Supplementary Information of

Phomopsischalins A–C, Polycyclic-fused Cytochalasins from the Endophytic Fungus *Phomopsis* sp. shj2 and Their Abilities to Induce Lysosomal Function

Rong Chen, [‡a] Li-Jing Guo, [‡b] Xue-Dan Li,[b] Xing-Ren Li,[a] Kun Hu,[a] Jian-Wei Tang,[a] Zhen-Nan Ye,*[b] Bing-Chao Yan,*[a] Pema-Tenzin Puno*[a]

[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Yunnan Key Laboratory of Natural Medicinal Chemistry, Kunming Institute of Botany, University of Chinese Academy of Sciences, Chinese Academy of Sciences, Kunming 650201, Yunnan, China

[b] Guangdong Provincial Key Laboratory of Autophagy and Major Chronic Non-communicable Diseases, Affiliated Hospital of Guangdong Medical University, Zhanjiang 524001, China

Contents of Supplementary Information

1. General experimental procedures

CD spectra were measured on an Applied Photophysics Chirascan spectrophotometer. Optical rotations were measured with a JASCO P-1020 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. Scanning IR spectroscopy was performed using a Bruker Tensor-27 spectrophotometer with KBr pellets. NMR spectra were recorded on Bruker AV III 500 and AV Ⅲ-600 spectrometers. ESIMS and HRESIMS experiments were performed on a Bruker HCT/Esquire spectrometer and a Waters AutoSpec Premier P776 spectrometer. Column chromatography (CC) was performed with silica gel (100–200 mesh, Qingdao Marine Chemical, Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40–63 μm, Merck, Darmstadt, Germany). Preparative HPLC and semi-preparative HPLC were performed on an Agilent 1200 liquid chromatograph with a Zorbax SB-C_{18} (9.4 mm × 25 cm) column. Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH. Petroleum ether (PE, 60-90 °C), EtOAc, $CHCl_3$, acetone, MeOH, and EtOH were of analytical grade and obtained from Sinopharm Chemical Reagent Co. Ltd, China. All solvents were distilled before use.

2. Fungal Material

The culture of *Phomopsis* sp. shj2 was isolated from the stems of *Isodon eriocalyx* var. *laxiflora* collected from Kunming Botanical Garden, Kunming, People's Republic of China, in December 2012. The isolate was identified based on sequence (GenBank Accession No. KU533636, CGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGATCATTGCTGGAACGCGCCCCTG GCGCACCCAGAAACCCTTTGTGAACTTATACCTTACTGTTGCCTCGGCGCAGGCCGTCC CCCATGGGGTCCCTCTGGAGACGGAGGAGCAGCCGGCCGGTGGCCAAATTAACTCTGT TTTTACACTGAAACTCTGAGTACAAAACATAAATGAATCAAAACTTTCAACAACGGAT CTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGC AGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGAGG GCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCCTGGCTTGGTGTTGGGGCACTGC CTGTGAAAGGGCAGGCCCTGAAATATAGTGGCGAGCTCGCCAGGACTCCGAGCGTAGT AGTTAAACCCTCGCTTTGGAAGGCCTGGCGGTGCCCTGCCGTTAAACCCCAACTTTTG

AAAATTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATC) analysis of the ITS region of the rDNA. The fungal strain was cultured on slants of potato dextrose agar at 25 °C for 7 days. Agar plugs were cut into small pieces (about 0.5 × 0.5 × 0.5 cm^3) under aseptic conditions, and 15 pieces were used to inoculate three Erlenmeyer flasks (250 mL), each containing 50 mL of media (0.4% glucose, 1% malt extract, and 0.4% yeast extract); the final pH of the media was adjusted to 6.5, and the flasks were sterilized by autoclave. Three flasks of the inoculated media were incubated at 28 °C on a rotary shaker at 180 rpm for five days to prepare the seed culture. Fermentation was carried out in 125 Fernbach flasks (500 mL), each containing 80 g of rice. Spore inoculum was prepared in sterile, distilled H_2O to give a final spore/cell suspension of 1 × 10^6/mL. Distilled H_2O (120 mL) was added to each flask, and the contents were soaked overnight before autoclaving at 15 psi for 30 min. After cooling to room temperature, each flask was inoculated with 5.0 mL of the spore inoculum and incubated at 28 °C for 42 days.

3. Bioassay

3.1 Antimicrobial activity

Table S1. Anti-migratory activities against MDA-MB-231 in vitro of compounds **1–3** (IC_{50} in μM)

Compounds	**1**	**2**	**3**	**4**	**Positive control (cytochalasins D)**
IC_{50}	>25	>25	>25	3.58	0.2

Table S2. The inhibitory ratio of tested sample against six strains of bacteria and *C. albicans*

Sample	Conc. (μM)	抑制率（%）					
		Escherichia coli	*Staphylococcus aureus subsp. aureus*	*Salmonella enterica subsp. enterica*	*Pseudomonas aeruginosa*	Methicillin-resistant *Staphylococcus aureus* (MRSA)	*C. albicans*
1	100	1.351±1.806	-43.155±2.445	-12.091±0	-11.295±3.261	-12.302±1.371	-2.859±1.183
2	100	9.582±2.353	27.261±0.26	-8±0.579	-29.966±0.557	-5.443±0.739	-1.443±0.438

3	100	-3.201±0.236	-3.39±1.039	-18.91±1.35	-6.796±0.239	-16.328±2.636	-5.885±0.492
4	100	3.793±0.707	99.756±0.001	-8.75±0.097	15.307±2.386	99.951±0.087	-2.833±0.219
Penicillin G Sodium	14		99.878±0.212				
	28			99.864±0.137			
Ceftazidime	4	99.964±0			100.225±0.001		
Vancomycin	0.7					99.901±0.15	
Amphotericin B	0.5						100.078±0.078

Table S3. The MIC_{50} of the tested samples against *Staphylococcus aureus* subsp. *aureus*

Sample	Conc. (μg/mL)	Inhibitory ratio (%)	MIC_{50} (μg/mL)
Penicillin G Sodium	14	100.237±0.001	2.629±0.003
	7	100.592±0	
	3.5	100.119±0.205	
	1.75	-21.159±0.739	
	0.875	27.542±1.003	
4	100	100.237±0.355	53.279±1.248
	50	44.918±2.006	
	25	38.889±1.505	
	12.5	21.336±3.762	
	6.25	-69.268±4.012	

Table S4. The MIC_{50} of the tested samples against MRSA

Sample	Conc. (μg/mL)	Inhibitory ratio (%)	MIC_{50} (μg/mL)
Vancomycin	0.7	99.879±0.105	0.468±0.001
	0.35	14.273±0.129	
	0.175	5.001±0.9	
	0.0875	-0.455±0.129	
	0.04375	-17.637±0.772	
4	100	100.091±0.129	36.791±0.27
	50	100±0	
	25	-13±2.7	
	12.5	-36.455±0.9	
	6.25	-68.91±2.058	

4. NMR, HRESIMS, CD, UV, OR, and IR spectra

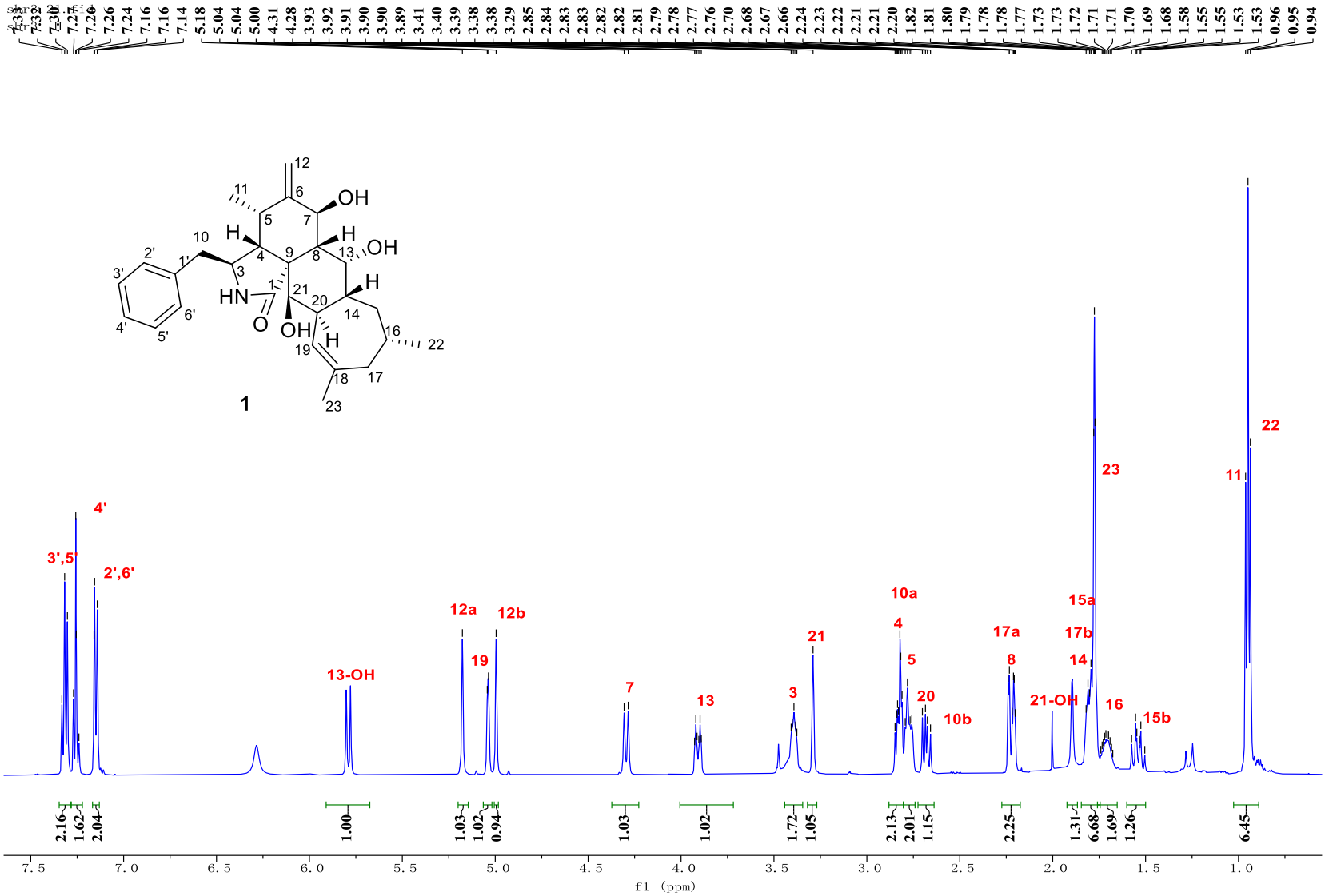


Figure S7. ^{1}H NMR spectrum of **1** (500 MHz, $CDCl_3$).

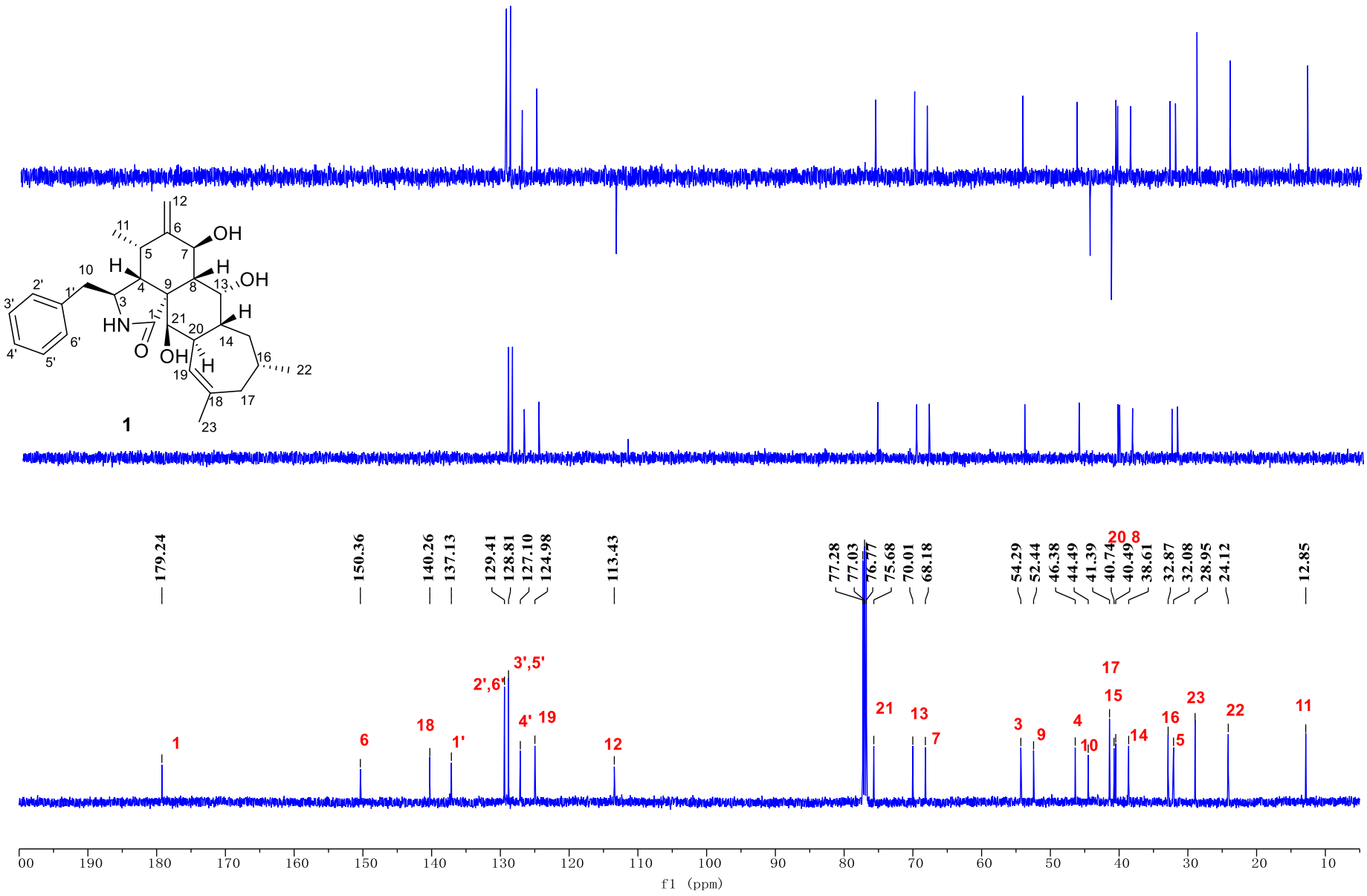


Figure S8. ^{13}C NMR spectrum of **1** (125 MHz, $CDCl_3$).

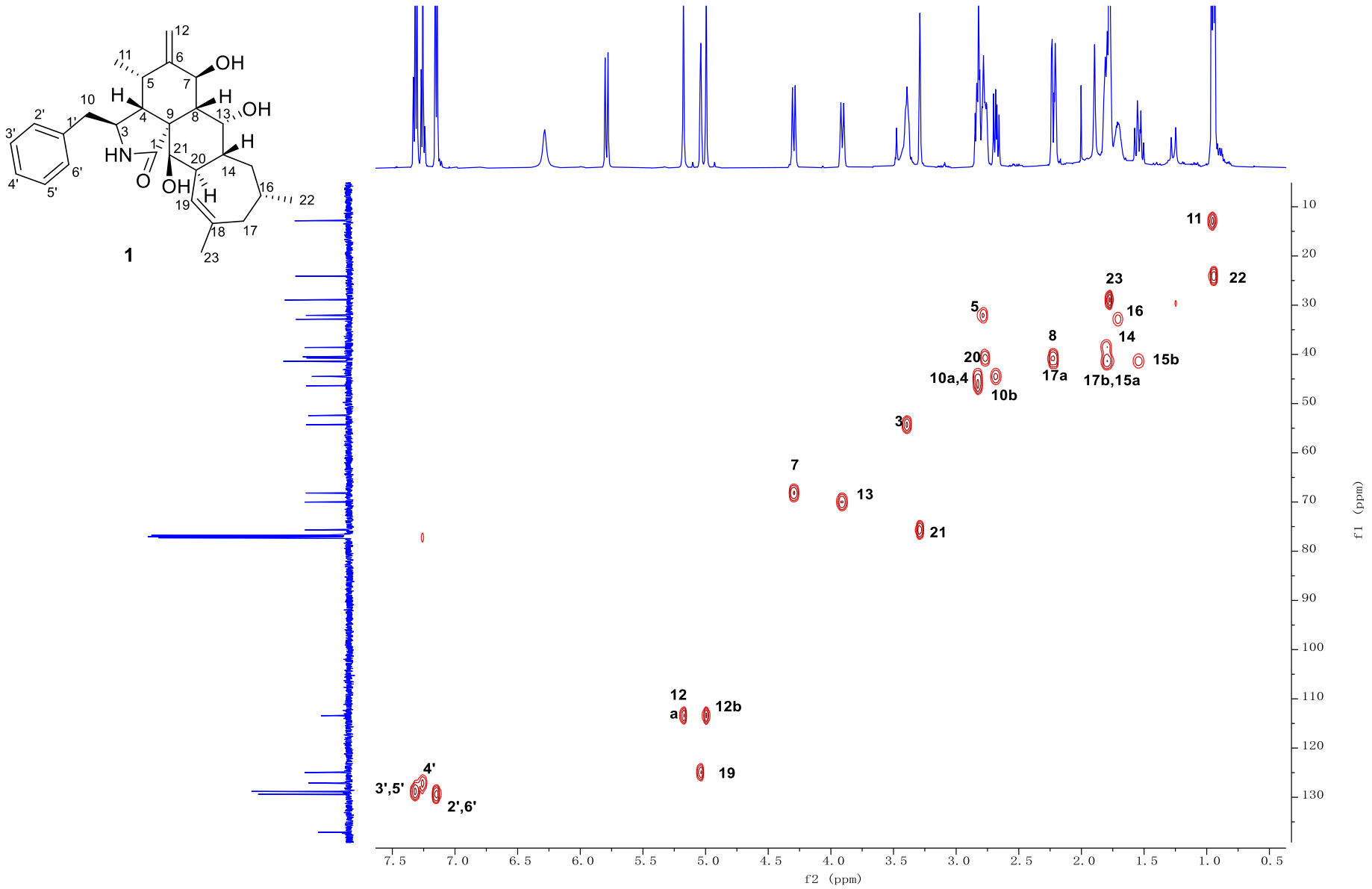


Figure S9. HSQC spectrum of **1** ($CDCl_3$).

