Support information

A naphthalimide based fast and selective fluorescent probe for hypochlorous acid/ hypochlorite and its application for bioimaging

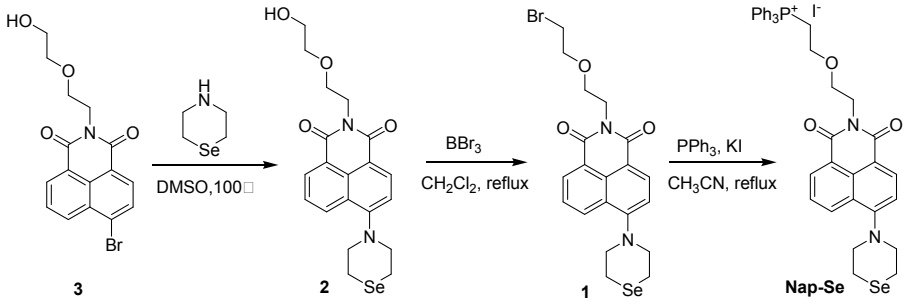
Xin Lv,* Xia Yuan, Yue Wang and Wei Guo*

School of Chemistry and Chemical Engineering, Shanxi University, Taiyuan 030006, China. E-mail: lvxin@sxu.edu.cn; guow@sxu.edu.cn

1. General information and methods.

All reagents and solvents were purchased from commercial suppliers and used without further purification unless otherwise stated. Deionized water was used throughout all experiments. All reactions were magnetically stirred and monitored by thin layer chromatography (TLC). Column chromatography was conducted over silica gel (mesh 200–300). Fluorescence spectra were taken on at room temperature on a Hitachi Fluorescence Spectrophotometer F-7000 with the excitation and emission slit widths at 5.0 and 10.0 nm respectively. Absorption spectra were recorded on Cary 4000 UV-vis Spectrophotometer. The ^{1}H NMR and ^{13}CNMR spectra were recorded at 600 and 150 MHz, respectively. High resolution mass spectra were obtained on a Varian QFT-ESI mass spectrometer. The fluorescence images were acquired through Olympus FluoView™ FV1000 confocal microscope unless otherwise specified.

2. Synthesis and Characterization of Compounds



Synthesis route of probe **Nap-Se**

Compound **3** is a known compound which was synthesized in three steps from the readily available acenaphthene by adapting the published procedures (*Chem. Commun.* 2013, **49**, 11430–11432).

Synthesis of compound 2

A mixture of compound 3 (0.363 g, 1.00 mmol), selenomorpholine (0.75 g, 5.00 mmol), and 10 ml DMSO was heated for 5 h at 100 °C in a sealed tube. After cooling, the mixture was poured into 100 ml water, extracted with ethyl acetate for 3 times. The organic phase was collected and dried with Na_2SO_4. Then, the solution was filtered and evaporated to dryness to afford a brown oil which was purified by silica gel chromatography (EtOAc : PE = 2 : 1) to yield **2** as a yellow solid (0.301 g, 0.69 mmol, yield 71%). ^{1}H-NMR (600 MHz, $CDCl_3$): δ 8.61 (d, 1H, J = 7.2 Hz), 8.54 (d, 1H, J = 7.8 Hz), 8.40 (d, 1H, J = 8.4 Hz), 7.74 (t, 1H, J = 8.4 Hz), 7.27 (d, 1H, J = 7.8 Hz), 4.46 (t, 2H, J=5.4Hz), 3.87 (t, 2H, J=5.4Hz), 3.71 (t, 2H, J=3.6Hz), 3.68 (t, 2H, J=3.6Hz), 3.65 (t, 4H, J=4.8Hz), 3.03 (t, 4H, J=4.2Hz);^{13}C-NMR ($CDCl_3$, 150 MHz): 164.77, 164.28, 157.47, 132.71, 131.45, 130.18, 129.94, 126.71, 126.01, 123.08, 116.95, 116.25, 72.21, 68.53, 61.87, 56.30, 39.39,18.56; HRMS: calculated for $[M+Na]^+$: 457.0637; found: 457.0629.

Synthesis of compound 1

compound 2 (0.353 g, 0.81 mmol) was dissolved in 10 ml ethyl acetate, and then BBr_3 (1.6 ml) was added dropwise to the solution. The mixture was refluxed under N_2 for 1.5 h. After cooling, the mixture was poured into 50 ml water, extracted with CH_2Cl_2 for 3 times. The organic phase was collected and washed with $NaHCO_3$ solution and brine for 3 times. The organic phase was collected and dried with Na_2SO_4. Then, the solution was filtered and evaporated to dryness to afford a brown oil which was purified by silica gel chromatography (DCM: PE = 2 : 1) to yield **1** as a yellow solid (0.190 g, 0.38 mmol, yield 48%). ^{1}H-NMR (600 MHz, $CDCl_3$): δ 8.61 (d, 1H, J=7.2 Hz), 8.54 (d, 1H, J=7.8 Hz), 8.40 (d, 1H, J=8.4 Hz), 7.74 (t, 1H, J=7.8 Hz), 7.27 (d, 1H, J=7.8 Hz), 4.46 (t, 2H, J=6 Hz), 3.87 (q, 4H, J=6 Hz), 3.66 (t, 4H, J=5.4 Hz), 3.45 (t, 2H, J=6 Hz), 3.02 (s, 4H); ^{13}C-NMR ($CDCl_3$, 150 MHz):164.51, 164.01, 157.36, 132.55, 131.33, 130.08, 129.93, 126.73, 125.99, 123.16, 117.07, 116.23, 70.54, 67.94,

56.31, 38.93, 30.44, 18.58; HRMS: calculated for $[M+Na]^+$: 518.9793; found: 518.9785.

