Electronic Supplementary Information

BODIPY-based Photosensitizers with Simultaneous Photodynamic Antitumor and Antibacterial Effects

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Characterization.

All optical spectra were recorded at room temperature. UV-Vis spectra were collected from Hitachi UV-3900 spectrophotometer. PL spectra were recorded on Hitachi F-4600 spectrophotometer. The photoluminescence quantum yield was determined on Hamamatsu C11347. Scanning electron microscope (SEM) images were obtained on Hitachi SU-8010. Dynamic light scattering (DLS) was tested by DynaPro NanoStar dynamic light scattering detector. ^{1}H and ^{13}C NMR spectra were measured on JEOL-400 or JEOL-600 spectrometers. High-resolution mass spectrometry (HRMS) data were recorded by Brucker Solarix XR Fourier Transform Ion Cyclotron Resonance Mass Spectrometer. Zeta potential was measured on Brookhaven 90 plus zeta. Confocal laser scanning microscope (CLSM) images were performed on Nikon AIR MP multiphoton microscopy. Cell viability test was performed on UMR-9600 microplate reader. Flow cytometric analysis was performed on Beckman Cytoflex Flow Cytometry. Irradiation was performed by using PLS-LED100B (660 nm, PerfectLight, Beijing, China). Preparation of **1a**-**5a** was performed on ultrasonic cleaning machine (SB-5200 DTD) and ultrasonic crusher (SCIENTZ JY92-IIN).

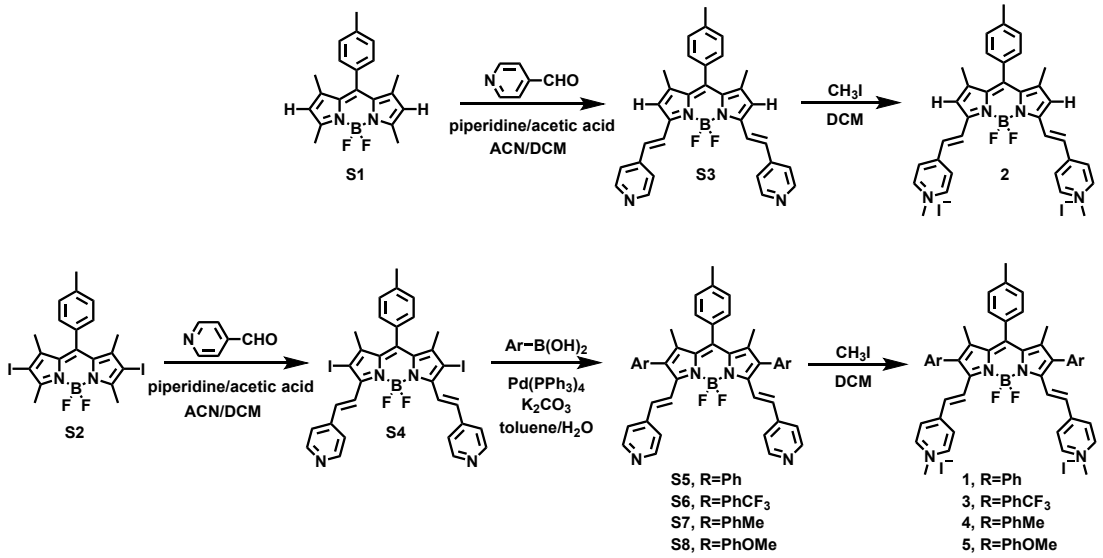
Measurement of $^{1}O_2$ quantum yield.

$^{1}O_2$ quantum yield was measured according to the literature.[1]

Cell incubation.

Cells were incubated in DMEM medium supplemented with 1% penicillin-streptomycin and 10% FBS. The culture atmosphere consists of 5% CO_2 and 95% air. All cells were cultured on 75 cm^2 culture flasks. When used for imaging, cells were subcultured on 35 mm glass-bottom culture dishes or 96-well plates.

Synthesis of Compounds 1-5.



Scheme S1. Synthetic routes of compounds 1-5.

S1 and S2 were synthesized according to the literature.[2]

Synthesis of Compound S3:

A mixture of S1 (300 mg, 0.89 mmol) and 4-pyridinaldehyde (0.3 ml, 3.1 mmol) in acetonitrile/dichloromethane (12 ml/2 ml) was added with acetic acid (0.1 ml) and piperidine (0.3 ml). The mixture was refluxed under nitrogen atmosphere for 2 h. After cooling to room temperature, the reaction mixture was diluted with dichloromethane, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on silica gel [eluted by petroleum ether/CH_2Cl_2/triethylamine from 300 ml/100 ml/4 ml to 50 ml/100 ml/10 ml] to provide a dark brown solid (100 mg, 22%).

^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.64 (d, *J* = 6.0 Hz, 4H), 7.89 (d, *J* = 16.8 Hz, 2H), 7.46 (d, *J* = 6.0 Hz, 4H), 7.34 (d, *J* = 7.8 Hz, 2H), 7.20 (d, *J* = 7.8 Hz, 2H), 7.16 (d, *J* = 16.8 Hz, 2H), 6.68 (s,2H), 2.47 (s, 3H), 1.50 (s, 6H).

Synthesis of Compound S4：

A mixture of S2 (300 mg, 0.51 mmol) and 4-pyridinaldehyde (0.3 ml, 3.1 mmol) in acetonitrile/dichloromethane (12 ml/2 ml) was added with acetic acid (0.1 ml) and piperidine (0.3 ml). The resulting mixture was refluxed under nitrogen atmosphere for 2 h. The reaction mixture was diluted with dichloromethane, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on silica gel [eluted by petroleum ether/CH_2Cl_2/triethylamine from 300 ml/100 ml/4 ml to 50 ml/100 ml/10 ml] to provide a dark solid

(170 mg, 44 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.67 (d, *J* = 6.4 Hz, 4H), 8.04 (d, *J* = 16.4 Hz, 2H), 7.82 (d, *J* = 16.4 Hz, 2H), 7.49 (d, *J* = 6.4 Hz, 4H), 7.37 (d, *J* = 7.6 Hz, 2H), 7.17 (d, *J* = 7.6 Hz, 2H), 2.49 (s, 3H), 1.50 (s, 6H).

Synthesis of Compound S5:

To a round-bottom flask were added compound S4 (100 mg, 0.13 mmol), Phenylboronic acid (195 mg, 1.6 mmol), $Pd(PPh_3)_4$ (46 mg, 0.04 mmol) and K_2CO_3 (166 mg, 1.2 mmol). The equipment was degassed and refilled with dried N_2. Then a mixture of Toluene/Ethanol/H_2O (10 ml/1 ml/5 ml) was added through a syringe under nitrogen atmosphere. The reaction was stirred at 90 ℃ for 6 h. After that, the crude of the reaction was poured into water (50 ml), extracted with dichloromethane (3×50 ml), dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (eluted by petroleum ether/CH_2Cl_2/triethylamine from 300 ml/100 ml/4 ml to 150 ml/150 ml/10 ml) to provide a brown solid (57 mg, 66 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.52 (d, *J* = 4.8 Hz, 4H), 7.89 (d, *J* = 16.8 Hz, 2H), 7.46-7.40 (m, 6H), 7.32 (d, *J* = 7.6 Hz, 2H), 7.26-7.24 (m, 6H), 7.14 (d, *J* = 4.8 Hz, 4H), 6.42 (d, *J* = 16.8 Hz, 2H), 2.43 (s, 3H), 1.28 (s, 6H).

Synthesis of Compound S6:

To a round-bottom flask were added compound S4 (100 mg, 0.13 mmol), 4-trifluoromethylphenylboronic acid (200 mg, 1.05 mmol), $Pd(PPh_3)_4$ (46 mg, 0.04 mmol) and K_2CO_3 (166 mg, 1.2 mmol). The equipment was degassed and refilled with dried N_2. Then a mixture of Toluene/Ethanol/H_2O (10 ml/1 ml/5 ml) was added through a syringe under nitrogen atmosphere. The reaction was stirred at 90 ℃ for 6 h. After that, the crude of the reaction was poured into water (50 ml), extracted with dichloromethane (3×50 ml), dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (eluted by petroleum ether/CH_2Cl_2/triethylamine from 300 ml/100 ml/4 ml to 150 ml/150 ml/10 ml) to provide a brown solid (60 mg, 58 %).

^{1}H NMR (400 MHz, Chloroform-d) δ 8.56 (d, J = 5.2 Hz, 4H), 7.87 (d, J = 16.8 Hz, 2H), 7.72 (d, J = 8.4 Hz, 4H), 7.41 (d, J = 8.4 Hz, 4H), 7.35 (d, J = 8.0 Hz, 2H), 7.25 (d, J = 8.0 Hz, 2H), 7.14 (d, J = 5.2 Hz, 4H), 6.38 (d, J = 16.8 Hz, 2H), 2.45 (s, 3H), 1.28 (s, 6H).

Synthesis of Compound S7：

To a round-bottom flask were added compound S4 (100 mg, 0.13 mmol), 4-methylphenylboronic acid (200 mg, 1.5 mmol), $Pd(PPh_3)_4$ (46 mg, 0.04 mmol) and K_2CO_3 (166 mg, 1.2 mmol). The equipment was degassed and refilled with dried N_2. Then a mixture of Toluene/Ethanol/H_2O (10 ml/1 ml/5 ml) was added through a syringe under nitrogen atmosphere. The reaction was stirred at 90 ℃ for 6 h. After that, the crude of the reaction was poured into water (50 ml), extracted with dichloromethane (3×50 ml), dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (eluted by petroleum ether/CH_2Cl_2/triethylamine from 300 ml/100 ml/4 ml to 150 ml/150 ml/10 ml) to provide a brown solid (60 mg, 66 %).

^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.53 (d, *J* = 6.0 Hz, 4H), 7.88 (d, *J* = 16.8 Hz, 2H), 7.31 (d, *J* = 7.8 Hz, 2H), 7.24 (d, *J* = 7.8 Hz, 4H), 7.24 (d, *J* = 7.8 Hz, 2H), 7.16 (d, *J* = 6.0 Hz, 4H), 7.12 (d, *J* = 7.8 Hz, 2H), 6.50 (d, *J* = 16.8 Hz, 2H), 2.43 (s, 3H), 2.42 (s, 6H) 1.26 (s, 6H).

Synthesis of Compound S8：

To a round-bottom flask were added compound S4 (100 mg, 0.13 mmol), 4-methoxyphenylboronic acid (218 mg, 1.6 mmol), $Pd(PPh_3)_4$ (46 mg, 0.04 mmol) and K_2CO_3 (166 mg, 1.2 mmol). The equipment was degassed and refilled with dried N_2. Then a mixture of Toluene/Ethanol/H_2O (10 ml/1 ml/5 ml) was added through a syringe under nitrogen atmosphere. After that, the crude of the reaction was poured into water (50 ml), extracted with dichloromethane (3×50 ml), dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (eluted by petroleum ether/CH_2Cl_2/triethylamine from 300 ml/100 ml/5 ml to 100 ml/100 ml/10 ml) to provide a brown solid (65 mg, 68 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.54 (d, *J* = 8.8 Hz, 4H), 7.89 (d, *J* = 16.6 Hz, 2H), 7.31 (d, *J* = 8.0 Hz, 2H), 7.24 (d, *J* = 8.0 Hz, 2H), 7.19 – 7.15 (m, 8H), 6.97 (d, *J* = 8.8 Hz, 4H), 6.53 (d, *J* = 16.6 Hz, 2H), 3.86 (s, 6H), 2.43(s, 3H), 1.26 (s, 6H).

Synthesis of Compound 1：

CH_3I (1 ml) was added to the solution of compound S5 (30 mg) in 10 ml dichloromethane. The solution was stirred at 40 ℃ for 24 h with the assistance of a nitrogen balloon. After that, the solvent and CH_3I were removed on a rotary evaporator under 50 ℃ to provide a black solid (40 mg, 95 %).

^{1}H NMR (400 MHz, Acetonitrile-d_3) δ 8.54 (d, *J* = 6.4 Hz, 4H), 8.07 (d, *J* = 16.4 Hz, 2H), 7.78 (d, *J* = 6.4 Hz, 4H), 7.53-7.48 (m, 6H), 7.44 (d, *J* = 8.0 Hz, 2H), 7.37 (d, *J* = 8.0 Hz, 2H), 7.31 (d, *J* =

7.2 Hz, 4H), 6.66 (d, *J* = 16.4 Hz, 2H), 4.22 (s, 6H), 2.43 (s, 3H), 1.34 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 150.10, 148.14, 145.62, 144.91, 141.46, 139.57, 135.60, 134.55, 132.59, 132.01, 130.62, 130.23, 130.00, 129.11, 128.38, 127.88, 127.65, 124.01, 47.33, 21.04, 12.54. HRMS(ESI): m/z calcd. for $C_{46}H_{41}BF_2N_4^{2+}$ $[M]^{2+}$ 349.1695, found: 349.1698.

