Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

for

FRET based fluorescent ratiometric probes for rapid detection of endogenous hydrogen sulphide in living cells

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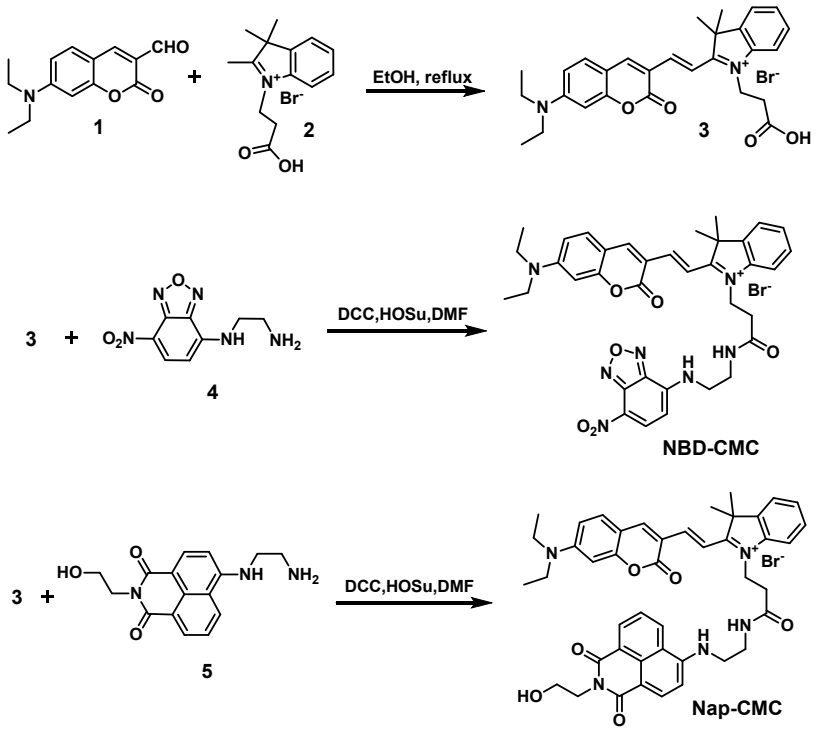
1. Materials and general methods.

All the solvents used in sensor preparation were of analytic grade, while the solvents used in spectroscopic detection were of HPLC grade, and triple-distilled water obtained from Millipore was used throughout the analytical experiments. The stock solutions of all the tested anions were prepared from $NaHCO_3$, $NaNO_3$, NaCl, NaBr, NaI, NaF, Na_2SO_4, Na_2CO_3, Na_2SO_3, Na_2SO_3, Na_2SCN, $[N(C_4H_9)_4]^+CN^-$ with doubly distilled water. The stock solutions of NaSH, cysteine, glutathione, and homocysteine were prepared in PBS solution (20 mM, pH=7.40). The ^1H NMR and ^{13}C NMR spectra were recorded on Bruker DRX-500 with TMS as internal reference. High resolution mass spectrometric data were determined with an Agilent 6540Q-TOF HPLC-MS spectrometer. Fluorescence measurements were performed on FluoroMax-4 Spectrofluorometer with 5 nm slit for both excitation and emission. Absorption spectra were measured on a Shimadzu UV-3100 spectrophotometer. All pH measurements were accomplished by a Model PHS-3C meter.

2. Synthesis and characterizations.



Scheme S1. Synthetic route of **CMCPM**, **CMCPP**, **NBD-CMC**, and **Nap-CMC**.

Synthesis of 3. Compound **1**[1] (245 mg, 1.0 mmol) and indolium derivative **2**[2] (312 mg, 1.0 mmol) were dissolved in 20 mL EtOH. The reaction mixture was refluxed with stirring for 12 h and monitored by TLC, then evaporated in vacuo. The residue was purified by column chromatography on silica gel (CH_2Cl_2/ MeOH, 20:1 v/v) to give **3** (275 mg,) as dark blue solid. Yield, 51%. ^1H NMR (500M, CD_3OD) δ 8.65 (*d*, 1H, *J* = 4.1 Hz), 8.38(*d*, 1H, *J* = 15.6 Hz), 8.05 (*d*, 1H, *J* =15.6 Hz), 7.75-7.80(*m*, 2H), 7.58-7.64 (*m*, 3H), 6.94 (*dd*, 1H, *J* = 2.0 Hz, *J* = 9.1 Hz), 6.64 (*d*, 1H, *J* = 2.0 Hz), 4.80 (*t*, 2H, *J* =6.9 Hz), 3.64 (*q*, 4H, *J* = 7.2 Hz), 2.99 (*t*, 2H, *J* = 6.9 Hz), 1.86 (*s*, 6H), 1.30 (*t*, 6H, *J* = 7.2 Hz). ^{13}C NMR (125M, CD_3OD) δ 181.9, 172.2, 160.2, 158.2, 154.8, 150.5, 149.8, 143.2, 140.8, 132.5, 129.0, 128.6, 122.5, 114.1, 112.4, 111.3, 110.2, 109.8,

96.5, 51.8, 45.1, 42.4, 32.2, 25.9, 11.5. ESI-HRMS for $[M]^+$: calc. 459.2284, found 459.2284.

