Supporting Information

Optimization, Structure-Activity Relationship and Mode of Action of Nortopsentin Analogues Containing Thiazole and Oxazole Moieties

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Contents

Detailed bio-assay procedures

Phytotoxic Activity. The growing 5–6 leaf stage tobaccos (*Nicotiana tabacum var Xanthi nc*) were selected. The compound solution (500 μg/mL) was smeared on the leaves and calculated the number of lesions after 3-4 days.

Antiviral Biological Assay.[1]

Purification of Tobacco Mosaic Virus.

Using Gooding's method[2], 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.

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

Protective Effect of Compounds against TMV in Vivo.

The compound solution was smeared on the left side and the solvent serving as control on the right side of growing *N. tabacum* L. leaves of the same ages. The leaves were then inoculated with the virus after 12 h. A brush was dipped in TMV of 6×10^{-3} mg/mL to inoculate the leaves, which were previously scattered with silicon carbide. The leaves were then washed with water and rubbed softly along the nervature once or twice. The local lesion numbers appearing 3-4 days after inoculation were counted.[3] There are three replicates for each compound.

Inactivation Effect of Compounds against TMV in Vivo.

The virus was inhibited by mixing with the compound solution at the same volume for 30 min. The mixture was then inoculated on the left side of the leaves of *N. tabacum* L., whereas the right side of the leaves was inoculated with the mixture of solvent and the virus for control. The local

lesion numbers were recorded 3-4 days after inoculation.[3] There are three replicates for each compound.

Curative Effect of Compounds against TMV in Vivo.

Growing leaves of *N. tabacum* L. of the same ages were selected. TMV (concentration of 6.0 × 10^{-3} mg/mL) was dipped and inoculated on the whole leaves. Then the leaves were washed with water and dried. The compound solution was smeared on the left side, and the solvent was smeared on the right side for control. The local lesion numbers were then counted and recorded 3-4 days after inoculation.[3] There are three replicates for each compound. The in vitro and in vivo inhibition rates of the compound were then calculated according to the following formula ("av" means average, and controls were not treated with compound).

Inhibition rate (%) = [(av local lesion no. of control − av local lesion no. of drug-treated)/av local lesion no. of control] × 100%

Mode of Action Studies.

In vitro TMV rod assembly inhibition： TMV purification was performed according to the instructions by Leberman.[4] 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.[5] 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 ℃ 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 compounds, 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 DMSO or the compound (10 μM). All treatments were repeated over time to validate the results.

In vitro 20S CP Disk assembly inhibition: For the inhibition tests with the compounds, TMV CP was first adjusted to 20.4 mg/mL with 0.1 M phosphate buffer (pH 7.0). In vitro 20S CP Disk assembly reactions were performed by adding 9.8 μL TMV CP (20.4 mg/mL) and 0.2 μL DMSO or the compound (10 μM). The assembly reaction was incubated at 20 °C for 12 h. The morphology of the 20S CP Disk was imaged via TEM at 200 keV on a CCD camera. All treatments were repeated over time for confirmation.

***Detailed bioassay procedures for the fungicidal activities*[6]**

In Vitro Antifungal Bioassay. The fungicidal activities of compounds were evaluated in mycelial growth tests conducted in artificial media against 14 plant pathogens at a rate of 50 μg/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 μg/mL. To each petri dish was added 1 mL of the test solution and 9 mL of culture medium to make a 50 μg/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±1°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.

In Vivo Antifungal Activity. The in vivo antifungal activity was evaluate by plant spray method. Azoxystrobin was evaluated as the positive controls at the same condition. Aqueous DMF（1%）containing 0.1% Tween 80 was set up as the blank control. Each tested compound was dissolved in DMF and then suspended in the distilled water (containing 0.1% Tween 80) to give the tested concentration of 200 $mg \cdot L^{-1}$. Three replicates were done for each compound. The inhibition percentage was expressed as the mean of values obtained in three independent experiments.

Healthy selected plants were sprayed with a solution of the tested compound at 200 $mg \cdot L^{-1}$ and evaporated under an ambient environment (~28 °C). After air drying, the leaves were inoculated by spraying the mycelial suspension of corresponding pathogens. Three days later, the symptoms were examined. The control efficacy of the target compounds was calculated as (*averaged disease index of blank controls – averaged disease index of drug treated*) / (*averaged disease index of blank controls*) × 100.

Reference:

(1) Wang, Z. W.; Wei, P.; Wang, L. Z.; Wang, Q. M. Design, synthesis, and anti-tobacco mosaic virus (TMV) activity of phenanthroindolizidines and their analogues. *J. Agric. Food Chem*. **2012**, *60*, 10212−10219.

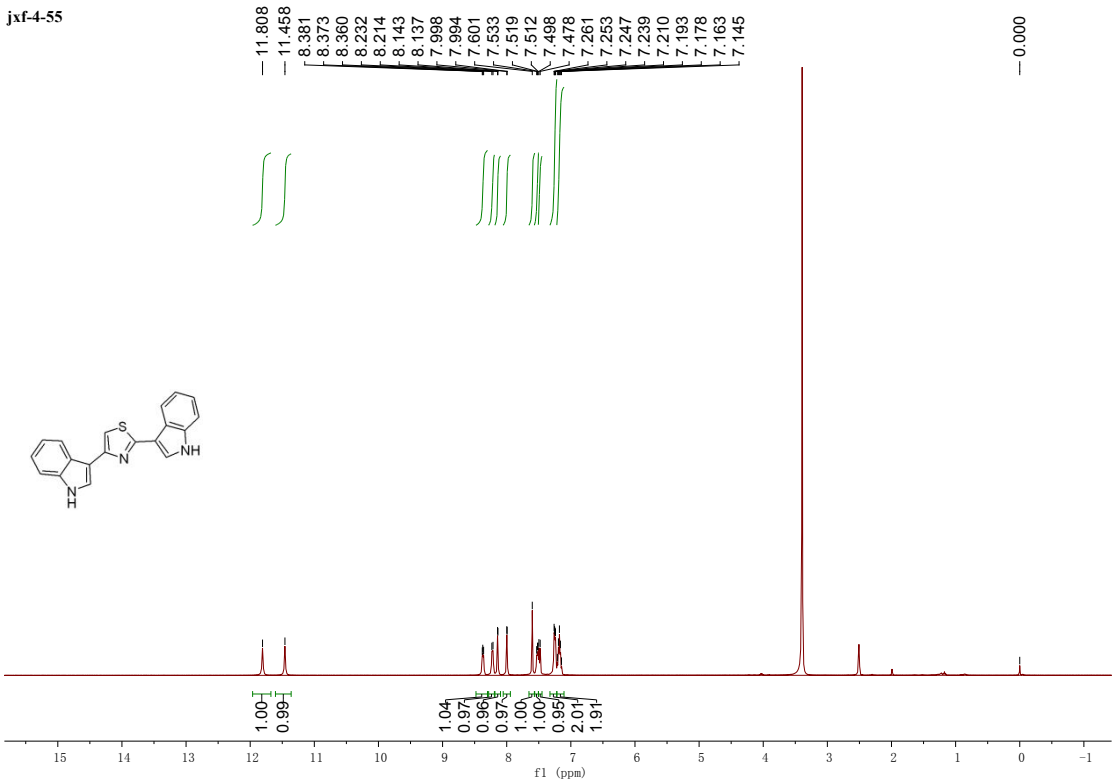
(2) Gooding, G. V., Jr.; Hebert, T. T. A simple technique for purification of tobacco mosaic virus in large quantities. *Phytopathology* **1967**, 57, 1285−1290.

(3) Li, S. Z.; Wang, D. M.; Jiao, S. M. In *Pesticide Experiment Methods-Fungicide Sector*; Li, S. Z., Ed.; Agriculture Press of China: Beijing, China, **1991**; 93−94.

(4) Leberman, R. Isolation of plant viruses by means of simple coacervates. *Virol* **1966**, *30*, 341–347.

(5) Fraenkel Conrat, H.; Williams, R. C. Reconstitution of active tobacco mosaic virus fromits inactive protein and nucleic acid components. *Proc Natl Acad Sci* U S A 1955, *41*, 690–698.

(6) Zhao, H. P.; Liu, Y. X.; Cui, Z. P.; Beattie, D.; Gu, Y. C.; Wang, Q. M. Design, synthesis, and biological activities of arylmethylamine substituted chlorotriazine and methylthiotriazine compounds. *J. Agric. Food Chem*. **2011**, *59*, 11711−11717.



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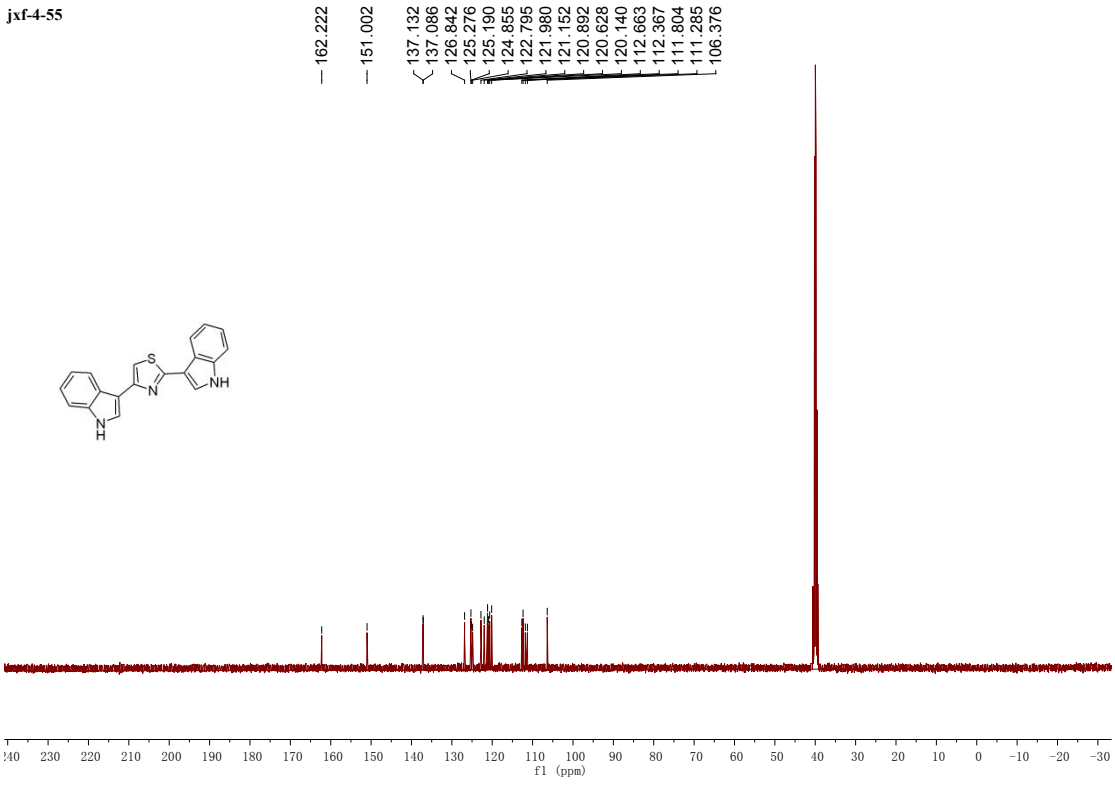

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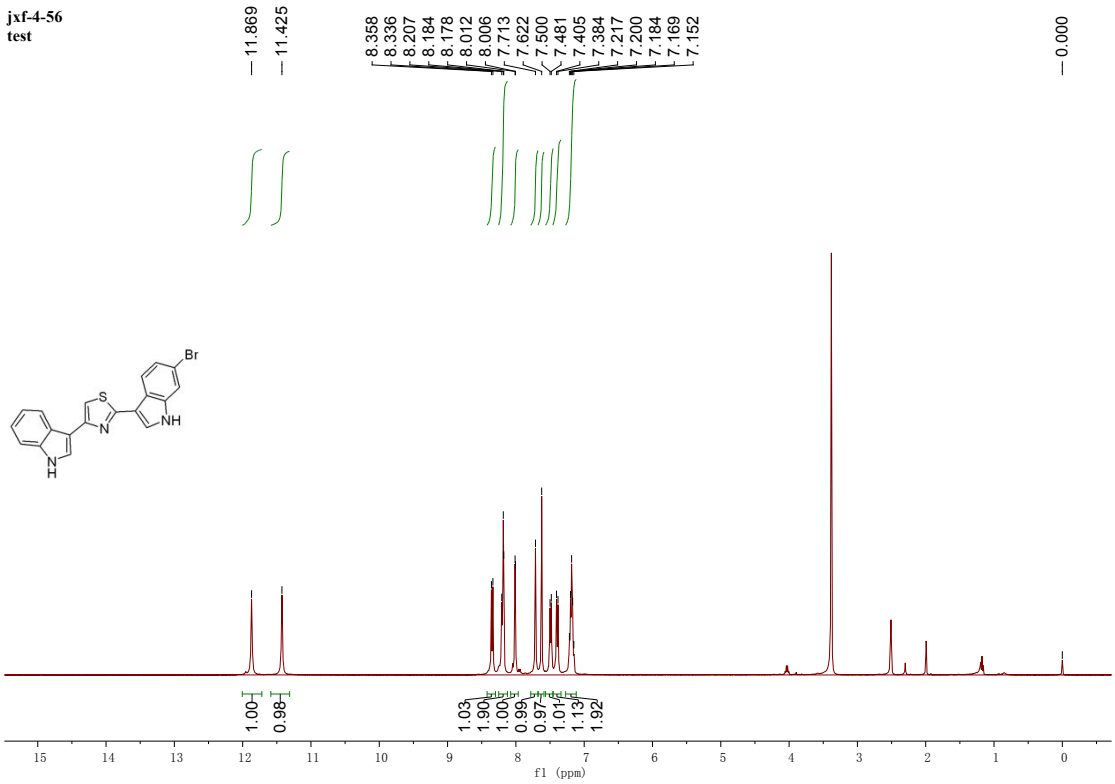

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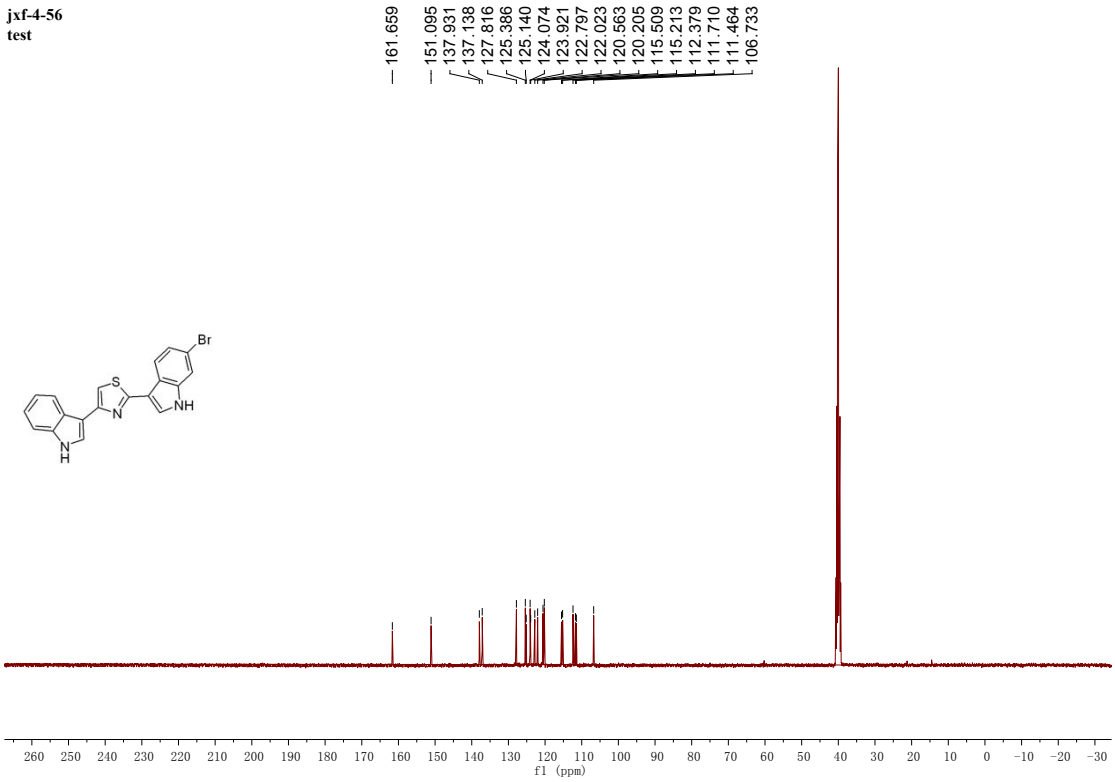

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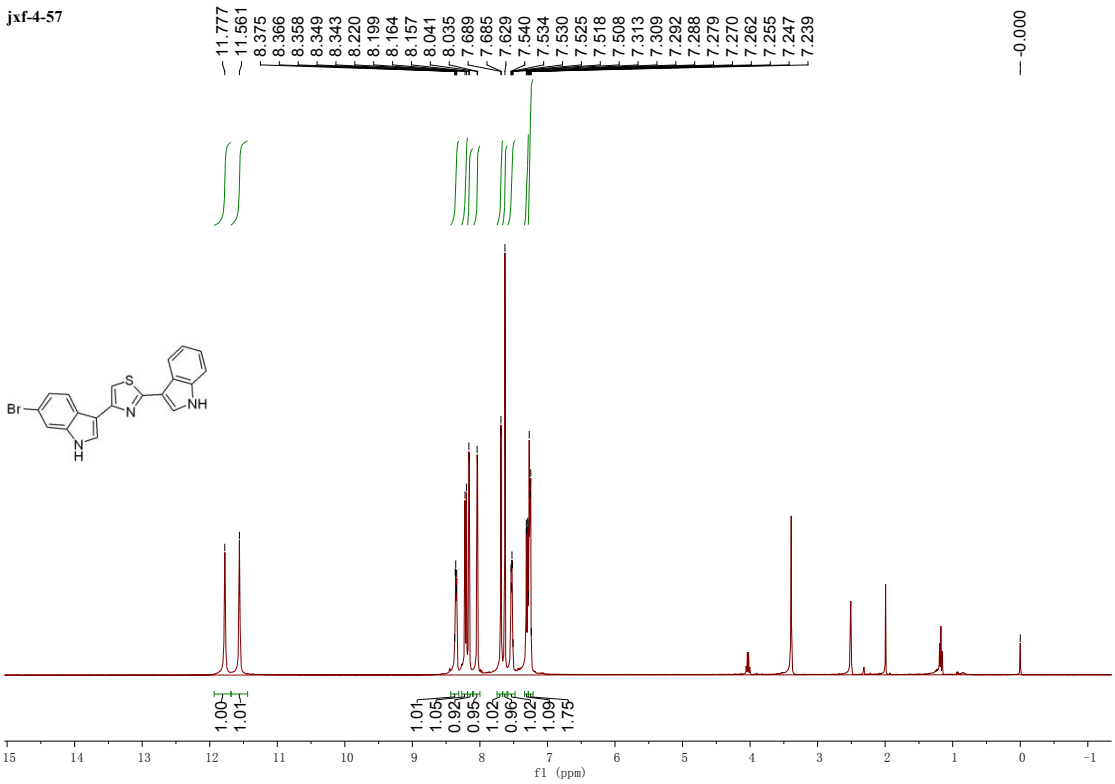

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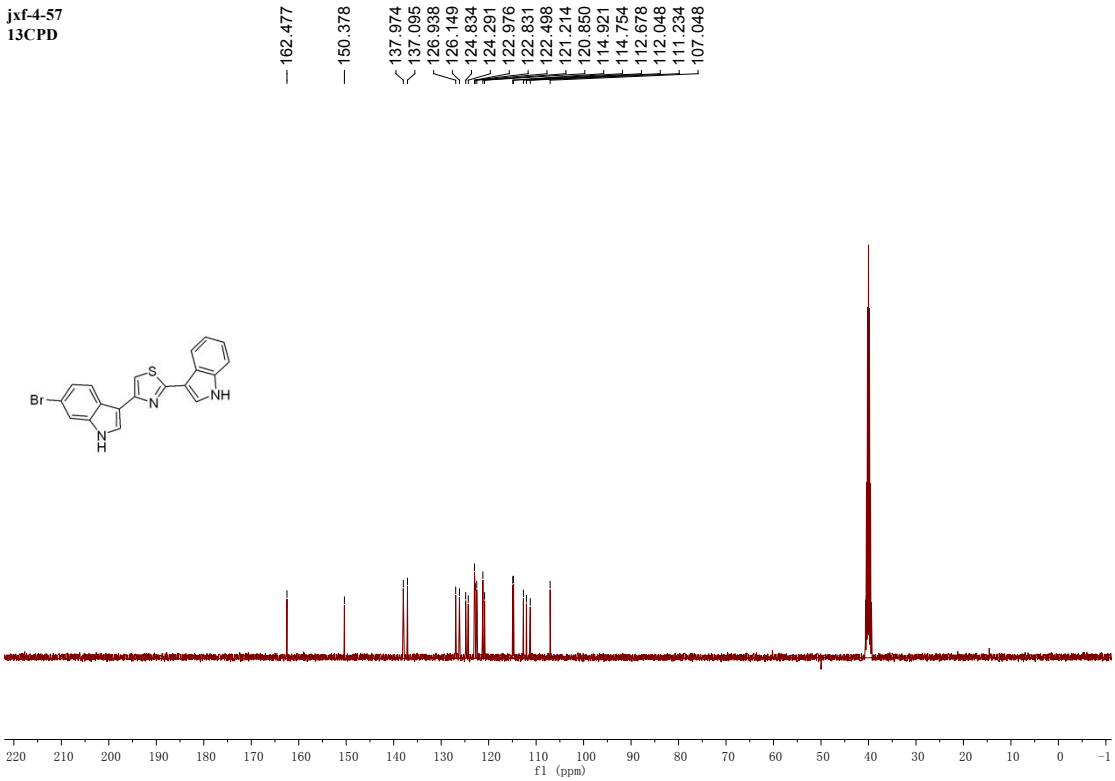

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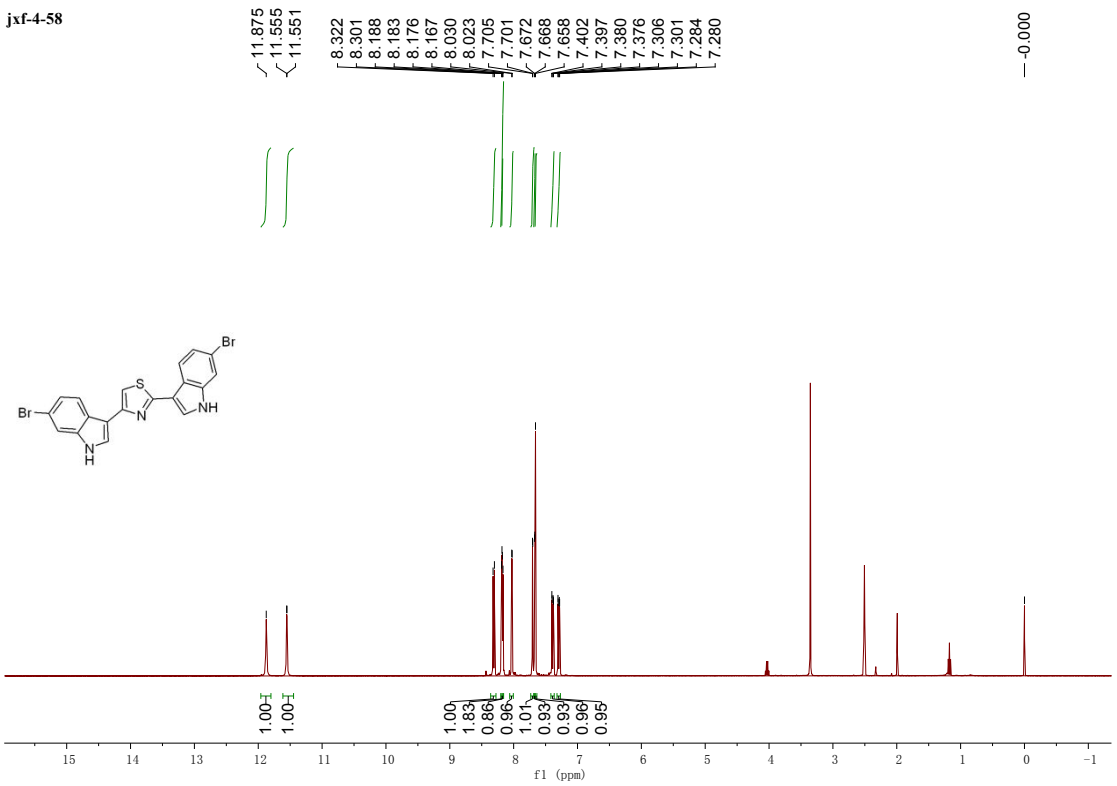

