

Support information

Improving Fluorescence Brightness of Distyryl Bodipys by Inhibiting Twisted Intramolecular Charge Transfer Excited State

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1. Experimental methods

1.1 Regents and solvents

All reagents and solvents were purchased from commercial suppliers and used without further purification unless otherwise stated.

1.2 Equipment and measurements

Thin-layer chromatography (TLC) was used to monitor the reactions and silica gel (200–300 mesh) was used to column chromatography. Absorption spectra were measured on Varian Cary 4000 spectrophotometer. Fluorescence spectra were measured on Hitachi F-7000 fluorescence spectrometer. The ^1H NMR and ^{13}C NMR spectra were measured on a Bruker spectrometer, and recorded at 400 and 100 MHz, respectively. High resolution mass spectra were obtained on a Thermo Scientific Q Exactive Mass spectrometer. The fluorescence imaging assays in cells were performed in a Zeiss LSM 880+ Airyscan Laser Scanning Confocal Microscope with a 60 \times oil-immersion objective lens. The living animal imaging assays were performed in a Bruker In Vivo FX Pro small animal optical imaging system with an excitation filter of 650 nm and an emission filter of 750 nm.

1.3 Spectra test of **BDP1-4**

The stock solutions of **BDP1-4** (2 mM) were prepared by directly dissolving the solid compound into CH₃CN. The test solutions of **BDP1-4** (2 μM) were prepared by diluting 2 μL of their stock solution to 2 mL with the corresponding solvents, including CH₂Cl₂, CH₃CN, MeOH and buffered aqueous solution (10 mM PBS, pH 7.4, containing 50% CH₃CN). The acquired test solutions were subjected to spectra test on a Hitachi F-7000 fluorescence spectrometer or Varian Cary 4000 spectrophotometer.

1.4 Determination of Quantum Yields

Fluorescence quantum yields of all samples were determined via the relative determination method by using Cy5.5 (Φ = 0.23 in PBS) as the reference.

The quantum yields were calculated using the following Equation:

$$\Phi_u = [(A_s FA_u \eta^2) / (A_u FA_s \eta_0^2)] \Phi_s$$

Where A_s and A_u are the absorbance of the reference and sample solution at the reference excitation wavelength, FA_s and FA_u are the corresponding integrated fluorescence intensity, and η and η₀ are the solvent refractive indexes of sample and reference, respectively. Absorbance of sample and reference at their respective excitation wavelengths was controlled to be lower than 0.05. Reported values are averages (n = 3).

1.5 Photostability test

The photostability test were carried out by irradiating the CH₃CN solution of the dyes (2 μM) by a LED source (660 nm, 50 mW/cm²) at room temperature for 10 min. The fluorescence intensities at the emission maxima of **BDP1-4**, **C-BDP** and **Cy5** were measured every one minute on a Hitachi F-7000 fluorescence spectrometer.

1.6 Preparation of the test solution for the selectivity test of **FP-BDP4**

The aqueous solutions of Na⁺, K⁺, Ca²⁺, Mg²⁺, Fe³⁺, Cu²⁺, Zn²⁺ were prepared from their chloride salts and the aqueous solutions of NO₃⁻, Cl⁻, Br⁻, CO₃²⁻ were prepared from their sodium salts. Hydrogen peroxide solution (H₂O₂) was prepared by dilution of commercial H₂O₂ solution in deionized water, and its concentration was determined by using an extinction coefficient of 43.6 M⁻¹cm⁻¹ at 240 nm. Hypochlorite solution (ClO⁻) was prepared by the dilution of commercial NaClO

solution in deionized water, and its concentration was determined using an extinction coefficient of $350 \text{ M}^{-1}\text{cm}^{-1}$ at 293 nm. Superoxide solution ($\text{O}_2^{\cdot-}$) was prepared by dissolving KO_2 to dry dimethyl sulfoxide. Hydroxyl radical ($\cdot\text{OH}$) was generated in situ through the Fenton reaction of $\text{Fe}(\text{ClO}_4)_2$ and H_2O_2 . Singlet oxygen ($^1\text{O}_2$) was generated in situ by adding ClO^- solution to 10 eq of H_2O_2 solution. Nitric oxide (NO) was generated from a commercial NO donor NOC-9 (dissolved in 0.1M NaOH solution). Peroxynitrite solution (ONOO^-) was prepared according to literature report^[1], and the concentration of ONOO^- was determined in 0.1 M NaOH by using an extinction coefficient of $1,670 \text{ M}^{-1} \text{ cm}^{-1}$ at 302 nm. The aqueous solutions of Cys, Hcy and GSH were freshly prepared by dissolving their solid in deionized water.

The test solution of **FP-BDP4** ($4 \mu\text{M}$) was prepared by diluting $4 \mu\text{L}$ of the **FP-BDP4** stock solution (2 mM in CH_3CN) into 2 mL buffer solution ($\text{PBS} / \text{CH}_3\text{CN} = 1:1$, 10 mM , $\text{pH} = 7.4$) containing various biological species. The resulting solutions were kept at 37°C for 60 min and then the fluorescence intensities were measured.

1.7 Cell Culture and Fluorescence Imaging.

All cell lines were purchased from GeneFull Biotech co., Ltd (China). Raw 264.7 cells were grown in RPMI 1640 medium and HeLa cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10 % FBS (Fetal Bovine Serum), 100 U/mL sodium penicillin G and $100 \mu\text{g/mL}$ streptomycin at 37°C in humidified environment of 5% CO_2 . Cells were plated on glass bottom cell culture dish (30 mm) and allowed to adhere for 12 hours.

For imaging experiments with **BDP1-4** and **C-BDP**, HeLa cells were treated with the corresponding dye ($1 \mu\text{M}$) in DMEM medium for 60 min . After washing the cells with PBS for three times, the fluorescence imaging assays were performed. Emissions were collected at $650\text{--}750 \text{ nm}$ ($\lambda_{\text{ex}} = 633 \text{ nm}$).

To evaluate the subcellular localization of **BDP2** and **BDP3**, HeLa cells were first incubated with **BDP2** or **BDP3** ($0.5 \mu\text{M}$) for 1 h in DMEM and then washed with PBS for three times, followed by treating with cytoplasm membrane dye **DiO** ($5 \mu\text{M}$) for 15 min . After washing the cells with PBS for three times, fluorescence imaging was performed. For **BDP2** and **BDP3**, emissions were collected at $650\text{--}750 \text{ nm}$ ($\lambda_{\text{ex}} = 633 \text{ nm}$); for **DiO**, at $500\text{--}600 \text{ nm}$ ($\lambda_{\text{ex}} = 488 \text{ nm}$).

For the selectivity test of probe **FP-BDP4** in cells, the Raw 264.7 macrophage cells were first

incubated with **FP-BDP4** (1 μ M) for 1 h at 37 °C and then washed with PBS for three times, followed by treating with various ROS/RNS (100 μ M). After 30 min, the cells were washed again and subjected to fluorescence imaging.

To image endogenous H₂O₂, Raw 264.7 macrophage cells were pretreated with PMA (2 μ g/mL) in culture medium for 12 h. The cells were washed with PBS and then incubated with **FP-BDP4** (1 μ M) for 60 min. After washing three times with PBS, the fluorescence imaging was performed. For the inhibition assay, the cells were pretreated with PMA (2 μ g/mL) for 12 h, washing with PBS and then incubated with NAC (2 mM) for 1 h. Then, the cells were washed three times with PBS, followed by incubating with **FP-BDP4** (1 μ M) for 60 min. After washing with PBS for three times, the imaging assays were performed.

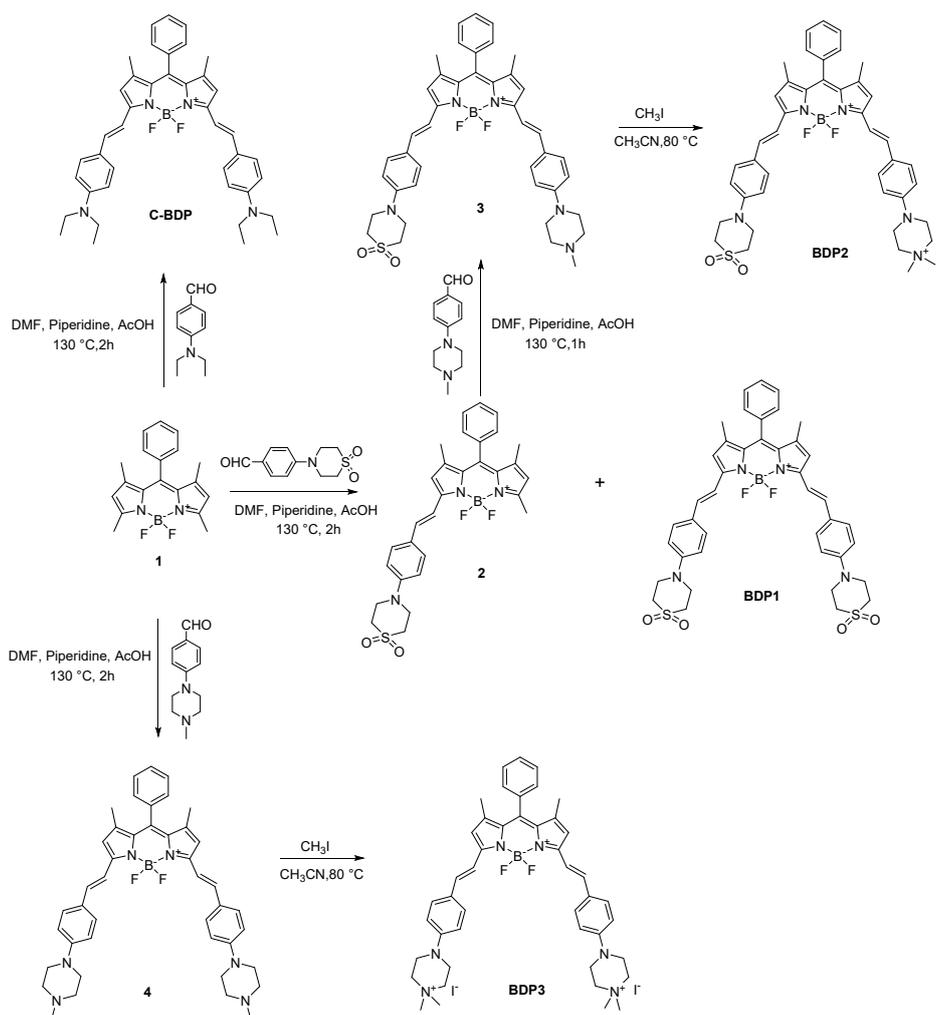
1.8 Imaging H₂O₂ in inflamed mouse models

Female BALB/c nude mice (6-8 weeks old) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. All the animal experiments were carried out in accordance with the relevant laws and guidelines issued by the Ethical Committee of Shanxi University. For imaging exogenous H₂O₂, the mice were first intraperitoneally (i.p.) injected with **FP-BDP4** (50 μ M, 100 μ L, 50% DMSO in saline) for 10 min, and then i.p. injected with H₂O₂ (2 mM, 100 μ L) for 60 min. For imaging endogenous H₂O₂, the mice were first i.p. injected with LPS (2 mg/mL, 100 μ L) for 24 h, and then i.p. injected with **FP-BDP4** (50 μ M, 100 μ L, 50% DMSO in saline) for 60 min.

1.9 Cytotoxicity Assays.

HeLa cells were seeded into a 96-well plate in DMEM medium supplemented with 10% FBS and 1% antibiotics at 37°C in a humidified environment of 5% CO₂. After 24 h of cell attachment, the cells were washed with PBS, followed by addition of increasing concentrations of the dye in DMEM medium. The final concentrations of the dyes were kept from 0 to 10 μ M. The cells were then incubated at 37 °C in an atmosphere of 5% CO₂ and 95% air for 24 h, followed by MTT assays. Untreated assays with DMEM medium (n = 6) were also conducted under the same conditions.

2. Synthesis and characterization of the compounds



Scheme S1. The synthetic routes of **BDP1-3** and **C-BDP**.

General procedures of Knoevenagel-type reactions for the synthesis of compound C-BDP, BDP1 and intermediate 2 and 4

BODIPY 1^[2] (1 equiv) and aromatic aldehyde (1-4 equiv) were dissolved in dry N, N-dimethylformamide (20 mL/mmol BODIPY). Glacial acetic acid (1 mL/mmol BODIPY) and piperidine (1 mL/mmol BODIPY) were added to the reaction mixture. Then, the reaction mixture was heated for 1-3 h at 130 °C. After cooling to room temperature, the mixture was poured into water and extracted with ethyl acetate for three times. The combined organic layer was dried over Na₂SO₄ and then filtered. The solvent was evaporated, and the residue was purified by silica gel chromatography.

C-BDP: Obtained according to the general procedure for Knoevenagel-type reactions using BODIPY 1 (162 mg, 0.5 mmol) and 4-(diethylamino) benzaldehyde (354 mg, 2.0 mmol). The crude

product was purified by column chromatography (PE: EA=5:1) affording a deep green solid (208 mg, 65%). ¹H-NMR (CDCl₃, 400 MHz) δ (ppm): 7.56 (s, 1H), 7.52-7.50 (m, 5H), 7.47-7.45 (m, 3H), 7.33-7.30 (m, 2H), 7.19 (s, 1H), 7.15 (s, 1H), 6.68-6.66 (d, *J* = 8.8 Hz, 4H), 6.58 (s, 2H), 3.43-3.38 (q, *J* = 7.2 Hz, 8H), 1.41 (s, 6H), 1.21-1.84 (t, *J* = 7.2 Hz, 12H); ¹³C NMR (100 MHz, CDCl₃) δ 152.8, 148.2, 140.7, 136.2, 135.9, 135.7, 132.9, 129.3, 128.8, 128.5, 124.2, 117.1, 114.4, 111.4, 44.5, 14.6, 12.7; ESI-MS [M+H]⁺ : calcd for 643.3778, Found 643.3787.

BDP1: Obtained according to the general procedure for Knoevenagel-type reactions using BODIPY **1** (162 mg, 0.5 mmol) and 4-(1,1-dioxidothiomorpholino) benzaldehyde (480 mg, 2.0 mmol). The crude product was purified by column chromatography (PE: EA=2:1) affording a dark blue solid (231 mg, 59%). ¹H-NMR (CDCl₃, 400 MHz) δ (ppm): 7.64-7.60 (d, *J* = 16 Hz, 2H), 7.57-7.55 (d, *J* = 8.8 Hz, 4H), 7.51-7.48 (m, 3H), 7.32-7.30 (m, 2H), 7.22-7.18 (d, *J* = 16 Hz, 2H), 6.90-6.88 (d, *J* = 8.8 Hz, 4H), 6.64 (s, 2H), 3.93-3.90 (t, *J* = 4.4 Hz, 8H), 3.10-3.07 (t, *J* = 4.4 Hz, 8H), 1.43 (s, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 152.5, 147.5, 141.9, 138.1, 135.3, 135.1, 133.2, 129.2, 129.1, 129.0, 128.9, 128.5, 125.5, 117.6, 117.2, 116.8, 115.6, 50.4, 46.9, 14.6; ESI-MS [M+Na]⁺ : calcd for 789.2523, Found 789.2528.

Compound 2: Obtained according to the general procedure for Knoevenagel-type reactions using BODIPY **1** (325 mg, 1.0 mmol) and 4-(1,1-dioxidothiomorpholino) benzaldehyde (239 mg, 1.0 mmol). The crude product was purified by column chromatography (DCM: EA=1:5) affording a dark blue solid (257 mg, 47%). ¹H-NMR (CDCl₃, 400 MHz) δ (ppm): 7.57-7.50 (m, 6H), 7.33-7.32 (d, *J* = 3.2 Hz, 2H), 7.20-7.17 (*J* = 10.8 Hz, 1H), 6.91-6.90 (d, *J* = 4.8 Hz, 2H), 6.61 (s, 1H), 6.02 (s, 1H), 3.95 (s, 4H), 3.13 (s, 4H), 2.61 (s, 3H), 1.44 (s, 3H), 1.40 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 154.7, 153.1, 147.5, 142.5, 142.4, 140.0, 135.4, 135.1, 132.8, 131.7, 129.2, 129.1, 128.9, 128.2, 121.1, 117.4, 117.1, 115.6, 50.4, 47.0, 14.6, 14.3; ESI-MS [M+Na]⁺ : calcd for 568.2012, Found 568.2014.