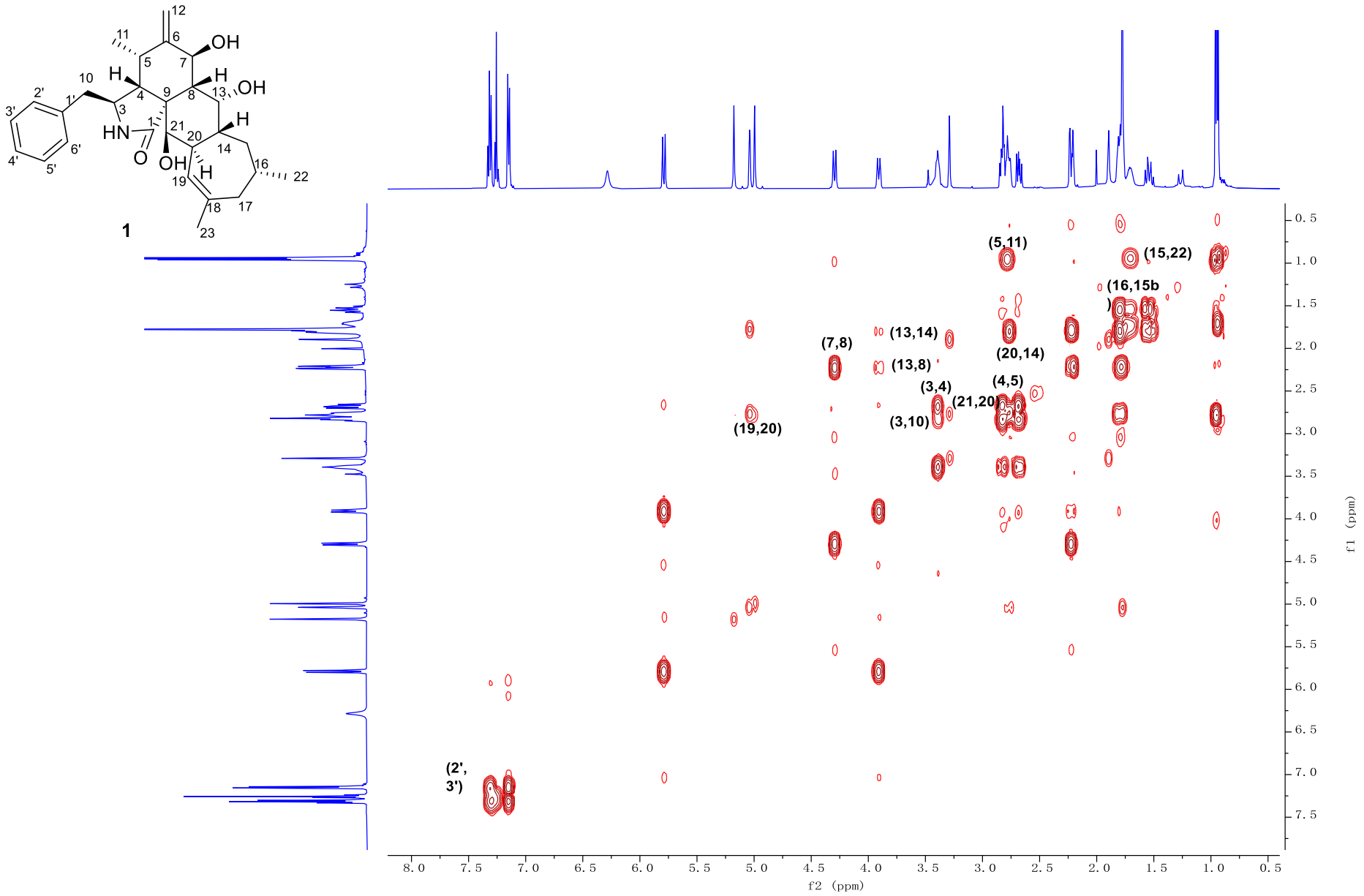


Figure S10. ^{1}H–^{1}H COSY spectrum of **1** ($CDCl_3$).

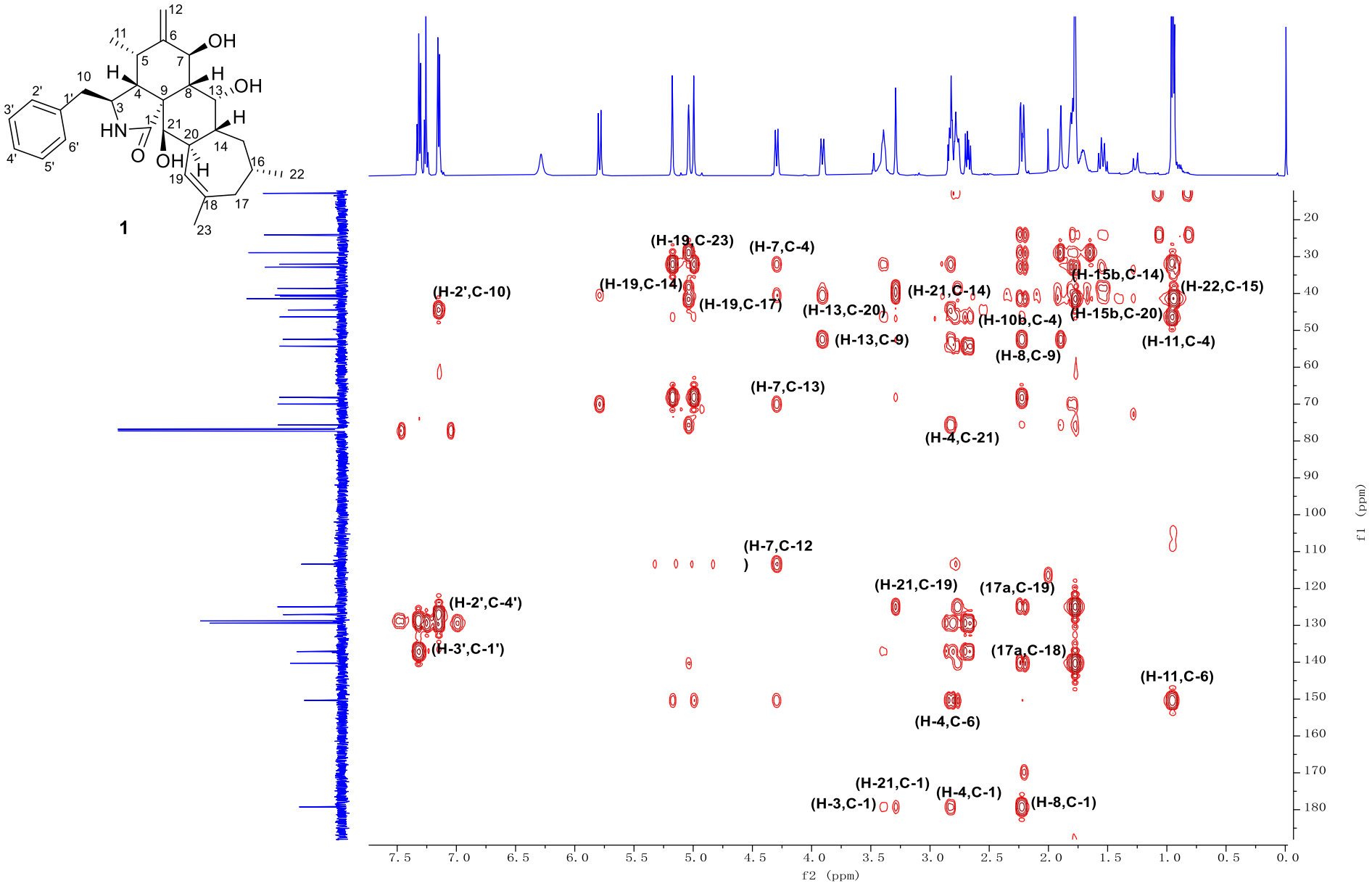


Figure S11. HMBC spectrum of **1** ($CDCl_3$).

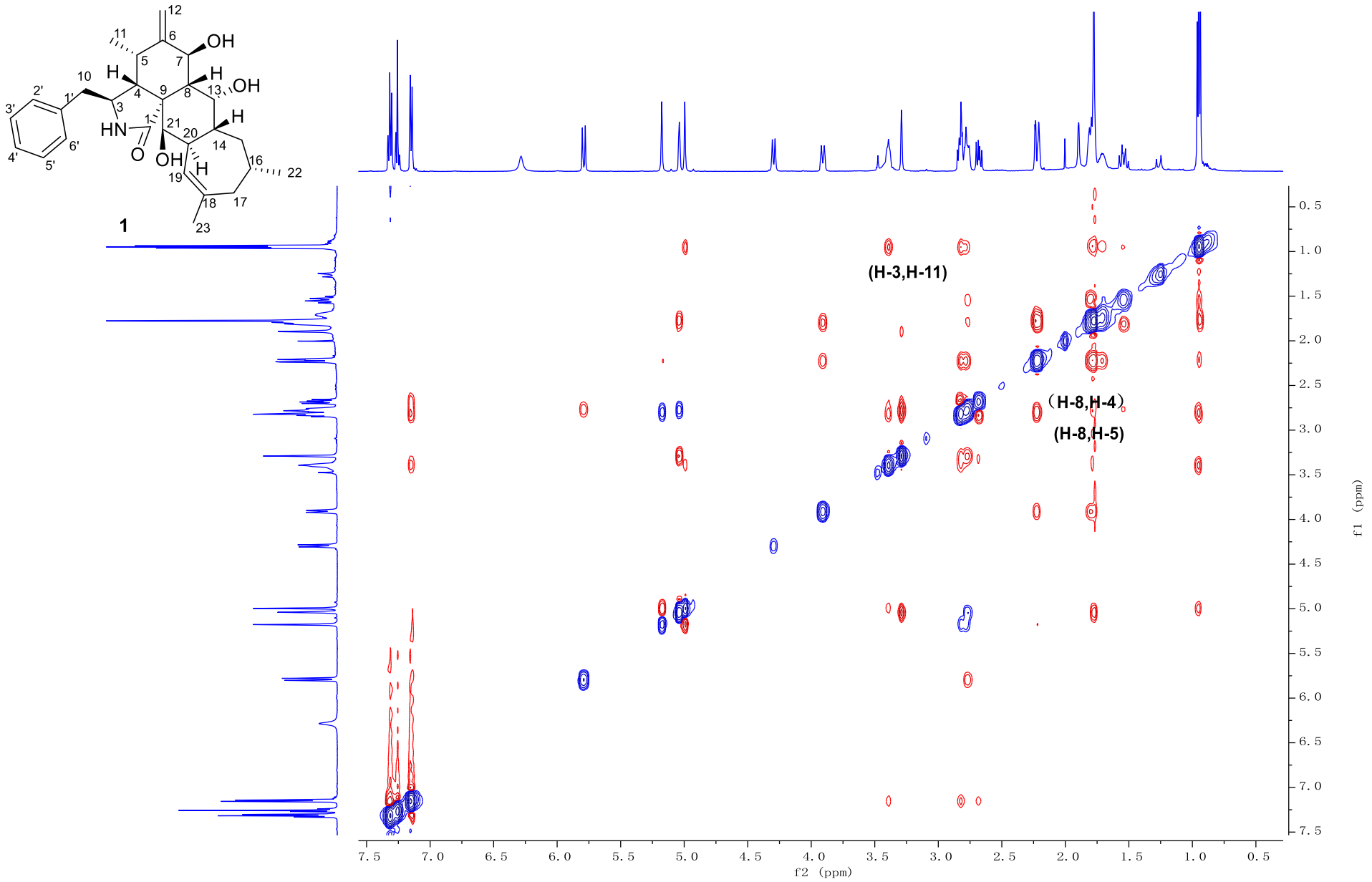


Figure S12. ROESY spectrum of **1** ($CDCl_3$).

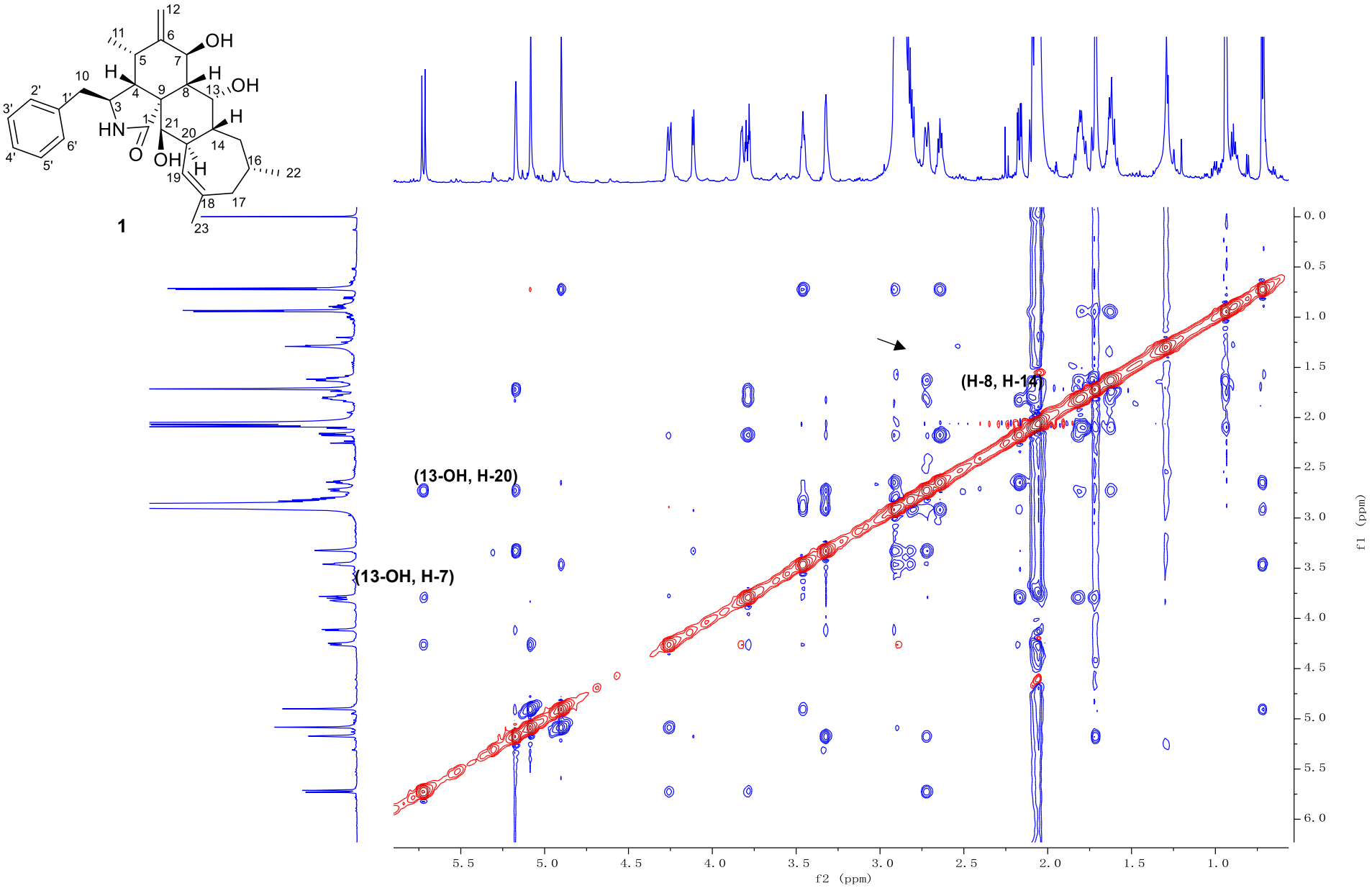


Figure S13. ROESY spectrum of **1** (Acetone-d_6).

