

Electronic supplementary information (ESI)

Natural Products for Biocides Discovery: Discovery of Arundine and Its Derivatives as Novel Antiviral and Anti-phytopathogenic-Fungus Agents

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Detailed bio-assay procedures

Antiviral Biological Assay.¹

Detailed bioassay procedures for the anti-TMV activities¹

To prepare the compound solutions, the compound was first dissolved within a suitable amount of DMF and diluted with water containing 0.1% TW-80 to make a concentration of 500 µg/mL, and the aqueous solution was diluted to 100 µg/mL.

Purification of Tobacco Mosaic Virus.

Using Gooding's method², the upper leaves of *Nicotiana tabacum* L. inoculated with TMV were selected and ground in phosphate buffer and then filtered through double-layer pledget. The filtrate was centrifuged at 10000 g, treated with PEG twice, and centrifuged again. The whole experiment was processed at 4 °C. Absorbance value was estimated at 260 nm by ultraviolet spectrophotometer.

$$\text{Virus concn} = (A_{260} \times \text{dilution ratio}) E /_{1\text{cm}}^{0.1\%, 260\text{nm}}$$

Protective Effect of Compounds against TMV in Vivo.

The compound solution was sprayed on growing *Nicotiana. tabacum* L. leaves (at least 3 leaves) of the same age. In another pot, the leaves were sprayed with the solvent as a control. After 12 h, the leaves were inoculated with TMV with the juice-leaf rubbing method and then washed with water. The total local lesion numbers appearing on the leaves 3–4 days after inoculation were recorded.³ The experiment was conducted with three replicates for each compound.

Inactivation Effect of Compounds against TMV.

To test viral inhibition, equal volumes of the virus and the compound solution were mixed together for 30 min. The mixture was then inoculated into the growing *N. tabacum* L leaves of the same age (at least 3 leaves), and another pot was inoculated with the mixture of solvent and the virus as the control. The local lesion numbers were recorded 3–4 days after inoculation.³

Curative Effect of Compounds against TMV

TMV (concentration of 6.0×10^{-3} $\mu\text{g/mL}$) was inoculated on the growing leaves of *N. tabacum* L. of the same age (at least 3 leaves). Then, the leaves were washed with water and dried. The compound solution was sprayed on the inoculated leaves, while inoculated leaves in another pot were sprayed with the solvent as a control. The local lesion numbers were recorded 3–4 days after inoculation.³ All assays were conducted using three replicates for each compound. The inhibition rates of the compound were calculated according to the following formula (“av” means average, and “controls” means not treated with compound): *inhibition rate (%) = [(av local lesion number of control – av local lesion number of drug treated)/av local lesion number of control] × 100%*.

Detailed bioassay procedures for the in vitro fungicidal activities⁴⁻⁶

The fungicidal activities of compounds were evaluated in mycelial growth tests conducted in artificial media against 14 plant pathogens at a rate of 50 $\mu\text{g/mL}$. Each test compound was dissolved in a suitable amount of acetone and diluted with water containing 0.1% TW-80 to a concentration of 500 $\mu\text{g/mL}$. To each petri dish was added 1 mL of the test solution and 9 mL of culture medium to make a 50 $\mu\text{g/mL}$ concentration of the test compound, while in another petri dish was added 1 mL distilled water containing 0.1% TW-80 and 9 mL of culture medium as a blank control. A 4 mm diameter of hyphal growth was cut using a hole puncher on a growing fungal culture and the hyphae were moved to the petri dish containing the test compound. Each assay was performed three times. The dishes were stored in controlled environment cabinets ($24 \pm 1^\circ\text{C}$) for 4 days, after which the diameter of mycelial growth was measured and the percentage inhibition was calculated using the following equation: *Percentage inhibition (%) = (averaged diameter of mycelia in blank controls – averaged diameter of mycelia in medicated tablets) / (averaged diameter of mycelia in blank controls – 4 mm) × 100*. The toxicity was ascertained by establishing the half maximal effective concentration (EC_{50}) of compounds with percentage inhibition over 80%.

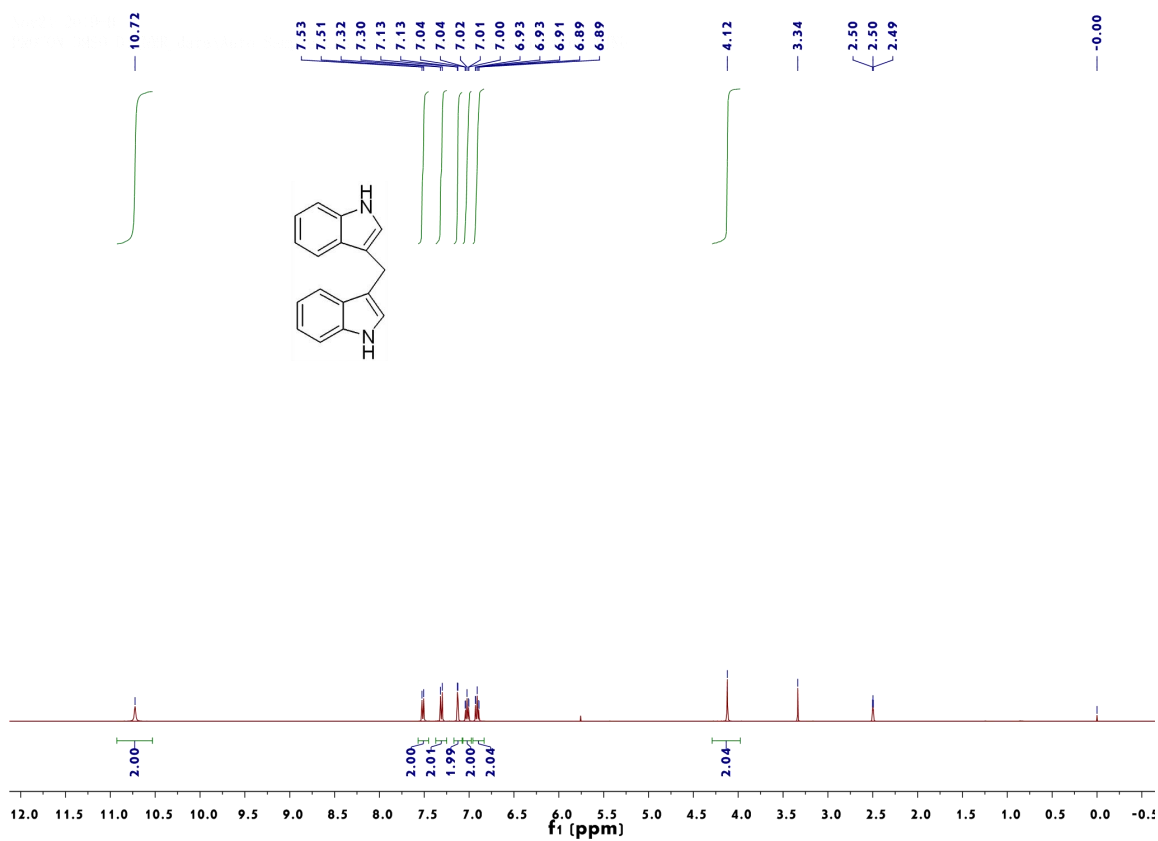
Mode of Action Studies.

In vitro TMV rod assembly inhibition : TMV purification was performed according to the instructions by Leberman.⁷ TMV RNA was purified by RNAPure virus kit (CoWin Biosciences) and TMV capsid protein (TMV CP) was isolated using glacial acetic acid as described by Fraenkel-Conrat.⁸ Before assembly, 20S CP Disk was prepared by incubating 20 mg/mL TMV CP in 0.1 M phosphate buffer (pH 7.0) at 20 °C for 12 h. After incubation, in vitro TMV reconstitution reactions were performed by adding 5 µL of phosphate buffer (0.1 M, pH 7.0), 4 µL of 20S Disk (2 mg/mL) and 1 µL of TMV RNA (200 ng/µL). The assembly reaction mixture was incubated at 20 °C for 12 h and could be then transferred into the copper grid for transmission electron microscopy (TEM) assay. The assembly reaction mixture (5 µL) was mixed with 5 µL 0.1 M phosphate buffer (pH 7.0) and dropped onto the copper film waiting for 5 minutes. After the incubation, the droplet was removed by filter paper and negatively stained by 2% phosphotungstic acid (pH 7.0) for three minutes. After removing the staining agent, the copper was placed at 37 °C for 2 h for drying. The morphology of the reconstituted TMV rods was imaged at 200 keV on a CCD camera. For the inhibition tests with the compound **6**, in vitro TMV reconstitution inhibition reactions were performed by adding 4.8 µL of phosphate buffer (0.1 M, pH 7.0), 4 µL of 20S Disk (2 mg/mL), 1 µL of TMV RNA (200 ng/µL) and 0.2 µL of compound **6** (10 µM). All treatments were repeated over time to validate the results.

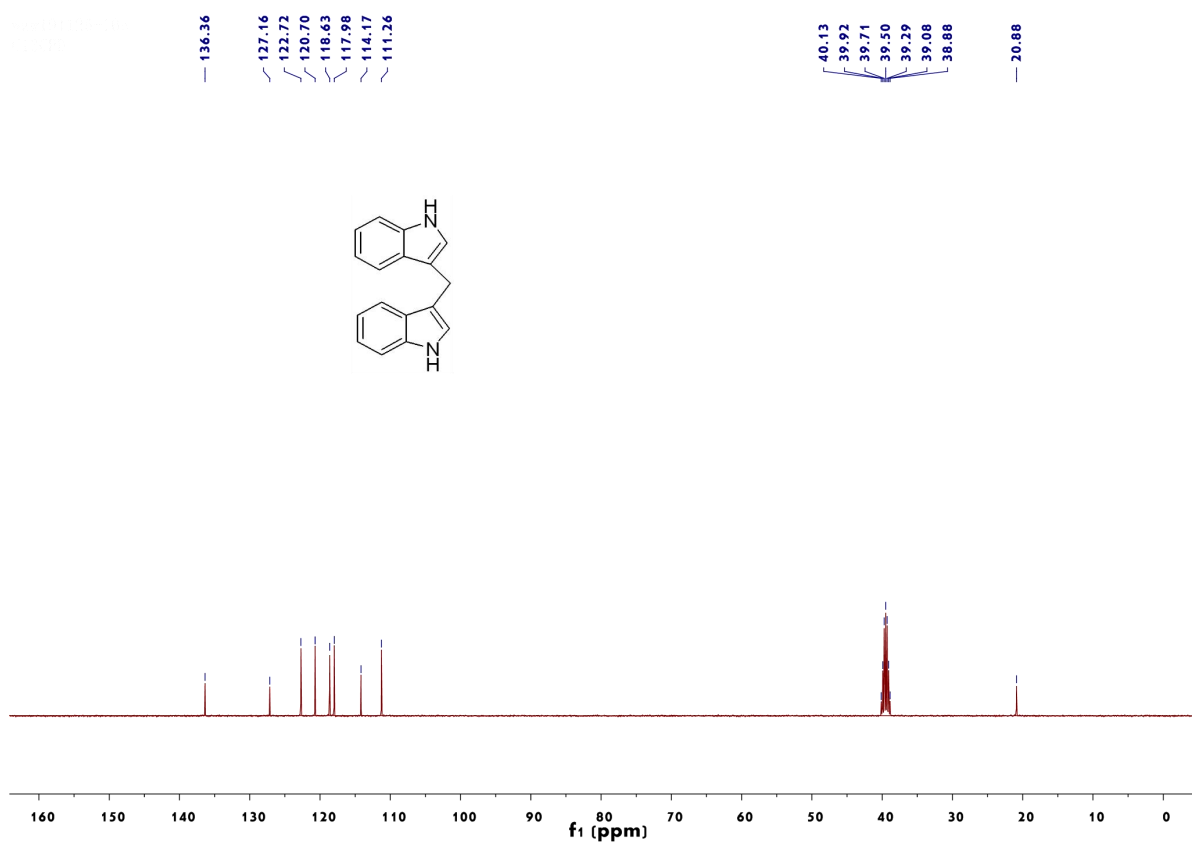
Reference:

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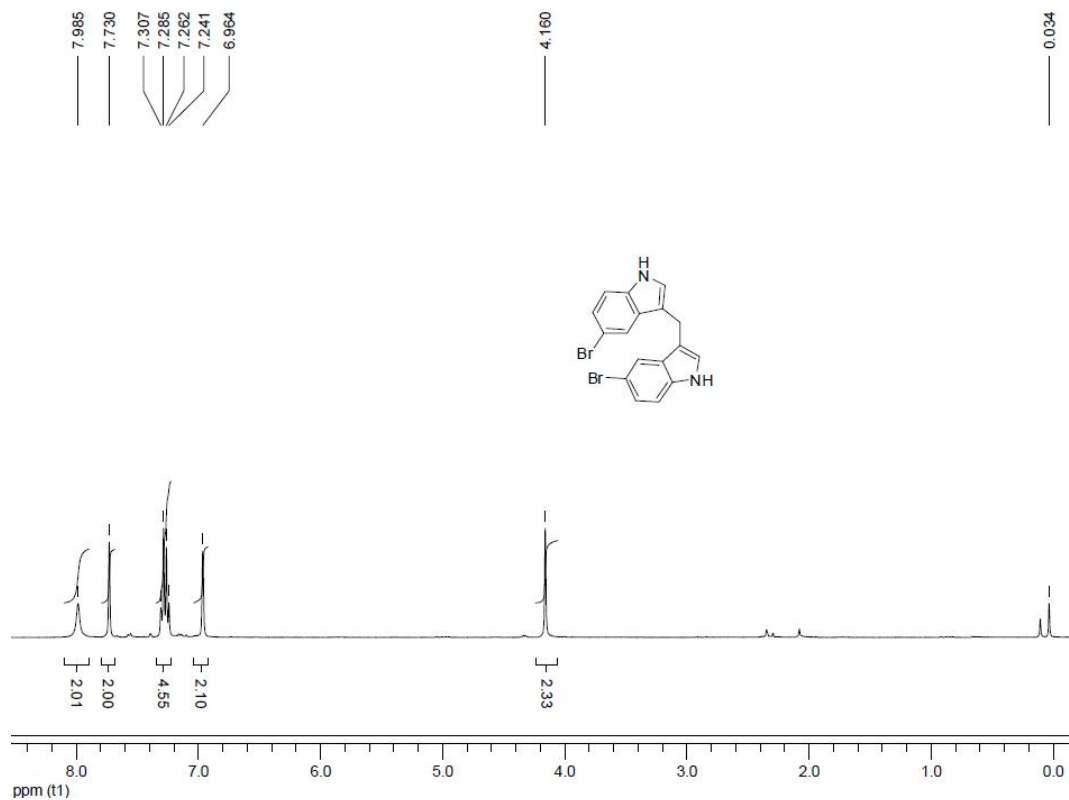
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6. Lv, P.; Chen, Y. L.; Zhao, Z.; Shi, T. Z.; Wu, X. W.; Li, Q. X.; Hua, R. M. Design, synthesis, and antifungal activities of 3-acyl thiotetronic acid derivatives: New fatty acid synthase inhibitors. *J. Agric. Food Chem.* **2018**, *66*, 1023–1032.
7. Leberman, R. Isolation of plant viruses by means of simple coacervates. *Virology* **1966**, *30*, 341–347.
8. Fraenkel Conrat, H.; Williams, R. C. Reconstitution of active tobacco mosaic virus from its inactive protein and nucleic acid components. *Proc Natl Acad Sci U S A* **1955**, *41*, 690–698.



