

## Supporting Information

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### Synthesis and biological activities of petromurin C nitrile derivatives

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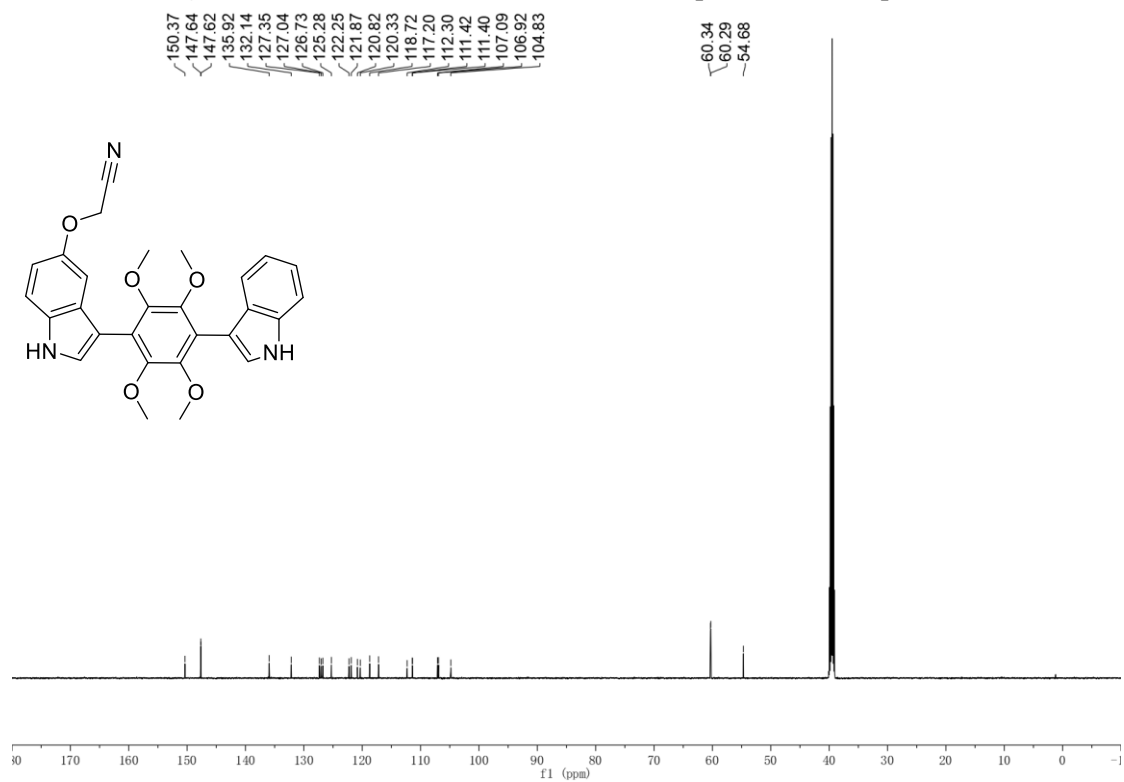
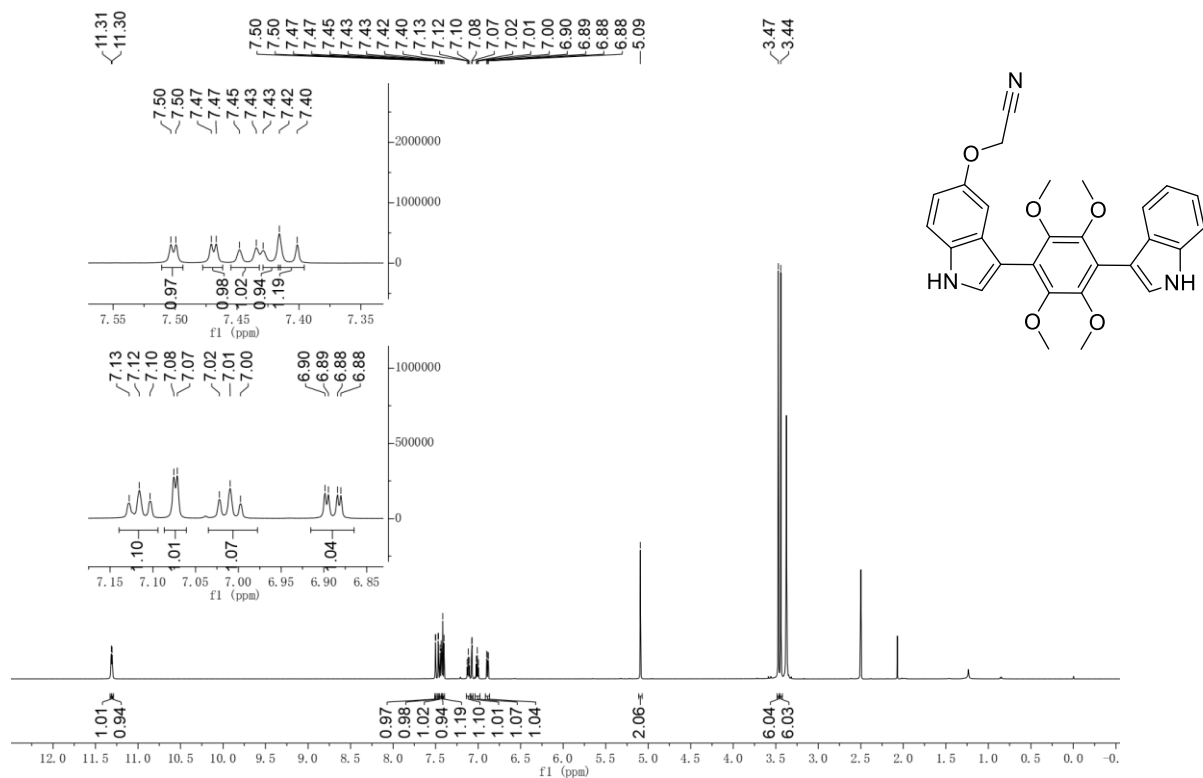
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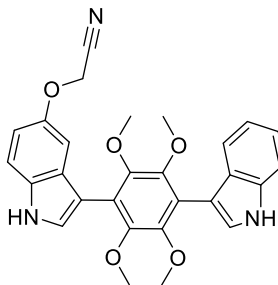
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Table of Contents	Page
<b>Figure S1:</b> <sup>1</sup> H-NMR (600 MHz, DMSO- <i>d</i> <sub>6</sub> ) spectrum of compound <b>1</b>	3
<b>Figure S2:</b> <sup>13</sup> C-NMR (150 MHz, DMSO- <i>d</i> <sub>6</sub> ) spectrum of compound <b>1</b>	3
<b>Figure S3:</b> HRESIMS spectrum of compound <b>1</b>	4
<b>Figure S4:</b> <sup>1</sup> H-NMR (600 MHz, DMSO- <i>d</i> <sub>6</sub> ) spectrum of compound <b>2</b>	4
<b>Figure S5:</b> <sup>13</sup> C-NMR (150 MHz, DMSO- <i>d</i> <sub>6</sub> ) spectrum of compound <b>2</b>	5
<b>Figure S6:</b> HRESIMS spectrum of compound <b>2</b>	5
<b>Figure S7:</b> <sup>1</sup> H-NMR (600 MHz, DMSO- <i>d</i> <sub>6</sub> ) spectrum of compound <b>3</b>	6
<b>Figure S8:</b> <sup>13</sup> C-NMR (150 MHz, DMSO- <i>d</i> <sub>6</sub> ) spectrum of compound <b>3</b>	6
<b>Figure S9:</b> HRESIMS spectrum of compound <b>3</b>	7
<b>Figure S10:</b> <sup>1</sup> H-NMR (600 MHz, DMSO- <i>d</i> <sub>6</sub> ) spectrum of compound <b>4</b>	7
<b>Figure S11:</b> <sup>13</sup> C-NMR (150 MHz, DMSO- <i>d</i> <sub>6</sub> ) spectrum of compound <b>4</b>	8
<b>Figure S12:</b> HRESIMS spectrum of compound <b>4</b>	8
<b>Figure S13:</b> <sup>1</sup> H-NMR (600 MHz, DMSO- <i>d</i> <sub>6</sub> ) spectrum of compound <b>5</b>	9
<b>Figure S14:</b> <sup>13</sup> C-NMR (150 MHz, DMSO- <i>d</i> <sub>6</sub> ) spectrum of compound <b>5</b>	9
<b>Figure S15:</b> HRESIMS spectrum of compound <b>5</b>	10
<b>Figure S16:</b> <sup>1</sup> H-NMR (600 MHz, DMSO- <i>d</i> <sub>6</sub> ) spectrum of compound <b>6</b>	10
<b>Figure S17:</b> <sup>13</sup> C-NMR (150 MHz, DMSO- <i>d</i> <sub>6</sub> ) spectrum of compound <b>6</b>	11
<b>Figure S18:</b> HRESIMS spectrum of compound <b>6</b>	11
<b>Figure S19:</b> <sup>1</sup> H-NMR (600 MHz, DMSO- <i>d</i> <sub>6</sub> ) spectrum of compound <b>7</b>	12
<b>Figure S20:</b> <sup>13</sup> C-NMR (150 MHz, DMSO- <i>d</i> <sub>6</sub> ) spectrum of compound <b>7</b>	12
<b>Figure S21:</b> HRESIMS spectrum of compound <b>7</b>	13
<b>Figure S22:</b> <sup>1</sup> H-NMR (600 MHz, DMSO- <i>d</i> <sub>6</sub> ) spectrum of compound <b>8</b>	13
<b>Figure S23:</b> <sup>13</sup> C-NMR (150 MHz, DMSO- <i>d</i> <sub>6</sub> ) spectrum of compound <b>8</b>	14
<b>Figure S24:</b> HRESIMS spectrum of compound <b>8</b>	14
<b>Figure S25:</b> <sup>1</sup> H-NMR (600 MHz, DMSO- <i>d</i> <sub>6</sub> ) spectrum of compound <b>9</b>	15

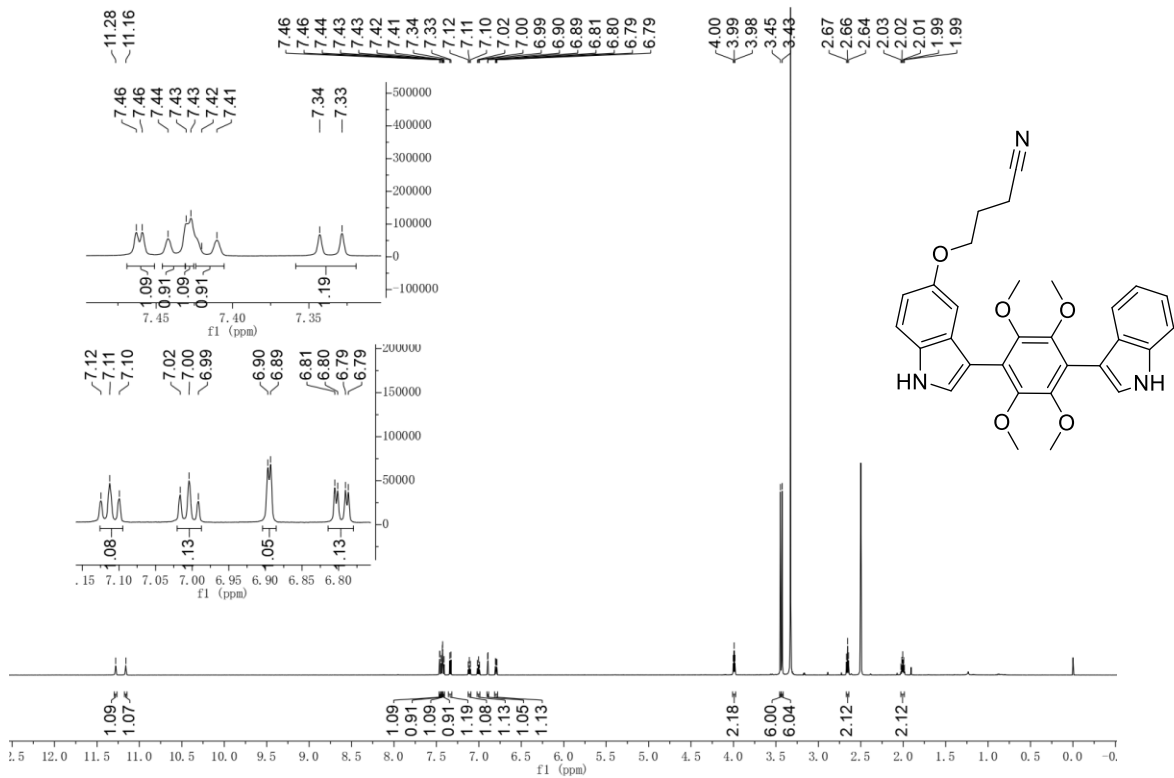
<b>Figure S26:</b> $^{13}\text{C}$ -NMR (150 MHz, DMSO- $d_6$ ) spectrum of compound <b>9</b>	15
<b>Figure S27:</b> HRESIMS spectrum of compound <b>9</b>	16
<b>Figure S28:</b> $^1\text{H}$ -NMR (600 MHz, DMSO- $d_6$ ) spectrum of compound <b>10</b>	16
<b>Figure S29:</b> $^{13}\text{C}$ -NMR (150 MHz, DMSO- $d_6$ ) spectrum of compound <b>10</b>	17
<b>Figure S30:</b> HRESIMS spectrum of compound <b>10</b>	17
<b>Figure S31:</b> $^1\text{H}$ -NMR (600 MHz, DMSO- $d_6$ ) spectrum of compound <b>11</b>	18
<b>Figure S32:</b> $^{13}\text{C}$ -NMR (150 MHz, DMSO- $d_6$ ) spectrum of compound <b>11</b>	18
<b>Figure S33:</b> HRESIMS spectrum of compound <b>11</b>	19
<b>Figure S34:</b> $^1\text{H}$ -NMR (600 MHz, DMSO- $d_6$ ) spectrum of compound <b>12</b>	19
<b>Figure S35:</b> $^{13}\text{C}$ -NMR (150 MHz, DMSO- $d_6$ ) spectrum of compound <b>12</b>	20
<b>Figure S36:</b> HRESIMS spectrum of compound <b>12</b>	20
<b>Figure S37:</b> $^1\text{H}$ -NMR (600 MHz, DMSO- $d_6$ ) spectrum of compound <b>13</b>	21
<b>Figure S38:</b> $^{13}\text{C}$ -NMR (150 MHz, DMSO- $d_6$ ) spectrum of compound <b>13</b>	21
<b>Figure S39:</b> HRESIMS spectrum of compound <b>13</b>	22
<b>Figure S40:</b> $^1\text{H}$ -NMR (600 MHz, DMSO- $d_6$ ) spectrum of compound <b>14</b>	22
<b>Figure S41:</b> $^{13}\text{C}$ -NMR (150 MHz, DMSO- $d_6$ ) spectrum of compound <b>14</b>	23
<b>Figure S42:</b> HRESIMS spectrum of compound <b>14</b>	23
<b>Figure S43:</b> $^1\text{H}$ -NMR (600 MHz, DMSO- $d_6$ ) spectrum of compound <b>15</b>	24
<b>Figure S44:</b> $^{13}\text{C}$ -NMR (150 MHz, DMSO- $d_6$ ) spectrum of compound <b>15</b>	24
<b>Figure S45:</b> HRESIMS spectrum of compound <b>15</b>	25
<b>Figure S46:</b> Infrared (IR) spectrum of compound <b>1</b> (KBr pellet)	25
<b>Figure S47:</b> Infrared (IR) spectrum of compound <b>2</b> (KBr pellet)	26
<b>Figure S48:</b> Infrared (IR) spectrum of compound <b>3</b> (KBr pellet)	26
<b>Figure S49:</b> Infrared (IR) spectrum of compound <b>4</b> (KBr pellet)	26
<b>Figure S50:</b> Infrared (IR) spectrum of compound <b>5</b> (KBr pellet)	27
<b>Figure S51:</b> Infrared (IR) spectrum of compound <b>6</b> (KBr pellet)	27
<b>Figure S52:</b> Infrared (IR) spectrum of compound <b>7</b> (KBr pellet)	27
<b>Figure S53:</b> Infrared (IR) spectrum of compound <b>8</b> (KBr pellet)	28
<b>Figure S54:</b> Infrared (IR) spectrum of compound <b>9</b> (KBr pellet)	28
<b>Figure S55:</b> Infrared (IR) spectrum of compound <b>10</b> (KBr pellet)	28
<b>Figure S56:</b> Infrared (IR) spectrum of compound <b>11</b> (KBr pellet)	29
<b>Figure S57:</b> Infrared (IR) spectrum of compound <b>12</b> (KBr pellet)	29
<b>Figure S58:</b> Infrared (IR) spectrum of compound <b>13</b> (KBr pellet)	29
<b>Figure S59:</b> Infrared (IR) spectrum of compound <b>14</b> (KBr pellet)	30
<b>Figure S60:</b> Infrared (IR) spectrum of compound <b>15</b> (KBr pellet)	30
<b>Figure S61:</b> Dose-response curves of $\text{IC}_{50}$ for compounds <b>1-4, 7-10</b> and positive control Cisplatin	31
<b>General</b>	32
<b>Apparatus and Reagents</b>	32
<b>Cytotoxic activity</b>	32
<b>Ant-Myco bacterium tuberculosis</b>	32-33
<b>References</b>	34



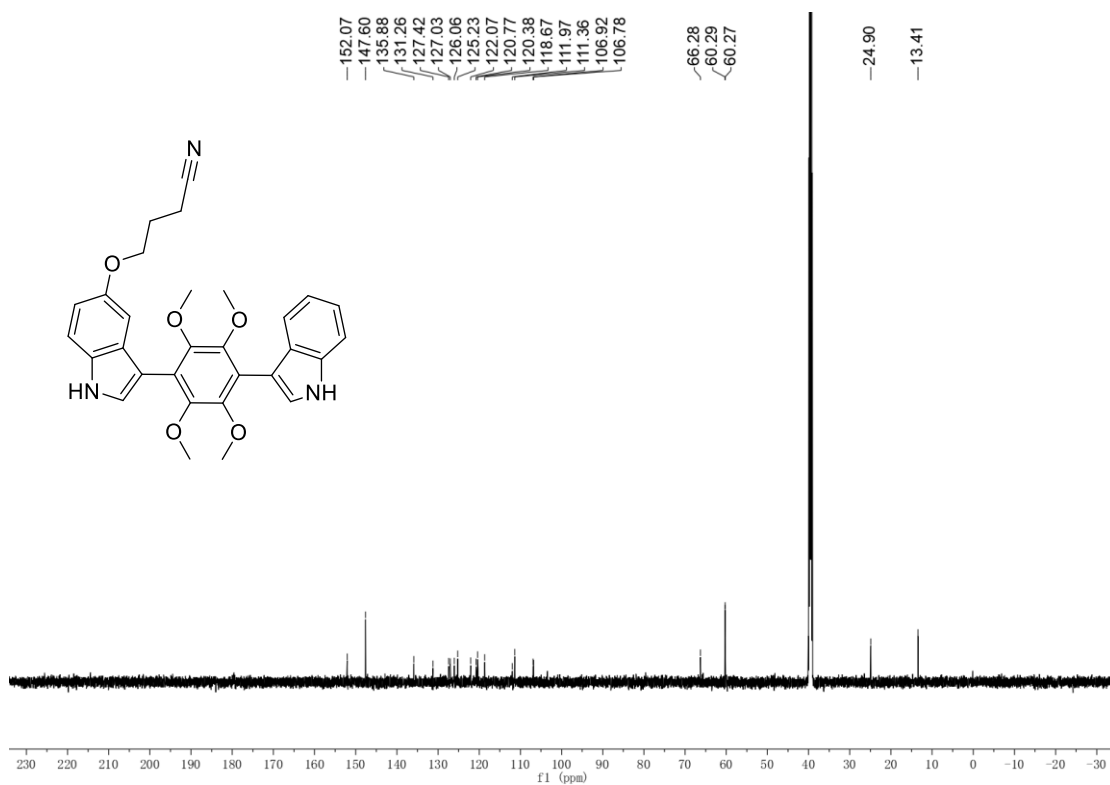
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**Figure S3: HRESIMS spectrum of compound 1**

