Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information

Modification of the SOCT-ISC type triphenylamine-BODIPY photosensitizer by multipolar dendrimer design for photodynamic therapy and two-photon fluorescence imaging

Lingfeng Wang，Ying Qian*
School of Chemistry and Chemical Engineering, Southeast University, Nanjing, 211189, China

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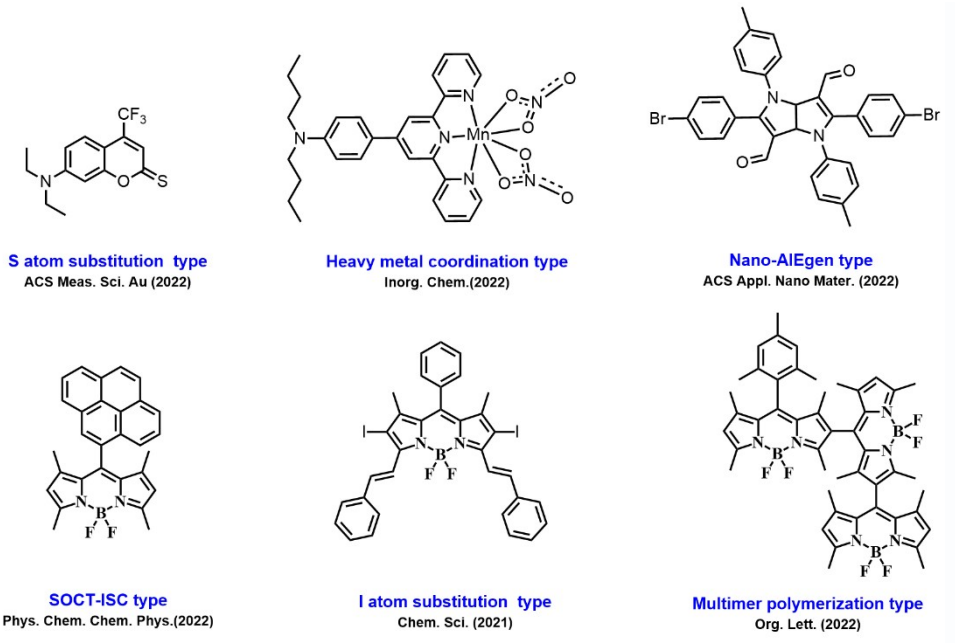
1. The main types of photosensitizer were widely reported recently.

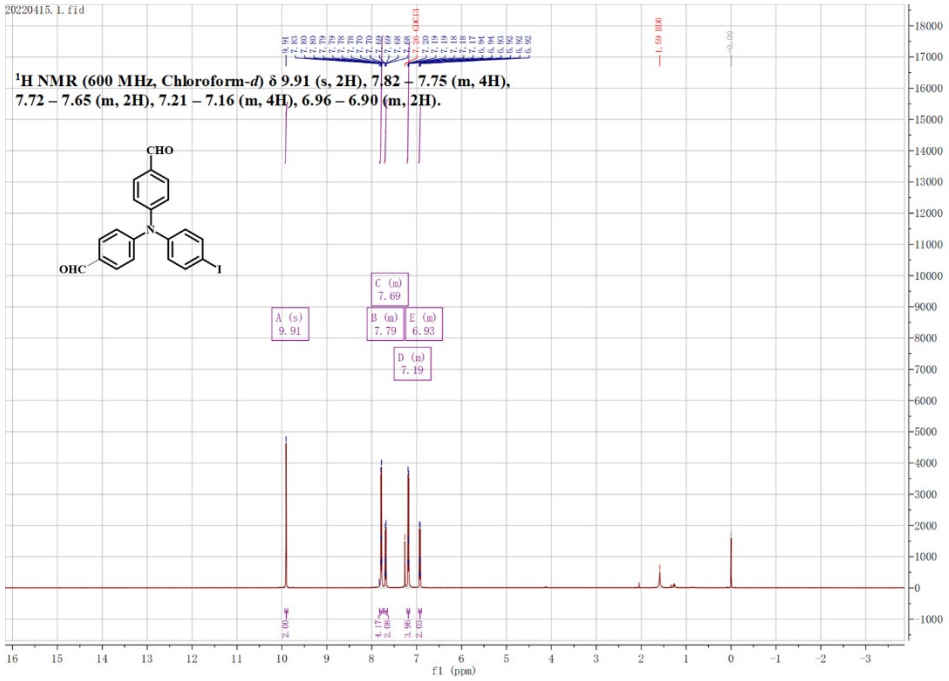


Scheme S1 The main types of photosensitizer were widely reported recently.

2. The ^{1}H NMR spectra of synthetic intermediate

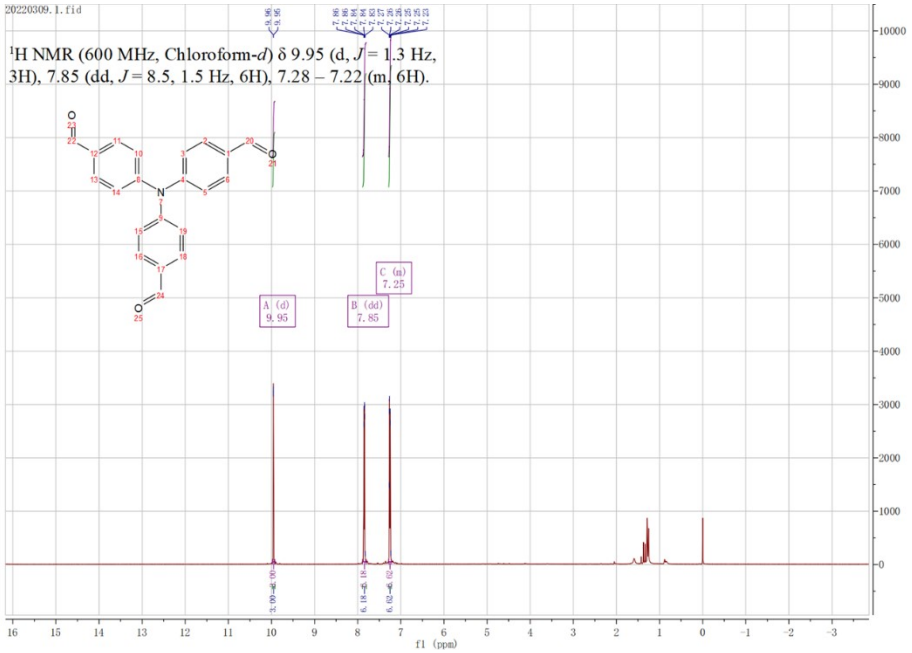
^{1}H NMR (600 MHz,$CDCl_3$, ppm). δ: 9.90 (s, 2H), 7.78 (m,4H), 7.40 (m,2H), 7.27 (m,1H), 7.19(m, 6H).

Fig S1. ^{1}H NMR spectra of 4,4'-(phenylazanediyl)dibenzaldehyde.



^{1}H NMR (600 MHz,$CDCl_3$, ppm). δ: 9.90 (s, 2H), 7.79 (m,4H), 7.69(m,2H) , 7.19(m, 4H), 6.93 (m,2H).

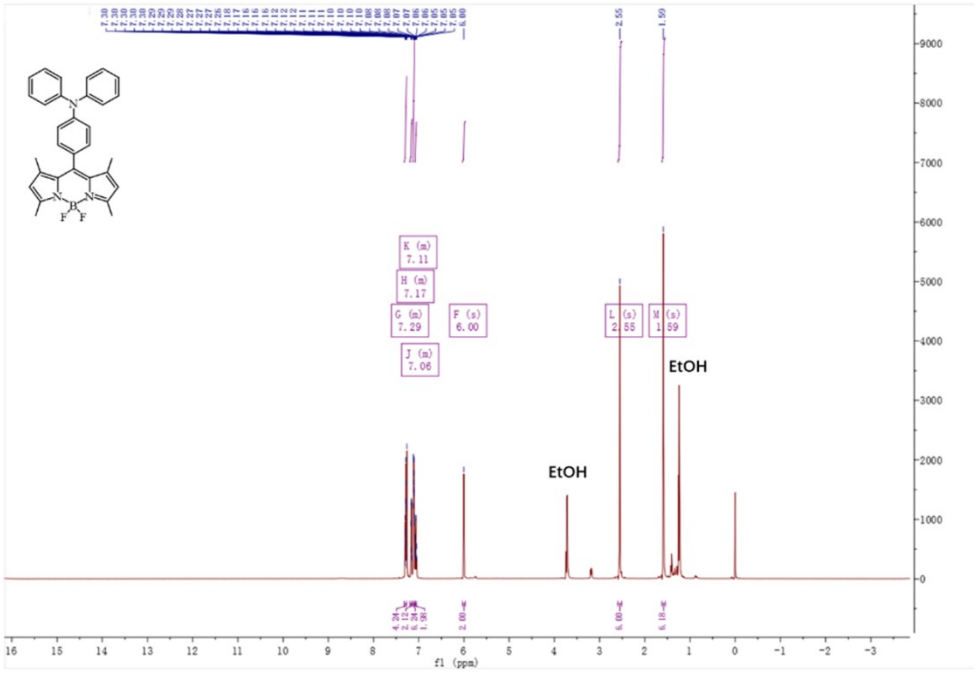
Fig S2. ^{1}H NMR spectra of 4,4'-((4-iodophenyl)azanediyl)dibenzaldehyde.



^{1}H NMR (600 MHz,$CDCl_3$, ppm). δ: 9.95 (s, 3H), 7.85 (dd, J=8.5,1.5Hz, 6H), 7.25(m,6H).

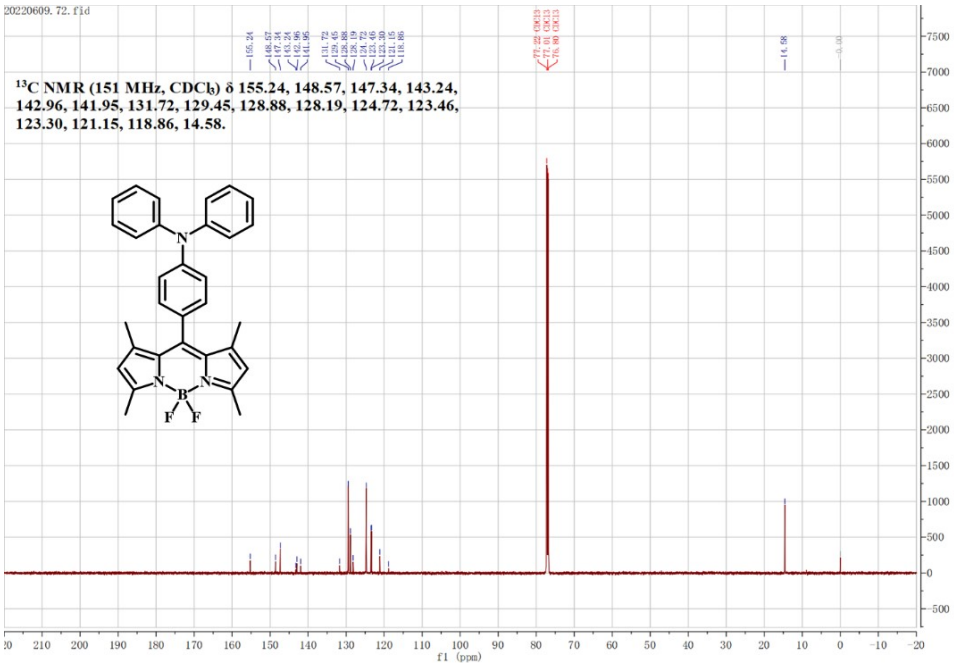
Fig S3. ^{1}H NMR spectra of 4,4',4''-nitrilotribenzaldehyde.

3. The ^{1}H NMR spectra, ^{13}C NMR spectra and mass spectrometry of T-BDP$_1$.



^{1}H NMR (600 MHz,$CDCl_3$, ppm). δ: 7.29 (m, 4H), 7.17 (m,2H), 7.11 (m,6H), 7.06 (m,2H), 6.00(s, 2H), 2.55(s, 6H), 1.59(s, 6H). CH_3CH_2OH= δ: 3.71 (q), δ: 1.25 (t).