Compound 4: Obtained according to the general procedure for Knoevenagel-type reactions using BODIPY **1** (162 mg, 0.5 mmol) and 4-(4-methylpiperazin-1-yl) benzaldehyde (408 mg, 2.0 mmol). The crude product was purified by column chromatography (CH₂Cl₂: Et₃N=30:1) affording a dark green solid, which was further washed with PE to give a pure product (130 mg, 37%). ¹H-NMR (CDCl₃, 400 MHz) δ (ppm): 7.61 (s, 1H), 7.57-7.53 (m, 5H), 7.48-7.47 (m, 3H), 7.33-7.31 (m, 2H), 7.20-7.16 (d, *J* = 16 Hz, 2H), 6.92-6.90 (d, *J* = 8.8 Hz, 4H), 6.60 (s, 2H), 3.33-3.30 (t, *J* = 4.8 Hz,

8H), 2.61-2.58 (t, $J = 4.8$ Hz, 8H), 2.37 (s, 6H), 1.42 (s, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 152.7, 151.4, 141.3, 135.9, 135.4, 128.9, 128.8, 128.7, 128.6, 127.8, 117.4, 116.3, 115.2, 54.9, 48.1, 46.1, 14.6; ESI-MS $[\text{M}+\text{H}]^+$: calcd for 697.3996, Found 697.3994.

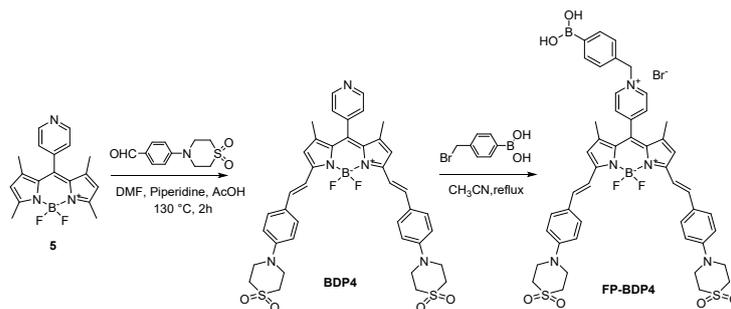
Compound 3: Compound **2** (100 mg, 0.18 mmol) and 4-(4-methylpiperazin-1-yl) benzaldehyde (75 mg, 0.36 mmol) were dissolved in dry N, N-dimethylformamide (10 mL). Glacial acetic acid (0.18 mL) and piperidine (0.18 mL) were added to the reaction mixture. Then, the reaction mixture was heated for 2 h at 130 °C. After cooling to room temperature, the mixture was poured into water and extracted with ethyl acetate for three times. The combined organic layer was dried over Na_2SO_4 and then filtered. The solvent was evaporated, and the residue was purified by silica gel chromatography (CH_2Cl_2 : MeOH=50:1) affording **3** as a dark green solid (35 mg, 27%). ^1H -NMR (CDCl_3 , 400 MHz) δ (ppm): 7.64-7.55 (m, 6H), 7.52-7.47 (m, 3H), 7.33-7.31 (m, 2H), 7.23-7.14 (d, $J = 16$ Hz, 2H), 6.92-6.89 (d, $J = 8.8$ Hz, 4H), 6.62-6.60 (d, $J = 8.0$ Hz, 2H), 3.94-3.92 (t, $J = 4.8$ Hz, 4H), 3.34-3.31 (t, $J = 4.8$ Hz, 4H), 3.12-3.10 (t, $J = 4.8$ Hz, 4H), 2.60 (s, 4H), 2.38 (s, 3H), 1.43 (s, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 153.5, 151.5, 147.3, 137.6, 136.5, 135.3, 134.5, 129.3, 129.2, 128.9, 128.8, 128.5, 127.6, 117.7, 117.5, 117.2, 116.1, 115.7, 115.2, 54.8, 50.4, 48.0, 47.1, 46.0, 14.6, 14.5; ESI-MS $[\text{M}+\text{H}]^+$: calcd for 732.3350, Found 732.3357.

BDP2: Compound **3** (30 mg, 0.04 mmol) was dissolved in CH_3CN (10 mL) followed by addition of CH_3I (0.5 mL). The reaction mixture was stirred at 80 °C for 12 h and then evaporated to dryness. The residue solid was washed with ethyl acetate to remove material and then collected as a dark green solid (31 mg, 88%). ^1H -NMR (CD_3CN , 400 MHz) δ (ppm): 7.62-7.57 (m, 8H), 7.54-7.50 (m, 1H), 7.43-7.41 (m, 3H), 7.38-7.37 (d, $J = 4.0$ Hz, 1H), 7.08-7.06 (d, $J = 8.8$ Hz, 4H), 6.78 (s, 2H), 3.96-3.93 (t, $J = 5.2$ Hz, 4H), 3.64-3.62 (t, $J = 4.4$ Hz, 4H), 3.55-3.52 (t, $J = 4.4$ Hz, 4H), 3.19 (s, 6H), 3.11-3.08 (t, $J = 6.0$ Hz, 4H), 1.47 (s, 6H); ^{13}C NMR (100 MHz, CD_3CN) δ 158.0, 157.4, 155.1, 153.7, 147.7, 143.7, 141.8, 141.3, 141.2, 140.1, 138.3, 138.2, 134.4, 134.3, 134.2, 134.1, 134.0, 133.9, 133.8, 132.9, 121.2, 121.0, 120.9, 120.7, 66.3, 56.5, 55.4, 51.6, 47.3, 19.1.; ESI-MS $[\text{M}]^+$: calcd for 746.3506, Found 746.3510.

BDP3: Compound **4** (28 mg, 0.04 mmol) was dissolved in CH_3CN (10 mL) followed by addition of CH_3I (0.5 mL). The reaction mixture was stirred at 80 °C for 12 h and then evaporated to dryness. The residue solid was washed with CH_2Cl_2 to remove material and then collected as a dark green

solid (38 mg, 97%). ¹H-NMR (CD₃CN, 400 MHz) δ (ppm): 7.63-7.60 (d, *J* = 8.8 Hz, 4H), 7.59-7.56 (m, 4H), 7.52 (s, 1H), 7.43-7.39 (m, 4H), 7.09-7.07 (d, *J* = 8.8 Hz, 4H), 6.80 (s, 2H), 3.64-3.63 (t, *J* = 4.8 Hz, 8H), 3.56-3.54 (t, *J* = 4.8 Hz, 8H), 3.20 (s, 12H), 1.47 (s, 6H); ¹³C NMR (100 MHz, CD₃CN) δ 152.4, 149.8, 142.3, 138.6, 136.2, 134.7, 133.0, 129.1, 128.6, 128.5, 115.8, 61.0, 51.2, 41.9, 13.8; ESI-MS [M]⁺: calcd for 853.3432, Found 853.3424.

Synthesis of compound BDP4 and FP-BDP4:



Scheme S2. The synthetic routes of **FP-BDP4**.

Compound BDP4: Obtained according to the general procedure for Knoevenagel-type reactions using BODIPY **5**^[3] (162 mg, 0.5 mmol) and 4-(1,1-dioxidothiomorpholino) benzaldehyde (360 mg, 1.5 mmol). The crude product was purified by column chromatography (PE: EA=2:1) affording a dark blue solid (231 mg, 60%). ¹H-NMR (CDCl₃, 400 MHz) δ (ppm): 8.80-8.79 (m, 2H), 7.62-7.57 (m, 6H), 7.37-7.36 (m, 2H), 7.23 (s, 1H), 7.19 (s, 1H), 6.92-6.90 (d, *J* = 8.8 Hz, 4H), 6.65 (s, 2H), 3.96-3.94 (t, *J* = 4.8 Hz, 8H), 3.13-3.10 (t, *J* = 4.8 Hz, 8H), 1.47 (s, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 153.1, 150.5, 147.6, 143.8, 141.1, 136.0, 133.7, 132.2, 129.4, 128.8, 123.9, 118.1, 117.0, 115.5, 50.4, 46.9, 14.9. ESI-MS [M+H]⁺: calcd for 768.2656, Found 768.2652.

Compound FP-BDP4: **BDP4** (192 mg, 0.25 mmol) and (4-(bromomethyl) phenyl) boronic acid (160 mg, 0.75 mmol) were dissolved in CH₃CN (30 mL) and then refluxed under N₂ for 24 h. After cooling to about 40 °C, the reaction mixture was filtered, and the filter cake was washed with a small amount of CH₃CN for several times. The residue solid was collected and dried in vacuum affording a dark green solid (200 mg, 82%). ¹H-NMR (CD₃CN, 400 MHz) δ (ppm): 8.91 (d, *J* = 4.0 Hz, 2H), 8.20-8.19 (d, *J* = 4.0 Hz, 2H), 7.89-7.88 (d, *J* = 5.2 Hz, 2H), 7.58-7.57 (d, *J* = 5.2 Hz, 4H), 7.42-7.41 (d, *J* = 1.6 Hz, 4H), 7.04-7.02 (d, *J* = 4.8 Hz, 4H), 6.80 (s, 2H), 6.26 (s, 2H), 5.85 (s, 2H), 5.42 (s, 2H), 3.94 (s, 8H), 3.07 (s, 8H), 1.46 (s, 6H); ¹³C NMR (100 MHz, DMSO) δ 153.6, 148.8, 146.4, 140.6, 138.4, 136.5, 135.3, 134.4, 131.2, 129.6, 127.7, 127.0, 125.8, 119.4, 115.7, 115.0, 64.0, 63.3, 50.3, 46.3, 15.4. ESI-MS [M]⁺: calcd for 902.3195, Found 902.3206.

3. Supplementary Figures

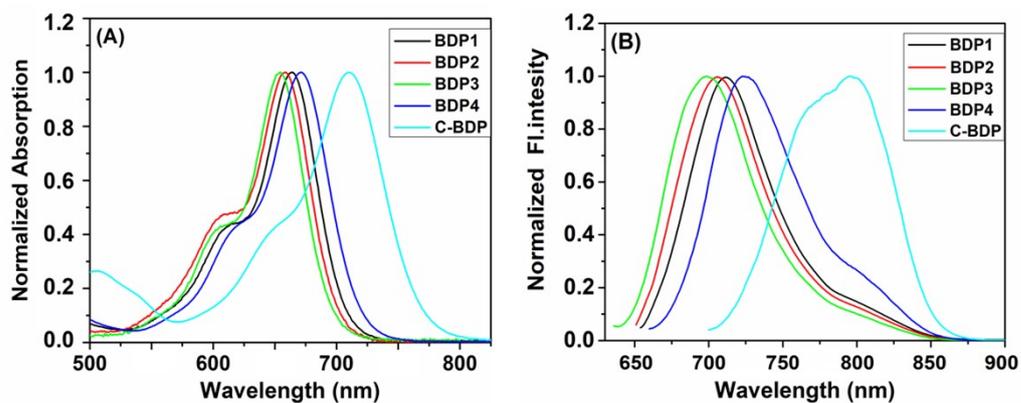


Figure S1. Normalized absorption (A) and emission (B) spectra of **BDP1-4** and **C-BDP** in CH_3CN at 25 °C. $\lambda_{\text{ex}} = 615$ nm; slits, 5/5 nm.

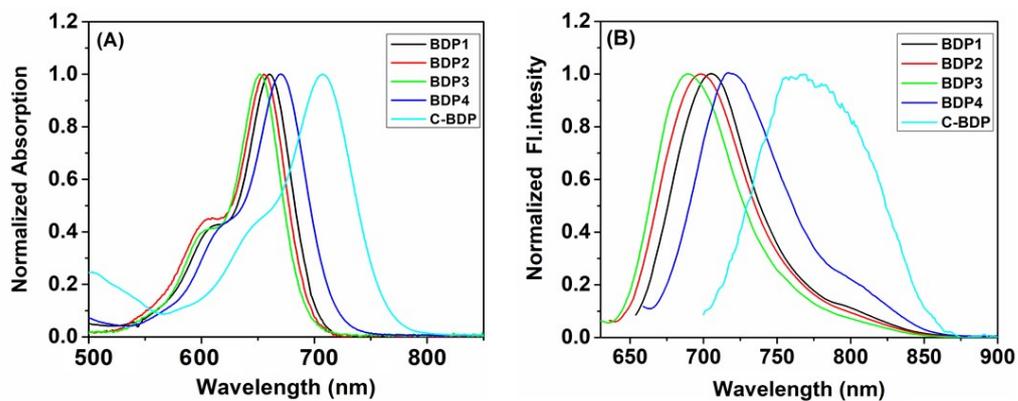


Figure S2. Normalized absorption (A) and emission (B) spectra of **BDP1-4** and **C-BDP** in CH_3OH at 25 °C. $\lambda_{\text{ex}} = 615$ nm; slits, 5/5 nm.

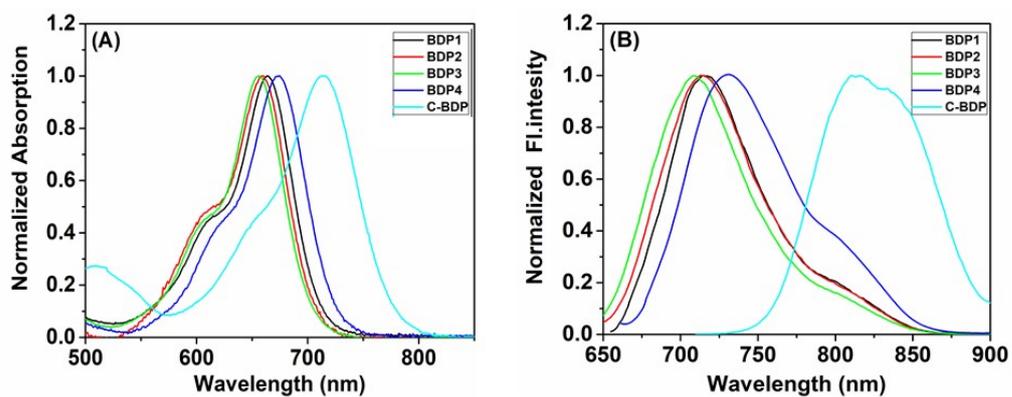


Figure S3. Normalized absorption (A) and emission (B) spectra of **BDP1-4** and **C-BDP** in buffered aqueous solution (10 mM PBS, pH 7.4, containing 50% CH₃CN) at 25 °C. λ_{ex} = 615 nm; slits, 5/5 nm.

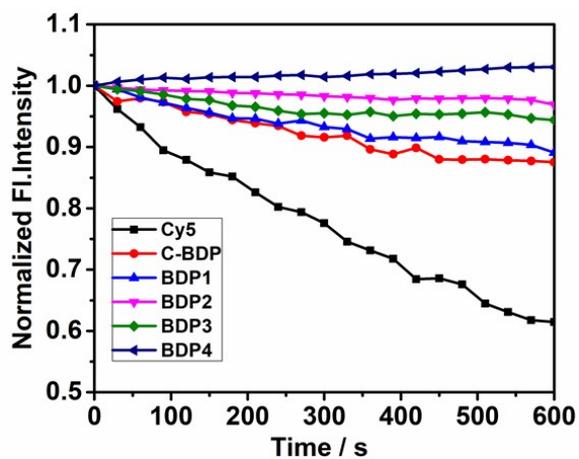


Figure S4. Fluorescence intensity changes of **C-BDP**, **BDP1-4**, and **Cy5** in CH₃CN when continuously irradiated by a LED source (660 nm, 50 mW/cm²) for 10 min.

