Supporting Information

Thiol-Thiosulfonate Reaction Providing Novel Strategy for turn-on Thiol Sensing

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Scheme S1 Synthesis of thiosulfonate probes.

Figure S1. Absorbance spectra of different probes in response to Cys.

Figure S2. Emission spectra of different probes in response to Cys.

Figure S3-14 Original NMR and MS spectra of probes.

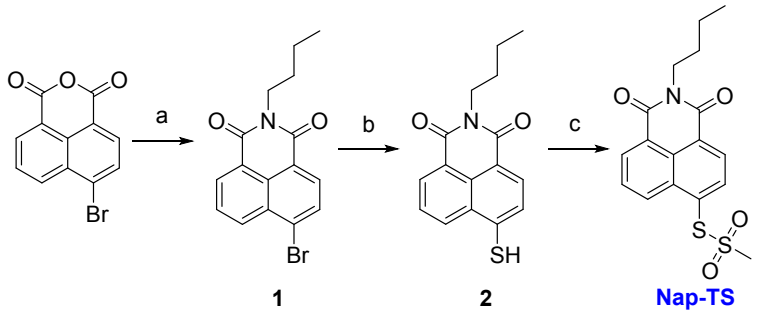
Figure S15-16 Original NMR and MS spectra of Cys-S-S-BODIPY.

Figure S17-19 Original NMR and MS spectra of BODIPY-SH.

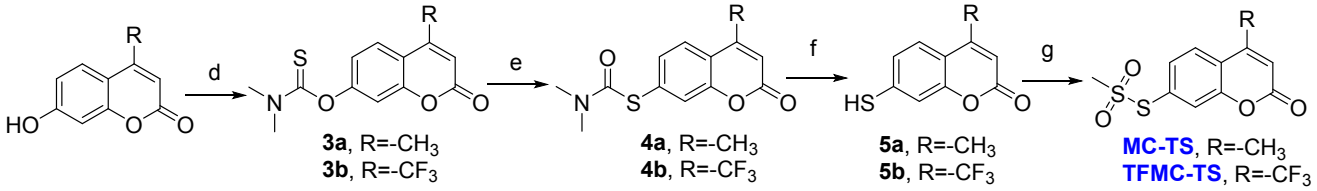
Experimental section

References

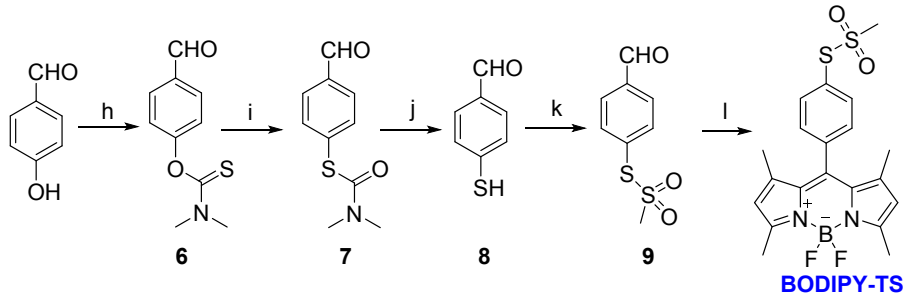
Scheme S1 Synthesis of thiosulfonate probes.



Reagents and conditions: (a) EtOH, n-butylamine, reflux; (b) Na_2S, DMF, 50 ^oC, 6 h; (c) NBS, CH_3CN, CH_3SO_2Na.



Reagents and conditions: (d) DMF, DMAP, DMTCC, 25 ^oC; (e) 210 ^oC; (f) CH_3OH, CH_3ONa; (g) NBS, CH_3CN, CH_3SO_2Na.



Reagents and conditions: (h) DMTCC, DABCO, DMF, 16 h, rt; (i) 200 ^oC, 3 h; (j) 5 M KOH, MeOH, reflux, 2.5 h; (k) NBS, CH_3CN, CH_3SO_2Na, rt; (l) 2,4-dimethylpyrrole, CF_3COOH, DDQ, Et_3N, BF_3 Et_2O, rt.

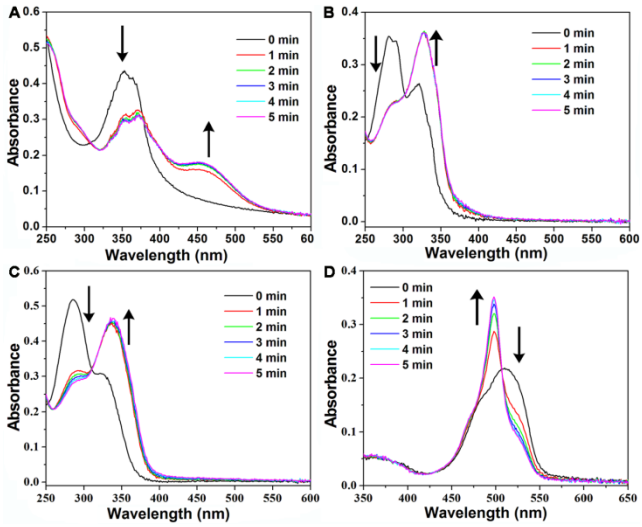


Figure S1. Absorbance spectra of different probes in responding to Cys. Thiosulfonate probes were incubated with the same concentration of Cys at room temperature in PBS, pH 7.4. The absorbance spectra were scanned every 1 min for 5 min. (A) Nap-TS (40 μM). (B) MC-TS (40 μM). (C) TFMC-TS (40 μM). (D) BODIPY-TS (10 μM).

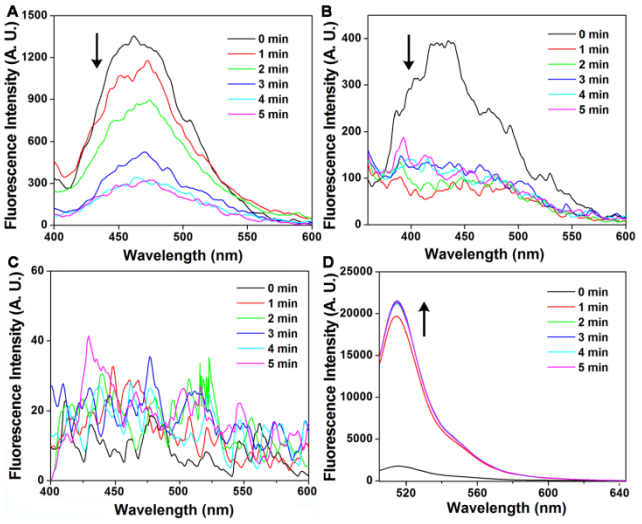


Figure S2. Emission spectra of different probes in responding to Cys. Thiosulfonate probes (10 μM) were incubated with the same concentration of Cys at room temperature in PBS, pH 7.4. The emission spectra were scanned every 1 min for 5 min. (A) Nap-TS (λ_{ex}=450 nm). (B) MC-TS (λ_{ex}=350 nm). (C) TFMC-TS (λ_{ex}=350 nm). (D) BODIPY-TS (λ_{ex}=490 nm).

