Supporting Information

Development of a far-red absorbing Se-Rhodamine photosensitizer and its application for bio-orthogonally activatable photodynamic therapy

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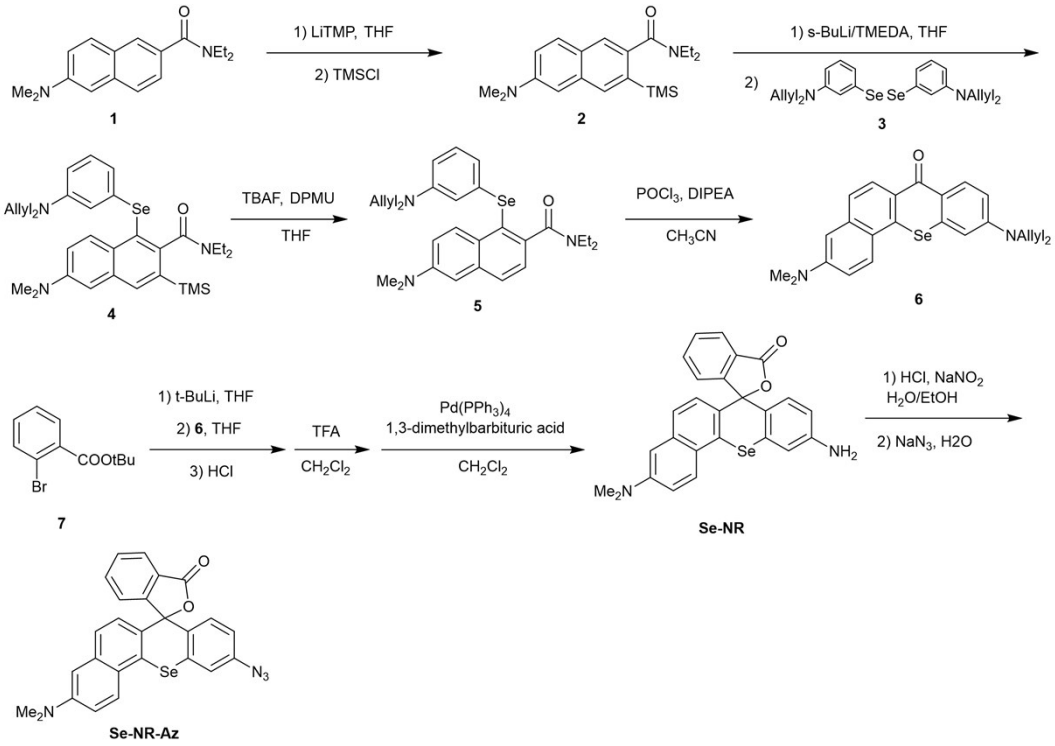
1. Materials and instruments

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. All aqueous solutions were prepared using ultrapure water. High-resolution mass spectra (HRMS) were obtained from DIONEX UltiMate 3000 & Bruker Compact TOF mass spectrometer. NMR spectra were recorded on a Bruker AVANCE III HD spectrometer, using TMS as an internal standard; Absorption spectra were measured on a Shimadzu UV 2550 UV-Vis spectrophotometer. The fluorescence spectra were measured on a Shimadzu RF-6000 fluorophotometer. MTT test was conducted on Thermo Scientific Multiskan Mk3 microplate reader. The fluorescence microscopy images of HeLa cells were acquired by PerkinElmer UltraVIEW VoX or LEICA M205FA microscope. The cell flow cytometry data was acquired by Beckman Counter CytoFlex S.

2. Synthesis and Characterization



Scheme S1. Synthetic route of **Se-NR** and **Se-NR-Az**

6-(dimethylamino)-*N*,*N*-diethyl-2-naphthamide (1),[S1] **3,3'-diselanediylbis(*N*,*N*-diallylaniline) (3)**[S2] and **tert-butyl 2-bromobenzoate (7)**[S3] was synthesized according to literature procedures.

6-(dimethylamino)-*N*,*N*-diethyl-3-(trimethylsilyl)-2-naphthamide (2). Under an argon atmosphere, *n*-BuLi (2.5 M in hexanes, 9 ml, 22.5 mmol) were added dropwise to a stired solution of TMP (3.8 ml, 22.5 mmol) in THF (16 ml) at 0 ℃. The resulting mixture was stirred for 15 min then transferred to a stirred solution of compound **1** (2 g, 7.4 mmol) in THF (20 ml) at -78 ℃. The resulting solution was stirred at −78 °C for 1.5 h and trimethylsilyl chloride (1.95 ml, 22.5 mmol) was added. The resulting mixture was stirred at −78 °C for 30 mins, after which it was warmed to room temperature. The reaction mixture was quenched with saturated NH_4Cl solution and extracted

with CH_2Cl_2, and the organic layer was washed with brine and dried with anhydrous Na_2SO_4 and evaporated. The residue was purified by column chromatography using silica gel and petroleum ether/EtOAc (8/1, v/v) as the eluent to give yellow solids (1.5 g, yield: 59 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.66 (d, J = 9.0 Hz, 1H), 7.52 (s, 1H), 7.20 (d, J = 8.2 Hz, 1H), 6.94 (s, 1H), 3.59 (d, J = 6.5 Hz, 2H), 3.25 (d, J = 6.6 Hz, 2H), 3.07 (s, 6H), 1.31 (t, J = 7.1 Hz, 3H), 1.11 (d, J = 7.2 Hz, 3H), 0.34 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.02, 149.10, 135.38, 135.27, 134.30, 134.14, 128.52, 125.64, 124.27, 117.21, 106.28, 40.82.

1-((3-(diallylamino)phenyl)selanyl)-6-(dimethylamino)-*N,N*-diethyl-3-(trimethylsilyl)-2-naphthamide (4). To a solution of **2** (1 g, 2.92 mmol) and TMEDA (568 μL, 3.79 mmol) in THF (15 ml) at −78 °C was added *s*-BuLi (1.3 M in *n*-hexane, 2.9 ml, 3.79 mmol). The resulting solution was stirred for 1 h at −78 °C and a solution of compound **3** (1.47 g, 2.92 mmol) in THF (9 ml) at −78 °C was added. The resulting mixture was stirred at −78 °C for 45 mins and then allowed to slowly warm up to -40 °C over 1.25 h. The mixture was further stirred at ambient temperature for 6 h. The reaction mixture was quenched with saturated NH_4Cl solution and extracted with CH_2Cl_2, and the organic layer was washed with brine and dried with anhydrous Na_2SO_4 and evaporated. The residue was purified by column chromatography using silica gel and petroleum ether/EtOAc (20/1, v/v) as the eluent to give yellow oils (1.15g, yield: 66 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, J = 9.3 Hz, 1H), 7.94 (s, 1H), 7.13 (dd, J = 9.4, 2.6 Hz, 1H), 6.91 (t, J = 8.0 Hz, 1H), 6.88 (d, J = 2.1 Hz, 1H), 6.56 (d, J = 7.6 Hz, 1H), 6.46 (s, 1H), 6.37 (dd, J = 8.2, 2.2 Hz, 1H), 5.66 – 5.54 (m, 2H), 4.96 – 4.84 (m, 4H), 3.96 – 3.86 (m, 1H), 3.67 (d, J = 4.6 Hz, 4H), 3.28 – 3.17 (m, 1H), 3.13 – 3.05 (m, 1H), 3.04 (s, 6H), 3.02 – 2.97 (m, 1H), 1.30 (t, J = 7.1 Hz, 3H), 0.90 (t, J = 7.2 Hz, 3H), 0.34 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.86, 148.95, 148.88, 136.68, 135.38, 134.55, 133.72, 133.69, 130.23, 129.18, 127.88, 117.93, 117.28, 115.85, 113.44, 109.77, 106.50, 52.71, 43.23, 40.69, 38.61, 13.43, 12.28, 0.26. HRMS m/z: calcd for $C_{32}H_{43}N_3NaOSeSi$ $[M+Na]^+$: 616.2233 found: 616.2236.

1-((3-(diallylamino)phenyl)selanyl)-6-(dimethylamino)-*N,N*-diethyl-2-naphthamide (5). To a solution of compound **4** (1.15 g, 1.94 mmol) in *N,N'*-dimethylpropyleneurea (DPMU, 5 ml) was added a solution of TBAF (1 M in THF, 10 ml, 10 mmol) at room temperature. After stirring for 24 h at 60 °C, water was added and the whole was extracted with EtOAc, and the organic layer was washed with brine and dried with anhydrous Na_2SO_4 and evaporated. The residue was purified by column chromatography using silica gel and petroleum ether/EtOAc (4/1, v/v) as the eluent to give yellow oils (0.85g, yield: 84 %).
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (d, J = 9.4 Hz, 1H), 7.72 (d, J = 8.4 Hz, 1H),7.25 (d, J = 8.4 Hz, 1H), 7.15 (dd, J = 9.4, 2.6 Hz, 1H), 6.94 – 6.84 (m, 2H), 6.51 (d, J = 7.7 Hz, 1H), 6.46 (s, 1H), 6.39 (dd, J = 8.3, 2.2 Hz, 1H), 5.67 – 5.53 (m, 2H), 4.98 – 4.86 (m, 4H), 3.88 – 3.74 (m, 1H), 3.69 (d, J = 4.8 Hz, 4H), 3.40 – 3.30 (m, 1H), 3.16 – 3.07 (m, 1H), 3.05 (s, 6H), 2.95 – 2.83 (m, 1H), 1.26 (t, J = 7.1 Hz, 3H), 0.93 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.61, 148.94, 148.86, 138.87, 135.27, 133.60, 133.46, 130.27, 129.28, 128.76, 128.03, 123.81, 123.64, 117.58, 117.44, 115.83, 113.34, 109.87, 106.31, 52.74, 42.67, 40.62, 38.53, 13.89, 12.55. HRMS m/z: calcd for $C_{29}H_{35}N_3NaOSe$ $[M+Na]^+$: 544.1838 found: 544.1841.

