Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

Imaging of intracellular sulfane sulfur expression changes under hypoxia stress via a selenium-containing near-infrared fluorescent probe

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Contents

1. General experimental section

Instruments: Absorption spectra were measured on NANO Drop 2000c UV-visible spectrophotometer (Thermo Fisher Scientific). Fluorescence spectra were obtained by a HORIBA Scientific FluoroMax-4 Spectrofluorometer with a Xenon lamp and 1.0-cm quartz cells. The pH measurements were performed with a pH-3C digital pH meter (Shanghai Lei Ci Device Works, Shanghai, China) with a combined glass–calomel electrode. Mass spectra were taken on LCQ Fleet LC-MS System (Thermo Fisher Scientific). ^{1}H NMR and ^{13}C NMR spectra were taken on a Bruker spectrometer. The fluorescence images of cells were taken using a confocal laser scanning microscope (Japan Olympus Co., Ltd) with an objective lens (×60). High performance liquid chromatography (HPLC) analysis were performed in Agilent Technologies 1260 Infinity II. High-resolution mass spectral (HRMS) analyses were measured with Hybrid Ion Trap-Orbitrap Mass Spectrometer (LTQ Orbitrap XL,Thermo).

Materials: aza-BODIPY was synthesized in our laboratory according to previous report with some modification.[1] 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and HEPES were purchased from Sigma-Aldrich. Human neuroblastoma (SH-SY5Y) cells, mouse macrophage (RAW 264.7) cells, human lung carcinoma (A549) cells, human cervical carcinoma (Hela) cells , Human embryonic kidney 293 (HEK 293) cells, human hepatocellular liver carcinoma (HepG2) cells, human hepatocellular liver carcinoma (SMMC7721) cells were obtained from the cell bank of the Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China). Monoclonal Antibody was obtained from Cell Signaling Technology. BD-*di*SeH (1.0 mM, 0.5 mL) was prepared in DMSO and stored at 4 ^{o}C in darkness. All other reagents and chemicals were from commercial sources and of analytical reagent grade, and used without further purification. Solvents used for spectroscopic studies were purified and dried by standard procedures before use. Ultrapure water (Millipore,

Bedford, MA, USA) was used throughout.

Absorption analysis: Absorption spectra were obtained with 1.0-cm glass cells. The probe (DMSO, 0.1 mL, 1.0 mM) was added to a 10.0-mL color comparison tube. After dilution to 10.0 μM with 10 mM HEPES (containing 20% fetal bovine serum, v/v, pH 7.4), Na_2S_4 (20 μM) was added. The mixture was equilibrated for 5 min before measurement.

Fluorescence analysis: Fluorescence spectra were obtained with a Xenon lamp and 1.0-cm quartz cells. The probe (DMSO, 0.1 mL, 1.0 mM) was added to a 10.0-mL color comparison tube. After dilution to 10.0 μM with 10 mM HEPES (containing 20% fetal bovine serum, v/v, pH 7.4), various concentration of Na_2S_4 (0-20 μM) was added. The mixture was equilibrated for 5 min before measurement.

Western blot. Cells were lysed using RIPA buffer supplemented with PMSF (Solarbio, China) and PHosSTOP (Roche, Germany) inhibitors for 30 min. Then the cells were scraped, sonicated, and centrifuged (20,000 × g at 4 °C) for 15 min. Protein concentrations in the supernatant were determined using the BCA Protein Assay kit (Biogot, China) and equal amounts of protein were electrophoresed on 6-12% SDS-polyacrylamide gels (Bio-Rad, USA) and transferred to PVDF menbranes (Millipore, Germany). Then the membranes were blocked with 7% milk and blotted with primary antibodies overnight at 4℃. A horseradish peroxidase (HRP)-conjugated secondary antibody was used to quantify protein and an enhance chemiluminescence (ECL) detection system was used to detect the signals.

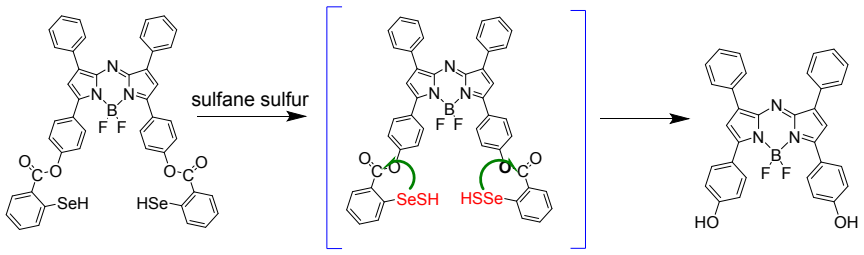
Visualization of sulfane sulfur in mice. BALB/c mice, 20-25 g, were obtained from Binzhou Medical University. Mice were group-housed on a 12:12 light–dark cycle at 22 °C with free access to food and water. Images were taken by Perkinelmer/IVIS Lumina XRMS Series Ⅲ. All surgical procedures were conducted in conformity with National Guidelines for the Care and Use of National Guidelines for the Care and Use of Laboratory Animals Laboratory Animals, and experimental protocols were approved by the Institutional Animal Care and Use Committee in Binzhou Medical University, Yantai, China. Approval Number: No.BZ2014-102R.

2. Preparation of analytes

Stock solutions of cysteine (Cys), homocysteine (Hcy), glutathione (GSH), cystine (CysSSCys), GSSG and NaHS were prepared in distilled water (2 mM, respectively). The stock solution of S_8 (200 mM) was prepared in CH_2Cl_2, and then used EtOH to dilute this solution, to get a 10 mM stock solution of S_8 in EtOH. The stock solution was diluted to desired concentrations when needed. Na_2S_2 and Cys-polysulfide were prepared according to reported procedures.[1-4] A heterogeneous mixture of Na (25 mg-atom) and S_8 (25 mg-atom) in 10 mL anhydrous 1,2-dimethoxyethane under Ar atmosphere. The heterogeneous mixture could immediately turn dark brown at room temperature. However, the reaction needs to be continued for 3 h at 70 ^oC to give anhydrous Na_2S_2. Filtrated then washed by ether to yield a yellow solid 30 mg, 60%. Na_2S_4 was also synthesized with Na (25 mg-atom) and S_8 (50 mg-atom) via the same method (yield 15%). Then the products were derived by benzyl chloride to convert sodium oligosulfide into dibenzyl oligosulfane. The Na_2S_2 (Na_2S_4) derived by benzyl chloride to convert Na_2S_2 into $PhCH_2S_2CH_2Ph$ ($PhCH_2S_4CH_2Ph$). After derived the samples by benzyl chloride, the purities of our products were up to 99.91% by GC. Retention time of $PhCH_2S_2CH_2Ph$ and $PhCH_2S_4CH_2Ph$ were at 12.1 min and 12.8 min, respectively. $PhCH_2S_2CH_2Ph$ ^1H NMR (500 MHz, DMSO-D6) δ(ppm): 7.43-7.37 (m, 4H), 7.33-7.20 (m, 6H), 3.81 (S, 4H). GC-MS (ESI^+): m/z $C_{14}H_{14}S_2$ calcd. 246.0537, found $[M^+]$ 246.0535. $PhCH_2S_4CH_2Ph$ ^1H NMR (500 MHz, DMSO-D_6) δ(ppm): 7.42-7.35 (m, 4H), 7.33-7.21 (m, 6H), 3.82 (S, 4H). GC-MS (ESI^+): m/z $C_{14}H_{14}S_4$ calcd. 309.9978, found $[M^+]$ 309.9978.

