

Chemo-enzymatic synthesis of (*E*)-2,3-diaryl-5-styryl-*trans*-2,3-dihydrobenzofuran-based scaffolds and their *in vitro* and *in silico* evaluation as a novel sub-family of potential allosteric modulators of the 90 kDa heat shock protein (Hsp90)

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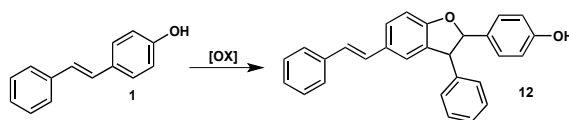
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SUPPORTING INFORMATION

Contents

Metal-mediated oxidation of 1	page S2
<i>In vitro</i> assay	page S4
Structures and activity data of Hsp90 modulators	page S5
¹ H, ¹³ C-NMR and mass spectra of compounds 1-28	page S11

Metal-mediated oxidation of compound **1**



The metal-mediated oxidations were conducted following published procedures on analogue compounds.¹ A water solution of the metal oxidant (4 M, 5 equiv.) was added to an ethanolic solution of the un-substituted (*E*)-4-hydroxystilbene **1** (50 mM). The resulting mixture was stirred at 35 °C for 24 - 48 h, monitoring the reaction by TLC analysis (CHCl₃ : acetone = 9 : 1)

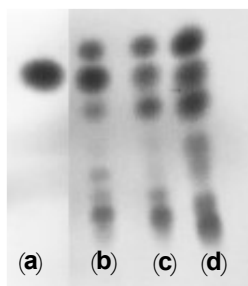


Figure S1: TLC plate of the reaction outcome of the CuCl₂ and FeCl₃-mediated oxidation of **1**: (a) standard of **1**; (b) overnight reaction with CuCl₂; (c) overnight and (d) 48 h reaction with FeCl₃.

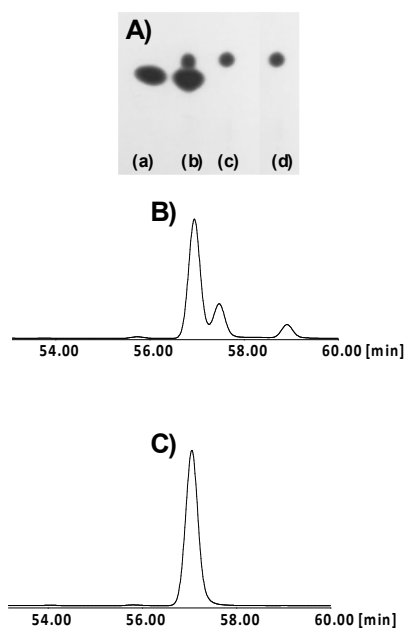
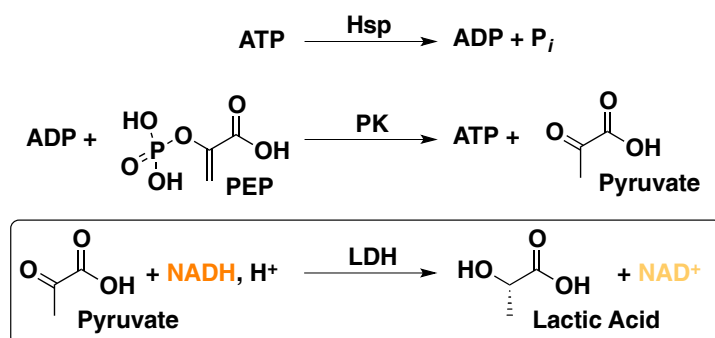


Figure S1b. A): TLC plate of: (a) standard of **1**; (b) co-chromatography of a and c; (c) MnO₂-mediated oxidation and (d) laccase-mediated oxidation after 24 h. B) and C): RP-HPLC chromatograms of the oxidation outcomes of **1** with MnO₂ and laccase, respectively.

¹ Takaya, Y.; Terashima, K.; Ito, J.; He, Y. H.; Tateoka, M.; Yamaguchi, N.; Niwa, M. *Tetrahedron* **2005**, *61* (43), 10285–10290

The oxidation with manganese(IV) oxide, run under the same experimental conditions, gave better results. The reaction proceeded smoothly and, as shown in **Figure 1Sb**, a single spot could be detected by TLC. The product(s) was isolated and its mass spectra indicated the formation of dimer(s). Unfortunately, the ¹H-NMR spectrum showed the presence of an inseparable mixture of oxidized regioisomeric compounds, as further confirmed by reverse-phase HPLC analysis (**Figure S1b**).

In vitro assay



Scheme S1. Enzymatic cascade reactions to evaluate by spectrophotometer the ATPase activity of the Hsp90 yeast homologous, Hsc82: the ADP produced by ATP hydrolysis during the chaperone cycle reacts with PEP (phosphoenolpyruvate) to form pyruvate in a reaction catalyzed by the enzyme pyruvate kinase (PK), whereby ATP is regenerated. Pyruvate then continues the cascade process and is reduced to lactic acid by action of lactate dehydrogenase (LDH), whereby NADH is oxidized to NAD⁺.

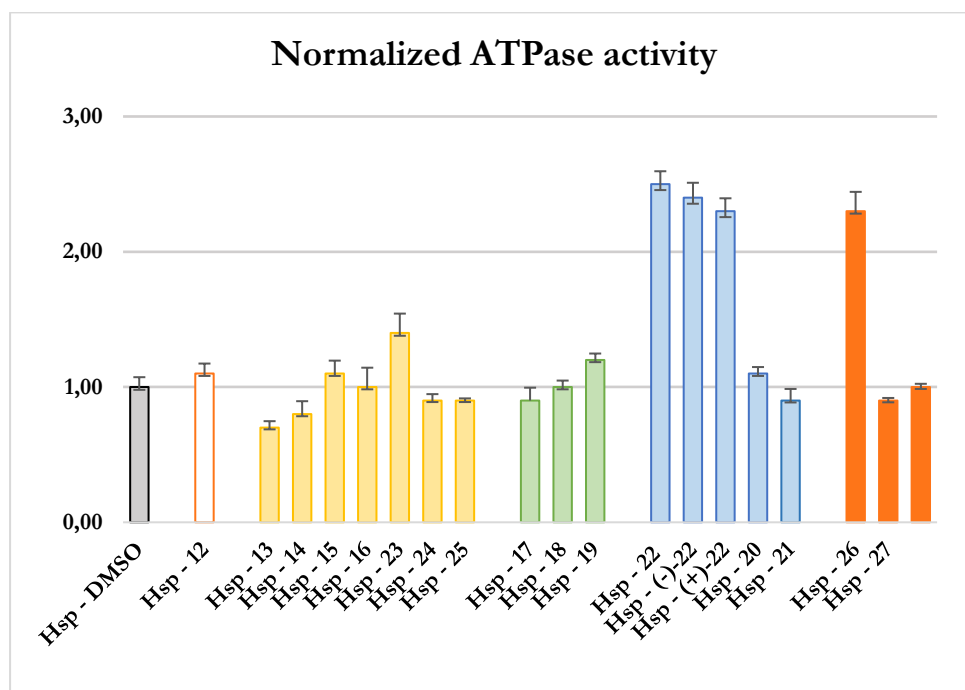
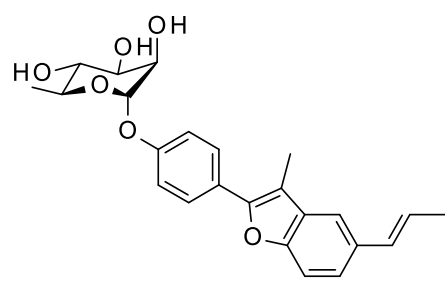
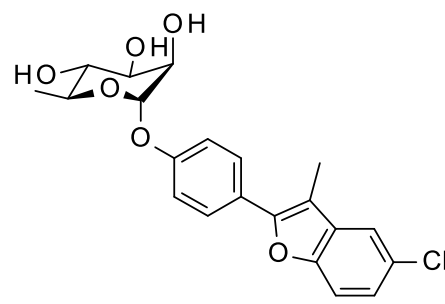
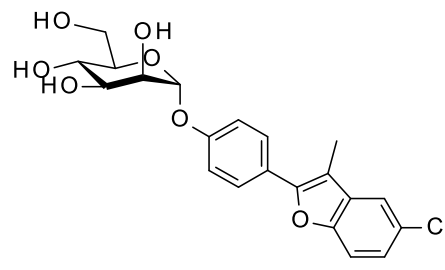
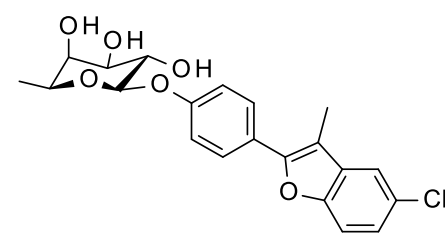
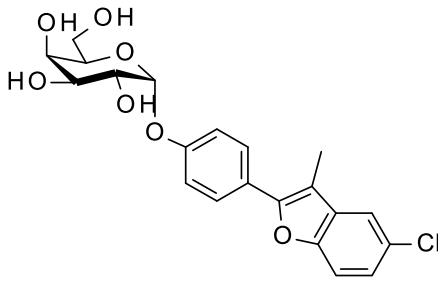
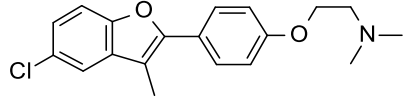
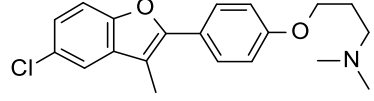
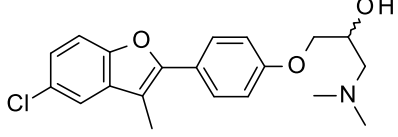
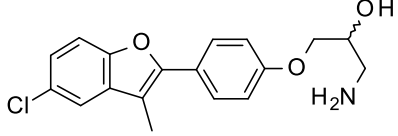
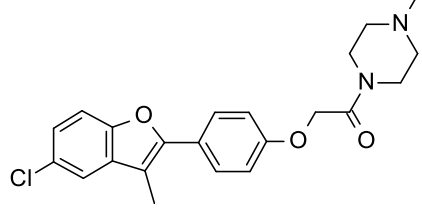
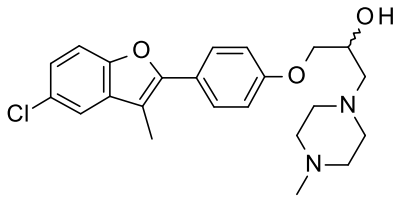
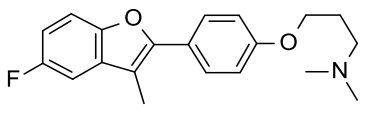
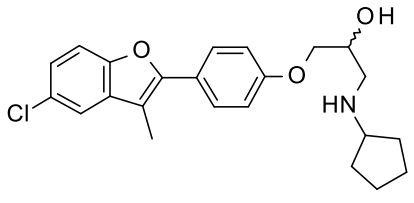
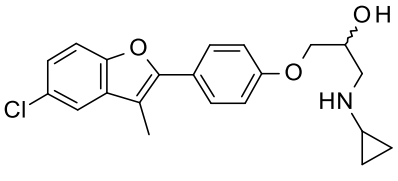
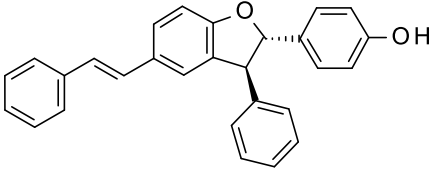
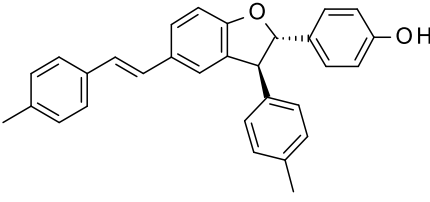


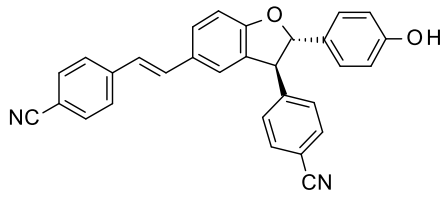
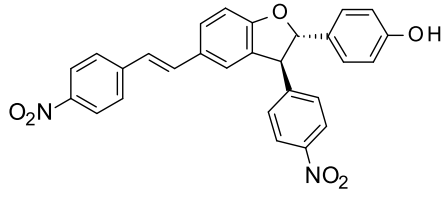
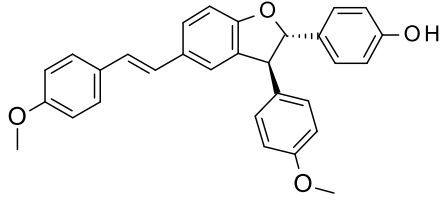
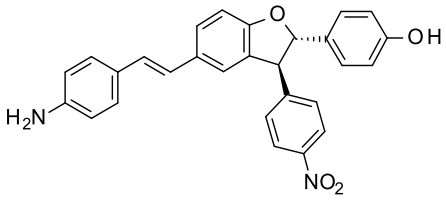
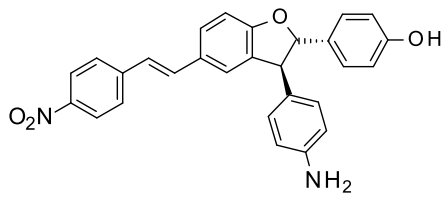
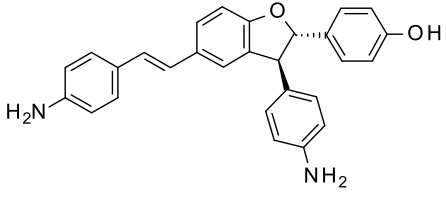
Figure S2: Normalized ATPase activities of Hsp incubated with all the different synthesized compounds.

Table S1: Structures and activity data of Hsp90 modulators

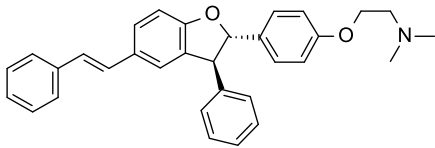
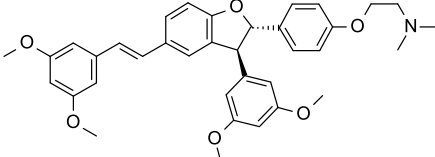
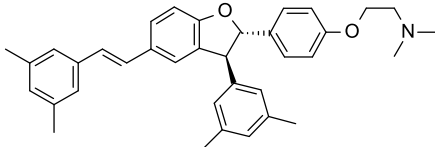
Normalized Hsp90's ATPase cycle activation	Ligand Efficiency	Compound, Structure
1.24	-0.26	<p>CheCosp-1</p> 
2.27	-0.27	<p>CheCosp-9</p> 
2.74	-0.26	<p>CheCosp-16</p> 
1.17	-0.26	<p>CheCosp-21</p> 

2.35	-0.26	<p style="text-align: center;">CheCosp-22</p> 
4.01	-0.32	<p style="text-align: center;">CheCosp26</p> 
5.3	-0.30	<p style="text-align: center;">CheCosp-27</p> 
4.15	-0.30	<p style="text-align: center;">CheCosp-30</p> 
4.52	-0.28	<p style="text-align: center;">CheCosp-33</p> 
1.86	-0.35	<p style="text-align: center;">CheCosp-35</p> 

2.39	-0.24	<p>CheCosp-36</p> 
4.24	-0.27	<p>CheCosp-57</p> 
2.23	-0.26	<p>CheCosp-43</p> 
1.55	-0.30	<p>CheCosp-42</p> 
1.1	-0.19	<p>Compound (±)-12</p> 
0.7	-0.17	<p>Compound (±)-13</p> 

0.8	-0.13	<p>Compound (±)-14</p> 
1.1	-0.23	<p>Compound (±)-15</p> 
1.0	-0.16	<p>Compound (±)-16</p> 
1.0	-0.17	<p>Compound (±)-24</p> 
0.90	-0.14	<p>Compound (±)-25</p> 
1.4	-0.18	<p>Compound (±)-23</p> 

0.9	-0.18	<p>Compound (±)-17</p>
1.0	-0.18	<p>Compound (±)-18</p>
1.2	-0.18	<p>Compound (±)-19</p>
2.5	-0.25	<p>Compound (±)-22</p>
1.1	-0.16	<p>Compound (±)-20</p>
0.9	-0.17	<p>Compound (±)-21</p>

2.3	-0.18	<p>Compound (\pm)-26</p> 
0.9	-0.16	<p>Compound (\pm)-27</p> 
1	-0.15	<p>Compound (\pm)-28</p> 

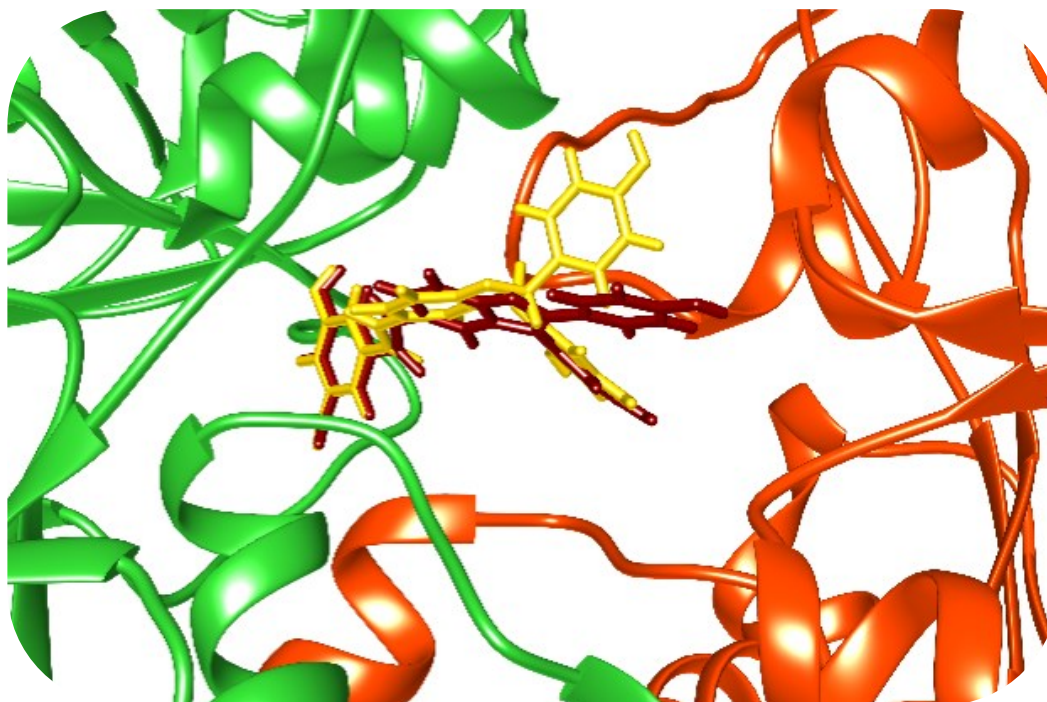
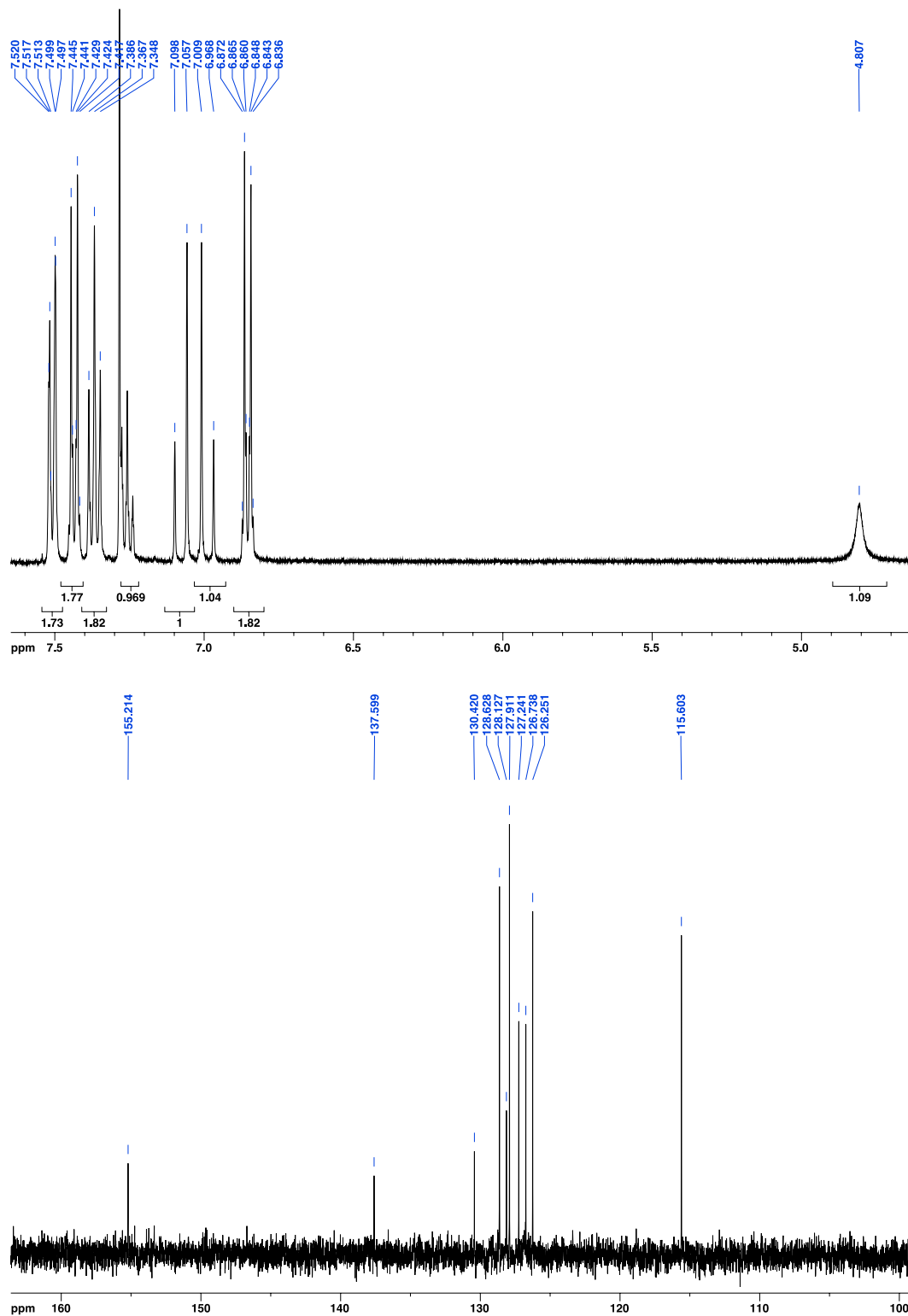
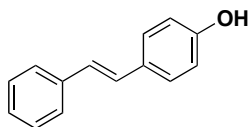


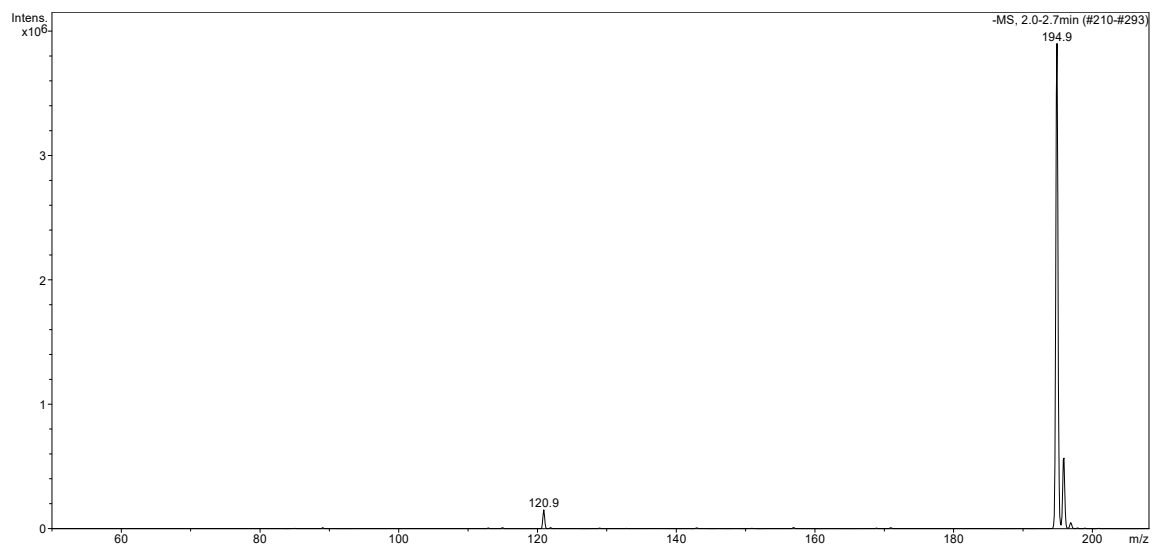
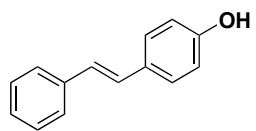
Figure S3: Superposition of the best pose for the two enantiomers (*R*) and (*S*) of compound **22** shown in red and yellow, respectively. Protomers A and B of Hsp90 are depicted in green and orange, respectively.