Synthesis of probe Nap-Se

The compound **1** (0.250 g，0.50 mmol) was added to KI (0.15 g, 1.00 mmol) in 20 ml dry acetone and the mixture was refluxed for 12 h. The solvent was evaporated and the residue was directly dissolved in 20 ml CH_3CN, and added PPh_3 (0.182g, 6.5 mmol). The resulting solution was stirred for 14 h under reflux. The solvent was evaporated and the residue was directly purified by silica gel column chromatography (DCM : MeOH = 10:1) to give compound **Nap-Se** as a yellow solid (0.230 g, 0.29 mmol, yield 58 %). ^{1}H-NMR (600 MHz, CD_3OD): δ 8.50 (m, 2H), 8.41(q, 1H, J=4.2 Hz)，7.81(q, 1H, J=8.4 Hz)， 7.73（m, 9H), 7.59 (m,6H), 7.38 (q, 1H, J=2.4 Hz), 4.17 (t, 2H, J=4.8 Hz), 3.88 (t, 2H, J=6 Hz), 3.85 (t, 2H, J=6 Hz), 3.76 (t, 2H, J=6 Hz), 3.73(d, 2H, J=6 Hz), 3.68 (t, 2H, J=5.4 Hz), 3.53(t, 4H, J=6 Hz); ^{13}C-NMR (CD_3OD, 150 MHz): 168.21, 167.76, 161.83, 138.42, 137.48, 137.42, 136.29, 135.76, 135.66, 134.82, 134.41, 133.73, 133.64, 132.64, 132.57, 132.52, 132.49, 130.48, 129.74, 129.71, 126.53, 123.20, 122.62, 119.98, 71.87, 67.80, 67.26, 67.21, 60.24, 42.33, 21.56; HRMS: calculated for $[M]^+$: 679.1623; found: 679.1620.

3. Preparation of the test solution

O_2^- was prepared by adding KO_2 and 18-Crown-6 (1 equiv) to dry dimethyl sulfoxide and stirring vigorously for 10 min. Hydroxyl radical (·OH) was generated through the Fenton reaction of $Fe(ClO_4)_2$ and H_2O_2 and its concentration was equal to the Fe(II) concentration. $^1O_2$ was generated in situ by adding NaClO solution into H_2O_2 solution (10 eq), and its concentration was equal to the NaClO concentration. NO was generated from a commercially available NO donor NOC-9 (dissolved in 0.1M NaOH solution). H_2O_2 solution was prepared by dilution of commercial H_2O_2 solution in deionized water, and its concentration was determined by using an extinction coefficient of 43.6 $M^{-1}cm^{-1}$ at 240 nm. HClO solution was prepared by the dilution of commercial NaClO solution in deionized water, and its concentration was determined

using an extinction coefficient of 350 $M^{-1}cm^{-1}$ at 293 nm. $ONOO^-$was generated from a commercially available $ONOO^-$donor SIN-1 (dissolved in 0.01 M NaOH solution). The aqueous solutions of Na^+, K^+, Ca^{2+}, Mg^{2+} were prepared from their chloride salts and the aqueous solutions of F^-, Cl^-, Br^-, I^-, NO_3^-, CO_3^{2-}, SO_3^{2-} were prepared from their sodium salts. The aqueous solutions of Cys, Hcy, GSH and H_2S were freshly prepared. Stock solution of **Nap-Se** in CH_3CN (5 mM) was used to prepare the working solutions in PBS (20 mM, pH 7.4, containing 1‰ CH_3CN) with a final concentration of 5 μM.

4. Quantum yield determination

Fluorescence quantum yields of **Nap-Se** before and after the treatment with NaClO were determined in PBS (20 mM, pH 7.4, containing 1‰ CH_3CN) with quinine sulphate as a reference (Φ_f= 0.55 in 0.5 M H_2SO_4). The quantum yield was calculated using Eq. (1):

$$\Phi u = [(A_s F A_u \eta^2)/ (A_u F A_s \eta_0^2)]\Phi s. \qquad \text{Eq.1}$$

Where A_s and A_u are the absorbance of the reference and sample solution at the reference excitation wavelength, FA_s and FA_u are the corresponding integrated fluorescence intensity, and η and η_0 are the solvent refractive indexes of sample and reference, respectively. Absorbance of sample and reference at their respective excitation wavelengths was controlled to be lower than 0.05.

5. **Cell culture**

The HeLa cells and Raw 264.7 macrophage cells were kindly provided by Key Laboratory of Chemical Biology and Molecular Engineering of Ministry of Education (China). Cells were grown in Dulbecco's modification of Eagle's medium (DMEM) supplemented with 10% FBS (Fetal Bovine Serum) and 1% antibiotics at 37℃ in humidified environment of 5% CO_2. Grow Cells in the exponential phase of growth on glass bottom cell culture dishes (Φ 15 mm) for 1 day.

