Electronic Supplementary Information

A sensitive colorimetric and ratiometric fluorescent probe for mercury species in aqueous solution and living cells

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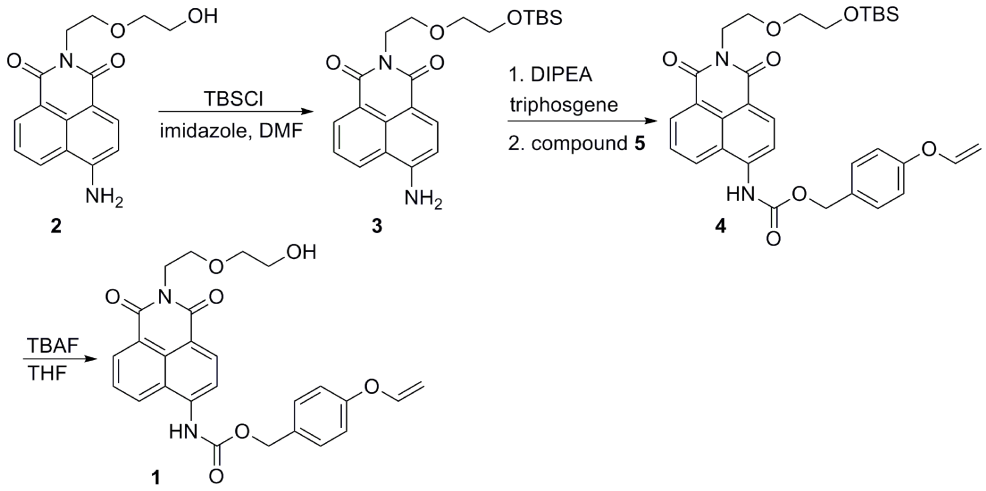
Contents

Materials and Methods: All reagents and solvents were obtained commercially and used without further purification unless otherwise noted. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX400 spectrometer and referenced to the solvent signals. Mass spectra (ESI) were performed on a LQC system (Finngan MAT, USA). All UV-visible spectra and fluorescence spectra were recorded using a Varian Cary 100 spectrophotometer and a Hitachi F-4500 luminescence spectrometer, respectively. Fluorescent quantum yields were determined to be 0.503 for probe **1** and 0.262 for compound **2** by an absolute method using an integrating sphere on FLS920 of Edinburgh Instrument.

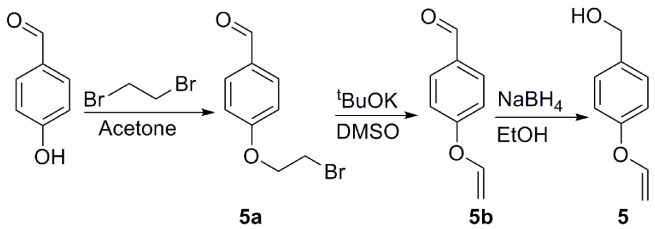
CAUTION: *Perchlorate salts with organic ligands can be potentially explosive and should be handled with care.*

Synthetic procedures:

Scheme S1 Synthetic procedures of probe **1**



Scheme S2 Synthetic procedures of compound **5**



Synthesis of compound **1** is summarized in Scheme S1. 4-Amino-N-(2-(2-hydroxyethoxy)ethyl)naphthalimide (compound **2**)[1] and [4-(vinyloxy)phenyl]methanol (compound **5**)[2] were synthesized according to the literature methods. Synthesis of other compounds is described as followed.

1. 1.1 Synthesis of probe 1 (1)

(4-(vinyloxy)phenyl)methanol (5) This compound was synthesized according to the literature procedure.[2] ESI-MS: m/z = 133.2 [M – H_2O + H^+].

^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.26 – 7.24 (m, 2H), 6.95 (dd, J = 2.0 Hz, 2H), 6.22 (dd, J = 13.6 Hz, J = 2.0 Hz, 1H), 4.75 (dd, J = 13.6 Hz, J = 1.6 Hz, 1H), 4.42 (dd, J = 6.0Hz, J = 1.6 Hz, 1H), 2.91 (s, 1H).

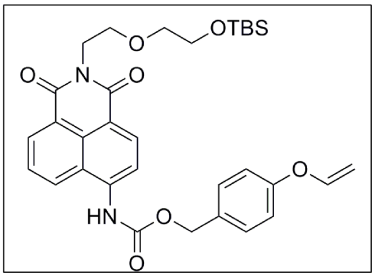
^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 156.1, 148.0, 135.6, 128.4, 116.9, 95.0, 64.6.

4-Amino-N-(2-(2-tert-Butyldimethylsilyloxy)ethyl)naphthalimide(3)

A solution of **2** (300 mg, 1 mmol) in anhydrous DMF (10 mL) was treated with imidazole (136 mg, 2 mmol) and chloro-tert-butyldimethylsilane (180 mg, 1.2 mmol) under N_2 atmosphere, heated to 50 °C, and stirred for 8 h. The reaction mixture was then cooled to room temperature, poured into brine (50 mL), and extracted with CH_2Cl_2 (200 mL). The combined organic layers were dried over $MgSO_4$, filtered, concentrated and chromatographed to provide compound **3** as orange powder. Yield: 372 mg, (90%). m.p.145.1 – 145.4 °C. ESI-MS: m/z = 415.3 [M + H^+].

^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 8.44 (d, J = 7.2 Hz, 1H), 8.16 (d, J = 8.0 Hz, 1H), 8.00 (d, J = 8.4 Hz, 1H), 7.49 (d, J = 8.0 Hz, 1H), 6.64 (d, J = 8.4 Hz, 1H), 5.28 (s, 2H), 4.41 (t, J = 6.0 Hz, 2H), 3.86 (t, J = 6.0 Hz, 2H), 3.79 (t, J = 5.2 Hz, 2H), 3.66 (t, J = 5.2 Hz, 2H), 0.84 (s, 9H), 0.02 (s, 6H).

^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 164.7, 164.0, 149.6, 133.7, 131.2, 129.5, 127.1, 124.5, 122.6, 120.0, 110.9, 109.1, 72.2, 68.2, 62.7, 38.9, 29.6, 25.9, 18.3.

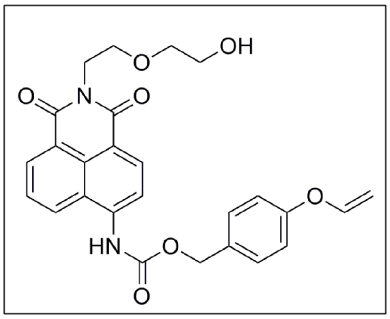


(Compound 4)

To a mixture of compound **3** (212 mg, 0.5 mmol), DIPEA (387 mg, 3 mmol) and DMAP(15 mg) in dry CH_2Cl_2 (10 mL) was added a solution of triphosgene (150 mg, 0.5 mmol) in CH_2Cl_2 dropwise in ice bath for 2 h under N_2. Then, the resulting solution was warmed to ambient temperature. After stirred for another 3 h, a solution of compound **5** (90 mg, 0.6 mmol) in CH_2Cl_2 was added into the mixture. At last, the reaction was quenched with water and extracted three times with CH_2Cl_2 after stirred overnight. The combined organic layers were dried, filtered, concentrated and chromatographed to provide compound **4** as white powder. Yield: 251 mg, (85%). m.p.109.3 – 111.2 °C. ESI-MS: m/z = 591.4 [M + H^+].