10-(diallylamino)-3-(dimethylamino)-7H-benzo[c]selenoxanthen-7-one (6). $POCl_3$ (1.82 ml,

19.56 mmol) was added dropwise to a solution of DIPEA (3.2 ml, 19.56 mmol) and compound **5** (0.85 g, 1.63 mmol) in CH_3CN (17 ml). The resulting mixture was heated at reflux for 12 h and then cooled to 0 °C. A solution of 2 M NaOH (30 mL) was added, and the resulting mixture was stirred for 2 h. Water was added and the whole was extracted with CH_2Cl_2. The organic layer was washed with brine and dried with anhydrous Na_2SO_4 and evaporated. The residue was purified by column chromatography using silica gel and CH_2Cl_2/Et_2O (20/1, v/v) as the eluent to give yellow solids (0.58 g, yield: 84 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (t, J = 9.1 Hz, 2H), 7.96 (d, J = 9.2 Hz, 1H), 7.59 (d, J = 8.9 Hz, 1H), 7.19 (dd, J = 9.3, 2.6 Hz, 1H), 6.91 (s, 1H), 6.84 – 6.79 (m, 2H), 5.95 – 5.82 (m, 2H), 5.28 – 5.16 (m, 4H), 4.03 (d, J = 4.6 Hz, 4H), 3.11 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.80, 150.71, 136.32, 135.97, 132.36, 132.34, 126.76, 126.24, 124.86, 120.38, 116.73, 115.55, 112.29, 108.11, 52.57, 40.46. HRMS m/z: calcd for $C_{25}H_{24}N_2NaOSe$ $[M+Na]^+$: 471.0946 found: 471.0947.

10-amino-3-(dimethylamino)-3'H-spiro[benzo[c]selenoxanthene-7,1'-isobenzofuran]-3'-one (Se-NR). Under an Ar atmosphere, *t*-BuLi (1.3 M in pentane, 3.4 mL, 4.42 mmol) was added dropwise to a solution of **7** (575 mg, 2.24 mmol) in THF (12 ml) at -78 °C. The solution was stirred for 30 min at -78 °C, and then a solution of compound **6** (400 mg, 0.89 mmol) in THF (8 ml) was added. The reaction mixture was warmed to room temperature, and stirred for 1 h, then conc. HCl (0.55 mL) was added. The solution was concentrated and neutralized with saturated $NaHCO_3$ and extracted with CH_2Cl_2. The organic solution was washed with brine, dried over Na_2SO_4 and evaporated. The residue was dissolved in 4 mL of CH_2Cl_2, and 4 mL of TFA was added. This mixture was stirred at 40 °C for 2 h. The solution was concentrated, then 2 M NaOH (20 ml) was added and stir for 30 mins. Water was added and the whole was extracted with CH_2Cl_2. The organic solution was washed with brine, dried over Na_2SO_4 and evaporated. The residue was roughly purified by column chromatography using silica gel and petroleum ether/EtOAc (8/1, v/v) as the eluent.

To 1,3-dimethylbarbituric acid (283 mg, 1.81 mmol) and $Pd(PPh_3)_4$ (84 mg, 0.073 mmol) under an Ar atmosphere were added a solution of the crude product in dry CH_2Cl_2 (16 ml), and the reaction mixture was stirred for 16 h at ambient temperature. A solution of 2 M NaOH (20 ml) was added, and the resulting mixture was extracted with CH_2Cl_2. The organic solution was washed with brine, dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography using silica gel and petroleum ether/EtOAc (2/1, v/v) as the eluent to give blue solids (320 mg, yield: 76 % in 3 steps).

^{1}H NMR (400 MHz, Acetone-*d6*) δ 7.98 (d, J = 9.3 Hz, 1H), 7.93 (d, J = 7.4 Hz, 1H), 7.87 (d, J = 7.7 Hz, 1H), 7.73 – 7.67 (m, 1H), 7.64 (td, J = 7.5, 1.0 Hz, 1H), 7.52 (d, J = 8.9 Hz, 1H), 7.36 (dd, J = 9.3, 2.7 Hz, 1H), 7.11 (d, J = 8.8 Hz, 1H), 7.07 (d, J = 2.4 Hz, 1H), 6.98 – 6.92 (m, 2H), 6.58 (dd, J = 8.6, 2.4 Hz, 1H), 3.07 (s, 6H). ^{13}C NMR (101 MHz, Acetone-*d6*) δ 169.66, 155.17, 149.43, 149.02, 134.88, 134.80, 131.84, 131.74, 129.74, 128.61, 128.05, 127.67, 126.30, 125.41, 125.27, 124.39, 123.93, 123.80, 123.64, 122.24, 116.72, 113.67, 113.02, 106.16, 39.61. HRMS m/z: calcd for $C_{26}H_{20}N_2NaO_2Se$ $[M+Na]^+$: 495.0582 found: 495.0593.

10-azido-3-(dimethylamino)-3'H-spiro[benzo[c]selenoxanthene-7,1'-isobenzofuran]-3'-one (Se-NR-Az). Conc. HCl (1.5 ml) and **Se-NR** (135 mg, 0.286 mmol) was dissolved in EtOH (2.5 ml). The blue solution was cooled to 0 °C, and a solution of sodium nitrite (40 mg, 0.58 mmol) in

water (1 ml) was added. The resulting solution was stirred for 30 mins at 0 °C. A solution of NaN_3 (57 mg, 0.87 mmol) in water (1 ml) was then added dropwise, and the reaction mixture was stirred for another 5 h at ambient temperature. Water was added and the whole was extracted with CH_2Cl_2. The organic solution was washed with brine, dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography using silica gel and petroleum ether/EtOAc (20/1, v/v) as the eluent to give faint yellow solids (123 mg, yield: 86 %).

^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.87 (d, J = 9.0 Hz, 2H), 7.83 (dd, J = 6.7, 1.6 Hz, 1H), 7.50 – 7.42 (m, 2H), 7.40 (d, J = 8.8 Hz, 1H), 7.33 (d, J = 2.3 Hz, 1H), 7.28 (d, J = 8.6 Hz, 1H), 7.18 (td, J = 6.0, 2.9 Hz, 2H), 6.81 (dd, J = 8.6, 2.3 Hz, 1H), 6.78 (d, J = 2.5 Hz, 1H), 2.98 (s, 6H). ^{13}C NMR (101 MHz, CD_2Cl_2) δ 170.04, 154.67, 149.28, 140.53, 134.88, 134.86, 131.83, 129.91, 128.88, 127.75, 127.39, 126.42, 125.81, 123.81, 123.53, 123.46, 123.22, 119.27, 117.91, 116.63, 105.95, 40.22. HRMS m/z: calcd for $C_{26}H_{19}N_4O_2Se$ $[M+H]^+$: 499.0668 found: 499.0679.

3. Methods

3.1 Fluorescence quantum yield determination

Fluorescence quantum yields for **Se-NR** and **Se-NR-Az** were determined by using Nile Blue (Φ_f = 0.25 in MeOH) as a fluorescence standard.[S4] The quantum yield was calculated using the Equation 1:

$$\Phi_{F(X)} = \Phi_{F(S)} (A_S F_X / A_X F_S) (n_X / n_S)^2 \qquad \text{Equation 1}$$

Where Φ_F is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, and n is the refractive index of the solvents used. Subscripts S and X refer to the standard and to the unknown, respectively. For compounds Se-NR, Se-NR-Az and Nile Blue, the excitation wavelength was at 600 nm while keeping the absorption below 0.05.

3.2 Singlet oxygen quantum yield determination

The singlet oxygen quantum yields for **Se-NR** and **Se-NR-Az** were determined by the 1,3-diphenylisobenzofuran (DPBF) bleaching method, evaluated using Equation 2, Methylene blue (Φ_Δ = 0.50) was used as a reference.[S5]

$$\Phi_{\Delta(X)} = \Phi_{\Delta(S)} (m_X / m_S) (F_S / F_X) \qquad \text{Equation 2}$$

Where Φ_Δ is the singlet oxygen quantum yield, m is the slope of difference in change in the absorbance of the trap molecule (at 411 nm) with irradiation time and F is the absorption correction factor, which is given by $F=1 - 10^{-OD}$ (OD at the irradiation wavelength). Subscripts S and X refer to the standard and to the unknown, respectively.

4. Cell experiments

4.1 Cell culture

HeLa cells were incubated in Dulbecco's modified Eagle's medium (DMEM) medium supplemented with 10% (v/v) fetal bovine serum (FBS), 100 mg/mL streptomycin, and 100 U/mL penicillin under 5% CO_2 and 95% air at 37 °C in a humidified atmosphere.

4.2 Intracellular $^1O_2$ detection

HeLa cells were seeded in confocal dishes for 24 h. Then the cells were incubated with **Se-NR-Az** (0 or 1 μM). Subsequently, **TPP** (0 or 200 μM) were added. After incubation for 6 h, the cells were stained with DCFH-DA (10 μM) for 30 min and then washed with PBS twice. After irradiation with a 610 nm LED light (0 mW/cm^2 or 10 mW/cm^2) for 5 min, cell images were acquired using a confocal laser scanning microscope (PerkinElmer UltraVIEW VoX). The probe was excited at 488 nm and the emission was collected between 500-550 nm.

4.3 *In vitro* PDT using MTT Assays

HeLa cells were seeded in 96-well plates with DMEM culture media. After 24 h, the cells were incubated with different concentrations of **Se-NR** or **Se-NR-Az**. Subsequently, appropriate concentrations of **TPP** were added. After 6 h incubation, the cells were washed with PBS twice, irradiated with a 610 nm LED light (0 mW/cm^2 or 10 mW/cm^2) for 10 min, and cultured for another 24 h. The cell viability was evaluated with the MTT method using a Thermo Scientific Multiskan Mk3 microplate reader.

4.4 *In vitro* PDT using confocal imaging

HeLa cells were seeded in confocal dishes for 24 h. Then the cells were incubated with **Se-NR-Az** (0 or 5 μM). Subsequently, **TPP** (0 or 200 μM) were added. After 6 h incubation, the cells were washed with PBS twice, irradiated with a 610 nm LED light (10 mW/cm^2) for 10 min, and cultured for another 24 h. Then, the cells were stained by the mixture of Calcein-AM (2 μM) and PI (4.5 μM) at 37 ºC for 30 min in the DMEM medium without FBS. The fluorescence images of the cells were immediately recorded by a confocal laser scanning microscope (LEICA M205FA), the excitation for Calcein-AM and PI were 488 nm and 561 nm, and the corresponding emission was collected between 500-550 nm and 580-650 nm.

