

# Supporting Information

## Aspeverin, a New Alkaloid from an Algicolous Strain of *Aspergillus versicolor*

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### List of Supporting Information

S2, Experimental Section;

S4,  $^1\text{H}$  NMR spectrum of compound **1** in  $\text{DMSO-}d_6$ ;

S5,  $^{13}\text{C}$  NMR and DEPT spectra of compound **1** in  $\text{DMSO-}d_6$ ;

S6, HSQC spectrum of compound **1** in  $\text{DMSO-}d_6$ ;

S7, HMBC spectrum of compound **1** in  $\text{DMSO-}d_6$ ;

S8,  $^1\text{H-}^1\text{H}$  COSY spectrum of compound **1** in  $\text{DMSO-}d_6$ ;

S9, NOESY spectrum of compound **1** in  $\text{DMSO-}d_6$ ;

S10,  $^1\text{H}$  NMR spectrum of compound **1** in  $\text{CDCl}_3$ ;

S11,  $^{13}\text{C}$  NMR and DEPT spectra of compound **1** in  $\text{CDCl}_3$ ;

S12, HSQC spectrum of compound **1** in  $\text{CDCl}_3$ ;

S13, HMBC spectrum of compound **1** in  $\text{CDCl}_3$ ;

S14,  $^1\text{H-}^1\text{H}$  COSY spectrum of compound **1** in  $\text{CDCl}_3$ ;

S15, NOESY spectrum of compound **1** in  $\text{CDCl}_3$ ;

S16, Positive ESIMS spectrum of compound **1**;

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S18, EIMS spectrum of compound **1**;

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S20, IR spectrum of compound **1**;

S21, Cartesian coordinates of the energy-minimized conformer of compound **1** optimized at the gas-phase B3LYP/6-31G(d) level;

S22, Correlation between experimental and calculated  $^{13}\text{C}$  NMR data for the C-6 epimer of compound **1**.

## EXPERIMENTAL SECTION

**General Experimental Procedures.** The NMR spectra were recorded at 500 and 125 MHz for  $^1\text{H}$  and  $^{13}\text{C}$ , respectively, on a Bruker Avance III 500 NMR spectrometer using TMS as an internal standard. The low resolution  $\text{ESI}^+$  and  $\text{ESI}^-$  mass spectra were measured on an LCQ Fleet mass spectrometer. The low and high resolution EI mass spectra were determined on an Autospec Premier P776 mass spectrometer. The IR spectrum was obtained on a JASCO FT/IR-4100 spectrometer. The UV spectrum was measured on a TU-1810 spectrophotometer. The optical rotation was determined on a JASCO P-1020 polarimeter. The ECD spectrum was recorded on a Chirascan CD spectrometer. Quantum chemical calculations were operated using Gaussian 09 software (IA32W-G09RevC.01). Column chromatography was performed with silica gel (200-300 mesh, Qingdao Haiyang Chemical Co., Qingdao, China) and Sephadex LH-20 (Pharmacia). TLC was carried out with precoated silica gel plates (GF-254, Qingdao Haiyang Chemical Co., Qingdao, China). All solvents were of analytical grade.

**Fungal Material and Fermentation.** The fungal strain *A. versicolor* dl-29 was isolated from the fresh tissue of surface-sterilized marine green alga *Codium fragile* collected from the coast of Dalian, China in September, 2010. The fungus was identified by morphological observation and analysis of the ITS regions of its rDNA, whose sequence data have been deposited at GenBank with the accession number JX401544. The strain was preserved at the Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences and China Center for Type Culture Collection (No. CCTCC M 2011421).

The initial cultures were maintained on the potato dextrose agar (PDA) plates. Pieces of mycelia were cut into small segments and aseptically inoculated into 50 Erlenmeyer flasks (1 L), each containing 300 mL potato dextrose broth (PDB) culture media. Static fermentations were performed at room temperature for 30 days.

**Extraction and Isolation.** The whole cultures (300mL  $\times$  50 flasks, 30 days) were filtered through cheesecloth to separate mycelia from broth. The broth was extracted with EtOAc to give an evaporated extract (9.2 g). The dried mycelia were homogenized and extracted with a mixture of  $\text{CHCl}_3$  and MeOH (1:1, v/v), and then the evaporated extract was partitioned between EtOAc and  $\text{H}_2\text{O}$  to yield an EtOAc-soluble extract (20.2 g). These two parts were combined for further separation based on their identical TLC profiles. The total EtOAc-soluble fraction (29.4 g) was subjected to step-gradient silica gel column chromatography (CC) with a solvent system consisting of 0–100% petroleum ether (PE)–EtOAc to afford 14 fractions (Frs. 1–14) based on TLC analysis. Fr.14 eluted with EtOAc and was further purified by CC on silica gel (PE/EtOAc, 2:1) and Sephadex LH-20 ( $\text{CHCl}_3$ /MeOH, 1:1) and preparative TLC ( $\text{CHCl}_3$ /EtOAc, 1:1) to yield compound **1** (8.6 mg).

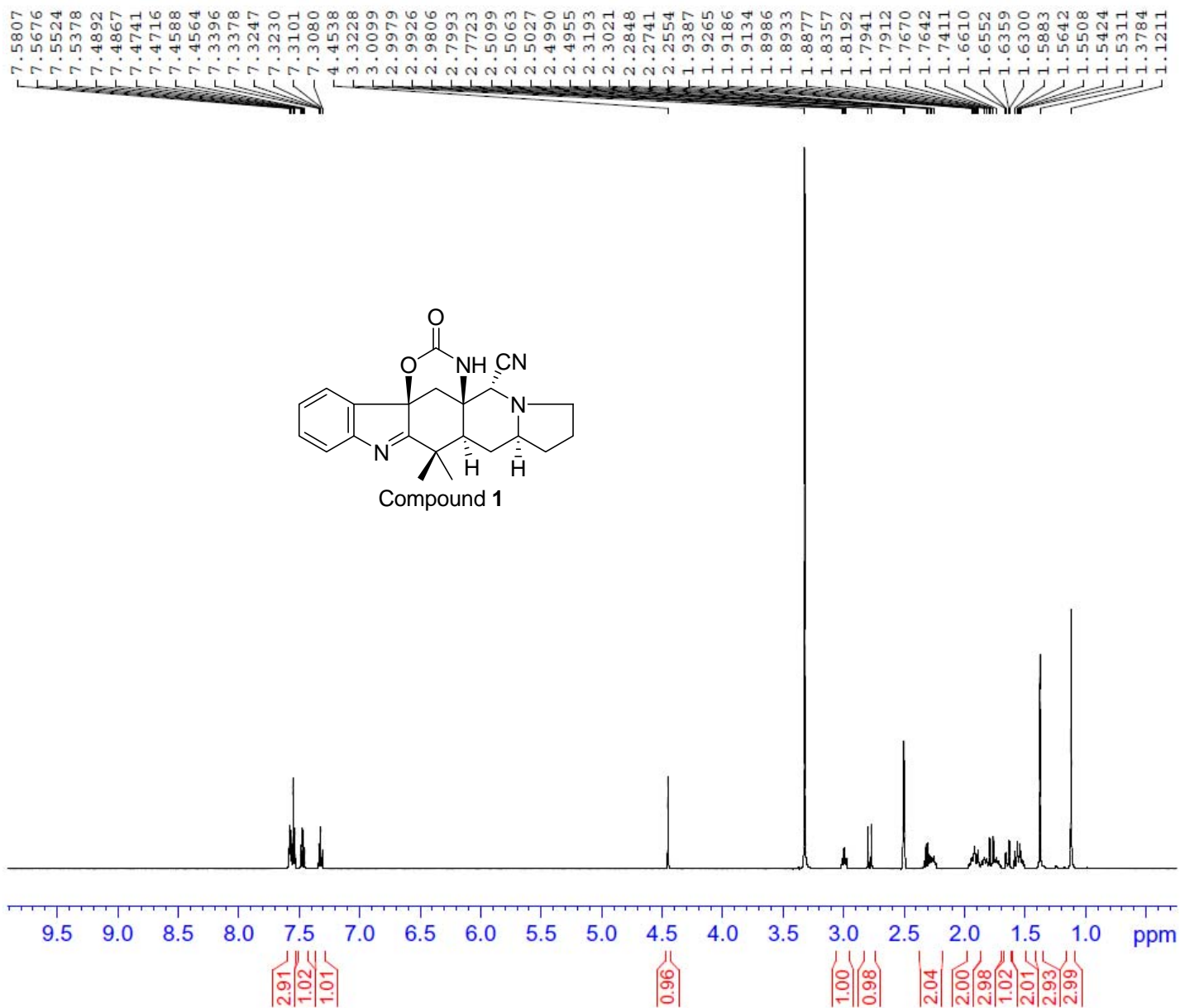
*Aspeverin (1)*: colorless gum;  $[\alpha]_D^{23} +17.8$  ( $c$  0.18, MeOH); UV (MeOH)  $\lambda_{\max}$  ( $\log \epsilon$ ) 220 (4.53), 280 (3.56) nm; ECD (MeOH)  $\lambda_{\max}$  ( $\Delta\epsilon$ ) 221 (-17.0), 256 (8.4), 278 (-1.2), 300 (2.5) nm; IR (KBr)  $\nu_{\max}$  3236, 2970, 2939, 2873, 2233, 1712, 1581, 1369, 1088, 756  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Table 1; ESI<sup>+</sup>MS  $m/z$  377  $[\text{M} + \text{H}]^+$ ; ESI<sup>-</sup>MS  $m/z$  375  $[\text{M} - \text{H}]^-$ ; EIMS  $m/z$  (%) 376 (100), 332 (34), 317 (24), 290 (20), 248 (50), 209 (38), 195 (30), 170 (25), 109 (48), 83 (98); HREIMS  $m/z$  376.1857  $[\text{M}]^+$  (calcd for  $\text{C}_{22}\text{H}_{24}\text{N}_4\text{O}_2$ , 376.1899).

