Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

A Reversible Fluorescence Probe Based on Se-BODIPY for the Redox Cycle between HClO Oxidative Stress and H_2S Repair in Living Cells

Bingshuai Wang, Peng Li, Fabiao Yu, Zhangrong Lou, Songqiu Yang, Ping Song, Xiaofei Sun, Zongjin Qu and Keli Han*

State Key Laboratory of Molecular Reaction Dynamics, Dalian Institute of Chemical Physics (DICP), Chinese Academy of Sciences (CAS), 457 Zhongshan Road, Dalian 116023, P. R. China

E-mail: klhan@dicp.ac.cn

Contents:

1. General Experimental Section

Materials: The solution of the probe MPhSe-BOD (acetonitrile, 1 mM) could be maintained in refrigerator at 4 °C. The $ONOO^-$ source used for chemical and biological experiments was the 3-morpholinosydnonimine hydrochloride (SIN-1, 1 mmol/mL).[1] NO is generated in form of 3-(Aminopropyl)-1-hydroxy-3-isopropyl-2-oxo-1-triazene (NOC-5, 50 μmol/ml). Methyl linoleate (MeLH) and 2,2'-azobis-(2,4-dimethyl)valeronitrile (AMVN) were used to produce MeLOOH.[2] Superoxide radicals ($O_2^{\cdot-}$) was generated by xanthine and xanthine oxidase. Xanthine oxidase was added first. Afterxanthine oxidase was dissolved, xanthine was added and the mixtures were stirred at 25 ^oC for 1 h.[3] *Tert*-butylhydroperoxide (*t*-BuOOH) and cumene hydroperoxide (CuOOH) could also use to induce ROS in biological systems.[4] HOCl was standardized at pH 12 ($\varepsilon_{292\ nm}$ = 350 $M^{-1}cm^{-1}$).[5] HOBr was prepared by adding HOCl to a small excess of NaBr in water and standardizing at pH 12 ($\varepsilon_{329\ nm}$ = 332 $M^{-1}cm^{-1}$).[6] ·OH was generated by Fenton reaction between Fe^{II}(EDTA) and H_2O_2 quantitively, and Fe^{II}(EDTA) concentrations represented ·OH concentrations.[7] The blue fluorescent Hoechst dyes are cell permeable nucleic acid stains. The fluorescence of these dyes is very sensitive to DNA conformation and chromatin state in cells. The regents were purchased from Invitrogen Corporation, and used according to the manufacturer's instructions. All other chemicals were from commercial sources and of analytical reagent grade, unless indicated otherwise. RAW264.7 cells (mouse macrophages cell line) were purchased from the Committee on type Culture Collection of Chinese Academy of Sciences. Ultrapure water was used throughout.

[1] (a) Kikuchi, K.; Hirata, M.; Nagaoka, A. *Jpn. J. Pharmacol.* **1970**, *20*, 102. (b) Nitz, R. E.; Fiedler, V. B.; *Pharmacotherapy* **1987**, *7*, 28. (c) Bassenge, E.; Kukovetz, W. R; *Cardiovascular Drug Reviews* **1984**, *2*, 177.

[2] a) Terao, J. A.; Nagao, A.; Park, D.-K. ; Lim, B. P. *Meth. Enzymol.* **1992**, *213*, 454-460. b) Chance, B.; Sies, H.; Boveris, A. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 3175-3179.

[3] a) D. Voet, J. Voet, *Biochemistry, 2nd ed.* John Wiley & Sons, New York: S04, **1995**; 816-820; b) Z. Sun, F. Liu, Y. Chen, P. Hang, D. Yang, *Org. Lett.* **2008,** *10*, 2171-2174.

[4] Nieminen, A. L.; Byrne, A. M.; Herman B.; Lemasters, J. *Am. J. Physiol. Cell Physiol.* **1997**, *272*, 1286-1294.

[5] Morris, J. C. *J. Phys. Chem.* **1966**, *70***,** 3798.

[6] Kumar, K.; Margerum, D. W. *Inorg. Chem.* **1987**, *26,* 2706-2711.

[7] Halliwell, B.; Gutteridge, J. M. C. *Arch. Biochem. Biophys.* **1986**, *246*, 501-514

Instruments: Fluorescence spectra were obtained by FluoroMax-4 Spectrofluorometer with a Xenon lamp and 1.0-cm quartz cells. Absorption spectra were measured on Lambda 35 UV-visible

spectrophotometer (PerkinElmer). ^{77}Se, ^{1}H and ^{13}C NMR spectra were taken on a Bruker spectrometer. The fluorescence images of cells were taken using a LTE confocal laser scanning microscope (Olympus FV1000 confocal laser-scanning microscope) with objective lens (×40).

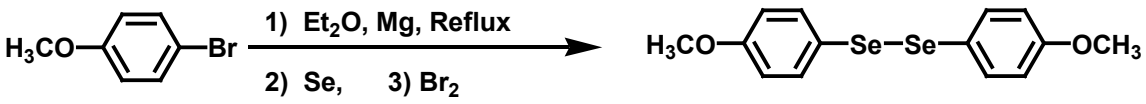
Absorption and Fluorescence Analysis: Absorption and fluorescence spectra were obtained with 1.0-cm glass cells. The probe MPhSe-BOD (acetonitrile, 0.10 mL, 1.0 mM) was added to a 10.0-mL color comparison tube. After dilution to 10 μM with 20 mM PBS buffers, the analytes were added. The mixture was equilibrated for 0.5 h before measurement.

Confocal Imaging: Florescent images were acquired on Olympus FV1000 confocal laser-scanning microscope with an objective lens ×40. The excitation wavelength was 488 nm. Prior to imaging, the medium was removed. Cell imaging was carried out after washing cells with 20 mM PBS for three times.

Cell Culture: Murine RAW264.7 macrophage cells (ATCC, USA) were maintained following protocols provided by the American Type Culture Collection. Cells were seeded at a density of 1×10^6 cells mL^{-1} for confocal imaging in RPMI 1640 Medium supplemented with 15% fetal bovine serum (FBS), $NaHCO_3$ (2 g/L), and 1% antibiotics (penicillin /streptomycin, 100 U/ml). Cultures were maintained at 37 °C under a humidified atmosphere containing 5% CO_2. The cells were subcultured by scraping and seeding on 20/12 mm Petri dishes according to the instructions from the manufacturer.

2. Synthesis and Characterization of Compounds

Caution! Most selenium compounds are toxic; care should be exercised to avoid contact with skin. All operations in this procedure should be conducted in a well-ventilated hood.



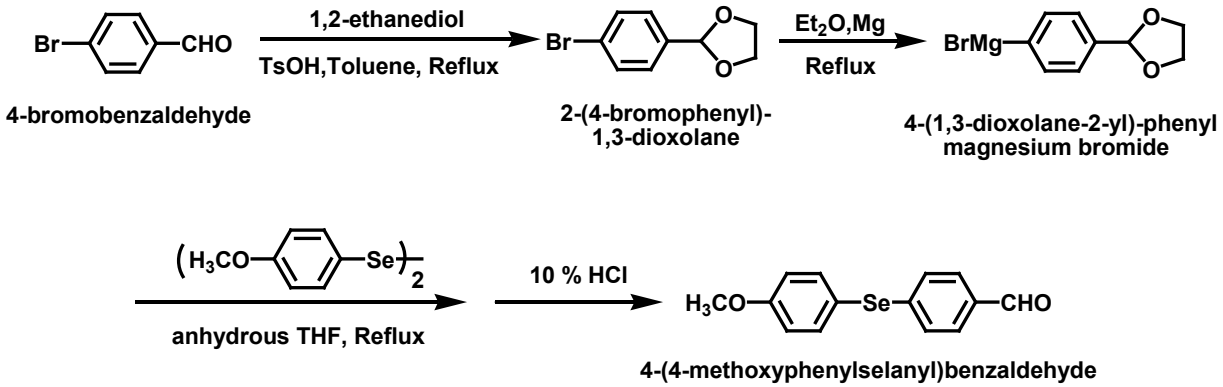
1-bromo-4-methoxybenzene **1,2-bis(4-methoxyphenyl)diselane**