4.5 Cell apoptosis and cecrosis detection by flow cytometer

HeLa cells were seeded in 6-well plate for 24 h. Then the cells were incubated with **Se-NR-Az** (0 or 1 μM). Subsequently, **TPP** (0 or 200 μM) were added. After 6 h incubation, the cells were washed with PBS twice, irradiated with a 610 nm LED light (10 mW/cm^2) for 5 min, and cultured for another 2 h. Then, the floating dead cells and adherent alive cells were all collected and stained with Annexin V-FITC/PI apoptosis detection kit (Beijing Solarbio Science & Technology Co., Ltd.) according to the manufacturer's instructions. The apoptosis and necrosis results were examined on a flow cytometer.

5. Additional figures

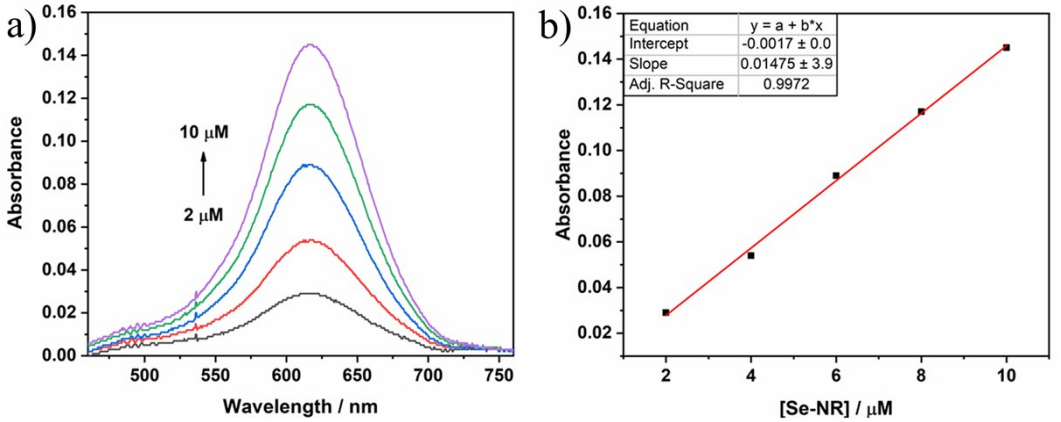


Figure S1. Absorption spectra (a) and concentration-absorbance curve (b) of **Se-NR** in buffer solution (PBS:MeCN = 4:1).

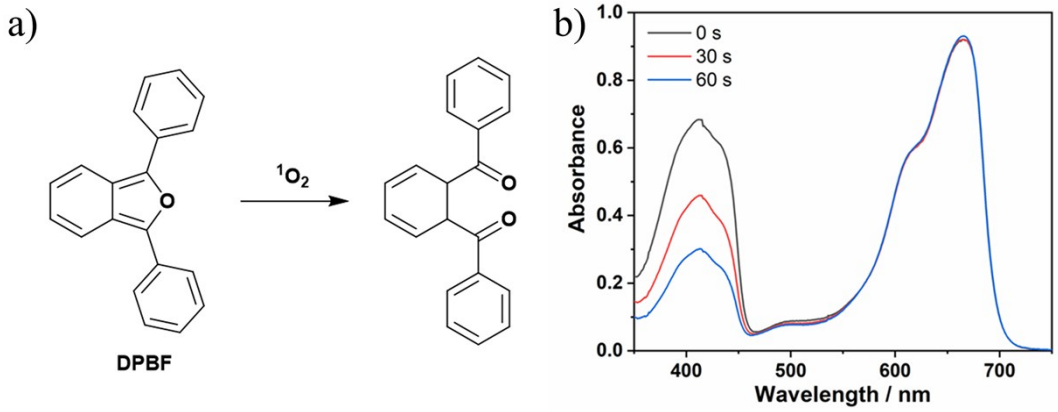


Figure S2. (a) Reaction mechanism of 1,3-diphenylisobenzofuran (DPBF) with $^1O_2$. (b) Time-dependent absorption spectral changes of 50 μM DMF solution of DPBF and 20 μM of Methylene Blue upon irradiation at 630 nm.

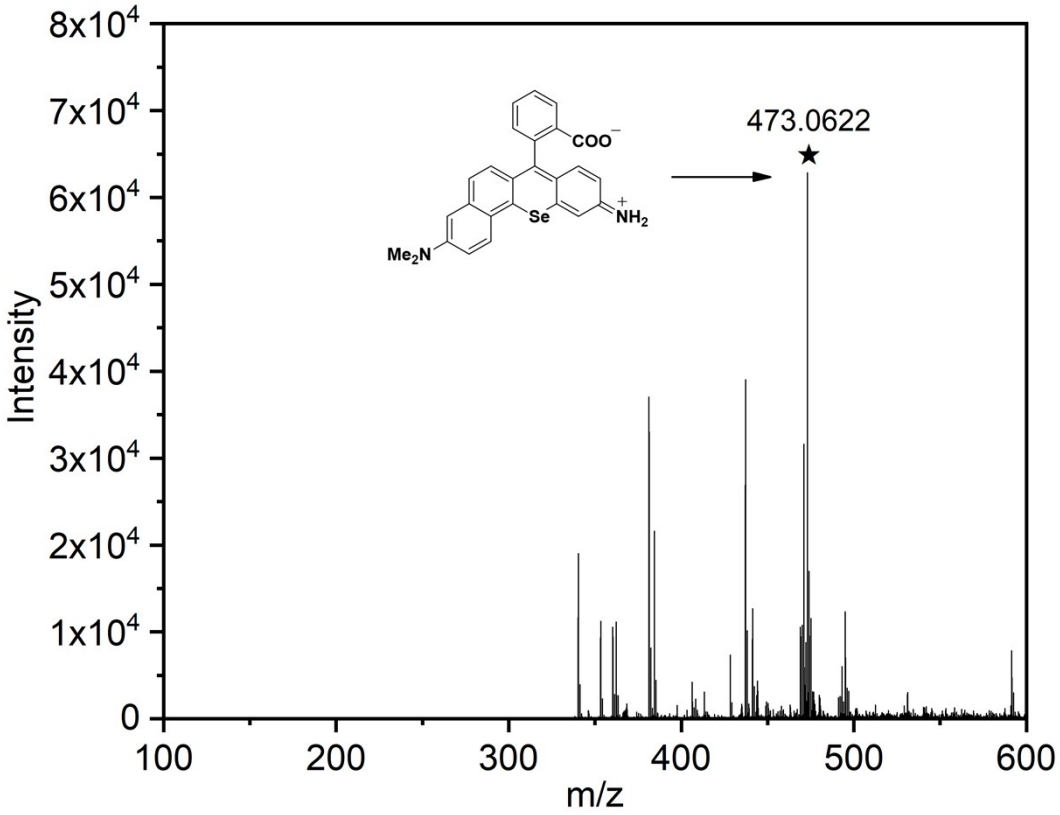


Figure S3. HRMS spectrum of **Se-NR-Az** and **TPP** reaction mixture in CH_3CN:H_2O = 4:1 for 4 h. The peak at m/z 473.0622 can be assigned to **Se-NR** (calcd for $C_{26}H_{21}N_2O_2Se$ $[M+H]^+$: 473.0768).

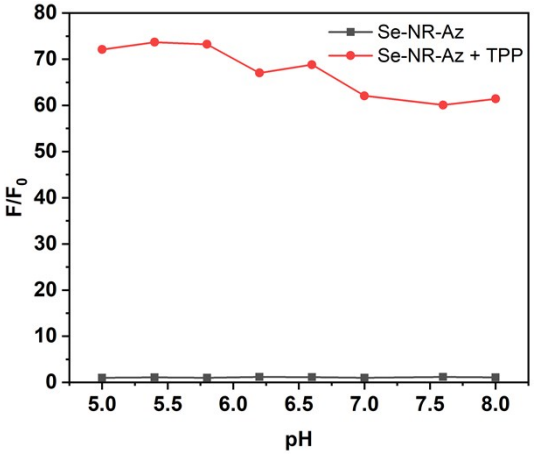


Figure S4. Effect of pH on the fluorescence of 10 μM Se-NR-Az before and after reacting with 0.5 mM TPP. The excitation wavelength was 610 nm, and the fluorescence intensity was measured at 740 nm in buffer solution (PBS:MeCN = 4:1).

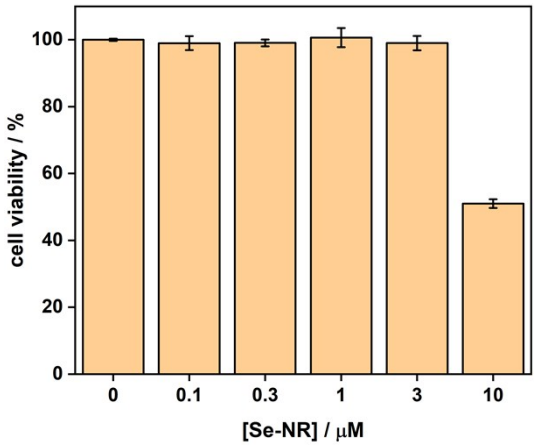


Figure S5. Dark cytotoxicity of **Se-NR**.

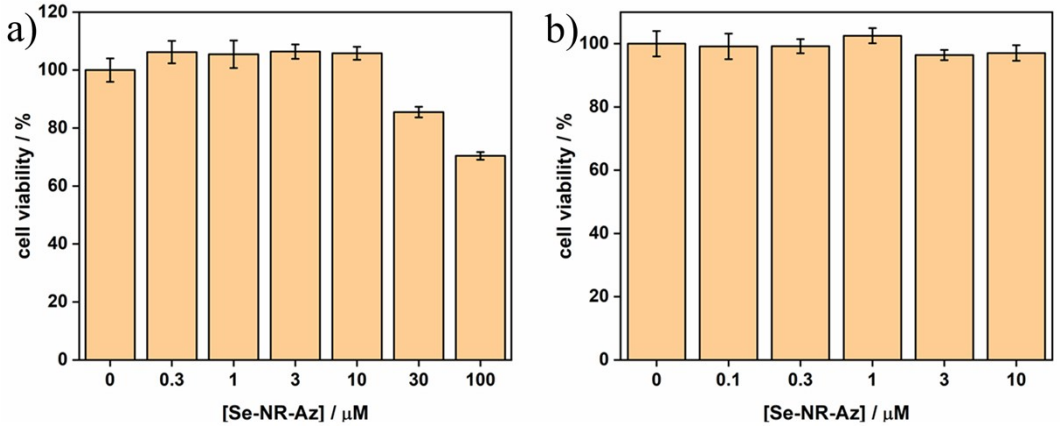


Figure S6. Dark cytotoxicity of (a) **Se-NR-Az** alone and (b) **Se-NR-Az** with 200 uM **TPP**.

