

## **The novel isoindigo compound with aggregation-induced emission: Br–Br bonding joint restriction of intramolecular motion and for cell imaging property**

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## Experimental section

### Materials and general instrumentation

All chemical reagents including di-tert-butyl dicarbonate (tBOC<sub>2</sub>O), N, N'-dimethylaminopyridine (DMAP), indolin-2-one, 2,3-Indolinedione were purchased from Sigma-Aldrich (USA) and used as received unless otherwise noted. Isoindigo and 6,6' dihalogen Isoindigo were prepared by the reported method.

<sup>1</sup>H NMR spectra were measured on the AVANCEIIIHD 500 MHz spectrometer (USA) with tetramethylsilane as the internal standard. Elemental analyses were performed on a flash EA 1112 spectrometer (Germany). The mass measurement was performed on Agilent 7890A-7000B. The size distribution of the resulting NPs was determined by dynamic light scattering (DLS) using a BI-200SM (Brookhaven, USA). The TEM images were collected on a field emission high-resolution 2100F transmission electron microscope (JEOL, Japan) operating at an acceleration voltage of 200 kV. Samples for TEM measurements were prepared by using the following method: for the NPs prepared in water, one droplet of the aliquot was cast on carbon-coated copper grids held by tweezers and dried in air at room temperature.

### Synthesis and characterization

TBOCII: Isoindigo (1.00 g, 3.82 mmol) was dispersed in dichloromethane (200 mL) at room temperature under continuous stirring. Then tBOC<sub>2</sub>O (1.67 g, 7.64 mmol) and DMAP (0.72 g, 5.72 mmol) were added and stirred continuously for 1 day. The reaction mixtures were filtrated and concentrated almost to dryness. The crude product was purified by column chromatography using silica gel with dichloromethane and hexane as the eluents to obtain the red brown solid. Yield: 1.07 g (60.5%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ: 8.95 (d, J = 8.0, 2 H), 7.81 (d, J = 8.0, 2 H), 7.43 (m, 2 H), 7.17 (m, 2 H), 1.68 (s, 18 H). Elemental analysis calcd: C 67.52%, H 5.67%, N 6.06%; found: C 67.48%, H 5.69%, N 5.61%. MS: m/z (%) = 462 [M - C<sub>10</sub>H<sub>18</sub>O<sub>4</sub>]<sup>+</sup>, 262.

Cl-TOBCII: 6,6' Dichloroisoindigo (1.00 g, 3.03 mmol) was dispersed in

dichloromethane (200 mL) at room temperature under continuous stirring. Then tBOC<sub>2</sub>O (1.32 g, 6.06 mmol) and DMAP (0.56 g, 4.55 mmol) were added and stirred continuously for 1 day. The reaction mixtures were filtrated and concentrated almost to dryness. The crude product was purified by column chromatography using silica gel with dichloromethane and hexane as the eluents to obtain the red brown. Yield: 0.95 g (59.2%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ: 8.92 (d, J = 8.5 Hz, 2H), 7.90 (d, J = 2.0 Hz, 2 H), 7.16 (m, 2 H), 1.68 (s, 18 H). Elemental analysis calcd: C 58.77%, H 4.55%, N 5.27%; found: C 58.29%, H 4.64%, N 5.35%. MS: m/z (%) = 530 [M – C<sub>10</sub>H<sub>18</sub>O<sub>4</sub>]<sup>+</sup>, 330.

Br-TOBCII: 6,6' Dibromoisindigo (1.00 g, 2.40 mmol) was dispersed in dichloromethane (200 mL) at room temperature under continuous stirring. Then tBOC<sub>2</sub>O (1.05 g, 4.80 mmol) and DMAP (0.45 g, 3.60 mmol) were added and stirred continuously for 1 day. The reaction mixtures were filtrated and concentrated almost to dryness. The crude product was purified by column chromatography using silica gel with dichloromethane and hexane as the eluents to obtain the red brown solid. Yield: 1.1 g (60.5%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ: 8.83 (d, J = 8.5 Hz, 2H), 8.07 (d, J = 2.0 Hz, 2 H), 7.30 (m, 2 H), 1.67 (s, 18 H). Elemental analysis calcd: C 50.34%, H 3.90%, N 4.52%; found: C 50.29%, H 3.94%, N 4.55%. MS: m/z (%) = 619 [M – C<sub>10</sub>H<sub>18</sub>O<sub>4</sub>]<sup>+</sup>, 419.

I-TOBCII: 6,6' Diiodoisindigo (0.1 g, 0.20 mmol) was dispersed in dichloromethane (20 mL) at room temperature under continuous stirring. Then tBOC<sub>2</sub>O (0.08 g, 0.39 mmol) and DMAP (0.04 g, 0.29 mmol) were added and stirred continuously for 1 day. The reaction mixtures were filtrated and concentrated almost to dryness. The crude product was purified by column chromatography using silica gel with dichloromethane and hexane as the eluents to obtain the red brown solid. Yield: 0.06 g (42.0%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ: 8.67 (d, J = 8.5 Hz, 2H), 8.28 (d, J = 2.0 Hz, 2 H), 7.53 (m, 2 H), 1.68 (s, 18 H). Elemental analysis calcd: C 43.72%, H 3.39%, N 3.92%; found: C 43.62%, H 3.45%, N 3.81%. MS: m/z (%) = 714 [M – C<sub>10</sub>H<sub>18</sub>O<sub>4</sub>]<sup>+</sup>, 514.

### **Single Crystal X-Ray Crystallography:**

Diffraction data were collected on an Agilent diffractometer, SUPERNOVA E MOVA010301. The structure was solved with direct methods using the SHELXTL programs. The corresponding CCDC reference number of Br-TBOCII (CCDC: 1914998) and the data can be obtained free of charge from The Cambridge Crystallographic Data Centre via [www.ccdc.cam.ac.uk/data\\_request/cif](http://www.ccdc.cam.ac.uk/data_request/cif).

### **Preparation of Br-TBOCII/F127 NPs**

A mixture of 2 mg of Br-TBOCII and 30 mg of Pluronic 127 (F127) was completely dissolved in 1 mL of THF for 2 h. Then 10 mL of deionized-water was quickly injected into the mixture under vigorous stirring at room temperature. After being stirred for 5 min, the dispersion was dialyzed against deionized-water by 3.5 KDa dialysis membranes for 48 h to remove THF. The Br-TBOCII/F127 aqueous solutions were separated by centrifugation at 10000 rpm for 5 min to remove unencapsulated surfactant and then redispersed in deionized-water before characterization and cell study.

