

## Electronic Supplementary Information (ESI):

### **A Real Time Bioimaging for Mitochondria by Taking Aggregating Process of Aggregation-Induced Emission Near-Infrared Dyes with Wash-Free Staining**

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## 1. Experimental

### 1.1 Materials

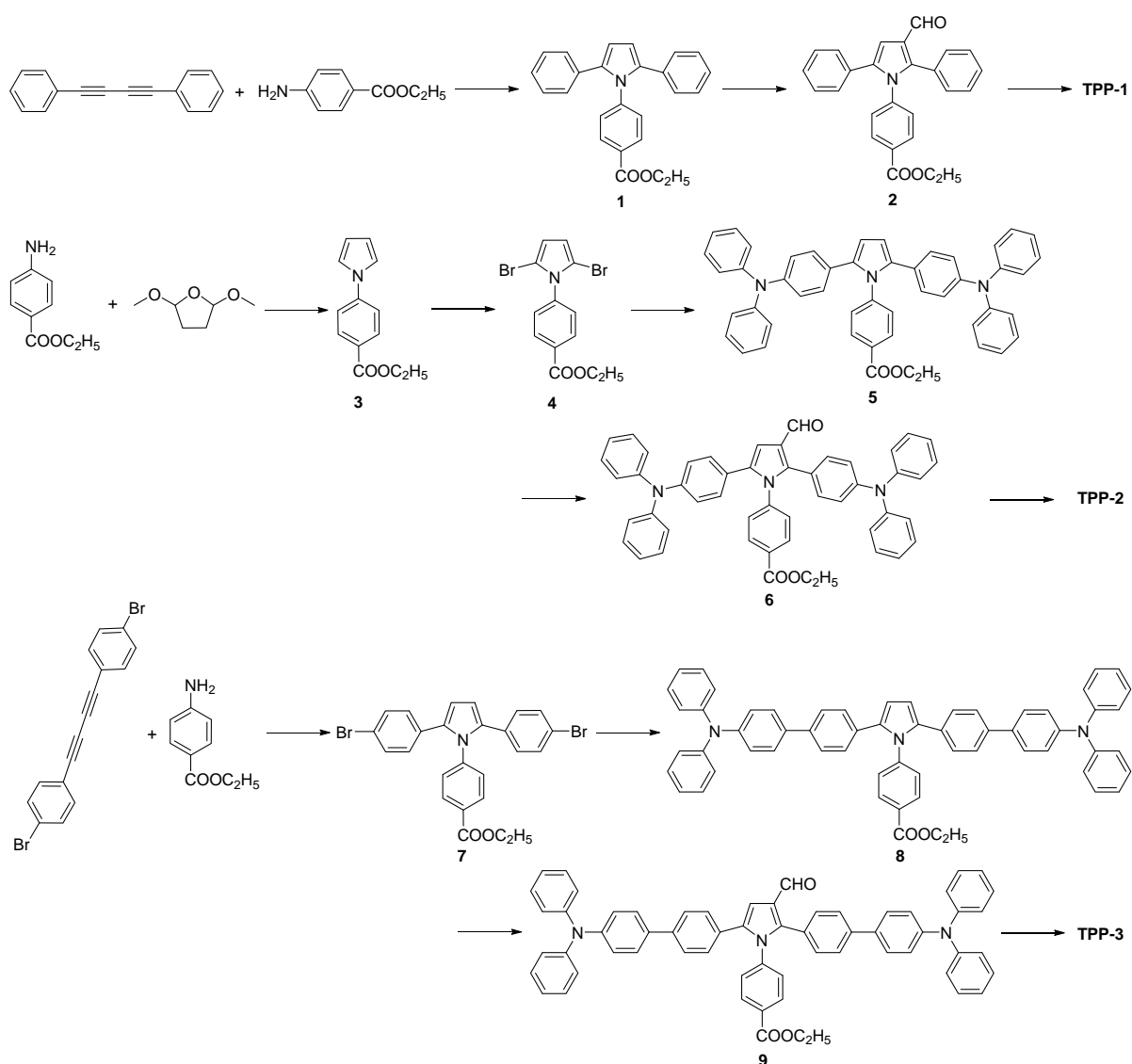
These compounds were obtained by conventional synthetic routes, as shown in Schemes S1~S12. All chemicals were used by commercial suppliers without further purification unless otherwise stated. CuCl, Pd(PPh<sub>3</sub>)<sub>4</sub> and ethyl 4-aminobenzoate were bought from J&K. POCl<sub>3</sub> was bought from Xiya Reagent. 2, 5-dimethoxytetrahydrofuran, (4-(diphenylamino)phenyl)boronic acid, and 1-bromopyrrolidine-2, 5-dione were bought from Energy Chemical. KPF<sub>6</sub> was bought from Aladdin. Chloroform-*d* and DMSO-*d*<sub>6</sub> were bought from Innochem. DMEM, PBS and trypsin were purchased from Coring Co., Ltd. MitoTracker Green, MitoTracker Red and Annexin V-FITC/PI Apoptosis Detection Kit were purchased from Beyotime technologies Co., Ltd. The deionized water was used for all experiments.

### 1.2 Equipments

<sup>1</sup>H and <sup>13</sup>C NMR spectra were measured on a Bruker AV 400 spectrometer. Mass spectra were collected by using a Finnigan BIFLEX III mass spectrometer. UV-Vis spectra were recorded on a TU-1901 double beam UV-Vis spectrophotometer. Fluorescence spectra were measured on a Hitachi F-7000 fluorescence spectrophotometer. The size of aggregates was measured by a Malvern ZEN3600 Zetasizer. PL quantum yields were measured by using an integrating sphere on Nanolog FL3-2iHR fluorescence spectrometer (Horiba Jobinyvon) and PL time-resolved decays were measured by Deltaflex ultrafast lifetime spectrofluorometer (Horiba Jobinyvon). Morphologies of the aggregates were imaged on a Hitachi S-4800

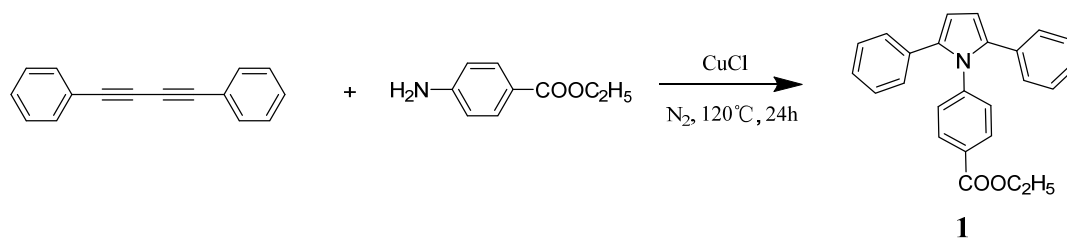
scanning electron microscope (SEM) operating at an accelerating voltage of 5 kV. The fluorescence imaging in living cell was recorded with a Leica TCS SP5 laser scanning confocal microscope. Cell apoptosis experiments were conducted with a Beckman FC500MCL flow cytometer.

### 1.3 Synthesis



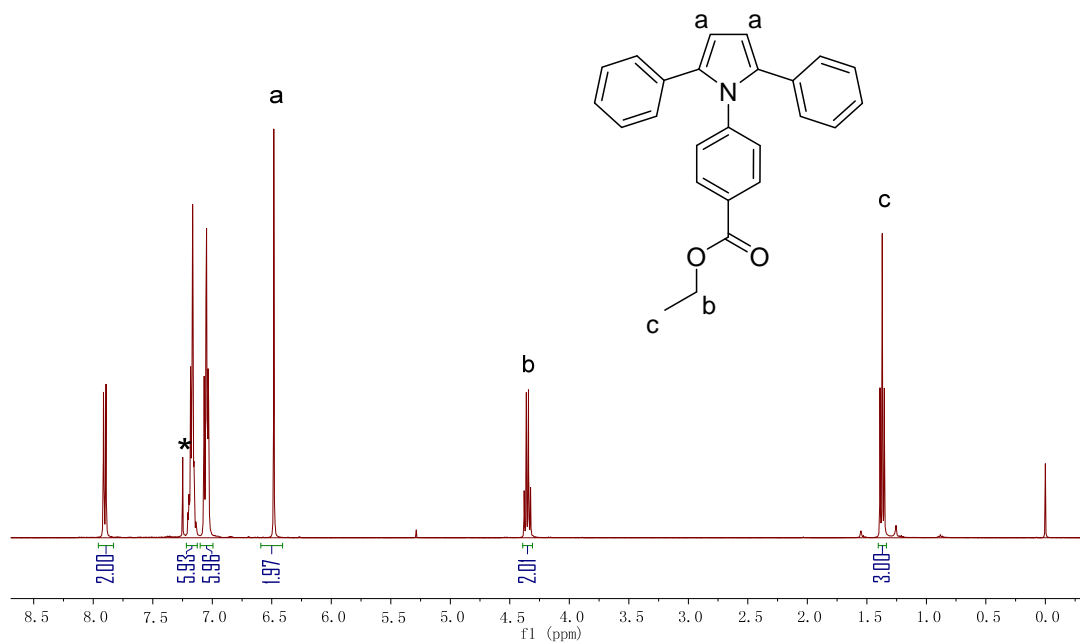
**Scheme S1.** Synthesis routes for TPP-1, TPP-2, and TPP-3

### 1.3.1 Preparation of TPP-1.

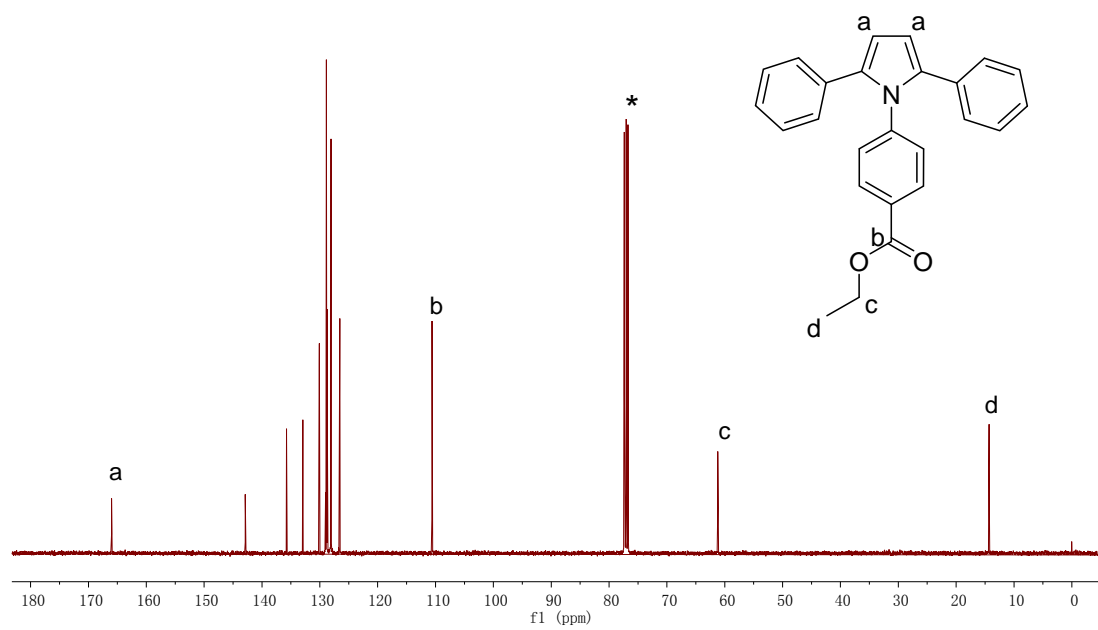


**Scheme S2.** The synthetic route to compound **1**

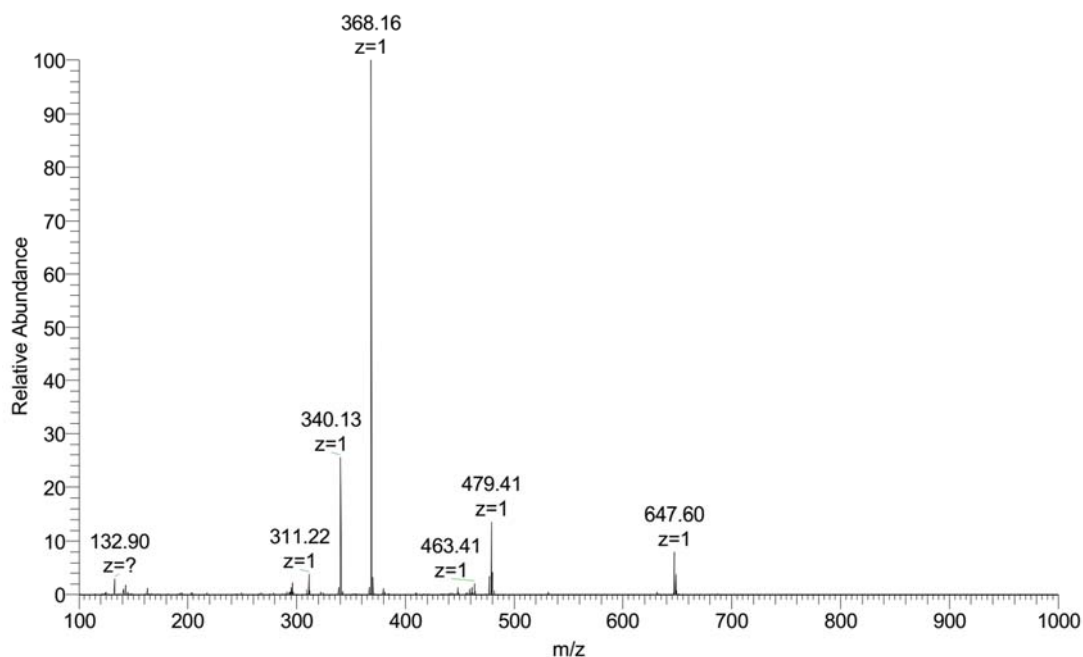
1,2-diphenylethynediene<sup>[1]</sup> (2.0104 g, 10.04 mmol), ethyl *p*-aminobenzoate (6.6340 g, 40.16 mmol) and CuCl (0.0983 g, 1.00 mmol) were added into a 100 ml polymerization tube reacted at 120 °C for 24 h under nitrogen. After the reaction was completed, the mixture was dissolved in dichloromethane, then washed three times with water and dilute hydrochloric acid. The solution was dried over anhydrous MgSO<sub>4</sub> and filtered by suction. The solvent was evaporated by vacuum distillation. The crude product was purified by gel chromatography using dichloromethane/petroleum ether mixture (1/5, V<sub>d</sub>/V<sub>p</sub>) as eluent. A white flocculent product **1** was obtained with a yield of 53.7%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ = 7.90 (d, *J* = 8.4 Hz, 2H), 7.22-7.12 (m, 6H), 7.09-7.00 (m, 6H), 6.48 (s, 2H), 4.35 (q, *J* = 7.2 Hz, 2H), 1.37 (t, *J* = 7.2 Hz, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ = 166.00, 142.89, 135.75, 132.93, 130.10, 129.01, 128.86, 128.71, 128.07, 126.48, 110.60, 61.20, 14.31. MS (ESI, *m/z*) Calcd for C<sub>25</sub>H<sub>21</sub>NO<sub>2</sub> [M+H]<sup>+</sup>: 368.15, found: 368.16.



**Figure S1.**  $^1\text{H}$  NMR spectrum of **1** in  $\text{CDCl}_3$



**Figure S2.**  $^{13}\text{C}$  NMR spectrum of **1** in  $\text{CDCl}_3$ .



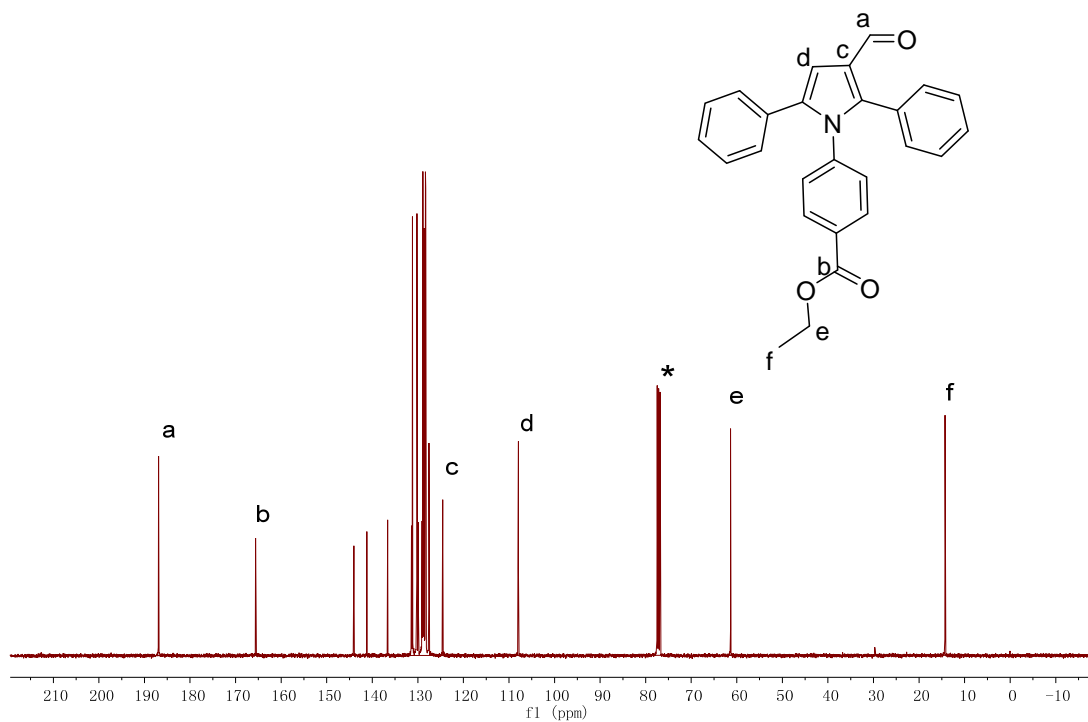
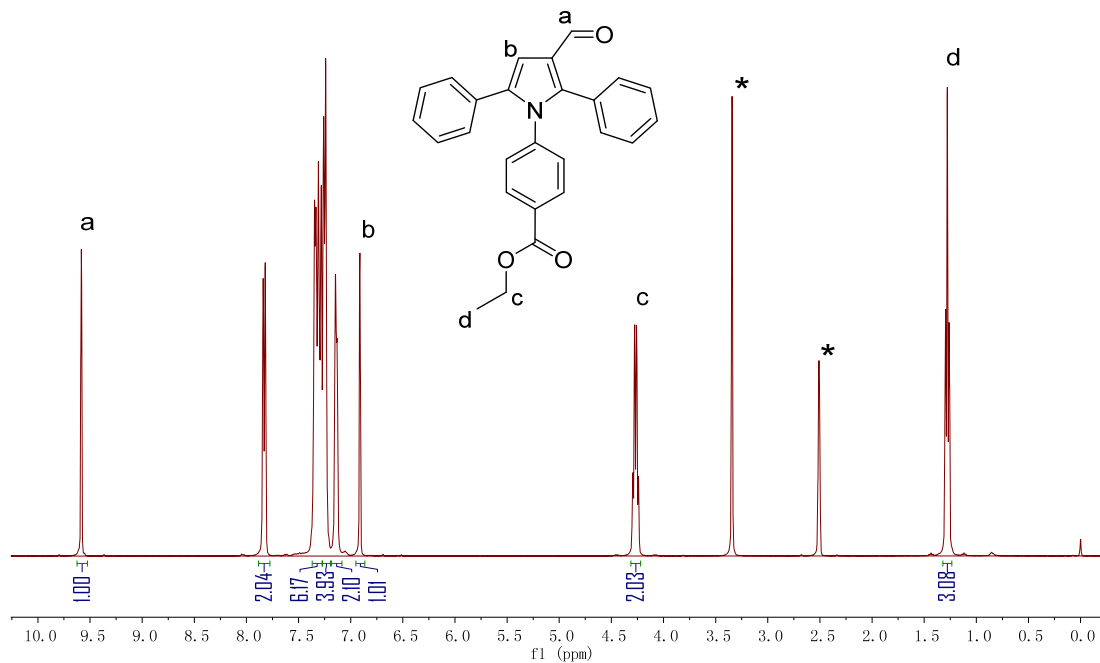
**Figure S3.** MS spectrum of **1**.



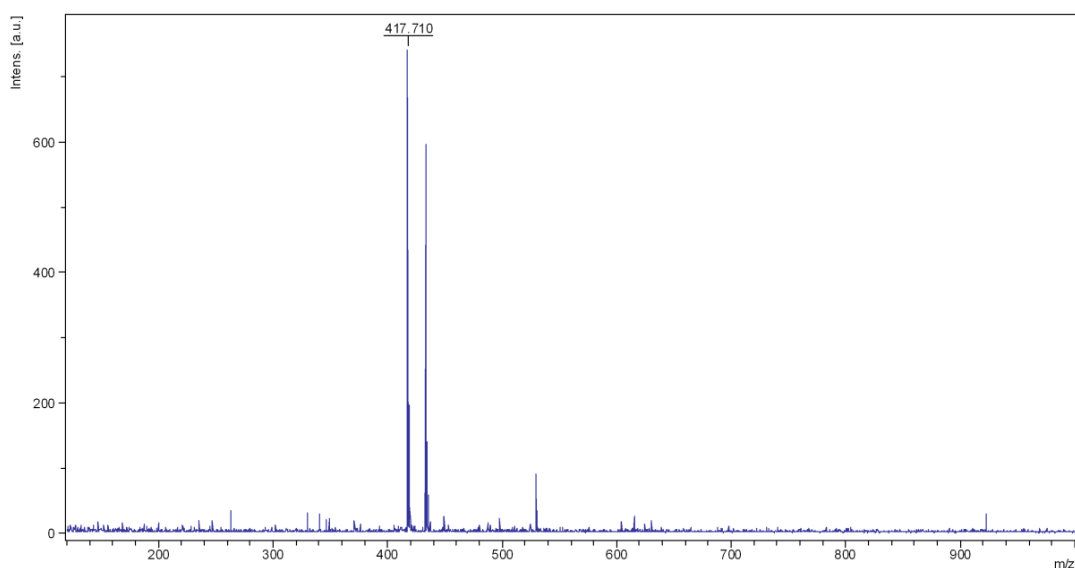
**Scheme S3.** The synthetic route to compound **2**

$\text{POCl}_3$  (200  $\mu\text{L}$ , 2.20 mmol) was dropped slowly into DMF (24 mL) at 0 °C and stirred for another 1 h at room temperature. To the above solution was added a dichloromethane solution of **1** (0.7343 g, 2.00 mmol). After the mixture was stirred for 12 h at room temperature. The residue was poured into water (250 mL) and extracted with dichloromethane; then the solution of dichloromethane was dried over anhydrous  $\text{MgSO}_4$  and filtered by suction. The solvent was evaporated by vacuum distillation. The crude product was purified by gel chromatography using dichloromethane/petroleum ether mixture (1/5,  $V_d/V_p$ ) as eluent. Product **2** was obtained with the yield of 83.2%.  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  = 9.58 (s, 1H), 7.83 (d,  $J$  = 7.9 Hz, 2H), 7.37-7.27 (m, 6H), 7.25 (d,  $J$  = 7.5 Hz, 4H), 7.14 (d,  $J$  = 5.6 Hz, 2H), 6.91 (s, 1H), 4.27 (q,  $J$  = 6.9 Hz, 2H), 1.28 (t,  $J$  = 6.9 Hz, 3H).  $^{13}\text{C}$  NMR (100 MHz,

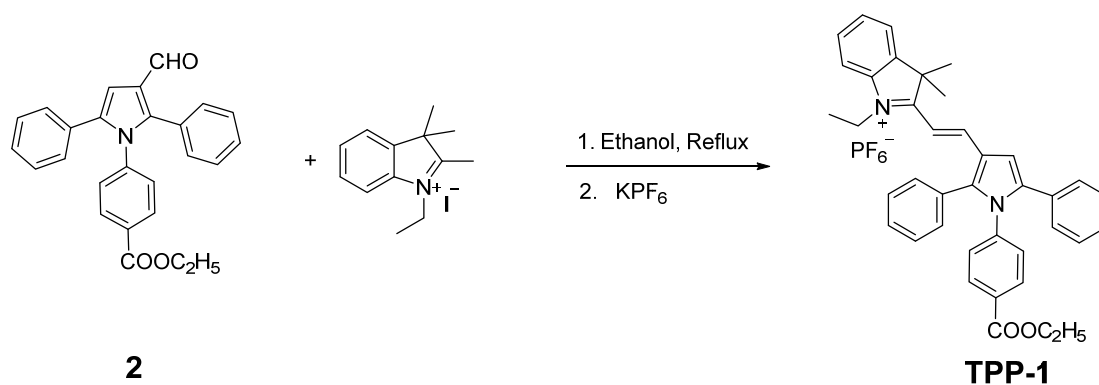
CDCl<sub>3</sub>):  $\delta$  = 186.91, 165.59, 141.19, 136.64, 131.38, 131.17, 130.21, 129.89, 129.06, 128.91, 128.81, 128.62, 128.32, 128.27, 127.58, 124.53, 107.93, 61.36, 14.28. MS (ESI,  $m/z$ ) Calcd for C<sub>26</sub>H<sub>21</sub>NO<sub>3</sub> [M+Na]<sup>+</sup>: 418.14, found: 417.71.



