

# Regio- and stereoselective synthesis and evaluation of densely functionalized bispiro[oxindole-isoxazole-indandione] hybrids as anticancer agents

Zhi-Gang Yin,<sup>a</sup> Xiong-Wei Liu,<sup>b</sup> Lin Chen,<sup>a</sup> Xiong-Li Liu,<sup>a,\*</sup> Bo-Wen Pan<sup>b</sup> and Ying Zhou<sup>b,\*</sup>

<sup>a</sup> Key laboratory of Plant Resource Conservation and Germplasm Innovation in Mountainous Region (Ministry of Education), Collaborative Innovation Center for Mountain Ecology & Agro-Bioengineering (CICMEAB), Institute of Agro-bioengineering/College of Life Sciences, Guizhou University, Guiyang 550025, China.

<sup>b</sup> College of Pharmaceutical Sciences, Guizhou University of Traditional Chinese Medicine, Guiyang, 550025.

\*E-mail address: xlliu1@gzu.edu.cn (Xiong-Li Liu) and zhouy@gzu.edu.cn (Ying Zhou)

## Table of Contents

Table of contents.....	S1
1. General information.....	S2
2. Synthesis of bispiro[oxindole-isoxazole-indandione] hybrids <b>4</b> .....	S2
3. Characterization data of hybrids <b>4</b> .....	S2
4. Scheme S1: gram scale synthesis of the products <b>4ba</b> .....	S13
5. X-ray crystal data for compounds <b>4ac</b> and <b>4bb</b> .....	S14
6. MTT assay.....	S16
7. Colony formation assay.....	S16
8. Cell morphology observation.....	S16
9. AO/EB staining.....	S16
10. Analysis for apoptosis by flow cytometry.....	S17
11. Analysis of the cell cycle using flow cytometry.....	S17
12. Western blot analysis.....	S17
13. The copies of <sup>1</sup> H NMR and <sup>13</sup> C NMR spectra for compounds <b>4</b> .....	S19

## 1. General information

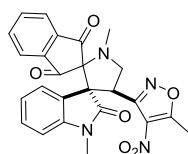
Reactions were monitored by thin layer chromatography using UV light to visualize the course of reaction. Purification of reaction products was carried out by flash chromatography on silica gel or just by simple filtration and washing.  $^1\text{H}$  and  $^{13}\text{C}$ NMR spectra were obtained using a Bruker DPX-400 spectrometer.  $^1\text{H}$  NMR chemical shifts are reported in ppm ( $\delta$ ) relative to tetramethylsilane (TMS) with the solvent resonance employed as the internal standard. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), coupling constants (Hz) and integration.  $^{13}\text{C}$  NMR chemical shifts are reported in ppm ( $\delta$ ) from tetramethylsilane (TMS) with the solvent resonance as the internal standard. Melting points were measured on an electrothermal digital melting point apparatus.

All cell lines were purchased from the Chinese Academy of Sciences, Kunming Cell Bank. All of which were cultured in RPMI-1640 or DMEM medium (Gibco, USA) supplemented with 10% foetal bovine serum, 1% glutamine, 100 U/mL penicillin and 100  $\mu\text{g}/\text{mL}$  streptomycin in a humidified atmosphere with 5%  $\text{CO}_2$  at 37°C. The synthetic compounds were placed at -20°C after dissolved in DMSO. Cisplatin purchased from Aladdin Company.

## 2. Synthesis of bispiro[oxindole-isoxazole-indandione] hybrids **4**

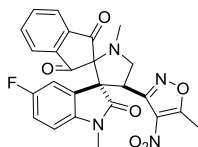
In a sealed tube equipped with a magnetic stirring bar, to 3.0 mL of toluene was added 3-methyl-4-nitro-5-isatylidenyl-isoxazole **1** (0.2 mmol), ninhydrin **2** (0.3 mmol) and sarcosine **3** (0.5 mmol). The reaction mixture was stirred at 60 °C for 4 h. After completion of the reaction, as indicated by TLC, purification by flash column chromatography (hexane/EtOAc, 5/1, v/v) was carried out to furnish the hybrids **4**.

## 3. Characterization data of hybrids **4**

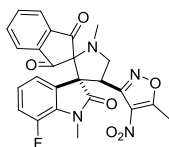


**4aa**: Light yellow solid, m.p. 214.4-215.5 °C; yield 90%, 19:1 dr;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$ : 2.26 (s, 3H), 2.41 (s, 3H), 2.79 (s, 3H), 3.96-4.01 (m, 1H), 4.20-4.24 (m, 1H), 5.25-5.29 (m, 1H), 6.46 (d,  $J = 7.6\text{Hz}$ , 1H), 6.87-6.91 (m, 1H), 7.06-7.10 (m, 1H), 7.32-7.34 (m, 1H), 7.55 (d,  $J$

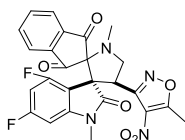
= 8.0 Hz, 1H), 7.62-7.66 (m, 1H), 7.73-7.77 (m, 1H), 7.90 (d,  $J = 7.6$  Hz, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$ : 10.4, 25.4, 34.4, 43.3, 52.9, 62.7, 78.6, 106.8, 121.2, 121.8, 122.0, 122.9, 126.7, 128.6, 129.6, 134.7, 135.6, 139.9, 140.4, 141.9, 154.6, 169.1, 171.6, 194.9, 197.6; HRMS (ESI-TOF)  $m/z$ : Calcd. for  $\text{C}_{25}\text{H}_{20}\text{N}_4\text{NaO}_6$   $[\text{M}+\text{Na}]^+$ : 495.1275; Found: 495.1271.



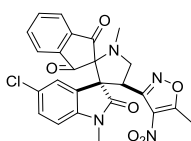
**4ab**: Light yellow solid, m.p. 275.4-275.8 °C; yield 88%, 18:1 dr;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$ : 2.28 (s, 3H), 2.39 (s, 3H), 2.77 (s, 3H), 3.99-4.04 (m, 1H), 4.09-4.13 (m, 1H), 5.32-5.36 (m, 1H), 6.42-6.45 (m, 1H), 6.81-6.85 (m, 1H), 7.28-7.30 (m, 1H), 7.60 (d,  $J = 7.6$  Hz, 1H), 7.66-7.70 (m, 1H), 7.77-7.81 (m, 1H), 7.94 (d,  $J = 7.6$  Hz, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$ : 10.4, 25.5, 34.3, 43.3, 52.7, 62.6, 77.9, 107.2 (d,  $J_{\text{CF}} = 8.1$  Hz), 114.9 (d,  $J_{\text{CF}} = 24.2$  Hz), 115.1 (d,  $J_{\text{CF}} = 26.0$  Hz), 121.9, 122.2, 124.8 (d,  $J_{\text{CF}} = 9.1$  Hz), 134.7, 135.9, 137.8, 139.9, 140.3, 154.6, 157.4 (d,  $J_{\text{CF}} = 240.2$  Hz), 168.5, 170.9, 194.3, 198.0; HRMS (ESI-TOF)  $m/z$ : Calcd. for  $\text{C}_{25}\text{H}_{19}\text{FN}_4\text{NaO}_6$   $[\text{M}+\text{Na}]^+$ : 513.1181; Found: 513.1184.



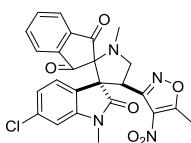
**4ac**: Light yellow solid, m.p. 243.3-243.9 °C; yield 87%, 11:1 dr;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$ : 2.30 (s, 3H), 2.40 (s, 3H), 2.98 (s, 3H), 3.98-4.03 (m, 1H), 4.14-4.18 (m, 1H), 5.27-5.31 (m, 1H), 6.80-6.88 (m, 2H), 7.20-7.23 (m, 1H), 7.60 (d,  $J = 7.6$  Hz, 1H), 7.66-7.70 (m, 1H), 7.77-7.81 (m, 1H), 7.93 (d,  $J = 8.0$  Hz, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$ : 11.4, 29.0, 35.3, 44.4, 53.9, 63.7, 79.5, 117.5 (d,  $J_{\text{CF}} = 19.3$  Hz), 122.7, 122.8 (d,  $J_{\text{CF}} = 19.4$  Hz), 123.2, 123.7, 123.8, 126.8, 129.6 (d,  $J_{\text{CF}} = 9.1$  Hz), 130.7, 135.8, 136.9, 140.9, 141.3, 147.5 (d,  $J_{\text{CF}} = 242.4$  Hz), 155.7, 169.8, 172.1, 195.5, 198.6; HRMS (ESI-TOF)  $m/z$ : Calcd. for  $\text{C}_{25}\text{H}_{19}\text{FN}_4\text{NaO}_6$   $[\text{M}+\text{Na}]^+$ : 513.1181; Found: 513.1185.



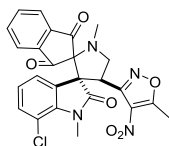
**4ad:** Light yellow solid, m.p. 264.8-265.2 °C; yield 80%, 10:1 dr; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ: 2.34 (s, 3H), 2.38 (s, 3H), 2.73 (s, 3H), 3.93-3.98 (m, 1H), 4.22-4.27 (m, 1H), 5.23-5.29 (m, 1H), 6.06-6.09 (m, 1H), 6.34-6.39 (m, 1H), 7.60 (d, *J* = 7.6 Hz, 1H), 7.67-7.71 (m, 1H), 7.77-7.81 (m, 1H), 7.90 (d, *J* = 7.6 Hz, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ: 11.5, 26.9, 35.3, 43.2, 54.8, 63.9, 80.8, 93.4, 93.5 (d, *J*<sub>CF</sub> = 24.0 Hz), 98.5, 98.8 (d, *J*<sub>CF</sub> = 26.1 Hz), 106.8 (d, *J*<sub>CF</sub> = 20.4 Hz), 122.7, 123.2, 130.6, 135.9, 136.8, 140.9, 145.8 (d, *J*<sub>CF</sub> = 24.2 Hz), 155.7, 159.4 (d, *J*<sub>CF</sub> = 269.0 Hz), 162.9, 164.6 (d, *J*<sub>CF</sub> = 236.3 Hz), 169.9, 172.2, 195.4, 197.8; HRMS (ESI-TOF) *m/z*: Calcd. For C<sub>25</sub>H<sub>18</sub>F<sub>2</sub>N<sub>4</sub>NaO<sub>6</sub> [M+Na]<sup>+</sup>: 531.1087; Found: 531.1089.



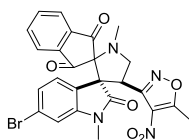
**4ae:** Light yellow solid, m.p. 270.6-271.6 °C; yield 82%, 12:1 dr; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ: 2.29 (s, 3H), 2.40 (s, 3H), 2.77 (s, 3H), 3.98-4.03 (m, 1H), 4.09-4.13 (m, 1H), 5.29-5.34 (m, 1H), 6.43 (d, *J* = 8.0 Hz, 1H), 7.08-7.10 (m, 1H), 7.47 (d, *J* = 2.0 Hz, 1H), 7.60 (d, *J* = 7.6 Hz, 1H), 7.66-7.70 (m, 1H), 7.77-7.81 (m, 1H), 7.93 (d, *J* = 8.0 Hz, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ: 10.4, 25.5, 34.3, 43.4, 52.7, 62.4, 77.9, 107.7, 121.9, 122.2, 124.8, 126.8, 127.2, 128.5, 134.8, 135.9, 139.9, 140.2, 140.4, 154.6, 168.5, 170.9, 194.3, 197.9; HRMS (ESI-TOF) *m/z*: Calcd. for C<sub>25</sub>H<sub>19</sub>ClN<sub>4</sub>NaO<sub>6</sub> [M+Na]<sup>+</sup>: 529.0885; Found: 529.0881.



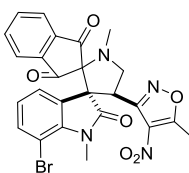
**4af:** Light yellow solid, m.p. 274.9-275.2 °C; yield 81%, 10:1 dr; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ: 2.29 (s, 3H), 2.39 (s, 3H), 2.77 (s, 3H), 3.97-4.02 (m, 1H), 4.13-4.17 (m, 1H), 5.26-5.30 (m, 1H), 6.48 (s, 1H), 6.88-6.90 (m, 1H), 7.34 (d, *J* = 8.4 Hz, 1H), 7.59 (d, *J* = 7.6 Hz, 1H), 7.66-7.70 (m, 1H), 7.77-7.81 (m, 1H), 7.92 (d, *J* = 8.0 Hz, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ: 10.4, 25.5, 34.3, 43.3, 52.9, 62.2, 78.3, 107.6, 121.2, 121.4, 121.9, 122.1, 127.8, 134.5, 134.8, 135.9, 139.8, 140.3, 143.0, 154.7, 168.7, 171.4, 194.6, 197.7; HRMS (ESI-TOF) *m/z*: Calcd. for C<sub>25</sub>H<sub>19</sub>ClN<sub>4</sub>NaO<sub>6</sub> [M+Na]<sup>+</sup>: 529.0885; Found: 529.0886.



**4ag:** Light yellow solid, m.p. 225.8-226.2 °C; yield 87%, 12:1 dr; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ: 2.30 (s, 3H), 2.39 (s, 3H), 3.12 (s, 3H), 3.98-4.03 (m, 1H), 4.11-4.15 (m, 1H), 5.29-5.33 (m, 1H), 6.84-6.88 (m, 1H), 7.02-7.05 (m, 1H), 7.40-7.43 (m, 1H), 7.60 (d, *J* = 7.6 Hz, 1H), 7.67-7.71 (m, 1H), 7.78-7.82 (m, 1H), 7.94 (d, *J* = 7.6 Hz, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ: 9.0, 27.5, 32.9, 42.1, 51.4, 60.9, 77.1, 112.7, 120.5, 120.6, 120.8, 124.2, 124.4, 129.5, 133.4, 134.5, 138.5, 138.9, 153.3, 167.2, 170.2, 192.9, 196.3; HRMS (ESI-TOF) *m/z*: Calcd. for C<sub>25</sub>H<sub>19</sub>ClN<sub>4</sub>NaO<sub>6</sub> [M+Na]<sup>+</sup>: 529.0885; Found: 529.0883.

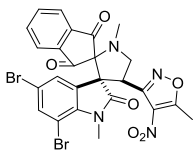


**4ah:** Light yellow solid, m.p. 257.8-258.2 °C; yield 83%, 11:1 dr; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ: 2.30 (s, 3H), 2.39 (s, 3H), 2.76 (s, 3H), 3.97-4.06 (m, 1H), 4.12-4.16 (m, 1H), 5.26-5.30 (m, 1H), 6.64 (s, 1H), 7.04-7.06 (m, 1H), 7.29 (d, *J* = 8.0 Hz, 1H), 7.59 (d, *J* = 7.6 Hz, 1H), 7.66-7.70 (m, 1H), 7.77-7.81 (m, 1H), 7.92 (d, *J* = 8.4 Hz, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ: 10.4, 25.5, 34.3, 43.2, 52.9, 62.2, 78.2, 110.4, 121.9, 122.0, 122.1, 122.5, 124.1, 128.1, 134.8, 135.9, 139.8, 140.3, 143.1, 154.7, 168.7, 171.3, 194.5, 197.7; HRMS (ESI-TOF) *m/z*: Calcd. for C<sub>25</sub>H<sub>19</sub>BrN<sub>4</sub>NaO<sub>6</sub> [M+Na]<sup>+</sup>: 573.0380; Found: 573.0385.

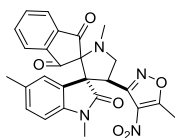


**4ai:** Light yellow solid, m.p. 234.5-235.2 °C; yield 83%, 10:1 dr; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ: 2.30 (s, 3H), 2.39 (s, 3H), 3.12 (s, 3H), 3.98-4.03 (m, 1H), 4.11-4.15 (m, 1H), 5.29-5.33 (m, 1H), 6.78-6.82 (m, 1H), 7.20-7.23 (m, 1H), 7.45-7.47 (m, 1H), 7.60 (d, *J* = 8.0 Hz, 1H), 7.67-7.71 (m, 1H), 7.78-7.82 (m, 1H), 7.94 (d, *J* = 7.6 Hz, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ: 11.4, 30.1, 35.3, 44.6, 53.8, 63.3, 79.5, 101.9, 123.0, 123.2, 123.4, 127.1, 127.2, 135.2, 135.8, 136.9, 140.1, 140.9,

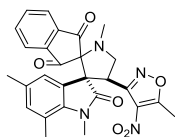
141.3, 155.7, 169.5, 172.8, 195.3, 198.7; HRMS (ESI-TOF)  $m/z$ : Calcd. for  $C_{25}H_{19}BrN_4NaO_6$   $[M+Na]^+$ : 573.0380; Found: 573.0383.



**4aj**: Light yellow solid, m.p. 270.4-271.2 °C; yield 85%, 11:1 dr;  $^1H$  NMR ( $CDCl_3$ , 400 MHz)  $\delta$ : 2.34 (s, 3H), 2.38 (s, 3H), 3.10 (s, 3H), 4.01-4.05 (m, 2H), 5.32-5.36 (m, 1H), 7.41 (d,  $J = 1.6$  Hz, 1H), 7.64-7.74 (m, 3H), 7.80-7.84 (m, 1H), 7.95 (d,  $J = 7.6$  Hz, 1H);  $^{13}C$  NMR ( $CDCl_3$ , 100 MHz)  $\delta$ : 10.4, 29.1, 34.2, 43.7, 52.6, 62.0, 77.7, 101.2, 114.2, 122.0, 122.2, 127.6, 129.3, 134.9, 136.0, 136.1, 138.3, 139.8, 140.2, 154.7, 168.0, 171.1, 193.8, 197.8; HRMS (ESI-TOF)  $m/z$ : Calcd. for  $C_{25}H_{18}Br_2N_4NaO_6$   $[M+Na]^+$ : 650.9485; Found: 650.9482.

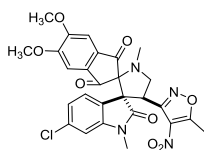


**4ak**: Light yellow solid, m.p. 264.1-264.8 °C; yield 83%, 11:1 dr;  $^1H$  NMR ( $CDCl_3$ , 400 MHz)  $\delta$ : 2.21 (s, 3H), 2.26 (s, 3H), 2.42 (s, 3H), 2.75 (s, 3H), 3.96-4.00 (m, 1H), 4.18-4.22 (m, 1H), 5.23-5.27 (m, 1H), 6.33 (d,  $J = 8.0$  Hz, 1H), 6.86 (d,  $J = 8.0$  Hz, 1H), 7.14 (s, 1H), 7.54 (d,  $J = 7.6$  Hz, 1H), 7.62-7.66 (m, 1H), 7.73-7.77 (m, 1H), 7.90 (d,  $J = 7.6$  Hz, 1H);  $^{13}C$  NMR ( $CDCl_3$ , 100 MHz)  $\delta$ : 10.3, 20.1, 25.4, 34.5, 43.4, 52.8, 62.9, 78.7, 106.4, 121.7, 122.0, 122.9, 127.4, 128.8, 130.7, 134.6, 135.6, 139.5, 140.0, 140.4, 154.5, 169.2, 171.5, 194.8, 197.7; HRMS (ESI-TOF)  $m/z$ : Calcd. for  $C_{26}H_{22}N_4NaO_6$   $[M+Na]^+$ : 509.1432; Found: 509.1437.

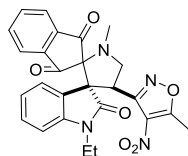


**4al**: Light yellow solid, m.p. 196.4-197.2 °C; yield 82%, 10:1 dr;  $^1H$  NMR ( $CDCl_3$ , 400 MHz)  $\delta$ : 2.16 (s, 3H), 2.18 (s, 3H), 2.27 (s, 3H), 2.41 (s, 3H), 3.00 (s, 3H), 3.94-3.99 (m, 1H), 4.17-4.21 (m, 1H), 5.21-5.25 (m, 1H), 6.59 (s, 1H), 6.98 (s, 1H), 7.54 (d,  $J = 7.6$  Hz, 1H), 7.62-7.66 (m, 1H), 7.73-7.77 (m, 1H), 7.90 (d,  $J = 8.0$  Hz, 1H);  $^{13}C$  NMR ( $CDCl_3$ , 100 MHz)  $\delta$ : 10.3, 17.7, 19.8, 28.8, 34.4, 43.6, 52.7, 62.6, 79.0, 117.7, 121.7, 122.0, 123.5, 125.2, 129.6, 130.4, 132.8, 134.5, 135.5,

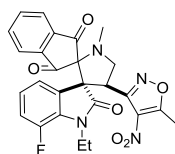
137.3, 140.0, 140.5, 154.5, 169.2, 172.3, 194.8, 197.7; HRMS (ESI-TOF)  $m/z$ : Calcd. for  $C_{27}H_{24}N_4NaO_6 [M+Na]^+$ : 523.1588; Found: 523.1591.



**4am**: Light yellow solid, m.p. 262.1-262.7 °C; yield 90%, 20:1 dr;  $^1H$  NMR ( $CDCl_3$ , 400 MHz)  $\delta$ : 2.29 (s, 3H), 2.36 (s, 3H), 2.82 (s, 3H), 3.86 (s, 3H), 3.93-3.98 (m, 4H), 4.11-4.15 (m, 1H), 5.29-5.33 (m, 1H), 6.51 (s, 1H), 6.88-6.91 (m, 1H), 6.96 (s, 1H), 7.27 (s, 1H), 7.38 (d,  $J = 8.0$  Hz, 1H);  $^{13}C$  NMR ( $CDCl_3$ , 100 MHz)  $\delta$ : 10.4, 25.6, 34.2, 43.3, 52.9, 55.7, 55.8, 61.8, 77.5, 101.8, 102.0, 107.5, 121.1, 121.8, 127.9, 129.6, 134.3, 135.1, 135.8, 143.0, 154.6, 155.1, 156.0, 168.8, 171.6, 193.3, 196.5; HRMS (ESI-TOF)  $m/z$ : Calcd. for  $C_{27}H_{23}ClN_4NaO_8 [M+Na]^+$ : 589.1097; Found: 589.11001.

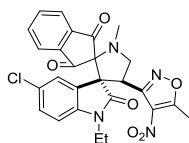


**4ba**: Light yellow solid, m.p. 241.1-241.9 °C; yield 85%, 15:1 dr;  $^1H$  NMR ( $CDCl_3$ , 400 MHz)  $\delta$ : 0.68-0.71 (m, 3H), 2.26 (s, 3H), 2.43 (s, 3H), 3.20-3.25 (m, 1H), 3.39-3.45 (m, 1H), 4.00-4.05 (m, 1H), 4.17-4.21 (m, 1H), 5.26-5.30 (m, 1H), 6.48 (d,  $J = 7.6$  Hz, 1H), 6.87-6.91 (m, 1H), 7.06-7.10 (m, 1H), 7.32-7.34 (m, 1H), 7.53 (d,  $J = 8.0$  Hz, 1H), 7.61-7.64 (m, 1H), 7.73-7.77 (m, 1H), 7.89 (d,  $J = 7.6$  Hz, 1H);  $^{13}C$  NMR ( $CDCl_3$ , 100 MHz)  $\delta$ : 10.4, 10.7, 33.8, 34.4, 42.8, 53.1, 62.8, 79.3, 106.9, 121.0, 121.7, 122.1, 123.2, 126.7, 128.5, 134.5, 135.5, 139.9, 140.5, 140.9, 154.6, 169.2, 170.9, 194.5, 197.5; HRMS (ESI-TOF)  $m/z$ : Calcd. for  $C_{26}H_{22}N_4NaO_6 [M+Na]^+$ : 509.1432; Found: 509.1427.

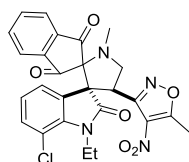


**4bb**: Light yellow solid, m.p. 250.3-250.8 °C; yield 80%, 10:1 dr;  $^1H$  NMR ( $CDCl_3$ , 400 MHz)  $\delta$ : 0.67-0.71 (m, 3H), 2.30 (s, 3H), 2.41 (s, 3H), 3.39-3.45 (m, 1H), 3.50-3.56 (m, 1H), 4.02-4.07 (m, 1H), 4.11-4.15 (m, 1H), 5.28-5.33 (m, 1H), 6.84-6.88 (m, 2H), 7.20-7.24 (m, 1H), 7.58 (d,  $J =$

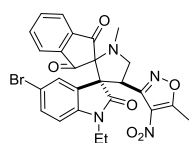
8.0 Hz, 1H), 7.64-7.68 (m, 1H), 7.77-7.81 (m, 1H), 7.92 (d,  $J = 8.0$  Hz, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$ : 11.5, 13.2, 35.4, 37.0, 43.9, 54.1, 63.8, 80.1, 117.6 (d,  $J_{\text{CF}} = 19.1$  Hz), 122.6, 122.7 (d,  $J_{\text{CF}} = 18.3$  Hz), 123.2, 123.7, 123.8, 127.1, 128.8 (d,  $J_{\text{CF}} = 8.3$  Hz), 135.7, 136.8, 140.9, 141.4, 146.7 (d,  $J_{\text{CF}} = 242.2$  Hz), 155.7, 169.8, 171.4, 195.2, 198.5; HRMS (ESI-TOF)  $m/z$ : Calcd. for  $\text{C}_{26}\text{H}_{21}\text{FN}_4\text{NaO}_6$   $[\text{M}+\text{Na}]^+$ : 527.1337; Found: 527.1333.



**4bc**: Light yellow solid, m.p. 225.6-226.0 °C; yield 89%, 15:1 dr;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$ : 0.65-0.69 (m, 3H), 2.30 (s, 3H), 2.41 (s, 3H), 3.19-3.24 (m, 1H), 3.36-3.41 (m, 1H), 4.02-4.11 (m, 2H), 5.30-5.34 (m, 1H), 6.44 (d,  $J = 8.4$  Hz, 1H), 7.08-7.10 (m, 1H), 7.46 (s, 1H), 7.58 (d,  $J = 7.6$  Hz, 1H), 7.64-7.68 (m, 1H), 7.77-7.80 (m, 1H), 7.92 (d,  $J = 7.6$  Hz, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$ : 11.4, 11.7, 35.0, 35.4, 43.9, 53.9, 63.5, 79.6, 108.8, 122.8, 123.2, 126.2, 127.6, 128.3, 129.5, 135.7, 136.8, 140.4, 140.9, 141.3, 155.7, 169.7, 171.3, 195.0, 198.8; HRMS (ESI-TOF)  $m/z$ : Calcd. for  $\text{C}_{26}\text{H}_{21}\text{ClN}_4\text{NaO}_6$   $[\text{M}+\text{Na}]^+$ : 543.1042; Found: 543.1042.



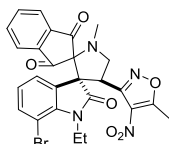
**4bd**: Light yellow solid, m.p. 161.2-162.0 °C; yield 84%, 10:1 dr;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$ : 0.65-0.68 (m, 3H), 2.31 (s, 3H), 2.41 (s, 3H), 3.68-3.73 (m, 2H), 4.02-4.13 (m, 2H), 5.31-5.35 (m, 1H), 6.86-6.90 (m, 1H), 7.05-7.07 (m, 1H), 7.41-7.43 (m, 1H), 7.59 (d,  $J = 7.6$  Hz, 1H), 7.65-7.69 (m, 1H), 7.78-7.82 (m, 1H), 7.94 (d,  $J = 7.6$  Hz, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$ : 11.5, 13.8, 35.3, 36.8, 44.0, 54.0, 63.3, 80.2, 114.7, 122.9, 123.3, 126.5, 127.2, 132.1, 135.7, 136.9, 138.0, 140.8, 141.4, 155.8, 169.7, 172.2, 195.0, 198.6; HRMS (ESI-TOF)  $m/z$ : Calcd. for  $\text{C}_{26}\text{H}_{21}\text{ClN}_4\text{NaO}_6$   $[\text{M}+\text{Na}]^+$ : 543.1042; Found: 543.1046.



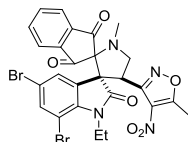
**4be**: Light yellow solid, m.p. 238.5-239.4 °C; yield 87%, 15:1 dr;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)



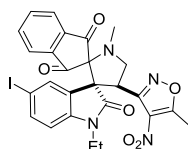
$\delta$ : 0.65-0.69 (m, 3H), 2.30 (s, 3H), 2.41 (s, 3H), 3.19-3.24 (m, 1H), 3.36-3.41 (m, 1H), 4.04-4.10 (m, 2H), 5.28-5.32 (m, 1H), 6.39 (d,  $J = 8.4$  Hz, 1H), 7.20-7.25 (m, 1H), 7.56-7.59 (m, 2H), 7.64-7.68 (m, 1H), 7.76-7.80 (m, 1H), 7.91 (d,  $J = 7.6$  Hz, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$ : 11.4, 11.7, 34.9, 35.4, 43.9, 53.9, 63.5, 79.6, 109.2, 114.8, 122.8, 123.2, 126.5, 131.0, 132.4, 135.7, 136.8, 140.9, 141.3, 155.7, 169.7, 171.3, 195.0, 198.7; HRMS (ESI-TOF)  $m/z$ : Calcd. for  $\text{C}_{26}\text{H}_{21}\text{BrN}_4\text{NaO}_6$   $[\text{M}+\text{Na}]^+$ : 587.0537; Found: 587.0541.



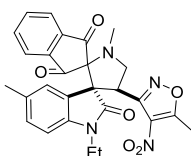
**4bf**: Light yellow solid, m.p. 188.7-189.4 °C; yield 81%, 10:1 dr;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$ : 0.64-0.67 (m, 3H), 2.31 (s, 3H), 2.41 (s, 3H), 3.70-3.77 (m, 2H), 4.02-4.12 (m, 2H), 5.31-5.35 (m, 1H), 6.79-6.83 (m, 1H), 7.20-7.25 (m, 1H), 7.45-7.48 (m, 1H), 7.58 (d,  $J = 7.6$  Hz, 1H), 7.65-7.69 (m, 1H), 7.78-7.82 (m, 1H), 7.94 (d,  $J = 8.0$  Hz, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$ : 11.5, 13.8, 35.3, 36.5, 44.0, 54.0, 63.2, 80.2, 101.6, 122.9, 123.3, 127.1, 127.5, 135.5, 135.6, 136.8, 139.4, 140.8, 141.4, 155.8, 169.6, 172.4, 194.9, 198.6; HRMS (ESI-TOF)  $m/z$ : Calcd. for  $\text{C}_{26}\text{H}_{21}\text{BrN}_4\text{NaO}_6$   $[\text{M}+\text{Na}]^+$ : 587.0537; Found: 587.0540.



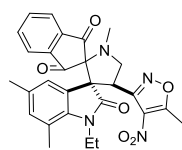
**4bg**: Light yellow solid, m.p. 241.8-242.2 °C; yield 83%, 10:1 dr;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$ : 0.62-0.65 (m, 3H), 2.34 (s, 3H), 2.40 (s, 3H), 3.67-3.74 (m, 2H), 3.99-4.08 (m, 2H), 5.33-5.37 (m, 1H), 7.43 (d,  $J = 2.0$  Hz, 1H), 7.62 (d,  $J = 7.6$  Hz, 1H), 7.67-7.68 (m, 1H), 7.70-7.72 (m, 1H), 7.80-7.84 (m, 1H), 7.95 (d,  $J = 7.6$  Hz, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$ : 11.5, 13.8, 35.3, 36.6, 44.1, 53.8, 62.9, 79.5, 101.9, 115.1, 122.9, 123.3, 129.1, 130.2, 135.8, 137.0, 137.3, 138.6, 140.8, 141.2, 155.8, 169.1, 171.8, 194.4, 198.7; HRMS (ESI-TOF)  $m/z$ : Calcd. For  $\text{C}_{26}\text{H}_{20}\text{Br}_2\text{N}_4\text{NaO}_6$   $[\text{M}+\text{Na}]^+$ : 664.9642; Found: 664.9645.



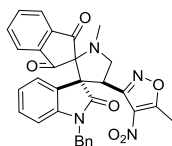
**4bh**: Light yellow solid, m.p. 234.5-235.2 °C; yield 80%, 11:1 dr; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ: 0.66-0.69 (m, 3H), 2.31 (s, 3H), 2.41 (s, 3H), 3.19-3.24 (m, 1H), 3.36-3.41 (m, 1H), 4.00-4.06 (m, 1H), 4.09-4.13 (m, 1H), 5.23-5.29 (m, 1H), 6.29 (d, *J* = 8.0 Hz, 1H), 7.40-7.44 (m, 1H), 7.58 (d, *J* = 7.6 Hz, 1H), 7.64-7.68 (m, 2H), 7.76-7.79 (m, 1H), 7.90-7.92 (m, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ: 10.4, 10.7, 33.9, 34.4, 42.9, 52.9, 62.4, 78.7, 83.5, 108.8, 121.8, 122.2, 125.6, 134.7, 135.5, 135.7, 137.3, 139.9, 140.3, 154.6, 168.8, 170.2, 194.1, 197.5; HRMS (ESI-TOF) *m/z*: Calcd. For C<sub>26</sub>H<sub>21</sub>IN<sub>4</sub>NaO<sub>6</sub> [M+Na]<sup>+</sup>: 635.0398; Found: 635.0396.



