

Supporting Information

Benzylic sp³ C-H Functionalization Reaction of 2-Methylazaarenes catalyzed by pepsin

Feng Ai, Guo-qing Chen, Jiu-jian Ji, Zhi-qiang Zhu, Zhang-gao Le*, and Zong-Bo

Xie*

Department of Applied Chemistry, East China University of Technology, Nanchang 330013, China

Email: zbxie@ecit.cn

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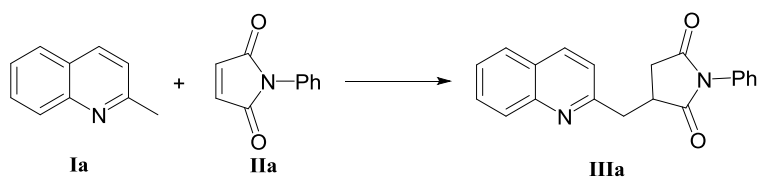
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1. General remarks

NMR spectra were obtained on a Bruker AV-600 MHz spectrometer. The ^1H NMR (600 MHz) chemical shifts were measured relative to CDCl_3 as the internal reference (CDCl_3 : $\delta = 7.26$ ppm). The ^{13}C NMR (150 MHz) chemical shifts were given using CDCl_3 as the internal standard (CDCl_3 : $\delta = 77.16$ ppm). High-resolution mass spectra (HRMS) were obtained with a LC-TOF MS-MS (ESI).

Unless otherwise noted, all reagents were obtained from commercial suppliers and used without further purification. All solvents were dried by refluxing, and freshly distilled prior to use.

2. General Procedure for the reaction



2-Methylquinoline **I a**(1.4 mmol) and *N*-phenylmaleimide **II a**(1 mmol) were dissolved in 2 mL of DMSO with 20 mg pepsin from pig gastric mucosa as catalyst. The mixture was stirred at 60 °C for 60 h, and then 10 mL water was added to the reaction mixture. The resulting mixture was extracted by ethyl acetate (10 mL) for 3 times. The combined organic phase was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give the coupling products **III a**.

3. Supplementary experiment details

3.1 Effect of water content on the yield of reaction

After exploring the effect of different reaction media on the catalytic effect of the enzyme, no doubt that both DMSO and H₂O have a good promoting effect. Besides, enzyme can be well dissolved in water, so the influence of the water content of the solvent on the reaction is also explored (**Fig. S1**). With the water content increased, the yield decreased continuously. Obviously, the addition of water does not promote the progress of the reaction but rather has a great inhibitory effect. Therefore, no water was added in DMSO.

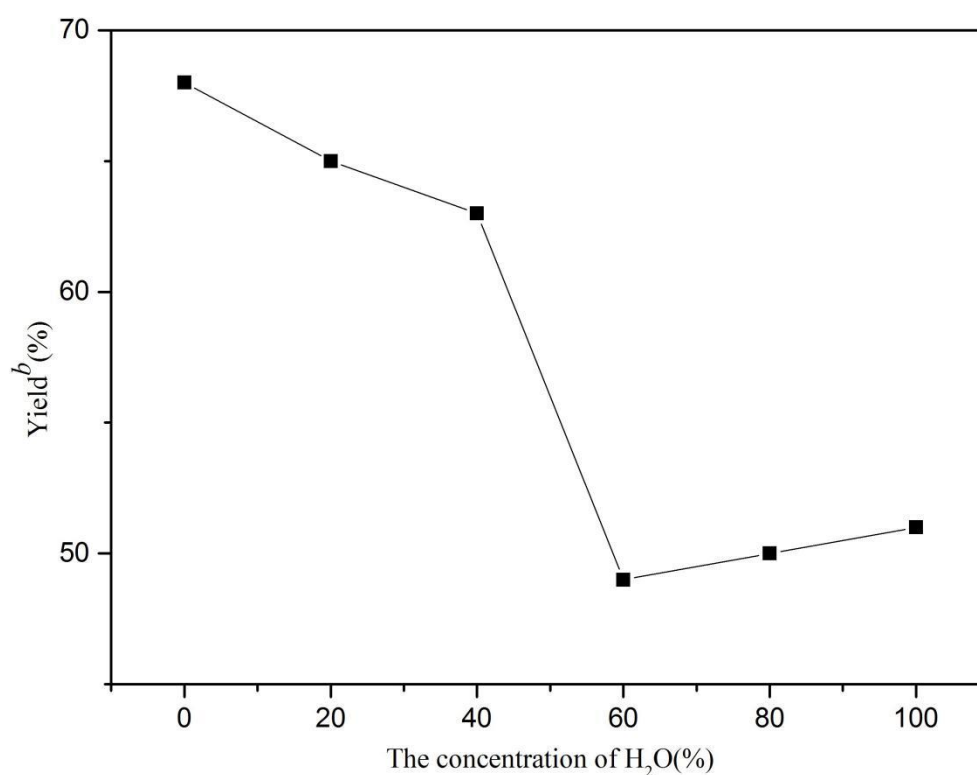


Fig. S1 Effect of water content on the yield of reaction

All reactions were carried out using N-phenylmaleimide (0.75 mmol), 2-methy quinoline (0.5 mmol), pepsin (20 mg) and DMSO (2 mL) at specified water content at 50 °C for 48 h. The yield refers to the isolated product after chromatography on silica gel.

3.2 Effect of enzyme loading on the yield of reaction

The effect of enzyme loading was also investigated (Fig. S2). The results showed that 10 mg/mL was the optimal quantity for the model reaction between 0.75 mmol *N*-phenylmaleimide and 0.5 mmol 2-methy quinoline under employed conditions. Higher enzyme loading did not result in the increase of yield, so 20 mg was chosen as the optimal enzyme loading.

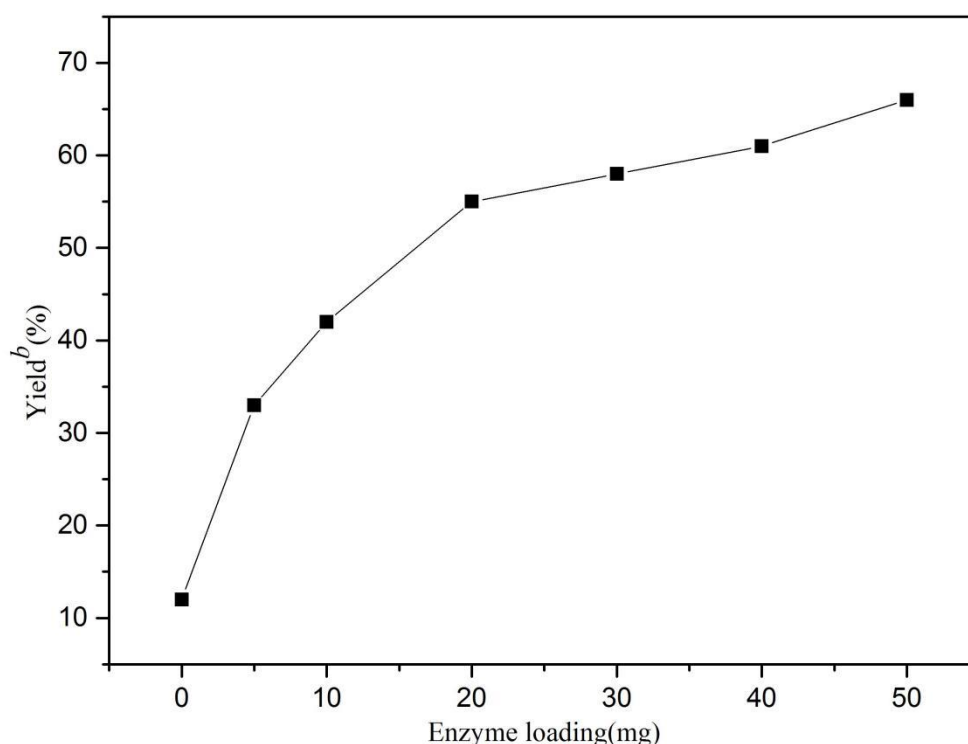


Fig. S2 Effect of enzyme loading on the yield of reaction

All reactions were carried out using N-phenylmaleimide (0.75 mmol), 2-methy quinoline (0.5 mmol), DMSO (2 mL) and specified amount of pepsin at 50 °C for 48 h. The yield refers to the isolated product after chromatography on silica gel.

3.3 Effect of temperature on the yield of reaction

Temperature is another important factor for the asymmetric enzyme-catalyzed reactions, due to their effects on the enzyme stability and reactivity. Thus, a temperature screen was performed using *N*-phenylmaleimide and 2-methy quinoline (Fig. S3). It was found that the activity of the enzymatic reaction could be enhanced

by increasing temperature, and reached the maximum yield of 73 % at 60 °C. However, the yield has no increase when the temperature surpassed 60 °C. Therefore, 60 °C was chosen as the optimal temperature for the reaction.

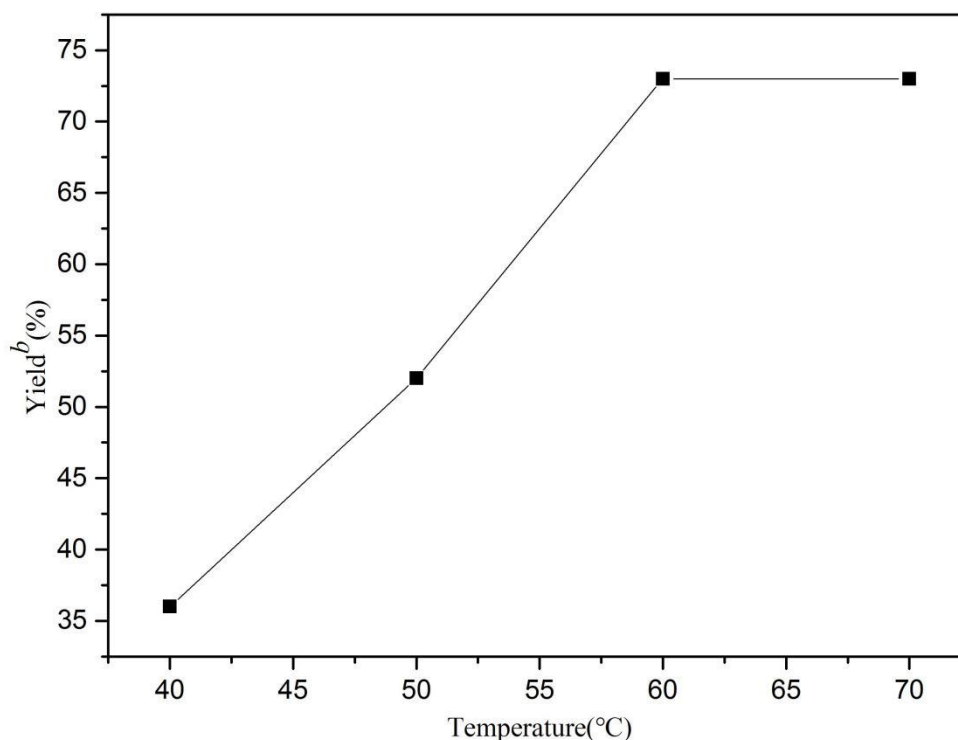


Fig. S3 Effect of temperature on the yield of reaction

All reactions were carried out using N-phenylmaleimide (0.75 mmol), 2-methyl quinoline (0.5 mmol), pepsin (20 mg) and DMSO (2 mL) at specified temperature for 48 h. The yield refers to the isolated product after chromatography on silica gel.

3.4 Effect of molar ratio of substrates on the yield of reaction

To optimize experimental conditions, we examined the effect of molar ratio of substrates on the yield of the reaction using 2-methyl quinoline and N-phenylmaleimide (**Table S1**). It was found that the yield was evidently affected by the molar ratio of substrates. When the excessive N-phenylmaleimide was used as the acceptor, the yield could be increased and reached a maximum. Thus, the molar ratio of 1.4 : 1 (acceptor/donor) was chosen as the optimal molar ratio of substrates (Table S1, entry 8).

Table. S1 Effect of molar ratio of substrates on the yield of reaction^a

Entry	Ia(mmol)	IIa(mmol)	Yield ^b /%
1	0.5	0.5	59
2	0.75	0.75	64
3	1	1	67
4	1.25	1.25	66
5	1.2	1.2	71
6	1	1.2	74
7	1	1.3	76
8	1	1.4	79
9	1	1.5	77

^aAll reactions were carried out using pepsin (20 mg), DMSO (2 mL) and specified amounts of *N*-phenylmaleimide and 2-methyl quinoline at 60 °C for 48 h. ^bYield of the isolated product after chromatography on silica gel.

3.5 Effect of reaction time on the yield of reaction

Since it was not determined whether the reaction reached equilibrium at 48 h, the reaction time was also investigated. When the reaction proceeded to 12 h, the yield was only 27%, and when the reaction reached 60 h, the equilibrium reached and the yield reached 84%. Therefore, we have chosen 60 h as the optimal reaction time.

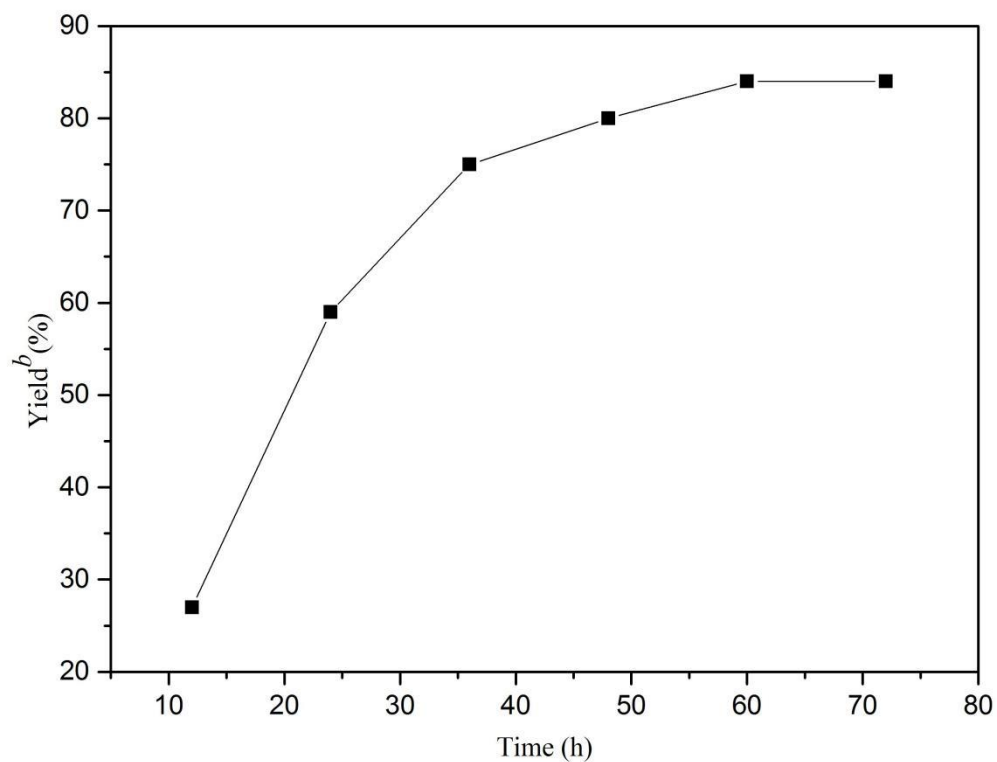
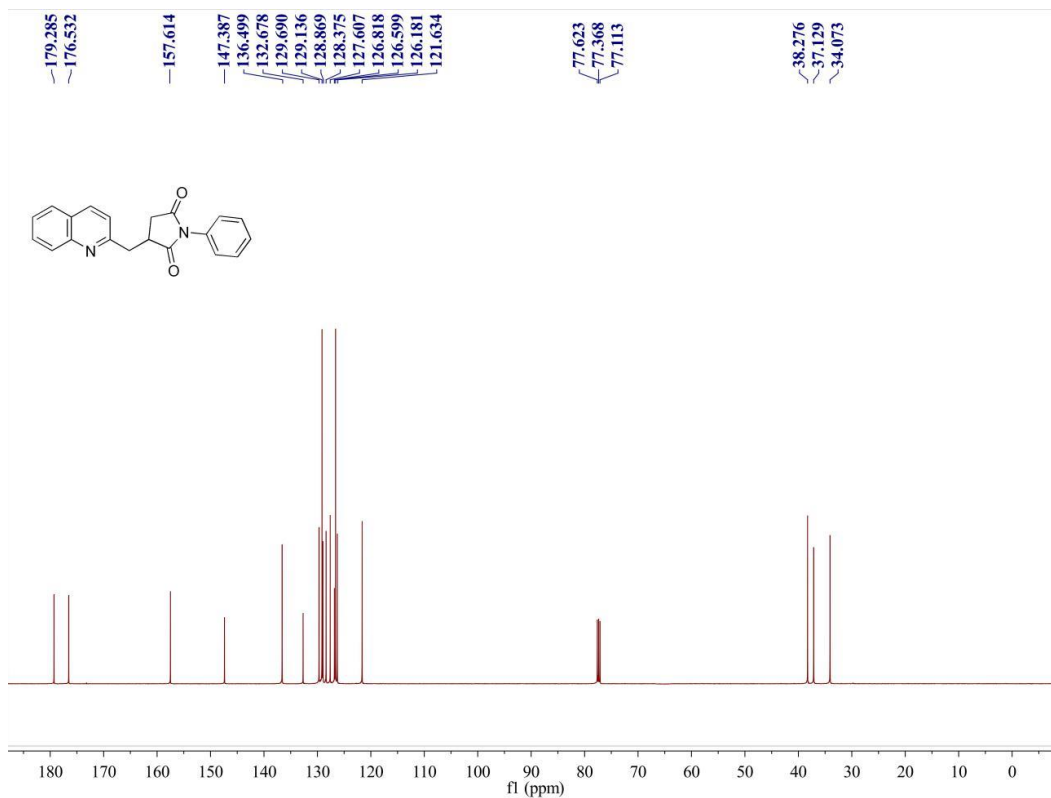


