Electronic Supplementary Information (ESI)

Schoberine B, Alkaloid with Unprecedented Straight C_5 Side Chain and Myriberine B from *Myrioneuron faberi*

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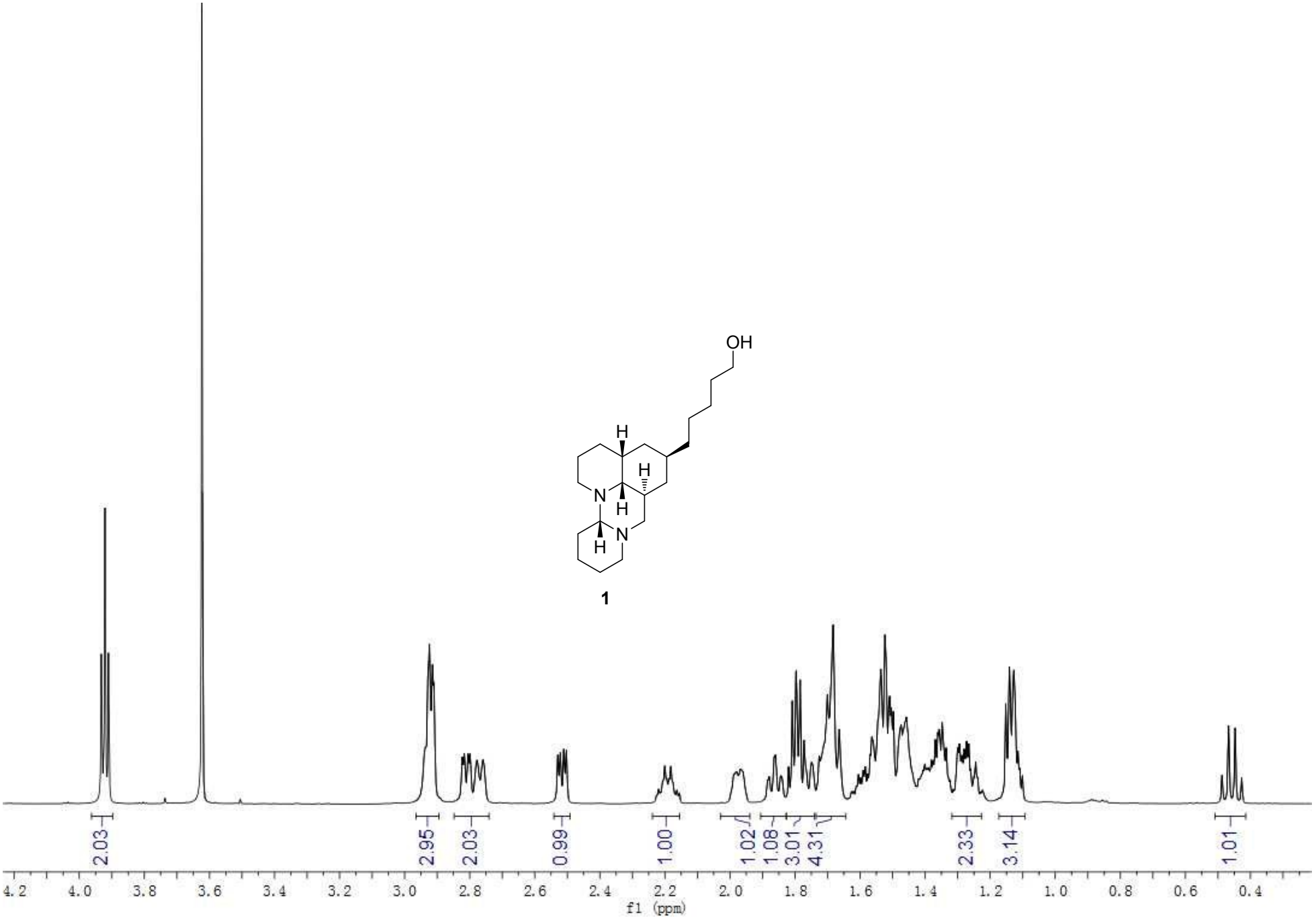
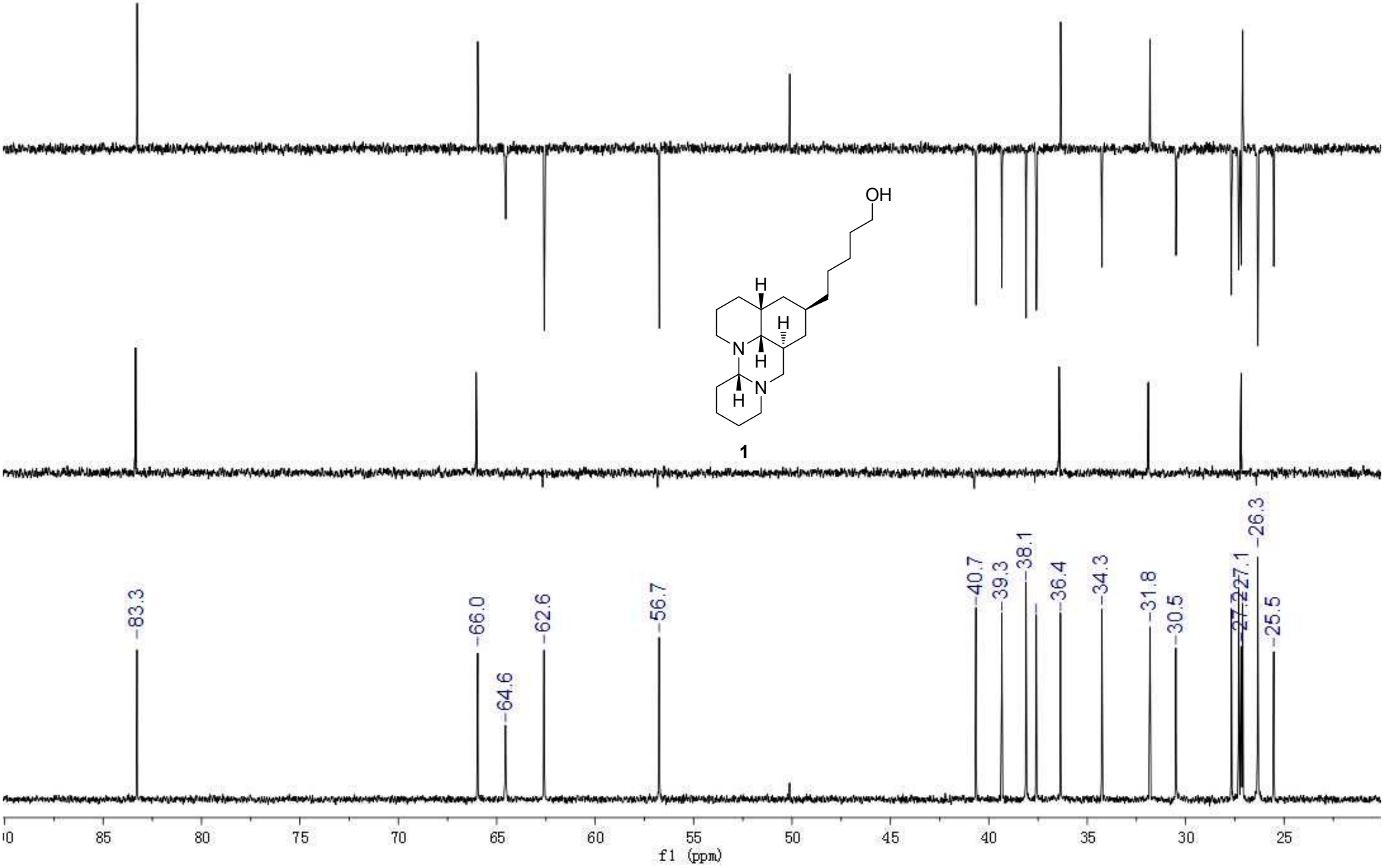
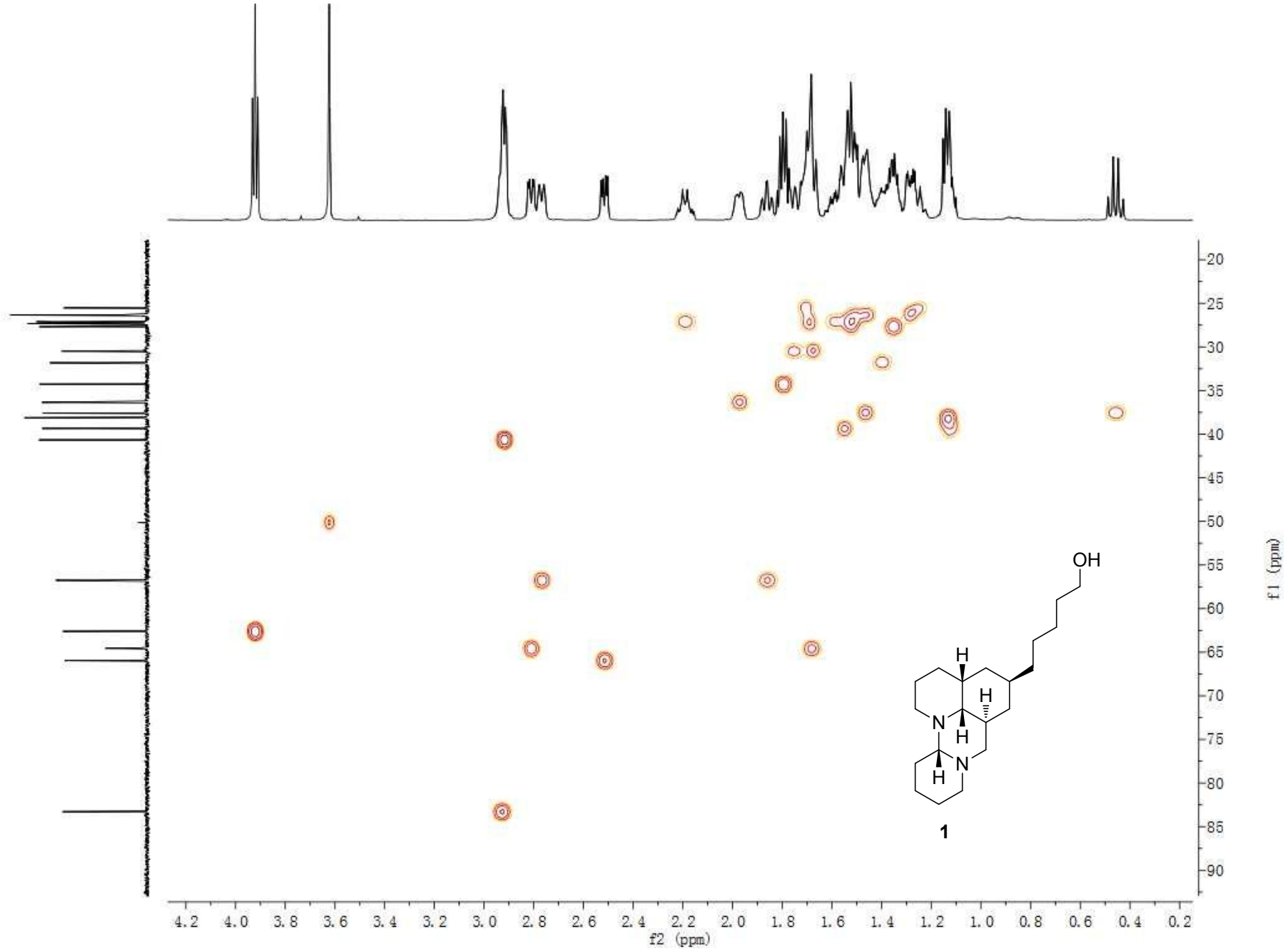
S1.1 ^{1}H NMR spectrum of schoberine B (**1**) in prydine-d_5 at 313K