^1H , ^{13}C -NMR and mass spectra

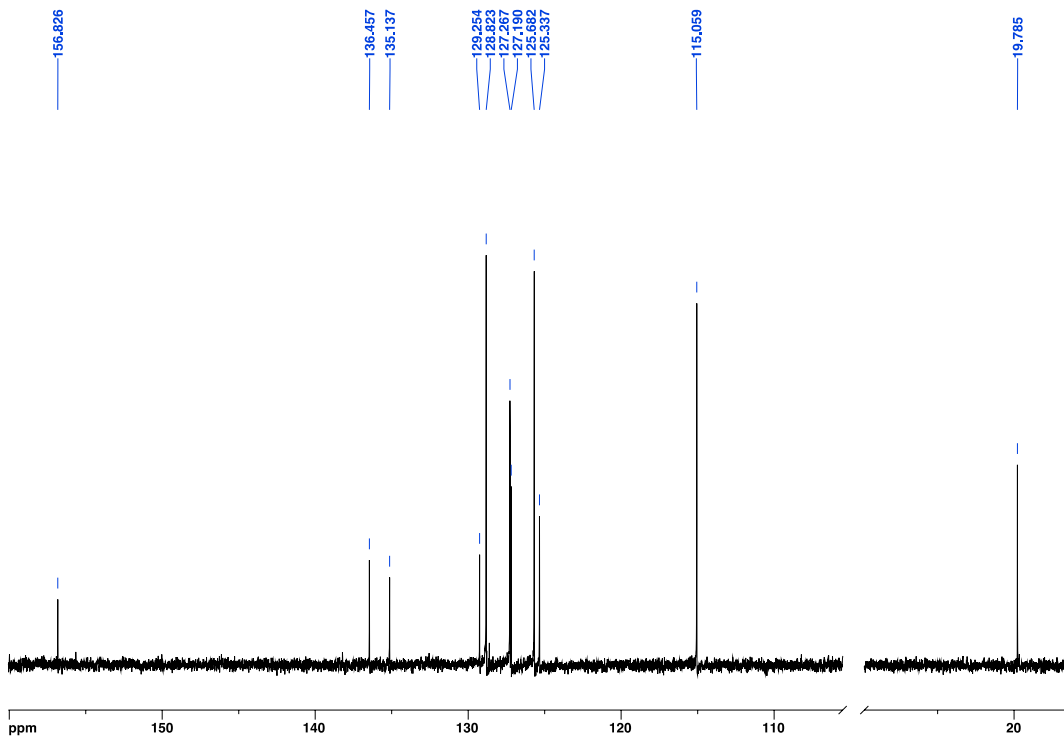
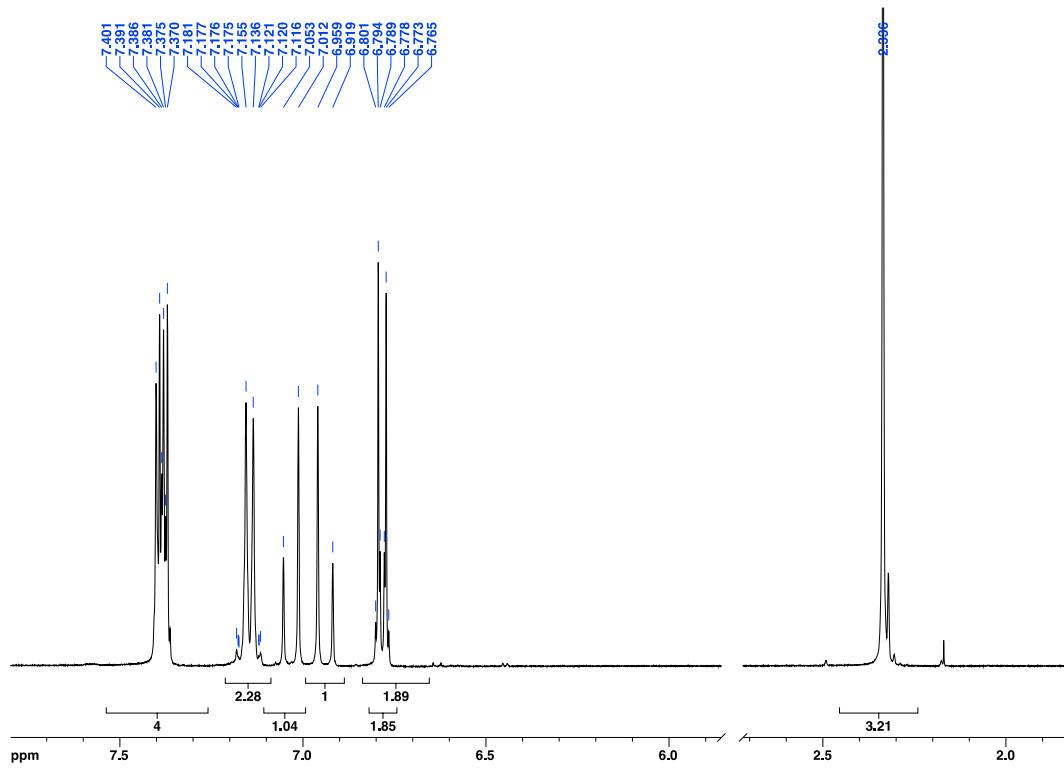
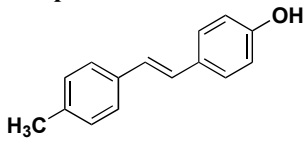
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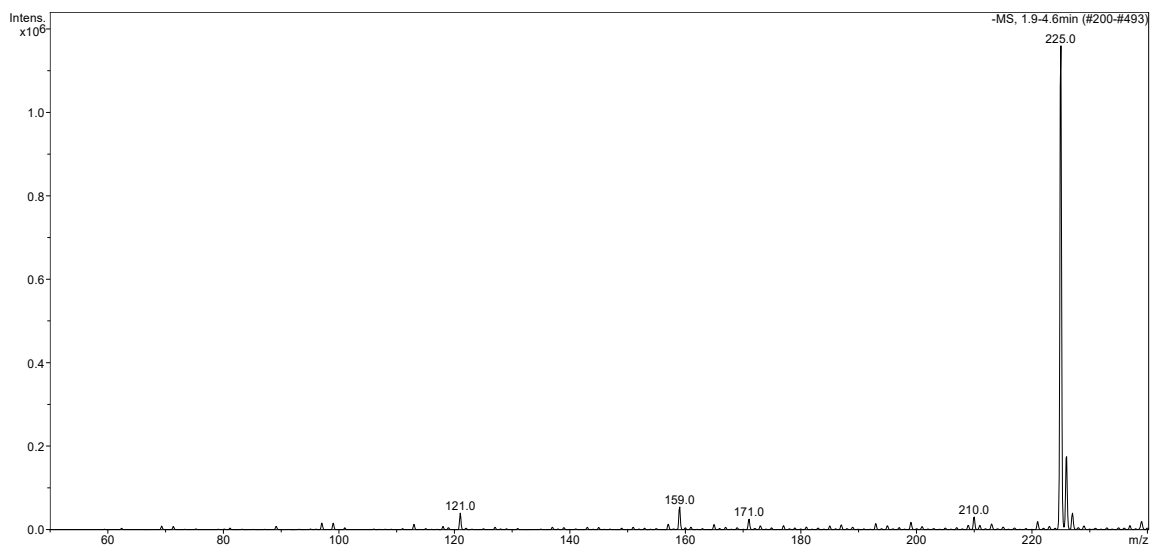
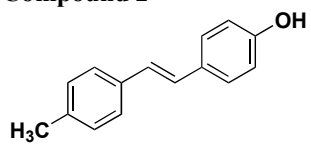
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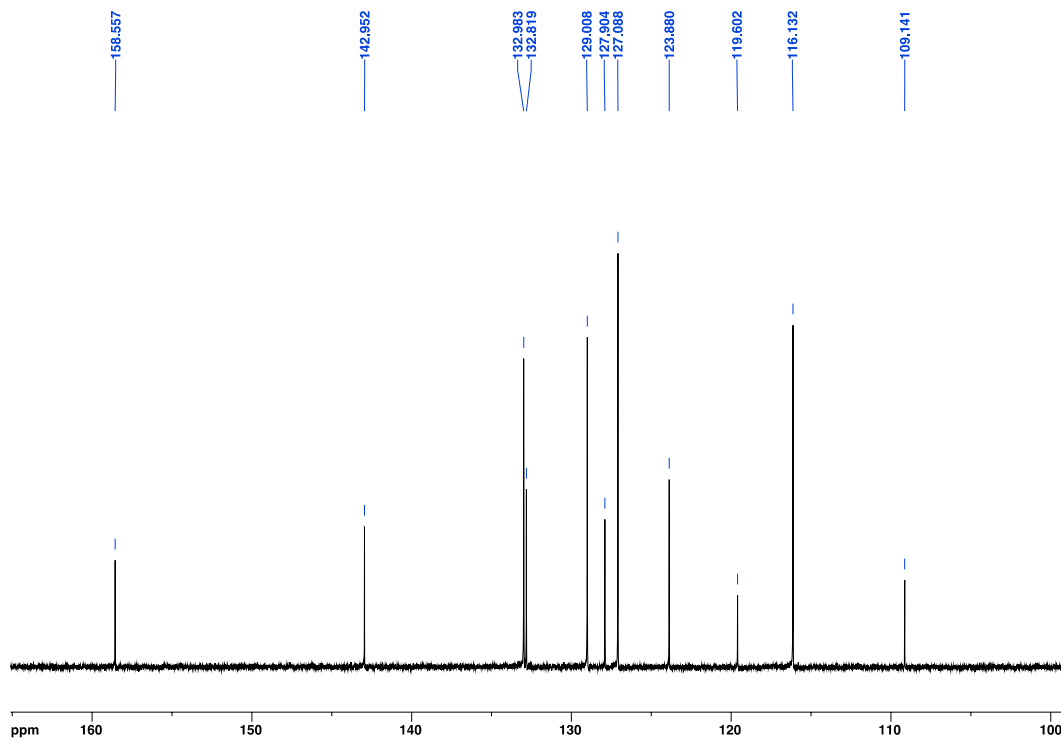
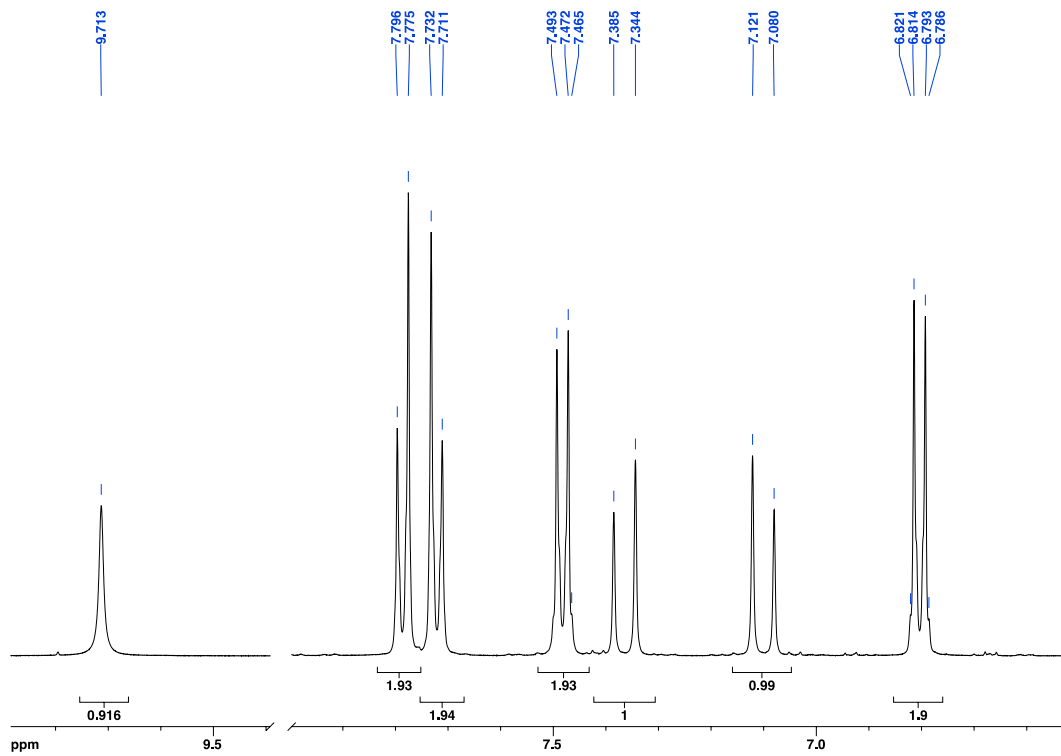
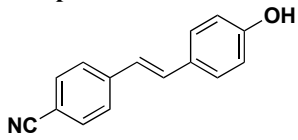
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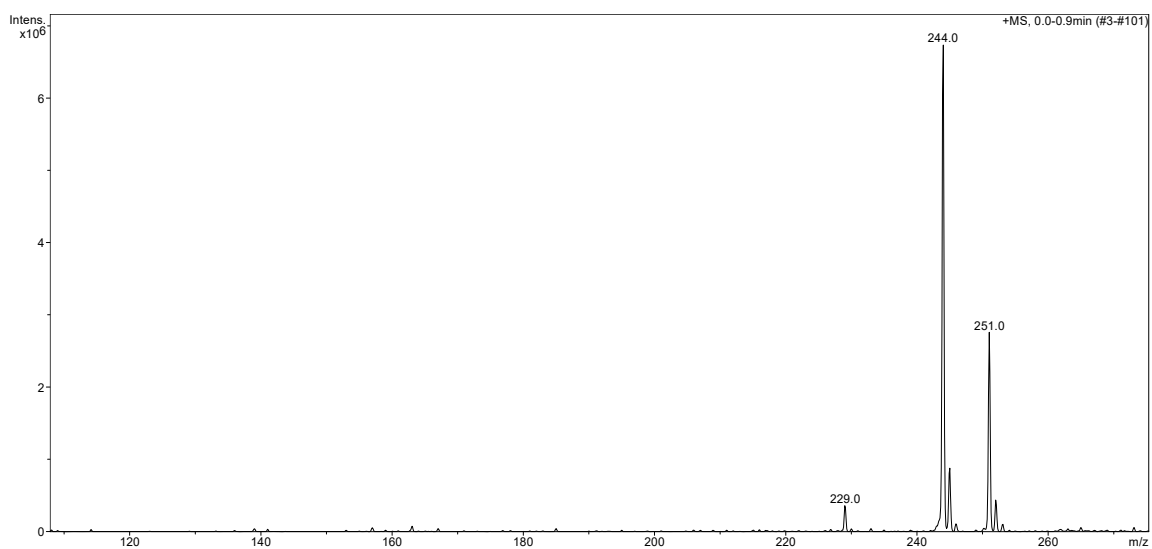
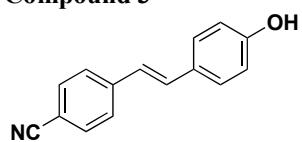
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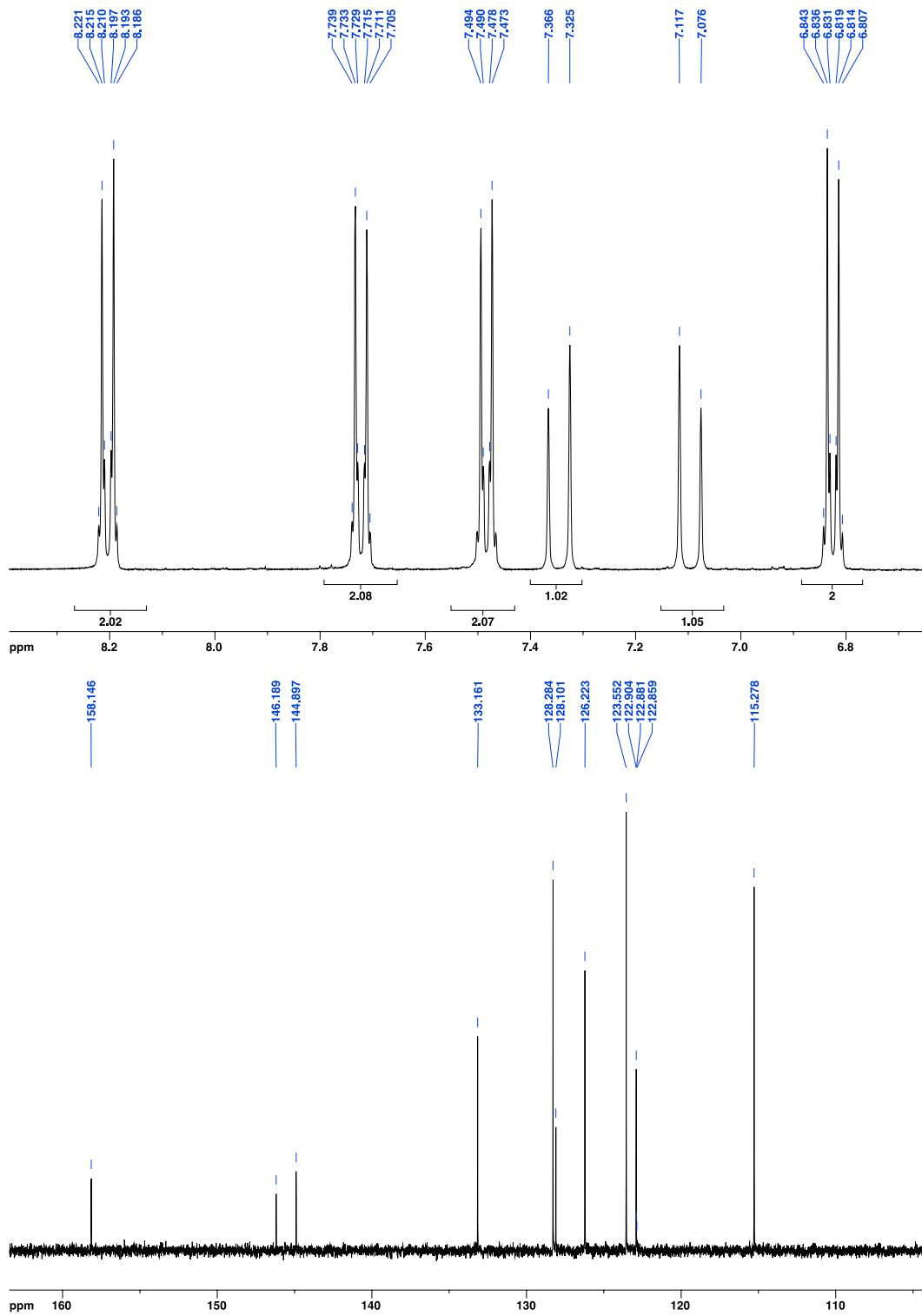
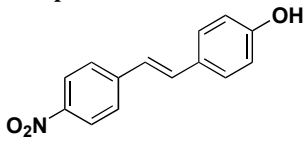
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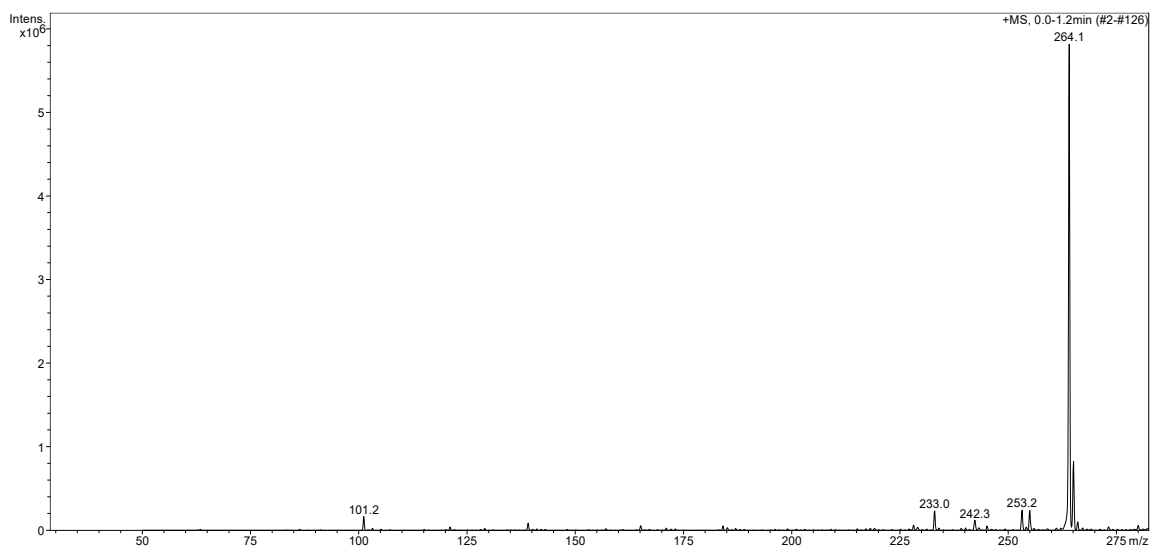
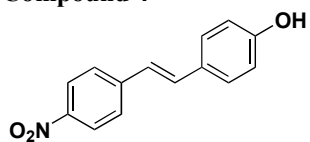
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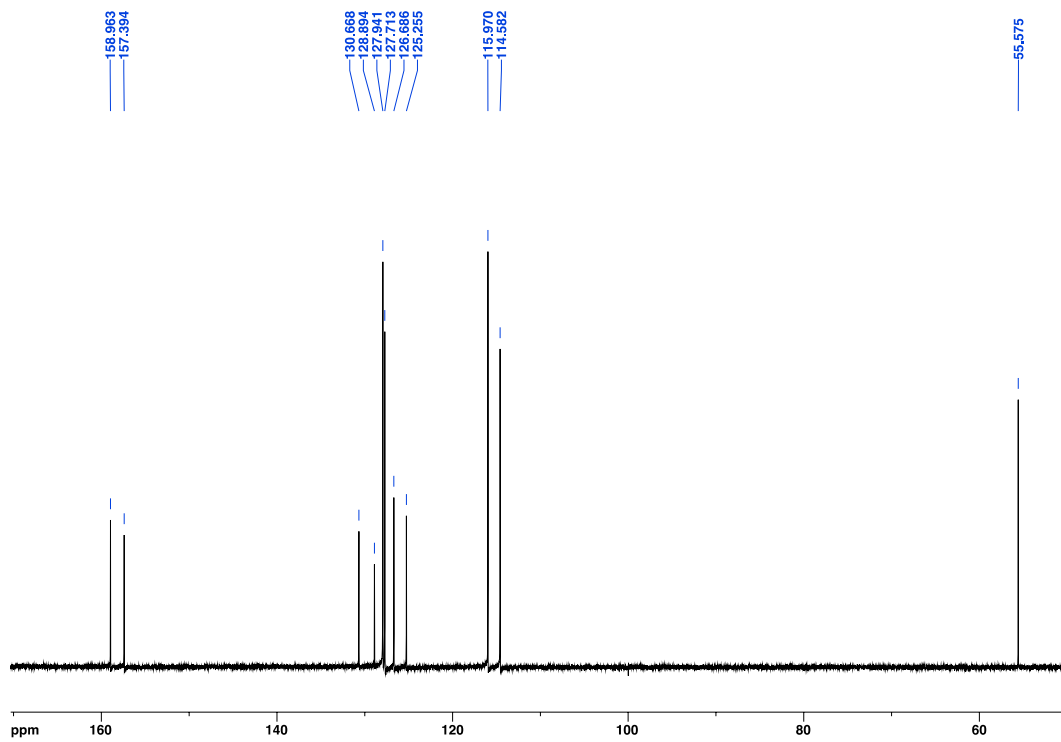
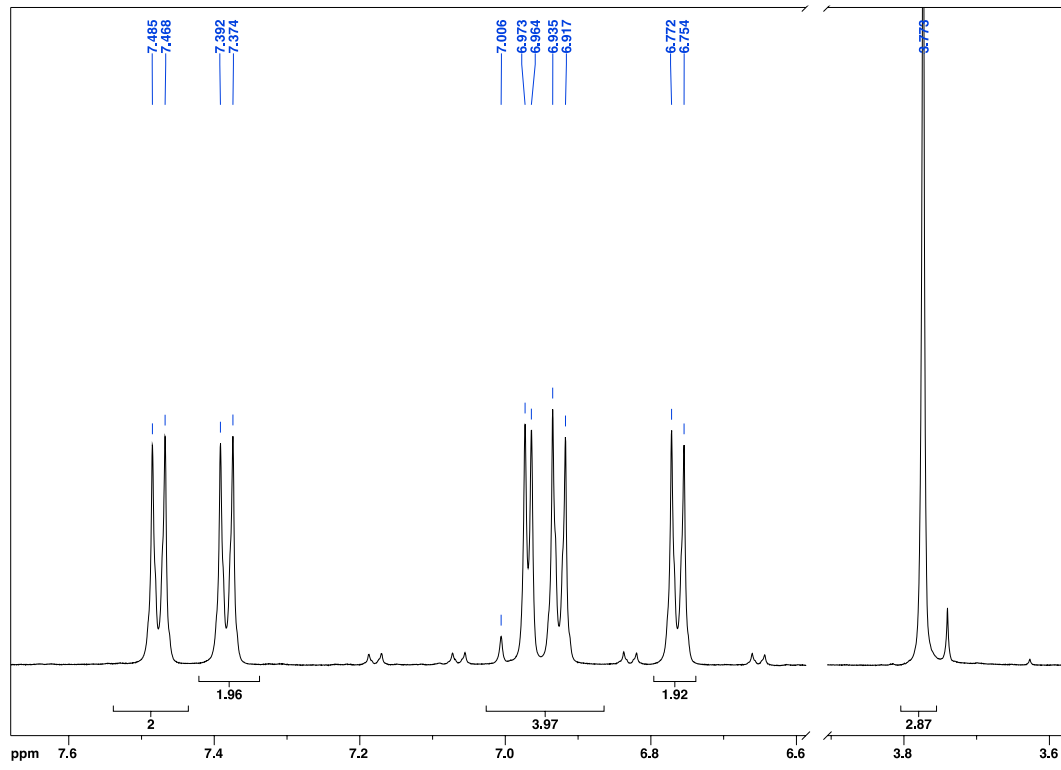
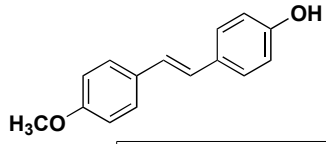
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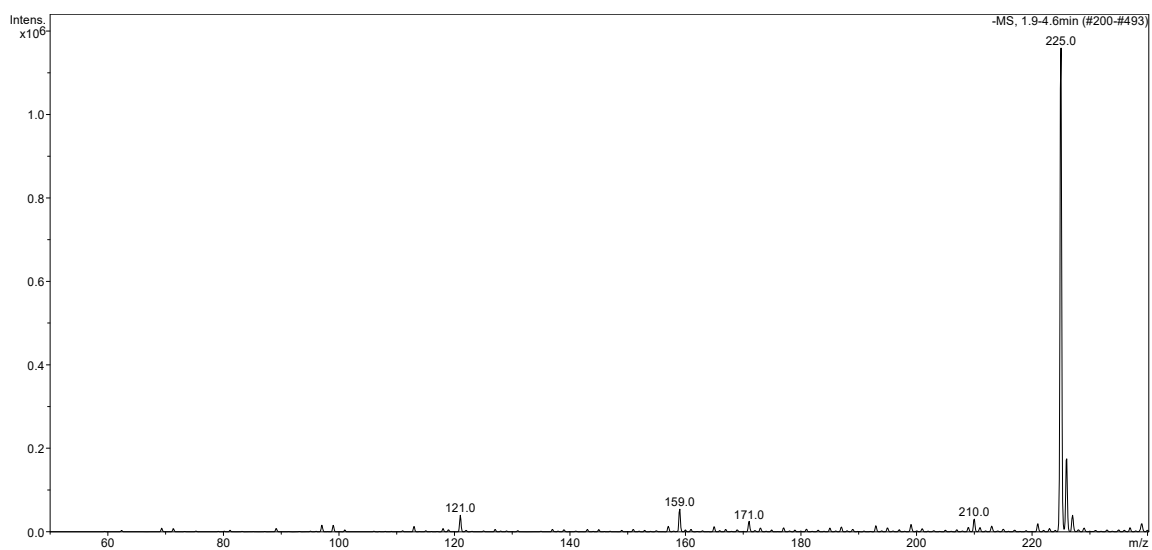
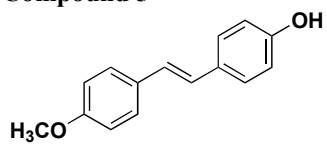
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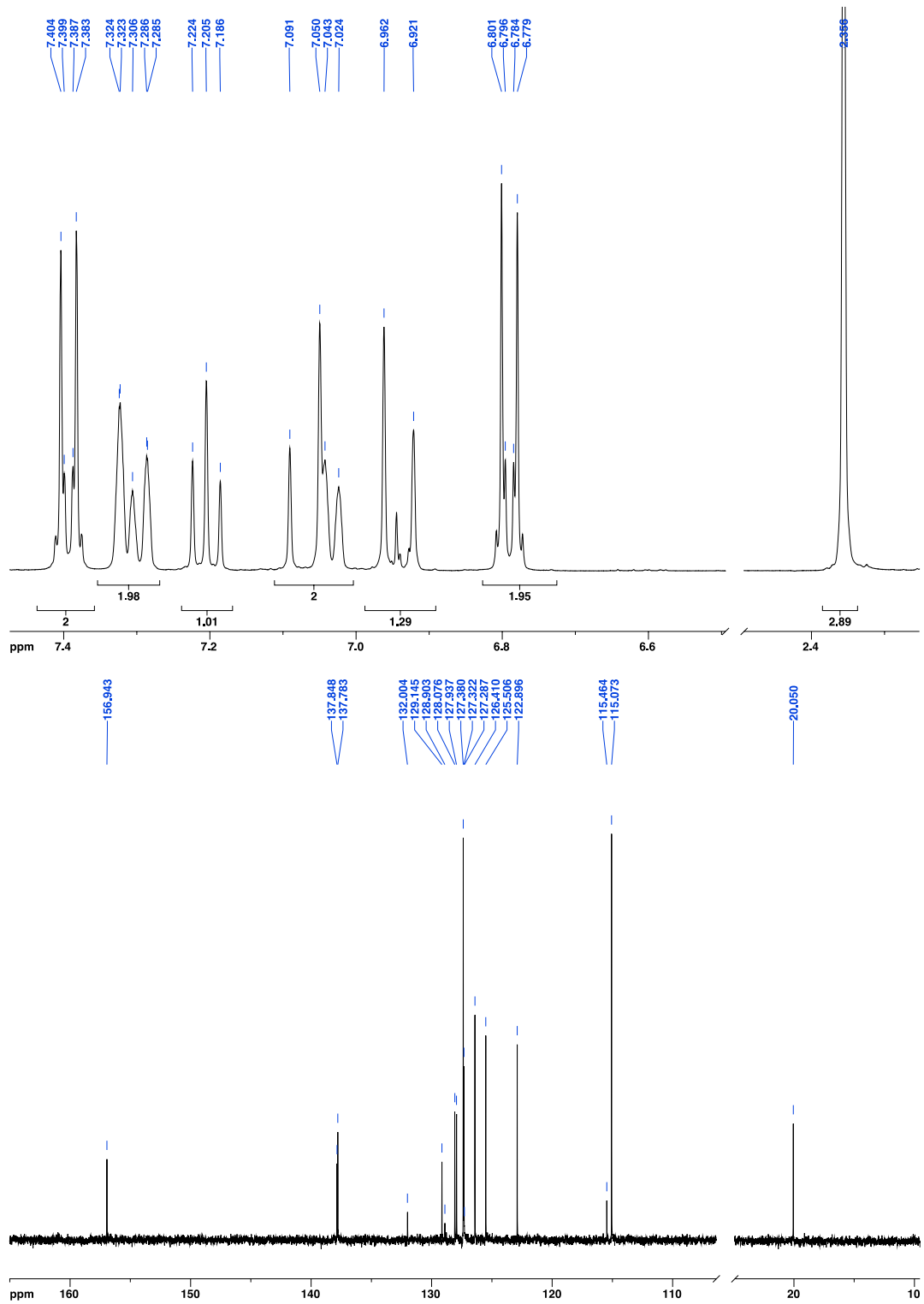
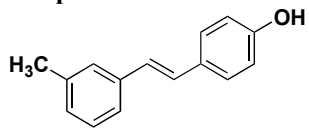
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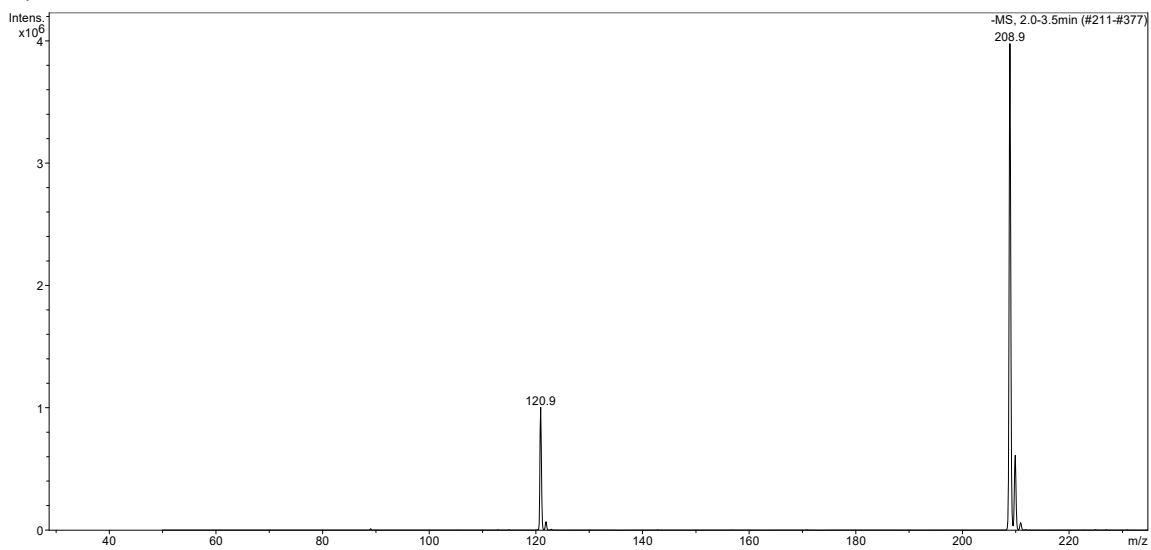
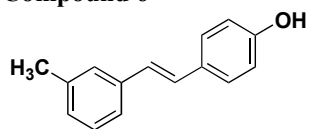
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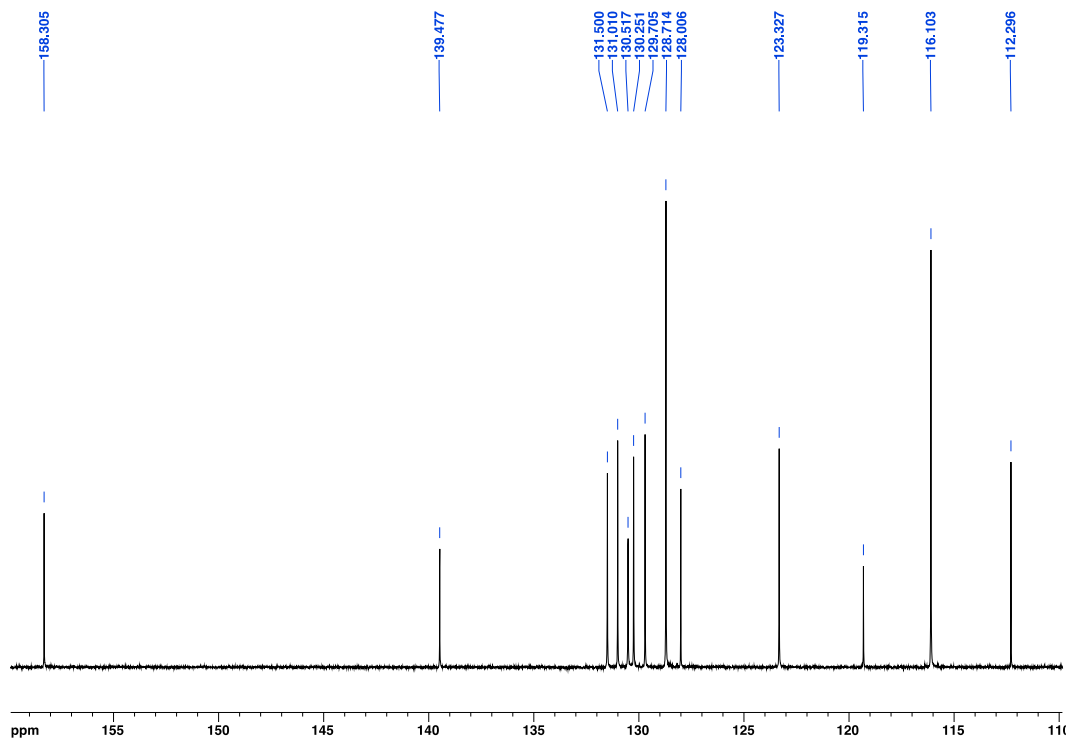
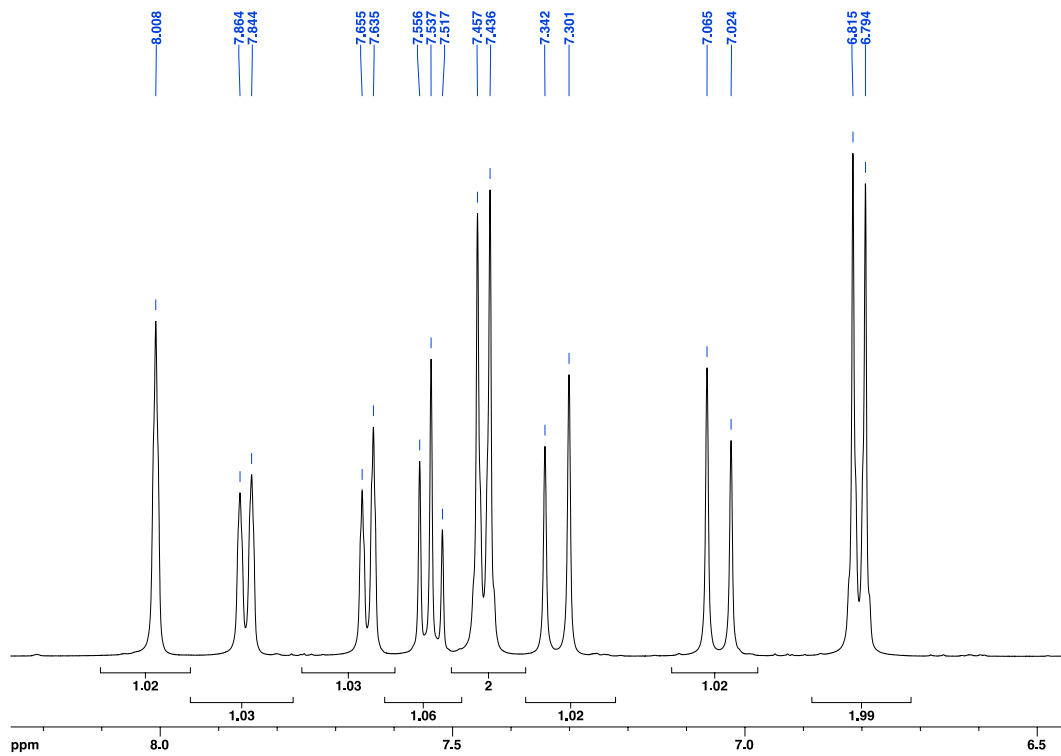
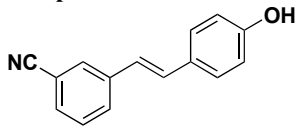
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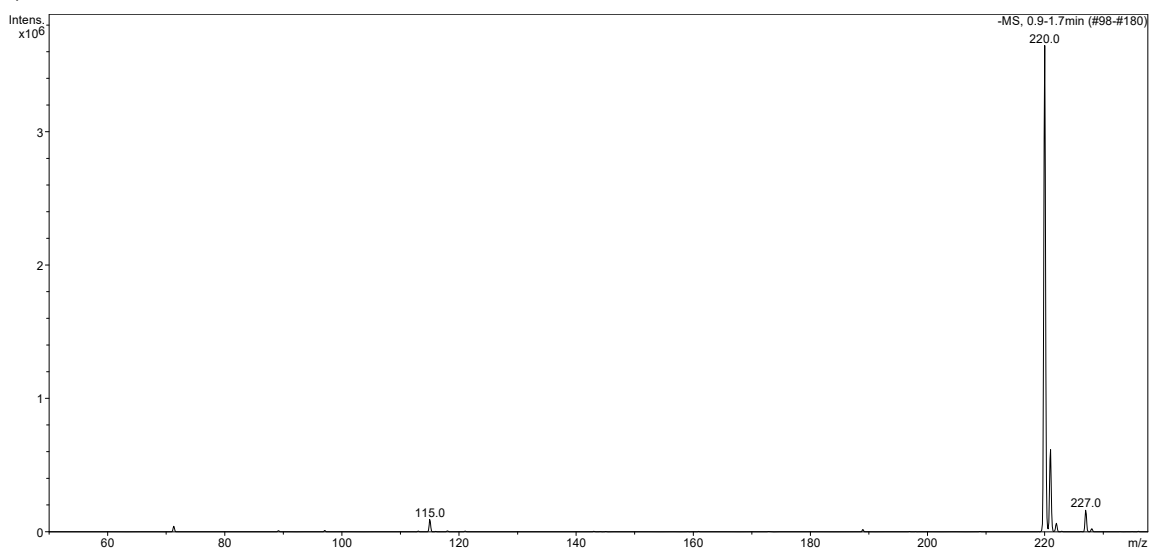
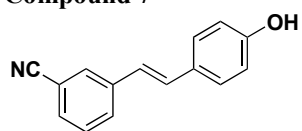
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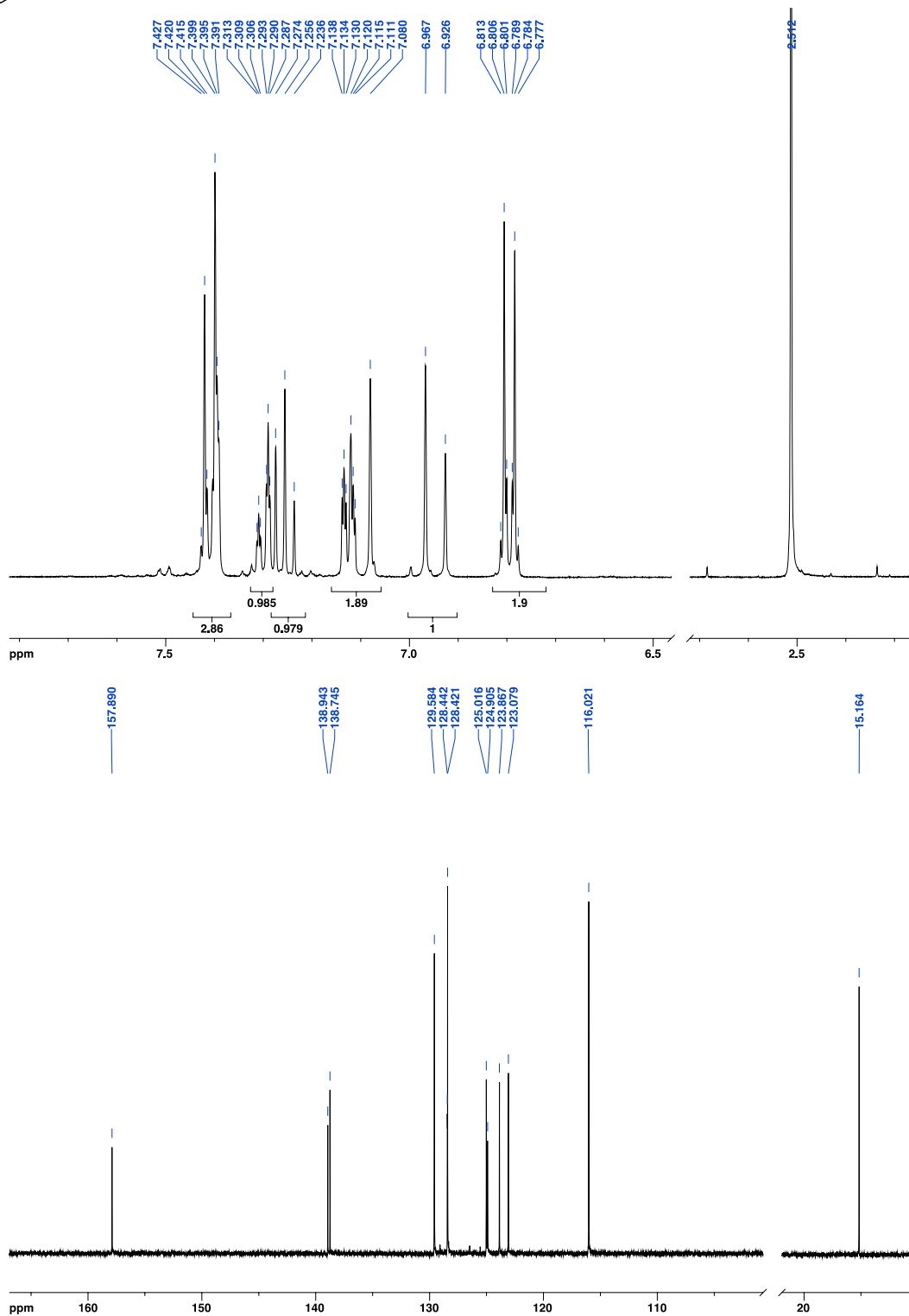
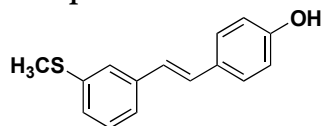
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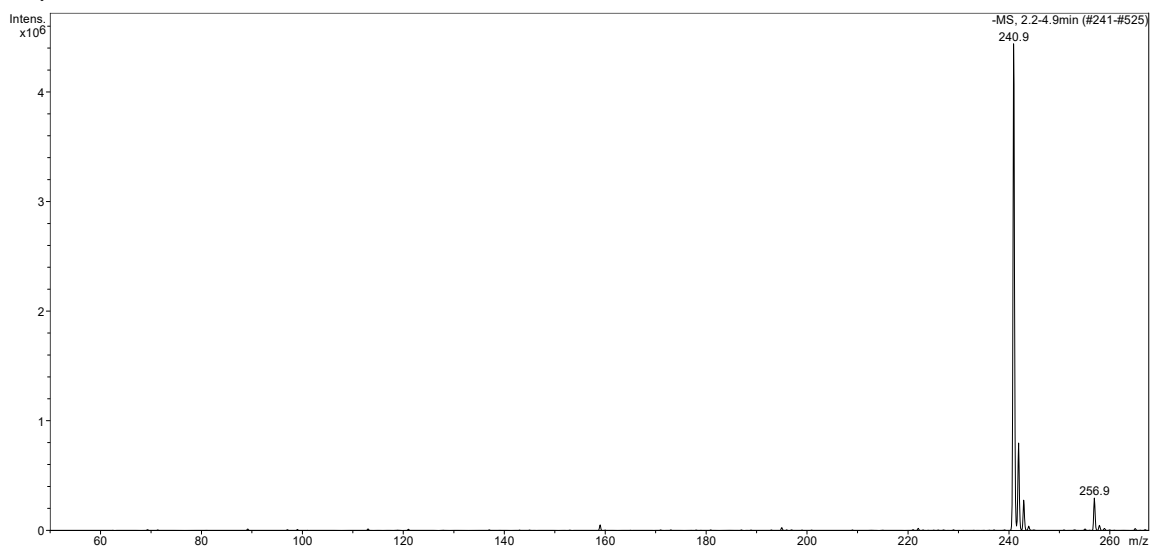
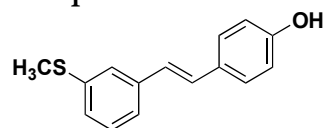
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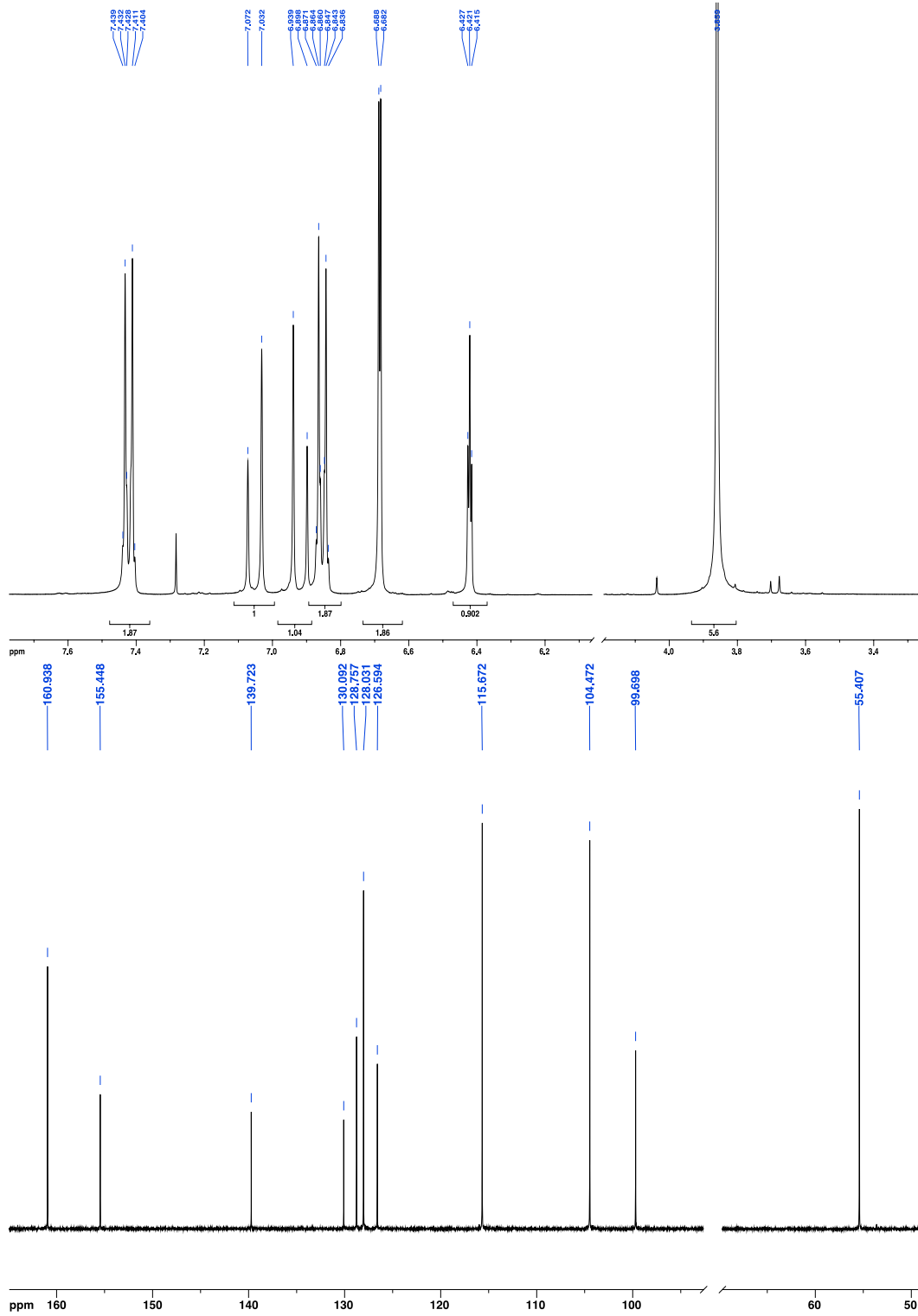
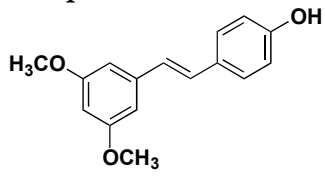
Compound 8



