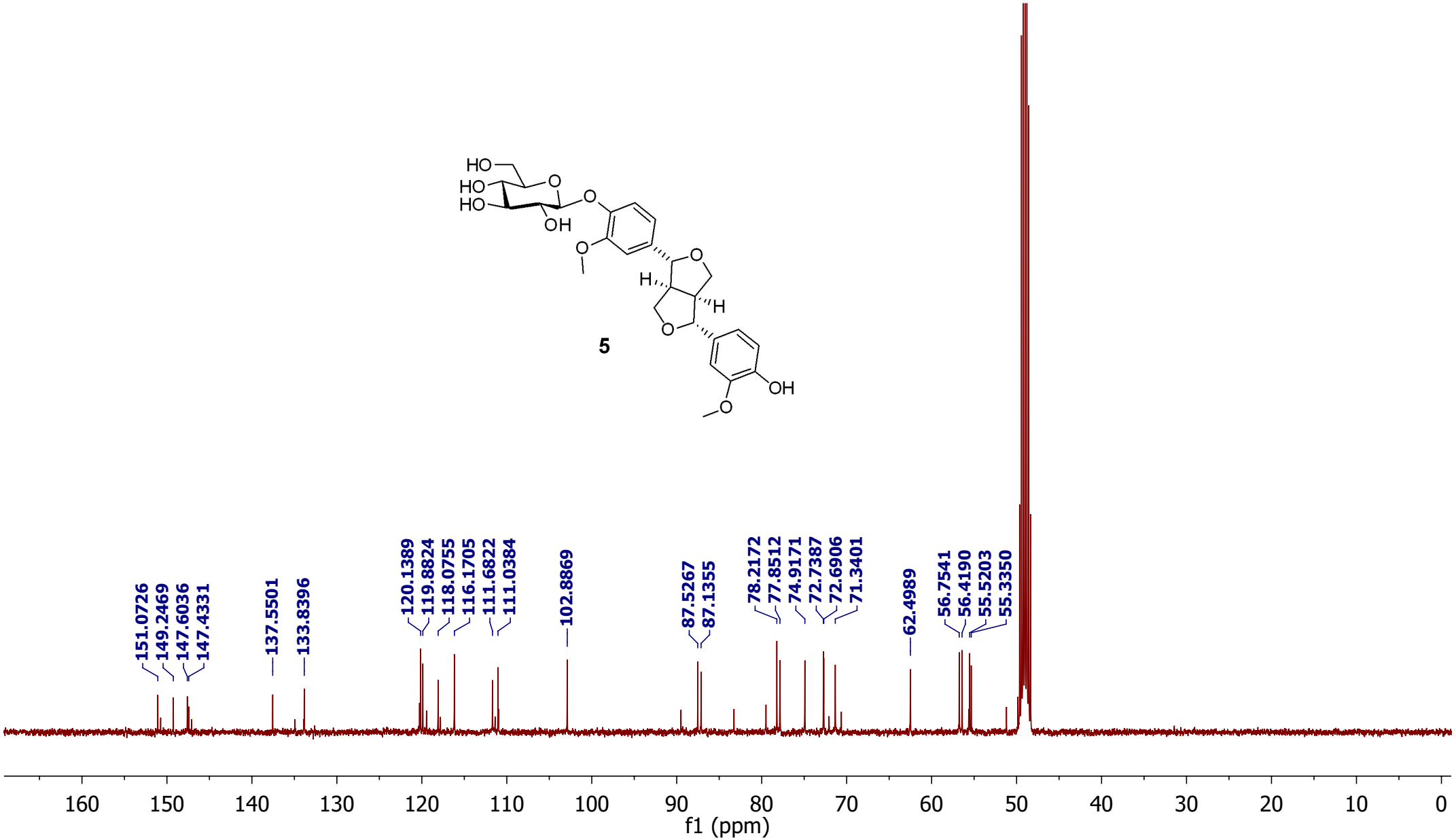


Figure S28. ^{13}CNMR spectrum of compound 5 (CD_3OD, 100 MHz)