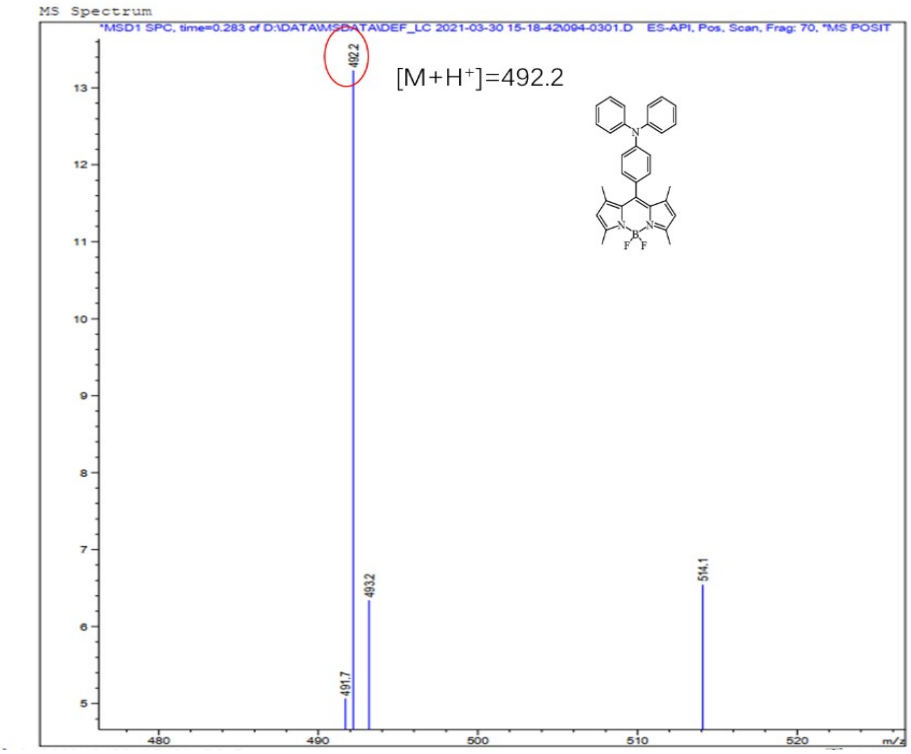
Fig S4. ^{1}H NMR spectra of T-BDP$_1$.



^{13}C NMR (151 MHz, $CDCl_3$) δ 155.24, 148.57, 147.34, 143.24, 142.96, 141.95, 131.72,

129.45, 128.88, 128.19, 124.72, 123.46, 123.30, 121.15, 118.86, 14.58.

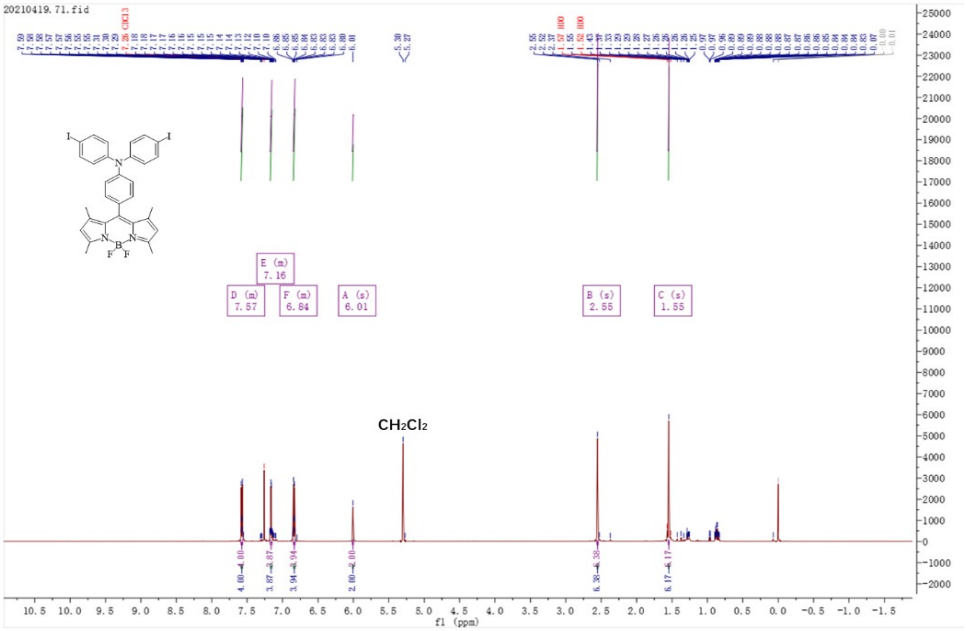
Fig S5. ^{13}C NMR spectra of T-BDP_1.



calcd for T-BDP_1 $[M+H]^+$=492.2, found $[M+H]^+$=492.2.

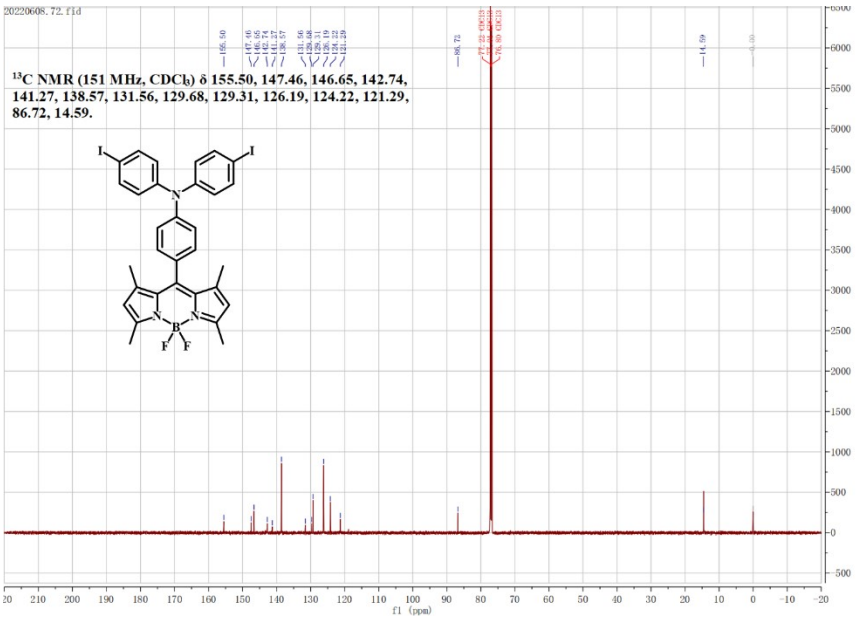
Fig. S6 Mass spectrometry of Compound T-BDP_1.

4. The ^1H NMR spectra, ^{13}C NMR spectra and mass spectrometry of T-BDP_1-I.



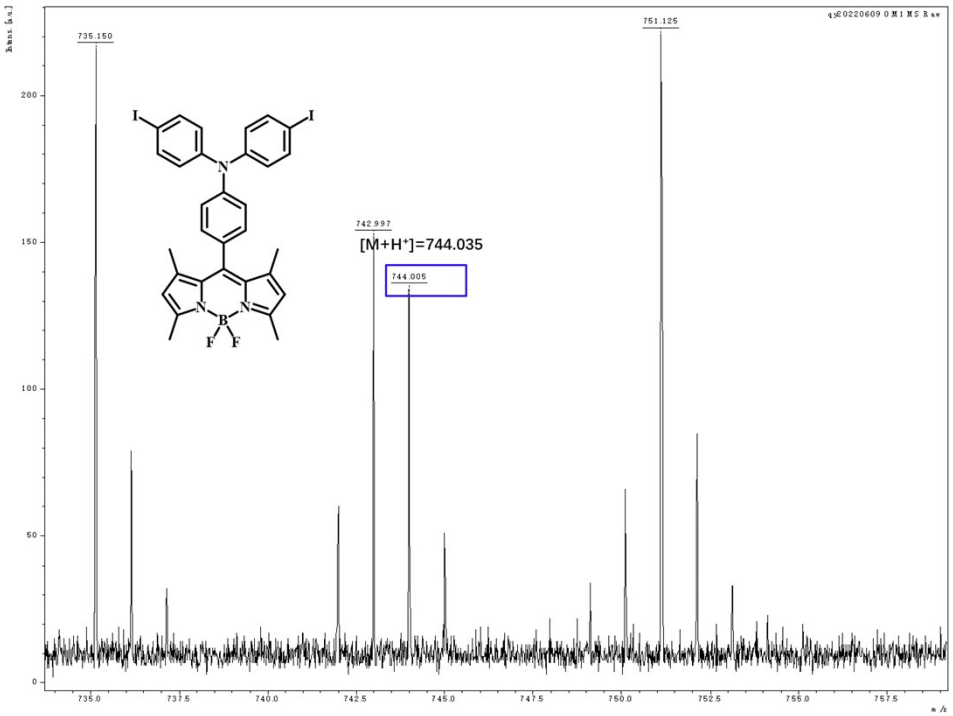
^{1}H NMR (600 MHz,$CDCl_3$, ppm). δ: 7.57 (m, 4H), 7.16 (m,4H), 6.84 (m,4H), 6.01(s, 2H), 2.55(s, 6H), 1.55(s, 6H). CH_2Cl_2=δ: 5.3(s).

Fig S7. ^{1}H NMR spectra of T-BDP_1-I.



^{13}CNMR(151MHz,$CDCl_3$)d:155.50,149.96,147.59,147.33,144.89,142.75,141.35,132.42,131.40,129.88, 129.33, 128.28, 124.91, 124.81, 124.24, 121.30, 120.73, 14.55.

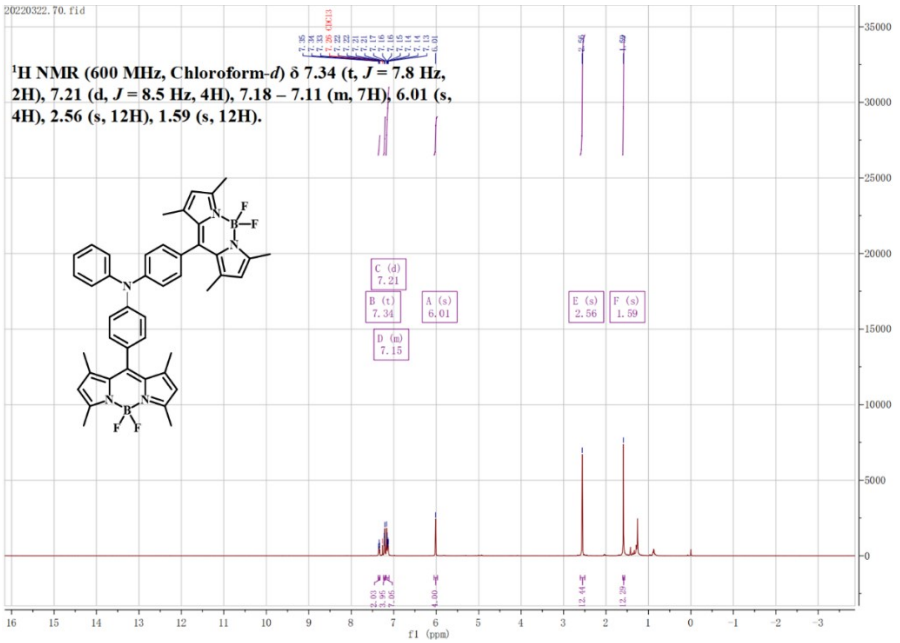
Fig S8. ^{13}C NMR spectra of T-BDP_1-I.



calcd for T-BDP$_1$-I $[M+H]^+$=744.035, found $[M+H]^+$=744.005

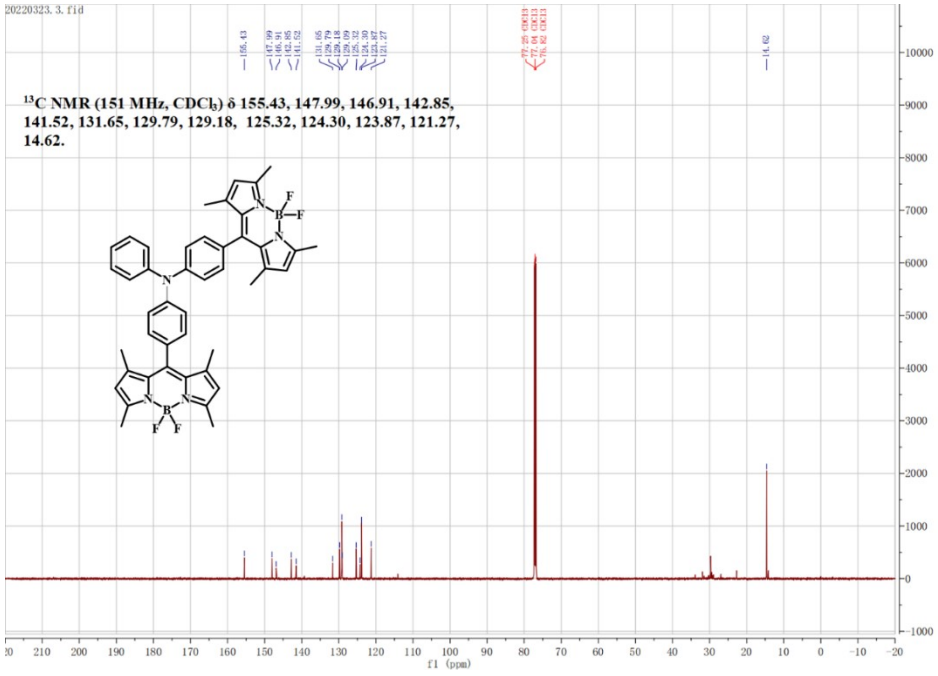
Fig. S9 Mass spectrometry of Compound T-BDP$_1$-I.

