

Supporting Information

Bisaspochalasin F: a Tetrahydrofuran Ring-Fused Cytochalasan Homodimer from an Endophytic *Aspergillus flavipes* as a Cav3.2 Inhibitor

Li Wang^{a,*}, Rui Liu^b, Zhiyin Yu^c, Chun-Jun Hou^a, Sheng-Xiong Huang^{b,c}, Yin Nian^{b,*}, Jing Yang^{d,*}

^a School of Chemical Science and Technology, Yunnan University, Kunming 650500, China

^b Key Laboratory of Phytochemistry and Natural Medicines, Kunming Institute of Botany,
Chinese Academy of Sciences, Kunming 650201, China

^c Institute of Herbgonomics and Innovative Institute of Chinese Medicine and Pharmacy,
Chengdu University of Traditional Chinese Medicine, Chengdu 611137, China

^d School of Modern Chinese Medicine Industry & Institute of Herbgonomics, Chengdu
University of Traditional Chinese Medicine, Chengdu 611137, China

*To whom correspondence should be addressed.

E-mail: wangli2024@ynu.edu.cn (L.W.)

nianyin@mail.kib.ac.cn (Y.N.)

yangjingc@cdutcm.edu.cn (J.Y.)

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General experimental procedures

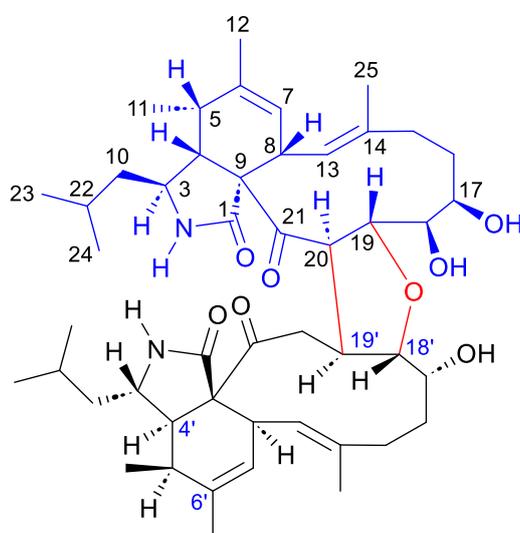
The melting point of compound **1** was measured using a RY-1G melting point apparatus (Jing Tuo Instrument, Tianjin, China). X-ray data for **1** were collected using a Bruker D8 Quest diffractometer (Bruker Corporation, Germany). The optical rotation of **1** was recorded in MeOH using an Autopol IVS2&Plus polarimeter (Rudolph Research Analytical, USA). The CD spectrum of **1** was recorded on an Applied Photophysics digital circular dichroism chiroptical spectrometer (Applied Photophysics Limited, United Kingdom). The UV spectrum of **1** was acquired in MeOH with a Shimadzu UV-2700 spectrophotometer (Shimadzu Corporation, Japan). The IR spectrum of **1** was measured on a Nicolet™ iS™ 10 FT-IR spectrometer with KBr disks (Thermo Fisher Scientific, USA). NMR spectra were recorded in CD₃OD (Energy Chemical, China) (δ_{H} 3.31 ppm, δ_{C} 49.00 ppm), using a Bruker AVANCE III-600 spectrometer (Bruker Corporation, Germany). The HR-ESI-MS spectrum of **1** was acquired on an Agilent 1290 LC/6545 Q-TOF mass instrument (Agilent Technologies, USA).

Column chromatography (CC) was performed using MCI gel CHP 20P (75–150 μm) and Sephadex LH-20 (18–111 μm , GE Healthcare, Sweden). Thin-layer chromatography (TLC) was performed using precoated silica gel GF254 plates (0.20–0.25 mm in thickness, Qingdao Marine Chemical Inc., China) with various solvent systems. Semipreparative HPLC was conducted on a HITACHI Chromaster system (Hitachi Ltd, Japan) equipped with a DAD detector, an Agilent ZORBAX SB-C18 column (150 mm \times 9.4 mm i.d., 5 μm), and a flow rate of 3.0 mL min^{-1} .

For patch-clamp recordings, the Double IPA system (an integrated patch clamp amplifier with a data acquisition system from Sutter Instrument, USA) was used for amplifying cell electrical signals. Currents were low-pass filtered at 2 kHz and sampled at 10 kHz. SutterPatch2.1 software (Sutter Instrument) was used for data acquisition and analysis. Pipettes were fabricated from borosilicate glass (Sutter Instrument, 2605321) using a micropipette puller (P-1000, Sutter Instrument), and were fire-polished to resistances of 4–6 M Ω for whole-cell recording. Z944 hydrochloride (SML2635) was obtained from Sigma-Aldrich.

Table S1. ^1H (600MHz) and ^{13}C (150MHz) NMR data of bisaspochalasin F (**1**) (CD_3OD , δ in ppm).

