Supporting Information

New sesquineolignan glycoside isomers from the aerial parts of *Leonurus japonicus* and their absolute configurations

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[1] Both authors contributed equally to this work.

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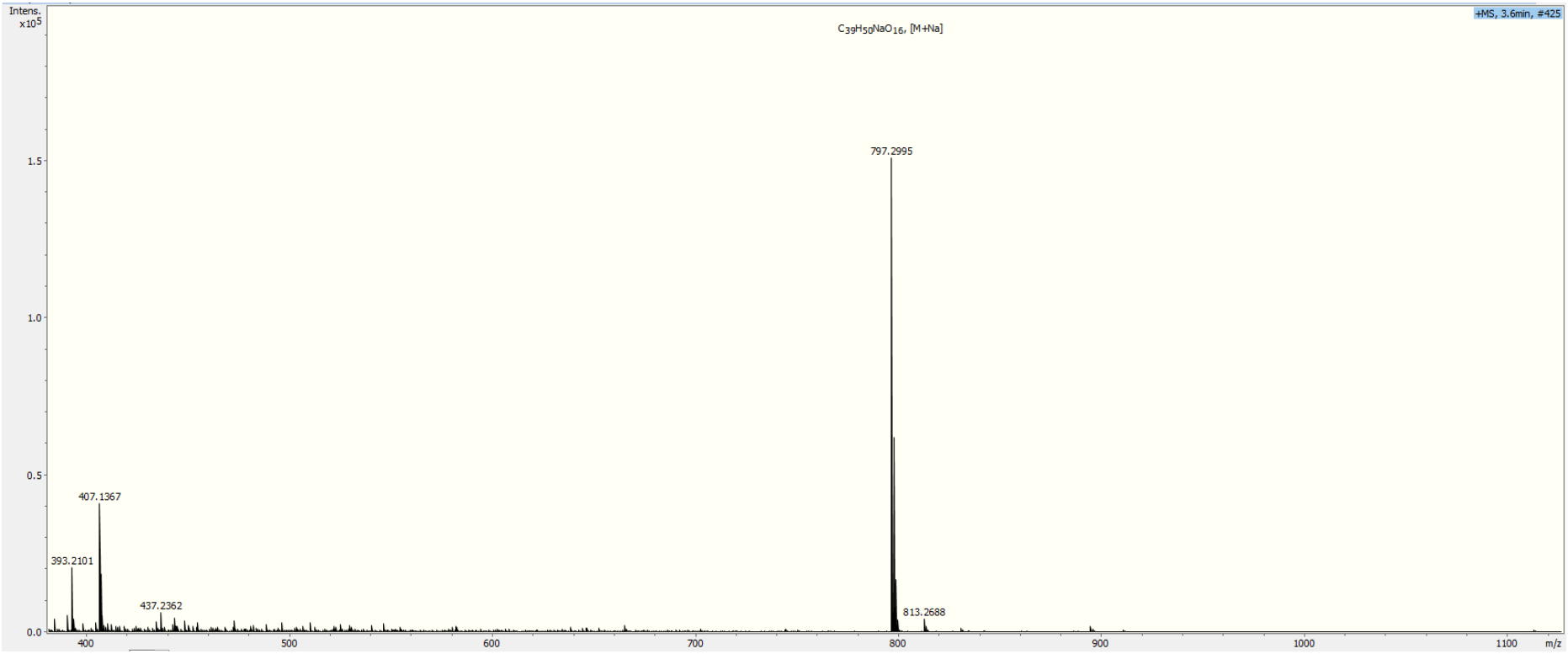
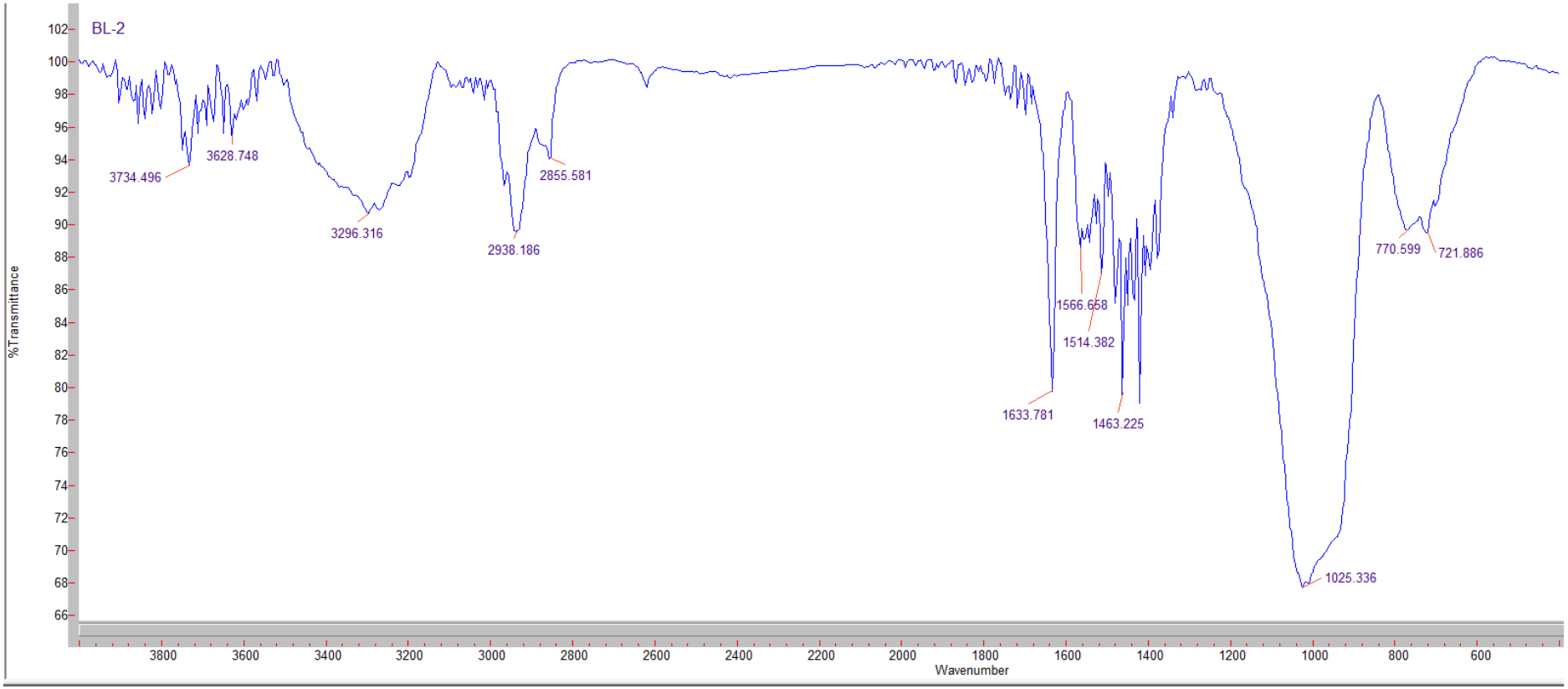


Figure S1. HR-ESI-MS spectrum of compound **1**.



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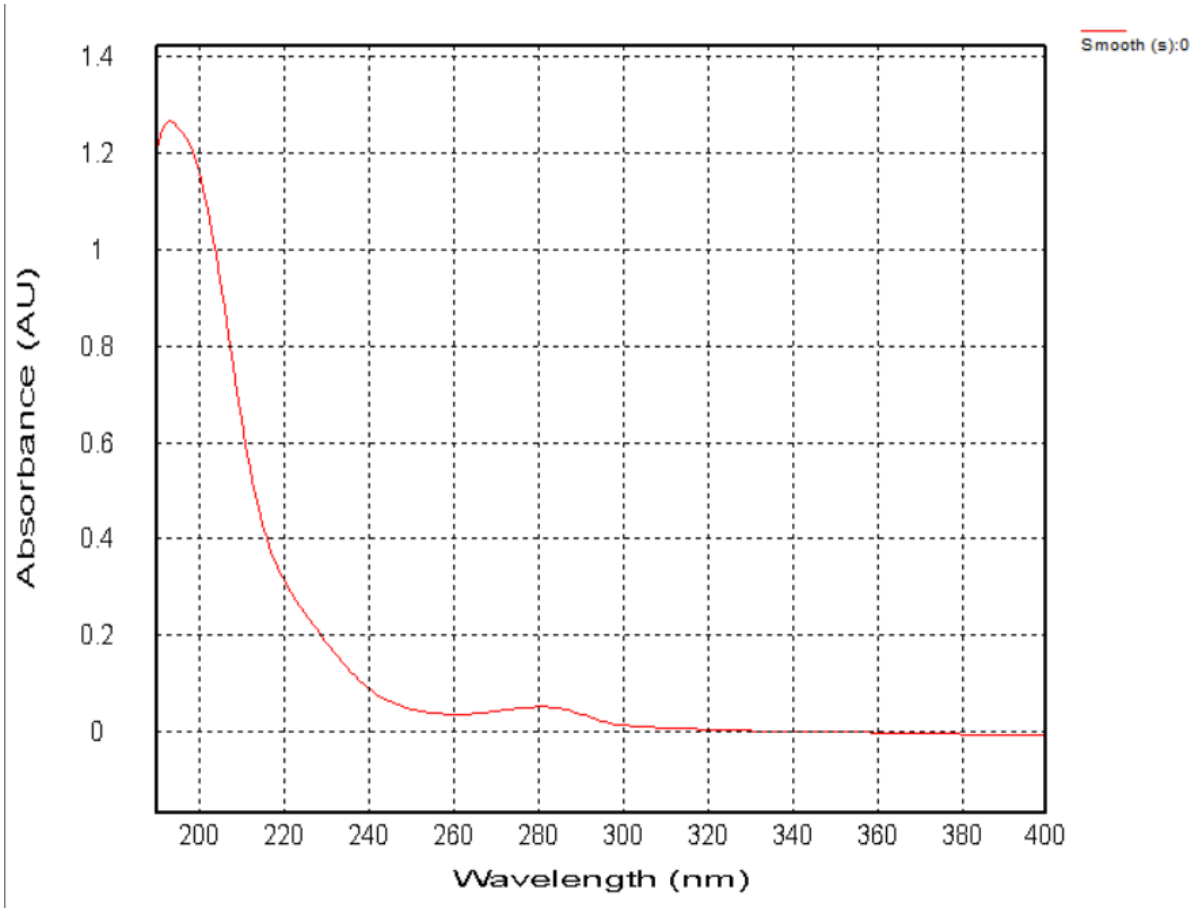
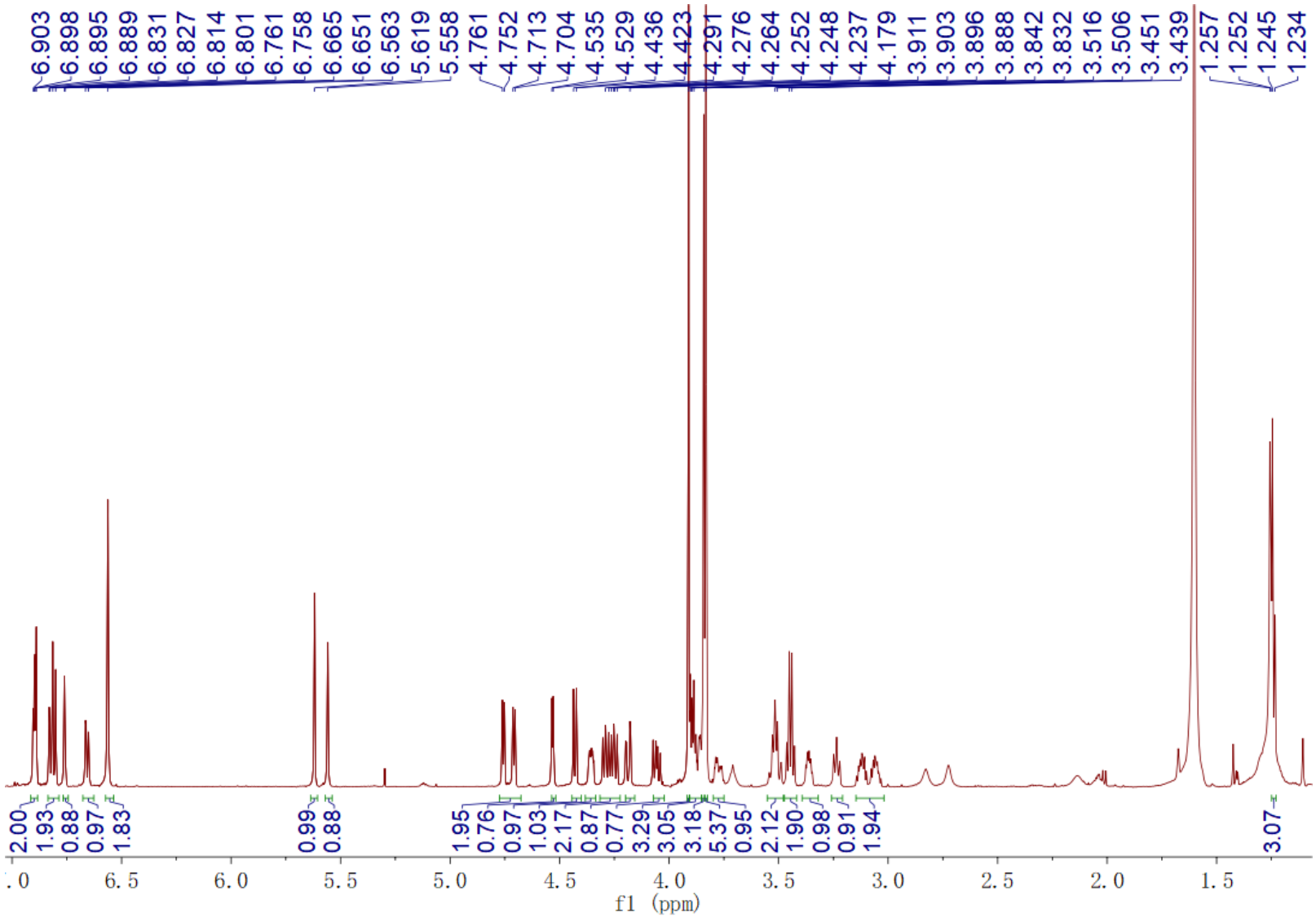


Figure S3. UV spectrum of compound **1**.



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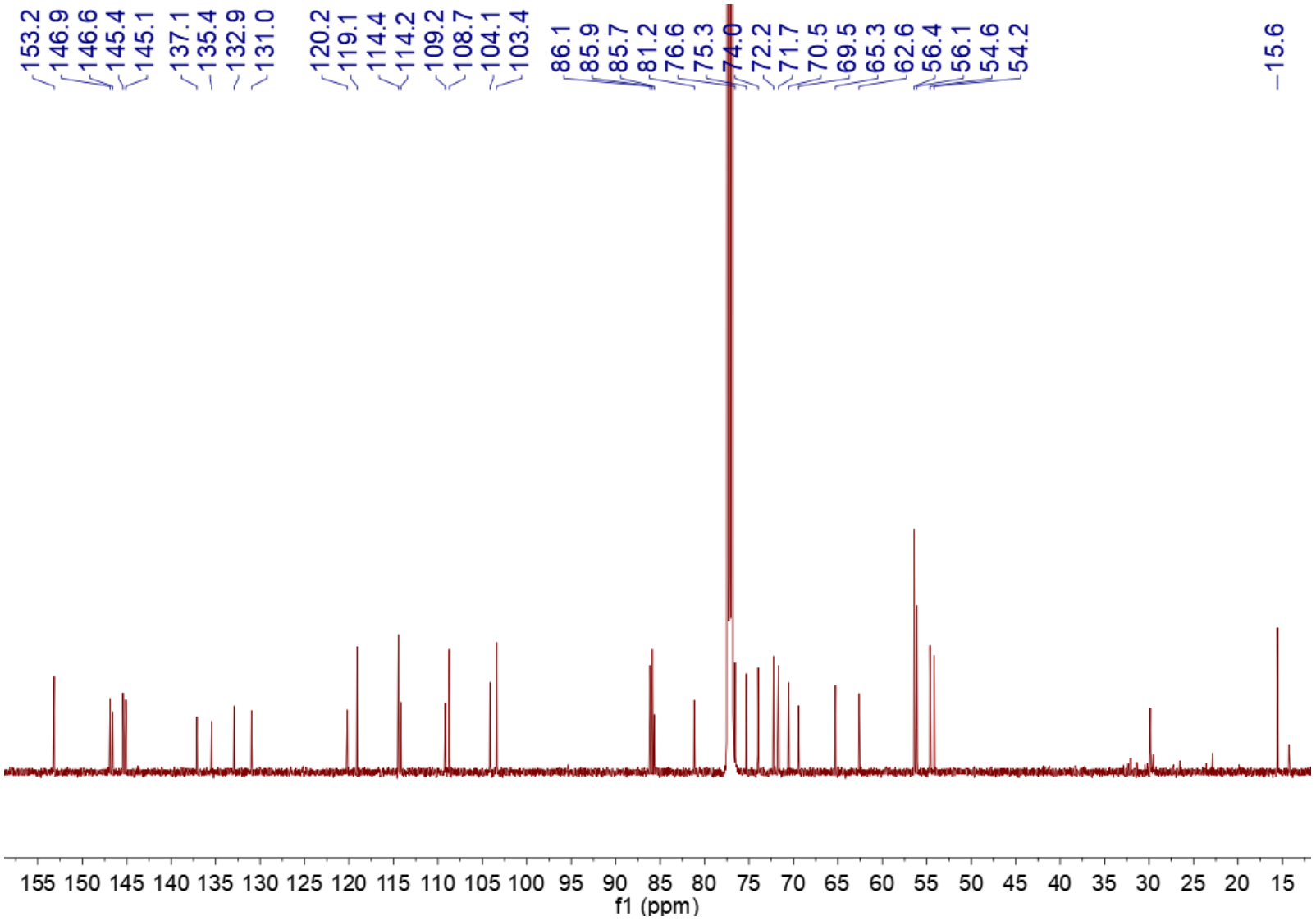

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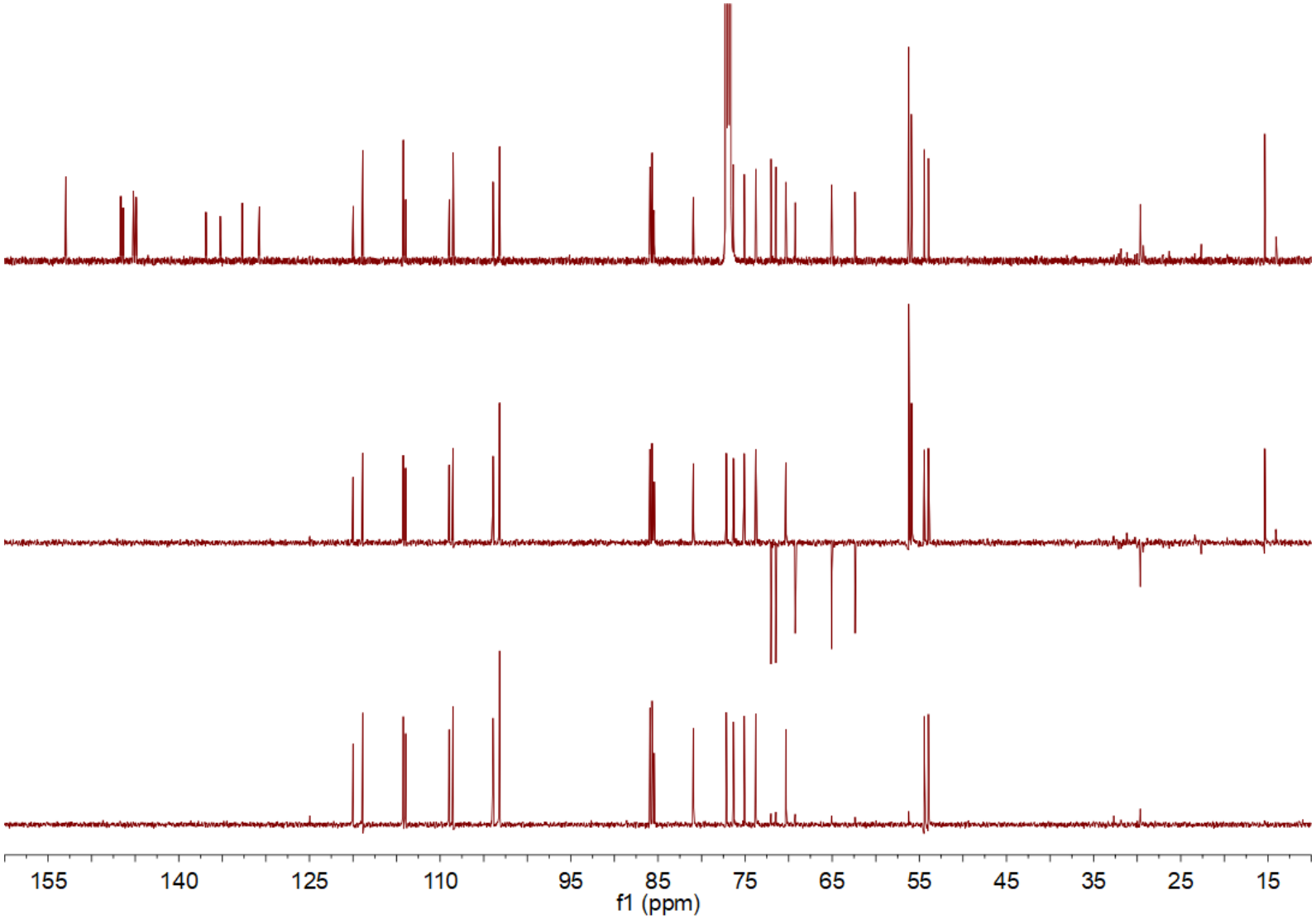


Figure S6. DEPT spectrum of compound **1** ($CDCl_3$).