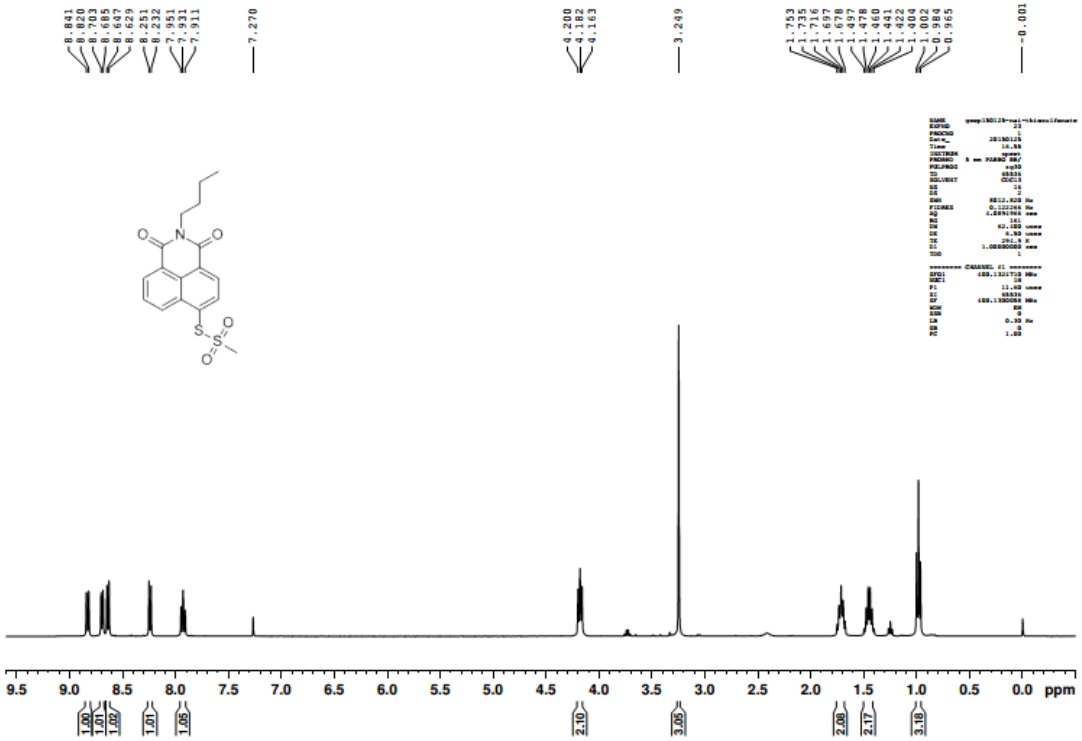


Figure S3. ^{1}H NMR Spectrum of Nap-TS in $CDCl_3$ (400 MHz)**.**

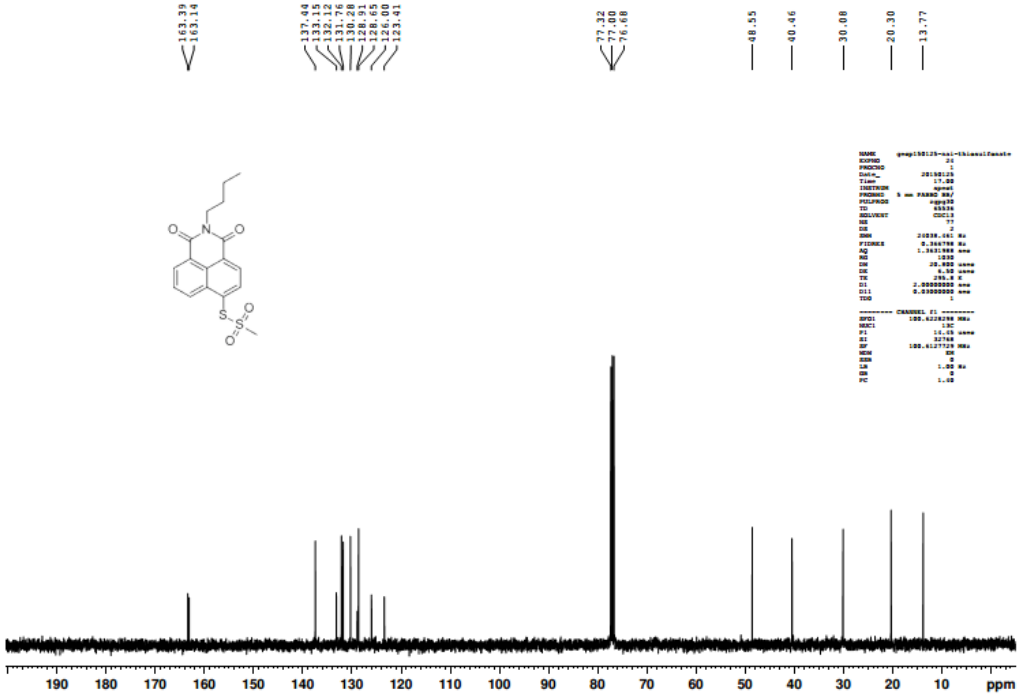


Figure S4. ^{13}C NMR Spectrum of Nap-TS in $CDCl_3$ (100 MHz)**.**

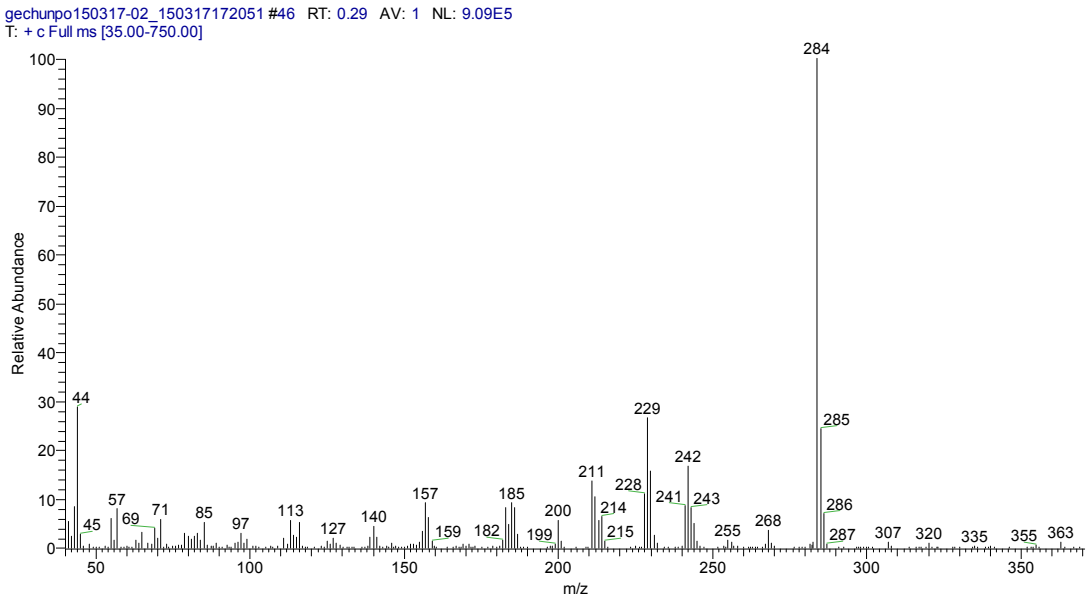


Figure S5. EI-Mass spectrum of Nap-TS (EI-MS).

