Supporting Information

Three Asymmetric BODIPY Derivatives as Fluorescent Probes for Highly Selective and Sensitive Detection of Cysteine in Living Cells

Jinlei Yue,‡[a] Nannan Wang,‡[a] Jiamin Wang,*[ac] Yuanfang Tao,[a] Han Wang,[a] Jinying Liu,[a] Jian Zhang,[a] Junrong Jiao*[a] and Weili Zhao*[ab]

[a] Key Laboratory for Special Functional Materials of Ministry of Education, School of Materials Science and Engineering
Henan University
Kaifeng 475004, P. R. China.

[b] School of Pharmacy, Institutes of Integrative Medicine
Fudan University
Shanghai, 201203, P. R. China.

[c] Key Laboratory of Natural Medicine and Immuno-Engineering of Henan Province, Henan University, Kaifeng 475004, P. R. China

*Corresponding Author

‡ These authors contributed equally.

E-mail:
jmwang@henu.edu.cn; jiaojr@vip.henu.edu.cn; zhaow@henu.edu.cn.

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1. General information

All chemicals and reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by dried and distilled from drying agents under an inert atmosphere. Double distilled water was utilized in all experiments. The NMR spectra were recorded on a Bruker AVANCE III HD 300 and 400 MHz spectrometer, using $SiMe_4$ as an internal standard. ^1H NMR data of chemical shifts (δ) were given in ppm (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet) using $CDCl_3$ (δ= 7.26 ppm) as reference. ^{13}C NMR data of chemical shifts (δ) were reported in ppm with $CDCl_3$ at δ 77.0 as internal standard. The HRMS were measured on the AB Sciex TripleTOF® 4600 high-resolution mass spectrometer and the MS were measured on the AmaZon SL mass spectrometer. The fluorescence spectra were measured using F97pro fluorescence spectrophotometer. The absorption spectra were measured using UV-1920 UV-vis spectrophotometer.

Preparation for Cys test. A stock solution of **BDP-S-Ph/BDP-S-ENE/BDP-S-R** (1 mM) was prepared in 100% CH_3CN and was subsequently diluted to prepare appropriate concentration solutions of the three probes in PBS buffer (10 mM, pH 7.4, containing 40% CH_3CN), respectively. Cys stock solutions were freshly prepared prior to each experiment.

Determination of the fluorescence quantum yield. Relative fluorescence quantum yields were determined by using rhodamine B (0.65 in EtOH) as the standard substance.[1] The quantum yield was calculated according to the formula:

$$\Phi_X = \Phi_S \frac{A_S \times F_X}{A_X \times F_S} \times \left(\frac{\eta_X}{\eta_S}\right)^2$$

where Φ is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the corresponding relative integrated fluorescence intensities, and η is the refractive index of the solvents used. Subscripts S and X refer to the standard and the unknown, respectively.

Detection limit. The detection limit was calculated based on the fluorescence titration. In the absence of Cys, the fluorescence emission spectrum of **BDP-S-**

Ph/BDP-S-ENE/BDP-S-R was measured by eight times and the standard deviation of blank measurement was achieved. To gain the slop, the fluorescence intensity at 539/558/561 nm was plotted to the concentration of Cys. The detection limit was calculated with the following equation:

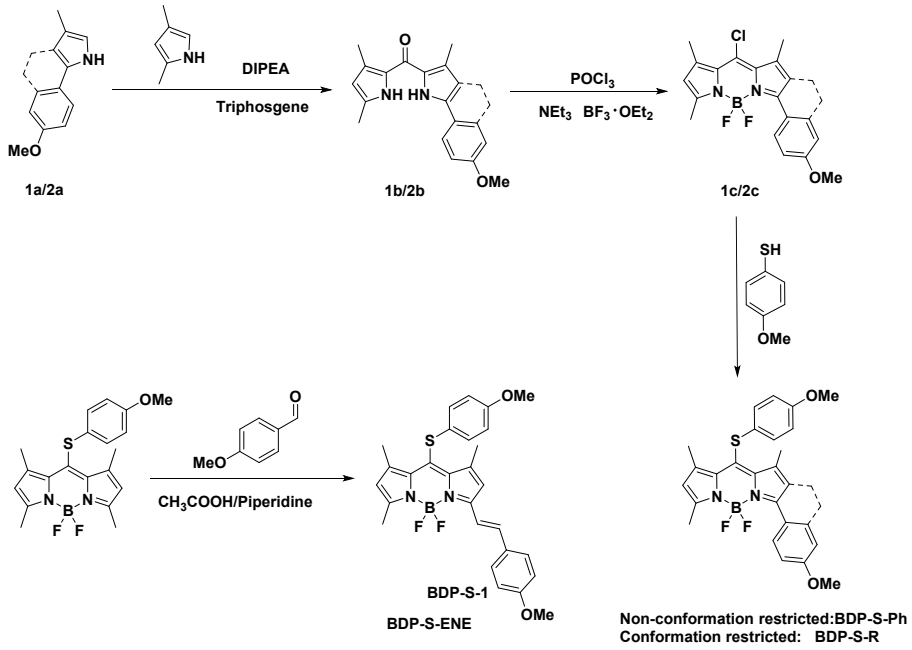
$$\text{Detection limit} = 3\sigma/k$$

Where σ is the standard deviation of blank measurement, k is the slop between the fluorescence intensity versus Cys concentration.

CCK-8 assay experiment. The cell viability was measured using the CCK-8 assay. Briefly, 5×10^3 Hela cells were incubated with different concentrations of probes in triplicate in a 96-well plate for 24 h at 37 °C in a final volume of 100 μL. The CCK-8 solution (10 μL) was added to each well and incubated with the cells for another 1 h. After thorough mixing, the absorbance was measured at 450 nm using a microplate reader. Each result was the average of three wells, and 100% viability was determined from untreated cells.

Cell Culture and Living Cell Imaging. The cells were imaged by using a Leica TCS SP8 + STED Laser Scanning Confocal Microscope. HeLa cells were incubated in DMEM with 10% (v/v) fetal bovine serum, 1% penicillin/streptomycin at 37 °C in 5% CO_2. HeLa cells were seeded at a density of 5×10^4 cells per well (200 μL) in a 6-well plate. Probes (1 mM, 10 μL) in DMSO were added to 1.0 mL of DMEM and incubated the cells at 37 °C for 30 min. Next, the cells were washed with PBS three times, the fluorescence images of cells were taken. Meanwhile, cells were pretreated with 200 μM NEM for 30 min under the same conditions, incubated with **BDP-S-Ph/BDP-S-ENE/BDP-S-R** (10 μM) for 30 min and then washed three times with PBS buffer for confocal imaging. Another group of the cells were pretreated with 200 μM NEM for 30 min under the same conditions, and treated with with 200 μM Cys for 30 min, then incubated with 10 μM **BDP-S-Ph/BDP-S-ENE/BDP-S-R** probes for 30 min and washed three times with PBS buffer for confocal imaging. The images were obtained with the emission in the range of 525-577 nm using 514 nm excitation filter; 585-680 nm using 561 nm excitation filter.

2. Synthesis



Scheme S1. Synthetic routes of **BDP-S-Ph**, **BDP-S-R** and **BDP-ENE**.

Synthesis of 1c:

In 100 mL flask under nitrogen, 2,4-dimethylpyrrole (0.44 mL, 4 mmol) dissolved in 1,2-dichloroethane (10 mL), N-ethyldiisopropylamine (0.64 mL, 4 mmol) was added, and then triphosgene (400 mg, 1.32 mmol) was slowly added at 0 °C, and then 1a (748 mg, 4 mmol) was added, the reaction mixture stirred for 1 h at 0 °C. The mixture was diluted with CH_2Cl_2, washed with saturated brine, dried over sodium sulfate. The organic layer was concentrated under reduced pressure and purification was carried out by silica gel column chromatography (petroleum ether/ethyl acetate, 6: 1, v/v) to yield **1b** of a pale-yellow solid (300 mg, 24.2%). To a solution of **1b** (308 mg, 1 mmol) in 1,2-dichloroethane (10 mL), phosphorus oxychloride (0.36 mL, 4 mmol) was added at 0 °C under nitrogen, overnight reaction; then cooled in an ice bath. triethylamine (0.84 mL, 6 mmol) was added, and the reaction was stirred at 0 °C for 10 min. Boron trifluoride etherate (1.02 mL, 8 mmol) was added dropwise into the mixture while maintaining the temperature at 0 °C. The reaction mixture was allowed to warm up to room temperature and stirred for additional 3 h. The mixture was diluted with CH_2Cl_2, washed with saturated brine, dried over sodium sulfate. The organic layer was concentrated under reduced pressure and purification was carried

out by silica gel chromatography (petroleum ether/CH_2Cl_2, 3: 1, v/v) to give **1c** as red crystalline solid (98 mg, 26%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (d, *J* = 8.9 Hz, 2H), 6.97 (d, *J* = 8.9 Hz, 2H), 6.40 (s, 1H), 6.12 (s, 1H), 3.86 (s, 3H), 2.54 (s, 3H), 2.49 (s, 3H), 2.48 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.64, 156.15, 155.17, 143.17, 142.22, 136.62, 130.97, 130.91, 130.86, 130.13, 124.86, 122.20, 122.15, 113.68, 55.29, 17.13, 16.77, 14.82.