Fig. S4 Effect of reaction time on the yield of reaction

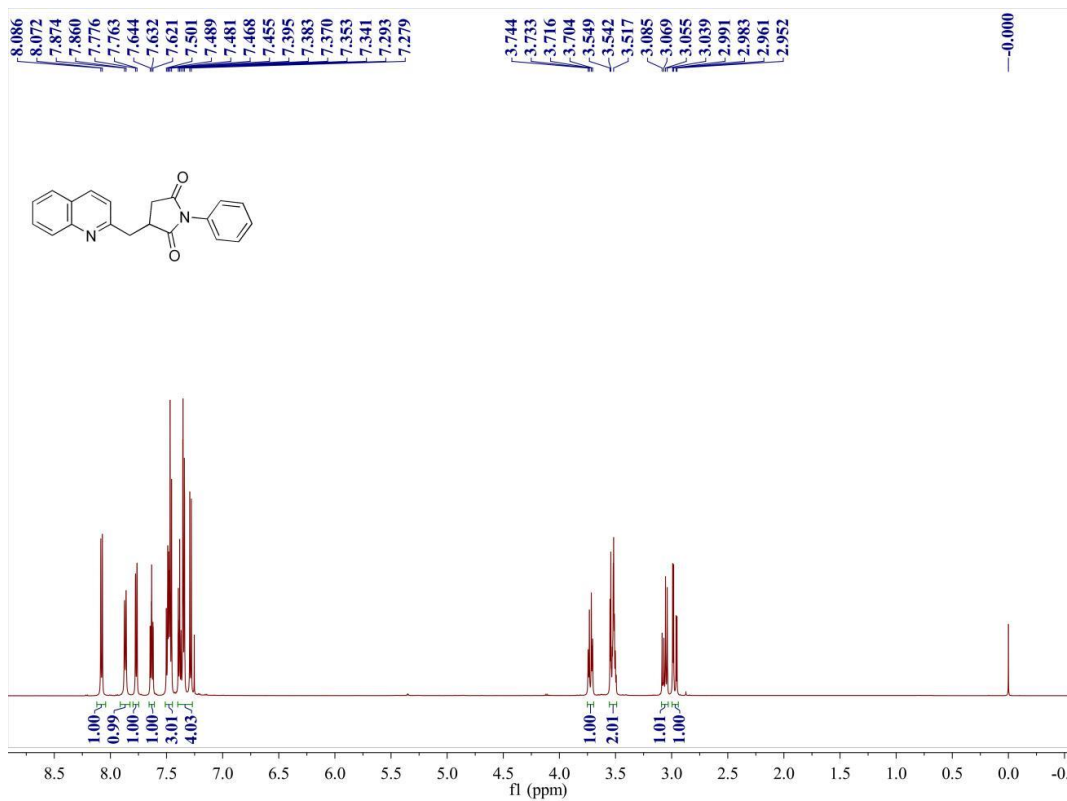
All reactions were carried out using N-phenylmaleimide (1.4 mmol), 2-methy quinoline (1 mmol), pepsin (20 mg) and DMSO (2 mL) at 60 °C for specified time. The yield refers to the isolated product after chromatography on silica gel.

