

## Supporting Information

# Novel bioluminescent coelenterazine derivatives with imidazopyrazinone C-6 extended substitution for *Renilla luciferase*

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## Bioluminescent properties

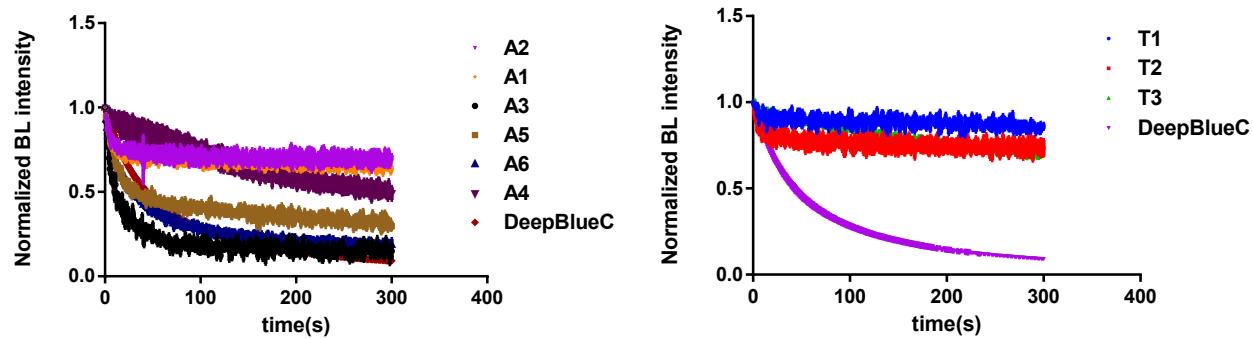


Figure S1: (Left): Kinetic profile of bioluminescence of A series derivatives with *Renilla* luciferase; (Right): Kinetic profile of bioluminescence of T series derivatives with *Renilla* luciferase.

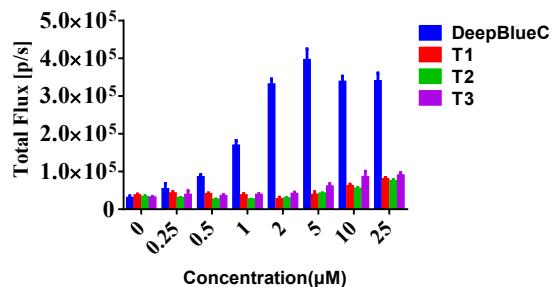


Figure S2: The comparison of bioluminescence intensities of T series derivatives and DeepBlueC in ES-2 cells expressing *Renilla* luciferase (Rluc) at various concentrations.

## Chemiluminescent and fluorescent properties

Table S1: Chemiluminescence maximum wavelength and fluorescence properties of novel compounds.

Compounds	Chemiluminescence Emission (nm)	Fluorescence Excitation (nm)	Fluorescence Emission (nm)
DeepBlueC	486	455	535
A1	477	420	530
A2	480	420	520
T1	490	335	405
T2	493	410	535
A6	482	420	545
T3	494	415	530

<b>A3</b>	484	415	545
<b>A4</b>	476	410	535
<b>A5</b>	479	330	400

## Mass solubility

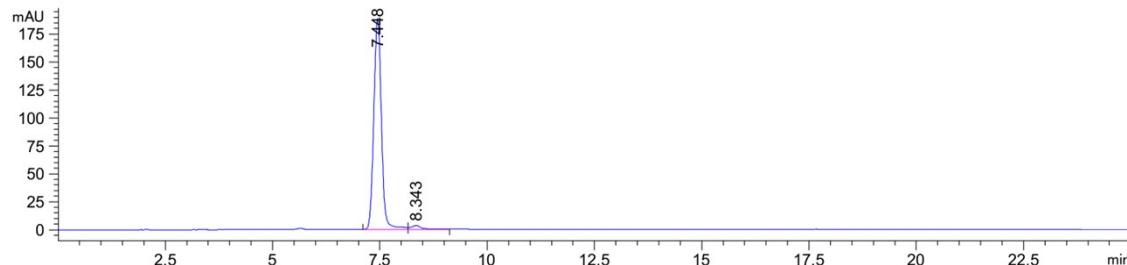
Table S2. Mass solubility data of compounds.

	<b>DeepBlueC</b>	<b>A4</b>	<b>A6</b>
Mass Solubility in Water (g/L, 25 °C)	$(3.25 \pm 0.03) \times 10^{-3}$	$(6.64 \pm 0.02) \times 10^{-2}$	$(4.45 \pm 0.01) \times 10^{-2}$

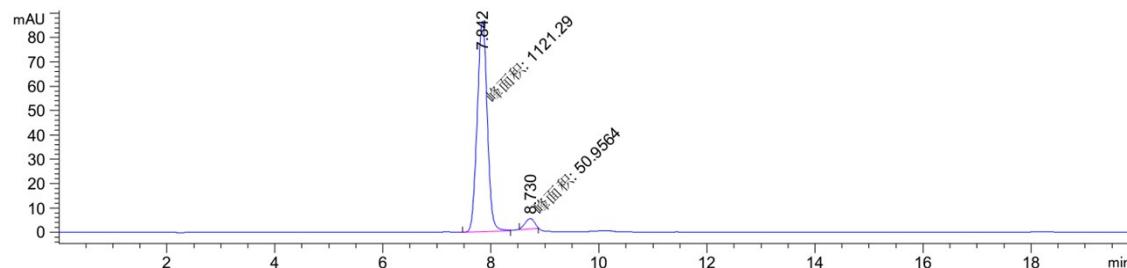
## HPLC spectra, NMR spectra and HR-MS spectra

### HPLC spectra

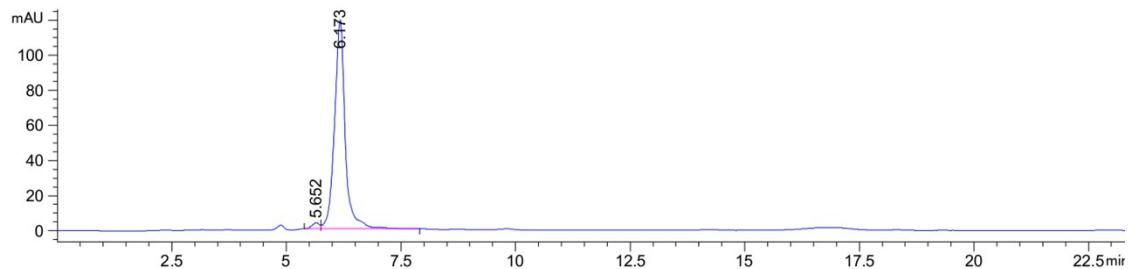
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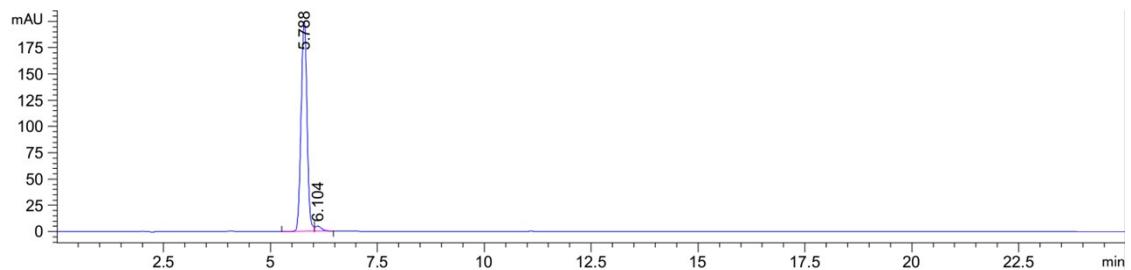
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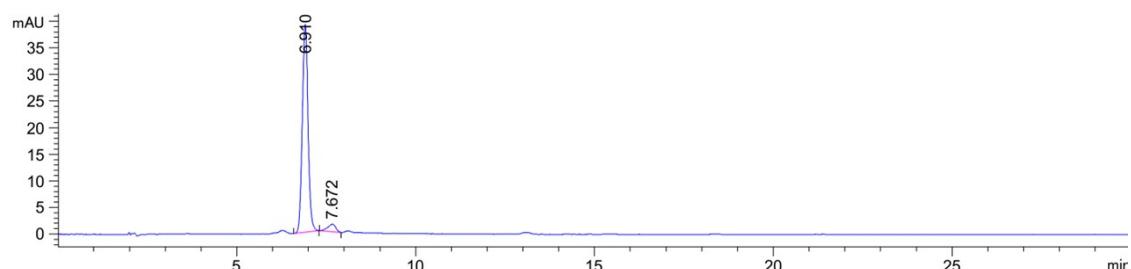
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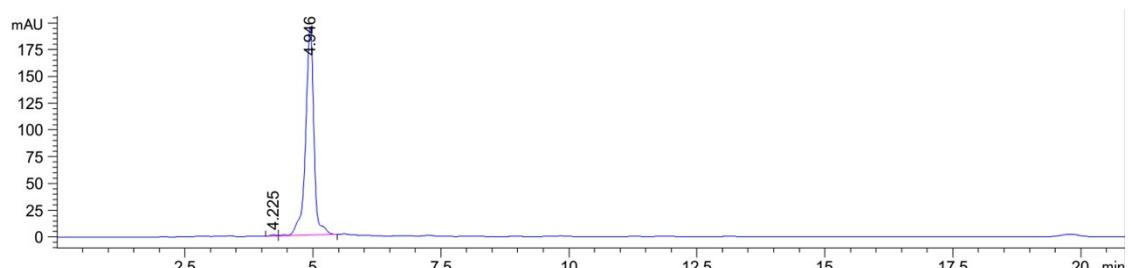
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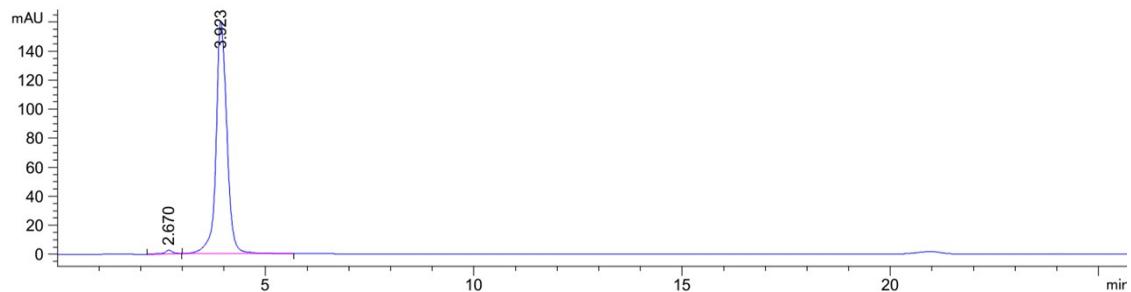
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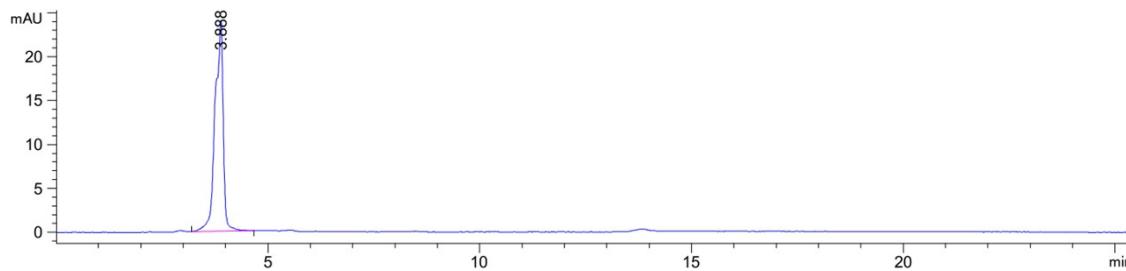
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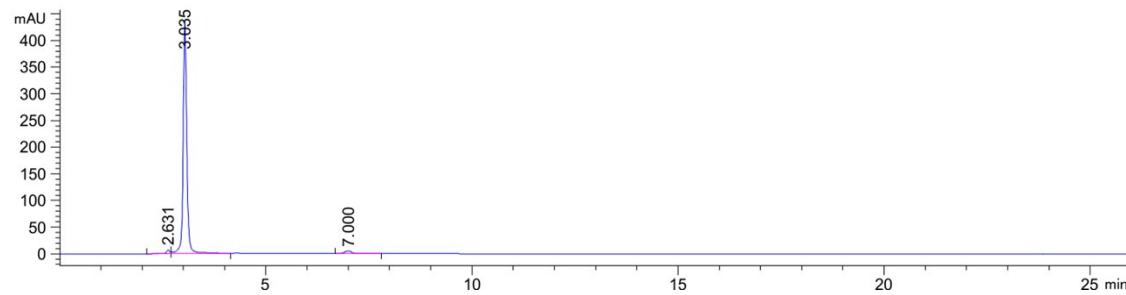
T1



T2

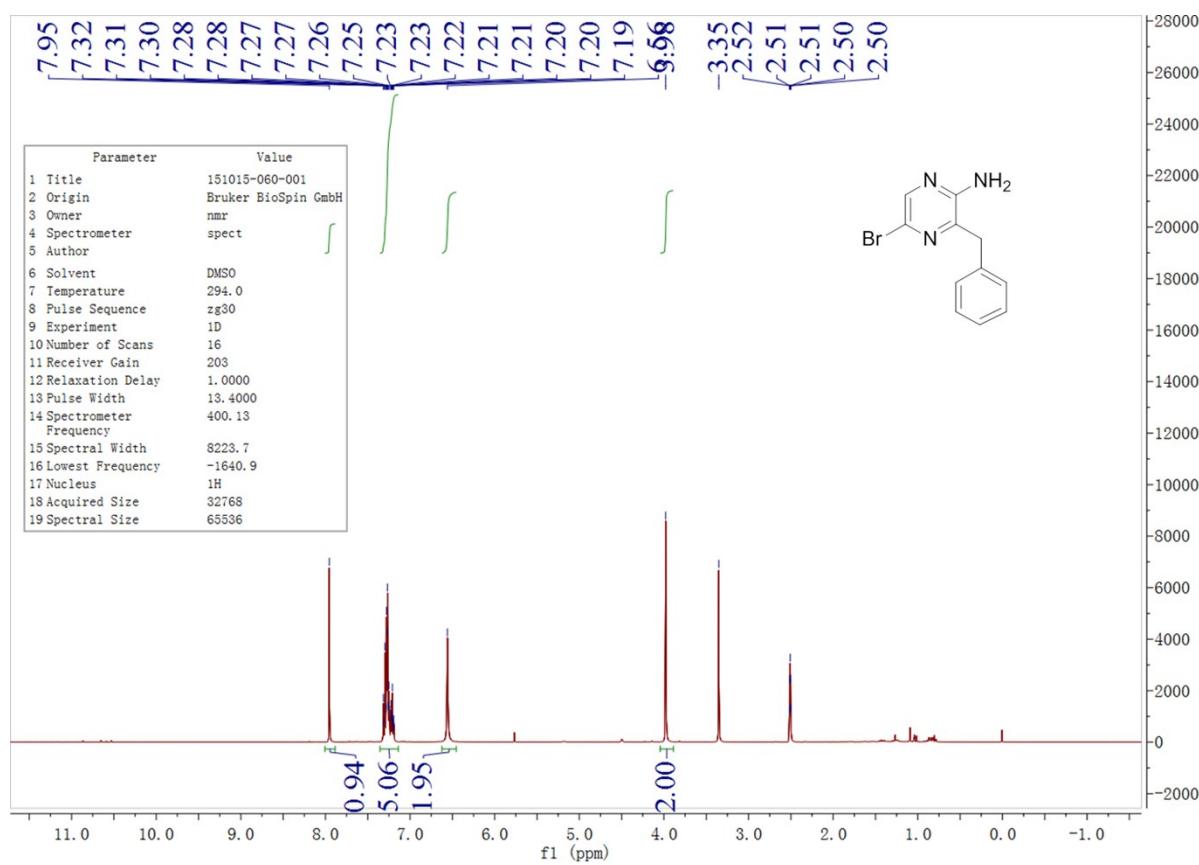


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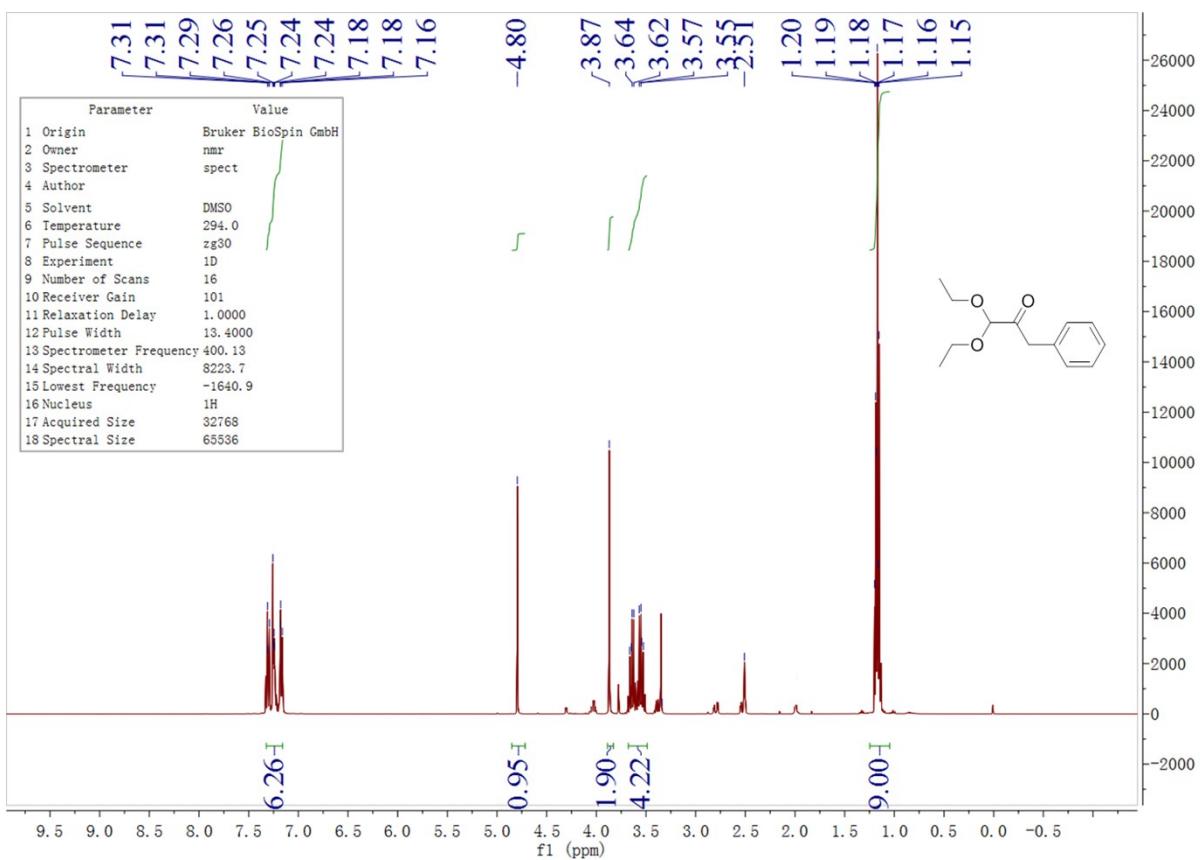


<sup>1</sup>H NMR and <sup>13</sup>C NMR

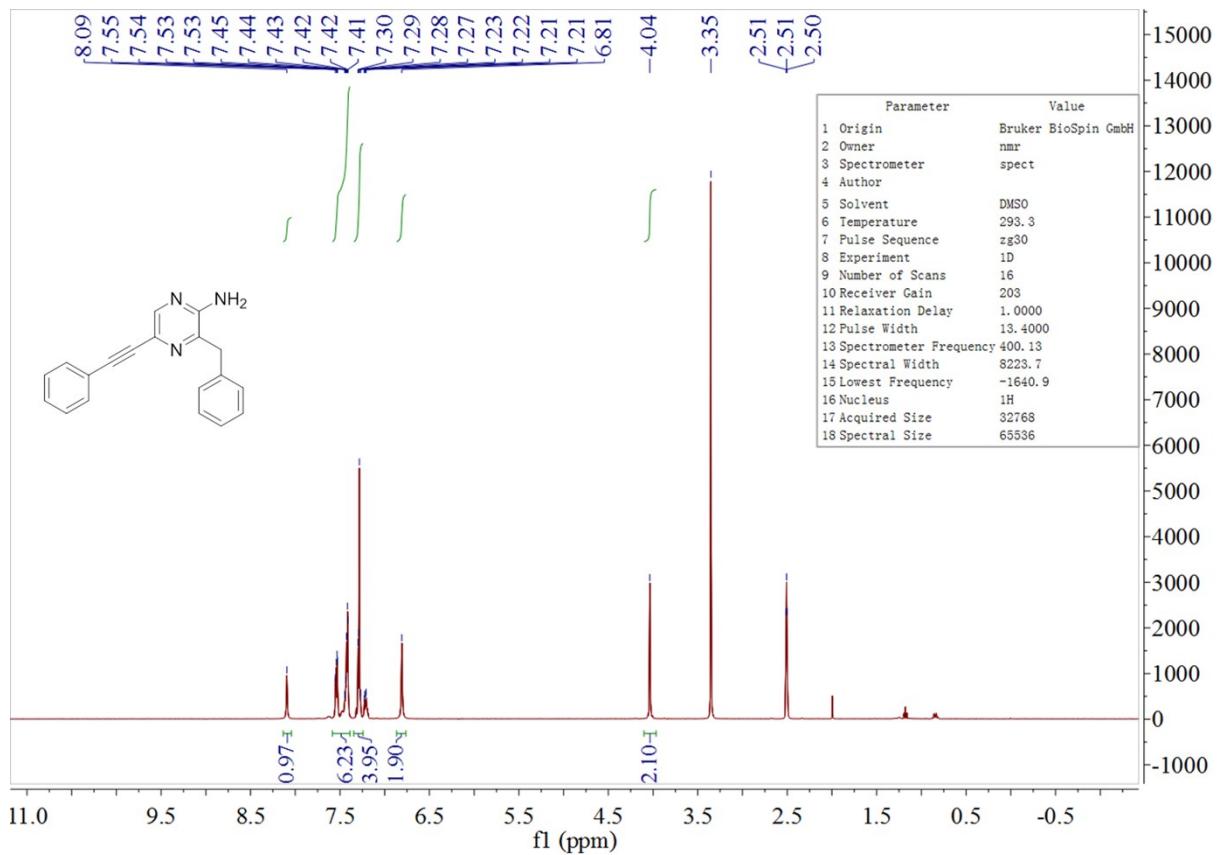
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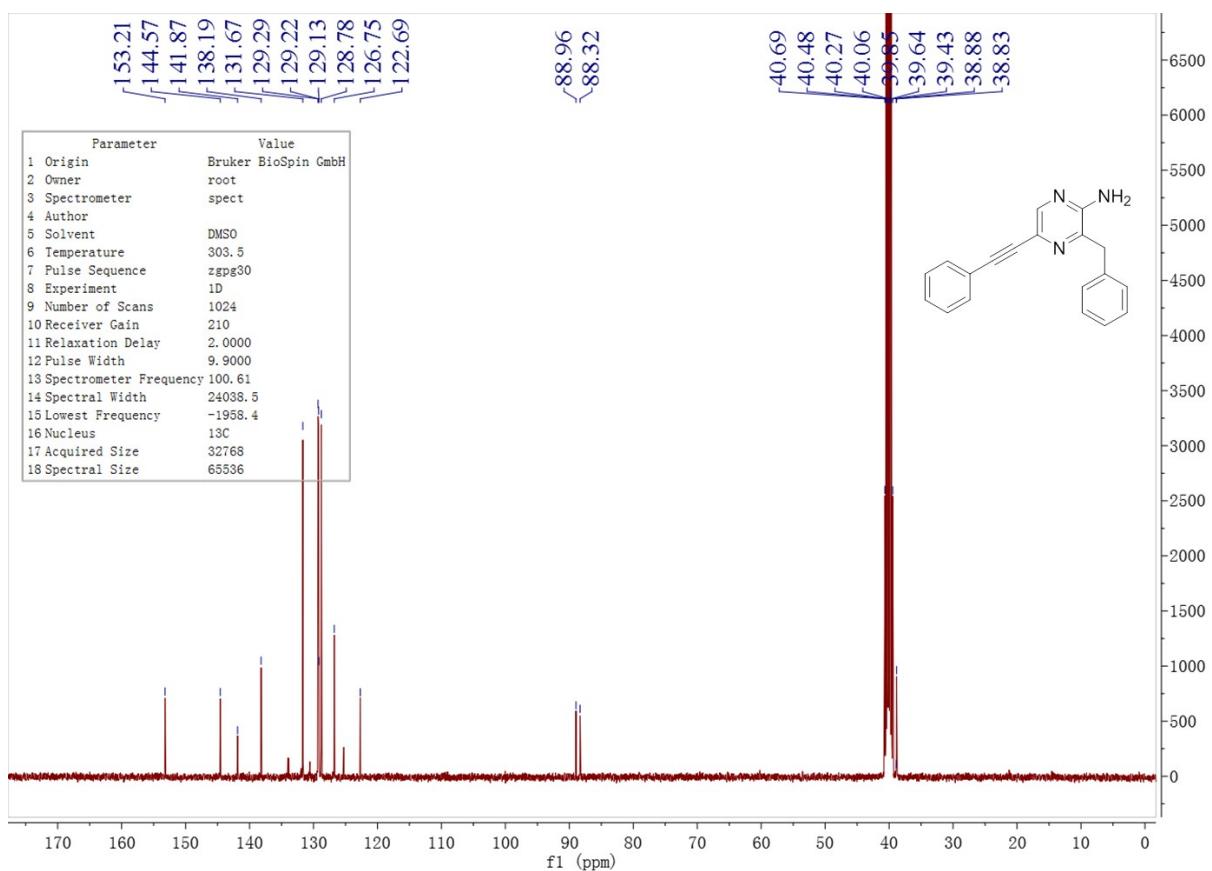


