Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Supporting Information

Alstolarines A and B, Two Unusual Monoterpenoid Indole Alkaloids with Acetal Moiety from *Alstonia scholaris*†

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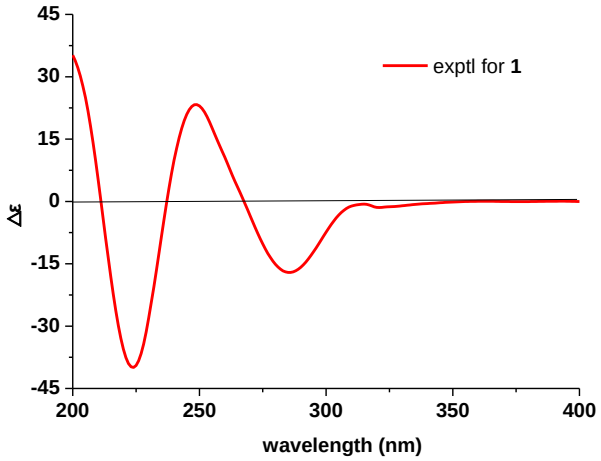


Figure S1. Experimental ECD spectrum of **1**.

X-ray Crystallographic Analysis of 1

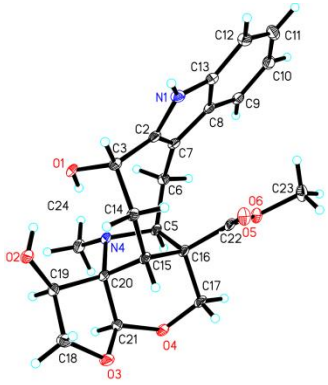


Figure S2. X-ray ORTEP drawing of **1** (thermal ellipsoid is scaled to the 30% probability level).

Compounds **1** were isolated as colorless block crystals via crystallization from CH_3OH. X-ray data were collected on an Agilent Gemini S Ultra CCD diffractometer with Cu Ka radiation (λ = 1.54178 Å). The structure was refined by full-matrix least squares on F^2 using SHELXL-97 package software. Crystallographic data for **1** have been deposited at the Cambridge Crystallographic Data Centre as CCDC 1999251.

Table S1. Crystal data and structure refinement for **1**.

Identification code	**1**
Empirical formula	$C_{22}H_{28}N_2O_7$
Formula weight	432.46
Temperature/K	100.00(10)
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a/Å	8.1650(2)
b/Å	14.1501(2)
c/Å	18.0594(3)
α/°	90
β/°	90
γ/°	90
Volume/Å^3	2086.50(7)
Z	4
ρ_{calc}g/cm^3	1.377
μ/mm^{-1}	0.857
F(000)	920.0
Crystal size/mm^3	0.15 × 0.13 × 0.12
Radiation	CuKα (λ = 1.54178)
2Θ range for data collection/°	7.938 to 147.768
Index ranges	$-9 \le h \le 10$, $-17 \le k \le 17$, $-22 \le l \le 22$
Reflections collected	27233
Independent reflections	4188 [R_{int} = 0.0810, R_{sigma} = 0.0346]
Data/restraints/parameters	4188/0/291
Goodness-of-fit on F^2	1.049
Final R indexes [I>=2σ (I)]	R_1 = 0.0507, wR_2 = 0.1349
Final R indexes [all data]	R_1 = 0.0511, wR_2 = 0.1355
Largest diff. peak/hole / e Å^{-3}	0.45/-0.35
Flack/Hooft parameter	-0.09(9)/-0.03(6)

Chemical calculation details for 2: The conformational analysis of compound **2** was performed in the SYBYL 8.1 program by using MMFF94s molecular force field, which afforded 35 conformers of **2**, with an energy cutoff of 10 kcal mol^{-1} to the global minima. All the obtained conformers were further optimized using DFT at the B3LYP/6-31+G(d) level in acetonitrile by using Gaussian09 software,[1] and 8 conformers of **2** were selected. All of the optimized stable conformers were used for TDDFT computation of the excited stats at the same levels, with the consideration of the first 30 excitations. The overall ECD curves of **2** were weighted by Boltzmann distribution of each conformer (with a half-bandwidth of 0.3 eV). The calculated ECD spectra of **2** were subsequently compared with the experimental one. The ECD spectra were produced by SpecDis 1.6 software.[2]

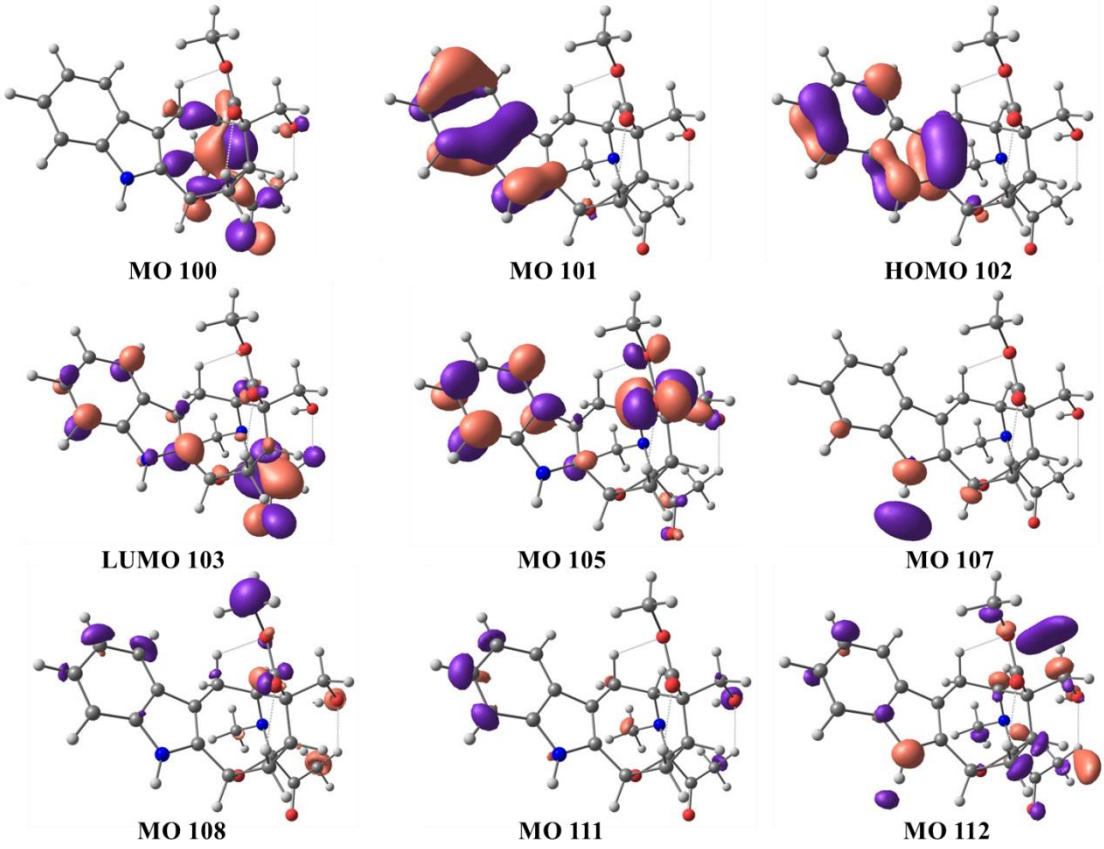


Figure S3. Key molecular orbitals involved in important transitions regarding the ECD spectra of conformer **2** in the gas phase at the B3LYP/6-31+G(d) level.

Table S2. Key transitions and their related rotatory and oscillator strengths of conformer **2** at the B3LYP/6-31+G(d) level in the gas phase.

HOMO is 102					
No.	**Energy (cm^{-1})**	**Wavelength (nm)**	**R (length)**	**Osc. Strength**	**Major contribs**
1	33953.5218	294.520258	6.5796	0.0001	H-3->LUMO (27%), H-2->LUMO (44%), H-2->L+1 (12%)
2	35129.47816	284.6612169	-7.7094	0.0244	HOMO->LUMO (68%), HOMO->L+1 (16%), HOMO->L+2 (12%)
3	35553.72578	281.2644745	6.0481	0.014	HOMO->LUMO (27%), HOMO->L+1 (53%), HOMO->L+2 (16%)
4	36583.69579	273.3458111	19.2242	0.0472	HOMO->L+1 (26%), HOMO->L+2 (61%)
5	37866.92389	264.0827132	-6.4077	0.0762	H-1->LUMO (37%), H-1->L+1 (20%), H-1->L+2 (10%), HOMO->L+6 (20%)
6	38967.06413	256.6269803	1.9254	0.0016	HOMO->L+3 (75%), HOMO->L+4 (21%)
7	39408.2494	253.7539767	-8.3238	0.0019	H-3->LUMO (22%), H-2->LUMO (22%), H-1->LUMO (22%), H-1->L+1 (21%)
8	39855.88711	250.9039624	12.5133	0.0119	H-1->L+1 (18%), H-1->L+2 (29%), HOMO->L+3 (11%), HOMO->L+4 (37%)
9	39951.06053	250.3062464	-6.2047	0.0024	H-1->L+1 (12%), H-1->L+2 (32%), HOMO->L+4 (38%)
10	40357.56396	247.7850251	0.938	0.0008	H-3->LUMO (16%), H-2->LUMO (22%), H-1->LUMO (28%), H-1->L+1 (11%), H-1->L+2 (11%)
11	40856.82116	244.7571719	-1.2242	0.0016	H-2->L+1 (71%), H-2->L+2 (11%)
12	42048.10205	237.8228627	9.2067	0.0044	HOMO->L+5 (87%)
13	42529.61504	235.1302731	-1.0356	0.0035	H-2->L+2 (73%)
14	42617.52947	234.6452299	-1.7947	0.0018	H-1->L+3 (44%), H-1->L+4 (37%)