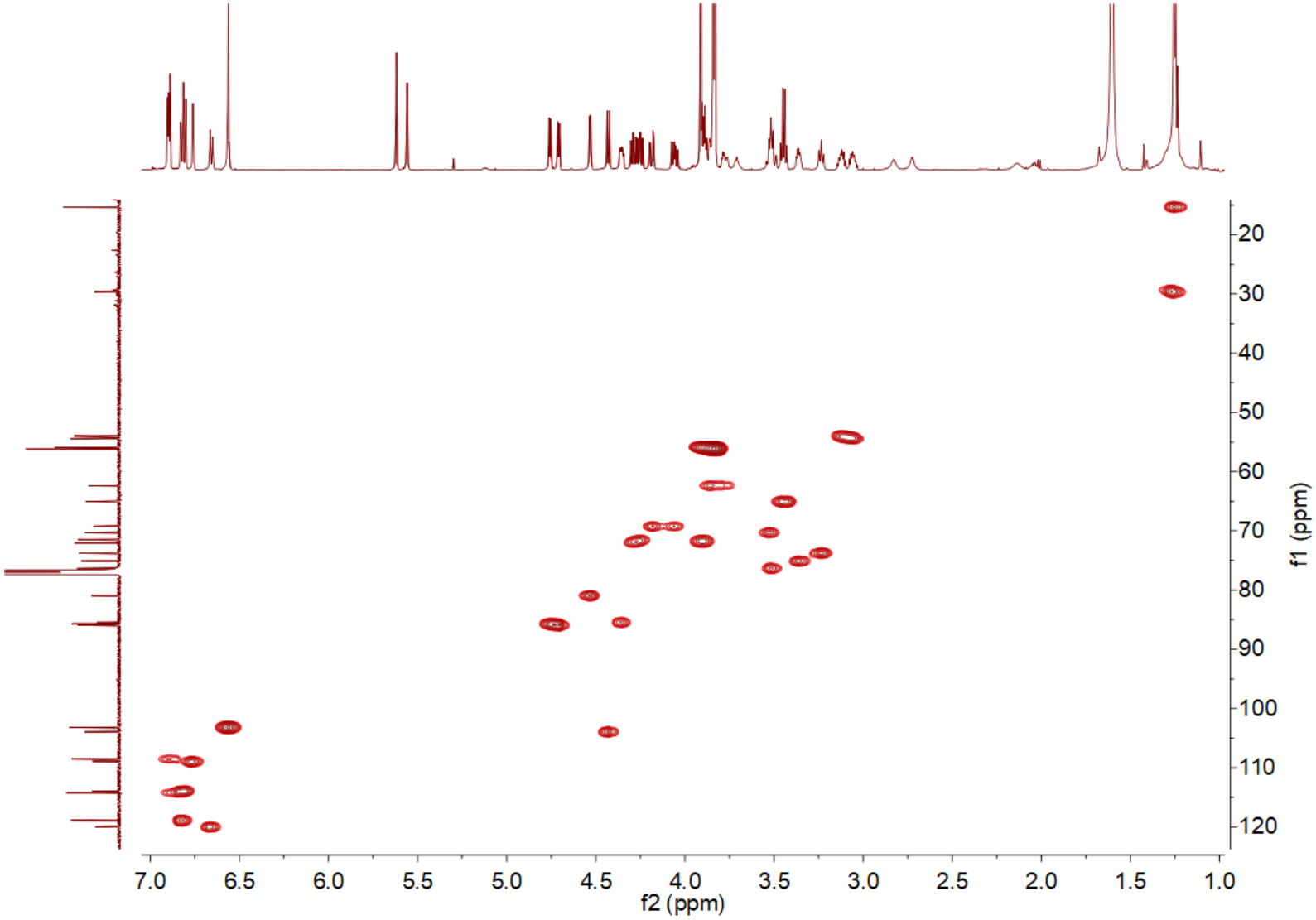


Figure S7. HSQC spectrum of compound **1** ($CDCl_3$).

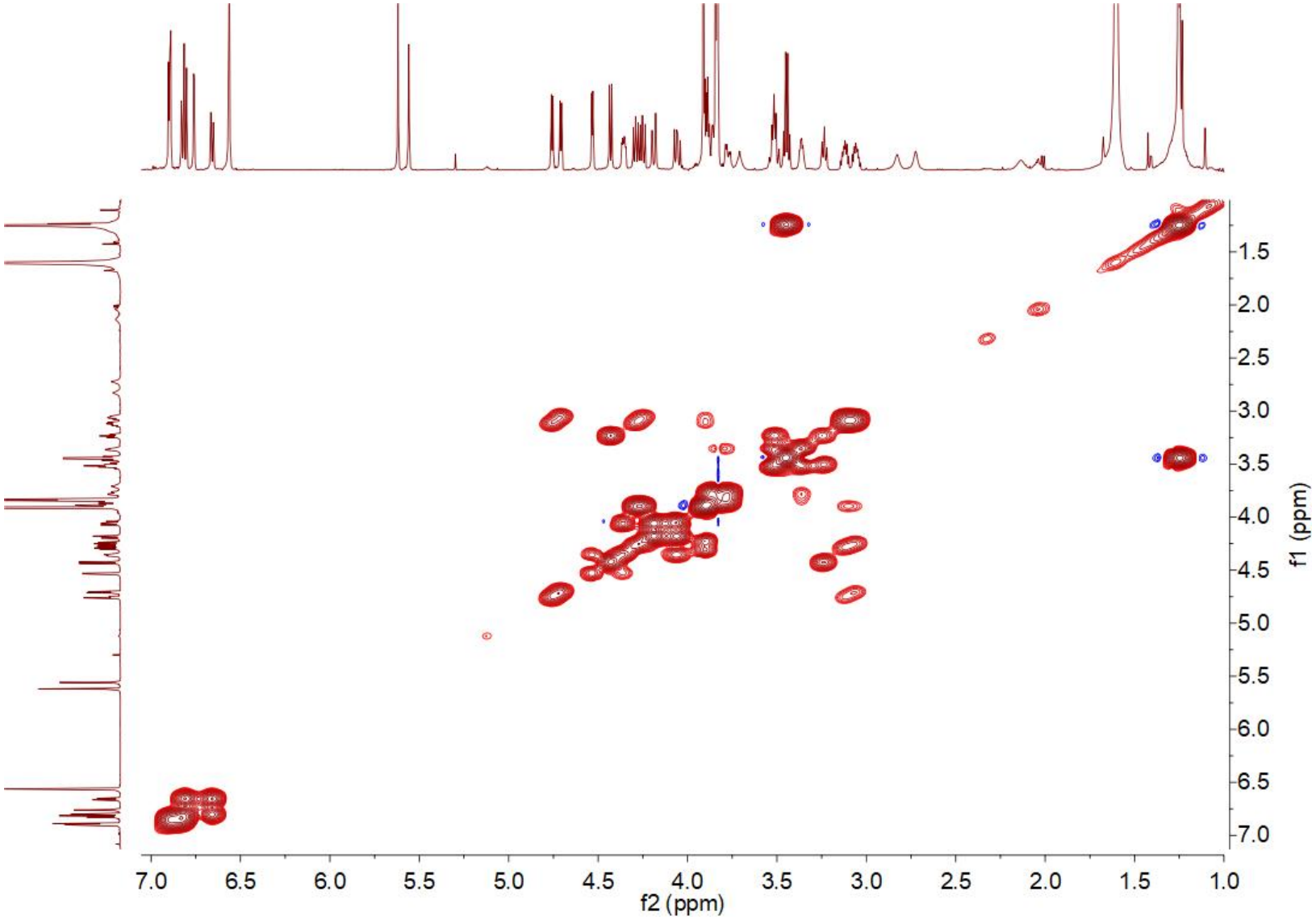


Figure S8. ^{1}H-^{1}H COSY spectrum of compound **1** ($CDCl_3$).

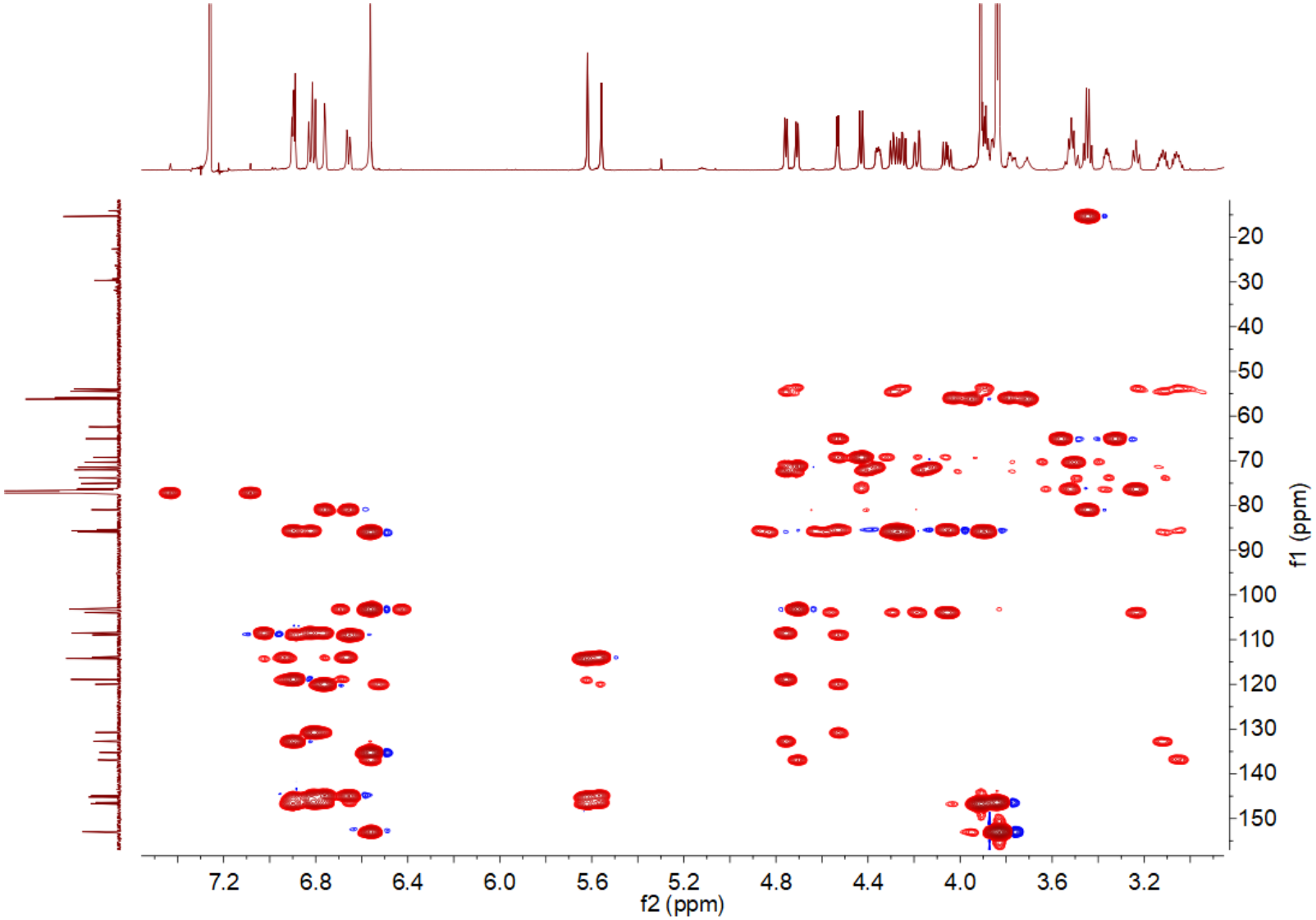


Figure S9. HMBC spectrum of compound **1** ($CDCl_3$).

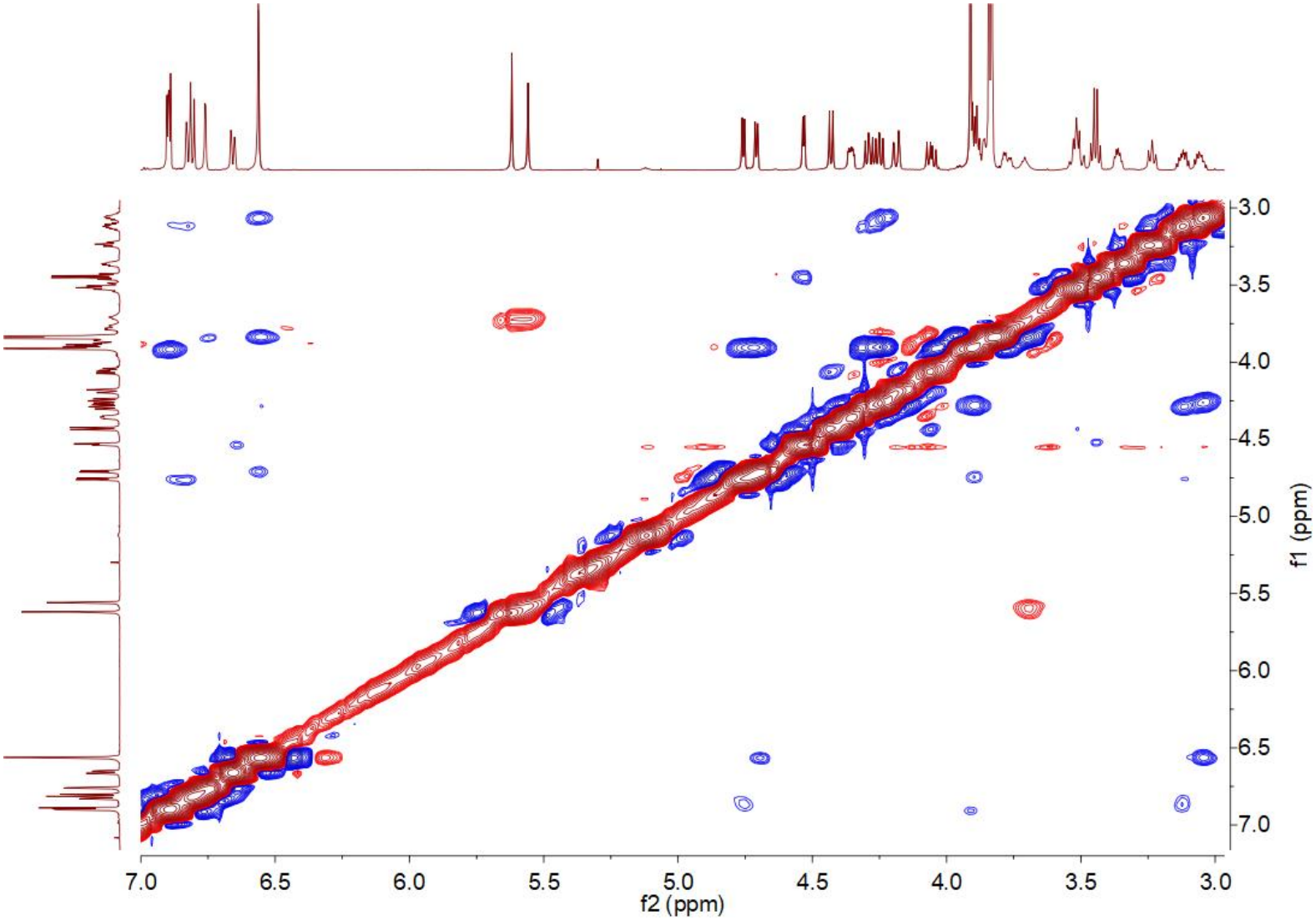


Figure S10. NOESY spectrum of compound **1** ($CDCl_3$).