Synthesis of NBD-CMC. Compound **3** (135 mg, 0.25 mmol), DCC (62 mg, 0.3 mmol), HOSu (49.5 mg, 0.3 mmol) and N^1-(7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)ethane-1,2-diamine[3] (**4**[3], 56 mg, 0.25 mmol) were added with 15 mL DMF. The solution was stirred for 12 h, and then the solvent was removed in vacuo. The residue was purified by silica gel column chromatography (CH_2Cl_2/ MeOH, 20:1 v/v), and pure compound **NBD-CMC** was obtained as blue powder (80 mg, 43%). ^{1}H NMR (500 M, DMSO-d_6) δ 9.22 (br, 1H), 8.66 (s, 1H), 8.48 (t, 1H, J = 5.5 Hz), 8.35 (d, 1H, J = 8.6 Hz), 8.20 (d, 1H, J = 15.7 Hz), 7.86-7.82 (m, 3H), 7.60-7.53 (m, 2H), 7.45 (d, 1H, J = 8.6 Hz), 6.85 (dd, 1H, J = 9.0 Hz, J = 1.8 Hz), 6.59 (d, 1H, J = 1.8 Hz), 6.26 (d, 1H, J = 9.0 Hz), 4.70 (t, 2H, J = 6.2 Hz), 3.56 (q, 4H, J = 7.0 Hz), 3.37 (br, 2H), 3.30 (m, 2H), 2.75 (d, 2H, J = 6.2 Hz), 1.76 (s, 6H), 1.20 (t, 6H, J = 7.0 Hz); ^{13}C NMR (124 M, DMSO-d_6) δ 181.9, 170.3, 159.6, 157.9, 154.5, 150.5, 150.2, 145.3, 144.6, 144.3, 143.6, 141.1, 138.0, 132.6, 129.3, 129.0, 123.3, 121.5, 115.0, 112.5, 111.8, 110.5, 109.8, 99.4, 96.9, 52.0, 45.4, 43.7, 43.5, 37.9, 33.8, 26.6, 12.9. HRMS: calc. 664.2884, found 664.2883 for $[M^+]$.

Synthesis of Nap-CMC. Coupound **3** (135 mg, 0.25 mmol), DCC (62 mg, 0.3 mmol), and 6-((2-aminoethyl)amino)-2-(2-hydroxyethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**5**[4], 75 mg, 0.25 mmol), HOSu (49.5 mg, 0.3 mmol) were added with 15 mL DMF. The solution was stirred for 12 h, and then the solvent was removed in vacuo. The residue was purified by silica gel column chromatography (CH_2Cl_2/ MeOH, 20:1 v/v), and pure compound **Nap-CMC** was obtained as blue powder (72 mg, 35%). ^{1}H NMR (500M, CD_3OD) δ 8.41(*d*, 1H, J =7.2 Hz), 8.26 (*d*, 1H, J =7.4 Hz), 8.12(*d*, 1H, J =7.4 Hz), 8.03-7.92(*m*, 3H), 7.76 (*t*, 2H, J =9.2 Hz), 7.64-7.54 (*m*, 3H), 7.23 (*d*, 1H, J =9.1 Hz), 6.73 (*d*, 1H, J =9.1 Hz), 6.64 (*d*, 1H, J =8.5 Hz), 6.31 (*s*, 1H), 4.81 (*t*, 2H, J =5.5 Hz), 4.24 (*t*, 2H, J =6.3 Hz), 3,80 (*t*, 2H, J =6.3 Hz), 3.57 (*q*, 4H, J =7.0 Hz), 3.52 (*t*, 2H, J =5.3 Hz), 3.37 (*t*, 2H, J =5.8 Hz), 2.94 (*t*, 2H, J =5.8 Hz), 1.86 (*s*, 6H), 1.30 (*t*, 6H, J =7.0 Hz). ^{13}C NMR (125M, CD_3OD) δ 182.2, 171.7, 164.7, 164.1, 159.6, 157.6, 154.6, 150.6, 149.4, 143.2, 140.6, 134.3, 132.0, 130.7, 129.5, 128.6, 127.7, 124.3, 122.6, 121.9, 120.2, 114.3, 111.9, 111.1, 109.8, 109.6, 108.3, 103.6, 96.2, 59.0, 51.8, 45.2, 44.1, 43.0, 41.4, 38.6, 33.3, 26.0, 11.6. HRMS: calc. 770.3448, found 770.3452 for $[M^+]$.