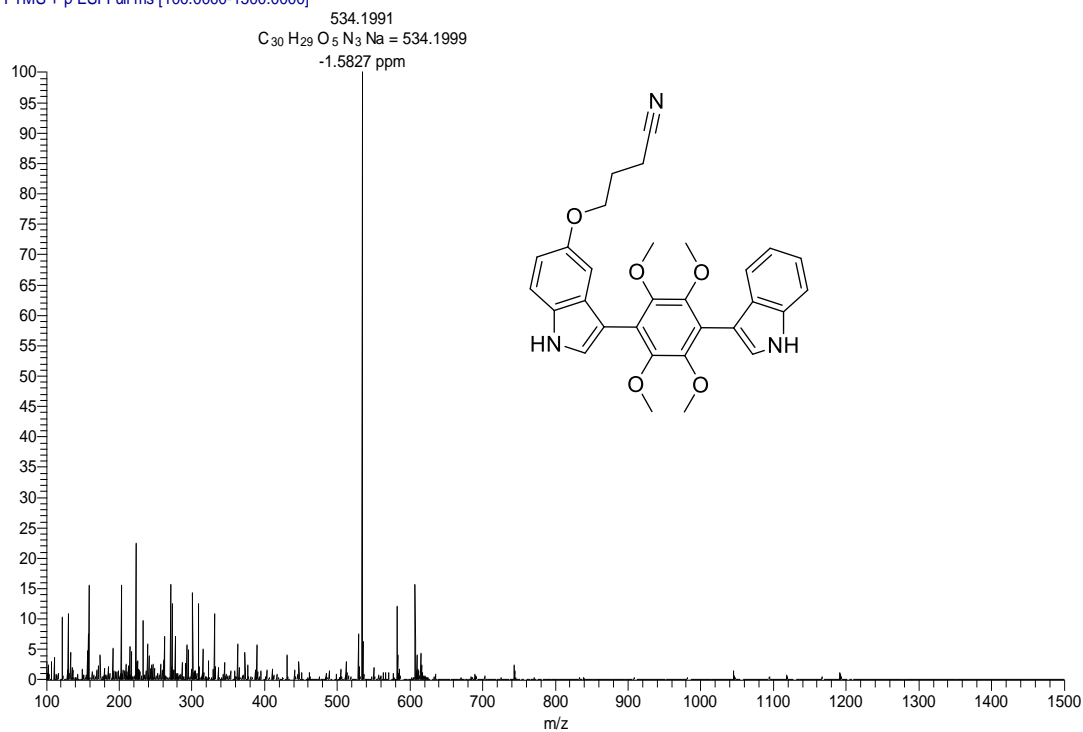


**Figure S4:**  $^1\text{H}$ -NMR (600 MHz,  $\text{DMSO-}d_6$ ) spectrum of compound **2**

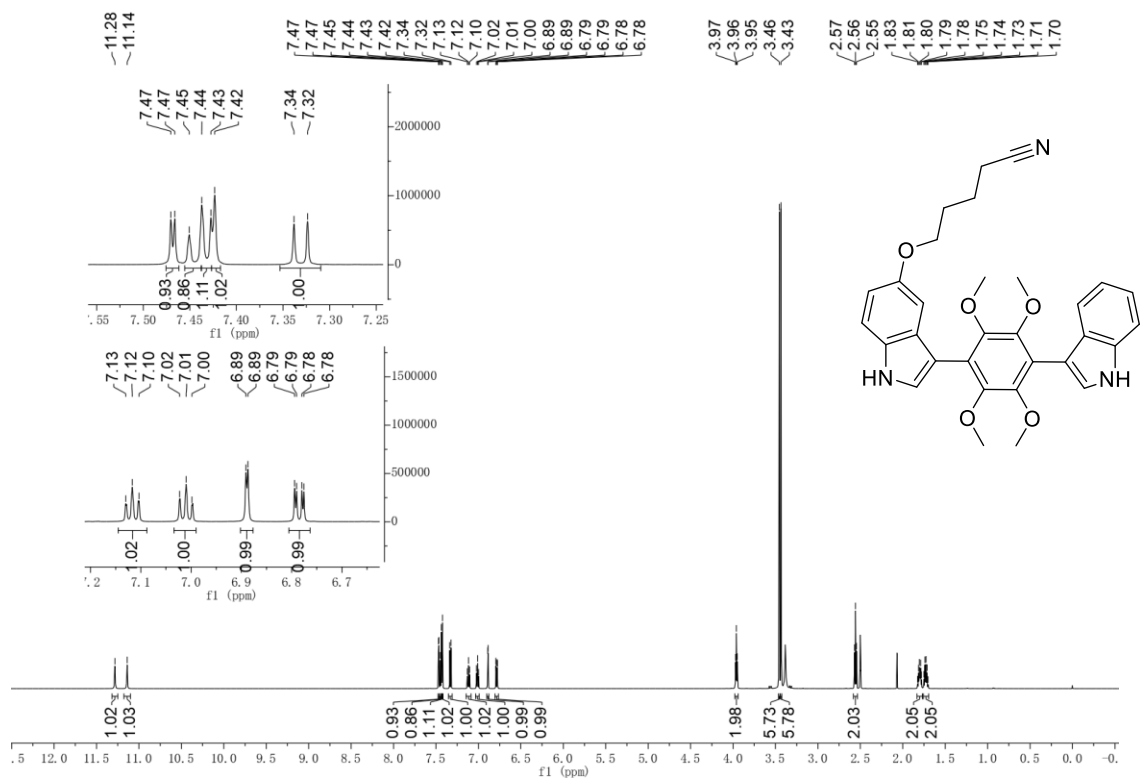


**Figure S5:** <sup>13</sup>C-NMR (150 MHz, DMSO-*d*<sub>6</sub>) spectrum of compound 2

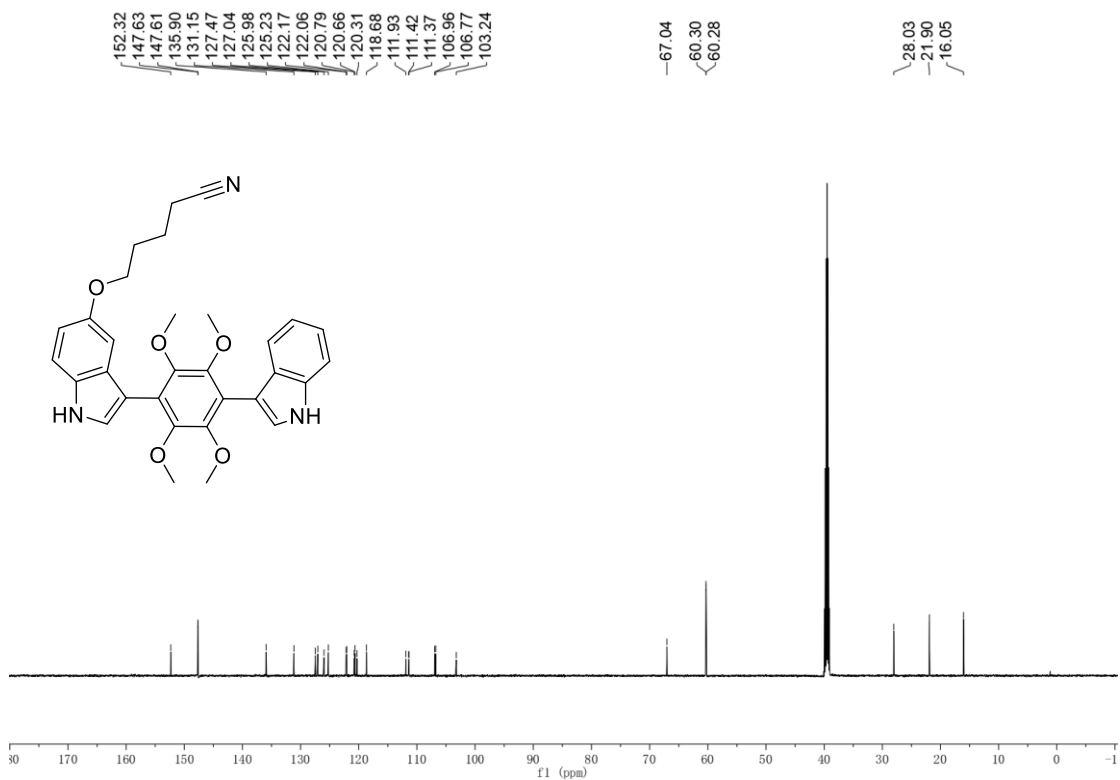
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T: FTMS + p ESI Full ms [100.0000-1500.0000]



**Figure S6:** HRESIMS spectrum of compound 2



**Figure S7:** <sup>1</sup>H-NMR (600 MHz, DMSO-*d*<sub>6</sub>) spectrum of compound **3**



**Figure S8:** <sup>13</sup>C-NMR (150 MHz, DMSO-*d*<sub>6</sub>) spectrum of compound **3**

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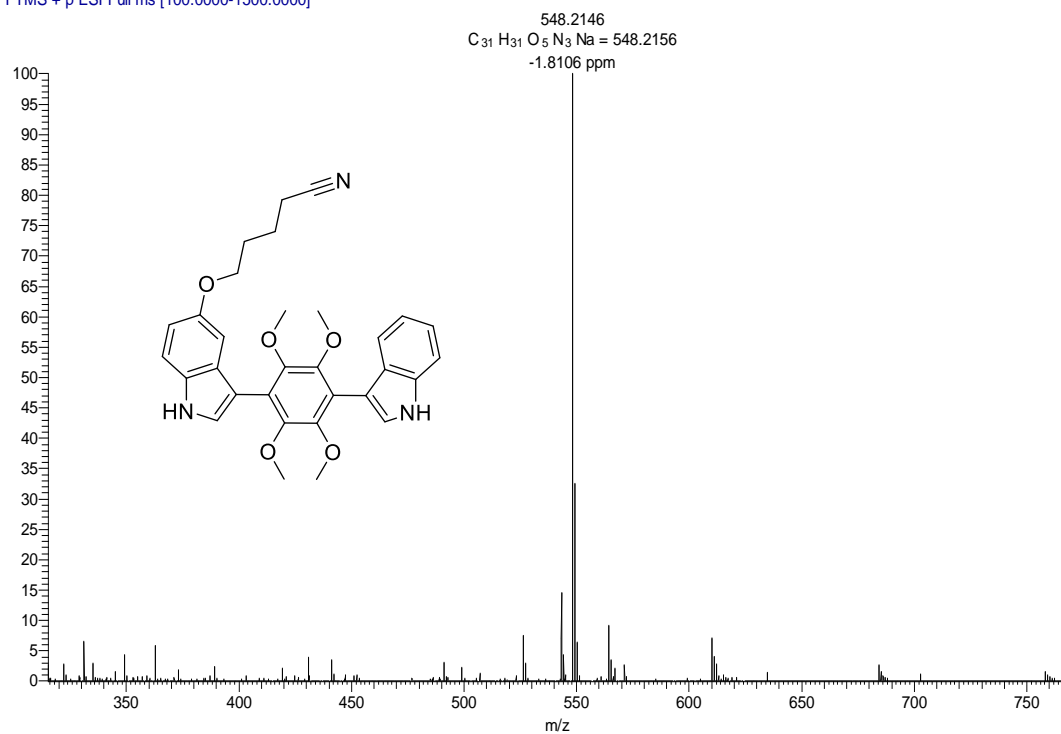


Figure S9: HRESIMS spectrum of compound 3

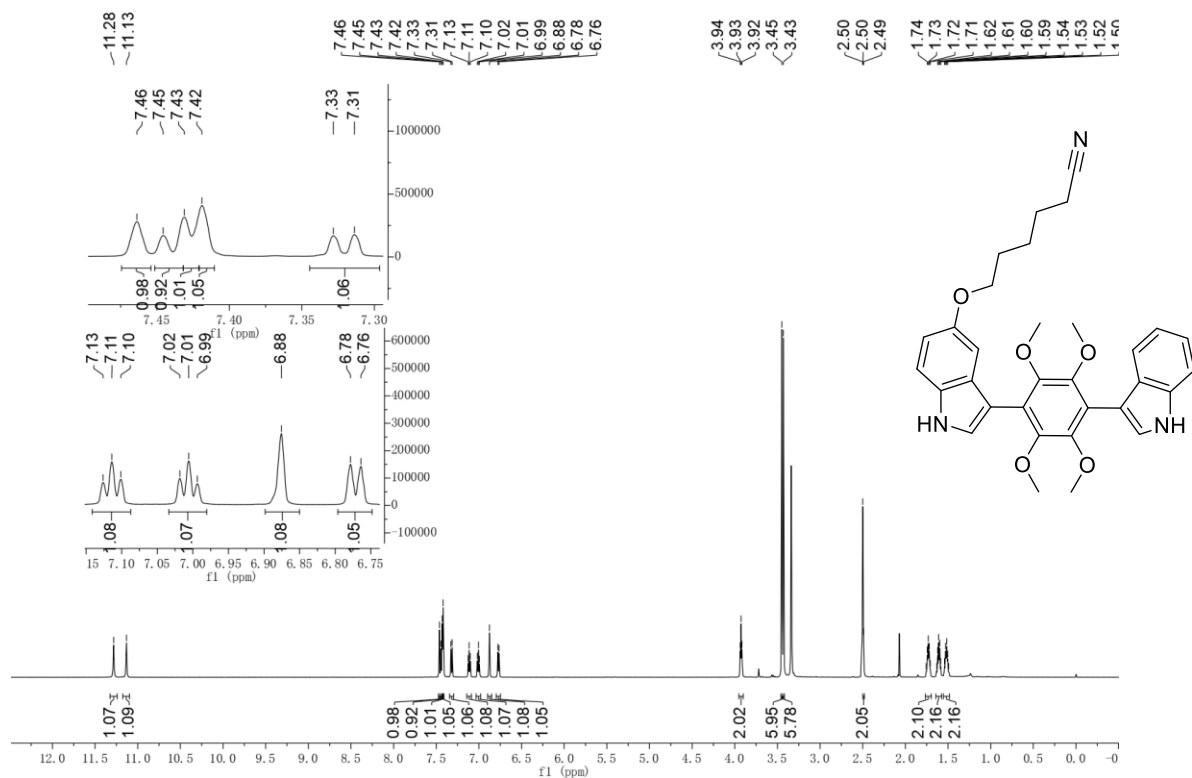
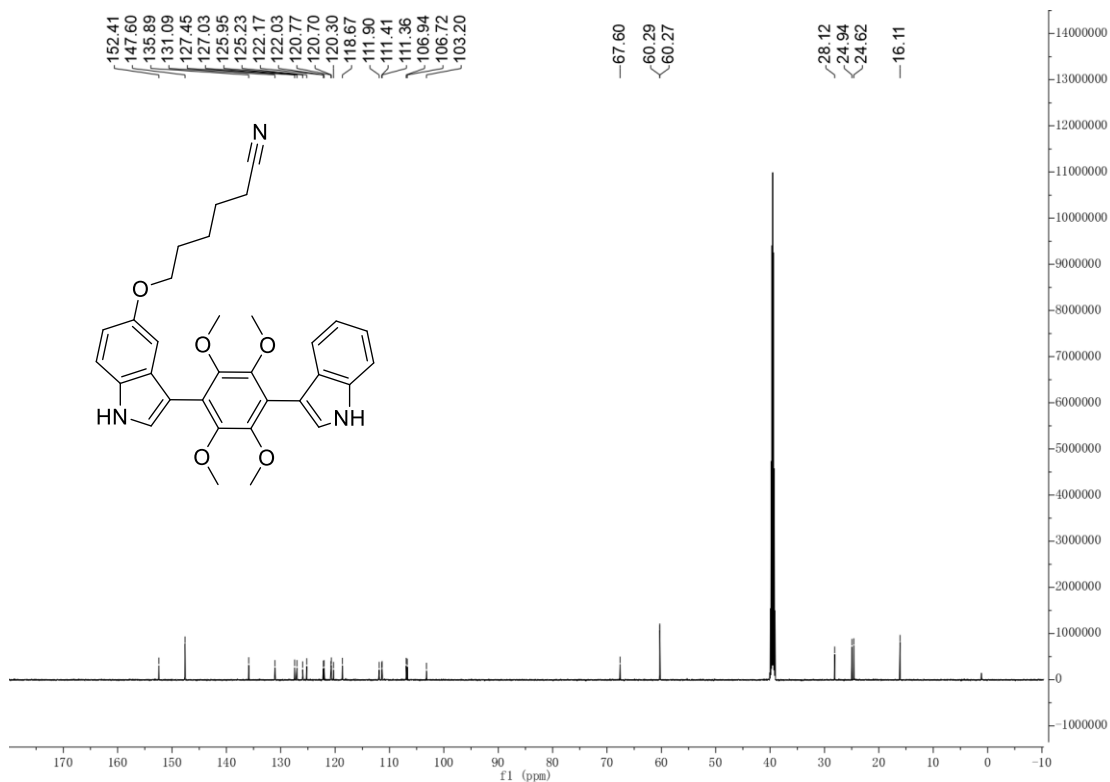
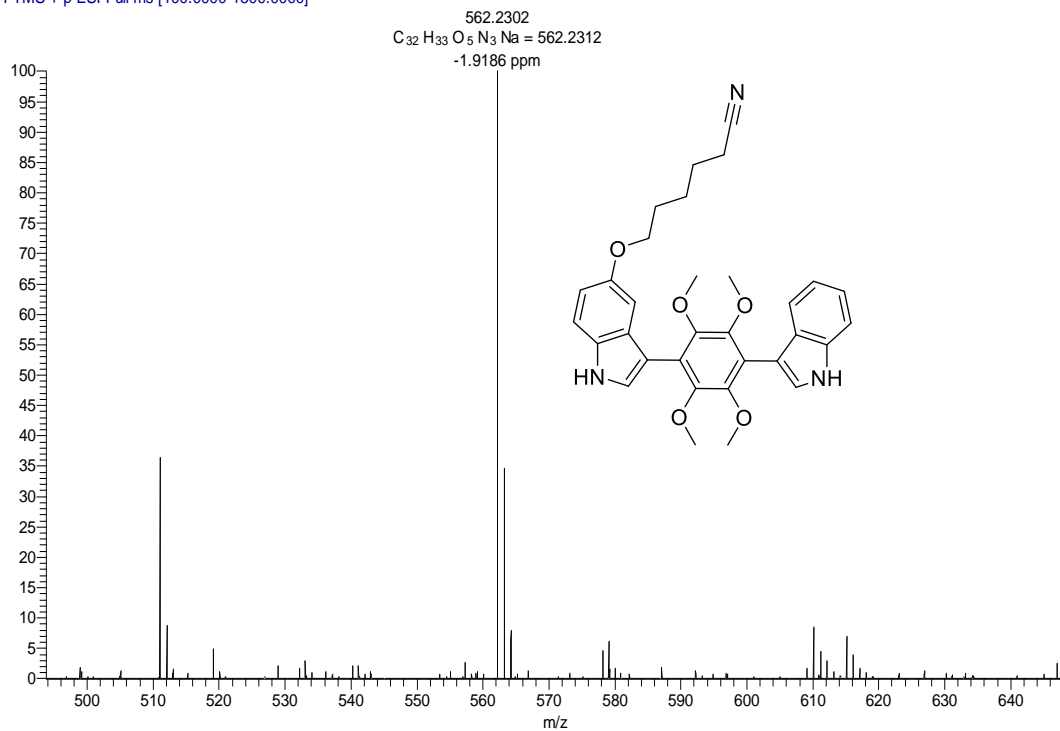


Figure S10: <sup>1</sup>H-NMR (600 MHz, DMSO-*d*<sub>6</sub>) spectrum of compound 4



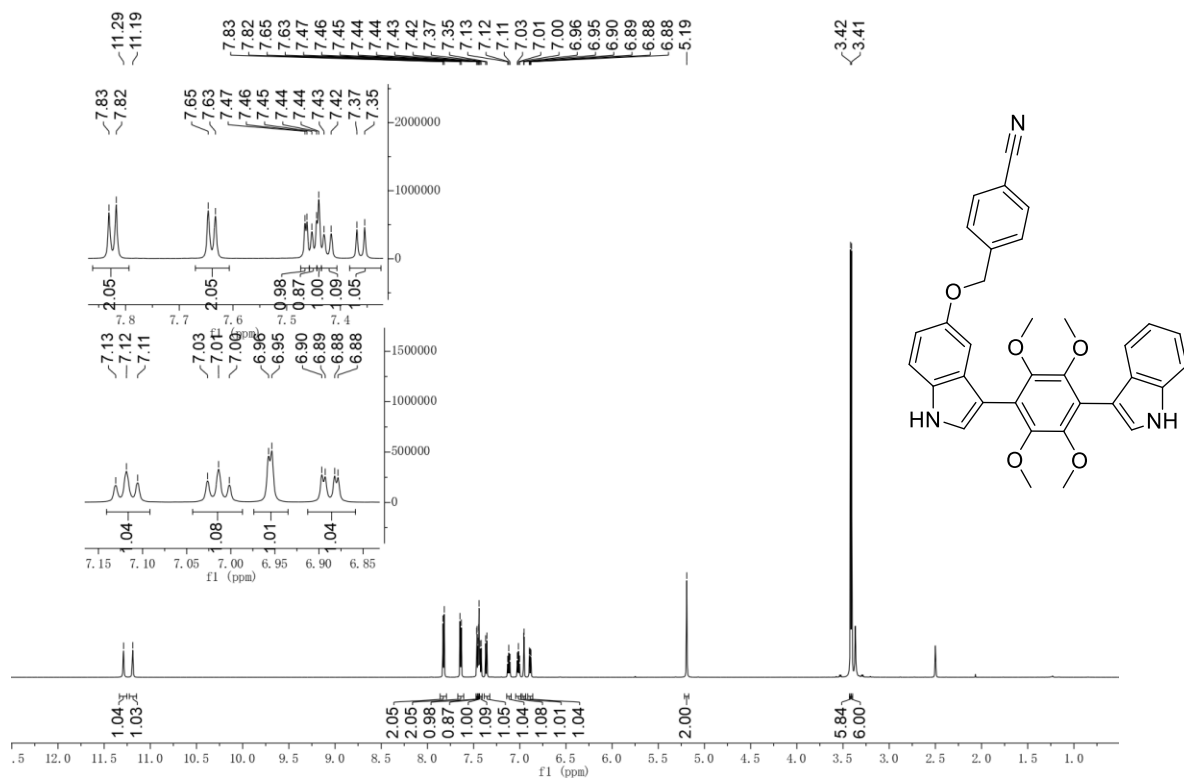
**Figure S11:**  $^{13}\text{C}$ -NMR (150 MHz,  $\text{DMSO}-d_6$ ) spectrum of compound **4**