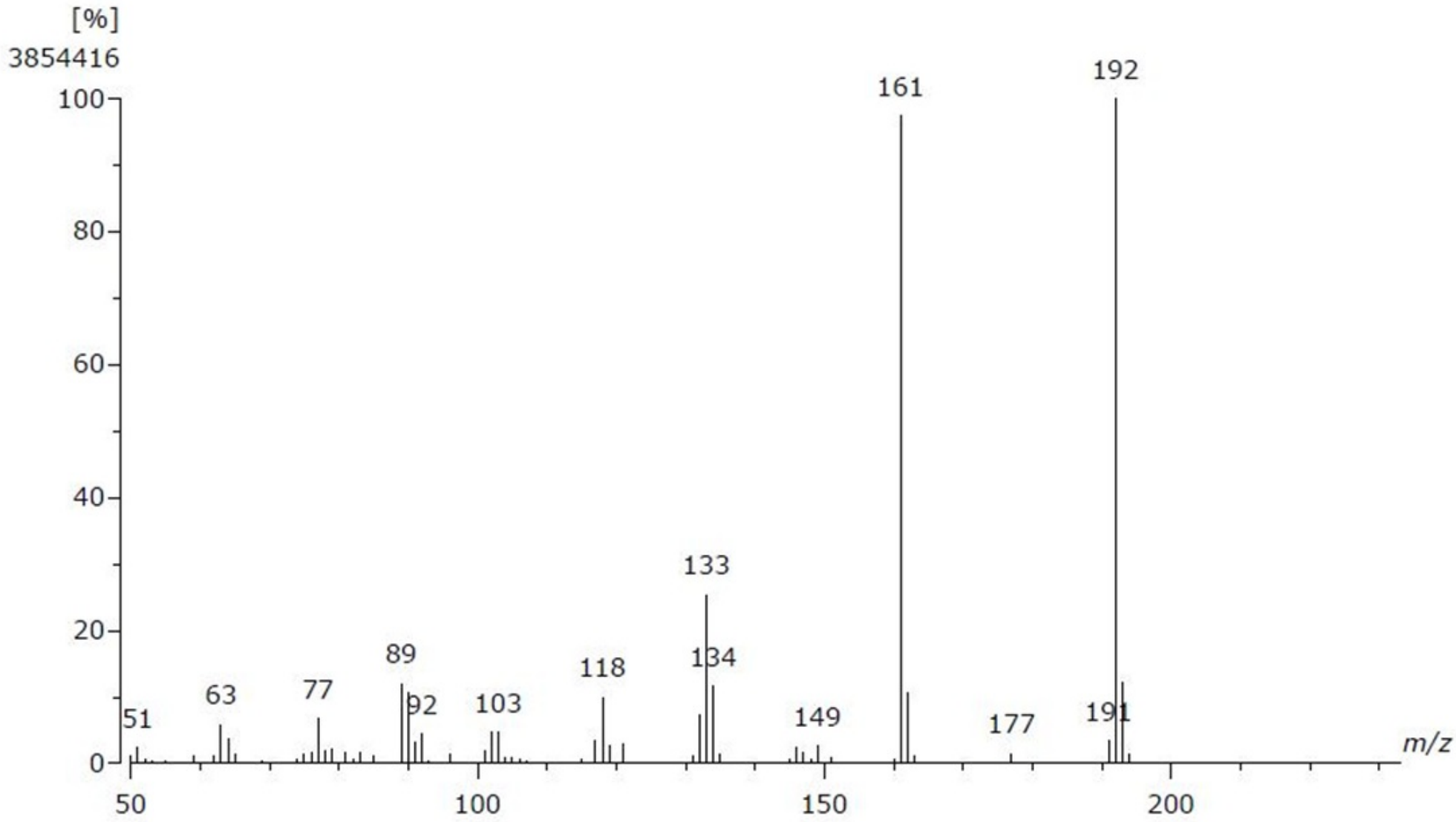


Figure S29. EI-MS spectrum of compound 6

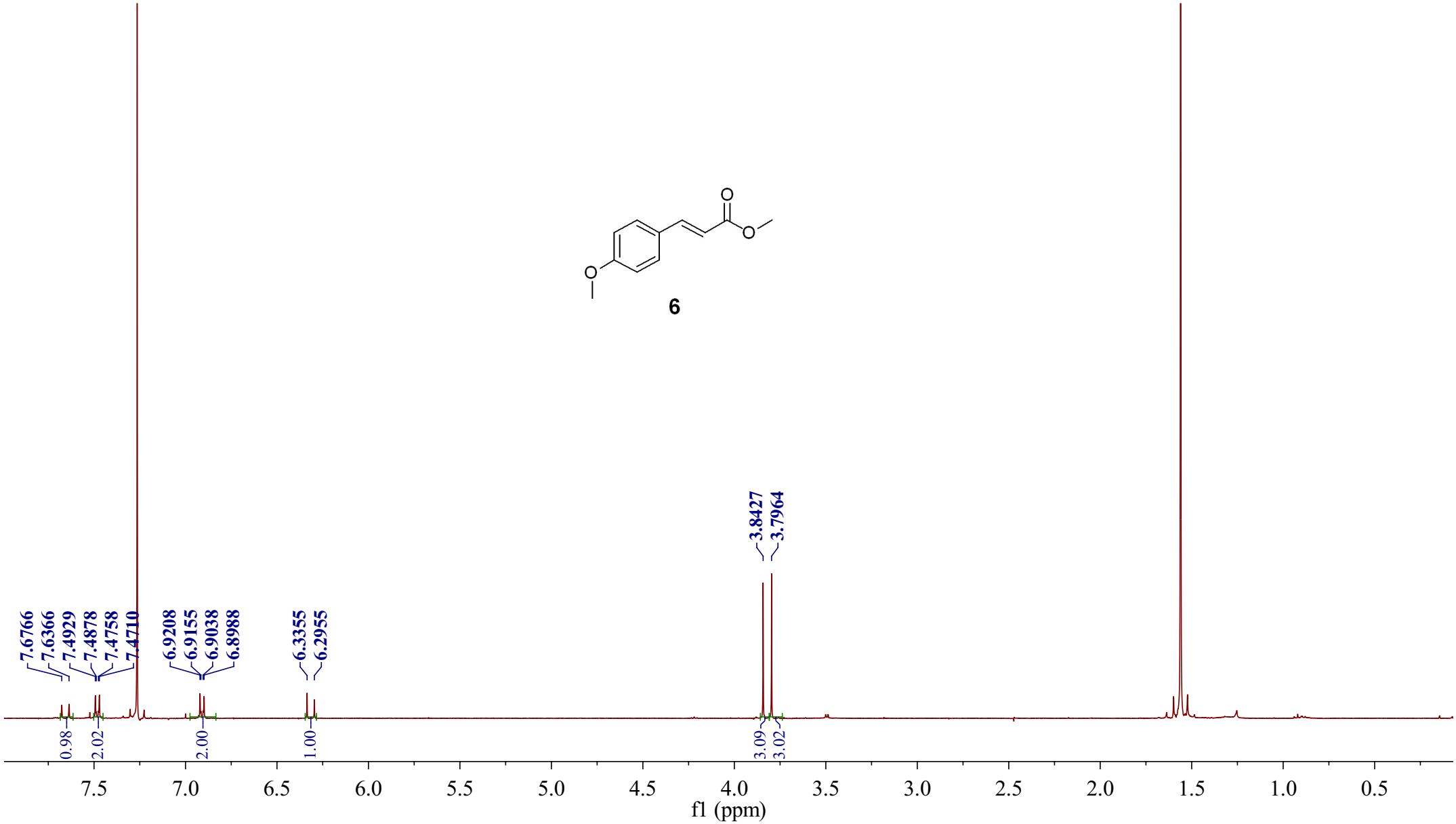


Figure S30. ^{1}HNMR spectrum of compound 6 ($CDCL_3$, 400 MHz)