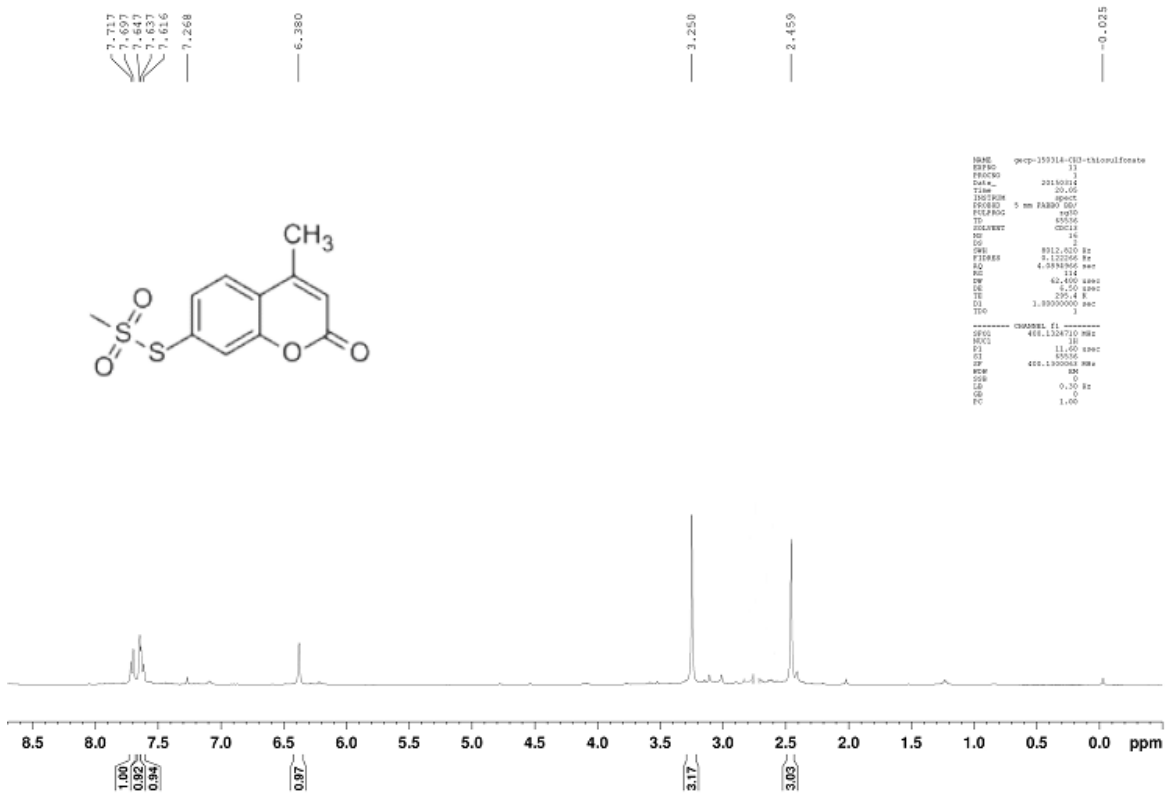


Figure S6. ^{1}H NMR Spectrum of MC-TS in $CDCl_3$ (400 MHz)**.**

Figure S7. ^{13}C NMR Spectrum of MC-TS in $CDCl_3$ (100 MHz)**.**

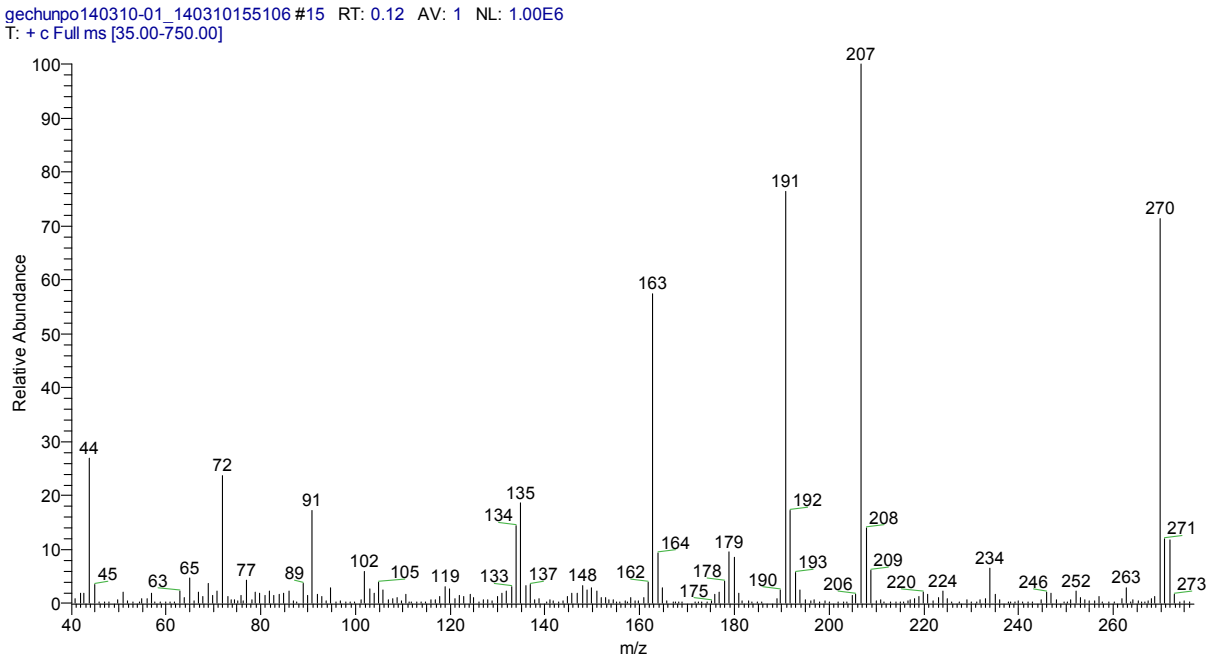


Figure S8. EI-Mass spectrum of MC-TS (EI-MS).

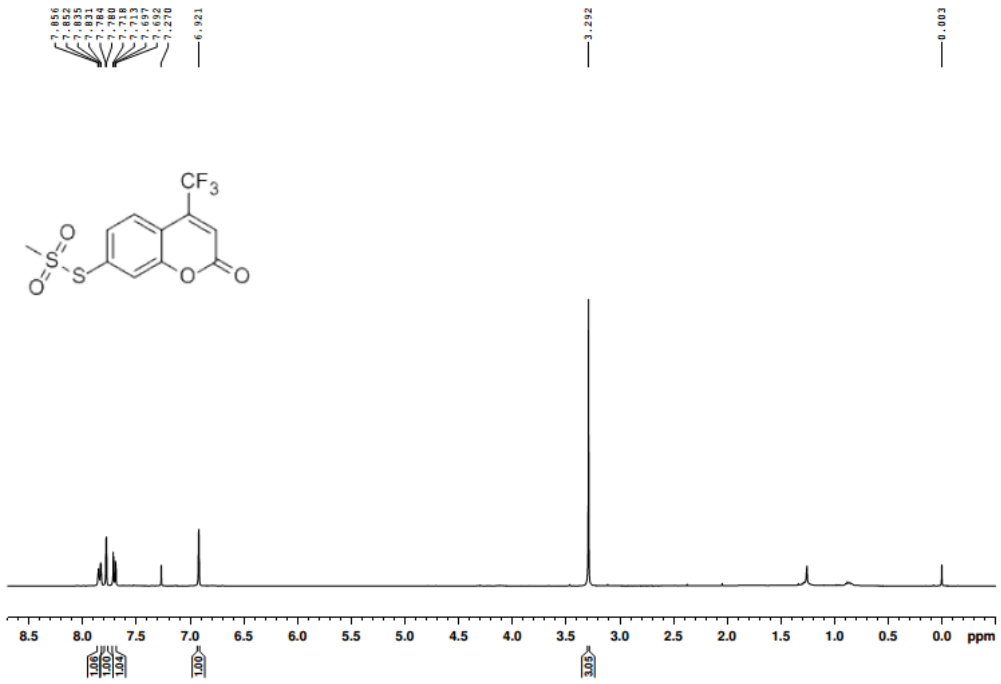


Figure S9. ^{1}H NMR Spectrum of TFMC-TS in $CDCl_3$ (400 MHz)**.**

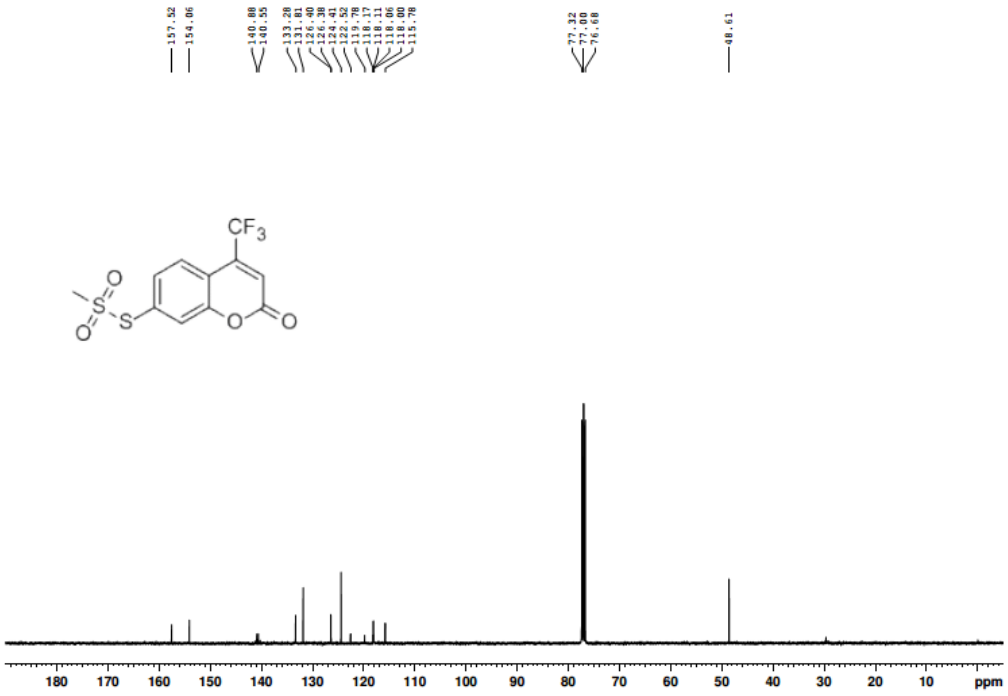


Figure S10. ^{13}C NMR Spectrum of TFMC-TS in $CDCl_3$ (100 MHz)**.**

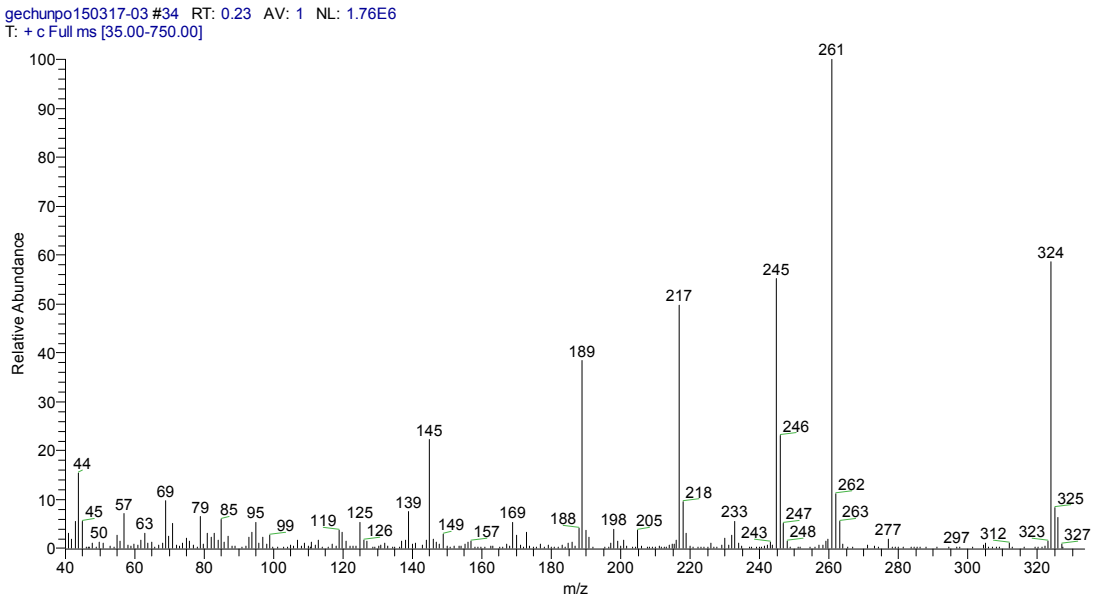


Figure S11. EI-Mass spectrum of TFMC-TS (EI-MS).

