Supporting information

An ESIPT fluorescence probe based on the double-switch recognition mechanism for selective and rapid detection of hydrogen sulfide in living cells

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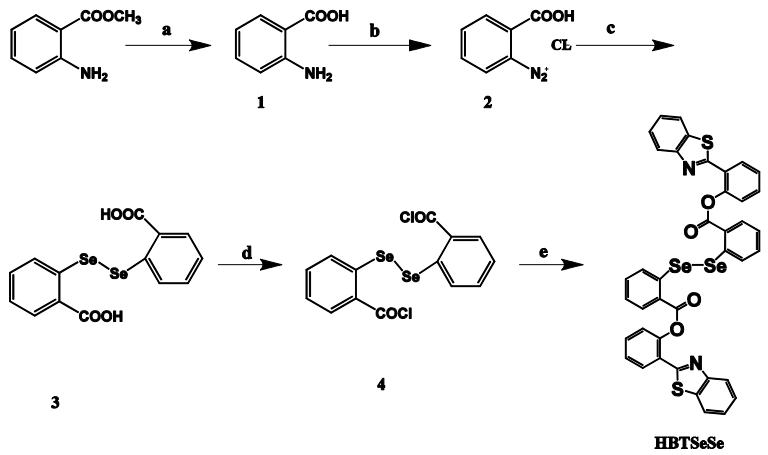
Contents



Scheme S1. The synthetic route of HBTSeSe.

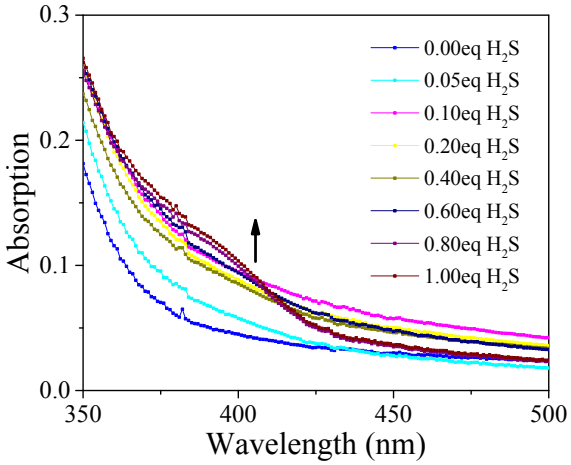


Figure S1. The absorption spectrum of the probe (10μM) react with different concentration of H_2S (DMSO/PBS = 1:1).

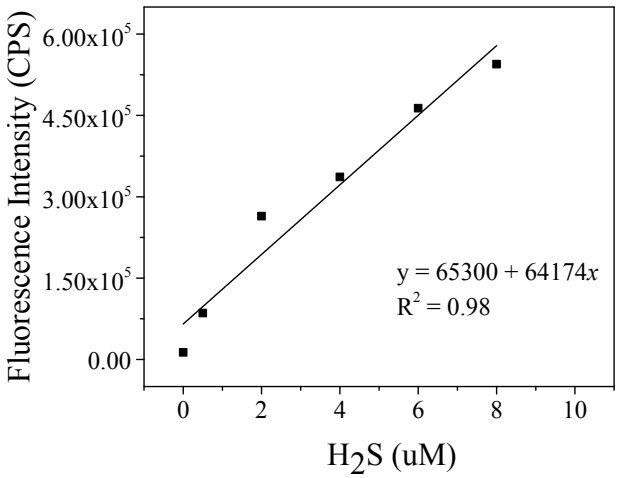


Figure S2. Linear relationship (R^2=0.98) between fluorescence intensity at 460nm of HBTSeSe and H_2S concentration.

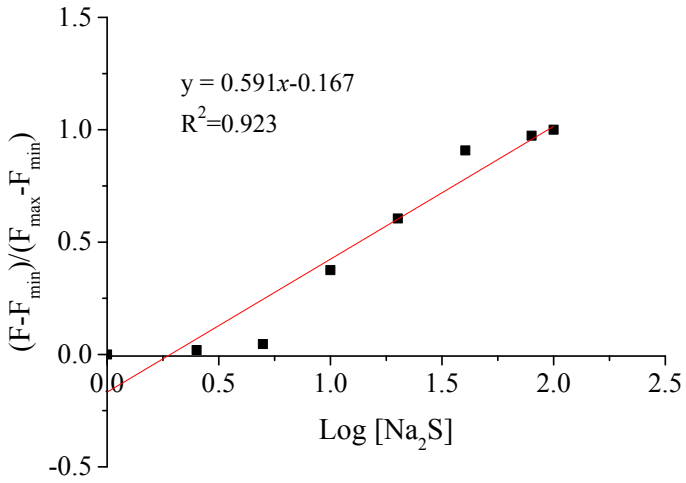


Figure S3. The limit of detection was calculated as 0.19 μM.