Synthesis of Compound 2：

Compound 2 was synthesized the same as compound 1. 60 mg of compound S3 provided 90 mg of compound 2. Yield: 97 %.

^{1}H NMR (600 MHz, DMSO-d6) δ 8.92 (d, J = 6.8 Hz, 4H), 8.22 (d, J = 6.8 Hz, 4H), 7.94 (d, J = 16.3 Hz, 2H), 7.86 (d, J = 16.3 Hz, 2H), 7.46 (d, J = 8.0 Hz, 2H), 7.37 (d, J = 8.0 Hz, 2H), 7.20 (s, 2H), 4.33 (s, 6H), 2.45 (s, 3H), 1.51 (s, 6H). ^{13}C NMR (151 MHz, DMSO-d6) δ 150.76, 150.32, 145.58, 143.96, 143.37, 139.39, 134.95, 131.60, 130.24, 130.04, 127.63, 127.26, 124.25, 120.71, 47.36, 20.98, 14.48. HRMS(ESI): m/z calcd. for $C_{34}H_{33}BF_2N_4^{2+}$ $[M]^{2+}$ 273.1381, found: 273.1386.

Synthesis of Compound 3：

Compound 3 was synthesized the same as compound 1. 20 mg of compound S6 provided 25 mg of compound 3. Yield: 93 %.

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.84 (d, *J* = 6.6 Hz, 4H), 8.00 (d, *J* = 6.6 Hz, 4H), 7.92 (d, *J* = 16.2 Hz, 2H), 7.84 (d, *J* = 8.4 Hz, 4H), 7.59 (d, *J* = 8.4 Hz, 4H), 7.48 (d, *J* = 7.8 Hz, 2H), 7.45 (d, *J* = 7.8 Hz, 2H), 6.70 (d, *J* = 16.2 Hz, 2H), 4.26 (s, 6H), 2.43 (s, 3H), 1.34 (s, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 150.06, 148.32, 145.64, 145.48 141.96, 139.75, 136.89, 134.45, 133.92, 132.57, 131.14, 130.43, 130.28, 128.47, 126.58 (q, *J* = 302 Hz, 129.63, 127.60, 125.82, 123.25), 125.06, 124.25, 124.14, 124.06, 47.34, 21.03, 12.64. HRMS(ESI): m/z calcd. for $C_{48}H_{39}BF_8N_4^{2+}$ $[M]^{2+}$ 417.1569, found: 417.1572.

Synthesis of Compound 4：

Compound 4 was synthesized the same as compound 1. 30 mg of compound S7 provided 42 mg of compound 4. Yield: 95 %.

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.82 (d, *J* = 6.6 Hz, 4H), 7.94-7.90 (m, 6H), 7.45 (d, *J* = 8.3 Hz, 2H), 7.43 (d, *J* = 8.3 Hz, 2H), 7.30 (d, *J* = 7.9 Hz, 4H), 7.20 (d, *J* = 7.9 Hz, 4H), 6.72 (d, *J* = 16.5 Hz, 2H), 4.27 (s, 6H), 2.42 (s, 3H), 2.37 (s, 6H), 1.30 (s, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 150.23, 148.15, 145.56, 144.68, 141.39, 139.50, 137.60, 135.59, 134.54, 131.85, 130.66, 130.17, 129.82, 129.64, 129.48, 128.00, 127.63, 124.00, 47.29, 21.00, 20.90, 12.49. HRMS (ESI): m/z

calcd. for $C_{48}H_{45}BF_2N_4^{2+}$ $[M]^{2+}$ 363.1851, found: 363.1856.

Synthesis of Compound 5:

Compound 5 was synthesized the same as compound 1. 30 mg of compound S8 provided 45 mg of compound 5. Yield: 99 %.

^{1}H NMR (400 MHz, Acetonitrile-d_3) δ 8.52 (d, J = 5.2 Hz, 4H), 8.05 (d, J = 17.0 Hz, 2H), 7.79 (d, J = 5.2 Hz, 4H), 7.40 (d, J = 5.6 Hz, 2H), 7.32 (d, J = 5.6 Hz, 2H), 7.18 (d, J = 7.6 Hz, 4H), 7.02 (d, J = 7.6 Hz, 4H), 6.72 (d, J = 17.0 Hz, 2H), 4.20 (s, 6H), 3.82 (s, 6H), 2.40 (s, 3H), 1.30 (s, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 159.06, 150.26, 148.18, 145.55, 144.50, 141.43, 139.47, 135.42, 134.55, 131.71, 131.20, 130.70, 130.15, 128.04, 127.65, 124.32, 124.00, 114.51, 55.17, 47.31, 21.00, 12.49. HRMS(ESI): m/z calcd. for $C_{48}H_{45}BF_2N_4O_2^{2+}$ $[M]^{2+}$ 379.1800, found: 379.1804.

NMR Spectra and HRMS

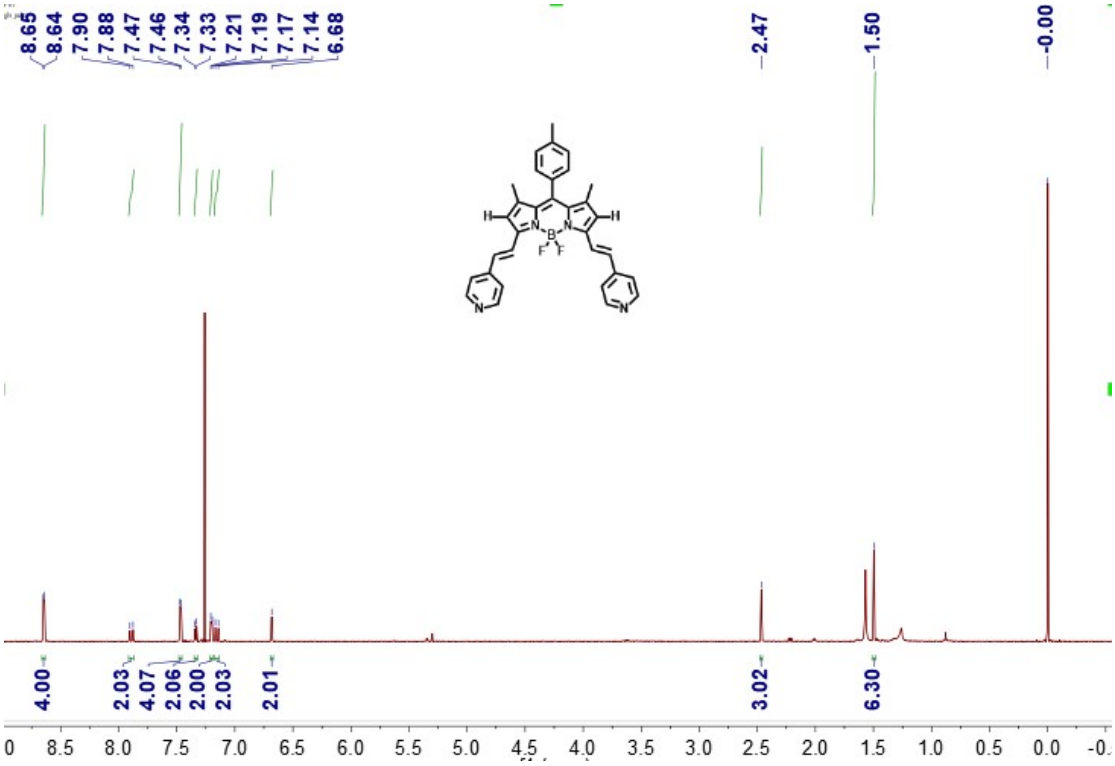


Figure S1. ^{1}H-NMR spectrum of Compound S3.

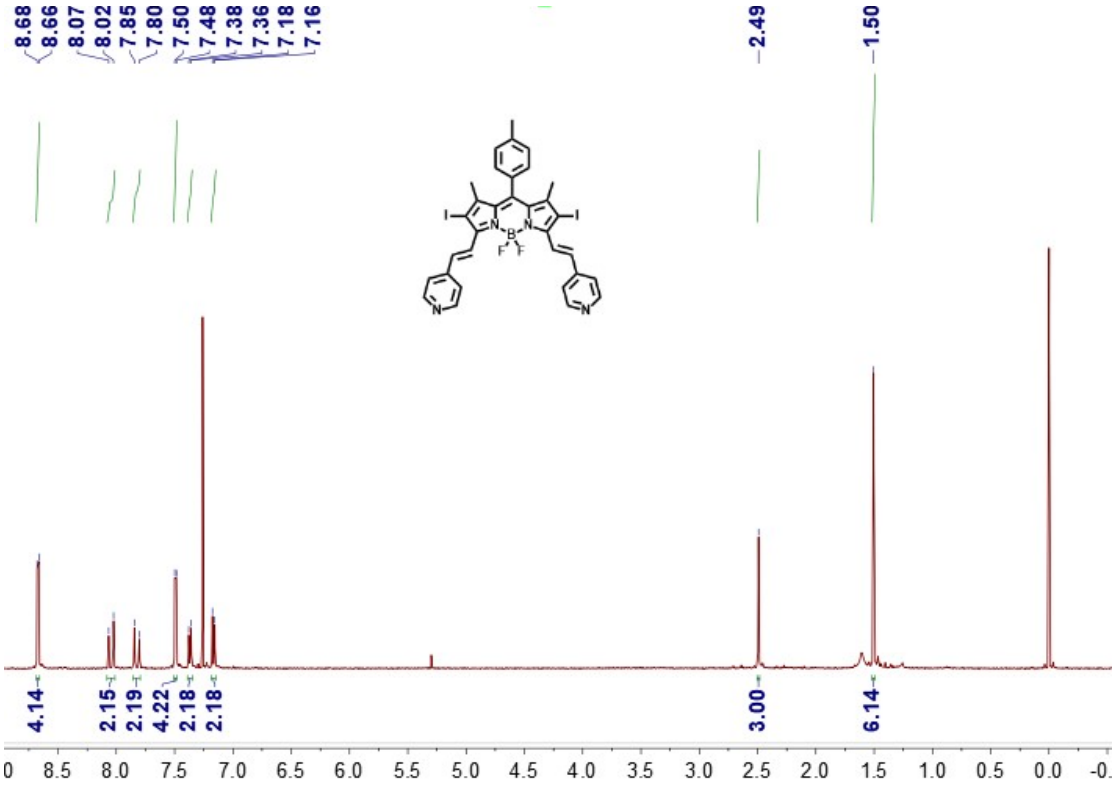


Figure S2. [1]H-NMR spectrum of Compound S4.

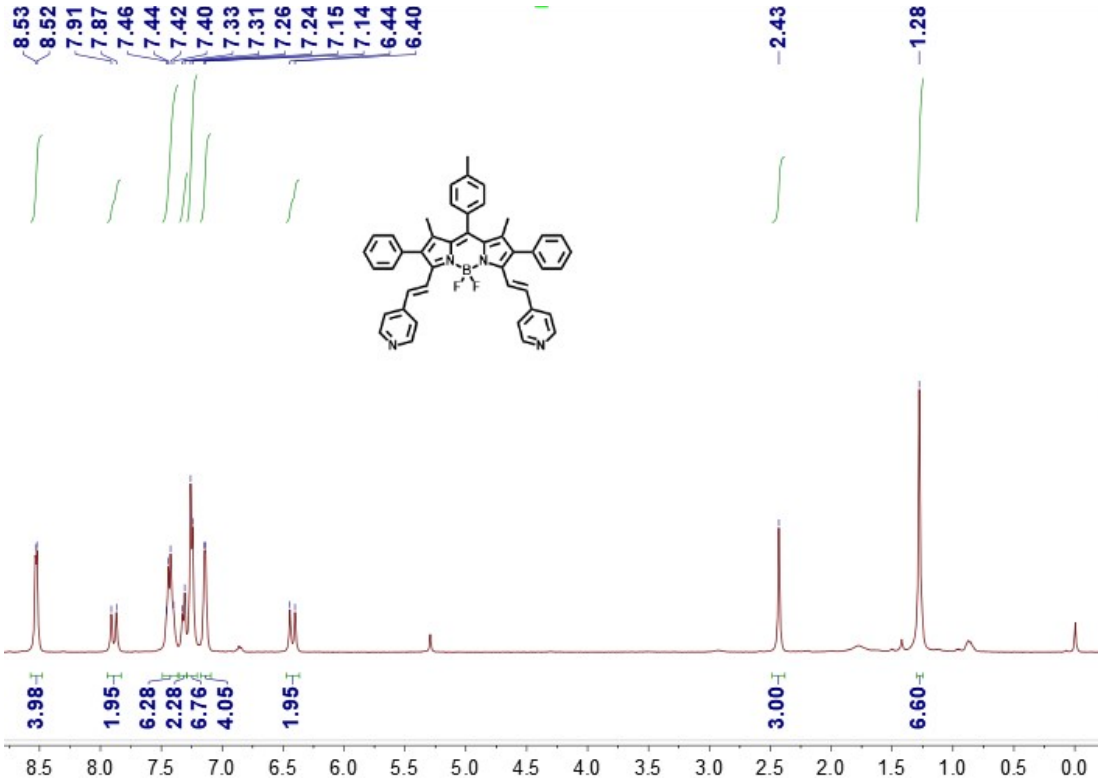


Figure S3. [1]H-NMR spectrum of Compound S5.