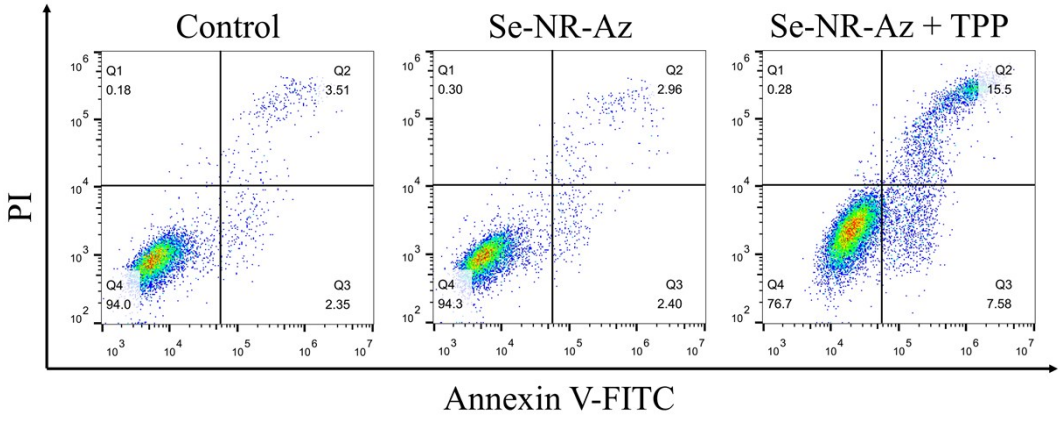


Figure S7. Annexin V-FITC/PI analysis of HeLa cells with different treatments.

6. NMR spectra and HRMS data

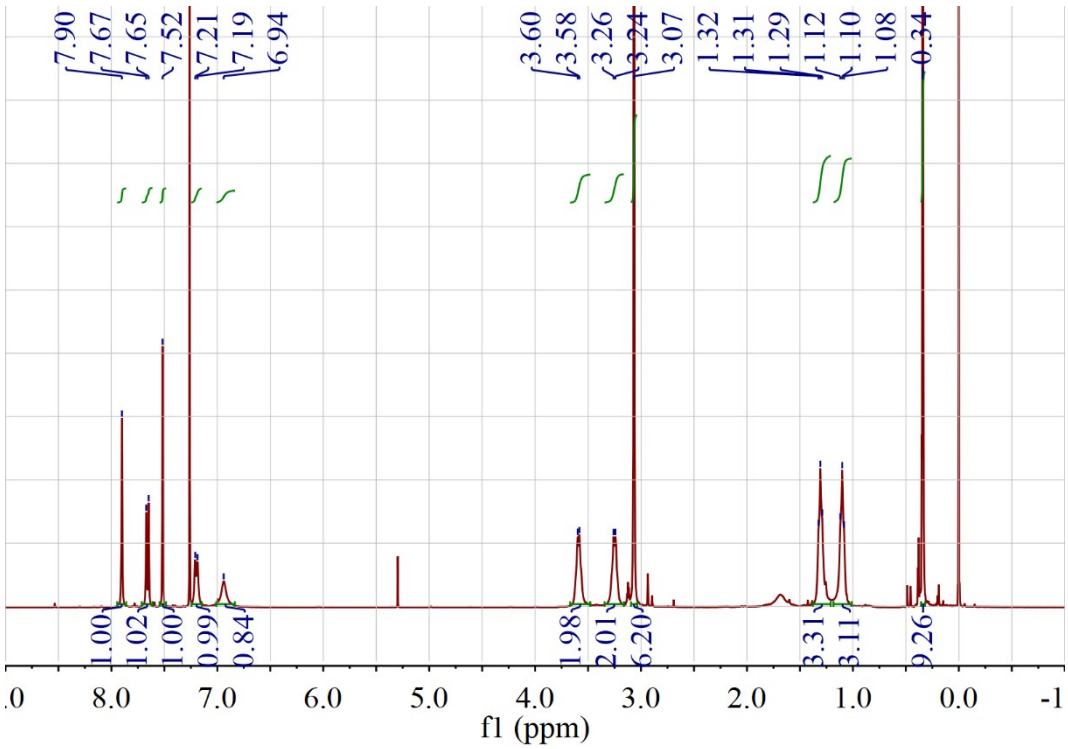


Figure S8. ^{1}H NMR spectrum of compound **2** ($CDCl_3$, 298K, 400 MHz).

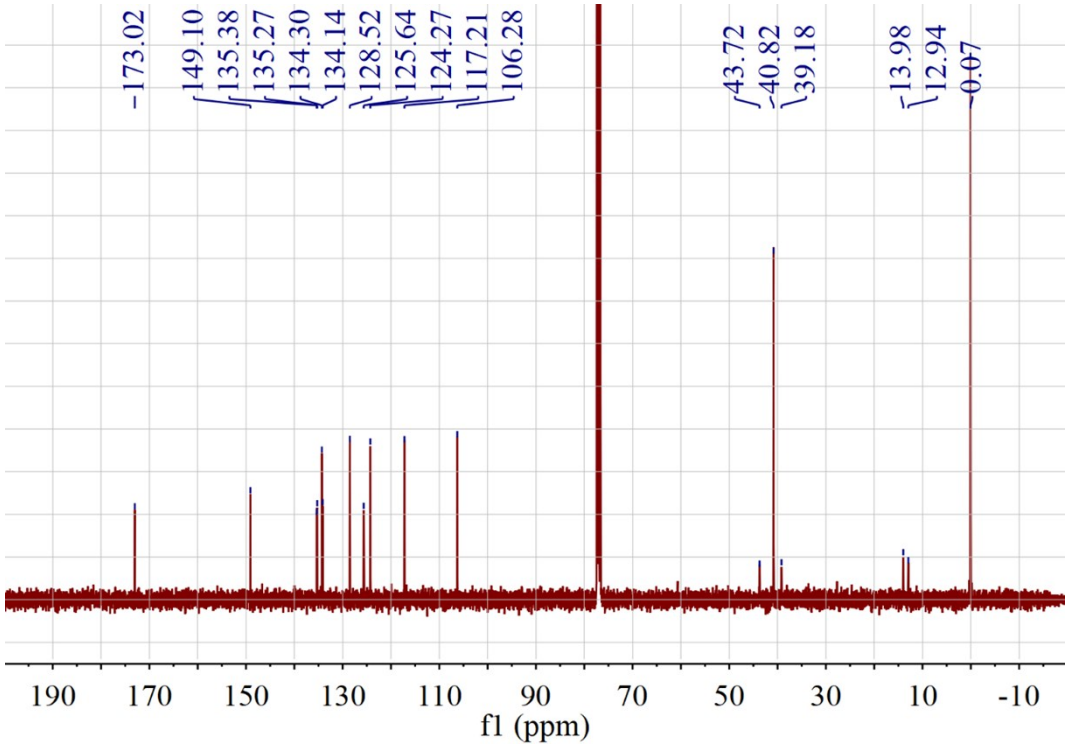


Figure S9. ^{13}C NMR spectrum of the compound **2** ($CDCl_3$, 298K, 101 MHz).

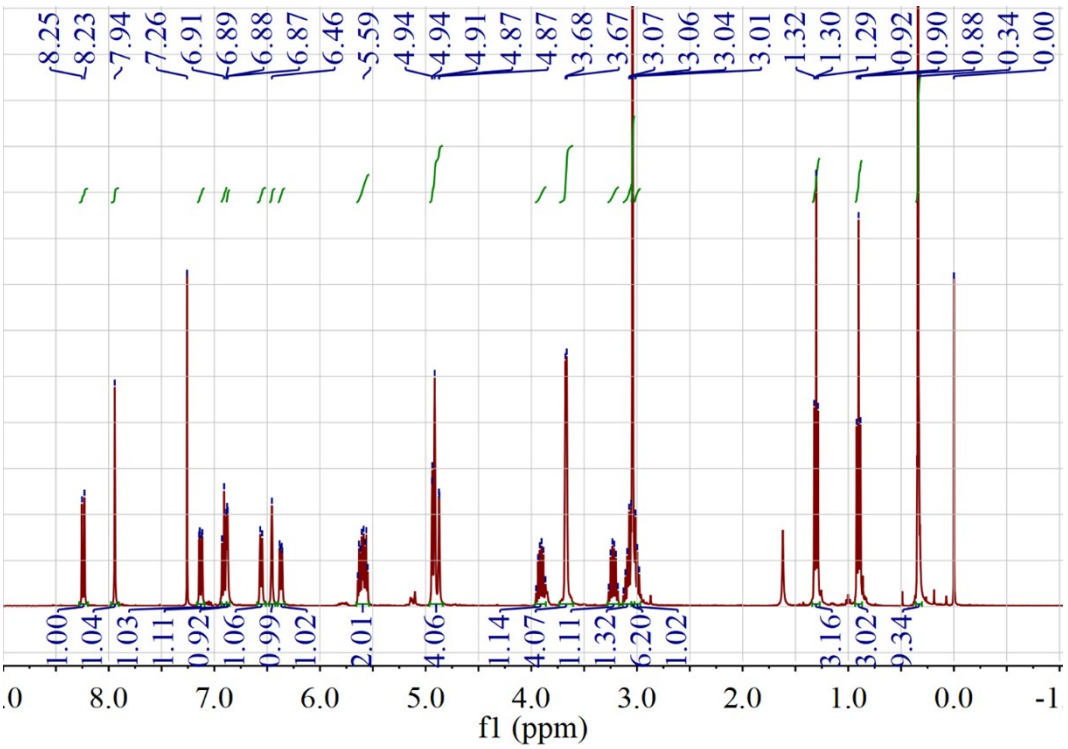


Figure S10. ^{1}H NMR spectrum of compound **3** ($CDCl_3$, 298K, 400 MHz).