Comment 1  
Comment 2



**Figure S6.** MS spectrum of **2**.

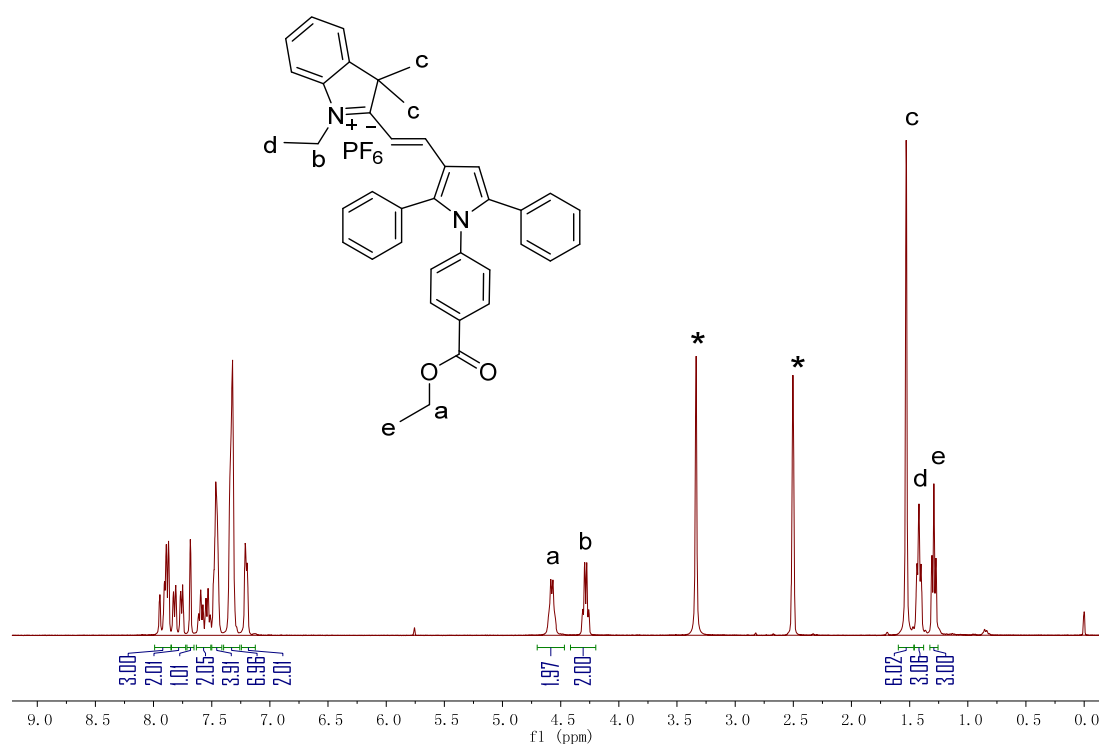


**Scheme S4.** The synthetic route to target compound **TPP-1**.

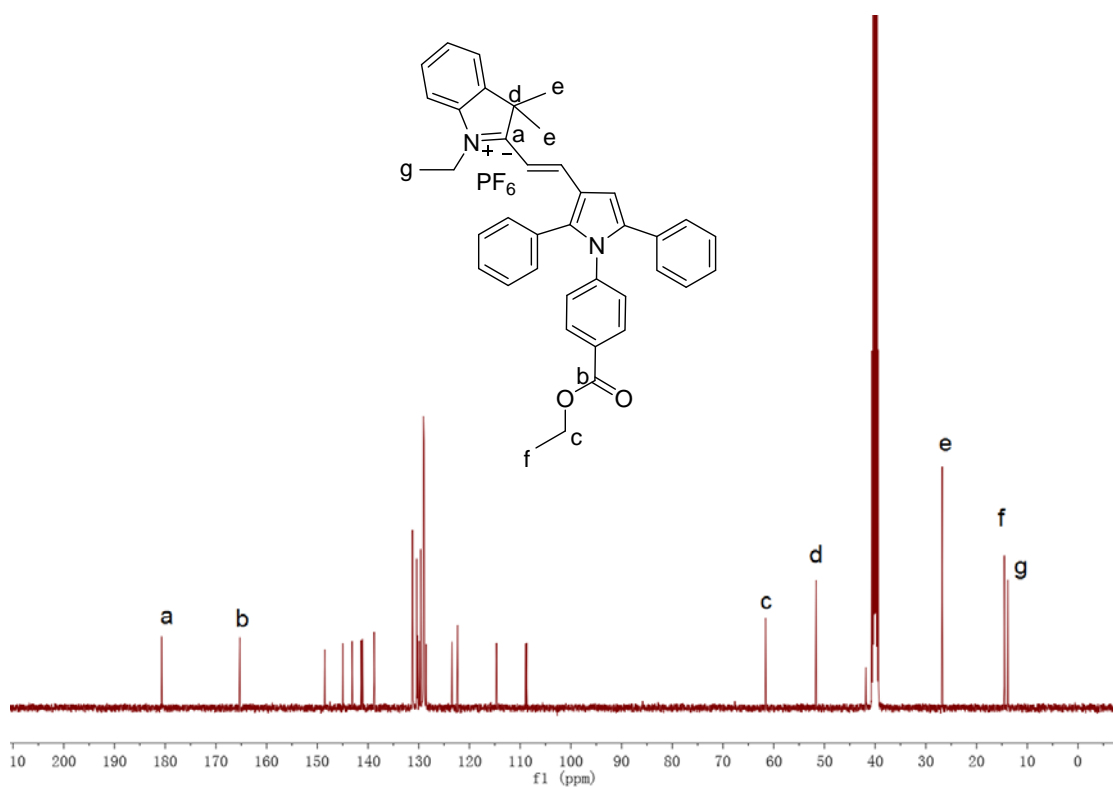
A solution of **2** (0.2377 g, 0.60 mmol) and indolium iodide salt<sup>[2]</sup> (0.2068 g, 0.65 mmol) in dry ethanol (15 mL) was refluxed for 24 h. After the reaction mixture was cooled to room temperature, the solvent was evaporated under reduced pressure. The solid was dissolved in THF (5 mL) and a saturated aqueous solution of KPF<sub>6</sub> (5 mL) was then added. After stirring for 30 min, the solvent was evaporated to get the crude product which was purified by a silica gel column chromatography using dichloromethane/methanol mixture (10/1, V<sub>d</sub>/V<sub>m</sub>) as eluent to give a red solid in 78.2% yield. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  = 7.94 (d, *J* = 16.0 Hz,



1H), 7.88 (d,  $J = 8.0$ , 2H), 7.82 (d,  $J = 8.0$ , 1H), 7.76 (d,  $J = 8.0$ , 1H), 7.68 (s, 1H), 7.61-7.47 (m, 6H), 7.32 (m, 7H), 7.20 (d,  $J = 6.9$  Hz, 2H), 4.58 (q,  $J = 7.3$  Hz, 2H), 4.28 (q,  $J = 8.9$ , 6.8 Hz, 2H), 1.53 (s, 6H), 1.42 (t,  $J = 7.3$  Hz, 3H), 1.29 (t,  $J = 6.8$  Hz, 3H).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta = 180.70, 165.27, 148.52, 143.14, 141.38, 141.10, 138.80, 131.24, 131.13, 130.39, 130.27, 129.89, 129.60, 129.10, 129.07, 129.04, 128.95, 128.87, 128.54, 123.44, 122.33, 114.66, 108.94, 108.73, 61.61, 51.63, 41.83, 26.78, 14.52, 13.82$ . HR-MS (ESI,  $m/z$ ) Calcd for  $\text{C}_{39}\text{H}_{37}\text{N}_2\text{O}_2^+$   $[\text{M}]^+$ : 565.2849, found: 565.2846, error -0.6 ppm.

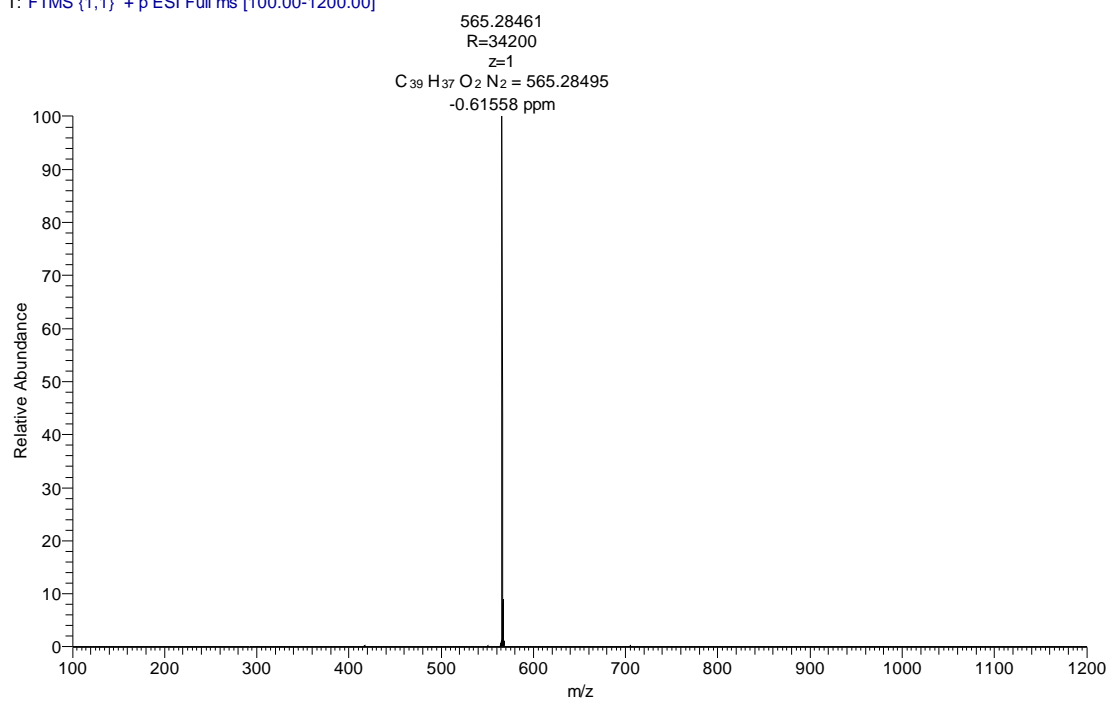


**Figure S7.**  $^1\text{H}$  NMR spectrum of TPP-1 in DMSO- $d_6$ .



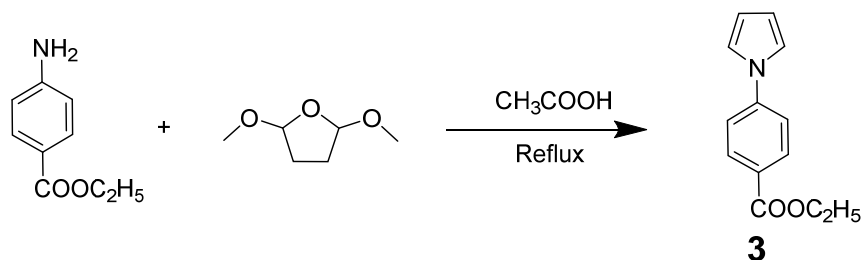
**Figure S8.**  $^{13}\text{C}$  NMR spectrum of **TPP-1** in  $\text{DMSO-}d_6$ .

RF-11\_180709111546 #31 RT: 0.39 AV: 1 NL: 2.47E7  
T: FTMS (1,1) + p ESI Full ms [100.00-1200.00]



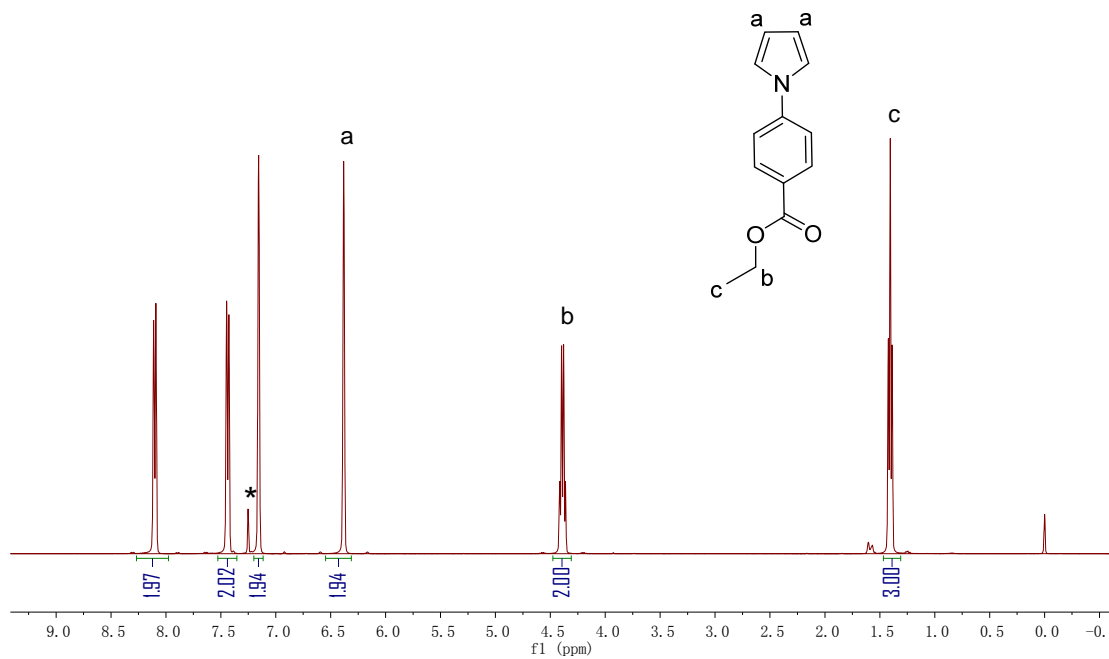
**Figure S9.** HR-MS spectrum of **TPP-1**.

### 1.3.2 Preparation of TPP-2.

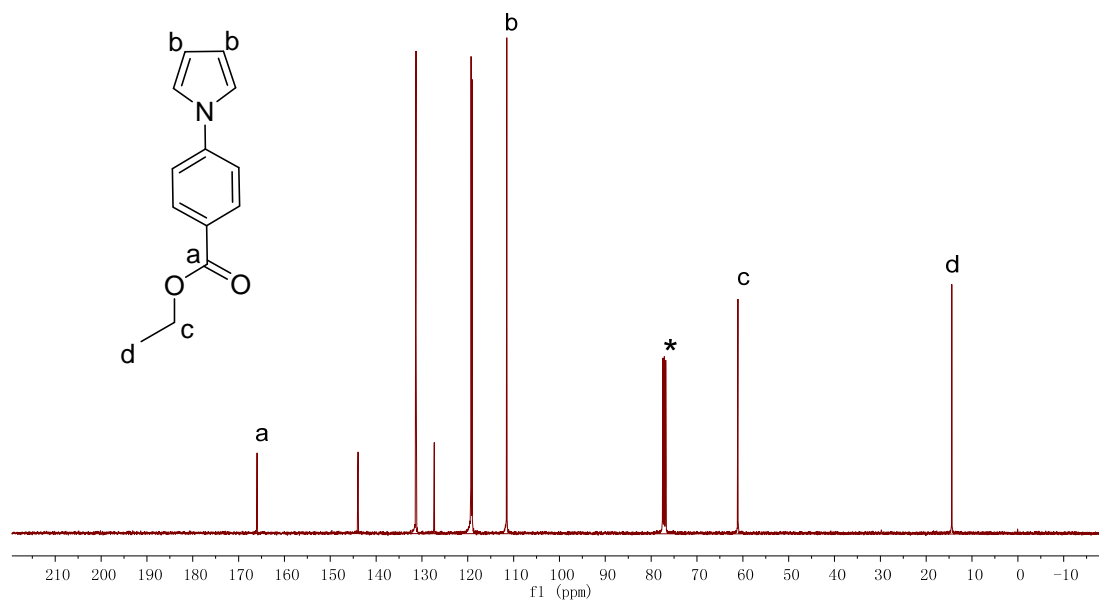


**Scheme S5.** The synthetic route to compound **3**.

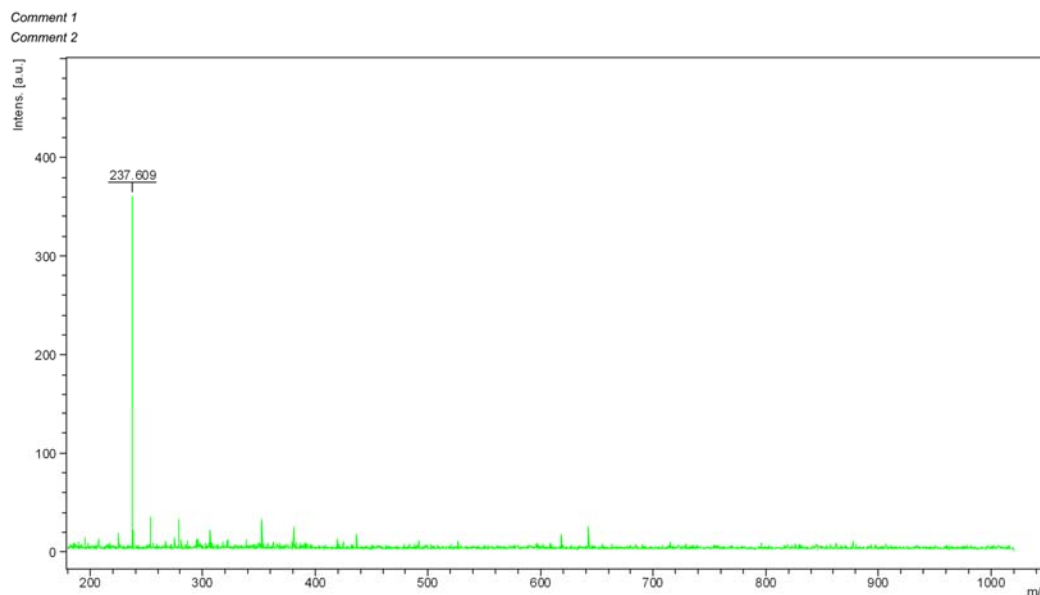
Ethyl 4-aminobenzoate (1.6519 g, 10.00 mmol) and 2, 5-dimethoxytetrahydrofuran (1.3216 g, 10.00 mmol) were dissolved in 25 mL acetic acid, then refluxed for 12 h. After cooled to room temperature, the solvent was evaporated under reduced pressure. The residue was dissolved in dichloromethane and washed with water for three times, followed by extraction with dichloromethane. Removed the water from the solution with anhydrous  $\text{MgSO}_4$ , then filtered by suction and evaporated by vacuum distillation. The crude product was purified by a silica gel column using dichloromethane/petroleum ether mixture (1/6,  $V_d/V_p$ ) as eluent. Product **3** was obtained with the yield of 83.2%.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 8.10 (d,  $J$  = 8.2 Hz, 2H), 7.44 (d,  $J$  = 8.1 Hz, 2H), 7.16 (s, 2H), 6.38 (s, 2H), 4.39 (q,  $J$  = 7.0 Hz, 2H), 1.41 (t,  $J$  = 7.1 Hz, 3H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 165.96, 143.94, 131.29, 119.51, 119.28, 119.07, 111.49, 61.07, 14.40. MS (MALDI,  $m/z$ ) Calcd for  $\text{C}_{13}\text{H}_{13}\text{NO}_2$   $[\text{M}+\text{Na}]^+$ : 238.08, found: 237.61.



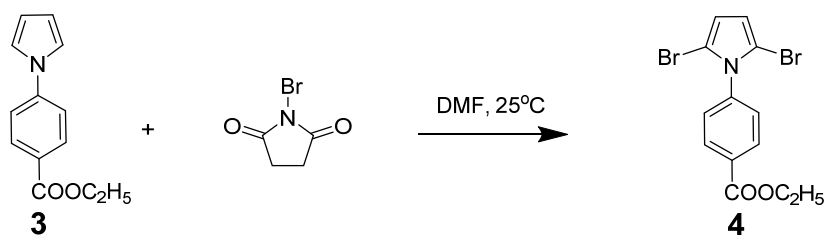
**Figure S10.**  $^1\text{H}$  NMR spectrum of **3** in  $\text{CDCl}_3$ .



**Figure S11.**  $^{13}\text{C}$  NMR spectrum of **3** in  $\text{CDCl}_3$ .



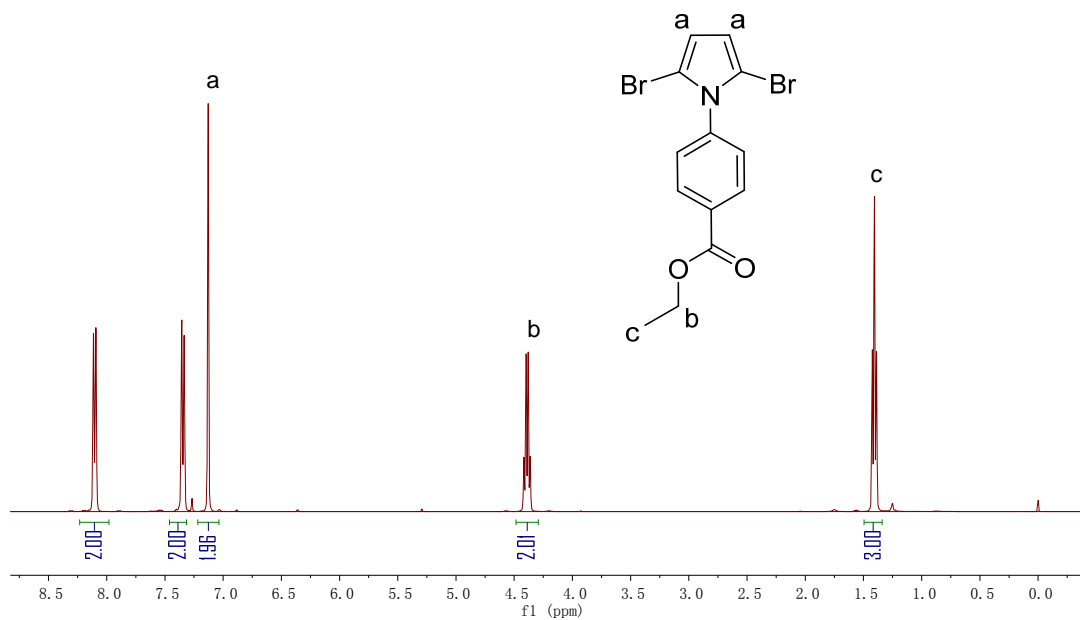
**Figure S12.** MS spectrum of **3**.