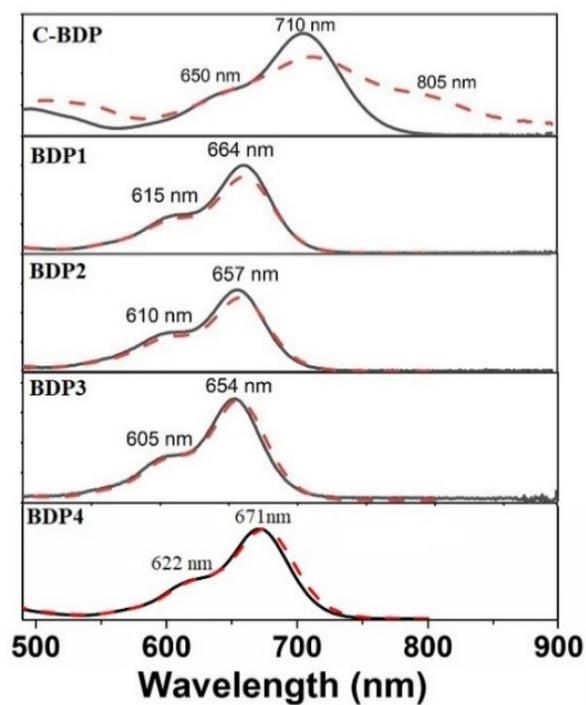


Figure S5. The solubility comparison of **BDP1-4** (10 μM) with **C-BDP** (10 μM). Absorbance spectra were obtained in CH_3CN (solid line) or $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ (v/v 1:1, dashed line).

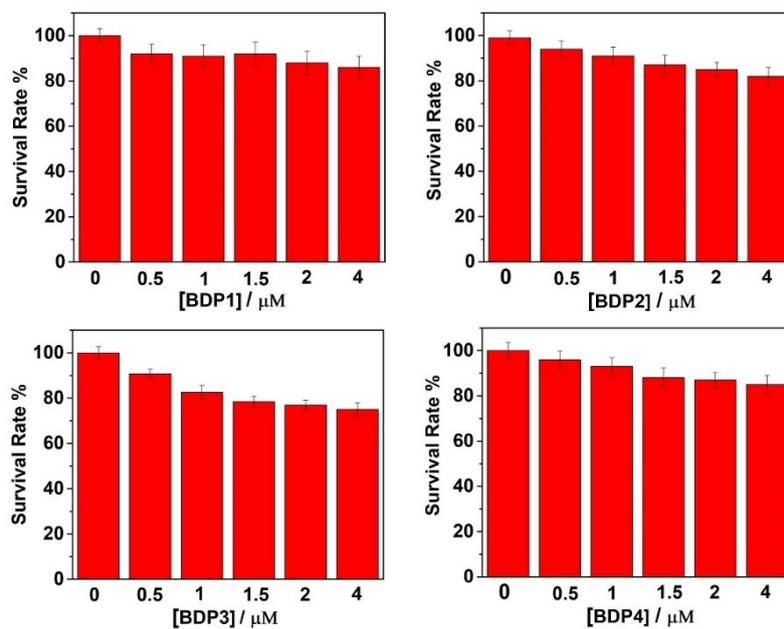


Figure S6. Percentage of viable HeLa cells after treatment with **BDP1-4**, respectively, at varied concentrations for 12 hr, which is measured by MTT assays.

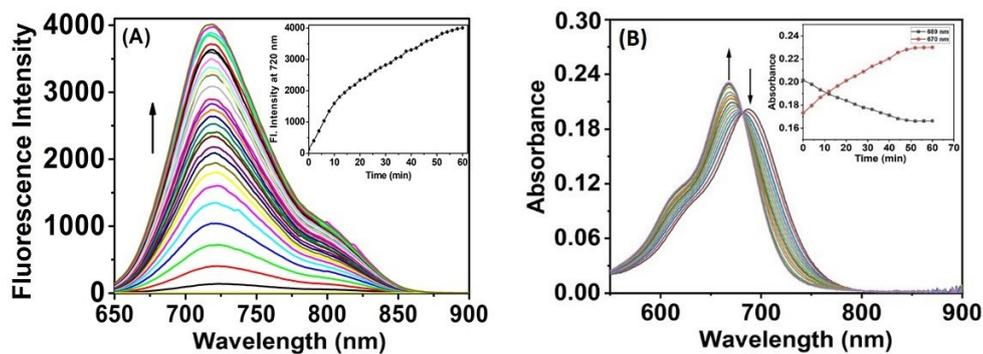


Figure S7. Time-dependent fluorescence (A) and absorption (B) changes of **FP-BDP4** ($4 \mu\text{M}$) upon treated with $100 \mu\text{M H}_2\text{O}_2$ in PBS (10 mM , $\text{pH} = 7.4$, containing $50\% \text{ CH}_3\text{CN}$). $\lambda_{\text{ex}} = 615 \text{ nm}$; slits: $5/5 \text{ nm}$.

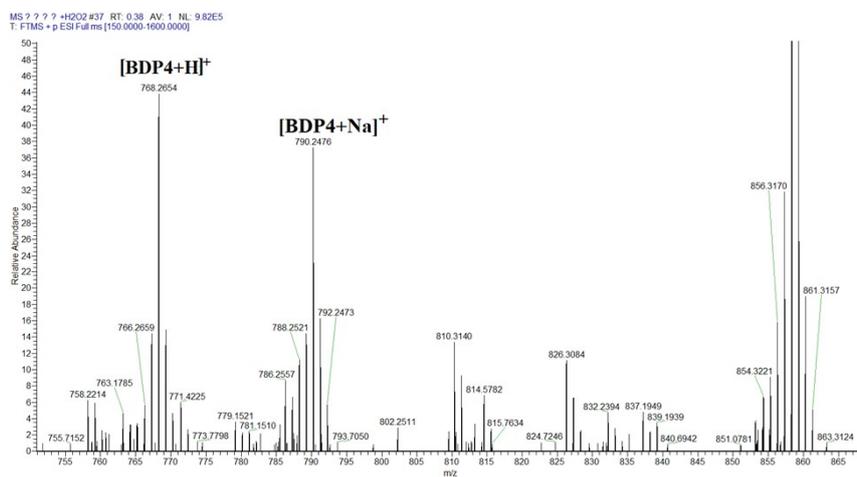


Figure S8. HRMS spectra of the mixture of **FP-BDP4** and H_2O_2 .

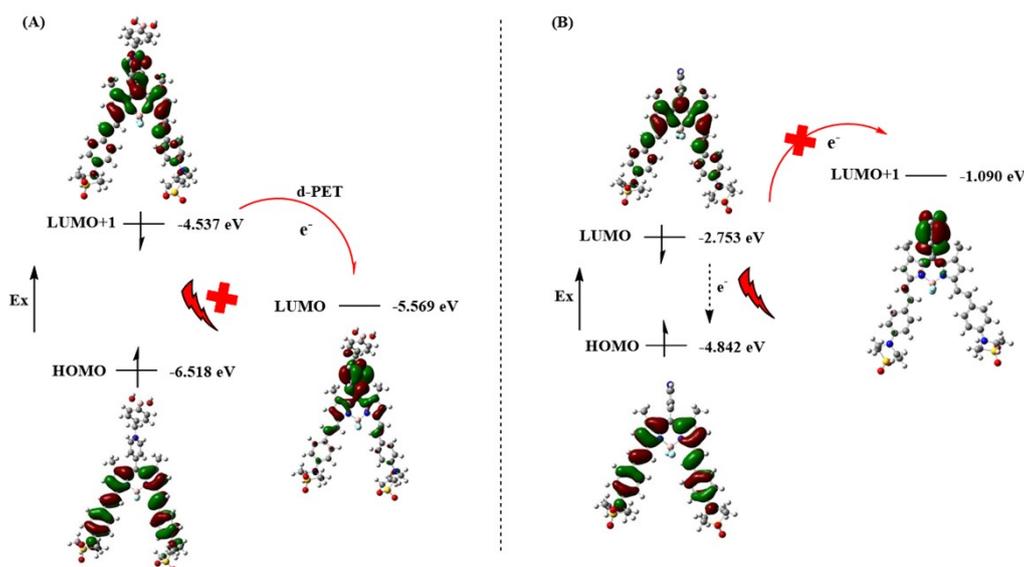


Figure S9. Prediction of the d-PeT effect in **FP-BDP4** and **BDP4** by DFT/TDDFT calculations. (A) Frontier orbital energy representation of the d-PeT process in **FP-BDP4**. (B) Frontier orbital energy representation of the d-PeT inhibition in **BDP4**. As showed in (A), the calculated LUMO level of the pyridinium unit lies between the HOMO and LUMO levels of **BDP** unit, thus allowing the electron transfer from the excited **BDP** unit to the pyridinium unit via d-PeT (Donor-excited PeT with fluorophore acts as the electron donor)^[4] that results in the fluorescence quenching. In contrast, as shown in (B), the calculated LUMO level of the pyridyl unit is higher than that of **BDP** unit, and thus the d-PeT is inhibited and the fluorescence is restored.

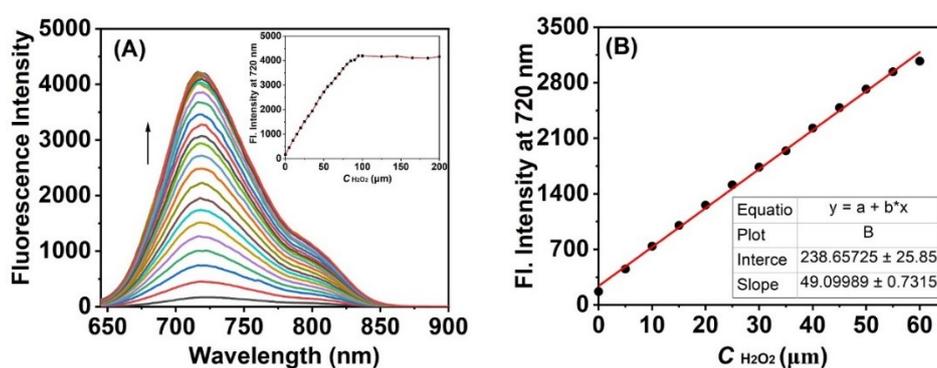


Figure S10. (A) Fluorescence spectra changes of **FP-BDP4** (4 μM) in PBS / CH_3CN ($v/v= 1:1$, 10 mM, pH = 7.4) upon addition of H_2O_2 (0-200 μM). The spectra were measured at 37 $^\circ\text{C}$ with a reaction time of 60 min. (B) The linear relationship between fluorescence intensity ($F_{720 \text{ nm}}$) and H_2O_2 concentration.

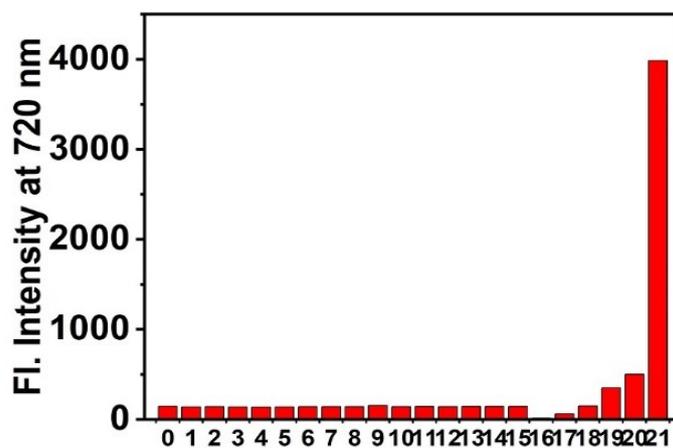


Figure S11. Fluorescence responses of **FP-BDP4** (4 μM) toward various biological species including various ions (1 mM), biothiols (1 mM), and ROS/RNS (100 μM). 0–21: blank, Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Fe^{3+} , Cu^{2+} , Zn^{2+} , NO_3^- , Cl^- , Br^- , CO_3^{2-} , Cys, GSH, Hcy, NO, HClO, $^1\text{O}_2$, ONOO^- , $\bullet\text{OH}$, O_2^- and H_2O_2 in PBS (10 mM, pH 7.4, containing 50% CH_3CN).

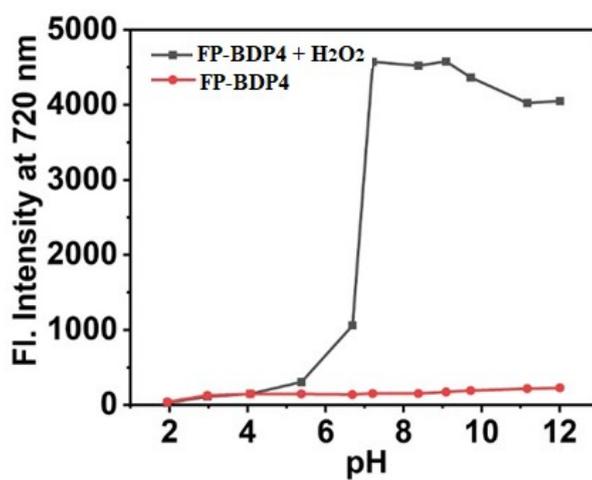


Figure S12. Fluorescence responses of **FP-BDP4** (4 μM) toward H_2O_2 (100 μM) at different pH values.

