Electronic Supplementary Material (ESI) for RSC Advances
This journal is © The Royal Society of Chemistry 2014

Supporting Information

Pegaharmalines A and B, two novel *β*-carboline alkaloids with unprecedented carbon skeletons from *Peganum harmala*

Kai-Bo Wang,[a] Chun-Mao Yuan,[b] Chun-Mei Xue,[a] Da-Hong Li,[a] Yong-Kui Jing,[c] Hong-Ping He,[b] Xiao-Jiang Hao,[b] Ying-Tong Di,*[b] Zhan-Lin Li,*[a] and Hui-Ming Hua*[a]

[a] Key Laboratory of Structure-Based Drug Design & Discovery, Ministry of Education, Shenyang Pharmaceutical University, Shenyang 110016, P. R. China.

[b] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Acad-emy of Sciences, Kunming 650201,Yunnan, P. R. China.

[c] Department of Medicine, Mount Sinai School of Medicine, New York, NY 10029, USA.

* Corresponding authors.
E-mail: huimhua@163.com. lzl1030@hotmail.com. diyt@mail.kib.ac.cn.

Contents

Experimental Section

General experimental procedures. Optical rotations were obtained on a Perkin-Elmer Model 341 polarimeter. UV spectrum was recorded using a Shimadzu UV-2201 spectrometer. CD spectra were measured on Bio-logic MOS 450 spectropolarimeter. ^{1}H, ^{13}C, and 2D-NMR spectra were recorded on Bruker AV-600 NMR spectrometers with TMS as an internal standard. Mass spectra were recorded on Varian QFT-ESI and Bruker micro-TOFQ-Q mass spectrometer (for HR-ESIMS). Column chromatography (CC) was performed with silica gel (Qingdao Marine Chemical-Co., Ltd.), ODS (50μm, YMC Co. Ltd., Kyoto, Japan) and Sephadex LH-20 (GE Healthcare). Preparative HPLC was conducted on a YMC ODS-A column (250 × 20mm I. D., 5 μm) equipped with a LC-6AD pump and a Shimadzu SPD-20A UV-Vis detector (Shimadzu Co., Ltd., Japan).

Plant Material. The plant material was purchased from Anguo medicines Ltd (Hebei), China, in July 2012 and was identified as the seeds of Peganum harmala L. by Prof. Jincai Lu of School of Traditional Chinese Meteria Medica, Shenyang Pharmaceutical University. The voucher sample (PH-20120705) was deposited in the Department of Natural Products Chemistry, Shenyang Pharmaceutical University, Shenyang, China.

Extraction and Isolation. The seeds of P. harmala L. (15.4 kg) were extracted under reflux with 95% ethanol (2×2 h×100 L) and 75% ethanol (1×2 h×100 L), respectively. The combined EtOH extracts were concentrated in vacuo to yield a residue (1.9 kg), which was suspended in water (13 L) and adjusted to pH 3 with 5% HCl. The acidic mixture was partitioned with CH_2Cl_2 (6×13 L), and the aqueous layer was then basified to pH 10 with 3 N NaOH, followed by exhaustive extraction with CH_2Cl_2 (6×13 L) to yield the crude alkaloids (420.2 g). The crude alkaloids were separated by a silica gel chromatography column (CC) using CH_2Cl_2/MeOH (1:0 → 0:1) as eluent, to give nine fractions (Fr. A-Fr. I). Fraction B, eluted with CH_2Cl_2/MeOH (100:1), was chromatographed on silica gel (CH_2Cl_2/acetone 1:0 → 0:1) to yield six subfractions (Fr. B1-Fr. B6). Fr. B2 was then separated by ODS CC, eluted with MeOH-H_2O (60:40) and was purified by preparative HPLC on a YMC C-18 column using MeOH-H_2O

(68:32) as the mobile phase to yield **2** (8.9 mg). Fraction C, eluted with CH_2Cl_2/MeOH (100:3), was chromatographed on silica gel (EtOAc/MeOH 1:0 → 0:1) to yield six subfractions (Fr. C1-Fr. C6). Fr. C1 was separated by ODS CC, eluted with MeOH-H_2O (60:40) and was then purified by preparative HPLC on a YMC C-18 column using MeOH-H_2O (70:30) as the mobile phase to yield **1** (3.4 mg).

Cytotoxic Assays. Cytotoxicity of isolated compound **1** was assayed by the trypan blue method[1] using the human leukaemia cell lines (HL-60), and the MTT assay[2] using the prostate cancer cell lines (PC-3), michigan cancer foundation-7 cell lines (MCF-7), a lung cancer cells (A549), and liver hepatocellular carcinoma cell lines (HepG-2). The cell lines were purchased from America Type Culture Collection, ATCC (Rockville, MD, USA) and cultured in RPMI-1640 medium (Gibco, New York, NY, USA) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 1 mM glutamine and 10 % heat-inactivated foetal bovine serum (Gibco).

In the trypan blue method, briefly, cells in logarithmic growth were seeded at a density of 4 × 104 cells/mL in 24-well microplates and incubated with various concentrations of the compounds under a humidified atmosphere of 5% CO_2 and 95% air at 37 °C for 3 days. The compounds were dissolved in DMSO and then diluted to the proper concentrations. Cell viability was determined after staining the cells with trypan blue and the total cell number was determined using a hematocytometer. 5-Fluorouracil (5-Fu) was used as a positive control.

In the MTT assay, briefly, cells suspensions, 200 μl, at a density of 5×104 cells/mL, were plated in 96-well microtiter plates and incubated for 24 h at 37 °C under 5% CO_2 and 95% air. Then 2 μl test compounds with different concentrations in DMSO were placed into each microtiter plates and further incubated for 72 h. Finally, 50 μl of a 0.4% MTT solution was added to each well and incubated for 4 h. Then, the MTT was removed from the wells and the fromazan crystals were dissolved in DMSO (200 μl). The plates were vibrated for 10 min. The absorbance was then determined on a microplate reader (Bio-RAD) at the wavelength of 570 nm. 5-Fluorouracil (5-Fu) was used as a positive control.

References:

[1] Wang F.; Hua H. M.; Pei Y. H.; Chen D.; Jing Y. K. *J. Nat. Prod.* **2006**, 69, 807-810.

[2] Mosmann, T. *J. Immunol. Methods*. **1983**, 65, 55-63.

Computational methods

The CONFLEX[1, 2] searches based on molecular mechanics with MMFF94S force fields were performed for (**1*S*)-1** and its enantiomer (**1*R*)-1**, which gave 8 stable conformers. Selected conformers of (**1*S*)-1** and (**1*R*)-1** with the lowest energy were further optimized by the density functional theory method at the B3LYP/6-31+G** level in Gaussian 03 program package,[3] which was further checked by frequency calculation and resulted in no imaginary frequencies. The ECD of the conformer of **1** was then calculated by the TDDFT method at the B3LYP/6-31+G** level, and at the B3LYP/6-311++G**//B3LYP/6-31+G** levels with the PCM model in methanol solution. The calculated ECD curve was generated using SpecDis 1.51[4] with σ = 0.16 ev, and UV shift -5 nm.

Computational methods for ECD of compound 1

Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	-5.071017	-0.698261	-0.839280
2	6	0	-4.695544	-1.479762	0.281564
3	6	0	-3.434575	-1.350845	0.863355
4	6	0	-2.559960	-0.416070	0.287310
5	6	0	-2.909430	0.376287	-0.838447
6	6	0	-4.193515	0.218320	-1.395184
7	7	0	-1.272838	-0.074677	0.651609

8	6	0	-0.794947	0.887654	-0.232161
9	6	0	-1.768972	1.201107	-1.144427
10	6	0	0.562711	1.539416	-0.140996
11	7	0	0.798575	2.451599	-1.280473
12	6	0	-0.396370	3.157899	-1.781616
13	6	0	-1.551530	2.230314	-2.212043
14	6	0	0.771477	2.231409	1.219933
15	6	0	1.776467	1.649803	1.909525
16	6	0	2.348894	0.575353	1.093483
17	7	0	1.654317	0.529607	-0.108038
18	7	0	3.328145	-0.206139	1.423687
19	6	0	3.708136	-1.150987	0.478299
20	6	0	3.084547	-1.253707	-0.792327
21	6	0	1.990291	-0.335836	-1.146683
22	6	0	4.760304	-2.036121	0.790937
23	6	0	5.171748	-2.988405	-0.131099
24	6	0	4.549887	-3.084851	-1.390617
25	6	0	3.514358	-2.219944	-1.717625
26	6	0	-0.084959	3.382766	1.637308
27	8	0	1.416238	-0.296430	-2.235420
28	6	0	2.336557	1.972397	3.260378
29	8	0	-5.660522	-2.346178	0.726271
30	6	0	-5.363980	-3.178803	1.835896
31	1	0	-6.065794	-0.844099	-1.248937
32	1	0	-3.131834	-1.939788	1.722166
33	1	0	-4.498287	0.804594	-2.258565
34	1	0	-0.734211	-0.515047	1.382967
35	1	0	1.146177	1.865529	-2.040440
36	1	0	-0.751111	3.843649	-1.005389
37	1	0	-0.061177	3.774976	-2.622626
38	1	0	-1.307063	1.747287	-3.170295
39	1	0	-2.458539	2.826944	-2.383338
40	1	0	5.233305	-1.946618	1.764437
41	1	0	5.984200	-3.665205	0.121635
42	1	0	4.880941	-3.833715	-2.104809
43	1	0	3.017395	-2.268508	-2.681766
44	1	0	0.052995	3.622869	2.695231
45	1	0	-1.146884	3.168800	1.467265
46	1	0	0.164686	4.280438	1.056443
47	1	0	3.354902	2.370852	3.170668
48	1	0	2.408802	1.068348	3.875655
49	1	0	1.726362	2.709756	3.788923
50	1	0	-6.258824	-3.780708	2.004986
51	1	0	-4.513765	-3.841200	1.623024
52	1	0	-5.148858	-2.585821	2.735561

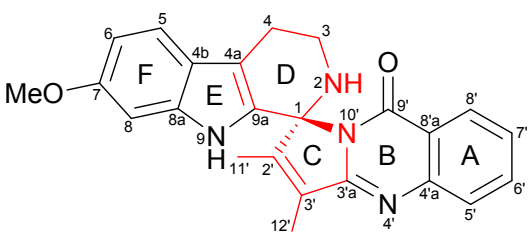
References

[1] Goto, H.; Osawa, E.; *J. Am. Chem. Soc.* 1989, ***111***, 8950–8951.

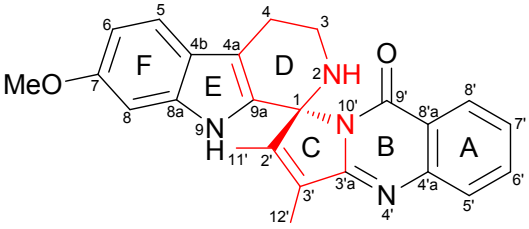
[2] Goto, H.; Osawa, E.; *J. Chem. Soc., Perkin Trans. 2*, **1993**, 187–198.

[3]. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb,M. A.; Cheeseman, J. R.; Montgomery, Jr., J. A.; Vreven, T.; Kudin, K. N.;Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.;Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.;Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.;Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li,X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.;Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.;Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.;Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich,S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.;Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.;Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz,P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.;Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson,B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03*,revision D.01; Gaussian, Inc.: Wallingford, CT, 2005.

[4]. Bruhn, T.; Hemberger, Y.; Schaumlöffel, A.; Bringmann, G. *Spec Dis*, version 1.51, University of Würzburg, Germany, 2010.

