A Near-infrared Fluorescent Probe for Selective Detection of HClO Based on Se-sensitized Aggregation of Heptamethine Cyanine Dye

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1. Materials and instruments

Unless otherwise specified, all materials were obtained from commercial suppliers and used without further purification. Lipopolysaccharides（LPS） and phorbol 12-myristate 13-acetate (PMA) were purchased from Sigma-Aldrich (USA). ^{1}H NMR and ^{13}C NMR spectra were recorded on a VARIAN INOVA-400 spectrometer, using TMS as an internal standard. ^{77}Se NMR spectra were recorded on a VARIAN INOVA-500 spectrometer, using CH_3SeCH_3 as an internal standard. Mass spectrometry data were obtained with a HP1100LC/MSD mass spectrometer and a LC/Q-TOF MS spectrometer. UV-visible spectra were collected on a Perkin Elmer Lambda 35 UV-Vis spectrophotometer. Fluorescence measurements were performed on a VAEIAN CARY Eclipse Fluorescence Spectrophotometer (Serial No. FL1109-M018).Institute of cancer research (ICR) mice and Nude mice, 4-6 weeks old, were obtained from Laboratory Animal Center of Dalian Medical University. All the animal experiments were carried out in accordance with the guidelines issued by the Ethical Committee of Dalian Medical University.

2. UV-vis and fluorescence emission spectra of different concentrations of Cy7Cl, SCy7 and SeCy7

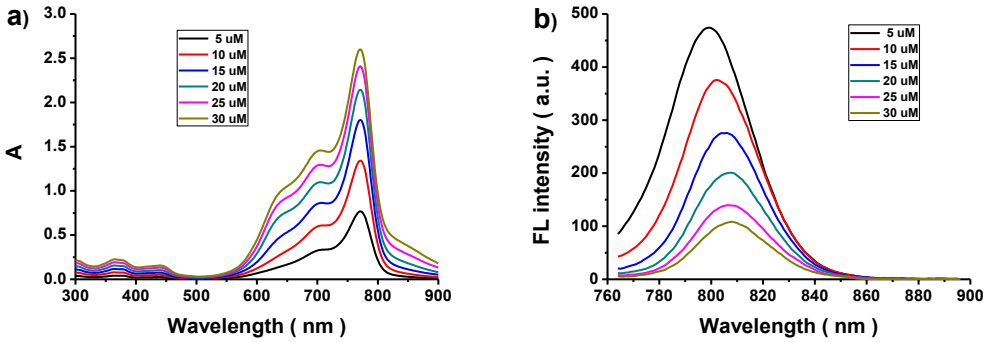


Fig. S1. UV-vis (a) and fluorescence emission (b) spectra of different concentrations of **Cy7Cl** (5-30 μM); λ_{ex} = 760 nm.

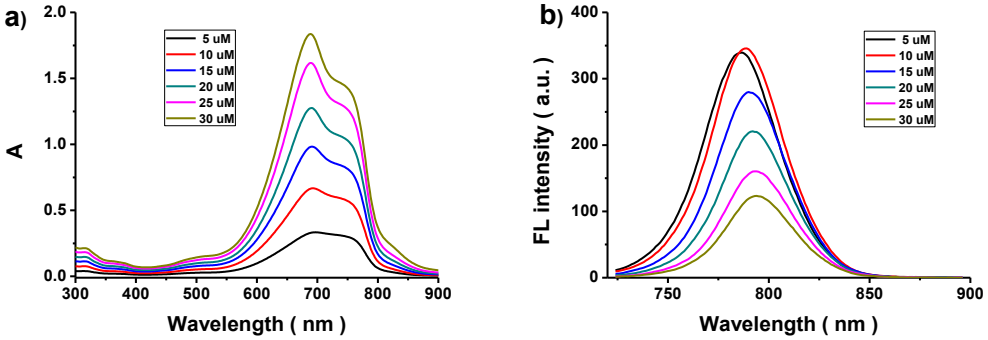


Fig. S2. UV-vis (a) and fluorescence emission (b) spectra of different concentrations of S**Cy7** (5-30 μM); λ_{ex} = 690 nm.

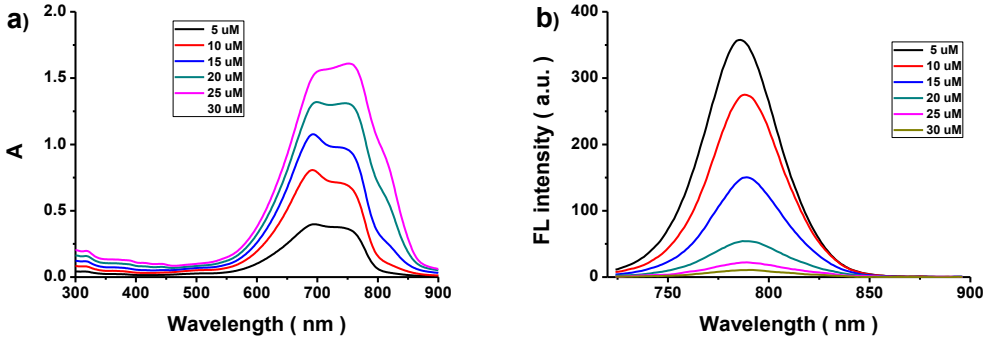


Fig. S3. UV-vis (a) and fluorescence emission (b) spectra of different concentrations of **SeCy7** (5-30 μM); λ_{ex} = 690 nm.

3. UV-vis spectra of SeCy7 with HClO

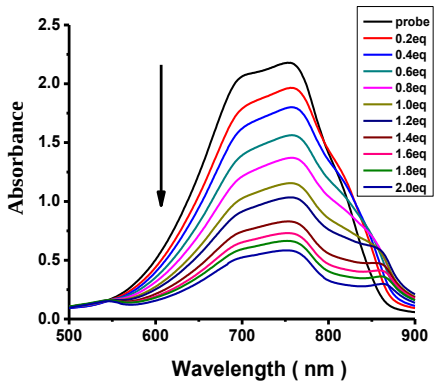


Fig. S4. UV-vis spectra of **SeCy7** (30 μM) upon the addition of increasing concentrations of sodium hypochlorite (0–2.0 equiv.) in PBS buffer (pH 7.4, 20 mM). The arrows indicate the changes in the absorption spectra with the increased sodium hypochlorite concentrations; λ_{ex} = 690 nm.