6. Imaging exogenously added HClO in raw 264.7 macrophage cells

Before the experiments, raw 264.7 macrophage cells were washed with PBS three times. Then, the cells were pretreated with 2.5 μM **Nap-Se** for 10 min in DMEM

medium at 37℃. After washed three times with PBS, the probe loaded cells were incubated with 5.0 μM NaClO solution in PBS for 10 min at 37℃. After that, the cells were washed with PBS three times, and then imaged.

7. Imaging endogenous HClO in raw 264.7 macrophage cells

For image of endogenous HClO, the cells were pretreated with LPS (1.0 μg/mL) for 12 h and then treated with PMA (1.0 μg/mL) for 1 h, followed by incubation with probe **Nap-Se** (2.5 μM) for 10 min in DMEM medium at 37℃. For inhibition assays, the cells were activated with LPS (1.0 μg/mL) for 12 h, and then incubated with PMA (1.0 μg/mL) in the presence of **ABAH** (200 μM) or GSH (2 mM) for 1 h, and then loaded with probe **Nap-Se** (2.5 μM) for 10 min. After each treatment, the cells were washed with PBS three times. Emission was collected at 450-560 nm (λex = 405 nm).

8. Cell costaining studies

To confirm that probe **Nap-Se** could specifically stain the mitochondria, HeLa cells were incubated in a sequence with **Nap-Se** (2.5 μM), MitoTracker Red FM (1.0 μM) or LysoTracker Red (1.0 μM) in DMEM media for 15 min, then washed three times with PBS, and then imaged. Emission was collected at 450-560 nm (λex = 405 nm) for probe **Nap-Se.** Emission was collected at 570-620 nm (λex = 561 nm) for MitoTracker Red FM or LysoTracker Red.

9. supplementary spectra.

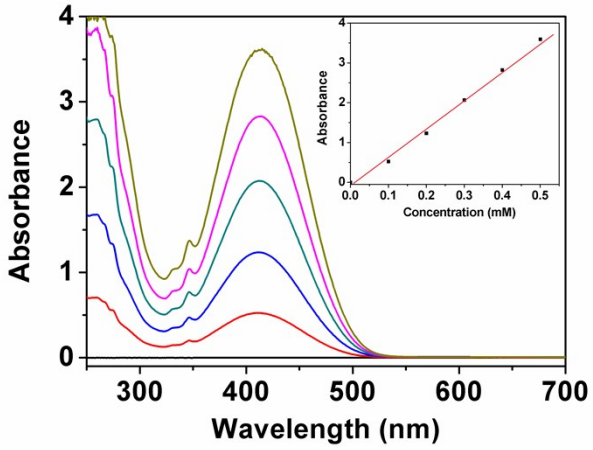


Fig. S1 Solubility evaluation of **Nap-Se** in pure water by absorption spectra. Inset: Plots of absorption intensity at 410 nm vs probe concentrations (0-0.5mM).

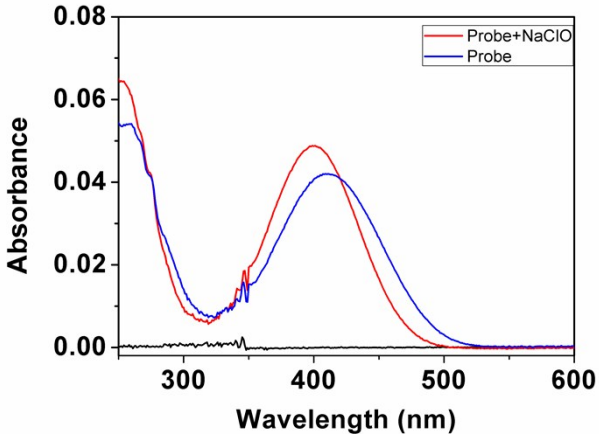


Fig.S2 Absorption spectra changes of **Nap-Se** (5 μM) upon addition of 1 equiv NaClO in PBS buffer (20 mM, pH 7.4).

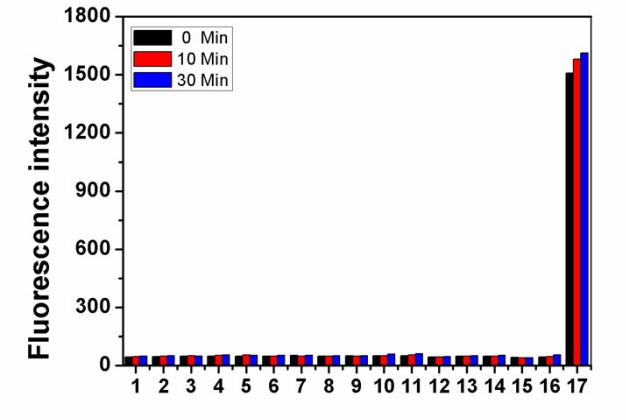


Fig. S3 Fluorescence intensities of (5 μM) upon addition of various species after 0, 10, and 30 min, respectively. (1) **Nap-Se** only. (2-11) K^+, Na^+,Ca^{2+}, Mg^{2+}, F^-, Cl^-, Br^-, I^-, CO_3^{2-}, NO_3^-(1 mM for each). (12) GSH (2 mM), (13-16) Cys, Hcy, H_2S, SO_3^{2-} (200 μM for each). (17) NaClO (5 μM). Condition: PBS buffer (20 mM, pH 7.4); 25 °C; λex = 405 nm, λem = 540 nm. Slits: 5/10 nm, voltage: 600 V.