No.	δ_{H}	δ_{C}	No.	δ_{H}	δ_{C}
1		177.66 (C)	1'		176.89 (C)
3	2.97 (1H, ddd, $J = 11.4, 4.8, 2.4$ Hz)	53.99 (CH)	3'	3.20 (1H, dt, $J = 9.6, 3.0$ Hz)	52.09 (CH)
4	2.00 (1H, m)	56.39 (CH)	4'	2.70 (1H, dd, $J = 6.6, 3.0$ Hz)	52.15 (CH)
5	2.62 (1H, m)	37.56 (CH)	5'	2.46 (1H, m)	36.63 (CH)
6		142.02 (C)	6'		141.64 (C)
7	5.34 (1H, overlapped)	126.64 (CH)	7'	5.35 (1H, overlapped)	126.76 (CH)
8	3.38 (1H, br d, $J = 10.8$ Hz)	46.62 (CH)	8'	2.99 (1H, br d, $J = 12.0$ Hz)	45.12 (CH)
9		70.21 (C)	9'		69.70 (C)
10	1.72 (1H, m); 0.99 (1H, m)	49.43 (CH_2)	10'	1.12 (2H, m)	51.50 (CH_2)
11	1.17 (3H, d, $J = 6.6$ Hz)	14.91 (CH_3)	11'	1.21 (3H, d, $J = 7.2$ Hz)	13.69 (CH_3)
12	1.77 (3H, br s)	20.62 (CH_3)	12'	1.77 (3H, br s)	19.99 (CH_3)
13	5.93 (1H, d, $J = 11.4$ Hz)	127.33 (CH)	13'	6.18 (1H, d, $J = 10.8$ Hz)	125.77 (CH)
14		136.88 (C)	14'		137.89 (C)
15a	2.21 (1H, m); 2.03 (1H, m)	36.84 (CH_2)	15'	2.16 (2H, m)	41.14 (CH_2)
16a	1.94 (1H, m)	31.66 (CH_2)	16'a	2.08 (1H, m)	29.79 (CH_2)
16b	1.61 (1H, m)		16'b	1.43 (1H, m)	
17	3.83 (1H, m)	77.63 (CH)	17'	3.73 (1H, m)	73.69 (CH)
18	4.72 (1H, br d, $J = 3.6$ Hz)	70.86 (CH)	18'	3.67 (1H, d, $J = 11.4$ Hz)	89.36 (CH)
19	3.66 (1H, d, $J = 6.6$ Hz)	84.14 (CH)	19'	1.44 (1H, m)	42.01 (CH)
20	4.33 (1H, dd, $J = 9.6, 6.6$ Hz)	53.29 (CH)	20'a	3.82 (1H, d, $J = 17.4$ Hz)	36.32 (CH_2)
			20'b	2.03 (1H, m)	
21		219.04 (C)	21'		212.99 (C)
22	1.77 (1H, m)	26.78 (CH)	22'	1.66 (1H, m)	25.95 (CH)
23	0.93 (3H, d, $J = 6.6$ Hz)	24.87 (CH_3)	23'	1.03 (3H, d, $J = 7.2$ Hz)	25.64 (CH_3)
24	0.92 (3H, d, $J = 6.6$ Hz)	21.75 (CH_3)	24'	0.94 (3H, d, $J = 6.6$ Hz)	21.51 (CH_3)
25	1.51 (3H, s)	15.71 (CH_3)	25'	1.47 (3H, br s)	15.91 (CH_3)



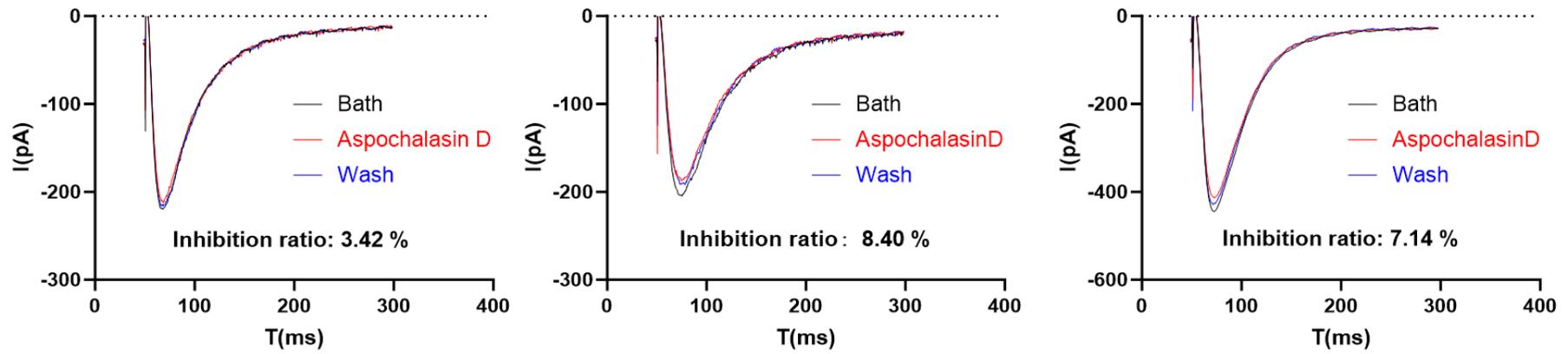


Figure S1. Inhibition of $Ca_v3.2$ peak current by aspochalasin D at a concentration of 10 μ M.

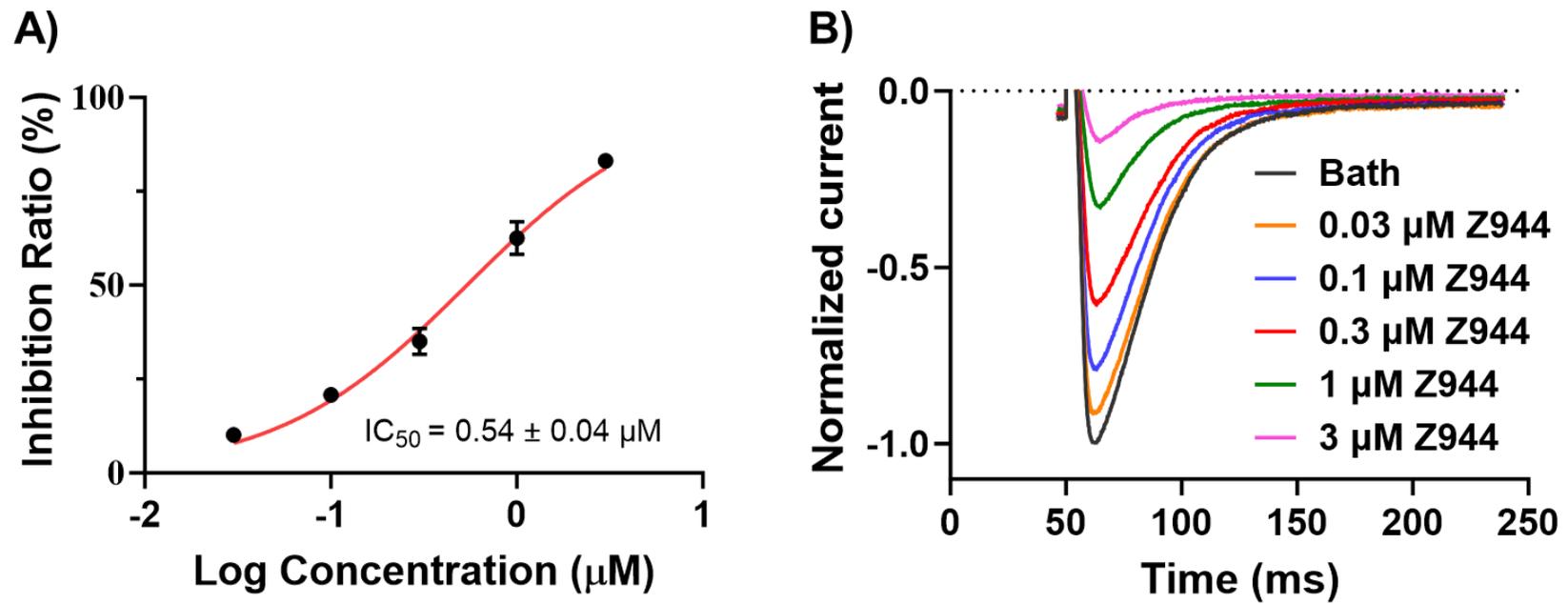


Figure S2. Inhibition of Cav3.2 expressed in HEK 293T cells by Z944. A) inhibition of Cav3.2 peak current by different concentrations of Z944. B) dose-response relationship of Cav3.2 peak current inhibition of Z944.