4. The ^{1}H NMR spectra, ^{13}C NMR spectra and mass spectrometry of T-BDP$_2$.



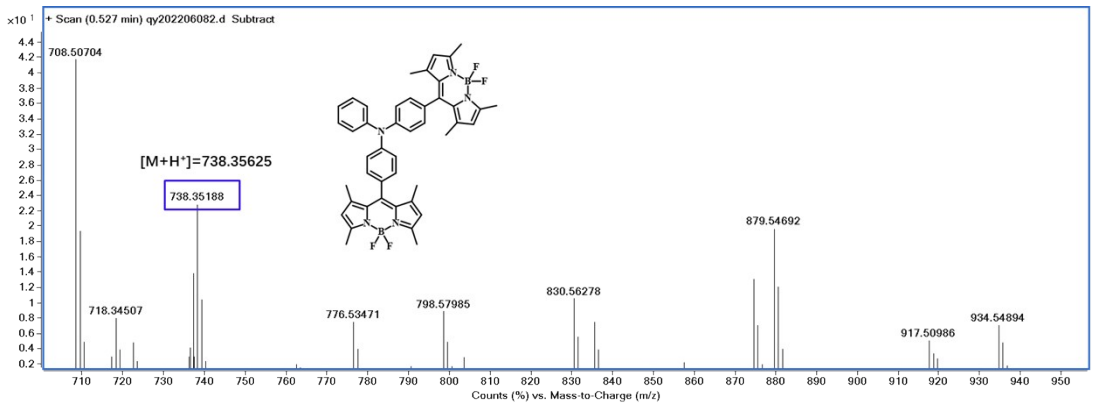
^{1}H NMR (600 MHz, Chloroform-d) δ 7.34 (t, J = 7.8 Hz, 2H), 7.21 (d, J = 8.5 Hz, 4H), 7.18 – 7.11 (m, 7H), 6.01 (s, 4H), 2.56 (s, 12H), 1.59 (s, 12H).

Fig S10. ^{1}H NMR spectra of T-BDP$_2$.



^{13}C NMR (151 MHz, $CDCl_3$) δ 155.43, 147.99, 146.91, 142.85, 141.52, 131.65, 129.79, 129.18, 125.32, 124.30, 123.87, 121.27, 14.62.

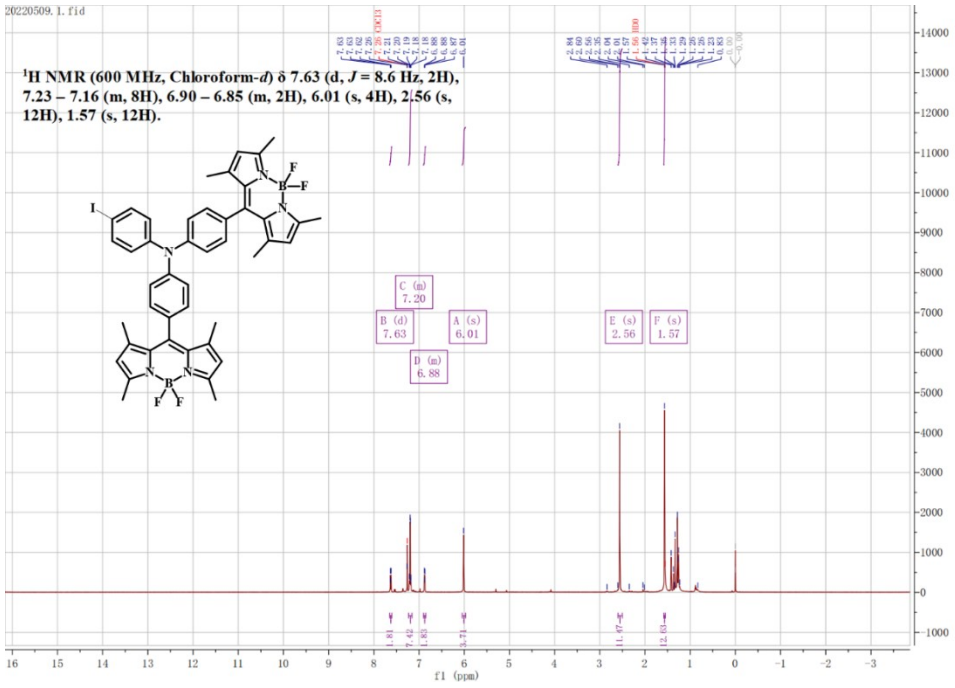
Fig S11. ^{13}C NMR spectra of T-BDP_2.



calcd for T-BDP_2 $[M+H]^+$=738.35625, found $[M+H]^+$=738.35188

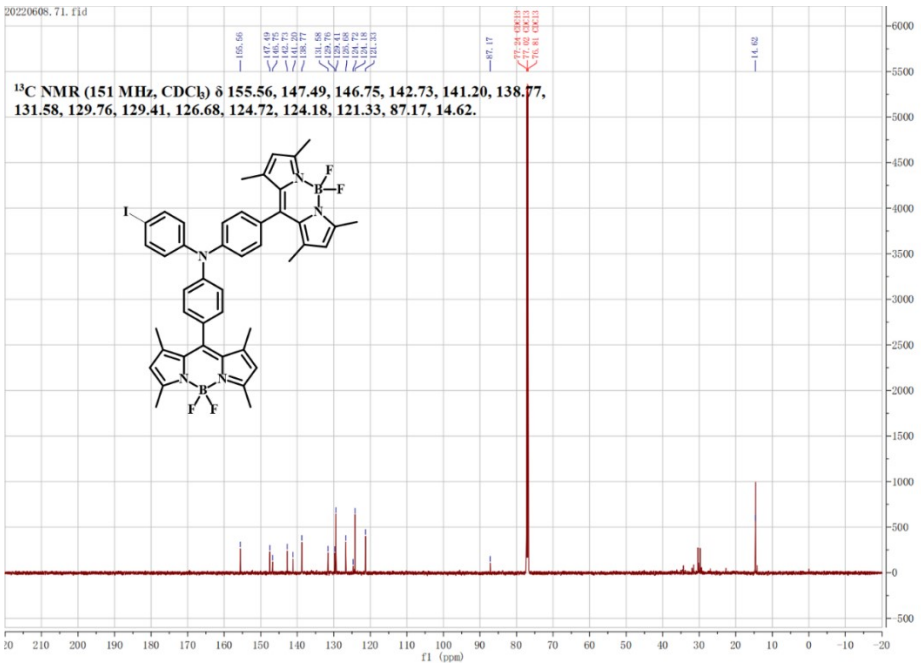
Fig. S12 Mass spectrometry of Compound T-BDP_2.

6. The ^{1}H NMR spectra, ^{13}C NMR spectra and mass spectrometry of T-BDP_2-I.



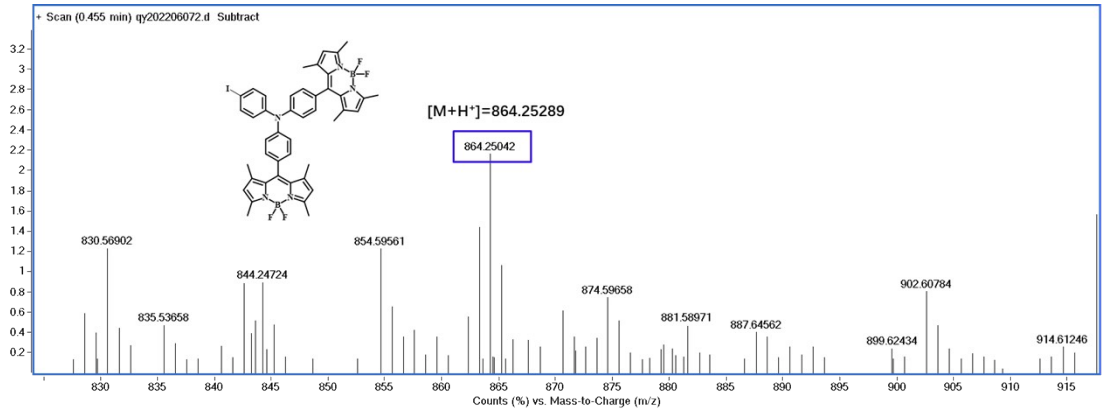
^{1}H NMR (600 MHz, Chloroform-d) δ 7.63 (d, J = 8.6 Hz, 2H), 7.23 – 7.16 (m, 8H), 6.90 – 6.85 (m, 2H), 6.01 (s, 4H), 2.56 (s, 12H), 1.57 (s, 12H).

Fig S13. ^{1}H NMR spectra of T-BDP_2-I.



^{13}C NMR (151 MHz, $CDCl_3$) δ 155.56, 147.49, 146.75, 142.73, 141.20, 138.77, 131.58, 129.76, 129.41, 126.68, 124.72, 124.18, 121.33, 87.17, 14.62.

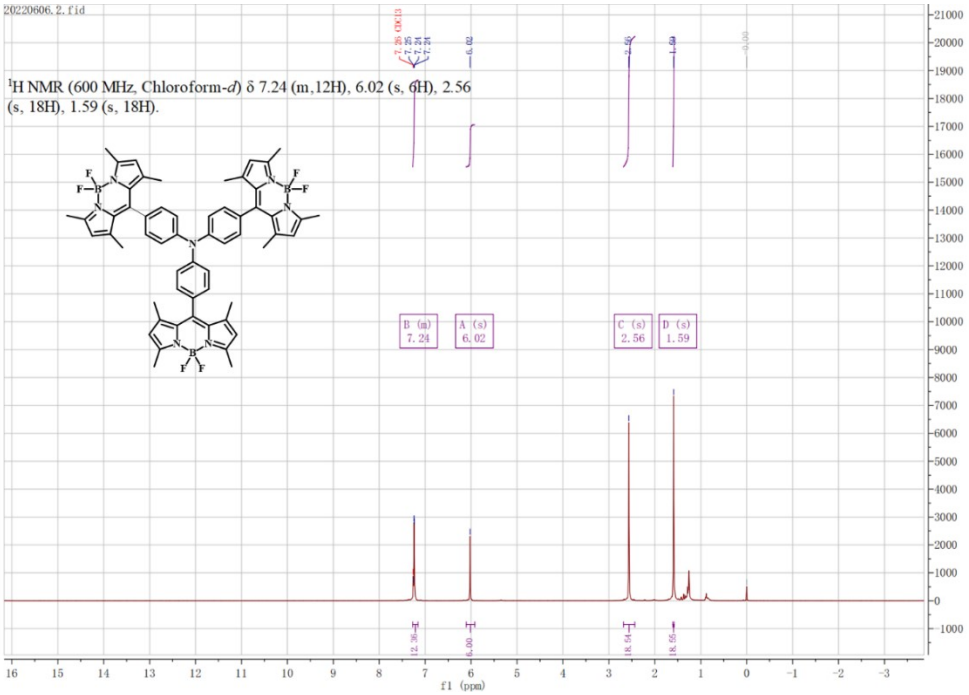
Fig S14. ^{13}C NMR spectra of T-BDP$_2$-I.



calcd for T-BDP$_2$-I [M+H]$^+$=864.25289, found [M+H]$^+$=864.25042

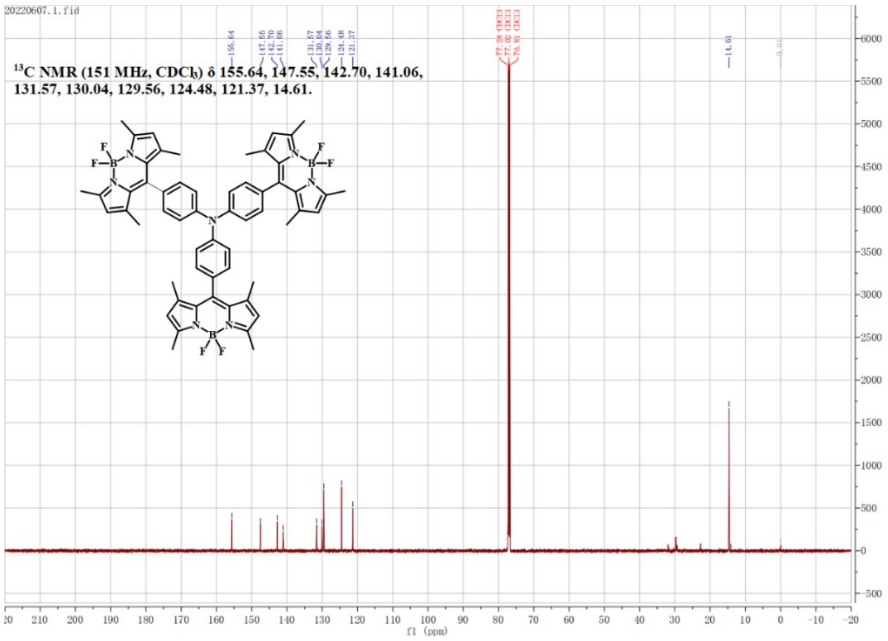
Fig. S15 Mass spectrometry of Compound T-BDP$_2$-I.