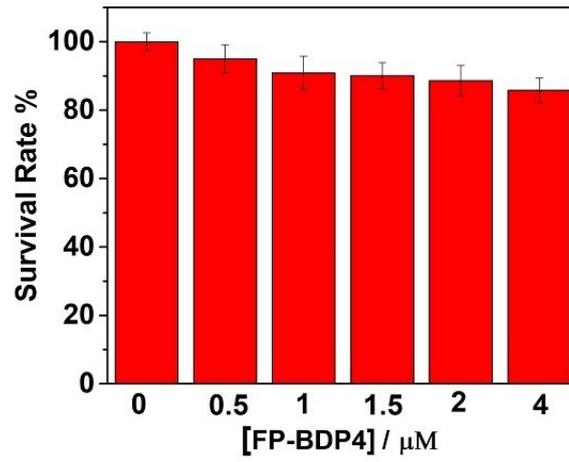
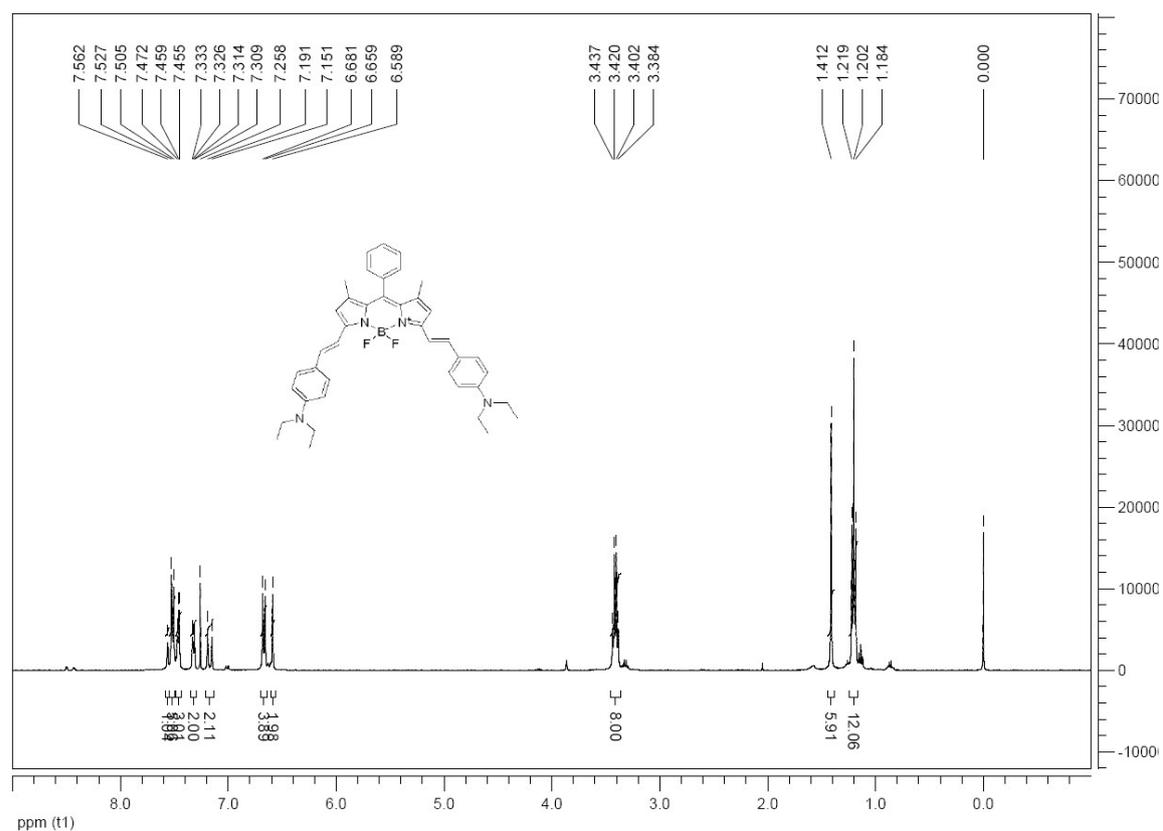
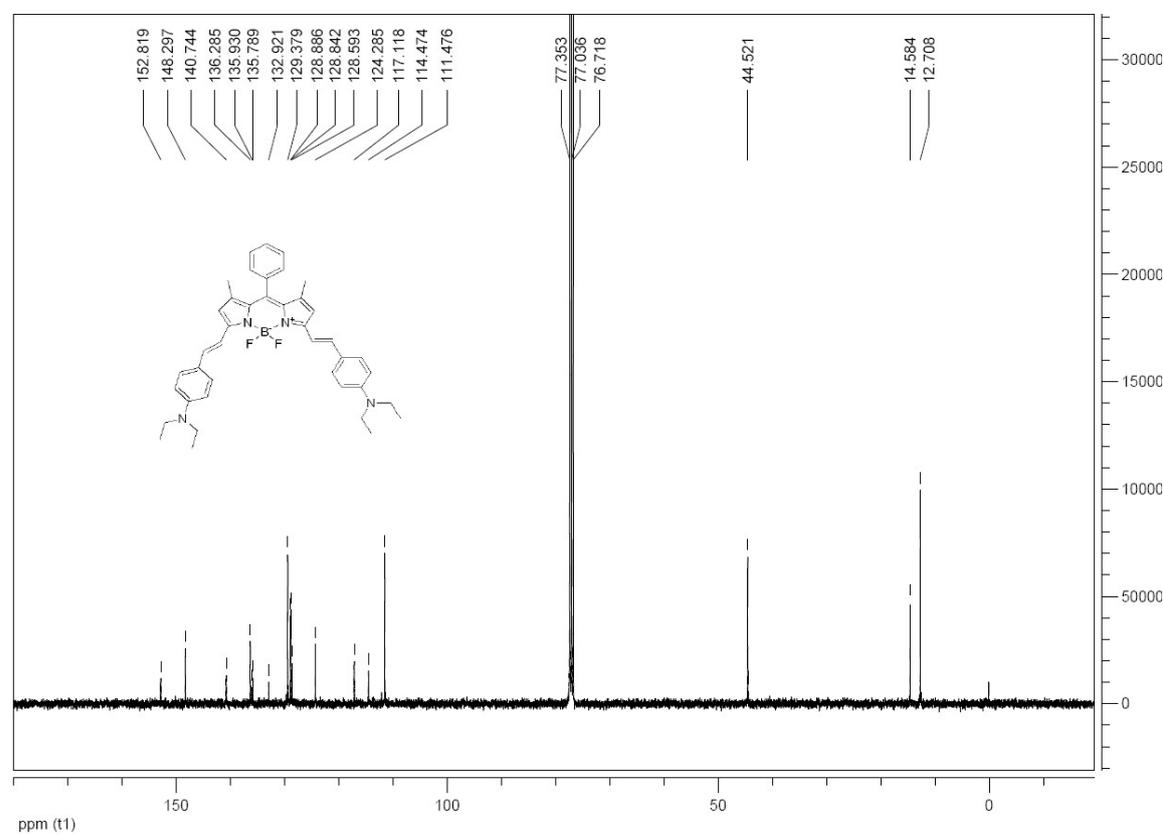


Figure S13. Percentage of viable HeLa cells after treatment with the indicated concentrations of **FP-BDP4** for 12 hours, which is measured by MTT assays.

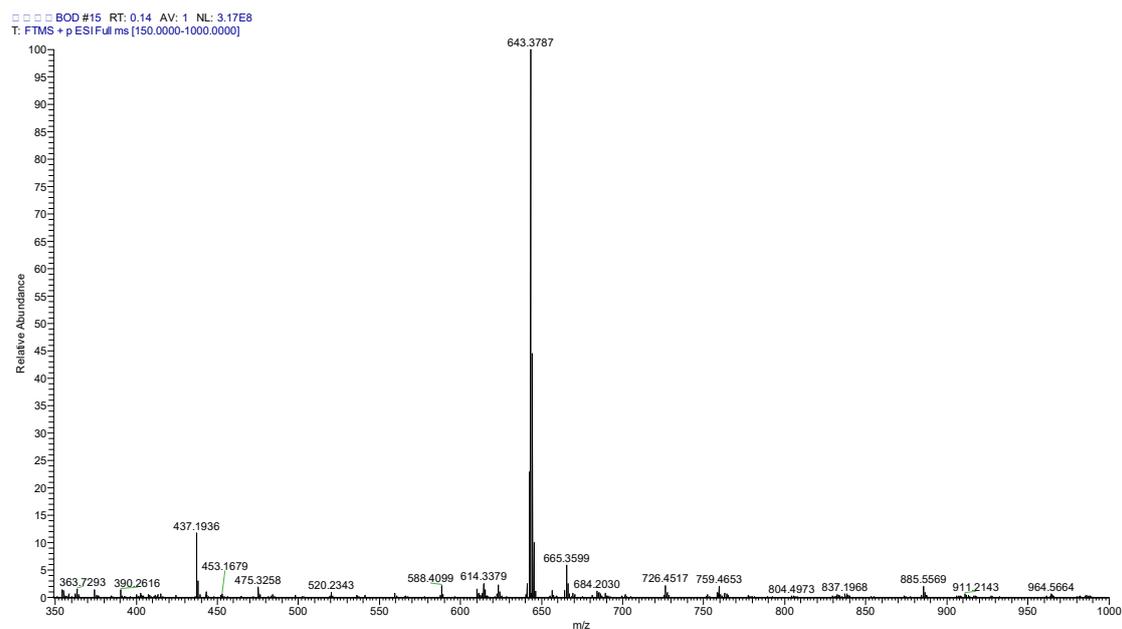
4. ¹H NMR, ¹³C NMR and HRMS spectra



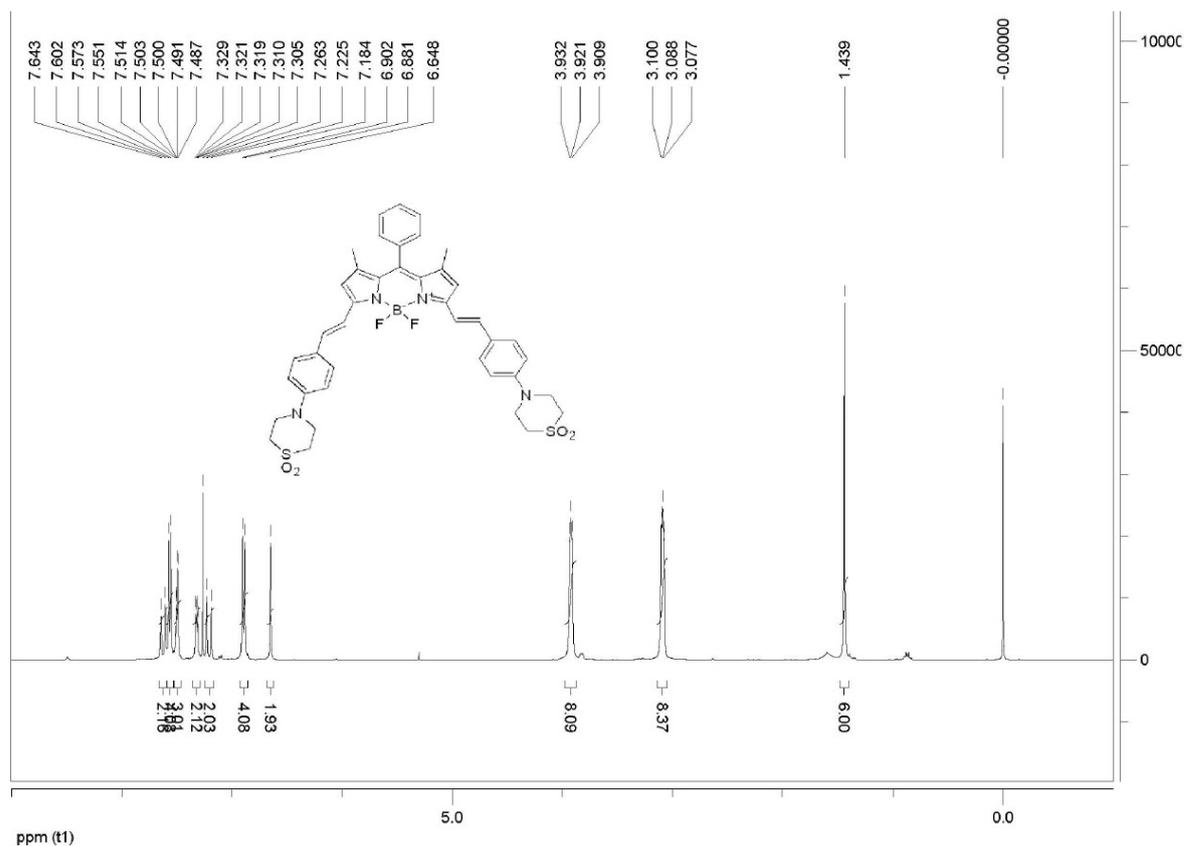
¹H NMR spectra of C-BDP in CDCl₃.



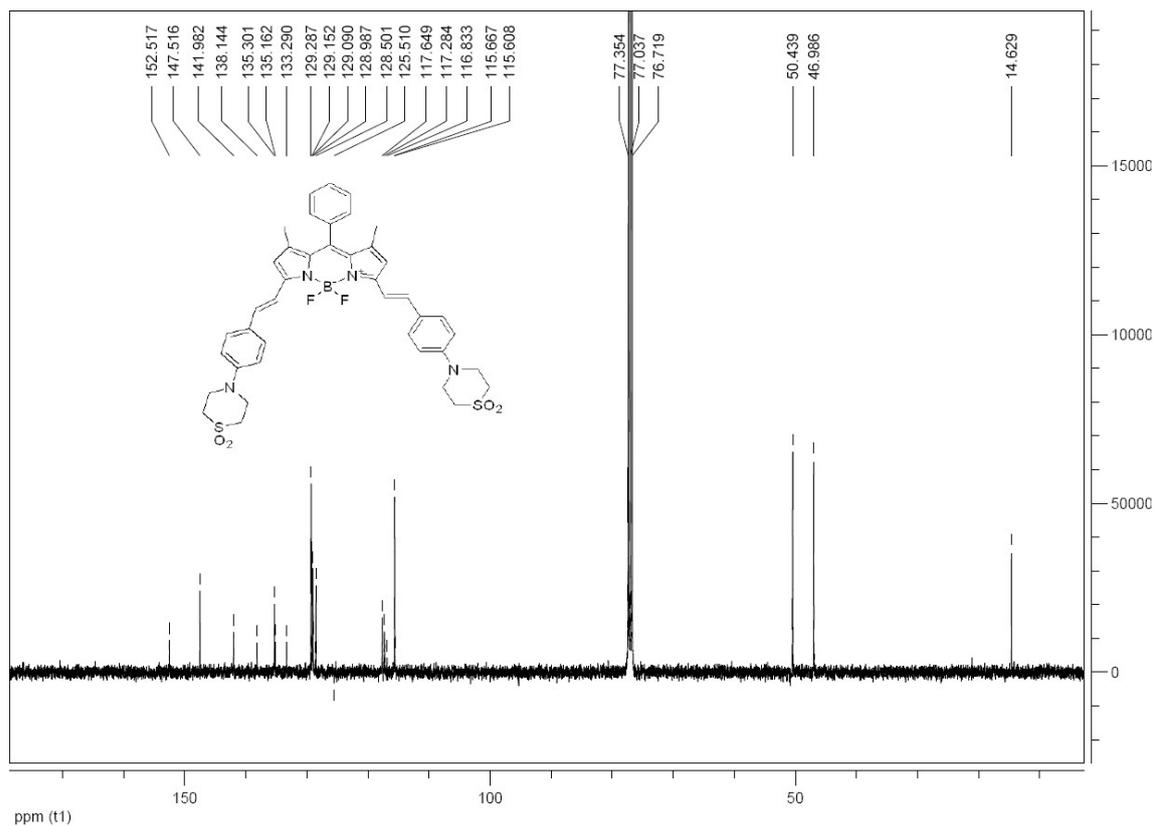
¹³C NMR spectra of C-BDP in CDCl₃.



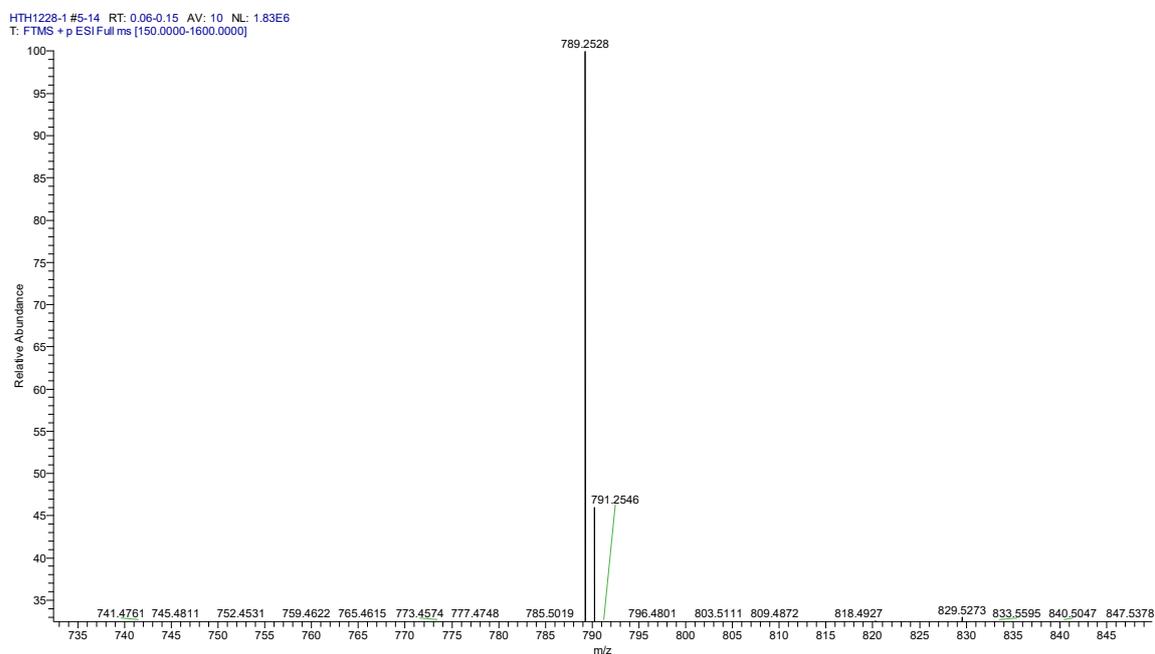
HRMS spectra of compound **C-BDP**.