**Computational Details.** A conformational search for **1** was performed via the Dreiding force field in MarvinSketch<sup>12</sup> (optimization limit = normal, diversity limit = 0.1) to give eight conformers, the geometries of which were further optimized at the gas-phase B3LYP/6-31G(d) level via Gaussian 09 software<sup>13</sup> to afford just one conformer within 3 kcal/mol energy threshold from the global minimum without vibrational imaginary frequencies. Then, this predominant conformer was subjected to the theoretical calculations of  $^{13}\text{C}$  NMR (GIAO method) and ECD (TD-DFT method) spectra at the gas-phase B3LYP/6-31G(d) level. The calculated ECD spectrum was drawn via SpecDic software<sup>17</sup> with sigma = 0.2 and UV shift = 0 nm.

**Bioassays and Results.** The inhibitory activities against marine zooplankton (*Artemia salina*) and phytoplankton (*Heterosigma akashiwo*) as well as four bacteria (*Vibrio ichthyenteri*, *Proteus mirabilis*, *Enterobacter cloacae*, and *Bacillus cereus*, at 30  $\mu\text{g}/\text{disk}$ ) isolated from seawater were assayed as described previously,<sup>18,19</sup> with positive controls of  $\text{K}_2\text{Cr}_2\text{O}_7$  and chloramphenicol, respectively. The results were shown in Table S1.

**Table S1.** Bioassay Results of Compound **1**

	<b>1</b>	$\text{K}_2\text{Cr}_2\text{O}_7$	chloramphenicol
<i>A. salina</i> (lethal rate at 100 $\mu\text{g}/\text{mL}$ ; 24h)	27.5%	100%	
<i>H. akashiwo</i> ( $\text{EC}_{50}$ , $\mu\text{g}/\text{mL}$ ; 24h)	6.3	13.5	
<i>H. akashiwo</i> ( $\text{EC}_{50}$ , $\mu\text{g}/\text{mL}$ ; 96h)	3.4	5.1	
<i>V. ichthyenteri</i> (inhibitory diameter, mm; 24h)	0		28
<i>P. mirabilis</i> (inhibitory diameter, mm; 24h)	7.0		26
<i>E. cloacae</i> (inhibitory diameter, mm; 24h)	6.5		22
<i>B. cereus</i> (inhibitory diameter, mm; 24h)	6.0		20



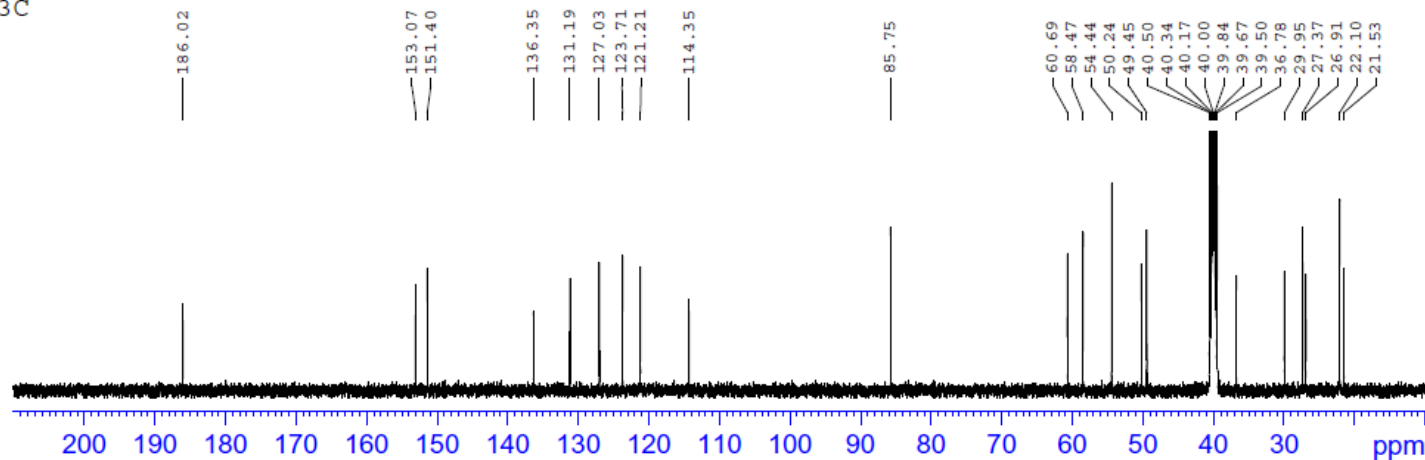
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FIDRES        0.157632 Hz
AQ            3.1719923 sec
RG            203
DW            48.400 usec
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TE            298.0 K
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TD0           1

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<sup>13</sup>C

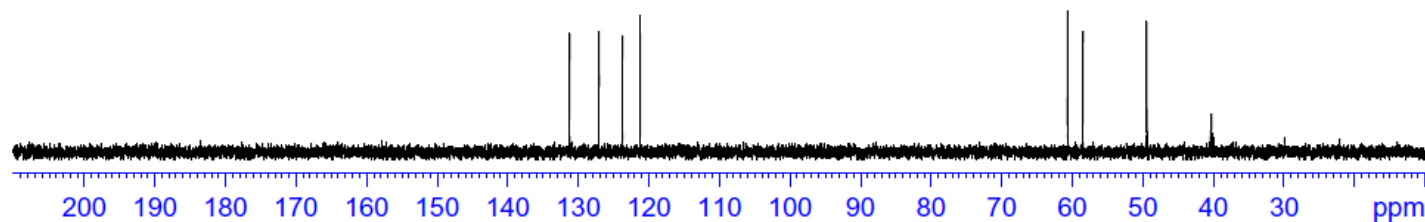


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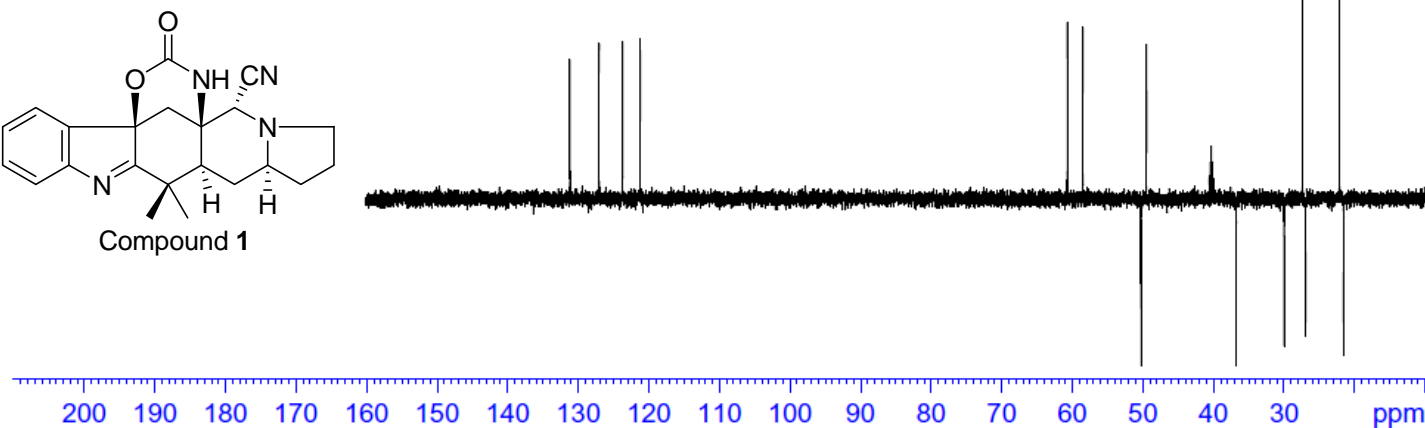