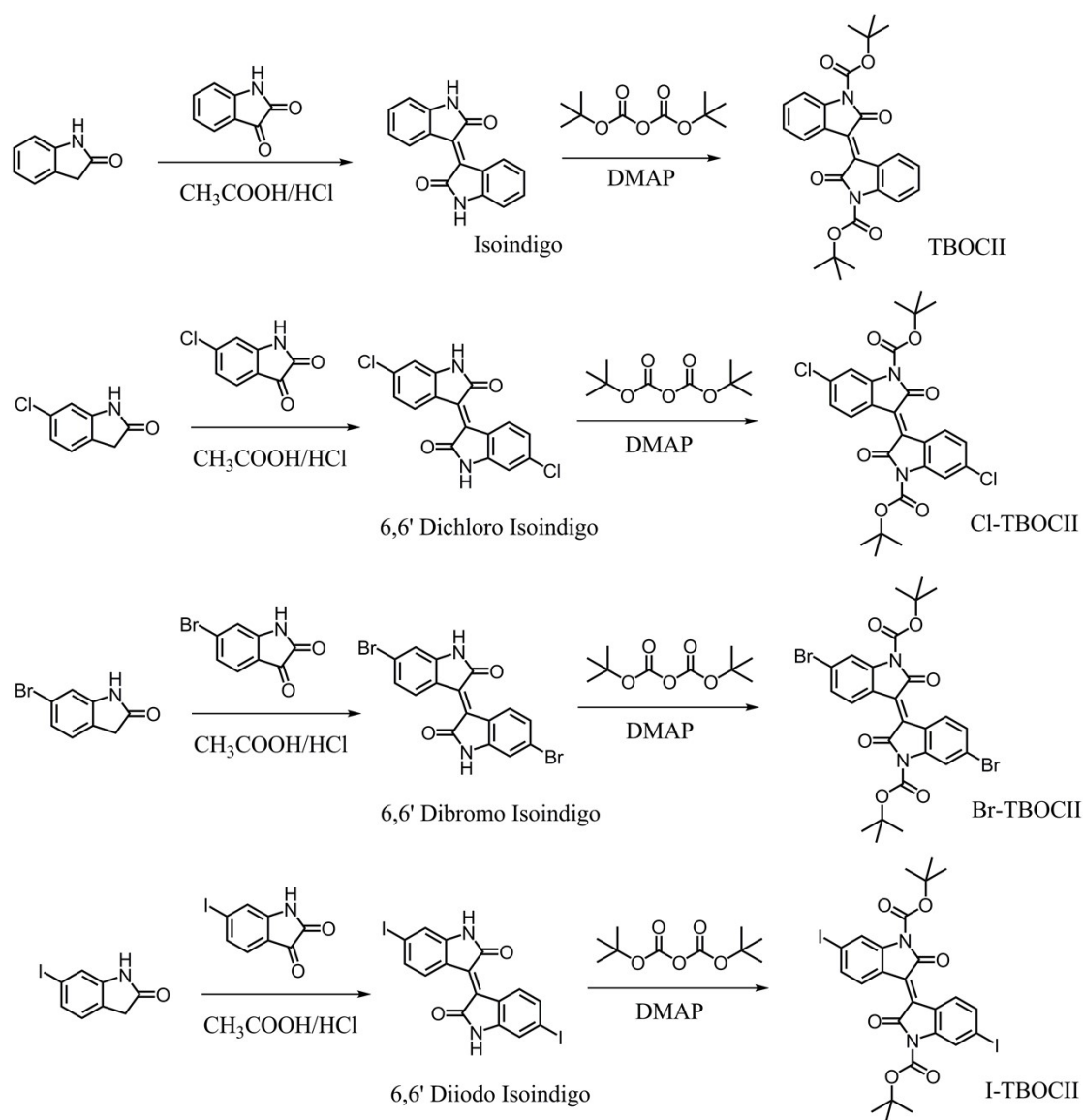
### **Cell labelling**

HeLa cells were regularly cultured and passaged using DMEM medium supplemented with 10% FBS and 1% penicillin–streptomycin at 37 °C with 5% CO<sub>2</sub> in a humidified incubator for 24 h. Subsequently, the cells were treated with **Br-TBOCII/F127** NPs (20 µg mL<sup>-1</sup>) and continued to incubate for another 4 h, washed three times using PBS buffer and then fixed using 4% of paraformaldehyde (20 min). The nucleus were then stained with DAPI (0.4 µg mL<sup>-1</sup>, 10 min) in order to track intracellular absorption.

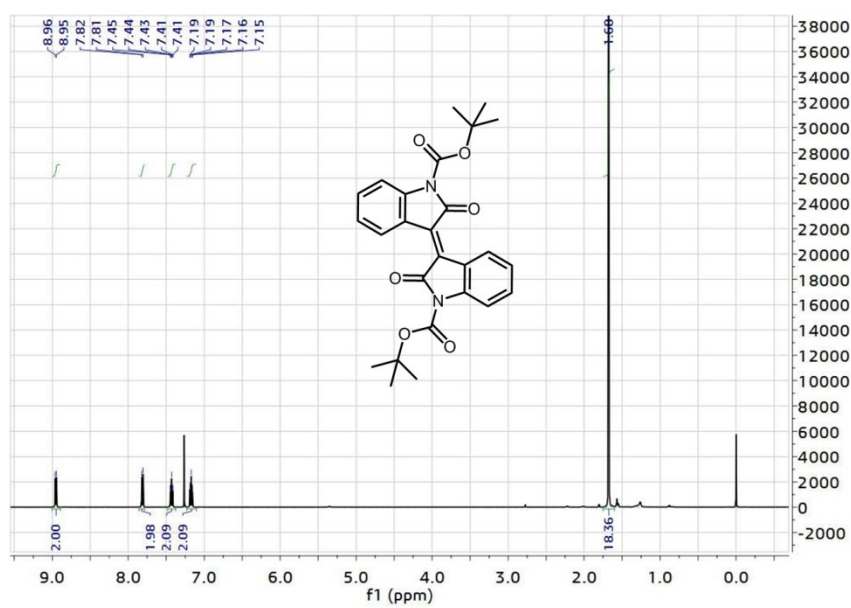
### **Cytotoxicity**

HeLa cells were seeded in a 96-well plate, preincubated for 12 h. And then incubated with Br-TBOCII/F127 for 24 h, 48 h and 72 h at concentrations ranging from 0 to 80.0 µg mL<sup>-1</sup>. Then DMEM medium was replaced with 20 µl 0.5 mg/ml MTT and after 4 h the MTT solution was replaced with 150 µl DMSO solution. Cell viability

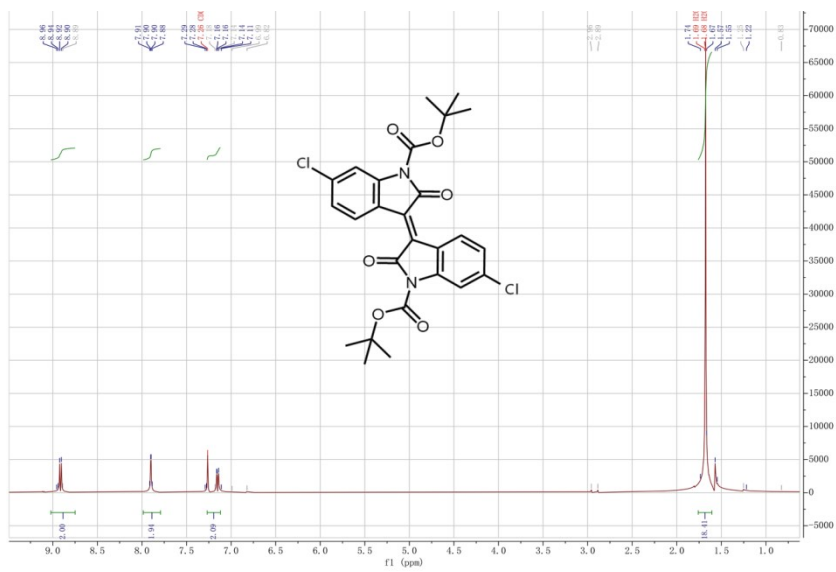
was measured at 490 nm by colorimetric assay (Rayto RT-2100C, Shenzhen, China).  
Cells without treatment in medium were used as control.