15	43618.46352	229.2607119	-12.7239	0.0125	H-1->L+3 (34%), H-1->L+4 (46%)
16	43908.82311	227.7446602	0.0646	0.0052	HOMO->L+6 (18%), HOMO->L+7 (70%)
17	43999.96377	227.2729144	4.8354	0.0037	H-2->L+3 (74%)
18	44656.49907	223.9315712	-14.7123	0.004	H-7->L+1 (13%), H-7->L+2 (15%), H-6->L+1 (14%), H-6->L+2 (15%), H-3->L+1 (12%)
19	44689.5678	223.7658696	-14.9504	0.0992	HOMO->L+6 (16%), HOMO->L+8 (58%)
20	44961.37664	222.4131187	-13.9725	0.2621	HOMO->L+6 (18%), HOMO->L+8 (32%)
21	45455.79451	219.9939547	15.8374	0.0192	H-1->L+5 (70%)
22	45667.11177	218.9759679	8.8397	0.0905	HOMO->L+9 (74%)
23	46074.42176	217.0401628	5.9131	0.0059	H-3->LUMO (16%), H-3->L+1 (47%)
24	46513.99392	214.9890637	3.1236	0.0905	HOMO->L+10 (68%)
25	46868.07131	213.3648712	5.454	0.0015	H-5->LUMO (51%), H-5->L+1 (12%), H-4->LUMO (14%)
26	47118.90974	212.2290192	-6.6826	0.0099	HOMO->L+10 (14%), HOMO->L+11 (76%)
27	47170.52923	211.9967735	9.0806	0.0116	H-2->L+4 (78%)
28	47484.2789	210.5960169	-4.5488	0.0079	H-3->L+2 (40%), H-2->L+4 (11%), H-1->L+6 (18%)
29	47523.80007	210.4208836	0.5218	0.0009	H-3->L+2 (29%), H-1->L+6 (16%), H-1->L+7 (32%)
30	48074.67674	208.009719	19.1143	0.0122	H-2->L+5 (49%), H-1->L+8 (17%)

Table S3. Cartesian coordinates of conformer **2**

Standard orientation:					
Imaginary frequencies	0				
E (Hartree)	-1300.31303				
Center Number	**Atomic Number**	**Atomic Type**	**Coordinates (Angstroms)**		
			X	**Y**	**Z**
1	6	0	5.085532	0.017661	1.182961
2	6	0	5.552311	-0.88389	0.190349
3	6	0	4.677782	-1.56965	-0.63284
4	6	0	3.29981	-1.33442	-0.44359
5	6	0	2.808236	-0.42684	0.549878
6	6	0	3.732933	0.248657	1.368188
7	7	0	2.189175	-1.86988	-1.10521
8	6	0	1.007296	-1.30727	-0.54738
9	6	0	1.355207	-0.42394	0.463683
10	6	0	-0.29592	-1.72888	-1.15647
11	7	0	-1.91893	-0.29905	1.382221
12	6	0	-0.89111	0.778069	1.184316
13	6	0	0.581454	0.406299	1.422517
14	6	0	-0.93293	-0.61221	-2.00069
15	6	0	-1.83798	0.145535	-1.02472
16	6	0	-2.16242	-0.93074	0.067991
17	6	0	-3.58568	-1.53921	-0.08141
18	6	0	-4.69886	-0.82871	0.616939
19	6	0	-1.6315	-1.27369	2.468503
20	8	0	-1.25744	-2.04145	-0.12058
21	6	0	-1.22123	1.341603	-0.24184
22	8	0	-3.7566	-2.50441	-0.78271
23	6	0	-0.01385	1.997673	-0.9002
24	8	0	0.425223	1.897929	-2.01932
25	8	0	0.527687	2.90584	-0.00777
26	6	0	1.717217	3.617617	-0.44689
27	6	0	-2.27318	2.489481	-0.1317
28	8	0	-3.55122	2.019949	0.284017
29	1	0	5.816855	0.528457	1.805419
30	1	0	6.626622	-1.0316	0.084653
31	1	0	5.027165	-2.2604	-1.39221
32	1	0	3.381391	0.936654	2.133134
33	1	0	2.216123	-2.5299	-1.85776
34	1	0	-0.2074	-2.6949	-1.7051
35	1	0	-1.13478	1.564889	1.952656
36	1	0	0.659471	-0.08746	2.420913
37	1	0	1.131658	1.375683	1.545051
38	1	0	-1.53939	-1.04681	-2.81886
39	1	0	-0.17863	0.027491	-2.50218
40	1	0	-2.7624	0.489392	-1.54309

41	1	0	-4.82073	0.198073	0.22549
42	1	0	-5.65706	-1.34938	0.476596
43	1	0	-4.51932	-0.7386	1.697558
44	1	0	-2.51634	-1.91719	2.608443
45	1	0	-1.44736	-0.73609	3.409704
46	1	0	-0.7738	-1.93714	2.25081
47	1	0	1.823469	4.400289	0.310419
48	1	0	2.562083	2.91983	-0.4423
49	1	0	1.563161	4.032166	-1.4489
50	1	0	-2.48503	2.943812	-1.12031
51	1	0	-1.92481	3.272388	0.569755
52	1	0	-3.47258	1.501071	1.12622

[1] Gaussian 09, Revision A.02, 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, 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, Inc., Wallingford CT, 2009.

[2] T. Bruhn, A. Schaumlöffel, Y. Hemberger, G. Bringmann, SpecDis version 1.60, University of Wuerzburg, Germany, 2012.

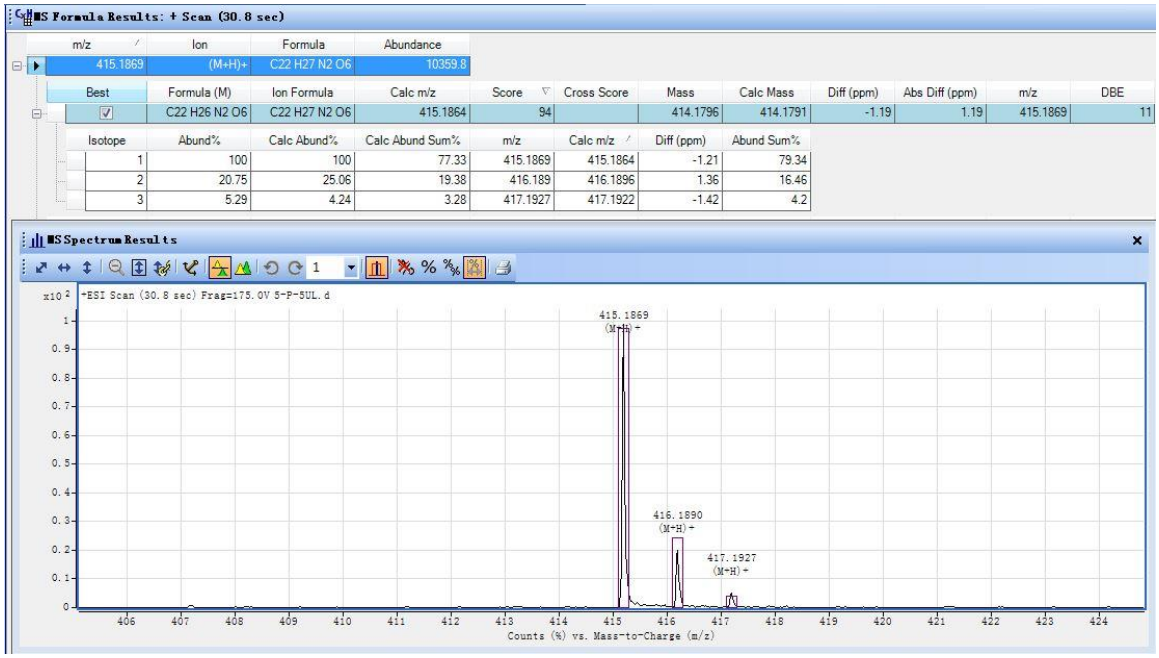
Bioactivity assays

Vasorelaxant assay

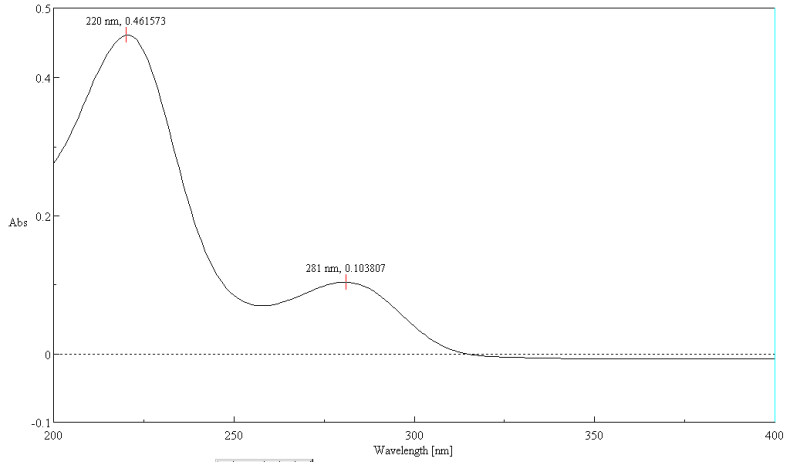
The vasorelaxant activity of the isolates against KCl-induced contractions of rat renal artery rings was measured as described previously. Renal arteries was removed rapidly out from SD rats, immediately placed into 4 ℃ oxygenated K-H solution, cleaned of its surrounding fat and connective tissues, and then cut into about 2 mm in length. Each segment was mounted in a Multi Myograph System (Danish Myo Technology A/S, Denmark), and bathed in K-H solution [composition (in mmol·L^{-1}): NaCl, 120; KCl, 4.6; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25; glucose, 10; $CaCl_2$, 2.5], bubbled with 95% O_2−5% CO_2 and maintained at 37 ℃. The isometric tension of renal artery rings was collected by four-channels psychological force transducers. All the rings were set to an optimal tension of 2 g and stabilized in normal K-H solution for 90 min. The rings were then contracted by 0.5 μmol·L^{-1} phenylephrine and challenged with 3 μmol·L^{-1} acetylcholine to confirm the integrity of the endothelium. Endothelium-intact rings contraction was evoked by a depolarizing KCl (60 m mol·L^{-1}) solution. The EC_{50} values of the test compounds and the positive control (phentolamine mesylate) were calculated from cumulative concentration-tension curves by linear regression.