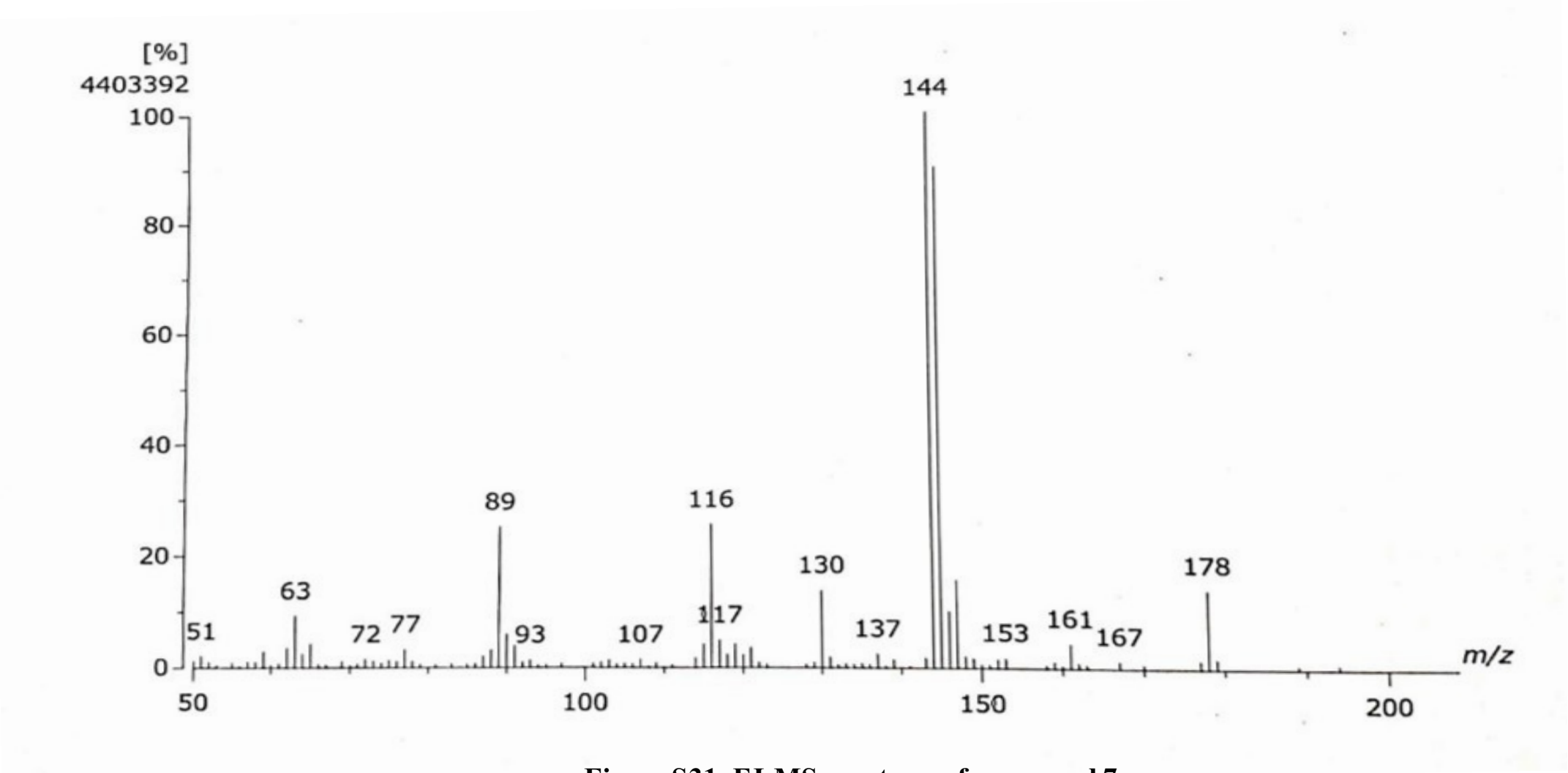


Figure S31. EI-MS spectrum of compound 7

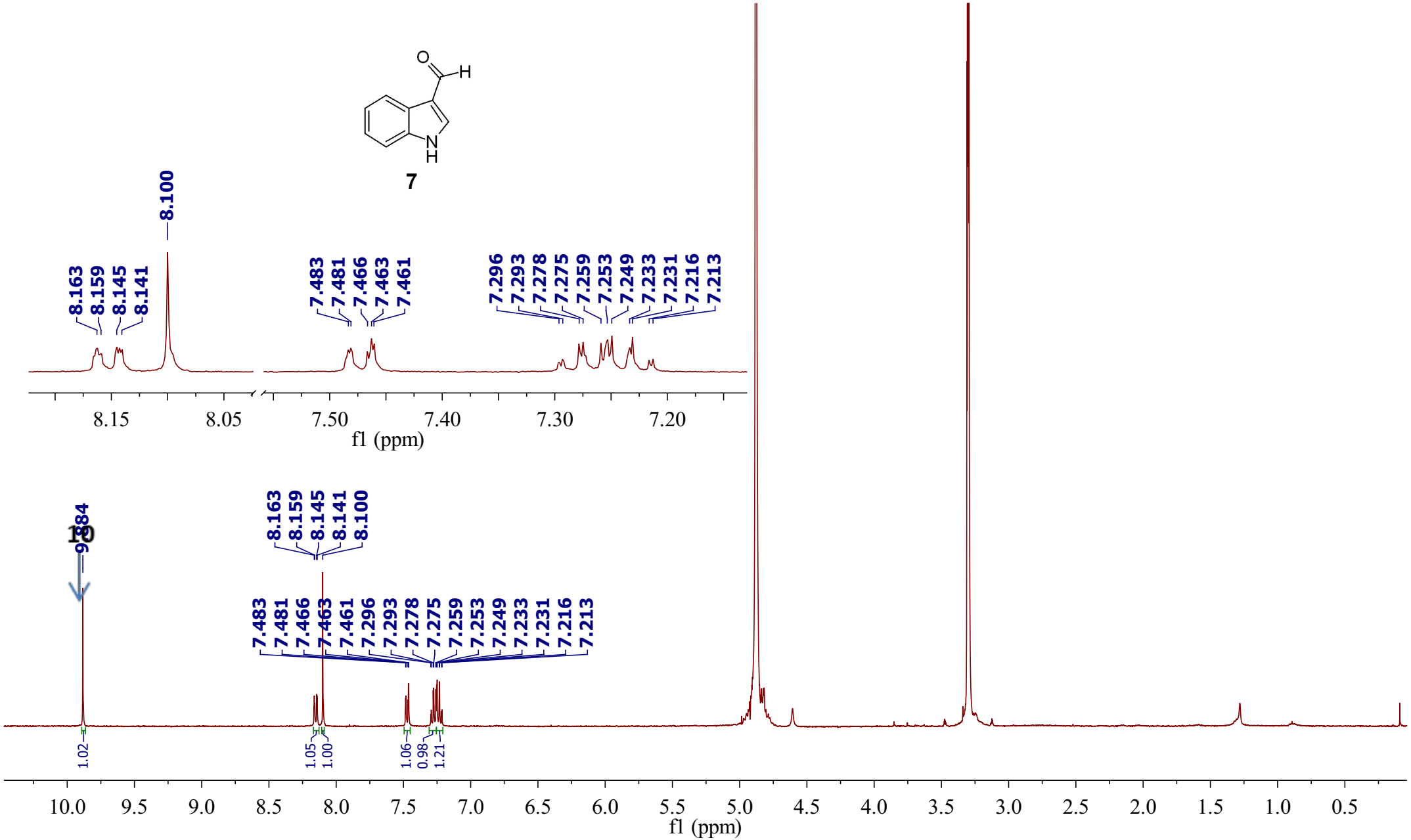


Figure S32. ^{1}HNMR spectrum of compound 7 (CD_3OD, 400 MHz)