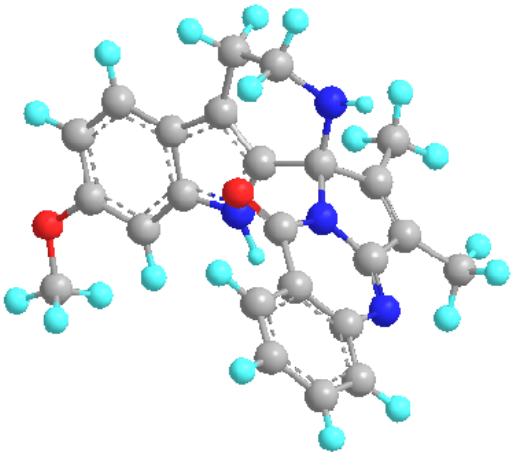


(1*S*)-1

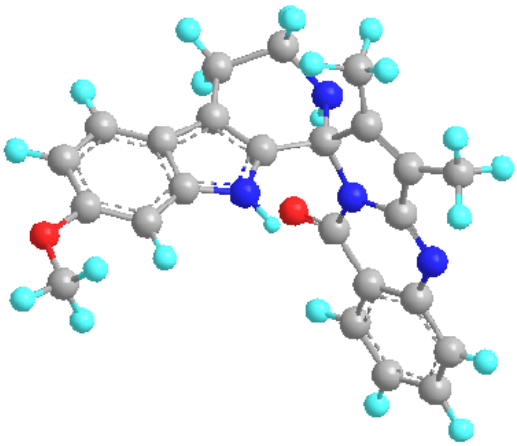


(1*R*)-1

Figure S1. Structures of (**1*S***)**-1** and (**1*R***)**-1.**



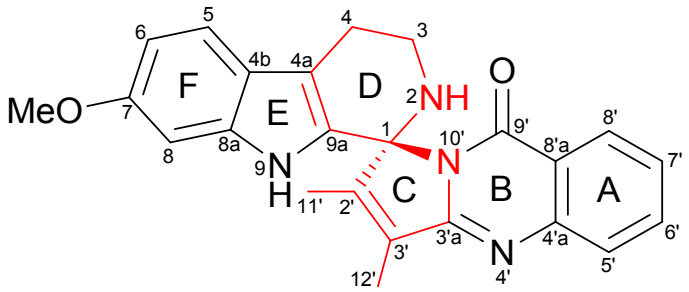
(**1*S***)**-1**



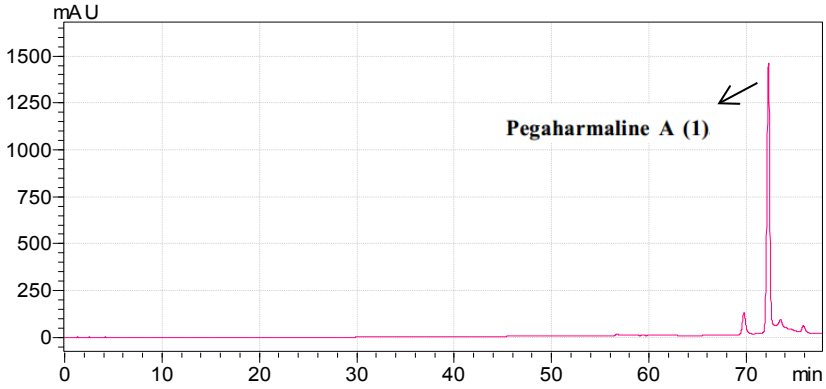
(**1*R***)**-1**

Figure S2. B3LYP/6-31+G** optimized lowest energy 3D conformer of (**1*S***)**-1** and (**1*R***)**-1**.

Fig. S3 The HPLC chromatograms of pegaharmaline A (**1**) and its corresponded UV spectrum. (MeOH:H_20 10% → 80% 80 min)



