Supporting information for

Ophiorrhines A and B, Two Immunosuppressive Monoterpenoid Indole Alkaloids from *Ophiorrhiza japonica*

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List of Supporting Information

1. Experimental Section

1.1. General experimental procedures. Melting points were obtained on an X-4 micro melting point apparatus. Optical rotations were measured with a Rudoph AUTOPOL IV polarimeter. UV data were afforded with a UH5300 UV-VIS Double Beam Spectrophotometer. IR spectra were obtained with a Shimadzu Fourier Transform Infrared Spectrometer using KBr pellets. 1D and 2D spectra were run on a Bruker Avance III 600 MHz spectrometer with TMS as an internal standard. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. Mass spectra were recorded on a Thermo Scientific Q Exactive Orbitrap MS system. Column chromatography (CC) was performed on silica gel (200-300 mesh, Qingdao Marine Chemical Ltd., Qingdao, People's Republic of China) and Sephadex LH-20 (Pharmacia Fine Chemical Co., Ltd., Sweden). Preparative High Performance Liquid Chromatography (prep-HPLC) was performed on an Agilent 1260 liquid chromatography system equipped with a Durashell C18 column (Agela Technologies, 5 μm, 10 mm × 150 mm) and a DAD detector. Fractions were monitored by TLC (GF 254, Qingdao Haiyang Chemical Co., Ltd. Qingdao), and spots were visualized by Dragendorff reagent or heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

1.2. Plant material. The whole plant of *Ophiorrhiza japonica* was collected from Hunan province, China, in 2016. It is identified by Ming-Qing Pan of Wuhan University. The voucher specimen (NO. HFG-P20160819-3.1) has been deposited at School of Pharmaceutical Sciences, South-Central University for Nationalities.

1.3. Extraction and isolation. Air dried and powdered plant (8 kg) of *O. japonica* was extracted with 80% ethanol (24 h × 3) to give a crude extract. The extract was partitioned between EtOAc and 0.5% HCl solution. The acidic water soluble materials, adjusted to pH 9–10 with 10% ammonia

solution, was extracted with EtOAc to give an alkaloidal extract (120 g). The extract was subjected to silica gel column chromatography (CC), eluted with a gradient solvent system of $CHCl_3$/MeOH (from 1:0 to 0:1) to give seven fractions A–G. Fraction C (3.8 g) was further separated by silica gel CC ($CHCl_3$/MeOH, 12:1) to give subfractions C1-C4. Fraction C2 (440 mg) was separated by Sephadex LH-20 (MeOH) to give subfractions C2a-C2e. Fraction C2b (68 mg) was purified by HPLC (MeCN/H_2O, v/v, from 20/80 to 40/60 in 30 min, flow speed: 8 mL/min) to yield **1** (23 mg, retention time = 22.6 min) and **2** (7 mg, retention time = 20.1 min).

*Ophiorrhine A (**1**):* Colorless needles, mp: 236-238. $[\alpha]_D^{27}$ -55.6, (c 0.14, MeOH); UV λnm (logε): 230 (4.34), 280 (3.85); IR (KBr) ν_{max}: 3425, 2926, 1694, 1646, 1447, 1373, 1299, 1263, 1094, 1021 cm^{-1} ; ^{1}H NMR (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz) spectroscopic data, see Table S1; HRESIMS (positive): *m/z* 579.1952 $[M + Na]^+$ (calcd for $C_{28}H_{32}N_2O_{10}Na$, 579.1949).

*Crystal data for cu_**1**_0m:* $C_{28}H_{32}N_2O_{10}$•5(H_2O), M = 646.63, a = 12.6319(3) Å, b = 9.5734(2) Å, c = 13.7927(3) Å, α = 90°, β = 115.8170(10)°, γ = 90°, V = 1501.47(6) $Å^3$, T = 100(2) K, space group $P21$, Z = 2, μ(CuKα) = 0.992 mm^{-1}, 14518 reflections measured, 4949 independent reflections (R_{int} = 0.0358). The final R_1 values were 0.0429 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1179 ($I > 2\sigma(I)$). The final R_1 values were 0.0431 (all data). The final $wR(F^2)$ values were 0.1185 (all data). The goodness of fit on F^2 was 1.063. Flack parameter = 0.09(4). CCDC: 1875337 (https://www.ccdc.cam.ac.uk).

*Ophiorrhine B (**2**):* Colorless needles, mp: 239-242. $[\alpha]_D^{27}$ -35.0, (c 0.12, MeOH); UV λnm (logε): 230 (4.34), 280 (3.95); IR (KBr) ν_{max}: 3430, 2956, 1694, 1779, 1669, 1447, 1373, 1299, 1081, 1043 cm^{-1} ; ^{1}H NMR (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz) spectroscopic data, see Table S1; HRESIMS (positive): *m/z* 609.2041 $[M + Na]^+$ (calcd for $C_{29}H_{34}N_2O_{11}Na$, 609.2055).

*Crystal data for cu_**2**_0m:* $C_{29}H_{34}N_2O_{11}$•3(H_2O), M = 640.63, a = 9.3751(3) Å, b = 13.2783(4) Å, c = 24.4319(8) Å, α = 90°, β = 90°, γ = 90°, V = 3041.41(17) Å^3, T = 100(2) K, space group P212121, Z = 4, μ(CuKα) = 0.952 mm^{-1}, 19180 reflections measured, 5439 independent reflections (R_{int} = 0.0297). The final R_1 values were 0.0402 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1001 ($I > 2\sigma(I)$). The final R_1 values were 0.0403 (all data). The final $wR(F^2)$ values were 0.1002 (all data). The goodness of fit on F^2 was 1.170. Flack parameter = 0.15(4). CCDC: 1875336 (https://www.ccdc.cam.ac.uk).

Table S1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for ophiorrhines A (**1**) and B (**2**) (Methanol-d_4, δ in ppm, J in Hz).

Position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
2		142.7, C		140.6, C
3		68.3, C		66.9, C
5		178.3, C		176.9, C
6	4.15, t (2.6)	41.9, CH		82.8, C
7		111.1, C		112.2, C
8		124.1, C		123.2, C
9	7.47, d (7.8)	117.1, CH	7.60, d (7.9)	118.6, CH
10	7.01, t (7.8)	119.3, CH	7.02, t (7.9)	119.5, CH
11	7.04, t (7.8)	120.6, CH	7.06, t (7.9)	120.8, CH
12	7.32, d (7.8)	111.7, CH	7.33, d (7.9)	111.7, CH
13		136.5, C		136.7, C
14a	3.25, m	33.1, CH_2	3.24, m	33.0, CH_2
14b	2.22, m		2.12, m	
15	3.33, m	31.1, CH	3.34, m	31.0, CH
16		113.3, C		113.2, C
17	7.37, s	149.6, CH	7.37, s	149.6, CH
18	1.85, ddd (6.8, 2.5 2.1)	26.6, CH_2	1.99, d (7.7)	31.0, CH_2
19	1.91, m	50.8, CH	1.92, m	51.1, CH
20	2.20, m	45.5, CH	2.20, m	45.4, CH
21	5.52, d (1.7)	94.2, CH	5.52, d (1.6)	94.1, CH
22		167.7, C		167.7, C
1′	4.59, d (8.0)	98.5, CH	4.58, d (8.0)	98.4, CH
2′	3.16, dd (9.2, 8.0)	73.2, CH	3.15, dd (9.2, 8.0)	73.2, CH
3′	3.35, m	76.5, CH	3.33, m	76.5, CH
4′	3.25, m	70.1, CH	3.24, m	70.1, CH
5′	3.28, m	76.9, CH	3.26, m	76.9, CH
6′a	3.88, dd (11.8, 1.8)	61.2, CH_2	3.87, dd (11.8, 1.9)	61.2, CH_2
6′b	3.65, dd (11.8, 5.7)		3.64, dd (11.8, 5.8)	
$COOCH_3$	3.76, s	50.5, CH_3	3.75, s	50.5, CH_3
N_4-CH_3	2.95, s	27.4, CH_3	2.98, s	27.7, CH_3
6-OMe			3.90, s	53.7, CH_3

