Supplementary Information (SI) for Organic & Biomolecular Chemistry.
This journal is © The Royal Society of Chemistry 2025

Supporting Information

Alstoscholarisine M, a 6,7-*seco*-monoterpenoid indole alkaloid with antimicrobial activity from *Alstonia scholaris*

Hao-Fei Yu, ‡ [a, b, c] Lan-Chun Zhang, ‡ [c] Khalid-Hassan Mohamed, ‡ [c] Lei Li, [c] Jian-Neng Yao, [c] Ya-Ping Liu, [b] Xiao-Nian Li, [b] Cai-Feng Ding, *[, c] Rong-Ping Zhang, *[, d] and Xiao-Dong Luo*[a,b]

[a] *Key Laboratory of Medicinal Chemistry for Natural Resource, Ministry of Education and Yunnan Province, School of Chemical Science and Technology, Yunnan University, Kunming, 650091, P. R. China*

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

[c] *School of Pharmaceutical Sciences, Yunnan College of Modern Biomedical Industry, Department of Zoology & Yunnan Key Laboratory of Pharmacology for Natural Products, Kunming Medical University, Kunming, 650500, P. R. China*

[d] *School of Chinese Materia Medica and Yunnan Key Laboratory of Southern Medicinal Resources, Yunnan University of Traditional Chinese Medicine, Kunming, 650500, P. R. China*

*Corresponding author.
E-mail addresses: xdluo@mail.kib.ac.cn (X. D. Luo), zhrpkm@163.com (R. P. Zhang), dingcaifeng@kmmu.edu.cn (C. F. Ding).
‡These authors contributed equally to this work.

Table of Contents

1 Computational methods

1.1 Conformational analysis

Conformational analysis for compound **1** (**Figure S1**) were performed using systematic algorithm by Confab at MMFF94 force field with RMSD threshold of 0.2 Å and energy window of 3 kcal/mol. The energies of all dominative conformers were provided in **Table S1**.

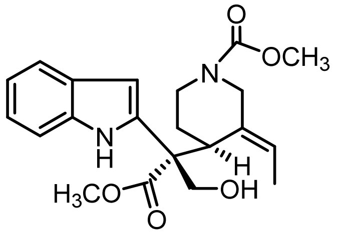


Figure S1 Chemical structure of compound **1**.

1.2 ECD calculation

The theoretical calculations were carried out using Gaussian 09. At first, all conformers were optimized at PM6. Room-temperature equilibrium populations were calculated according to Boltzmann distribution law, based on which dominative conformers of population over 0.1% were kept. The chosen conformers were further optimized at B3LYP/6-31G(d) in gas phase. Vibrational frequency analysis confirmed the stable structures. ECD calculations were conducted at B3LYP/6-311G(d,p) level in methanol with IEFPCM model using Time-dependent Density functional theory (TD-DFT). Rotatory strengths for 30 excited states were calculated. The ECD spectrum was simulated using the ECD/UV analysis tool by overlapping Gaussian functions for each transition according to (2).

$$\frac{N_i}{N} = \frac{g_i e^{-\frac{E_i}{k_B T}}}{\sum g_i e^{-\frac{E_i}{k_B T}}} \qquad \textbf{(1)}$$

where N_i is the number of conformer i with energy E_i and degeneracy g_i at temperature T, and k_B is Boltzmann constant.

$$\Delta\varepsilon(E)=\frac{1}{2.297\times10^{-39}}\times\frac{1}{\sqrt{2\pi\sigma}}\sum_{i}^{A}\Delta E_i R_i e^{-\left(\frac{E-E_i}{2\sigma}\right)^2} \qquad \textbf{(2)}$$

where σ represents the width of the band at $1/e$ height, while ΔE_i and R_i are the excitation energies and rotatory strengths for transition *i*, respectively.

The σ and UV-shift values were set 0.3 eV and 9 nm, respectively. Spectra of the enantiomers were produced directly by mirror inversion about the horizontal axis.

1.3 NMR calculation

The structures were directly derived from the previous ECD calculations. NMR calculations were carried out following the protocol adapted from Michael *et al.* (**Table S1**) using the Gauge-Including Atomic Orbitals (GIAO) method at mPW1PW91/6-311+G(2d,p) level in $CDCl_3$ simulated by the IEFPCM model. The TMS-corrected NMR chemical shift values were averaged according to Boltzmann distribution and fitted to the experimental values by linear regression. The calculated ^{13}C- and ^{1}H-NMR chemical shift values of TMS in $CDCl_3$ were 187.1781 and 31.7347 ppm, respectively.

1.4 References

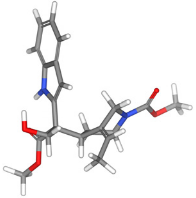
1. N. M. OBoyle, T. Vermeersch, C. J. Flynn, A. R. Maguire, , and G. R. Hutchison. Confab-systematic generation of diverse low-energy conformers. *Journal of Cheminformatics*, **2011**, 3: 3–8.
2. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox. Gaussian 09 Revision D.01. Gaussian Inc. Wallingford CT **2009**.
3. M. W. Lodewyk, M. R. Siebert, and D. J. Tantillo. Computational prediction of ^{1}H and ^{13}C chemical shifts: a useful tool for natural product, mechanistic, and synthetic organic chemistry. *Chem. Rev.*, **2012**, *112* (3): 1839–1862.

2 Energies and Coordinates

2.1 Energies at B3LYP theory level

Structures for ECD calculation were shown in **Table S1**.

Table S1 Energies of configuration **1** at B3LYP/6-311G(d,p) in methanol.

Configuration	Conformer	Structure	E (Hartree)	E (kcal/mol)	Population (%)
1	1		-1301.89040	-816948.55	100

2.2 Coordinates at B3LYP theory level

Table S2 Standard orientations of configuration **1** for ECD calculation.

Conformer **1**-1

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms)		
			X	Y	Z
1	6	0	-2.386257	2.010164	-0.662196
2	6	0	-2.243379	2.163347	0.743698
3	6	0	-2.591508	3.391859	1.334034
4	6	0	-3.077345	4.410878	0.526379
5	6	0	-3.223717	4.232372	-0.866063
6	6	0	-2.88053	3.033784	-1.479099
7	7	0	-1.958415	0.73975	-0.985603
8	6	0	-1.589244	0.062001	0.165533
9	6	0	-1.744077	0.914059	1.243352
10	6	0	-1.072171	-1.368428	0.170123
11	6	0	1.071184	-0.976758	-1.238798
12	6	0	0.519911	-1.454924	0.132145
13	6	0	1.237522	-0.696484	1.244792
14	6	0	1.759071	0.667047	0.834533
15	7	0	2.75495	0.493656	-0.239514
16	6	0	2.557375	-0.618282	-1.175539
17	6	0	3.927966	1.184461	-0.153981
18	8	0	4.184478	2.047476	0.671285
19	8	0	4.799604	0.794602	-1.129737
20	6	0	6.040387	1.509397	-1.133212
21	6	0	-1.574571	-2.169136	-1.048764
22	6	0	-1.63717	-2.064226	1.440246
23	8	0	-2.085301	-1.721025	-2.059229
24	8	0	-1.325503	-3.481803	-0.900105
25	6	0	-1.75527	-4.329161	-1.98175
26	8	0	-3.052422	-2.102034	1.429746
27	6	0	1.575459	-1.202502	2.441829
28	6	0	1.3115	-2.571913	3.00139
29	1	0	-2.484164	3.538667	2.405787
30	1	0	-3.351051	5.364211	0.970209
31	1	0	-3.609282	5.048964	-1.470342
32	1	0	-2.988656	2.897305	-2.55179
33	1	0	-2.1228	0.243351	-1.852479
34	1	0	-1.483108	0.697249	2.27
35	1	0	0.928078	-1.757489	-1.993997
36	1	0	0.515417	-0.097357	-1.582484
37	1	0	0.75121	-2.521088	0.23399
38	1	0	0.946458	1.315522	0.48511
39	1	0	2.255641	1.175533	1.661494
40	1	0	3.147115	-1.495499	-0.867541
41	1	0	2.915997	-0.320288	-2.163502
42	1	0	6.56932	1.376165	-0.185613
43	1	0	5.874456	2.577909	-1.296516
44	1	0	6.618091	1.083859	-1.955485
45	1	0	-1.248628	-1.55513	2.330014
46	1	0	-1.307186	-3.102799	1.469481
47	1	0	-2.83708	-4.253282	-2.112959
48	1	0	-1.472953	-5.339479	-1.686033
49	1	0	-1.258562	-4.044458	-2.912645
50	1	0	-3.353368	-1.176875	1.446547
51	1	0	2.146261	-0.550067	3.103864
52	1	0	0.605667	-2.532609	3.842272
53	1	0	2.2421	-2.998761	3.396825
54	1	0	0.907622	-3.270455	2.264028

3 Experimental and calculated ECD spectra

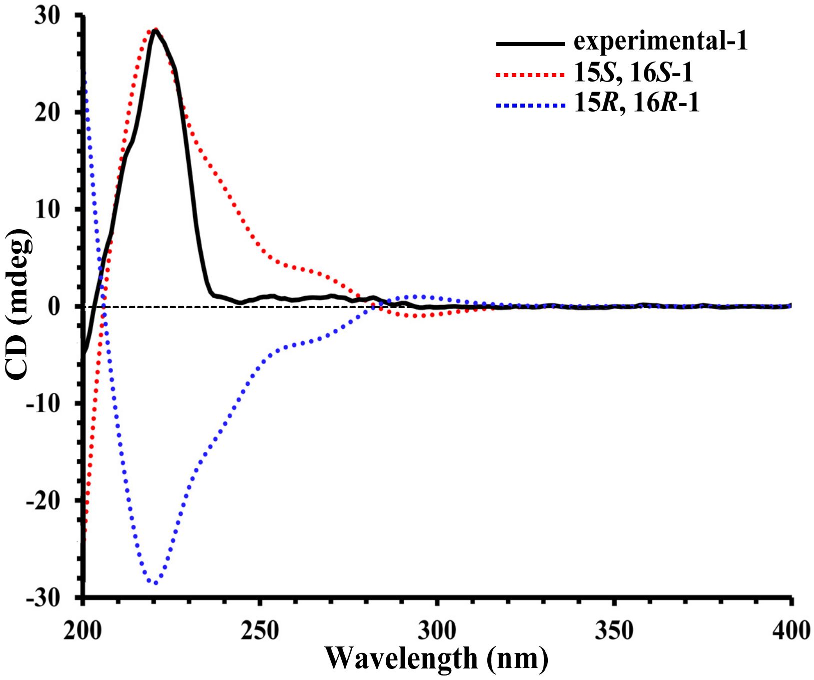


Figure S2 Calculated ECD spectra of compound **1** were compared with the experimental.

4 Experimental and Computed NMR Chemical Shifts

4.2 ^{13}C- and ^{1}H-NMR chemical shifts

The TMS-corrected computed ^{13}C- and ^{1}H-NMR chemical shifts of compound **1** were fitted to the experimental values by Ordinary Least Squares (OLS) Linear Regression method in order to remove systematic error that results from the conformational search and random error from experimental conditions (**Table S3**).

Table S3 Experimental and computed chemical shifts of **1**.

^{13}C-NMR:

Position	Experimental	Calculated
2	134.52	142.0175
3	40.62	43.1427
6	155.51	161.5237
7	101.71	104.8452
8	127.53	132.9519
9	120.04	124.5127
10	120.4	124.3558
11	122.18	126.1792
12	111.37	115.4242
13	136.16	141.0943
14	26.26	27.8403
15	40.13	45.9802
16	58.49	65.3559
17	66.15	71.7572
18	14.15	16.8833
19	129.06	139.1628
20	132.23	143.239
21	50.03	50.7059
$\underline{CO_2}Me$	174.27	186.1177
$CO_2\underline{Me}$	52.94	54.857
6-OMe	52.33	53.4448

^{1}H-NMR:

Position	**Experimental**	**Calculated**
1	9.88	11.021
3a	3.1	2.7808
3b	3.1	3.3758
7	6.46	6.7069
9	7.11	7.5624
10	7.57	7.9767
11	7.2	7.6374
12	7.41	7.8646
14a	1.84	1.9862
14b	1.73	1.6364
15	3.39	3.4864
17a	4.39	4.5615
17b	4.23	4.4216
18	1.61	1.6801
19	5.77	6.2922
21a	3.01	2.4827
21b	3.94	3.9019
CO2Me	3.86	4.0158
6-OMe	3.59	3.5624

Relatively higher R^2 and lower CMAD and CLAD values were shown in both ^{13}C- and ^{1}H-NMR Ordinary Least Squares Linear Regression (OLS-LR) for configuration **1**, which indicated that this configuration was the correct structure instead of **1**.

Table S4 Statistics of Ordinary Least Squares Linear Regression (OLS-LR) of experimental and computed ^{13}C- and ^{1}H-NMR chemical shifts.