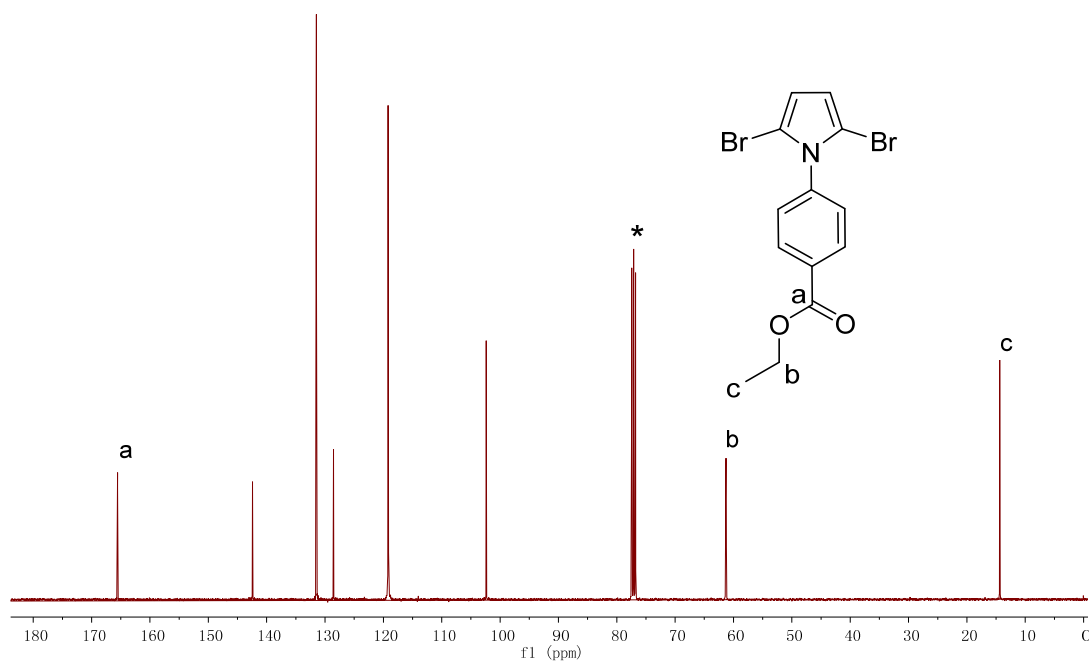
**Scheme S6.** The synthetic route to compound **4**.

The compound **3** (0.9024 g, 5.10 mmol) was added into 25 mL DMF. 1-Bromopyrrolidine-2, 5-dione (1.0755 g, 5.00 mmol ) was dissolved in 5 mL DMF, and then slowly added dropwise into the DMF solution of compound **3** with stirring. The mixture reacted at room temperature for 12 h. The solution was washed by water and extracted with dichloromethane. The dichloromethane solution was dried over anhydrous  $\text{MgSO}_4$ , then filtered by suction and evaporated by vacuum distillation. The crude product was purified by gel chromatography using dichloromethane/petroleum ether mixture (1/6,  $V_d/V_p$ ) as eluent. Product **4** was obtained with 78.2% yield.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 8.10 (d,  $J$  = 8.1 Hz, 2H), 7.34 (d,  $J$  = 8.1 Hz, 2H), 7.13 (s, 2H), 4.39 (d,  $J$  = 7.2 Hz, 2H), 1.41 (t,  $J$  = 7.1 Hz, 3H).  $^{13}\text{C}$  NMR (100 MHz,

CDCl<sub>3</sub>):  $\delta$  = 165.56, 142.43, 131.48, 128.56, 119.19, 119.16, 102.37, 61.29, 14.38. MS (APCI,  $m/z$ ) Calcd for C<sub>13</sub>H<sub>11</sub>Br<sub>2</sub>NO<sub>2</sub> [M+H]<sup>+</sup>: 373.91, found: 373.92.

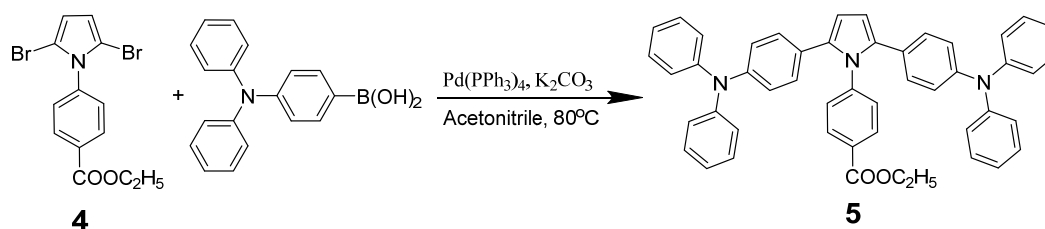
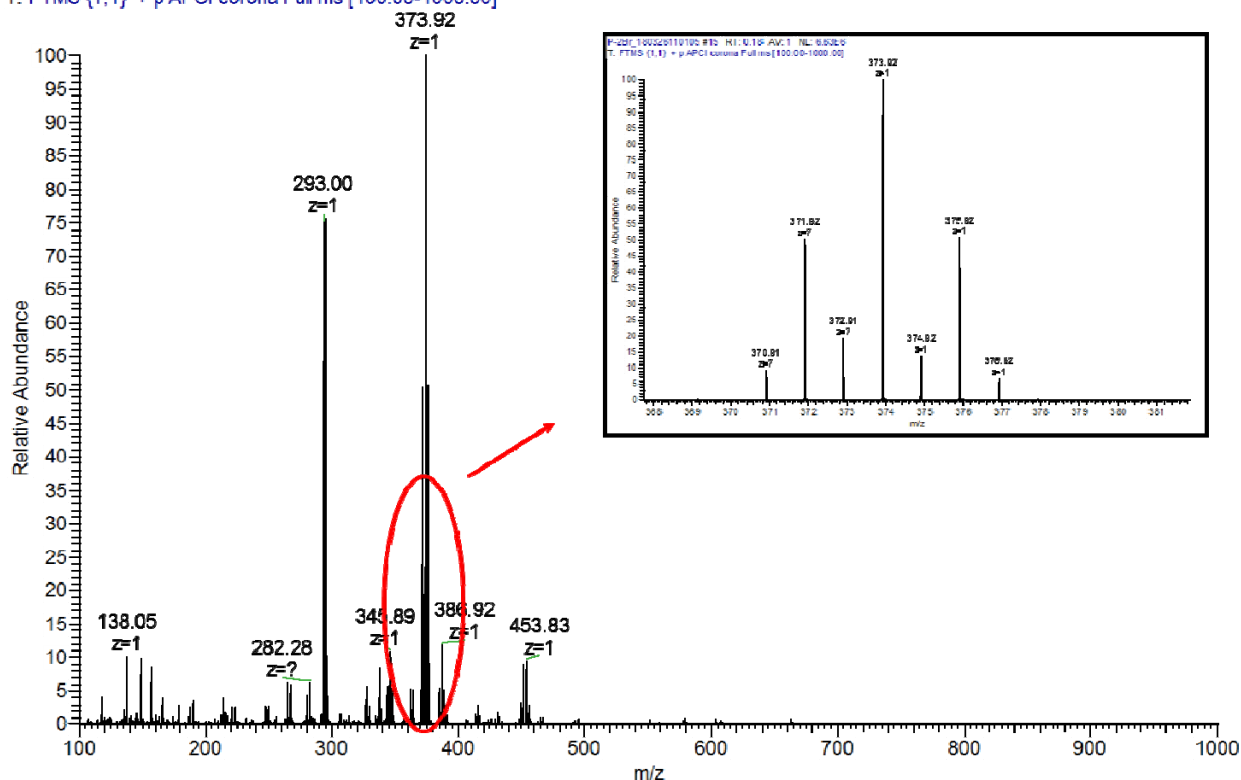


**Figure S13.** <sup>1</sup>H NMR spectrum of **4** in CDCl<sub>3</sub>.



**Figure S14.** <sup>13</sup>C NMR spectrum of **4** in CDCl<sub>3</sub>.

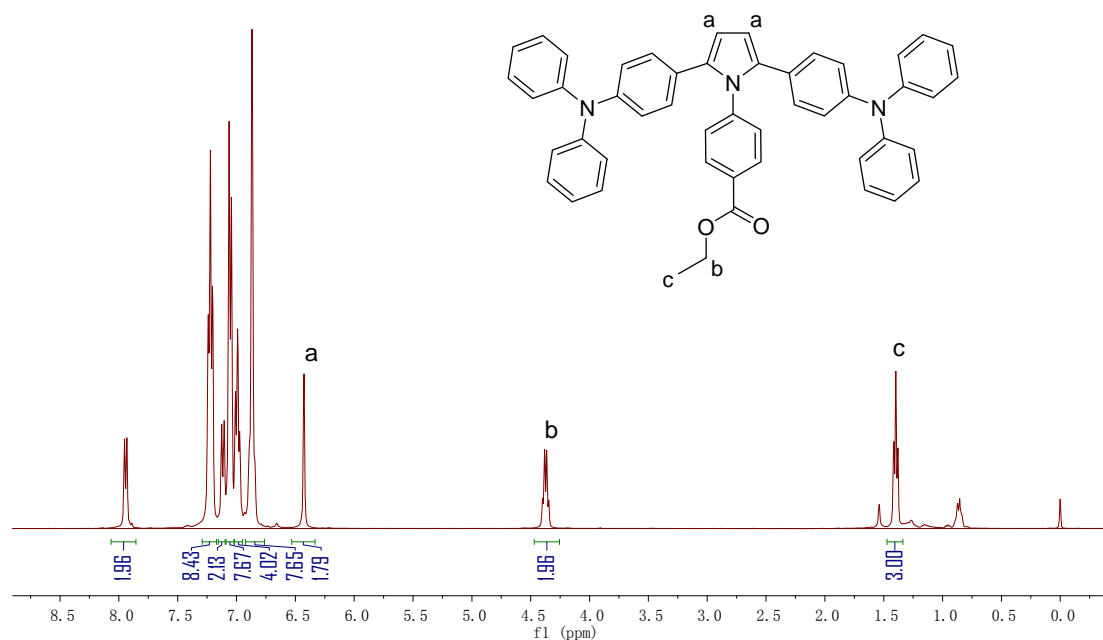
P-2Br\_180326110105 #15 RT: 0.18 AV: 1 NL: 6.63E6  
T: FTMS {1,1} + p APCI corona Full ms [100.00-1000.00]



**Scheme S7.** The synthetic route to compound 5.

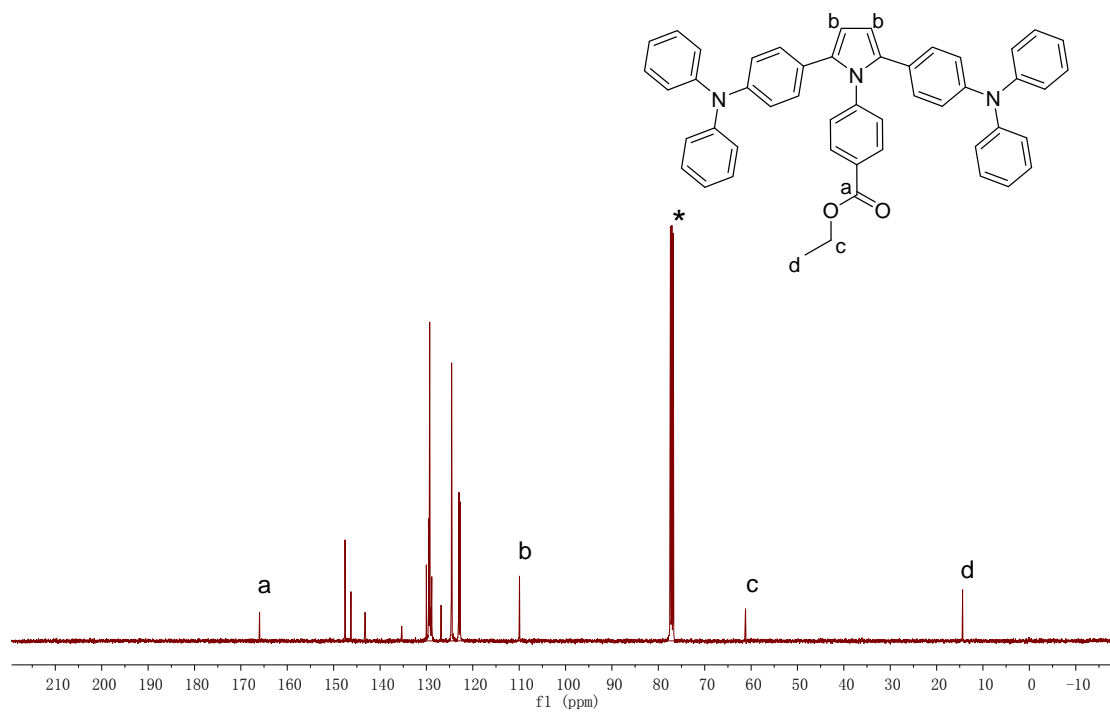
Compound **4** (0.7458 g, 2.00 mmol), (4-(diphenylamino)phenyl)boronic acid (1.3011 g, 4.50 mmol) and Pd(PPh<sub>3</sub>)<sub>4</sub> (0.0924 g, 0.08 mmol) were dissolved in degassed acetonitrile (20 ml) and then a saturated aqueous solution of K<sub>2</sub>CO<sub>3</sub> (5 mL) was added. After stirring for 24 h at 80 °C under nitrogen protection, the solution was cooled to room temperature, and then removed the solvent under reduced pressure. The residue was extracted with dichloromethane and washed with water, then the organic layer was dried over anhydrous MgSO<sub>4</sub>. The solvent was removed under reduced pressure, and the crude product was purified by silica gel column

using dichloromethane/petroleum mixture (1/6,  $V_d/V_p$ ) as eluent to give the compound **5** with 59.5% yield.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 7.94 (d,  $J$  = 8.0 Hz, 2H), 7.29-7.17 (m, 8H), 7.12 (d,  $J$  = 8.1 Hz, 2H), 7.09-7.02 (m, 8H), 7.02-6.95 (m, 4H), 6.92-6.76 (m, 8H), 6.43 (s, 2H), 4.37 (q,  $J$  = 7.2 Hz, 2H), 1.40 (t,  $J$  = 6.8 Hz, 3H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 165.98, 147.56, 146.25, 143.23, 135.32, 130.01, 129.50, 129.27, 128.85, 124.56, 122.99, 122.73, 109.93, 61.21, 14.38. MS (MALDI,  $m/z$ ) Calcd for  $\text{C}_{49}\text{H}_{39}\text{N}_3\text{O}_2$   $[\text{M}]^+$ : 701.30, found: 701.07.



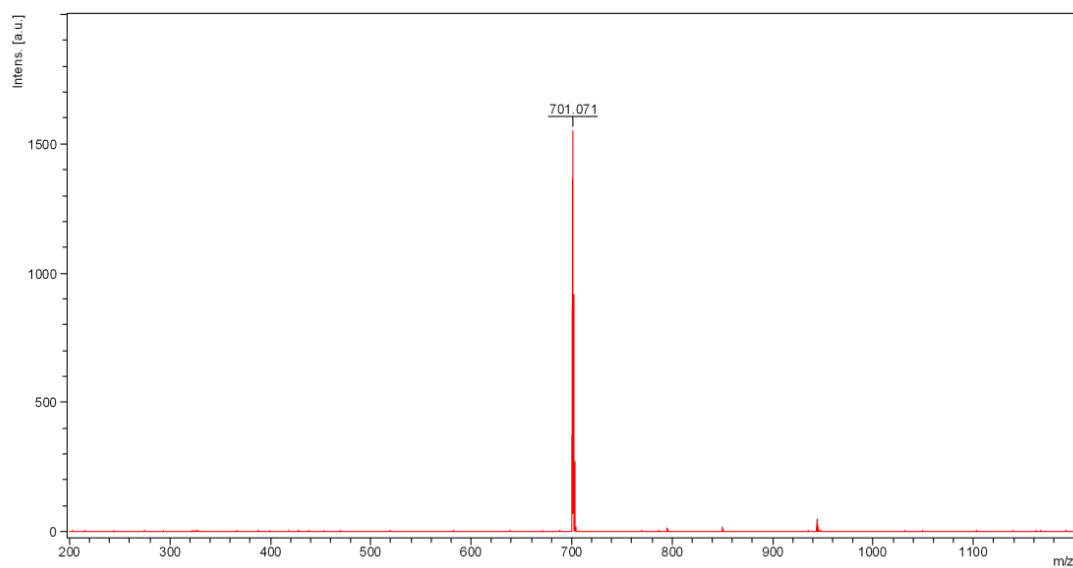
**Figure S16.**  $^1\text{H}$  NMR spectrum of **5** in  $\text{CDCl}_3$ .



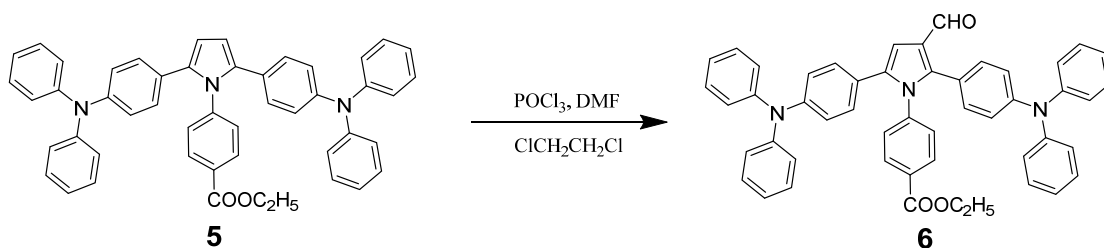


**Figure S17.**  $^{13}\text{C}$  NMR spectrum of **5** in  $\text{CDCl}_3$ .

Comment 1  
Comment 2

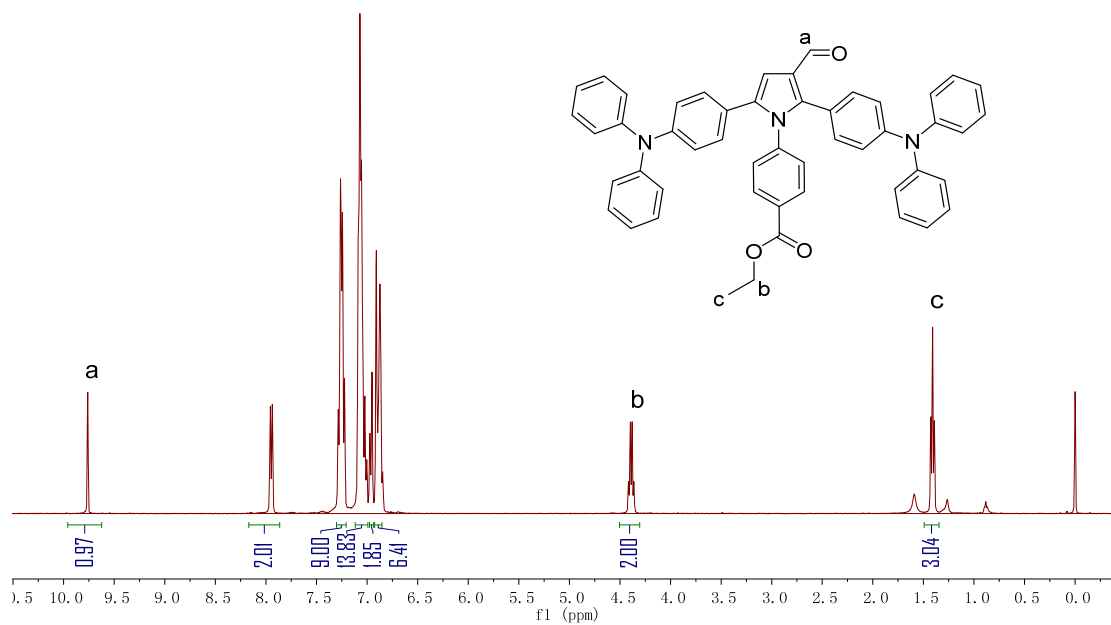


**Figure S18.** MS spectrum of **5**.

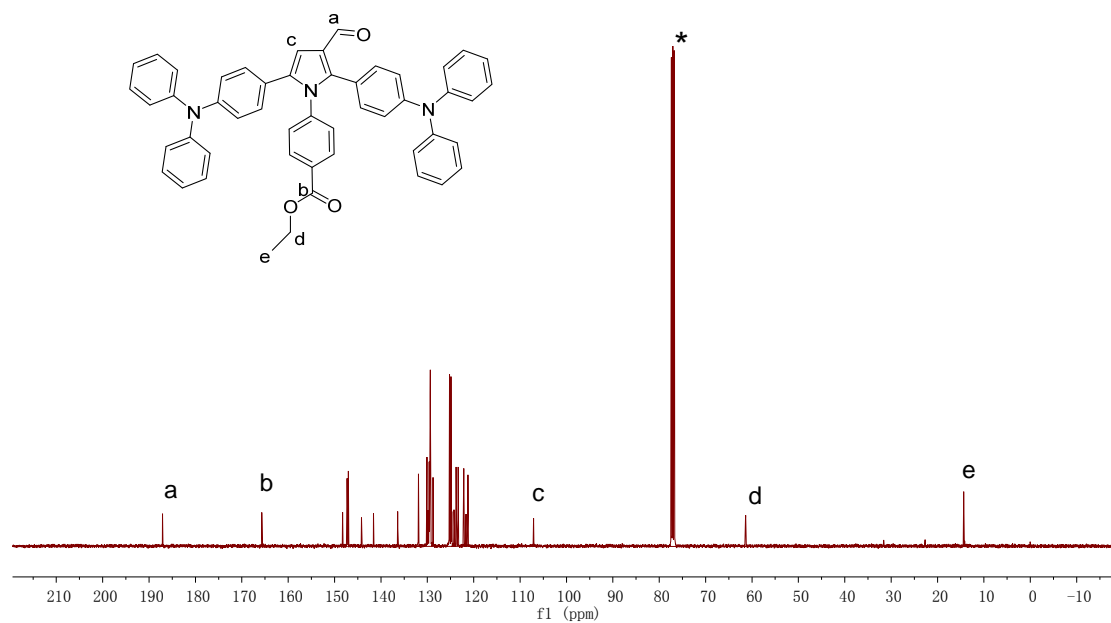


**Scheme S8.** The synthetic route to compound **6**.

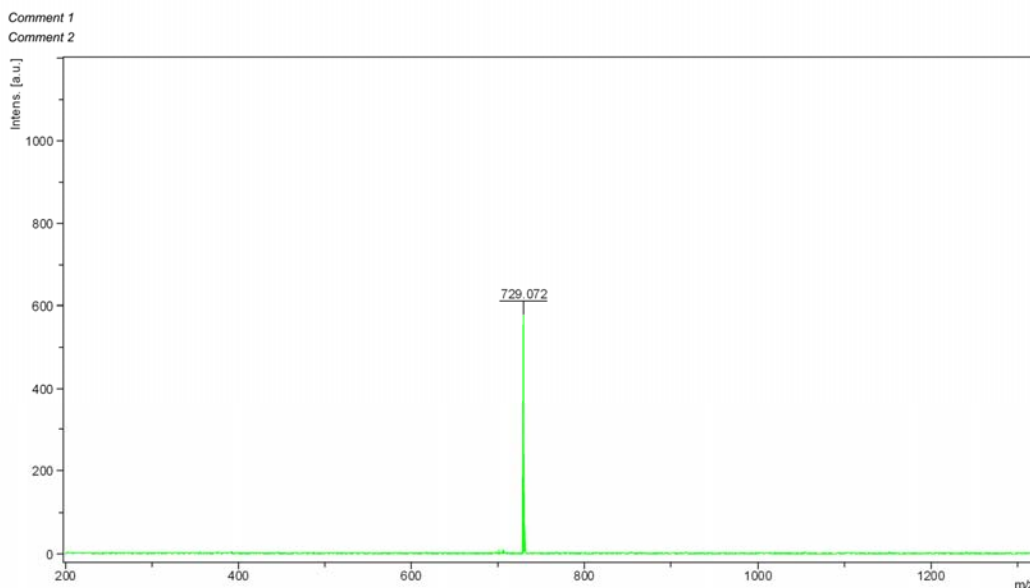
POCl<sub>3</sub> (50 μL, 0.55 mmol) was slowly dropped into DMF (24 mL) at 0 °C, then the mixture was stirred for 1 h at room temperature. To the above solution was added a dichloromethane solution of compound **5** (0.3507 g, 0.50 mmol). After the mixture was stirred for 12 h at room temperature, the solution was poured into water (250 mL) and extracted with dichloromethane. The dichloromethane solution was dried over anhydrous MgSO<sub>4</sub> and filtered by suction, and then the solvent was evaporated by vacuum distillation. The residue was purified by gel chromatography using dichloromethane/petroleum mixture (1/2, V<sub>d</sub>/V<sub>p</sub>) as eluent. Product **6** was obtained with a yield of 73.2%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ = 9.76 (s, 1H), 7.95 (d, *J* = 8.0 Hz, 2H), 7.30-7.21 (m, 9H), 7.12-6.99 (m, 14H), 6.96 (d, *J* = 8.3 Hz, 2H), 6.93-6.85 (m, 6H), 4.39 (q, *J* = 7.1 Hz, 2H), 1.41 (t, *J* = 7.2 Hz, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ = 187.08, 165.68, 148.28, 147.31, 147.21, 147.03, 141.60, 136.38, 131.92, 130.09, 129.83, 129.60, 129.47, 129.35, 128.77, 125.23, 124.89, 124.26, 123.81, 123.36, 122.16, 121.67, 121.26, 107.09, 61.38, 14.36. MS (MALDI, *m/z*) Calcd for C<sub>50</sub>H<sub>39</sub>N<sub>3</sub>O<sub>3</sub> [M]<sup>+</sup> : 729.30, found: 729.07.