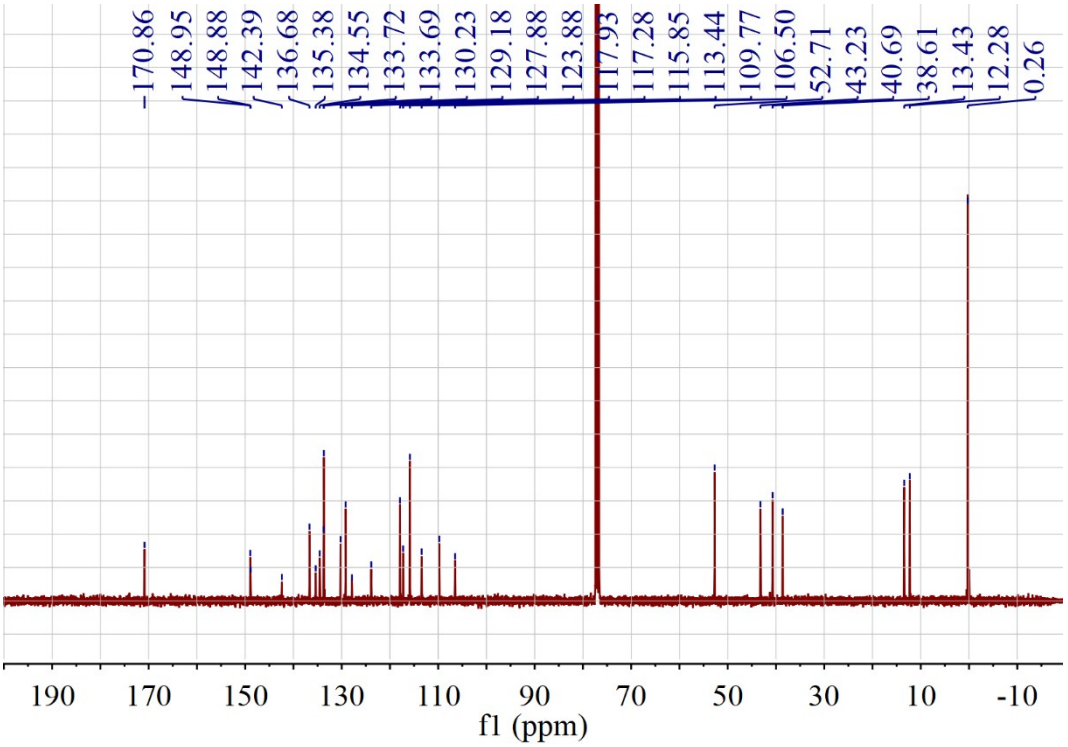


Figure S11. ^{13}C NMR spectrum of the compound **3** ($CDCl_3$, 298K, 101 MHz).

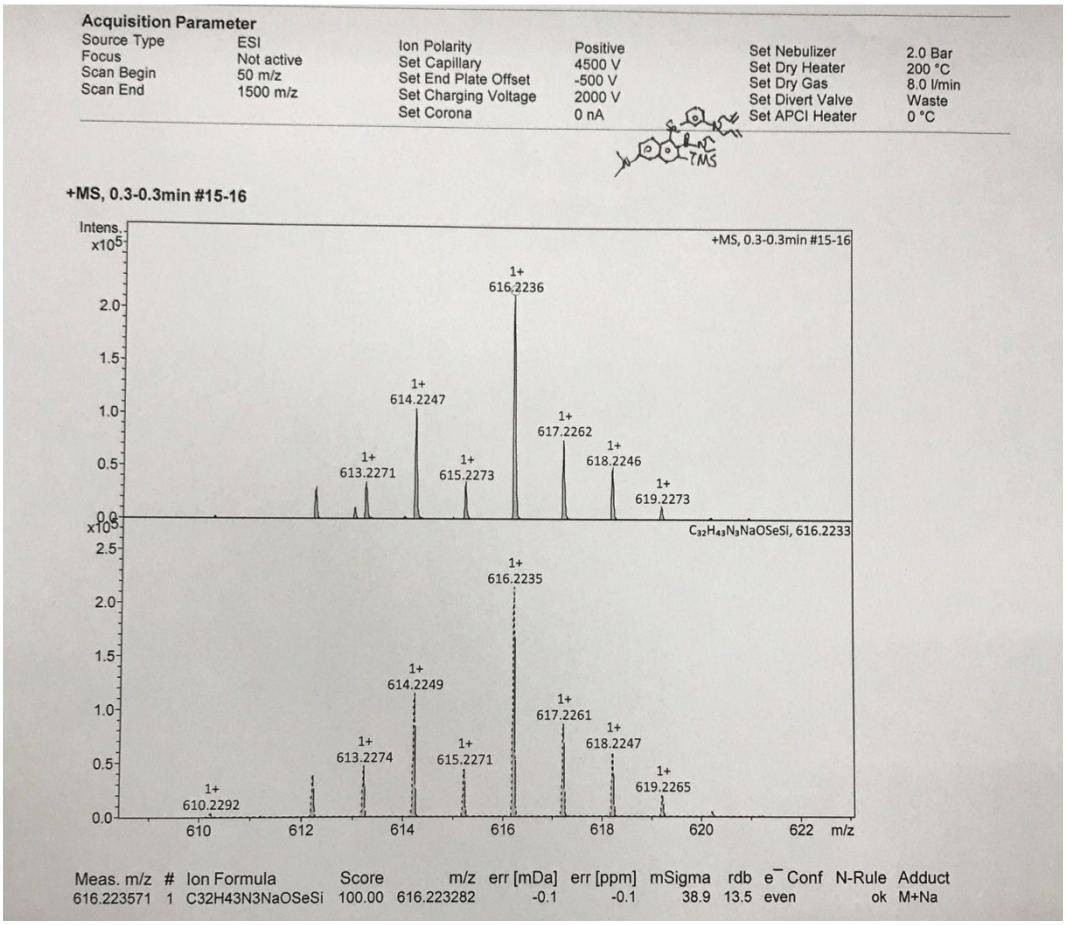


Figure S12. HRMS spectrum of the compound **3**.

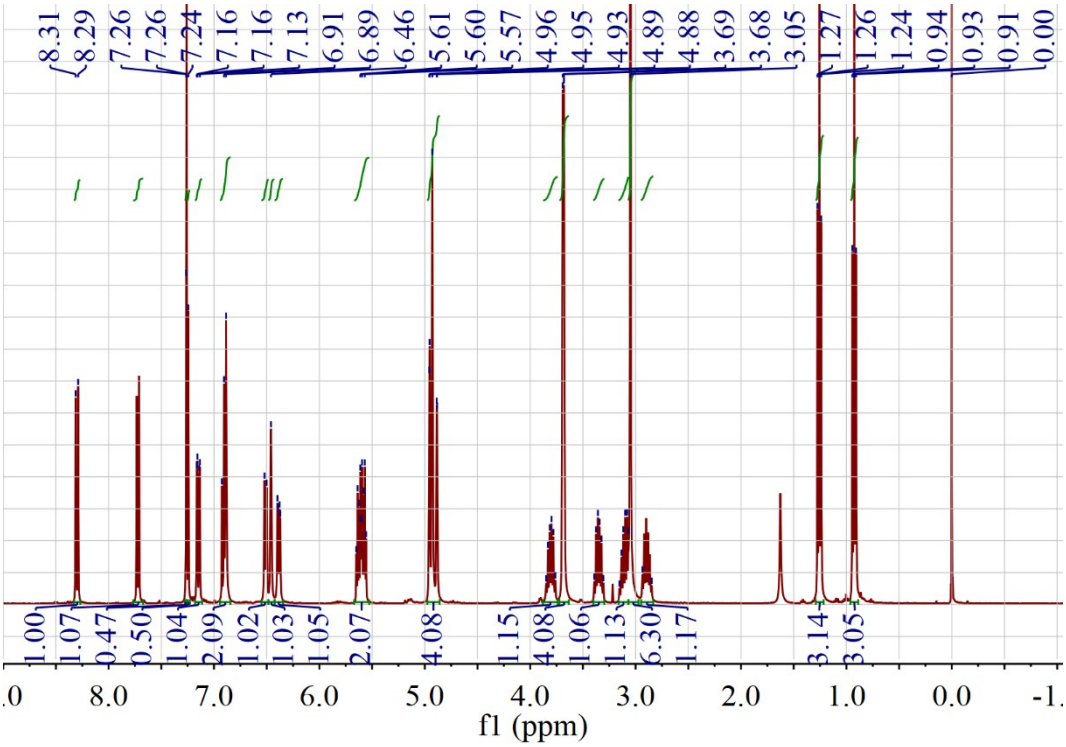


Figure S13. ^{1}H NMR spectrum of compound **4** (CDCl3, 298K, 400 MHz).

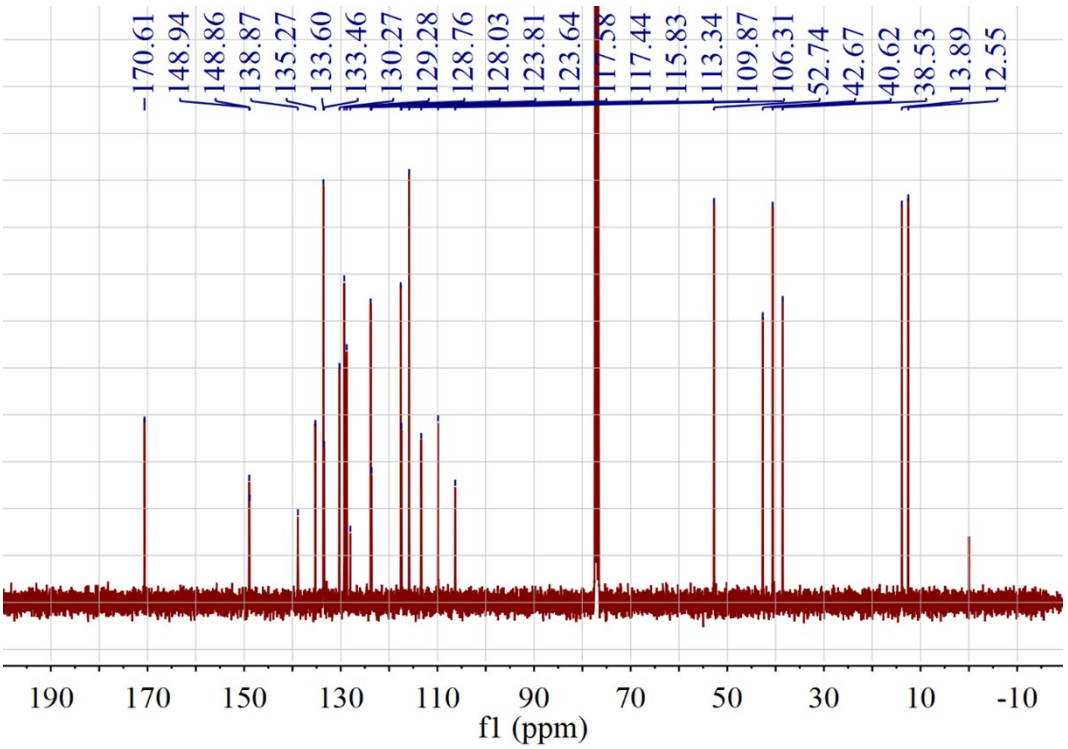


Figure S14. ^{13}C NMR spectrum of compound **4** ($CDCl_3$, 298K, 101 MHz).