Scheme S1. Synthesis of 1,2-bis(4-methoxyphenyl)diselane

Synthesis of 1,2-bis(4-methoxyphenyl)diselane: A 250 mL, three-necked, round-bottomed flask was equipped with a magnetic stirrer, a pressure-equalizing dropping funnel, a condenser distillation column and a economical allihn condenser connected to a nitrogen source. The apparatus is vacuumized，then a stream of nitrogen was passed through the system. In the cooled flask a solution of 4-methoxyphenyl magnesium bromide was prepared from 38 g (0.203 mol) of 4-methoxy bromobenzene, 4.8 g (0.2 mol) of magnesium, and 30 ml of anhydrous diethyl ether. 14 g (0.177 mol) of selenium was added through the condenser distillation column. The selenium was added in portions at a rate sufficient to maintain a vigorous reflux. The addition requires 15–30 minutes, after which the mixture was stirred and heated at reflux for another 30 minutes. 0.6 mL water was added to hydrolyze any excess Grignard reagent. The mixture was stirred and cooled in an ice bath while 5 mL bromine was added dropwise at a rate such that the ether does not reflux. Cooling and stirring were continued as a solution of 10.7 g of ammonium chloride in 28 mL water was added slowly[8]. The mixture was filtered by vacuum, and the granular precipitate was washed thoroughly with three 100-mL portions of ether. The combined filtrates were dried with anhydrous sodium sulfate and then evaporated on a rotary evaporator until dry. The remaining solid was purified on silica gel chromatography (200-300 mesh) eluted with dichloromethane: petroleum ether = 1:1 (v/v), the bright yellow product was collected. After evaporated the eluent, it afforded orange solid 26.3 g (yield: 79.8 %) of 4-methoxy diphenyl diselenide.

^{1}H NMR (400 MHz, $CDCl_3$, ppm): 3.80 (s, 6H, O*CH*$_3$), 6.80 (d, 4H, *Ar-H*, *J* = 8.0), 7.50 (d, 4H, *Ar-H*, *J* = 8.0). ^{77}Se NMR (95 MHz, $CDCl_3$, ppm): 503.77. HRMS (TOF-LD+): *m*/*z* $C_{14}H_{14}O_2Se_2$ Calc. 373.9324, found 373.9307.

[8] Reich, H. J.; Cohen, M. L.; Clark. P. S. *Organic Syntheses,* **1979**, *59,* 141; **1988**, *6,* 533.



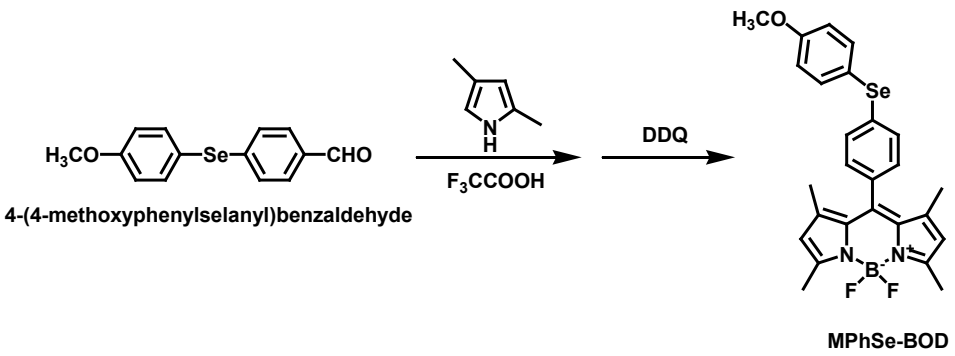
Scheme S2. Synthesis of 4-(4-methoxyphenylselanyl)benzaldehyde

Synthesis of 2-(4-bromophenyl)-1,3-dioxolane: 4-bromobenzaldehyde (10 g, 0.054 mol), 4-methylbenzenesulfonic acid (0.25 g, 1.45 mmol) were dissolved in 150 mL of toluene. Ethylene glycol (6 mL) was added, and the solution was refluxed with a Dean-Stark trap to azeotropically remove water[9]. After 10 hour, the solution was allowed to cool to ambient temperature and washed twice with saturated Na_2CO_3 solution. The mixture was extracted three times with ethyl acetate then the combined organic solution was dried with anhydrous sodium sulfate and evaporated to yield yellow oil. The remaining oil was purified on silica gel chromatography (200-300 mesh) eluted with dichloromethane: petroleum ether = 1:1 (v/v), the product was collected according to the determination of thin layer chromatography (TLC). After evaporated the eluent, it afforded yellowy oil (white solid at -20 °C) 12 g (yield: 97.6 %) of 2-(4-bromophenyl)-1,3-dioxolane.

Synthesis of 4-(4-methoxyphenylselanyl)benzaldehyde: Under N_2, A slowly refluxing solution of 4-(1,3-dioxolane-2-yl)-phenyl magnesium bromide was prepared from 10 g (0.044 mol) of 2-(4-bromophenyl)-1,3-dioxolane, 1.26 g (0.053 mol) of magnesium, and 90 mL of anhydrous THF. Then 19.5 g (0.052 mol) of 1,2-bis(4-methoxyphenyl)diselane in 50 mL of dichloromethane was added dropwise at a rate sufficient to maintain a vigorous reflux. After that the mixture was stirred and heated at reflux for another 2 h. 3 mL of water was added to hydrolyze any excess Grignard reagent and then the solvent was removed on a rotary evaporator. The remaining oil was dissolved in 100 mL of dichloromethane and the solution was cooled in an ice bath while 30 mL of hydrochloric acid is added slowly. The mixture was stirred until the deprotection was finished. The organic solution was dried over anhydrous Na_2SO_4 and then evaporated. The remaining solid was purified on silica gel chromatography (200-300 mesh) eluted with dichloromethane: petroleum ether = 1:1 (v/v), the product was collected. After evaporated the eluent, it afforded yellowy solid 4.94 g (yield: 49 %) of 4-(4-methoxyphenylselanyl)benzaldehyde.

^{1}H NMR (400 MHz, $CDCl_3$, ppm): 3.84 (s, 3H, O*CH*$_3$), 6.93 (d, 2H, *Ar-H*, *J* = 8.0), 7.3 (d, 2H, *Ar-H*, *J* = 8.0), 7.57 (d, 2H, *Ar-H*, *J* = 8.0), 7.65 (d, 2H, *Ar-H*, *J* = 8.0), 9.88 (s, 1H, *CHO*). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): 55.35, 115.59, 117.35, 128.93, 130.01, 134.09, 138.02, 144.35, 160.63, 191.33. ^{77}Se NMR (95MHz, $CDCl_3$, ppm): 419.14. HRMS (TOF LD+) $[M+H]^+$ calcd. for $C_{14}H_{13}O_2Se$ 309.0030, found 309.0005.

[9] Briggs, J. R.; Klosin, J.; Whiteker. G. T. *Org. Lett.* **2005**, *7*, 4795-4798.



Scheme S3. Synthesis of MPhSe-BOD

Synthesis of MPhSe-BOD: 0.5 g (1.7 mmol) of 4-(4-methoxyphenylselanyl)benzaldehyde, and 0.35 g (3.4 mmol) of 2,4-dimethylpyrrole were dissolved in 250 mL of anhydrous dichloromethane under nitrogen atmosphere. Five drops of trifluoroacetic acid was added and the solution stirred at room temperature until TLC-control showed complete consumption of the aldehyde. At this point, 0.39 g (1.7 mmol) dichlorodicyanobenzoquinone (DDQ) was added, and stirring was continued for 15 min followed by addition of 1.2 g (11.9 mmol) of triethylamine and 2.65 g (18.7 mmol) of $BF_3 \cdot Et_2O$[10]. After stirring for another 3 h the reaction mixture was washed with water and dried, and the solvent was evaporated. The residue was purified on preparative TLC (0.5 mm thick) eluted with dichloromethane: petroleum ether = 1:1 (v/v) and the orange solid was obtained. 0.7 g, Yield: 76 %. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): 1.42 (s, 6H, *CH*$_3$), 2.54 (s, 6H, *CH*$_3$), 3.83 (s, 3H, O*CH*$_3$), 5.97 (s, 2H, *CH*), 6.9 (d, 2H, Ar-*H*, *J* = 8.0), 7.11 (d, 2H, *J* = 8.0, Ar-*H*), 7.39 (d, 2H, *J* = 8.0, Ar-*H*), 7.54 (d, 2H, *J* = 8.0, Ar-*H*). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): 14.57, 29.71, 55.35, 115.39, 119.07, 121.28, 128.72, 130.87, 131.44, 132.92, 135.11, 136.84, 141.11, 143.03, 155.56, 160.14. ^{77}Se NMR (95 MHz, DMSO-d6, ppm): 396.82. HRMS (TOF LD+) calcd. for $C_{26}H_{25}BF_2N_2OSe$ 510.1193, found 510.1173.