**Scheme S1.** The synthetic route of Isoindigo derivatives.

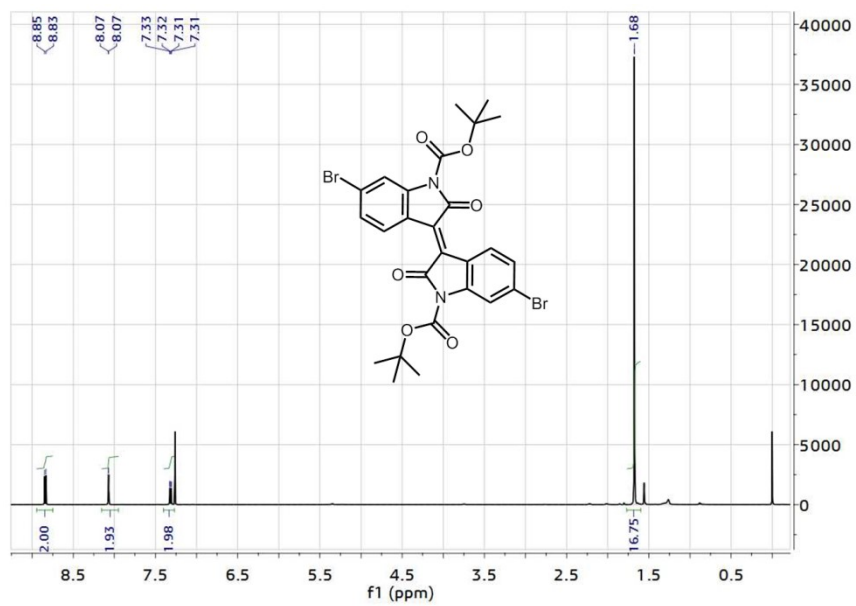


**Figure S1.** The <sup>1</sup>H NMR spectrum (500 MHz) of TBOCII in CDCl<sub>3</sub>.

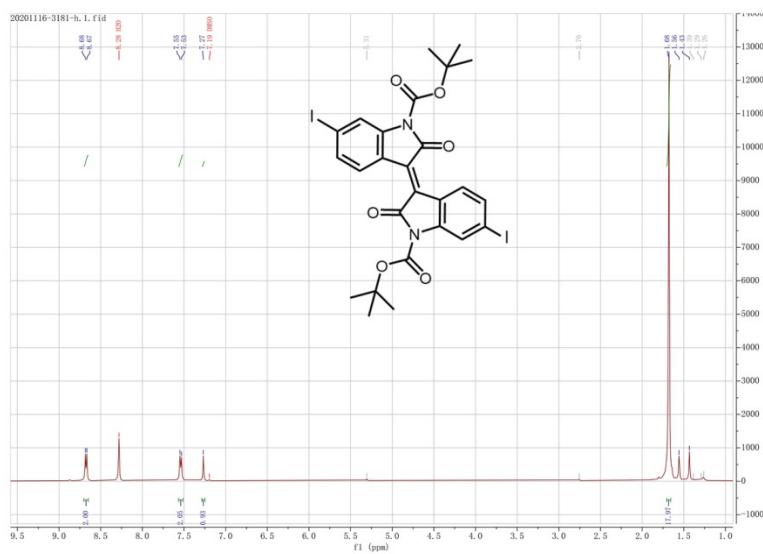


**Figure S2.** The <sup>1</sup>H NMR spectrum (500 MHz) of Cl-TBOCII in CDCl<sub>3</sub>.

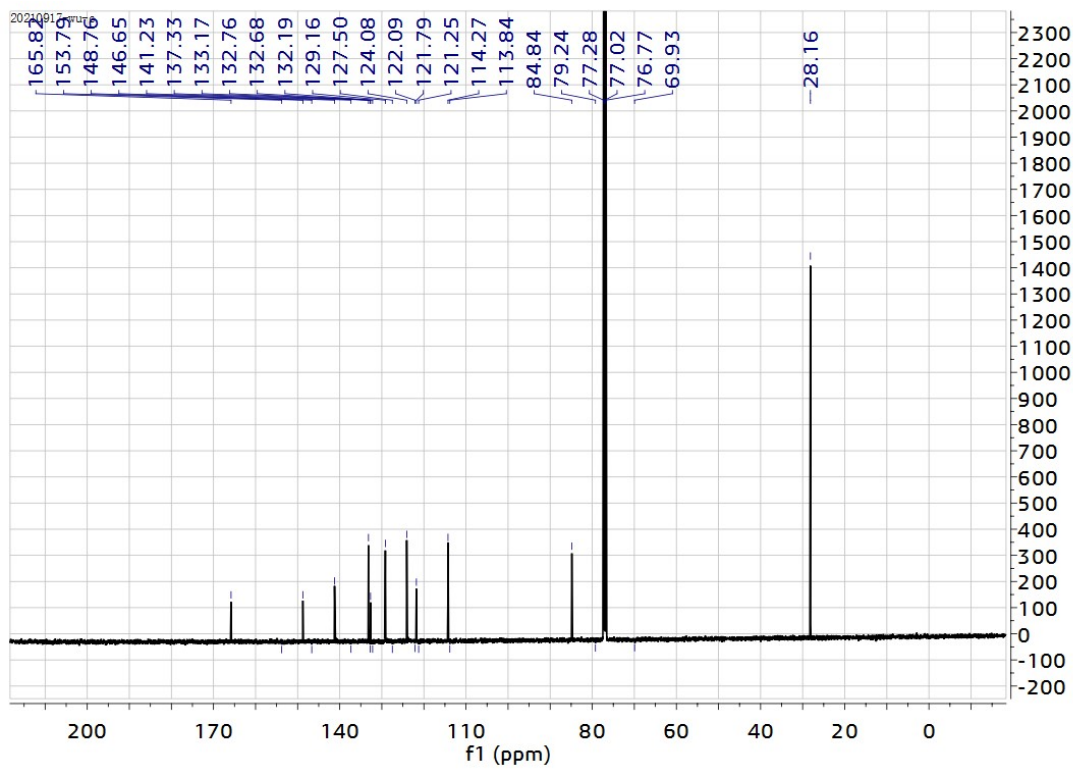