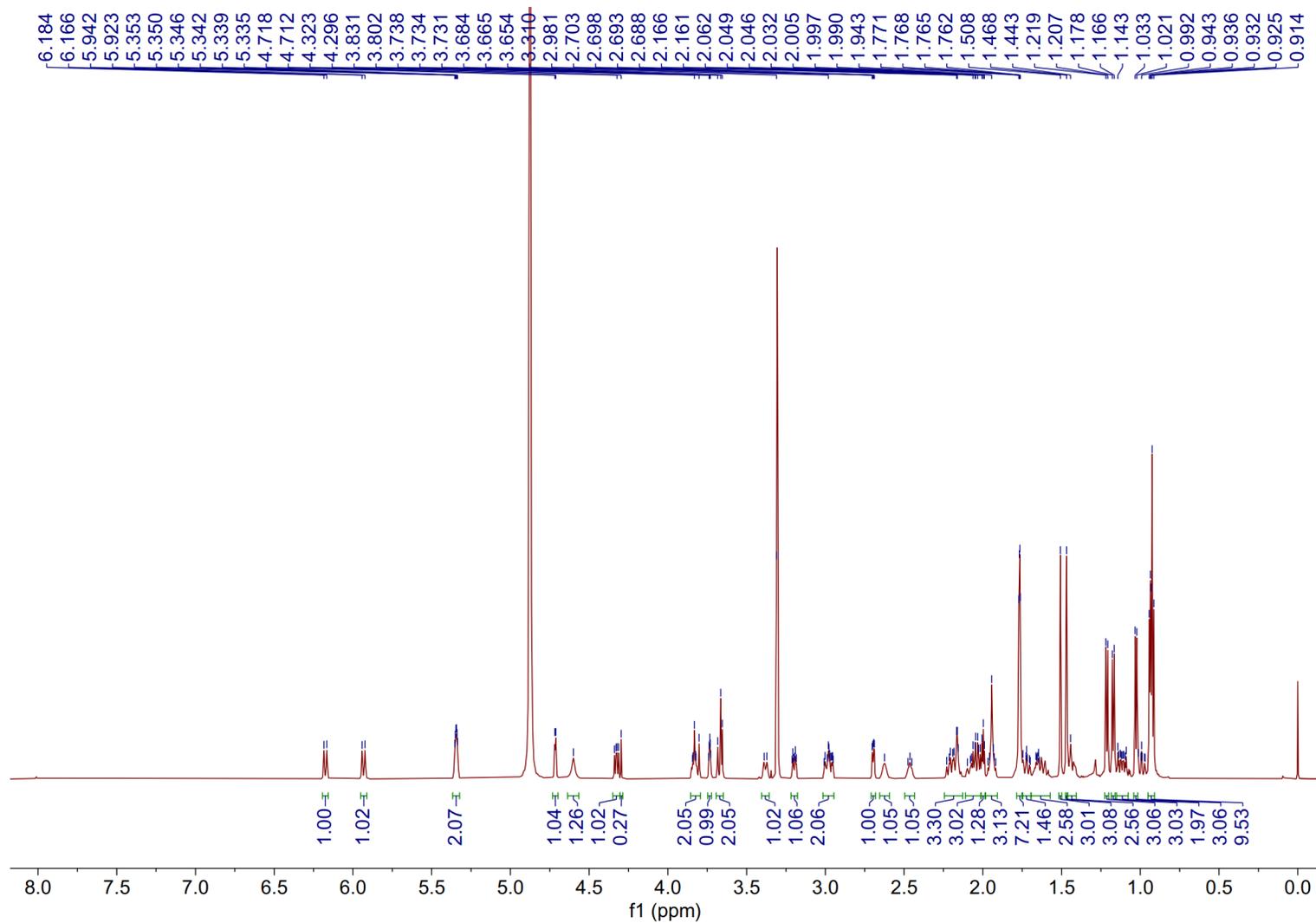


Figure S3. ^1H NMR (600 MHz) spectrum of **1** in CD_3OD .

