Supporting Information

1. Materials and instruments

All chemicals were purchased from Bide Pharmatech Ltd (Shanghai, China). Unless otherwise noted, all solvents and reagents were obtained from local distributor and used directly. Distilled water was processed with the ultra-purification system. All chemical reactions were monitored by thin layer chromatography (TLC). ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AVANCE-600 MHz spectrometer in DMSO-d_6 and tetramethylsilane was used as internal standard. HRMS spectra were tested on the Thermo Scientific Q Exactive mass spectrometer system. The UV-vis absorption and fluorescence spectra were carried out with Hitachi U-3900 spectrophotometer and Hitachi F-4600 fluorescence spectrophotometer respectively. Cells and zebrafish imaging were measured by the Zeiss LSM880 Airyscan confocal laser scanning microscope.

2. General measurements

The stock solution of **CS-O-NBD** (2 mM) was prepared in DMSO. The stock solutions of different analytes (0.1 mM) were prepared in deionized water. Na_2SO_3 and NaHS were picked as the source of SO_2 and H_2S, respectively. All tests were measured in PBS buffer (pH 7.4, 30% DMSO, v/v).The tested analytes included biothiols (Cys, Hcy, GSH), amino acids (Tyr, Val, Thr, Leu, Arg, Phe, Pro, Glu, Lys, His, Ala, Asp, Ser, Ile, Gly, Trp, Asn, Met), cations (Fe^{3+}, K^{+}, Mg^{2+}, Na^{+}, Ca^{2+}) and anion (SO_4^{2-}, NO_3^{-}, NO_2^{-}, Cl^{-}, CO_3^{2-}, N_3^{-}, CH_3COO^{-}, $S_2O_3^{2-}$, SO_3^{2-}, HS^{-}). The fluorescence spectra were measured at a slit width of 10 nm/10 nm upon excitation at 570 nm. The slit width was set at 5 nm/5 nm upon excitation at 395 nm and 481 nm.

3. Imaging of cells and zebrafish

HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS and 5% CO_2 at 37 °C for 24 h. The cells were separated into 7 groups. The first group of cells were cultured with **CS-O-NBD** (10 μM) for 30 min and then washed three times with PBS in order to detect endogenous RSS. The second group of cells were pretreated with NEM (1.0 mM) and then incubated with

CS-O-NBD (10 μM) for 30 min. The other five groups of cells were incubated with 1.0 mM NEM for 30 min, rinsed with PBS three times, subsequently treated with Cys/Hcy/GSH/H_2S/SO_3^{2-} (500 μM) for 30 min, washed with PBS three times, finally incubated with **CS-O-NBD** (10 μM) for another 30 min. A commercial mitochondrial Marker, Mito-Tracker Green, was chosen as the costaining reagent with **CS-O-NBD** to carry out the colocalization experiments. Before imaging, all cells should be washed with PBS.

Similar to imaging of cells, the imagings of zebrafish were also divided into 7 groups. The first group of zebrafish was cultured with **CS-O-NBD** (20 μM) for 30 min. The second group of zebrafish was precultured with 1.0 mM NEM for 30 min, subsquently treated with 20 μM **CS-O-NBD** for 30 min. The other five groups were treated with 1.0 mM NEM for 30 min, subsequently incubated with Cys/Hcy/GSH/H_2S/SO_3^{2-} (500 μM) for 30 min, then finally cultured with **CS-O-NBD** (20 μM) for 30 min.

4. Supplementary Figures

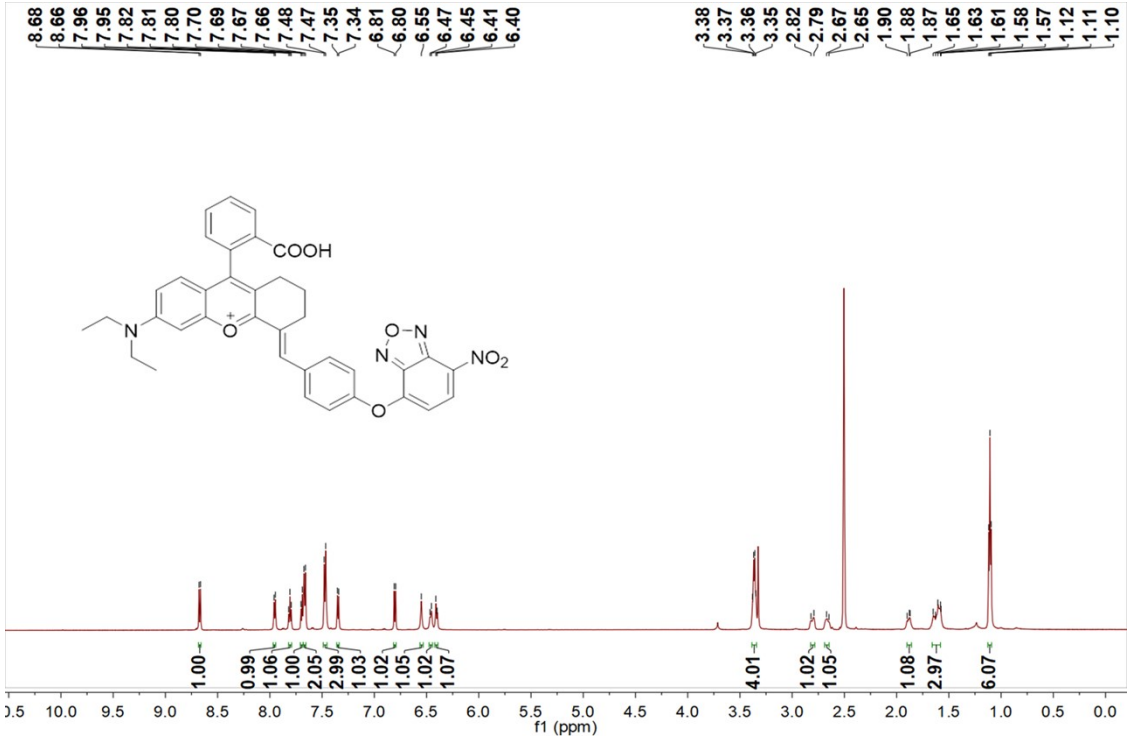


Fig. S1. ^{1}H NMR spectrum of **CS-O-NBD** in DMSO-d_6.