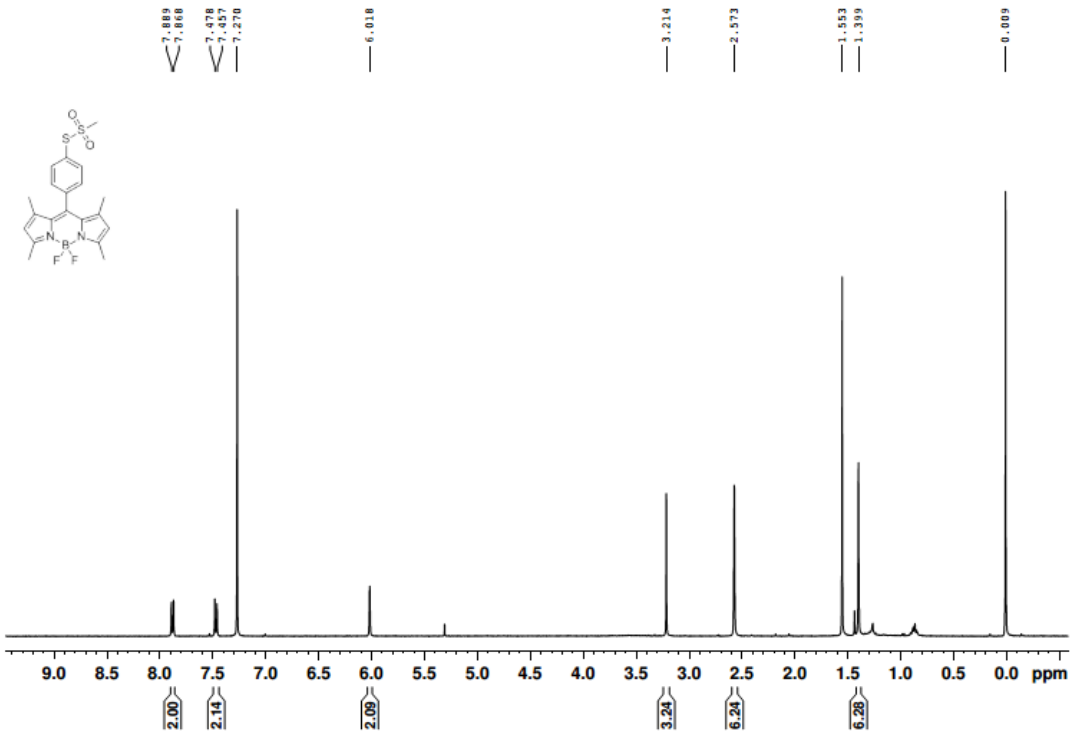


Figure S12. ^{1}H NMR Spectrum of BODIPY-TS in $CDCl_3$ (400 MHz)**.**

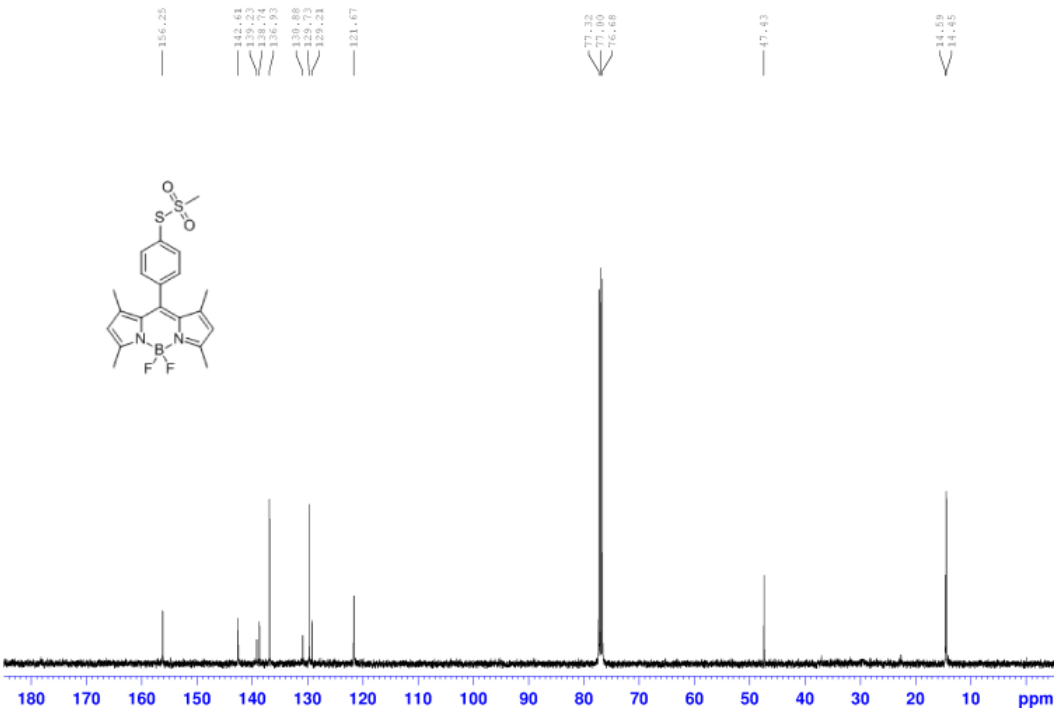


Figure S13. ^{13}C NMR Spectrum of BODIPY-TS in $CDCl_3$ (100 MHz)**.**

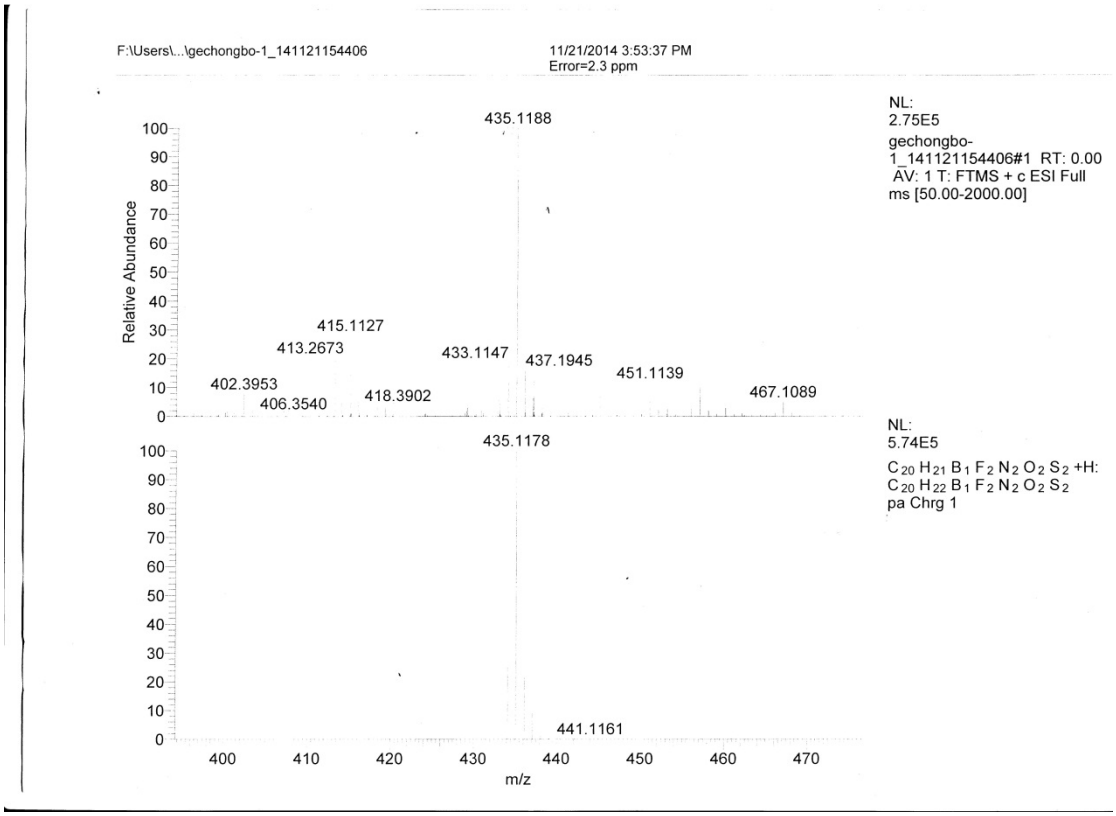


Figure S14. HRMS spectrum of BODIPY-TS (ESI).

