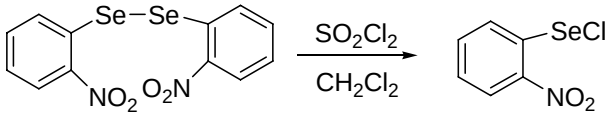
Supplementary Information

1. Synthesis of Rh-Se-2

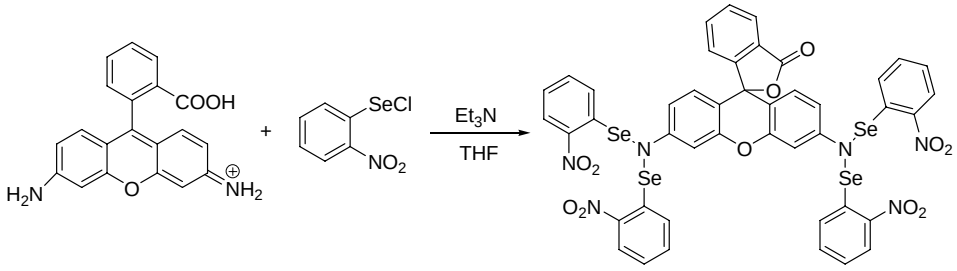
All reactions were carried out under nitrogen with standard vacuum-line techniques. Solvents were dried by distillation under N_2 over sodium (for THF), CaH_2 (for CH_2Cl_2, triethylamine and hexane) and Mg (for methanol). Bis(*o*-nitrophenyl) diselenide was obtained from *o*-nitroaniline by the classical method.[1]

Scheme S1. Synthesis of *o*-Nitrophenyl Selenochlorid



To a solution of Bis(*o*-nitrophenyl) diselenide (0.402 g, 1 mmol) in CH_2Cl_2 (5 mL) at room temperature was added a solution of SO_2Cl_2 (80 μL, 1 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was stirred for 1 h at room temperature. The resulting solution was concentrated to give a saffron yellow powder, which was recrystallized from a methanol/hexane mixture to give red needle crystals.[2] Yield: 0.4106 g (87%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.38 (1H, d, J = 8.29), 8.21 (1H, d, J = 8.27), 7.80 (1H, t), 7.27-7.60 (1H, t).

Scheme S2. Synthesis of Rh-Se-2



To a 3 mL THF solution mixture of 73.2 mg (0.2 mmol) rhodamine 110 and 417 μL

(3 mmol) triethylamine, a 5 mL THF solution of 0.4106 g (1.7 mmol) *o*-nitrophenyl selenochlorid was added dropwise at 0 ºC. After being stirred at room temperature for 4h, the reaction mixture was filtered and evaporated under reduced pressure. The crude product was purified by silica gel chromatography (R_f= 0.2; $CHCl_3$/THF 120:1) as a red solid.[3] Yield: 186 mg (82.3%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.42 (4H, d, J = 8.19), 7.75 (1H, d, J = 7.43), 7.46-7.72 (14H, m), 7.25 (1H, d, J = 12.83), 7.08 (2H, s), 6.92 (2H, d, J = 7.63), 6.59 (2H, d, J = 8.59); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 169.06, 154.08, 152.43, 143.32,139.19, 135.36,134.88, 129.79, 129.04, 127.22, 126.41, 126.19, 125.07, 123.97, 113.97, 112.12, 105.34, 82.89, 77.45, 77.23, 77.03, 76.61; ^{77}Se-NMR (57 MHz, $CDCl_3$): δ 1007.37, 1006.31, 1005.43, 1004.44; IR (KBr, cm^{-1}): 1764.41 (carbonyl, C=O); MS (API-ES): m/z calcd 1133.9, found 1133.0, 1131.1.

2. Fluorescence Spectra of Excitation and Emission

Excitation and emission spectra were recorded at 25 °C in PBS (pH 7.40, 15 mM). As expected, Rh-Se-2 exhibits no fluorescence in the absence of GSH. Upon reaction with GSH for 5 min, a significant increase in fluorescence intensity was observed, indicating the generation of the highly fluorescent Rh110 (Fig. S1).