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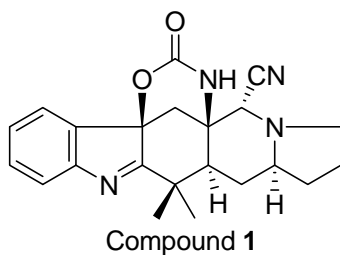
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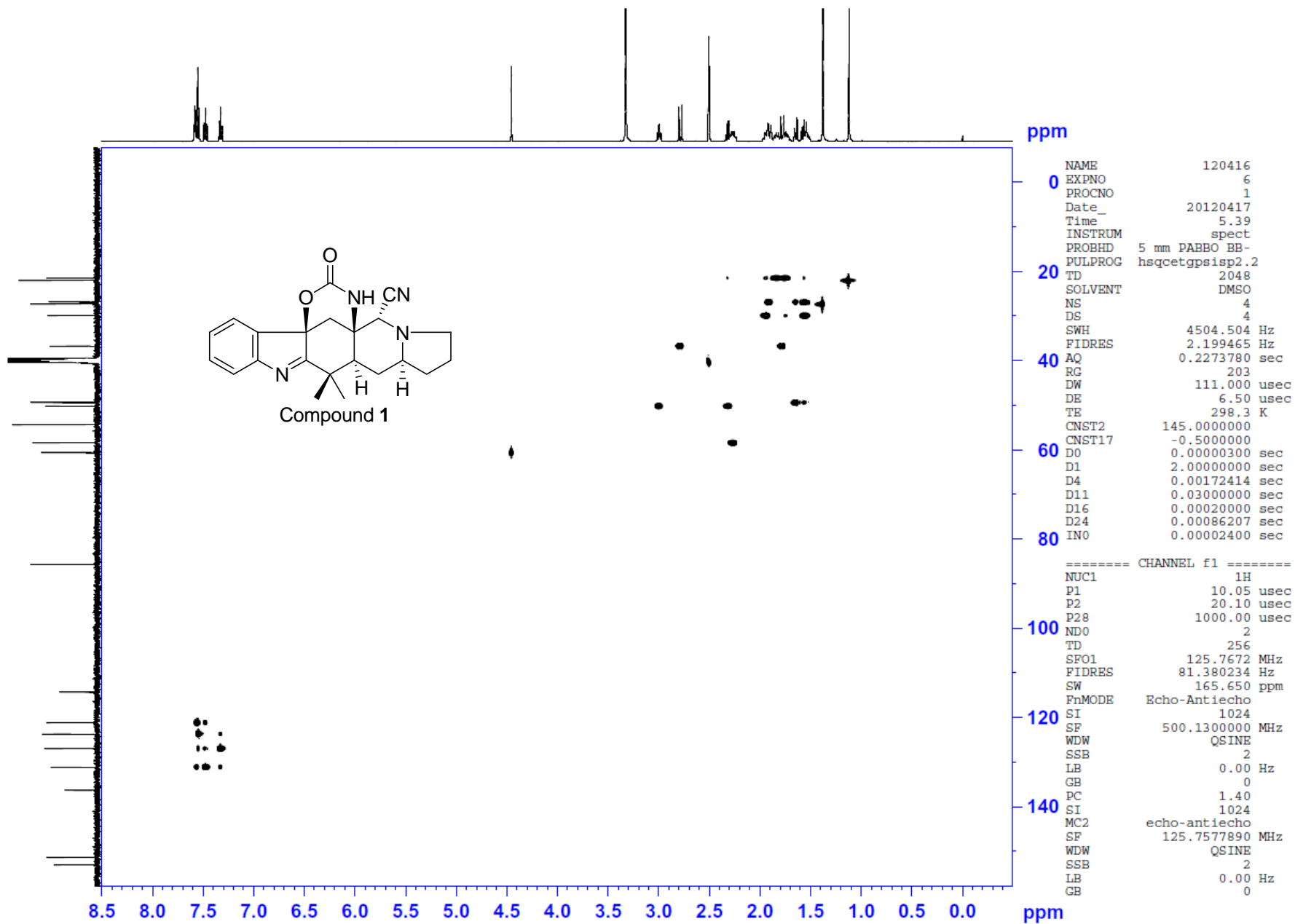


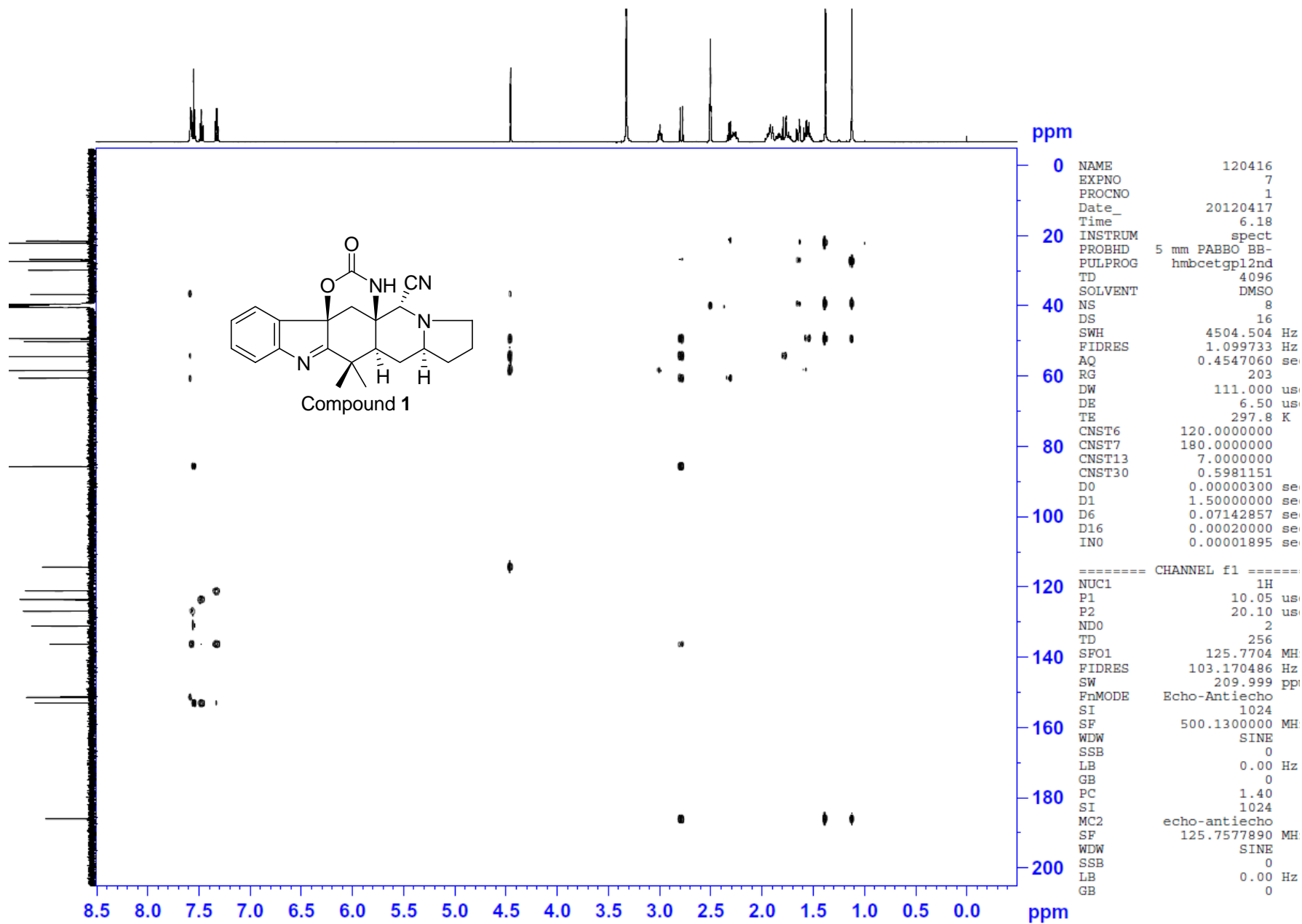
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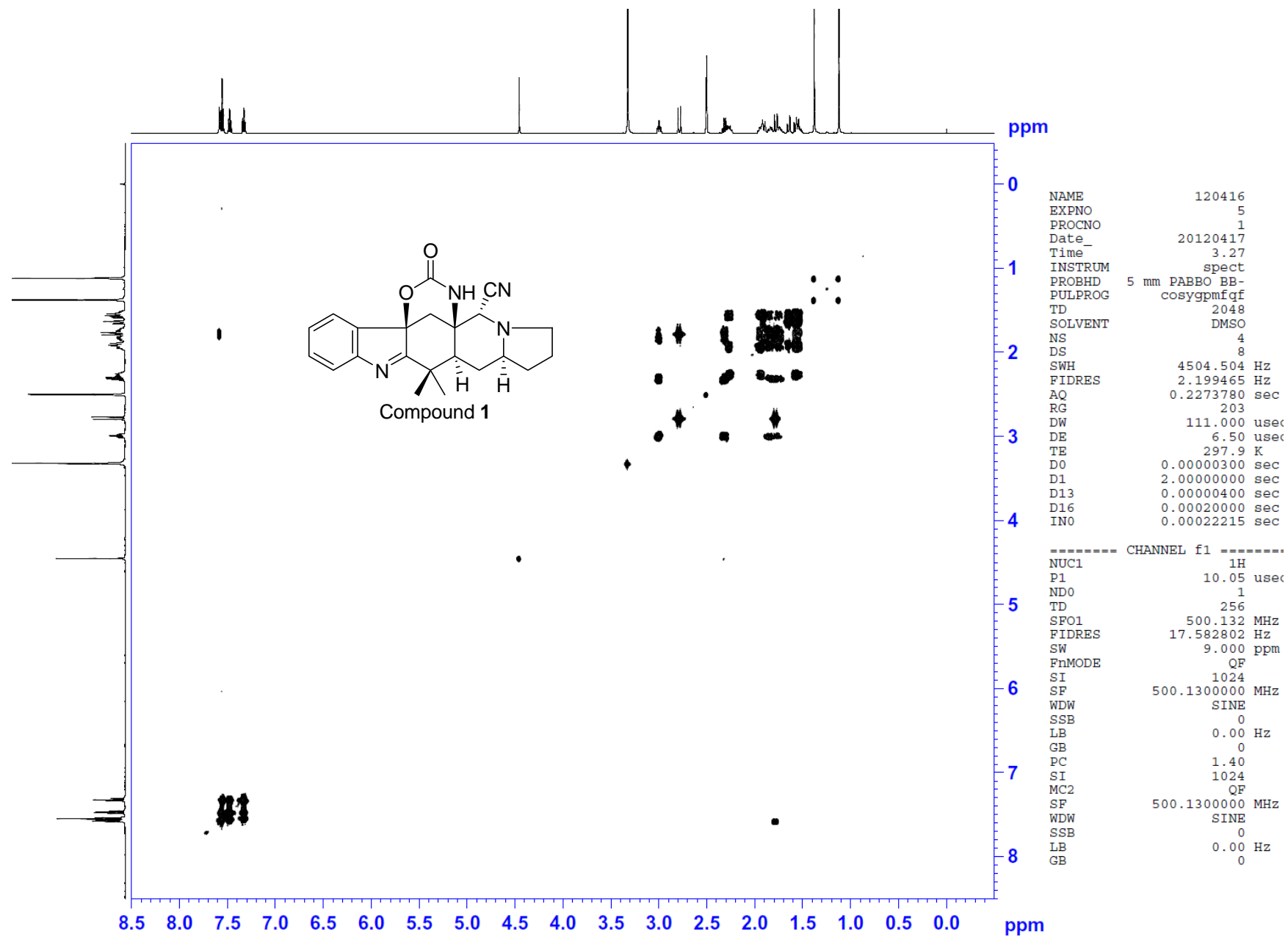
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RG         203
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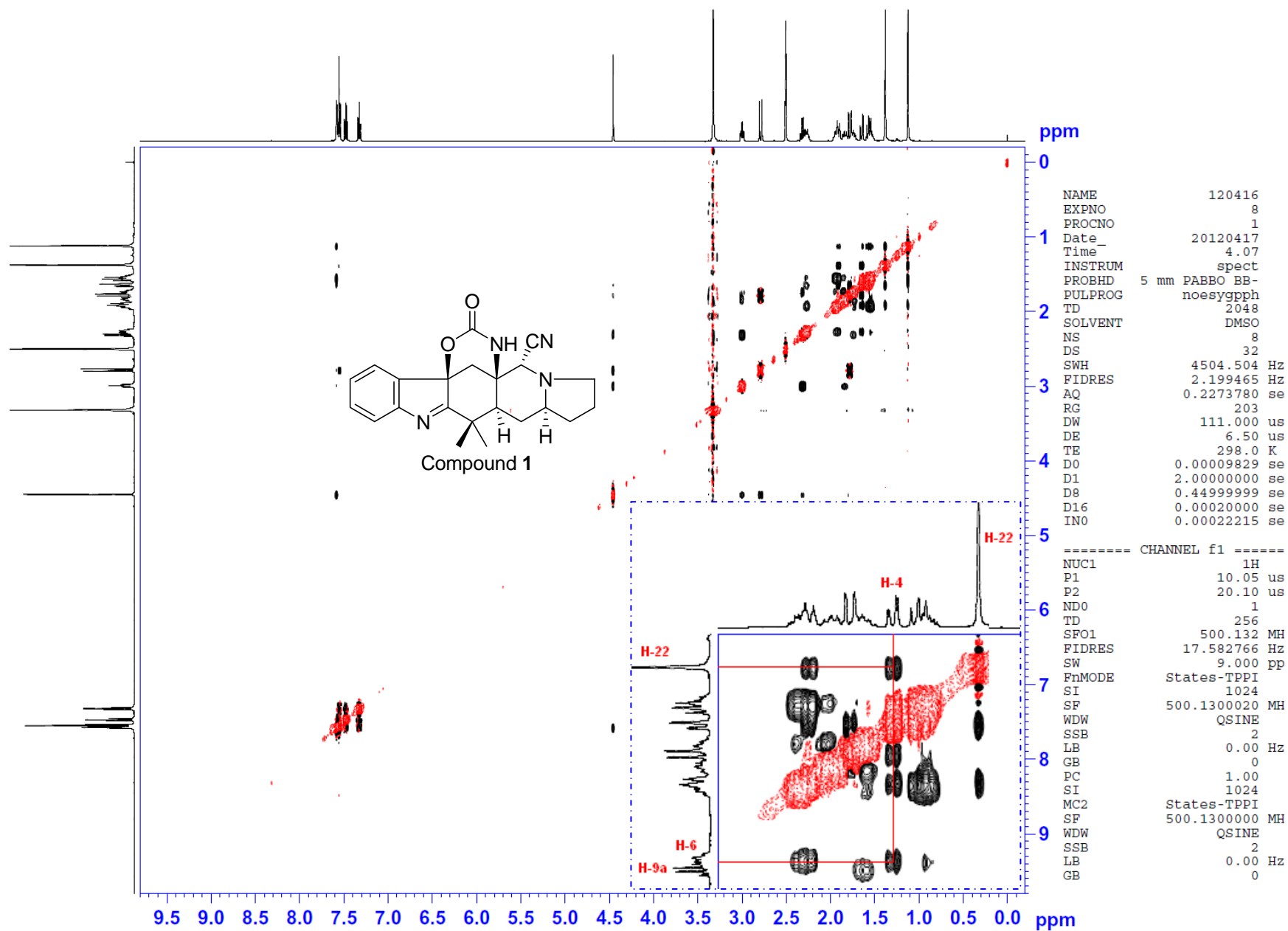