4. Characterization of compounds

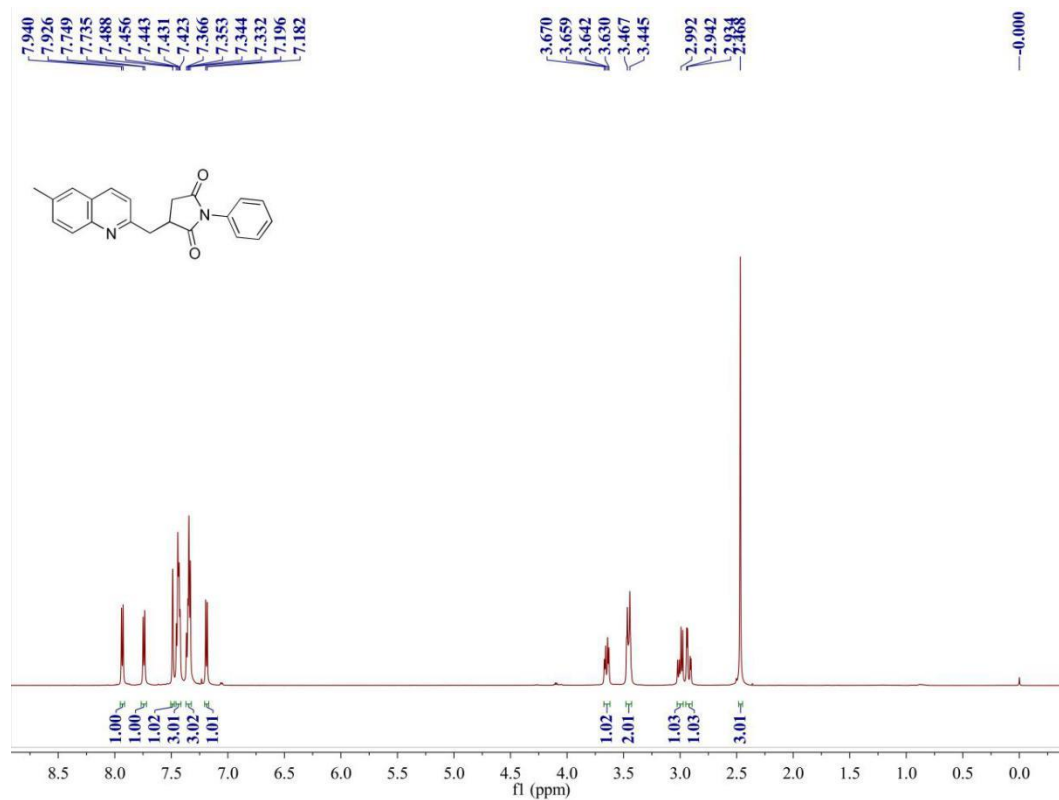
^1H NMR spectra of compound IIIa



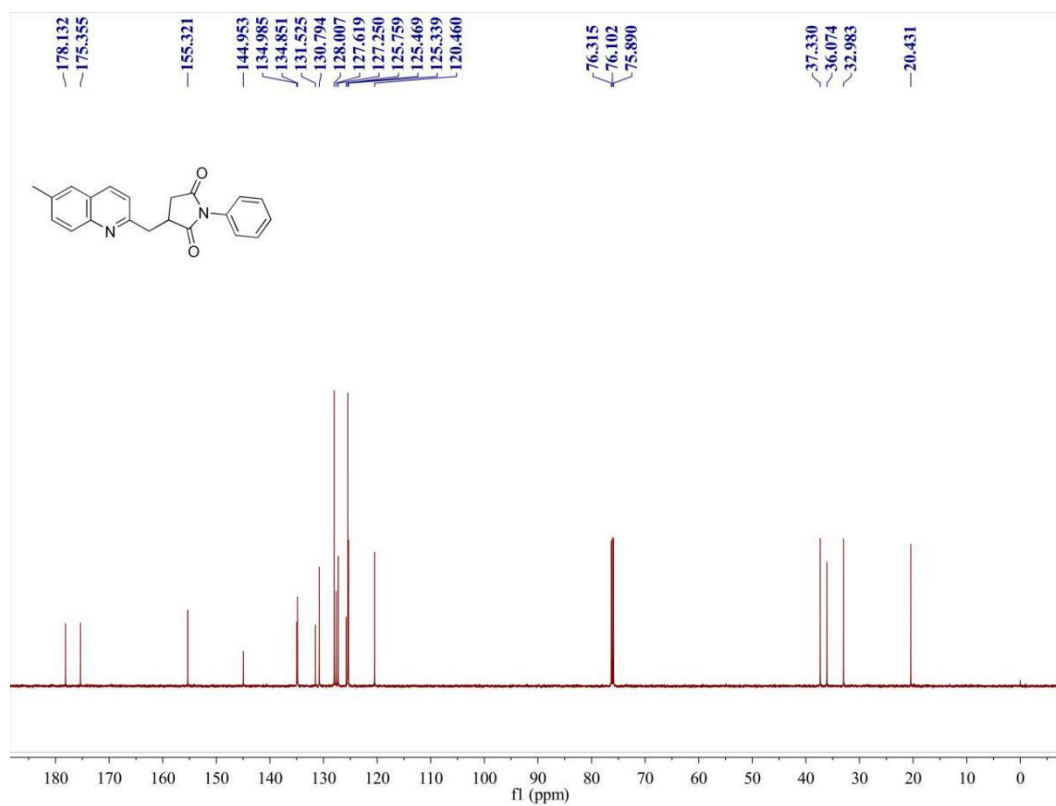
^{13}C NMR spectra of compound IIIa



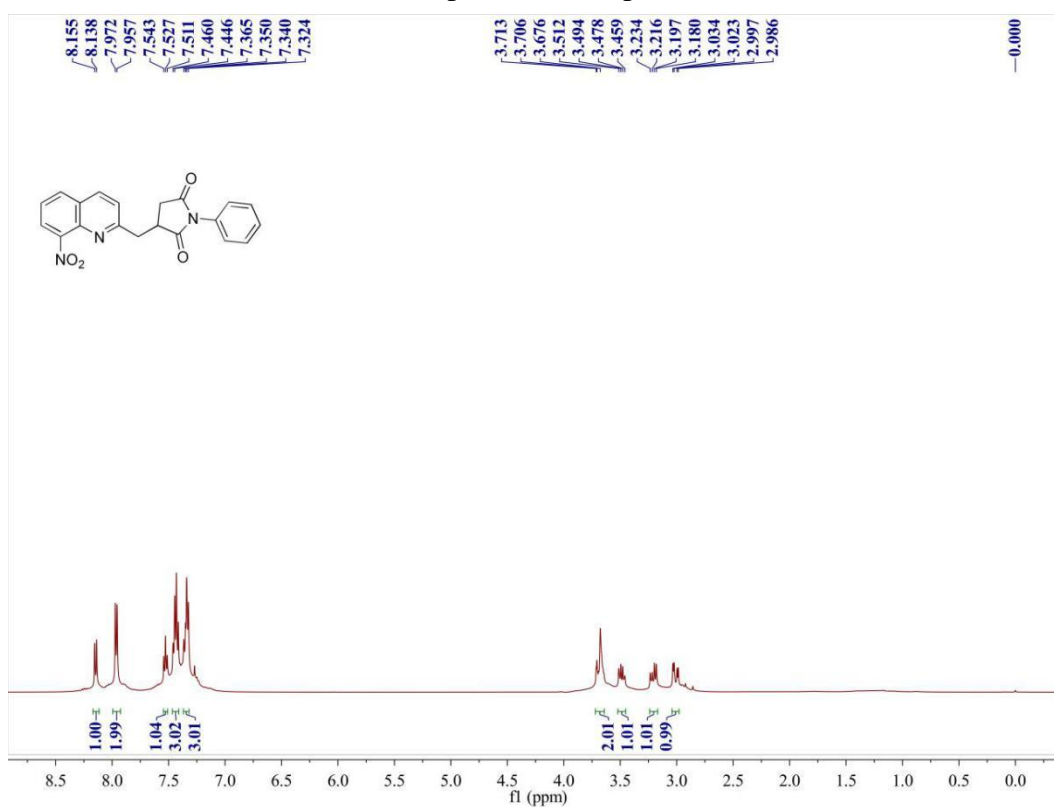
¹H NMR spectra of compound **IIIb**



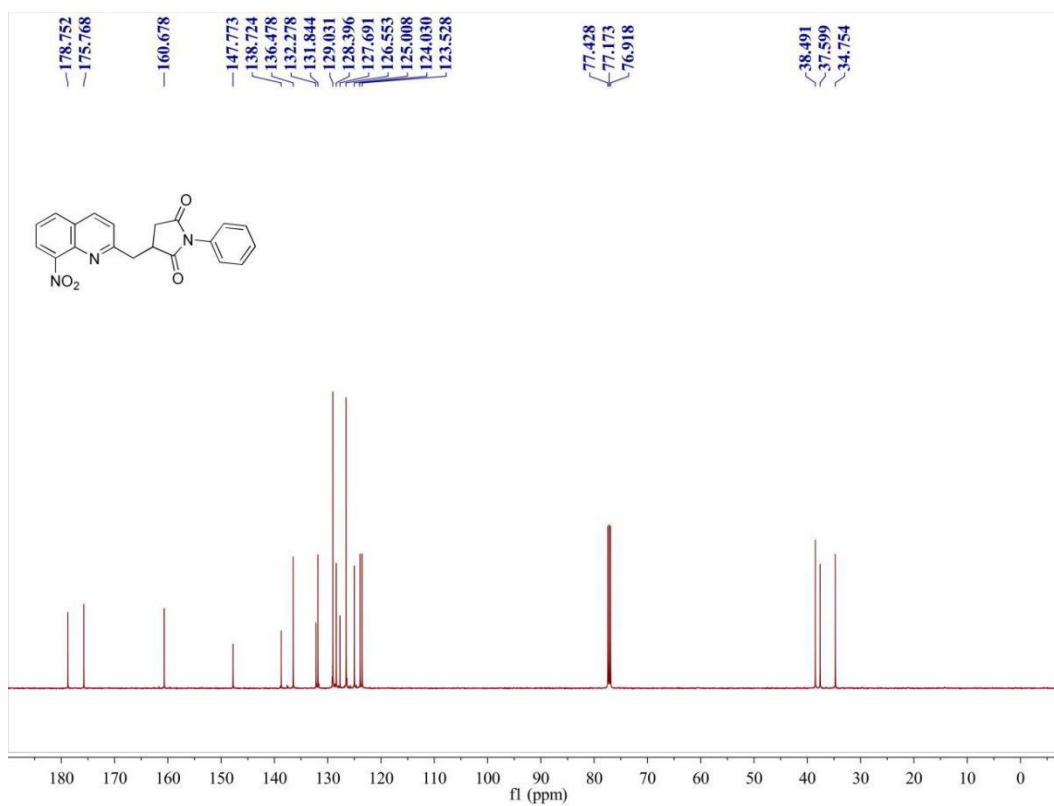
¹³C NMR spectra of compound **IIIb**



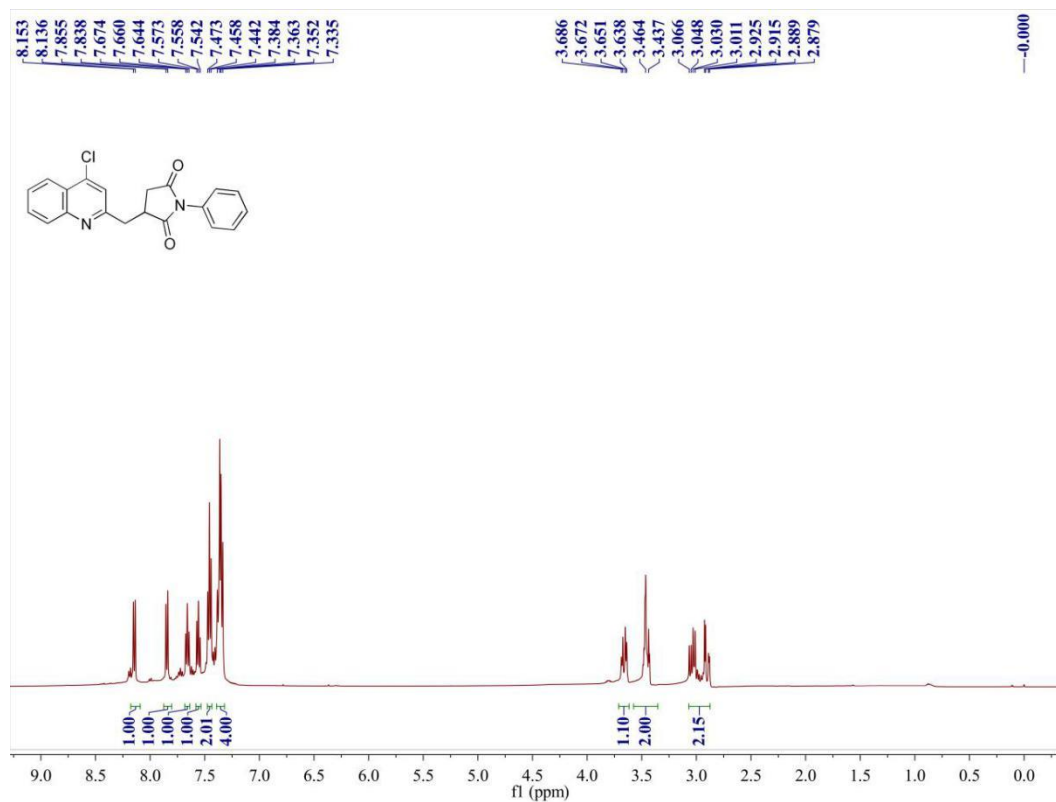
¹H NMR spectra of compound IIIc



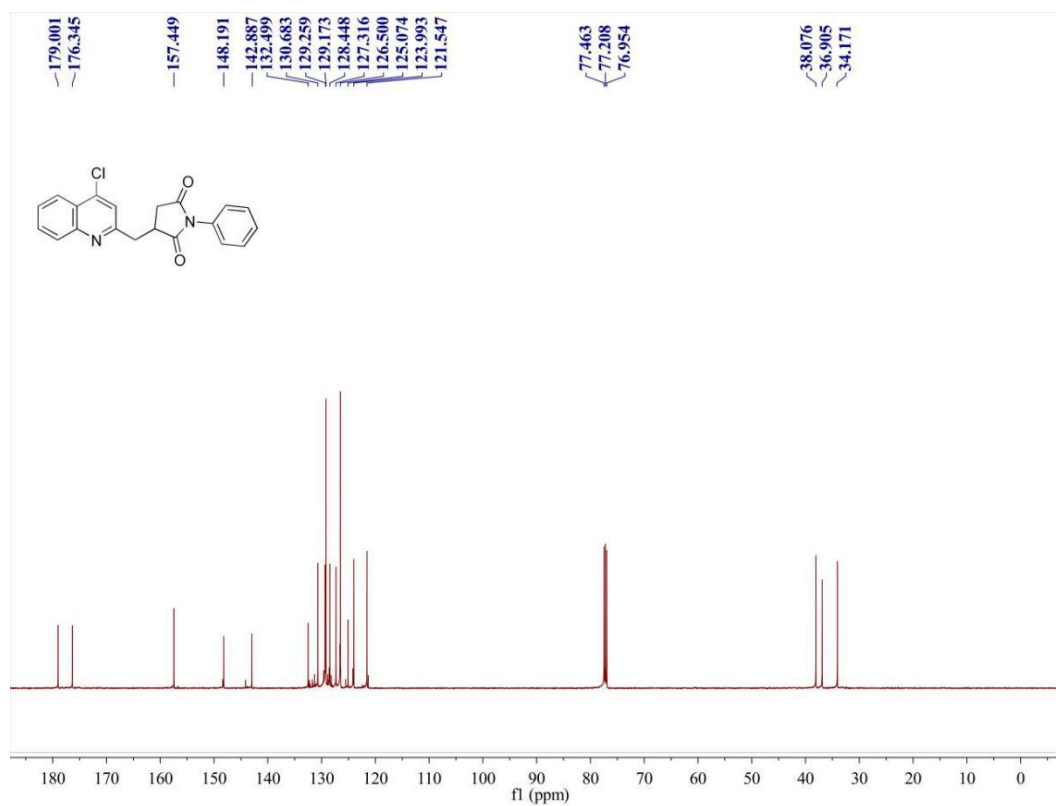
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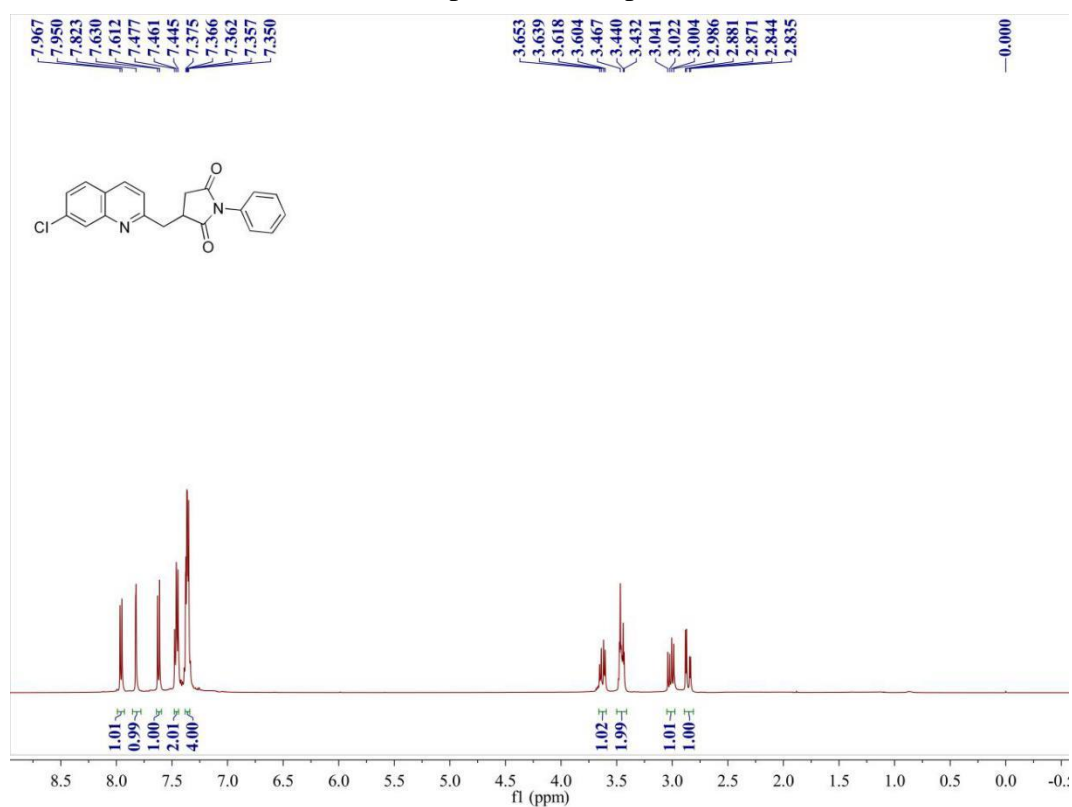
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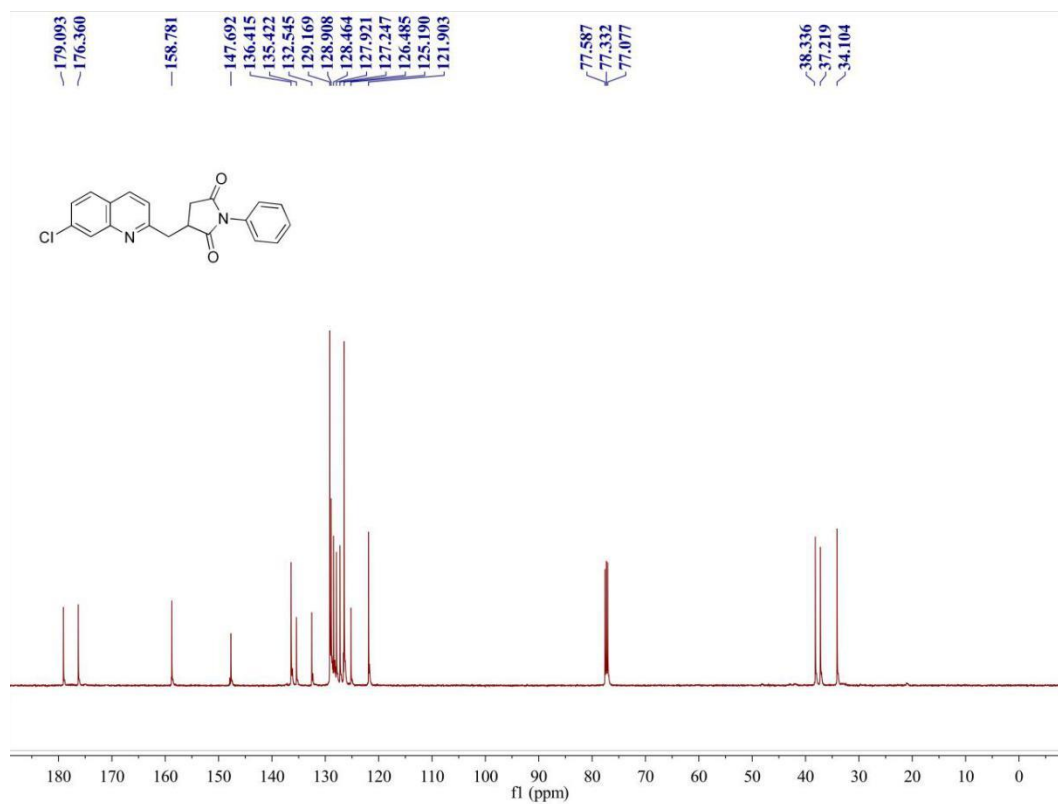
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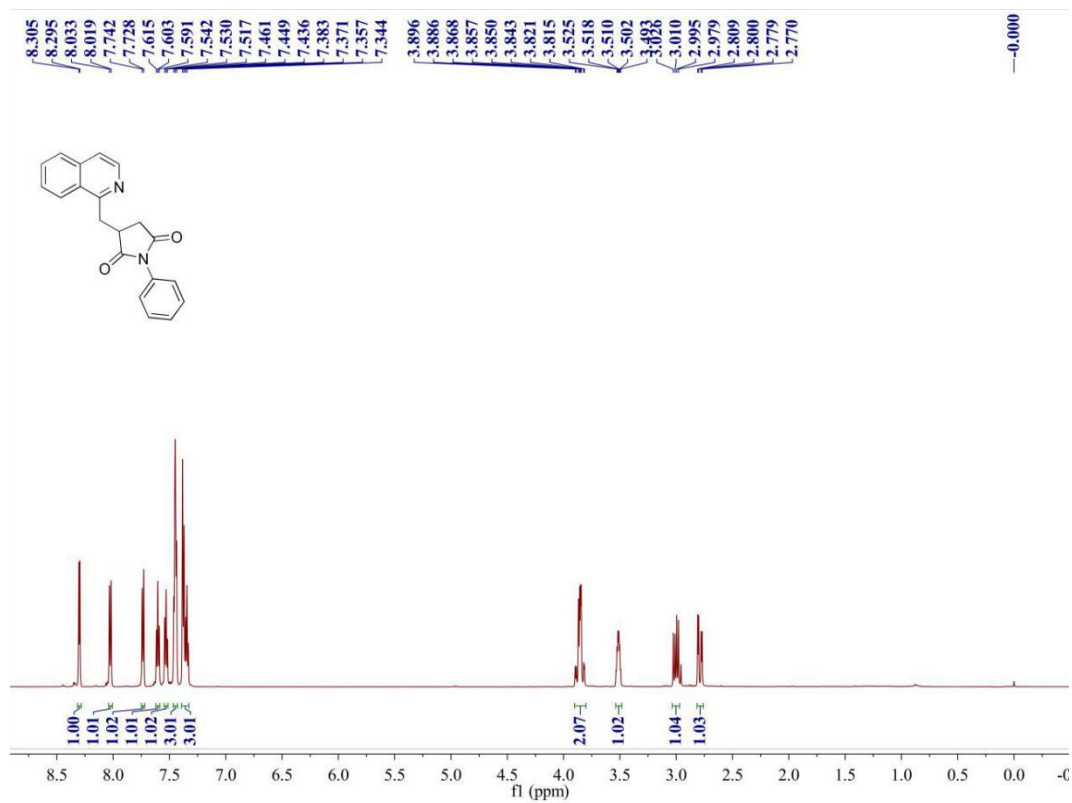
¹H NMR spectra of compound **IIIe**