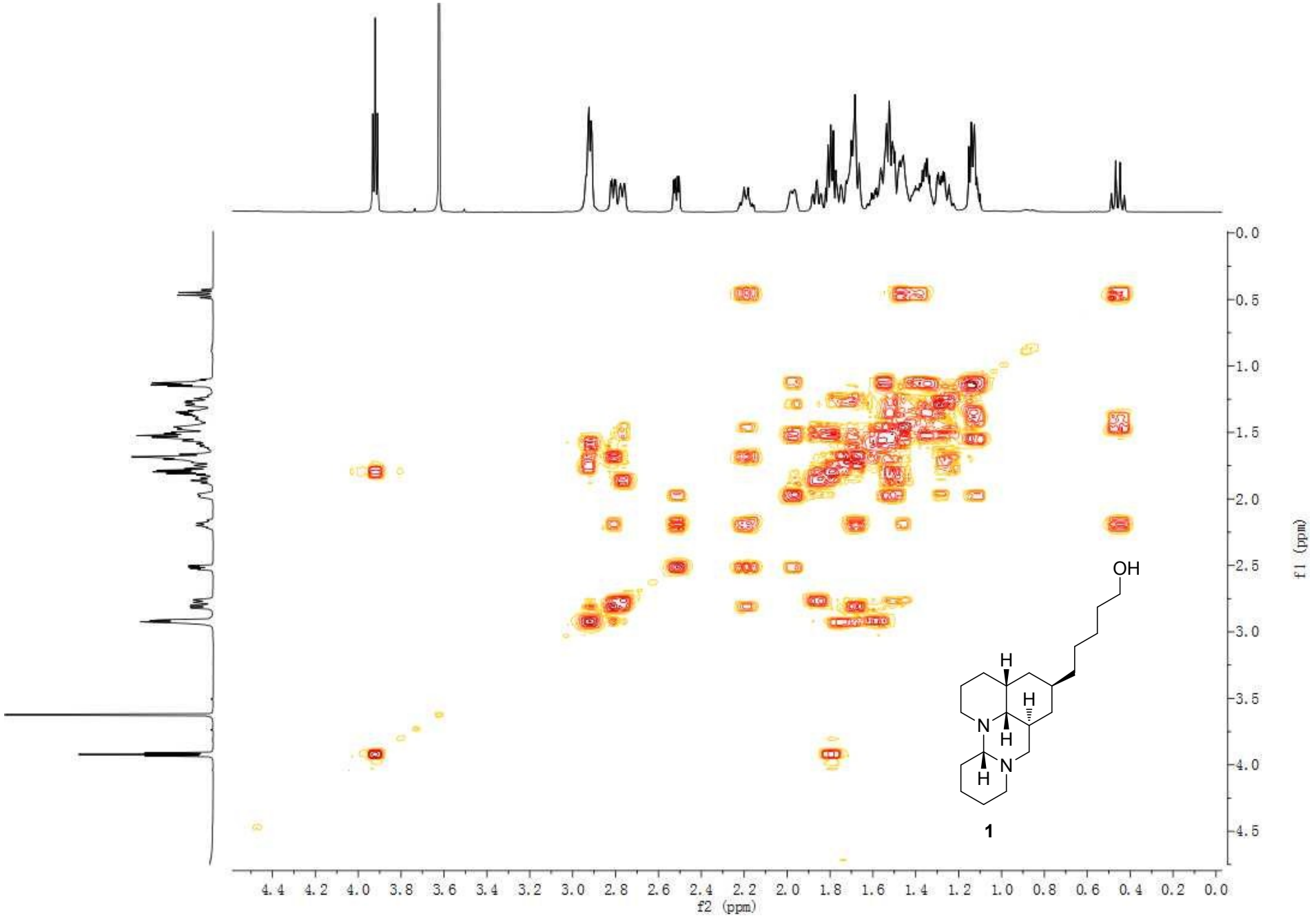
S1.2 ^{13}C NMR spectrum of schoberine B (**1**) in prydine-d_5 at 313K



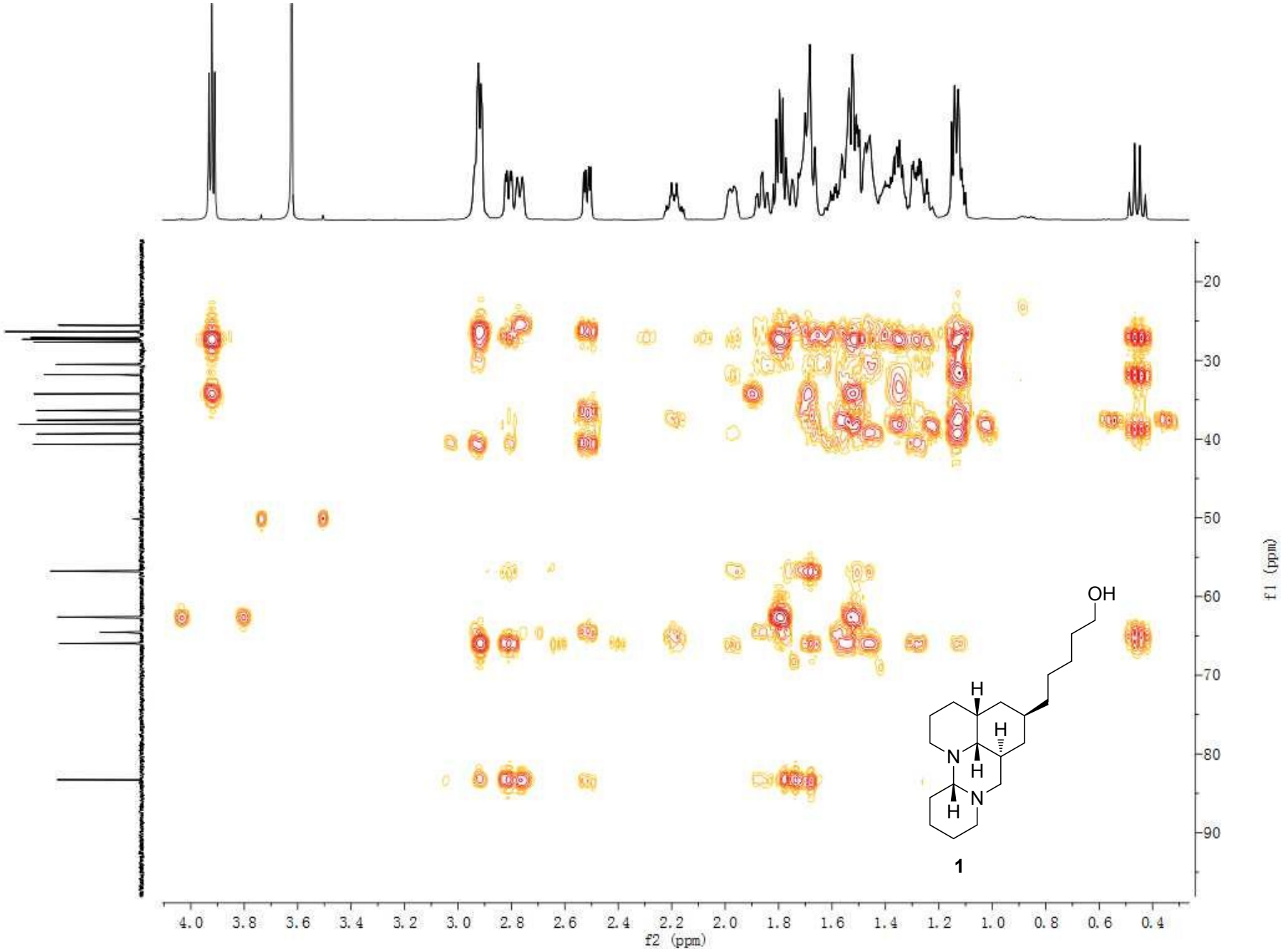
S1.3 HSQC spectrum of schoberine B (**1**) in prydine-d_5 at 313K



