Chalcogen Modification: One-Step Strategy for Tuning the Photophysical Properties and NIR Phototherapy of Iodinated BODIPY

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Supporting Information

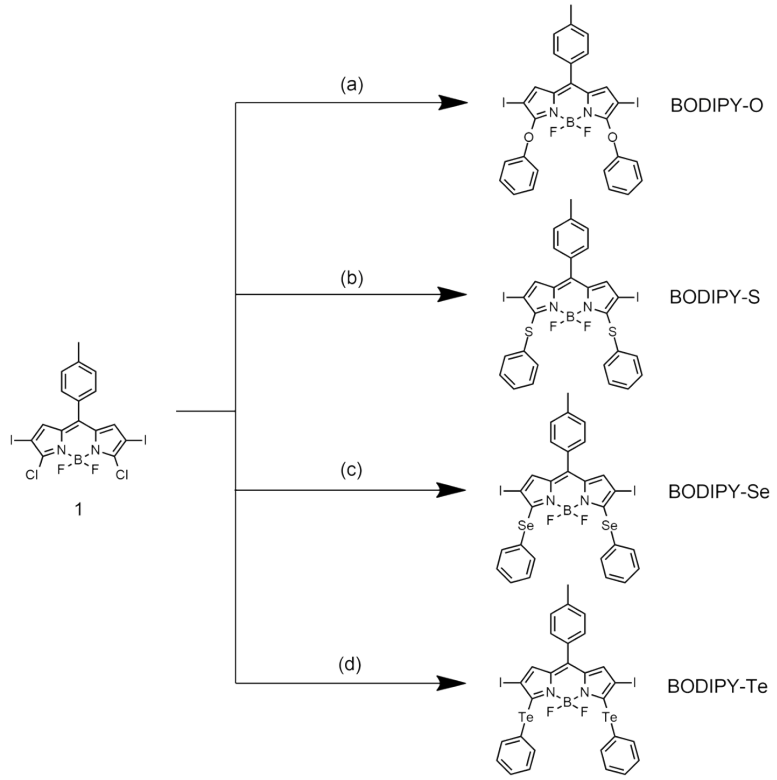
Experimental Procedures

1. Materials and methods

All reagents and deuterated solvents were commercially available and used without further purification. Pluronic-127 (F-127), phenol, diphenyl disulfide, diphenyl diselenide, and diphenyl ditelluride are all purchased from J&K Scientific. 2',7'-Di-chlorodihydrofluorescein diacetate (DCFH-DA), 3-[4,5-dimethylthiazol-2-yl]-2,5-di-phenyltetrazolium bromide (MTT), Annexin V-FITC, dulbecco's modified eagle medium (DMEM), phosphate buffered solution (PBS) and fetal bovine serum (FBS) were purchased from Beyotime Biotechnology. NMR spectra were recorded with a 500 MHz NMR spectrometer (AVANCE, Bruker, Germany). ^{1}H and ^{13}C NMR chemical shifts were reported relative to residual solvent signals. The molecular weights of the samples were determined by mass spectrometer (Agilent, USA). The UV-Vis absorption spectra were measured by a spectrophotometer (U-5300, HAITACHI, Japan). The fluorescent emission spectra were recorded on a fluorescence spectrophotometer (F-7000, HITACHI, Japan). Transmission electron microscope (TEM, JEM-2100F, HITACHI, Japan) images were captured at an accelerating voltage of 200 kV. Dynamic light scattering (DLS) experiments were performed on a Size Analyzer (NanoBrook-90 Plus, Brookhaven Instrument Corporation, USA) with a He-Ne laser (633 nm) and 90° collecting optics.

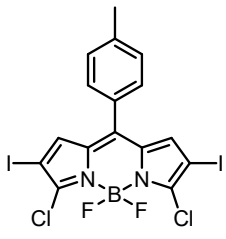
2. Synthesis

2.1 Synthesis of BODIPY-X



Scheme S1 Synthesis of compounds BODIPY-X (X = O, S, Se, and Te). Reaction conditions: (a) $NaBH_4$, K_2CO_3, CH_3CN, refluxed for 12 h, 50.0%. (b) Diphenyl disulfide, $NaBH_4$, THF, refluxed for 1 h; then **1**, THF, room temperature for 10 min, 50.0 %; (c) Diphenyl diselenide , $NaBH_4$, C_2H_5OH; then **1**, THF, room temperature for 1h, 48.0 %; (d) Diphenyl ditelluride, $NaBH_4$, C_2H_5OH; then **1**, THF, room temperature for 30 min 32.0 %.

*2.1.1 Synthesis of compound **1***



Compound **1** was synthesized as the reported literature and the ^{1}H NMR of **1** matched well with the reported data.[S1] ^{1}H NMR (500 MHz, $CDCl_3$, 298 K) δ (ppm): 7.36 (d, J = 8.3 Hz, 2H), 7.38 (d, J = 8.3 Hz, 2H), 7.07 (s, 2H), 2.48 (s, 3H).

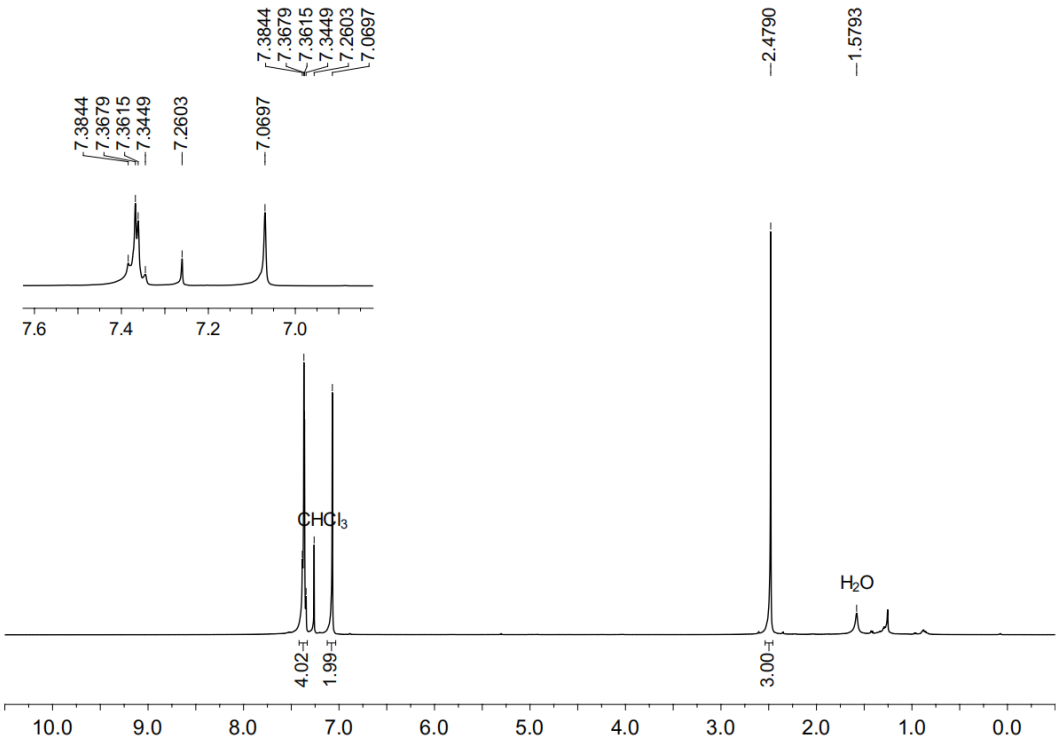
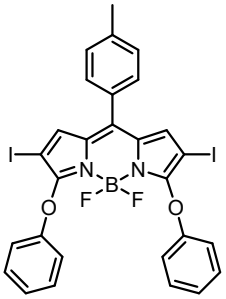


Figure S1 ^{1}H NMR spectrum (500 MHz, $CDCl_3$, 298 K) recorded for **1**.

2.1.2 Synthesis of compound BODIPY-O



Compound **1** (250 mg, 0.41 mmol), phenol (82 mg, 1.05 mmol) and K_2CO_3 (145 mg, 1.25 mmol) were added into CH_3CN (100 mL). The mixture solution was heated at reflux for 12 h under nitrogen. After the solvent was evaporated, the residue was extracted with dichloromethane. The organic layer was dried by anhydrous Na_2SO_4 and the solvent was removed under vacuum. The crude product was purified by silica gel flash column chromatography (petroleum ether/dichloromethane, 30:1, *v*/*v*) to afford BODIPY-O as a red solid (150 mg, 50.4%). ^{1}H NMR (500 MHz, $CDCl_3$, 298 K) δ (ppm): 7.44 (d, J = 7.9 Hz, 2H), 7.36 (d, J = 8.4 Hz, 6H), 7.13 (t, J = 7.4 Hz, 2H), 7.10 – 7.05 (m, 6H), 2.48 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$, 298K) δ (ppm): 160.3, 155.8, 141.7, 141.5, 138.1, 130.5, 129.9, 129.7, 129.5, 129.4, 124.4, 118.1, 62.0, 21.5. MS (MALDI-TOF): calcd for $C_{28}H_{19}O_2N_2BF_2I_2$ [M^+] 717.9600; found: 717.9603.