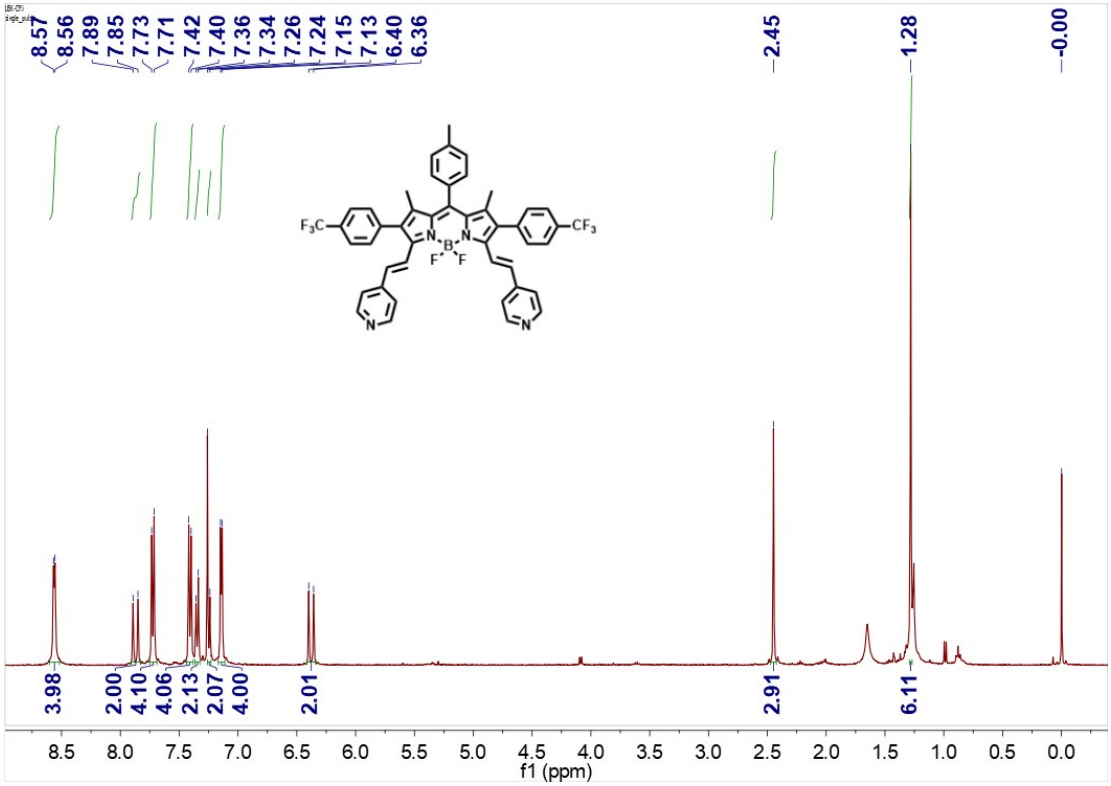


Figure S4. [1]H-NMR spectrum of Compound S6.

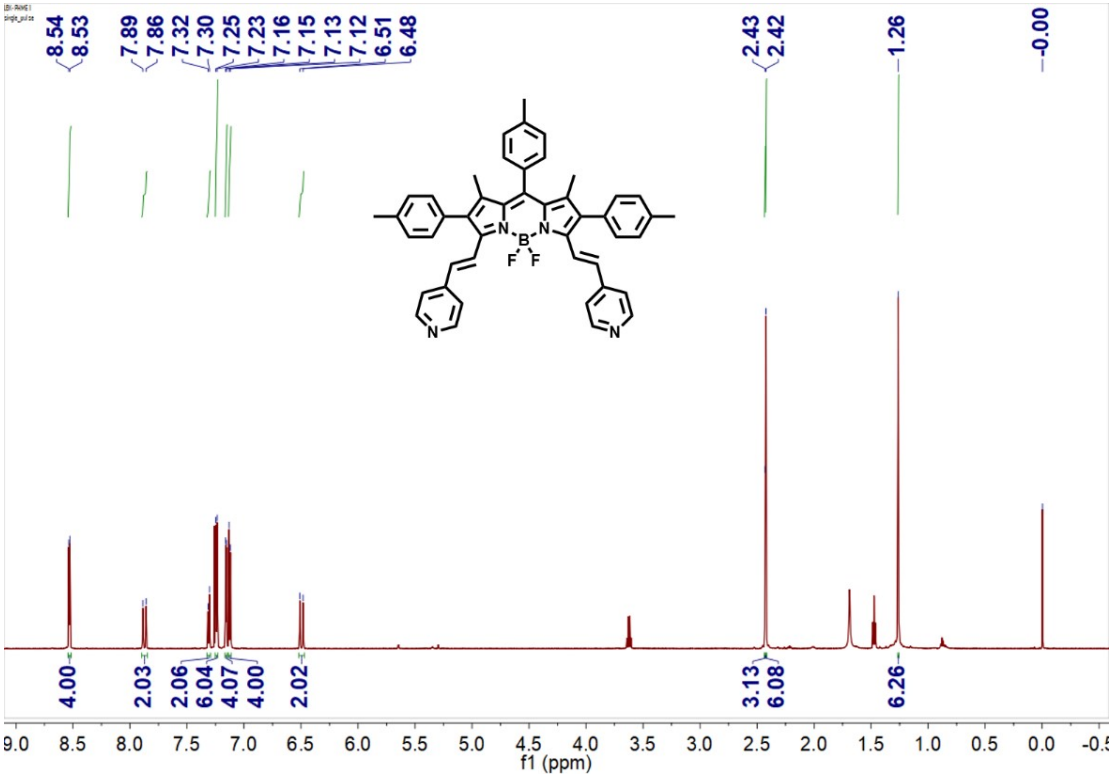


Figure S5. [1]H-NMR spectrum of Compound S7.

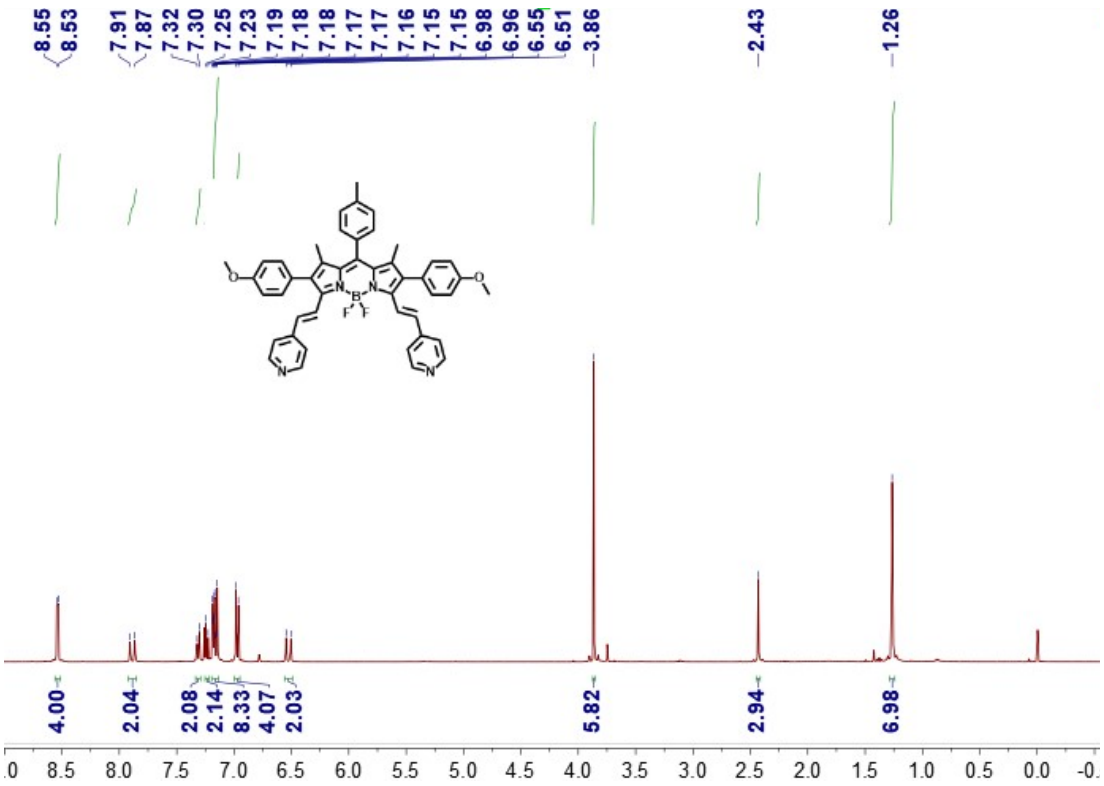


Figure S6. [1]H-NMR spectrum of Compound S8.

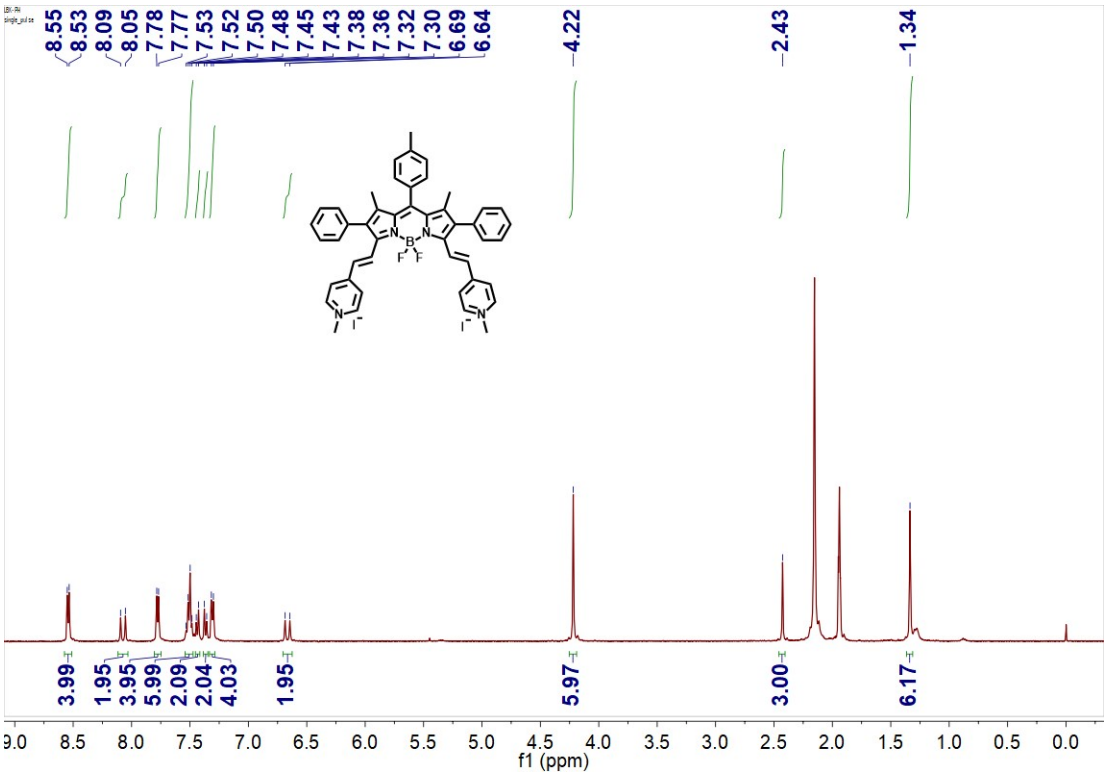


Figure S7. [1]H-NMR spectrum of Compound 1.