3. Detection limit.

The emission spectrum of 10 μM **NBD-CMC** and **Nap-CMC** in PBS buffer (20 mM, pH = 7.40, 1% DMSO, v/v) was collected for 20 times to determine the background noise σ_1 and σ_2. Then the solution was treated with various concentrations of NaHS from 0-10 μM, and all spectra were collected after adequate mixing for 60 s (for **NBD-CMC**) or 90 s (for **Nap-CMC**). Linear regression curves were then fitted according to the emission ratios, and the slope of the curves S_1 and S_2 were obtained, respectively. The detection limit (3σ/slope) was then determined to be ~0.5 μM for probe **NBD-CMC** and ~1.0 μM for probe **Nap-CMC**.

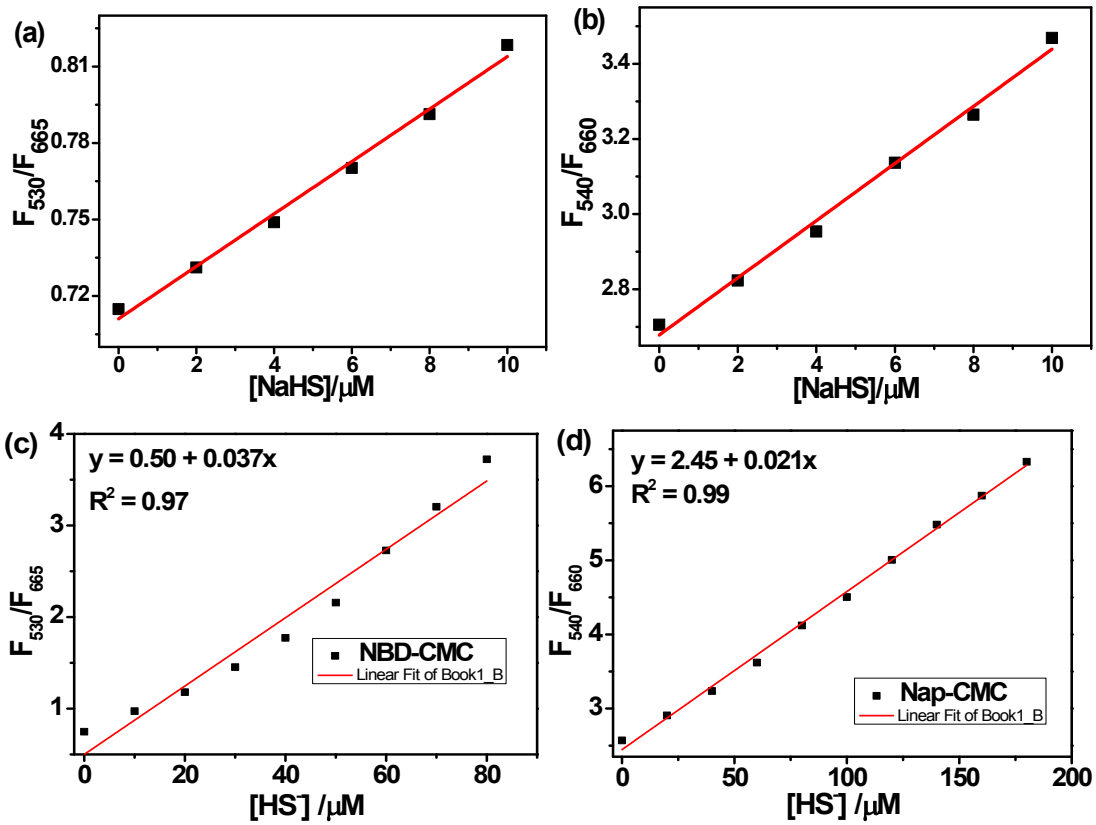


Figure S1. Emission ratios of 10 μM **NBD-CMC** (a) and **Nap-CMC** (b) in PBS buffer (20mM, pH = 7.40) containing 2% DMSO (v/v) as a function of HS^- concentration in the range of 0-10 μM. (c) Emission ratios of 10 μM **NBD-CMC** in PBS buffer (20mM, pH = 7.40) containing 2% DMSO (v/v) as a function of HS^- concentration in the range of 0-80 μM. (d) Emission ratios of 10 μM **Nap-CMC** in PBS buffer (20mM, pH = 7.40) containing 2% DMSO (v/v) as a function of HS^- concentration in the range of 0-180 μM.

4. Absorption HS^- titration of probe NBD-CMC.

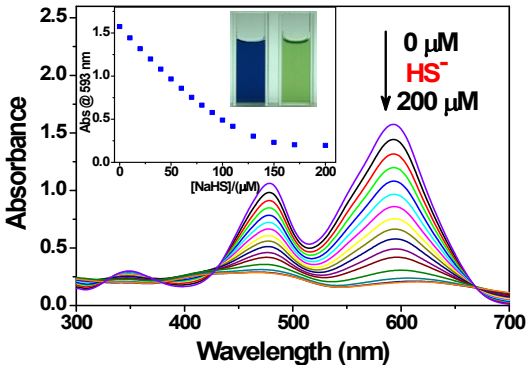


Figure S2. Absorption spectra of probe **NBD-CMC** (20 μM) in PBS solution (20 mM, pH=7.40, 2% DMSO) in response to different concentration of HS^-. Inset: absorbance of **NBD-CMC** at 593 nm against HS^- concentration and the colour change of **1** in response to HS^-.