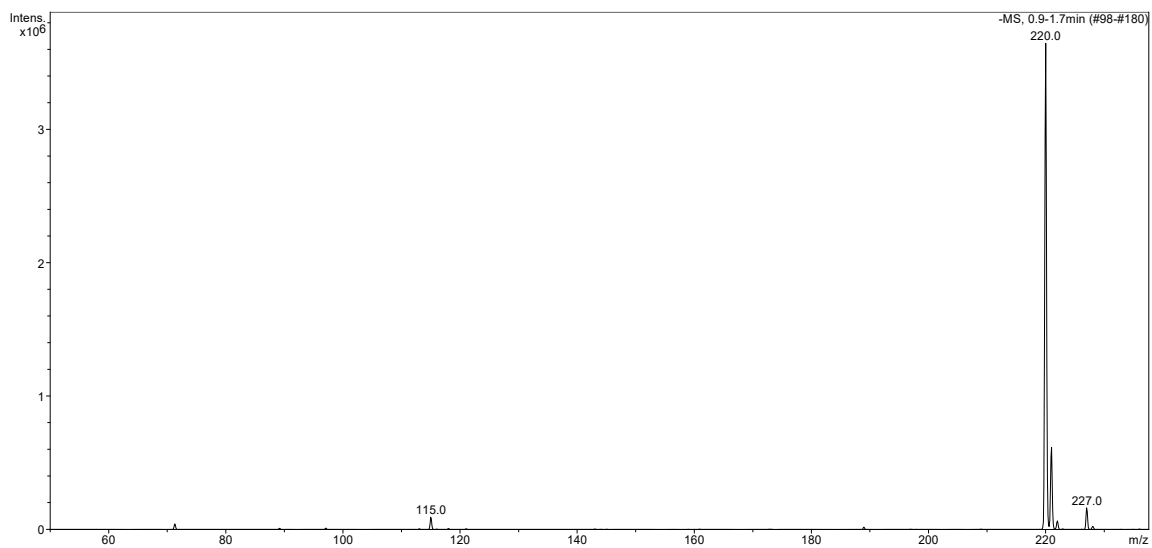
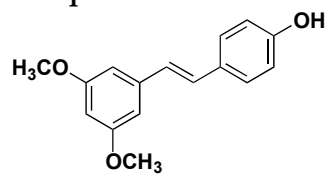
Compound 8