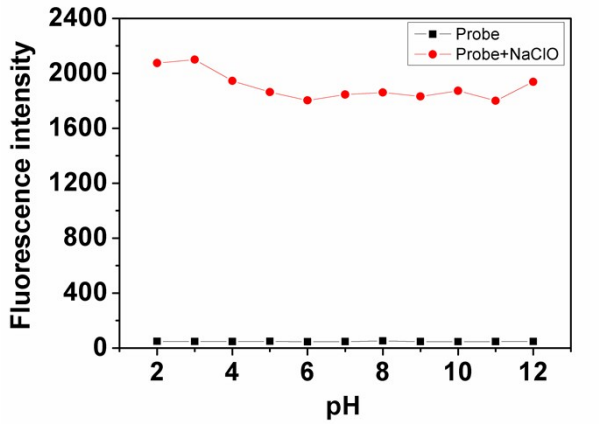


Fig. S4 pH-dependent experiments of **Nap-Se** (5 μM) with NaClO (10 μM) at different pH values.

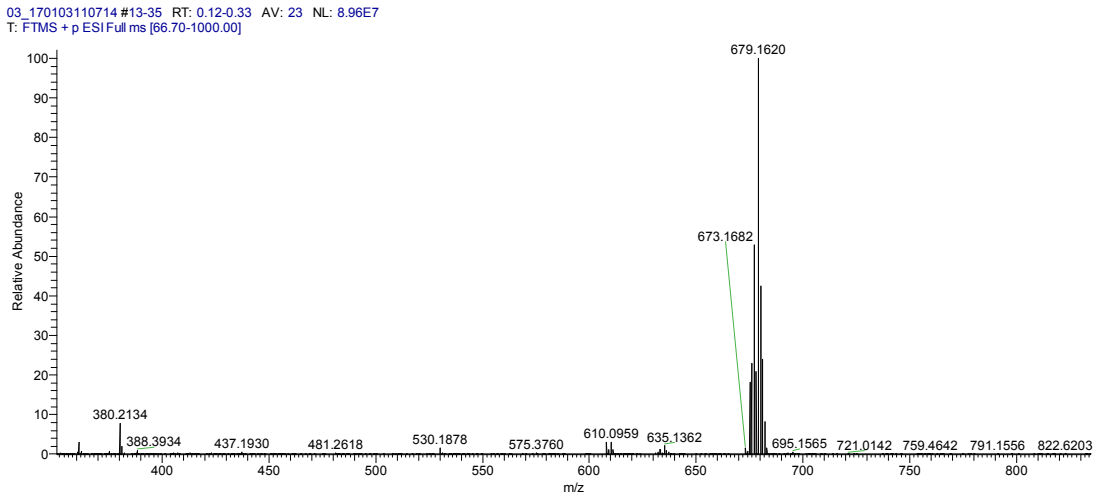