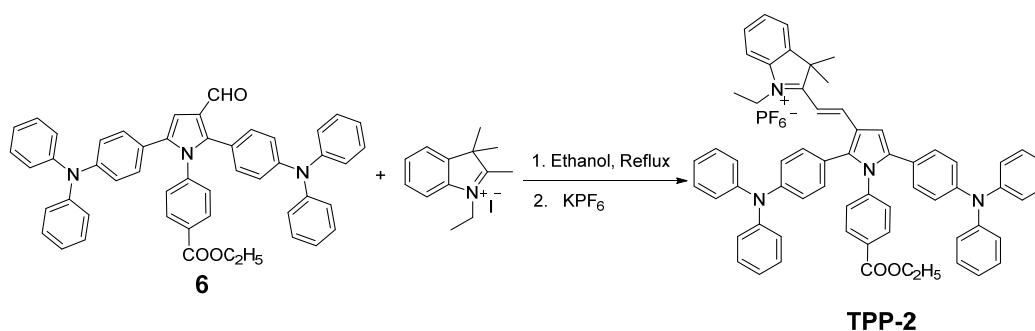
**Figure S19.** <sup>1</sup>H NMR spectrum of **6** in CDCl<sub>3</sub>.



**Figure S20.** <sup>13</sup>C NMR spectrum of **6** in CDCl<sub>3</sub>.



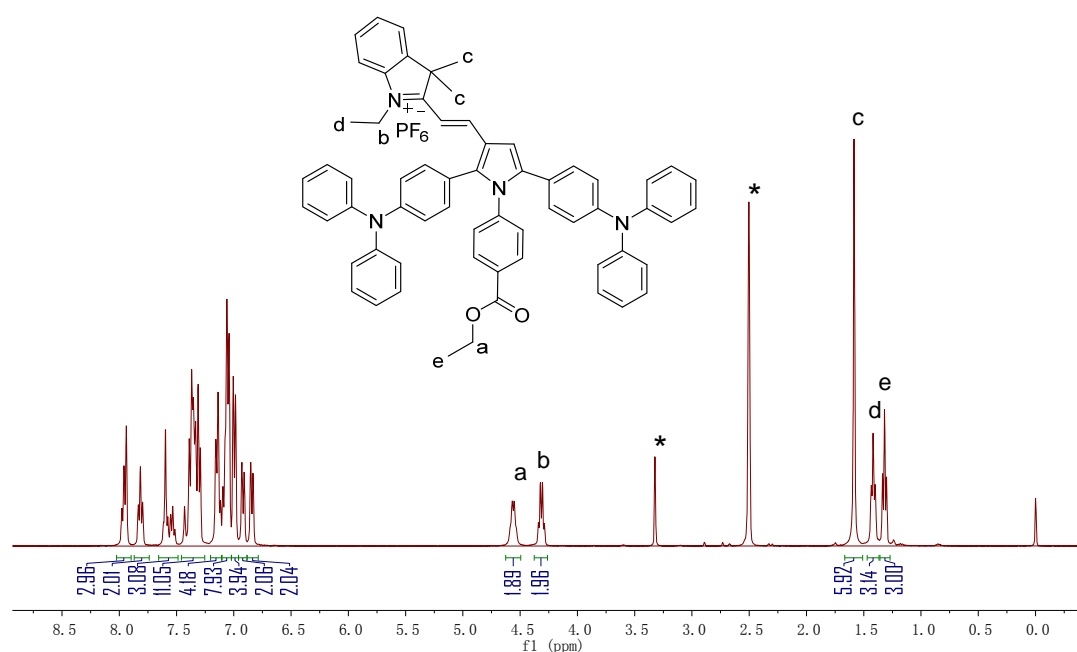
**Figure S21.** MS spectrum of **6**.



**Scheme S9.** The synthetic route to target compound **TPP-2**.

A solution of **6** (0.2917 g, 0.40 mmol) and indolium iodide salt (0.1553 g, 0.45 mmol) in ethanol (15 mL) was refluxed for 24 h. After the reaction mixture was cooled to room temperature, the solvent was evaporated under reduced pressure. The residue was dissolved in THF (5 mL) and a saturated aqueous solution of KPF<sub>6</sub> (5 mL) was then added. After stirring for 30 min, the solution was evaporated to get crude product that was purified by a silica gel column chromatography using dichloromethane/methanol mixture (10/1, V<sub>d</sub>/V<sub>m</sub>) as eluent to give a red solid in 52.2% yield. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ = 7.98 (d, *J* = 16.0 Hz, 1H), 7.94 (s, 2H), 7.82 (t, *J* = 7.5 Hz, 2H), 7.66-7.49 (m, 3H), 7.46-7.25 (m, 11H), 7.20-7.02 (m, 12H), 6.99 (d, *J* = 7.8 Hz, 4H), 6.92 (d, *J* = 8.0 Hz, 2H), 6.84 (d, *J* = 8.1 Hz, 2H), 4.56 (q, *J* =

7.0 Hz, 2H), 4.31 (q,  $J = 6.9$  Hz, 2H), 1.59 (s, 6H), 1.42 (t,  $J = 6.9$  Hz, 3H), 1.32 (t,  $J = 7.0$  Hz, 3H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO-}d_6$ ):  $\delta = 180.43, 165.35, 148.62, 147.12, 146.84, 145.20, 143.03, 141.52, 141.17, 138.66, 132.32, 130.45, 130.30, 130.13, 130.08, 129.58, 125.40, 124.95, 124.69, 124.53, 124.15, 123.54, 122.46, 122.23, 121.54, 121.31, 108.33, 108.17, 61.63, 51.56, 26.87, 14.57, 13.77$ . HR-MS (ESI,  $m/z$ ) Calcd for  $\text{C}_{64}\text{H}_{56}\text{N}_3\text{O}_2^+$   $[\text{M}+\text{H}]^+$ : 899.4320, found: 899.4293, error -3.0 ppm.



**Figure S22.**  $^1\text{H}$  NMR spectrum of TPP-2 in  $\text{DMSO-}d_6$ .

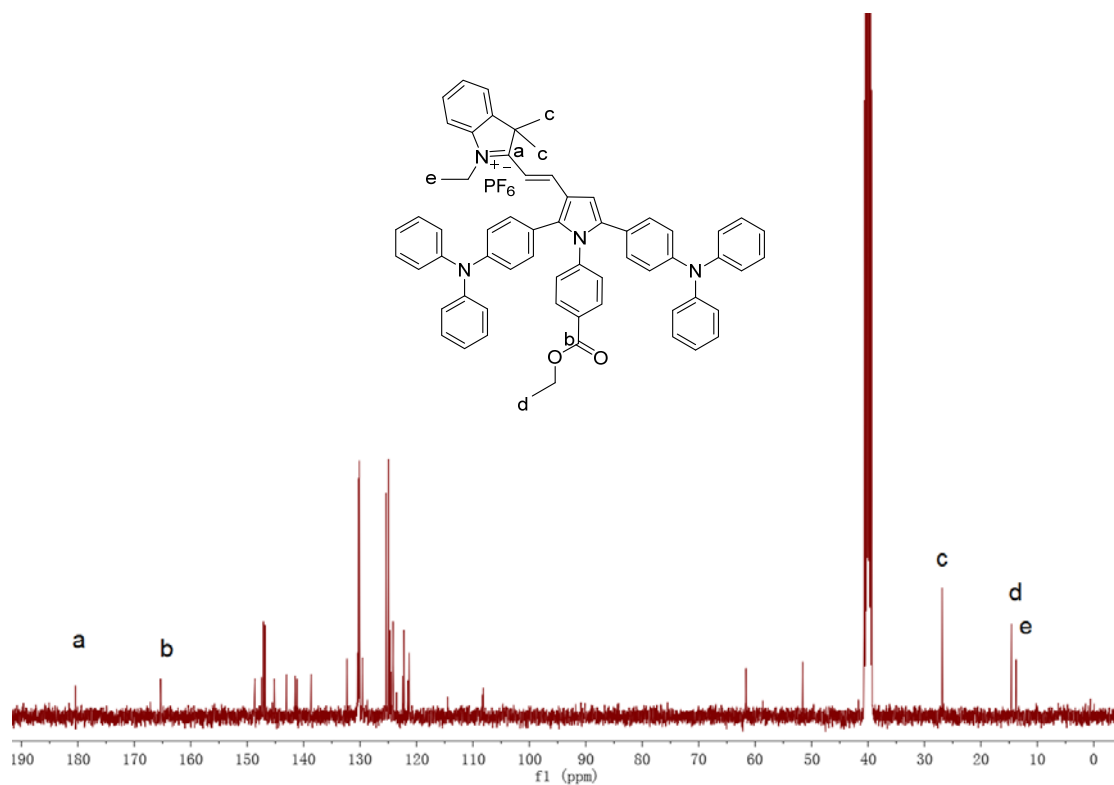


Figure S23.  $^{13}\text{C}$  NMR spectrum of TPP-2 in  $\text{DMSO}-d_6$ .

P-2PA-YD 180417105504#9 RT: 0.09 AV: 1 NL: 7.86E7  
T: FIMS+p ESI Full ms [100.0000-1500.0000]

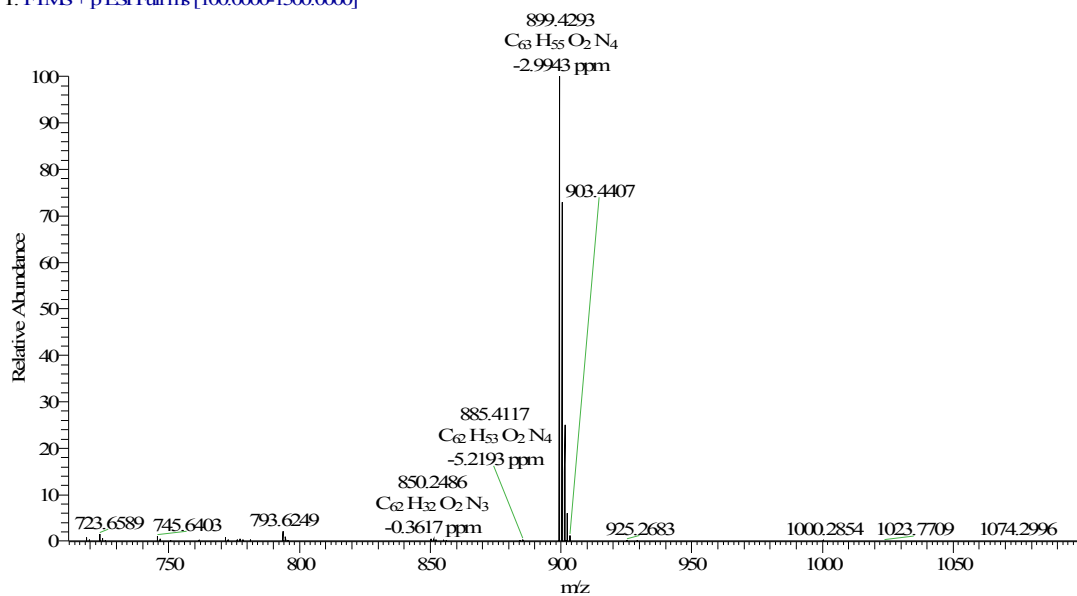
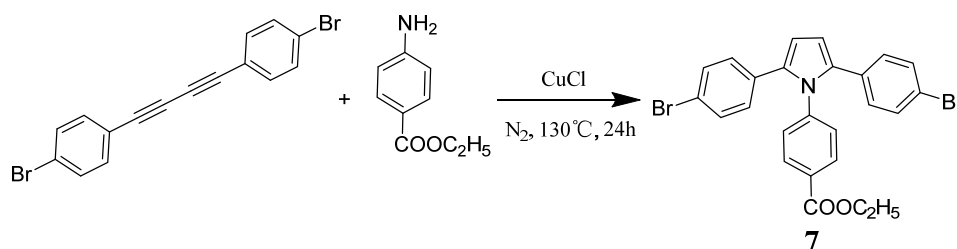


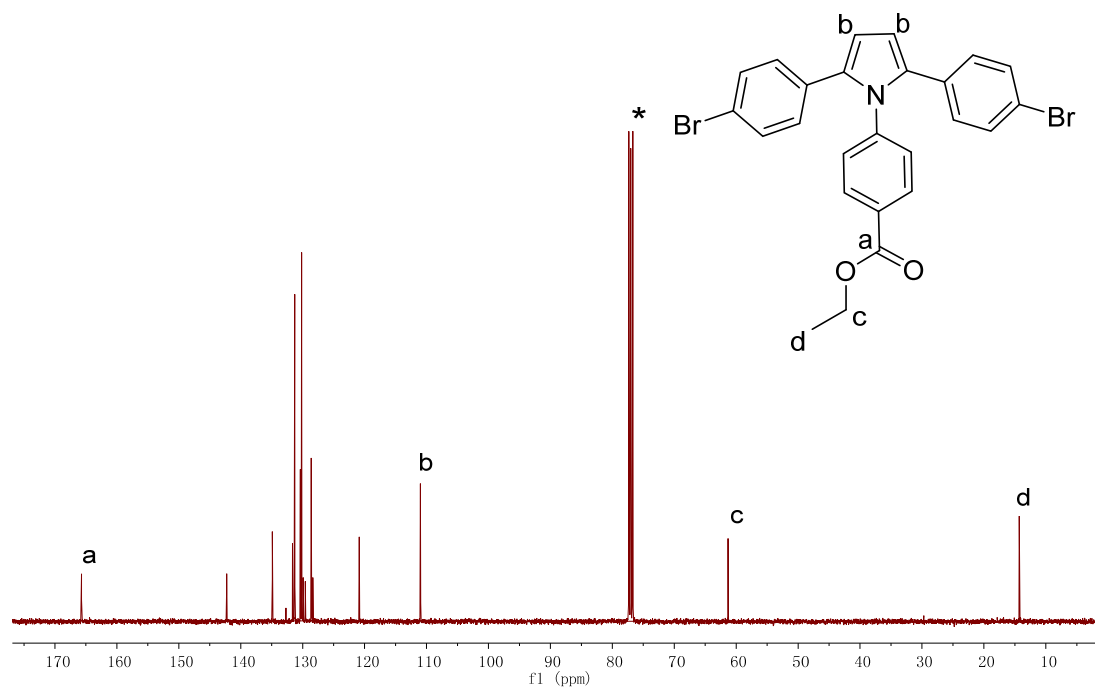
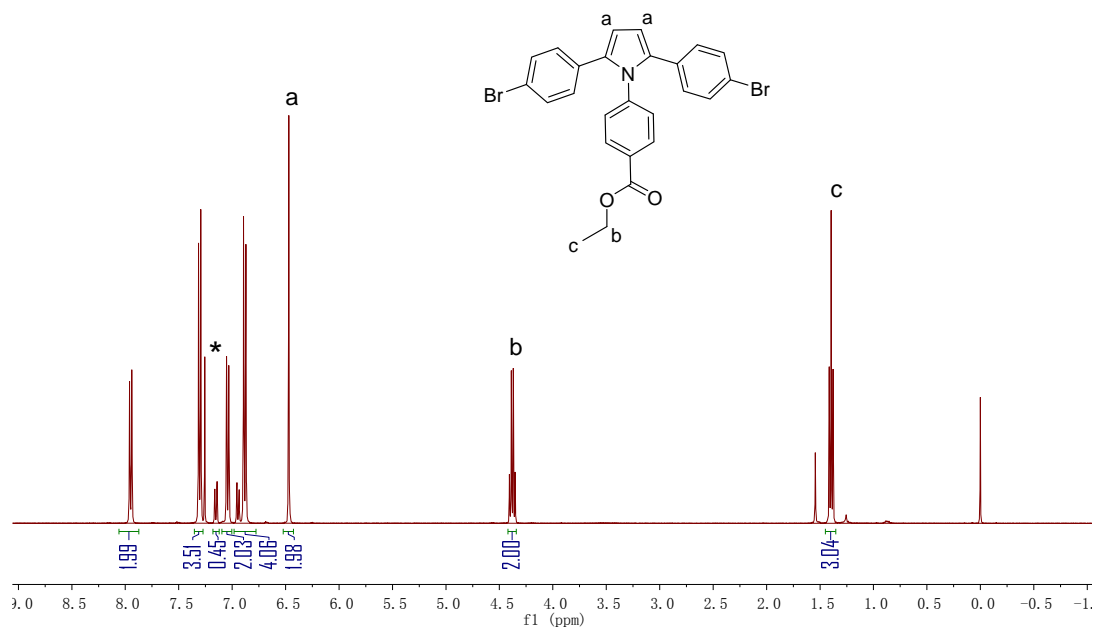
Figure S24. HR-MS spectrum of TPP-2.

### 1.3.3 Preparation of TPP-3.

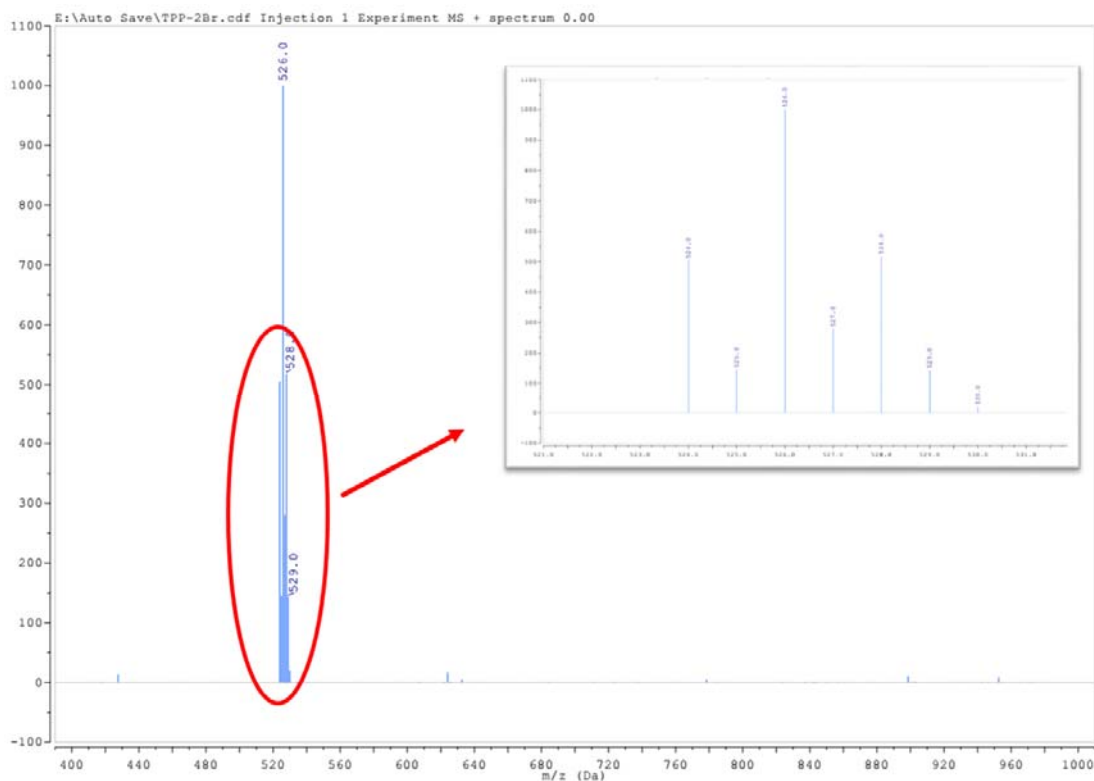


**Scheme S10.** The synthetic route to compound **7**.

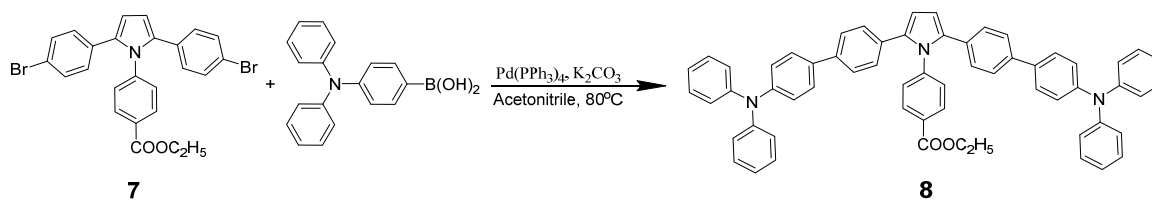
1,4-Bis(4-bromophenyl) buta-1,3-diyne<sup>[3]</sup> (3.5990 g, 10.00 mmol), ethyl p-aminobenzoate (6.6076 g, 40.00 mmol) and CuCl (0.0983 g, 1.00 mmol) were added into a 100 ml Schlenk tube reacted at 130 °C for 24 h under nitrogen. After the reaction was completed, dichloromethane was added into the tube and then washed with dilute hydrochloric acid for three times. The organic solution was dried over anhydrous MgSO<sub>4</sub> and filtered by suction, and then the solvent was evaporated by vacuum distillation. The crude product was purified by gel chromatography using dichloromethane/petroleum ether mixture (1/5, V<sub>d</sub>/V<sub>p</sub>) as eluent. Product **7** was obtained with a yield of 50.6%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ = 7.94 (d, *J* = 8.0 Hz, 2H), 7.38-7.30 (m, 4H), 7.07 (d, *J* = 8.4 Hz, 2H), 7.01-6.80 (m, 4H), 6.50 (s, 2H), 4.40 (q, *J* = 7.2 Hz, 2H), 1.42 (t, *J* = 7.1 Hz, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ = 165.72, 134.89, 131.29, 130.39, 130.18, 129.90, 129.57, 128.62, 120.86, 110.99, 61.32. MS (APCI, *m/z*) Calcd for C<sub>25</sub>H<sub>19</sub>Br<sub>2</sub>NO<sub>2</sub> [M+H]<sup>+</sup>: 526.0, found: 526.0.