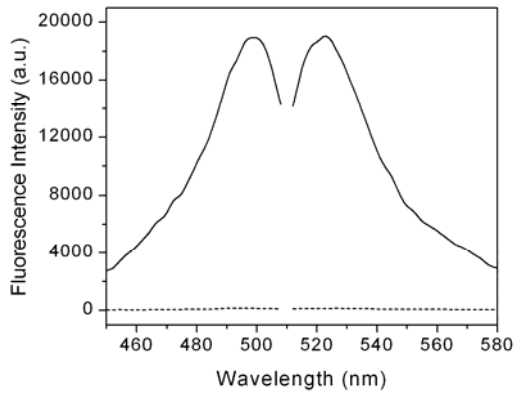


Fig. S1. Excitation and emission spectra of Rh-Se-2 (0.50 μM). Dashed line and solid line were recorded before and after the addition of GSH (1.0 μM), respectively ($\lambda_{ex}/\lambda_{em}$= 499/522 nm).

3. Optimization of Experimental Variables

The effect of some experimental variables was investigated for the reaction of Rh-Se-2 and GSH.

3.1 Effect of pH

In the experiment, pH of the medium has great effect on the fluorescence intensity. As

is shown in Fig. S2, a pH range of 7.20 to 7.80 is appropriate for the detection of GSH. In order to achieve low blank and the high signal-to-noise (the following optimum experimental variables were also chosen according to this principle), PBS (pH 7.40, simulation of physiological condition) was employed throughout the experiment.

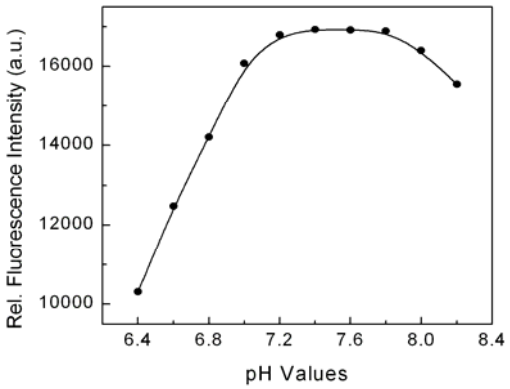


Fig. S2. Effect of pH values (probe concentration: 0.50 μM; GSH concentration: 1.0 μM).

3.2 Effect of Buffer Concentration

We tested the effect of different buffer concentrations. The results in Fig. S3 showed that Rh-Se-2 gave maximum response to GSH at a buffer concentration of 15 mM.

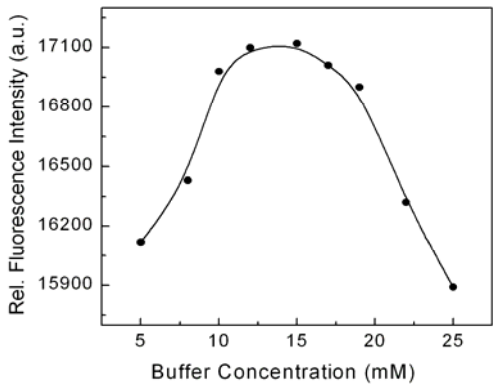


Fig. S3. Effect of buffer concentration (probe concentration: 0.50 μM; GSH concentration: 1.0 μM; PBS: pH 7.40).

3.3 Effect of Probe Concentration

We also tested the effect of probe concentrations. The results in Fig. S4 showed that Rh-Se-2 gave maximum response to GSH at the concentration of 0.50 μM.

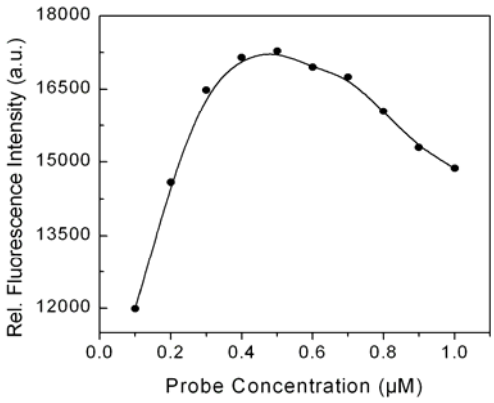


Fig. S4. Effect of Probe Concentration (GSH concentration: 1.0 μM; PBS: pH 7.40, 15 mM).