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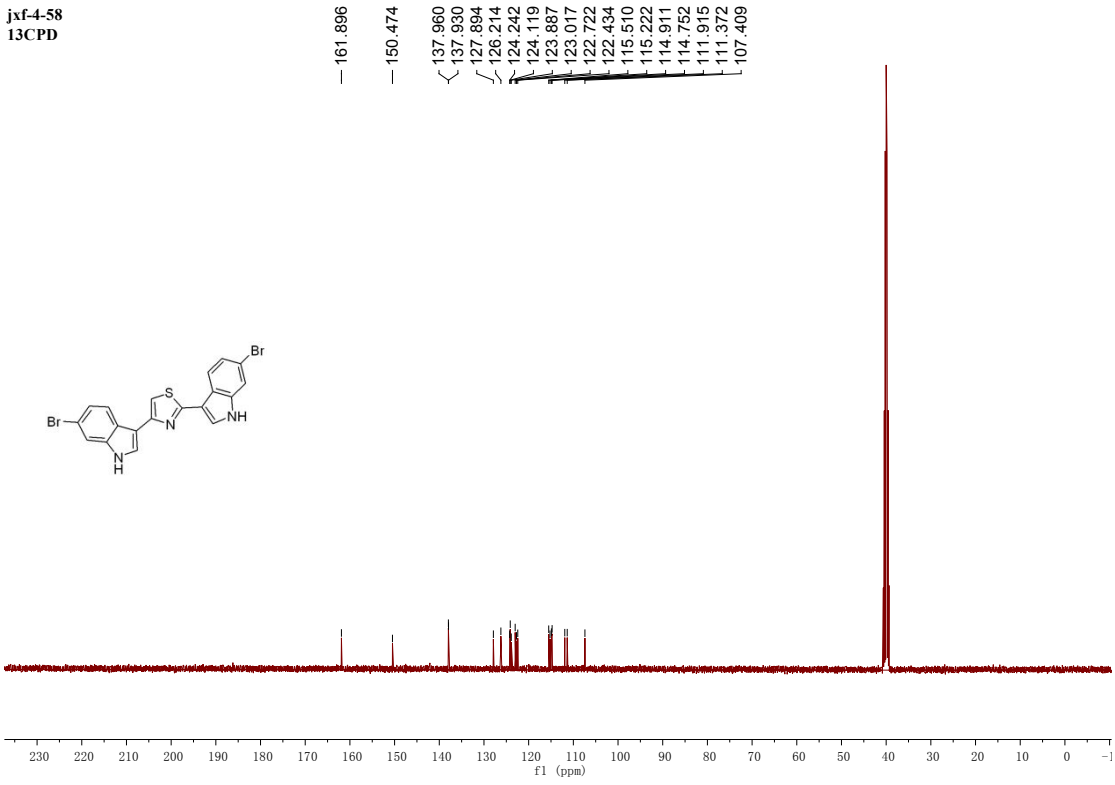

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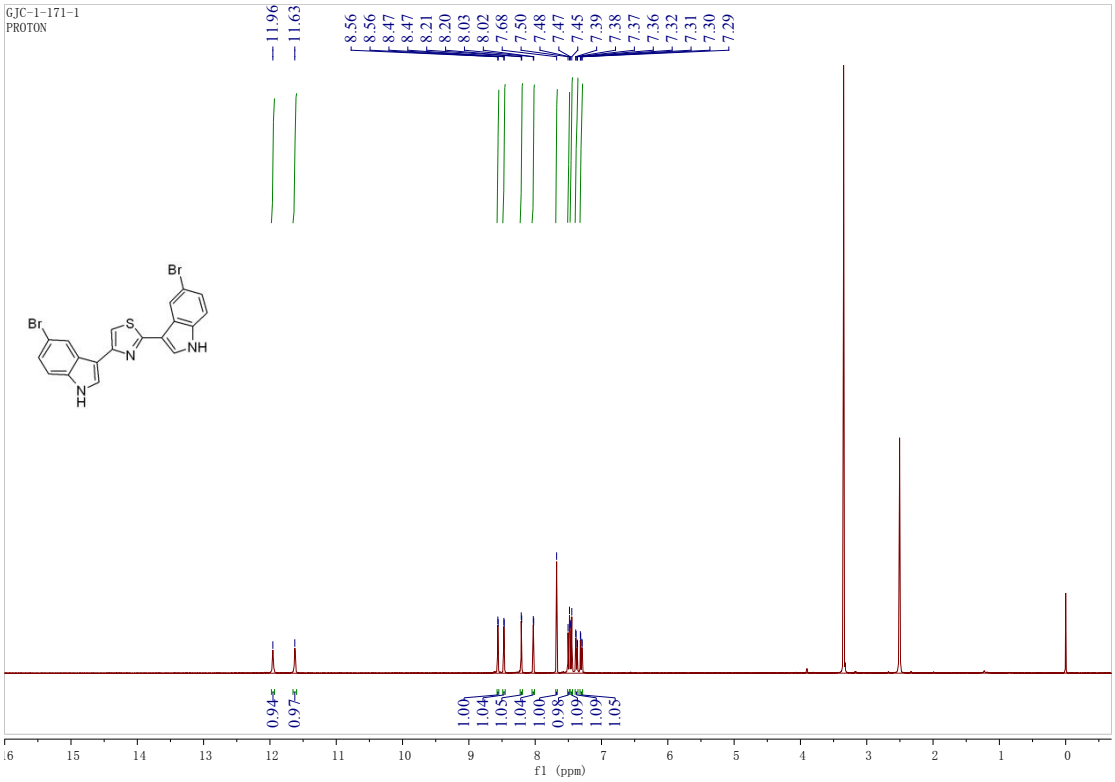

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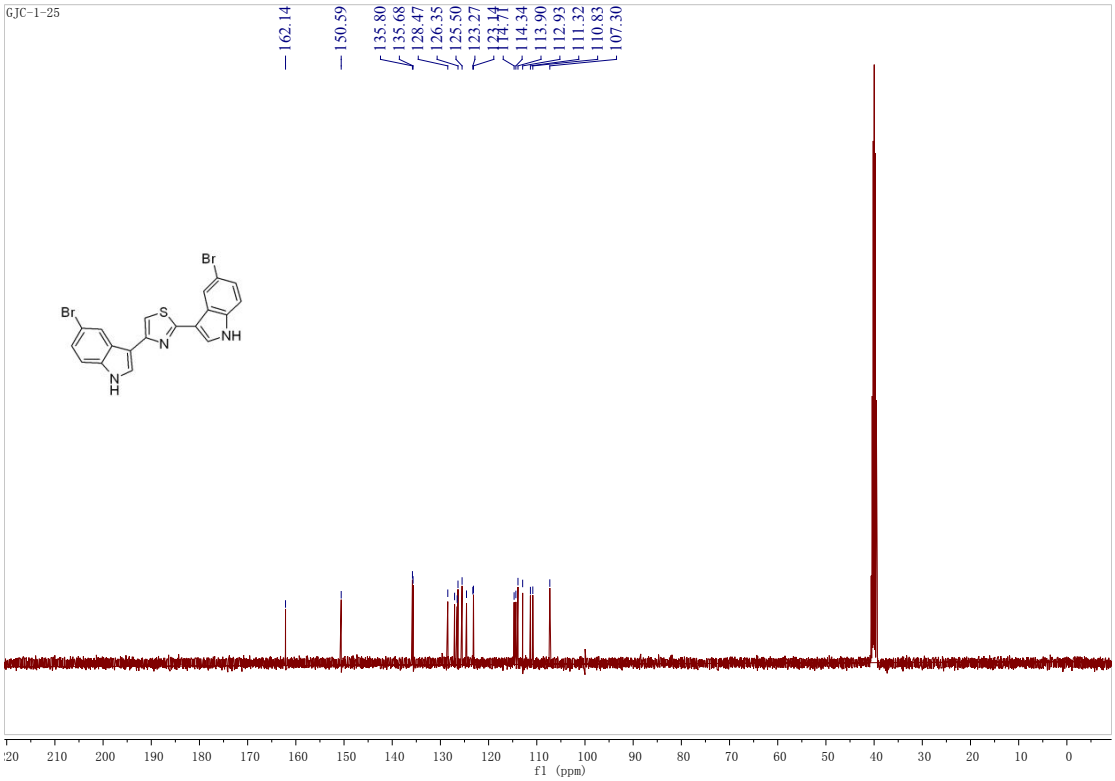

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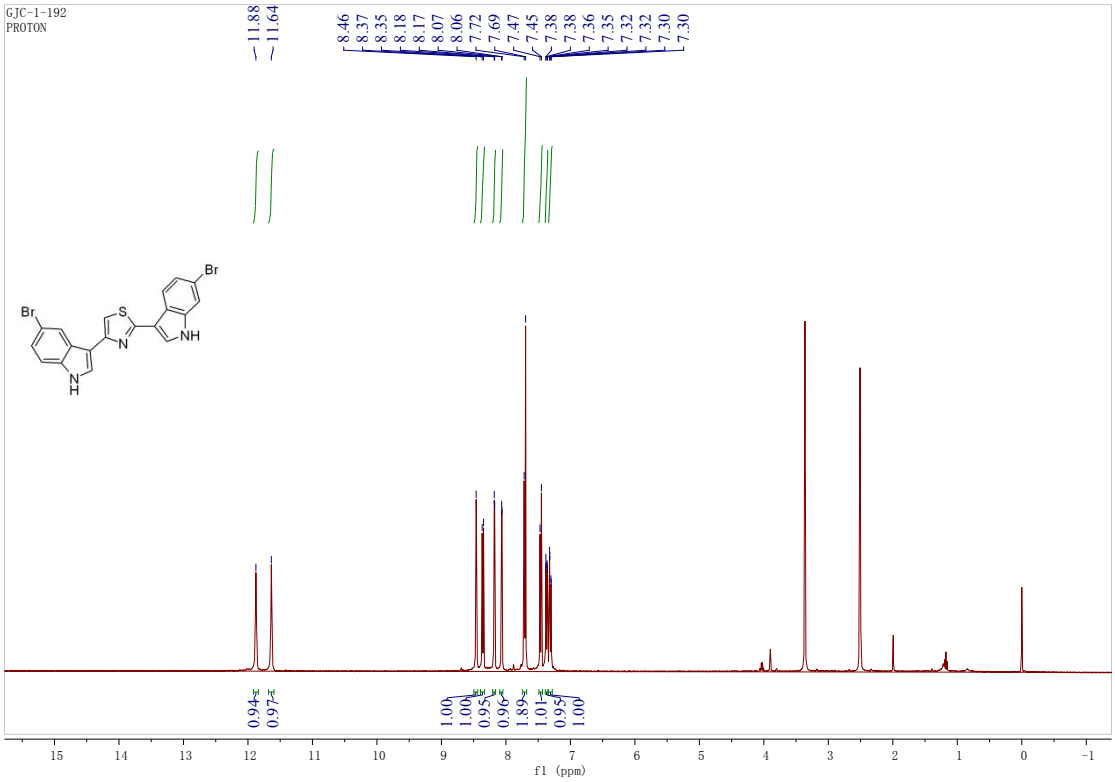

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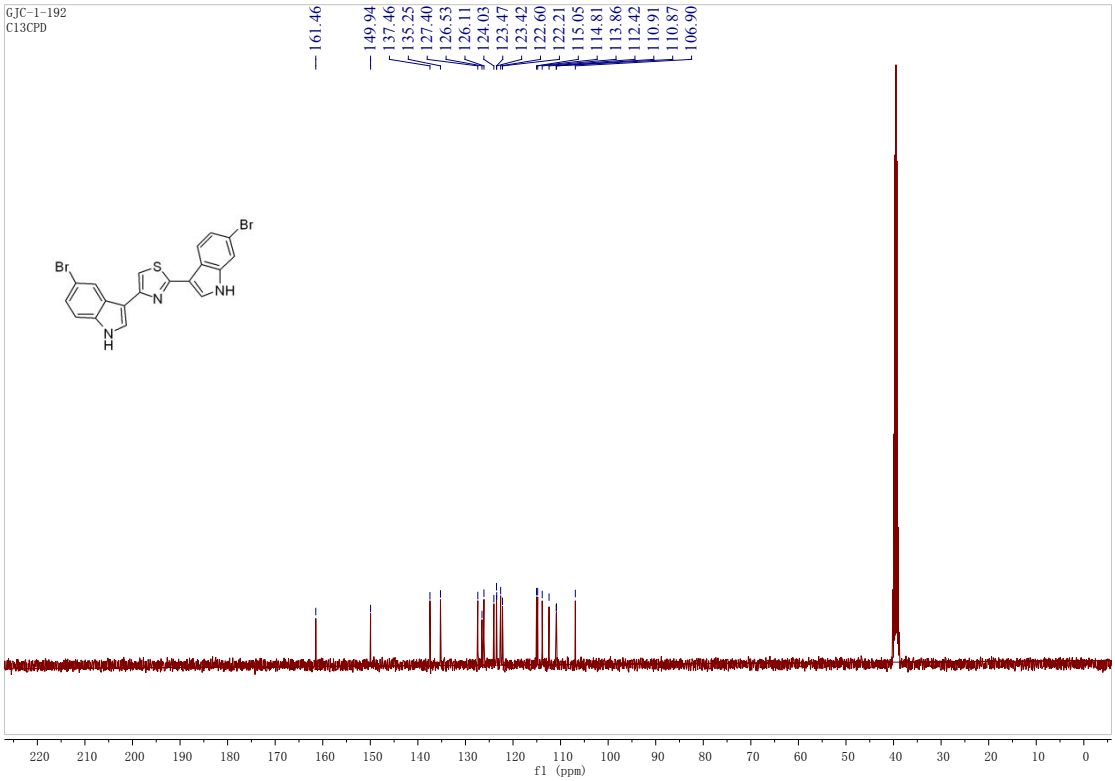

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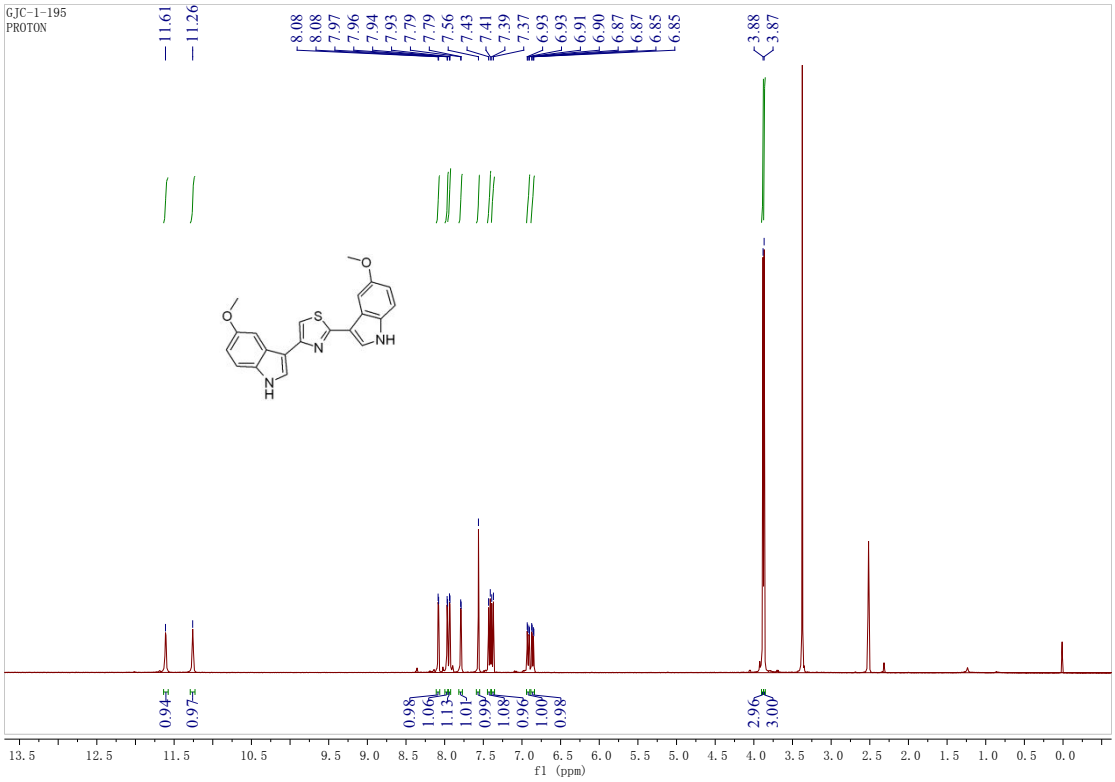

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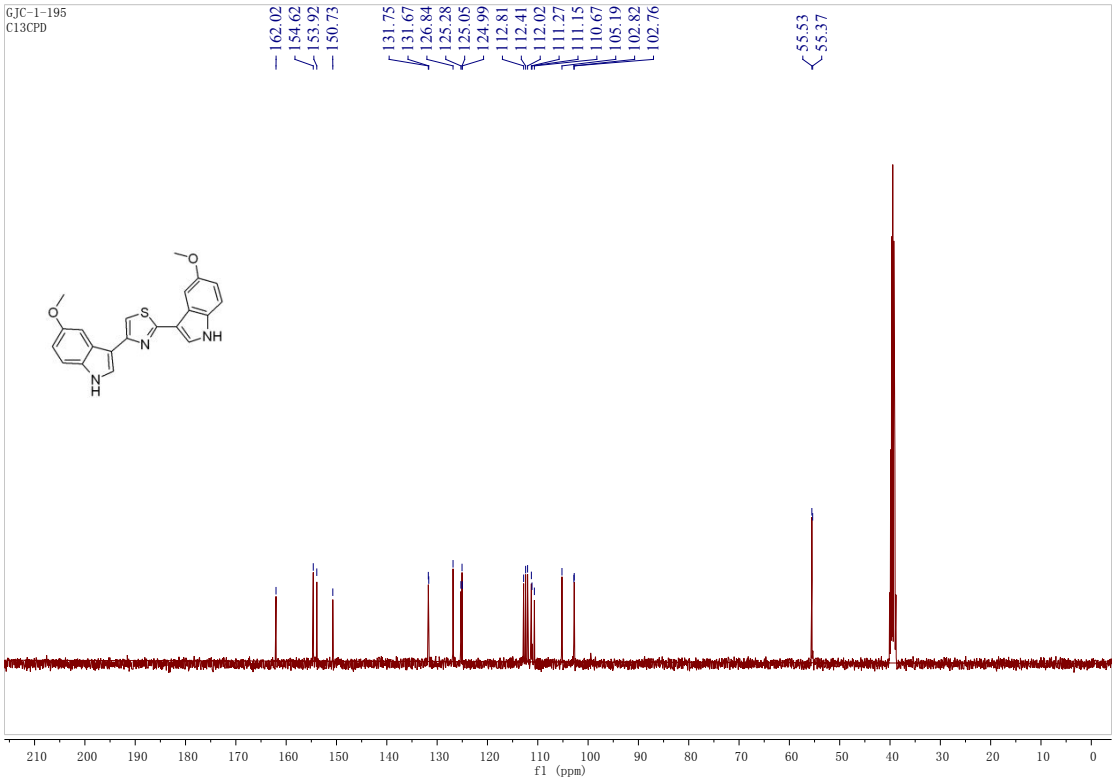

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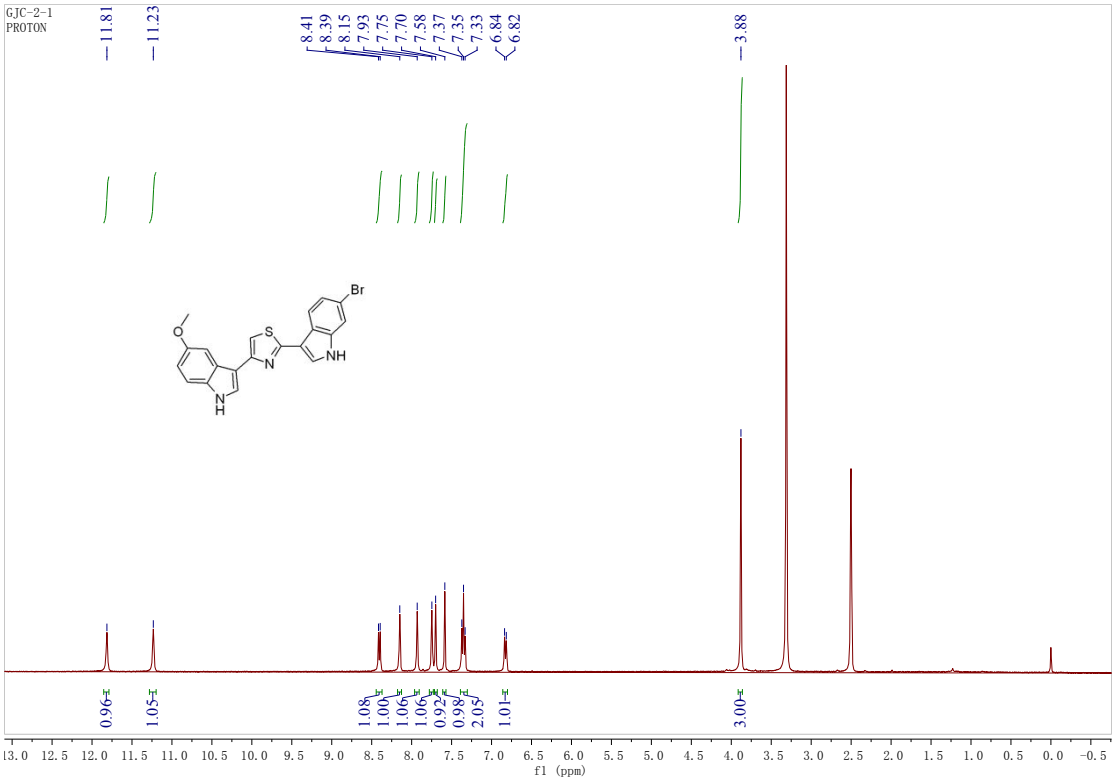

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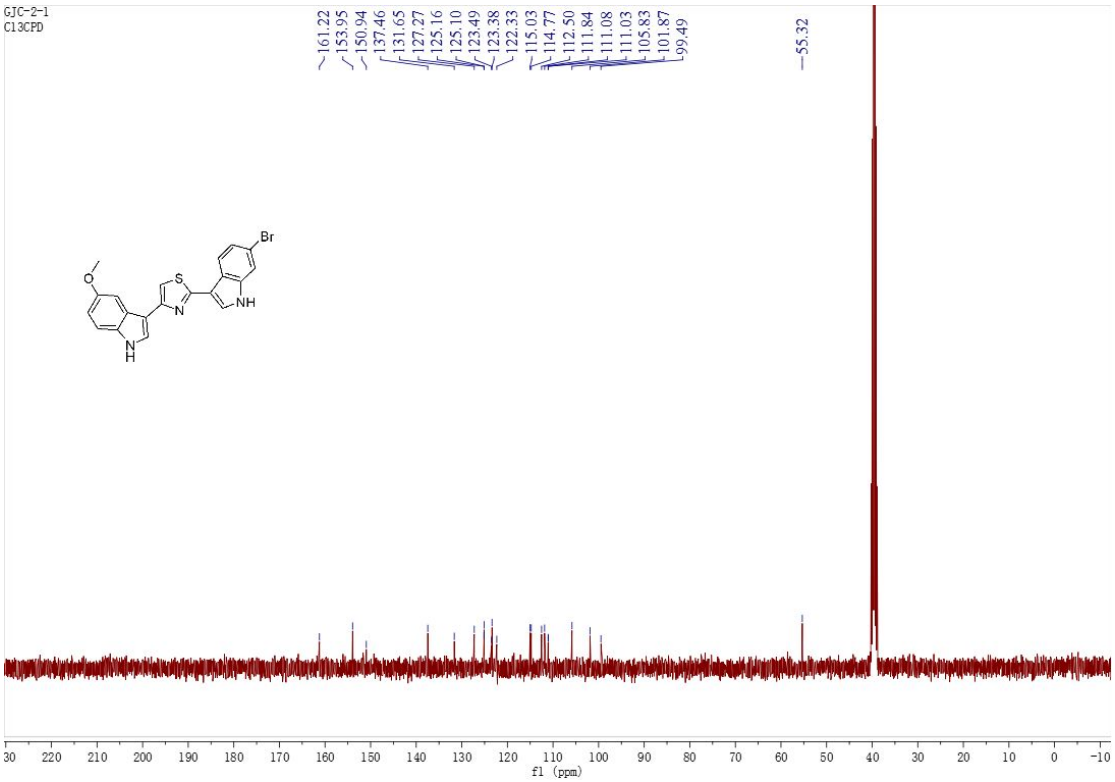