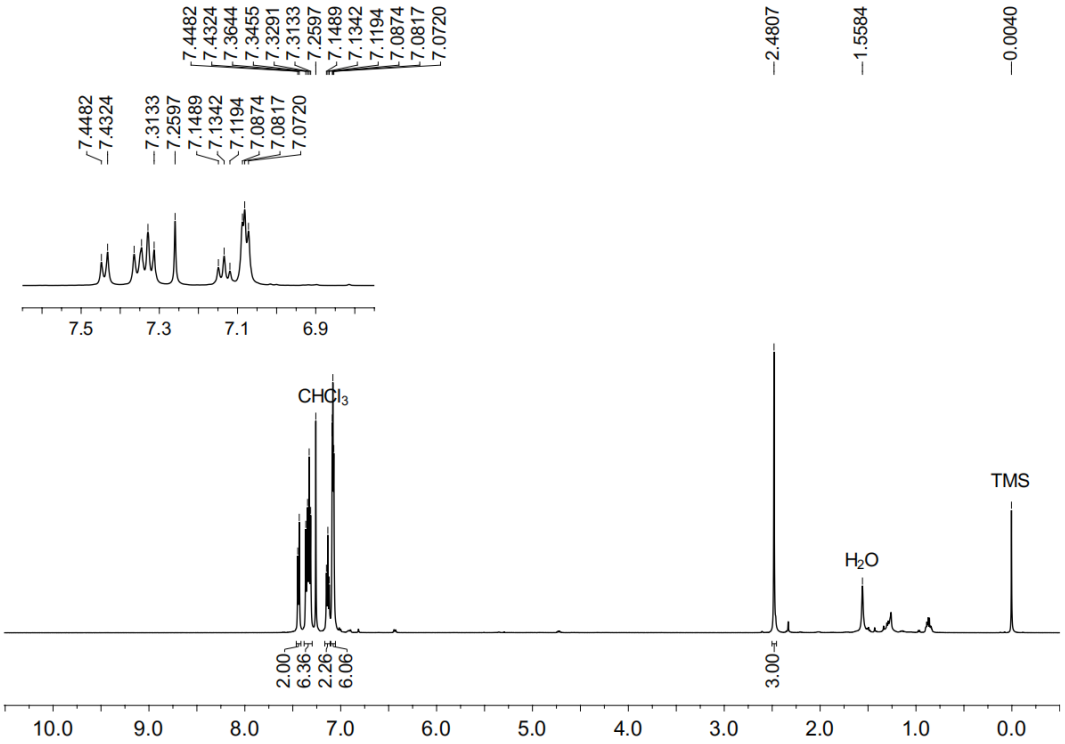


Figure S2 ^{1}H NMR spectrum (500 MHz, $CDCl_3$, 298 K) recorded for BODIPY-O.

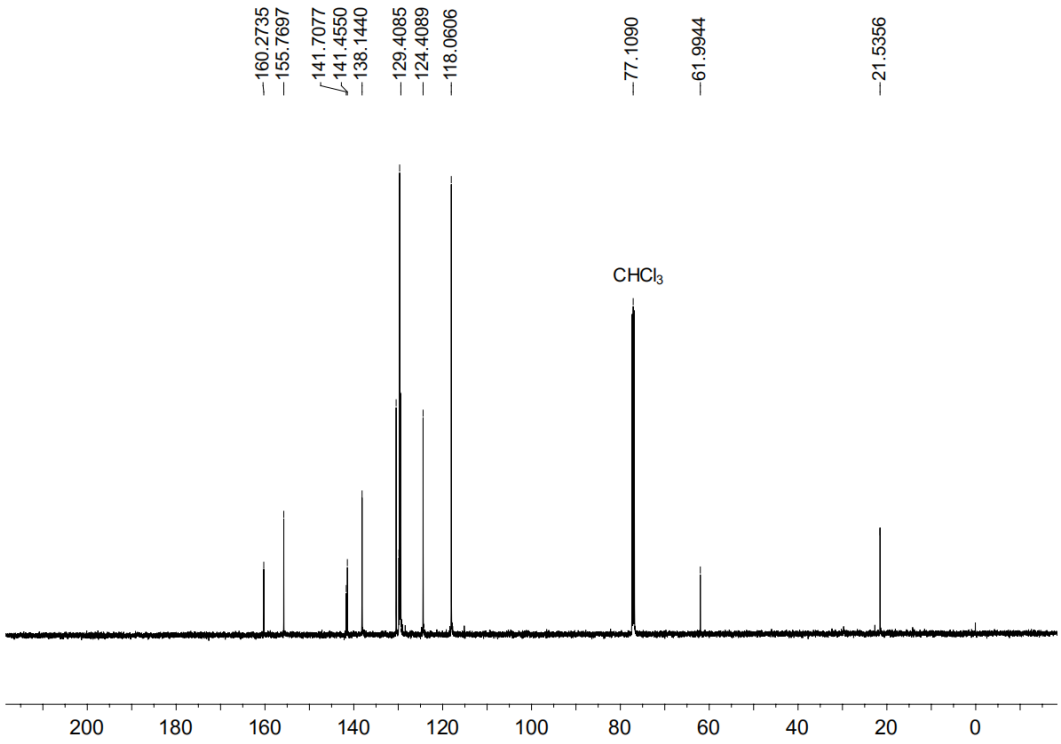


Figure S3 ^{13}C NMR spectrum (126 MHz, $CDCl_3$, 298 K) recorded for BODIPY-O.

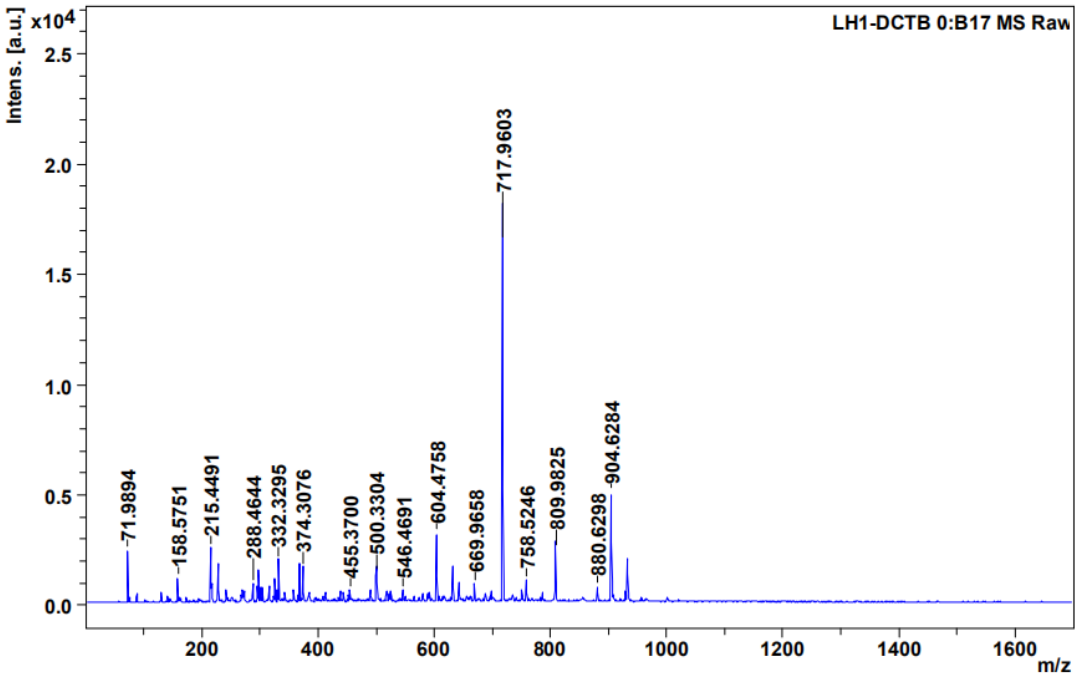
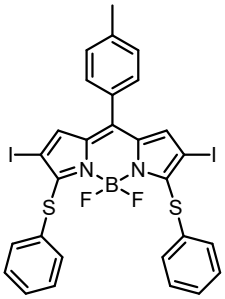


Figure S4 MALDI-TOF spectrum of BODIPY-O.

2.1.3 Synthesis of compound BODIPY-S



Diphenyl disulfide (108 mg, 0.5 mmol) and sodium borohydride (216 mg, 1.5 mmol) were dissolved in THF (100 mL), and the mixture was heated at reflux for 1 h under nitrogen. Then a solution of compound **1** (218 mg, 0.3 mmol) in THF (20 mL) was added to the above mixture. The mixture solution was further stirred for 10 minutes at room temperature. After the solvent was evaporated, the residue was extracted with dichloromethane. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under vacuum. The crude product was purified by silica gel flash column chromatography (petroleum ether/dichloromethane, 30:1, *v*/*v*) to afford BODIPY-S as a red solid (112 mg, 41.3%). ^{1}H NMR (500 MHz, $CDCl_3$, 298 K) δ (ppm): 7.42 (d, J = 7.6 Hz, 6H), 7.35 (d, J = 7.7 Hz, 2H), 7.30 (t, J = 7.3 Hz, 4H), 7.24 (s, 2H), 7.13 (s, 2H), 2.47 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$, 298K) δ (ppm): 153.3, 141.9, 141.7, 138.7, 137.1, 133.5, 131.2, 130.6, 129.9, 129.5, 129.2, 127.6, 84.1, 21.6. MS (MALDI-TOF): calcd for $C_{28}H_{19}N_2BF_2S_2I_2$ [M^+] 741.9103; found: 741.9100.

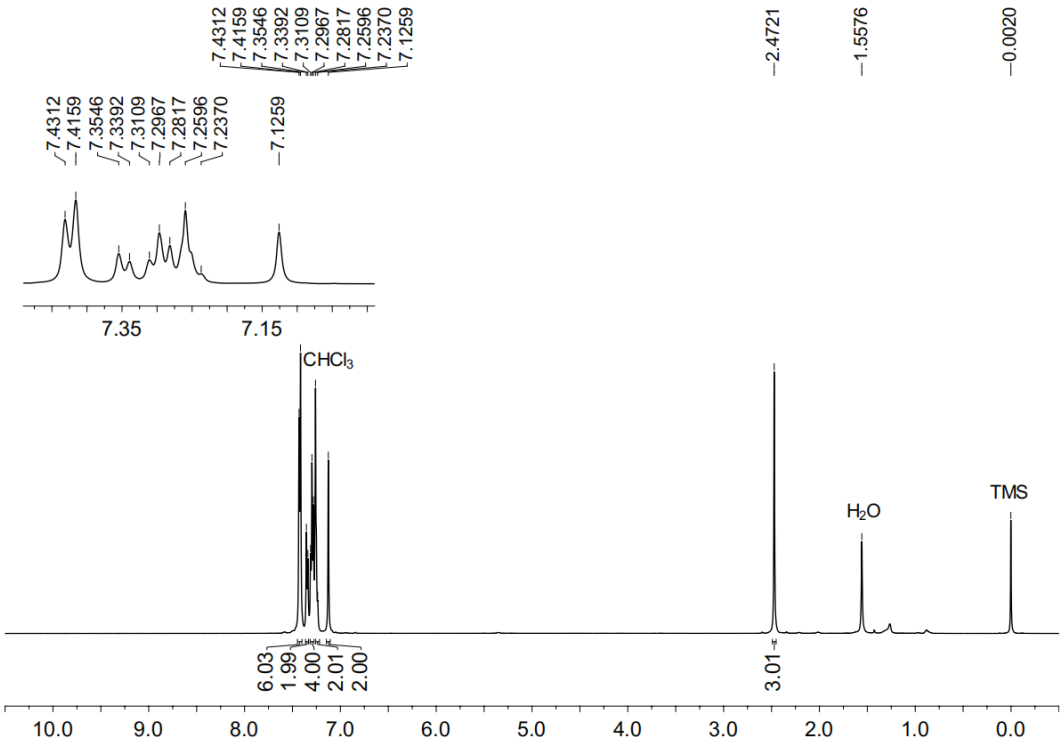


Figure S5 ^{1}H NMR spectrum (500 MHz, $CDCl_3$, 298 K) recorded for BODIPY-S.

