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Near-Infrared Fluorescent Dyes with Large Stokes Shifts: Light Generation in BODIPYs Undergoing the Excited State Intramolecular Proton Transfer

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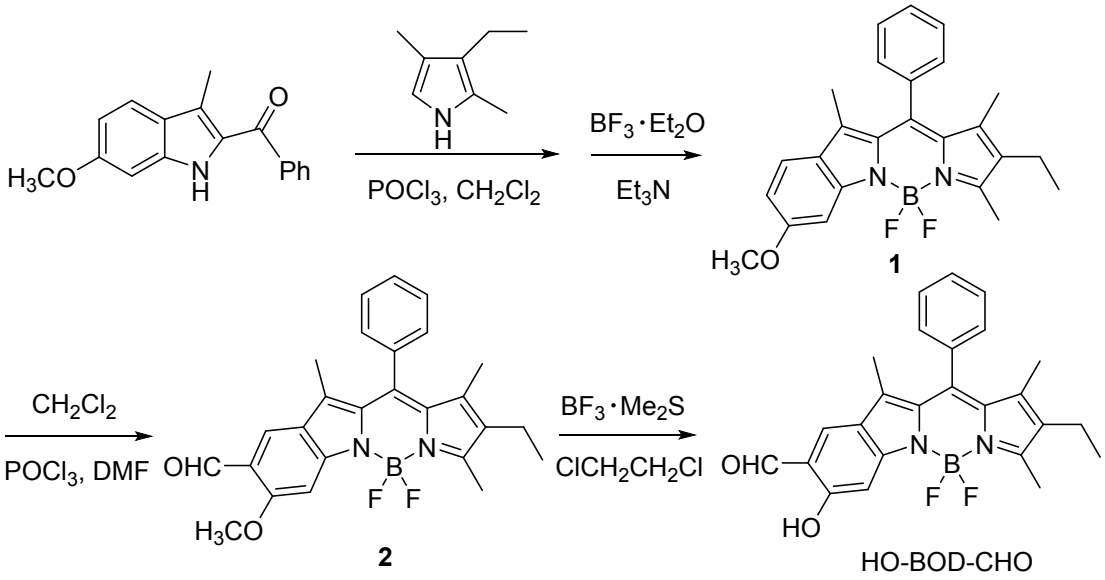
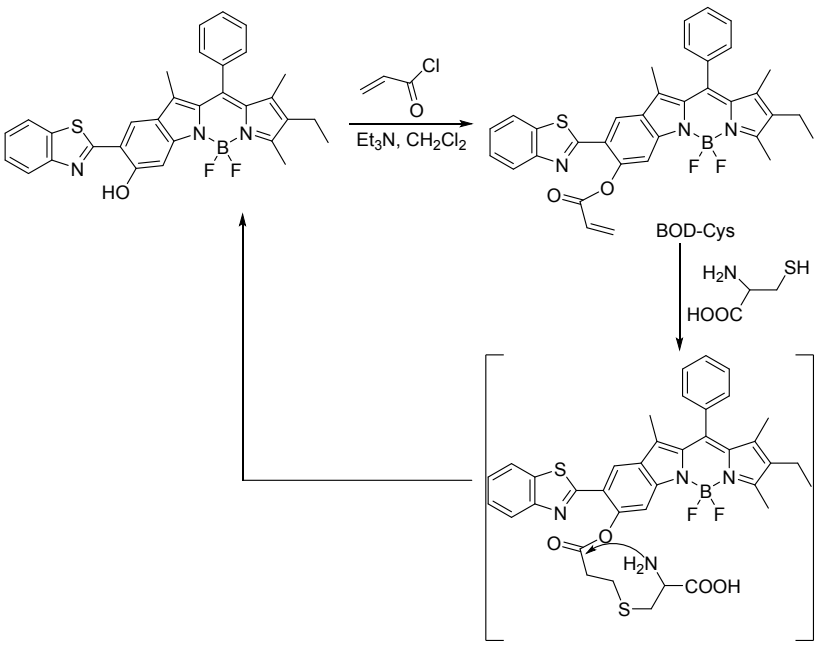
1. Materials and Instruments

All chemical reagents and solvents for synthesis were purchased from commercial suppliers and were used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV-400 spectrometer with chemical shifts reported in ppm at room temperature. Mass spectra were measured on a HP 1100 LC-MS spectrometer. UV-vis absorption spectra were recorded on a Varian Cary 100 spectrophotometer. Fluorescence spectra were measured with a Varian Cary Eclipse Fluorescence spectrophotometer.

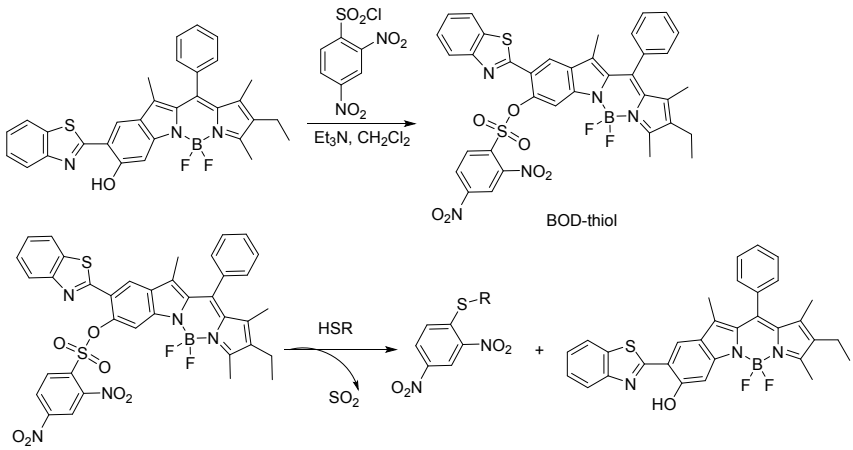
2. Synthesis



Scheme S1. The synthesis of HO-BOD-CHO, which was obtained acoording to the procedure in J. Cao, C. Zhao, X. Wang, Y. Zhang and W. Zhu, *Chem. Commun.*, **2012**, *48*, 9897–9899. The yields of synthetic compounds were 51 % (**1**), 53 % (**2**) and 70 % (HO-BOD-CHO).



Scheme S2. The synthesis of BOD-Cys and its reaction with thiols.



Scheme S3. The synthesis of BOD-thiol and its reaction with thiols.

Synthesis of NIR-BODI. Compound HO-BOD-CHO (130 mg, 0.3 mmol), 2-aminothiophenol (75 mg, 0.6 mmol) and sodium metabisulfite (58 mg, 0.3 mmol) were dissolved in dry DMF (10 mL). The resulted mixture was stirred for 1 h at room temperature. Then the reaction mixture was heated to 130 °C and stirred for another 2 h, followed by cooling to room temperature. Then the solution was diluted with CH_2Cl_2 and washed with water (3 × 100 mL). The organic layer was dried over anhydrous Na_2SO_4, and evaporated under vaccum. The residue was purified by silica gel chromatography (petroleum ether - ethyl acetate) to offer a reddish brown solid (86 mg, 53 %). ^{1}H NMR (d_6-DMSO, 400 MHz, ppm) δ 11.92 (s, 1H), 8.46 (s, 1H), 8.11 (d, 1H, J = 7.6 Hz), 8.04 (d, 1H, J = 8.0 Hz), 7.65-7.64 (m, 3H), 7.53-7.48 (m, 3H), 7.41 (t, 1H, J = 8.0 Hz), 7.14 (s, 1H), 2.65 (s, 3H), 2.40 (q, 2H, J = 7.6 Hz), 1.65 (s, 3H), 1.38 (s, 3H), 1.00 (t, 3H, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 170.0, 163.7, 159.2, 151.9, 147.4, 142.0, 141.0, 137.7, 136.5, 135.0, 134.3, 132.3, 132.2, 129.3, 128.3, 126.6, 126.4, 125.3, 122.2, 122.0, 121.3, 114.0, 100.1, 17.2, 14.2, 13.6, 12.2, 11.3. HRMS (ESI): calcd for $C_{31}H_{27}BF_2N_3OS$: 538.1936; found: 538.1941 $[M+H]^+$.