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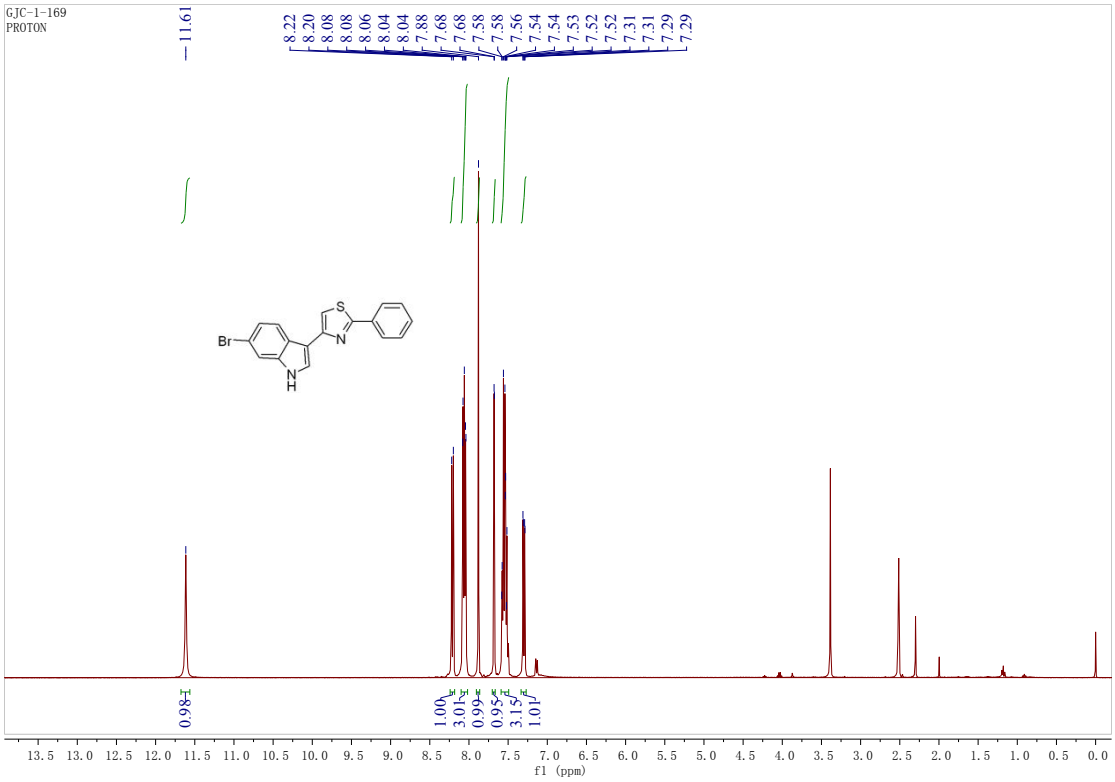

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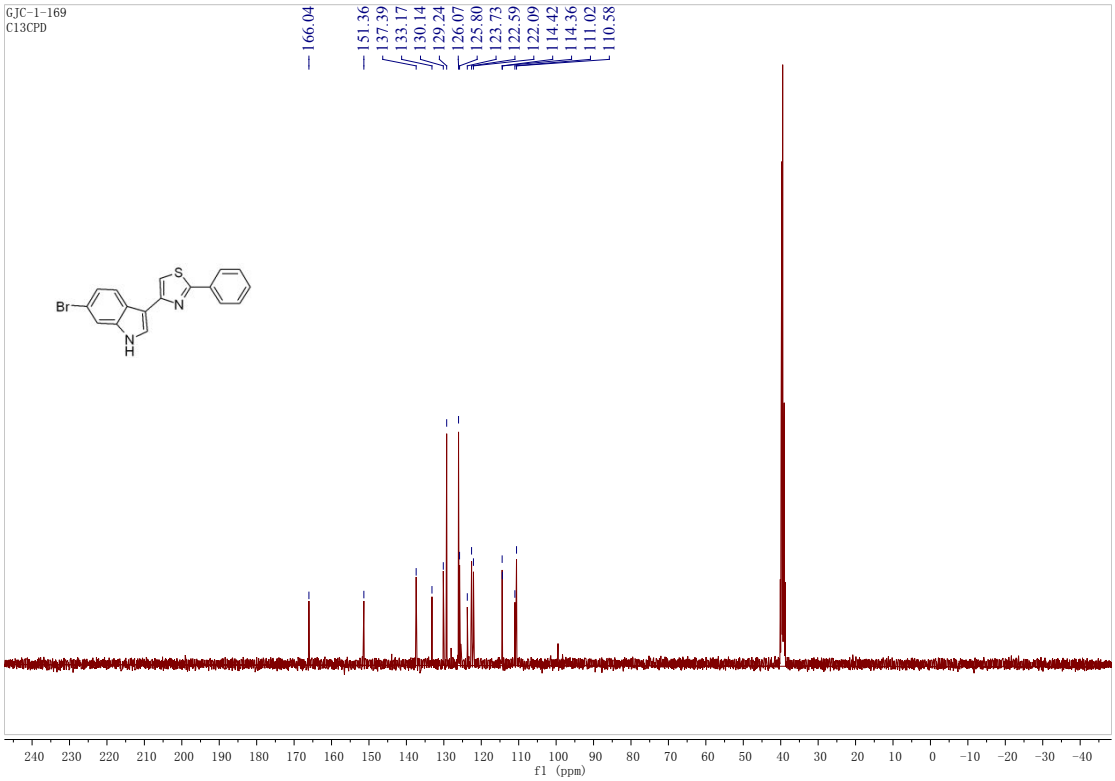

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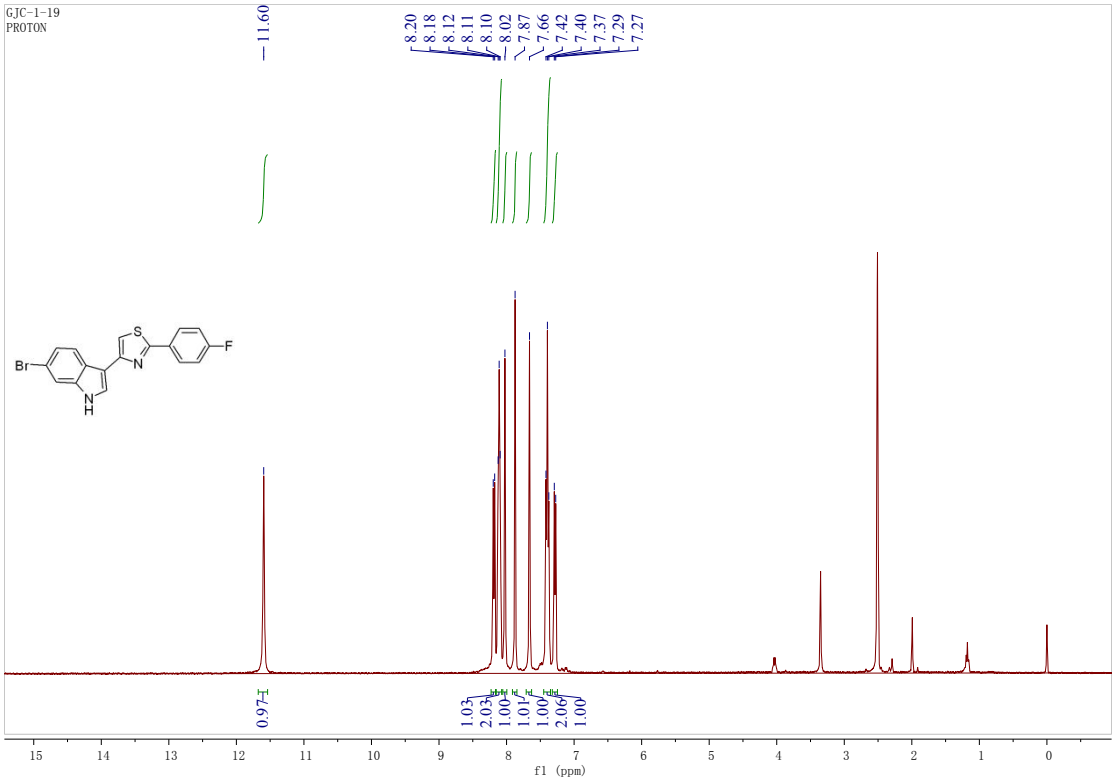

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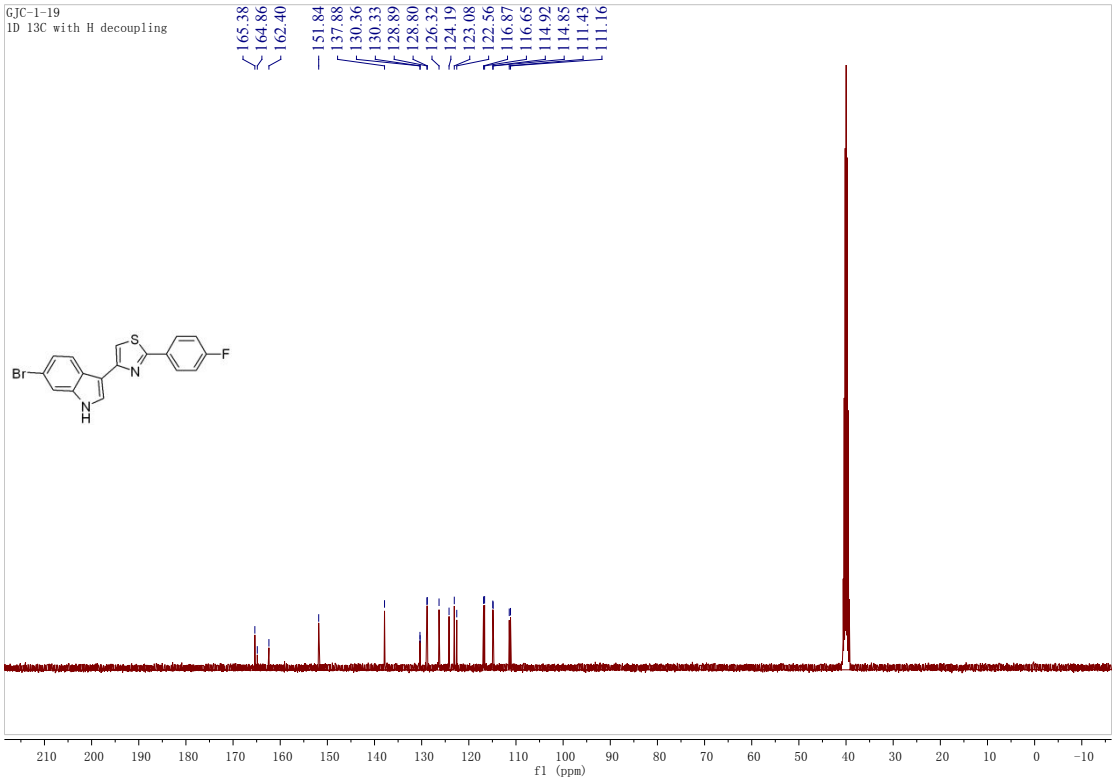

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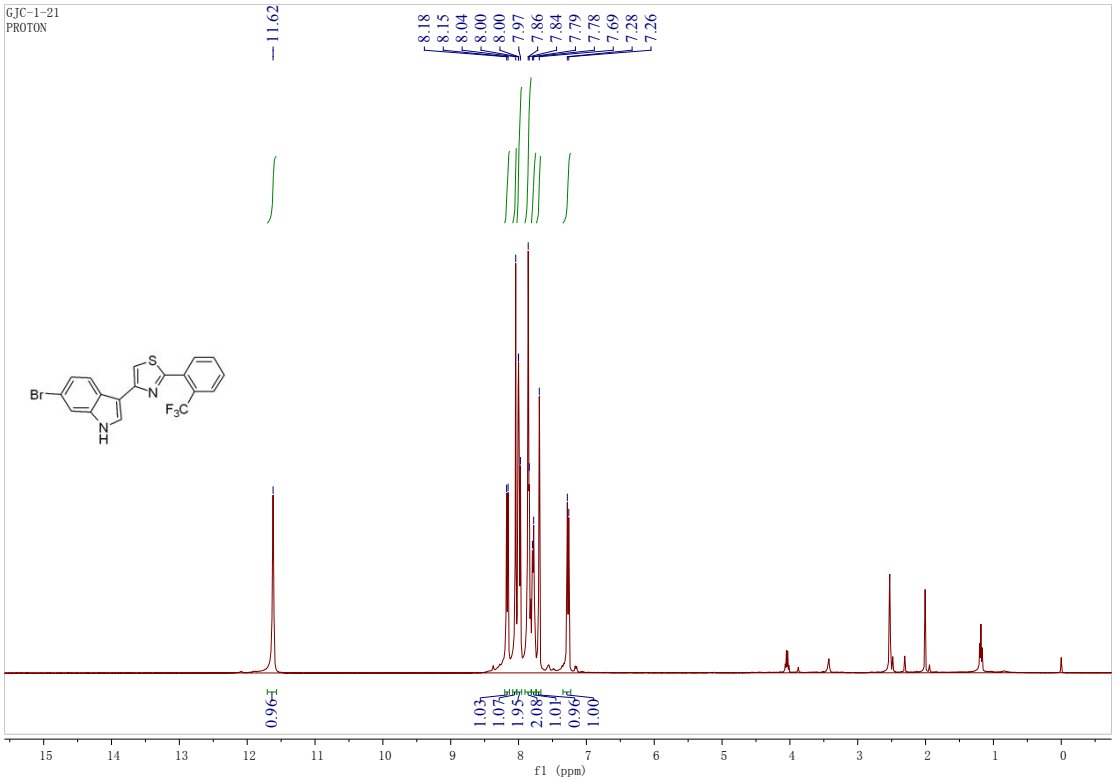

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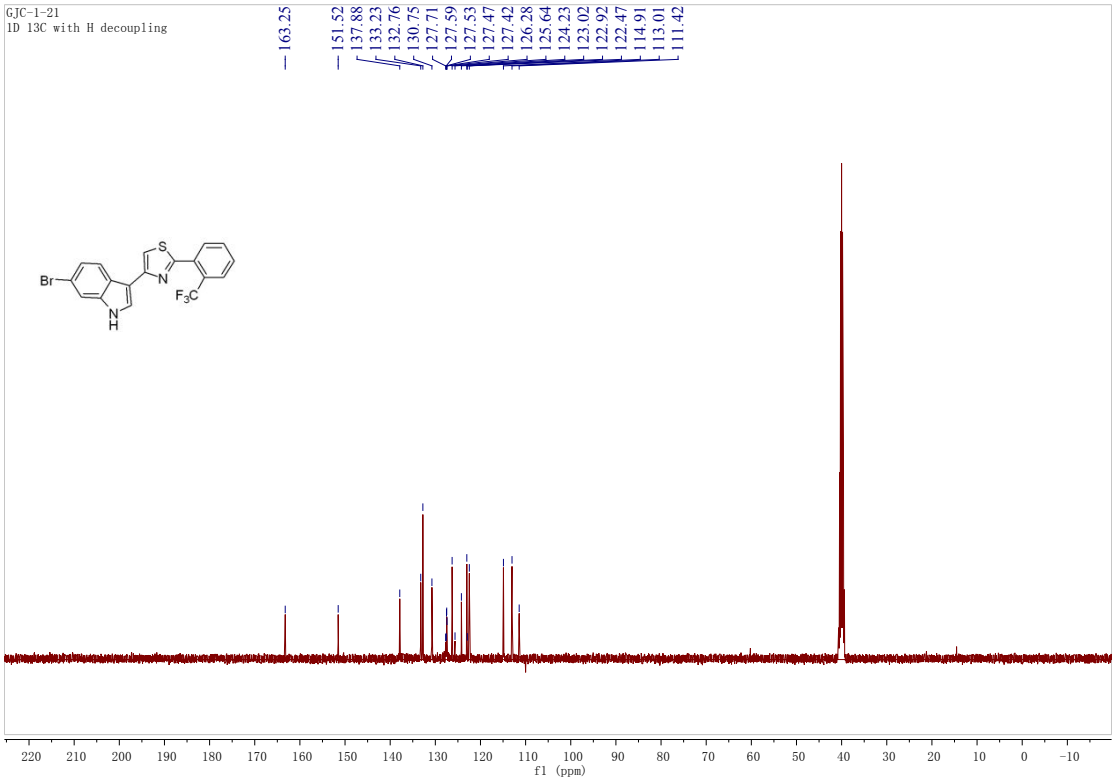

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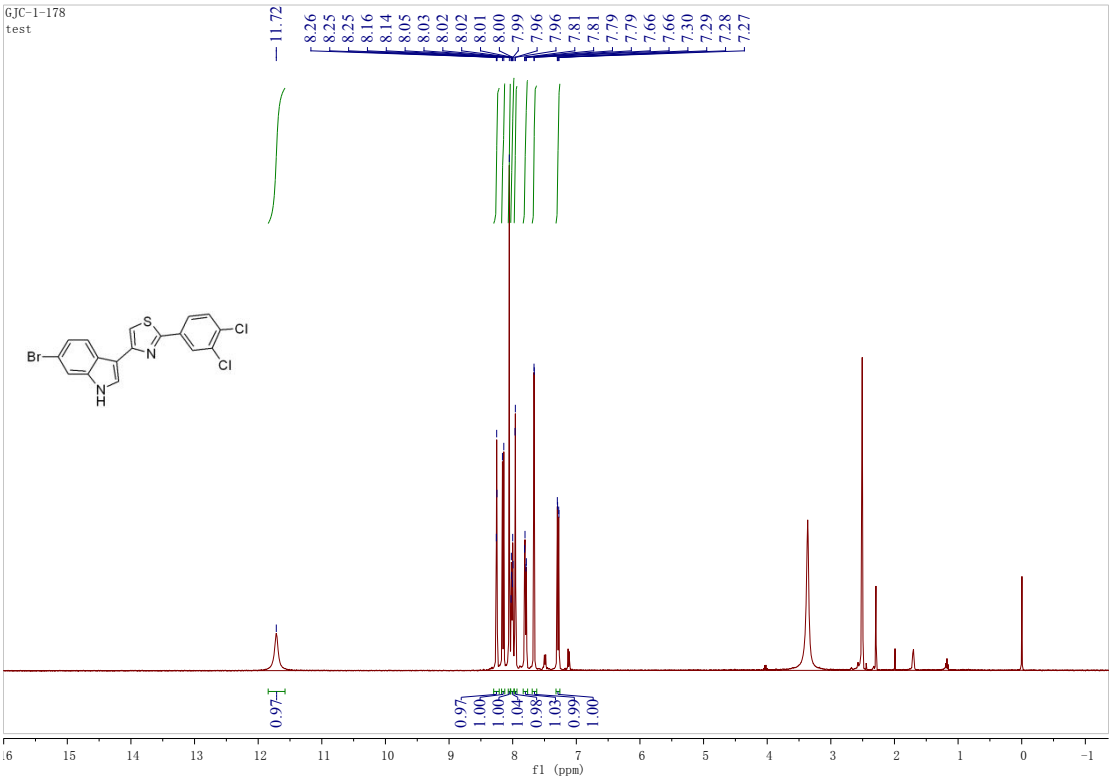

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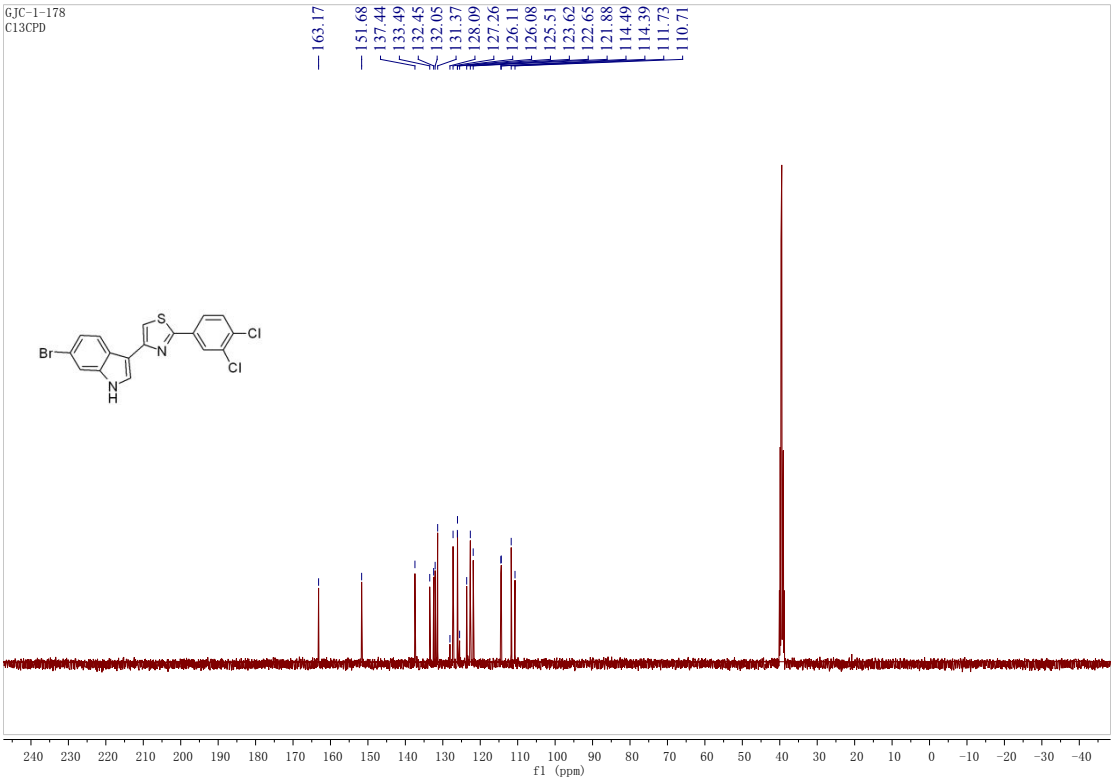

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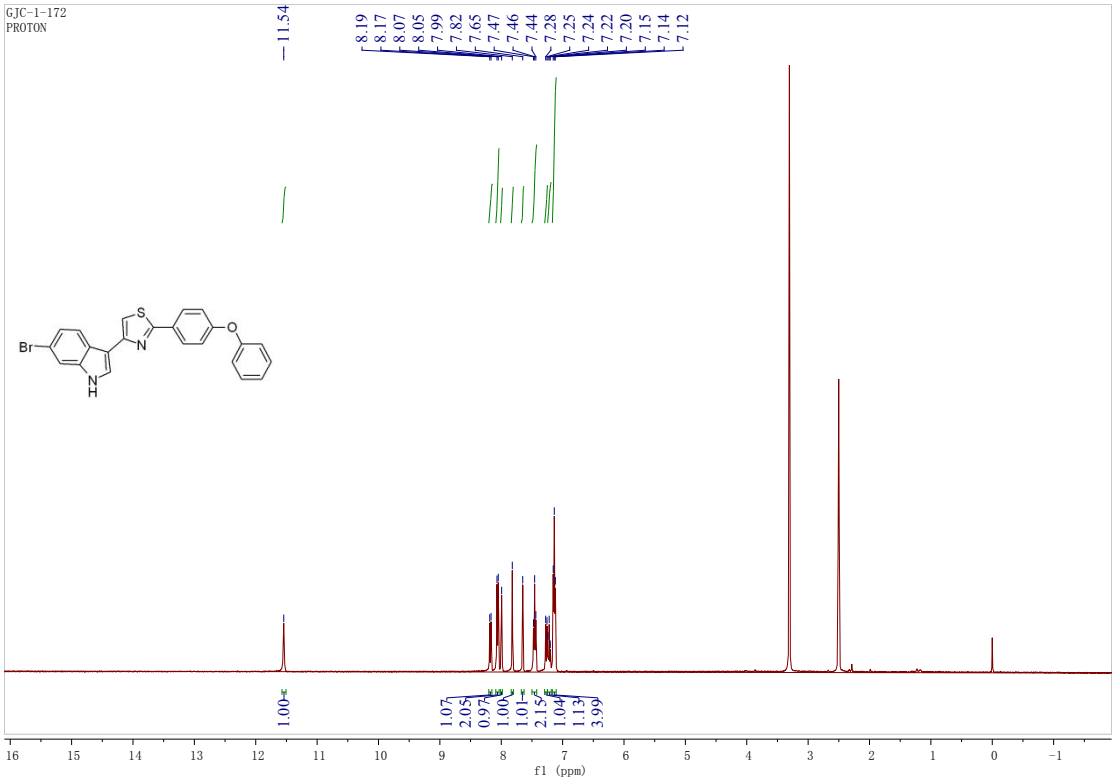