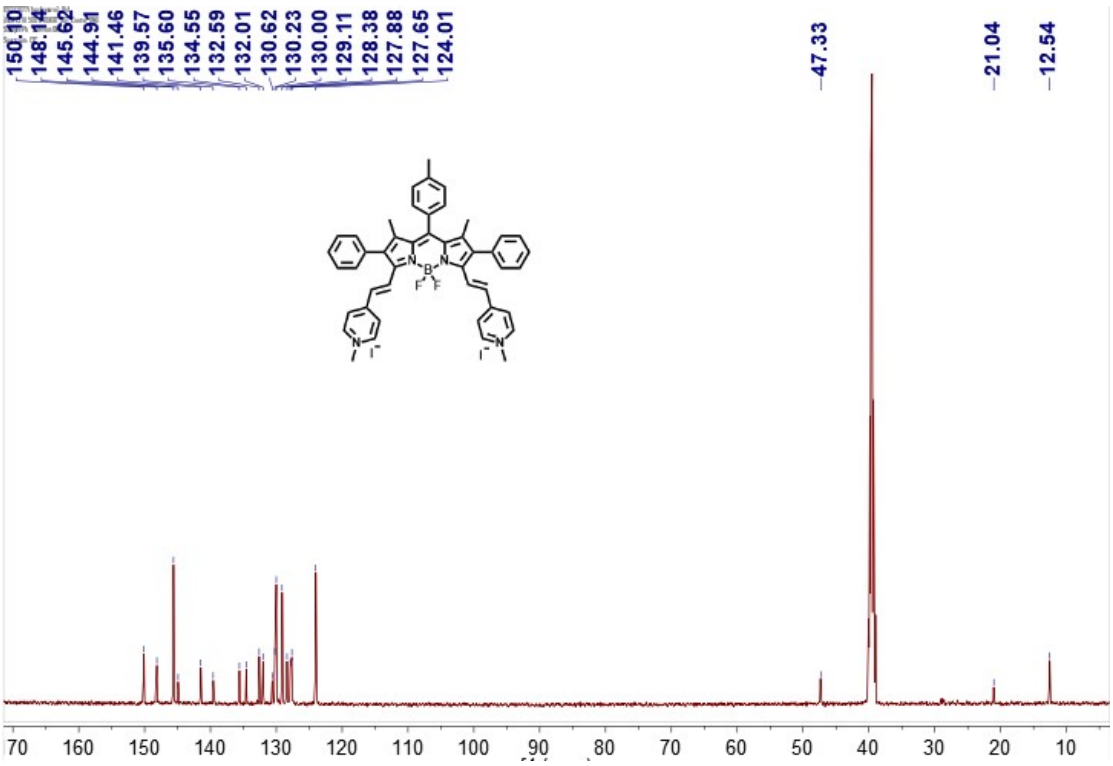


Figure S8. ^{13}C-NMR spectrum of Compound 1.

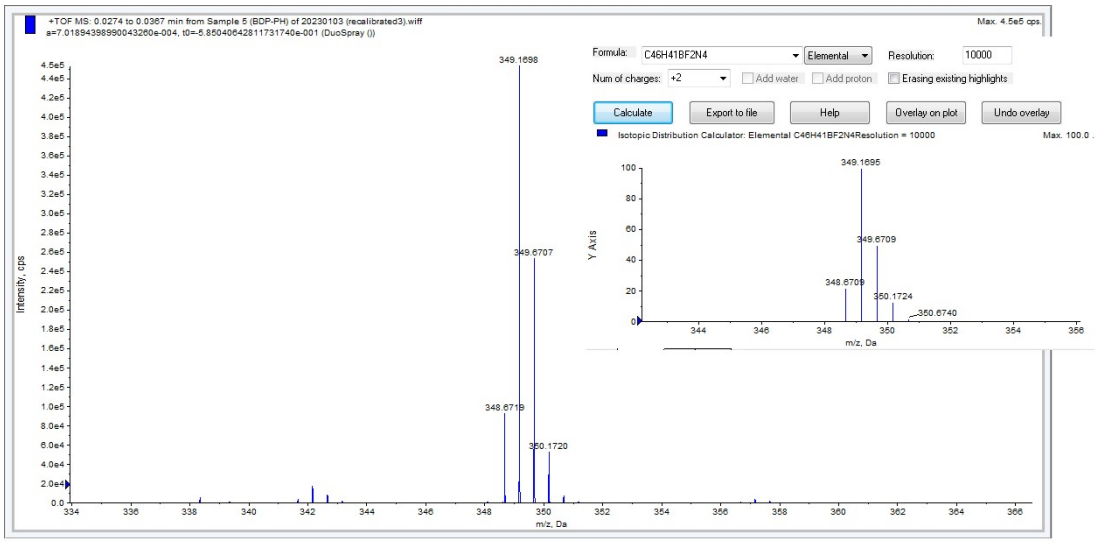


Figure S9. HRMS spectrum of Compound 1.

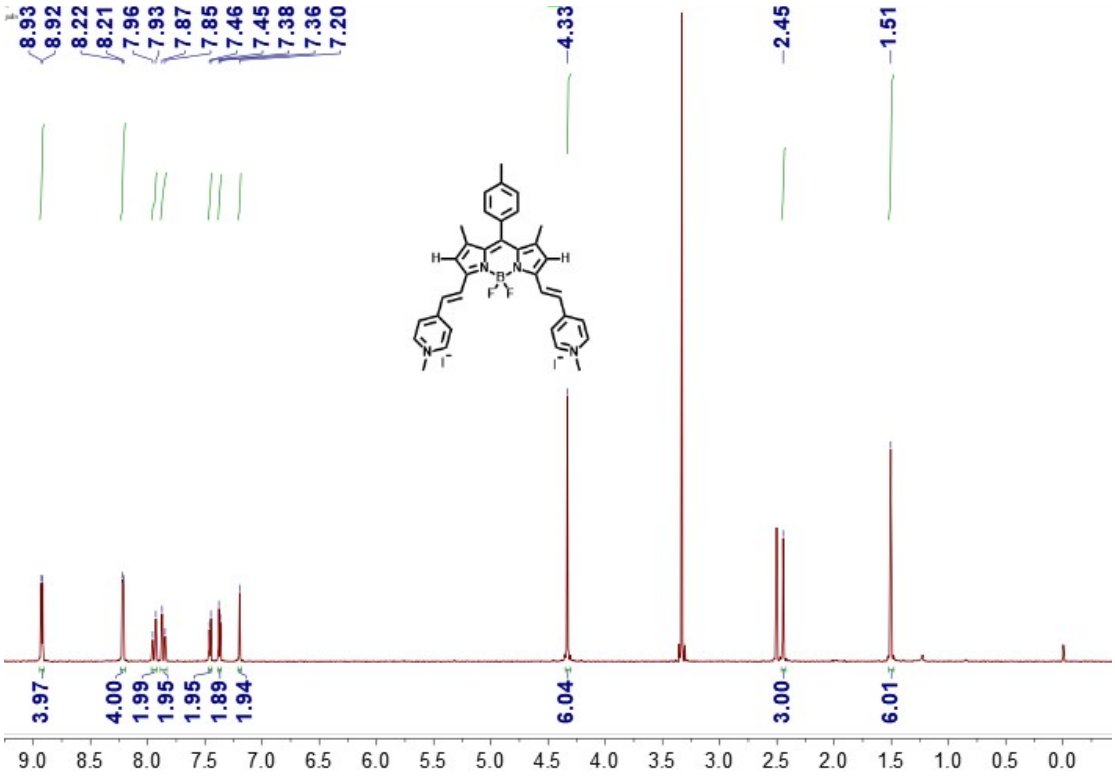


Figure S10. ^{1}H-NMR spectrum of Compound 2.

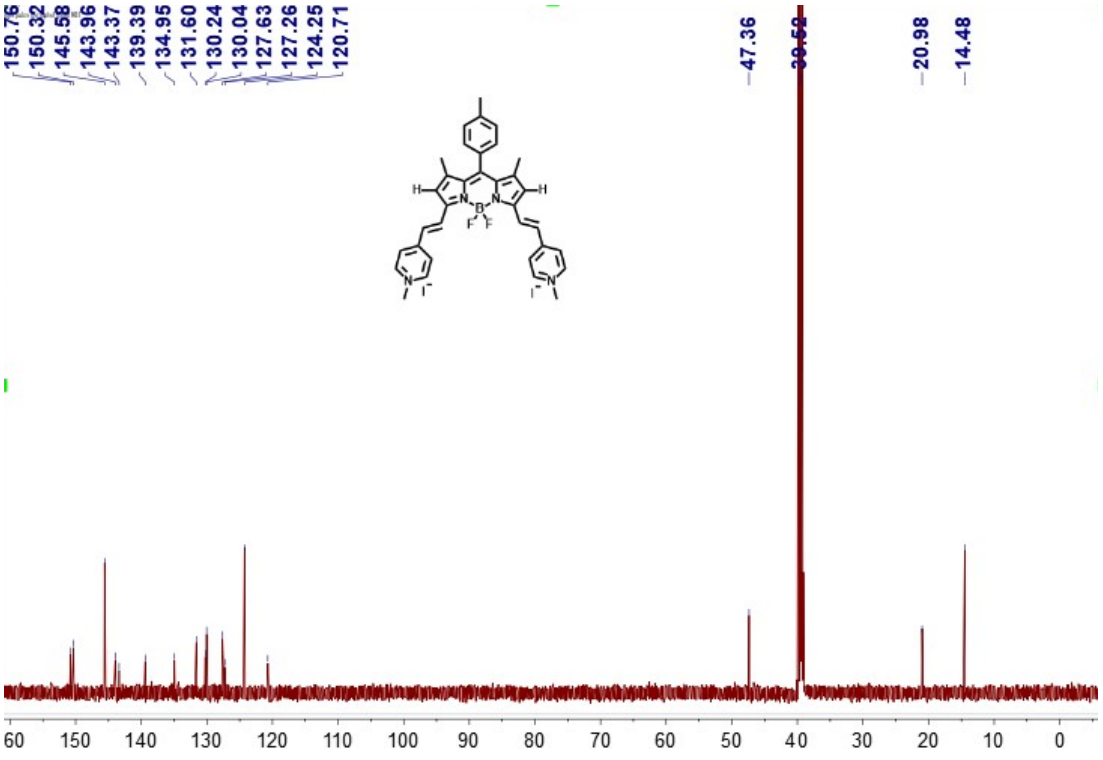


Figure S11. ^{13}C-NMR spectrum of Compound 2.

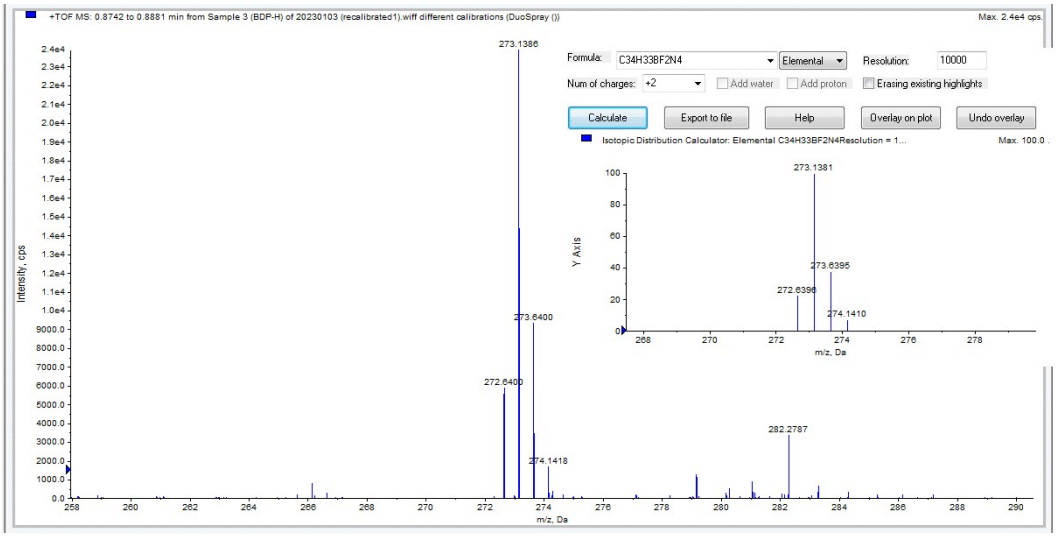


Figure S12. HRMS spectrum of Compound 2.