Synthesis of NIR-BODII. To a solution of Compound HO-BOD-CHO (100 mg, 0.23 mmol) in dry ethanol (10 mL) was added 1,2-dimethyl-1H-imidazol-5(4H)-one (257 mg, 2.3 mmol). Then the reaction mixture was refluxed for 18 h. Then the mixture was cooled to room temperature, and the solvent was removed under vaccum. The residue was purified by silica gel chromatography (petroleum ether - dichloromethane - ethyl acetate) to give a black blue solid (44 mg, 36 %). ^{1}H NMR (d_6-DMSO, 400 MHz, ppm) δ 11.98 (s, 1H), 8.65 (s, 1H), 7.63-7.62 (m, 3H), 7.48-7.45 (m, 2H), 7.31 (s, 1H), 6.95 (s, 1H), 3.10 (s, 3H), 2.62 (s, 3H), 2.38 (q, 2H, J = 7.2 Hz), 2.35 (s, 3H), 1.56 (s, 3H), 1.37 (s, 3H), 0.99 (t, 3H, J = 7.2 Hz) . HRMS (ESI): calcd for $C_{30}H_{30}BF_2N_4O_2$: 527.2430; found: 527.2430 $[M+H]^+$.

Synthesis of BOD-Cys. Compound NIR-BODI (50 mg, 0.093 mmol) was dissolved in anhydrous dichloromethane (10 mL) and the resulted mixture was cooled to 0 °C. Et_3N (64.5 μL, 0.47 mmol) was then added to the above solution. The obtained mixture was stirred for 5 min, followed by the addition of acryloyl chloride (37.6 μL, 0.47 mmol). The reaction mixture was further stirred for 10 min at 0 °C and was then warmed to room temperature. The reaction was quenched with water, and extracted with CH_2Cl_2, washed with deionised water and saturated brines, respectively. Then the organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by silica gel chromatography (petroleum ether - ethyl acetate) to afford a reddish solid (30 mg, 55 %). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 8.52 (s, 1H), 8.05 (d, 1H, J = 8.0 Hz), 7.87 (d, 1H, J = 7.6 Hz), 7.62-7.56 (m, 4H), 7.46 (t, 1H, J = 7.6 Hz), 7.37-7.33 (m, 3H), 6.71 (d, 1H, J = 17.2 Hz),

6.54-6.47 (m, 1H), 6.12 (d, 1H, J = 11.6 Hz), 2.71 (s, 3H), 2.38 (q, 2H, J = 7.6 Hz), 1.71 (s, 3H), 1.41 (s, 3H), 1.04 (t, 3H, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 166.5, 164.3, 163.9, 152.9, 148.3, 144.7, 143.4, 141.9, 138.7, 137.5, 135.4, 134.7, 133.2, 131.2, 129.8, 129.6, 129.5, 128.1, 126.1, 124.7, 123.3, 122.8, 121.2, 120.1, 108.8, 100.0, 17.2, 14.0, 13.8, 12.4, 11.3. HRMS (ESI): calcd for $C_{34}H_{27}BF_2N_3O_2S$: 590.1885; found: 590.1885 $[M-H]^-$.

Synthesis of BOD-thiol. Et_3N (25.8 μL, 0.19 mmol) was added to a solution of NIR-BODI (50 mg, 0.093 mmol) in dry dichloromethane, and the reaction mixture cooled to 0 °C and stirred for 10 min. Then 2, 4-dinitrobenzenesulfonyl chloride (50.7 mg, 0.19 mmol) dissolved in dry CH_2Cl_2 was added dropwise to the system. Then the reaction was stirred for 1 h at room temperature. The reaction was quenched with water and diluted with CH_2Cl_2, and washed with deionised water and brine, respectively. The obtained organic layer was dried with anhydrous Na_2SO_4, filtered, removed under reduced pressure. The residue was purified by silica gel chromatography (petroleum ether - dichloromethane - ethyl acetate) to yield a red solid (28 mg, 39 %). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 8.86 (s, 1H), 8.61 (d, 1H, J = 2.4 Hz), 8.36 (dd, 1H, J_1 = 8.4 Hz, J_2 = 2.0 Hz), 8.29 (d, 1H, J = 8.0 Hz), 8.06 (d, 1H, J = 8.8 Hz), 7.92 (d, 1H, J = 7.6 Hz), 7.59-7.56 (m, 4H), 7.49 (t, 1H, J = 7.4 Hz), 7.34-7.32 (m, 2H), 7.07 (s, 1H), 2.62 (s, 3H), 2.39 (t, 2H, J = 7.6 Hz), 1.74 (s, 3H), 1.43 (s, 3H), 1.06 (t, 3H, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 168.5, 164.0, 150.5, 148.4, 147.5, 145.8, 144.2, 143.8, 143.2, 141.6, 139.5, 138.2, 135.2, 134.1, 134.0, 133.4, 130.5, 130.1, 129.8, 129.6, 127.9, 126.7, 126.3, 125.7, 124.7,

122.5, 121.5, 120.0, 109.1, 17.2, 13.9, 13.8, 12.5, 11.1. HRMS (ESI): calcd for $C_{37}H_{29}BF_2N_5O_7S_2$: 768.1570; found: 768.1572 $[M+H]^+$.

3. **Cells culture and imaging.** In a humidified atmosphere of 5/95 CO_2/air incubator, Hela cells were cultured in Roswell Park Memorial Institute 1640 medium (RPMI-1640) supplemented with 10 % fetal bovine serum (FBS) at 37 °C. For fluorescence imaging, cells were seeded in glass bottom dishes for 24 h. (1) Cells were pretreated with 1 mM N-methylmaleimide for 10 min, followed by incubation with BOD-Cys (10 μM) for 30 min. (2) Cells pretreated with N-ethylmaleimide were incubated with 500 μM Cys for 20 min, then were stained with BOD-Cys for 30 min. (3) Cells were treated with BOD-thiol (10 μM) for 30 min. (4) Cells pretreated with 1 mM N-ethylmaleimide were incubated with BOD-thiol (10 μM) for 30 min. The confocal imaging was carried out using Nikor AIR with a 60 × oil objective. The excitation wavelength was 561 nm and emission was collected at 720-750 nm.

4. The optical spectra of NIR-BODI.

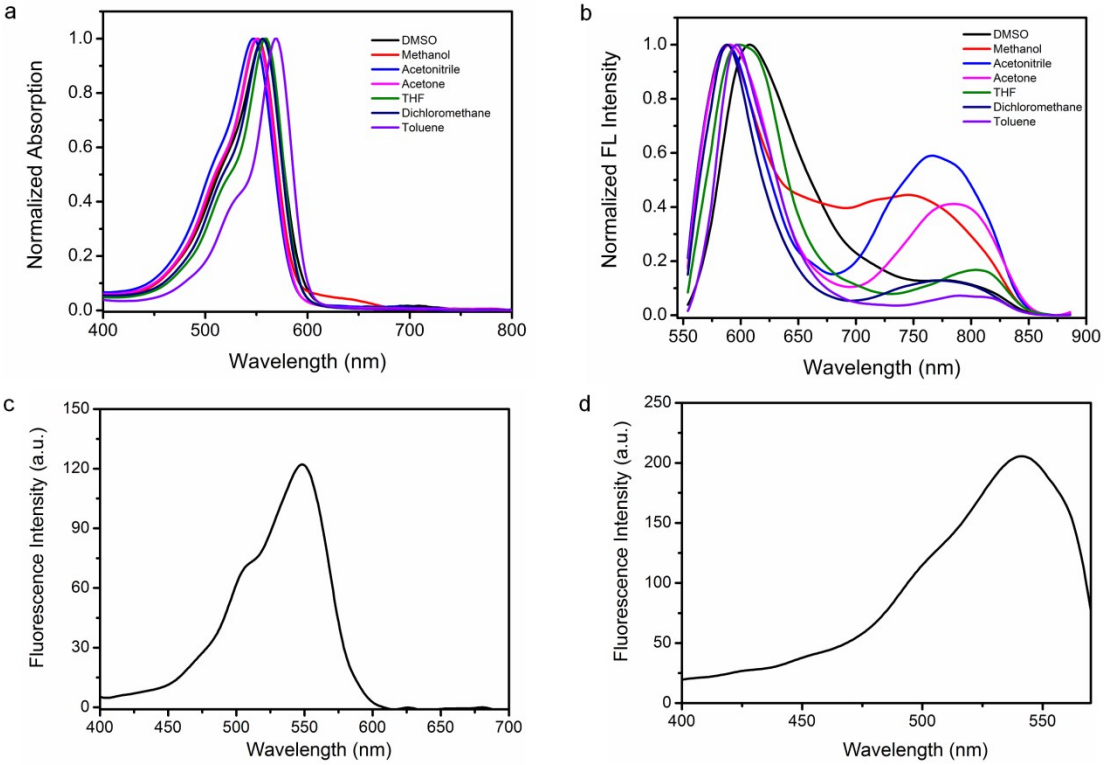


Fig. S1 The absorption (a) and emission (b) spectra of NIR-BODI in various solvents, λ_{ex} = 546 nm. The excitation spectra with monitoring the emission at (c) 765 nm and (d) 586 nm in CH_3CN.