1



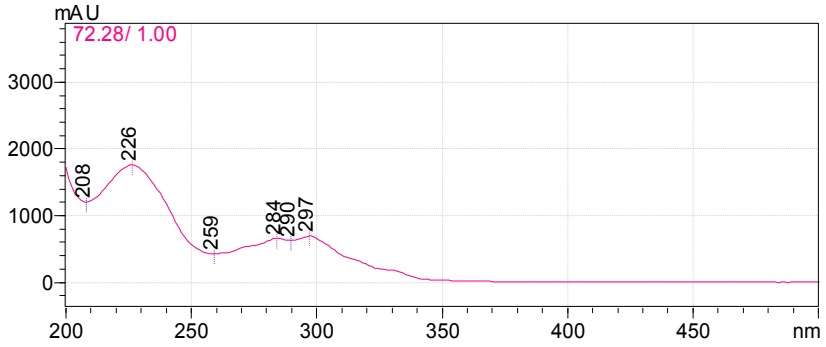


Fig. S4 The ^{1}H-NMR spectrum of pegaharmaline A (**1**) in DMSO-d_6

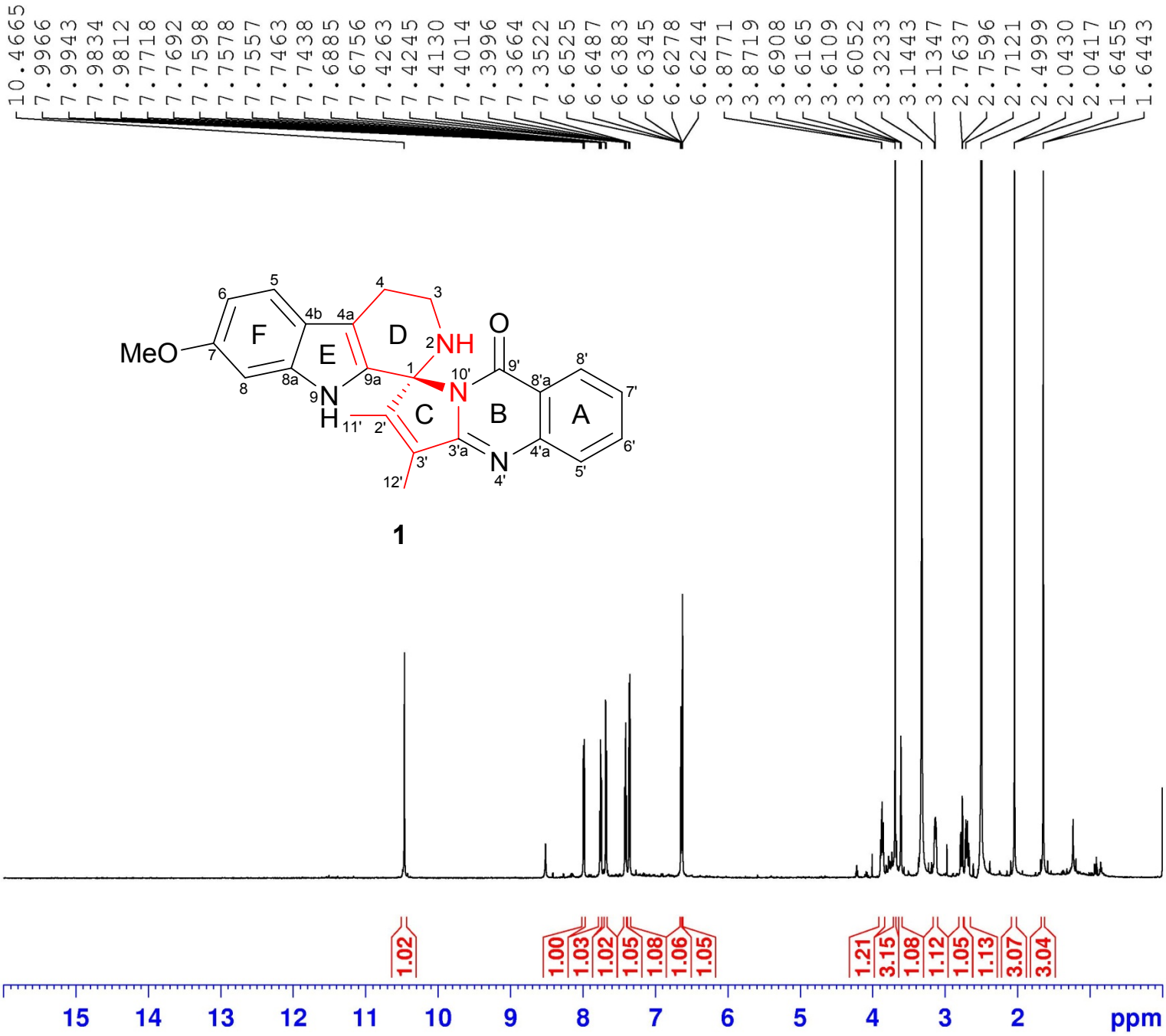


Fig. S5 Expanded ^{1}H-NMR spectrum of pegaharmaline A (**1**) in DMSO-d_6

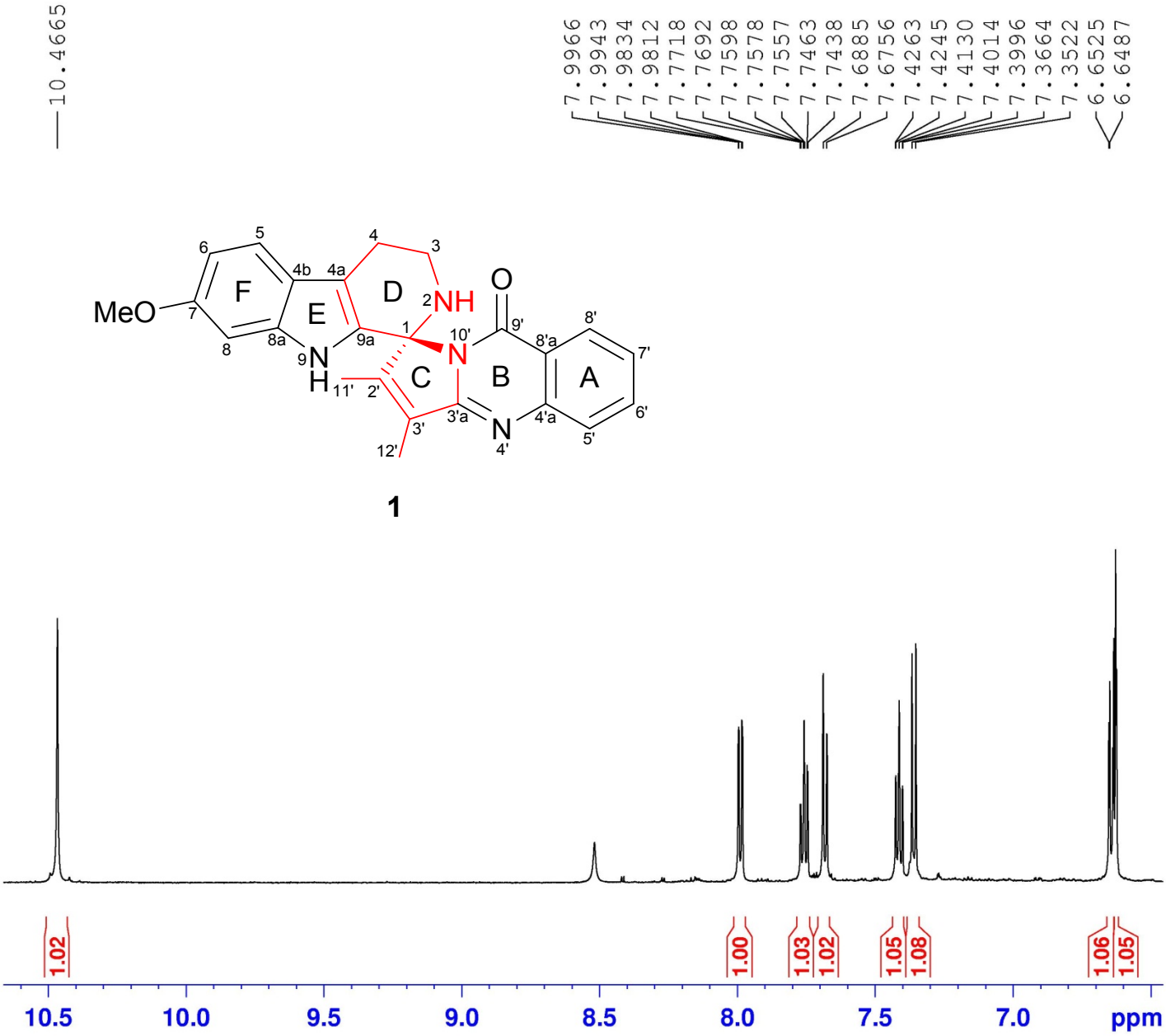


Fig. S6 Expanded ^{1}H-NMR spectrum of pegaharmaline A (**1**) in DMSO-d_6

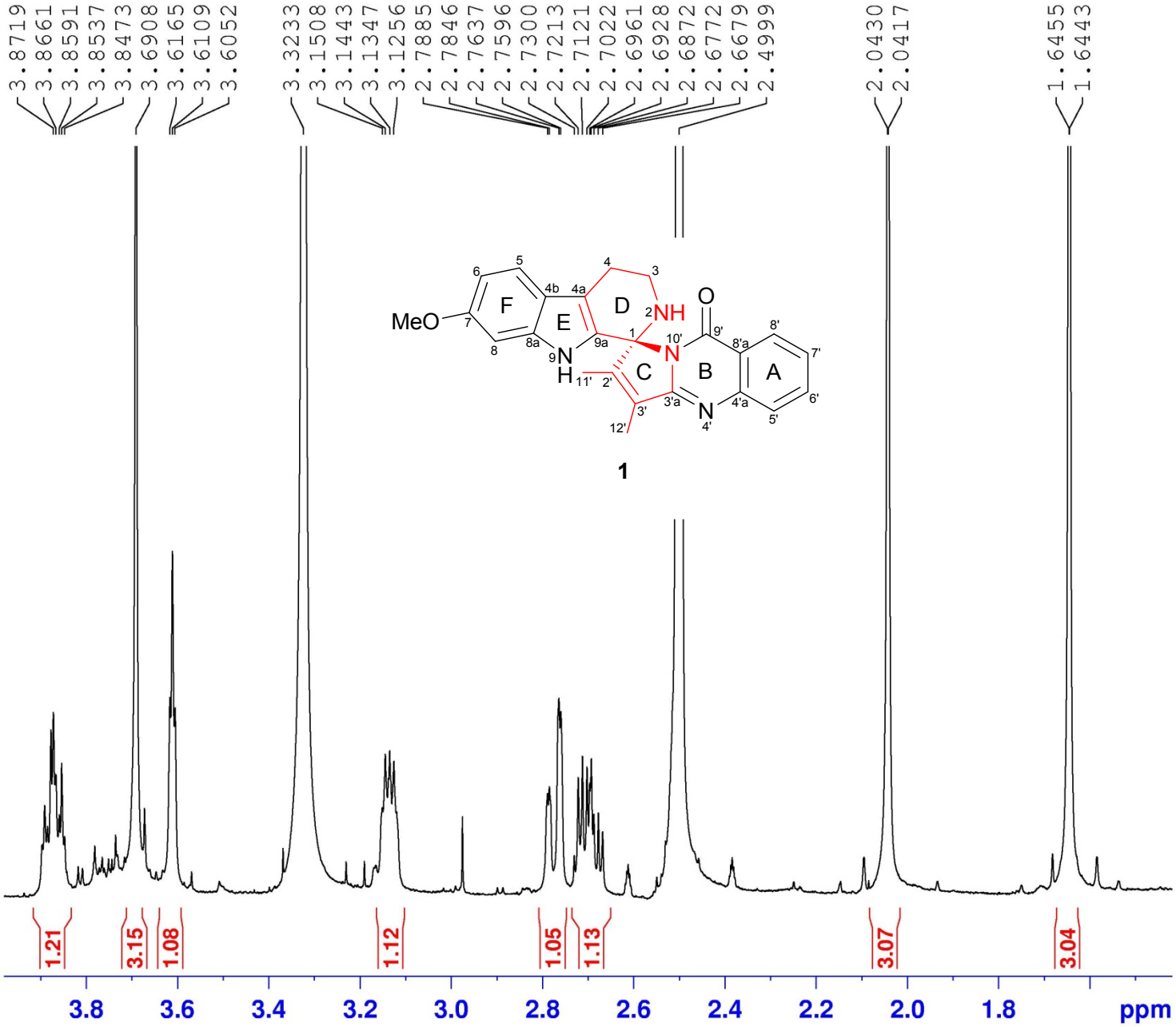


Fig. S7 The ^{13}C-NMR spectrum of pegaharmaline A (**1**) in DMSO-d_6

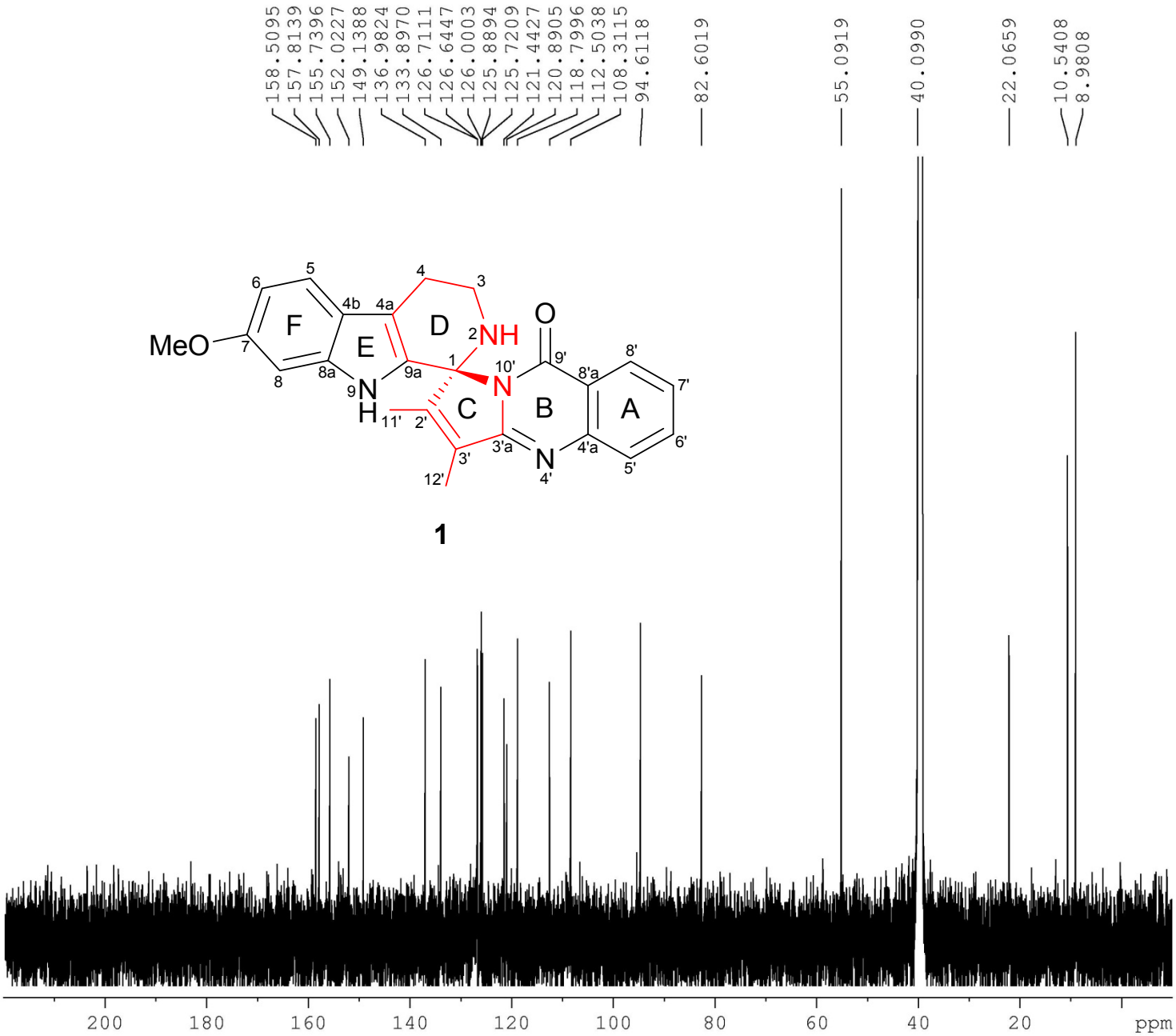


Fig. S8 The ^{1}H-^{1}H COSY spectrum of pegaharmaline A (**1**) in DMSO-d_6

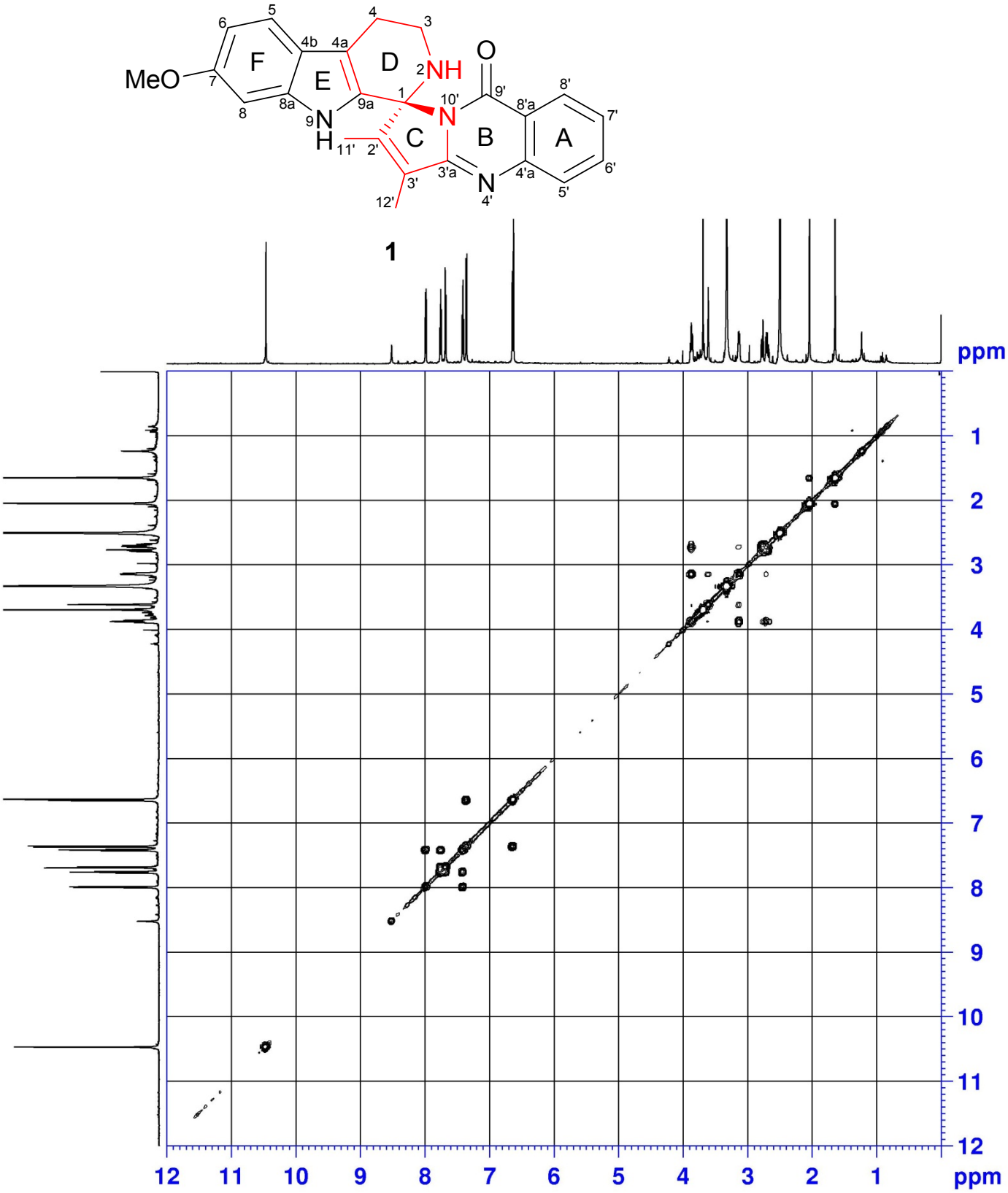
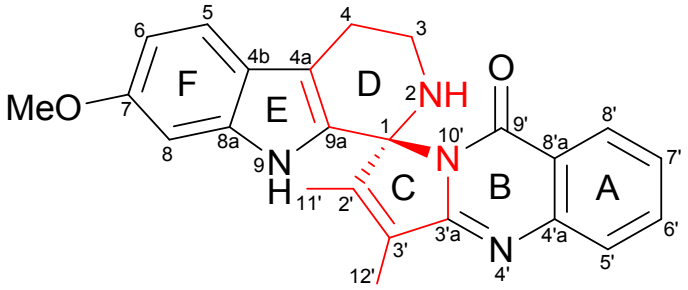