3. Characterization of the reaction between BD-*di*SeH and sulfane sulfur

A solution of BD-*di*SeH (0.1 mmol) was treated with Na_2S_4 (0.2 mmol) in 10 mM HEPES (containing 20% fetal bovine serum, v/v, pH 7.4). The mixture was stirred for 30 min at 37 ^oC. Then solvent was extracted by CH_2Cl_2 and washed the organic phase with H_2O, the organic layer evaporated to dryness. The resulted residue was purified by column chromatography. Compound BODIPY was obtained as a blue solid. The formation of BODIPY was confirmed by HRMS. LC-MS (ESI^-): m/z $C_{32}H_{22}BF_2N_3O_2$ calcd. 529.1773, found $[M-H]^-$ 528.1695.



BD-*di*SeH **BODIPY**

4. Fluorescence quantum yield of BD-*di*SeH

The fluorescence quantum yield of BD-*di*SeH was calculated according to the following formula:

$$\varphi_u = \frac{(\varphi_s)(FA_u)(A_s)(\lambda_{exs})(\eta_u^2)}{(FA_s)(A_u)(\lambda_{exu})(\eta_s^2)}$$

Where φ is fluorescence quantum yield; the subscripts u and s refer to the unknown and the standard, respectively; F is integrated fluorescence intensity under the corrected emission spectra; A is the absorbance at the excitation wavelength; η is the refractive index of the solution.[5] We used tera-tert-butyl-29*H*, 31*H*-phtalocyanine as a standard (quantum yield of 0.44).[6] The quantum yield of BD-*di*SeH was determined to be 0.002.

5. Effect of pH values

Standard fluorescence pH titrations were performed in the solution at a probe concentration of 10 μM. As shown in Fig. S1, the probe itself and the reaction product almost had no effect on the fluorescence by the pH of mediums within the range from 2.0 to 7.4. These results indicated that the probe could work under physiological conditions.

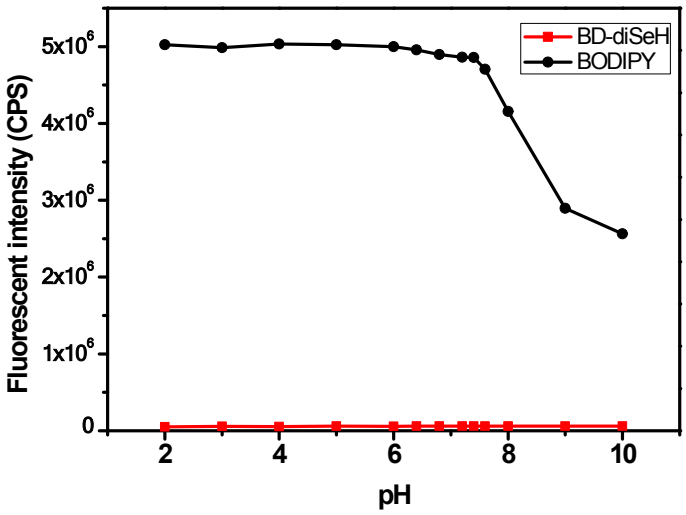


Fig. S1. Effect of pH on the fluorescent intensity of BD-*di*SeH (10.0 μM) and its metabolite BODIPY (10.0 μM) in 10 mM HEPES. pH values: 2.0, 3.0, 4.0, 5.0, 6.0, 6.4, 6.8, 7.0, 7.2, 7.4, 7.6, 8.0, 9.0, 10.0. Excitation wavelengths for BD-*di*SeH and its metabolite were 702 nm and 707 nm, respectively.

6. MTT assay

Prior to the bioimaging of sulfane sulfur in living cells, MTT assays were performed to evaluate the cytotoxicity of BD-*di*SeH towards A549 cells with probe concentrations from 2 μM to 30 μM. A549 cells were cultured in RPMI 1640 Medium supplemented with 10% FBS at 37 ^{o}C in a humidified atmosphere containing 5% CO_2. Cells were planted into 96-well cell culture plate at 4000 /well, cultured at 37 ^{o}C and 5% CO_2 for 48 h, and then different concentrations of BD-*di*SeH (0, 2.0, 5.0, 10.0, 20.0 and 30 μM, final concentration) were added to the wells and the cultures were maintained for 24 h at 37 ^{o}C and 5% CO_2. Subsequently, 20 μL MTT (5 mg/mL) was added to each well and incubated for an additional 4 h at 37 ^{o}C under 5% CO_2. After 4 h incubation, the remaining MTT was removed. DMSO was added to each well to dissolve the formazan crystals. Finally, the amount of MTT formazan was qualified by determining the absorbance at 570 nm using a microplate reader (Tecan, Austria). The result showed BD-*di*SeH exhibited relatively low toxicity towards cell cultures under experimental conditions.

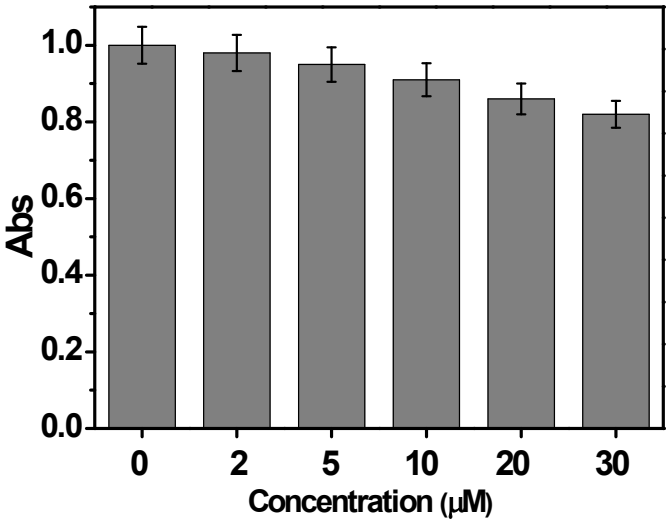


Fig. S2. MTT assays of BD-*di*SeH with different concentrations in A549 cells. The experiment was repeated three times and the data are shown as mean (±S.D.).