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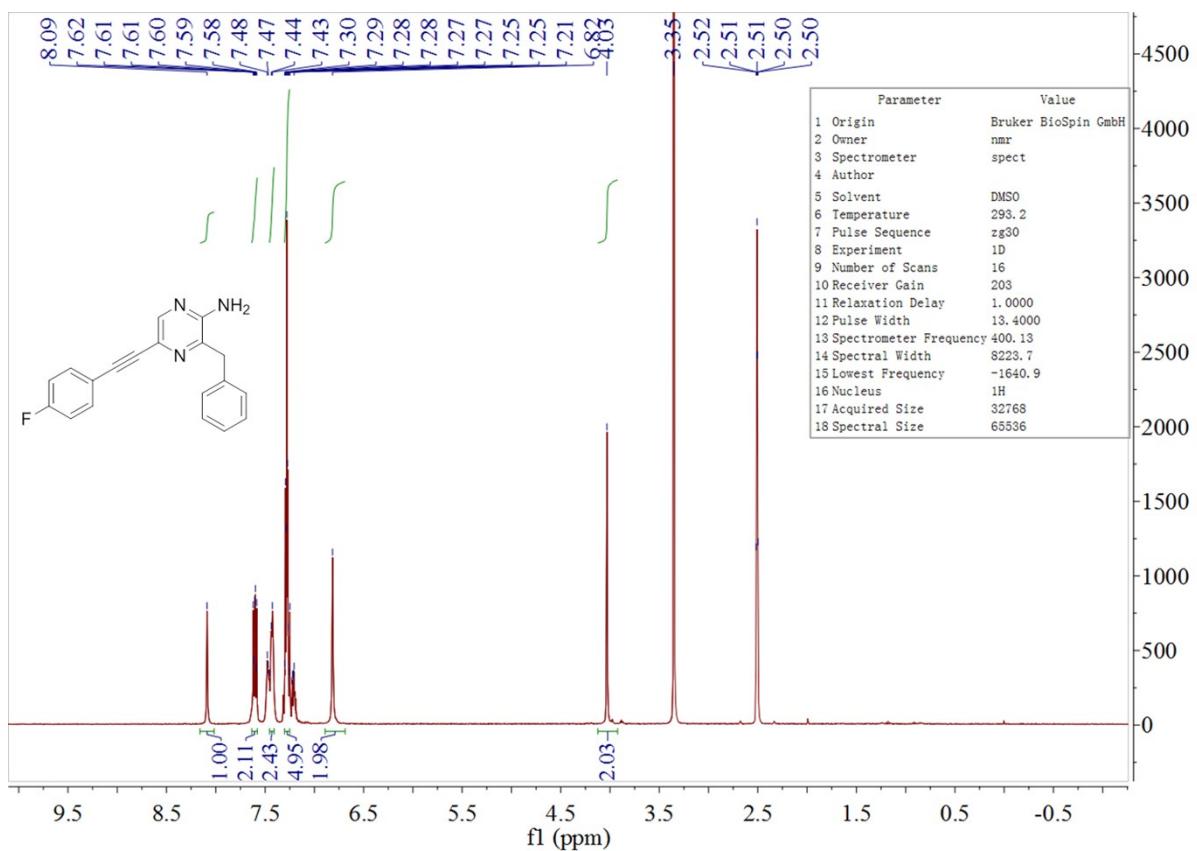


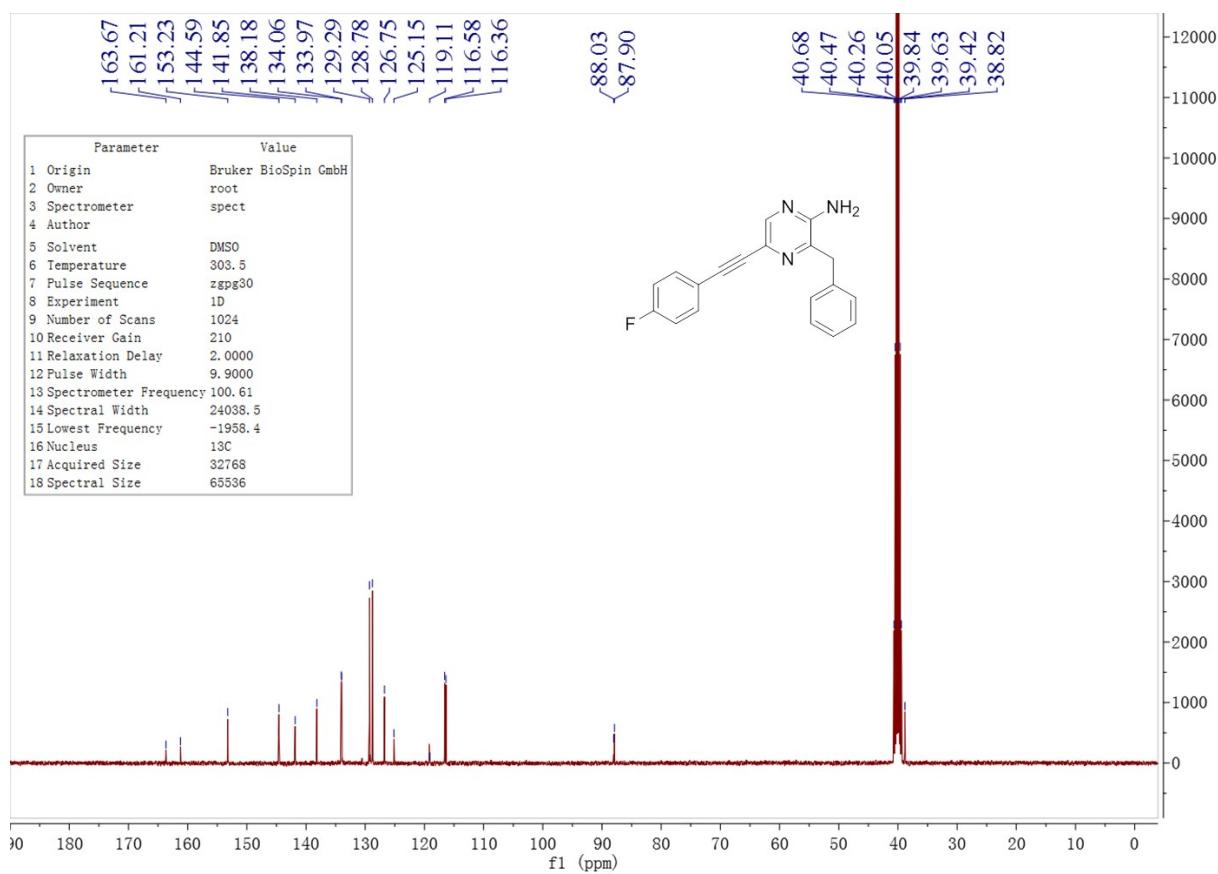
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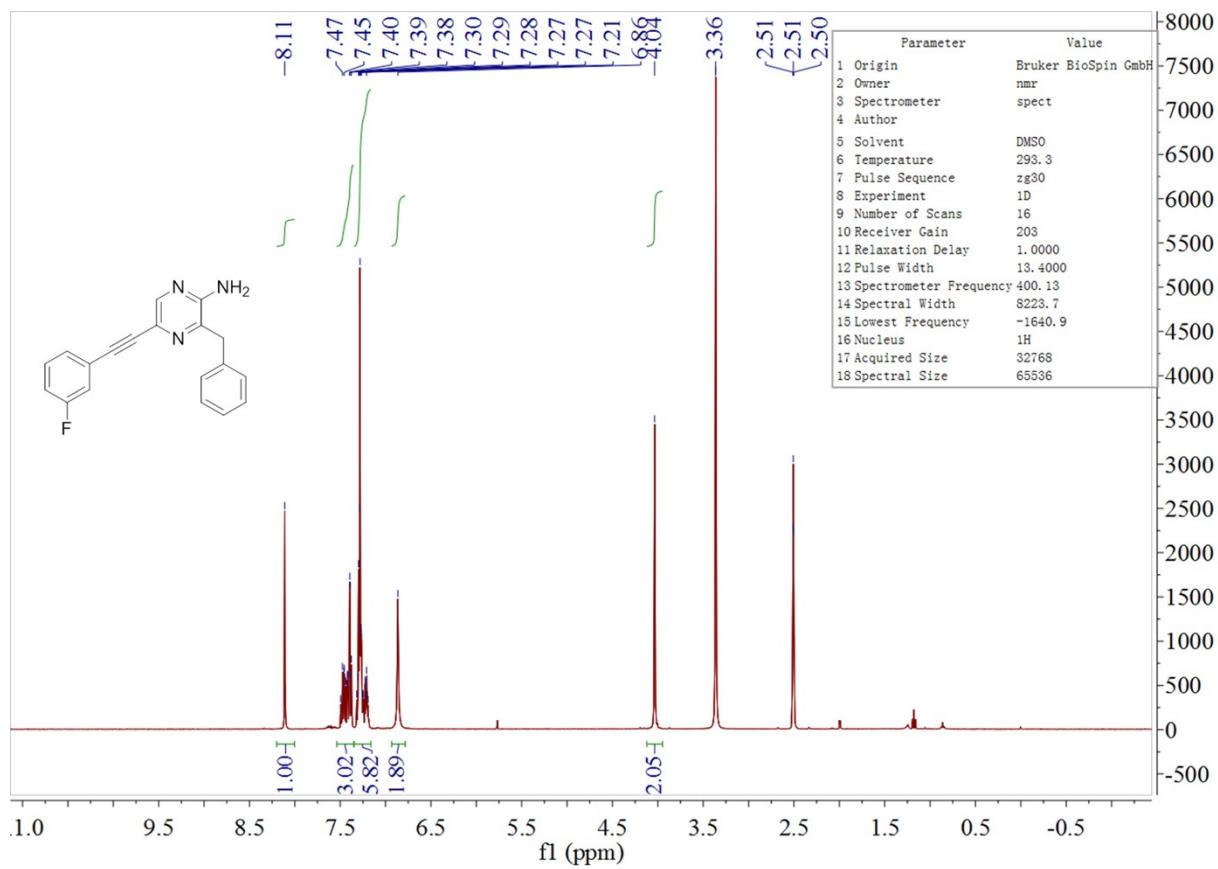


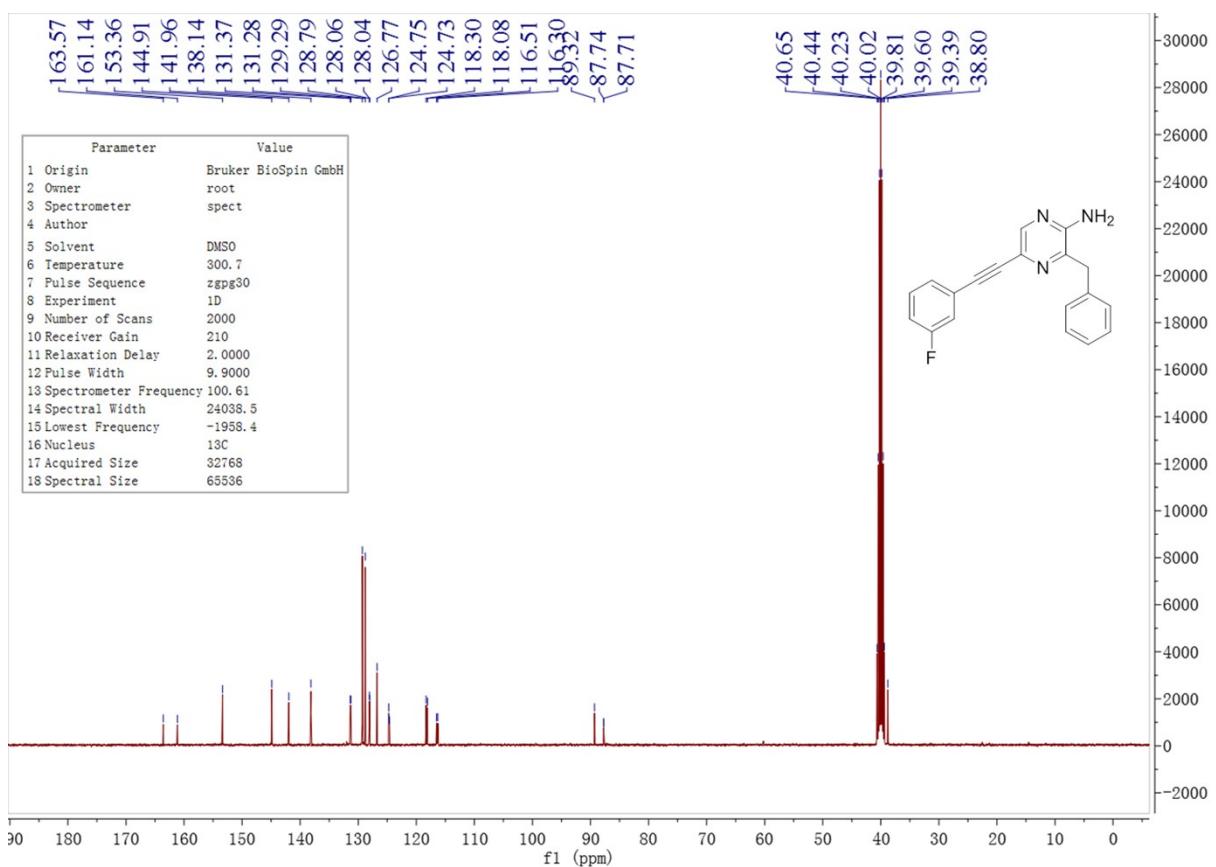
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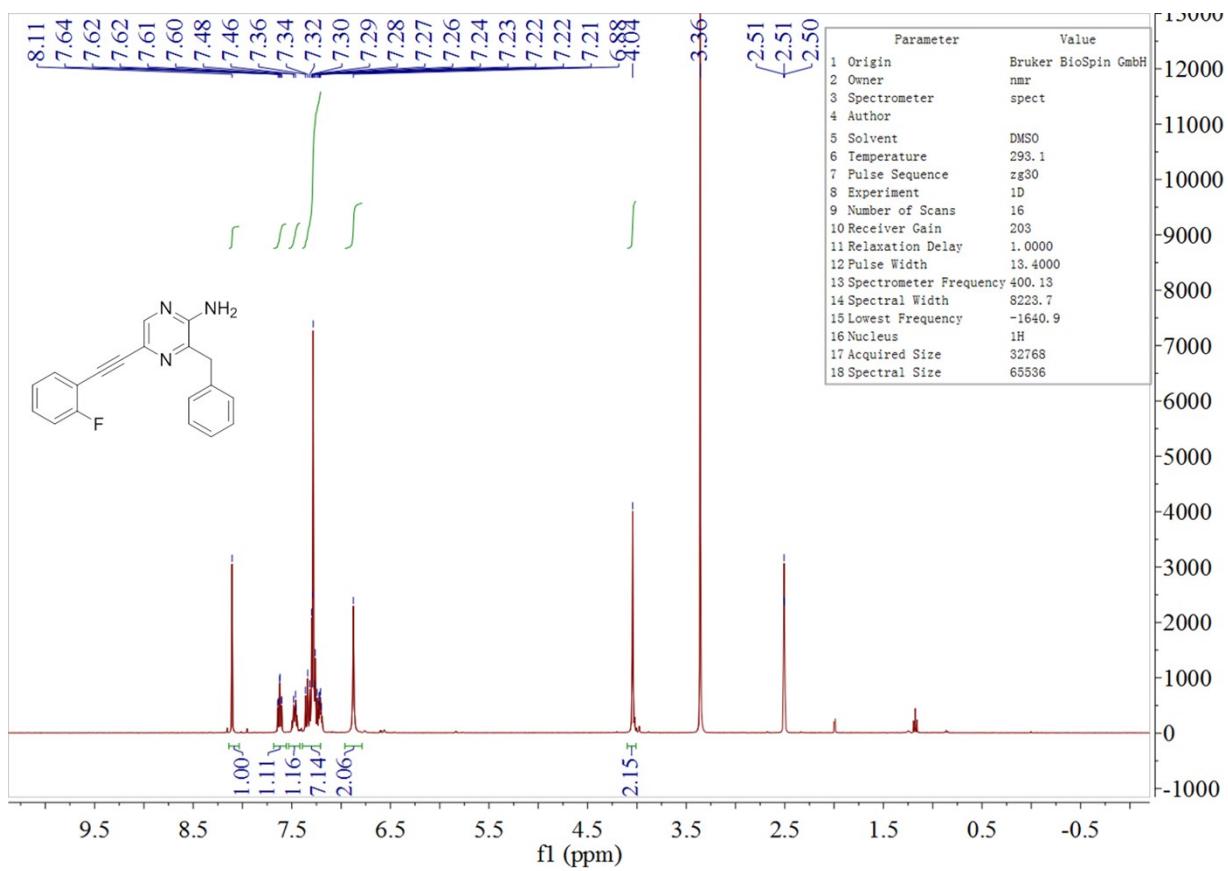


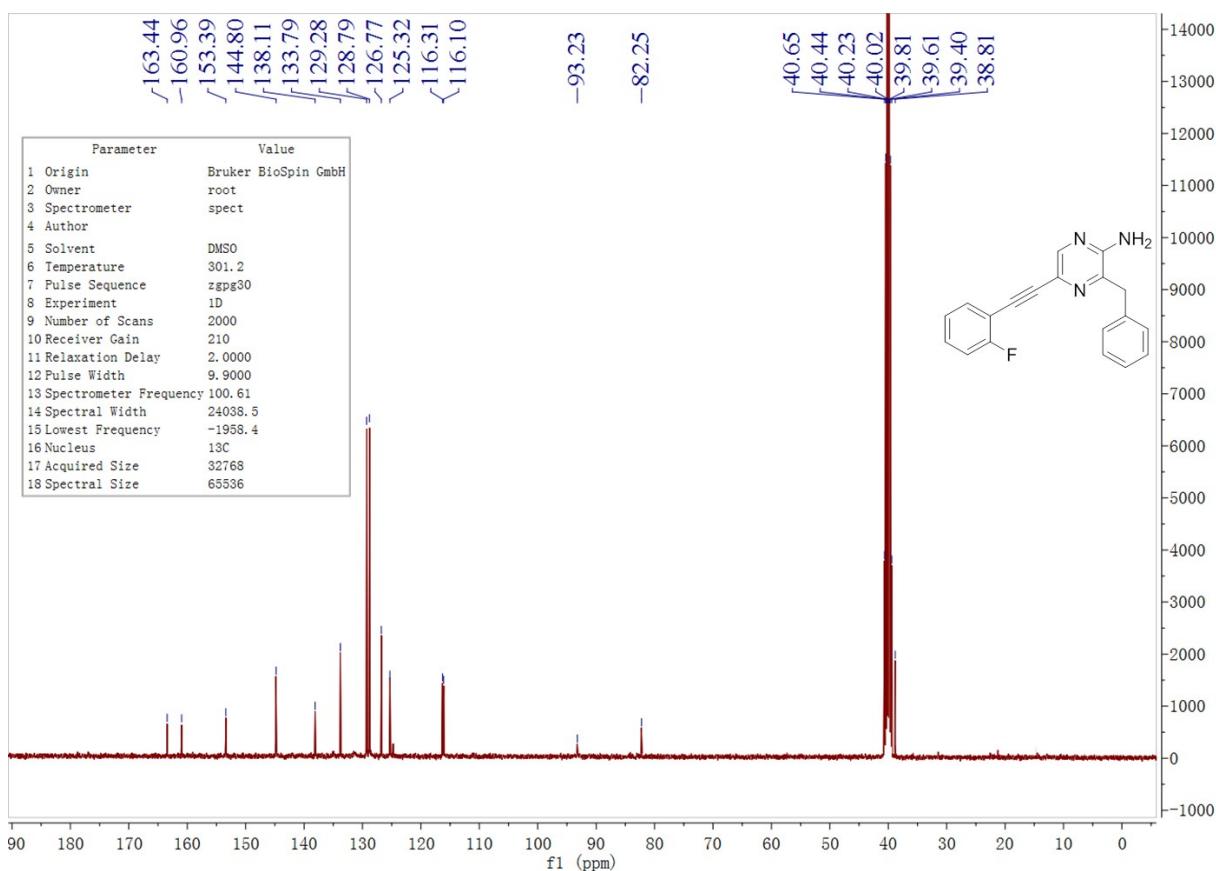
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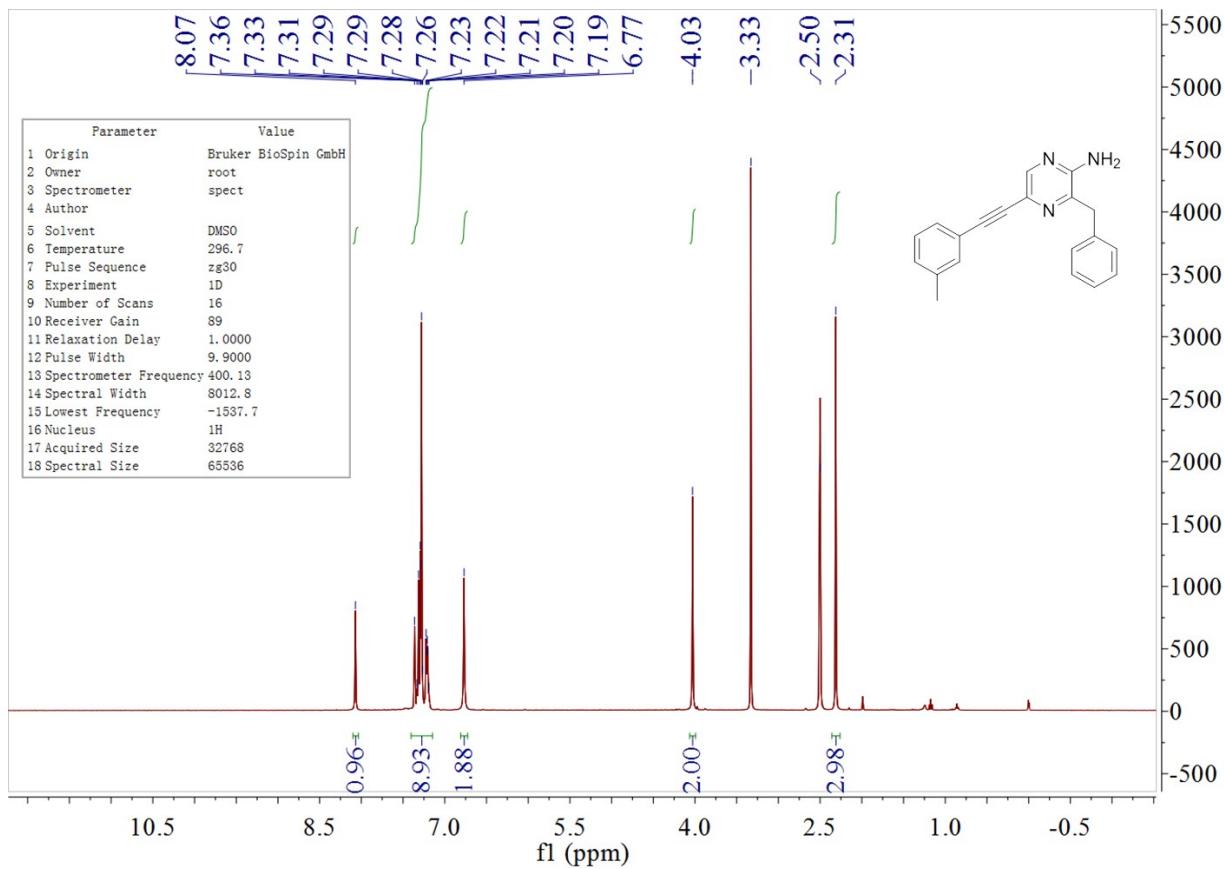