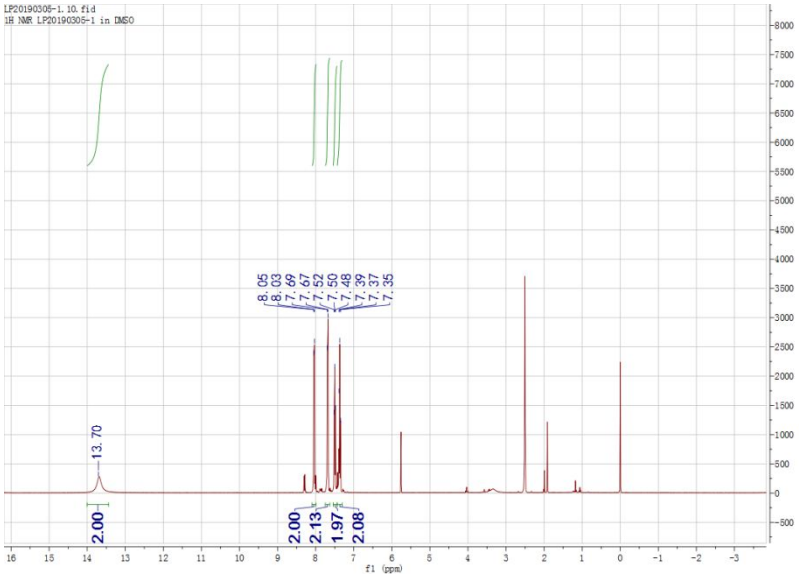


Figure S4. [1]H-NMR (400MHz, DMSO-d6) of Compound 3

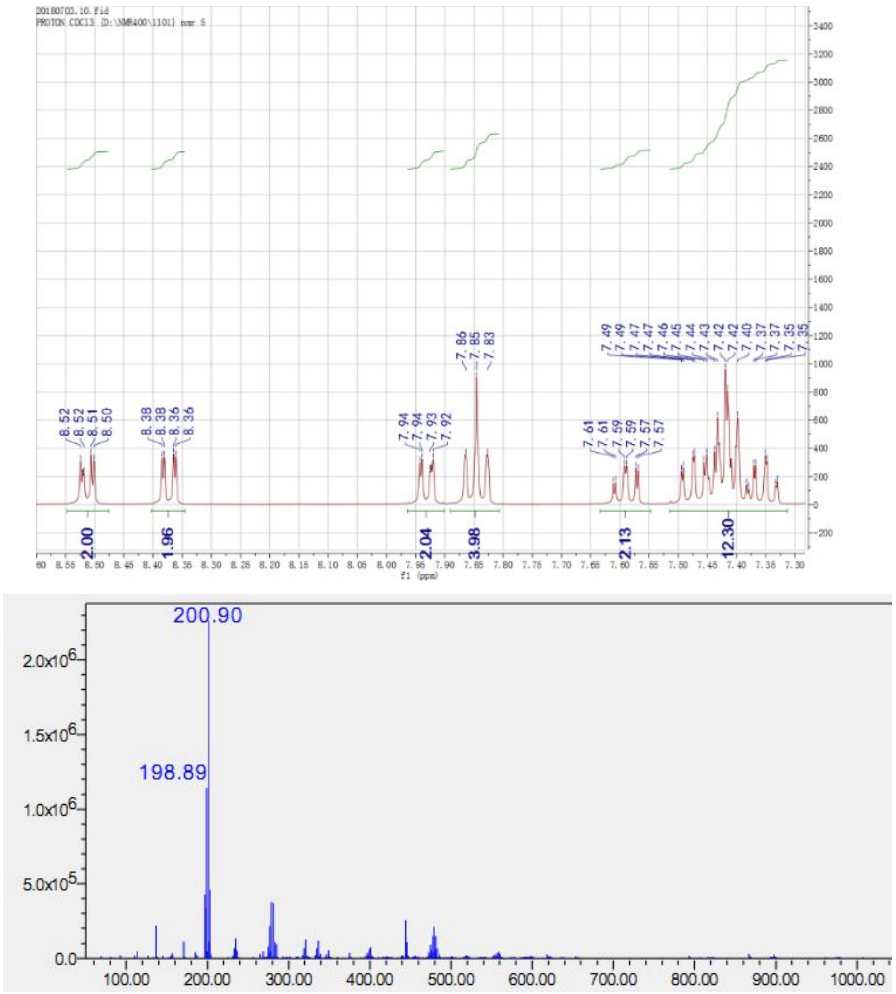


Figure S5. ^{1}H-NMR (400MHz, $CDCl_3$) of HBTSeSe and mass spectra (ESI-) of $C_{14}H_{10}O_4Se_2$