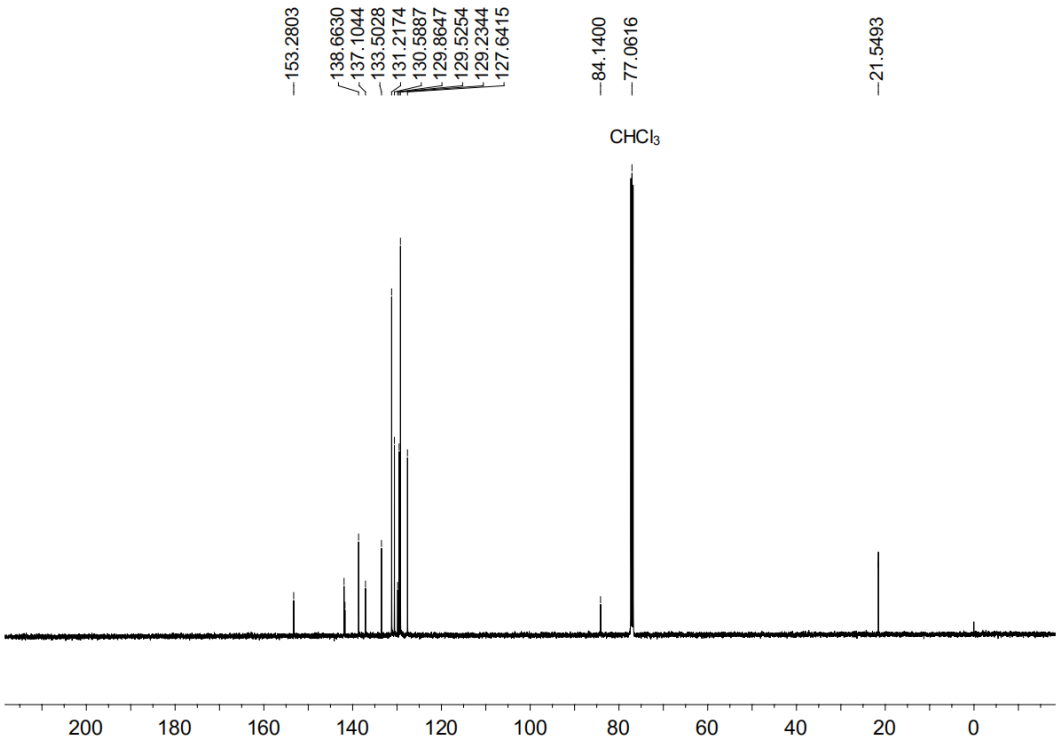


Figure S6 ^{13}C NMR spectrum (126 MHz, $CDCl_3$, 298 K) recorded for BODIPY-S.

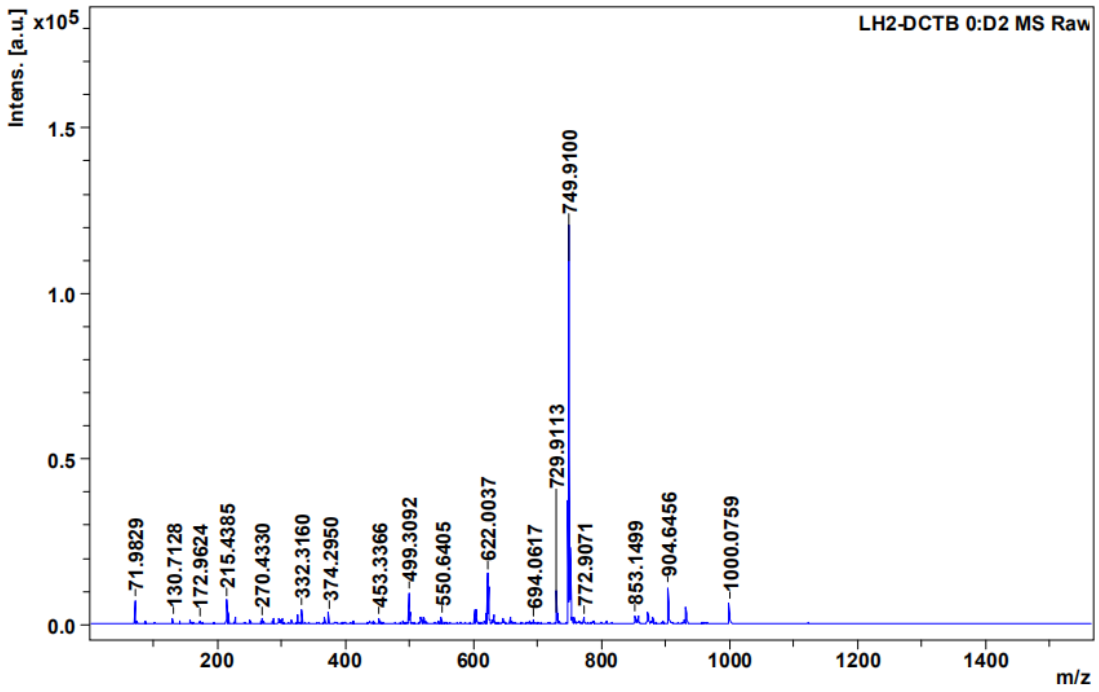
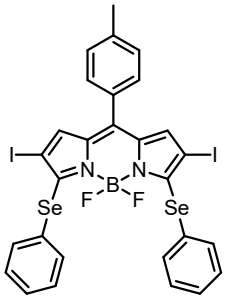


Figure S7 MALDI-TOF spectrum of BODIPY-S.

2.1.4 Synthesis of compound BODIPY-Se



Diphenyl diselenyl ether (100 mg, 0.32 mmol) was dissolved in ethanol (5 mL), and then the sodium borohydride (21.4 mg, 0.64 mmol) was slowly added to the mixture until the solution was colorless. After that, compound **1** (211 mg, 0.25 mmol) in THF (20 mL) was added into the above mixture, and the mixture solution was further reacted at room temperature for 1 h under nitrogen. After the solvent was evaporated, the residue was extracted with dichloromethane. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under vacuum. The crude product was purified by silica gel flash column chromatography (petroleum ether/dichloromethane, 30:1, *v*/*v*) to afford BODIPY-Se as a blue solid (124 mg, 42.0%). ^{1}H NMR (500 MHz, $CDCl_3$, 298 K) δ (ppm): 7.59 (s, 4H), 7.41 (d, J = 7.8 Hz, 2H), 7.33 (d, J = 7.8 Hz, 2H), 7.28 (s, 6H), 7.08 (s, 2H), 2.46 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$, 298K) δ (ppm): 151.1, 141.9, 141.4, 138.5, 137.8, 133.7, 130.5, 130.0, 129.9, 129.5, 129.4, 128.0, 86.8, 21.5. MS (MALDI-TOF): calcd for $C_{28}H_{19}N_2BF_2Se_2I_2$ [M^+] 845.8009; found: 846.7970.

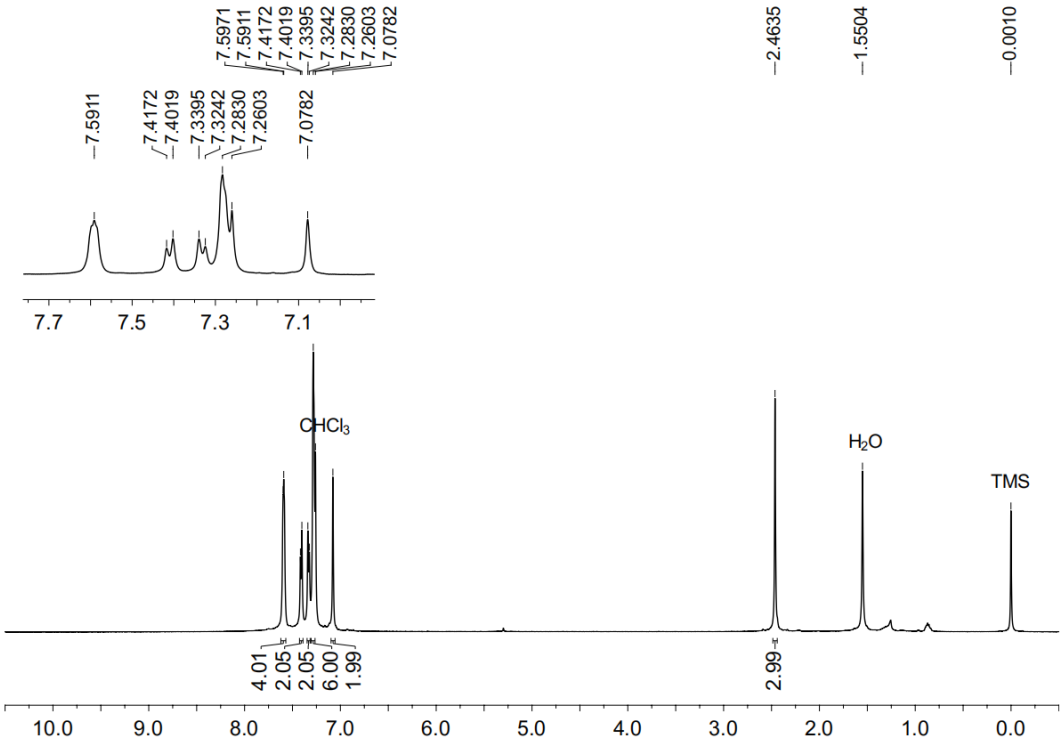


Figure S8 ^{1}H NMR spectrum (500 MHz, $CDCl_3$, 298 K) recorded for BODIPY-Se.