3.4 Effect of GSH Concentration

We detected the effect of GSH concentration. Rh-Se-2 responded to GSH in the concentration range from 0.015 μM to 2.0 μM and leveled off thereafter.

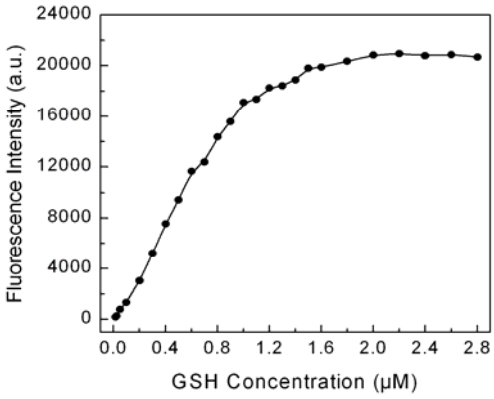


Fig. S5. Effect of GSH Concentration (probe concentration: 0.50 μM; PBS: pH 7.40, 15 mM; GSH concentration: 0.015, 0.025, 0.050, 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.80, 0.90, 1.0, 1.1, 1.2, 1.3, 1.4, 1.6, 1.8, 1.9, 2.0, 2.2, 2.4, 2.6, 2.8 μM).

4. Reactivity of Rh-Se-2 with Thiol-containing Compounds

The reactivity of Rh-Se-2 to several thiol-containing compounds (including protein thiols and non-protein thiols) was investigated. Non-protein thiols detected included 2-mercaptoethanol (β-ME), *N*-acetylcysteine (NAC), Cys, dithiothreitol (DTT) and GSH at the concentration of 1.0 μM and 5.0 mM, respectively, as GSH has an intracellular level of 1~10 mM.[4] As can be seen from Fig. S6, Rh-Se-2 showed the highest response to GSH among the tested non-protein thiols.

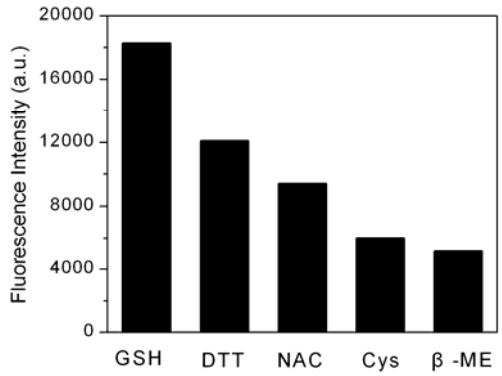


Fig. S6. Fluorescence responses of 0.50 μM Rh-Se-2 toward non-protein thiols (1.0 μM) in PBS (pH 7.40, 15 mM). All data were obtained after incubation at 25 °C for 5 min ($\lambda_{ex}/\lambda_{em}$ = 499 /522 nm).

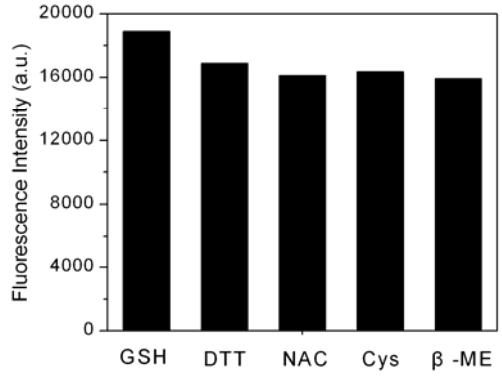


Fig. S7. Fluorescence responses of Rh-Se-2 (0.50 μM) toward non-protein thiols (5.0 mM) in PBS (pH 7.40, 15 mM). All data were obtained after incubation at 25 °C for 5 min ($\lambda_{ex}/\lambda_{em}$ = 499 /522 nm).