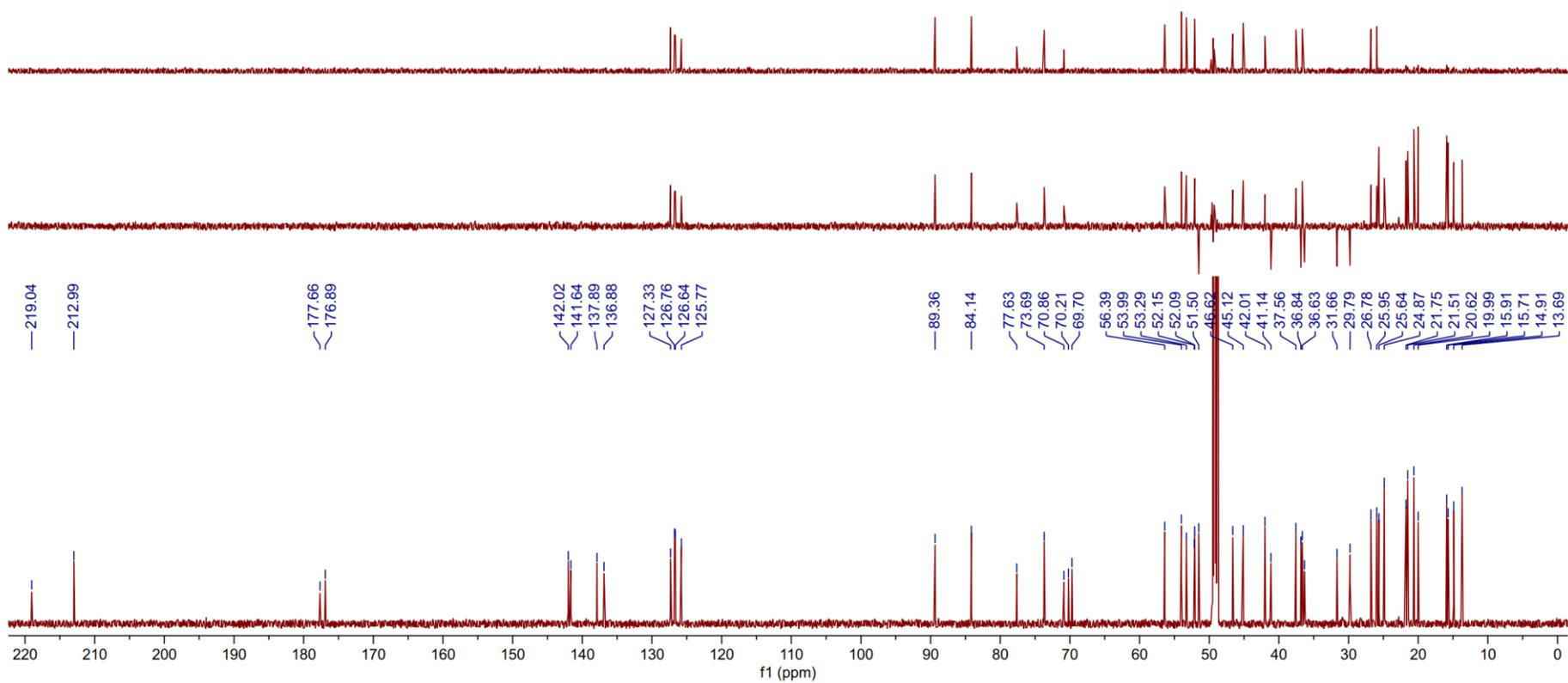


Figure S4. ^{13}C NMR (150 MHz) spectrum of **1** in CD_3OD .

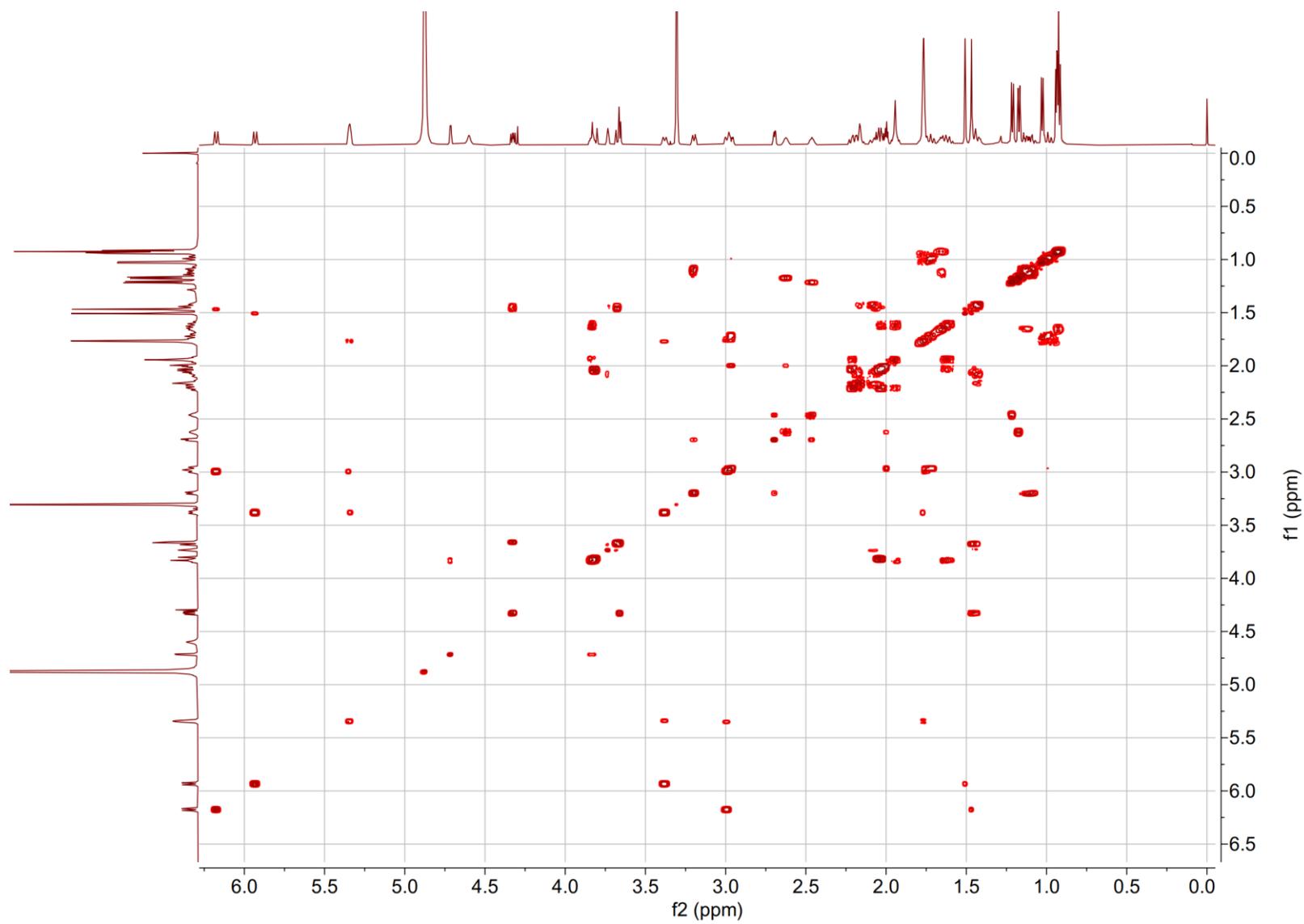


Figure S5. ^1H - ^1H COSY (600 MHz) spectrum of **1** in CD_3OD .