Fig. S9 The NOESY spectrum of pegaharmaline A (**1**) in DMSO-d_6



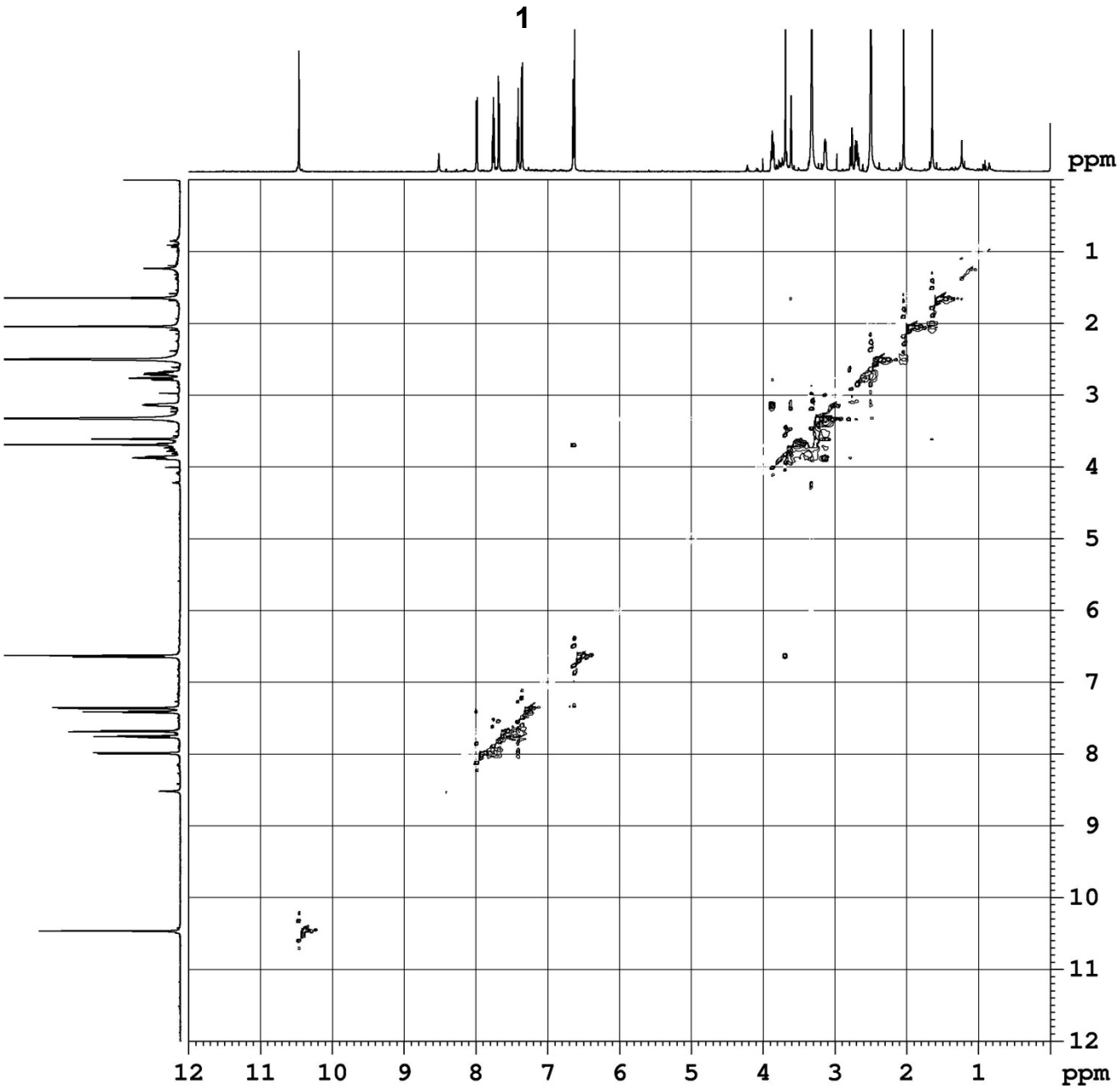


Fig. S10 Expanded NOESY spectrum of pegaharmaline A (**1**) in DMSO-d_6

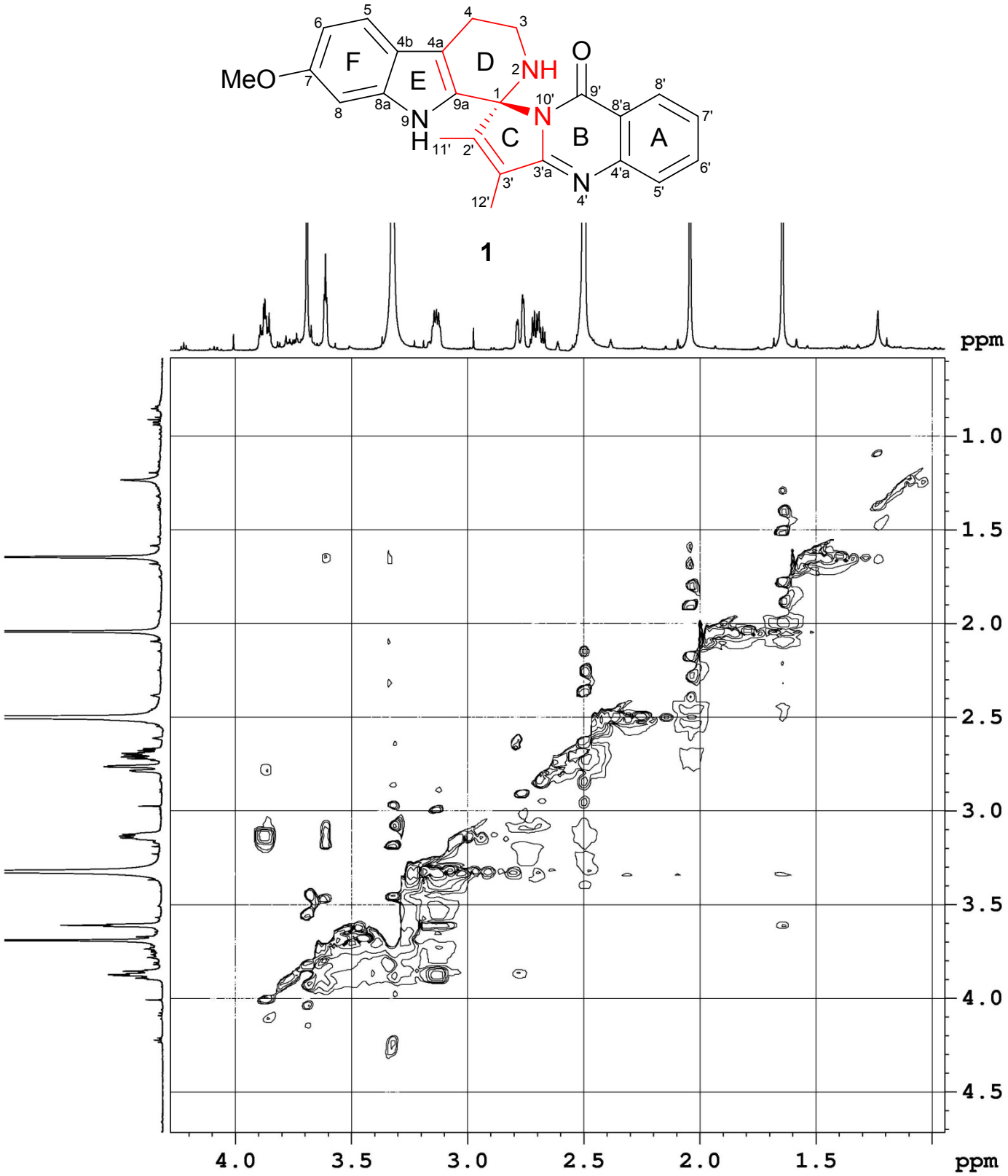


Fig. S11 The HSQC spectrum of pegaharmaline A (**1**) in DMSO-d_6

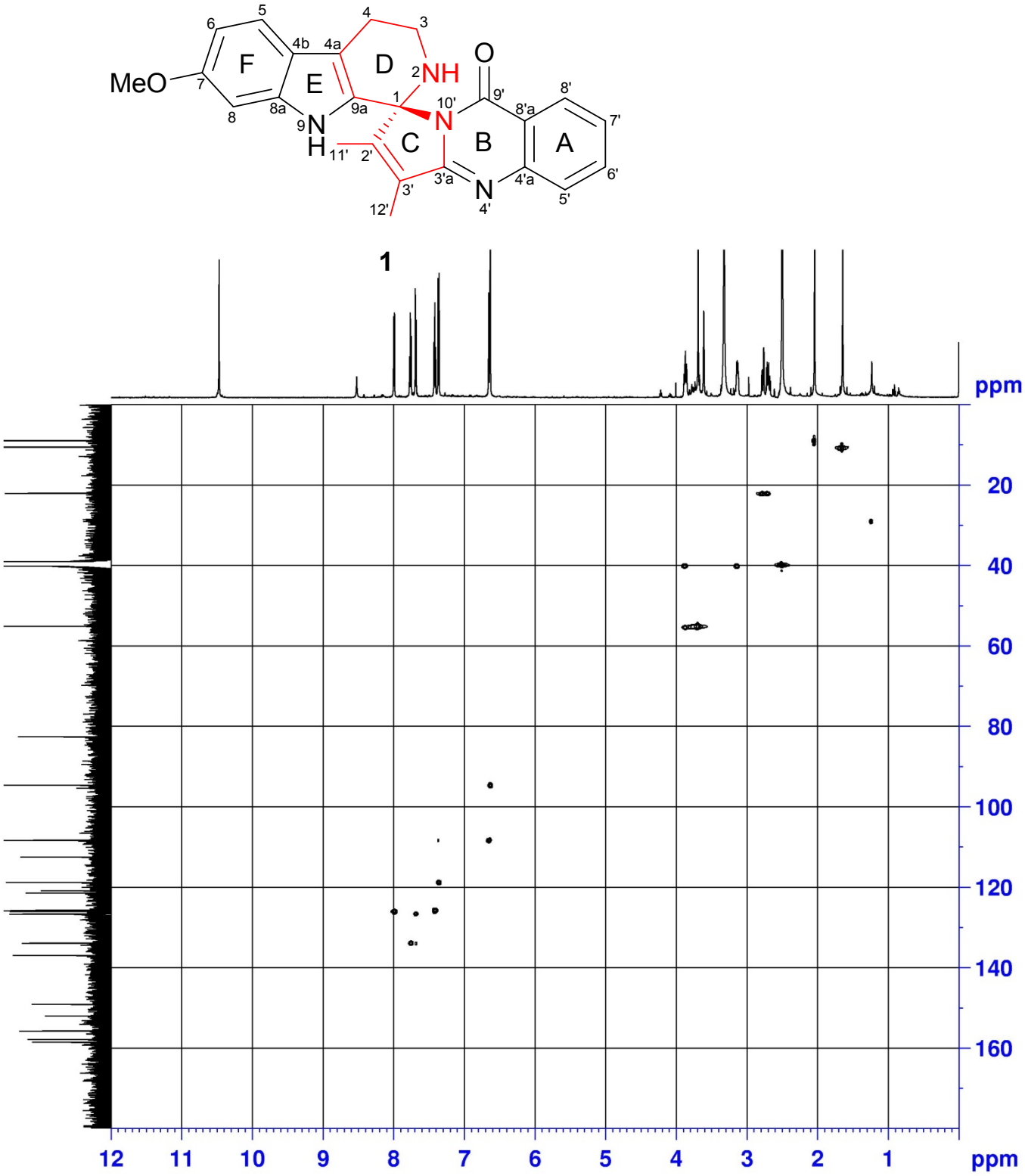


Fig. S12 The HMBC spectrum of pegaharmaline A (**1**) in DMSO-d_6

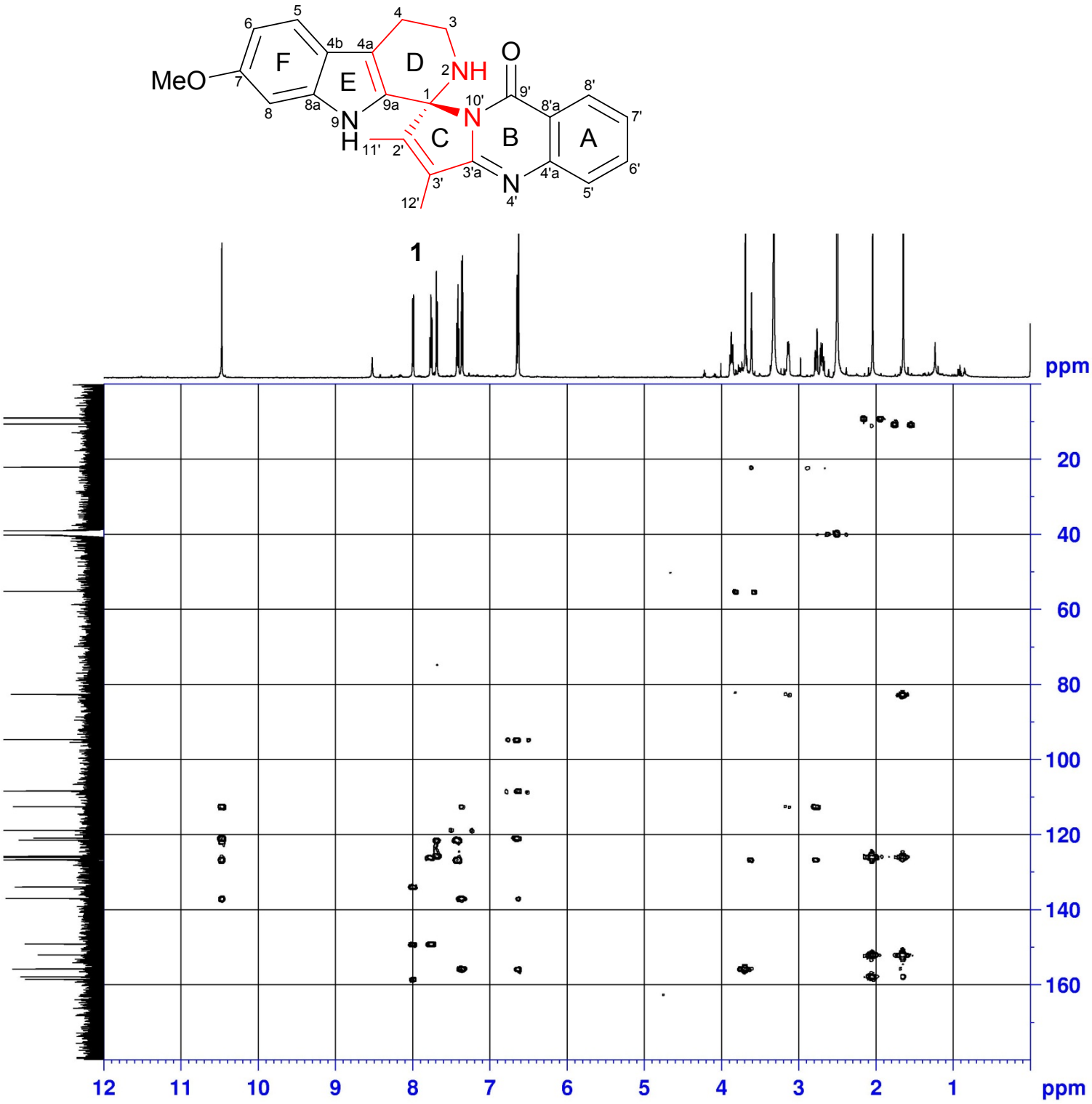
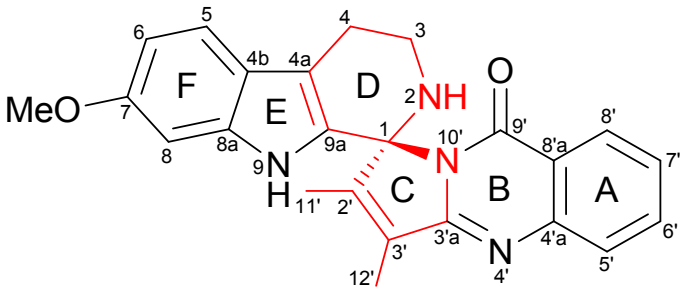


Fig. S13. The HR-ESIMS spectrum of pegaharmaline A (**1**) in CH_3OH



1

Mass Spectrum Molecular Formula Report

Analysis Info

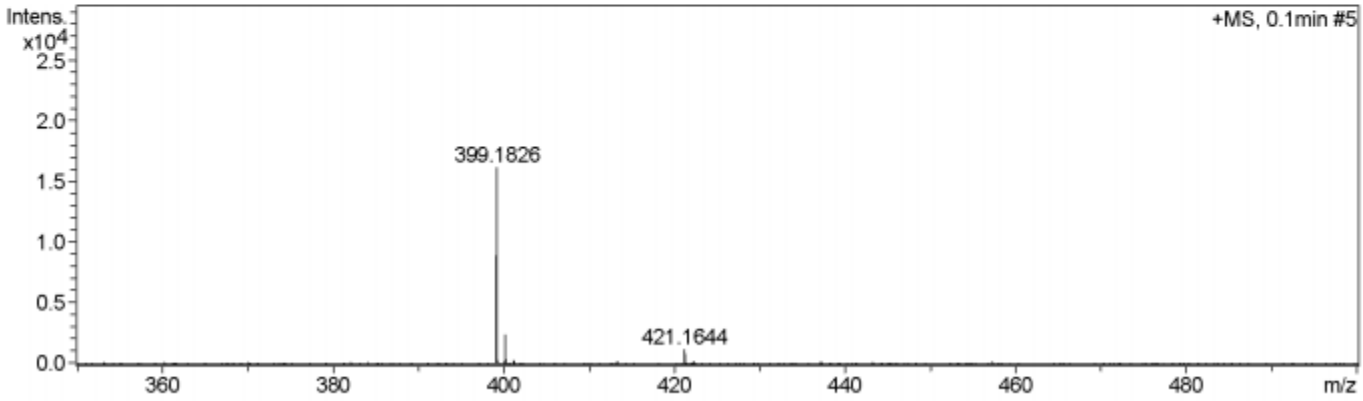
Analysis Name	D:\Data\20140514ceyang\WKB-53.d	Acquisition Date	5/14/2014 2:02:04 PM
Method	Liu_low_20131025.m	Operator	Bruker Customer
Sample Name	WKB-53	Instrument / Ser#	micrOTOF-Q 125
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.3 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1000 m/z	Set Collision Cell RF	300.0 Vpp	Set Divert Valve	Source

Generate Molecular Formula Parameter