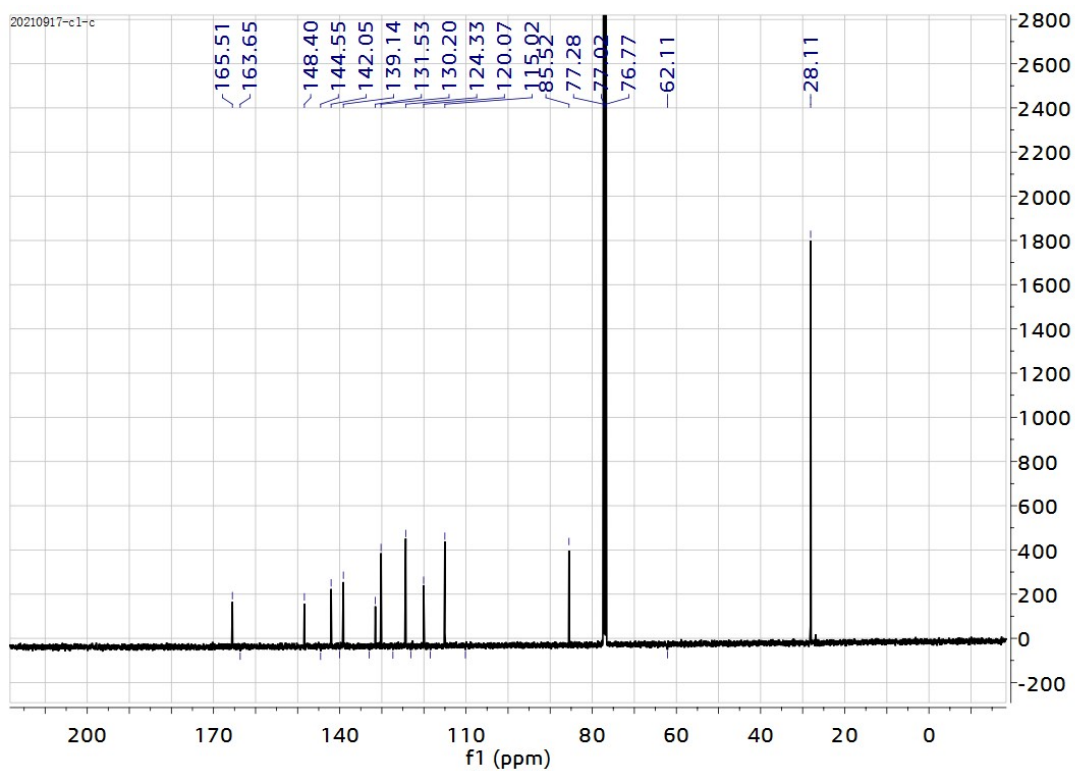
**Figure S3.** The <sup>1</sup>H NMR spectrum (500 MHz) of Br-TBOCII in CDCl<sub>3</sub>.



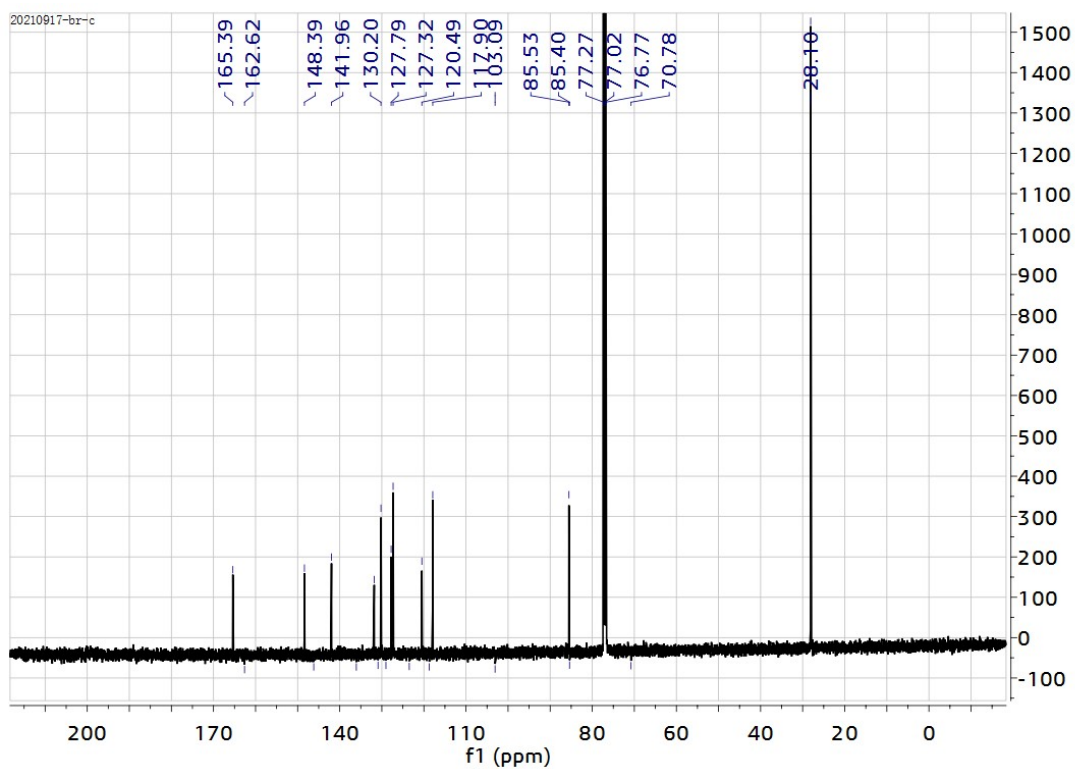
**Figure S4.** The <sup>1</sup>H NMR spectrum (500 MHz) of I-TBOCII in CDCl<sub>3</sub>.



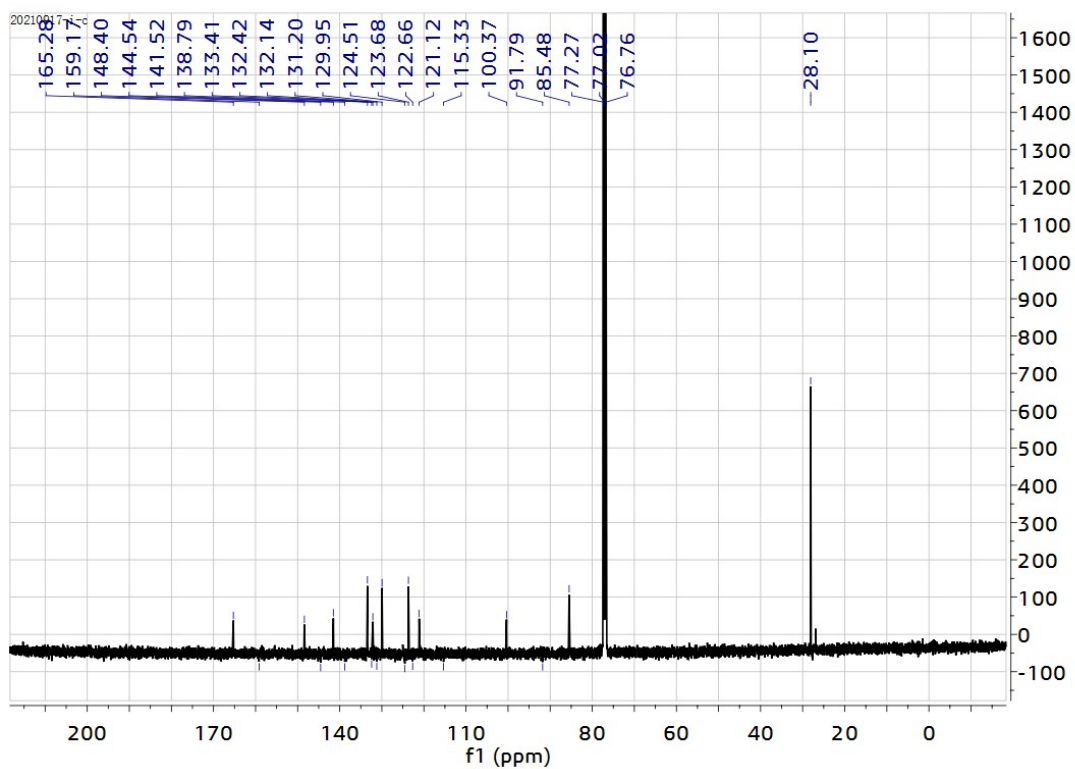
**Figure S5.** The  $^{13}\text{C}$  NMR spectrum (125 MHz) of TBOCII in  $\text{CDCl}_3$ .