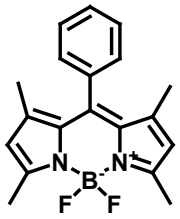
[10] Kollmannsberger, M.; Rurack, K.; Resch-Genger, U.; Daub, J. *J. Phys. Chem. A* **1998,** *102,* 10211-10220.

Characterization of MPhSeO-BOD: MPhSe-BOD 1 mmol was dissolved in 10 mL of water containing 30 % acetonitrile. Subsequently, 100 equiv. of ClO^- was added. After 0.5 h, **MPhSeO-BOD** was characterized by ^{77}Se NMR and MS. ^{77}Se NMR (95 MHz, DMSO-d6, ppm): 858.50. HRMS (TOF LD+) $[M+H^+]^+$calcd. for $C_{26}H_{25}BF_2N_2O_2Se$ 527.1221, found 527.1264.

Characterization of the reductive product in the presence of H_2S: The oxidative product of 1 mM MPhSe-BOD by 100 equiv. of ClO^- was dissolved in 10 mL of water containing 30 % acetonitrile, then, 100 equiv. of NaHS was added. After 1 h, the reductive product was characterized by MS. HRMS (TOF LD+) M^+ calcd. for $C_{26}H_{25}BF_2N_2OSe$ 510.1193, found 510.1179.

3. Structure of BOD and fluorescence lifetime decay of MPhSe-BOD, MPhSeO-BOD, BOD

Time-resolved fluorescence studies would provide invaluable information about the system when more than one emitting species contribute to steady-state fluorescence intensities.[11,12] Therefore, we carried out fluorescence lifetime studies to confirm the involvement of PET process in the fluorescence probe. The fluorescence decay of the probe MPhSe-BOD obeyed biexponential fit (τ = 0.3 ns 89 % and 3.3 ns 11 %). The shorter lifetime component was due to the PET promoted charge transfer.[11] After response to HClO, the shorter lifetime component decreased until it disappeared (Figure S2a). The experimental results indicate that the PET process was blocked. The single exponential decay (τ = 3.1 ns) of the oxidation product (MPhSeO-BOD) was similar to that of fluorophore BOD (τ = 3.8 ns, Figure S1 and S2b), which wouldn't produce PET process. This experimental evidence demonstrates that the quenching mechanism of the fluorescence probe is due to the PET process.



BOD

Figure S1. Structure of BOD.

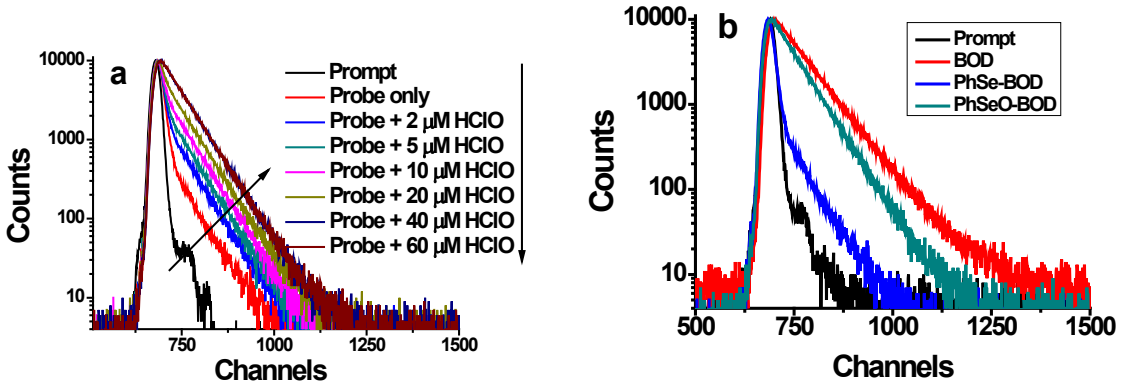


Figure S2. a) Fluorescence lifetime decay of MPhSe-BOD (10 μM) upon the addition of HClO in 20 mM PBS pH 7.4 containing 30 % acetonitrile (λ_{ex} = 465 nm, λ_{em} = 510 nm). b) Fluorescence lifetime decay of MPhSe-BOD, MPhSeO-BOD, BOD (10 μM) in 20 mM pH = 7.4 PBS containing 30 % acetonitrile (λ_{ex} = 465 nm, λ_{em} : MPhSe-BOD, 510 nm; MPhSeO-BOD, 510 nm; BOD, 508 nm).

[11] Ashokkumar, P.; Ramakrishnan, V. T.; Ramamurthy P. *J. Phys. Chem. A* **2011**, *115*, 14292–14299.

[12] Royzen, M.; Durandin, A.; Young Jr., V. G.; Geacintov, N. E.; Canary, J. W. *J. Am. Chem. Soc.* **2006**, *128*, 3854-3855.

4. The absorption spectra of MPhSe-BOD with HClO in different concentration

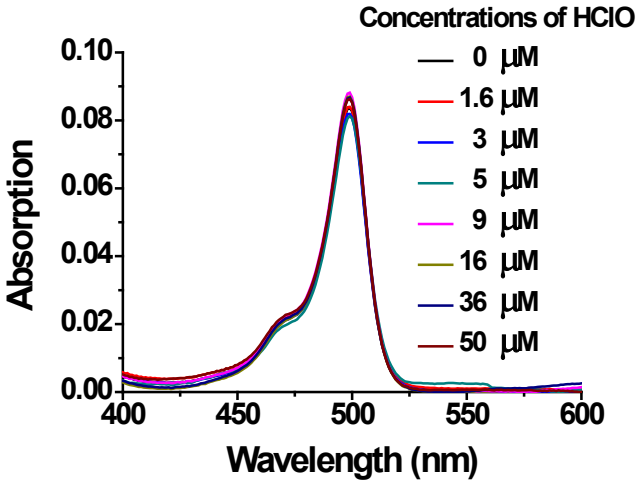


Figure S3. Absorption response of 10 μM MPhSe-BOD to HClO in 20 mM pH 7.40 PBS containing 30 % acetonitrile.

5. Effect of pH Values

We investigated the effect of pH on the fluorescence emission. the probe **MPhSe-BOD** and the oxidition product **MPhSeO-BOD** had nearly no effect on fluorescence intensity by the pH of the mediums within the range from 4.0 to 12.0.

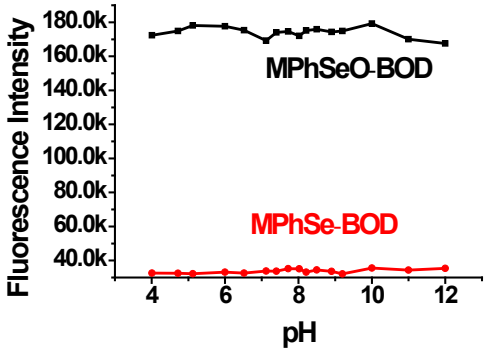


Figure S4. Fluorescence intensity of probe (10 μM) with absence and presence of 100 μM HClO in various pH PBS containing 30 % acetonitrile (λ_{ex}: 460 nm, λ_{em}: 510 nm). pH values: 4.01, 4.72, 5.12, 6.0, 6.52, 7.12, 7.4, 7.72, 8.02, 8.21, 8.5, 8.9, 9.2, 10, 11, 12.

6. Time-dependent fluorescence spectra of MPhSeO-BOD with different oxidants

We also tested the influence of HBrO, but HBrO in a lower concentration (30 μM) can decrease quickly and remarkably the fluorescence of MPhSeO-BOD (data no shown). The reason may be the strong oxidizability of HBrO destroys the structure of the fluorophore.