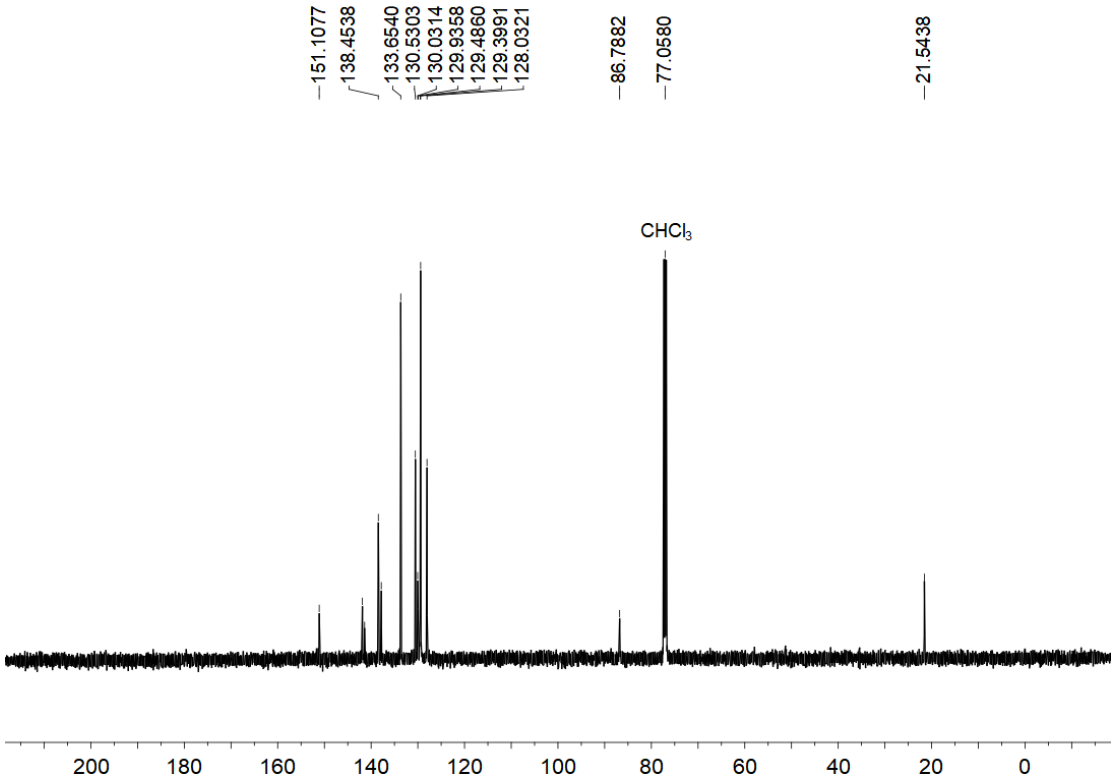


Figure S9 ^{13}C NMR spectrum (126 MHz, $CDCl_3$, 298 K) recorded for BODIPY-Se.

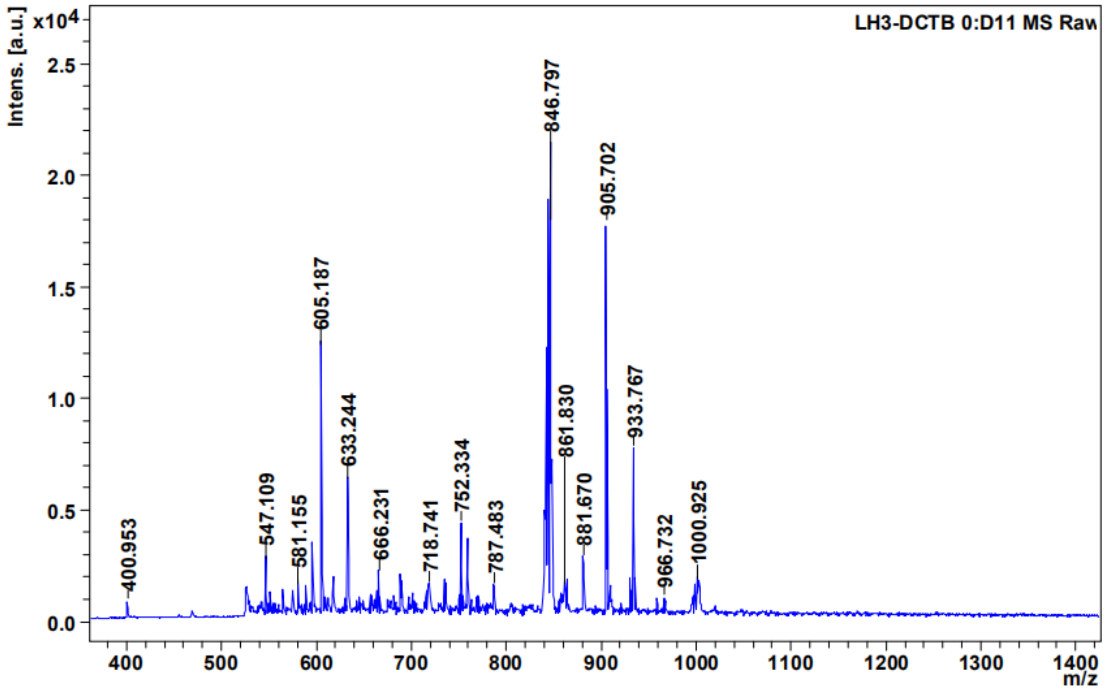
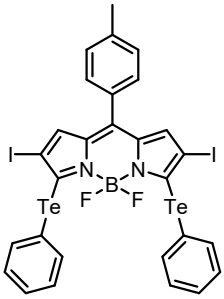


Figure S10 MALDI-TOF spectrum of BODIPY-Se.

2.1.5 Synthesis of compound BODIPY-Te



Diphenylditellurium (164 mg, 2.1 mmol) was dissolved in ethanol (5 mL), and then the sodium borohydride (289 mg, 2.5 mmol) was slowly added to the mixture until the solution was colorless. After that, compound **1** (200 mg, 0.24 mmol) in THF (20 mL) was added into the above mixture, and the mixture solution was further reacted at room temperature for 30 minutes under nitrogen. After the solvent was evaporated, the residue was extracted with dichloromethane. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under vacuum. The crude product was purified by silica gel flash column chromatography (petroleum ether/dichloromethane, 30:1, *v*/*v*) to afford BODIPY-Te as a blue solid (99 mg, 31.7%). ^{1}H NMR (500 MHz, CD_2Cl_2, 298 K) δ (ppm): 7.84 (dd, J = 8.1, J = 1.2 Hz, 4H), 7.42 (d, J = 8.1 Hz, 2H), 7.33 (m, 4H), 7.26 (t, J = 7.4 Hz, 4H), 7.08 (s, 2H), 2.45 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$, 298K) δ (ppm): 141.7, 139.8, 139.5, 139.30, 137.9, 130.5, 130.1, 129.6, 129.4, 128.6, 115.5, 92.7, 29.7, 21.5. MS (ESI-HRMS): $[M+3Na]^{3+}$ calcd. for 340.5935; found 340.5955.

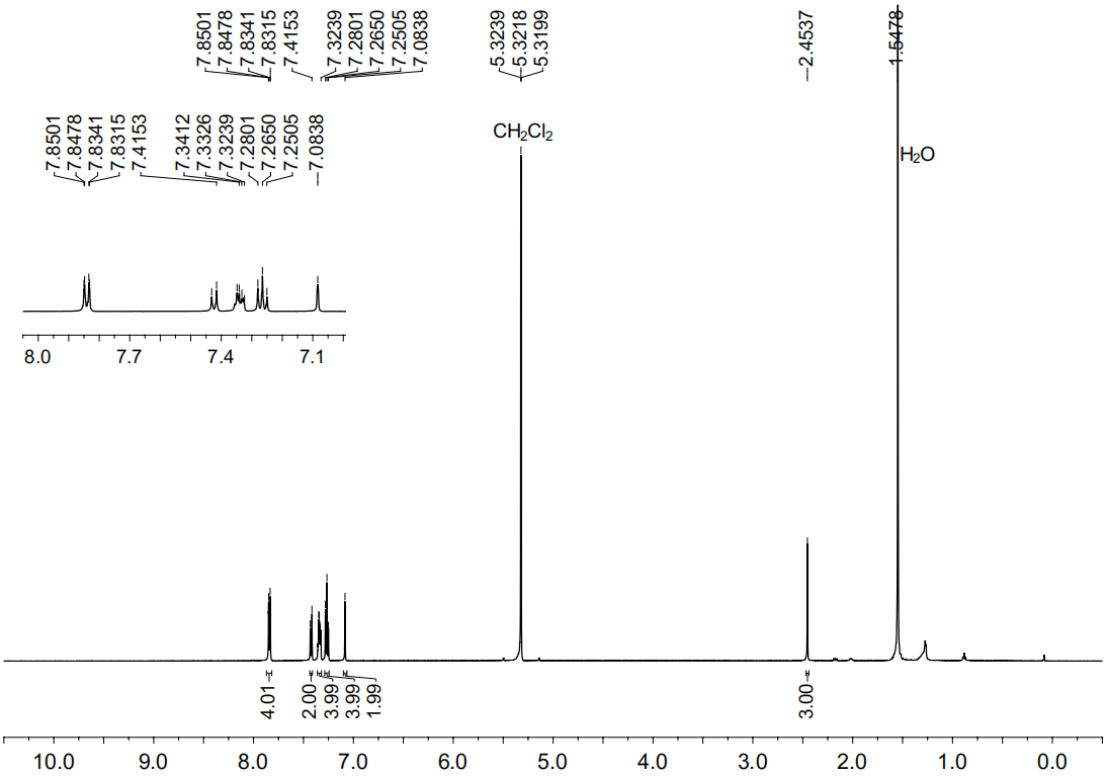


Figure S11 ^{1}H NMR spectrum (500 MHz, $CDCl_3$, 298 K) recorded for BODIPY-Te.