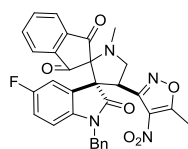
**4bi**: Light yellow solid, m.p. 164.1-165.0 °C; yield 89%, 15:1 dr; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ: 0.65-0.68 (m, 3H), 2.23 (s, 3H), 2.27 (s, 3H), 2.43 (s, 3H), 3.16-3.21 (m, 1H), 3.36-3.42 (m, 1H), 3.99-4.04 (m, 1H), 4.16-4.20 (m, 1H), 5.24-5.28 (m, 1H), 6.36 (d, *J* = 8.0 Hz, 1H), 6.86-6.88 (m, 1H), 7.14 (s, 1H), 7.53 (d, *J* = 8.0 Hz, 1H), 7.60-7.64 (m, 1H), 7.72-7.76 (m, 1H), 7.90 (d, *J* = 7.6 Hz, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ: 11.5, 11.8, 21.2, 34.8, 35.6, 43.9, 54.1, 64.0, 80.4, 107.6, 122.6, 123.1, 124.3, 128.4, 129.8, 131.5, 134.5, 136.5, 139.5, 141.0, 141.5, 155.6, 170.4, 171.8, 195.6, 198.6; HRMS (ESI-TOF) *m/z*: Calcd. for C<sub>25</sub>H<sub>19</sub>ClN<sub>4</sub>NaO<sub>6</sub> [M+Na]<sup>+</sup>: 529.0885; Found: 529.0889.



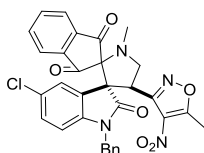
**4bj**: Light yellow solid, m.p. 246.5-247.2 °C; yield 82%, 10:1 dr; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ: 0.61-0.65 (m, 3H), 2.15 (s, 3H), 2.18 (s, 3H), 2.28 (s, 3H), 2.42 (s, 3H), 3.40-3.46 (m, 1H), 3.54-3.59 (m, 1H), 3.98-4.03 (m, 1H), 4.13-4.17 (m, 1H), 5.23-5.28 (m, 1H), 6.62 (s, 1H), 7.01 (s, 1H), 7.53 (d, *J* = 7.6 Hz, 1H), 7.60-7.64 (m, 1H), 7.73-7.77 (m, 1H), 7.90 (d, *J* = 8.0 Hz, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ: 11.4, 13.6, 18.6, 20.8, 35.5, 36.6, 44.1, 54.0, 63.6, 80.7, 118.3, 122.6, 123.1, 125.0, 126.3, 130.6, 131.3, 134.0, 135.3, 136.4, 137.4, 141.0, 141.6, 155.5, 170.3, 172.8, 195.4, 198.7; HRMS (ESI-TOF) *m/z*: Calcd. For C<sub>28</sub>H<sub>26</sub>N<sub>4</sub>NaO<sub>6</sub> [M+Na]<sup>+</sup>: 537.1745; Found: 537.1746.



**4ca:** Light yellow solid, m.p. 221.1-221.8 °C; yield 88%, 19:1 dr;  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$ : 2.25 (s, 3H), 2.43 (s, 3H), 4.02-4.15 (m, 2H), 4.17-4.21 (m, 1H), 4.82 (d,  $J = 15.6$  Hz, 1H), 5.34-5.38 (m, 1H), 6.40 (d,  $J = 7.6$  Hz, 1H), 6.63 (d,  $J = 7.2$  Hz, 2H), 6.87-6.91 (m, 1H), 6.97-7.04 (m, 3H), 7.06-7.10 (m, 1H), 7.41-7.43 (m, 1H), 7.53 (d,  $J = 7.6$  Hz, 1H), 7.63-7.67 (m, 1H), 7.73-7.77 (m, 1H), 7.88 (d,  $J = 7.6$  Hz, 1H);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 100 MHz)  $\delta$ : 10.4, 34.4, 43.0, 43.4, 53.1, 62.6, 79.0, 107.8, 121.3, 121.9, 122.2, 126.1, 126.5, 126.9, 127.6, 128.5, 134.3, 134.5, 135.6, 139.9, 154.6, 169.0, 171.5, 194.4, 197.7; HRMS (ESI-TOF)  $m/z$ : Calcd. for  $\text{C}_{31}\text{H}_{24}\text{N}_4\text{NaO}_6$   $[\text{M}+\text{Na}]^+$ : 571.1588; Found: 571.1589.

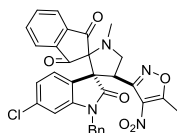


**4cb:** Light yellow solid, m.p. 179.4-179.9 °C; yield 87%, 17:1 dr;  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$ : 2.28 (s, 3H), 2.42 (s, 3H), 4.04-4.09 (m, 2H), 4.13 (d,  $J = 15.6$  Hz, 1H), 4.76 (d,  $J = 15.6$  Hz, 1H), 5.40-5.45 (m, 1H), 6.34-6.37 (m, 1H), 6.58 (d,  $J = 7.2$  Hz, 2H), 6.71-6.76 (m, 1H), 6.99-7.03 (m, 2H), 7.06-7.10 (m, 1H), 7.32-7.35 (m, 1H), 7.58 (d,  $J = 7.6$  Hz, 1H), 7.68-7.72 (m, 1H), 7.78-7.82 (m, 1H), 7.94 (d,  $J = 8.0$  Hz, 1H);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 100 MHz)  $\delta$ : 11.4, 35.3, 44.1, 44.3, 54.0, 63.6, 79.4, 109.3 (d,  $J_{\text{CF}} = 8.2$  Hz), 116.0 (d,  $J_{\text{CF}} = 23.4$  Hz), 116.2 (d,  $J_{\text{CF}} = 26.1$  Hz), 123.0, 123.4, 125.9 (d,  $J_{\text{CF}} = 8.4$  Hz), 127.0, 127.6, 128.7, 134.9, 135.7, 136.9, 137.8, 140.9, 141.3, 155.6, 158.6 (d,  $J_{\text{CF}} = 240.2$  Hz), 169.4, 171.9, 194.9, 199.0; HRMS (ESI-TOF)  $m/z$ : Calcd. for  $\text{C}_{31}\text{H}_{23}\text{FN}_4\text{NaO}_6$   $[\text{M}+\text{Na}]^+$ : 589.1494; Found: 589.1492.

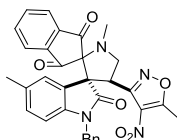


**4cc:** Light yellow solid, m.p. 258.8-259.2 °C; yield 91%, 19:1 dr;  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$ : 2.29 (s, 3H), 2.42 (s, 3H), 4.06-4.09 (m, 2H), 4.13 (d,  $J = 15.6$  Hz, 1H), 4.76 (d,  $J = 15.6$  Hz, 1H), 5.39-5.43 (m, 1H), 6.35 (d,  $J = 8.4$  Hz, 1H), 6.59 (d,  $J = 8.4$  Hz, 2H), 6.98-7.03 (m, 3H), 7.06-7.10

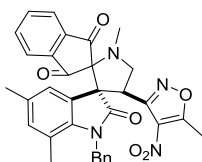
(m, 1H), 7.54 (d,  $J = 2.0$  Hz, 1H), 7.58 (d,  $J = 7.6$  Hz, 1H), 7.68-7.72 (m, 1H), 7.78-7.82 (m, 1H), 7.93 (d,  $J = 7.6$  Hz, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$ : 11.5, 35.4, 44.1, 44.4, 54.0, 63.4, 79.3, 109.7, 123.0, 123.4, 127.0, 127.7, 127.9, 128.4, 128.7, 129.5, 134.8, 135.7, 136.9, 155.6, 169.4, 171.8, 194.8, 198.9; HRMS (ESI-TOF)  $m/z$ : Calcd. for  $\text{C}_{31}\text{H}_{23}\text{ClN}_4\text{NaO}_6$   $[\text{M}+\text{Na}]^+$ : 605.1198; Found: 605.1198.



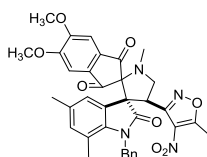
**4cd**: Light yellow solid, m.p. 233.4-234.1 °C; yield 88%, 10:1 dr;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$ : 2.29 (s, 3H), 2.41 (s, 3H), 4.03-4.14 (m, 3H), 4.77 (d,  $J = 15.6$  Hz, 1H), 5.35-5.39 (m, 1H), 6.41 (d,  $J = 1.6$  Hz, 1H), 6.60 (d,  $J = 7.6$  Hz, 2H), 6.87-6.90 (m, 1H), 7.01-7.05 (m, 2H), 7.08-7.11 (m, 1H), 7.42 (d,  $J = 8.0$  Hz, 1H), 7.57 (d,  $J = 7.6$  Hz, 1H), 7.67-7.71 (m, 1H), 7.77-7.80 (m, 1H), 7.91 (d,  $J = 7.6$  Hz, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$ : 10.4, 34.3, 43.1, 43.4, 53.0, 62.1, 78.7, 108.4, 121.4, 122.0, 122.3, 126.0, 126.7, 127.7, 128.0, 133.7, 134.4, 134.7, 135.9, 154.7, 168.6, 171.3, 194.0, 197.7; HRMS (ESI-TOF)  $m/z$ : Calcd. for  $\text{C}_{31}\text{H}_{23}\text{ClN}_4\text{NaO}_6$   $[\text{M}+\text{Na}]^+$ : 605.1198; Found: 605.1201.



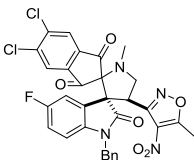
**4ce**: Light yellow solid, m.p. 208.3-208.9 °C; yield 90%, 20:1 dr;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$ : 2.21 (s, 3H), 2.25 (s, 3H), 2.44 (s, 3H), 4.02-4.11 (m, 2H), 4.15-4.19 (m, 1H), 4.80 (d,  $J = 15.6$  Hz, 1H), 5.32-5.37 (m, 1H), 6.27 (d,  $J = 8.0$  Hz, 1H), 6.60 (d,  $J = 7.2$  Hz, 2H), 6.77-6.79 (m, 1H), 6.98-7.02 (m, 2H), 7.05-7.08 (m, 1H), 7.23 (s, 1H), 7.53 (d,  $J = 8.0$  Hz, 1H), 7.63-7.67 (m, 1H), 7.73-7.77 (m, 1H), 7.89 (d,  $J = 7.6$  Hz, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$ : 9.4, 19.1, 33.5, 42.0, 42.5, 52.0, 61.8, 78.1, 106.5, 120.8, 121.3, 122.1, 125.1, 125.4, 126.5, 127.8, 129.8, 133.4, 133.5, 134.6, 137.6, 153.5, 168.1, 170.4, 193.4, 196.8; HRMS (ESI-TOF)  $m/z$ : Calcd. for  $\text{C}_{32}\text{H}_{26}\text{N}_4\text{NaO}_6$   $[\text{M}+\text{Na}]^+$ : 585.1745; Found: 585.1748.



**4cf:** Light yellow solid, m.p. 266.4-267.1 °C; yield 87%, 10:1 dr; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ: 1.91 (s, 3H), 2.20 (s, 3H), 2.28 (s, 3H), 2.44 (s, 3H), 4.05-4.16 (m, 2H), 4.42 (d, *J* = 16.8 Hz, 1H), 4.95 (d, *J* = 16.8 Hz, 1H), 5.32-5.37 (m, 1H), 6.40 (d, *J* = 7.2 Hz, 2H), 6.58 (s, 1H), 6.94-6.98 (m, 2H), 7.01-7.04 (m, 1H), 7.23 (s, 1H), 7.66-7.75 (m, 3H), 7.90 (d, *J* = 7.2 Hz, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ: 10.4, 17.5, 19.8, 34.4, 44.1, 44.3, 52.9, 62.4, 79.1, 117.8, 121.9, 122.3, 123.9, 124.3, 125.6, 125.9, 127.5, 130.7, 133.1, 134.3, 135.7, 136.0, 136.7, 140.0, 140.5, 154.5, 169.0, 172.4, 194.2, 198.2; HRMS (ESI-TOF) *m/z*: Calcd. For C<sub>33</sub>H<sub>28</sub>N<sub>4</sub>NaO<sub>6</sub> [M+Na]<sup>+</sup>: 599.1901; Found: 599.1905.

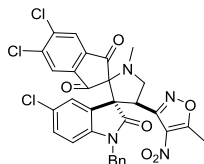


**4cg:** Light yellow solid, m.p. 196.4-196.9 °C; yield 85%, 15:1 dr; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ: 1.96 (s, 3H), 2.20 (s, 3H), 2.29 (s, 3H), 2.43 (s, 3H), 3.88 (s, 3H), 3.92 (s, 3H), 4.04-4.14 (m, 2H), 4.44 (d, *J* = 16.4 Hz, 1H), 4.99 (d, *J* = 16.4 Hz, 1H), 5.31-5.36 (m, 1H), 6.44 (d, *J* = 7.6 Hz, 2H), 6.59 (s, 1H), 6.91-6.95 (m, 2H), 7.01-7.05 (m, 2H), 7.24 (d, *J* = 6.8 Hz, 2H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ: 11.5, 18.7, 20.9, 35.4, 45.1, 45.5, 54.0, 56.6, 56.8, 63.1, 79.7, 103.0, 103.2, 118.7, 125.3, 125.7, 126.7, 127.0, 128.3, 131.6, 134.0, 136.2, 137.0, 137.3, 137.8, 155.5, 155.8, 156.8, 170.3, 173.7, 193.7, 197.8; HRMS (ESI-TOF) *m/z*: Calcd. for C<sub>35</sub>H<sub>32</sub>N<sub>4</sub>NaO<sub>8</sub> [M+Na]<sup>+</sup>: 659.2112; Found: 659.2108.

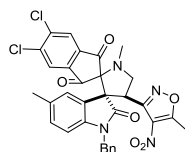


**4ch:** Light yellow solid, m.p. 248.6-248.9 °C; yield 89%, 20:1 dr; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ: 2.28 (s, 3H), 2.38 (s, 3H), 4.03-4.05 (m, 2H), 4.18 (d, *J* = 15.6 Hz, 1H), 4.74 (d, *J* = 15.6 Hz, 1H), 5.37-5.41 (m, 1H), 6.46-6.49 (m, 1H), 6.67 (d, *J* = 7.2 Hz, 2H), 6.77-6.82 (m, 1H), 7.03-7.07 (m, 2H), 7.12-7.16 (m, 1H), 7.34-7.37 (m, 1H), 7.57 (s, 1H), 7.99 (s, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100

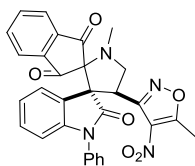
MHz)  $\delta$ : 10.4, 34.2, 43.1, 43.2, 52.8, 62.7, 78.6, 108.3 (d,  $J_{CF} = 8.1$  Hz), 115.2 (d,  $J_{CF} = 26.1$  Hz), 115.3 (d,  $J_{CF} = 24.2$  Hz), 123.6, 124.0, 124.7 (d,  $J_{CF} = 9.2$  Hz), 126.2, 126.9, 127.6, 133.8, 138.2, 140.1, 154.6, 157.7 (d,  $J_{CF} = 240.3$  Hz), 168.0, 170.5, 191.5, 196.0; HRMS (ESI-TOF)  $m/z$ : Calcd. for  $C_{31}H_{21}Cl_2FN_4NaO_6 [M+Na]^+$ : 657.0714; Found: 657.0710.



**4ci**: Light yellow solid, m.p. 228.6-229.6 °C; yield 89%, 18:1 dr;  $^1H$  NMR ( $CDCl_3$ , 400 MHz)  $\delta$ : 2.29 (s, 3H), 2.38 (s, 3H), 4.03-4.05 (m, 2H), 4.18 (d,  $J = 15.2$  Hz, 1H), 4.73 (d,  $J = 15.2$  Hz, 1H), 5.35-5.39 (m, 1H), 6.48 (d,  $J = 8.4$  Hz, 1H), 6.68 (d,  $J = 7.2$  Hz, 2H), 7.03-7.08 (m, 3H), 7.12-7.16 (m, 1H), 7.56 (d,  $J = 2.8$  Hz, 2H), 7.99 (s, 1H);  $^{13}C$  NMR ( $CDCl_3$ , 100 MHz)  $\delta$ : 10.4, 34.2, 43.2, 52.8, 62.5, 78.5, 108.7, 123.7, 124.0, 124.7, 126.3, 126.9, 127.1, 127.4, 127.6, 128.7, 133.7, 138.6, 154.6, 168.0, 170.4, 191.4, 196.0; HRMS (ESI-TOF)  $m/z$ : Calcd. for  $C_{31}H_{21}Cl_3N_4NaO_6 [M+Na]^+$ : 673.0419; Found: 673.0413.



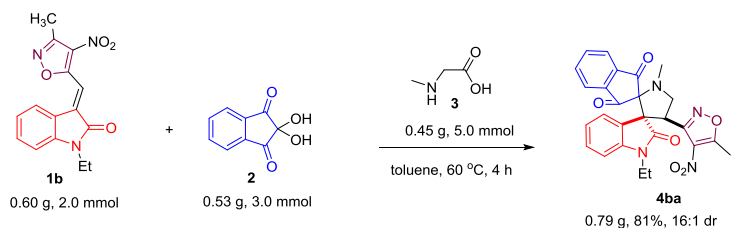
**4cj**: Light yellow solid, m.p. 238.1-238.8 °C; yield 87%, 10:1 dr;  $^1H$  NMR ( $CDCl_3$ , 400 MHz)  $\delta$ : 2.24 (s, 3H), 2.26 (s, 3H), 2.40 (s, 3H), 3.99-4.04 (m, 1H), 4.11-4.15 (m, 2H), 4.77 (d,  $J = 15.2$  Hz, 1H), 5.29-5.33 (m, 1H), 6.41 (d,  $J = 8.0$  Hz, 1H), 6.70 (d,  $J = 7.2$  Hz, 2H), 6.85 (d,  $J = 8.0$  Hz, 1H), 7.02-7.06 (m, 2H), 7.11-7.14 (m, 1H), 7.26 (s, 1H), 7.52 (s, 1H), 7.93 (s, 1H);  $^{13}C$  NMR ( $CDCl_3$ , 100 MHz)  $\delta$ : 11.4, 21.2, 35.4, 44.1, 44.4, 53.9, 64.0, 80.3, 108.5, 123.9, 124.6, 125.0, 127.4, 127.7, 128.5, 128.6, 130.1, 132.1, 135.3, 139.3, 139.5, 155.6, 169.7, 172.0, 193.0, 196.9; HRMS (ESI-TOF)  $m/z$ : Calcd. for  $C_{32}H_{24}Cl_2N_4NaO_6 [M+Na]^+$ : 653.0965; Found: 653.0968.



**4da**: Light yellow solid, m.p. 209.1-209.8 °C; yield 86%, 16:1 dr;  $^1H$  NMR ( $CDCl_3$ , 400 MHz)  $\delta$ : 2.30 (s, 3H), 2.47 (s, 3H), 4.04-4.09 (m, 1H), 4.28-4.32 (m, 1H), 5.26-5.30 (m, 1H), 6.38 (d,  $J =$

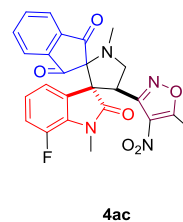
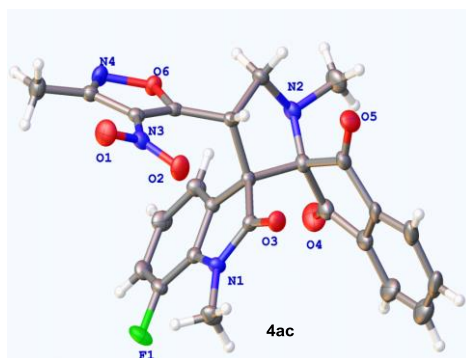
7.6 Hz, 1H), 6.85-6.89 (m, 3H), 6.95-6.99 (m, 1H), 7.19-7.24 (m, 2H), 7.27-7.31 (m, 2H), 7.58 (d,  $J = 7.2$  Hz, 1H), 7.62-7.66 (m, 1H), 7.70-7.74 (m, 1H), 7.86 (d,  $J = 7.6$  Hz, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$ : 10.0, 34.1, 42.8, 52.6, 62.7, 79.2, 107.7, 121.1, 121.2, 121.8, 122.3, 124.6, 126.1, 126.8, 128.1, 132.2, 132.3, 135.2, 154.3, 169.2, 171.0, 194.5, 196.6; HRMS (ESI-TOF)  $m/z$ : Calcd. for  $\text{C}_{30}\text{H}_{22}\text{N}_4\text{NaO}_6$   $[\text{M}+\text{Na}]^+$ : 557.1432; Found: 557.1435.

#### 4. Scheme S1: gram scale synthesis of the products **4ba**



In a sealed tube equipped with a magnetic stirring bar, to 20 mL of toluene was added 3-methyl-4-nitro-5-isatylidenyl-isoxazole **1b** (0.60 g, 2.0 mmol), ninhydrin **2** (0.53 g, 3.0 mmol) and sarcosine **3** (0.45 g, 5.0 mmol). The reaction mixture was stirred at 60 °C for 4 h. After completion of the reaction, as indicated by TLC, purification by flash column chromatography (hexane/EtOAc, 5/1, v/v) was carried out to furnish the hybrid **4ba** (0.79 g, 81%, 16:1 dr).

## 5. X-ray crystal data for compounds 4ac and 4bb

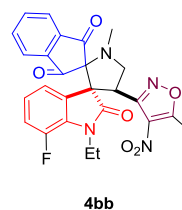
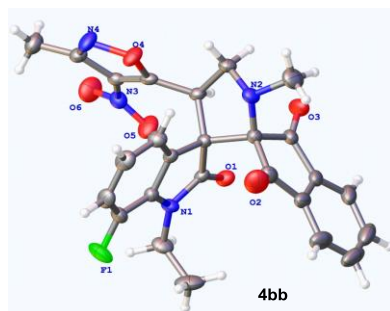


**Table S1 Crystal data and structure refinement for 4ac**

Identification code	<b>4ac</b>
Empirical formula	C <sub>25</sub> H <sub>19</sub> FN <sub>4</sub> O <sub>6</sub>
Formula weight	490.44
Temperature/K	199.92(16)
Crystal system	monoclinic
Space group	P2 <sub>1</sub> /c
a/Å, b/Å, c/Å	12.6086(7), 8.8642(5), 20.3547(14)
α/°, β/°, γ/°	90, 92.860(5), 90
Volume/Å <sup>3</sup>	2272.1(2)
Z	4
ρ <sub>calc</sub> /cm <sup>3</sup>	1.434
μ/mm <sup>-1</sup>	0.110
F(000)	1016.0
Radiation	Mo Kα (λ = 0.71073)
Crystal size/mm <sup>3</sup>	0.15 × 0.13 × 0.12
2θ range for data collection/°	4.008 to 49.994
Index ranges	-12 ≤ h ≤ 14, -8 ≤ k ≤ 10, -24 ≤ l ≤ 24
Reflections collected	9504
Independent reflections	3994 [R <sub>int</sub> = 0.0241, R <sub>sigma</sub> = 0.0329]
Data/restraints/parameters	3994/0/328
Goodness-of-fit on F <sup>2</sup>	1.062
Final R indexes [I >= 2σ(I)]	R <sub>1</sub> = 0.0490, wR <sub>2</sub> = 0.1200
Final R indexes [all data]	R <sub>1</sub> = 0.0620, wR <sub>2</sub> = 0.1309
Largest diff. peak/hole / e Å <sup>-3</sup>	0.43/-0.37

**Crystal Data** for C<sub>25</sub>H<sub>19</sub>FN<sub>4</sub>O<sub>6</sub> (*M* = 490.44 g/mol): monoclinic, space group P2<sub>1</sub>/c (no. 14), *a* = 12.6086(7) Å, *b* = 8.8642(5) Å, *c* = 20.3547(14) Å, β = 92.860(5)°, *V* = 2272.1(2) Å<sup>3</sup>, *Z* = 4, *T* = 199.92(16) K, μ(Mo Kα) = 0.110 mm<sup>-1</sup>, *D*<sub>calc</sub> = 1.434 g/cm<sup>3</sup>, 9504 reflections measured (4.008° ≤ 2θ ≤ 49.994°), 3994 unique (*R*<sub>int</sub> = 0.0241, *R*<sub>sigma</sub> = 0.0329) which were used in all calculations. The final *R*<sub>1</sub> was 0.0490 (*I* > 2σ(*I*)) and *wR*<sub>2</sub> was 0.1309 (all data).





**Table S2 Crystal data and structure refinement for 4bb**

Identification code	<b>4bb</b>
Empirical formula	C <sub>26</sub> H <sub>21</sub> FN <sub>4</sub> O <sub>6</sub>
Formula weight	504.47
Temperature/K	199.99(10)
Crystal system	monoclinic
Space group	P2 <sub>1</sub> /c
a/Å, b/Å, c/Å	12.3686(8), 8.7434(7), 21.3869(12)
α/°, β/°, γ/°	90, 90.743(6), 90
Volume/Å <sup>3</sup>	2312.7(3)
Z	4
ρ <sub>calc</sub> /cm <sup>3</sup>	1.449
μ/mm <sup>-1</sup>	0.110
F(000)	1048.0
Radiation	Mo Kα (λ = 0.71073)
Crystal size/mm <sup>3</sup>	0.15 × 0.12 × 0.11
2θ range for data collection/°	5.004 to 49.996
Index ranges	-14 ≤ h ≤ 13, -8 ≤ k ≤ 10, -21 ≤ l ≤ 25
Reflections collected	10333
Independent reflections	4067 [R <sub>int</sub> = 0.0293, R <sub>sigma</sub> = 0.0393]
Data/restraints/parameters	4067/0/337
Goodness-of-fit on F <sup>2</sup>	1.040
Final R indexes [I >= 2σ (I)]	R <sub>1</sub> = 0.0426, wR <sub>2</sub> = 0.0911
Final R indexes [all data]	R <sub>1</sub> = 0.0573, wR <sub>2</sub> = 0.0994
Largest diff. peak/hole / e Å <sup>-3</sup>	0.18/-0.20

**Crystal Data** for C<sub>26</sub>H<sub>21</sub>FN<sub>4</sub>O<sub>6</sub> (*M* = 504.47 g/mol): monoclinic, space group P2<sub>1</sub>/c (no. 14), *a* = 12.3686(8) Å, *b* = 8.7434(7) Å, *c* = 21.3869(12) Å, β = 90.743(6)°, *V* = 2312.7(3) Å<sup>3</sup>, *Z* = 4, *T* = 199.99(10) K, μ(Mo Kα) = 0.110 mm<sup>-1</sup>, *D*<sub>calc</sub> = 1.449 g/cm<sup>3</sup>, 10333 reflections measured (5.004° ≤ 2θ ≤ 49.996°), 4067 unique (*R*<sub>int</sub> = 0.0293, *R*<sub>sigma</sub> = 0.0393) which were used in all calculations. The final *R*<sub>1</sub> was 0.0426 (*I* > 2σ(*I*)) and *wR*<sub>2</sub> was 0.0994 (all data).

## 6. MTT assay

Cells were plated into 96-well plates at a density of approximate  $4.5 \times 10^3$  cells/well, incubated for 24 h at 37°C in a CO<sub>2</sub> incubator. After incubation, cells were treated with test compounds for 48 h. Next, 10 μL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (5 mg/mL) was added to each well to react with the mitochondria of living cells for approximately 4 h. Then the supernatant from each well was carefully removed, formazon crystals were dissolved in 150 μL DMSO and absorbance at 490 nm wavelength was recorded.

## 7. Colony formation assay

K562 cells were seeded into 6-well plates at a density of 300 cells/mL, and 1 mL of cell suspension was added to each well. After 24 h, another 1 mL of medium containing compound **4ah** with different concentrations was added to the 6-well plate to make the final concentration reach the specified concentration, and continue to be cultured for a week. The control group was treated with 0.1% DMSO. After a week of treatment, colonies were photographed and counted.

## 8. Cell morphology observation

K562 cells were seeded into 6-well plates at a density of  $1 \times 10^5$  cells/mL, and 1 mL of cell suspension was added to each well. After 24 h, another 1 mL of medium containing compound **4ah** with different concentrations was added to the 6-well plate to make the final concentration reach the specified concentration, and the culture was continued for 24 h, and the changes of cell morphology were observed under phase contrast microscope (Leica Inc., Germany).

## 9. AO/EB staining

K562 cells were seeded into 6-well plates at a density of  $1 \times 10^5$  cells/mL, and 1 mL of cell suspension was added to each well. After 24 h, another 1 mL of medium containing compound **4ah** with different concentrations was added to the 6-well plate to make the final concentration reach the specified concentration, and the culture was

continued for another 24 h, and 0.1% DMSO was used to culture control cells. Cells were harvested and washed with ice-cold PBS and stained with 500  $\mu$ L AO-EB stain (containing 1 mg/mL AO and 1 mg/mL EB in PBS) at room temperature for 5 min without light. Cells were harvested and washed with ice-cold PBS again, and fluorescence was examined under a fluorescence microscope (Leica Inc., Germany).

#### **10. Analysis for apoptosis by flow cytometry**

K562 cells were seeded into 6-well plates at a density of  $1 \times 10^5$  cells/mL, and 1 mL was added to each well. After 24 h, 1 mL of medium containing compound **4ah** with different concentrations was added to make the final concentration at the specified concentration, and the culture was continued for 24 h. And the control group was treated with 0.1% DMSO. After harvested and washed in PBS, the collected cells were stained with Annexin V-PE/7-AAD according to the instructions of the Annexin V-PE/7-AAD apoptosis kit (MultiSciences, China). Finally, cells were suspended and analyzed by flow cytometry (ACEA Novocyte, USA).

#### **11. Analysis of the cell cycle using flow cytometry**

K562 cells were seeded into 6-well plates at a density of  $1 \times 10^5$  cells/mL, and 1 mL was added to each well. After 24 h, 1 mL of medium containing compound **4ah** with different concentrations was added to make the final concentration at the specified concentration, and the culture was continued for 24 h, and the control group was treated with 0.1% DMSO. Then the cells were harvested and washed with PBS. After fixation in 1 mL 70% ethyl alcohol at 4°C for more than 24 h, the cells were washed in PBS incubated in PI/RNase Staining Buffer (BD Biosciences, USA) for another 15 min at room temperature in the dark, and analyzed using flow cytometry (ACEA Novocyte, USA).