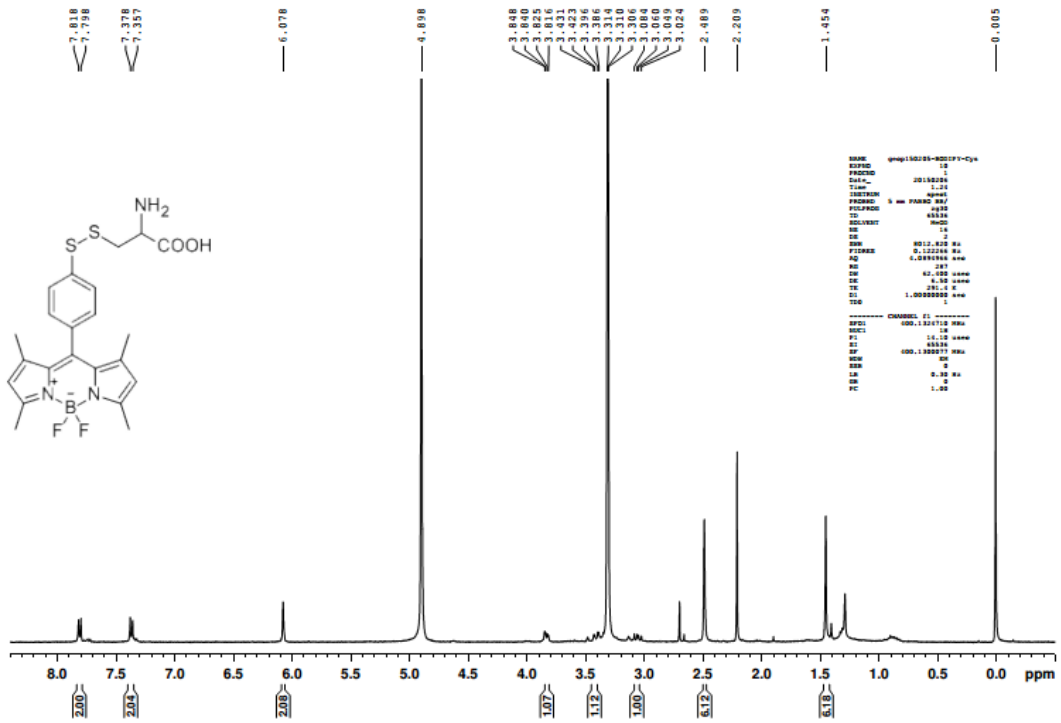


Figure S15. ^{1}H NMR Spectrum of Cys-S-S-BODIPY in CD_3 OD (400 MHz)**.**

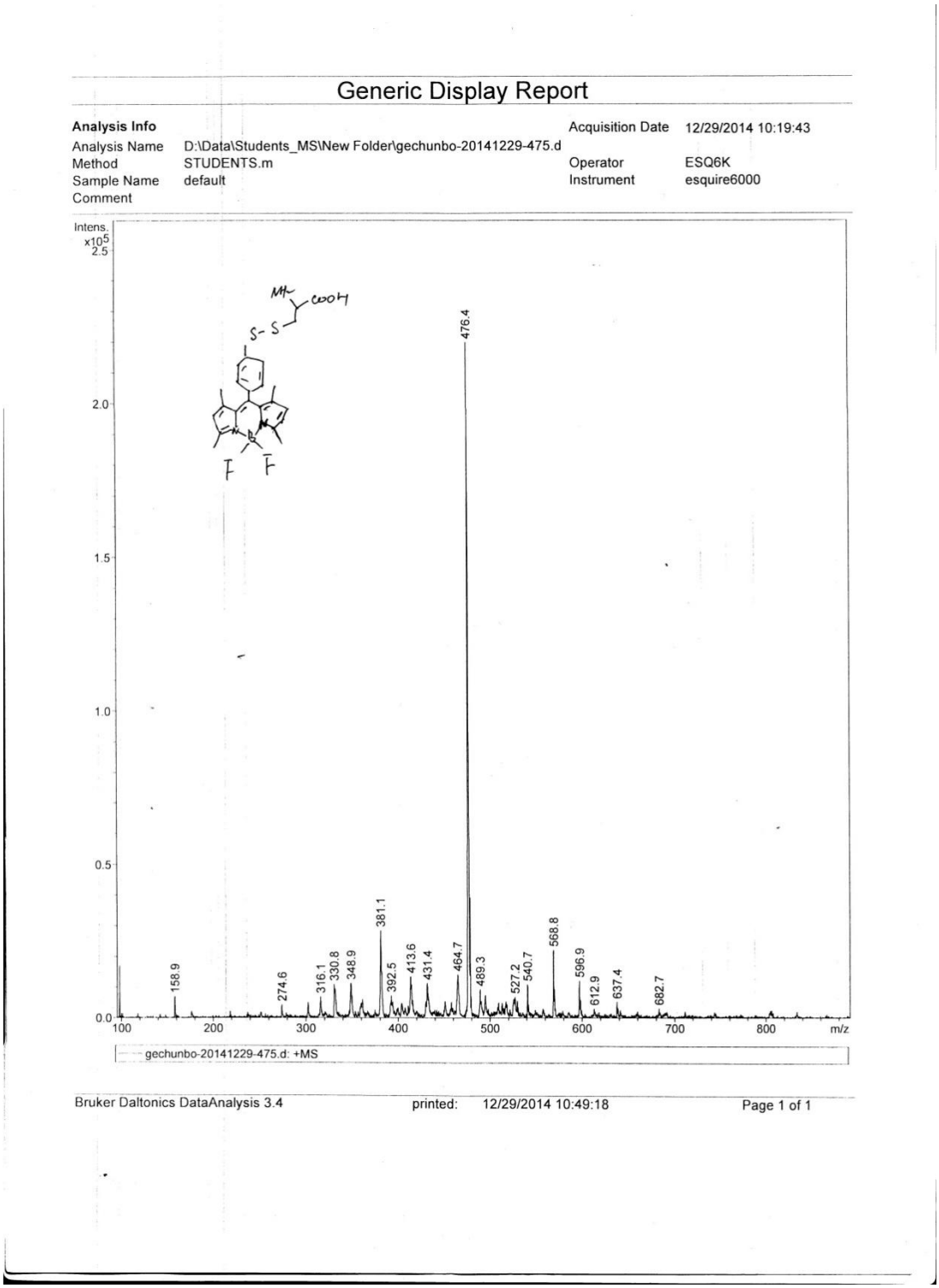


Figure S16. ESI-Mass spectrum of Cys-S-S-BODIPY (ESI-MS).