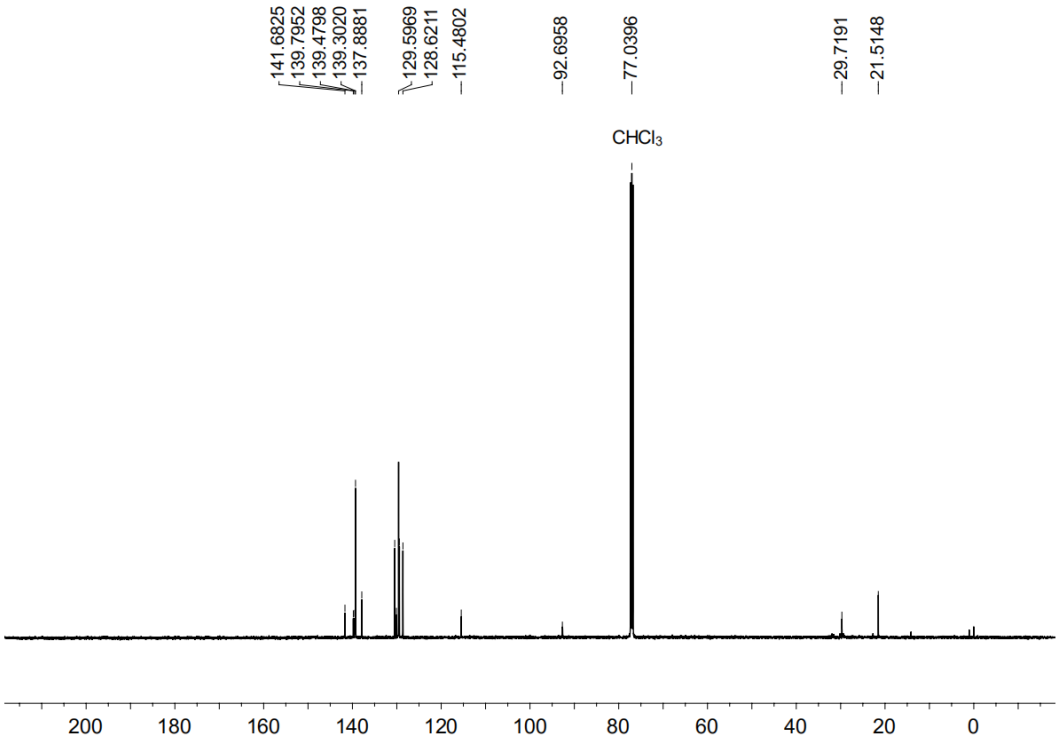


Figure S12 ^{13}C NMR spectrum (126 MHz, $CDCl_3$, 298 K) recorded for BODIPY-Te.

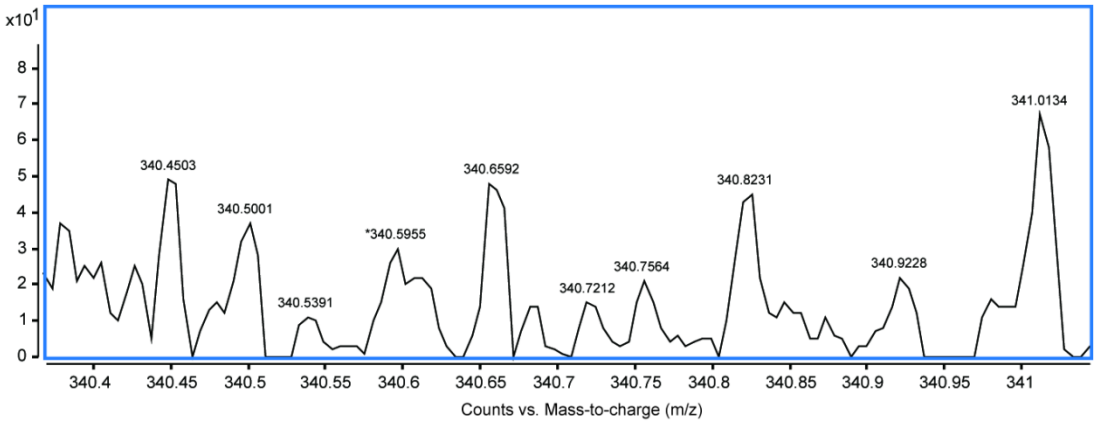
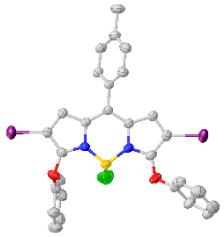


Figure S13 ESI-HRMS spectrum of BODIPY-Te.

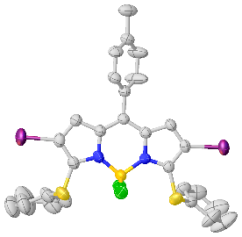
Table S1 Crystal data and structure refinement for BODIPY-O.



CCDC NO.	2202487
Empirical formula	$C_{28}H_{19}BF_2I_2N_2O_2$
Formula weight	718.06
Temperature/K	296.15
Crystal system	triclinic
Space group	P-1
a/Å	9.6753(16)
b/Å	12.238(2)
c/Å	13.120(2)
α/°	64.097(3)
β/°	81.872(3)
γ/°	71.312(3)
Volume/Å^3	1323.8(4)
Z	2
ρ_{calc}g/cm^3	1.801
μ/mm^{-1}	2.418
F(000)	692.0
Crystal size/mm^3	0.15 × 0.15 × 0.12
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	3.45 to 61.168

Index ranges	$-13 \le h \le 13, -16 \le k \le 16, -16 \le l \le 18$
Reflections collected	13423
Independent reflections	6843 [R_{int} = 0.0249, R_{sigma} = 0.0338]
Data/restraints/parameters	6843/0/335
Goodness-of-fit on F^2	1.038
Final R indexes [I>=2σ (I)]	R_1 = 0.0392, wR_2 = 0.0913
Final R indexes [all data]	R_1 = 0.0579, wR_2 = 0.1017
Largest diff. peak/hole / e $Å^{-3}$	1.42/-1.49

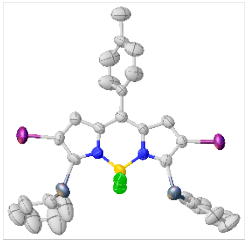
Table S2 Crystal data and structure refinement for BODIPY-S.



CCDC NO.	2202488
Empirical formula	$C_{28}H_{19}BF_2I_2N_2S_2$
Formula weight	754.08
Temperature/K	296.15
Crystal system	monoclinic
Space group	$P2_1/n$
a/Å	14.6906(9)
b/Å	11.0622(7)
c/Å	18.6098(12)
α/°	90
β/°	108.0770(10)
γ/°	90
Volume/$Å^3$	2875.0(3)
Z	4
ρ_{calc}g/cm^3	1.742
μ/mm^{-1}	2.367
F(000)	1457.0
Crystal size/mm^3	0.15 × 0.15 × 0.12
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	3.104 to 60.926
Index ranges	$-20 \le h \le 19, -15 \le k \le 15, -25 \le l \le 25$
Reflections collected	29998

Independent reflections	8057 [R_{int} = 0.0299, R_{sigma} = 0.0306]
Data/restraints/parameters	8057/374/367
Goodness-of-fit on F^2	1.023
Final R indexes [I>=2σ (I)]	R_1 = 0.0474, wR_2 = 0.1253
Final R indexes [all data]	R_1 = 0.0805, wR_2 = 0.1424
Largest diff. peak/hole / e $Å^{-3}$	1.28/-0.71

Table S3 Crystal data and structure refinement for BODIPY-Se.



CCDC NO.	2202489
Empirical formula	$C_{28}H_{19}BF_2I_2N_2Se_2$
Formula weight	843.98
Temperature/K	296.15
Crystal system	monoclinic
Space group	$P2_1/n$
a/Å	14.779(2)
b/Å	10.9831(18)
c/Å	18.764(2)
α/°	90
β/°	108.115(4)
γ/°	90
Volume/$Å^3$	2894.9(8)
Z	4
$\rho_{calc}g/cm^3$	1.936
μ/mm^{-1}	4.720
F(000)	1592.0
Crystal size/mm^3	0.15 × 0.15 × 0.12
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	3.082 to 50.996
Index ranges	-17 ≤ h ≤ 17, -13 ≤ k ≤ 12, -22 ≤ l ≤ 22
Reflections collected	19245
Independent reflections	5390 [R_{int} = 0.0540, R_{sigma} = 0.0565]
Data/restraints/parameters	5390/116/311

Goodness-of-fit on F^2	1.064
Final R indexes [I>=2σ (I)]	R_1 = 0.0660, wR_2 = 0.1955
Final R indexes [all data]	R_1 = 0.1140, wR_2 = 0.2145
Largest diff. peak/hole / e $Å^{-3}$	2.79/-0.71

3. Cell culture and mice

U87 cells were cultured in Dulbecco's modified Eagle medium (DMEM) with 10 % fetal bovine serum (FBS), 1 wt.% penicillin and streptomycin in a humidified atmosphere incubator with 5 % CO_2 at 37 °C. Female BALB/c mice (5 weeks) were purchased from the Experimental Animal Center of Zhejiang University, China. All animals were maintained in a pathogen-free environment under controlled humidity and temperature. The animal experiments were performed in accordance with the China Animal Protection Law.

4. Density functional theory (DFT)

The molecular geometries of BODIPY-O, BODIPY-S, BODIPY-Se, and BODIPY-Te in the ground, S1, and T1 states were calculated and optimized at the TD-B3LYP/def2-SVP level with the solution described by the IEFPCM model with the dielectric constant of THF. The HOMO and LUMO energy levels were determined at the B3LYP/def2-TZVP level. The electronic energies of the S1 and T1 states were further calculated at the TD-B3LYP/def2-TZVP level. All calculations were performed using the Gaussian 16 program. The ΔE_{ST}, HOMO, and LUMO energy levels of BODIPY-X compounds are listed in Table S4.

Table S4 ΔE_{ST}, HOMO, and LUMO energy levels of BODIPY-X compounds.

Compounds	**ΔE_{ST} (eV)**	**HOMO (eV)**	**LUMO (eV)**
BODIPY-O	1.01	−6.02	−3.25
BODIPY-S	0.58	−6.05	−3.49
BODIPY-Se	0.48	−6.01	−3.47
BODIPY-Te	0.27	−5.82	−3.44

5. Preparation of F127/BODIPY-X nanoparticles

F127/BODIPY-X (X = O, S, Se, and Te) nanoparticles were prepared by nanoprecipitation method with the assistance of a triblock copolymer Pluronic F127. BODIPY-X (3 mg) in CH_2Cl_2 (1.0 mL) was dropwise added to an aqueous solution of F127 (10 mg) in water (5.0 mL) and then the mixture solution was stirred overnight.