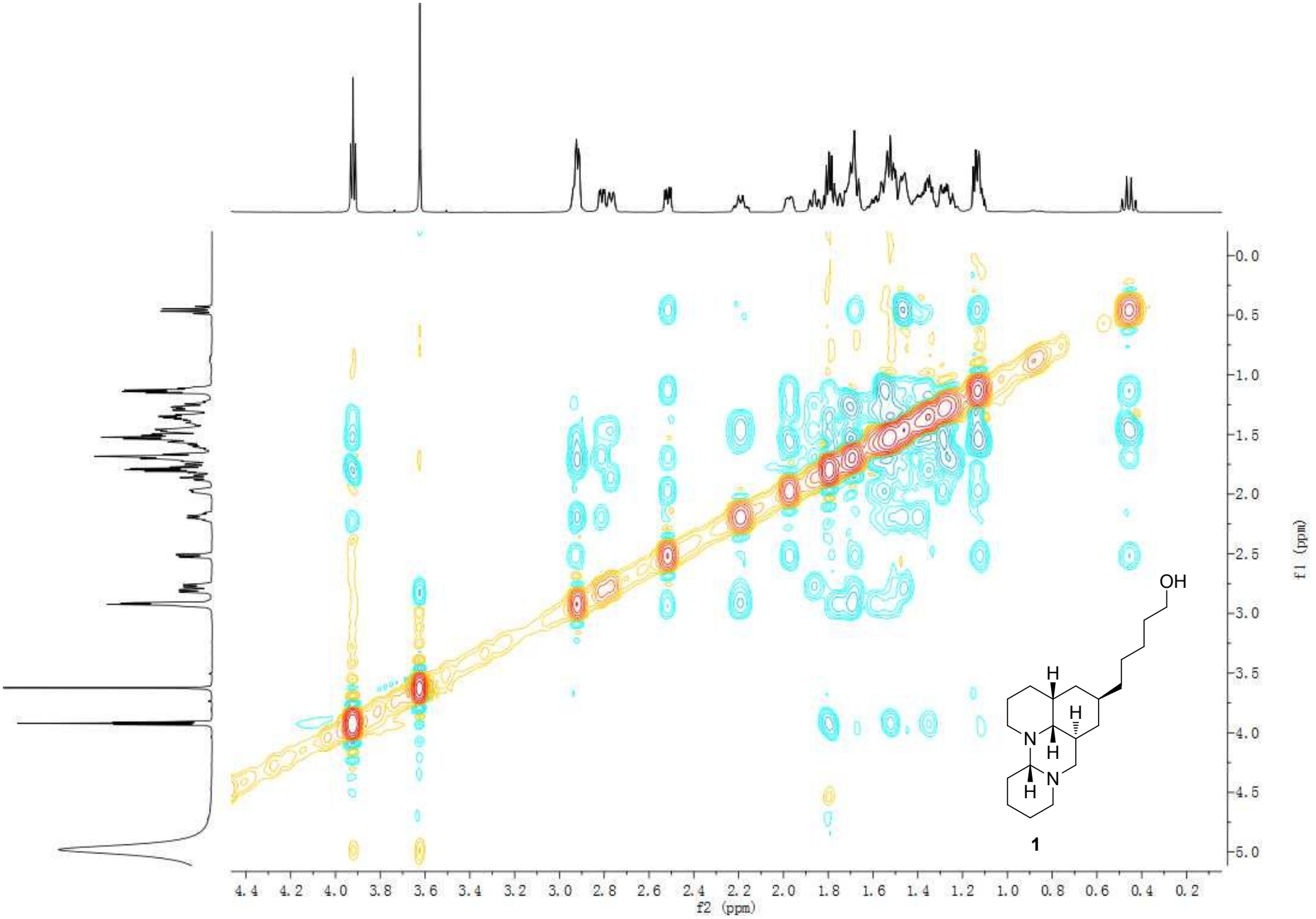
S1.4 COSY spectrum of schoberine B (**1**) in prydine-d_5 at 313K



S1.5 HMBC spectrum of schoberine B (**1**) in prydine-d_5 at 313K



S1.6 ROESY spectrum of schoberine B (**1**) in prydine-d_5 at 313K



S1.7 ESIMS and HREIMS spectrums of schoberine B (**1**)

Mass Spectrum List Report

Analysis Info

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Sample Name	hm9	Operator	Bruker
		Instrument	HCT

Acquisition Parameter

Ion Source Type	ESI	Ion Polarity	Positive	Alternating Ion Polarity	off
Mass Range Mode	Ultra Scan	Scan Begin	100 m/z	Scan End	1000 m/z
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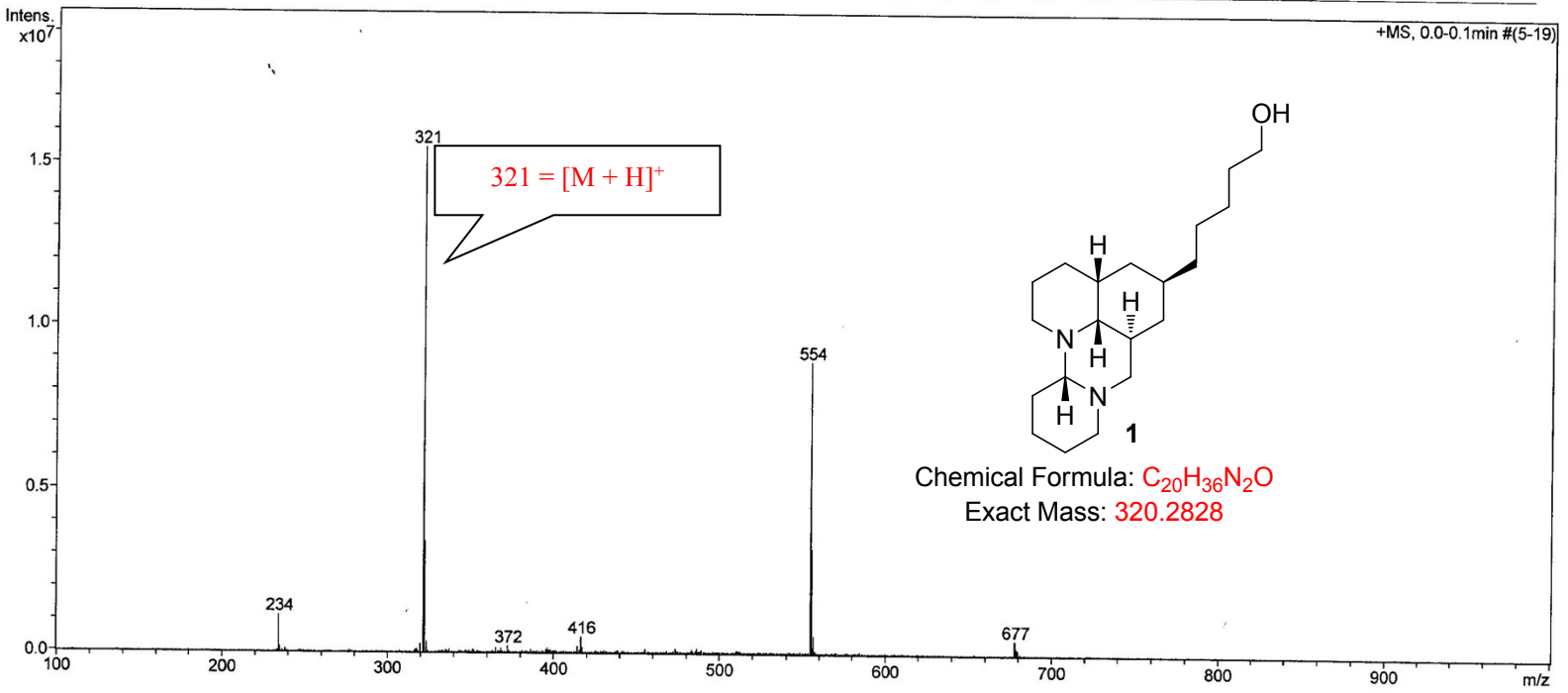