5. The optical spectra of NIR-BODII.

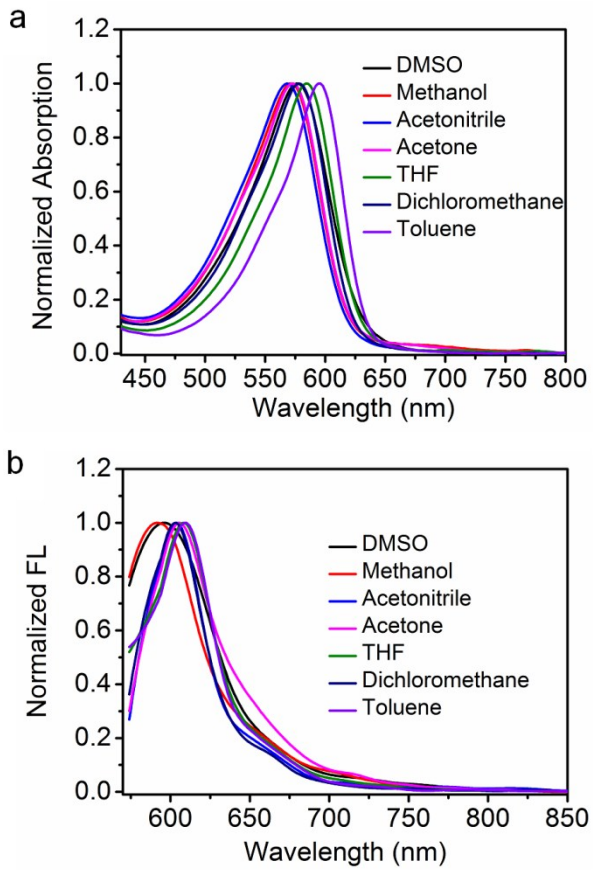


Fig. S2 The absorption (a) and emission (b) spectra of NIR-BODII in various solvents, λ_{ex} = 568 nm.

6. The absorption and large Stokes shifted ESIPT tautomer emission of NIR-BODI.

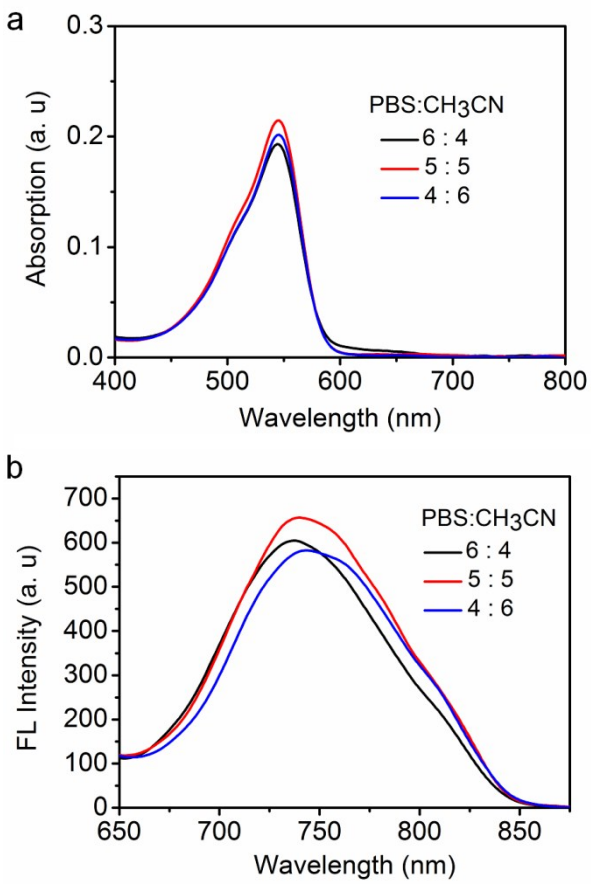


Fig. S3 The absorption (a) and large Stokes shifted ESIPT tautomer emission (b) spectra of NIR-BODI (5 μM) in a series of aqueous buffer solutions with varying acetonitrile content (acetonitrile/PBS buffer, pH 7.2, 37 °C), λ_{ex} = 544 nm.

7. pH effect on the optical spectra of NIR-BODI.

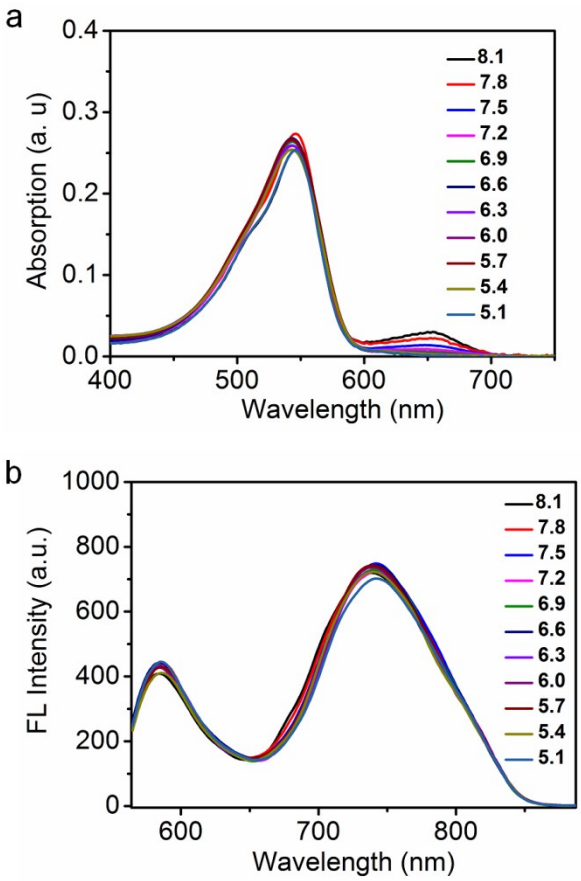


Fig. S4 The absorption (a) and emission (b) spectra profies of NIR-BODI (5 μM) in a series of aqueous buffer solutions with varying pH value (acetonitrile/PBS buffer, v/v, 1 : 1, 37 °C), λ_{ex} = 544 nm.