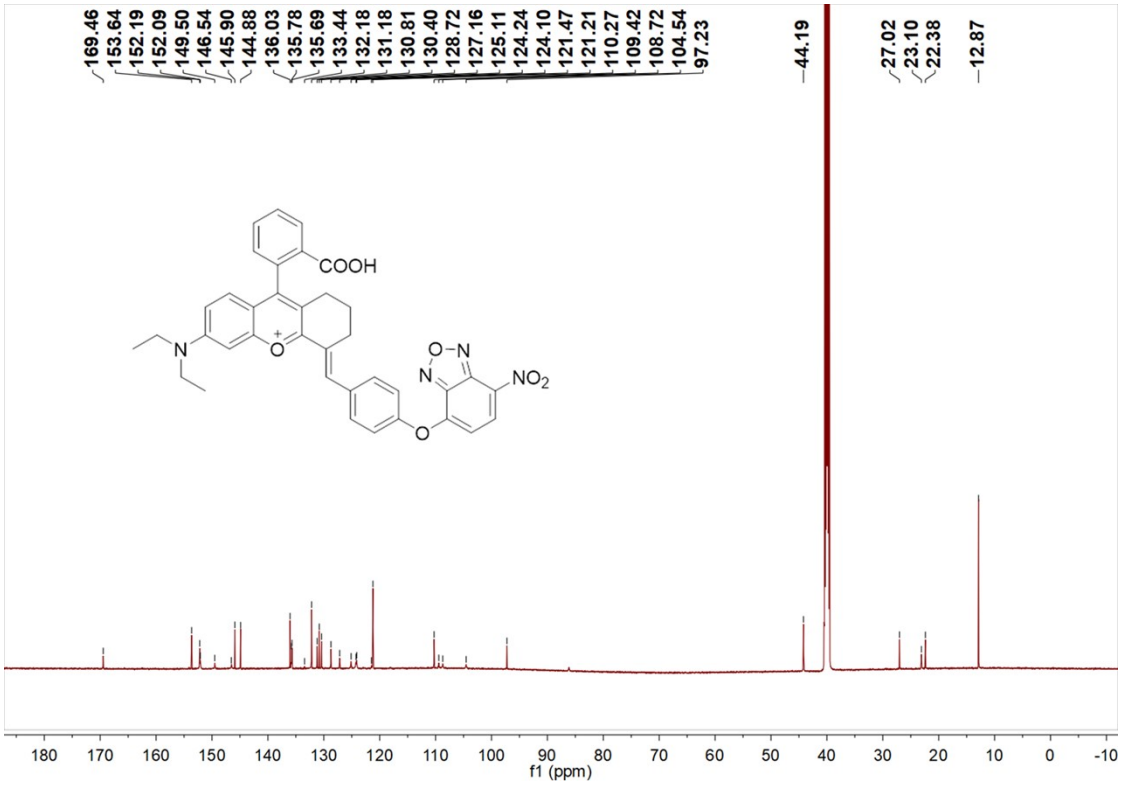


Fig. S2. ^{13}C NMR spectrum of **CS-O-NBD** in DMSO-d_6.

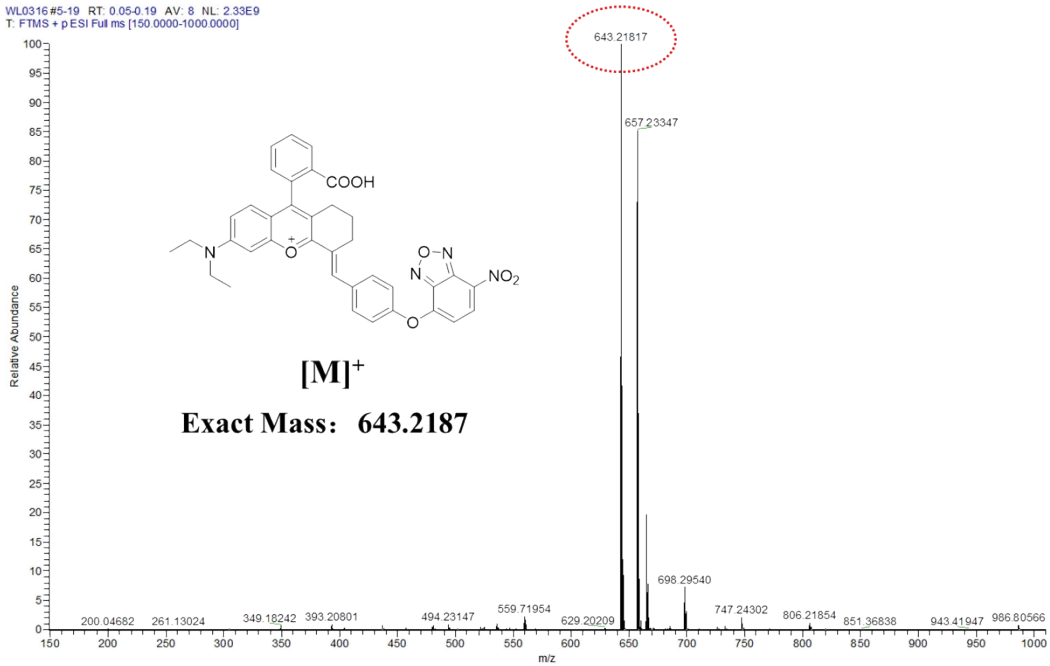


Fig. S3. HRMS spectrum of **CS-O-NBD**.