7. Photostability of BD-*di*SeH

Photostability of fluorescent probe is a key factor in practical applications. Thus, we evaluated the photostability of probe using a time-dependent fluorescence measurement. The solution of probe and sulfane sulfur was excited continuously at 635 nm for 600 s, and fluorescence intensity was measured every 30 s. The results showed that stable fluorescence intensity was observed, indicating BD-*di*SeH was stable and could be applied for long-time cells imaging.

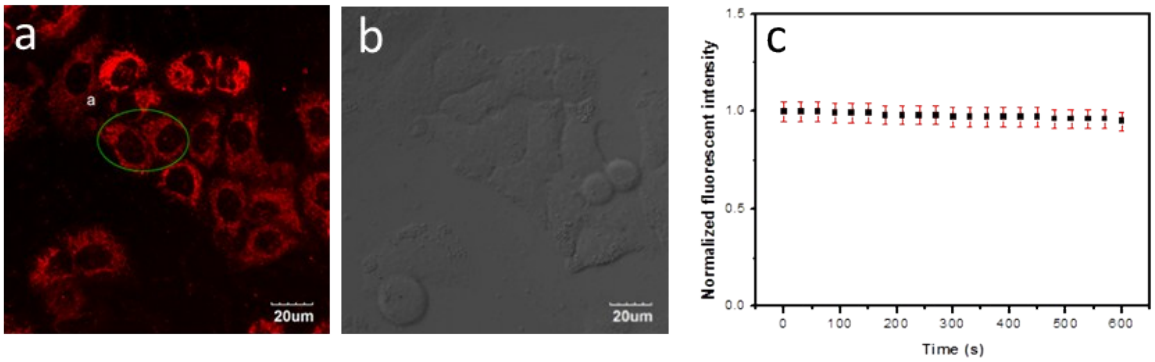


Fig. S3. The photostability test of BD-*di*SeH (10 μM) in A549 cells. A549 cells were first incubated with Na_2S_4 (20 μM) for 15 min. (a) After A549 cells were incubated with BD-*di*SeH for 15 min, the fluorescence images were obtained every 30 s. (b) Bright-field of (a). (c) Normalized fluorescence intensity of the selected regions of (a) from 0 to 600 s.

8. Imaging sulfane sulfur in different cell lines

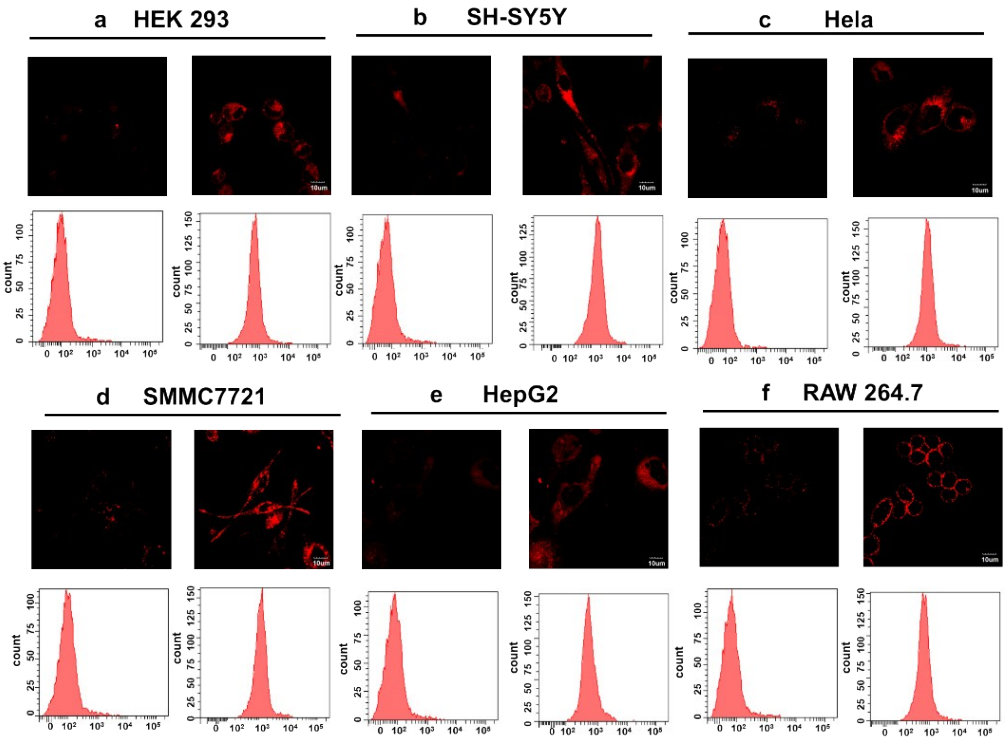


Fig. S4. Confocal microscopy images and flow cytometry analysis of various cell lines for imaging sulfane sulfur using BD-*di*SeH (1 μM), including HEK 293 cells (a), SH-SY5Y cells (b), Hela cells (c), SMMC7721 cells (d), HepG2 cells (e) and RAW 264.7 cells (f). The cells in each group: the left cells were as controls, and the right cells were treated with Na_2S_4 (1 μM) for 15 min at 37 °C. All cells preincubated with BD-*di*SeH for 15 min and washed with PBS. Images displayed represent emission intensities collected in optical windows between 680 and 780 nm upon excitation at 635 nm for BD-*di*SeH.

9. Bright-Field images for Fig. 3, 4, and 6a.

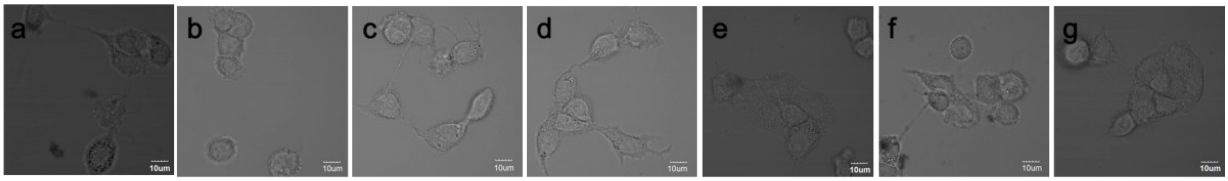


Fig. S5. Bright-field images of cells in Fig. 2 (a) – (g), respectively.