Type	Compound	CMAD[a]	CLAD[b]	R^2	*RMSD*	*F*	*p* value
^{13}C NMR	**1**	1.95	4.03	0.9977	2.3007	8311.92	< 0.01
^{1}H NMR	**1**	0.15	0.45	0.9932	0.1971	2501.14	< 0.01

[a] CMAD = corrected mean absolute deviation, computed as $(1/n)\sum_i^n|\delta_{\text{calc}} - \delta_{\text{exp}}|$, where δ_{calc} and δ_{exp} refer to the calculated and experimental chemical shifts. [b] CLAD = corrected largest absolute deviation, computed as $\max(|\delta_{\text{calc}} - \delta_{\text{exp}}|)$.

(1) Regression analysis of experimental *vs* calculated ^{13}C- NMR chemical shifts of 1

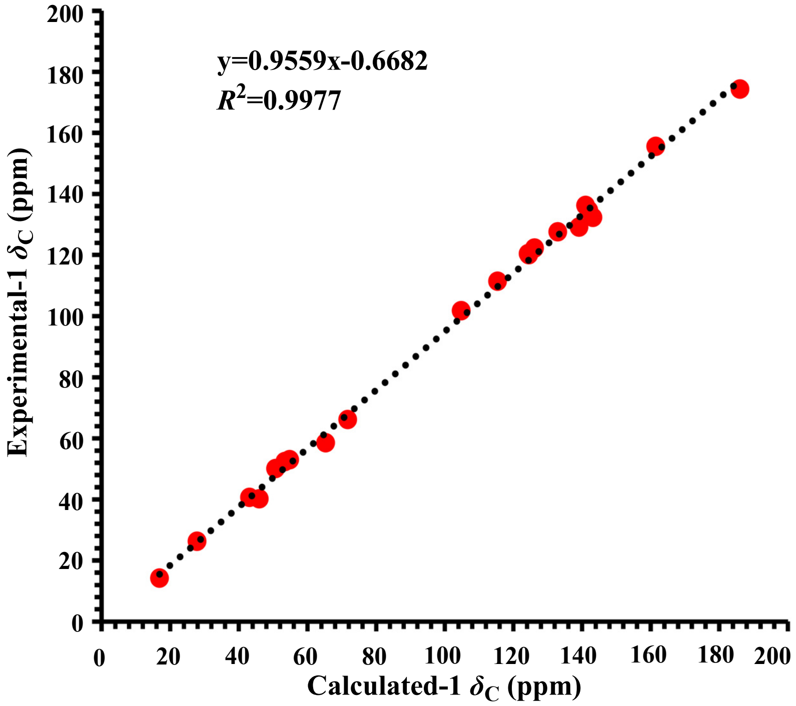


Figure S3 Calculated ^{13}C-NMR of compound **1** were compared with the experimental.

(2) Regression analysis of experimental *vs* calculated ^{1}H-NMR chemical shifts of 1

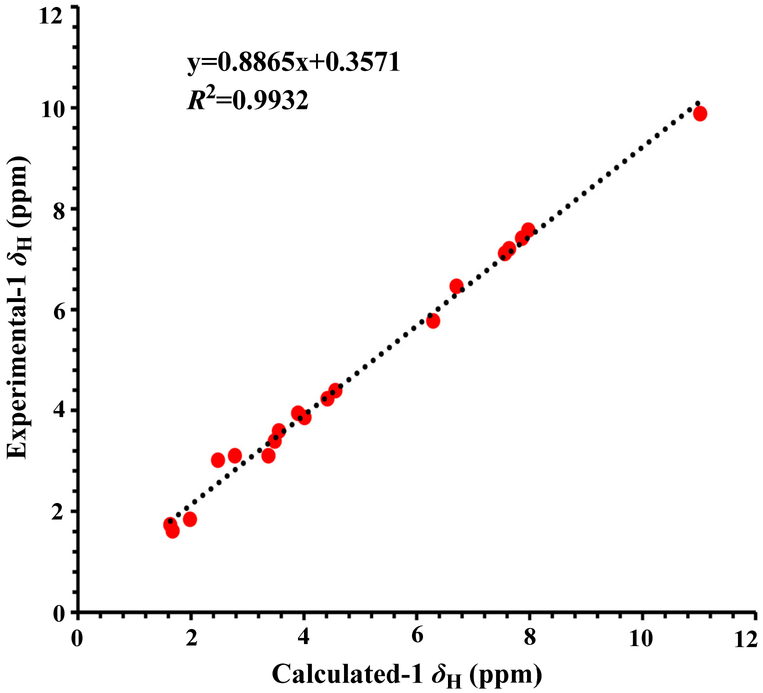
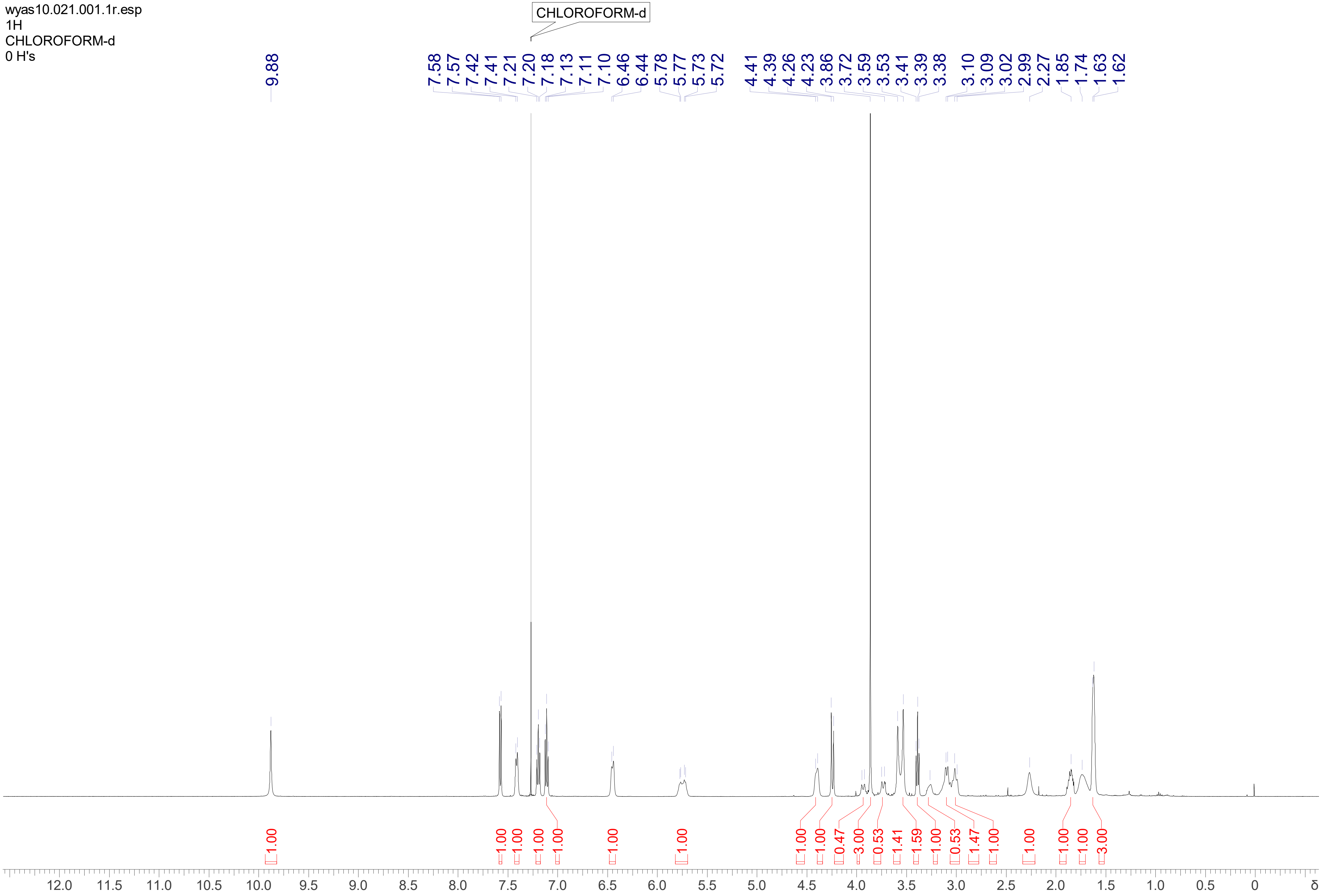


Figure S4 Calculated ^{1}H-NMR of compound **1** were compared with the experimental.

Fig. S5 ^{1}H NMR spectrum of alstoscholarisine M (1) in $CDCl_3$ (500 MHz)



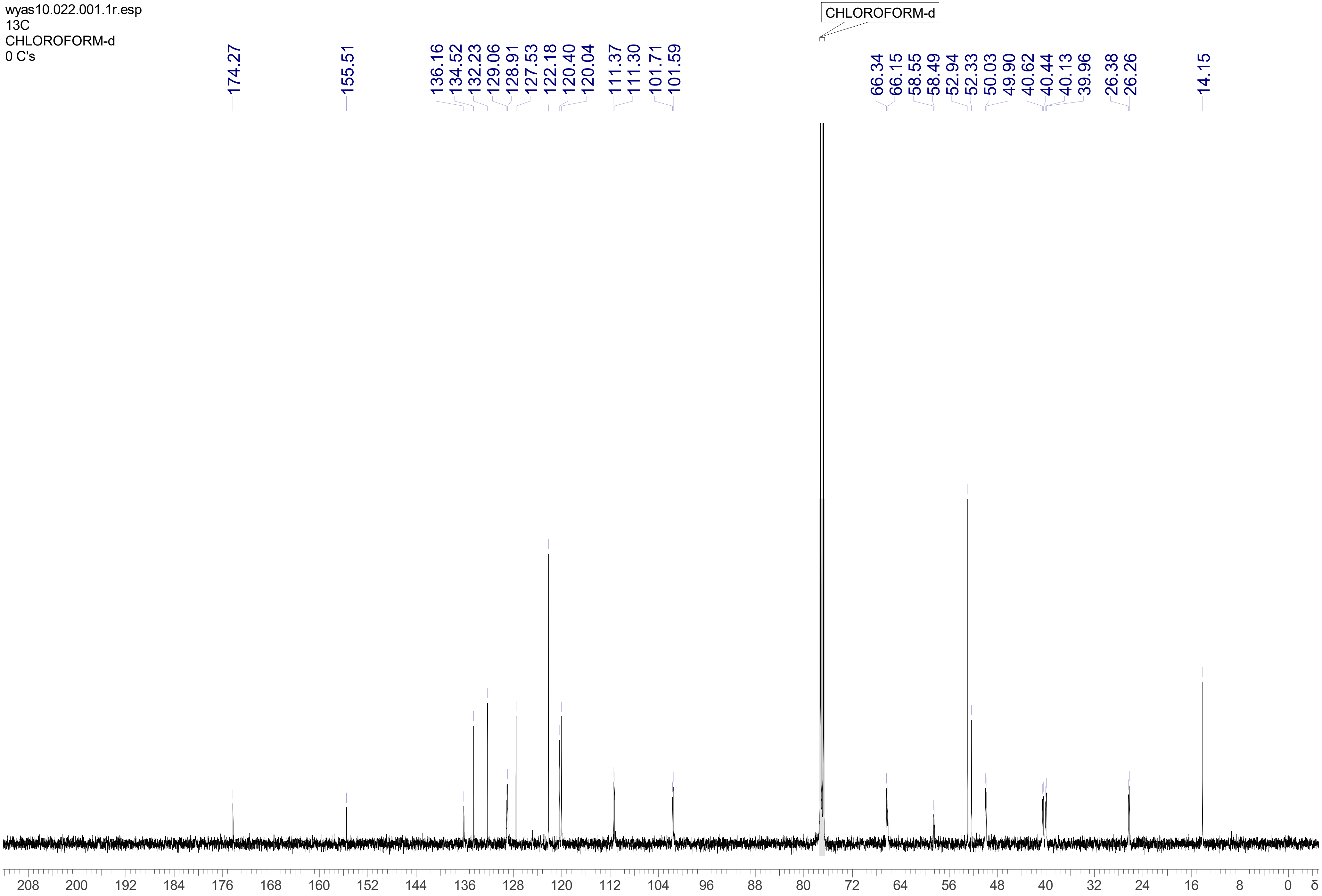


Fig. S6 ^{13}C NMR spectrum of alstoscholarisine M (1) in $CDCl_3$ (125 MHz)

Fig. S7 The partially enlarged ^{13}C NMR spectrum of alstoscholarisine M (1) in $CDCl_3$ (125 MHz)