Fig. S5 HRMS spectra of probe **Nap-Se**

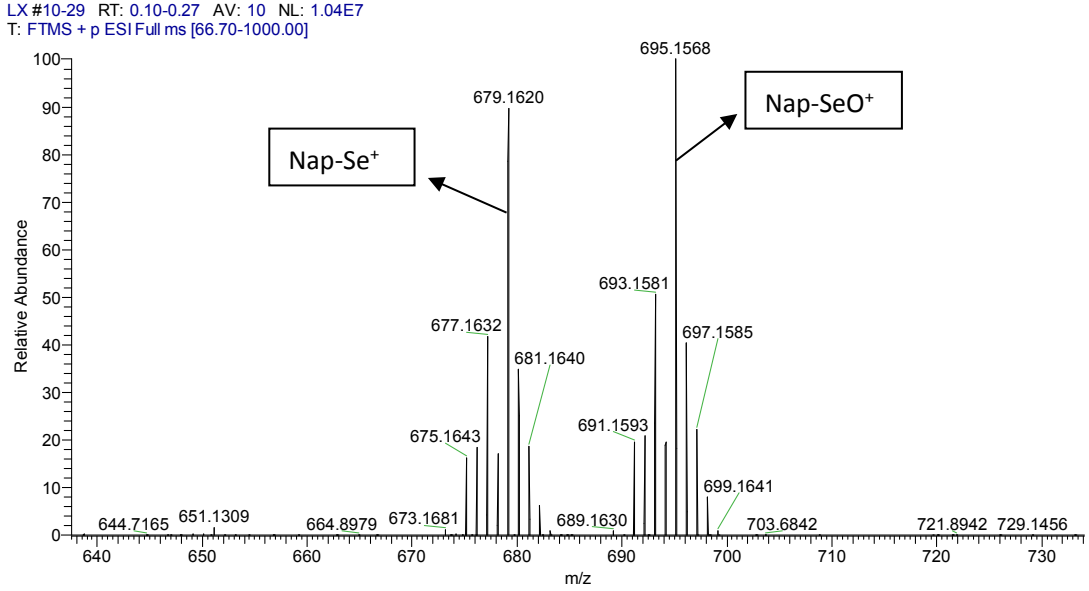


Fig. S6 HRMS spectra of probe **Nap-Se+NaClO**

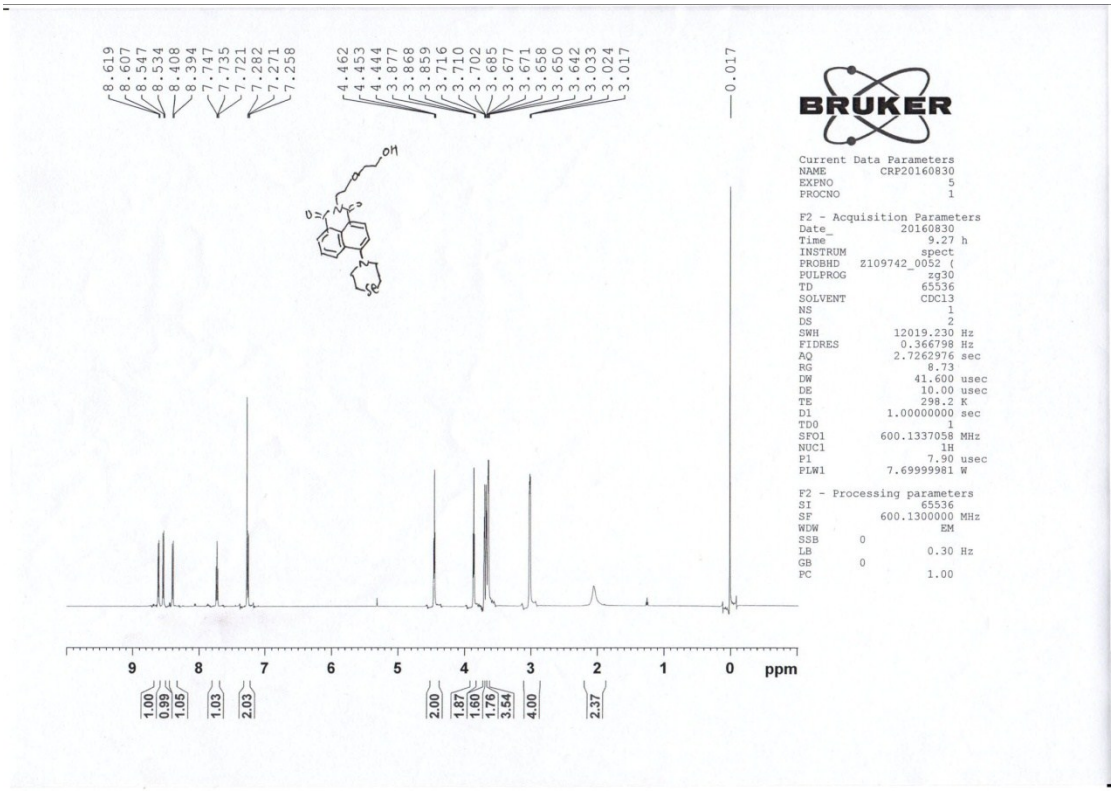


Fig. S7 ^{1}H-NMR spectra of compound 2