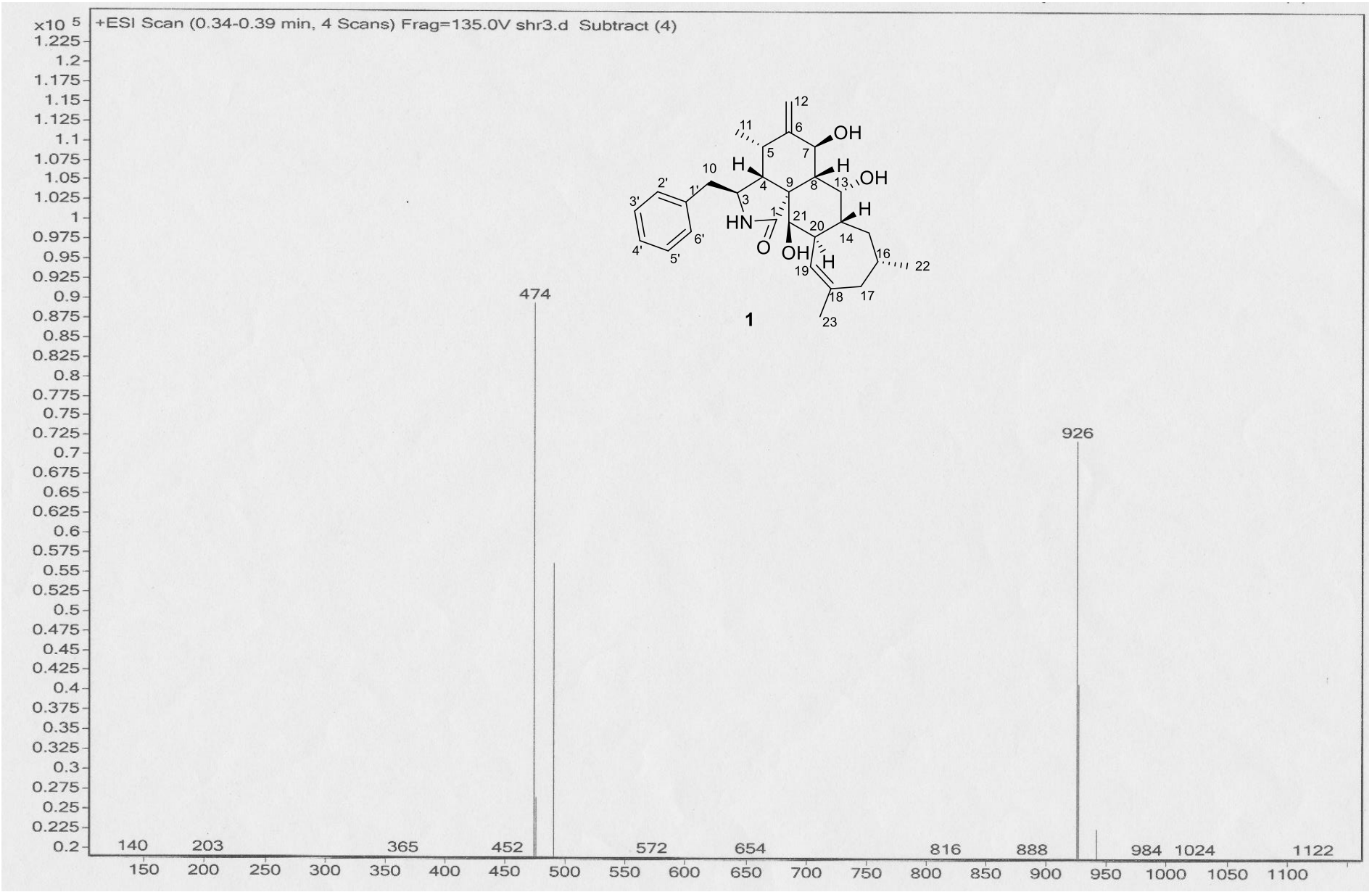
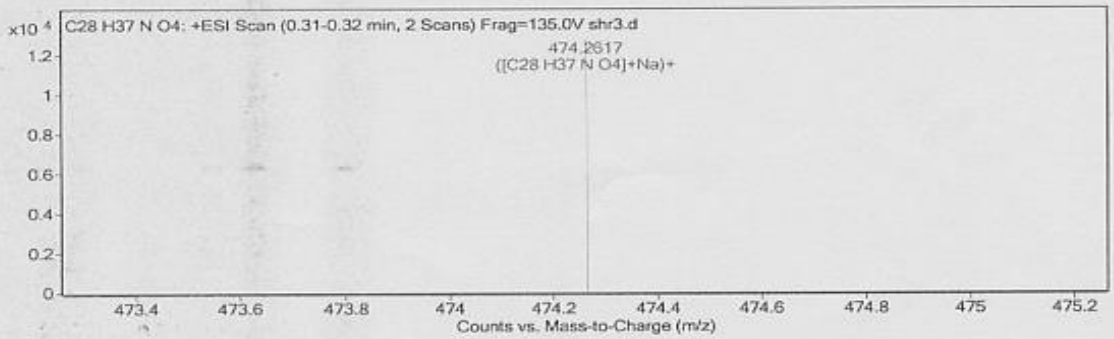


Figure S14. ESIMS spectrum of **1**.

Sample Group	Info.
Acquisition SW	6200 series TOF/6500 series
Version	Q-TOF B.05.01 (B5125.2)

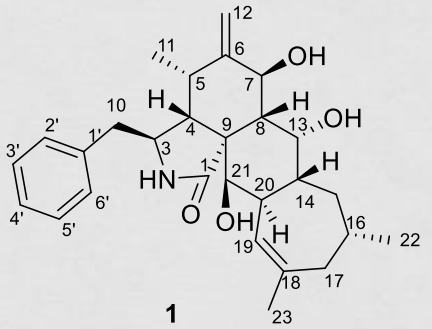
User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	ESI



Peak List

m/z	z	Abund	Formula	Ion
128.9537		2753.73		
239.1614	1	2514.73		
245.078	1	2732.77		
474.2617	1	12673.1	C28 H37 N O4	(M+Na)+
475.2648	1	3789.87	C28 H37 N O4	(M+Na)+
490.2358	1	16934.91		
491.2399	1	5061.51		
922.0098	1	3916.07		
925.5339	1	4006.89		
959.9661	1	6612.91		



1

Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	120
O	0	30
N	0	10

Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C28 H37 N O4	451.2723	474.2615	474.2617	-0.2	-0.3	11.0000

Figure S15. HRESIMS spectrum of **1**.

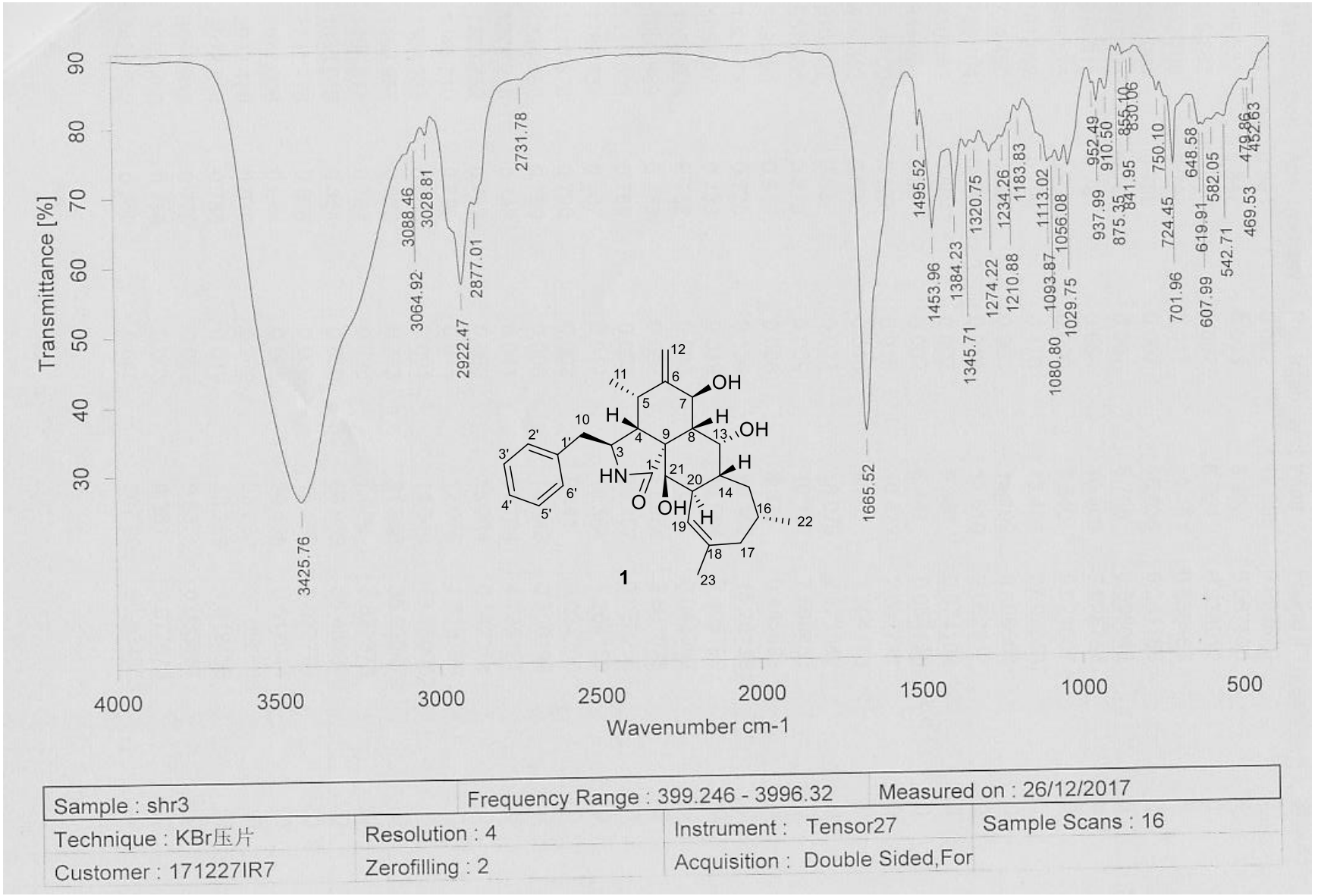


Figure S16. IR spectrum of **1**.

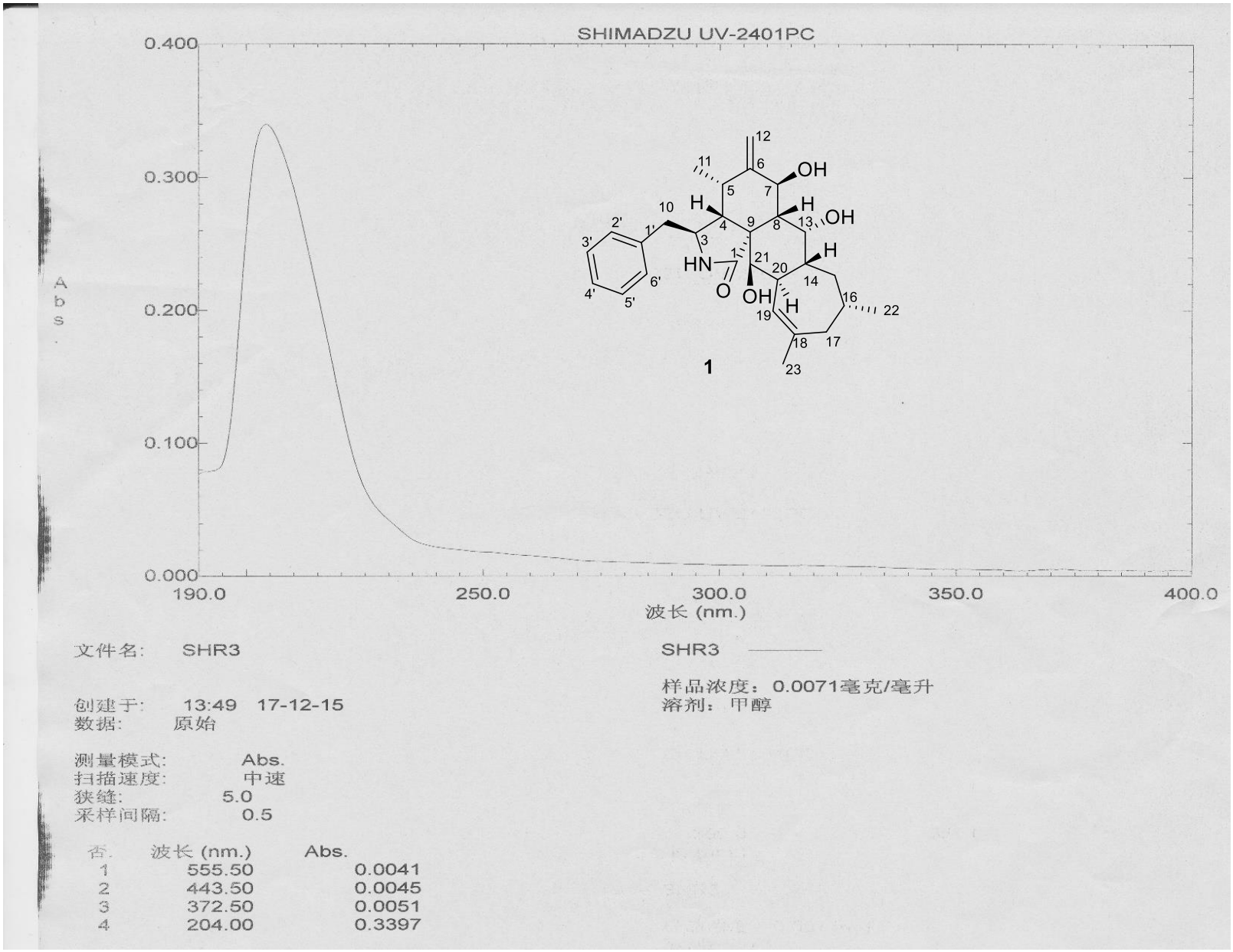


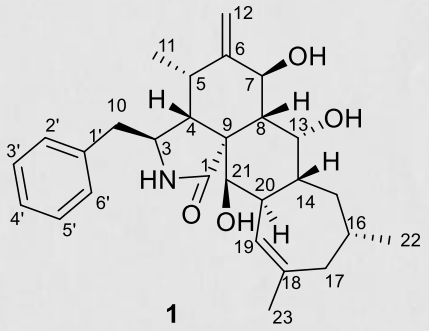
Figure S17. UV spectrum of **1**.

Optical rotation measurement

Model : P-1020 (A060460638)

No.	Sample	Mode	Data	Monitor Blank	Temp. Cell Temp Point	Date Comment Sample Name	Light Filter Operator	Cycle Time Integ Time
No.1	18 (1/3)	Sp.Rot	57.3170	0.0235 0.0000	20.7 50.00 Cell	Thu Nov 23 03:19:47 2017 0.00082g/mL MeOH SHR3	Na 589nm	2 sec 2 sec
No.2	18 (2/3)	Sp.Rot	54.3900	0.0223 0.0000	20.7 50.00 Cell	Thu Nov 23 03:19:52 2017 0.00082g/mL MeOH SHR3	Na 589nm	2 sec 2 sec
No.3	18 (3/3)	Sp.Rot	52.9270	0.0217 0.0000	20.7 50.00 Cell	Thu Nov 23 03:19:57 2017 0.00082g/mL MeOH SHR3	Na 589nm	2 sec 2 sec

+74.8°



1

Figure S18. ORD spectrum of **1**.

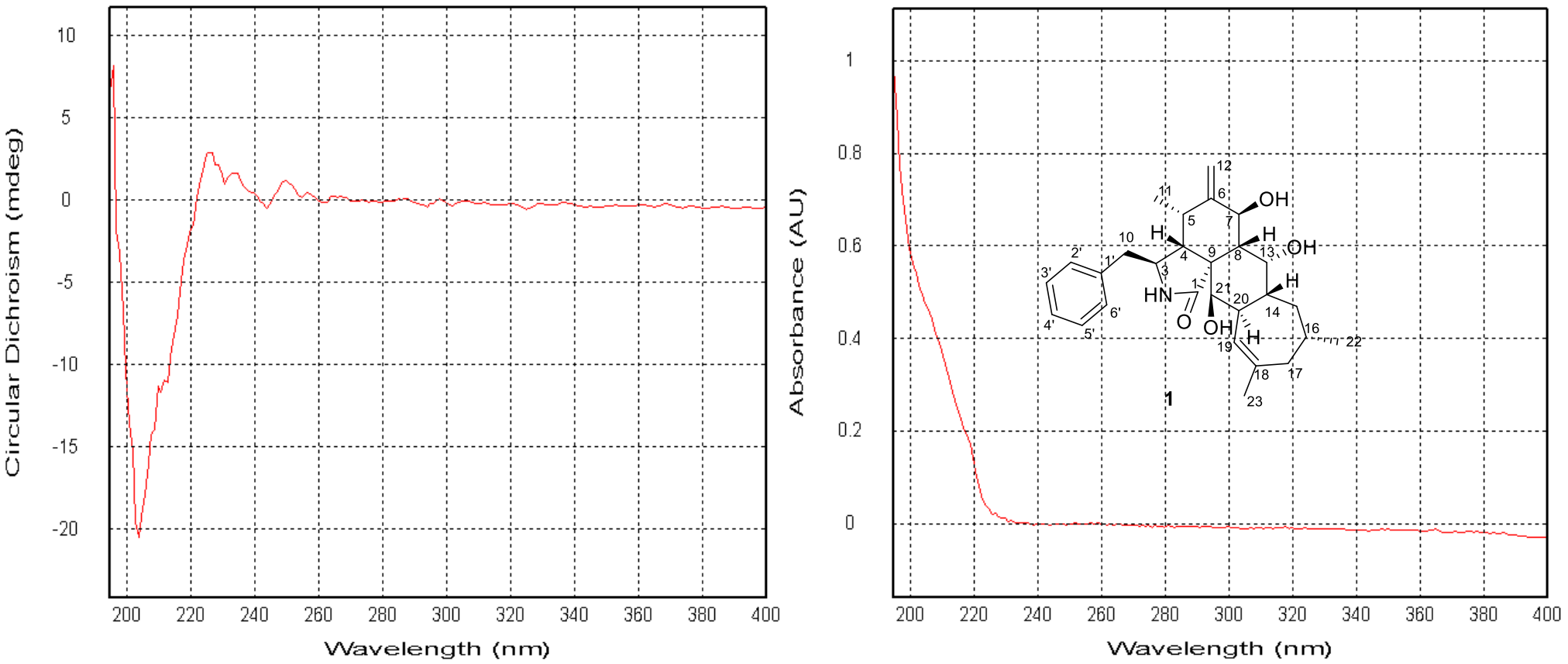


Figure S19. CD spectrum of **1**.