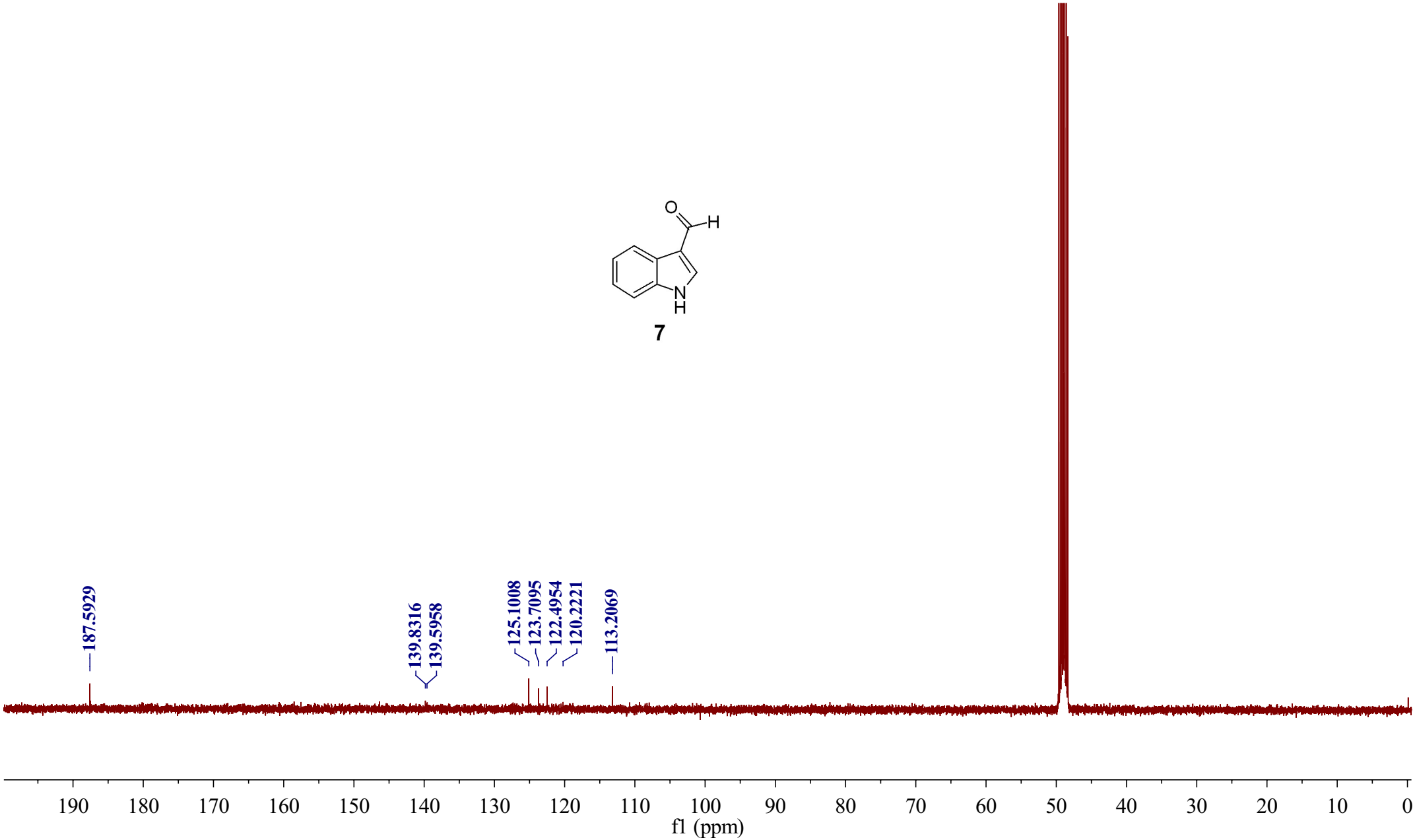


Figure S33. ^{13}CNMR spectrum of compound 7 (CD_3OD, 100 MHz)