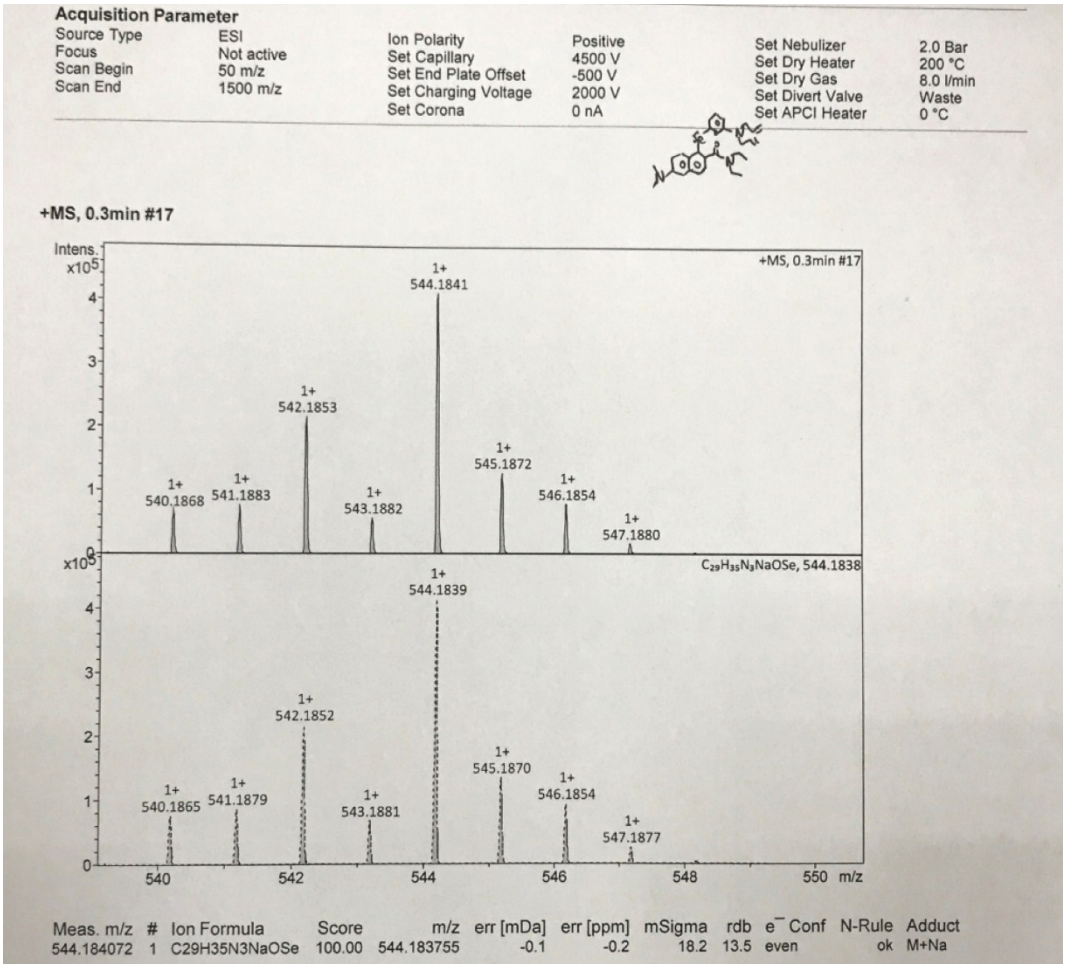


Figure S15. HRMS spectrum of compound **4**.

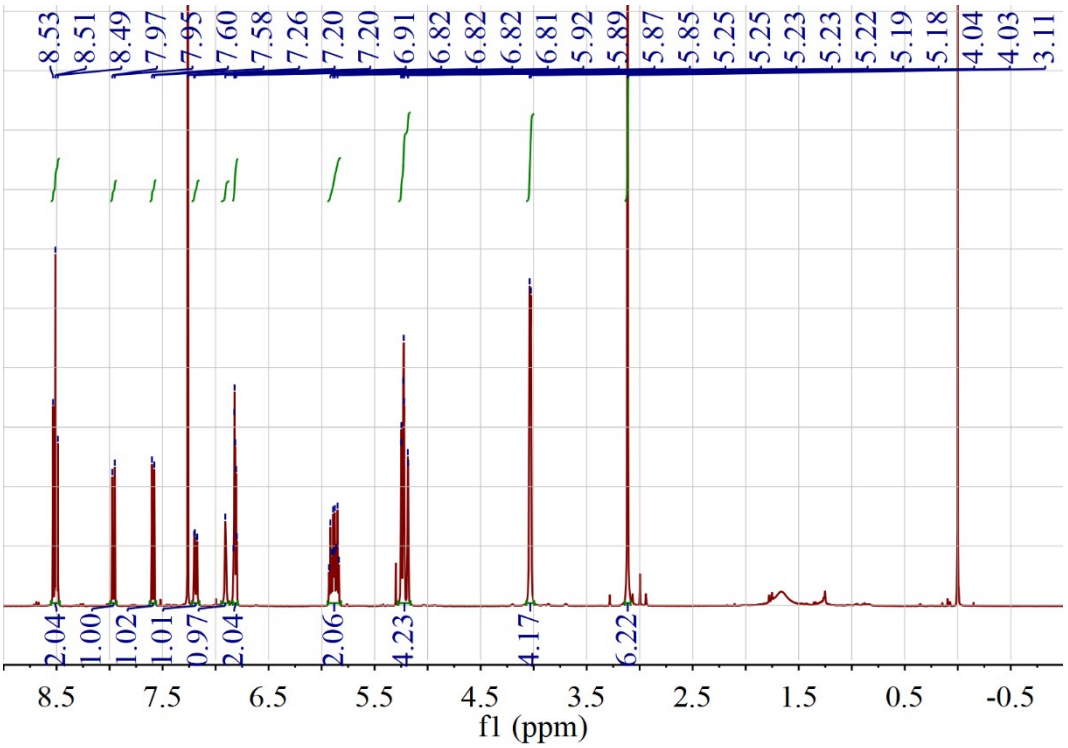


Figure S16. ^{1}H NMR spectrum of compound **6** ($CDCl_3$, 298K, 400 MHz).

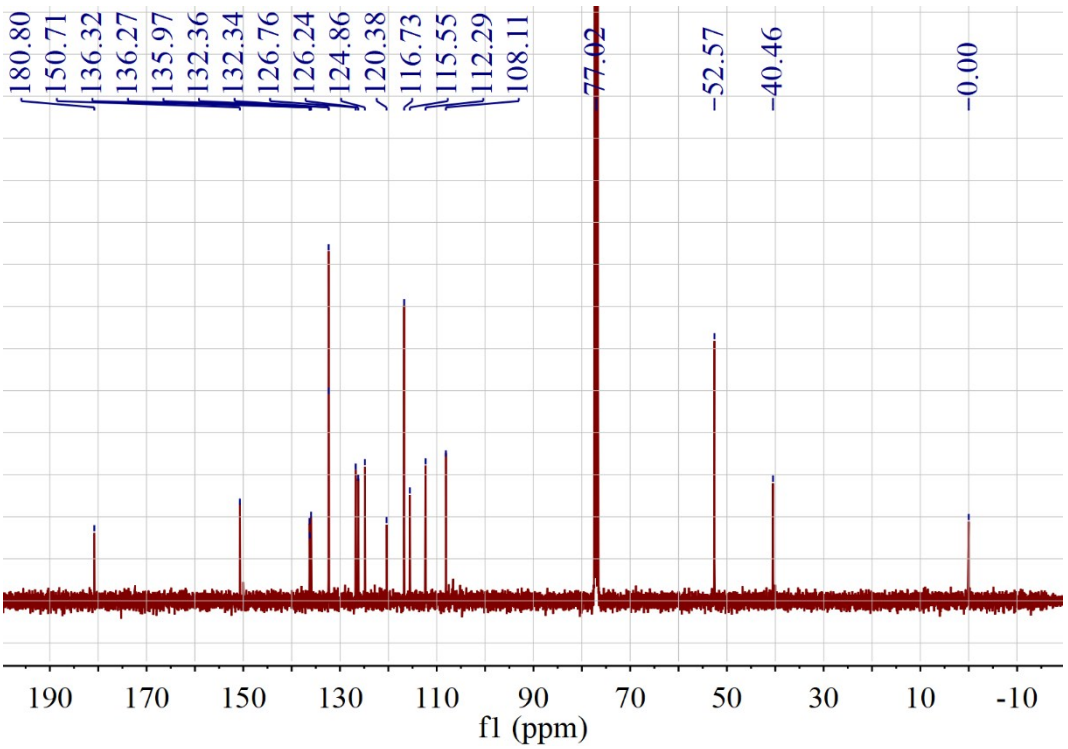


Figure S17. ^{13}C NMR spectrum of compound **6** ($CDCl_3$, 298K, 101 MHz).