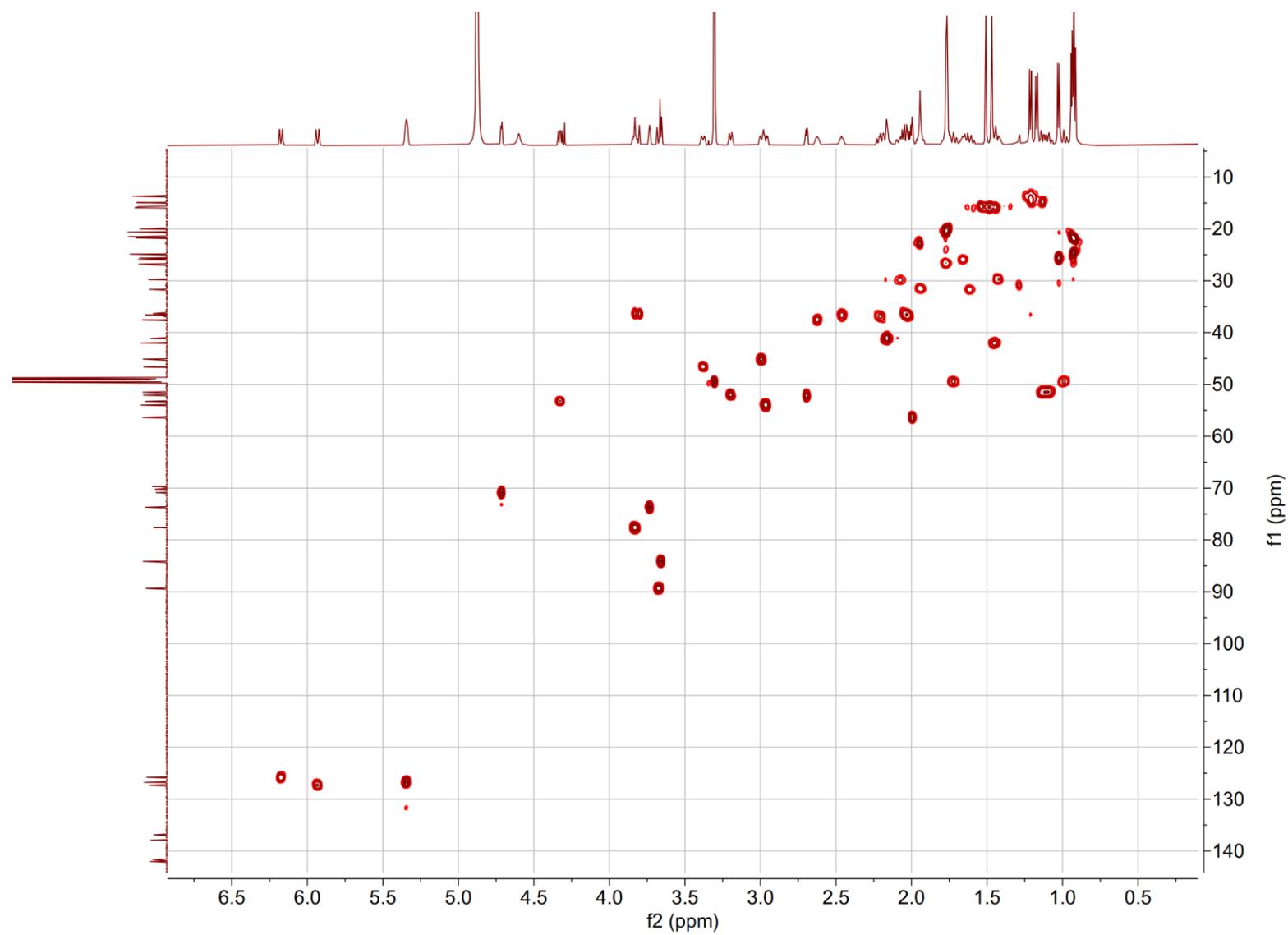


Figure S6. HSQC (600 MHz) spectrum of **1** in CD_3OD .