4. Time-dependent fluorescence spectra of SeCy7 with HClO

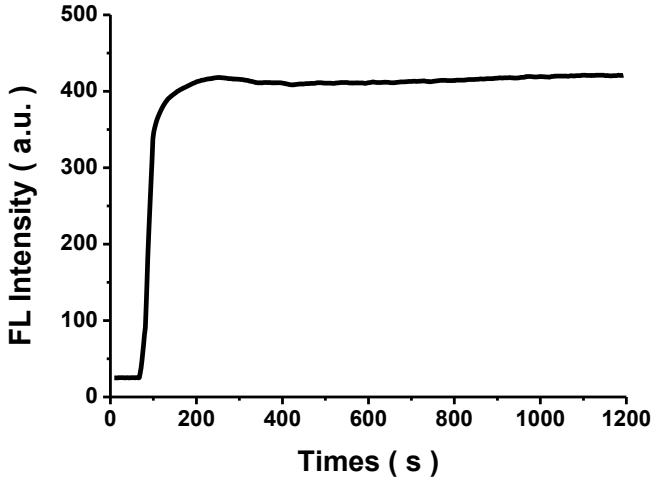


Fig. S5. Time dependent fluorescence intensity changes of probe **SeCy7** (30 μM) with 2 equiv. NaClO in PBS buffer (pH 7.4, 20 mM).(λ_{ex}= 690 nm,λ_{em}= 786 nm).

5. The linear relationship between fluorescence intensity and the concentrations of HClO

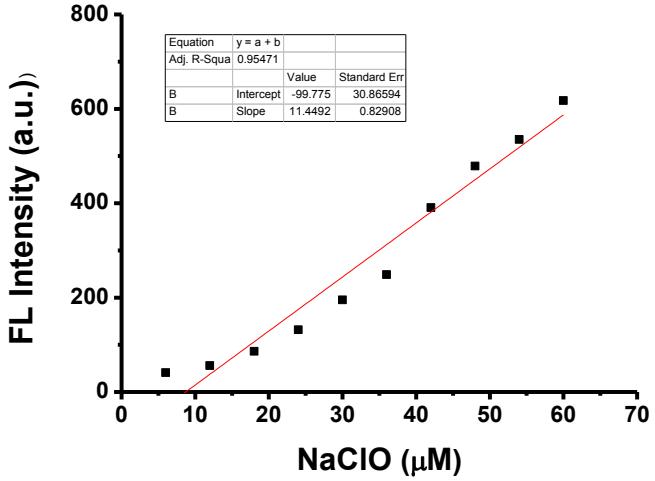


Fig. S6. The linear relationship between the concentrations of sodium hypochlorite and fluorescence intensity