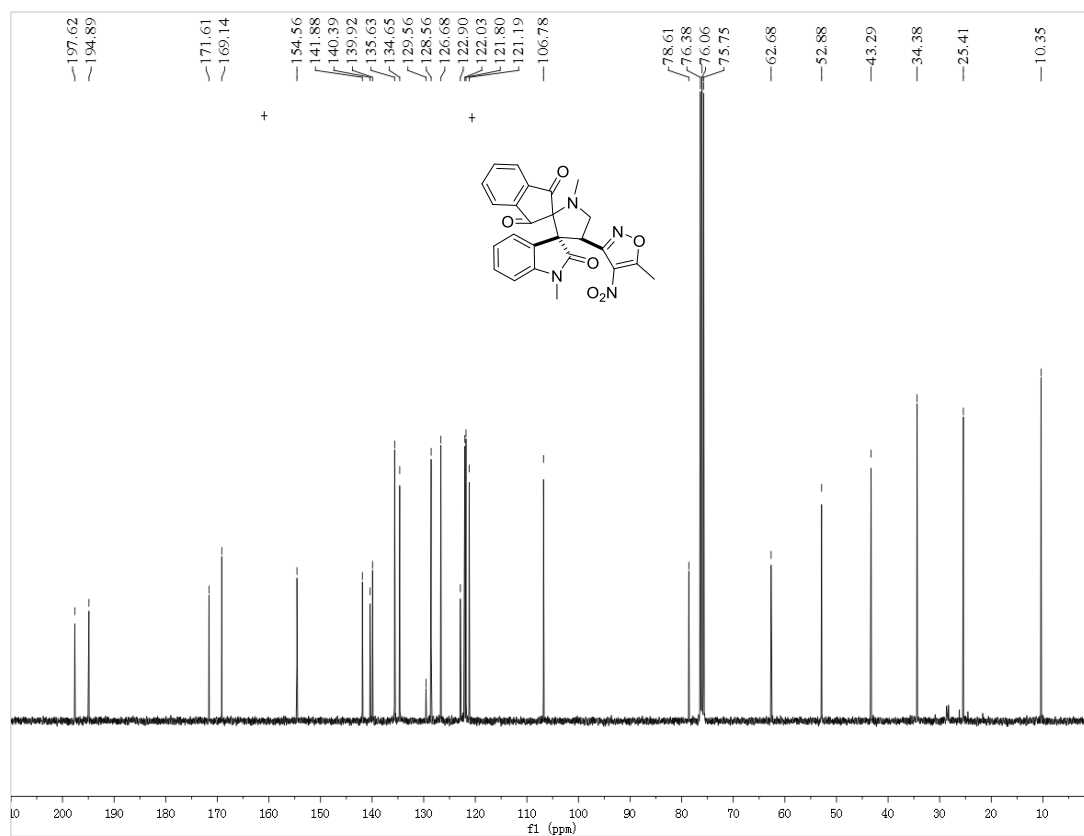
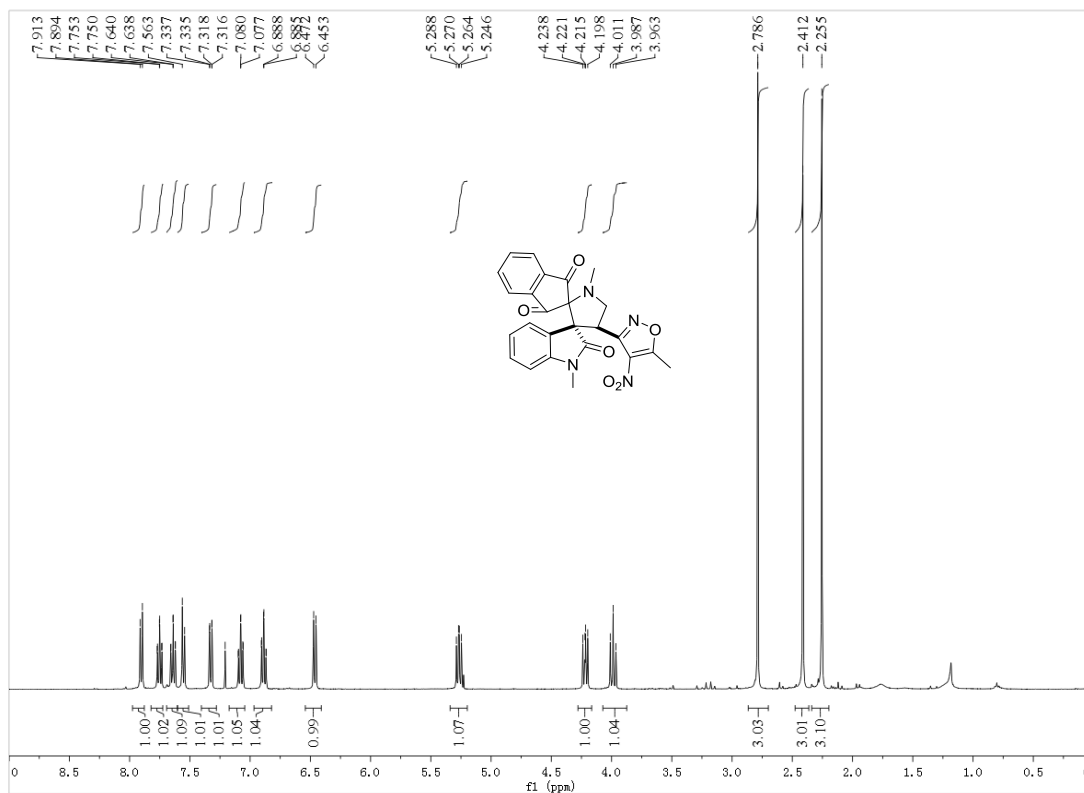
#### **12. Western blot analysis**

K562 cells were seeded into 6-well plates at a density of  $1 \times 10^5$  cells/mL, and 1 mL was added to each well. After 24 h, 1 mL of medium containing compound **4ah** with different concentrations was added to make the final concentration at the specified

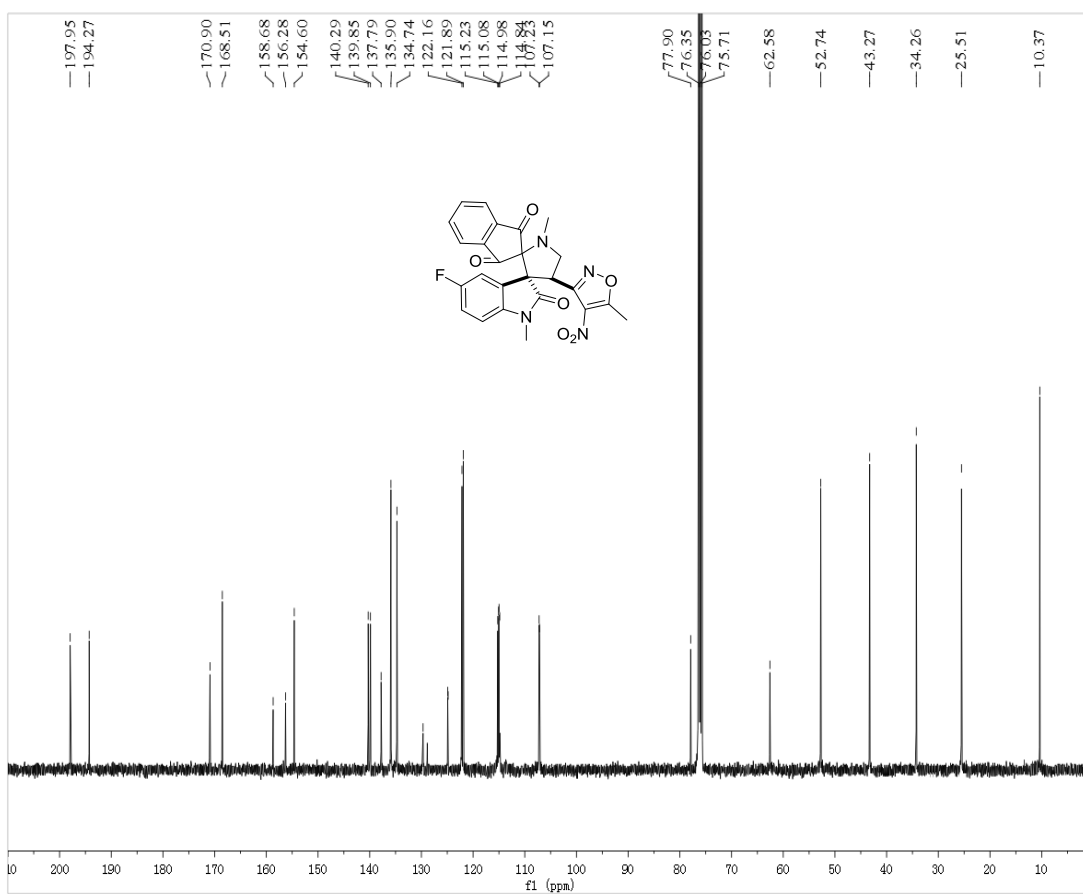
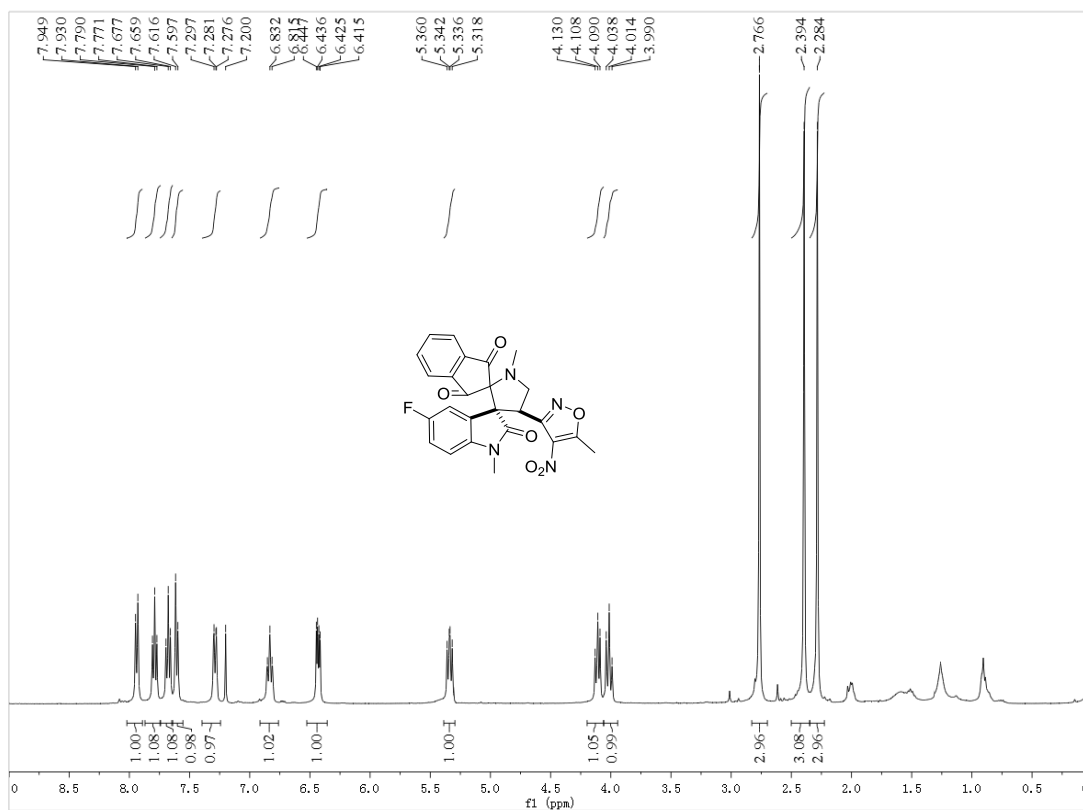
concentration, and the culture was continued for 24 h, and the control group was treated with 0.1% DMSO. After harvested and washed in PBS, total cell protein was extracted by RIPA lysis buffer (Beyotime, China) with PMSF. Nuclear and cytosolic proteins were extracted with the Nuclear/Cytosol Fractionation Kit (Beyotime, China) according to the manufacturer's protocol, and then supernatant was collected and quantified by BCA Protein Assay Kit (Beyotime, China), and boiled with 5×loading buffer for 5 min at 100°C. Subsequently, all protein samples were separated using 8%-12% SDS-PAGE gels and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, USA). After blocking in 5% defatted milk at room temperature for 1 h, and then incubated with primary antibodies ( PARP (#9532S, Cell Signaling, USA); Cleaved-PARP (#5625 Cell Signaling, USA); Caspase-3 (ab32351, Abcam, USA);  $\beta$ -actin (#4970, Cell Signaling, USA); AIF (#67791-1-Ig, Proteintech, China); Histone-H3 (#17168-1-AP, Proteintech, China)) at 4°C overnight. The second day, membranes were washed in TBST thrice and then were incubated with corresponding second antibodies (HRP-linked anti-rabbit IgG (#7074, Cell Signaling, USA); HRP-linked anti-mouse IgG (#7076, Cell Signaling, USA)) at room temperature for 1 h. Subsequently, membranes were washed in TBST thrice and ECL substrate was added to the surface of the membranes, and then the chemiluminescent signals were observed and collected by ChemiDoc<sup>TM</sup> Touch Imaging System (BIO-RAD, USA). Finally, the protein bands were analyzed using Image Lab software.

13. The copies of  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra for compounds 4

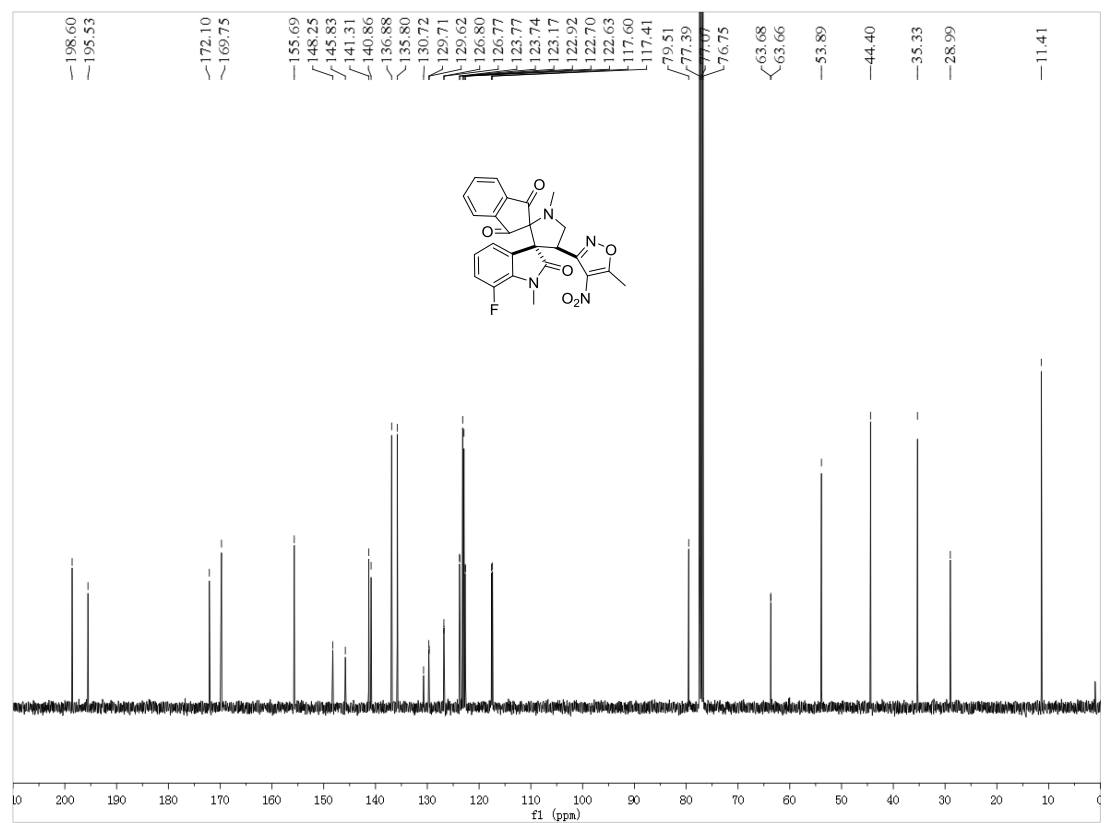
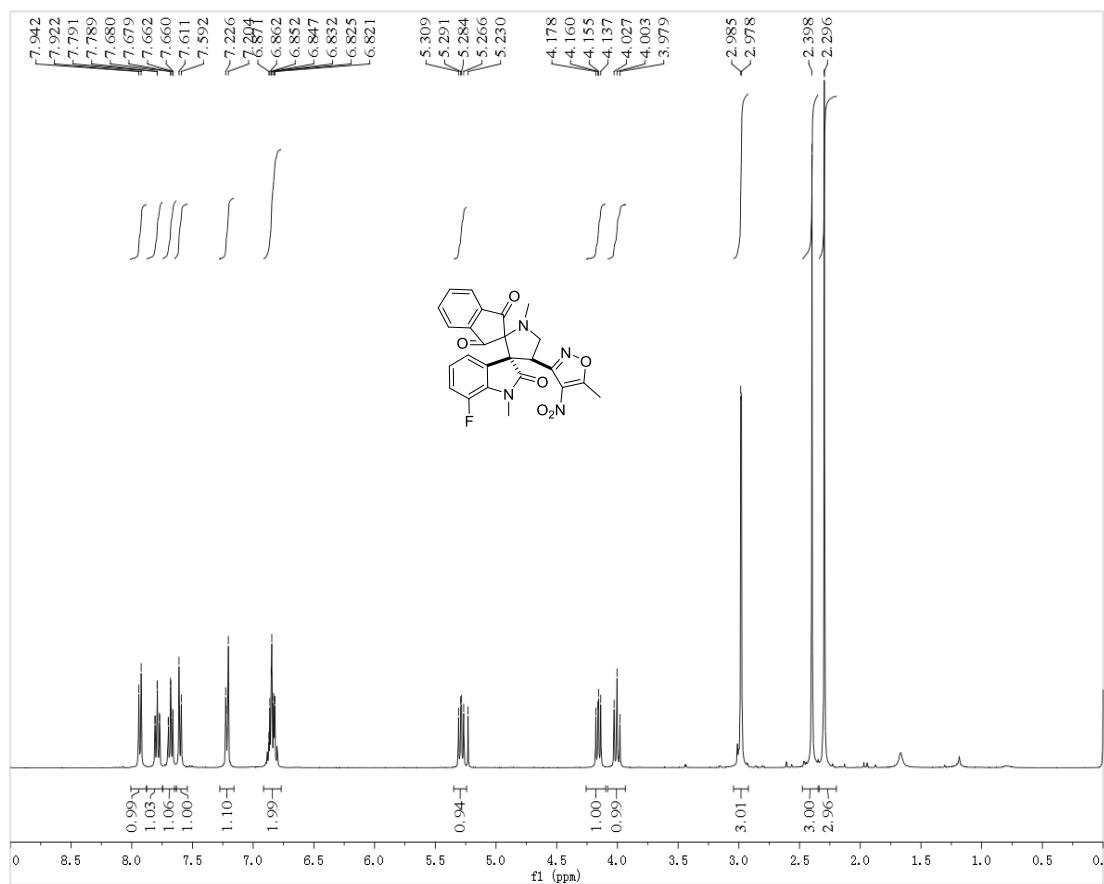
$^1\text{H}$  and  $^{13}\text{C}$  NMR of 4aa



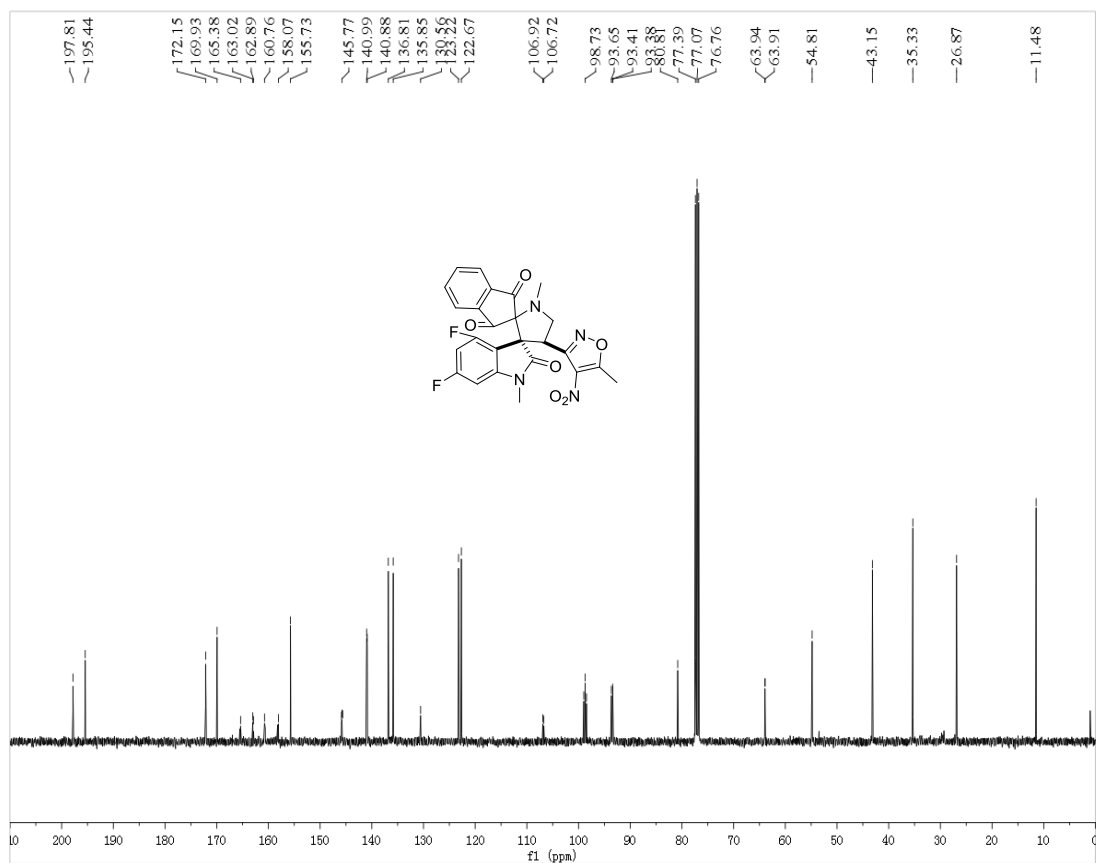
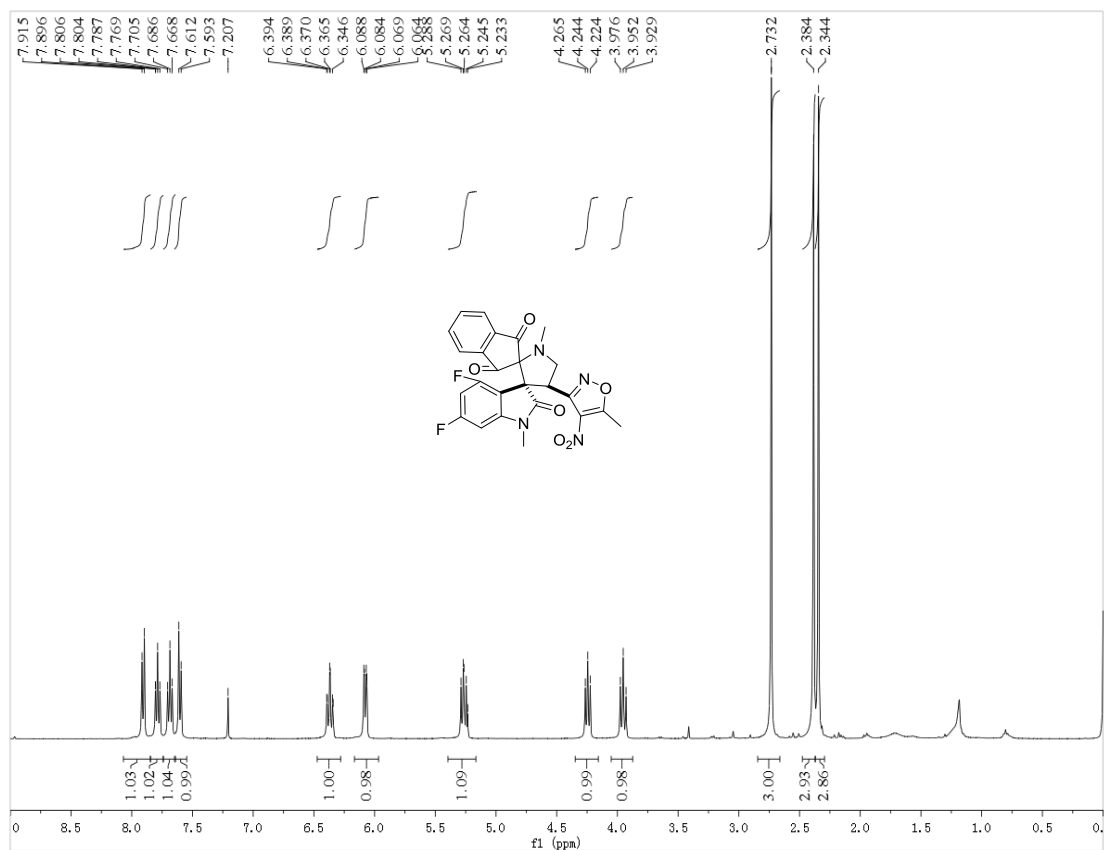
# <sup>1</sup>H and <sup>13</sup>C NMR of 4ab



# $^1\text{H}$ and $^{13}\text{C}$ NMR of 4ac

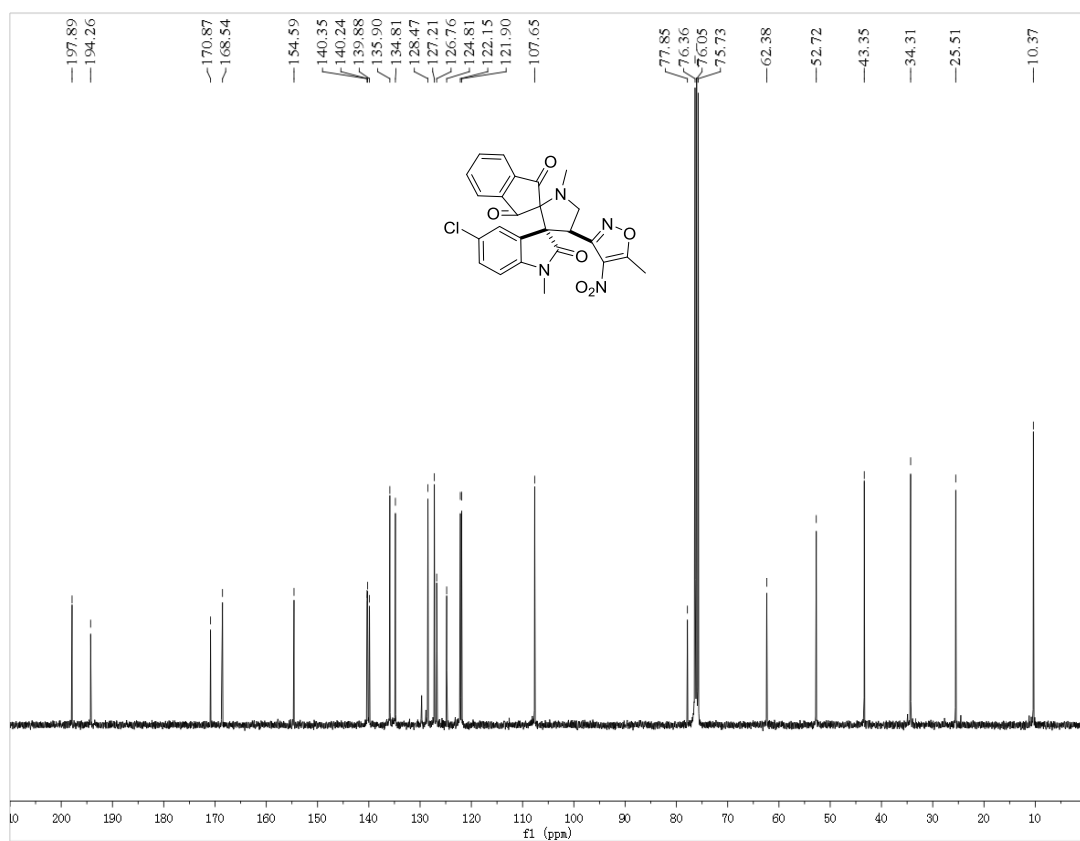
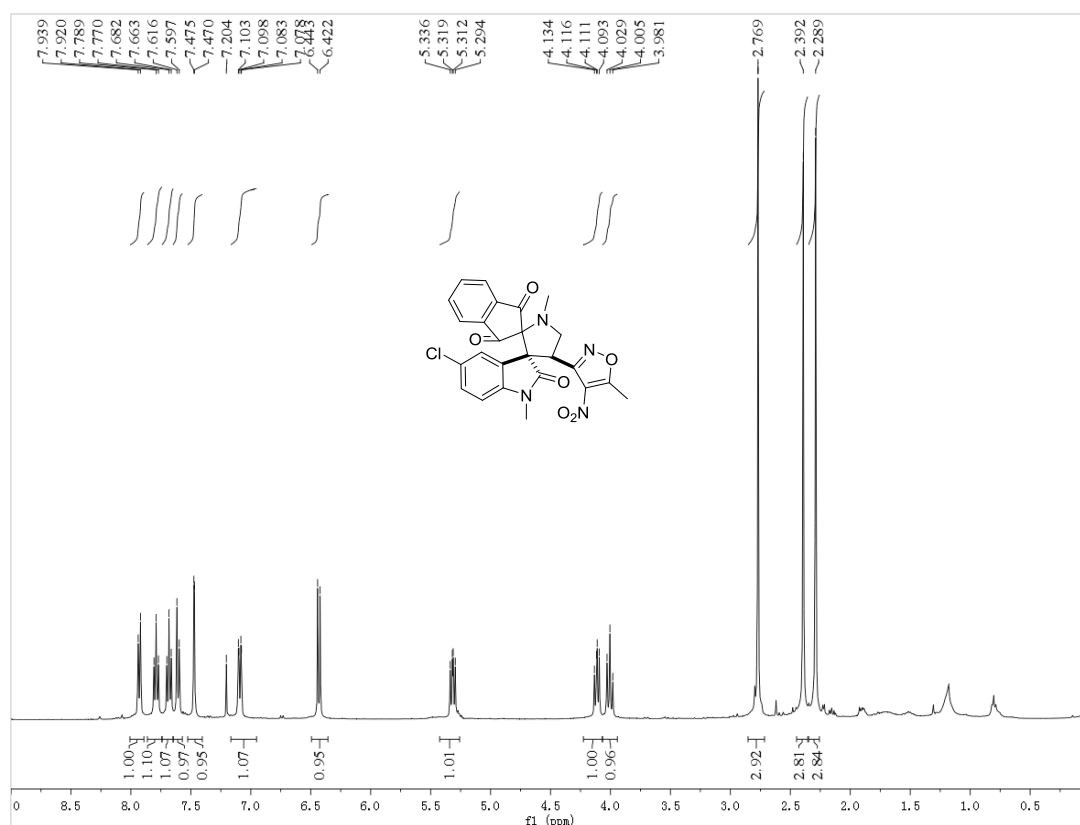


# $^1\text{H}$ and $^{13}\text{C}$ NMR of 4ad

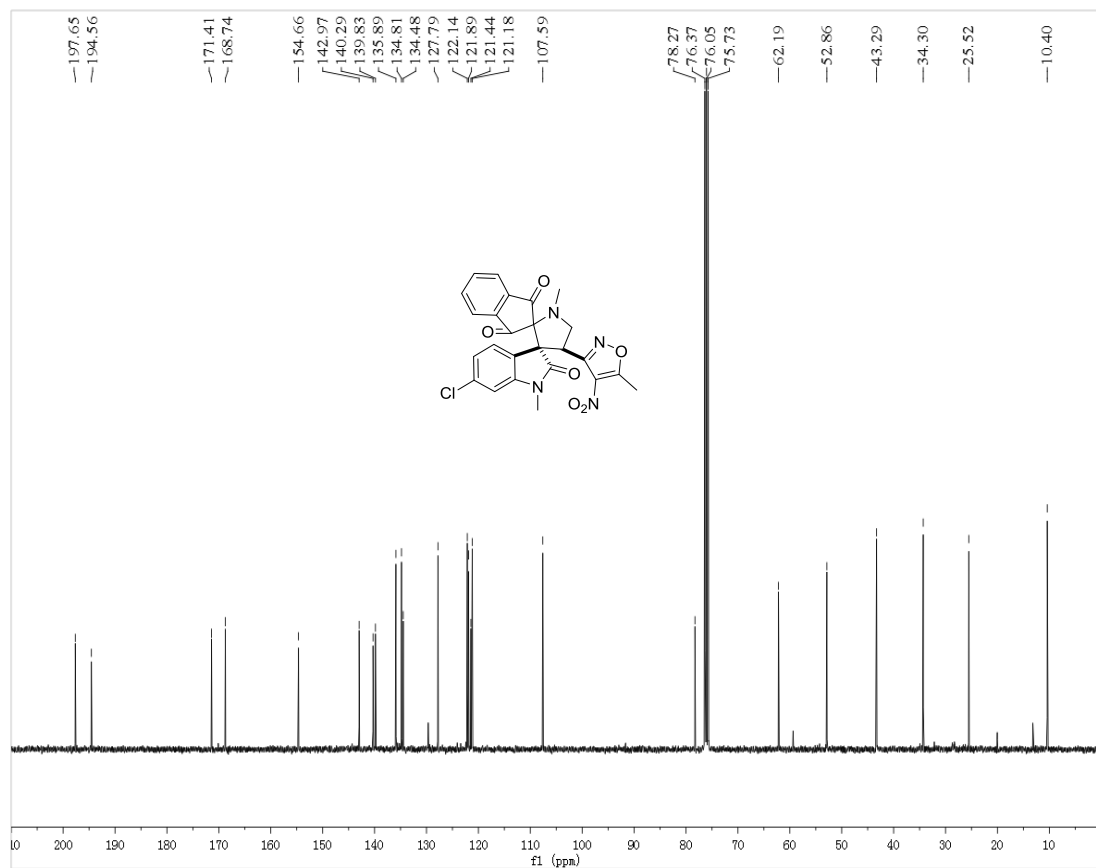
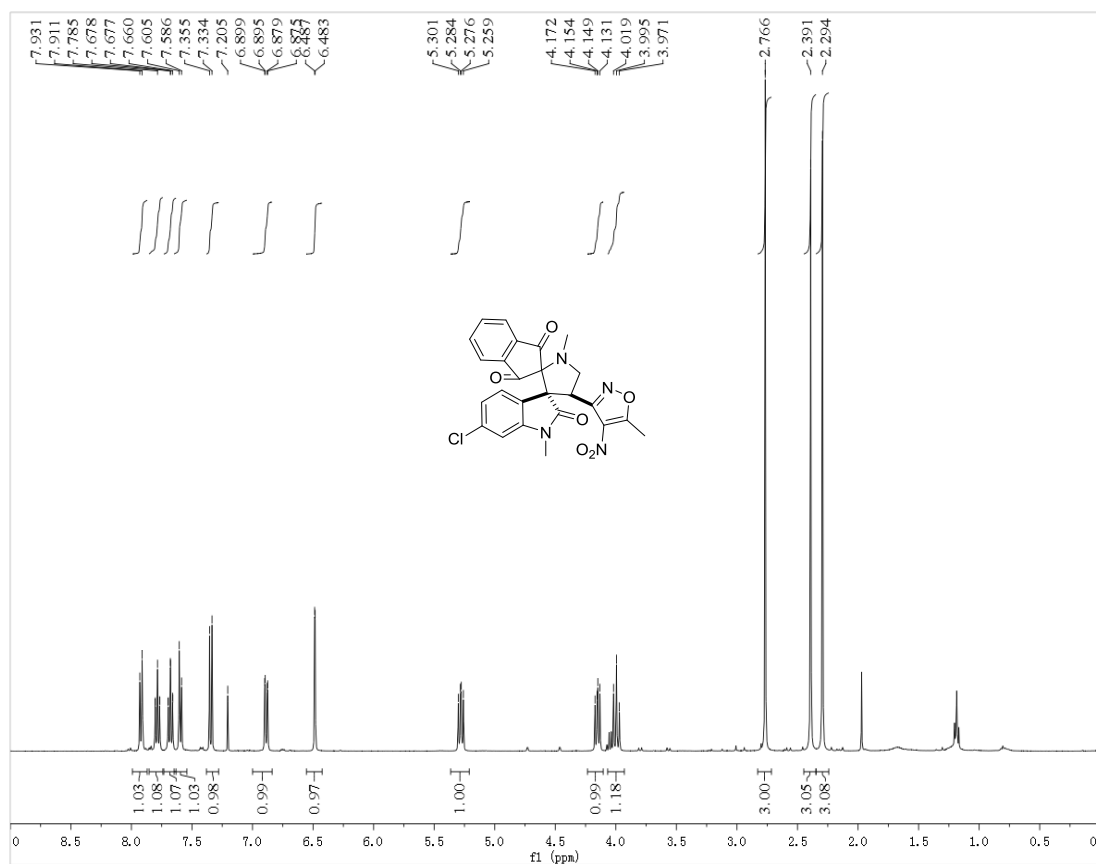