1.4. Cytotoxicity Assay

Five human cancer cell lines, breast cancer MCF-7, hepatocellular carcinoma SMMC-7721, human myeloid leukemia HL-60, colon cancer SW480, and lung cancer A-549 cells, were used in the cytotoxic assay. All the cells were cultured in RPMI-1640 or DMEM medium (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA) in 5% CO_2 at 37 °C. The cytotoxicity assay was performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method in 96-well microplates. Briefly, 100 μL adherent cells were seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition with initial density of 1×10^5 cells mL^{-1}. Each tumor cell line was exposed to the test compound at concentrations of 0.0625, 0.32, 1.6, 8, and 40 μm in triplicates for 48 h, with cisplatin (sigma, USA) as a positive control. After compound treatment, cell viability was detected and cell growth curve was graphed. IC_{50} value was calculated by Reed and Muench's method.

1.5. Immunosuppressive Activities Assay

Preparation of spleen cells from mice. Female BALB/c mice were sacrificed by cervical dislocation, and the spleens were removed aseptically. Mononuclear cell suspensions were prepared after cell debris, and clumps were removed. Erythrocytes were depleted with ammonium chloride buffer solution. Lymphocytes were washed and resuspended in RPMI 1640 medium supplemented with 10% FBS, penicillin (100 U mL^{-1}), and streptomycin (100 mg mL^{-1}).

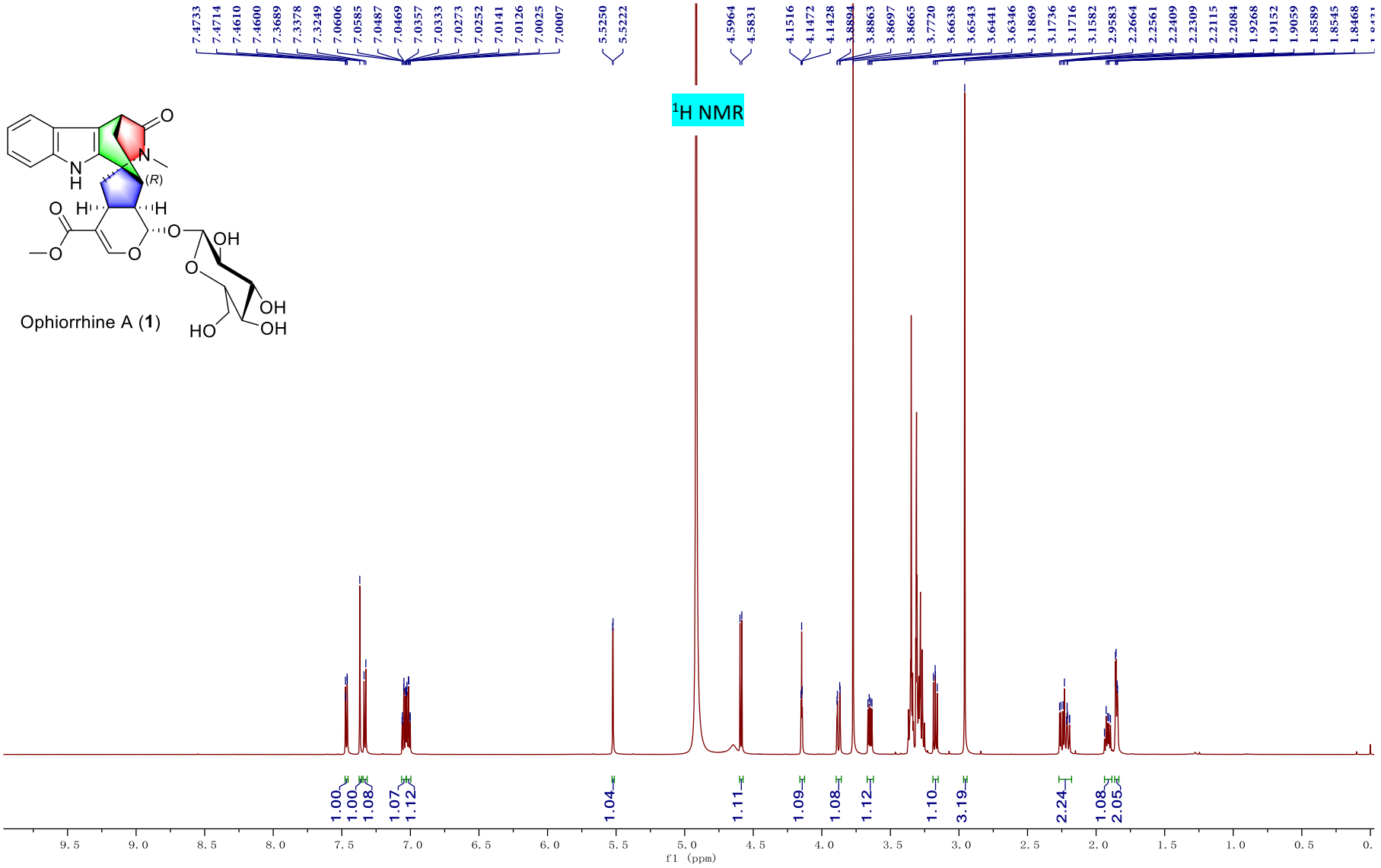
Cytotoxicity assay. Cytotoxicity was tested with Cell Counting Kit-8 (CCK-8) assay. Briefly, fresh spleen cells were gained from female BALB/c mice (18–20 g). Spleen cells (1×10^6 cells) were seeded in triplicate in 96-well flat plates and cultured at 37 °C for 48 h in 96-well flat plates, in the presence

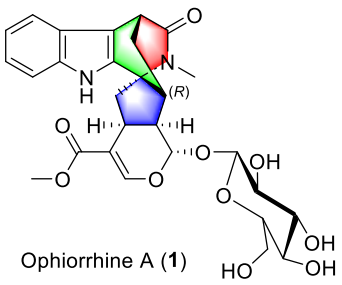
or absence of various concentrations of compounds, in a humidified and 5% CO_2-containing incubator. A certain amount of CCK-8 was added to each well at the final 8-10 h of culture. To the end of the culture, we measured the OD values with microplate reader (Bio Rad 650) at 450 nm. Cyclosporin A (CsA) an immunosuppressive agent, was used as positive compound with definite activity, and the OD values from medium only culture were used as background. The cytotoxicity of each compound was expressed as the concentration of compound that reduced cell viability to 50% (CC_{50}).

T cell and B cell function assay. Fresh spleen cells were obtained from female BALB/c mice (18–20g). The 5×10^5 spleen cells were cultured at the same conditions as those mentioned above. The cultures, in the presence or absence of various concentrations of compounds, were stimulated with 5 μg mL^{-1} of ConA or 10 μg mL^{-1} of LPS to induce T cells or B cells proliferative responses, respectively. Proliferation was assessed in terms of uptake of [^{3}H]-thymidine during 8 h of pulsing with 25μL/well of [^{3}H]-thymidine, and then cells will be harvested onto glass fiber filters. The incorporated radioactivity was counted using a Beta scintillation counter (MicroBeta Trilux, PerkinElmer Life Sciences). Cells treated without any stimuli were used as negative control. The immunosuppressive activity of each compound was expressed as the concentration of compound that inhibited ConA-induced T cell proliferation or LPS-induced B cell proliferation to 50% (IC_{50}) of the control value. Both the cytotoxicity and proliferation assessment repeated twice.