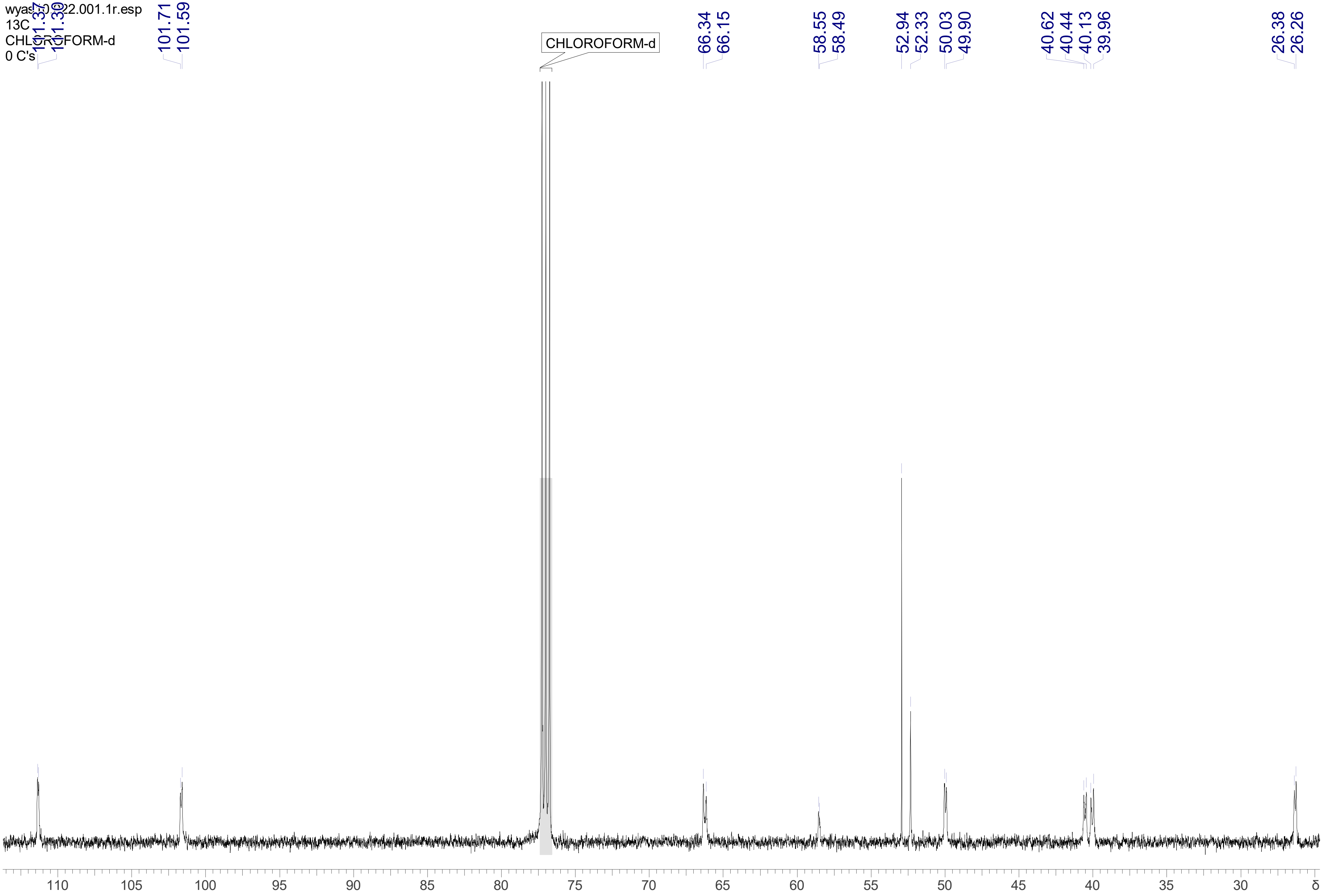


Fig. S8 DEPT 90 spectrum of alstoscholarisine M (1) in $CDCl_3$ (125 MHz)

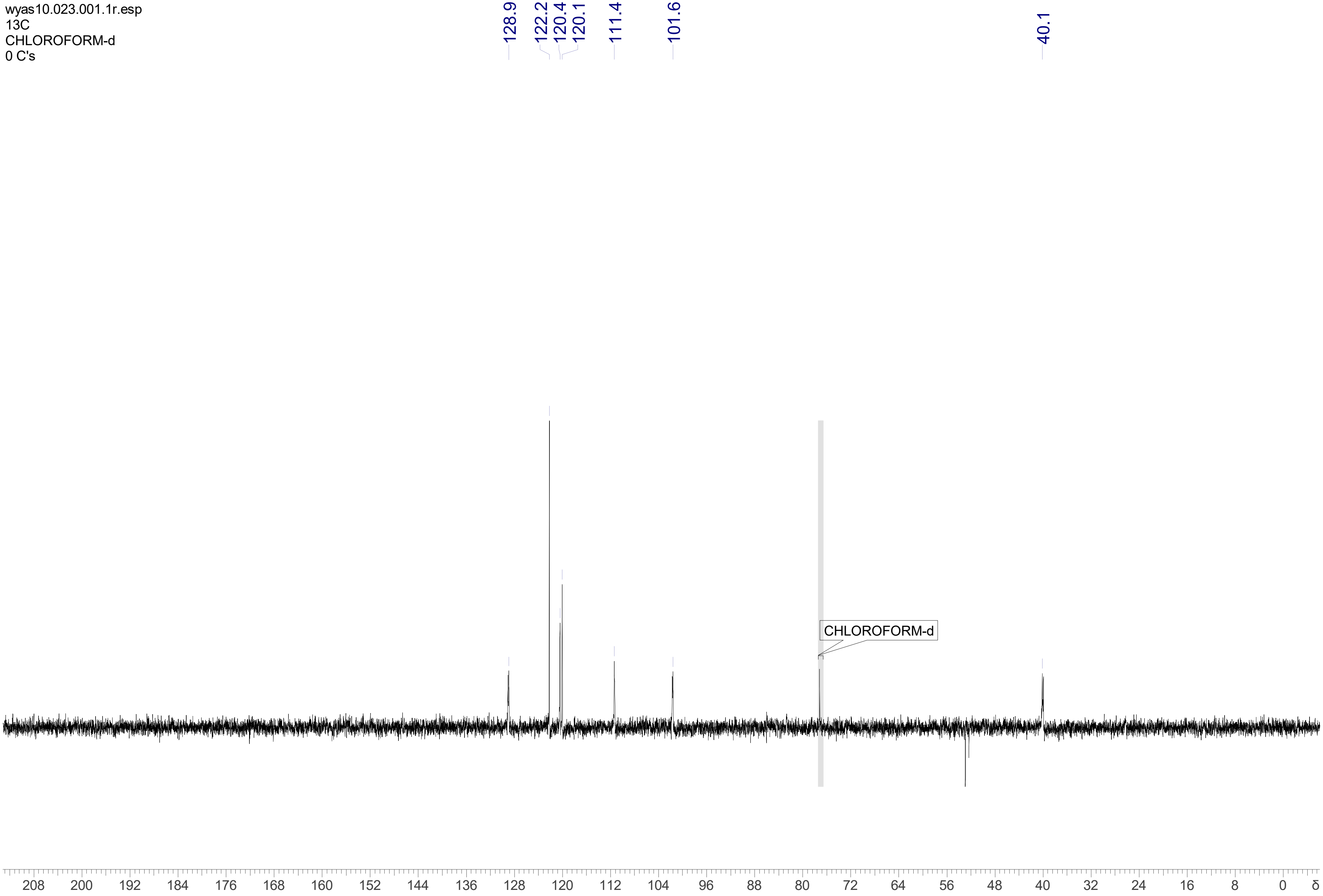


Fig. S9 DEPT 135 spectrum of alstoscholarisine M (1) in CDCl$_3$ (125 MHz)