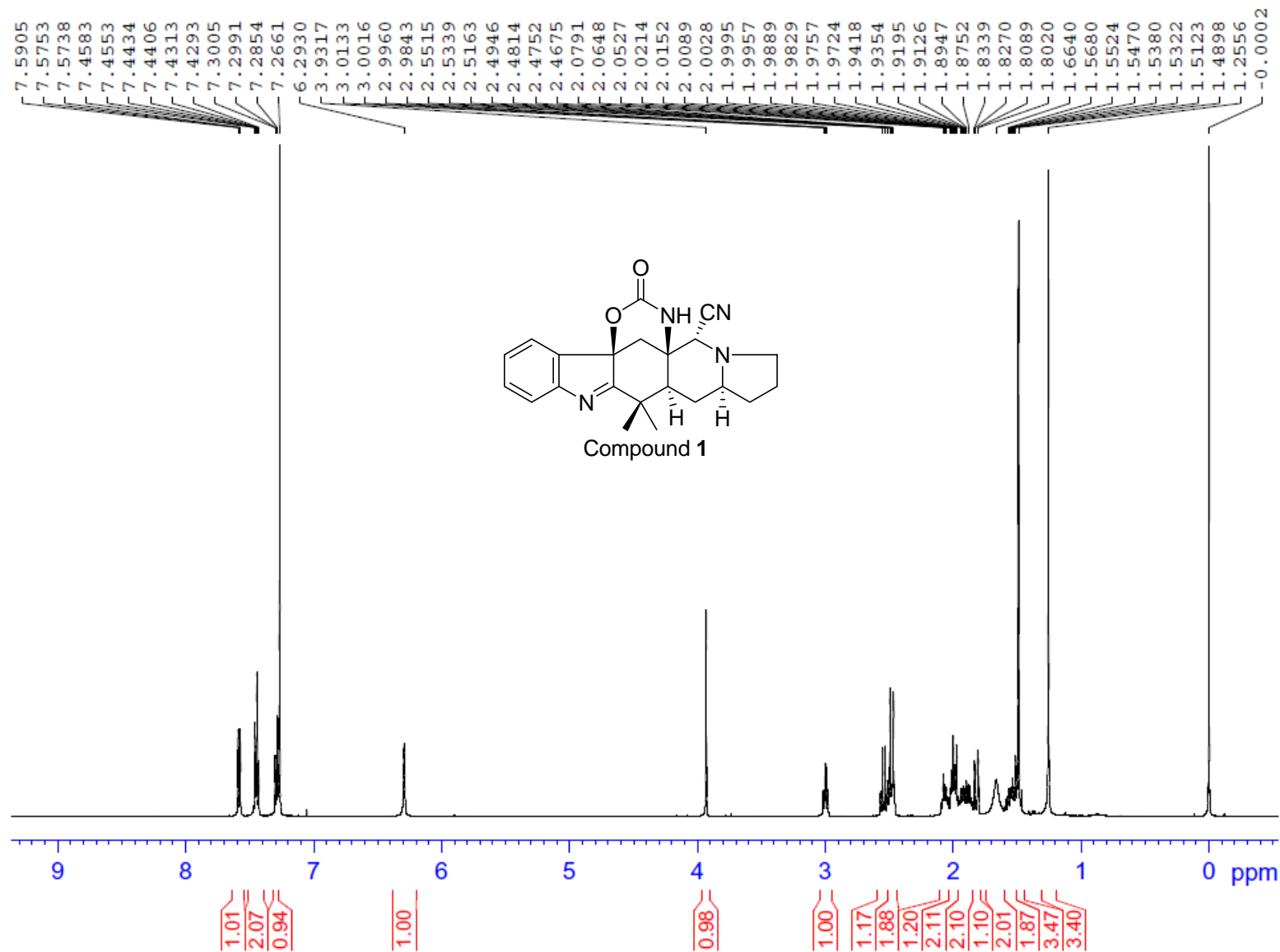










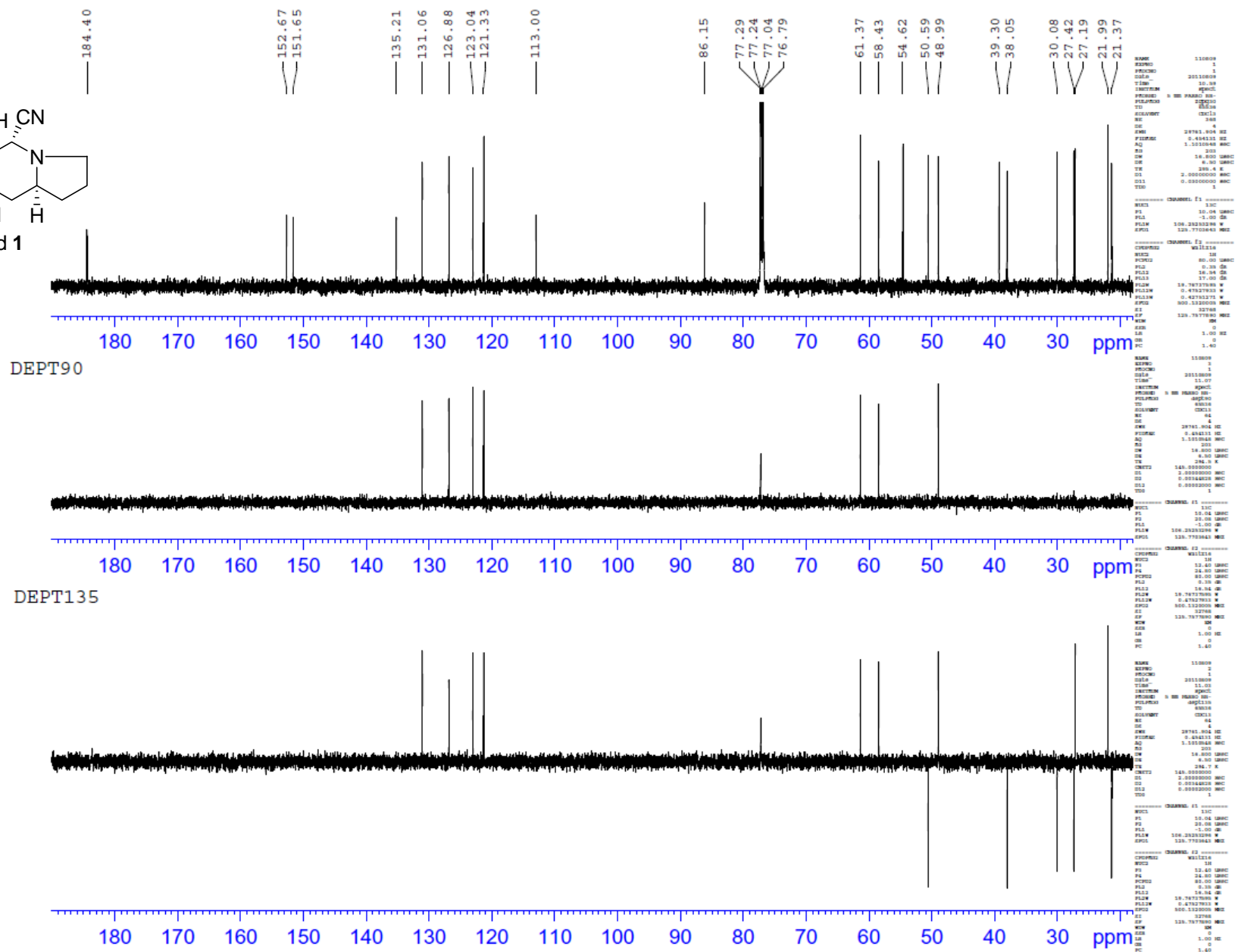
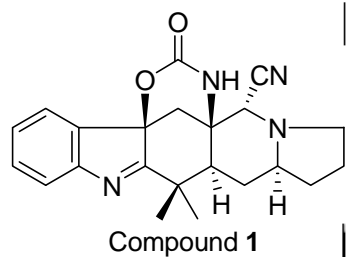