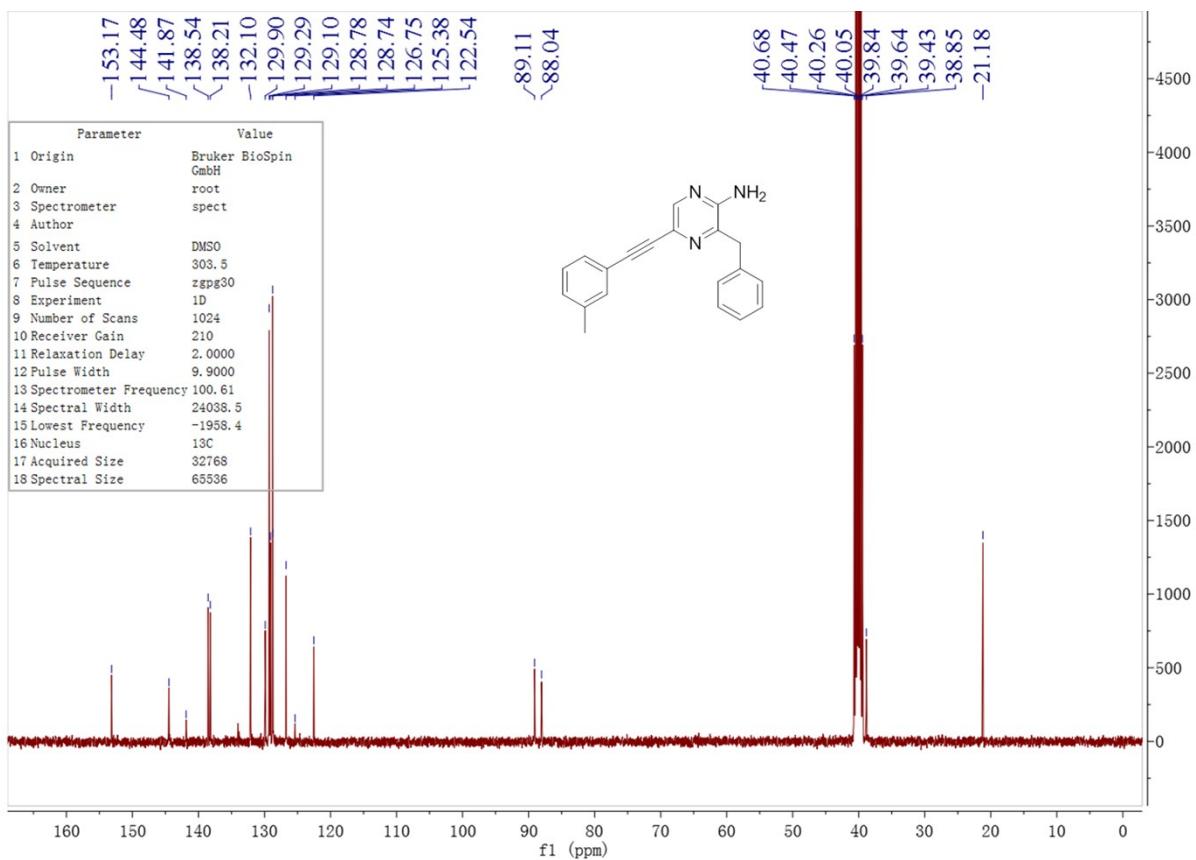
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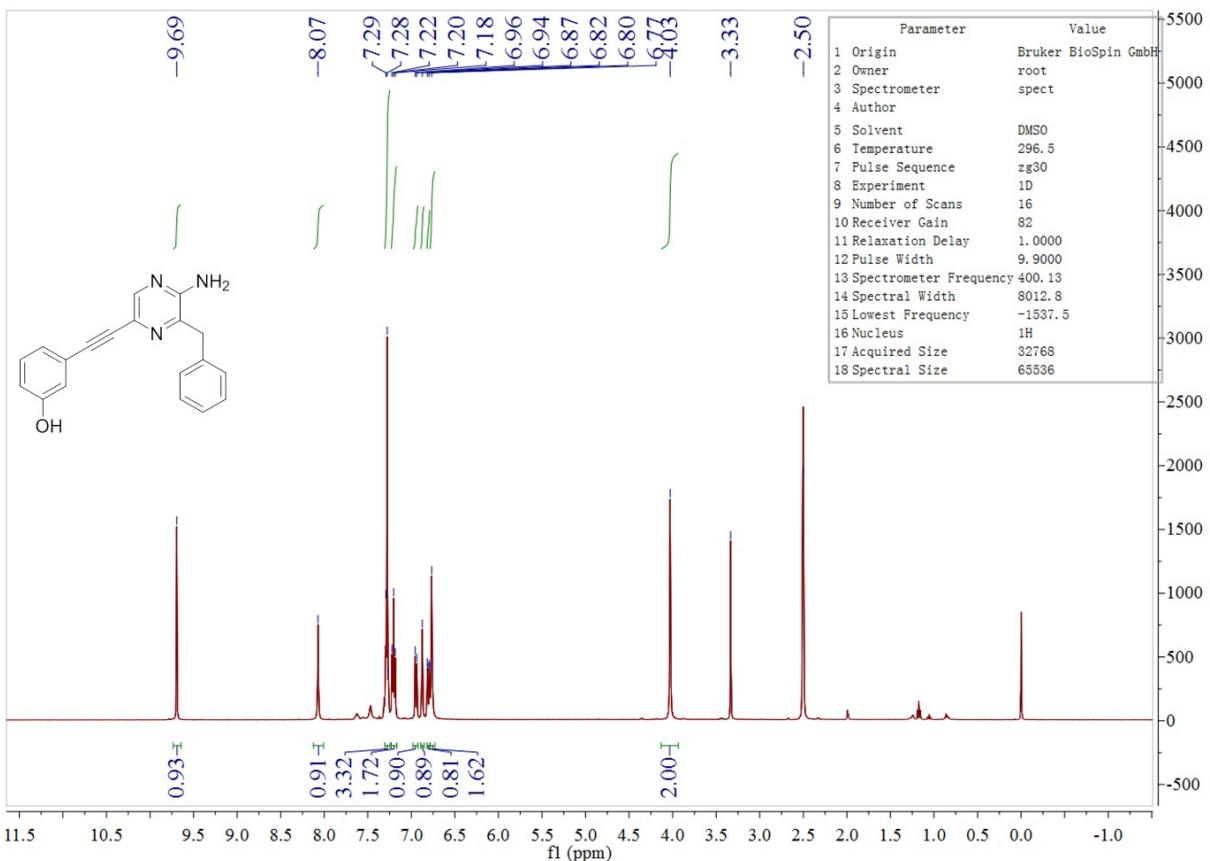


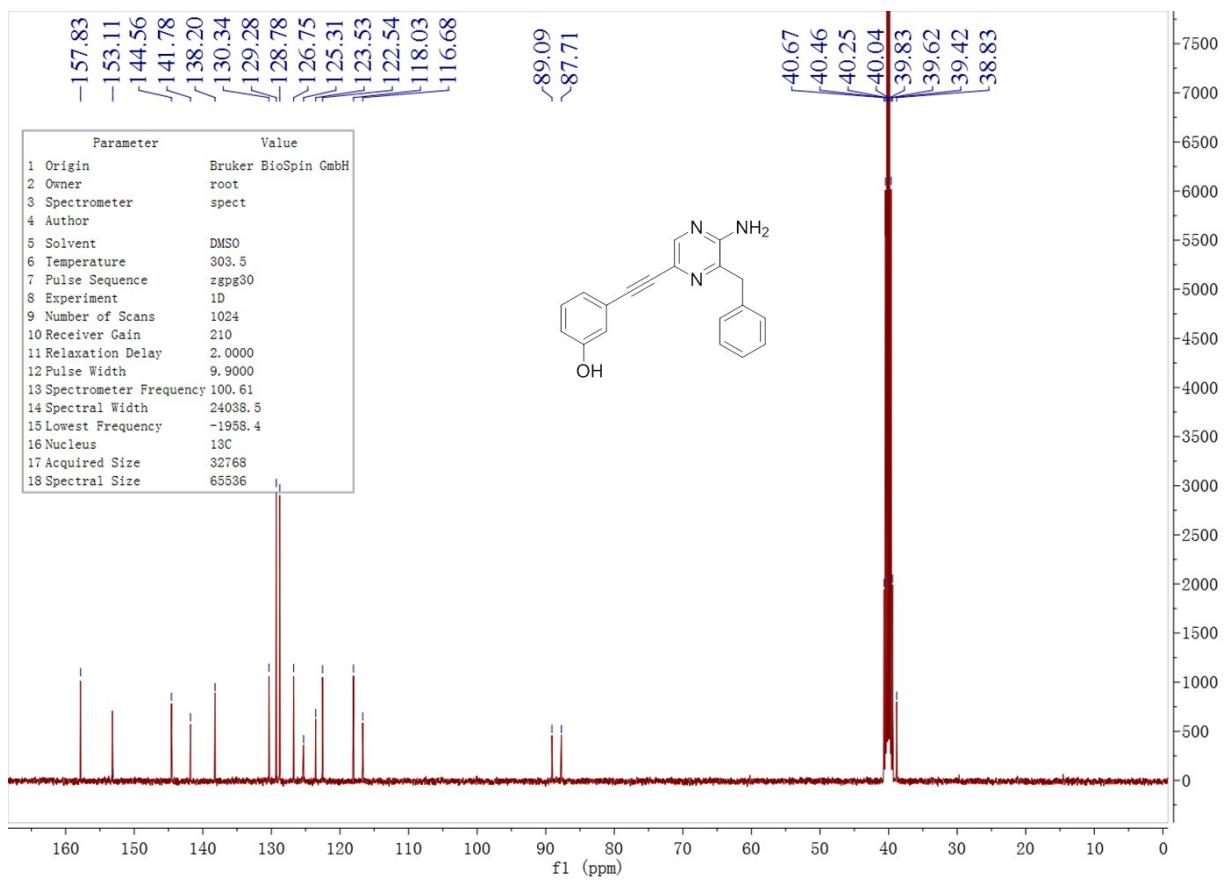
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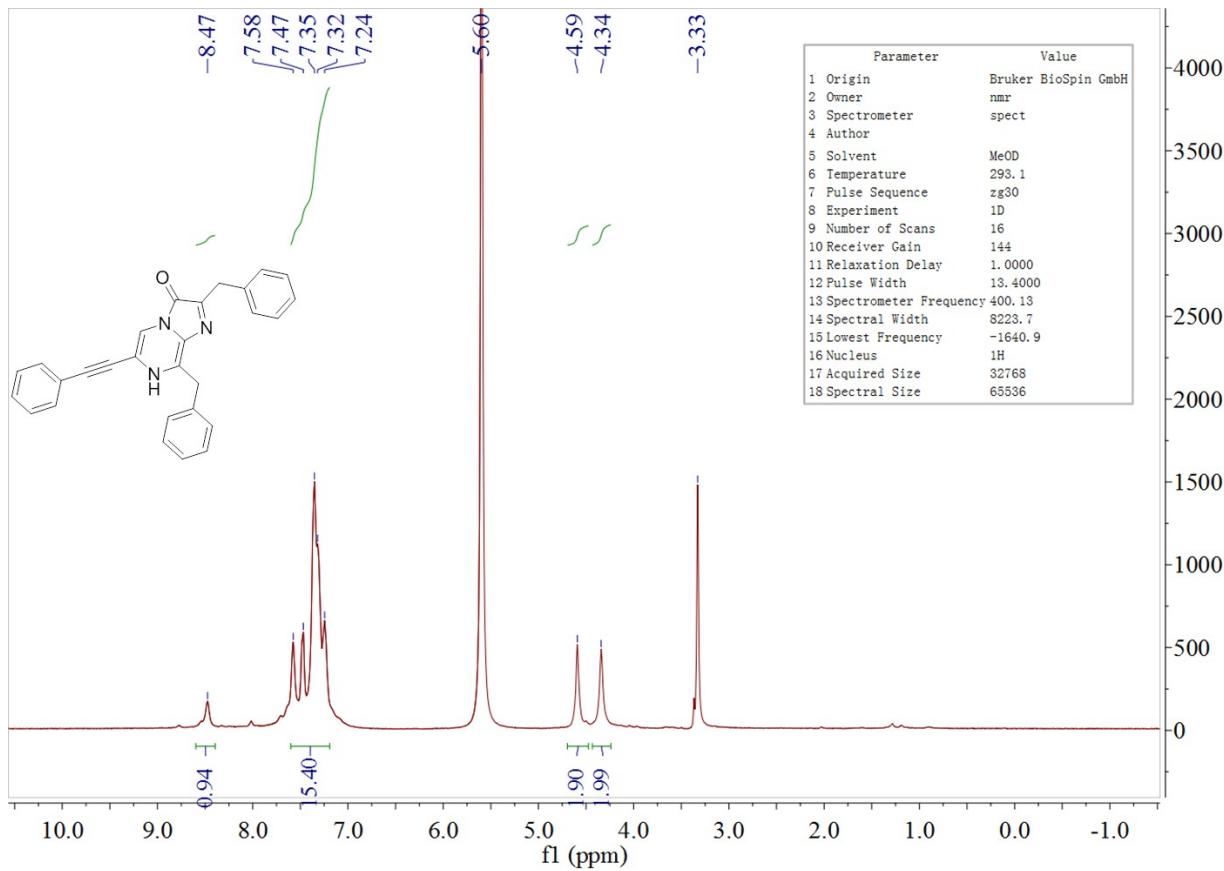


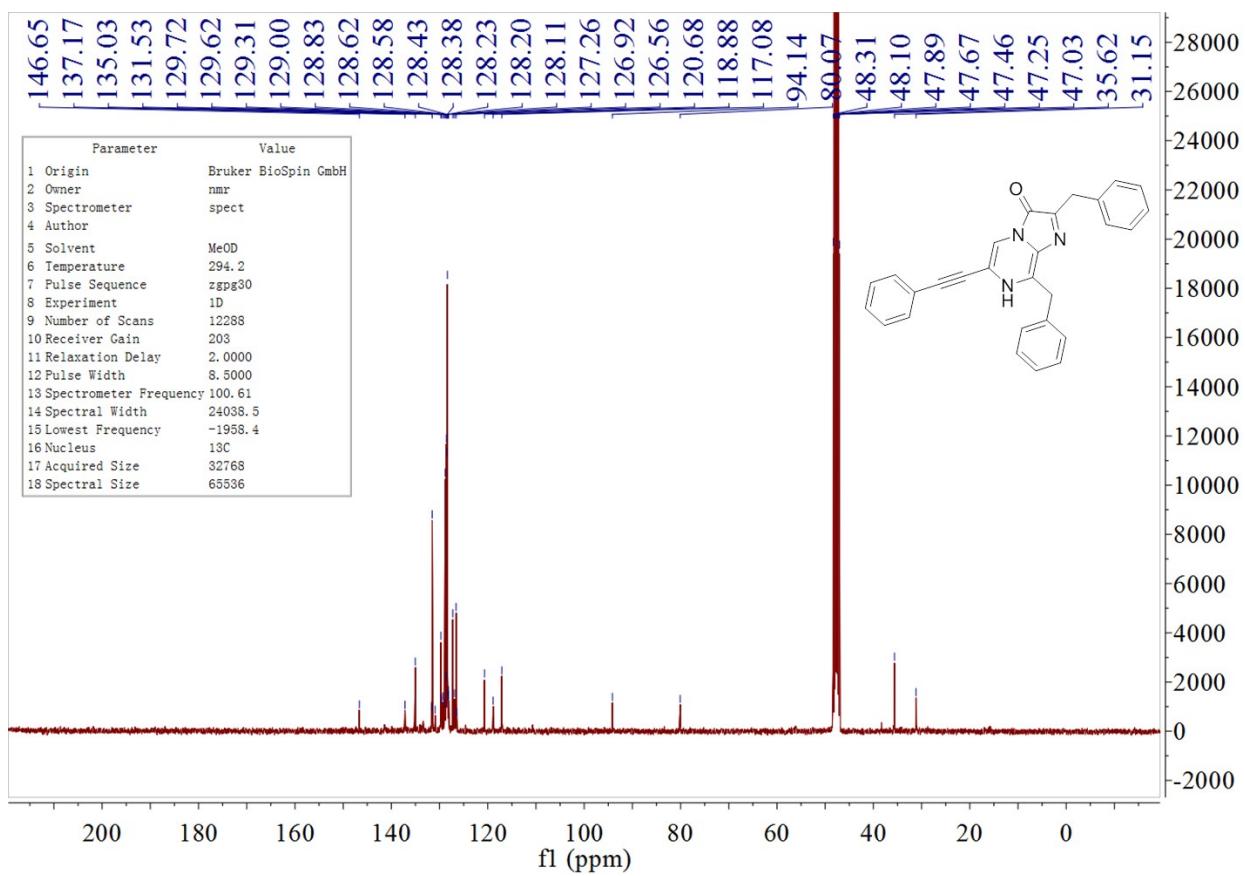
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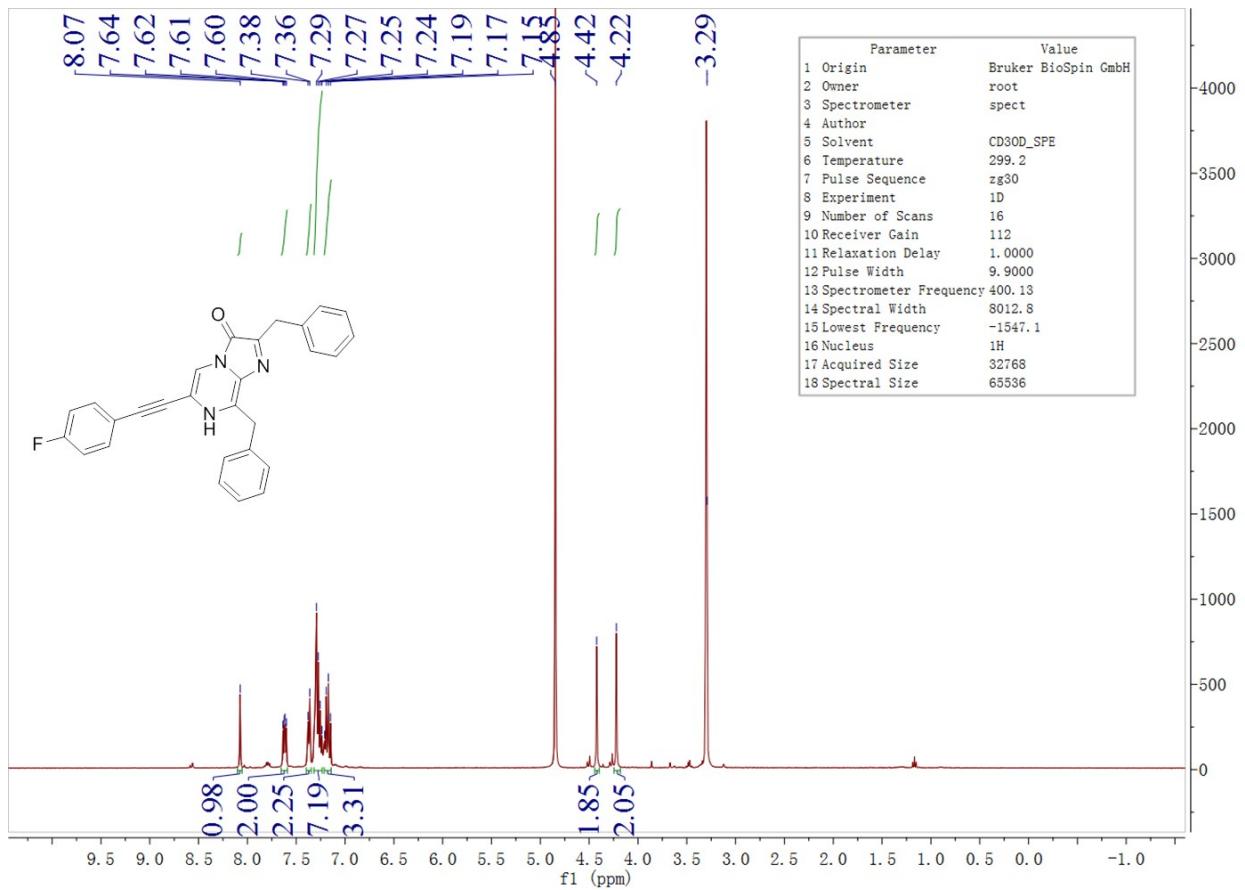


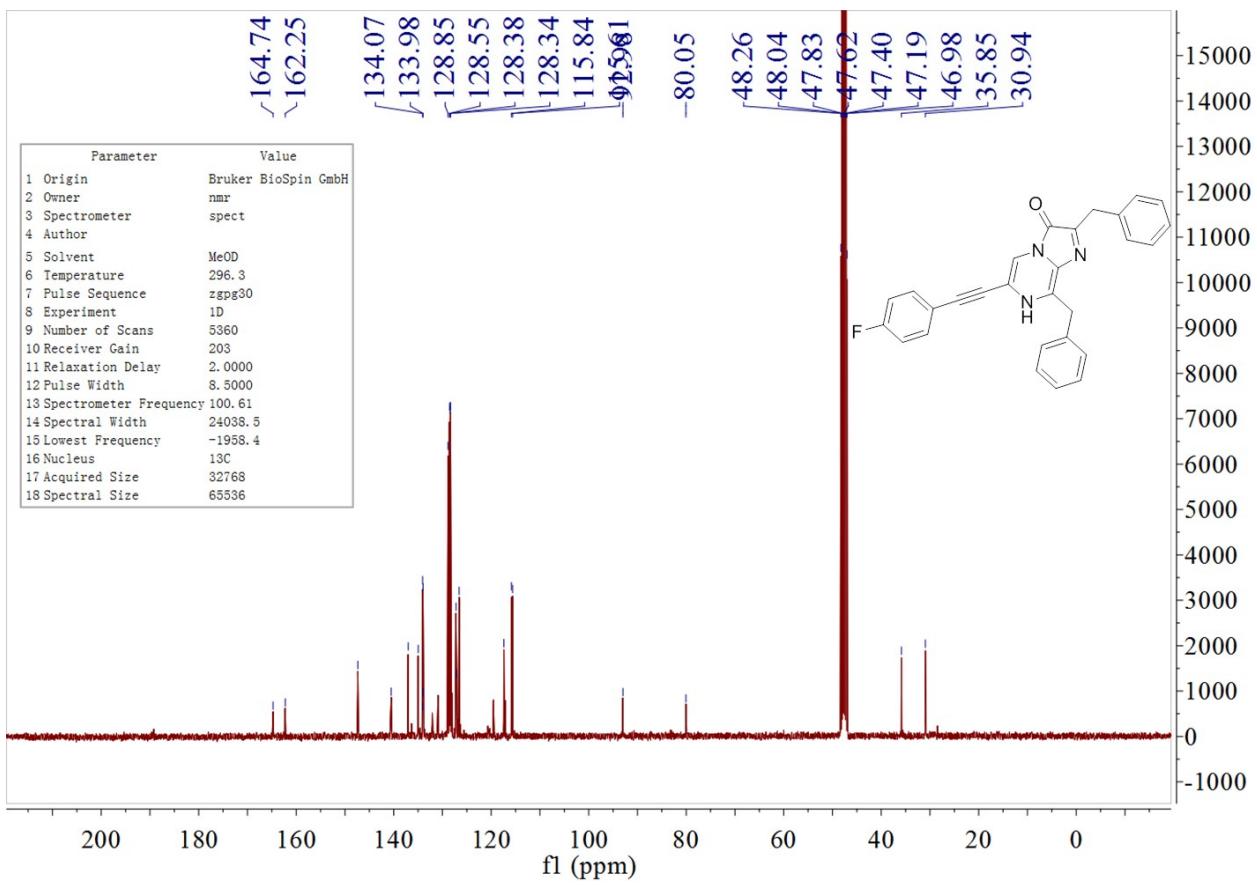
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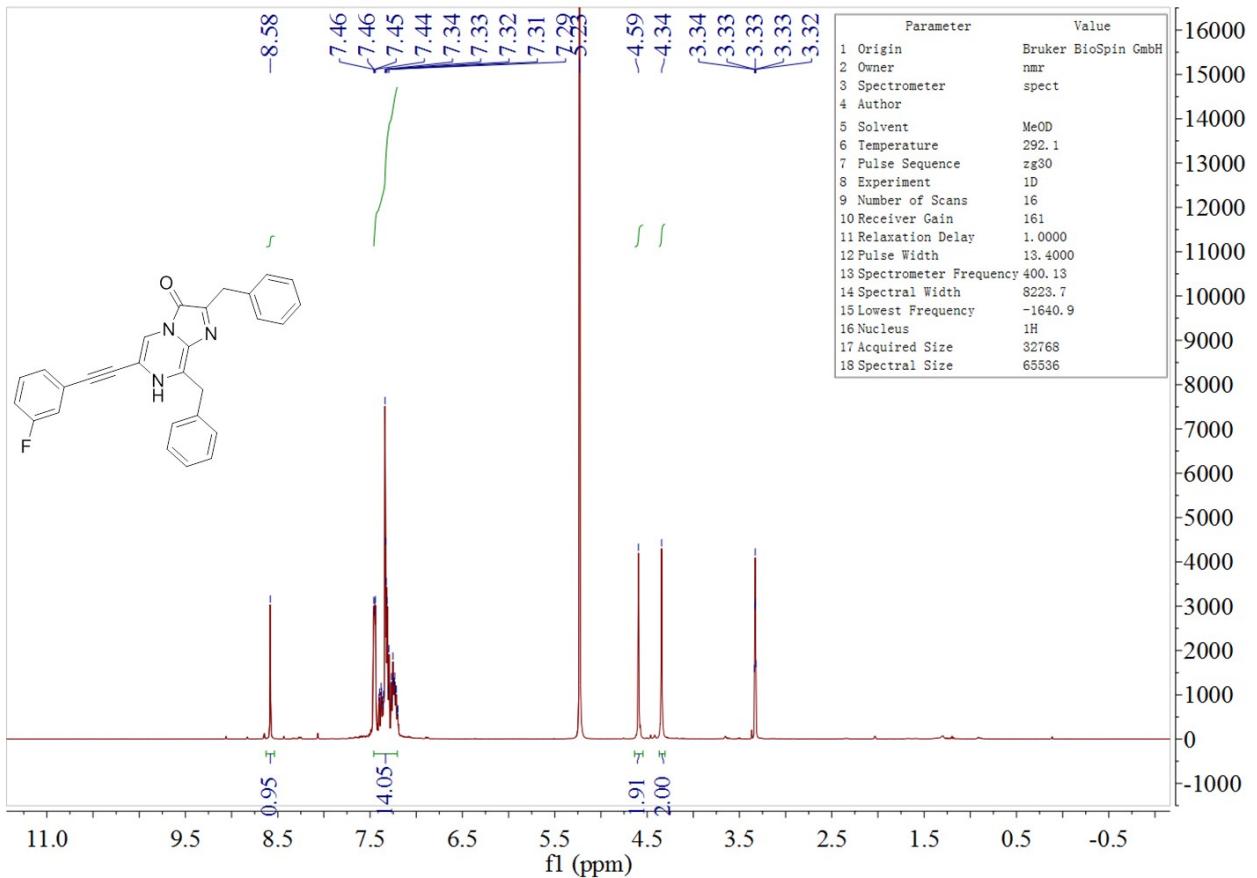