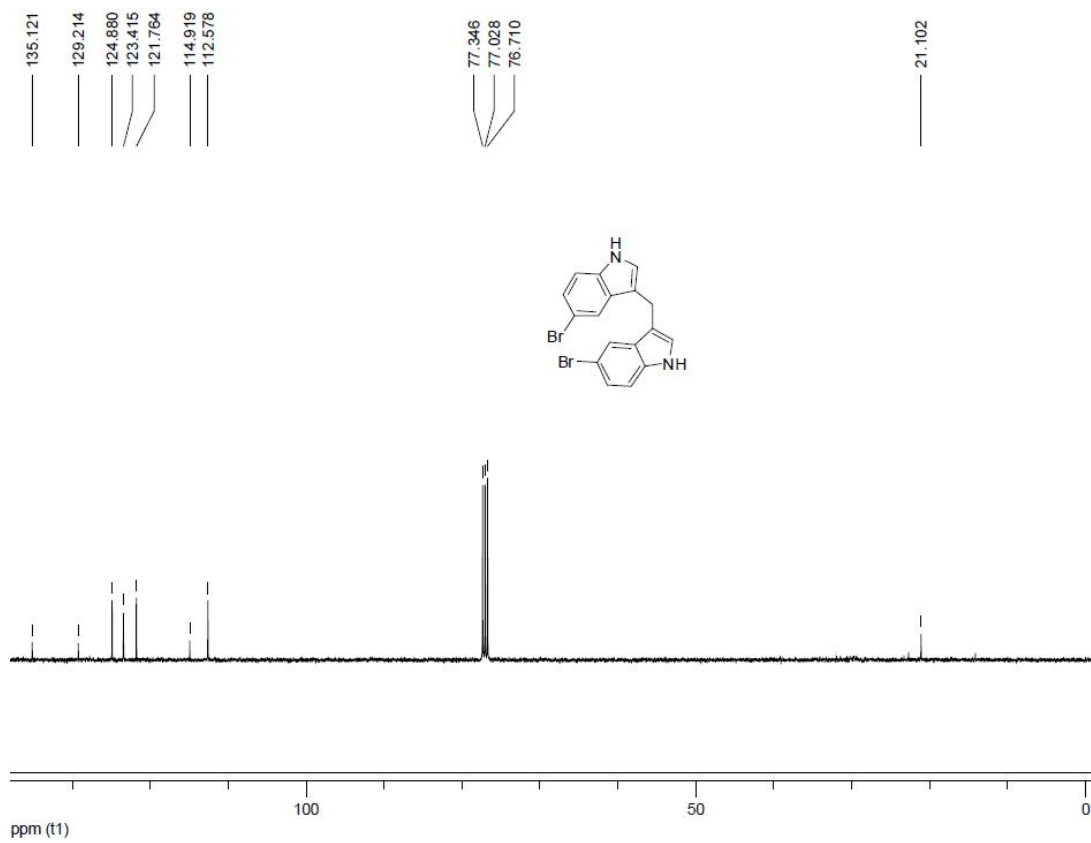
¹H NMR spectrum of 1



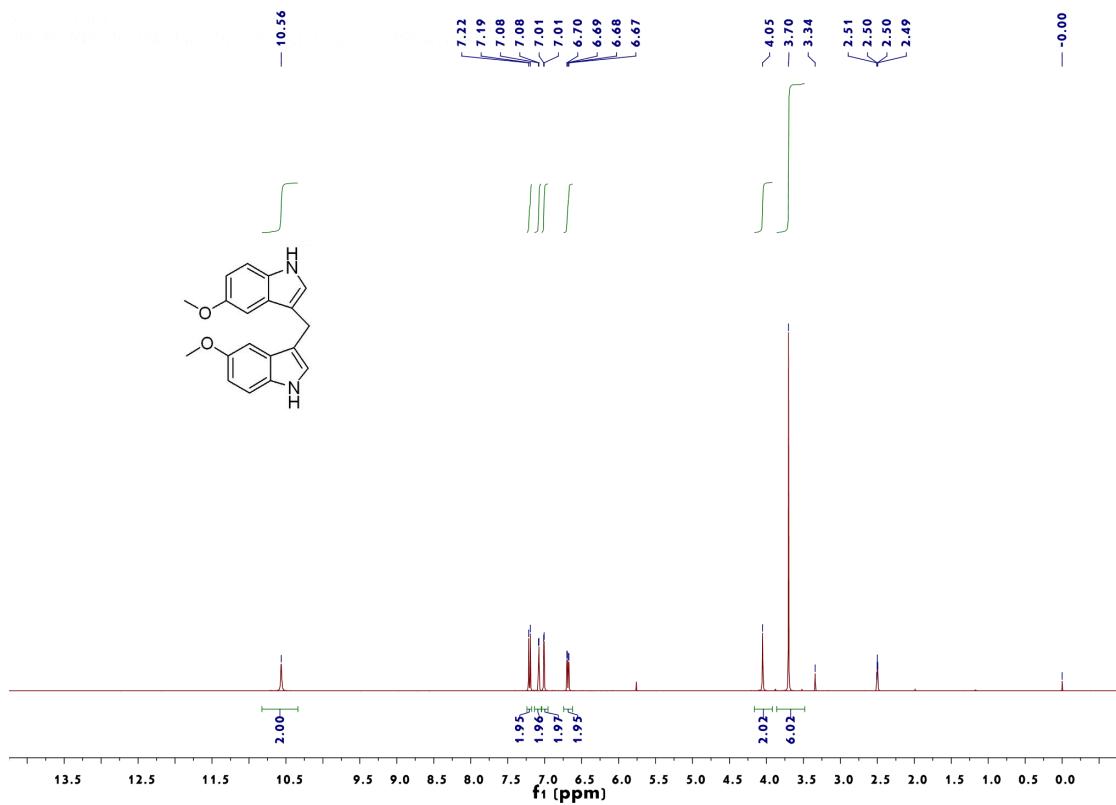
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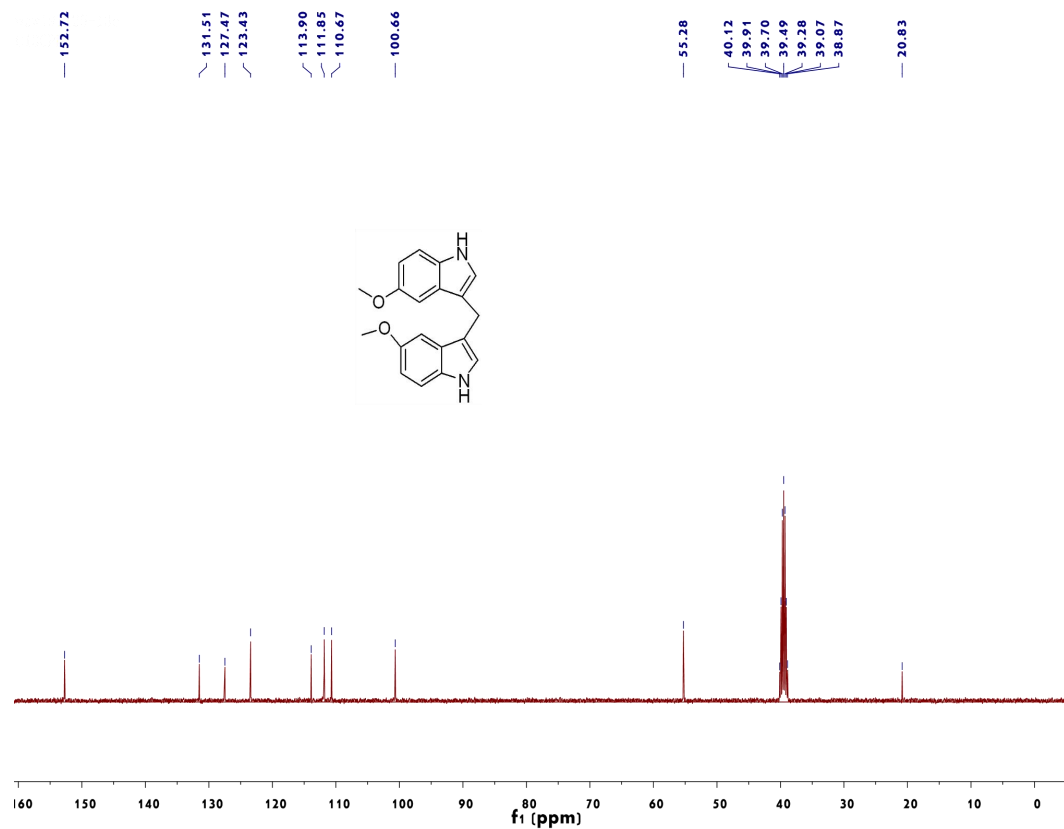
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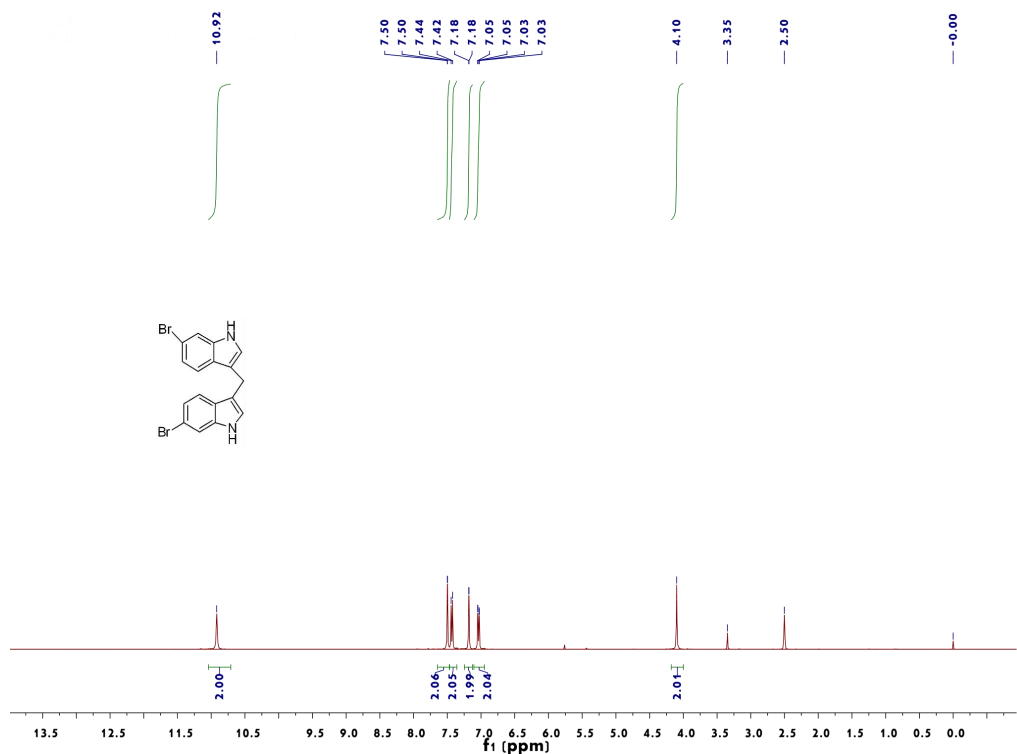
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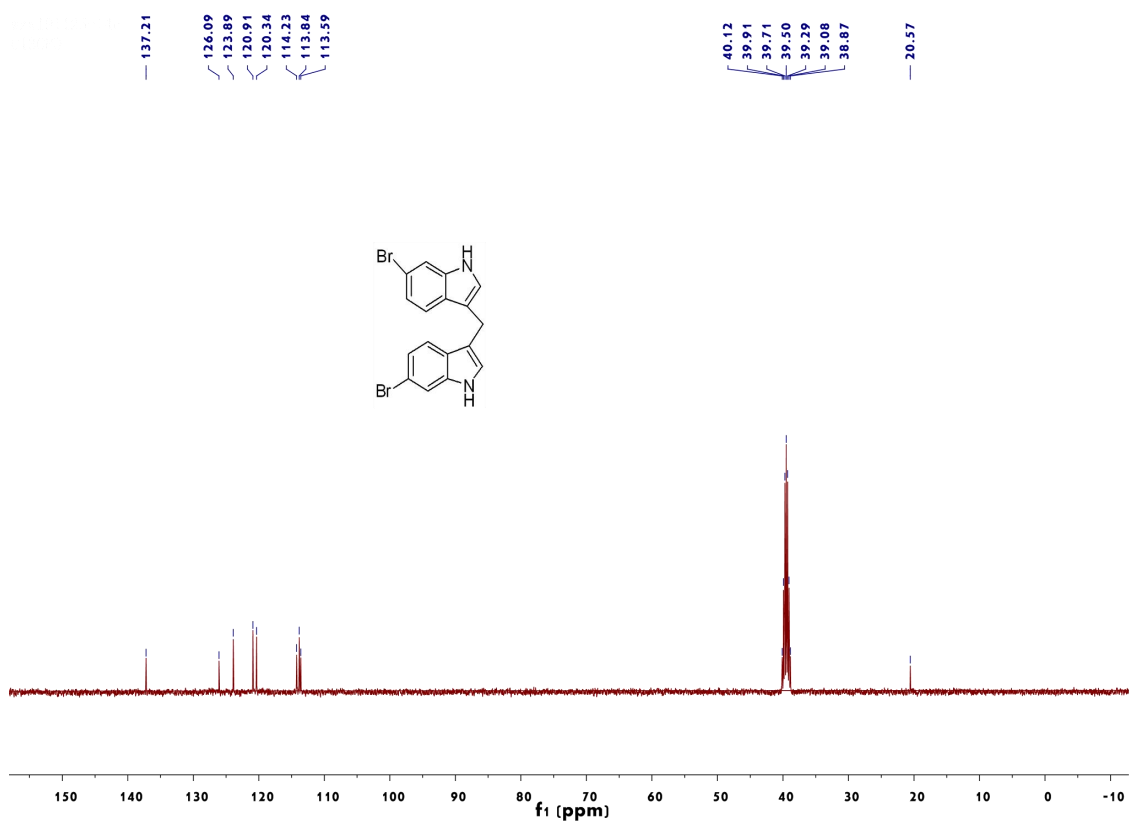
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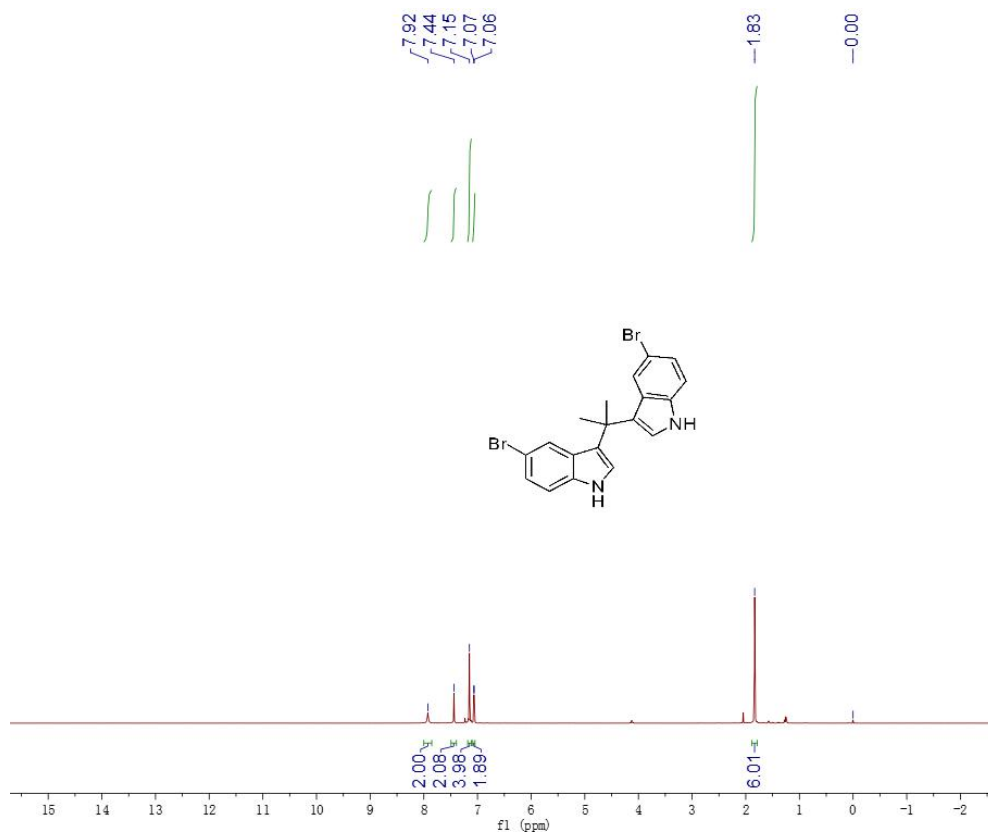
^{13}C NMR spectrum of **3**