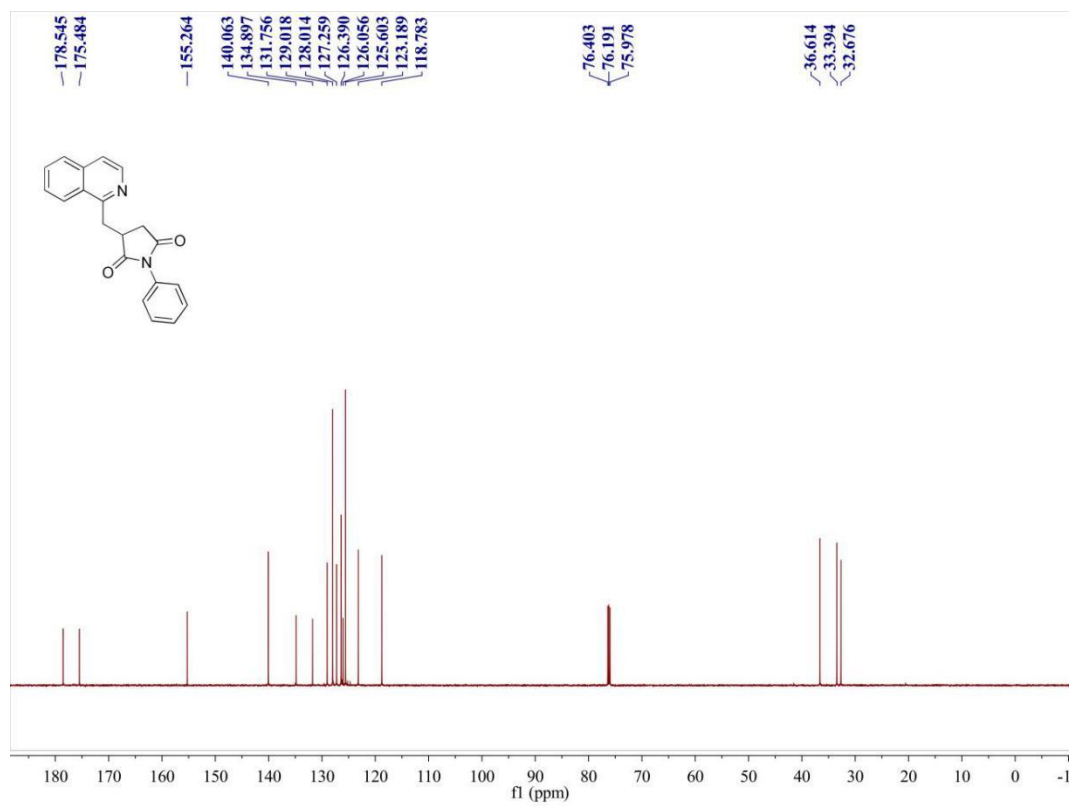
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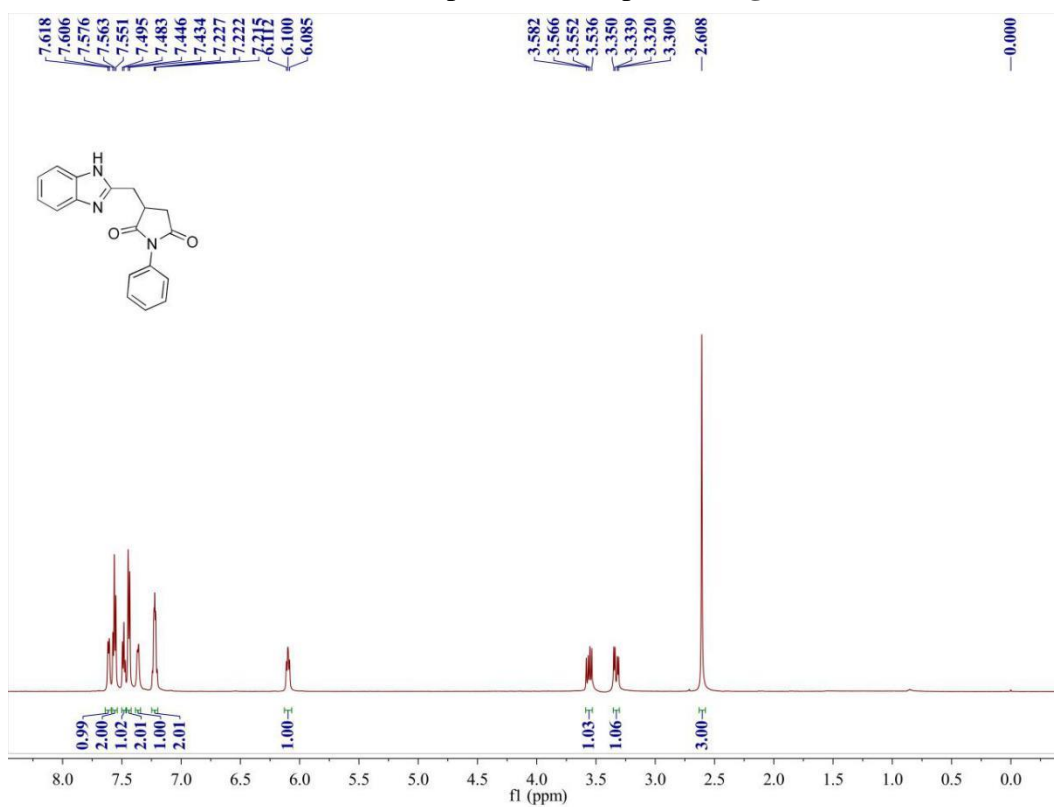
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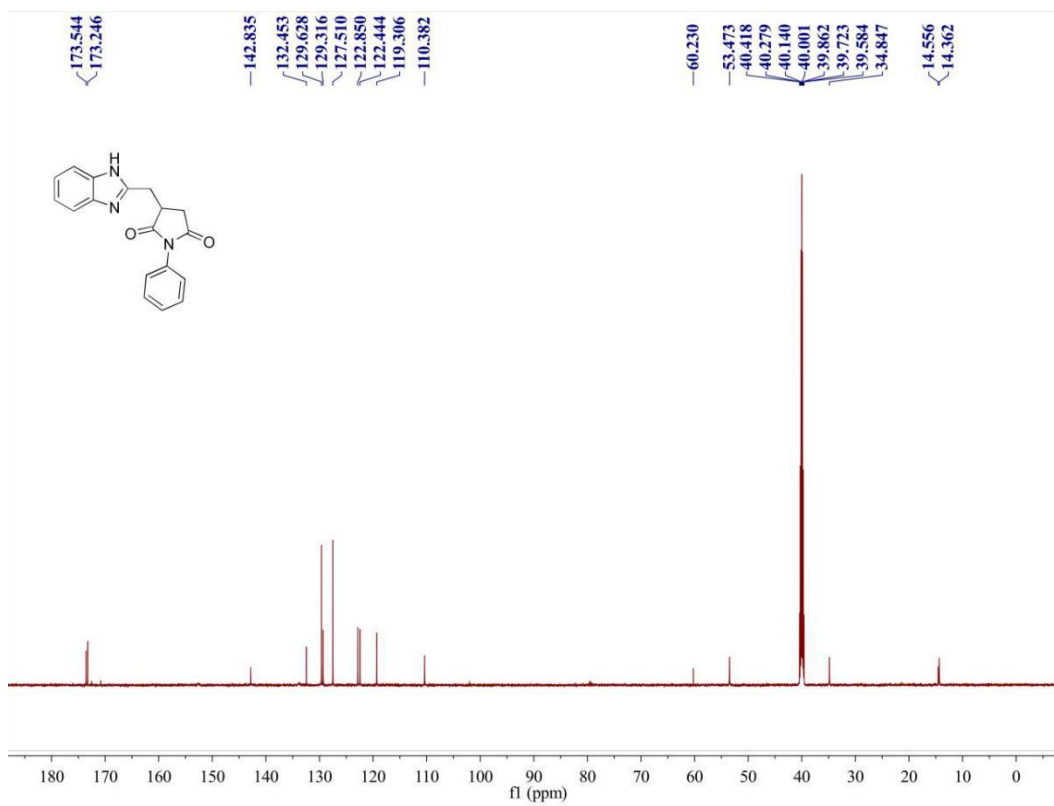
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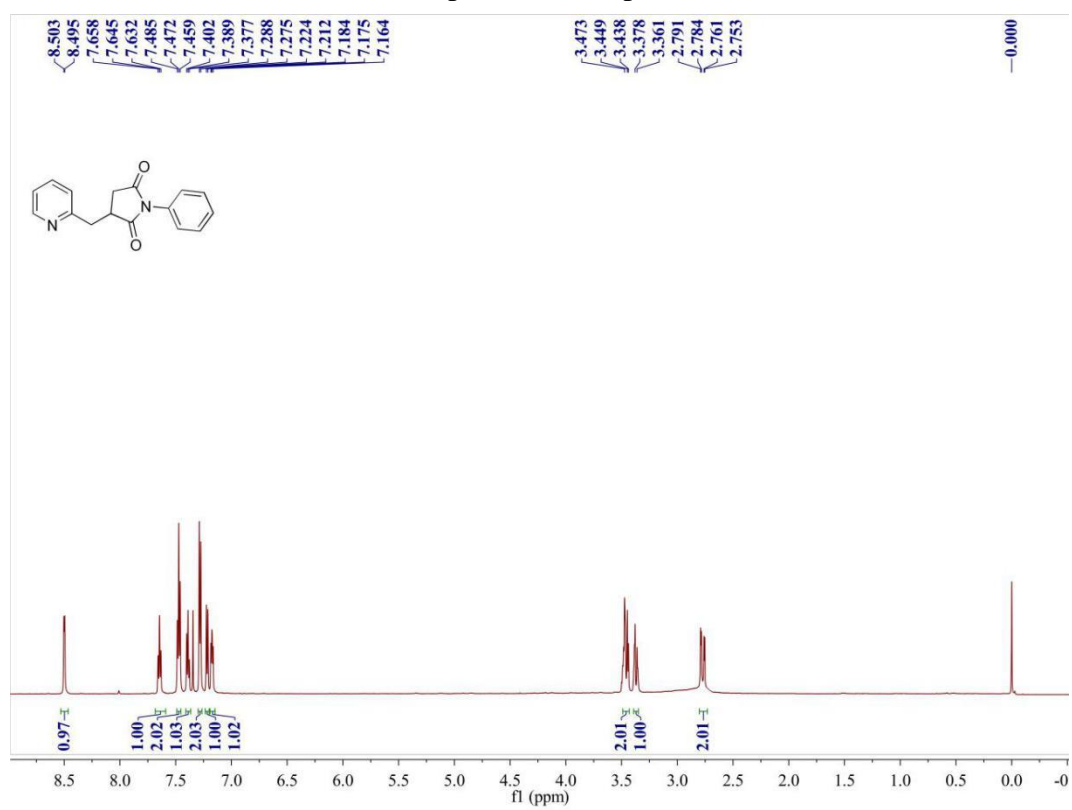
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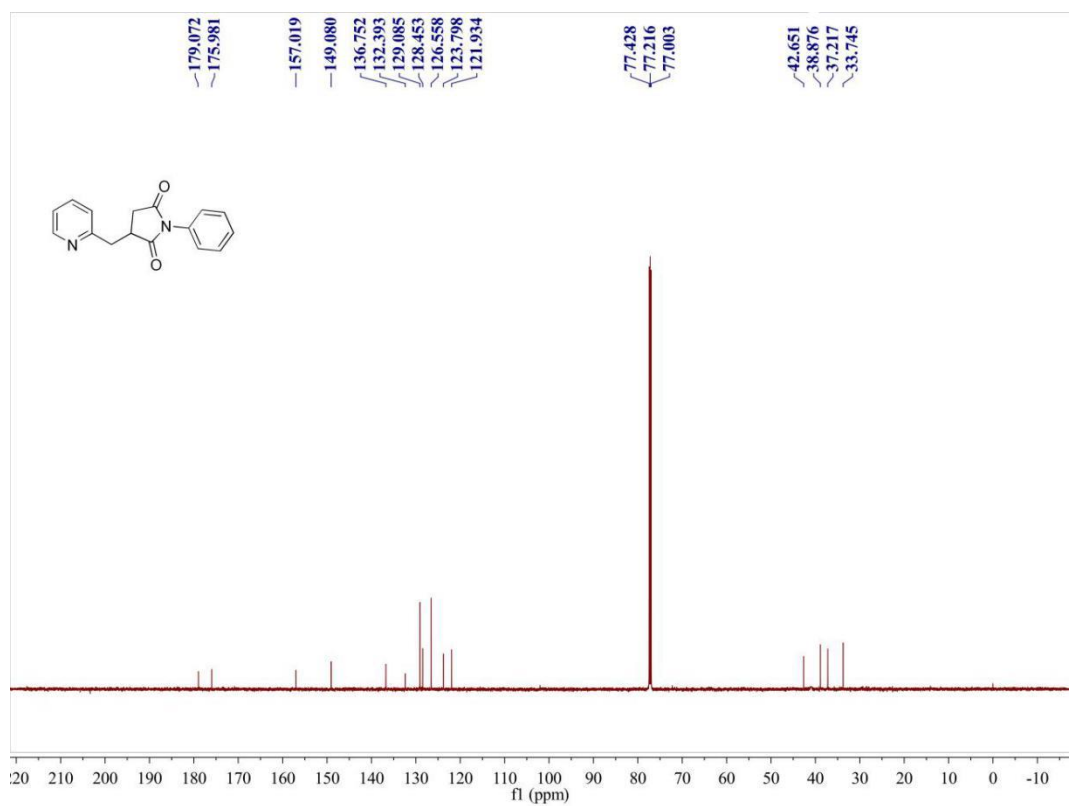
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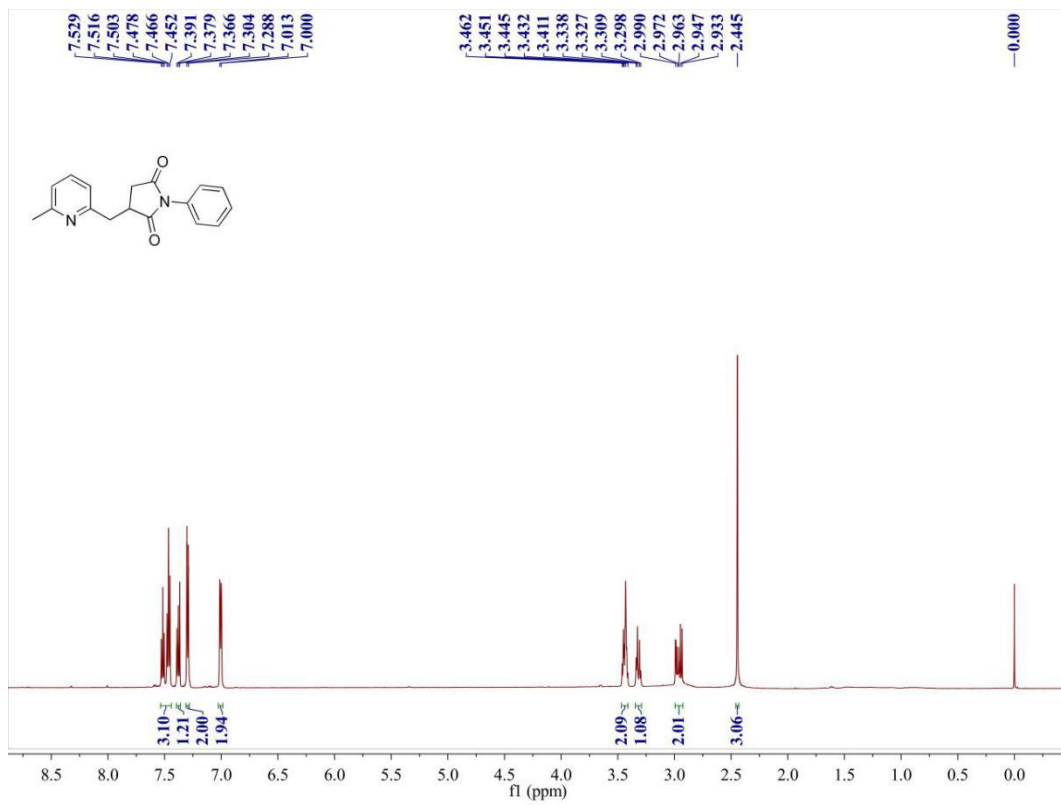
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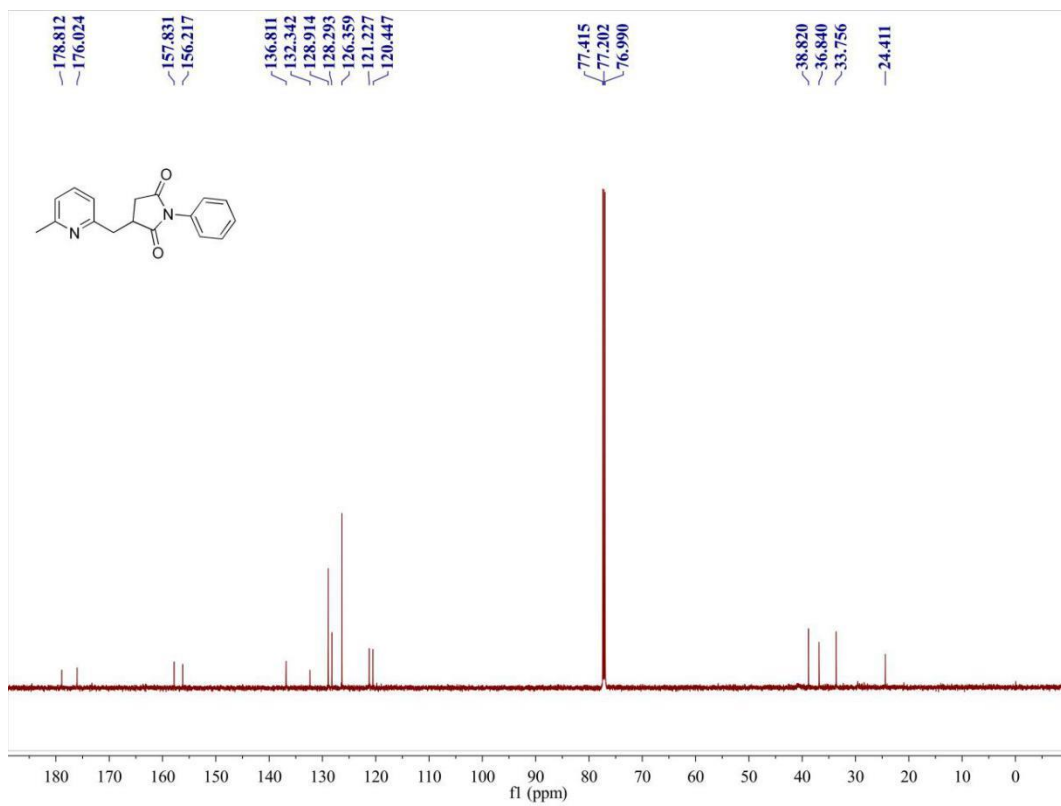