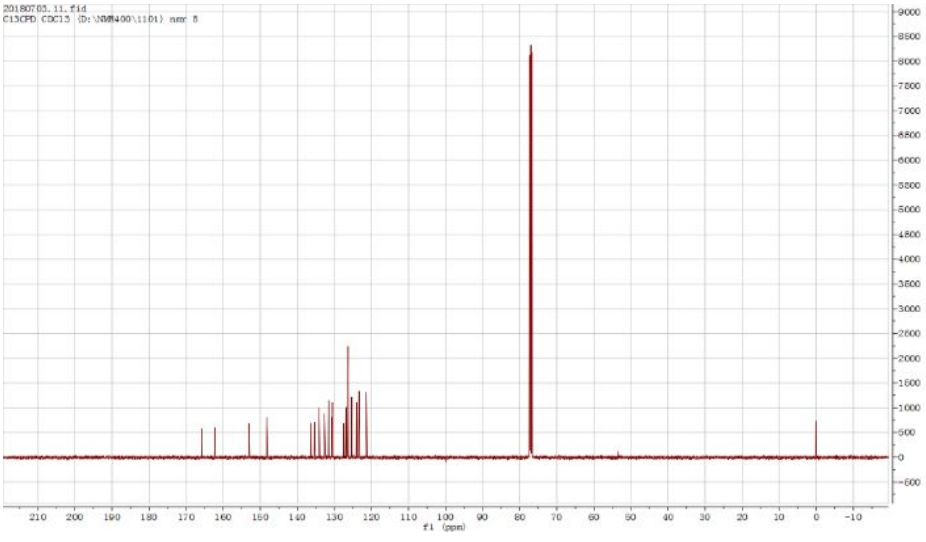


Figure S6. ^{13}C-NMR (400MHz, CDCl3) of HBTSeSe

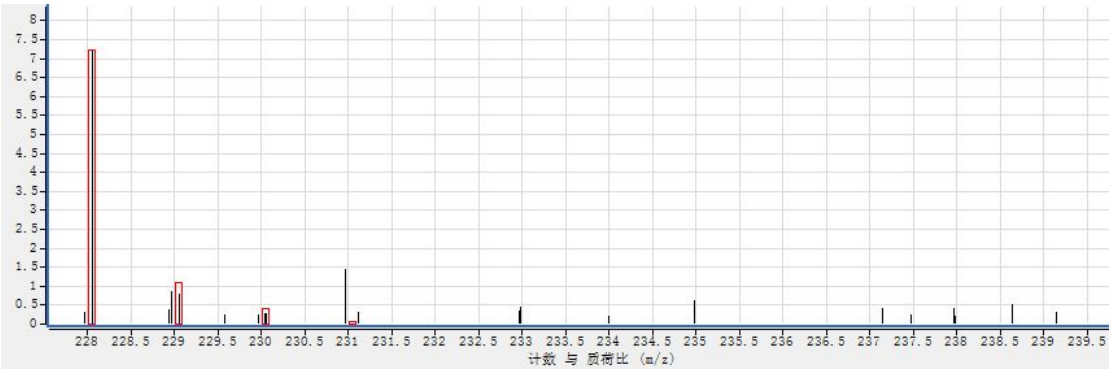


Figure S7. HRMS (ESI+) of the mixture contains HBTSeSe and Na_2S

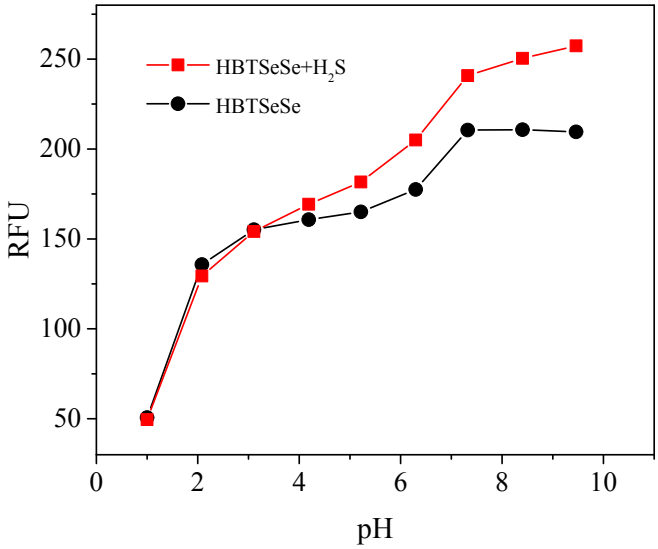


Figure S8. HBTSeSe probe fluorescence intensity before and after adding H_2S with changes in PH