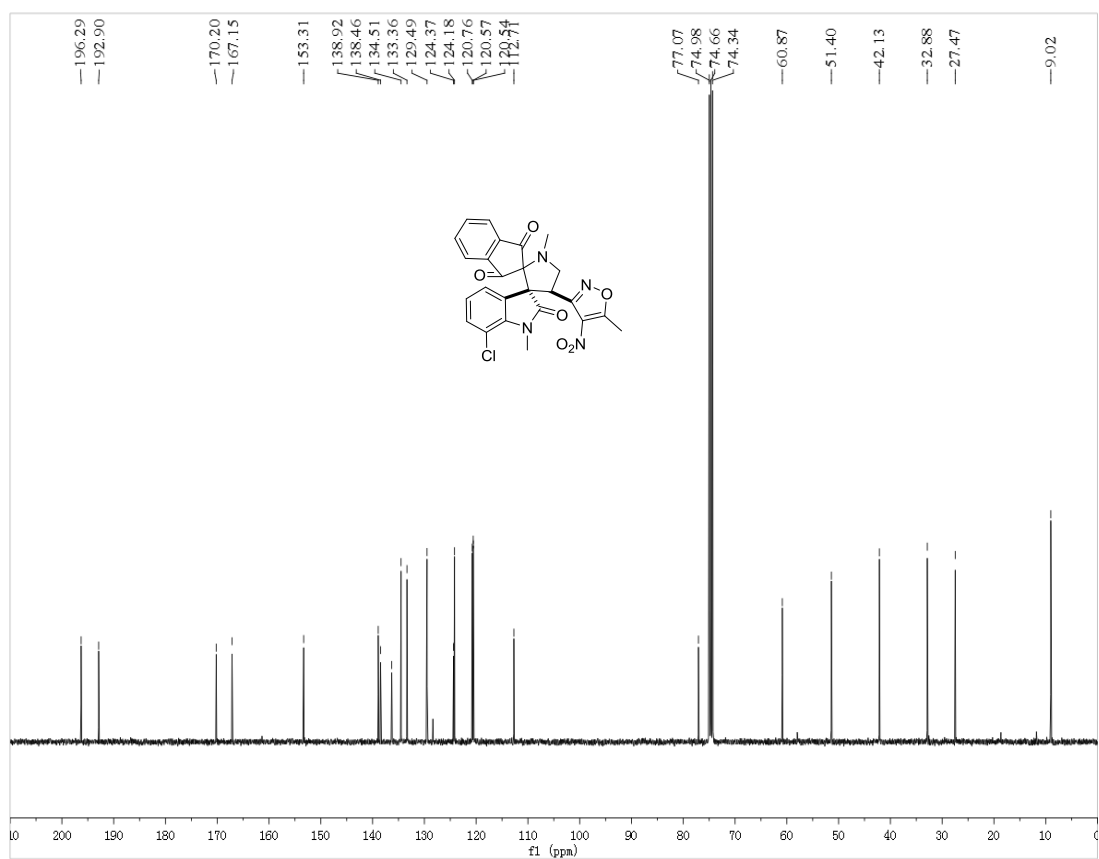
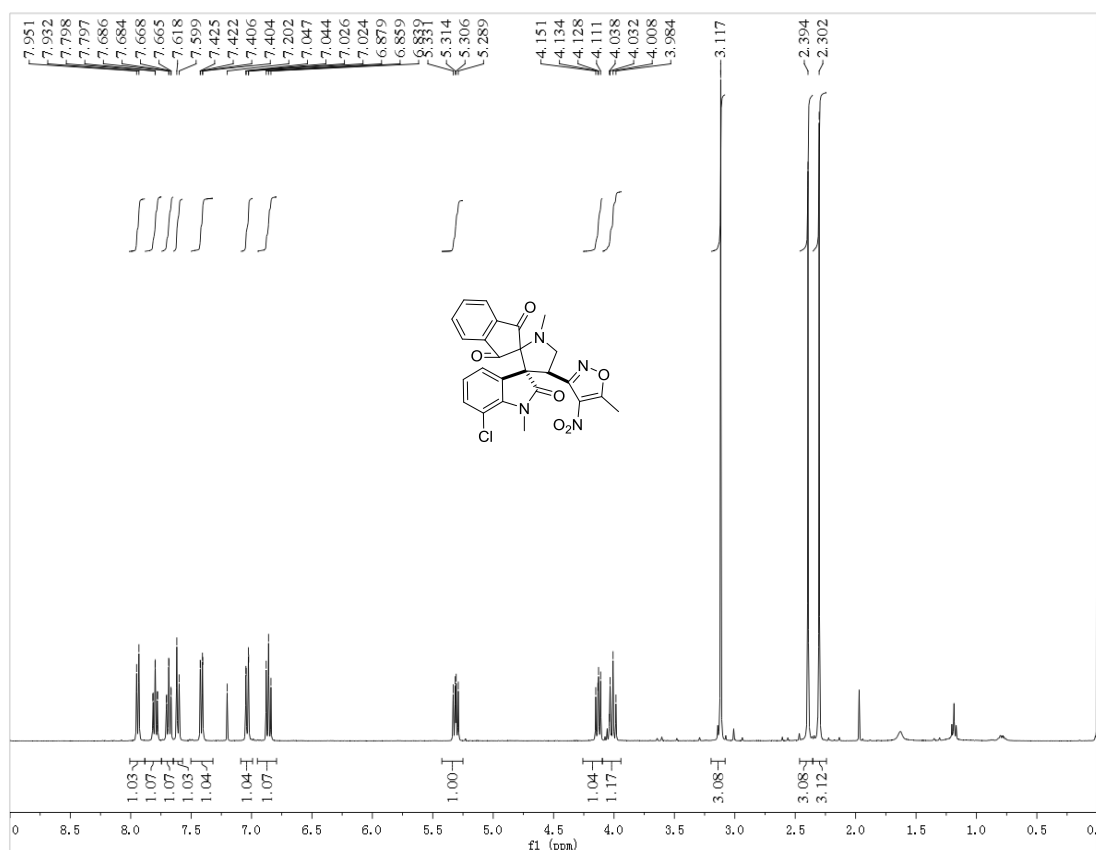
# $^1\text{H}$ and $^{13}\text{C}$ NMR of 4ae



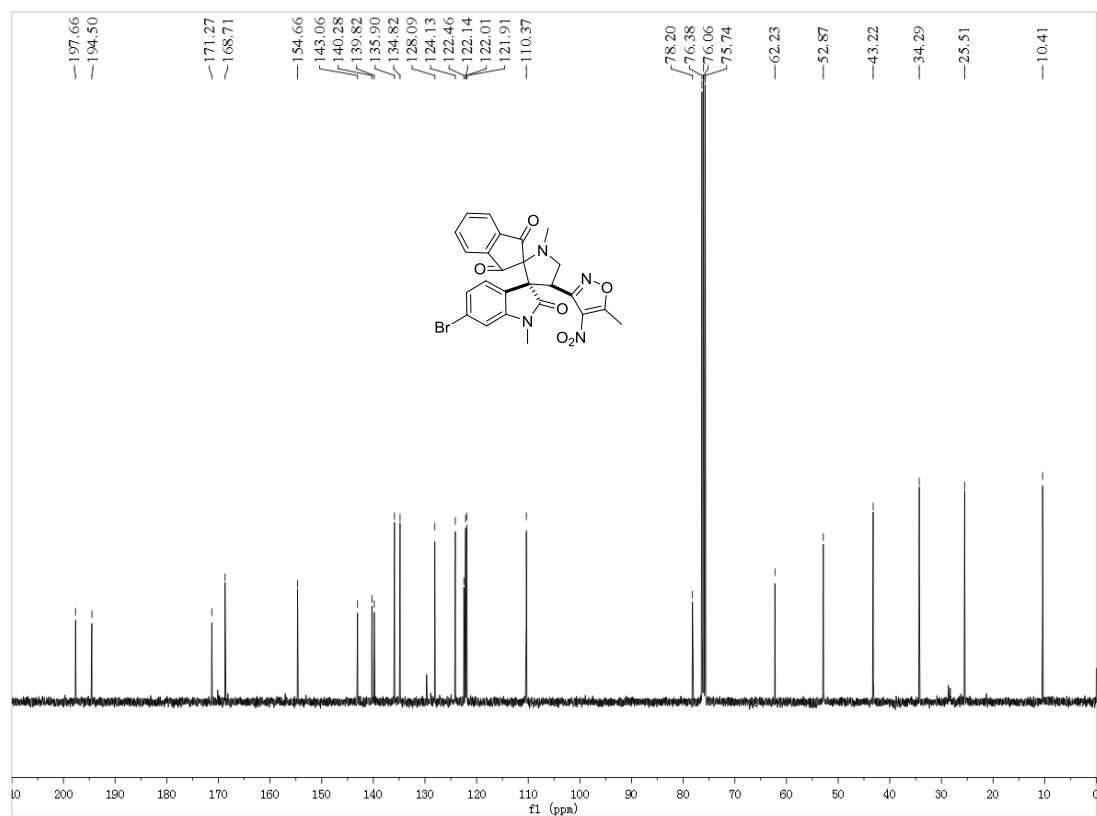
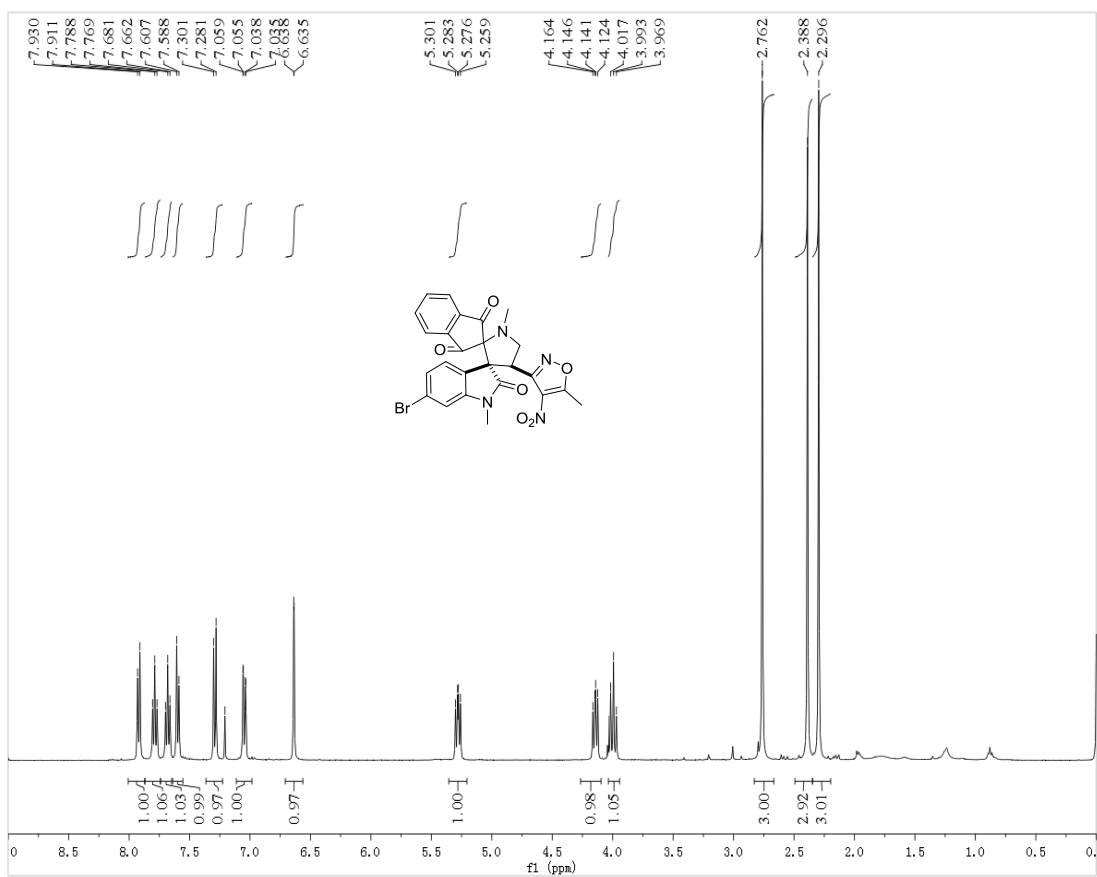
# $^1\text{H}$ and $^{13}\text{C}$ NMR of 4af



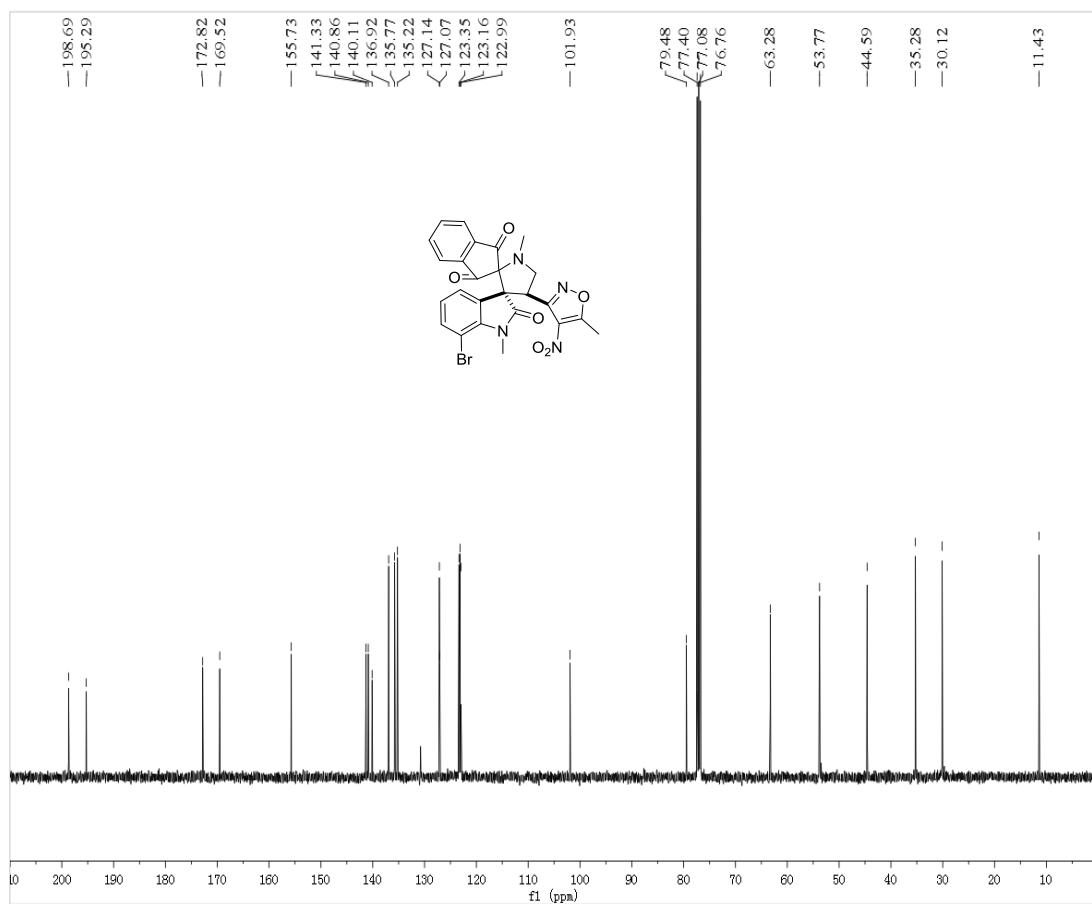
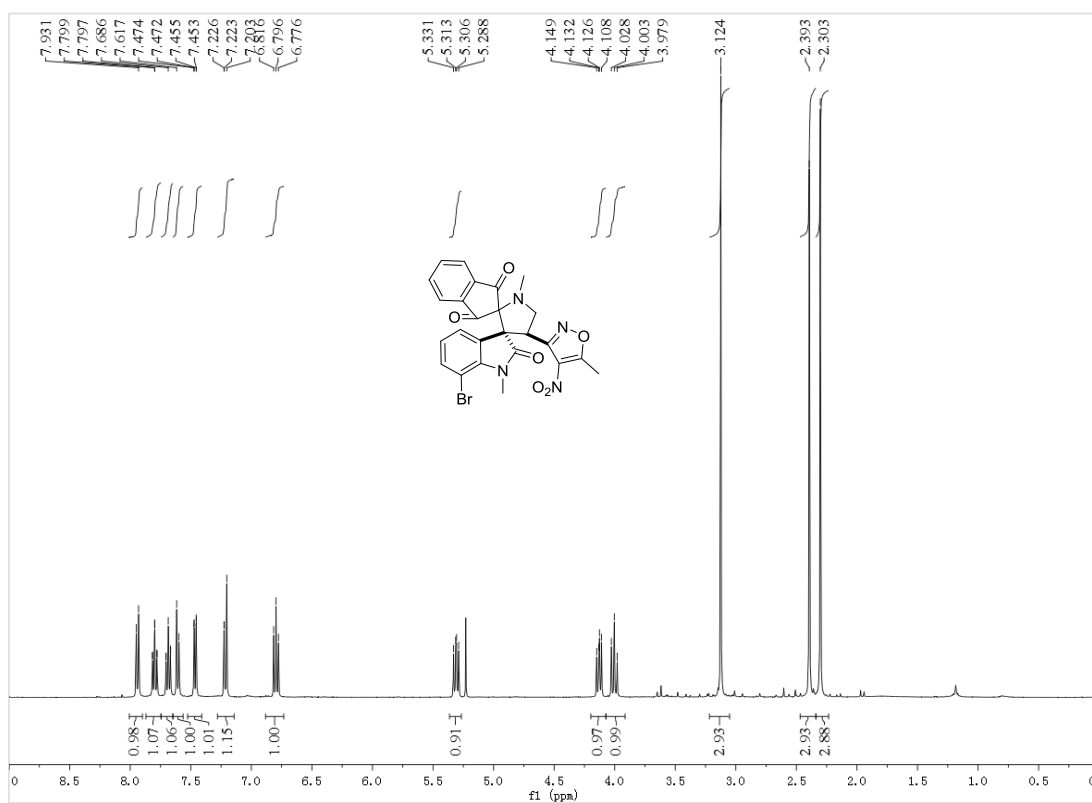
# <sup>1</sup>H and <sup>13</sup>C NMR of 4ag



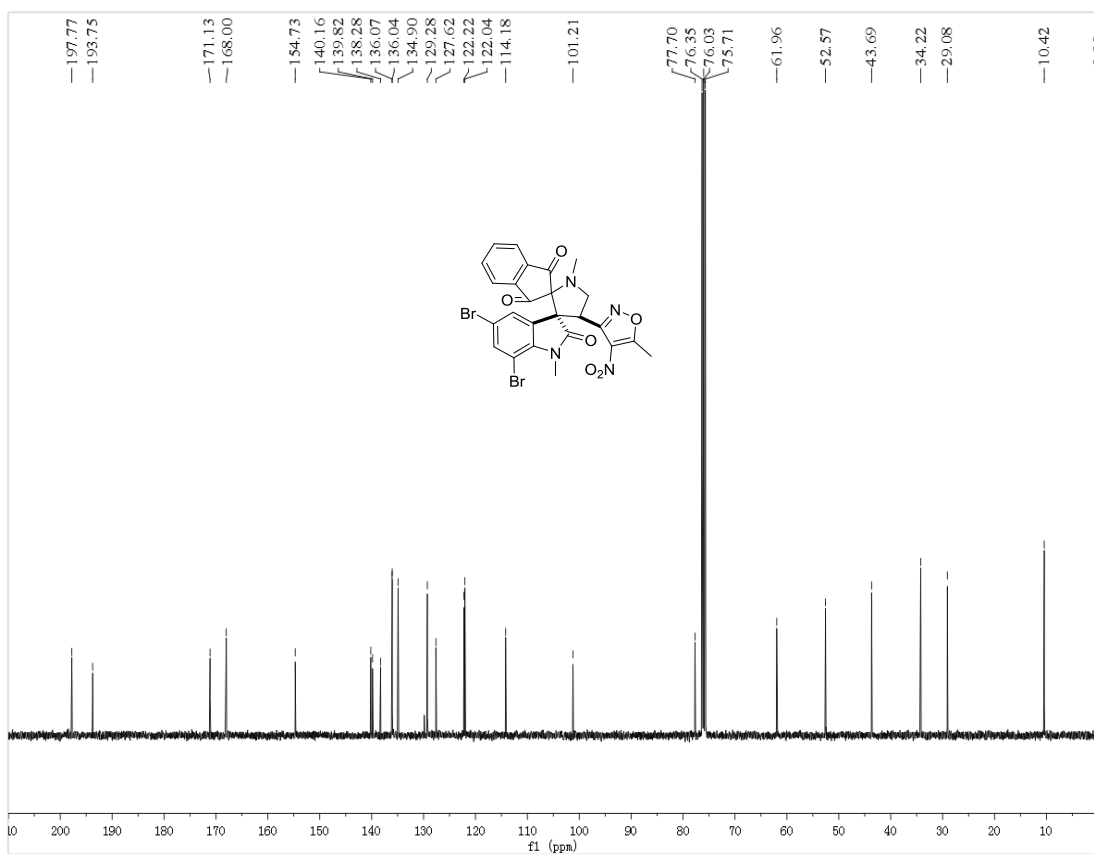
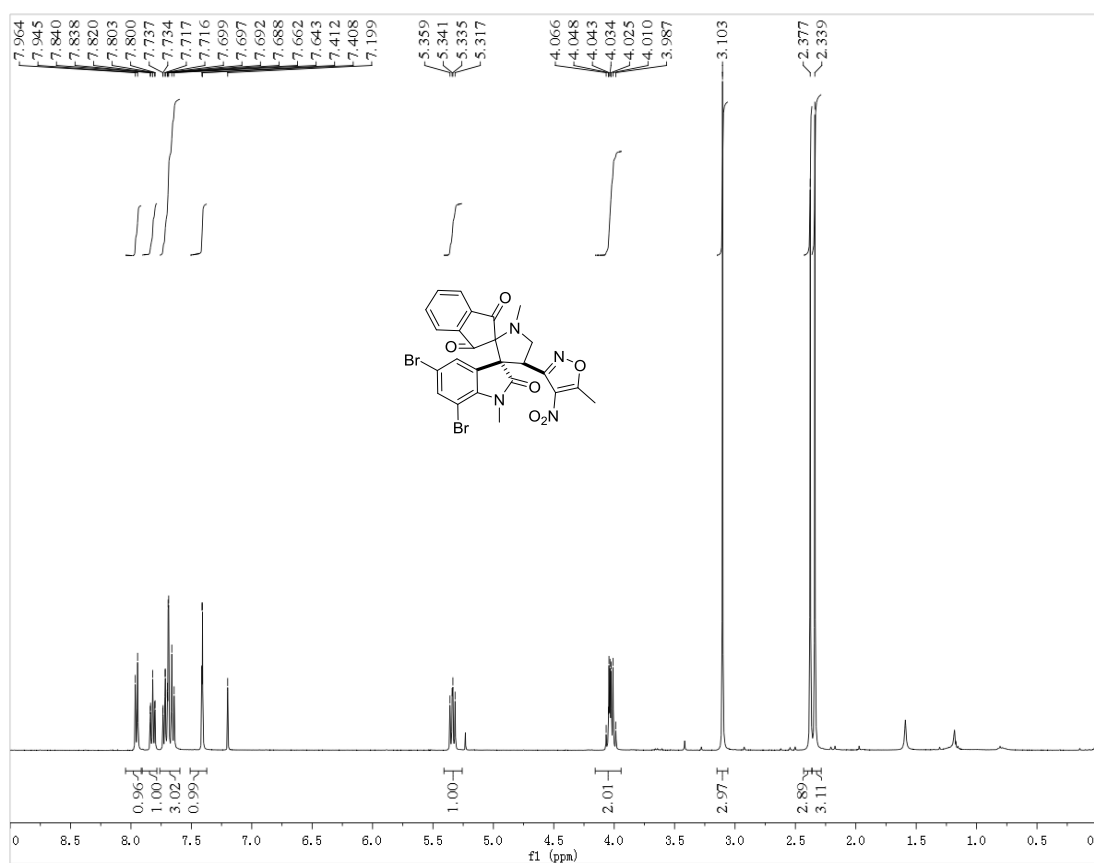
# <sup>1</sup>H and <sup>13</sup>C NMR of 4ah



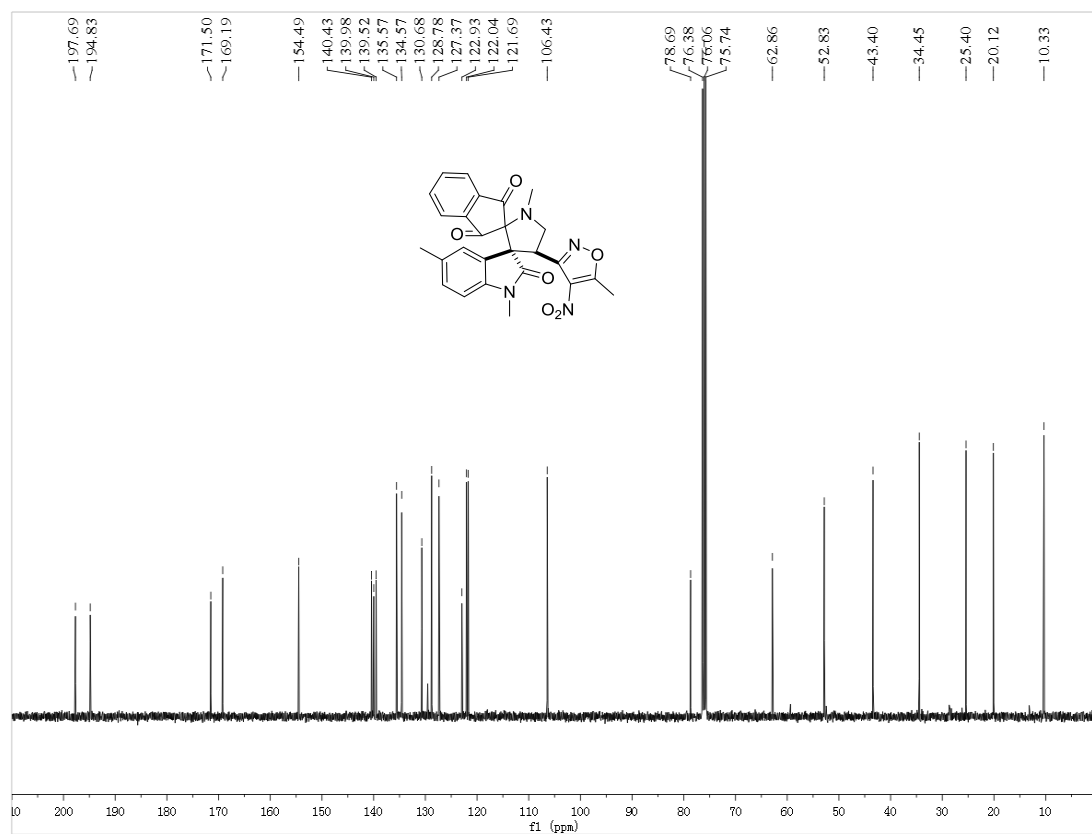
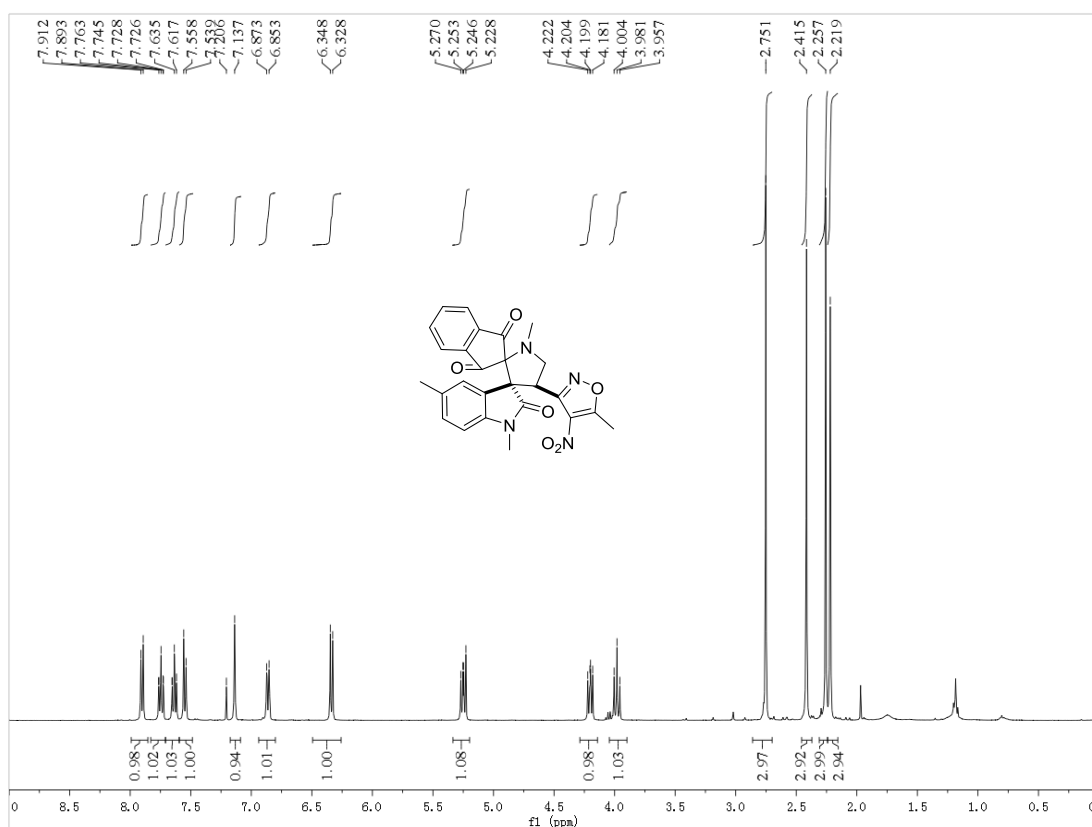
# <sup>1</sup>H and <sup>13</sup>C NMR of 4ai