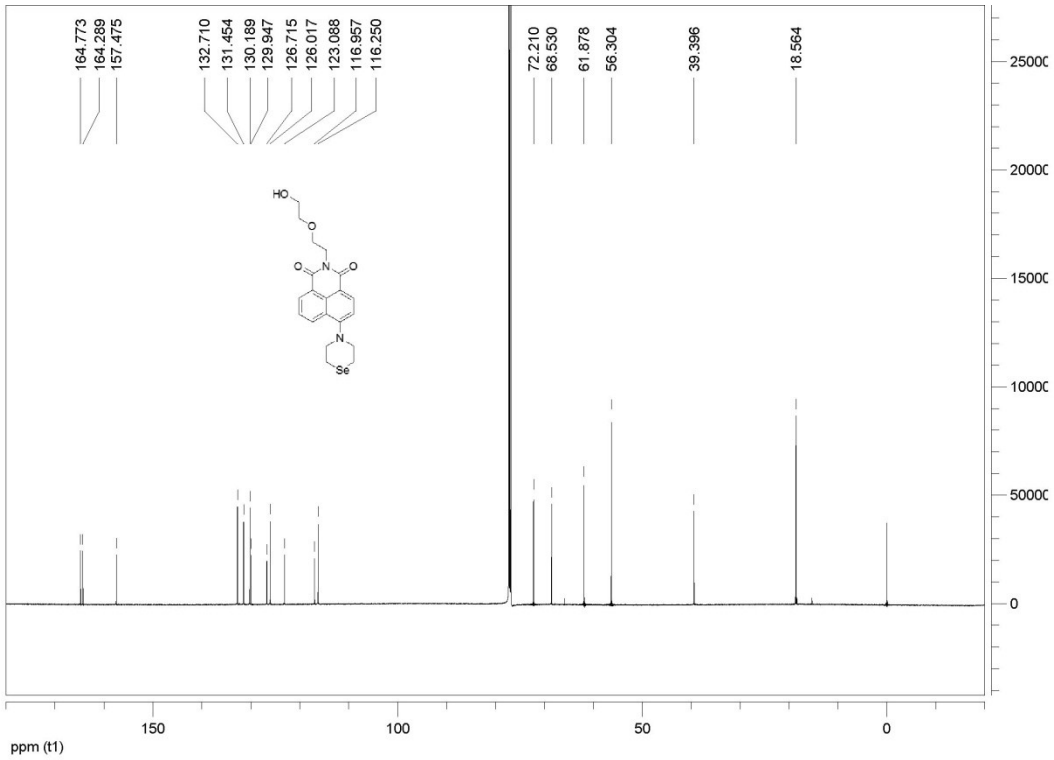


Fig. S8 ^{13}C-NMR spectra of compound 2

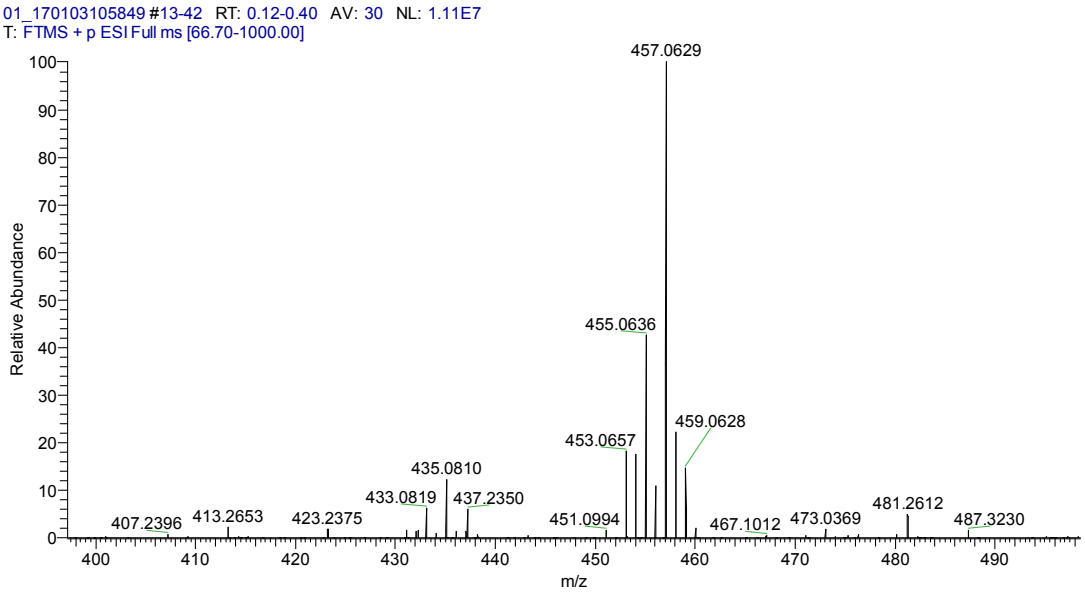


Fig. S9 HRMS spectra of compound 2

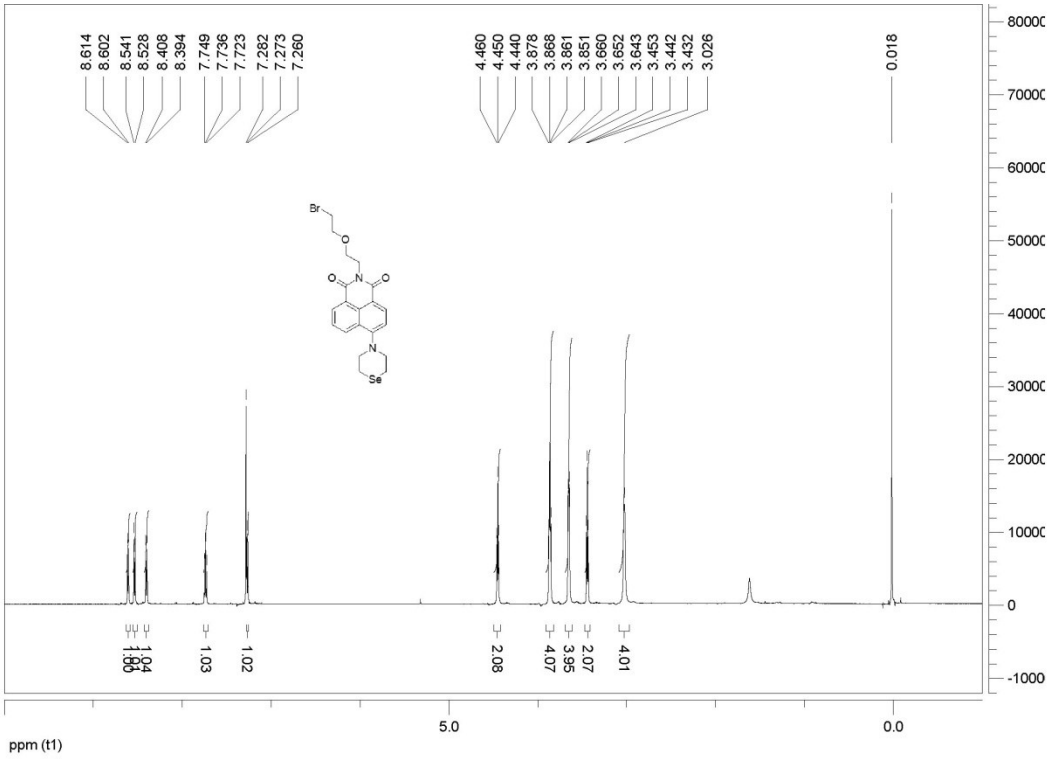