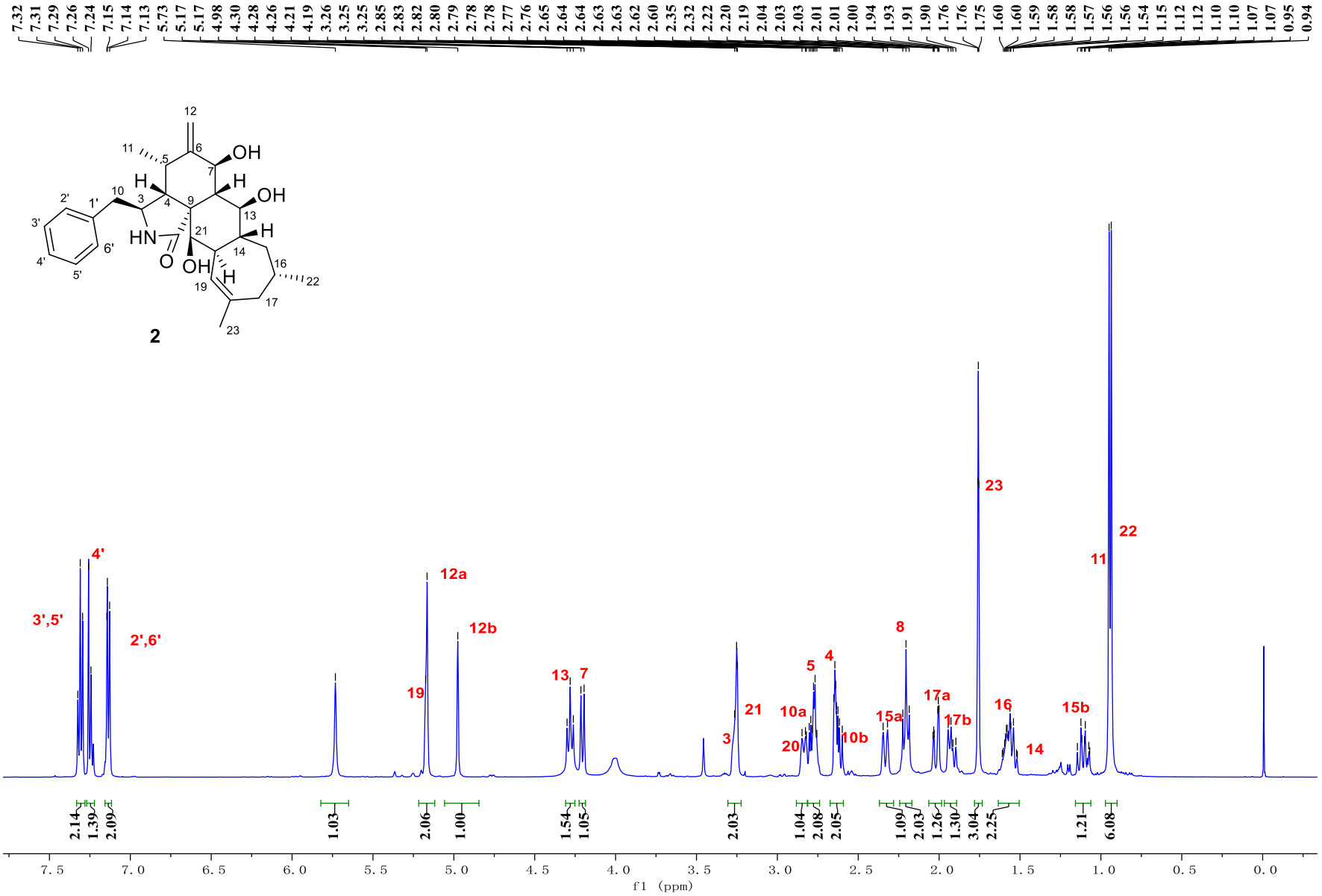


Figure S20. ^{1}H NMR spectrum of **2** (500 MHz, $CDCl_3$).

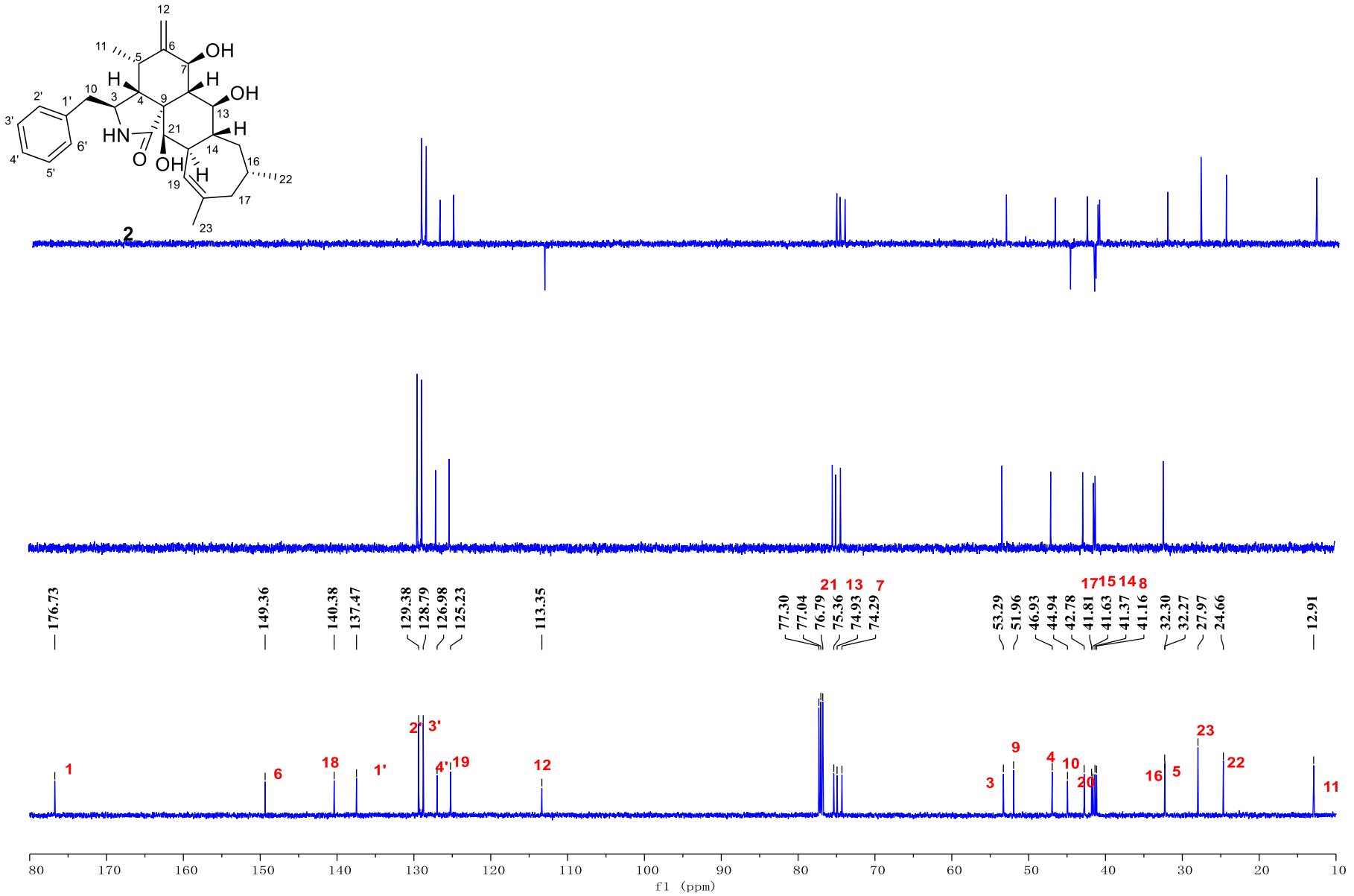


Figure S21. ^{13}C NMR spectrum of **2** (125 MHz, $CDCl_3$).

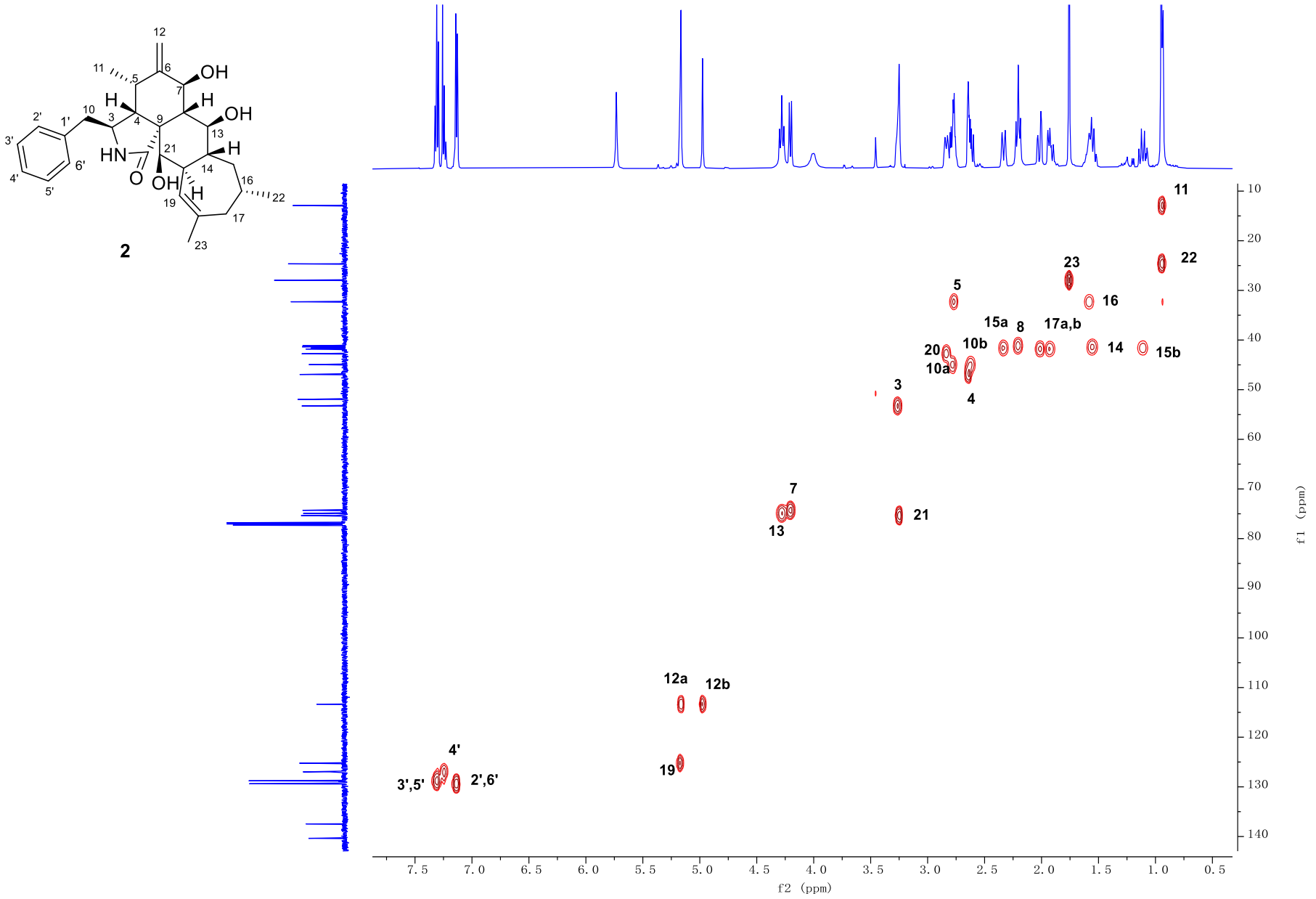


Figure S21. HSQC spectrum of **2** ($CDCl_3$).

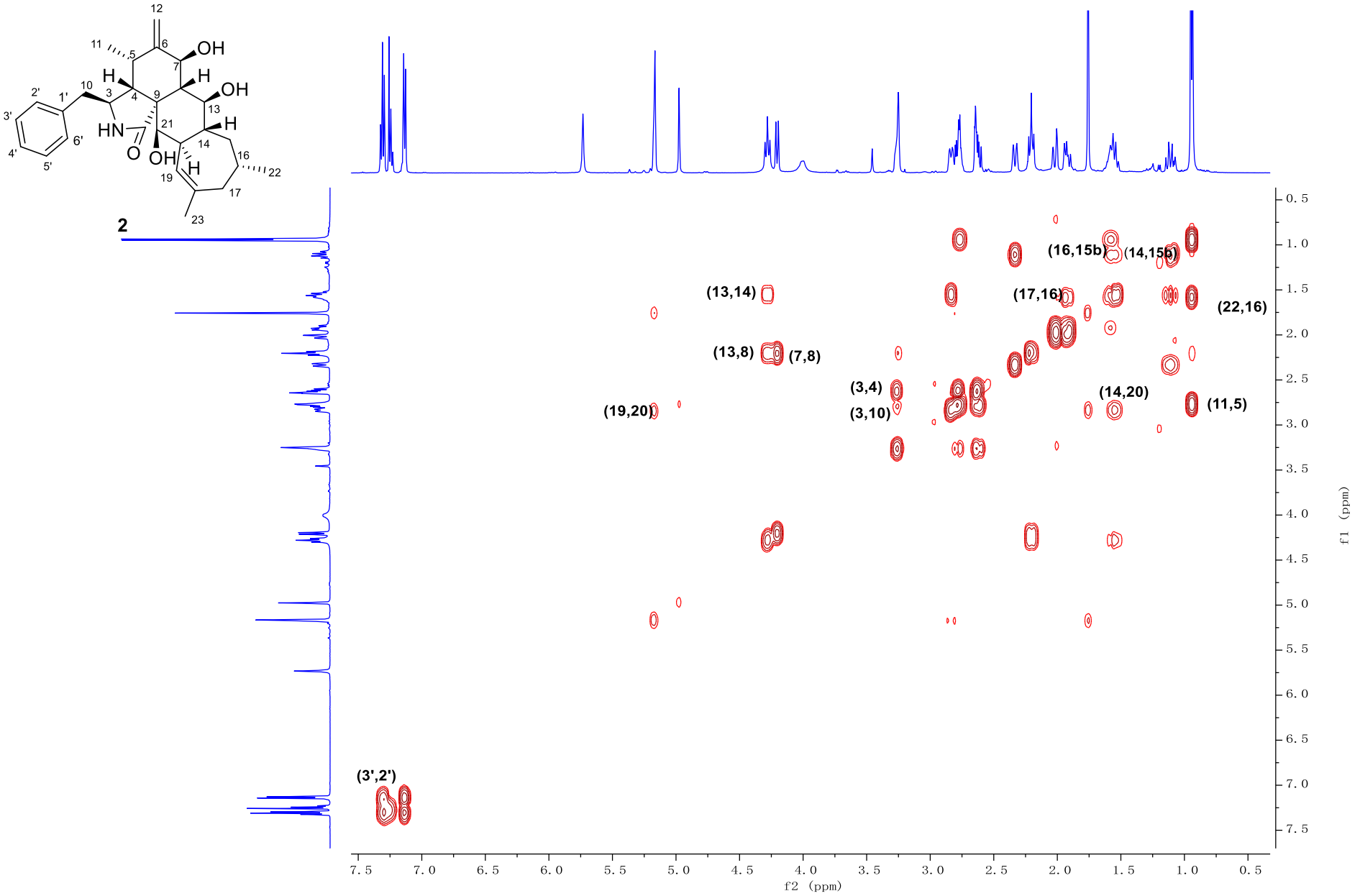


Figure S22. ^{1}H–^{1}H COSY spectrum of **2** ($CDCl_3$).

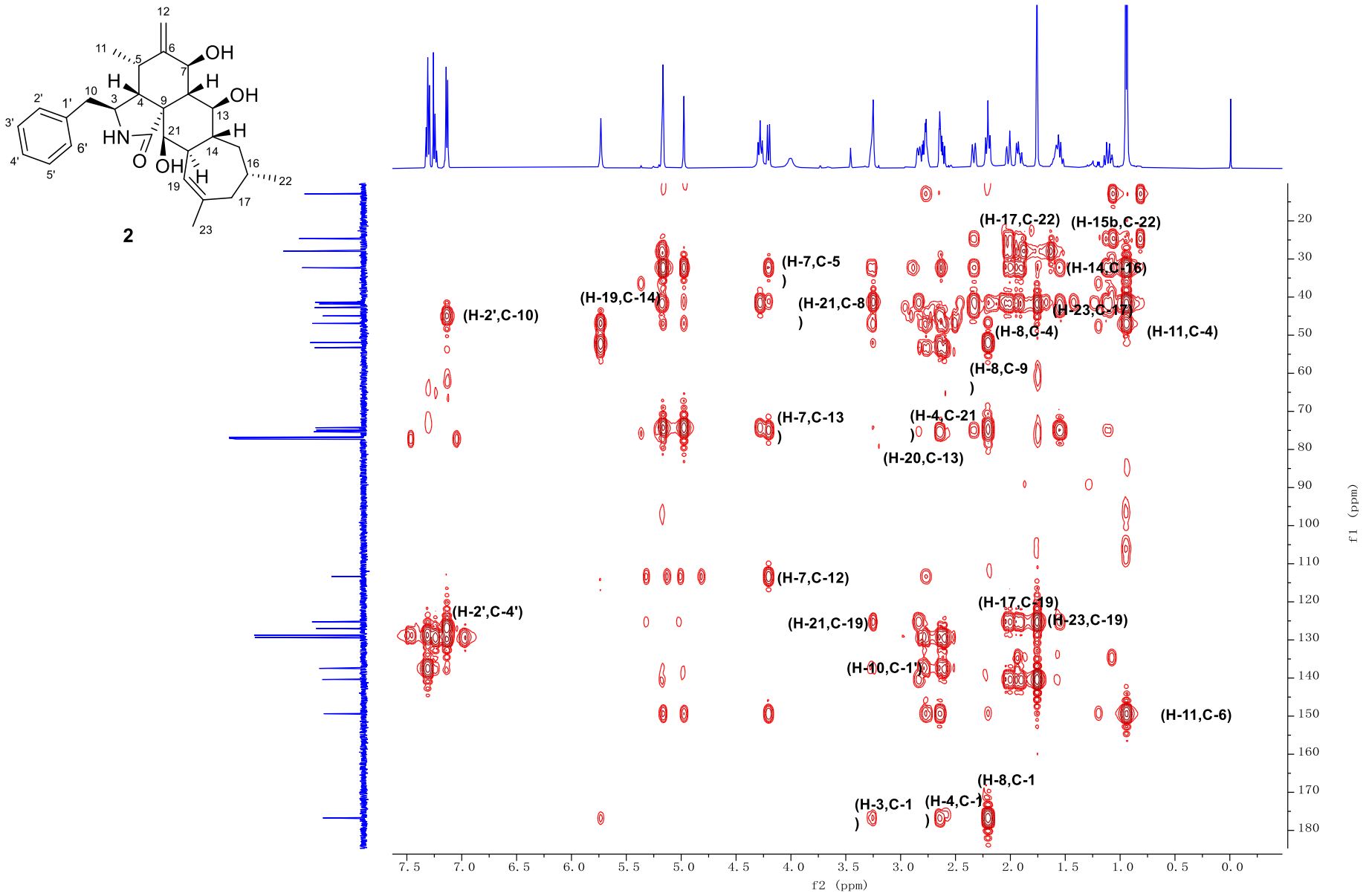


Figure S23. HMBC spectrum of **2** ($CDCl_3$).