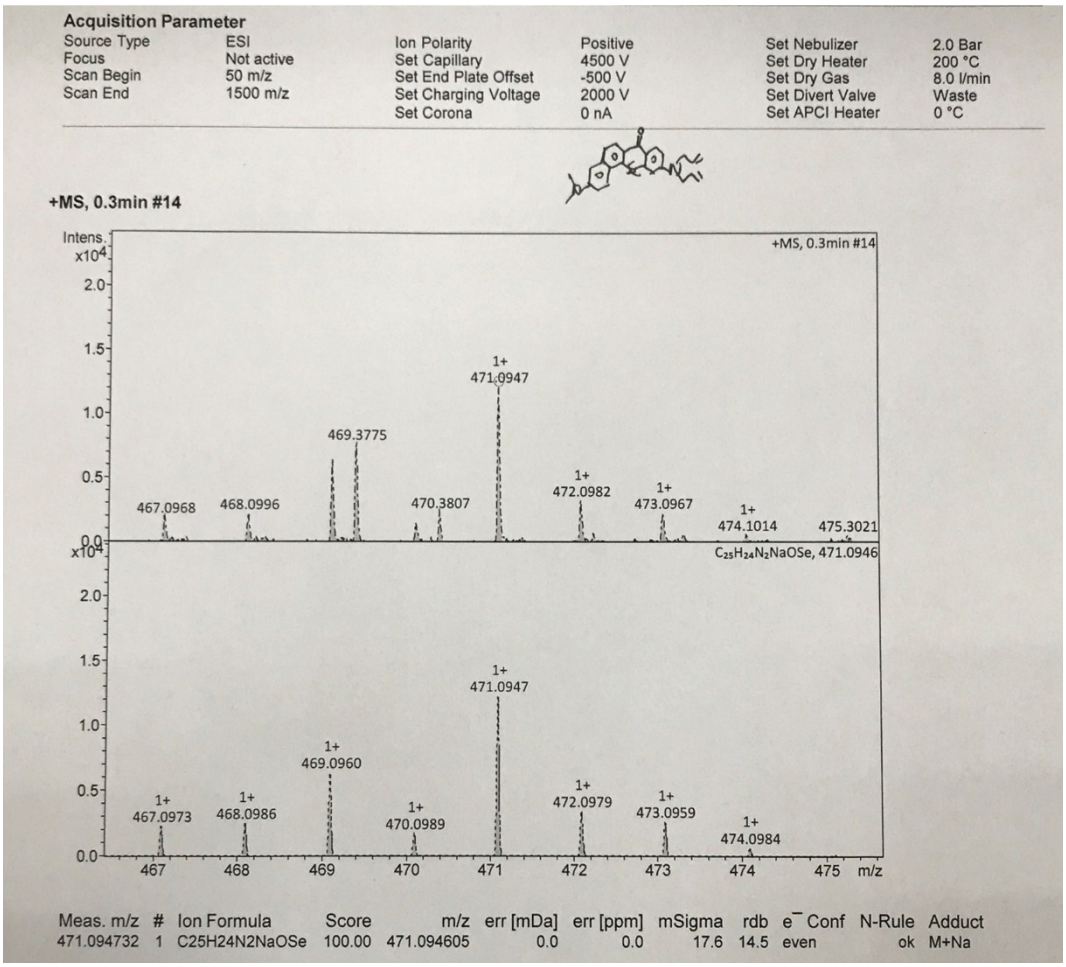


Figure S18. HRMS spectrum of compound **6**.

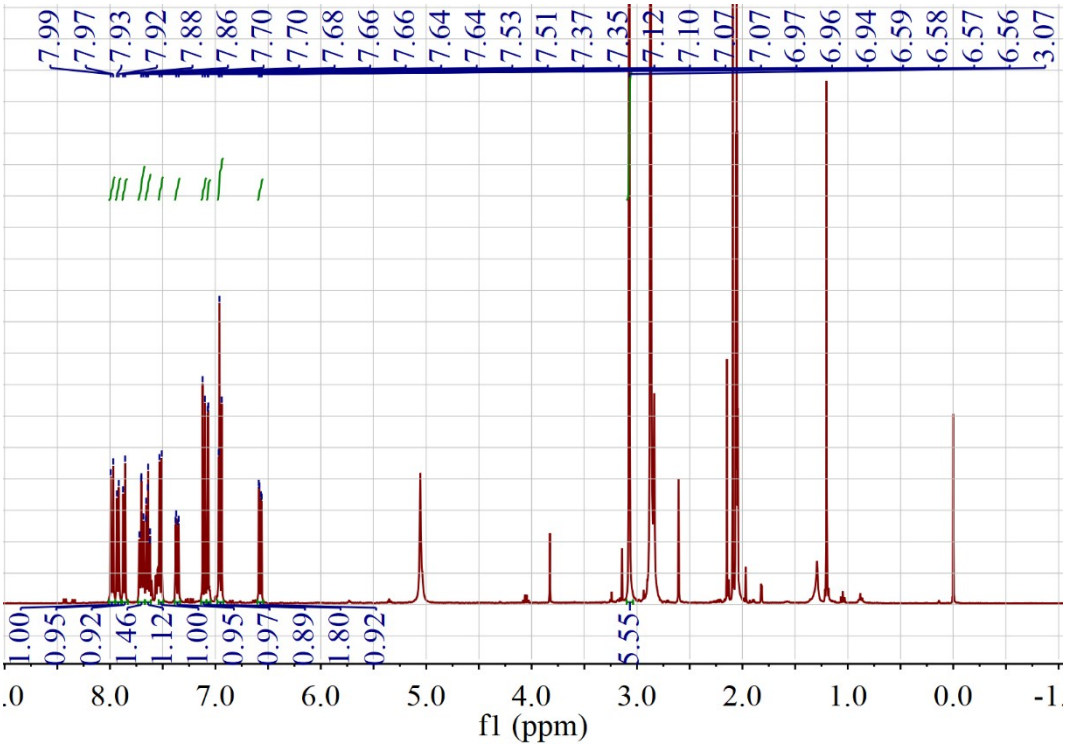


Figure S19. ^{1}H NMR spectrum of compound **Se-NR** (Acetone-*d6*, 298K, 400 MHz).

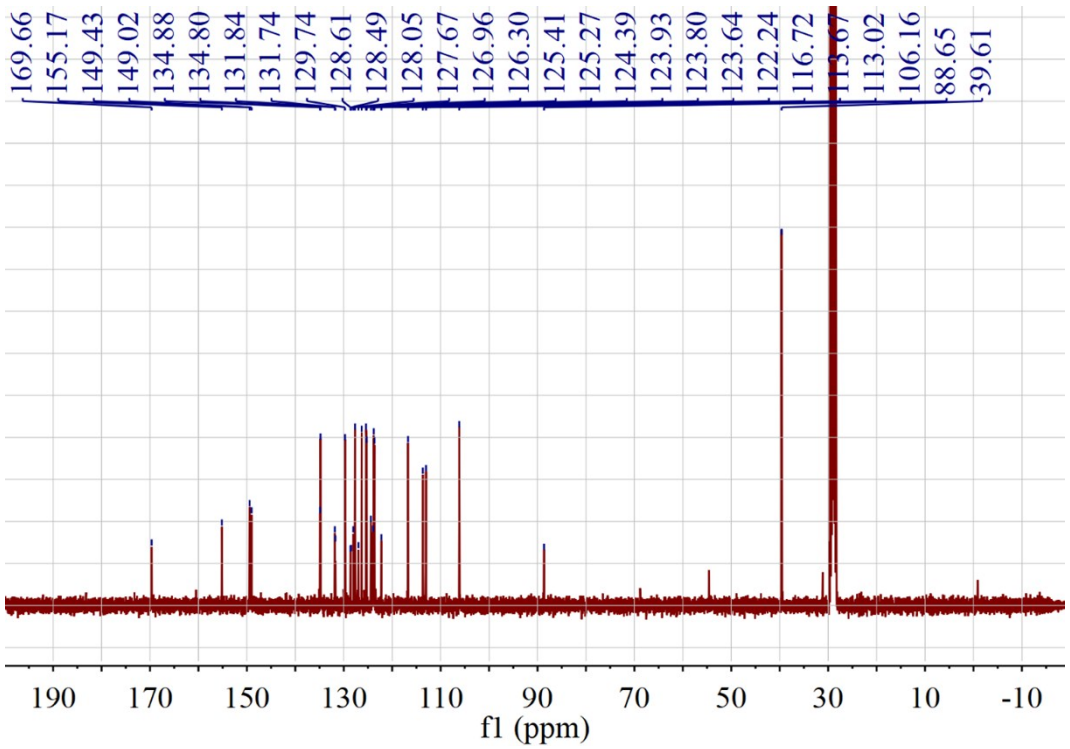


Figure S20. ^{13}C NMR spectrum of compound **Se-NR** (Acetone-*d6*, 298K, 101 MHz).

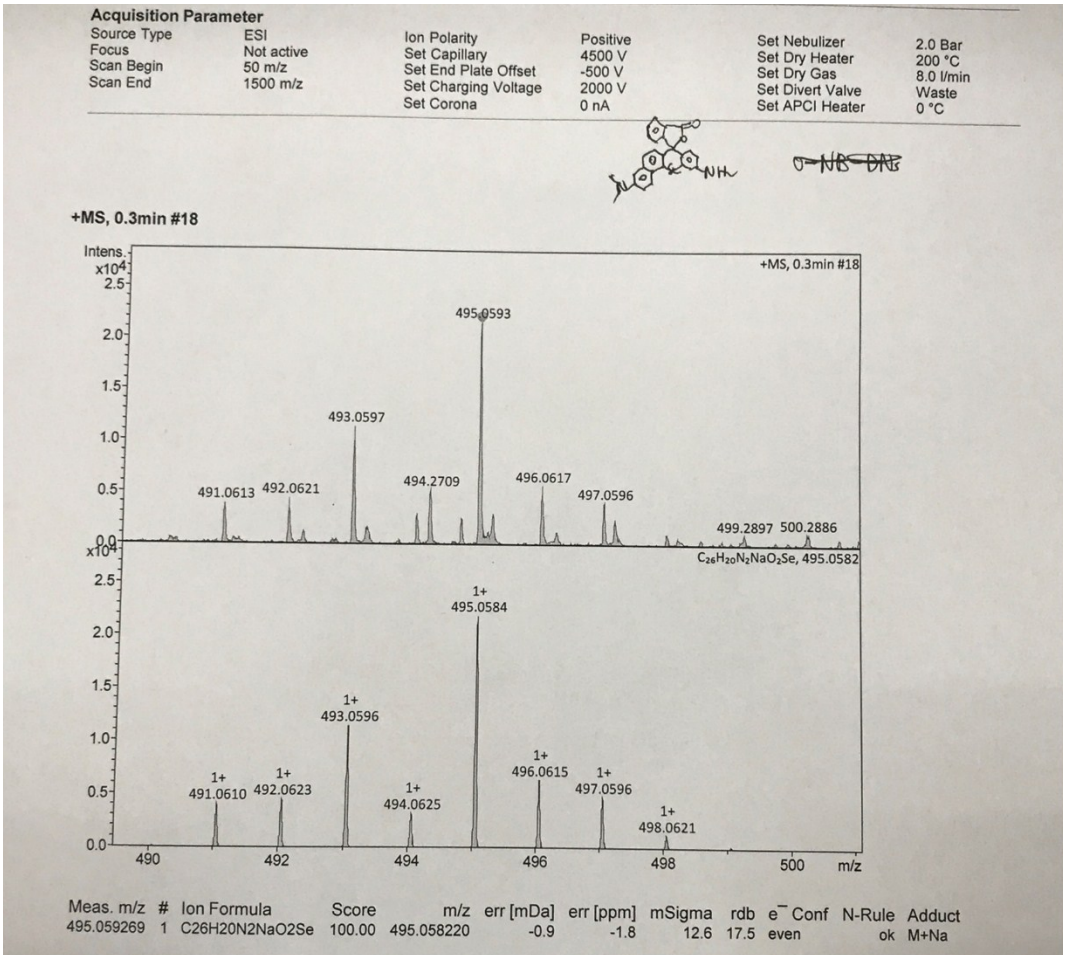


Figure S21. HRMS spectrum of compound **Se-NR**.