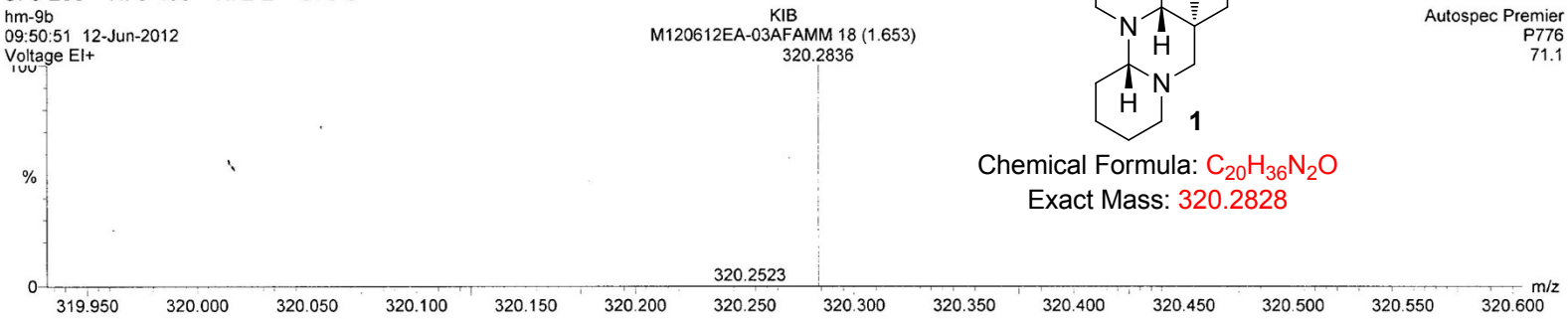
Chemical Formula: $C_{20}H_{36}N_2O$

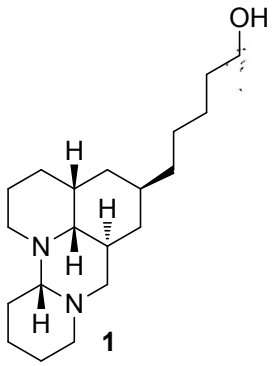
Exact Mass: 320.2828

Elemental Composition Report

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -10.0, max = 120.0
Selected filters: None

Monoisotopic Mass, Odd and Even Electron Ions
22 formula(e) evaluated with 1 results within limits (up to 51 closest results for each mass)
Elements Used:
C: 0-200 H: 0-400 N: 2-2 O: 0-3





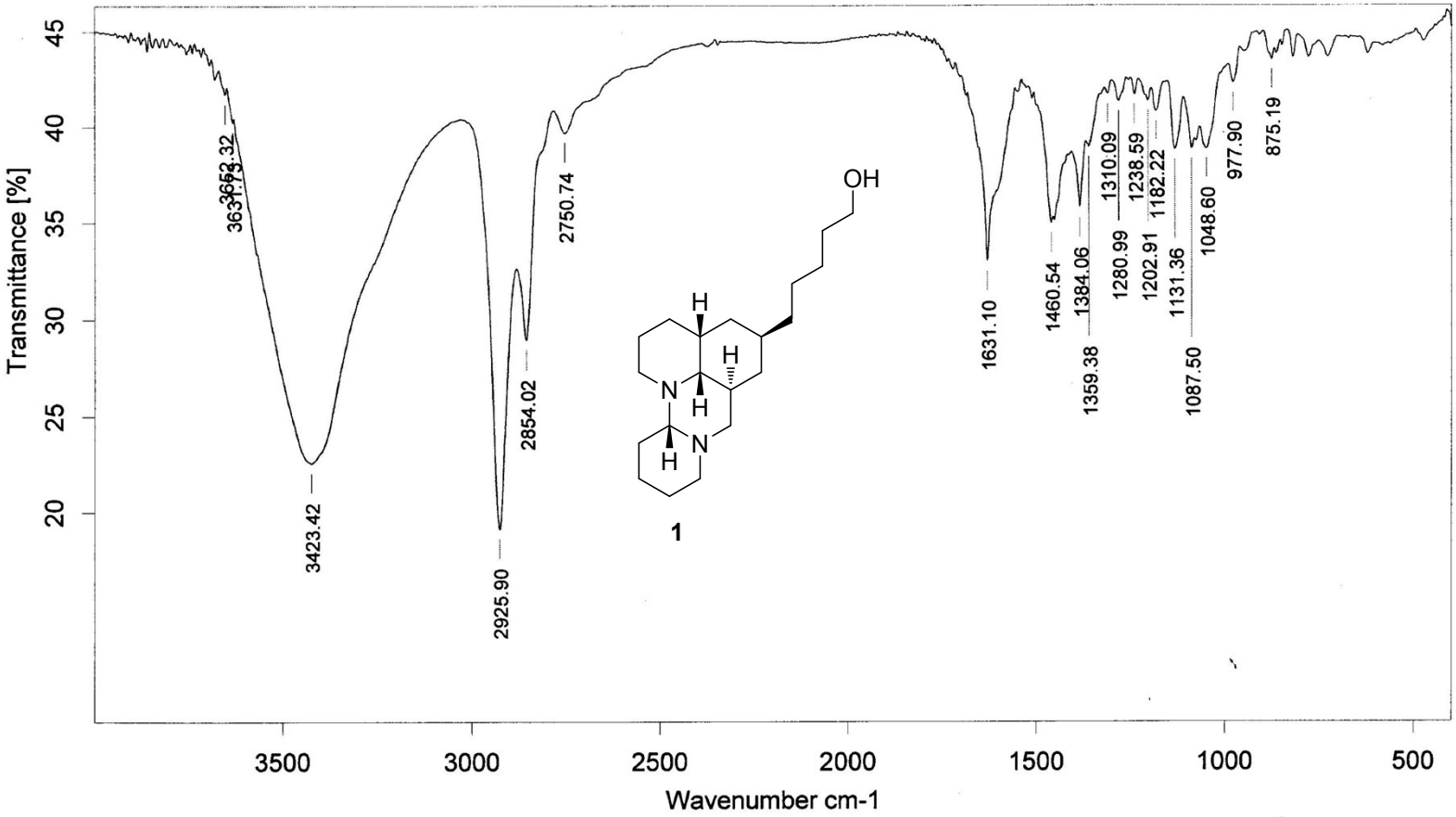
1

Autospec Premier
P776
71.1

Chemical Formula: $C_{20}H_{36}N_2O$
Exact Mass: 320.2828

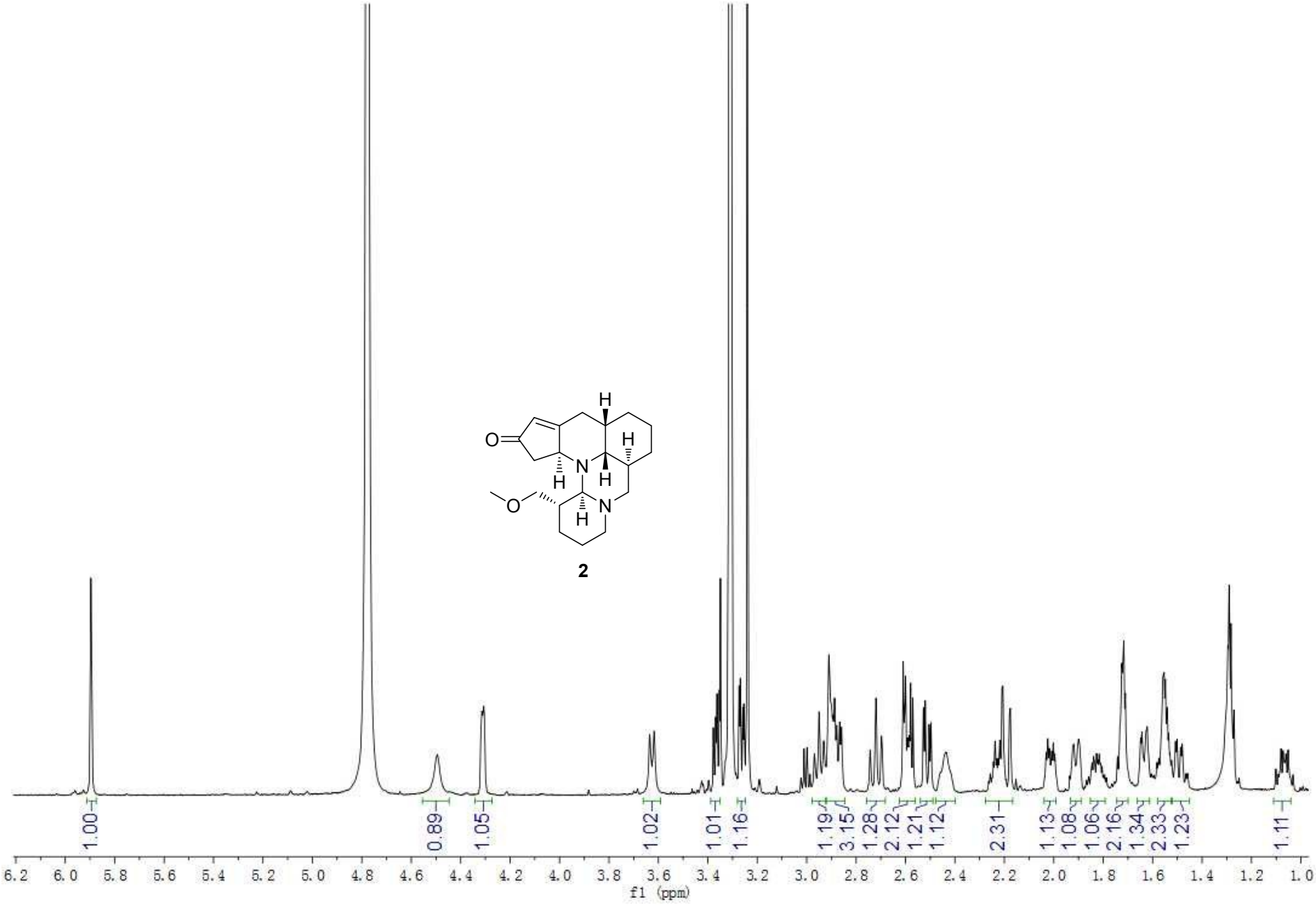
Minimum:				-10.0		
Maximum:		100.0	10.0	120.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
320.2836	320.2828	0.8	2.5	4.0	5546050.0	C20 H36 N2 O