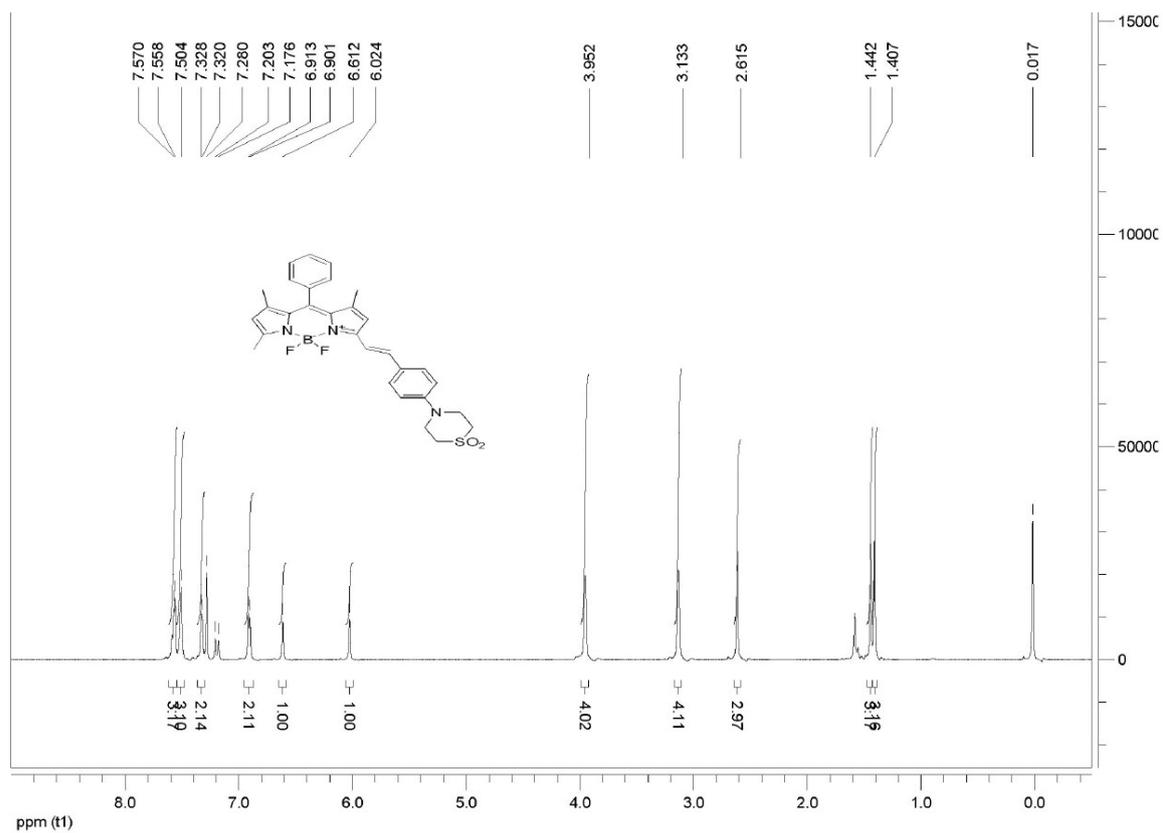
^1H NMR spectra of **BDP1** in CDCl_3 .



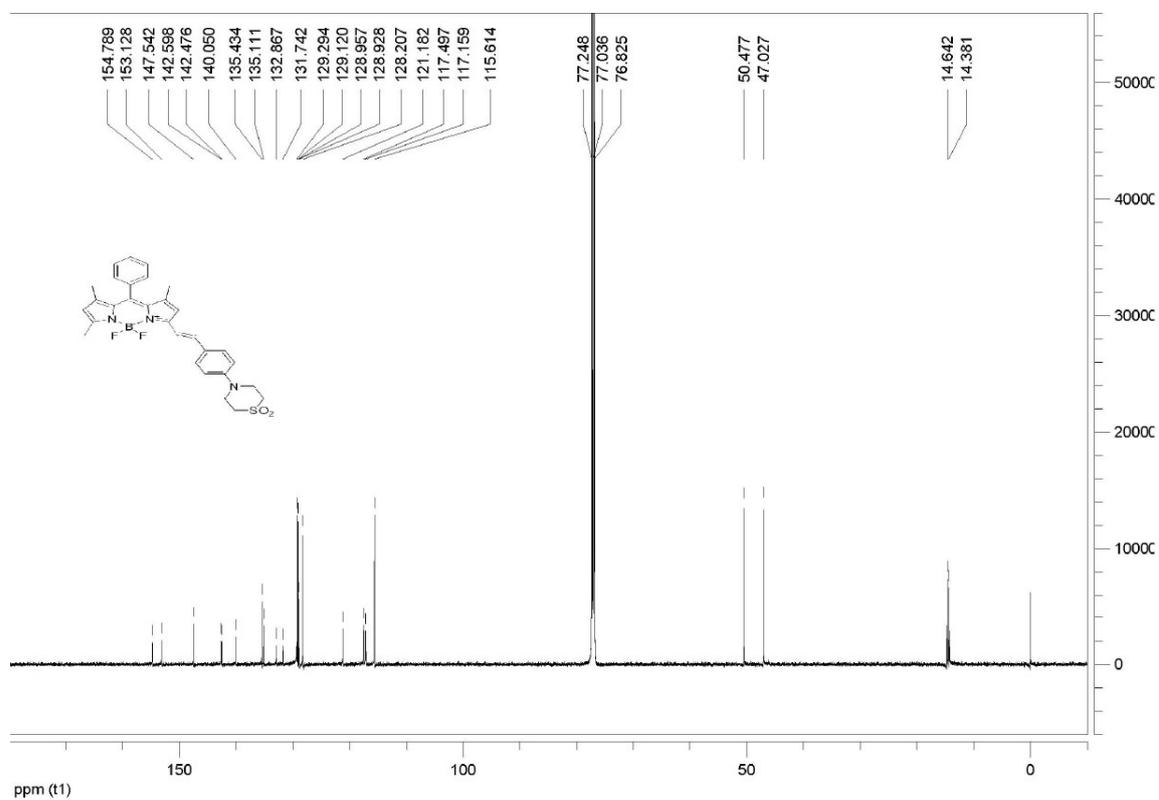
^{13}C NMR spectra of **BDP1** in CDCl_3 .



HRMS spectra of compound **BDP1**.

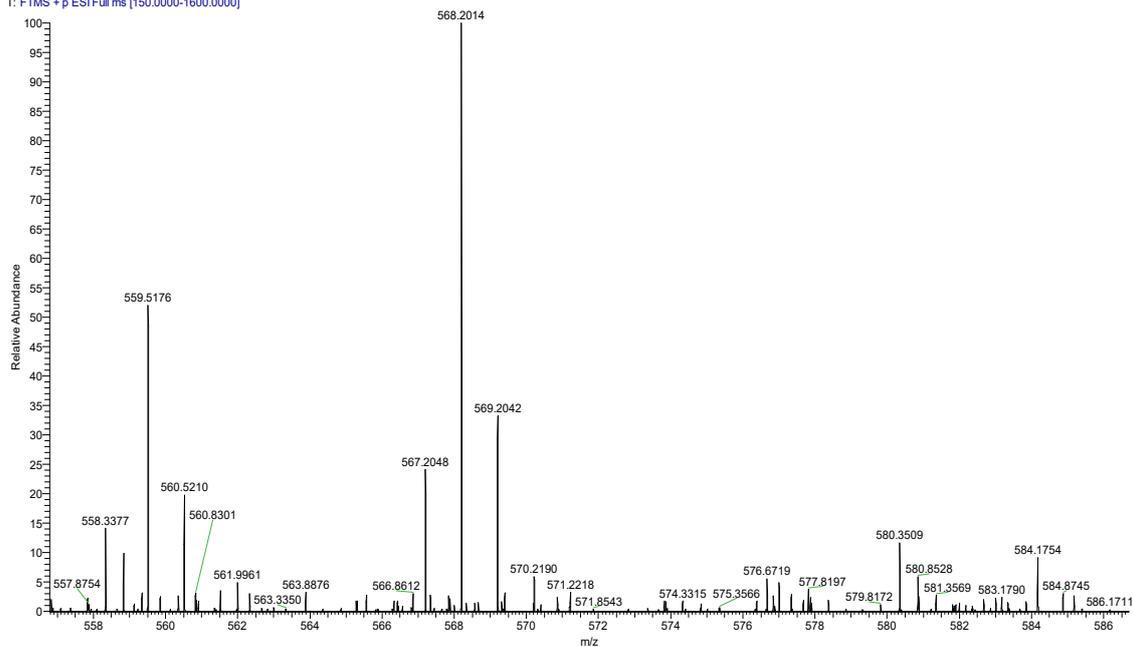


¹H NMR spectra of compound **2** in CDCl₃.

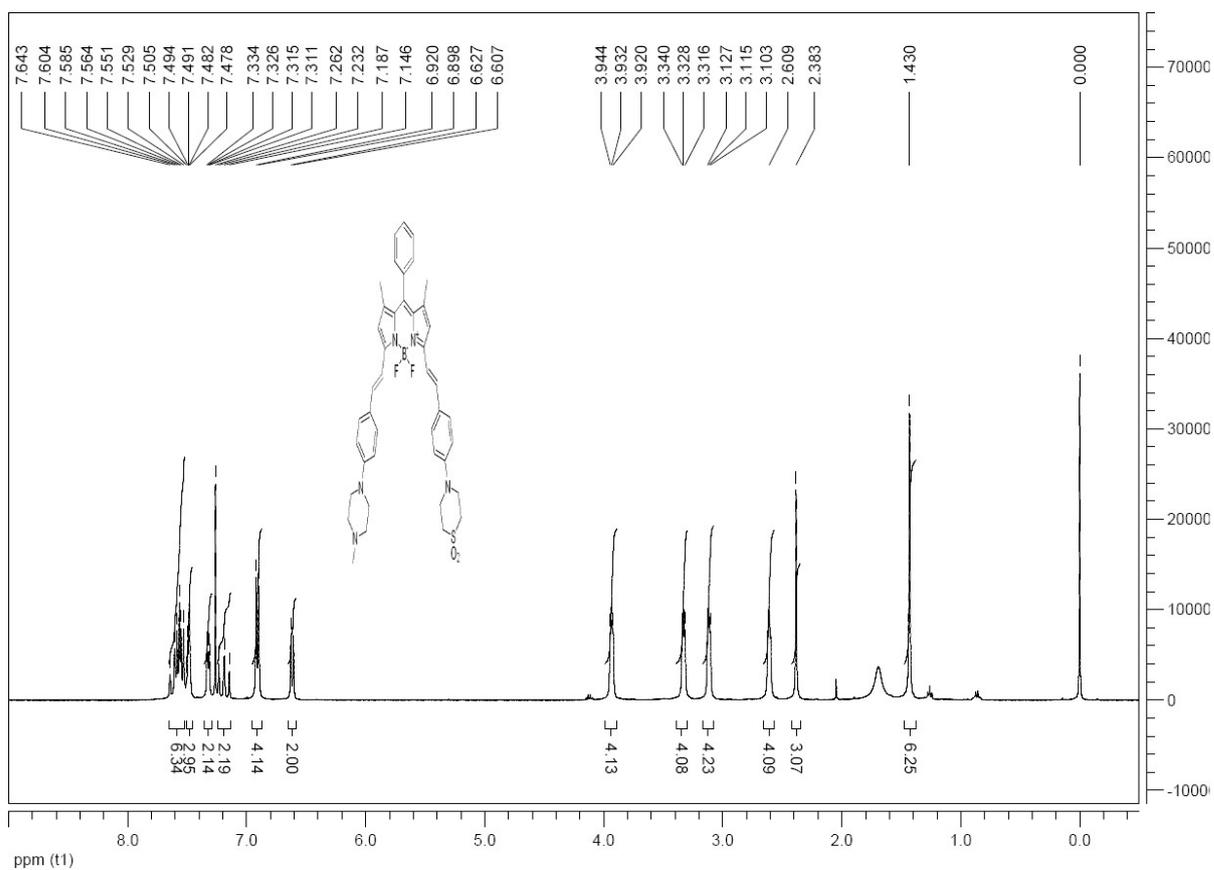


¹³C NMR spectra of compound **2** in CDCl₃.

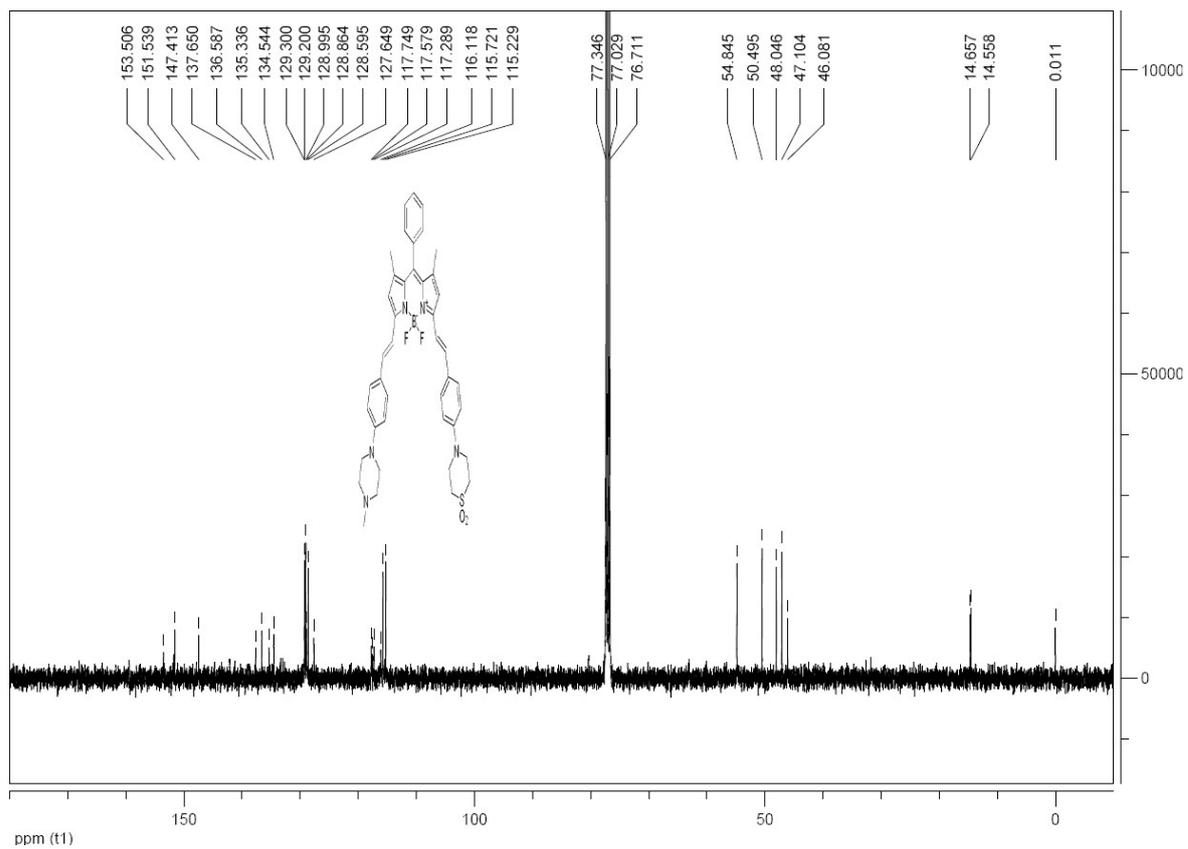
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T: FTMS + p ESI Full ms [150.0000-1600.0000]