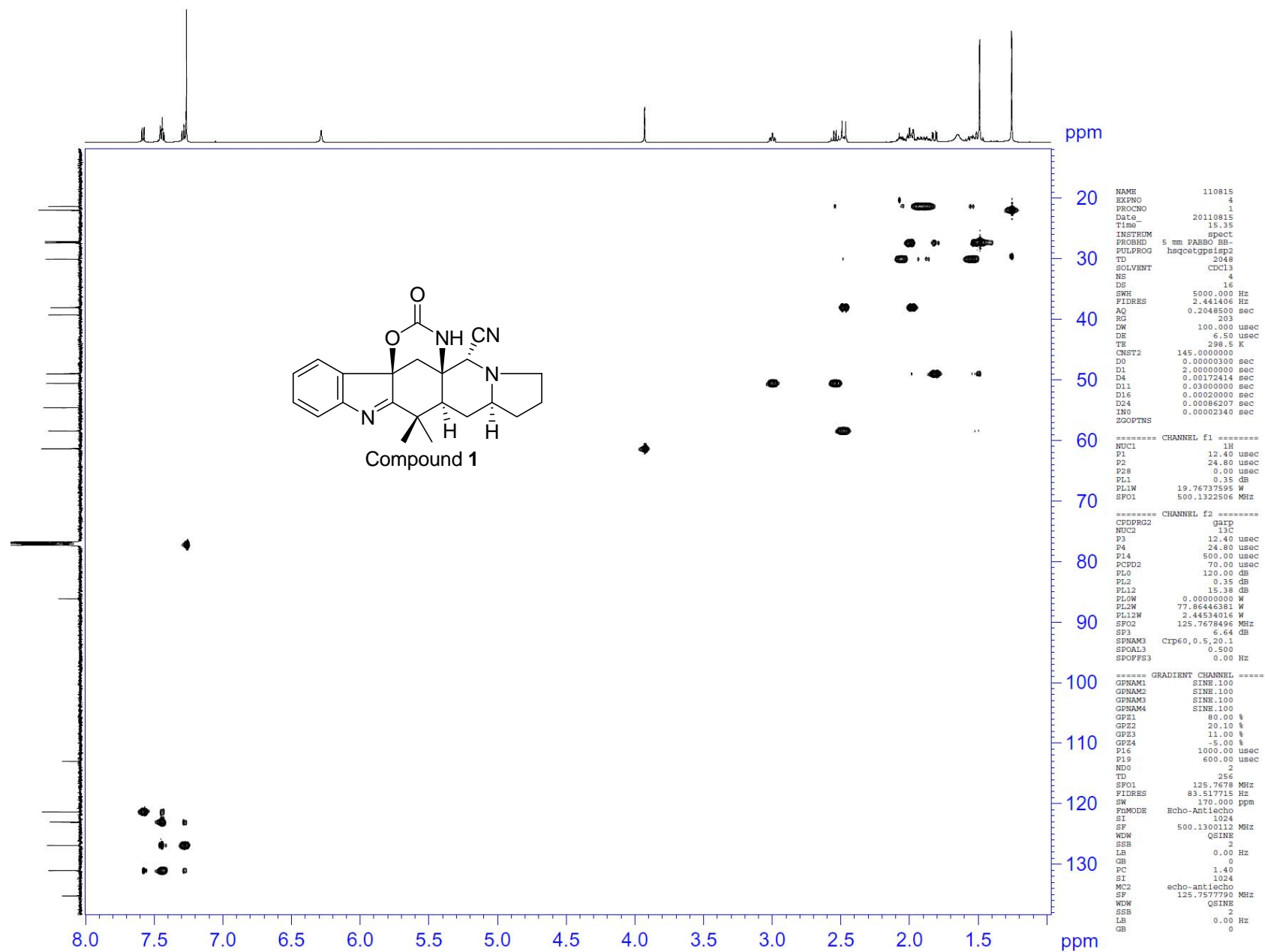
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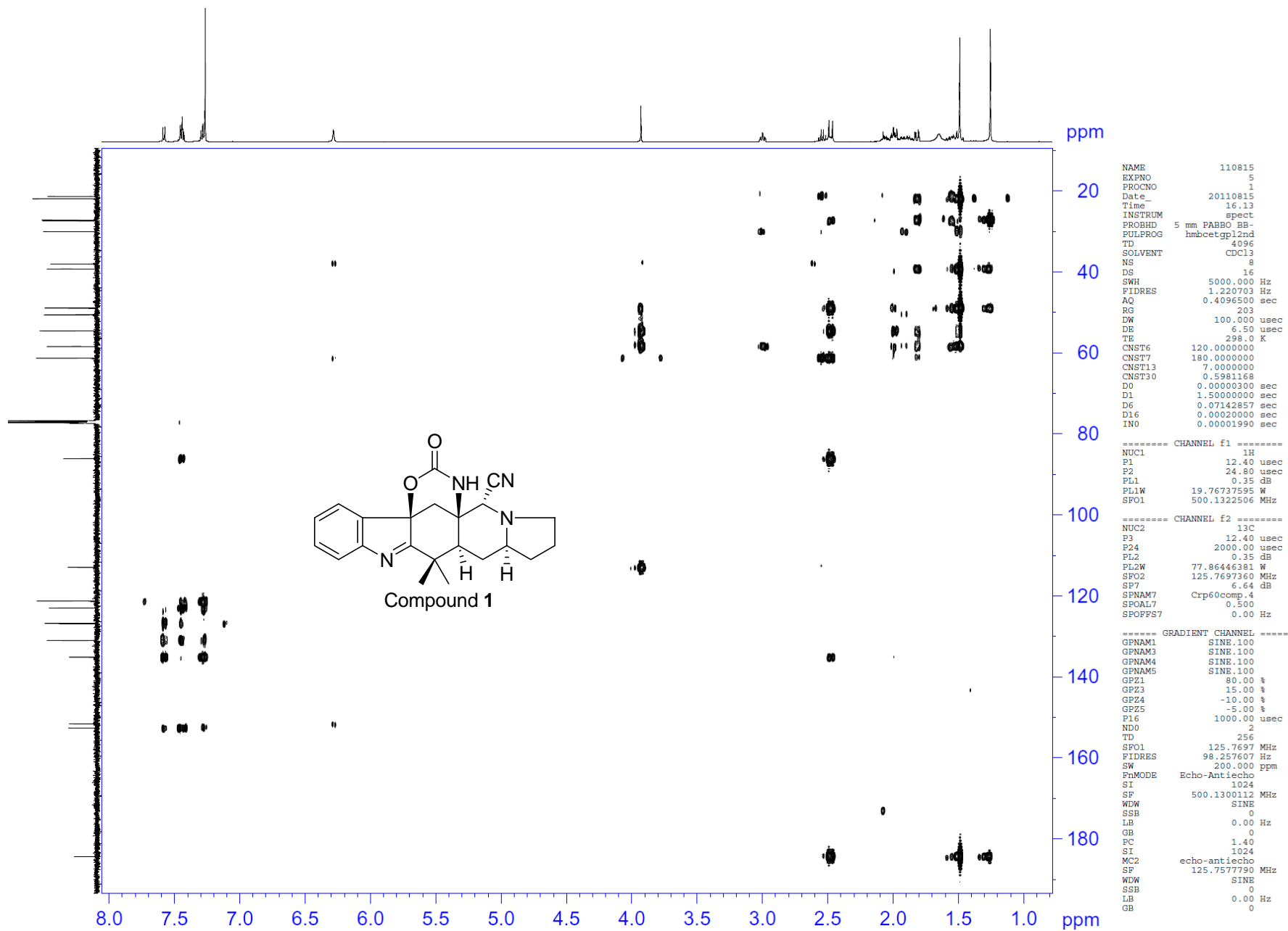
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FIDRES              0.157632 Hz
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RG                   128