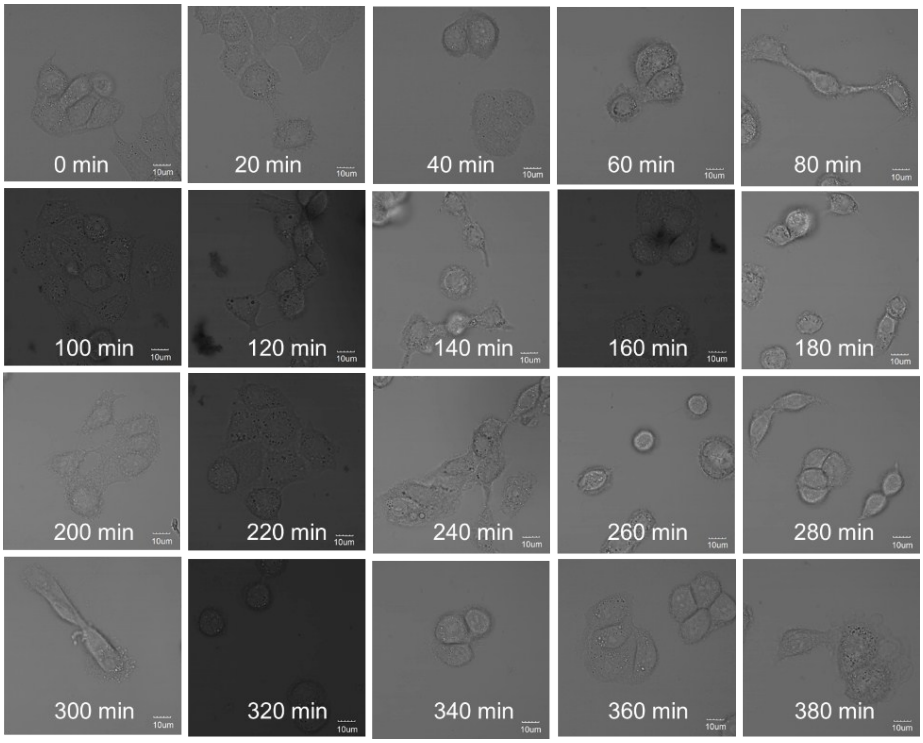


Fig. S6. Bright-field images of Fig. 3.

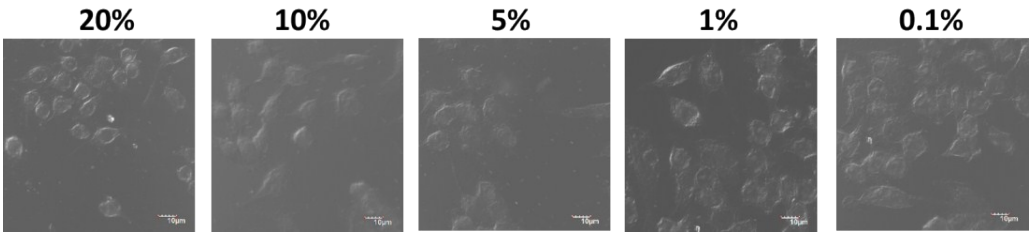


Fig. S7. Bright-field images of cells in Fig. 4 under different level of oxygen concentration (20%, 10%, 5%, 1%, and 0.1%).

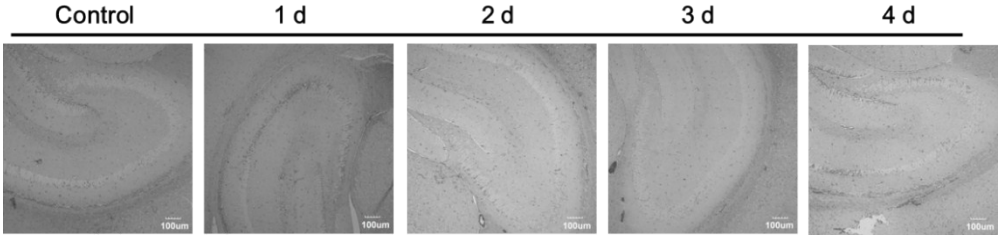


Fig. S8. Bright-field images of Fig. 6a.

10. Quantitative of proteins in Fig. 6c

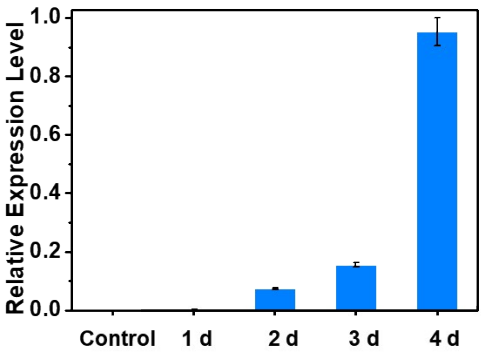


Fig. S9. The relative expression level of HIF-1α in Fig. 6c was quantified. Data are presented as means ± SD (n = 5).

11. Stability tests for BD-*di*SeH

The selenol (-SeH) can be oxidized to form diselenide (-Se-Se-). However, the oxidation reaction requires proper conditions, and it depends on its reaction thermodynamics and kinetics. For examples, the -SeH can be oxidized to form -Se-Se- by sodium perborate at room temperature.[7] Or the $-Se^-$ can be oxidized to form -Se-Se- by bromine at 25 ^oC.[8] The other oxidation way to diselenides is by a solution of pyridine, water, benzoic acid, and trichloroisocyanuric acid in acetonitrile and methylene chloride.[9] That is, the oxidation of –SeH in air is not fast. Therefore, the selenol is difficult to be oxidized in the air at room temperature. In order to further avoid the oxidation reaction of selenol, our design strategy for the probe involves the electron-withdrawing bridge between the response moiety and fluorophore to govern the reactivity and stability of our probe. Moreover, the fluorophore BODIPY is also considered to be an electron withdrawing group for selenol. Electron withdrawing properties can reduce the electron density of benzenselenol, which will prevent the benzenselenol moieties (-SeH) to be further oxidized. Therefore, although the probe, BD-diSeH, contains two benzenselenol moieties (-SeH), the selenol (-SeH) does not exhibit a rapidly oxidation in air to form diselenide (-Se-Se-).