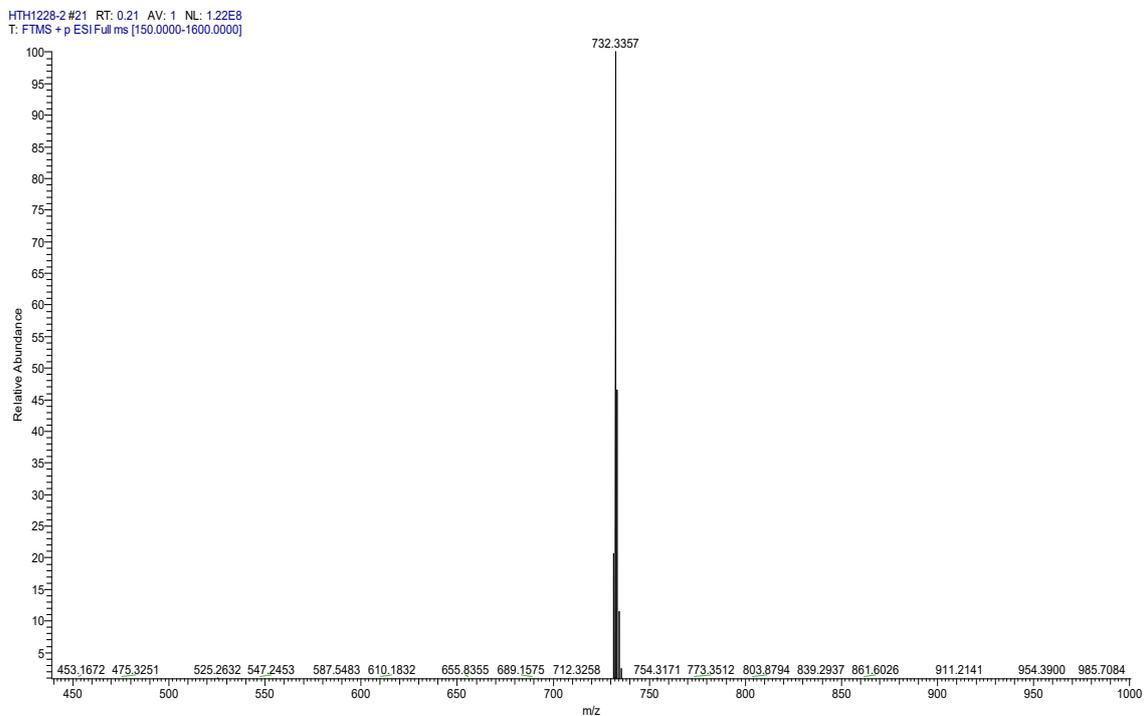
HRMS spectra of compound 2.



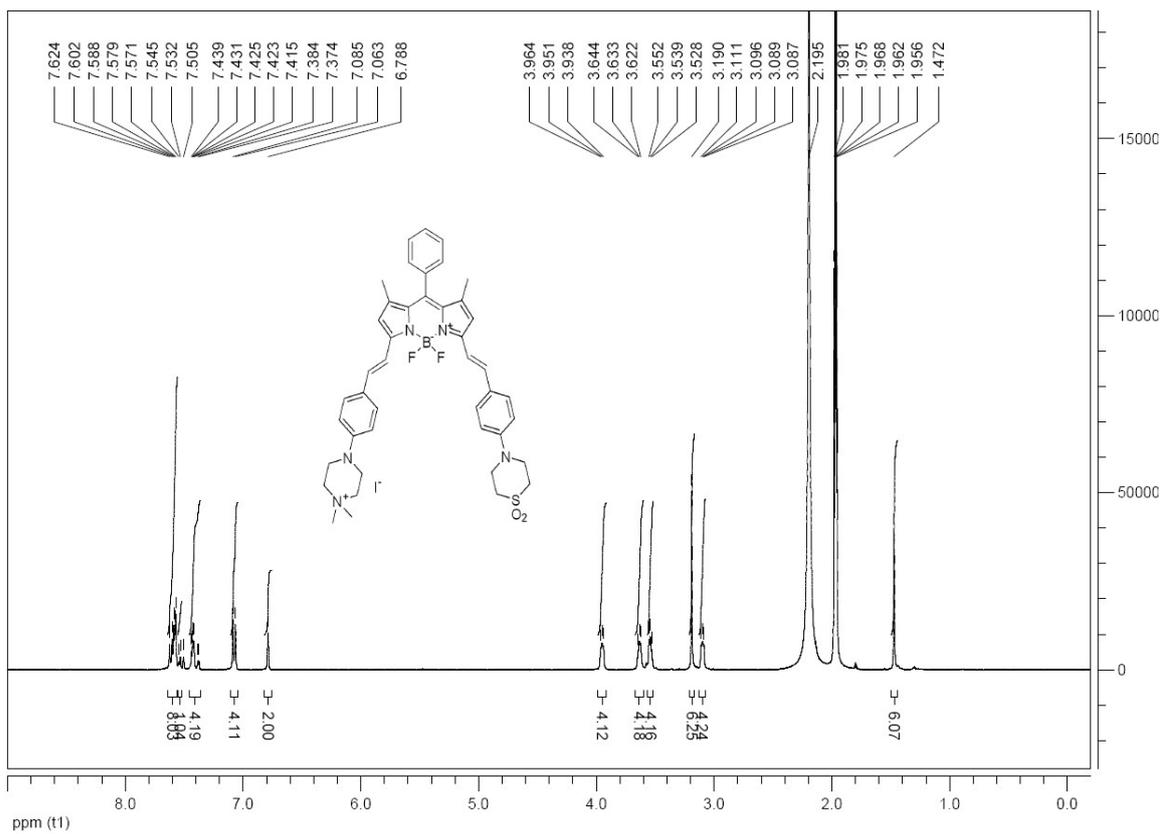
¹H NMR spectra of compound 3 in CDCl₃.



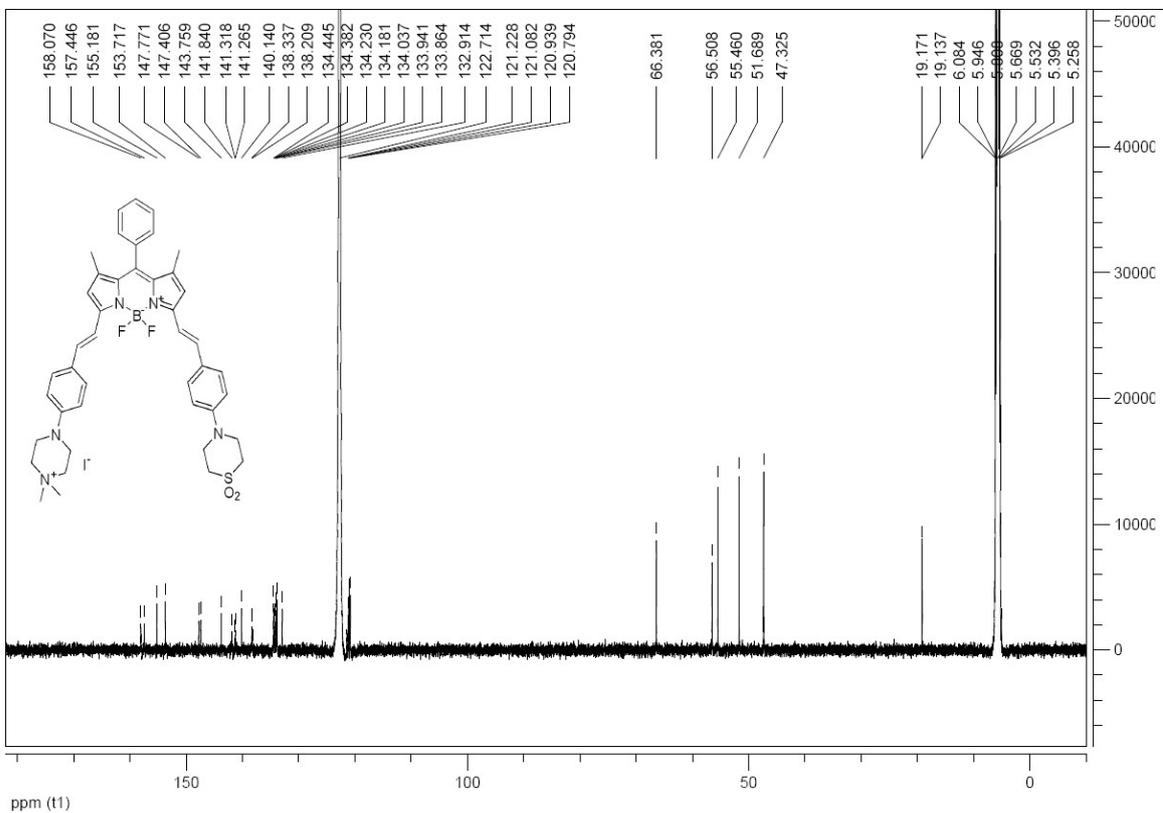
¹³C NMR spectra of compound **3** in CDCl₃.



HRMS spectra of compound **3**.

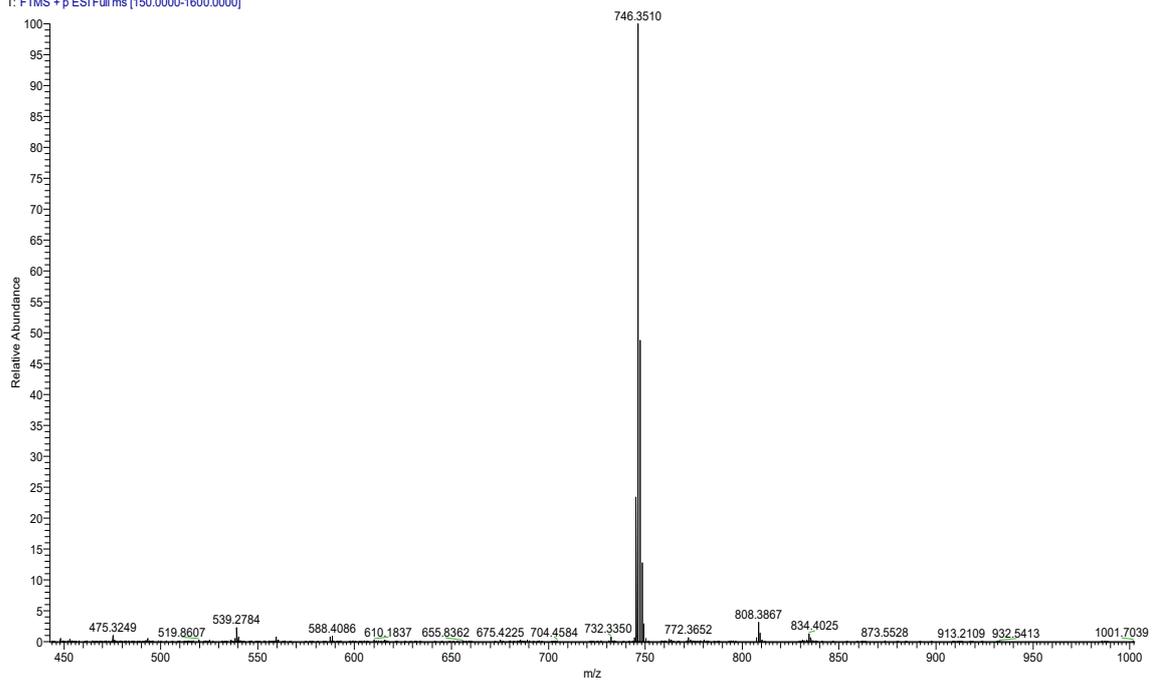


¹H NMR spectra of BDP2 in CD₃CN.

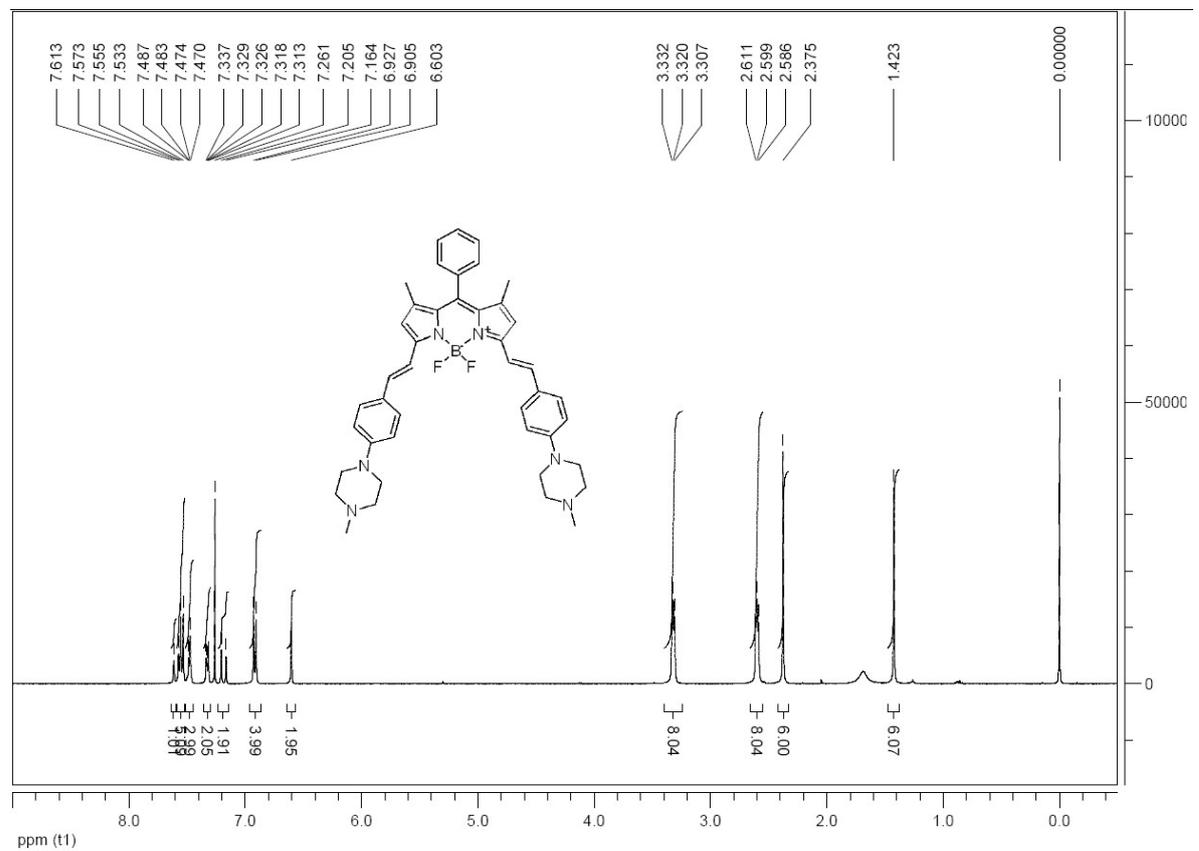


¹³C NMR spectra of BDP2 in CD₃CN.