7. The ^{1}H NMR spectra, ^{13}C NMR spectra and mass spectrometry of T-BDP$_3$.



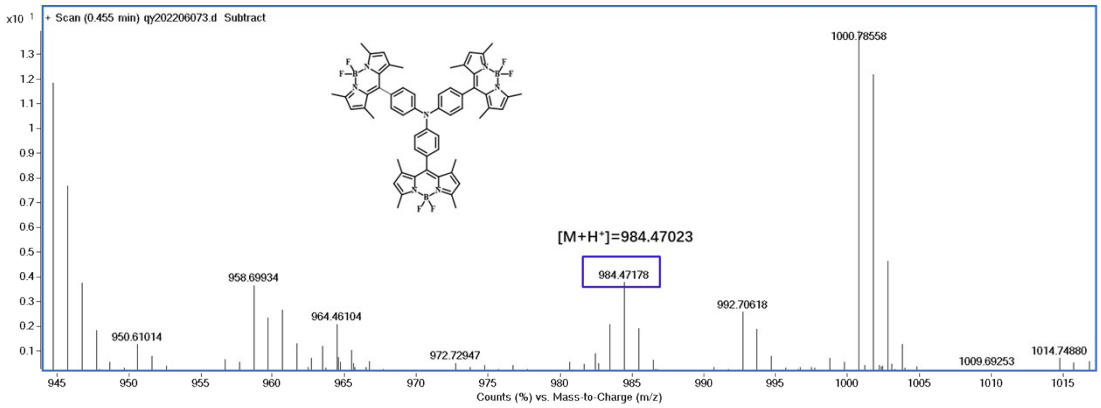
^{1}H NMR (600 MHz, Chloroform-d) δ 7.24 (m,12H), 6.02 (s, 6H), 2.56 (s, 18H), 1.59 (s, 18H).

Fig S16. ^{1}H NMR spectra of T-BDP$_3$.



^{13}C NMR (151 MHz, $CDCl_3$) δ 155.64, 147.55, 142.70, 141.06, 131.57, 130.04, 129.56, 124.48, 121.37, 14.61.

Fig S17. ^{13}C NMR spectra of T-BDP$_3$.



calcd for T-BDP_3 $[M+H]^+$=984.47023, found $[M+H]^+$=984.47178

Fig. S18 Mass spectrometry of Compound T-BDP_3.

8. The experiment procedure of singlet oxygen yield[1], superoxide radical($O_2^{\cdot-}$) [2, 3], fluorescence quantum yield[9,10]

Firstly, the absorbance of the photosensitizer was adjusted to about 0.2~0.3. Then, the suitable DPBF solution was added to the above solution and make the absorbance of DPBF near 1.0. Afterward, the mixed solution was exposed to corresponding green monochromatic light for a different interval seconds and the ultraviolet spectra was recorded by ultraviolet absorption spectrometer immediately. Taking the decrease in max absorbance of DPBF as the horizontal coordinate and the time interval as vertical coordinate to obtained the slope for the calculation of the singlet oxygen yield. The value of pearson coefficient of fitted line was used to verify whether the concentration of oxygen or DPBF is saturated during the period of experiments. The reference also was tested in the same method. The singlet oxygen quantum yield was calculated according to the following equation:

$$\phi_{\triangle} = \phi_r \times \frac{k_s}{k_r} \times \frac{1 - 10^{-OD_r}}{1 - 10^{-OD_s}}$$

Where $\boldsymbol{\phi}_{\triangle}$ represent the singlet oxygen yield, the "r" represent the reference sample, "s" represent the test sample, "k" was the slope of absorbance decrease of DPBF with the time interval and "OD" was stand for absorbance correction factor.

In the superoxide radical tests, the DHR123 was used as the fluorescent indicator of superoxide radical($O_2^{\cdot-}$)[2, 3]. When DHR123 reacts with superoxide radical($O_2^{\cdot-}$)., its green fluorescence will be significantly enhanced. The detection method is to add 10 μM DHR123 into the solution of a little drop of T-BDP_3. After different time illumination, the fluorescence intensity of DHR123 was detected through fluorescence emission spectra to determinate whether the superoxide radical ($O_2^{\cdot-}$) was produced.

In the fluorescence quantum yield test, the fluorescein in 0.1 M sodium hydroxide solution ($\boldsymbol{\phi}_r$=0.95) was selected as the reference. The absorbance of both sample and reference was adjusted to about 0.05, then the wavelength at the intersection of absorption spectra (496/498 nm) was used to excite the sample and reference, and the fluorescence quantum yield was calculated by the following formula.

$$\phi_f = \phi_r \times \frac{A_r}{A_s} \times \frac{F_s}{F_r} \times \frac{n_s^2}{n_r^2}$$

Where $\boldsymbol{\phi}_f$ is stand for the fluorescence quantum yield, the "r" represent the reference sample, "s" represent the test sample A is stand for the absorbance, F is stand for integration of fluorescence spectra, n is stand for the refractive index.

9. The experiment procedure of photo/dark cytotoxicity test[4, 5]

After the cultivation of A-549 cells in three 96-well plate (about 1×10^4 cells/well) for 24 h, the photosensitizer with different concentrations (0-5 μM) were added into two 96- well plates and same dosage PBS was added into another 96-well plate. After another 24 h incubation, the medium tested samples were replaced to remove dead cells and excess photosensitizer. The phototoxic cell experiment group and the PBS control group were conducted by irradiating the 96-well plates with green monochromatic light for 10 minutes. Dark toxicity tested sample still was placed in the incubator. Afterward, after 12 h incubation, the MTT solution (thiazolyl blue ,10 μL; 5 mg/mL) was added into both three 96-well plates and these three 96-well plates were incubated at proper environment for 4 h. At last, the MTT solution was replaced with

150 μL DMSO in each well. The absorbance at 570 nm of each well was measured with the enzyme-labeled instrument.

Cell viability = (Mean absorbance of test wells – Mean absorbance of medium control wells) / (Mean absorbance of untreated wells - Mean absorbance of medium control well) ×100%

10. The experiment procedure of fluorescence imaging in cells[2, 6, 7]

In the cell fluorescence imaging, the A-549 cells were seeded into petri dish with 2 mL 1640 culture medium. After 24 h of cell cultivation, 10 μL photosensitizer (10^{-3} M) solution was added into the petri dish and incubate with cells for 4 h. Then, the fluorescence imaging was perform on Olympus FV3000 laser scanning confocal microscope.

In the superoxide radical($O_2^{\cdot-}$) detection experiment, the A-549 cells were seeded into petri dish with 2 mL 1640 culture medium. After 24 h of cell cultivation, 10 μL T-BDP_3 photosensitizer (10^{-3} M) solution was added into the petri dish and incubate with cells for 4 h. Once the confocal microscope confirms that the photosensitizer has entered the cell, then, the DHE 10 uL (10^{-3}M) was added into the petri dish and incubate with cells for 10 min in dark condition. After the corresponding irradiated treatment of the cells, the Olympus FV-3000 laser scanning confocal microscope was used to detect the superoxide radical($O_2^{\cdot-}$) generation.

In the intracellular photodynamic experiments, the AO/EB stained experiment was perform on A-549 cell and the AO/EB was used to indicate the apoptosis condition of the cells. Firstly, the petri dish containing cells was placed under illumination for 10 min, and the 10 time-diluted 5 μL AO and 5 μL EB was added to detect cell survival situation. Then, the cells was washed with PBS three times. Afterward, the 10 μL T-BDP_3 photosensitizer (10^{-3} M) was added to the petri dish and incubate for 4 hours and the 10 time-diluted 5 μL AO and 5 μL EB was added to detect cell survival situation. Finally, the 10 μL T-BDP_3 photosensitizer (10^{-3} M) photosensitizer was added to another petri dish containing A-549 cells and incubated for 4 h. After the

corresponding irradiated treatment of the cells, the 10 time-diluted 5 μL AO and 5 μL EB were added to the dishes. Then, according to the excitation wavelength on the instruction manual of AO/EB, the Olympus FV-3000 laser scanning confocal microscope was used to analyzing the apoptosis condition of A-549 cells.

11. Singlet oxygen test of T-BDP$_1$ in different solvent system.

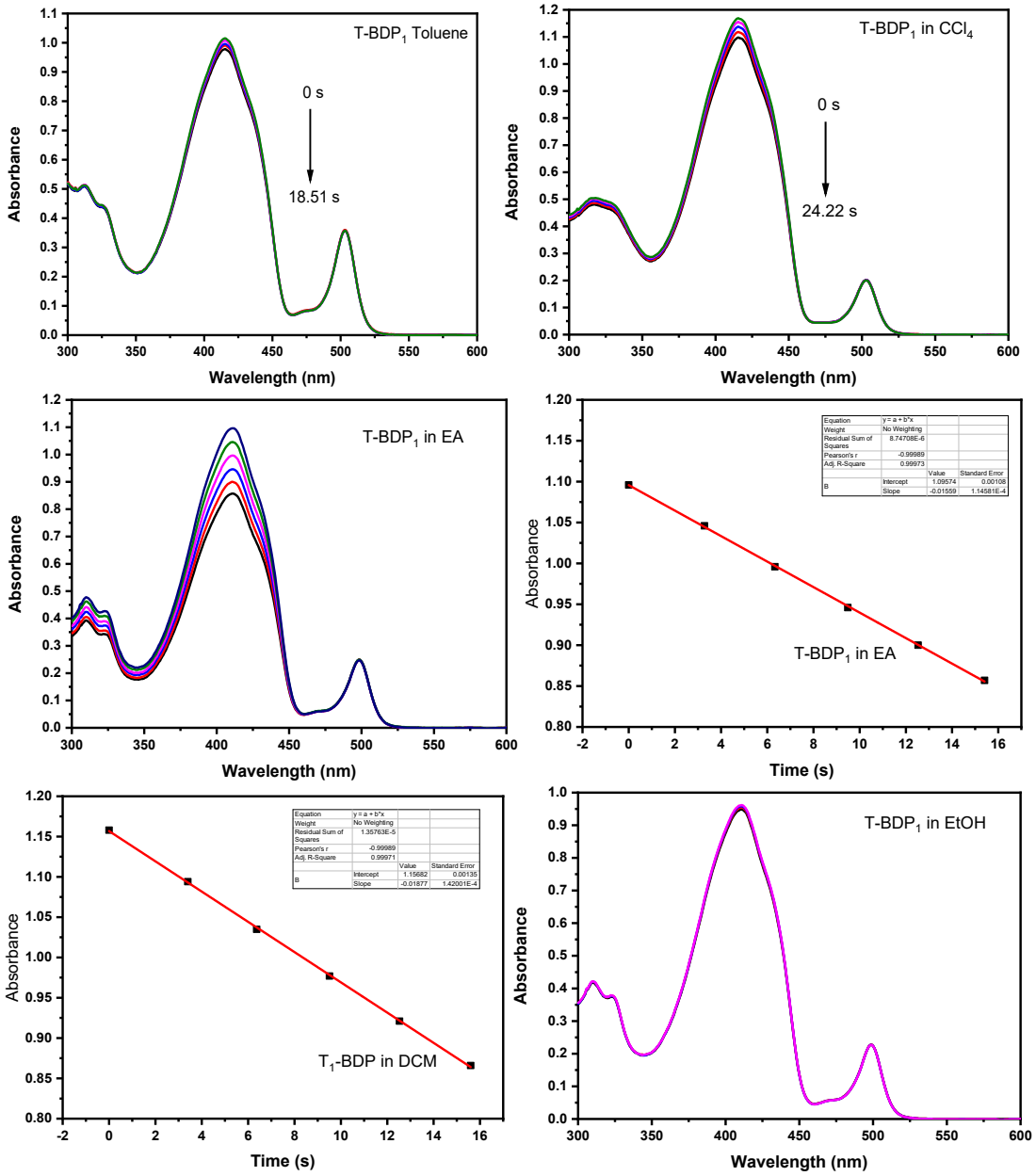


Fig. S19 Singlet oxygen test of T-BDP$_1$ in different solvent system.