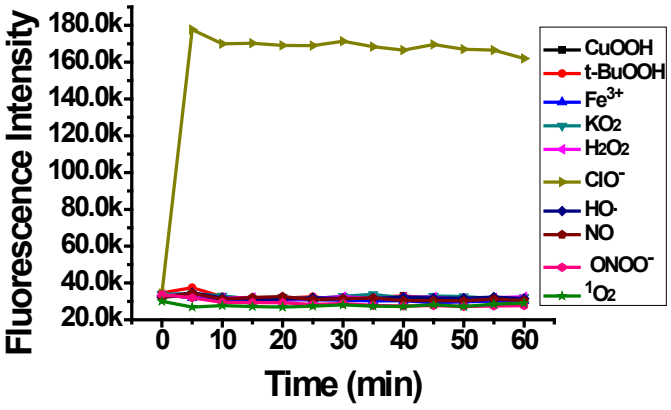


Figure S5. Time dependent fluorescence intensity changes of probe MPhSe-BOD (10 μM) with 100 μM ROS in 20 mM PBS (acetonitrile 30 %) at pH = 7.4 (λ_{ex}: 460 nm, λ_{em}: 510 nm).

7. Selectivity of MPhSeO-BOD to the reductants

To evaluate the specific nature of **MPhSeO-BOD** towards reductants, the selectivity of MPhSeO-BOD to the reductants, including glutathione (GSH), H_2S, cysteine (Cys), dithiothreitol (DTT), Fe^{2+}, $Na_2S_2O_4$, vitamin C (Vc), $NaHSO_3$, homocysteine (Hcys), alpha lipoic acid (ALA), was investigated. The fluorescence intensity changes of **MPhSeO-BOD** with various oxidants within 30 min were shown in Figure S6. **MPhSeO-BOD** was quickly reduced by H_2S and the fluorescence intensity recovered to the original level, whereas the fluorescence intensity decreased slowly upon the addition of the other reductants.

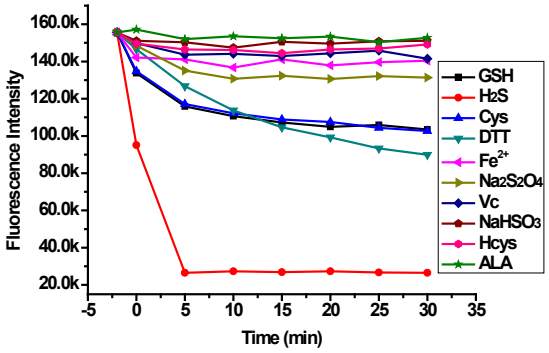


Figure S6. Time dependent fluorescence intensity changes of MPhSeO-BOD (10 μM) with 100 μM reductants in 20 mM PBS (acetonitrile 30 %) at pH = 7.4 (λ_{ex}: 460 nm, λ_{em}: 510 nm).

8. Comparison of fluorescence lifetime in the redox cycle

The fluorescence lifetime decay of MPhSeO-BOD reduced by H_2S was carried out and compared with that of probe MPhSe-BOD. Figure S8 showed two decays were very similar, which also corroborate MPhSeO-BOD were reduced to original state.

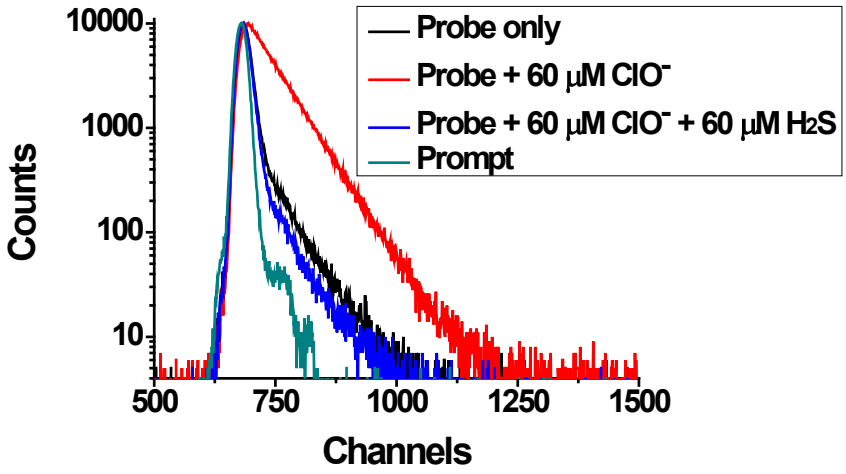


Figure S7. Fluorescence lifetime decay of MPhSe-BOD, MPhSeO-BOD, MPhSeO-BOD with H_2S in 20 mM pH = 7.4 PBS containing 30 % acetonitrile (λ_{ex} = 465 nm, λ_{em} : 510 nm).

9. The redox cycles

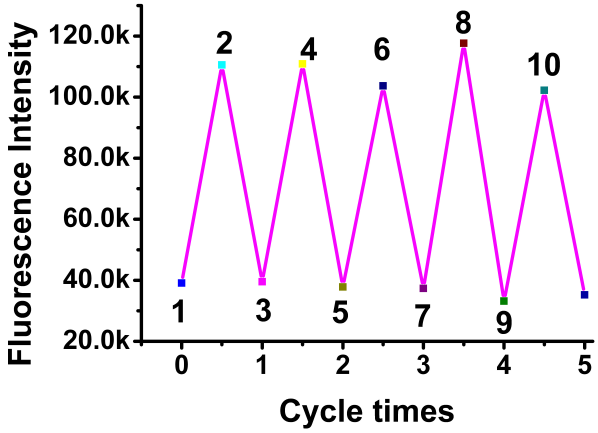


Figure S8. Fluorescence responses of MPhSe-BOD (10 μM) to redox cycles. MPhSe-BOD was oxidized by HClO, After 20 min, the solution was treated with H_2S. When fluorescence returned to starting levels, another portion of HClO was added. The redox cycles were repeated five times. All spectra were measured in 20 mM PBS pH 7.4, λ_{ex} = 460 nm and λ_{em} = 510 nm. The following represent the numbers shown in Figure 5: 1, 50 μM HClO; 2, 50 μM H_2S; 3, 90 μM HClO; 4, 90 μM H_2S; 5,180 μM HClO; 6, 90 μM H_2S; 7, 180 μM HClO; 8, 90 μM H_2S; 9, 180 μM HClO; 10, 90 μM H_2S.

10 The calculation of the detection limit

The detection limit (DL) can be calculated with the equation[11,13], DL = $3S_0/m$, where "m" is the calibration sensitivity of the fluorescence intensity change ($\Delta F = F_0 - F$) versus [ClO^-] or [H_2S],

and "S_0" is the standard deviation of the blank signal (F_0) obtained without ClO^- or H_2S. From this, the detection limits of the probe for ClO^- and H_2S were found to be 0.98 and 1.08 μM under the testing conditions, respectively.

[13] (a) Long, G.; Winefordner, J. *Anal. Chem.* 1983, **55**, 712A–724A. (b) Pandey, S.; Azam, A.; Pandey, S.; Chawla, H. *Org. Biomol. Chem.* 2009, **7**, 269–279.

11 MTT Assay

To investigate cytotoxicity of the probe, MTT assay were carried out when the probes existed. RAW264.7 macrophage cells (1×106 cells/well) were dispersed within replicate 96-well microtiter plates to atotal volume of 200 μL $well^{-1}$. Plates were maintained at 37 °C in a 5% CO2/95% air incubator for 4 h.The probe was diluted to different concentrations （10^{-3}，10^{-4}，10^{-5}，10^{-6}，10^{-7}，10^{-8} M）of solution with medium and added to each well after the original medium has been removed. Macrophages were incubated with different probe concentrations for 24 h. And then 100 μL MTT solution (5 mg mL^{-1} in PBS) was added to each well. After 4 h, the remaining MTT solution was removed, and 150 μL of DMSO was added to each well to dissolve the formazan crystals. The absorbance was measured at 570 nm in a TRITURUS microplate reader. Calculation of IC50 values was done according to Huber and Koella[14].

From this, IC50 value is calculated to be 120 μM, and clearly demonstrated that the probe is of low toxicity to cultured cell lines under the experimental conditions at the concentration of 10 μM.

[14] Huber W.; Koella J. *Acta Trop.*, 1993, **55**, 257-261.

12 The effect of MPO inhibitor and taurine

We also performed a trial to investigate the effect of MPO inhibitor and taurine. Living RAW264.7 cells loaded with 10 μM MPhSe-BOD for 10 min showed weak fluorescence (Fig. S9a). When the cells were firstly incubated with 100 μM MPO inhibitor 4-aminobenzoic acid hydrazide (4-ABAH) [15] for 00 min, and then stimulated with 1.5 μg/mL PMA for 30 min. No increase in the intracellular fluorescence was observed (Fig. S9b), which showed MPO had important impact. Probe-loaded, PMA-treated cells (Fig. S9c) were treated with taurine, there is not obvious change in the intracellular fluorescence intensity (Fig. S9d), which indicated taurine hadn't any effect in the fluorescent properties of the probe. The results confirmed that MPhSe-BOD was oxidized selectively by HClO in the living cells.