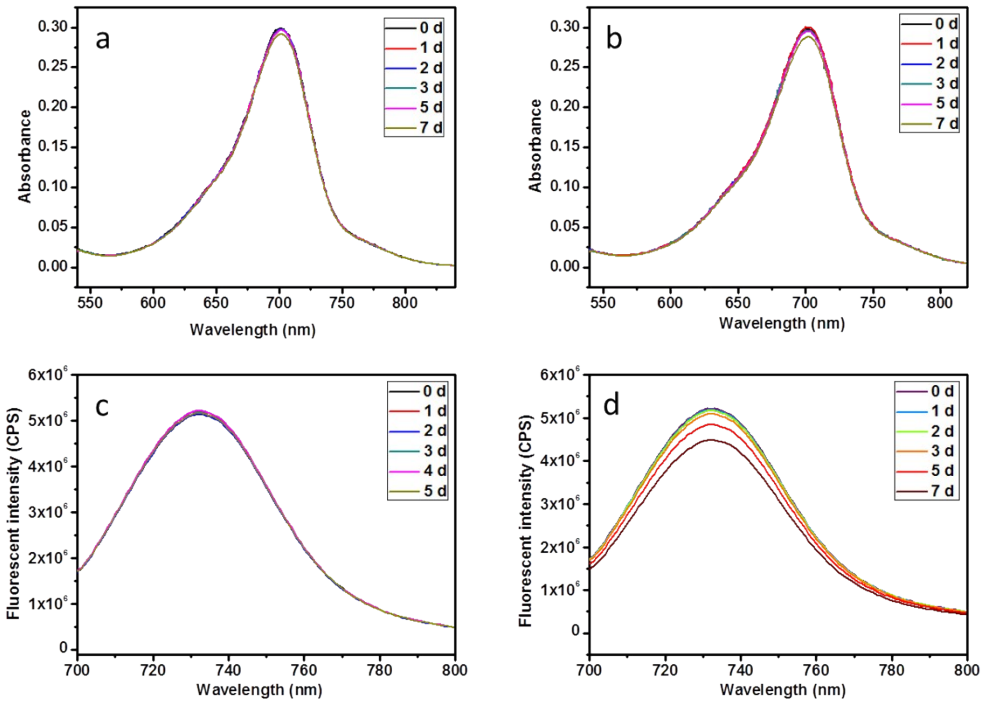


Fig. S10. The stability test of BD-*di*SeH. a) and b): The changes of absorbance and fluorescence response of BD-*di*SeH stored in solution. A dosage of BD-*di*SeH was dissolved in DMSO as stock solution (1 mM). The stock solution were protected from light at 20 °C for 0, 1, 2, 3, 5, 7 days. Then the solution was diluted to 10 μM for absorbance detection. Subsequently, added Na_2S_4 (20 μM) to the tested probe solution (10 μM) to further detection of the fluorescence changes. c) and d): The changes of absorbance and fluorescence response of BD-*di*SeH solid exposed to the air for 0, 1, 2, 3, 5, 7 days. Then the solids were dissolved in DMSO to form six stock solutions (1 mM) and diluted to 10 μM for absorbance detection. Then added Na_2S_4 (20 μM) to the tested probe solution (10 μM) to further detection of the fluorescence changes. All spectra were acquired in 10 mM HEPES (20% fetal bovine serum, v/v, pH 7.4).

In order to comprehensively evaluate the stability of BD-*di*SeH, we performed experiments by investigating the spectral properties of BD-*di*SeH and high performance liquid chromatography (HPLC) analysis. 1) Assessment the stability of the BD-*di*SeH in solution. The probe BD-*di*SeH was dissolved in DMSO as stock solution (1 mM) and protected from light at 20 °C for 0, 1, 2, 3, 5, 7 days. The probe was diluted to 10 μM with 10 mM HEPES for detecting the absorbance changes. Then added Na_2S_4 (20 μM) to the tested probe solution (10 μM) to further detection of the fluorescence changes. As shown in Fig. S10a and S10b, no obvious interference on absorption and fluorescence spectra were observed. The purity was also analyzed by HPLC as ≥ 99% on the 7th day (Fig. S11). 2) Evaluation the stability of the BD-*di*SeH solid. The solid of BD-*di*SeH was exposed in

the air for 0, 1, 2, 3, 5, 7 days from light at 20 °C. And then the solid was dissolved in DMSO as 1 mM stock solutions. Then the stock solutions were diluted to 10 μM with 10 mM HEPES for tests, respectively. Then added Na_2S_4 (20 μM) to the tested probe solution (10 μM) to further detection of the fluorescence changes. As displayed in Fig. S10c and S10d, the fluorescence spectra showed a decrease in fluorescence response from the 3nd day to the 7th day. The result indicated that the solid of our probe could be oxidized when exposed to air. But the solid could not be severe oxidized within 48 h. Furthermore, the photoability of BD-*di*SeH was also investigated in cells (Fig. S3). In general, our probe was stable for the detection of sulfane sulfur under the given experimental conditions.

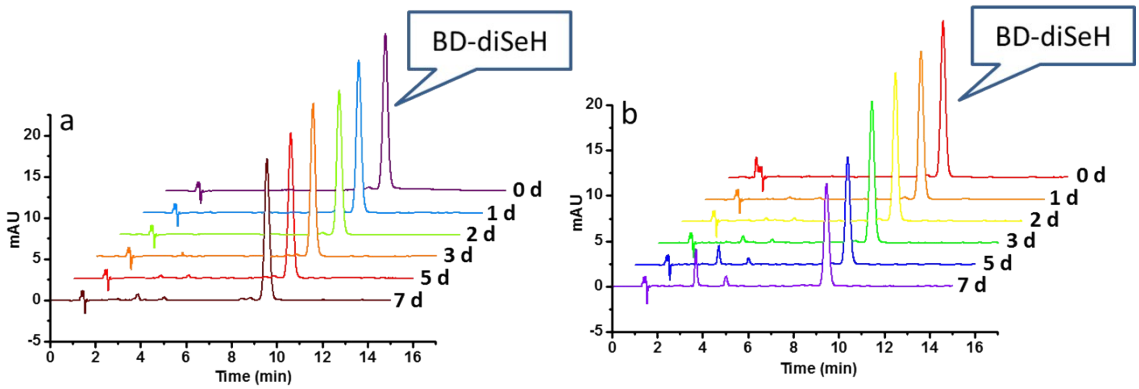


Fig. S11. HPLC tests for the purity of BD-*di*SeH in stock solutions in Fig. S10. Method: a ZORBAX Eclipse Plus C18 (4.6 mm × 150 mm, 5 μm) column and mobile phase of methanol-water (70:30) were used. The flow rate was 1 mL/min. the detection wavelength was at 310 nm. The detection temperature was 25 °C.

12. Selectivity of BD-*di*SeH for ROS, metal ions and amino acids

We examined the selectivity of BD-*di*SeH for various biological relevant reactive oxygen species. As shown in Fig. S12, in contrast to strong fluorescent intensity induced by Na_2S_4, BD-*di*SeH showed a negligible fluorescent response upon addition of same concentration of biological relevant reactive oxygen species including $O_2^{\bullet-}$, $^{\bullet}OH$, H_2O_2, methyl linoleate hydroperoxide (MeLOOH), cumene hydroperoxide (CuOOH), $ONOO^-$, NO and *tert*-butyl hydroperoxide (*t*-BuOOH). These results showed that our probe exhibited high selectivity for sulfane sulfur over relevant reactive oxygen species at physiological concentrations.