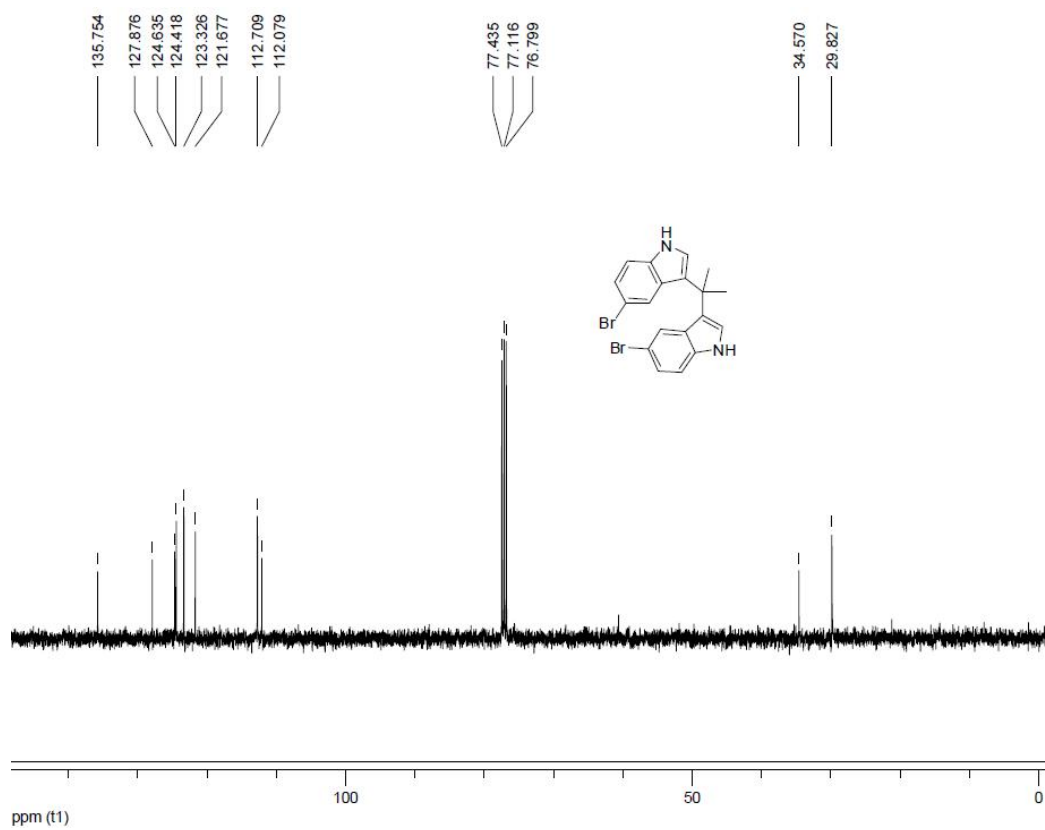
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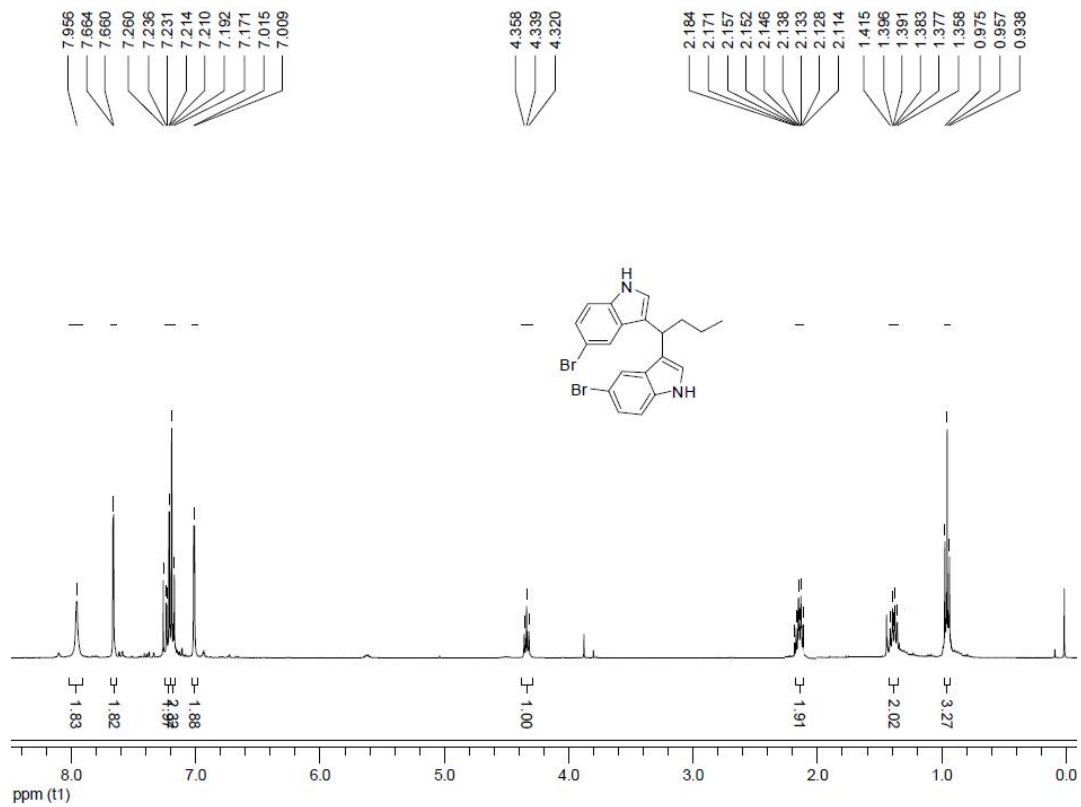
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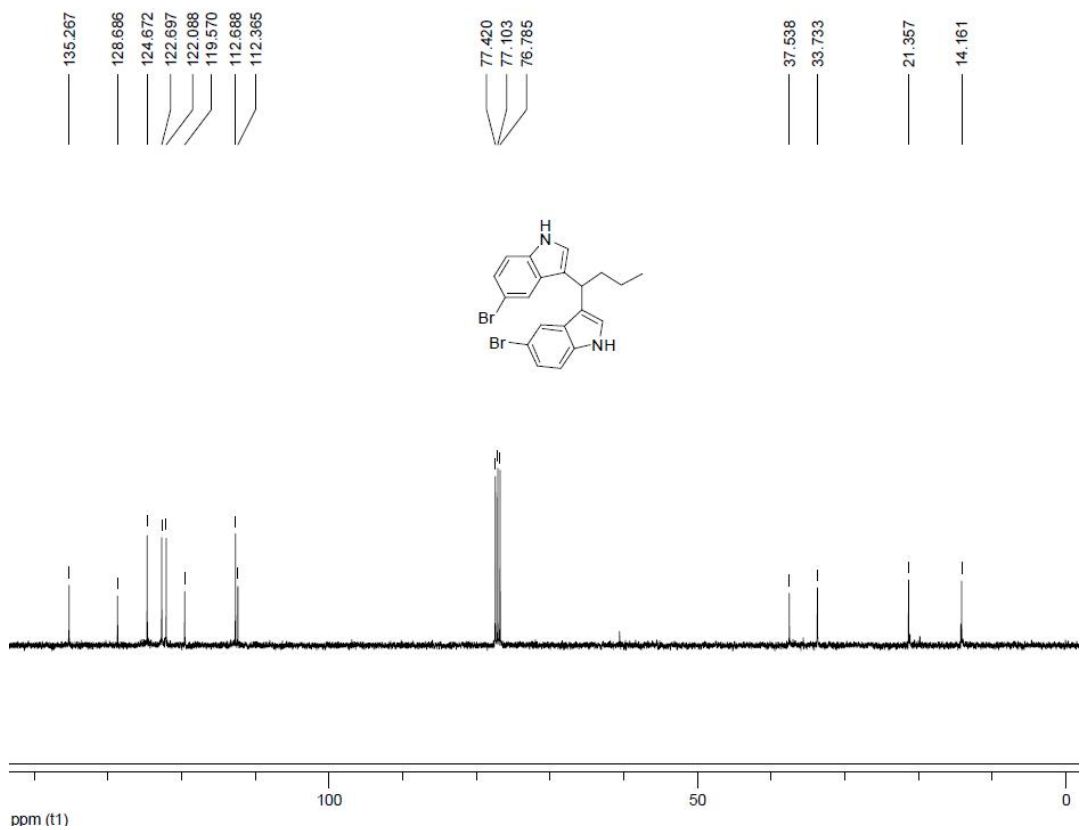
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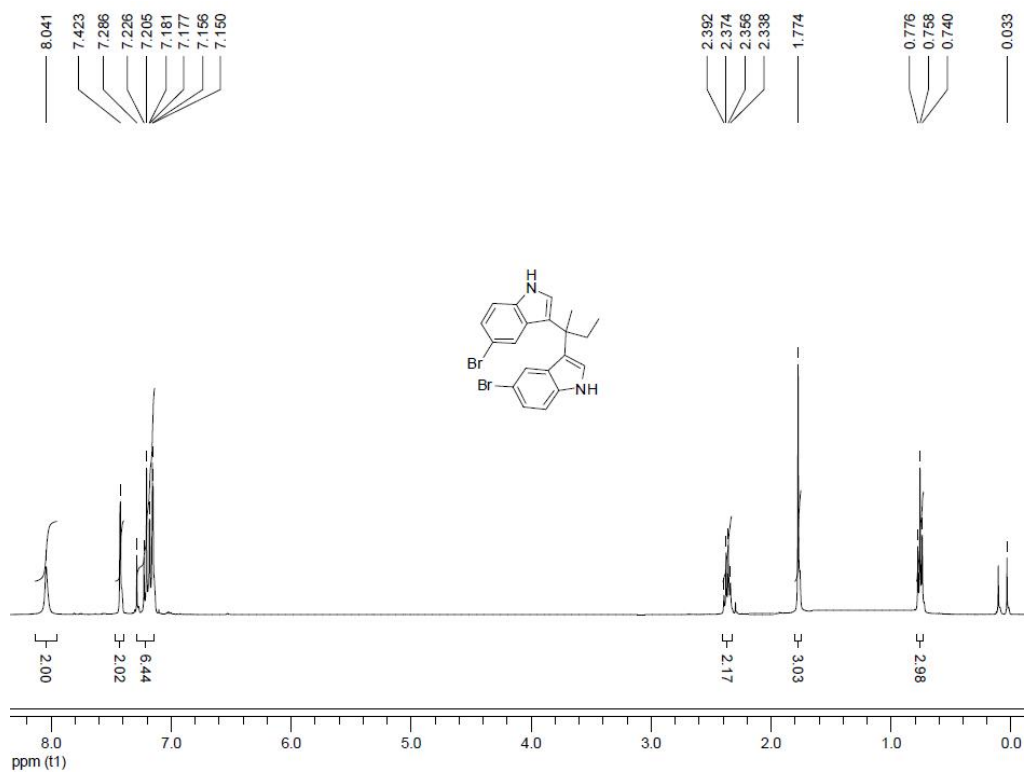
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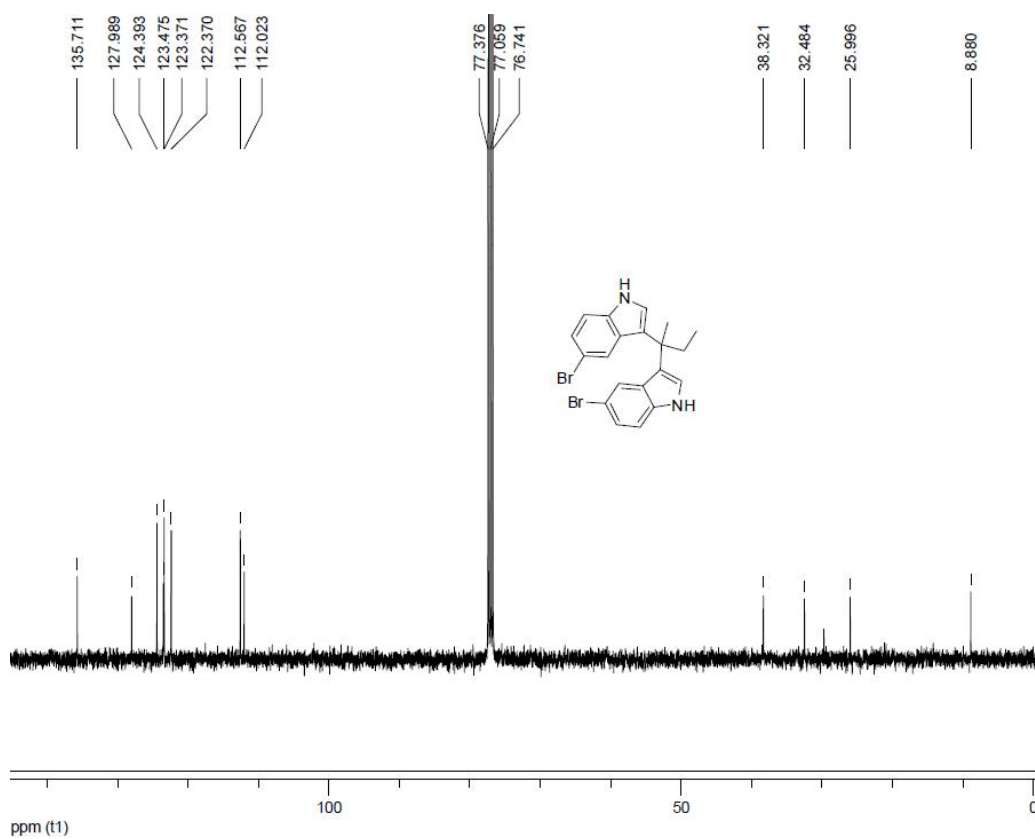
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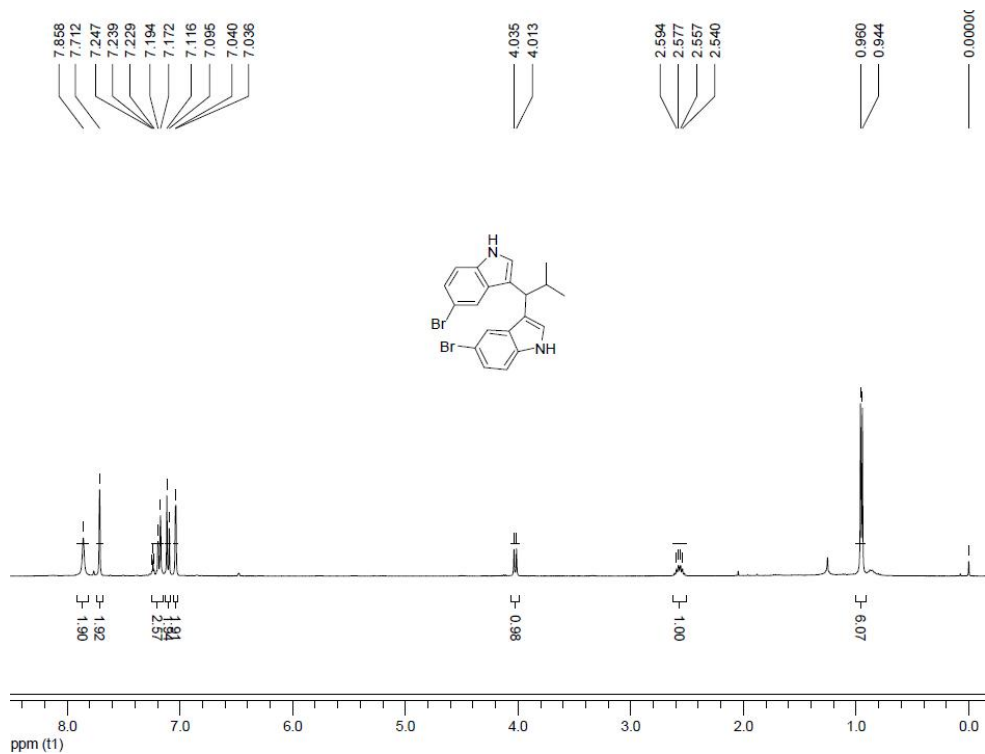