Several kinds of protein thiols such as thioredoxin (Trx), glutathione reductase (GR) and metallothionein (MT) were detected in a range of concentrations according to their intracellular parameters: the Km value of E.coli Trx for human thioredeoxin reductase was 20 μM to 35 μM; [5] the activities of GR were approximately 10 μmol/mg protein/min (10 U/mg protein) in HepG2 cells and different tissues in male mice.[6] MT was in micromolar concentration range according to the study of Michael A Lynes *et al.*[7] From the results of our experiments (Fig. S8~S10), Rh-Se-2 was found to show a good response to all tested thiol-containing compounds.

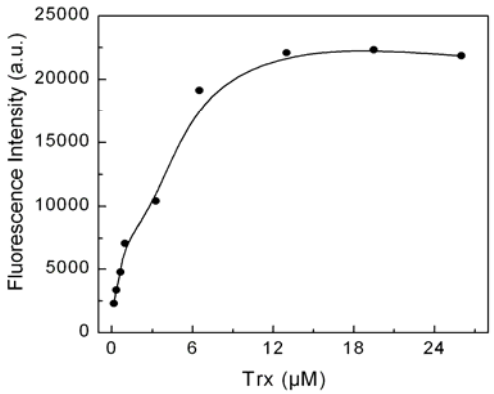


Fig. S8. Fluorescence responses of 0.50 μM Rh-Se-2 toward Trx (0.163, 0.325, 0.650, 0.975, 3.25, 6.50, 13.0, 19.5, 26.0 μM) in PBS (pH 7.40, 20 mM) after incubation at 25 °C for 5 min ($\lambda_{ex}/\lambda_{em}$ = 499/522 nm).

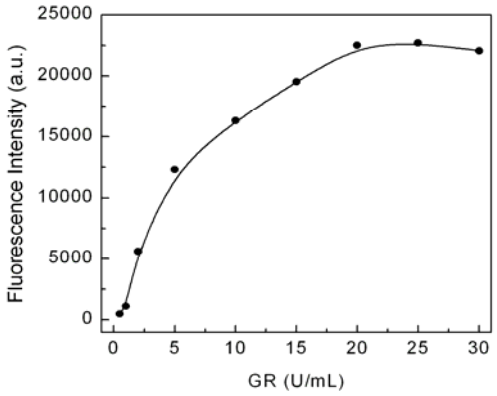


Fig. S9. Fluorescence responses of 0.50 μM Rh-Se-2 toward GR (0.50, 1.0, 2.0, 5.0, 10, 15, 20, 25, 30 U/mL) in PBS (pH 7.40, 20 mM) after incubation at 25 °C for 5 min ($\lambda_{ex}/\lambda_{em}$ = 499/522 nm).

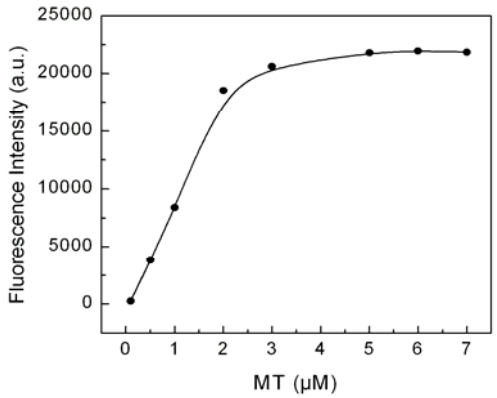


Fig. S10. Fluorescence responses of 0.50 μM Rh-Se-2 toward MT (0.10, 0.50, 1.0, 2.0, 3.0, 5.0, 6.0, 7.0 μM) in PBS (pH 7.40, 20 mM) after incubation at 25 °C for 5 min ($\lambda_{ex}/\lambda_{em}$ = 499/522 nm).

5. Cell Culture and confocal fluorescent image assay

Cell Culture Human normal liver cell line (HL-7702) and the human hepatoma cell line (HepG2) were maintained following protocols provided by the American Type Tissue Culture Collection. Cells were seeded at a density of 1×10^6 cells mL^{-1} in high glucose Dulbecco's Modified Eagle Medium (DMEM, 4.5 g of glucose/L) supplemented with 10% fetal bovine serum (FBS), $NaHCO_3$ (2.0 g/L) and 1 % antibiotics (penicillin/streptomycin, 100 U/mL). Cultures were maintained at 37 °C under a humidified atmosphere containing 5% CO_2.