S1.8 IR spectrum of schoberine B (**1**)

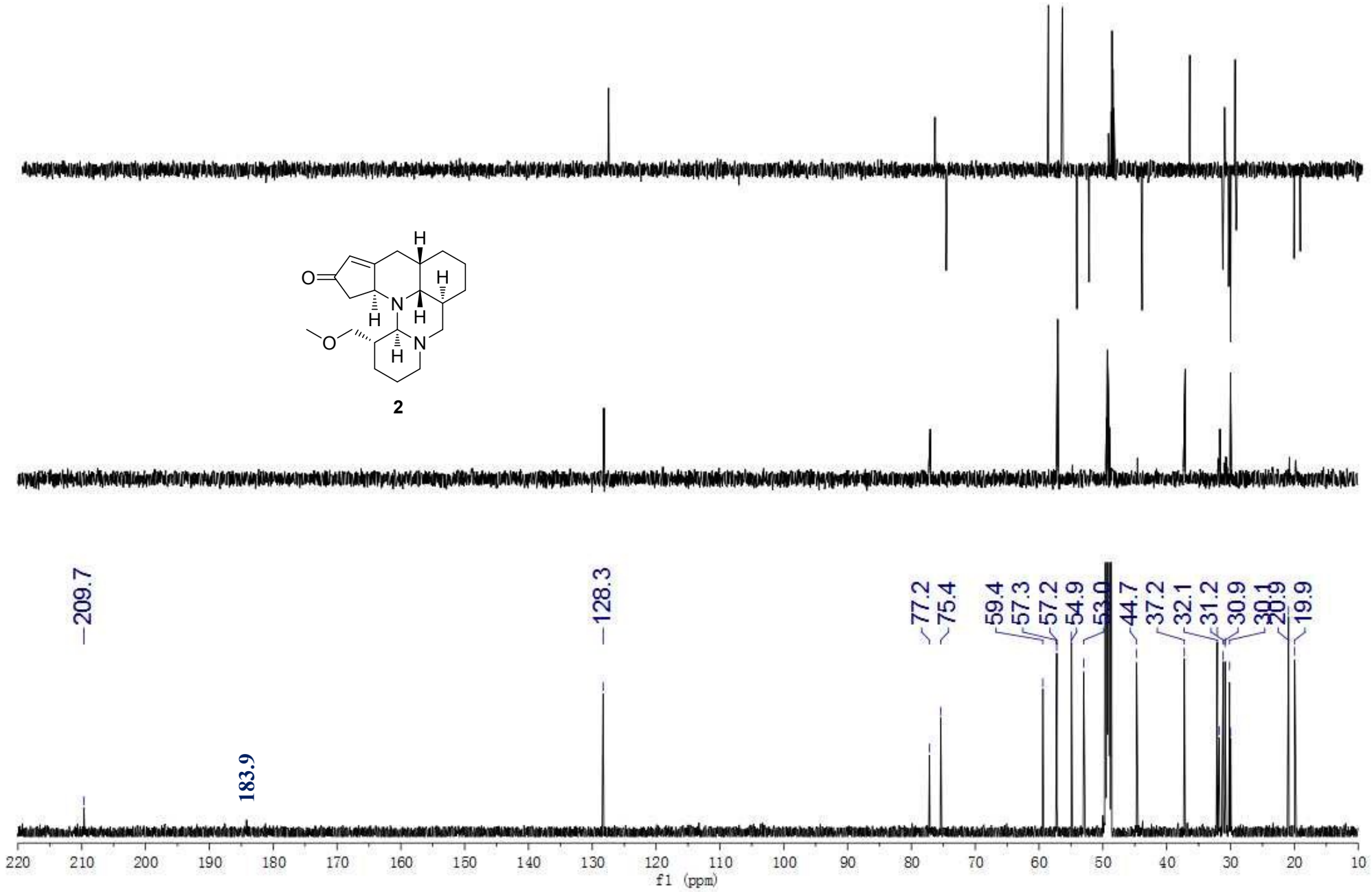


Sample : hm-9b	Frequency Range : 399.246 - 3996.32	Measured on : 05/07/2012	
Technique : KBr压片	Resolution : 4	Instrument : Tensor27	Sample Scans : 16
Customer : 120705IR0	Zerofilling : 2	Acquisition : Double Sided,For	