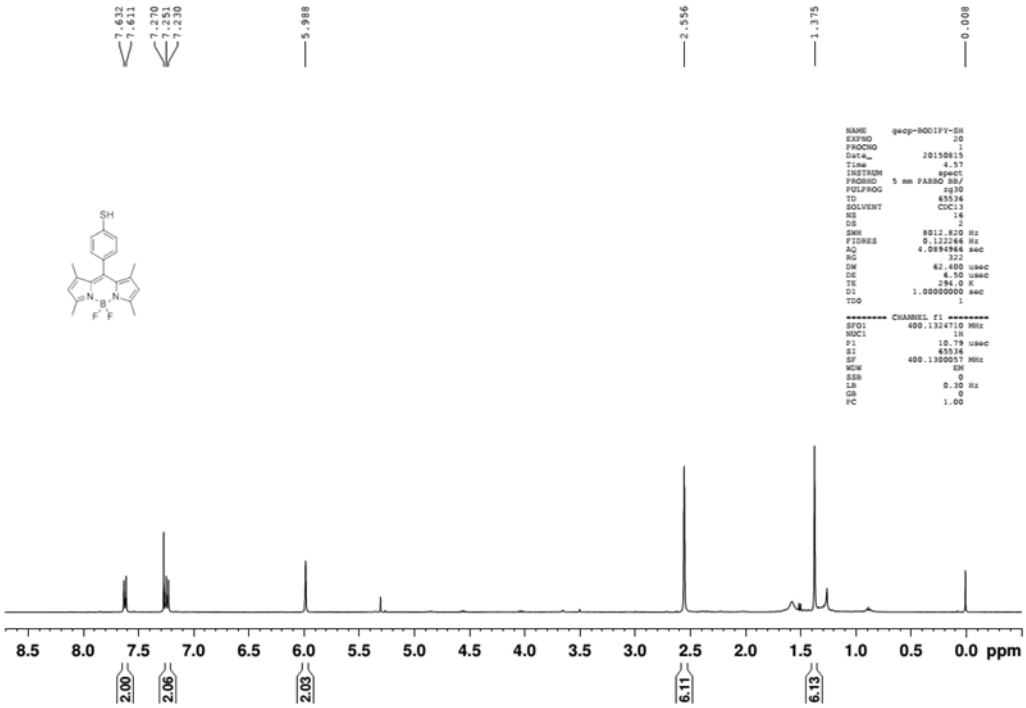


Figure S17. ^{1}H NMR Spectrum of BODIPY-SH in $CDCl_3$ (400 MHz)**.**

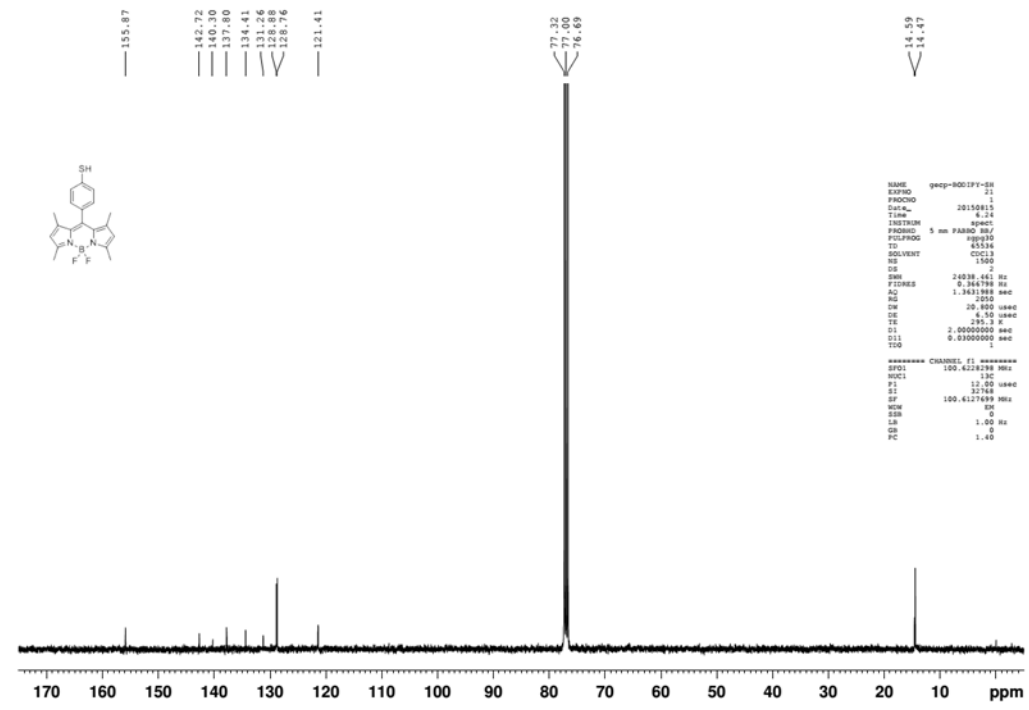


Figure S18. ^{13}C NMR Spectrum of BODIPY-SH in $CDCl_3$ (75 MHz)**.**

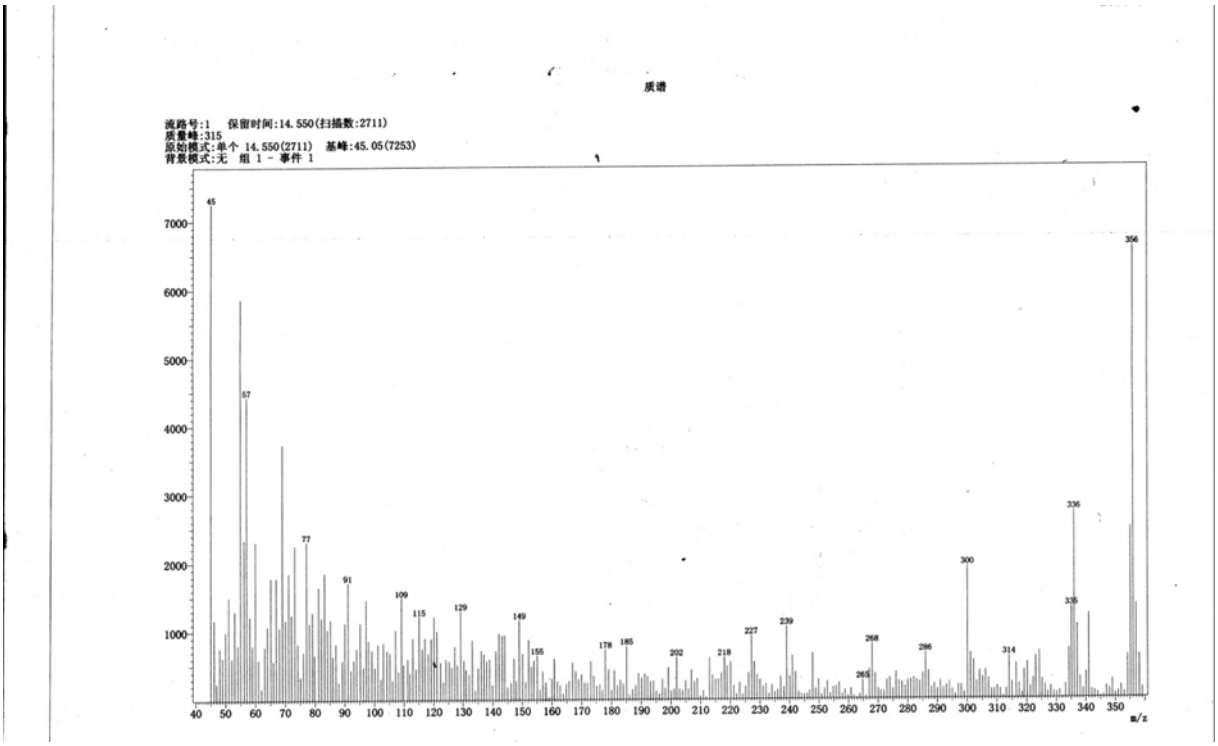


Figure S19. EI-Mass spectrum of BODIPY-SH (EI-MS).

EXPERIMENTAL SECTION

Materials and Instruments.

The HepG2 and HeLa cells were obtained from the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. Dulbecco's modified Eagle's medium (DMEM), GSH, Papain, dimethyl sulfoxide (DMSO), bovine insulin, N-α-Benzoyl-L-arginine 4-nitroanilide hydrochloride (BAPNA), DNCB, IAM and NEM were obtained from Sigma-Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS) was obtained from Sijiqing (Hangzhou, China). Thioredoxin (Trx) from *E. coli* was prepared in our lab.[1] Penicillin and streptomycin were obtained from Sangon (Shanghai, China). Sephadex G-25 was from GE Healthcare Life Sciences. All other reagents were of analytical grade and were purchased from commercial supplies. Melting points (mp) were determined on WRS-2 Melting Point Apparatus (Shanghai Precision & Scientific Instrument, Shanghai, China) and were uncorrected. Absorption spectra were recorded on UV−vis spectrometer evolution 200 (Thermo Scientific). Fluorescence studies were carried out using a LUMINA fluorescence spectrofluorometer (Thermo Scientific) at room temperature. The slit width was 5 nm for both excitation and emission. MS spectra were recorded on Trace DSQ GC-MS spectrometer or Bruker Daltonics esquire 6000 mass spectrometer. HRMS was obtained on Orbitrap Elite (Thermo Scientific). The absolute quantum yields (ϕ) of BODIPY-4-TS with and with Cys were determined on FLS920 spectrometer (Edinburgh Instruments, U.K.) with λ_{ex}=490 nm. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Advance 400, and tetramethylsilane (TMS) was used as a

reference.

Chemical synthesis

*Synthesis of N-butyl-4-bromo-1, 8-naphthalimide (**1**).*[2, 3] 4-Bromo-1, 8-naphthalic anhydride (0.5 g, 1.8 mmol) was dissolved in 25 mL of EtOH, and *n*-butylamine (0.18 mL, 1.8 mmol) was added. The mixture was heated at reflux for 6 hours and monitored by TLC. After cooling to room temperature, 50 mL of water were added and the solution was extracted with EtOAc. The organic phase was separated and dried with Na_2SO_4 and filtered. The filtrate was concentrated and the resulting residue was purified by column chromatography (petroleum ether/ethyl acetate=5:1) to provide the compound **1** as light yellow solid (520 mg, 87%). ^{1}HNMR (400 MHz, $CDCl_3$), δ（ppm）8.63-8.61 (dd, J_1 = 8 Hz, J_2 = 0.8 Hz, 1H, Ar-H), 8.53- 8.51 (dd, J_1 = 8 Hz, J_2 = 0.8 Hz, 1H, Ar-H), 8.39-8.37 (d, J = 8 Hz, 1H, Ar-H), 8.01-7.99 (d, J = 8 Hz, 1H, Ar-H), 7.84-7.78 (q, J = 8 Hz, 1H, Ar-H), 4.16 (t, J = 8 Hz, 2H, N–CH_2), 1.75-1.67 (m, 2H, -CH_2), 1.49-1.40 (m, 2H, -CH_2), 0.98 (t, J = 8 Hz, 3H, -CH_3); ^{13}CNMR (100 MHz, $CDCl_3$), δ（ppm）163.6, 163.5, 133.1, 131.9, 131.1, 131.0, 130.5, 130.1, 128.9, 128.0, 123.1, 122.2, 40.3, 30.1, 20.3, 13.8.

*Synthesis of N-butyl-4-mercapto-1, 8-naphthalimide (**2**).*[4] *N*-Butyl-4-bromo-1, 8-naphthalimide (**1**, 330 mg, 1 mmol) and $Na_2S.9H_2O$ (600 mg, 2.5 mmol) were stirred in DMF (20 mL) at room temperature for 6 h. The mixture was poured into H_2O (50 mL), an appropriate volume of 2 N HCl was added to adjust the pH value of the resulting mixture between 1 and 2, and the precipitate was collected by filtration and dried to obtain the crude product as a yellow solid ,which was used in next reaction

without further purification.