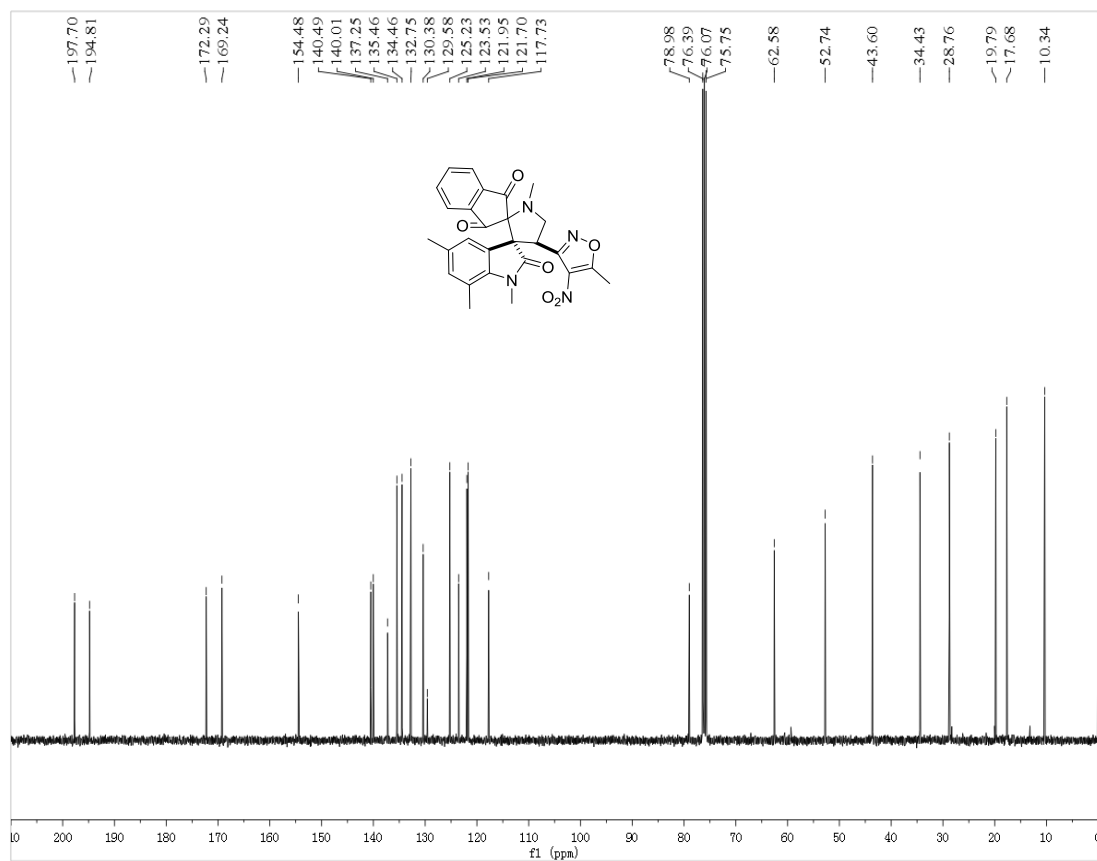
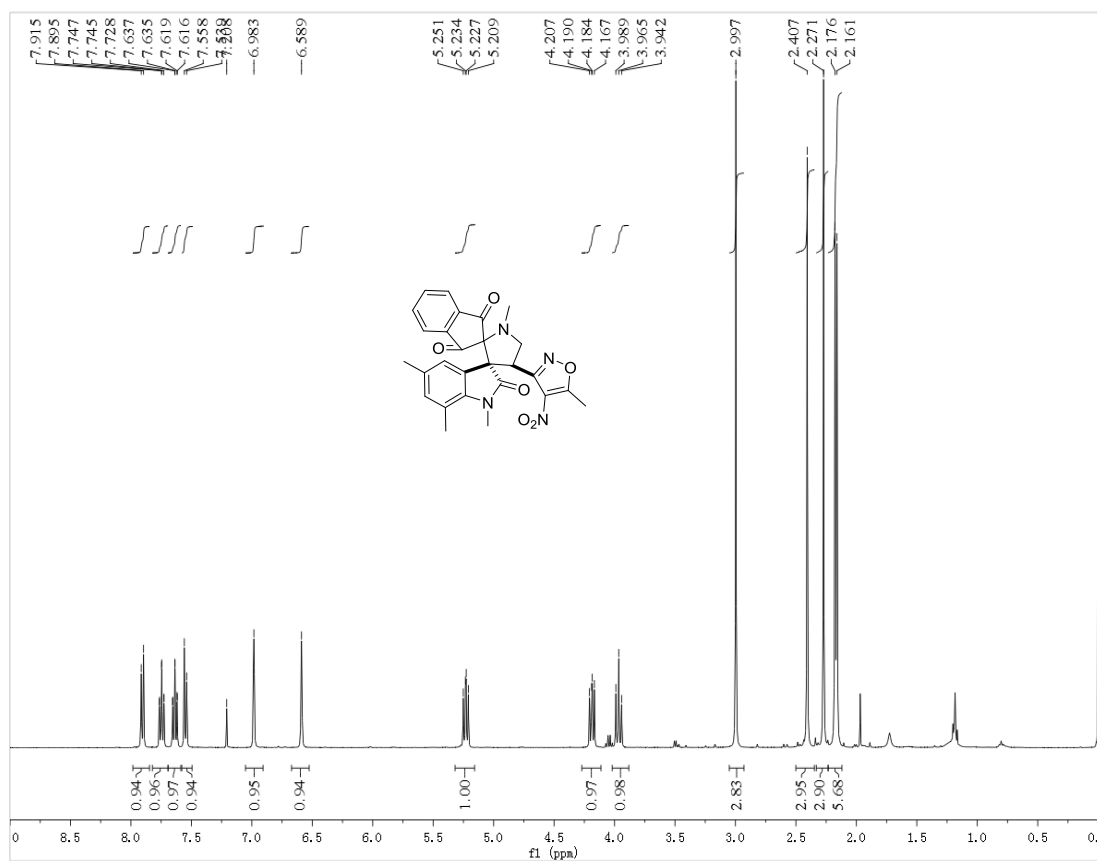
# <sup>1</sup>H and <sup>13</sup>C NMR of 4aj



# $^1\text{H}$ and $^{13}\text{C}$ NMR of 4ak

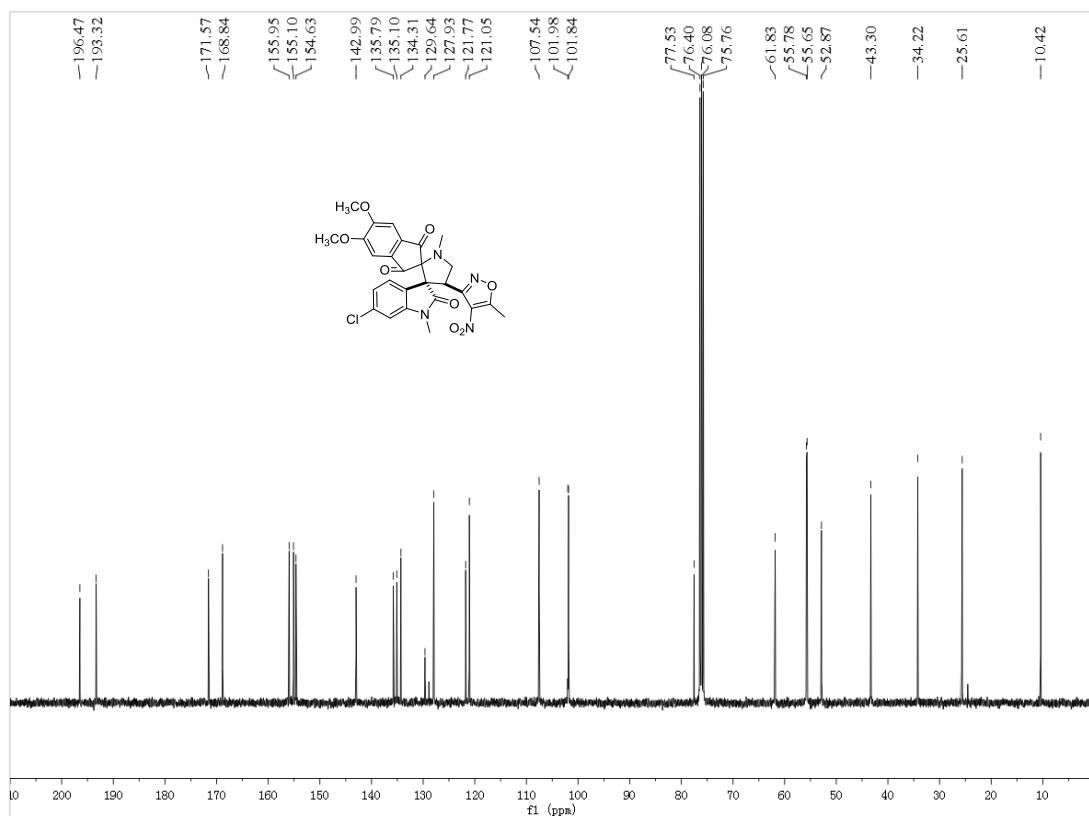
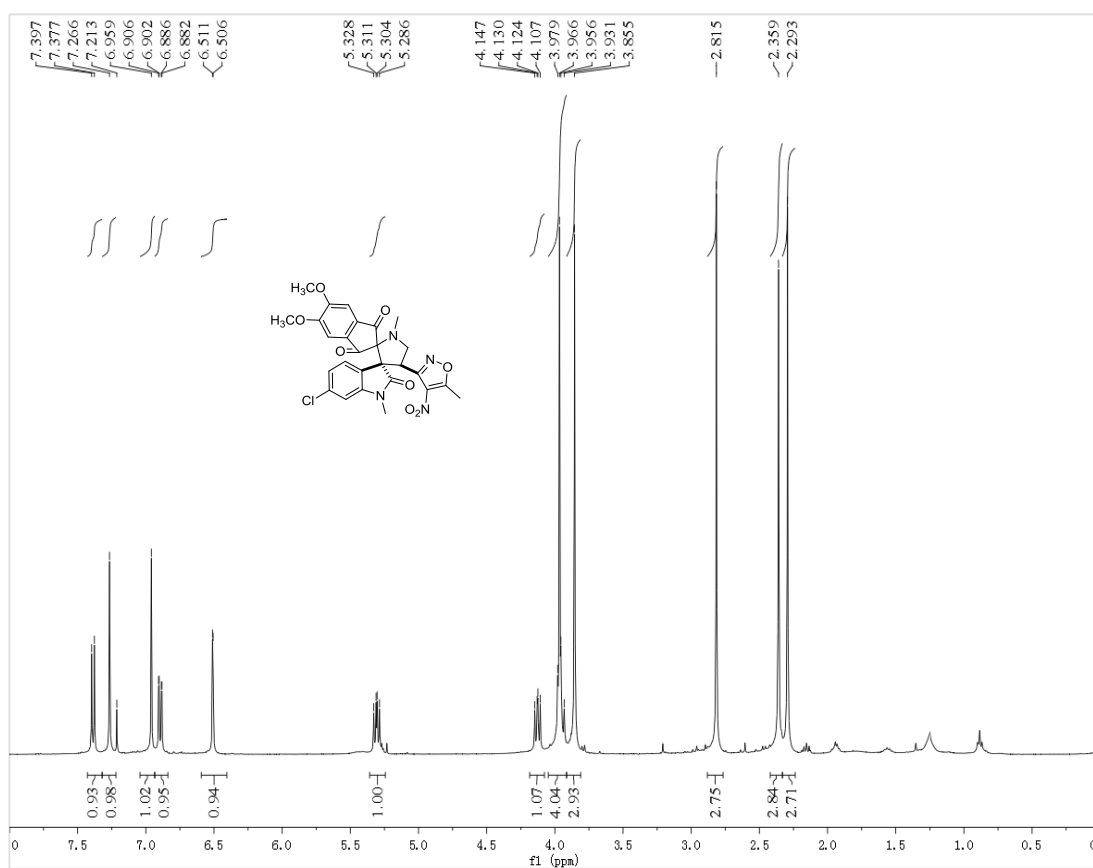


# <sup>1</sup>H and <sup>13</sup>C NMR of 4al

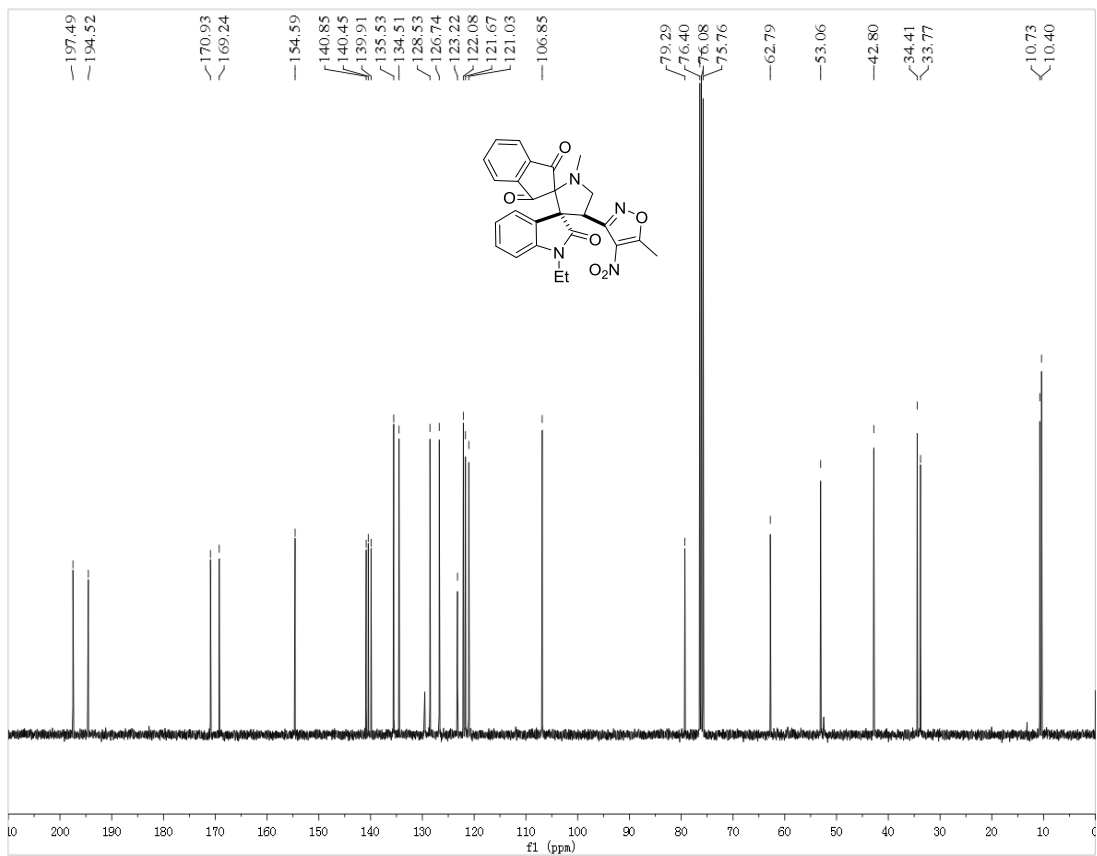
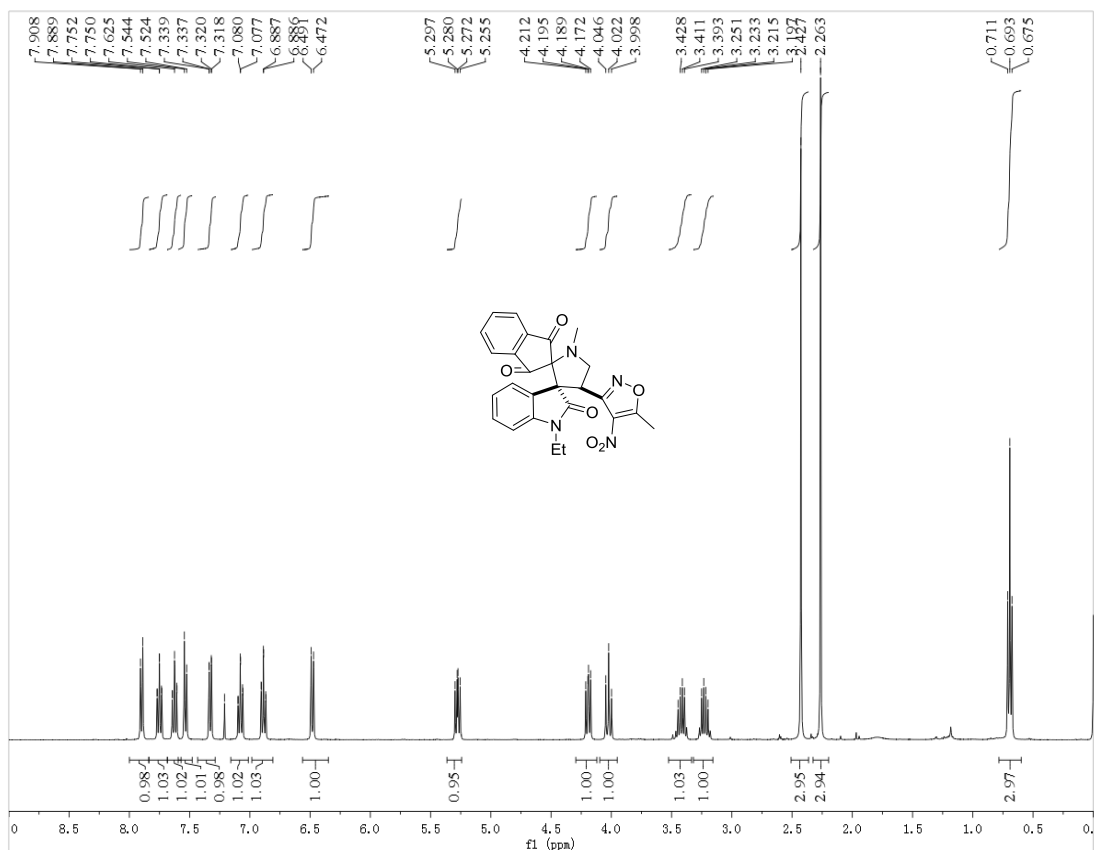




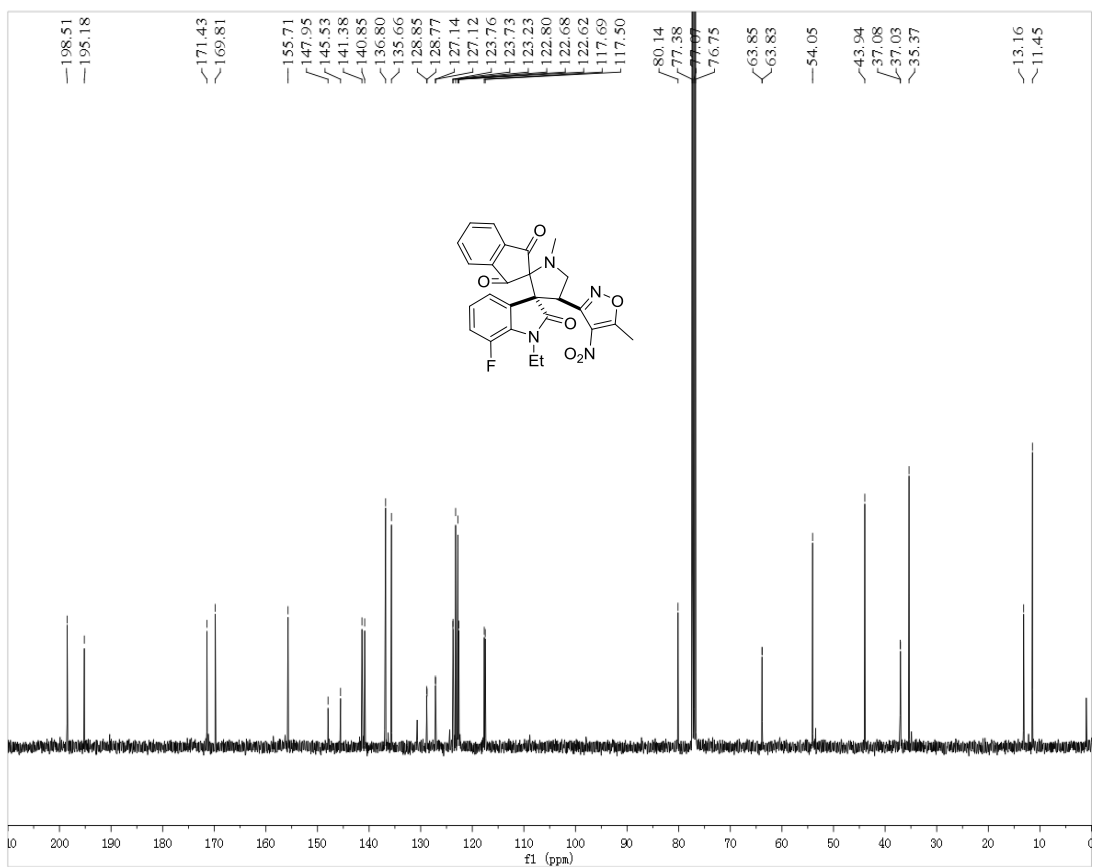
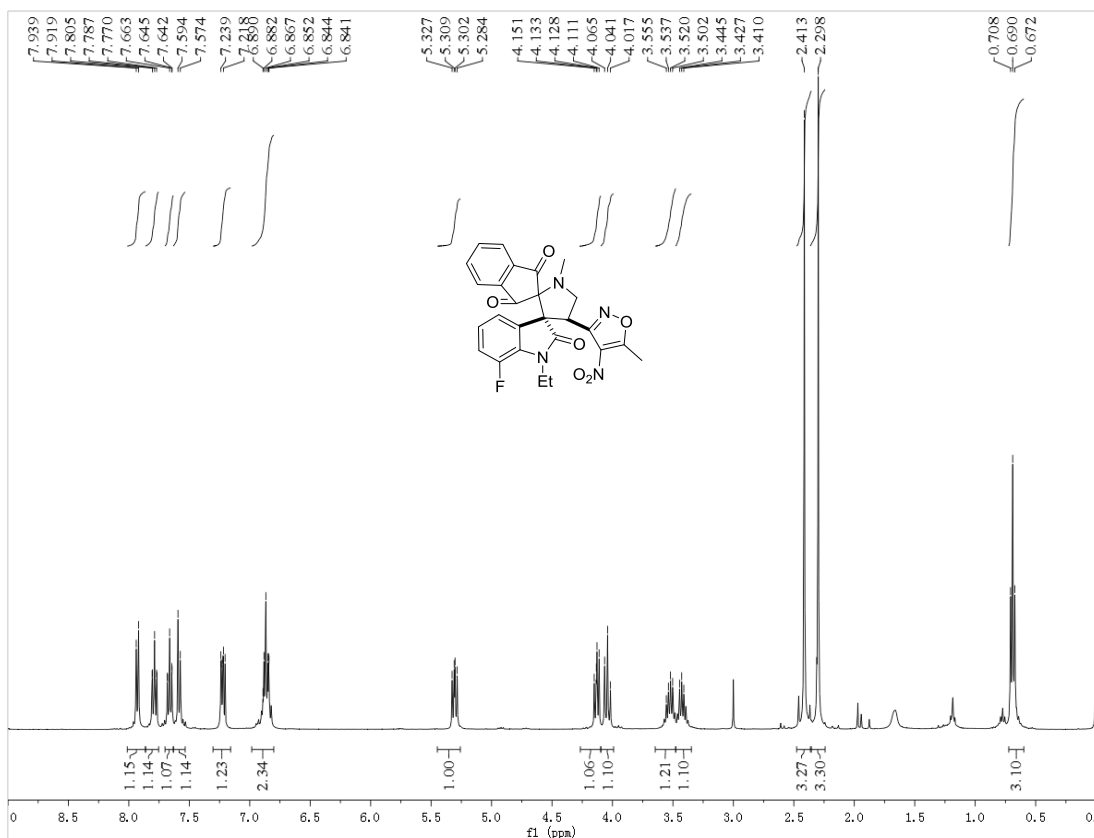
# $^1\text{H}$ and $^{13}\text{C}$ NMR of 4am



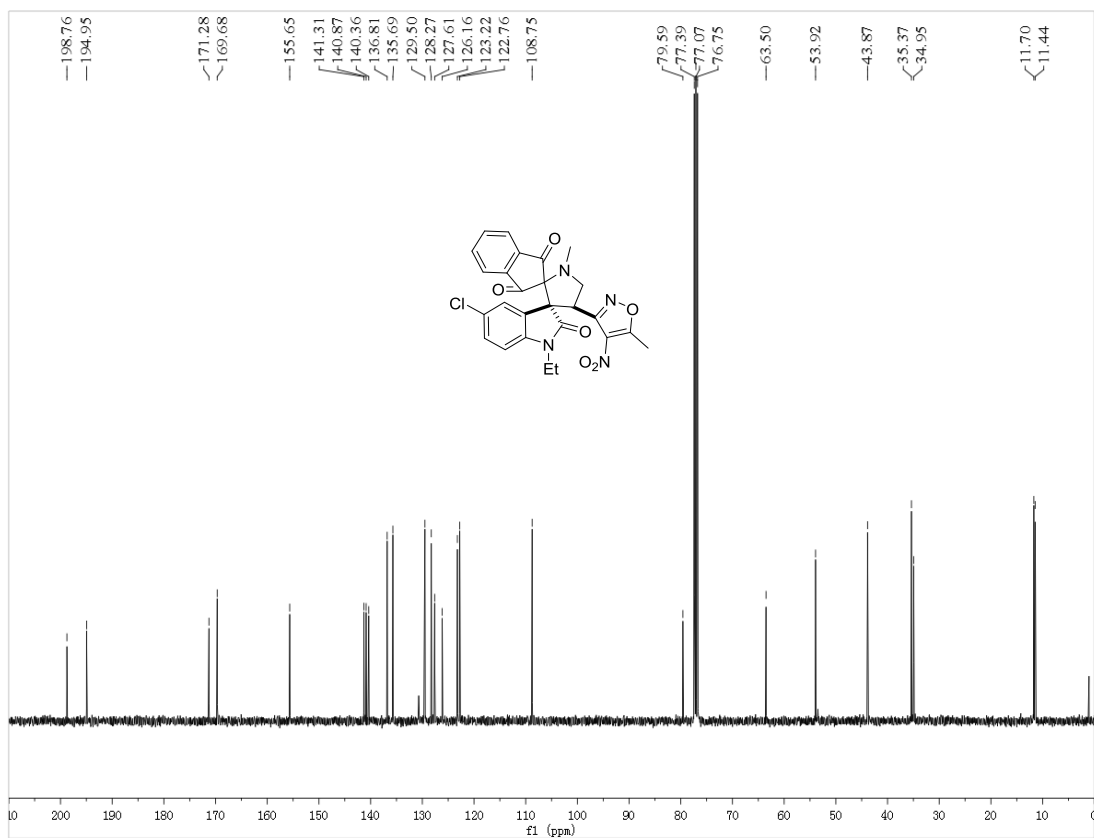
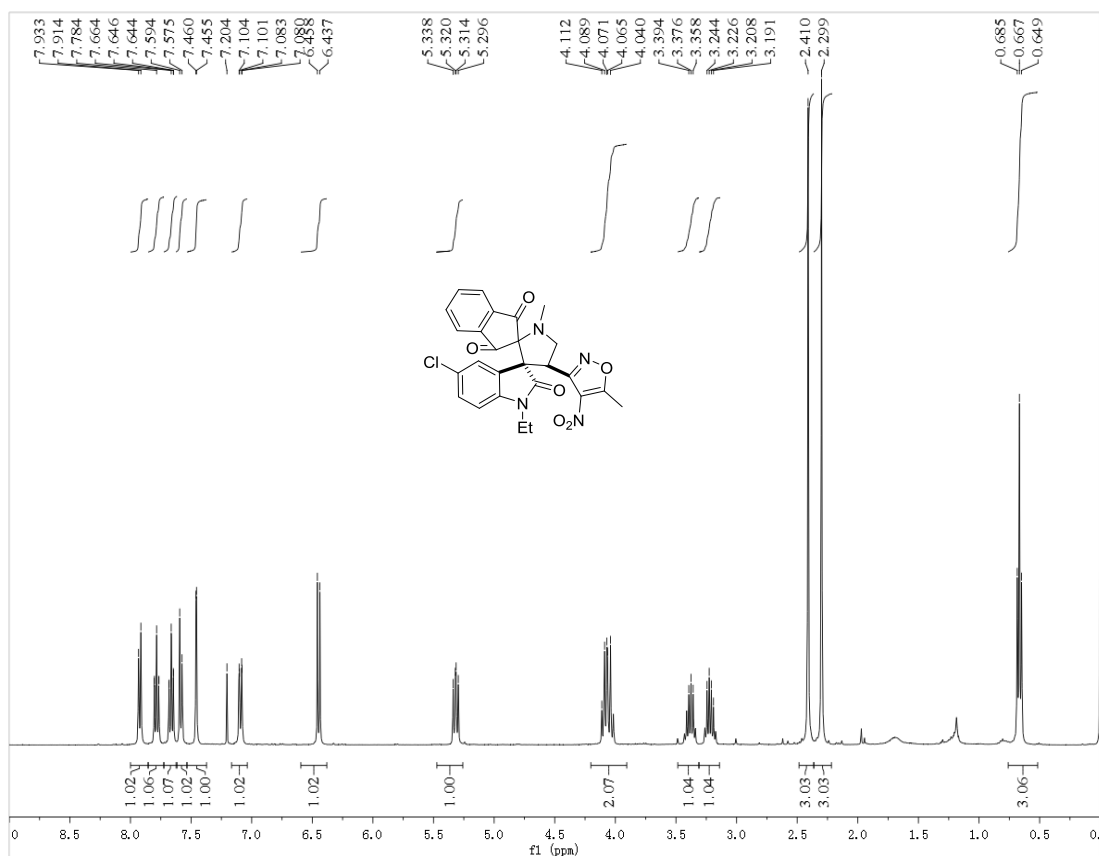
# <sup>1</sup>H and <sup>13</sup>C NMR of 4ba



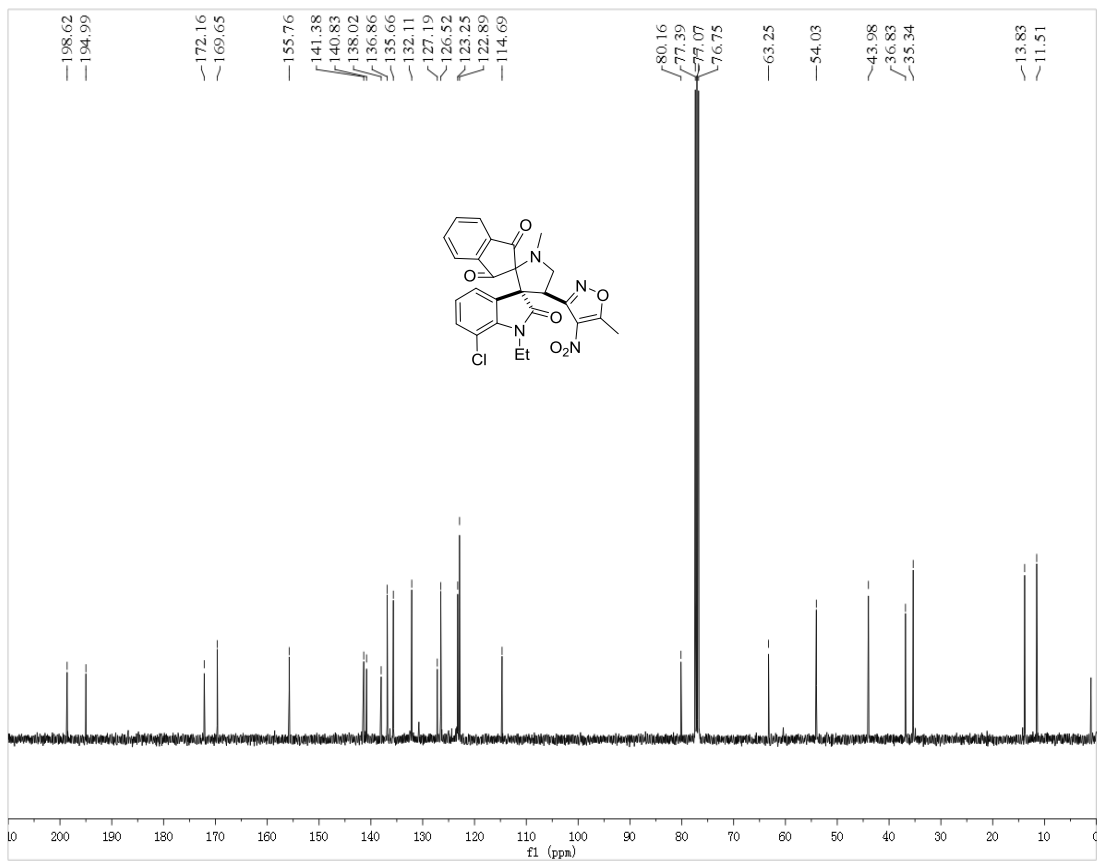
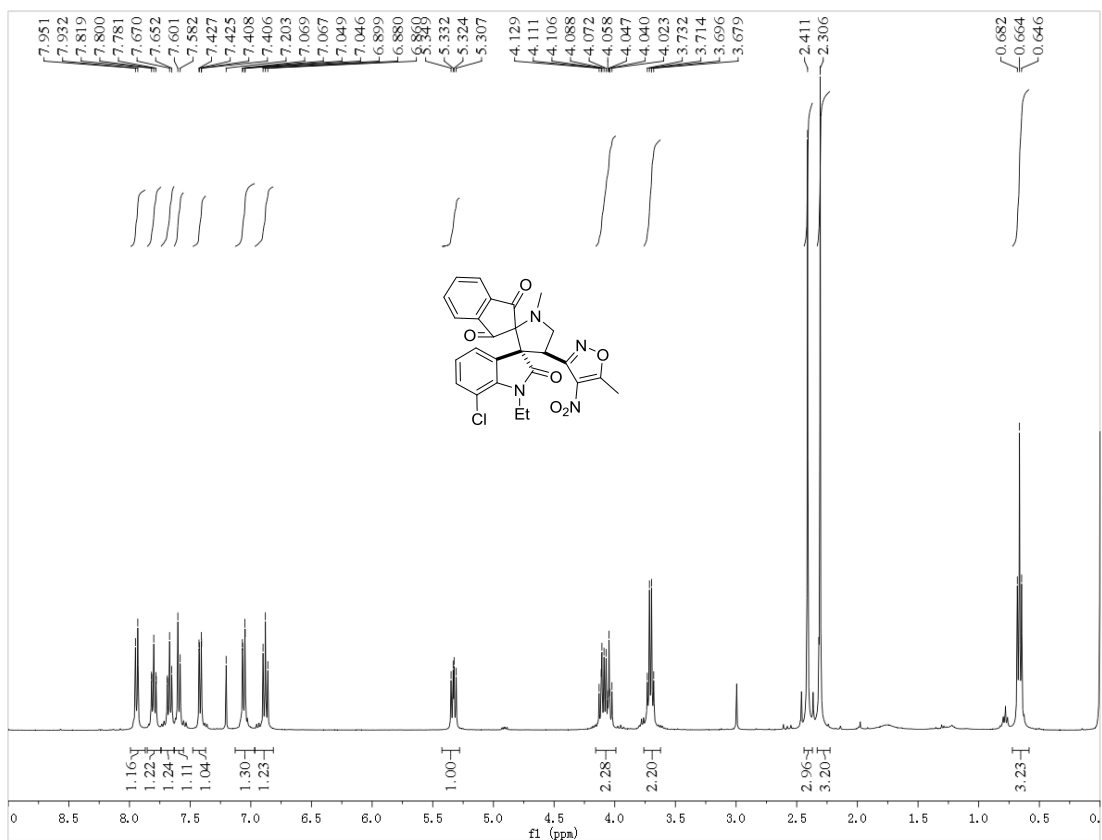
# $^1\text{H}$ and $^{13}\text{C}$ NMR of 4bb