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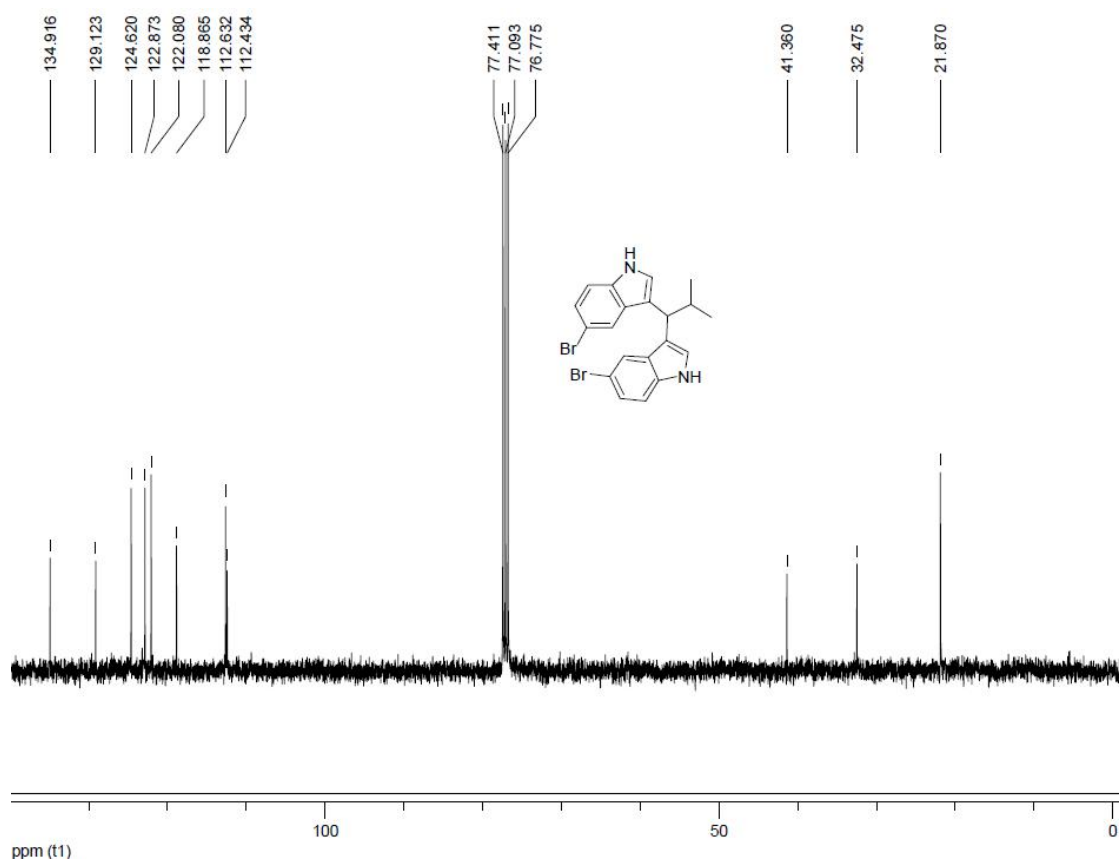
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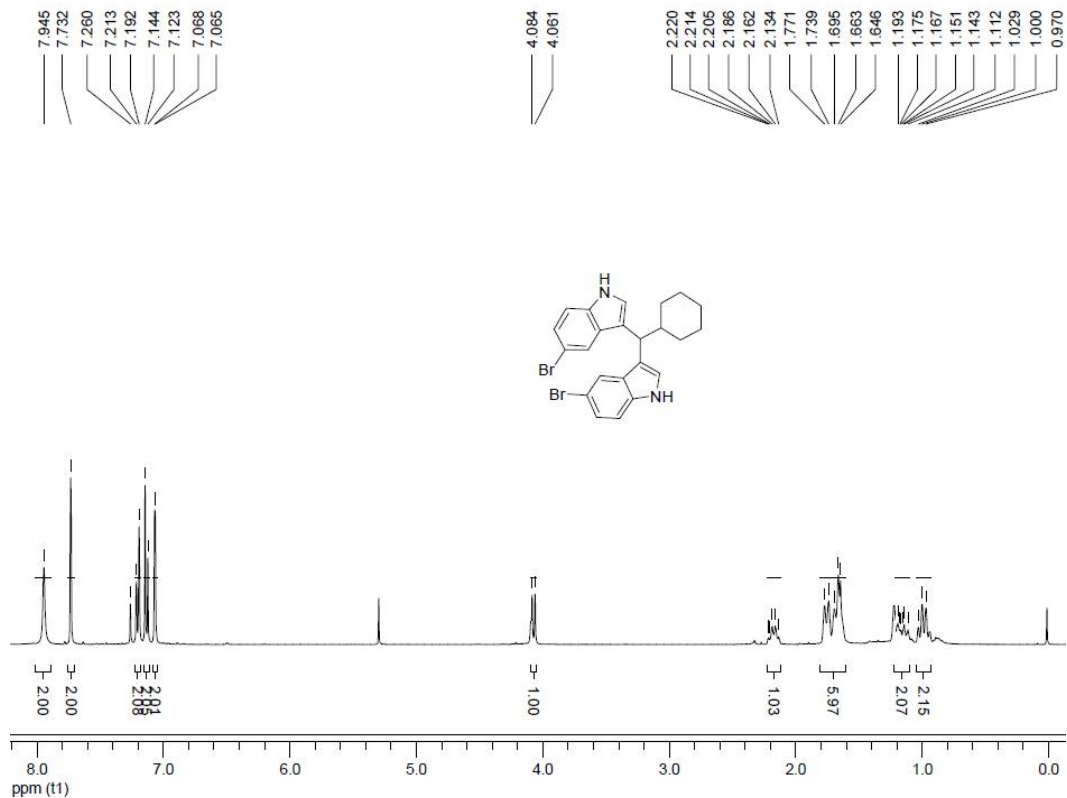
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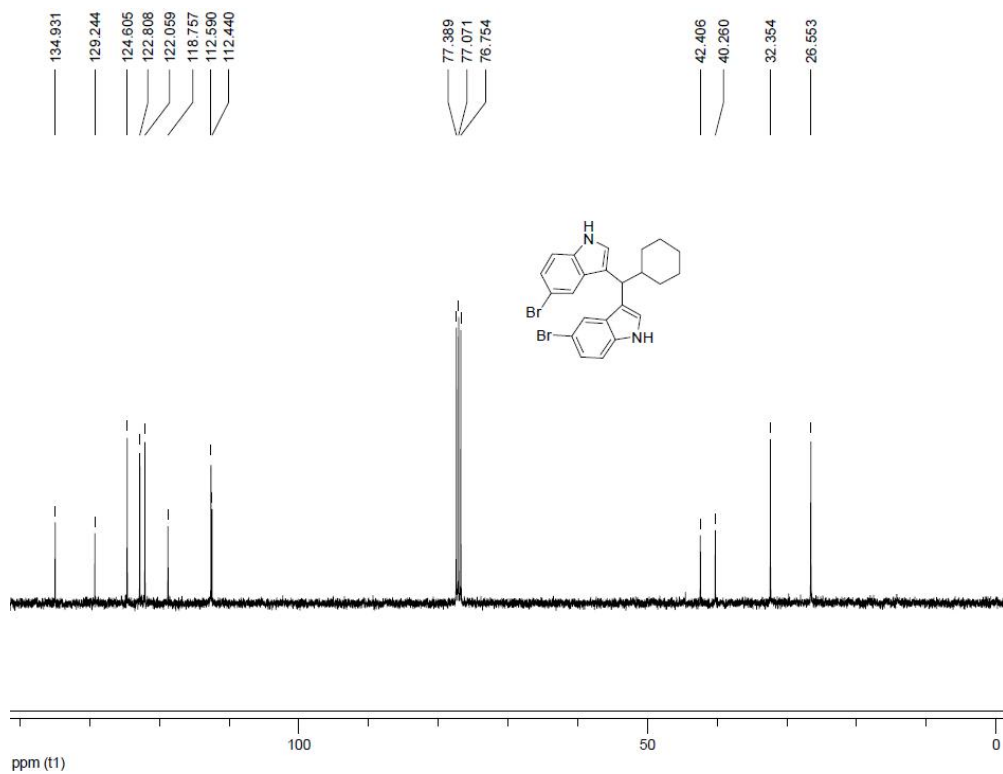
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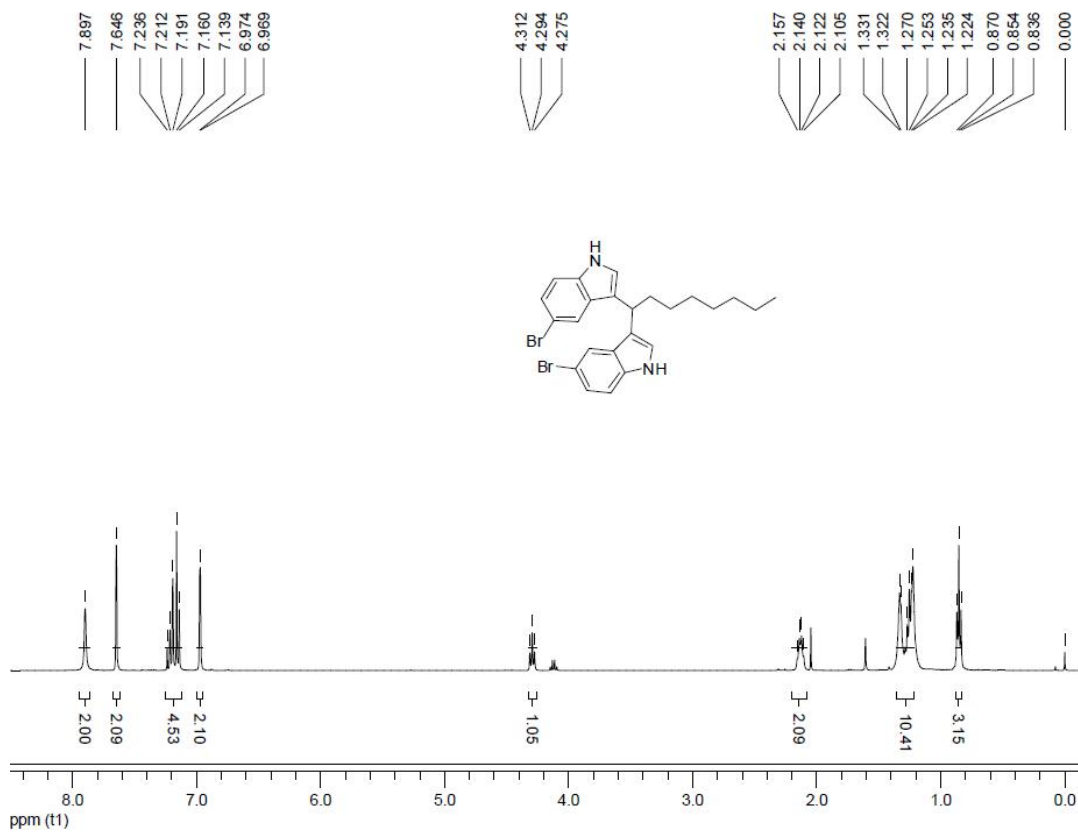
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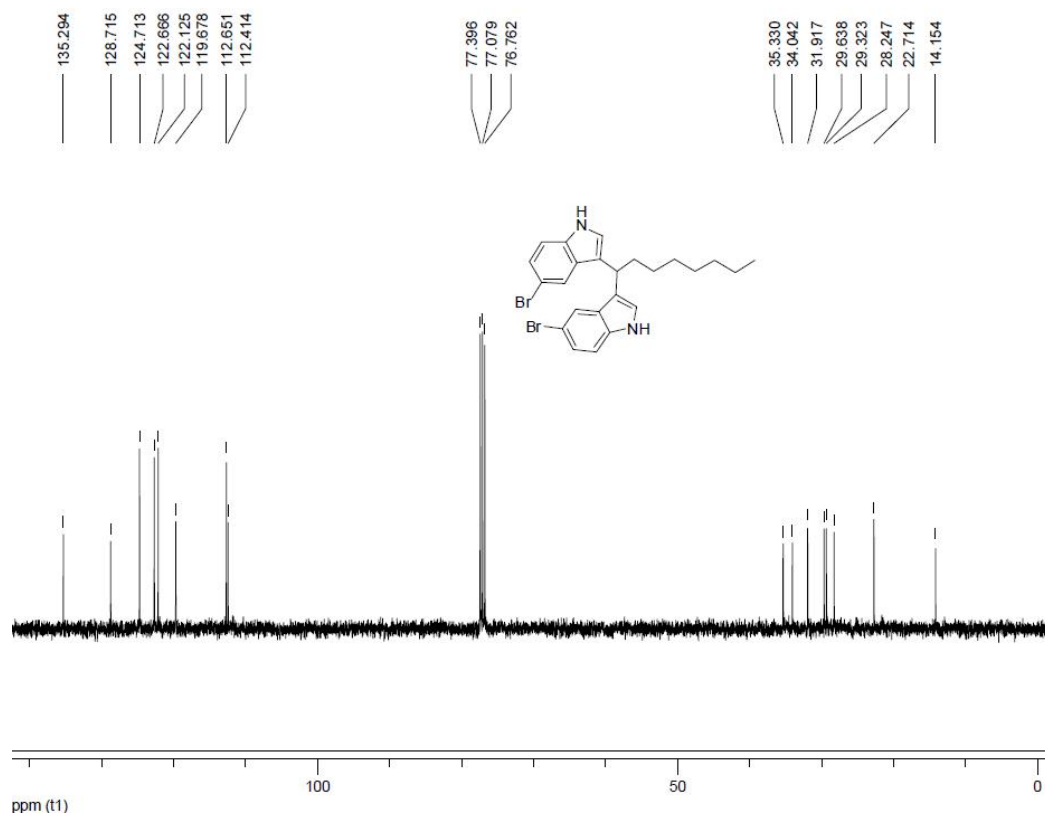
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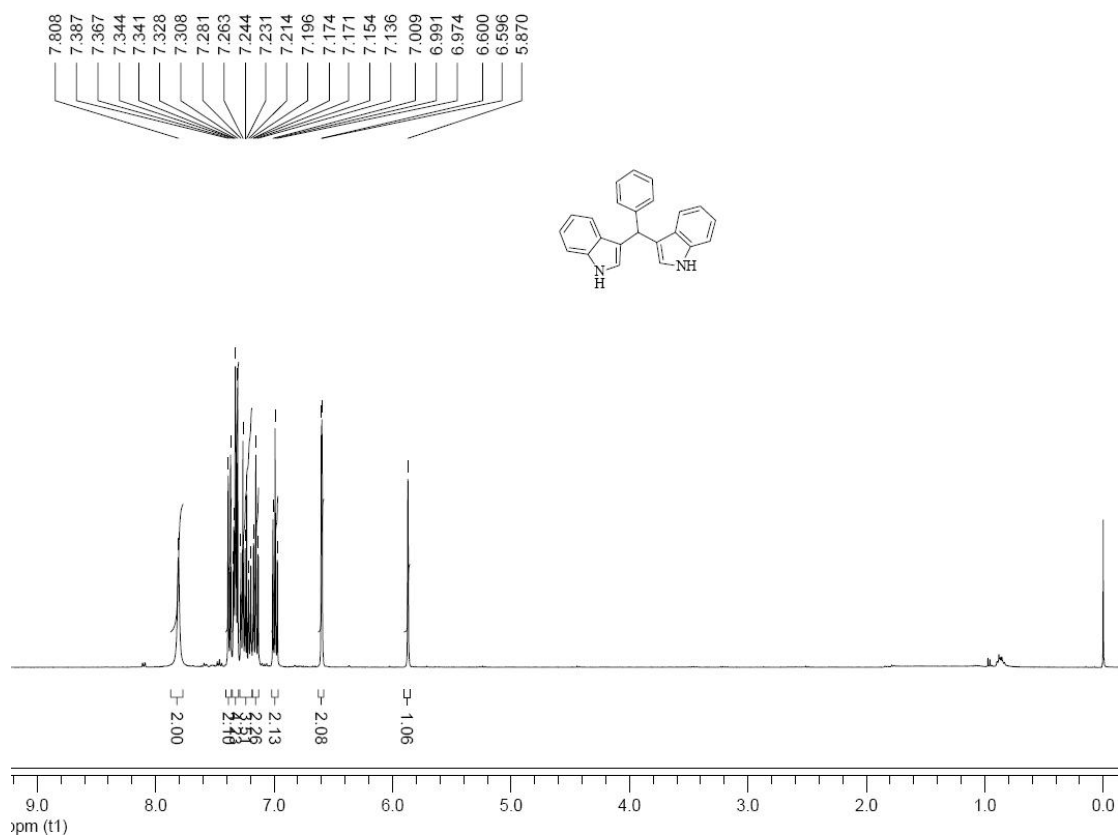