Formula, min.	C24H22N4O2Na						
Formula, max.							
Measured m/z	421.164	Tolerance	5	ppm	Charge	1	
Check Valence	no	Minimum	0		Maximum	0	
Nirogen Rule	no	Electron Configuration	both				
Filter H/C Ratio	no	Minimum	0		Maximum	3	
Estimate Carbon	yes						



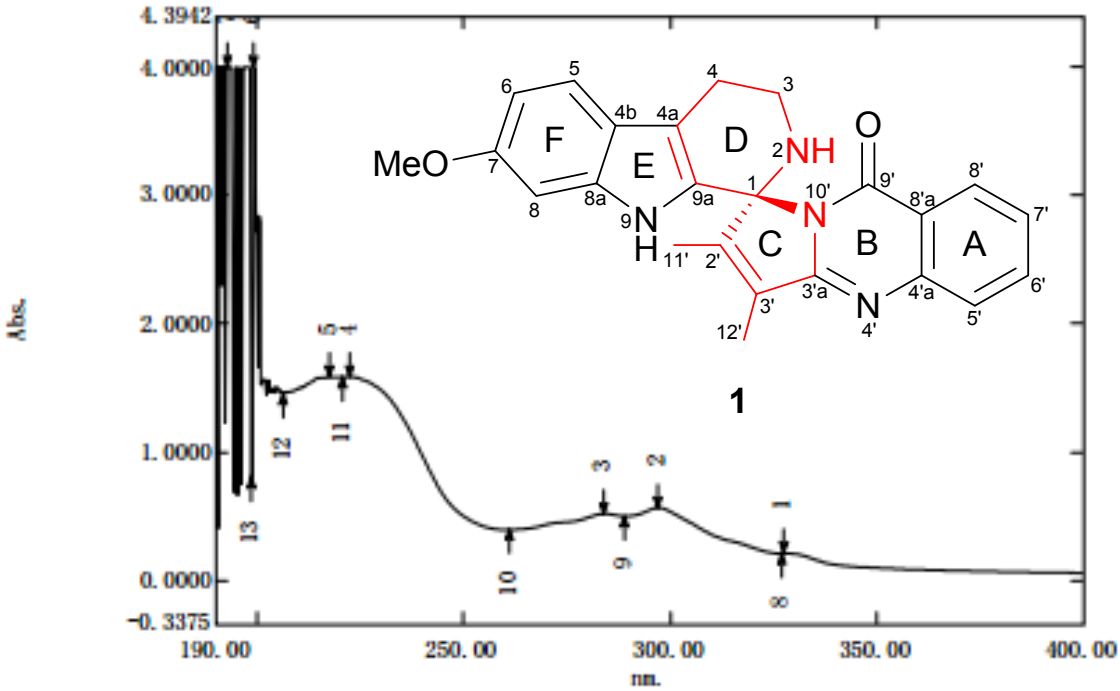
Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	Err [mDa]	rdb	N Rule	e⁻
C 24 H 22 N 4 Na 1 O 2	0.060	421.1635	-2.07	-4.40	-0.87	15.50	ok	even

Fig. S14. The UV spectrum of pegaharmaline A (**1**) in CH_3OH

Spectrum Peak Pick Report

FIELD FIELD TEXT

Data Set: 没有



测定属性
波长范围（nm.）: 190.00到400.00
扫描速度: 低速
采样间隔: 0.2
自动采样间隔: 启用
扫描模式: 自动

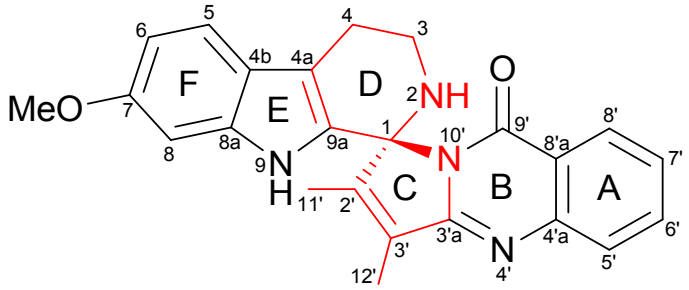
试样准备属性
重量:
体积 :
稀释:
光程长:
附加信息:

仪器属性
仪器类型: UV-1700
测定方式: 吸收值
狭缝宽: 1.0 nm
光源改变波长: 340.8 nm
S/R 转换: 标准

附件属性
附件: 无

No.	P/V	Wavelength	Abs.	描述
1	⊕	327.80	.2086	
2	⊕	297.00	.5663	
3	⊕	284.20	.5193	
4	⊕	222.20	1.5907	
5	⊕	217.40	1.5897	
6	⊕	199.20	3.9999	
7	⊕	192.60	3.9999	
8	⊖	327.20	.2083	
9	⊖	289.00	.4995	
10	⊖	261.20	.3939	
11	⊖	220.40	1.5815	
12	⊖	206.00	1.4606	
13	⊖	198.60	.8016	