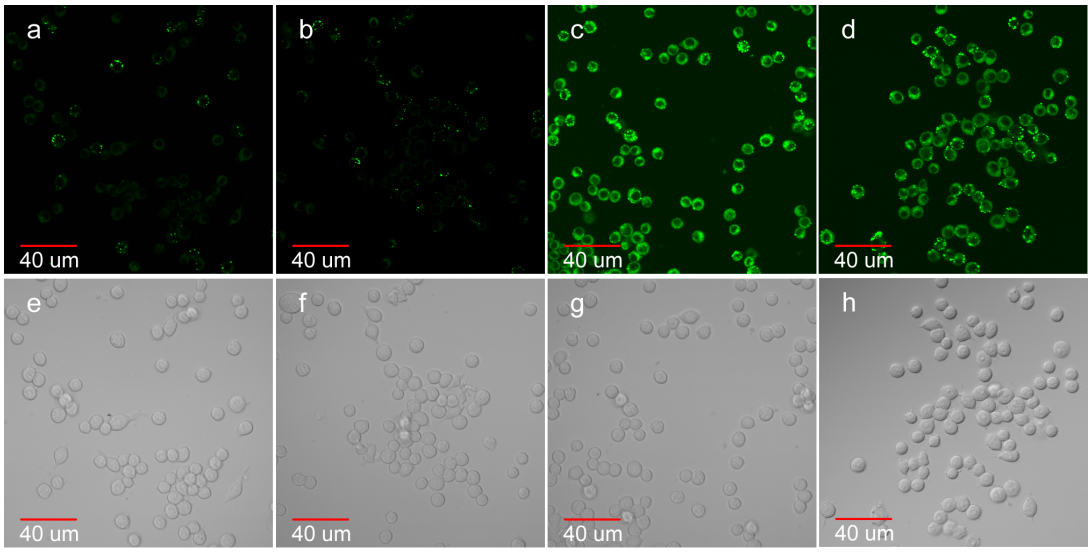


Figure S9. Confocal fluorescence images of the effect of MPO inhibitor and taurine in RAW264.7 cells. Cell images were obtained using an excitation wavelength of 488 nm and emission band from 500 nm to 600 nm. (a) Control. (b) Probe-loaded cells incubated with 4-ABAH (100 μM) for 20 min, then, PMA (1.5 μg/mL) was added for 30 min. (c) Probe-loaded, incubated with PMA (1.5 μg/mL) was added for 30 min.. (d) Probe-loaded, PMA-treated cells treated with taurine (5 μg/mL) for 20 min. e) - h) are the bright-field images of a) – d), respectively.

[15] a) M.Knaapen A.; P.F.Schins R.; J.A.Borm P; J.van Schooten F. *Carcinogenesis*, 2005, **26**,1642–1648, b) Kumar S.; Patel S.; Jyoti A.; Keshari R.;Verma A.; Barthwal M.; Dikshit M. *Cytometry Part A*, 2010,**77A**, 1038-1048.

13 Time dependent H_2S release associated with decomposition of H_2S Donor

5-(4-hydroxyphenyl)-3H-1,2-dithiol-3-thione (ADT-OH) was chosen as the hydrogen sulfide donor model[16], and NaHS was chosen as a control. As shown in Fig. S10, the probe could respond to the released H_2S which was decomposed by H_2S donor in living cells.

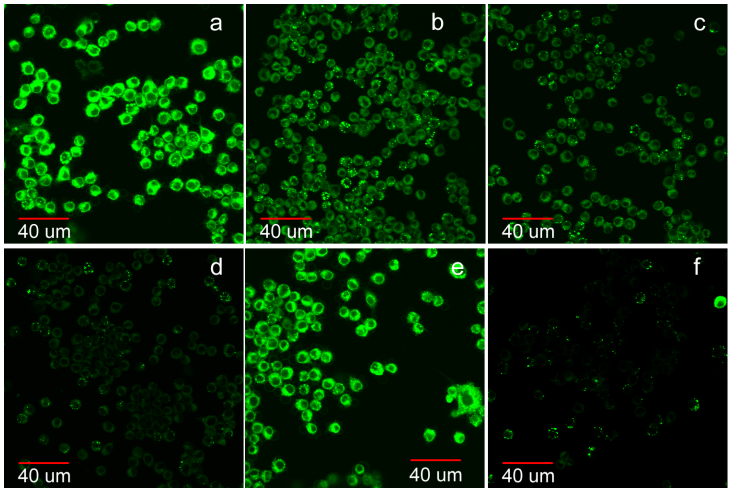
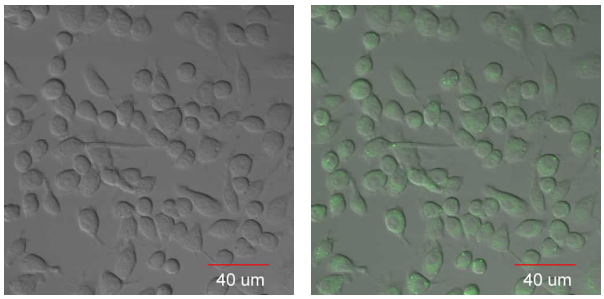


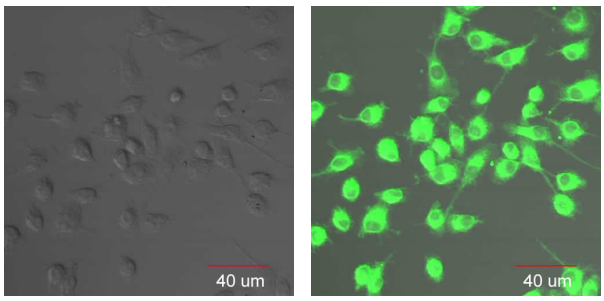
Figure S10. Confocal fluorescence images of the effect of hydrogen sulfide donors in RAW264.7 cells. (a) (e) Probe-loaded cells incubated with PMA (1.5 μg/mL) for 30 min. (b) Probe-loaded, PMA-treated cells incubated with ADT-OH (500 μM) for 30 min, (c) 60 min, (d) 120 min, (f) Probe-loaded, PMA-treated cells incubated with H_2S (100 μM) for 10 min.

[16] F. Yu, P. Li, P. Song, B. Wang, J. Zhao and Han, K. *Chem. Commun.*, 2012, **48**, 2852-2854

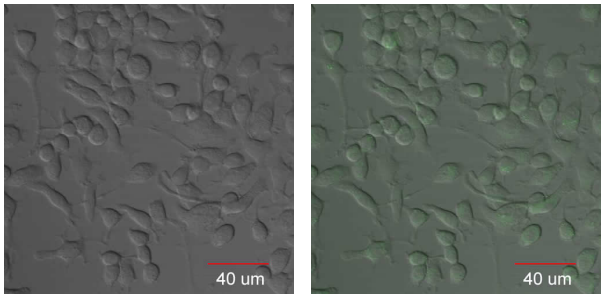
14. Bright-Field Images of Figure 3



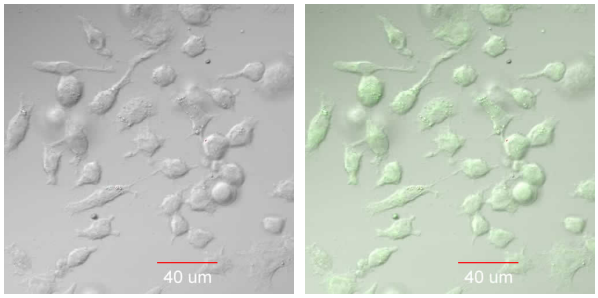
Bright-field (left), overlay of bright-field and fluorescence images (right) of Figure 3a.



Bright-field (left), overlay of bright-field and fluorescence images (right) of Figure 3b.