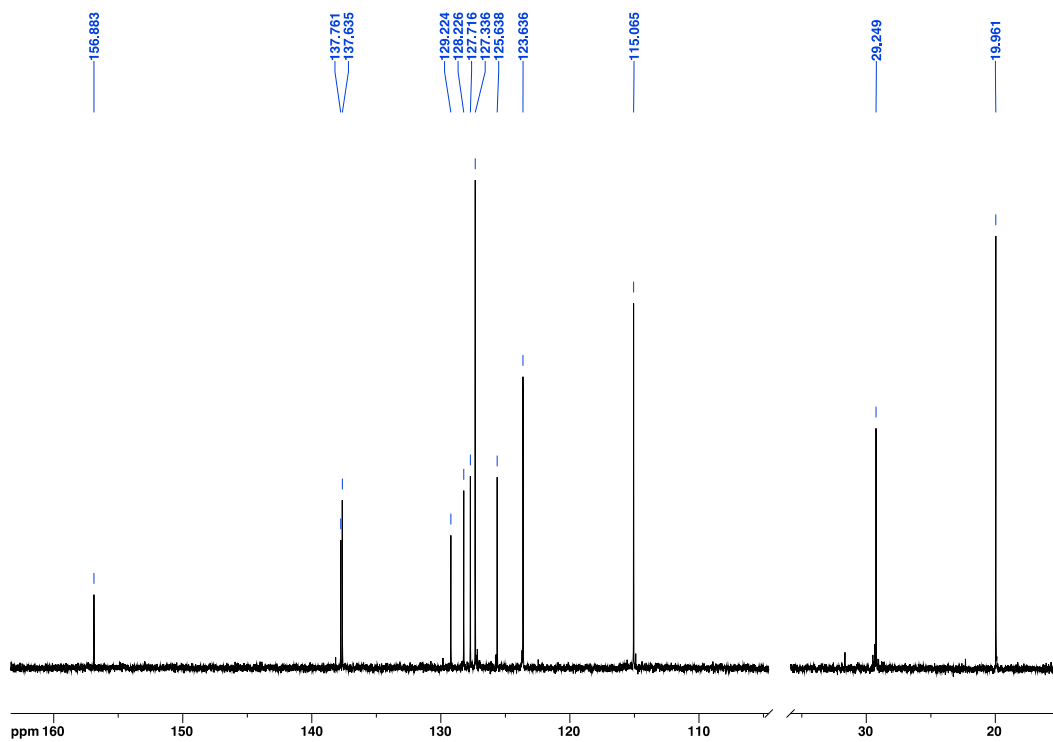
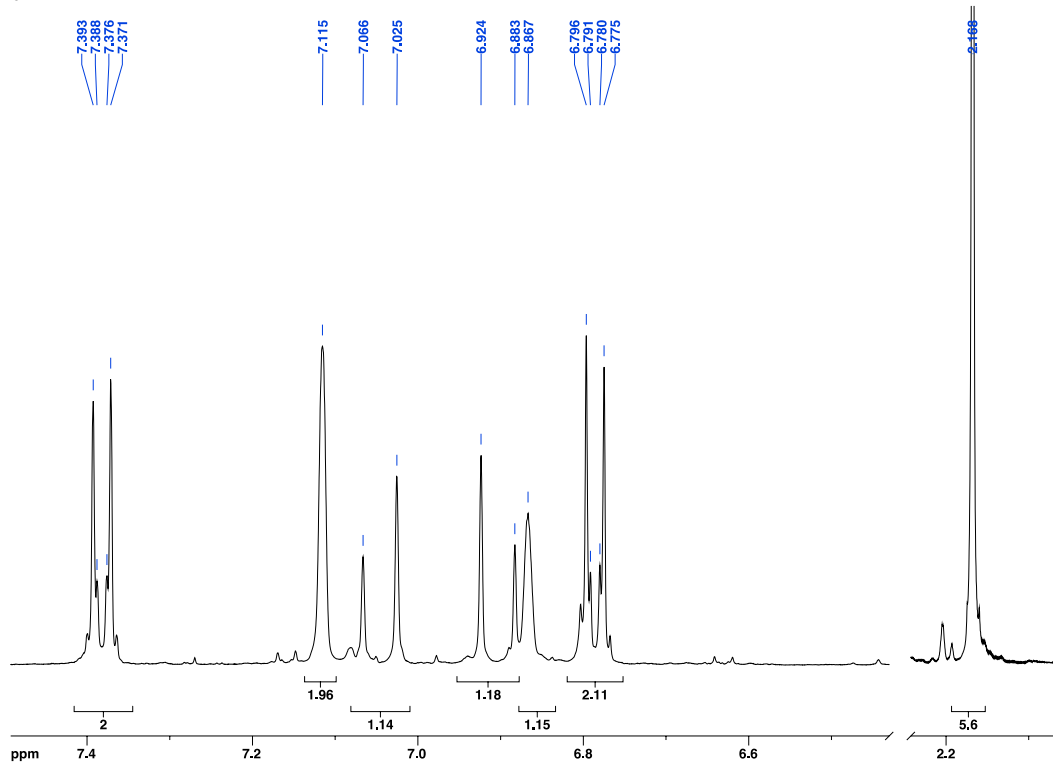
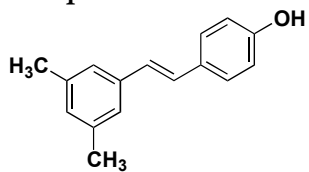
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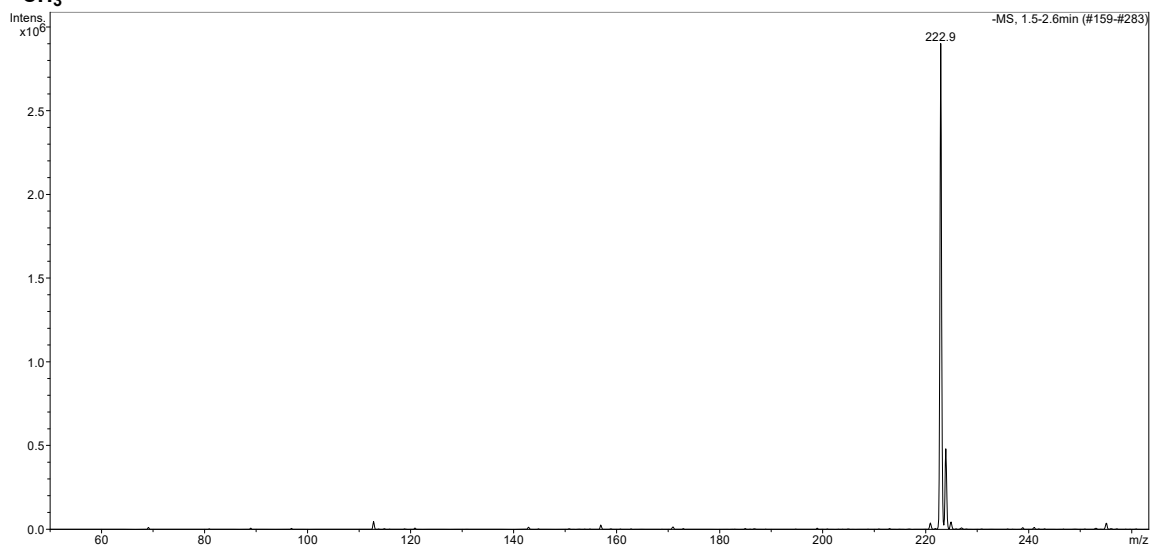
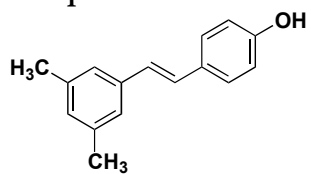
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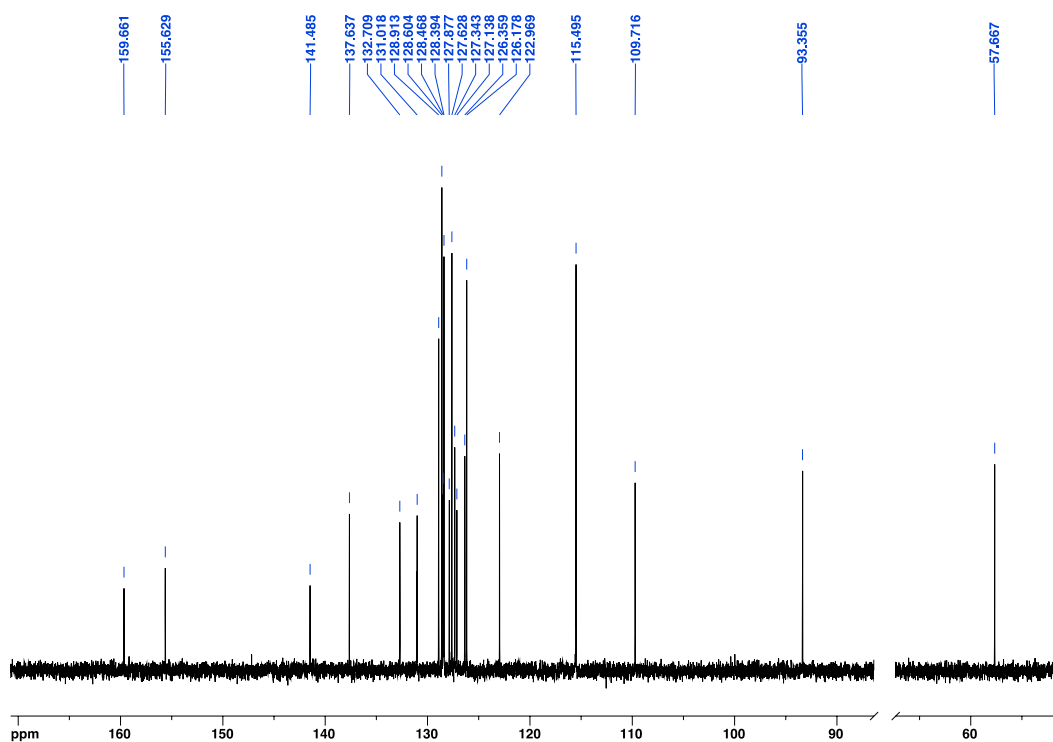
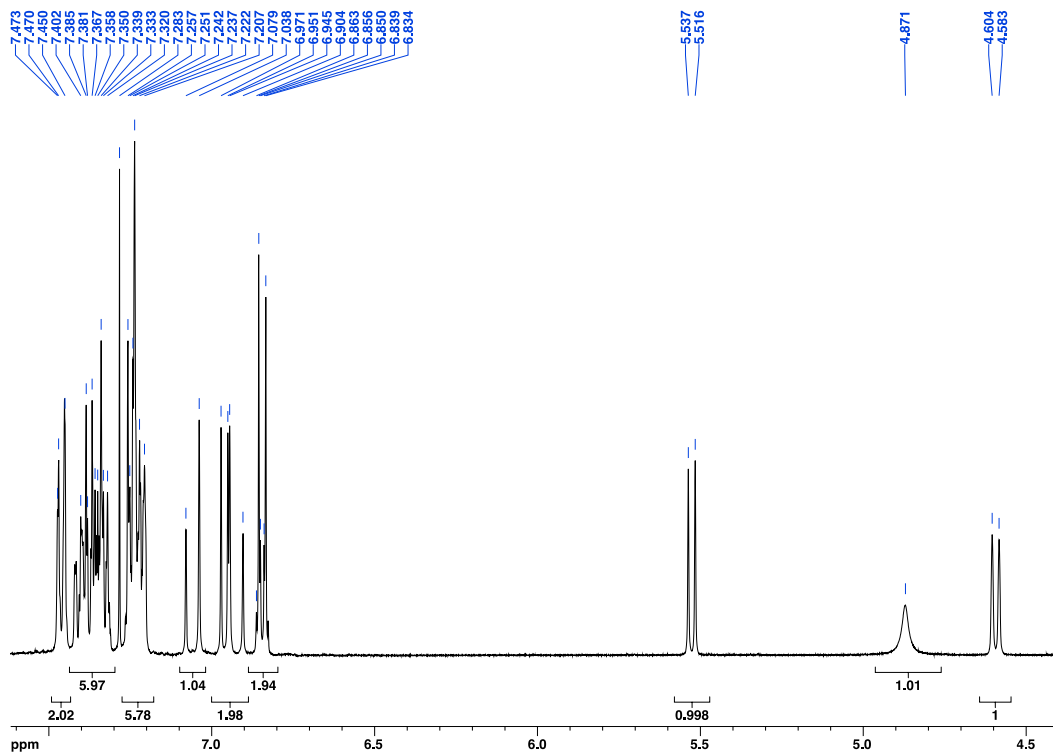
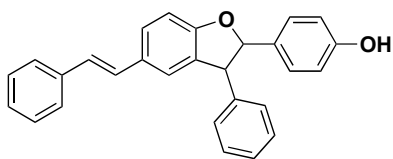
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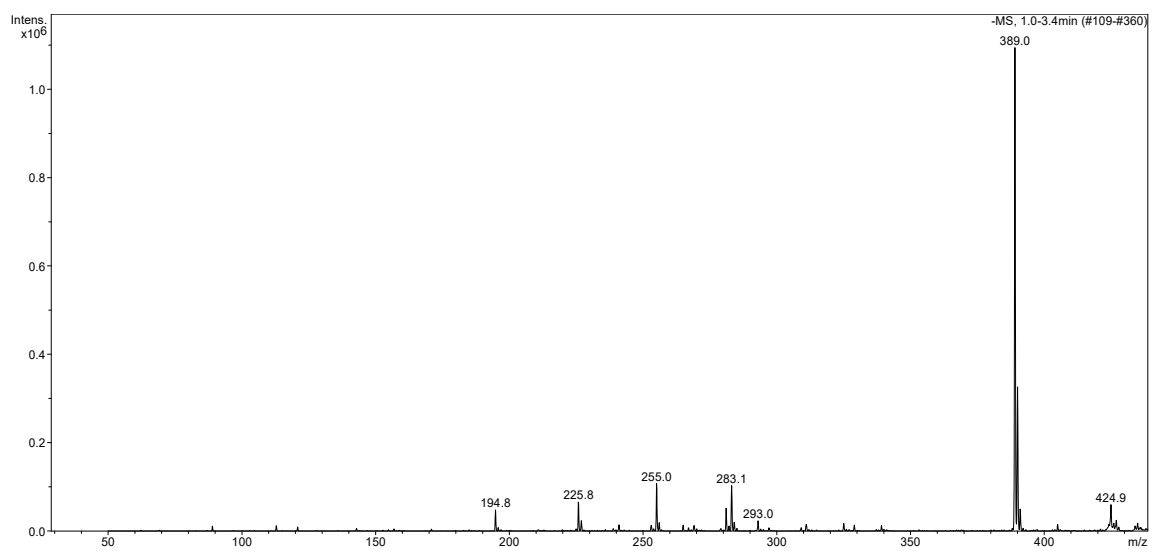
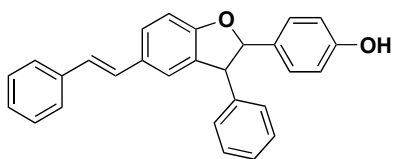
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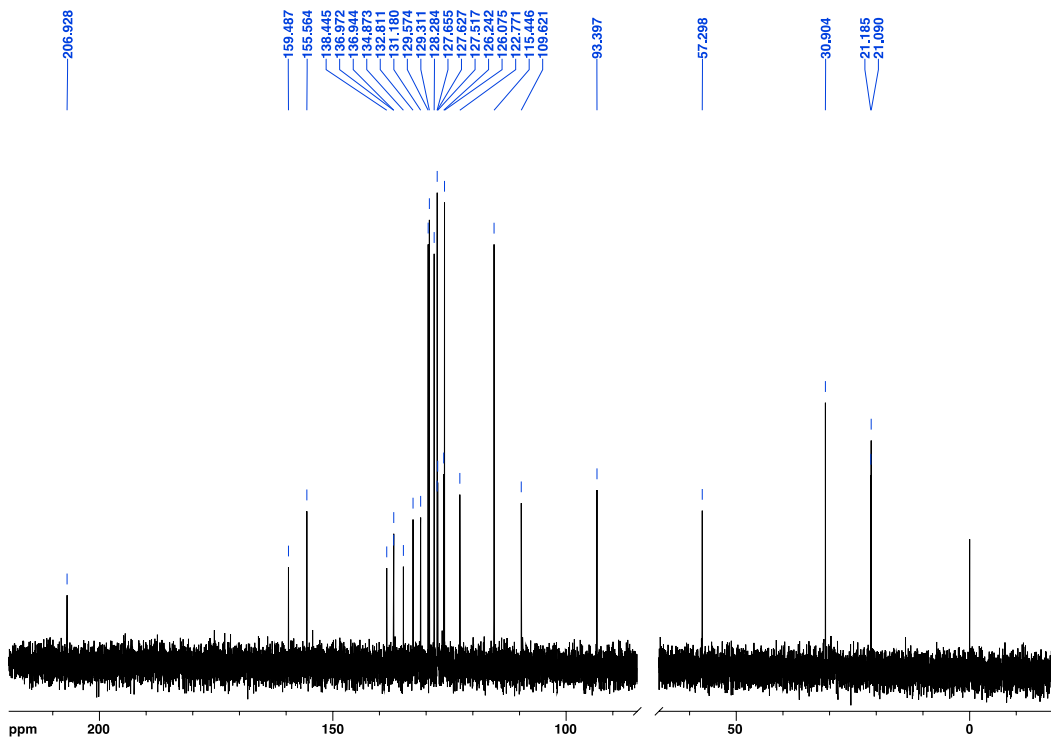
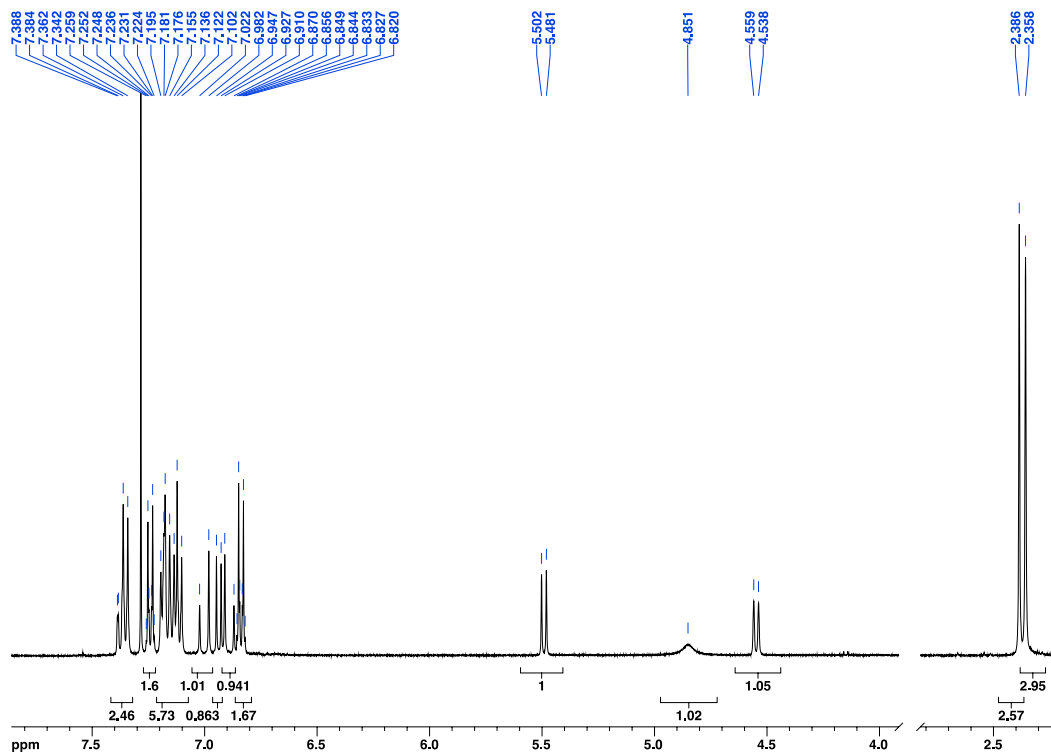
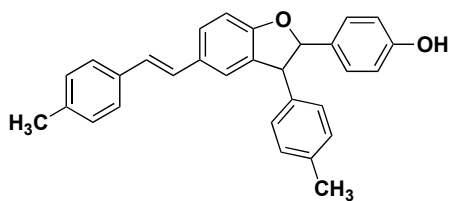
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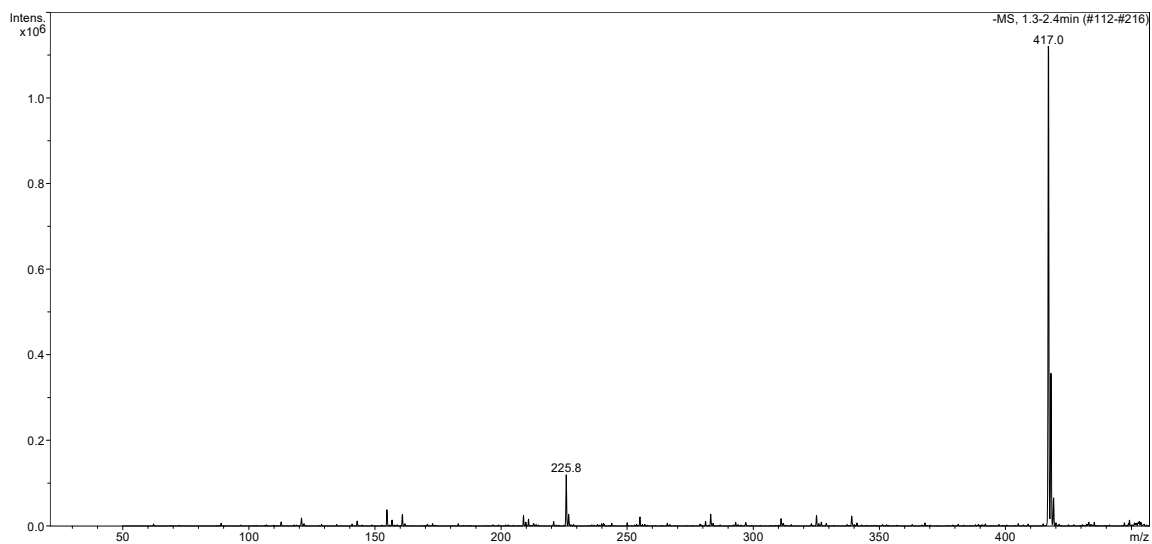
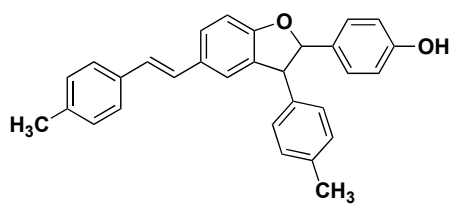
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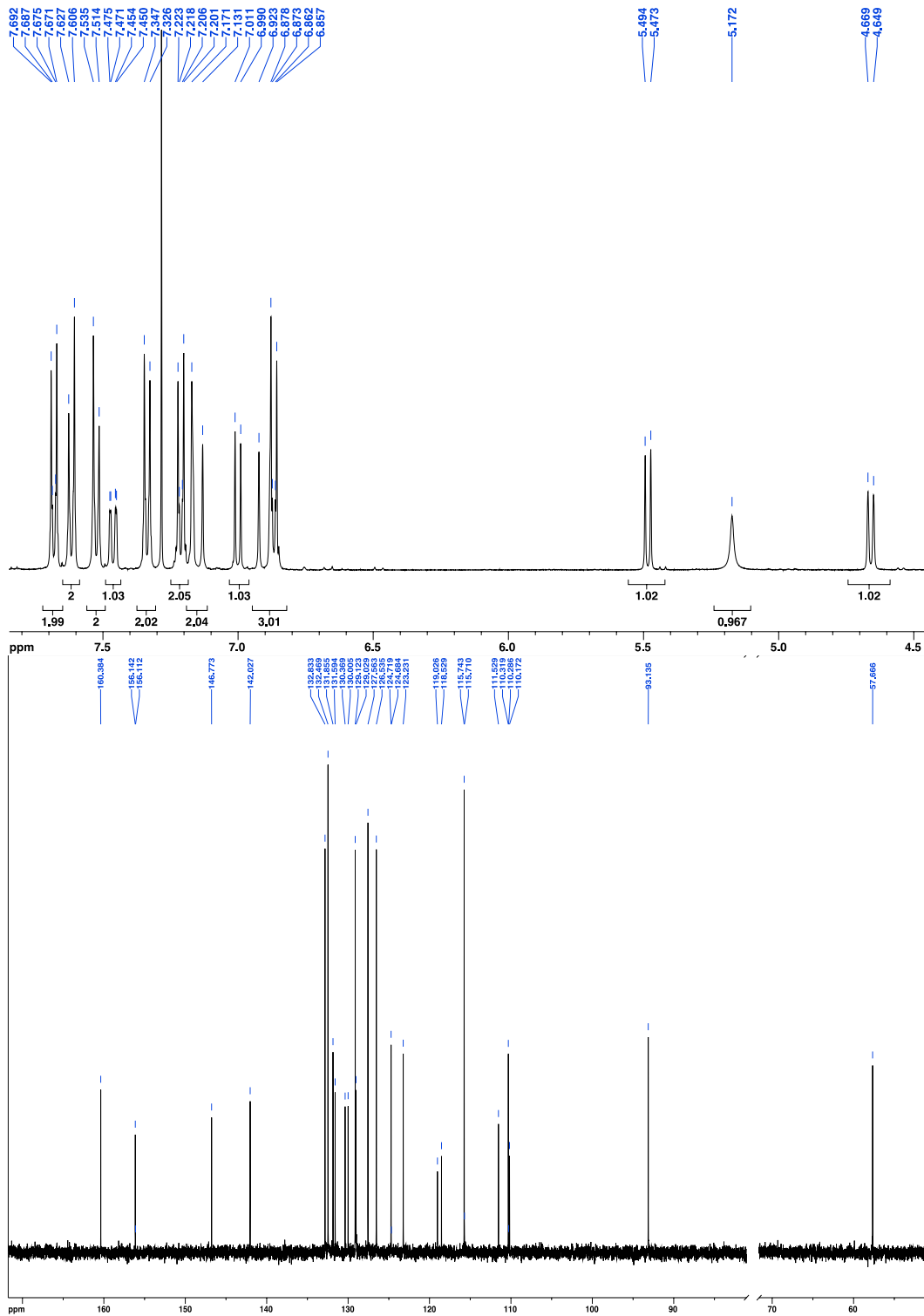
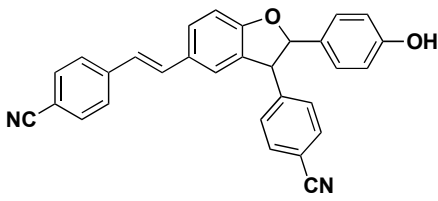
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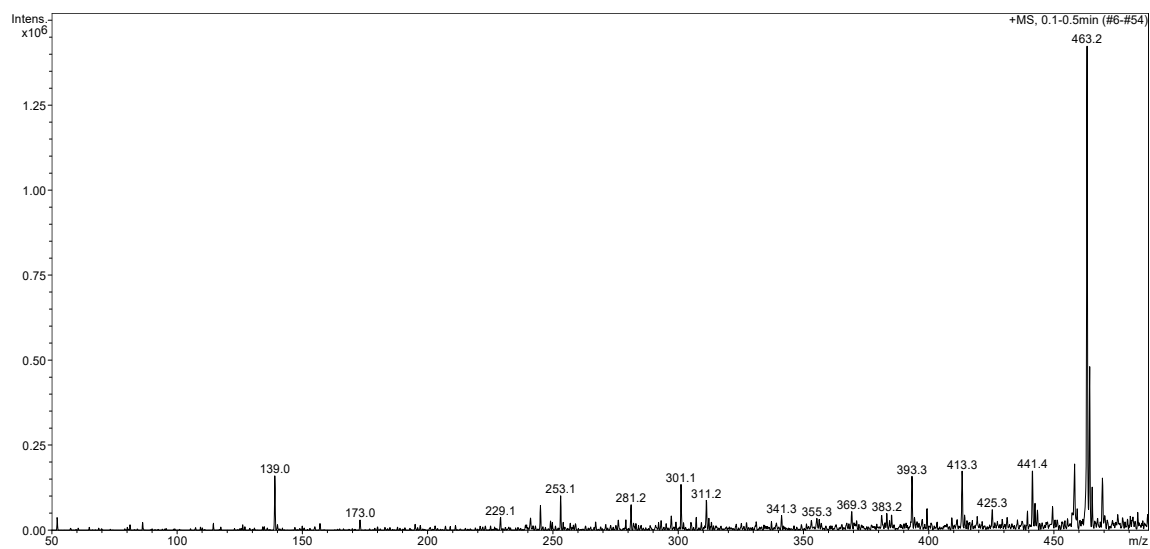
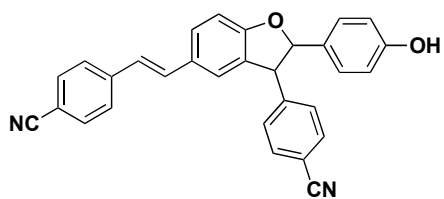
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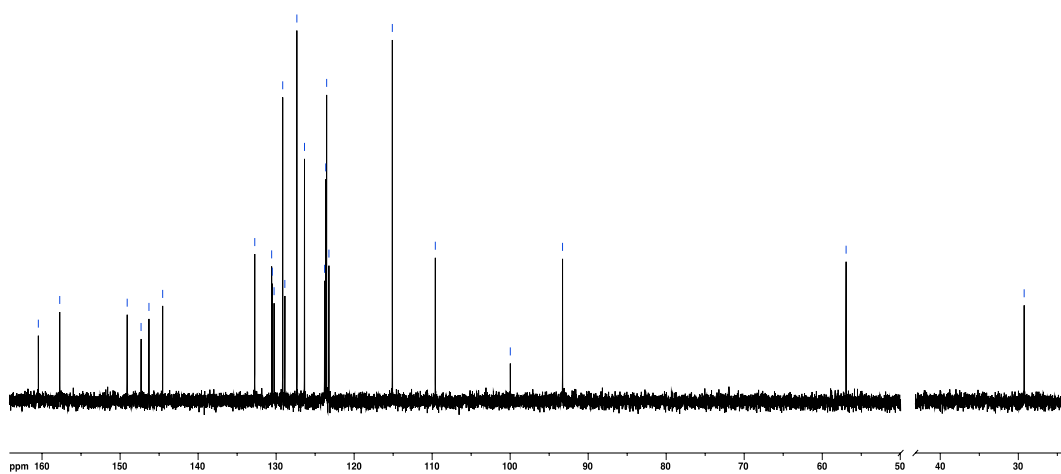
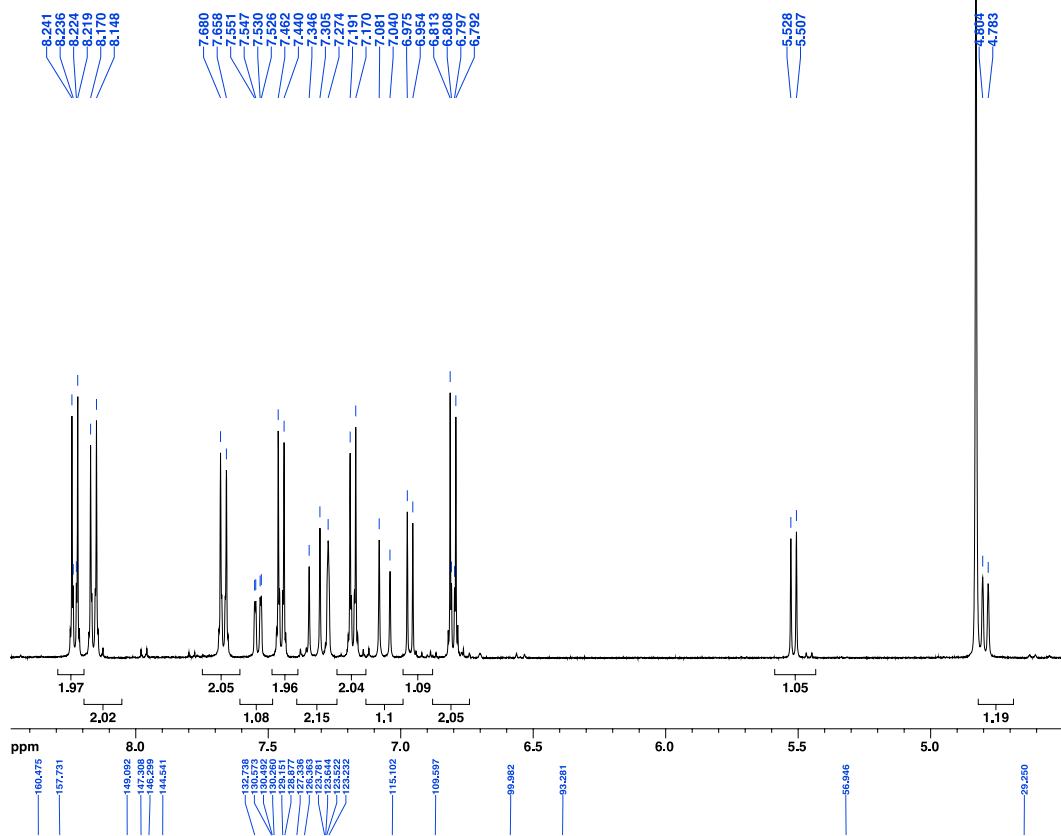
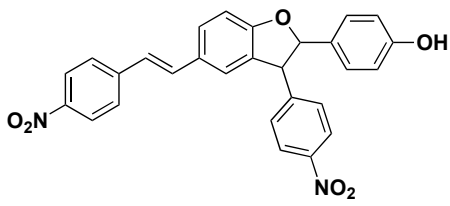
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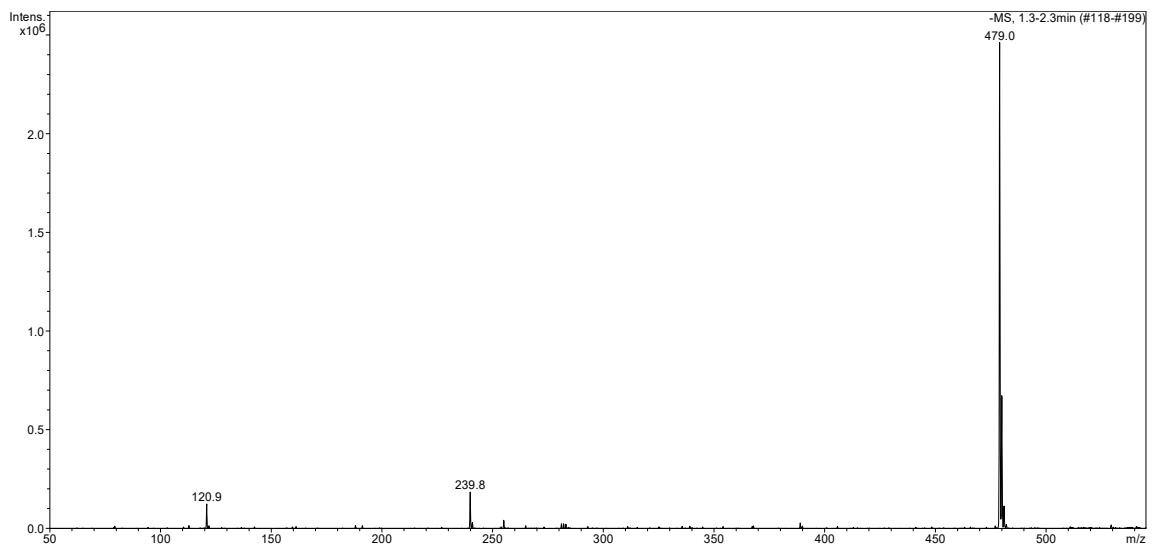
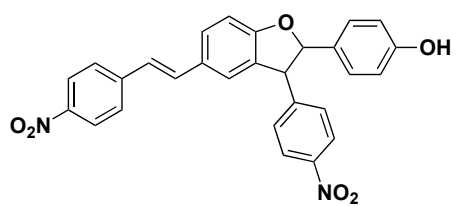
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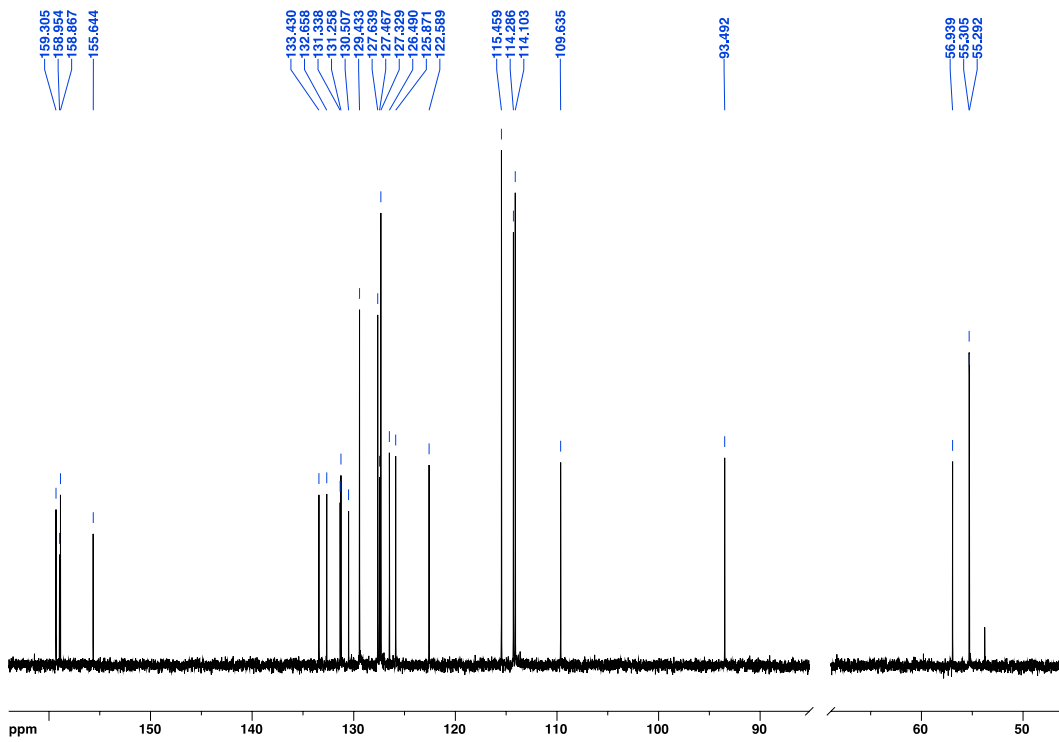
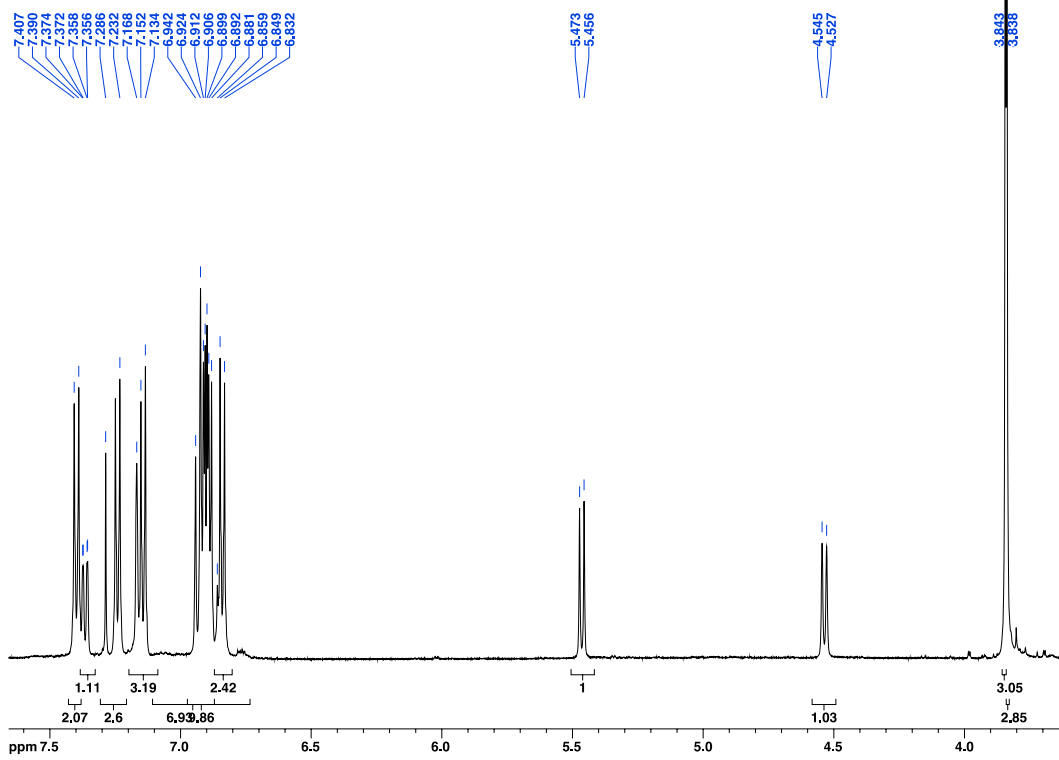
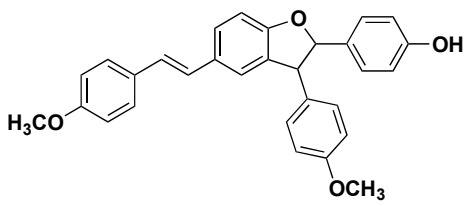
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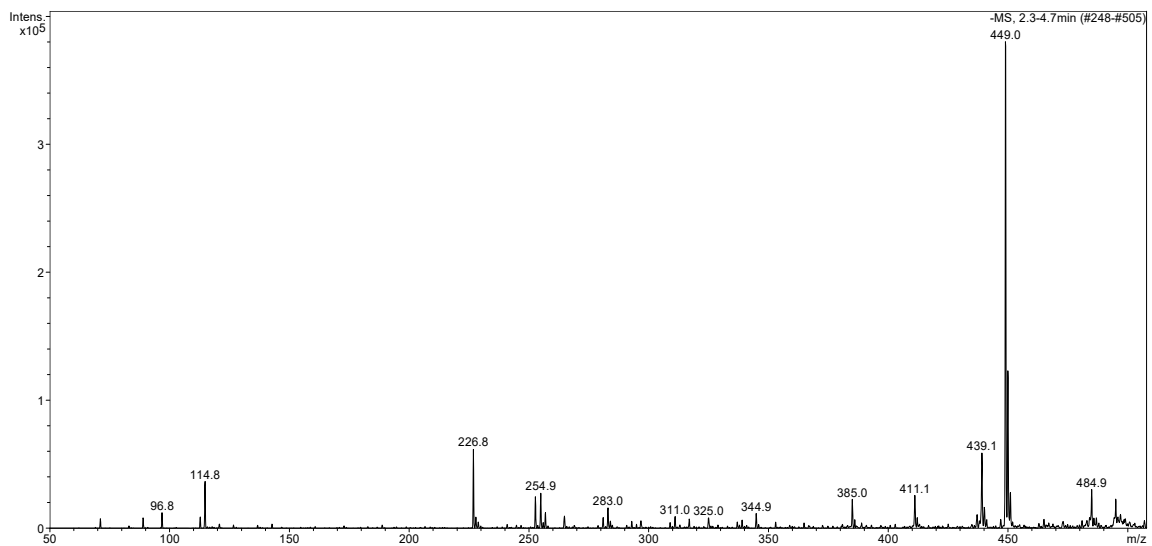
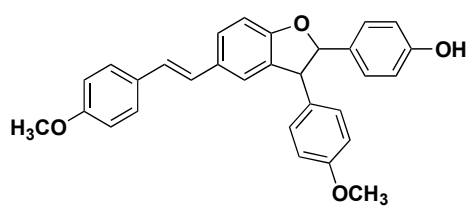
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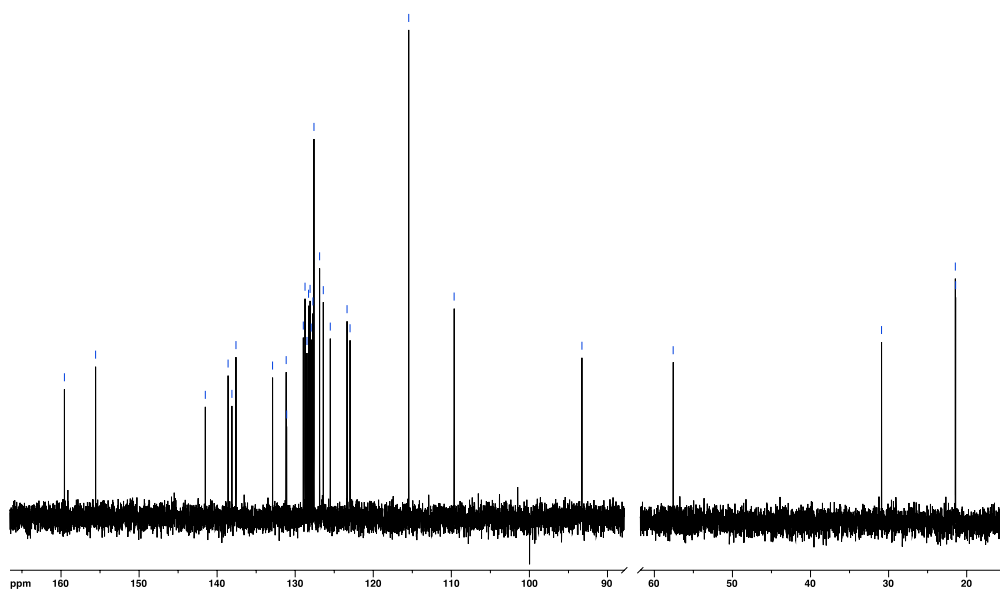
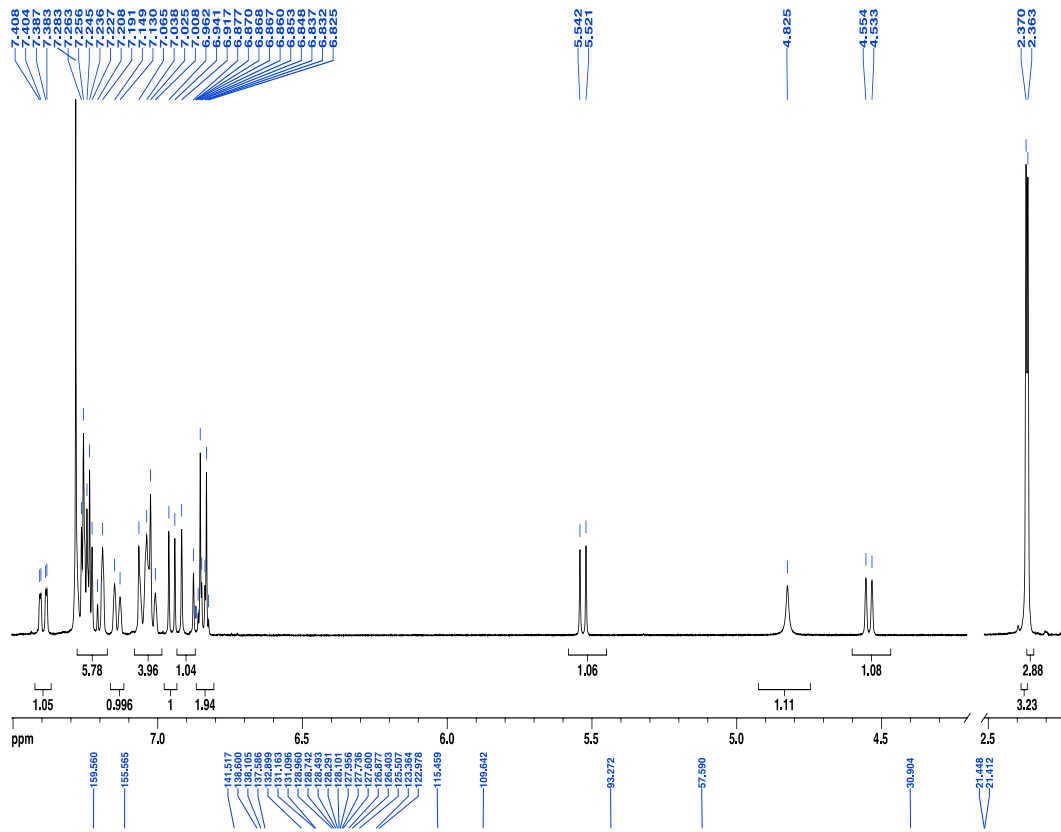
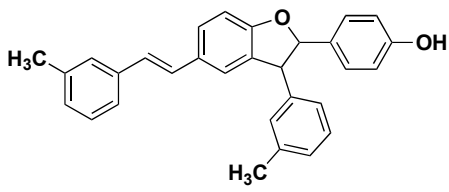
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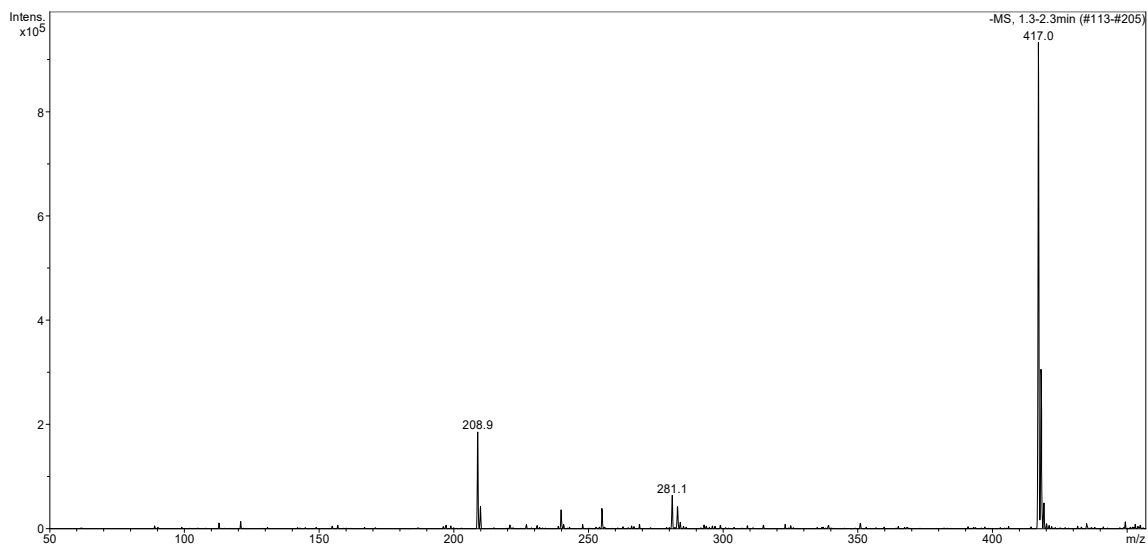
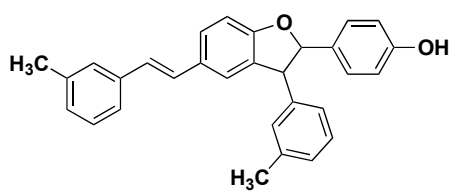
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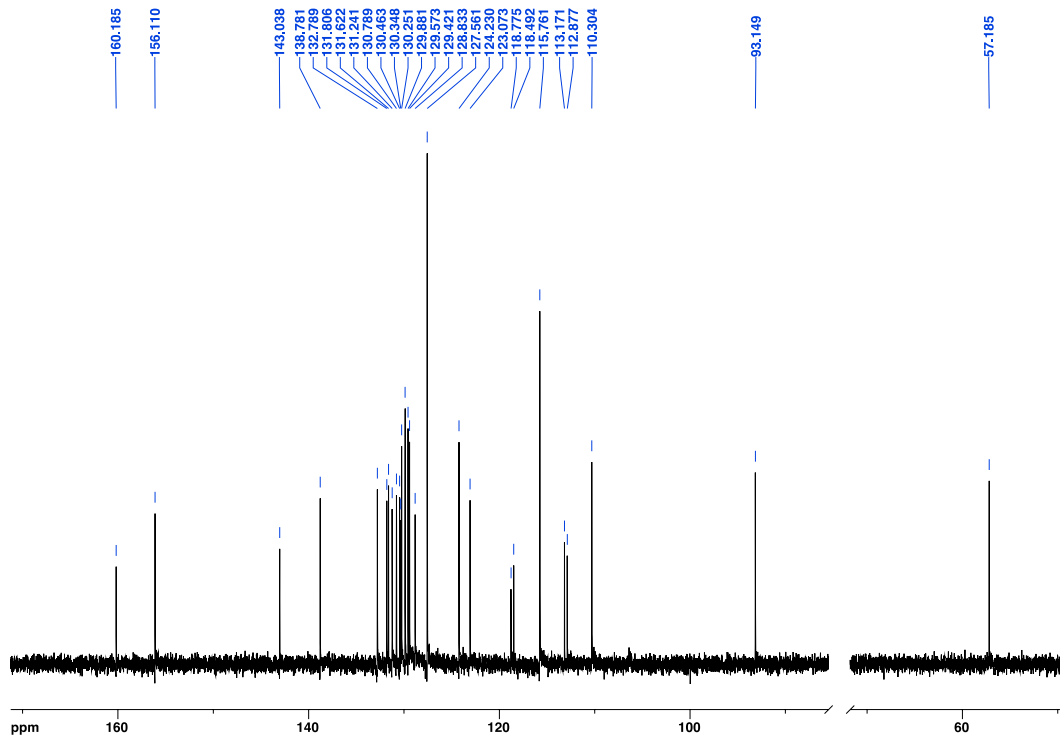
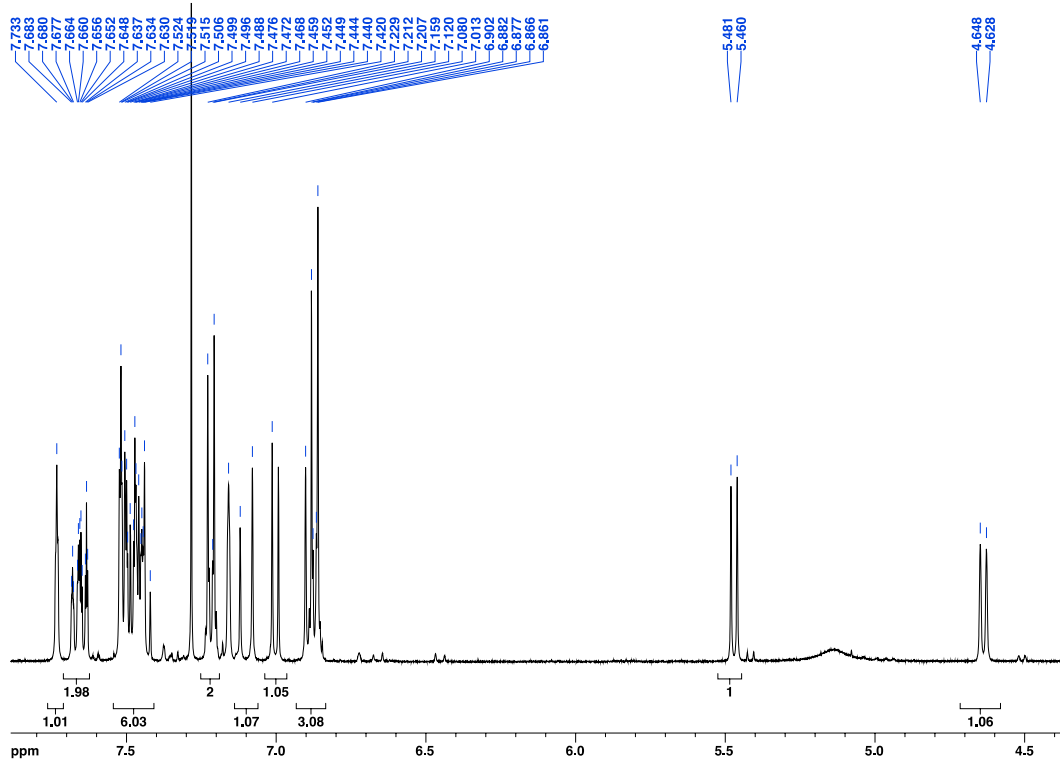
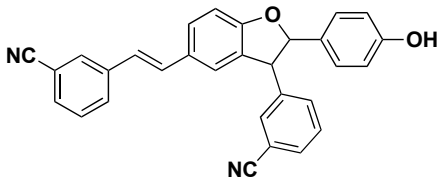
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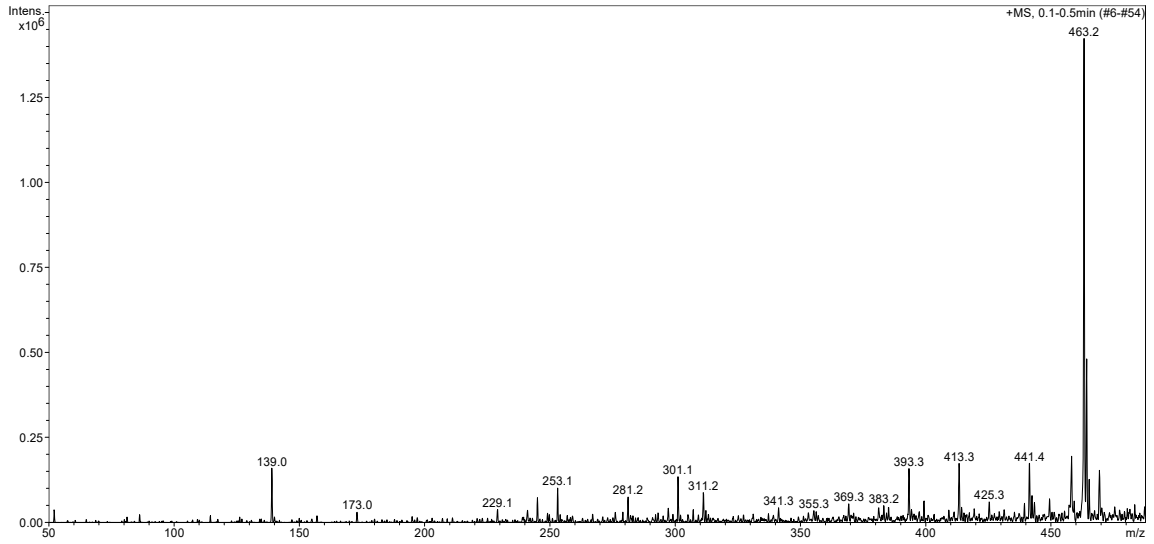
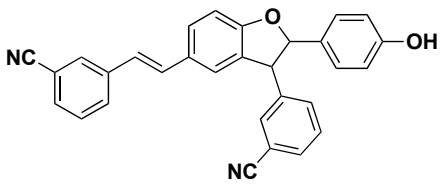
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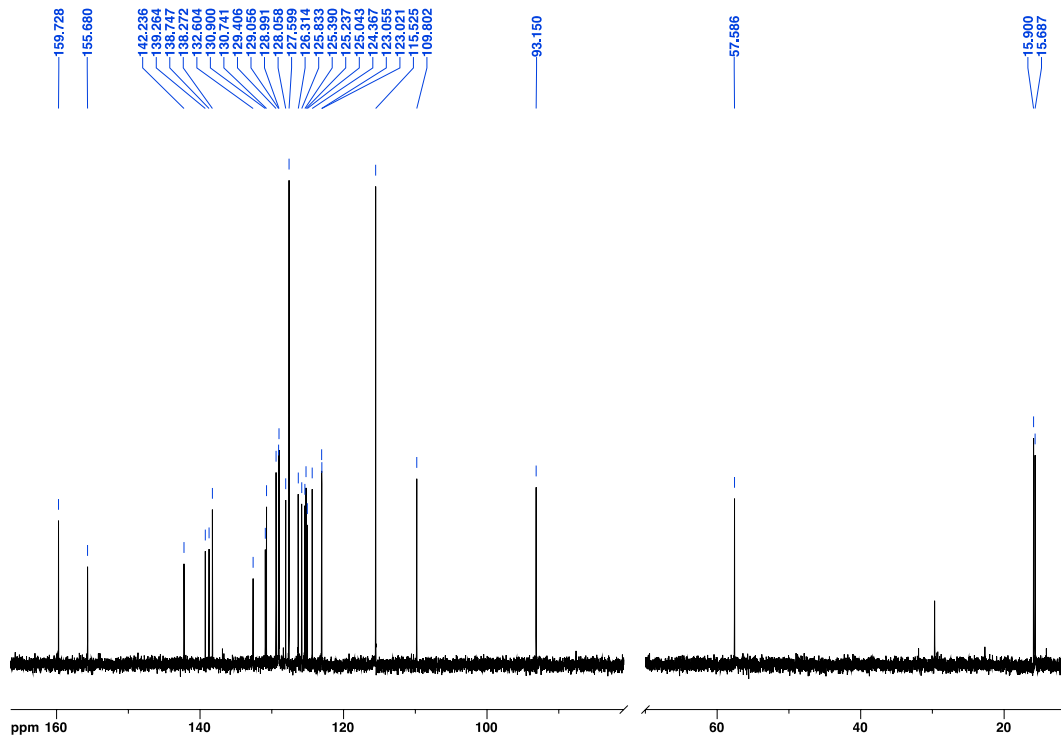
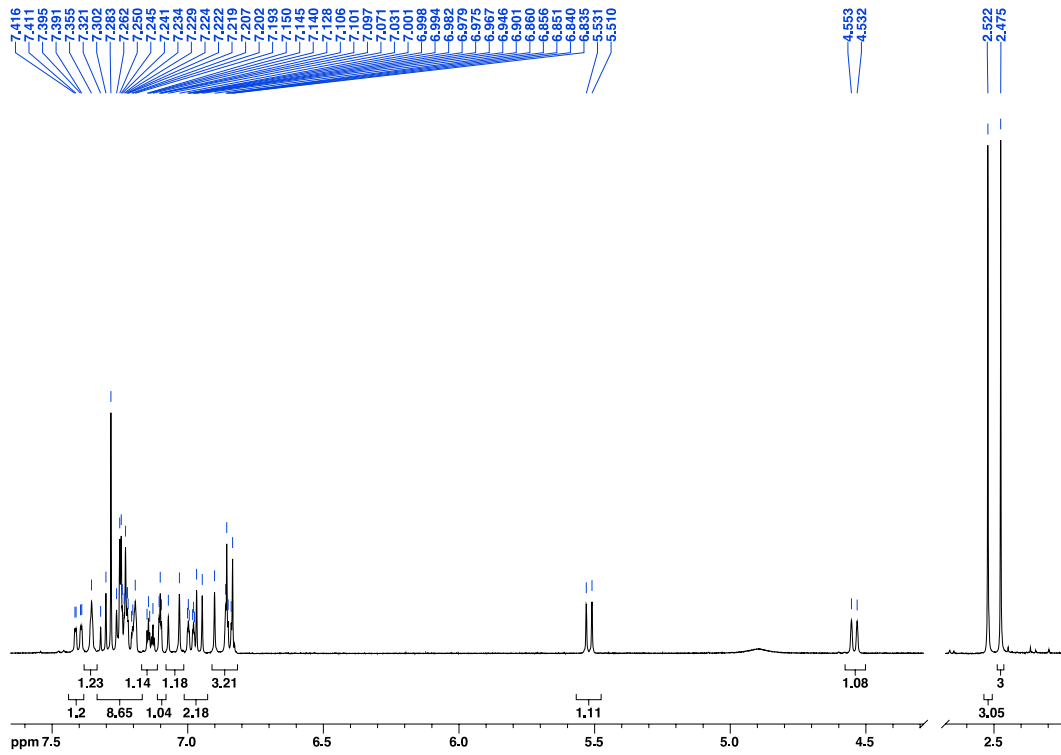
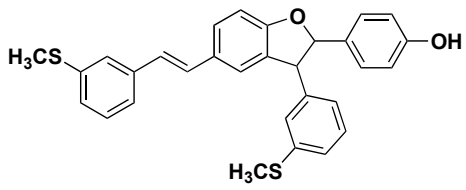
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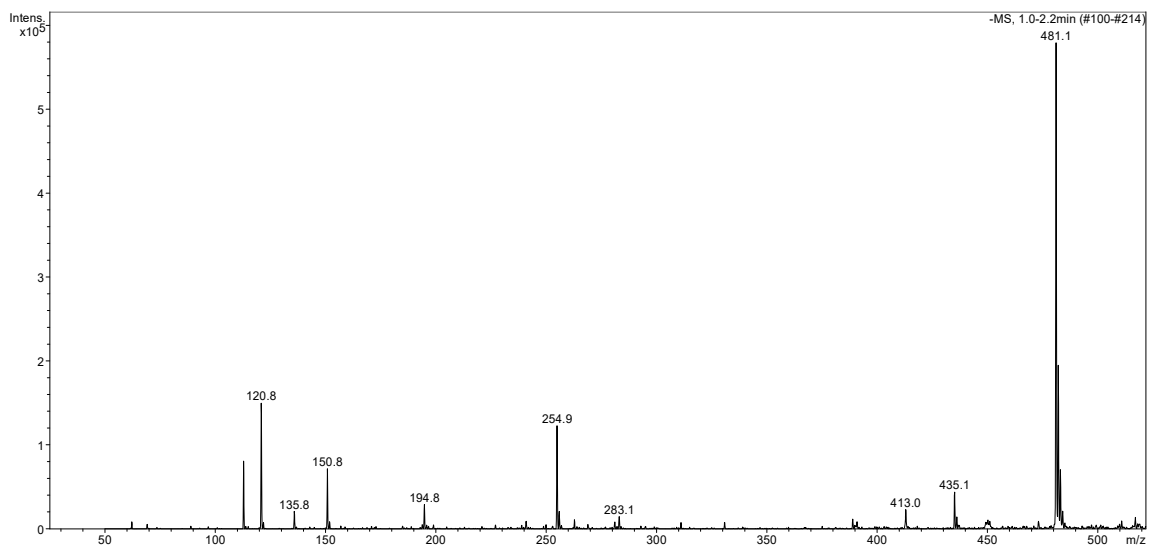
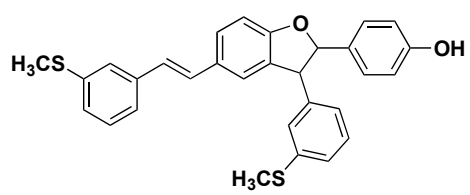
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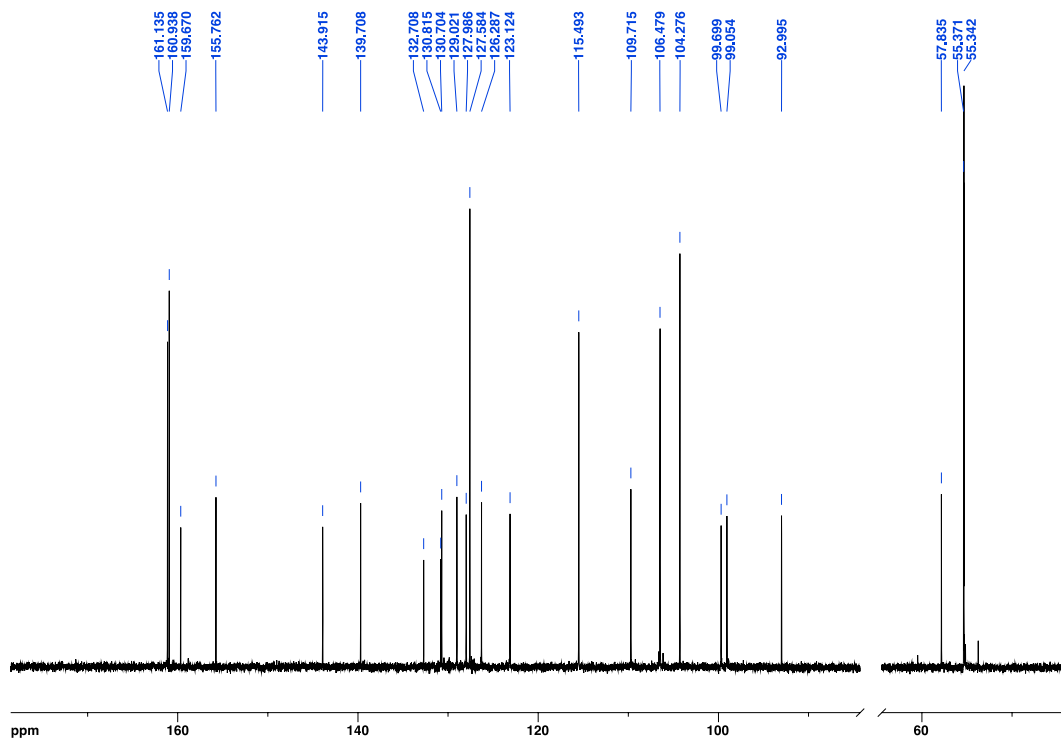
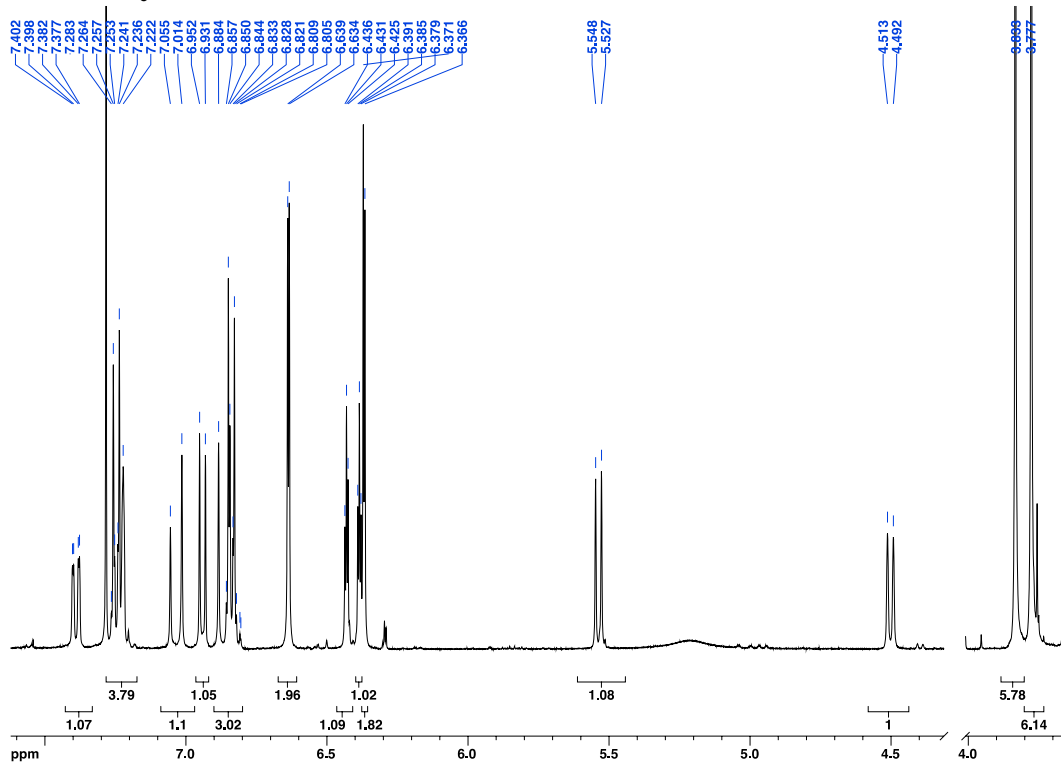
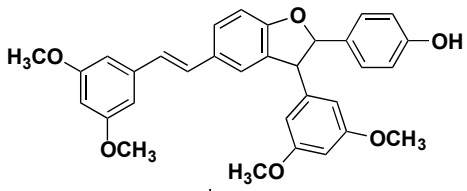
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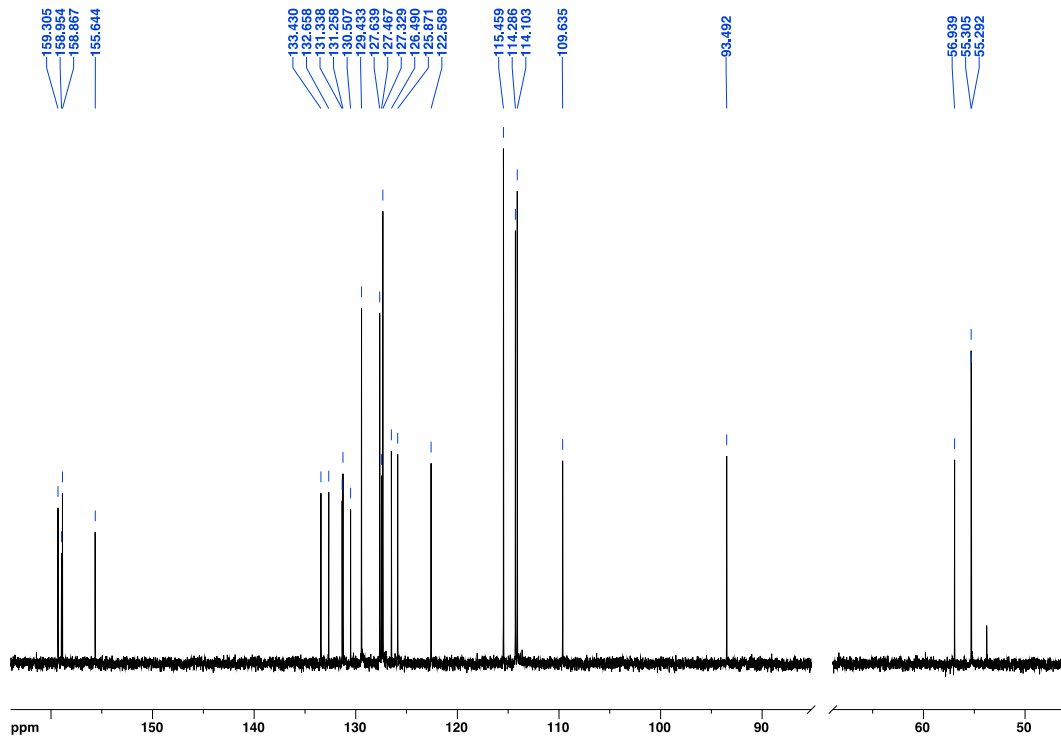
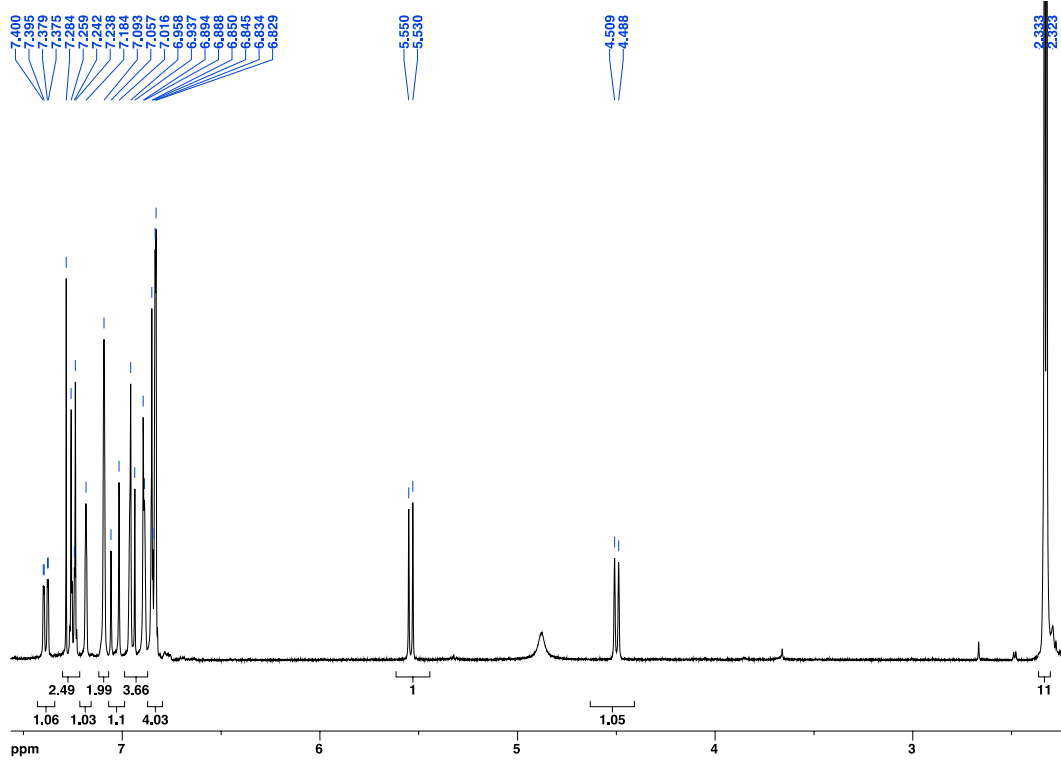
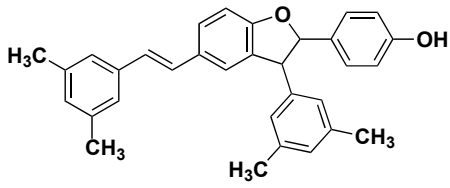
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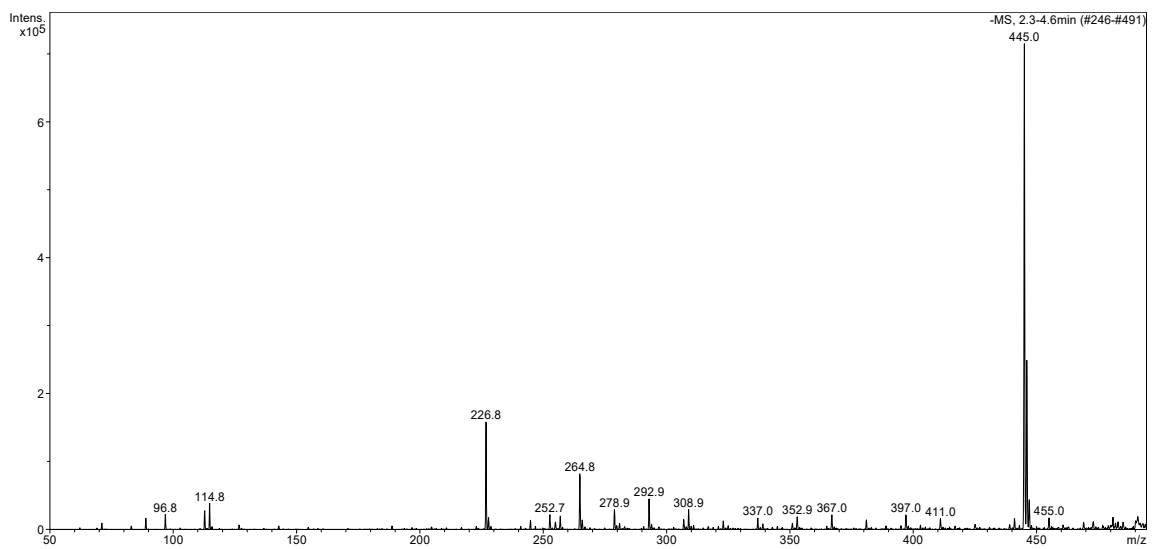
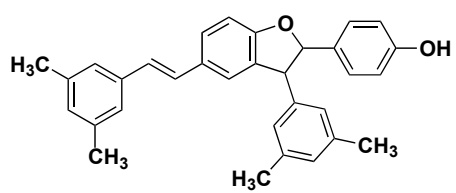
Compound 20