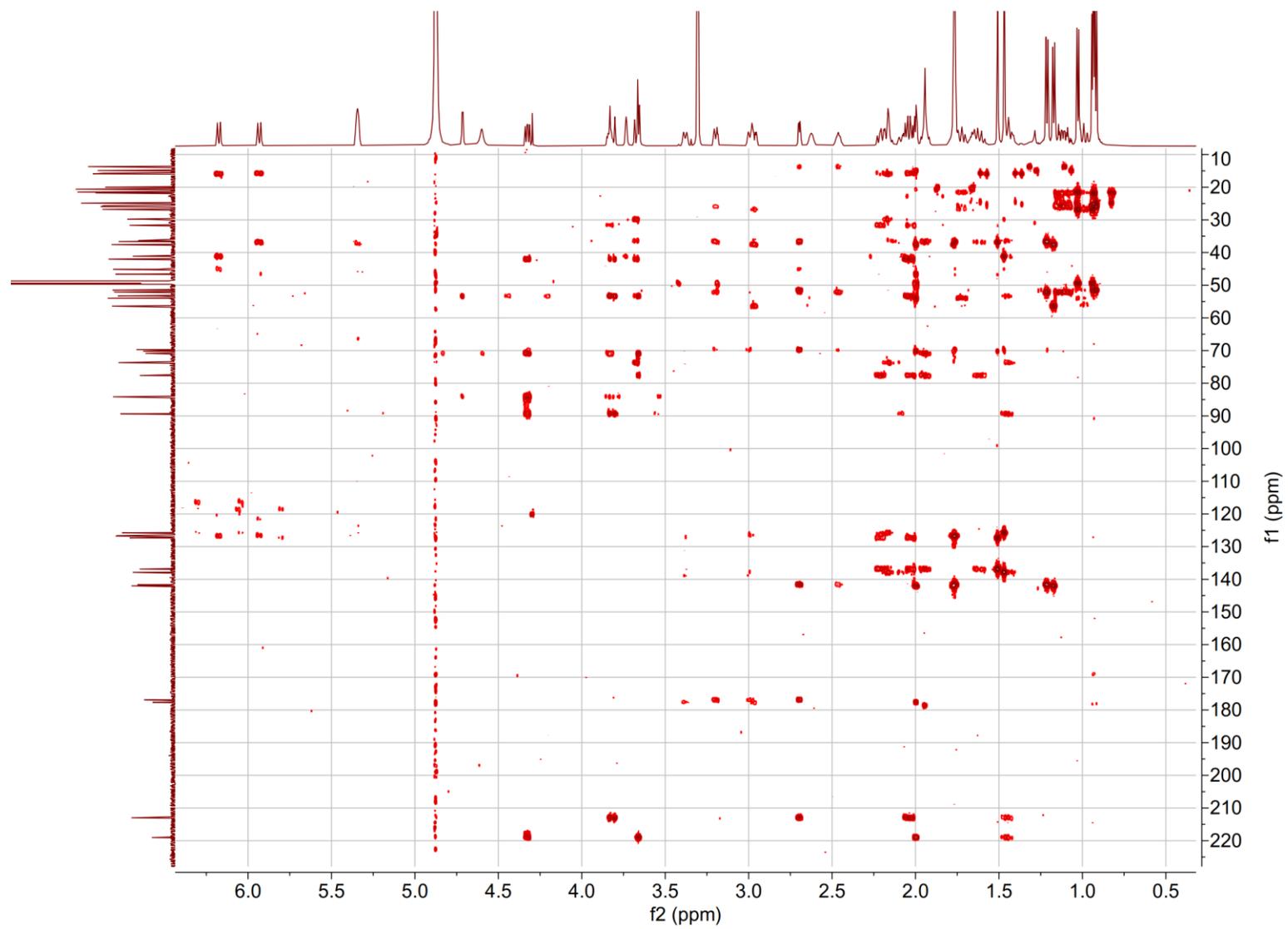


Figure S7. HMBC (600 MHz) spectrum of **1** in CD_3OD .

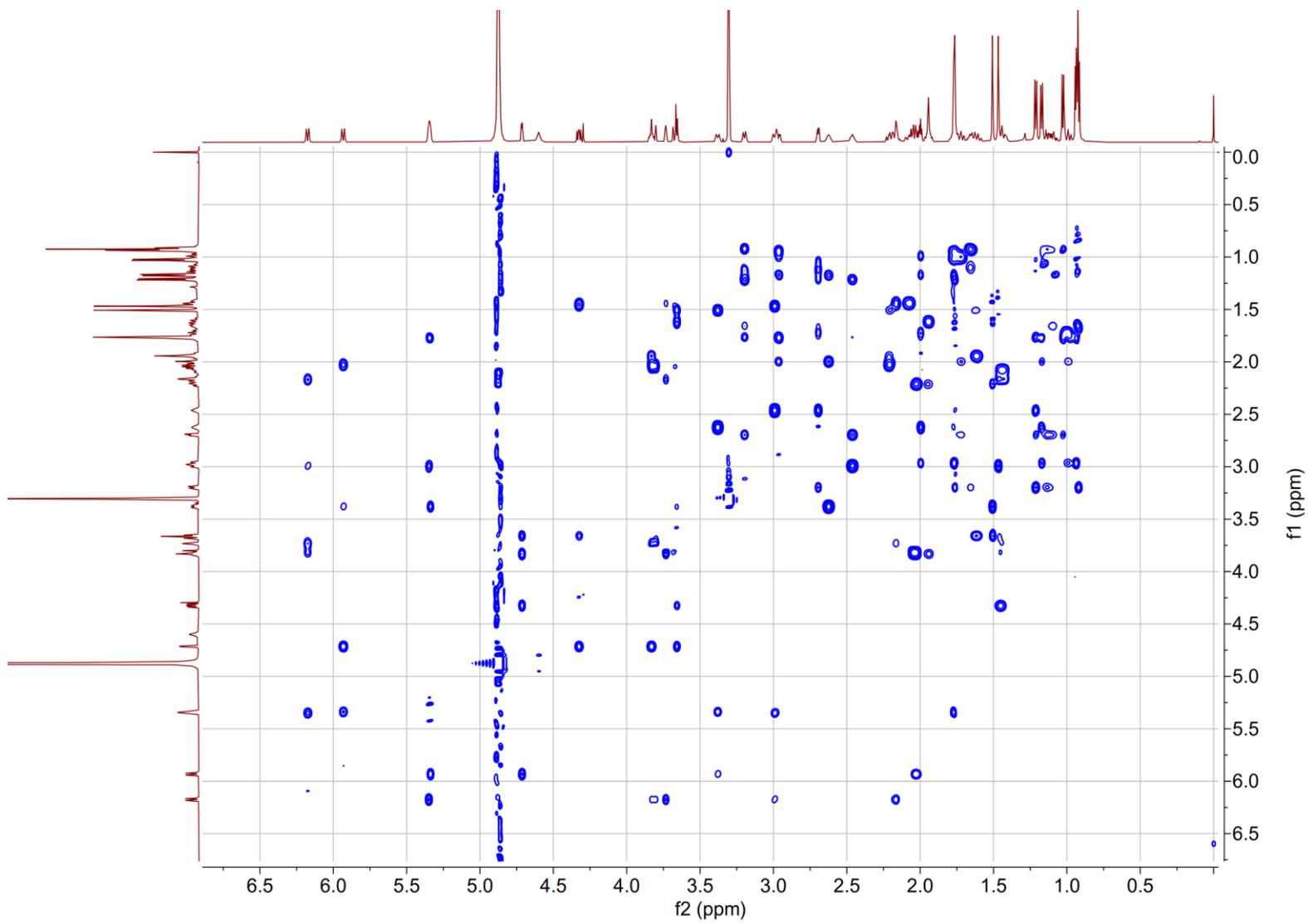
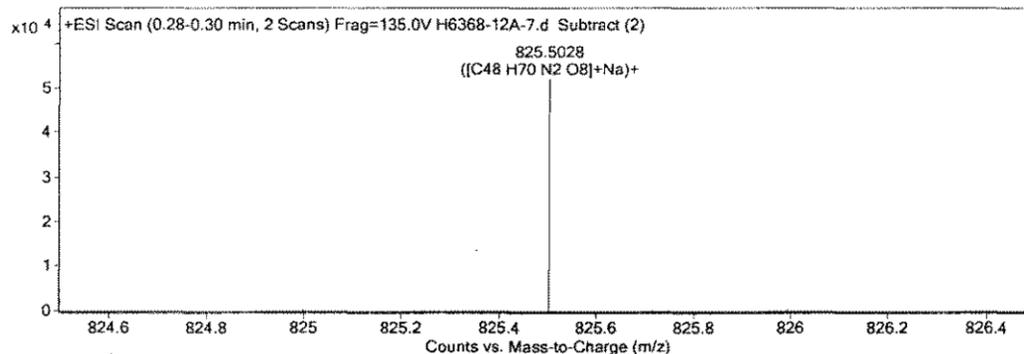


Figure S8. ROESY (600 MHz) spectrum of **1** in CD₃OD.