6. ^{77}Se NMR spectra of the SeCy7 and SeOCy7

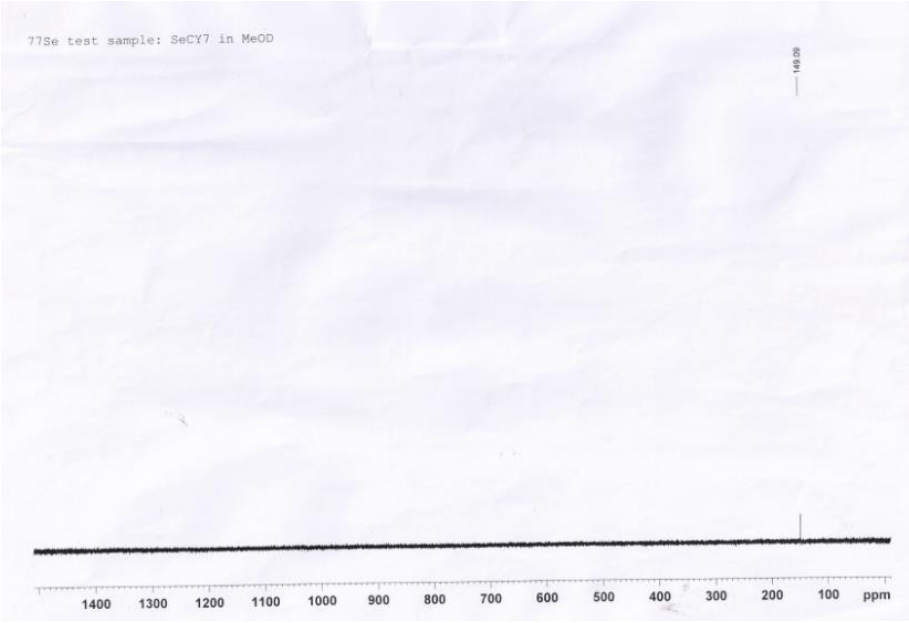


Fig. S7. ^{77}Se NMR of **SeCy7** in CD_3OD

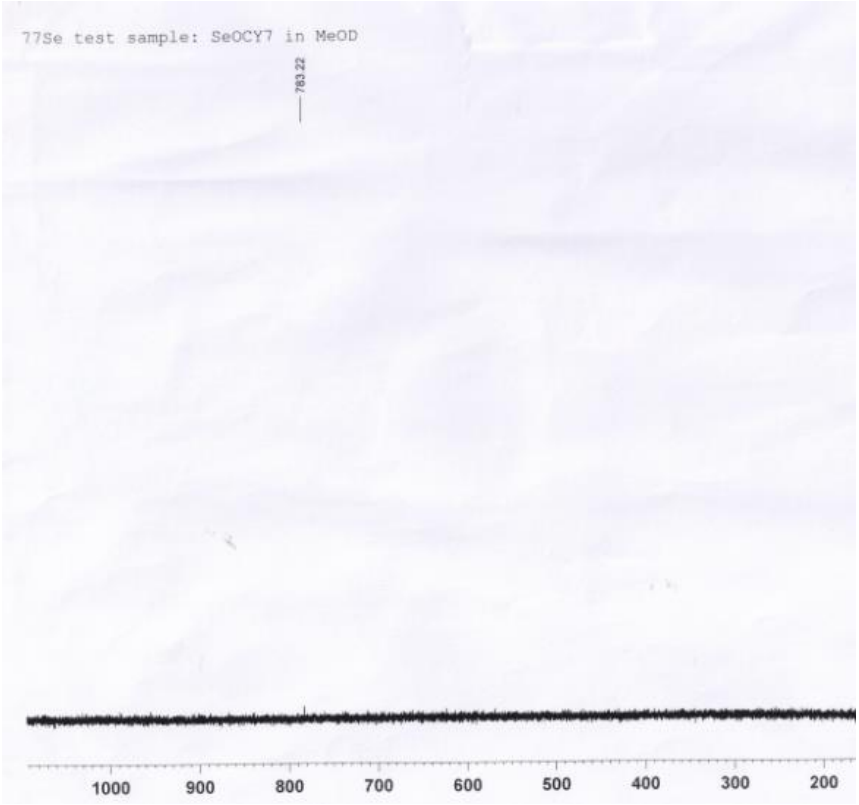


Fig. S8. ^{77}Se NMR of **SeOCy7** in CD_3OD

7. HRMS of SeOCy7

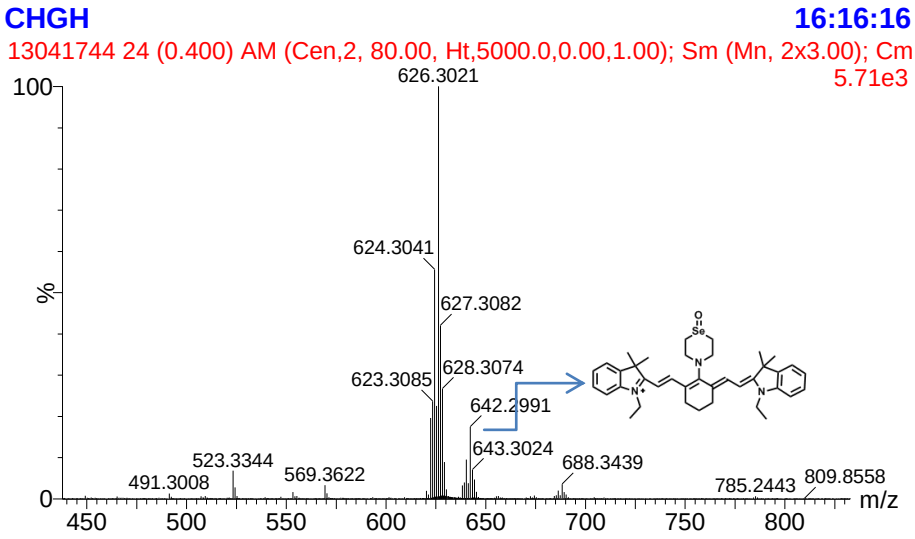


Fig. S9. The HRMS of **SeCy7** after addition of HClO

Elemental Composition Report

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
642.2991	642.2963	2.8	4.4	17.5	0.5	$C_{38}H_{48}N_3OSe$
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
626.3021	626.3013	0.8	1.3	17.5	0.6	$C_{38}H_{48}N_3Se$

8. Effect of pH values

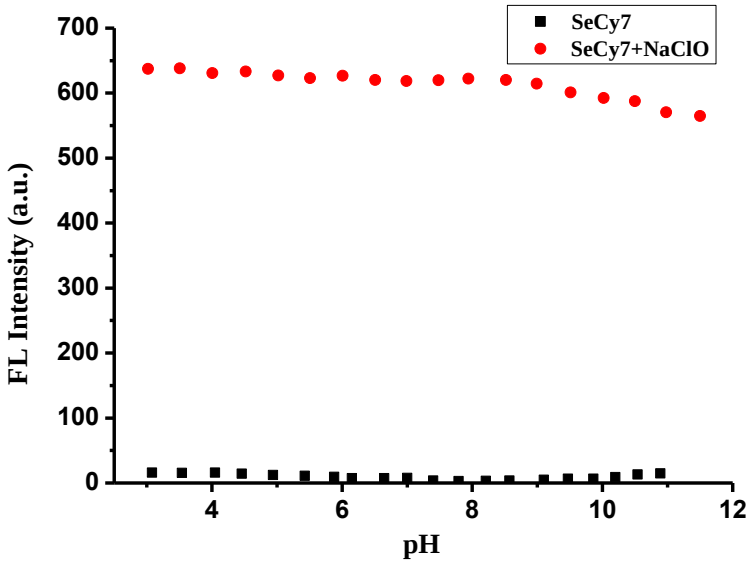


Fig. S10. pH-dependent fluorescence intensity of **SeCy7**(30 μM) in the absence (black) and presence (red) of NaClO (2 equiv.).

9. Fluorescence spectra of SeCy7 with HClO in fetal bovine serum.

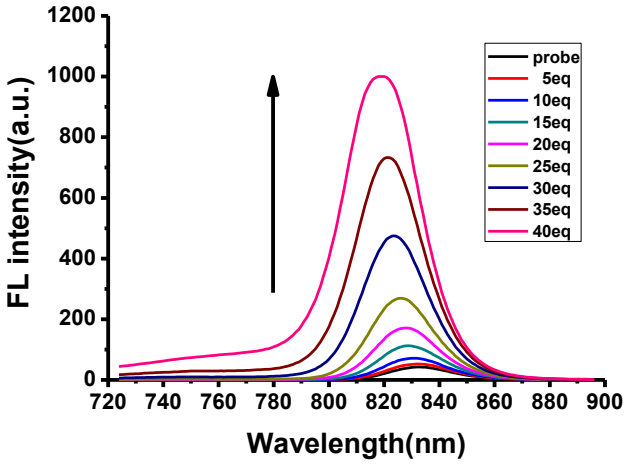


Fig. S11. Fluorescence emission spectra of **SeCy7** (60 μM) upon the addition of increasing concentrations of sodium hypochlorite (0–40 equiv.) in fetal bovine serum. The arrows indicate the changes in the emission intensities with the increased sodium hypochlorite concentration; λ_{ex} = 690 nm.