Confocal fluorescent image assay Florescent images were acquired on a LSM510 confocal laser-scanning microscope (Carl Zeiss Co., Ltd.) 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 PBS (pH 7.40, 0.10 M) for three times.

6. References

(1) (a) Z. Casar, I. Leban, A. M. -L. Maréchal and D. Lorcy, *J. Chem. Soc*., *Perkin Trans. 1*, 2002, 1568; (b) K. B. Sharpless and M. W. Young, *J. Org. Chem.*, 1975, **40**, 947; (c) T. Hori and K. B. Sharpless, *J. Org. Chem.*, 1978, **43**, 1689.

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(3) (a) C. Paulmier, P. Lerouge and F. Outurquin, *Magn. Reson. Chem.*, 1987, **25**, 955; (b) Y. Miura and O. Tsumori, *Bull. Chem. Soc. Jpn.*, 1987, **60**, 4154.

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(5) (a) J. E. Oblong, M. Berggren, P. Y. Gasdaska and G. Powis, *J. Biol. Chem.*, 1994, **269**, 11714; (b) S. Gromer, L. D. Arscott, C. H. Williams, Jr., R. H. Schirmer and K. Becker, *J. Biol. Chem.*, 1998, **273**, 20096; (c) K. C. Das, L.-M. Yvette and C. W. White, *Am. J. Respir. Cell Mol. Biol.*, 1997, **17**, 713.

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7. ^{1}H-NMR, ^{13}C-NMR, ^{77}Se-NMR, MS and IR spectra of adducts