8. The reaction of BOD-Cys with thiols.

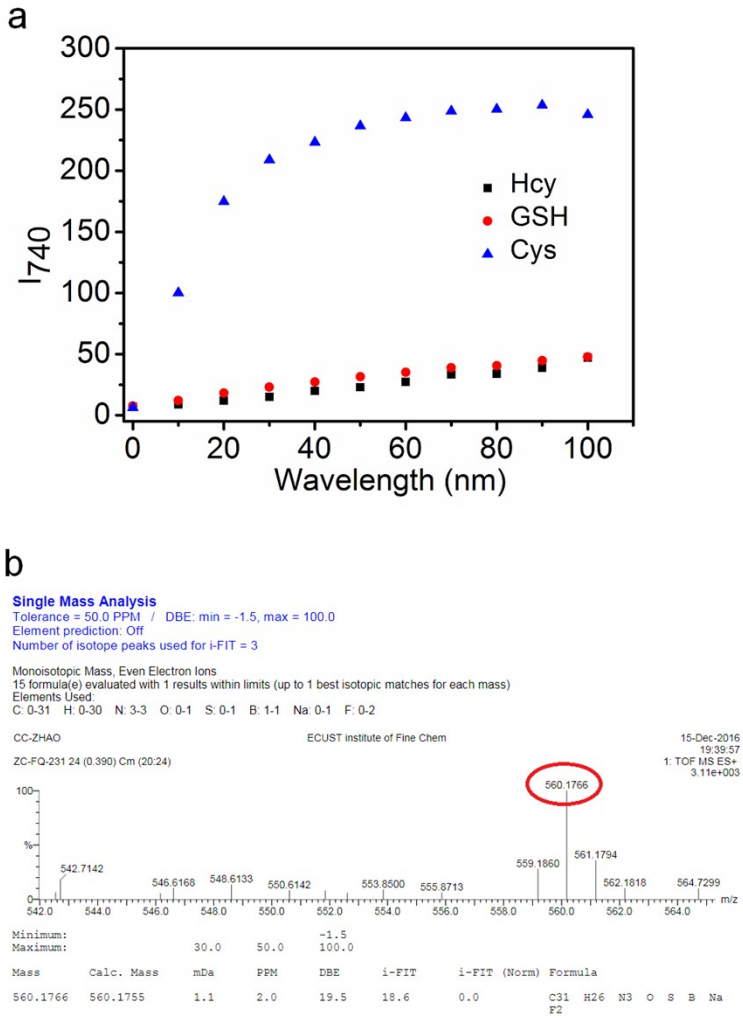


Fig. S5 (a) Time-dependent fluorescence changes of BOD-Cys (5 μM) in the presence of Cys (500 μM), Hcy (500 μM) and GSH (500 μM) in a phiological condition (acetonitrile/PBS buffer, v/v, 1 : 1, 37 °C), (b) HRMS spectrum of BOD-Cys +Cys.

9. **Selectivity of BOD-Cys.**

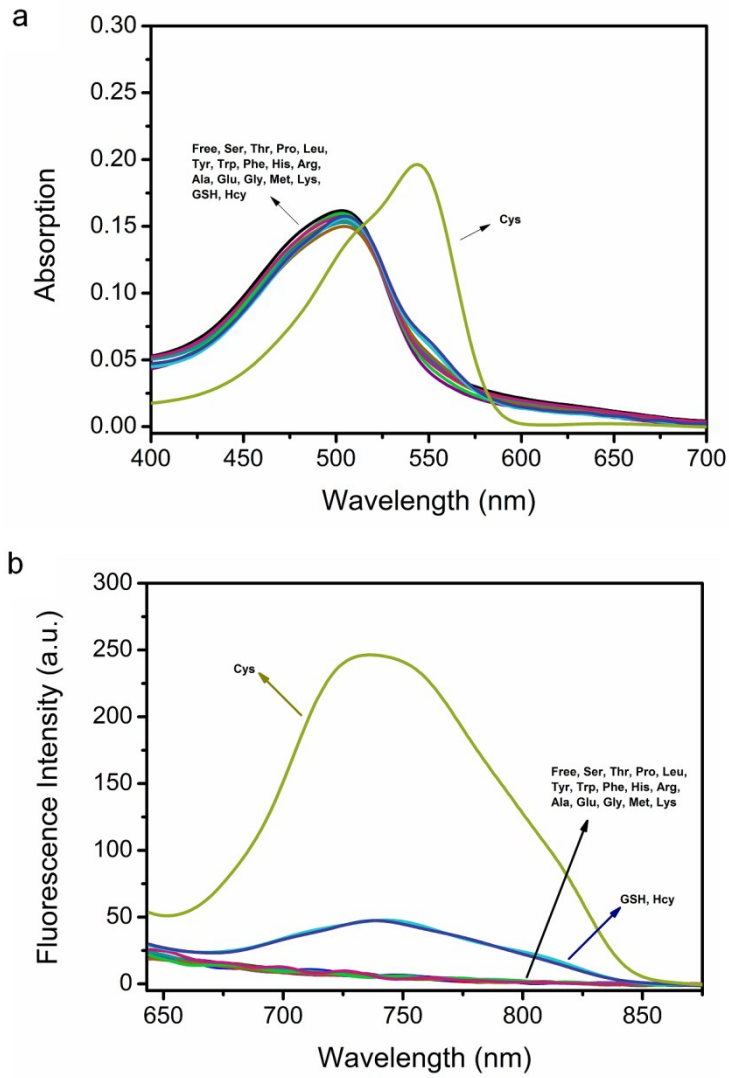


Fig. S6 The absorption (a) and emssion (b) changes of BOD-Cys (5 μM) in the presence of 500 μM biologically relevant amino acids under physiological condition (acetonitrile/PBS buffer, 1:1, v/v, pH 7.2, 37 °C). λ_{ex} = 544 nm.

10. Titration experiment.

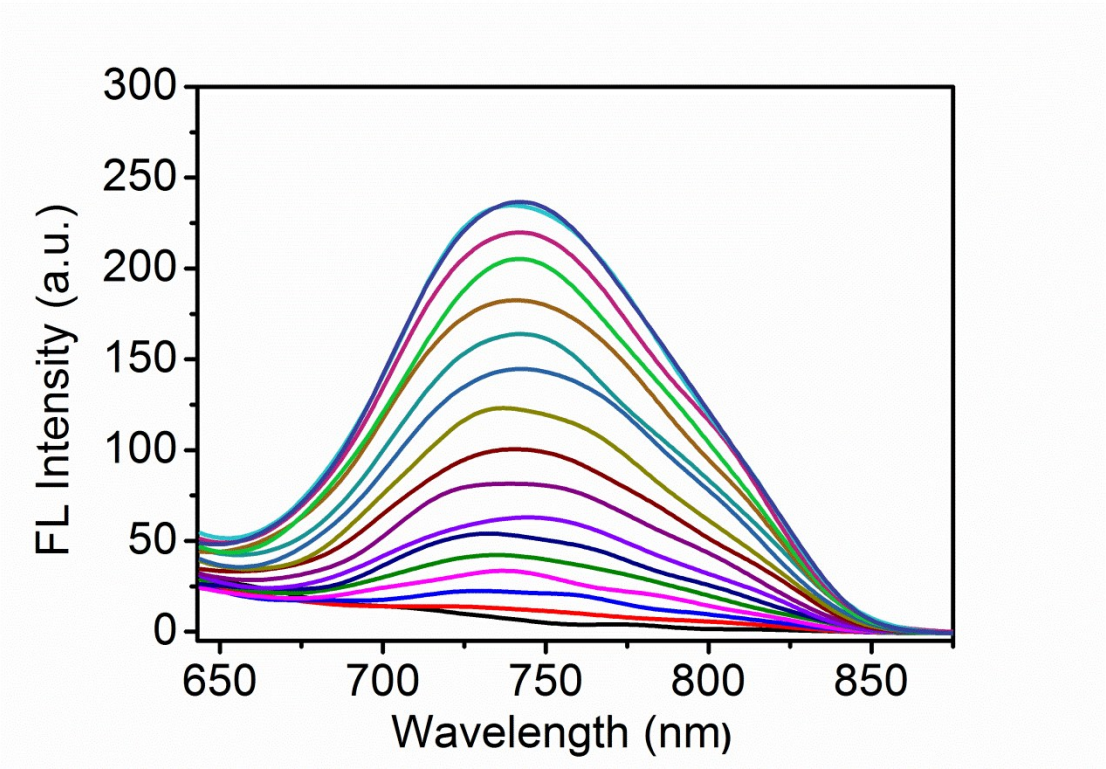


Fig. S7 Fluorescence spectra of BOD-Cys (5 μM) in the presence of various concentrations of Cys (0, 5, 10, 15, 20, 25, 30, 40, 50, 75, 100, 150, 200, 250, 300, 400, 500 μM, respectively) under physiological condition (acetonitrile/PBS buffer, 1:1, v/v, pH 7.2, 37 °C). λ_{ex} = 544 nm.