5. Emission and absorption HS^- titration of probe Nap-CMC.

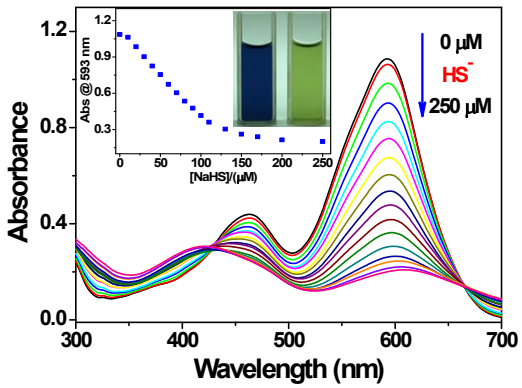


Figure S3. Absorption spectra of probe **Nap-CMC** (20 μM) in PBS solution (20 mM, pH=7.40, 2% DMSO) in response to different concentration of HS^-. Inset: absorbance of **Nap-CMC** at 593 nm against HS^- concentration and the colour change of **2** in response to HS^-.

6. Selectivity of probe Nap-CMC

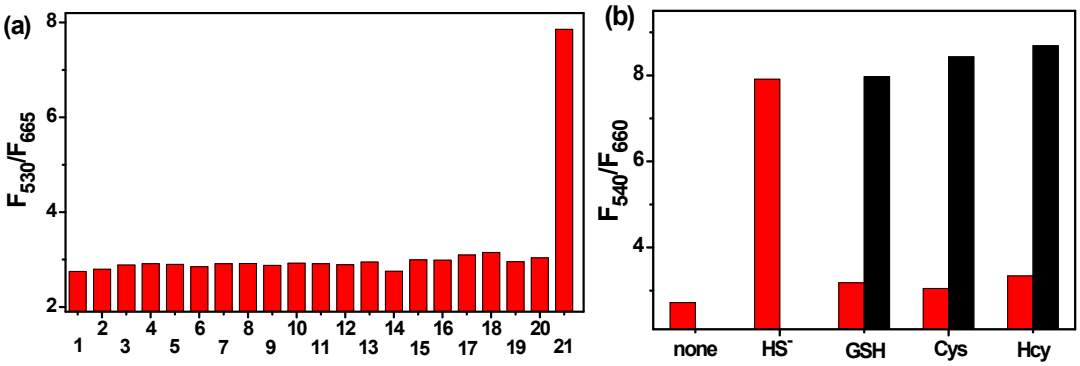


Figure S4. (a) Emission ratio F_{530}/F_{665} of 10 μM **Nap-CMC** in PBS buffer (20 mM, pH=7.40, 2% DMSO, v/v) in the presence of HS^-, various anions and bio-related species. 1. Blank, 2. NO_3^-, 3. HCO_3^-, 4. Cl^-, 5. Br^-, 6. I^-, 7. CN^-, 8. F^-, 9.$P_2O_7^{4-}$, 10. SO_4^{2-}, 11. SO_3^{2-}, 12.$S_2O_3^{2-}$, 13. SCN-, 14. $EtNH_2$, 15. EtOH, 16. NO, 17. S-nitrosoglutathione, 18. H_2O_2, 19.ClO^-, 20. O_2^-, (2-20: 1 mM), 21. HS^- (200 μM). (b) Emission ratio F_{530}/F_{665} of 10 μM **Nap-CMC** in the presence of HS^- (200 μM), Cys (5 mM), GSH (5 mM), Hcy (200 μM). For (b): Red bar for free **Nap-CMC** or **Nap-CMC** with HS^- or marked biothiols; black bar for **Nap-CMC** in the presence of both HS^- (200 μM) and the marked biothiols.

7. pH dependence.

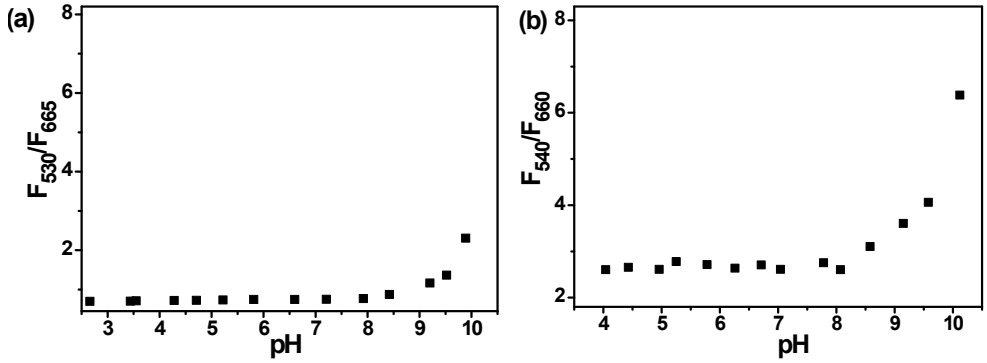


Figure S5. The emission ratios of 10 μM probe **NBD-CMC** (a) and **Nap-CMC** (b) at different pH in aqueous solution with 1% DMSO.