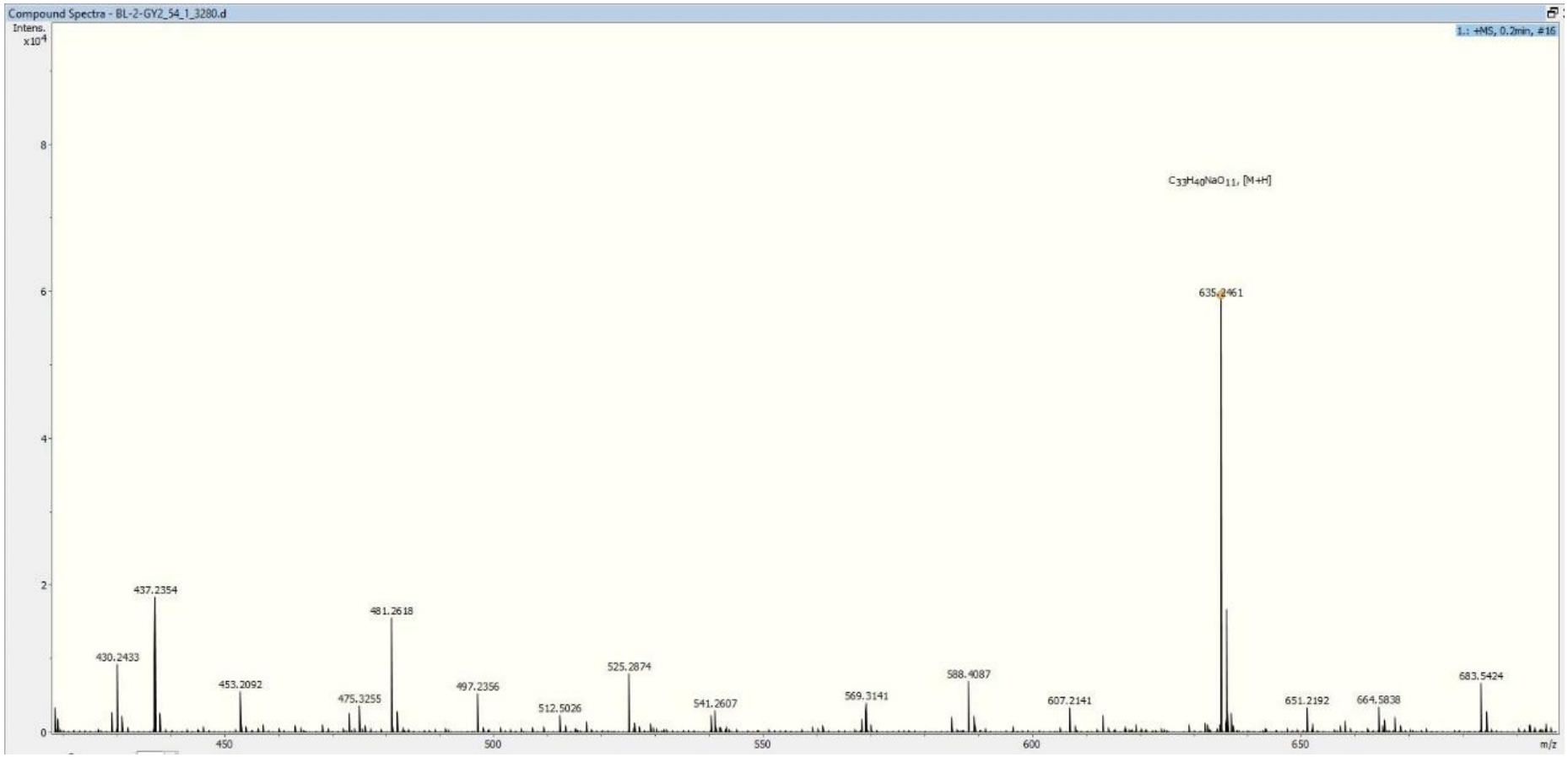


Figure S11. HR-ESI-MS spectrum of compound **1a**.

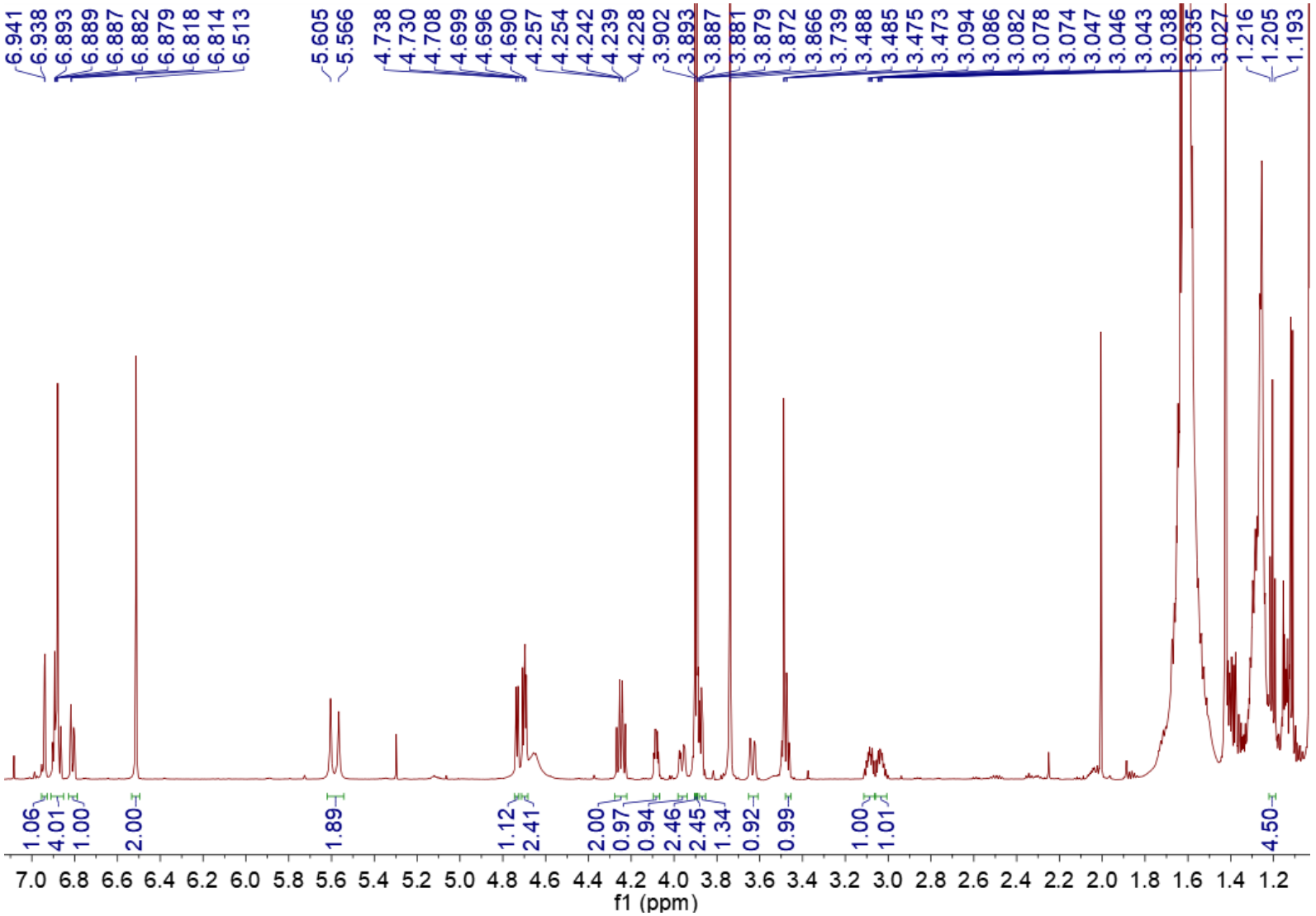


Figure S12. ^{1}H NMR spectrum of compound **1a** (600 MHz, $CDCl_3$).

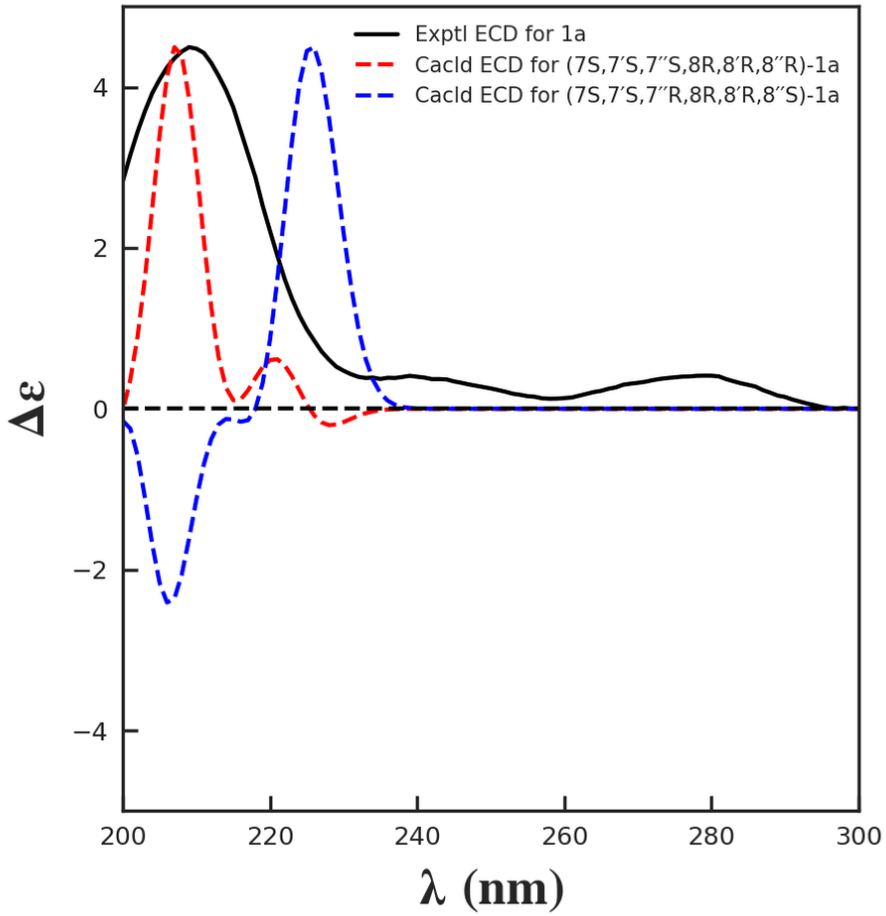


Figure S13. Experimental and calculated ECD spectra of compound **1a**.

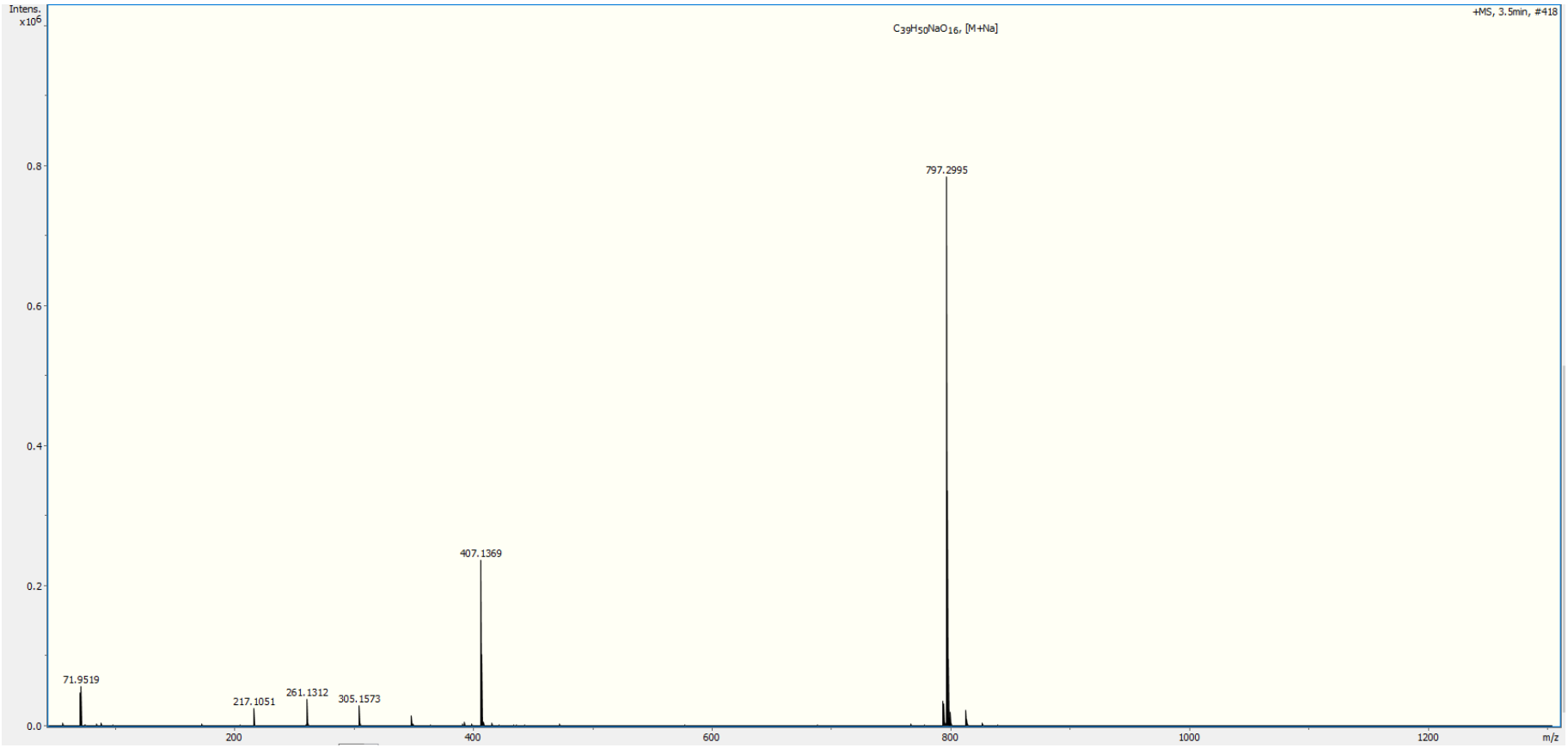


Figure S14. HR-ESI-MS spectrum of compound **2**.

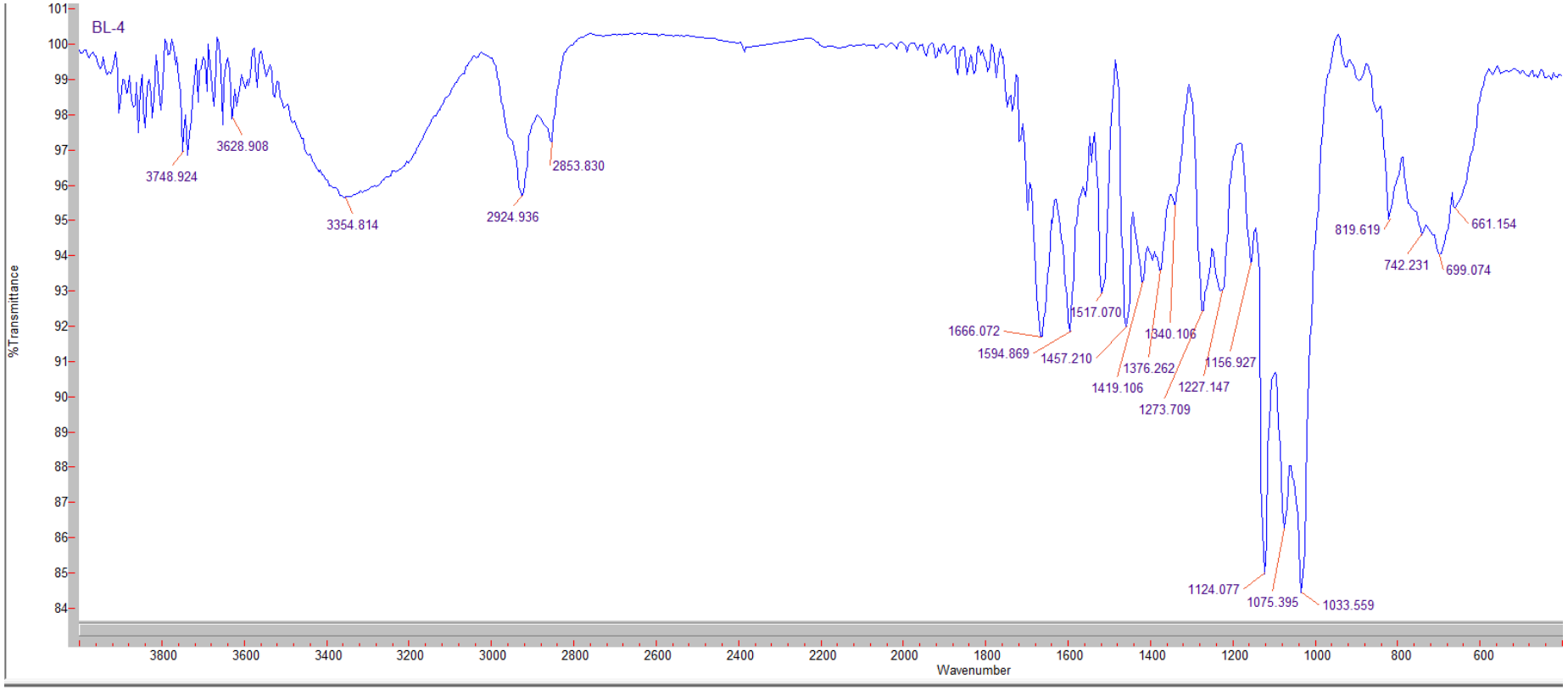


Figure S15. IR spectrum of compound **2**.

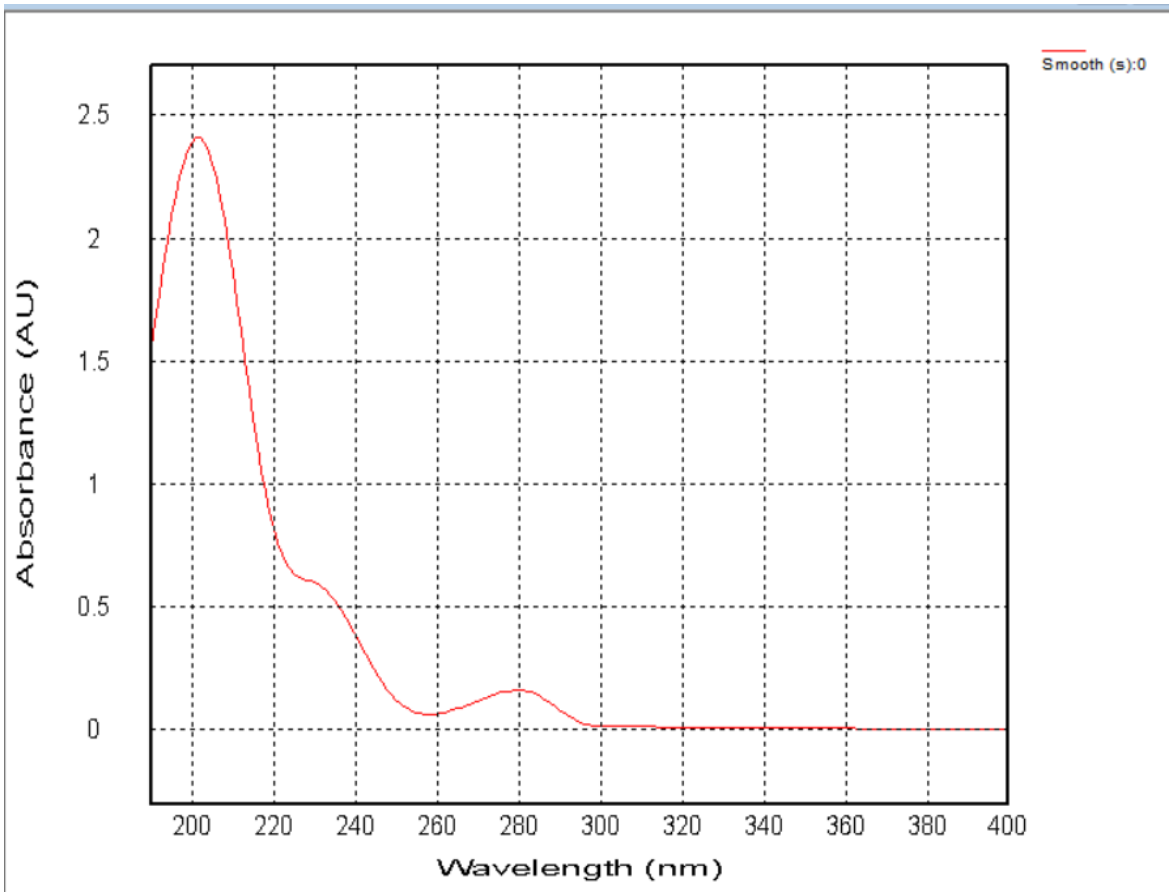


Figure S16. UV spectrum of compound **2**.