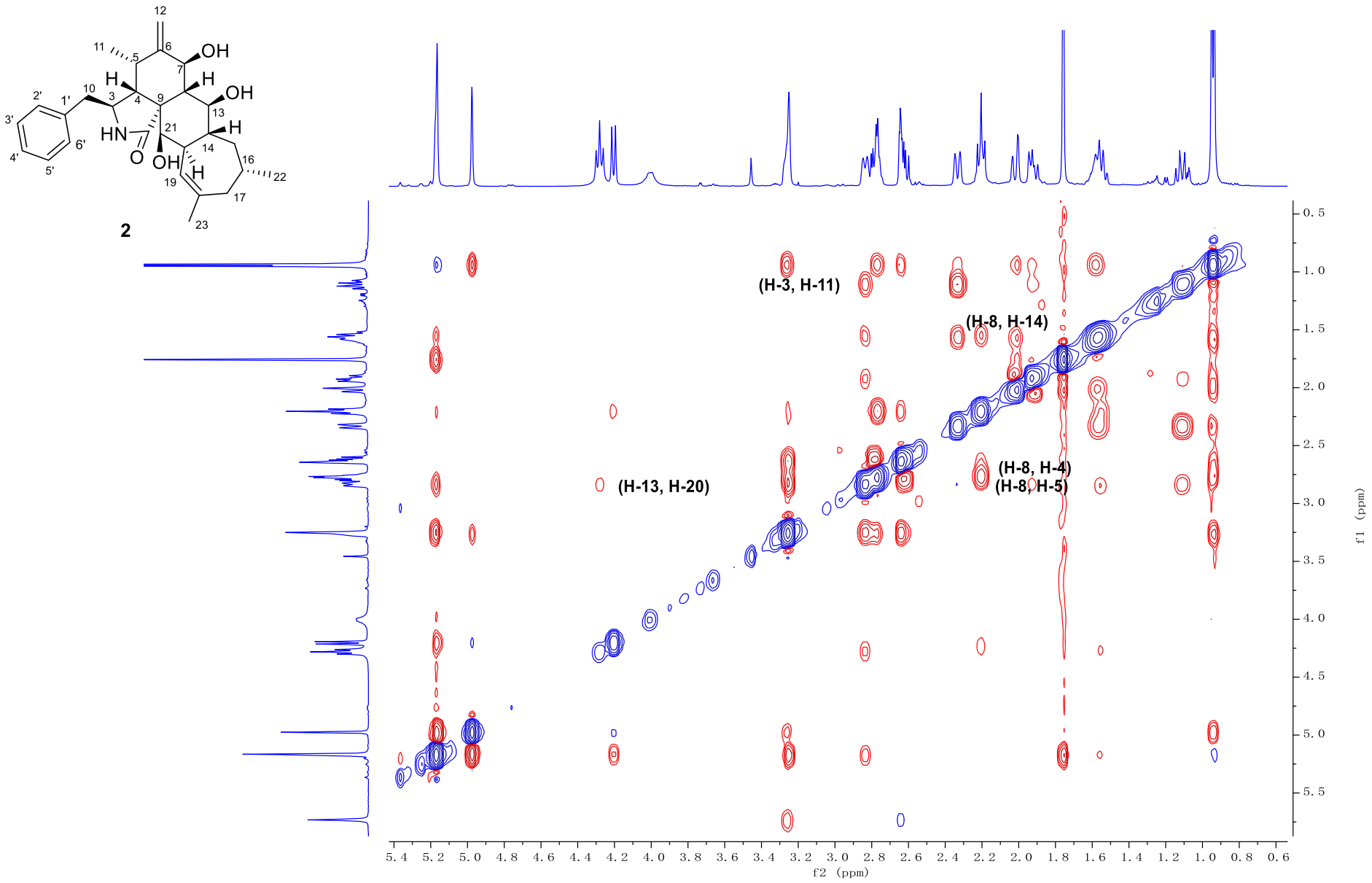


Figure S24. ROESY spectrum of **2** ($CDCl_3$).

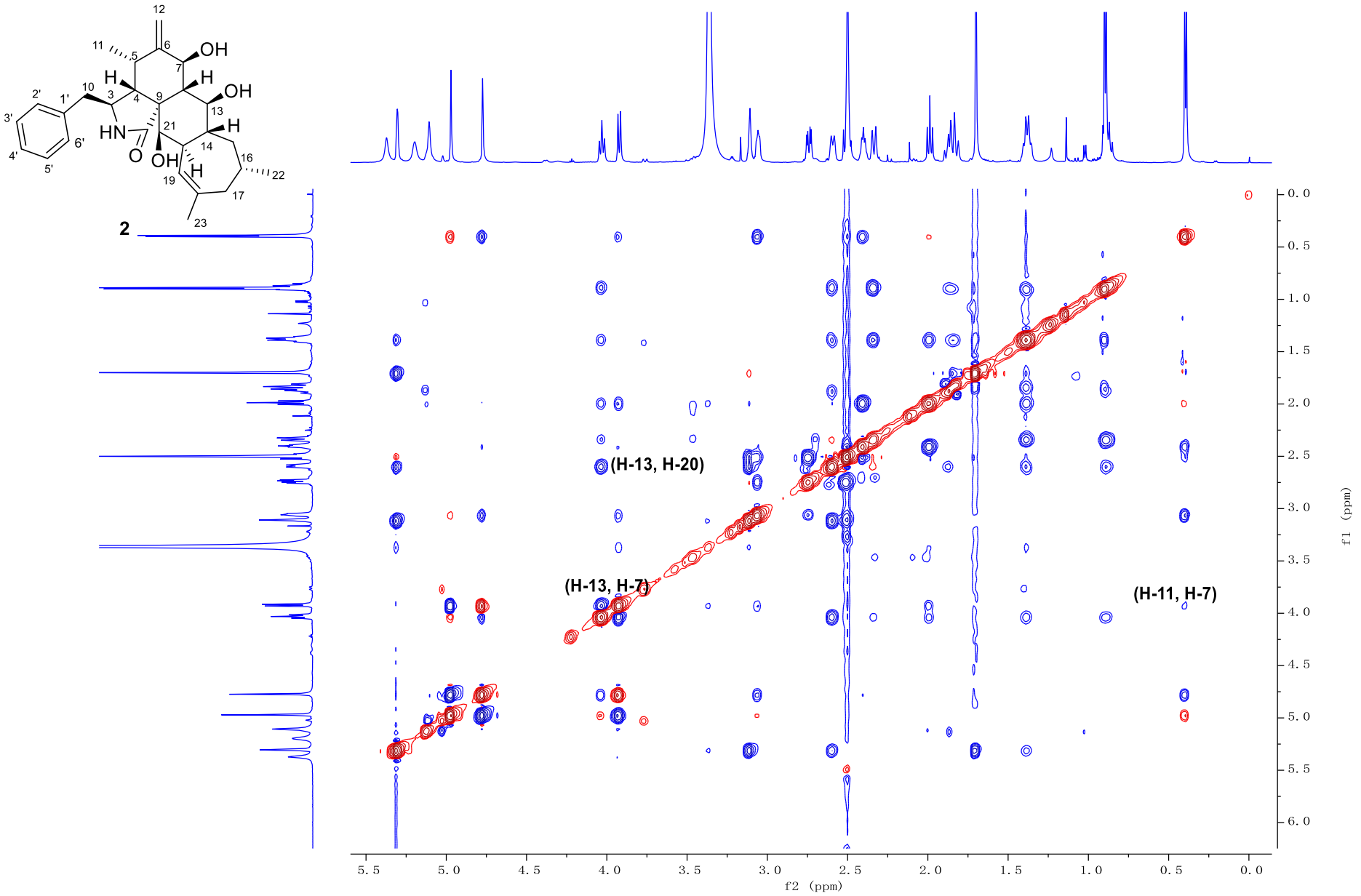


Figure S25. ROESY spectrum of **2** (DMSO-d_6).

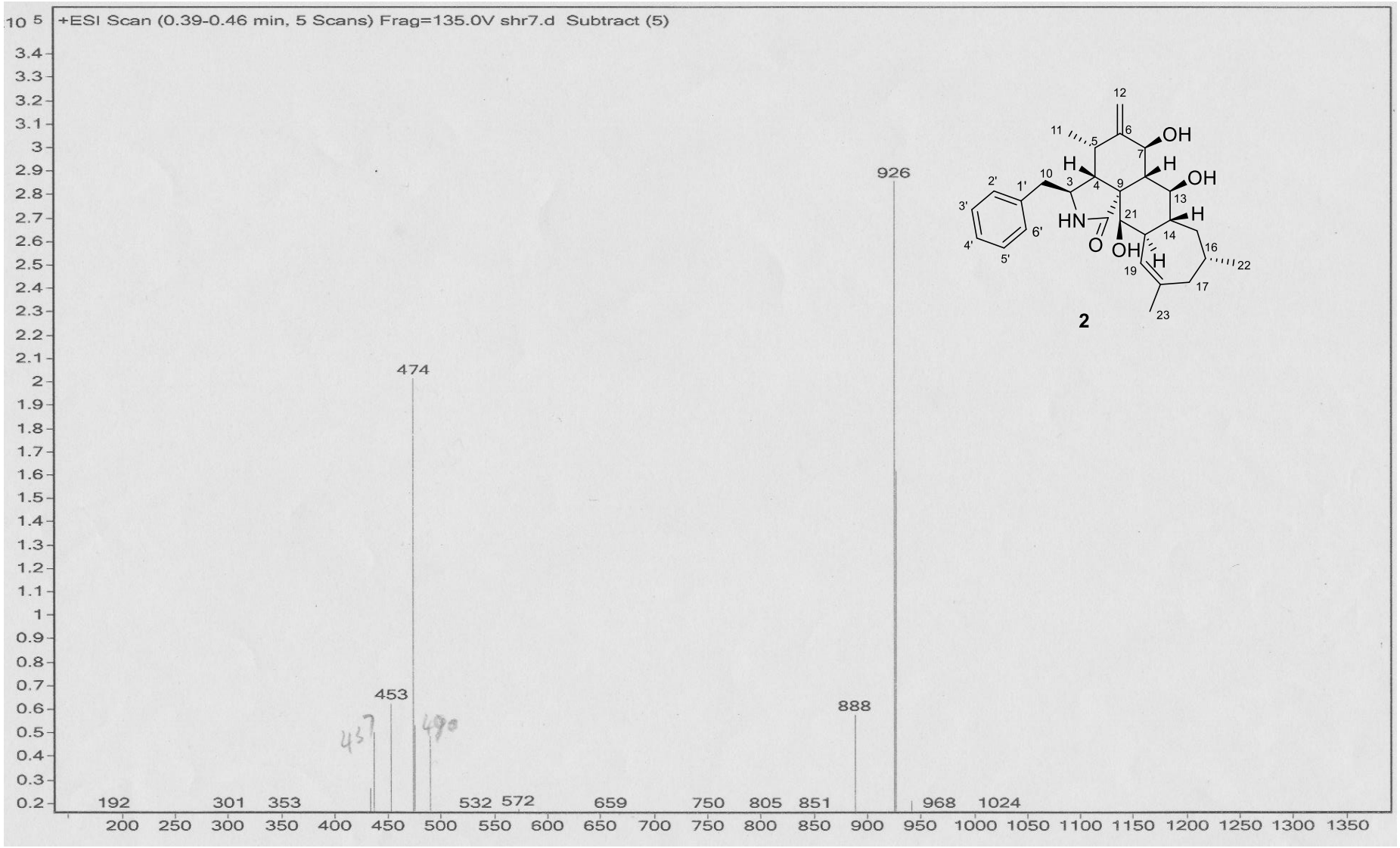
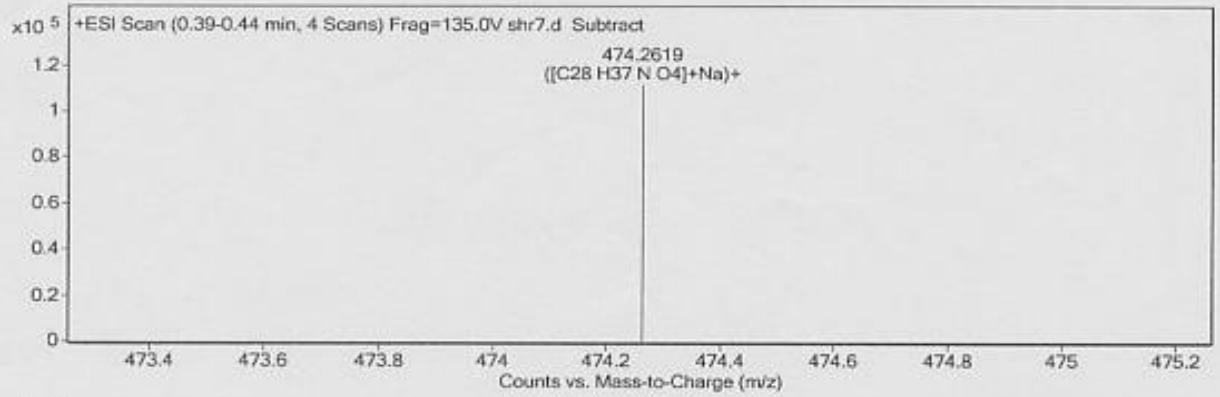


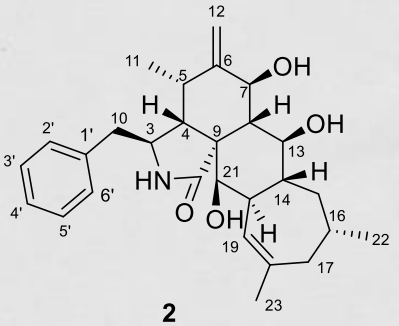
Figure S26. ESIMS spectrum of **2.**

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	ESI



Peak List

m/z	z	Abund	Formula	Ion
434.2693	1	23460.51		
437.1935	1	33573.46		
453.1676	1	62882.1		
474.2619	1	111414.45	C28 H37 N O4	(M+Na)+
475.265	1	29755.84	C28 H37 N O4	(M+Na)+
888.4665	1	73997.81		
889.4698	1	38417.85		
925.5349	1	164423.02		
926.5379	1	93533.14		
927.5405	1	26599.6		



2

Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	120
O	0	30
N	0	10

Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C28 H37 N O4	451.2723	474.2615	474.2619	-0.4	-0.8	11.0000