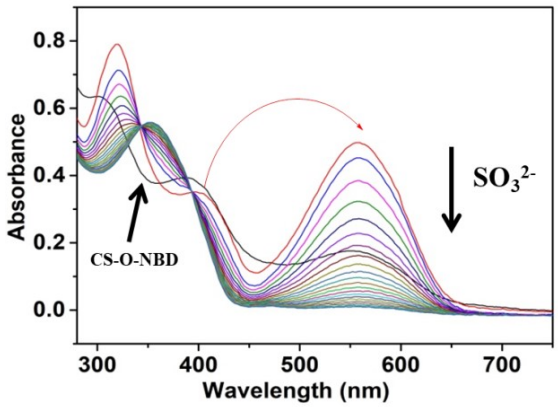


Fig. S4. Time-dependent UV-vis spectra changes of **CS-O-NBD** (10 μM) in the presence of SO_3^{2-} (10 eq.) in PBS buffer (pH 7.4, 30% DMSO, v/v).

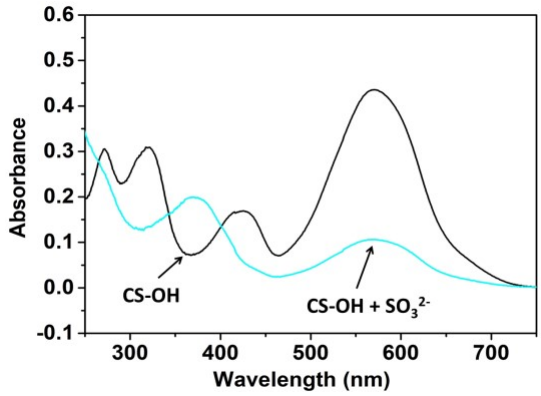


Fig. S5. UV-vis spectra of **CS-OH** (10 μM) before and after the addition of SO_3^{2-} (10 eq.) in PBS buffer (pH 7.4, 30% DMSO, v/v).

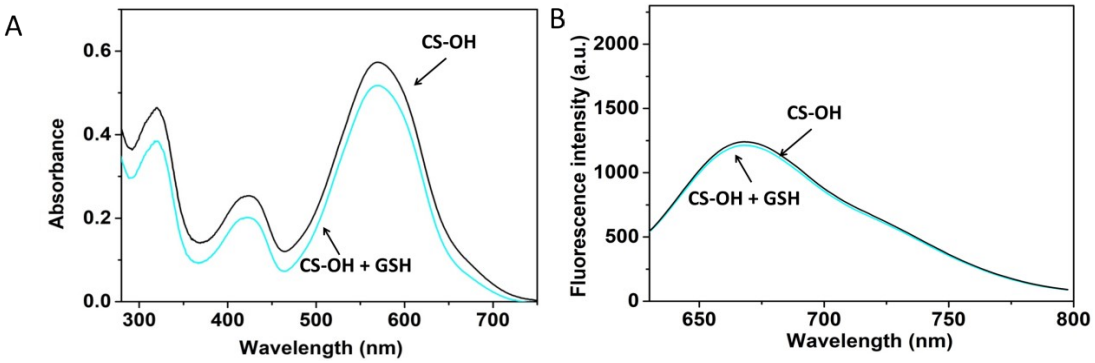


Fig. S6. UV-vis (A) and fluorescence spectra (B) of **CS-OH** (10 μM) before and after the addition of GSH (10 eq.) in PBS buffer (pH 7.4, 30% DMSO, v/v), λ_{ex} = 570 nm.

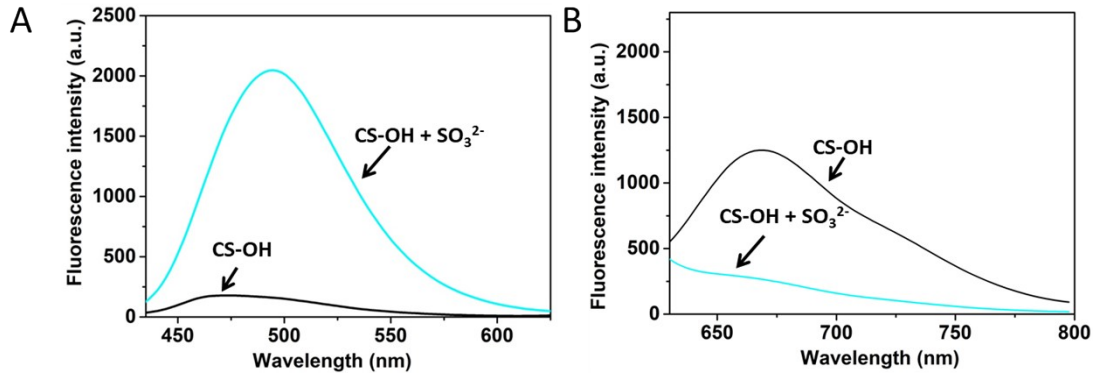


Fig. S7. Fluorescence spectra of **CS-OH** (10 μM) before and after the addition of SO_3^{2-} (10 eq.) in PBS buffer (pH 7.4, 30% DMSO, v/v). (A) λ_{ex} = 395 nm, (B) λ_{ex} = 570 nm.