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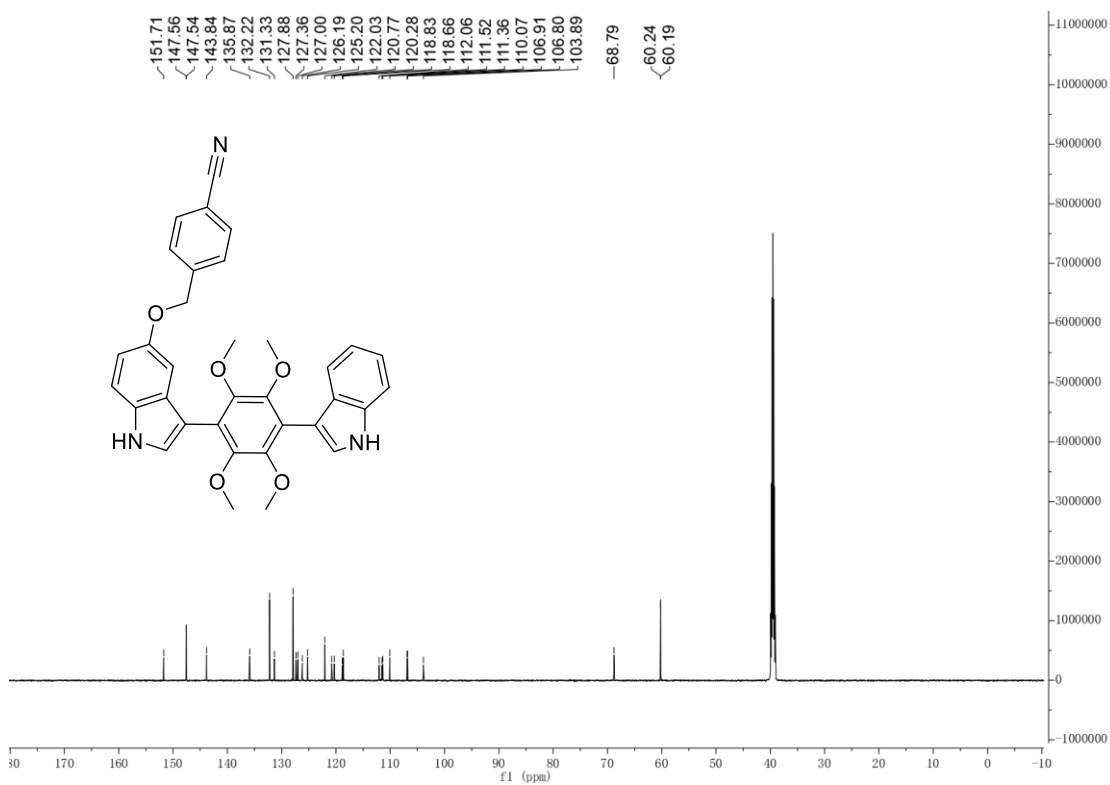


**Figure S12:** HRESIMS spectrum of compound **4**





**Figure S13:** <sup>1</sup>H-NMR (600 MHz, DMSO-*d*<sub>6</sub>) spectrum of compound **5**



**Figure S14:** <sup>13</sup>C-NMR (150 MHz, DMSO-*d*<sub>6</sub>) spectrum of compound **5**

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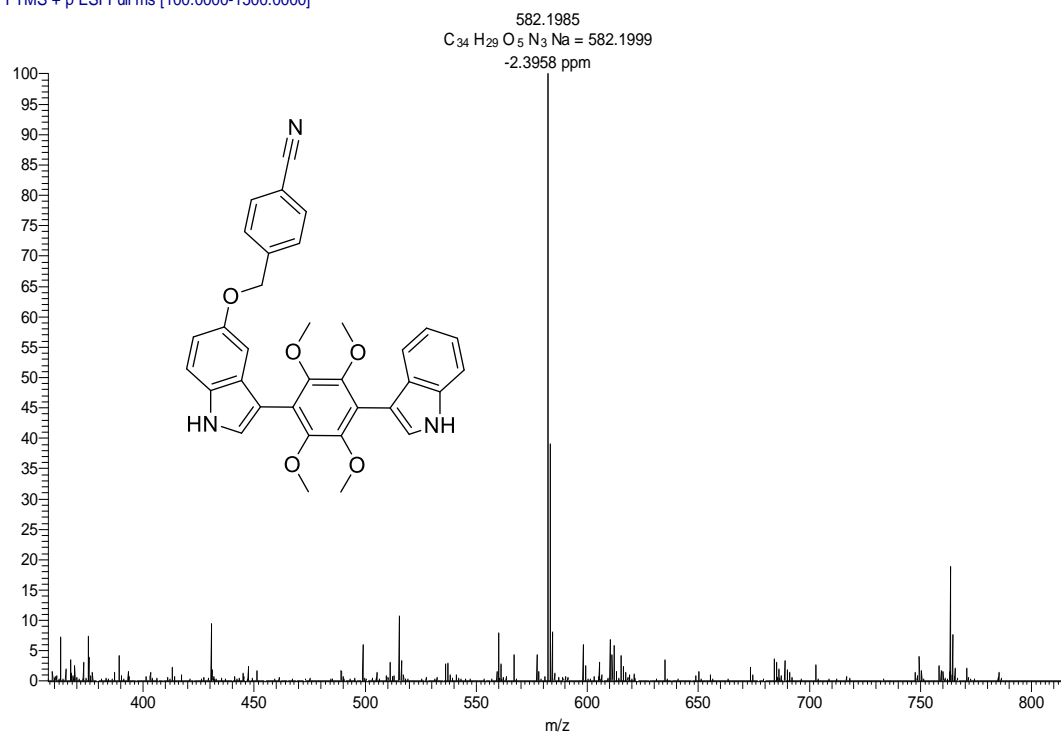


Figure S15: HRESIMS spectrum of compound 5

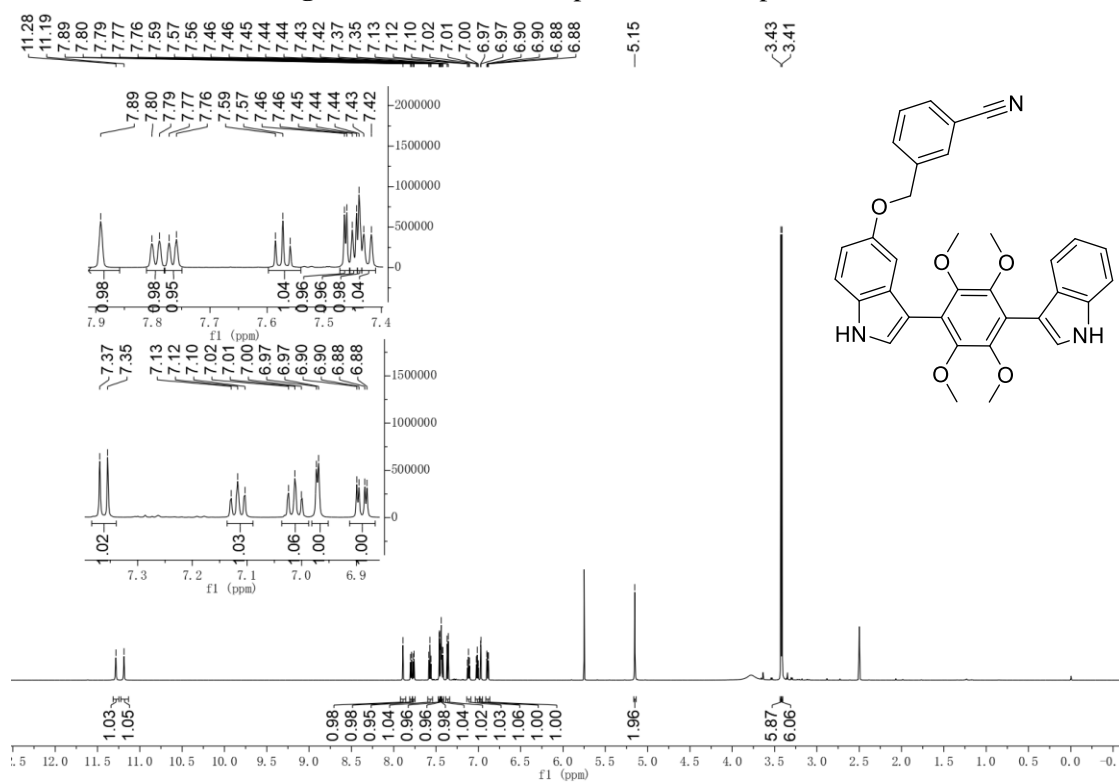
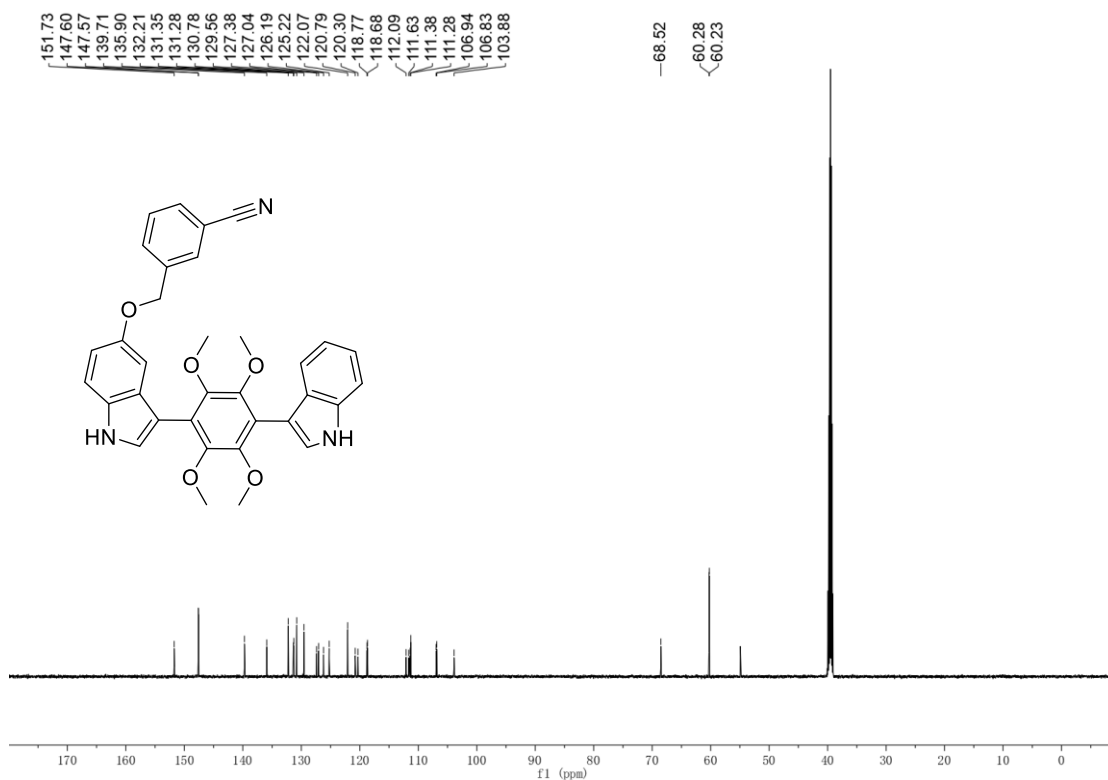
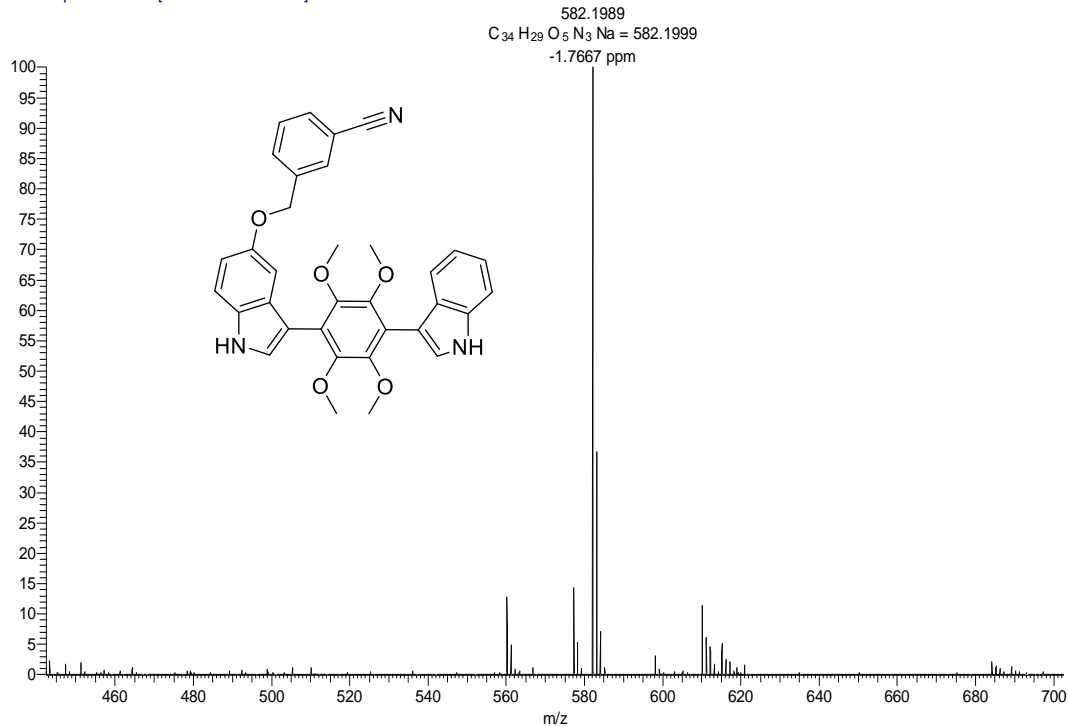


Figure S16: <sup>1</sup>H-NMR (600 MHz, DMSO-d<sub>6</sub>) spectrum of compound 6

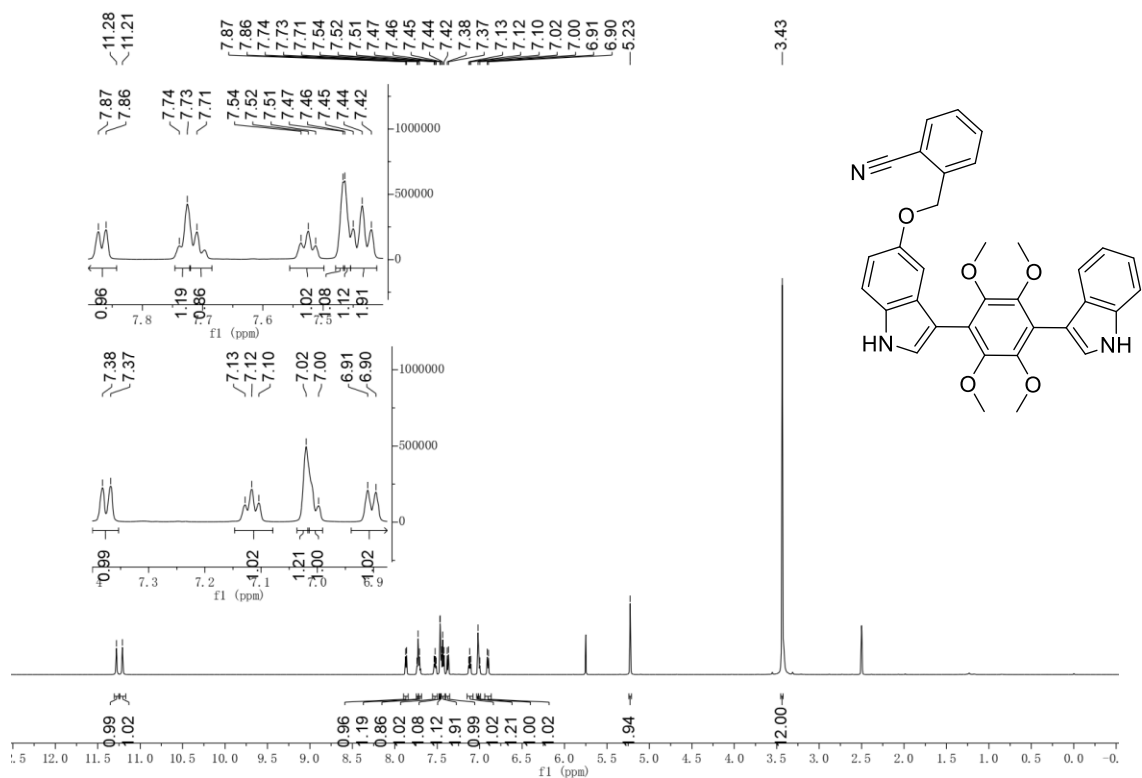


**Figure S17:** <sup>13</sup>C-NMR (150 MHz, DMSO-*d*<sub>6</sub>) spectrum of compound **6**

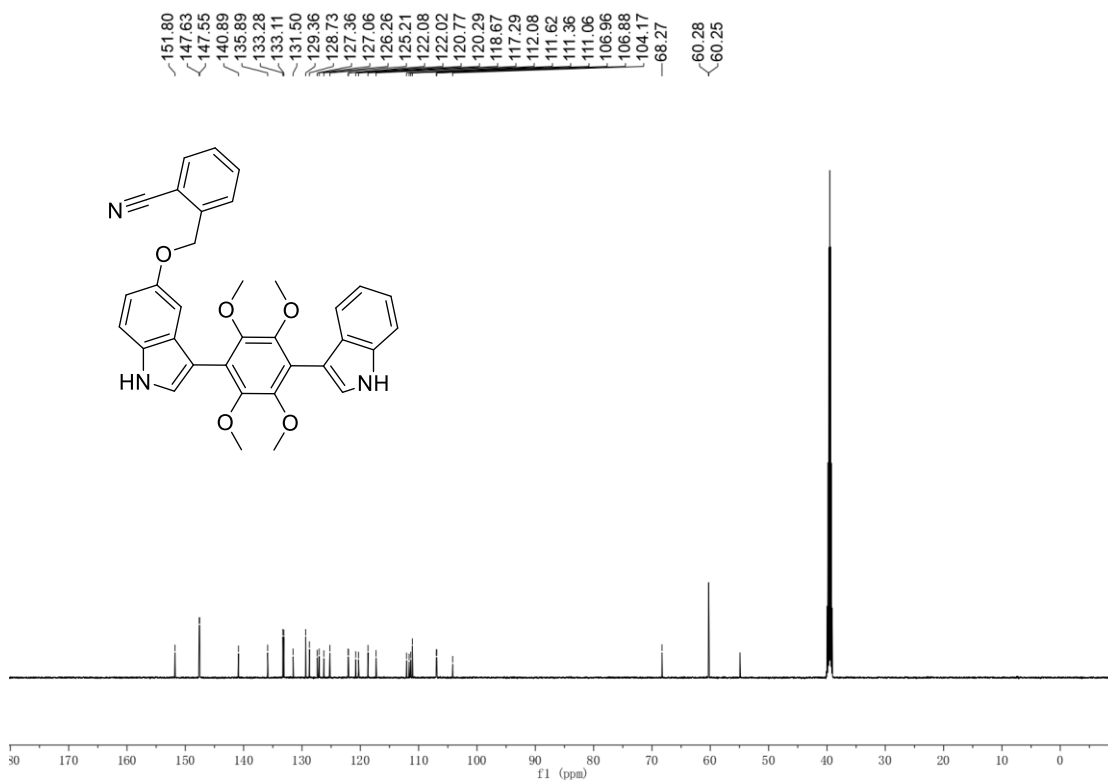
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T: FTMS + p ESI Full ms [100.0000-1500.0000]



**Figure S18:** HRESIMS spectrum of compound **6**



**Figure S19:**  $^1\text{H}$ -NMR (600 MHz,  $\text{DMSO-}d_6$ ) spectrum of compound **7**



**Figure S20:**  $^{13}\text{C}$ -NMR (150 MHz,  $\text{DMSO-}d_6$ ) spectrum of compound **7**

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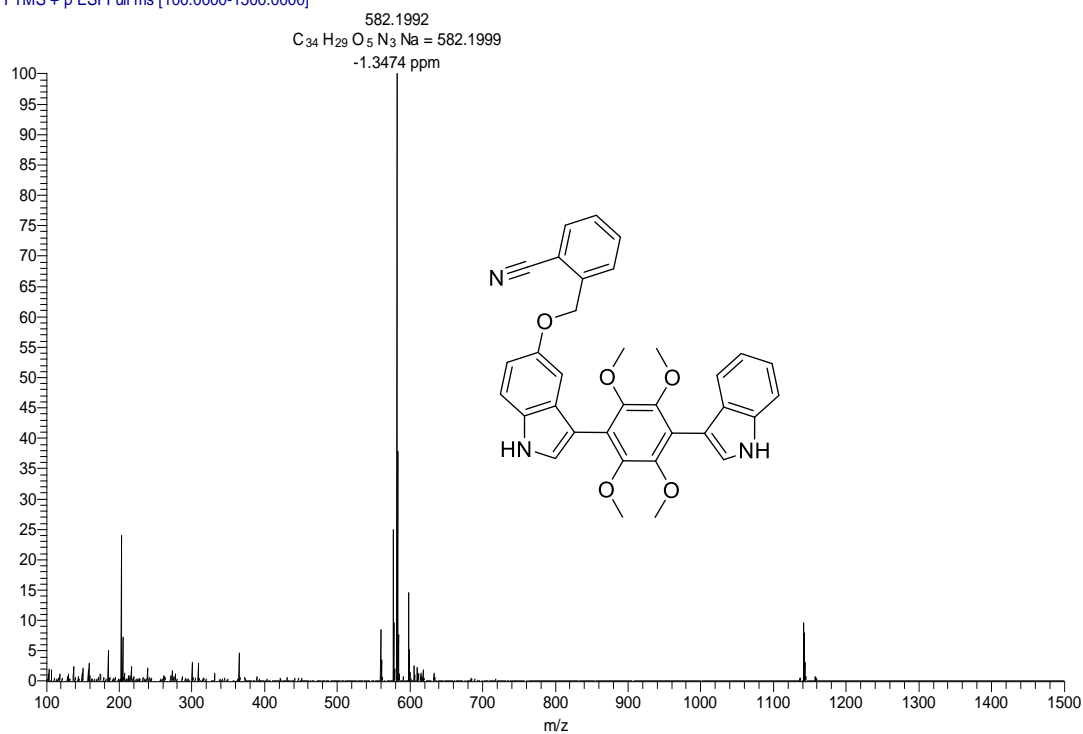