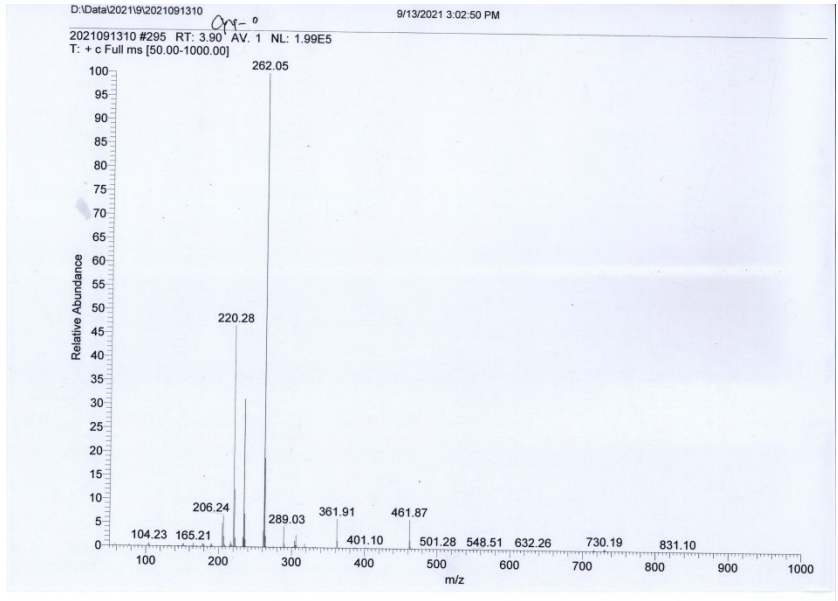
**Figure S6.** The  $^{13}\text{C}$  NMR spectrum (125 MHz) of Cl-TBOCII in  $\text{CDCl}_3$ .



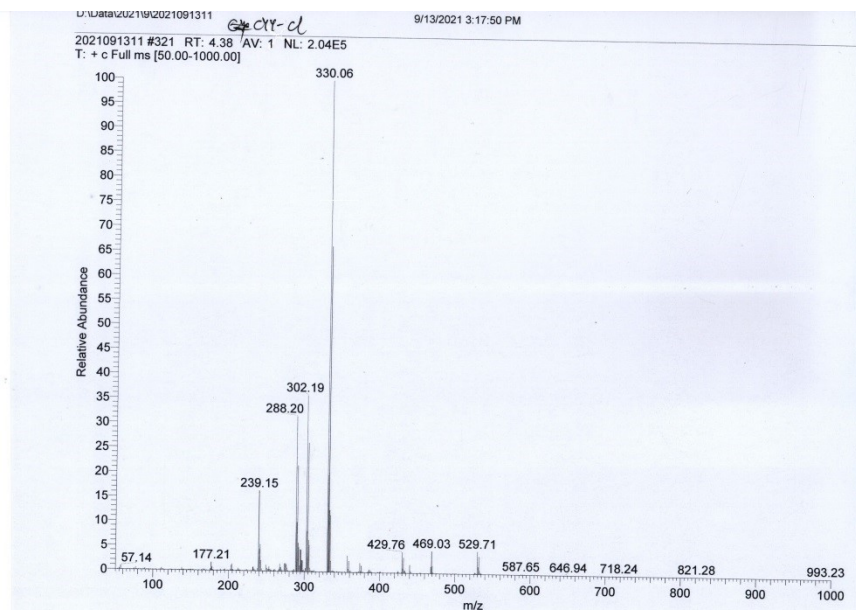
**Figure S7.** The  $^{13}\text{C}$  NMR spectrum (125 MHz) of Br-TBOCII in  $\text{CDCl}_3$ .