8. Confocal fluorescent imaging

HepG-2 cells were cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 and 95% air at 37 °C. The ratiometric imaging of HepG-2 cells was imaged by laser scanning confocal fluorescence microscope (Zeiss LSM710) via the dual emission mode. The excitation wavelength was 488 nm, the band path for green images was 500-600 nm and that for red images was 640-750 nm.

Cells were treated with 10 μM **NBD-CMC** or **Nap-CMC** for 1 h and the images were collected at each channel. Then, NaHS (200 μM) was added and incubated for 30 min and the images were collected at each channel.

As for the endogenous H_2S imaging assay, HepG-2 cells were pretreated with 200 μM Cys or GSH in the absence and presence of 50 mg/L PAG, respectively. After 30 min incubation, the cells were stained with probe **NBD-CMC** for 1 h and then the images of each channel were collected.

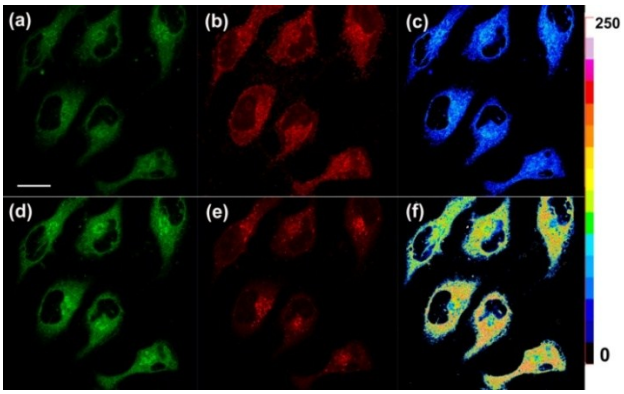


Fig. S6 Confocal fluorescent microscopic images of 10 μM **Nap-CMC** stained HepG-2 cells in the absence (a-c) and presence (d-f) of 250 μM NaHS. (a, d) Green channel: 500-600 nm; (b, e) red channel: 640-750 nm; (c, f) pseudo colour ratio images obtained from green channel and red channel. Scale bar: 20 μm.

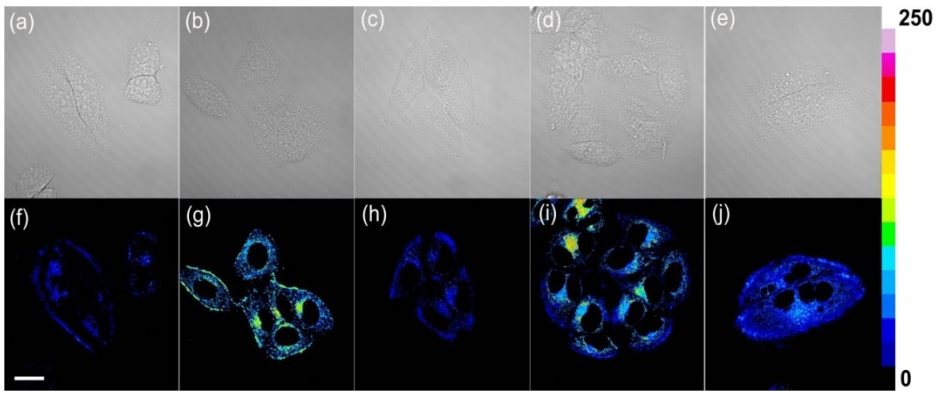


Fig. S7 Bright field images (a-e) and pseudo colour ratio images (f-j) of HepG-2 cells. (a, f) Control cells only

stained with 10 μM **NBD-CMC** for 1h; (b, g) cells pretreated with 200 μM Cys for 30 min, then stained with 10 μM **NBD-CMC** for 1h; (c, h) cells pretreated with 200 μM Cys and PAG for 30 min, then stained with 10 μM **NBD-CMC** for 1h; (d, i) cells pretreated with 200 μM GSH for 30 min, then stained with 10 μM **NBD-CMC** for 1h; (e, j) cells pretreated with 200 μM GSH and PAG for 30 min, then stained with 10 μM **NBD-CMC** for 1h. Scale bar: 20 μm.