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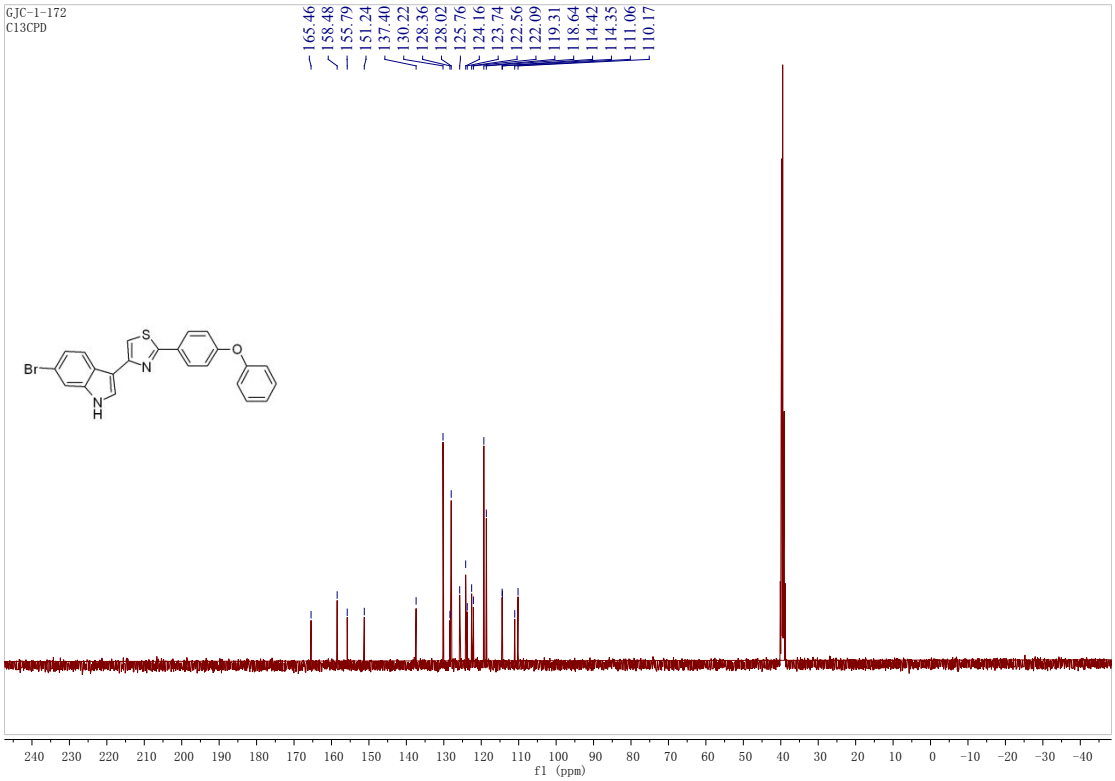

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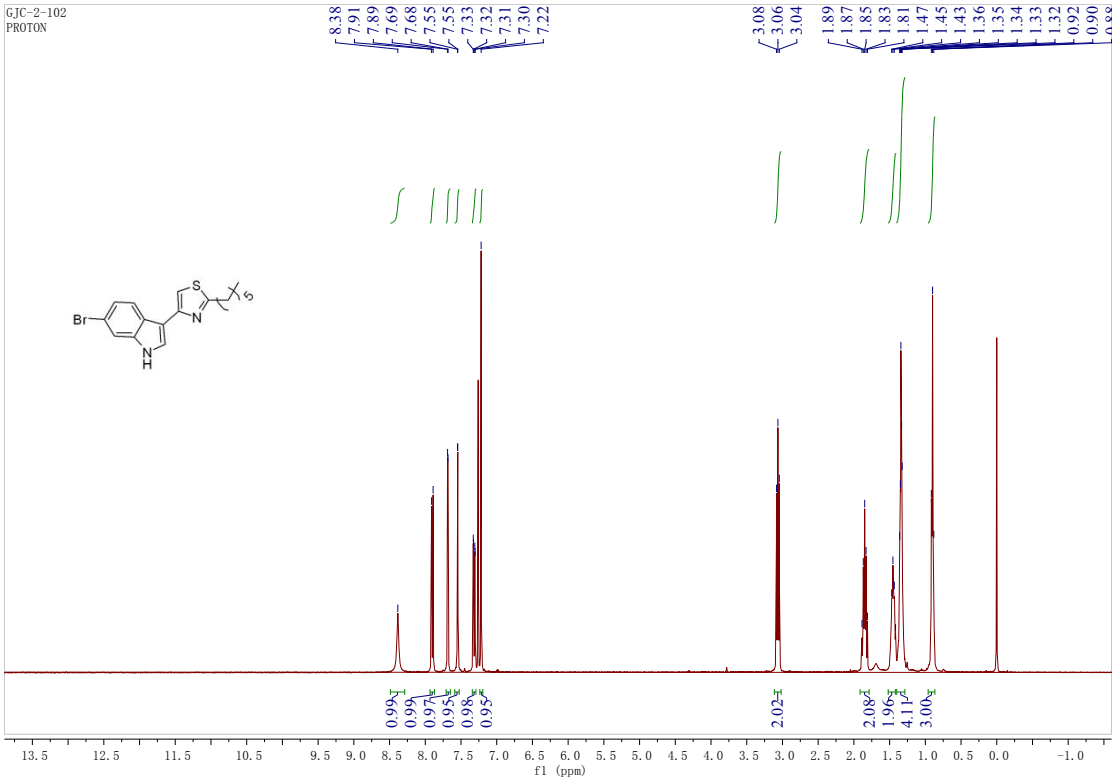

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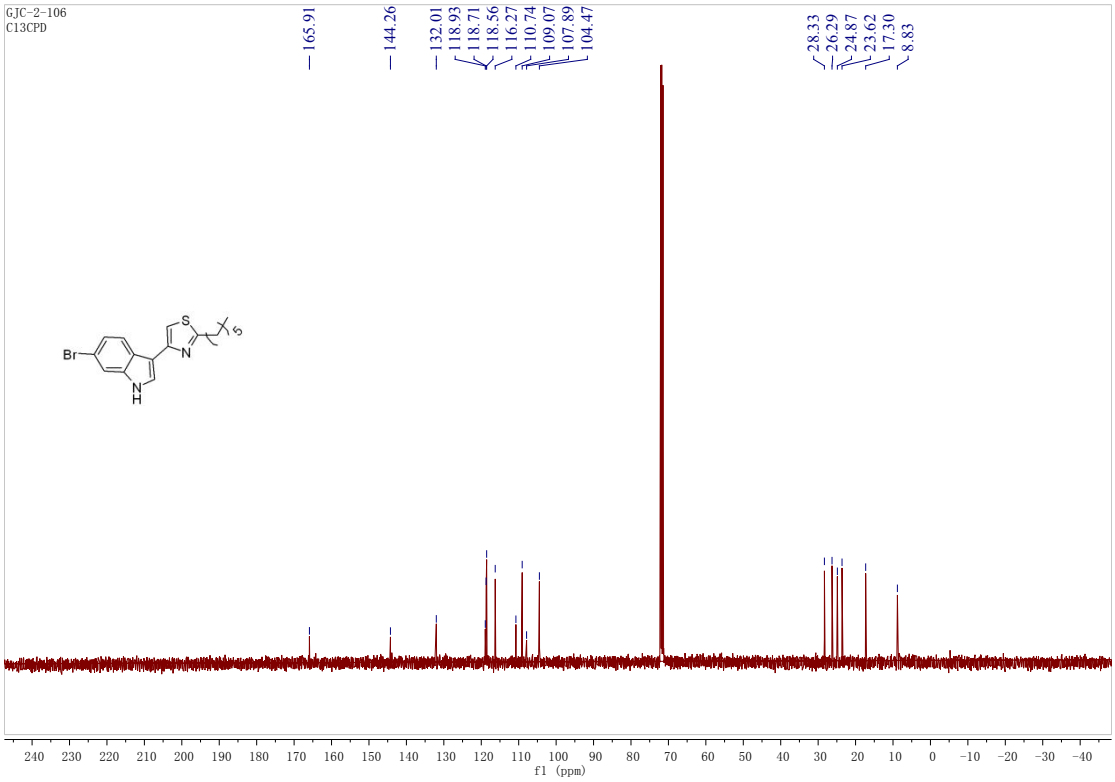

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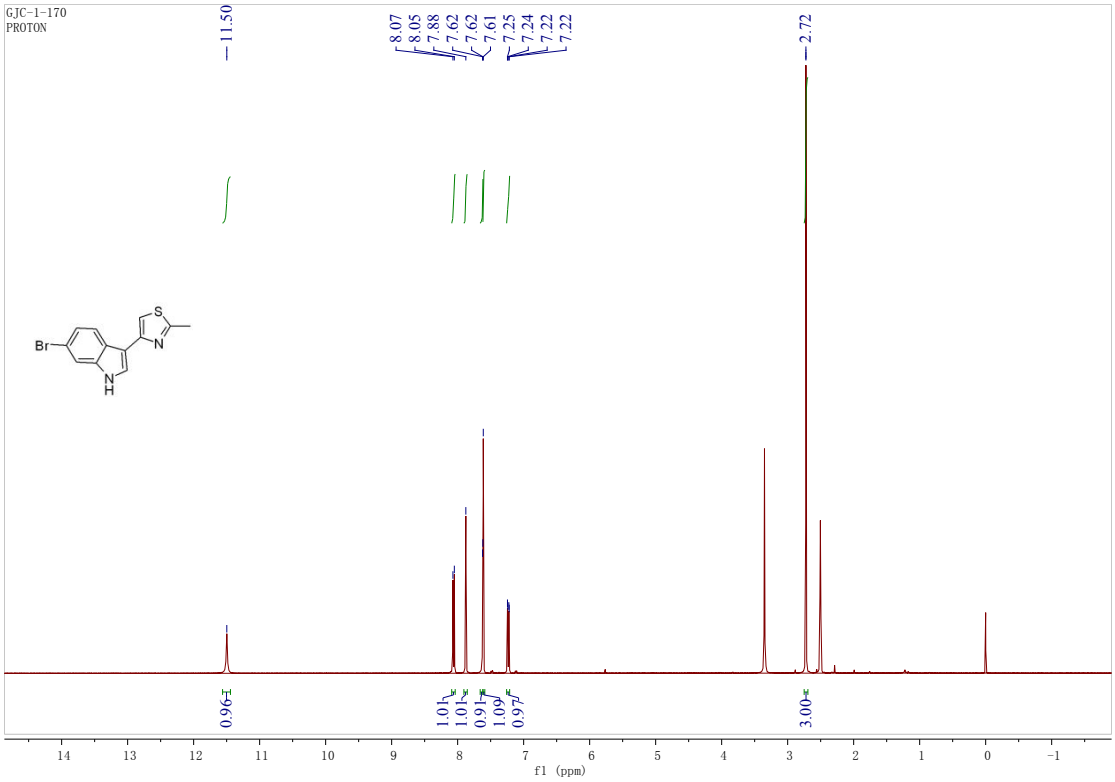

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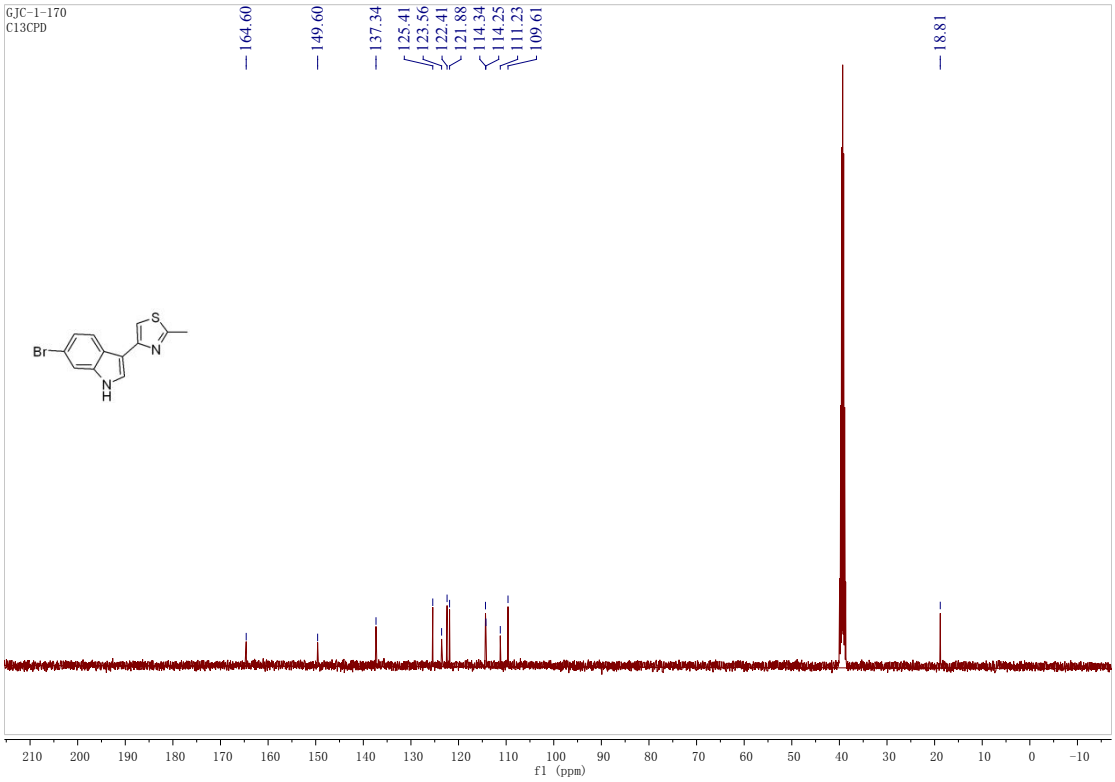

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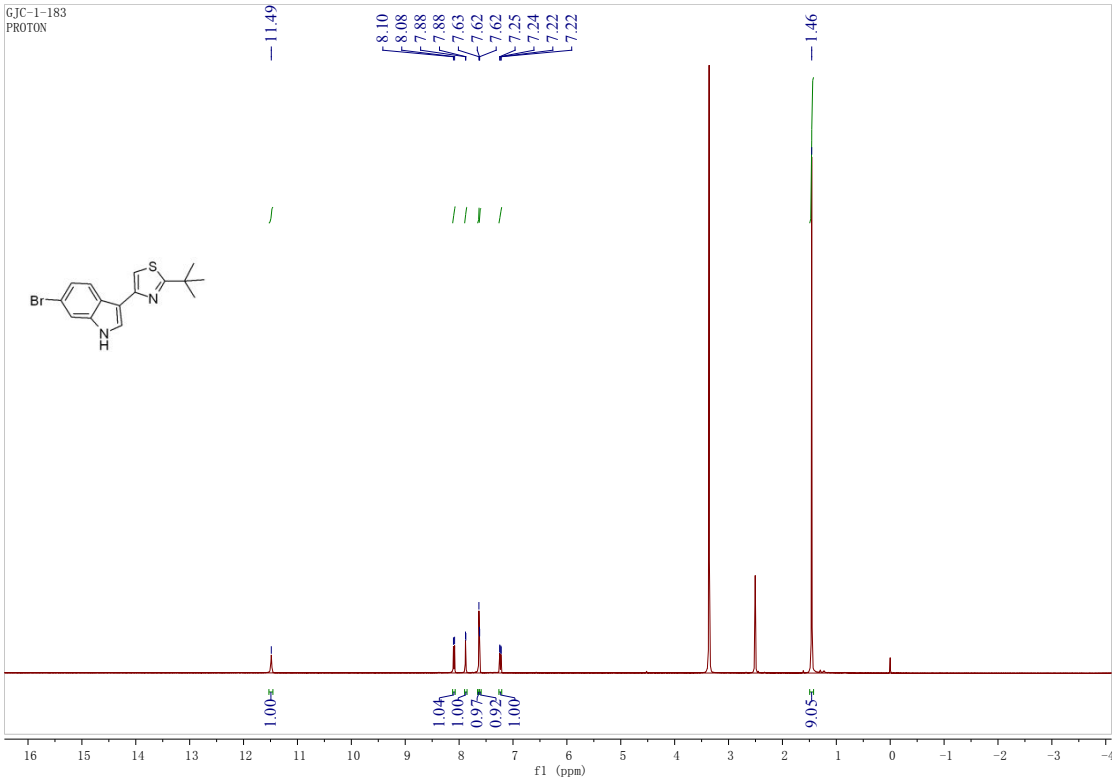

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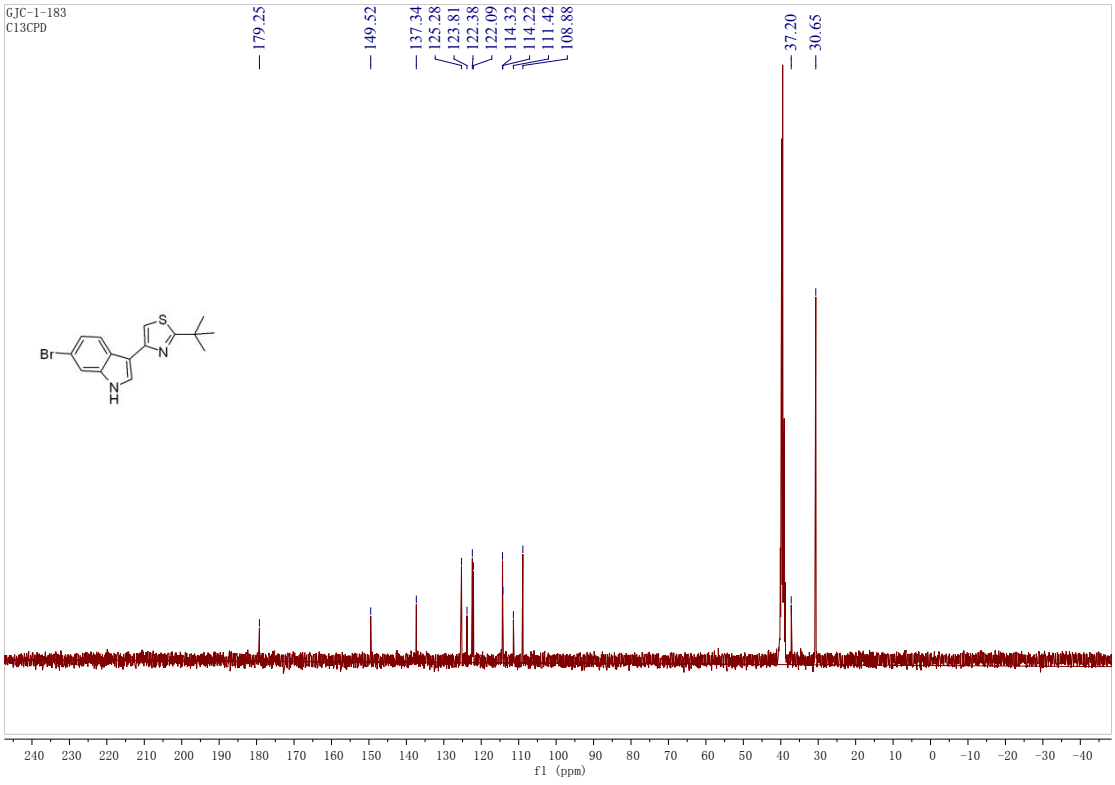

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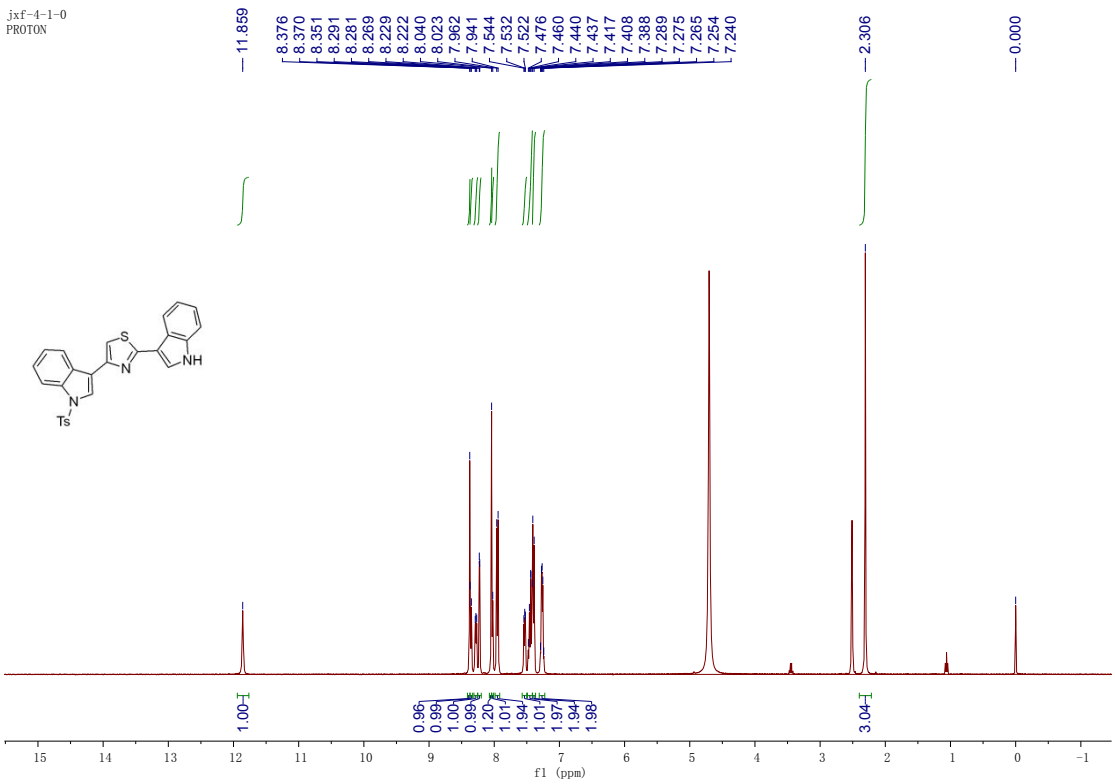

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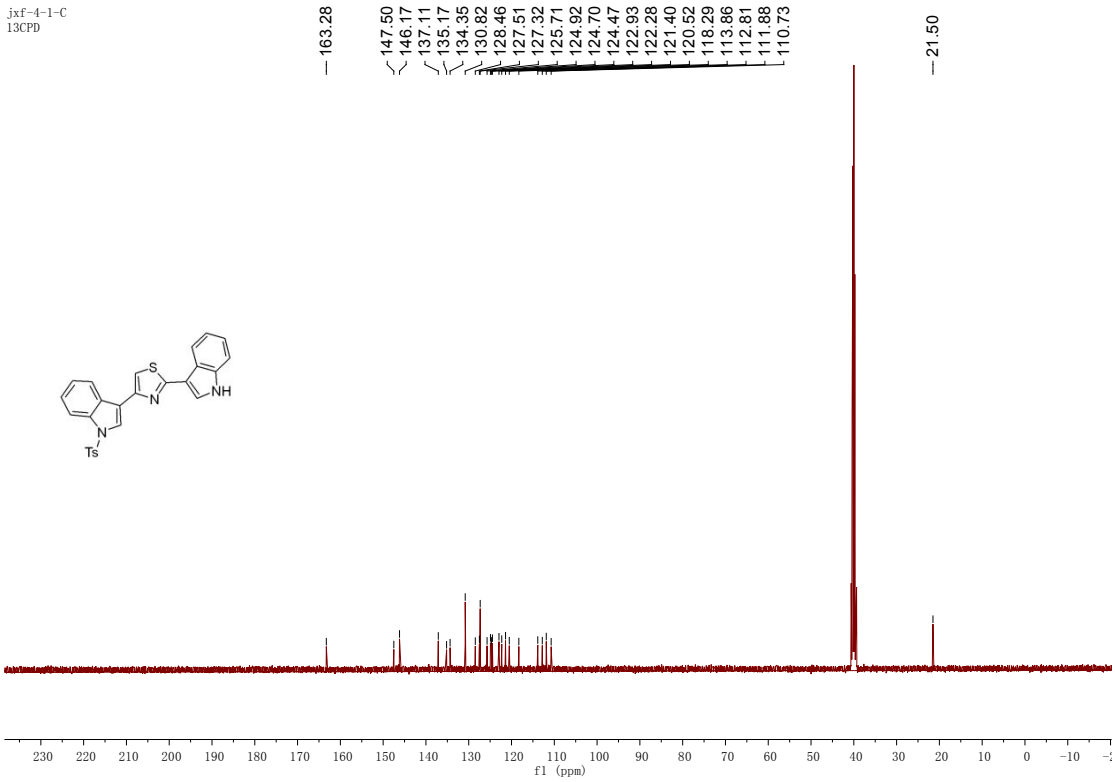