10. Experiment of ICR mouse

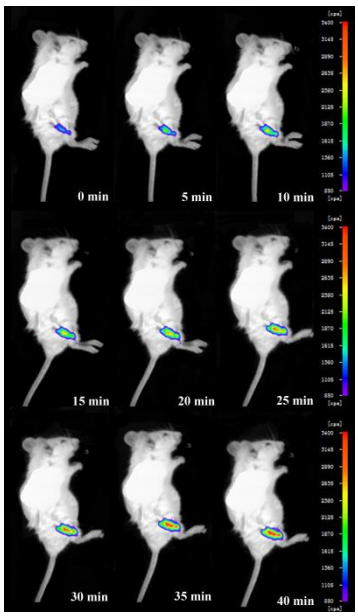
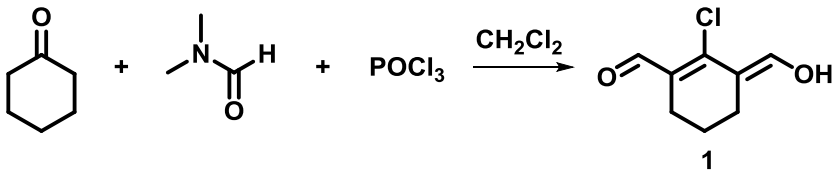


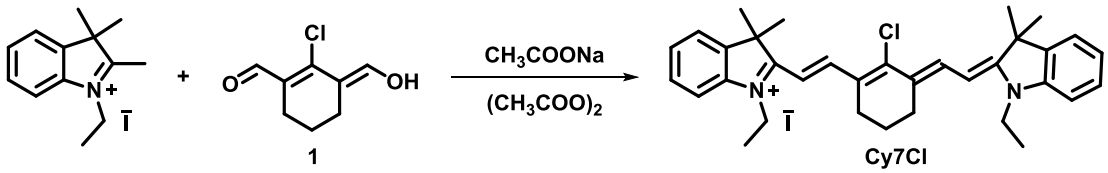
Fig. S12. Representative fluorescence images (pseudocolor) of an ICR mouse given a skin-pop injection of **SeCy7** (25 μL, 60 μM) and a subsequent (15 minutes later) skin-pop injection of NaClO (2.0 equiv.) at the same region. Images were taken after incubation for 0, 5, 10, 15 , 20 , 25 , 30 , 35 and 40 min, respectively. Images were taken with an excitation laser of 690 nm and an emission filter of 820±20 nm.

11. Synthesis and characterization of compounds



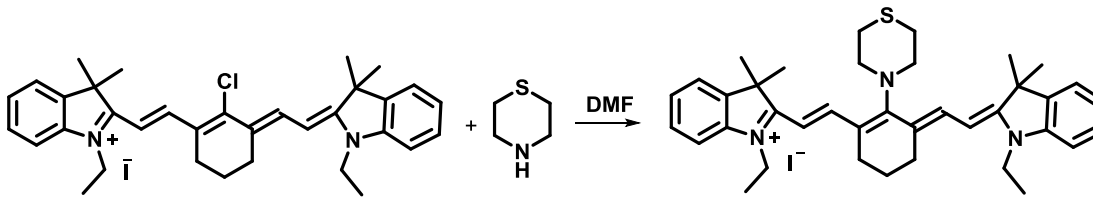
1

A solution of N,N-dimethylformamide (DMF,40mL) and dichloromethane (40mL) was cooled to -10℃ and phosphorus oxychloride (38 mL,0.407 mol) was added dropwise with vigorous stirring, followed by cyclohexanone (10.00g, 0.102 mol). The mixture was refluxed for 4 h. After cooled, the solution was poured into 500 g ice and allowed to stand overnight. A yellow solid **1** was collected (8.45 g, 48.0%). The product was used directly for the next reaction without purification.



1 Cy7Cl

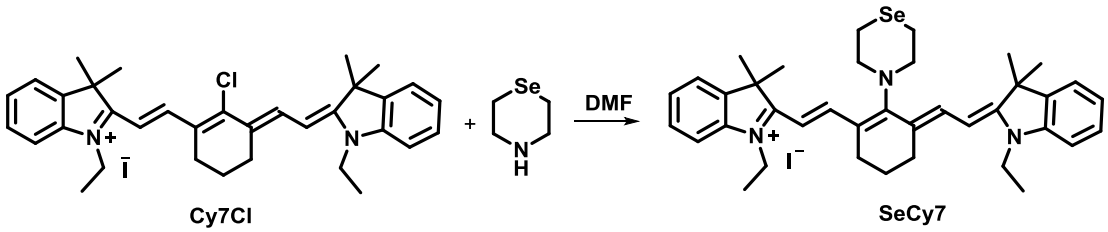
Under the nitrogen atmosphere, to a round bottom flask containing compound **1**(1.00 g, 5.79mmol) in 15 ml acetic anhydride, 1-ethyl-2,3,3-trimethyl-*3H*-indol-1-ium iodide (3.65g,11.6 mmol) and sodium acetate (0.95 g, 36 mmol) were added. The solution was heated to 130℃for 1 h. The generated mixture was filtered and the precipitate was washed thoroughly with diethyl ether and potassium iodide aqueous solution. A dark green solid (**Cy7Cl**) with metallic luster was obtained (3.35g,90.5%).^{1}H NMR (400 MHz, CD_3OD) δ8.46(d,2H), 7.28-7.55(m,8H), 6.30 (d, 2H),4.24(q,4H),2.75(t,4H),1.95(m,2H),1.74(s,12H),1.42(t,6H). ^{13}C NMR (100 MHz, CD_3OD) δ 172.47, 154.26, 149.77, 144.28, 141.77, 141.35, 128.54, 126.50, 125.17, 122.17, 110.64, 100.53, 38.95, 26.82, 25.96, 20.73, 11.08.TOF LD$^+$ ($C_{34}H_{40}N_2Cl^+$) calc. m/z = 511.2880, found m/z = 511.2899.