User Spectra

Fragmentor Voltage 135 Collision Energy 0 Ionization Mode ESI



Peak List

m/z	z	Abund	Formula	Ion
433.7623	2	8956.46		
441.7517	2	8074.65		
825.5028	1	51989.09	C48 H70 N2 O8	(M+Na)+
826.5055	1	27635.61	C48 H70 N2 O8	(M+Na)+
827.5087	1	8776.33	C48 H70 N2 O8	(M+Na)+
841.4768	1	14600.64		
842.4799	1	7650.62		
1628.0148	1	24551.76		
1629.0174	1	28418.23		
1630.0202	1	14562.92		

Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	120
O	0	30
N	0	5

Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C48 H70 N2 O8	802.5132	825.5024	825.5028	-0.40	-0.48	15.0000

Figure S9. HR-ESI-MS spectrum of 1.

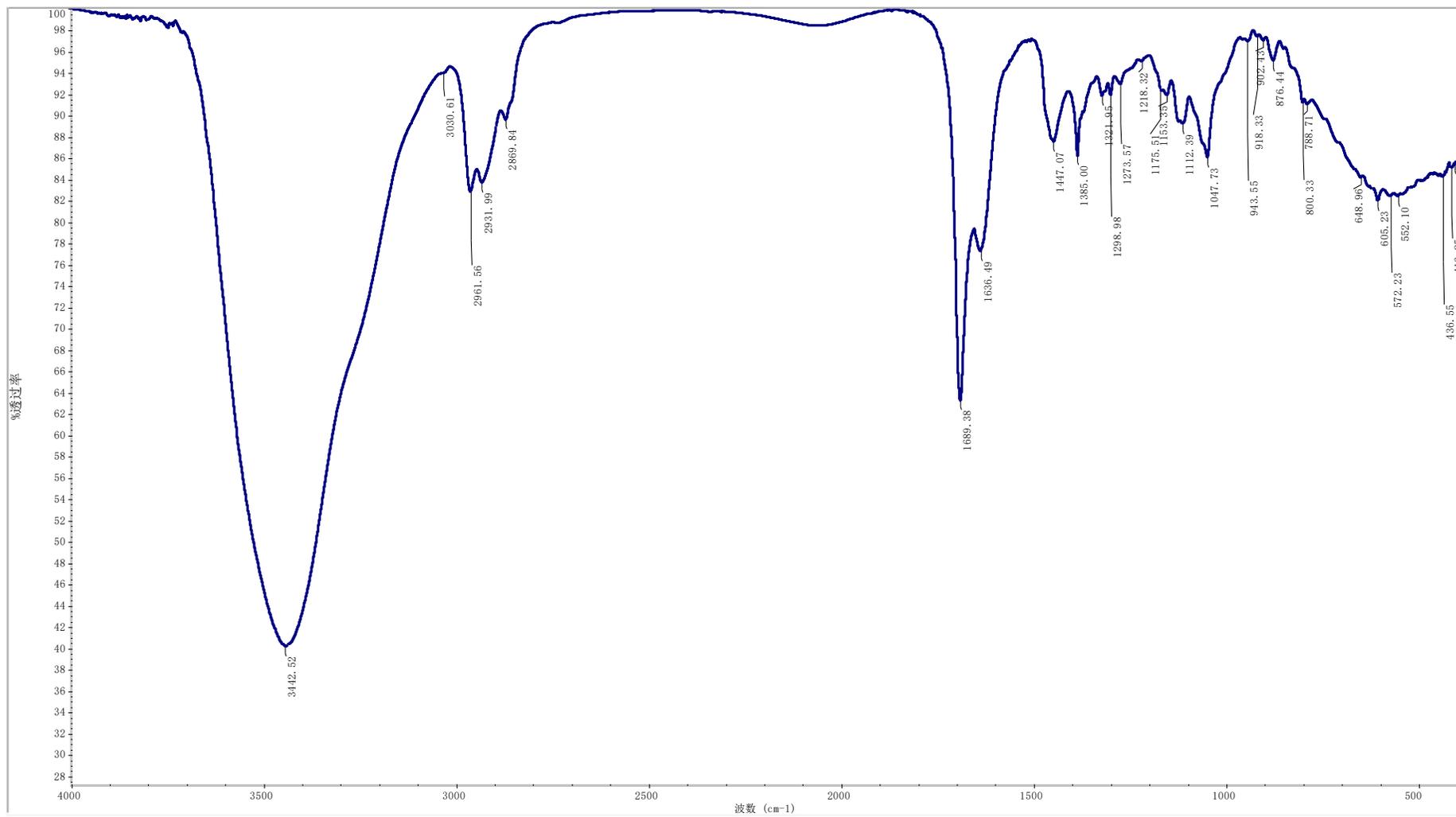


Figure S10. IR spectrum of 1.