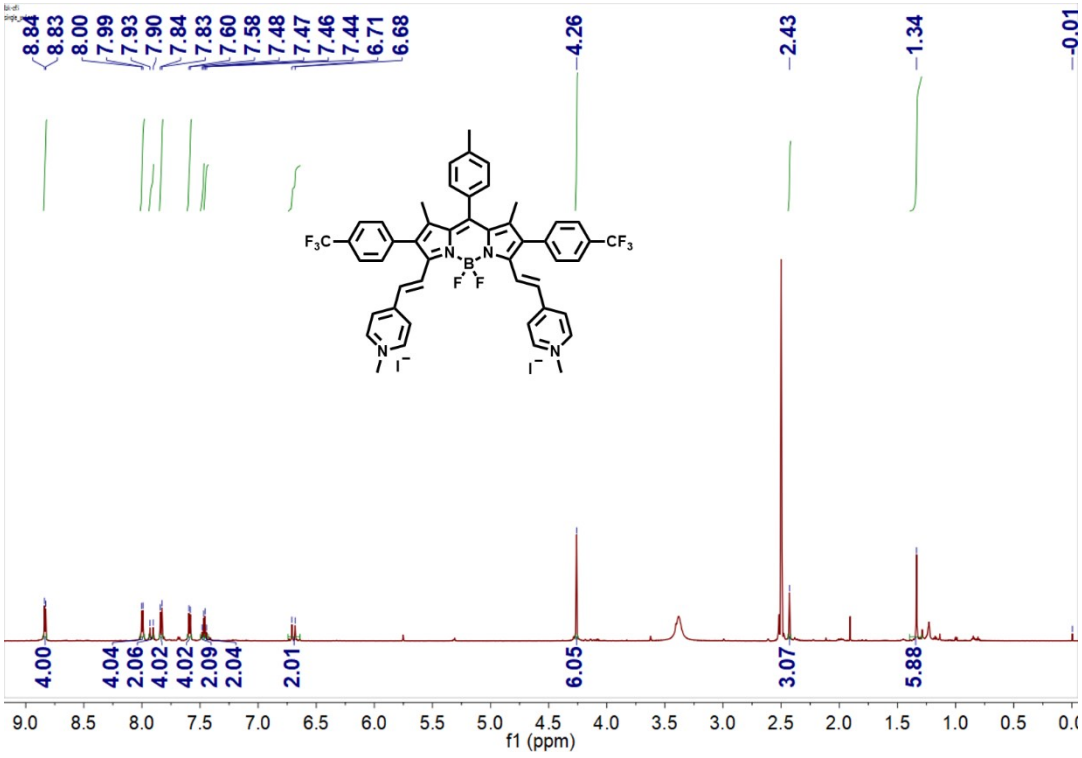


Figure S13. ^{1}H-NMR spectrum of Compound 3.

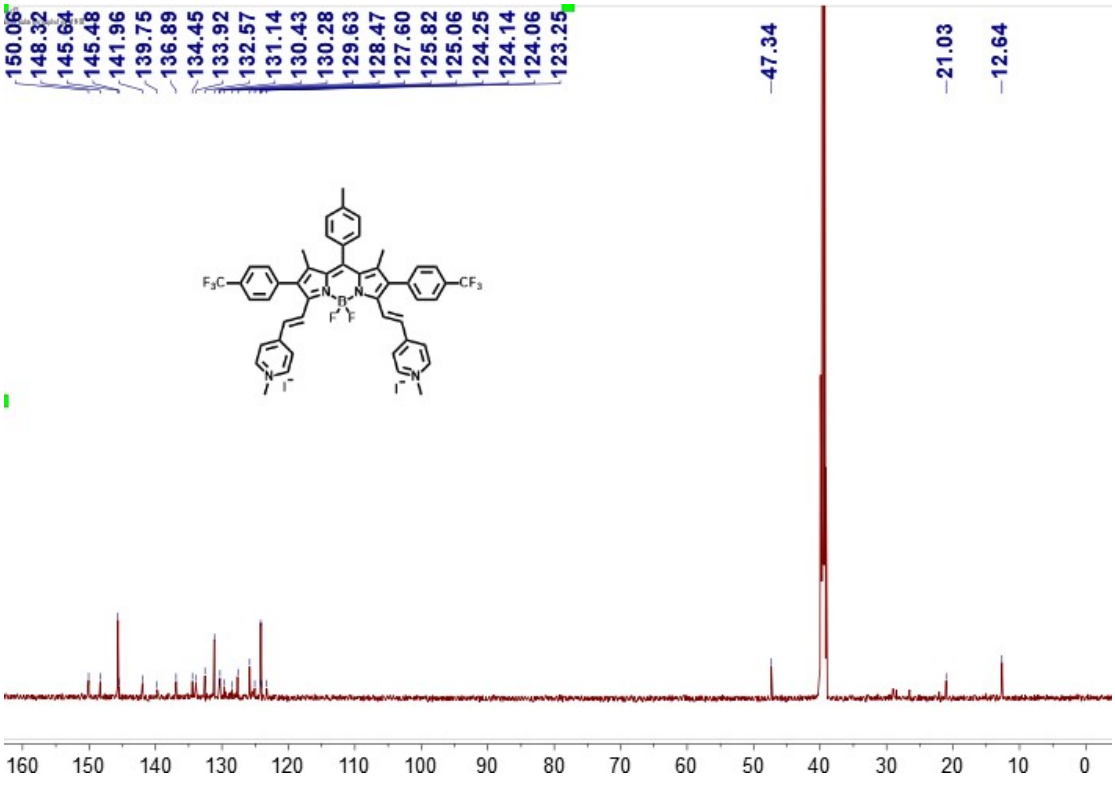


Figure S14. ^{13}C-NMR spectrum of Compound 3.

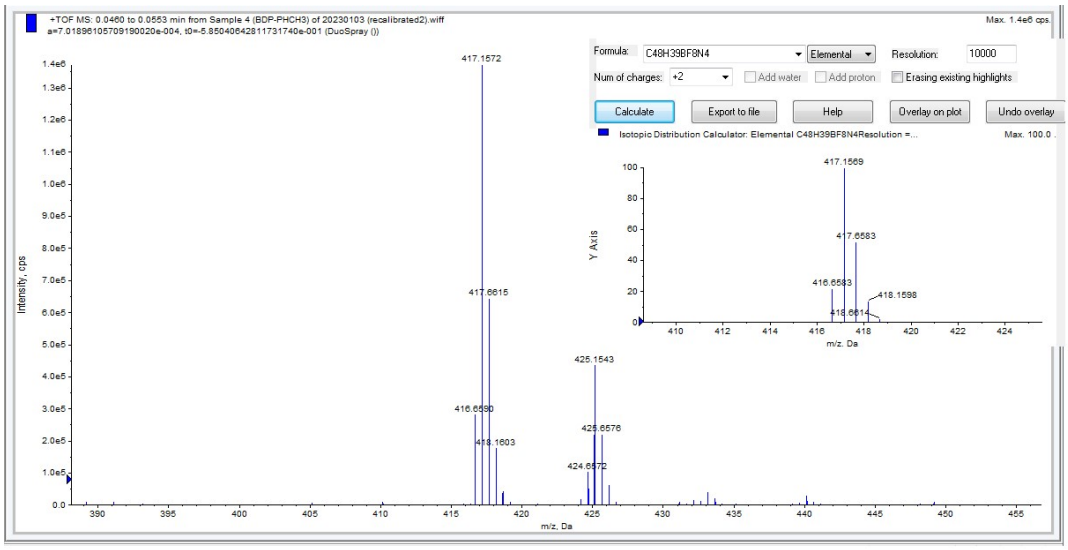


Figure S15. HRMS spectrum of Compound 3.

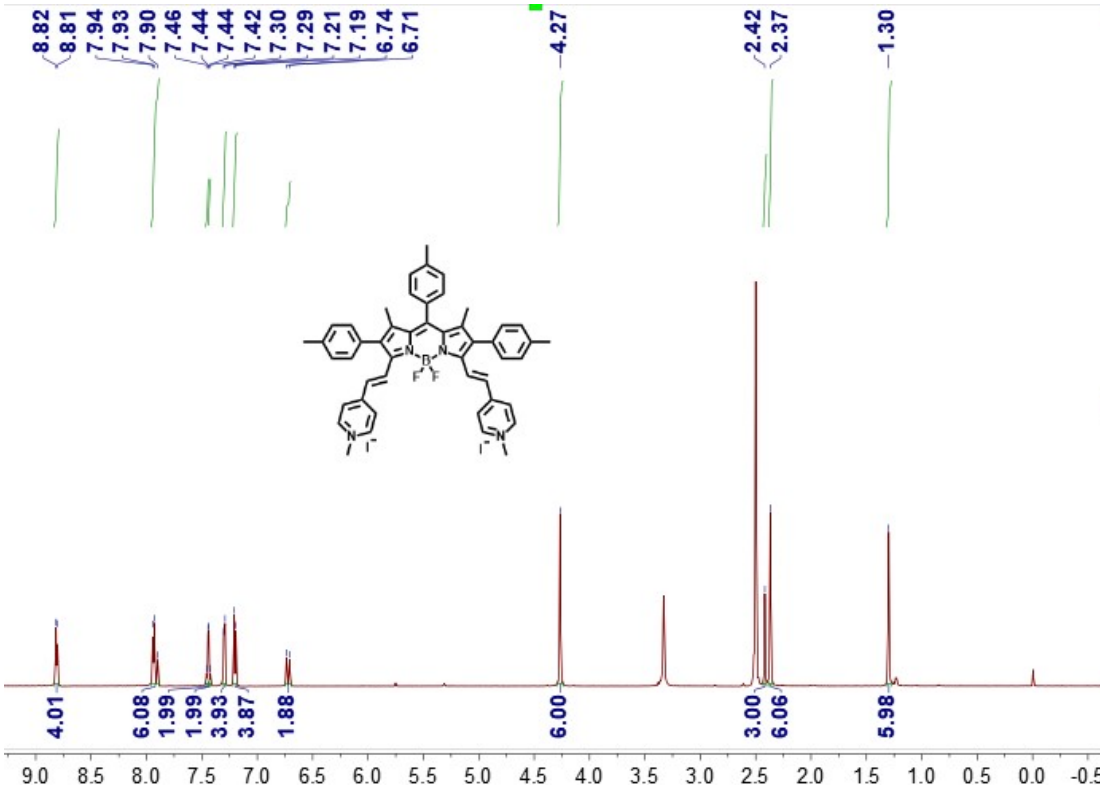


Figure S16. ^{1}H-NMR spectrum of Compound 4.

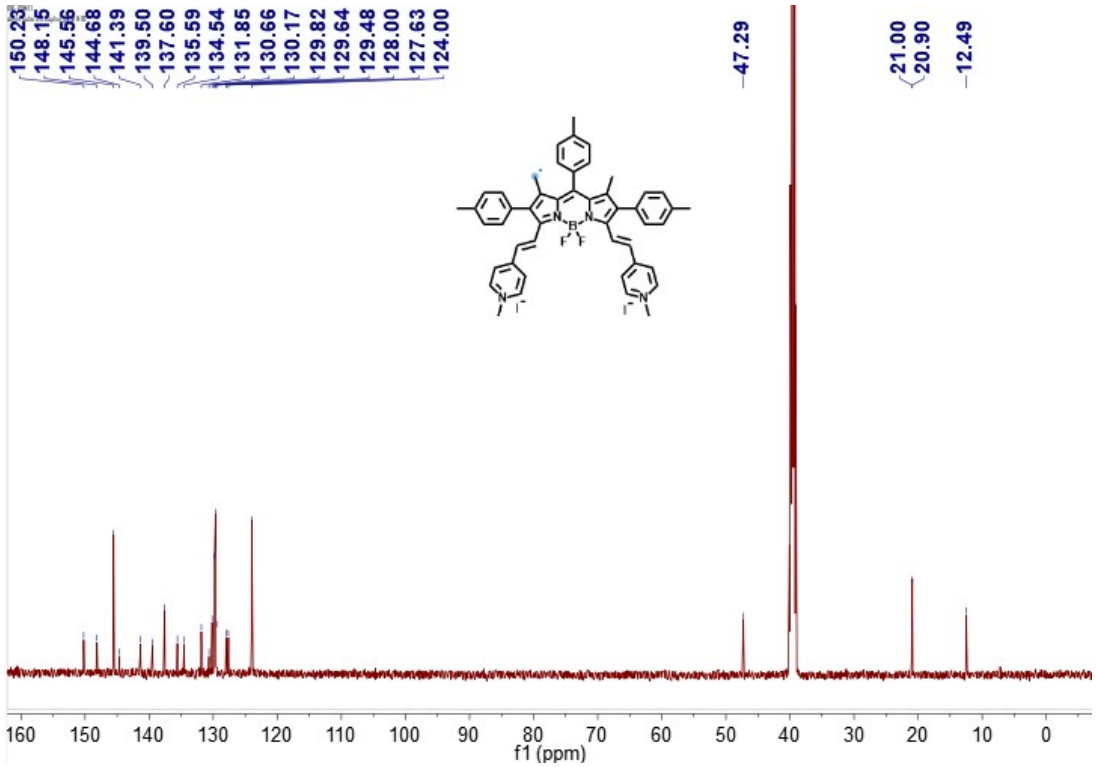


Figure S17. ^{13}C-NMR spectrum of Compound 4.