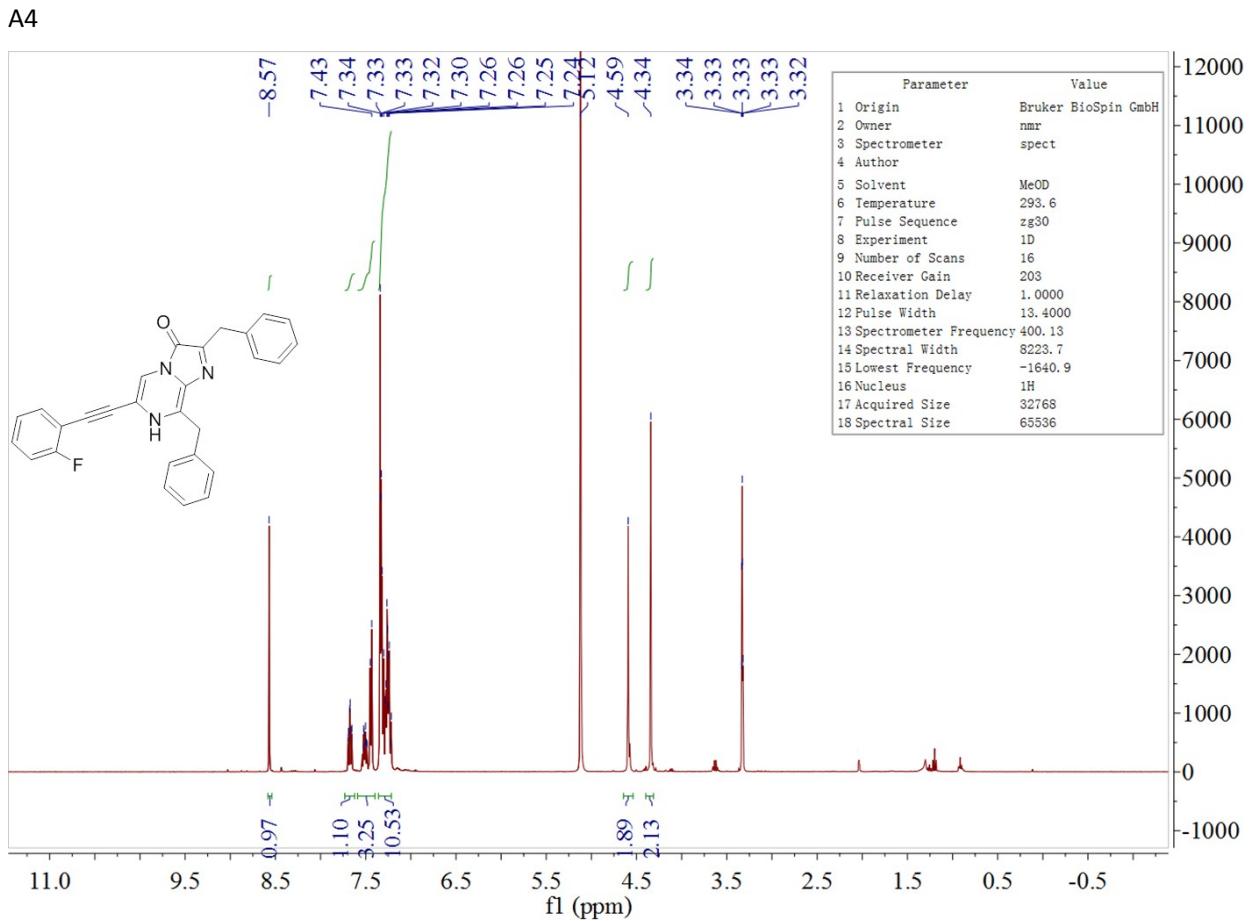
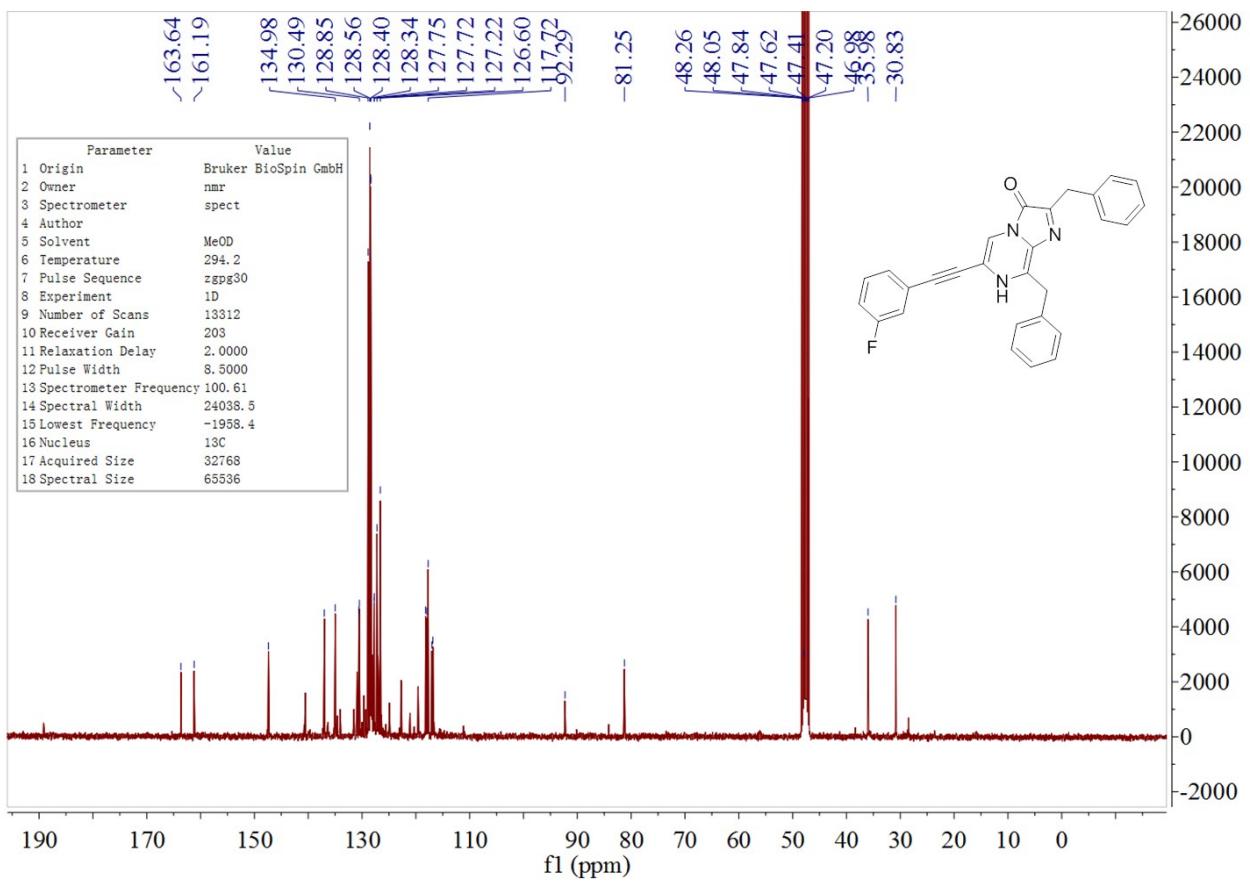
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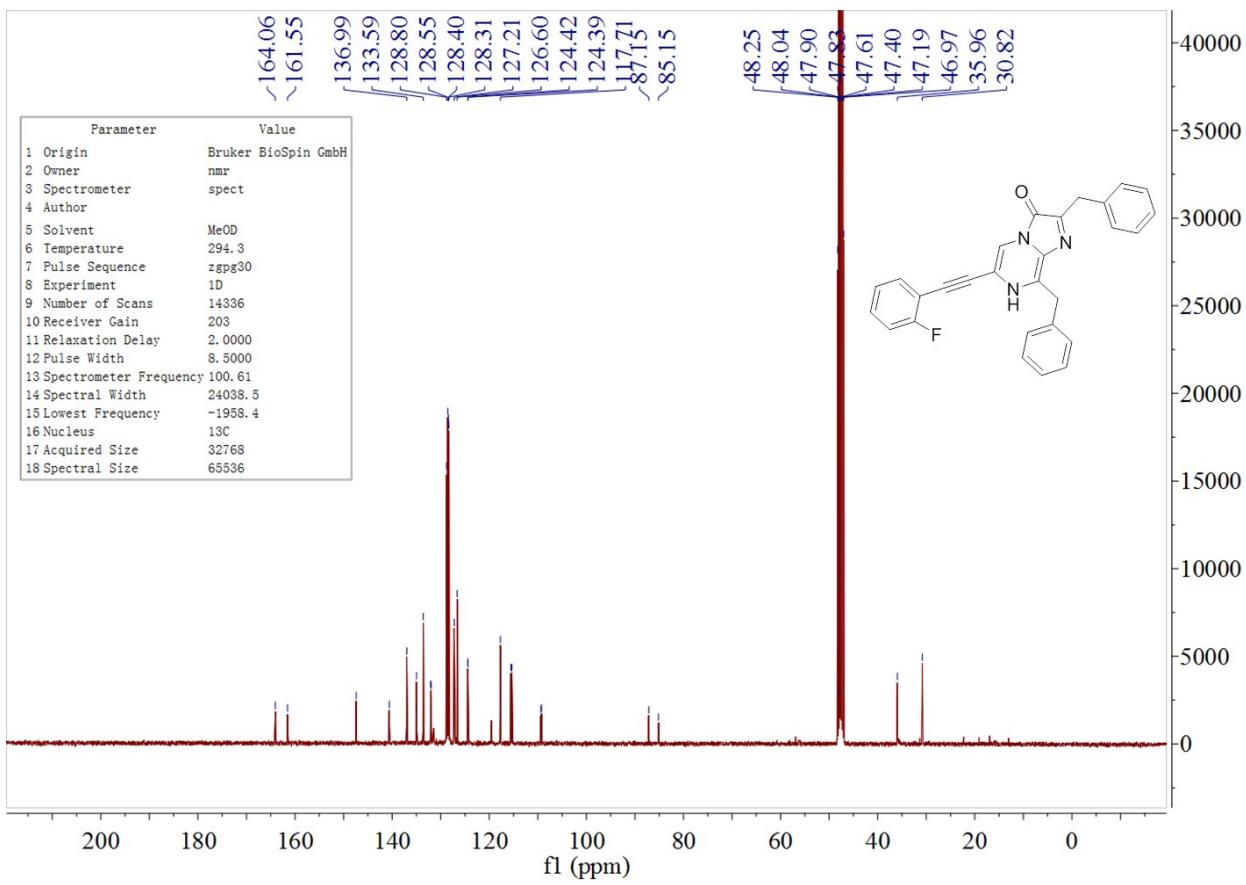




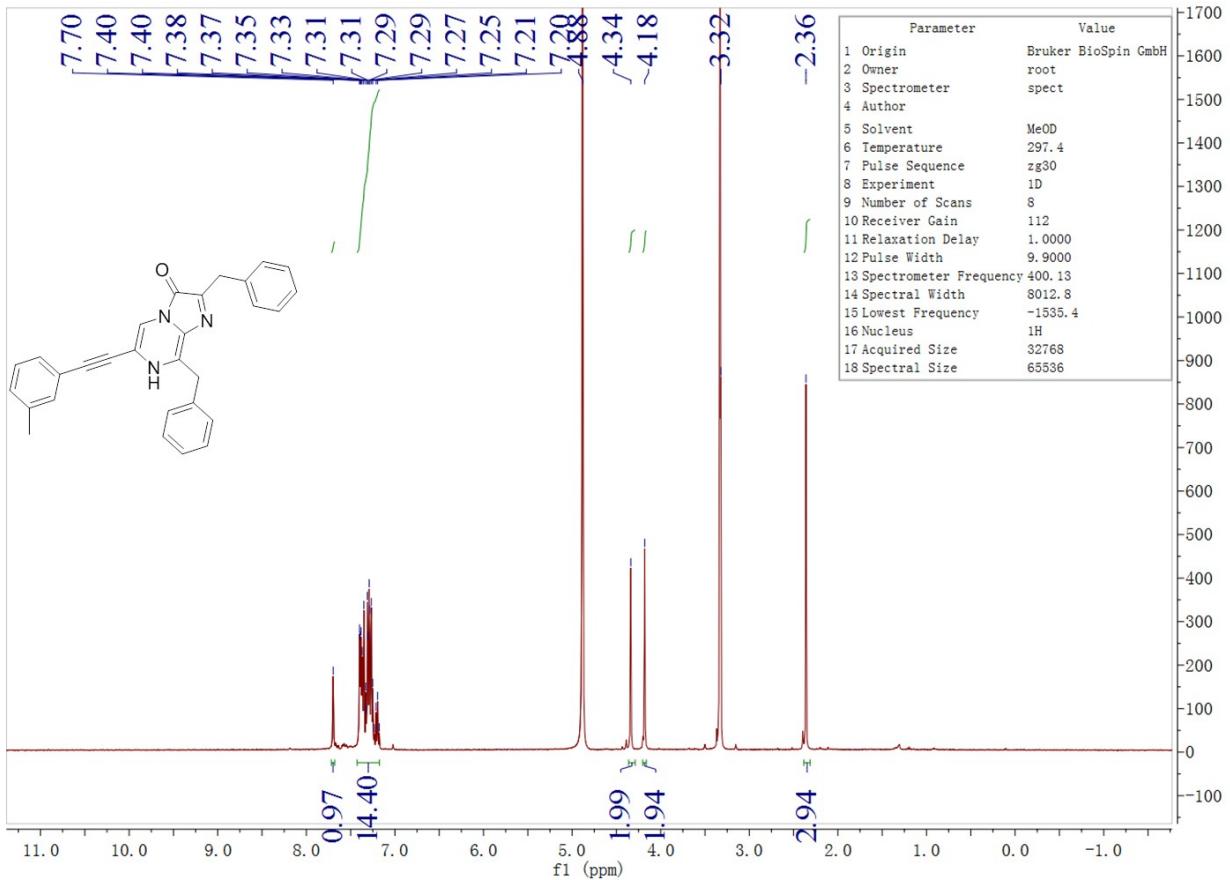
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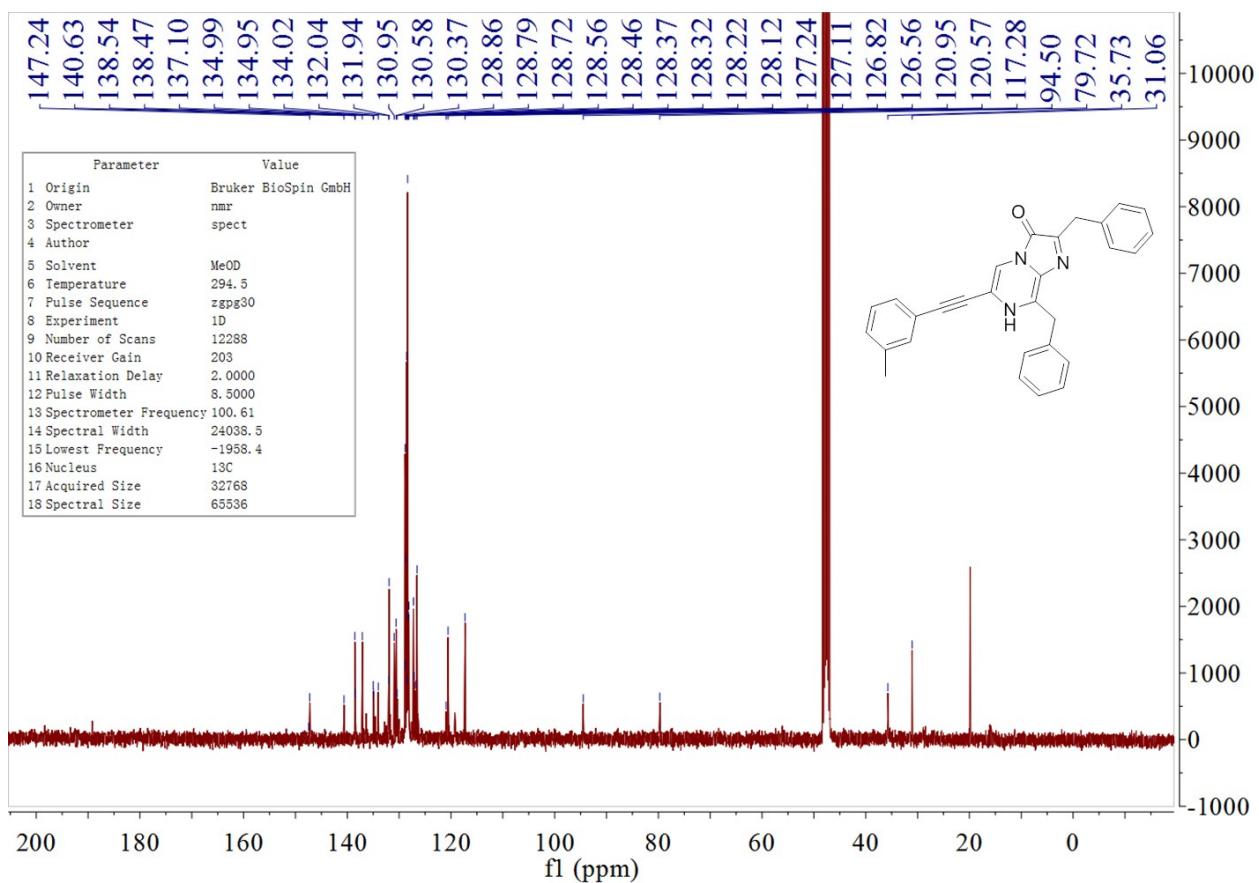




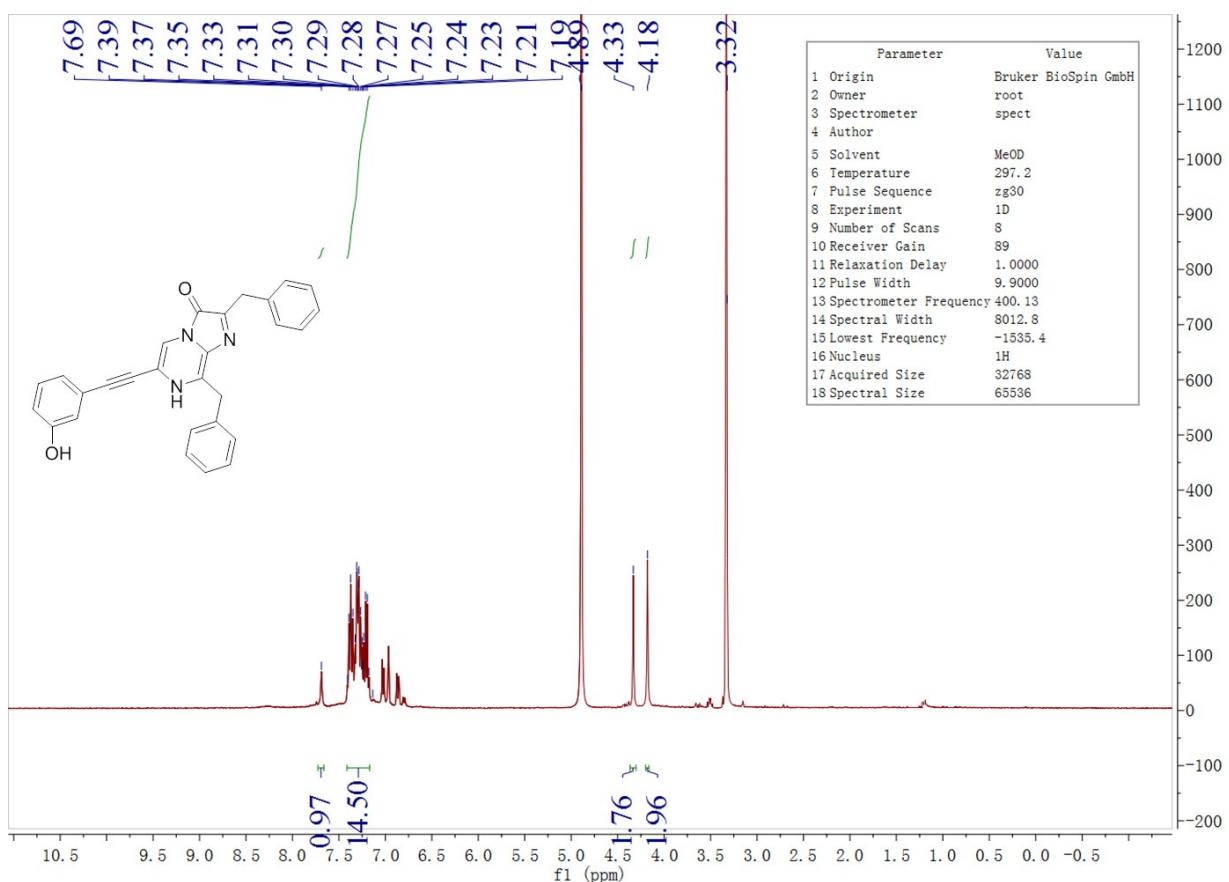


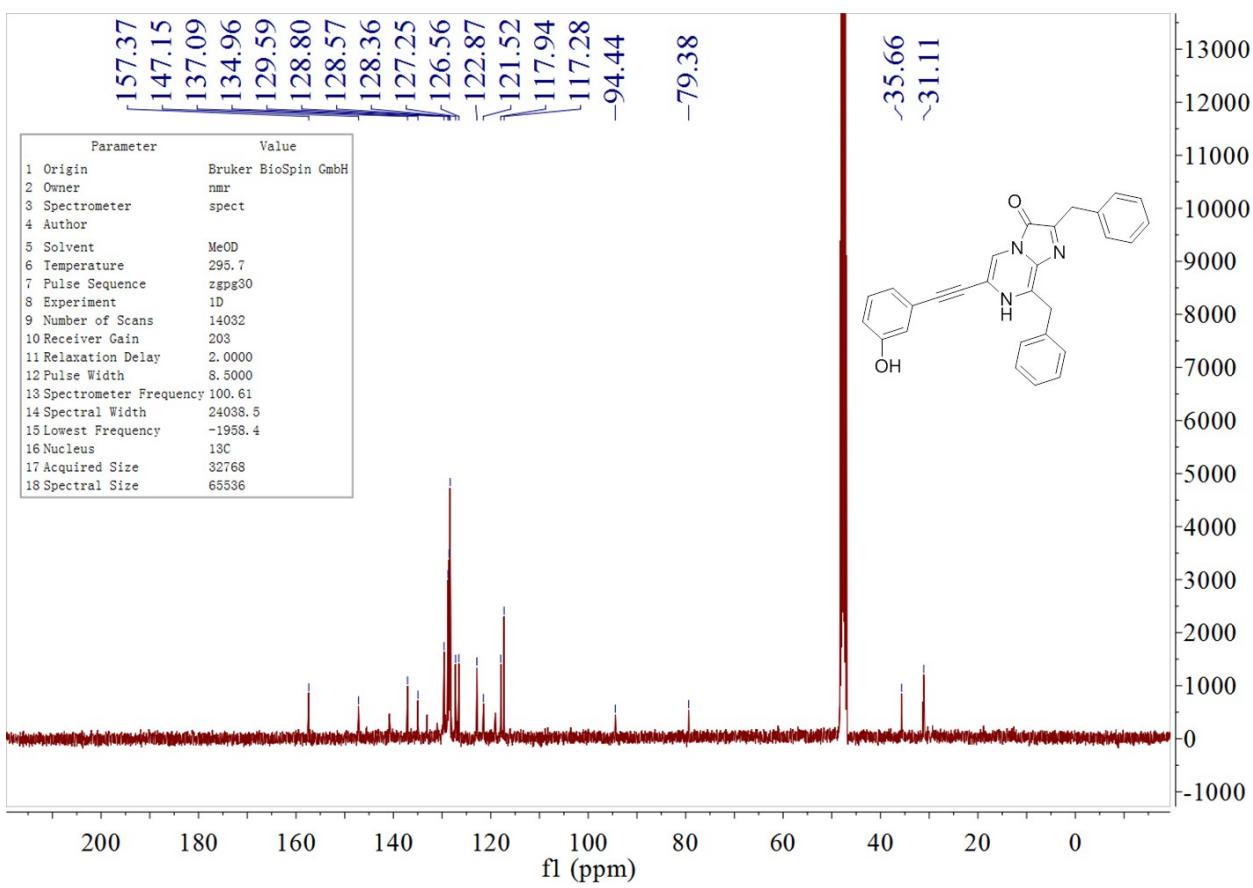
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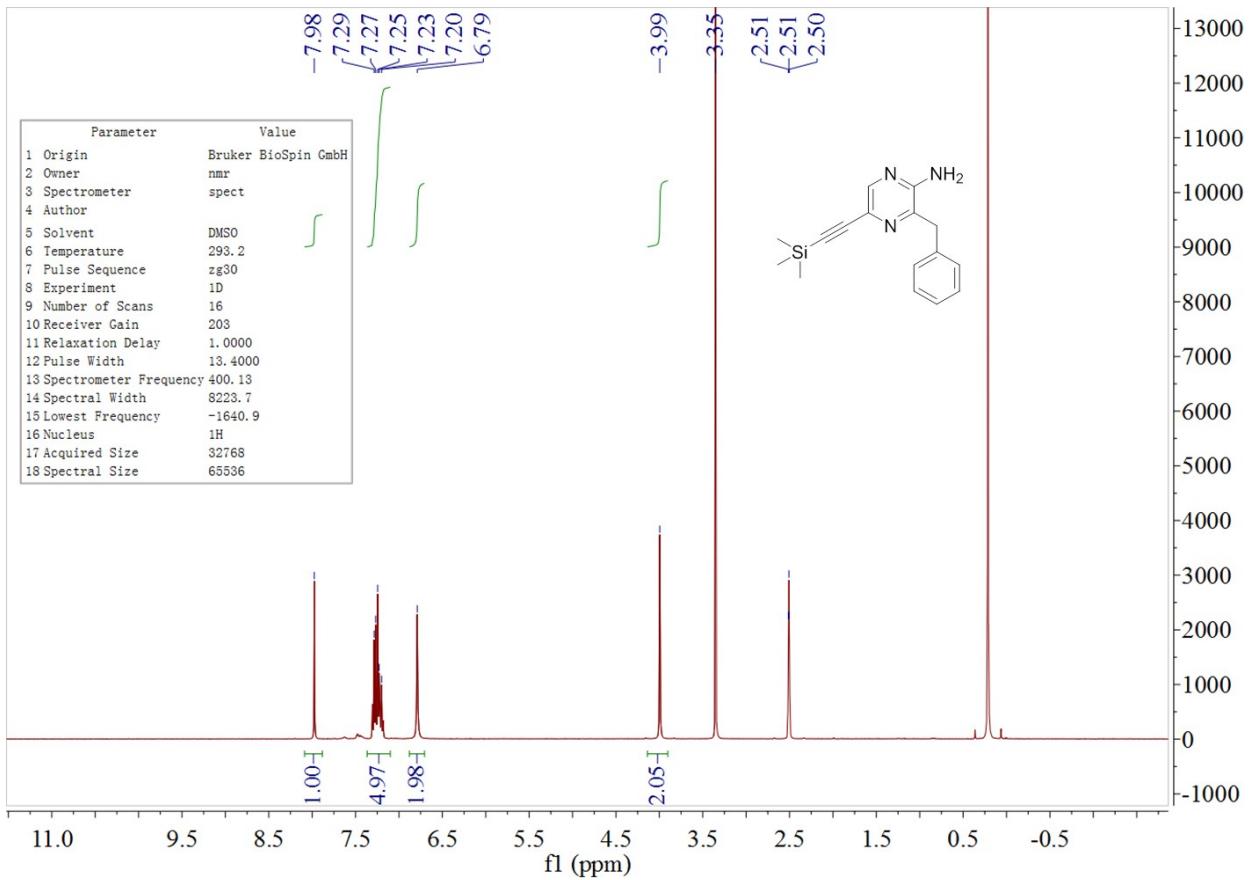