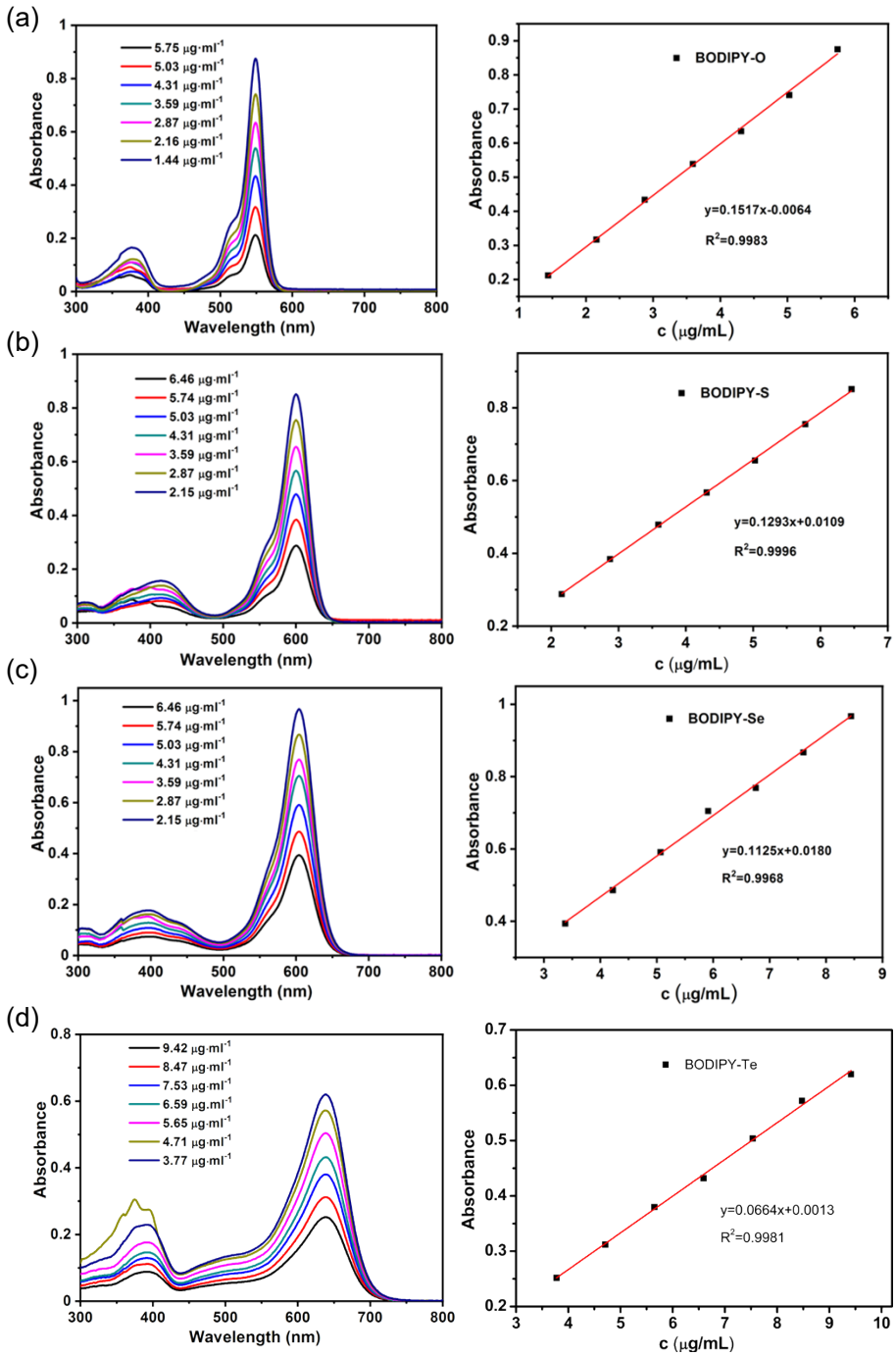


Figure S14 Concentration-dependent absorbance of (a) F127/BODIPY-O, (b) F127/BODIPY-S, (c) F127/BODIPY-Se and (d) F127/BODIPY-Te in water.

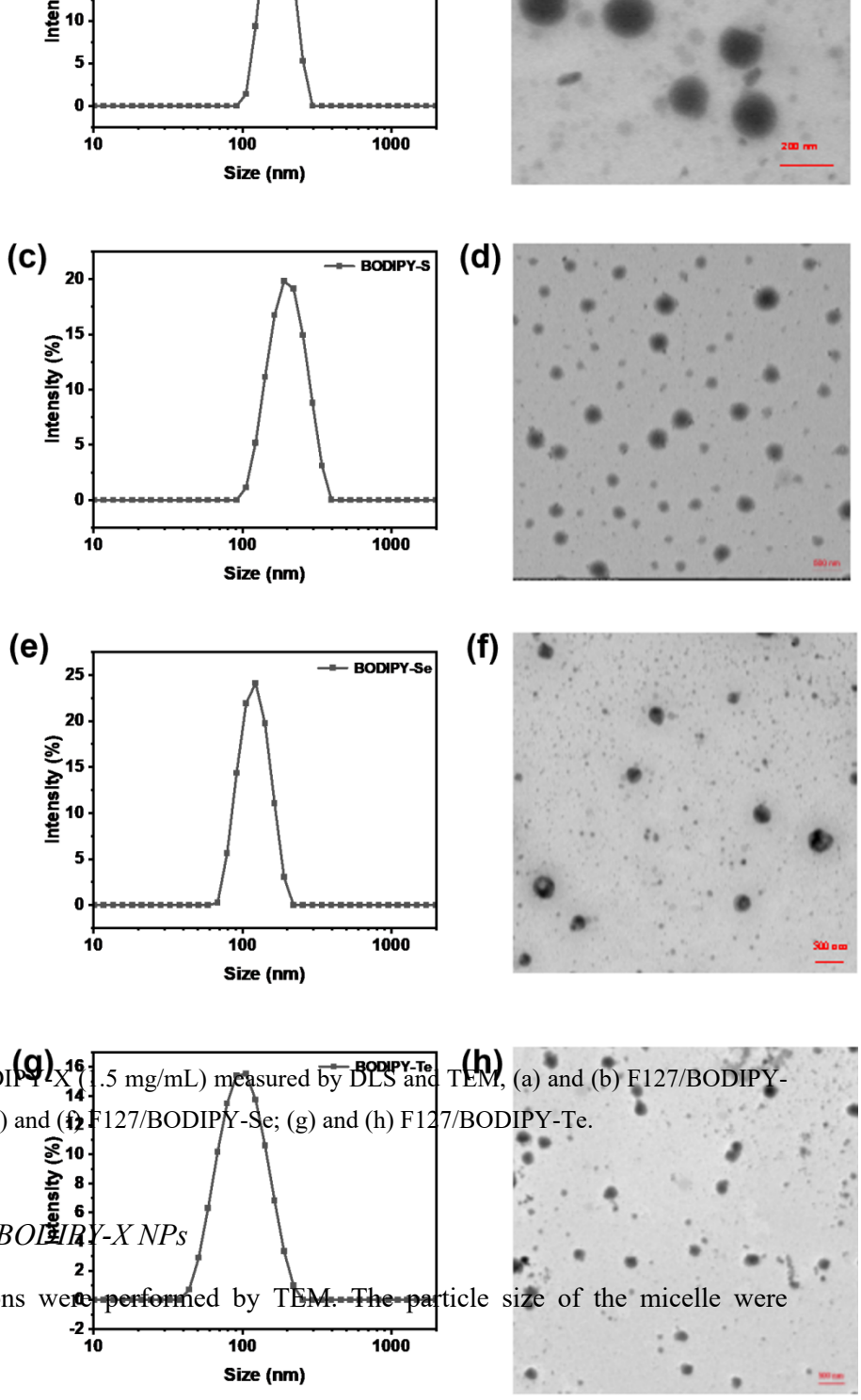


Figure S15 The size of F127/BODIPY-X (1.5 mg/mL) measured by DLS and TEM, (a) and (b) F127/BODIPY-O; (c) and (d) F127/BODIPY-S; (e) and (f) F127/BODIPY-Se; (g) and (h) F127/BODIPY-Te.

6. Characterization of F127/BODIPY-X NPs

The morphological examinations were performed by TEM. The particle size of the micelle were measured by DLS at 25 ℃.

7. Nanoparticles Stability

F127/BODIPY-X NPs was suspended in water. After incubation for a week, the diameters of F127/BODIPY-X NPs were monitored by DLS.

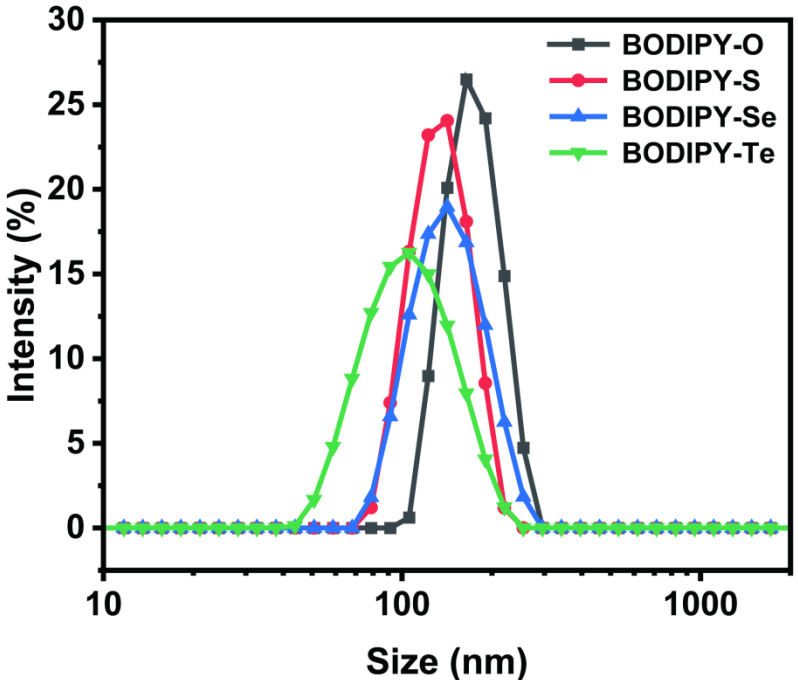


Figure S16 Size distribution of F127/BODIPY-X NPs (1.5 mg/mL) in water after storage for a week.