S2.1 ^{1}H NMR spectrum of myriberine B (**2**) in methanol-d_4 at 313K

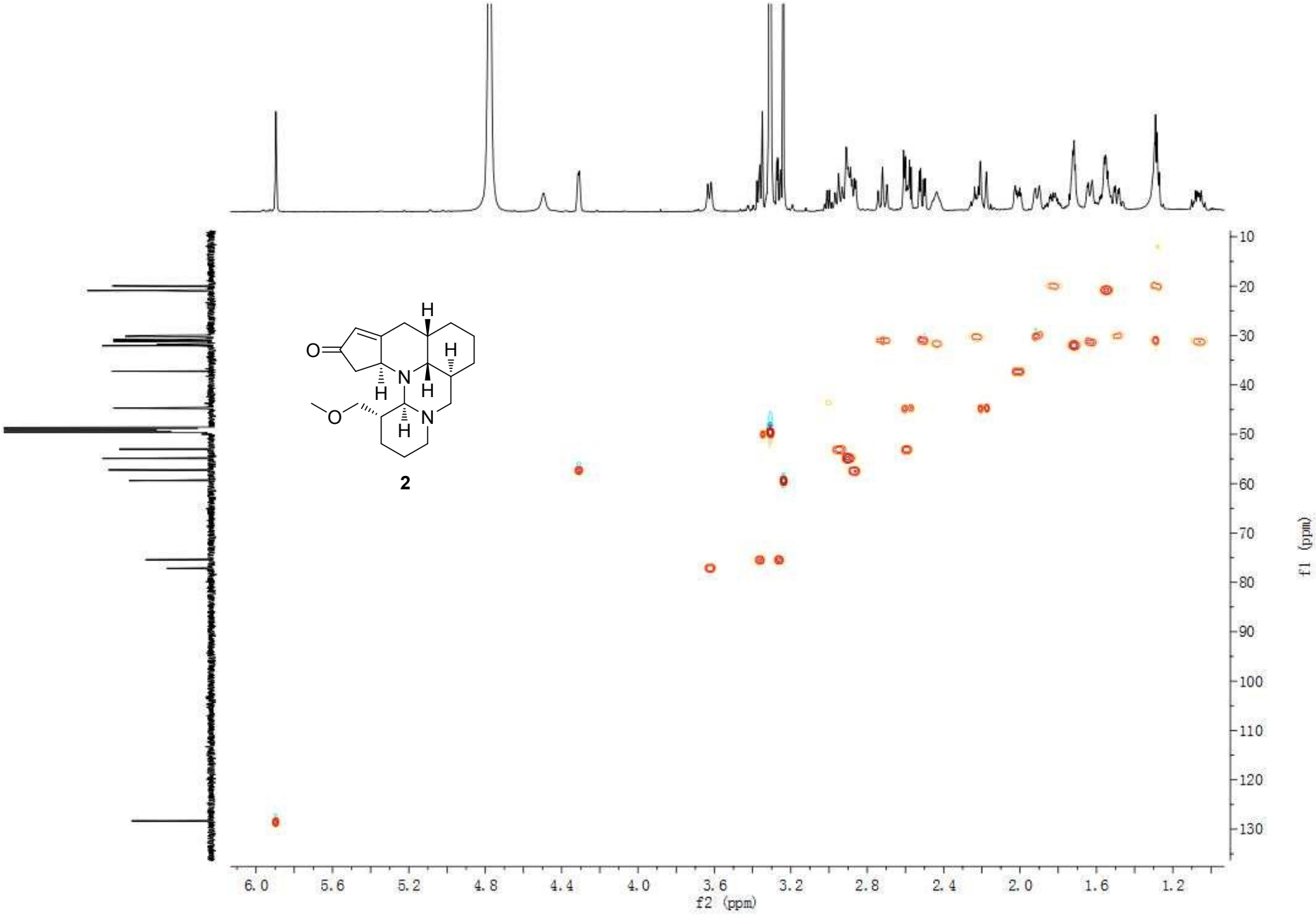


S2.2 ^{13}C NMR spectrum of myriberine B (**2**) in methanol-d_4 at 313K

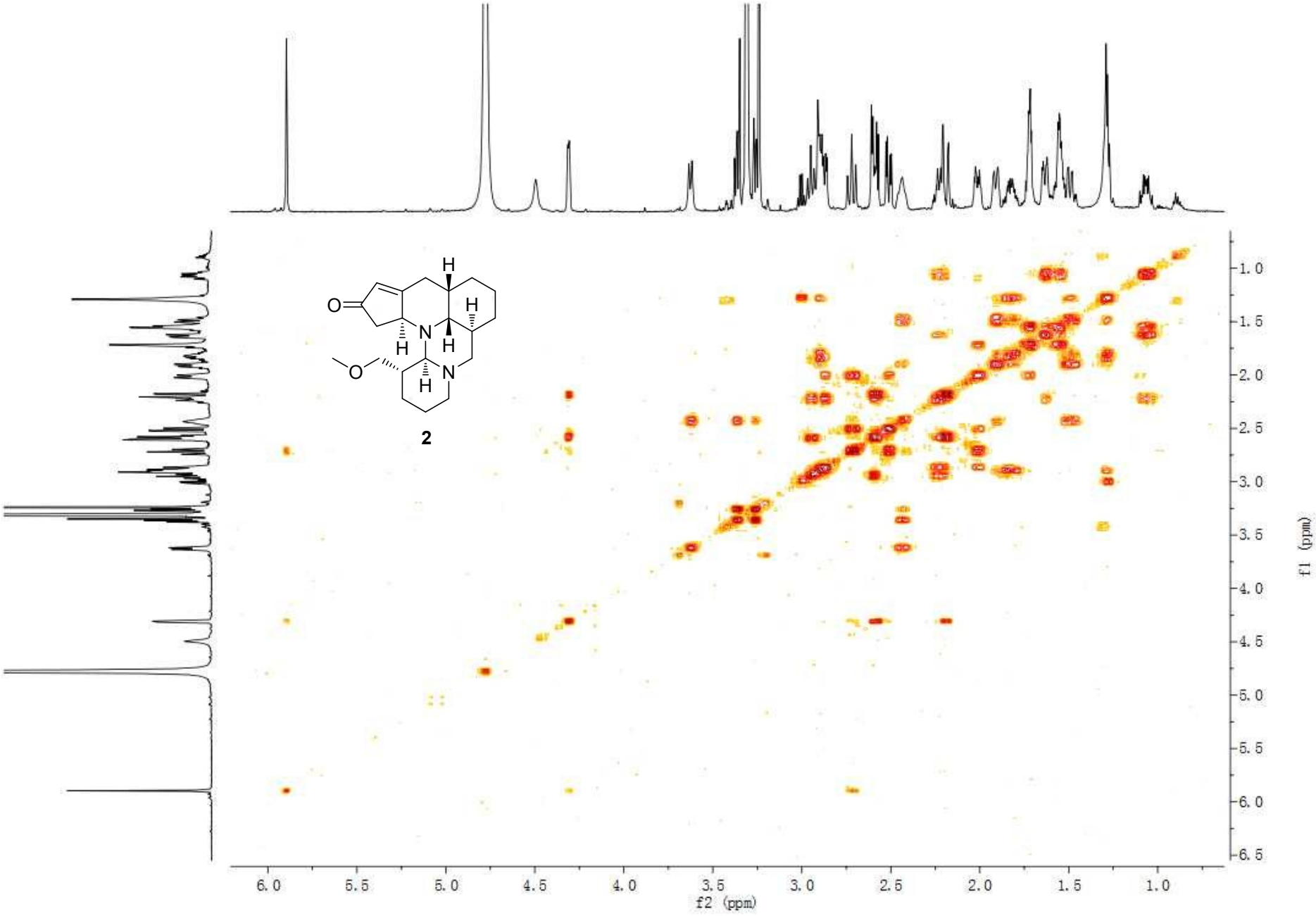


2

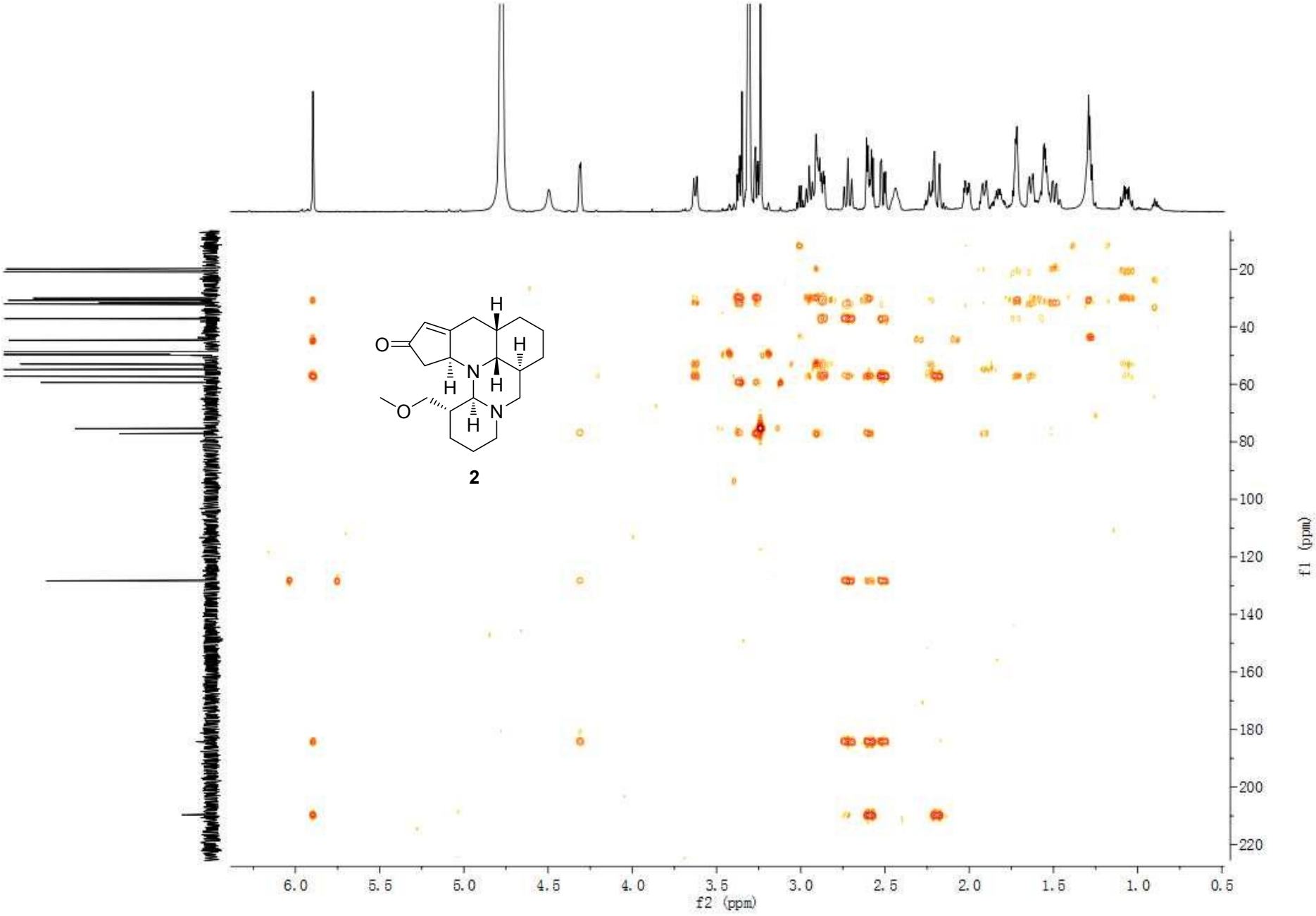
S2.3 HSQC spectrum of myriberine B (**2**) in methanol-d_4 at 313K



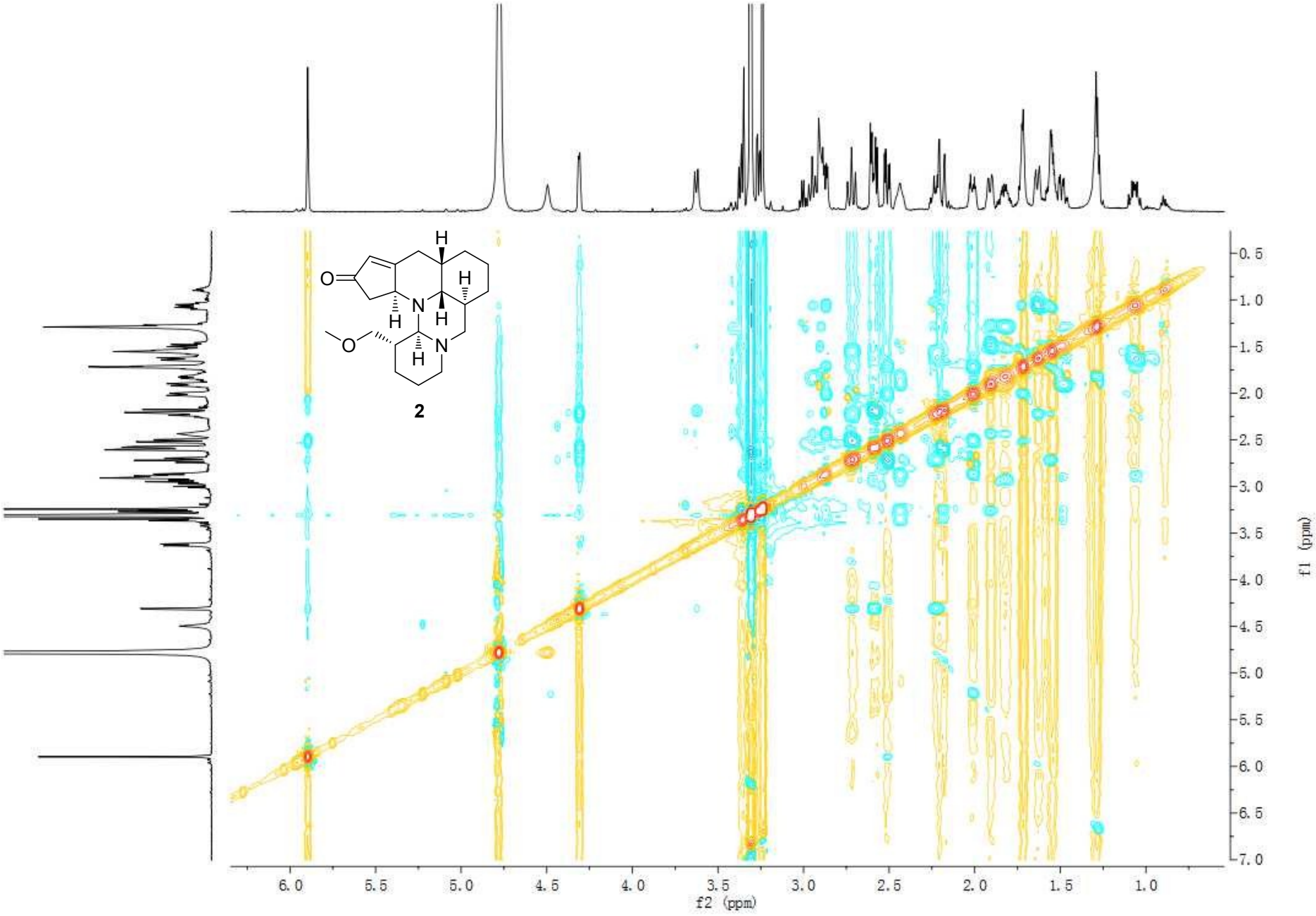
S2.4 COSY spectrum of myriberine B (**2**) in methanol-d_4 at 313K