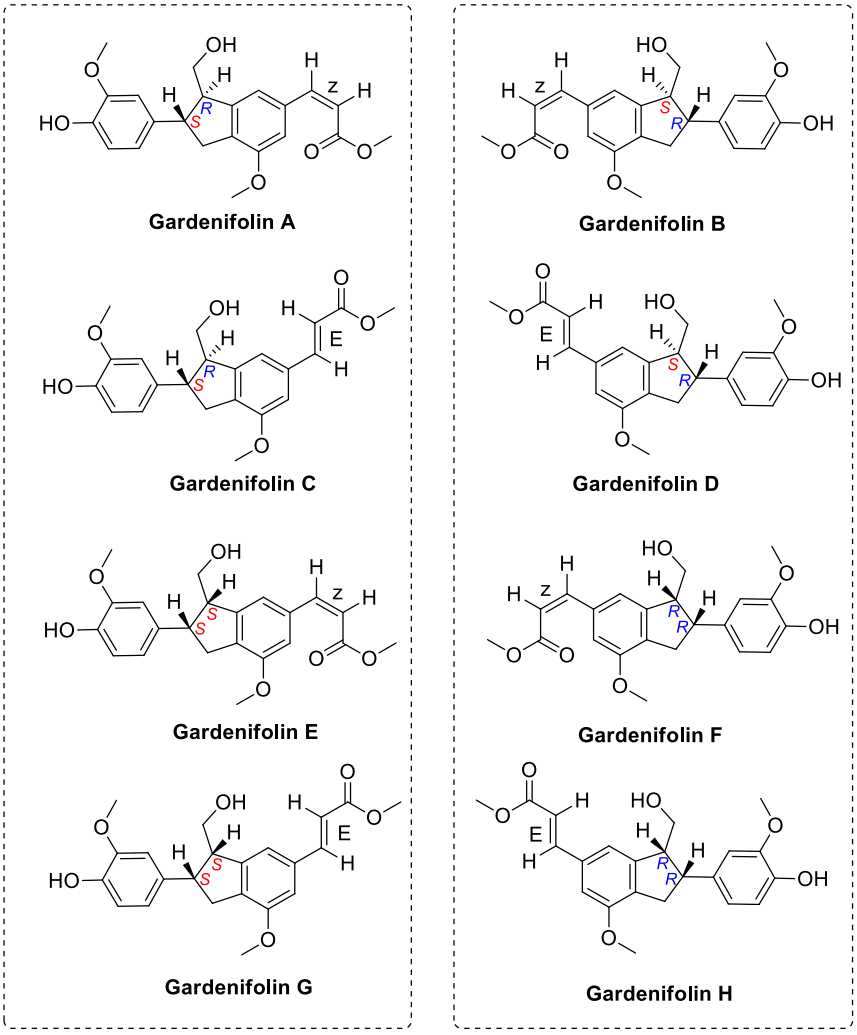


Figure S34. Structures of gardenifolins A, C, E, and G and their respective mirror images gardenifolins B, D, F, and H [S1].

Spectroscopic data of the isolated known compounds **2–7**.

- Pinoresinol (**2**): A colorless needles, ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 6.87 (2H, d, *J* = 2.5 Hz, H-2 and H-2′), 6.86 (2H, d, *J* = 8.1 Hz, H-5 and H-5′), 6.80 (2H, dd, *J* = 8.1, 1.8 Hz, H-6 and H-6′), 4.71 (2H, d, *J* = 4.3 Hz, H-7 and H-7′), 3.08 (2H, m, H-8 and H-8′), 4.22 (2H, dd, *J* = 9.1, 6.9 Hz, H_2-9 and H_2-9′), 3.86 (2H, d, *J* = 3.6, Hz, H_2-9 and H_2-9′), and 3.88 [6H, s, $(O\text{-}CH_3)_2$]. ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 133.0 (2C, C-1 and C-1′), 108.7 (2C, C-2, and C-2′), 146.9 (2S, C-3 and C-3′), 145.4 (2C, C-4 and C-4′), 114.4 (2C, C-5 and C-5′), 119.1 (2C, C-6 and C-6′), 86.0 (2C, C-7 and C-7′), 54.2 (2C, C-8 and C-8′), 71.7 (2C, C-9 and C-9′), and 56.0[2C, $(O\text{-}CH_3)_2$]. EI-MS *m*/*z* 358 $[M]^+$ [S2].

- Epipinoresinol (**3**): A colorless needles, ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 6.95 (1H, d, *J* = 1.6 Hz, H-2), 6.88 (1H, d, *J* = 8.4 Hz, H-5), 6.84 (1H, dd, *J* = 8.4, 1.6 Hz, H-6), 4.43 (1H, d, *J* = 7.2 Hz, H-7), 2.90 (1H, m, H-8), 4.12 (1H, d, *J* = 9.1 Hz, H_2-9), 3.83 (1H, d, *J* = 6.4, Hz, H_2-9), 6.90 (1H, d, *J* = 1.6 Hz, H-2′), 6.88 (1H, d, *J* = 8.4 Hz, H-5′), 6.78 (1H, dd, *J* = 8.2, 1.6 Hz, H-6′), 4.85 (1H, d, *J* = 5.3 Hz, H-7′), 3.32 (1H, m, H-8′), 3.33 (1H, m, H_2-9′), 3.85 (1H, d, *J* = 6.2 Hz, H_2-9′), 3.89 (3H, s, O-CH_3), and 3.91 (3H, s, O-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 133.0 (C-1), 108.5 (C-2), 146.7 (C-3), 145.3 (C-4), 114.2 (C-5), 119.2 (C-6), 87.7 (C-7), 54.4 (C-8), 70.9 (C-9), 130.3 (C-1′), 108.3 (C-2′), 146.4 (C-3′), 144.6 (C-4′), 114.2 (C-5′), 118.4 (C-6′), 82.0 (C-7′), 50.0 (C-8′), 69.6 (C-9′) 55.8 (O-CH_3), and 55.9 (O-CH_3). EI-MS *m*/*z* 358 $[M]^+$ [3].

- Phillyrin (**4**): A yellowish white powder, ^{1}H NMR (DMSO-d_6, 400 MHz): δ_H 6.96 (^{1}H, brs, H-2), 7.05 (1H, d, *J* = 8.5 Hz, H-5), 6.90 (1H, br.d, *J* = 8.5, Hz, H-6), 4.37 (1H, d, *J* = 6.6 Hz, H-7), 2.83 (1H, dd, *J* = 14.7, 6.7 Hz, H-8), 4.09 (1H, d, *J* = 9.1 Hz, H_2-9), 3.79 (1H, m, H_2-9), 6.92 (1H, brs, H-2′), 6.87 (1H, d, *J* = 8.5 Hz, H-5′), 6.90 (1H, brd, *J* = 8.5 Hz, H-6′), 4.79 (1H, d, *J* = 5.7 Hz, H-7′), 3.30 (1H, m, H-8′), 3.35 (1H, m, H_2-9′), 3.79 (1H, m, H_2-9′), 4.88 (1H, d, *J* = 6.1 Hz, H-1″), 3.25 (2H, brs, H-2″, H-3″), 3.05 (1H, brs, H-4″), 3.06 (1H, t, *J* = 8.5 Hz, H-5″), 3.45 (1H, d, *J* = 5.9 Hz, H_2-6″), 3.65 (1H, m, H_2-6″), 3.74 (3H, s, O-CH_3), 3.75 (3H, s, O-CH_3), and 3.76 (3H, s, O-CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C 135.9 (C-1), 111.0 (C-2), 149.6 (C-3), 146.6 (C-4), 115.8 (C-5), 118.8 (C-6), 87.3 (C-7), 54.6 (C-8), 70.9 (C-9), 131.8 (C-1′), 110.0 (C-2′), 149.1 (C-3′), 148.3 (C-4′), 112.1 (C-5′), 118.2 (C-6′), 81.8 (C-7′), 49.8 (C-8′), 69.5 (C-9′), 100.7 (C-1″), 73.8 (C-2″), 77.4 (C-3″), 70.2

(C-4″), 77.6 (C-5″), 61.2 (C-6″), 56.0 [2C, (O-CH_3)$_2$], and 56.2 (O-CH_3). Positive FABMS *m*/*z* 557 $[M+Na]^+$ [4].