Acetylcholinesterase (AChE) inhibitory activity assay

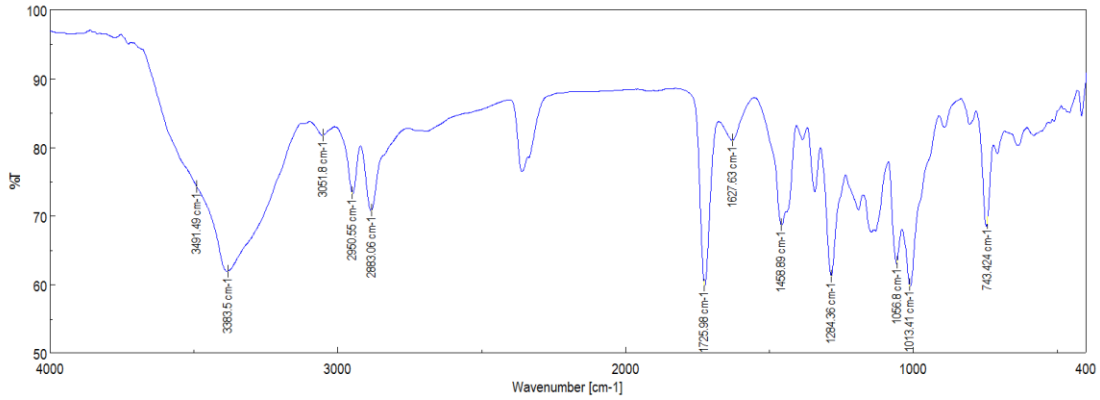
The AChE inhibitory activities of the compounds were assayed by a modified Ellman's method. Compounds and positive control were dissolved in 1‰ DMSO. The phosphate buffer (PH 8.0), tacrine, test compounds, and acetylcholinesterase (0.02 U mL^{-1}) were added in sequence to 96-well plates and incubated for 20 min (30 ℃). The reaction was initiated by the addition of 20 μL of 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) (0.625 mM) and 20 μL of acetylthiocholine iodide (0.625 mM) for the AChE inhibitory activity assay respectively. The optical density was measured at 405 nm by an ELISA microplate reader. Tacrine (IC_{50} 0.33 μM) was used as positive control. All the reactions were performed in triplicate. The percentage inhibition (I %) was calculated as follows: I % = (1−S)/E × 100 (S is the absorbance of the test compound-containing reaction, and E is the absorbance of the control reaction).



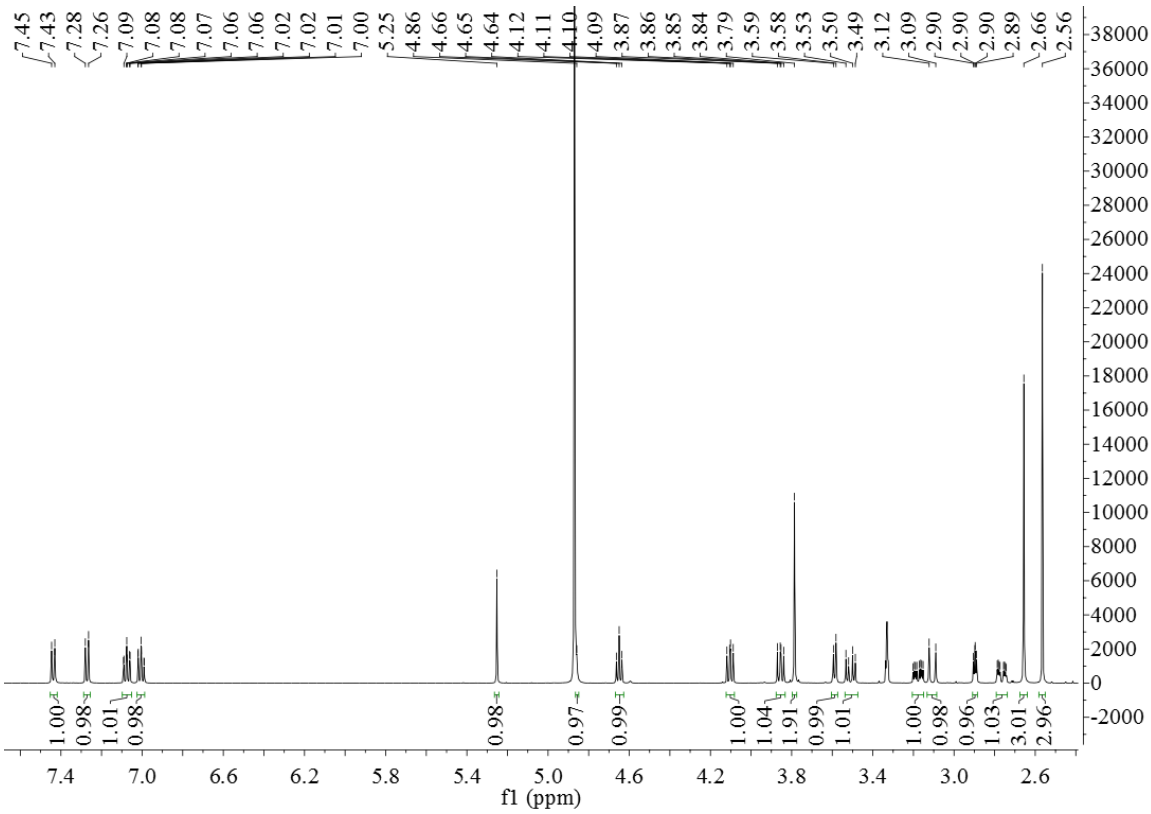
HR-ESI-MS spectrum of **1**



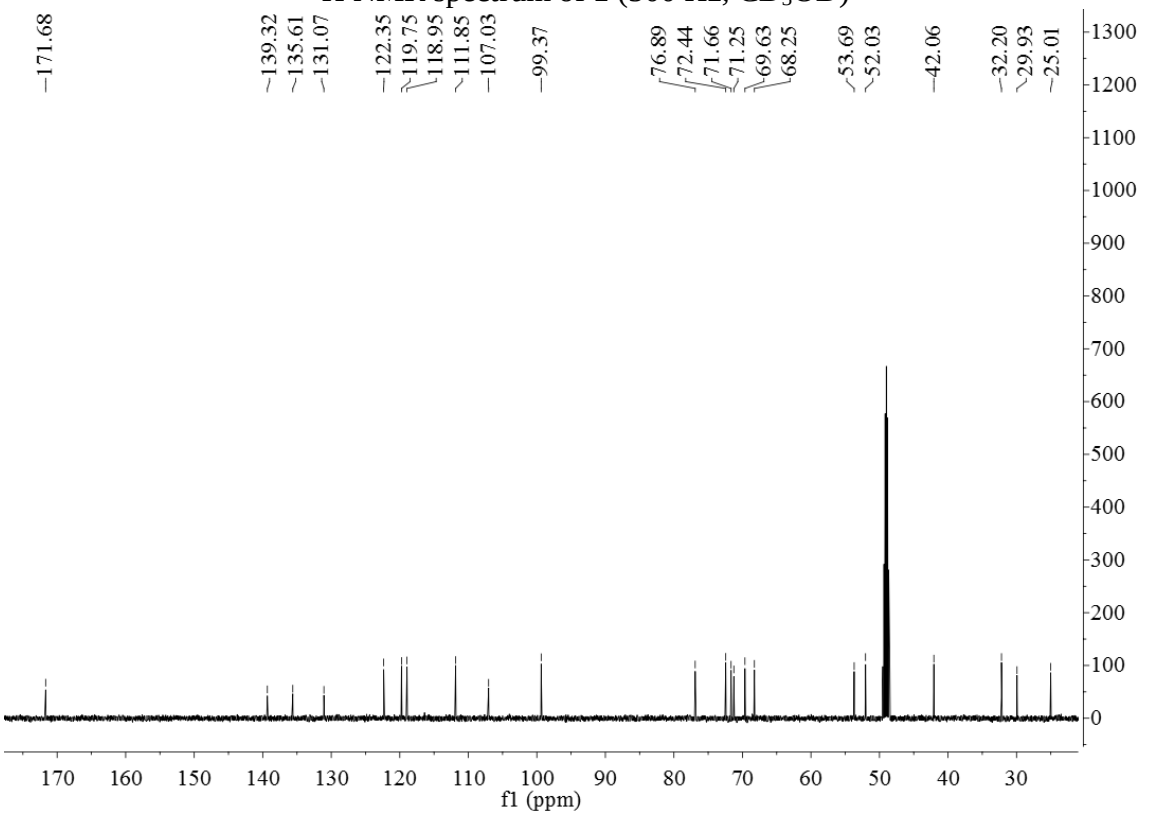
UV spectrum of **1** (CH_3OH)