Fig. S15. The IR spectrum of pegaharmaline A (**1**) in CH_3OH



1

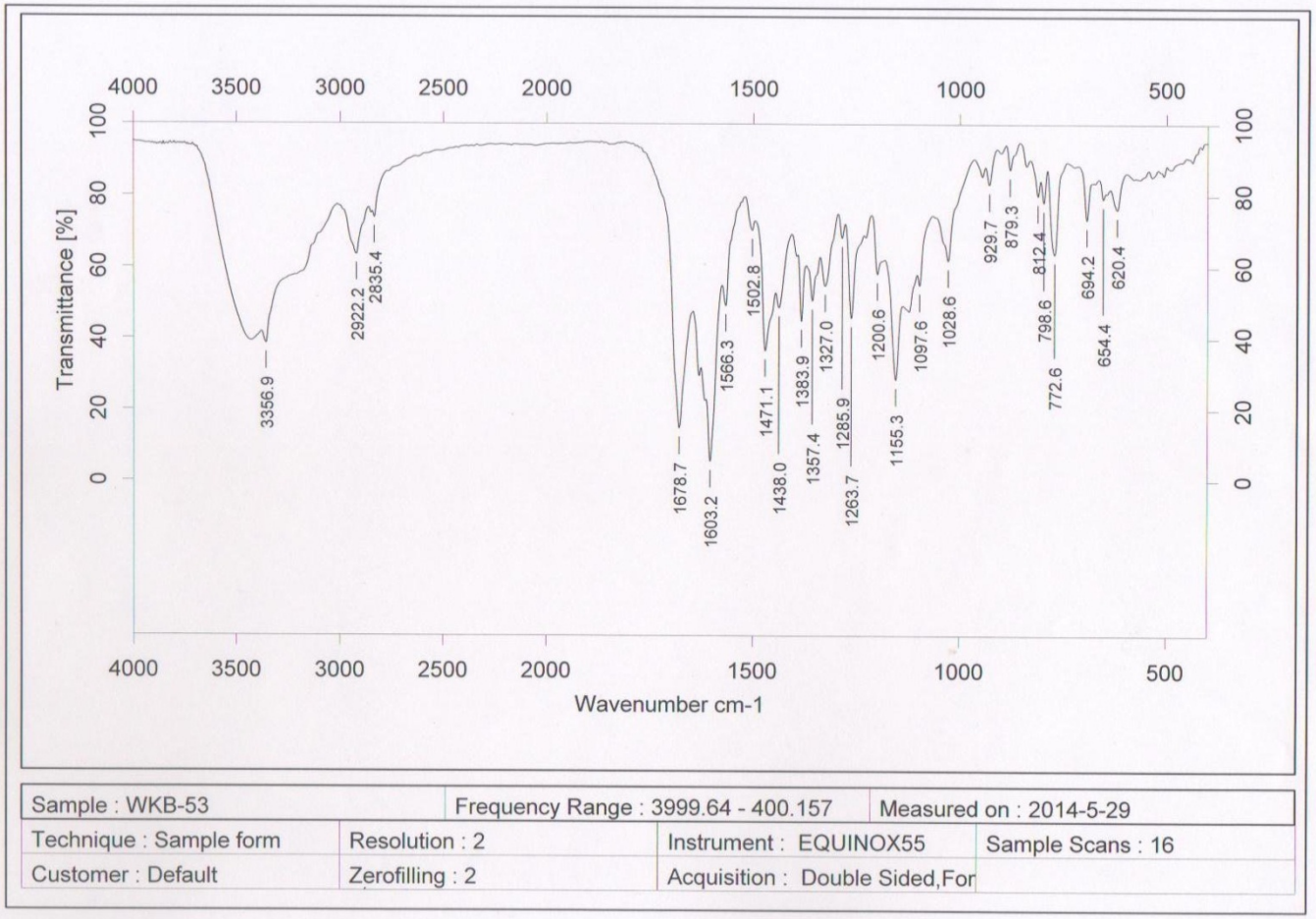


Fig. S16 The ^{1}H-NMR spectrum of pegaharmaline B (**2**) in DMSO-d_6

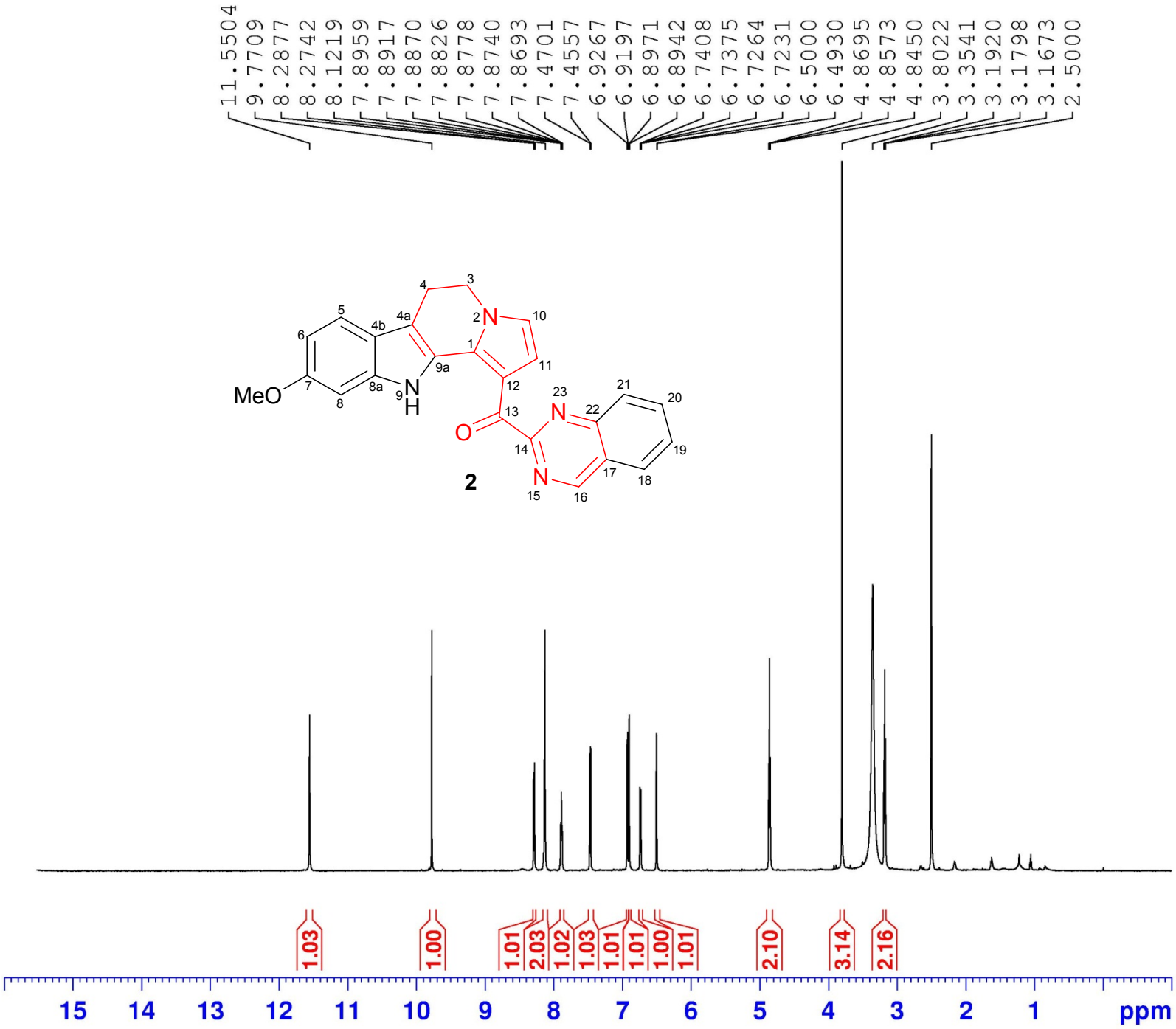


Fig. S17 Expanded ^{1}H-NMR spectrum of pegaharmaline B (**2**) in DMSO-d_6

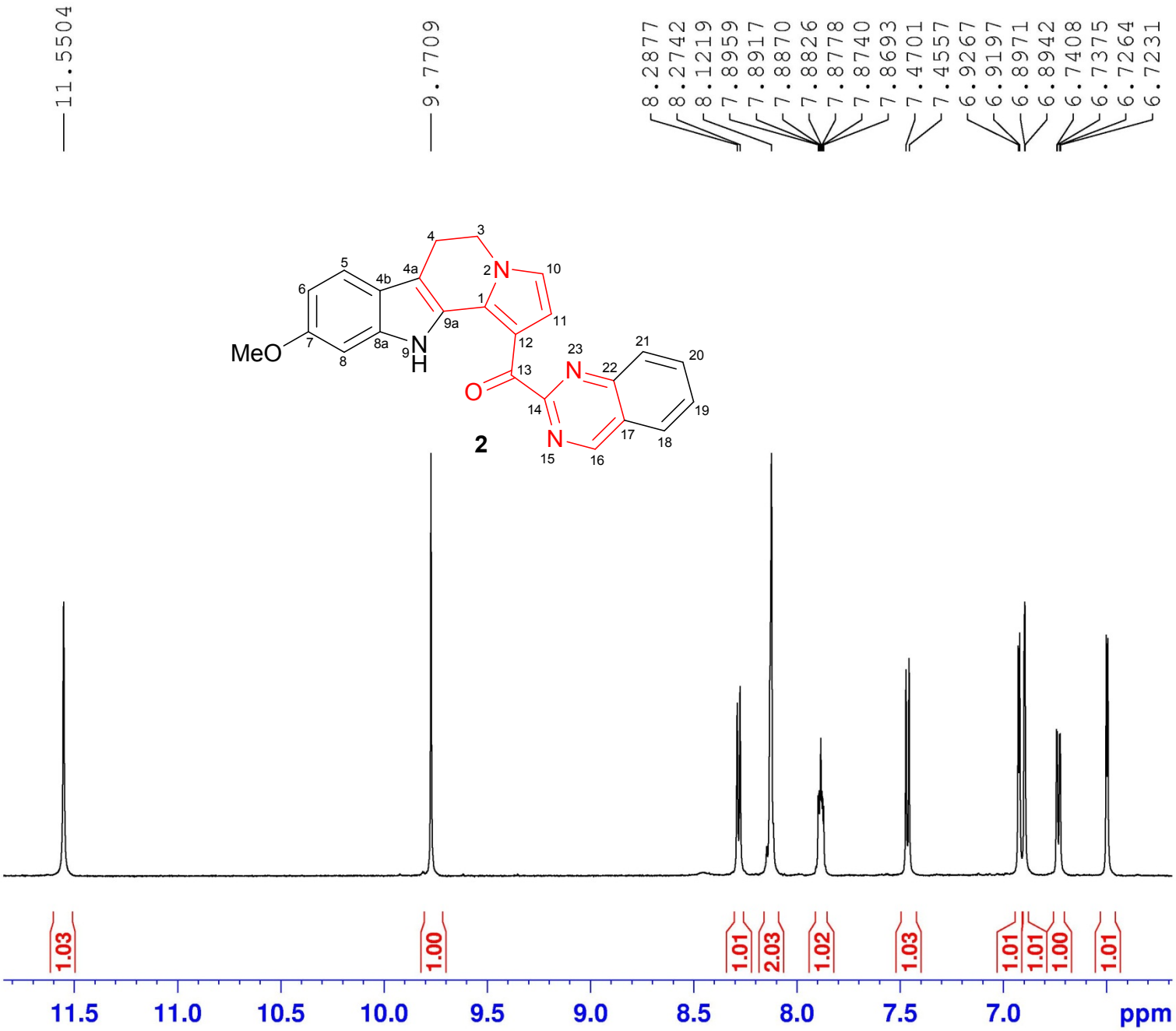


Fig. S18 Expanded ^{1}H-NMR spectrum of pegaharmaline B (**2**) in DMSO-d_6

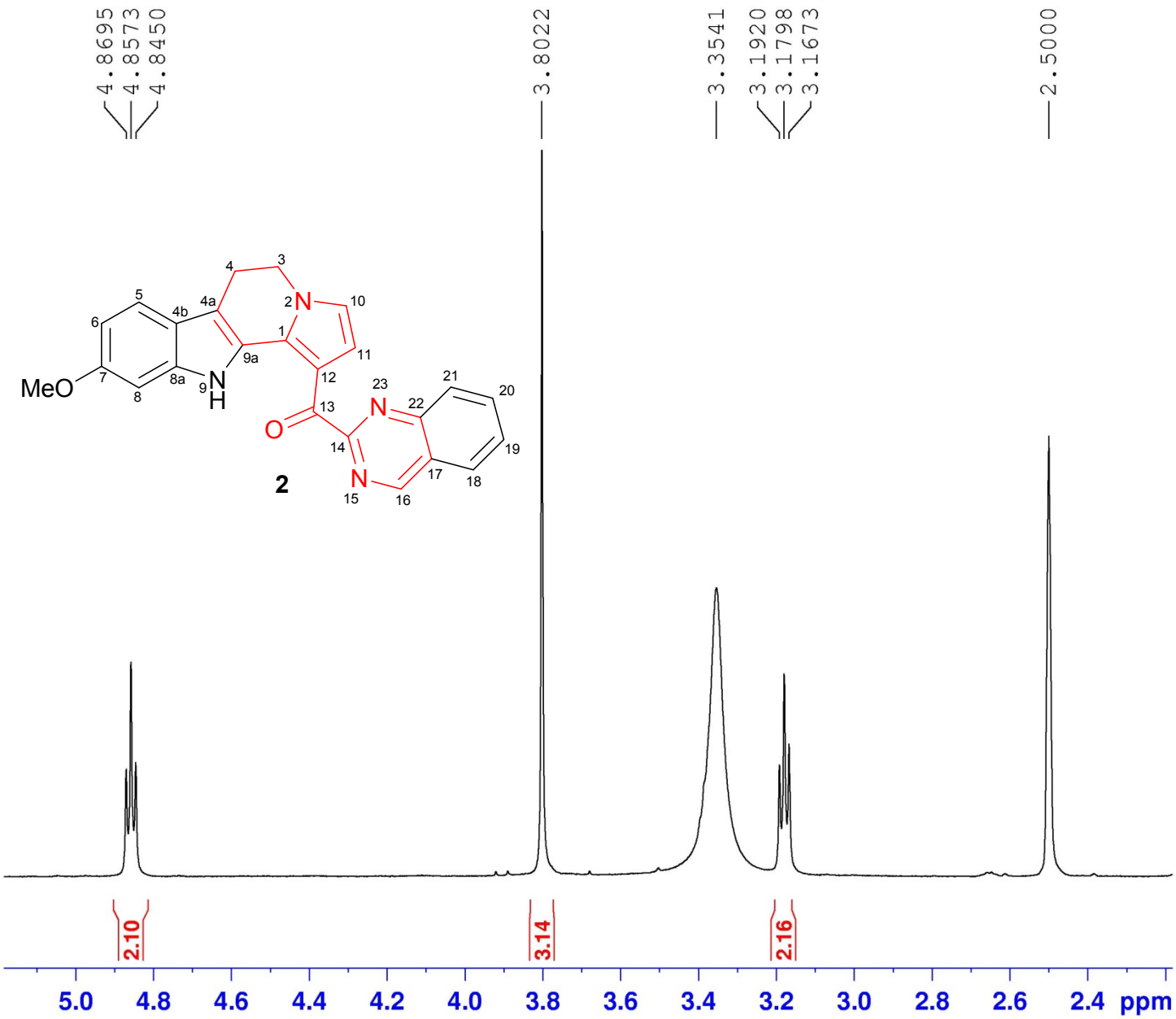


Fig. S19 The ^{13}C-NMR spectrum of pegaharmaline B (**2**) in DMSO-d_6

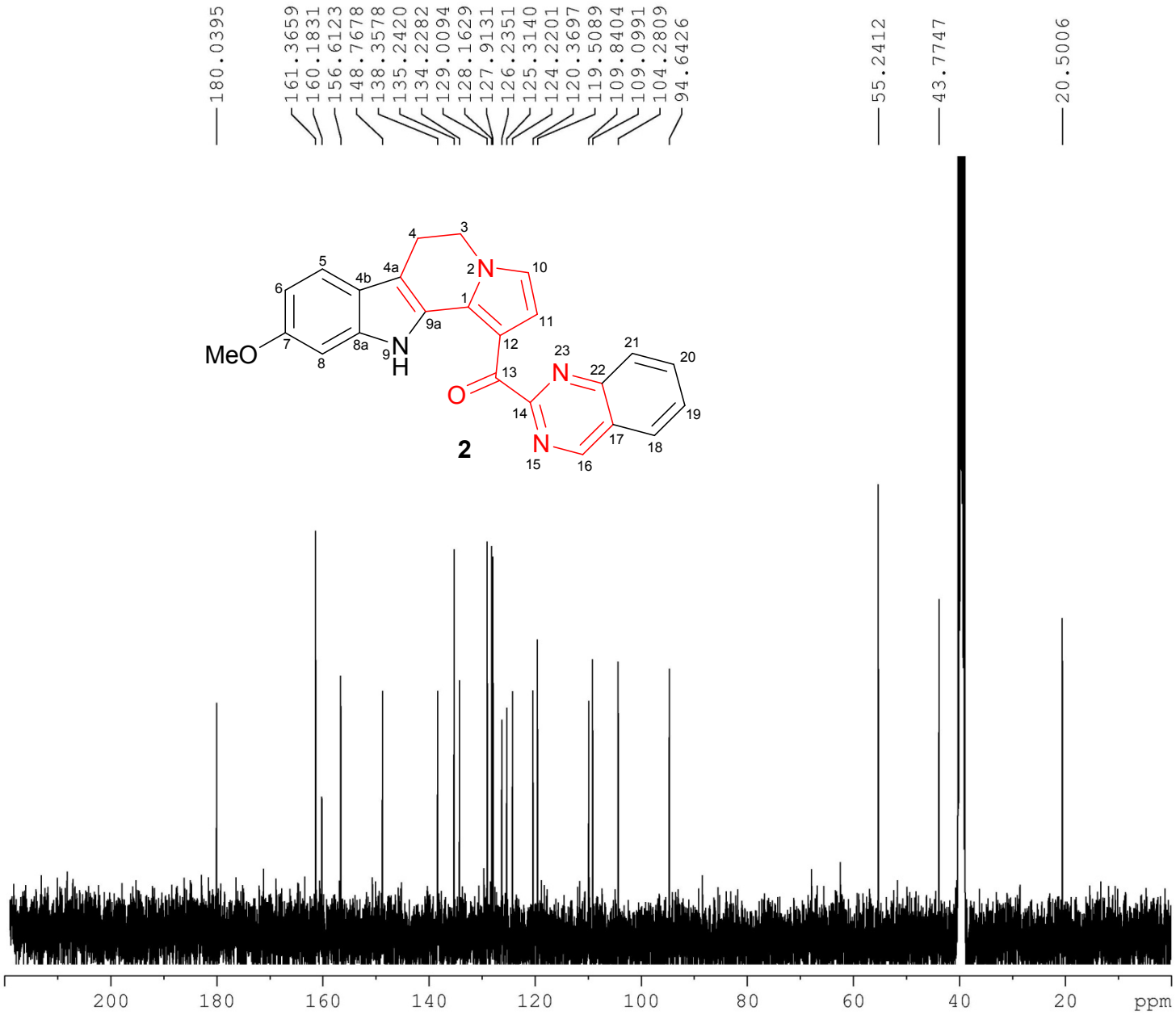


Fig. S20 The ^{1}H-^{1}H COSY spectrum of pegaharmaline B (**2**) in DMSO-d_6

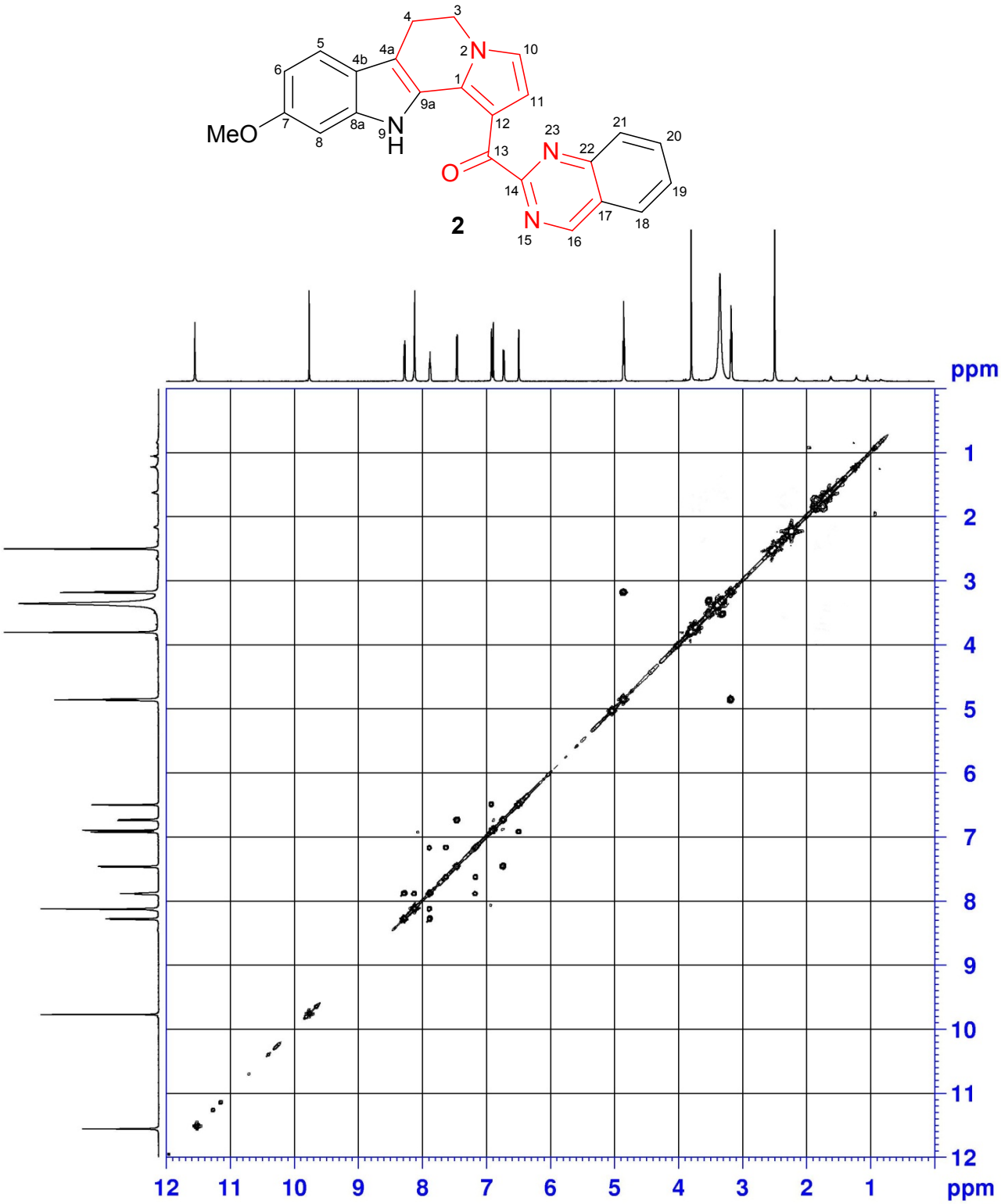


Fig. S21 The HSQC spectrum of pegaharmaline B (**2**) in DMSO-d_6

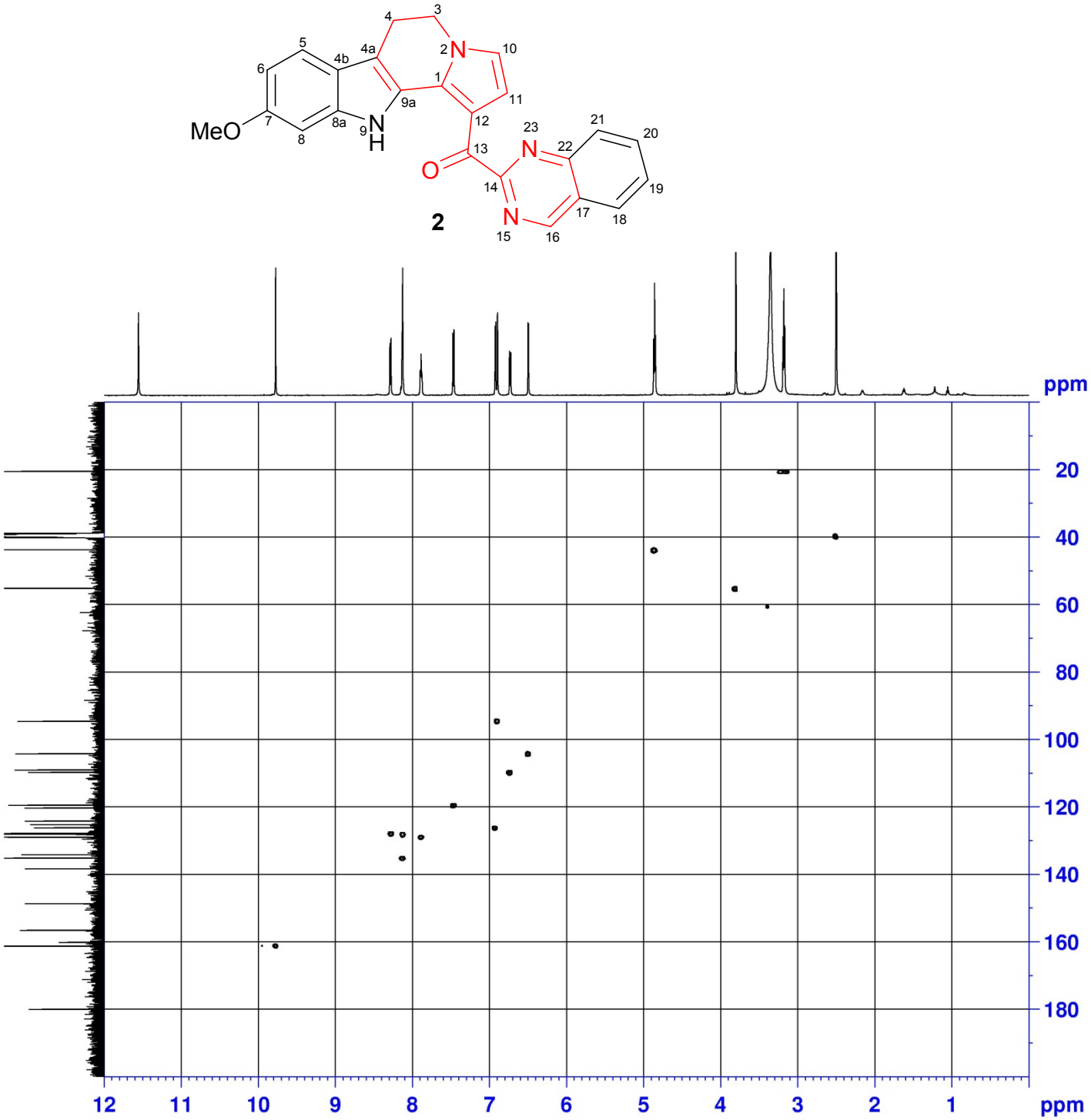
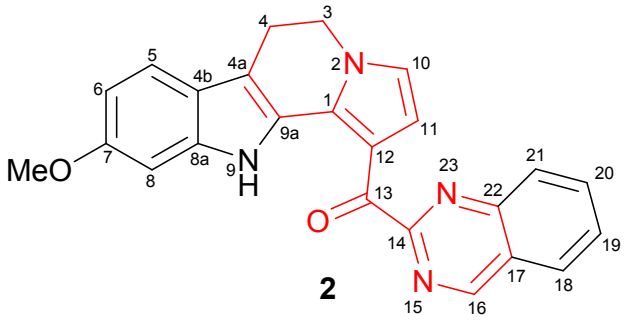