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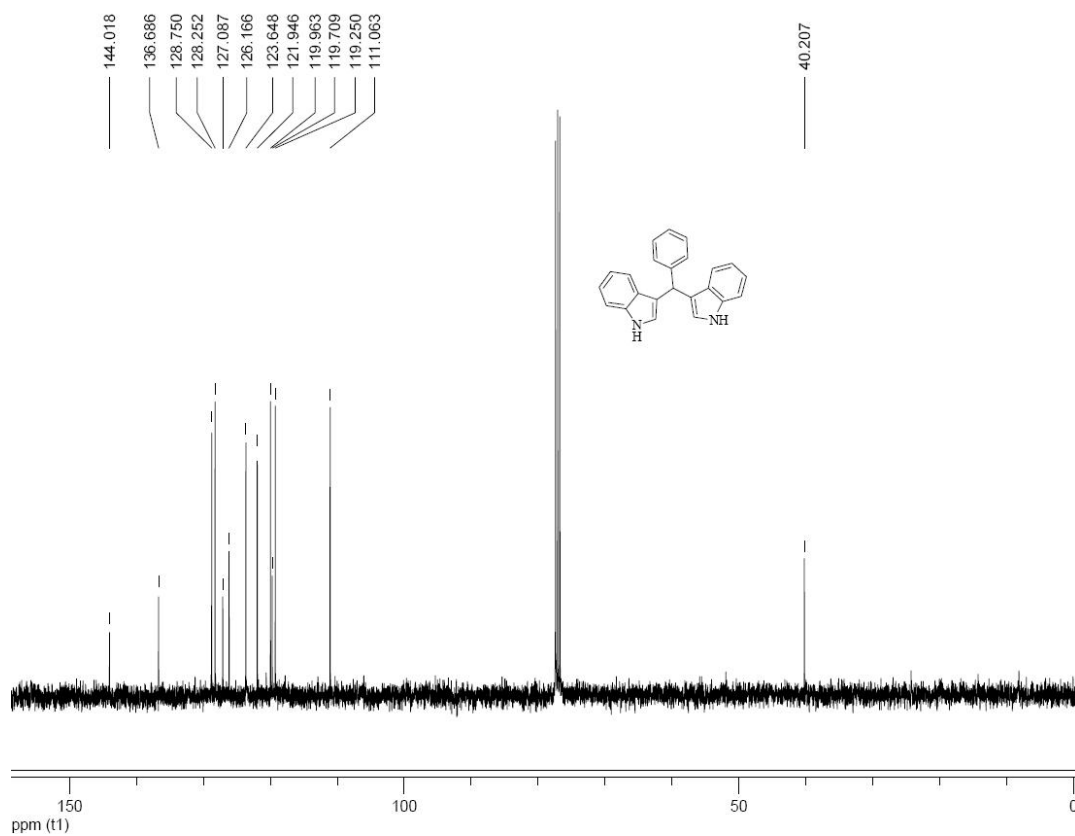
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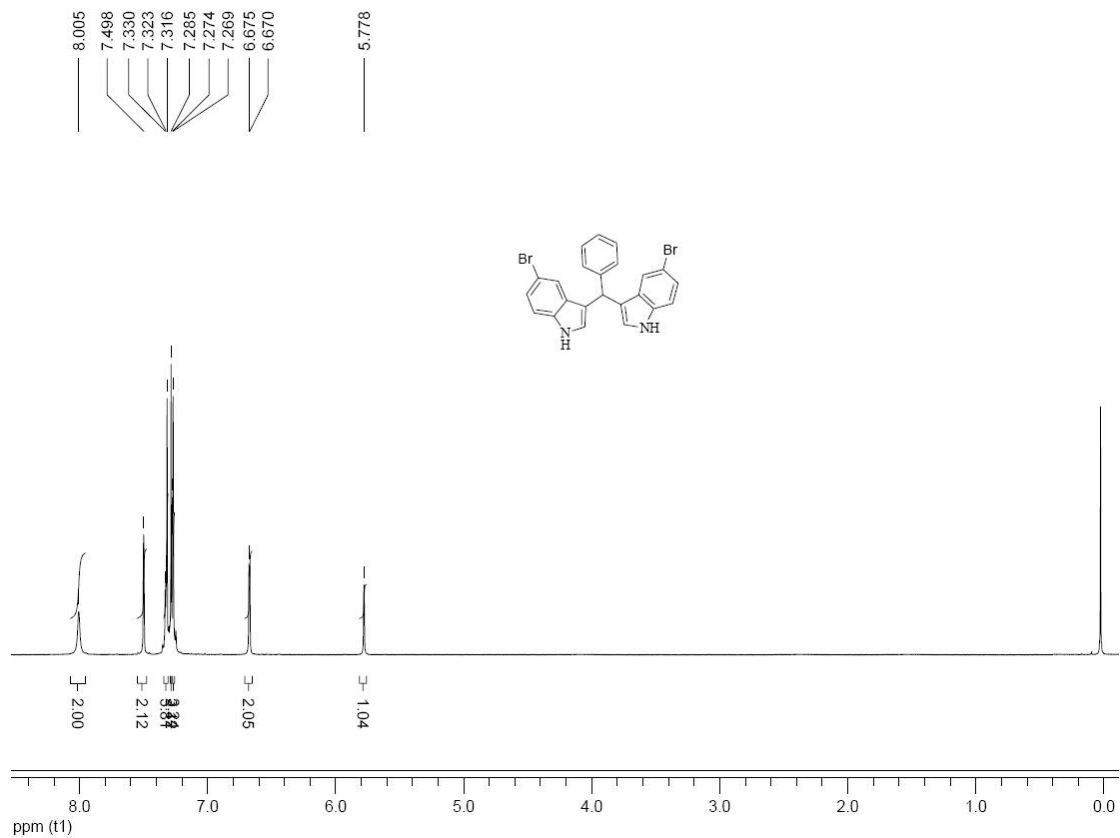
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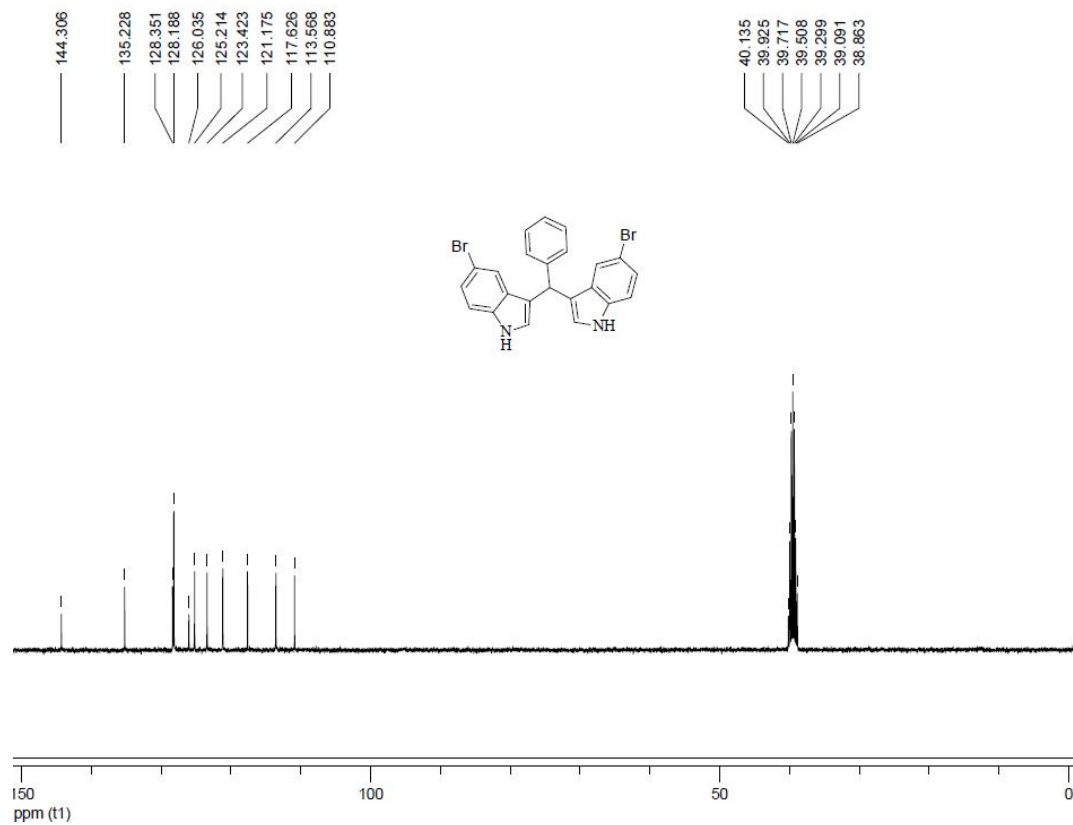
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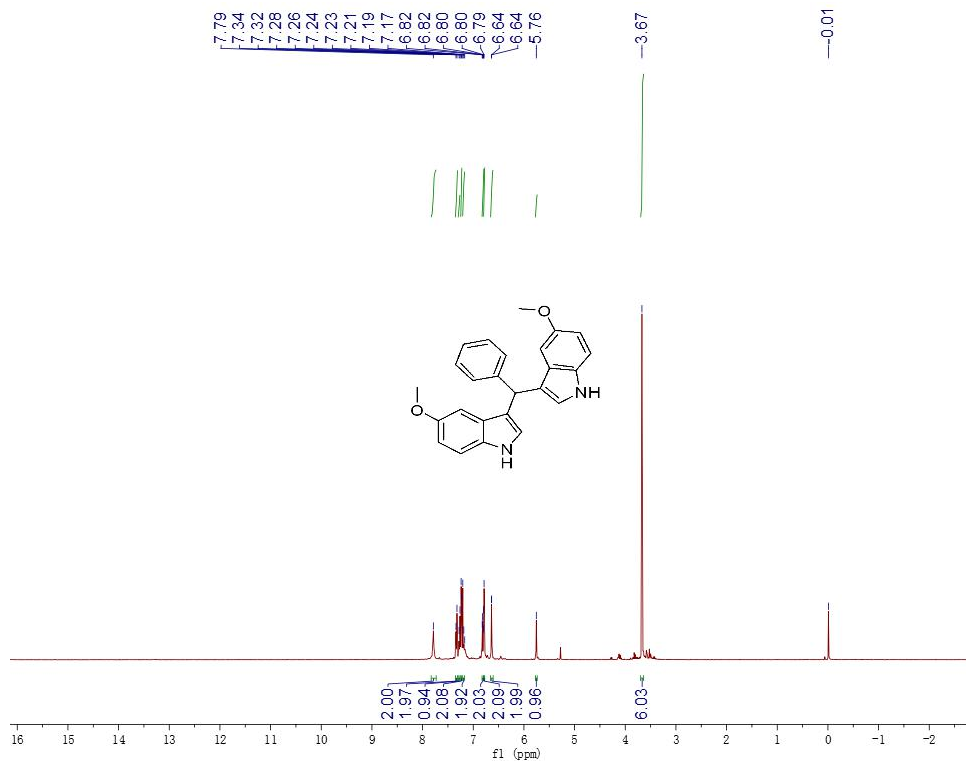
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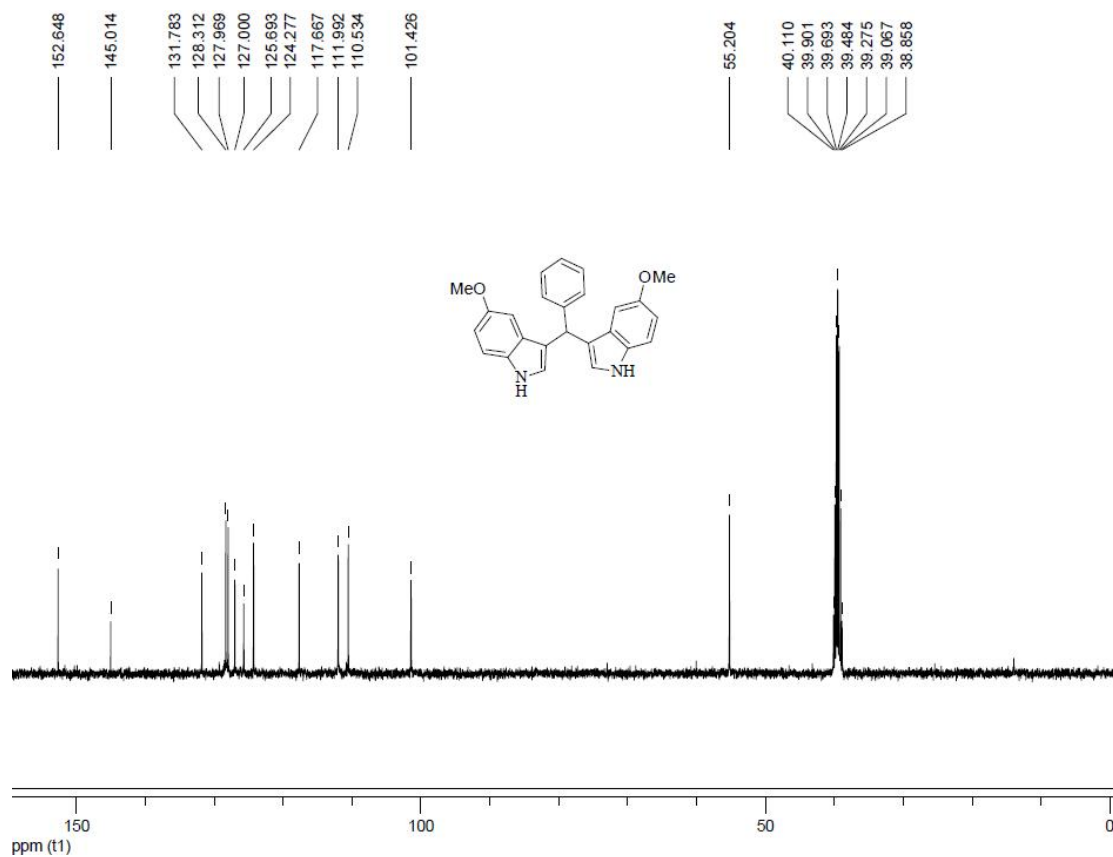
^1H NMR spectrum of **12**