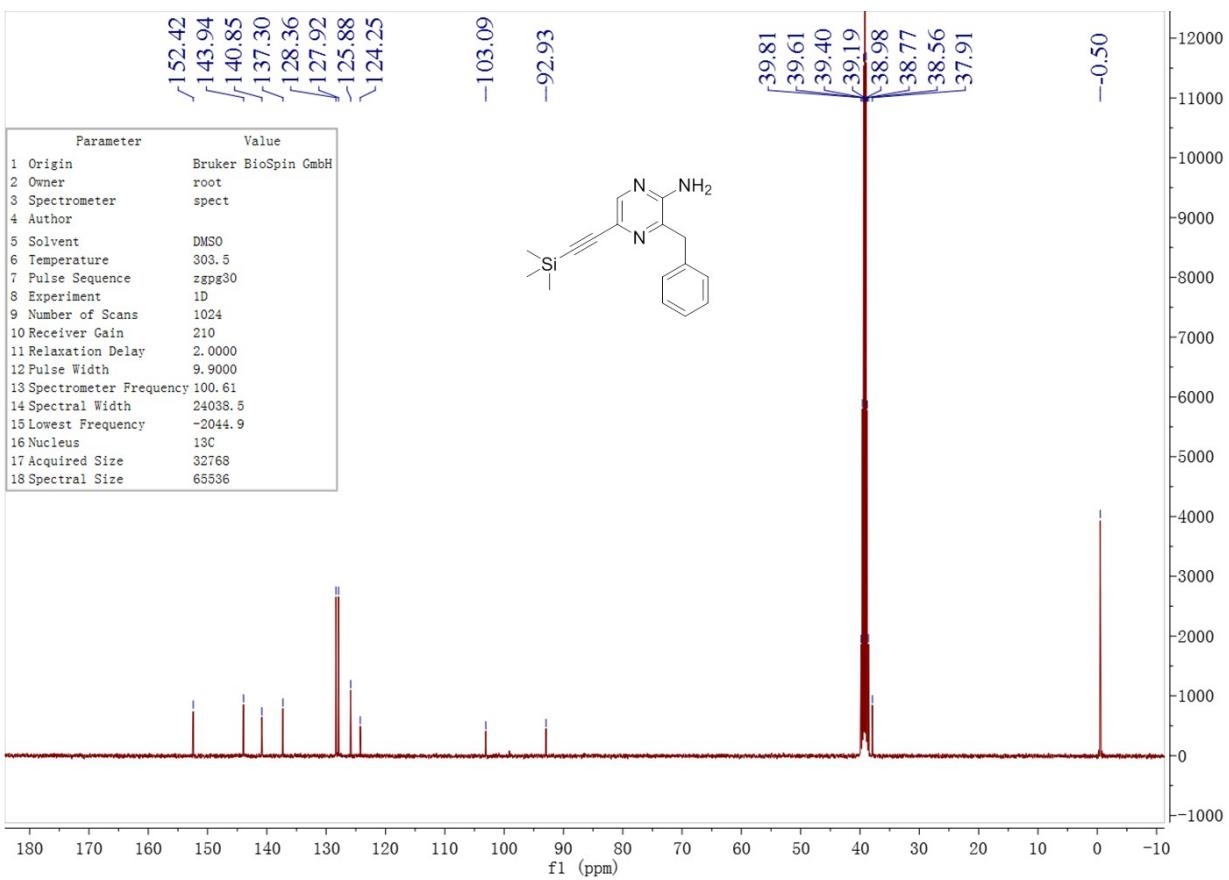
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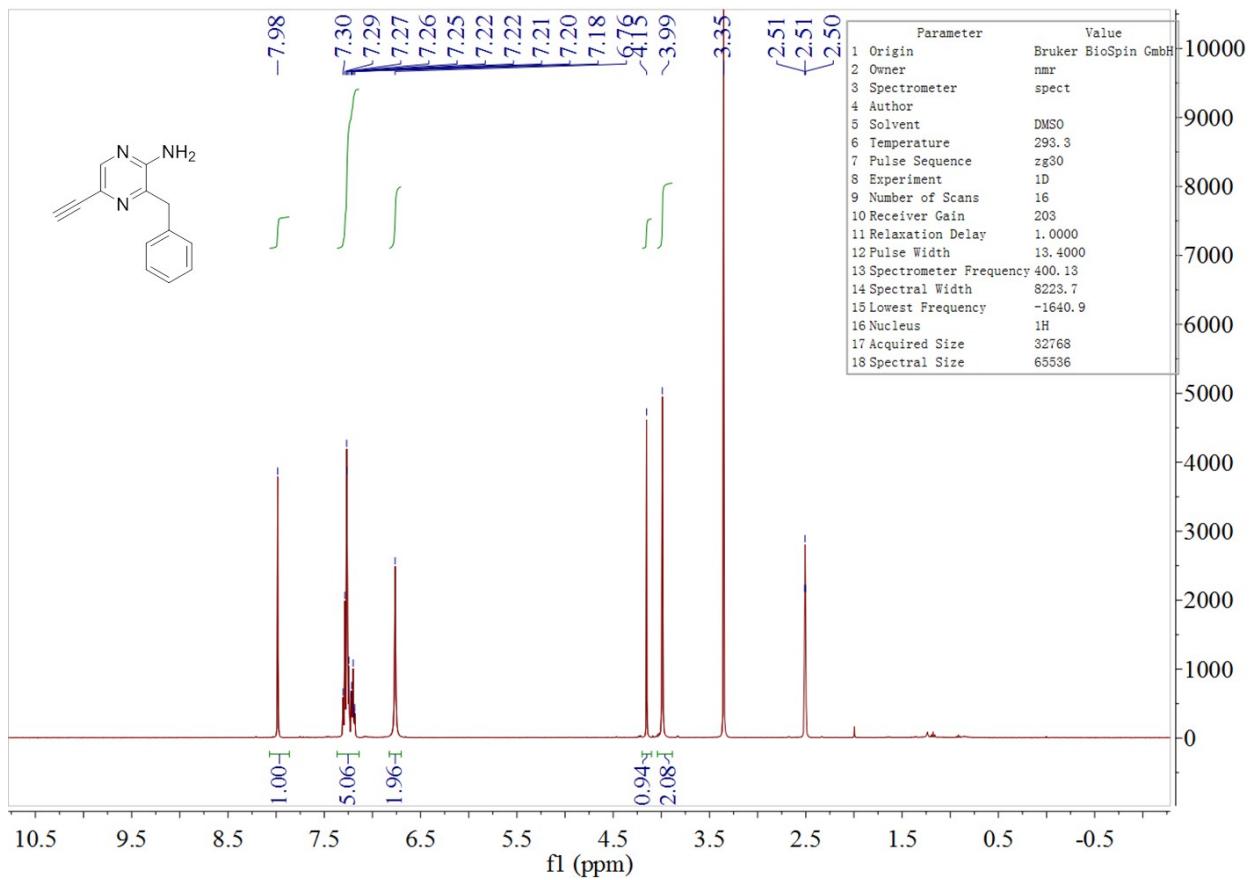


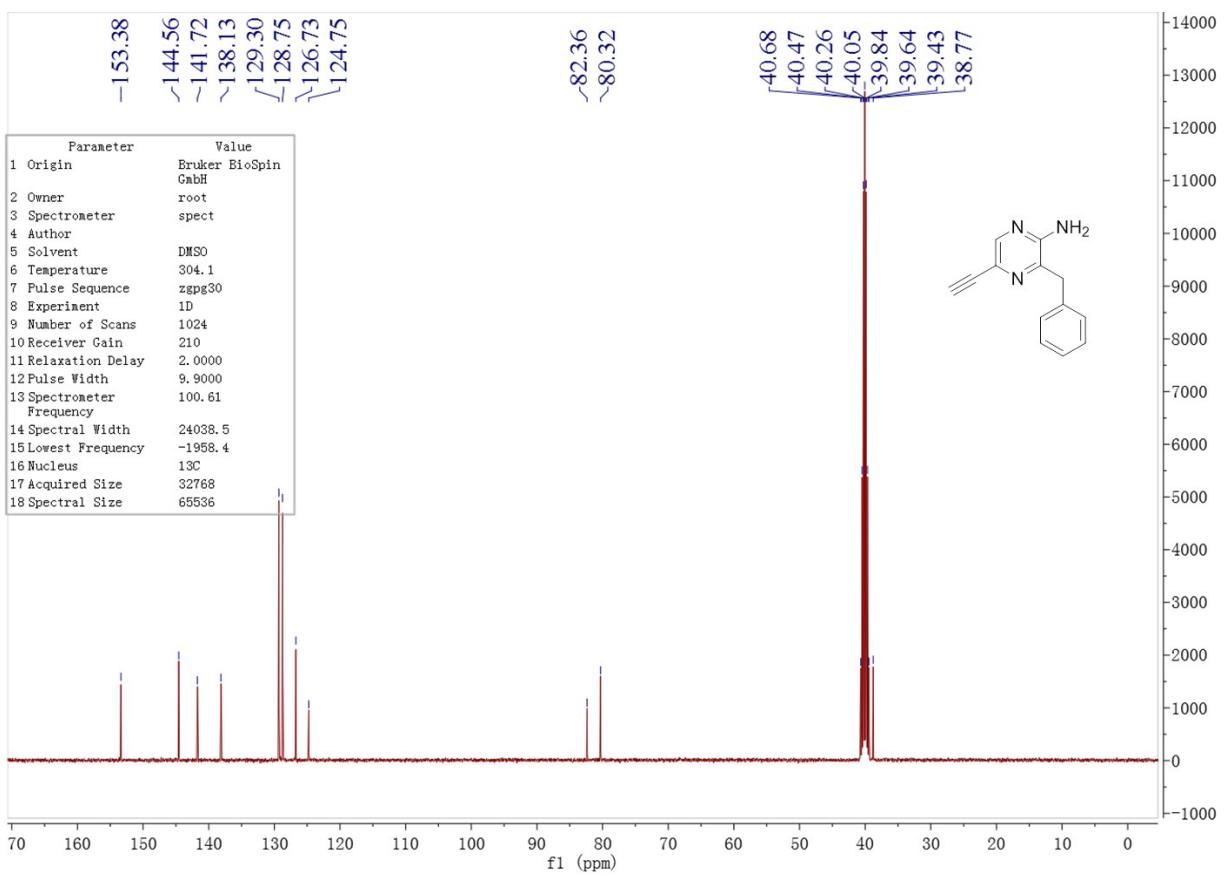
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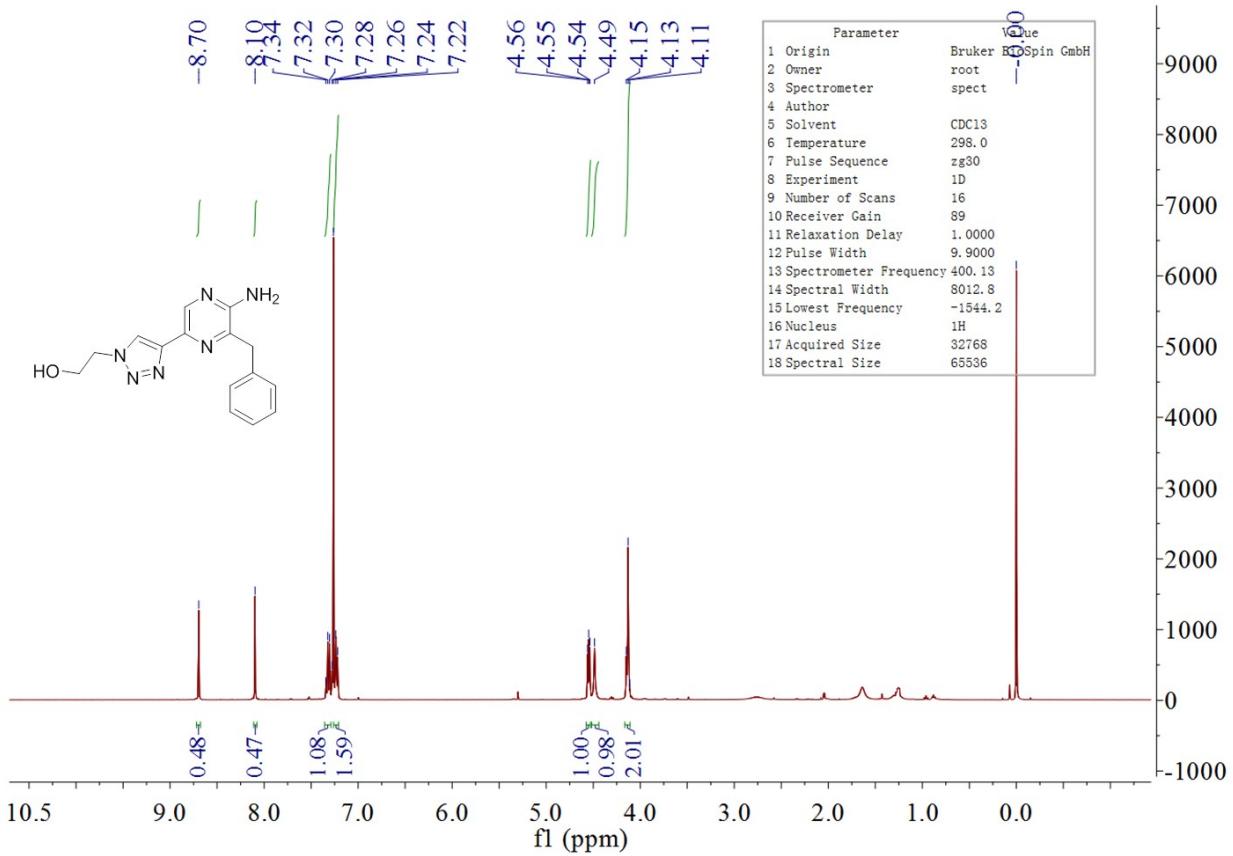


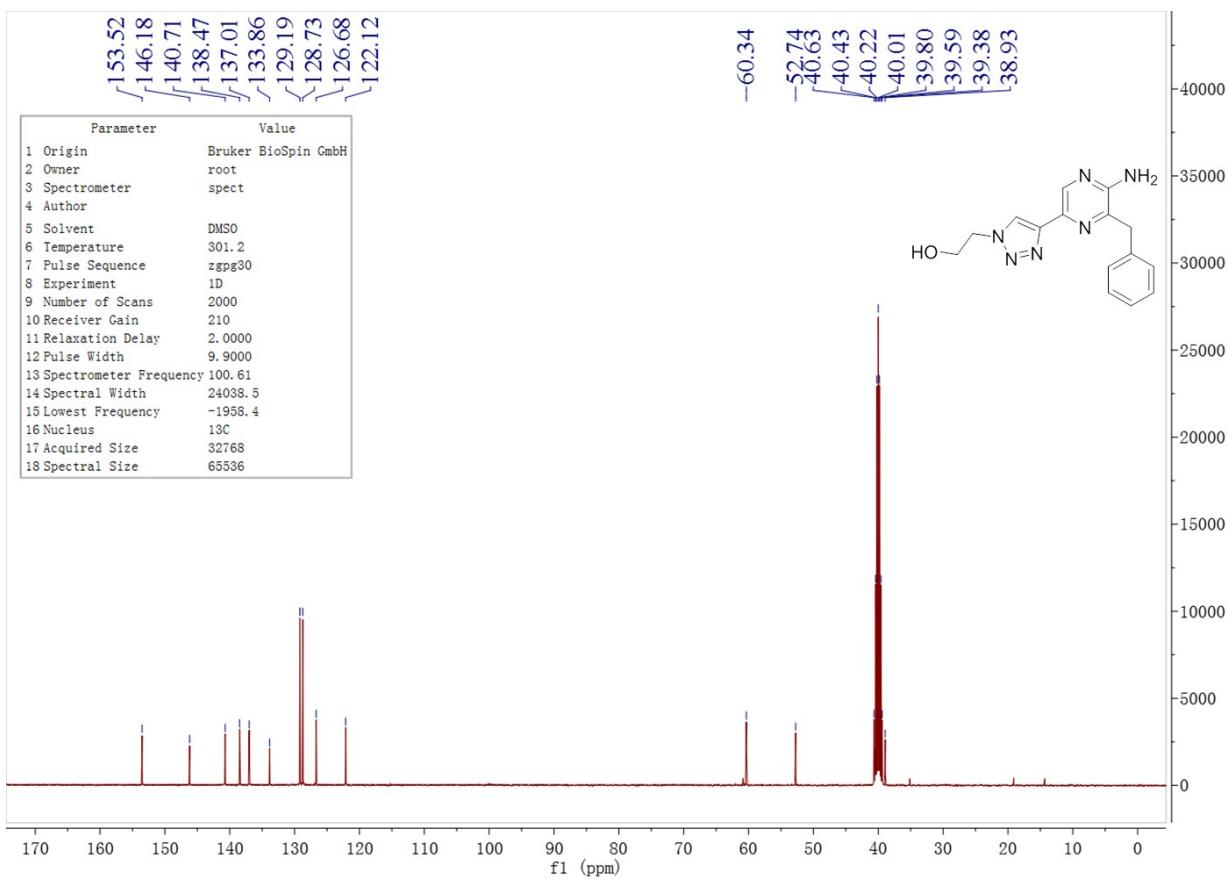
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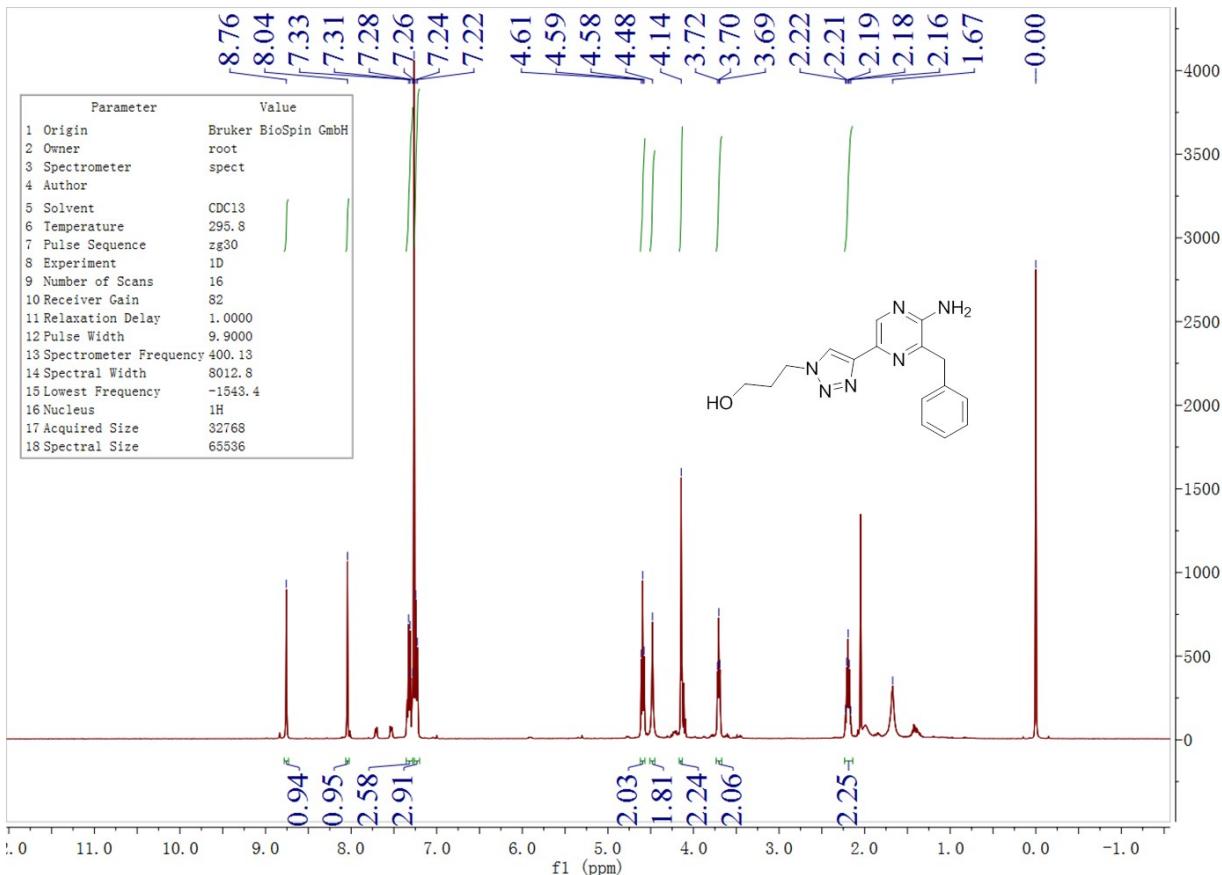