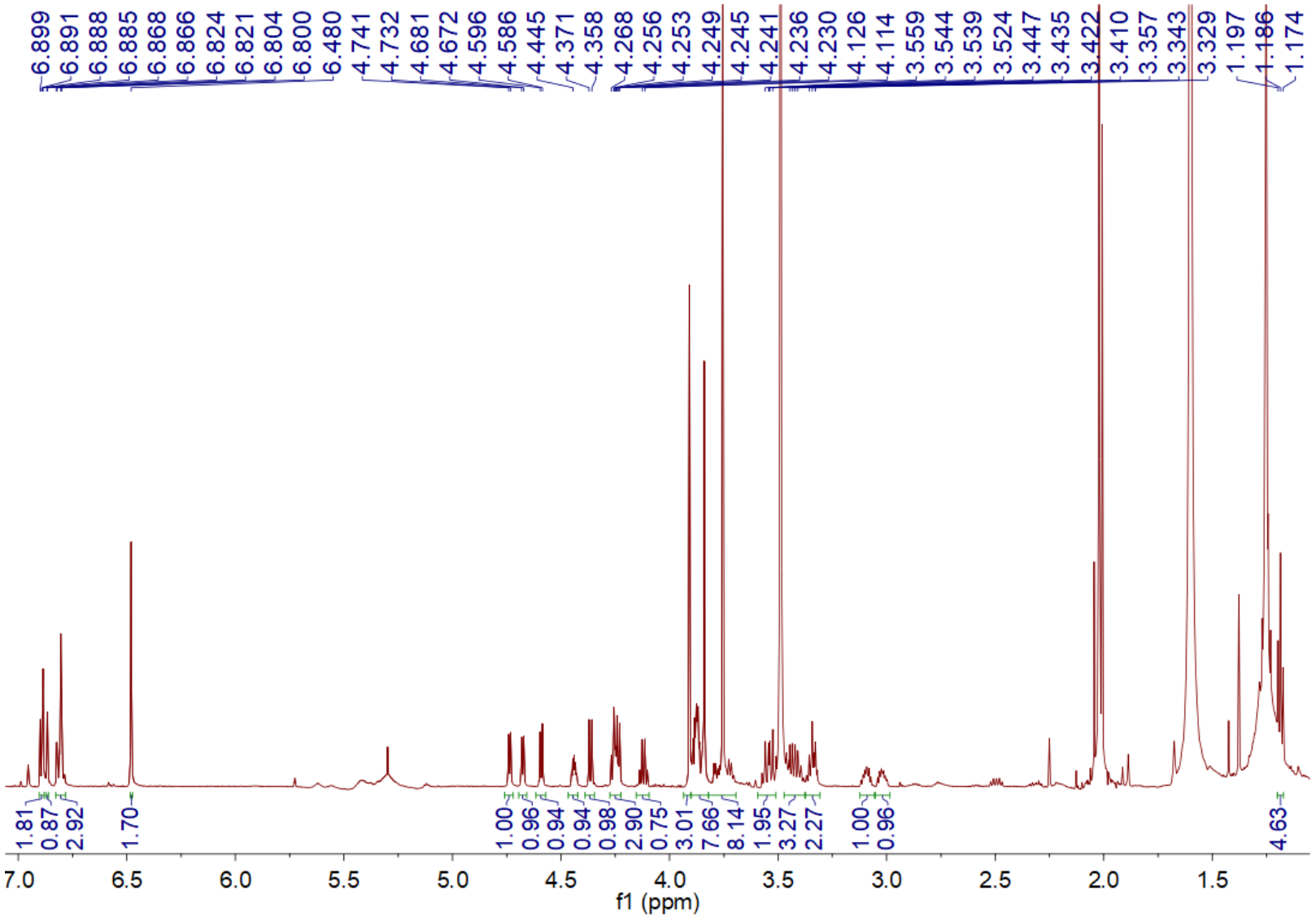


Figure S17. ^{1}H NMR spectrum of compound **2** (600 MHz, $CDCl_3$).

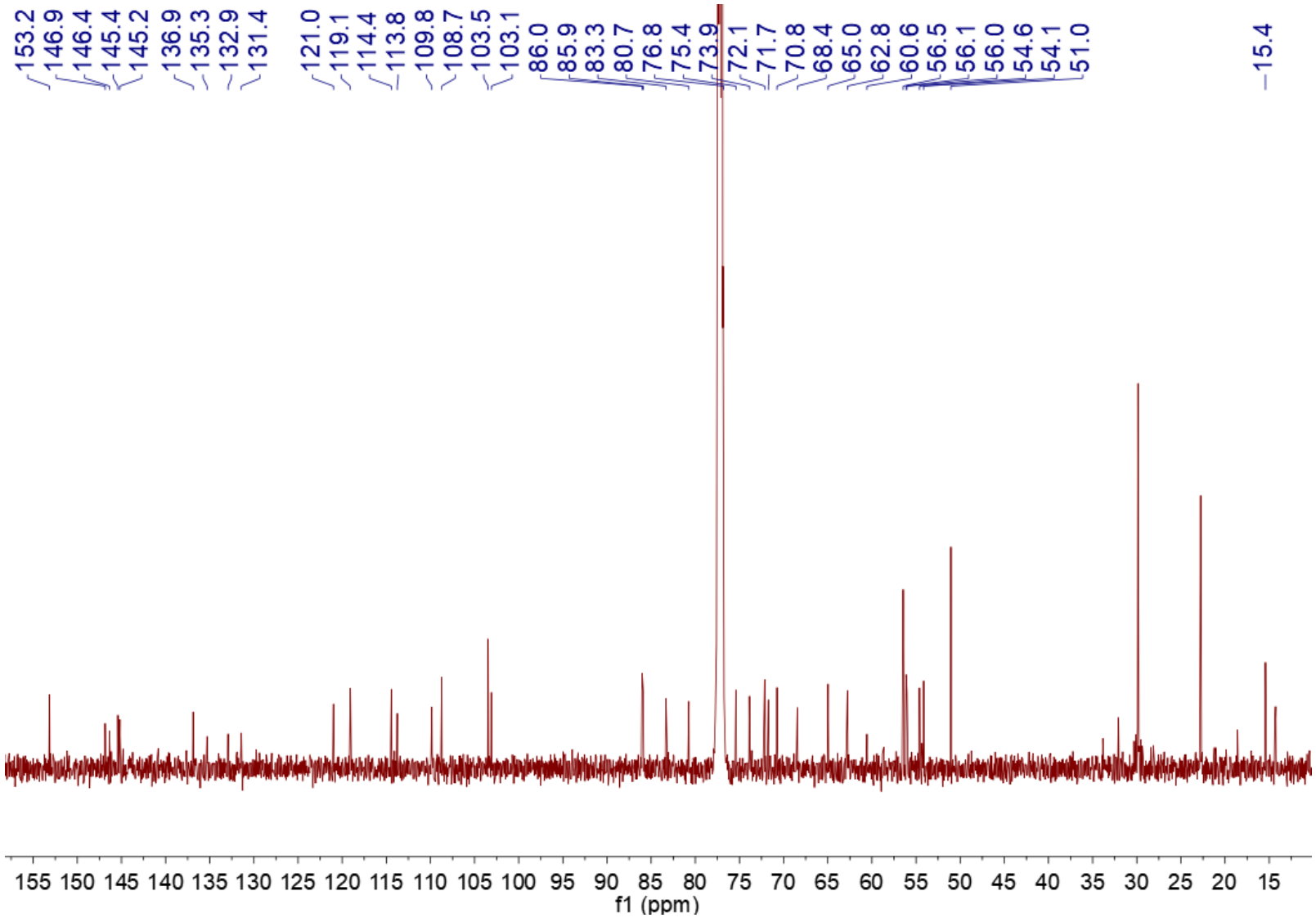


Figure S18. ^{13}C NMR spectrum of compound **2** (150 MHz, $CDCl_3$).

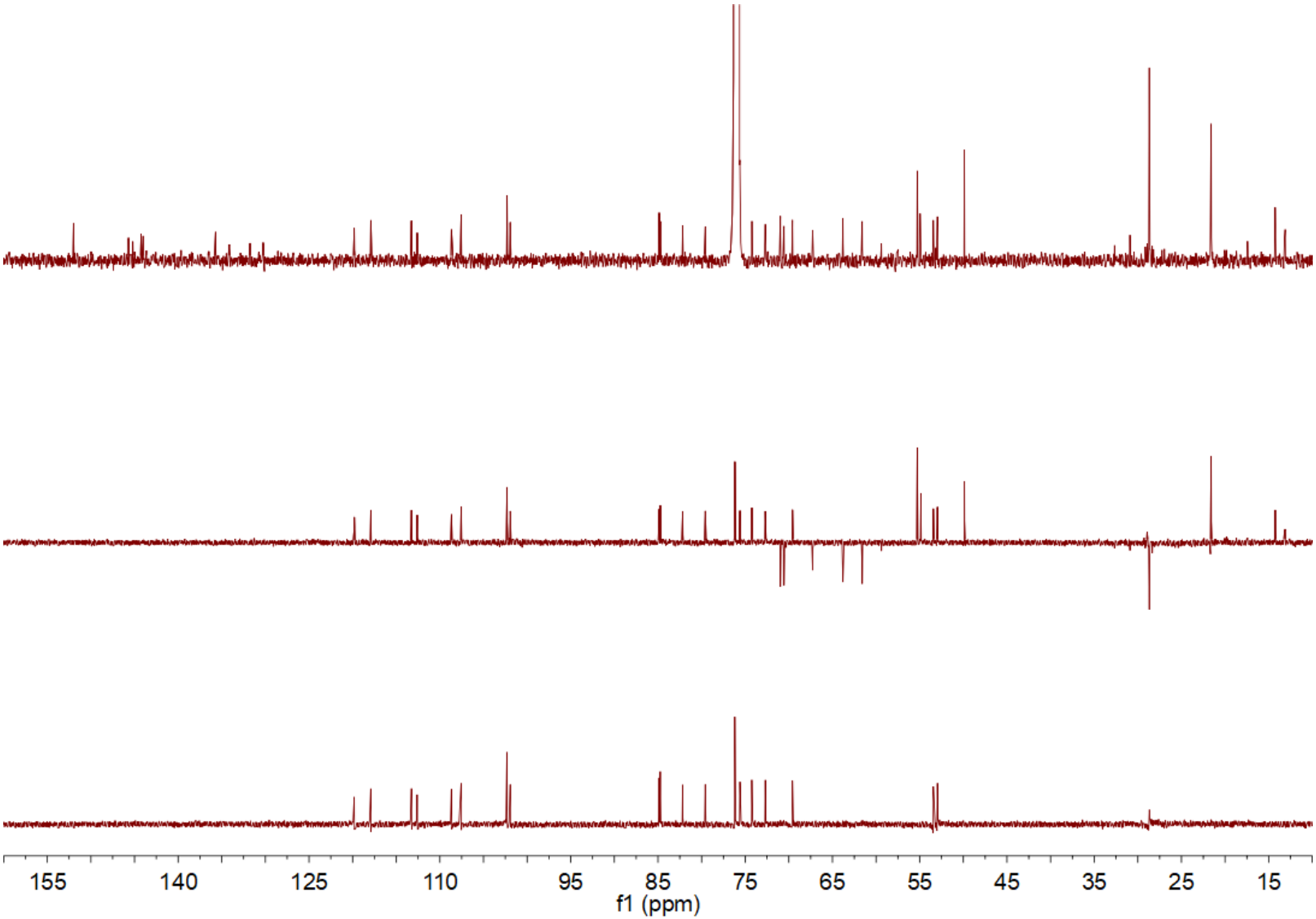


Figure S19. DEPT spectrum of compound **2** ($CDCl_3$).

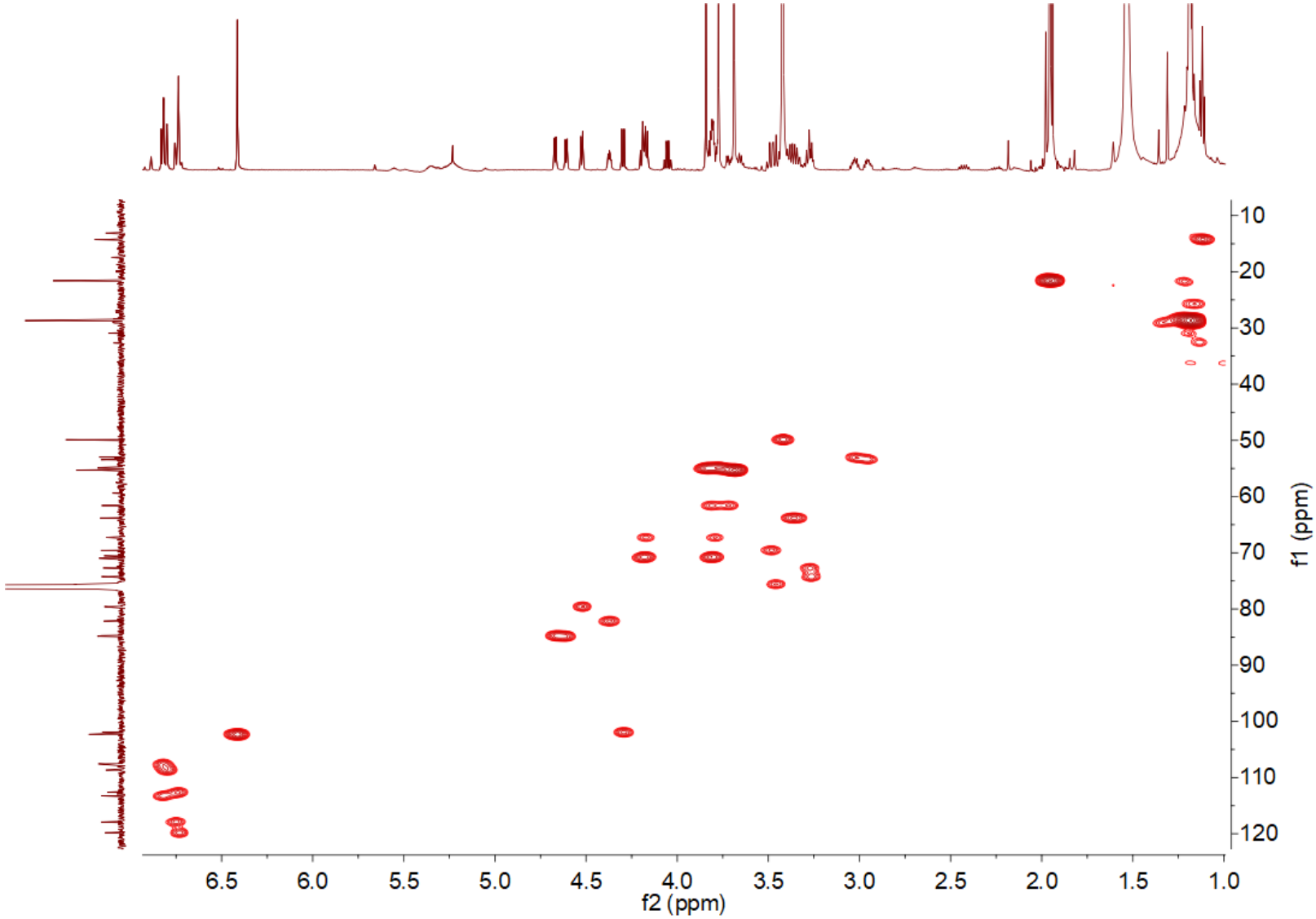


Figure S20. HSQC spectrum of compound **2** ($CDCl_3$).

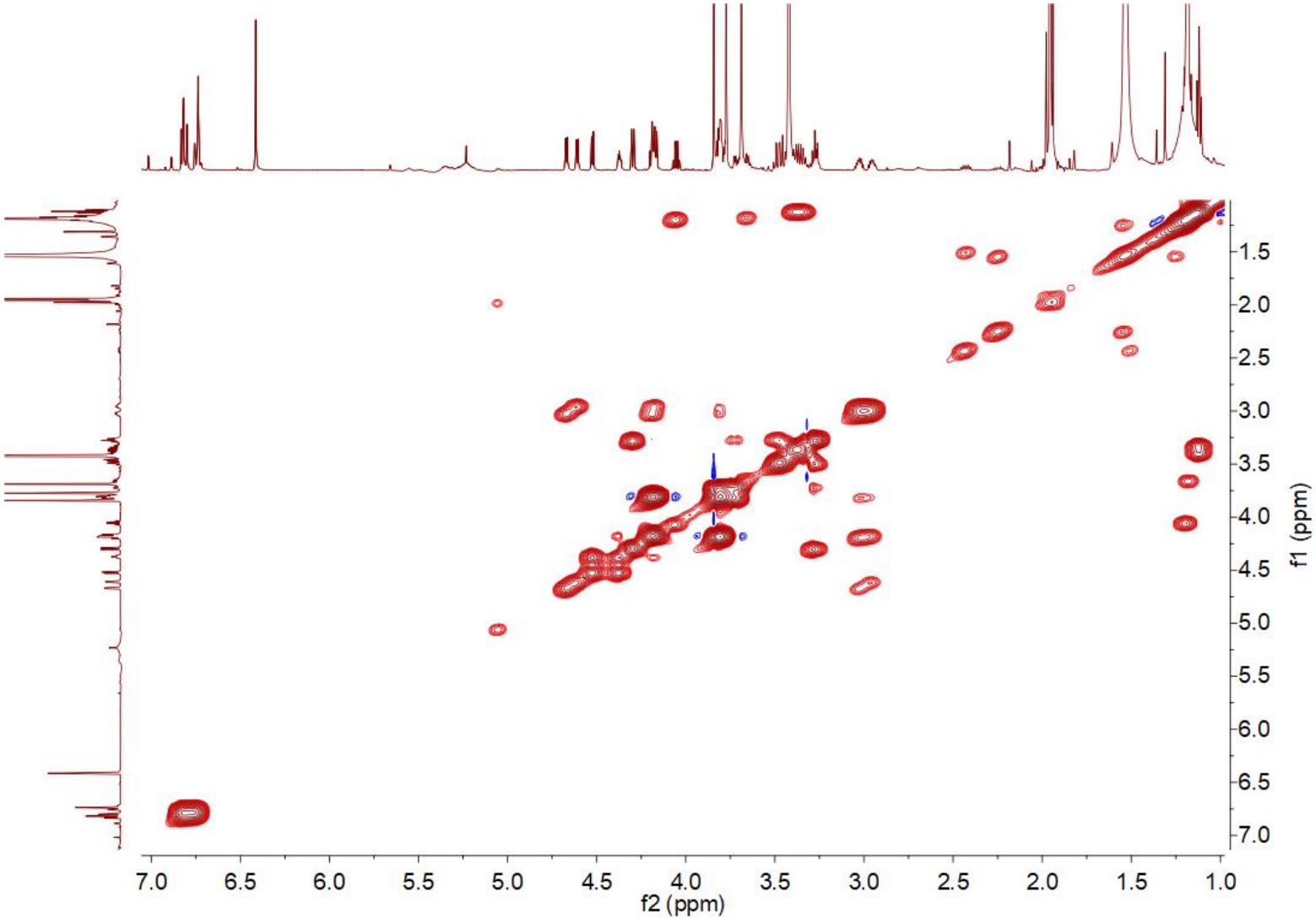


Figure S21. ^{1}H-^{1}H COSY spectrum of compound **2** ($CDCl_3$).