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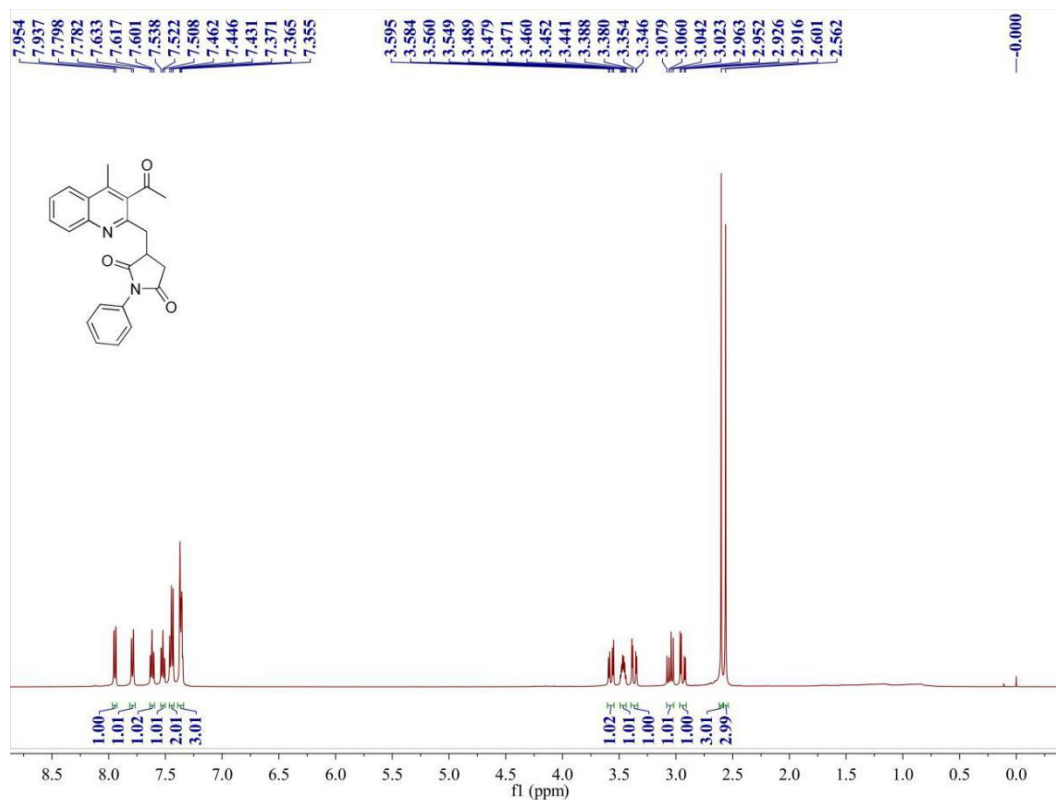
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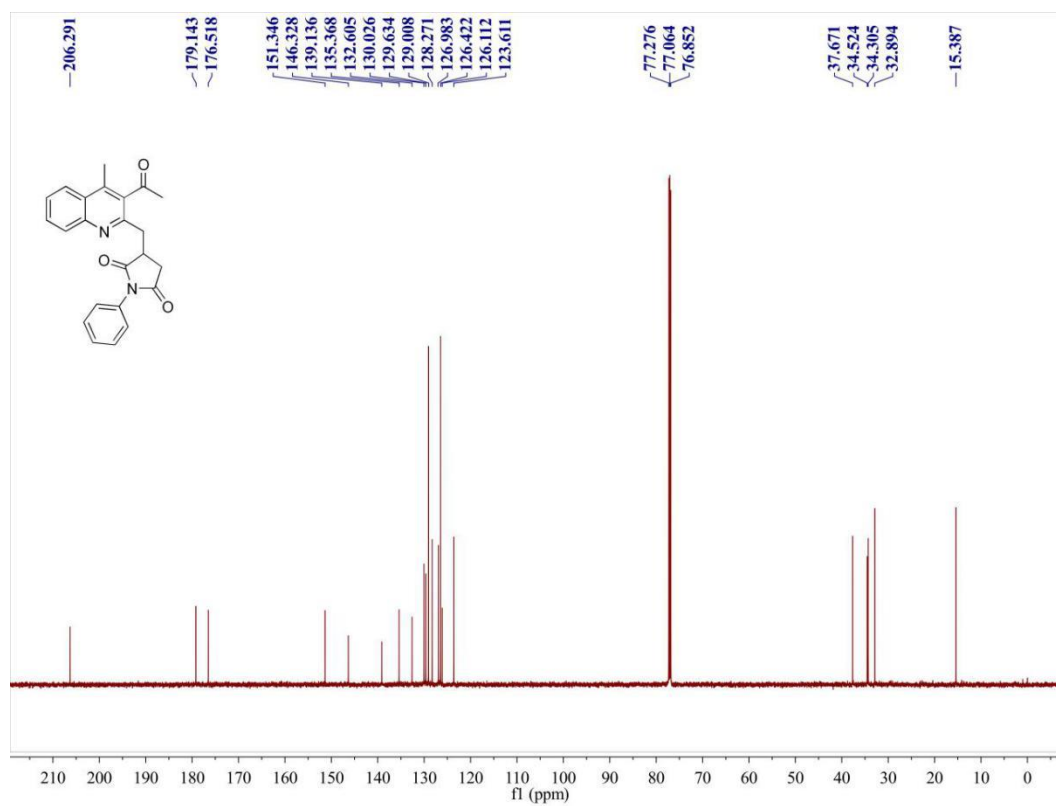
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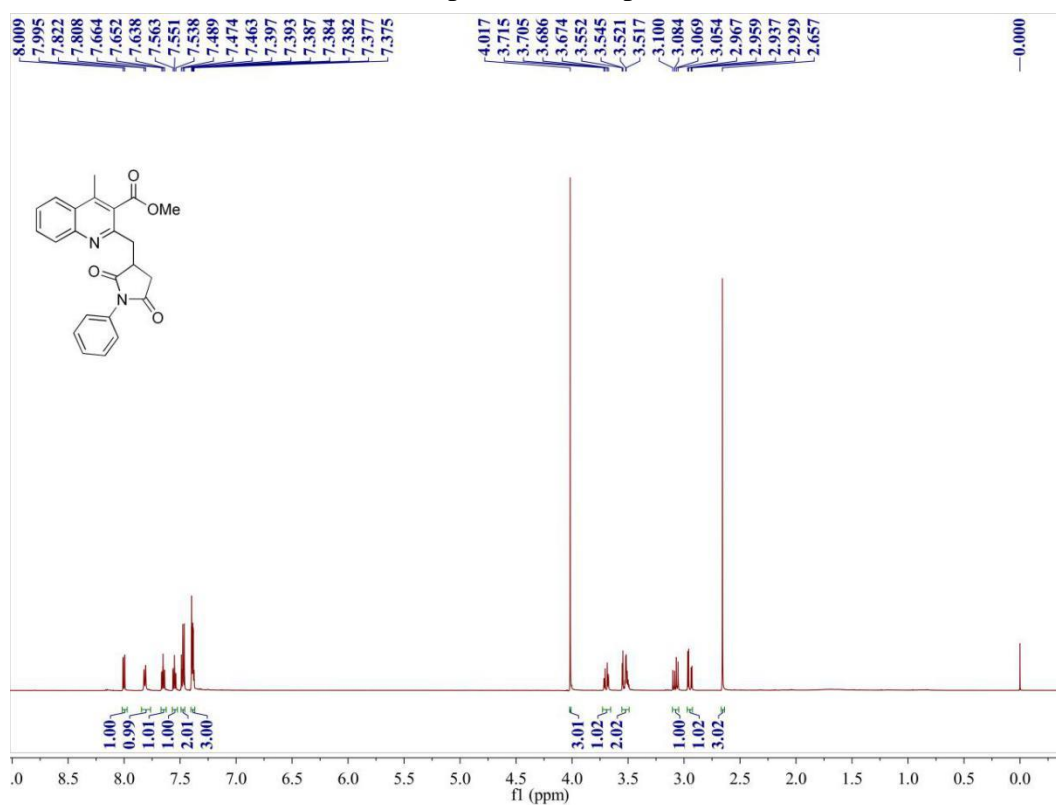
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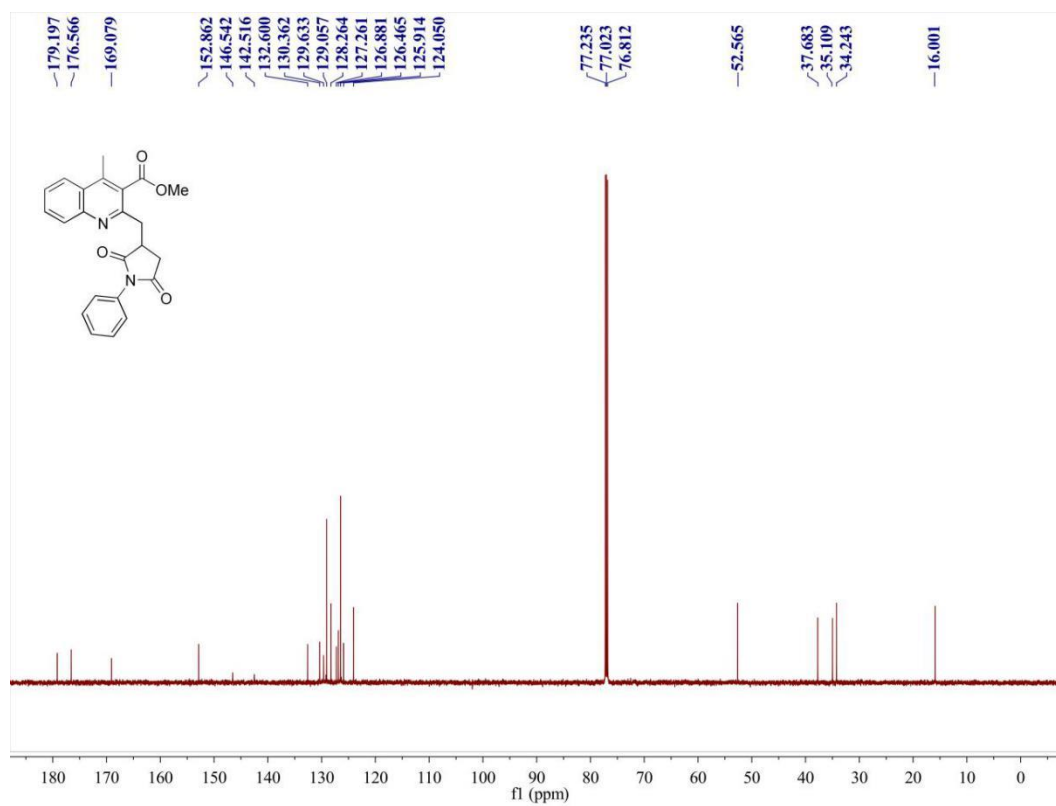
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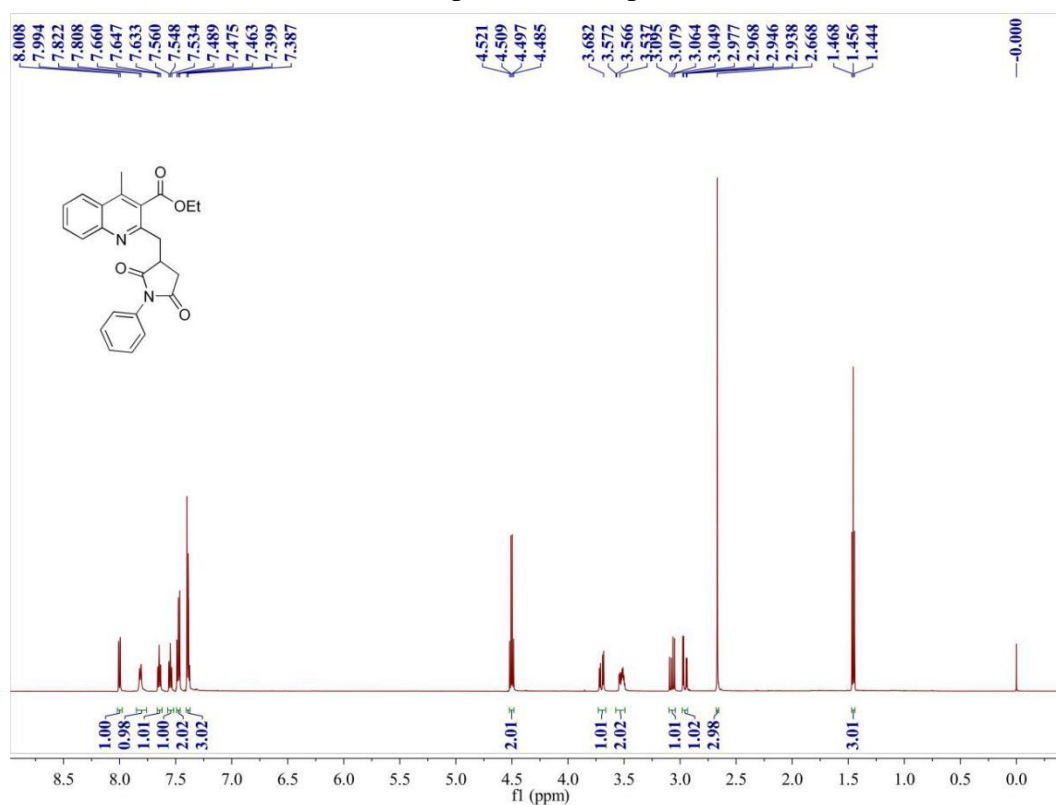
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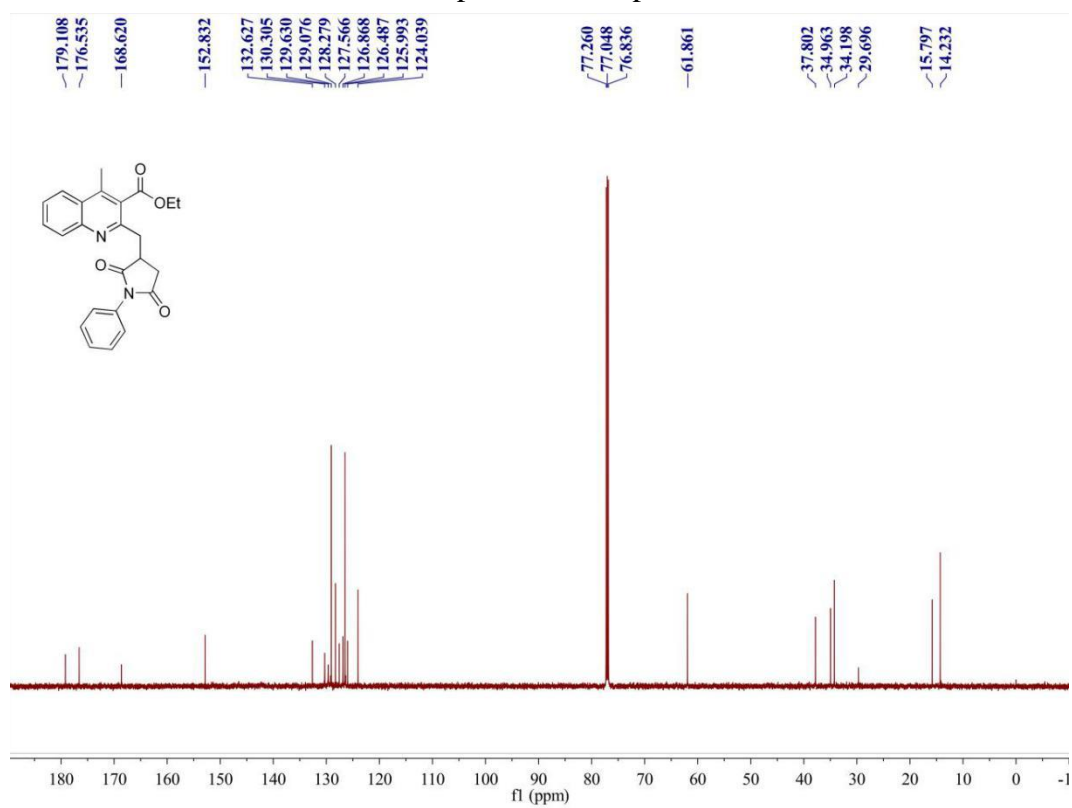
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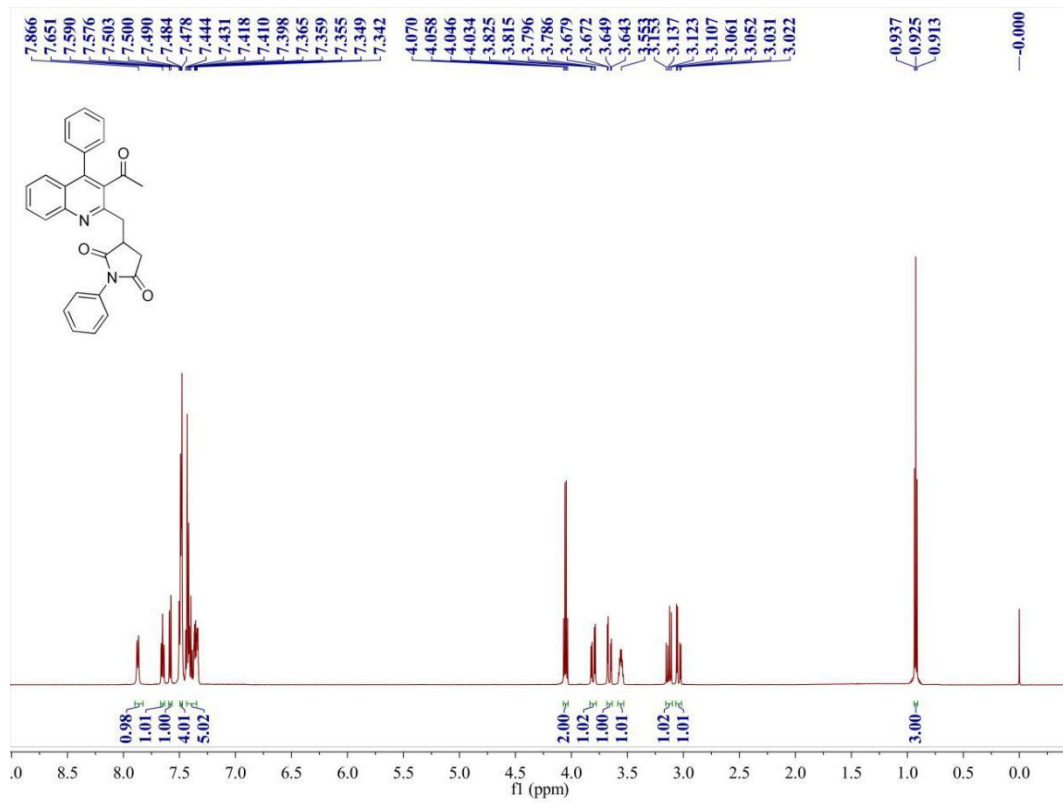