Figure S21: HRESIMS spectrum of compound 7

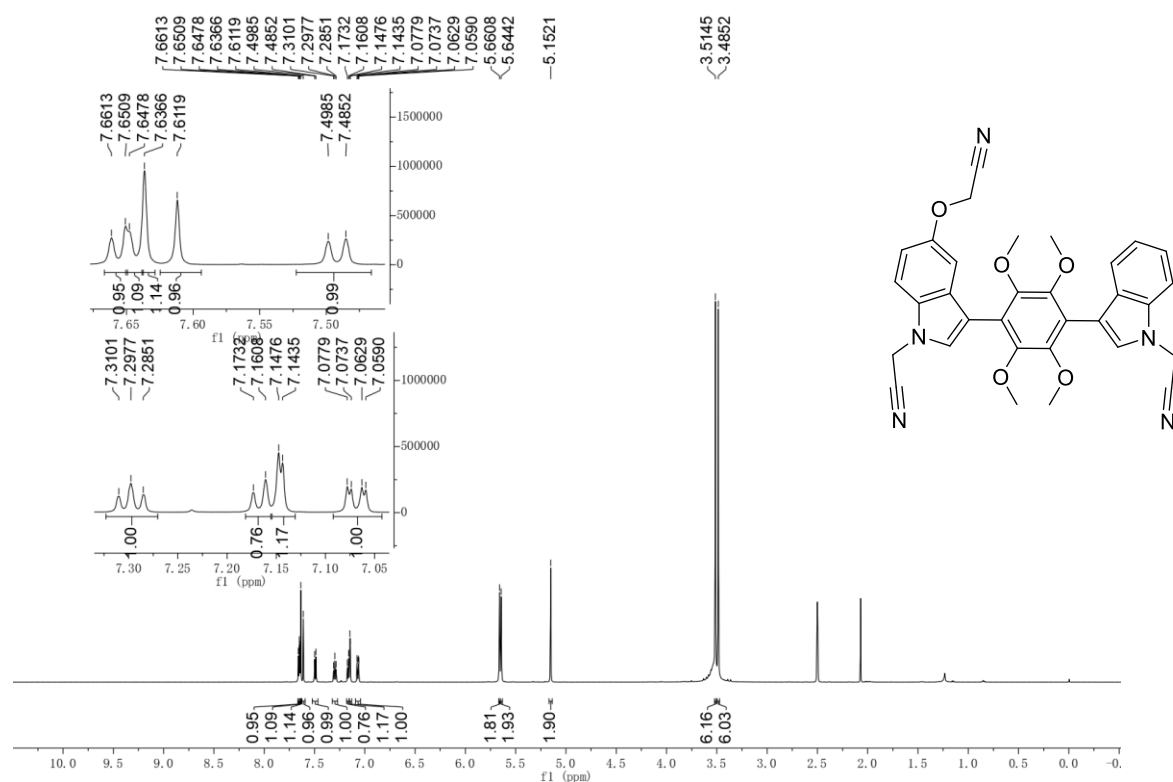
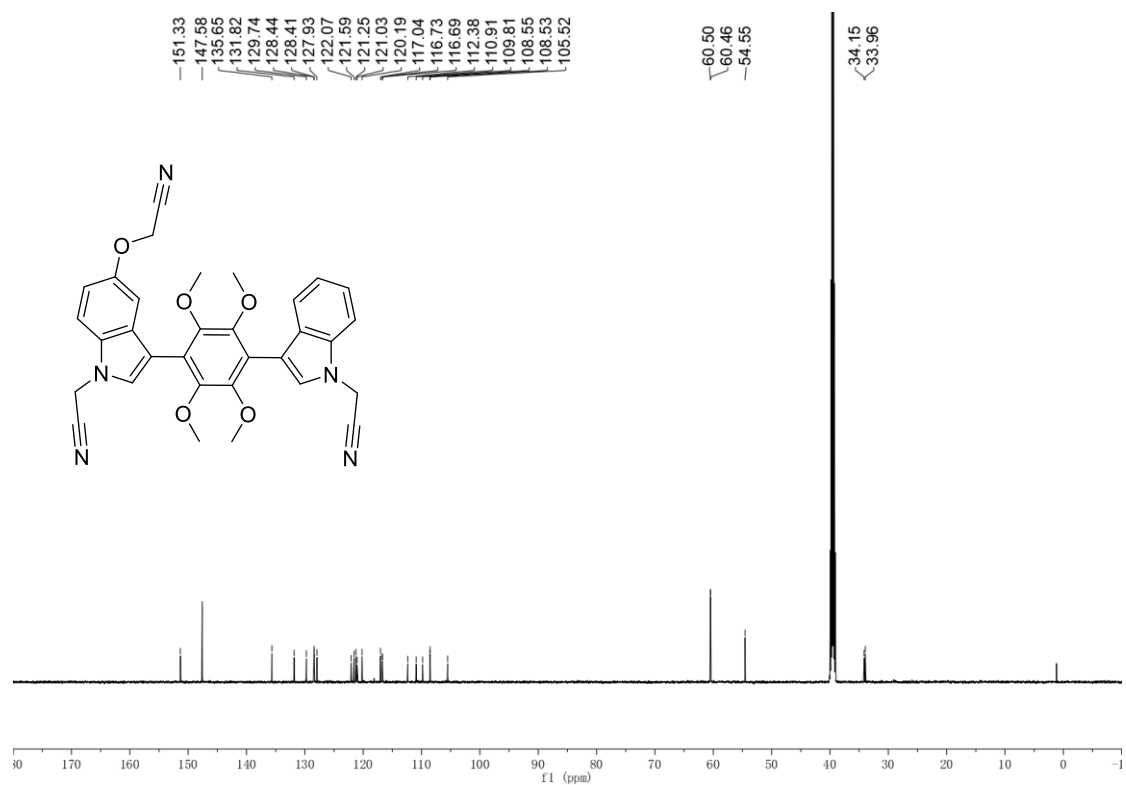
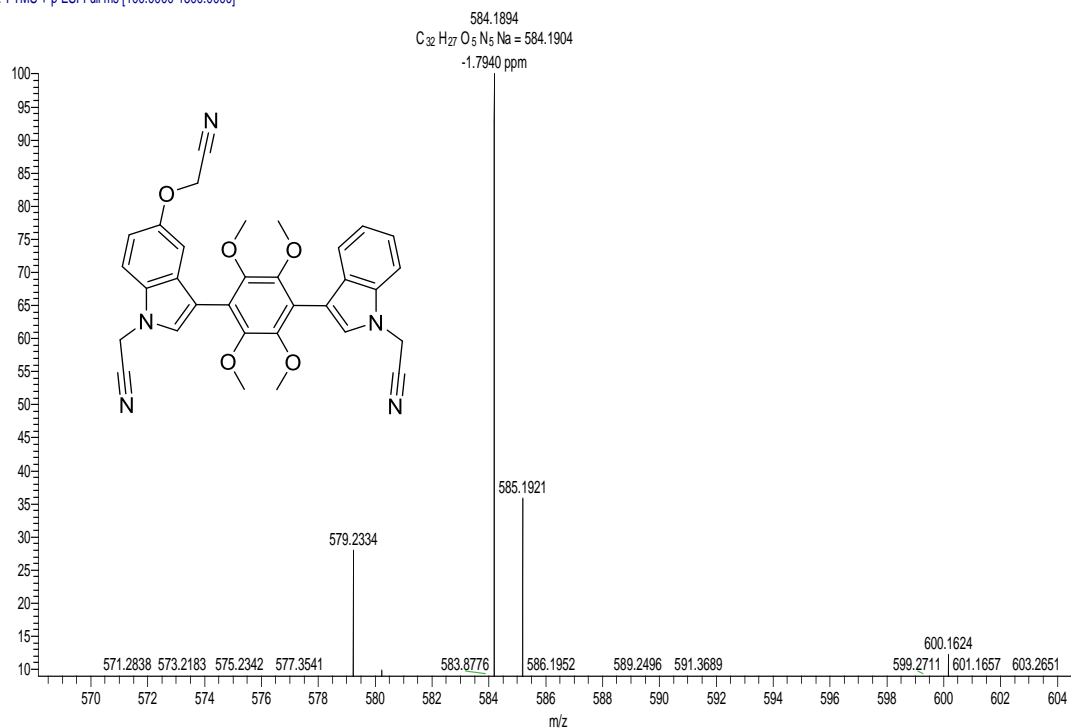


Figure S22: <sup>1</sup>H-NMR (600 MHz, DMSO-*d*<sub>6</sub>) spectrum of compound 8

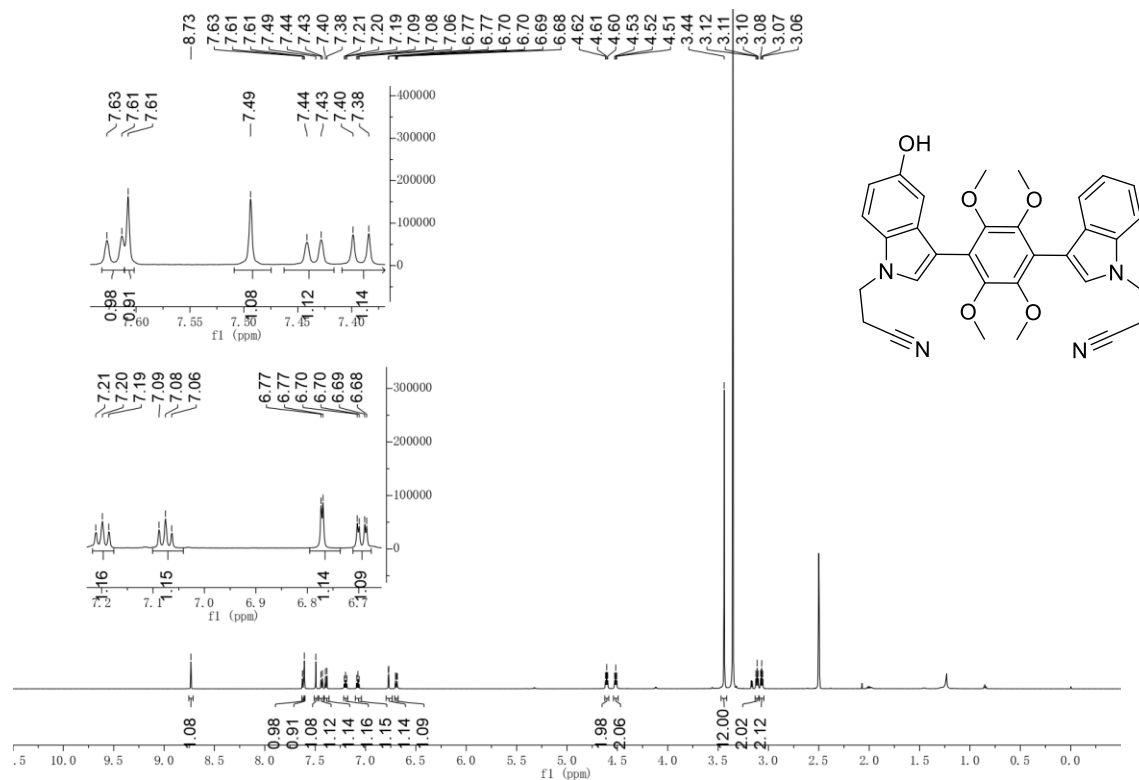


**Figure S23:** <sup>13</sup>C-NMR (150 MHz, DMSO-*d*<sub>6</sub>) spectrum of compound 8

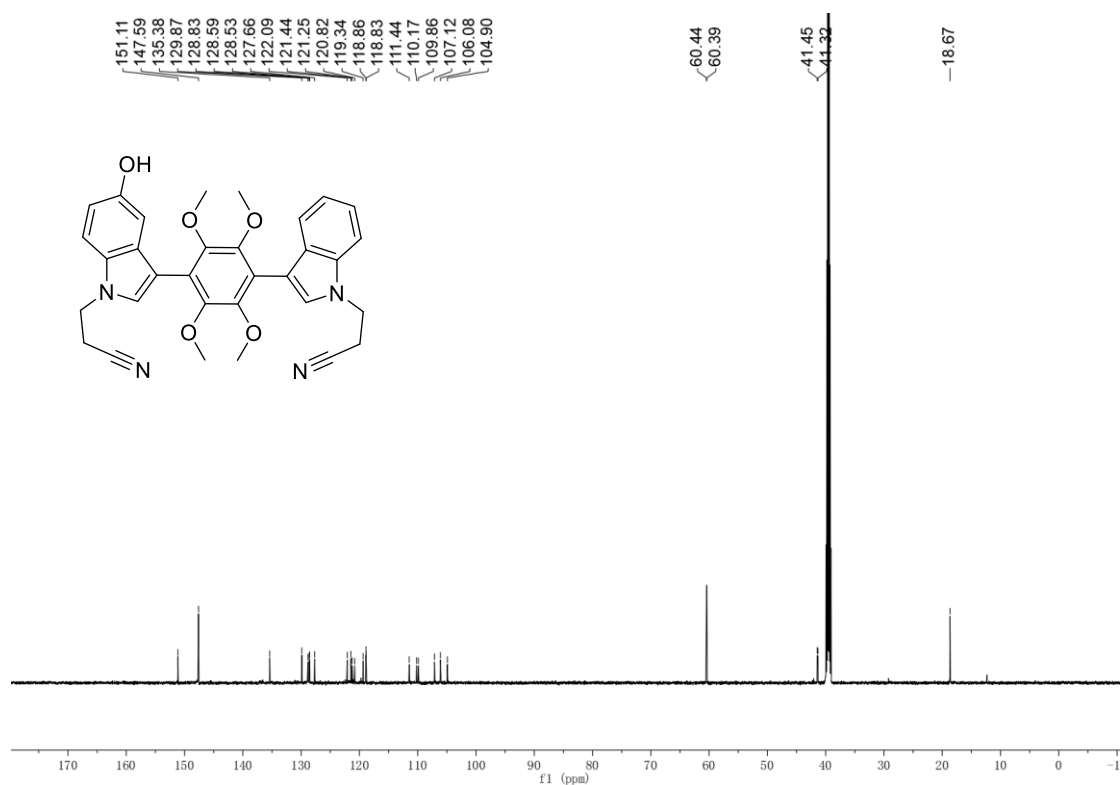
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T: FTMS + p ESI Full ms [100.0000-1500.0000]



**Figure S24:** HRESIMS spectrum of compound 8



**Figure S25:** <sup>1</sup>H-NMR (600 MHz, DMSO-*d*<sub>6</sub>) spectrum of compound **9**



**Figure S26:** <sup>13</sup>C-NMR (150 MHz, DMSO-*d*<sub>6</sub>) spectrum of compound **9**

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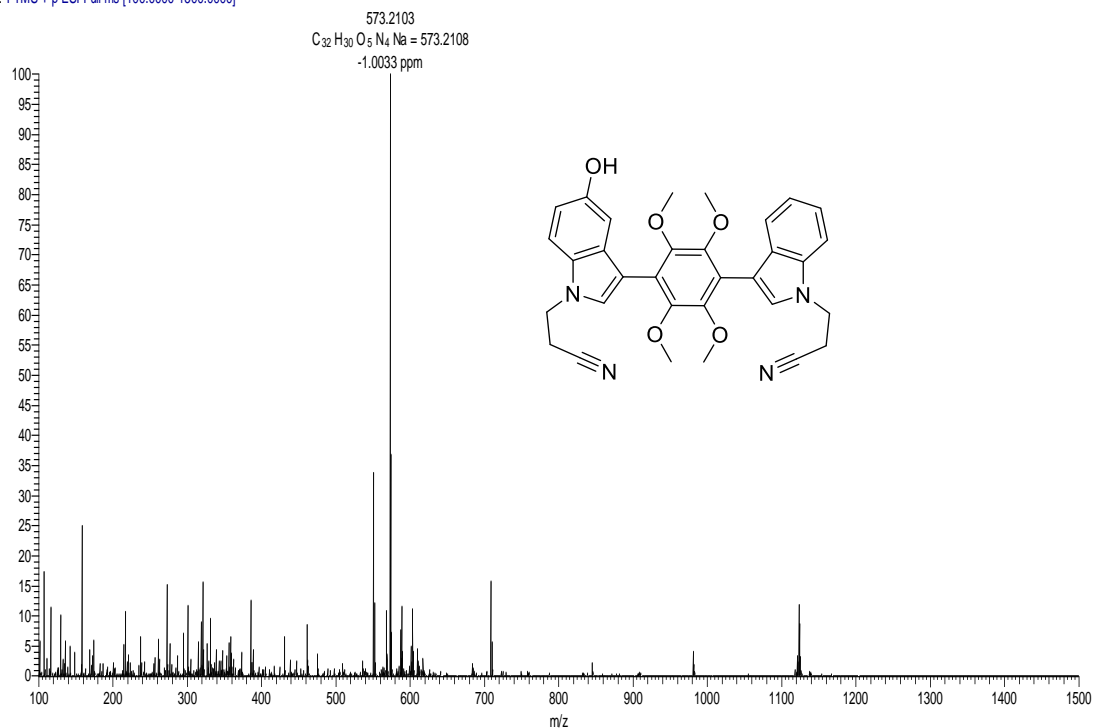


Figure S27: HRESIMS spectrum of compound **9**

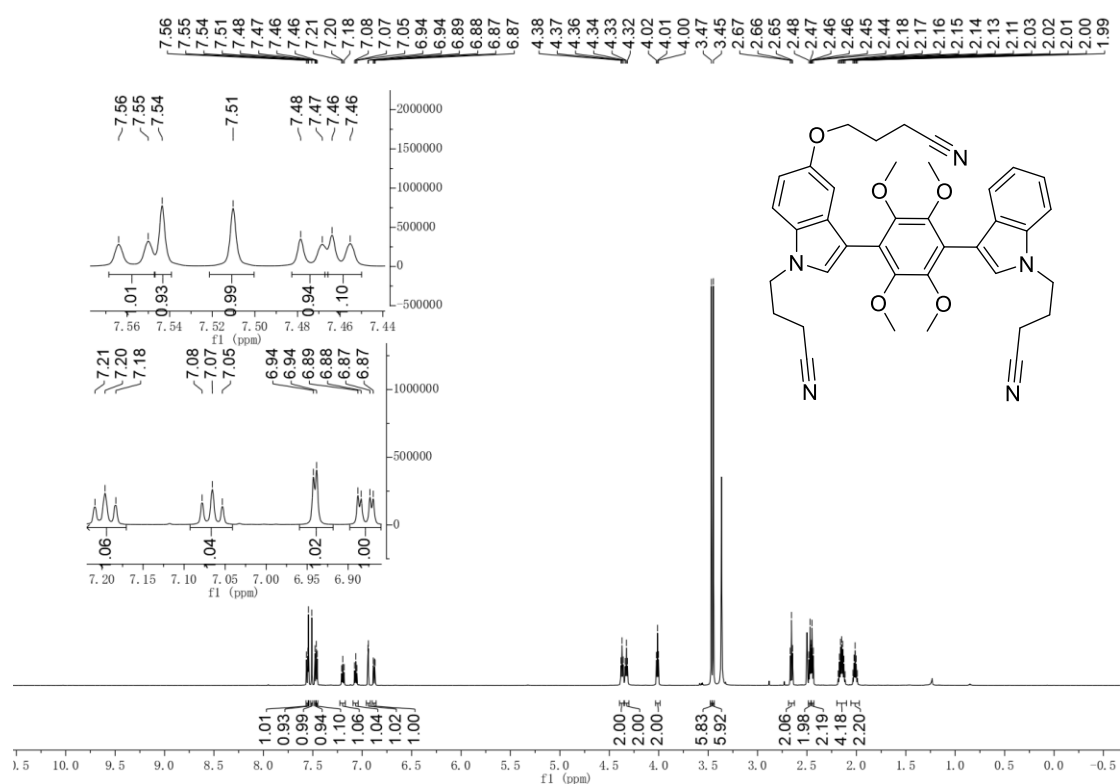
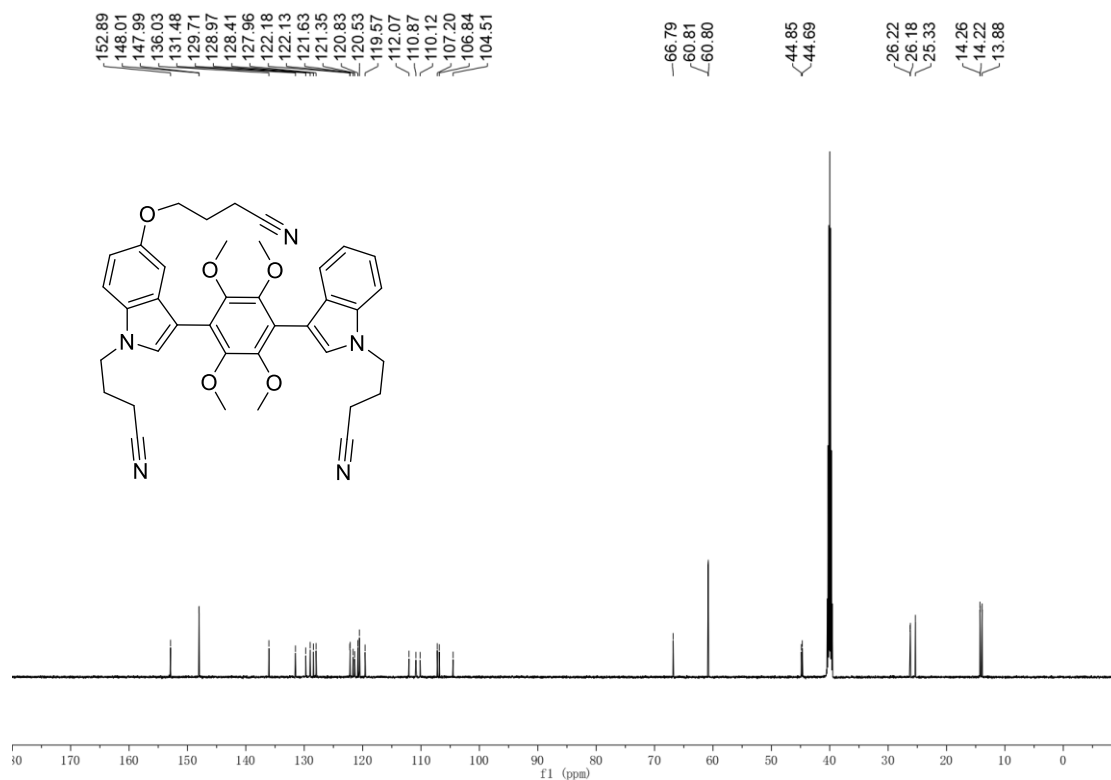