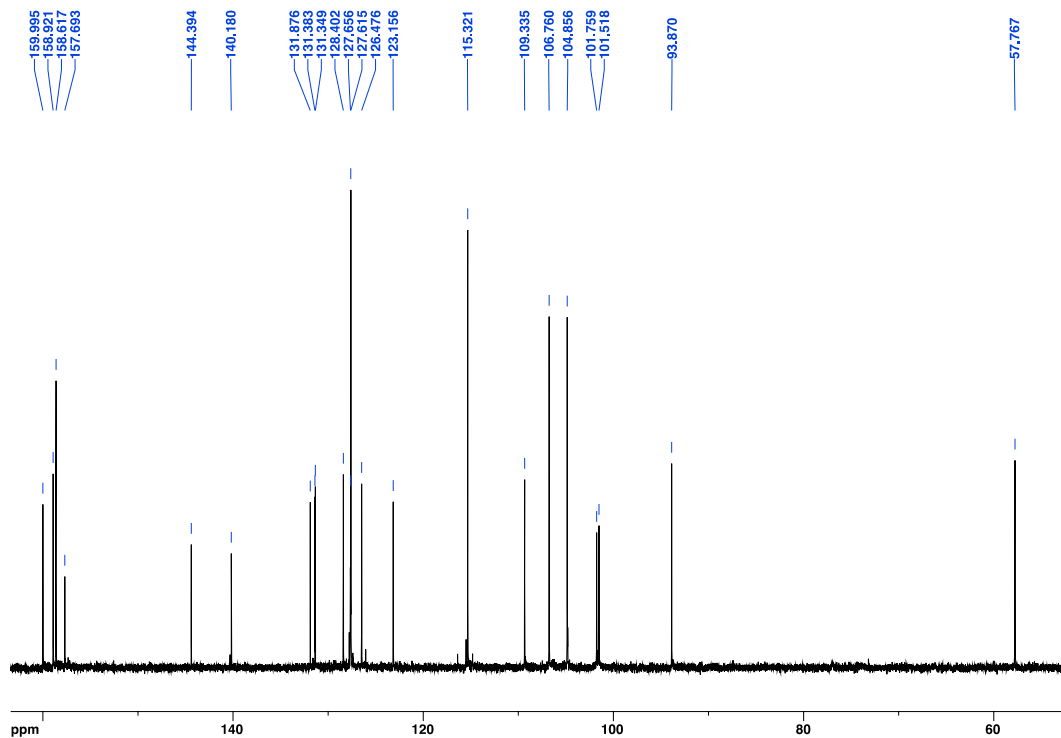
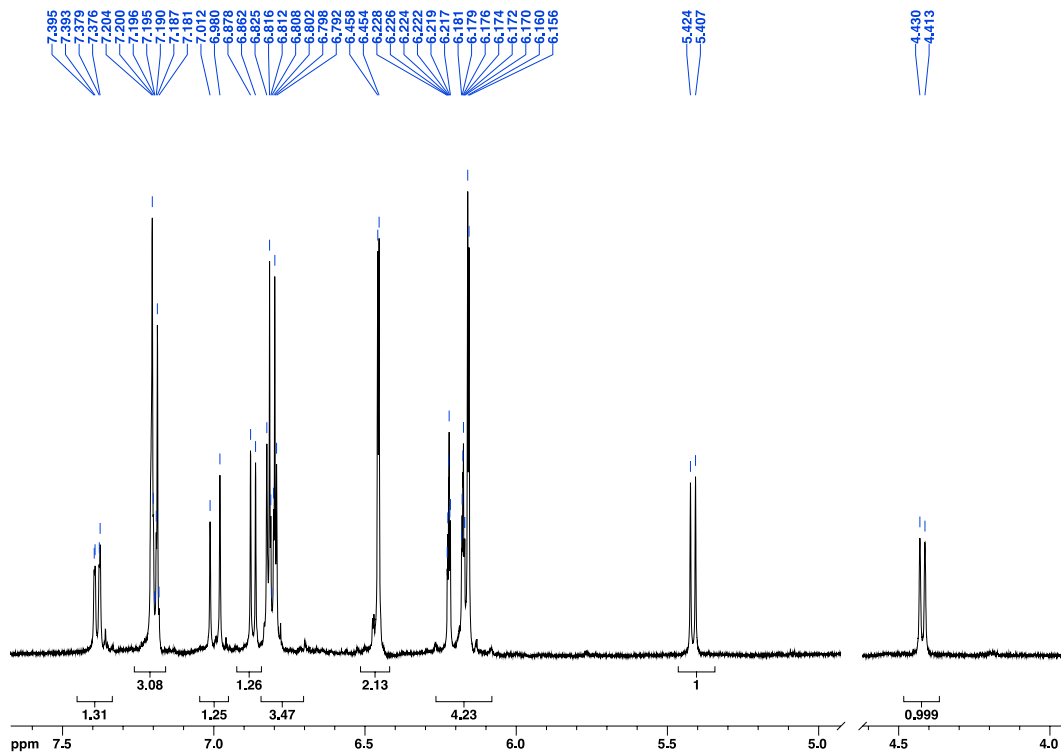
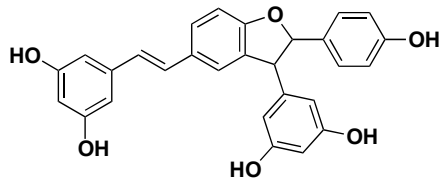
Compound 21