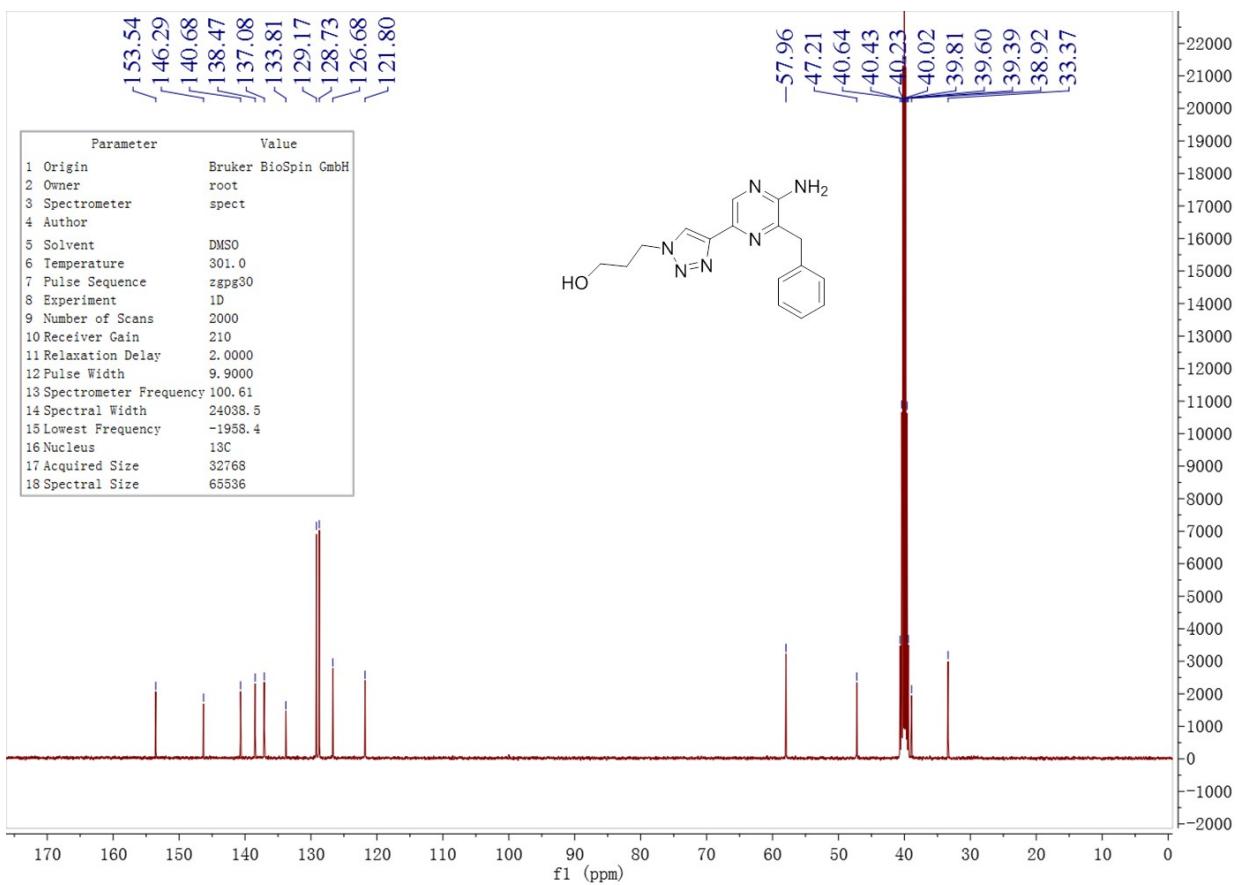
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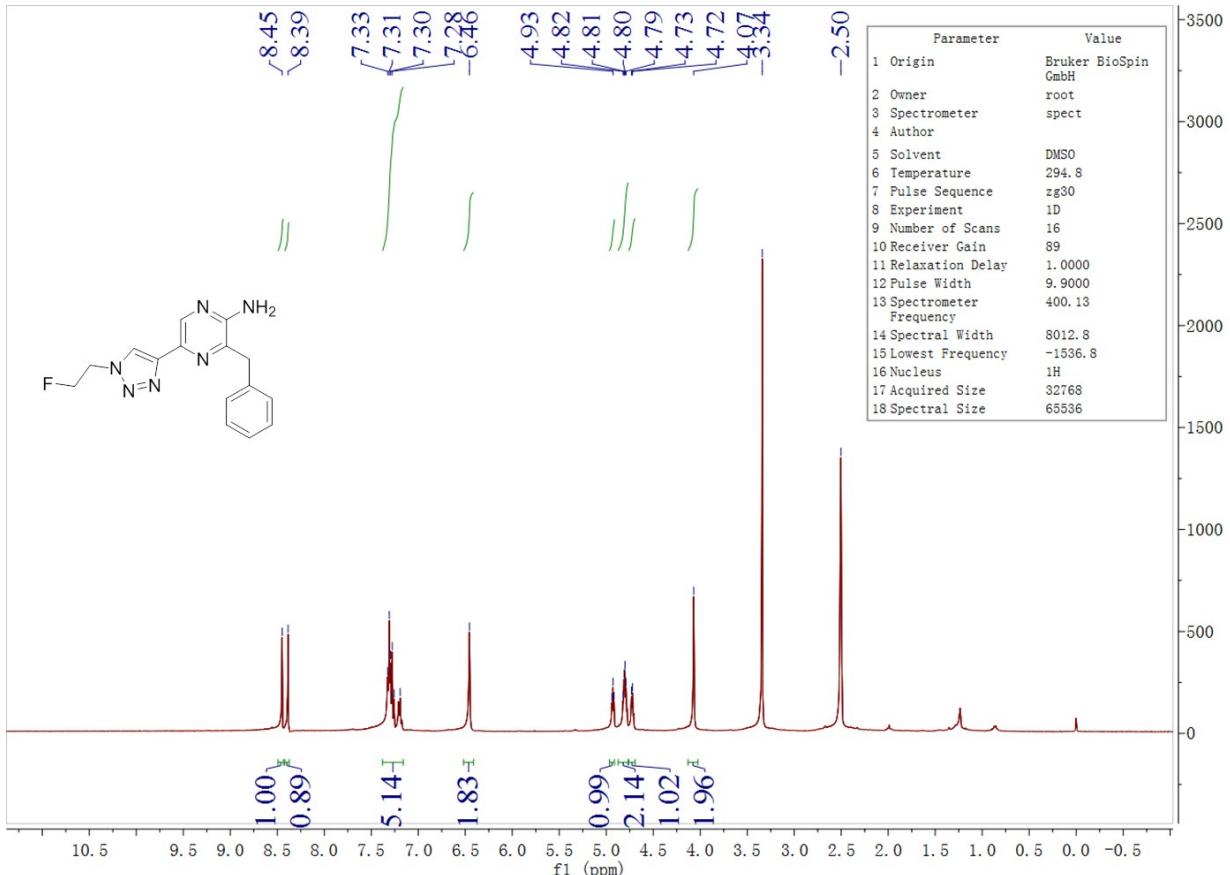


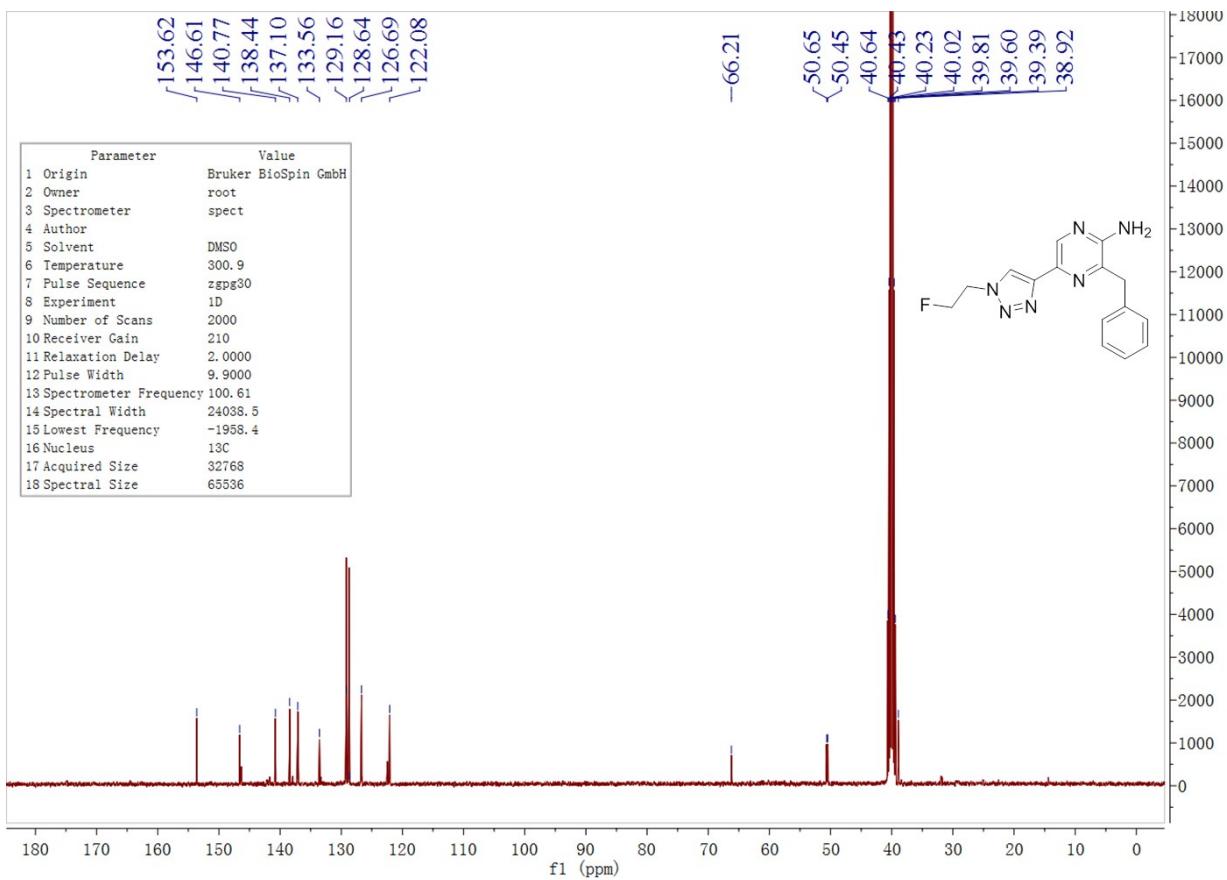
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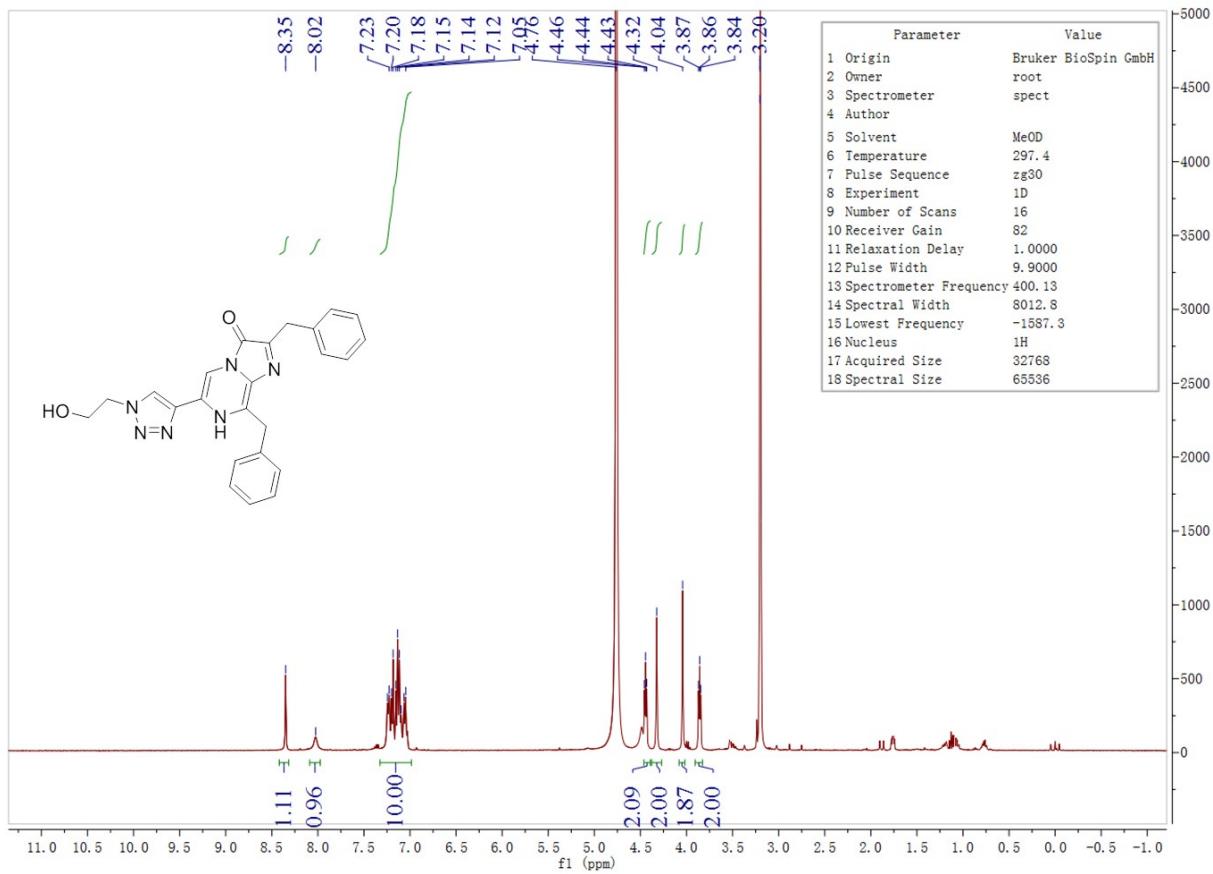


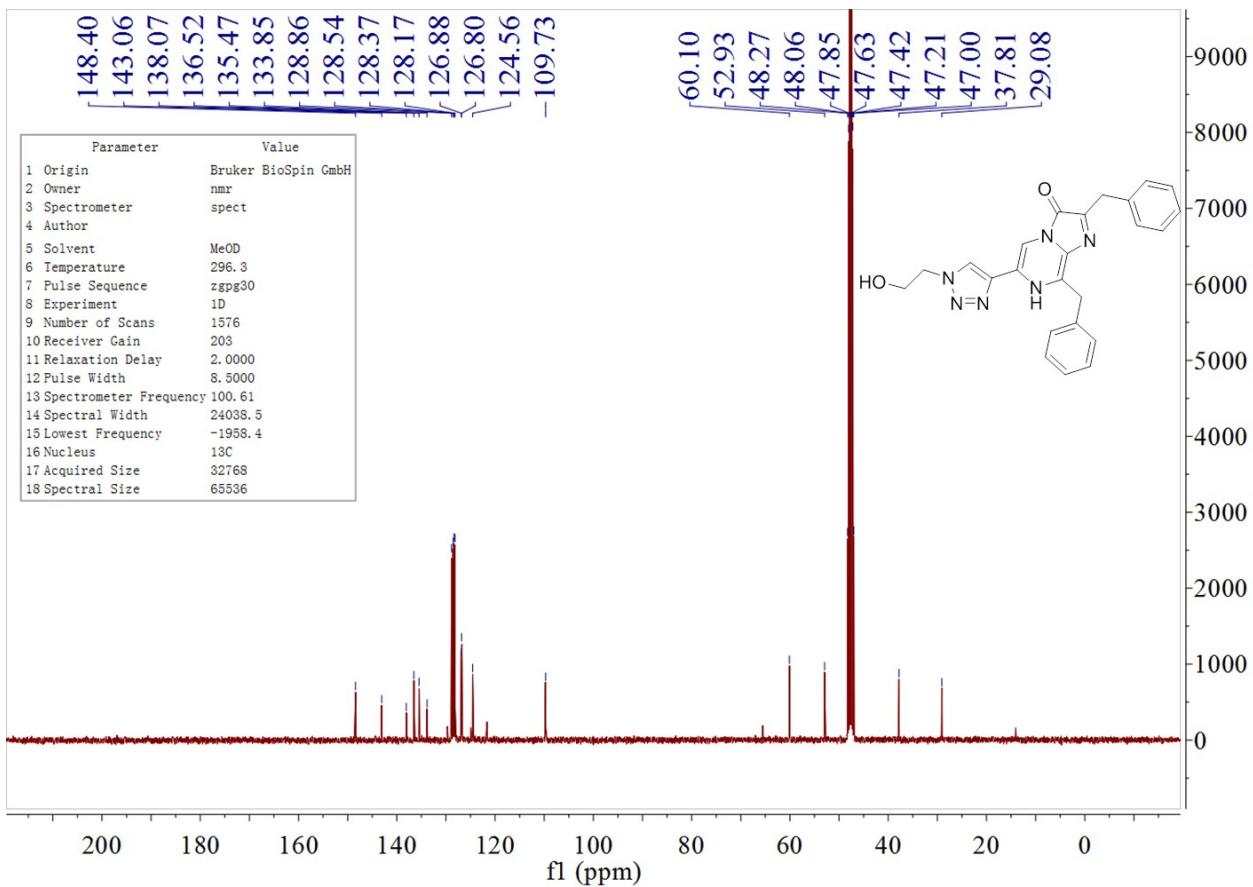
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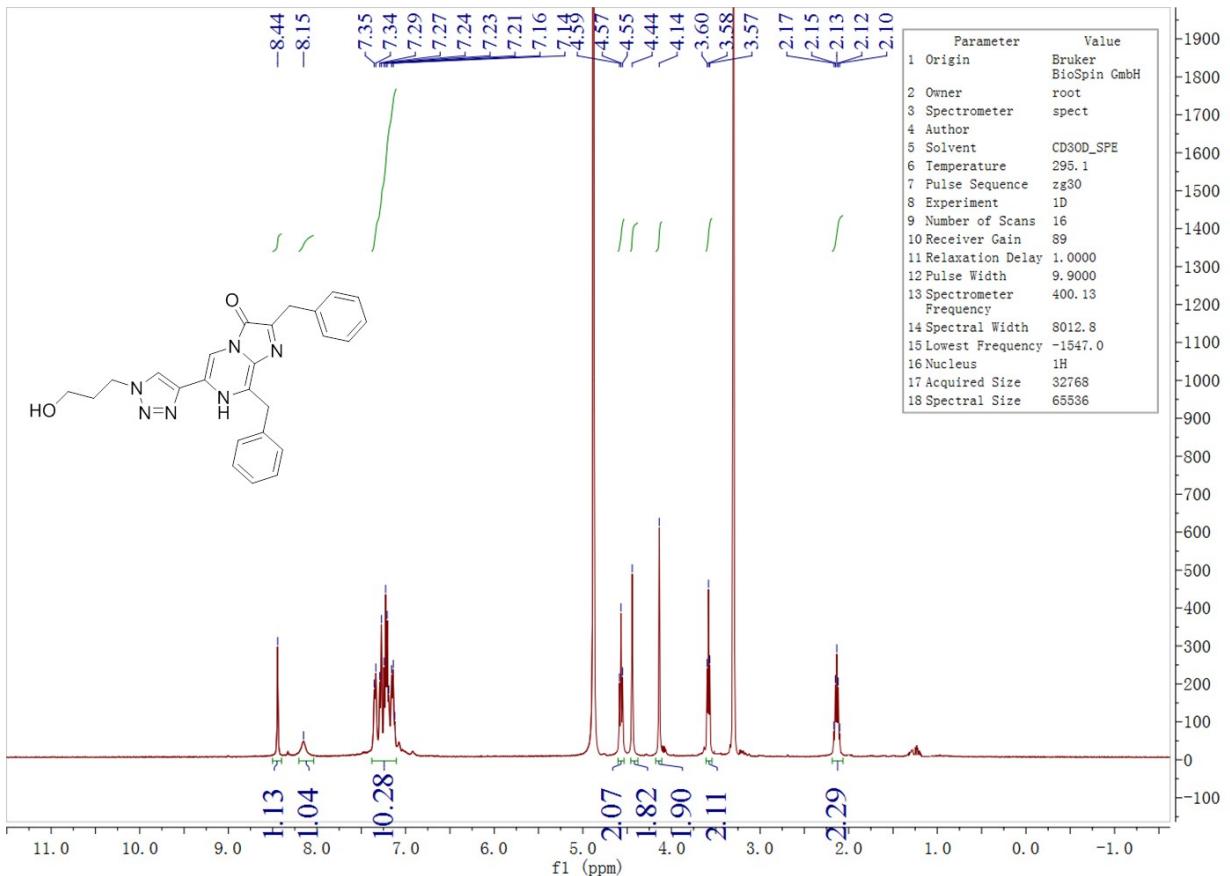


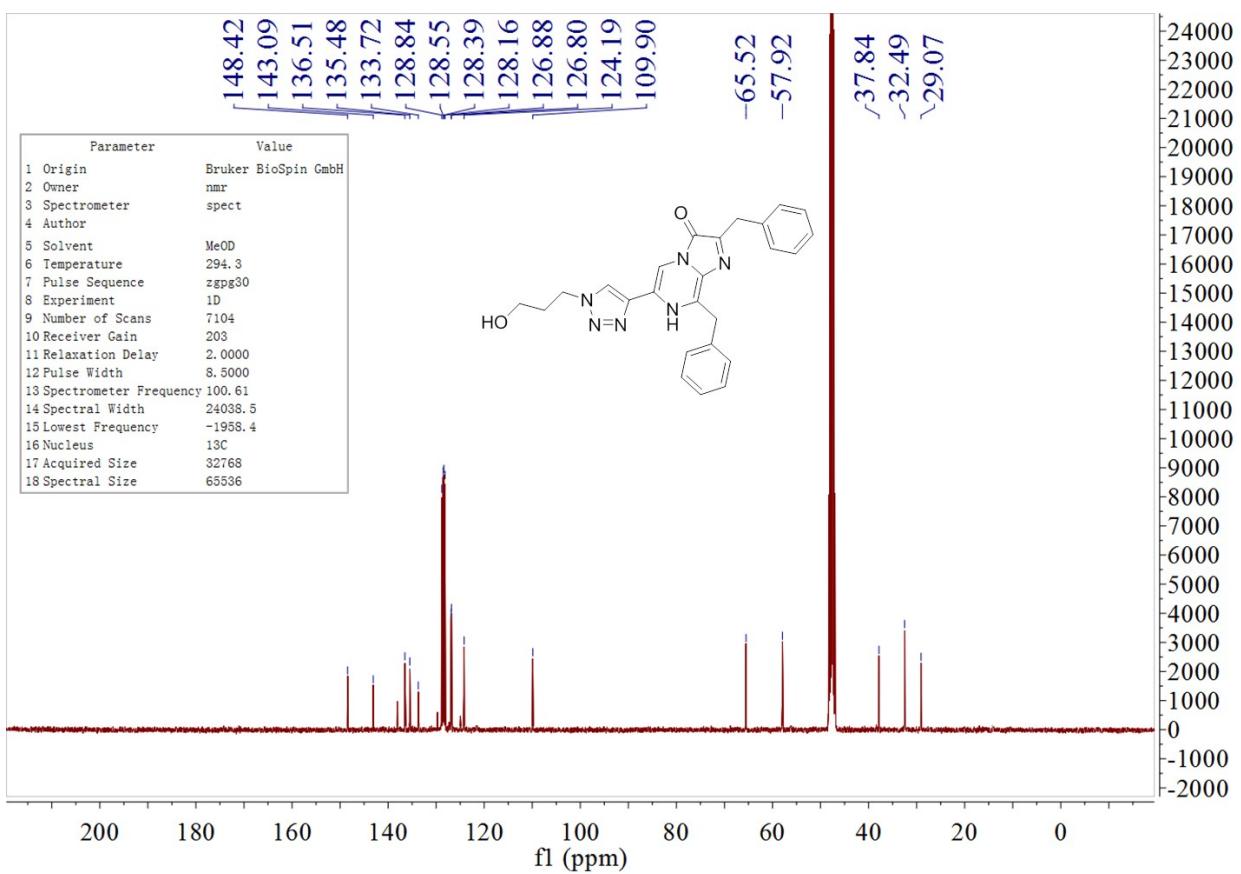
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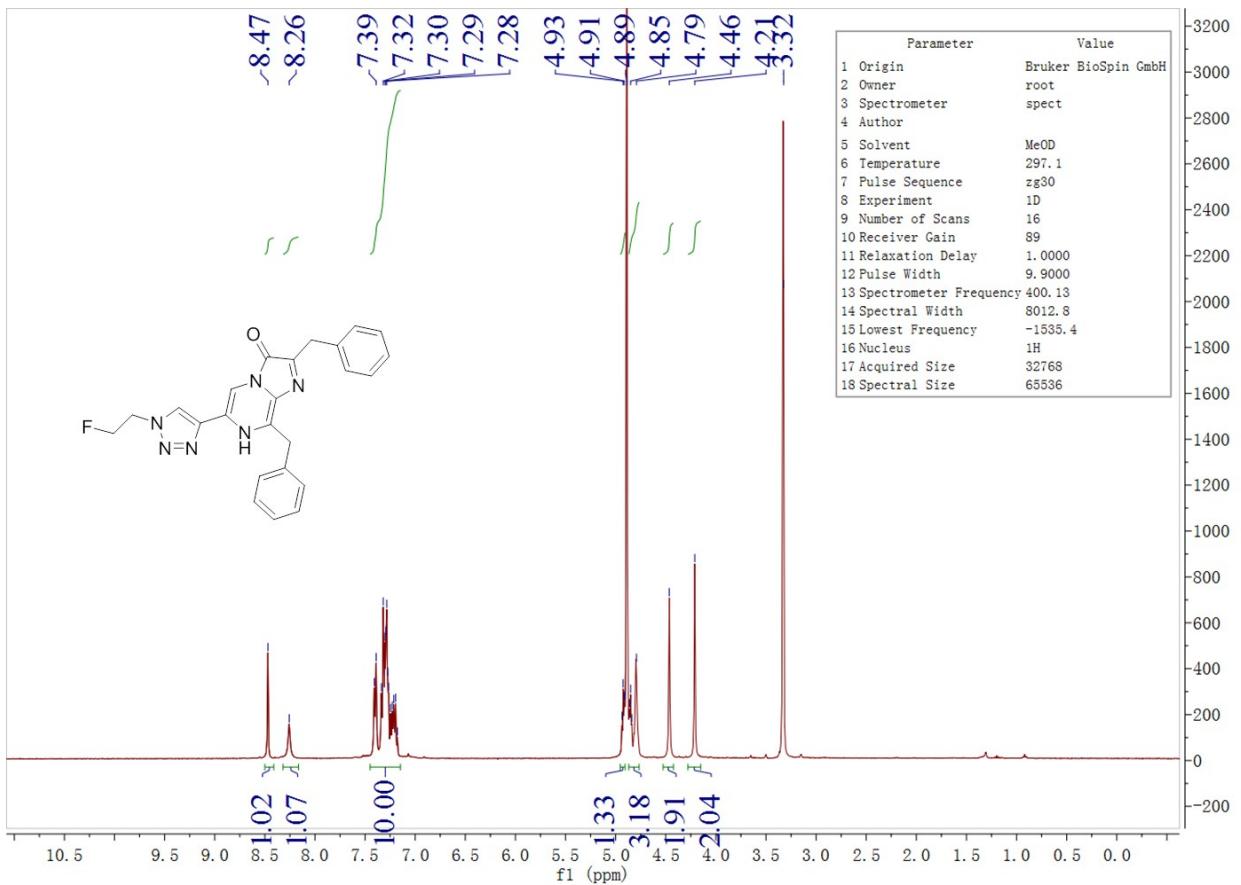