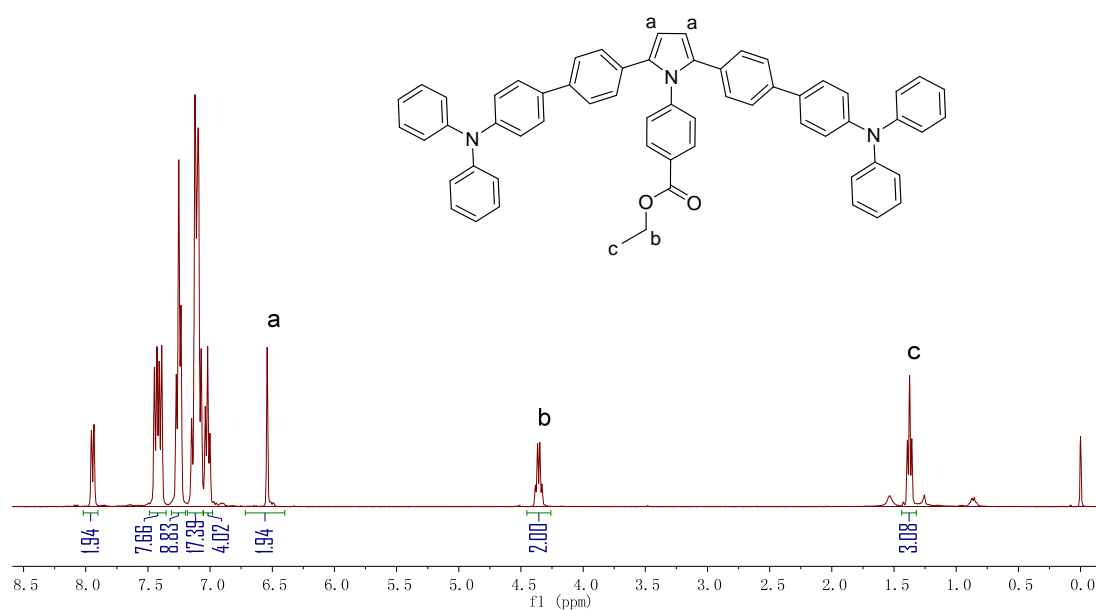
**Figure S27.** MS spectrum of **7**.



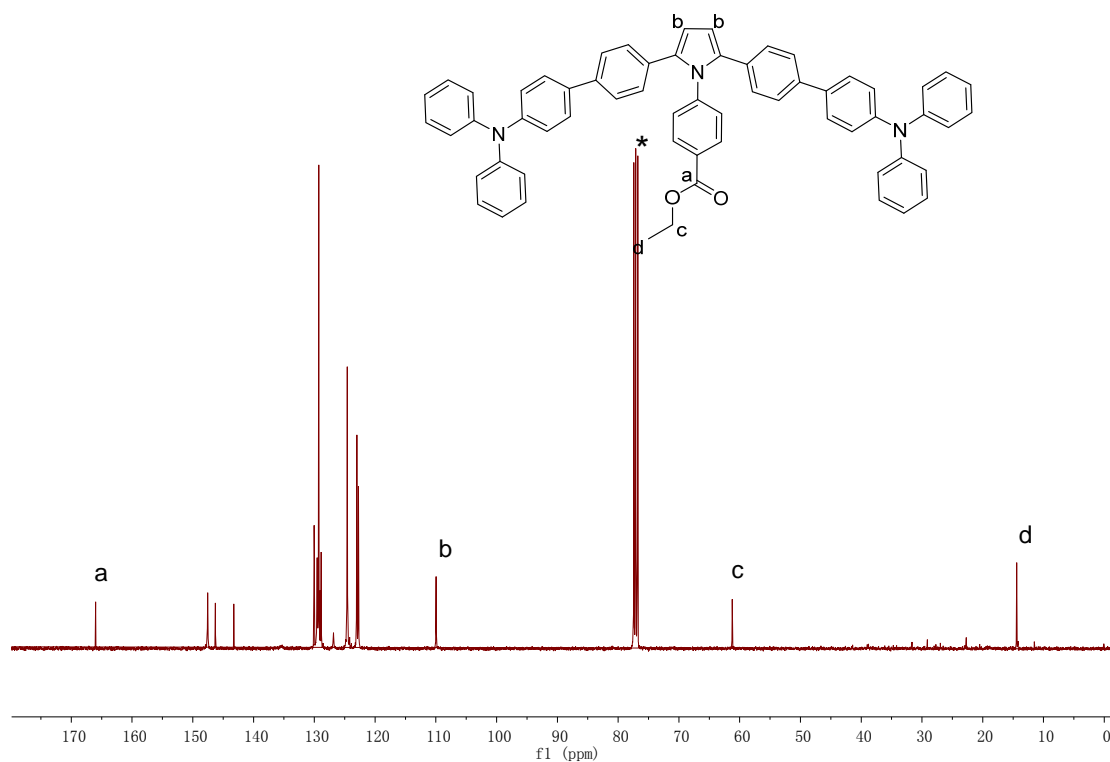
**Scheme S11.** The synthetic route to compound **8**.

Compound **7** (1.0500 g, 2.00 mmol), (4-(diphenylamino)phenyl)boronic acid (1.3011 g, 4.50 mmol), Pd(PPh<sub>3</sub>)<sub>4</sub> (0.0924 g, 0.08 mmol) were dissolved in degassed acetonitrile (20 ml) and a saturated aqueous solution of K<sub>2</sub>CO<sub>3</sub> (5 mL) was then added. After stirring for 24 h at 80 °C under nitrogen, the solution was cooled to room temperature, removed the solvent under reduced pressure. The residue was extracted by dichloromethane and then washed with water. The organic solution was dried over anhydrous MgSO<sub>4</sub>, and then the solvent was removed under reduced pressure. The crude product was purified by silica gel column using dichloromethane/petroleum mixture (1/6, V<sub>d</sub>/V<sub>p</sub>) as eluent to give compound **8** with 68.5%

yield.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 7.94 (d,  $J$  = 8.0 Hz, 2H), 7.43 (dd,  $J$  = 8.0, 15.2 Hz, 8H), 7.31-7.20(m, 9H), 7.18-7.06 (m, 17H), 7.02 (t,  $J$  = 7.5 Hz, 4H), 6.54 (s, 2H), 4.36 (q,  $J$  = 7.2 Hz, 2H), 1.37 (t,  $J$  = 7.2 Hz, 3H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 166.00, 147.55, 146.28, 143.25, 130.03, 129.54, 129.28, 129.05, 128.86, 124.59, 123.01, 122.74, 109.96, 61.22, 14.40. MS (APCI,  $m/z$ ) Calcd for  $\text{C}_{61}\text{H}_{47}\text{N}_3\text{O}_2$   $[\text{M}+\text{H}]^+$ : 854.37, found: 854.37.

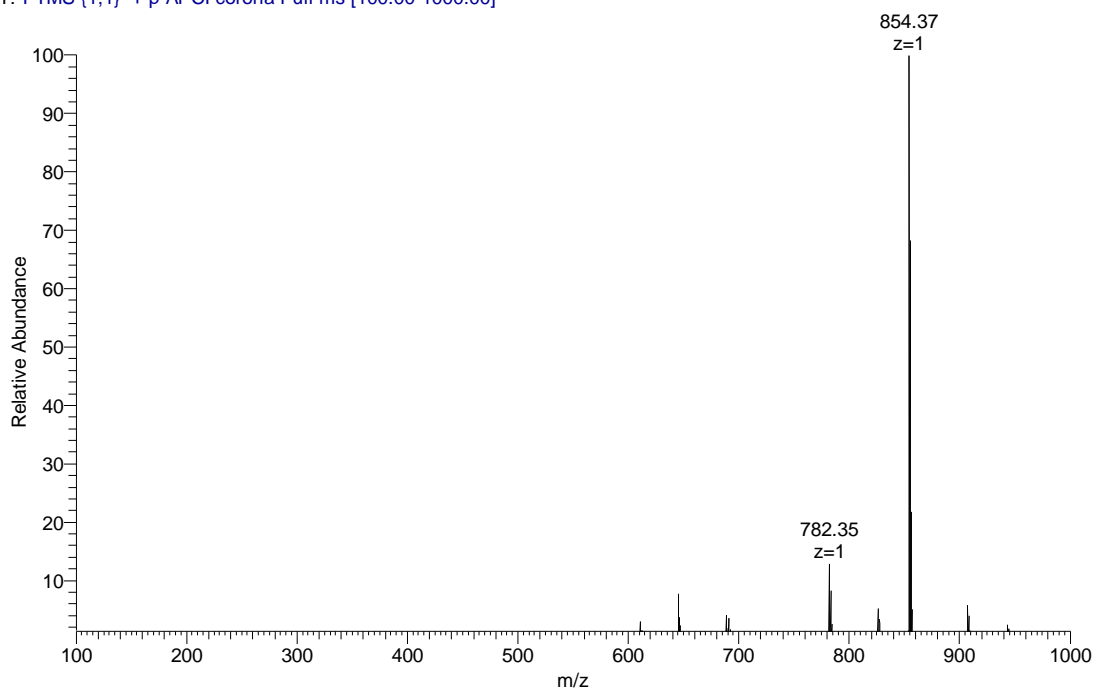


**Figure S28.**  $^1\text{H}$  NMR spectrum of **8** in  $\text{CDCl}_3$ .

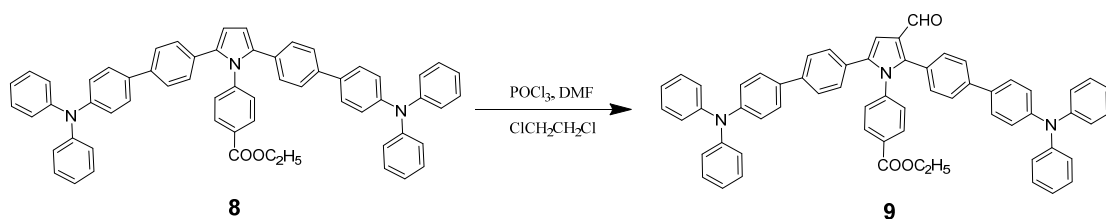


**Figure S29.**  $^{13}\text{C}$  NMR spectrum of **8** in  $\text{CDCl}_3$ .

TPP-2TPN #7 RT: 0.08 AV: 1 NL: 1.41E8  
 T: FTMS {1,1} + p APCI corona Full ms [100.00-1000.00]



**Figure S30.** MS spectrum of **8**.



**Scheme S12.** The synthetic route to compound **9**.

POCl<sub>3</sub> (100 μL, 1.10 mmol) was slowly dropped into DMF (24 mL) at 0 °C and stirred for 1 h at room temperature. Then dichloromethane solution of compound **8** (0.8534 g, 1.00 mmol) was added into the above solution. After the mixture was stirred for 12 h at room temperature, the resultant solution was poured into water (250 mL) and extracted by dichloromethane. The dichloromethane solution was dried over anhydrous MgSO<sub>4</sub> and filtered by suction, and then the solvent was evaporated by vacuum distillation. The crude product was purified by gel chromatography using dichloromethane/petroleum mixture (1/2, V<sub>d</sub>/V<sub>p</sub>) as eluent. Product **9** was obtained with a yield of 73.8%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ = 9.79 (s, 1H), 7.92 (d, *J* = 8.1 Hz, 2H), 7.53-7.46 (m, 3H), 7.46-7.38 (m, 5H), 7.32-7.17 (m, 11H), 7.16-7.07 (m, 16H), 7.07-6.97 (m, 5H), 4.34 (q, *J* = 7.2 Hz, 2H), 1.36 (t, *J* = 7.2 Hz, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ D= 186.98, 165.64, 147.62, 147.56, 147.50, 141.35, 133.91, 131.59, 130.39, 129.99, 129.74, 129.39, 129.23, 128.75, 127.72, 127.60, 127.29, 126.39, 126.26, 124.68, 124.63, 124.54, 123.82, 123.72, 123.22, 123.10, 108.10, 61.42, 14.34. MS (APCI, *m/z*) Calcd for C<sub>62</sub>H<sub>47</sub>N<sub>3</sub>O<sub>3</sub> [M+H]<sup>+</sup>: 882.36, found: 882.37.

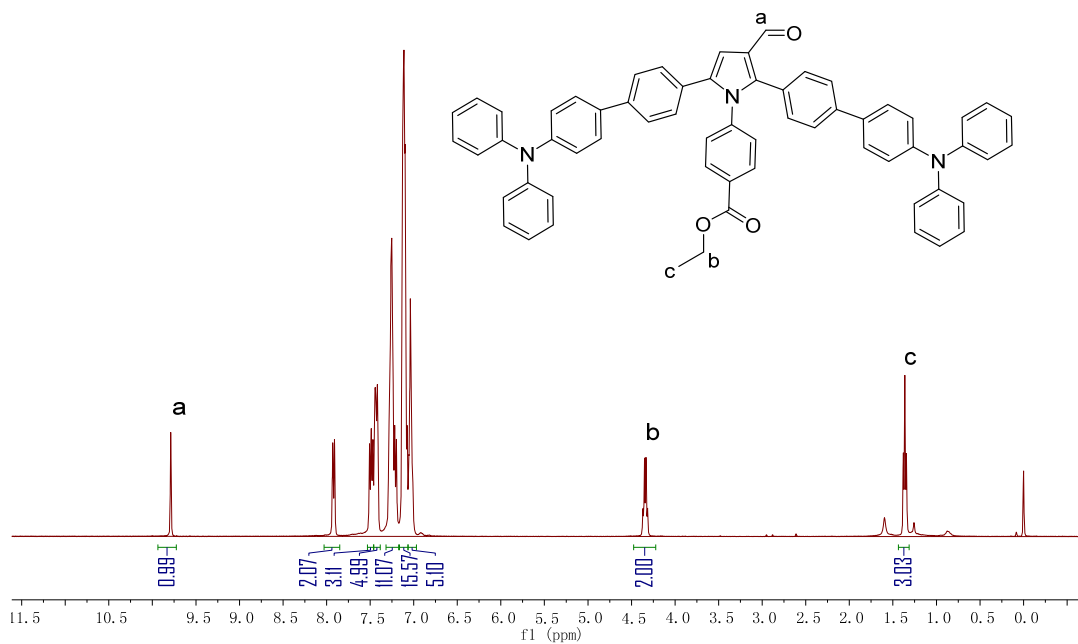


Figure S31.  $^1\text{H}$  NMR spectrum of **9** in  $\text{CDCl}_3$ .

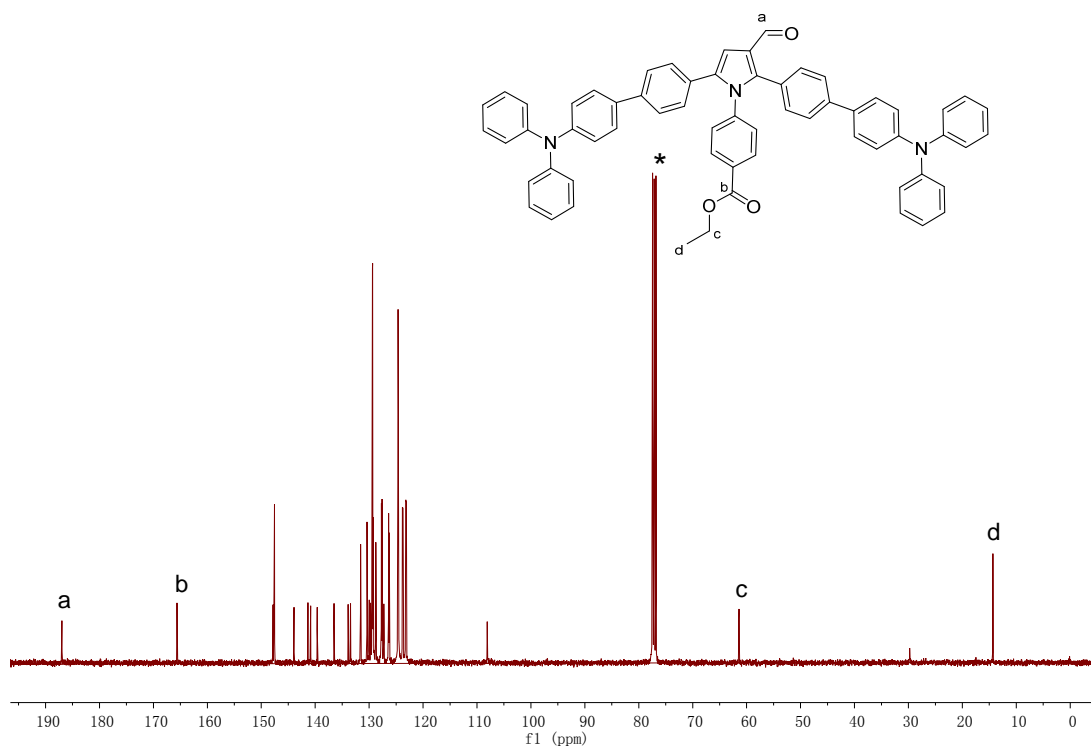
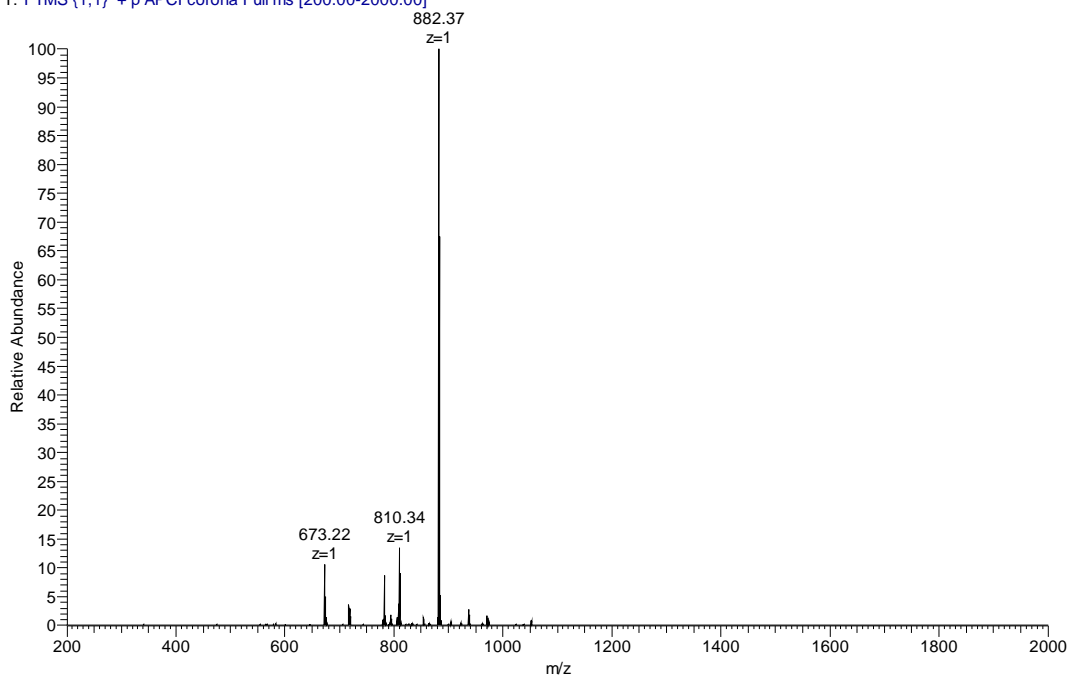
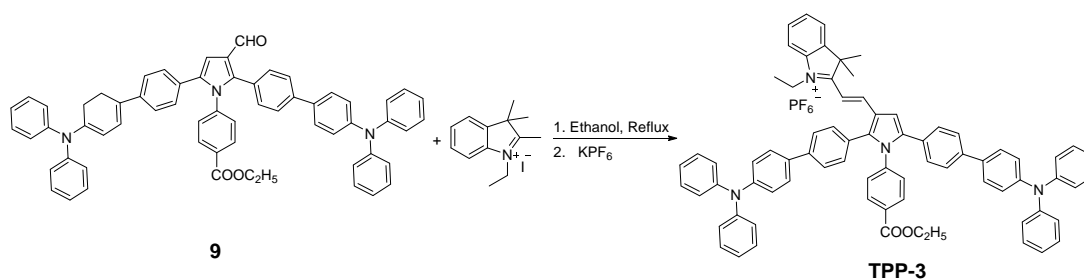


Figure S32.  $^{13}\text{C}$  NMR spectrum of **9** in  $\text{CDCl}_3$ .

TPP-2TPA-CHO #7 RT: 0.09 AV: 1 NL: 4.07E8  
T: FTMS {1,1} + p APCI corona Full ms [200.00-2000.00]



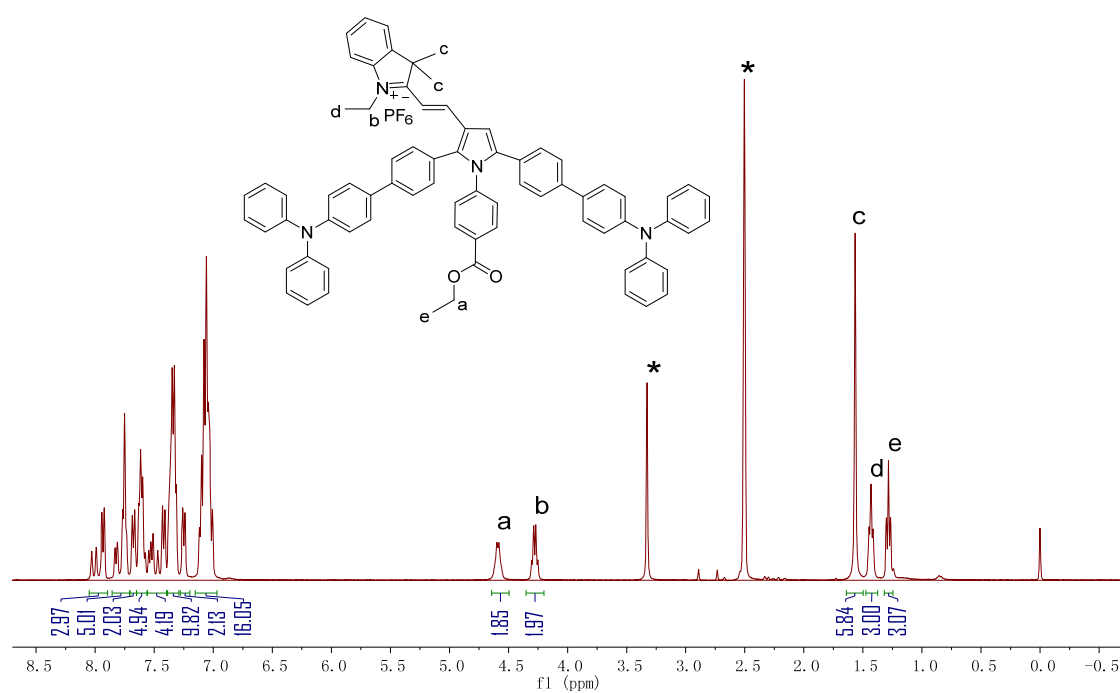
**Figure S33.** MS spectrum of **9**.