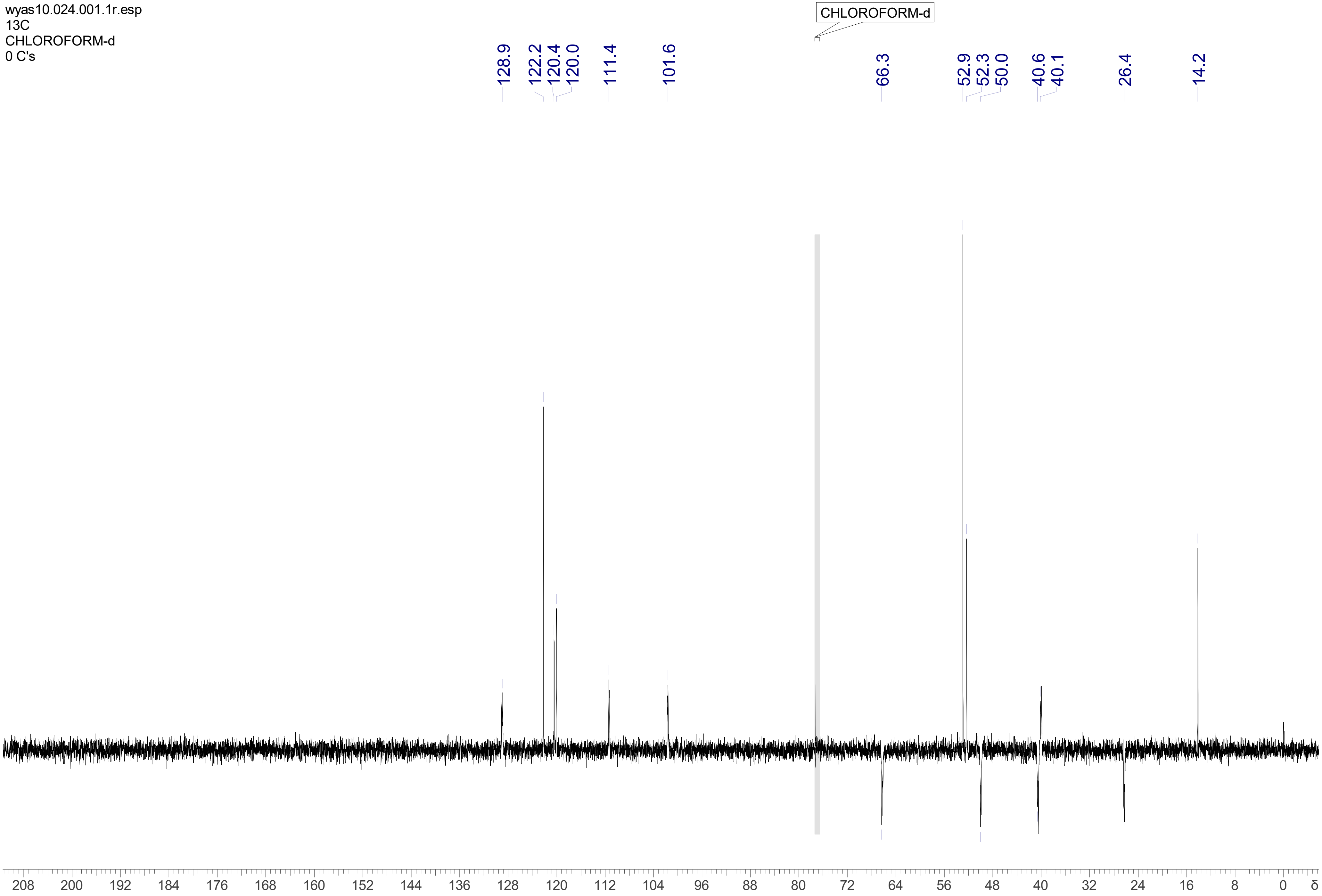


Fig. S10 The ^{1}H-^{1}H COSY spectrum of alstoscholarisine M (1) in CDCl$_3$

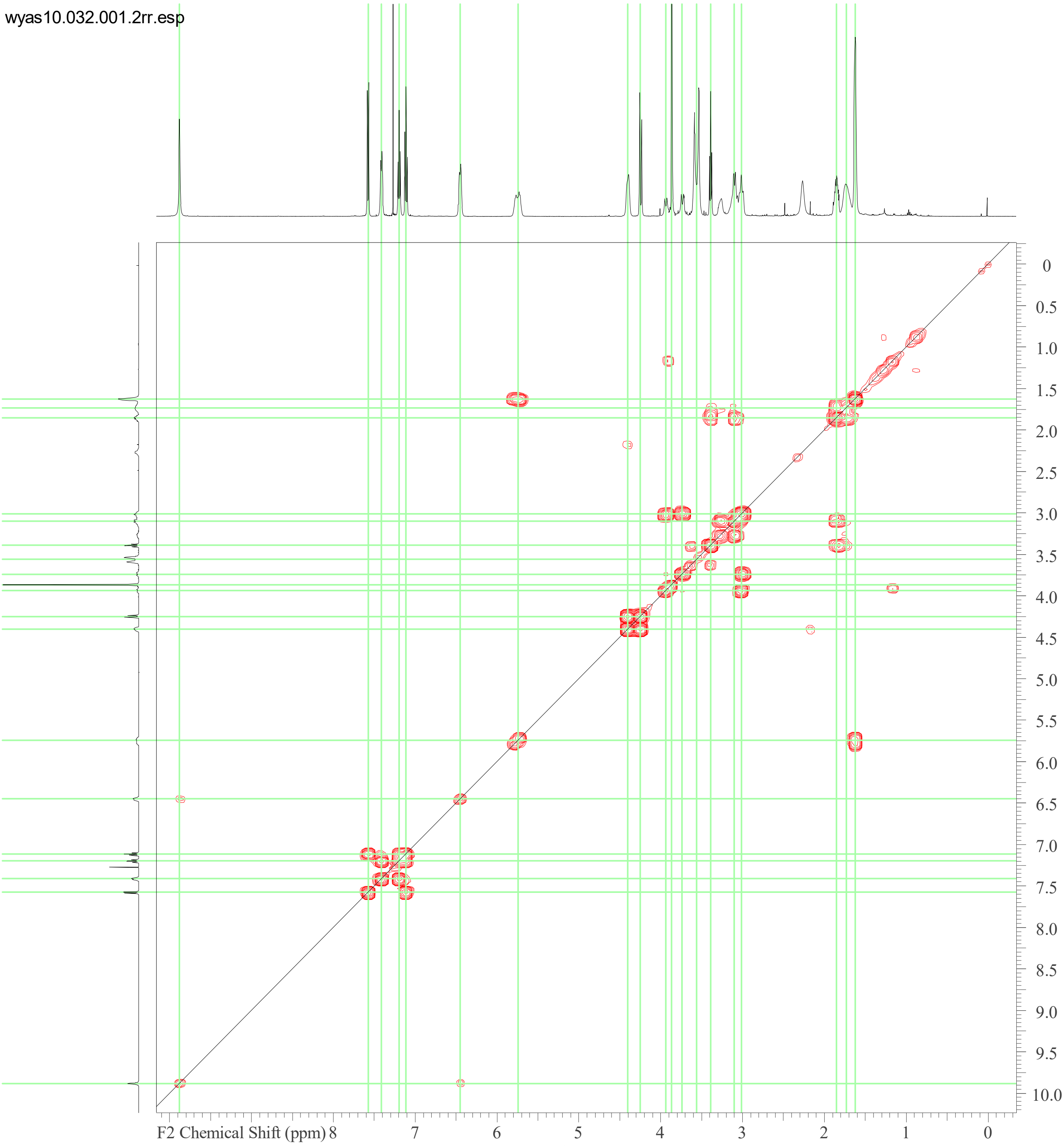


Fig. S11 The HSQC spectrum of alstoscholarisine M (1) in CDCl$_3$

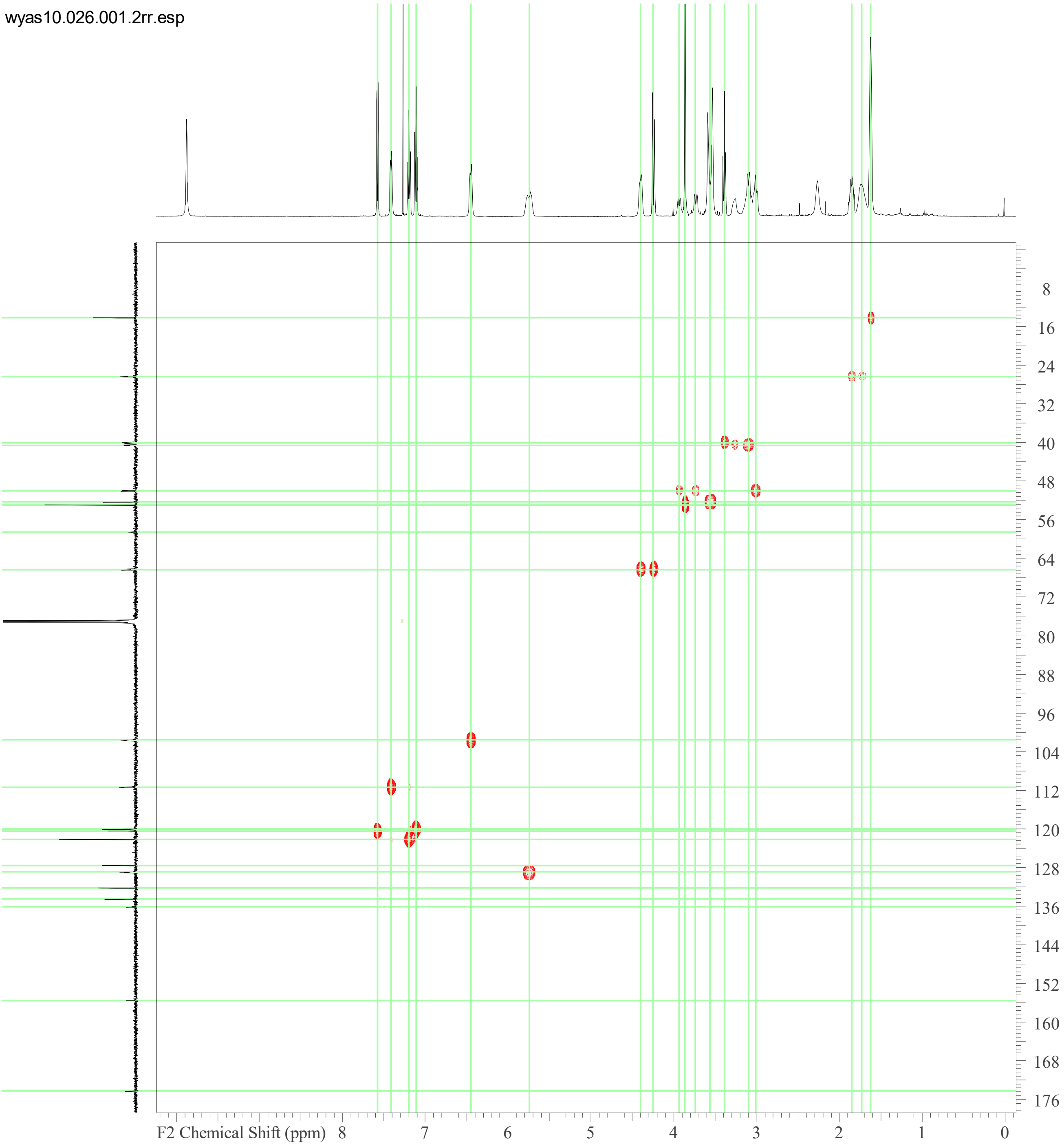


Fig. S12 The HMBC spectrum of alstoscholarisine M (1) in $CDCl_3$

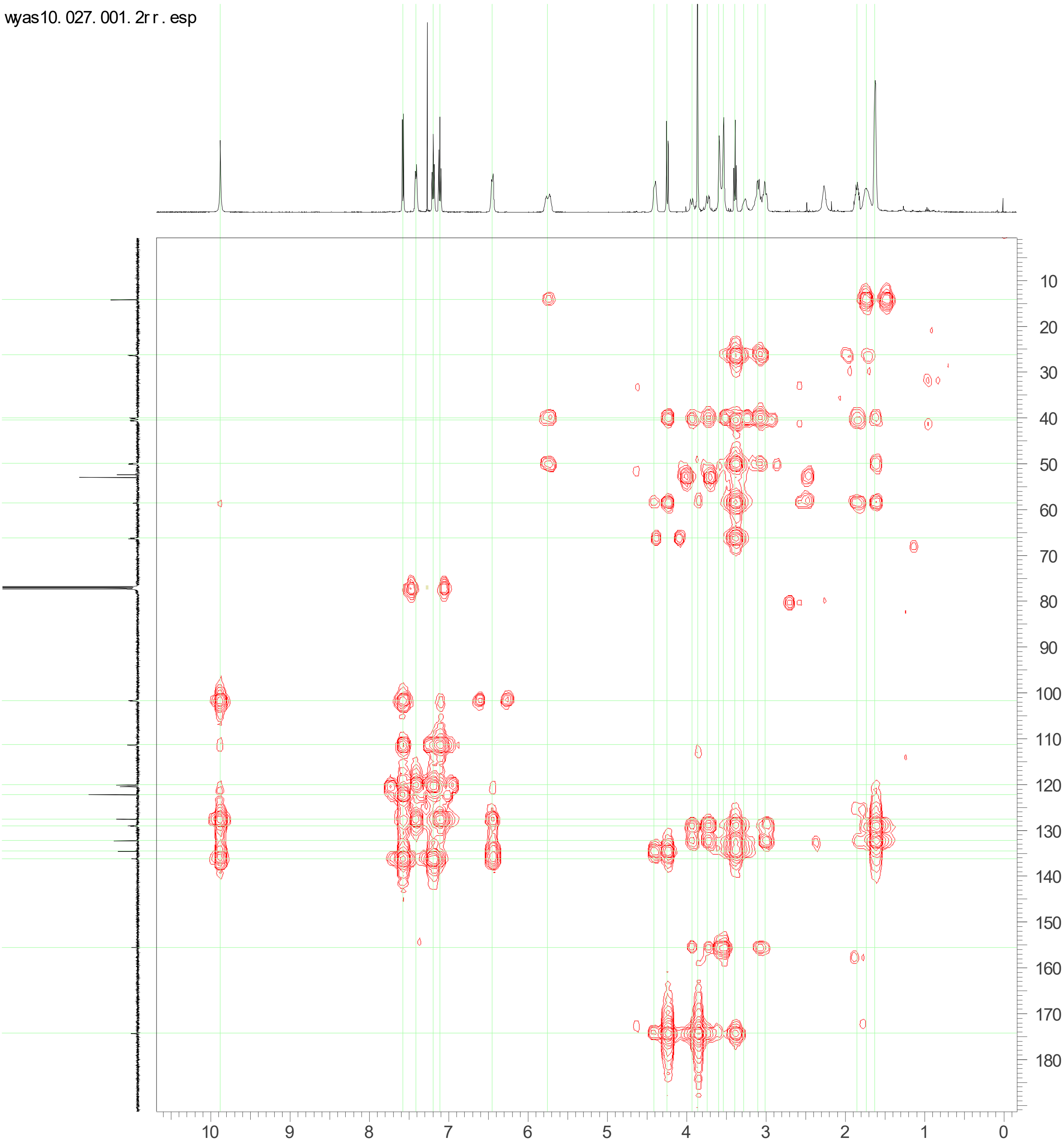


Fig. S13 The partially enlarged HMBC spectrum of alstoscholarisine M (1) in $CDCl_3$