Fig. S10 ^{1}H-NMR spectra of compound 1

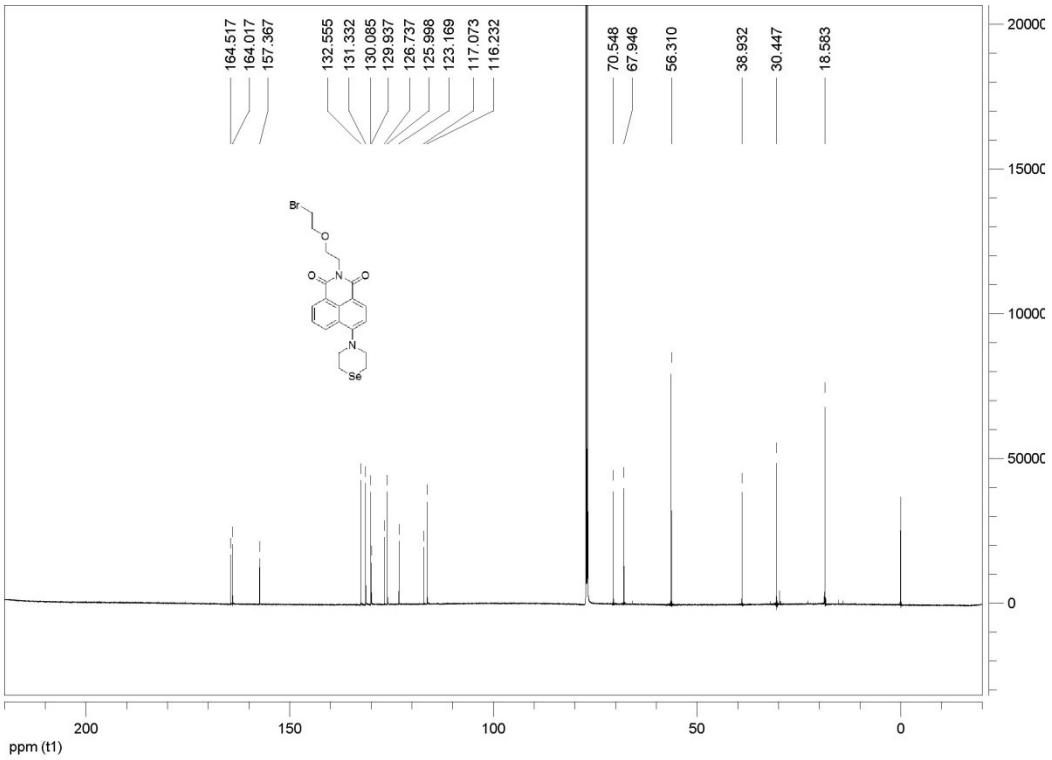


Fig. S11 ^{13}C-NMR spectra of compound 1

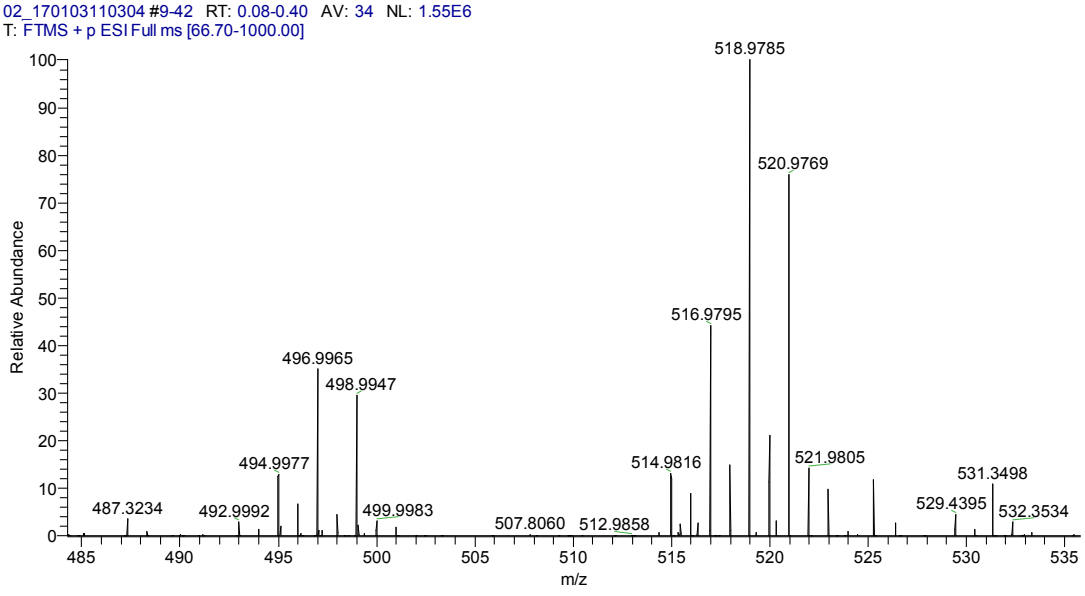