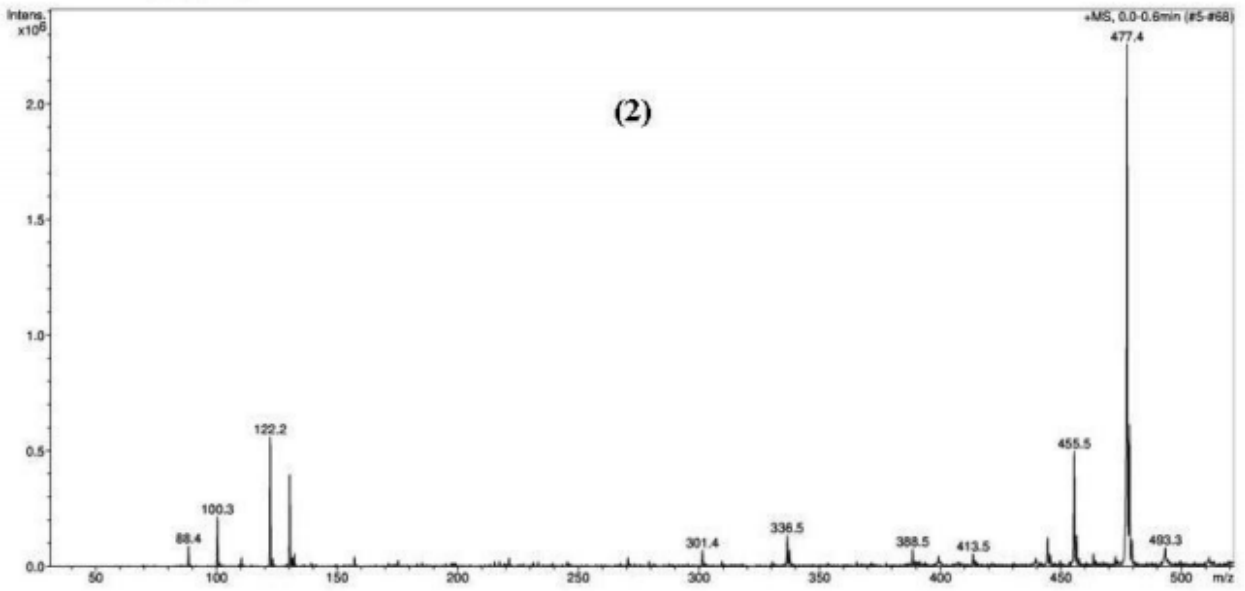
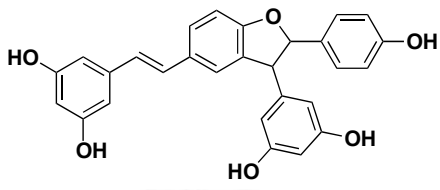
Compound 21