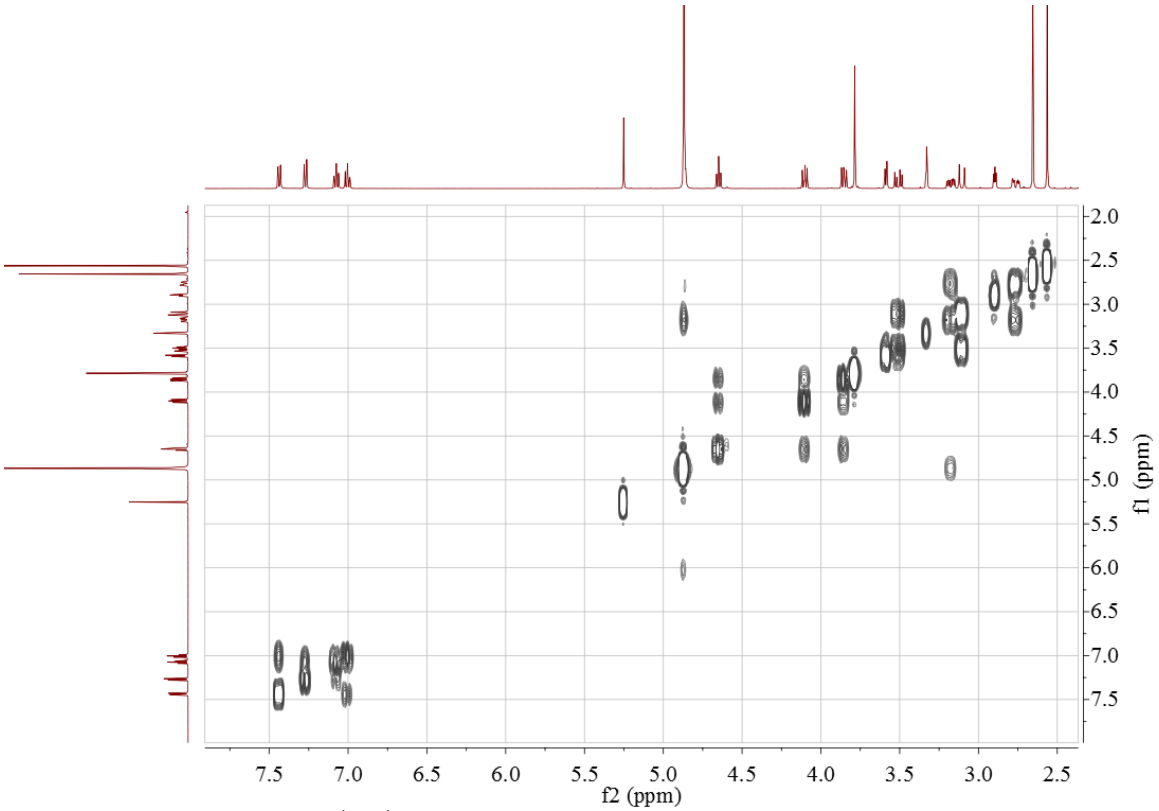
IR spectrum of **1** (KBr disc)



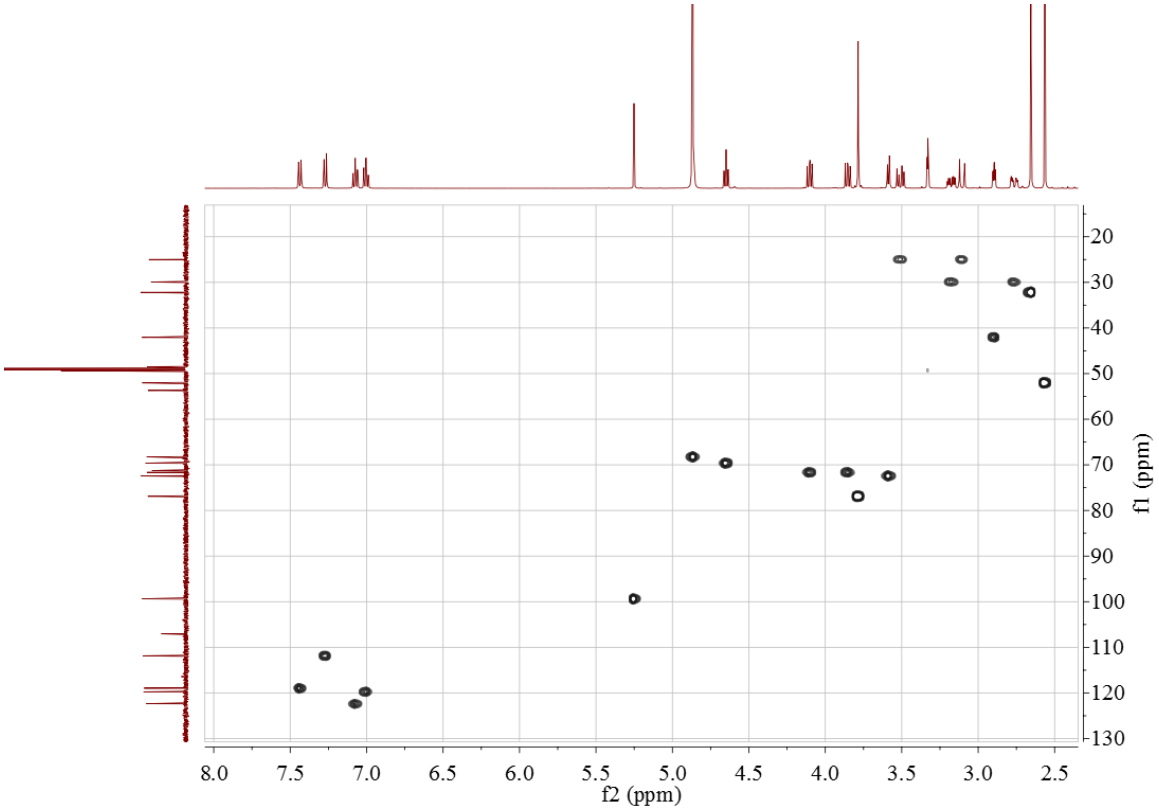
^{1}H NMR spectrum of **1** (500 Hz, CD_3OD)



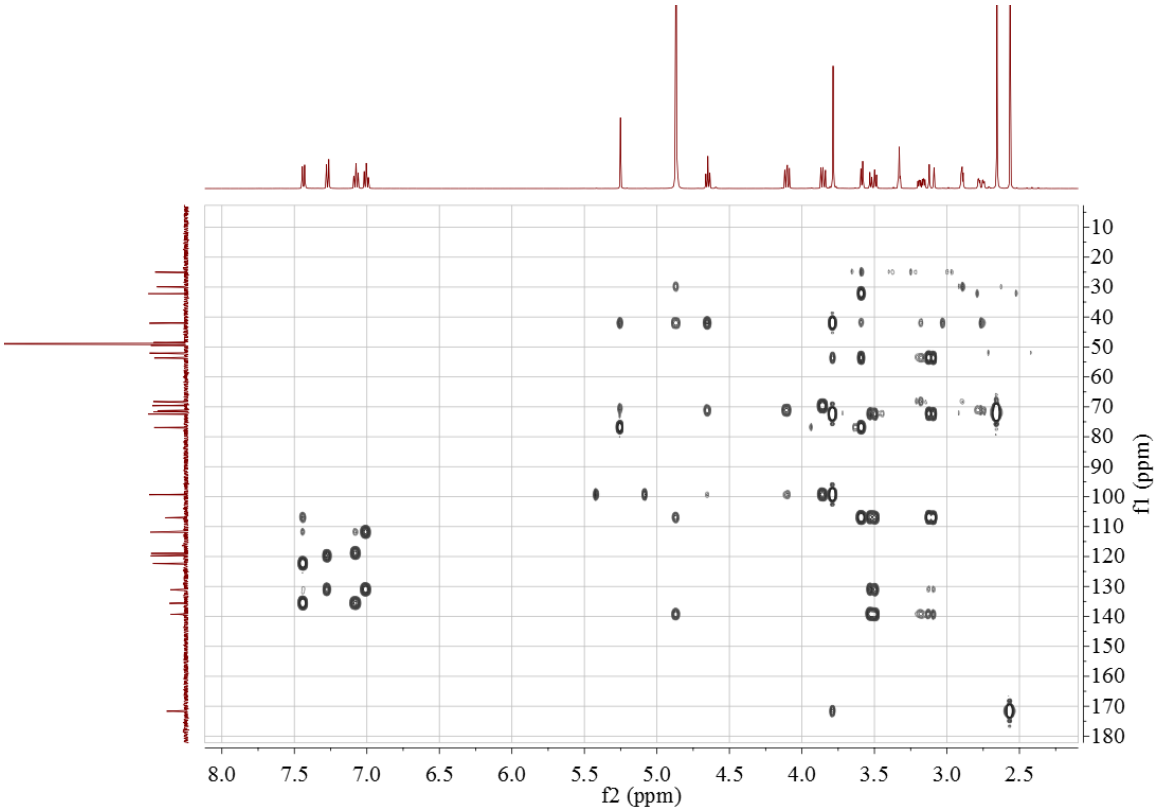
^{13}C NMR spectrum of **1** (125 Hz, CD_3OD)