Synthesis of N-butyl-4-methanesulfonothioate-1, 8-naphthalimide (Nap-TS).[5] A mixture of sodium methanesulfinate (0.43 g, 4.4 mmol), the compound **2** (0.3 g, 1.1 mmol), and NBS (0.4 g, 2.2 mmol) in CH_3CN (50 mL) was stirred at room temperature for 8 h. After the completion of the reaction, the reaction mixture was washed with water and extracted with ethyl acetate. The organic phase was separated and dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated and the resulting residue was purified by column chromatography (petroleum ether/ethyl acetate=2:1) to provide Nap-TS as light yellow solid (255 mg, 70%). ^{1}HNMR (400 MHz, $CDCl_3$), δ（ppm）8.84- 8.82 (d, *J* = 8Hz, 1H, Ar-H), 8.71-8.69 (d, *J* = 8Hz, 1H, Ar-H), 8.65-8.63 (d, *J* = 8Hz, 1H, Ar-H), 8.25-8.23 (d, *J* = 8Hz, 1H, Ar-H), 7.93 (t, *J* = 8Hz, 1H, Ar-H), 4.18 (t, *J* = 8 Hz, 2H, -NCH_2), 1.75-1.69 (m, 2H, -CH_2), 1.49-1.41 (m, 2H, -CH_2), 0.98 (t, *J* = 8 Hz, 3H, -CH_3); ^{13}CNMR (100 MHz, $CDCl_3$), δ（ppm）163.4, 163.1, 137.4, 133.2, 132.1, 131.8, 130.3, 128.9, 128.7, 126.0, 123.4, 48.6, 40.5, 30.1, 20.3, 13.8; m. p.: 134.5-135.7 ^{o}C. EI-MS (m/z) (%): 363 (M^+, 1.2), 284 (100), 229 (26.5)

*Synthesis of 4-methyl-7-[(N, N-dimethyl)-thiocarbamoyloxy]-coumarin (**3a**).*[6] 4-Methyl-7-hydroxylcoumarin (1.76g, 10 mmol), which was readily prepared from rescorcinol following our published procedure,[2] and DMAP (2.43 g, 20 mmol) were dissolved in dry DMF (20 mL) in a 100 mL flask. The mixture was allowed to stir for 5 min, then N, N-dimethylthiocarbamoyl chloride (DMTCC) (2.46 g, 20 mmol) was added and the mixture was stirred at room temperature overnight. The reaction was

quenched by pouring on ice. The solid was collected via filtration, washed with water (×3), and then dried to obtain the crude product as a white solid, which was used in next reaction without further purification.

*4-Trifluoromethyl-7-[(N, N-dimethyl)-thiocarbamoyloxy]-coumarin (**3b**).*[6] Compound **3b** was synthesized from 4-trifluoromethyl-7-hydroxylcoumarin, which was readily prepared by our published procedure,[2] following the same procedures as the preparation of **3a**, and was used directly for the next step.

*Synthesis of 4-methyl-7-[(N, N-dimethyl)-carbamoylmercapto]-coumarin (**4a**).*[6] The crude product (**3a**) (1.0 g, 3.8 mmol) was placed in a round bottom flask that was fitted with a reflux condenser. The apparatus was maintained under an argon atmosphere and the reaction heated in an oil bath to 210 ~ 220 °C. The reaction was not stopped until TLC analysis indicated that the starting material had been consumed. The flask was cooled and the brown solid dissolved in $CHCl_3$, and purified with slica gel column chromatography (petroleum ether/ethyl acetate=3:1) to afford compound (**4a**). ^{1}HNMR (400 MHz, $CDCl_3$),7.58-7.56 (d, J = 8Hz, 1H, Ar-H), 7.45 (s, 1H, Ar-H), 7.41-7.39 (d, J = 8Hz, 1H, Ar-H), 6.27 (s, 1H, =CH), 3.10 (s, 3H, N-H), 3.01 (s, 3H, N-H), 3.40 (s, 3H, -CH_3); ^{13}CNMR (100 MHz, $CDCl_3$), δ（ppm）165.1, 160.1, 152.9, 151.8, 133.3, 130.9, 124.4, 123.2, 120.1, 115.6, 36.8, 18.5.

*Synthesis of 4-trifluoromethyl-7-[(N, N-dimethyl)-carbamoylmercapto]-coumarin (**4b**).*[6] This compound was prepared according to the same procedure for the synthesis of **4a** using **3b** as starting material. ^{1}HNMR (400 MHz, $CDCl_3$), δ（ppm）7.73-7.71 (dd, J_1 = 8 Hz, J_2 = 1.6 Hz, 1H, Ar-H),7.59 (d, J = 1.6 Hz, 1H, Ar-H), 7.51-7.49 (dd,

J_1 = 8 Hz, J_2 = 1.6 Hz, 1H, Ar-H), 6.83 (s, 1H, = CH), 3.12 (s, 3H, N-CH_3), 3.06 (s, 3H, N-CH_3); ^{13}CNMR (100 MHz, $CDCl_3$), δ（ppm）164.7, 158.4, 153.8, 135.7, 131.5, 125.2, 125.1, 123.8, 116.6, 116.5, 113.7, 36.9.

*Synthesis of 4-methyl-7-mercaptocoumarin (**5a**).*[6] Compound **4a** (1.0g, 3.8 mmol) was hydrolyzed in the presence of $NaOCH_3/CH_3OH$ (5mL, 25% w/v) in 60mL of MeOH under nitrogen for 8 h. The reaction mixture was then acidified with HCl. The solution was extracted with $CHCl_3$ (30 mL×3), and dried with Na_2SO_4. The compound was obtained by removal of the solvent and was used directly for the synthesis of **6a** without further purification.

*Synthesis of 4-trifluoromethyl-7-mercaptocoumarin (**5b**).*[6] Compound **5b** was synthesized from **4b** following the same procedures as the preparation of **5a**, and was used directly for the next step without further purification.

Synthesis of 4-methyl-7-methanesulfonothioatecoumarin (MC-TS).[5] A mixture of sodium methanesulfinate (1.55 g, 15.2 mmol), the crude product **5a** (0.73 g, 3.8 mmol), and NBS (1.35 g, 7.6 mmol) in CH_3CN (50 mL) was stirred at room temperature for 8 h. After the completion of the reaction, the reaction mixture was washed with water and extracted with ethyl acetate. The organic phase was separated and dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated and the resulting residue was purified by column chromatography (petroleum ether/ethyl acetate=3:1) to provide compound MC-TS as light yellow solid (760 mg, 75% yield). ^{1}HNMR (400 MHz, $CDCl_3$), δ（ppm）7.72-7.70 (d, J = 8Hz, 1H, Ar-H), 7.65 (s, 1H, Ar-H), 7.64-7.62 (d, J = 8Hz, 1H, Ar-H), 6.38 (s, 1H, =CH), 3.25 (s, 3H, -CH_3), 2.46

(s, 3H, -CH_3); ^{13}CNMR (100 MHz, $CDCl_3$), δ（ppm）159.5, 153.2, 151.2, 131.4, 131.2, 125.8, 123.8, 122.3, 116.9, 48.1, 18.6; mp: 149.3-152.5 °C; EI-MS (m/z) (%): 270 (M^+, 71.2), 207 (100), 191 (76.3).

4-Trifluoromethyl-7-methanesulfonothioatecoumarin (TFMC-TS).[5] This compound was prepared according to the same procedure for the synthesis of MC-TS using **5b** as starting material.^{1}HNMR (400 MHz, $CDCl_3$), δ（ppm）7.86-7.83 (dd, J_1 = 8 Hz, J_2 = 1.6 Hz, 1H, Ar-H), 7.78 (d, J = 1.6 Hz, 1H, Ar-H), 7.72-7.69 (dd, J_1 = 8 Hz, J_2 = 2 Hz, 1H, Ar-H), 6.92 (s, 1H, = CH) , 3.29 (s, 3H, -CH_3); ^{13}CNMR (100 MHz, $CDCl_3$), δ（ppm）157.5, 154.1, 140.8(q, CF_3, J = 132 Hz), 133.3, 131.8, 126.4, 124.4, 122.5, 119.8, 118.1(q, CF_3-C, J = 24 Hz), 115.8, 48.6; mp: 159.5-160.0 °C; EI-MS (m/z) (%): 324 (M^+, 58.4), 261 (100), 245 (55.1).

*Synthesis of O-(4-formylphenyl)-dimethylcarbamothioate (**6**).*[7] Commercially available 4-hydroxybenzaldehyde (1.22 g, 10 mmol) and 1, 4 diazabicyclo-[2.2.2]-octane (DABCO) (2.24 g, 20 mmol) were dissolved in dry DMF (20 mL) in a 100 mL flask. The mixture was allowed to stir for 5 min, and then N, N-dimethylthiocarbamoyl chloride (DMTCC) (2.46 g, 20 mmol) was added and the mixture was stirred at room temperature overnight. The reaction was quenched by pouring on ice. The solid was collected via filtration, washed with water (×3), and then dried to obtain the crude product as a white solid (1.65 g). The crude product was pure by ^{1}H and ^{13}C NMR analysis and was not further purified. ^{1}HNMR (300 MHz, $CDCl_3$), δ (ppm) 10.00 (s, 1H, -CHO), 7.94-7.91 (d, J = 9 Hz, 2H, Ar-H), 7.26-7.23 (d, J = 9 Hz, 2H, Ar-H), 3.45 (s, 3H, N-H), 3.36 (s, 3H, N-H); ^{13}CNMR (75 MHz, $CDCl_3$),

δ(ppm)190.8, 186.4, 158.4, 133.8, 130.7, 123.6, 43.1, 38.8.

*Synthesis of S-(4-formylphenyl) dimethylcarbamothioate (**7**).*[7] The crude product (**6**) (1.0 g, 4.8 mmol) was placed in a round bottom flask that was fitted with a reflux condenser. The apparatus was maintained under an argon atmosphere and the reaction heated in an oil bath to 200 °C. The reaction was not stopped until TLC analysis indicated that the starting material had been consumed. The flask was cooled and the brown solid dissolved in dichloromethylene, and directly purified with silica gel column chromatography (petroleum ether/ethyl acetate=3:1) to afford the compound (750 mg, 75 % yield). 1HNMR (400 MHz, CDCl3), δ（ppm）10.04 (s, 1H, -CHO),7.89-7.87 (d, 2H, J = 8 Hz, Ar-H), 7.69 - 7.67 (d, 2H, J = 8 Hz, Ar-H), 3.12 (s, 3H, N-H), 3.05 (s, 3H, N-H); 13CNMR (100 MHz, CDCl3), δ（ppm） 191.4, 164.9, 136.5, 135.8, 135.3, 129.4, 36.7.