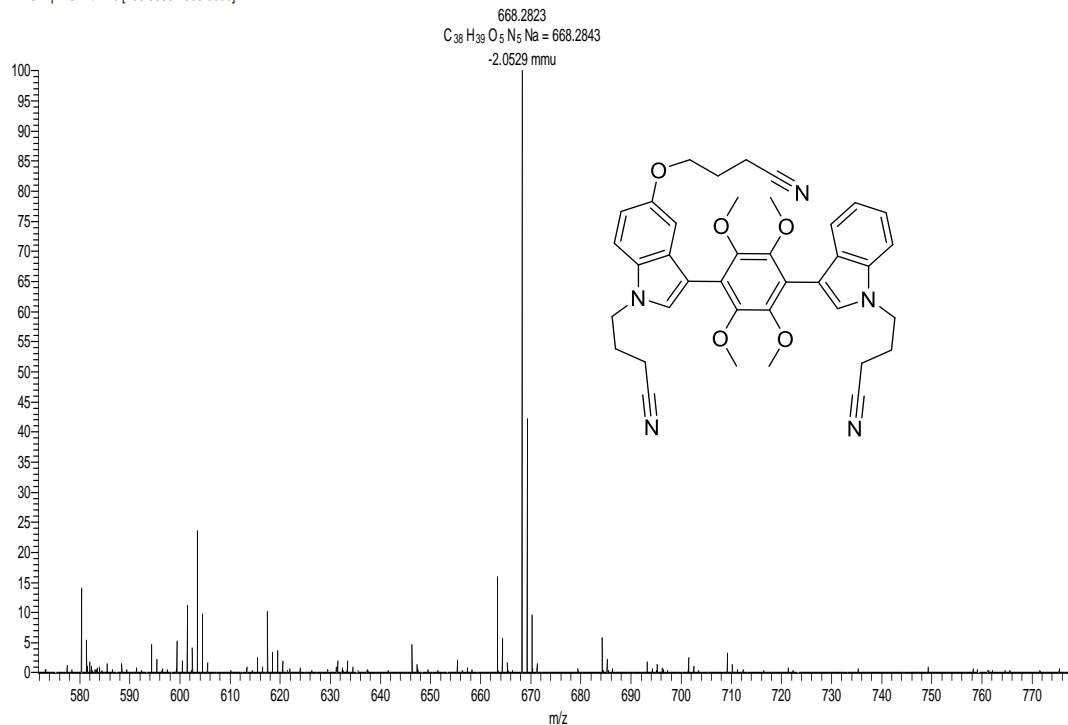
Figure S28: <sup>1</sup>H-NMR (600 MHz, DMSO-*d*<sub>6</sub>) spectrum of compound **10**



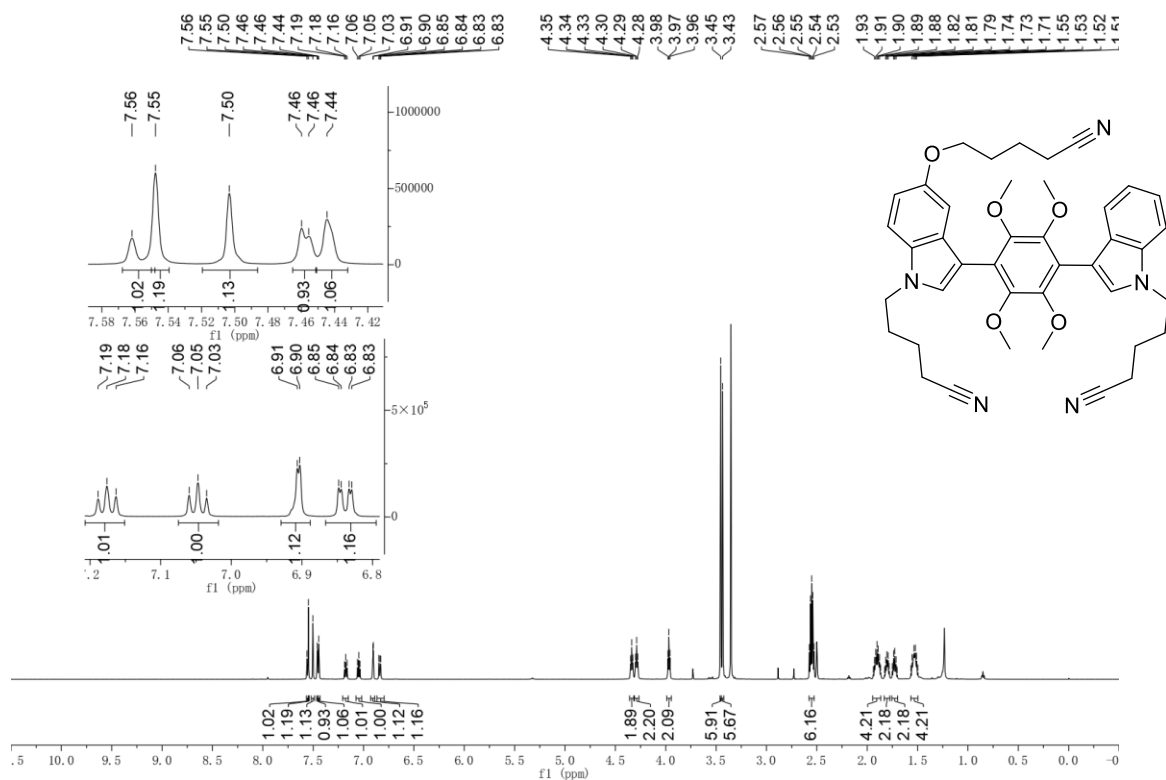


**Figure S29:**  $^{13}\text{C}$ -NMR (150 MHz,  $\text{DMSO}-d_6$ ) spectrum of compound **10**

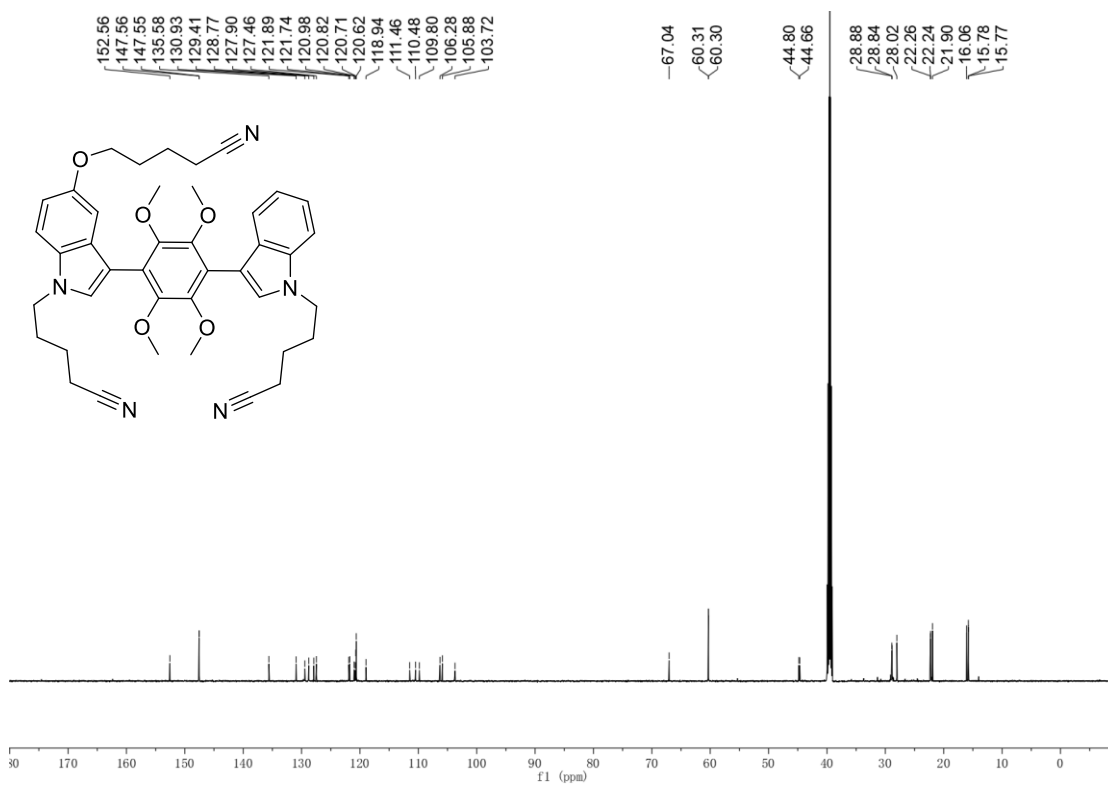
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**Figure S30:** HRESIMS spectrum of compound **10**

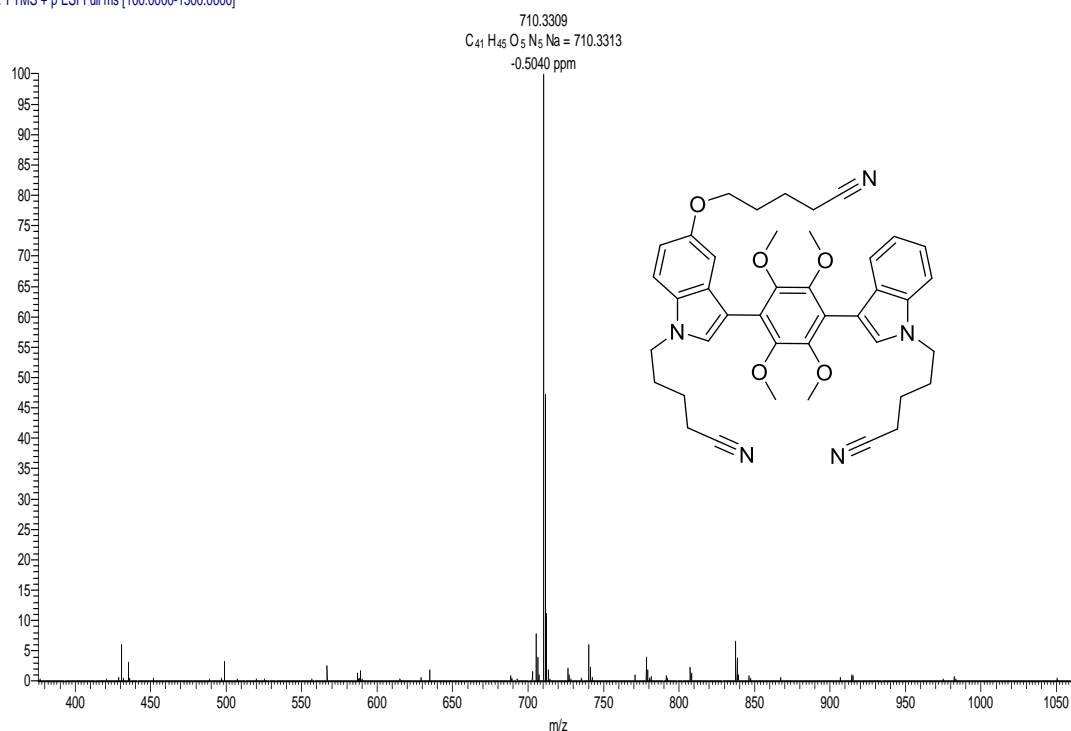


**Figure S31:**  $^1\text{H}$ -NMR (600 MHz,  $\text{DMSO}-d_6$ ) spectrum of compound **11**

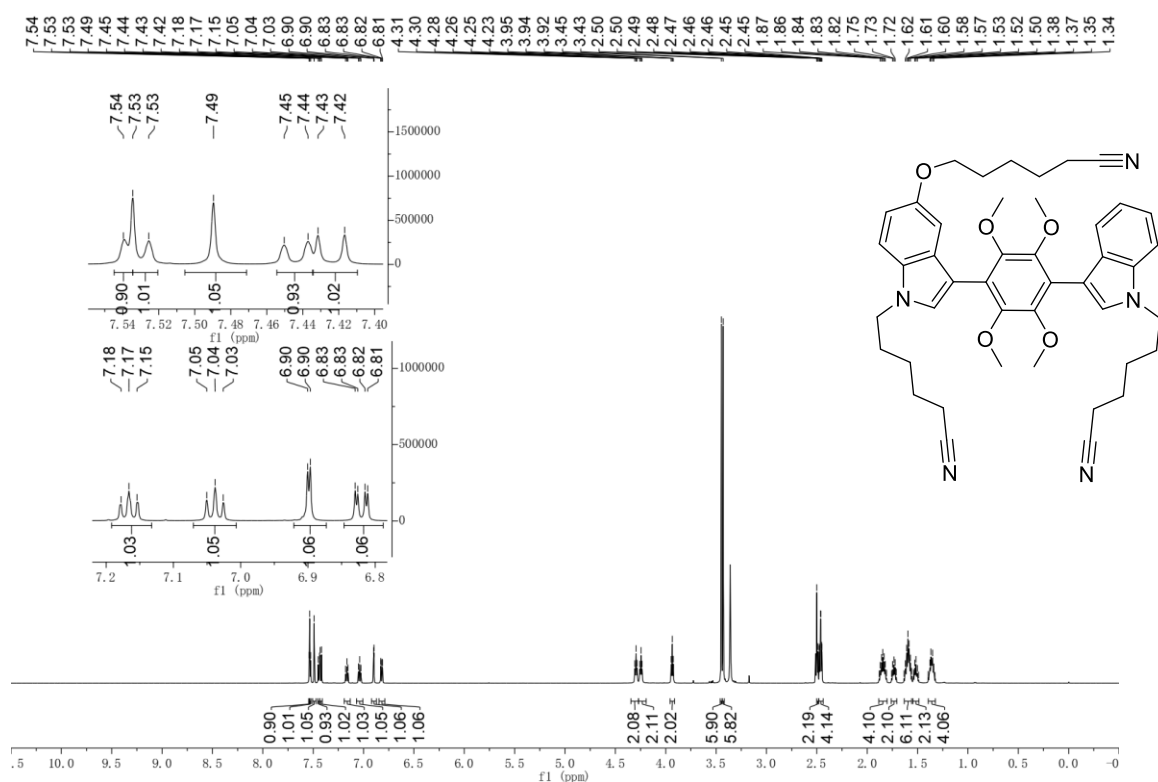


**Figure S32:**  $^{13}\text{C}$ -NMR (150 MHz,  $\text{DMSO}-d_6$ ) spectrum of compound **11**

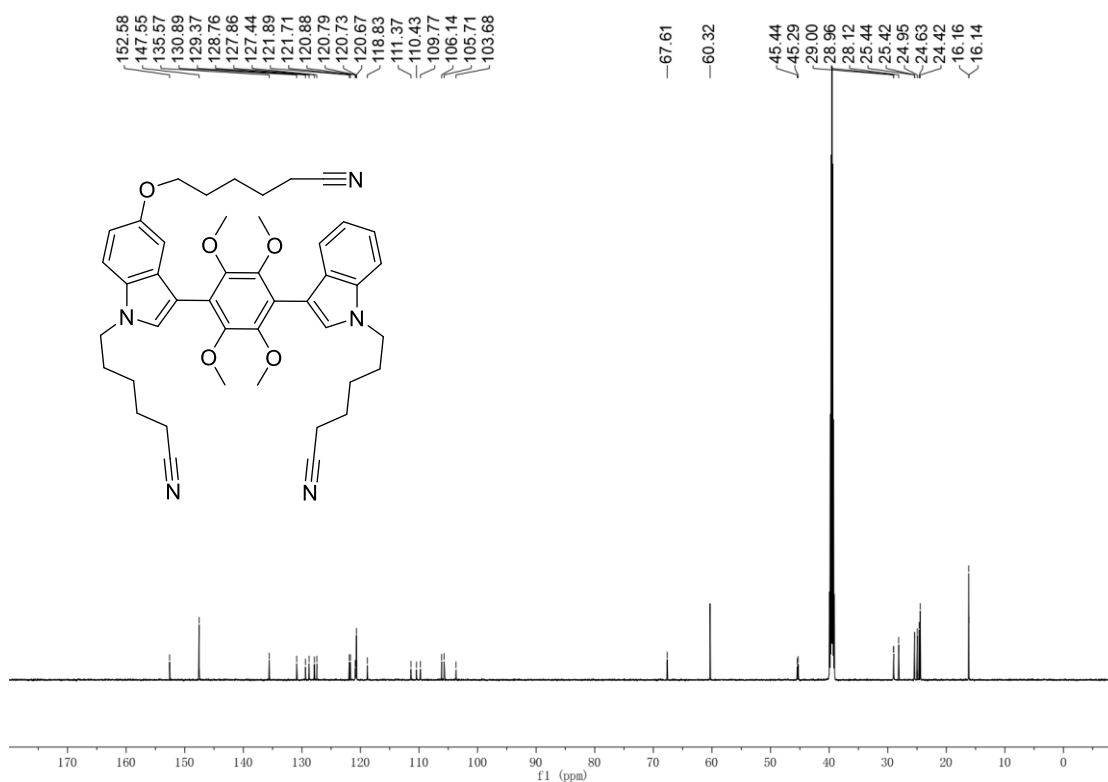
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**Figure S33:** HRESIMS spectrum of compound **11**