9. Characterization spectra of the sensors

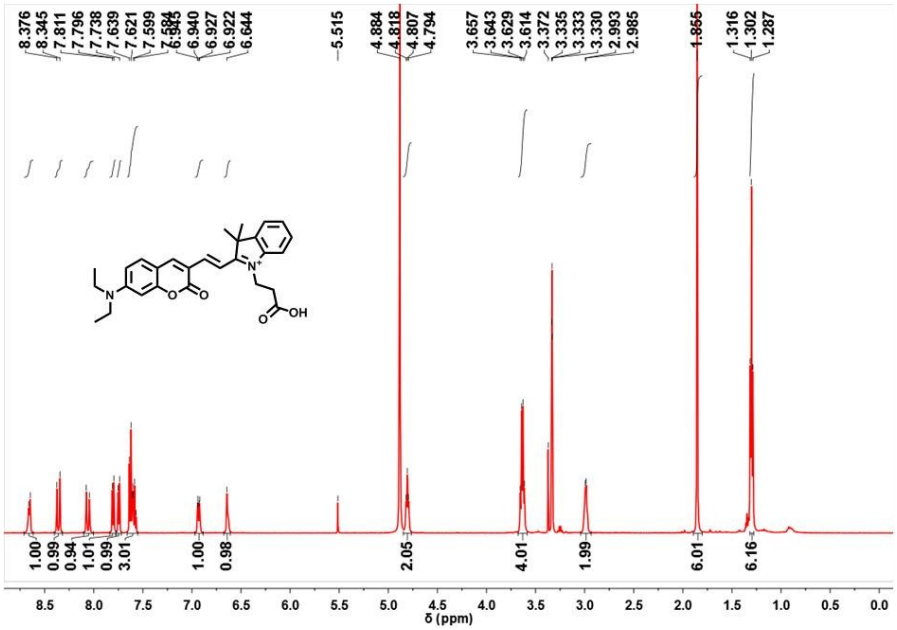


Figure S8. ^{1}H NMR spectrum of compound **3** in CD_3OD.

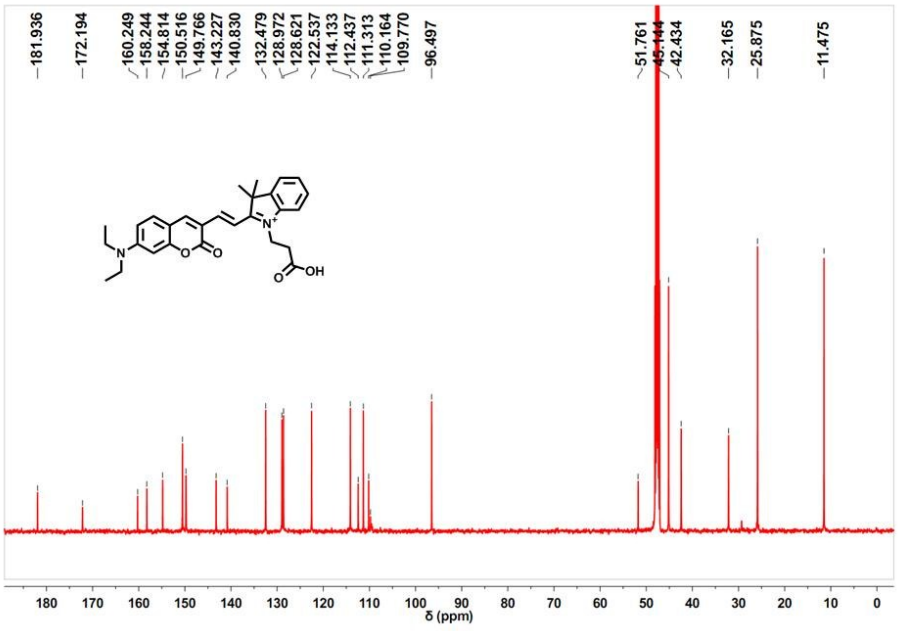


Figure S9. ^{13}C NMR spectrum of compound **3** in CD_3OD.

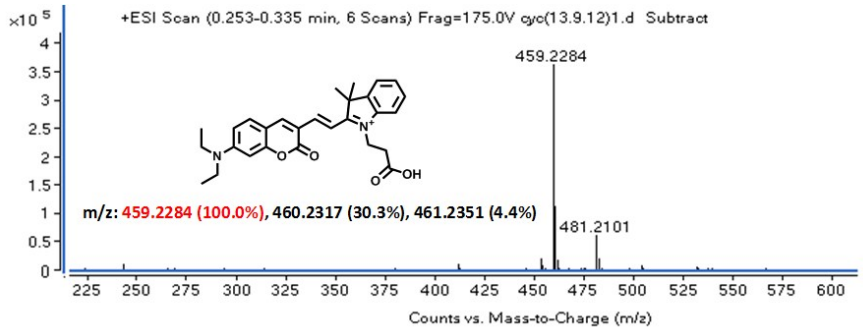


Figure S10. HRMS spectrum of compound **3**.