Cy7Cl SCy7

Cy7Cl (500 mg,0.782 mmol) and thiomorpholine (242 mg, 2.35 mmol) were

dissolved in 8 mL of anhydrous DMF in a 25mL round bottom flask under Ar for 1 h at 90℃.Then the solvent was poured into 200 mL anhydrous diethyl ether. The generated mixture was filtered and the precipitate was washed thoroughly with diethyl ether.The solid was purified by silica gel chromatography usingethyl acetate/methanol=10/1 as eluent to afford**SCy7** (385 mg) with metallic yellow-green powder.Yield: 69.7 %.^{1}H NMR (400 MHz, CD_3OD) δ 8.03(d,2H),7.49(d,2H), 7.39(t,2H),7.21 (m,4H), 6.09(d,2H), 4.14(q,4H),3.77(t,4H),2.92(t,4H), 2.57(t,4H), 1.87(m,2H), 1.76(s,12H), 1.38(t,6H) . ^{13}C NMR (100 MHz, CD_3OD) δ171.68, 170.16, 142.41, 142.06, 140.72, 128.35, 126.85, 124.04, 121.99, 109.69, 56.28, 38.25, 28.76, 27.39, 24.78, 21.56, 10.75. TOF LD$^+$ ($C_{38}H_{48}N_3S^+$) calc. m/z = 578.3569, found m/z = 578.3565.



Cy7Cl (500 mg,0.782 mmol) andselenomorpholine (470 mg, 3.13 mmol,The compound was synthesized according to previously reported literature.[S1]) were dissolved in 8 mL of anhydrous DMF in a 25mL round bottom flask under Ar for 1 h at 90℃.Then the solvent was poured into 200 mL anhydrous diethyl ether. The generated mixture was filtered and the precipitate was washed thoroughly with diethyl ether.The solid was purified by silica gel chromatography usingethyl acetate/methanol=10/1 as eluent to afford SeCy7 (358 mg) with metallickhaki powder.Yield: 60.8 %.^{1}H NMR (400 MHz, CD_3OD)δ 8.04(d,2H), 7.50(d,2H), 7.42(t,2H),7.23(m,4H),6.12(d,2H),4.16(q,4H),3.86(t,4H),2.96(t,4H),2.59(t,4H), 1.87 (m,2H), 1.78(s,12H), 1.40(t,6H).^{13}C NMR (100 MHz, CD_3OD) δ171.82, 170.32, 142.62, 142.05, 140.73, 128.37, 127.44, 124.13, 122.01, 109.76, 97.89, 56.77, 38.31, 27.30, 24.86, 21.54, 18.65，10.78.^{77}Se NMR (95 MHz, CD_3OD)δ149.09. TOF LD$^+$ ($C_{38}H_{48}N_3Se^+$) calc. m/z = 626.3013, found m/z = 626.3049.

Ref

S1 Hu L., Chen Z., Lu S., Li X., Liu Z and Xu H; *Phosphorus, Sulfur, and Silicon and the Related Elements*, 2004, **179**, 1065.