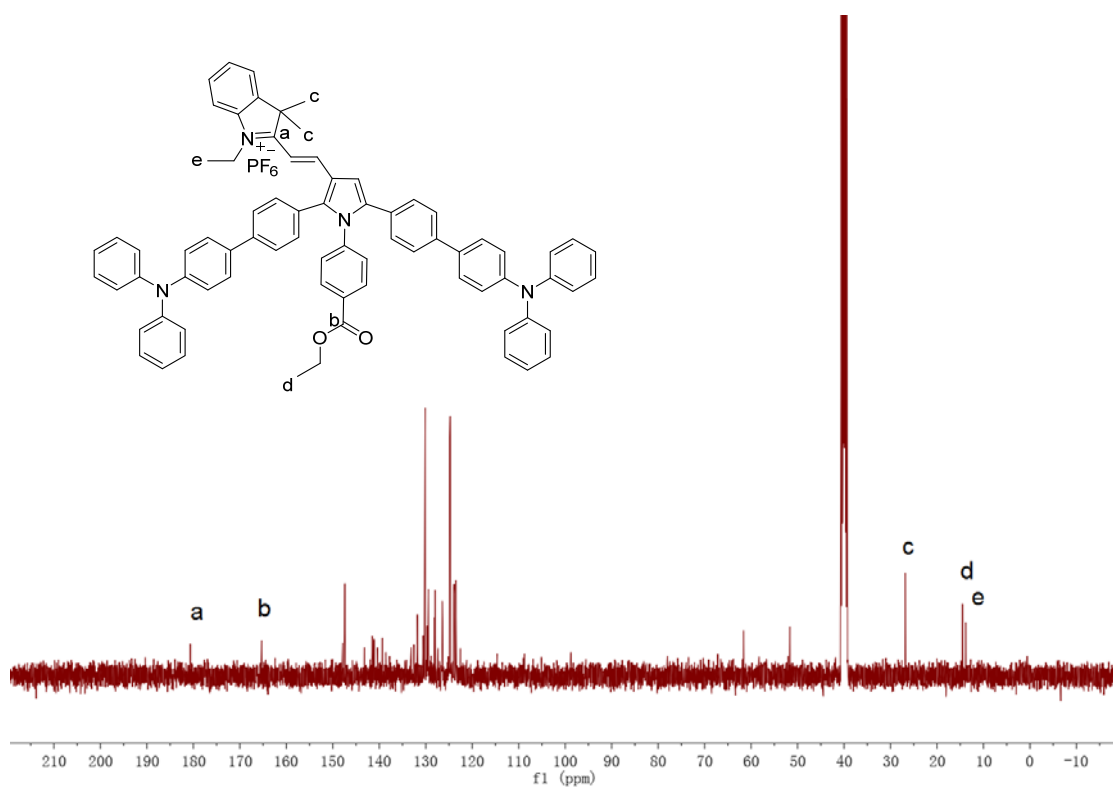
**Scheme S13.** The synthetic route to target compound **TPP-3**.

A solution of **9** (0.2377 mg, 0.60 mmol) and indolium iodide salt (0.2068 mg, 0.65 mmol) in dry ethanol (15 mL) were fluxed for 24 h. After the reaction mixture was cooled to room temperature, the solvent was evaporated under reduced pressure. The residue was dissolved in THF (5 mL) and a saturated aqueous solution of KPF<sub>6</sub> (5 mL) was then added. After stirring for 30 min, the solvent was removed to give the crude product. The crude product was purified by a silica gel column chromatography using dichloromethane/methanol mixture (10/1, V<sub>d</sub>/V<sub>m</sub>) as eluent to give a red solid in 78.2% yield. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ = 8.02 (d, *J* = 16.0 Hz, 1H), 7.93 (d, *J* = 8.0 Hz, 2H), 7.82 (d, *J* = 8.0 Hz, 1H) 7.75 (t, *J* = 7.5 Hz, 4H), 7.68 (d, *J* = 8.0 Hz, 2H), 7.63-7.47 (m, 7H), 7.42 (d, *J* = 8.0 Hz, 2H), 7.37-7.31 (m,

10H), 7.25 (d,  $J = 7.6$  Hz, 2H), 7.11-7.01 (m, 16H), 4.59 (q,  $J = 6.4$  Hz, 2H), 4.28 (q,  $J = 6.4$  Hz, 2H), 1.57 (s, 6H), 1.43 (t,  $J = 6.4$  Hz, 3H), 1.28 (t,  $J = 7.2$  Hz, 3H).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta = 180.66, 165.29, 147.52, 147.40, 147.35, 141.51, 140.41, 139.33, 132.54, 131.80, 130.57, 130.37, 130.15, 130.11, 129.67, 129.43, 128.12, 127.99, 126.44, 126.35, 124.83, 124.73, 124.00, 123.88, 123.52, 123.44, 61.63, 51.65, 26.83, 14.52, 13.84$ . HR-MS (ESI,  $m/z$ ) Calcd for  $\text{C}_{75}\text{H}_{63}\text{N}_4\text{O}_2$   $[\text{M}]^+$ : 1051.4945, found: 1051.4933, error -1.2 ppm.

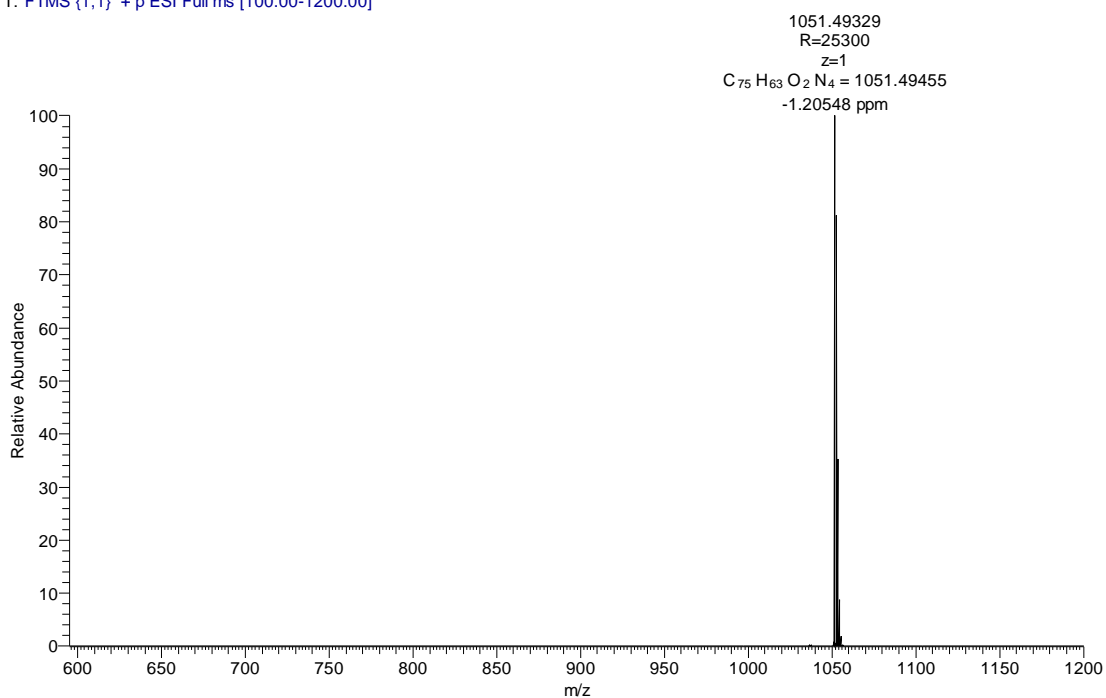


**Figure S34.**  $^1\text{H}$  NMR spectrum of TPP-3 in DMSO- $d_6$ .



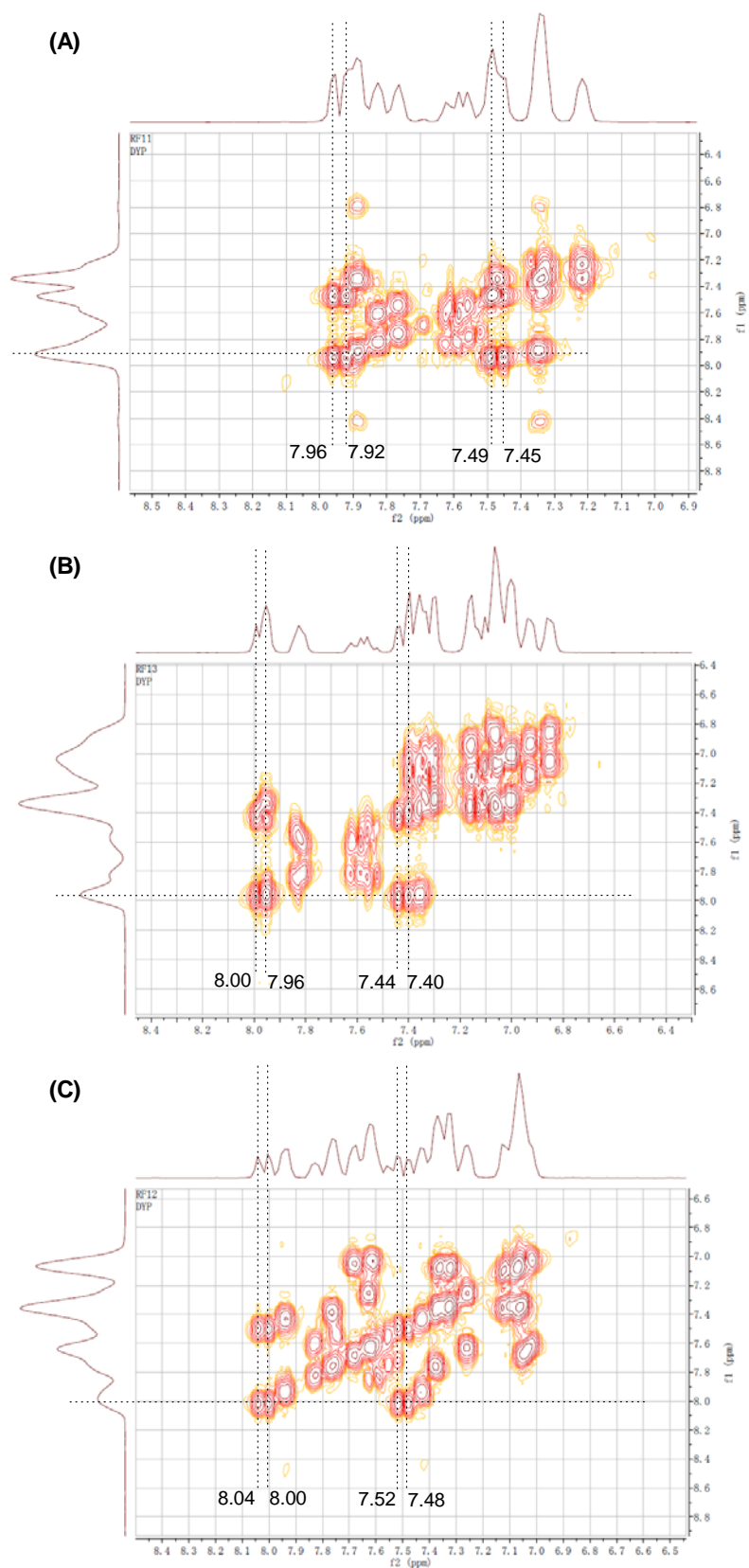
**Figure S35.**  $^{13}\text{C}$  NMR spectrum of **TPP-3** in  $\text{DMSO-}d_6$ .

RF-12\_180709112624 #33 RT: 0.44 AV: 1 NL: 4.80E6  
T: FTMS {1,1} + p ESI Full ms [100.00-1200.00]



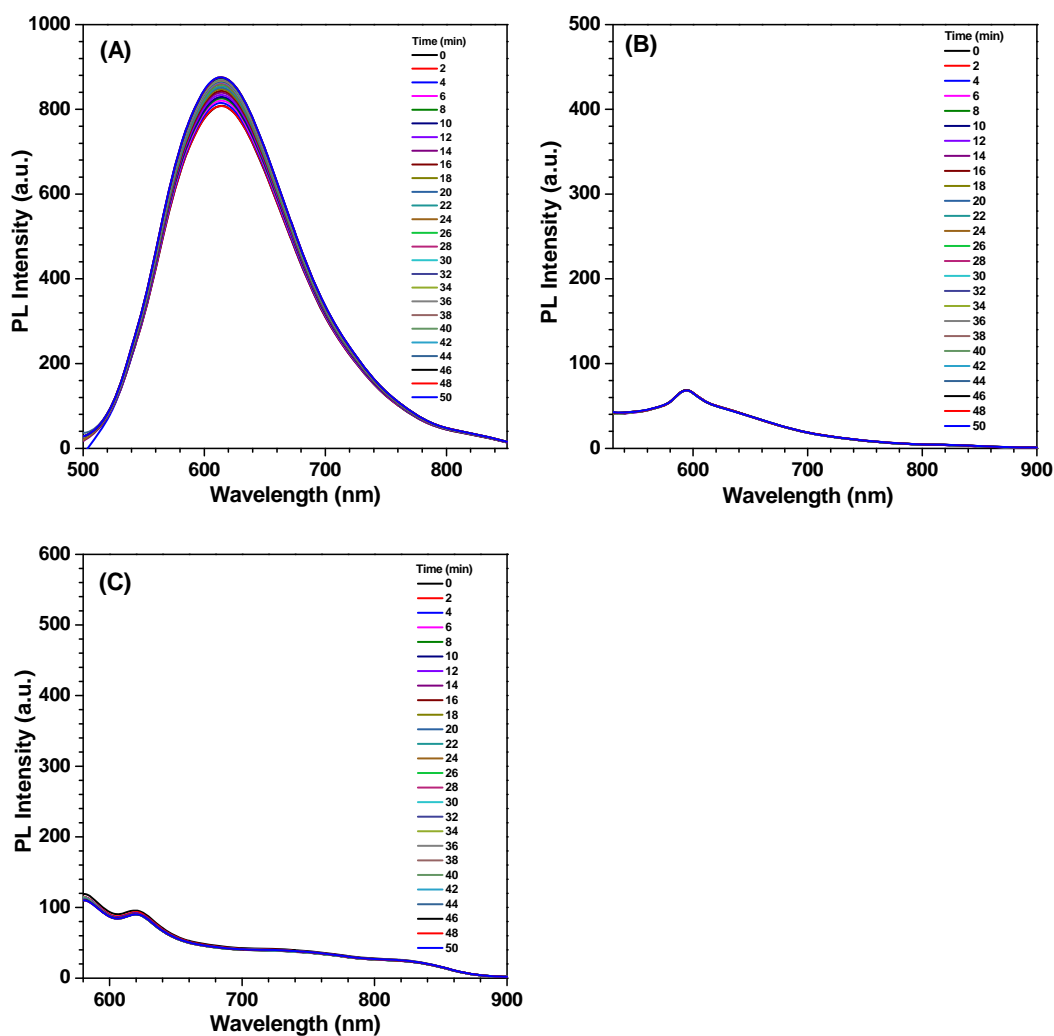
**Figure S36.** HR-MS spectrum of **TPP-3**.



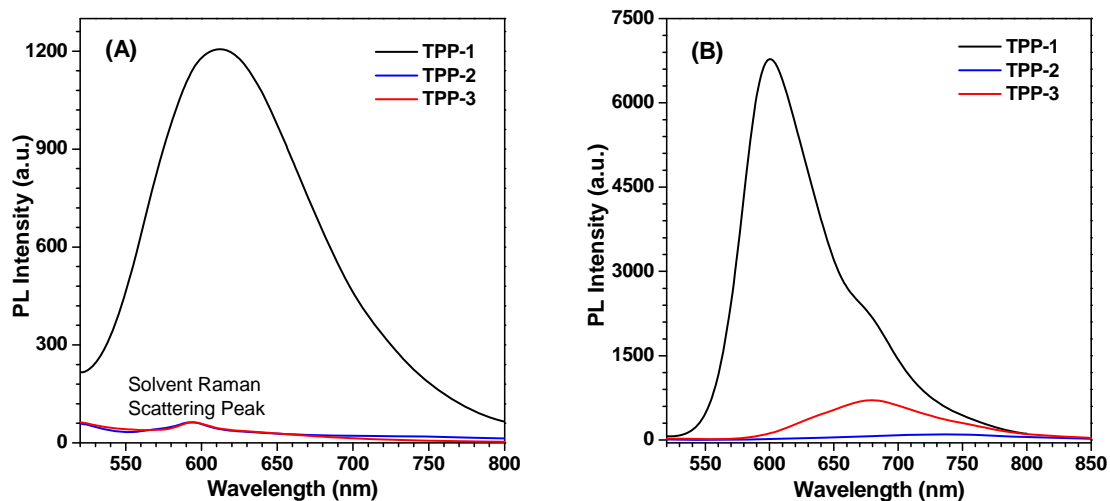


**Figure S37.** <sup>1</sup>H-<sup>1</sup>H COSY NMR spectra of (A) TPP-1, (B) TPP-2, and (C) TPP-3 in DMSO-*d*<sub>6</sub>.

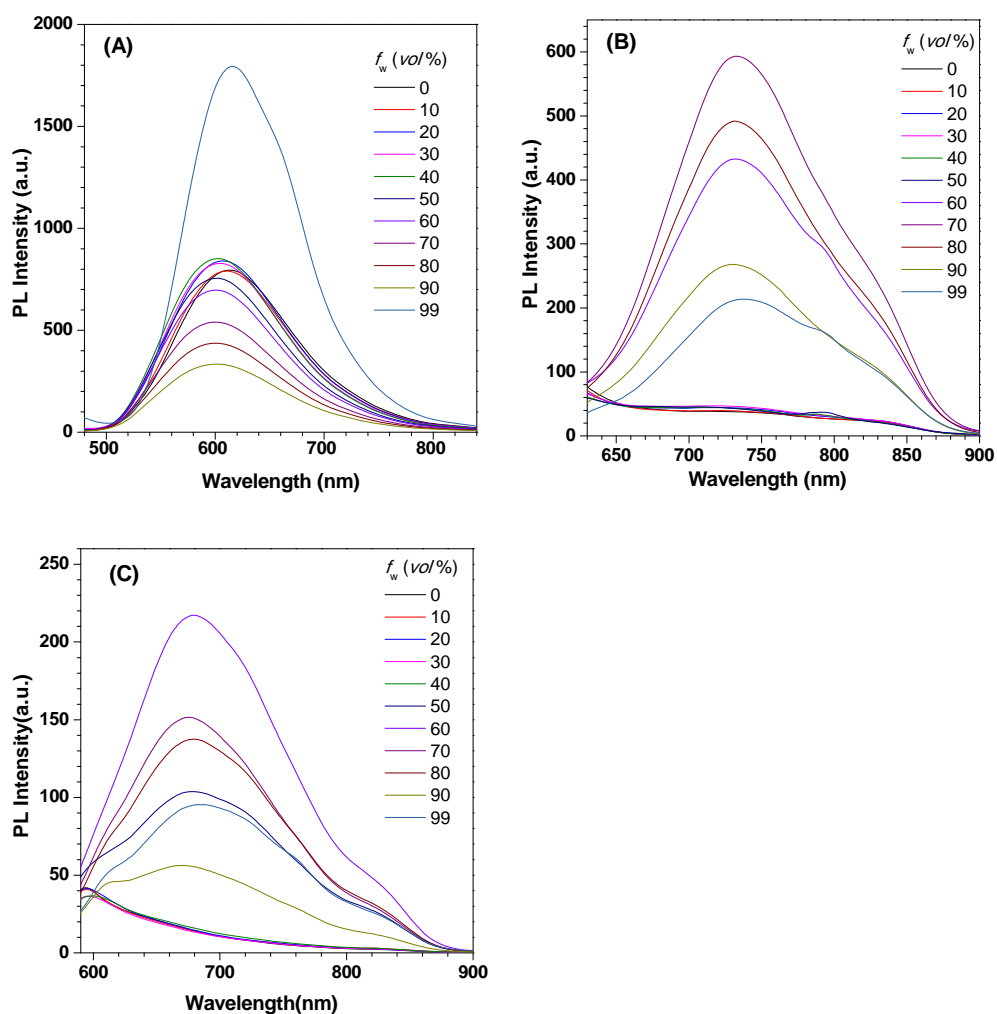
## 2. PL spectra of TPP-1~3



**Figure S38.** PL spectra of DMSO solution of (A) TPP-1, (B) TPP-2 and (C) TPP-3 at different time. Excitation wavelengths:  $\lambda_{\text{ex}} = 460$  nm for TPP-1, 520 nm for TPP-2, and 500 nm for TPP-3.  $[\text{TPP-1}] = [\text{TPP-2}] = [\text{TPP-3}] = 1.0 \times 10^{-5}$  mol/L.

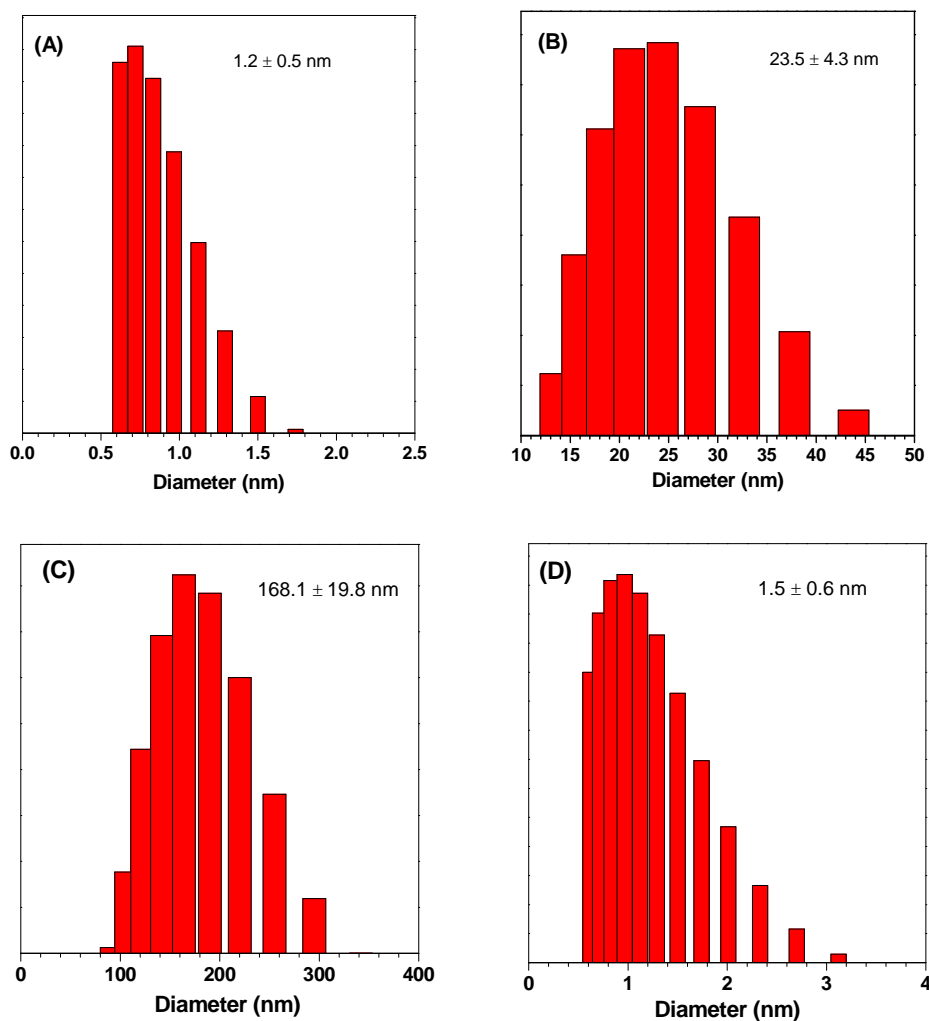


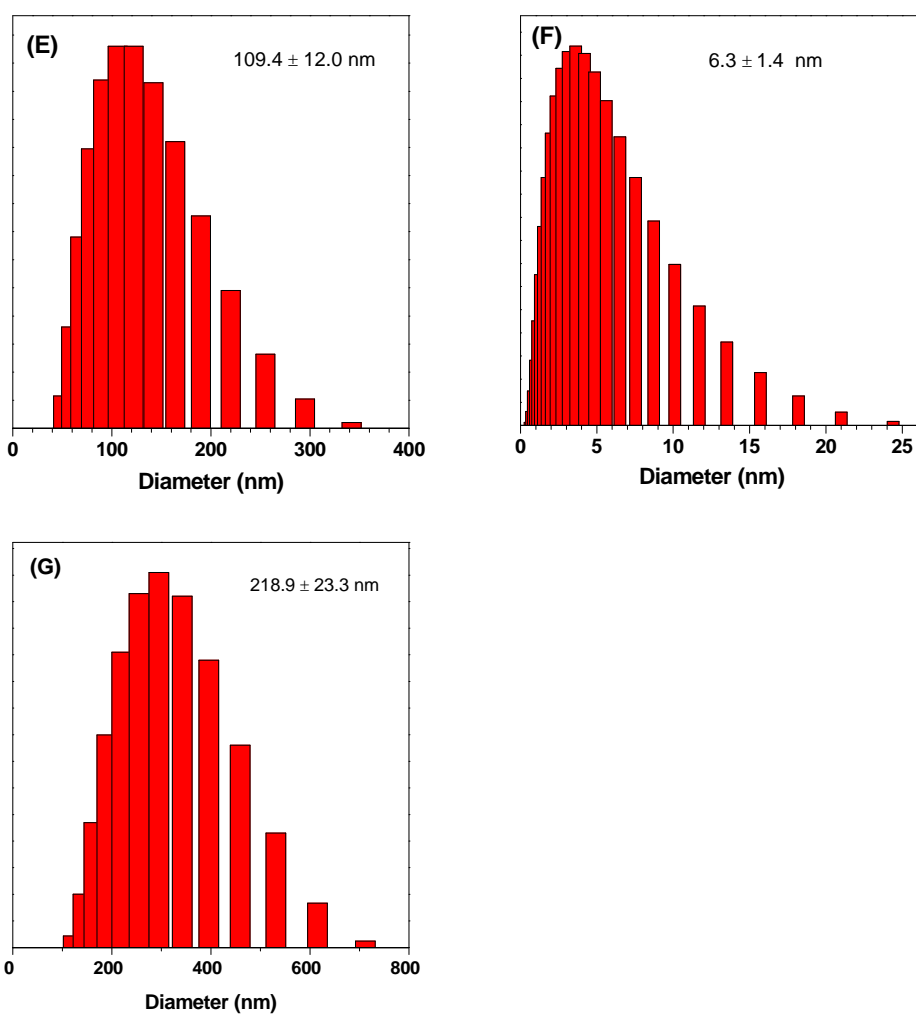
**Figure S39.** (A) PL spectra of organic solutions of TPP-1~3 in DMSO ( $[TPP-1] = [TPP-2] = [TPP-3] = 1.0 \times 10^{-5}$  mol/L). (B) PL spectra of TPP-1~3 in solid. Excitation wavelength:  $\lambda_{ex} = 500$  nm.