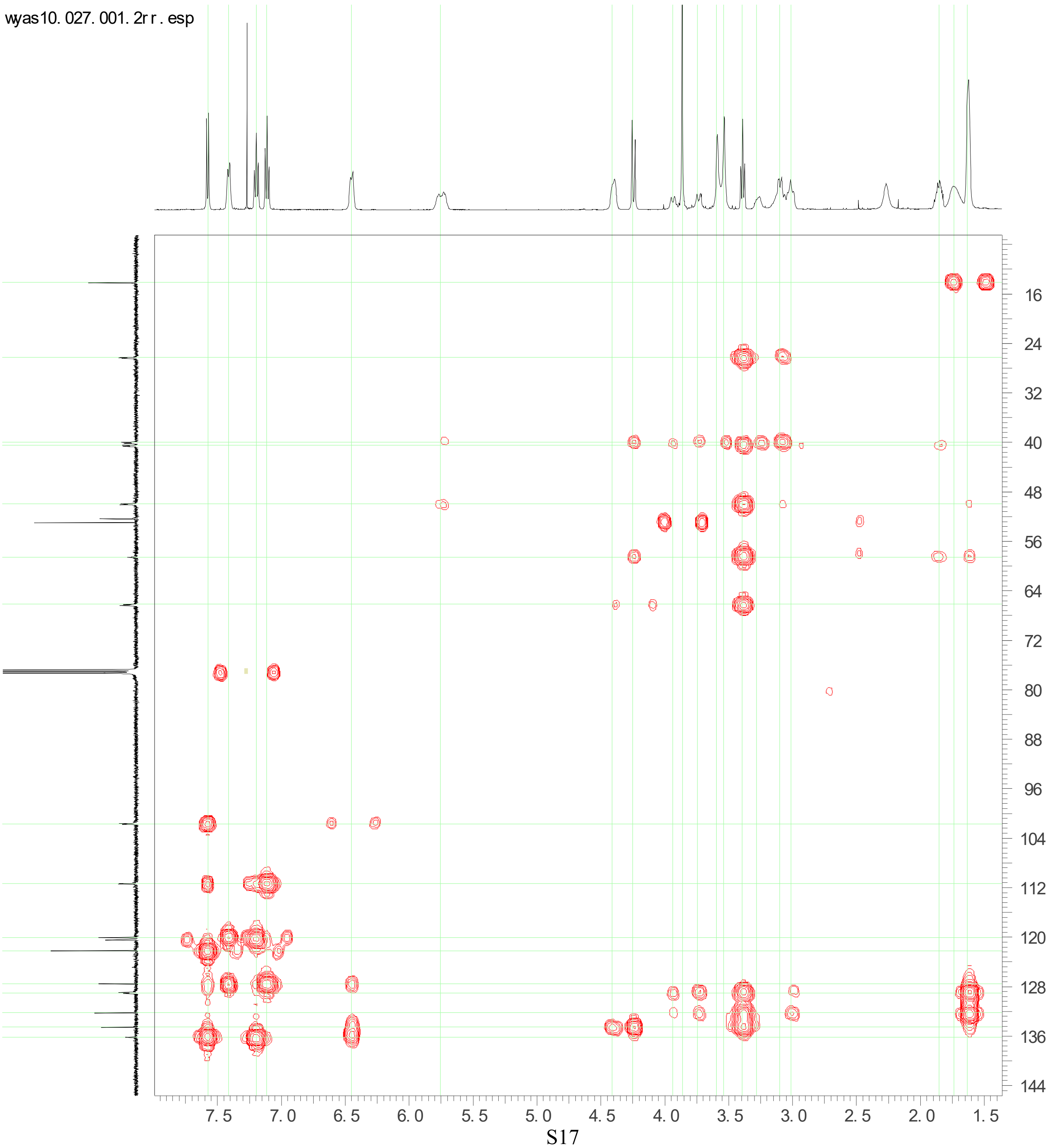
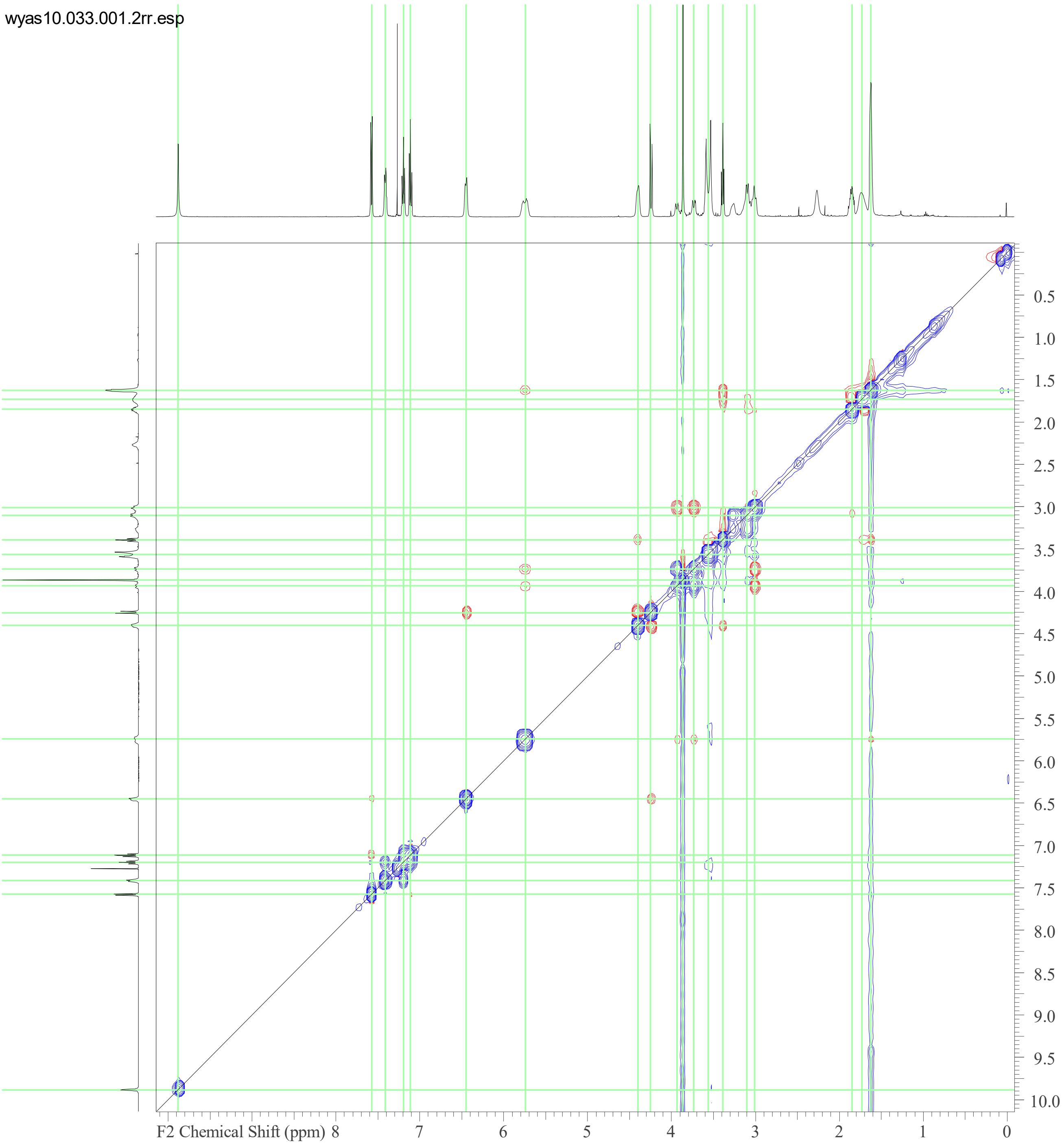


Fig. S14 The ROESY spectrum of alstoscholarisine M (1) in $CDCl_3$



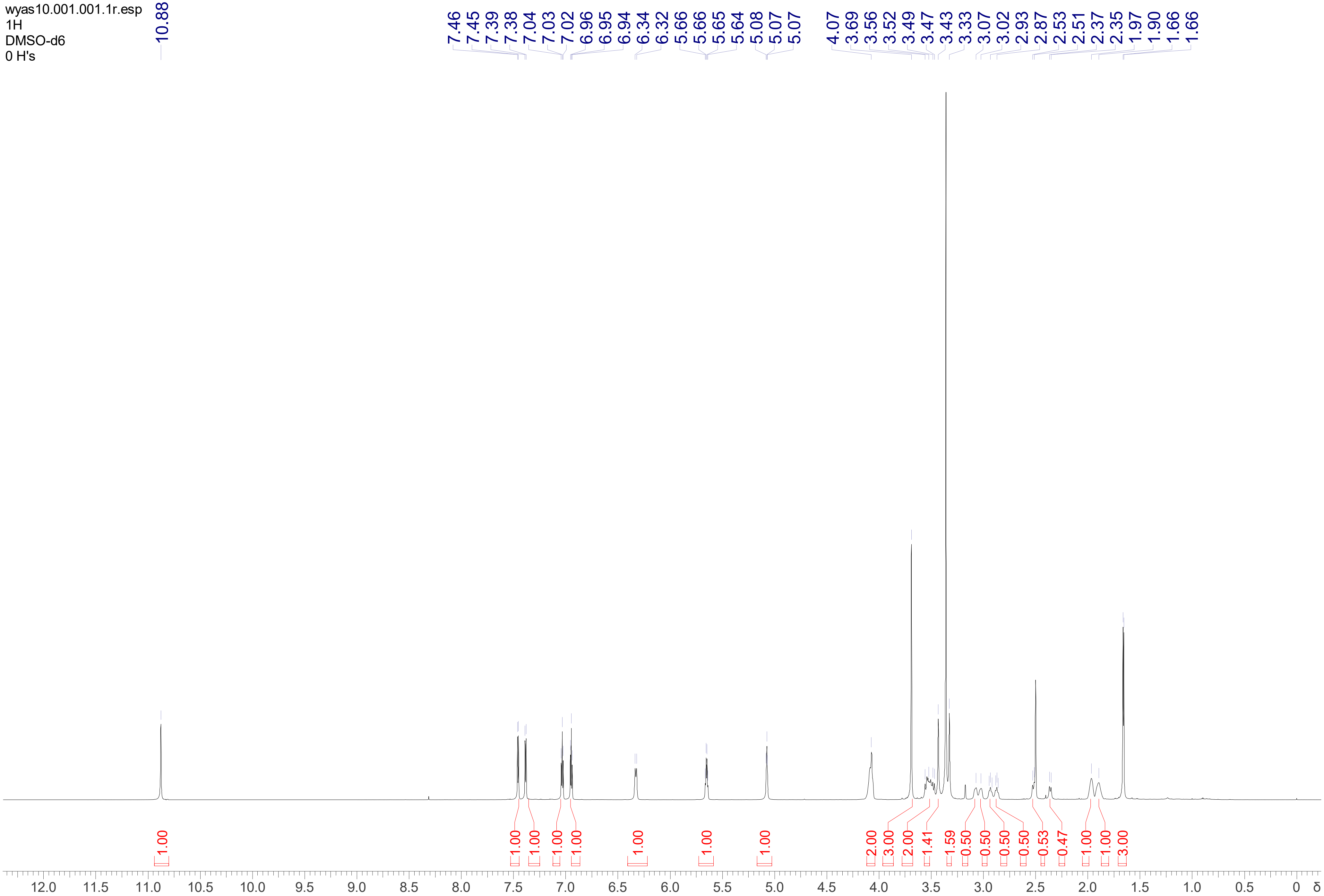


Fig. S15 ^{1}H NMR spectrum of alstoscholarisine M (1) in DMSO-d_6 (800 MHz)

Fig. S16 ^{13}C NMR spectrum of alstoscholarisine M (1) in DMSO-d_6 (200 MHz)

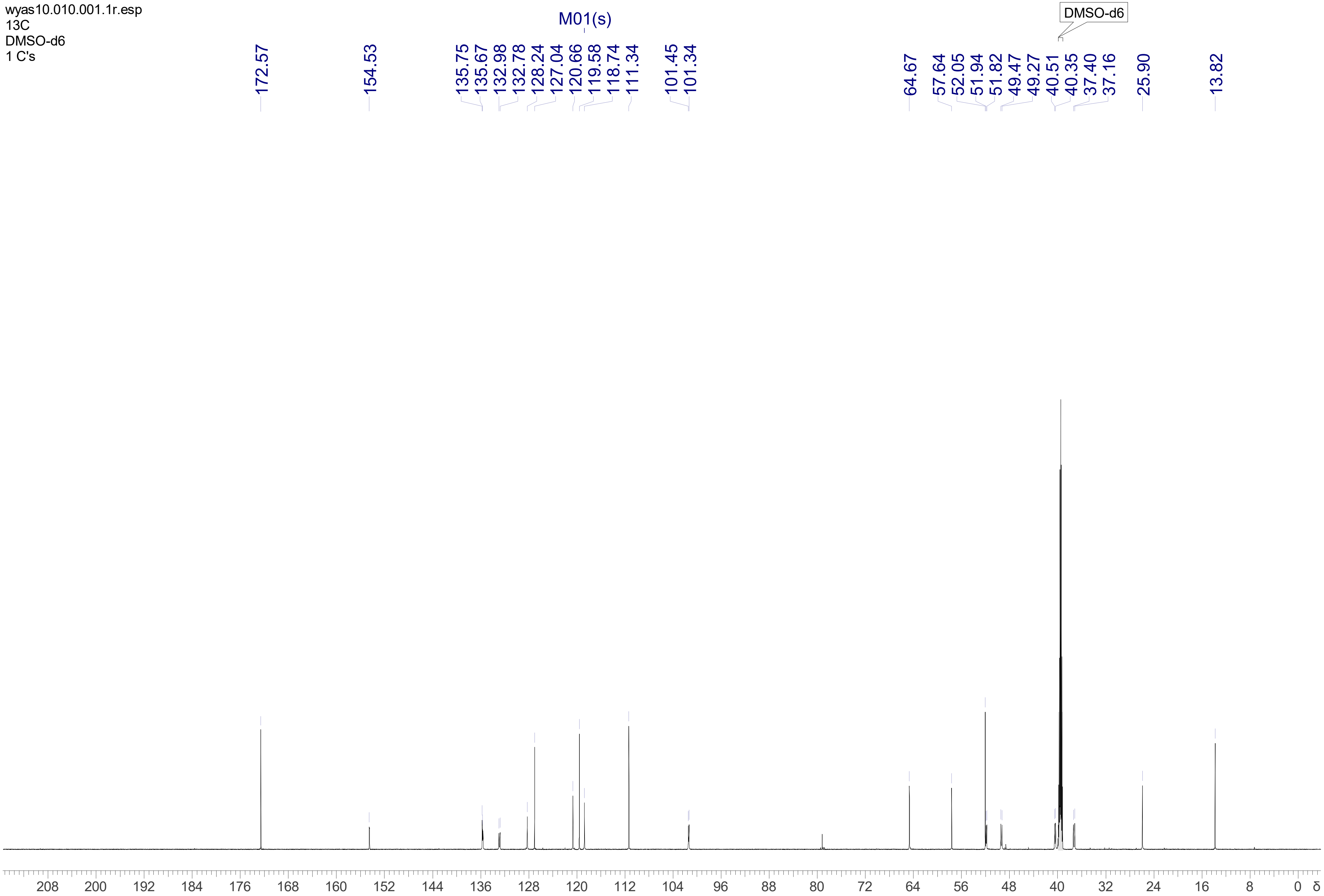


Fig. S17 The partially enlarged ^{13}C NMR spectrum of alstoscholarisine M (1) in DMSO-d_6 (200 MHz)