12. ^{1}H NMR, ^{13}C NMR spectra of the compounds

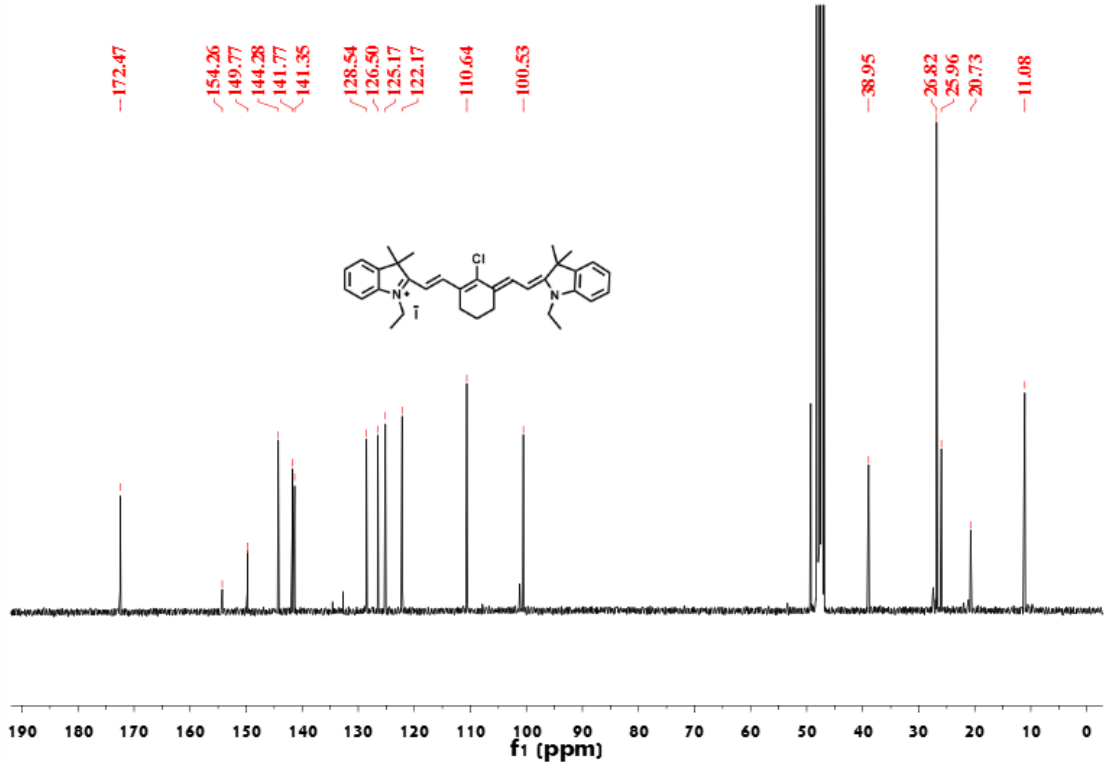


Fig. S13. ^{13}C NMR of **Cy7Cl** in CD_3OD

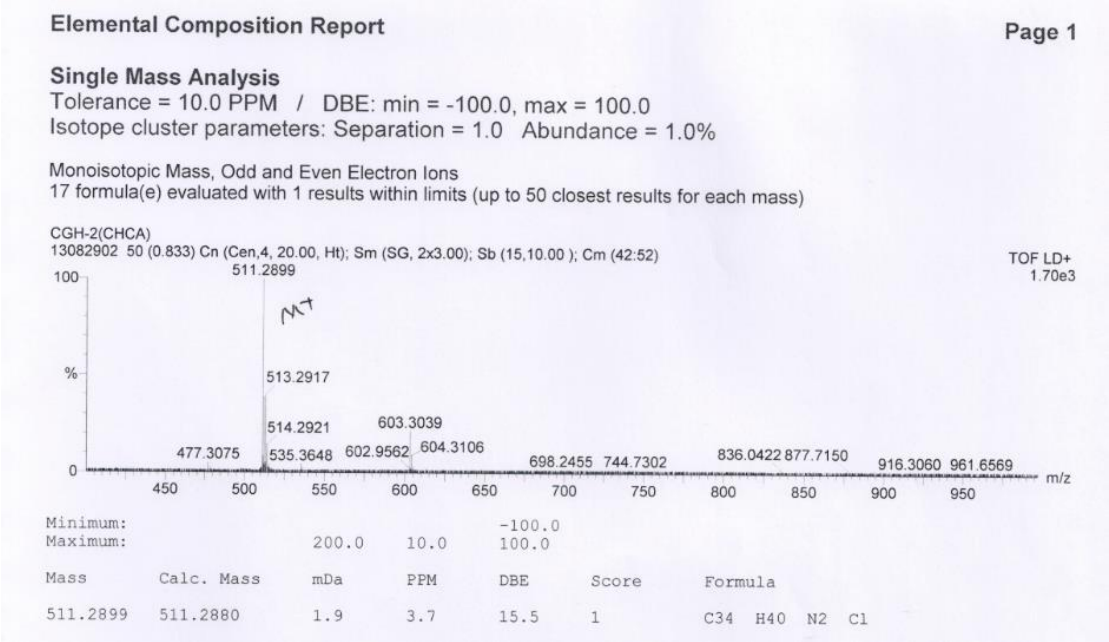


Fig. S14. TOF-MS of compound **Cy7Cl**

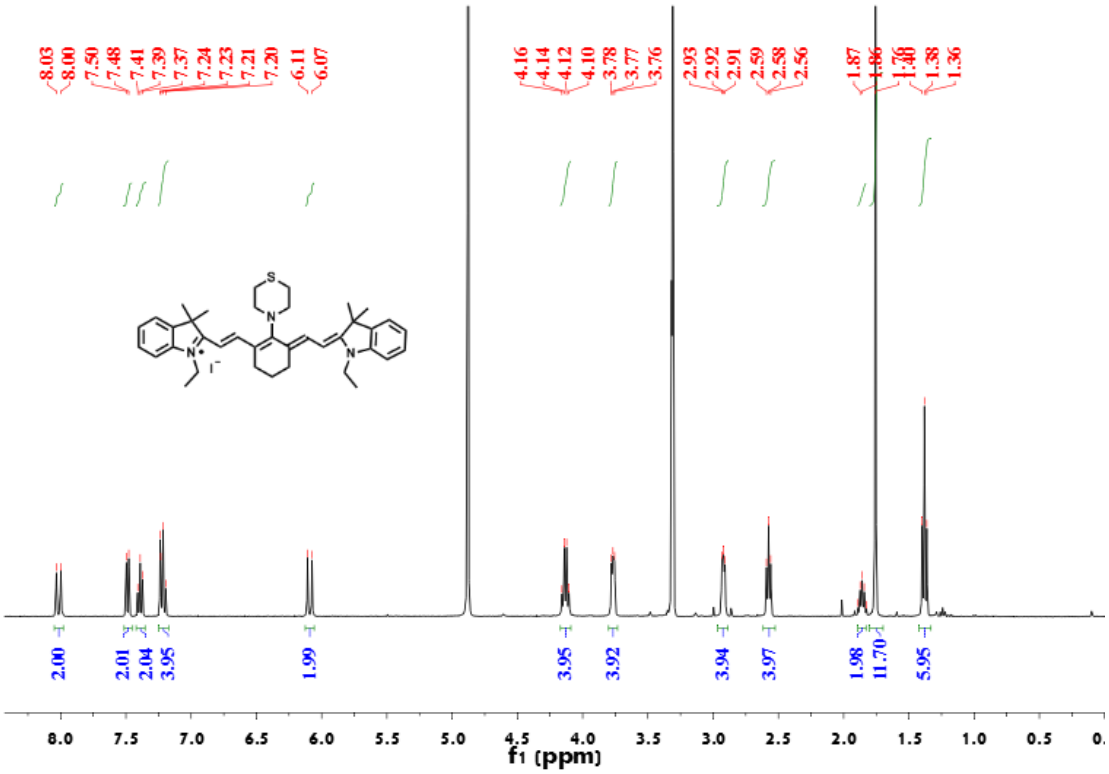