Synthesis of 2c:

In 100 mL flask under nitrogen, 2,4-dimethylpyrrole (0.55 mL, 5 mmol) dissolved in 1,2-dichloroethane (15 mL), N-ethyldiisopropylamine (0.8 mL, 5 mmol) was added, and then triphosgene (500 mg, 1.65 mmol) was slowly added at 0 °C, and then 2a (1.06 g, 5 mmol) was added, the reaction mixture stirred for 1 h at 0 °C. The mixture was diluted with CH_2Cl_2, washed with saturated brine, dried over sodium sulfate. The organic layer was concentrated under reduced pressure and purification was carried out by silica gel column chromatography (petroleum ether/ethyl acetate, 5: 1, v/v) to yield **2b** of a pure yellow solid (381 mg, 22.8%). To a solution of **2b** (334 mg, 1 mmol) in 1,2-dichloroethane (15 mL), phosphorus oxychloride (0.36 mL, 4 mmol) was added at 0 °C under nitrogen, overnight reaction; then cooled in an ice bath, triethylamine (0.84 mL, 6 mmol) was added, and the reaction was stirred at 0 °C for 10 min. Boron trifluoride etherate (1.02 mL, 8 mmol) was added dropwise into the mixture while maintaining the temperature at 0 °C. The reaction mixture was allowed to warm up to room temperature and stirred for additional 3 h. The mixture was diluted with CH_2Cl_2, washed with saturated brine, dried over sodium sulfate. The organic layer was concentrated under reduced pressure and purification was carried out by silica gel chromatography (petroleum ether/CH_2Cl_2, 2: 1, v/v) to yield **2c** of a purple solid (113 mg, 28%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (d, *J* = 9.2 Hz, 1H), 6.92 (dd, *J* = 8.9, 2.7 Hz, 1H), 6.80 (d, *J* = 2.6 Hz, 1H), 6.08 (s, 1H), 3.86 (s, 3H), 2.88 (t, *J* = 7.0 Hz, 2H), 2.68–2.60 (t, 2H), 2.55 (s, 3H), 2.47 (s, 3H), 2.45 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.05, 153.01, 151.55, 143.43, 140.60, 136.67, 134.20, 132.06, 131.38, 130.67, 130.56, 130.44, 129.20, 121.01, 120.63, 114.17, 112.26, 55.29, 30.65, 20.36, 16.55, 14.59, 14.37.

3. Supplementary Figures

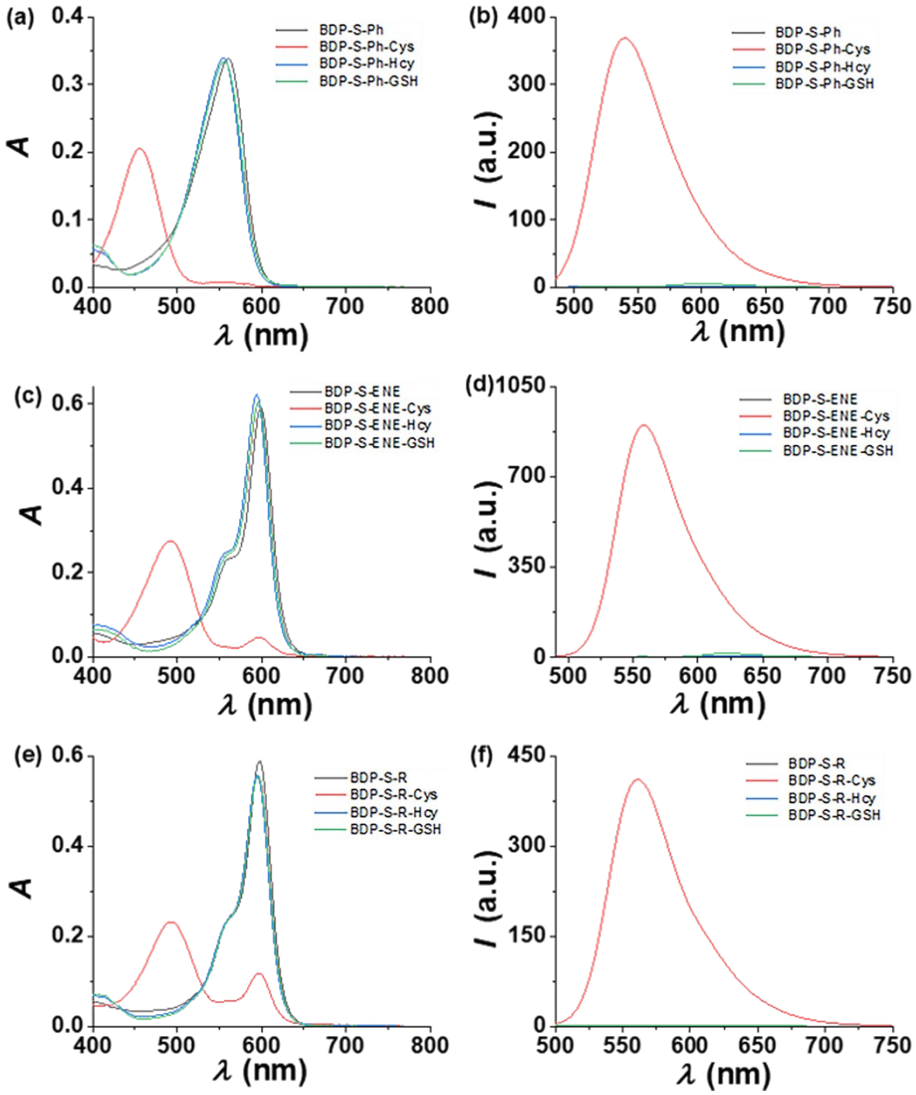
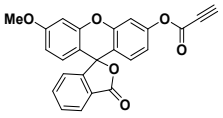
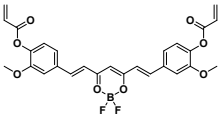
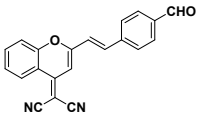
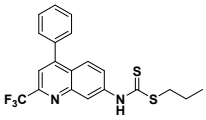
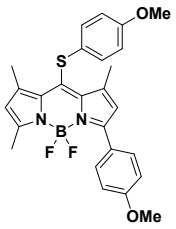
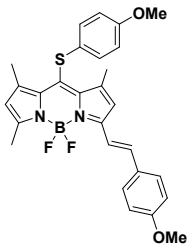
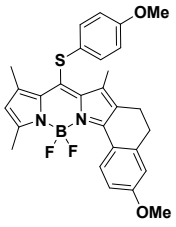


Fig. S1. Absorption and fluorescence spectra changes of 10 μM **BDP-S-Ph**, **BDP-S-ENE** and **BDP-S-R** upon treated with 100 μM Cys, Hcy and GSH, respectively. Conditions: 10 mM PBS buffer (pH 7.4, containing 40% CH_3CN, 37 °C).

Table S1 Performance comparison with other Cys sensors.

No.	Sensors	Buffer solution (v/v)	$\lambda_{ex}/\lambda_{em}$ (nm)	Φ	Response time (min)	LOD (nM)	Ref.
1		CH_3CN: PBS = 6:4	450/515	–	30	182	[55]
2		Ethanol: PBS = 4:1	510/615	0.024	80	1550	[56]
3		DMF: PBS = 4:1	350/440	0.1363	50	63	[57]
4		Ethanol: PBS = 3:7	270,350/ 515	–	30	105	[58]
5		CH_3CN: PBS = 4:6	455/539	0.1609	15	50	This work
6		CH_3CN: PBS = 4:6	491/558	0.4479	30	28	This work
7		CH_3CN: PBS = 4:6	493/561	0.2006	50	87	This work

The data in the table are all measured after the probe reacts with cysteine.

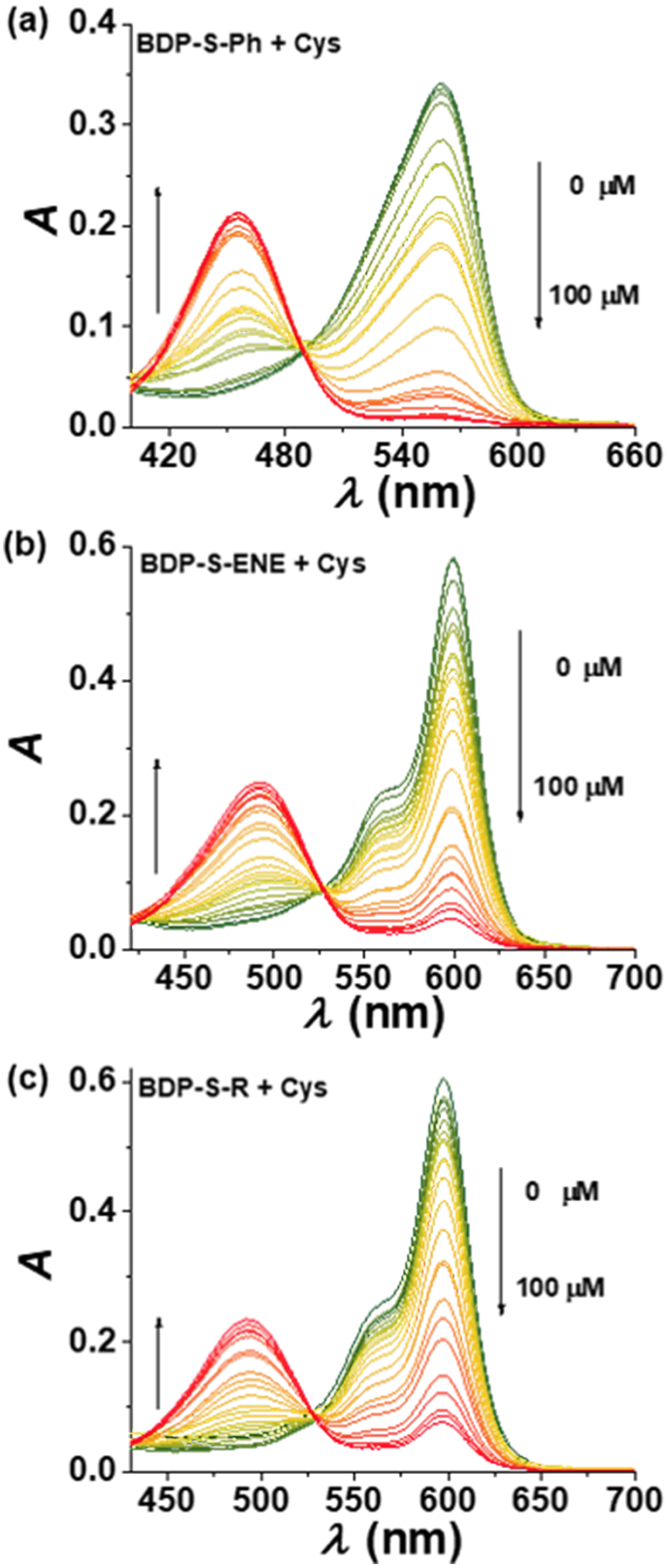


Fig. S2. Concentration-dependent absorption spectra of (a) **BDP-S-Ph** (10 μM) with 0–100 μM Cys for 15 min; (b) **BDP-S-ENE** (10 μM) with 0–100 μM Cys for 30 min; (c) **BDP-S-R** (10 μM) with 0–100 μM Cys for 50 min. Conditions: 10 mM PBS buffer (pH 7.4, containing 40% CH_3CN, 37 °C).