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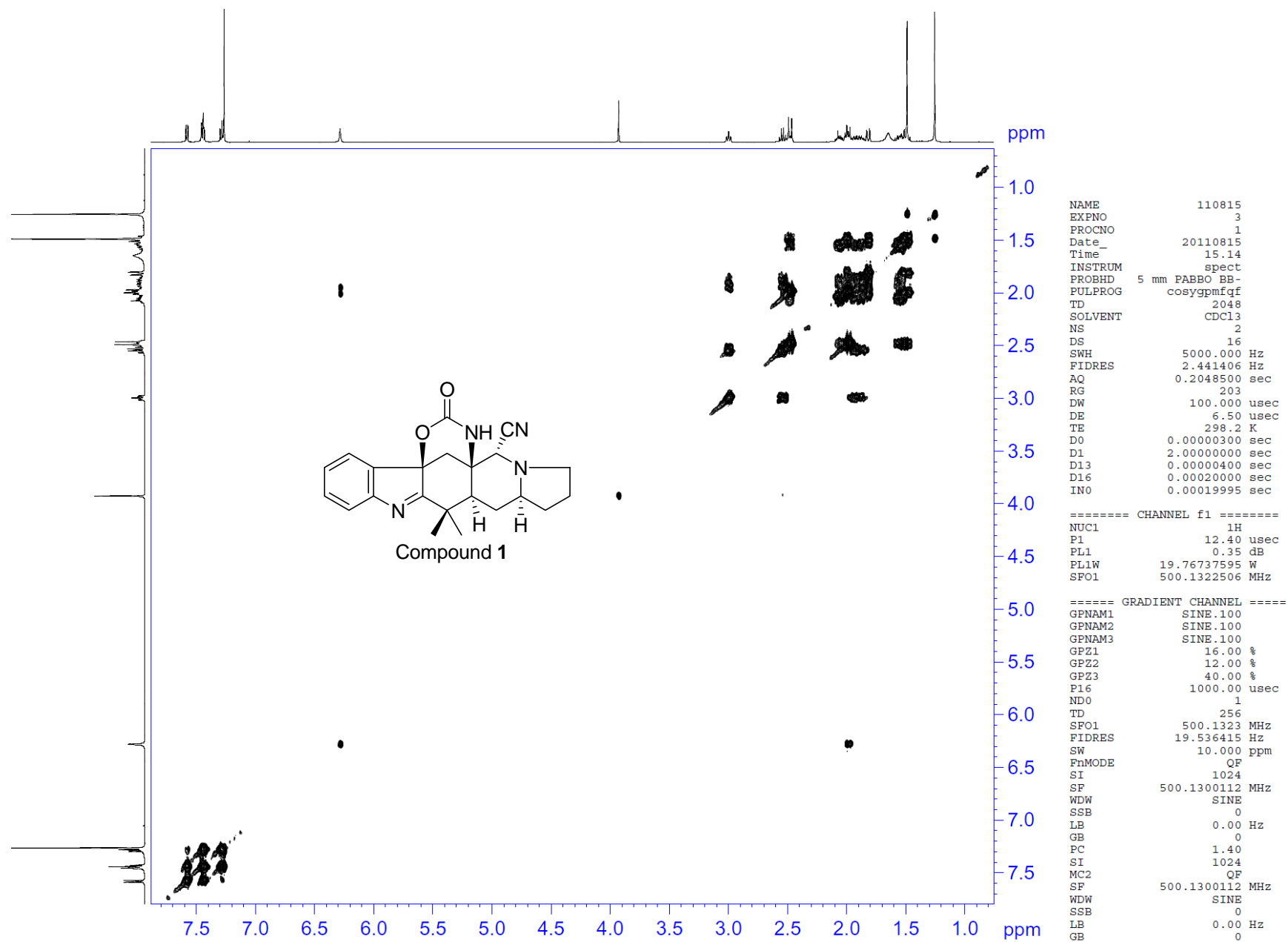
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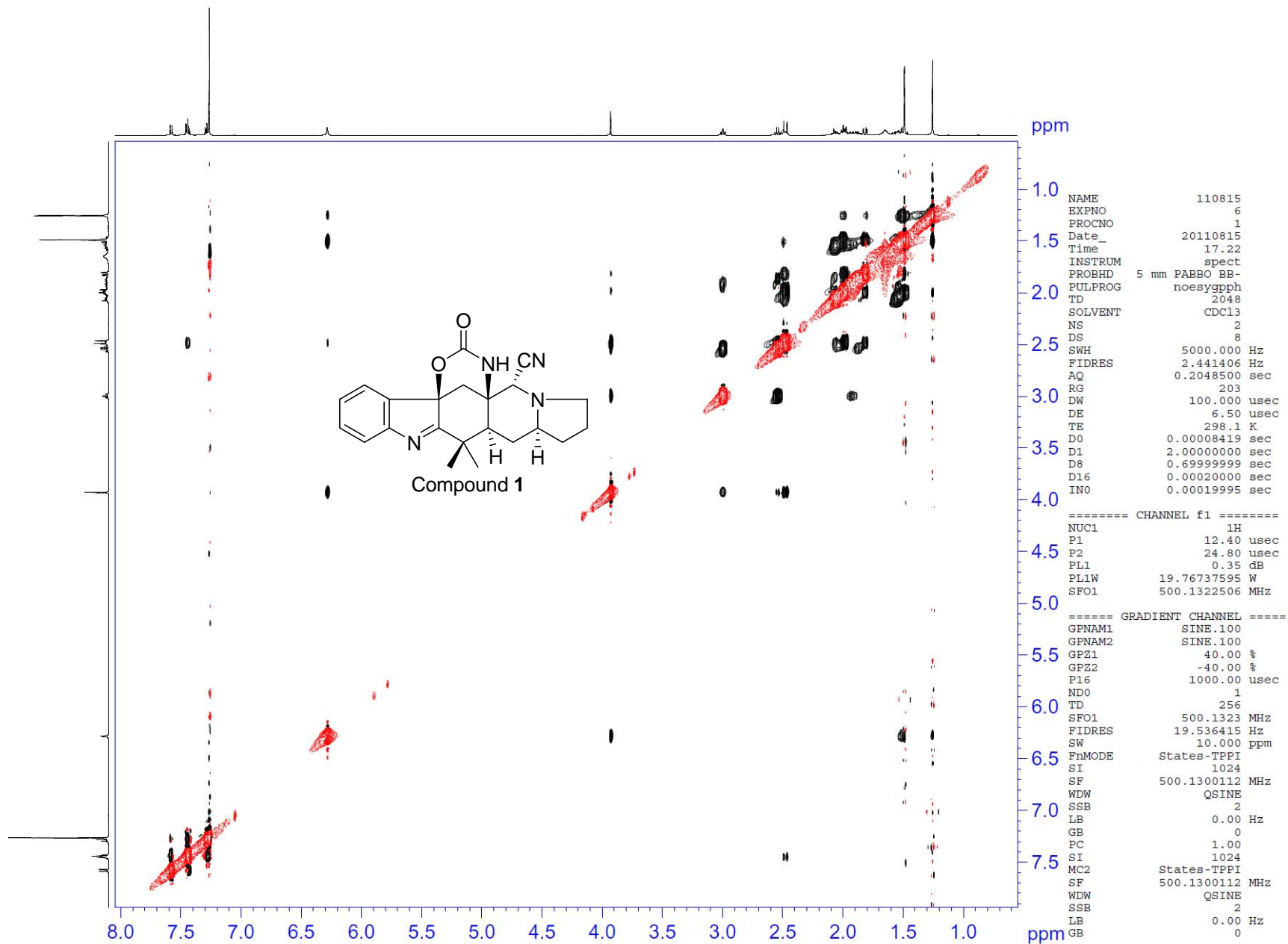
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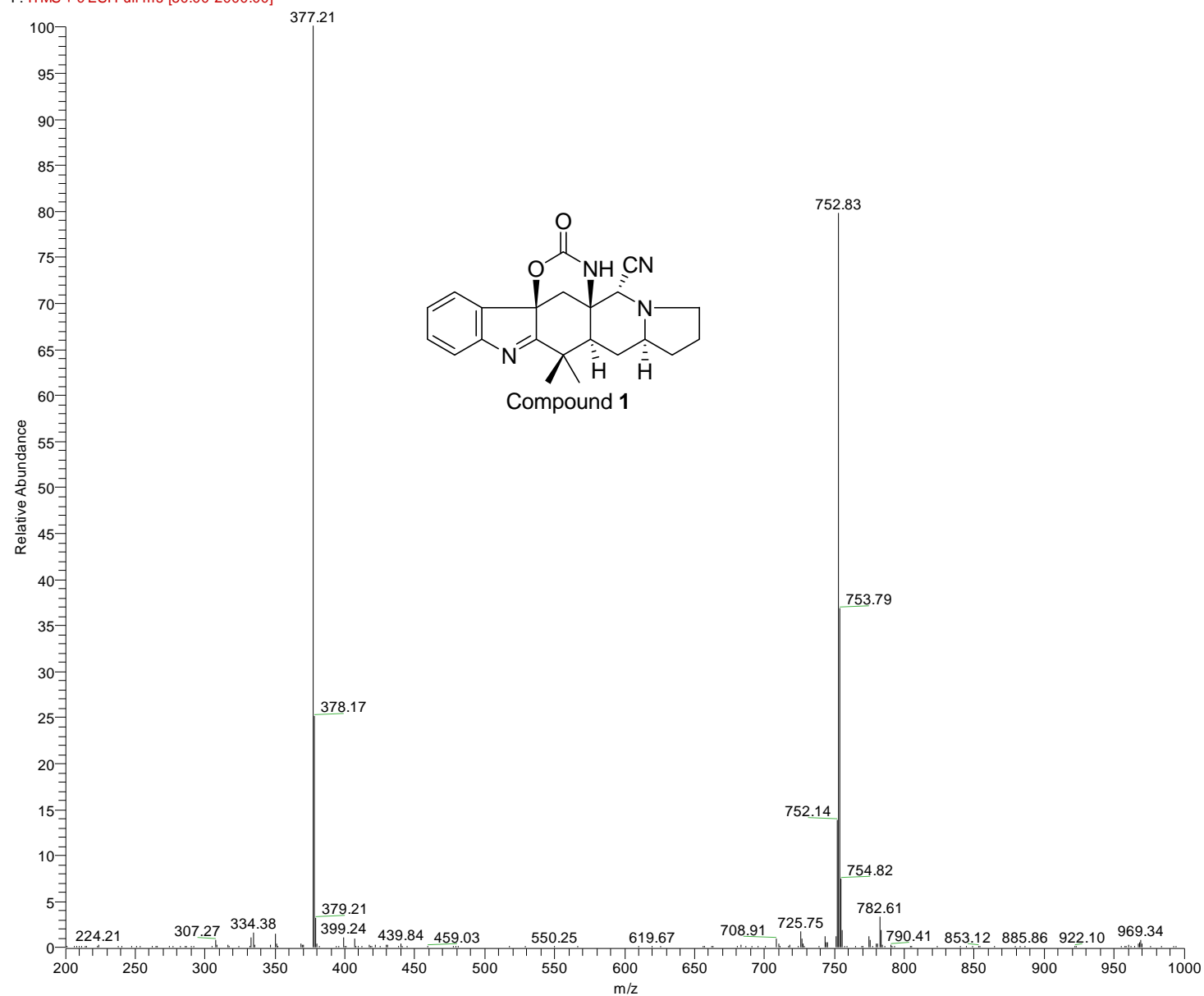