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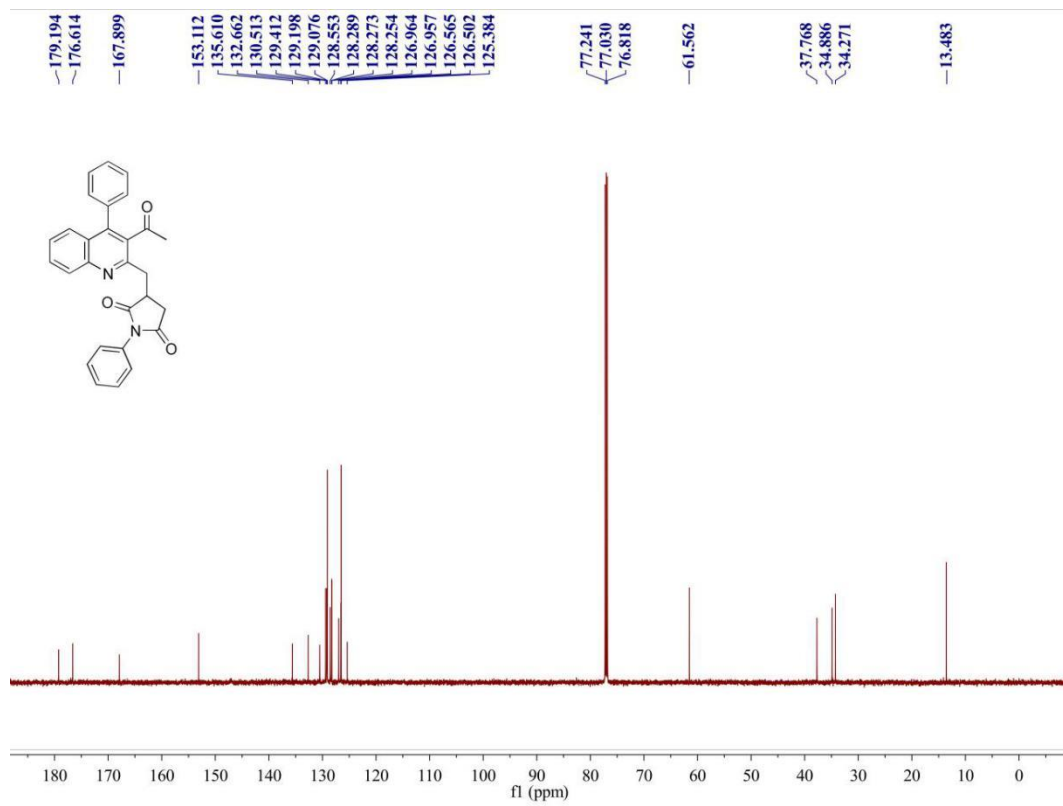
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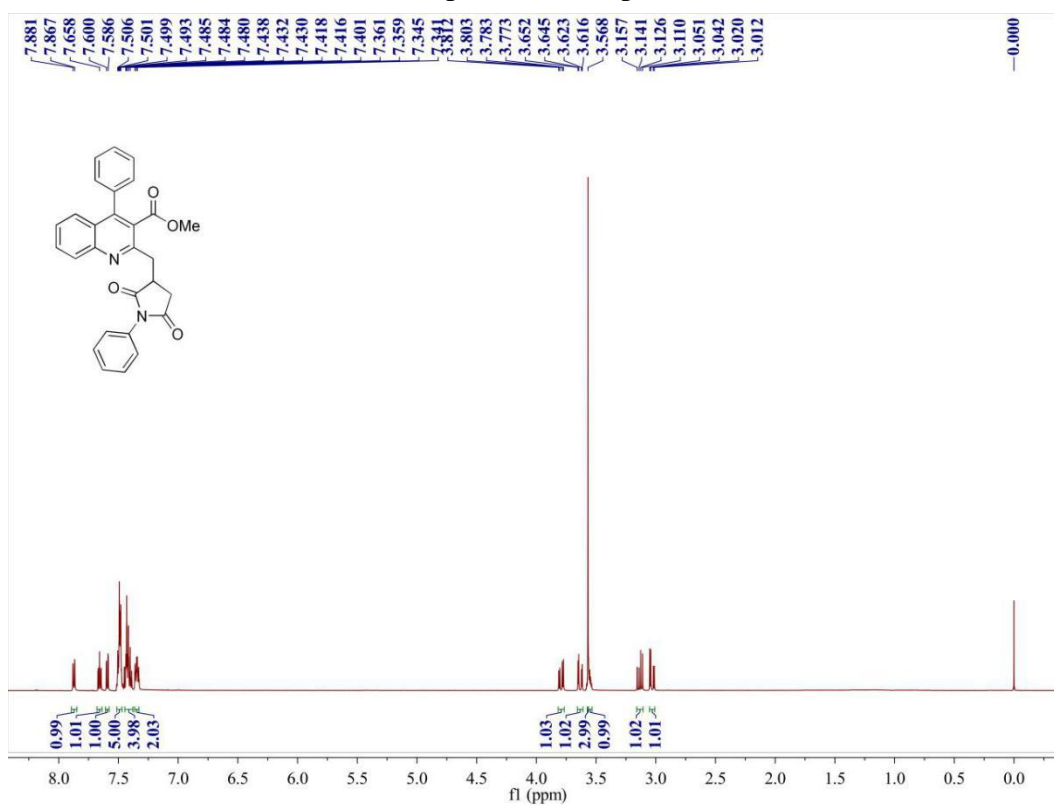
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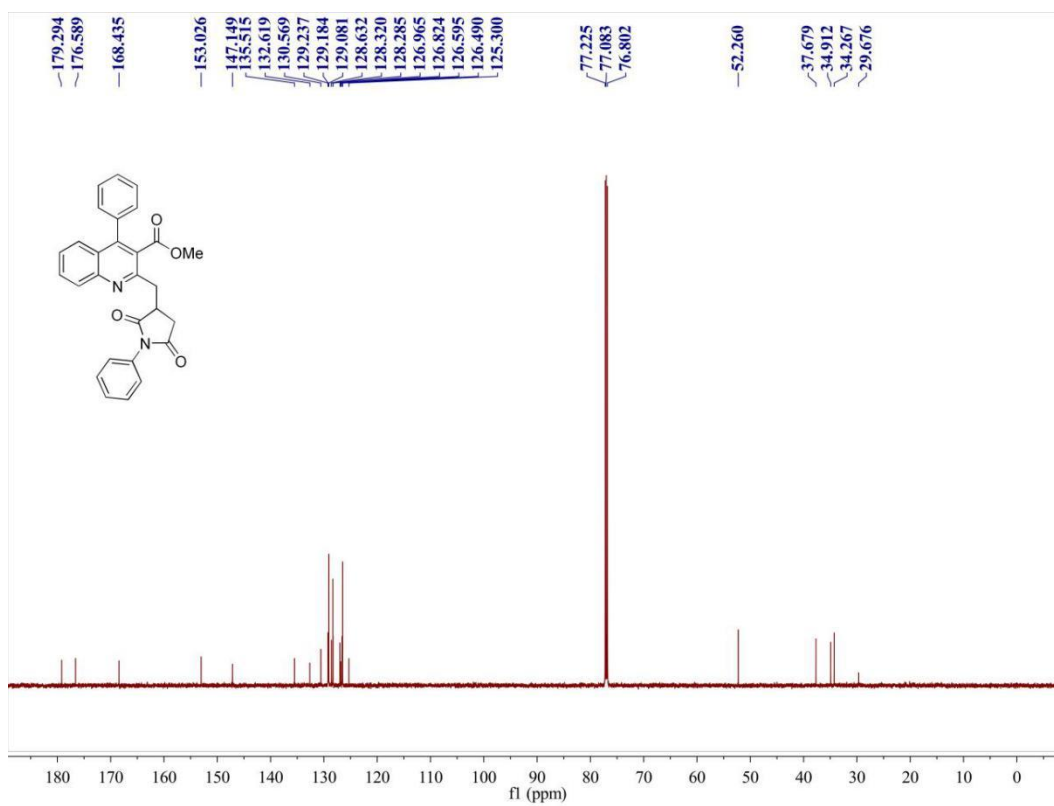
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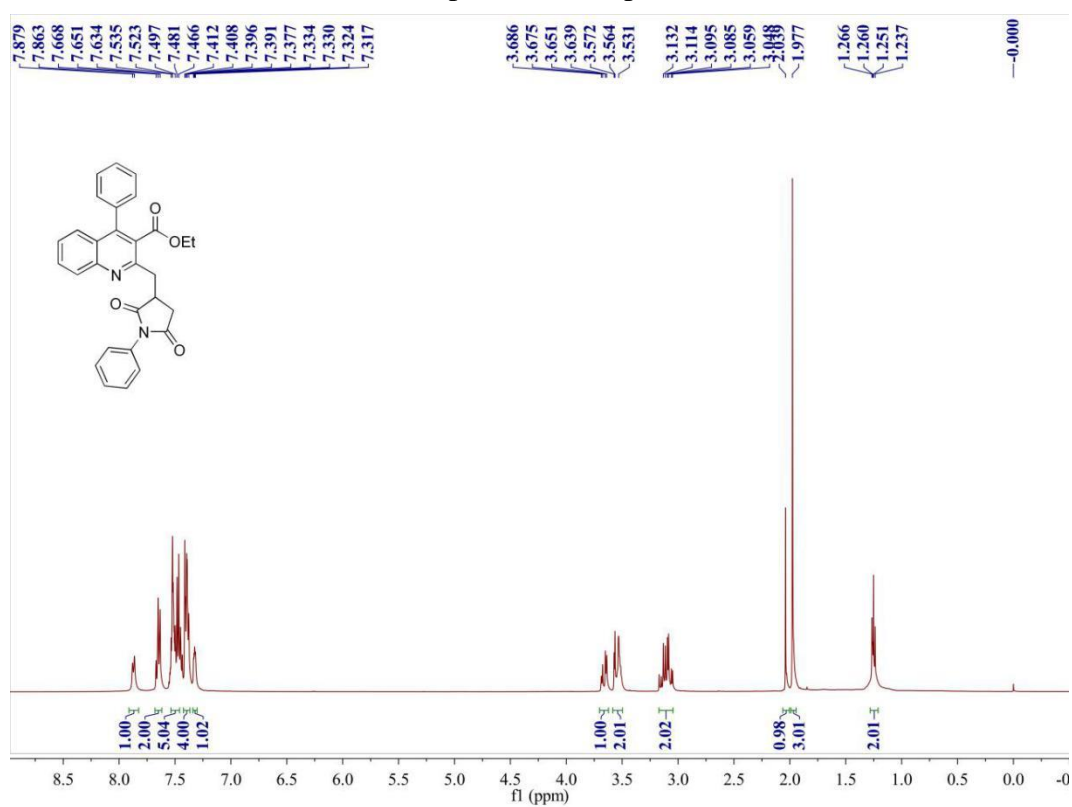
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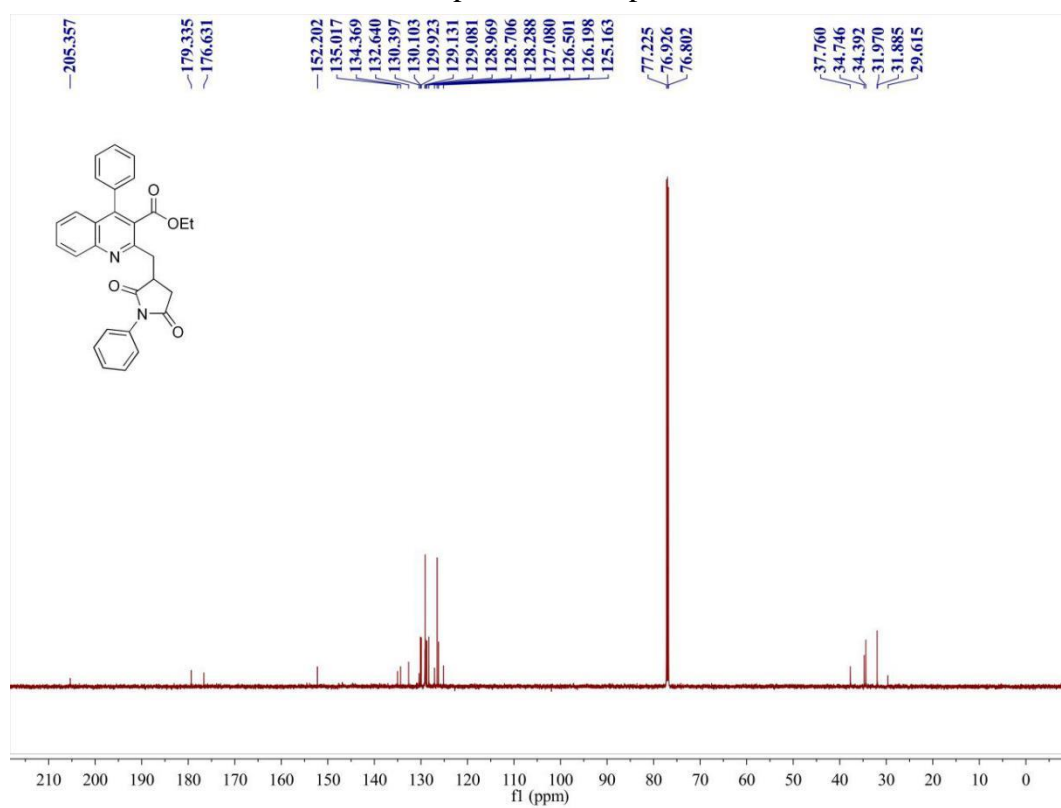
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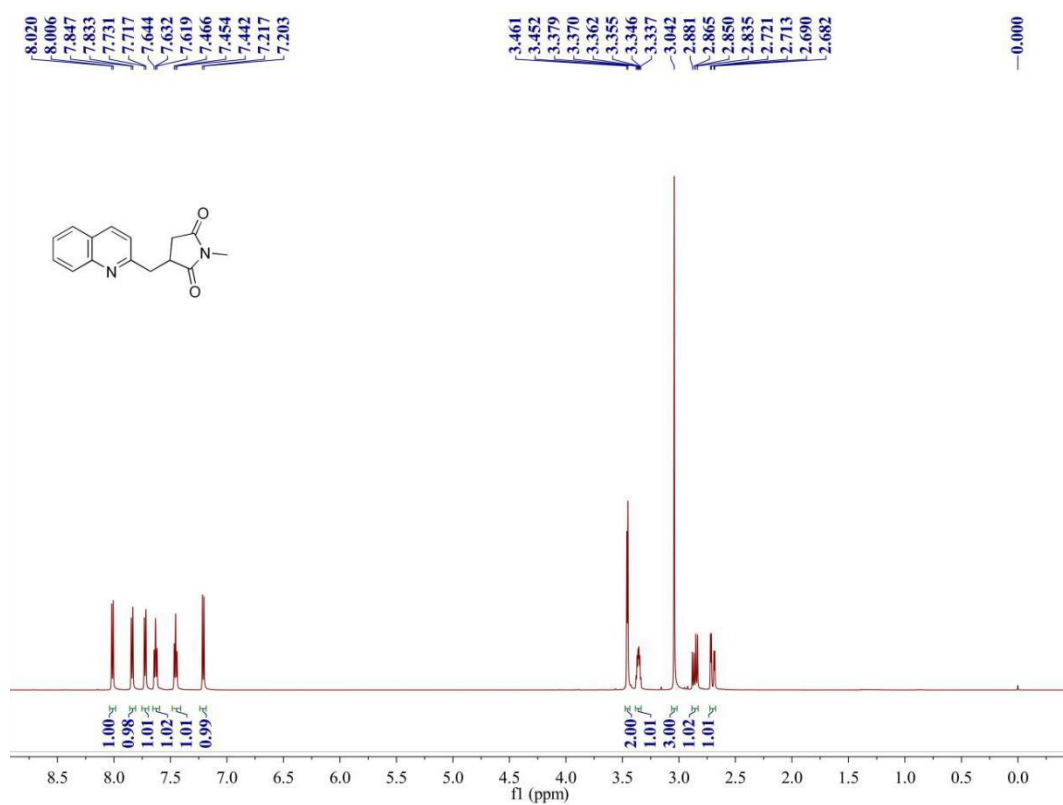
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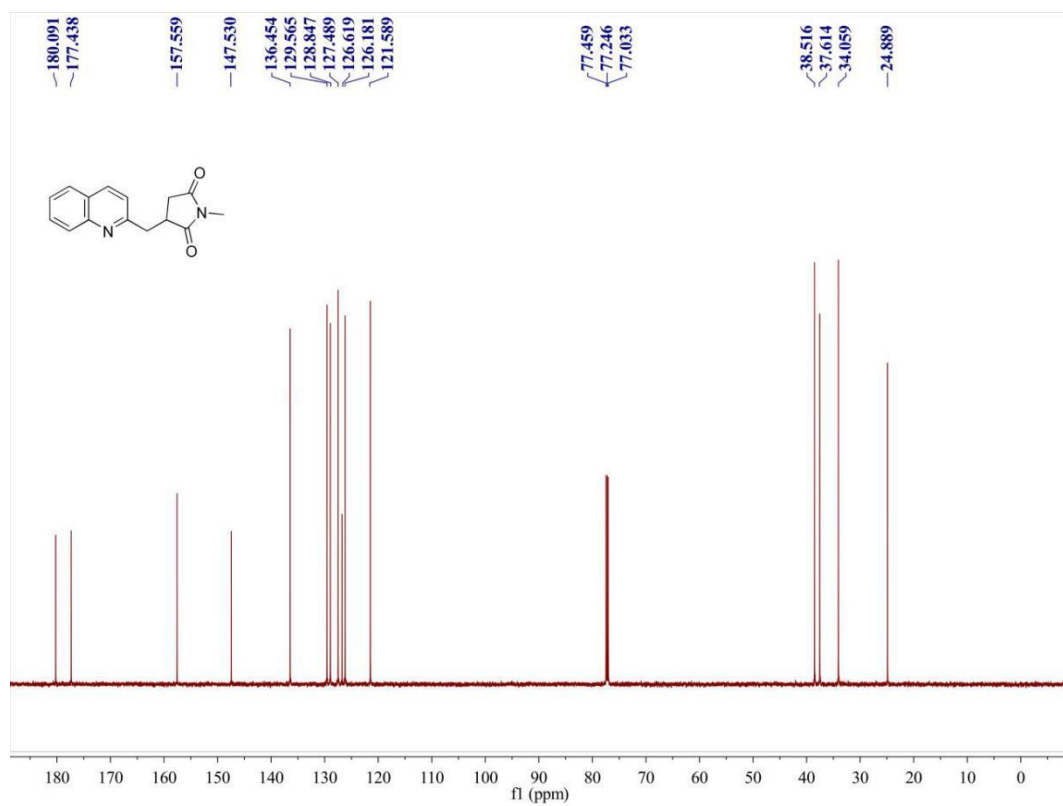