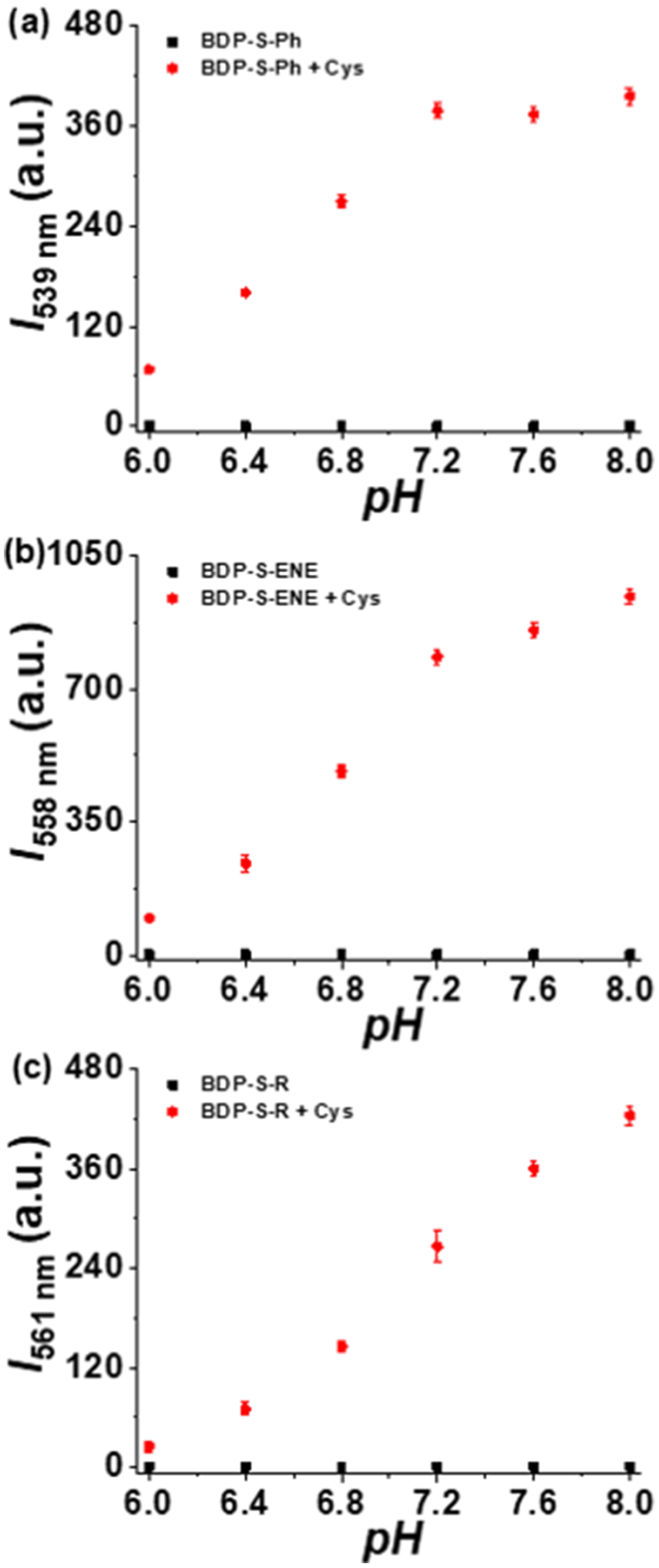


Fig. S3. Fluorescence intensities of 10 μM (a) **BDP-S-Ph**, (b) **BDP-S-ENE**, (c) **BDP-S-R** in the absence (black) and presence (red) of 10 equiv. of Cys at various pH values.

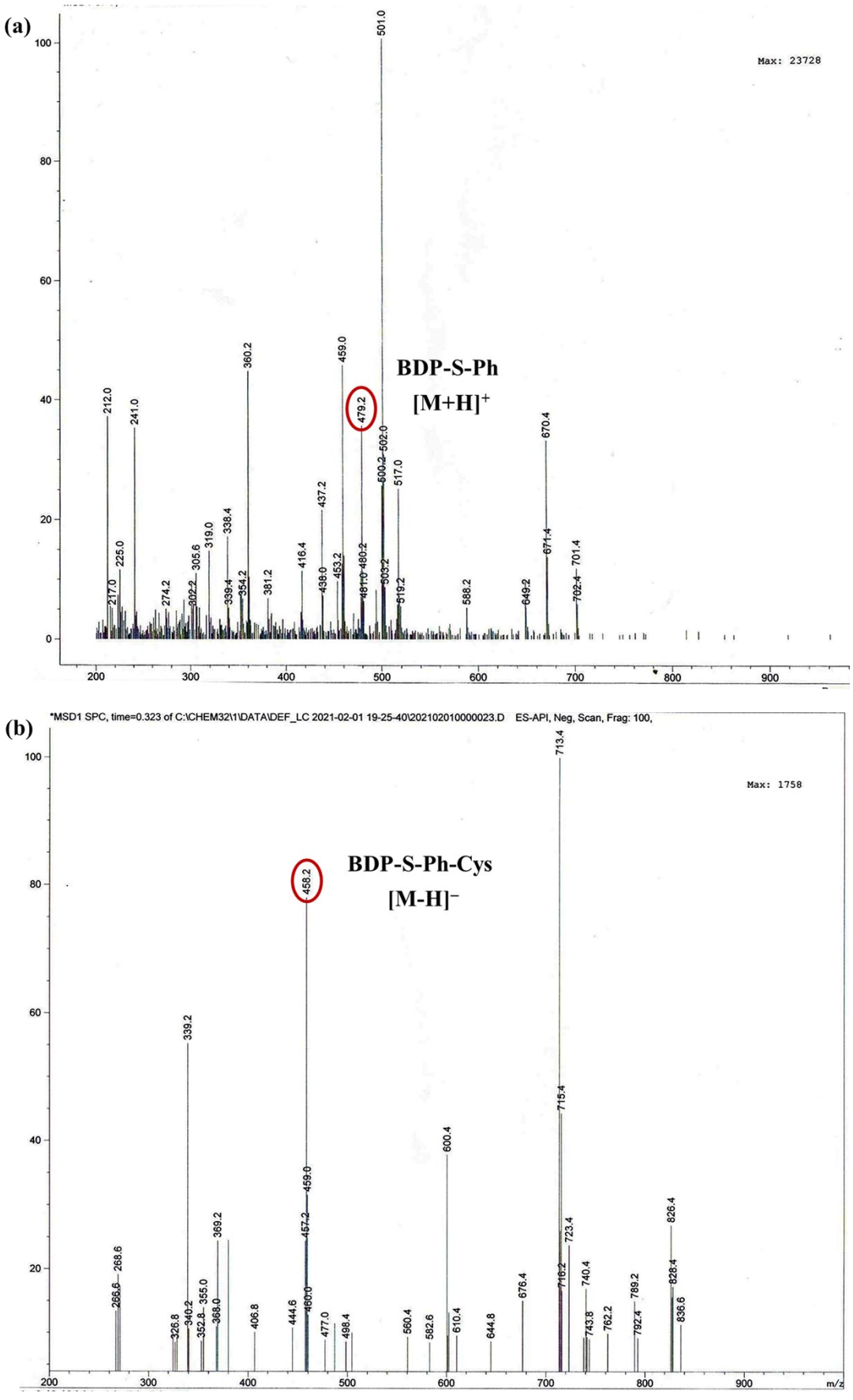


Fig. S4. The MS of **BDP-S-Ph** upon addition of Cys.

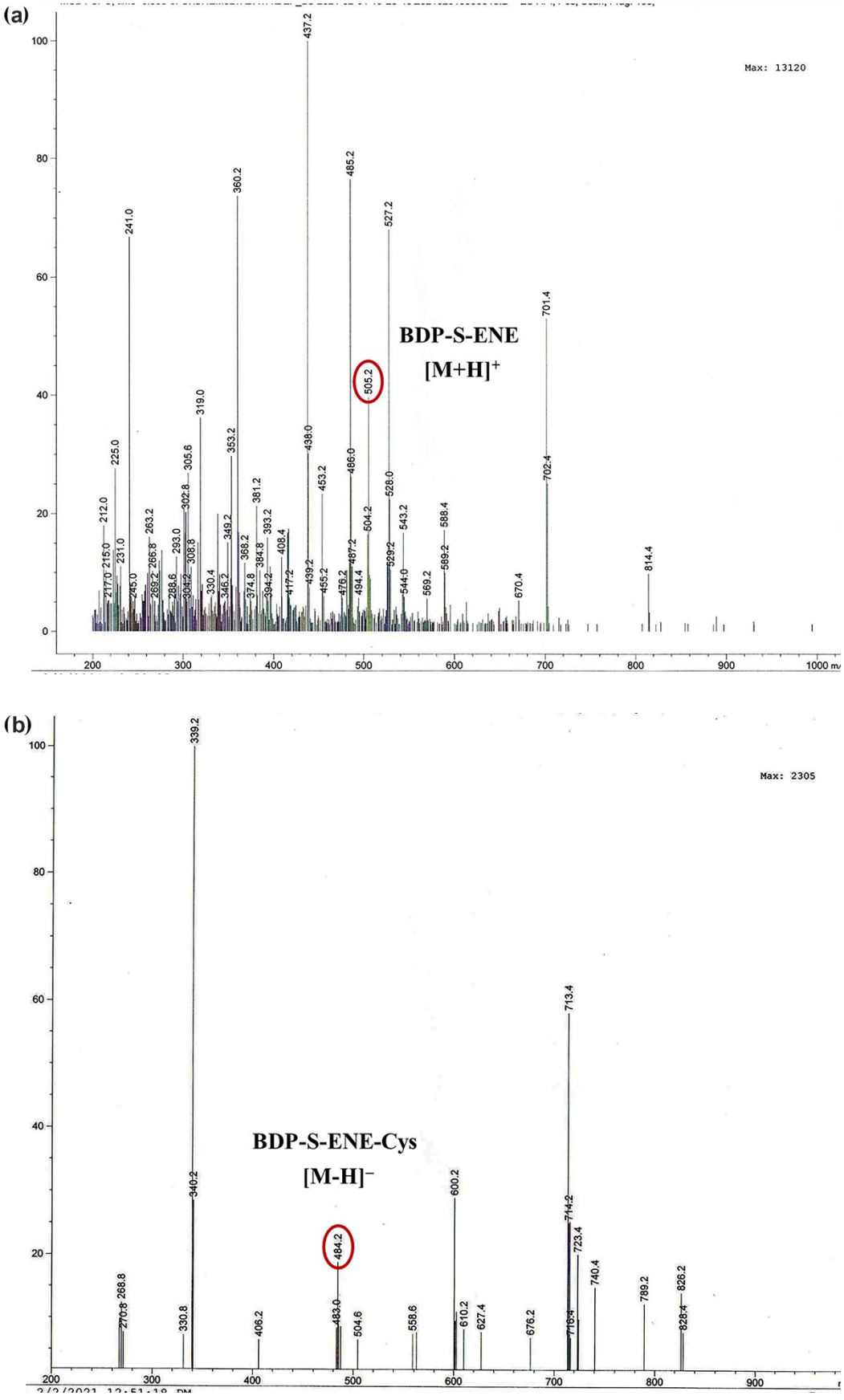


Fig. S5. The MS of **BDP-S-ENE** upon addition of Cys.