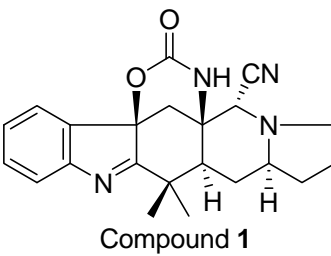
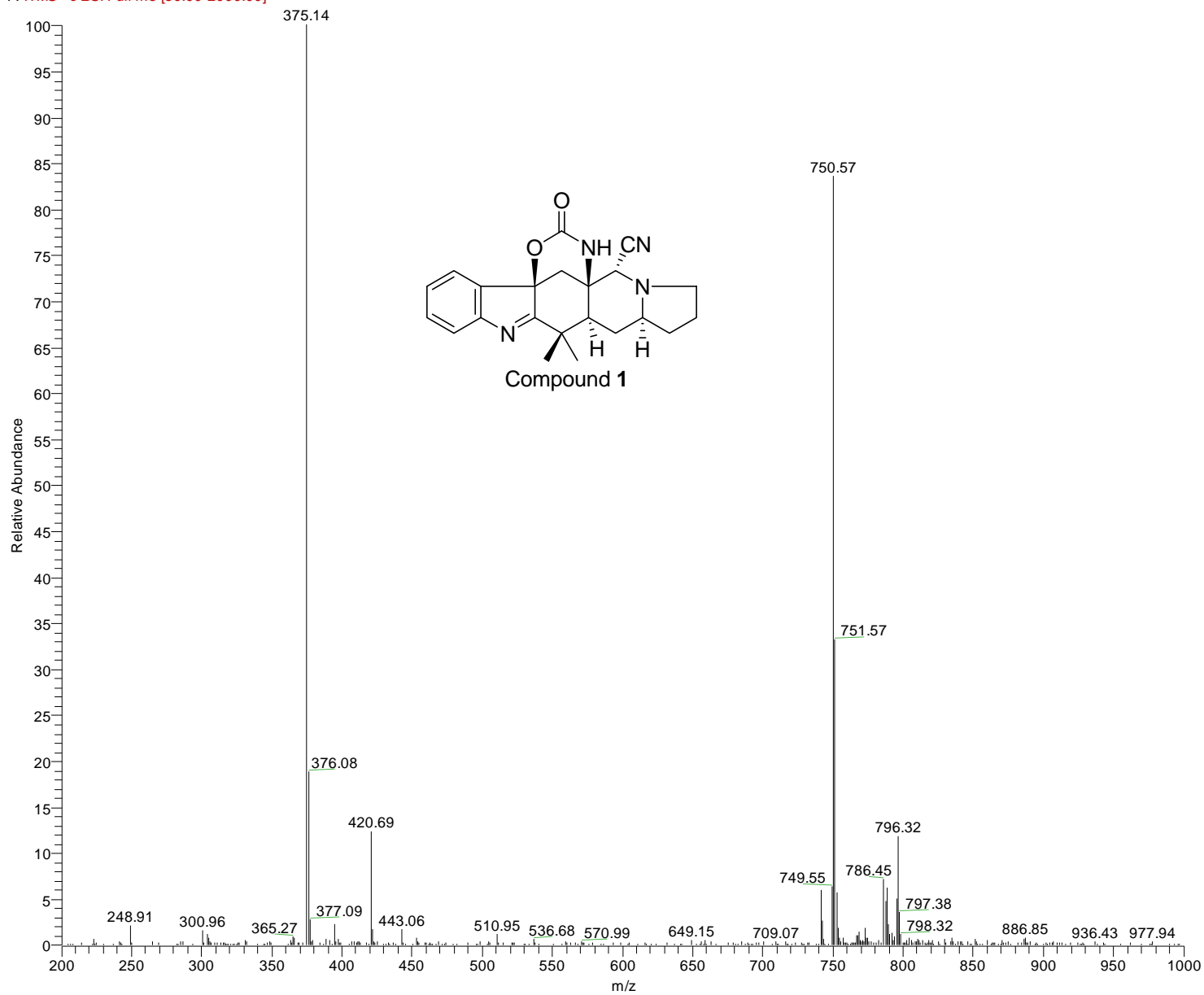