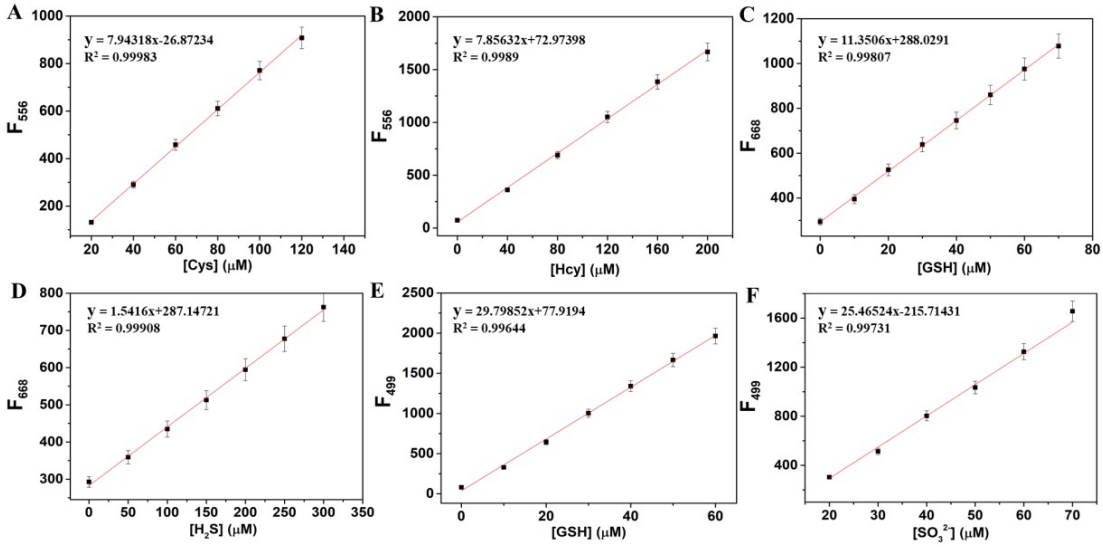


Fig. S8. (A-B) The linear relationship between fluorescence intensities at 556 nm of **CS-O-NBD** (10 μM) and concentrations of Cys/Hcy, λ_{ex} = 481 nm. (C-D) The linear relationship between fluorescence intensities at 668 nm of **CS-O-NBD** (10 μM) and concentrations of GSH/H_2S, λ_{ex} = 570 nm. (E-F) The linear relationship between fluorescence intensity at 499 nm of **CS-O-NBD** (10 μM) and concentrations of GSH/SO_3^{2-}, λ_{ex} =395 nm.

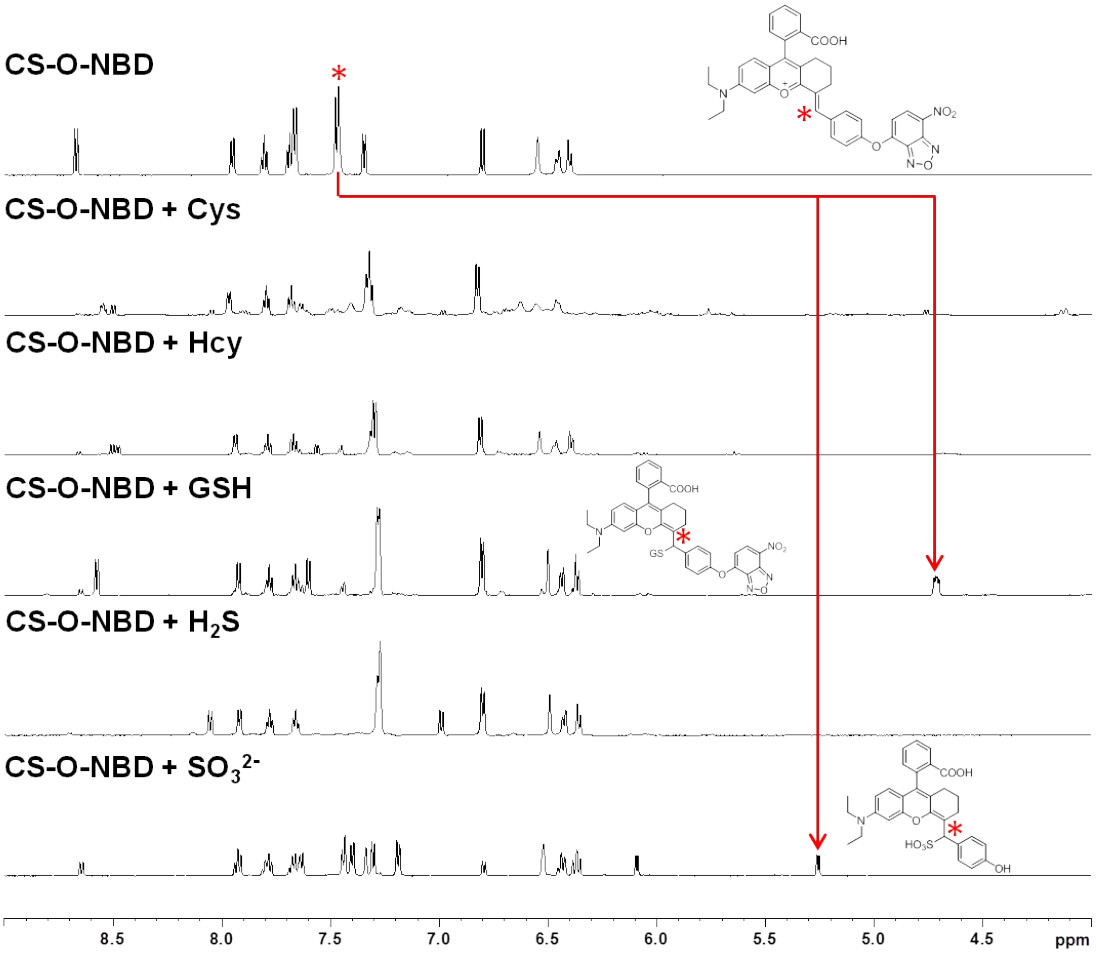


Fig. S9. ^{1}H NMR spectra of **CS-O-NBD** in the absence or presence of different RSS (Cys, Hcy, GSH, H_2S and SO_3^{2-}) in DMSO-d_6.