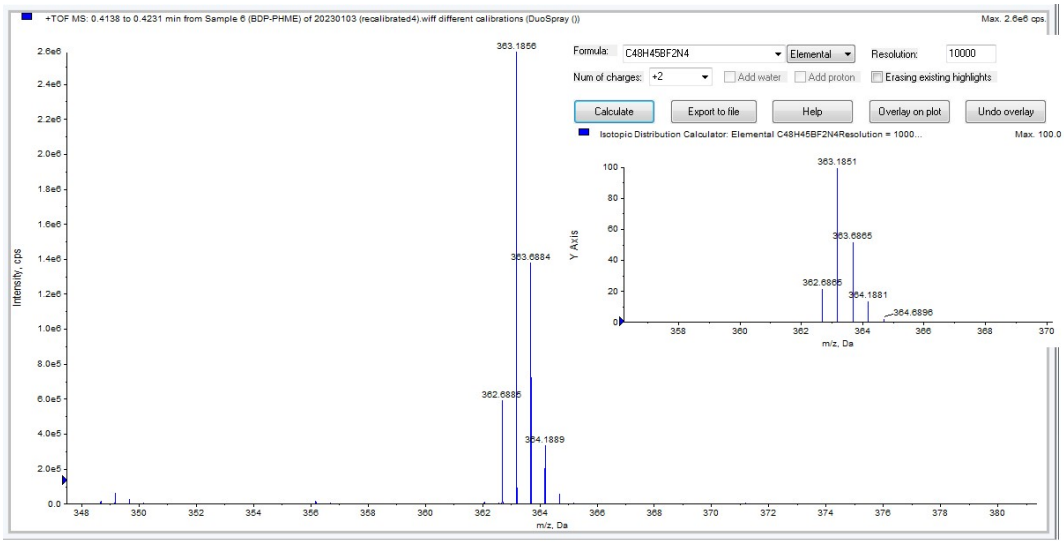


Figure S18. HRMS spectrum of Compound 4.

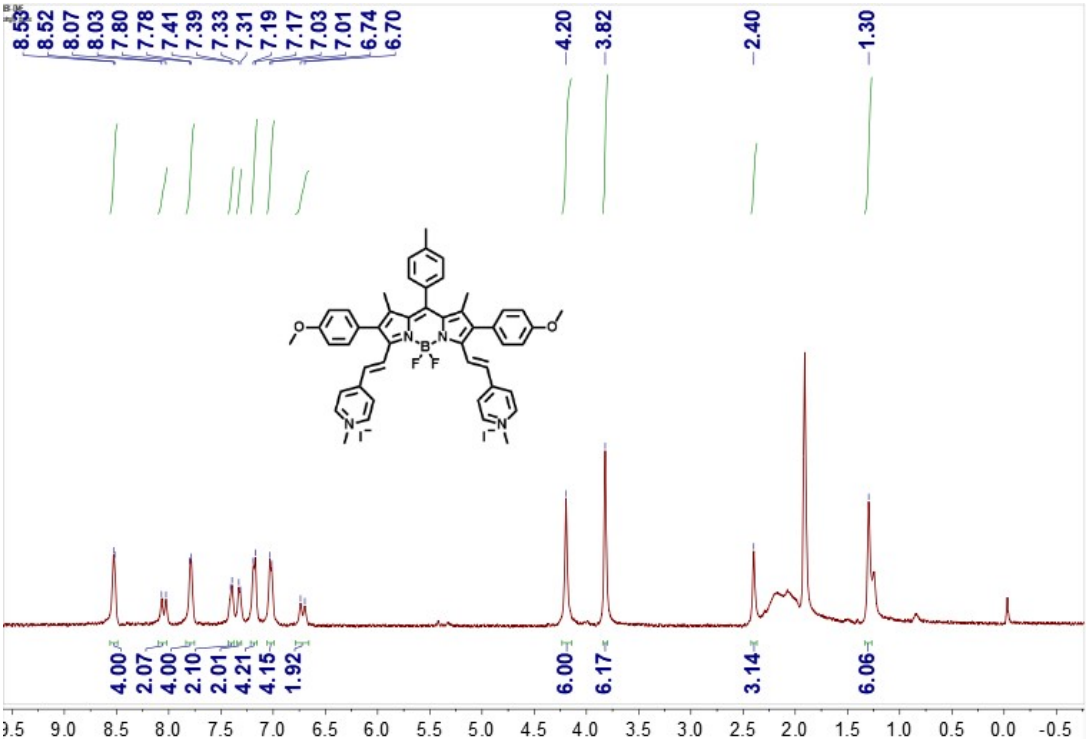


Figure S19. ^{1}H-NMR spectrum of Compound 5.

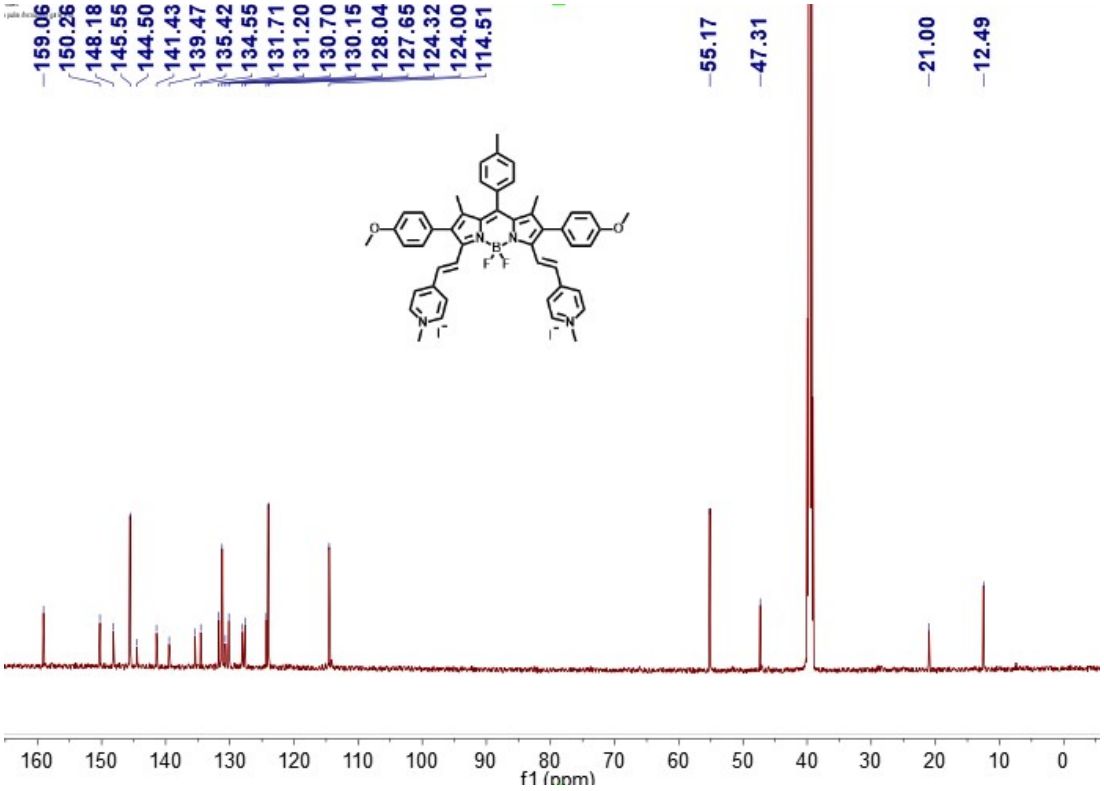


Figure S20. ^{13}C-NMR spectrum of Compound 5.

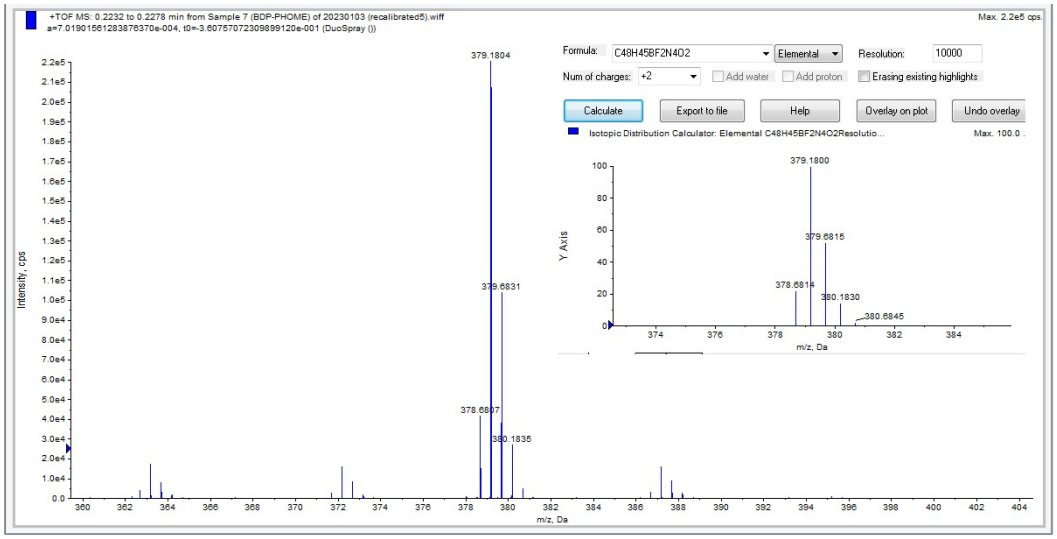


Figure S21. HRMS spectrum of Compound 5.

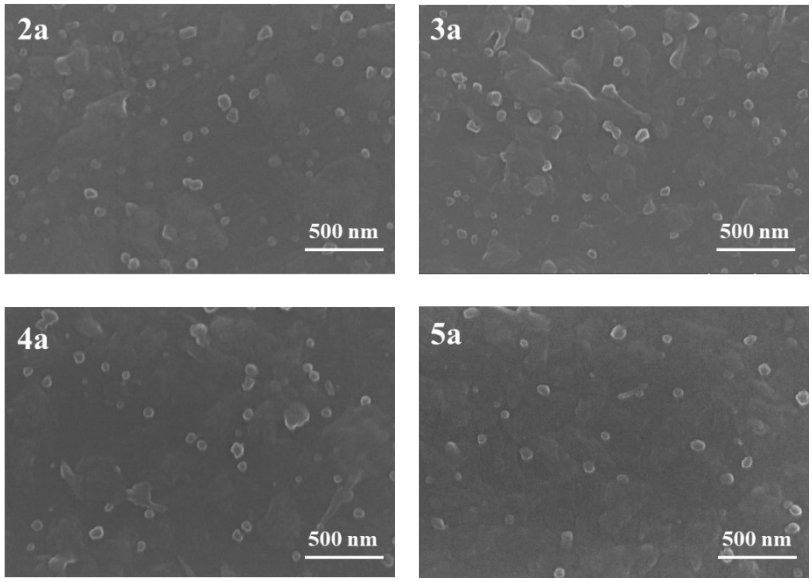


Figure S22. SEM images of **2a-5a**.

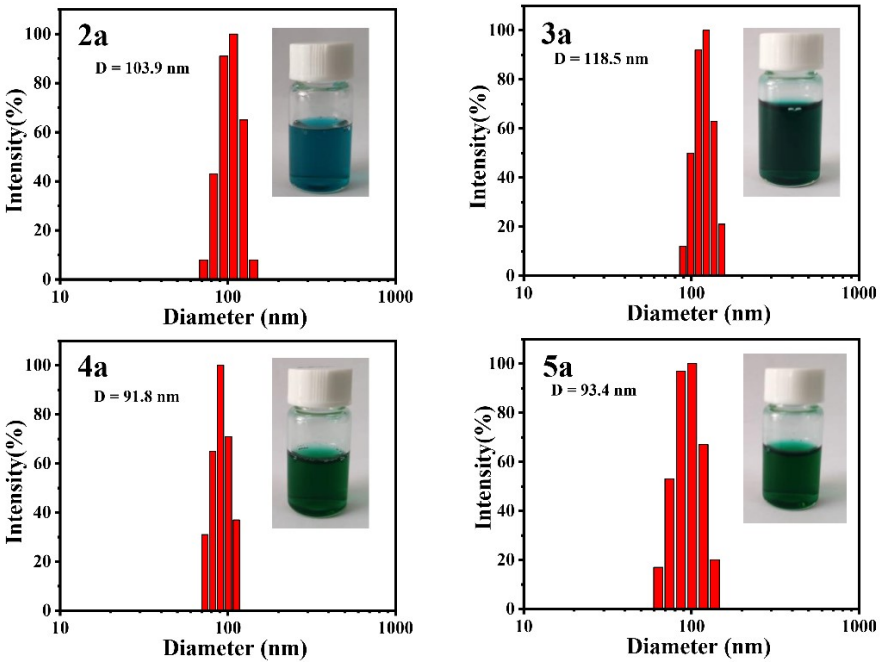


Figure S23. Particle size distributions of **2a-5a**. Inset: the picture of corresponding aqueous solution.