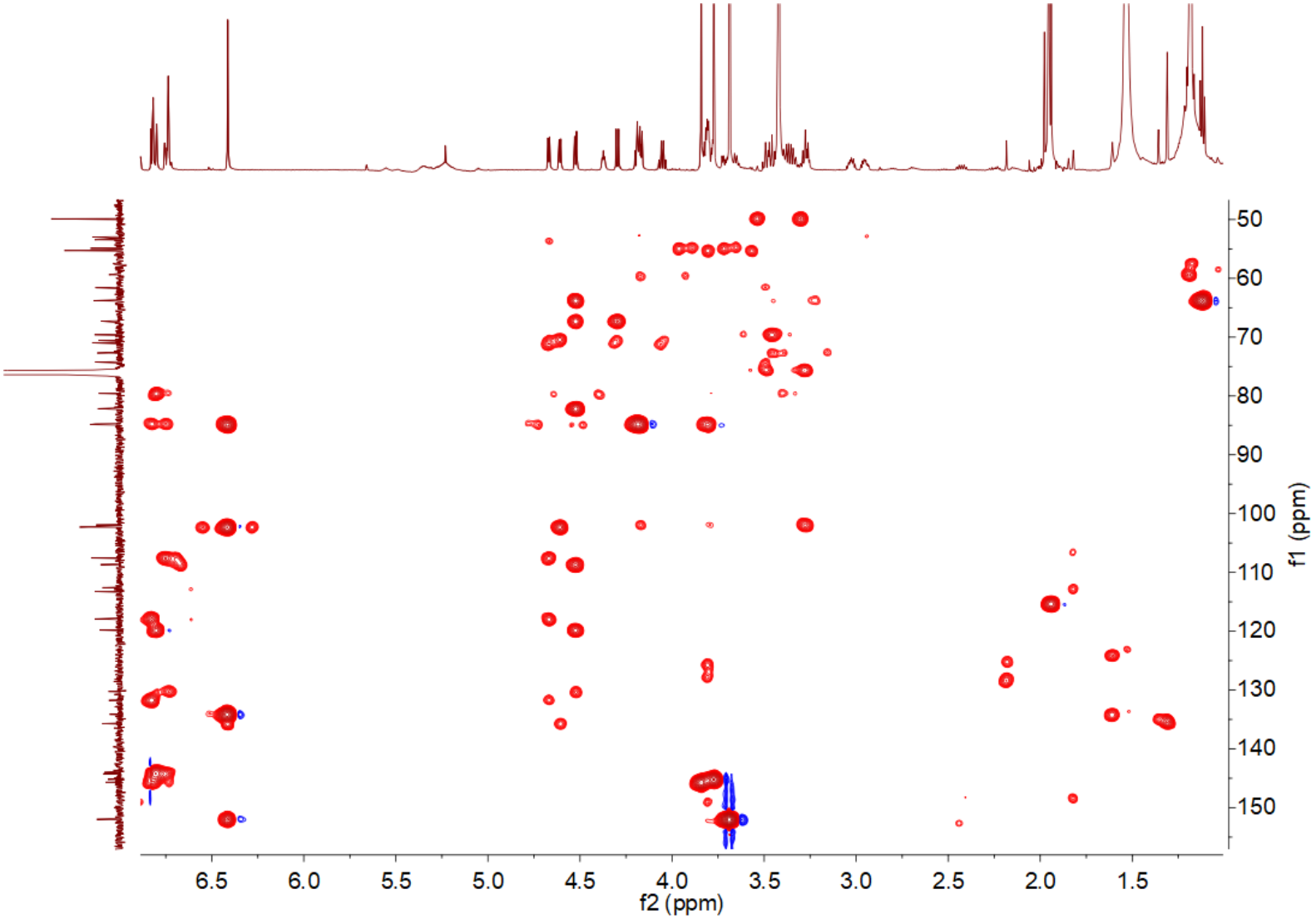


Figure S22. HMBC spectrum of compound **2** ($CDCl_3$).

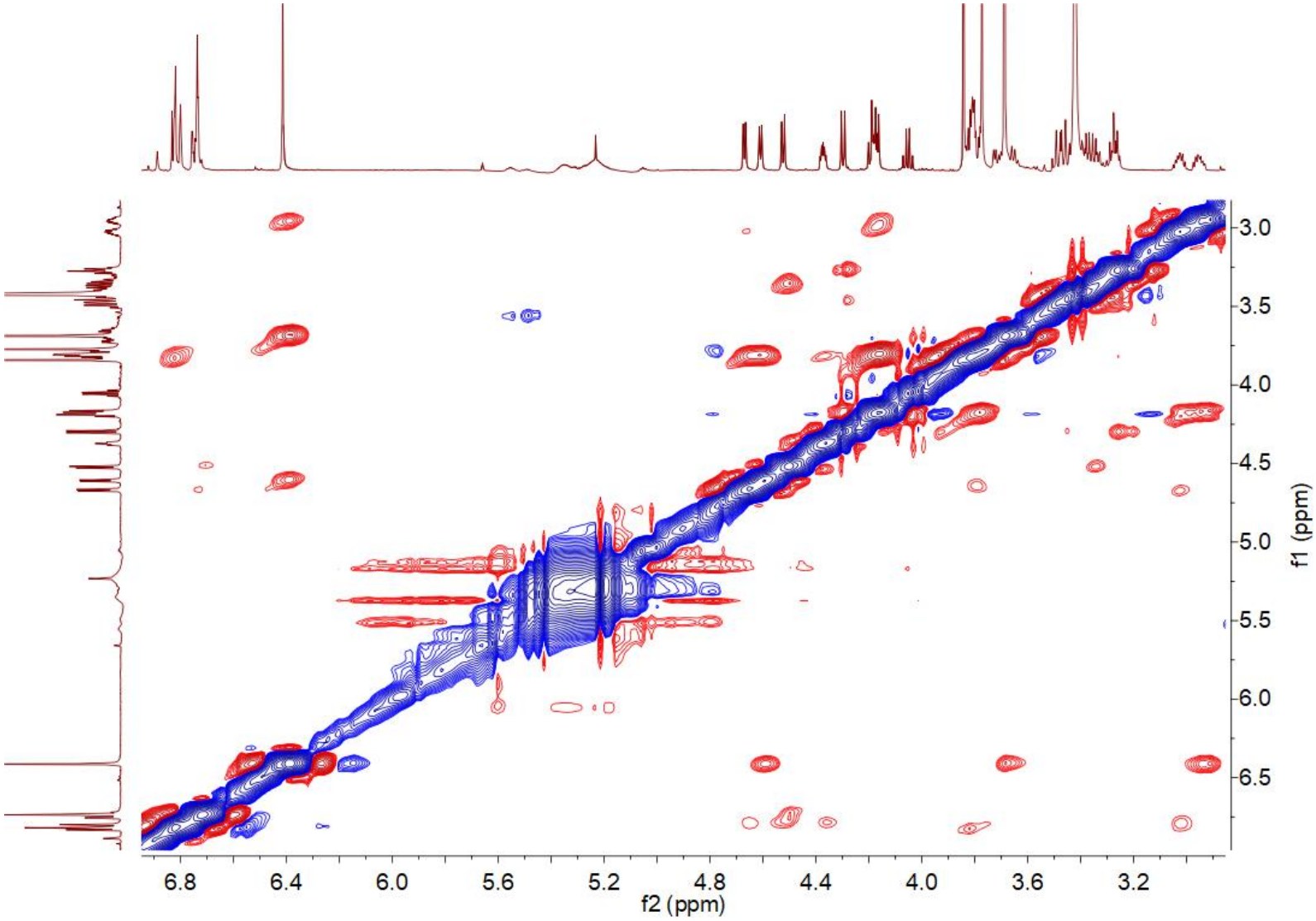


Figure S23. NOESY spectrum of compound **2** ($CDCl_3$).

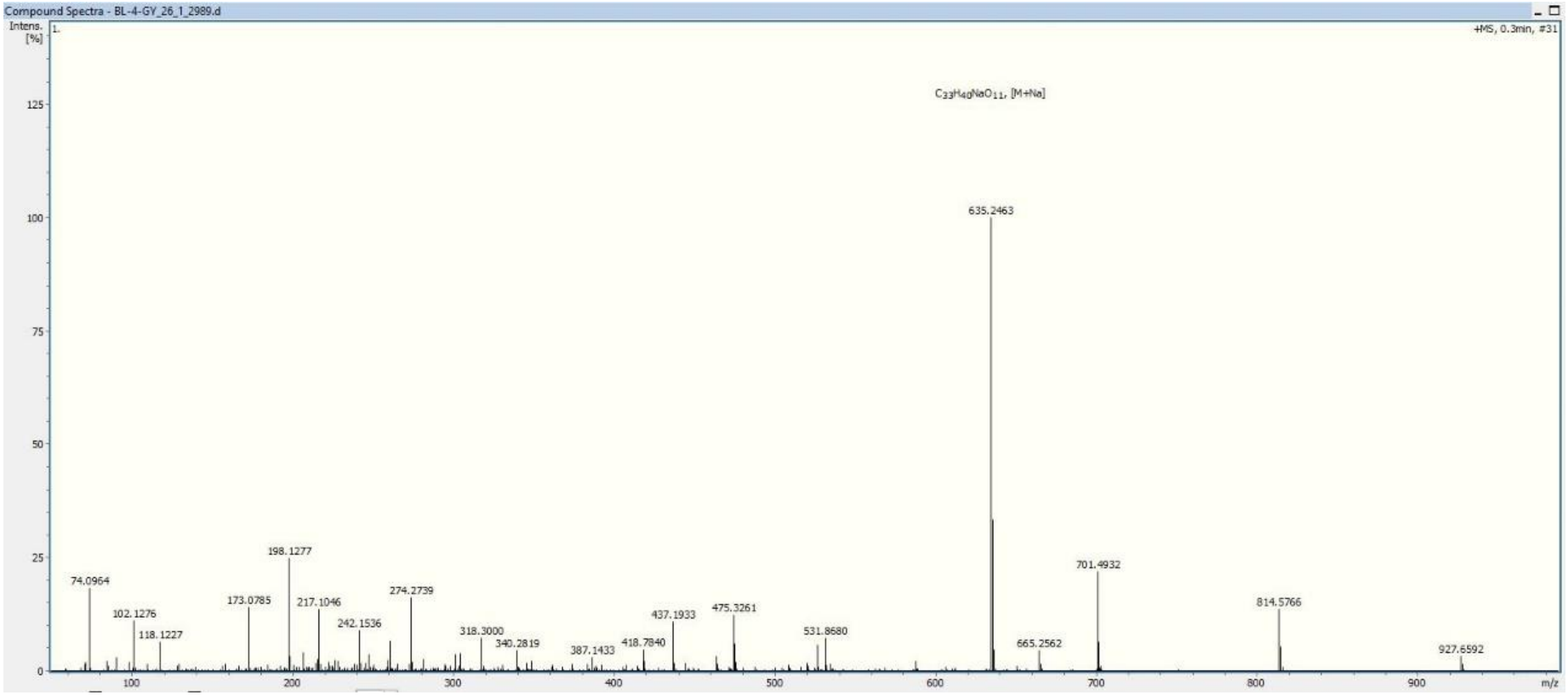


Figure S24. HR-ESI-MS spectrum of compound **2a**.

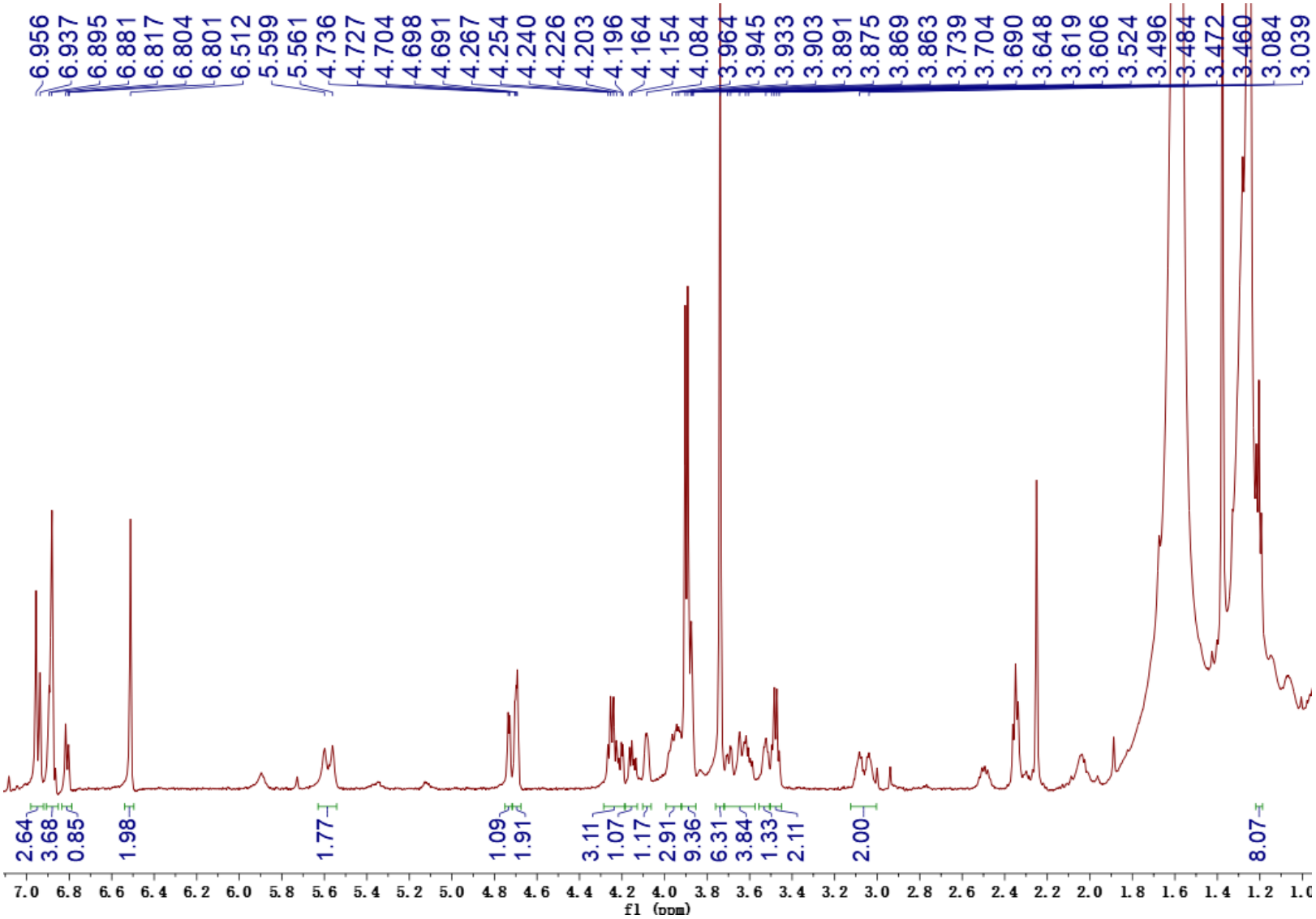


Figure S25. ^{1}H NMR spectrum of compound **2a** (600 MHz, $CDCl_3$).

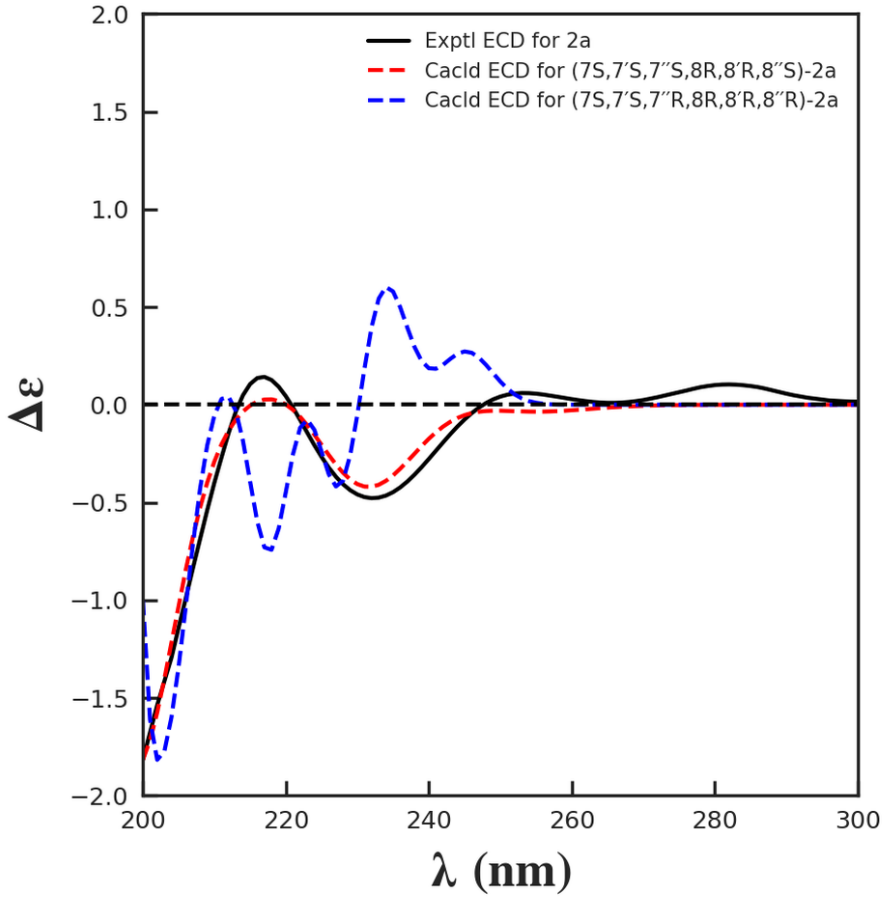


Figure S26. Experimental and calculated ECD spectra of compound **2a**.