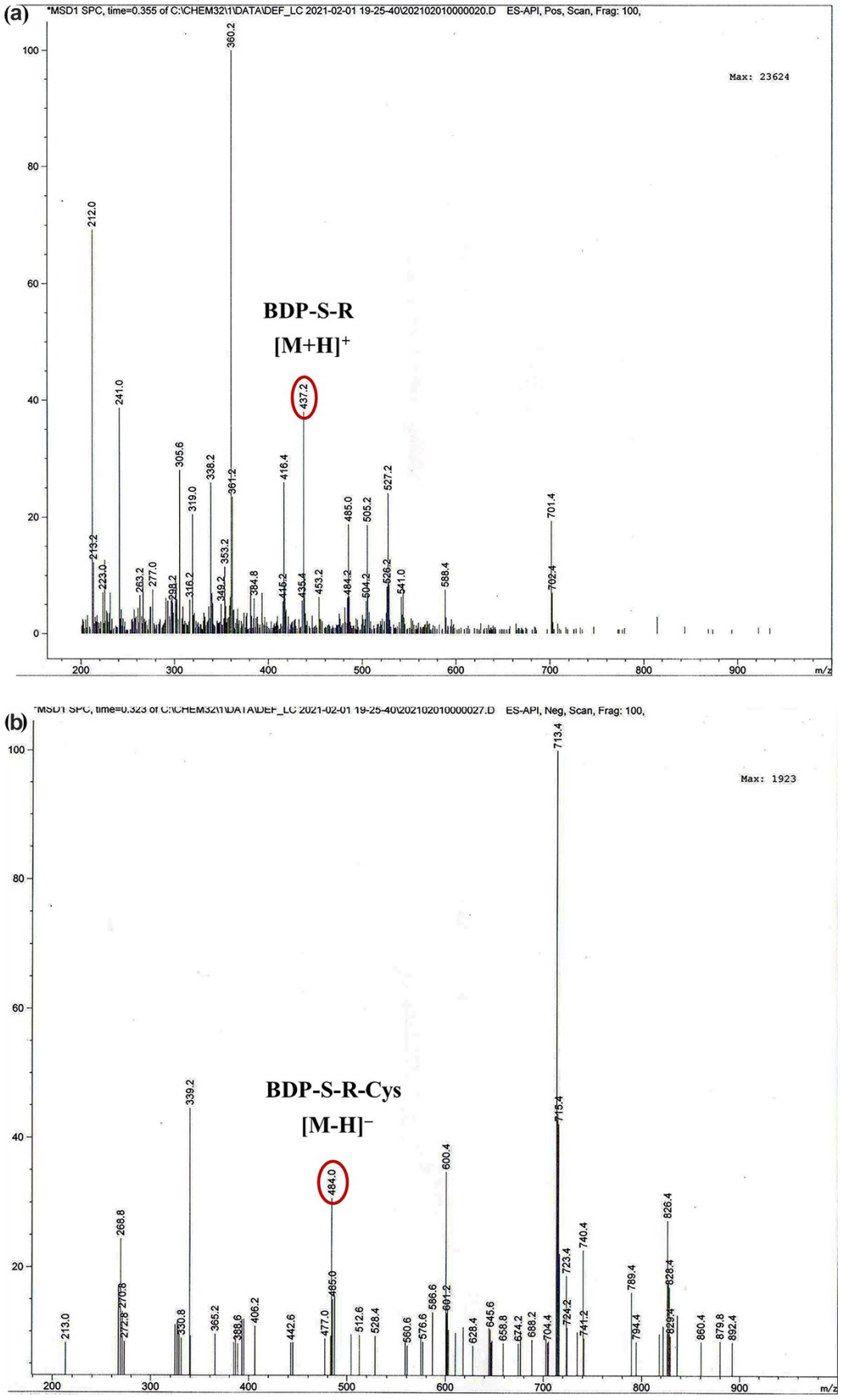


Fig. S6. The MS of **BDP-S-R** upon addition of Cys.

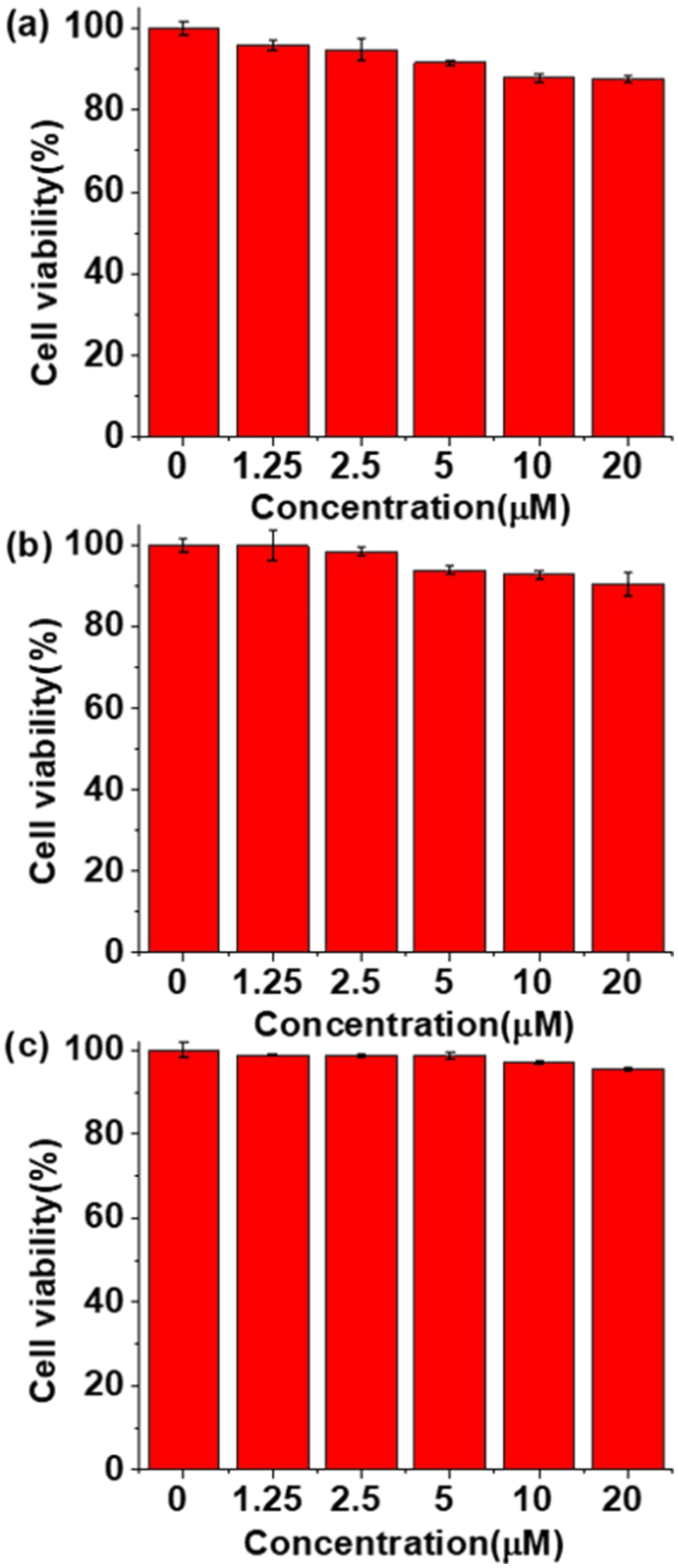


Fig. S7. Cell viability assay of (a) **BDP-S-Ph**, (b) **BDP-S-ENE** and (c) **BDP-S-R** in HeLa cells. The cells were incubated with the probes for 24 h, and the cell viability was observed via CKK-8 assays.