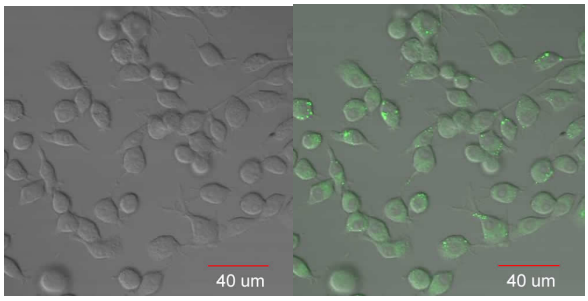


Bright-field (left), overlay of bright-field and fluorescence images (right) of Figure 3c.

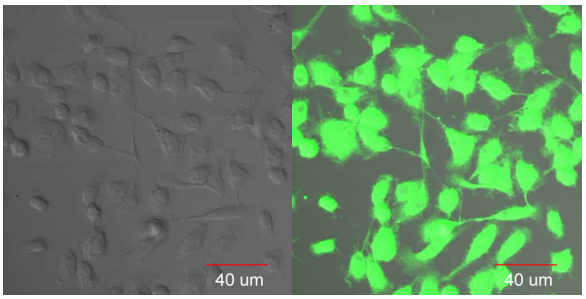


Bright-field (left), overlay of bright-field and fluorescence images (right) of Figure 3d.

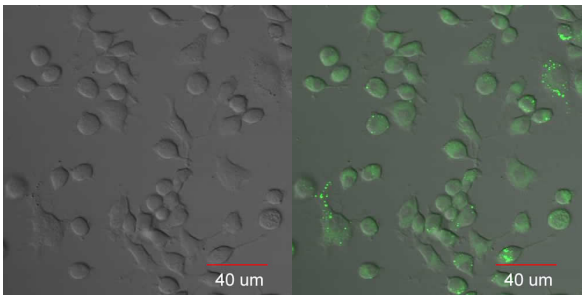
15. Bright-Field and Blue Channel Images of Figure 4



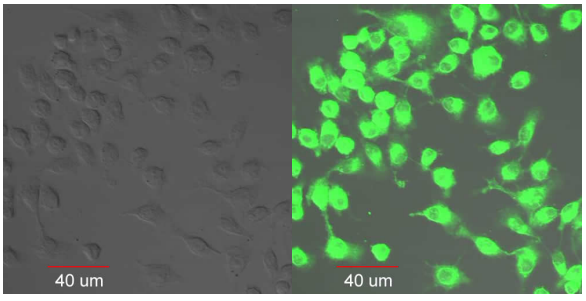
Bright-field (left), overlay of bright-field and fluorescence images (right) of Figure 4a.



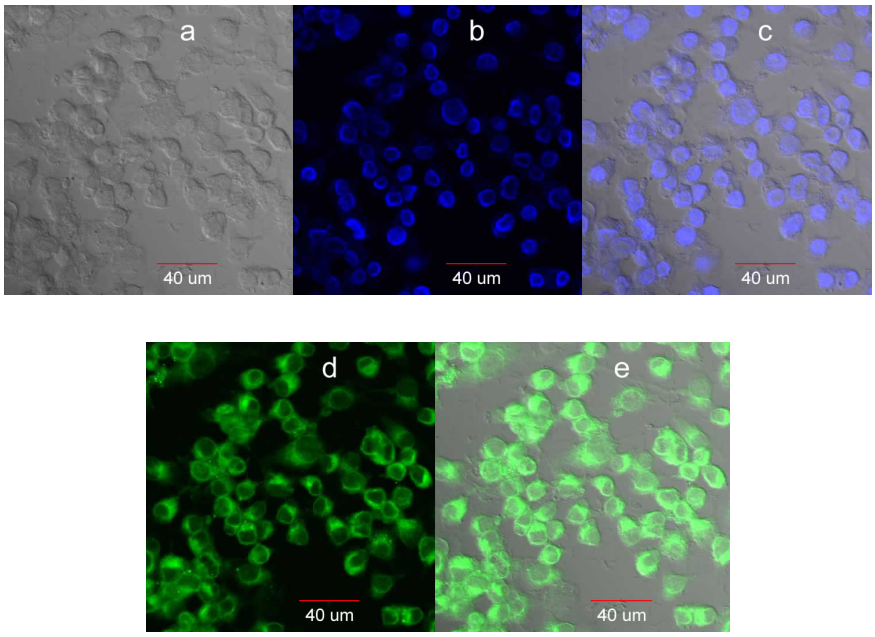
Bright-field (left), overlay of bright-field and fluorescence images (right) of Figure 4b.



Bright-field (left), overlay of bright-field and fluorescence images (right) of Figure 4c.

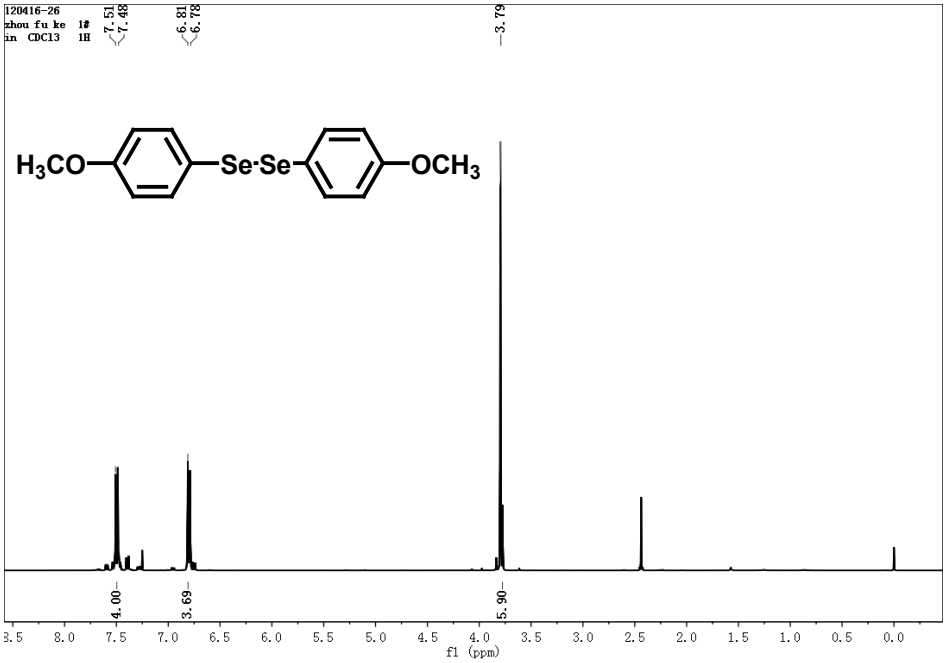


Bright-field (left), overlay of bright-field and fluorescence images (right) of Figure 4d.

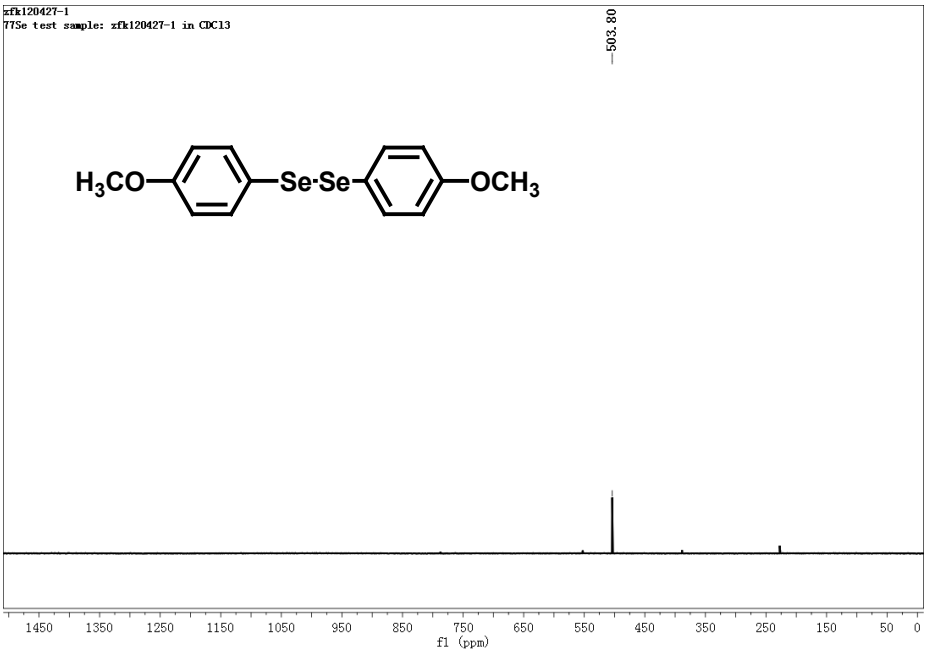


Bright-Field Images and Blue Channel of Figure 4e. a) bright field; b) blue channel (Hoechst dye); c) overlay of bright field and blue channel images; d) green channel (probe); e) overlay of bright field and green channel images.