^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 8.44 ~ 8.47 (m, 2H), 8.27 (d, J = 8.4 Hz, 1H), 8.16 (d, J = 8.4 Hz, 1H), 7.75 (s, 1H), 7.62 (t, J = 8.0 Hz, 1H), 7.40 (d, J = 8.0 Hz, 2H), 6.99 (d, J = 8.4 Hz, 2H), 6.63 (dd, J = 13.6 Hz, J = 6.0 Hz, 1H), 5.23 (s, 2H), 4.80 (d, J = 13.6 Hz, 1H), 4.75 (d, J = 6.0 Hz, 1H), 4.38 (t, J = 6.0 Hz, 2 H), 3.81 (t, J = 6.0 Hz, 2H), 3.72 (t, J = 7.2 Hz, 2H), 3.59 (t, J = 7.2 Hz, 2H), 0.81 (s, 9H), 0.02 (s, 6H).

^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 164.0, 163.6, 157.1, 153.1, 147.6, 139.1, 132.4, 131.1, 130.4, 130.0, 128.7, 126.4, 126.2, 123.0, 117.5, 117.1, 116.7, 95.8, 72.3, 68.0, 67.4, 62.7, 39.2, 29.9, 18.3, -5.38.



(probe 1)

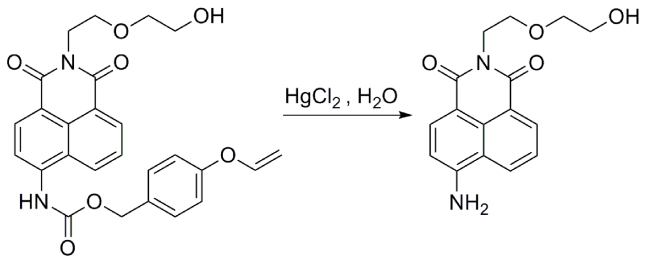
A mixture of compound **3** (295 mg, 0.5 mmol) and TBAF (131 mg, 0.5 mmol) in THF (10 mL) was stirred at room temperature for 30 min until it completed. The reaction mixture was concentrated in vacuo and the residue was dissolved in CH_2Cl_2, washed with brine, dried over

$MgSO_4$, filtered, and concentrated in vacuo and chromatographed to provide probe **1** as white powder. Yield: 226 mg, (95%). m.p.132.1 – 133.2 °C. ESI-MS: m/z = 477.2 [M + H^+].

^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 8.44 ~ 8.47 (m, 2H), 8.25 (d, J = 8.4 Hz, 1H), 8.11 (d, J = 8.4 Hz, 1H), 7.80 (s, 1H), 7.62 (t, J = 8.4 Hz, 1H), 7.43 (d, J = 8.4 Hz, 2H), 7.03 (d, J = 8.4 Hz, 2H), 6.65 (dd, J = 13.6 Hz, J = 6.0 Hz, 1H), 5.25 (s, 2H), 4.82 (dd, J = 13.6 Hz, J = 1.6 Hz, 1H), 4.49 (dd, J = 6.0 Hz, J = 1.6 Hz, 1H), 4.39 (t, J = 5.2 Hz, 2 H), 3.86 (t, J = 5.2 Hz, 2H), 3.69 (t, J = 7.2 Hz, 4H), 2.72 (s, 1H).

^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 164.4, 163.9, 157.1, 153.0, 147.6, 139.2, 132.6, 131.3, 130.5, 130.0, 128.7, 126.4, 126.2, 122.9, 117.3, 117.1, 116.7, 95.9, 72.2, 68.4, 67.5, 61.8, 39.4.

1.2 Formation of compound 2 from probe 1



probe **1** **2**

A solution of compound **1** (95.2 mg, 0.2 mmol) treated with $HgCl_2$ (27.2 mg, 0.1 mmol) in 20 mL DMSO-H_2O (1:9, v/v) was stirred at room temperature. The course of the reaction was monitored by thin layer chromatography TLC assay until its completion. The mixture solution was extracted with EtOAc, dried over Mg_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel flash chromatography to afford compound **2** as an orange solid. Yield: 54.0 mg, (90%). m.p.204.9 – 206.2 °C. ESI-MS: m/z = 301.2 [M + H^+].

^{1}H NMR (d_6-DMSO, 400 MHz, ppm): δ 8.61 (d, J = 8.4 Hz, 1H), 8.41 (d, J = 7.2 Hz, 1H), 8.18 (d, J = 8.4 Hz, 1H), 7.64 (t, J = 8.0 Hz, 1H), 7.46 (s, 2H), 6.85 (d, J = 8.4 Hz, 1H), 4.59 (s, 1H), 4.21 (t, J = 6.4 Hz, 2 H), 3.63 (t, J = 6.4 Hz, 2H), 3.48 (s, 4H).

^{13}C NMR (d_6-DMSO, 100 MHz, ppm): δ 163.8, 162.9, 152.7, 134.0, 131.0, 129.7, 129.3, 123.9, 121.7, 119.3, 108.2, 107.4, 72.1, 67.1, 60.2, 38.3.

2. Absorption and fluorescence spectra

Preparation of stock solutions.

Solution	Reagent	Quantity	Solvent(10mL)	Conc.
1	probe **1**	4.76 mg (0.010 mmol)	DMSO	1.0 mM
2	$PdCl_2$	1.8 mg (10 μmol)	1:3 brine/MeOH	1.0 mM
3	$PtCl_2$	2.7 mg (10 μmol)	DMSO	1.0 mM
4	$AuCl_3$	3.0 mg (10 μmol)	DMSO	1.0 mM

Notes:

The solution of chloromethyl mercury (1.0 mM) was prepared in methanol. All the other cation solutions were prepared from $LiClO_4$, NaCl, KCl, $CaCl_2$, $MgCl_2$, $CrCl_3$, $MnCl_2$, $FeCl_3$, $FeCl_2$, $CoCl_2$, $NiCl_2$, $CuCl_2$, $ZnCl_2$, $CdCl_2$, $HgCl_2$, $Hg(OAc)_2$, $Hg(ClO_4)_2$, $Hg(NO_3)_2$ and $AgNO_3$ in distilled water, with a concentration of 1 mM, respectively.

All the anion solutions were prepared from NaF, NaCl, KCNS, $NaNO_3$, $NaClO_4$, NaAcO, Na_2SO_4, and Na_2CO_3 in distilled water, with a concentration of 10 mM, respectively.

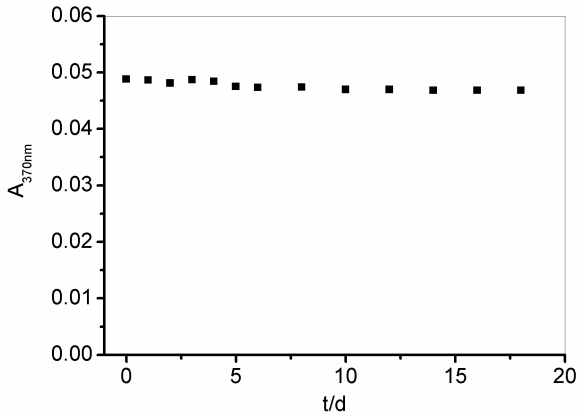


Figure S1. UV-vis absorption at 370 nm of probe **1** in PBS buffer solutions (pH = 7.4, containing 0.5% DMSO). At t = 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16 and 18 d, 10 μL (10 nmol) aliquots were taken from solution **1** and diluted to 2 mL with PBS buffer solution for UV-vis absorption spectra measurement.