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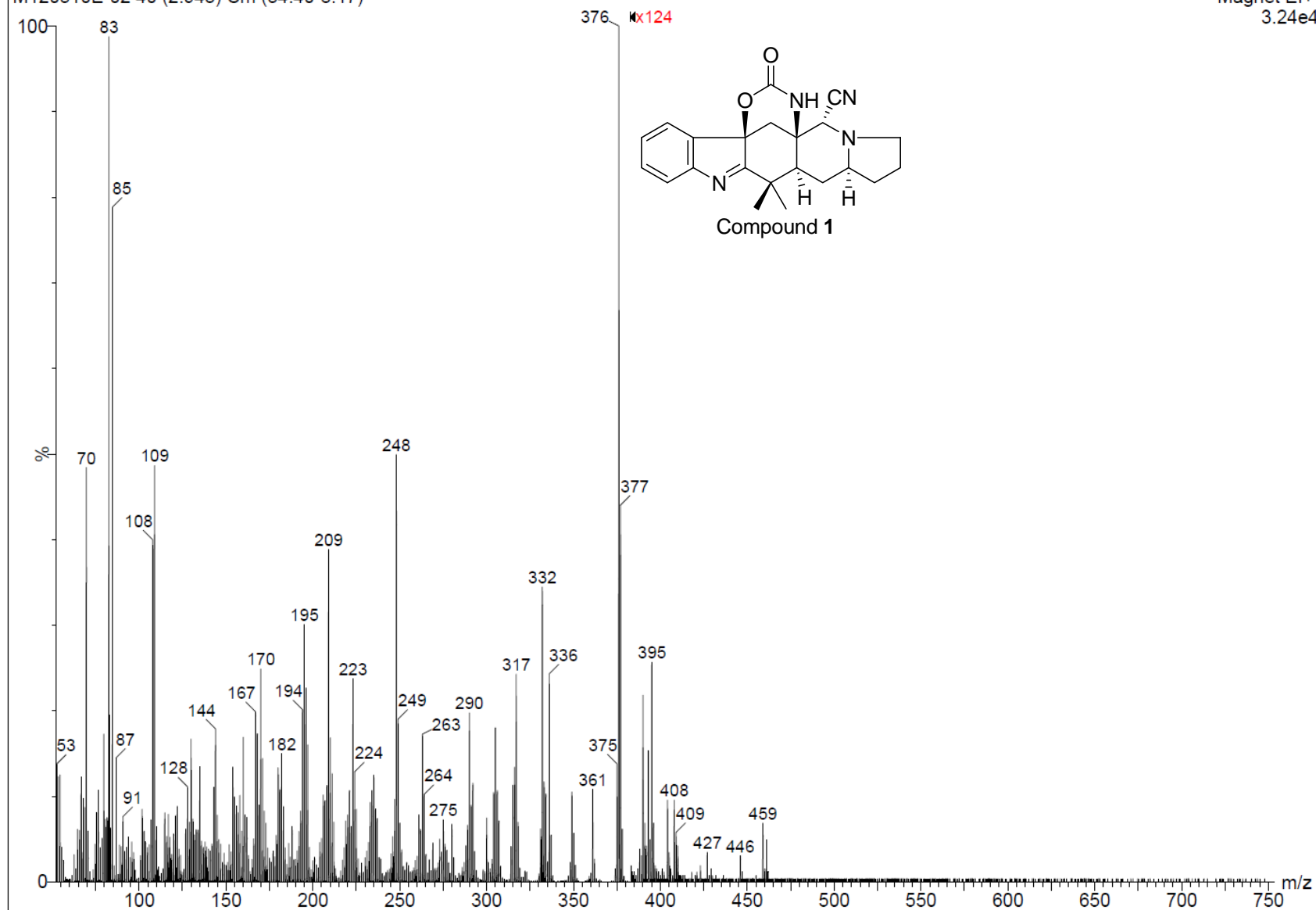
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## Elemental Composition Report

### Single Mass Analysis

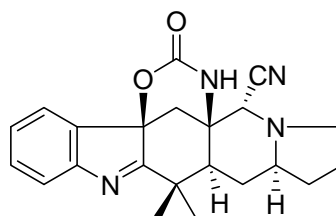
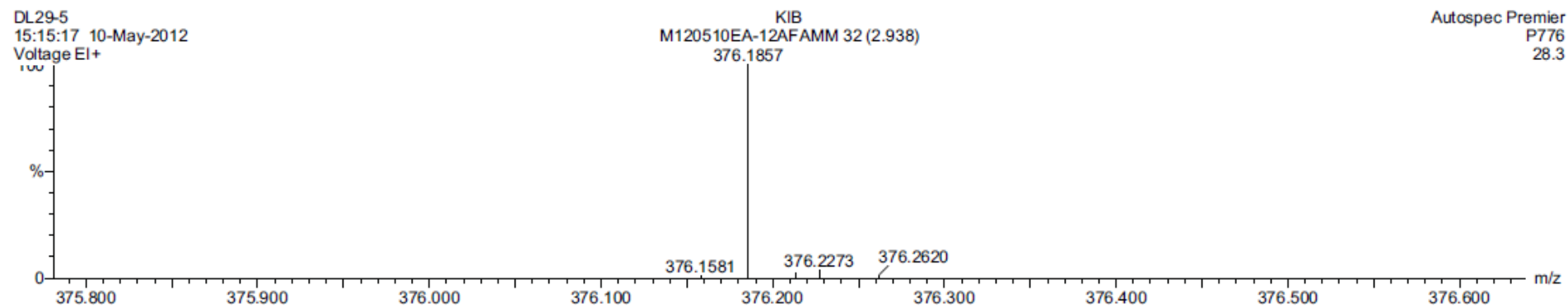
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Compound 1



Cartesian coordinates of the energy-minimized conformer of compound **1** optimized at the gas-phase B3LYP/6-31G(d) level

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms)		
			X	Y	Z
1	6	0	5.197100	-1.728079	-0.157001
2	6	0	5.699298	-0.637278	-0.872601
3	6	0	4.902297	0.484821	-1.136101
4	6	0	3.597197	0.475319	-0.661601
5	6	0	3.086898	-0.623981	0.044200
6	6	0	3.874600	-1.730580	0.313000
7	7	0	2.630896	1.512018	-0.814501
8	6	0	1.559596	1.133817	-0.209601
9	6	0	1.658098	-0.290883	0.387600
10	6	0	0.264295	1.919715	-0.195101
11	6	0	-0.929004	0.926013	-0.496601
12	6	0	-0.763302	-0.515686	0.092400
13	6	0	0.583699	-1.131185	-0.301701
14	6	0	-2.313104	1.493311	-0.117401
15	6	0	-3.434903	0.543710	-0.535601
16	7	0	-3.198301	-0.792690	0.038300
17	6	0	-1.953401	-1.407588	-0.386001
18	6	0	-4.844504	0.865808	-0.016700
19	6	0	-5.551702	-0.516593	0.075300
20	6	0	-4.433800	-1.558891	-0.168701
21	6	0	0.140494	2.655915	1.169100
22	6	0	0.307194	2.993215	-1.303001
23	8	0	1.493198	-0.345284	1.823300
24	7	0	-0.811602	-0.546187	1.550700
25	1	0	-0.922204	0.786713	-1.587801
26	1	0	-3.462303	0.481810	-1.640901
27	6	0	-1.895100	-1.662288	-1.853301
28	6	0	0.256398	-0.438385	2.401700
29	8	0	0.154698	-0.421185	3.608600
30	7	0	-1.906300	-1.849588	-2.999601
31	1	0	5.838101	-2.582578	0.040500
32	1	0	6.726298	-0.657977	-1.227001
33	1	0	5.284196	1.338921	-1.686401
34	1	0	3.489901	-2.573581	0.880800
35	1	0	0.727799	-1.106185	-1.385901
36	1	0	0.644800	-2.174885	0.029900
37	1	0	-2.464706	2.468111	-0.594101
38	1	0	-2.372805	1.650111	0.965600
39	1	0	-1.855899	-2.379488	0.113700
40	1	0	-4.775904	1.327508	0.974400
41	1	0	-5.367605	1.566908	-0.673401
42	1	0	-6.011202	-0.653393	1.058000
43	1	0	-6.344302	-0.621594	-0.670801
44	1	0	-4.493100	-1.954591	-1.196801
45	1	0	-4.472499	-2.408891	0.521000
46	1	0	1.031293	3.273216	1.323100
47	1	0	-0.727007	3.323814	1.161400
48	1	0	0.045395	1.991815	2.029500
49	1	0	-0.621007	3.574714	-1.313901
50	1	0	0.448194	2.541715	-2.290001
51	1	0	1.140493	3.680116	-1.135901
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Correlation between experimental (in CDCl<sub>3</sub>) and calculated (in gas phase) <sup>13</sup>C NMR data for the C-6 epimer of compound **1**