11. Time-dependent spectra changes of BOD-thiol in the presence of Cys.

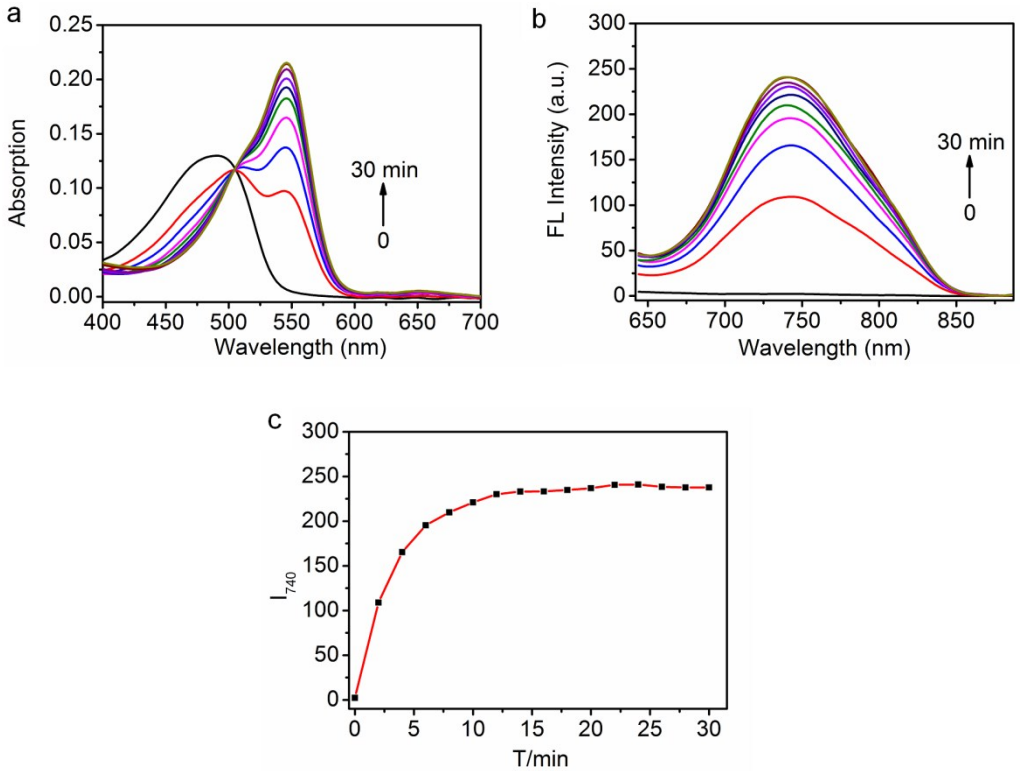


Fig. S8 Time-dependent absorption (a) and emission (b) changes of BOD-thiol (5 μM) in the presence of 500 μM Cys, λ_{ex} = 544 nm. (c) Plots of the fluorescence intensity of BOD-thiol (5μM) at 740 nm as a function of time in the presence of 500 μM Cys, λ_{ex} = 544 nm.

12. HRMS spectrum of BOD-thiol +Cys.

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
15 formula(e) evaluated with 1 results within limits (up to 1 best isotopic matches for each mass)
Elements Used:
C: 0-31 H: 0-30 B: 1-1 N: 3-3 O: 0-1 F: 0-2 Na: 0-1 S: 0-1

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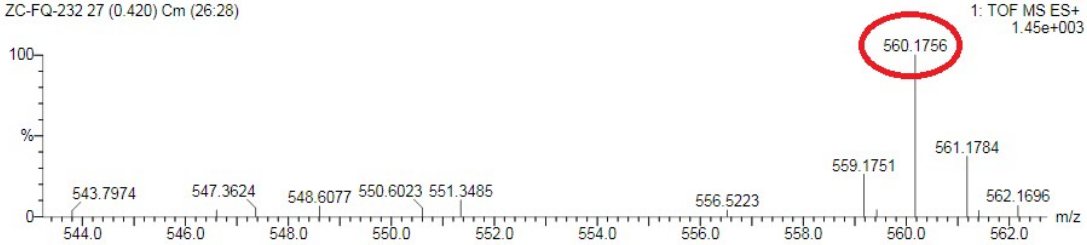

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Maximum:		30.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
560.1756	560.1755	0.1	0.2	19.5	24.3	0.0	C31 H26 B N3 O F2 Na S

Fig. S9 HRMS spectrum of BOD-thiol +Cys.

13. Selectivity of BOD-thiol.

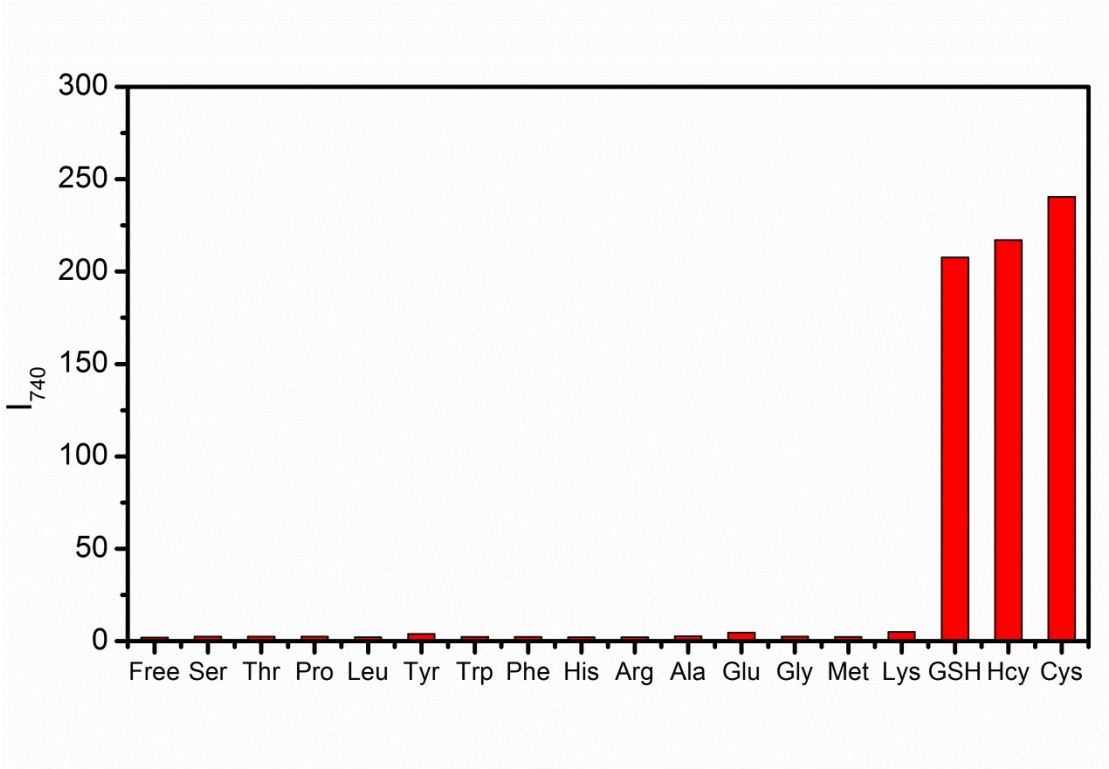

Fig. S10 Fluorescence changes of BOD-thiol in the presence of 500 μM biologically relevant amino acids under physiological condition (acetonitrile/PBS buffer, 1:1, v/v, pH 7.2, 37 °C). λ_{ex} = 544 nm.

14. Titration experiment.

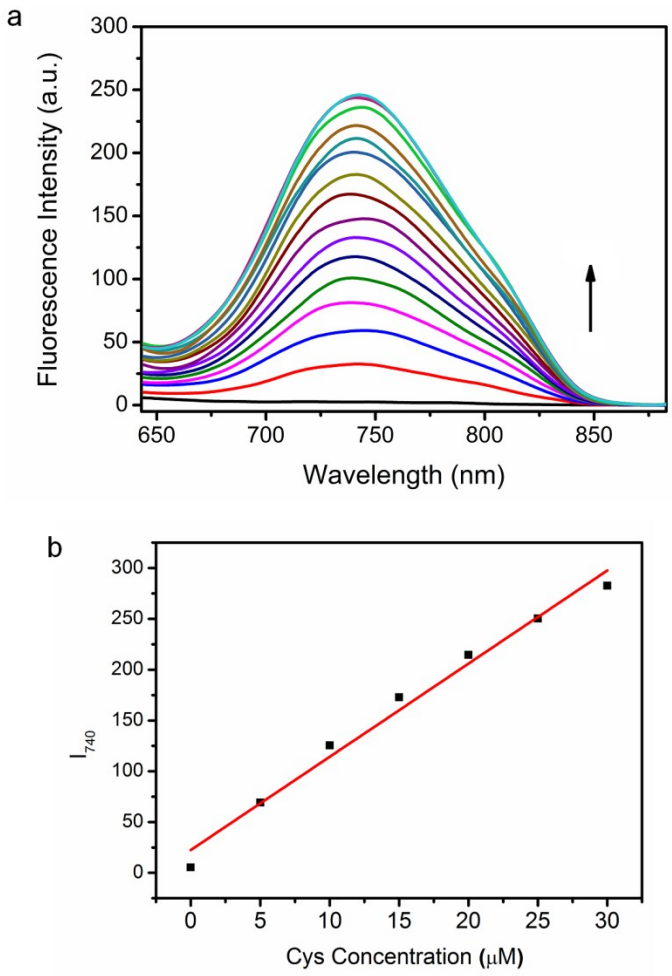

Fig. S11 (a) Fluorescence spectra of BOD-thiol (5 μM) in the presence of various concentrations of Cys (0, 5, 10, 15, 20, 25, 30, 40, 50, 75, 100, 125, 150, 200, 300, 400 μM, respectively) under physiological condition (acetonitrile/PBS buffer, 1:1, v/v, pH 7.2, 37 °C). (b) Fluorescence intensity at 740 nm as a function of Cys concentrations. λ_{ex} = 544 nm.