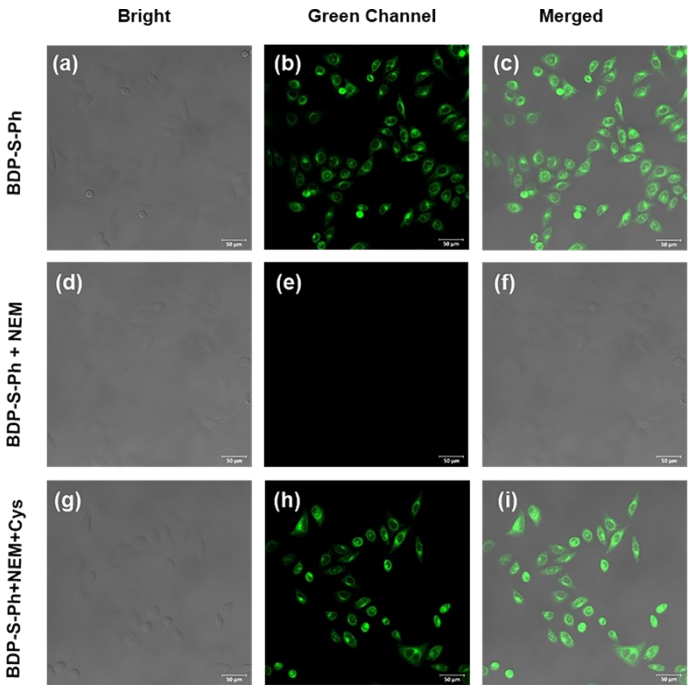
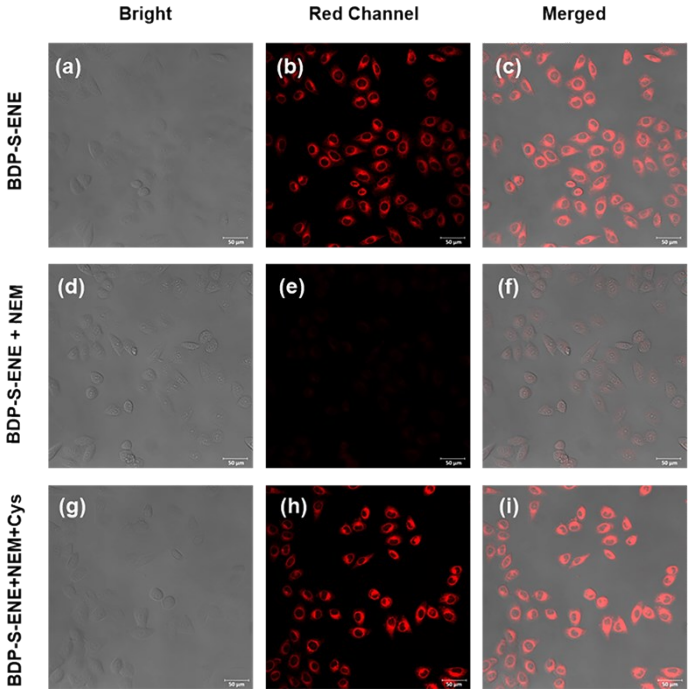


Fig. S8. Confocal fluorescence images. Cells were (a-c) incubated with **BDP-S-Ph** (10 μM, 30 min); (d-f) pretreated with NEM (200 μM, 30 min) followed by **BDP-S-Ph** (10 μM, 30 min); (g-i) preincubated with NEM (200 μM, 30 min), then incubated with Cys (200 μM, 30 min), further treated with **BDP-S-Ph** (10 μM, 30 min) (green channel: λ_{ex} = 514 nm, λ_{em} = 525-577 nm, scale bar: 50 μm).



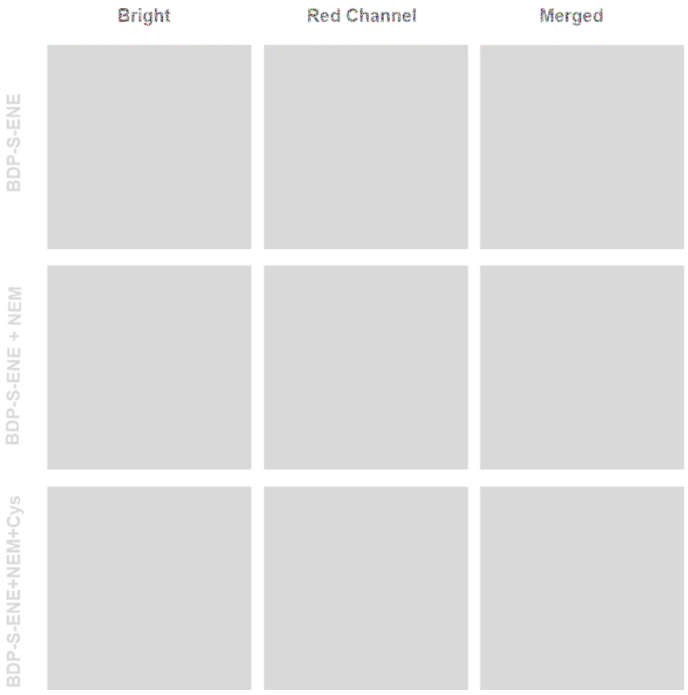


Fig. S9. Confocal fluorescence images. Cells were (a-c) incubated with **BDP-S-ENE** (10 μM, 30 min); (d-f) pretreated with NEM (200 μM, 30 min) followed by **BDP-S-ENE** (10 μM, 30 min); (g-i) preincubated with NEM (200 μM, 30 min), then incubated with Cys (200 μM, 30 min), further treated with **BDP-S-ENE** (10 μM, 30 min) (red channel: λ_{ex} = 561 nm, λ_{em} = 585-680 nm, scale bar: 50 μm).

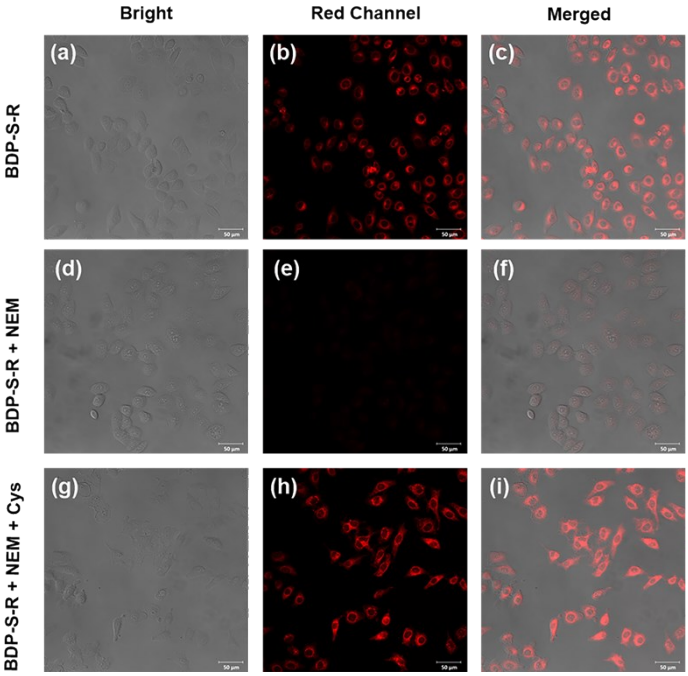


Fig. S10. Confocal fluorescence images. Cells were (a-c) incubated with **BDP-S-R** (10 μM, 30 min); (d-f) pretreated with NEM (200 μM, 30 min) followed by **BDP-S-R** (10 μM, 30 min); (g-i) preincubated with NEM (200 μM, 30 min), then incubated with Cys (200 μM, 30 min), further treated with **BDP-S-R** (10 μM, 30 min) (red channel: λ_{ex} = 561 nm, λ_{em} = 585-680 nm, scale bar: 50 μm).

4. NMR and HRMS data

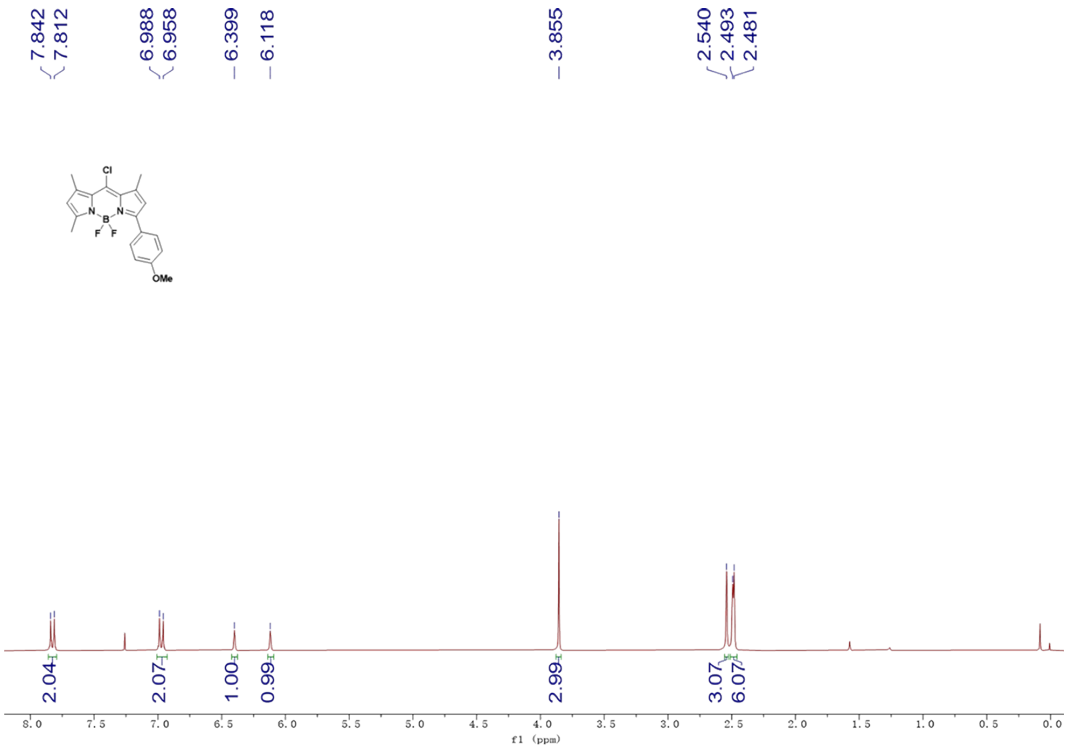


Fig. S11. ^{1}H NMR spectrum (300 MHz) of **1c** in $CDCl_3$.