**Figure S8.** The  $^{13}\text{C}$  NMR spectrum (125 MHz) of I-TBOCII in  $\text{CDCl}_3$ .

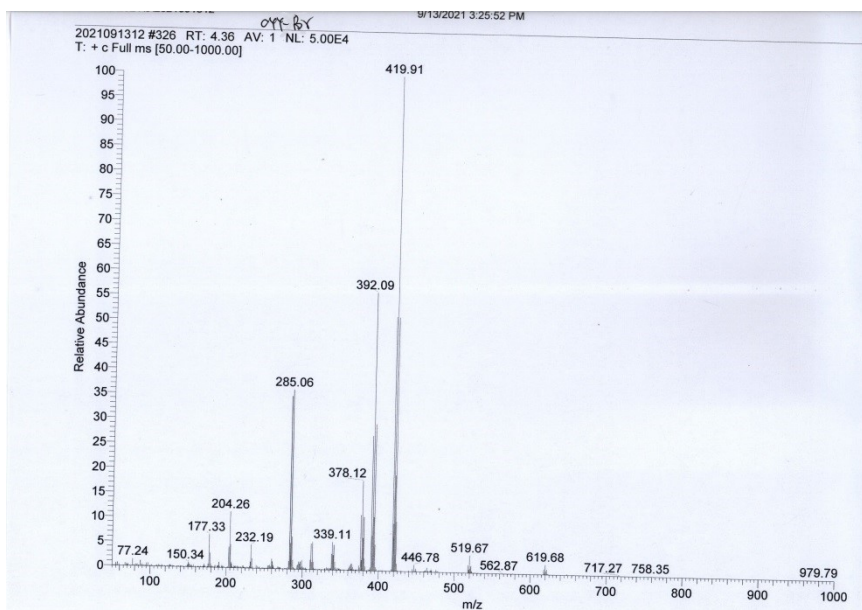


**Figure S9. The mass spectrum of TBOCII.**

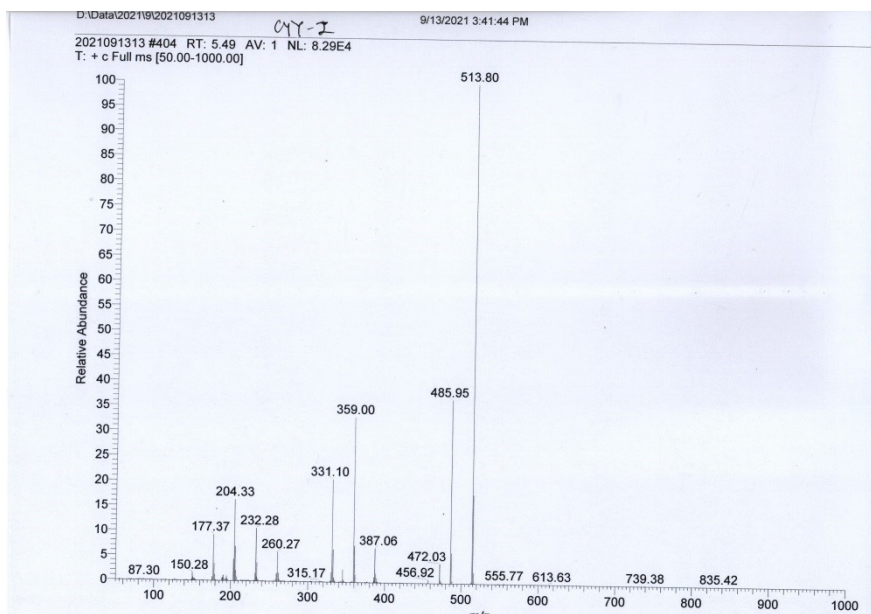


**Figure S10. The mass spectrum of Cl-TBOCII.**

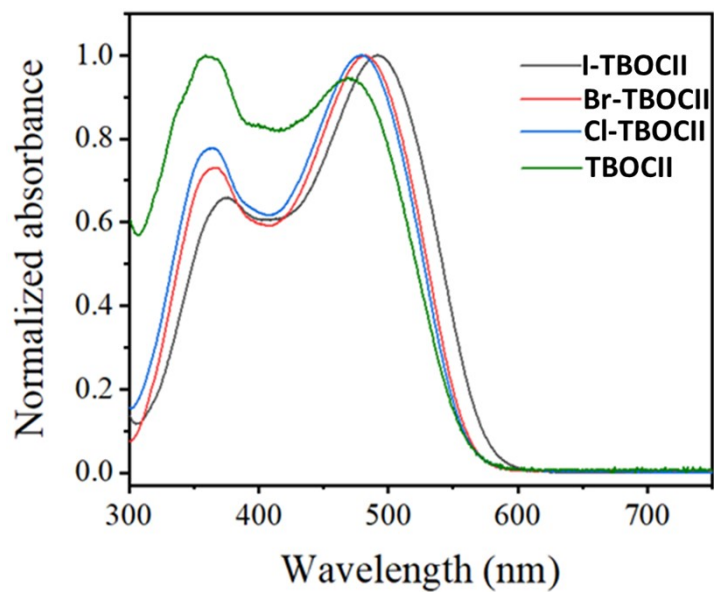




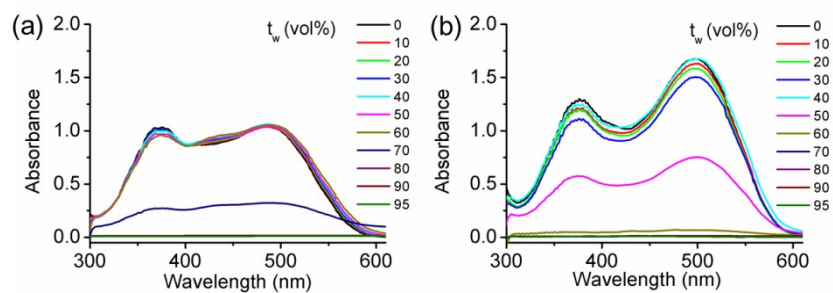
**Figure S11. The mass spectrum of Br-TBOCII.**



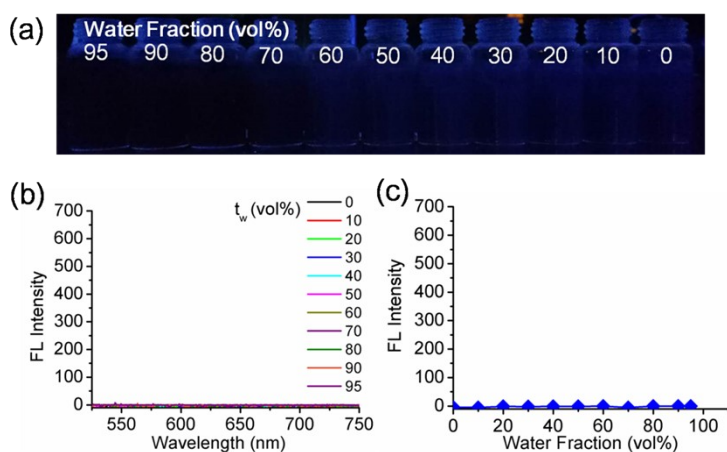
**Figure S12. The mass spectrum of I-TBOCII.**



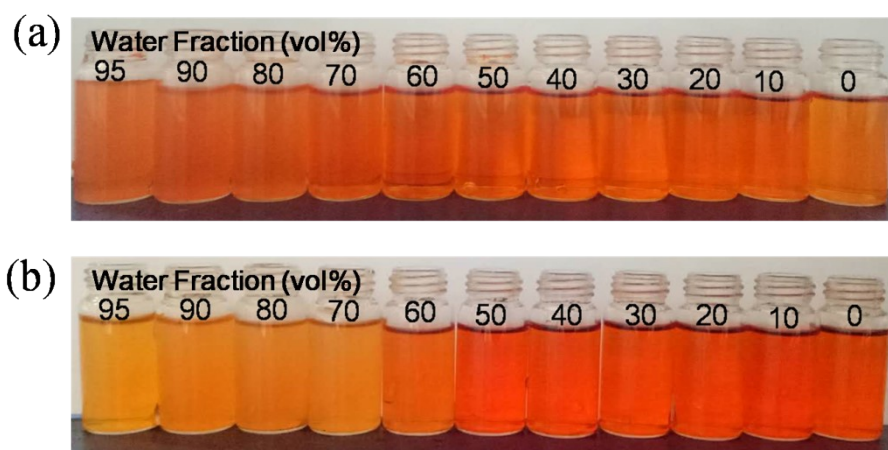
**Figure S13.** The UV-vis absorption spectrum of TBOCII, Cl-TBOCII, Br-TBOCII and I-TBOCII in THF ( $20 \mu\text{g mL}^{-1}$ ).