12. Singlet oxygen test of T-BDP$_1$-I in different solvent system.

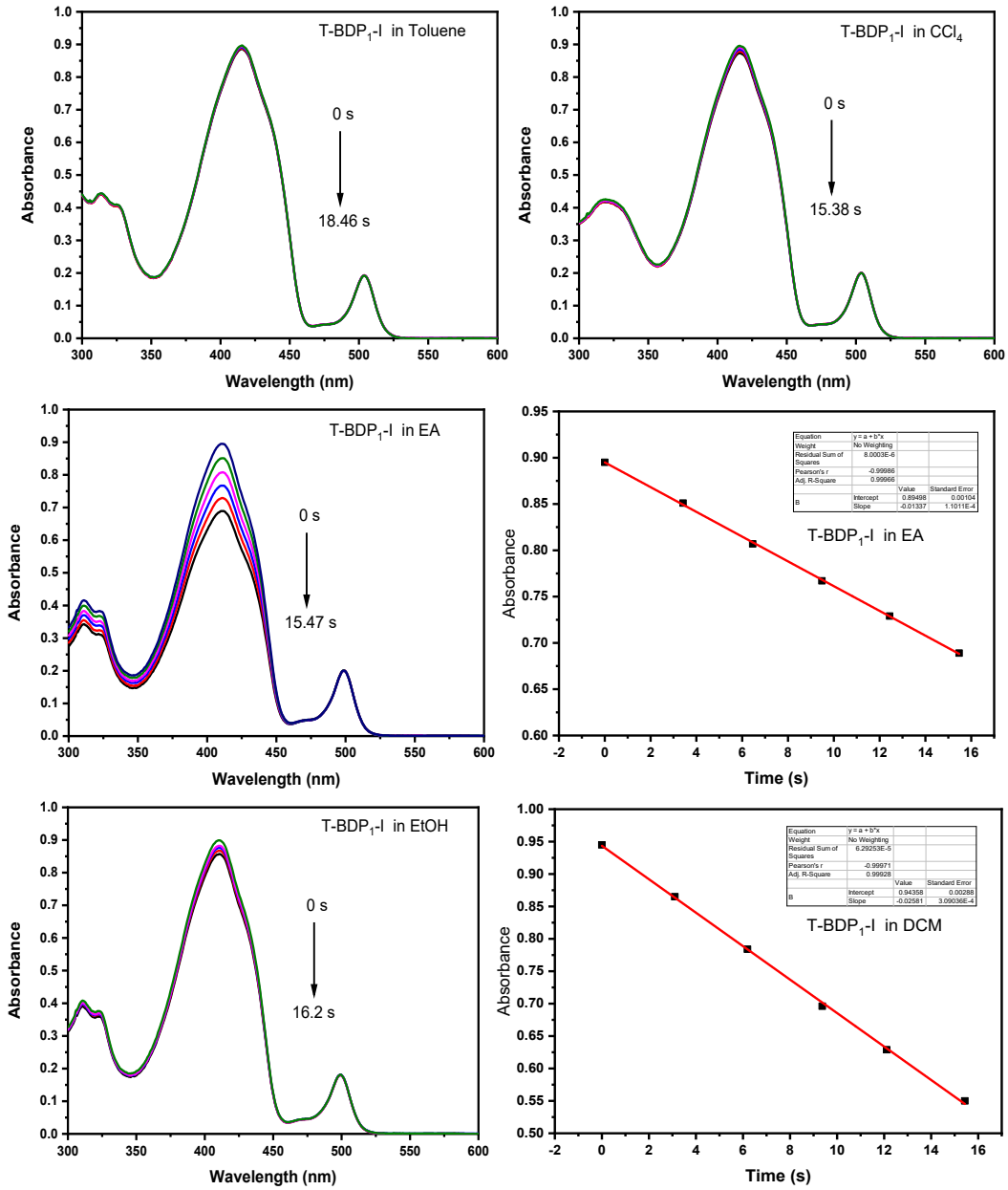


Fig. S20 Singlet oxygen test of T-BDP$_1$-I in different solvent system.

13. Singlet oxygen test of T-BDP$_2$ in different solvent system.

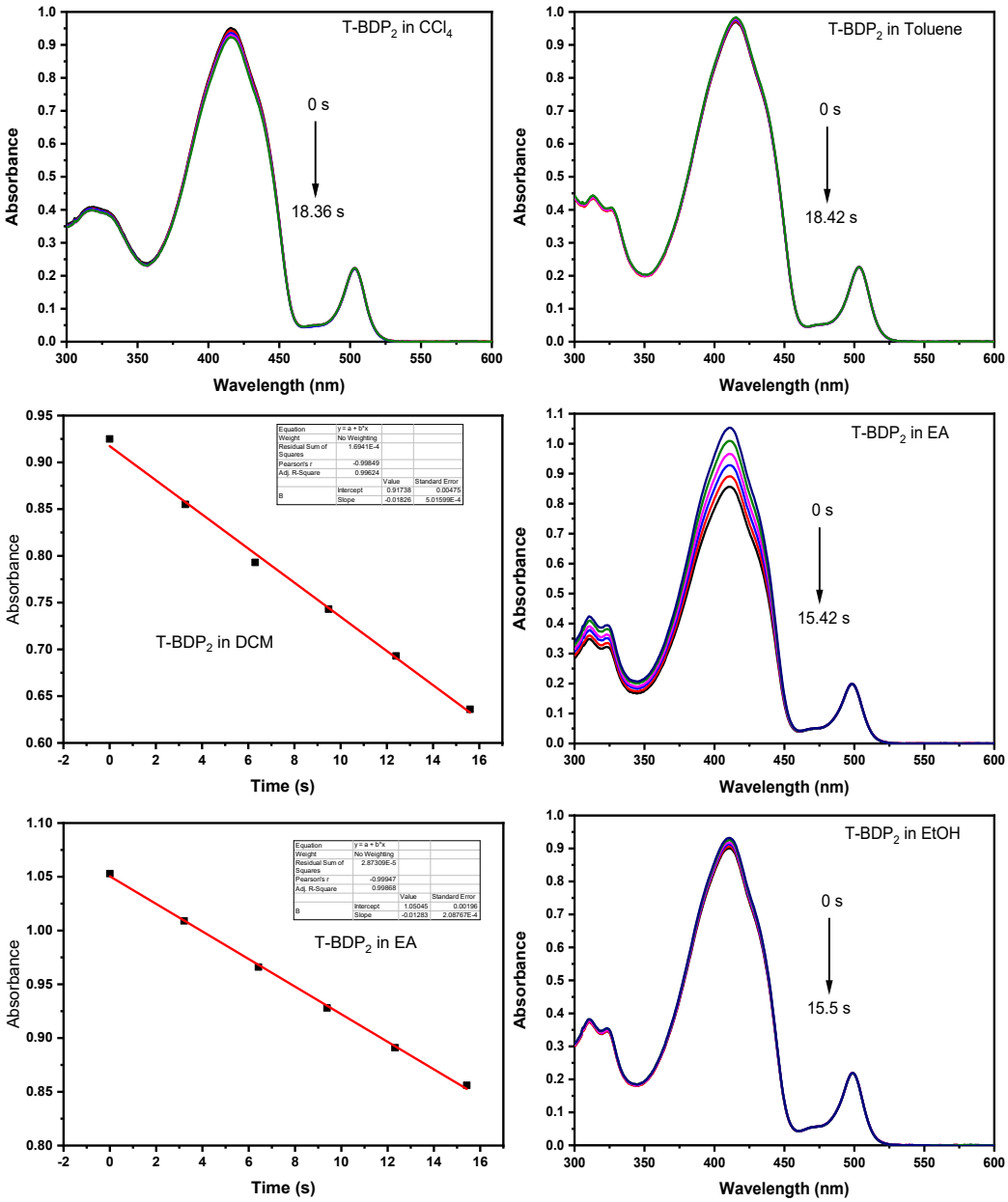


Fig S21. Singlet oxygen test of T-BDP$_2$ in different solvent system.

14. Singlet oxygen test of T-BDP$_2$-I in different solvent system.

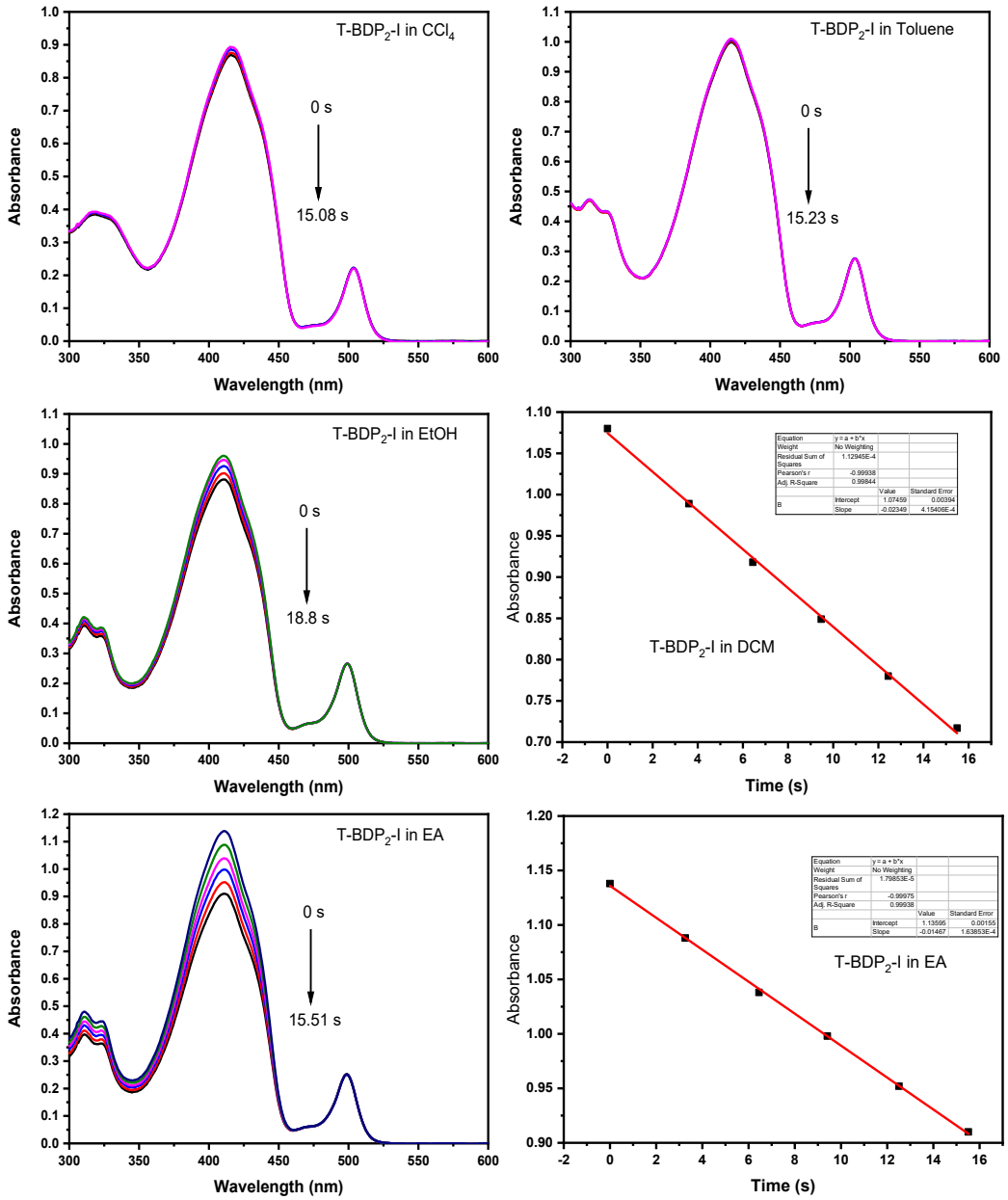


Fig S22. Singlet oxygen test of T-BDP$_2$-I in different solvent system.