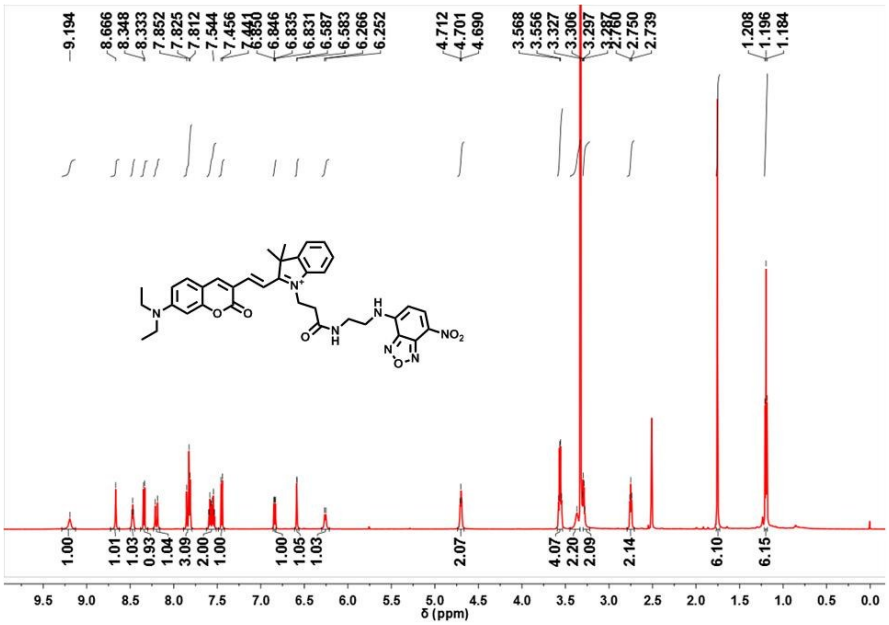


Figure S11. [1]H NMR spectrum of compound **NBD-CMC** in DMSO-*d*6.

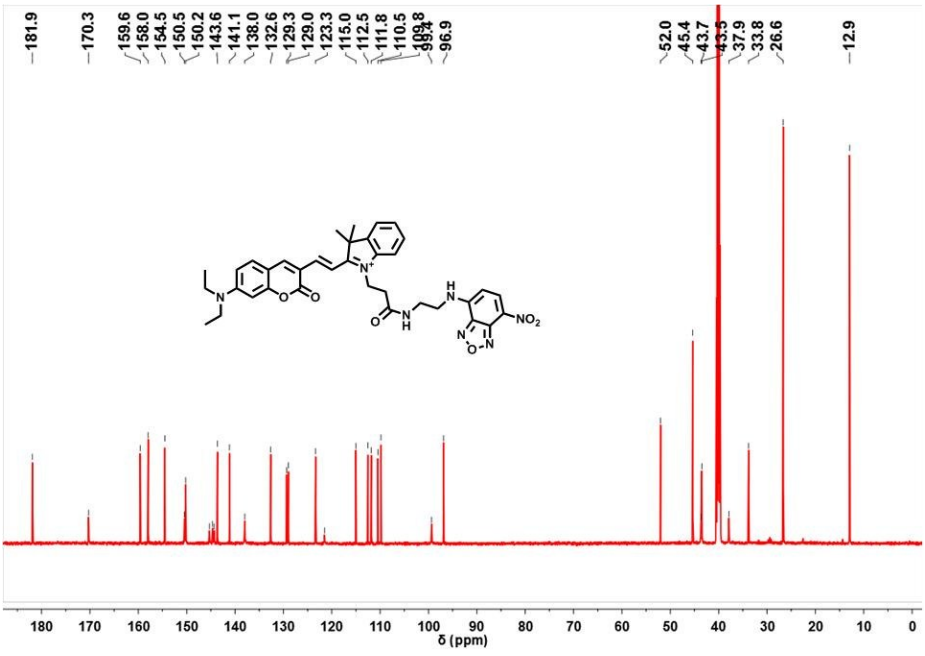


Figure S12. [13]C NMR spectrum of compound **NBD-CMC** in DMSO-*d*6.

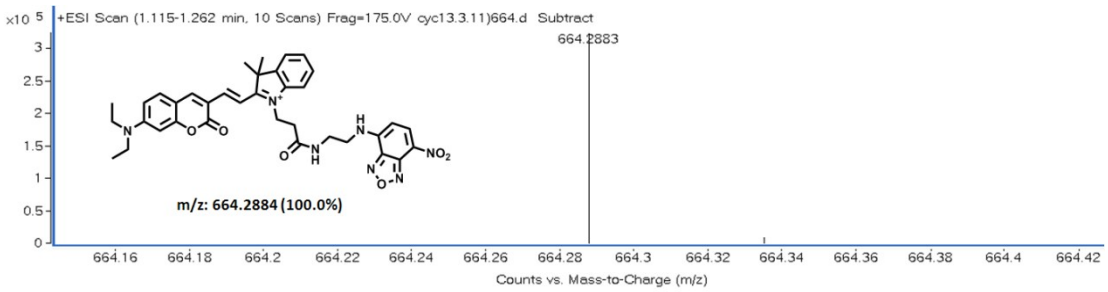


Figure S13. HRMS spectrum of compound **NBD-CMC**.