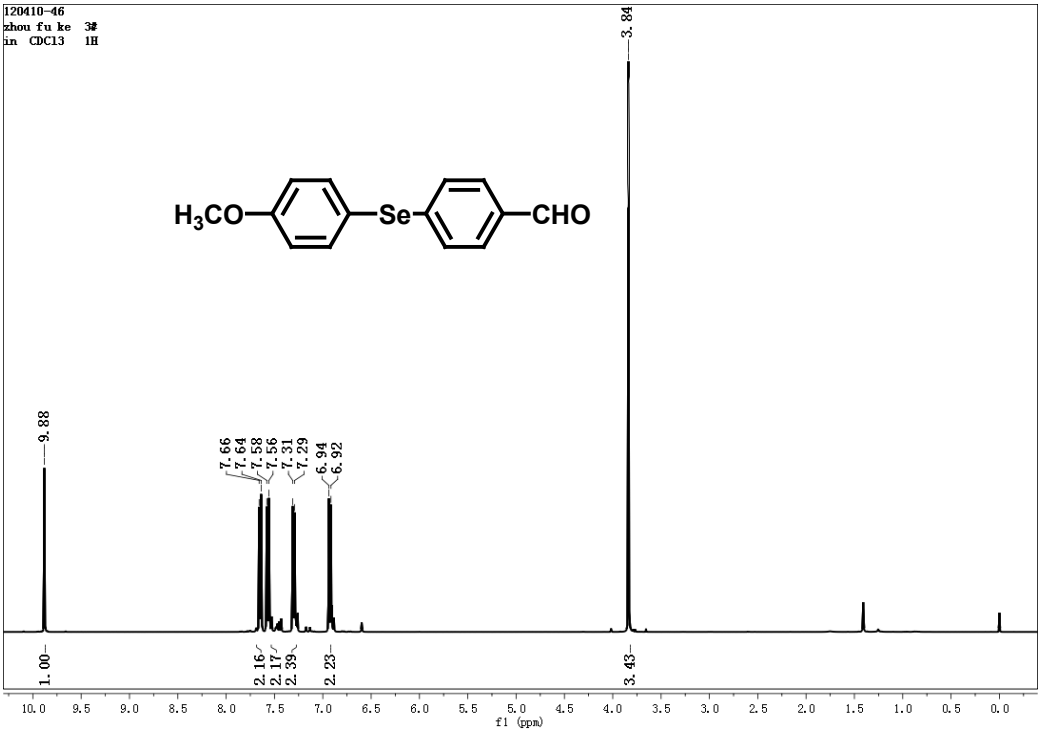
16. ^{1}H-NMR, ^{13}C-NMR, and ^{77}Se-NMR spectra of the compounds



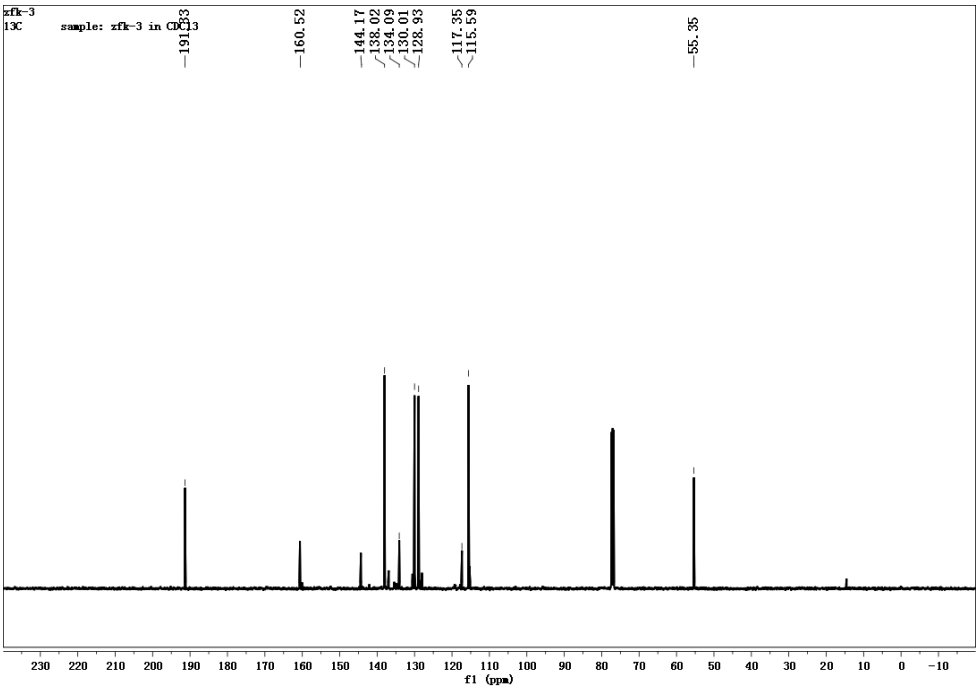
^{1}H-NMR of 1,2-bis(4-methoxyphenyl)diselane



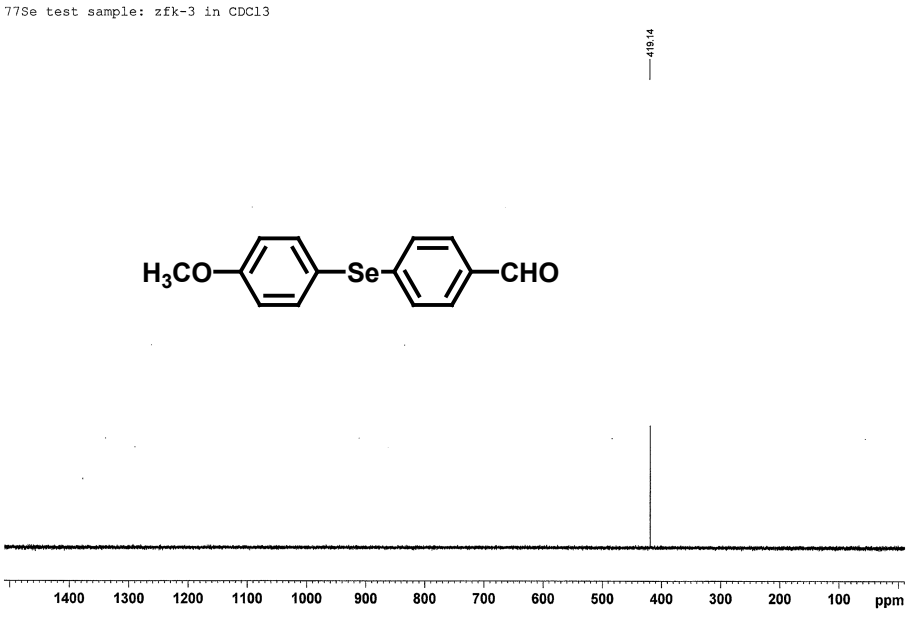
^{77}Se-NMR of 1,2-bis(4-methoxyphenyl)diselane



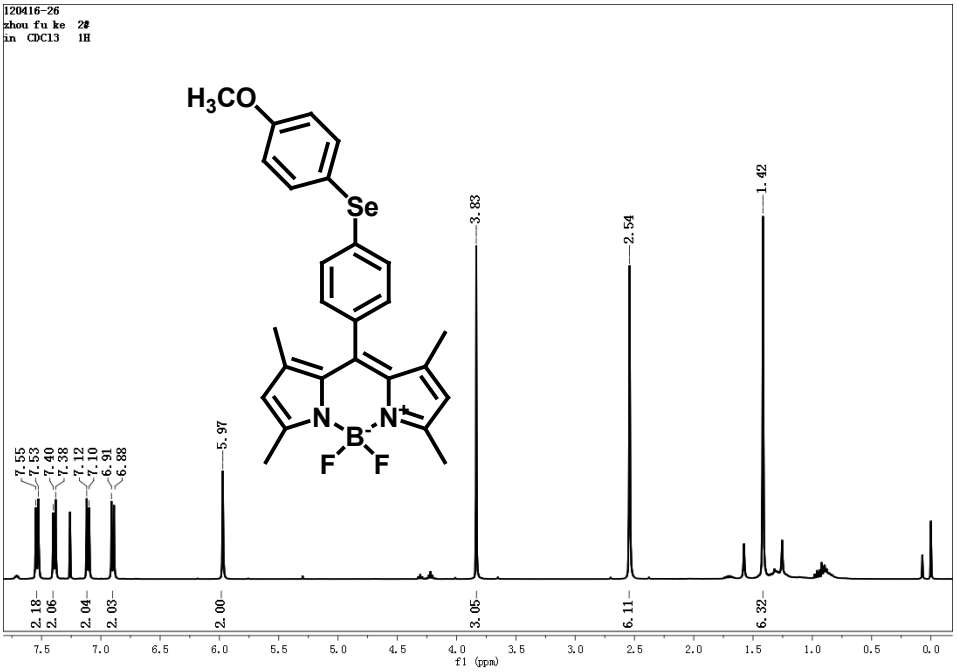
^{1}H-NMR of 4-(4-methoxyphenylselanyl)benzaldehyde