15. Singlet oxygen test of T-BDP$_3$ in different solvent system.

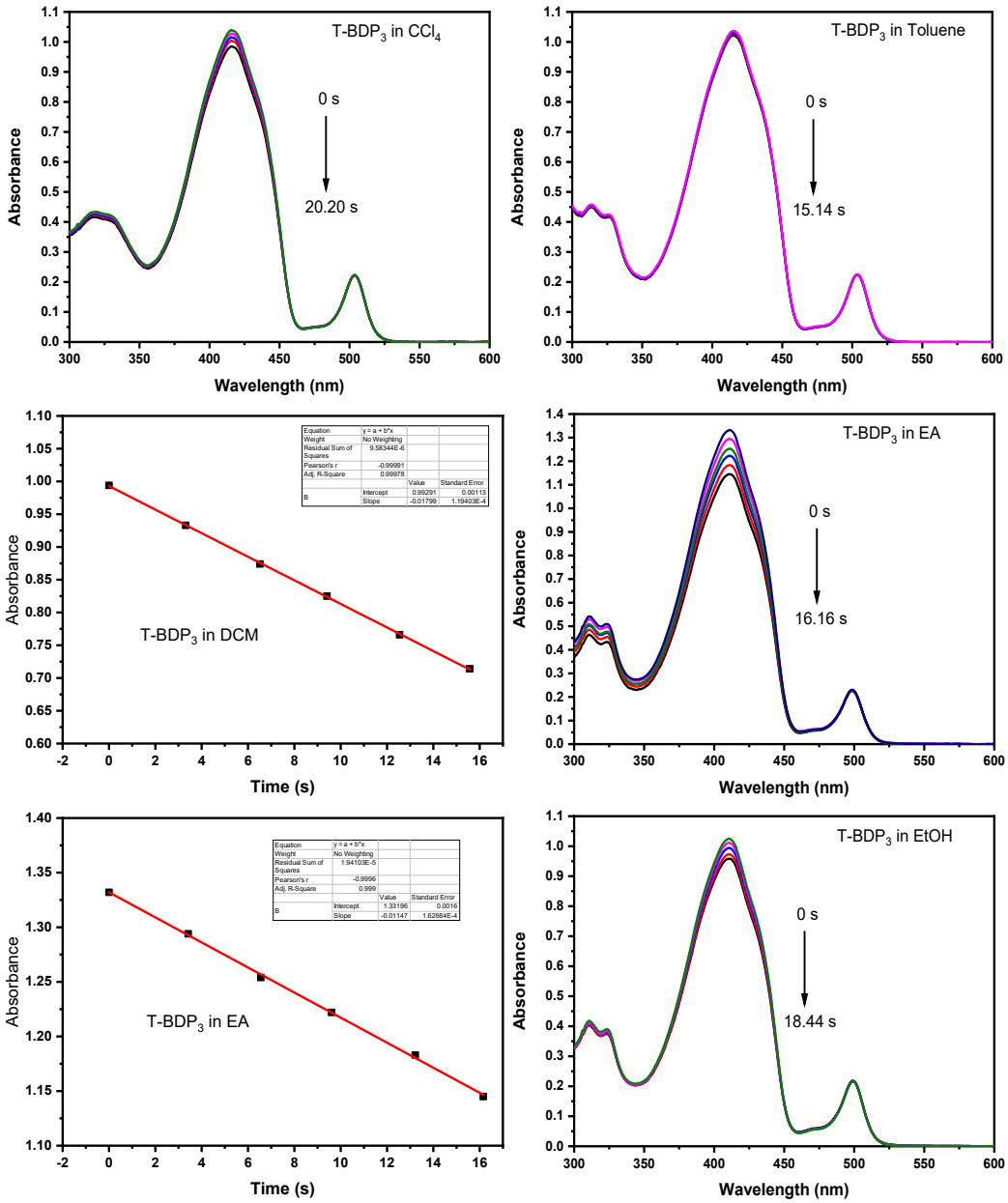


Fig. S23 Singlet oxygen test of T-BDP$_3$ in different solvent system.

16. Singlet oxygen test of Compound 2I-Ph-BDP(The reference) in DCM.

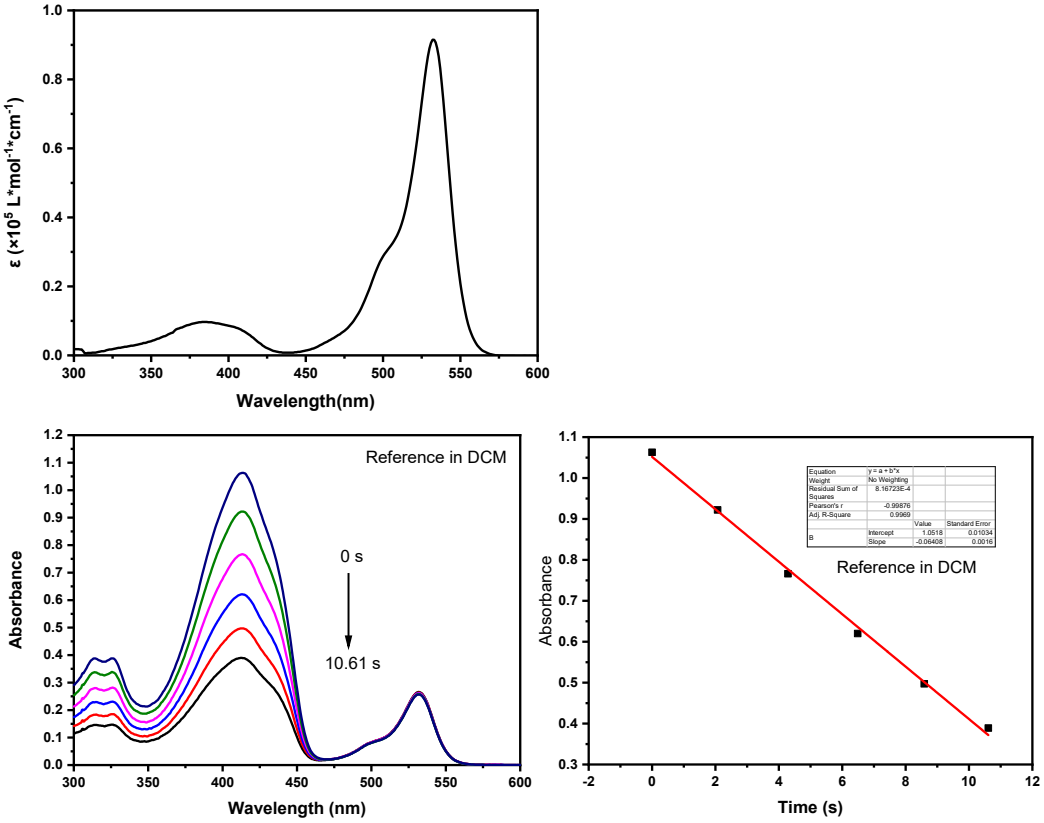


Fig. S24 The molar extinction coefficient and singlet oxygen test of 2I-Ph-BDP in DCM.

17. The detailed synthesis methods of these compounds

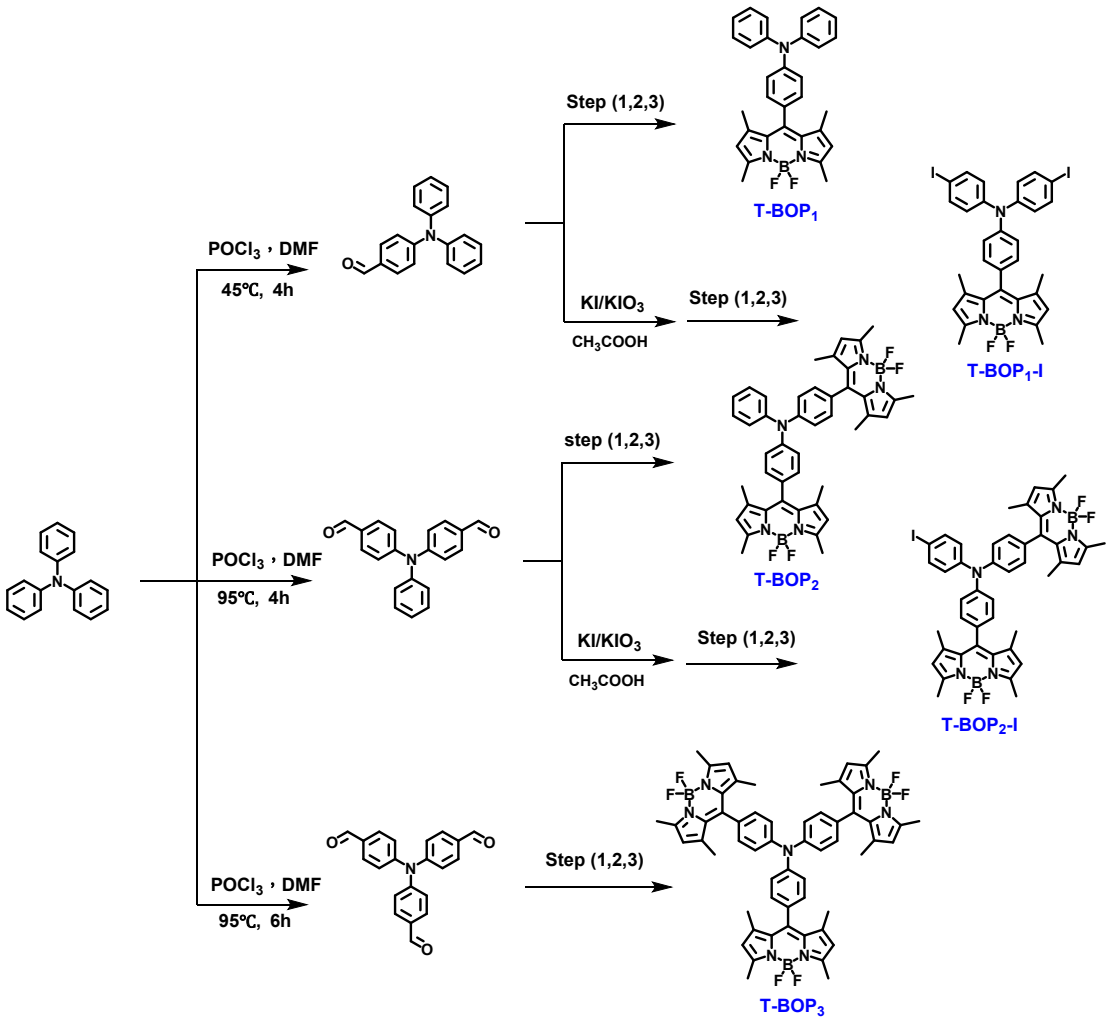


Fig. S25 The synthesis method of a series of triphenylamine-BODIPY compounds. (Note: Step1:2,4 Dimethylpyrrole, TFA,DCM,r.t. Step2: TCQ, DCM, r.t. Step3: $BF_3{\cdot}OEt_2$,Et_3N,DCM, r.t.)

The general procedure of formylation of triphenylamine：

In a 250mL flask, the DMF solvent was added. Then, the phosphorus trichloride was dropwise added in an ice water bath. After the addition of phosphorus trichloride, the solution continue to stir in an ice bath for 1 h. The color of solution turn pink yellow. At this point, triphenylamine was added and the reaction was transferred to the oil bath. The reaction was heat to different temperature. The solution gradually changed from yellow to red. After the reaction is complete, the reaction system cool to room temperature and was poured the solution into the 200 mL water. A large amount of earthy solid was precipitated. Then, the saturated sodium hydroxide

solution was used to adjust the PH to 7~8`. After filtration, the precipitation was dissolved with ethyl acetate and a series of triphenylaldehyde compounds was obtained by column separation.

, ,.