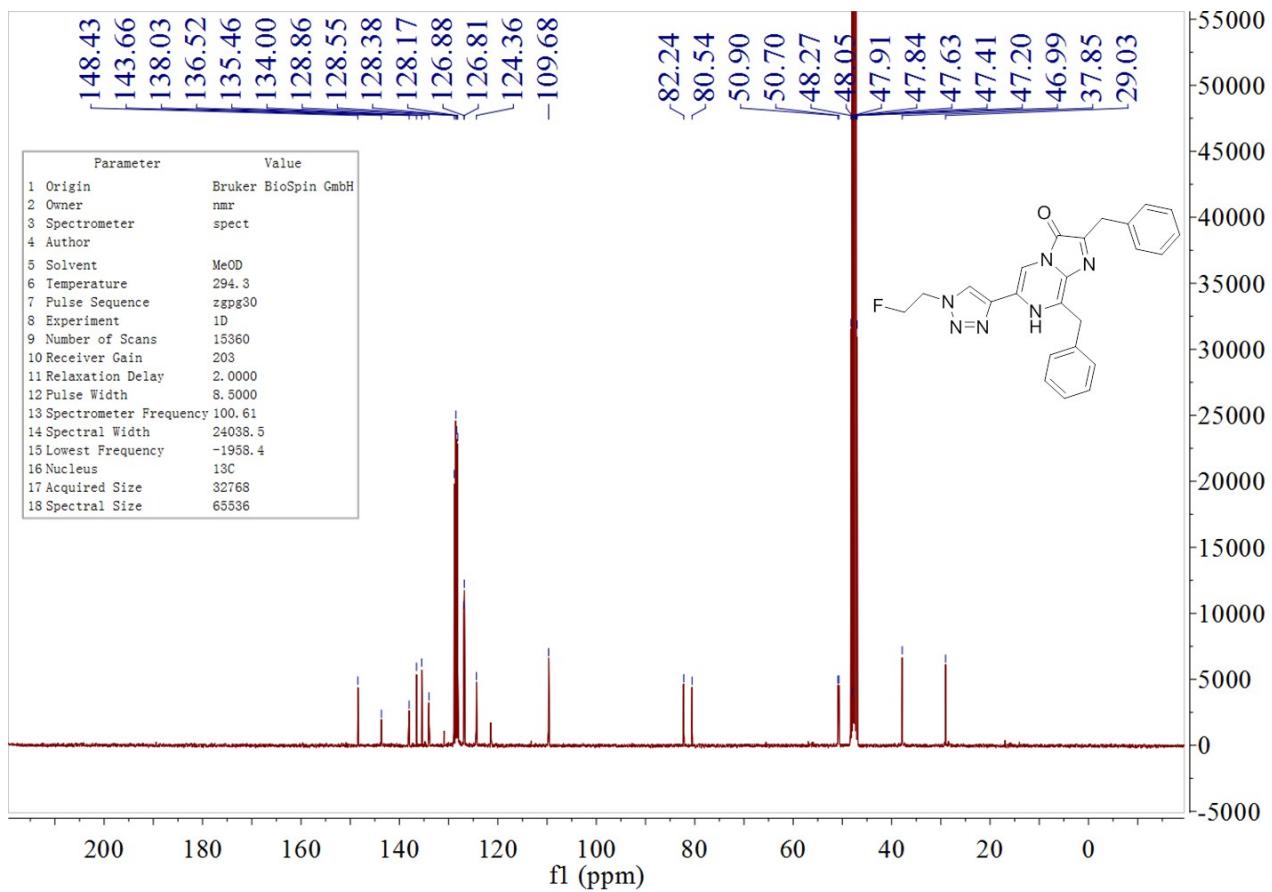
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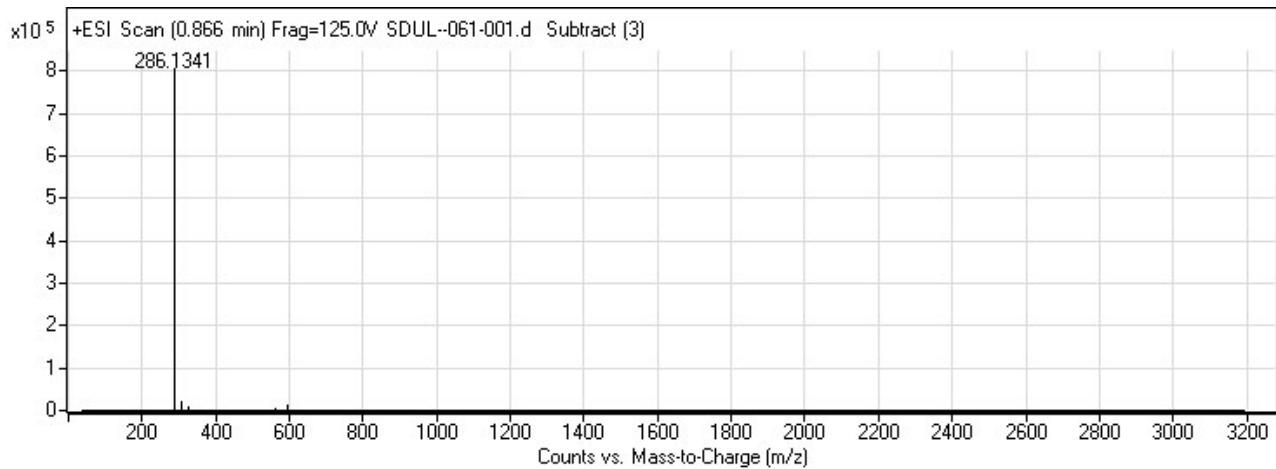
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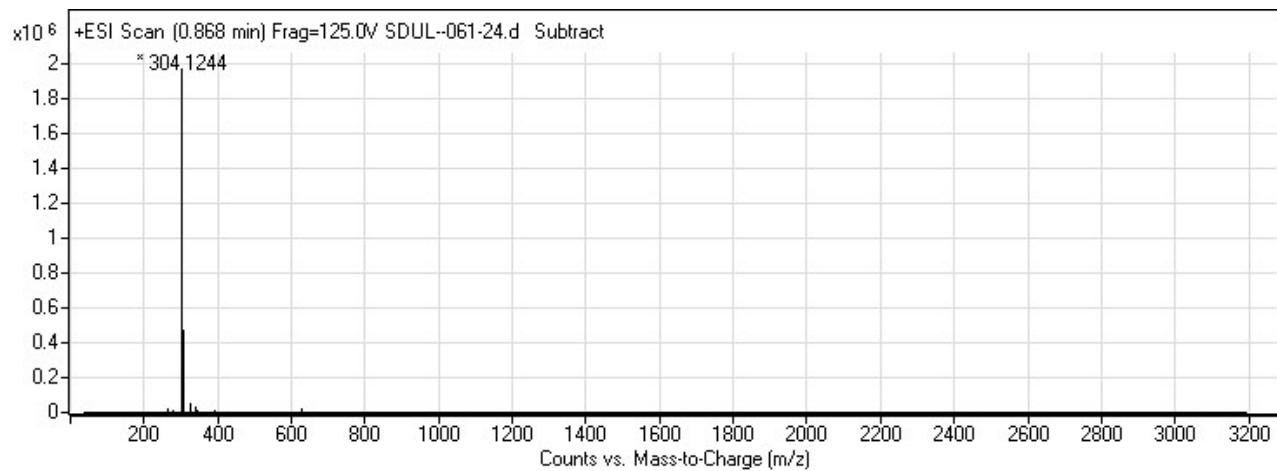