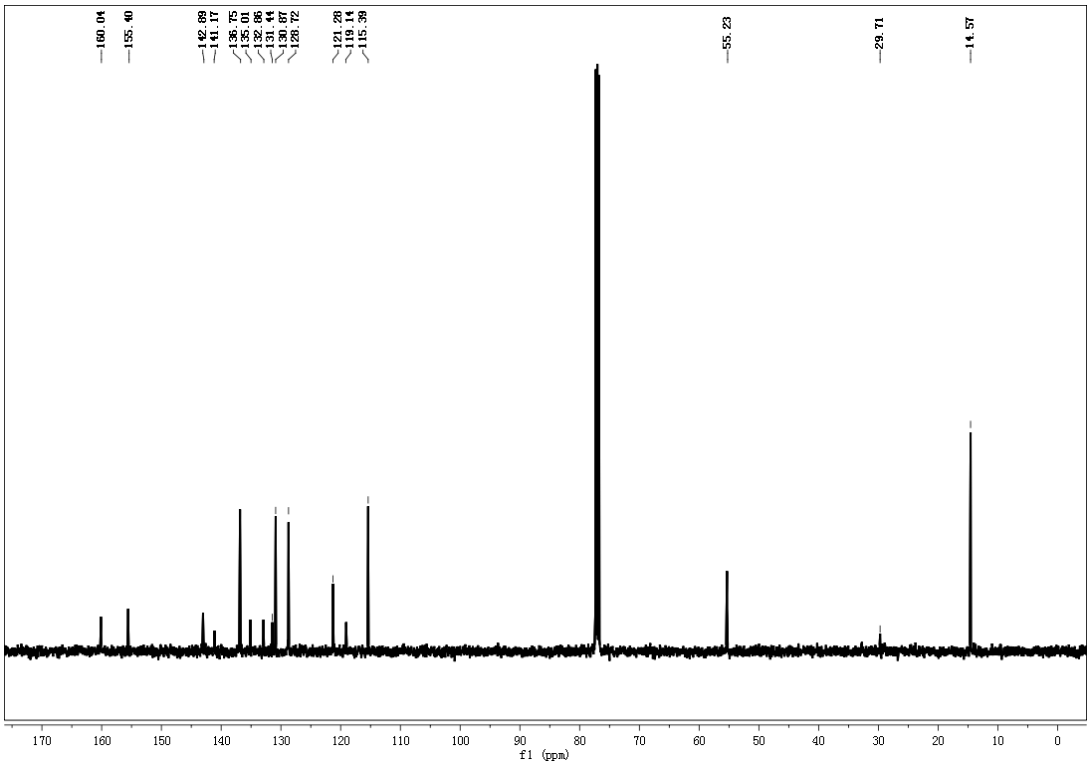
^{13}C-NMR of 4-(4-methoxyphenylselanyl)benzaldehyde



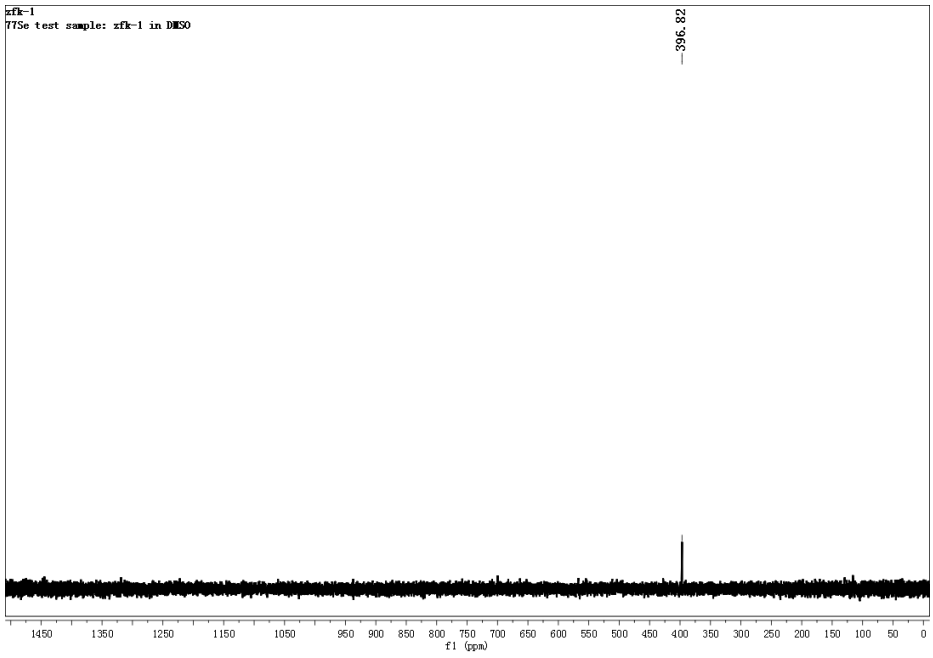
^{77}Se -NMR of 4-(4-methoxyphenylselanyl)benzaldehyde



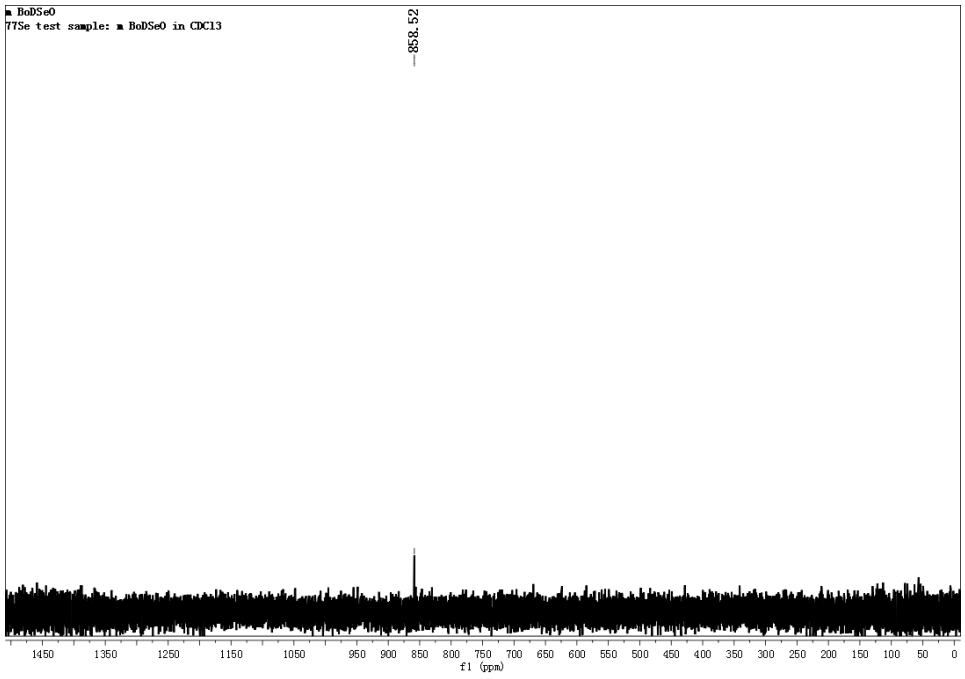
^{1}H-NMR of MPhSe-BOD



^{13}C-NMR of MPhSe-BOD



^{77}Se-NMR of MPhSe-BOD



^{77}Se-NMR of MPhSeO-BOD