Reaction equivalent:

For 4-(diphenylamino)benzaldehyde : DMF 4 mL (51.9 mmol), $POCl_3$ 14.7 mL (168.5 mmol) , 5 g (20.4 mmol) triphenylamine, 45℃, 4 h. obtain: 4.58 g, yield: 82.1%

For 4,4'-(phenylazanediyl)dibenzaldehyde : DMF 18.2 mL (236.1 mmol), $POCl_3$ 23.75 mL (272.2 mmol) , 2.5 g (10.2 mmol) triphenylamine, 95℃, 4 h. obtain: 1.92 g,yield: 62.5%

For 4,4',4''-nitrilotribenzaldehyde : DMF 18.2 mL (236.1 mmol), $POCl_3$ 23.75 mL (272.2 mmol) , 2.5 g (10.2 mmol) triphenylamine, 95℃, 6 h. obtain: 0.68 g yield: 20.4%

The general procedure of Iodide reaction of triphenylamine:

In 100mL round-bottomed flask, the correspond triphenylamine aldehyde, KI and KIO_3 was add in turn and, afterward, 10 mL of glacial acetic acid was added. The mixed solution was react at 70 degrees for 3 h. When the reaction completed, the solution was pour into 100 mL of ice water and the yellow flocculent solid precipitates immediately. After filtration, the product was obtained by flash column separation.

Reaction equivalent:

For 4-(bis(4-iodophenyl)amino)benzaldehyde: 4-(diphenylamino)benzaldehyde 100 mg (0.37 mmol), KIO_3 158.4 mg (0.74 mmol), KI 122.8 mg (0.74 mmol) obtain: 186 mg yield: 96.1%

For 4,4'-((4-iodophenyl)azanediyl)dibenzaldehyde: 4,4'-(phenylazanediyl) dibenzaldehyde 100 mg (0.33 mmol), KIO_3 71.1 mg (0.33 mmol), KI 55.1 mg (0.33 mmol) obtain: 135 mg yield: 95.3%

The general procedure of triphenylamine BODIPY structure:

In a 250 mL flask, 100 mL of anhydrous dichloromethane, 2,4-dimethylpyrrole (1.2 mmol) and the corresponding aldehyde (1 mmol) was added. After the reaction system was stirred evenly, 1 drop of TFA was added as a catalyst. The reaction system turn from pale yellow to dark red (in the case of triphenylamine monoaldehyde) or purple (in the case of triphenylamine polyaldehyde). After 3 hours of reaction at room temperature, tetrachlorobenzoquinone (TCQ) was added to the solution, and the reaction was continued for 1 hour and the reaction system turn to black. Next, trimethylamine (Et_3N) was added and, after 15 minutes, boron trifluoride ether ($BF_3{\cdot}OEt_2$) was added, and the reaction was stirred for 12 h. After the reaction was completed, the reaction solution was poured into 100 mL of water, extracted with DCM three times, and the corresponding BODIPY photosensitizer was obtained by column separation.

Reaction equivalent:

For T-BDP_1 and T-BDP_1-I: 4-(diphenylamino)benzaldehyde 273 mg (4-(bis(4-iodophenyl)amino)benzaldehyde 525 mg), 2,4-dimethylpyrrole 228 mg, TCQ,245 mg, Et_3N, 2 mL, $BF_3{\cdot}OEt_2$ 3 mL. obtain: 97.2 mg (134.5 mg) yield: 19.8 % (18.1%)

For T-BDP_2 and T-BDP_2-I: 4,4'-(phenylazanediyl)dibenzaldehyde 301 mg (4,4'-((4-iodophenyl)azanediyl)dibenzaldehyde 427 mg), 2,4-dimethylpyrrole 456 mg, TCQ, 490 mg, Et_3N, 4 mL, $BF_3{\cdot}OEt_2$ 6 mL. obtain: 62.7mg (62.1 mg) yield: 8.5 % (7.2%)

For T-BDP_3: 4,4',4''-nitrilotribenzaldehyde 329 mg, 2,4-dimethylpyrrole 684 mg, TCQ, 735 mg, Et_3N, 6 mL, $BF_3{\cdot}OEt_2$ 9 mL. obtain: 24.6 mg yield: 2.5 %

18. The photostability of DHR123 in pure water solvent

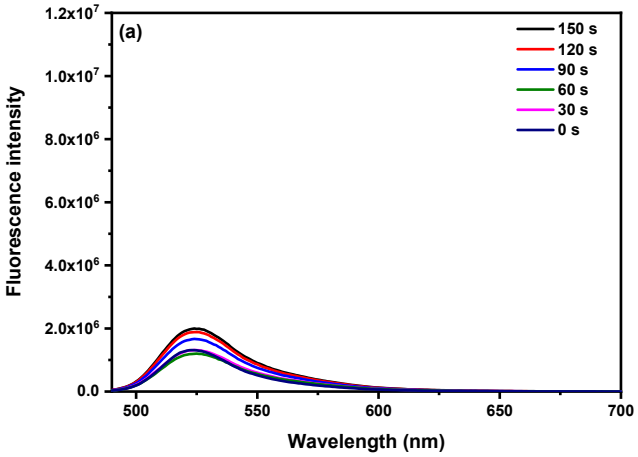


Fig S26. The photostability of DHR123 in pure water solvent

19. The Electron hole transfer situation of T-BDP$_1$ and T-BDP$_2$

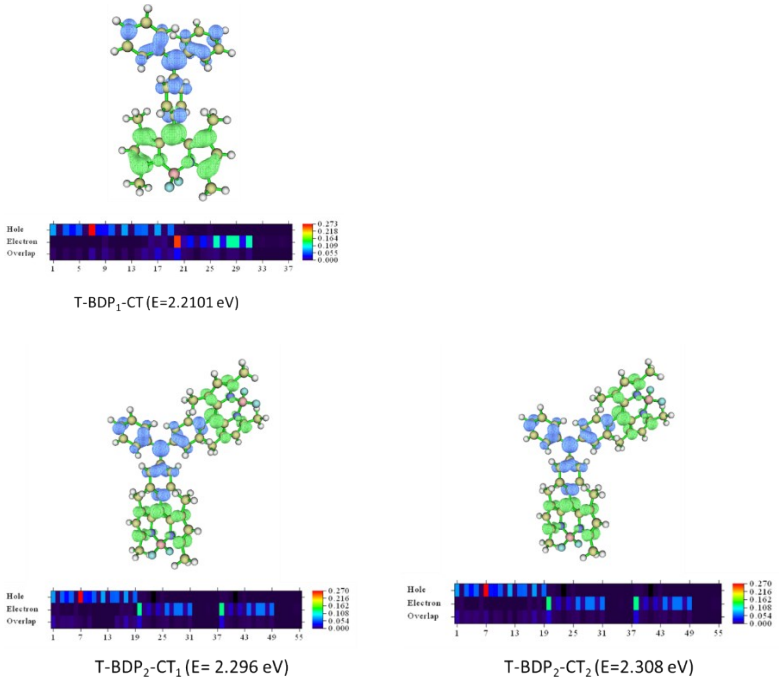


Fig S27**.** The electron (green) and hole (blue) transfer situation of the CT state of the T-BDP$_1$ and T-BDP$_2$. (The atomic number of the heat map is range from the number of the first non-hydrogen element.)

20. The Electron hole transfer situation of the LE state of T-BDP$_3$

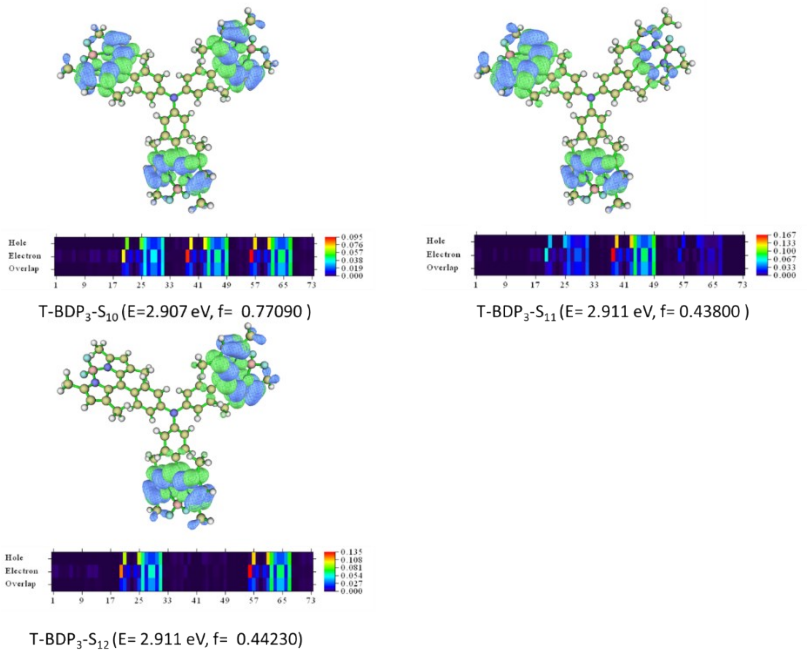
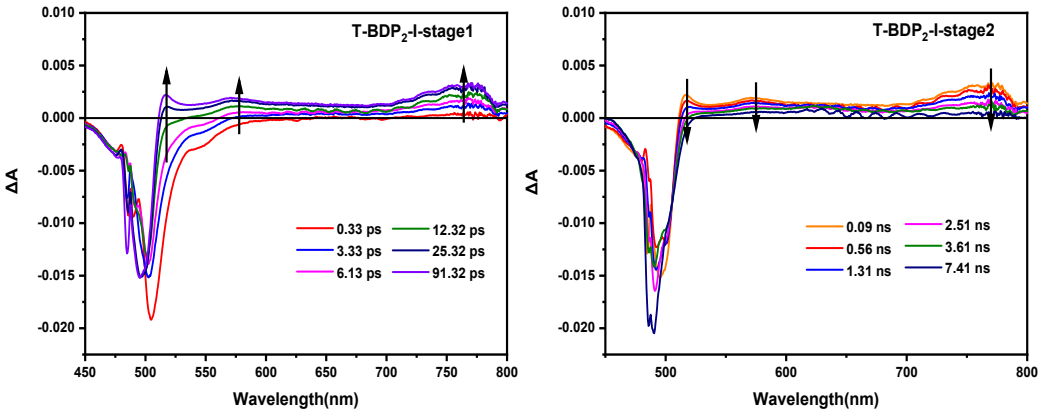


Fig S28**.** The electron (green) and hole (blue) transfer situation of the LE state of T-BDP$_3$. (The atomic number of the heat map is range from the number of the first non-hydrogen element.)

21. The fs-transient absorption spectra of T-BDP$_2$-I



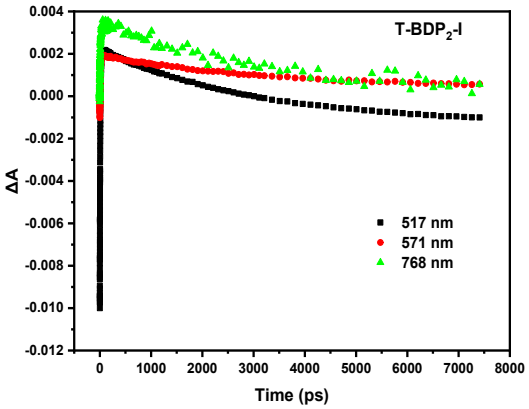


Fig. S29 Transient absorption spectra of T-BDP_2-I and attenuation curves of major absorption peaks.