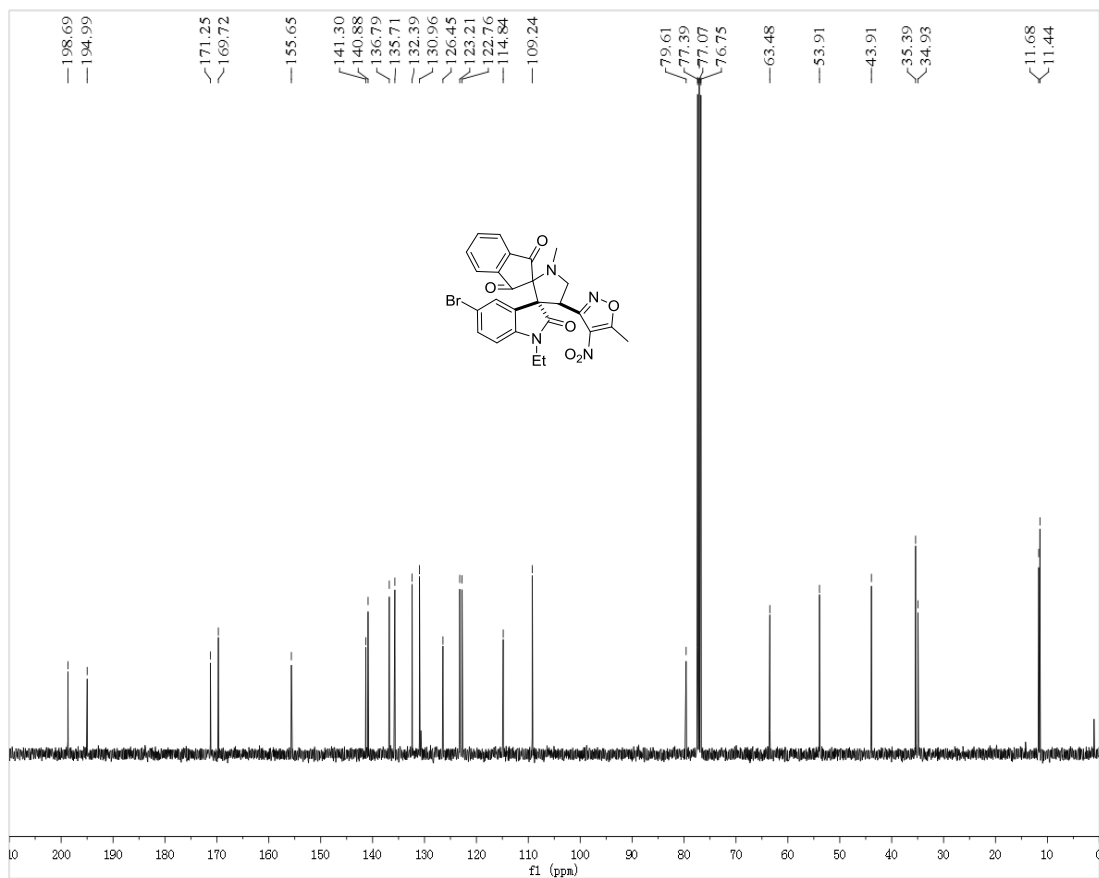
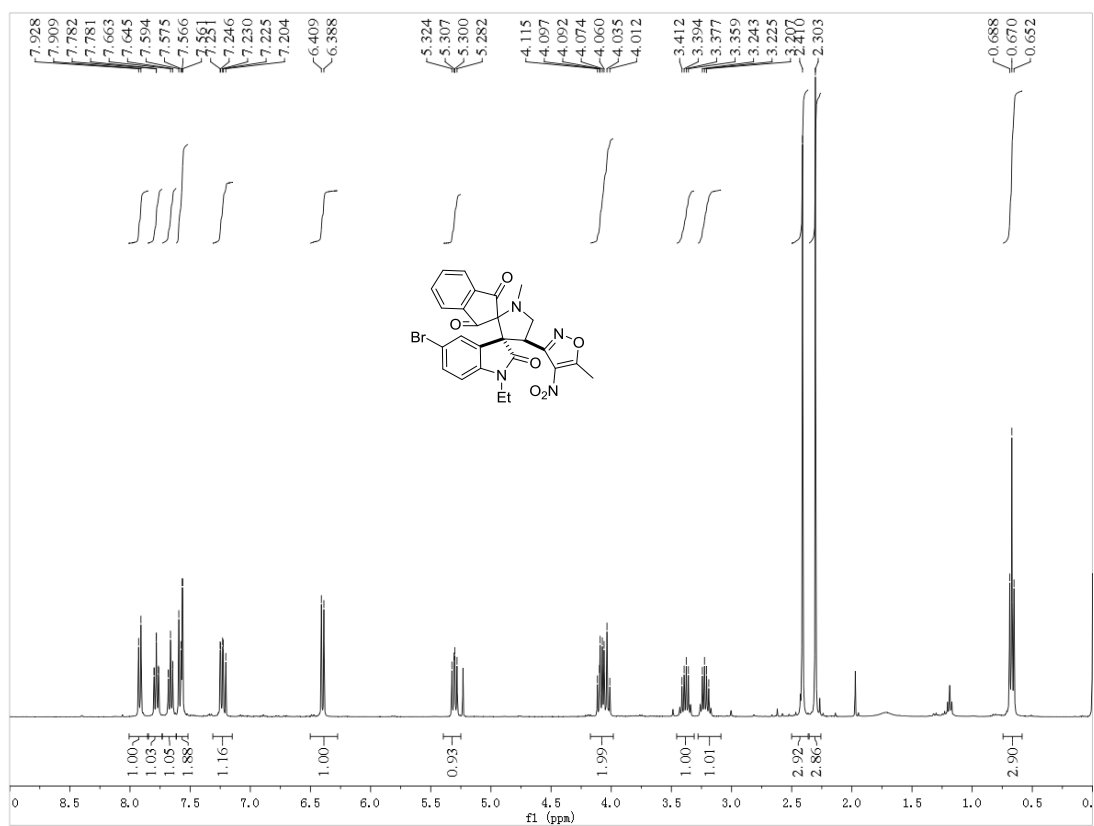
# <sup>1</sup>H and <sup>13</sup>C NMR of 4bc



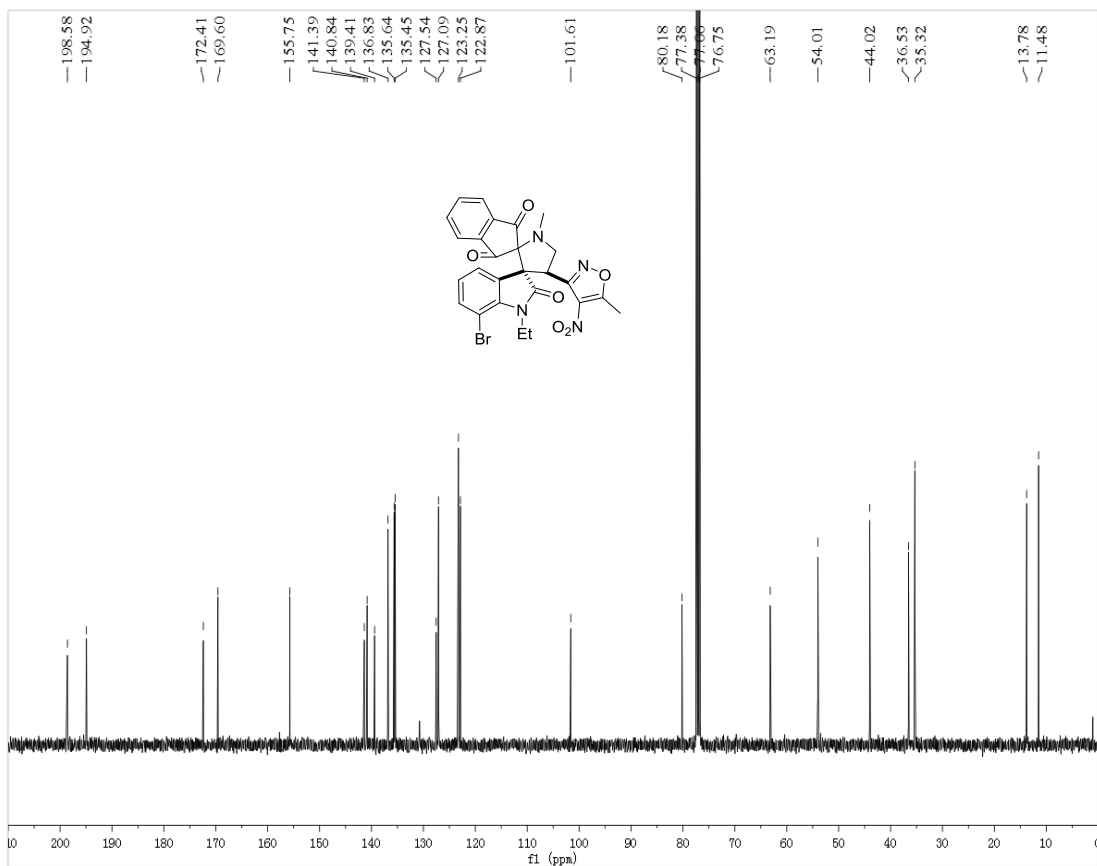
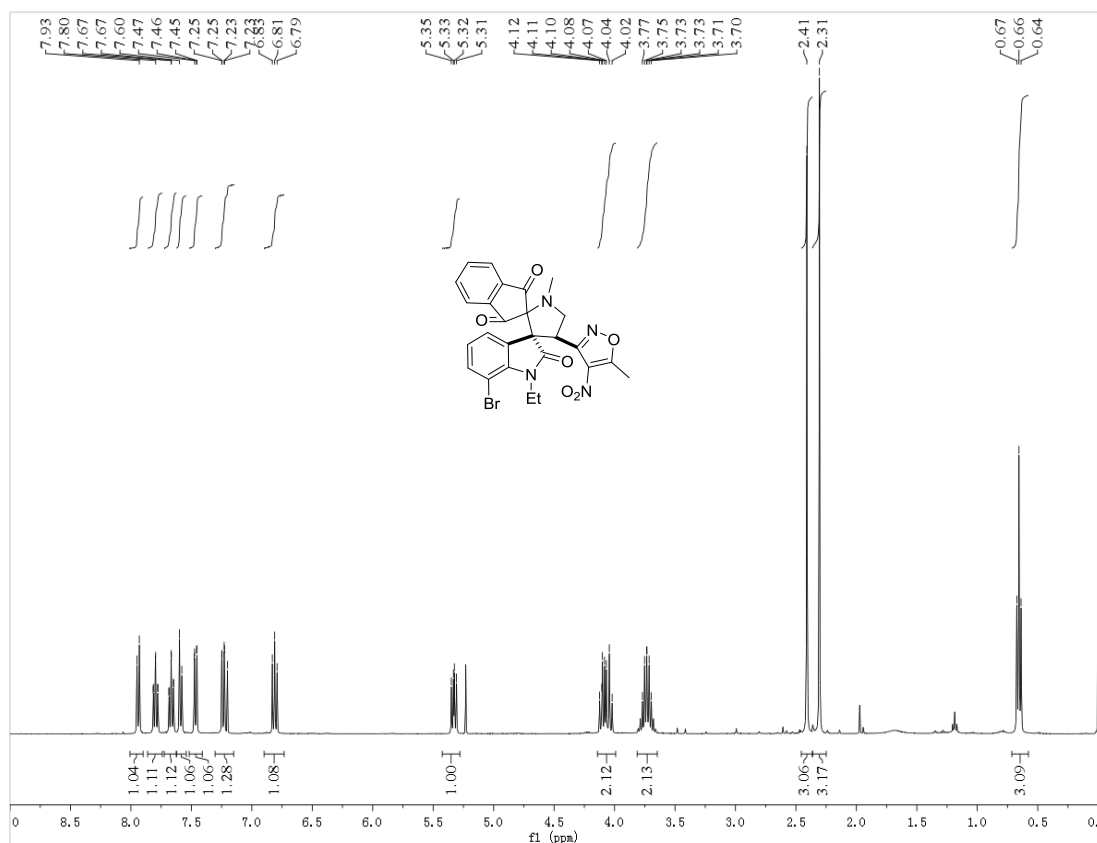
# <sup>1</sup>H and <sup>13</sup>C NMR of 4bd



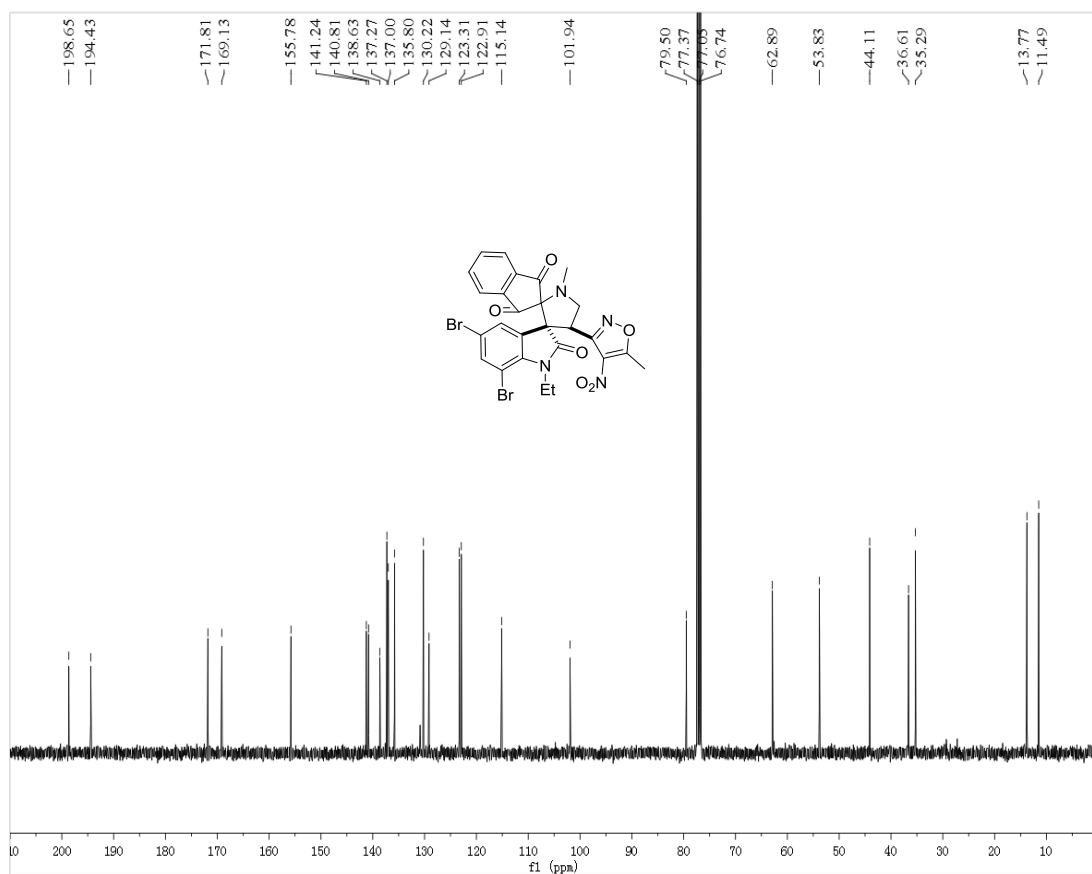
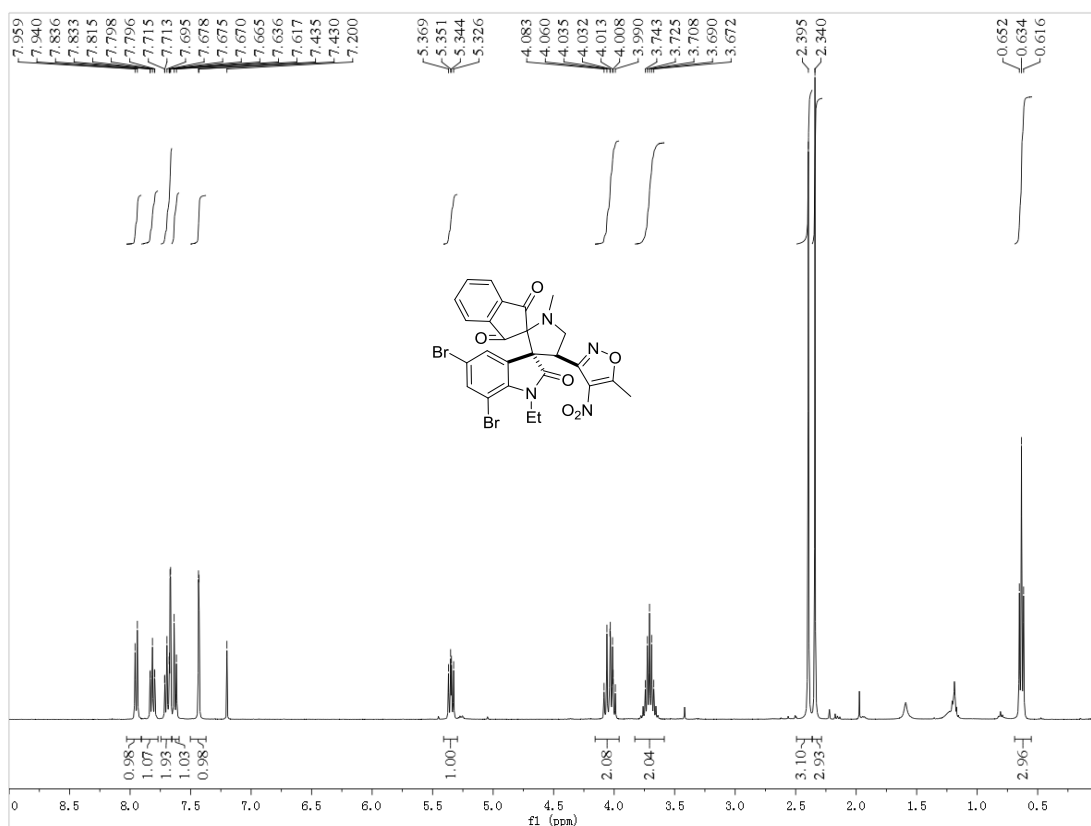
# <sup>1</sup>H and <sup>13</sup>C NMR of 4be



# <sup>1</sup>H and <sup>13</sup>C NMR of 4bf

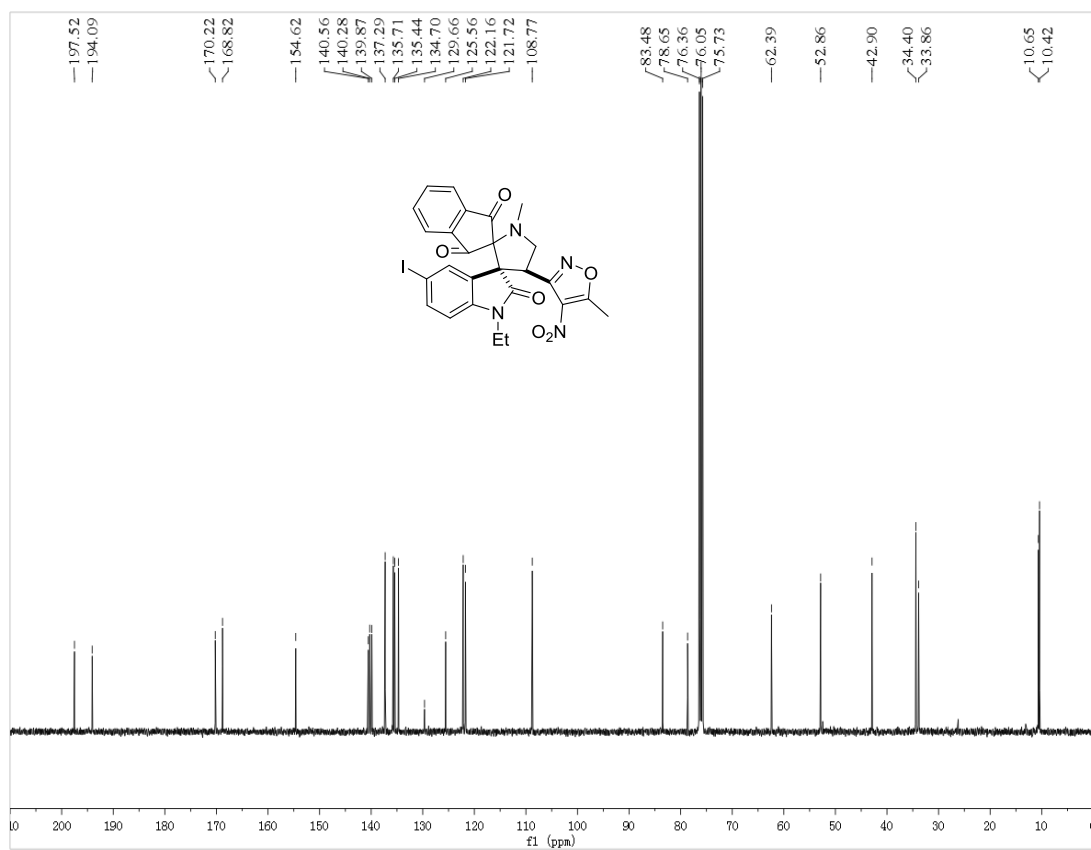
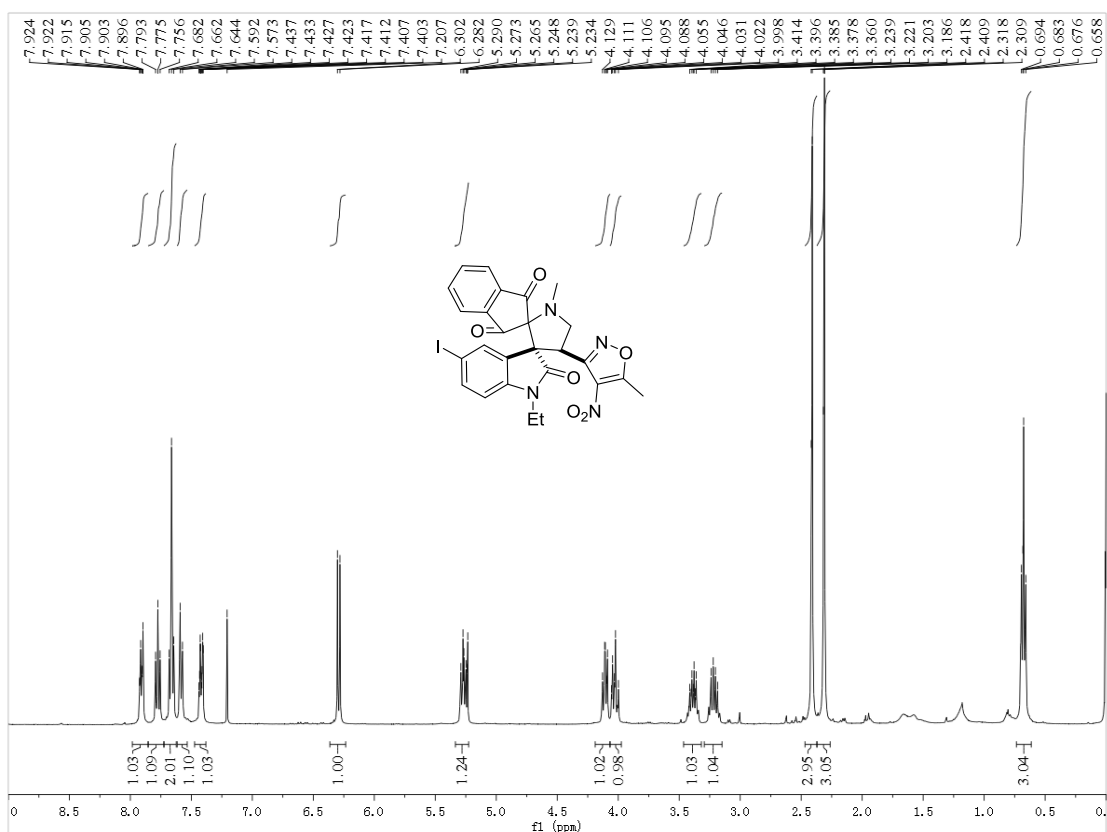


# <sup>1</sup>H and <sup>13</sup>C NMR of 4bg

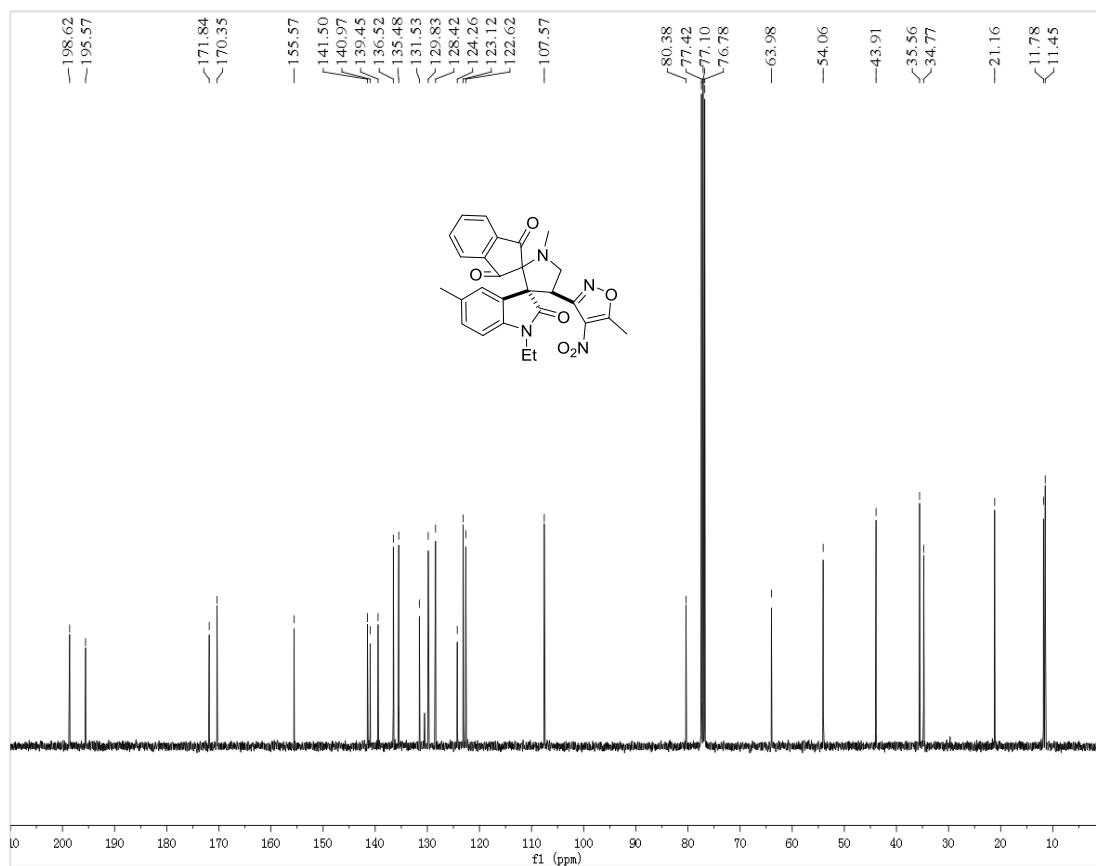
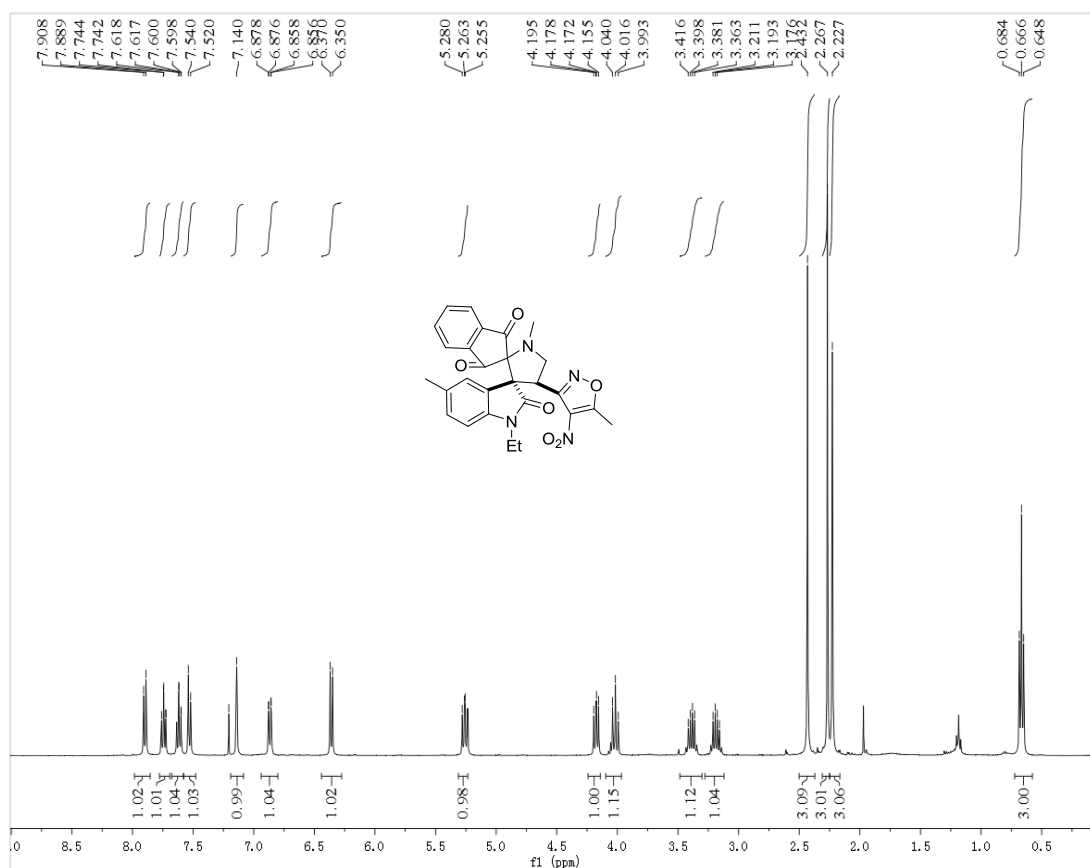