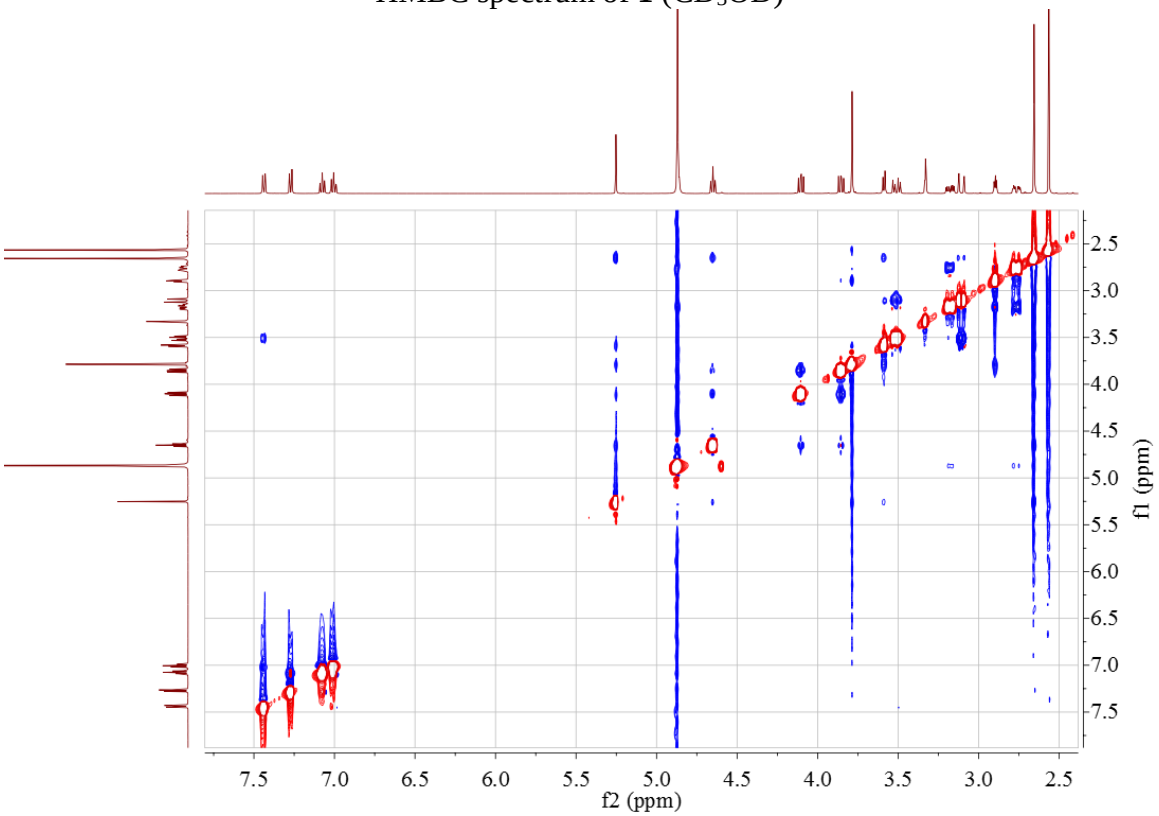
^{1}H-^{1}H COSY spectrum of **1** (CD_3OD)



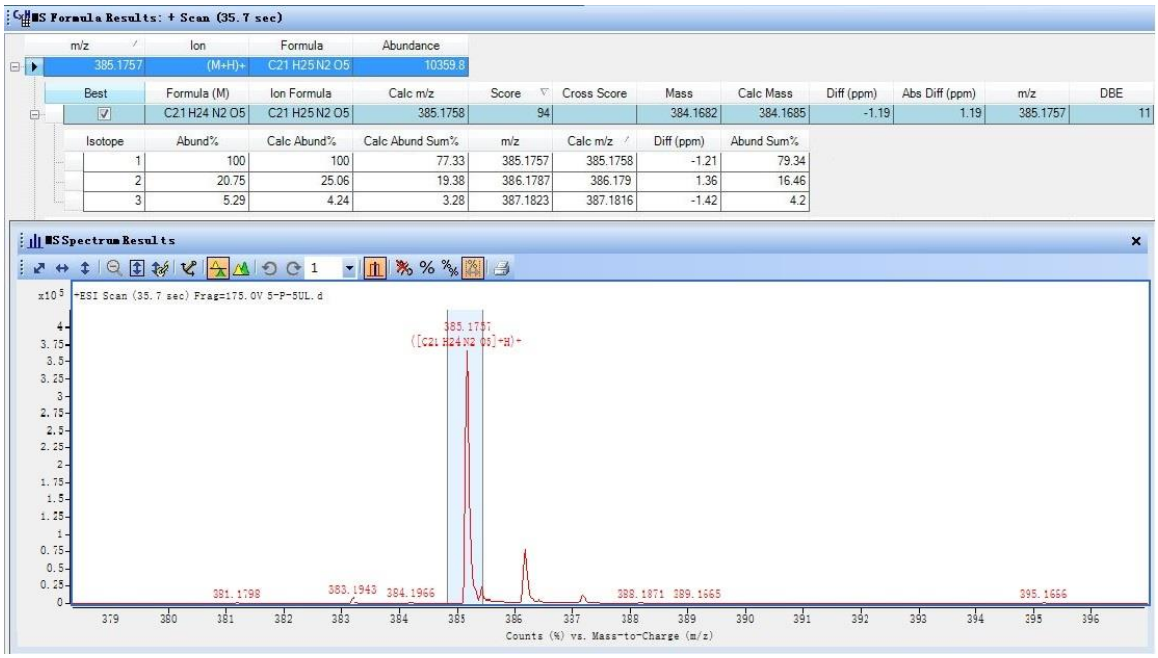
HSQC spectrum of **1** (CD_3OD)



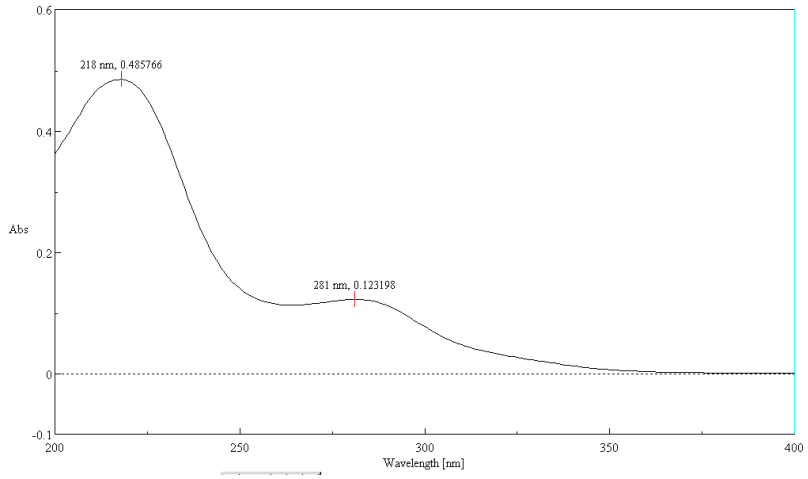
HMBC spectrum of **1** (CD_3OD)



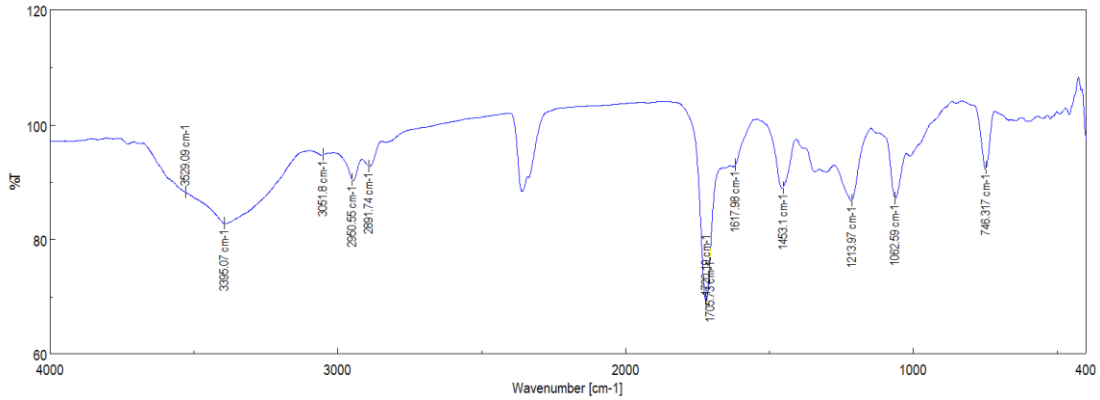
NOESY spectrum of **1** (CD_3OD)