8. In vitro release of F127/BODIPY-X NPs

The *in vitro* release profiles were studied by dialyzing the F127/BODIPY-X NPs suspension in PBS (pH 7.4) under horizontal shaking (100 rpm). The BODIPY-X-loaded NPs (2.0 mL) was dialyzed against a 10.0 mL solution (MWCO = 1000 Da). At predetermined time intervals, all of the medium outside of the dialysis tube were collected. The samples were analyzed by UV-Vis spectroscopy.

9. Singlet oxygen detection

The singlet oxygen generations of BODIPY-X and F127/BODIPY-X were detected with DPBF as $^1O_2$ fluorescent probe in PBS solution at pH 7.4. Briefly, 20 μM DPBF was added to NPs solution with the absorbance around 0.2-0.3. Then the mixture was irradiated with laser (660 nm, 1.0 W/cm^2) for 300 s. The oxidation of DPBF (at 414 nm) *vs* irradiation time was monitored by UV-Vis-NIR spectrophotometer.

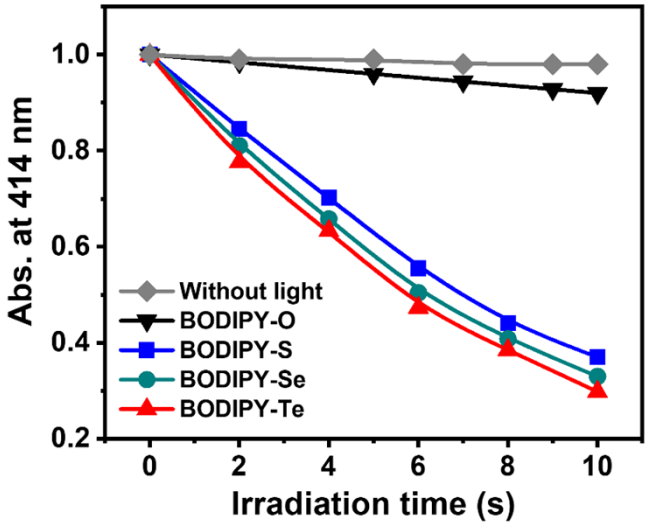


Figure S17 Photodegradation kinetics of the DPBF solution in the presence of BODIPY-X under irradiation of 660 nm laser at 1.0 W/cm^2.

ESR analysis was performed to monitor the generation of $^1O_2$. ESR spectra of BODIPY-X solution (10 μM) containing TEMP (100 mM) were obtained by using 660 nm laser illumination (0.6 W/cm^2) with various illumination time.

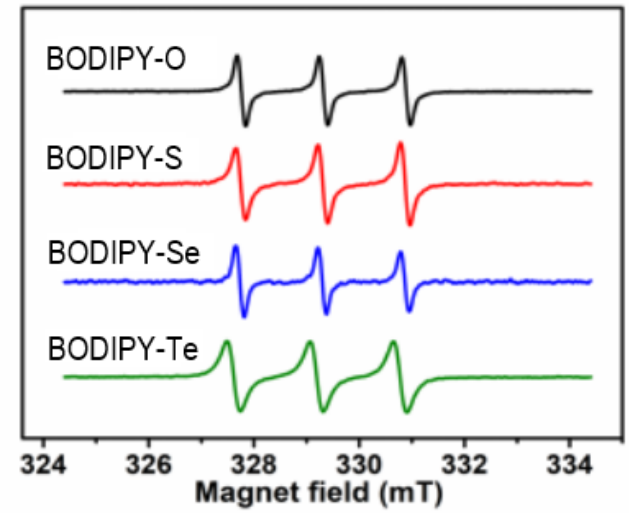


Figure S18 ESR spectra of BODIPY-X (O, S, Se, Te) incubated with TEMP served as a spin trap.

10. In vitro photothermal performance

The formulations of F127/BODIPY-Te were added into 1.5 mL tube. All the samples were treated with NIR laser (660 nm, 1.0 W/cm^2) for 10 min, following with infrared imaging at each 1 min. The sample was irradiated with laser light (660 nm, 1.0 W/cm^2) for 10 min, and then cooled down to the initial temperature after turning off the laser. The process was carried out for 5 cycles.

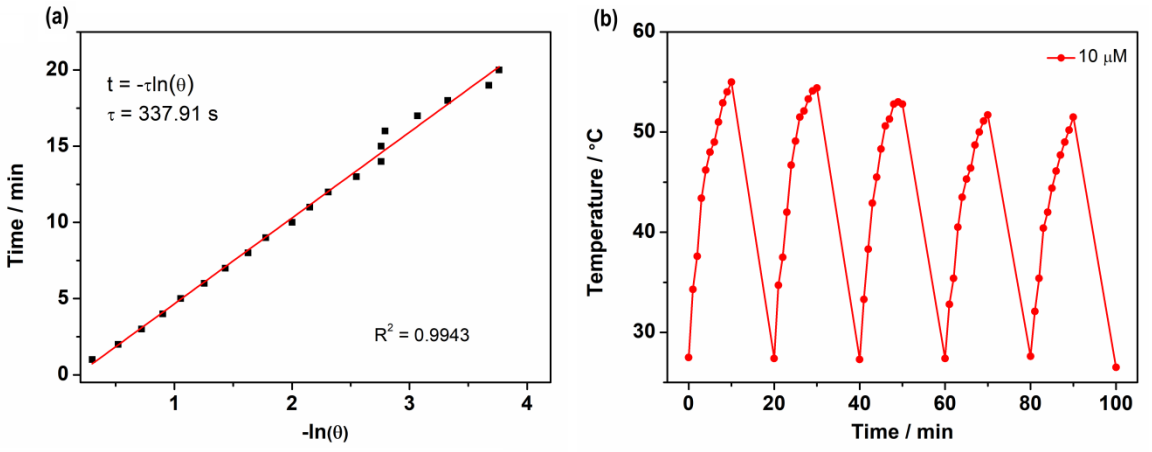


Figure S19 (a) Linear relationship between the F127/BODIPY-Te NPs cooling period and ln(θ). (b) Temperature variation of F127/BODIPY-Te NPs dispersed in water during the 5 consecutive on-off cycles of irradiation. Laser power density: 1.0 W/cm^2.

11. In vitro ROS detection

The generation of ROS in U87 cells was measured using ROS fluorescence indicator 2,7-dichlorodihydroflfluorescein diacetate (DCFH-DA) by CLSM and FCM. After acetate cleavage by cellular esterase, DCFH-DA can be oxidized to 2′,7′-dichloroflfluorescein (DCF) by ROS, exhibiting strong green fluorescence that can be detected by fluorescent microscopy or flow cytometry. U87 cells (1×10^5 /mL) were seeded into 24-well plates and incubated for 16 h in the dark. The cells were then treated with BODIPY-X and F127/BODIPY-X and labeled as dark or blue light groups. After 24 h of incubation under normoxia condition, the media were refreshed and the cells were treated with DCFH-DA (20 μM) for 30 min at 37 °C. After that, the control group was irradiated with 660 nm light for 1 min. The levels of intracellular ROS were examined using the FACSCalibur flow cytometer (BD Biosciences) and a confocal laser scanning microscope (CLSM, Radiance 2100, Bio-Rad).

12. PDT and PTT-mediated apoptosis assay

The PDT and PTT-mediated apoptosis was studied by Annexin V-FITC assay using flow cytometer. U87 cells were incubated in microscope slides or in 6-well culture plate with BODIPY-X and F127/BODIPY-X. After 4 h incubation, the cells were treated with or without 560 nm light for 5 min and incubated for another 12 h. Then the cells were washed with PBS and stained with Annexin V-FITC

(green fluorescence, Ex: 488 nm, Em: 505-540 nm) for 15 min before observed via CLSM or collected for flow cytometry.

13. In vitro cytotoxicity evaluation

The cytotoxicity was assessed by MTT assay. The U87 or HEK293 cells were seeded in 96-well plates at a density of 1×10^4 cells/well for 18 h. The culture medium was then replaced by a serum-free medium containing various concentrations of BODIPY-X and F127/BODIPY-X. After 24 h of incubation, the medium was replaced with the MTT solution (0.5 mg/mL in serum free DMEM medium) for 4 h. After the removal of MTT medium, the formazan crystals were dissolved in 100 μL of DMSO and the microplates were agitated for 10 s at a medium rate prior to the spectrophotometrical measurement at a wavelength of 570 nm on an ELISA reader (Model 680, Bio-Rad). The untreated cells served as the 100% cell viability control, and the completely died cells served as the blank. All experiments were carried out with three replicates. The relative cell growth (%) related to control cells cultured in the medium without the polymer was calculated by the following formula:

$$\text{Cell viability (\%)} = \frac{\text{OD}_{\text{sample}} - \text{OD}_{blank}}{\text{OD}_{\text{control}} - \text{OD}_{\text{blank}}} \times 100\%$$

In the formula, $\text{OD}_{\text{sample}}$ is the absorbance of the wells culturing the treated cells, OD_{blank} is the absorbance of the blank, and $\text{OD}_{\text{control}}$ is the absorbance of the wells culturing untreated cells.

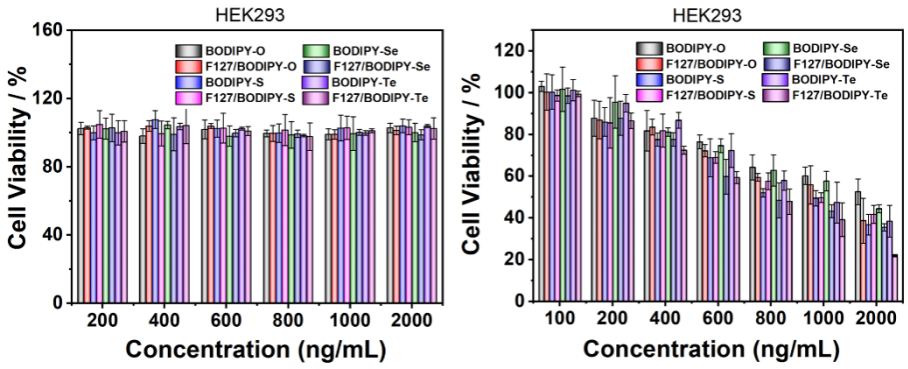


Figure S20 Dose-dependent cytotoxicity of BODIPY-X (X = O, S, Se, Te) and F127/BODIPY-X NPs to HEK293 cells under normal incubation condition without laser irradiation (left) or with 660 nm laser irradiation for 10 minutes (right). Laser power density: 1.0 W/cm^2.