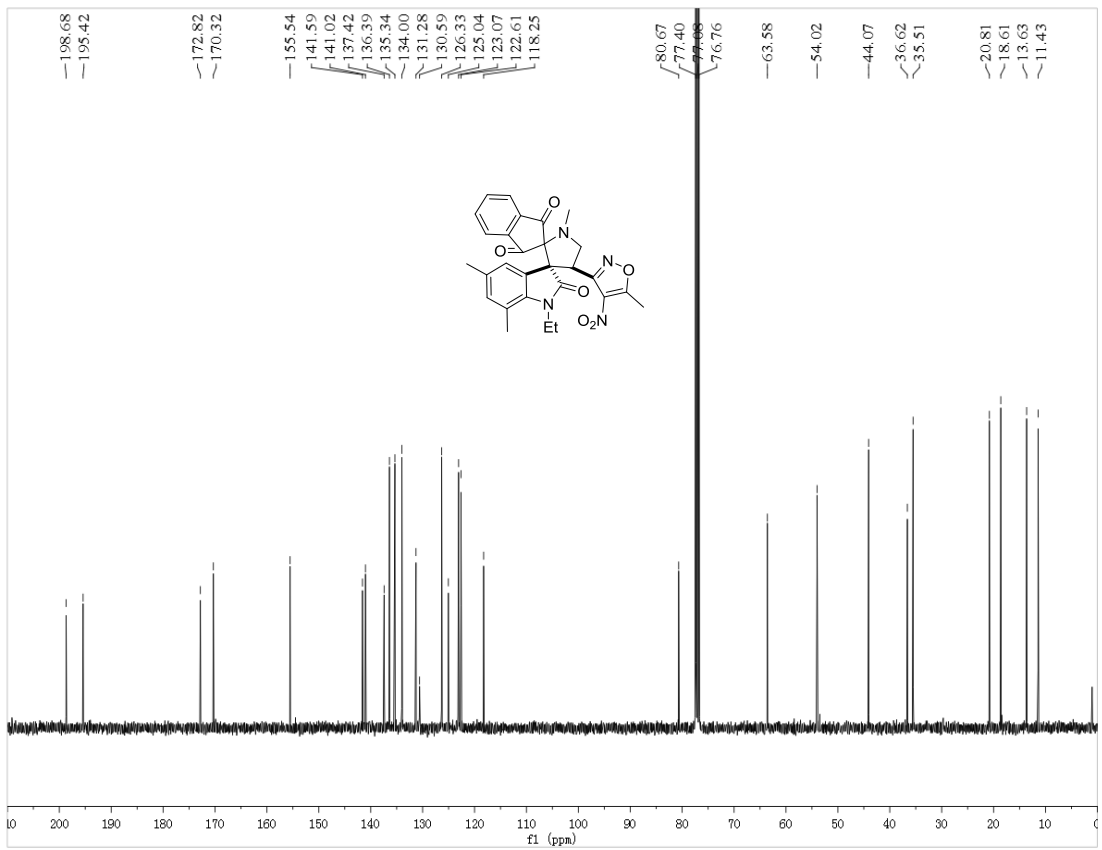
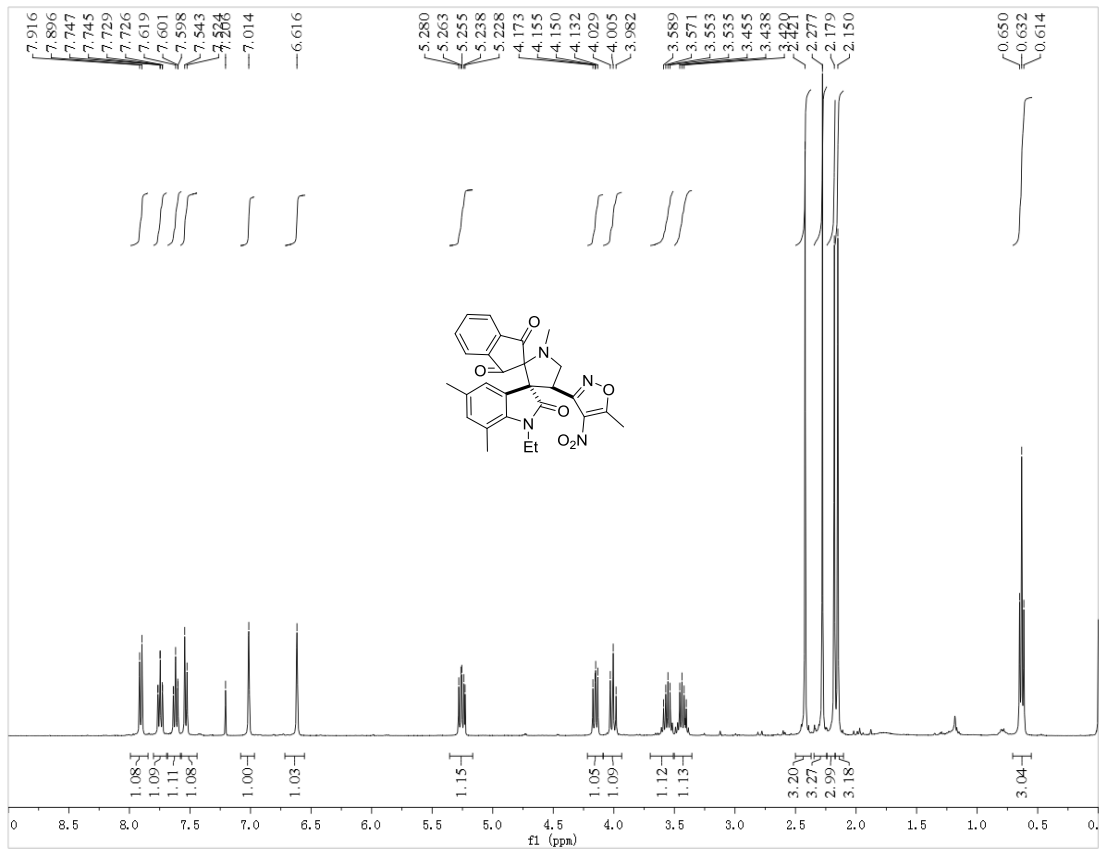
<sup>1</sup>H and <sup>13</sup>C NMR of 4bh



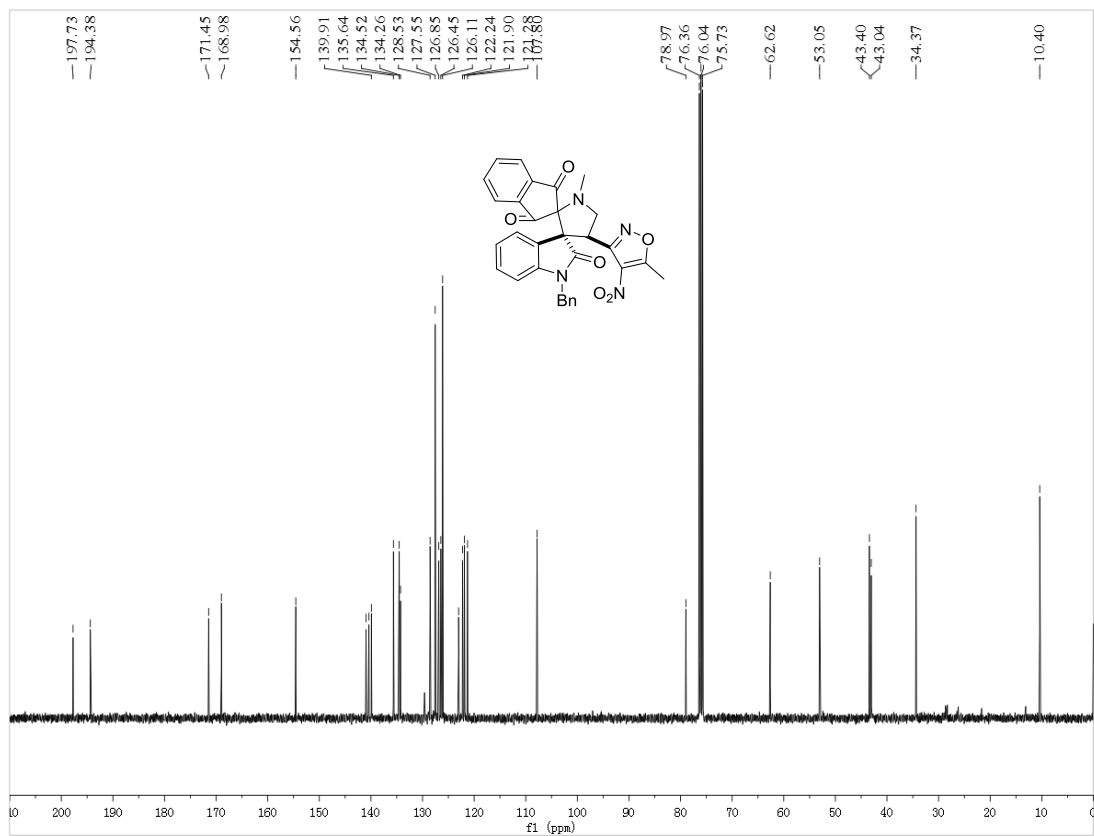
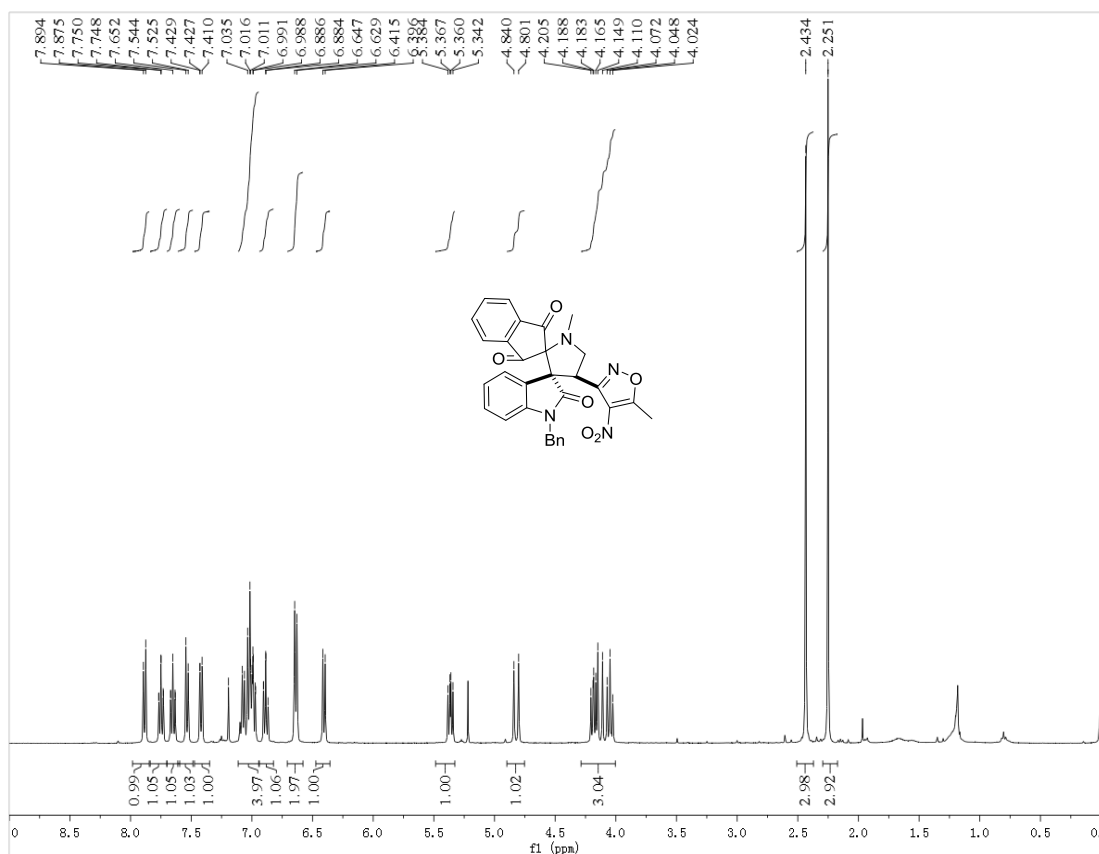
# <sup>1</sup>H and <sup>13</sup>C NMR of 4bi



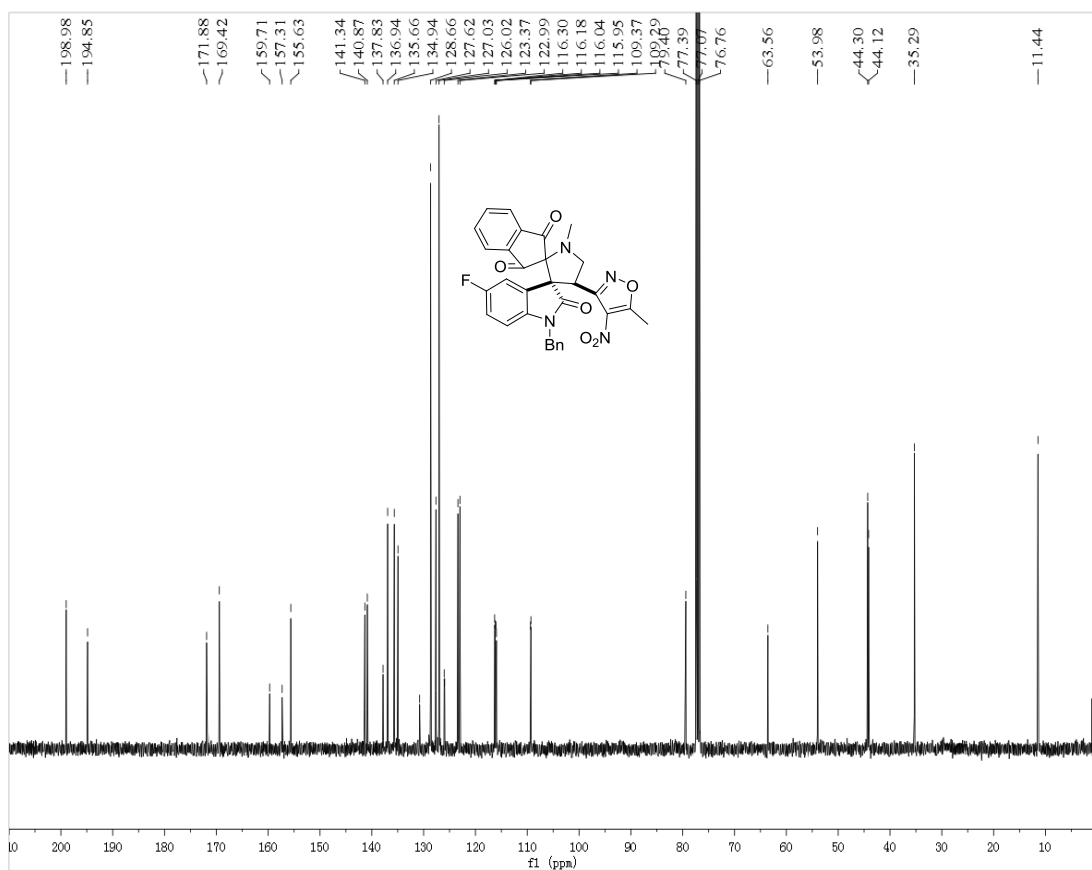
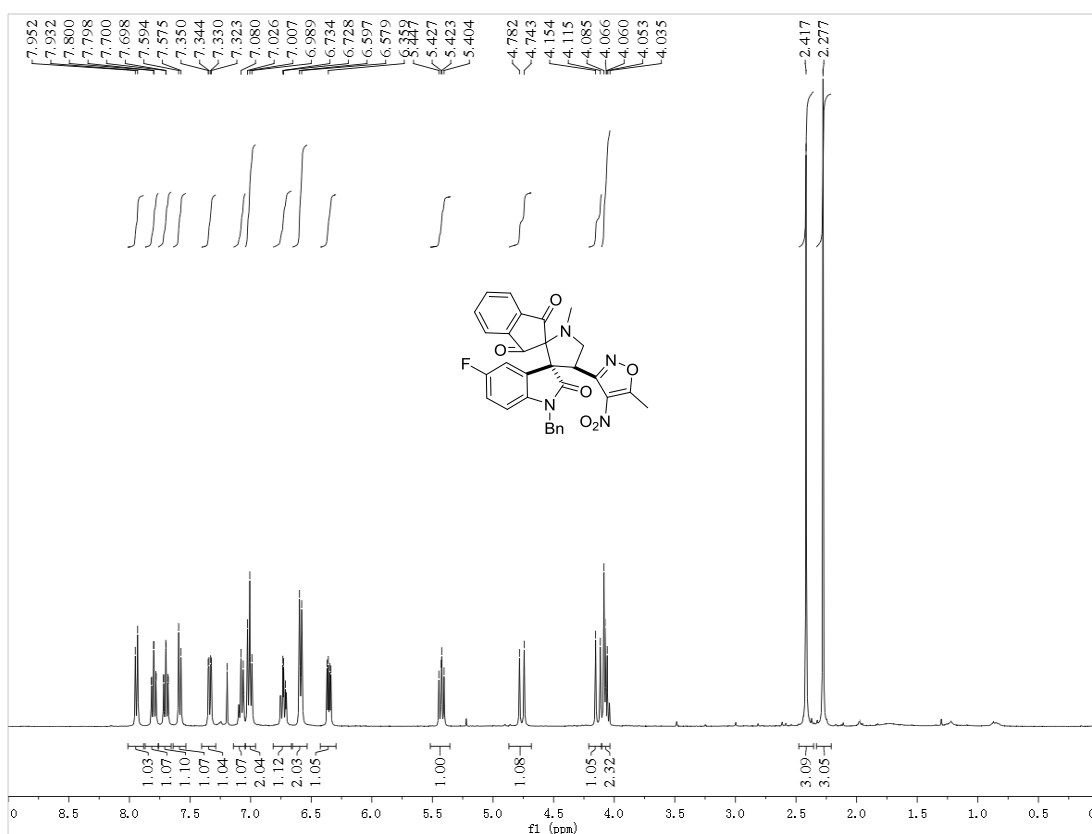
**<sup>1</sup>H and <sup>13</sup>C NMR of 4bj**



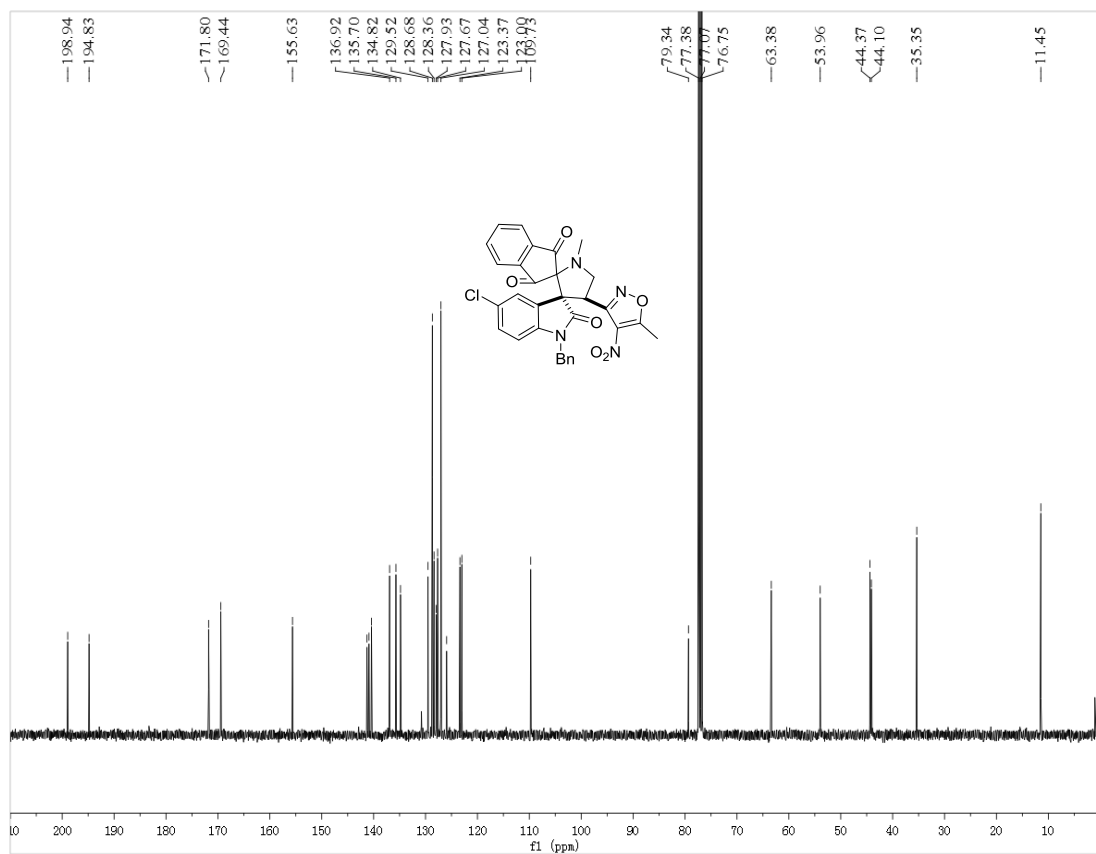
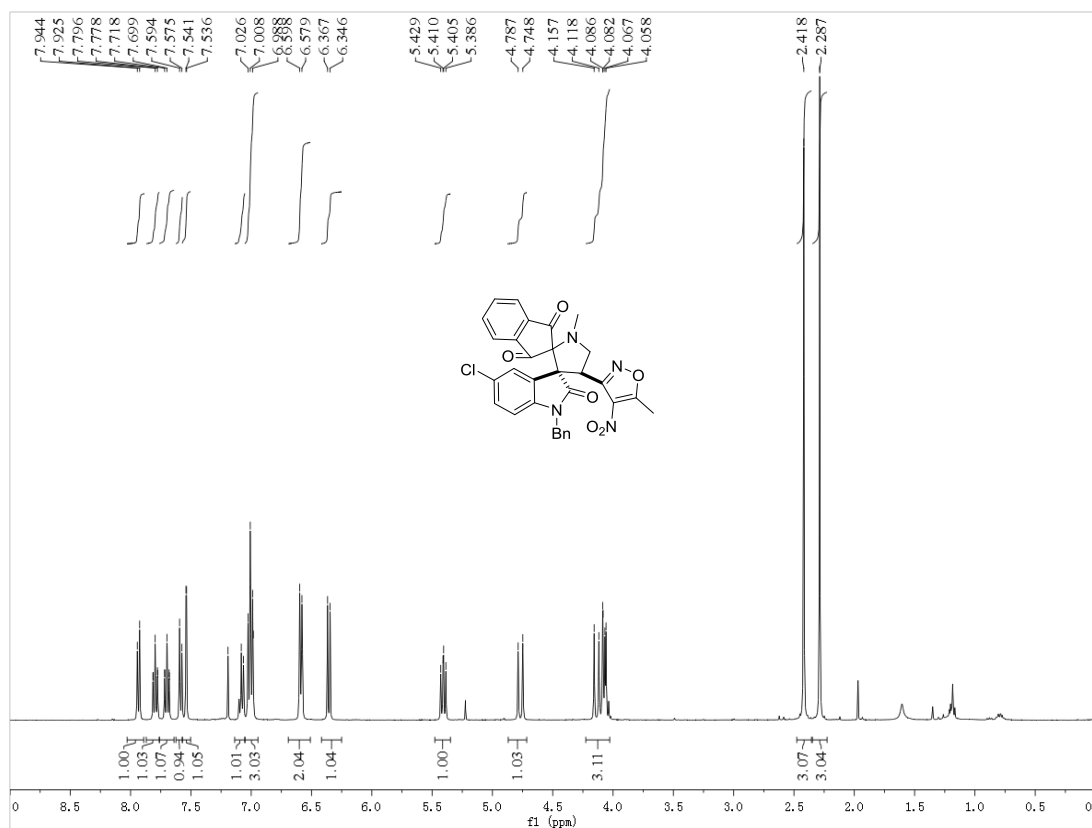
# <sup>1</sup>H and <sup>13</sup>C NMR of 4ca



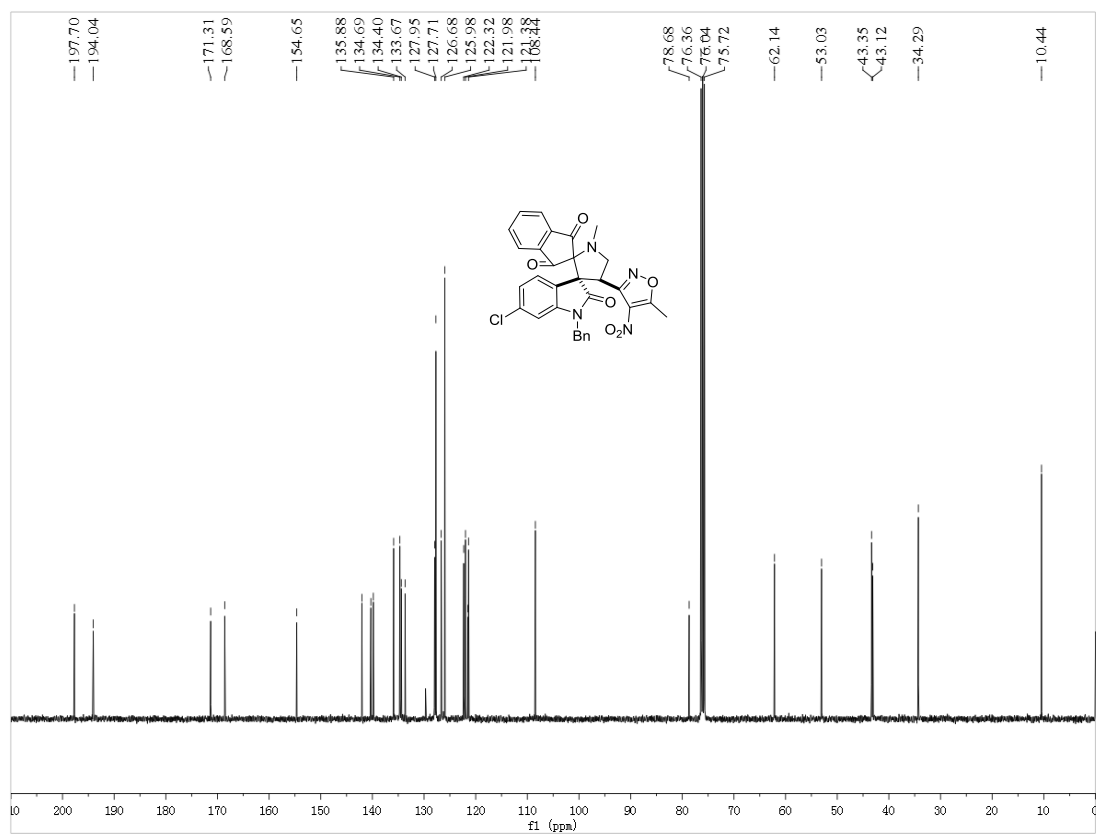
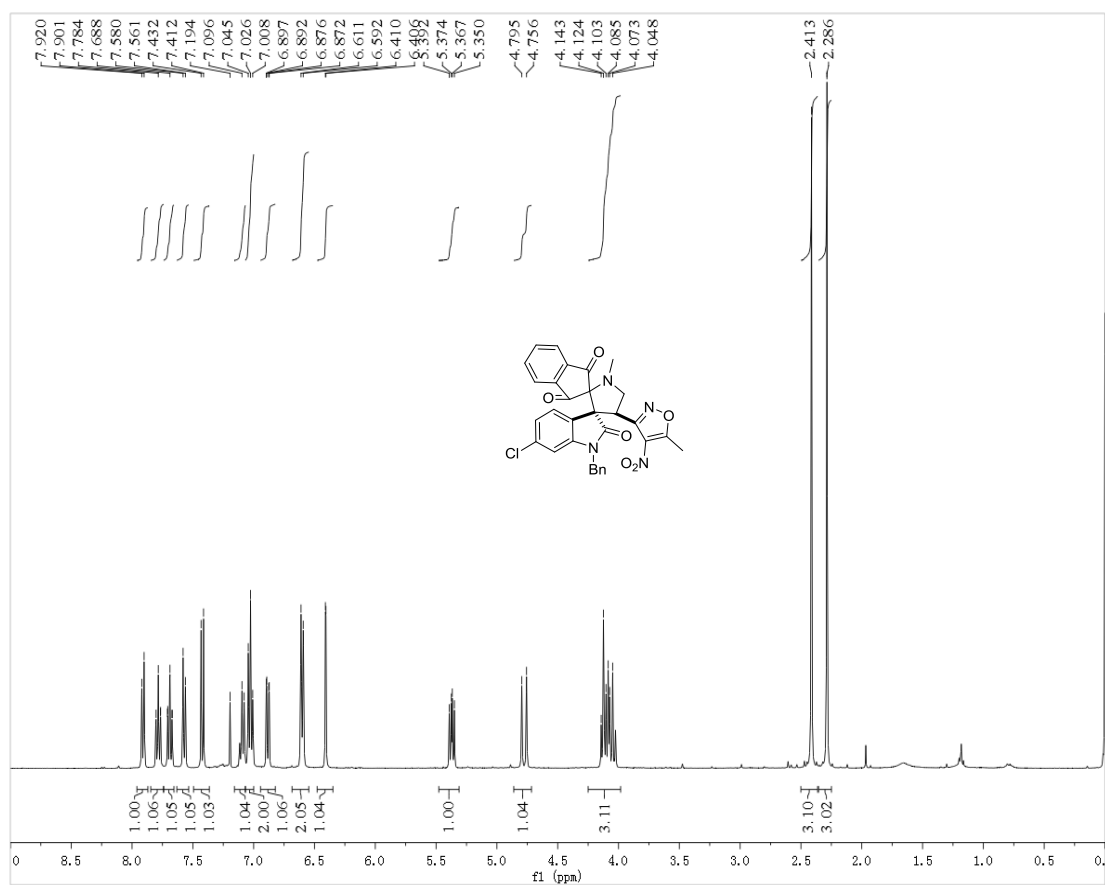
# $^1\text{H}$ and $^{13}\text{C}$ NMR of 4cb



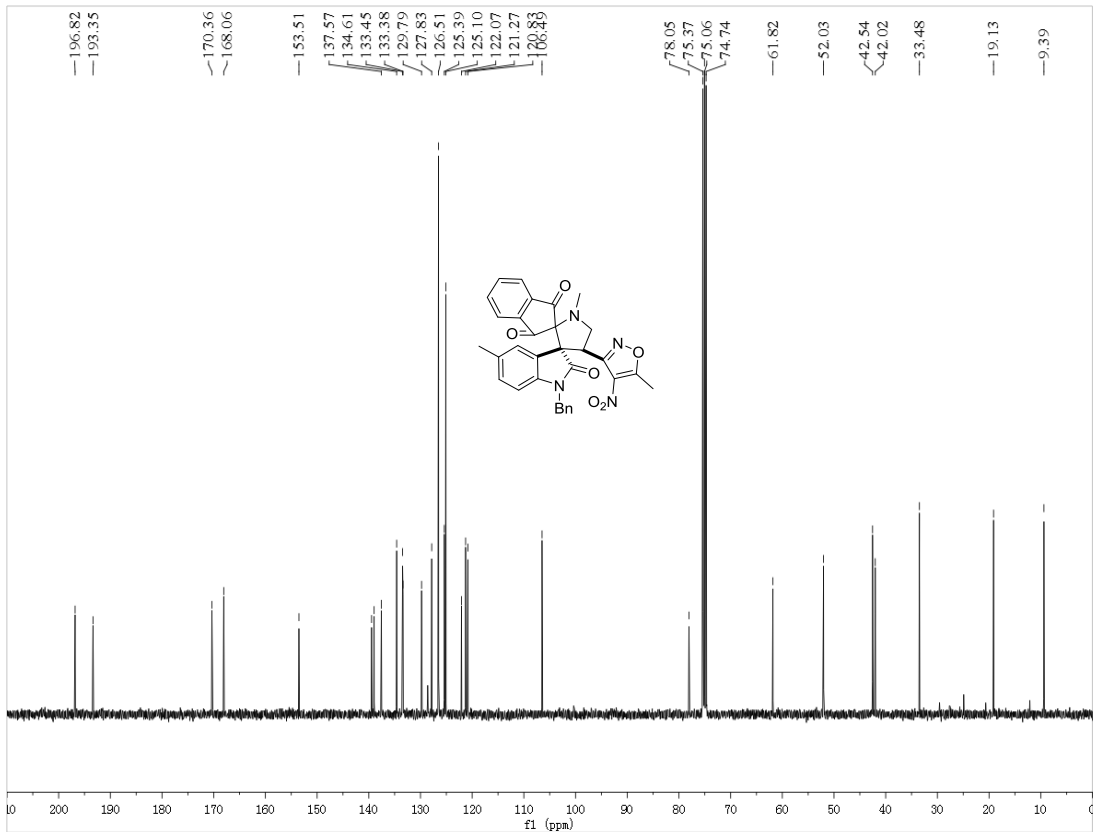
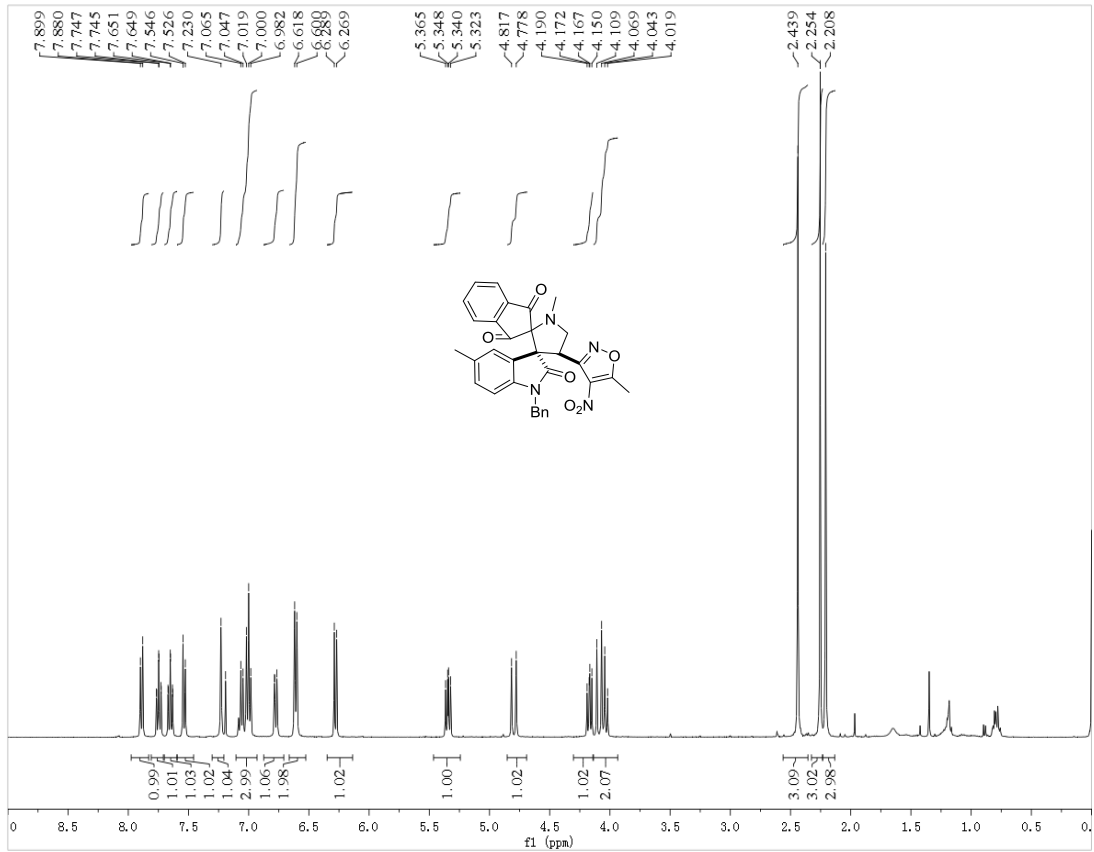
# <sup>1</sup>H and <sup>13</sup>C NMR of 4cc