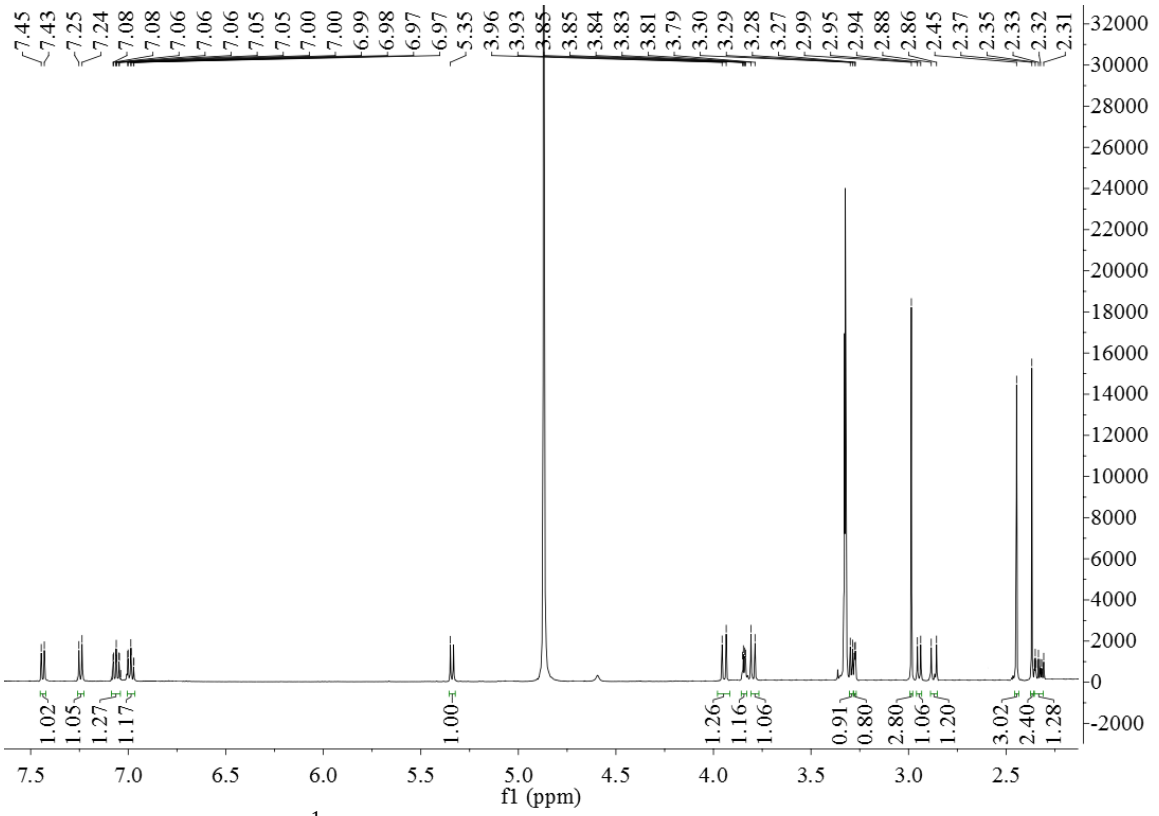
HR-ESI-MS spectrum of **2**



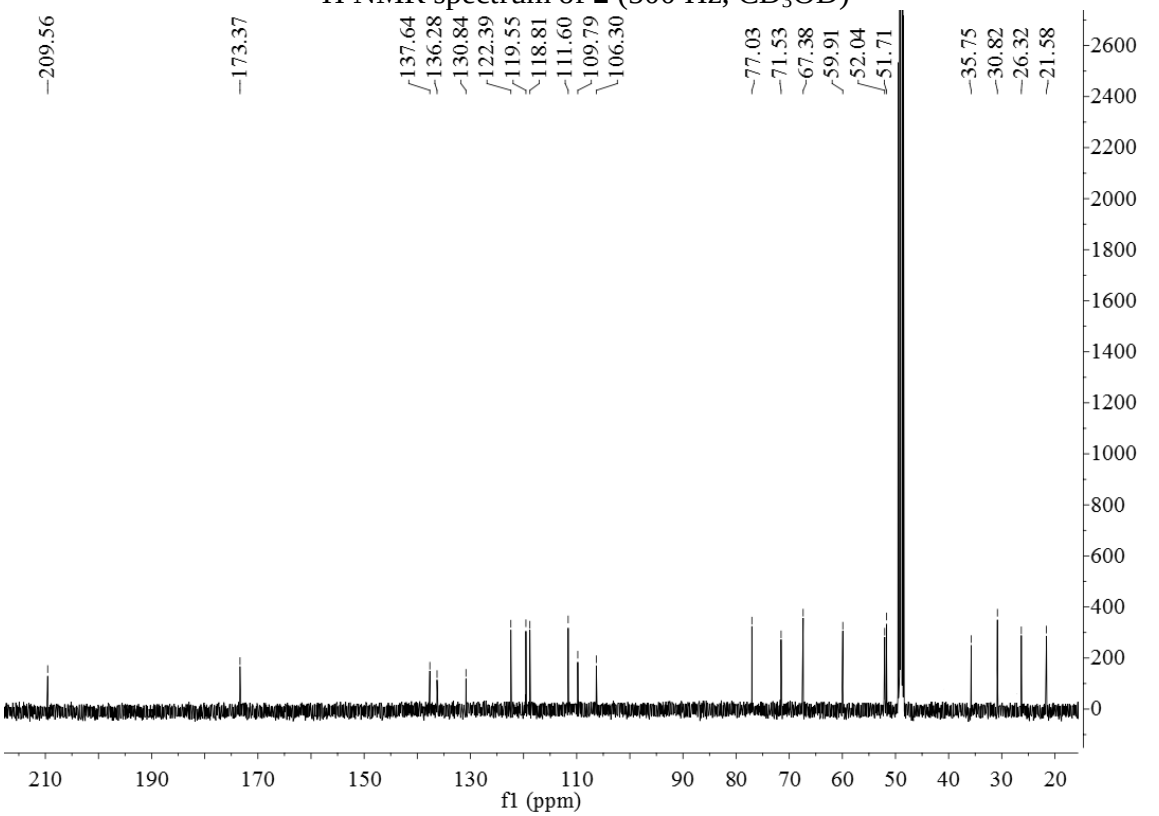
UV spectrum of **2** (CH_3OH)



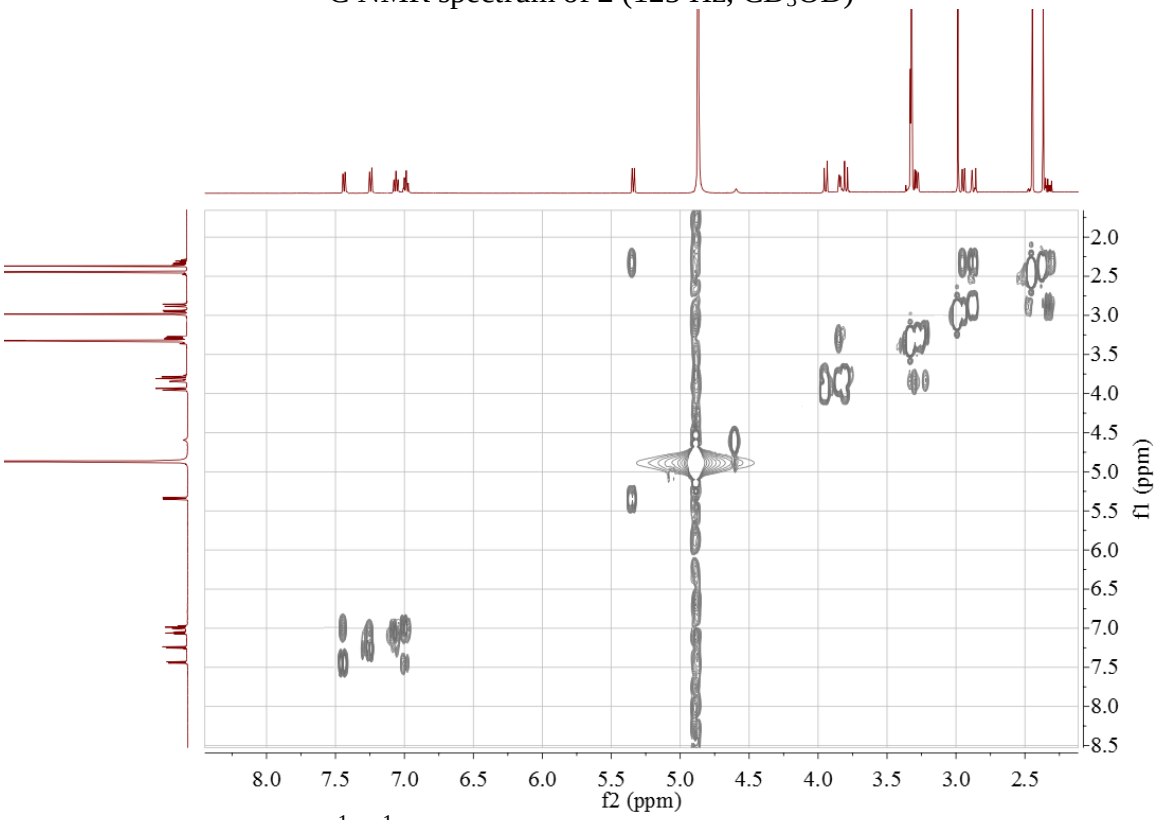
IR spectrum of **2** (KBr disc)