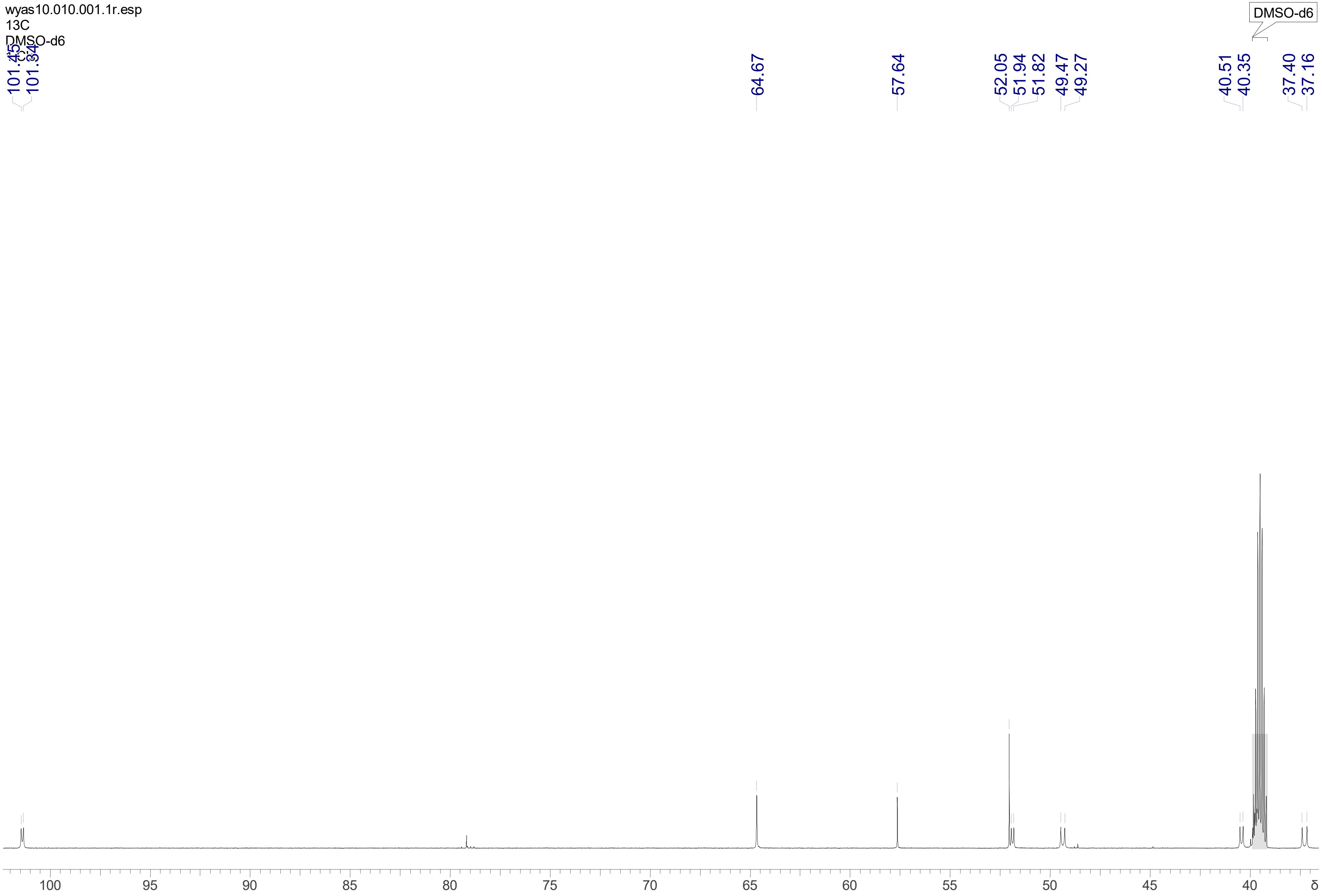


Fig. S18 The HSQC spectrum of alstoscholarisine M (1) in DMSO-d_6

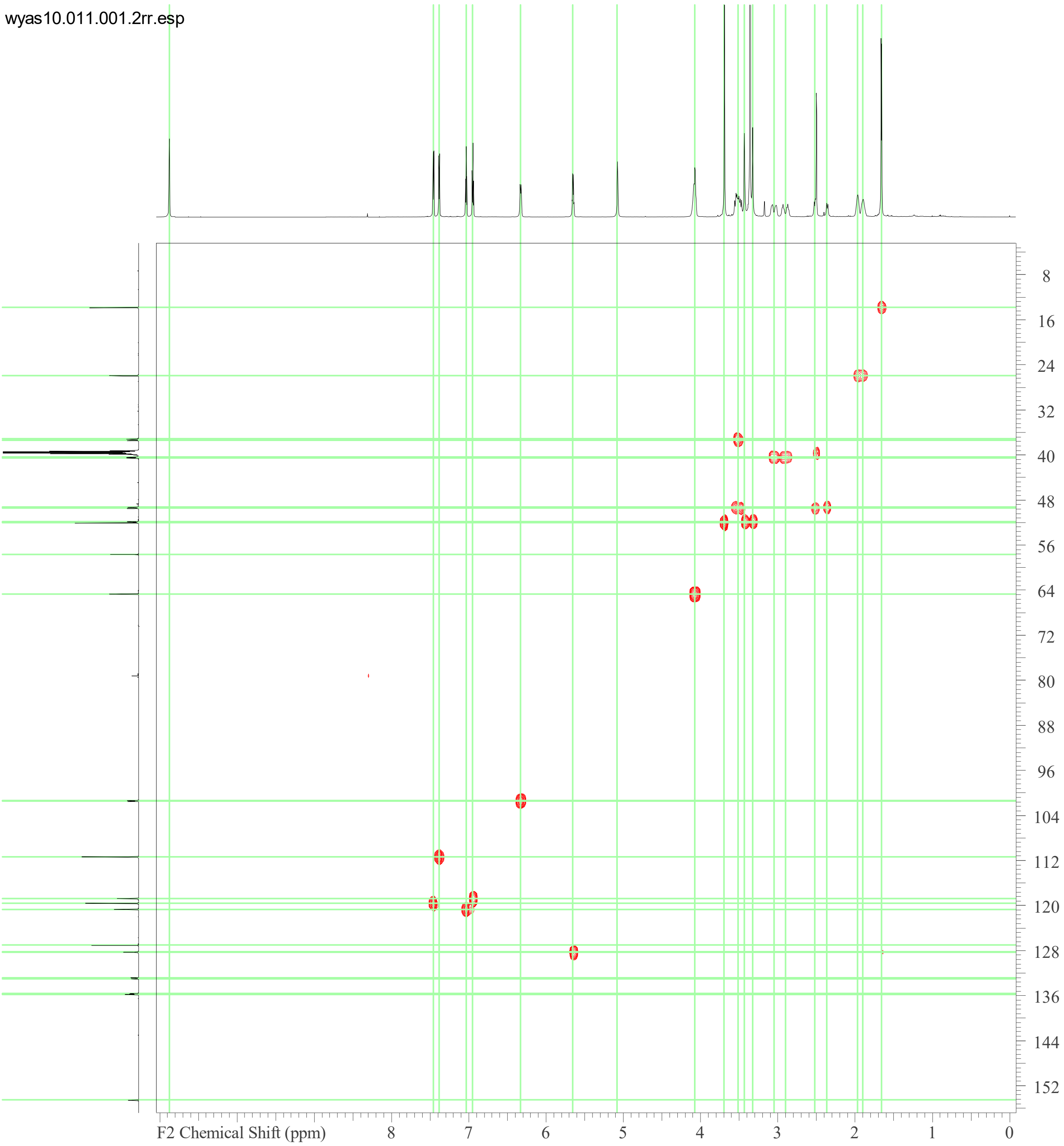


Fig. S19 The HMBC spectrum of alstoscholarisine M (1) in DMSO-d_6

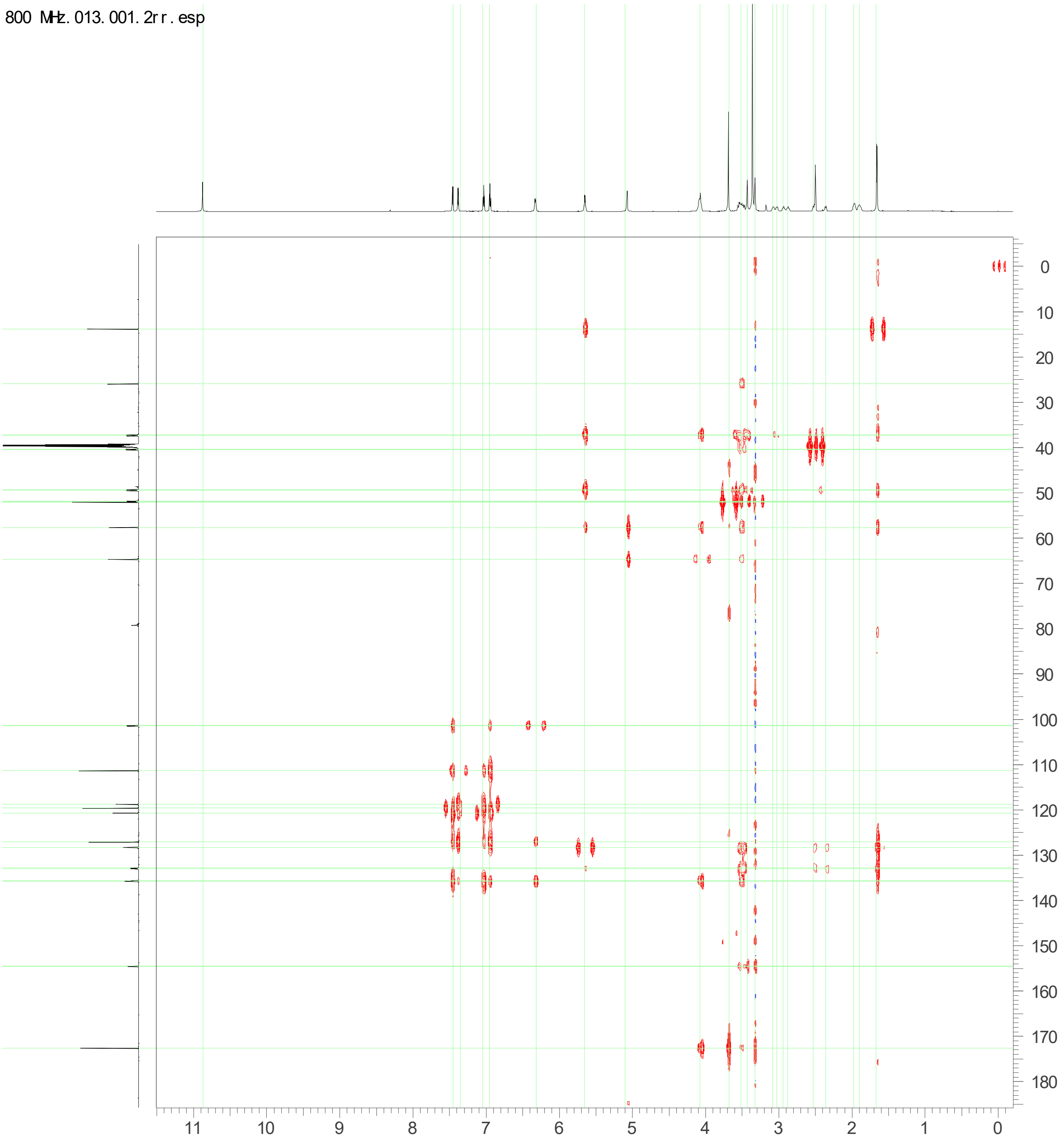
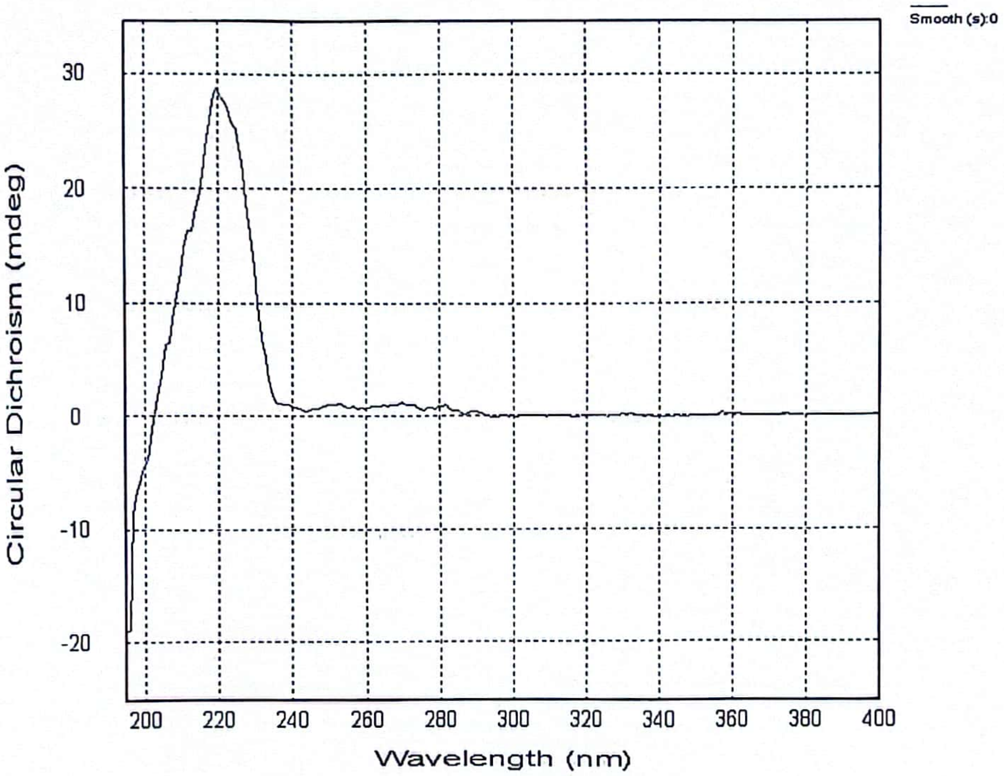
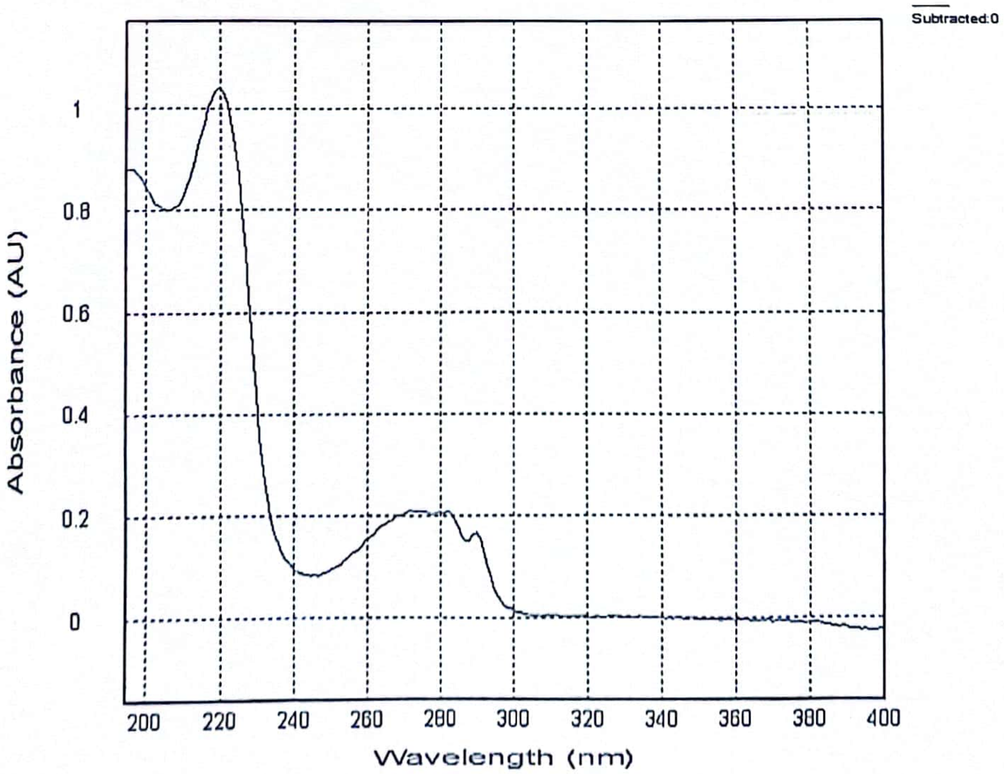