Figure S27. HRESIMS spectrum of **2.**

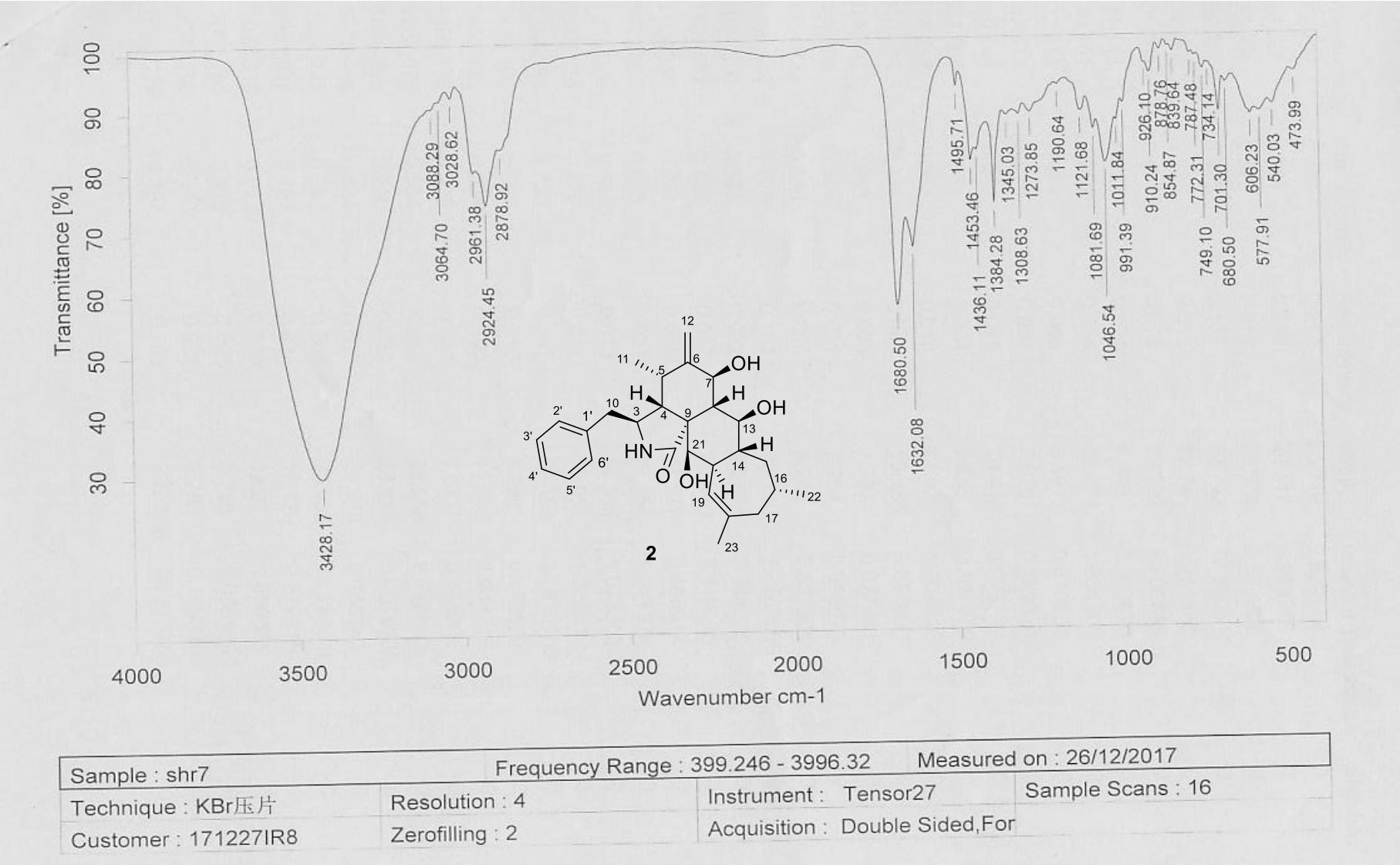


Figure S28. IR spectrum of **2.**

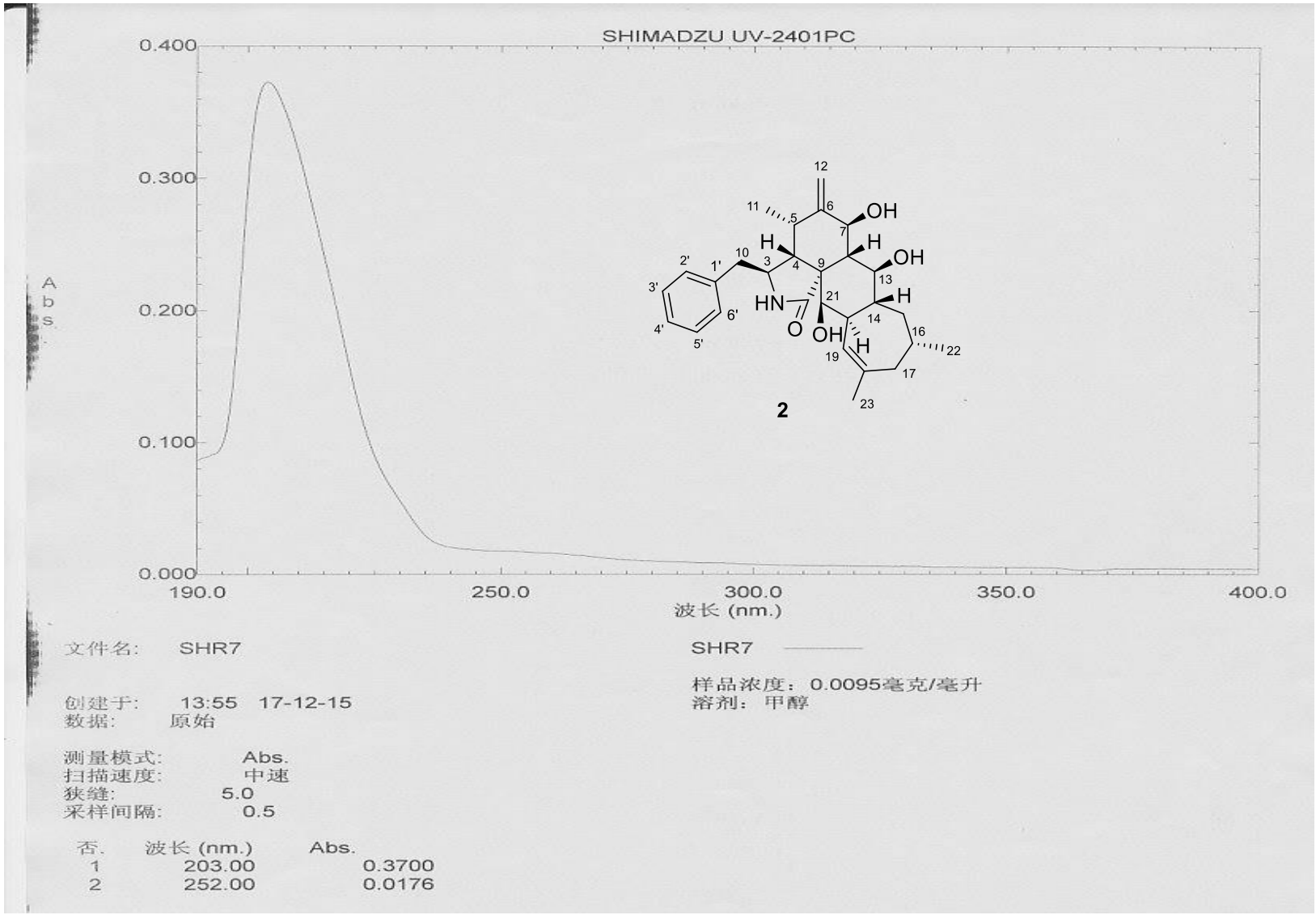
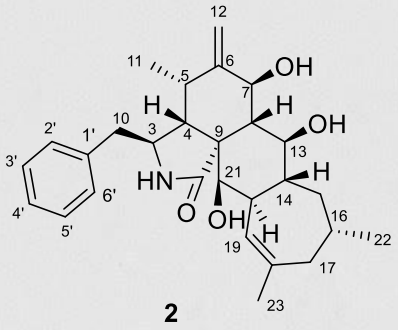


Figure S29. UV spectrum of **2.**

Optical rotation measurement

Model : P-1020 (A060460638)

No.	Sample	Mode	Data	Monitor Blank	Temp. Cell Temp Point	Date Comment Sample Name	Light Filter Operator	Cycle Time Integ Time
No.1	21 (1/3)	Sp.Rot	30.3330	0.0182 0.0000	18.3 50.00 Cell	Fri Nov 24 19:59:50 2017 0.00120g/mL MeOH SHR7	Na 589nm	2 sec 2 sec
No.2	21 (2/3)	Sp.Rot	33.6670	0.0202 0.0000	18.3 50.00 Cell	Fri Nov 24 19:59:56 2017 0.00120g/mL MeOH SHR7	Na 589nm	2 sec 2 sec
No.3	21 (3/3)	Sp.Rot	29.8330	0.0179 0.0000	18.3 50.00 Cell	Fri Nov 24 20:00:01 2017 0.00120g/mL MeOH SHR7	Na 589nm	2 sec 2 sec



2

Figure S30. OR spectrum of **2.**

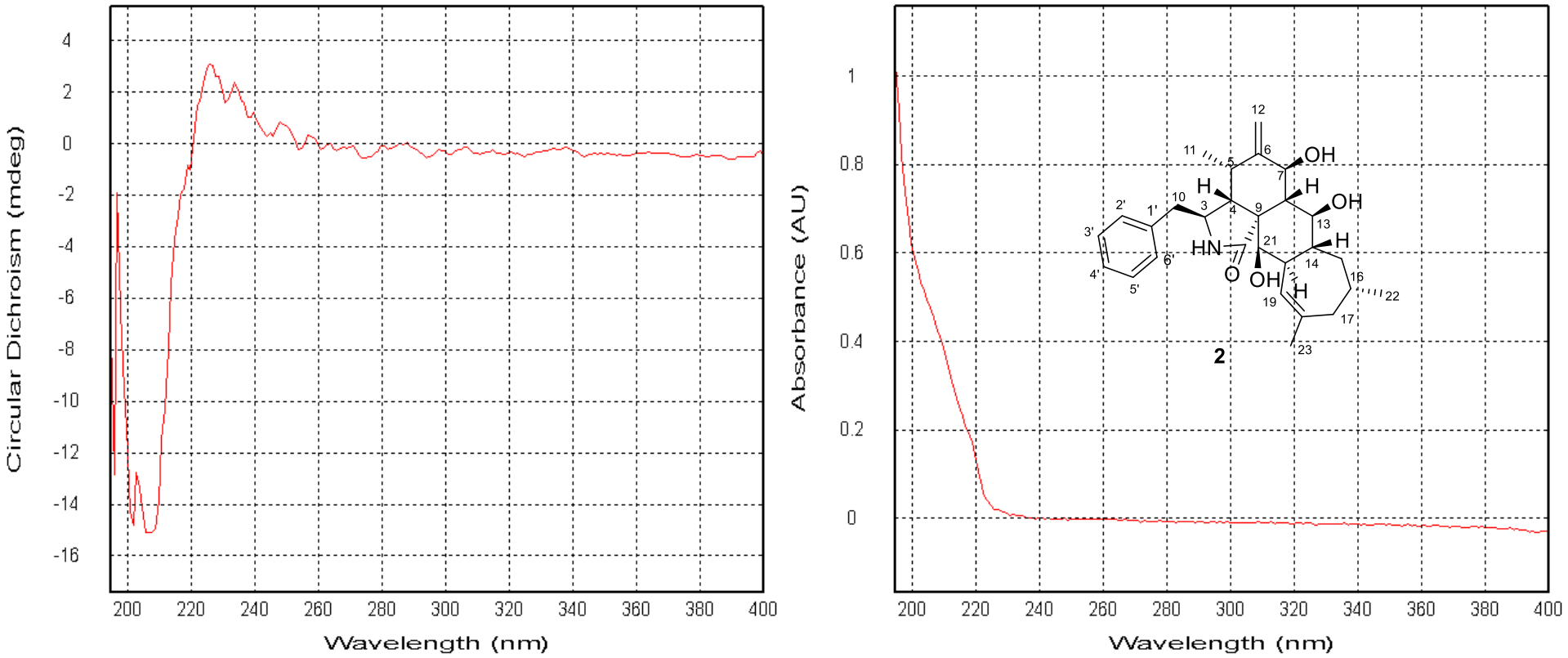


Figure S31. CD spectrum of **2.**

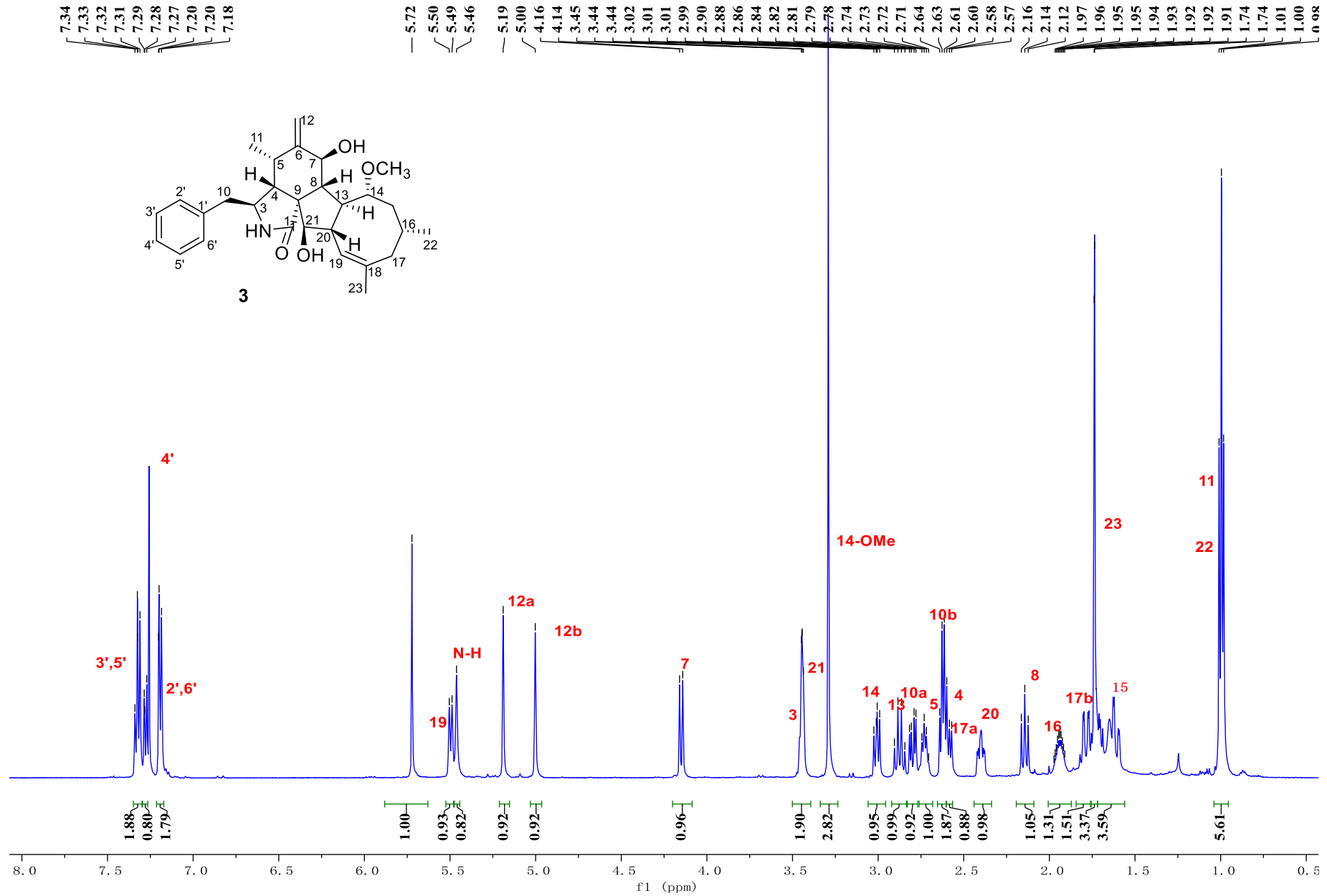


Figure S32. ^{1}H NMR spectrum of **3** (500 MHz, $CDCl_3$).

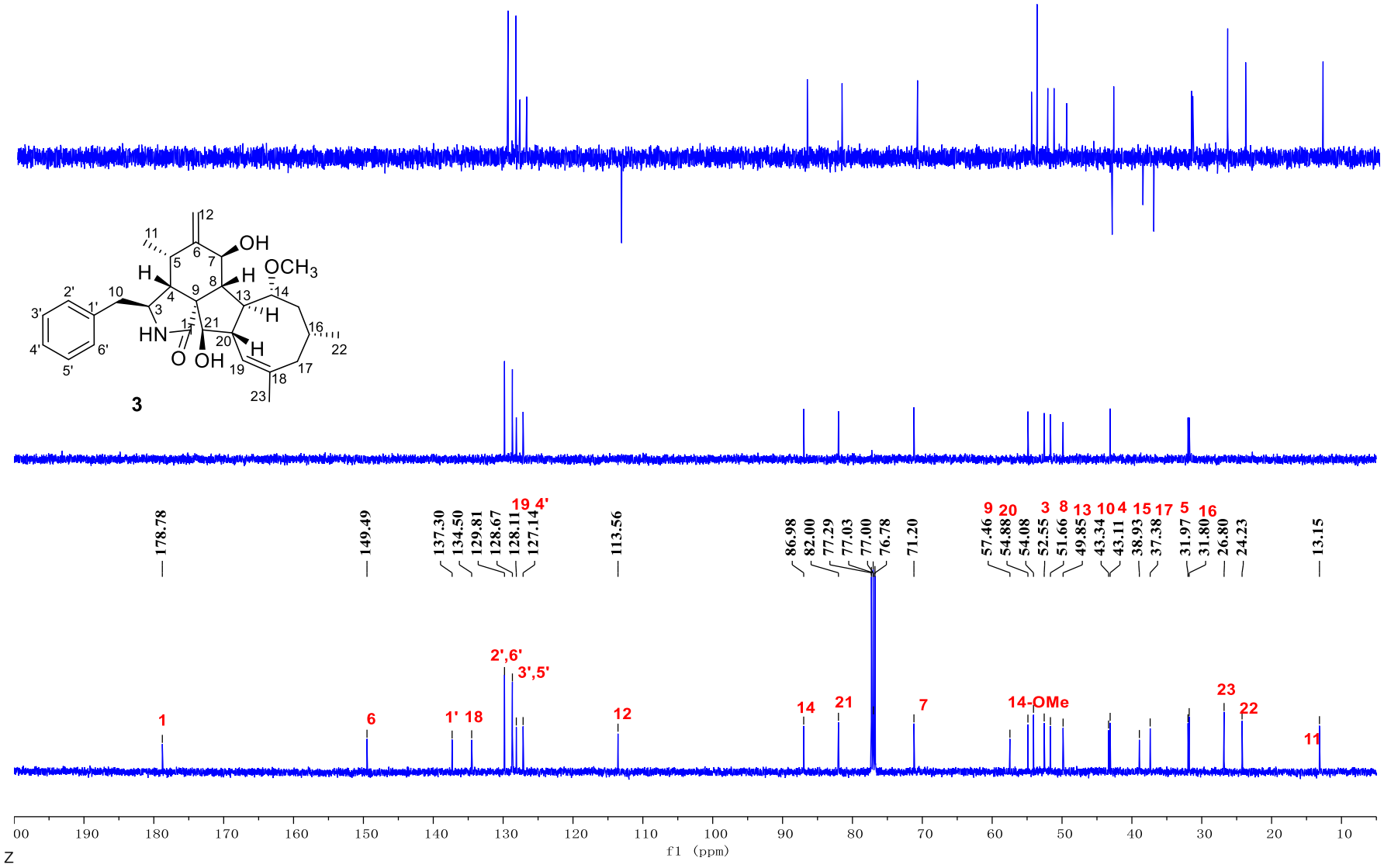


Figure S33. ^{13}C NMR spectrum of **3** (125 MHz, $CDCl_3$).