15. Detection of thiols in HeLa cells by BOD-thiol.

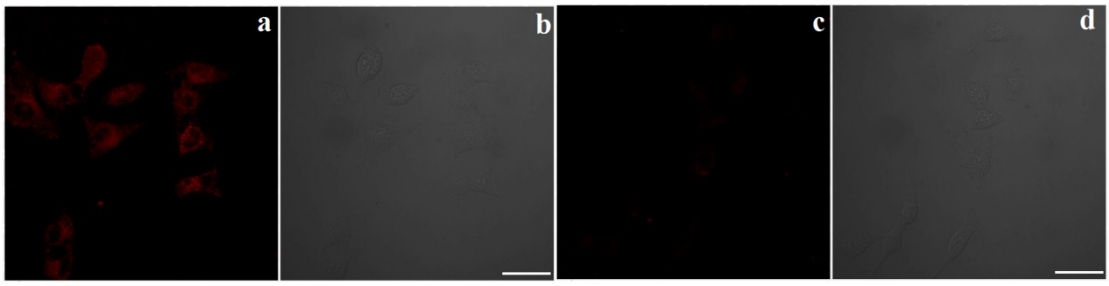

Fig. S12 Detection of thiols in HeLa cells using confocal fluorescence imaging. (a) Cells were incubated BOD-thiol (10 μM) for 30 min. (c) Cells pretreated with N-methylmaleimide (for 10 min) were incubated with BOD-thiol for 30 min. (b) and (d) are the bright field images. Scale bar represents 50 μm.

16. NMR and HRMS spectra

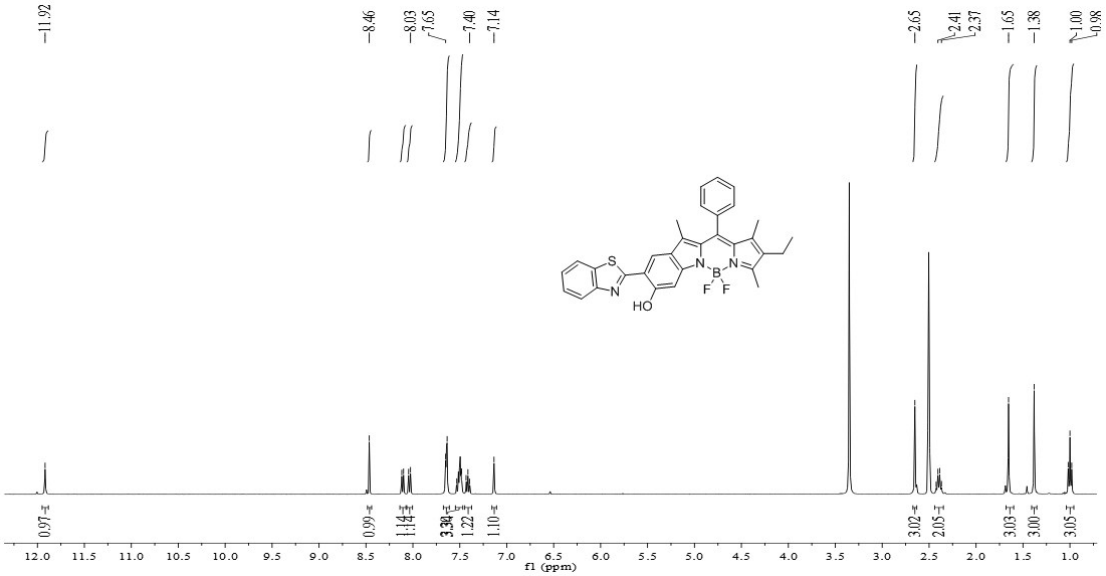

Fig. S13 ^{1}H NMR spectrum (in d_6-DMSO) of NIR-BODI.

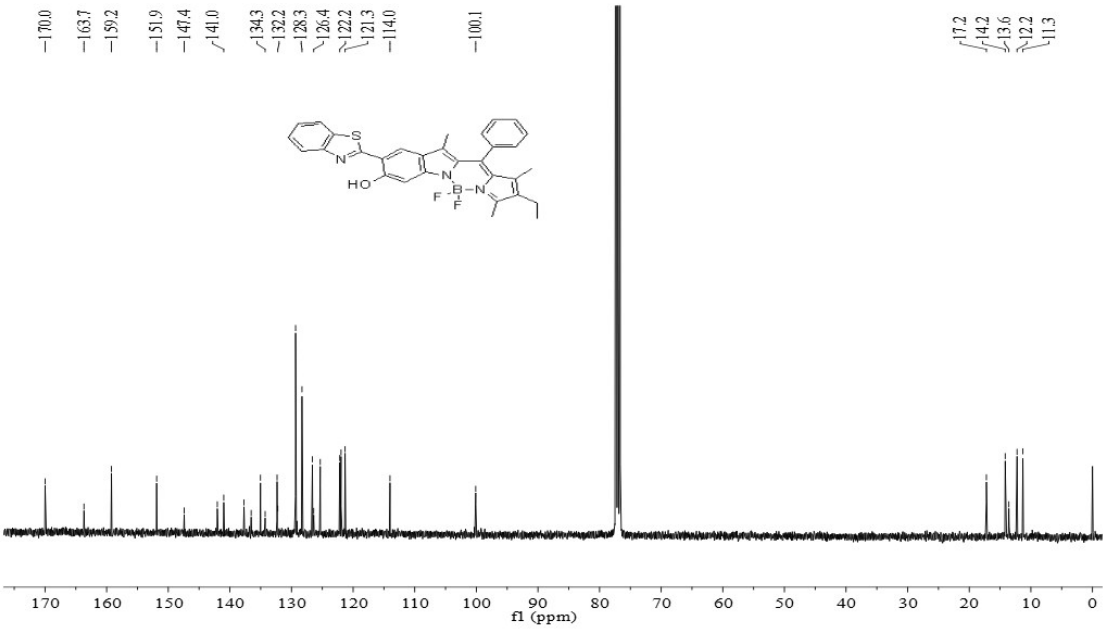

Fig. S14 ^{13}C NMR ($CDCl_3$, 100 MHz) spectrum of NIR-BODI.

Elemental Composition Report **Page 1**

Single Mass Analysis
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Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
71 formula(e) evaluated with 1 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-31 H: 0-27 B: 0-1 N: 0-3 O: 0-1 F: 0-2 S: 0-1

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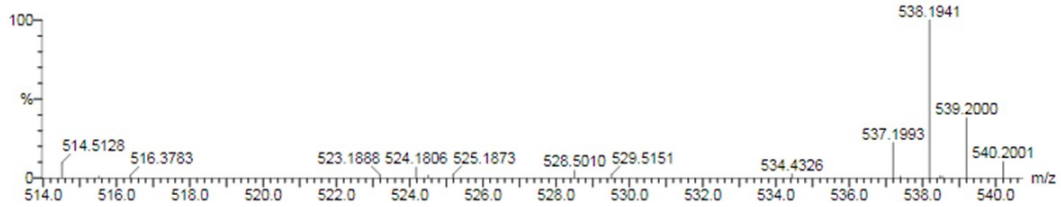

Minimum:				-1.5									
Maximum:		300.0	50.0	100.0									
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula						
538.1941	538.1936	0.5	0.9	19.5	39.0	0.0	C31	H27	B	N3	O	F2	S

Fig. S15 HRMS spectrum of NIR-BODI.