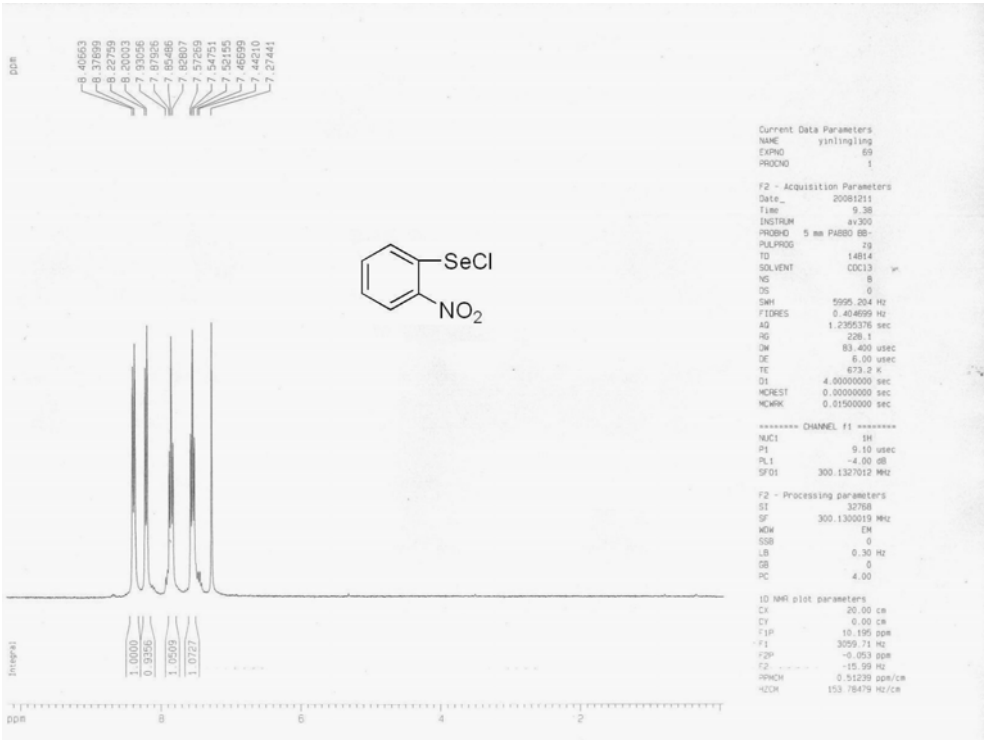

Fig. S11. ^{1}H-NMR spectra of *o*-nitrophenyl selenochlorid

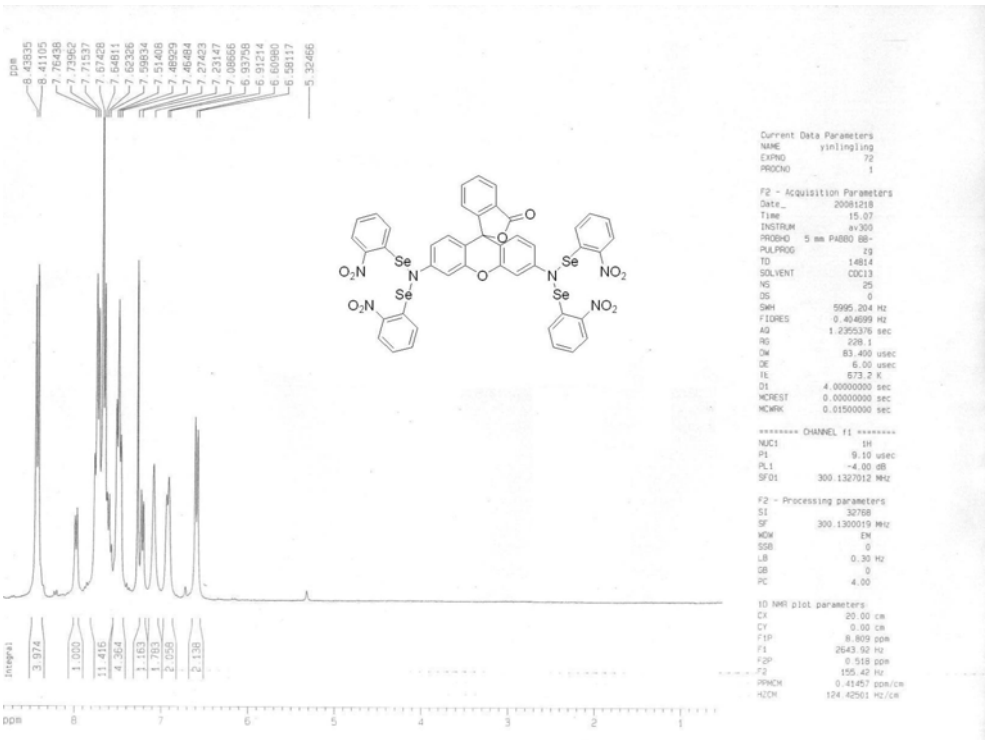

Fig. S12. ^{1}H-NMR spectra of Rh-Se-2