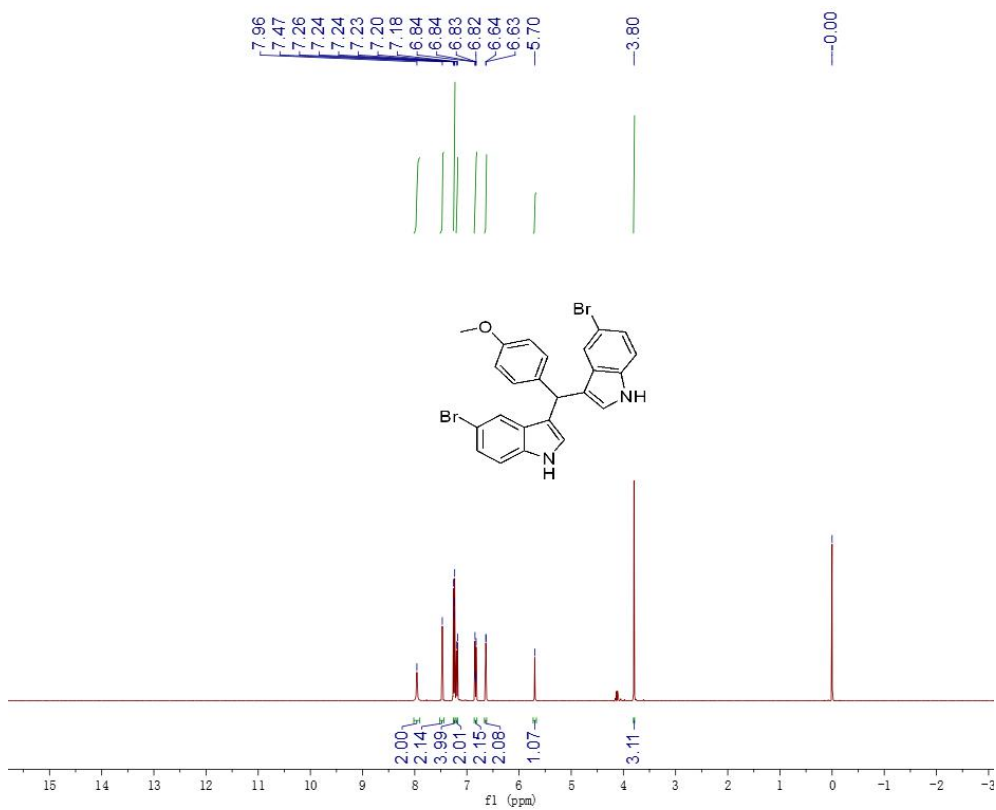
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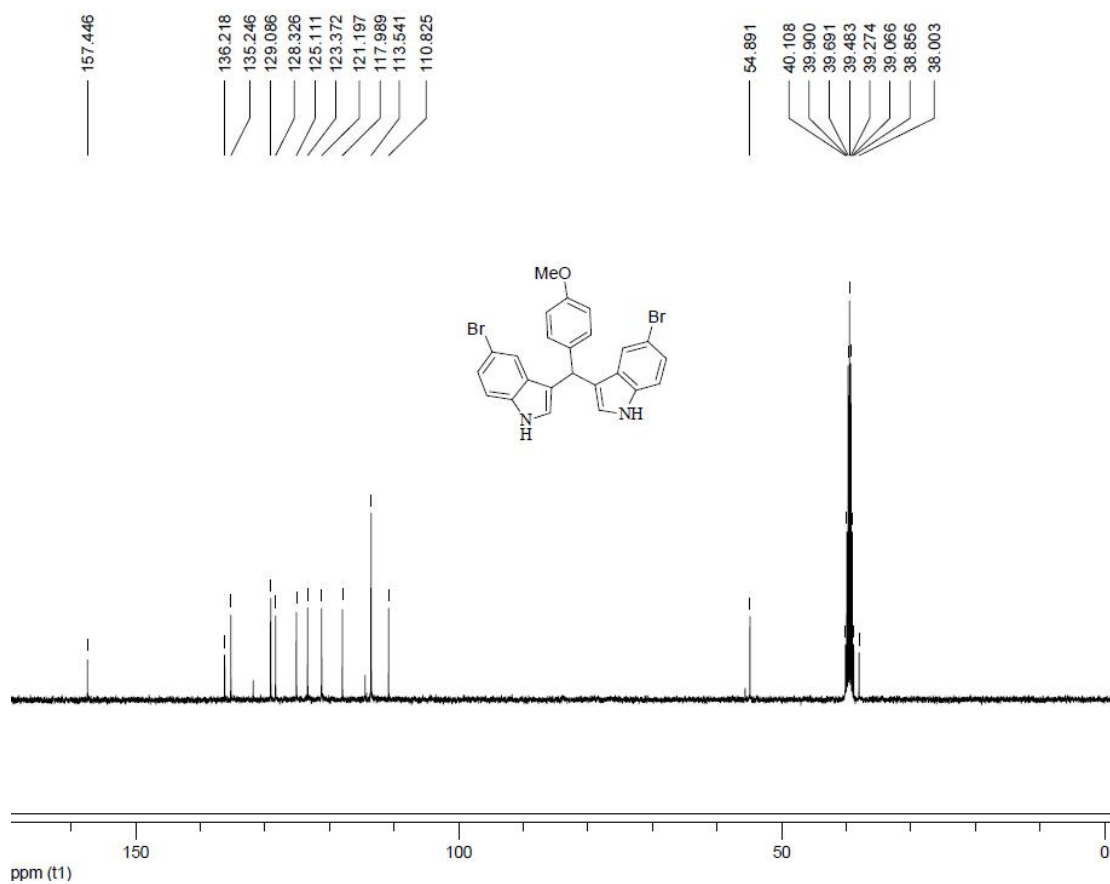
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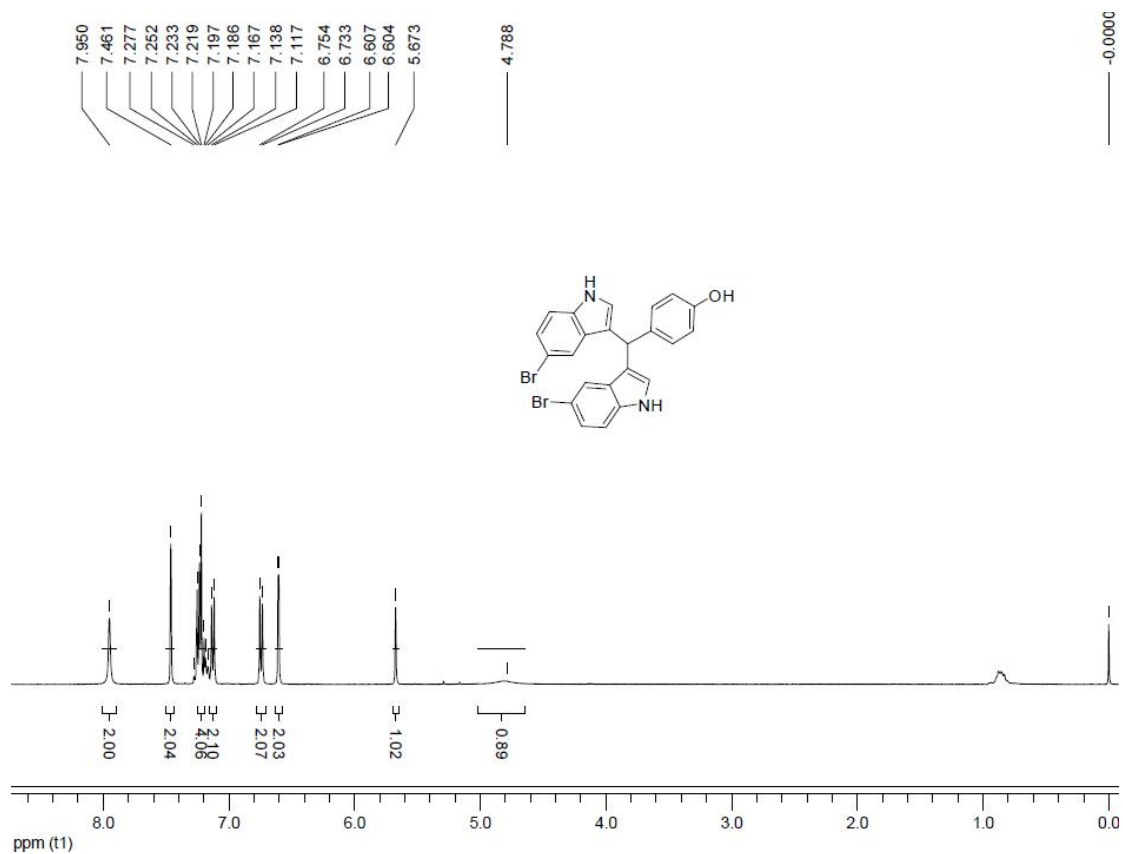
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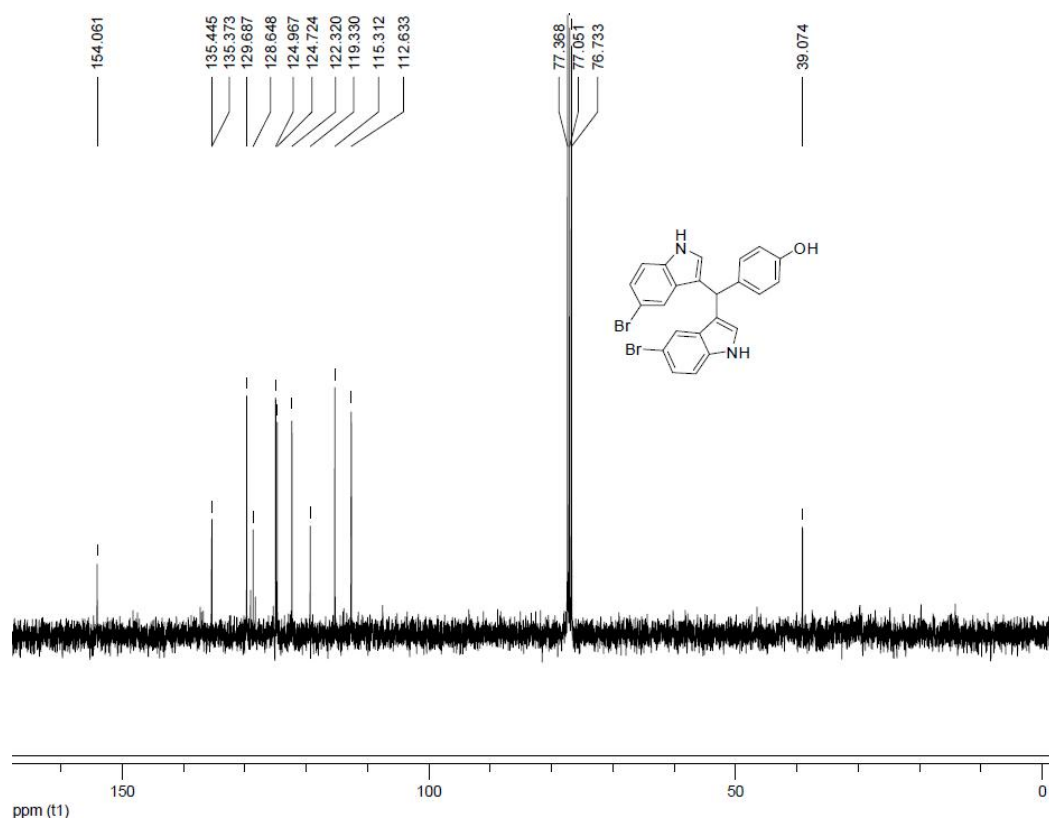
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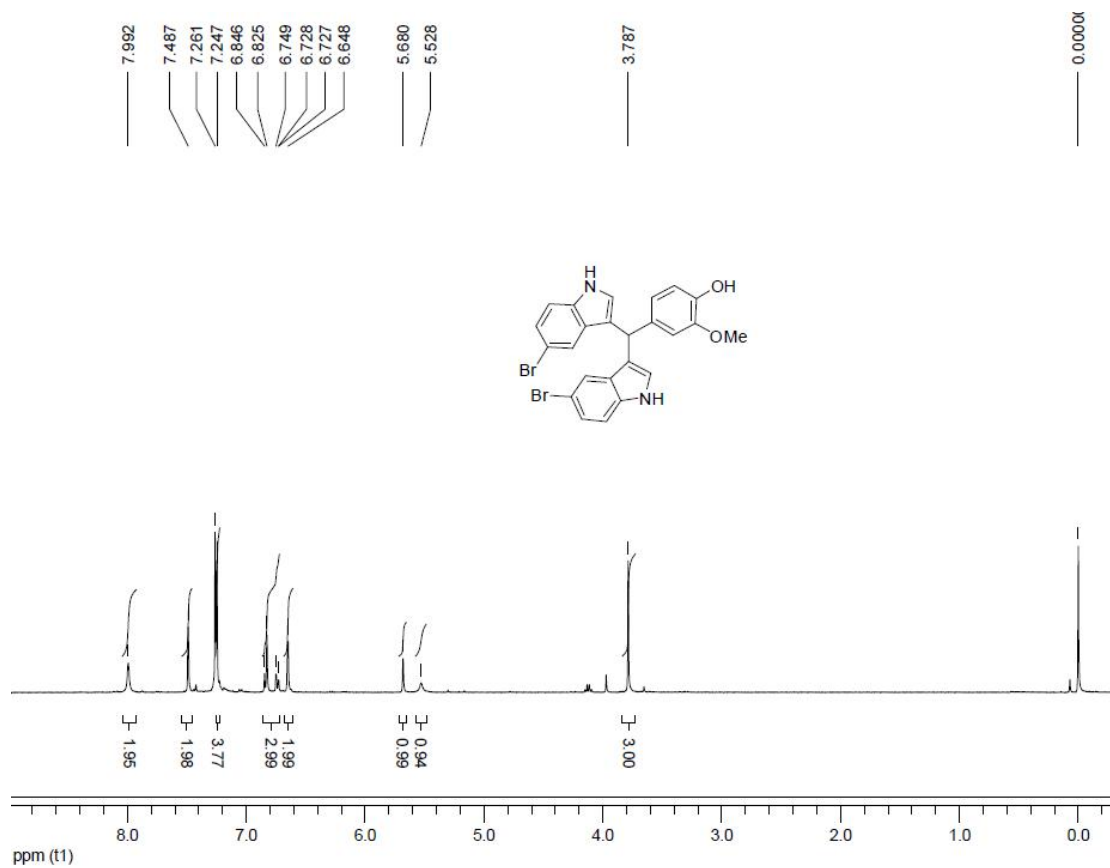
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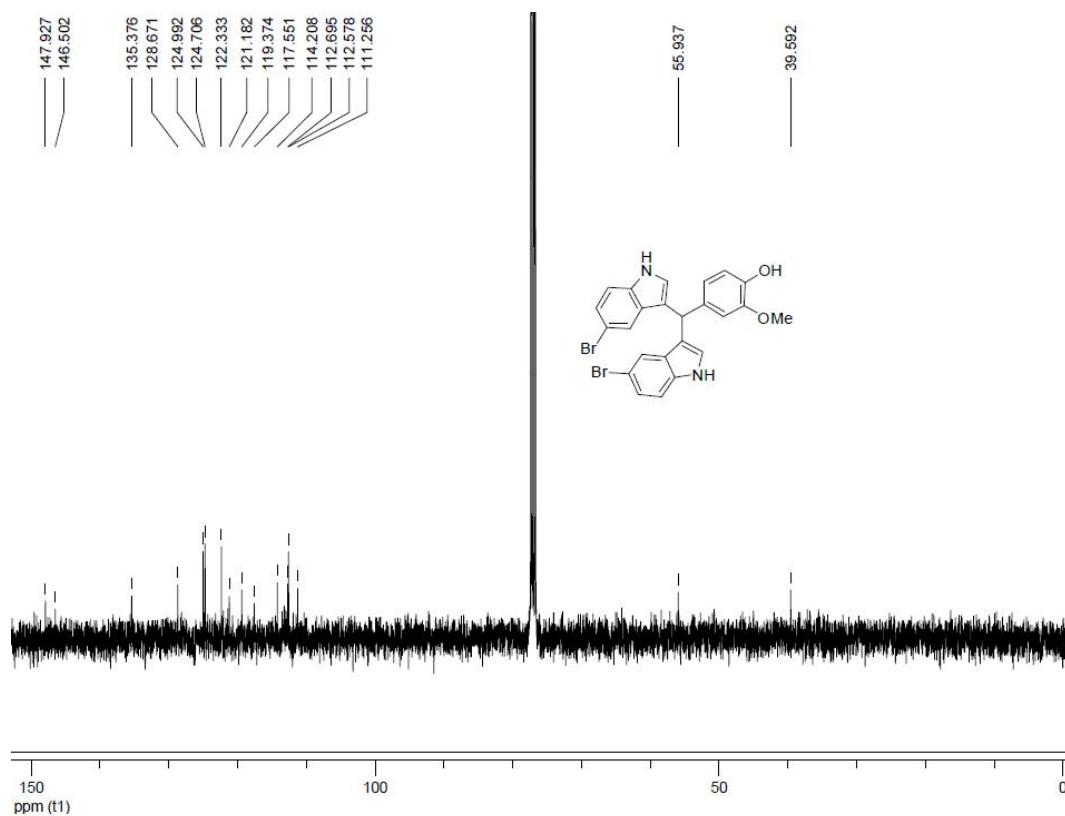
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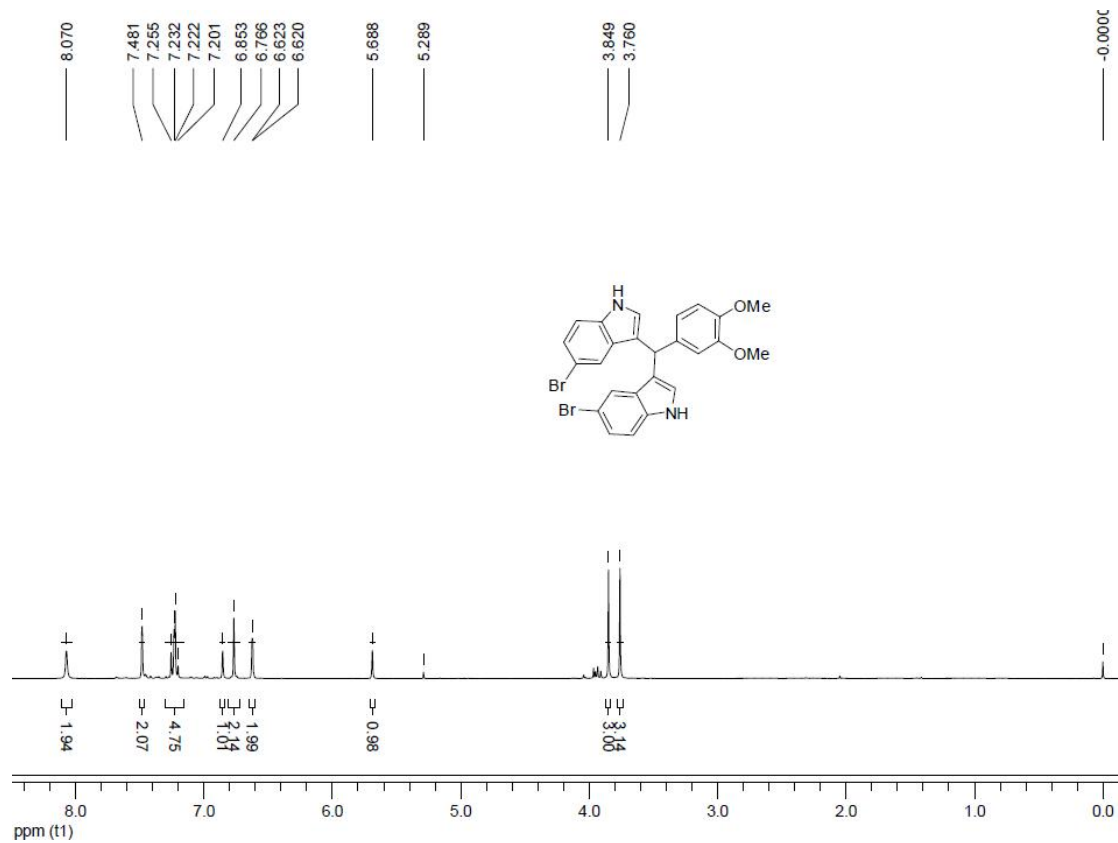
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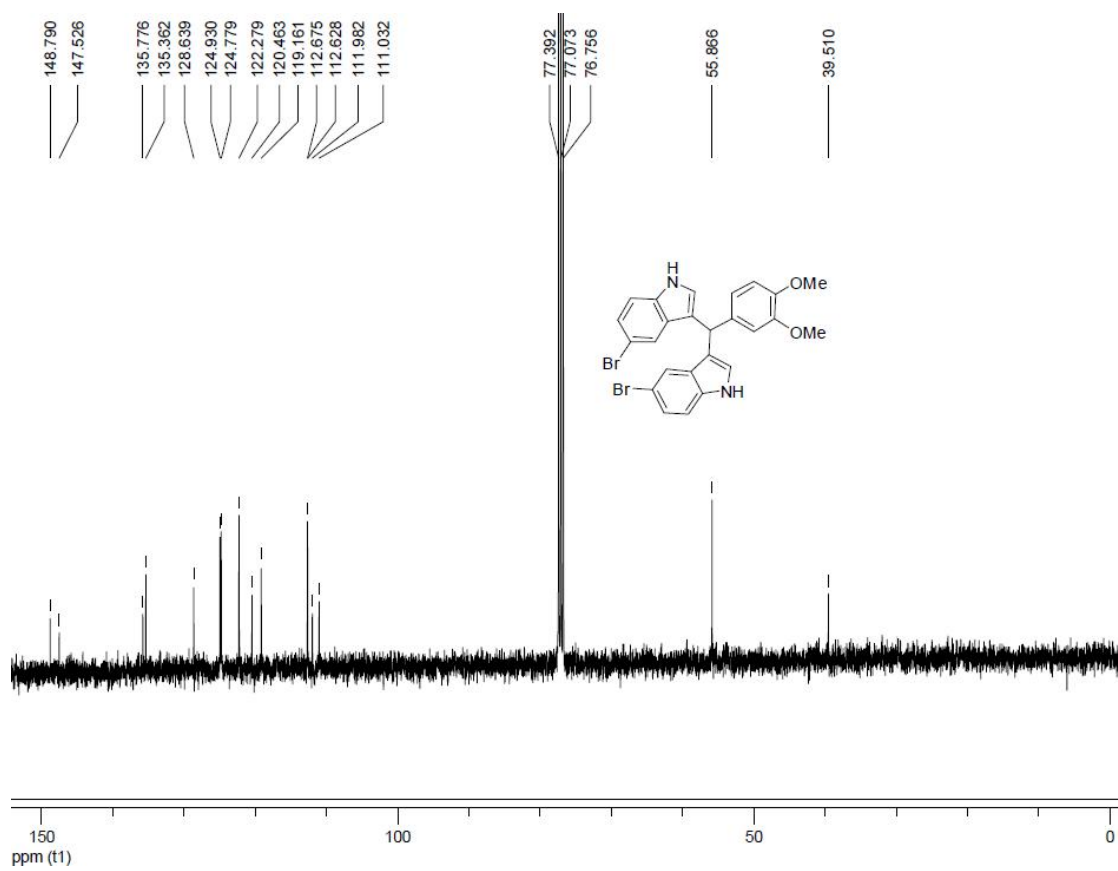