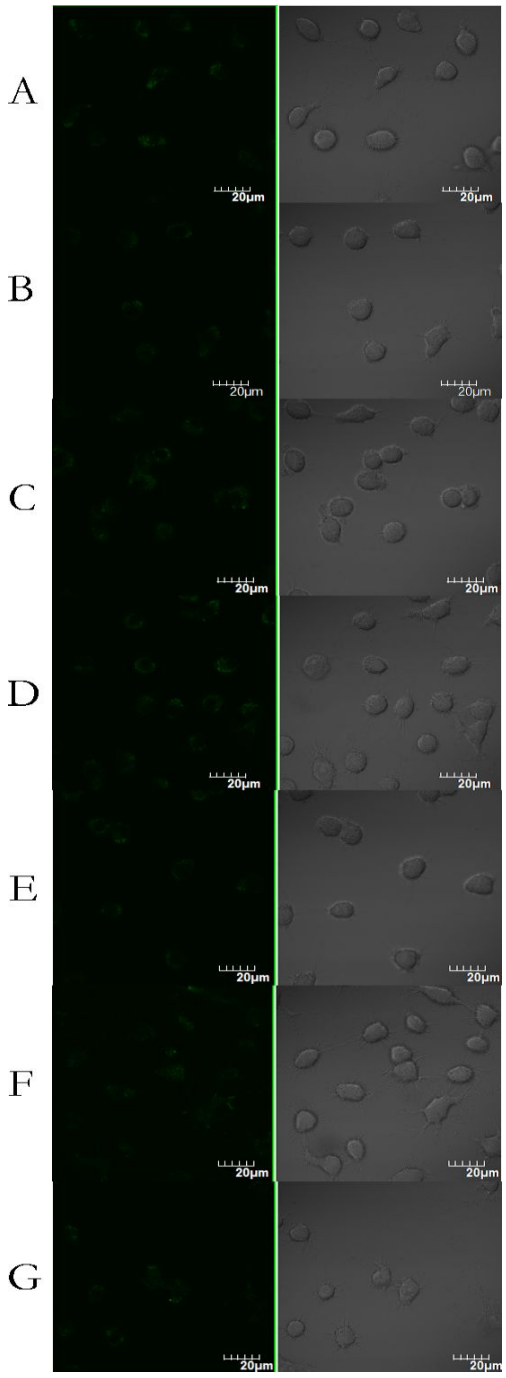


Figure S9. (**A**-**G**) Confocal fluorescence images of RAW264.7 incubated by HBTSeSe (10μM) for 20 min and subsequently incubated by different concentration PMA for 30 min. Panel A, 0.0 μg/ml PMA; Panel B, 0.05μg/ml PMA; Panel C, 0.1μg/ml PMA; Panel D, 0.2μg/ml PMA; Panel E, 0.4μg/ml PMA; Panel F, 0.8μg/ml PMA; Panel G, 1.0 μg/ml PMA.(λ_{ex} = 405nm, scale bar = 20μM). All images were collected at the same microscopy settings.

Table S1. The absorbance of MTT assay with different HBTSeSe concentration.

Test Number	HBTSeSe Concentration（μM）									
	0	0.01	0.1	1	5	10	25	50	100	200
1	0.629	0.637	0.684	0.695	0.593	0.736	0.734	0.837	0.86	0.852
2	0.639	0.663	0.643	0.715	0.647	0.698	0.737	0.856	0.849	0.900
3	0.621	0.640	0.642	0.683	0.712	0.700	0.735	0.897	0.854	0.898
4	0.600	0.619	0.610	0.647	0.735	0.687	0.708	0.759	0.863	0.892
5	0.555	0.636	0.579	0.721	0.669	0.654	0.751	0.718	0.784	0.829
Average	0.6088	0.639	0.6316	0.6922	0.6712	0.695	0.733	0.8134	0.842	0.8742

The toxicity of the HBTSeSe probe was tested by MTT assay before the probe is subjected to cell testing. The RAW264.7 cells grown in the log phase were first counted using a cell counter and adjusted to a concentration of 1×10^6 cells/mL. The cells were seeded directly on 96-well plates at a volume of 200 μL per well. The 96-well microplate was placed in a CO_2 incubator for 24 hours, and the culture solution was subtracted. Then add different concentrations of probes (0, 0.01, 0.1, 1, 5, 10, 25, 50,100,200 μM) and continue to incubate RAW264.7 cells for 24 h, deducting the probe solution. 150 μL of MTT solution at a concentration of 0.5 mg/mL was added to each well for 4 h to remove the remaining MTT solution. Add 150 μL of DMSO to each well to dissolve the formazan, and then measure the absorbance of the solution at 460 nm in each well on a microplate reader. From the experiment data, we can know that it is nontoxicity for cells when the concentration of HBTSeSe was 10 μM.