22. Theoretical calculation result of fluorescence emission

T-BDP_1:

```
Excited State   1:      Singlet-A      2.0960 eV  591.53 nm  f=0.0002  <S**2>=0.000
    257 -> 258        0.70319
This state for optimization and/or second-order correction.
Total Energy, E(TD-HF/TD-DFT) =  -3262.02544738
```

T-BDP_2:

```
Excited State   1:      Singlet-A      2.0098 eV  616.90 nm  f=0.0009  <S**2>=0.000
    193 ->194         0.70353
This state for optimization and/or second-order correction.
Total Energy, E(TD-HF/TD-DFT) =  -2424.56016044
```

T-BDP_3:

```
Excited State   1:      Singlet-A      1.9175 eV  646.59 nm  f=0.0093  <S**2>=0.000
    129 ->130         0.70409
This state for optimization and/or second-order correction.
Total Energy, E(TD-HF/TD-DFT) =  -1587.25825385
```

Fig. S30 The theoretical calculation result of fluorescence emission

23. Theoretical calculation result of optimized ground state structure

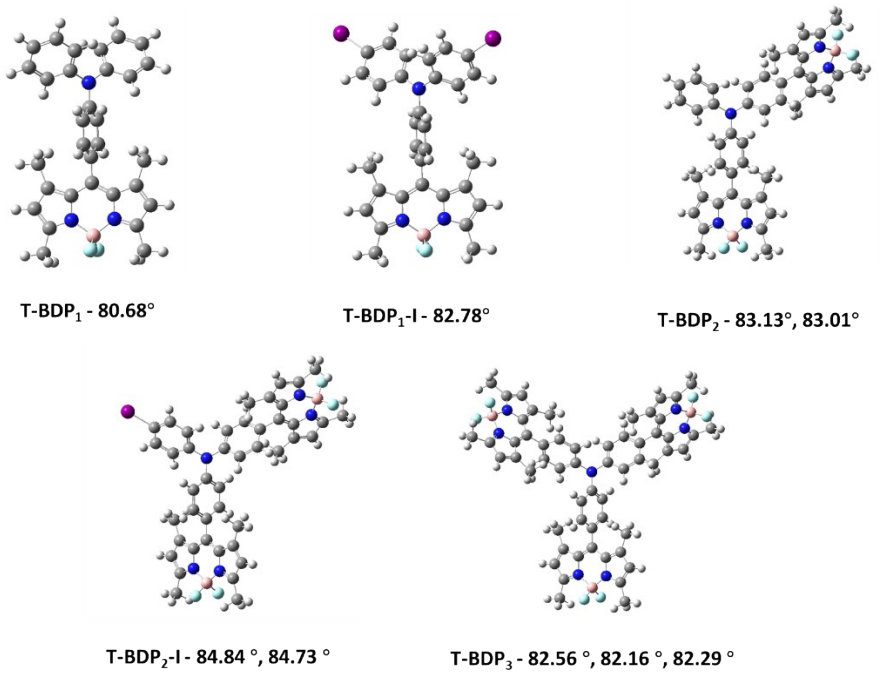


Fig. S31 Theoretical calculation result of optimized ground state structure

24. The fluorescence imaging experiment of T-BDP$_3$ in zebrafish

In fluorescence imaging of zebrafish[8], the purchased zebrafish seedling was incubated in melanin inhibitor containing medium at 28.5℃ environment and zebrafish egg will become fish-shaped within 24-48 hours. Then, the 20 μL T-BDP$_3$ photosensitizer (10^{-3} M) was added to the zebrafish containing medium and incubated for 5 hours. After that, 30 μL MS222 anesthetic was added to that petri dish and incubated for another 15 minutes. Until no obvious swimming was observed, the zebrafish was used to fluorescence imaging. The detection of $O_2^{\cdot-}$ (Superoxide radical) was similar to the method used in cells and the dosage is 20 μL photosensitizer (10^{-3} M).

25. The cytotoxicity test of T-BDP_3 through MTT experiment in A-549 cells

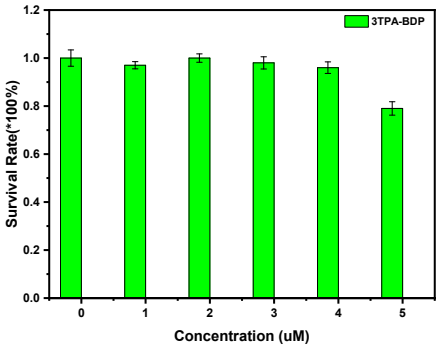
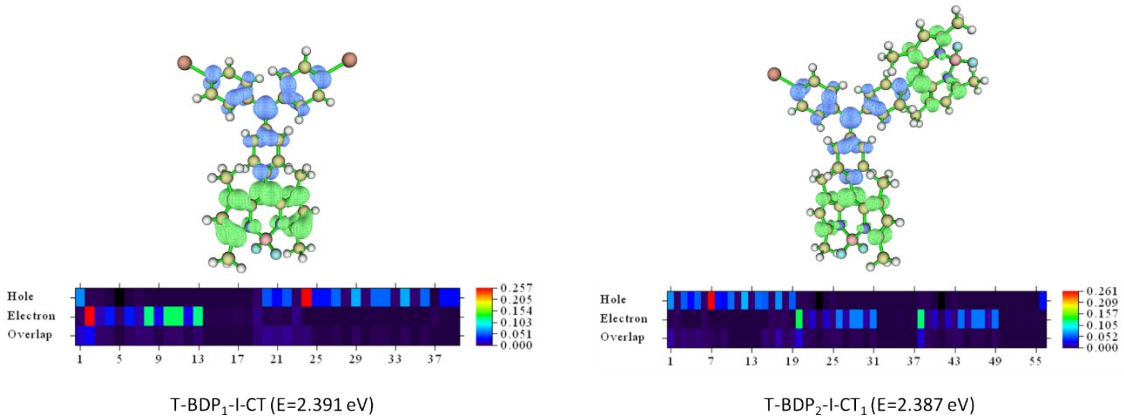


Fig. S32 The cytotoxicity test of T-BDP_3 in cells.

26. The electron (green) and hole (blue) transfer situation of T-BDP_1-I and T-BDP_2-I



T-BDP_1-I-CT (E=2.391 eV)

T-BDP_2-I-CT_1 (E=2.387 eV)

Fig. S33 The electron (green) and hole (blue) transfer situation of T-BDP_1-I and T-BDP_2-I. (The atomic number of the heat map is range from the number of the first non-hydrogen element.)

27. The fluorescence imaging of T-BDP$_3$ in zebrafish.

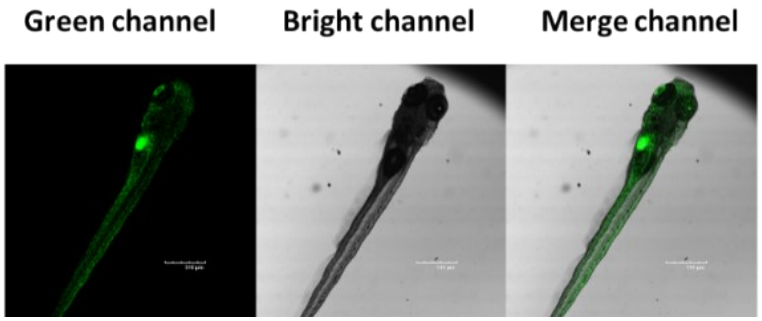


Fig. S34 The fluorescence imaging of T-BDP$_3$ in zebrafish (7 day age).

28. The calculation of the power density of the monochrome LED light at green waveband

The illuminance at 15 cm away from the light was about 1.6w lux, the power density of the monochrome green light was 2.3 mW/cm^2 according the transformational relation at green waveband.

29. The solubility and stability of T-BDP$_3$ in pure water condition

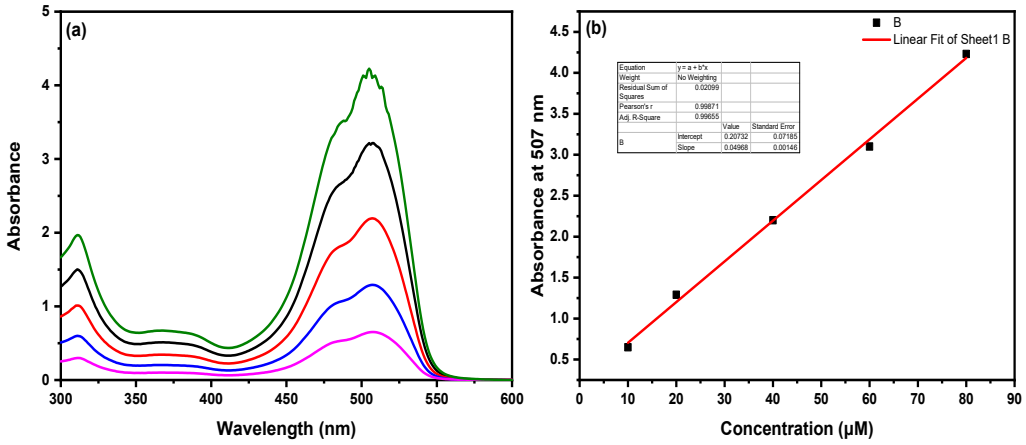


Fig S35 The solubility and stability of T-BDP$_3$ in pure water condition.

30. The photostability of T-BDP$_3$ in pure water condition

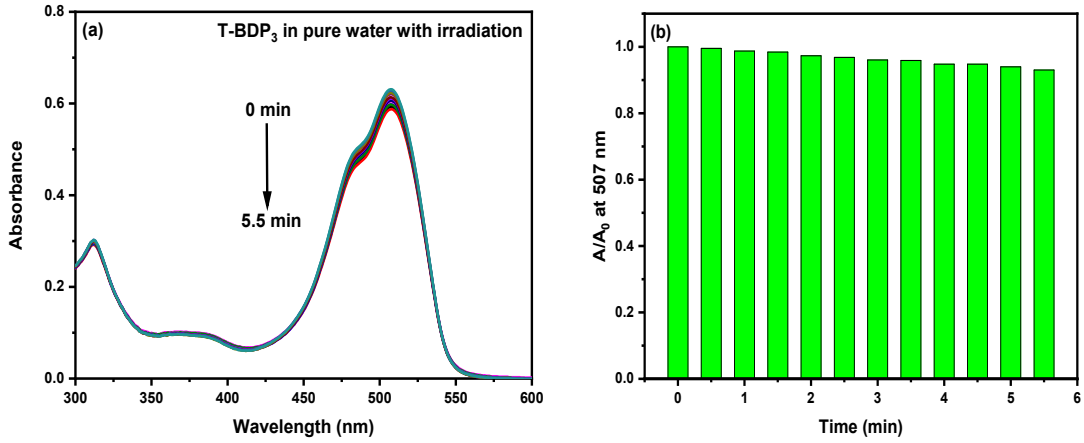


Fig S36 The photostability of of T-BDP$_3$ in pure water condition

31. The fluorescence spectra of T-BDP$_3$ in pure water condition

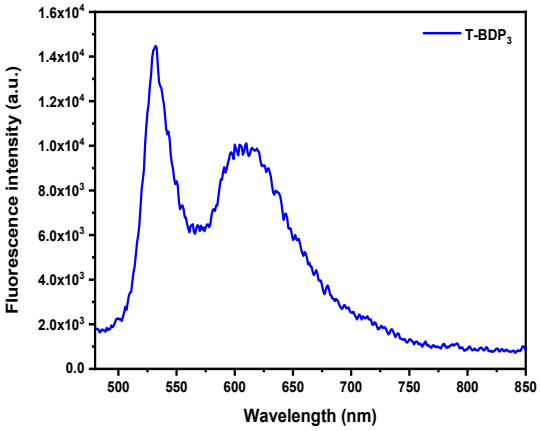


Fig. S37 The fluorescence spectra of T-BDP$_3$ in pure water condition

32. The fs-transient state spectra of T-BDP$_3$ through SVD analysis.

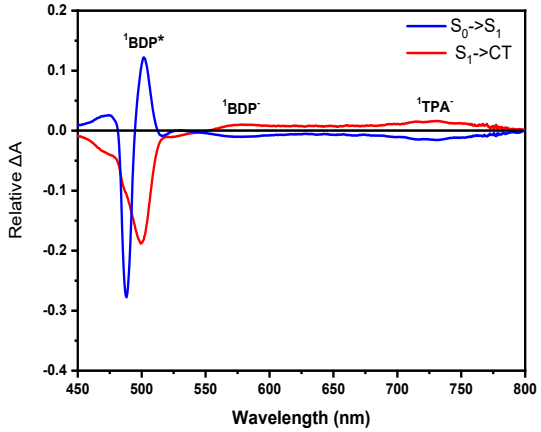


Fig. S38 The fs-transient state spectra of T-BDP$_3$ through SVD analysis.

33. The co-solvent methods for the application of organic material in biological system.

At first, the T-BDP$_3$ photosensitizer dissolved in DMSO solvent to obtain the mother liquor with the concentration at 10^{-3} M. Then, only a small fixed amount mother liquor, like 5 μL or 10 μL, was used for the biological experiment in 2 mL culture medium. Because the DMSO with 0.25% or 0.5% volume content will not cause damage to cells or zebrafish and the DMSO has good compatibility with water, thus, the T-BDP$_3$ photosensitizer could dispersed in the water system evenly. Then, the T-BDP$_3$ photosensitizer could be absorb by the cell or zebrafish through endocytosis process.

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