Fig. S15. ^{1}H NMR of **SCy7** in CD_3OD

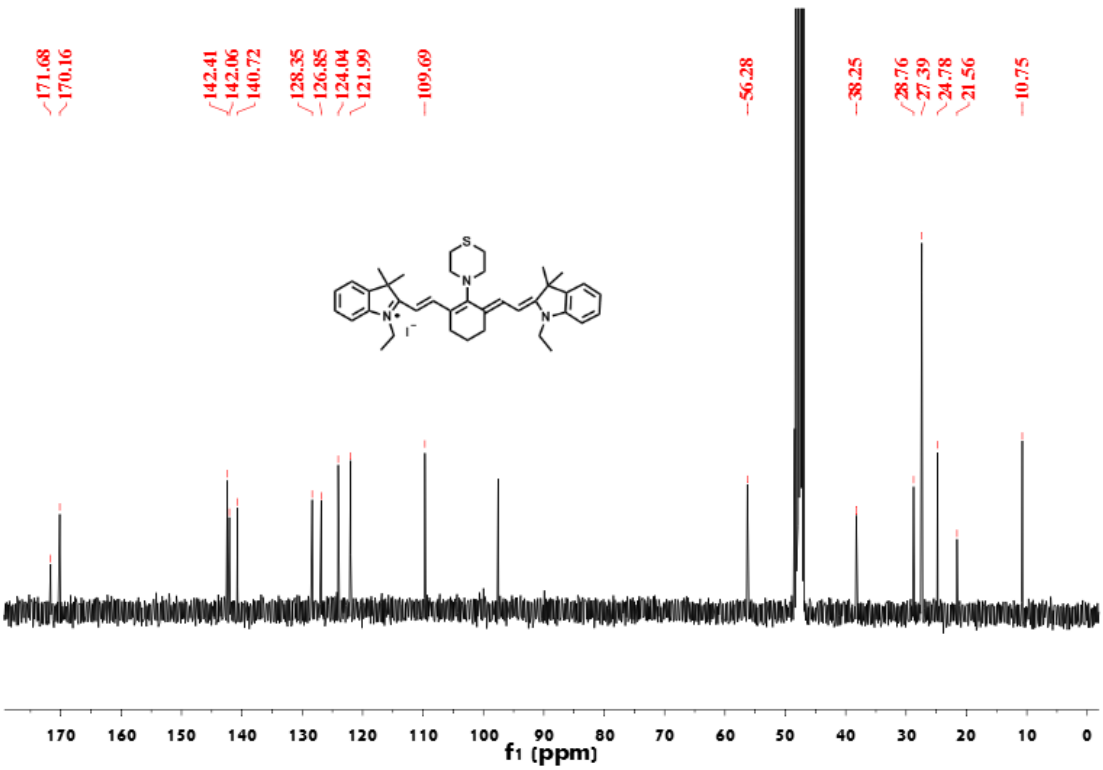


Fig. S16. ^{13}C NMR of **SCy7** in CD_3OD

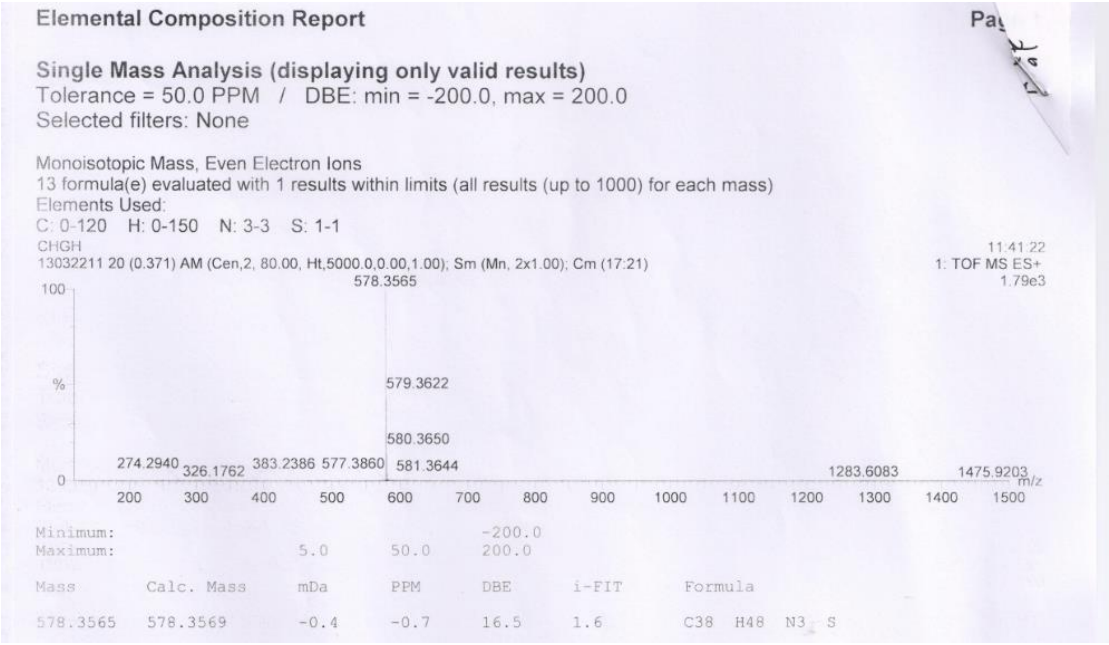


Fig. S17. TOF-MS of compound **SCy7**