2. NMR spectra and HRESIMS for ophiorrhines A (1) and B (2)

2.1 NMR spectra and HRESIMS for ophiorrhine A (1)





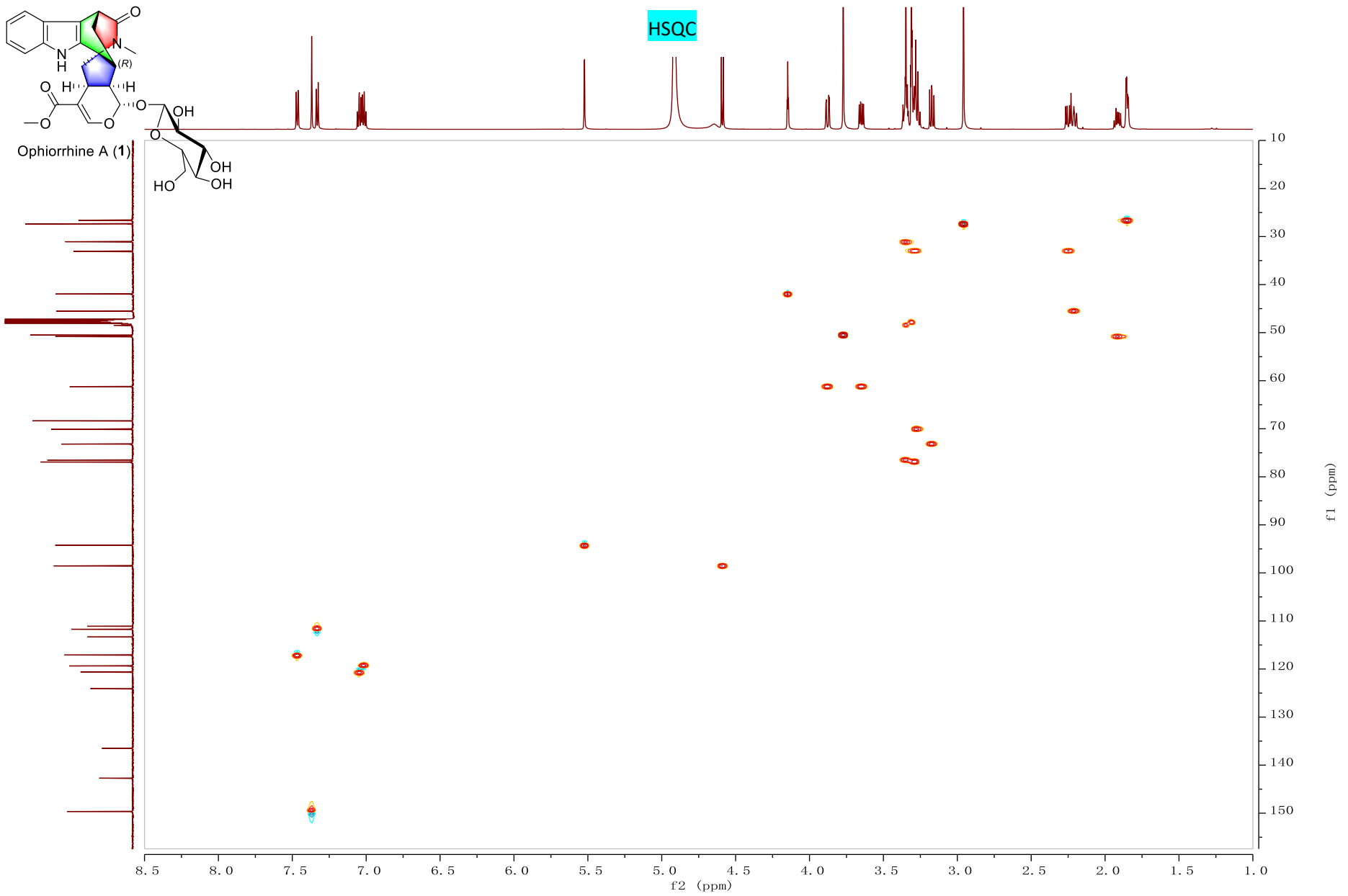
Ophiorrhine A (**1**)