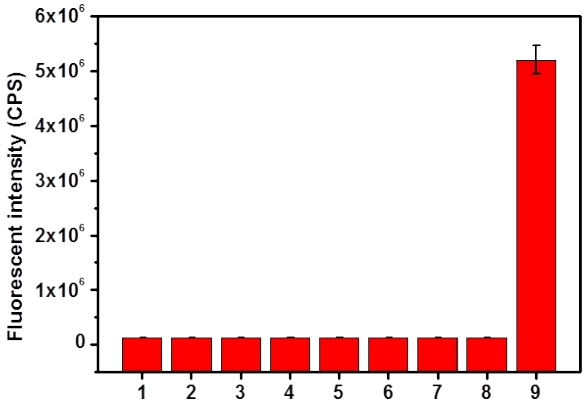


Fig. S12. Fluorescence responses of BD-*di*SeH to reactive oxygen species. (1) $O_2^{\bullet-}$ (20 μM), (2) $^{\bullet}OH$ (20 μM), (3) H_2O_2 (20 μM), (4) methyl linoleate hydroperoxide (MeLOOH, 20 μM), (5) cumene hydroperoxide (CuOOH, 20 μM), (6) $ONOO^-$ (20 μM), (7) NO (20 μM), (8) *tert*-butyl hydroperoxide (*t*-BuOOH, 20 μM), (9) Na_2S_4 (20 μM). Data were obtained in 10 mM HEPES buffer (20% fetal bovine serum, v/v, pH 7.4) at 37 °C for 15 min. λ_{ex} = 707 nm, λ_{em} = 737 nm.

We then examined the selectivity of BD-*di*SeH for various important biological relative metal ions and amino acids. BD-*di*SeH (10 μM) was treated with various analytes in HEPES buffer. As shown in Figure S13, the K^+ , Na^+ , Ca^{2+}, Mg^{2+}, Zn^{2+} and Cu^{2+} did not induce any fluorescent enhancement. BD-*di*SeH showed a negligible fluorescent response upon addition of biological amino acid including alanine, glutamine, glycine, histidine. Therefore, these results showed high selectivity of sulfane sulfur over important biological relative metal ions and amino acid.

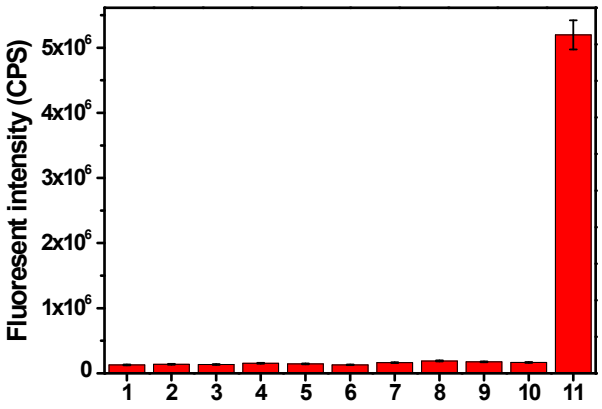


Fig. S13. Fluorescence responses of BD-*di*SeH to metal ions and amino acids. (1) K^+ (1 mM), (2) Na^+ (1 mM), (3) Ca^{2+} (1 mM), (4) Mg^{2+} (1 mM), (5) Zn^{2+} (1 mM), (6) Cu^{2+} (1 mM), (7) alanine (200 μM), (8) glutamine (200 μM), (9) glycine (200 μM), (10) histidine (200 μM), (11) Na_2S_4 (20 μM). Data were obtained in 10 mM HEPES buffer (20% fetal bovine serum, v/v, pH 7.4) at 37 °C for 15 min. λ_{ex} = 707 nm, λ_{em} = 737 nm.

13. Response ability of BD-*di*SeH towards sulfane sulfur

To investigate whether the response ability of BD-*di*SeH towards sulfane sulfur was effected by pH, Na_2S_4 (20 μM) was added to BD-*di*SeH (10 μM) at pH 10.0, the fluorescent intensity was then determined (a). Subsequently, the pH of solution adjusted to 7.4, and then fluorescent intensity was determined (b). As shown in Figure S14, the fluorescent intensity was increased after the pH of solution adjusted to 7.4, which was consistent with that of the reaction under pH 7.4 (c). The results indicated that pH values did not affect the response ability of BD-*di*SeH towards sulfane sulfur.

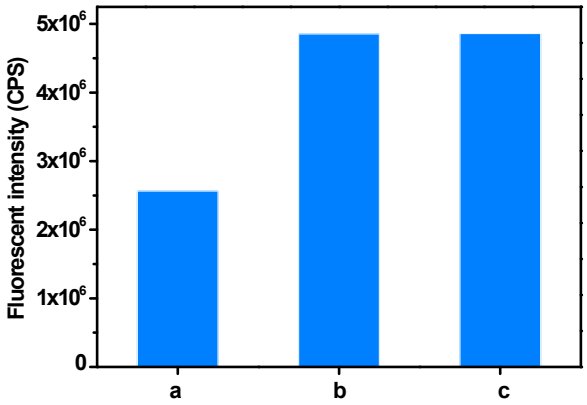


Fig. S14. Fluorescent intensity of BD-*di*SeH (10.0 μM) towards sulfane sulfur (20.0 μM) in 10 mM HEPES under different pH values. (a) Na_2S_4 (20 μM) was added to BD-*di*SeH (10 μM) at pH 10.0. (b) Na_2S_4 (20 μM) was added to BD-*di*SeH (10 μM) at pH 10.0, and then adjusted the solution to pH 7.4. (c) Na_2S_4 (20 μM) was added to BD-*di*SeH (10 μM) at pH 7.4.

14. The characteristics of the previously published probes for sulfane sulfur detection

Table S1. The characteristics of the previously published probes.

Fluorescent probe	λ_{ex}	λ_{em}	Limit of detection	Response time	Selectivity	Application (In the cells)	Application (In vivo)
BD-*di*SeH	707 nm	737 nm	2.3 nM	80 s	High	A549 cells, Hek 293 cells, SHSY5Y cells, HepG2 cells, RAW 264.7 cells, HeLa cells, SMMC-7721 cells	Imaging sulfane sulfur in mice and in zebrafish under hypoxic condition
SSP1 [4]	380 nm	458 nm	73 nM	10 min	High	H9c2 cells, HeLa cells	No
SSP2 [4]	482 nm	518 nm	32 nM	10 min	High	H9c2 cells, HeLa cells	No
Mito-SeH [10]	680 nm	720 nm	3.1 nM	100 s	High	VSM cells	Imaging sulfane sulfur in mice
BD-diSH [11]	710 nm	736 nm	10 nM	2 min	High	RAW 264.7 cells, A549 cells, Hek cells, HeLa cells, RH-35 cells, SMMC-7721 cells, SHSY5Y cells	Imaging sulfane sulfur in mice

15. Identification of the product from the reaction by HPLC and HRMS

To characterization of the product from the reaction between BD-*di*SeH and sulfane sulfur, a solution of BD-*di*SeH (0.1 mM) treated with Na_2S_4 (0.2 mM) in 10 mM HEPES (containing 20% fetal bovine serum, v/v, pH 7.4) was performed. The mixture was stirred for 30 min at 37 °C. Then the reaction product was identified by HPLC and HRMS. LC-MS (ESI^-): m/z $C_{32}H_{22}BF_2N_3O_2$ calcd. 529.1773, found $[M+H]^+$ 530.1843.