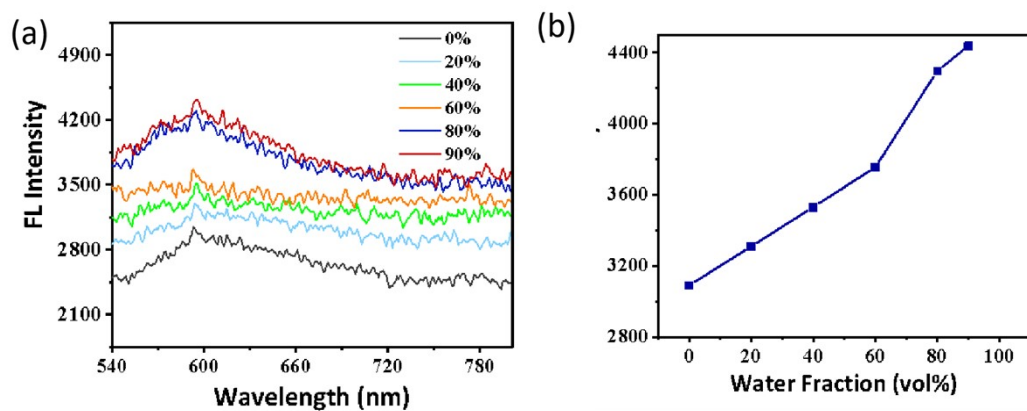
**Figure S14.** The UV-vis absorption spectra of TBOCII and Br-TBOCII in THF/water mixture. (a) TBOCII ( $100 \mu\text{g mL}^{-1}$ ); (b) Br-TBOCII ( $100 \mu\text{g mL}^{-1}$ ).



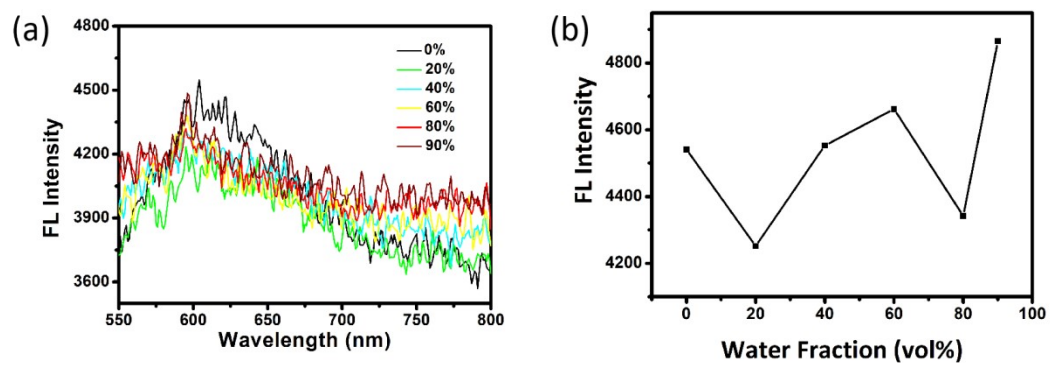
**Figure S15.** (a) A digital photograph under the UV light (365 nm) irradiation of TBOCII ( $100 \mu\text{g mL}^{-1}$ ) in THF/water mixtures; (b) FL spectra of TBOCII ( $100 \mu\text{g mL}^{-1}$ ) in THF/water mixture; (c) FL relative intensity ( $\lambda = 599 \text{ nm}$ ) of TBOCII versus the water volume fraction in THF/water mixtures.



**Figure S16.** A digital photograph under fluorescent lamp of TBOCII ( $100 \mu\text{g mL}^{-1}$ ) (a), Br- TBOCII ( $100 \mu\text{g mL}^{-1}$ ) (b) in THF/water mixtures.

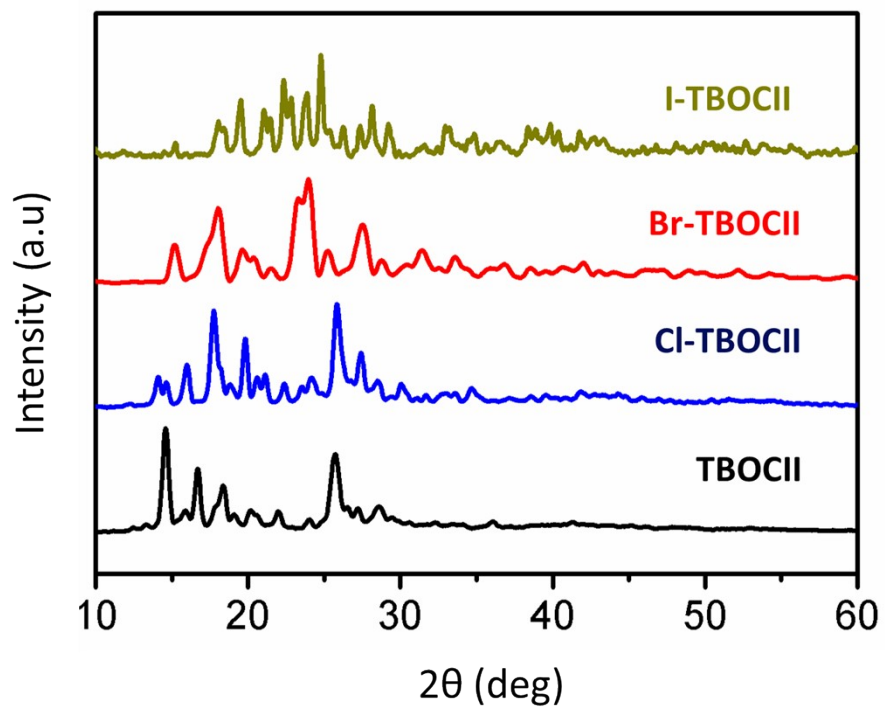


**Figure S17.** (a) FL spectra of Cl-TBOCII ( $100 \mu\text{g ml}^{-1}$ ) in THF/water mixture; (b) FL relative intensity ( $\lambda_{\text{em}} = 578 \text{ nm}$ ) of Cl-TBOCII versus the water volume fraction in THF/water mixtures.

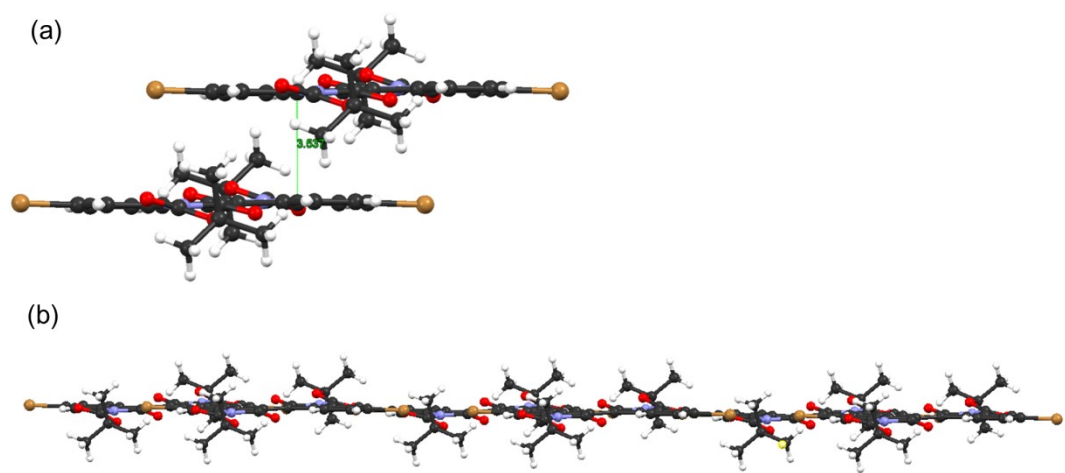


**Figure S18.** (a) FL spectra of I-TBOCII ( $100 \mu\text{g ml}^{-1}$ ) in THF/water mixture; (b) FL relative intensity ( $\lambda_{\text{em}} = 606 \text{ nm}$ ) of I-TBOCII versus the water volume fraction in THF/water mixtures.