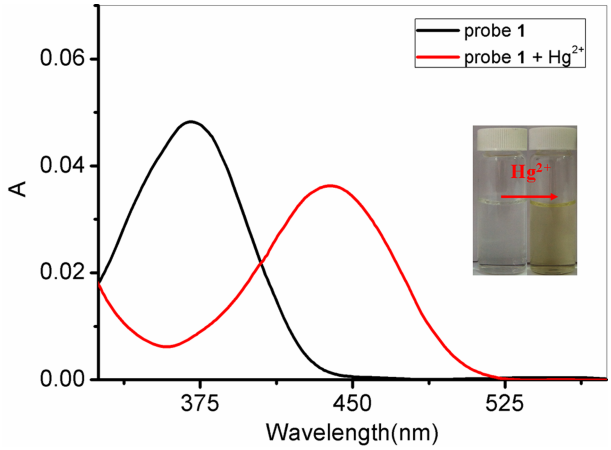


Figure S2. UV-vis absorption spectra of probe **1** in the absence and presence of Hg^{2+} in PBS buffer solutions (pH = 7.4, containing 0.5% DMSO). [Hg^{2+}] = 5 μM, [probe **1**] = 5 μM. Inset: Color changes in probe **1** upon addition of Hg^{2+}.

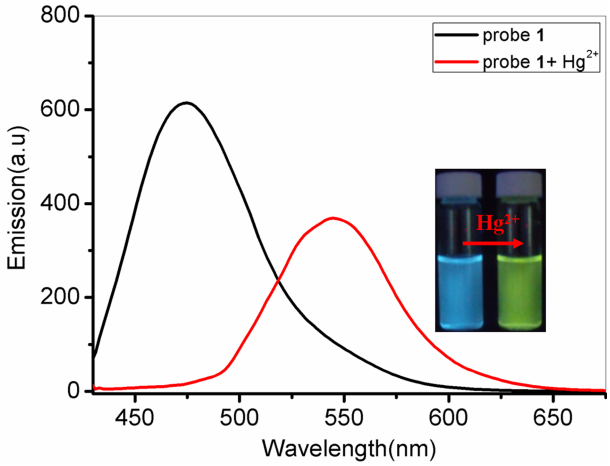


Figure S3. Fluorescence spectra of probe **1** in the absence and presence of Hg^{2+} in PBS buffer solutions (pH = 7.4, containing 0.5% DMSO). Ex = 408 nm. Slit: 5.0 nm/5.0 nm. [Hg^{2+}] = 5 μM, [probe **1**] = 5 μM. Inset: Fluorescence changes excited by UV lamp (365 nm) in probe **1** upon addition of Hg^{2+}.

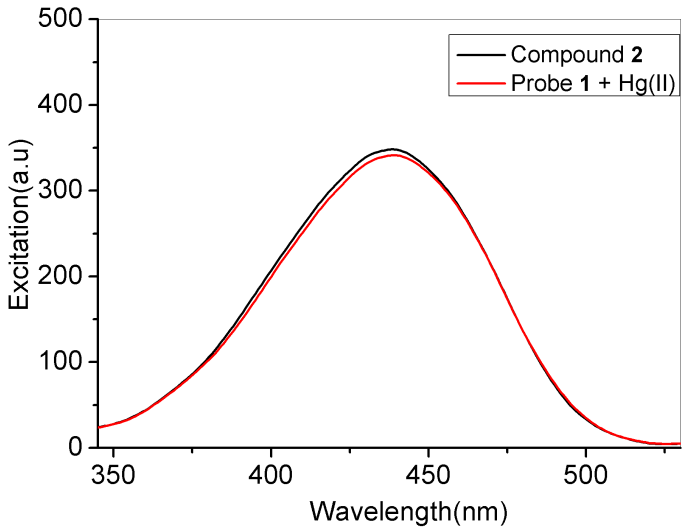


Figure S4. Excitation spectra for probe **1** (5 μM) in the presence of Hg^{2+} (5 μM) (red line) and compound **2** (black line, 5 μM) in PBS buffer solutions (pH = 7.4, containing 0.5% DMSO). Em = 541 nm. Slit: 5.0 nm/5.0 nm.

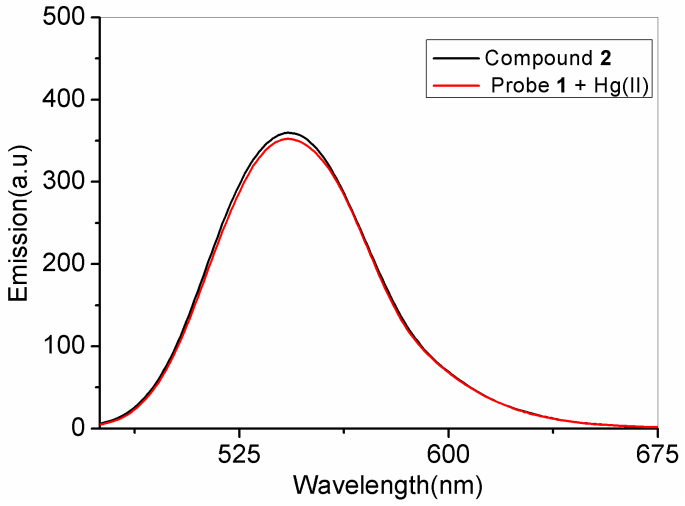


Figure S5. Emission spectra for probe **1** (5 μM) in the presence of Hg^{2+} (5 μM) (red line) and compound **2** (black line, 5 μM) in PBS buffer solutions (pH = 7.4, containing 0.5% DMSO). Ex = 408 nm. Slit: 5.0 nm/5.0 nm.

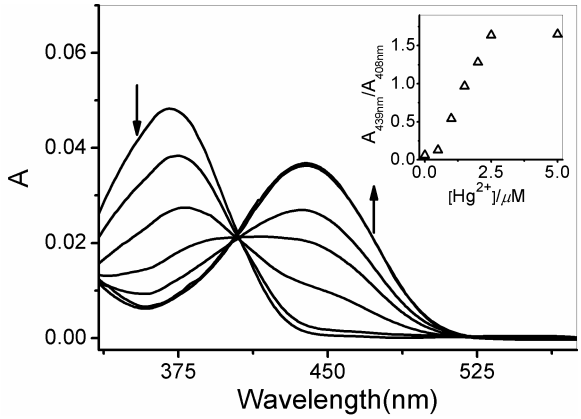


Figure S6. UV-vis absorption spectra of probe **1** in PBS buffer solutions (pH = 7.4, containing 0.5% DMSO) upon the titration of Hg^{2+}. Hg^{2+} = 0 – 5 μM, [probe **1**] = 5 μM. Inset: Ratiometric calibration curve $A_{439\ nm}$ / $A_{408\ nm}$ as a function of Hg^{2+}.