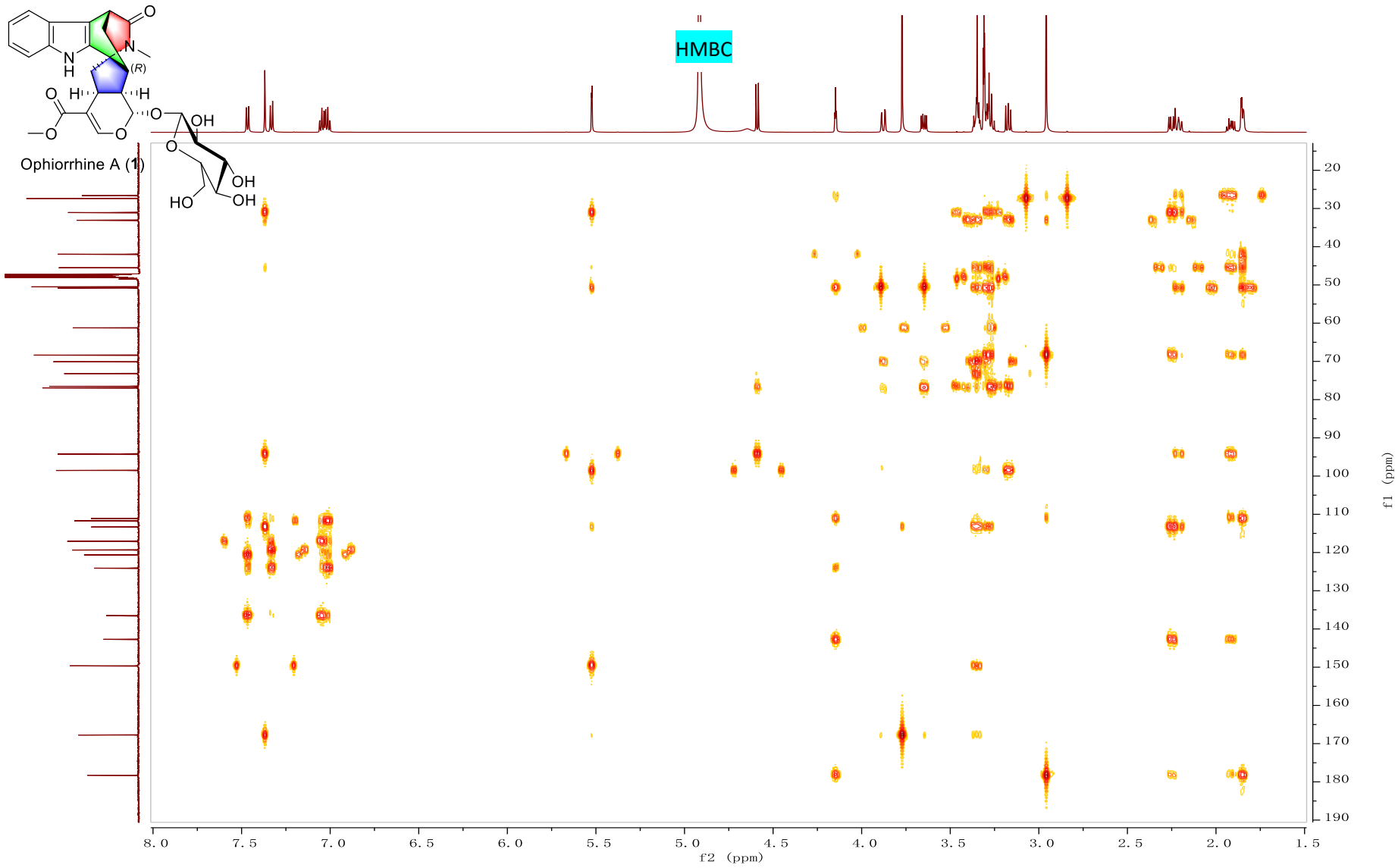
^{13}C NMR

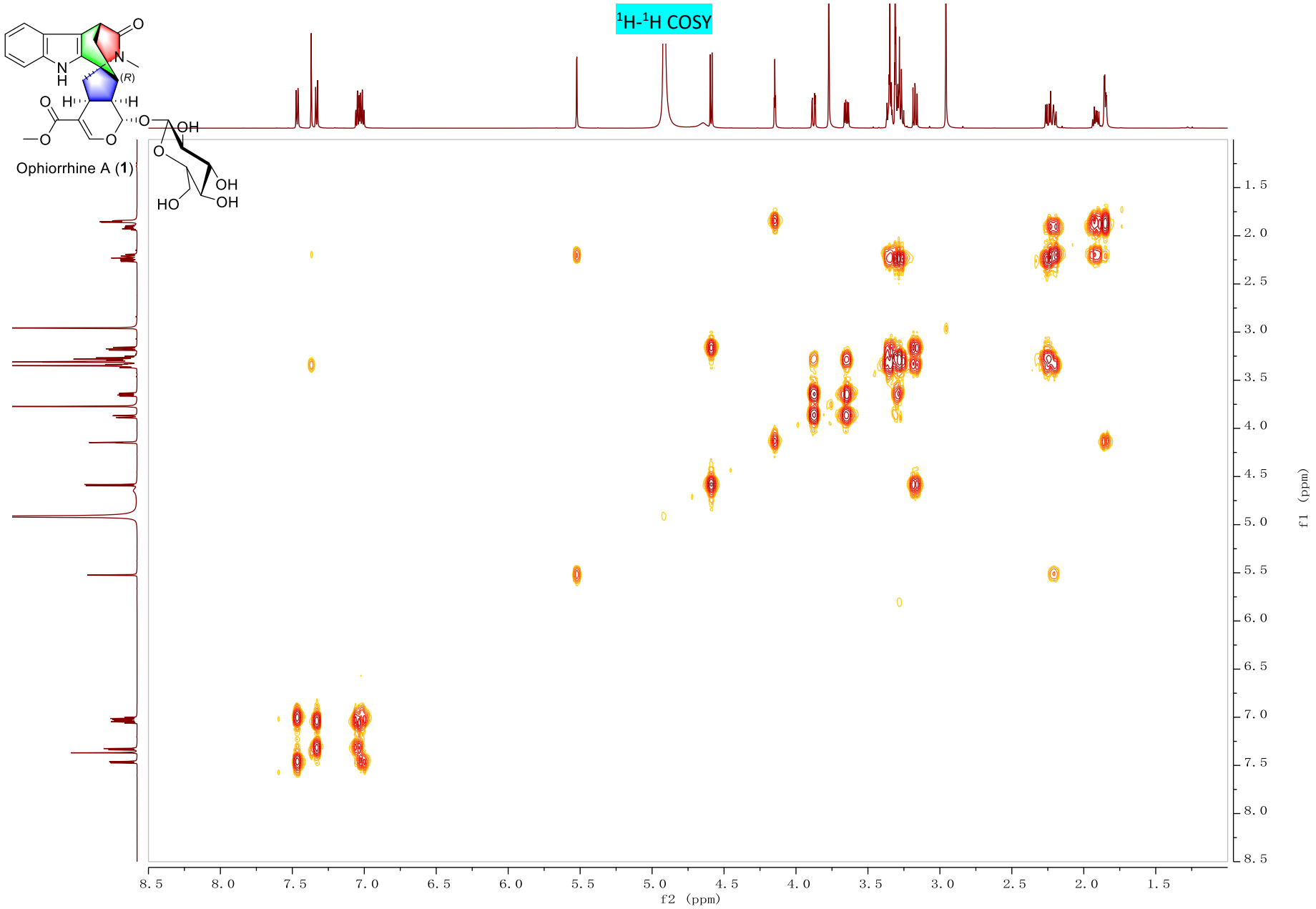
178.28
167.76
149.65
142.71
136.50
124.09
120.62
119.35
117.08
113.33
111.71
111.09
98.53
94.25
76.92
76.51
73.19
70.09
68.36
61.22
50.81
50.50
45.51
41.97
33.08
31.07
27.38
26.61

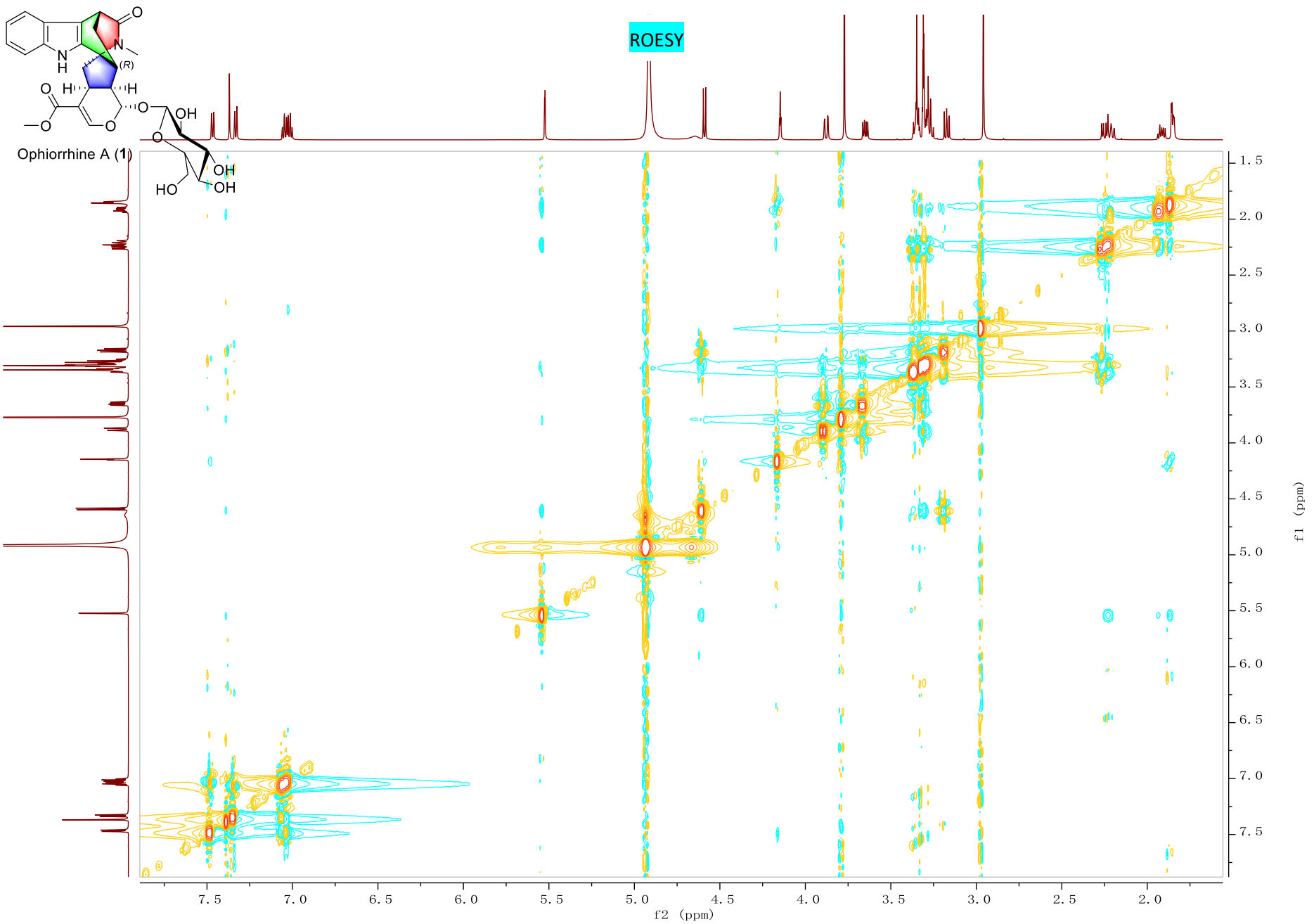
20 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10