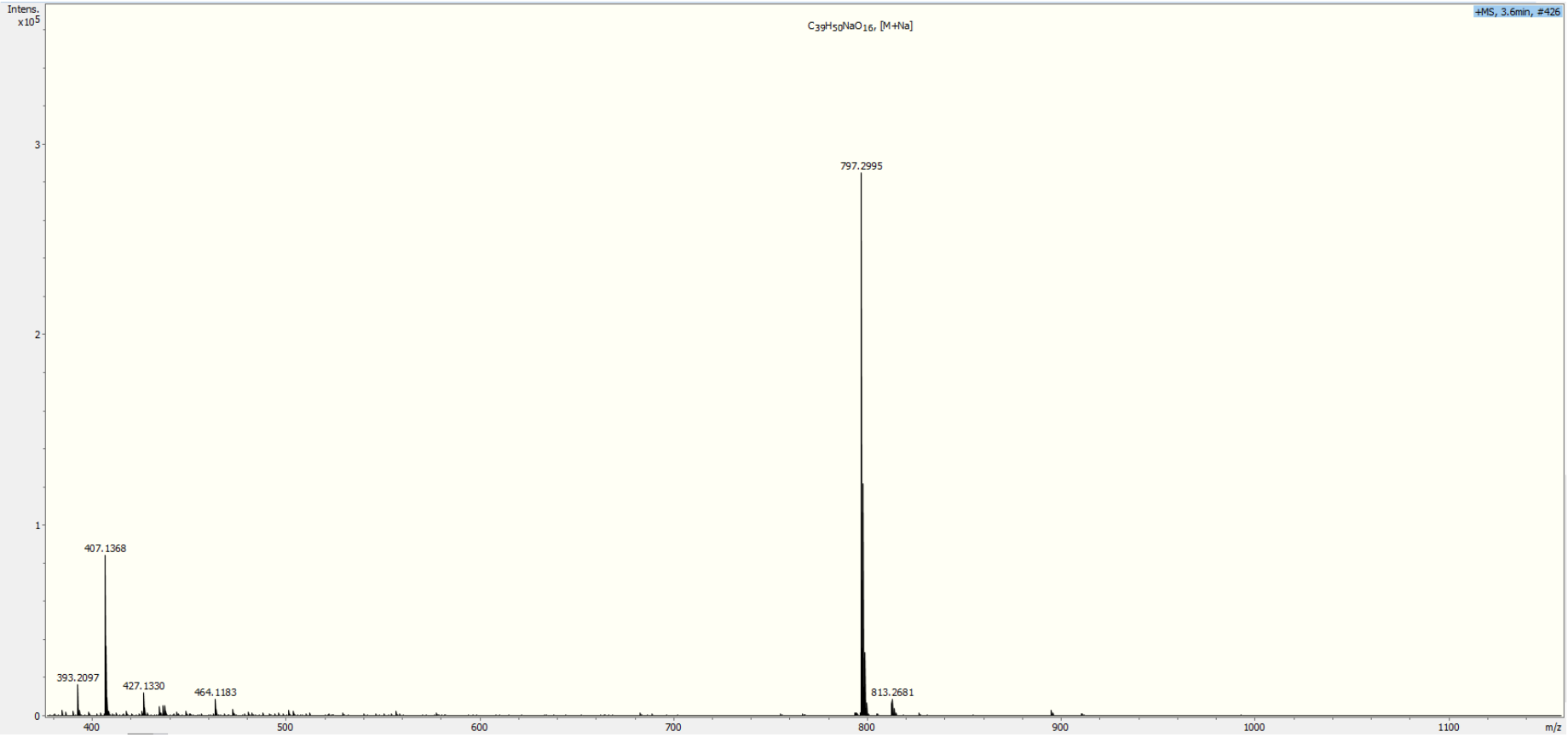


Figure S27. HR-ESI-MS spectrum of compound **3**.

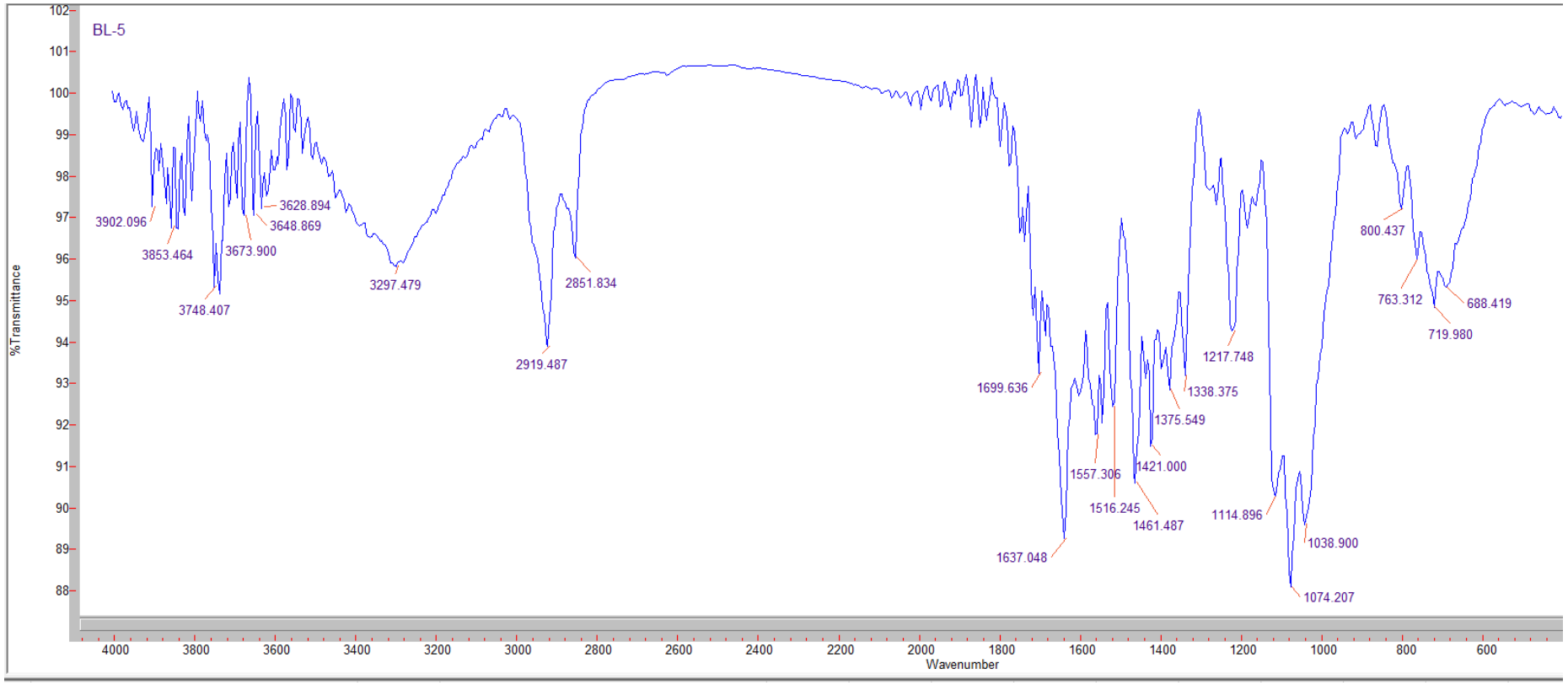


Figure S28. IR spectrum of compound **3**.

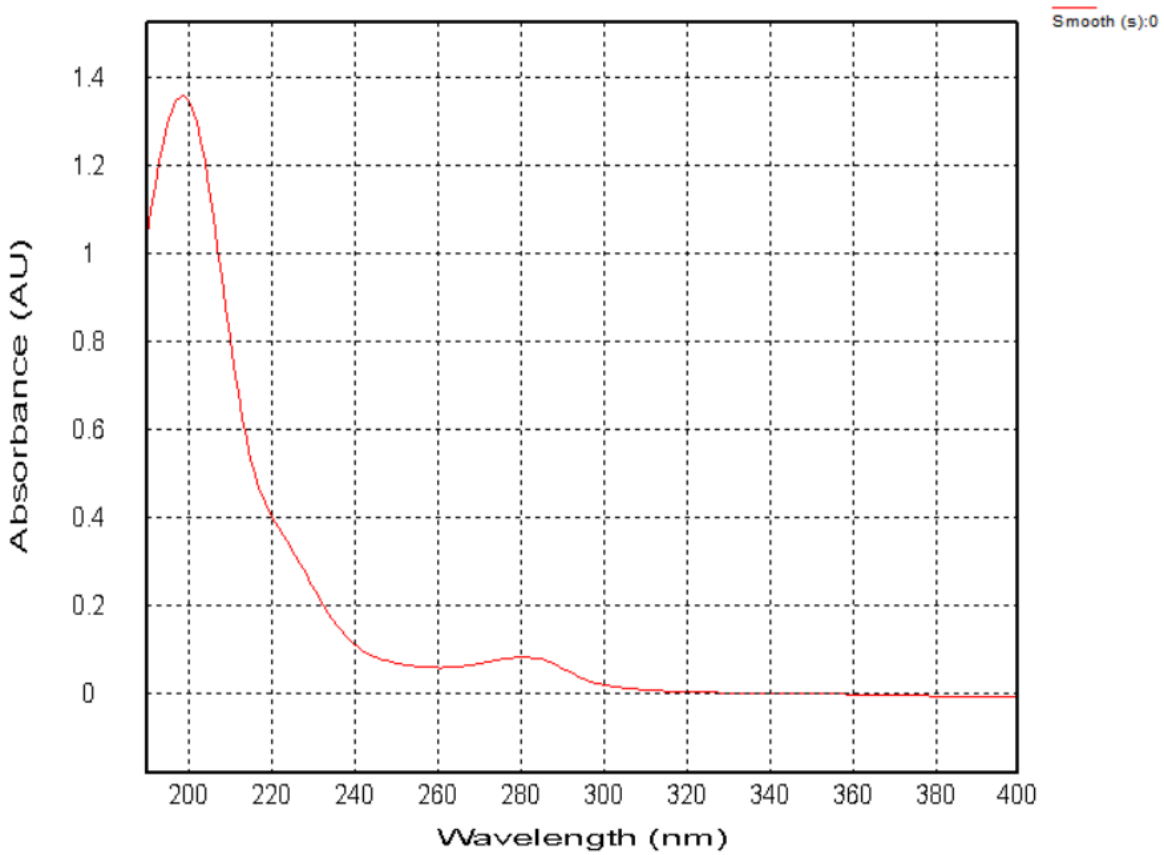


Figure S29. UV spectrum of compound **3.**

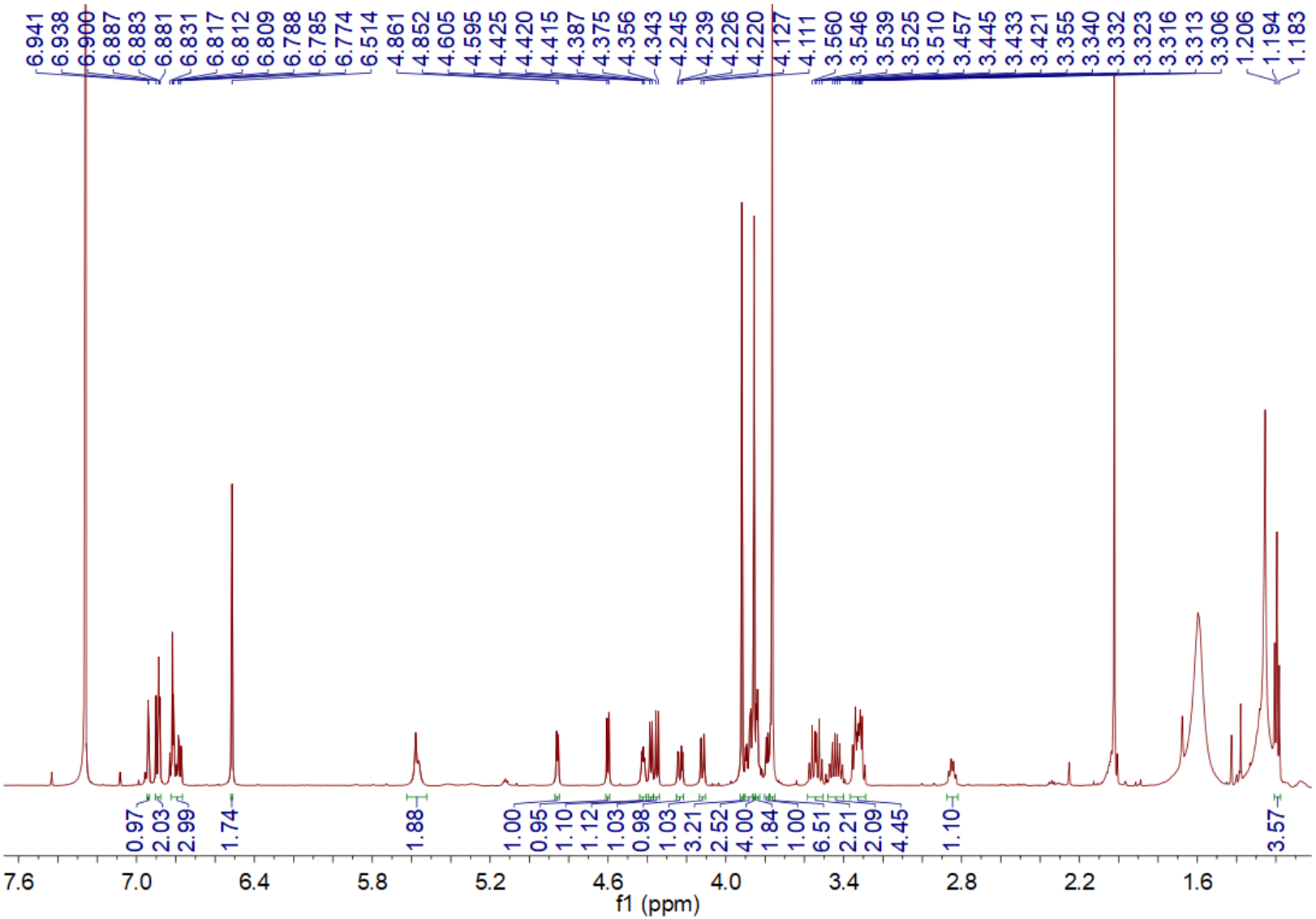


Figure S30. ^{1}H NMR spectrum of compound **3** (600 MHz, $CDCl_3$).

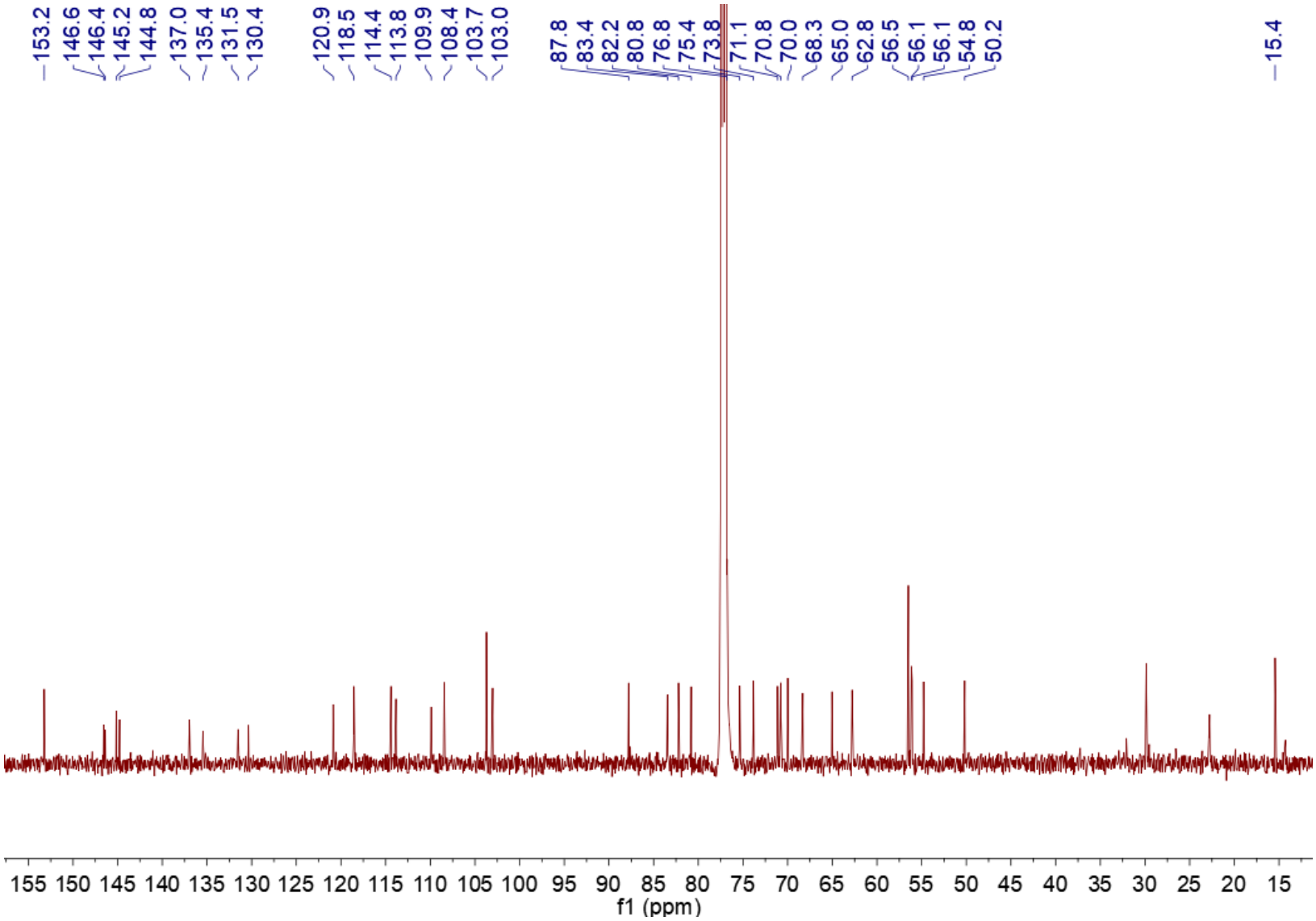


Figure S31. ^{13}C NMR spectrum of compound **3** (150 MHz, $CDCl_3$).