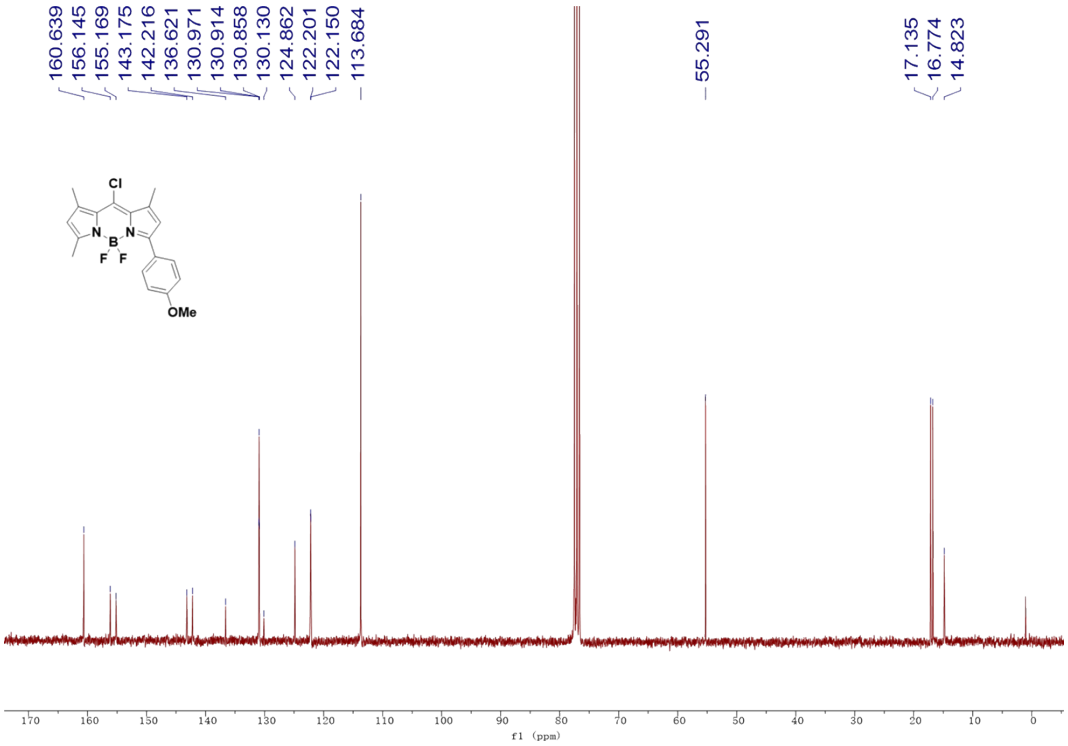


Fig. S12. ^{13}C NMR spectrum (75 MHz) of **1c** in $CDCl_3$.

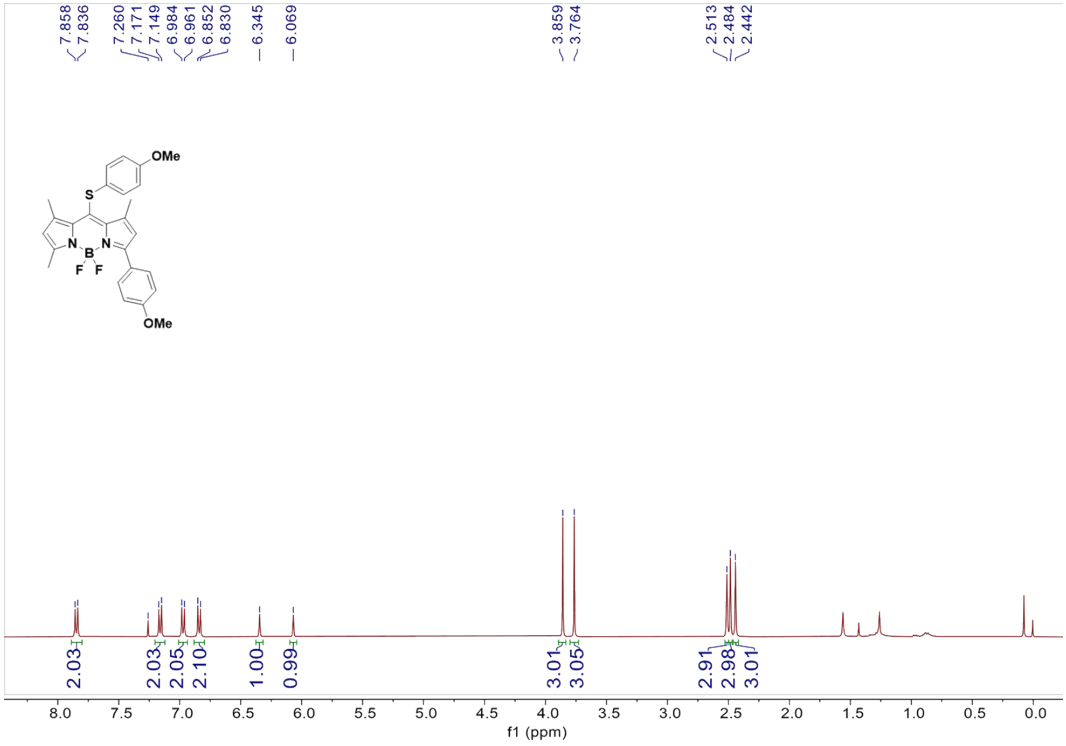


Fig. S13. ^{1}H NMR spectrum (400 MHz) of **BDP-S-Ph** in $CDCl_3$

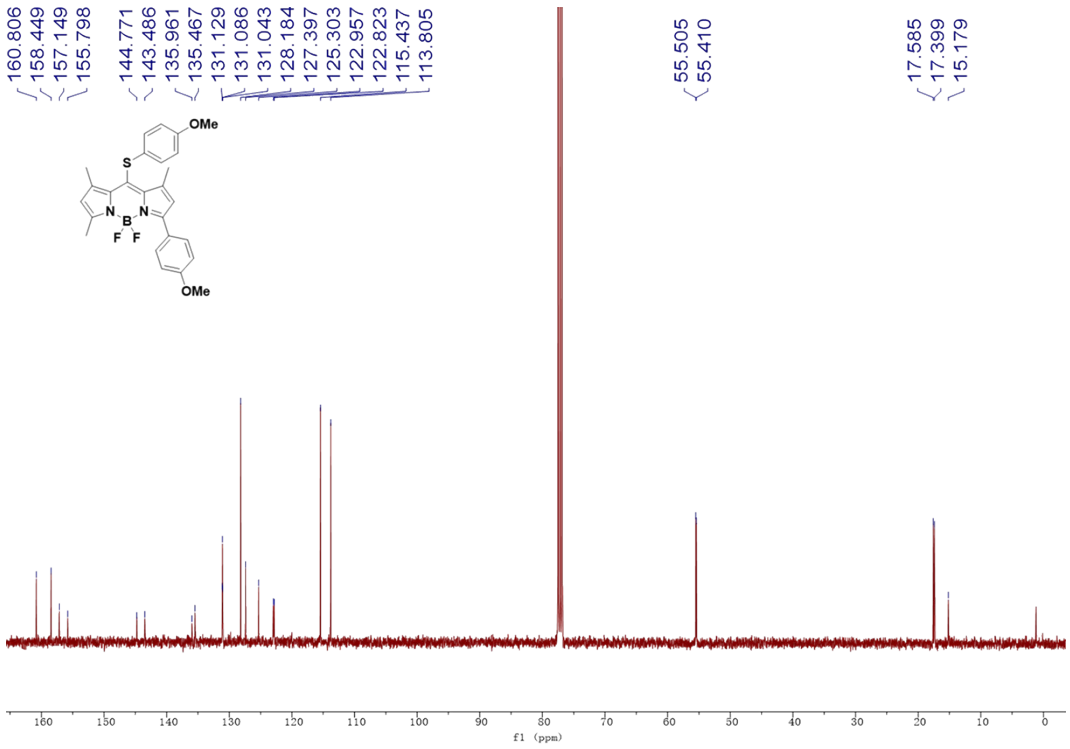


Fig. S14. ^{13}C NMR spectrum (100 MHz) of **BDP-S-Ph** in $CDCl_3$.

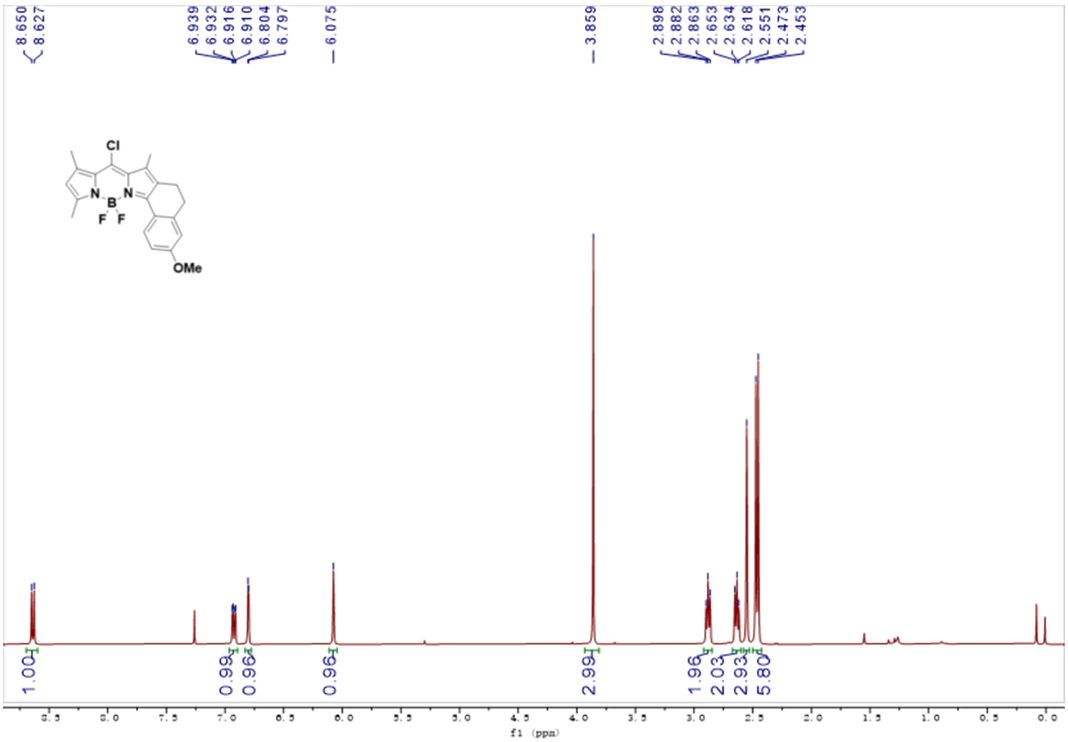


Fig. S15. ^{1}H NMR spectrum (400 MHz) of **2c** in $CDCl_3$.