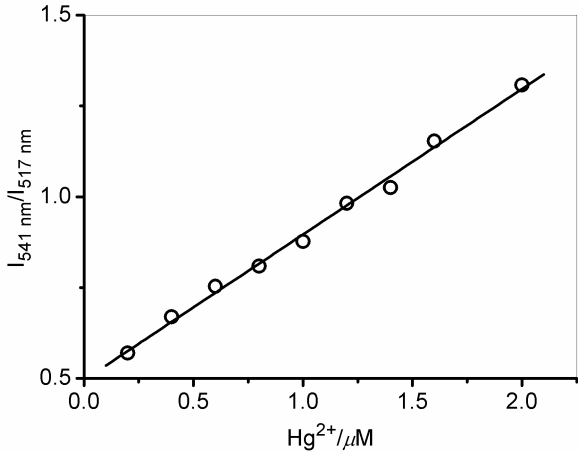


Figure S7. Proof-of-concept experiment with probe **1** for Hg^{2+} detection in drinking water (containing 0.5 % DMSO) at μM levels. The water sample was collected, filtered and pretreated with different amount of $HgCl_2$ (0 – 2 μM). And then probe **1** (10 μL in DMSO, $[\text{probe } \mathbf{1}]_{final}$ = 5 μM) was added into the mixture. Ex = 408 nm. Slit: 5.0 nm/5.0 nm.

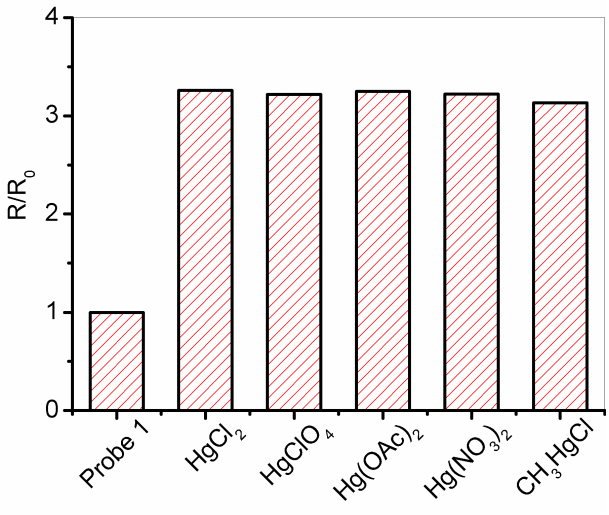


Figure S8. Fluorescence responses of probe **1** (5 μM) to mercury metal sources: $HgCl_2$ (5 μM), $Hg(ClO_4)_2$ (5 μM), $Hg(OAc)_2$ (5 μM), $Hg(NO_3)_2$ (5 μM) and CH_3HgCl (5 μM) in PBS buffer solutions (pH = 7.4, containing 0.5% DMSO), measured after 200 min at room temperature. Bars represent the ratio of the fluorescence intensity ratio in the presence (R) and absence (R_0) of mercury metal sources. $R = I_{541\ nm}/I_{517\ nm}$, λ_{ex} = 408 nm. Slit: 5.0 nm/5.0 nm.

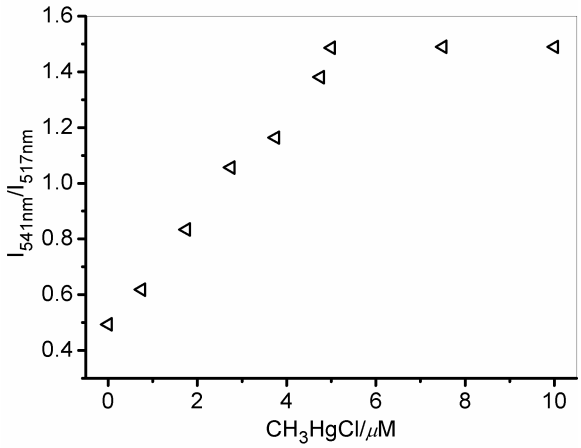


Figure S9. Ratiometric calibration curve $I_{541\ nm}/I_{517\ nm}$ as a function of CH_3HgCl in PBS buffer solutions (pH = 7.4, containing 0.5% DMSO) at 50℃, measured after 20 min. [CH_3HgCl] = 0 – 10 μM, [probe **1**] = 5 μM.

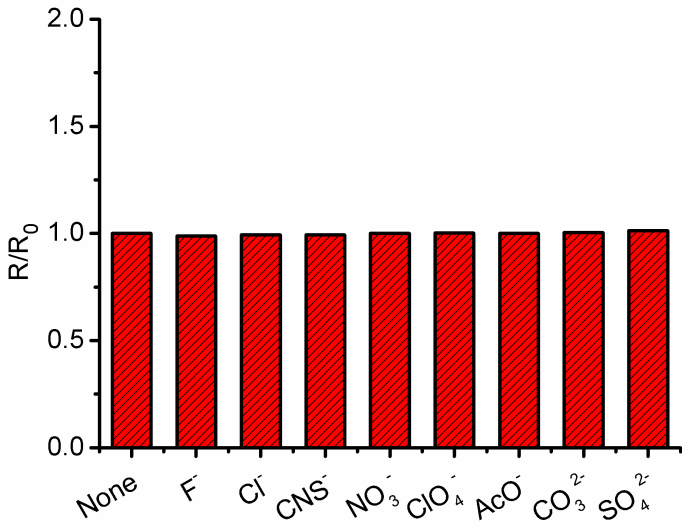


Figure S10. Fluorescence spectra of probe **1** (5 μM) in the absence and presence of different anions (10 equiv): F^-, Cl^-, CNS^-, NO_3^-, ClO_4^-, AcO^-, CO_3^{2-} and SO_4^{2-} (as their Na^+ or K^+ salts) in PBS buffer solutions (pH = 7.4, containing 0.5% DMSO). Bars represent the ratio of the fluorescence intensity ratio in the presence (R) and absence (R_0) of various anions. $R = I_{541\ nm}/I_{517\ nm}$, λ_{ex} = 408 nm. Slit: 5.0 nm/5.0 nm.

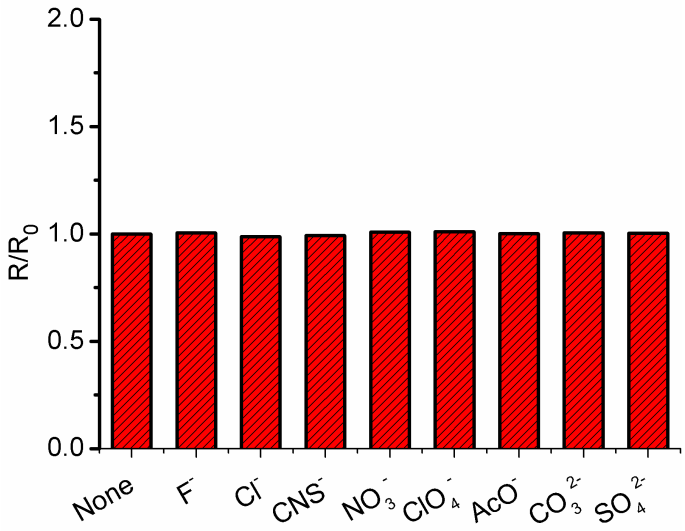


Figure S11. Fluorescence responses of probe **1**-Hg^{2+} system (5 μM) in the presence of 10 equiv various anions: F^-, Cl^-, CNS^-, SO_4^{2-}, CO_3^{2-}, NO_3^-, ClO_4^- and AcO^- (as their Na^+ or K^+ salts) in PBS buffer solutions (pH = 7.4, containing 0.5% DMSO). Bars represent the ratio of the fluorescence intensity ratio in the presence (R) and absence (R_0) of various anions. $R = I_{541\ nm}/I_{517\ nm}$, λ_{ex} = 408 nm. Slit: 5.0 nm/5.0 nm.