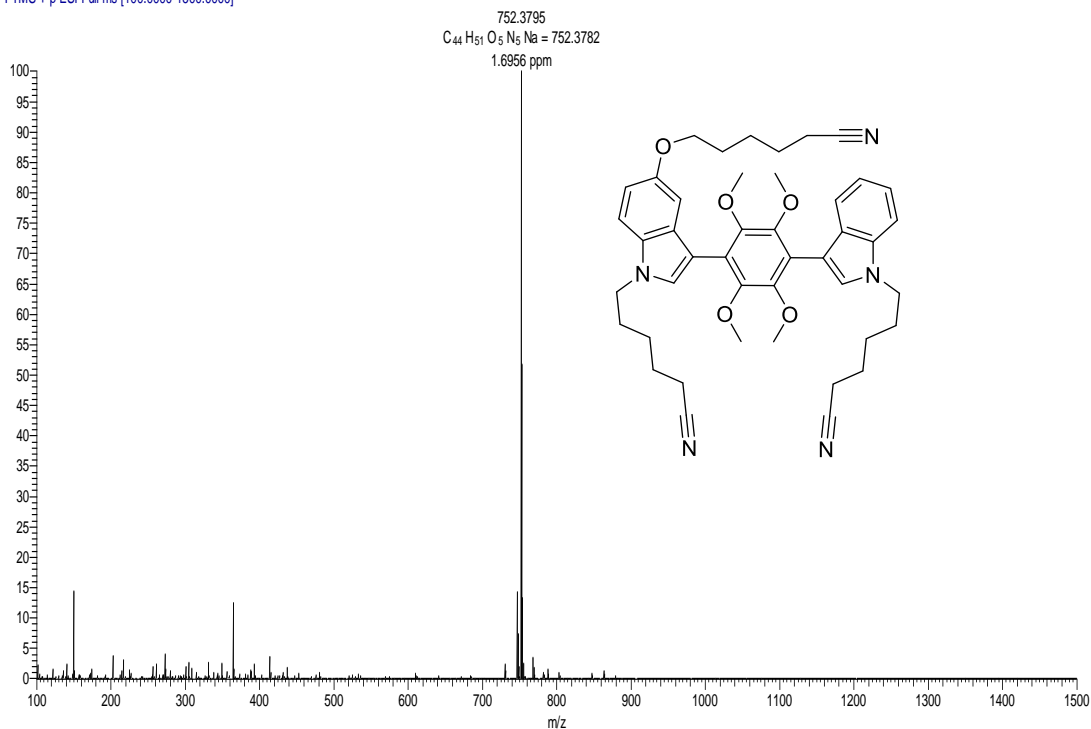


**Figure S34:** <sup>1</sup>H-NMR (600 MHz, DMSO-*d*<sub>6</sub>) spectrum of compound **12**

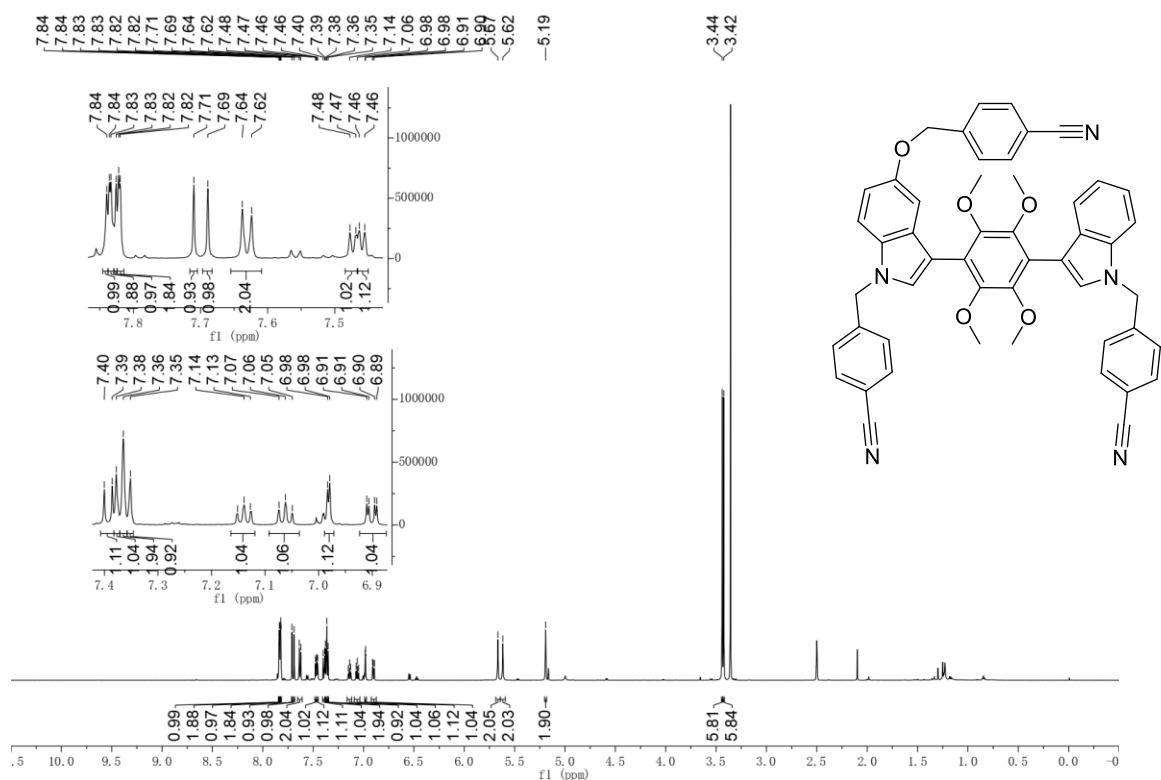


**Figure S35:**  $^{13}\text{C}$ -NMR (150 MHz,  $\text{DMSO}-d_6$ ) spectrum of compound **12**

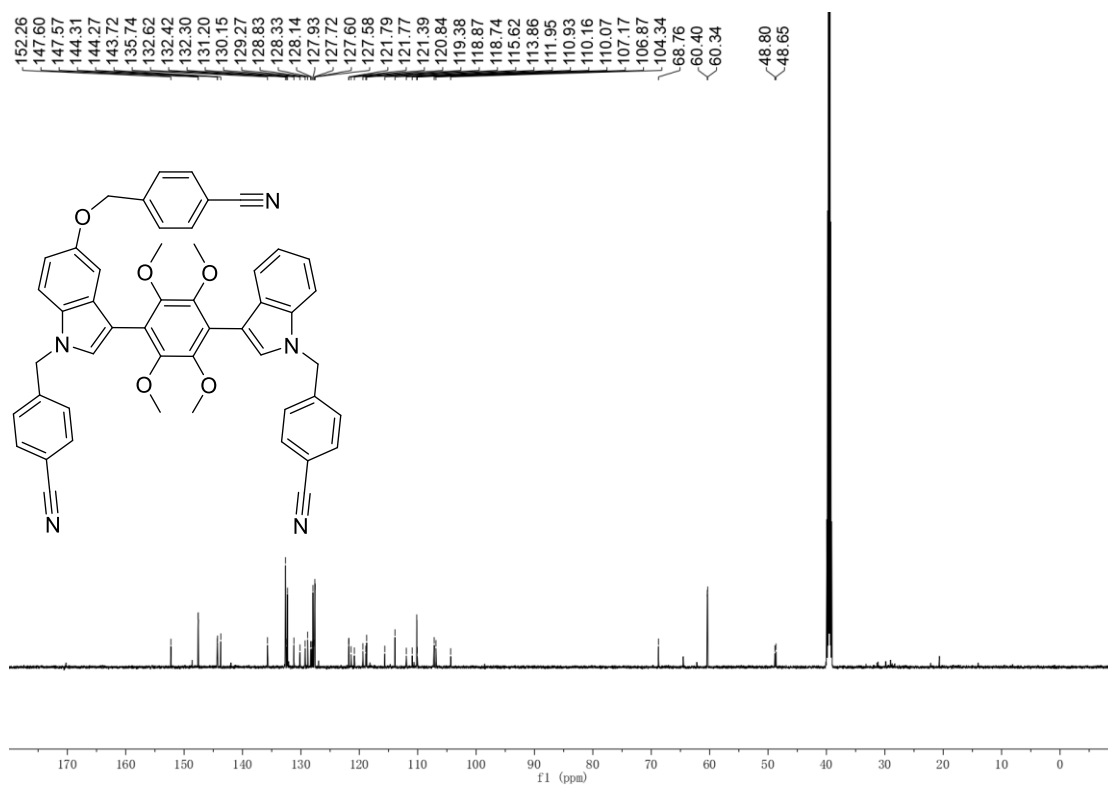
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**Figure S36:** HRESIMS spectrum of compound **12**

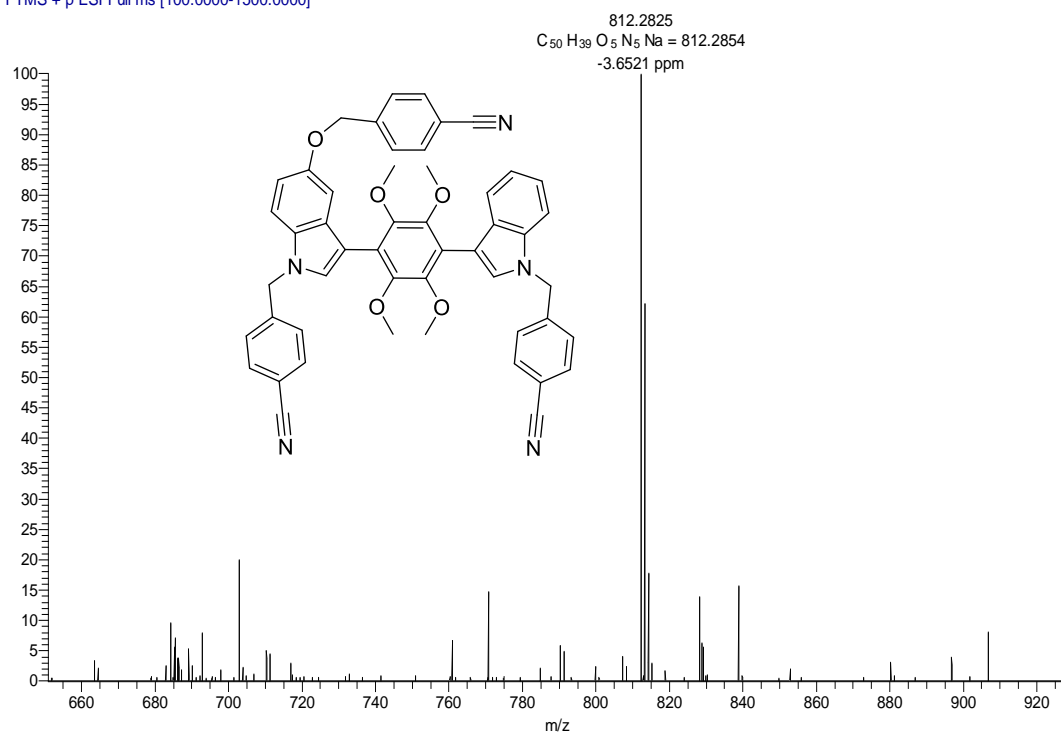


**Figure S37:** <sup>1</sup>H-NMR (600 MHz, DMSO-*d*<sub>6</sub>) spectrum of compound **13**

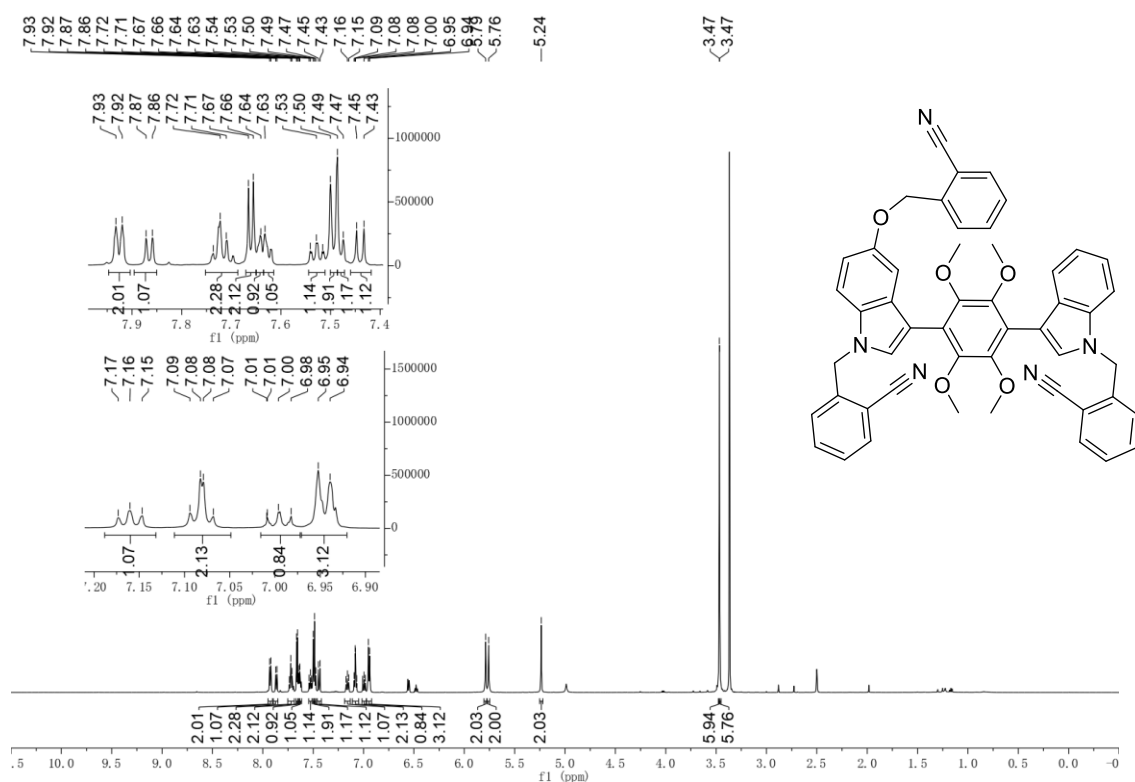


**Figure S38:** <sup>13</sup>C-NMR (150 MHz, DMSO-*d*<sub>6</sub>) spectrum of compound **13**

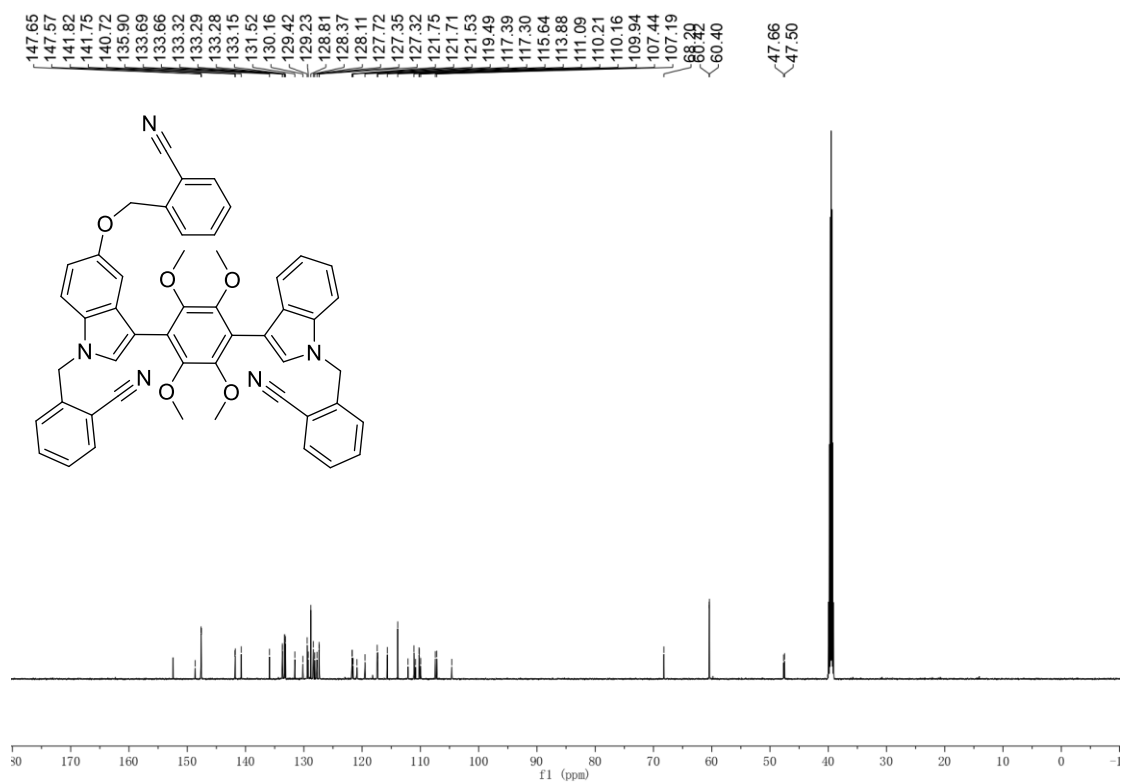
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**Figure S39:** HRESIMS spectrum of compound 13

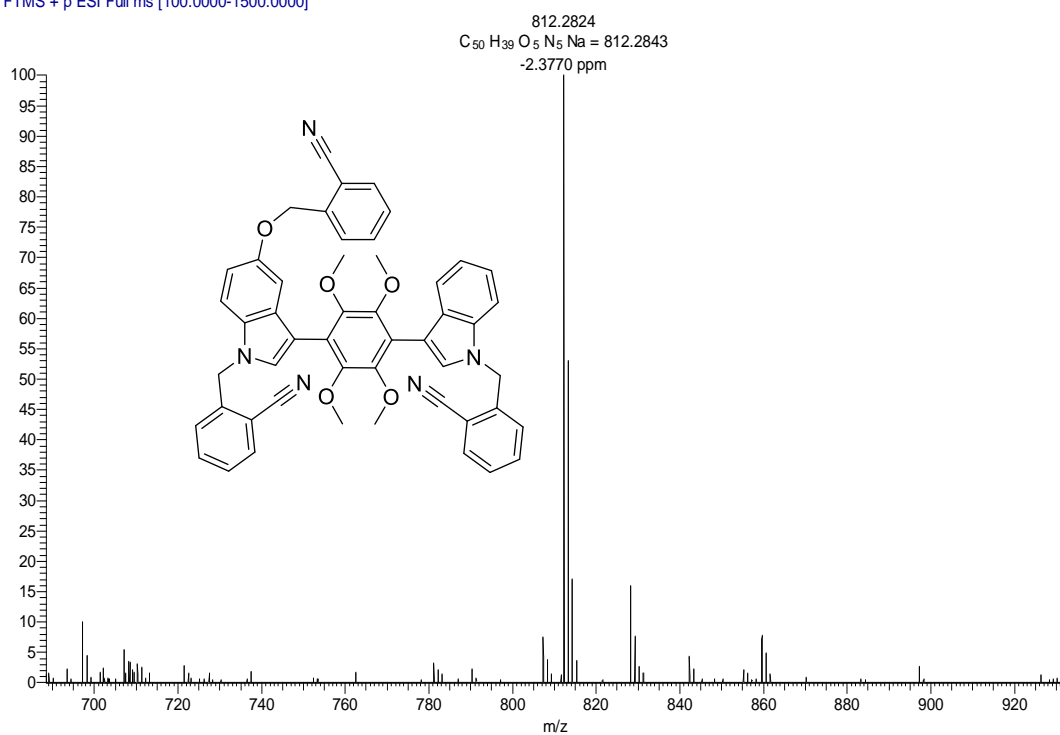


**Figure S40:** <sup>1</sup>H-NMR (600 MHz, DMSO-*d*<sub>6</sub>) spectrum of compound 14

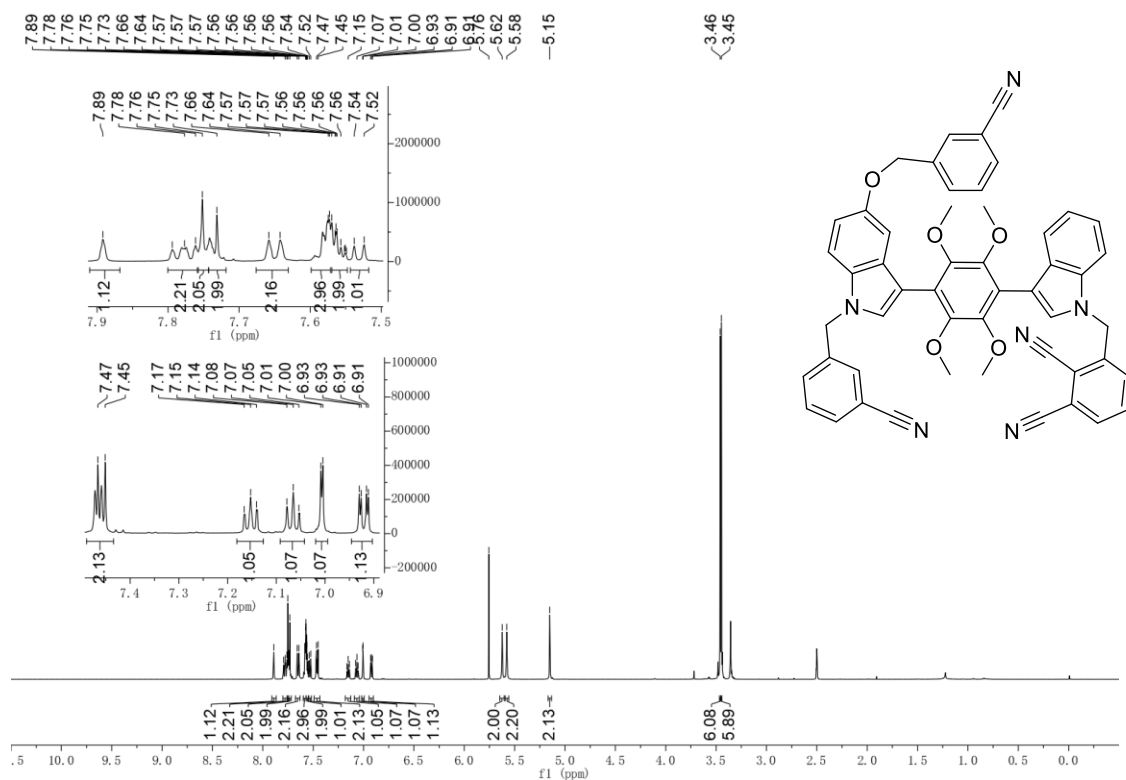


**Figure S41:** <sup>13</sup>C-NMR (150 MHz, DMSO-*d*<sub>6</sub>) spectrum of compound 14

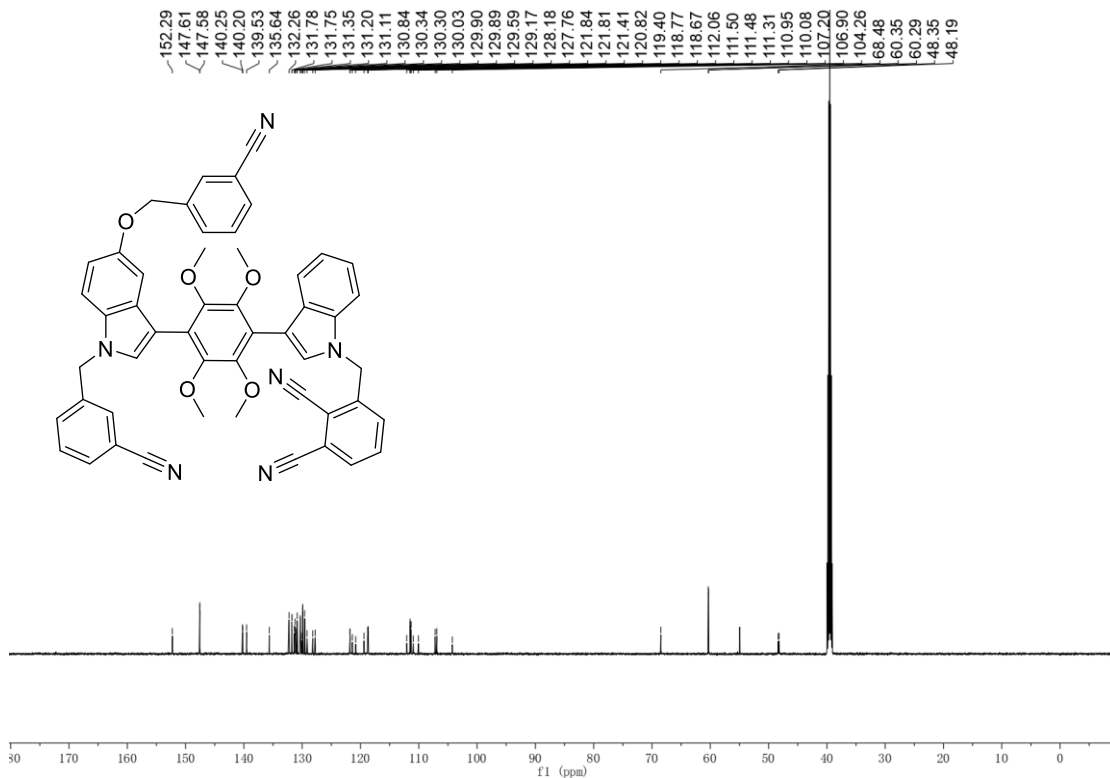
AL-1-88 #19 RT: 0.08 AV: 1 NL: 1.95E6  
T: FTMS + p ESI Full ms [100.0000-1500.0000]