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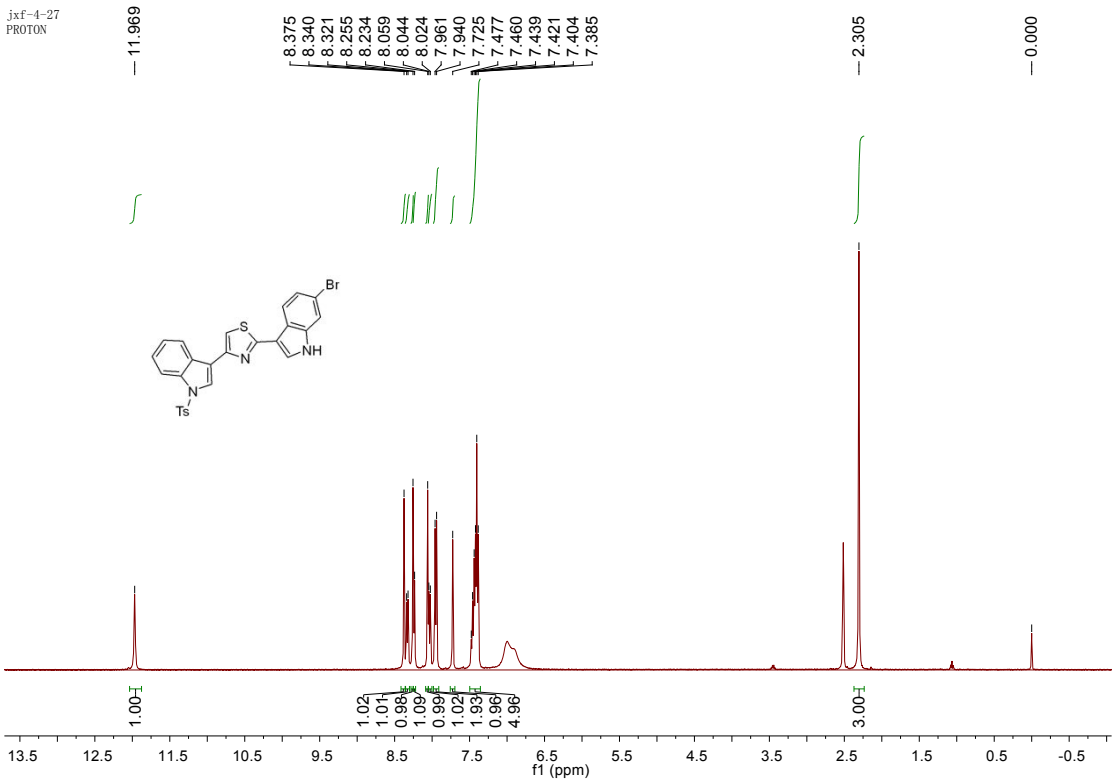

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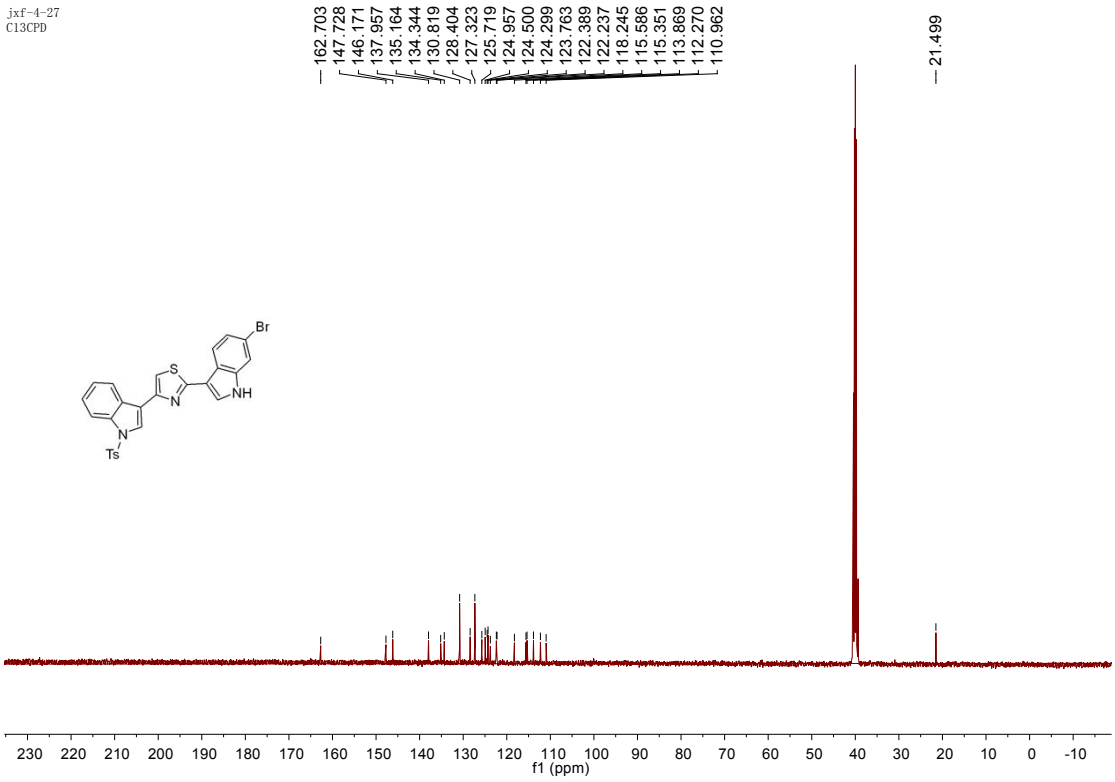

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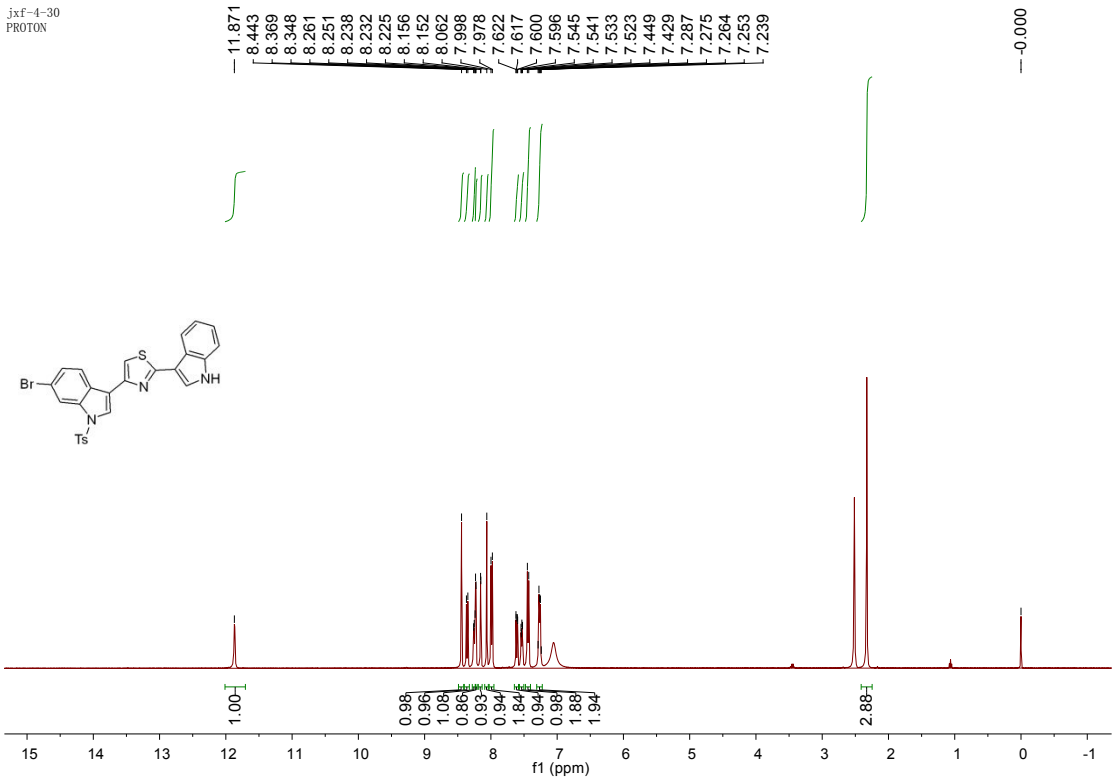

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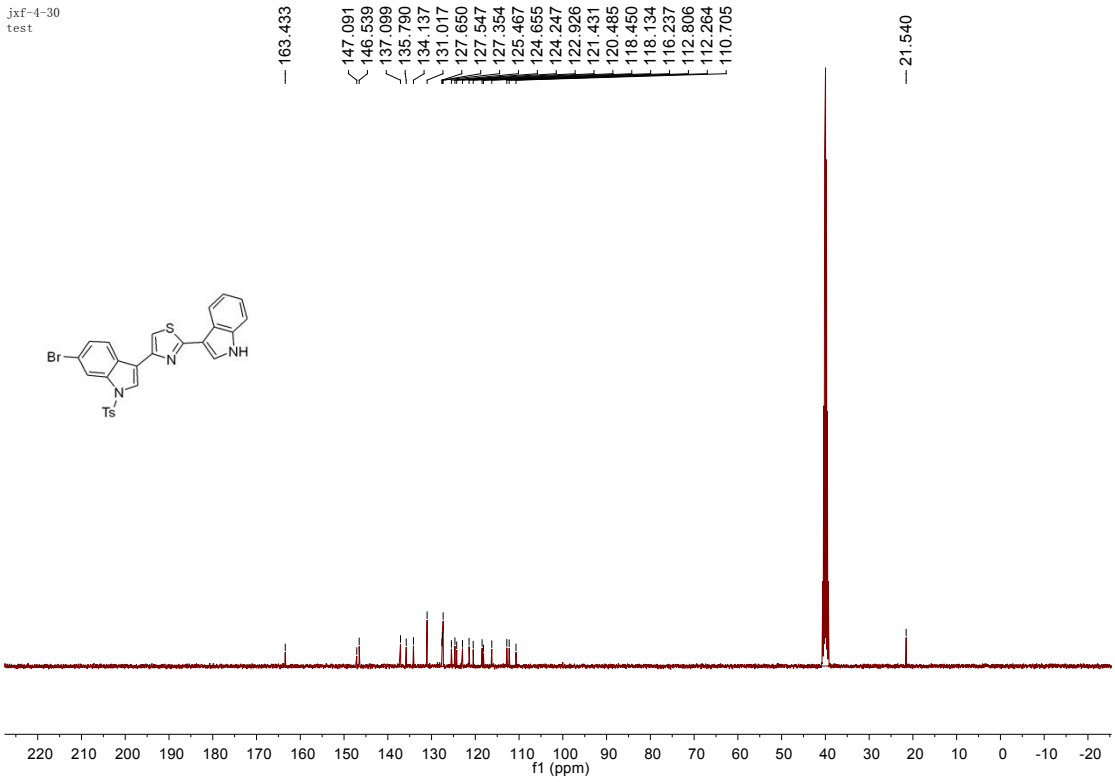

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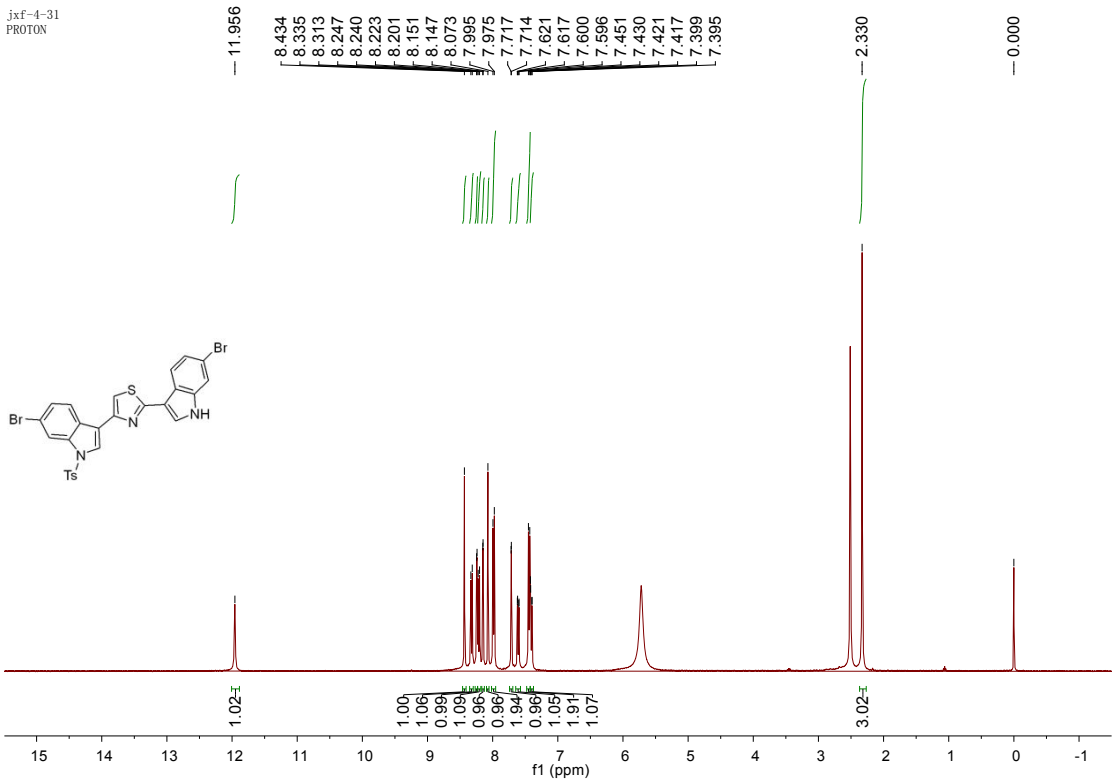

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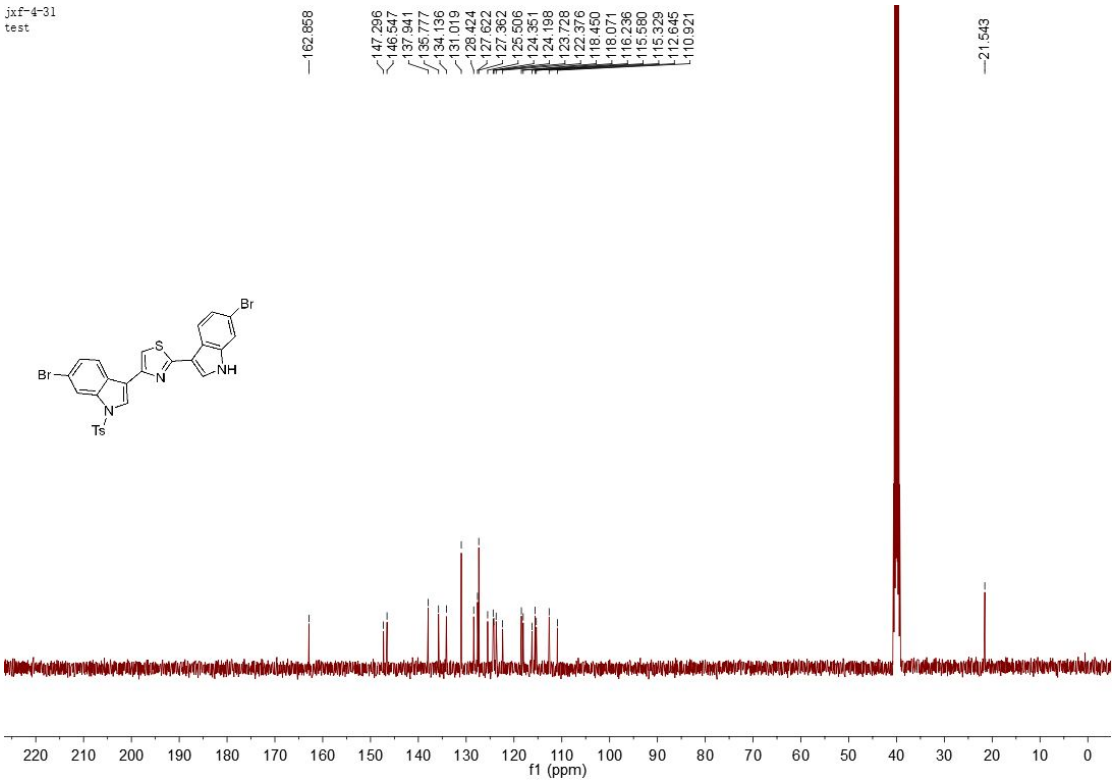
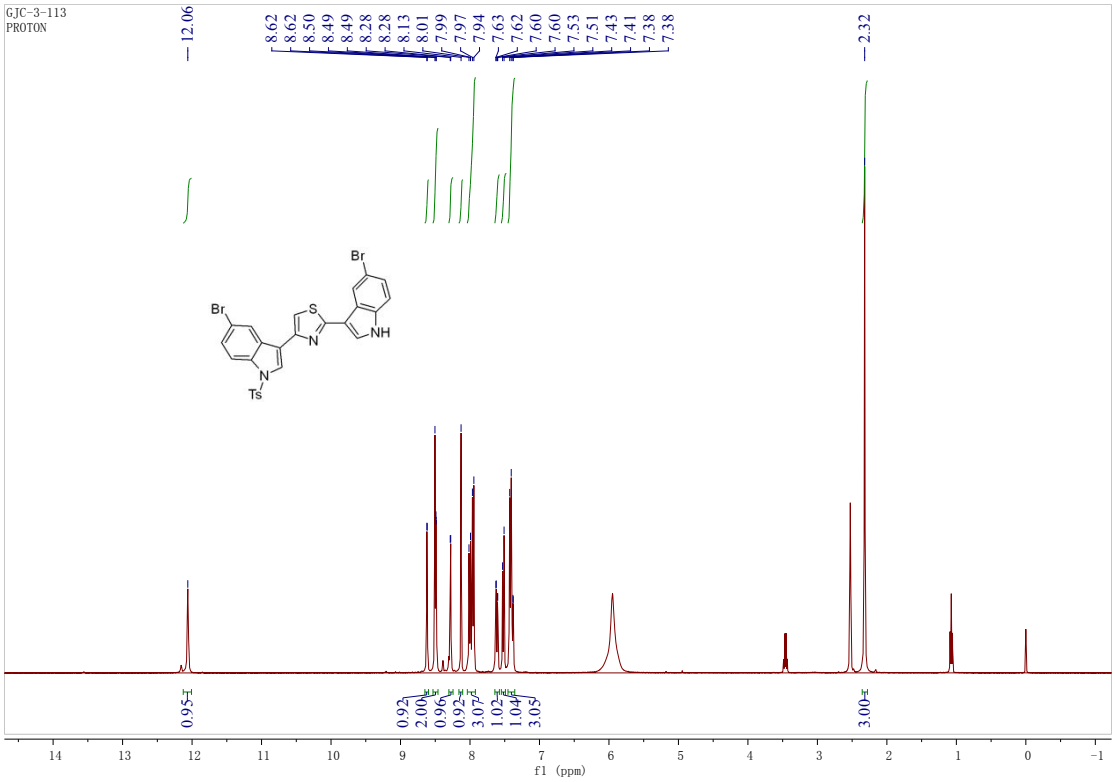
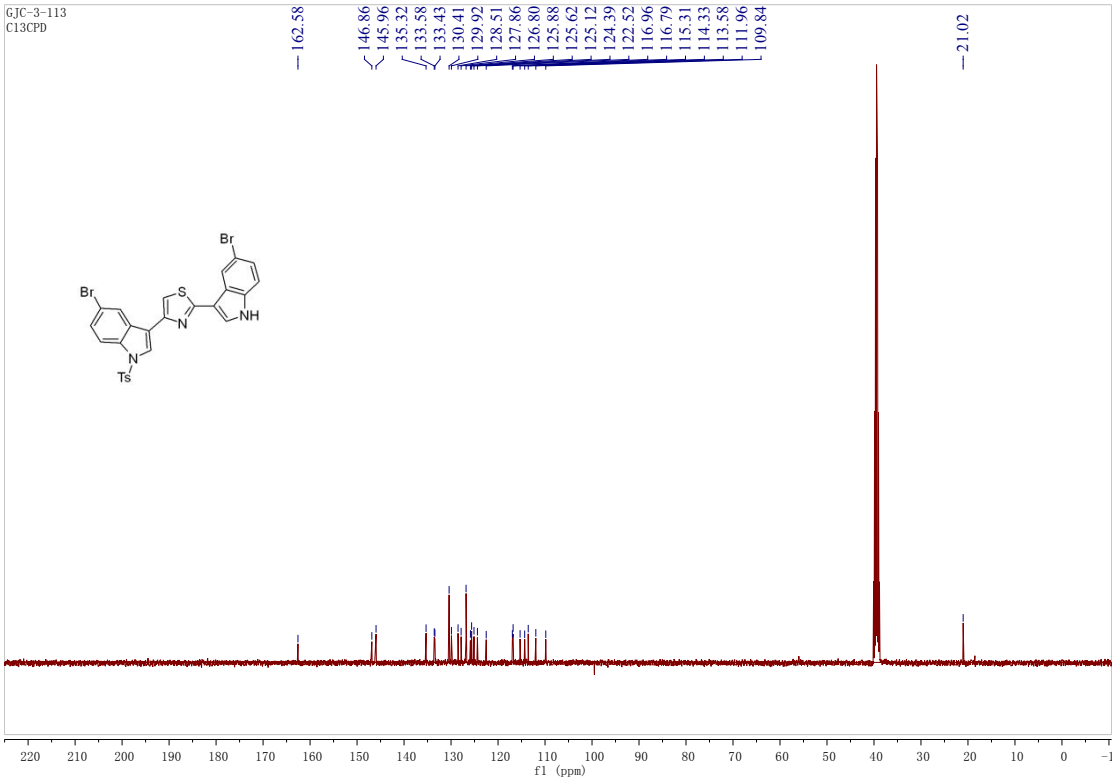