**Figure S40.** PL spectra of (A) TPP-1, (B) TPP-2 and (C) TPP-3 in the mixture of DMSO/H<sub>2</sub>O with different water fractions ( $f_w$ ). Excitation wavelengths:  $\lambda_{ex} = 460$  nm for TPP-1, 520 nm for TPP-2, and 500 nm for TPP-3.  $[TPP-1] = [TPP-2] = [TPP-3] = 1.0 \times 10^{-5}$  mol/L.

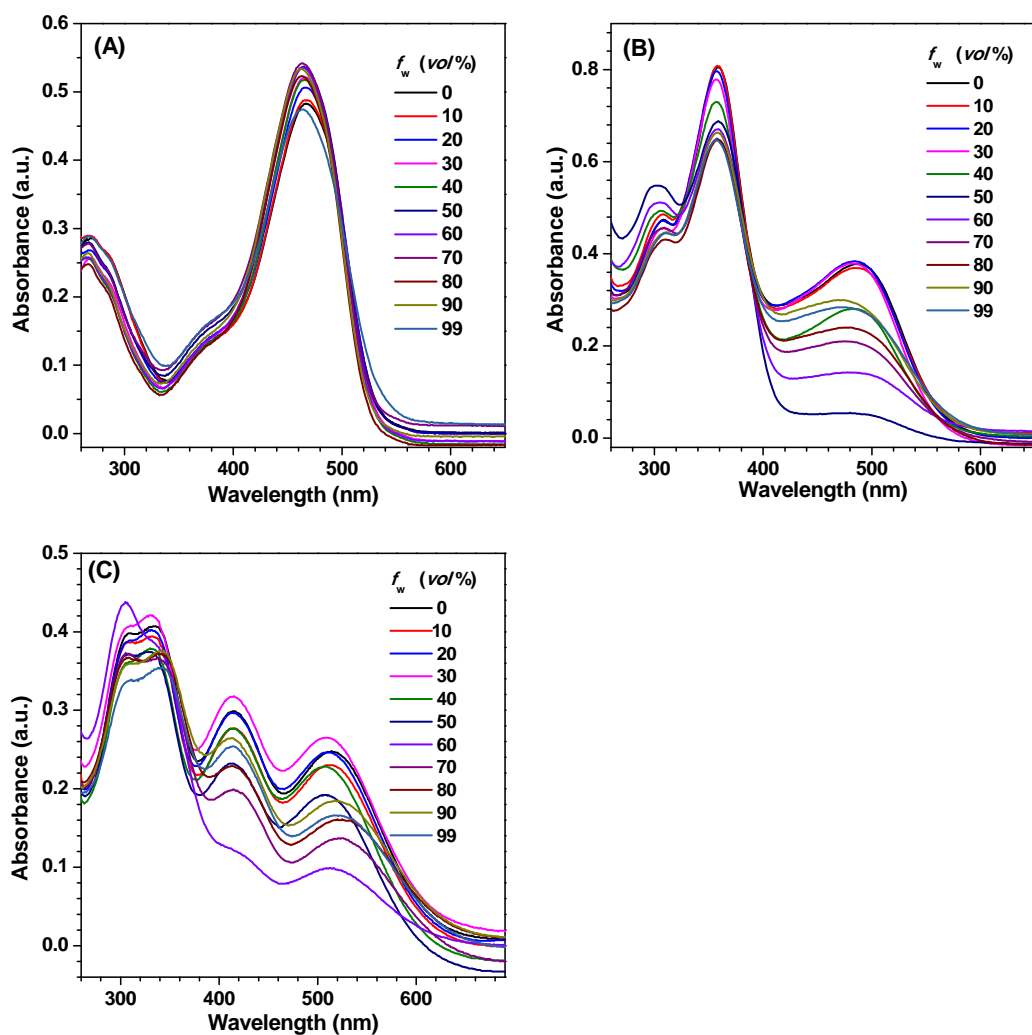
### 3. Particle diameter size distributions





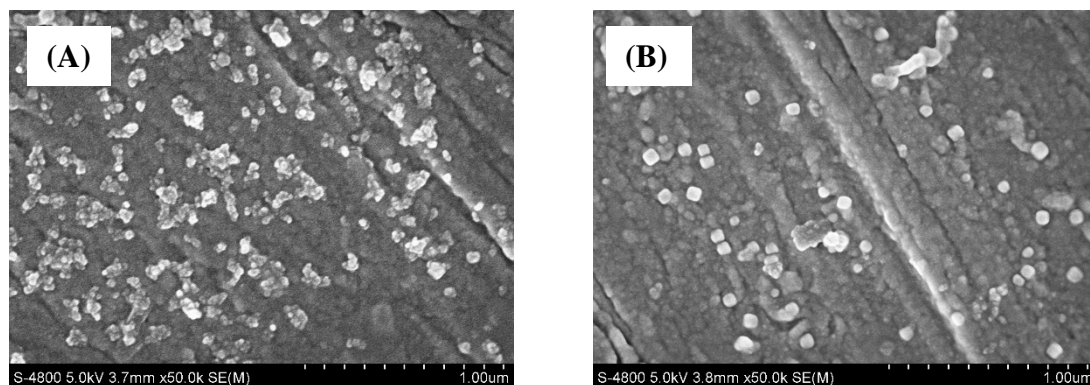
**Figure S41.** Particle diameter size distributions of (A) TPP-1 in DMSO, (B) TPP-1 in DMSO/H<sub>2</sub>O (1:9), (C) TPP-1 in DMSO/H<sub>2</sub>O (1:99), (D) TPP-2 in DMSO, (E) TPP-2 in DMSO/H<sub>2</sub>O (3:7), (F) TPP-3 in DMSO, (G) TPP-3 in DMSO/H<sub>2</sub>O (4:6). [TPP-1] = [TPP-2] = [TPP-3] =  $1.0 \times 10^{-5}$  mol/L.

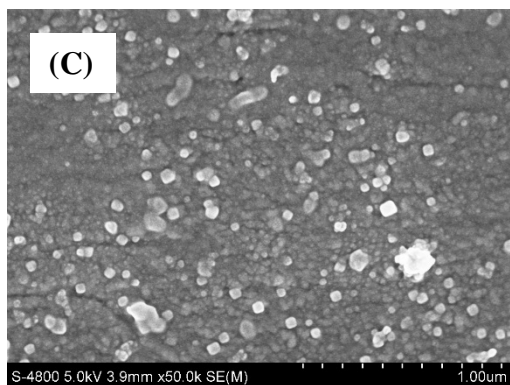
#### 4. UV-visible spectral changes on addition of H<sub>2</sub>O



**Figure S42.** Absorption spectra of (A) TPP-1, (B) TPP-2 and (C) TPP-3 in the mixture of DMSO/H<sub>2</sub>O with different  $f_w$ . [TPP-1] = [TPP-2] = [TPP-3] =  $1.0 \times 10^{-5}$  mol/L.

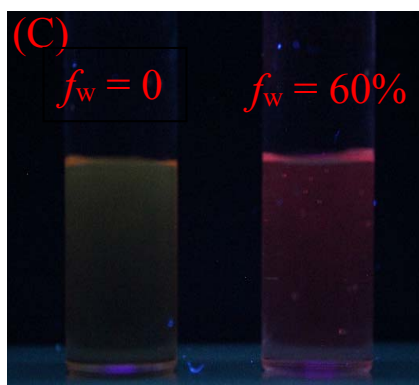
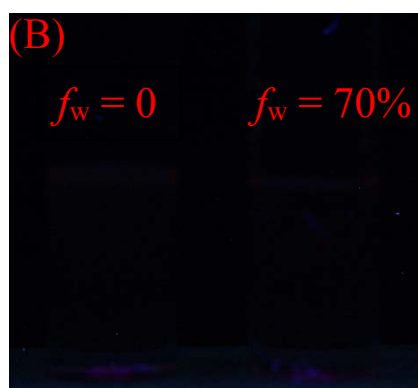
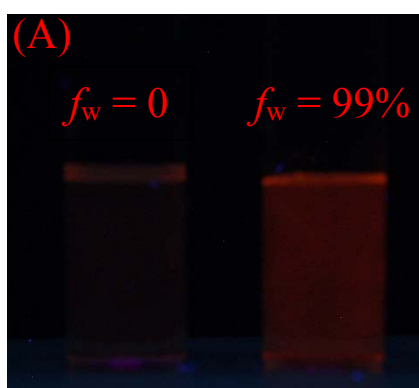
## 5. SEM images of the aggregates in the mixture of DMSO/H<sub>2</sub>O





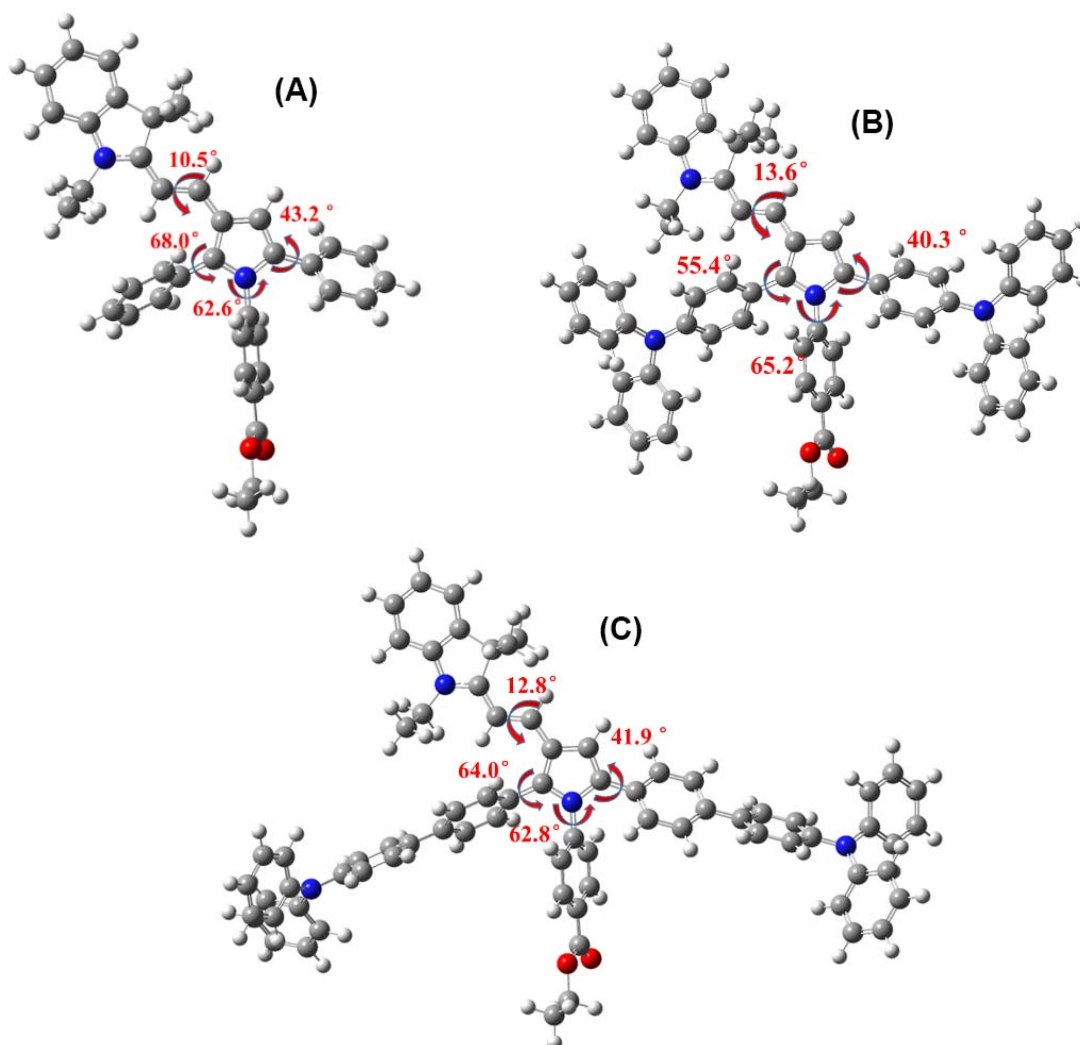
**Figure S43.** SEM images of the aggregates of (A) TPP-1, (B) TPP-2 and (C) TPP-3. The aggregates were formed in the mixture of DMSO/H<sub>2</sub>O (1:99 for TPP-1, 3:7 for TPP-2, 4:6 for TPP-3) and then filtered by pure carbon coated grids and dried in vacuum at room temperature.

## 6. Color changes in the molecular and aggregate state



**Figure S44.** Photographs of fluorescent (A) TPP-1, (B) TPP-2 and (C) TPP-3 in the mixture of DMSO/H<sub>2</sub>O with different  $f_w$  under UV radiation at 365 nm. [TPP-1] = [TPP-2] = [TPP-3] =  $1.0 \times 10^{-5}$  mol/L.

## 7. The simulated molecular conformation



**Figure S45.** The optimized molecular conformation of (A) TPP-1, (B) TPP-2 and (C) TPP-3 by using the B3LYP/6-31G basis set with G09 program.



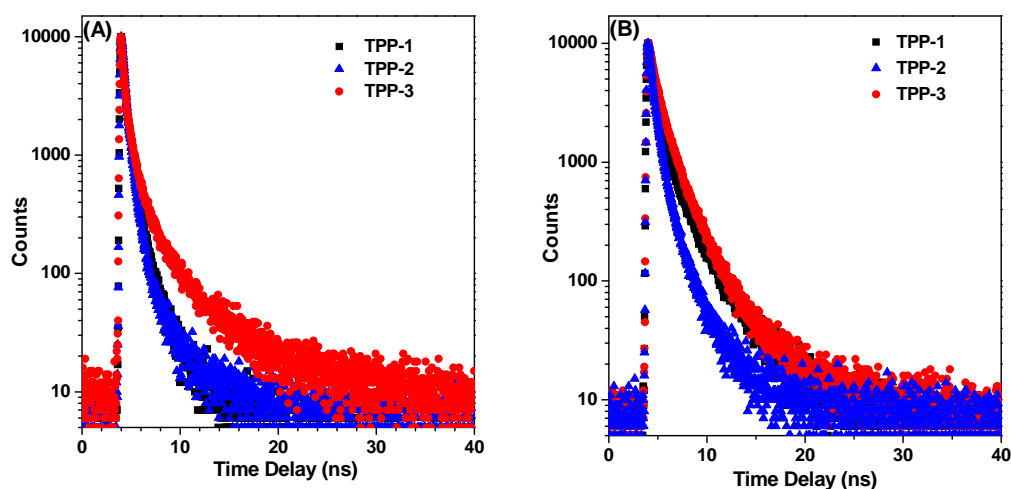
## 8. Fluorescence decay data and curves

**Table S1.** The original lifetime data of TPP-1~3 in solution

Compounds	Solution (DMSO $10^{-5}$ M)					
	$\tau_1$ (ns)	$\tau_2$ (ns)	B <sub>1</sub>	B <sub>2</sub>	$\chi^2$	$\tau_{ave}$ (ns)
TPP-1	0.98	1.34	0.80	0.20	1.58	1.05
TPP-2	-	-	-	-	-	-
TPP-3	-	-	-	-	-	-

**Table S2.** The original lifetime data of TPP-1~3 in aggregation and solid state

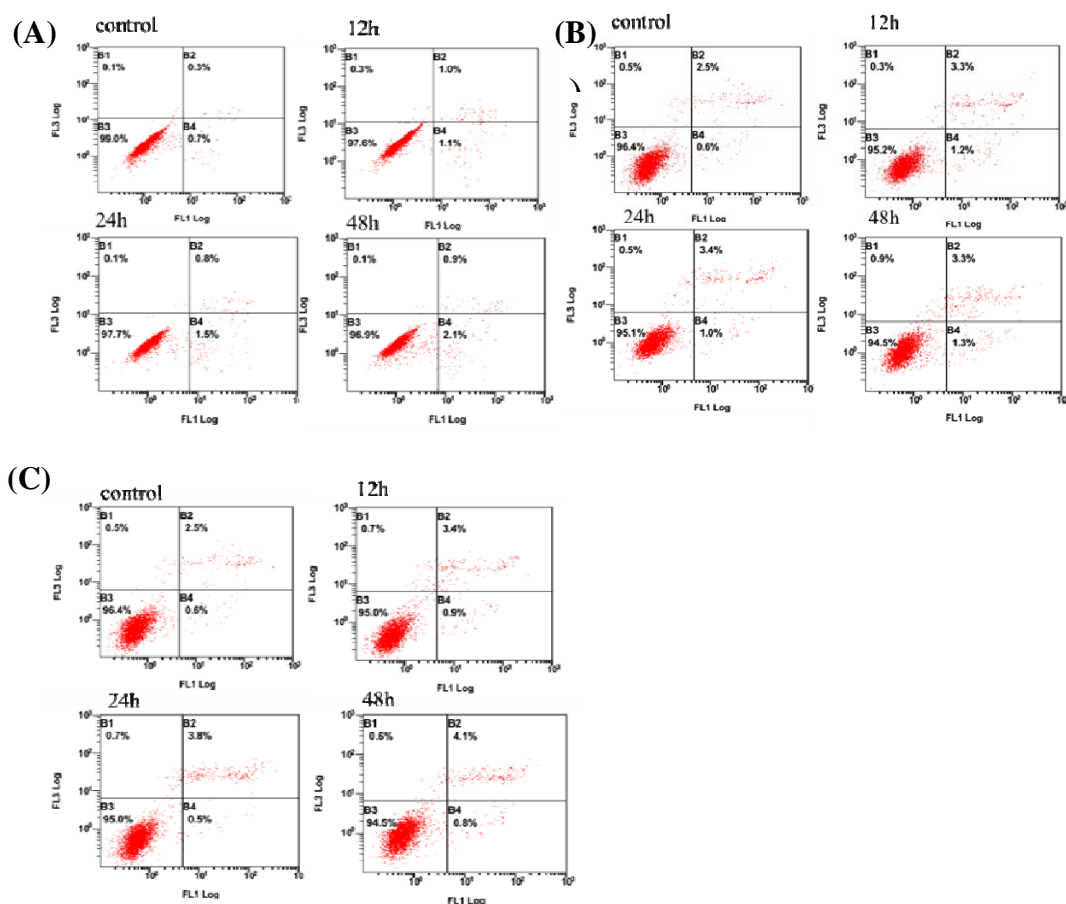
Compounds	Aggregation						Solid					
	$\tau_1$ (ns)	$\tau_2$ (ns)	B <sub>1</sub>	B <sub>2</sub>	$\chi^2$	$\tau_{ave}$ (ns)	$\tau_1$ (ns)	$\tau_2$ (ns)	B <sub>1</sub>	B <sub>2</sub>	$\chi^2$	$\tau_{ave}$ (ns)
TPP-1	0.58	2.08	0.89	0.11	1.73	0.75	0.78	2.99	0.77	0.23	1.67	1.30
TPP-2	0.52	2.31	0.92	0.08	1.85	0.65	0.63	2.04	0.82	0.18	1.90	0.89
TPP-3	0.74	3.60	0.82	0.18	1.78	1.25	1.06	2.88	0.77	0.23	1.61	1.49



**Figure S46.** The fluorescence decay curves of TPP-1, TPP-2 and TPP-3 in aggregated state (A) and in solid (B). The aggregates were obtained from the mixture of DMSO and water at  $f_w = 99 \text{ vol}\%$  for TPP-1,  $f_w = 70 \text{ vol}\%$  for TPP-2, and  $f_w = 60 \text{ vol}\%$  for TPP-3 and  $[\text{TPP-1}] = [\text{TPP-2}] = [\text{TPP-3}] = 1.0 \times 10^{-5} \text{ mol/L}$ .

## 9. Cell apoptosis test

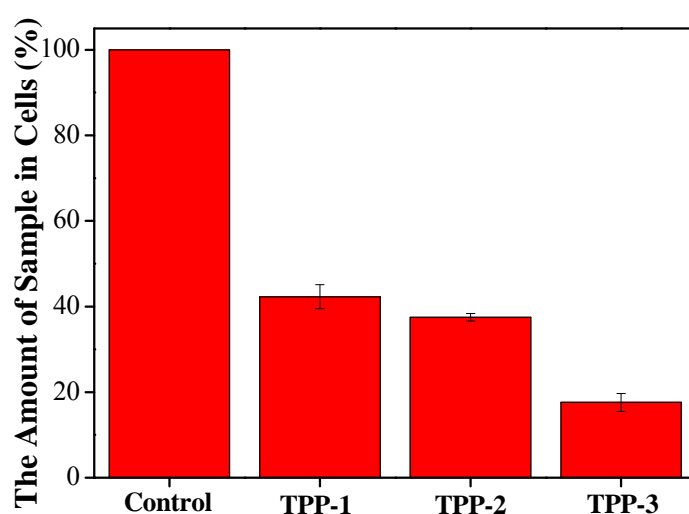
HeLa cells were stained with  $1.0 \times 10^{-7}$  mol/L of TPP-1, TPP-2 and TPP-3 solution for 12 h, 24 h, and 48 h, respectively. Cell apoptosis was measured by Annexin V-FITC/PI Apoptosis Detection Kit. Cells were digested with 0.05% Trypsin and stained with annexin V-FITC and propidium iodide in the dark at room temperature according to the manufacturer's instructions. After treatment at a given time, 400 $\mu$ L 1 x Annexin V binding solution was added into each sample. Apoptotic cells were measured by a flow cytometer.