Fig. S20 CD and UV spectra of alstoscholarisine M (1)

WYAS10





File: CD WYAS10-1mm(195-400)17061303.dsx
ProBinaryX
Attributes :
- Time Stamp :Tue Jun 13 15:23:38 2017

- File ID : {6ED2B9E8-10CD-4146-B4C4-18144F7D666B}
- Is CFR Compliant : false
- Original unaltered data

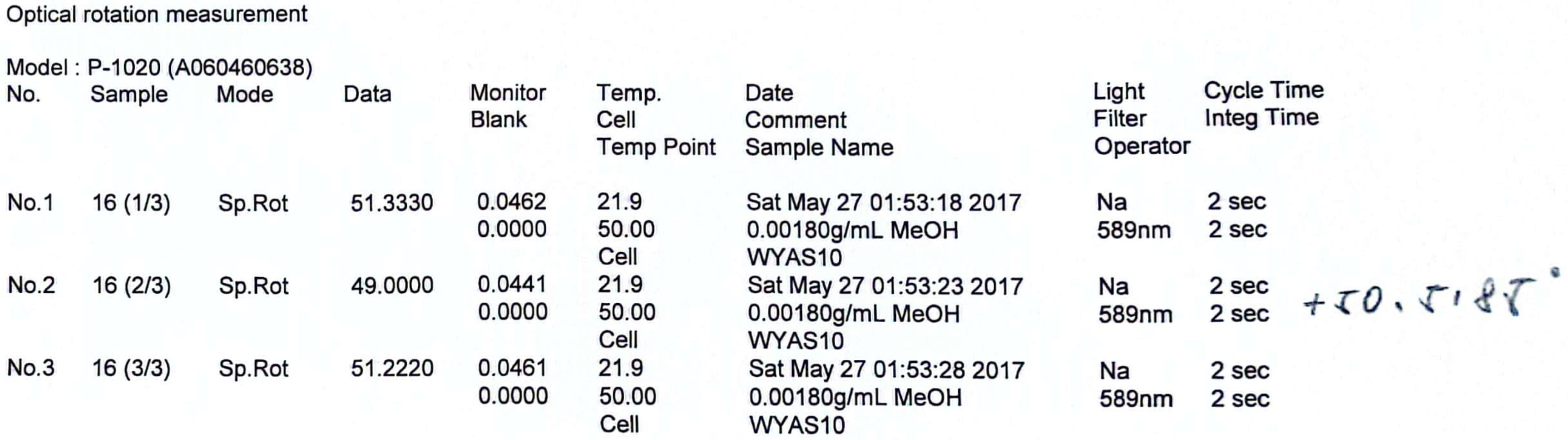
Remarks:

- HV (CDDC channel): 0 v
- Time per point: 1 s
- Description: Sample 1
- Concentration: 0.1026 mg/mL MeOH
- Pathlength: 1 mm

Settings:

- Time-per-point: 1s (25us x 40000)
- Wavelength: 195nm - 400nm
- Step Size: 1nm
- Bandwidth: 1nm

Fig. S21 Optical rotation measurement of alstoscholarisine M (1)



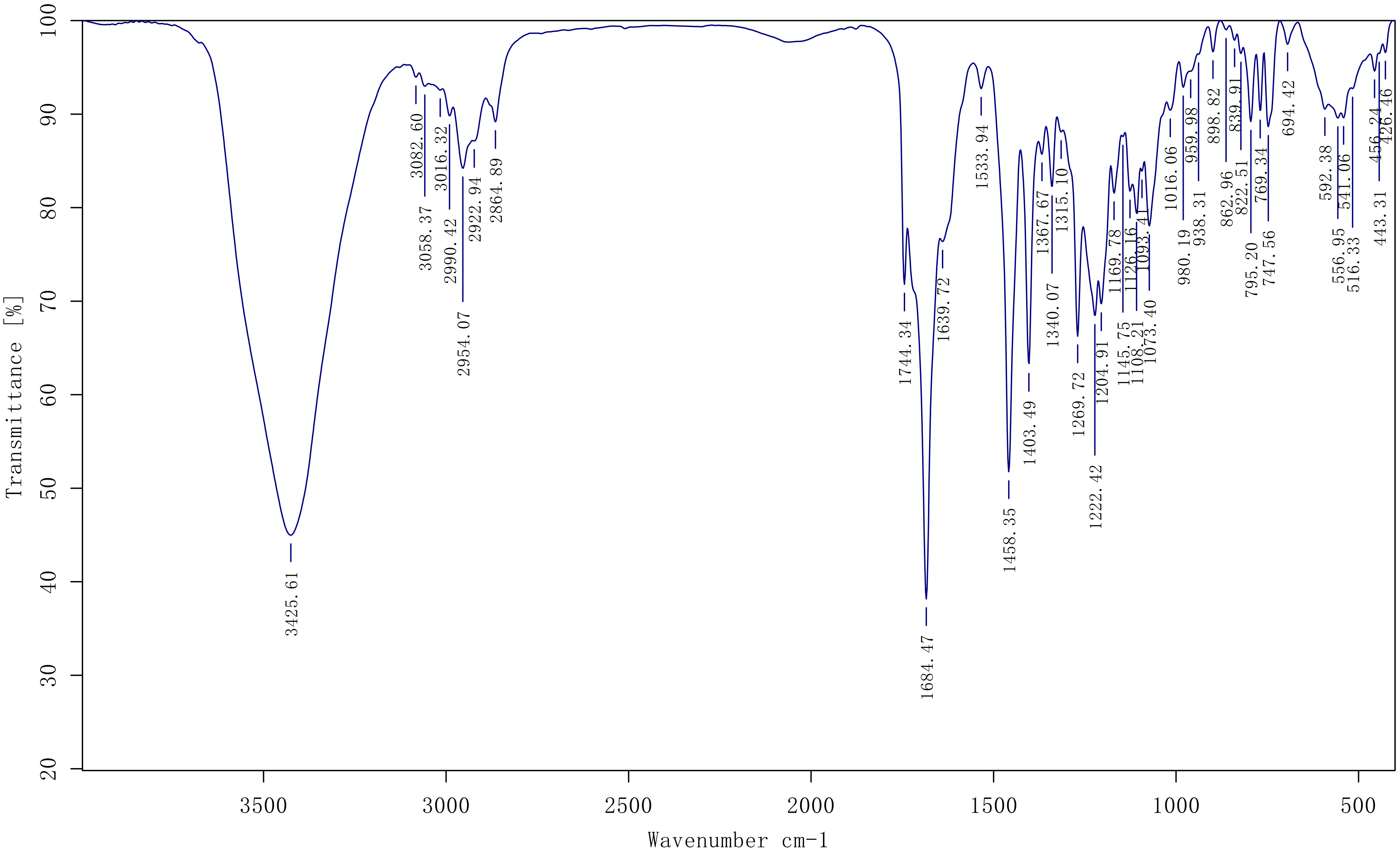
```
Optical rotation measurement

Model : P-1020 (A060460638)
No.     Sample     Mode      Data       Monitor   Temp.        Date                          Light     Cycle Time
                                        Blank     Cell         Comment                       Filter    Integ Time
                                                  Temp Point   Sample Name                   Operator

No.1    16 (1/3)   Sp.Rot    51.3330    0.0462    21.9         Sat May 27 01:53:18 2017      Na        2 sec
                                        0.0000    50.00        0.00180g/mL MeOH              589nm     2 sec
                                                  Cell         WYAS10
No.2    16 (2/3)   Sp.Rot    49.0000    0.0441    21.9         Sat May 27 01:53:23 2017      Na        2 sec
                                        0.0000    50.00        0.00180g/mL MeOH              589nm     2 sec
                                                  Cell         WYAS10
No.3    16 (3/3)   Sp.Rot    51.2220    0.0461    21.9         Sat May 27 01:53:28 2017      Na        2 sec
                                        0.0000    50.00        0.00180g/mL MeOH              589nm     2 sec
                                                  Cell         WYAS10
```

+50.5185°

Fig. S22 IR spectrum of alstoscholarisine M (1)



Sample Name: wyas-10
Sample Form: KBr
Path of File: E:\data
Date of Measurement: 2025/2/27

Resolution: 4
Aperture Setting: 6 mm
Number of Background Scans: 16
Number of Sample Scans: 16

Beamsplitter Setting: KBr
Source Setting: MIR
Instrument Type: BRUKER VERTEX 70
Soft Version: OPUS8.1

Fig. S23 ESIMS spectrum of alstoscholarisine M (1)

==== LCMSsolution Data Report ====

Sample Information

Acquired by : Admin
Date Acquired : 2017/3/16 16:10:14
Sample Name : wyas10
Data File : wyas10.lcd
Method File : 阻尼管一级20151028-100-1500.lcm

System Configuration

<<Instrument>> : LC-IT-TOF

<Spectrum>

Retention Time:0.400(Scan#:81)
Spectrum:Averaged 0.320-0.480(65-97)
Background:Averaged 0.000-0.323(1-65) MS Stage:MS Polarity:Pos Segment1 - Event1 Precursor:----- Cutoff:

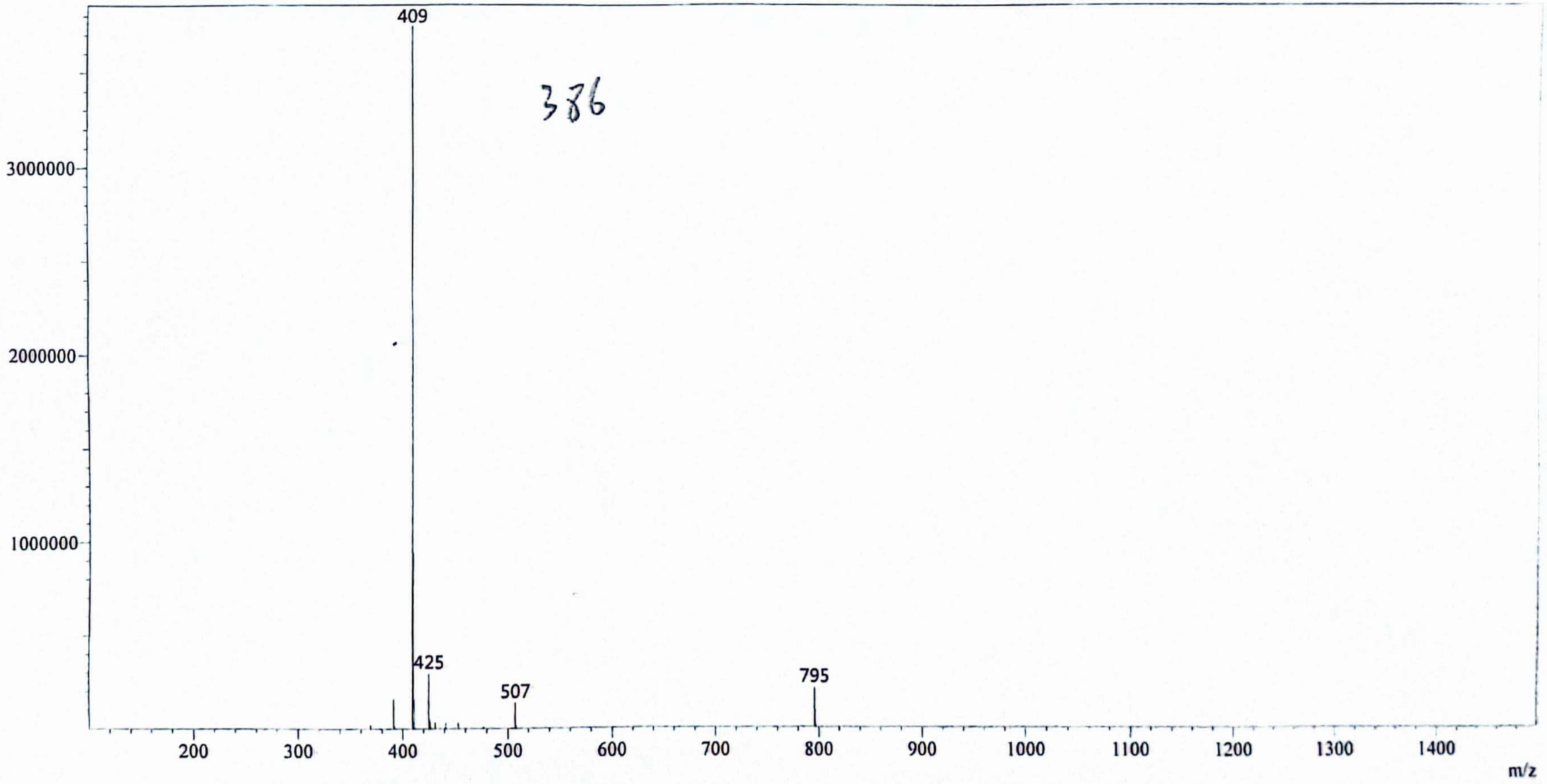
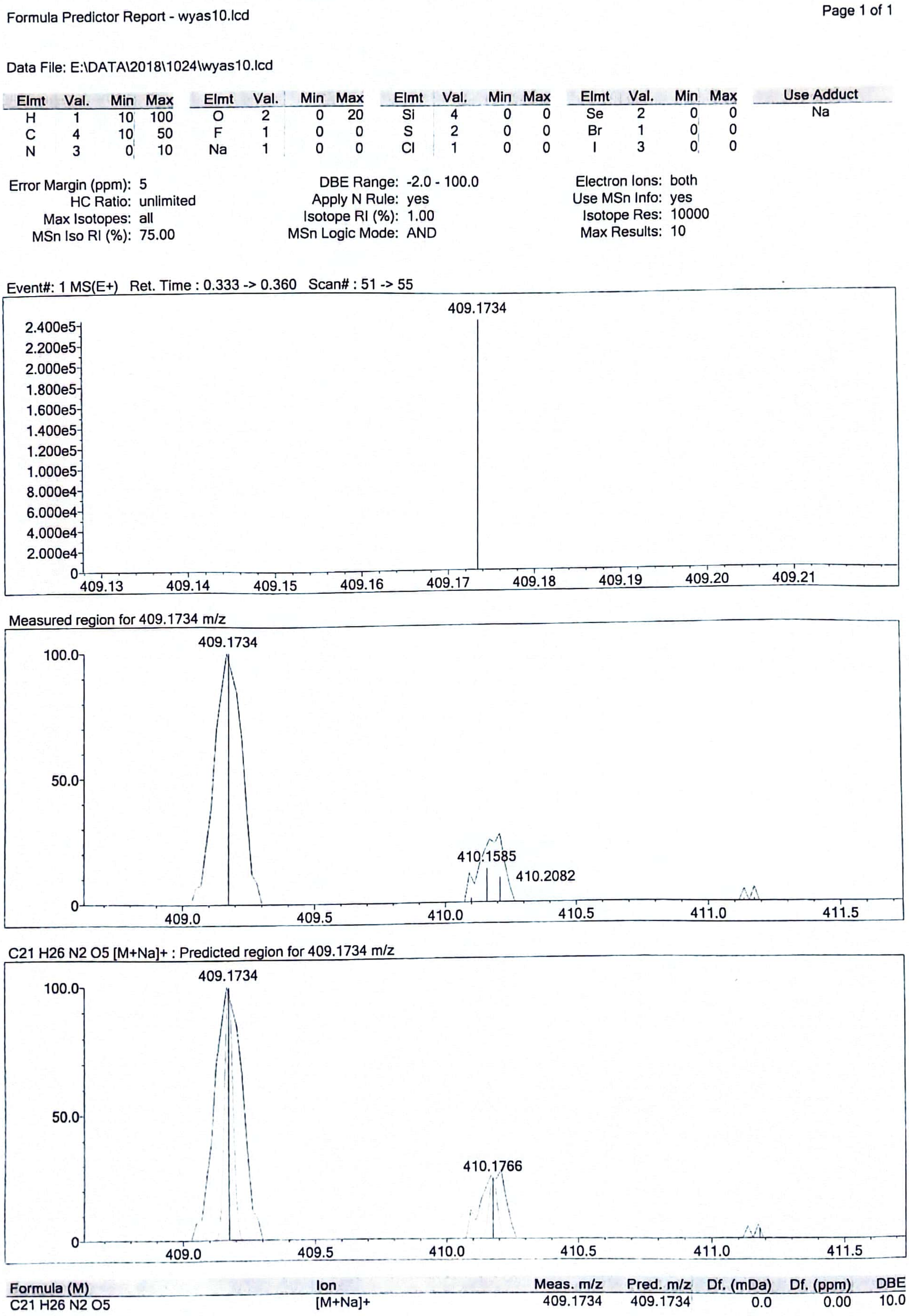


Fig. 24 HR-ESIMS spectrum of alstoscholarisine M (1)



Formula Predictor Report - wyas10.lcd

Page 1 of 1

Data File: E:\DATA\2018\1024\wyas10.lcd

Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	100	O	2	0	20	Si	4	0	0	Se	2	0	0	Na
C	4	10	50	F	1	0	0	S	2	0	0	Br	1	0	0	
N	3	0	10	Na	1	0	0	Cl	1	0	0	I	3	0	0	

Error Margin (ppm): 5
HC Ratio: unlimited
Max Isotopes: all
MSn Iso RI (%): 75.00

DBE Range: -2.0 - 100.0
Apply N Rule: yes
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: yes
Isotope Res: 10000
Max Results: 10

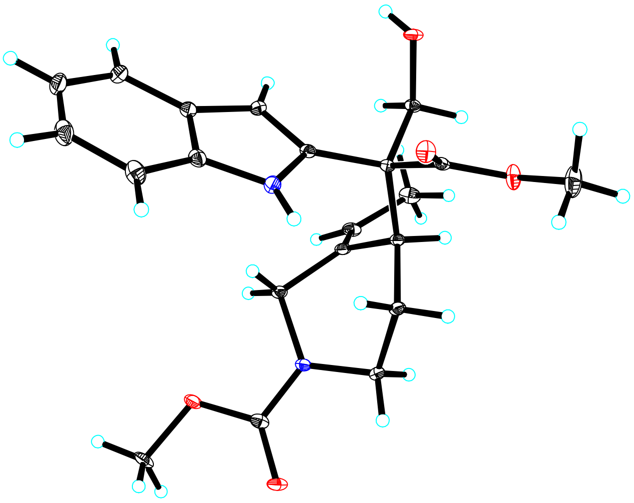
Event#: 1 MS(E+) Ret. Time : 0.333 -> 0.360 Scan# : 51 -> 55

Measured region for 409.1734 m/z

C21 H26 N2 O5 [M+Na]+ : Predicted region for 409.1734 m/z

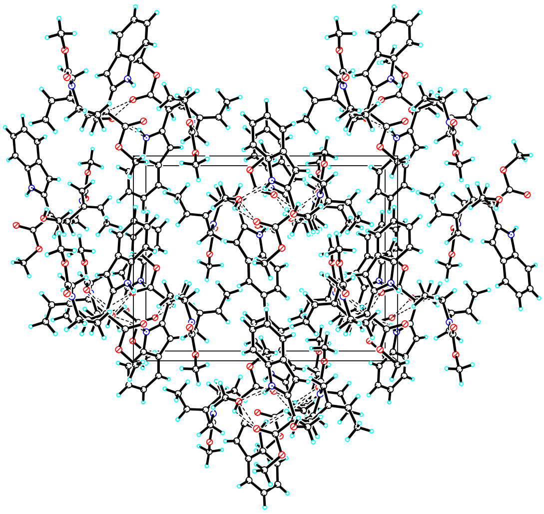
Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	DBE
C21 H26 N2 O5	[M+Na]+	409.1734	409.1734	0.0	0.00	10.0

Crystal data for wyas10: $C_{21}H_{26}N_2O_5$, M = 386.44, a = 9.6537(4) Å, b = 12.6770(5) Å, c = 16.3750(6) Å, α = 90°, β = 90°, γ = 90°, V = 2003.97(14) Å^3, T = 100.(2) K, space group P212121, Z = 4, μ(Cu Kα) = 0.753 mm^{-1}, 17575 reflections measured, 3930 independent reflections (R_{int} = 0.0282). The final R_1 values were 0.0277 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0969 ($I > 2\sigma(I)$). The final R_1 values were 0.0282 (all data). The final $wR(F^2)$ values were 0.0996 (all data). The goodness of fit on F^2 was 0.935. Flack parameter = 0.02(4).



View of a molecule of wyas10 with the atom-labelling scheme.

Displacement ellipsoids are drawn at the 30% probability level.



View of the pack drawing of wyas10.

Hydrogen-bonds are shown as dashed lines.

Table 1. Crystal data and structure refinement for wyas10_0m.

Identification code	global	
Empirical formula	C21 H26 N2 O5	
Formula weight	386.44	
Temperature	100(2) K	
Wavelength	1.54178 Å	
Crystal system	Orthorhombic	
Space group	$P2_12_12_1$	
Unit cell dimensions	a = 9.6537(4) Å	α= 90°.
	b = 12.6770(5) Å	β= 90°.
	c = 16.3750(6) Å	γ = 90°.
Volume	2003.97(14) Å^3	
Z	4	
Density (calculated)	1.281 Mg/m^3	
Absorption coefficient	0.753 mm^{-1}	
F(000)	824	
Crystal size	0.580 x 0.520 x 0.320 mm^3	
Theta range for data collection	5.32 to 72.39°.	
Index ranges	-11<=h<=9, -15<=k<=13, -20<=l<=20	
Reflections collected	17575	
Independent reflections	3930 [R(int) = 0.0282]	
Completeness to theta = 72.39°	98.9 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.80 and 0.66	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3930 / 0 / 258	
Goodness-of-fit on F^2	0.935	
Final R indices [I>2sigma(I)]	R1 = 0.0277, wR2 = 0.0969	
R indices (all data)	R1 = 0.0282, wR2 = 0.0996	
Absolute structure parameter	0.02(4)	
Largest diff. peak and hole	0.245 and -0.171 e.Å^{-3}	