# $^1\text{H}$ and $^{13}\text{C}$ NMR of 4cd

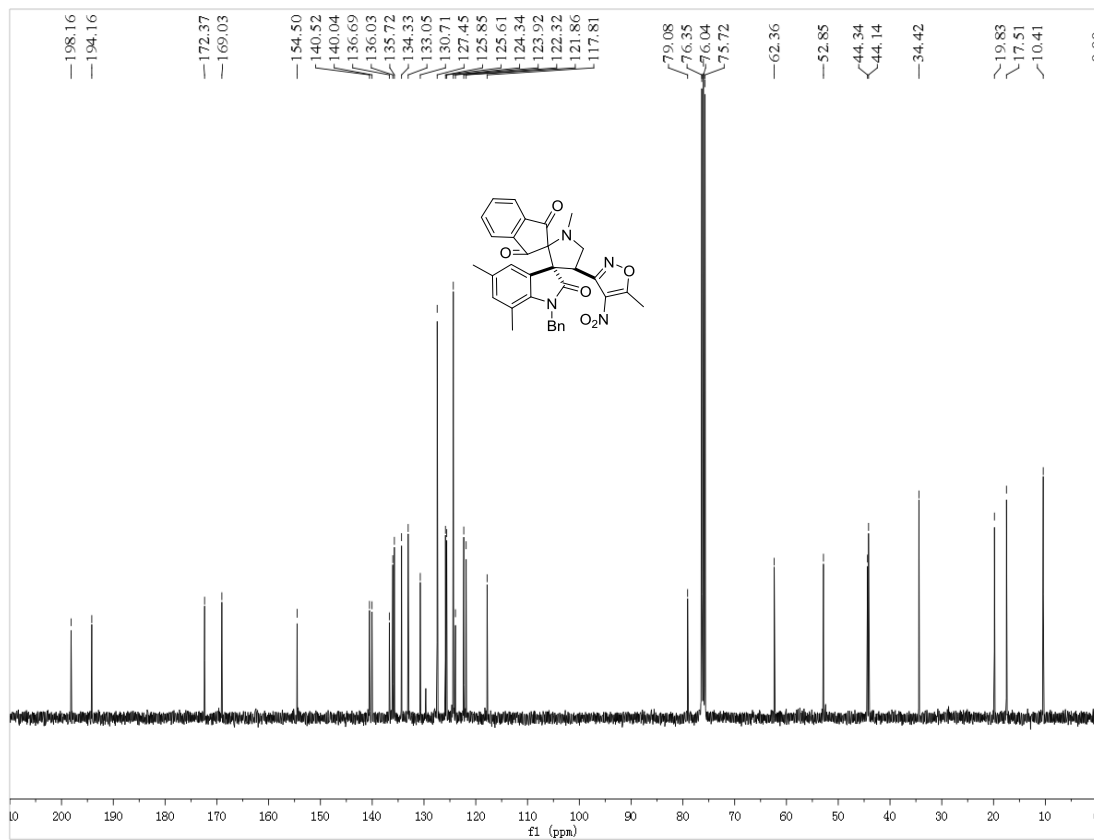
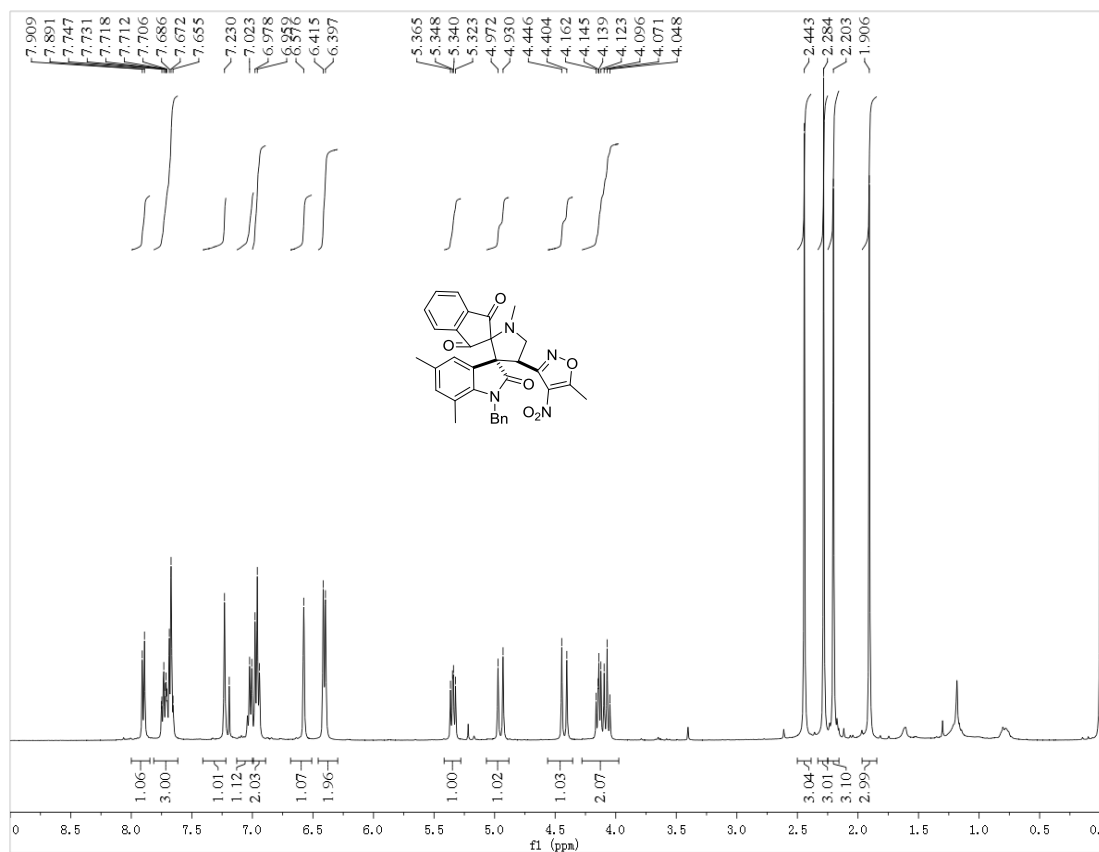


# <sup>1</sup>H and <sup>13</sup>C NMR of 4ce

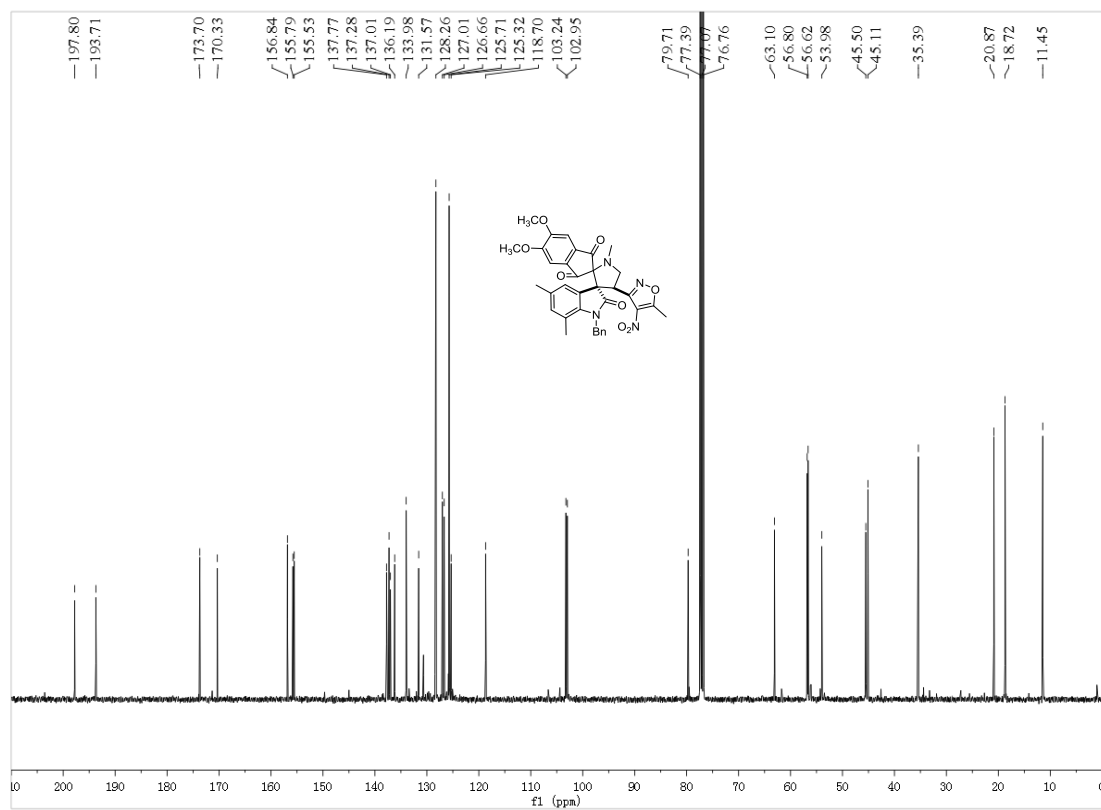
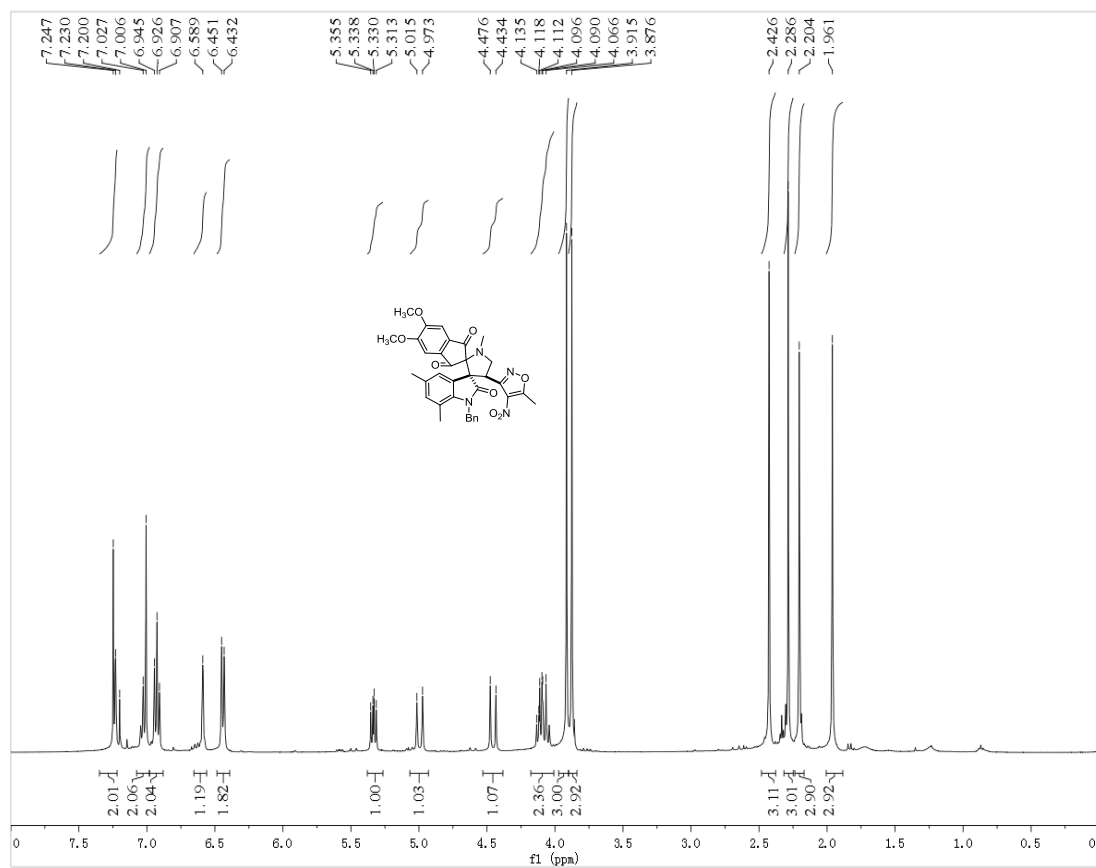




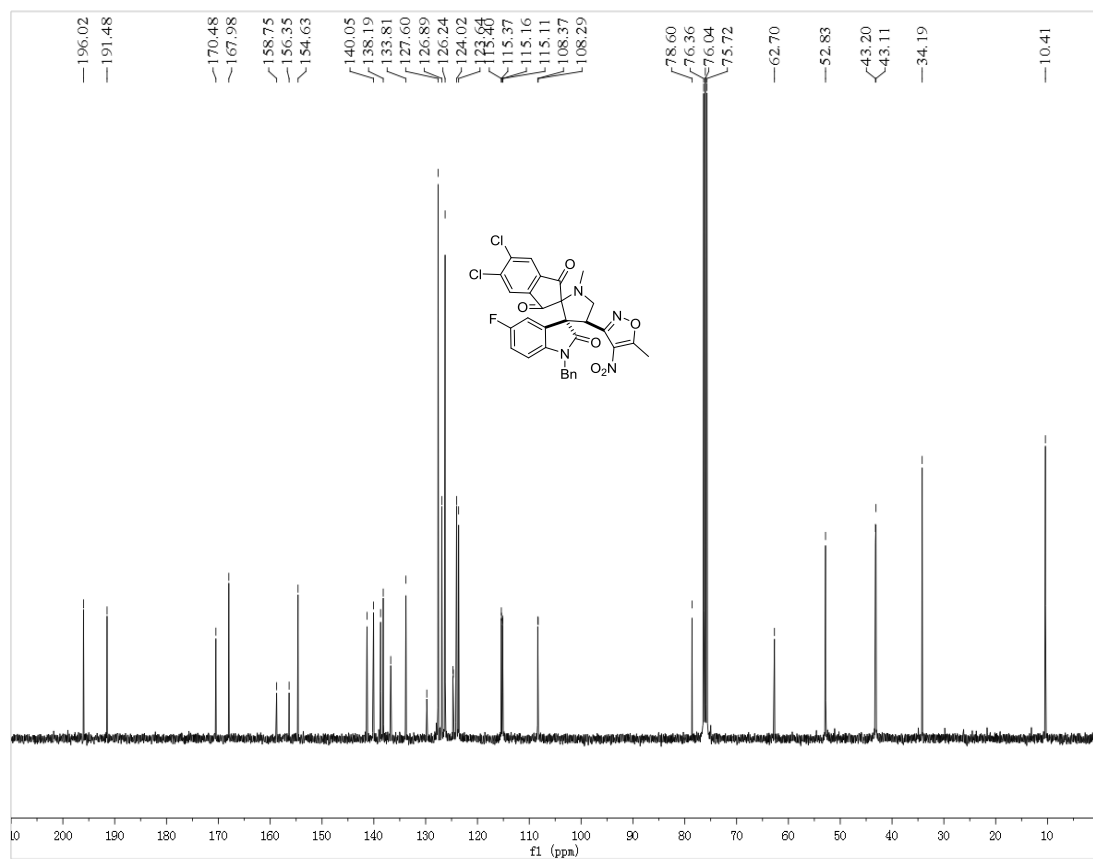
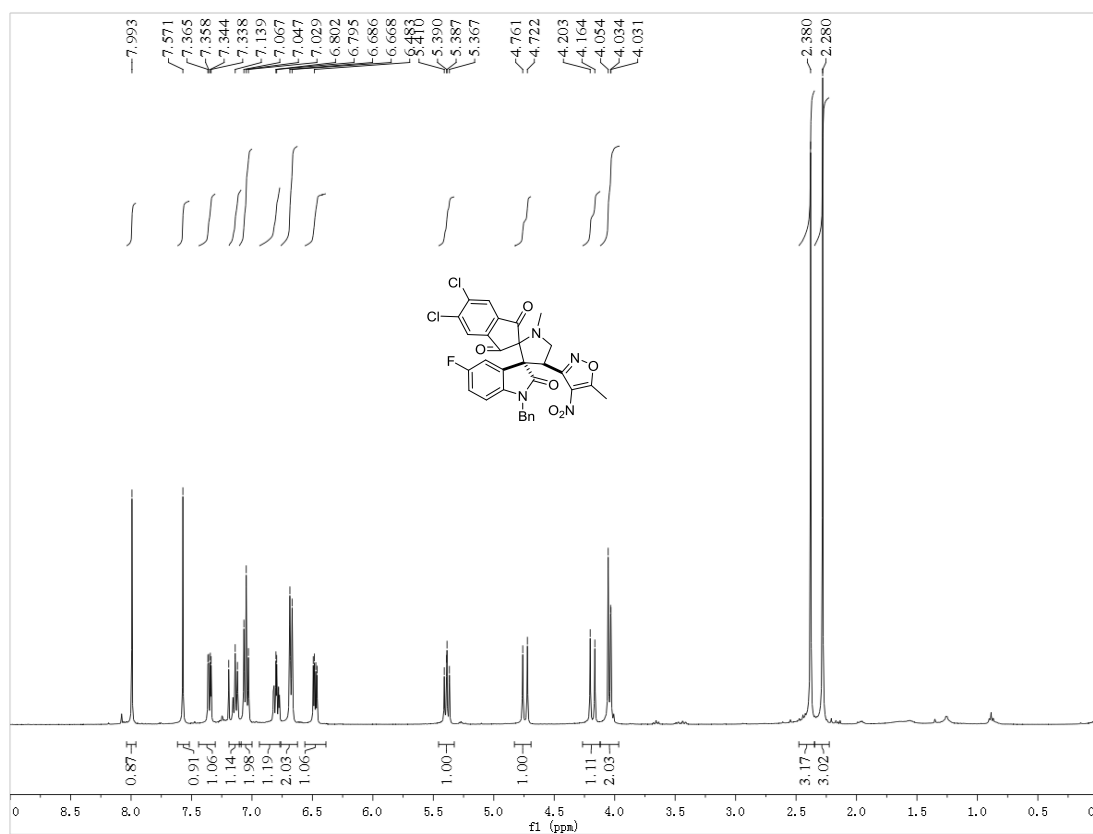
# <sup>1</sup>H and <sup>13</sup>C NMR of 4cf



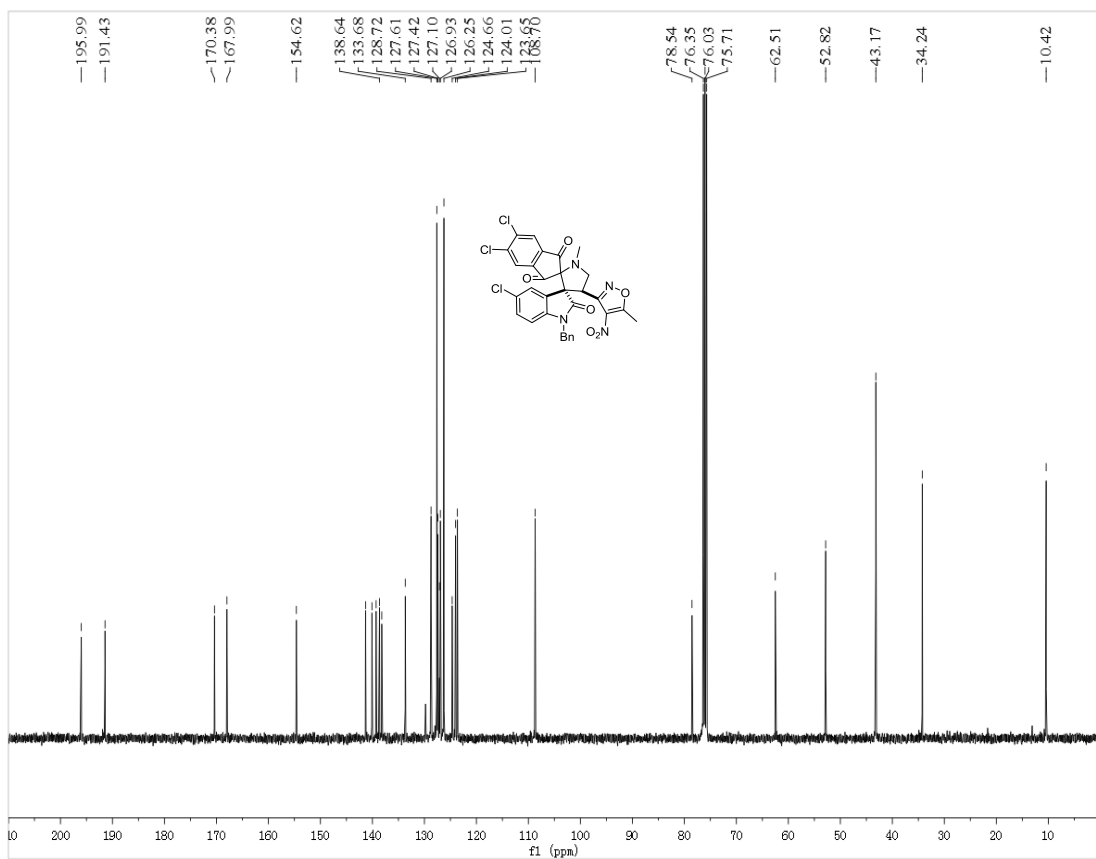
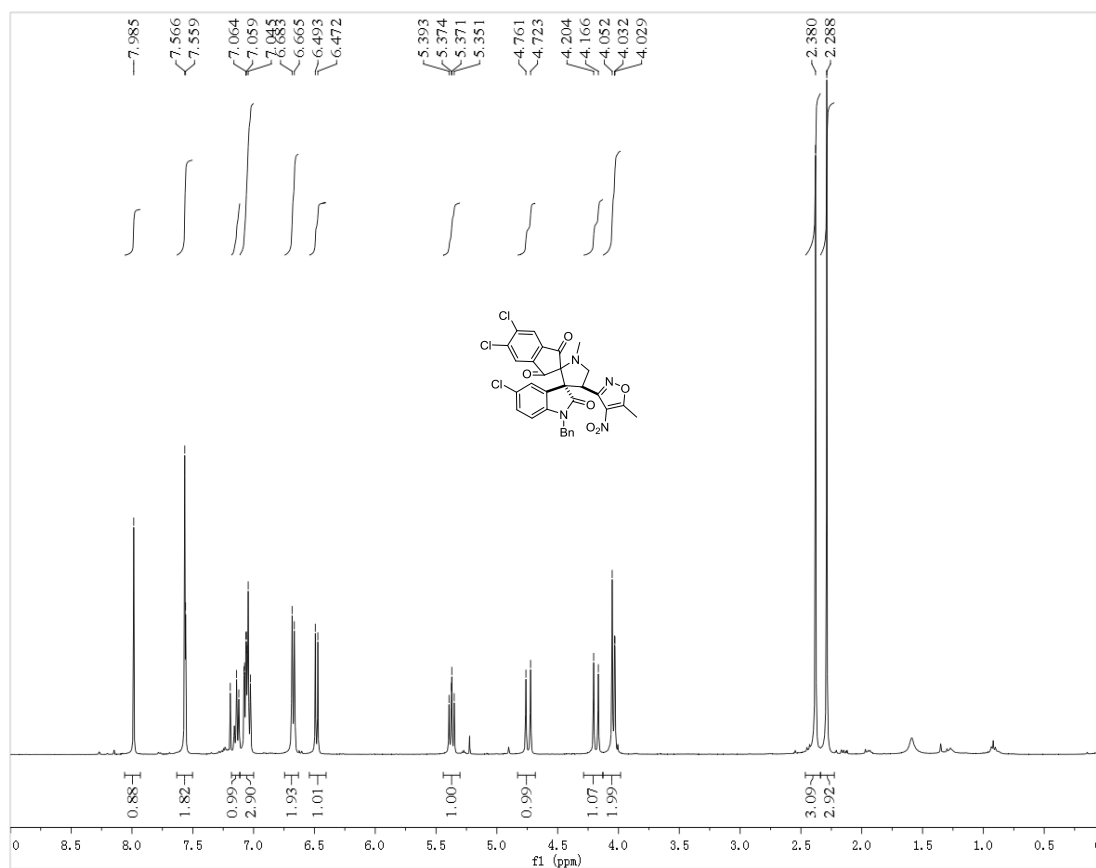
# <sup>1</sup>H and <sup>13</sup>C NMR of 4cg



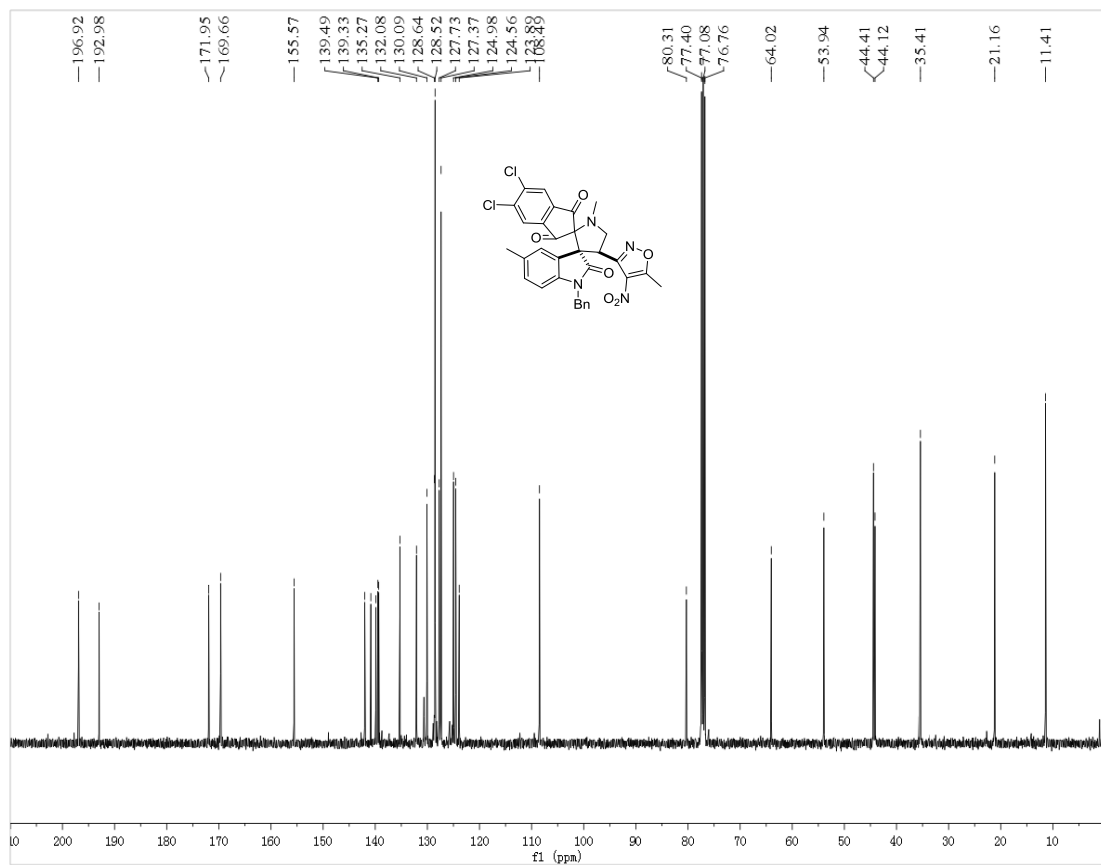
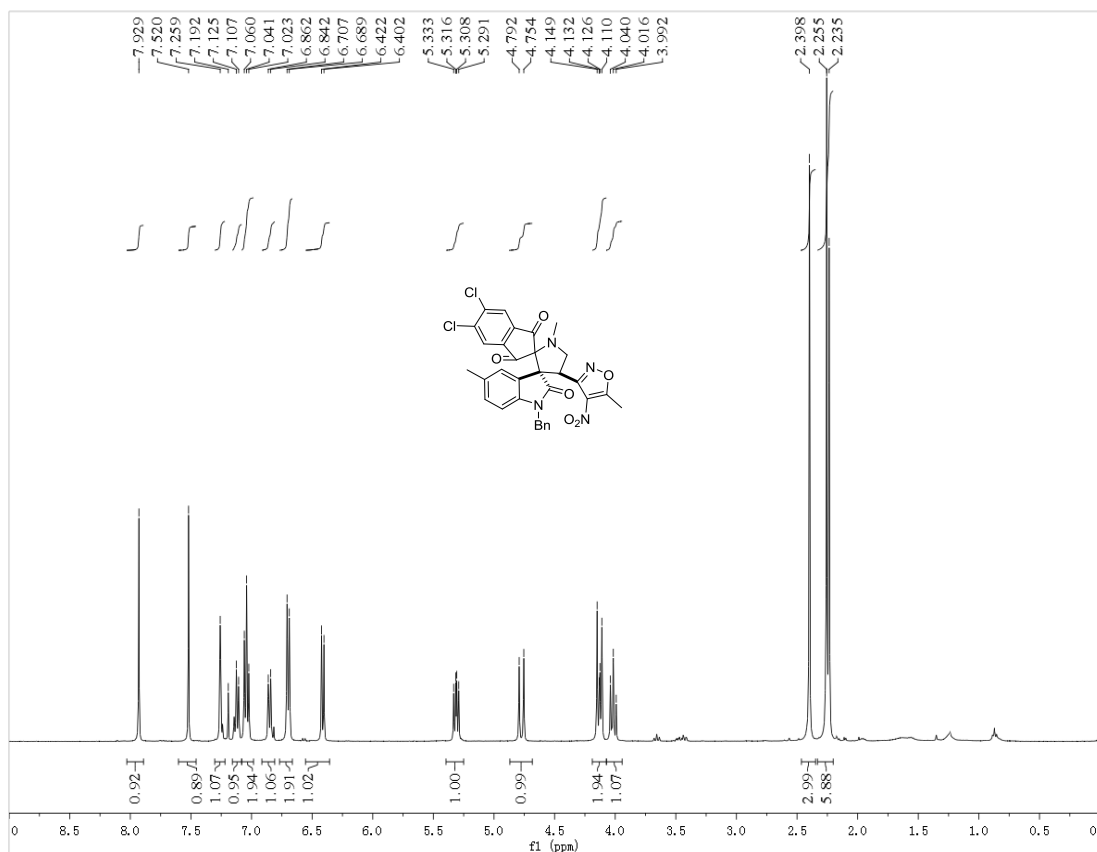
# $^1\text{H}$ and $^{13}\text{C}$ NMR of 4ch



# <sup>1</sup>H and <sup>13</sup>C NMR of 4ci



# <sup>1</sup>H and <sup>13</sup>C NMR of 4cj



# $^1\text{H}$ and $^{13}\text{C}$ NMR of 4da