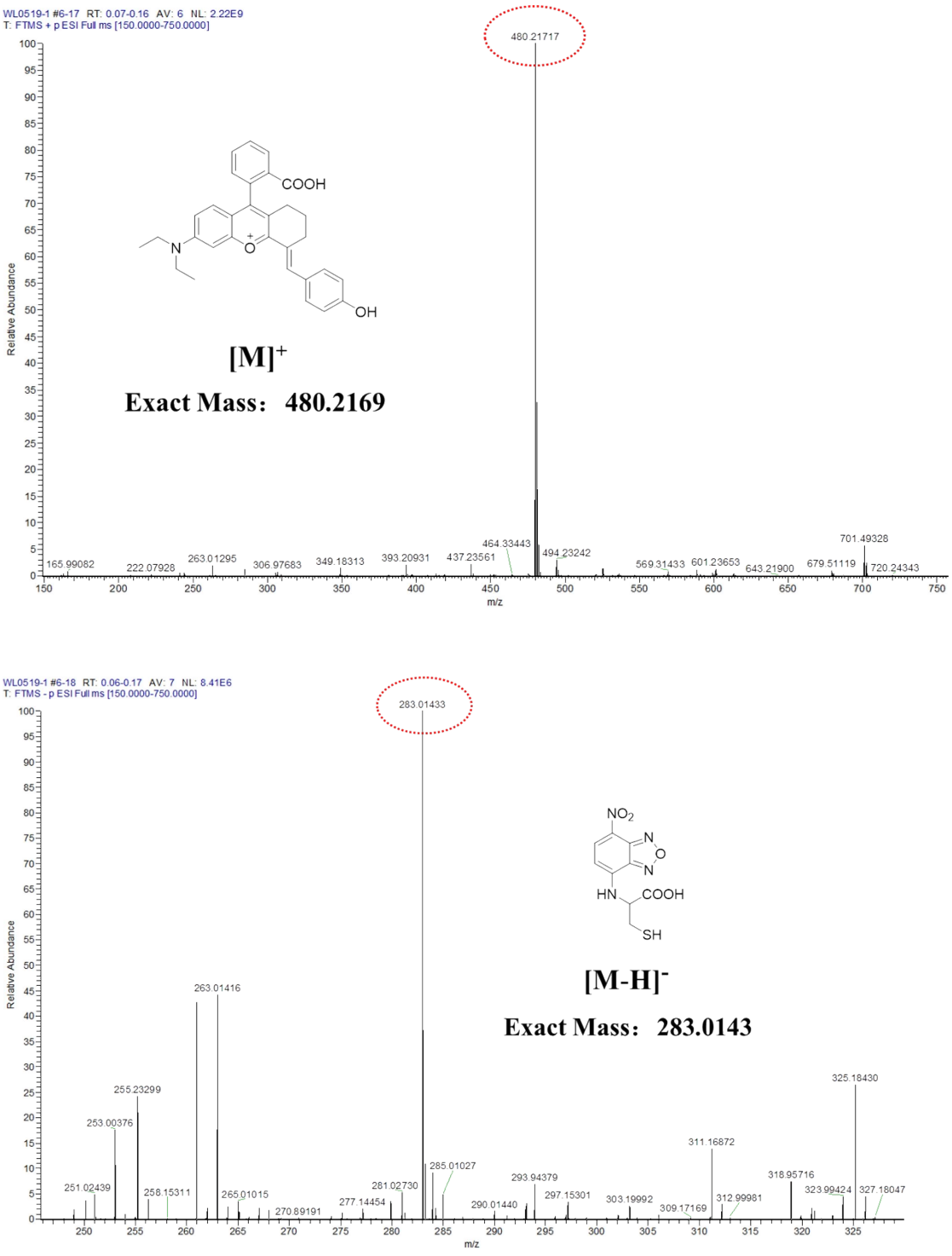


Fig. S10. HRMS spectrum after reaction of **CS-O-NBD** with Cys.