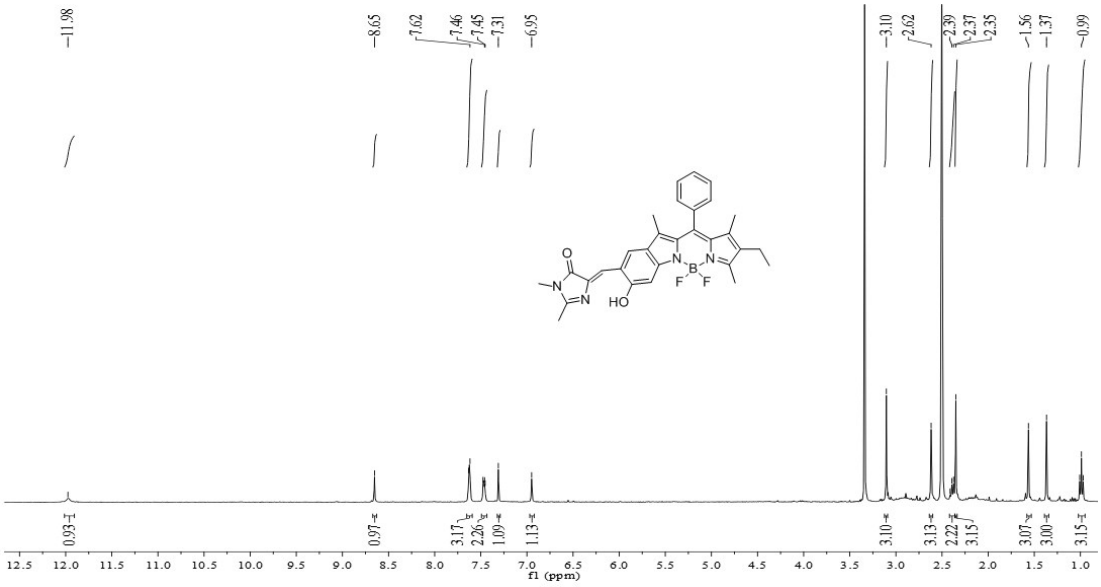

Fig. S16 ^{1}H NMR spectrum (in d_6-DMSO) of NIR-BODII.

Elemental Composition Report **Page 1**

Single Mass Analysis
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Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
72 formula(e) evaluated with 1 results within limits (up to 1 best isotopic matches for each mass)
Elements Used:
C: 0-30 H: 0-33 11B: 0-1 N: 0-4 O: 0-2 F: 0-2

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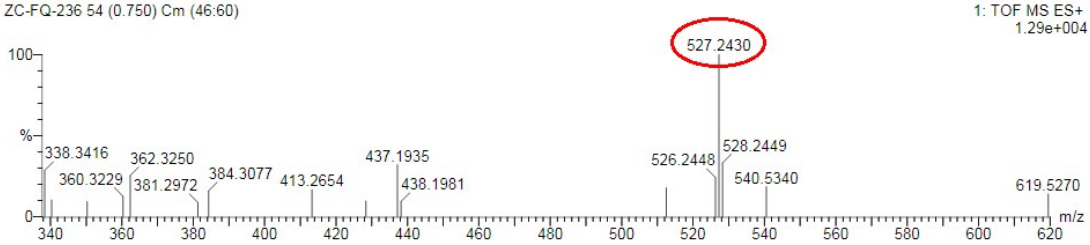


Minimum:				-1.5			
Maximum:		30.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
527.2430	527.2430	0.0	0.0	17.5	28.8	0.0	C30 H30 11B N4 O2 F2

Fig. S17 HRMS spectrum of NIR-BODI.

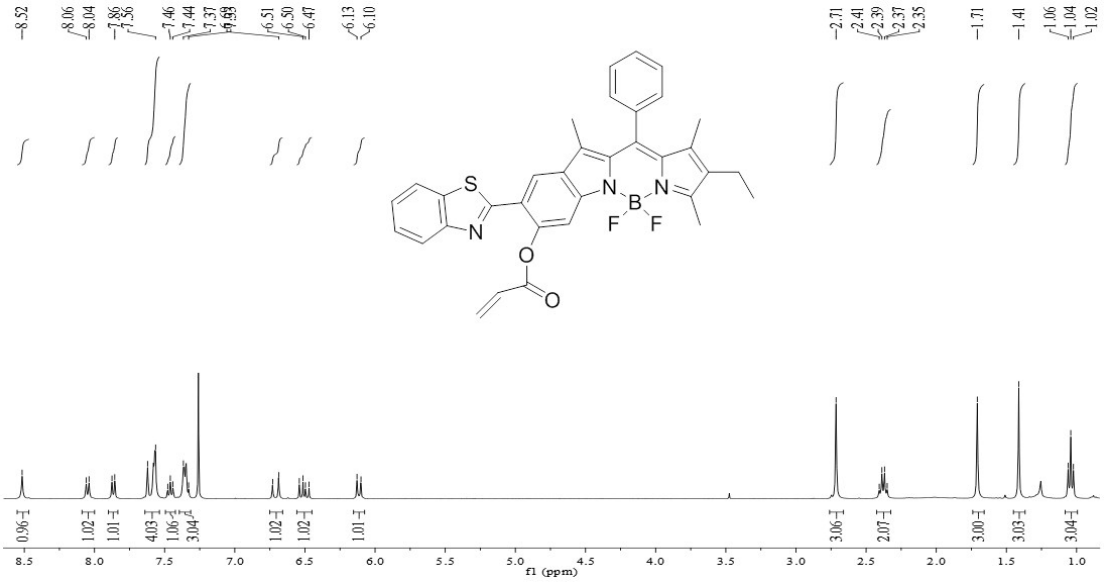


Fig. S18 ^{1}H NMR spectrum (in $CDCl_3$) of BOD-Cys.

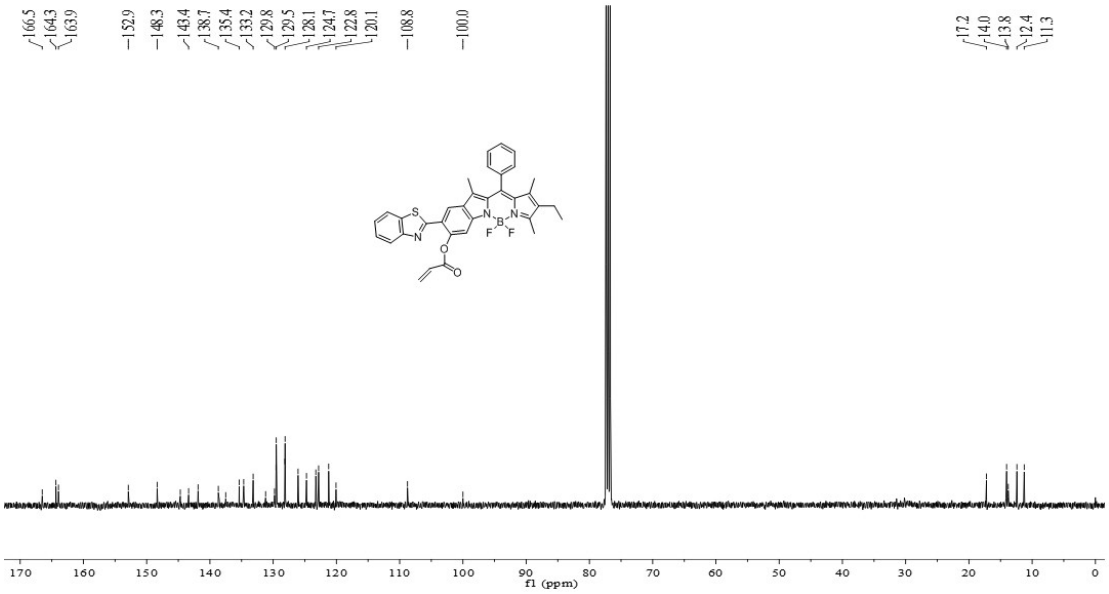


Fig. S19 ^{13}C NMR ($CDCl_3$, 100 MHz) spectrum of BOD-Cys.