- Pinoresinol-4-*O*-β-D-glucoside (**5**): A yellowish residue, ^{1}H NMR (CD_3OD, 400 MHz): δ_H 7.02 (1H, d, *J* = 1.8 Hz, H-2), 7.13 (1H, d, *J* = 8.4 Hz, H-5), 6.91 (1H, dd, *J* = 8.4, 1.6 Hz, H-6), 4.74 (1H, d, *J* = 4.4 Hz, H-7), 3.19 (1H, m, H-8), 3.84 (1H, H_2-9), 4.22 (1H, dd, *J* = 9.0, 6.7 Hz, H_2-9), 6.94 (1H, d, *J* = 1.6 Hz, H-2′), 6.75 (1H, d, *J* = 8.5 Hz, H-5′), 6.79 (1H, dd, *J* = 8.5, 1.6 Hz, H-6′), 4.69 (1H, d, *J* = 4.3 Hz, H-7′), 3.11 (1H, m, H-8′), 3.84 (1H, m, H_2-9′), 4.22 (1H, dd, *J* = 9.0, 6.7 Hz H_2-9′), 4.88 (1H, d, *J* = 7.4 Hz, H-1″), 3.47 (1H, m, H-2″) 3.46(1H, m, H-3″), 3.39 (2H, m, H-4″, H-5″), 3.68 (1H, dd, *J* = 12.0, 5.3 Hz, H_2-6″), 3.85 (1H, m, H_2-6″), 3.86 (3H, s, O-CH_3), and 3.84 (3H, s, O-CH_3). ^{13}C NMR (CD_3OD, 100 MHz): δ_C 137.5 (C-1), 111.6 (C-2), 147.6 (C-3), 151.0 (C-4), 118.0 (C-5), 119.8 (C-6), 87.1 (C-7), 55.5 (C-8), 72.6 (C-9), 133.8 (C-1′), 111.0 (C-2′), 147.4 (C-3′), 149.2 (C-4′), 116.1 (C-5′), 120.1 (C-6′), 87.5 (C-7′), 55.3 (C-8′), 72.7 (C-9′), 102.8 (C-1″), 74.9 (C-2″), 77.8 (C-3″), 71.3 (C-4″), 78.2 (C-5″), 62.4 (C-6″), 56.4 (O-CH_3), and 56.7 (O-CH_3). Positive FABMS *m*/*z* 543 $[M+Na]^+$ [5].

- *p*-Methoxy-*trans*-methyl cinnamate (**6**): A white powder, ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 7.48 (2H, d, *J* = 8.8 Hz, H-2/H-6), 6.91 (2H, d, *J* = 8.8 Hz, H-3/H-5), 7.65 (1H, d, *J* = 16.0 Hz, H-7), 6.31(1H, d, *J* = 16.0 Hz, H-8), 3.49 (3H, s, O-CH_3), and 3.84 (3H, s, O-CH_3). EI-MS *m*/*z* 192 $[M]^+$ [6].

- 1H-indole-3-carboxaldehyde (**7**): A white powder, ^{1}H NMR (DMSO-d_6, 400 MHz): δ_H 8.10 (1H, s, H-2), 8.15 (1H, brd, J = 7.2 Hz, H-4), 7.28 (1H, ddd, J = 8.8, 7.2, 1.2 Hz, H-5), 7.23 (1H, ddd, J = 8.0, 7.2, 1.2 Hz, H-6), 7.47 (1H, brd, J = 7.2 Hz, H-7), and 9.88 (1H, H-10). EI-MS m/z 145 $[M]^+$ and positive FABMS m/z 146 $[M+H]^+$ [7,8].

code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
Compound 1		10	4	82.67538	30	0	30	0.136	0	0.136	0.026167	20.78954	120
		1	3	74.77739	30	0	30	0.198	0	0.198	0.026167	30.26713	120
		0.1	2	60.89222	30	0	30	0.307	0	0.307	0.026167	46.92934	120
		0.01	1	24.58695	30	0	30	0.592	0	0.592	0.026167	90.49566	120
EC				0	30	0	30	0.785	0	0.785	0.026167	120	120

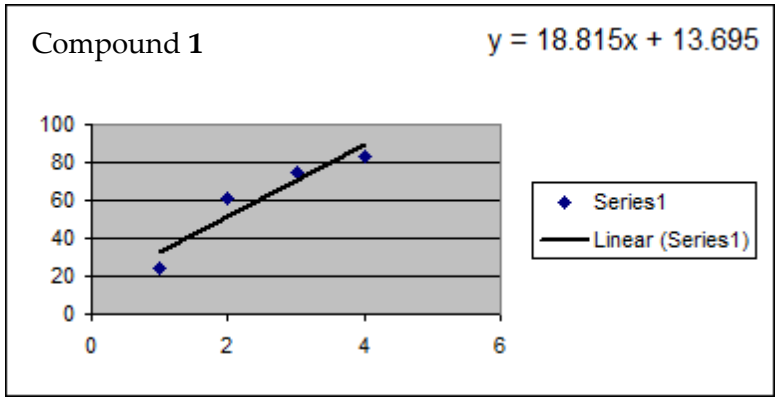
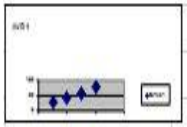


Figure S35. Dose-response curve of the anti-AChE assay of compound **1**.

code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
Compound 2		10	4	75.54171	30	0	30	0.192	0	0.192	0.026167	29.34994	120
		1	3	60.00051	30	0	30	0.314	0	0.314	0.026167	47.99939	120
		0.1	2	40.76509	30	0	30	0.465	0	0.465	0.026167	71.0819	120
		0.01	1	25.9882	30	0	30	0.581	0	0.581	0.026167	88.81416	120
EC				0	30	0	30	0.785	0	0.785	0.026167	120	120

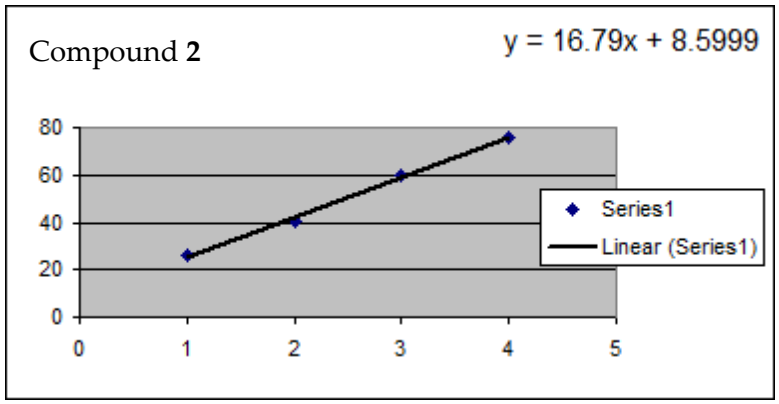
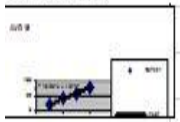