Fig. S22 The HMBC spectrum of pegaharmaline B (**2**) in DMSO-d_6



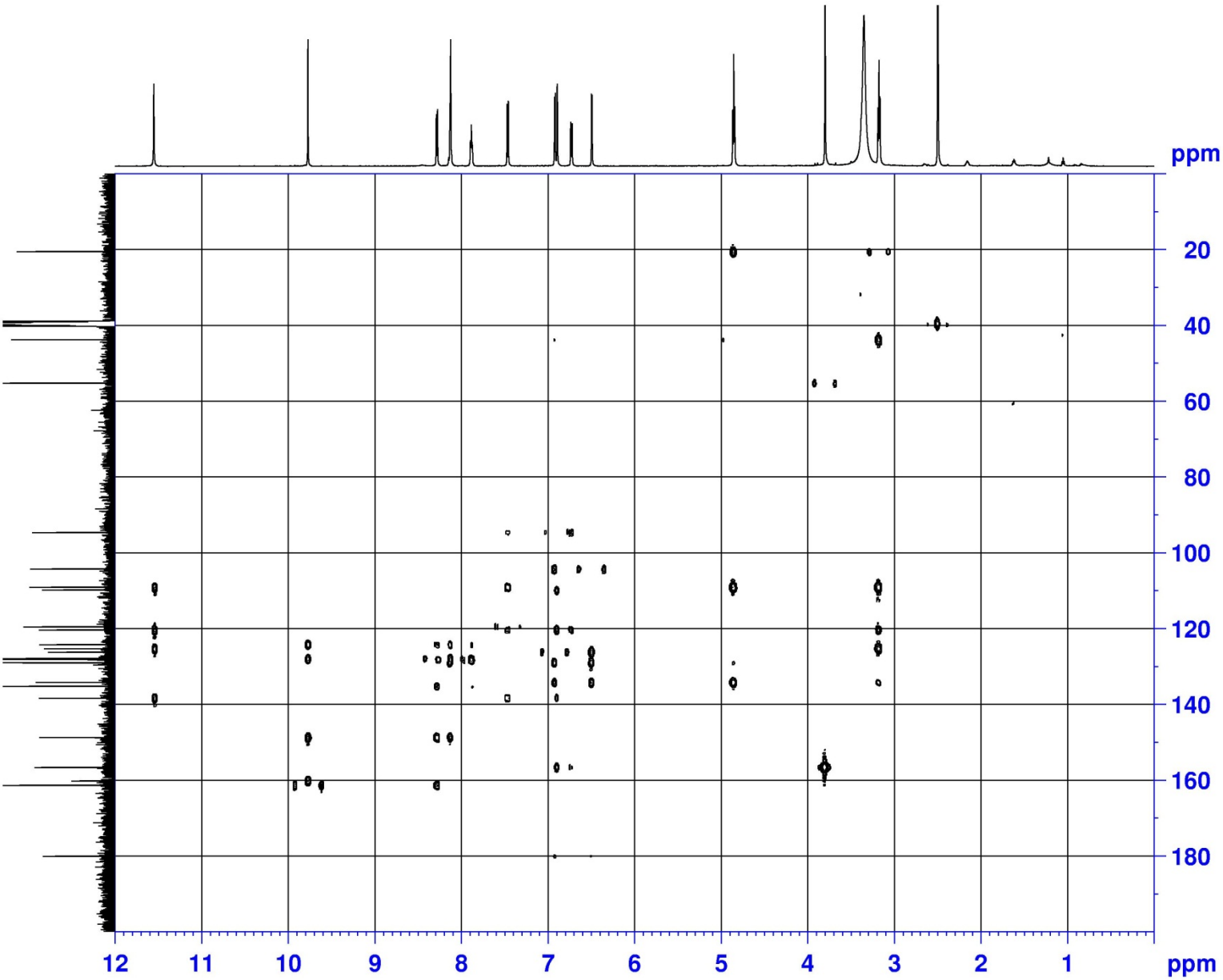


Fig. S23. The HR-ESIMS spectrum of pegaharmaline B (**2**) in CH_3OH

Mass Spectrum Molecular Formula Report

Analysis Info

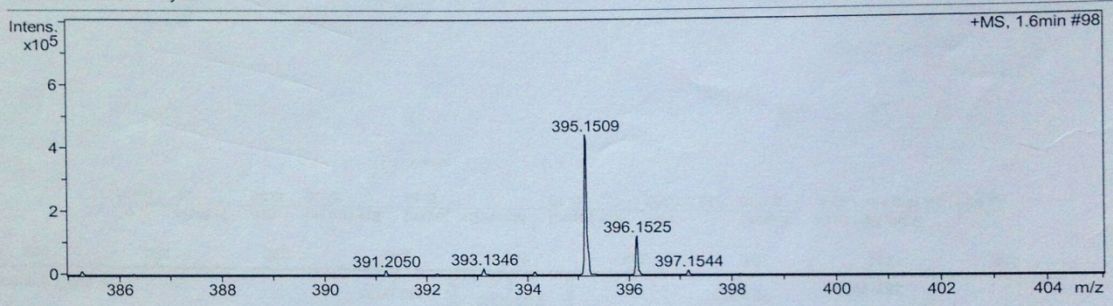
Analysis Name	D:\Data\20130622\WKB-17.d	Acquisition Date	6/22/2013 5:18:34 PM
Method	yujia-shuijie.m	Operator	Bruker Customer
Sample Name	WKB-17	Instrument / Ser#	micrOTOF-Q 125
Comment			

Acquisition Parameter

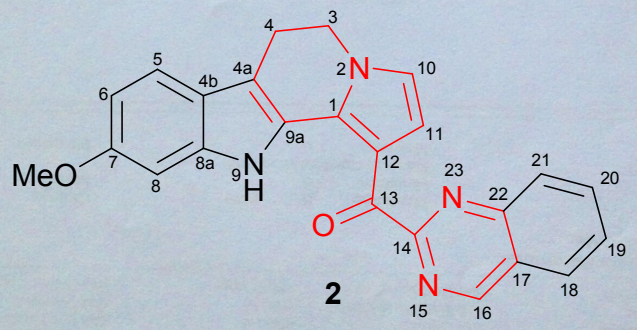
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.3 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	1.2 l/min
Scan End	3000 m/z	Set Collision Cell RF	450.0 Vpp	Set Divert Valve	Source

Generate Molecular Formula Parameter

Formula, min.	C24H18O2N4H				
Formula, max.					
Measured m/z	395.151	Tolerance	5 ppm	Charge	1
Check Valence	no	Minimum	0	Maximum	0