*Synthesis of 4-mercaptobenzaldehyde (**8**).*[7] Compound **7** (297 mg, 1.4 mmol) was dissolved in KOH (5N, 3.2 mL) and MeOH (25.0 mL) in a 100 mL round bottom flask. The reaction was heated to reflux for 2-2.5 h, and the yellow solution was cooled and the MeOH was evaporated. HCl (3 M) was then added to acidify the solution. The yellow solution was extracted with EtOAc (20 mL×2), and the organic layers were combined, washed with brine, dried with Na_2SO_4, filtered and concentrated. The light brown oil obtained was directly used for the next step without further purification.

*Synthesis of S-(4-formylphenyl) methanesulfonothioate (**9**).*[5] A mixture of sodium methanesulfinate (0.8 mmol), the crude product **8**, and NBS (0.4 mmol) in CH_3CN (15 mL) was stirred at room temperature for 8 h. After the completion of the reaction, the

reaction mixture was washed with water and extracted with ethyl acetate. The organic phase was separated and dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated and the resulting residue was purified by column chromatography (petroleum ether/ethyl acetate=3:1) to provide compound **9** as a write solid. ^{1}HNMR (400 MHz, $CDCl_3$), δ（ppm）10.07(s, 1H, -CHO), 7.99-7.97 (d, 2H, *J* = 8 Hz, Ar-H), 7.90-7.88 (d, 2H, *J* = 8 Hz, Ar-H), 3.23 (s, 3H, -CH_3); ^{13}CNMR (100 MHz, $CDCl_3$), δ（ppm）191.0, 137.8, 136.4, 134.3, 130.4, 48.1.

Synthesis of BODIPY-TS.[8] 2, 4-Dimethylpyrrole (190 mg, 2 mmol) and **9** (216 mg, 1 mmol) were dissolved in CH_2Cl_2 with a catalytic amount of TFA (1-2 drops). The mixture was stirred for 16 h at room temperature. Then a solution of 2, 3-dichloro-5, 6-dicyanobenzoquinone (DDQ) (227 mg, 1 mmol) in CH_2Cl_2 was added dropwisely, and the mixture was stirred for 30 min. Finally, $BF_3 \cdot OEt_2$ (2 mL, excess) and triethylamine (2 mL, excess) were added, and the mixture was stirred for 3 h at room temperature. The crude mixture was diluted with CH_2Cl_2 and washed with H_2O. The organic extracts were dried over $MgSO_4$, filtered and evaporated under reduced pressure. Flash chromatography (hexane/CH_2Cl_2=1:1) afforded 75 mg of BODIPY-TS as an orange solid (17% yield). ^{1}HNMR (400 MHz, $CDCl_3$), δ（ppm）7.89 – 7.87 (d, 2H, *J* = 8 Hz, Ar-H), 7.48- 7.46 (d, 2H, *J* = 8 Hz, Ar-H), 6.02 (s, 2H, =CH), 3.21 (s, 3H, CH_3), 2.57 (s, 6H, CH_3), 1.39 (s, 6H, CH_3); ^{13}CNMR (100 MHz, $CDCl_3$), δ（ppm）156.3, 142.6, 139.2, 138.7, 136.9, 130.9, 129.7, 129.2, 121.7, 47.4, 14.6, 14.5; mp: 159.4-163.8 ^{o}C; HRMS (m/z): $[M+H]^+$ calcd. for 435.1178, found 435.1188.

Reaction of BODIPY-TS with Cys. BODIPY-TS (30 mg, 0.07 mmol) were

dissolved in 10 mL of EtOH, and Cys (7.9 mg, 0.065 mmol) which were dissolved in 2 mL of H_2O was added. The mixture was stirred at room temperature. The reaction was allowed to progress until TLC analysis indicated that the starting material had been consumed (~5 min). The mixture was adsorbed on silica gel and directly purified with column chromatography (CH_2Cl_2/CH_3OH=3:1) to afford compound Cys-S-S-BODIPY (26 mg, 85% yield). ^{1}HNMR (400 MHz, CD_3OD), δ（ppm）7.82-7.80 (d, 2H, *J* = 8 Hz, Ar-H), 7.38-7.36(d, 2H, *J* = 8 Hz, Ar-H), 6.08 (s, 2H, =CH), 3.85-3.82 (m, 1H, N-CH), 3.43-3.89 (m, 1H, S-CH), 3.08-3.02 (m, 1H, S-CH), 2.49 (s, 6H, -CH_3), 1.45 (s, 6H, -CH_3); ESI-MS(m/z): $[M+H]^+$ 476.4.

Reaction of BODIPY-TS with excess Cys. To a solution of BODIPY-TS (20 mg, 0.047 mmol, in 5 mL EtOH) was added Cys (30 mg, 0.25 mmol, in 5 mL H_2O). The reaction mixture was stirred at room temperature, and the reaction progress was monitored by TLC. After 30 min, the starting material disappeared. The solvent was removed in vacuo, and resulting mixture was purified by silica gel column chromatography (hexane/CH_2Cl_2=1:1) to afford compound BODIPY-SH (13 mg, 78% yield). ^{1}HNMR (400 MHz, $CDCl_3$), δ（ppm）7.63-7.61 (d, 2H, *J* = 8 Hz, Ar-H), 7.25-7.23(d, 2H, *J* = 8 Hz, Ar-H), 5.99 (s, 2H, =CH), 2.56 (s, 6H, -CH_3), 1.38 (s, 6H, -CH_3); ^{13}CNMR (75 MHz, $CDCl_3$), δ (ppm) 155.8, 142.7, 140.3, 137.8, 134.4, 131.3, 128.9, 128.8, 121.4, 14.6, 14.5. EI-MS (m/z) (%): 356 (M^+, 100), 341 (20.7), 336 (41.8), 300 (31.3).

Absorbance and fluorescence spectra measurement

Different probes were incubated with equal concentration of Cys at room temperature in PBS. The absorbance and emission spectra were scanned every 1 min. For the absorbance spectra, the concentrations of Nap-TS, MC-TS, TFMC-TS and BODIPY-TS were 40 μM, 40 μM, 40 μM and 10 μM, respectively. For the fluorescence spectra, the concentration of all probes is 10 μM. The excitation wavelengths of Nap-TS, MC-TS, TFMC-TS and BODIPY-TS were 450 nm, 350 nm, 350 nm and 490 nm, respectively.

Response of BODIPY-TS to Cys

The time-dependent absorbance spectra and fluorescence spectra (λ_{ex}=490 nm) were acquired with the following procedures: BODIPY-TS (5 μM) was incubated with Cys (5μM) at room temperature in PBS. The spectra were scanned every 1 min for 10 min. To acquire the emission spectra of BODIPY-TS toward different concentrations of Cys, BODIPY-TS (5 μM) was incubated with different concentrations of Cys at room temperature in PBS. The emission spectra (λ_{ex}=490 nm) were recorded after 5 min. The selectivity of BODIPY-TS toward different analytes was studied as the following procedures: The fold of fluorescence increase at 515 nm (λ_{ex}=490 nm) was determined after mixing BODIPY-TS with various analytes for 5 min in PBS. The concentrations of thiol compounds were 5 μM, while the concentrations of other analytes were 50 μM.

pH-Dependent fluorescence response of BODIPY-TS to Cys

The folds of fluorescence change at 515 nm (λ_{ex}=490 nm) were determined after mixing BODIPY-TS (5 μM) with Cys (5 μM) at room temperature for 5 min in

different buffers (pH 2 −12).

Imaging Thiols in Live HepG2 Cells

HepG2 cells were cultured in DMEM supplemented with 10% FBS, 2 mM glutamine, and 100 units/mL penicillin/streptomycin and maintained in an atmosphere of 5% CO_2 at 37 °C. The cells were seeded in 12-well plates at 2×10^4 cells per well in 1 mL growth medium and incubated at 37 °C for 24 h, and then the cells were exposed to IAM (50 μM) or control PBS buffers for 30 min. The cells were further incubated with BODIPY-TS (0.5 μM) for 10 min at 37°C. After washing the cells with PBS three times, the fluorescence images were acquired with Floid cell imaging station (life technology).

Cytotoxicity Assay [9]

The cytotoxicity of NEM, IAM, DNCB and BODIPY-TS was determined by the MTT assay. Cells (5×10^3) were incubated with varying concentrations of the compounds in triplicate in a 96-well plate at 37 °C in a final volume of 100 μL. At the end of the treatment (20 h), 10 μL of MTT (5 mg/mL) was added to each well and incubated for an additional 4 h at 37 °C. An extraction buffer (100 μL, 10% SDS, 5% isobutanol, 0.1% HCl) was added, and the cells were incubated overnight at 37 °C. The absorbance was measured at 570 nm on Multiskan GO (Thermo Scientific). The cell viability was expressed as the percentage of the control (cells without drug treatment).

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