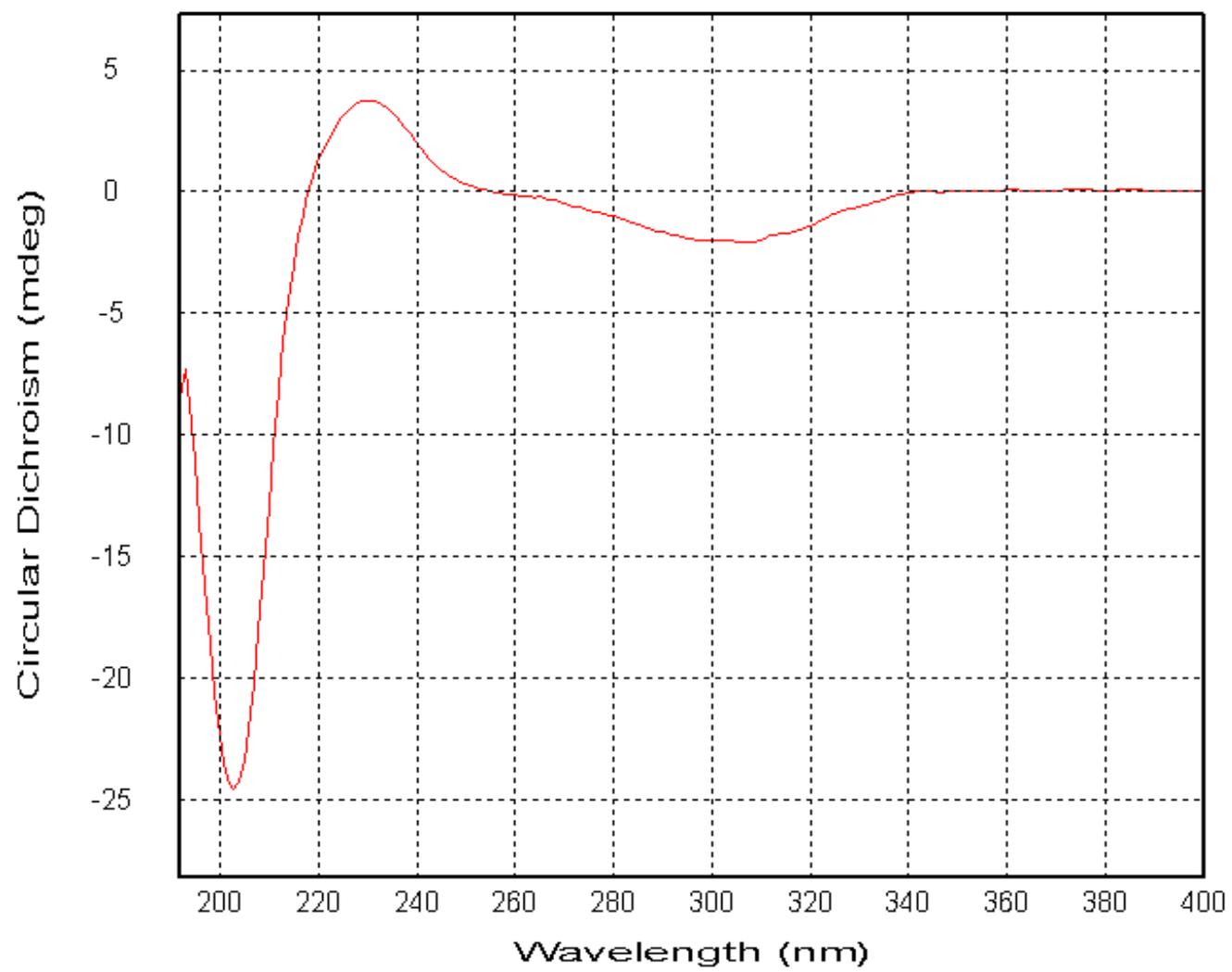


Figure S11. CD spectrum of **1**.

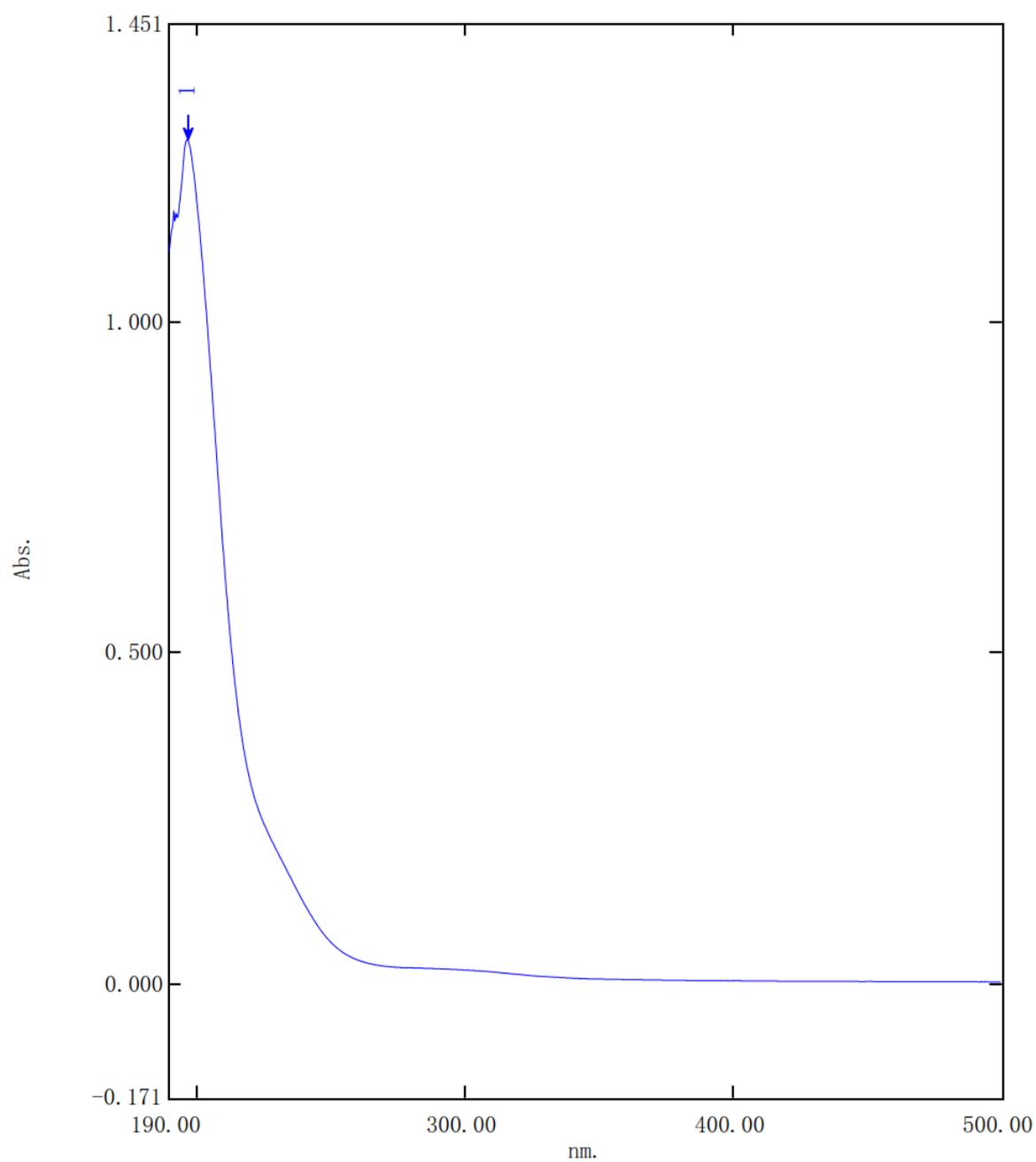


Figure S12. UV spectrum of **1**.