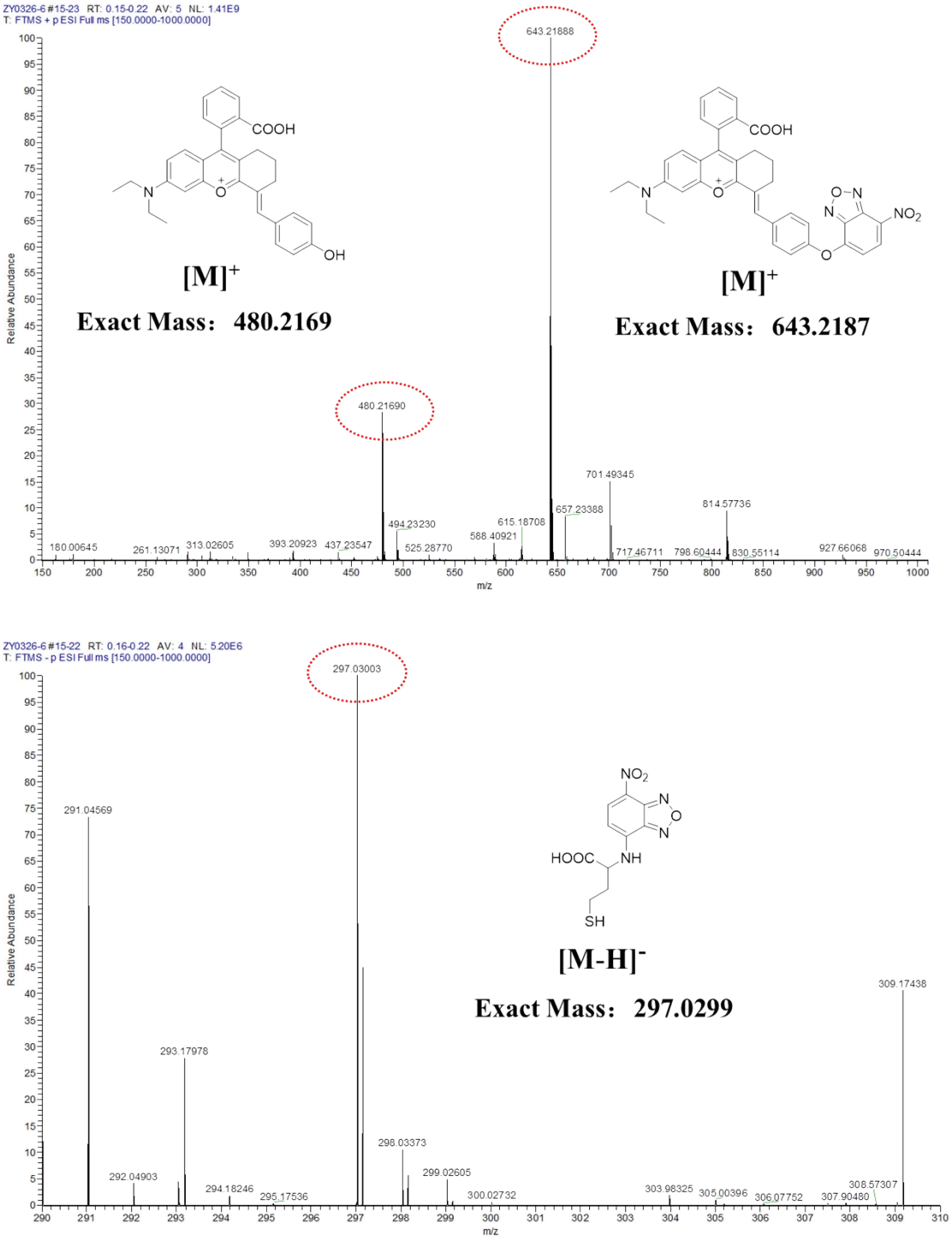
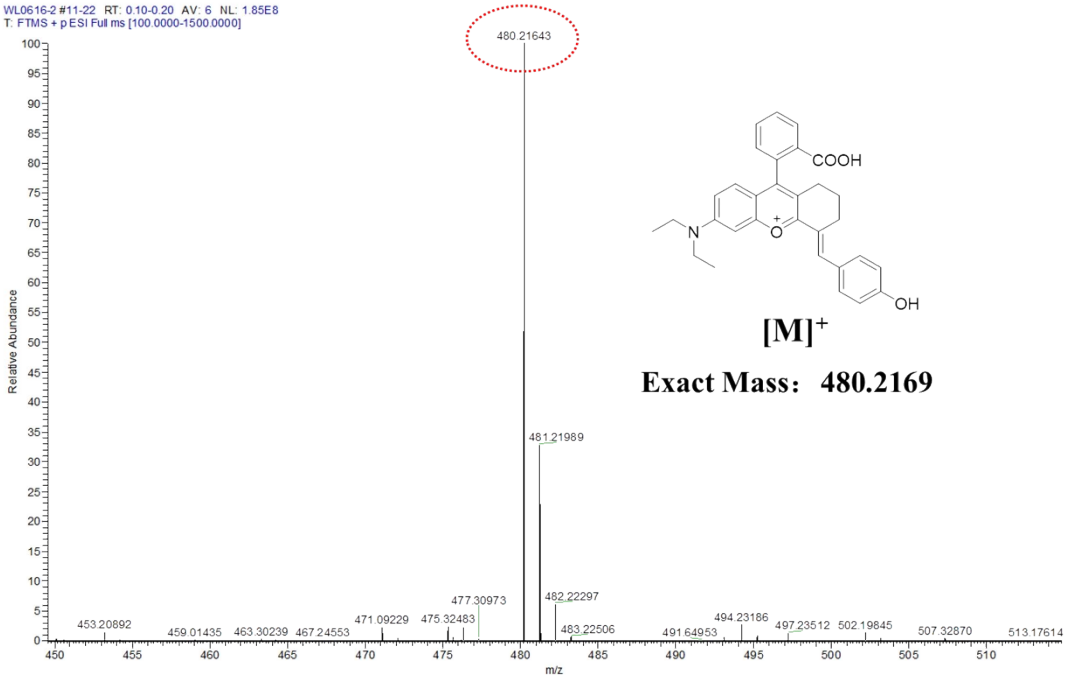
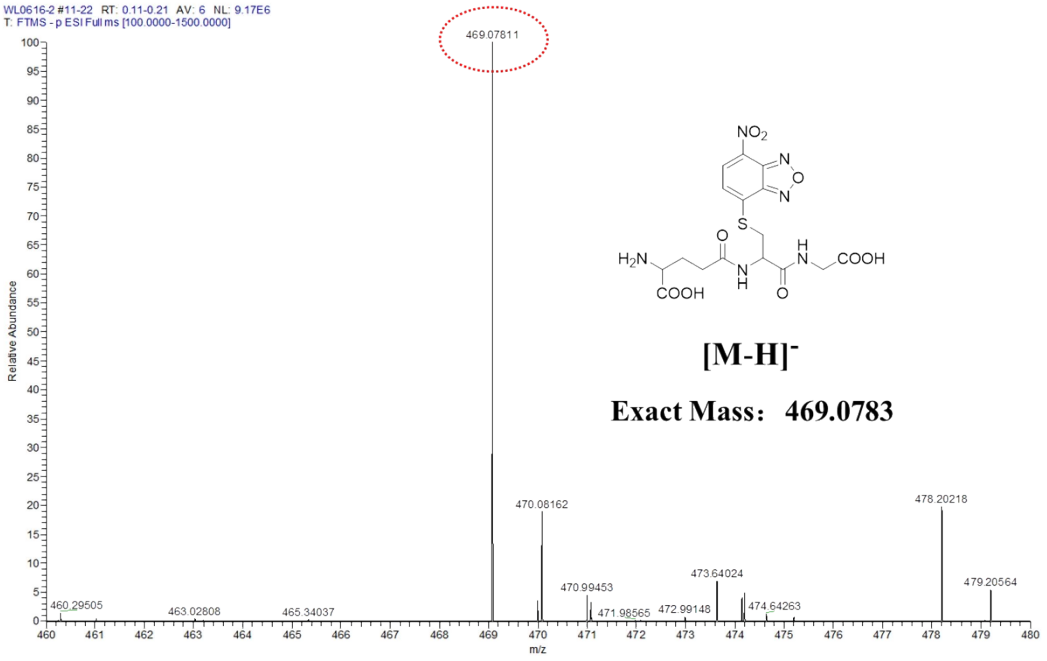


Fig. S11. HRMS spectrum after reaction of **CS-O-NBD** with Hcy.