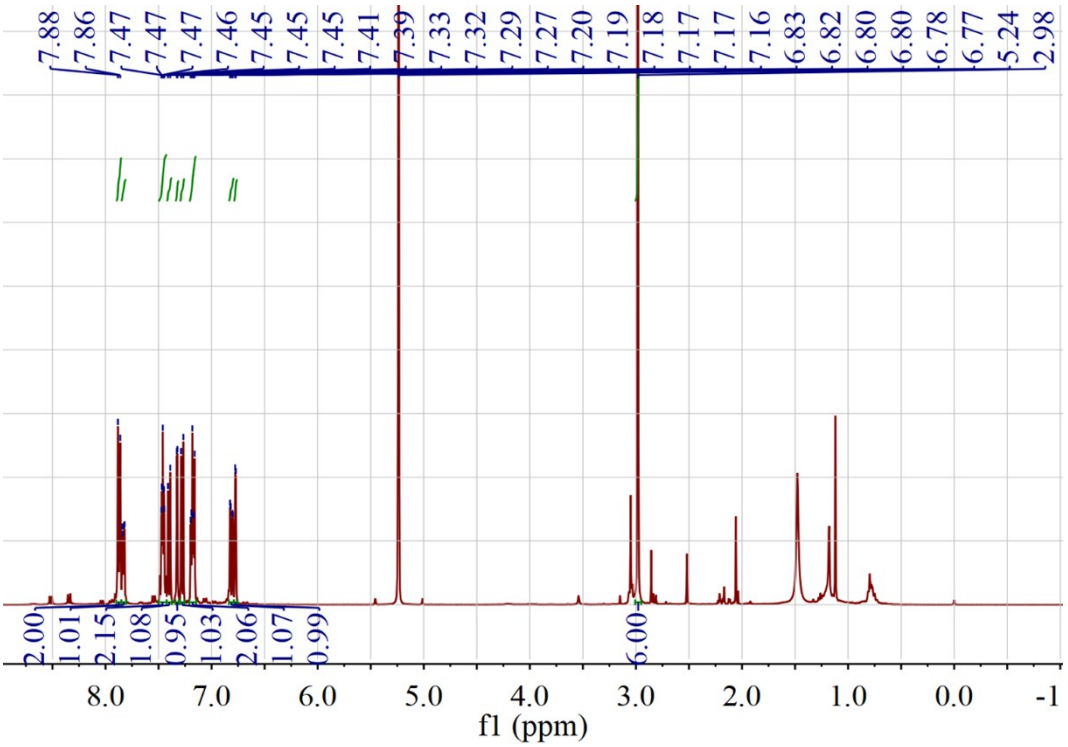


Figure S22. ^{1}H NMR spectrum of compound **Se-NR-Az** (CD_2Cl_2, 298K, 400 MHz).

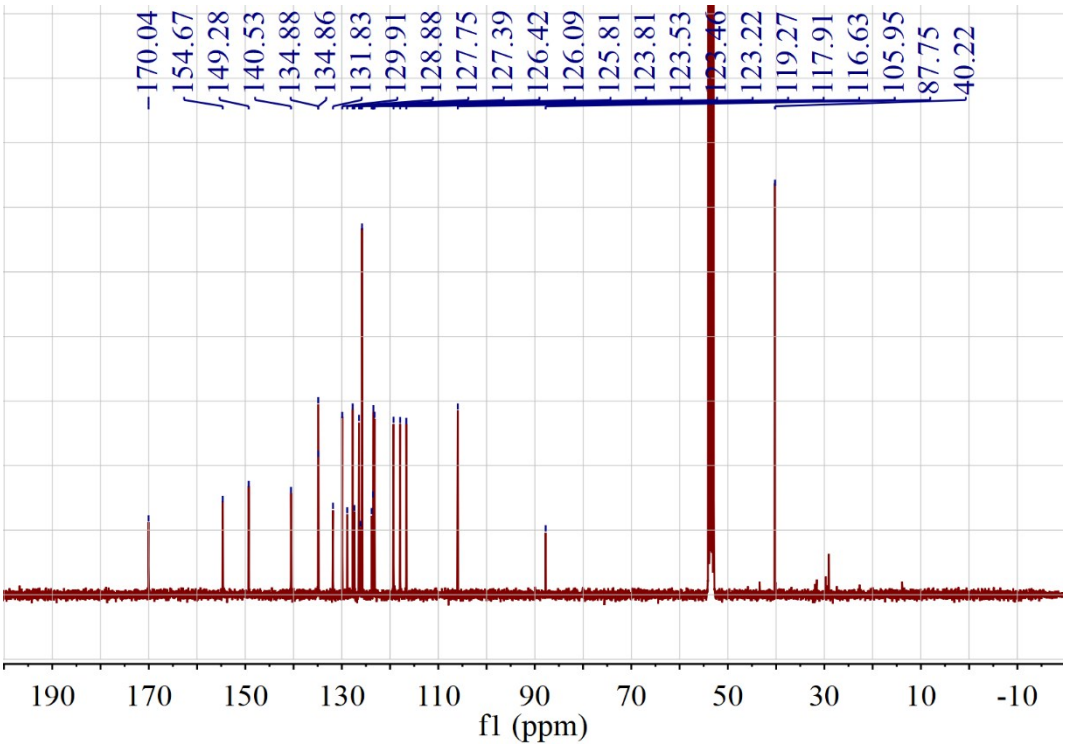


Figure S23. ^{13}C NMR spectrum of compound **Se-NR-Az** (CD_2Cl_2, 298K, 101 MHz).

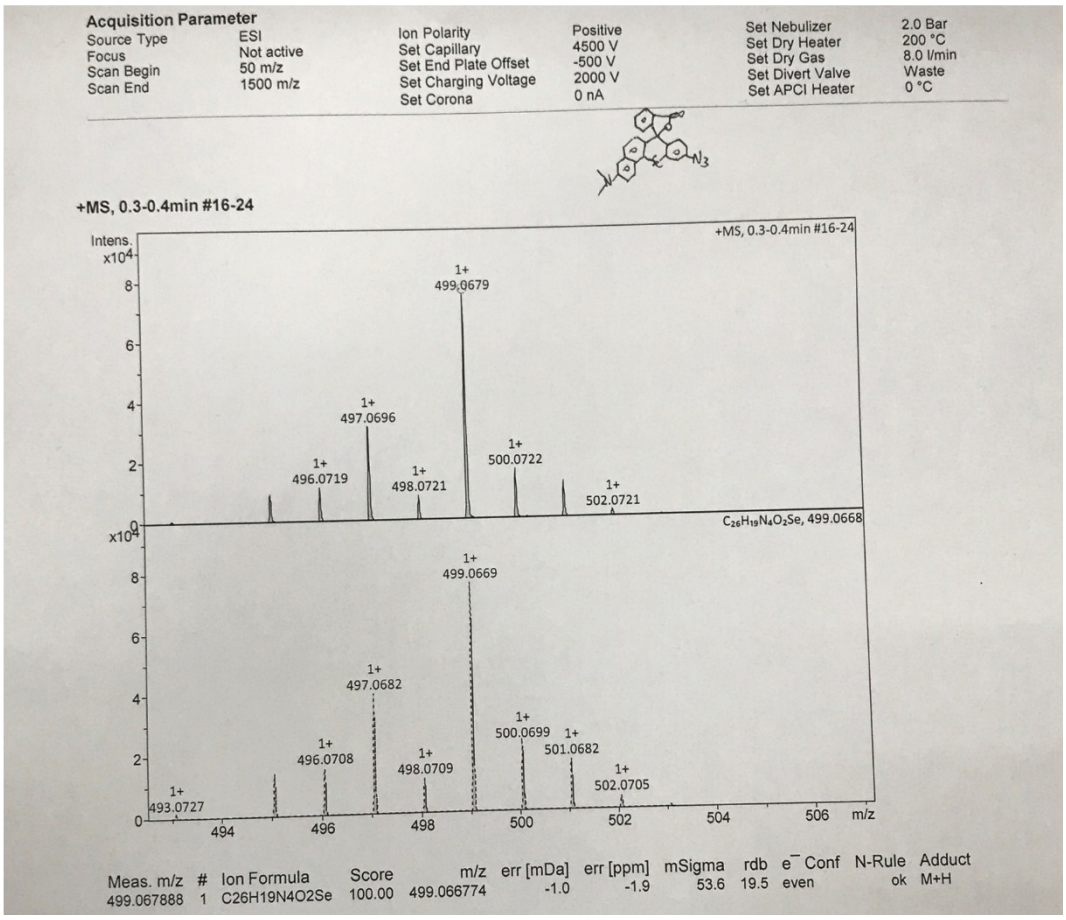


Figure S24. HRMS spectrum of compound **Se-NR-Az**.

7. Reference

S1 M. W. Kryman, T. M. McCormick and M. R. Detty, *Organometallics*, 2016, **35**, 1944-1955.

S2 M. Chiba, Y. Ichikawa, M. Kamiya, T. Komatsu, T. Ueno, K. Hanaoka, T. Nagano, N. Lange and Y. Urano, *Angew. Chem. Int. Ed.*, 2017, **56**, 10418-10422.

S3 K. Hirabayashi, K. Hanaoka, T. Takayanagi, Y. Toki, T. Egawa, M. Kamiya, T. Komatsu, T. Ueno, T. Terai and K. Yoshida, *Anal. Chem.*, 2015, **87**, 9061-9069.

S4 J. Jose and K. Burgess, *Tetrahedron*, 2006, **62**, 11021-11037.

S5 X. Zheng, U. W. Sallum, S. Verma, H. Athar, C. L. Evans and T. Hasan, *Angew. Chem. Int. Ed.*, 2009, **48**, 2148-2151.