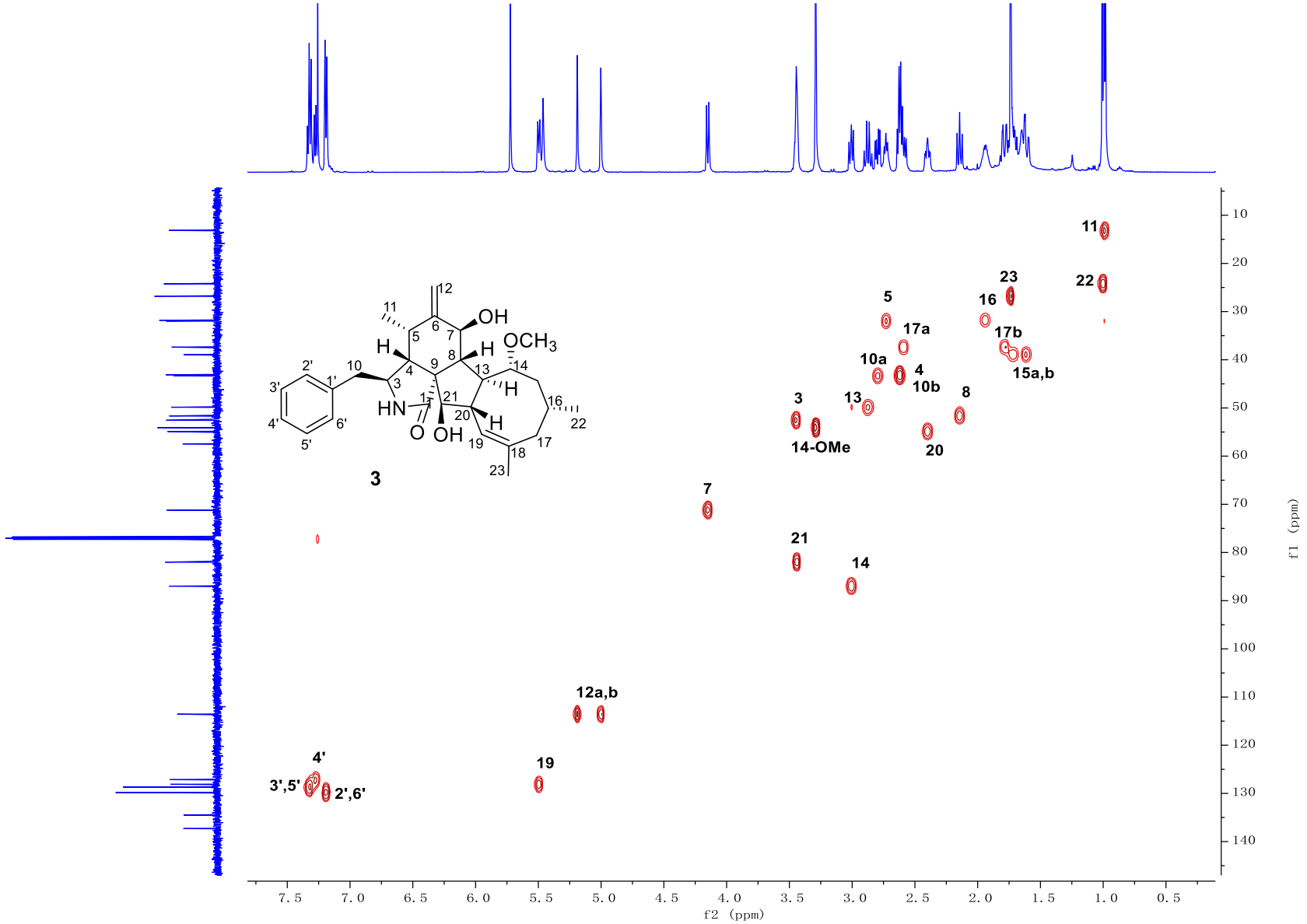


Figure S34. HSQC spectrum of **3** ($CDCl_3$).

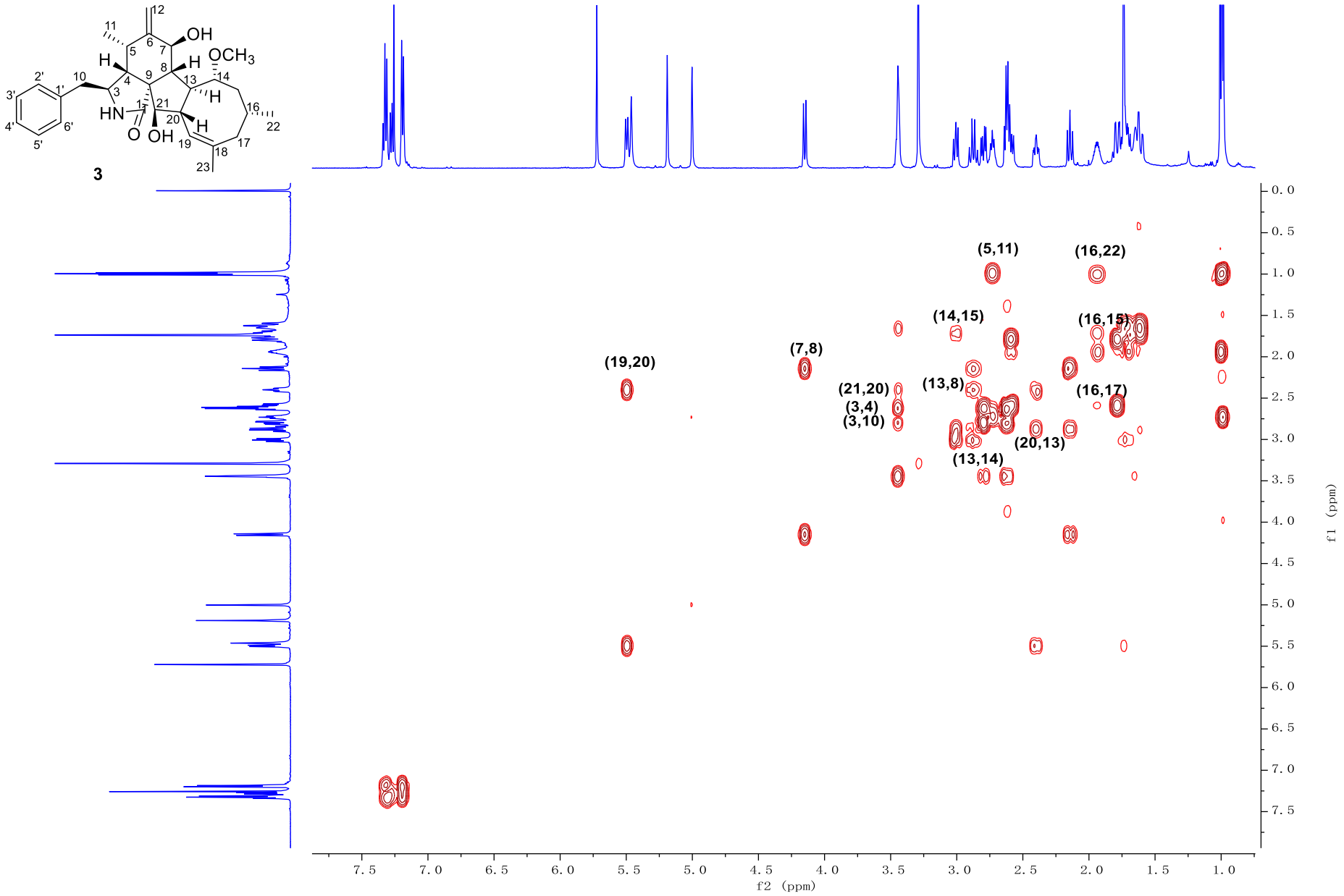


Figure S35. ^{1}H–^{1}H COSY spectrum of **3** ($CDCl_3$).

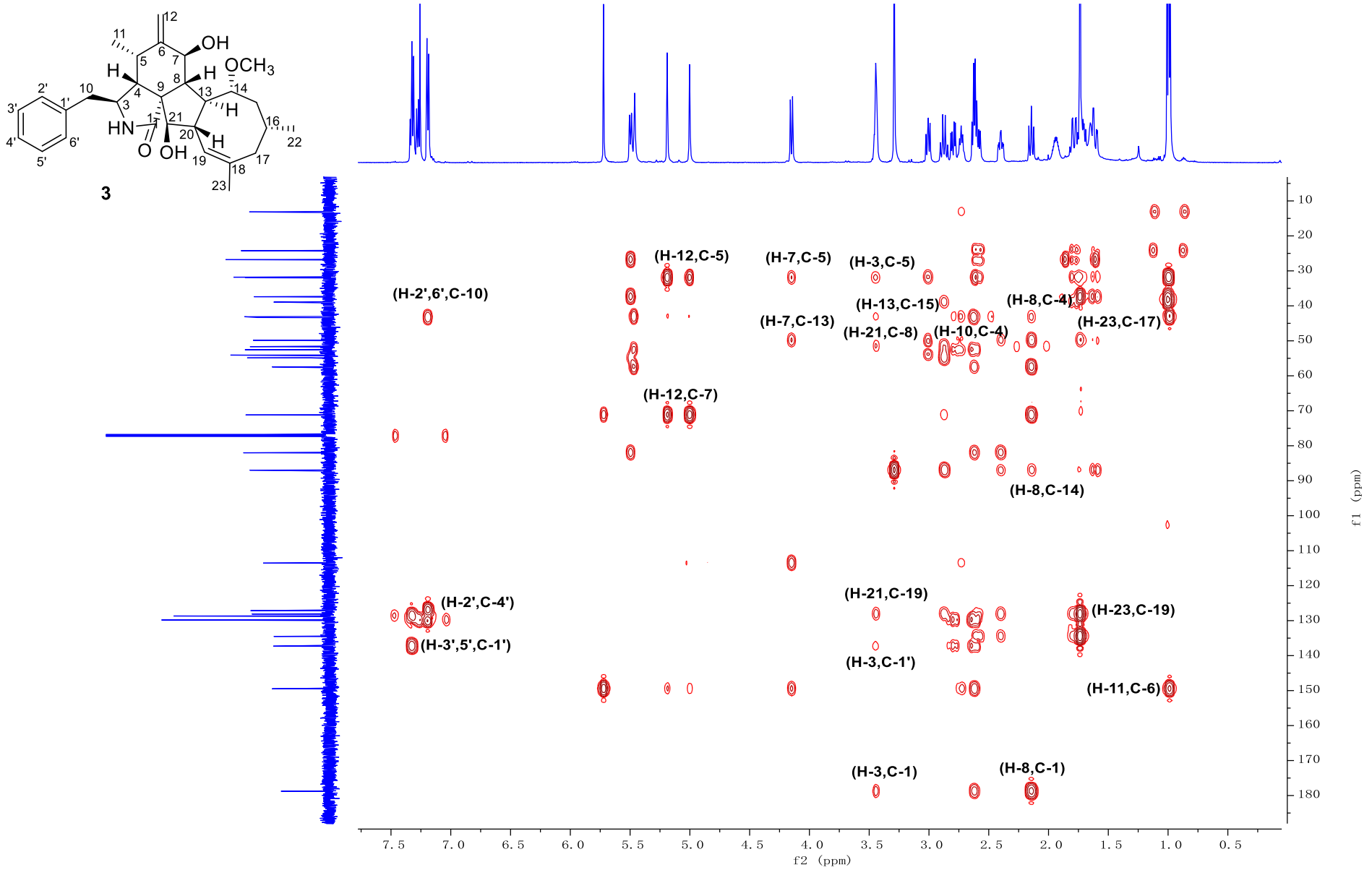


Figure S36. HMBC spectrum of **3** ($CDCl_3$).

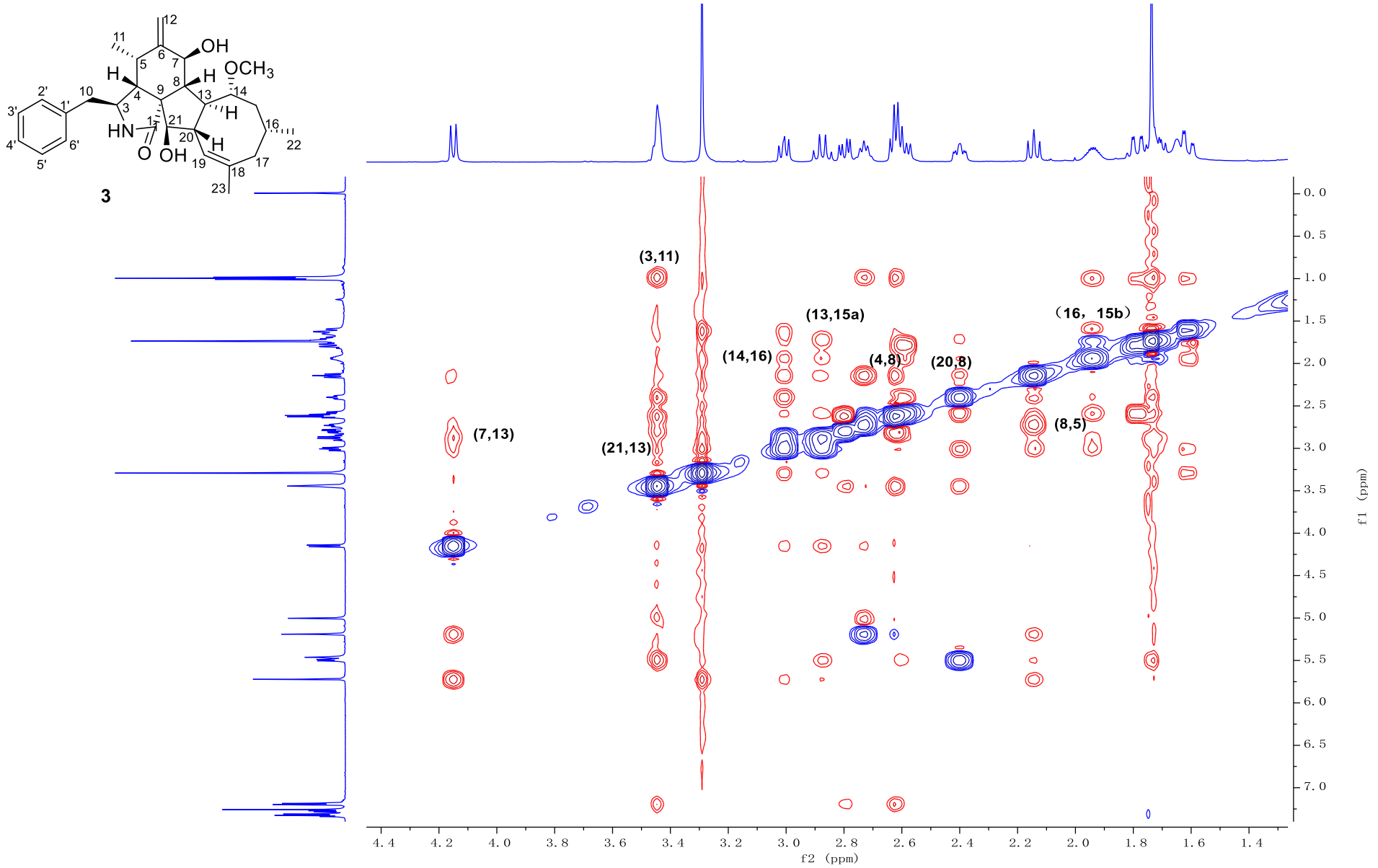


Figure S37. ROESY spectrum of **3** ($CDCl_3$).

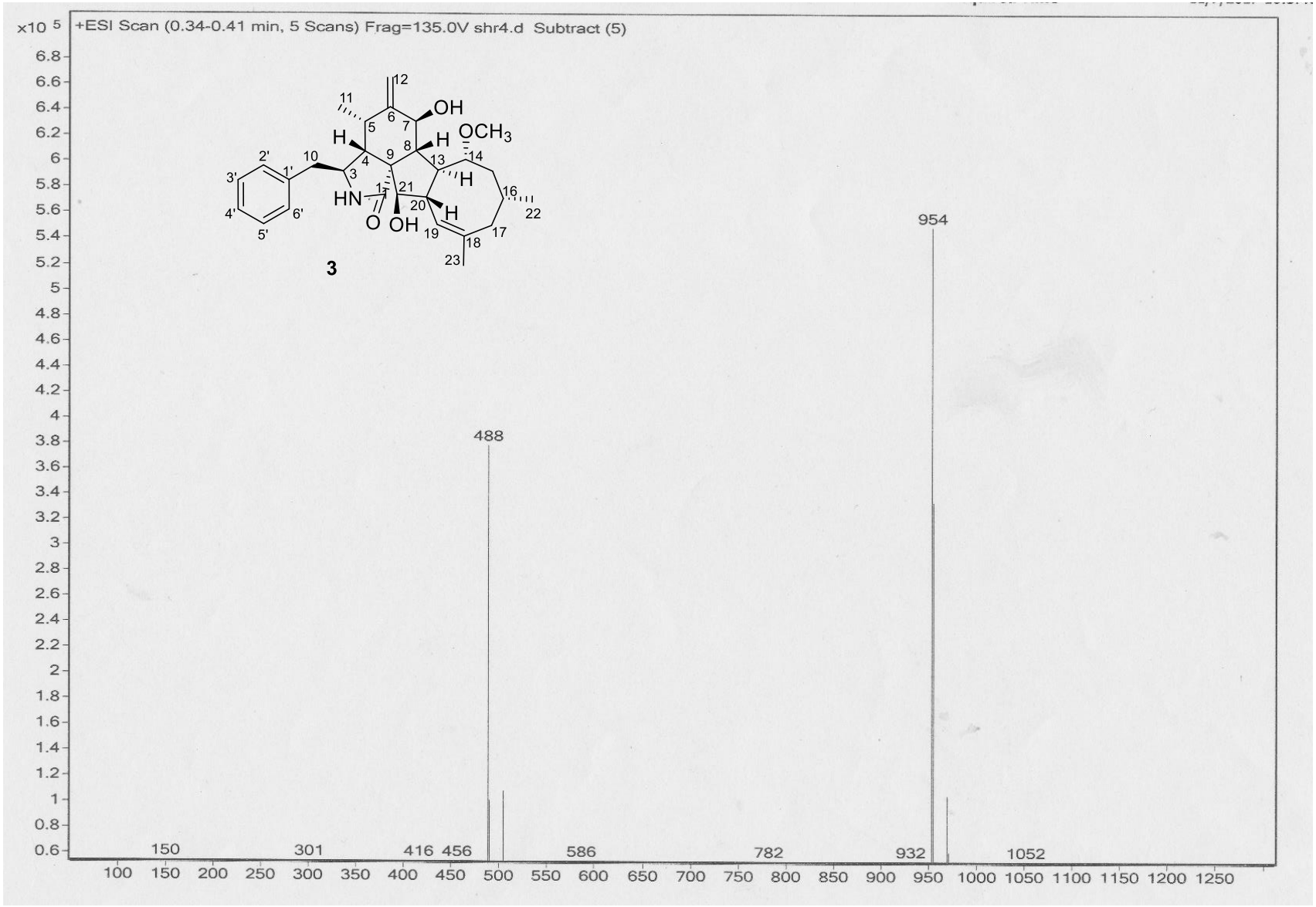
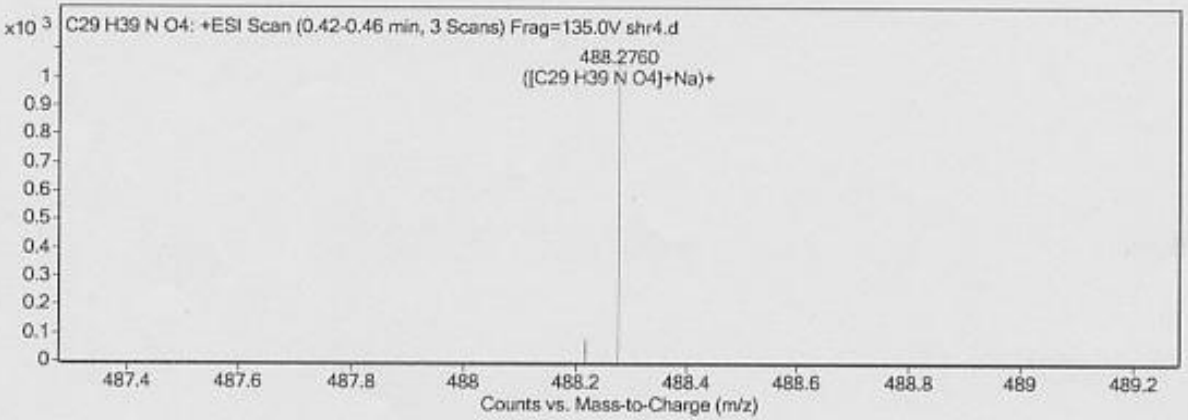
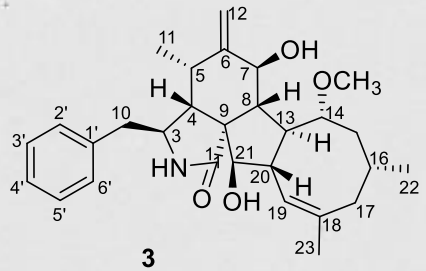


Figure S38. ESIMS spectrum of **3**.