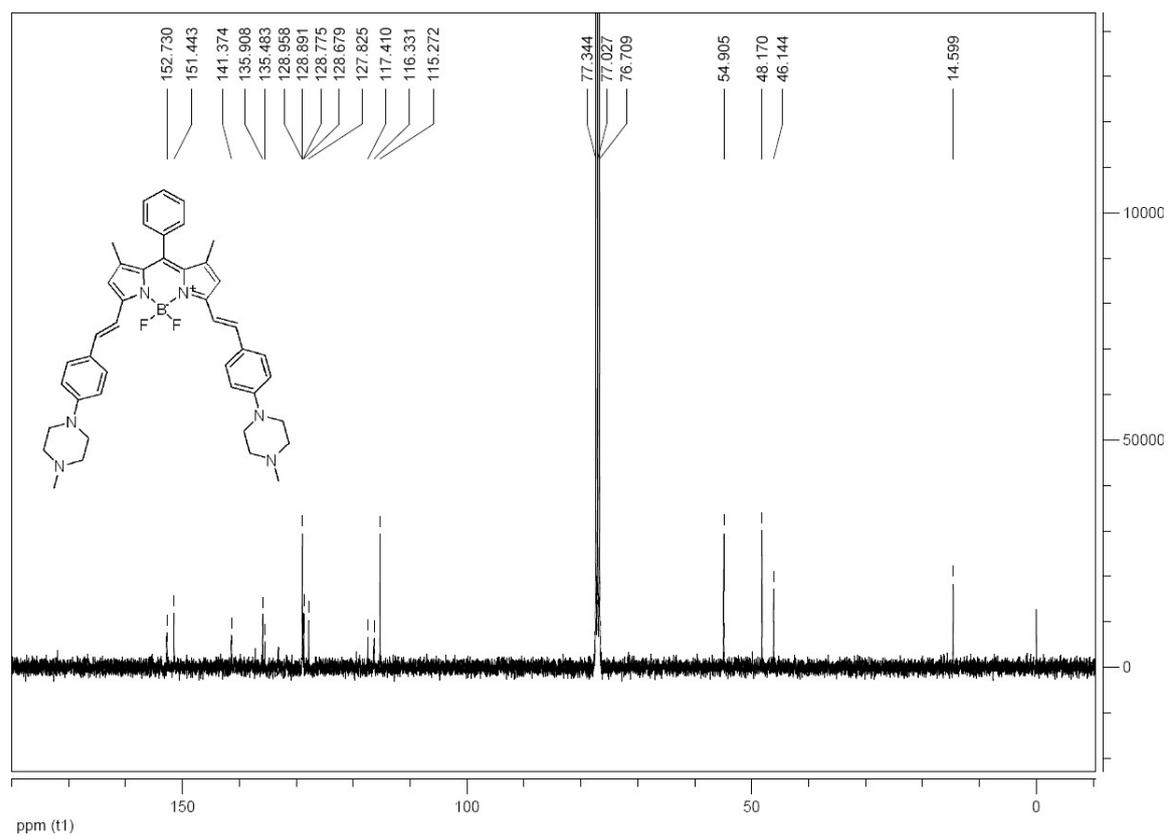
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T: FTMS + p ESI/Full ms [150.0000-1600.0000]



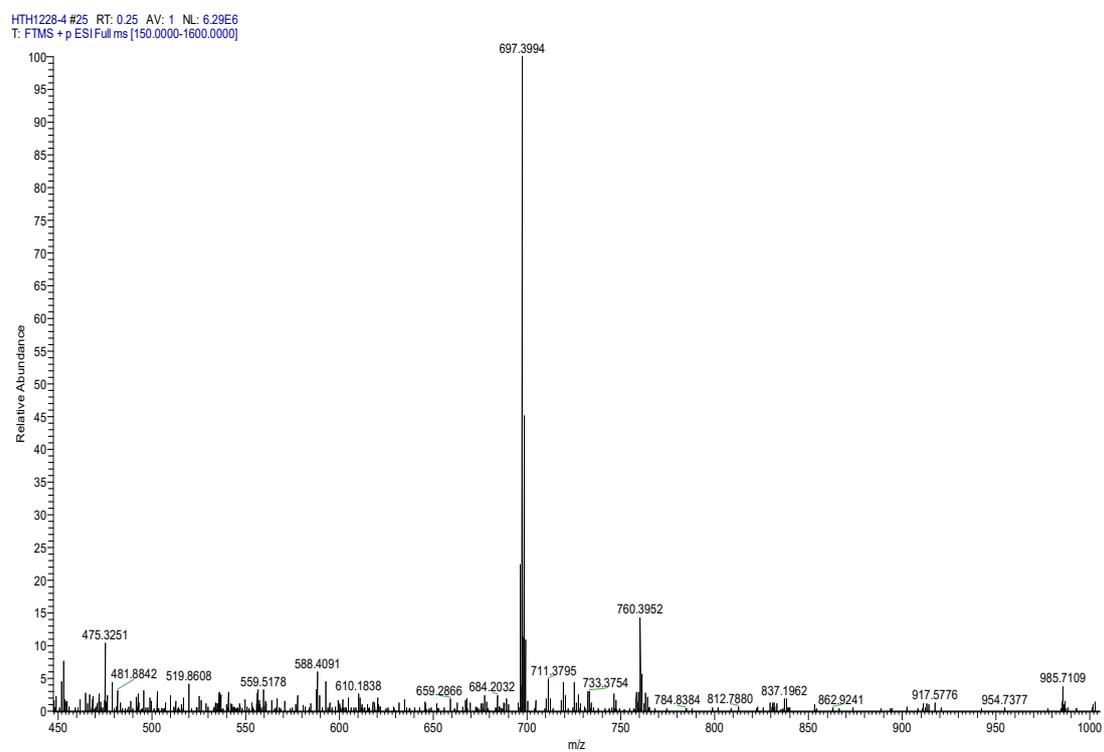
HRMS spectra of BDP2.



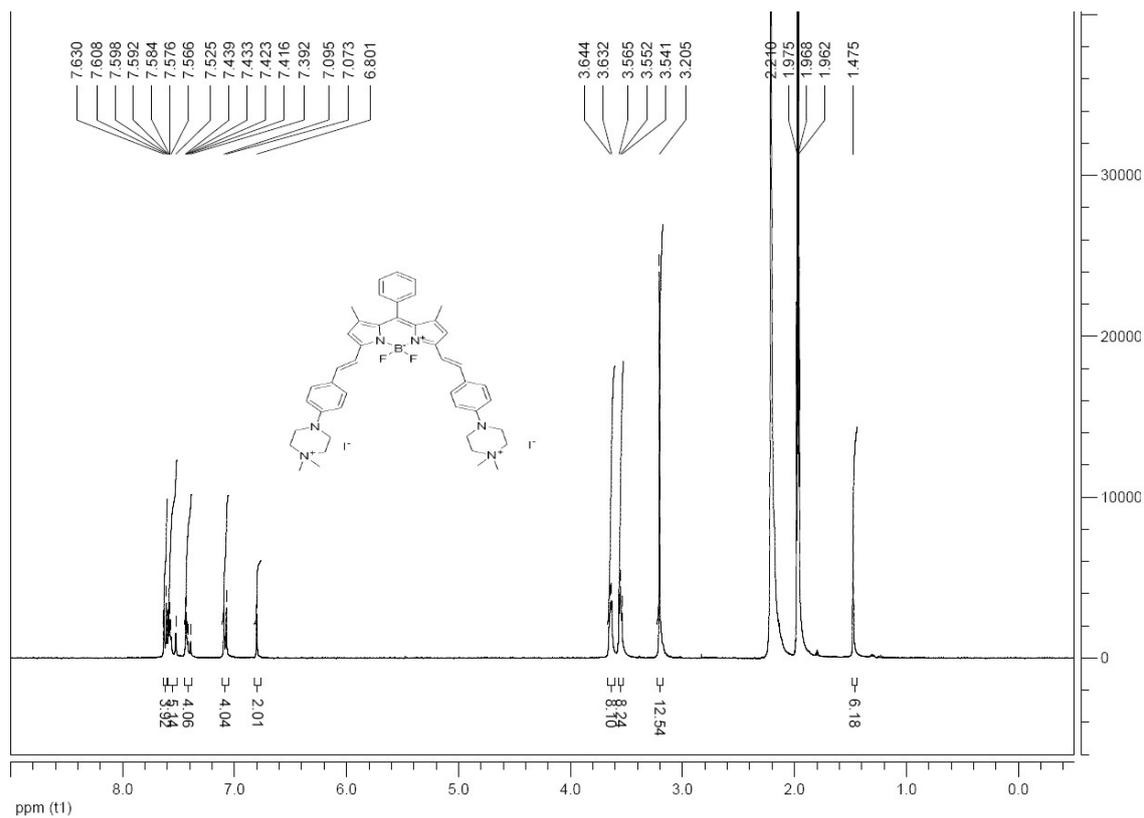
¹H NMR spectra of compound 4 in CDCl₃.



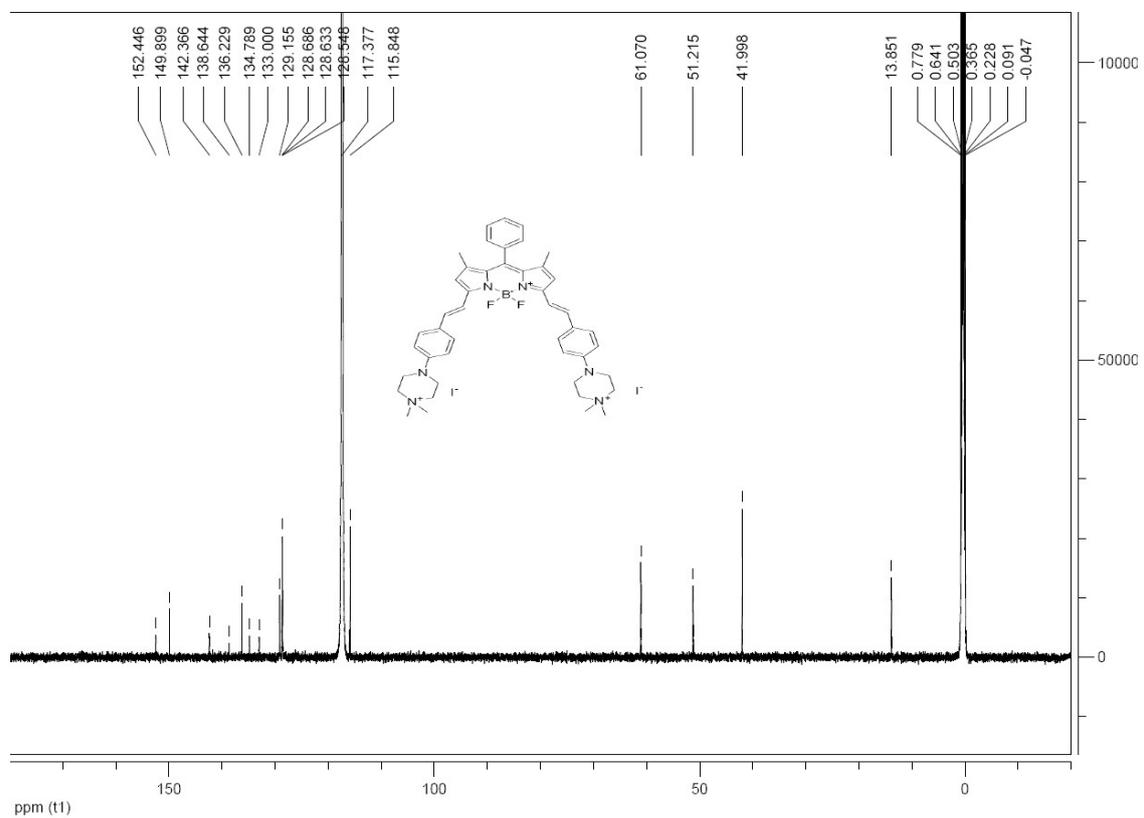
¹³C NMR spectra of compound 4 in CDCl₃.



HRMS spectra of compound 4.

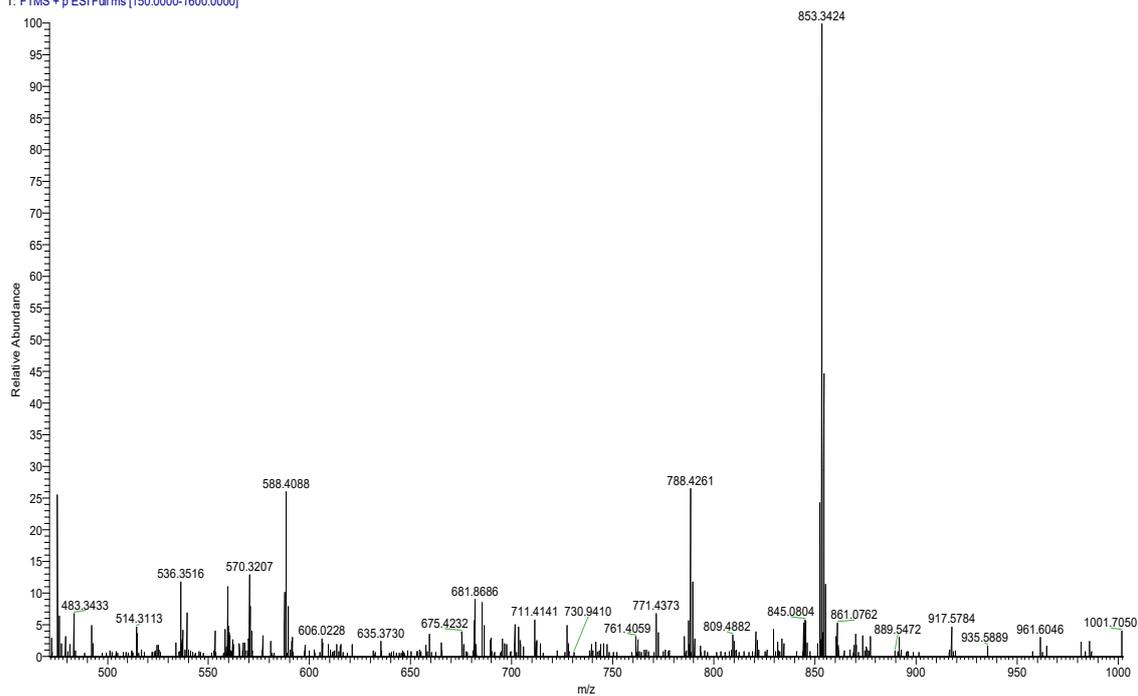


¹H NMR spectra of BDP3 in CD₃CN.

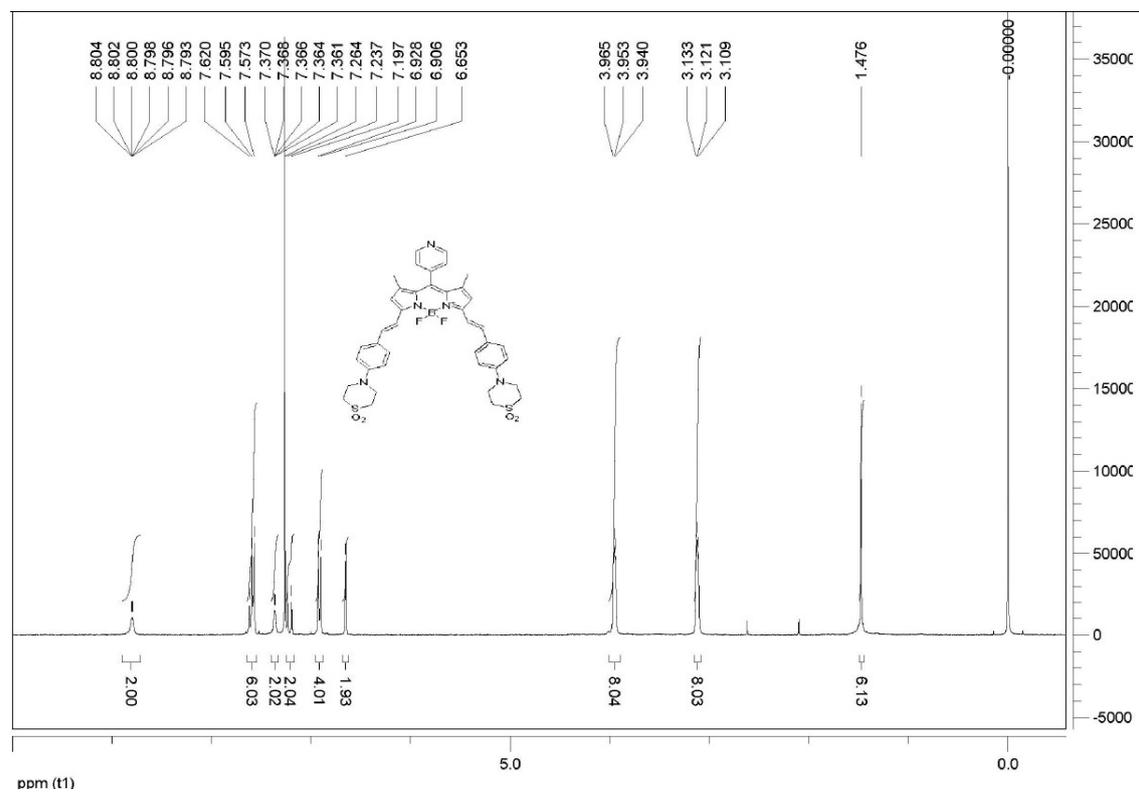


¹³C NMR spectra of BDP3 in CD₃CN.