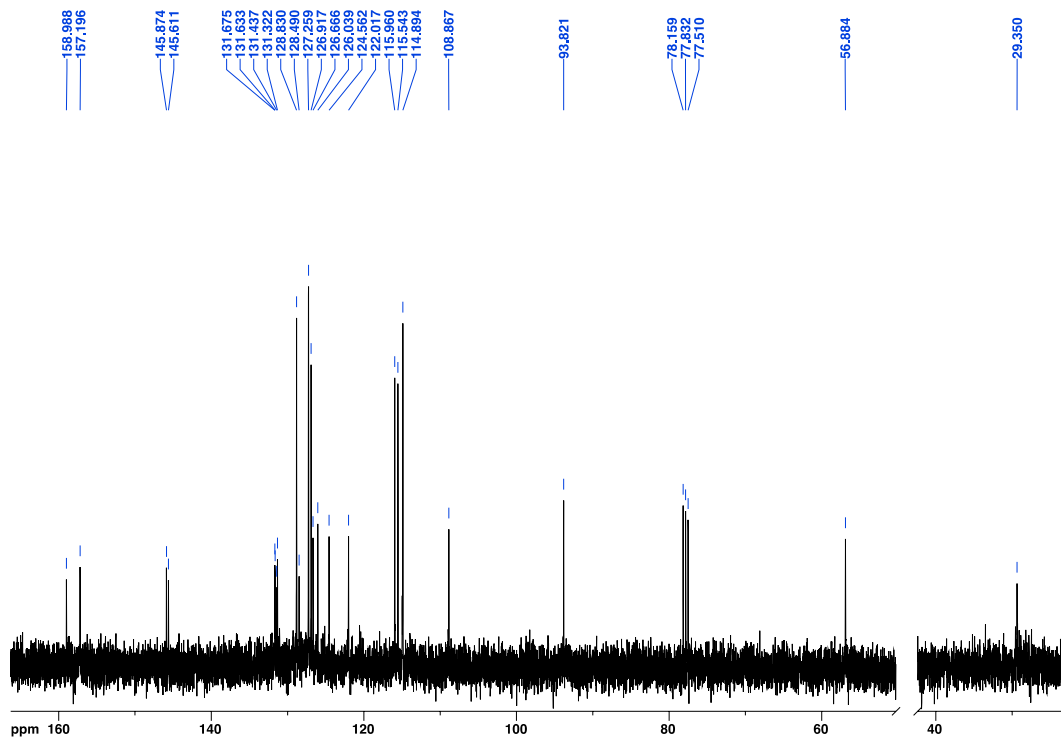
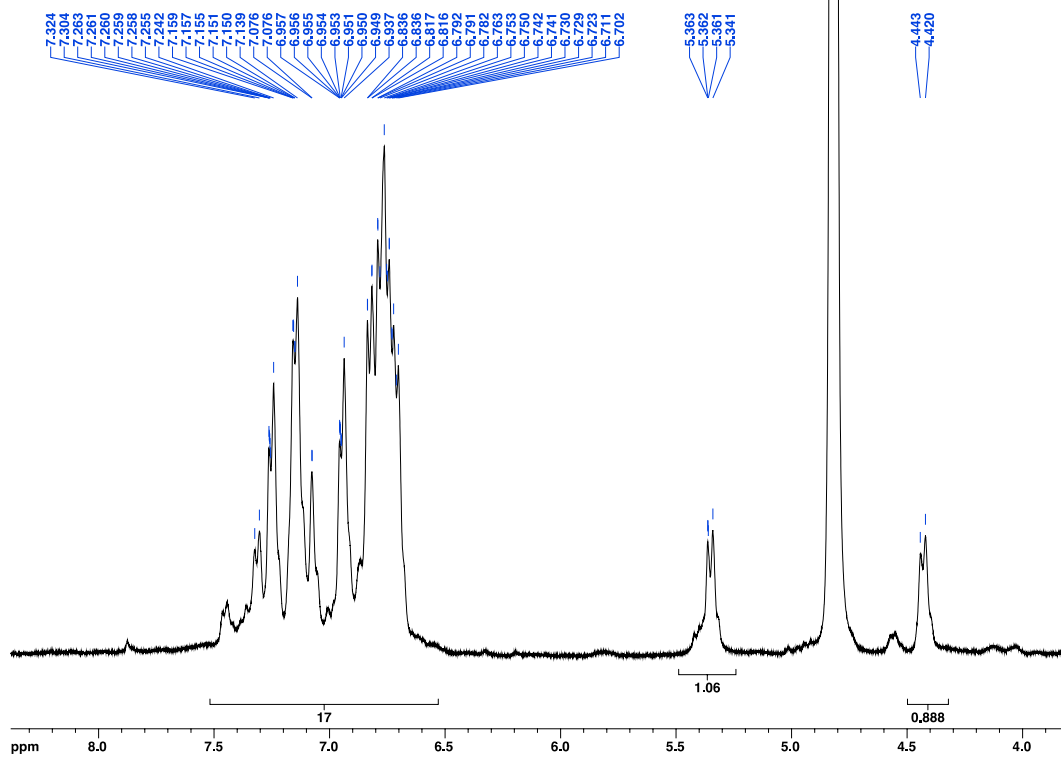
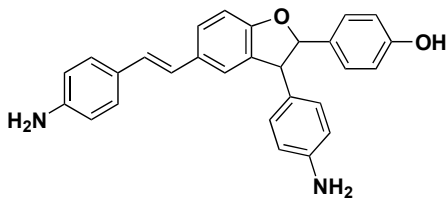
Compound 22



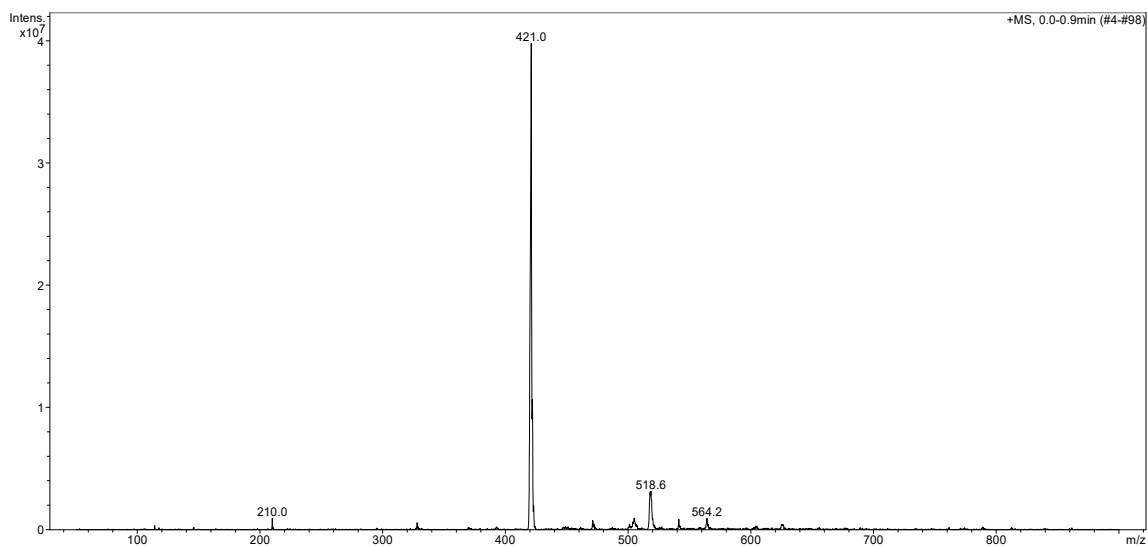
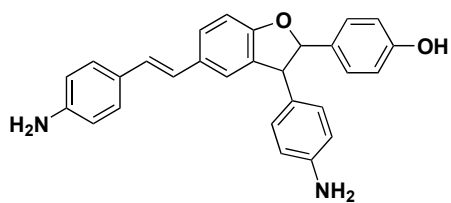
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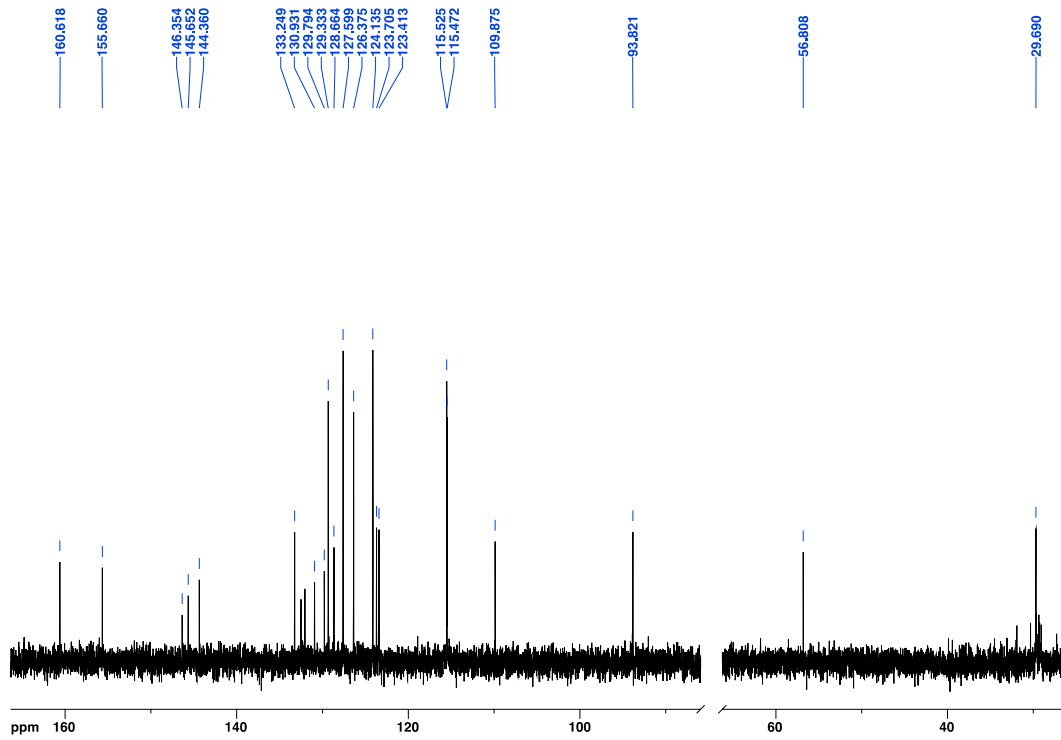
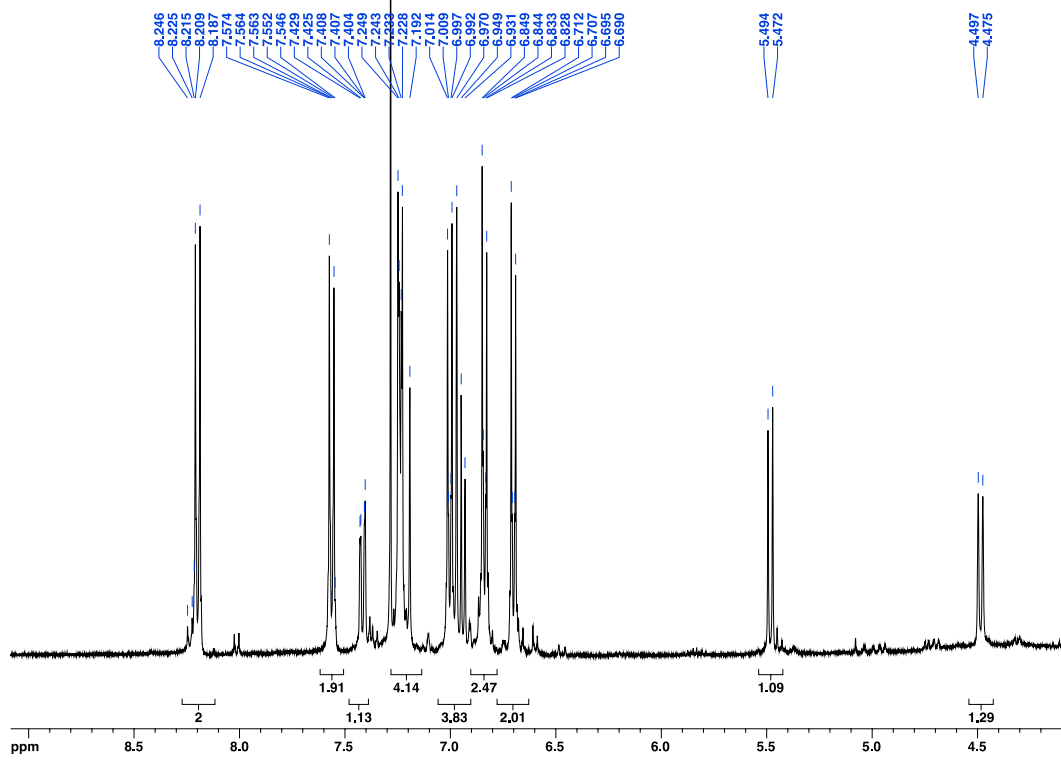
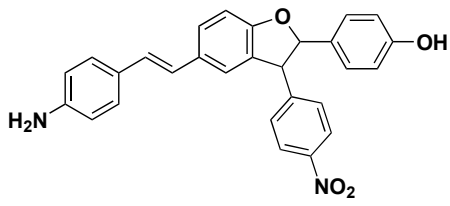
Compound 23



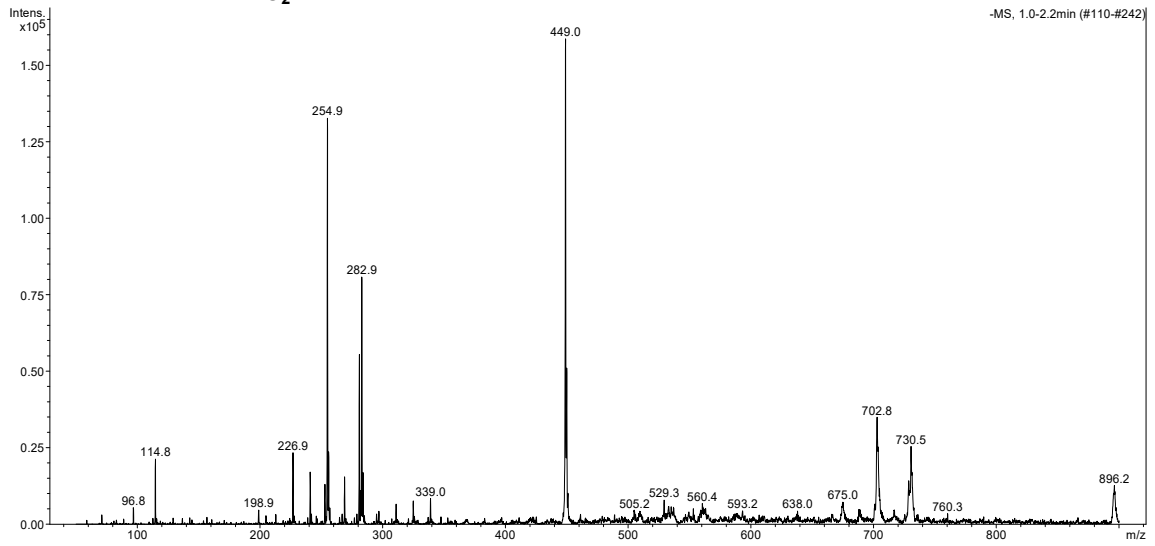
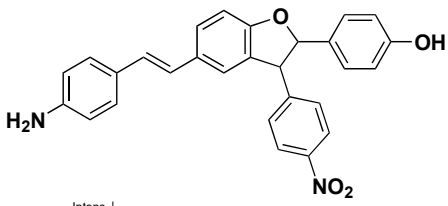
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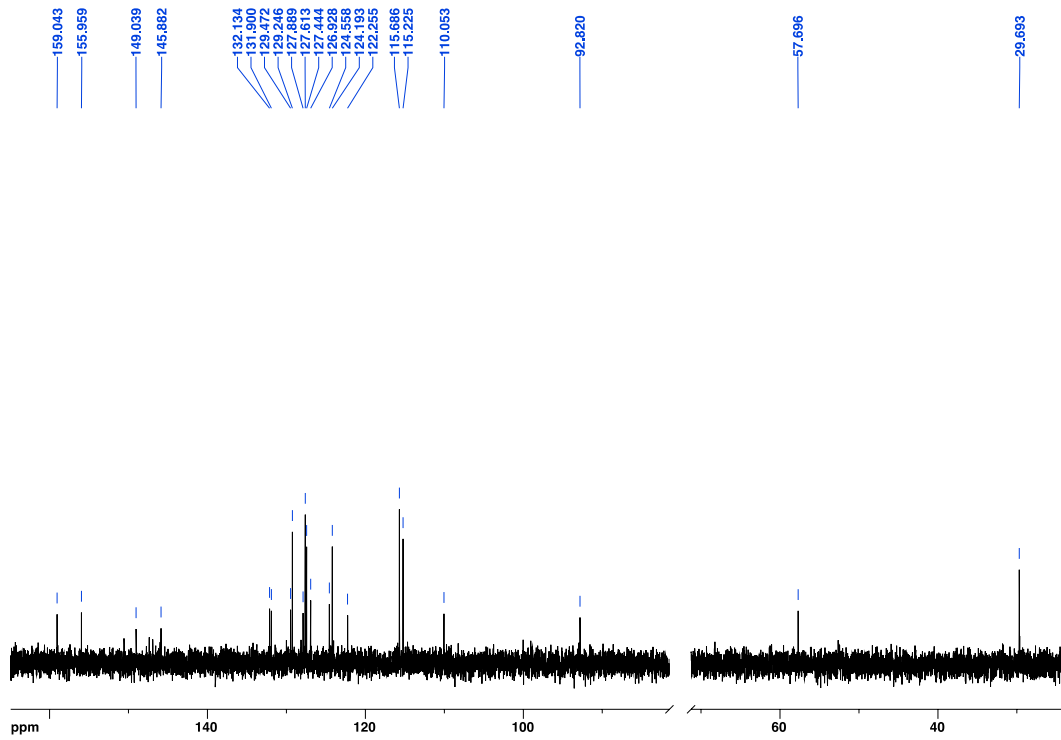
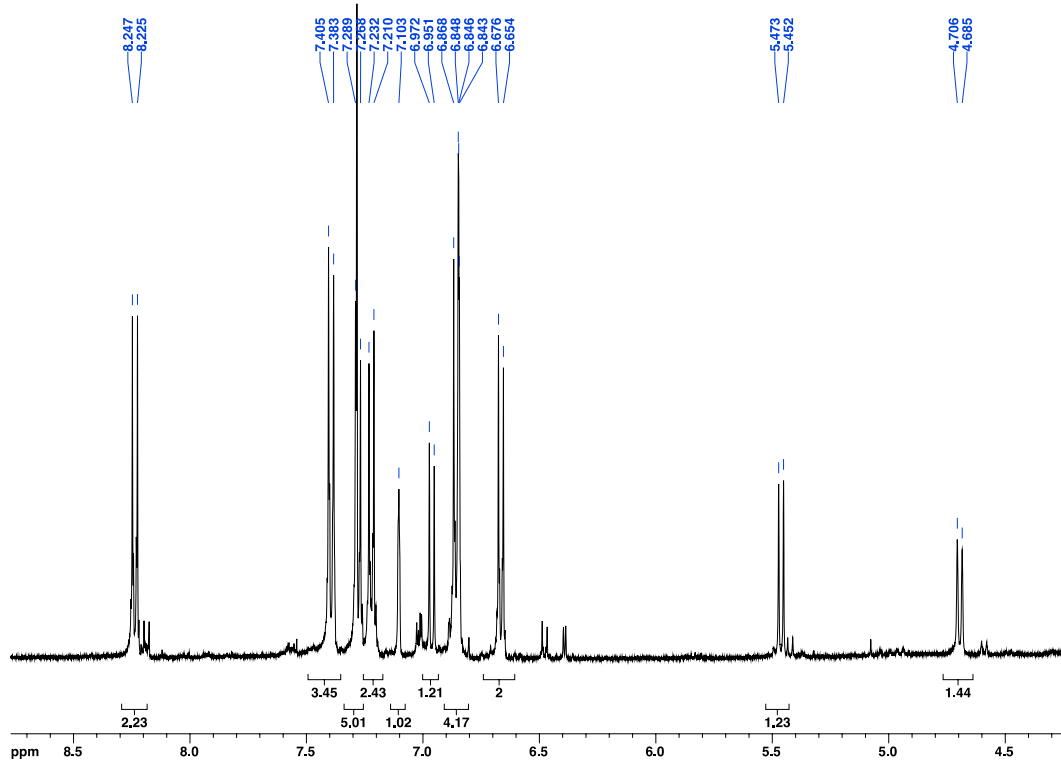
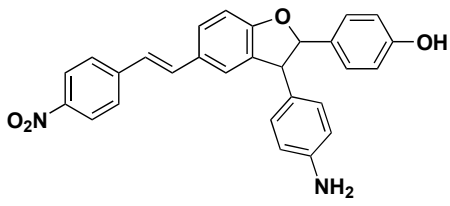
Compound 24