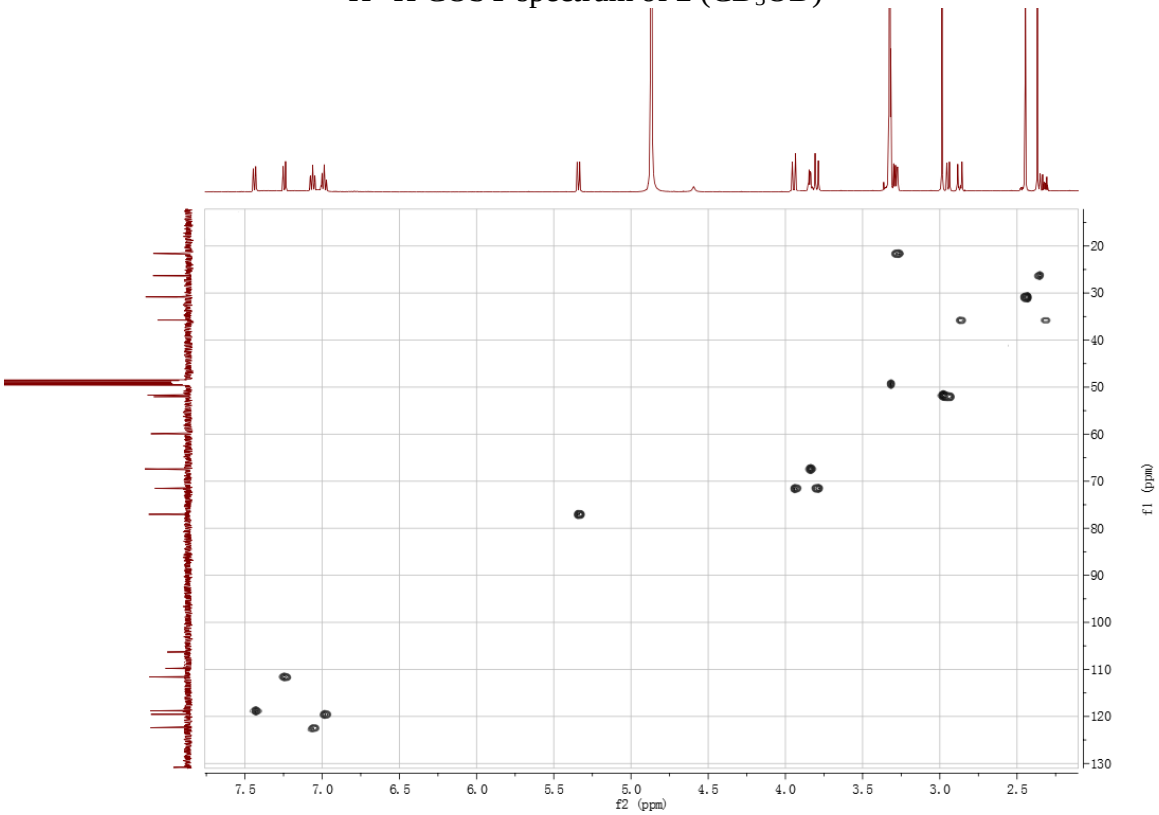
^{1}H NMR spectrum of **2** (500 Hz, CD_3OD)



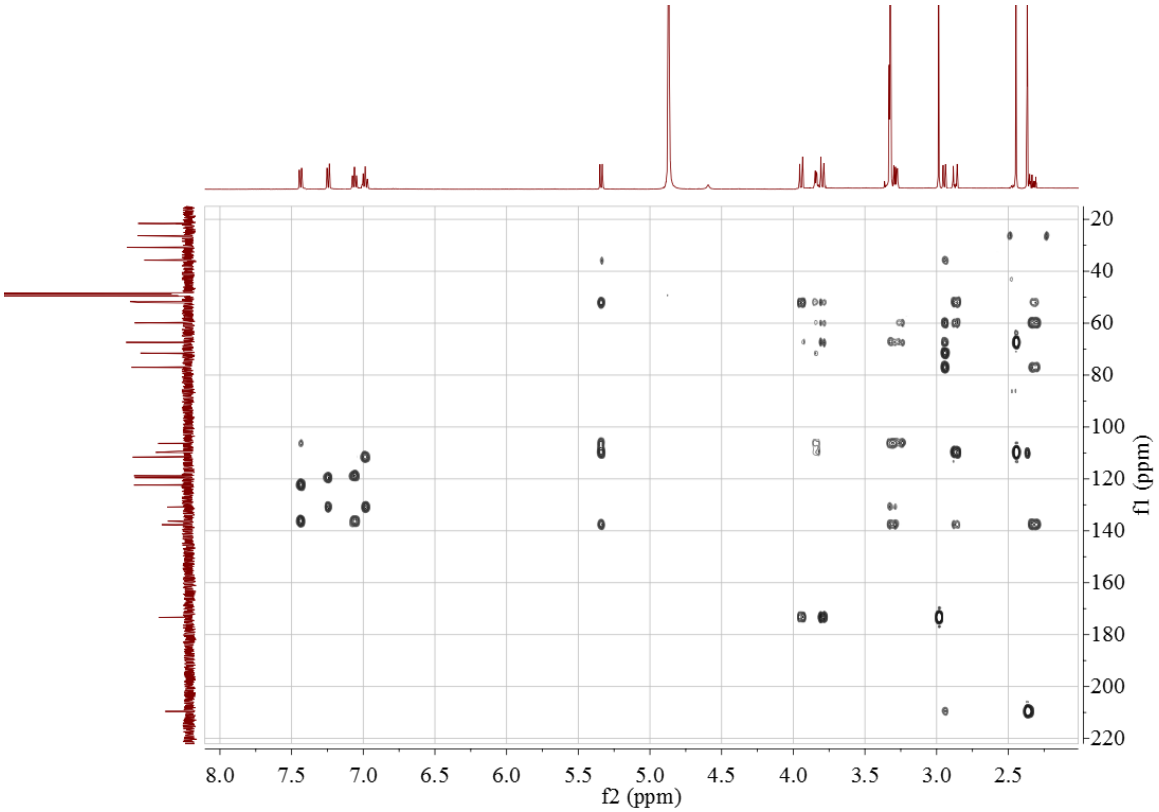
^{13}C NMR spectrum of **2** (125 Hz, CD_3OD)



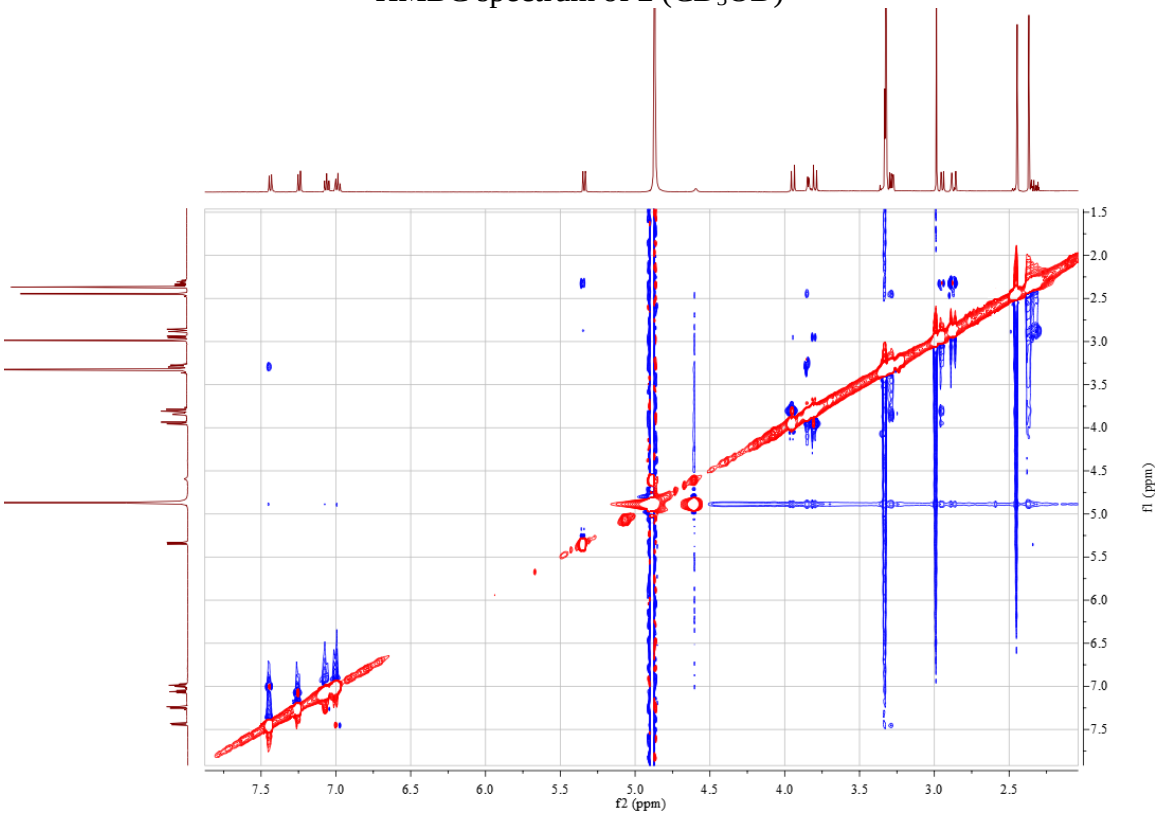
^{1}H-^{1}H COSY spectrum of **2** (CD_3OD)



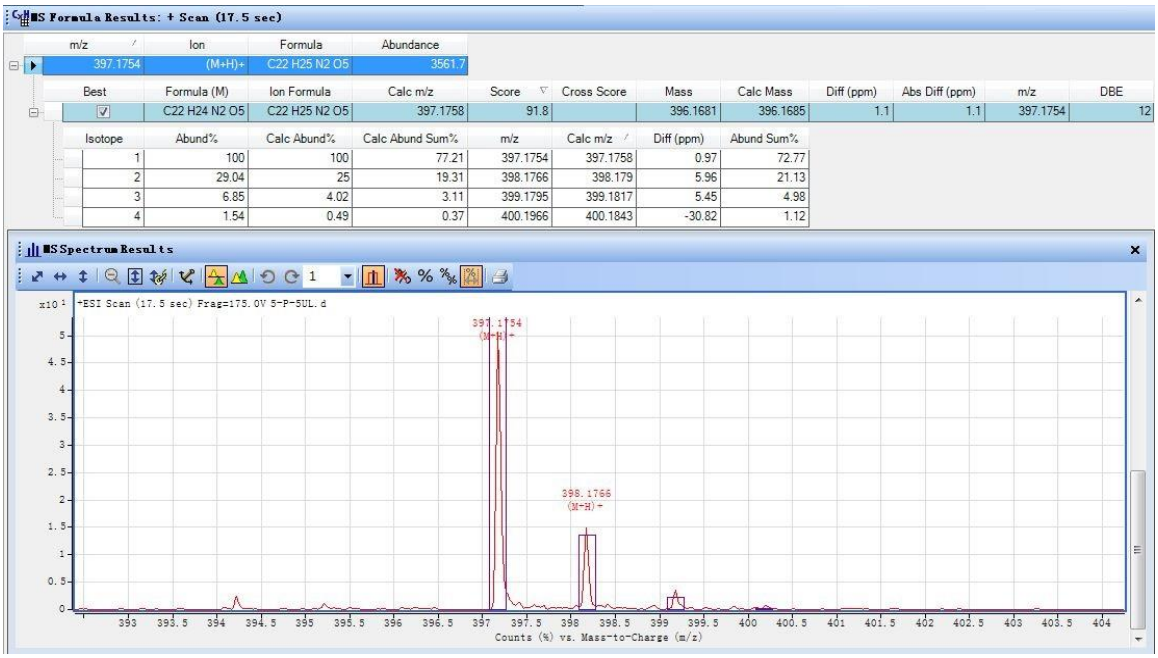
HSQC spectrum of **2** (CD_3OD)