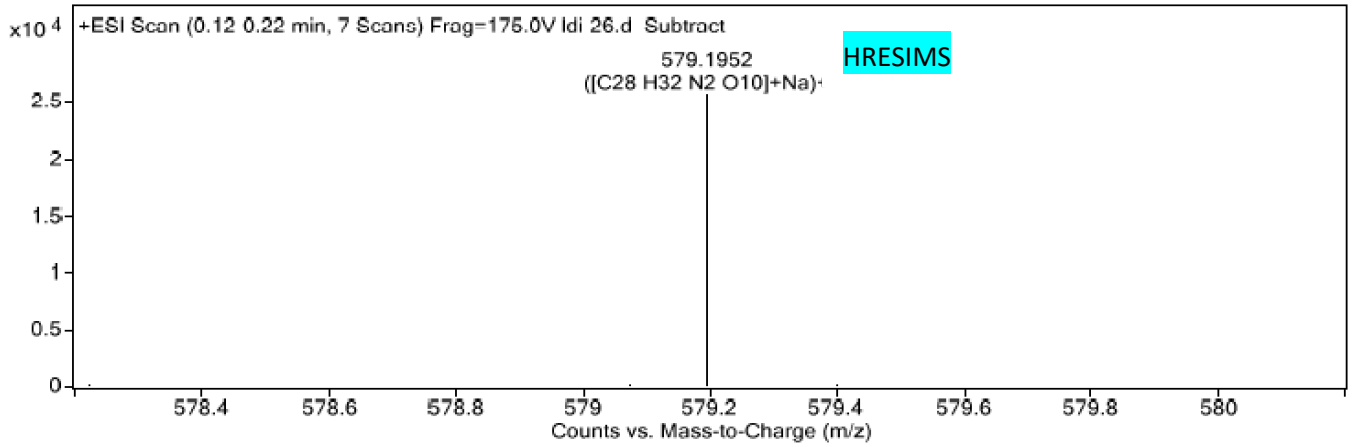
f1 (ppm)


HSQC
Ophiorrhine A (1)
f2 (ppm)
f1 (ppm)


HMBC
Ophiorrhine A (1)
f2 (ppm)
f1 (ppm)

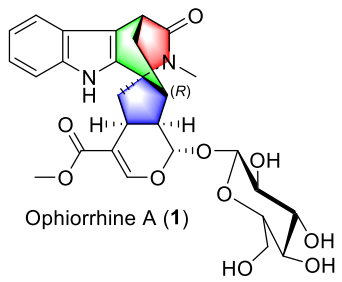

^{1}H-^{1}H COSY
Ophiorrhine A (1)
f2 (ppm)
f1 (ppm)


ROESY
Ophiorrhine A (1)
f2 (ppm)
f1 (ppm)



Peak List

m/z	z	Abund	Formula	Ion
274.2742	1	5663.46		
453.1677	1	5928.37		
557.2131	1	9730.42		
558.2172	1	2953.76		
579.1952	1	25646.44	C28 H32 N2 O10	(M+Na)+
580.1992	1	7672.65	C28 H32 N2 O10	(M+Na)+
595.1685	1	12923.1		
596.1712	1	3714.91		
1135.3976	1	8106.62		
1136.4001	1	5250.77		



Ophiorrhine A (**1**)