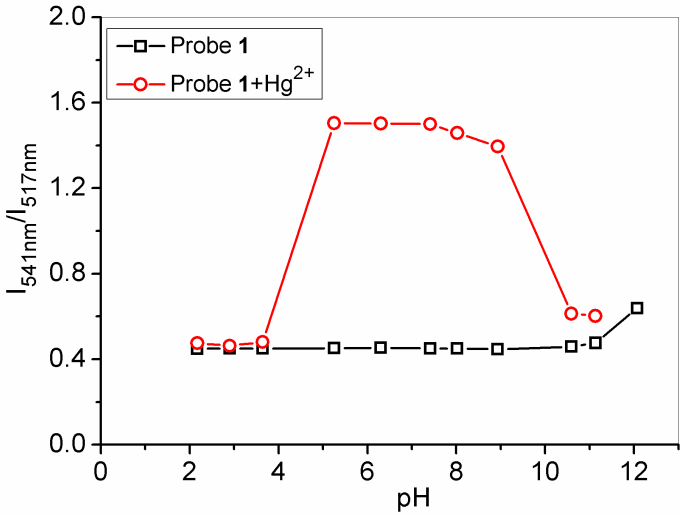


Figure S12. The fluorescence intensity ratio of probe **1** (5 μM) as a function of pH in PBS buffer solutions (pH = 7.4, containing 0.5% DMSO) (□). The fluorescence intensity ratio of probe **1** (5 μM) in the presence of Hg^{2+} (2.5 μM) as a function of pH in PBS buffer solutions (pH = 7.4, containing 0.5% DMSO) (○). λ_{ex} = 408 nm. Slit: 5.0 nm/5.0 nm.

3. Determination of the detection limit [3]

The detection limit was calculated based on the fluorescence titration. The fluorescence emission spectrum of probe **1** was measured by ten times and the standard deviation of blank measurement was achieved. To gain the slope, the ratio of the fluorescence intensity at 541 nm to the fluorescence intensity at 517 nm ($I_{541\ nm}/I_{517\ nm}$) was plotted as a concentration of Hg^{2+}. So the detection limit was calculated with the following equation:

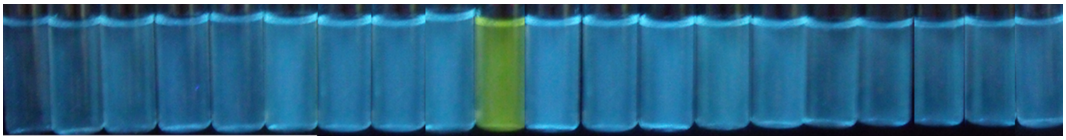
Detection limit = $3\sigma/k$

Where σ is the standard deviation of blank measurement, k is the slope between the fluorescence intensity ratios versus Hg^{2+} concentration.

The detection limits for Hg^{2+} were deduced to be 4.9 *n*M (1.0 ppb).

4. Fluorescence and color changes by naked eyes

a)



b)

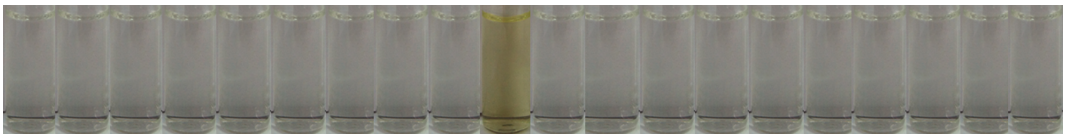


Figure S13. Fluorescence changes **a)** excited by UV lamp (Ex = 365 nm) and color changes **b)** of probe **1** upon addition of various metal cations in PBS buffer solutions (pH = 7.4, containing 0.5 % DMSO). From left to right: Li^{+}, Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Cr^{3+}, Mn^{2+}, Fe^{3+}, probe **1** only, Hg^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Fe^{2+}, Ag^{+}, Au^{3+}, Pd^{2+} and Pt^{2+}. [probe **1**] = 5 μM, [M^{n+}] = 5 μM.

5. Cell incubation and imaging

The Hep G2 cells were grown in H-DMEM (Dulbecco's Modified Eagle's Medium, High Glucose) supplemented with 10% FBS (Fetal Bovine Serum) in an atmosphere of 5% CO_2, 95% air at 37 °C. Cells (5×10^8/L) were plated on 18 mm glass coverslips and allowed to adhere for 24 hours. Then the cells were incubated with 10 μM probe **1** for 2 h at 37 °C under 5% CO_2, washed with PBS three times before incubating with 10 μM CH_3HgCl for another 2 h and rinsed with PBS three times again. Fluorescence imaging of intracellular CH_3HgCl was then carried out on a Zeiss Leica inverted epifluorescence /reflectance laser scanning confocal microscope. The Hep G2 cell only incubated with 10 μM probe **1** for 1 h at 37 °C under 5% CO_2, was as a control.

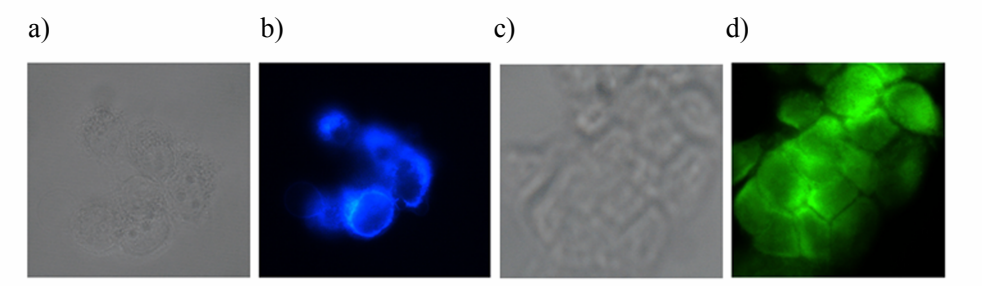


Figure S14. Fluorescence image of Hep G2 cells incubated with 10 μM probe **1** for 2 h (b) and then further incubated with 10 μM CH_3HgCl for 2 h (d). (a), (c) Bright-field transmission image of Hep G2 cells in (b) and (d) (Zeiss Leica DM 4000B microscope, 40 × objective lens).