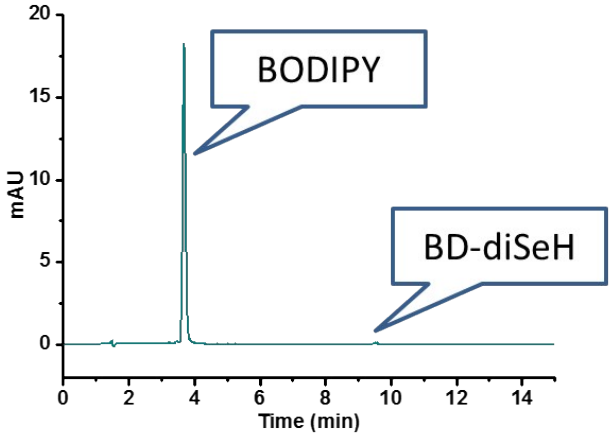
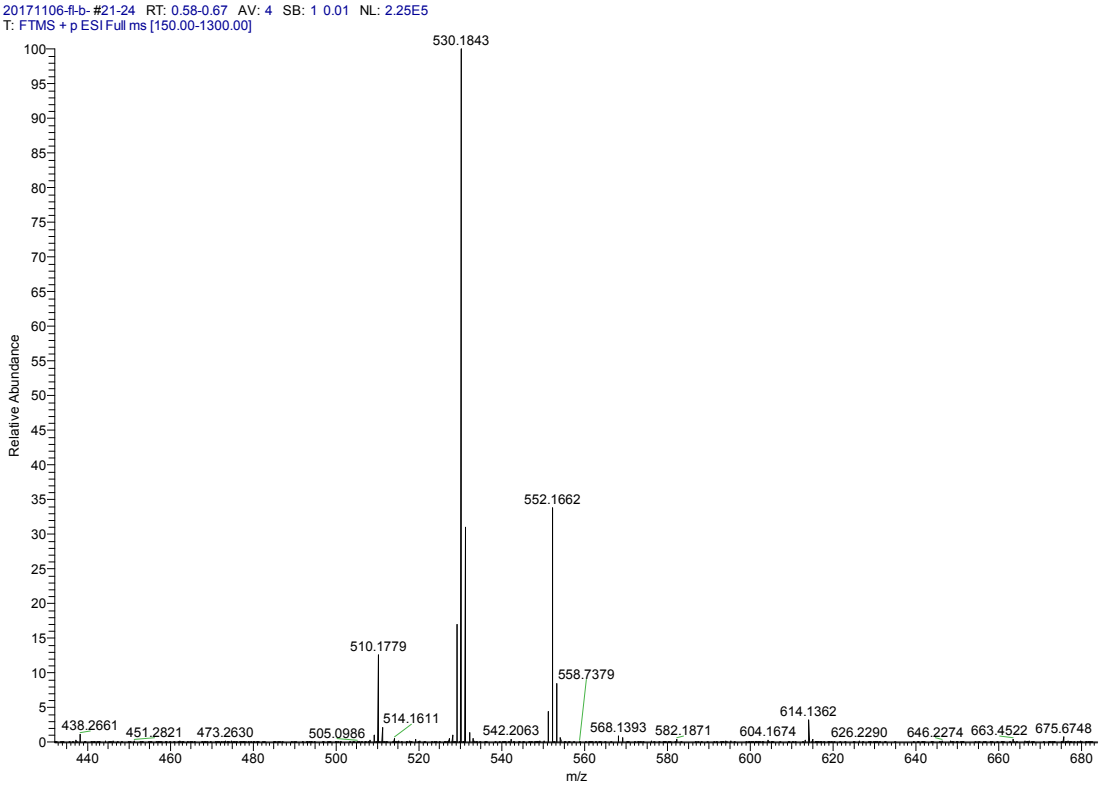
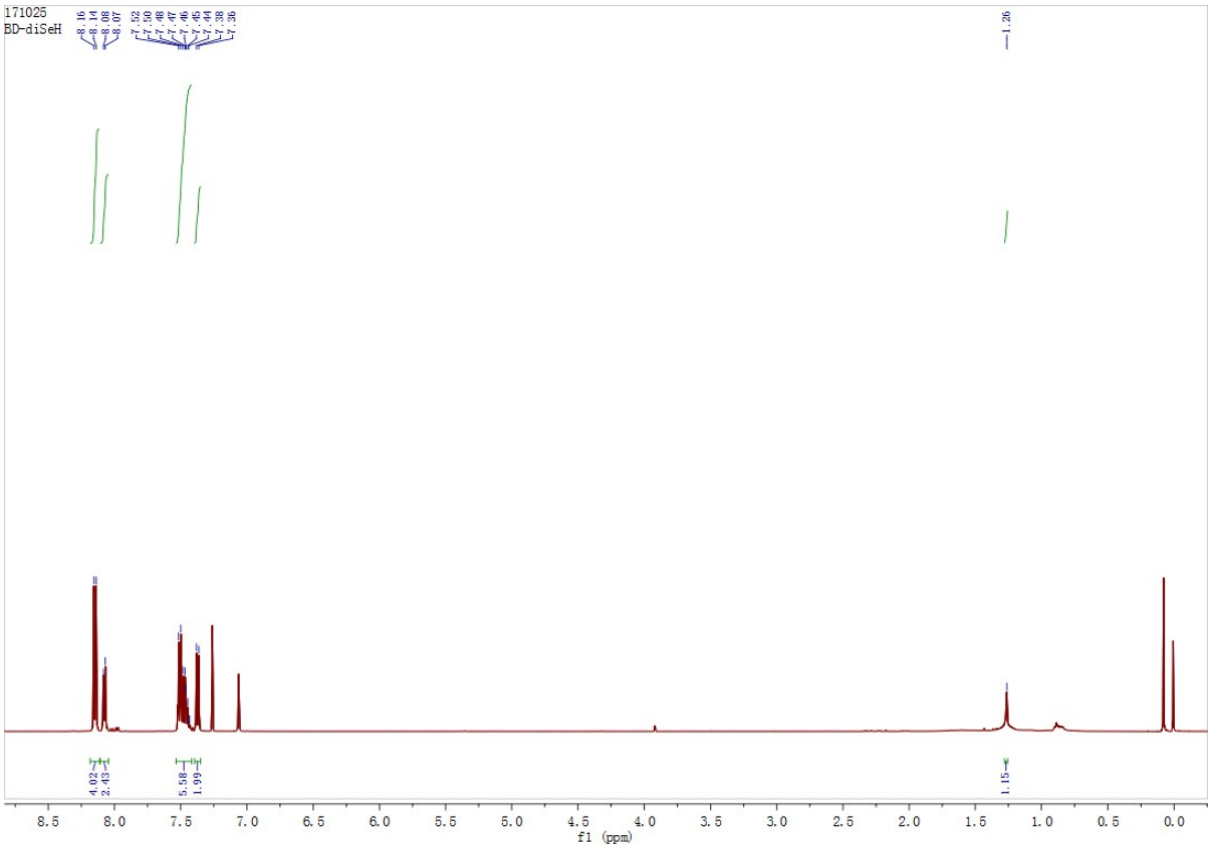
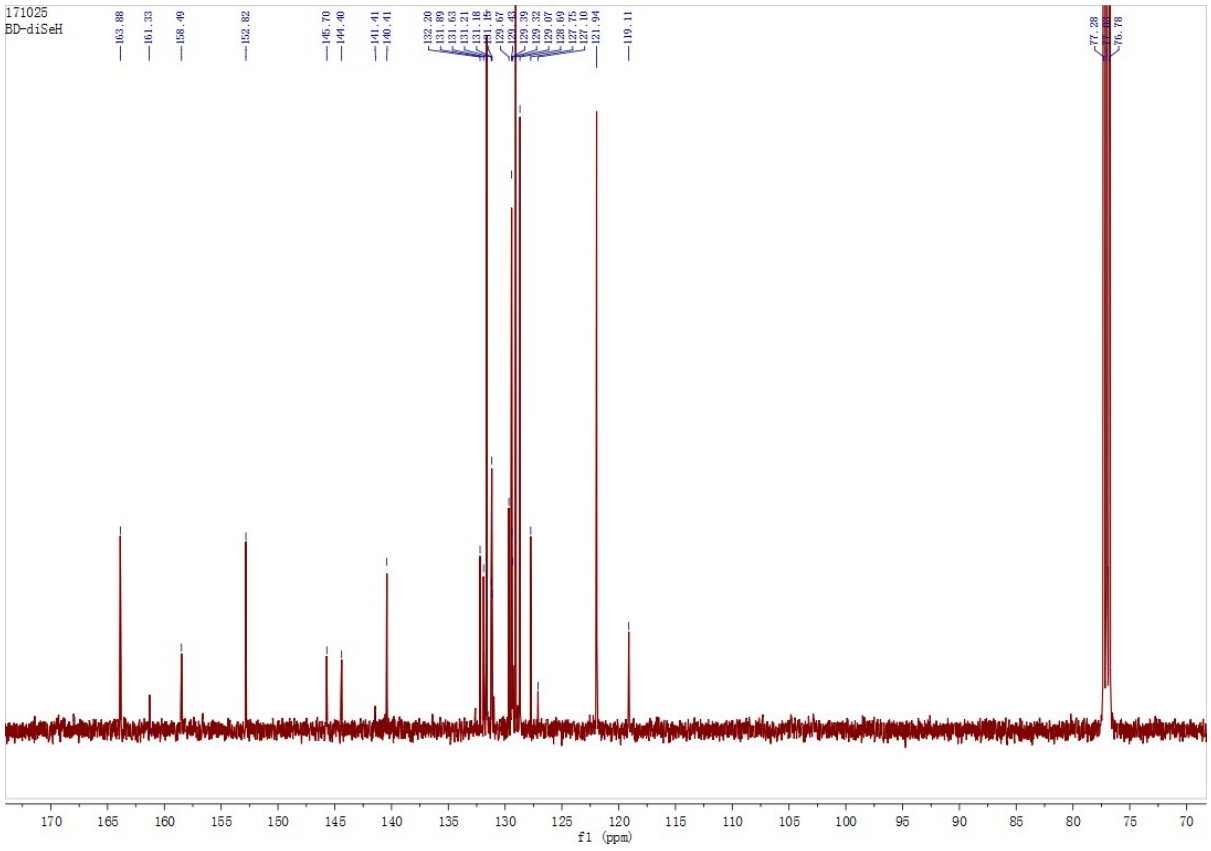