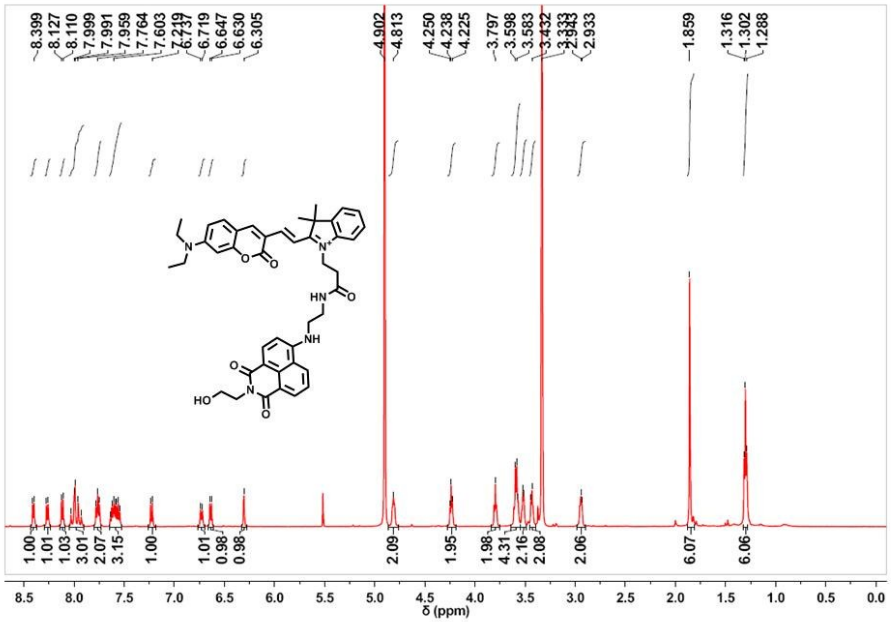


Figure S14. ^{1}H NMR spectrum of compound **Nap-CMC** in CD_3OD.

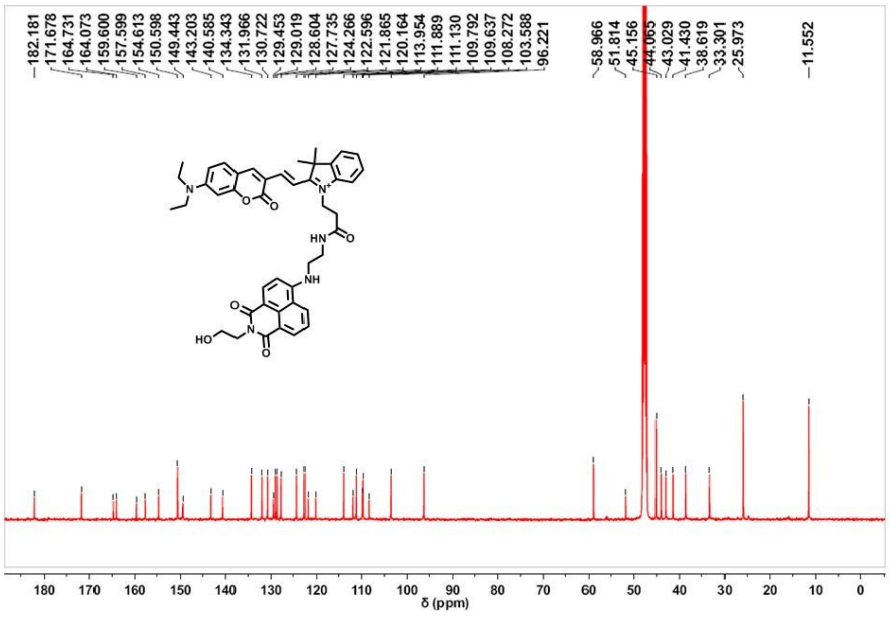


Figure S15. ^{13}C NMR spectrum of compound **Nap-CMC** in CD_3OD.

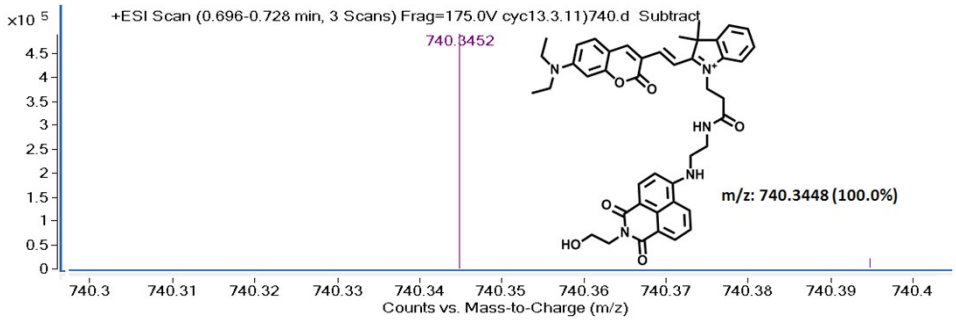


Figure S16. HRMS spectrum of compound **Nap-CMC**.

10. References

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