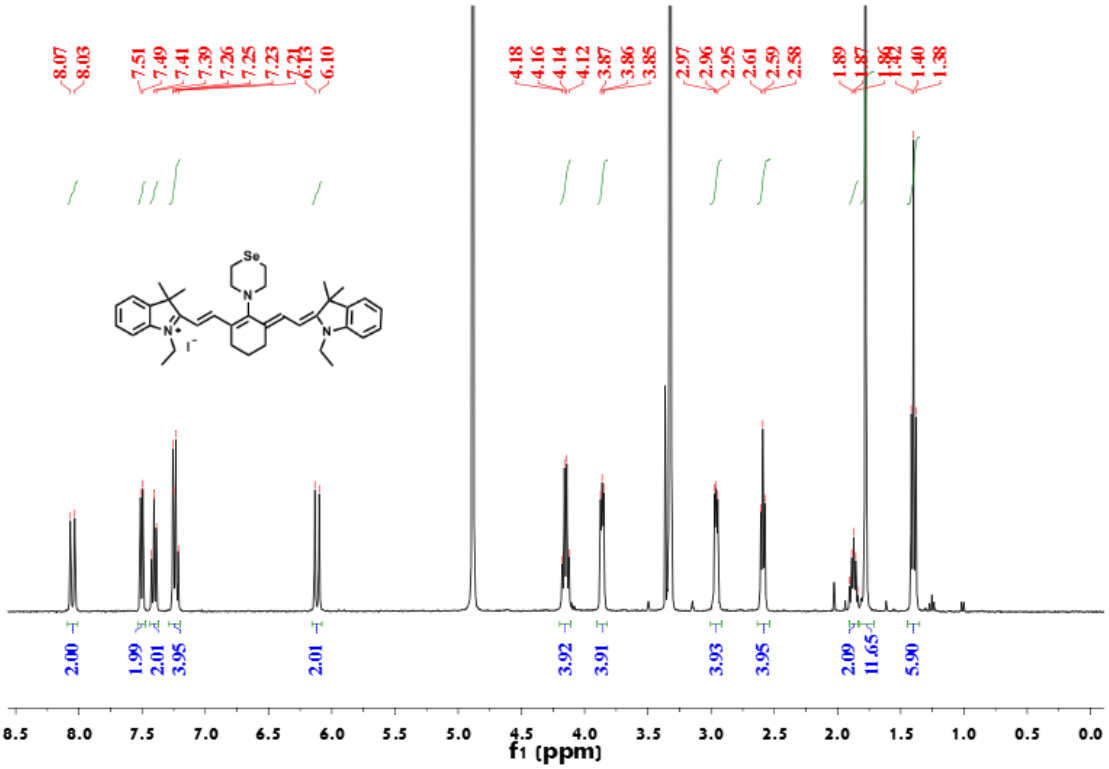


Fig. S18. ^{1}H NMR of **SeCy7** in CD_3OD

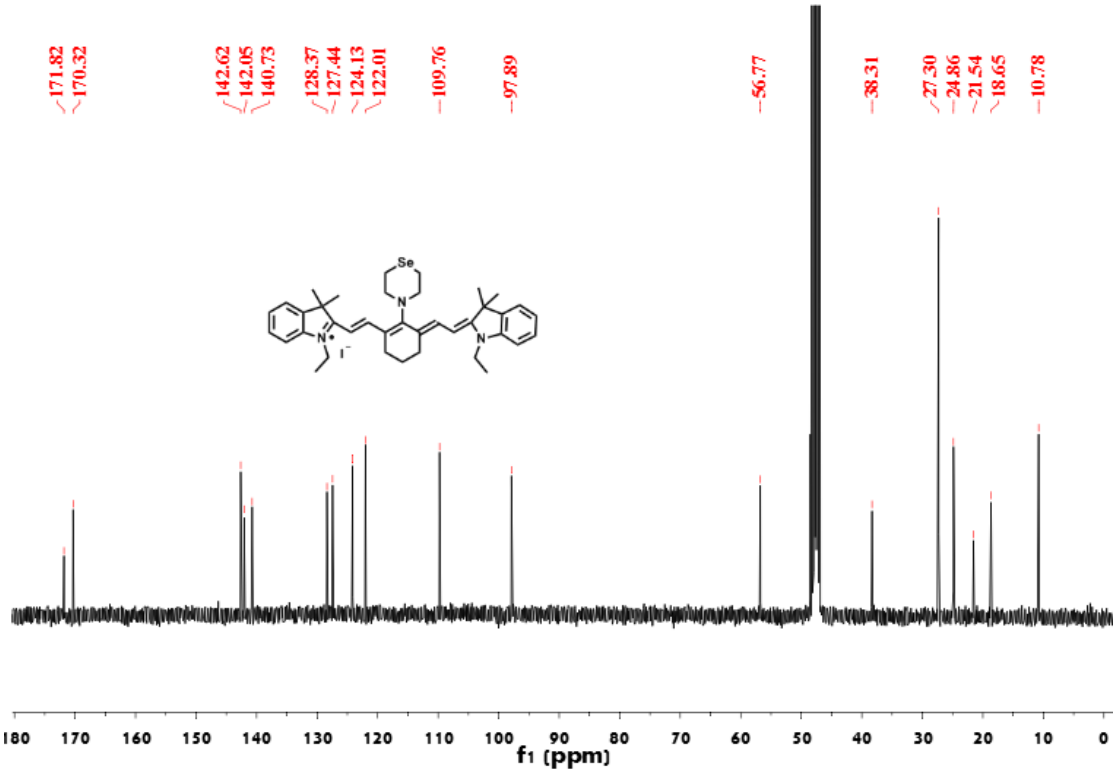


Fig. S19. ^{13}C NMR of **SeCy7** in CD_3OD

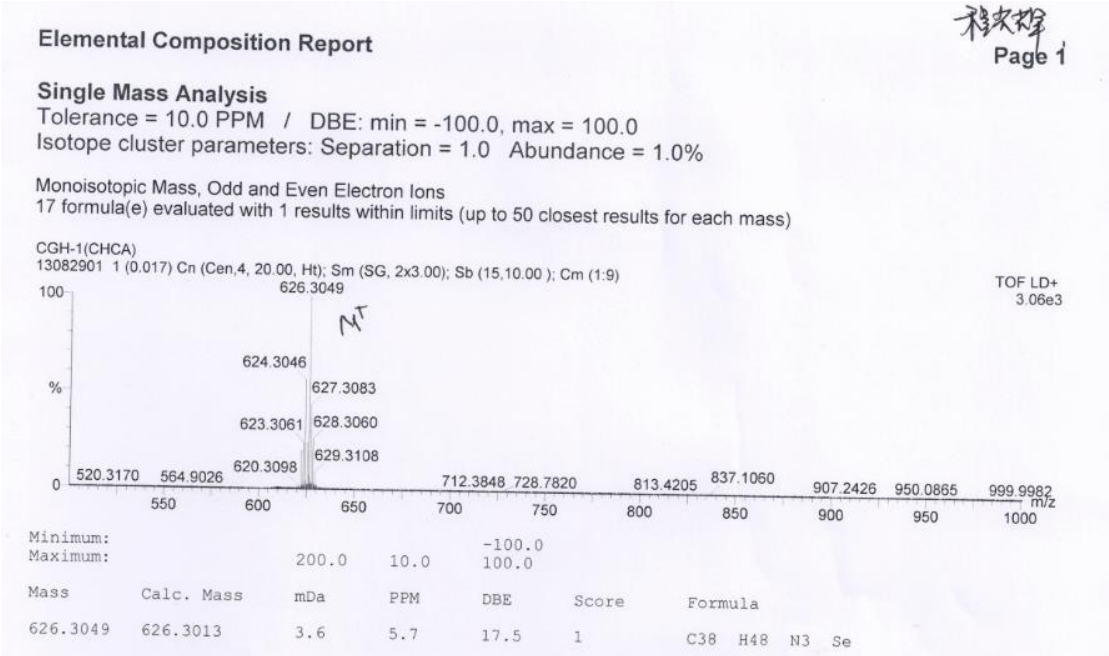


Fig. S20. TOF-MS of compound **SeCy7**