[M]$^+$

Exact Mass：480.2169



[M-H]$^-$

Exact Mass：469.0783

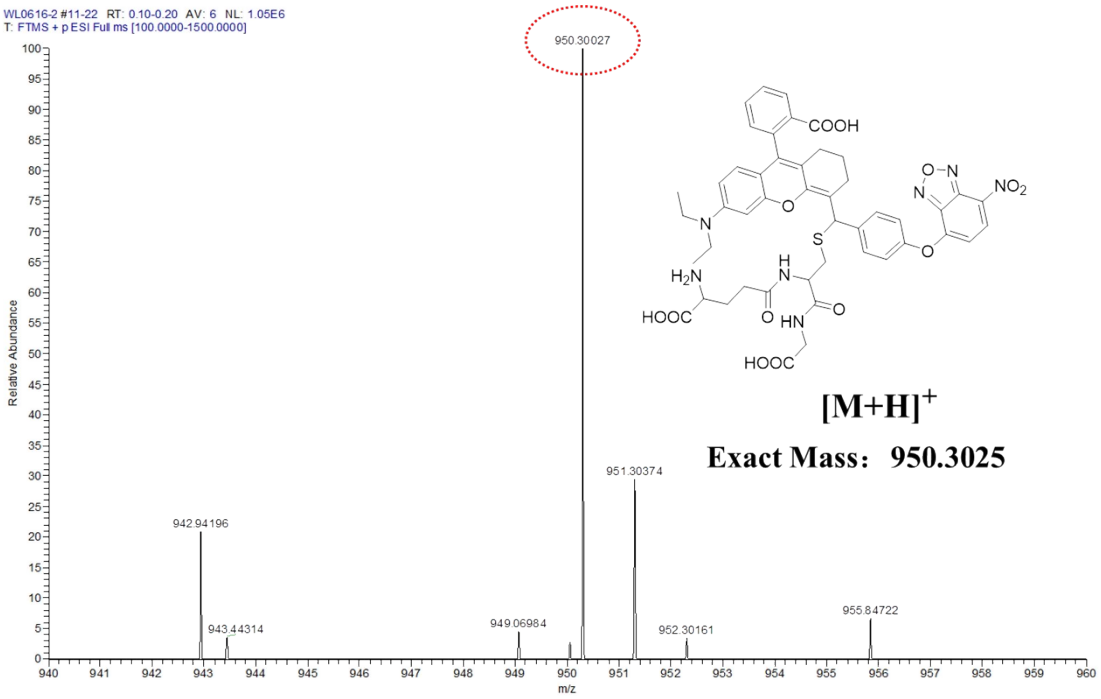


Fig. S12. HRMS spectrum after reaction of **CS-O-NBD** with GSH.

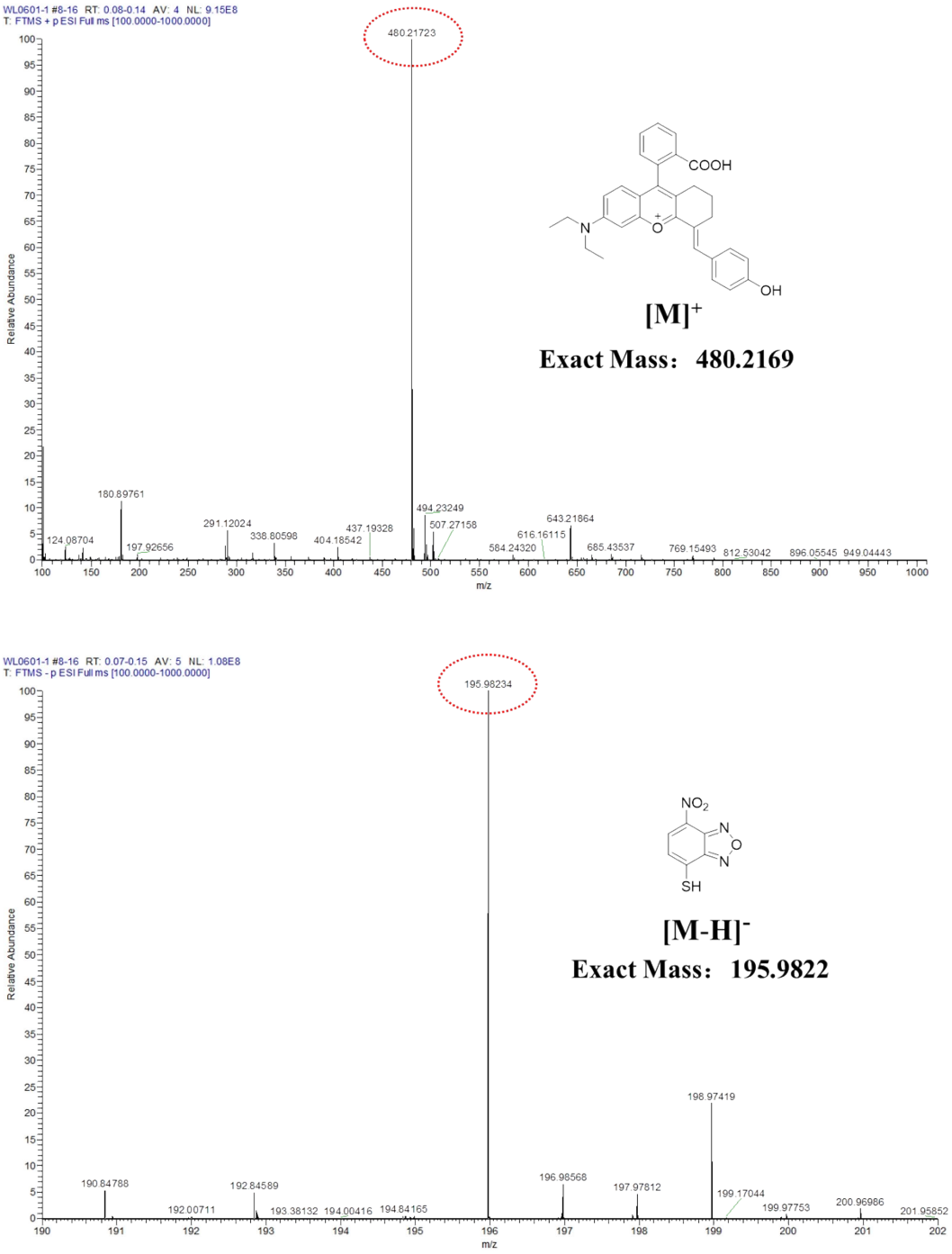


Fig. S13. HRMS spectrum after reaction of **CS-O-NBD** with H_2S.