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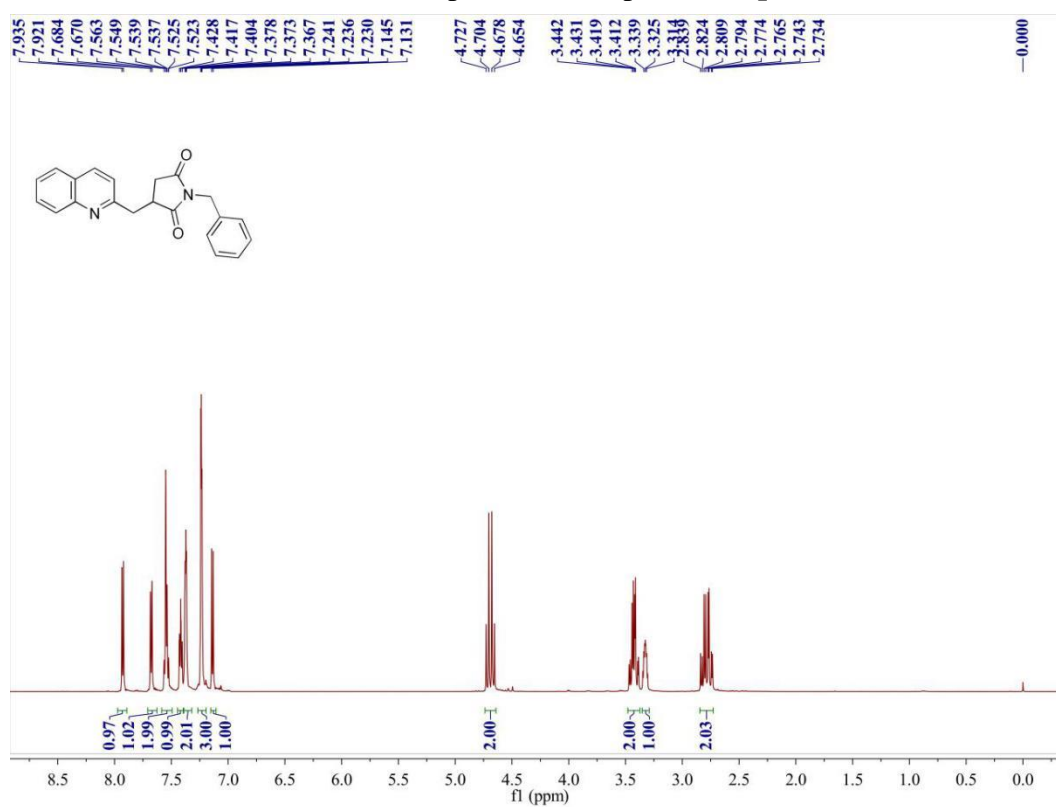
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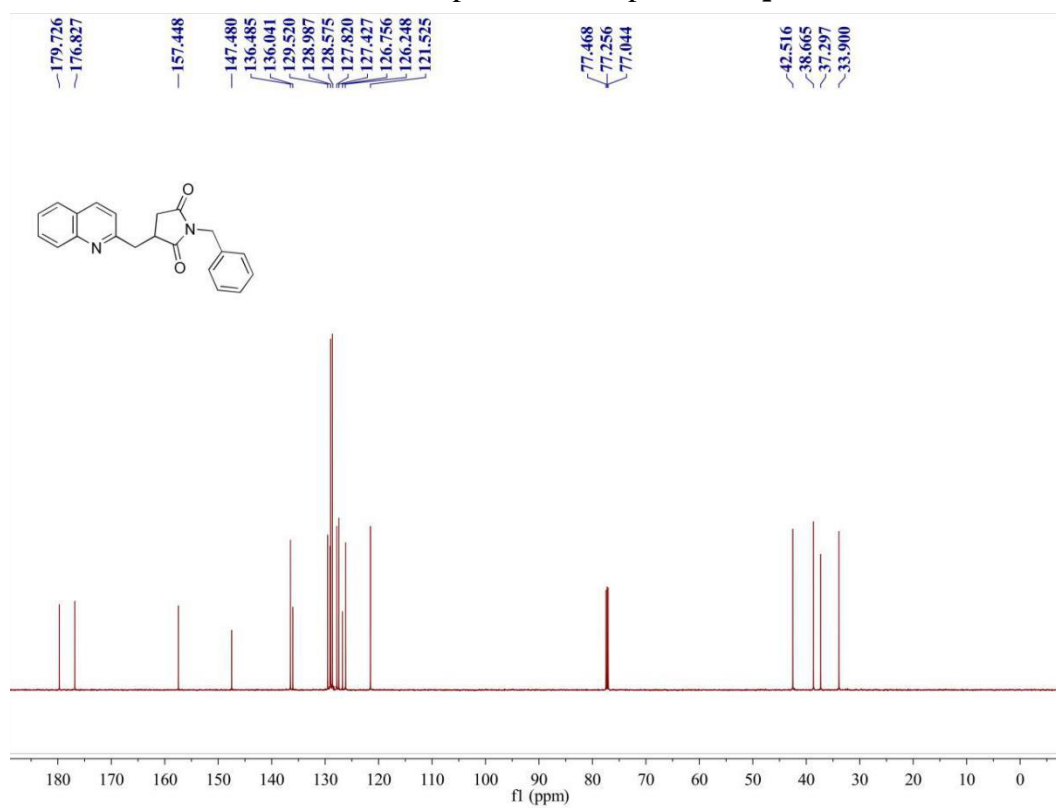
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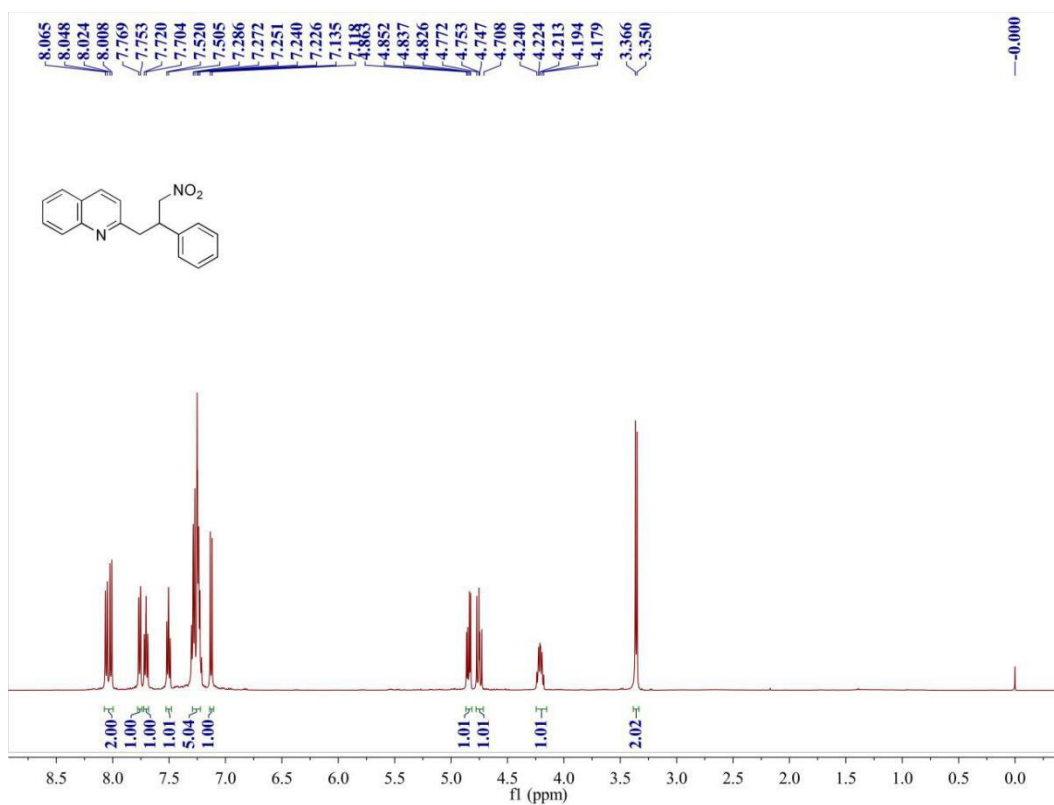
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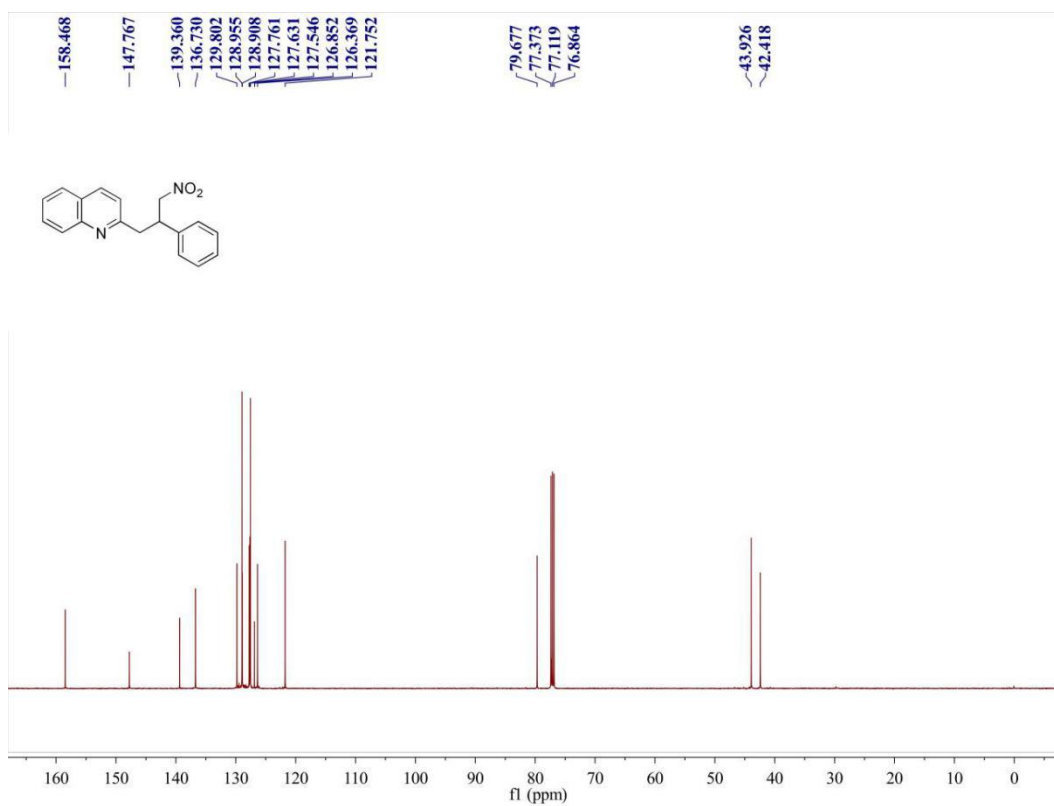
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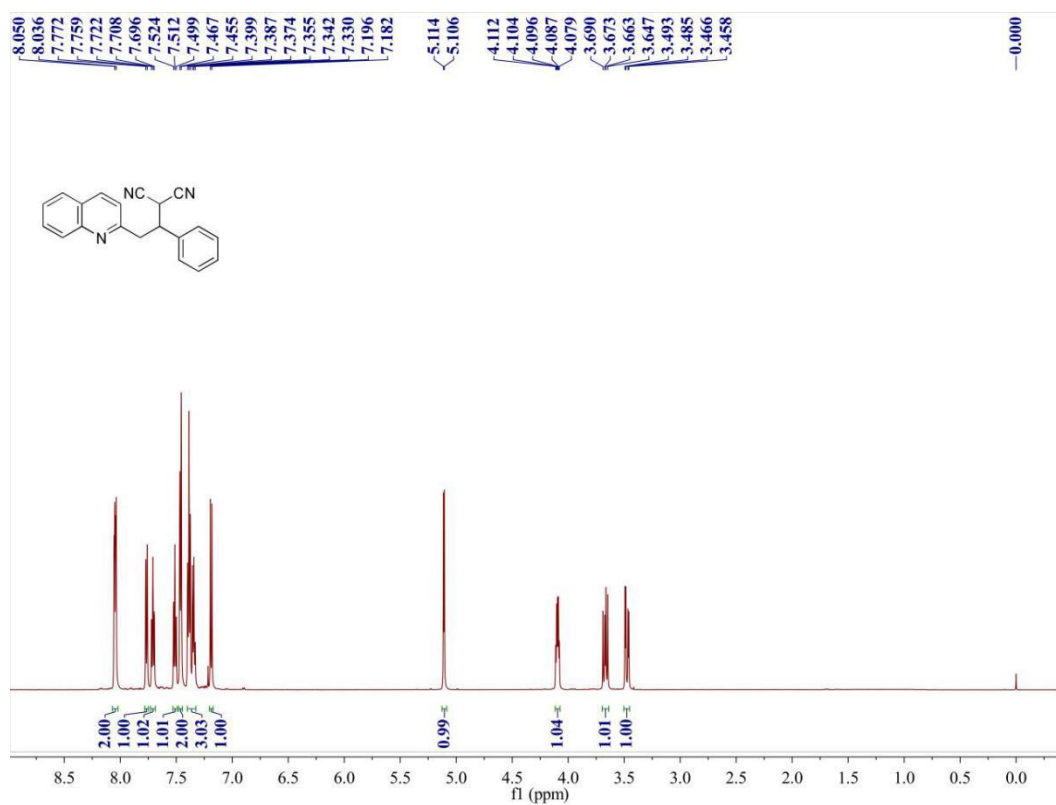
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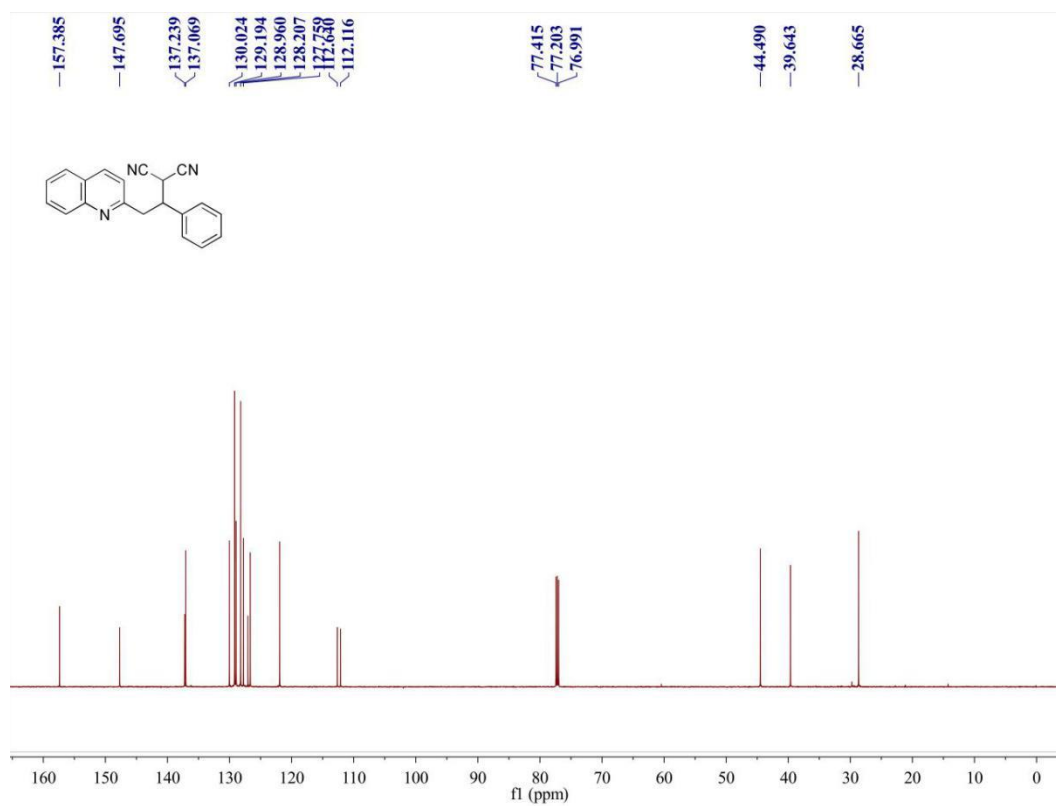
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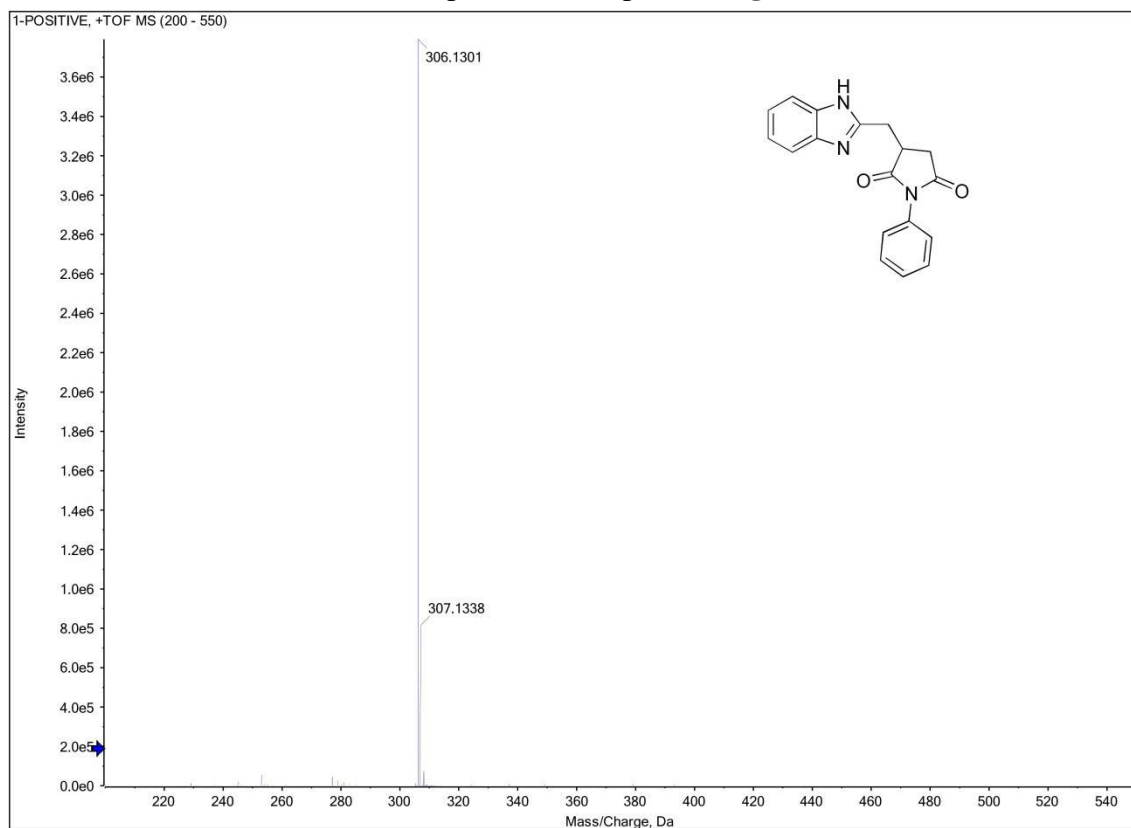