**Figure S42:** HRESIMS spectrum of compound 14



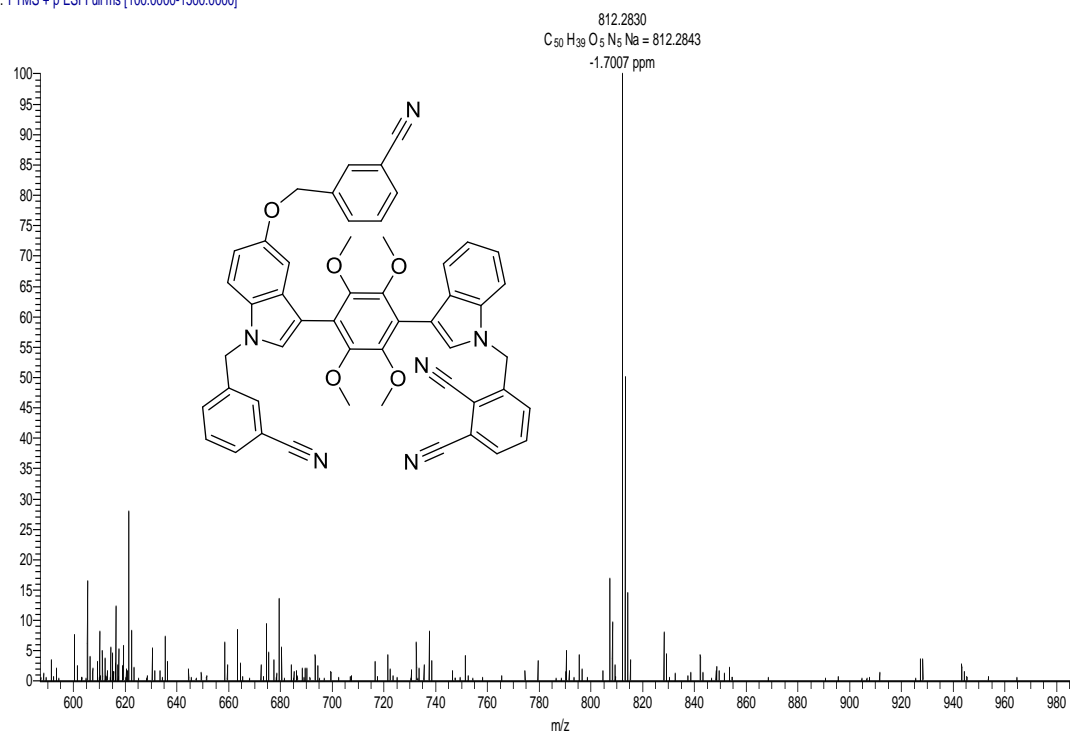
**Figure S43:** <sup>1</sup>H-NMR (600 MHz, DMSO-*d*<sub>6</sub>) spectrum of compound **15**



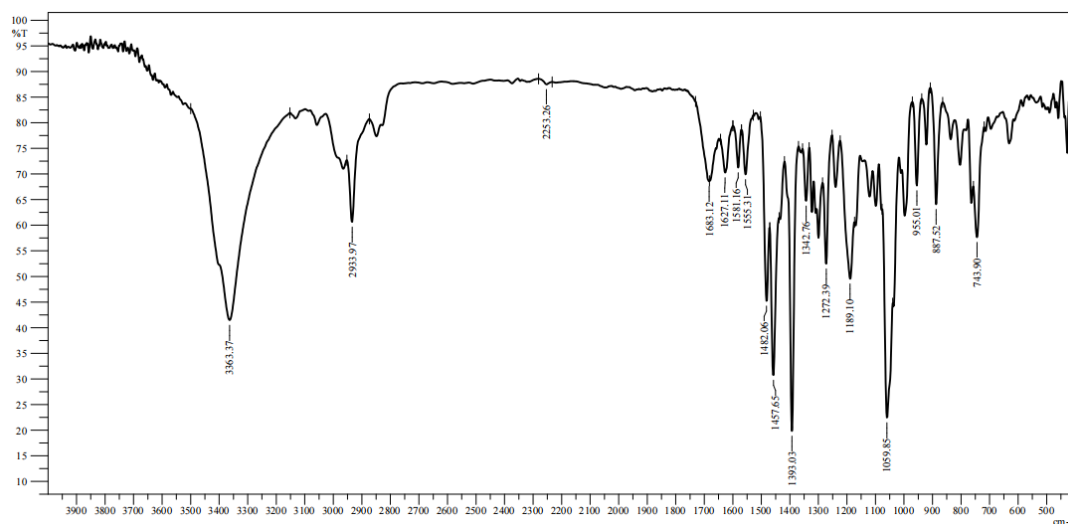
**Figure S44:** <sup>13</sup>C-NMR (150 MHz, DMSO-*d*<sub>6</sub>) spectrum of compound **15**



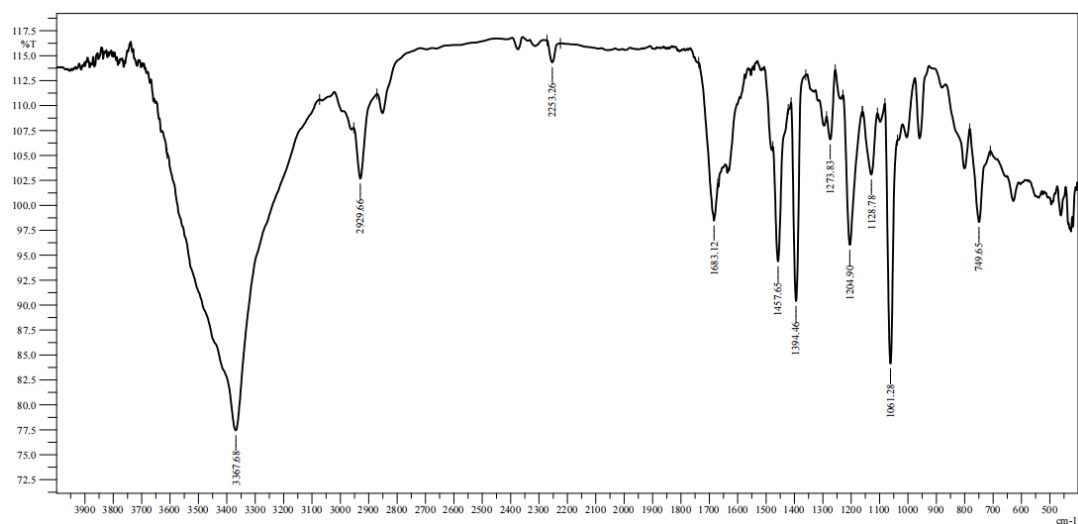
AL-1-91 #19 RT: 0.08 AV: 1 NL: 3.22E7  
T: FTMS + p ESI Full ms [100.0000-1500.0000]



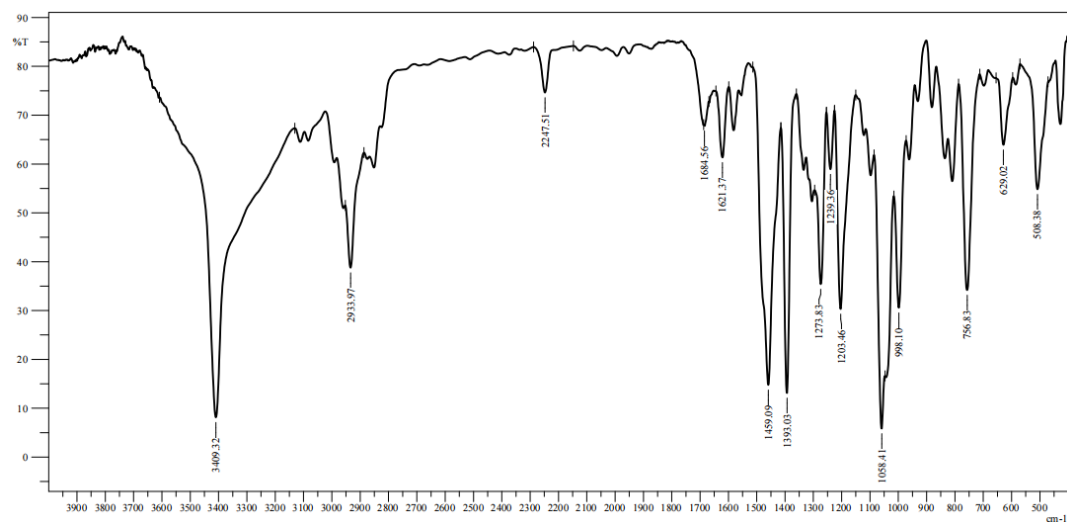
**Figure S45:** HRESIMS spectrum of compound **15**



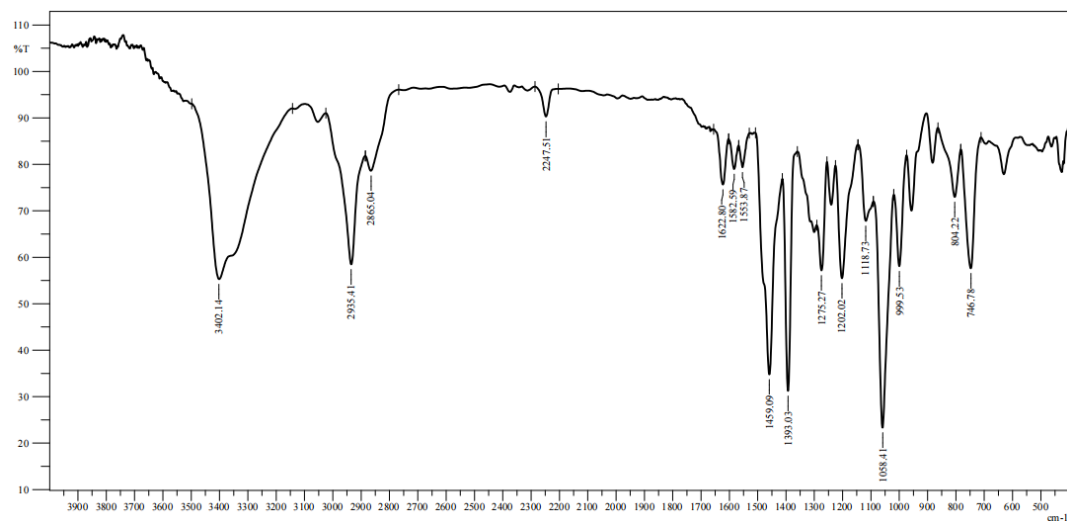
**Figure S46:** Infrared (IR) spectrum of compound **1** (KBr pellet)



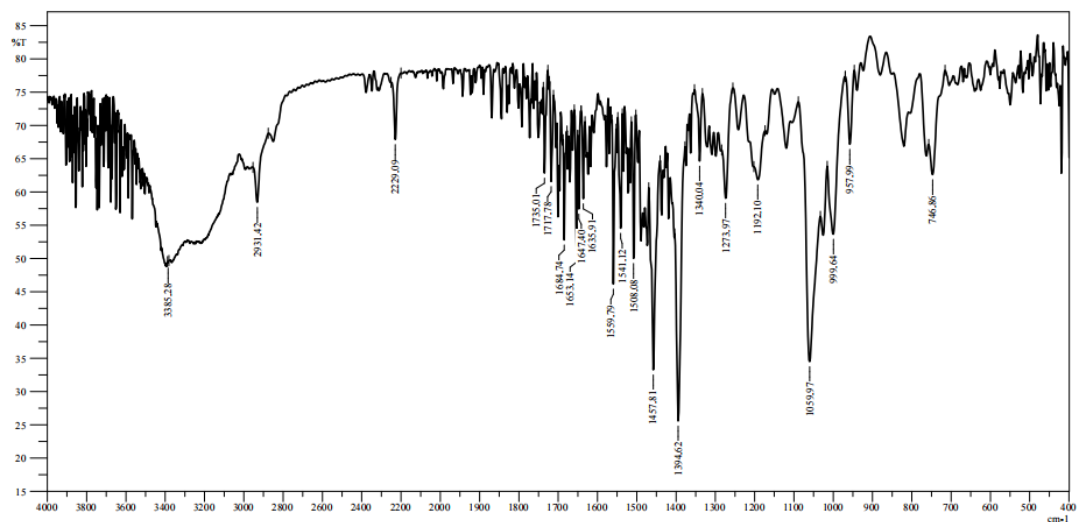
**Figure S47:** Infrared (IR) spectrum of compound **2** (KBr pellet)



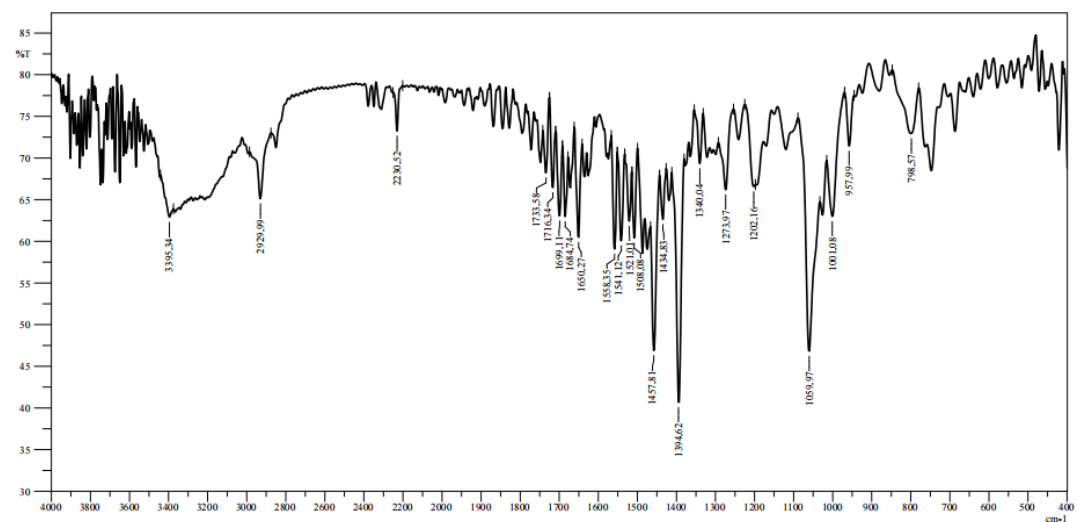
**Figure S48:** Infrared (IR) spectrum of compound **3** (KBr pellet)



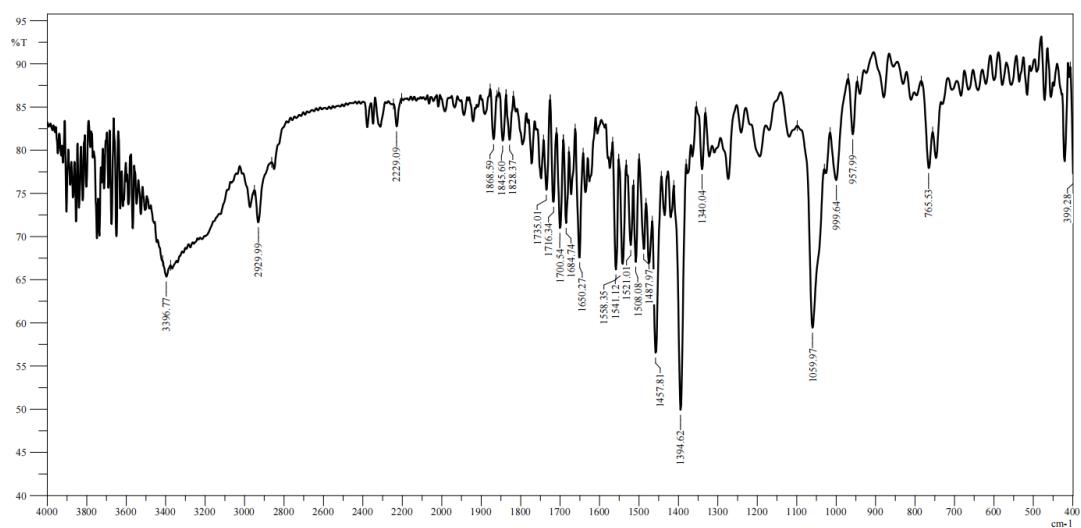
**Figure S49:** Infrared (IR) spectrum of compound **4** (KBr pellet)



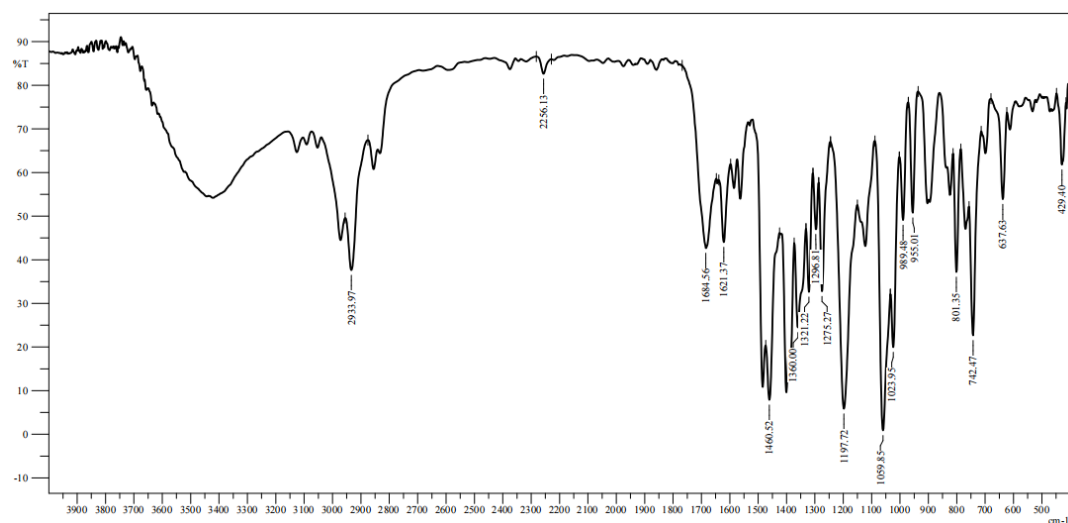
**Figure S50:** Infrared (IR) spectrum of compound **5** (KBr pellet)



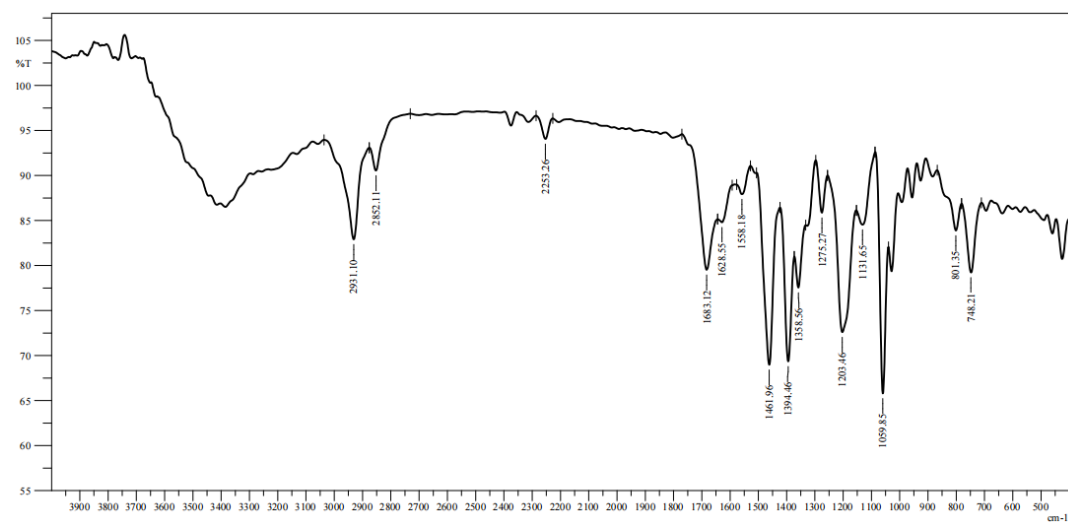
**Figure S51:** Infrared (IR) spectrum of compound **6** (KBr pellet)