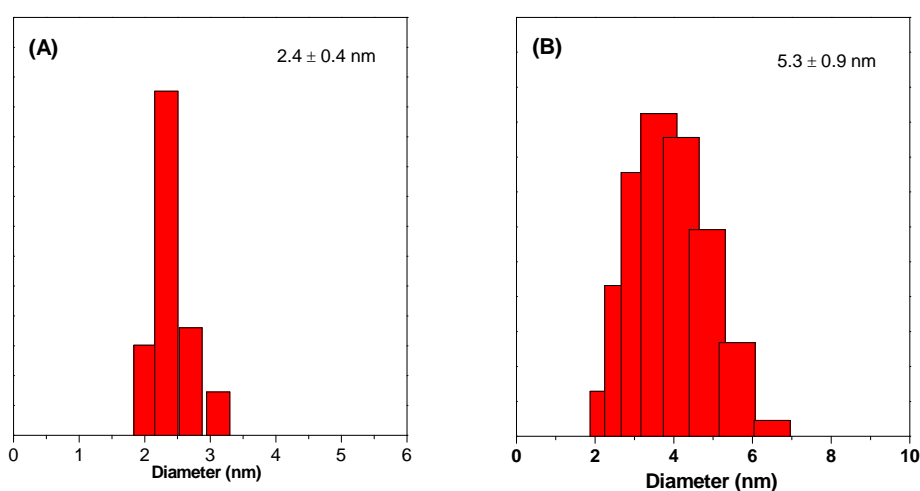
**Figure S47.** The apoptosis assay of (A) TPP-1, (B) TPP-2 and (C) TPP-3 evaluated on HeLa cells. [TPP-1] = [TPP-2] = [TPP-3] =  $1.0 \times 10^{-7}$  mol/L.

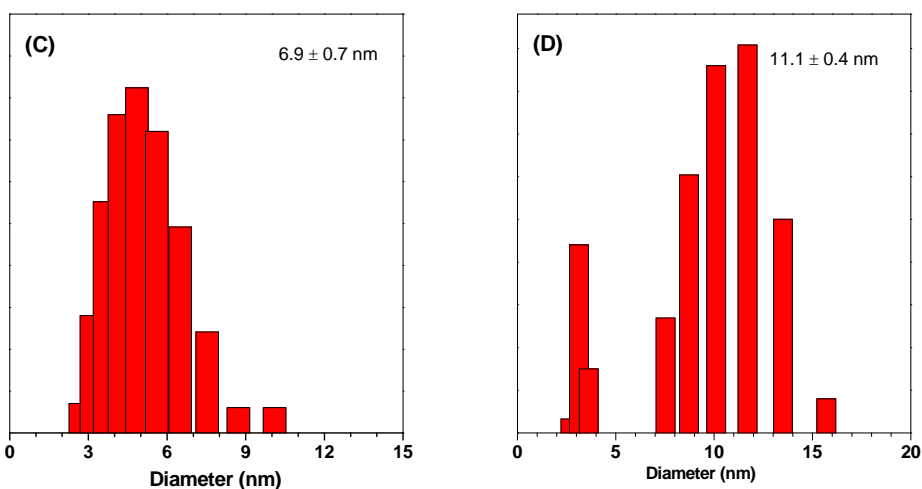
## 10. The test of amount diffused in HeLa cells

Cells were cultured after overnight culture in a humidified incubator at 37°C with 5% CO<sub>2</sub>. 1.0 × 10<sup>-6</sup> mol/L of TPP-1, TPP-2 and TPP-3 (1 mL) was added into HeLa cells with 9 mL of DMEM. 1.0 × 10<sup>-6</sup> mol/L of TPP-1, TPP-2 and TPP-3 (1 mL) was added into 9 mL DMEM without HeLa cells as control group. After 15 min incubation, all solutions were taken out and the UV tests were measured.

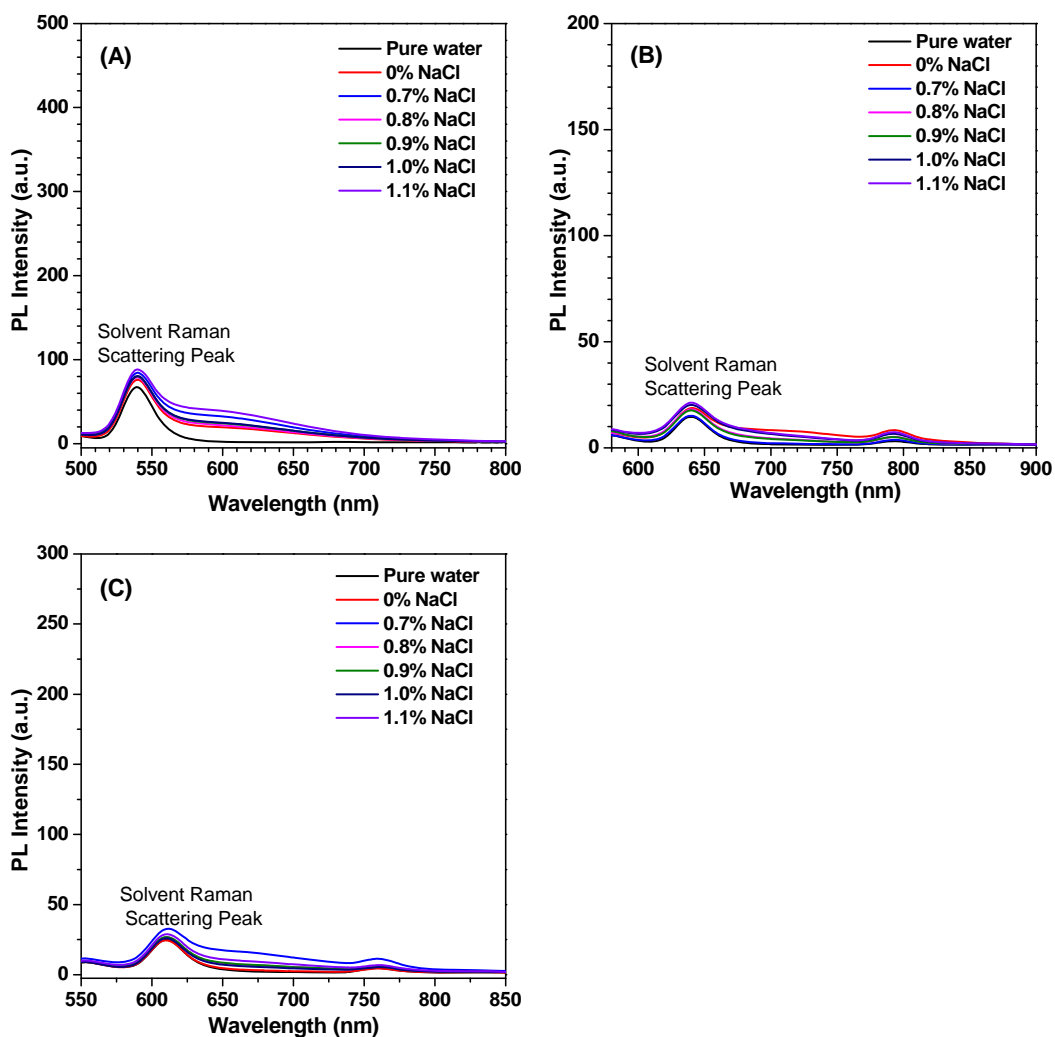


**Figure S48.** The amount of TPP-1, TPP-2 and TPP-3 in HeLa cells after 15 min incubation in the same concentration at [TPP-1] = [TPP-2] = [TPP-3] = 1.0 × 10<sup>-7</sup> mol/L.





**Figure S49.** Particle diameter size distributions of (A) DMEM, (B) TPP-1 in DMEM, (C) TPP-2 in DMEM, (D) TPP-3 in DMEM at  $[TPP-1] = [TPP-2] = [TPP-3] = 1.0 \times 10^{-7}$  mol/L.

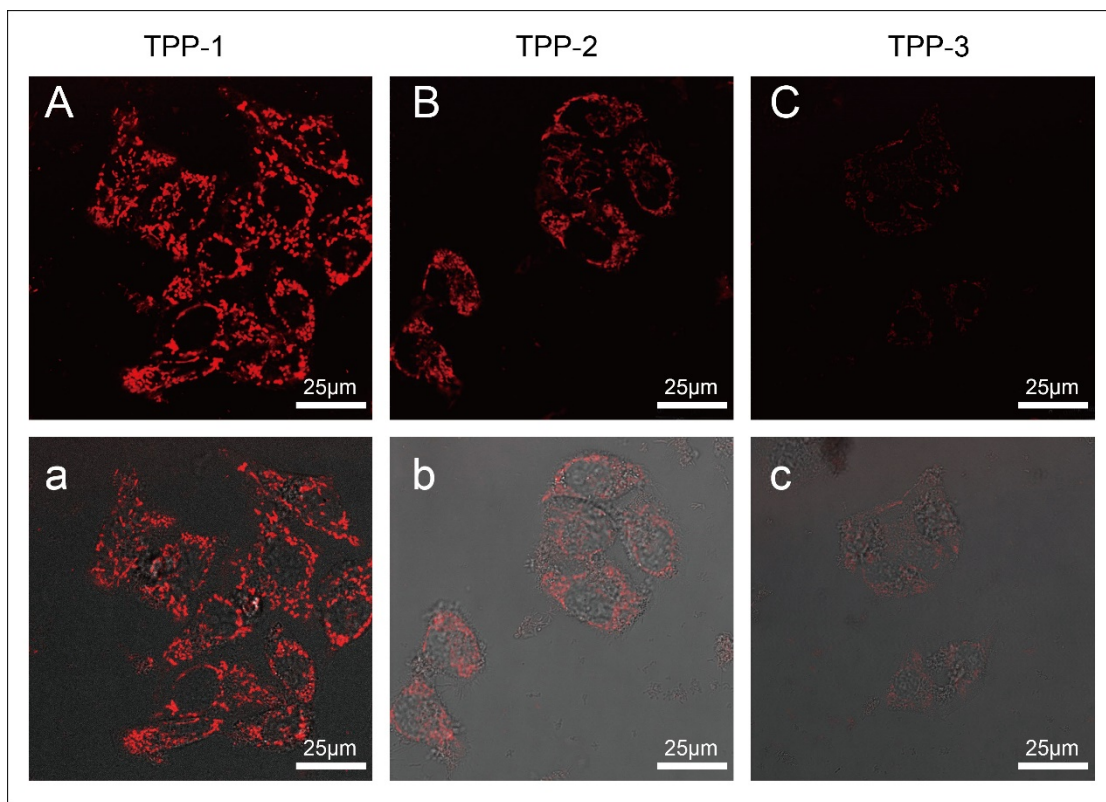


**Figure S50.** PL spectra of (A) TPP-1, (B) TPP-2 and (C) TPP-3 in DMSO/ aqueous solution of NaCl (1:99) with different NaCl contents ( $wt\%$ ). Excitation wavelengths:  $\lambda_{ex} = 460$  nm for

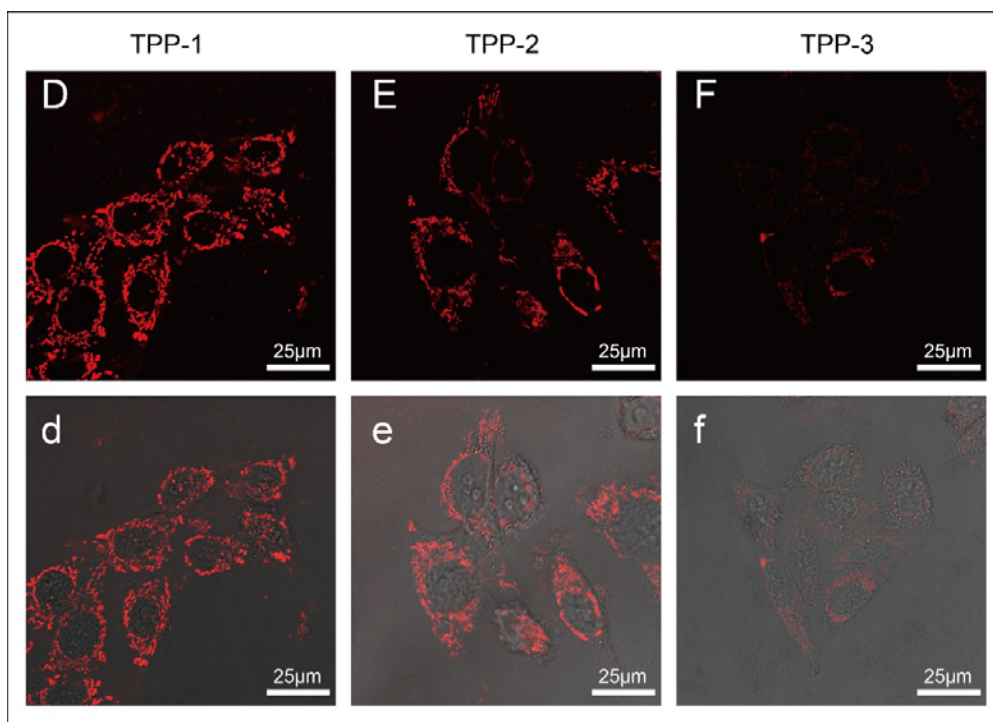
TPP-1, 520 nm for TPP-2, and 500 nm for TPP-3.  $[TPP-1] = [TPP-2] = [TPP-3] = 1.0 \times 10^{-7}$  mol/L.

## **11. Confocal fluorescent images of TPP-1, TPP-2 and TPP-3 in living cells**

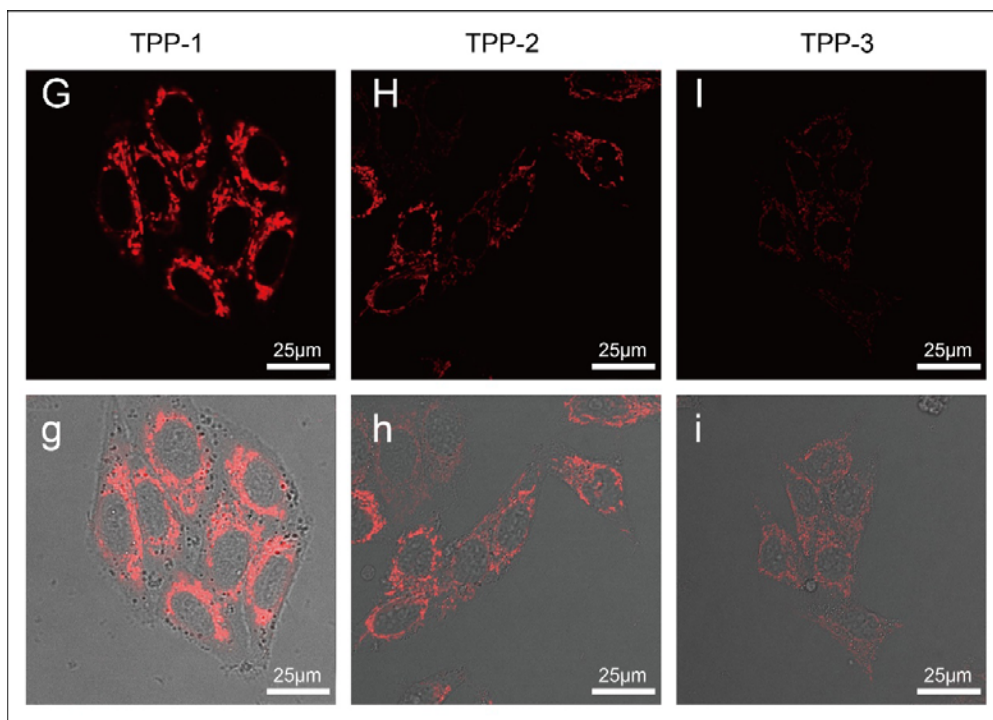
HeLa, MCF-7, HepG2 and NIH3T3 cells were seeded in  $\Phi 20$  mm glass bottom cell culture dishes ( $7.2 \times 10^5 \pm 0.05 \times 10^5$  cells in each dish). After overnight culture in a humidified incubator at 37 °C with 5% CO<sub>2</sub>, culture medium was removed and cells were stained with  $1.0 \times 10^{-7}$  mol/L of TPP-1, TPP-2 and TPP-3 for 5 min in DMSO/DMEM (1% DMSO) solution at 37 °C. Before imaging, no washing processed were needed. The fluorescence imaging of TPP-1, TPP-2 and TPP-3 in living cells was recorded with a Leica TCS SP5 laser scanning confocal microscope.



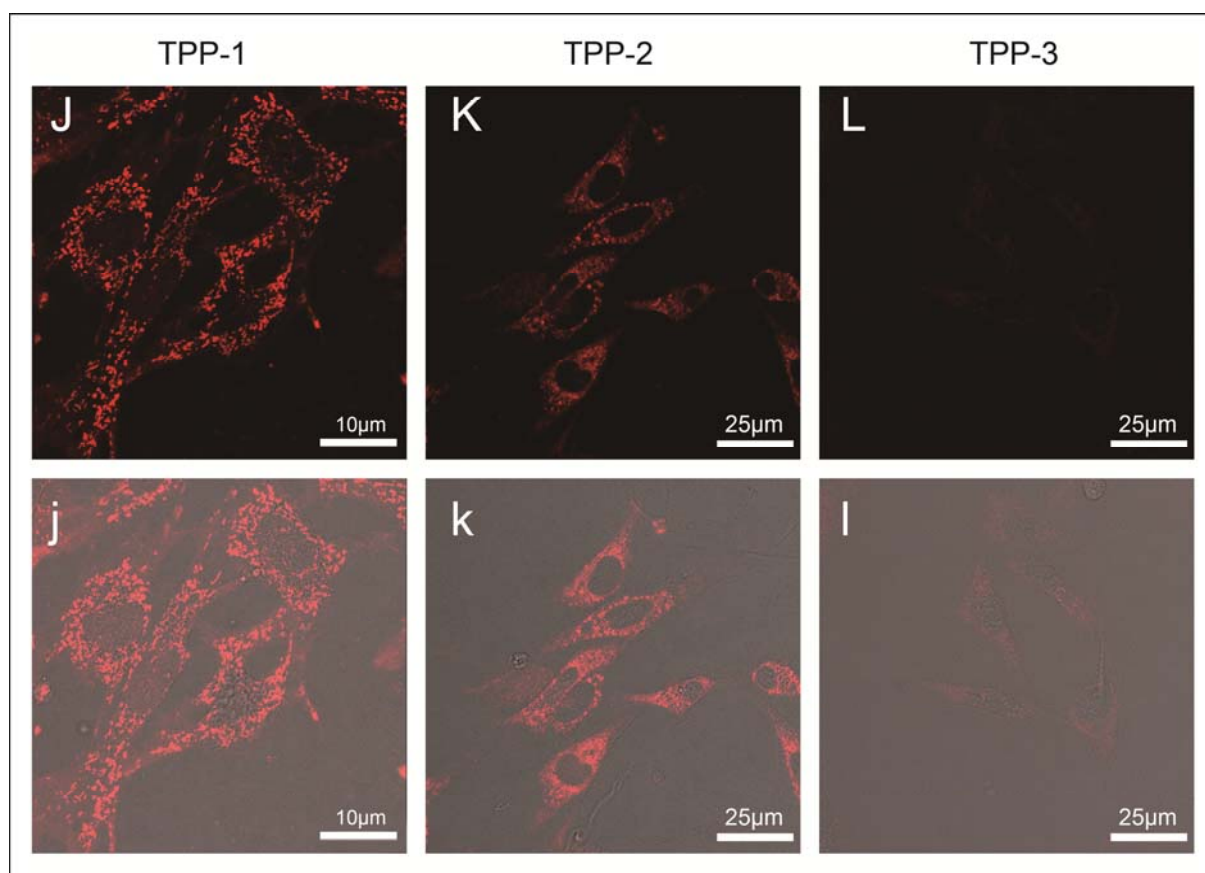
**Figure S51.** Fluorescent images of TPP-1, TPP-2 and TPP-3 in HeLa cells after 5 min incubation in the same concentration at  $[TPP-1] = [TPP-2] = [TPP-3] = 1.0 \times 10^{-7}$  mol/L.



**Figure S52.** Fluorescent images of TPP-1, TPP-2 and TPP-3 in HepG2 cells after 5 min incubation in the same condition at  $[TPP-1] = [TPP-2] = [TPP-3] = 1.0 \times 10^{-7}$  mol/L.



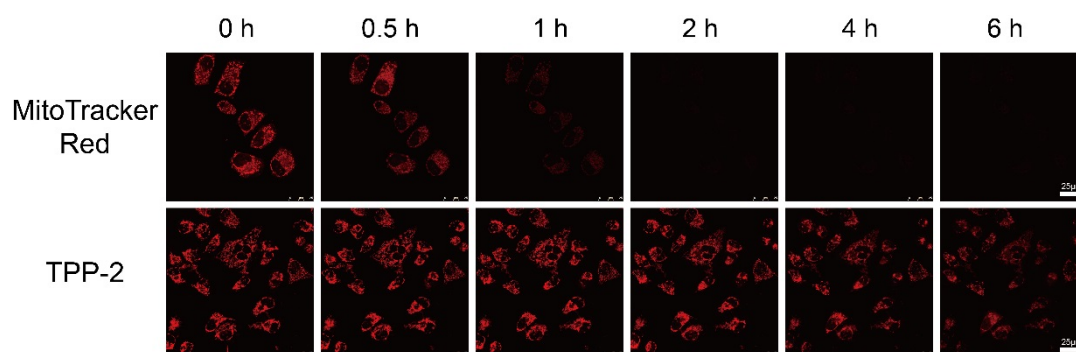
**Figure S53.** Fluorescent images of TPP-1, TPP-2 and TPP-3 in MCF-7 cells after 5 min incubation in the same condition at  $[TPP-1] = [TPP-2] = [TPP-3] = 1.0 \times 10^{-7}$  mol/L.



**Figure S54.** Fluorescent images of TPP-1, TPP-2 and TPP-3 in NIH 3T3 cells after 5 min incubation in the same condition at  $[TPP-1] = [TPP-2] = [TPP-3] = 1.0 \times 10^{-7}$  mol/L.

## 12. Photostability of TPP-2

HeLa Cells were incubated in 5% CO<sub>2</sub> and  $1.0 \times 10^{-7}$  mol/L of TPP-2 at 37 °C for 5 min. In colocalization experiments, the cells were first washed with PBS, and then incubated with  $1.0 \times 10^{-7}$  mol/L of MitoTracker Red at 37 °C for 30 min. The medium was removed and the cells were rinsed with PBS. The cells were imaged immediately and at an interval by using Leica TCS SP5 laser scanning confocal microscope.



**Figure S55.** Fluorescent images of MitoTracker Red and TPP-2 after 6 h continuous irradiation. The concentration of MitoTracker Red and TPP-2 were both  $1.0 \times 10^{-7}$  mol/L.

## 13. References

- [1] T. Han, X. Feng, B. Tong, J. Shi, L. Chen, J. Zhi, Y. Dong, *Chem. Commun.* 2012, 48, 416.
- [2] N. Zhao, S. Chen, Y. Hong, B. Z. Tang, *Chem. Commun.* 2015, 51, 13599.
- [3] G. Liu, D. Chen, L. Kong, J. Shi, B. Tong, J. Zhi, X. Feng, Y. Dong, *Chem. Commun.* 2015, 51, 8555.