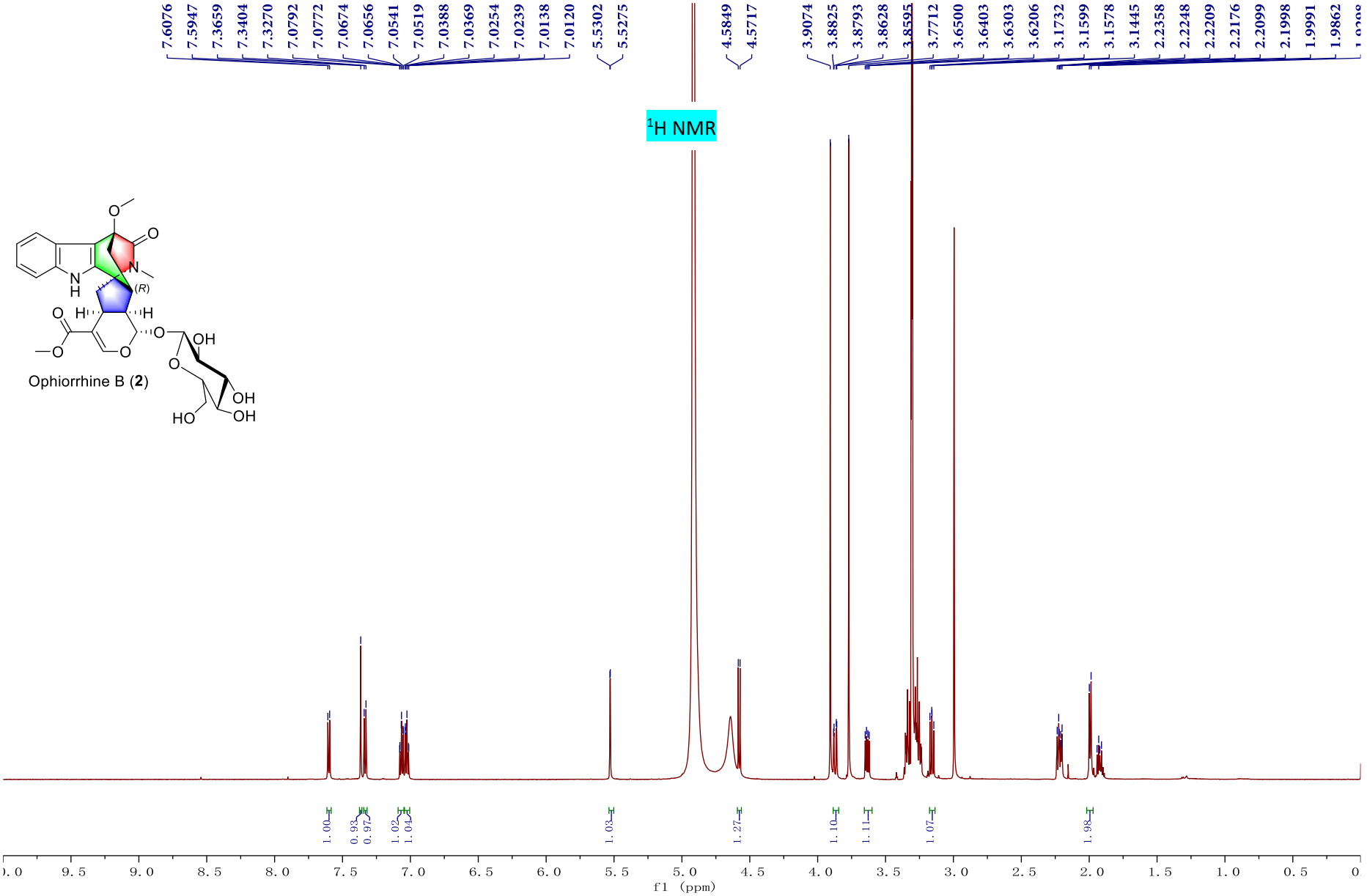
Formula Calculator Element Limits

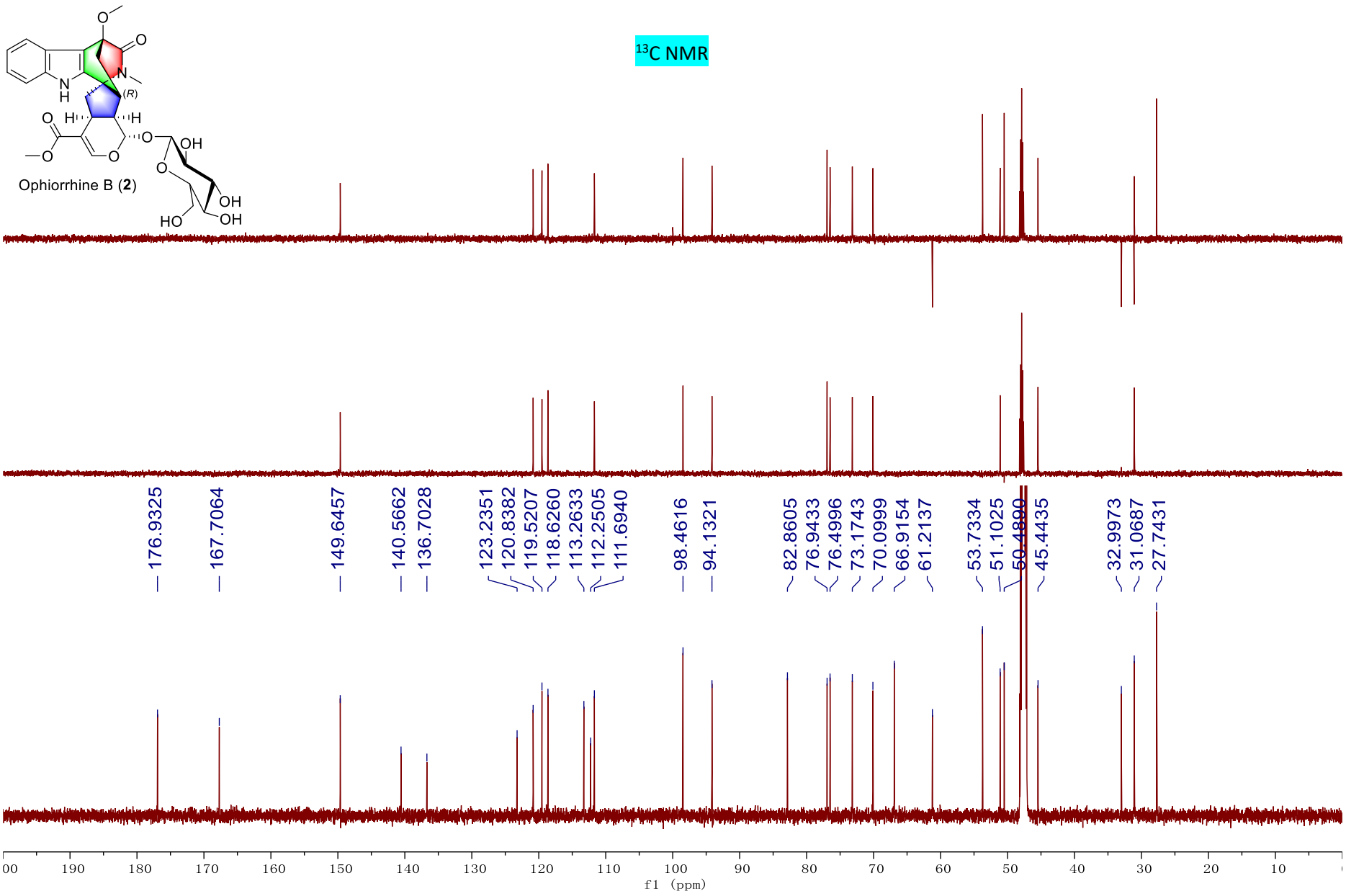
Element	Min	Max
C	3	60
H	0	120
O	0	30
N	0	5

Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C28 H32 N2 O10	556.2057	579.1949	579.1952	-0.5	-0.9	14.0000