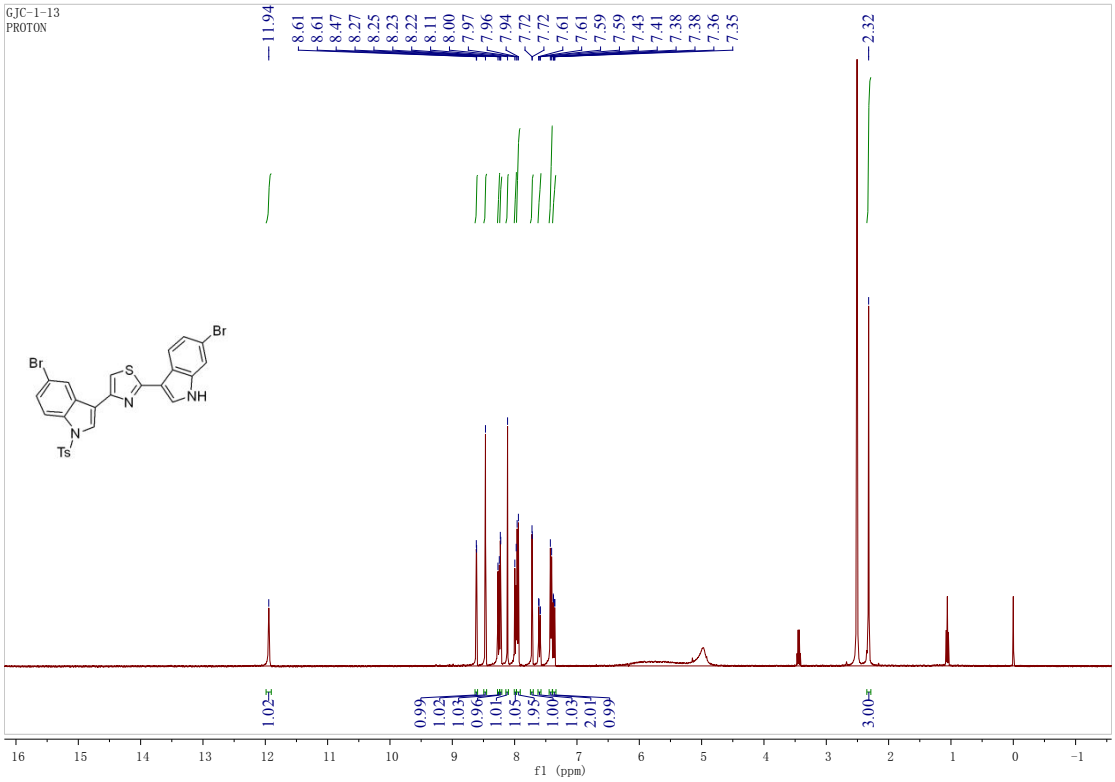
^{13}C NMR spectra of **2d**



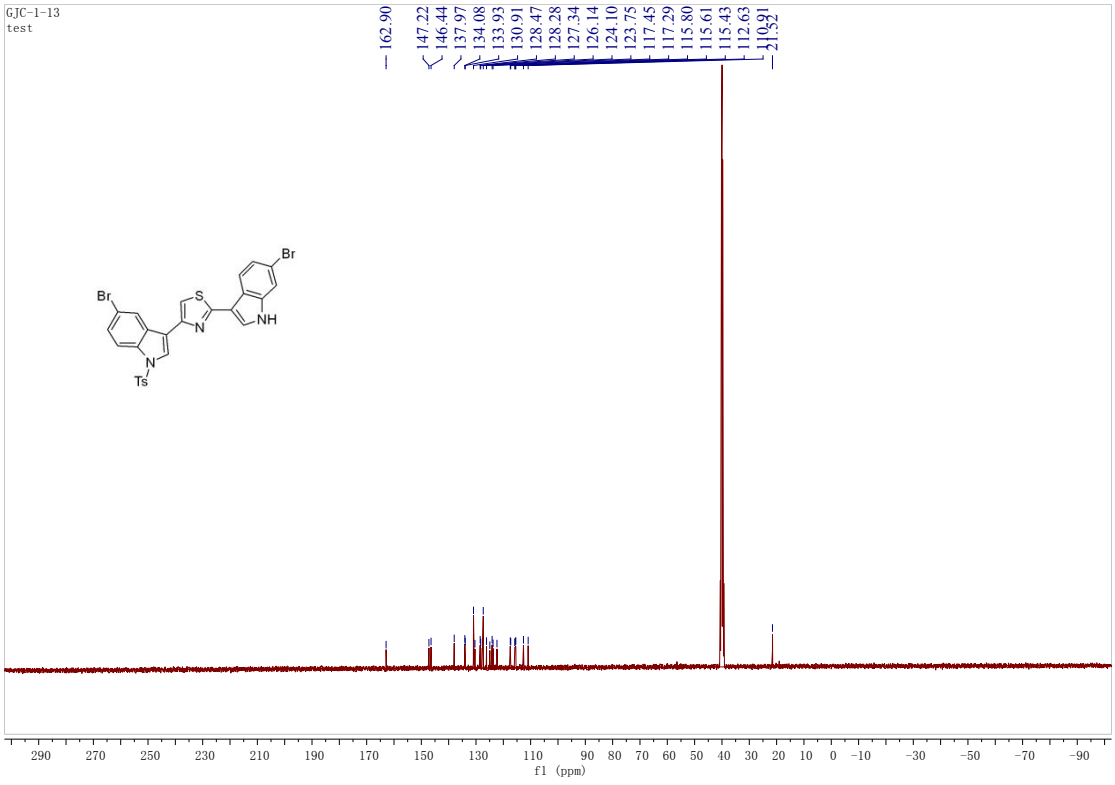
^{1}H NMR spectra of **2e**



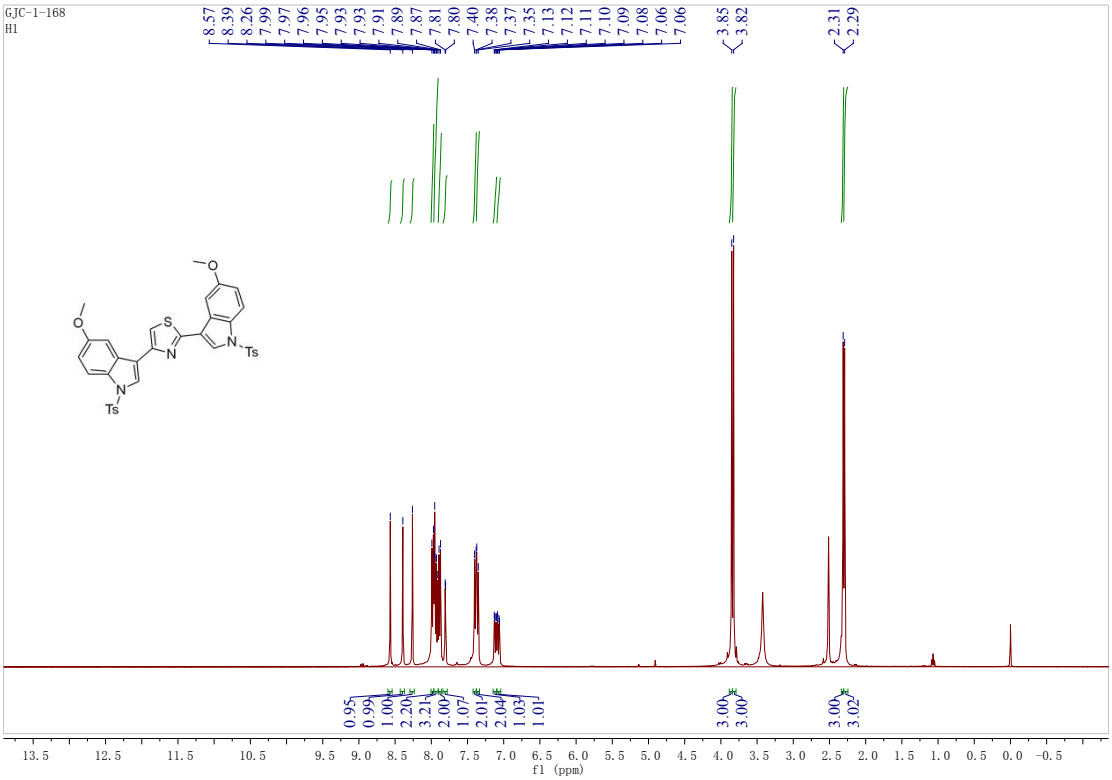
^{13}C NMR spectra of **2e**



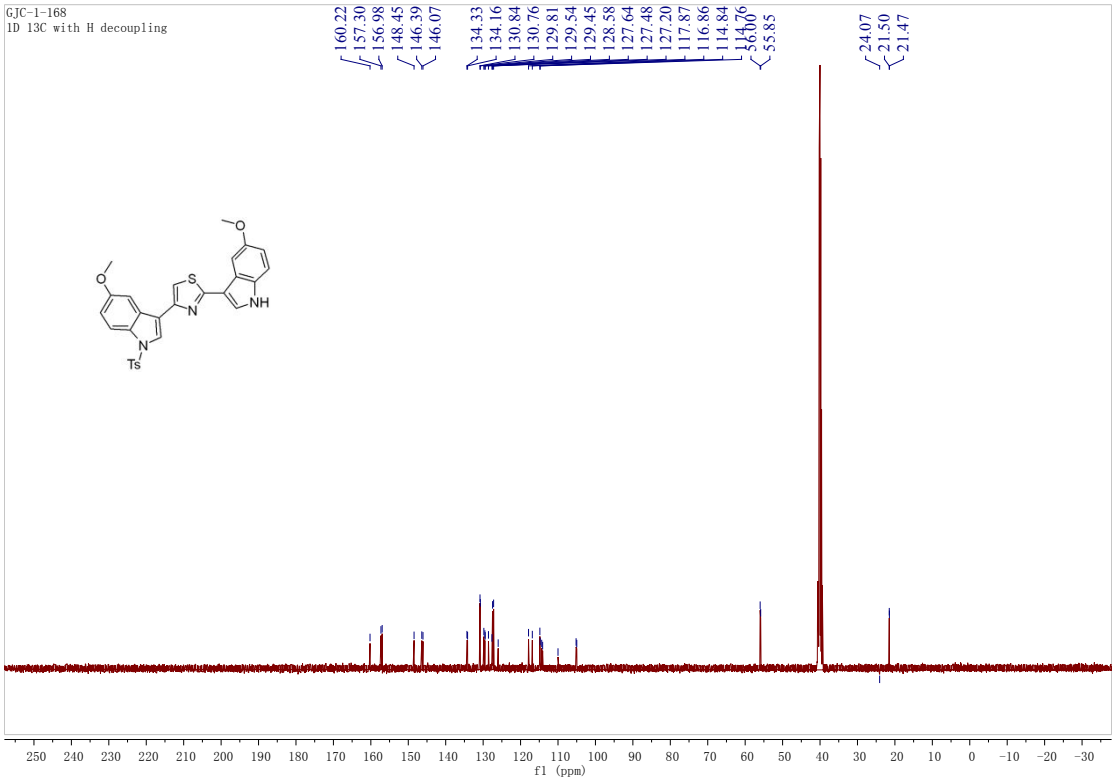
^{1}H NMR spectra of **2f**



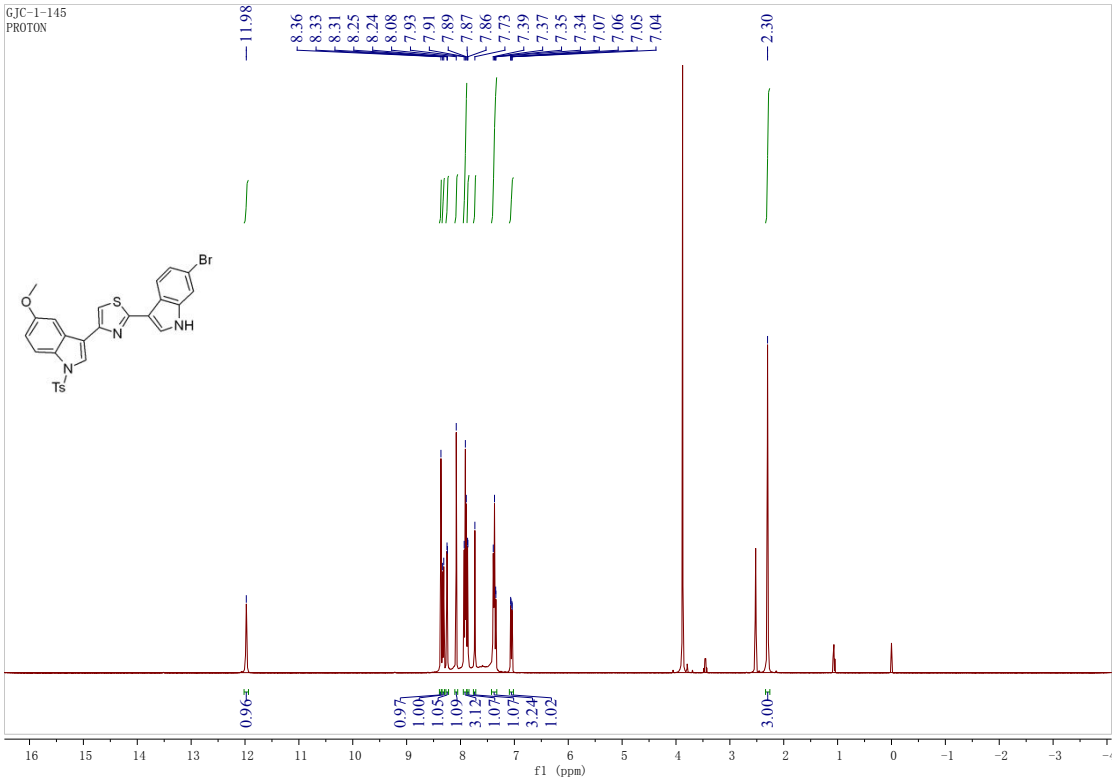
^{13}C NMR spectra of **2f**



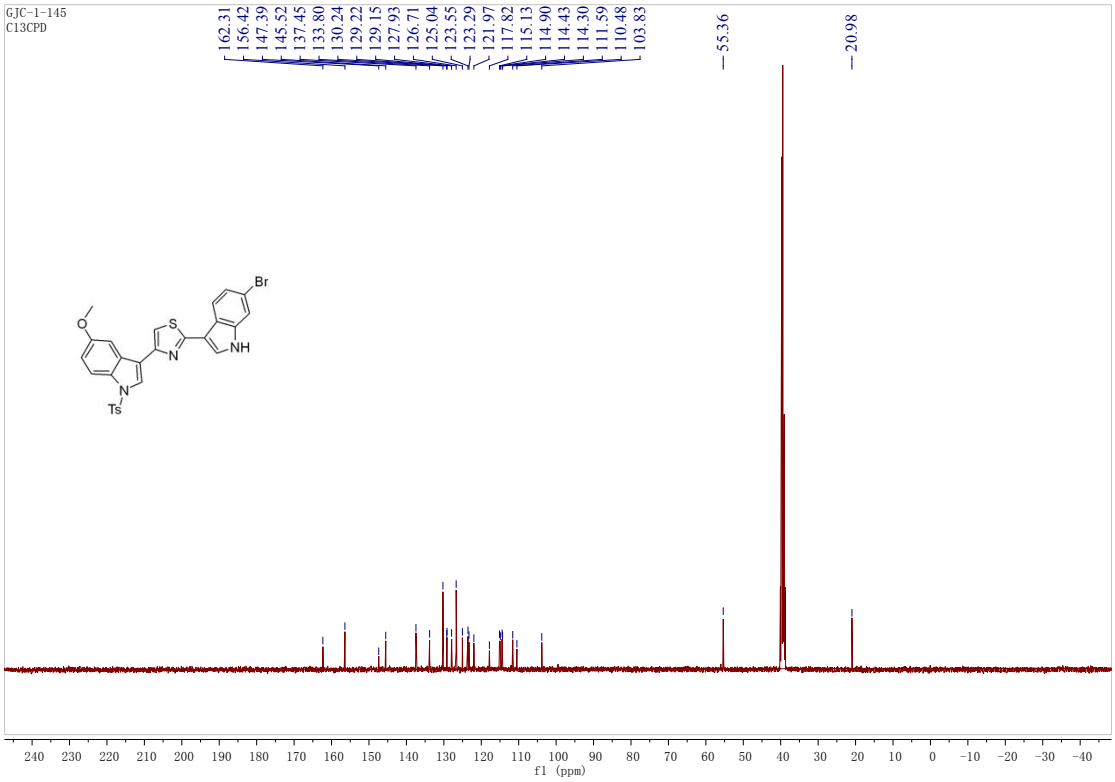
^{1}H NMR spectra of **2g**



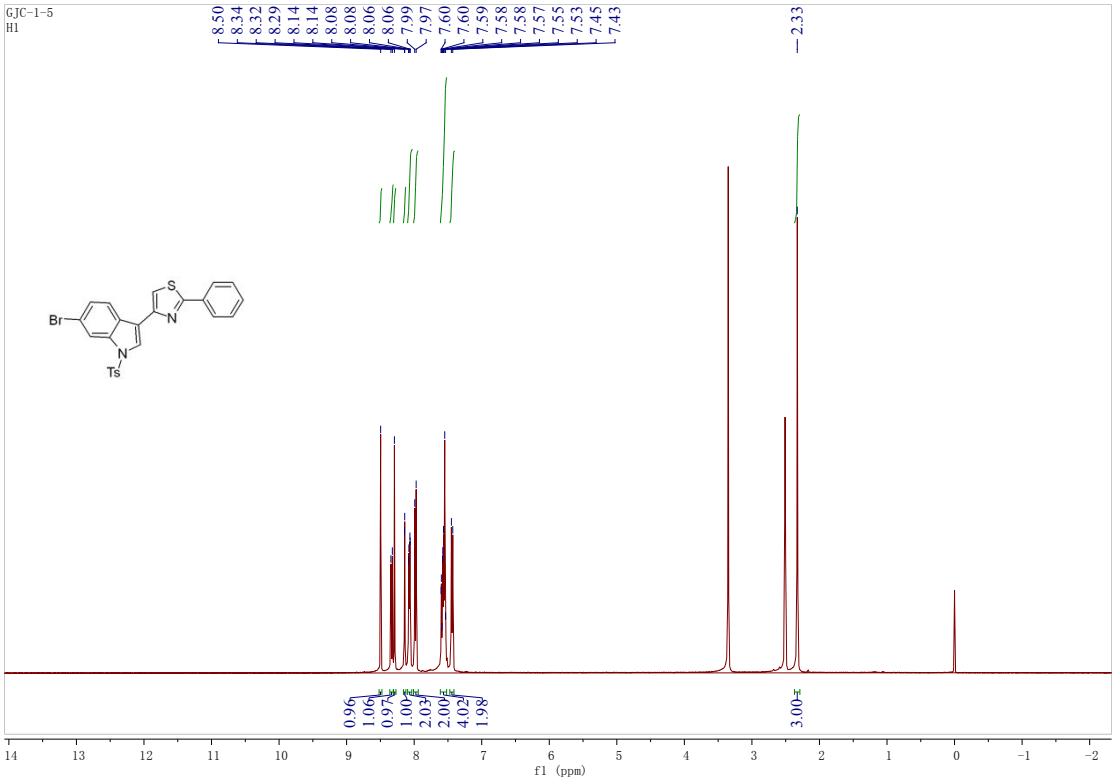
^{13}C NMR spectra of **2g**



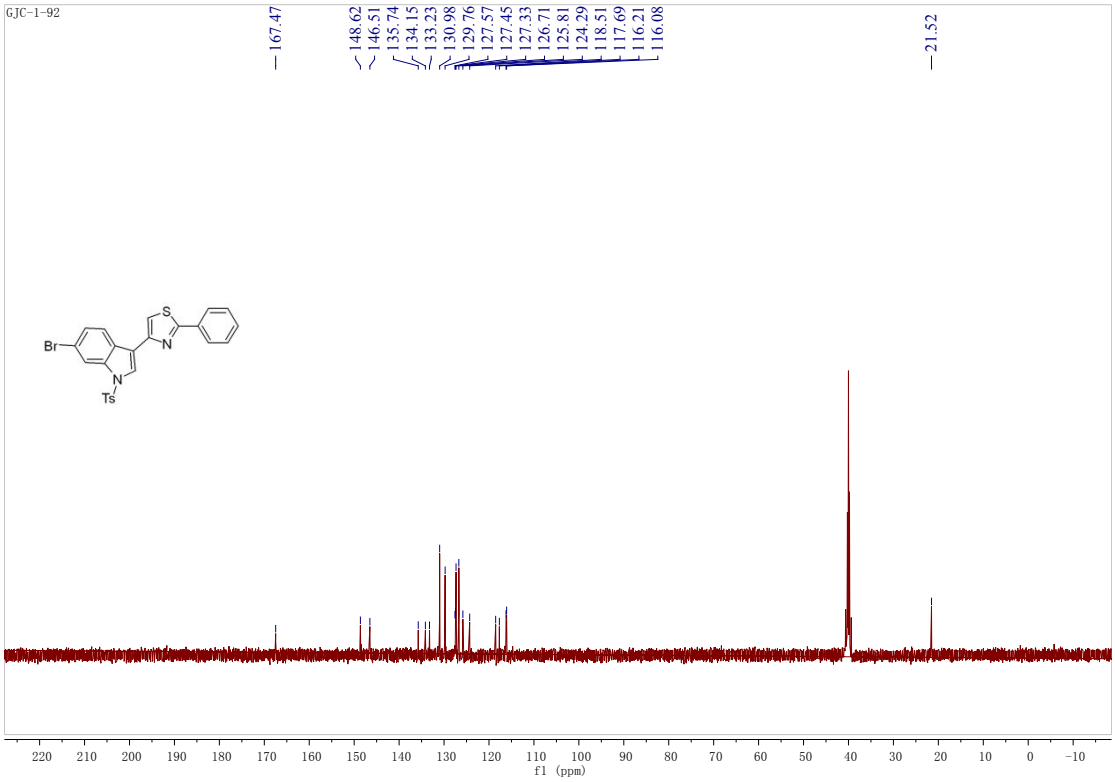
^{1}H NMR spectra of **2h**



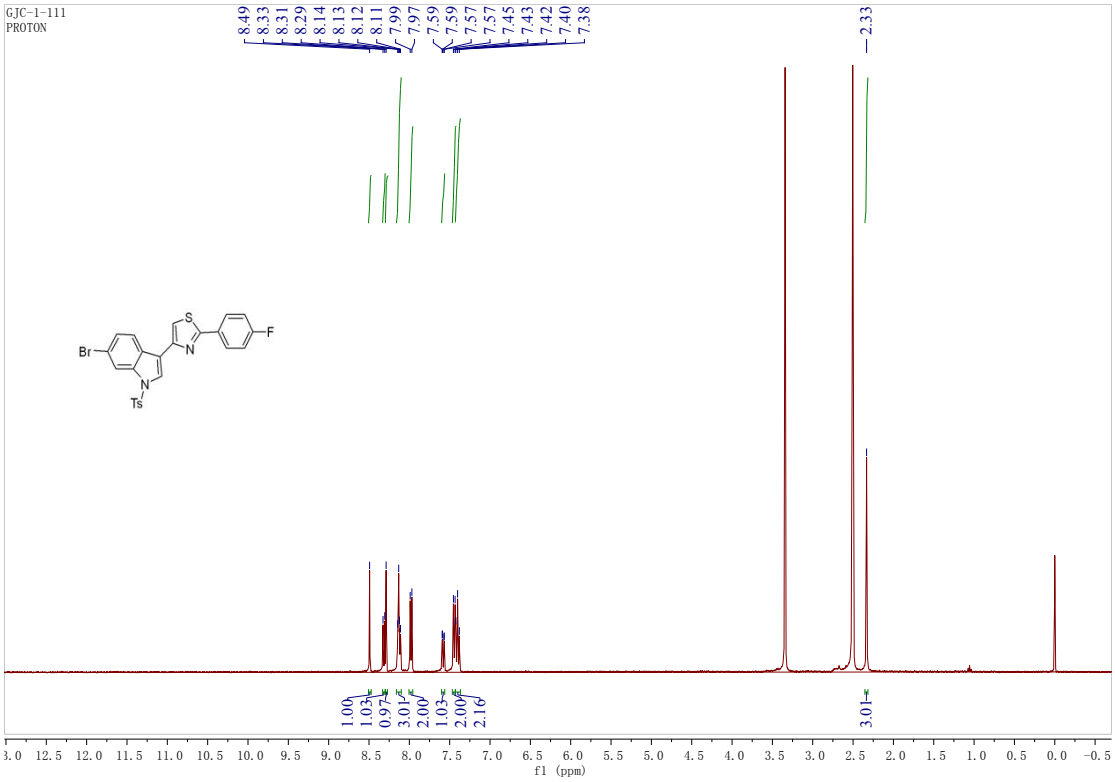
^{13}C NMR spectra of **2h**



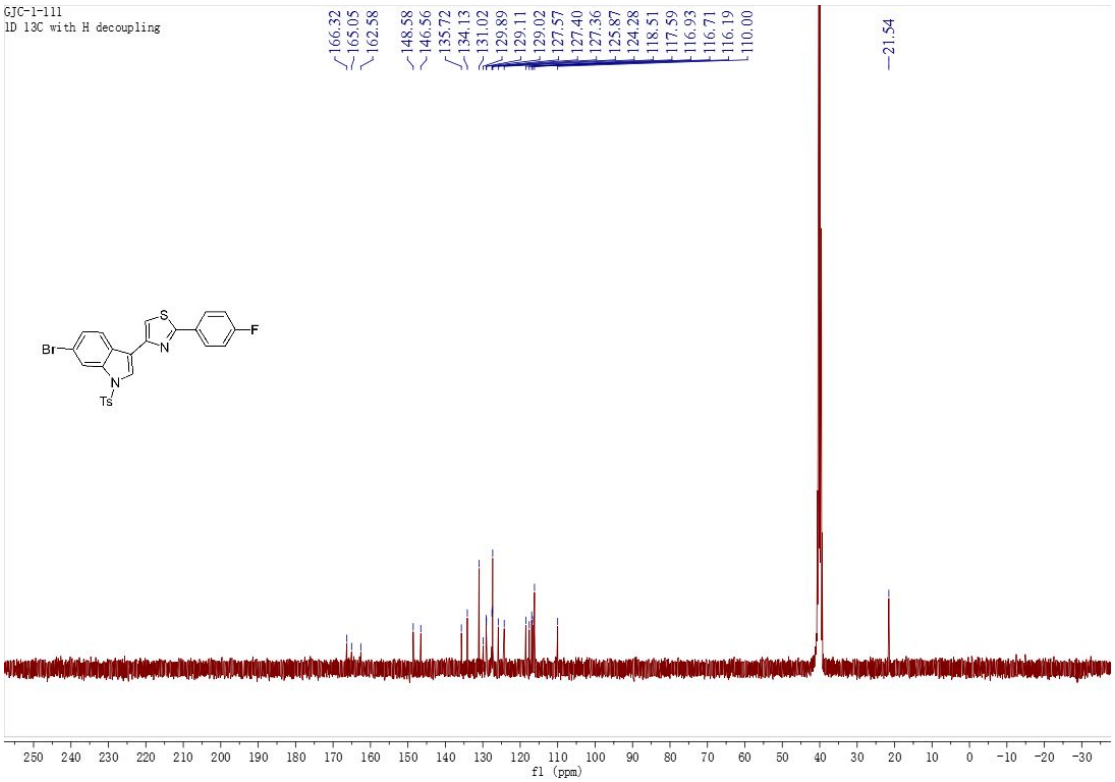