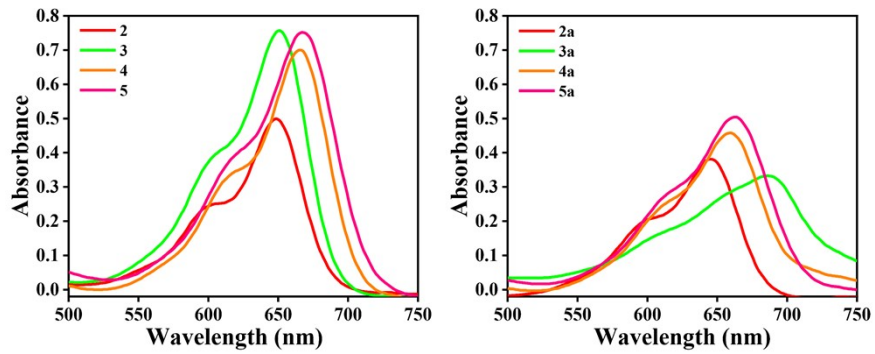


Figure S24. Absorption spectra of **2-5** (10 μM) in acetonitrile (left) and **2a-5a** (10 μM) in water (right).

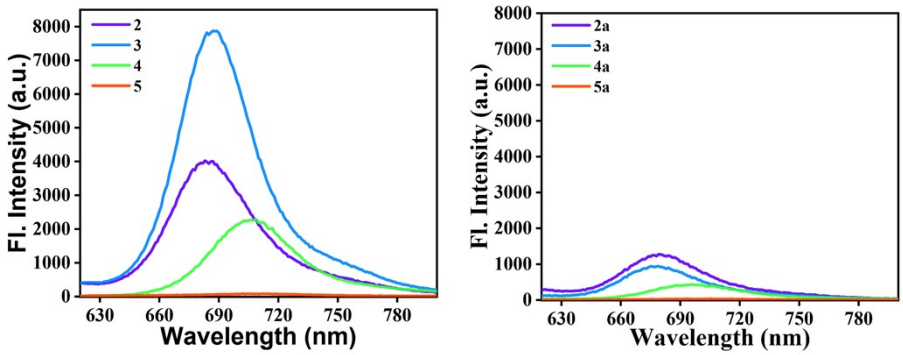


Figure S25. Fluorescence spectra of **2-5** (10 μM) in acetonitrile (left) and **2a-5a** (10 μM) in water (right). (Ex: 580 nm)

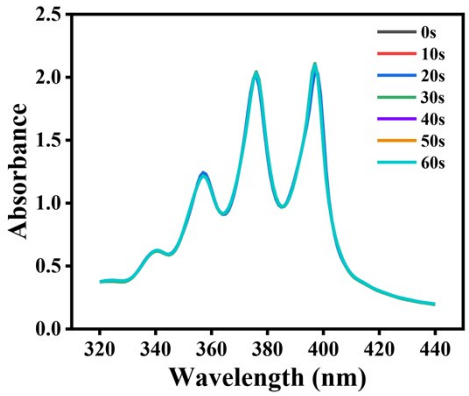


Figure S26. Absorption spectrum of ABDA in pure water under 660 nm light irradiation (30 mW cm^{-2}).

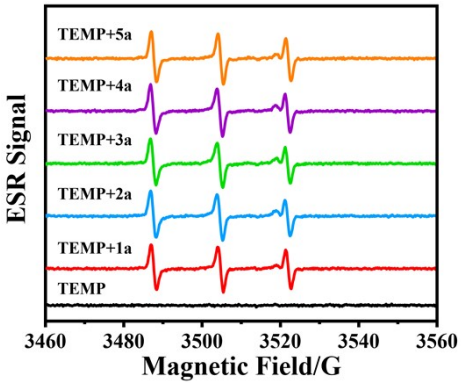


Figure S27. ESR spectra to detect $^1O_2$ generated by **1a-5a** under light exposure (white light, 100 mW cm^{-2}), using TEMP as a spin trap agent.

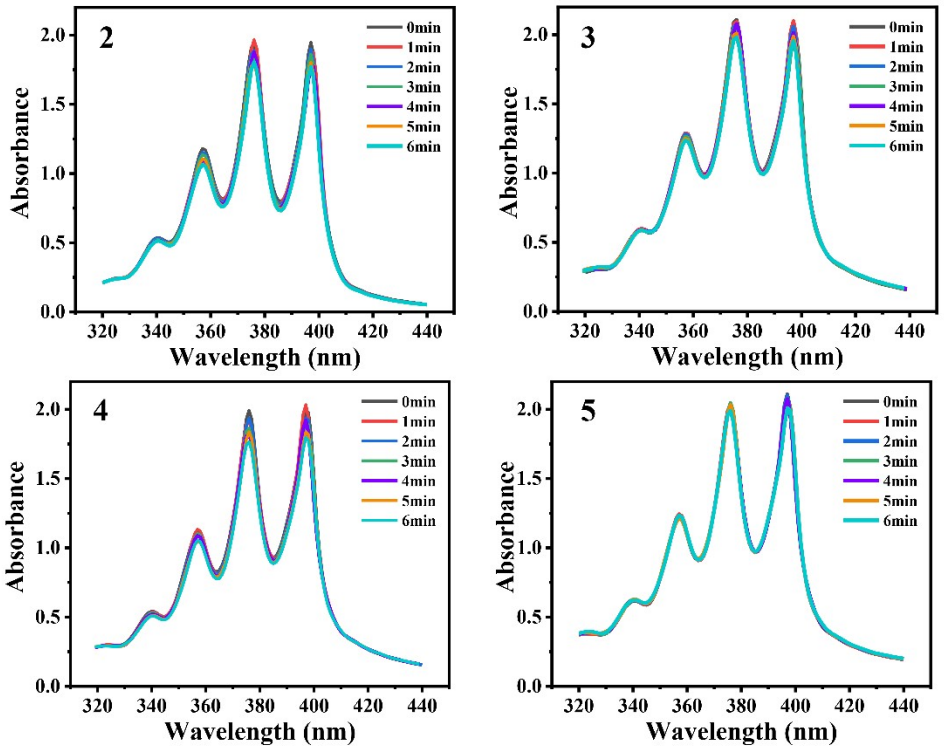


Figure S28. Degradation of ABDA in the presence of **2-5** (10 μM) in acetonitrile under 660 nm light irradiation (30 mW cm^{-2}).

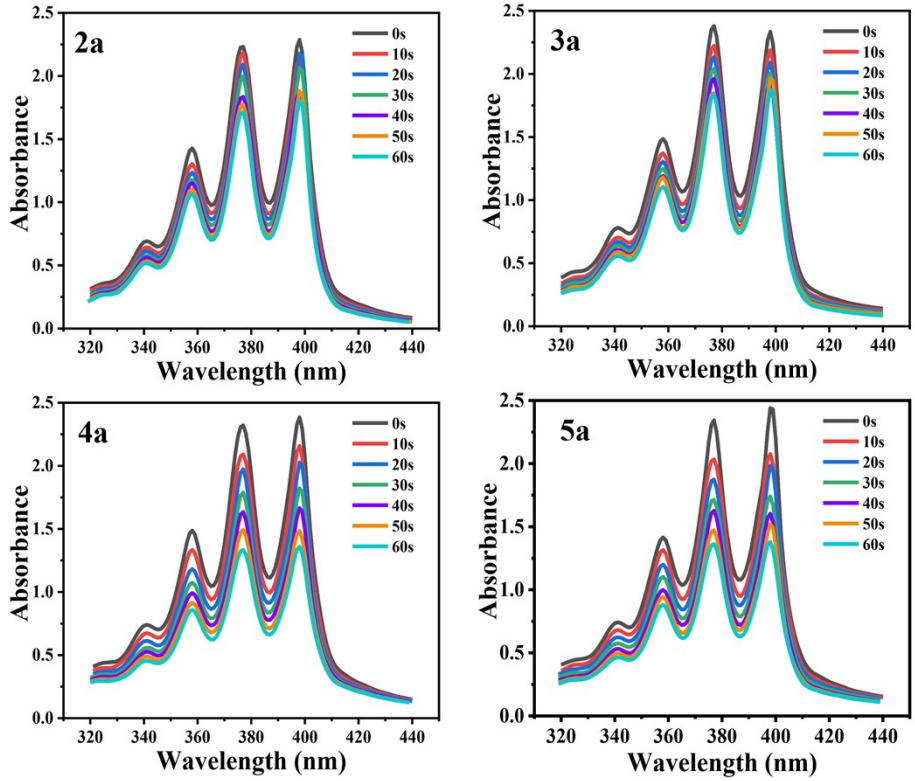


Figure S29. Degradation of ABDA in the presence of **2a-5a** (10 μM) in water under 660 nm light irradiation (30 mW cm^{-2}).

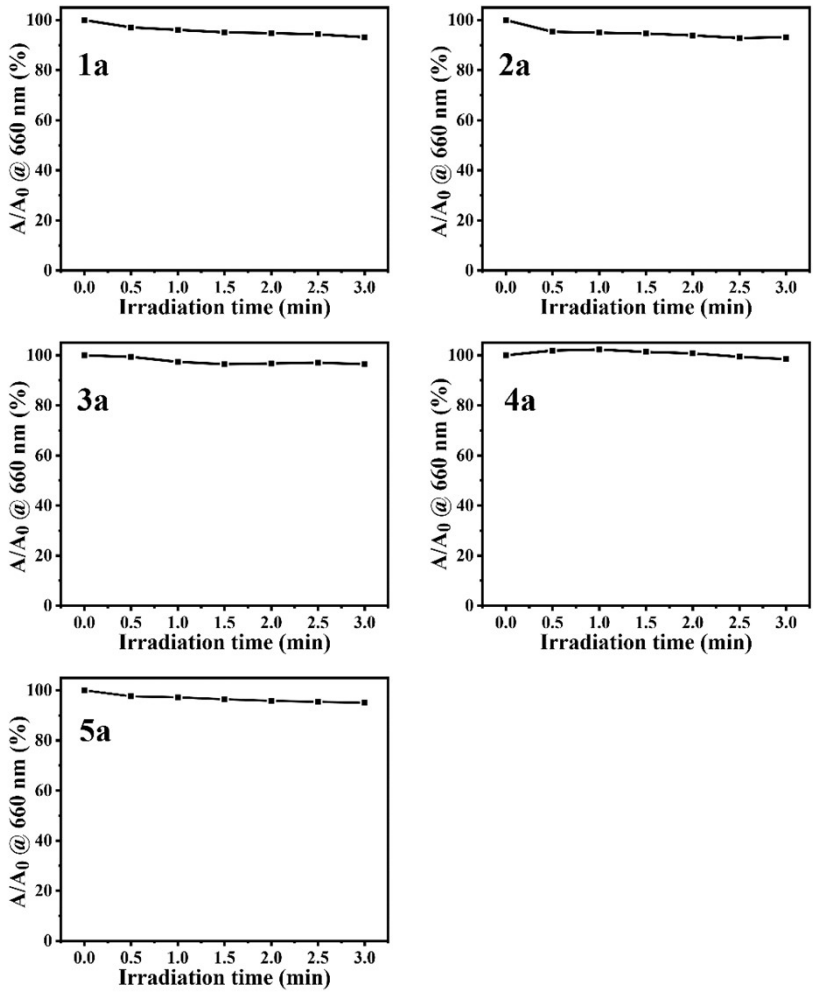


Figure S30. Absorption of **1a-5a** at 660 nm under 660 nm light irradiation (50 mW cm^{-2})

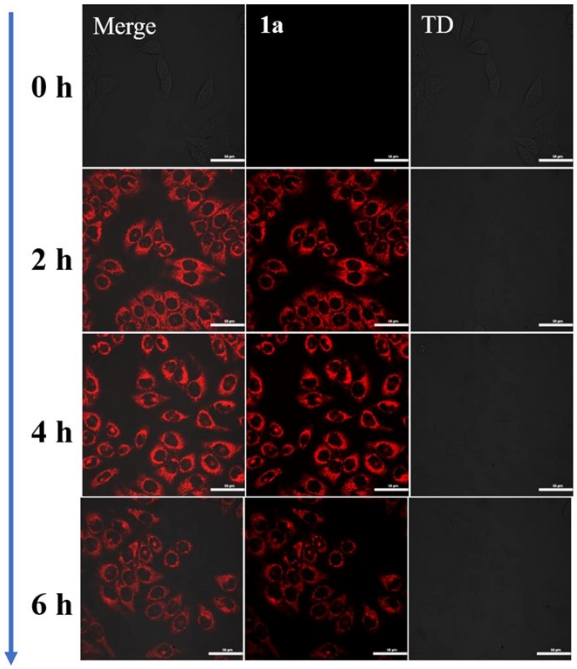


Figure S31. Confocal fluorescence images of Hela cells incubated with **1a** (2 μM) for different time. Scale bars: 25 μm.