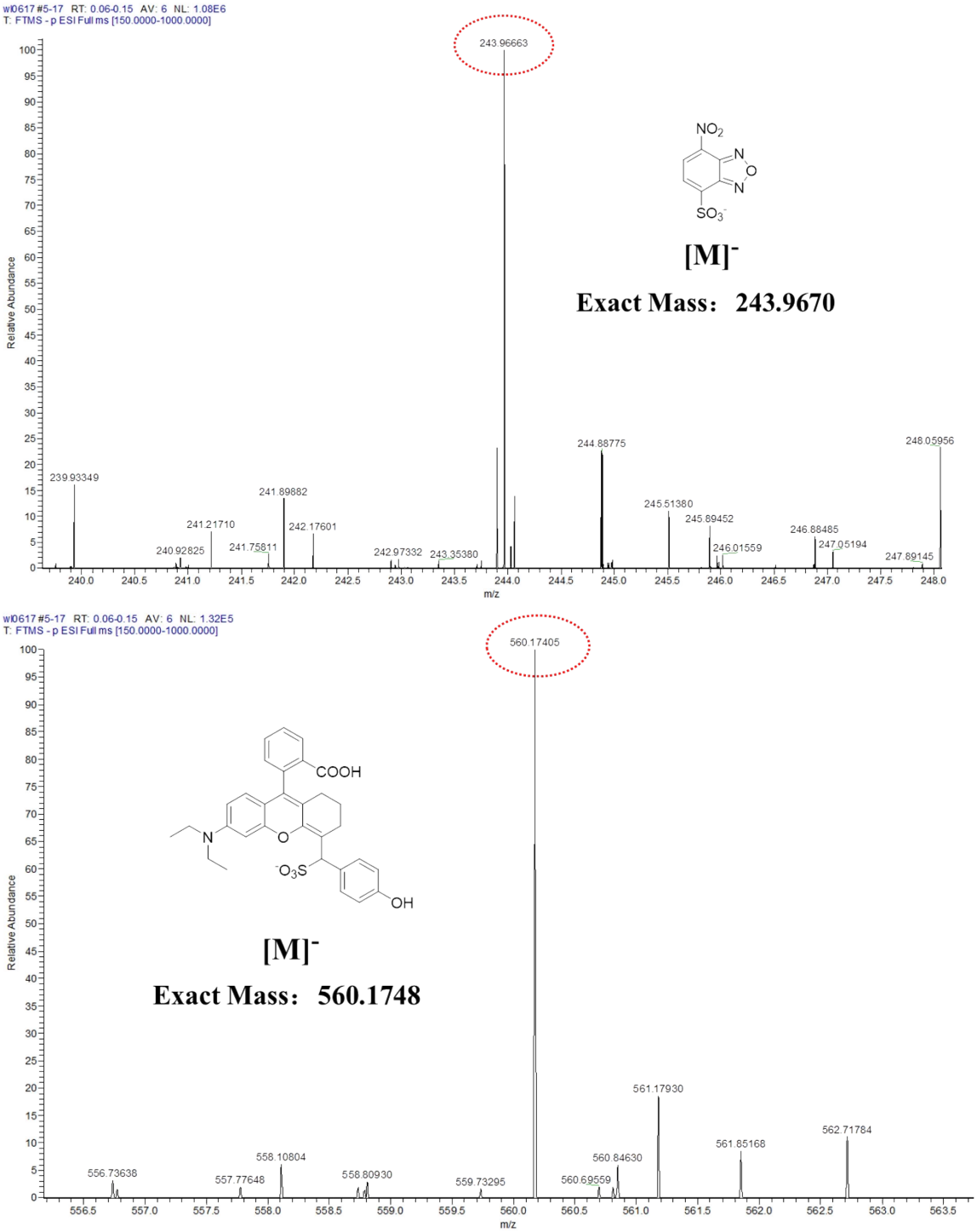


Fig. S14. HRMS spectrum after reaction of **CS-O-NBD** with SO_3^{2-}.

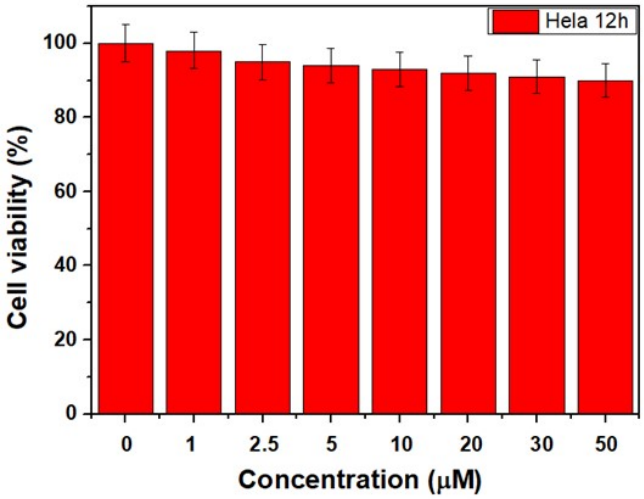


Fig. S15. Cell viability estimated by MTT assay with Hela cells, which were cultured in the presence of **CS-O-NBD** (0-50 μM) for 12 h.