2.2. NMR spectra and HRESIMS for trichothecrotocin B (2)




Ophiorrhine B (2)
13C NMR
176.9325
167.7064
149.6457
140.5662
136.7028
123.2351
120.8382
119.5207
118.6260
113.2633
112.2505
111.6940
98.4616
94.1321
82.8605
76.9433
76.4996
73.1743
70.0999
66.9154
61.2137
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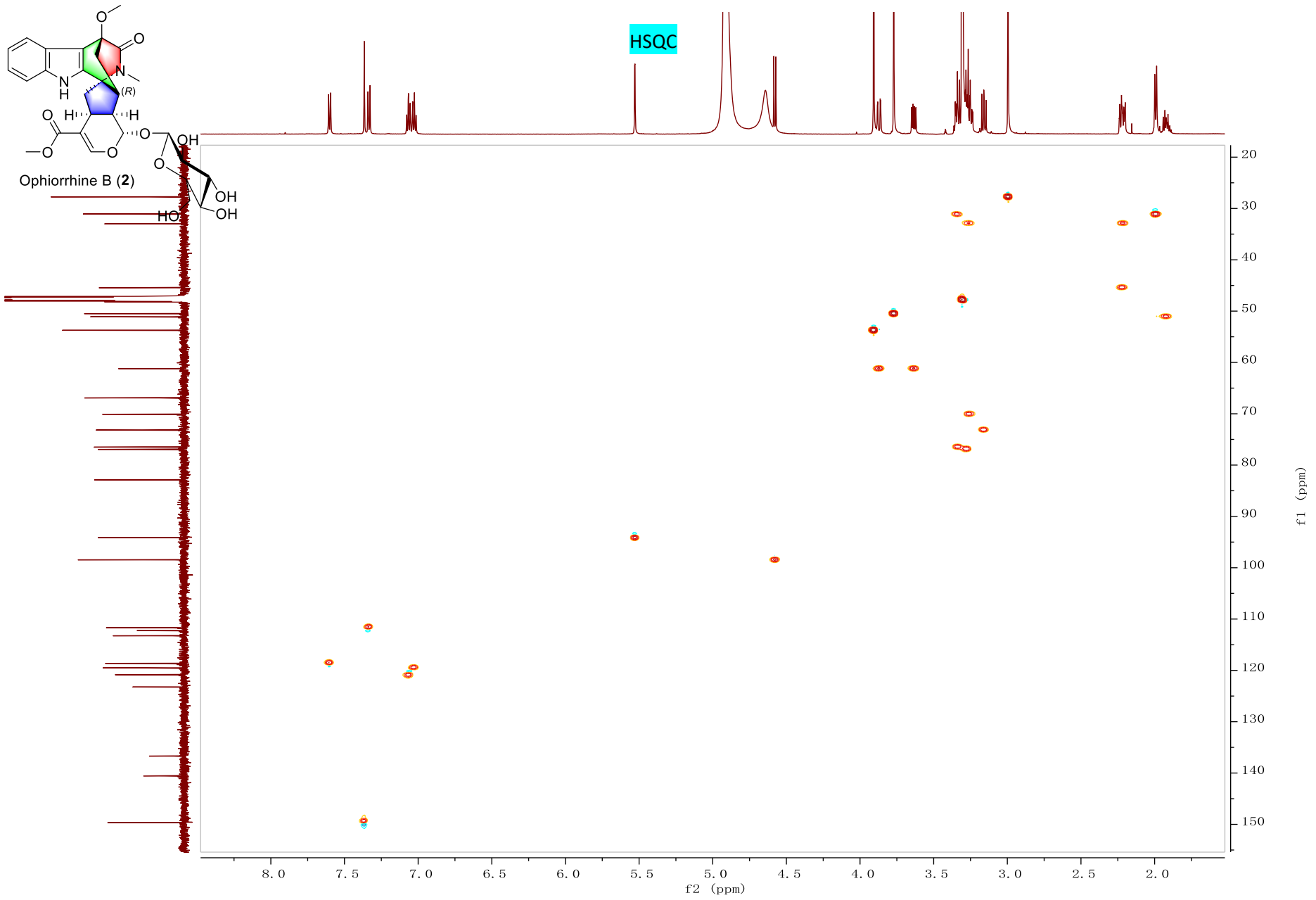
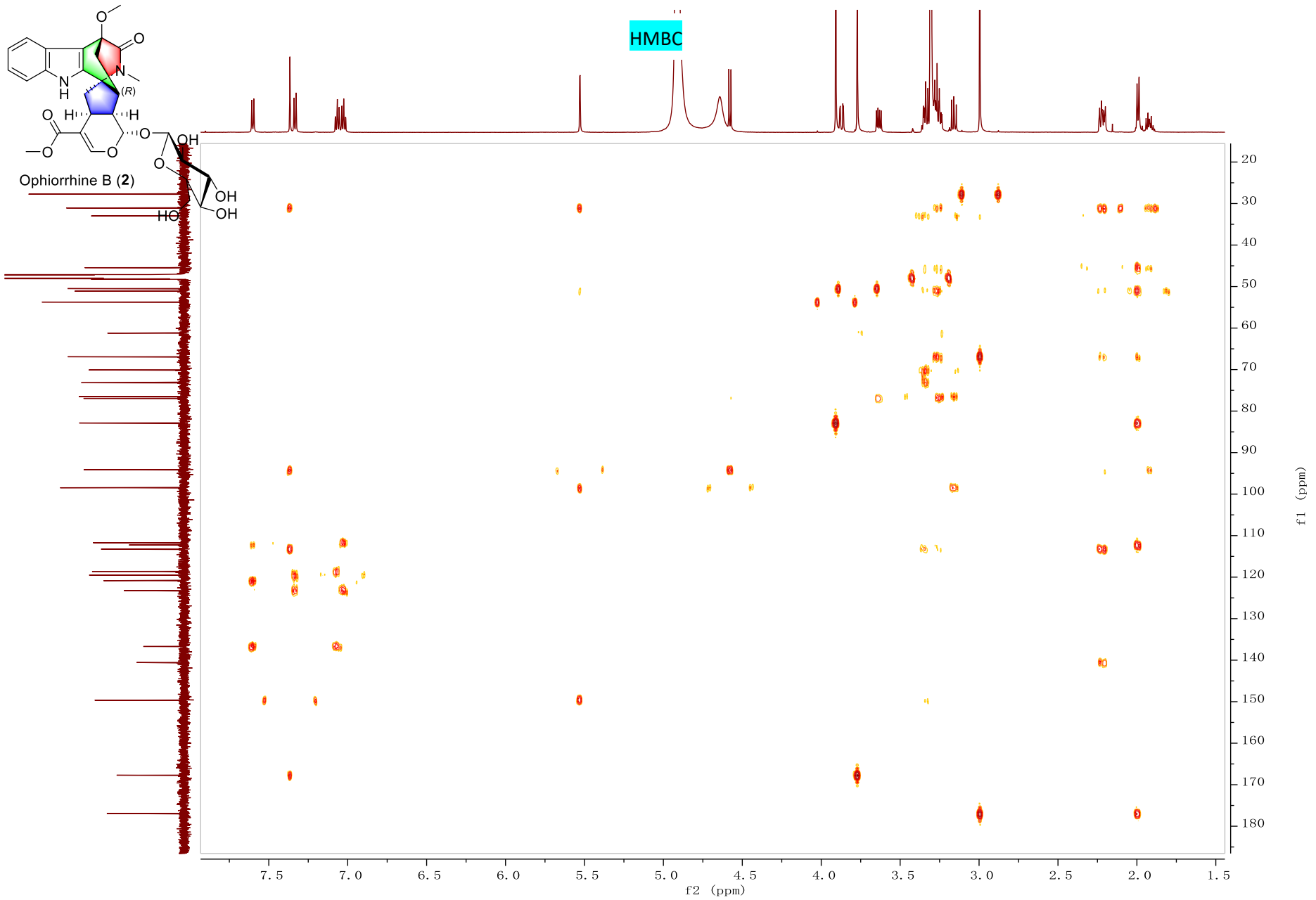
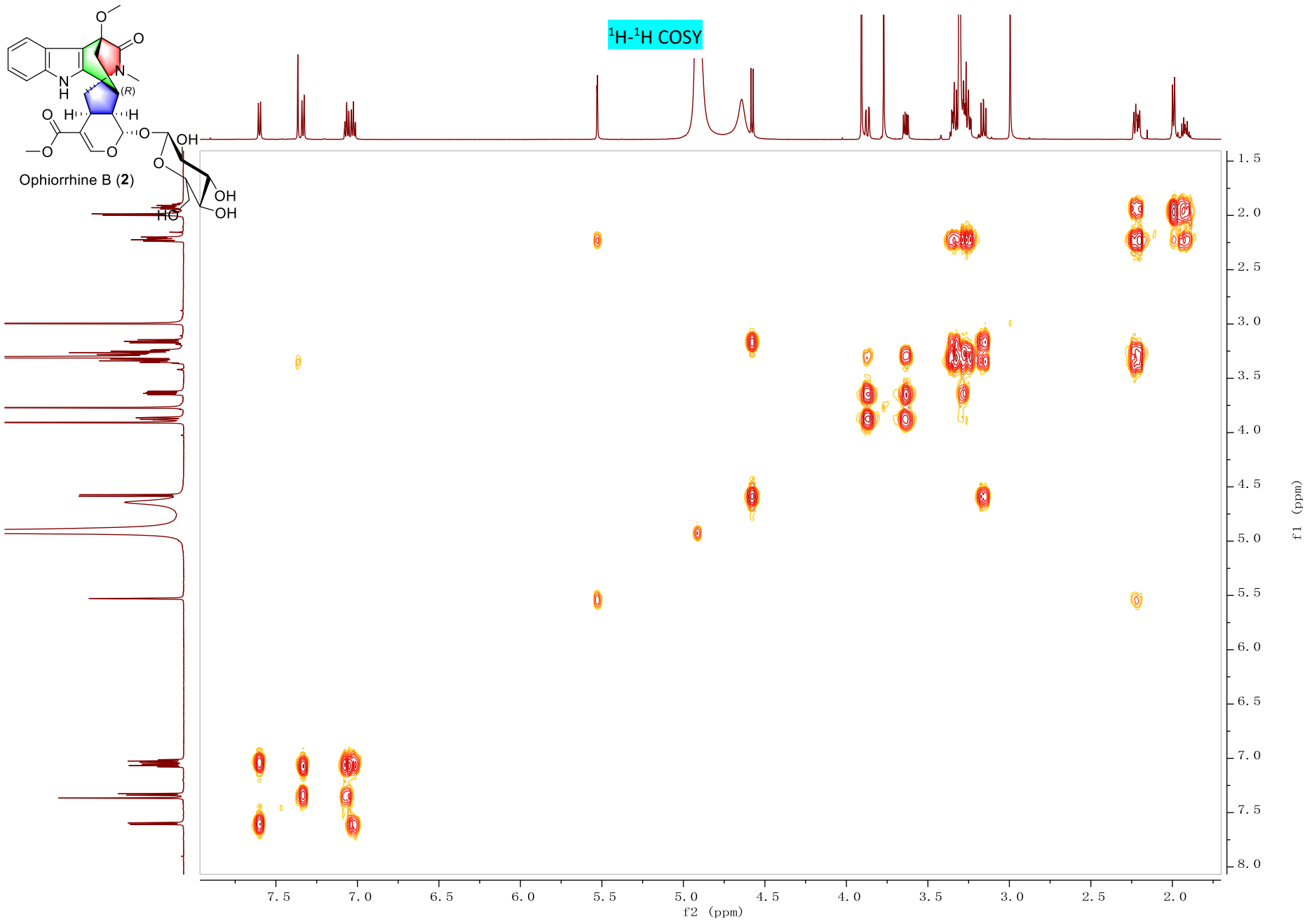
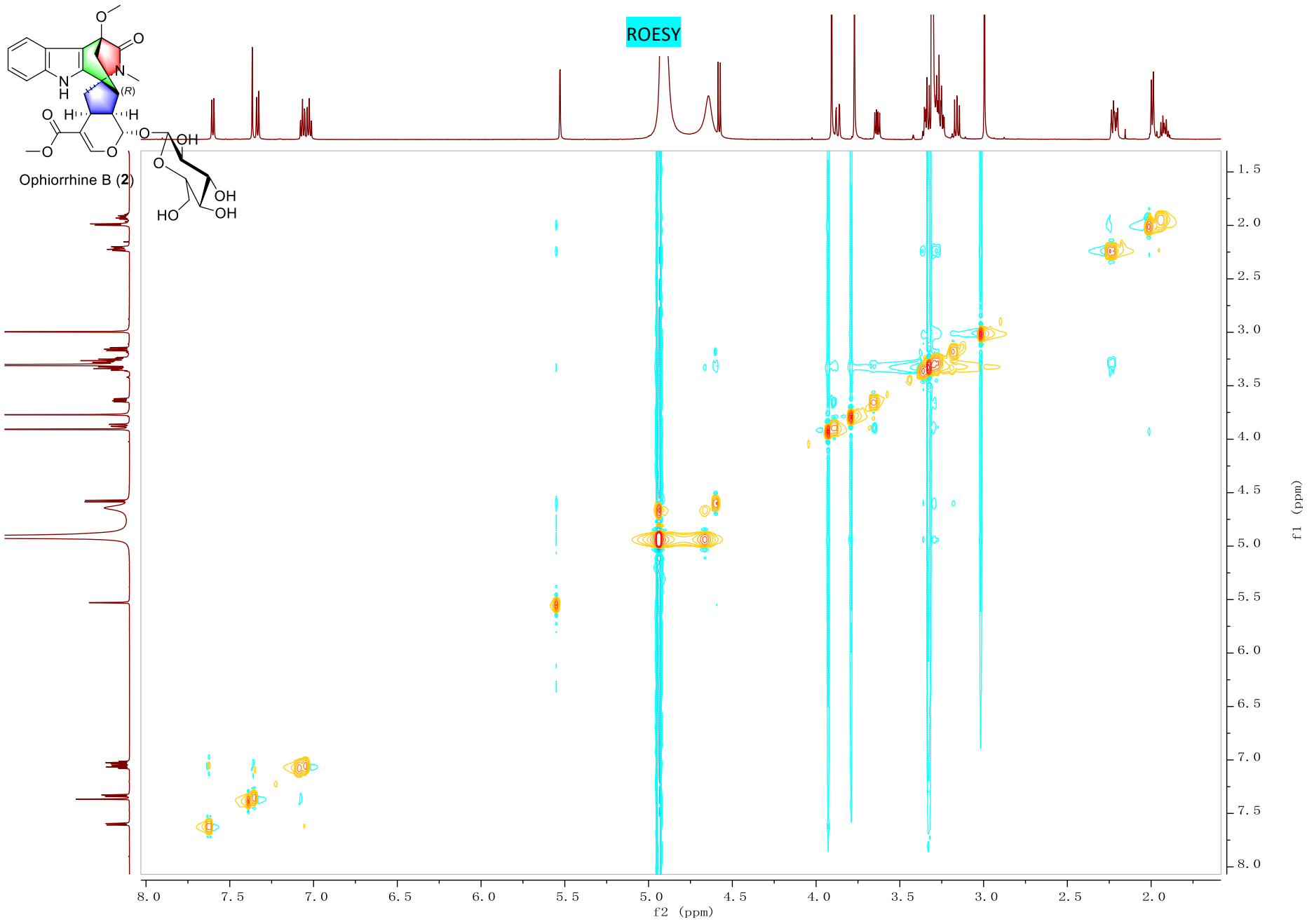
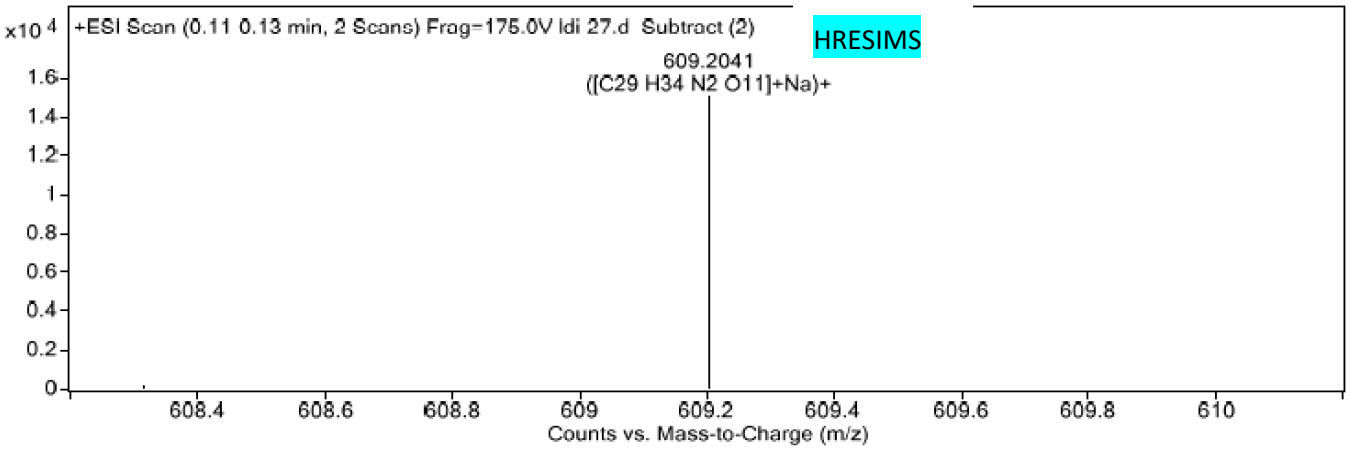
32.9973
31.0687
27.7431
f1 (ppm)