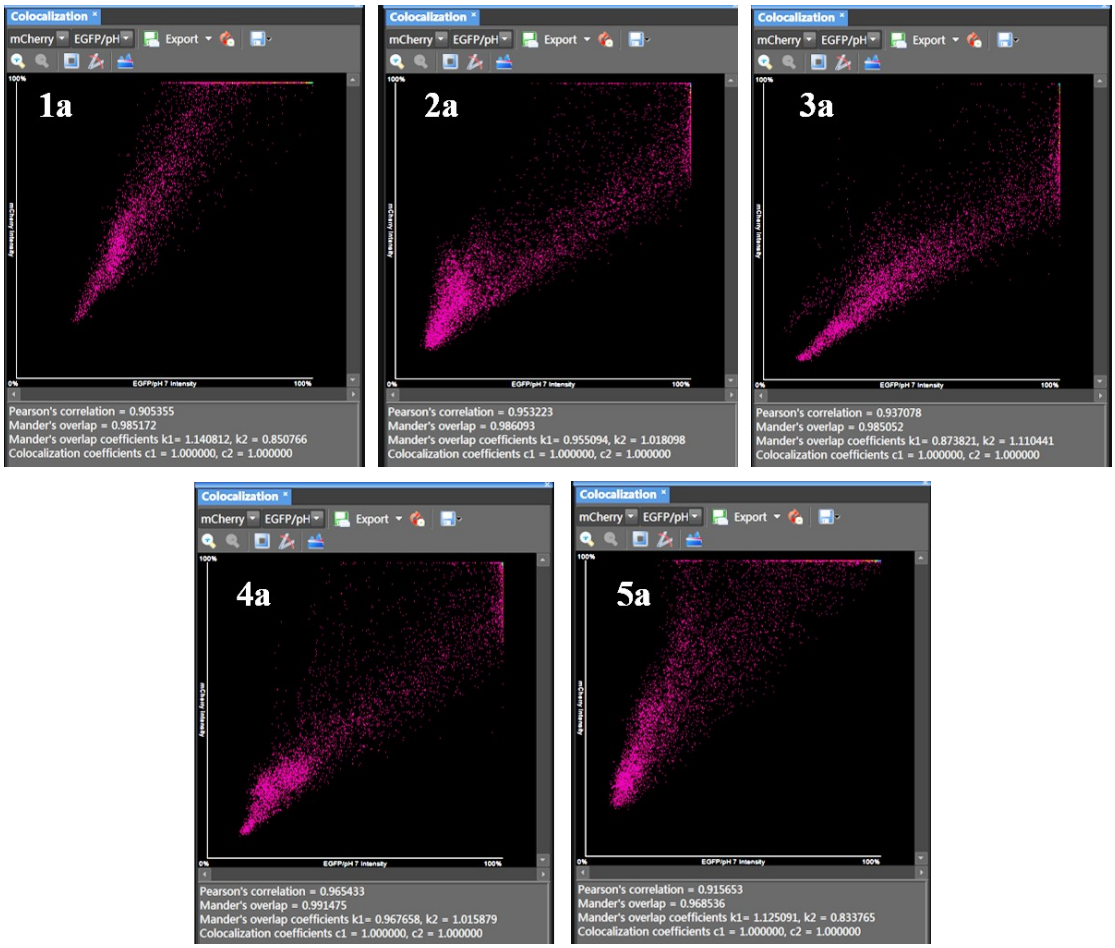
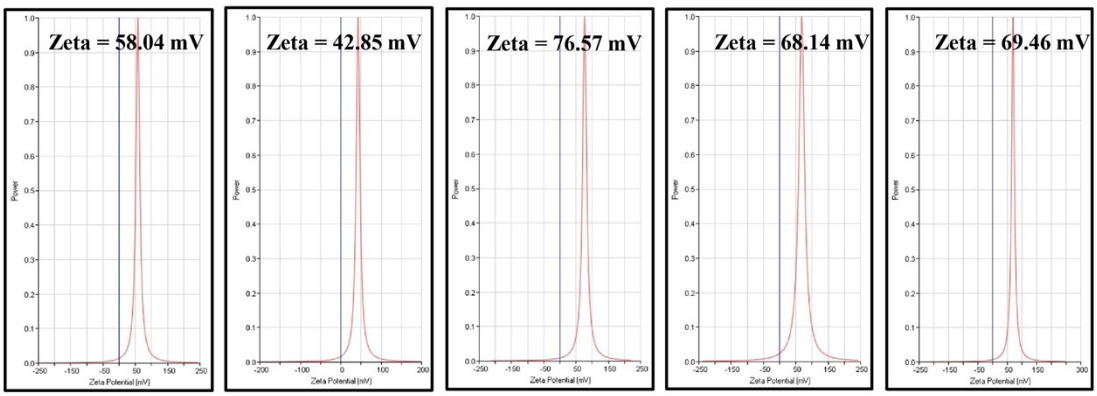


Figure 32. Mitochondrial colocalization coefficients of **1a-5a** in Hela cells.



1a **2a** **3a** **4a** **5a**

Figure S33. Zeta potentials of **1a-5a** in deionized water.

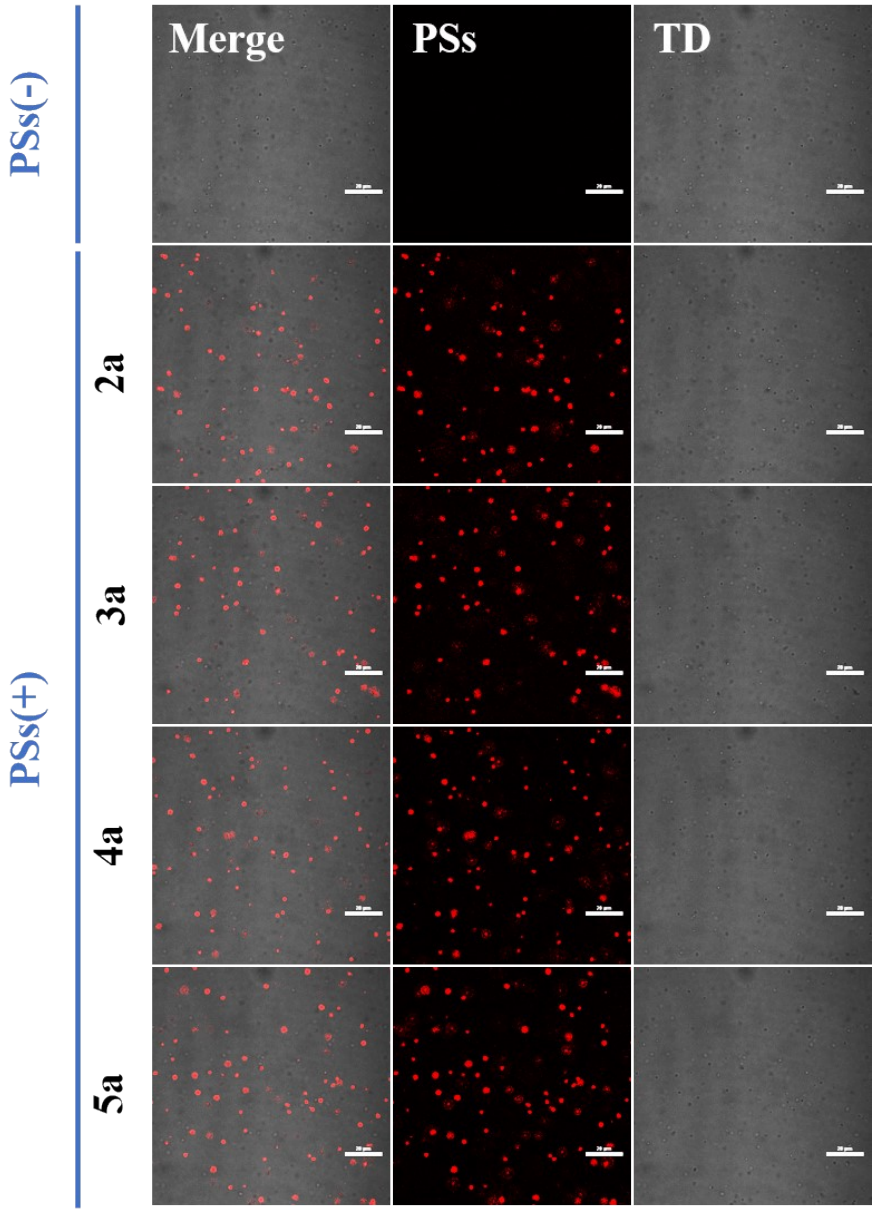


Figure S34. Confocal fluorescence images of MRSA incubated with **2a-5a (**4 μM) for 2 h. Scale bars: 20 μm.

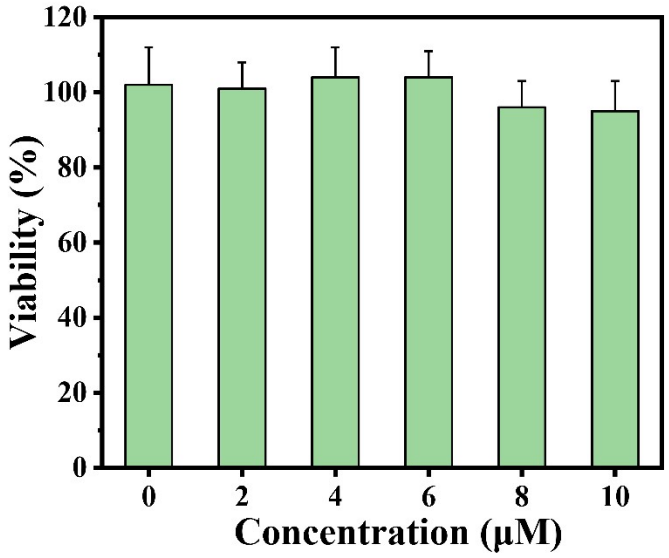


Figure S35. Cell viability of HUVEC cells incubated with **1a** at different concentrations in the dark.

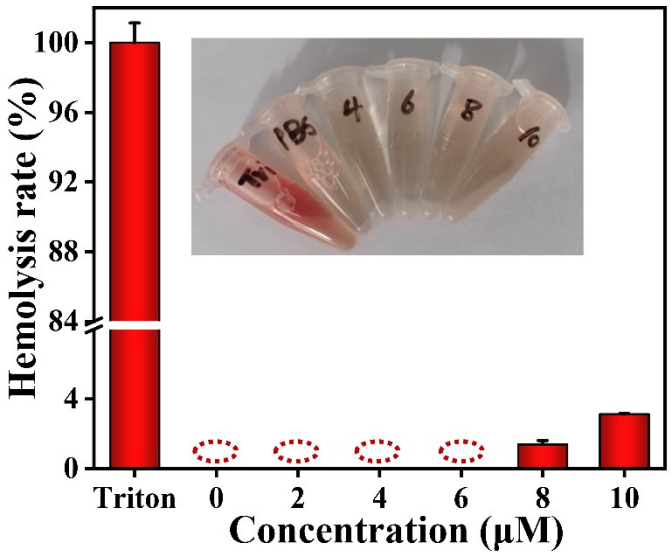


Figure S36. Hemolysis ratio of RBCs incubated with **1a** at different concentrations.

Table S1. Photophysical properties of **1**-**5** in acetonitrile and **1a**-**5a** in water.

Compound	λ_{abs} [nm][a] (ε [$\times 10^4$ M^{-1} cm^{-1}])	λ_{FL} [nm][b]	ε@660 nm [$\times 10^4$ M^{-1} cm^{-1}]	Φ_{FL} [%][c]	Φ_{Δ} [%][d]
1	659 (6.63)	700	6.63	29.4	nd
2	648 (4.99)	684	4.08	29.2	nd
3	651 (7.57)	687	6.81	31.5	nd
4	666 (7.00)	708	6.78	25.3	nd
5	668 (7.51)	718	7.16	1.9	nd
1a	655(6.30)	692	6.13	10.6	56
2a	644 (3.81)	680	2.78	9.2	31
3a	686 (3.32)	679	2.86	3.6	24
4a	660 (4.58)	696	4.58	5.3	48
5a	662 (5.04)	708	5.01	0.5	56

[a] Absorption maxima. [b] Fluorescence maxima. [c] Absolute fluorescence quantum yield. [d] Absolute $^1O_2$ quantum yield. [nd] Too low to measure accurately.

Reference

1. X. Zhao, Q. Yao, S. Long, W. Chi, Y. Yang, D. Tan, X. Liu, H. Huang, W. Sun, J. Du, J. Fan and X. Peng, An Approach to Developing Cyanines with Simultaneous Intersystem Crossing Enhancement and Excited-State Lifetime Elongation for Photodynamic Antitumor Metastasis, *J. Am. Chem. Soc.*, 2021, **143**, 12345-12354.
2. X. Zhang, M. Ivanov, Z. Wang, M. H. E. Bousquet, X. Liu, Y. Wan, J. Zhao, A. Barbon, D. Escudero, D. Jacquemin and M. Fedin, Confinement of the Triplet States in π-Conjugated BODIPY Dimers Linked with Ethynylene or Butadiynylene Bridges: A Different View on the Effect of Symmetry, *Angew. Chem., Int. Ed.*, 2022, **61**, e202210419.