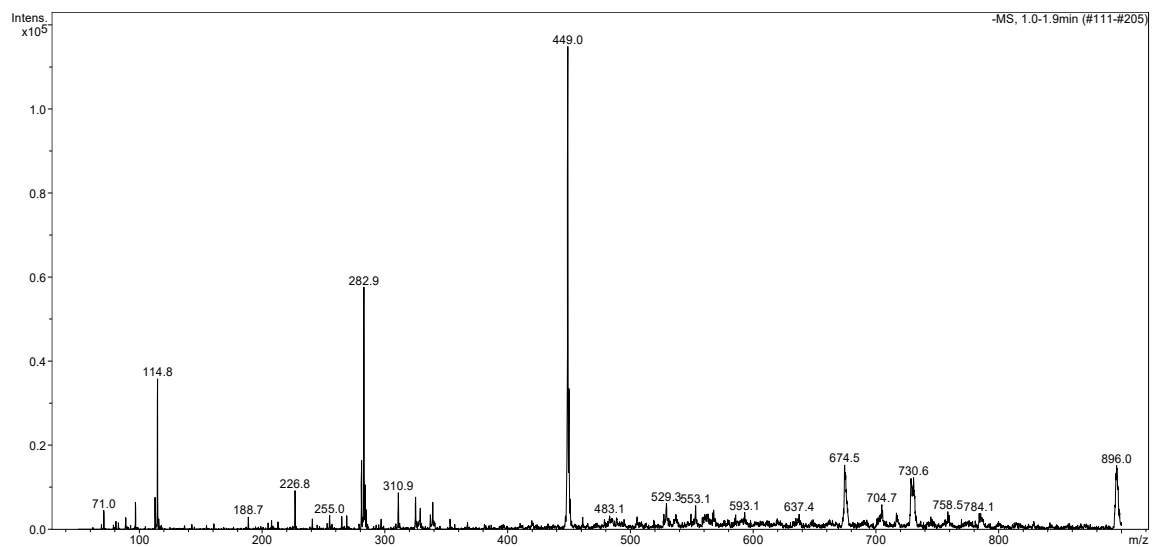
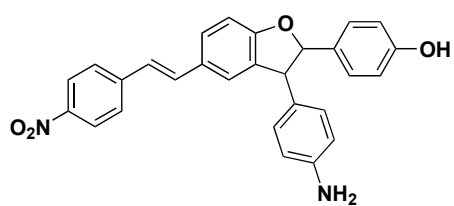
Compound 24



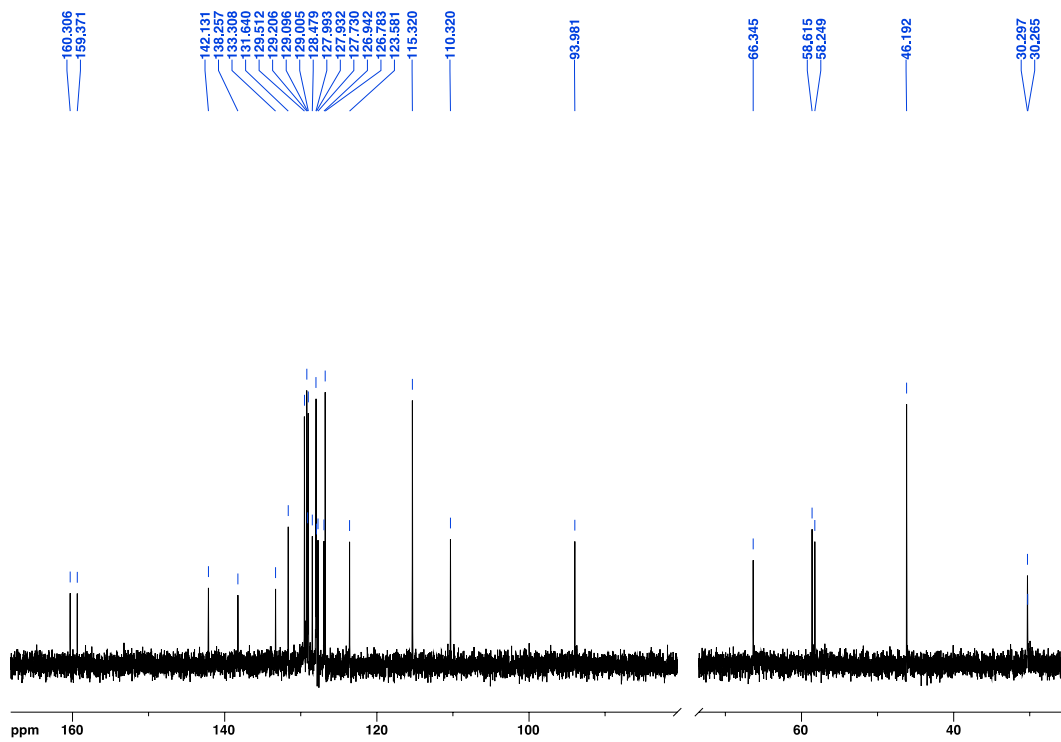
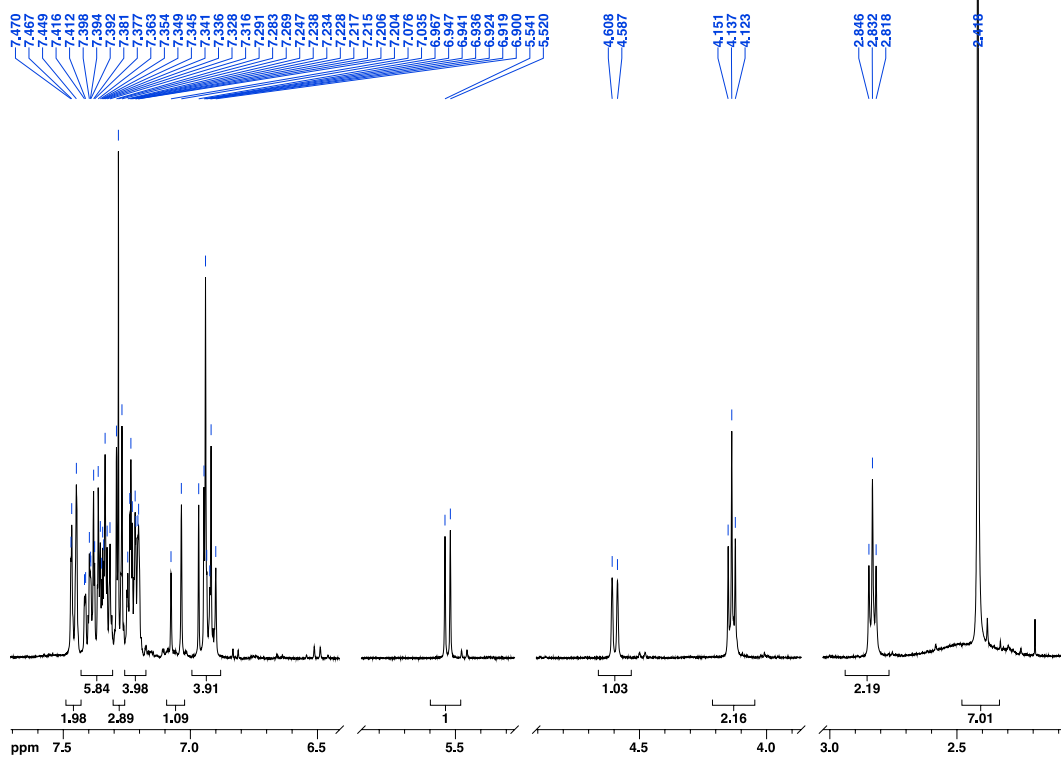
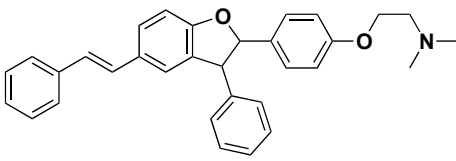
Compound 25



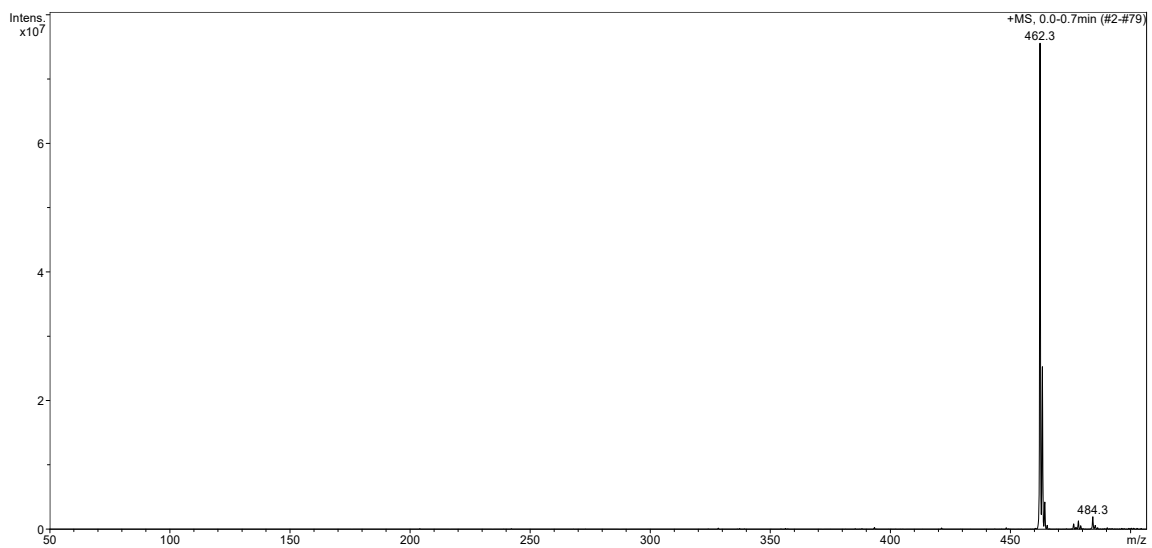
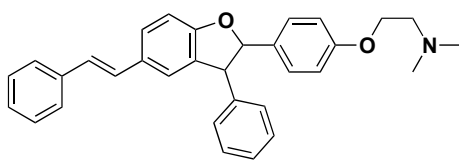
Compound 25



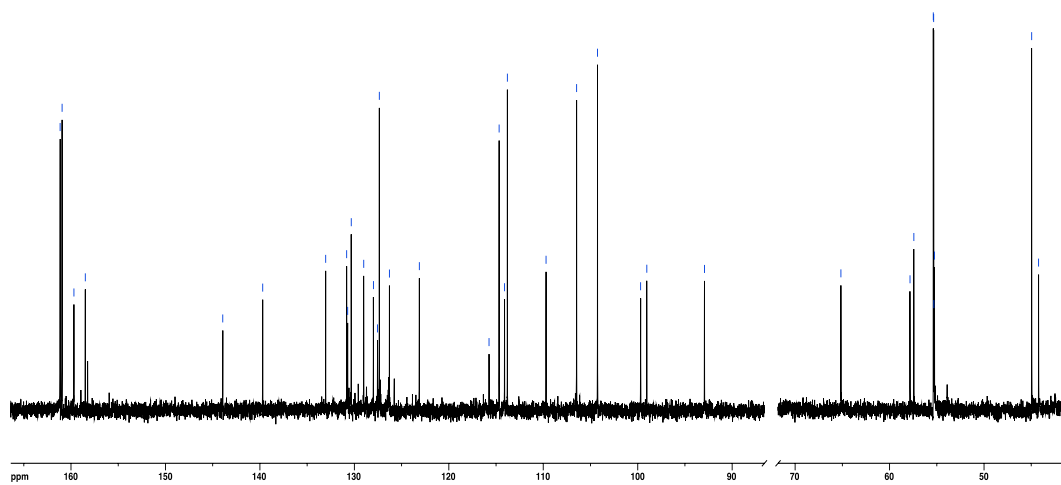
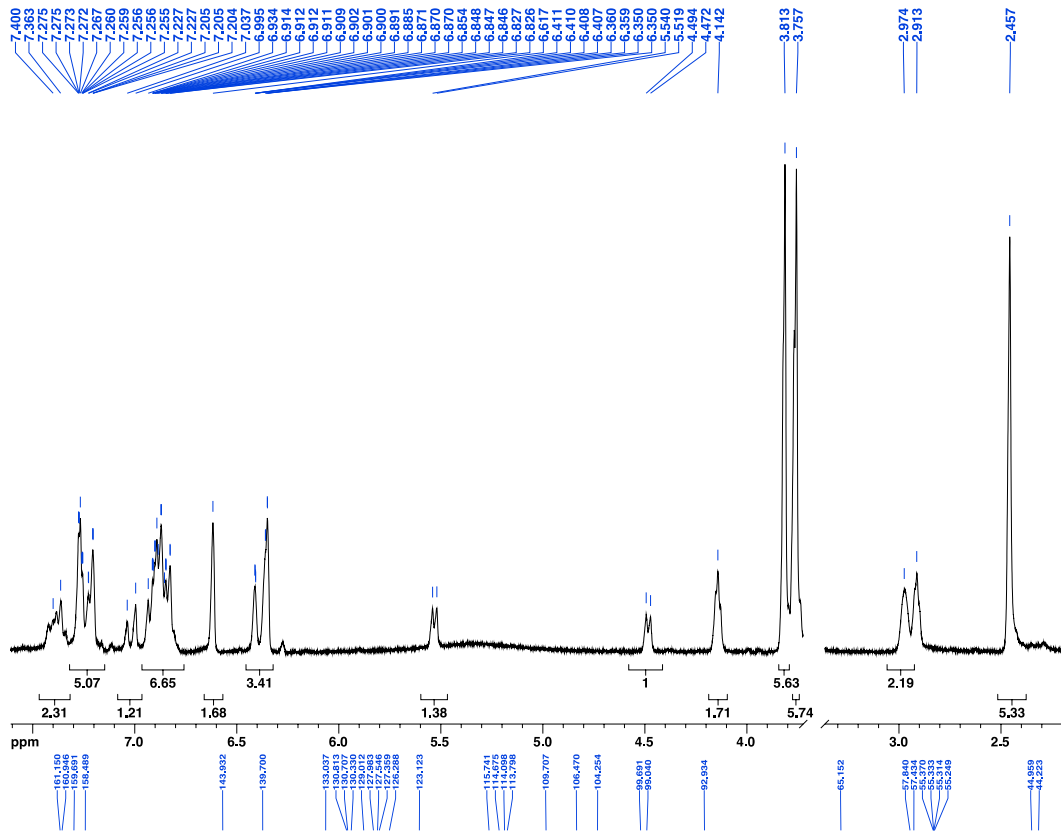
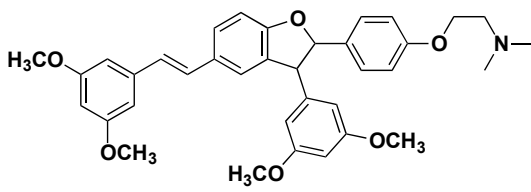
Compound 26



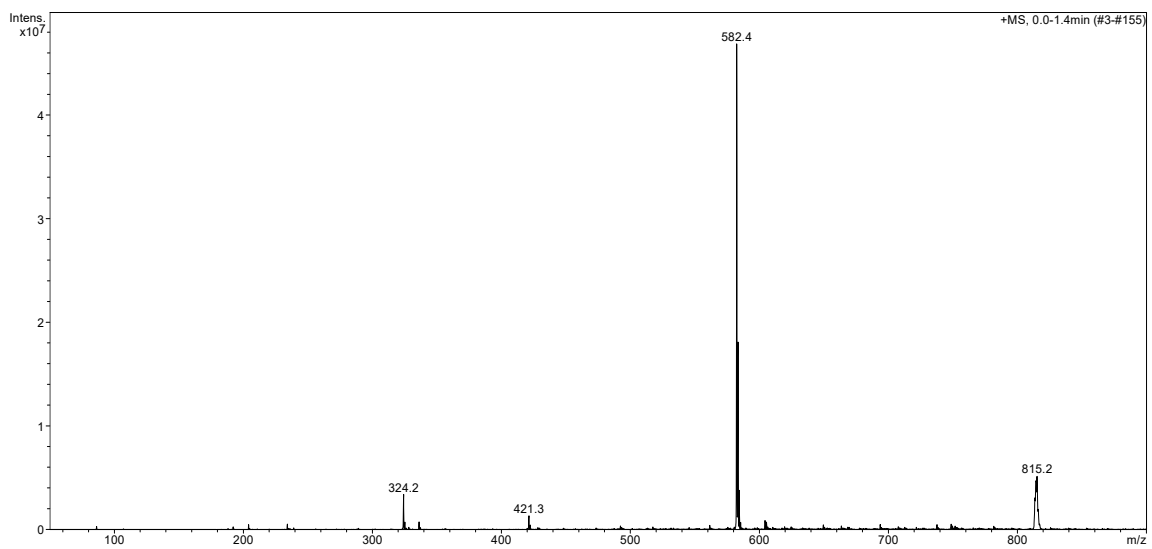
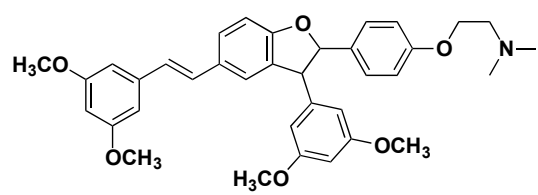
Compound 26



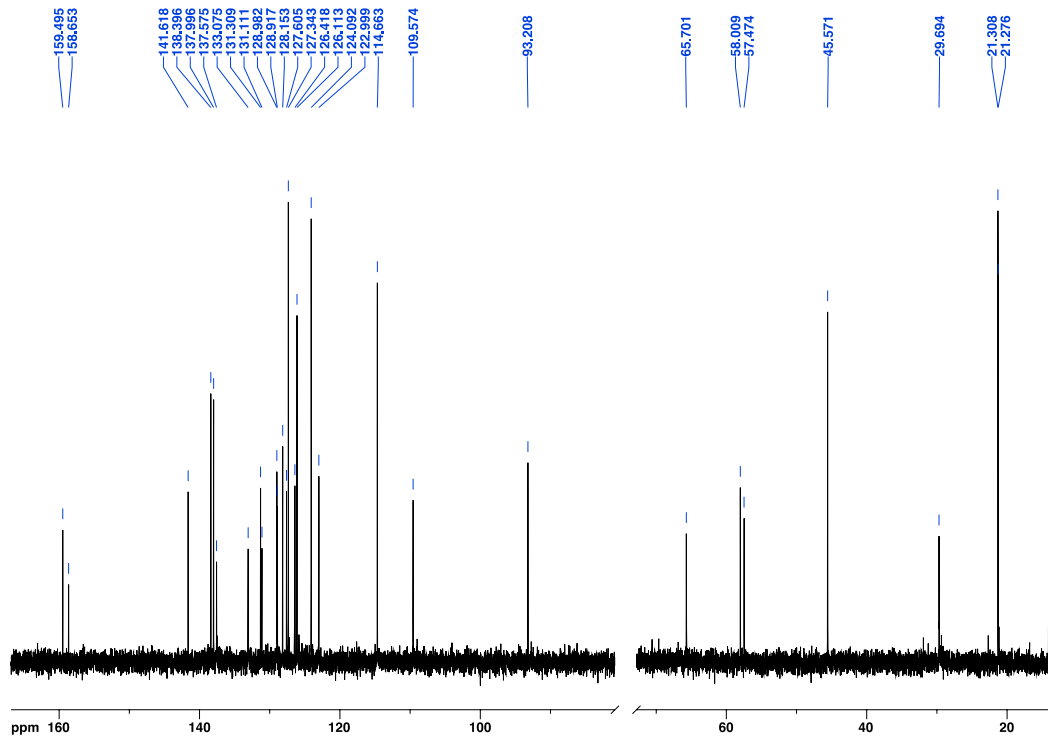
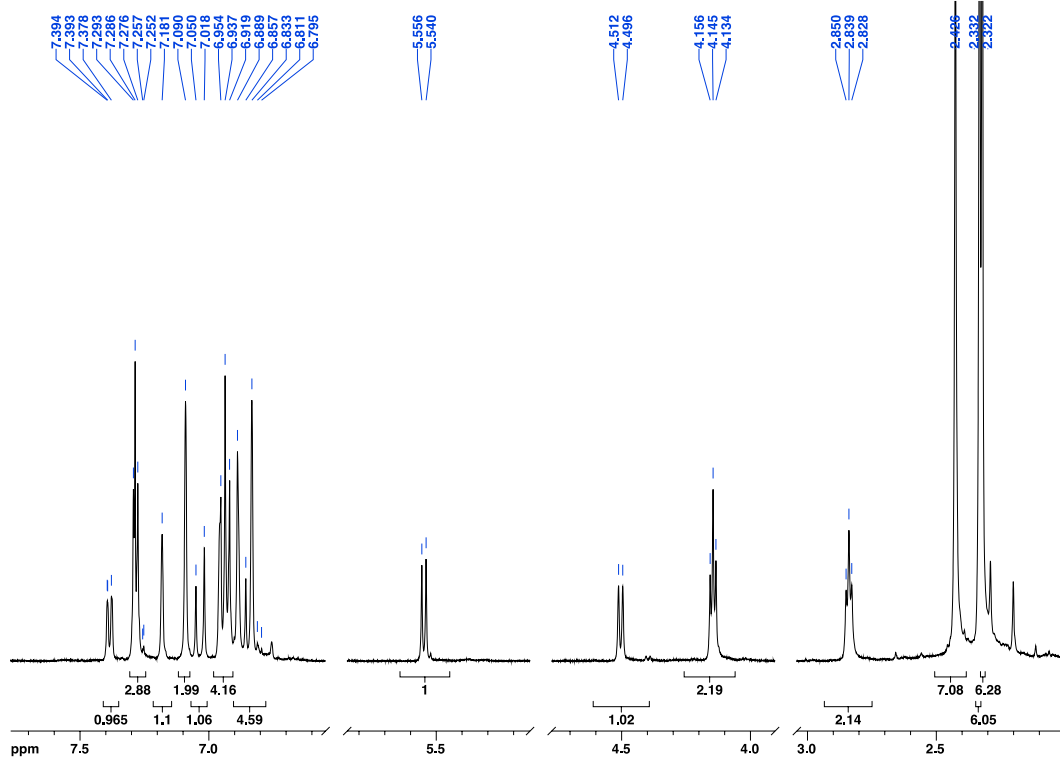
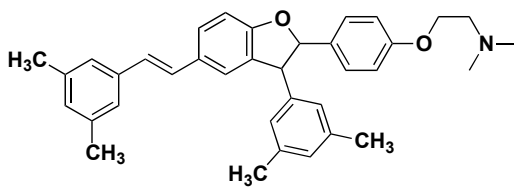
Compound 27



Compound 27



Compound 28



Compound 28