Figure S36. Dose-response curve of the anti-AChE assay of compound **2**.

code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
Compound 3		10	4	81.78367	30	0	30	0.143	0	0.143	0.026167	21.85959	120
		1	3	60.38267	30	0	30	0.311	0	0.311	0.026167	47.5408	120
		0.1	2	43.5676	30	0	30	0.443	0	0.443	0.026167	67.71888	120
		0.01	1	23.18569	30	0	30	0.603	0	0.603	0.026167	92.17717	120
EC				0	30	0	30	0.785	0	0.785	0.026167	120	120

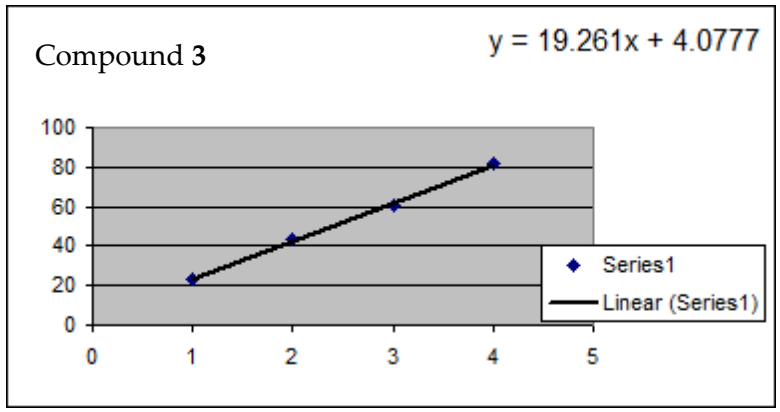


Figure S37. Dose-response curve of the anti-AChE assay of compound **3**.

code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
Compound 4		10	4	72.86659	30	0	30	0.213	0	0.213	0.026167	32.56009	120
		1	3	63.69473	30	0	30	0.285	0	0.285	0.026167	43.56632	120
		0.1	2	40.6377	30	0	30	0.466	0	0.466	0.026167	71.23476	120
		0.01	1	26.37036	30	0	30	0.578	0	0.578	0.026167	88.35556	120
EC				0	30	0	30	0.785	0	0.785	0.026167	120	120

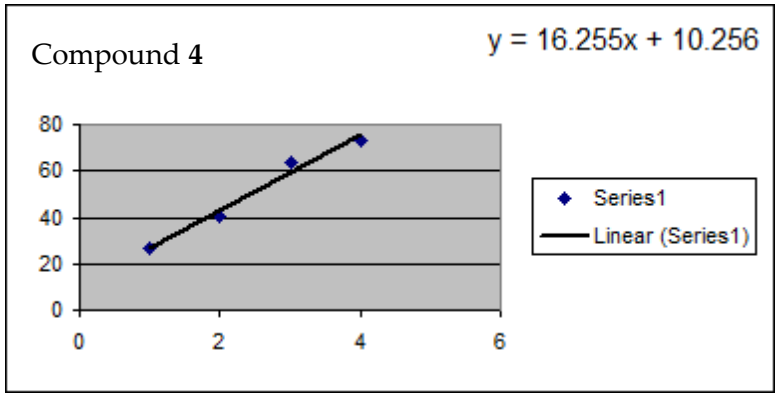


Figure S38. Dose-response curve of the anti-AChE assay of compound **4**.

code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
Compound 5		10	4	87.77086	30	0	30	0.096	0	0.096	0.026167	14.67497	120
		1	3	78.85377	30	0	30	0.166	0	0.166	0.026167	25.37547	120
		0.1	2	59.87312	30	0	30	0.315	0	0.315	0.026167	48.15225	120
		0.01	1	28.15378	30	0	30	0.564	0	0.564	0.026167	86.21546	120
EC				0	30	0	30	0.785	0	0.785	0.026167	120	120

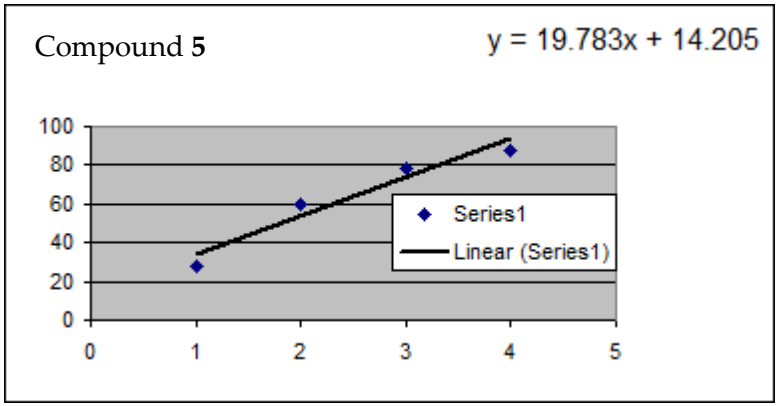


Figure S39. Dose-response curve of the anti-AChE assay of compound **5**.