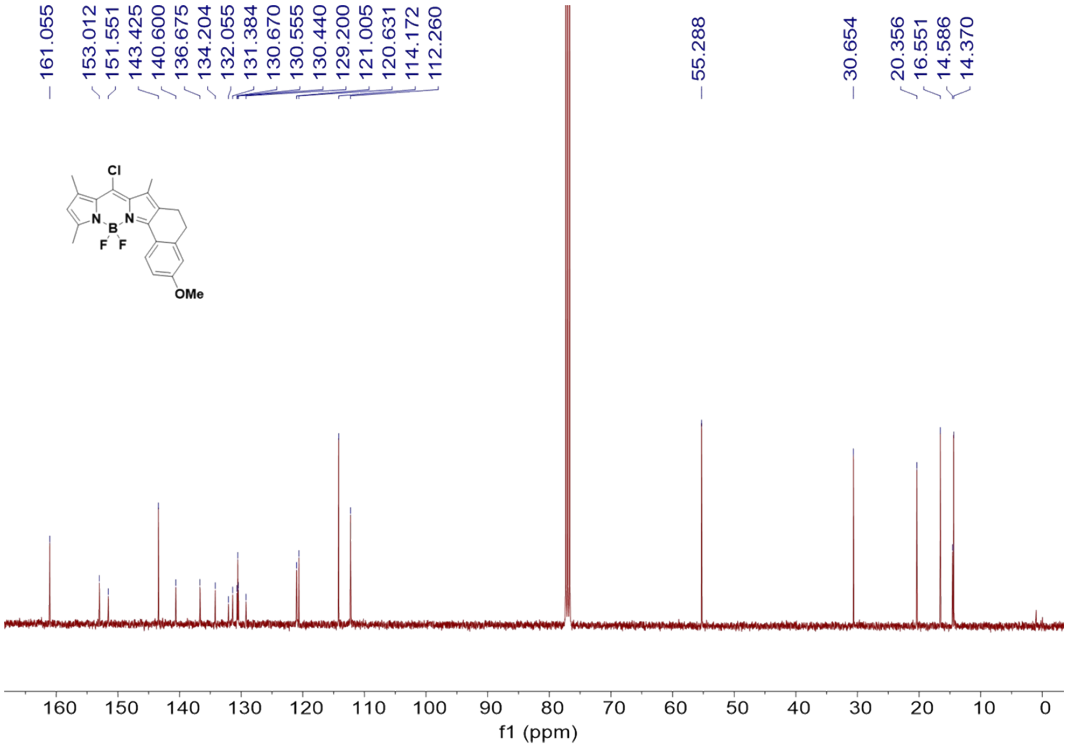


Fig. S16. ^{13}C NMR spectrum (100 MHz) of **2c** in $CDCl_3$.

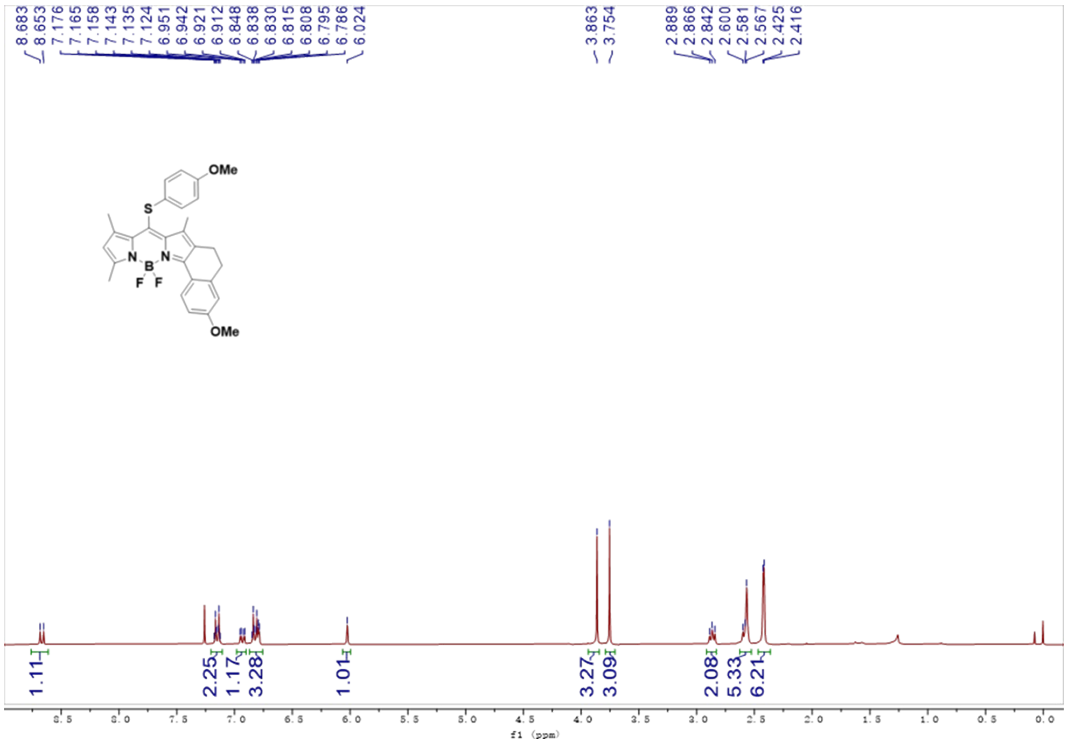


Fig. S17. ^{1}H NMR spectrum (300 MHz) of **BDP-S-R** in $CDCl_3$.

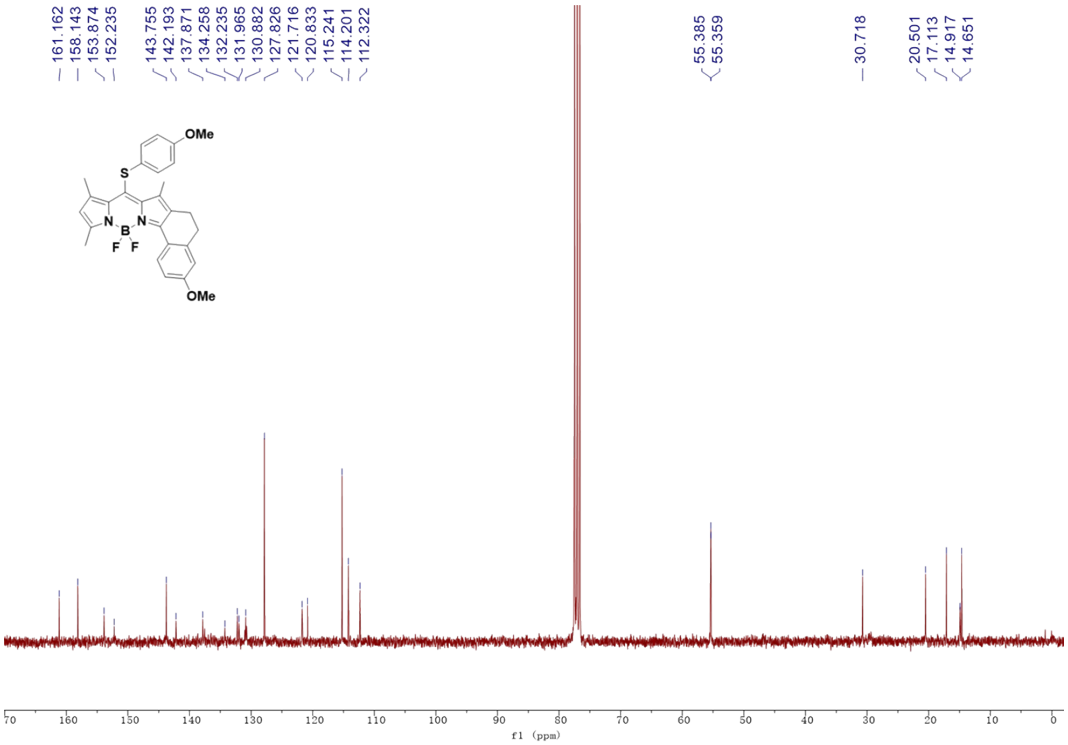


Fig. S18. ^{13}C NMR spectrum (75 MHz) of **BDP-S-R** in $CDCl_3$.

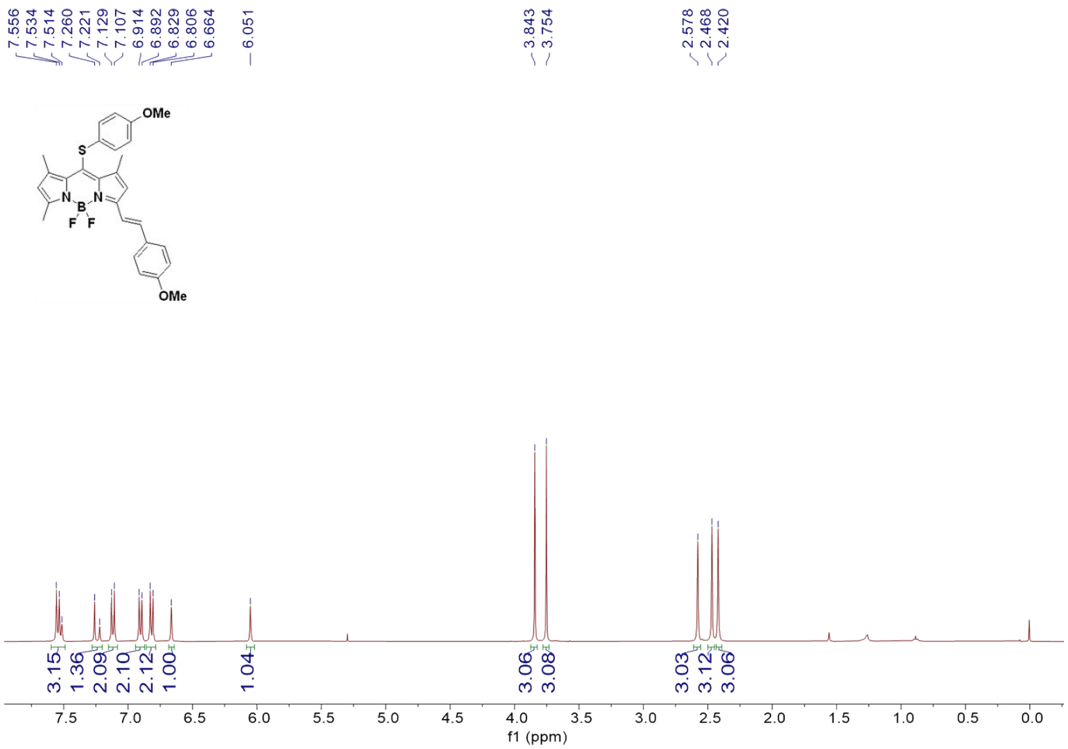


Fig. S19. ^{1}H NMR spectrum (400 MHz) of **BDP-S-ENE** in $CDCl_3$.