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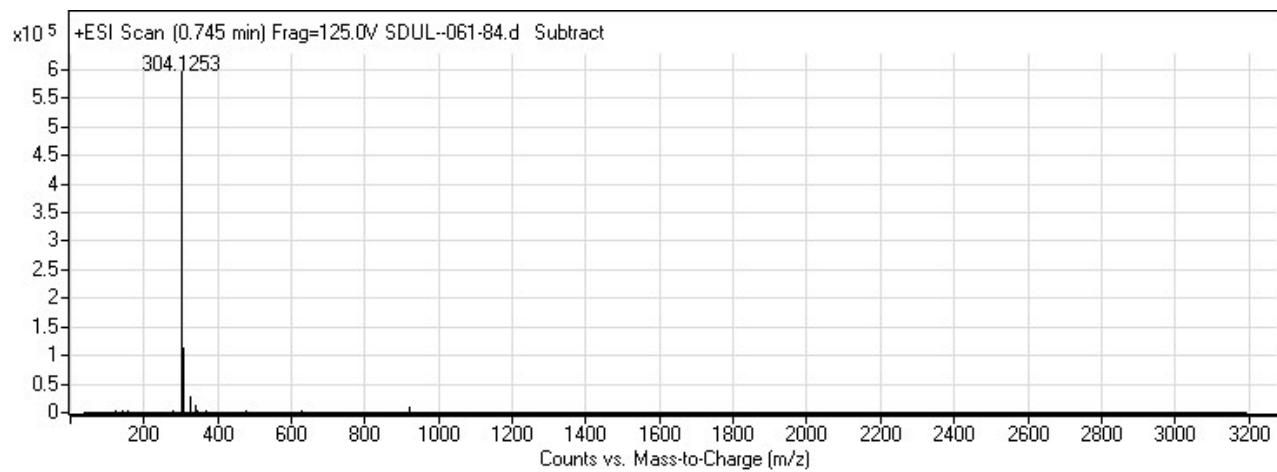
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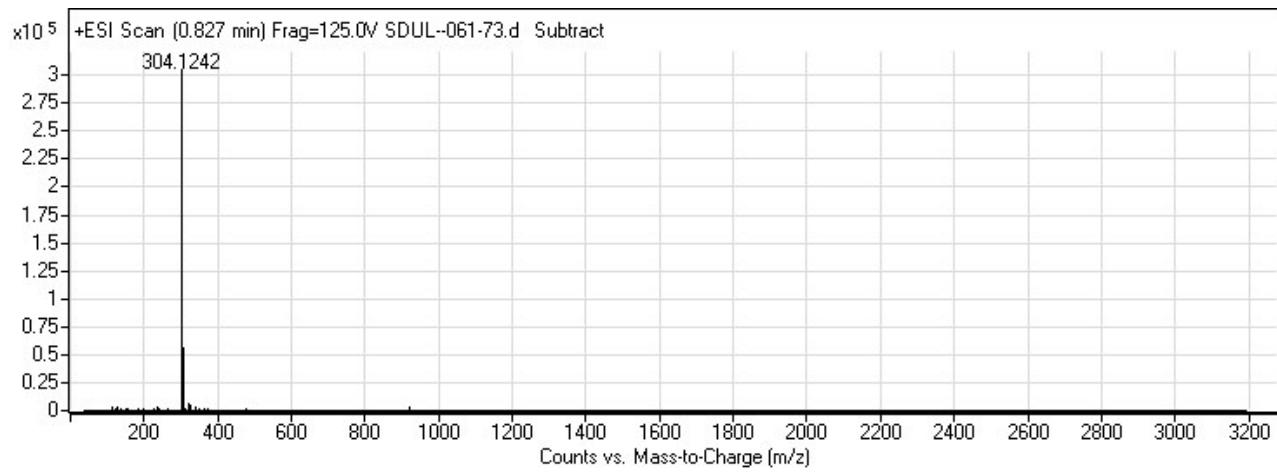
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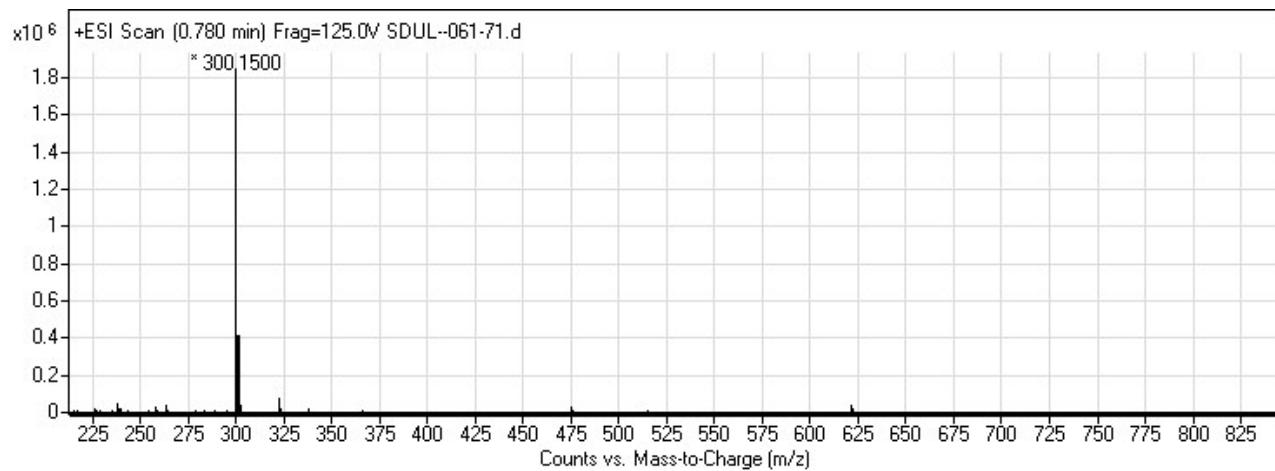
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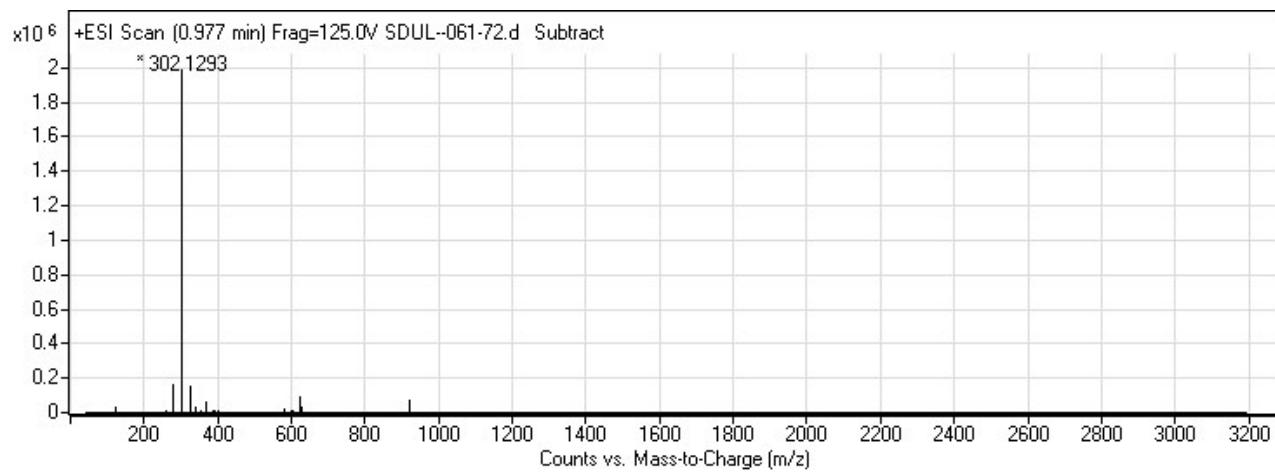
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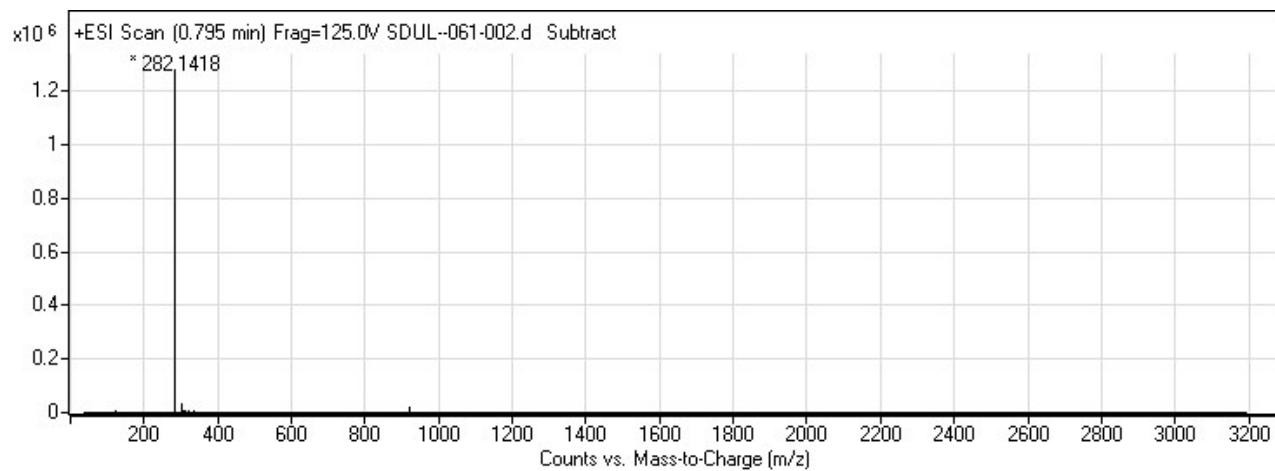
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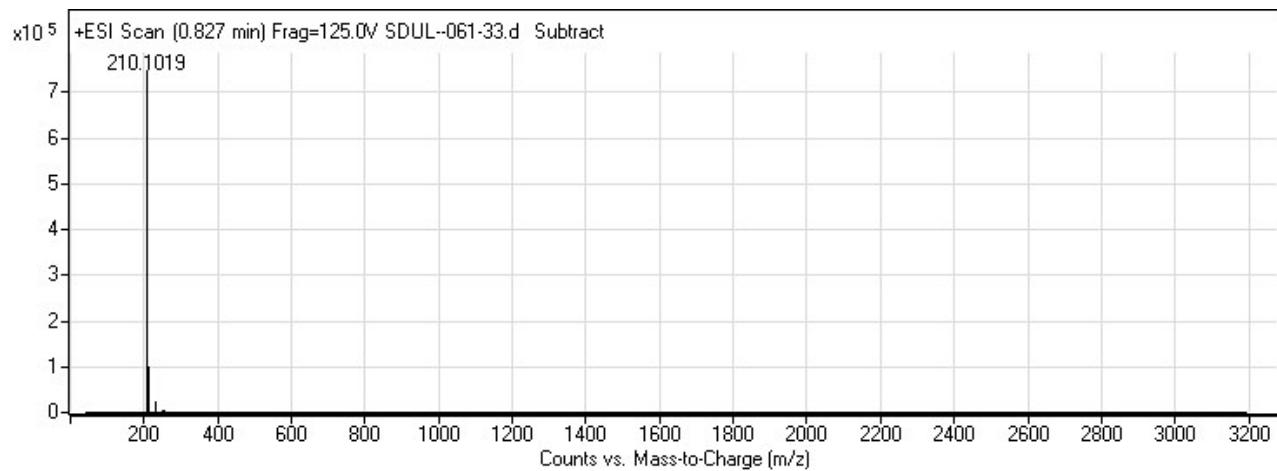
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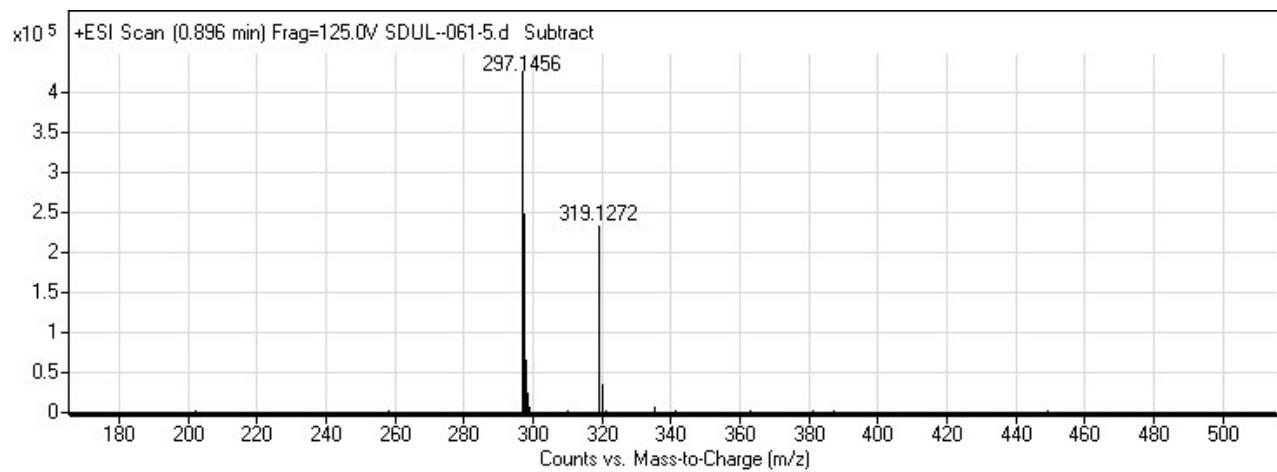
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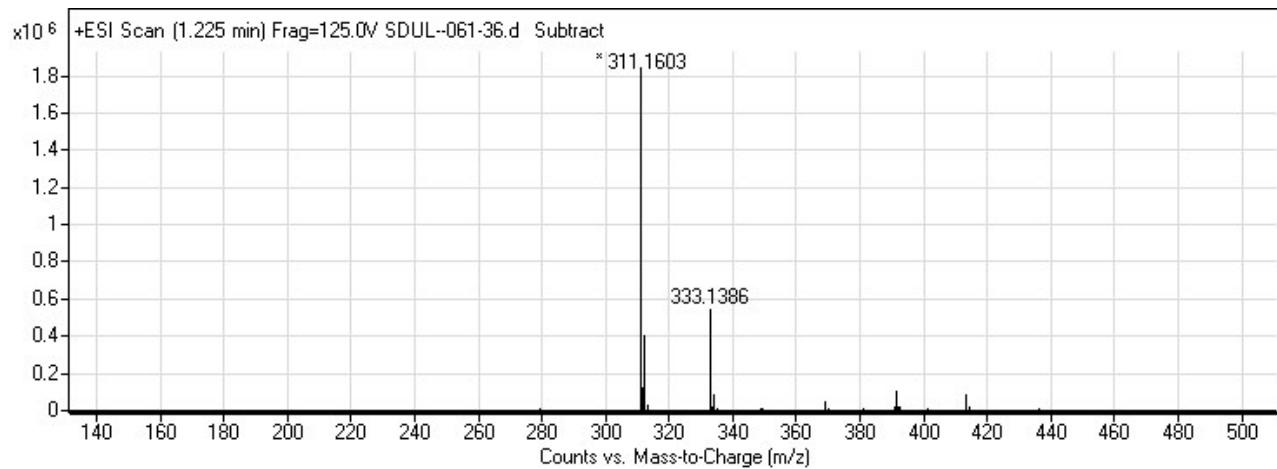
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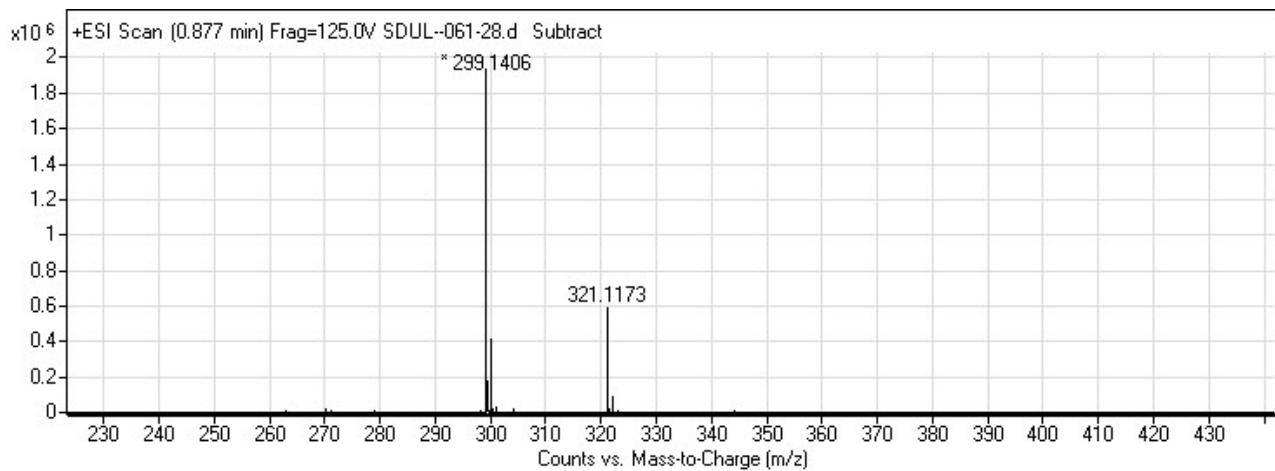
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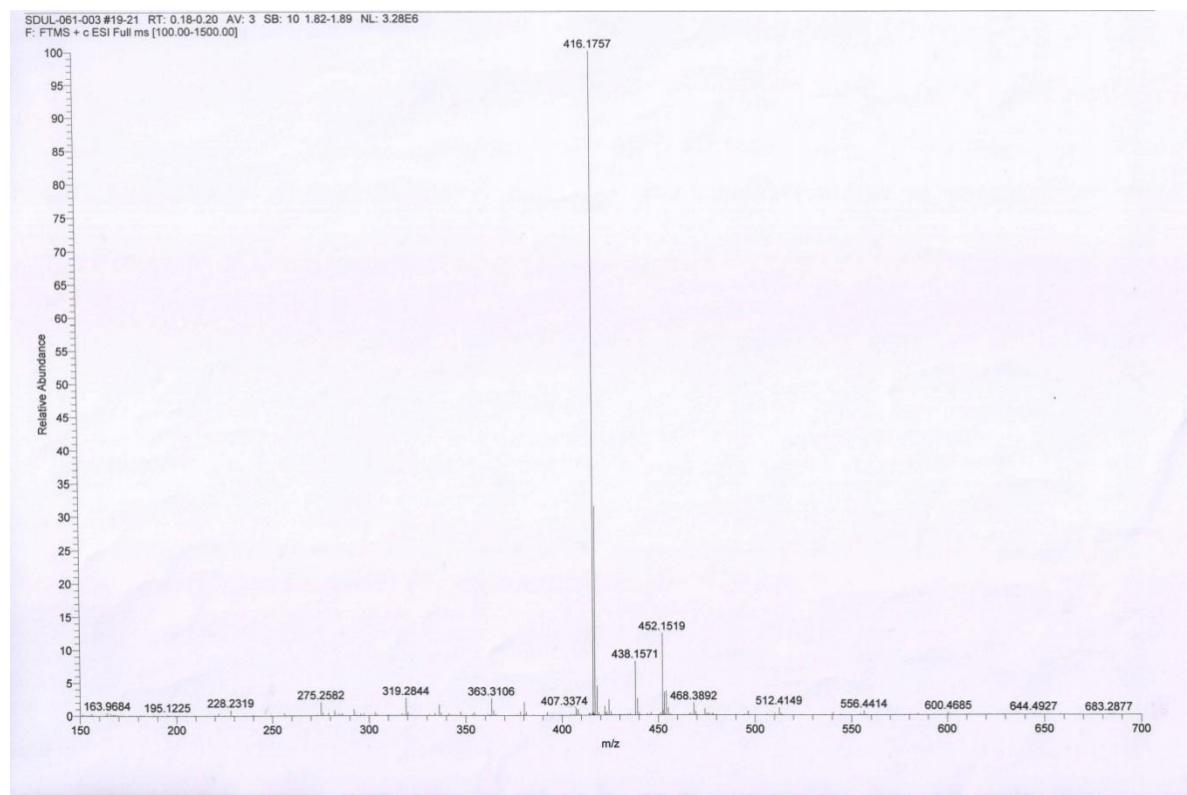
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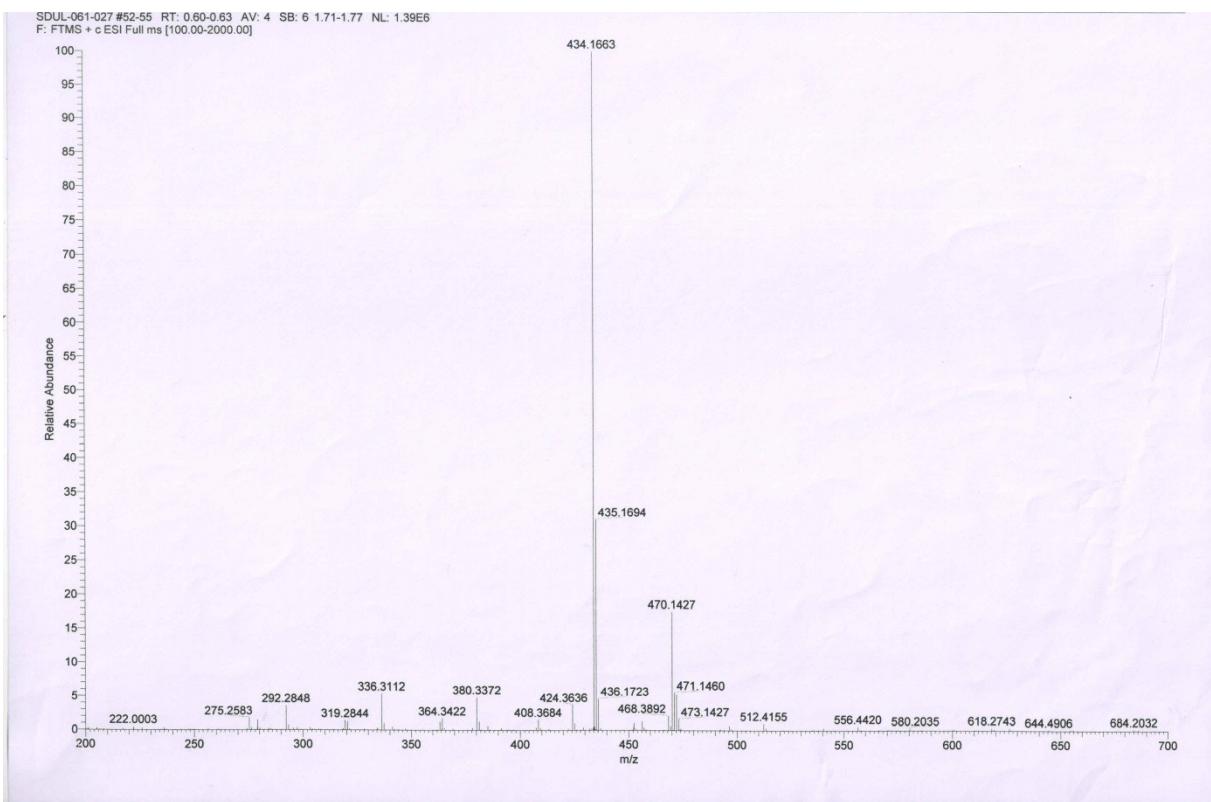
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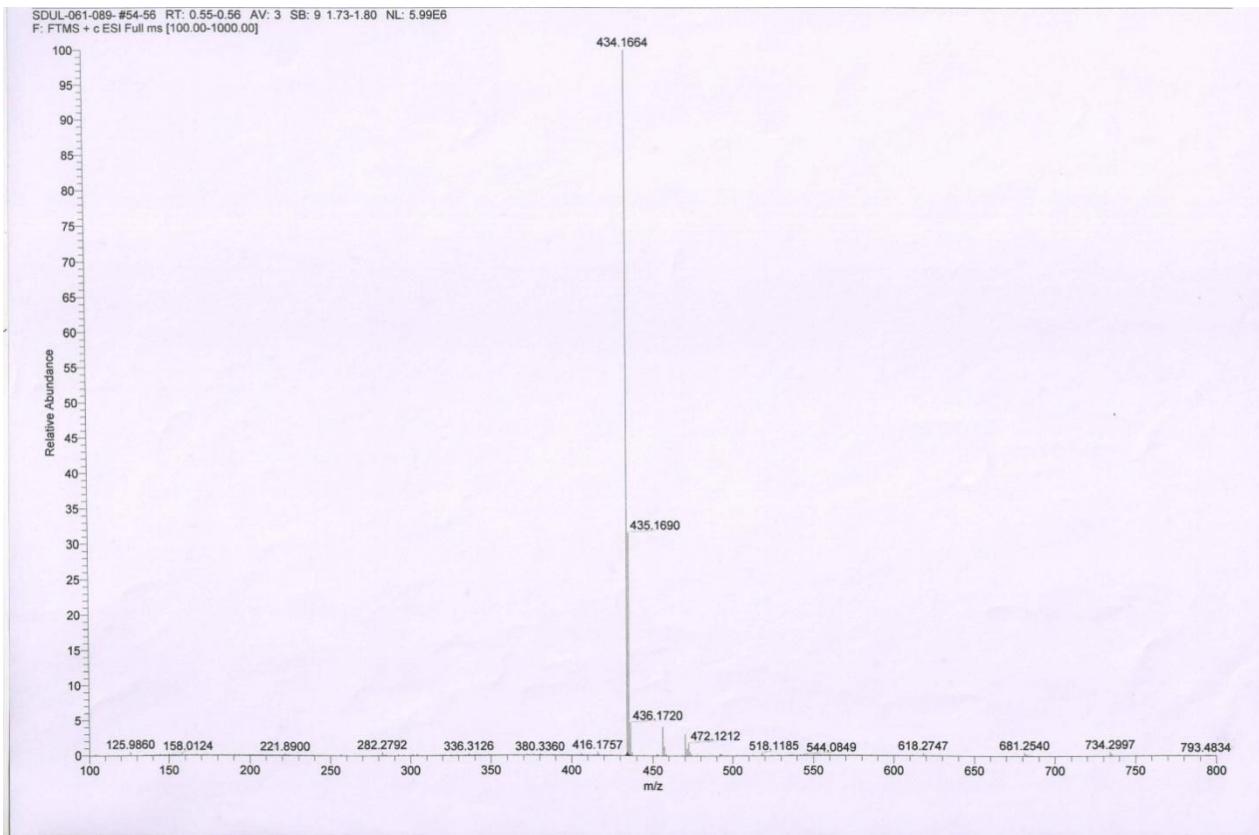
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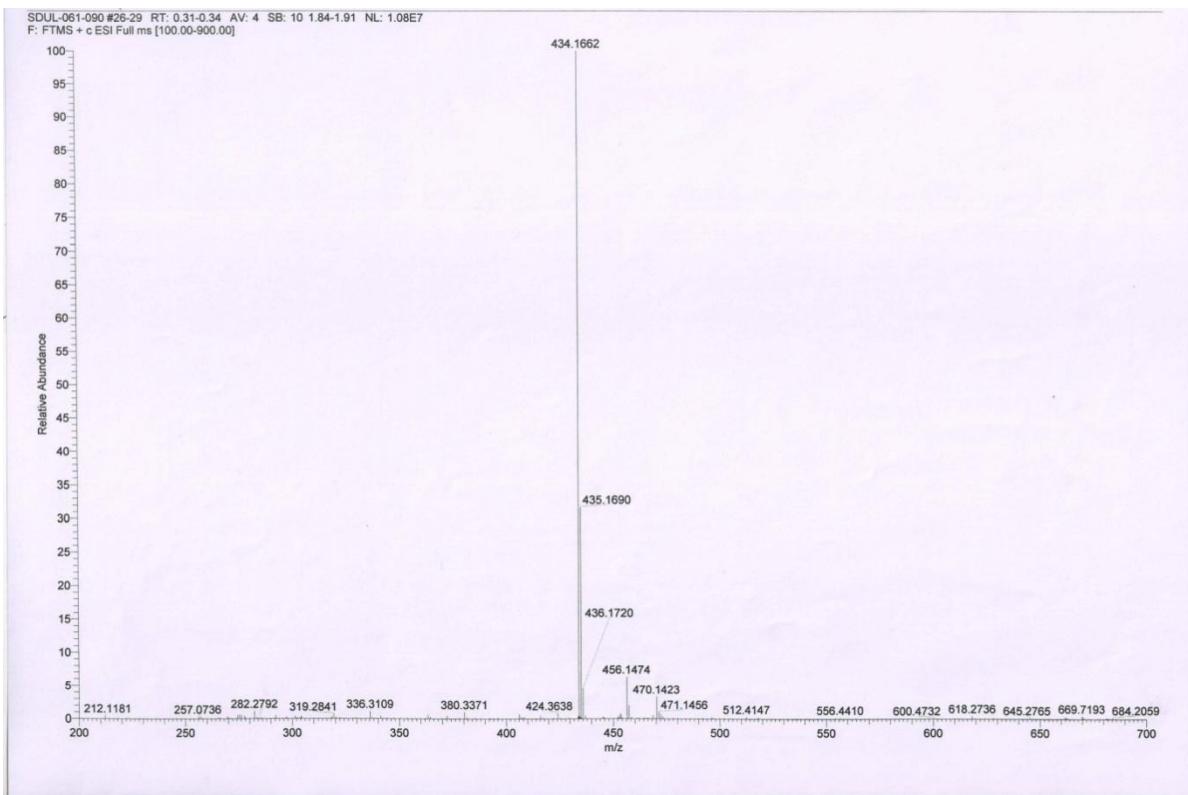
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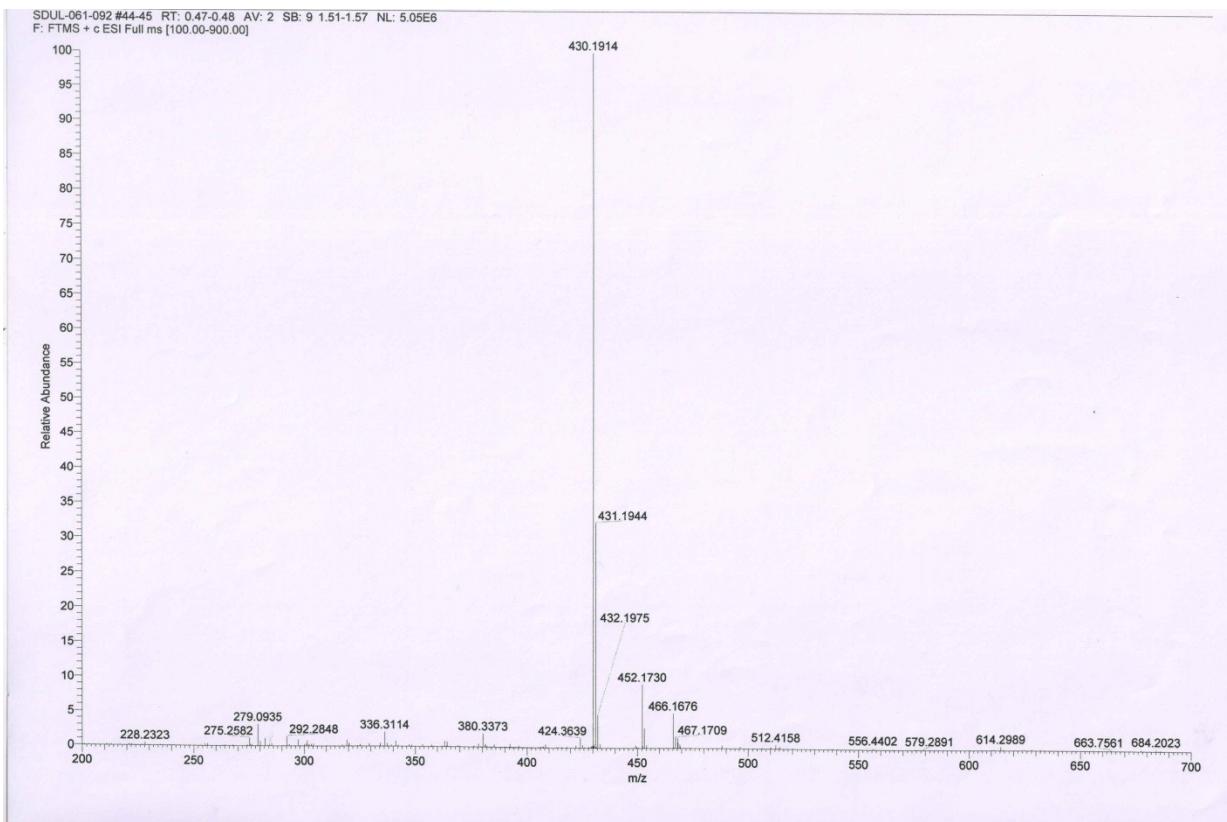
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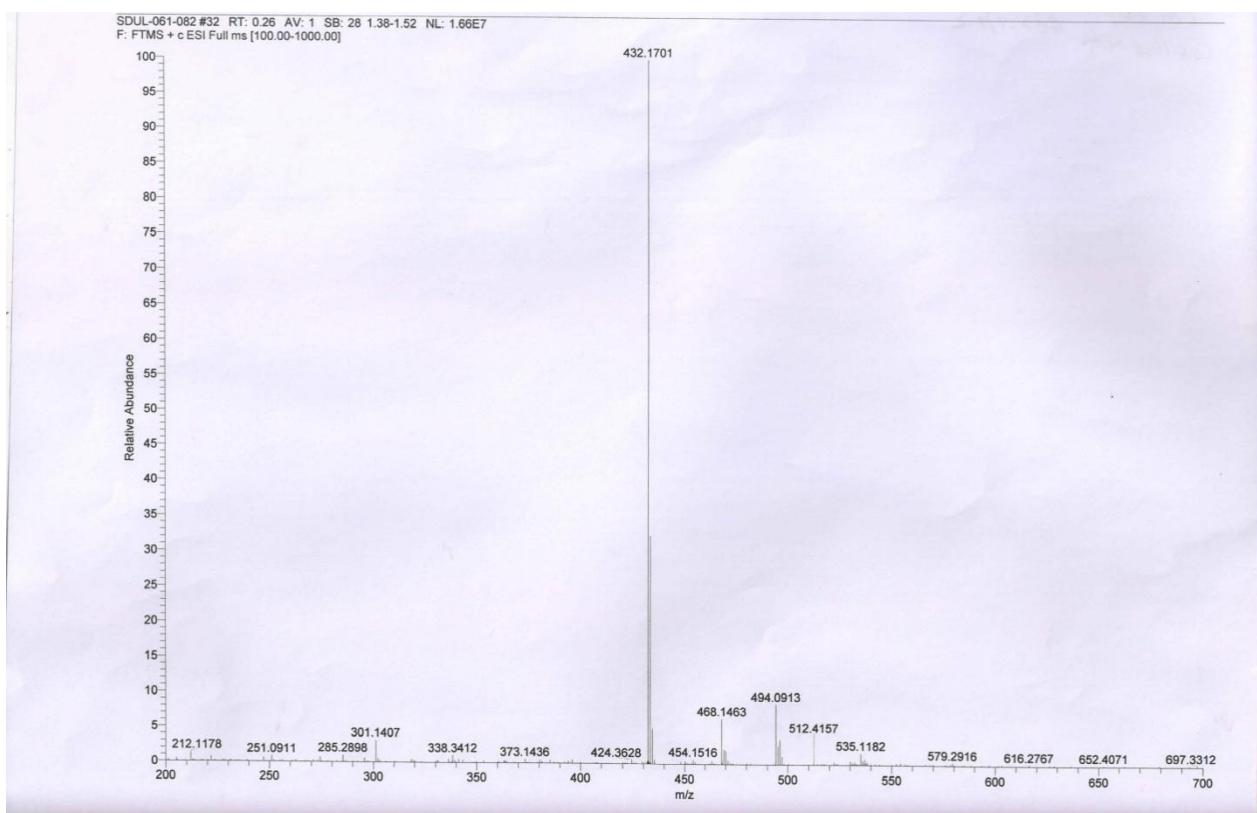
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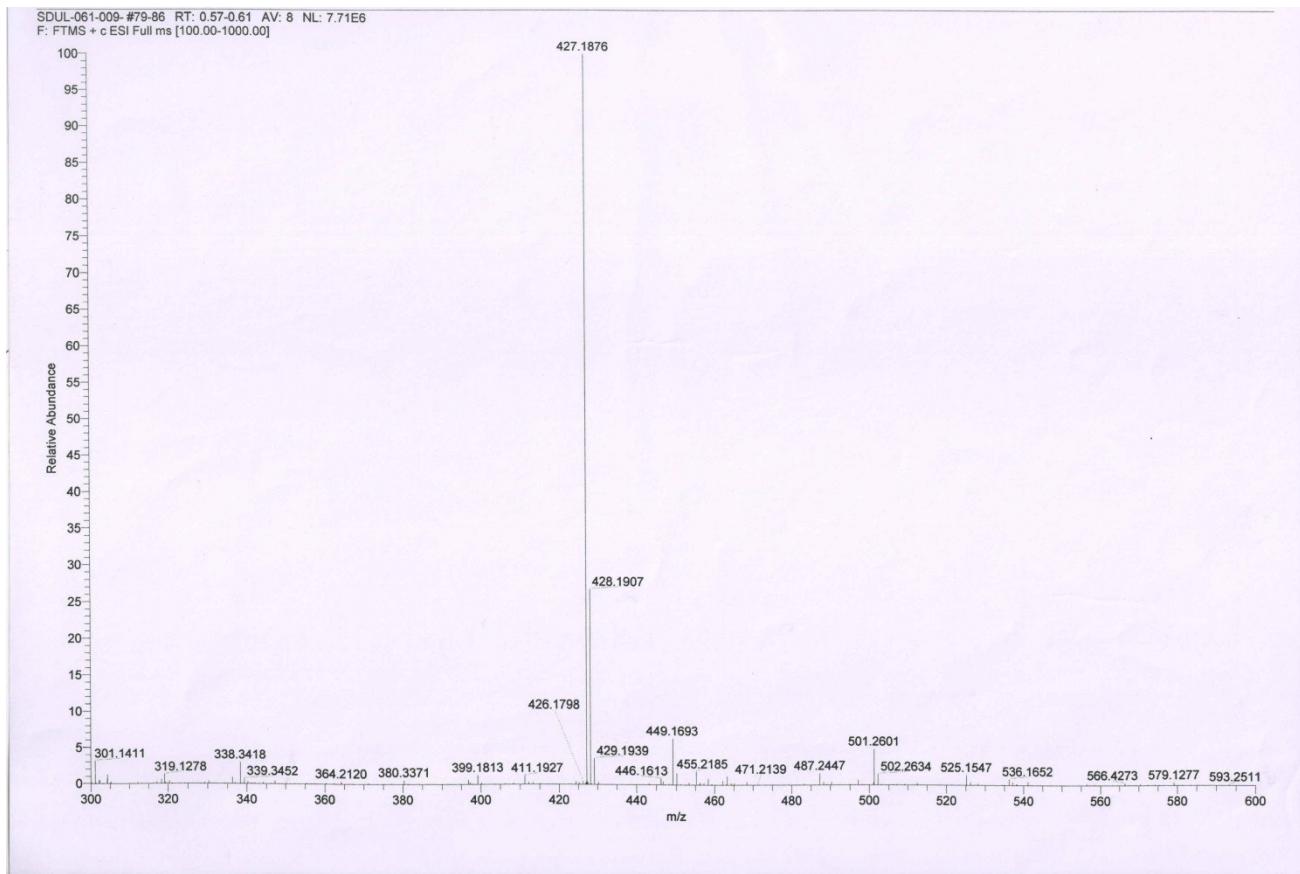
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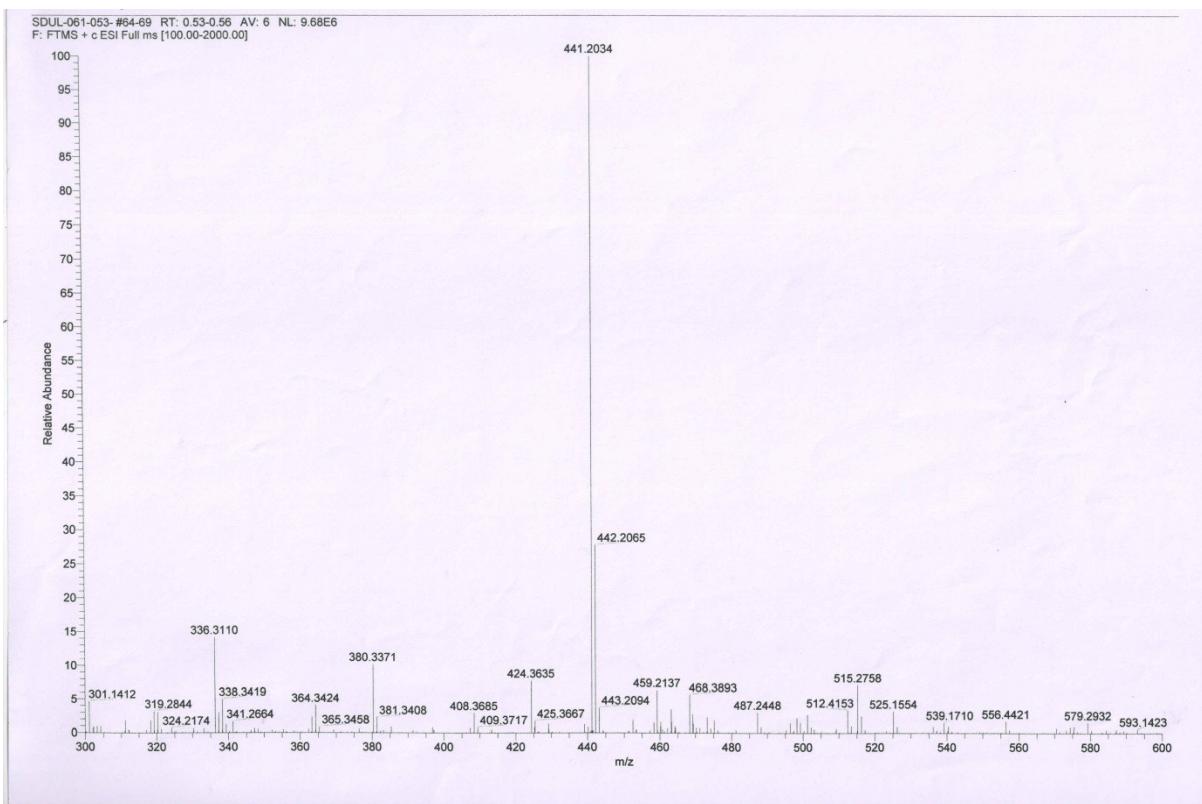
A6



T1



T2



T3