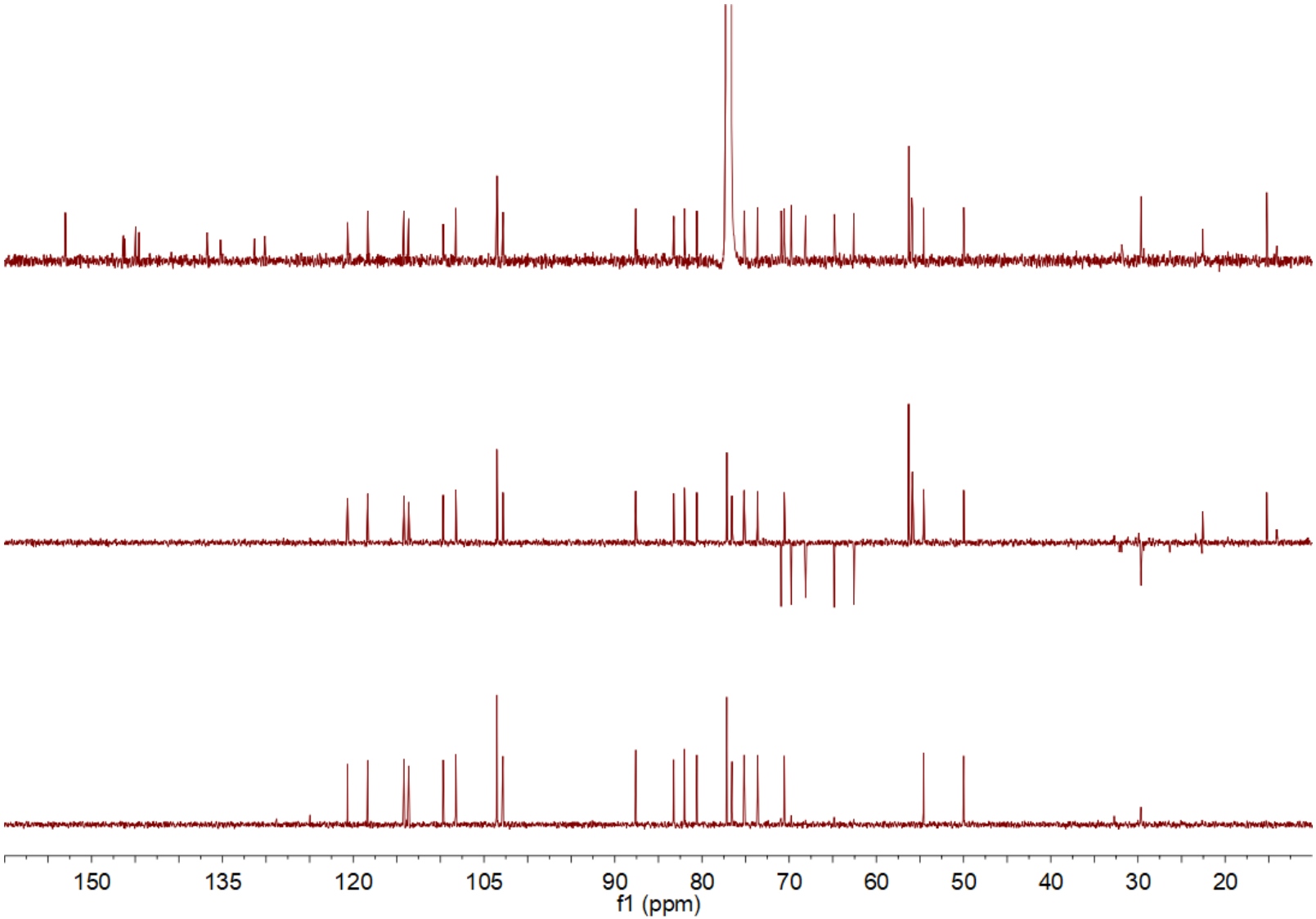


Figure S32. DEPT spectrum of compound **3** ($CDCl_3$).

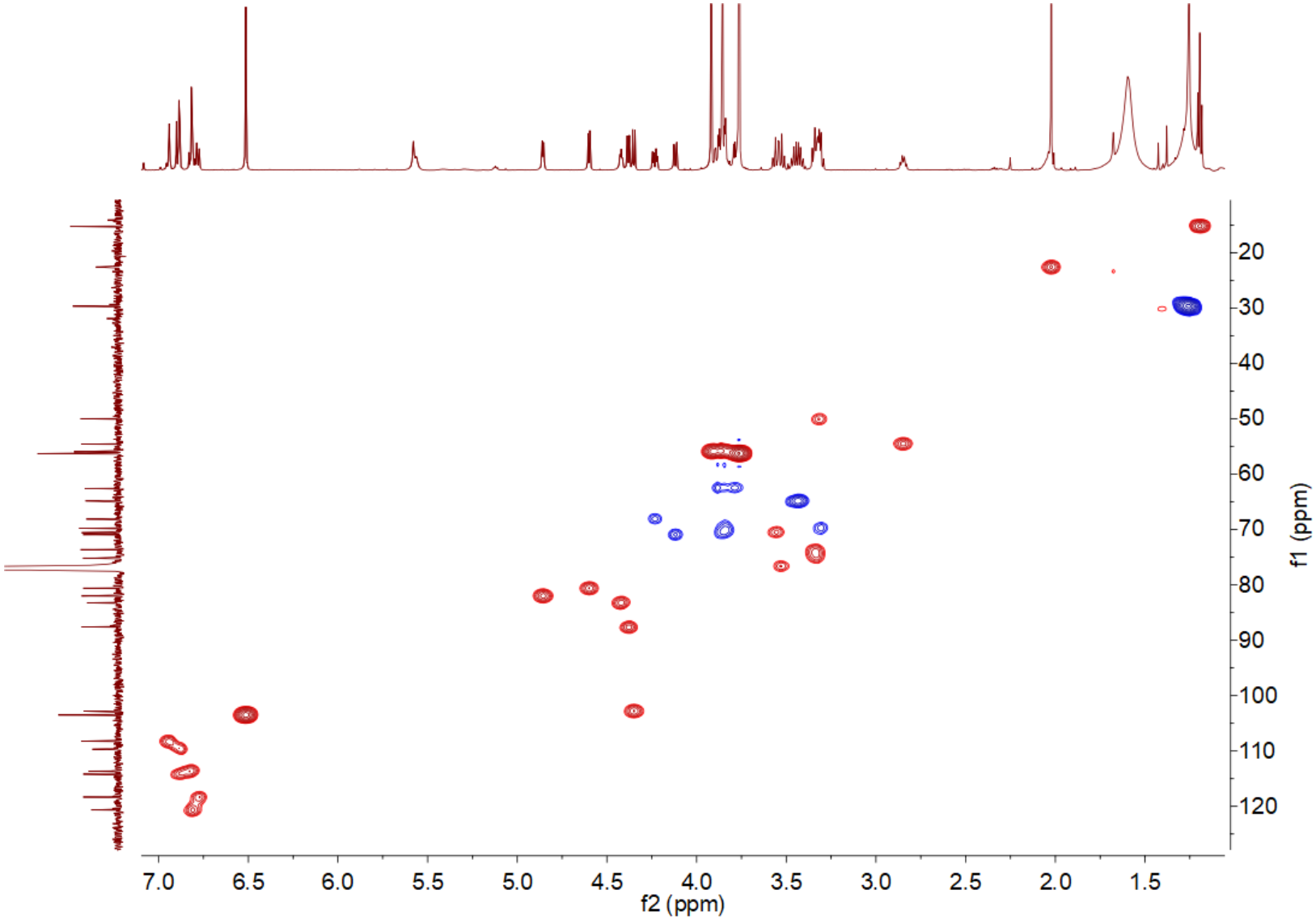


Figure S33. HSQC spectrum of compound **3** ($CDCl_3$).

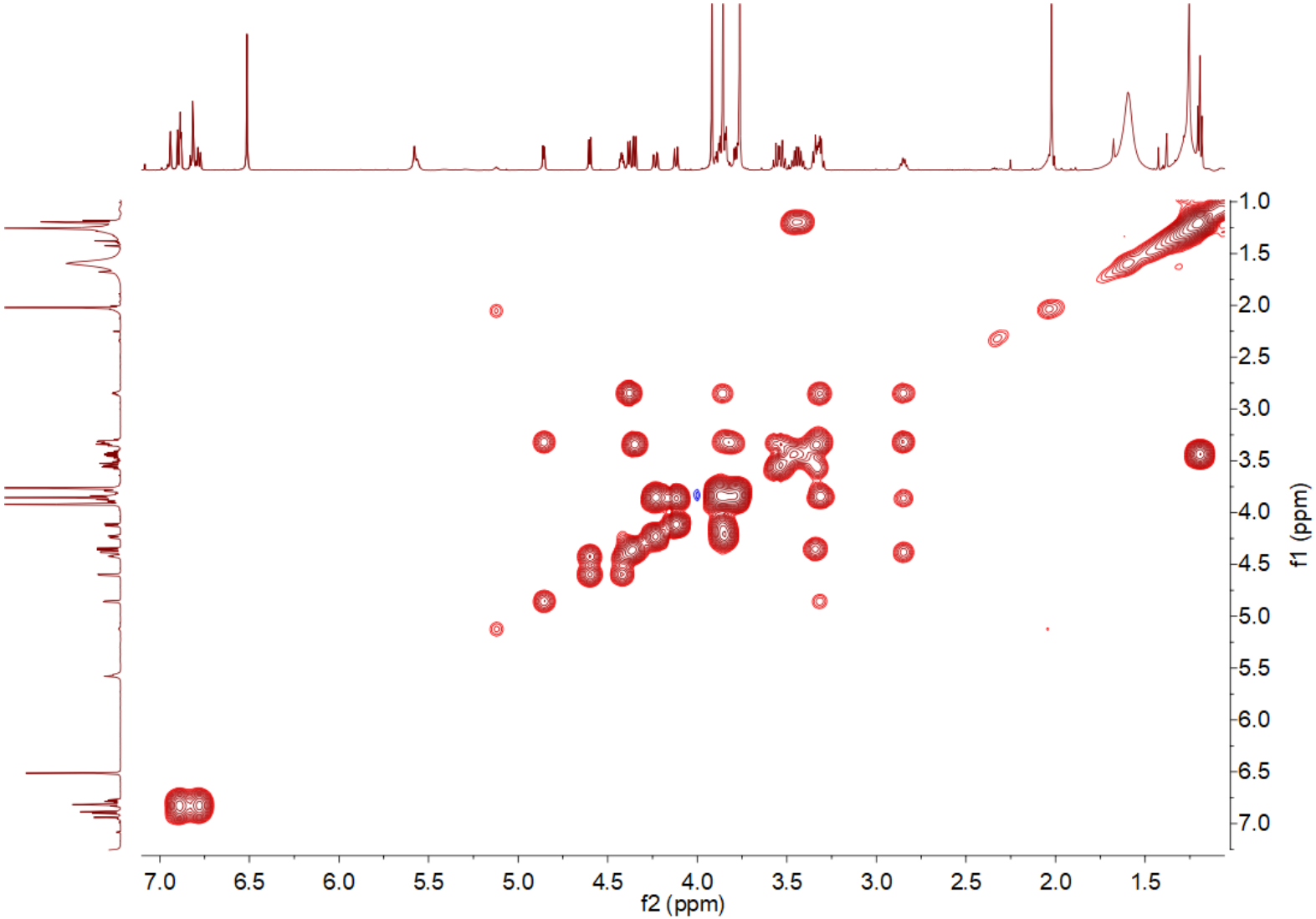


Figure S34. ^{1}H-^{1}H COSY spectrum of compound **3** ($CDCl_3$).

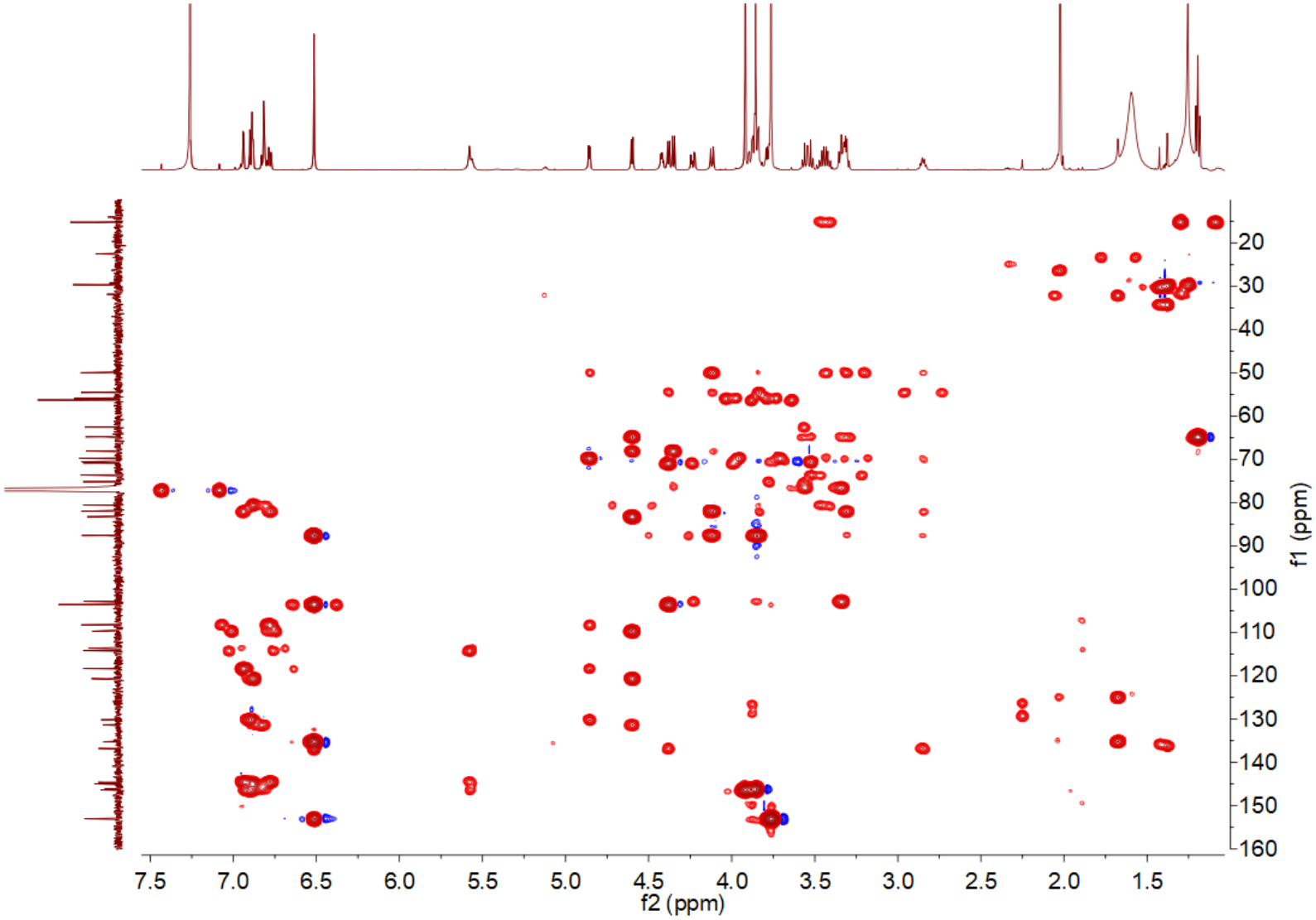


Figure S35. HMBC spectrum of compound **3** ($CDCl_3$).

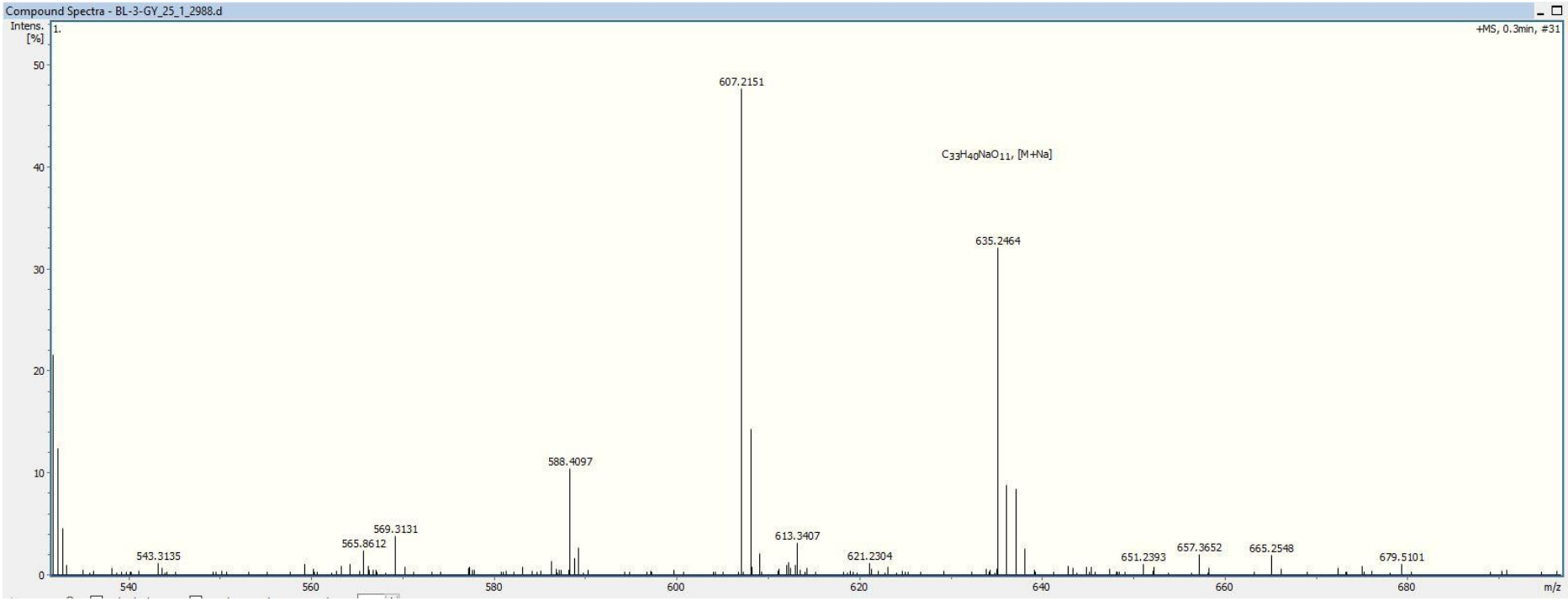


Figure S36. HR-ESI-MS spectrum of compound **3a**.

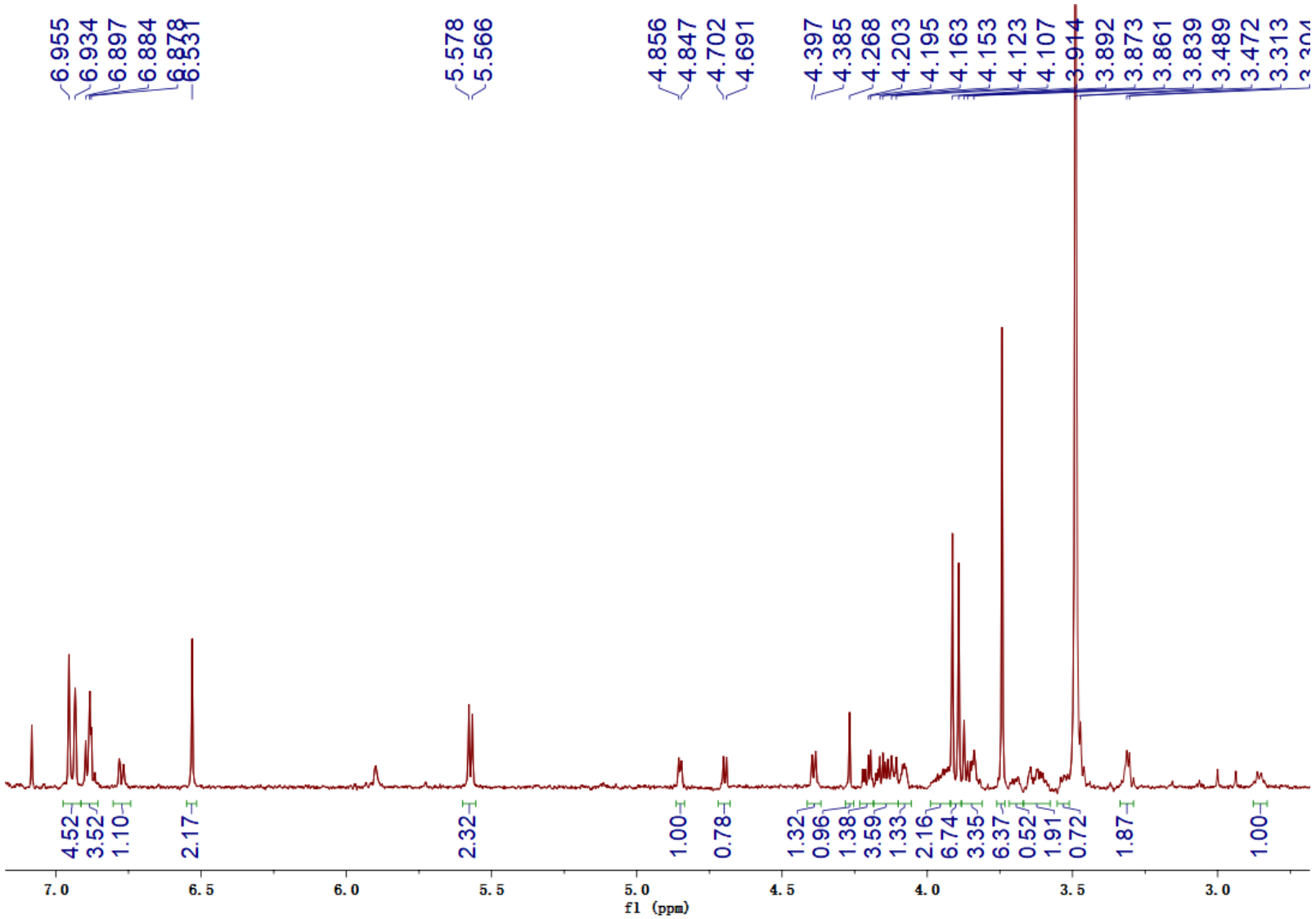


Figure S37. ^{1}H NMR spectrum of compound **3a** (600 MHz, $CDCl_3$).