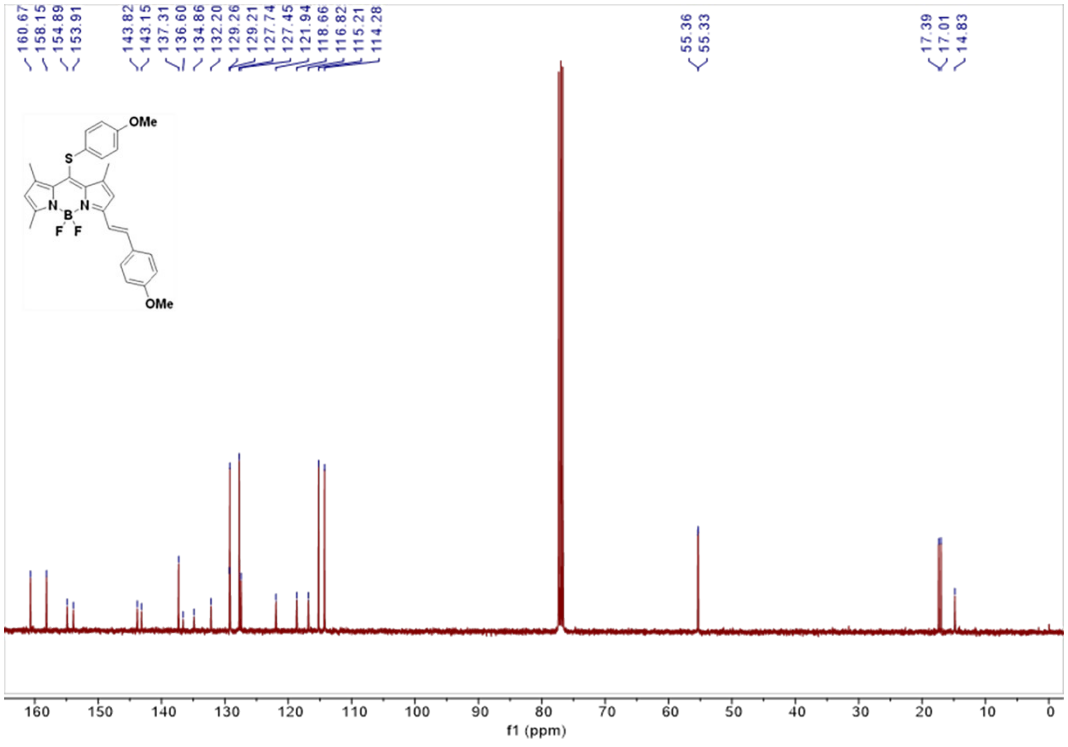


Fig. S20. ^{13}C NMR spectrum (100 MHz) of **BDP-S-ENE** in $CDCl_3$.

Data		Acquisition	
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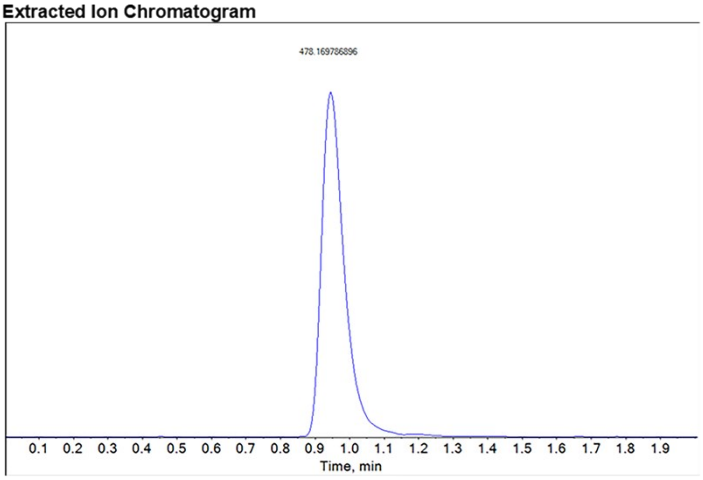
		Operator	COMPUTERNAME\Administrator
Batch	D:\Analyst Data\Projects\mw\2013_12_19\Batch\New Batch.dab		
Sample		Results	
		Result Table	
		Library Search	
Dilution Factor	1.0000	Library	All Libraries
Vial	0	Mass Tolerance	0.4
Injection Volume	µL	Purity Threshold	0.05

Extracted Ion Chromatogram

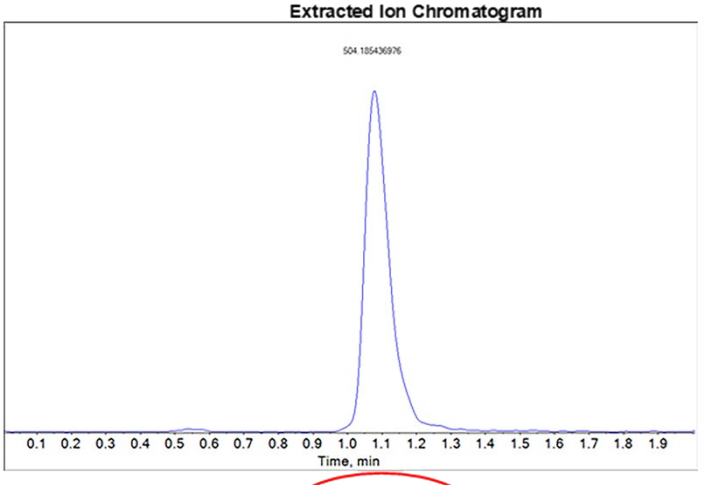


Compound Name (Library Hit)	Formula	Intensity	Threshold	Expected m/z	Found at m/z	Error (ppm)	Expected RT (min)	Found RT (min)	RT Delta (min)	Isotope Diff (%)	Purity (%)
478.169786896	C26H25BF2N2O2S	84570	5	479.1771	479.1774	0.7	0.00	0.96	0.96	18.7%	N/A

Fig. S21. The HRMS of **BDP-S-Ph**.

Data		Acquisition	
Data File	20210226-02.wiff (sample 1)	Method	MW-TOF.dam
Date and Time	Friday, February 26, 2021 (12:24:10 PM)	Instrument Serial #	BN21481307
		Operator	COMPUTERNAME\Administrator
Batch	D:\Analyst Data\Projects\mw\2013_12_19\Batch\New Batch.dab		
Sample		Results	
		Result Table	
		Library Search	
Dilution Factor	1.0000	Library	All Libraries
Vial	0	Mass Tolerance	0.4
Injection Volume	µL	Purity Threshold	0.05

Extracted Ion Chromatogram



Compound Name (Library Hit)	Formula	Intensity	Threshold	Expected m/z	Found at m/z	Error (ppm)	Expected RT (min)	Found RT (min)	RT Delta (min)	Isotope Diff (%)	Purity (%)
504.185436976	C28H27BF2N2O2S	31637	5	505.1927	505.1923	0.8	0.00	1.08	1.08	201.1%	N/A

Fig. S22. The HRMS of **BDP-S-ENE**.

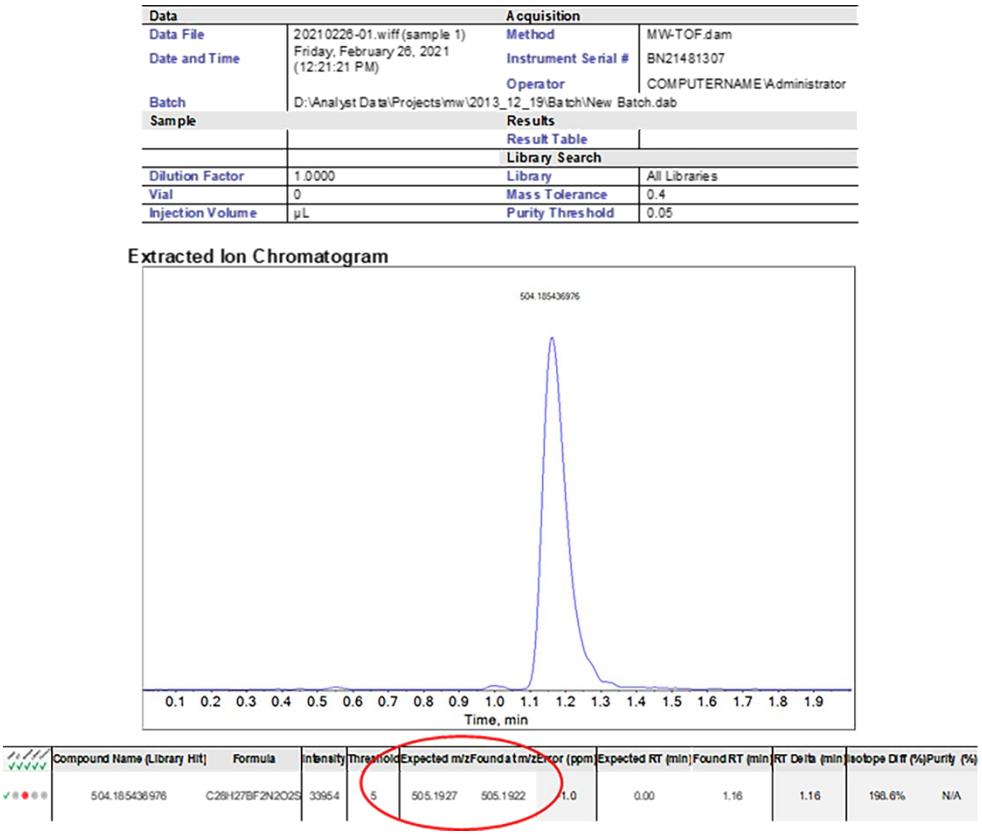


Fig. S23. The HRMS of **BDP-S-R**.

5. References

[1] R. F. Kubin and A. N. Fletcher, *J. Lumin.*, 1982, **27**, 455–462.