^{1}H NMR spectra of **2i**



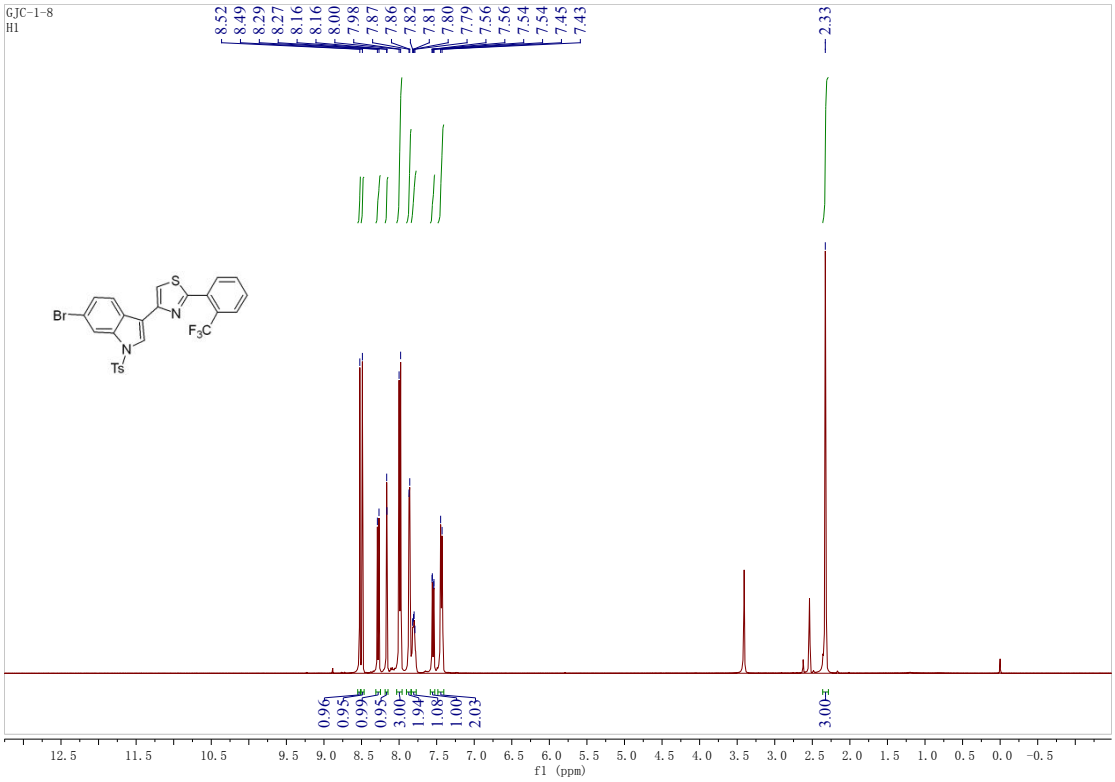
^{13}C NMR spectra of **2i**



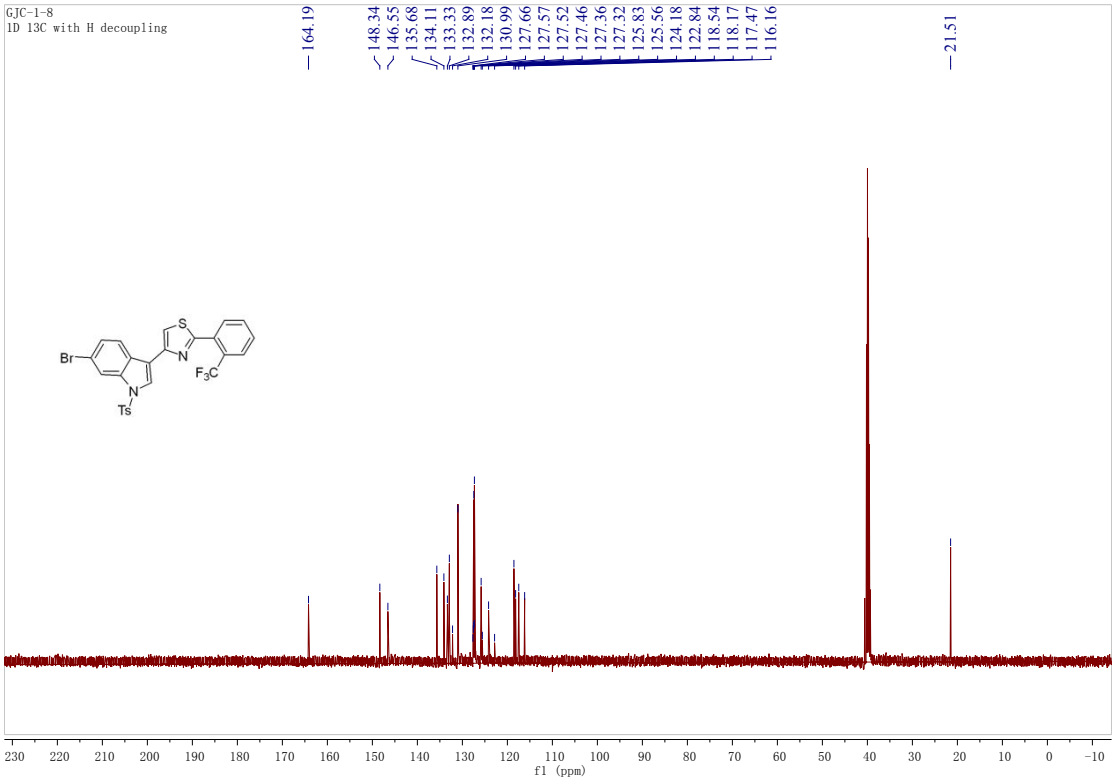
^{1}H NMR spectra of **2j**



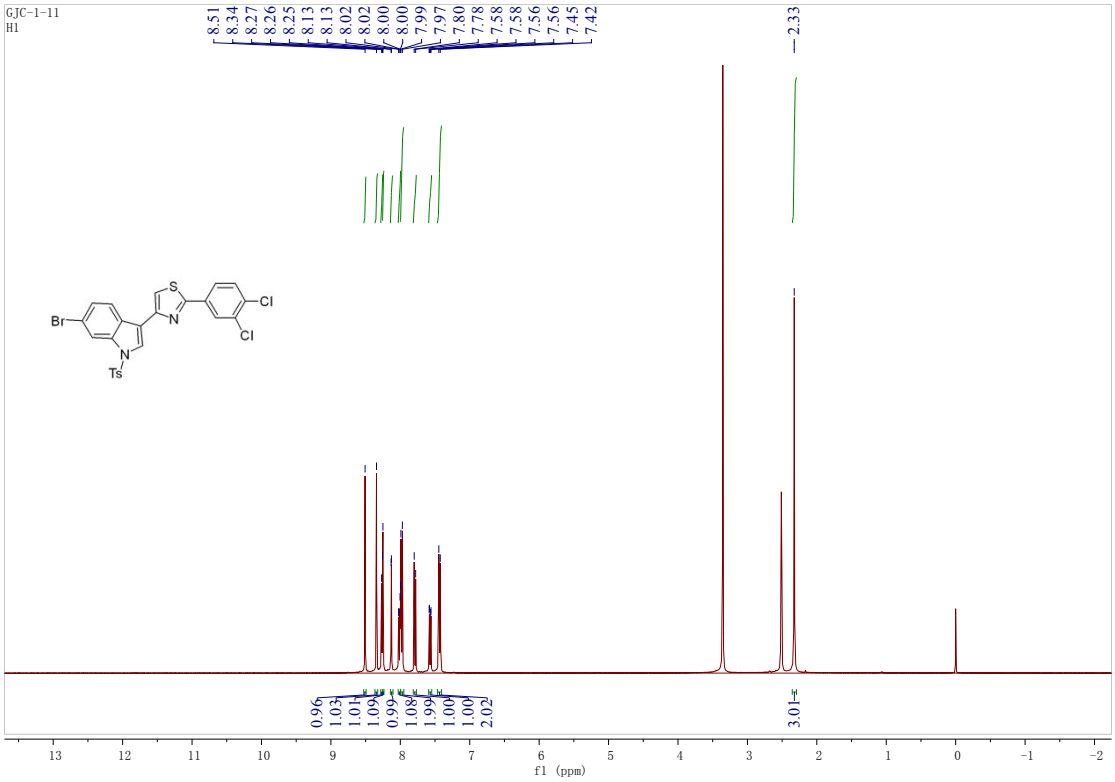
^{13}C NMR spectra of **2j**



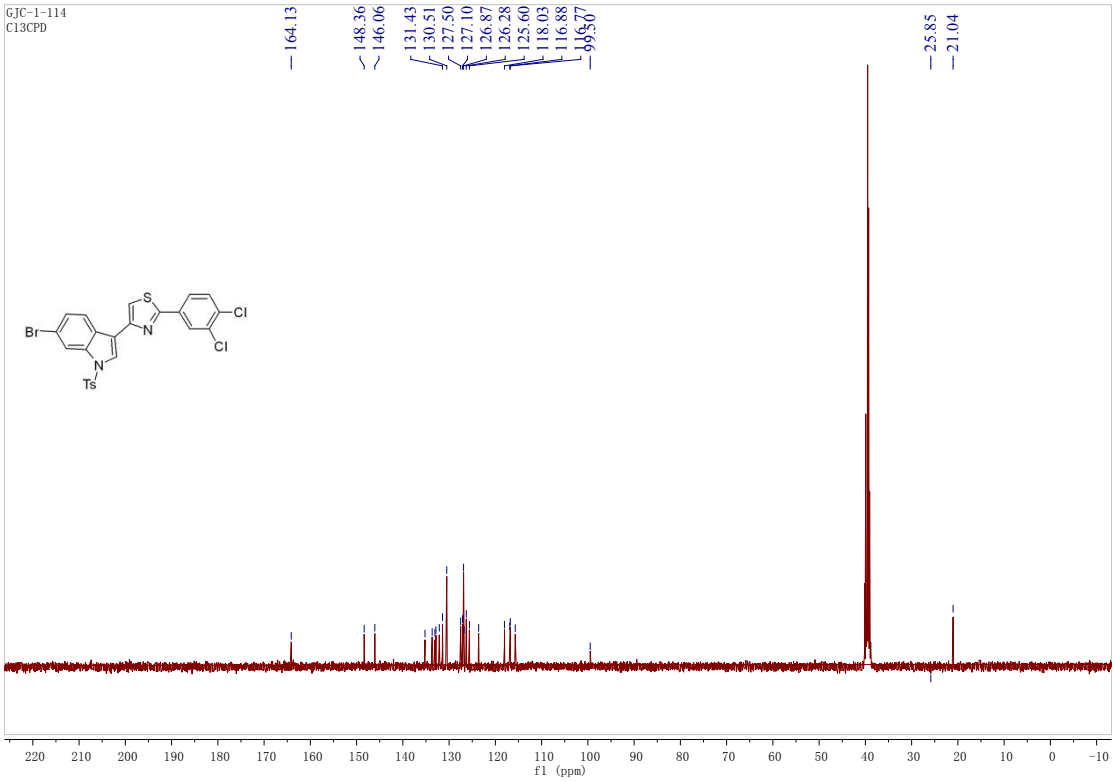
^{1}H NMR spectra of **2k**



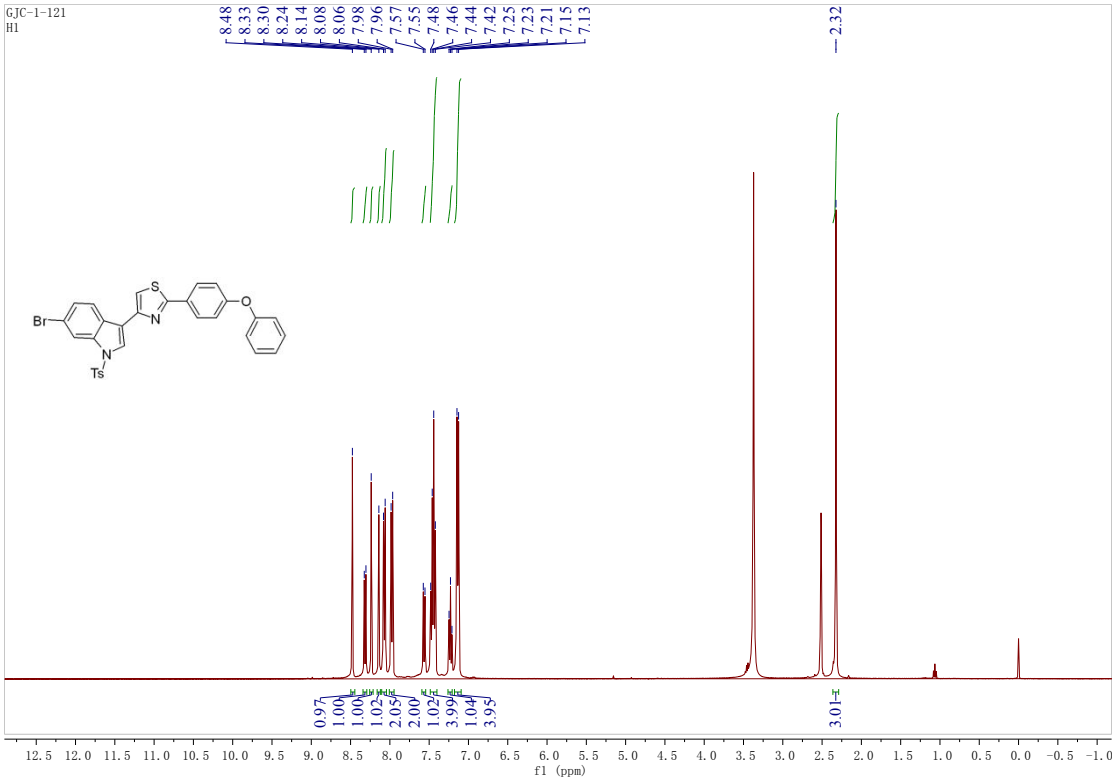
^{13}C NMR spectra of **2k**



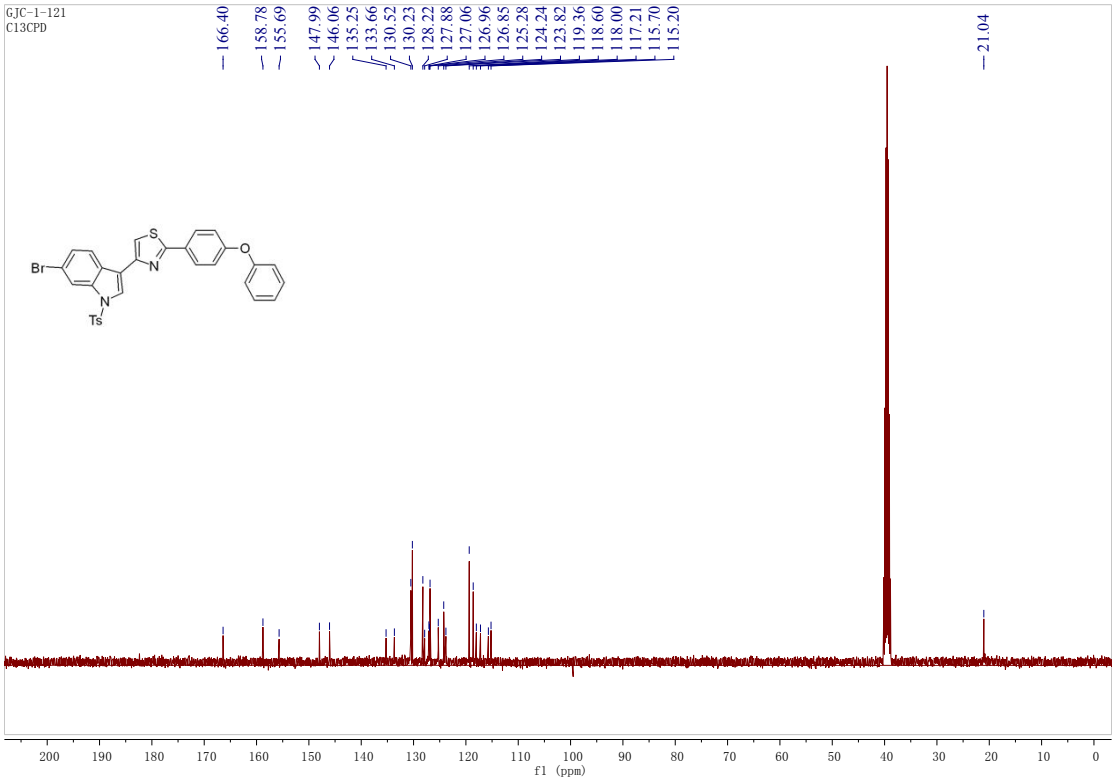
^{1}H NMR spectra of **2l**



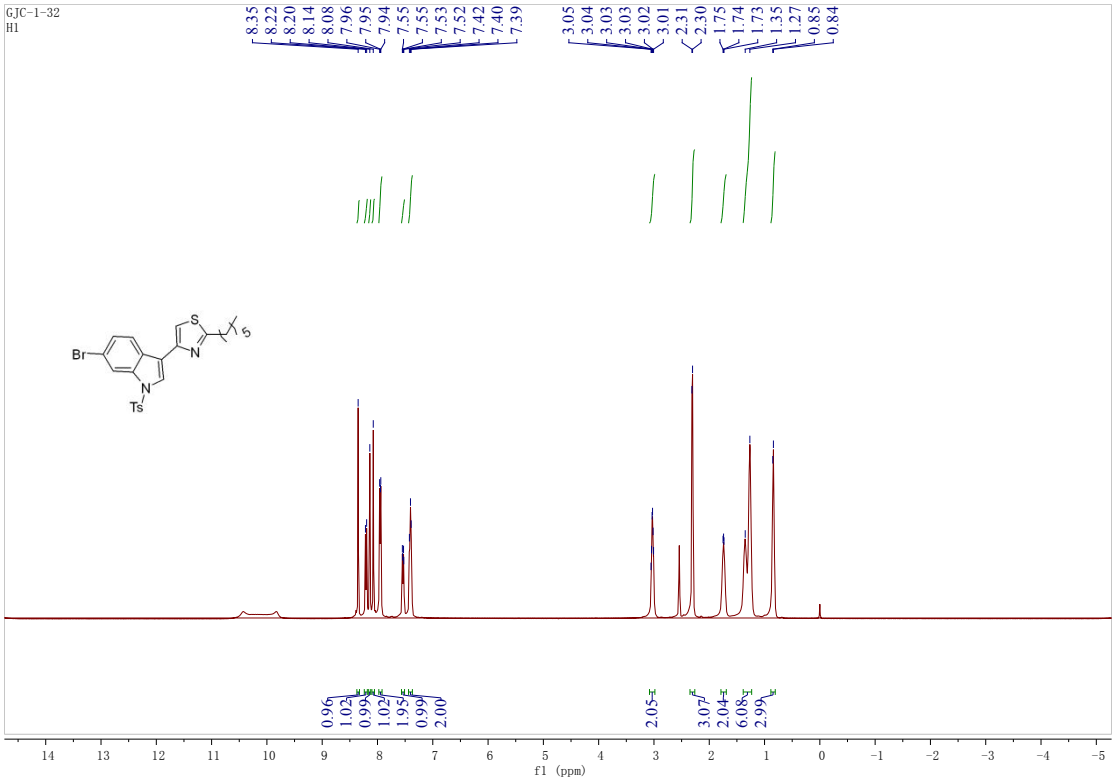
^{13}C NMR spectra of **2l**



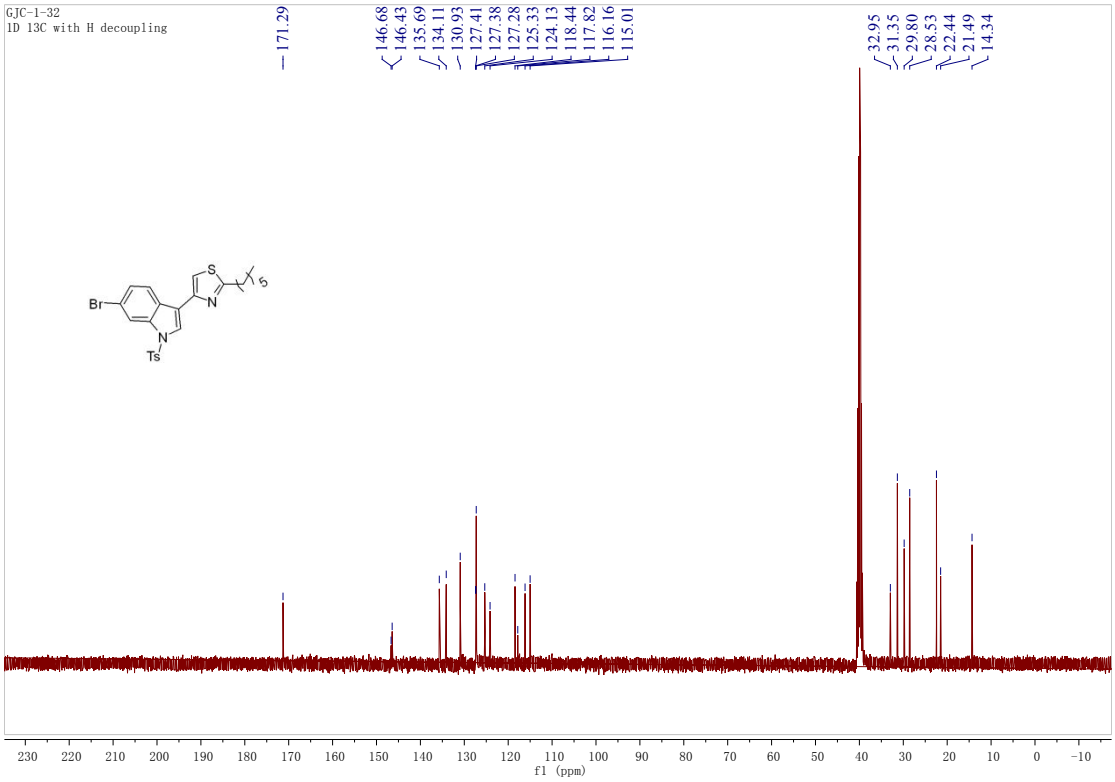
^{1}H NMR spectra of **2m**



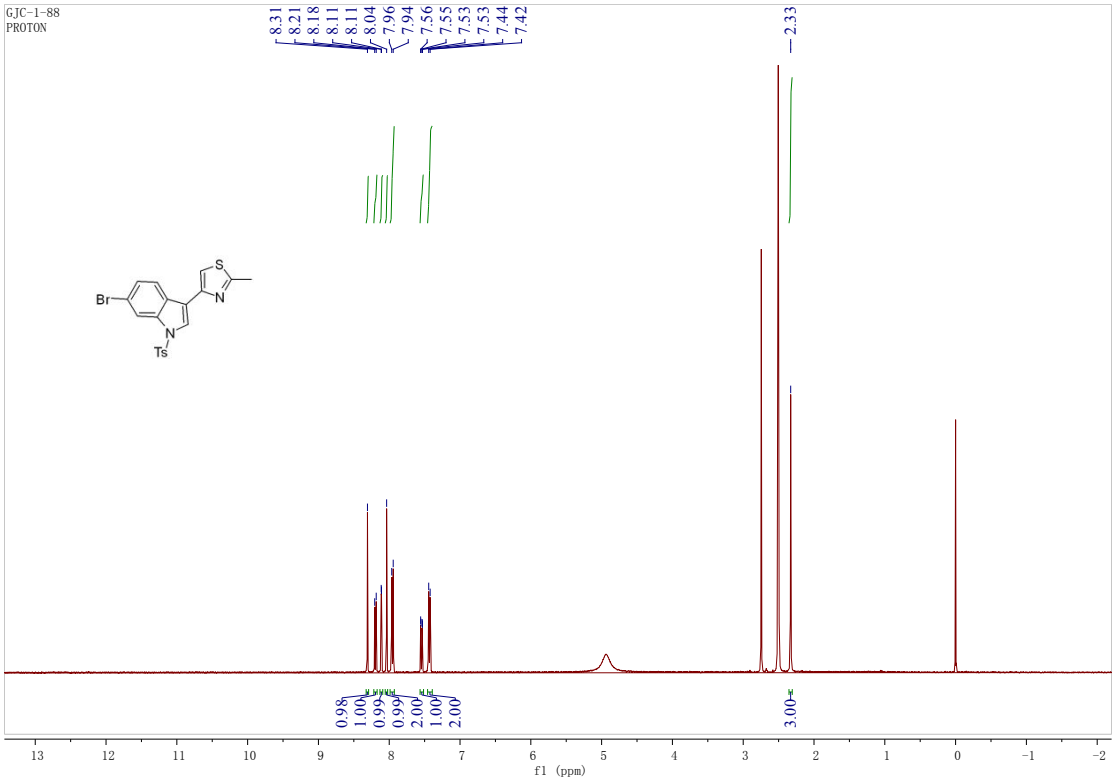
^{13}C NMR spectra of **2m**



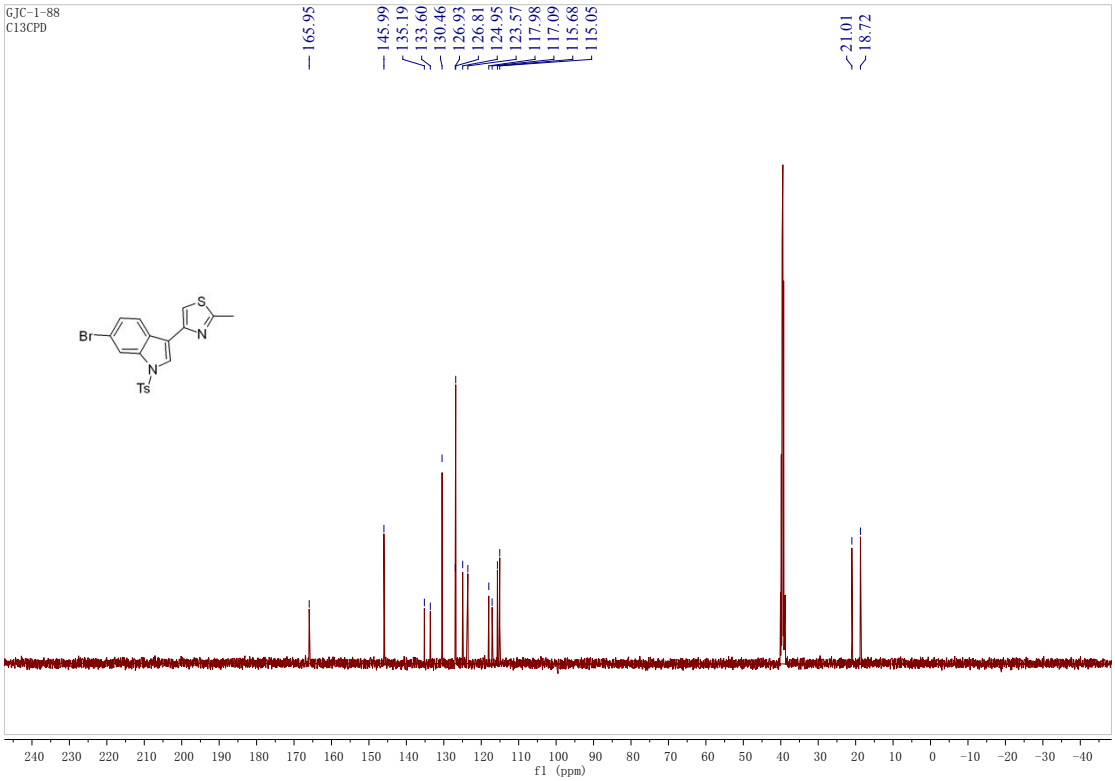