14. Cellar uptake

The cell uptake and distribution were examined by confocal laser scanning microscopy. U87 cells were seeded onto 24-well plates and grown for 18 h, then treated with BODIPY-X and F127/BODIPY-X at the same BODIPY-X concentration of 40 μg/mL. The media were removed after incubation 4 h, then the cells were washed twice with PBS and fixed with 4 % formaldehyde for 10 min and treated with 200 μL DAPI for 5 min. The confocal images were acquired on a confocal laser scanning microscope (CLSM, Radiance 2100, Bio-Rad). Flow cytometry was used to quantitatively evaluate the cellular uptake. 1×10^5 cells/well of U87 cells were seeded in 6-well plates and cultured at 37 ℃ for 18 h. BODIPY-X and F127/BODIPY-X were added to each well (BODIPY-X dose: 40 μg/mL). The drug-free culture medium was applied as a blank control. After 4 h incubation at 37 ℃, cells were rinsed with PBS, harvested by trypsinization, centrifuged (1000 rpm, 10 min), and resuspended in PBS. The BODIPY-X fluorescence intensity was measured by flow cytometer.

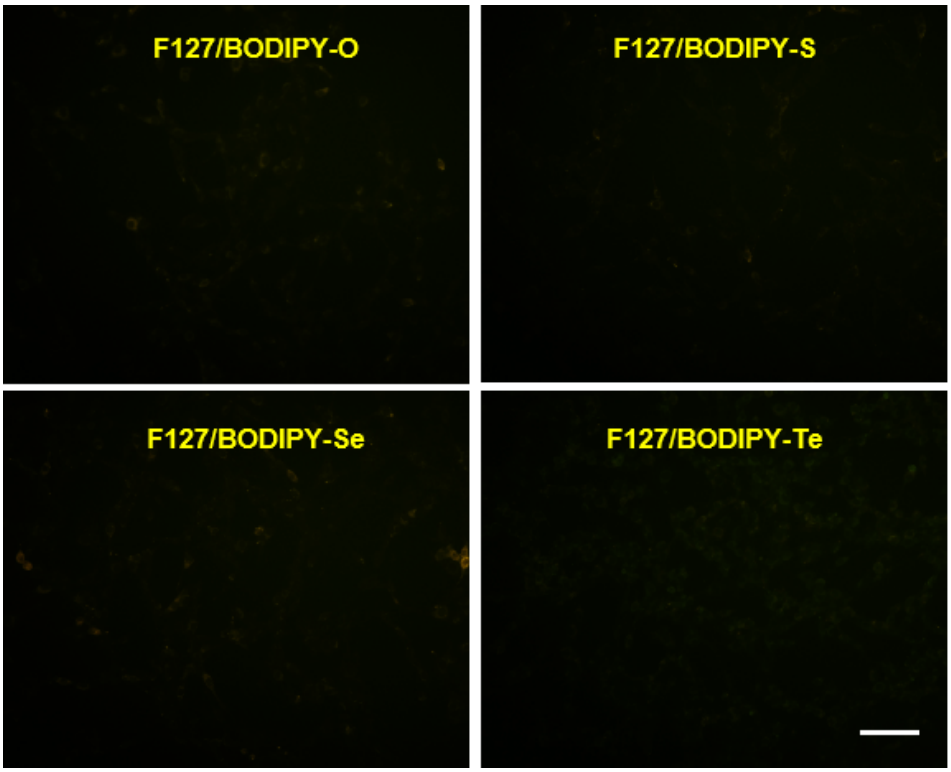


Figure S21 Fluorescence microscope images of intracellular ROS generation in U87 cells exposed to F127/BODIPY-X without irradiation. Scale bar: 100 μm.

15. In vivo fluorescence imaging

When the tumor volume reached up to 150 mm^3, the mice were injected with BODIPY-X and F127/BODIPY-X via the tail vein. Whole-body optical images were captured on the IVIS Kinetic imaging system with an excitation filter of 560 nm and an emission filter of 630 nm.

16. In vivo photothermal imaging

U87 tumor-bearing mice injected with the F127/BODIPY-Te NPs solution (100 μg/mL) was irradiated by 660 nm laser (1.0 W/cm^2) for 10 min, and the temperature of tumor was recorded by an FLIR thermal camera.

17. Antitumor effect experiment

Female BALB/c nude mice (4-6 weeks old, weighting about 16-18 g) were purchased from Charles River Laboratory China Branch (Zhejiang, China) with production license number SCXK (Zhejiang) 2019-0001 and certificate number 20211228Abzz0619072327. The mice were housed at the Laboratory Animal Center of Hangzhou Normal University with use license number SYXK (Zhejiang) 2020-0026, and cultivated in a pathogen-free environment with appropriate humidity and temperature. All animal procedures were performed in accordance with the animal care and use guidelines of the Organizational Animal Care and Use Committee. U87 tumor mice models were randomly divided into four groups when the tumors grew to around 80-100 mm^3. Samples were injected intravenously via tail vein four times with 3 days intervals. Tumor volume (mm^3) was calculated as $(width)^2 \times (length) \times 1/2$. Body weight and tumor volume were measured every 3 days until the end of the experiment. After that, the mice were sacrificed and tumor tissues were fixed with 4 % paraformaldehyde overnight at 4 °C and embedded in paraffin for analysis. Tissue sections (3 μm) were stained with hematoxylin/eosin (H&E). The terminal transferase dUTP nickend labeling (TUNEL) assay was applied to further detect the cell apoptosis in tumor tissues according to the manufacturer's instructions (Roche, Basel, Switzerland). The expression levels of Ki-67 in tumor tissues after different treatments were conducted by immunohistochemical staining. Major organs including the lungs, liver, spleen, and kidneys were extracted for histological analyses. The mice blood was collected by enucleation of mouse eyes before sacrificed for further blood biochemistry and haematology analysis. The indexes include total protein

(TP, reference value: 65-85 g/L), albumin (ALB, reference value: 40-55 g/L), white cell ratio (A/G, reference value: 1.2-2.4), alanine aminotransferase (ALT, reference value: 0-50 U/L), aspartate aminotransferase (AST, reference value: 0-40 U/L), aspartate/alanine ratio (AST/ALT), urea nitrogen (BUN, reference value: 3.1-8.0 mmol/L), uric acid (UA, reference value: 214-488 μml/L).

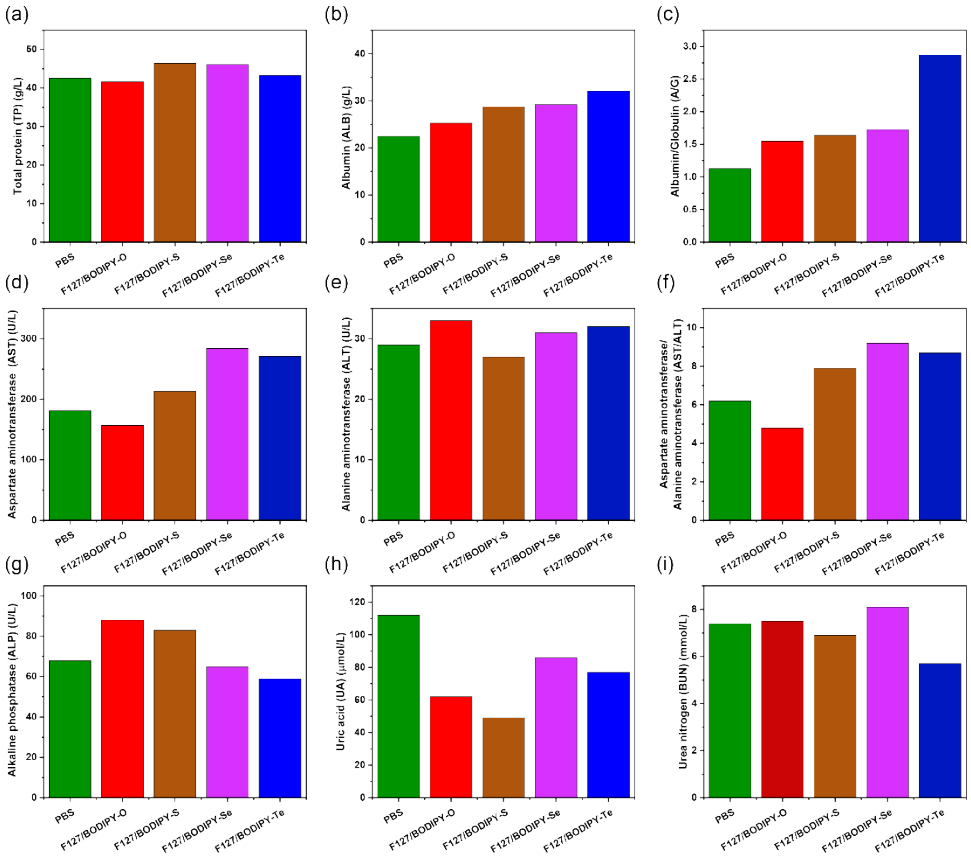


Figure S22 The related indexes of blood biochemistry and hematology analysis after different treatments.

18. Statistical analysis

All the experiments were repeated at least three times and the data were presented as means standard deviation. The statistical significance ($p < 0.05$) was evaluated by the student *t*-test when only two groups were compared.

Reference

[S1] Ortiz, M. J.; Agarrabeitia, A. R.; Duran-Sampedro, G.; Bañuelos Prieto, J.; Lopez, T. A.; Massad, W. A.; Montejano, H. A.; García, N. A.; Lopez Arbeloa, I. Synthesis and functionalization of new

polyhalogenated BODIPY dyes. Study of their photophysical properties and singlet oxygen generation, *Tetrahedron* **2012**, *68*, 1153-1162.