6. NMR Data

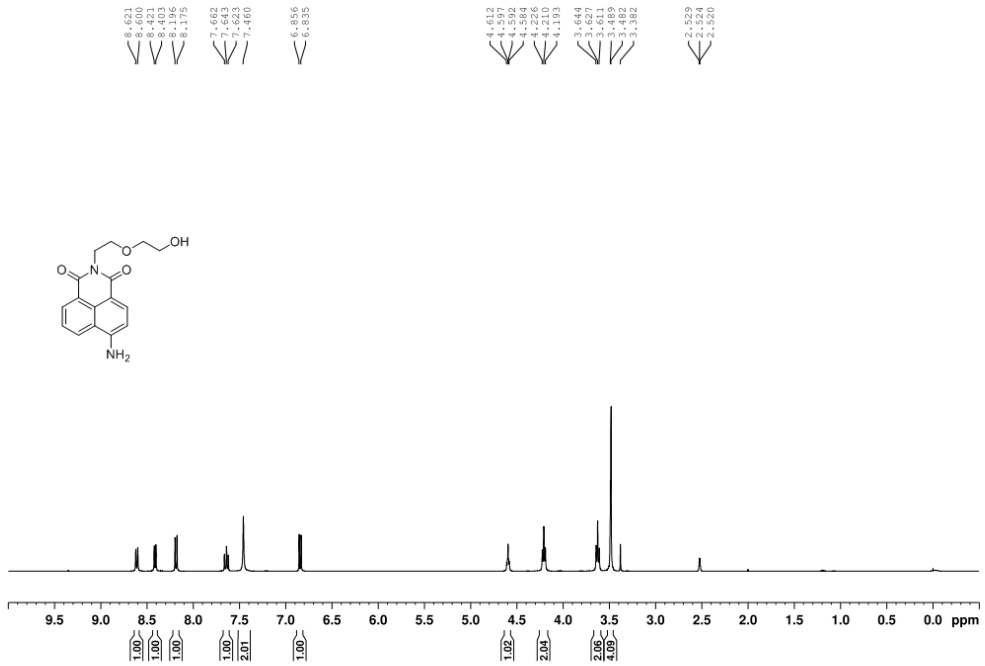


Figure S15. ^{1}H NMR spectrum of compound **2** (d_6-DMSO)

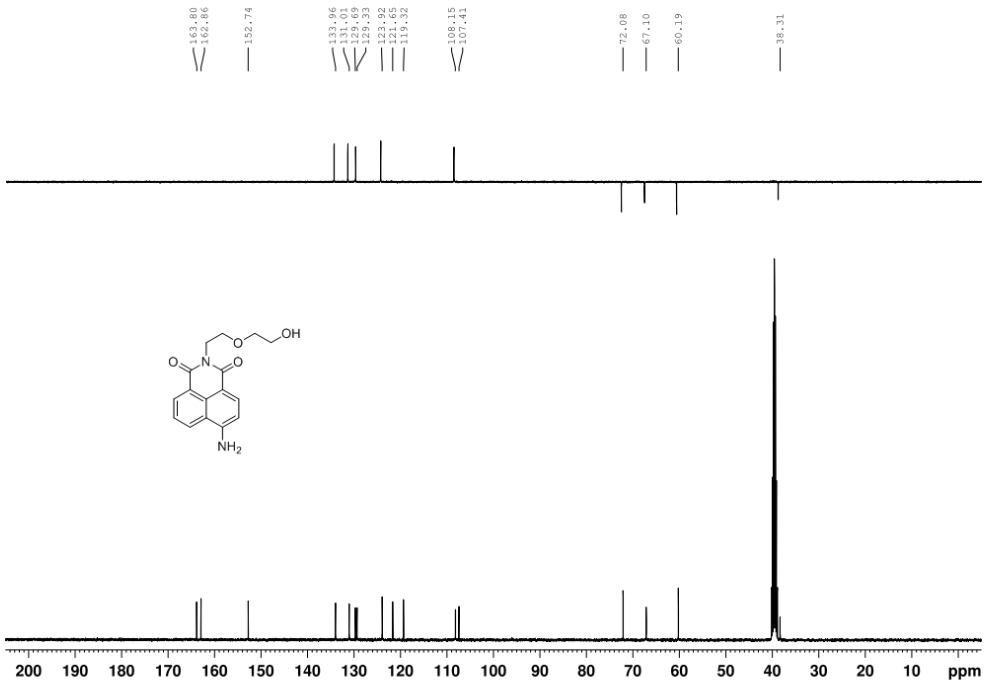


Figure S16. ^{13}C NMR spectrum of compound **2** (d_6-DMSO)

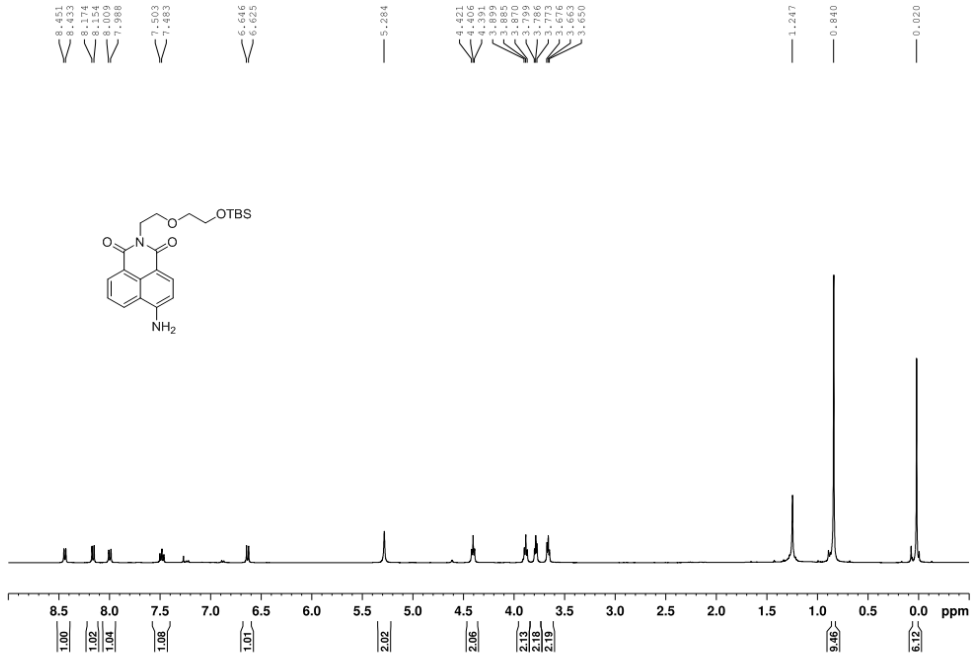


Figure S17. ^{1}H NMR spectrum of compound **3** ($CDCl_3$)

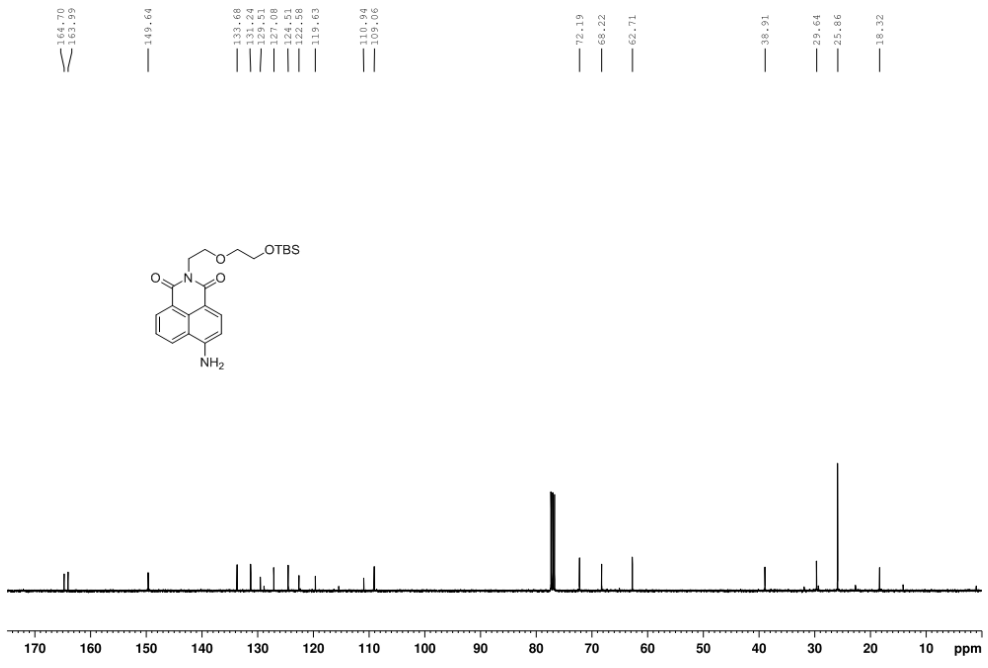


Figure S18. ^{13}C NMR spectrum of compound **3** ($CDCl_3$)