Nirogen Rule	yes	Electron Configuration	both		
Filter H/C Ratio	yes	Minimum	0	Maximum	3
Estimate Carbon	yes				



Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	Err [mDa]	rdb	N Rule	e⁻
C 24 H 19 N 4 O 2	0.006	395.1503	-1.64	-0.67	-0.65	17.50	ok	even



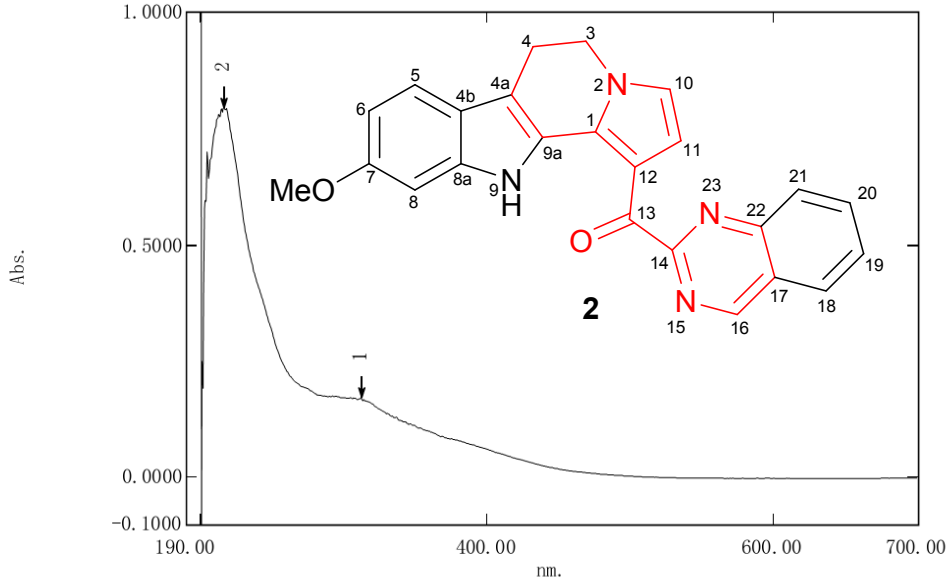
Bruker Daltonics DataAnalysis 3.4 printed: 6/24/2013 6:51:04 PM Page 1 of 1

Fig. S24. The UV spectrum of pegaharmaline B (**2**) in CH_3OH

Spectrum Peak Pick Report

FIELD FIELD TEXT

Data Set: 没有



测定属性
波长范围 (nm.): 190.00到700.00
扫描速度: 高速
采样间隔: 0.5
自动采样间隔: 启用
扫描模式: 自动

No.	P/V	Wavelength	Abs.	描述
1	↑	312.00	.1709	
2	↑	216.00	.7946	
3	↑	196.50	3.9999	
4	↓	309.50	.1672	
5	↓	200.50	-.1232	

试样准备属性
重量:
体积 :
稀释:
光程长:
附加信息:

仪器属性
仪器类型: UV-1700
测定方式: 吸收值
狭缝宽: 1.0 nm
光源改变波长: 340.8 nm
S/R 转换: 标准

附件属性
附件: 无

FIELD TEXT

Fig. S25. The IR spectrum of pegaharmaline B (**2**) in CH_3OH