S2.5 HMBC spectrum of myriberine B (**2**) in methanol-d_4 at 313K



S2.6 ROESY spectrum of myriberine B (**2**) in methanol-d_4 at 313K



S2.7 ESIMS and HREIMS spectrums of myriberine B (**2**)

Mass Spectrum List Report

Analysis Info

Analysis Name	D:\DATA\2012file\1204\120427\hm-5100.d	Acquisition Date	4/26/2012 4:06:54 PM
Method	ms_ptservice.m	Operator	Bruker
Sample Name	hm-51	Instrument	HCT

Acquisition Parameter

Ion Source Type	ESI	Ion Polarity	Positive	Alternating Ion Polarity	off
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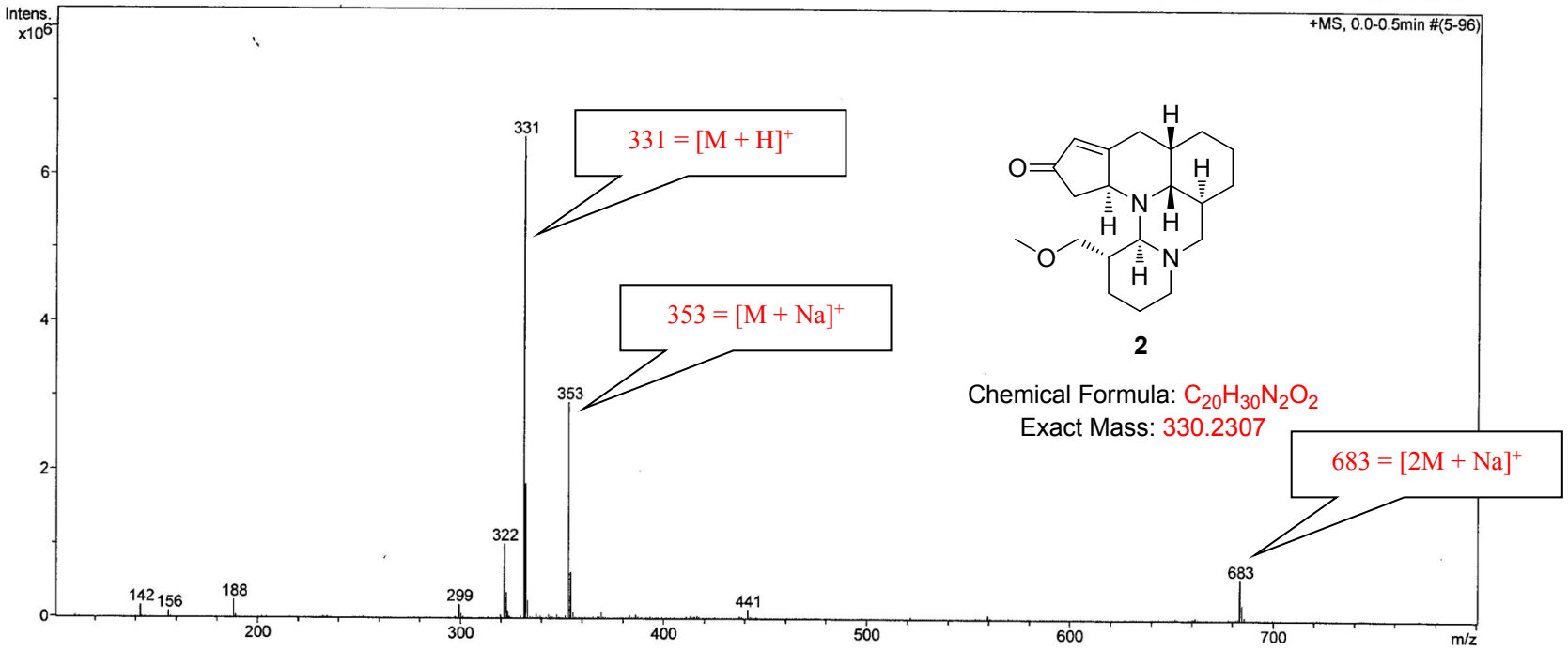
Accumulation Time	1062 µs	Averages	5 Spectra	Auto MS/MS	off



2

Chemical Formula: $C_{20}H_{30}N_2O_2$

Exact Mass: 330.2307

Elemental Composition Report

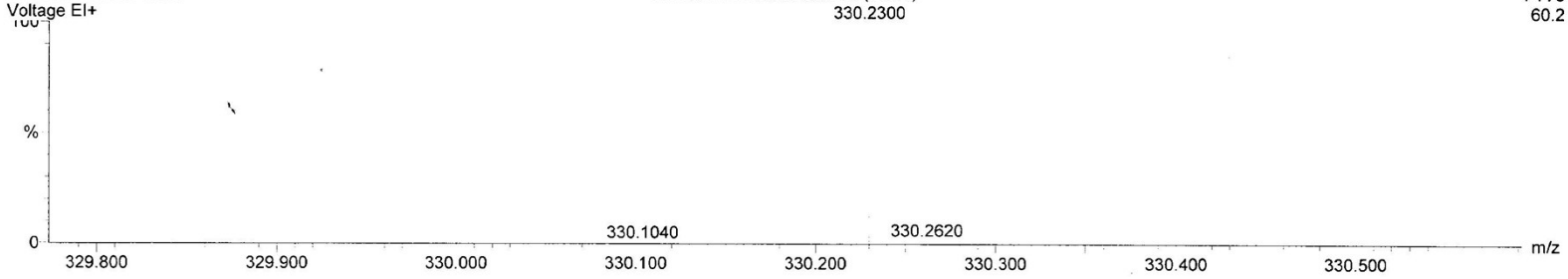
Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -10.0, max = 120.0
Selected filters: None

Monoisotopic Mass, Odd and Even Electron Ions
22 formula(e) evaluated with 1 results within limits (up to 51 closest results for each mass)
Elements Used:
C: 0-200 H: 0-400 N: 2-2 O: 0-3

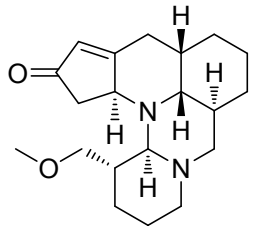
hm-51
09:45:29 12-Jun-2012
Voltage EI+

KIB
M120612EA-02AFAMM 13 (1.194)
330.2300

Autospec Premier
P776
60.2



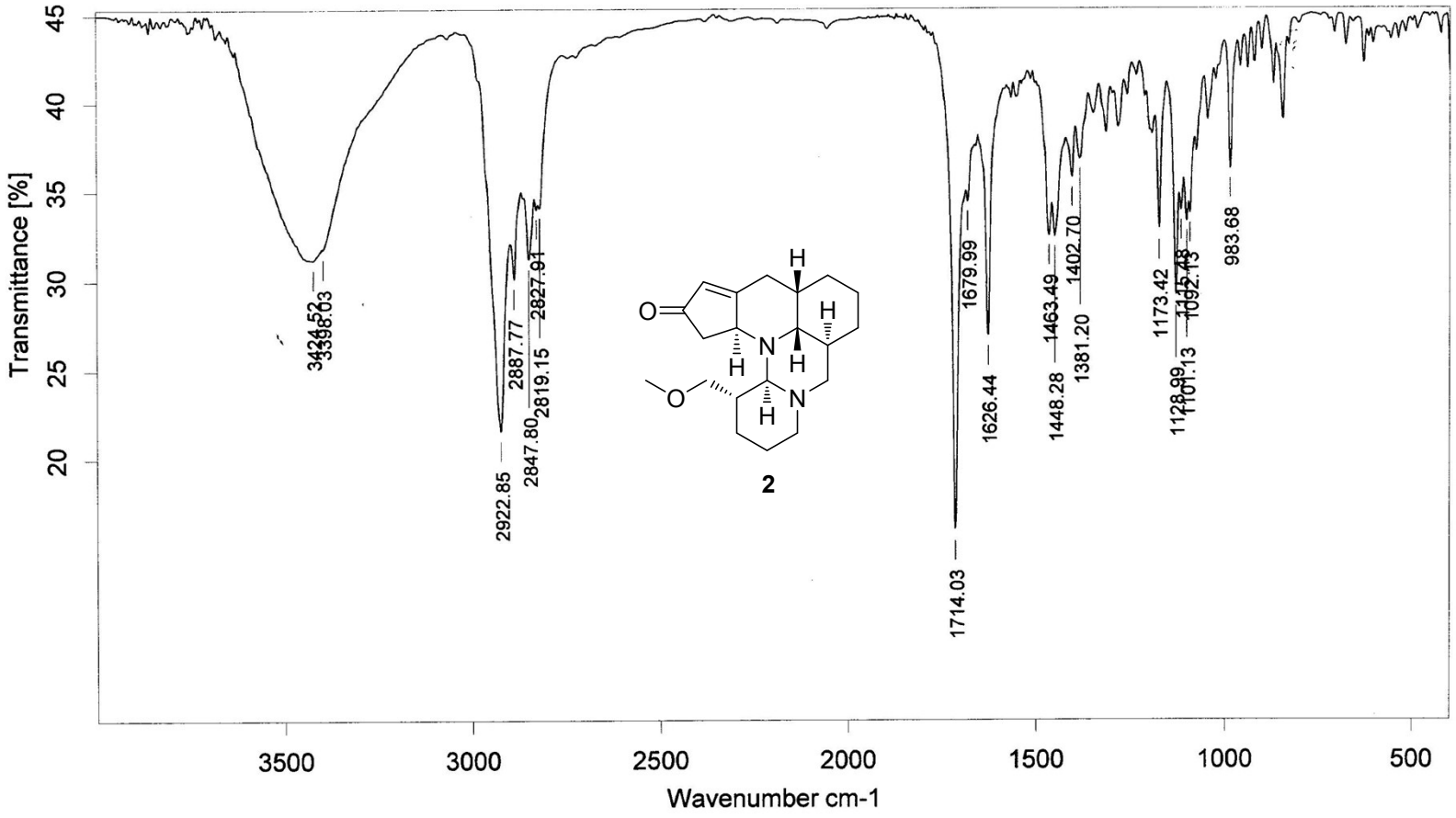
Minimum:				-10.0		
Maximum:		100.0	10.0	120.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
330.2300	330.2307	-0.7	-2.1	7.0	5546045.0	C20 H30 N2 O2