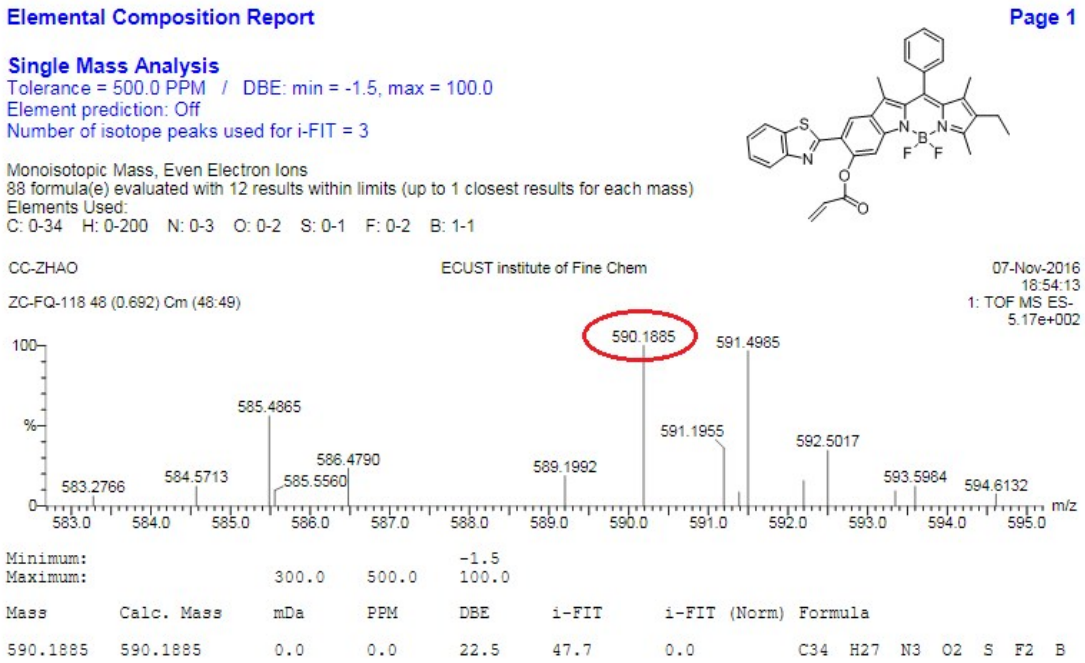


Fig. S20 HRMS spectrum of BOD-Cys.

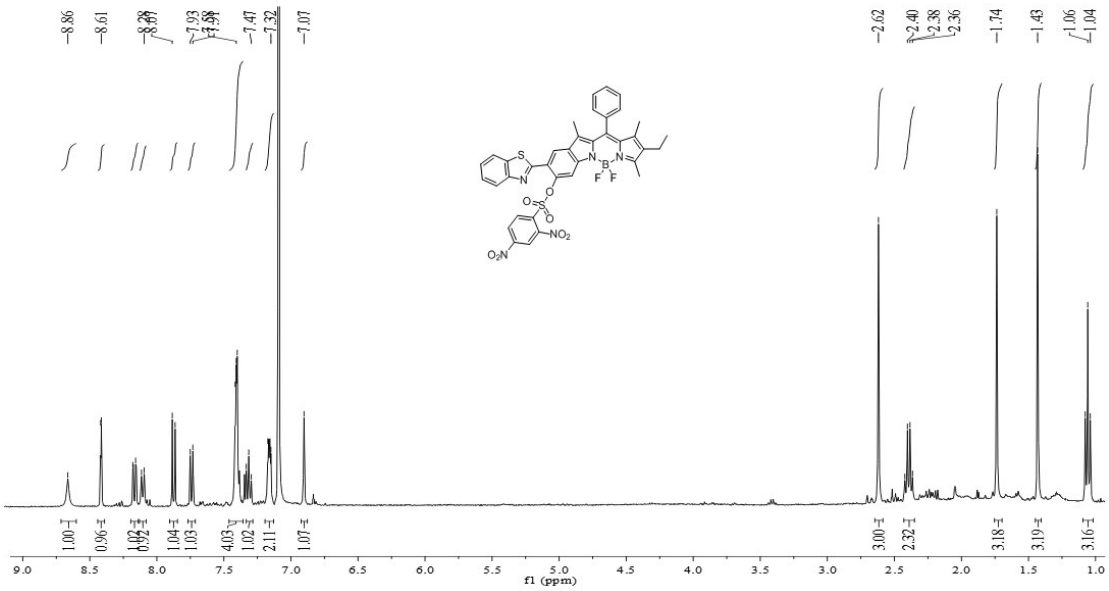


Fig. S21 ^{1}H NMR spectrum (in $CDCl_3$) of BOD-thiol.

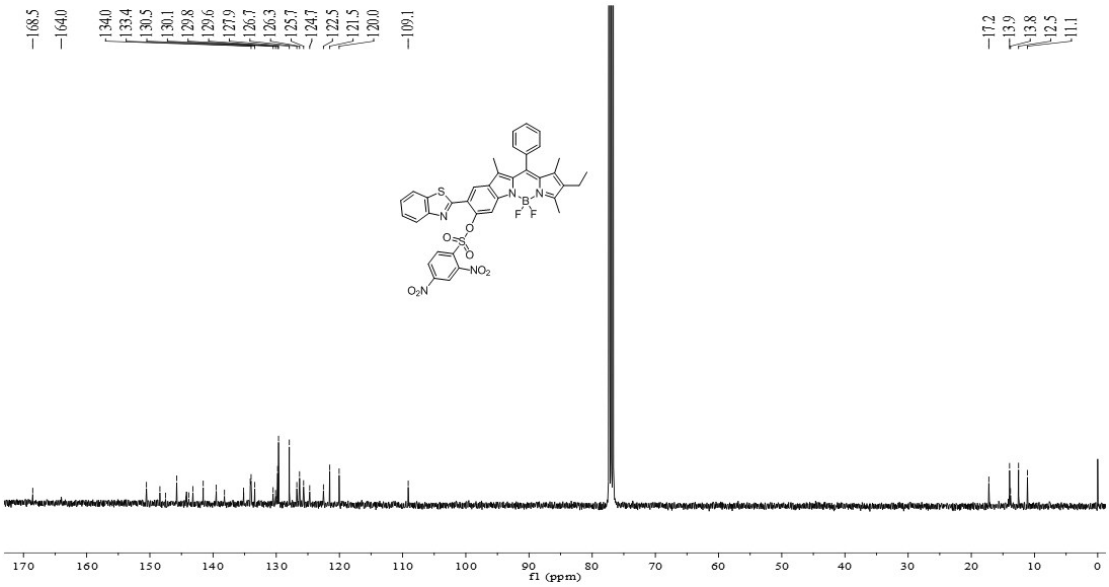
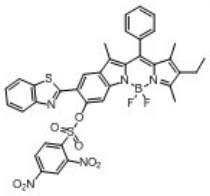


Fig. S22 ^{13}C NMR ($CDCl_3$, 100 MHz) spectrum of BOD-thiol.

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 500.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3



Monoisotopic Mass, Even Electron Ions
412 formula(e) evaluated with 4 results within limits (up to 1 best isotopic matches for each mass)
Elements Used:
C: 0-37 H: 0-50 B: 1-1 N: 0-5 O: 0-7 F: 0-2 S: 0-2

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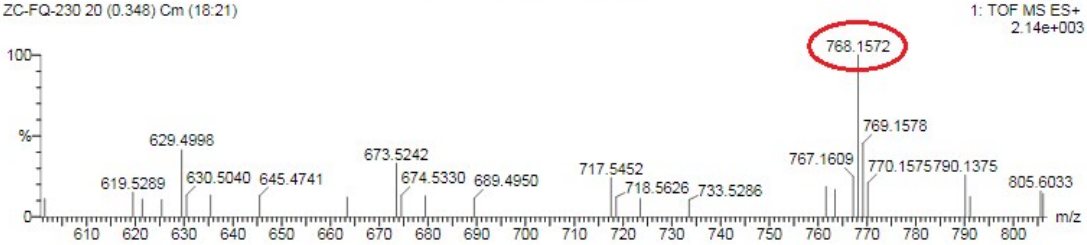
ZC-FQ-230 20 (0.348) Cm (18:21) 1: TOF MS ES+ 2.14e+003



Minimum:				-1.5			
Maximum:		300.0	500.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
768.1572	768.1570	0.2	0.3	25.5	20.4	0.0	C37 H29 B N5 O7 F2 S2

Fig. S23 HRMS spectrum of BOD-thiol.