HSQC
Ophiorrhine B (2)
f2 (ppm)
f1 (ppm)


HMBC
Ophiorrhine B (2)
f2 (ppm)
f1 (ppm)


Ophiorrhine B (2)
^{1}H-^{1}H COSY
f2 (ppm)
f1 (ppm)


ROESY
Ophiorrhine B (2)
f2 (ppm)
f1 (ppm)



Peak List

m/z	z	Abund	Formula	Ion
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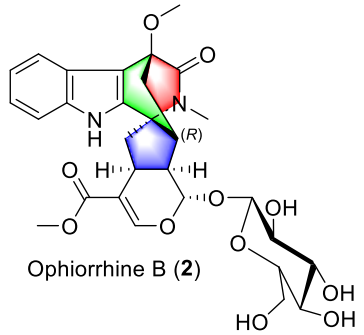
318.3009	1	3762.01		
453.1685	1	4436.43		
587.2237	1	5241.88		
609.2041	1	15044.65	C29 H34 N2 O11	(M+Na)+
610.2081	1	5336.13	C29 H34 N2 O11	(M+Na)+
625.1795	1	8949.68		
626.1847	1	2666.99		
675.2586	1	2499.61		



Ophiorrhine B (**2**)

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
C29 H34 N2 O11	586.2163	609.2055	609.2041	1.1	1.8	14.0000