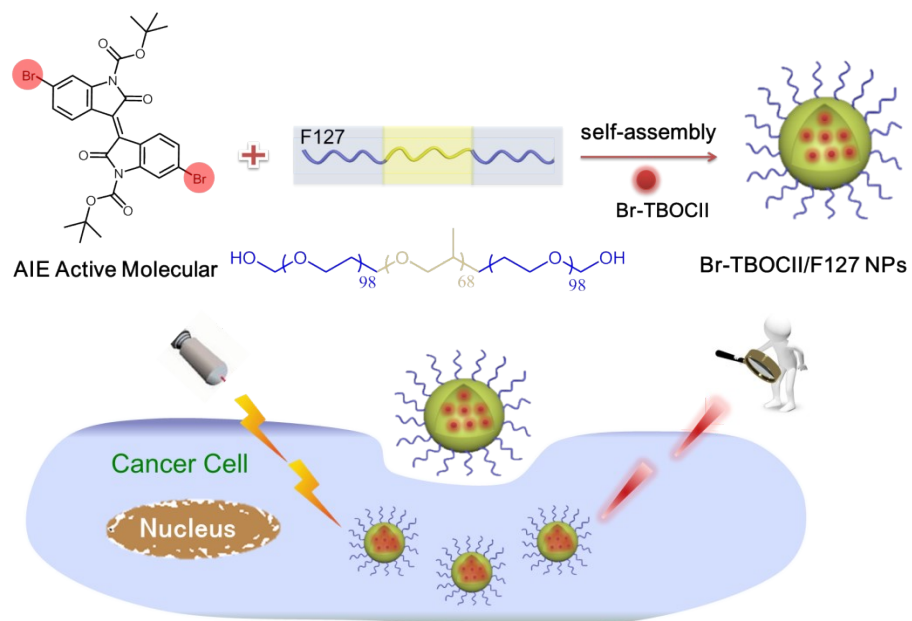




**Figure S19.** The XRD patterns of the Cl-TBOCII, Br-TBOCII and I-TBOCII powders.



**Figure S20.** The crystal structure of Br-TBOCII AIE system (molecular packing mode).



**Scheme 2.** The chemical structure of Br-TBOCII and preparation of Br-TBOCII/F127 NPs; (b) schematic illustration of cell imaging.

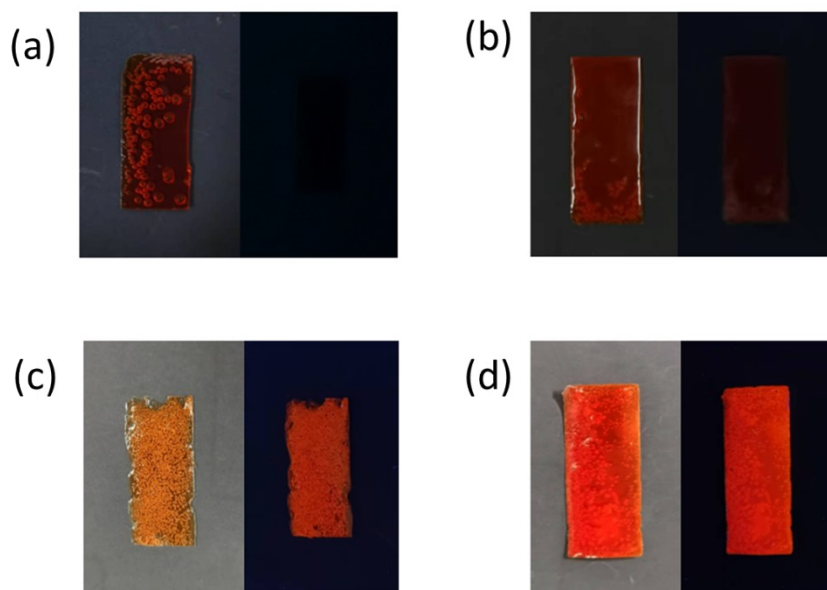
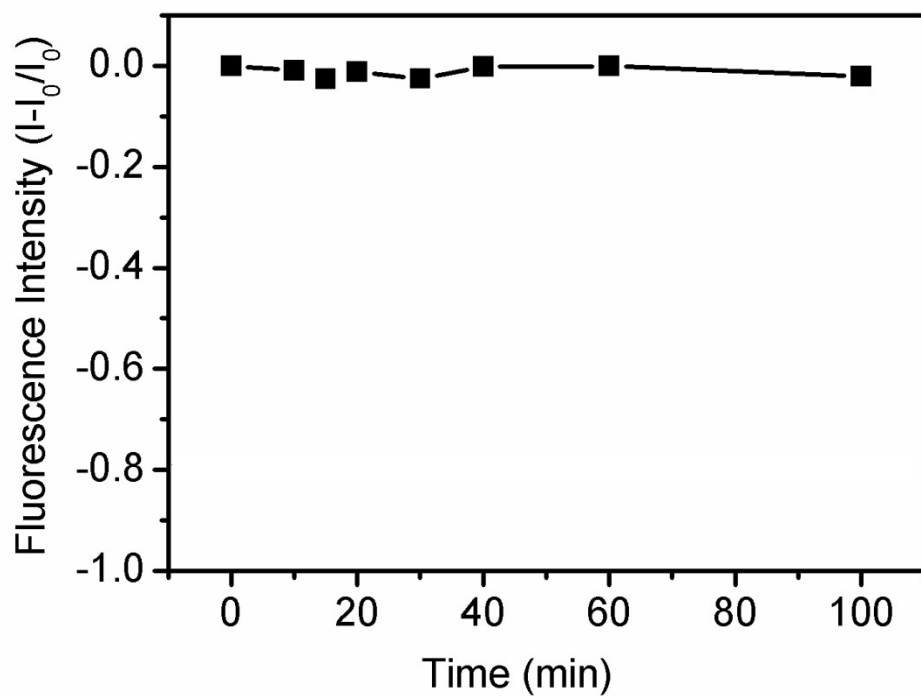


Figure S21. The images of the TBOCII, Br-TBOCII doped into the polymer polymethyl methacrylate (PMMA) and polystyrene (PS) with the concentration of 1% (w/w) under daylight lamp and 356 nm UV lamp. (a) TBOCII doped into PMMA; (b) TBOCII doped into PS; (c) Br-TBOCII doped into PMMA; (d) Br-TBOCII doped into PS.



**Figure S22.** Fluorescence intensity of Br-TBOCII/F127 micelle at 599 nm upon irradiation with blue LED light (20 W) in aqueous solution. I is fluorescence intensity after blue LED irradiation and  $I_0$  is fluorescence intensity without blue LED irradiation.