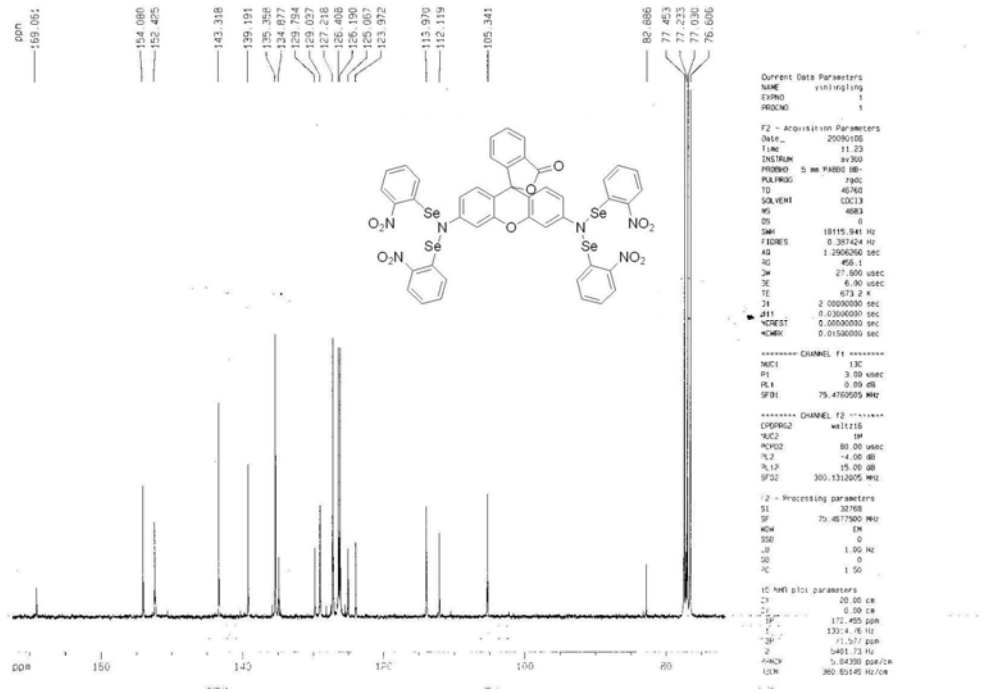

Fig. S13. ^{13}C-NMR spectra of Rh-Se-2

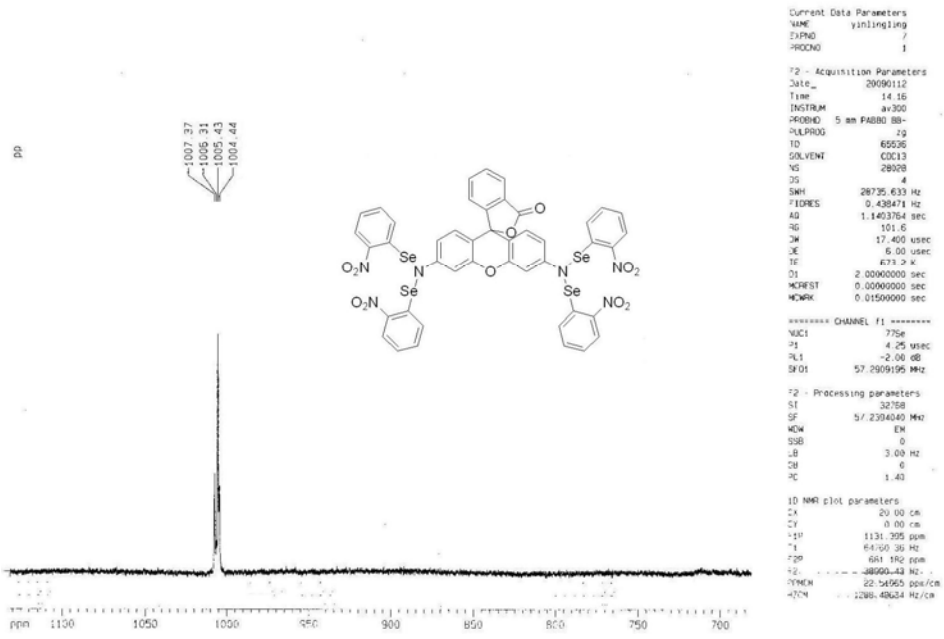

Fig. S14. ^{77}Se-NMR spectra of Rh-Se-2