2

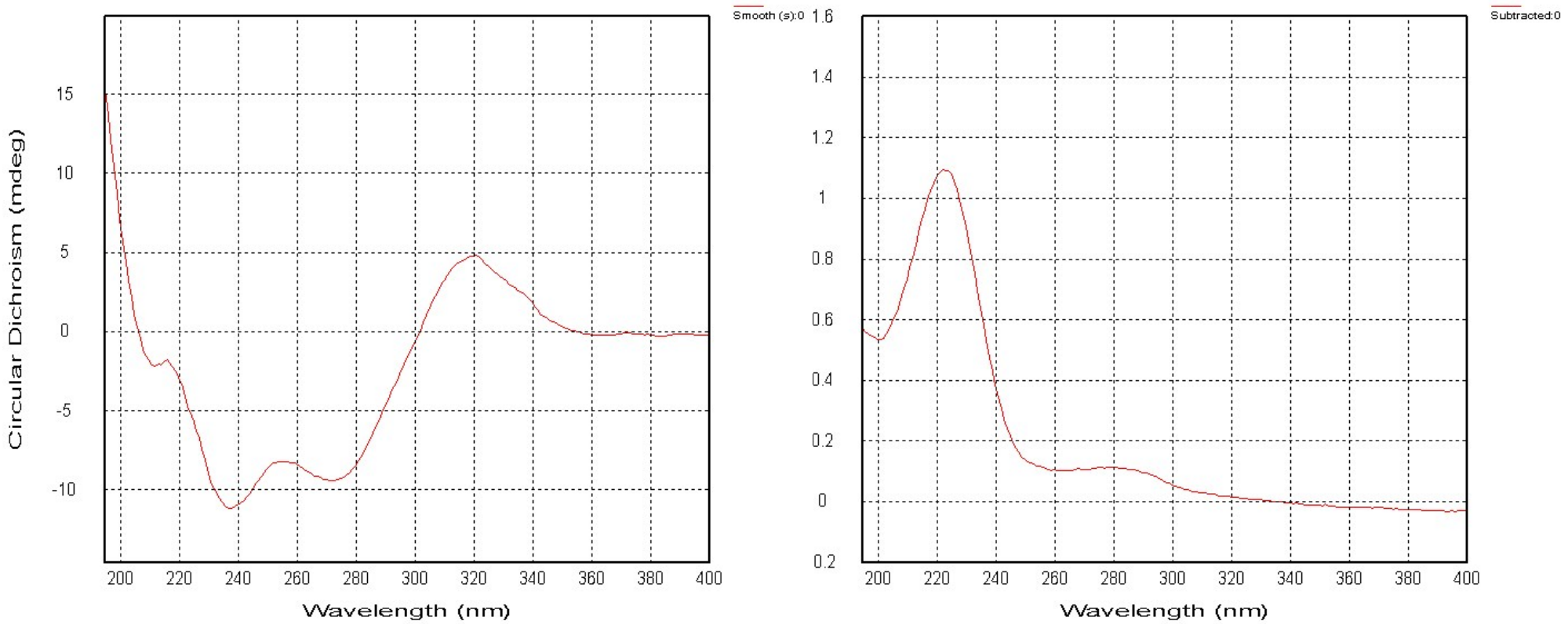
Chemical Formula: $C_{20}H_{30}N_2O_2$
Exact Mass: 330.2307

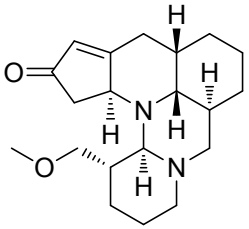
S2.8 IR spectrum of myriberine B (**2**)



Sample : hm-51	Frequency Range : 399.246 - 3996.32	Measured on : 26/06/2012	
Technique : KBr压片	Resolution : 4	Instrument : Tensor27	Sample Scans : 16
Customer : 120626IR18	Zerofilling : 2	Acquisition : Double Sided,For	

S2.9 ECD spectrum of myriberine B (**2**) in methanol





2

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a Jasco P-1020 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. ECD spectra were recorded with an Applied Photophysics Chirascan spectrometer. A Tenor 27 spectrophotometer was used for the recording of IR spectra as KBr pellets. 1D and 2D NMR spectra were recorded using Bruker AVANCEIII-600 spectrometer. HREIMS was performed with an API QSTAR time-of-flight spectrometer. Semi-preparative HPLC was performed using an Agilent 1100 liquid chromatograph with a Waters X-Bridge C18 (4.6×250 mm) column. Detectors for HPLC analysis applied DAD and ELSD. Column chromatography (CC) was performed using silica gel (200-300 mesh and 300-400 mesh, Qingdao Marine Chemical, Inc., Qingdao, P. R. China).

Plant Material. The aerial parts of *M. faberi* were collected in October 2011 from Sichuan Province, the People's Republic of China, after its flowering phase. The plant samples were identified by Prof. Xun Gong of the State Key Laboratory of Phytochemistry and Plant Resource in West China, Kunming Institute of Botany (KIB), Chinese Academy of Sciences (CAS). A voucher specimen was deposited at KIB, CAS under the accession number KIB H20111001.

Extraction and Isolation. The air-dried, powdered leaves and stems (30 kg) of *M. faberi* were extracted three times with 50 L of 95% EtOH. After removing saccharides using a macroporous resin (D101), the crude alkaloids (223 g) were subjected to normal-phase silica gel chromatography (200-300 mesh; $CHCl_3$/MeOH, 20:1→0:1), yielding four fractions (Fr 1-4). Fraction 1 (Fr 1, 12.9 g) was further subjected to normal-phase Si gel (200-300 mesh; PE/EtOAc = 5:1) to give Fr. 1A-1C. Fr. 1C was separated using two steps of normal-phase Si gel and then subjected to a Waters X-Bridge C_{18} column (4.6×250 mm) (MeCN/H_2O = 60:40) to give **2** (2 mg). Fraction 2 (Fr 2, 32.4 g) was subjected to

normal-phase Si gel (200-300 mesh; PE/EtOAc = 5:1) to yield four fractions (Fr 2A-2D). Fr 2C (1.9 g) was repeatedly purified on normal-phase Si gel, resulting in **1** (2 mg) after Waters X-Bridge C_{18} (4.6×250 mm) chromatography (MeCN/H_2O = 50:50). The retention time of **1** on HPLC was detected by evaporative light-scattering detector.

Bioassay.

Cells:

Huh7.5 human liver cells (kindly provided by Vertex Pharmaceuticals, Boston, MA) were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen, CA) supplemented with 10% inactivated fetal bovine serum (Invitrogen) and 1% penicillin-streptomycin (Invitrogen). The cells were cultured at 37°C in air atmosphere (containing 5% CO2).

HCV Infection and Treatment:

The Huh7.5 cells were seeded into 96-well plates (Costar) at a density of 3×10^4 cells/cm^2; after 24 hrs, the cells were infected with HCV viral stock (chimeric HCV FL-J6/JFH/JC1, approximately 45 IU per cell) and simultaneously treated with compounds **1** and **2** or solvent as the control. The culture medium was removed at 72 hrs after inoculation, and intracellular RNA was extracted with RNeasy Mini Kit (Qiagen). The intracellular HCV RNA and internal control gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were quantified using AgPath-ID™ One-Step RT-PCR Kit (Applied Biosystems). The results were calculated with $2^{\Delta\Delta CT}$. The half maximal effective concentration (EC_{50}) was calculated using the Reed & Muench method.[1]

[1] Z. G. Peng, B. Fan, N. N. Du, Y. P. Wang, L. M. Gao, Y. H. Li, Y. H. Li, F. Liu, X. F. You, Y. X. Han, Z. Y. Zhao, S. Cen, J. R. Li, D. Q. Song, J. D. Jiang, *Hepatology*, 2010, **52**, 845-853.