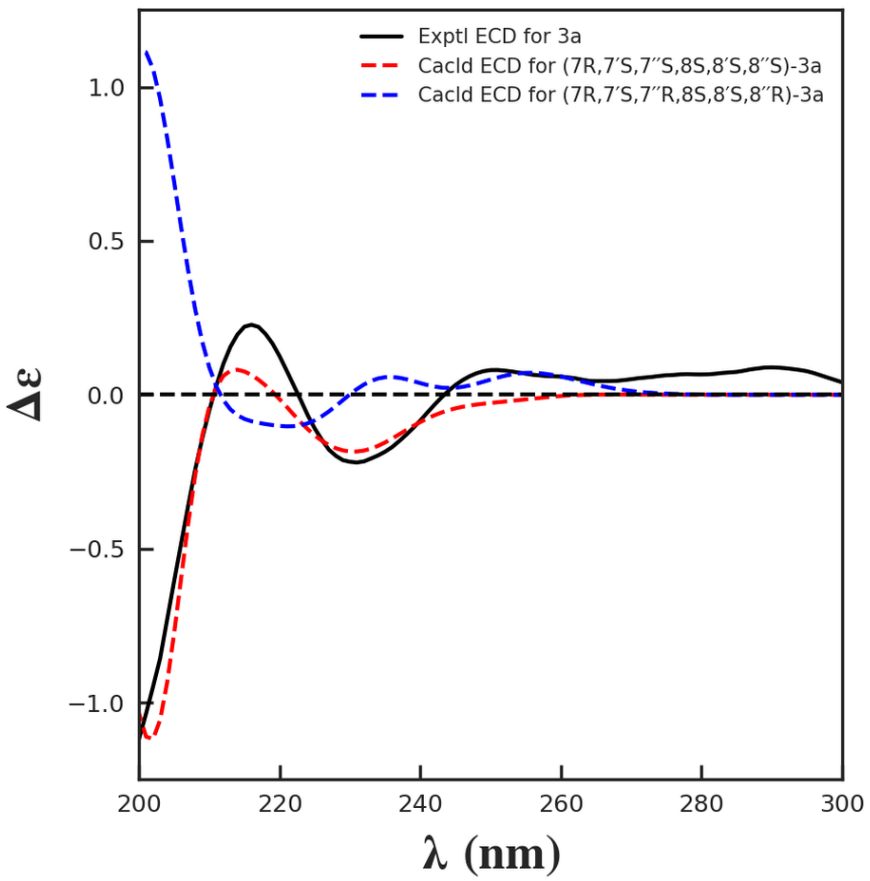


Figure S38. Experimental and calculated ECD spectra of compound **3a**.

Computational methods

1. Conformational analysis

Conformational analysis for compounds **1a**–**3a** were performed in Yinfo Cloud Platform (https://cloud.yinfotek.com/) using systematic algorithm by Confab [1] at the MMFF94 force field with a RMSD threshold of 0.5 Å and an energy window of 7 kcal/mol.

2. ECD calculation

The theoretical ECD calculations were carried out using GAUSSIAN 09. At first, all conformers were optimized at PM6. Room-temperature equilibrium populations were calculated according to the Boltzmann distribution law 错误!未找到引用源。), based on which dominative conformers of population over 1% were kept. The chosen conformers were further optimized at the B3LYP/6-31G(d) in gas phase. Vibrational frequency analysis confirmed the stable structures. ECD calculations were conducted at the B3LYP/6-311G(d,p) level in acetonitrile with a CPCM model using the Time-dependent Density functional theory (TD-DFT). Rotatory strengths for 11/4 (**1a**), 2/4 (**2a**), and 8/4 (**3a**) excited states were calculated. The ECD spectrum was simulated using the ECD/UV analysis tool in Yinfo Cloud Computing Platform (https://cloud.yinfotek.com/) by overlapping Gaussian functions for each transition according to (2).

$$\frac{N_i}{N} = \frac{g_i e^{-\frac{E_i}{k_B T}}}{\sum g_i e^{-\frac{E_i}{k_B T}}} \qquad \textbf{(1)}$$

where N_i is the number of conformer i with energy E_i and degeneracy g_i at temperature T, and k_B is Boltzmann constant.

$$\Delta\varepsilon(E) = \frac{1}{2.297\times10^{-39}} \times \frac{1}{\sqrt{2\pi\sigma}} \sum_{i}^{A} \Delta E_i R_i e^{-\left(\frac{E-E_i}{2\sigma}\right)^2} \qquad \textbf{(2)}$$

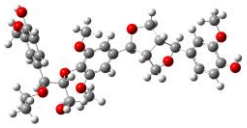
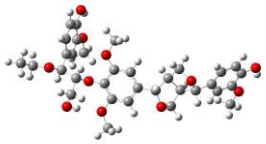
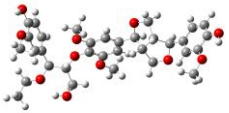
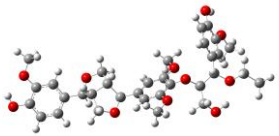
where σ represents the width of the band at 1/e height, while ΔE_i and R_i are the excitation energies and rotatory strengths for transition i, respectively.

The σ and UV-shift values were set 0.49/0.27 eV and 13/10 nm (compound **1a**), 0.13/0.21 eV and −30/−5 nm (compound **2a**), and 0.2/0.32 eV and 9/10 nm (compound **3a**), respectively.

References

[1] O'Boyle NM, Vandermeersch T, Flynn CJ, *et al*. Confab - Systematic generation of diverse low-energy conformers[J]. *J Cheminform*, 2011, **3**: 8. doi: 10.1186/1758-2946-3-8.

Table S1. ECD calculation energies of (7*S*,7′*S*,7″*S*,8*R*,8′*R*,8″*R*)-**1a** at B3LYP/6-311G(d,p) in acetonitrile.

Configuration	Conformer	Structure	E (Hartree)	E (kcal/mol)	Population (%)
(7*S*,7′*S*,7″*S*,8*R*,8′*R*,8″*R*)	1		-2109.540814	-1323756.836	77.53
(7*S*,7′*S*,7″*S*,8*R*,8′*R*,8″*R*)	2		-2109.539233	-1323755.844	14.54
(7*S*,7′*S*,7″*S*,8*R*,8′*R*,8″*R*)	3		-2109.537062	-1323754.481	1.46
(7*S*,7′*S*,7″*S*,8*R*,8′*R*,8″*R*)	4		-2109.536407	-1323754.071	0.73
(7*S*,7′*S*,7″*S*,8*R*,8′*R*,8″*R*)	5		-2109.535942	-1323753.779	0.45

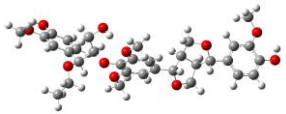
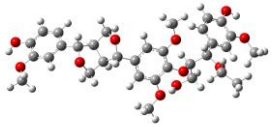
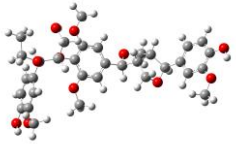
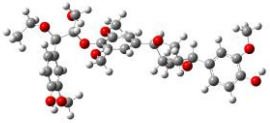
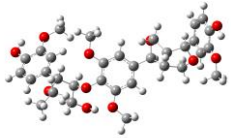
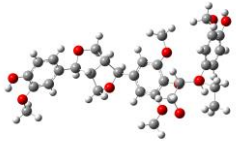
(7*S*,7′*S*,7″*S*,8*R*,8′*R*,8″*R*)	6		-2109.536224	-1323753.956	0.6
(7*S*,7′*S*,7″*S*,8*R*,8′*R*,8″*R*)	7		-2109.538168	-1323755.176	4.7

Table S2. ECD calculation energies of (7*S*,7′*S*,7″*R*,8*R*,8′*R*,8″*S*)-**1a** at B3LYP/6-311G(d,p) in acetonitrile.

Configuration	Conformer	Structure	E (Hartree)	E (kcal/mol)	Population (%)
(7*S*,7′*S*,7″*R*,8*R*,8′*R*,8″*S*)	1		-2109.540104	-1323756.39	4.33
(7*S*,7′*S*,7″*R*,8*R*,8′*R*,8″*S*)	2		-2109.54231	-1323757.775	44.75

(7*S*,7′*S*,7″*R*,8*R*,8′*R*,8″*S*)	3		-2109.540241	-1323756.476	5
(7*S*,7′*S*,7″*R*,8*R*,8′*R*,8″*S*)	4		-2109.53919	-1323755.817	1.64
(7*S*,7′*S*,7″*R*,8*R*,8′*R*,8″*S*)	5		-2109.539679	-1323756.124	2.76
(7*S*,7′*S*,7″*R*,8*R*,8′*R*,8″*S*)	6		-2109.535944	-1323753.78	0.05
(7*S*,7′*S*,7″*R*,8*R*,8′*R*,8″*S*)	7		-2109.539899	-1323756.262	3.48
(7*S*,7′*S*,7″*R*,8*R*,8′*R*,8″*S*)	8		-2109.539506	-1323756.015	2.3

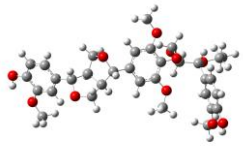
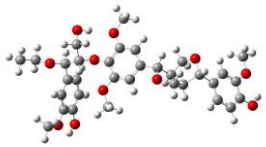
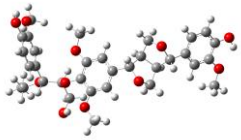
(7*S*,7′*S*,7″*R*,8*R*,8′*R*,8″*S*)	9		-2109.538885	-1323755.625	1.19
(7*S*,7′*S*,7″*R*,8*R*,8′*R*,8″*S*)	10		-2109.541982	-1323757.569	31.63
(7*S*,7′*S*,7″*R*,8*R*,8′*R*,8″*S*)	11		-2109.539718	-1323756.148	2.87

Table S3. ECD calculation energies of (7*S*,7′*S*,7″*S*,8*R*,8′*R*,8″*S*)-**2a** at B3LYP/6-311G(d,p) in acetonitrile.

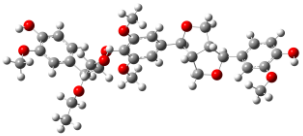
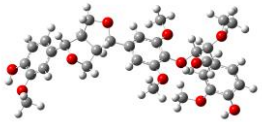
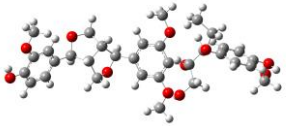
Configuration	Conformer	Structure	E (Hartree)	E (kcal/mol)	Population (%)
(7*S*,7′*S*,7″*S*,8*R*,8′*R*,8″*S*)	1		-2109.539214	-1323755.832	13.2
(7*S*,7′*S*,7″*S*,8*R*,8′*R*,8″*S*)	2		-2109.537515	-1323754.766	2.18
(7*S*,7′*S*,7″*S*,8*R*,8′*R*,8″*S*)	3		-2109.539324	-1323755.901	14.83
(7*S*,7′*S*,7″*S*,8*R*,8′*R*,8″*S*)	4		-2109.540786	-1323756.819	69.78

Table S4. ECD calculation energies of (7*S*,7′*S*,7″*R*,8*R*,8′*R*,8″*R*)-**2a** at B3LYP/6-311G(d,p) in acetonitrile.

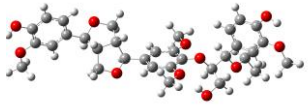
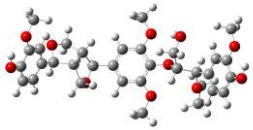
Configuration	Conformer	Structure	E (Hartree)	E (kcal/mol)	Population (%)
(7*S*,7′*S*,7″*R*,8*R*,8′*R*,8″*R*)	1		-2109.538522	-1323755.398	23.89
(7*S*,7′*S*,7″*R*,8*R*,8′*R*,8″*R*)	2		-2109.539616	-1323756.084	76.11

Table S5. ECD calculation energies of (7*R*,7′*S*,7″*S*,8*S*,8′*S*,8″*S*)-**3a** at B3LYP/6-311G(d,p) in acetonitrile.

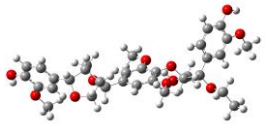
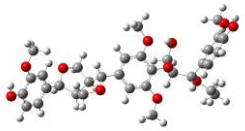
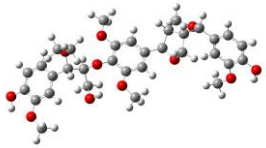
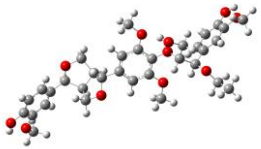
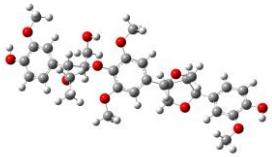
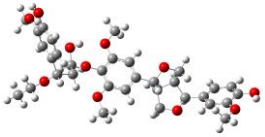
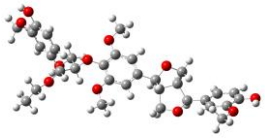
Configuration	Conformer	Structure	E (Hartree)	E (kcal/mol)	Population (%)
(7*R*,7′*S*,7″*S*,8*S*,8′*S*,8″*S*)	1		-2109.539747	-1323756.166	58.61
(7*R*,7′*S*,7″*S*,8*S*,8′*S*,8″*S*)	2		-2109.53842	-1323755.334	14.38
(7*R*,7′*S*,7″*S*,8*S*,8′*S*,8″*S*)	3		-2109.538278	-1323755.245	12.37
(7*R*,7′*S*,7″*S*,8*S*,8′*S*,8″*S*)	4		-2109.538134	-1323755.154	10.61
(7*R*,7′*S*,7″*S*,8*S*,8′*S*,8″*S*)	5		-2109.537218	-1323754.579	4.02

Table S6. ECD calculation energies of (7*R*,7′*S*,7″*R*,8*S*,8′*S*,8″*R*)-**3a** at B3LYP/6-311G(d,p) in acetonitrile.

Configuration	Conformer	Structure	E (Hartree)	E (kcal/mol)	Population (%)
(7*R*,7′*S*,7″*R*,8*S*,8′*S*,8″*R*)	1		-2109.54055	-1323756.67	18.83
(7*R*,7′*S*,7″*R*,8*S*,8′*S*,8″*R*)	2		-2109.537828	-1323754.962	1.05
(7*R*,7′*S*,7″*R*,8*S*,8′*S*,8″*R*)	3		-2109.539452	-1323755.981	5.89
(7*R*,7′*S*,7″*R*,8*S*,8′*S*,8″*R*)	4		-2109.538546	-1323755.413	2.26
(7*R*,7′*S*,7″*R*,8*S*,8′*S*,8″*R*)	5		-2109.537904	-1323755.01	1.14

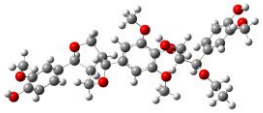
(7*R*,7′*S*,7″*R*,8*S*,8′*S*,8″*R*)	6		-2109.541314	-1323757.15	42.32
(7*R*,7′*S*,7″*R*,8*S*,8′*S*,8″*R*)	7		-2109.540941	-1323756.916	28.5

Table S7. The images of original western blots in three repetitions for Figure 3.

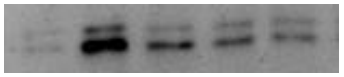
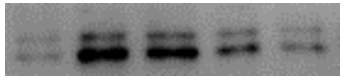
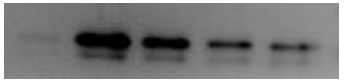
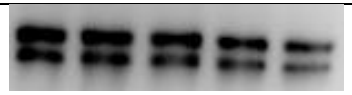
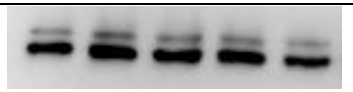
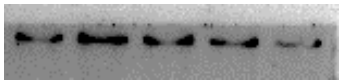
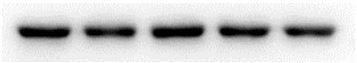
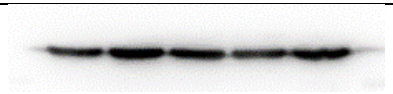
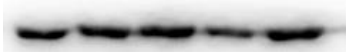
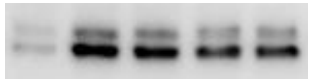
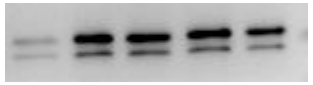
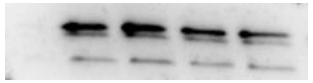
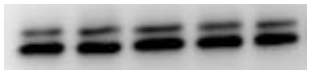
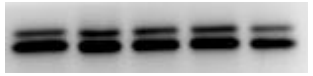
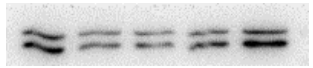
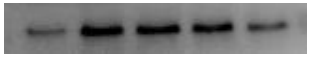
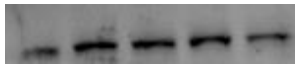
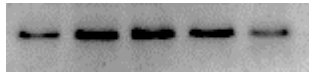
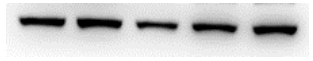
	n1	n2	n3
p-ERK1/2			
ERK1/2			
iNOS			
β-actin			

Table S8. The images of original western blots in three repetitions for Figure 7.

	n1	n2	n3
p-ERK1/2			
ERK1/2			
p-NF-κB			
NF-κB			
β-actin			