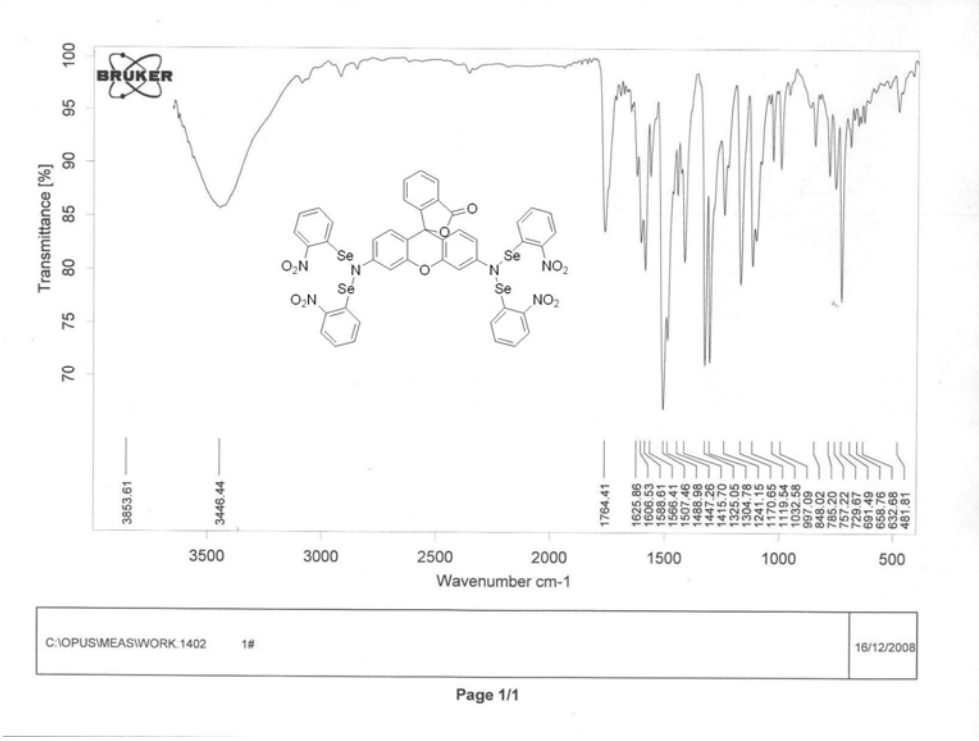

Fig. S15. IR spectra of Rh-Se-2

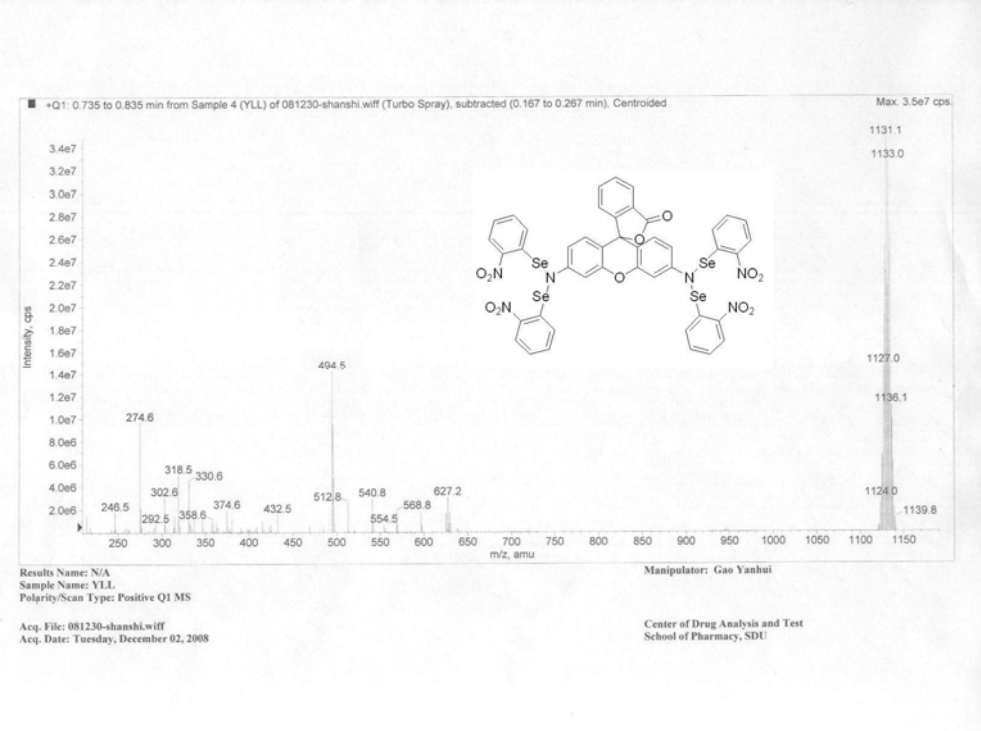

Fig. S16. MS spectra of Rh-Se-2