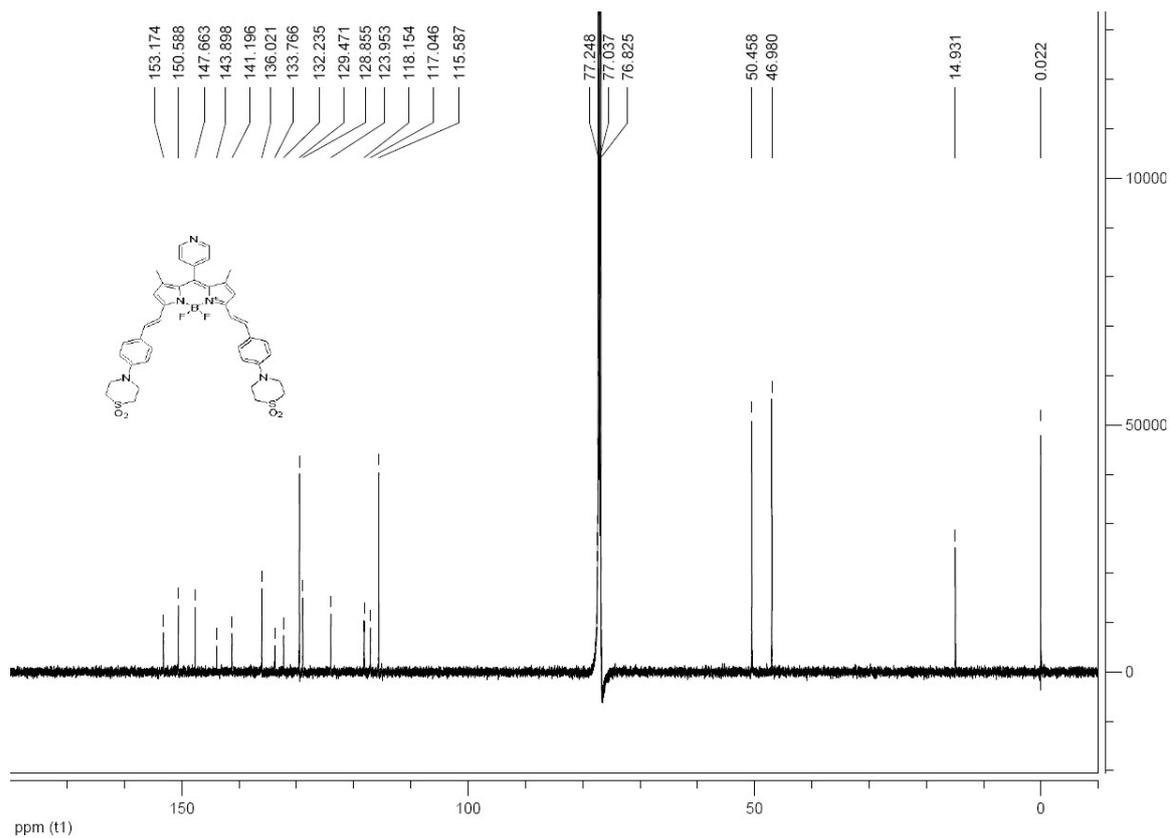
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T: FTMS + p ESI Full ms [150.0000-1600.0000]



HRMS spectra of **BDP3**.

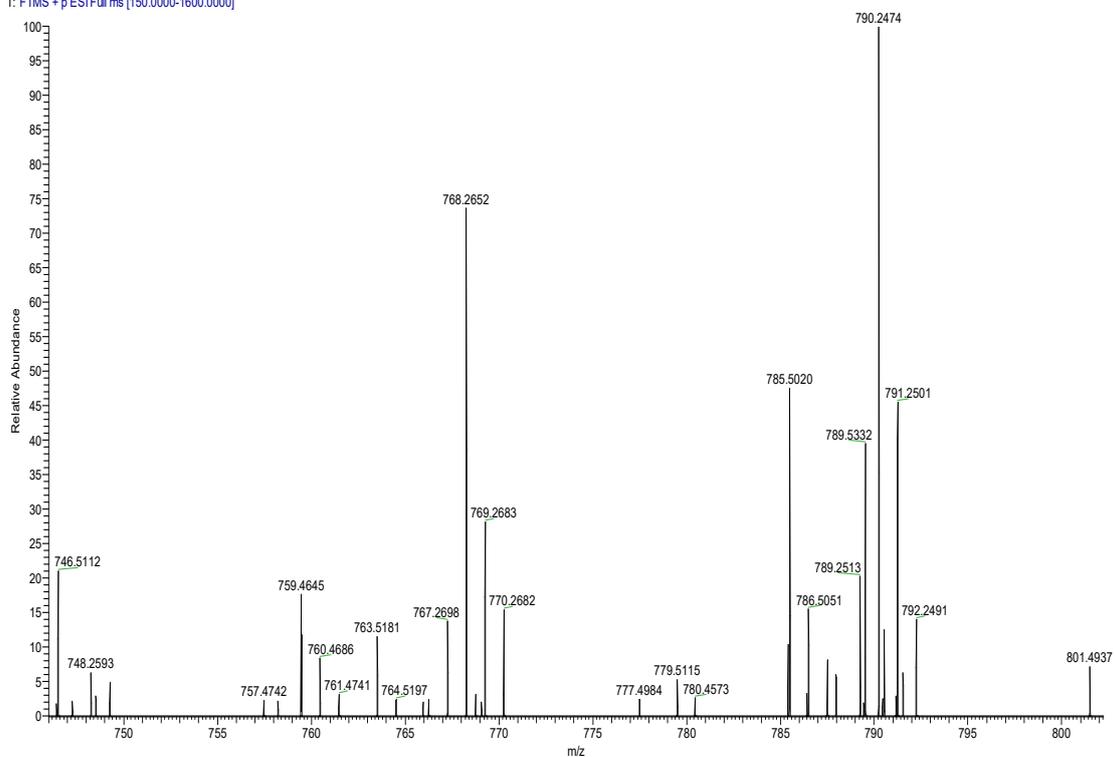


¹H NMR spectra of **BDP4** in CDCl₃.

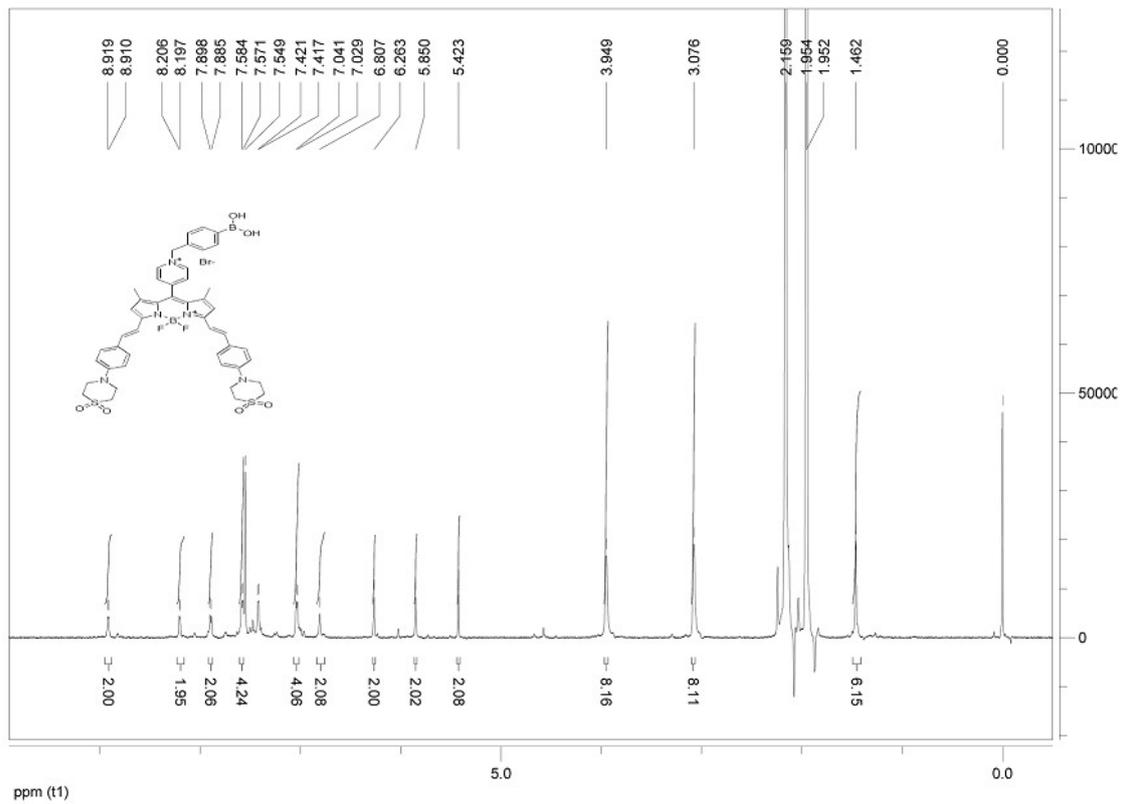


¹³C NMR spectra of **BDP4** in CDCl₃.

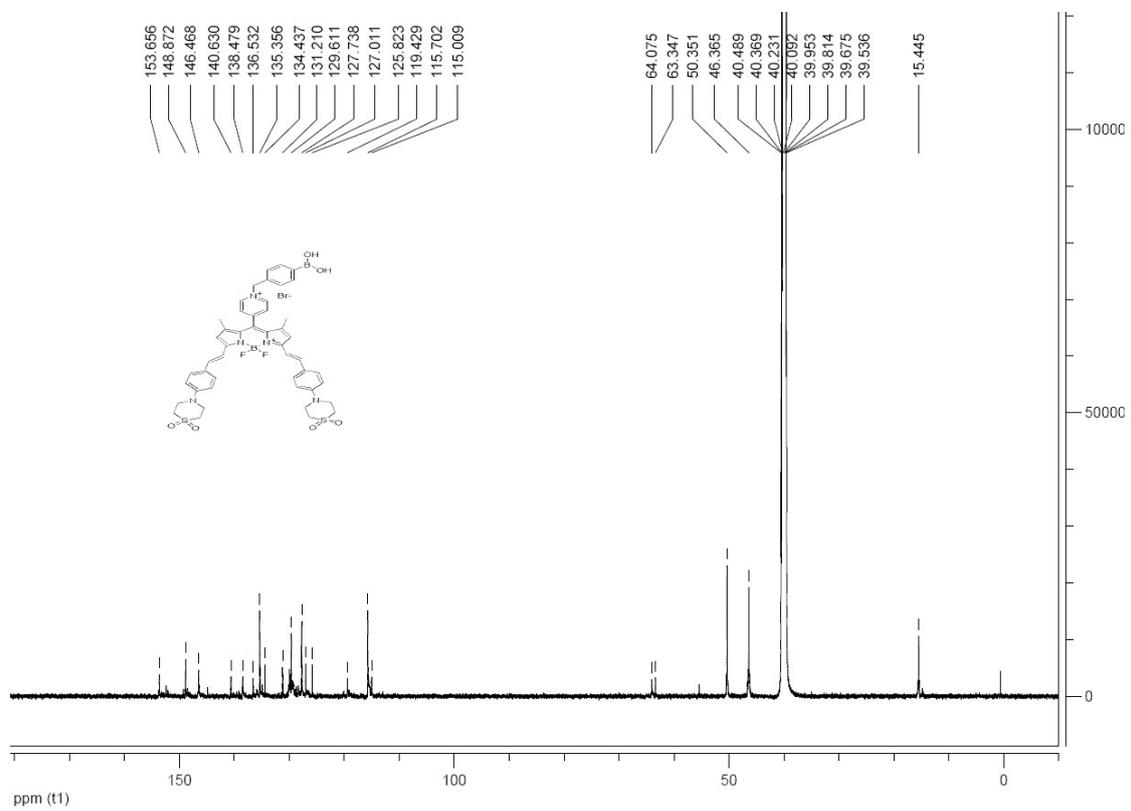
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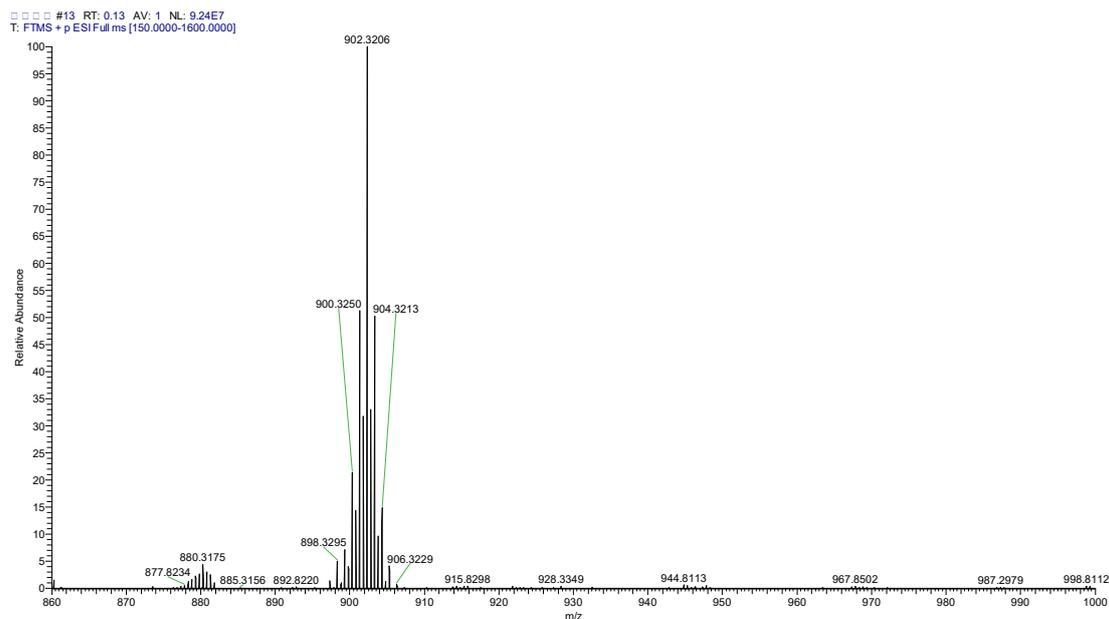
HRMS spectra of **BDP4**.



^1H NMR spectra of **FP-BDP4** in CD_3CN .



^{13}C NMR spectra of **FP-BDP4** in $\text{DMSO}-d_6$.



HRMS spectra of **FP-BDP4**.

5. Reference

- [1] R. M. Uppu, W. A. Pryor, Synthesis of peroxyxynitrite in a two-phase system using isoamyl nitrite and hydrogen peroxide, *Anal. Biochem.* 1996, **236**, 242-249.
- [2] X. Wang, Shu, Yu, C. Wang, D. Xue, J. Xiao, BODIPY catalyzed amide synthesis promoted by BHT and air under visible light, *Org. Biomol. Chem.* 2016, **14**(29), 7028-7037.
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- [4] T. Ueno, Y. Urano, K. Setsukinai, H. Takakusa, H. Kojima, K. Kikuchi, K. Ohkubo, S. Fukuzumi, and T. Nagano, Rational Principles for Modulating Fluorescence Properties of Fluorescein, *J. Am. Chem. Soc.* 2004, **126**, 14079-14085.