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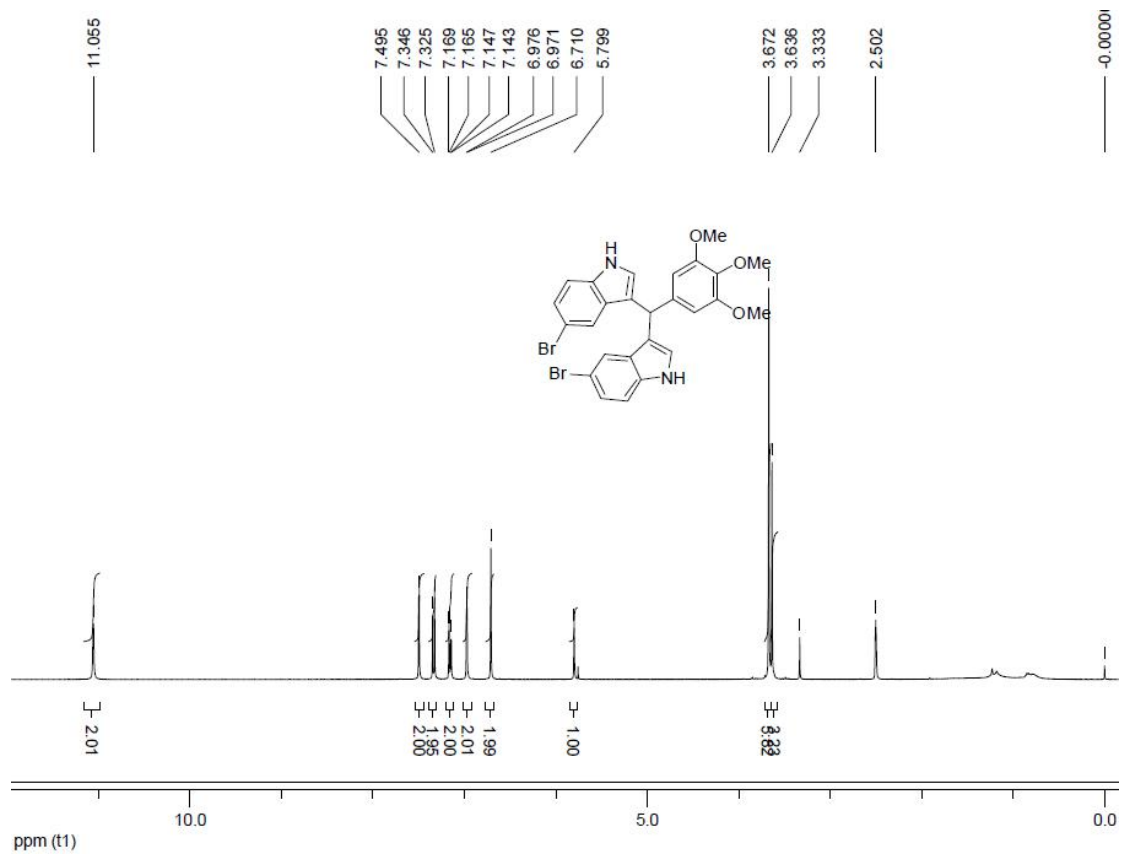
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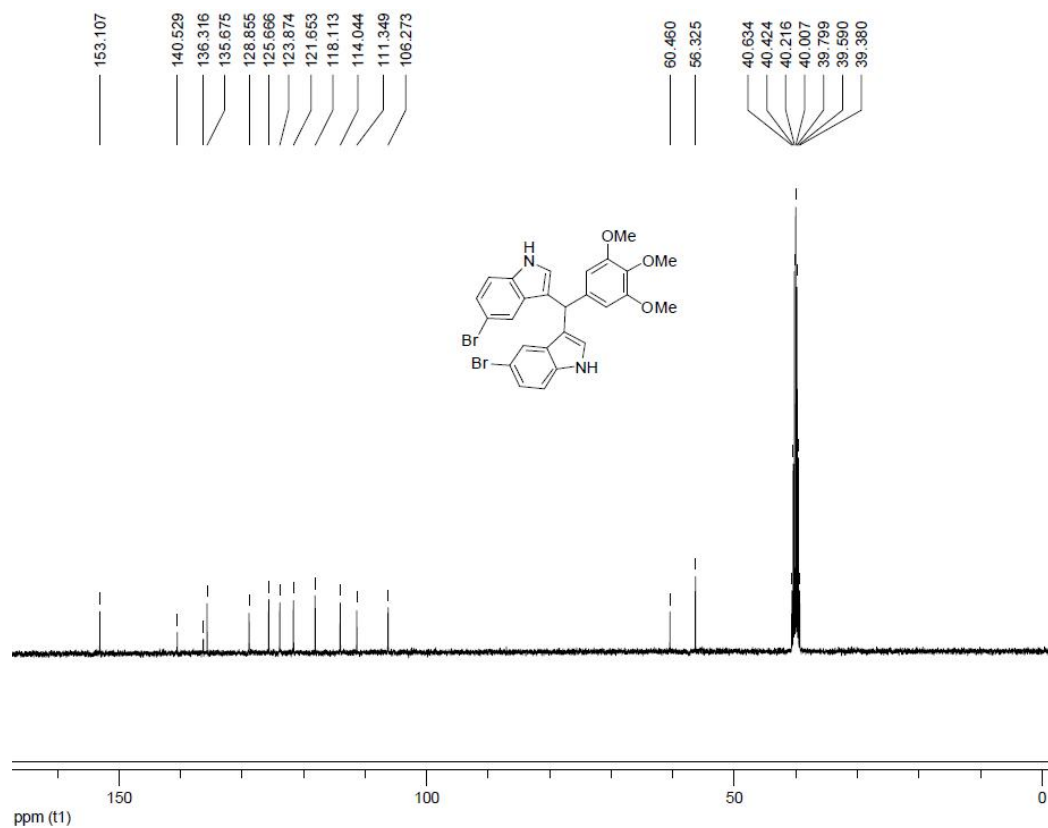
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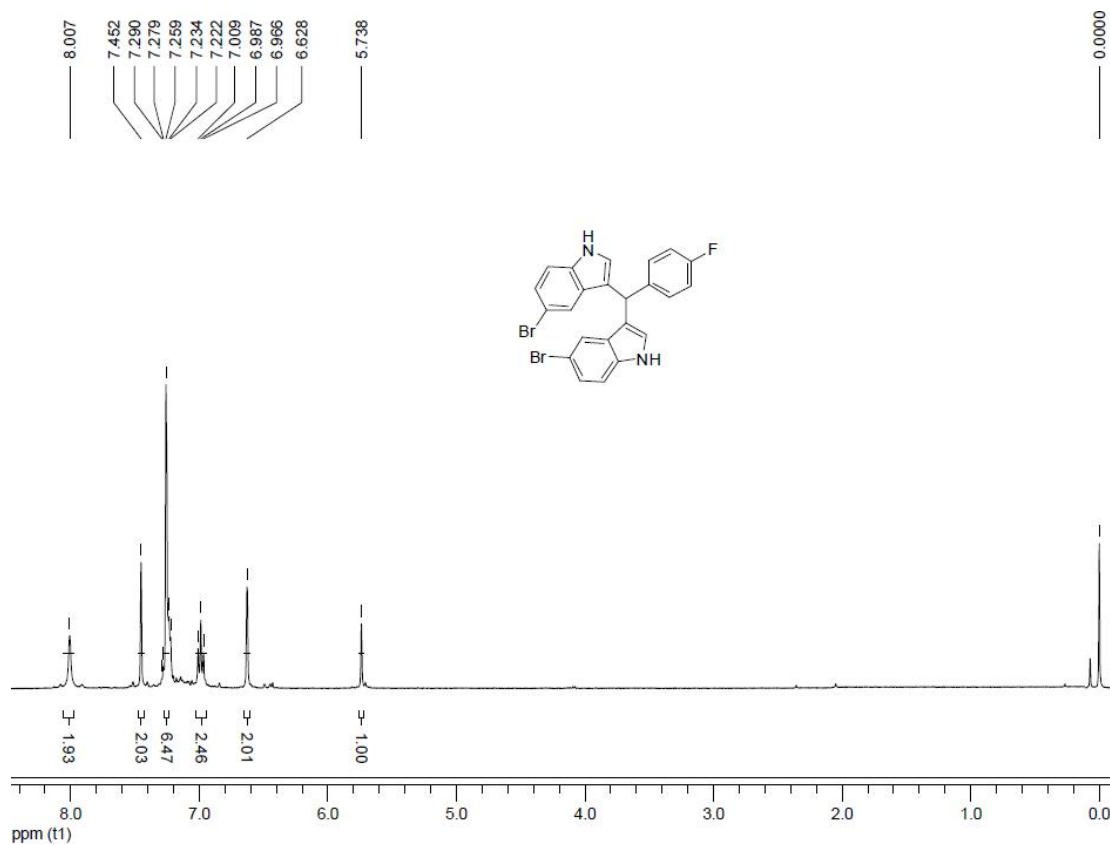
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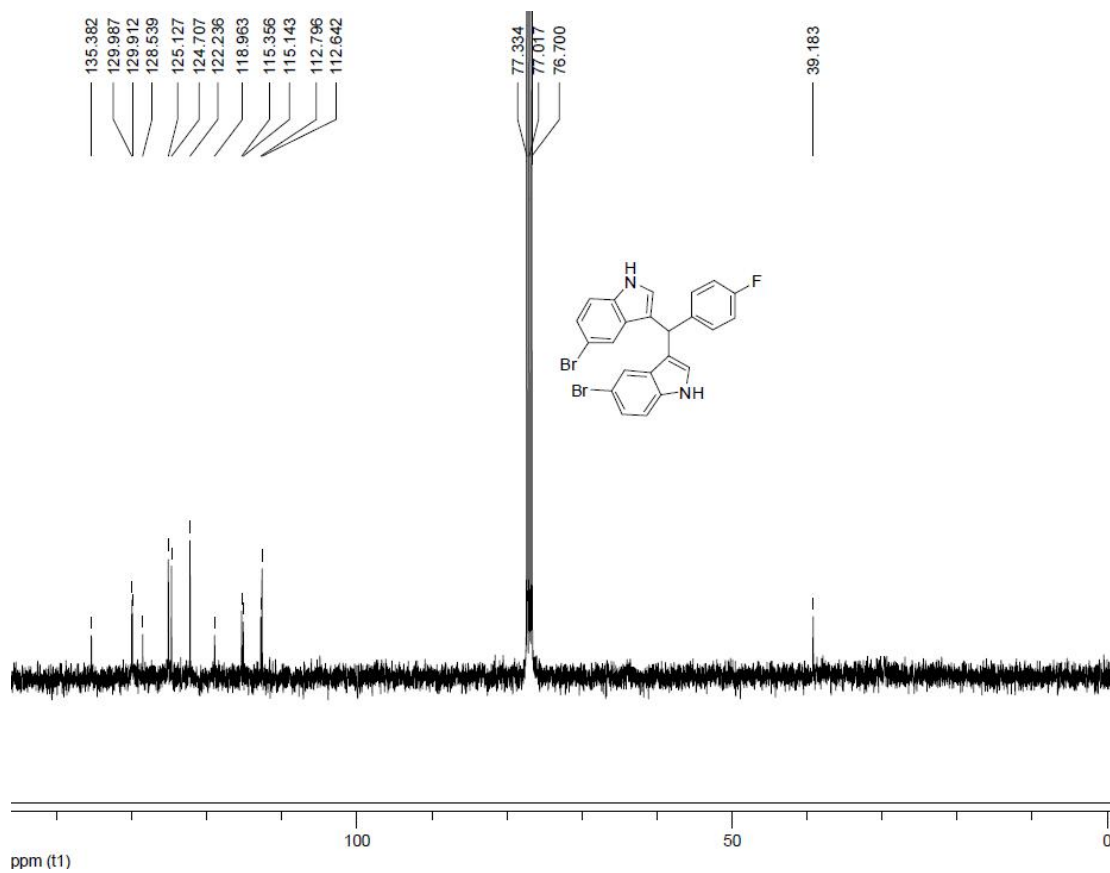
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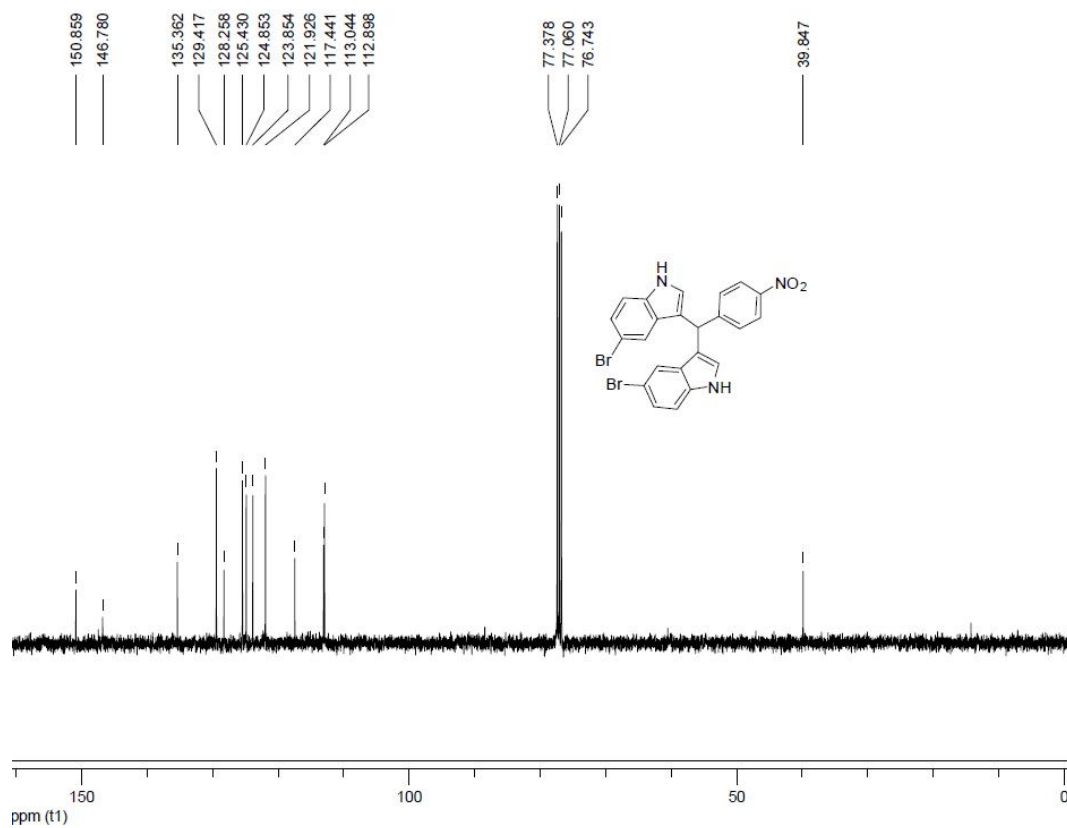
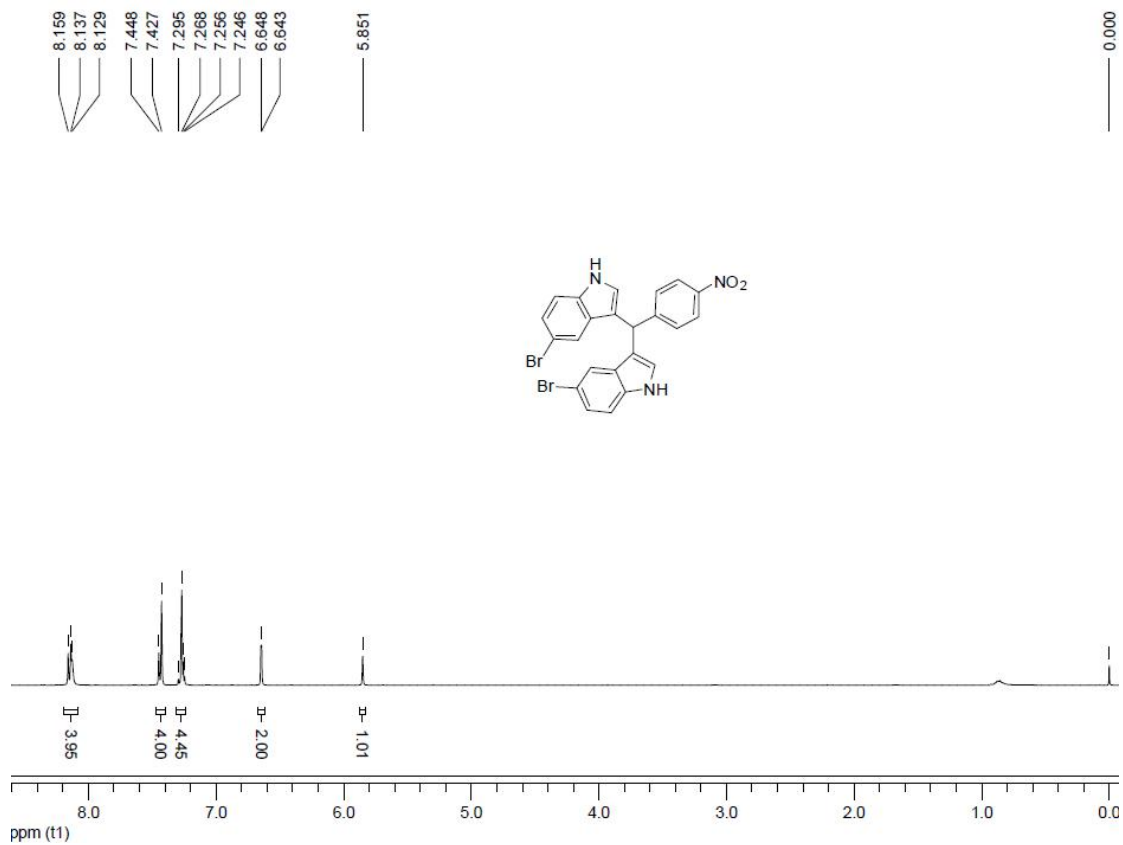
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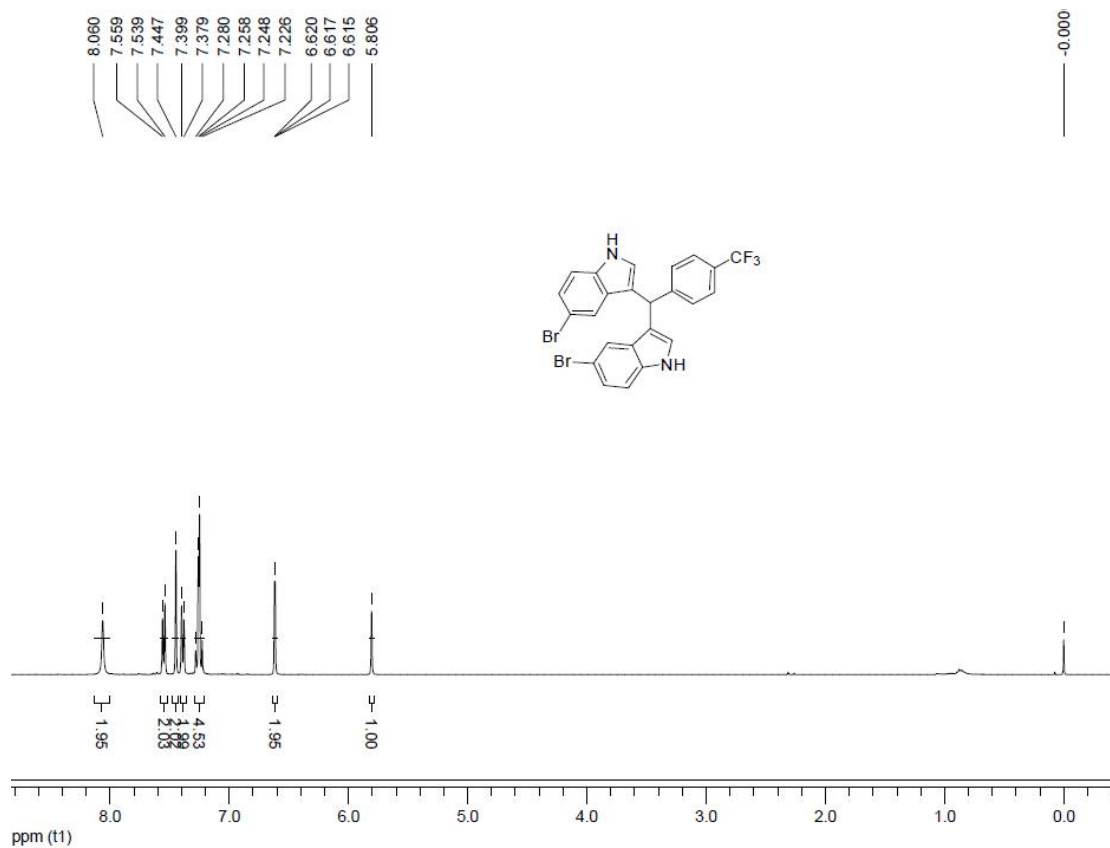
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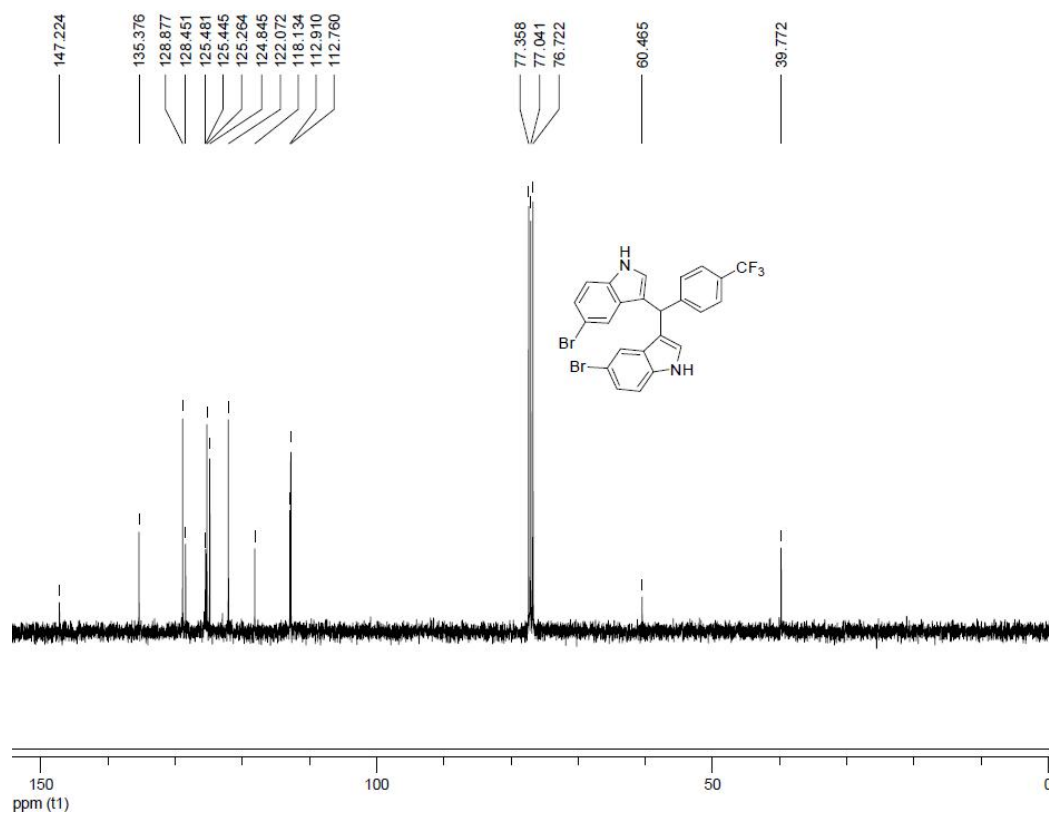