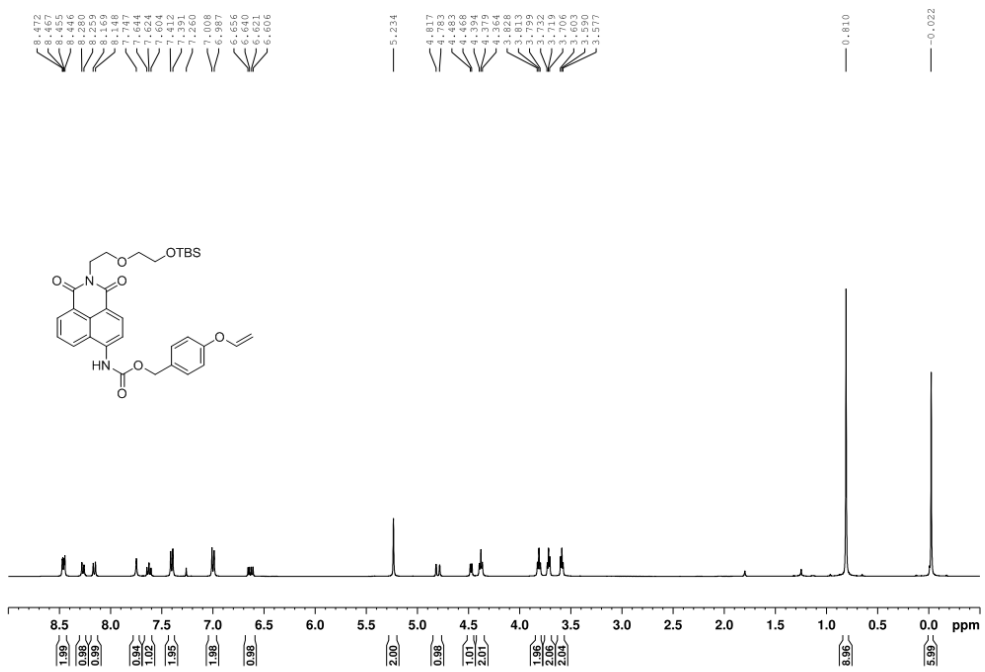


Figure S19. ^{1}H NMR spectrum of compound **4** ($CDCl_3$)

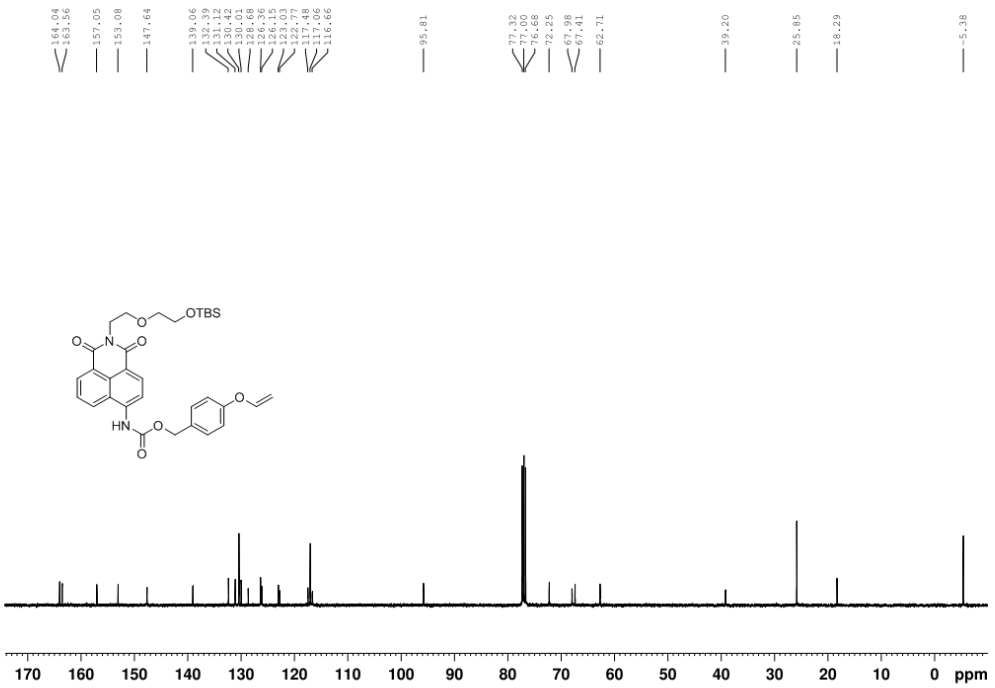


Figure S20. ^{13}C NMR spectrum of compound **4** ($CDCl_3$)

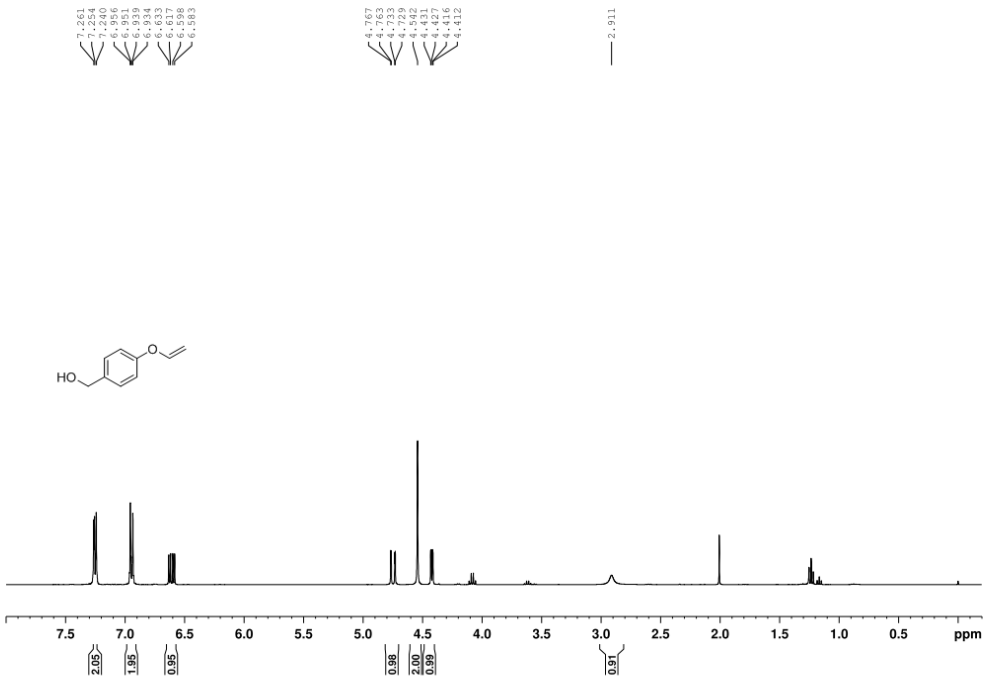


Figure S21. ^{1}H NMR spectrum of compound **5** ($CDCl_3$)