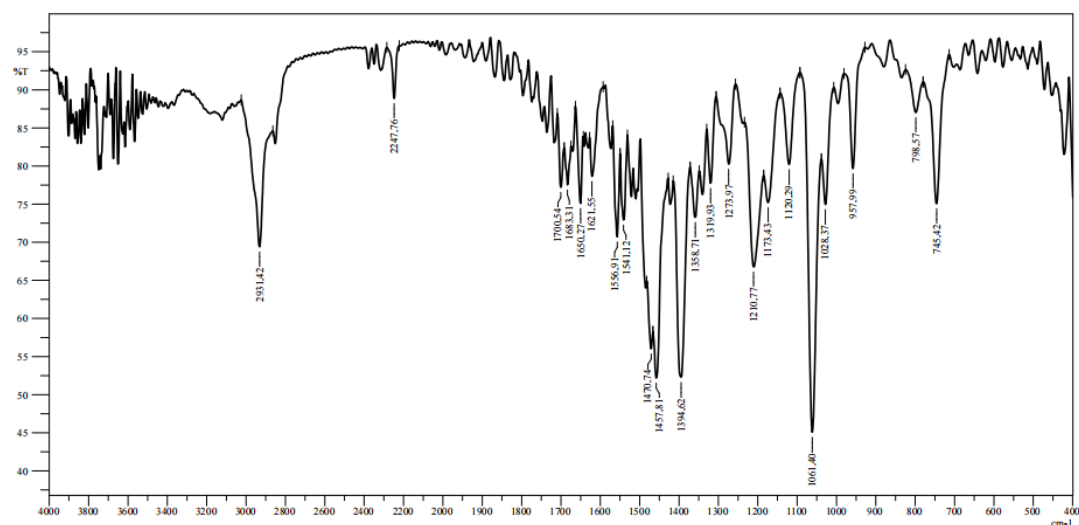
**Figure S52:** Infrared (IR) spectrum of compound **7** (KBr pellet)



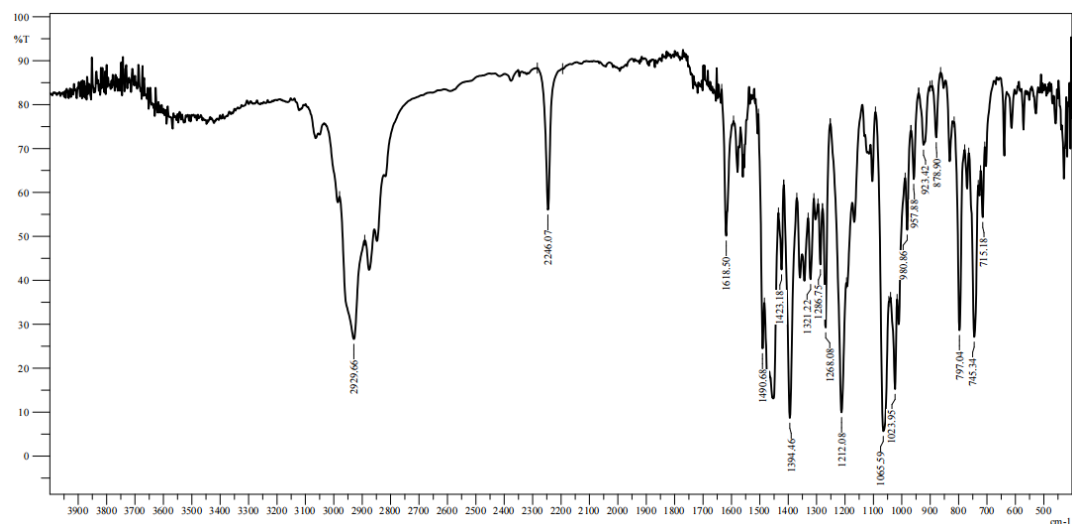
**Figure S53:** Infrared (IR) spectrum of compound **8** (KBr pellet)



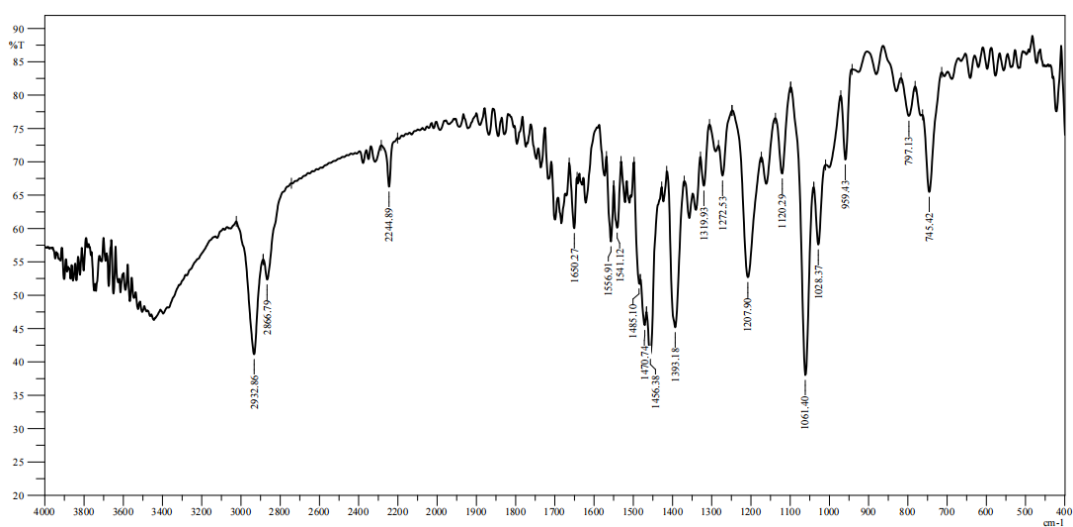
**Figure S54:** Infrared (IR) spectrum of compound **9** (KBr pellet)



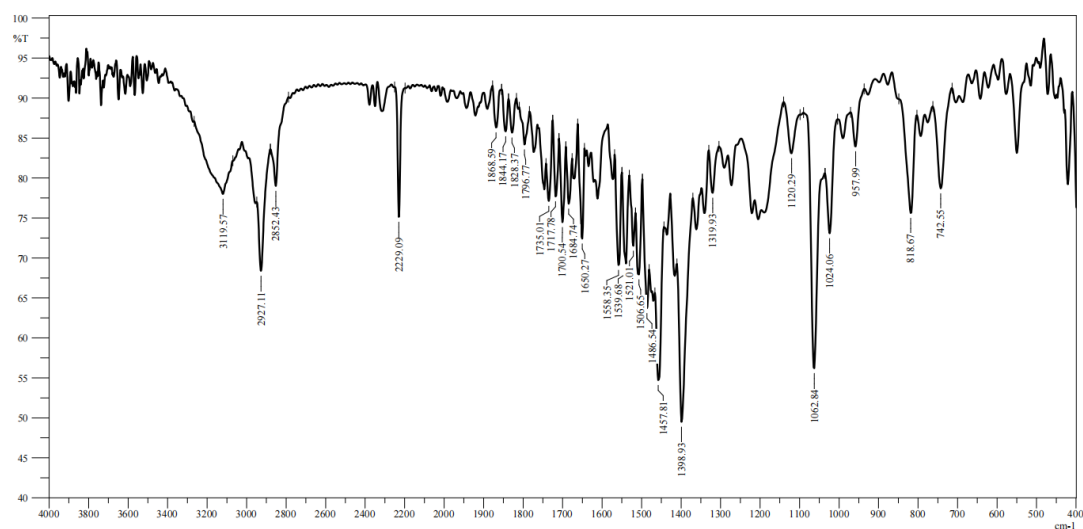
**Figure S55:** Infrared (IR) spectrum of compound **10** (KBr pellet)



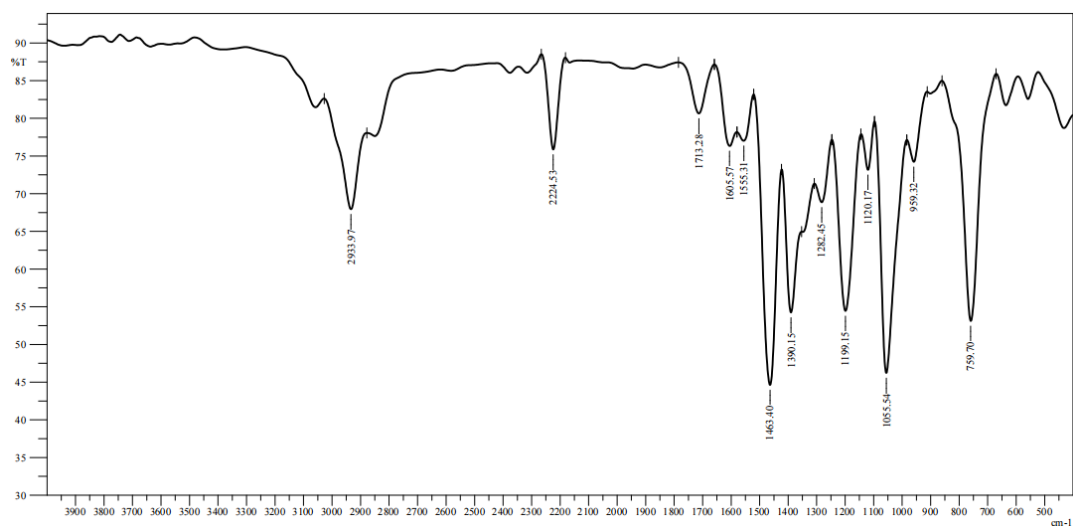
**Figure S56:** Infrared (IR) spectrum of compound **11** (KBr pellet)



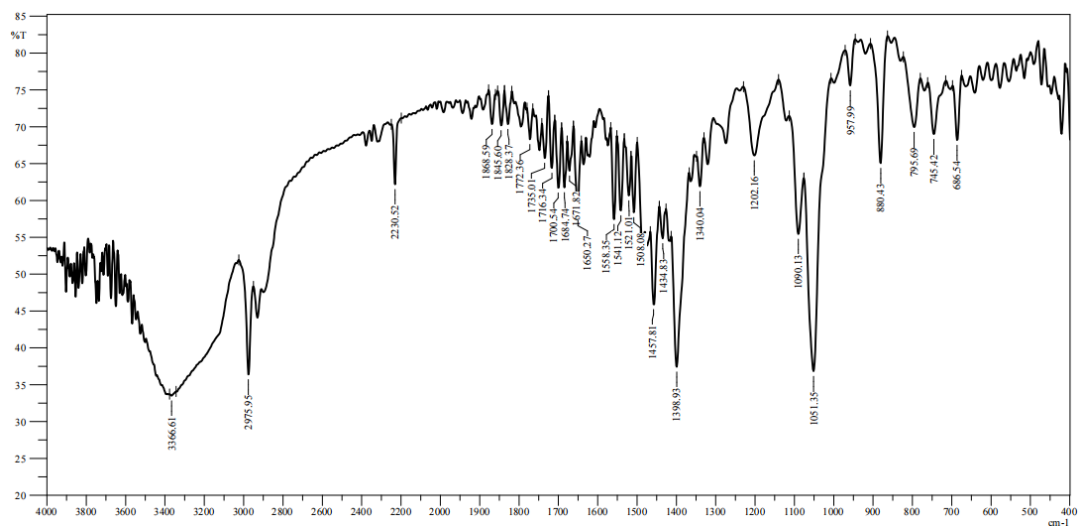
**Figure S57:** Infrared (IR) spectrum of compound **12** (KBr pellet)



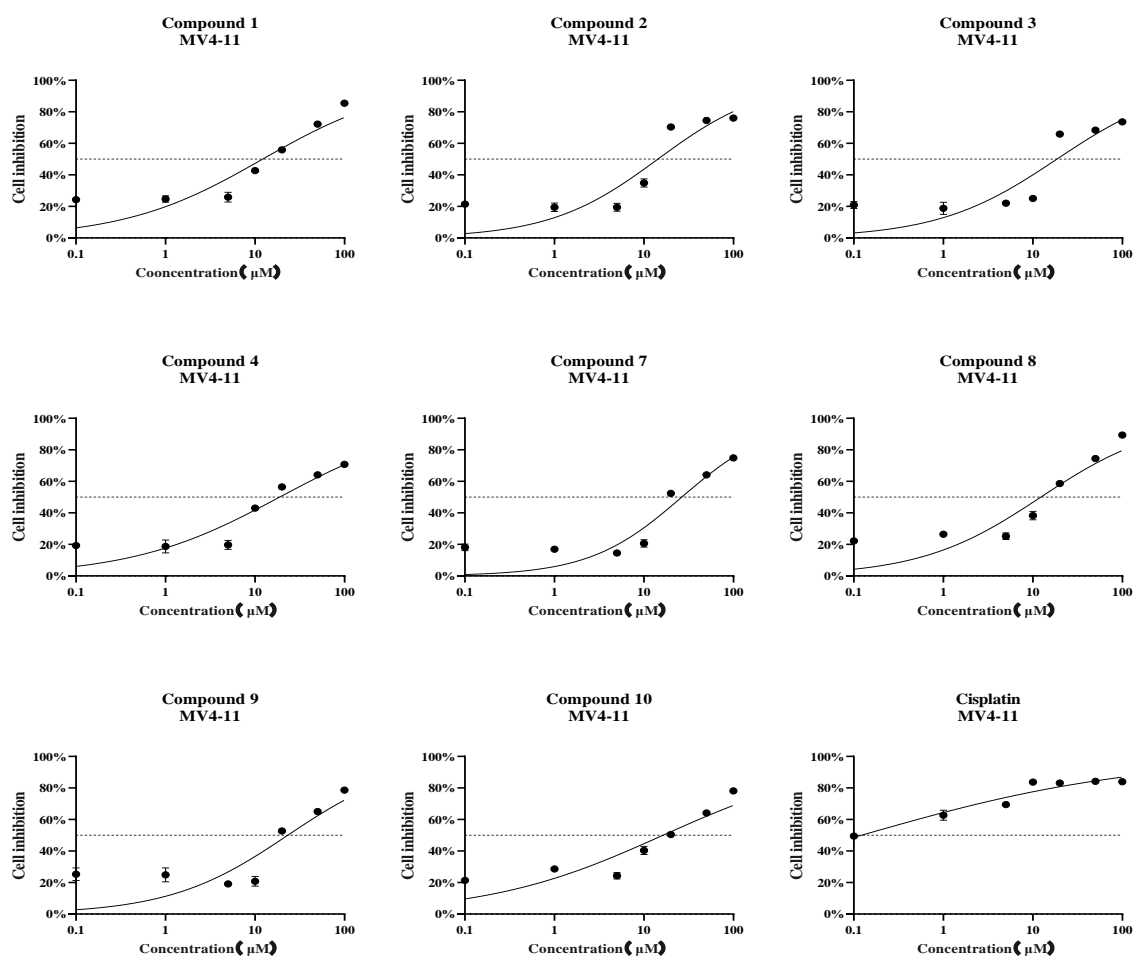
**Figure S58:** Infrared (IR) spectrum of compound **13** (KBr pellet)



**Figure S59:** Infrared (IR) spectrum of compound **14** (KBr pellet)



**Figure S60:** Infrared (IR) spectrum of compound **15** (KBr pellet)



**Figure S61: Dose-response curves of IC<sub>50</sub> for compounds 1-4, 6-10  
and positive control Cisplatin**

## General

Optical rotations, NMR spectra, ESIMS spectra, and HRESIMS spectra were measured on an AUTOPOL1 polarimeter, Bruker 600 MHz (TMS was used as an internal standard), Waters Xevo TQS, and Agilent Technologies 6530 Accurate-Mass Q-TOF LC/MS, respectively. Semipreparative HPLC separation was performed on a Hitachi Primaide with an ODS column (*YMC-pack ODS-A*, 10 × 250 mm, 5 μm, 4 mL/min). Synthetic compounds were also purified using a SepaBean machine equipped with SepaFlash columns (Santai Technologies Inc.). Column chromatography was performed on silica gel (200-300 mesh; Qingdao Puke Parting Materials Co., Ltd.) and Sephadex LH-20 (Amersham Biosciences).

## Apparatus and Reagents

UV absorbances were measured on a Beckman DU 640 spectrophotometer. IR spectra were measured as a thin film on a KBr disk using an Anton Paar MCP5100 spectrophotometer. <sup>1</sup>H, <sup>13</sup>C NMR spectra were carried out on a Bruker-600MHz spectrometer with tetramethylsilane as the internal standard. HRESIMS data were obtained using a Waters Xevo TQS and Agilent Technologies 6530 Accurate-Mass Q-TOF LC/MS. A binary gradient Hitachi Primaide HPLC system with an evaporative light scattering detector and a 1430 diode array detector were used for purifications with an ODS column (*YMC-Pack ODS-A*, 5 μm, 250 × 10 mm). TLC was performed on plates precoated with silica gel GF<sub>254</sub> (10 - 40 μm).

## Cytotoxic activity<sup>s1</sup>

The cell line sources and ATCC codes: Human myelomonocytic leukemia cell line MV4-11 (ATCC; CRL-9591), human chronic myelogenous leukemia cell line K562 (ATCC; CCL-243), human acute promyelocytic leukemia cell line HL-60 (ATCC; CCL-240), and human embryonic kidney cell line 293T (ATCC; CRL-3216).

The Cytotoxic activity of compounds was evaluated by CCK-8 method. The samples were dissolved in the cell grade DMSO after accurately weighing to generate the 10 mM drug solution. The drug solution was further diluted to tenfold detection concentration by cell culture medium. Inoculate cells: The cells were prepared into single cell suspension with the culture medium containing 10% fetal bovine serum, and the 96 well plates were inoculated with 90 μL cell culture medium (Adherent cell viewed 5×10<sup>4</sup>/mL and Suspension cell viewed 9×10<sup>4</sup>/mL) per well, then cultured at 5% CO<sub>2</sub> and 37 °C for 24 hours. Add the sample solution to be tested: Add 10μL sample solution to each well. One concentration was set for each sample during preliminary screening and three multiple holes were set for each concentration. Seven concentration gradients (100, 50, 20, 10, 5, 1 and 0.1 μM) were set for each sample for IC<sub>50</sub> determination and three multiple holes were set for each concentration. The 96 well plates were cultured at 5% CO<sub>2</sub> and 37 °C for 48 hours. The experiment was divided into blank group, control group and drug group. Color development: The old culture medium and drug solution of adherent cells was sucked out, then 100μL of CCK-8 solution (diluted ten times with the basic medium) was added and the suspension cells was directly added 10μL of CCK-8 stock solution. Culture at 37 °C with 5% CO<sub>2</sub> for 1-4 h (dark operation, real-time observation). Result detection: The absorbance was measured at 450nm with an enzyme labeling instrument and the original data and results were recorded. The toxicity is expressed by cell inhibition, and the calculation formula is as follow:

Cell inhibition (%) = (ODControl-ODDrug)/(ODControl-ODBlank)×100%. The IC<sub>50</sub> was calculated by software graphpad prism 8 (version 8.0.2, from GraphPad Software Inc), and the experimental results are expressed in ± SD. Positive control: Cisplatin.

## Ant-*Mycobacterium tuberculosis*<sup>s2</sup>

The MIC values were determined using a modified microdilution method in 96-well U-bottom plates. *Mycobacterium tuberculosis* (Mtb) cultures in logarithmic growth phase were adjusted to 1×10<sup>7</sup> CFU/mL using 7H9-OADC broth. Sterile distilled water (200 μL) was added to all peripheral wells to minimize evaporation. For drug dilution, 198 μL of bacterial suspension was dispensed into



column 2 (B2-G2), followed by 100  $\mu$ L aliquots in subsequent wells (columns 3-10). 2  $\mu$ L of test compound was added to column 2 and mixed thoroughly. Serial two-fold dilutions were performed by transferring 100  $\mu$ L sequentially from column 2 to column 10, with the final 100  $\mu$ L being discarded from column 10. Column 11 (B11-G11) served as growth control without antimicrobial agent. Plates were incubated statically at 37°C for 12 days. MIC were determined as the lowest drug concentration preventing visible bacterial pellet formation as assessed by direct visual inspection under ambient light.

#### References:

- [s1] Tominaga, H.; Ishiyama, M.; Ohseto, F.; Sasamoto, K.; Hamamoto, T.; Suzuki, K.; Watanabe, M. A water-soluble tetrazolium salt useful for colorimetric cell viability assay. *Anal. Commun.* **1999**, 36, 47-50.
- [s2] Wiegand, I.; Hilpert, K.; Hancock, R. E. W. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nat. Protoc.* **2008**, 3, 163-175.