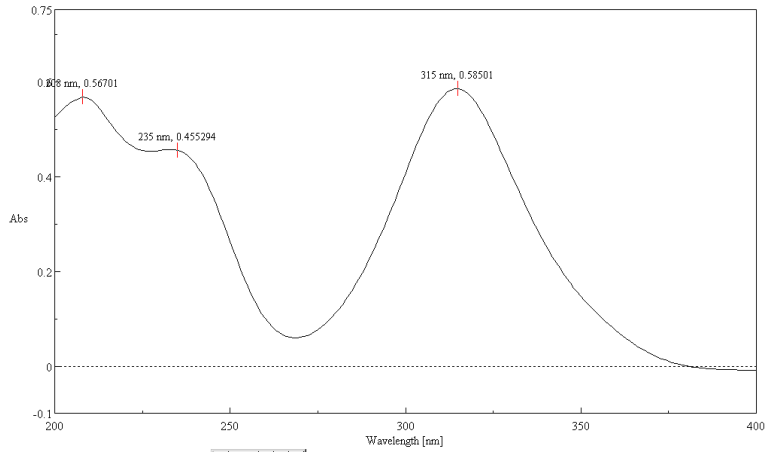
HMBC spectrum of **2** (CD_3OD)



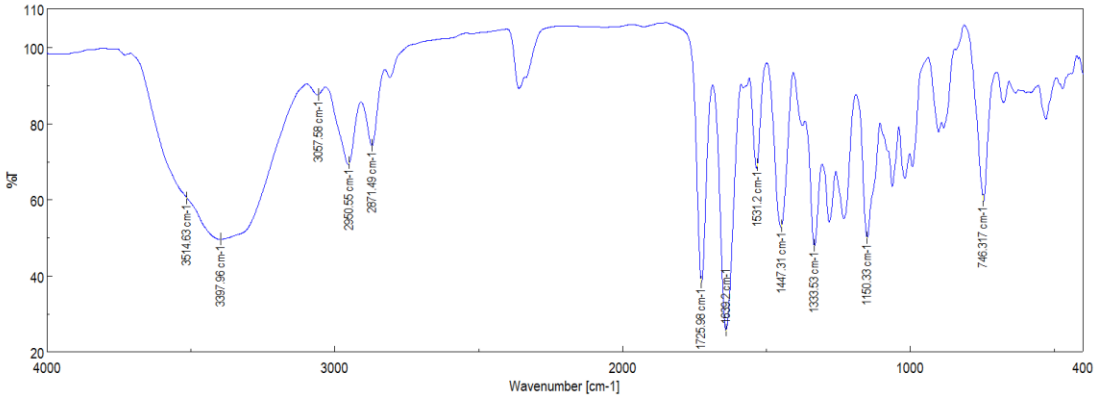
NOESY spectrum of **2** (CD_3OD)



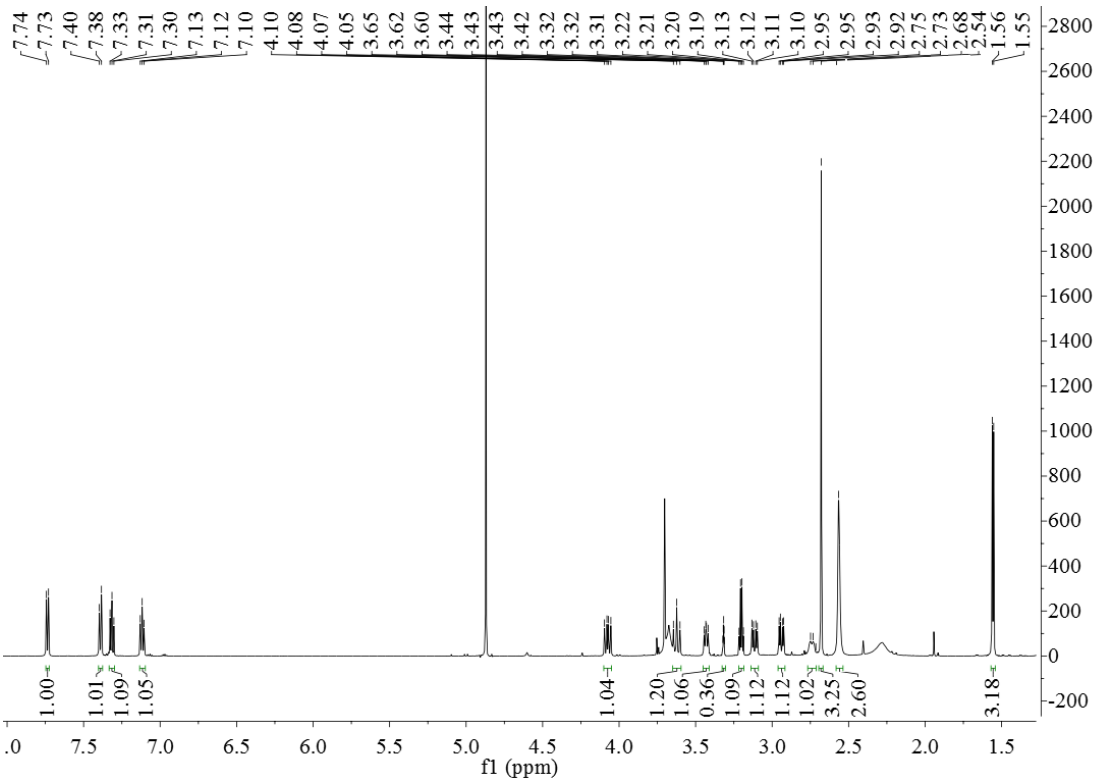
HR-ESI-MS spectrum of **3**



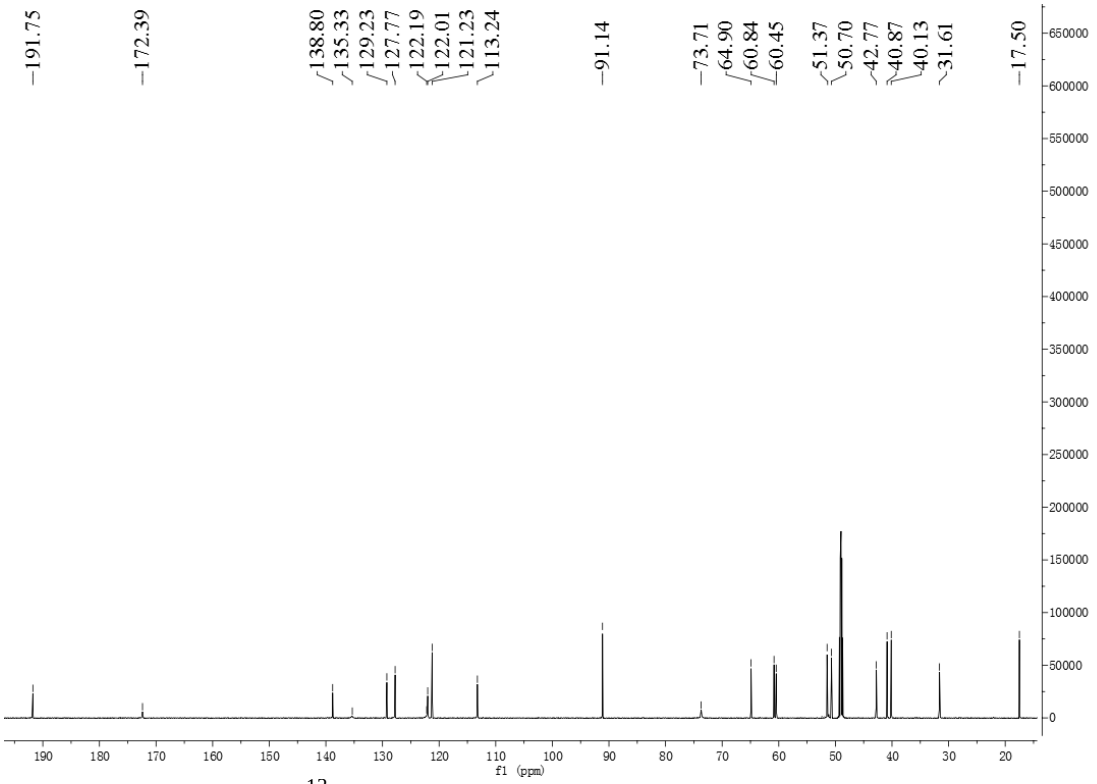
UV spectrum of **3** (CH_3OH)



IR spectrum of **3** (KBr disc)



^{1}H NMR spectrum of **3** (600 Hz, CD_3OD)



^{13}C NMR spectrum of **3** (150 Hz, CD_3OD)