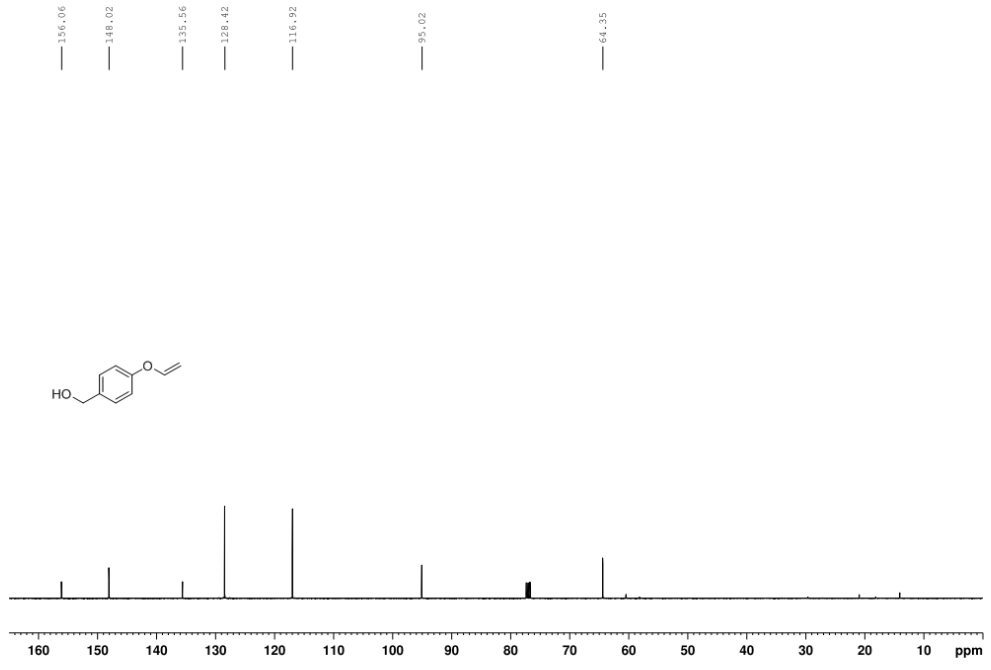


Figure S22. ^{13}C NMR spectrum of compound **5** ($CDCl_3$)

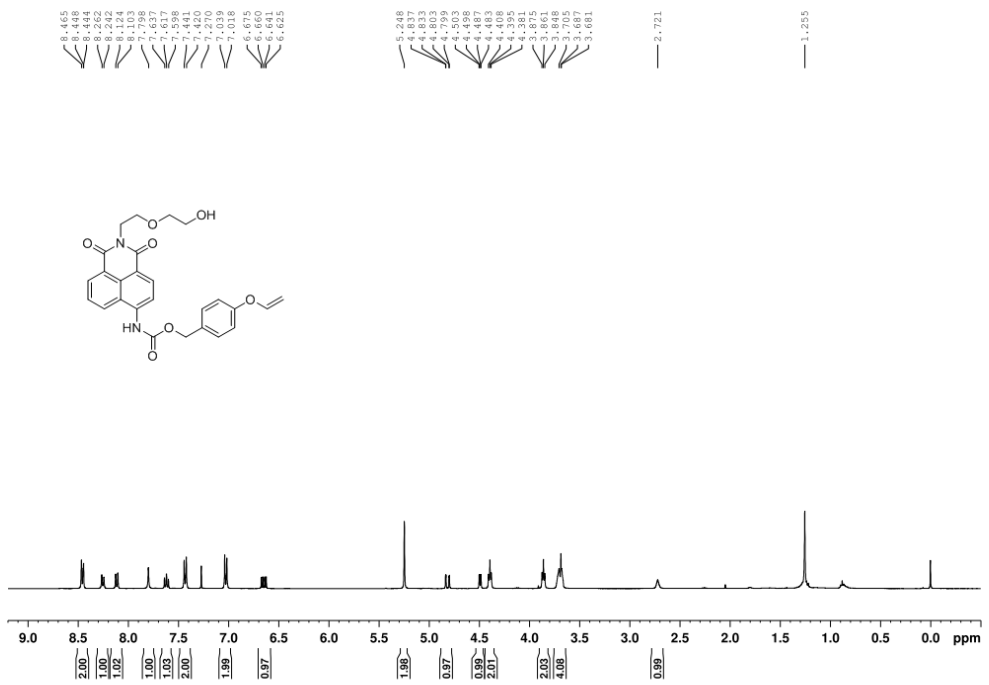


Figure S23. ^{1}H NMR spectrum of probe **1** ($CDCl_3$)

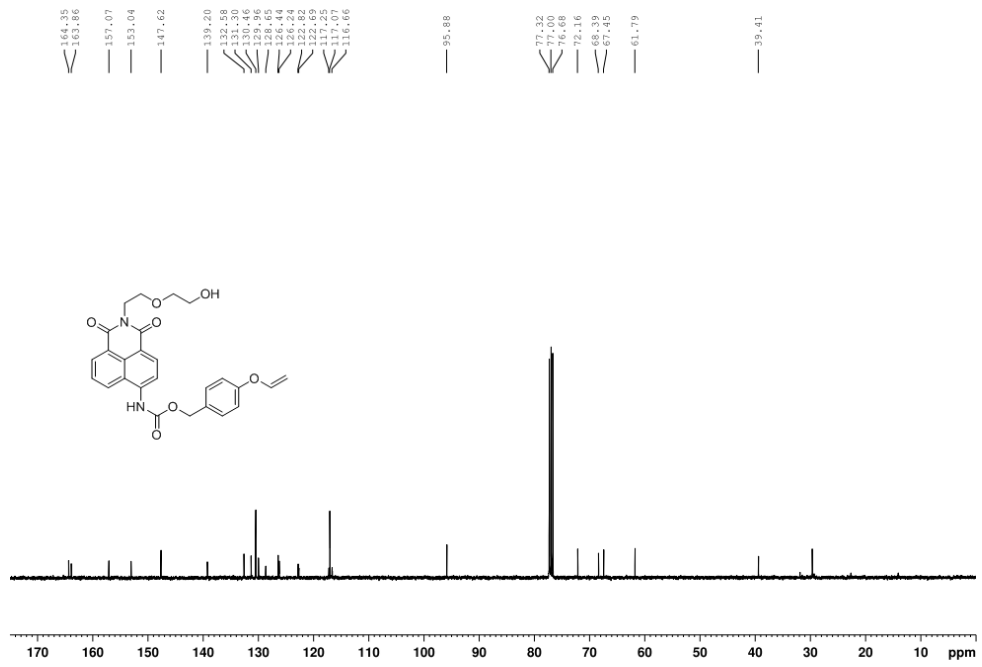


Figure S24. ^{13}C NMR spectrum of probe **1** ($CDCl_3$)

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