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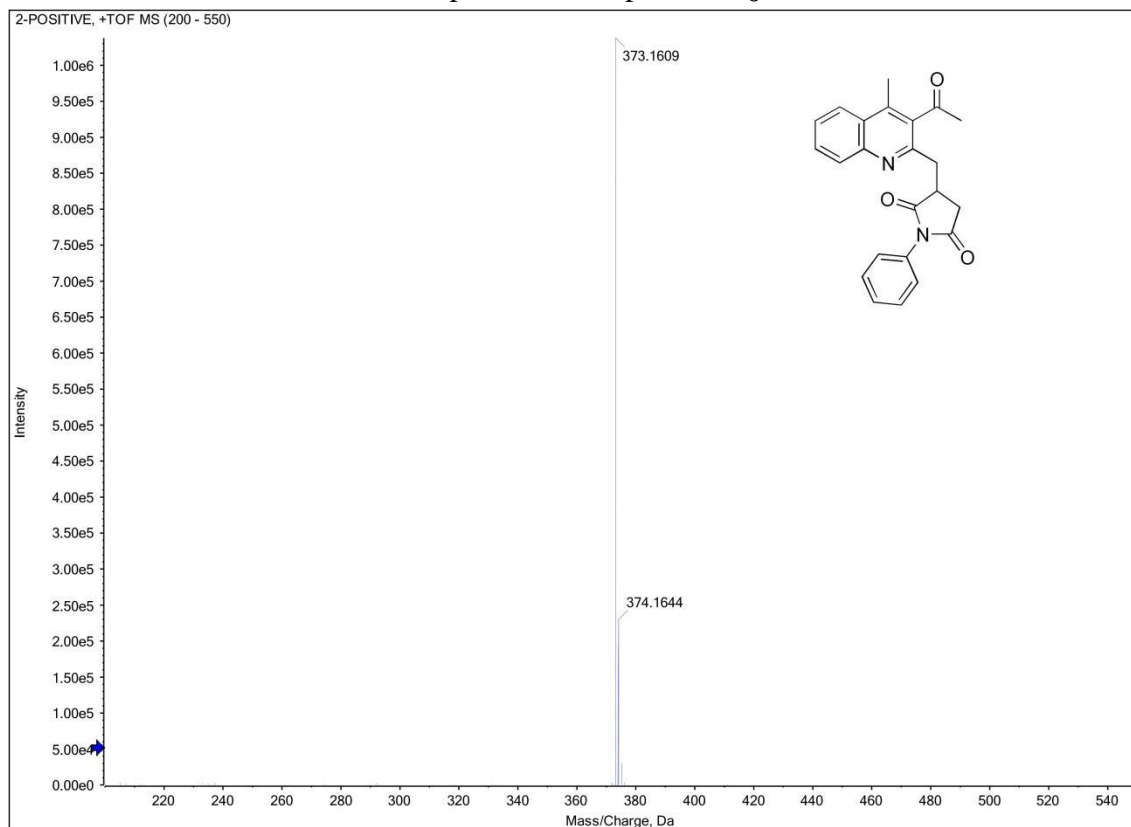
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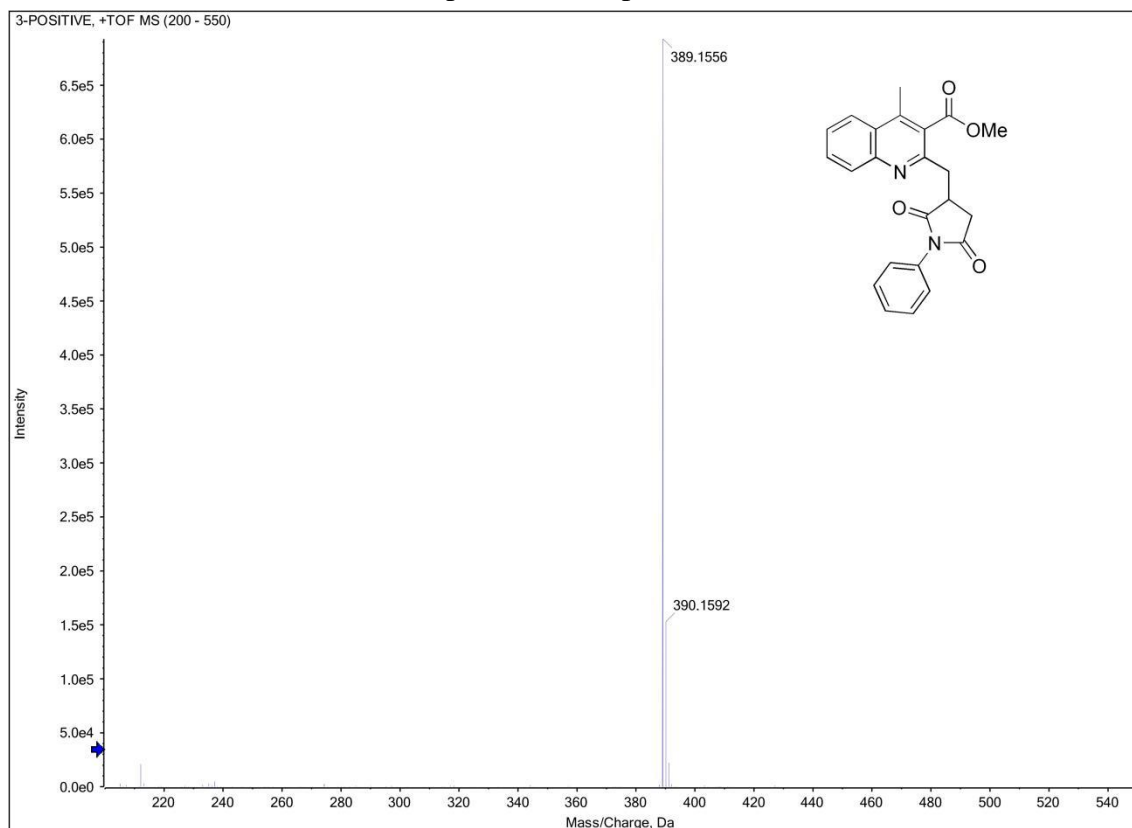
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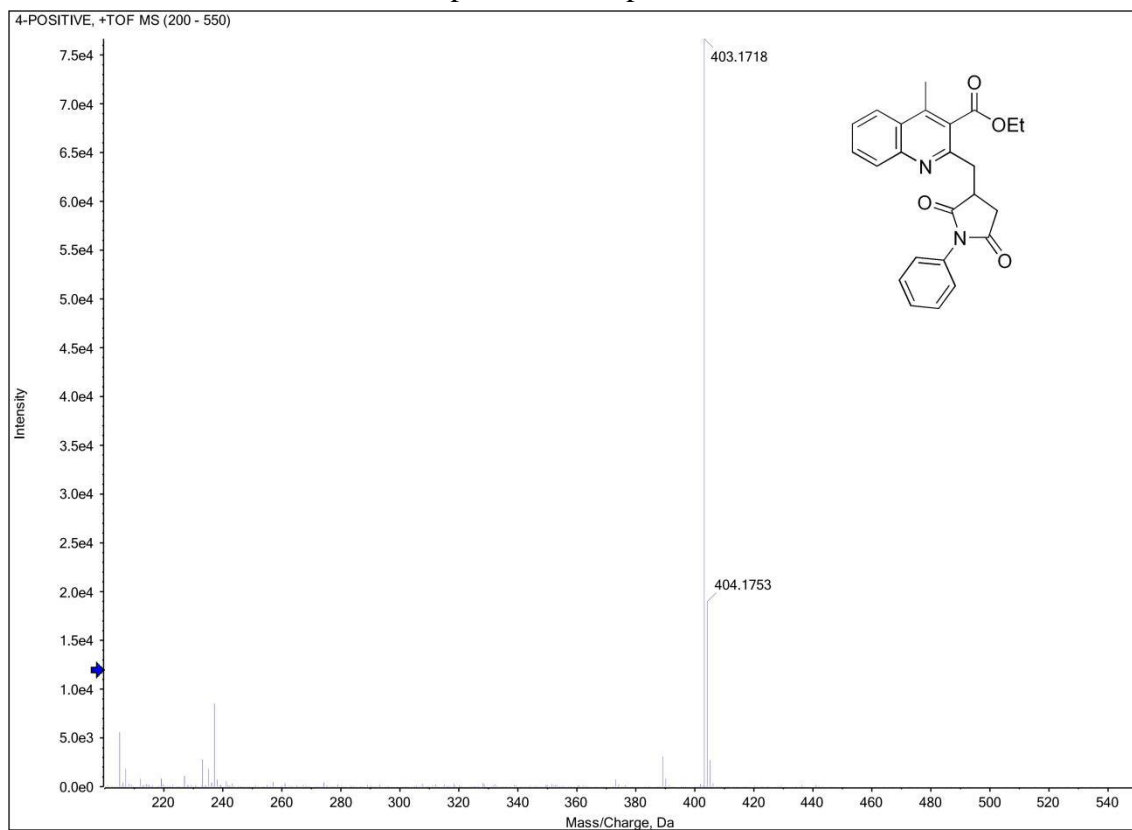
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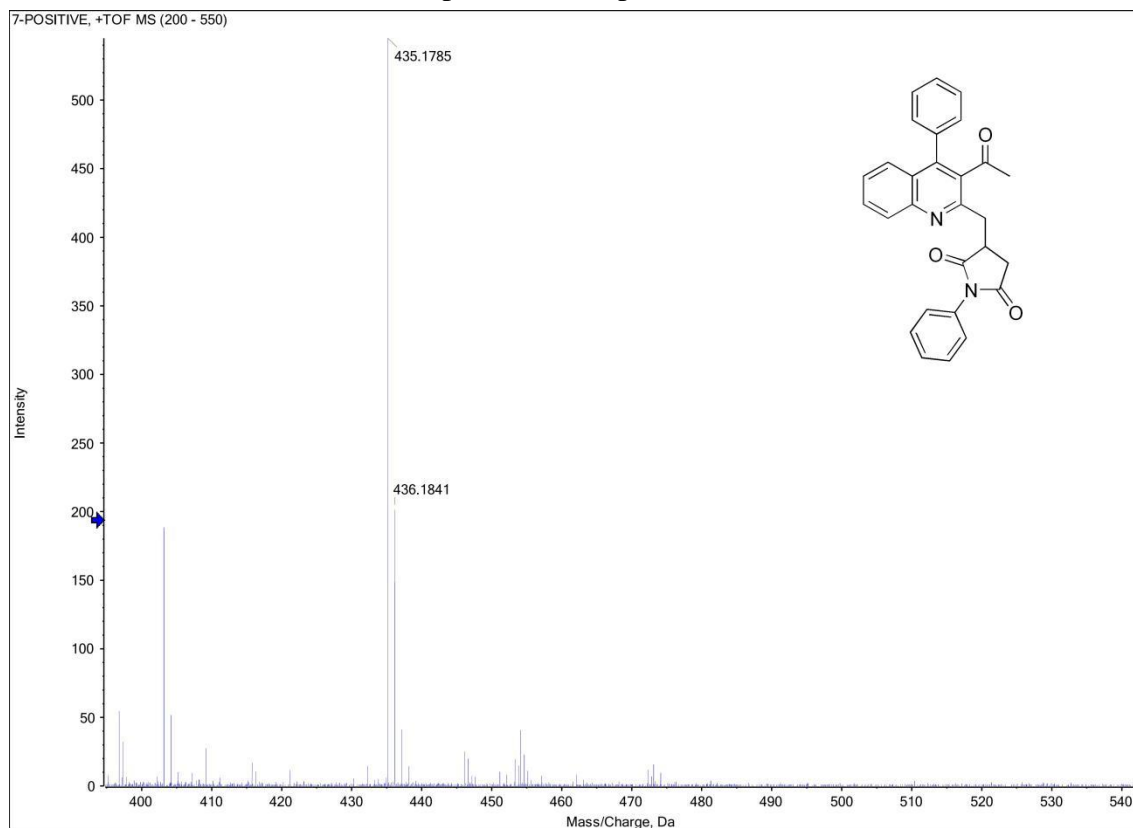
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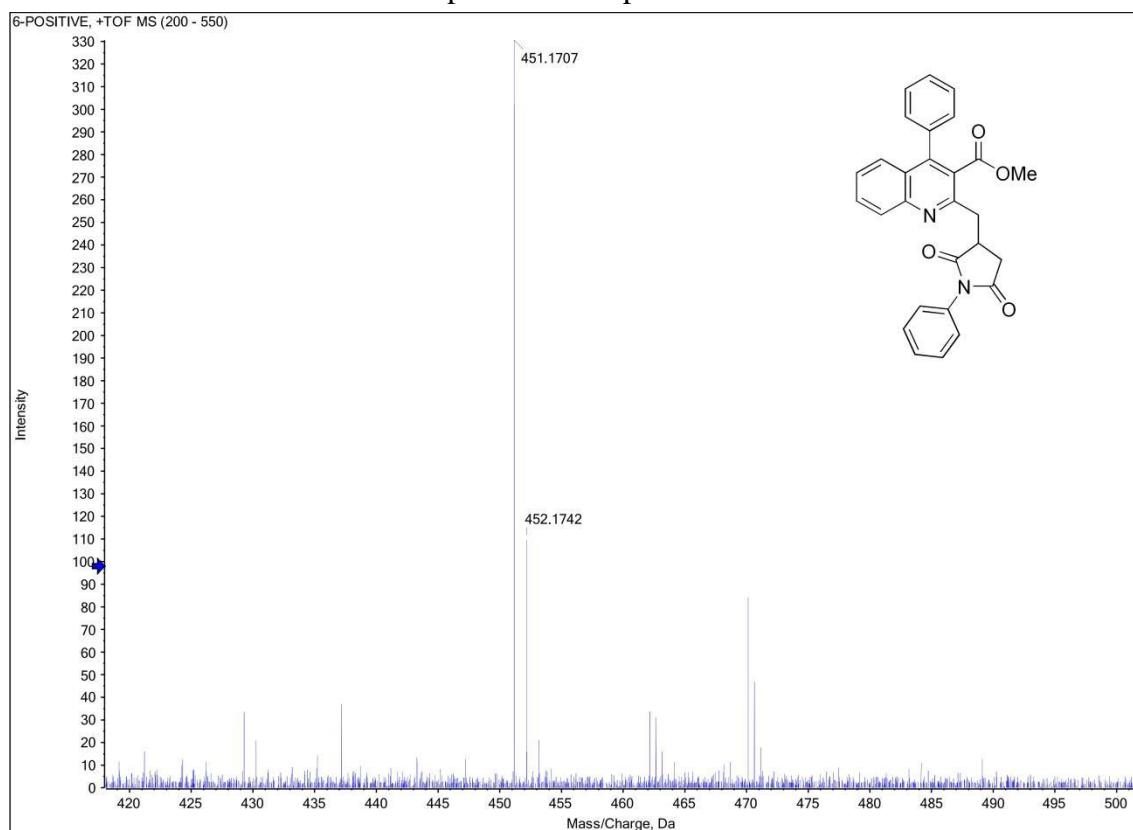
Mass spectra of compound IIIl



Mass spectra of compound IIIm



Mass spectra of compound IIIn



Mass spectra of compound **IIIo**