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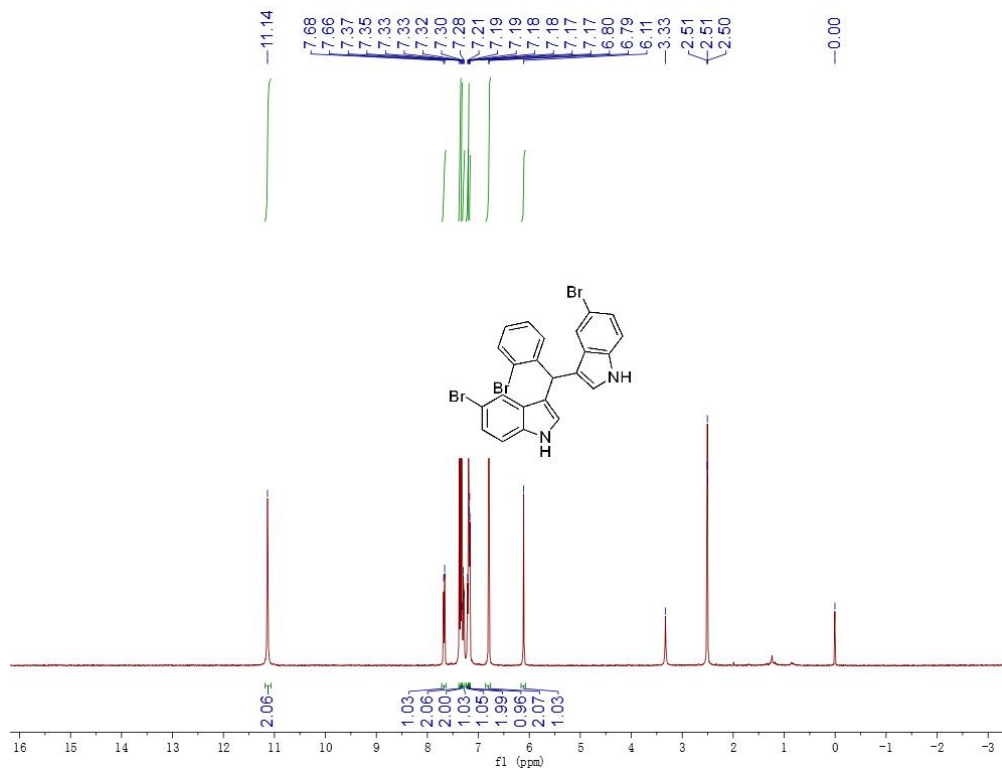
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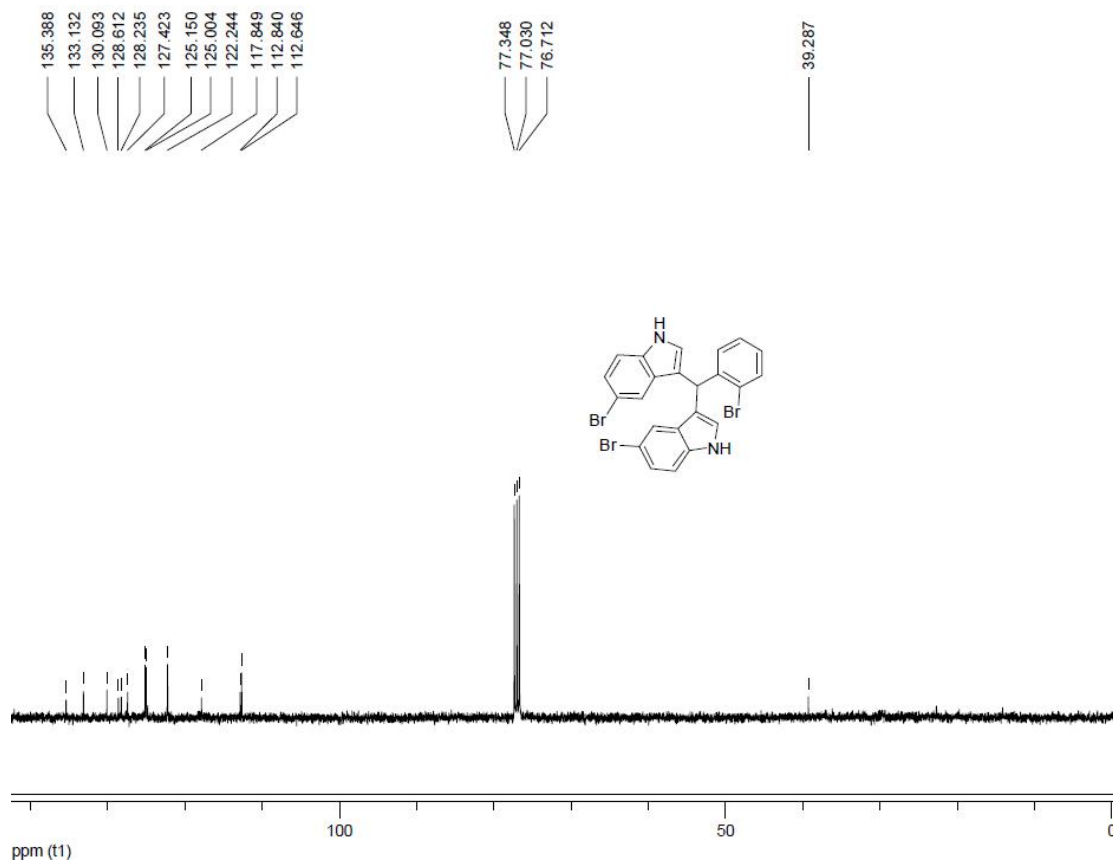
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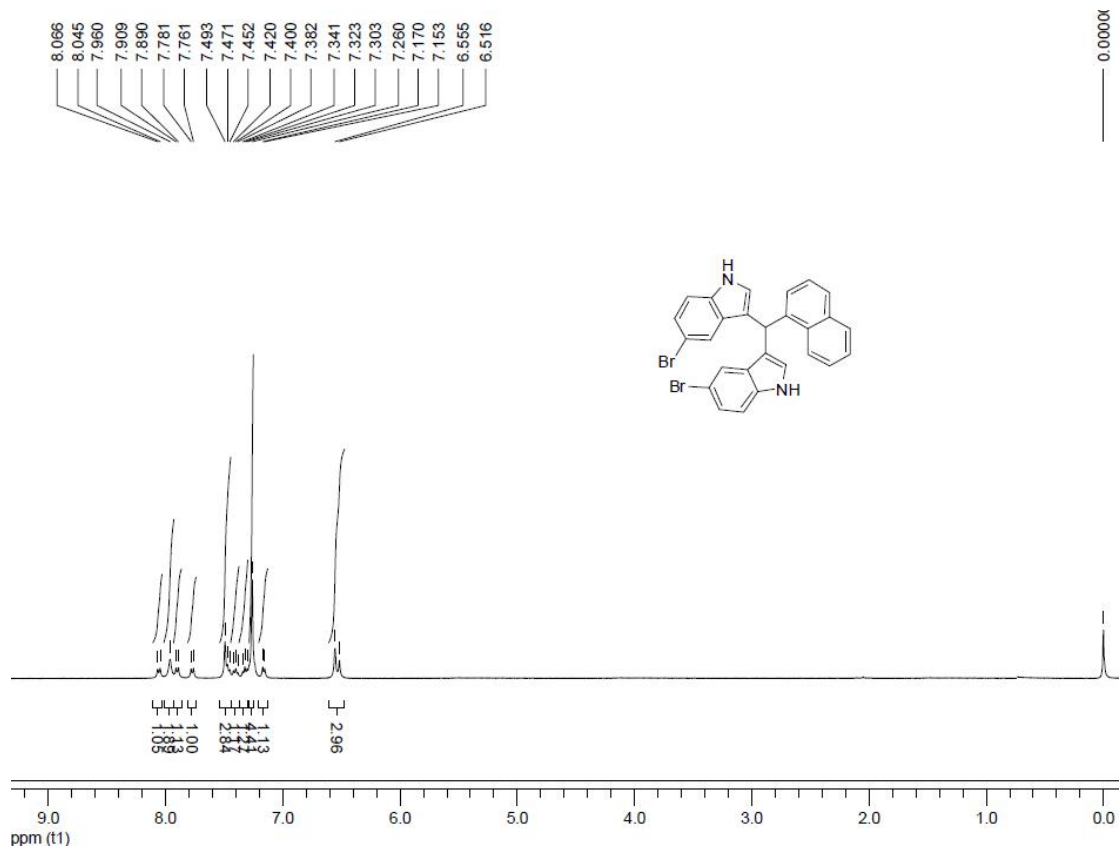
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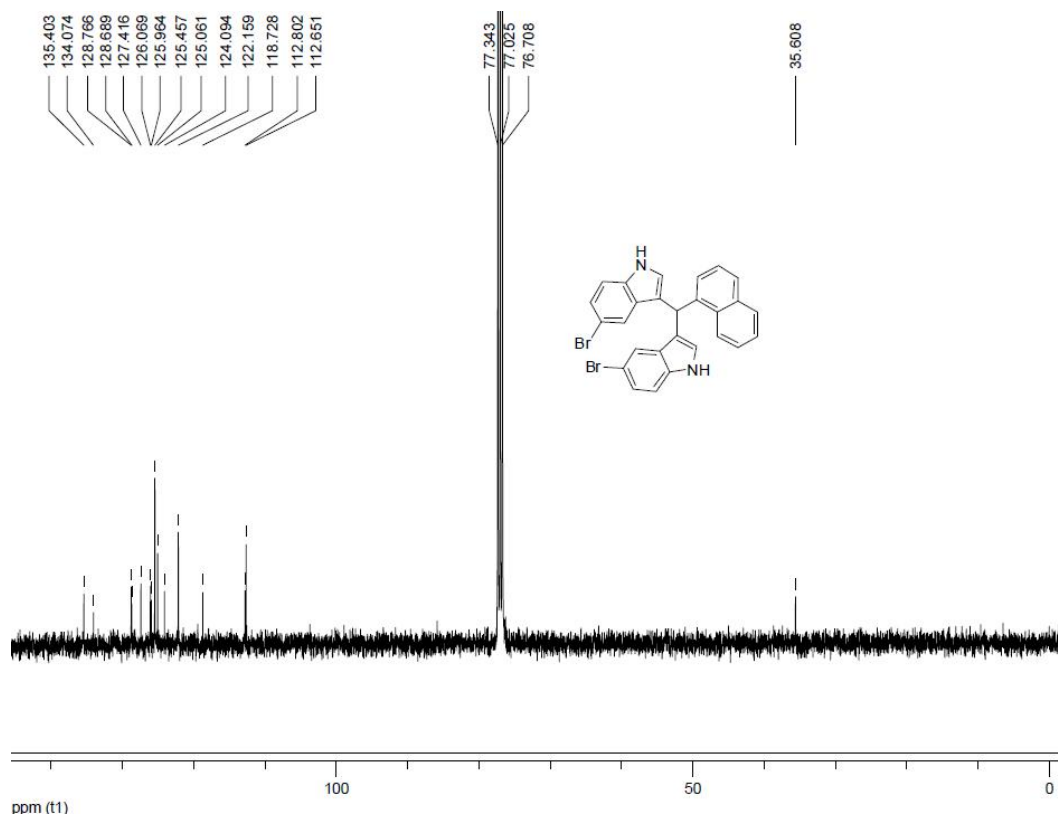
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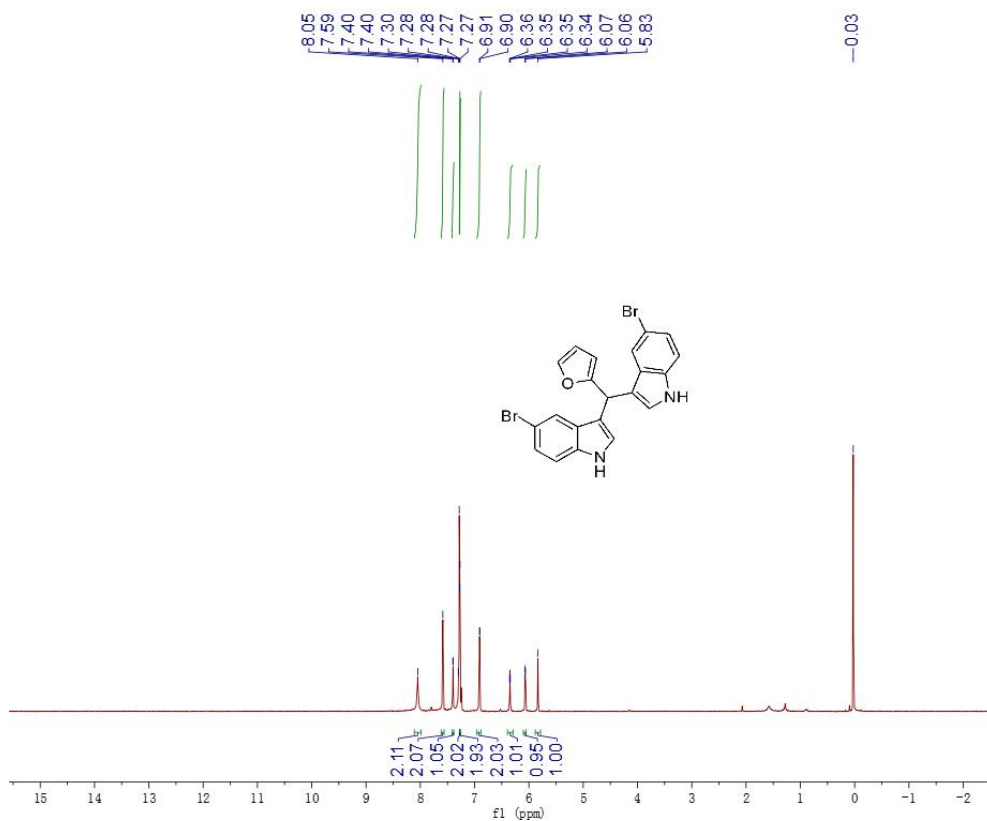
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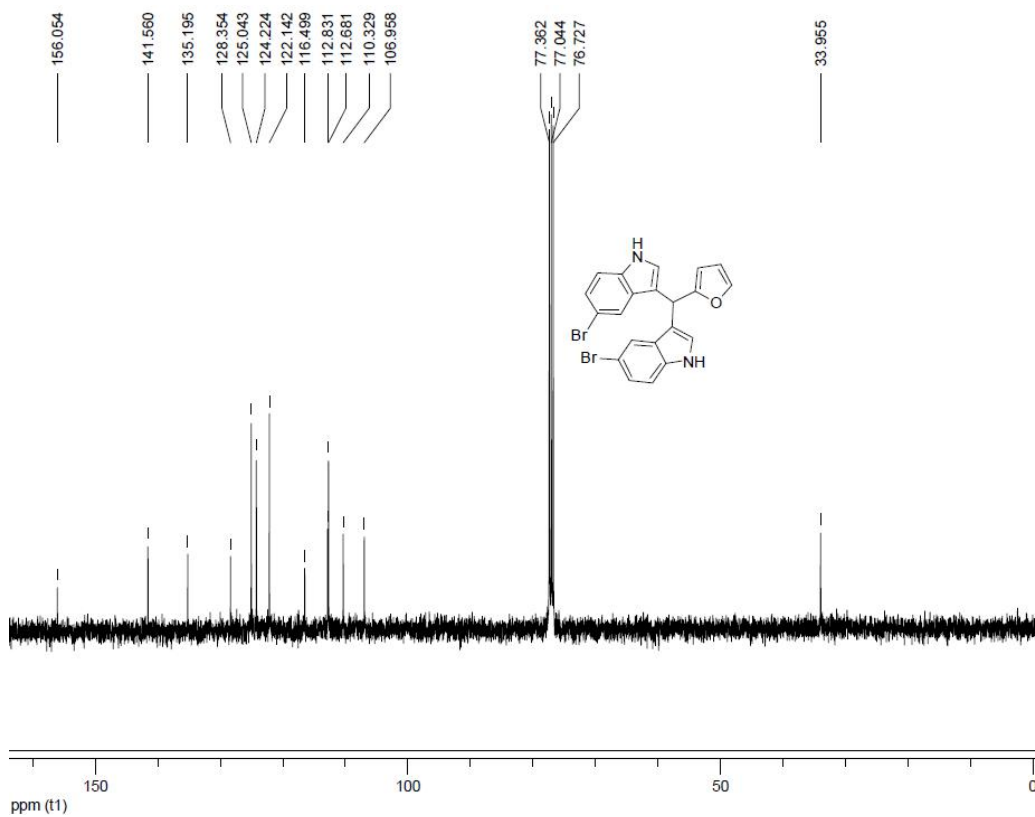
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¹³C NMR spectrum of **23**



¹H NMR spectrum of **25**



¹³C NMR spectrum of **25**