Fig. S12 HRMS spectra of compound 1

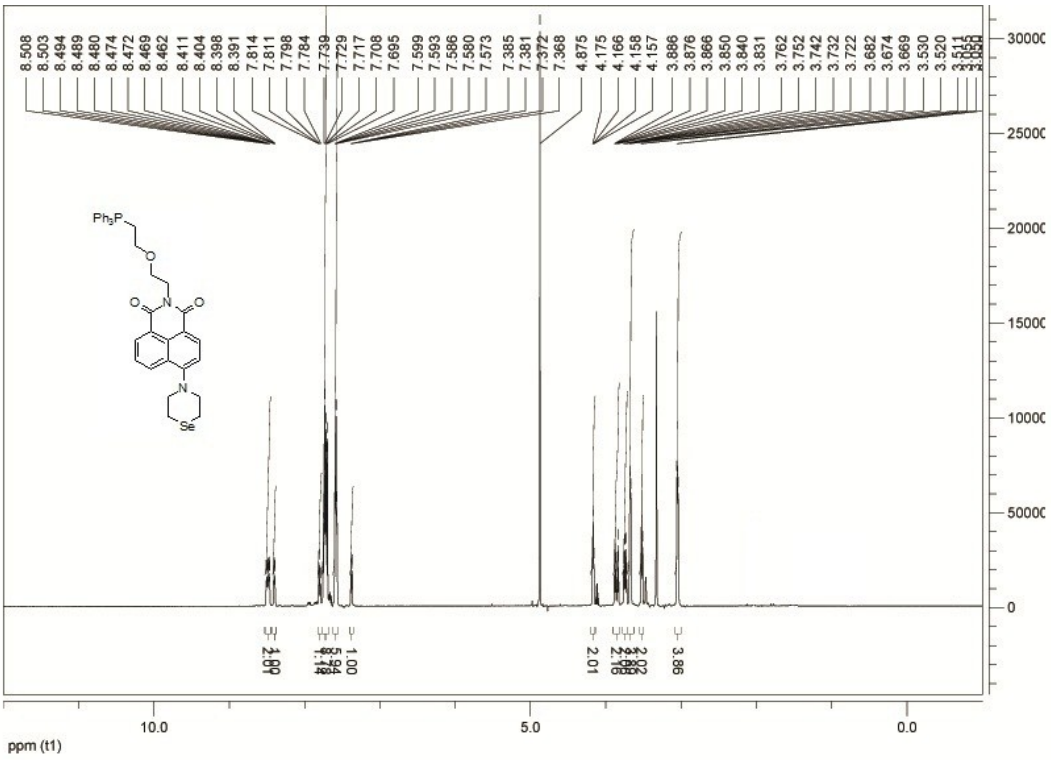


Fig. S13 ^{1}H-NMR spectra of compound Nap-Se

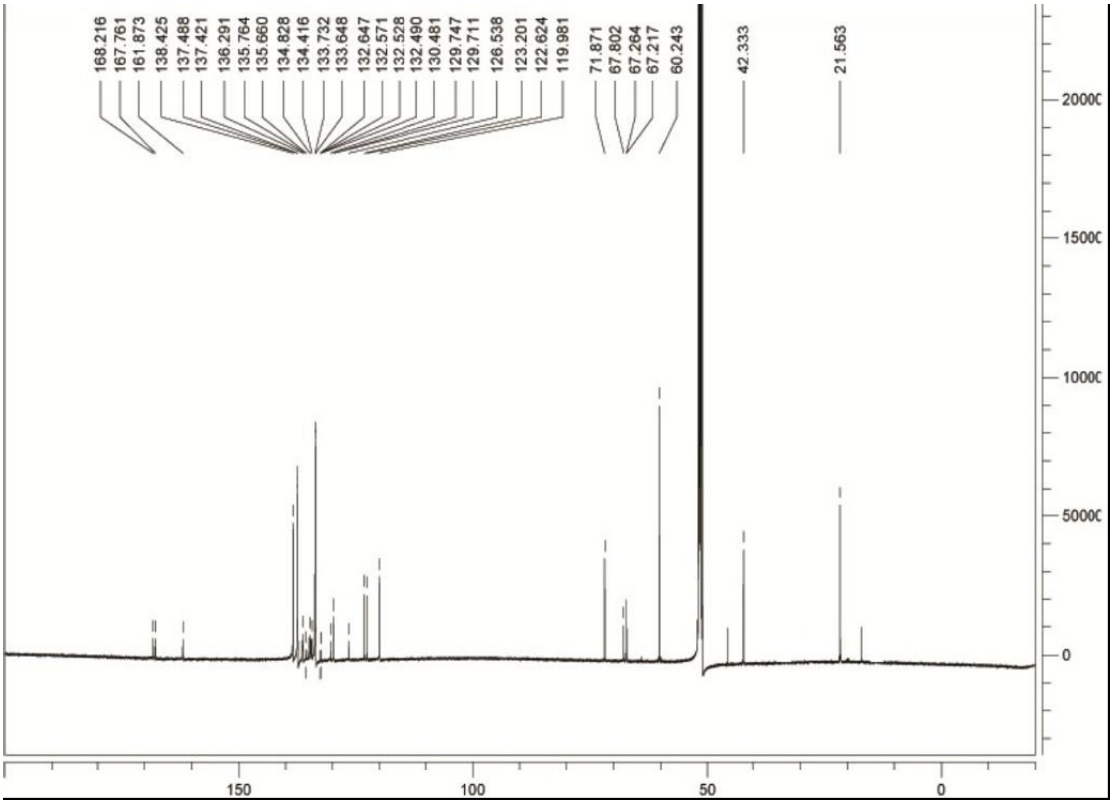


Fig. S14 ^{13}C-NMR spectra of compound Nap-Se