^{1}H NMR spectra of **2n**



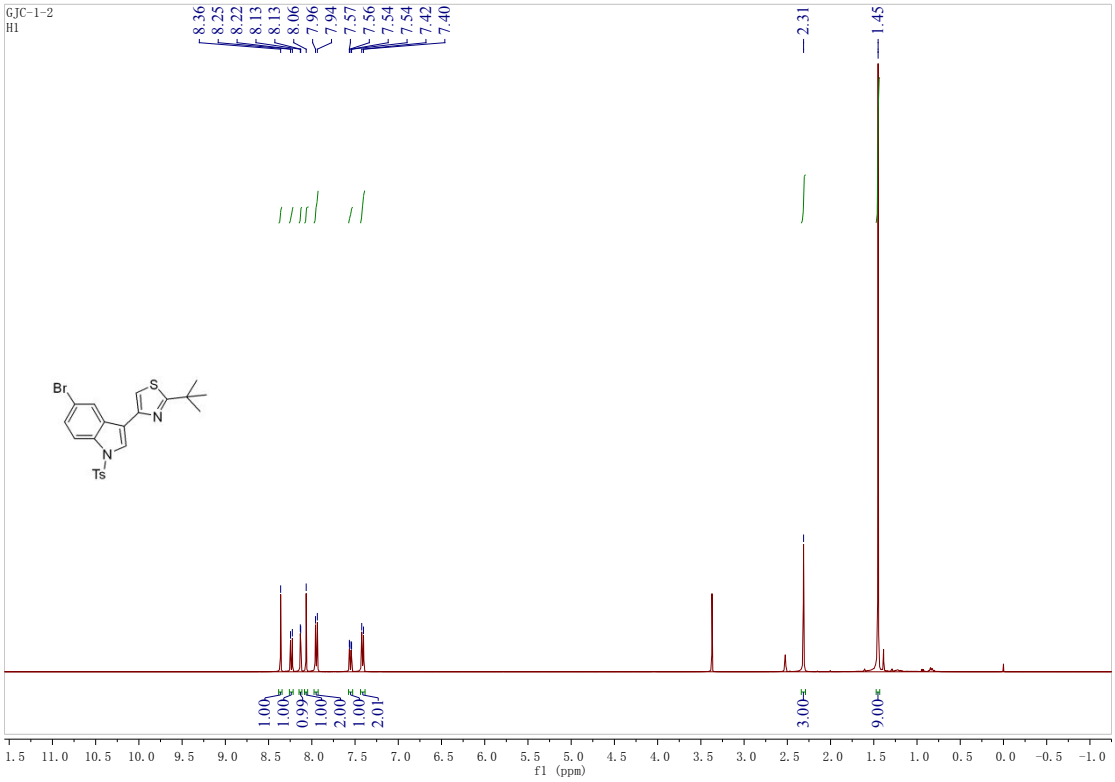
^{13}C NMR spectra of **2n**



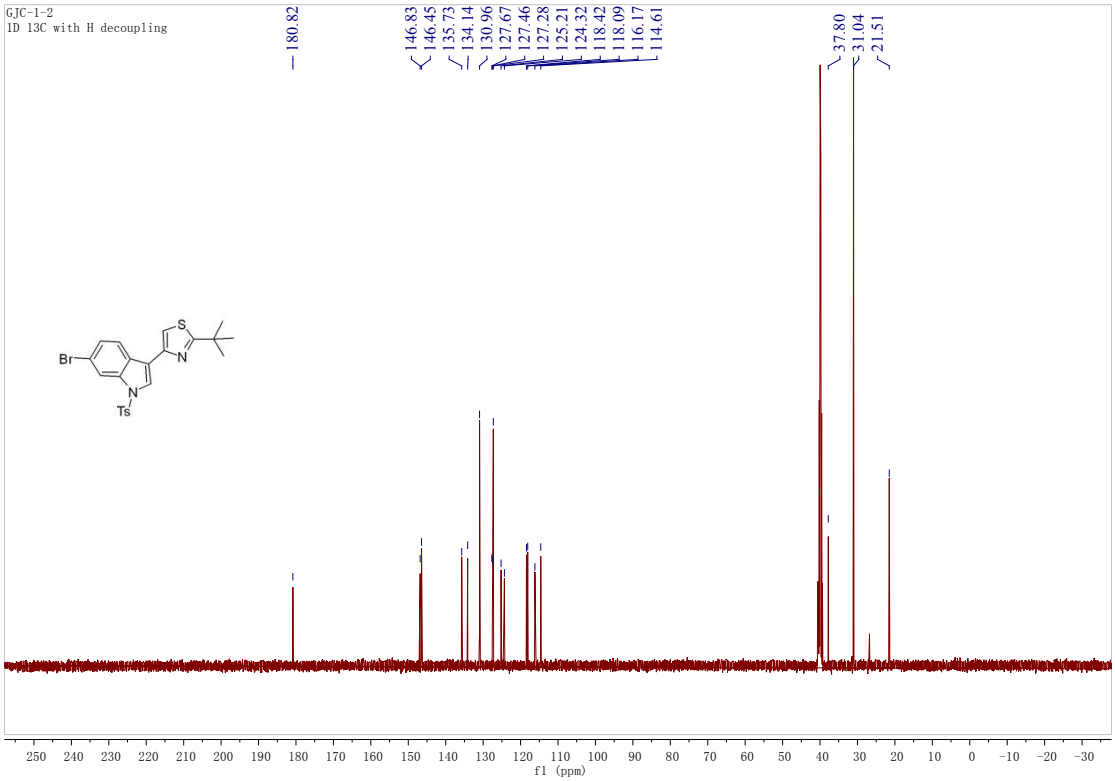
^{1}H NMR spectra of **2o**



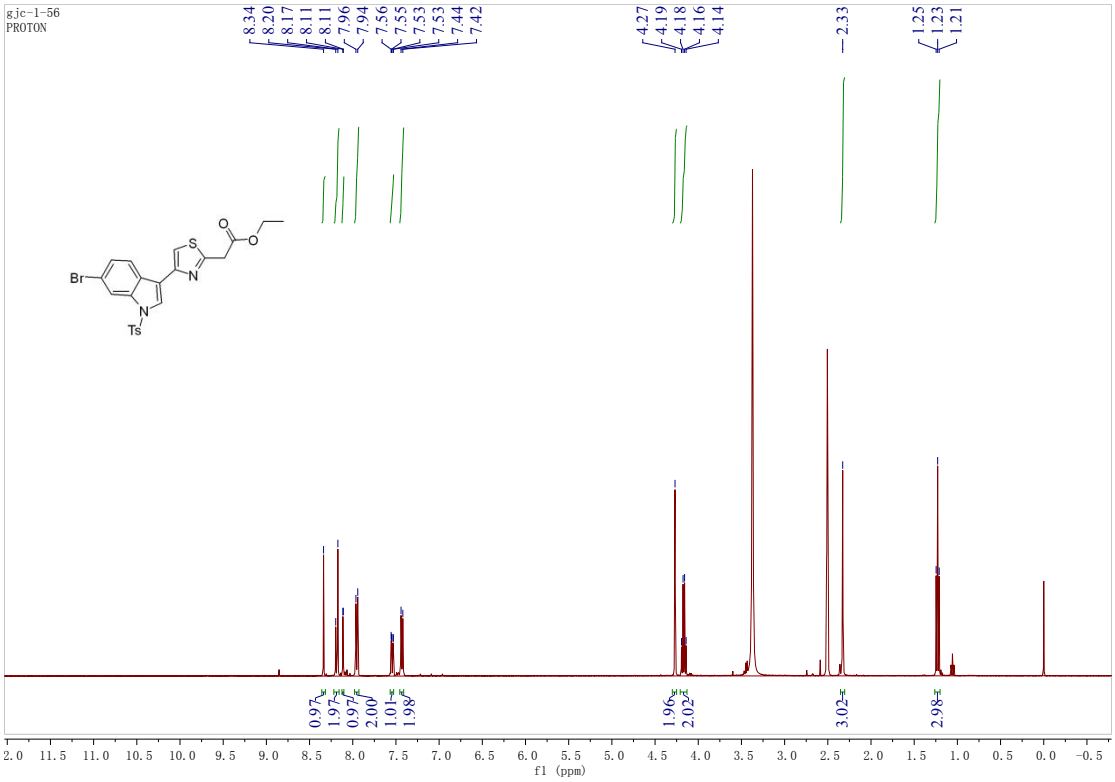
^{13}C NMR spectra of **2o**



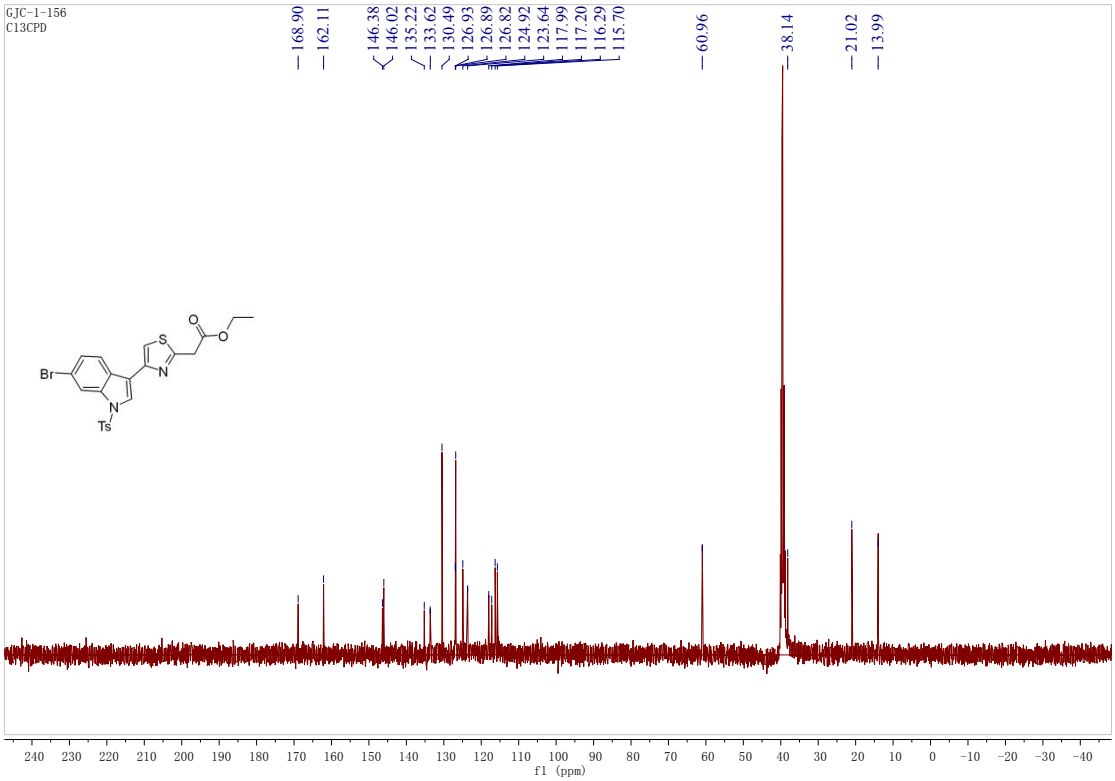
^{1}H NMR spectra of **2p**



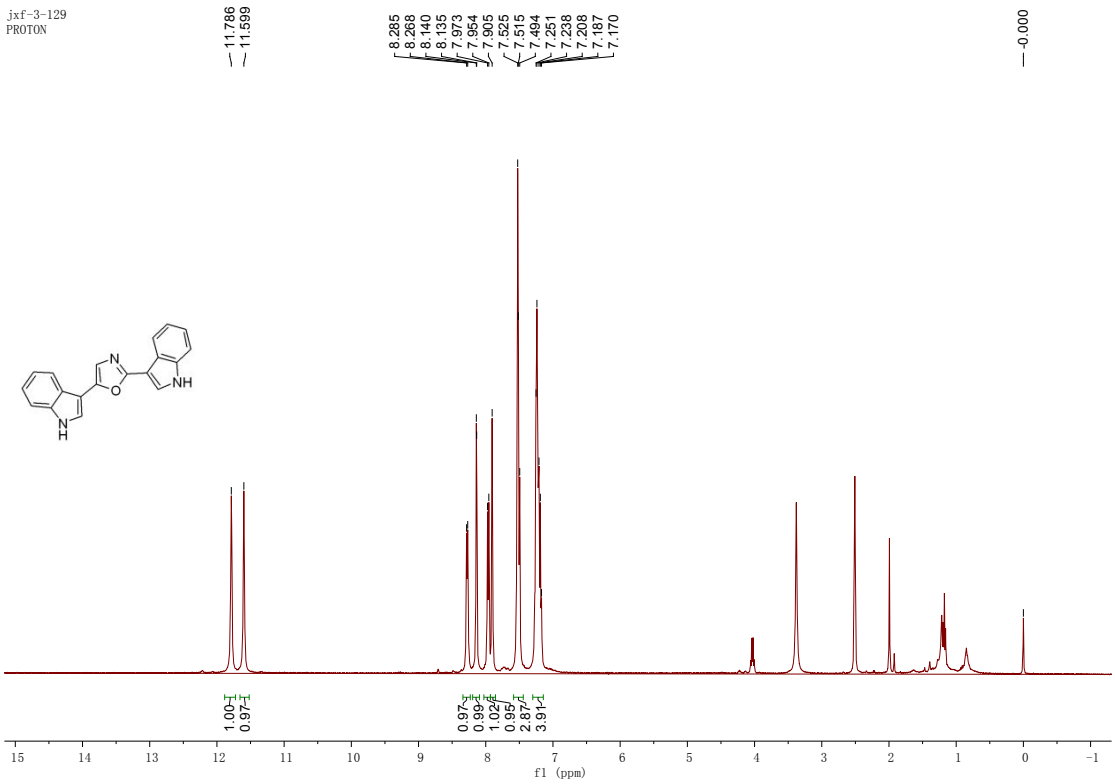
^{13}C NMR spectra of **2p**



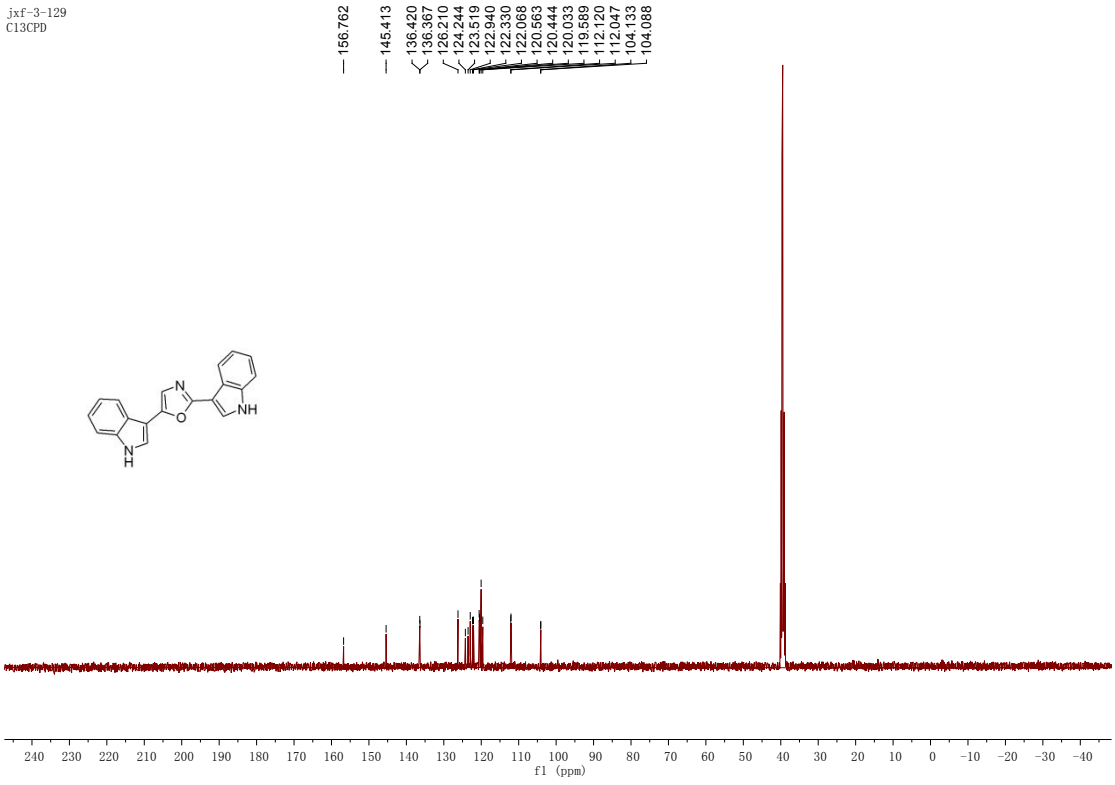
^{1}H NMR spectra of **2q**



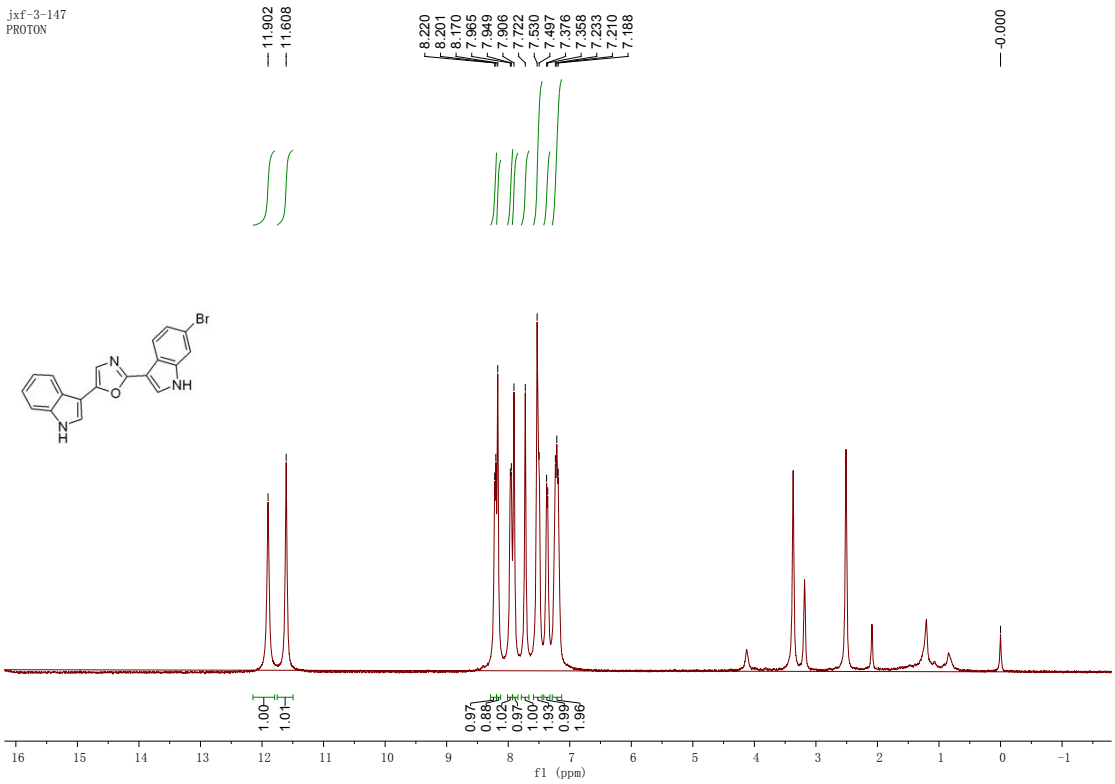
^{13}C NMR spectra of **2q**



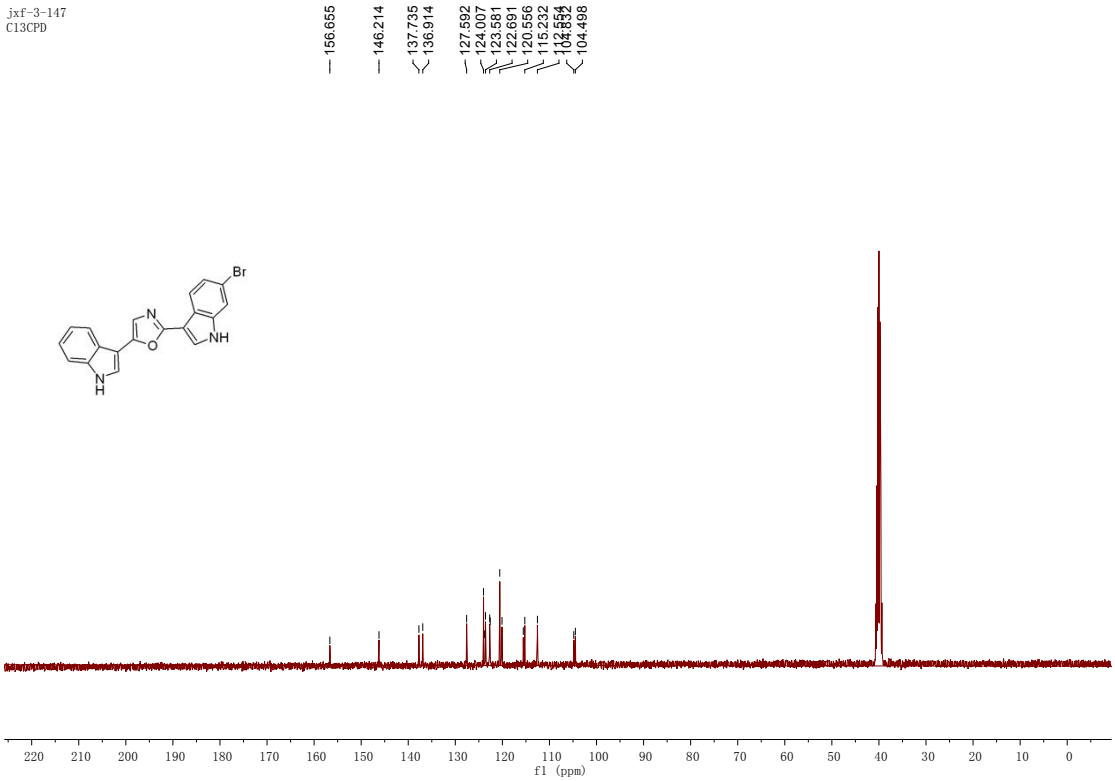
^{1}H NMR spectra of **12a**



^{13}C NMR spectra of **12a**



^{1}H NMR spectra of **12b**



^{13}C NMR spectra of **12b**