Peak List

m/z	z	Abund
102.1278		3106.06
107.041		3166.07
121.0509	1	5591.72
128.9536		3024.99
175.1441		3056.36
245.0781	1	3708.04
472.2252	1	2333.29
548.2403	1	3488.01
922.0098	1	7629.48
959.9649	1	4919.65



3

Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	120
O	0	30
N	0	10

Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C29 H39 N O4	465.2879	488.2771	488.2760	0.3	0.7	11.0000

Figure S39. HRESIMS spectrum of **3**.

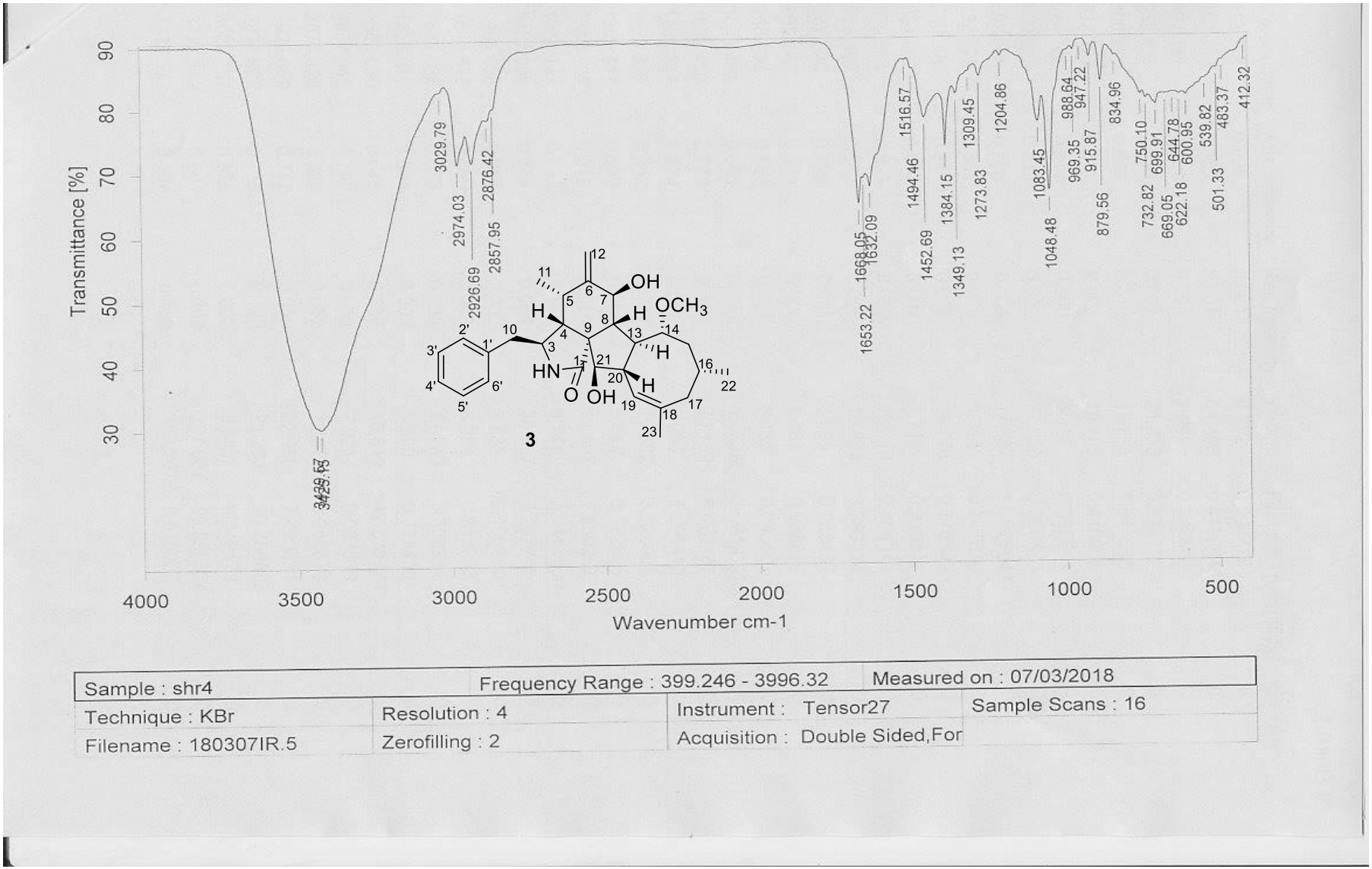


Figure S40. IR spectrum of **3**.

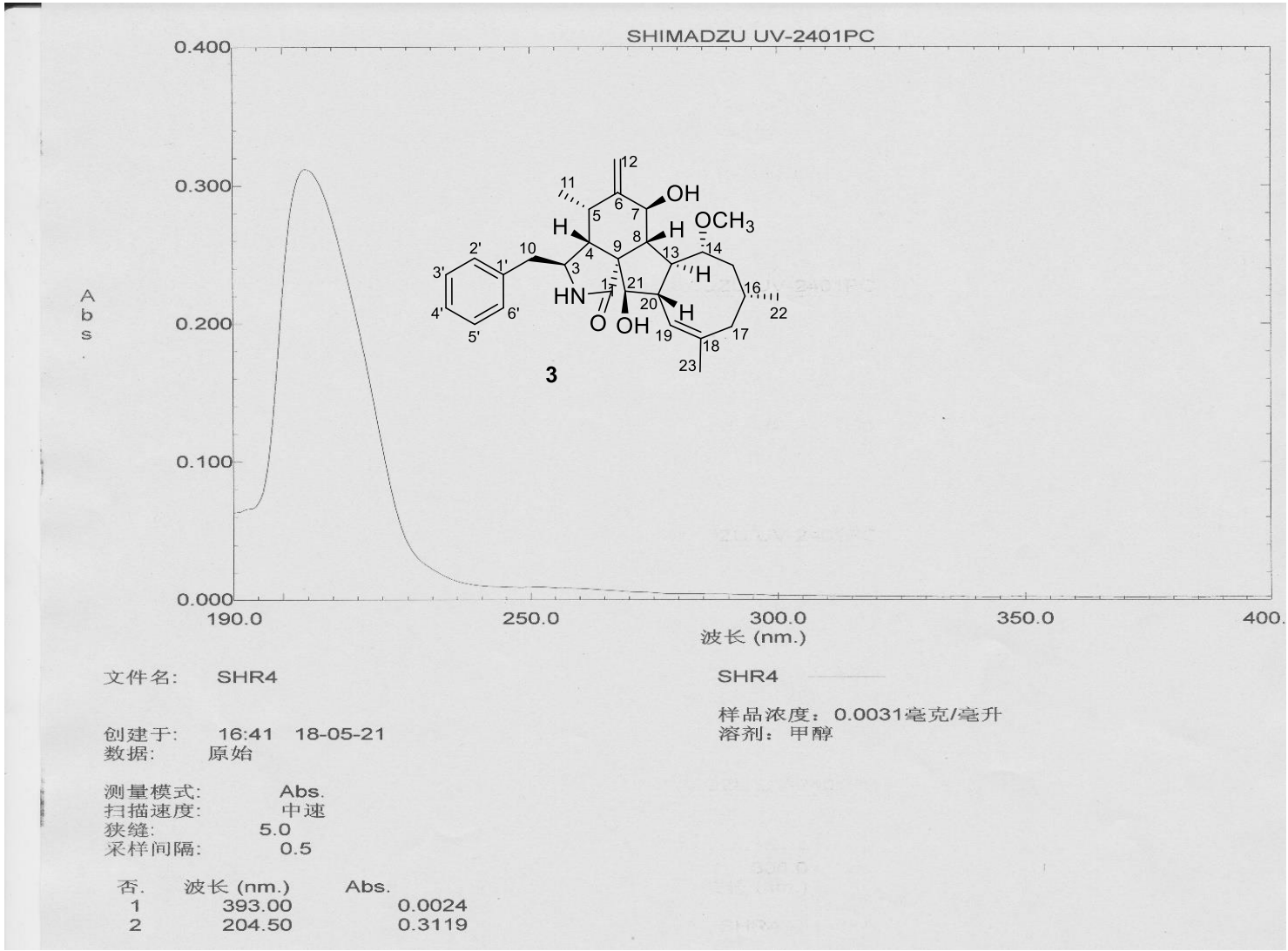


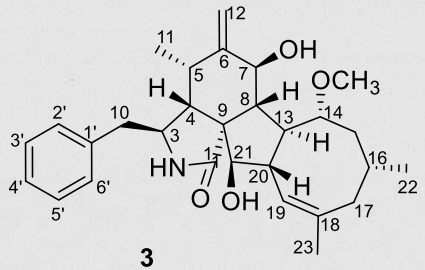
Figure S41. UV spectrum of **3**.

Optical rotation measurement

Model : P-1020 (A060460638)

No.	Sample	Mode	Data	Monitor Blank	Temp. Cell Temp Point	Date Comment Sample Name	Light Filter Operator	Cycle Time Integ Time
No.1	7 (1/3)	Sp.Rot	35.4550	0.0039 0.0000	25.1 10.00 Cell	Mon May 14 14:00:30 2018 0.00110g/mL MeOH SHR4	Na 589nm	2 sec 2 sec
No.2	7 (2/3)	Sp.Rot	37.2730	0.0041 0.0000	25.1 10.00 Cell	Mon May 14 14:00:35 2018 0.00110g/mL MeOH SHR4	Na 589nm	2 sec 2 sec
No.3	7 (3/3)	Sp.Rot	35.4550	0.0039 0.0000	25.1 10.00 Cell	Mon May 14 14:00:40 2018 0.00110g/mL MeOH SHR4	Na 589nm	2 sec 2 sec

+36.0606°



3

Figure S42. ORD spectrum of **3**.

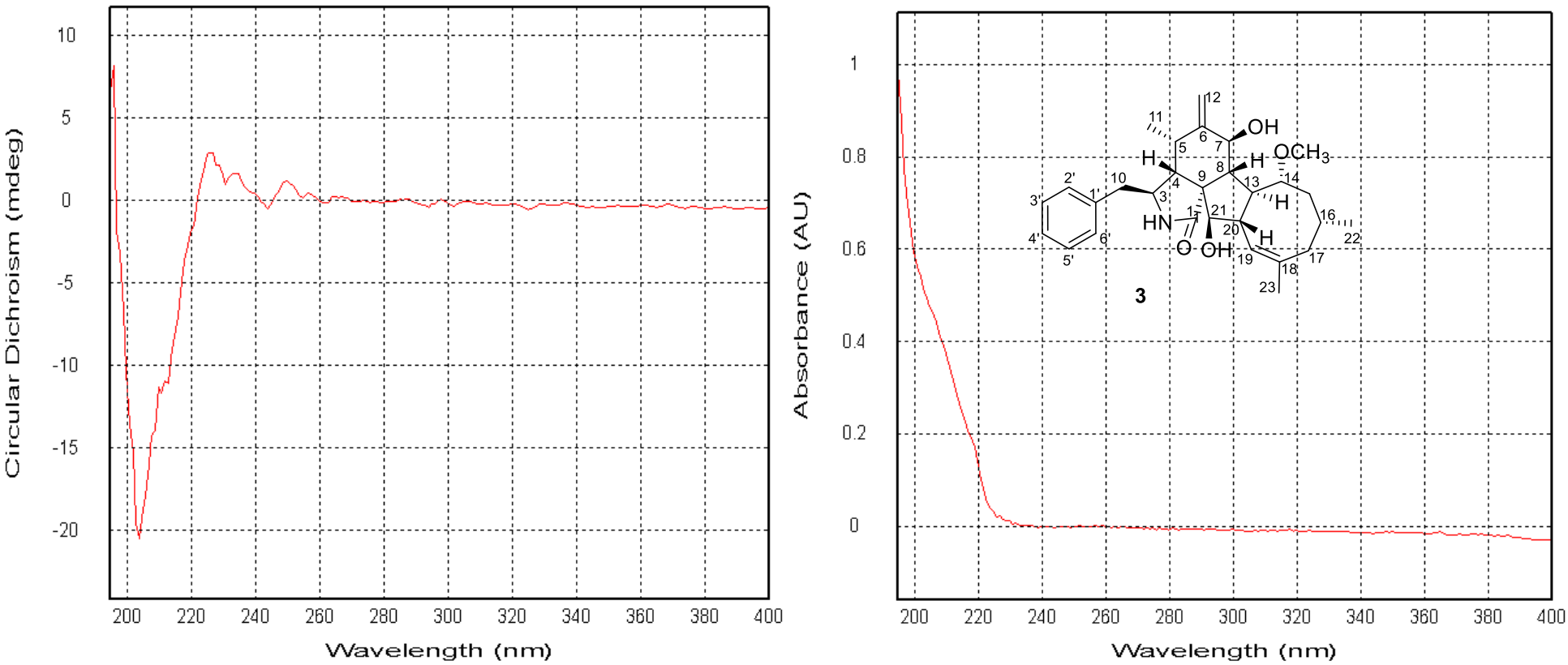


Figure S43. CD spectrum of **3**.

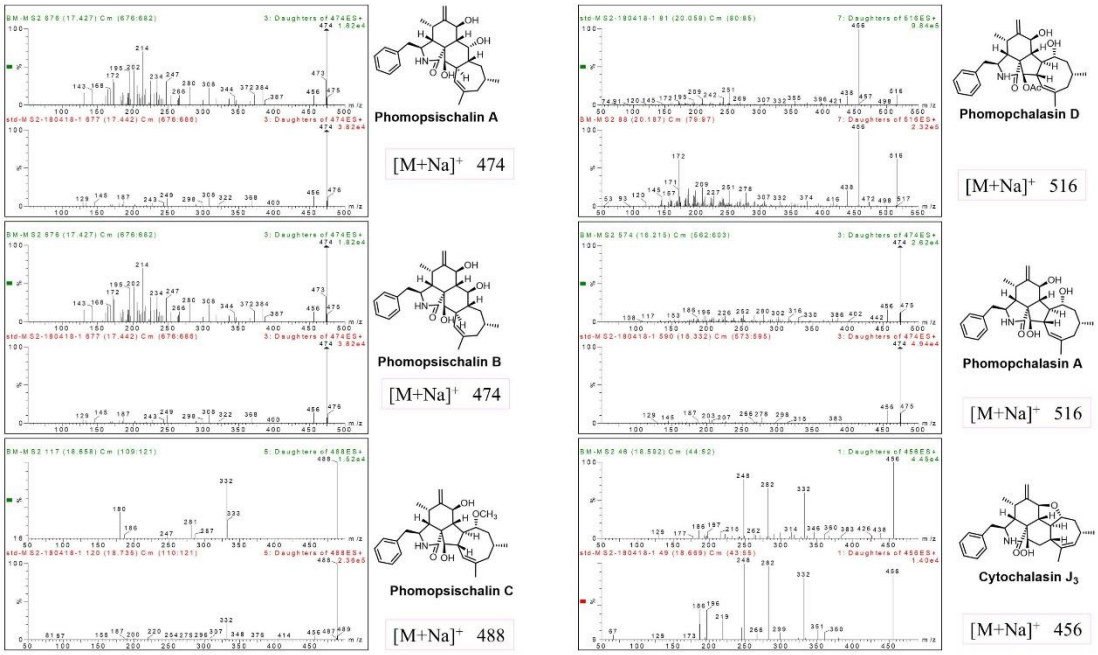


Figure S44. LC-MS/MS profiles for phomopsischalins A–C, phomopchalasins A, D, and cytochalasin J_3 (**1**–**3**) in the EtOAc extracts. (Green: LC–MS/MS result of EtOAc extracts, red: LC–MS/MS result of standards)