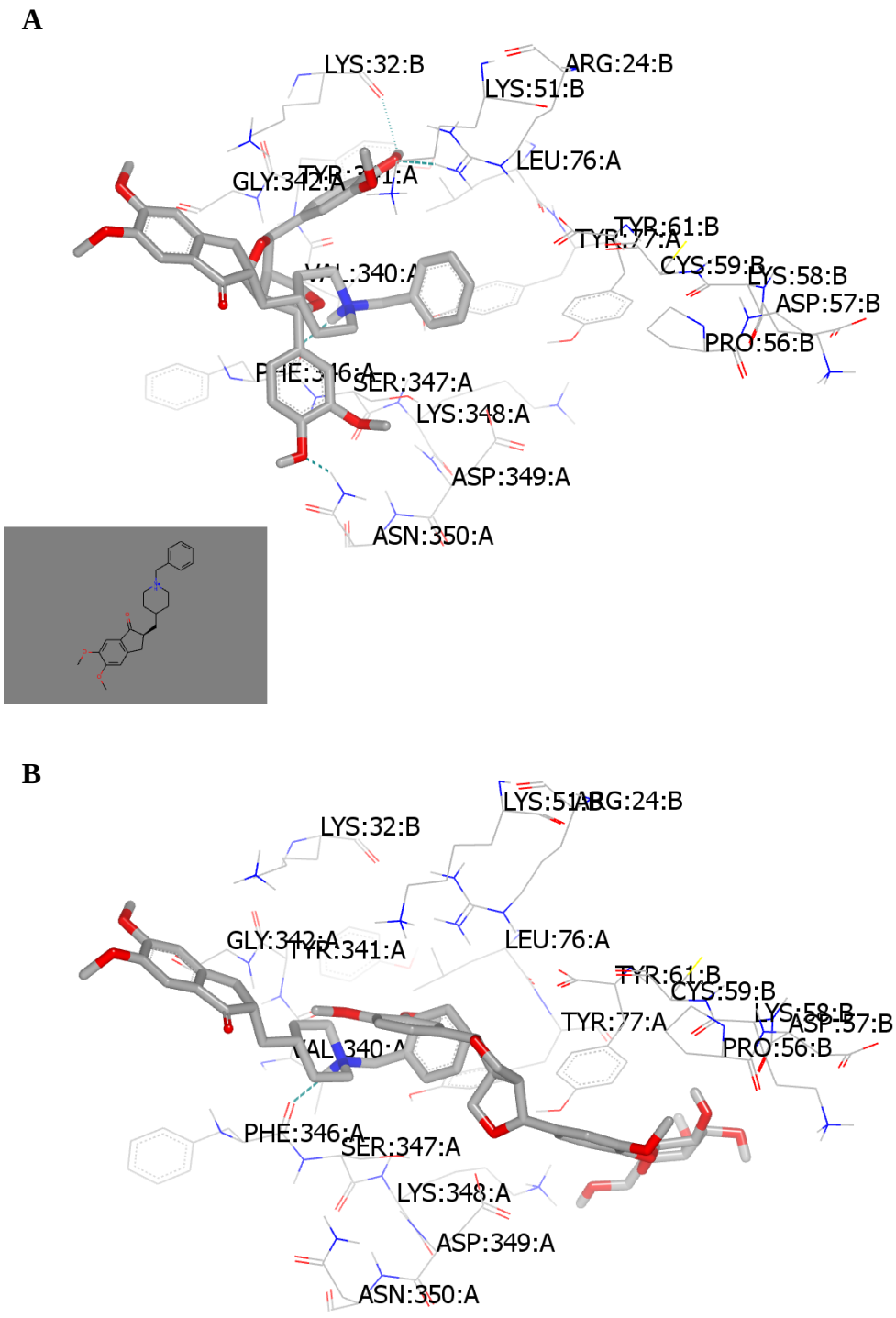


Figure S40: The overlay of donepezil with compounds **3** (A) and **4** (B).

Table S1. ^{1}H and ^{13}C NMR spectral data of compound **1** (CD_3OD, 500 and 125 MHz, respectively) and pinoresinol (**2**) ($CDCl_3$, 400 and 100, respectively).

Position	δ_H in ppm, multiplicity (*J* in Hz)		δ_C in ppm	
	1	**2**	**1**	**2**
1			136.1	133.0
2	6.95, d (*J* = 1.5)	6.87 (d, *J* = 2.5)	110.9	108.7
3			149.1	146.9
4			147.3	145.4
5	6.77, d (*J* = 8.0)	6.86 (d, *J* = 8.1)	116.0	114.4
6	6.92, dd (*J* = 8.0, 2.0)	6.80 (dd, *J* = 8.1, 1.8)	119.3	119.1
7	4.74[a], d (*J* = 4.5)	4.71 (d, *J* = 4.3)	87.6	86.0
8	3.17[b], m	3.08, m	55.6	54.2
9	4.23[c], ddd (*J* = 11.5, 7.0, 2.0) 3.85* (ov)[d]	4.22 (dd, *J* = 9.1, 6.9) 3.86 (d, *J* = 3.6)	72.7	71.7
1′	-		133.7	133.0
2′	6.92, brs.	6.87 (d, *J* = 2.5)	112.1	108.7
3′			145.5	146.9
4′			149.0	145.4
5′		6.86 (d, *J* = 8.1)	129.9	114.4
6′	6.90, brs.	6.80 (dd, *J* = 8.1, 1.8)	116.0	119.1
7′	4.72[a], d (*J* = 4.5)	4.71 (d, *J* = 4.3)	87.4	86.0
8′	3.15[b], m	3.08, m	55.3	54.2
9′	4.25[c], ddd (*J* = 11.5, 7.0, 2.0) 3.84* (ov)[d]	4.22 (dd, *J* = 9.1, 6.9) 3.84 (d, *J* = 3.6)	72.5	71.7
1″	-		138.1	
2″	7.03, d (*J* = 1.6)		111.1	
3″	-		150.9	
4″	-		147.6	
5″	7.14, d (*J* = 8.5)		118.0	
6″	6.82, dd (*J* = 8.5, 1.5)		120.0	
7″	5.58, d (*J* = 5.5)		88.6	
8″	3.48, m		55.6	
9″	3.78, dd (*J* = 11.0, 7.0) 3.85* (ov)[d]		64.9	
1‴	4.89, d (*J* = 7.5)		102.7	
2‴	3.47, dd (*J* = 10, 7.5)		74.9	
3‴	3.39, t (*J* = 10)		78.2	
4‴	3.39, m		71.3	
5‴	3.45, m		77.9	
6‴	3.68, ddd (*J* = 12.0, 5.5,1.5) 3.87* (ov)		62.4	
3′-OCH_3	3.85, s	3.88, s	56.4	56.0
3″-OCH_3	3.83, s		56.7	
3-OCH_3	3.88, s	3.88, s	56.8	56.0

*Multiplicity was not calculated because signals partially overlapped with methoxy signals.
[a,b,c] exchangeable.
(ov)[d] mean overlapped

Table S2. Chemical shift differences of H_2-9 and H_2-9′ (ΔδH-9 and ΔδH-9′) of compounds **1**–**5**.

Compound	$\Delta\delta_{H\text{-}9}$	$\Delta\delta_{H\text{-}9'}$	Deuterated solvent
1	0.38	0.41	CD_3OD
2	0.36	0.38	$CDCl_3$
3	0.29	0.52	$CDCl_3$
4	0.30	0.44	DMSO-d_6
5	0.38	0.38	CD_3OD

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