Fig. S15. HPLC test for confirming the product from the reaction between probe and sulfane sulfur. Method: a ZORBAX Eclipse Plus C18 (4.6 mm× 150 mm, 5 μm) column and mobile phase of methanol-water (70:30) were used. The flow rate was 1 mL/min. the detection wavelength was at 310 nm. The detection temperature was 25 °C.



16. ^{1}H NMR and ^{13}C NMR for BD-*di*SeH





17. Reference

(1) M. Gao, R. Wang, F. Yu, J. You and L. Chen, A near-infrared fluorescent probe for the detection of hydrogen polysulfides biosynthetic pathways in living cells and in vivo. *Analyst*, 2015, **140,** 3766-3772.

(2) T. Takata, D. Saeki, Y. Makita, N. Yamada and N. Kihara, Aromatic hydrocarbon-catalyzed direct reaction of sulfur and sodium in a heterogeneous system: selective and facile synthesis of sodium monosulfide and disulfide. *Inorg. Chem.*, 2003, **42**, 3712-3714.

(3) N. Yamada, M. Furukawa, N. Nishi and T. Takata, Direct preparation of anhydrous sodium oligosulfides from metal sodium and elemental sulfur in aprotic organic media directed toward synthesis of silane coupling agent. *Chem. Lett.,* 2002, **4**, 454-455.

(4) W. Chen, C. Liu, B. Peng, Y. Zhao, A. Pacheco and Xian, M. New fluorescent probes for sulfane sulfurs and the application in bioimaging. *Chem. Sci.,* 2013, **4**, 2892-2896.

(5) T. Karstens and K. Kobs, Rhodamine B and rhodamine 101 as reference substances for fluorescence quantum yield measurements. *J. Phys. Chem.,* 1980, **84**, 1871.

(6) W. Freyer, S. Mueller and K. Teuchner, Photophysical properties of benzoannelated metal-free phthalocyanines. *J. Photoch. Photobio. A.,* 2004, **163**, 231–240.

(7) A. McKillop, D. Koyunçu, A. Krief, W. Dumont, P. Renier and M. Trabelsi, Efficicient, high yield, oxidation of thiols and selenols to disulphides and diselenides. *Tetrahedron Lett.,* 1990, **31**, 5007-5010.

(8) H. J. Reich, M. L. Cohen and P. S. Clark, Reagents for synthesis of organoselenium compounds: diphenyl diselenide and benzeneselenenyl chloride. *Org. Synth.,* 1979, **50**, 533-537.

(9) P. Zhong, M. P. and Guo, Preparation of diselenides by the oxidation of selenols using trichloroisocyanuric acid. *Synthetic Commun.,* 2001, **31**, 1507-1510.

(10) M. Gao, R. Wang, F. Yu and L. Chen, Evaluation of sulfane sulfur bioeffects via a mitochondria-targeting selenium-containing near-infrared fluorescent probe，*Biomaterials,* 2017, **160**, 1-14.

(11) X. Han**,** X. Song, B. Li, F. Yu and L. Chen. A near-infrared fluorescent probe for sensitive detection and imaging of sulfane sulfur in living cells and in vivo. *Biomater. Sci.*, 2018, **6**, 672-682.