Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Supporting Information for

A versatile fluorescent dye based on naphthalimide: highly selective detection of Hg^{2+} in aqueous solution and living cells and its aggregation-induced emission behaviour

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Contents:

1. General information

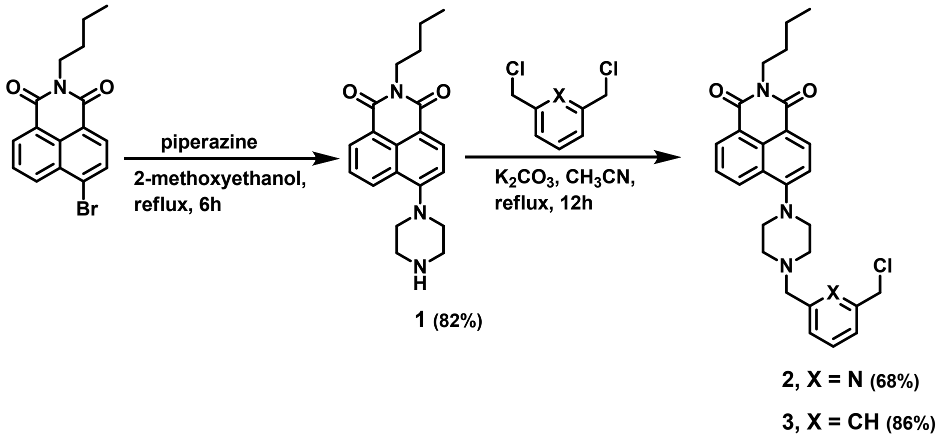
Unless otherwise mentioned, all the reagents were of analytic grade. ^1H NMR and ^{13}C NMR spectra were measured on a Bruker AM-400 spectrometer with chemical shifts reported as ppm (in $CDCl_3$). Mass spectrometry data were obtained with a HP 5989A spectrometer. Absorption spectra were determined on a Varian Cary 100 Spectrophotometer. Fluorescence spectra were determined on a Varian Cary Eclipse.

The metal salts used were $Fe(ClO_4)_2$, $Zn(ClO_4)_2\cdot 6H_2O$, $Co(ClO_4)_2\cdot 6H_2O$, $Ni(ClO_4)_2\cdot 6H_2O$, $Ba(ClO_4)_2\cdot 3H_2O$, $Pb(ClO_4)_2\cdot 3H_2O$, $Cd(ClO_4)_2\cdot 6H_2O$, $Cu(ClO_4)_2\cdot 6H_2O$, $Mn(ClO_4)_2\cdot 6H_2O$, $LiClO_4\cdot 3H_2O$, $NaClO_4\cdot H_2O$, $AgClO_4\cdot H_2O$, $Hg(ClO_4)_2\cdot 3H_2O$, $Mg(ClO_4)_2\cdot 6H_2O$, $Al(ClO_4)_3\cdot 9H_2O$.

Hela cells were obtained from Institute of Basic Medical Sciences (IBMS) of Chinese Academy of Medical Sciences (CAMS), and grown in DMEM (High glucose) medium supplemented with 10% FBS. Cells were incubated in a 5% CO_2 humidified incubator at 37 °C and typically passaged with sub-cultivation ratio of 1:3 every two days.

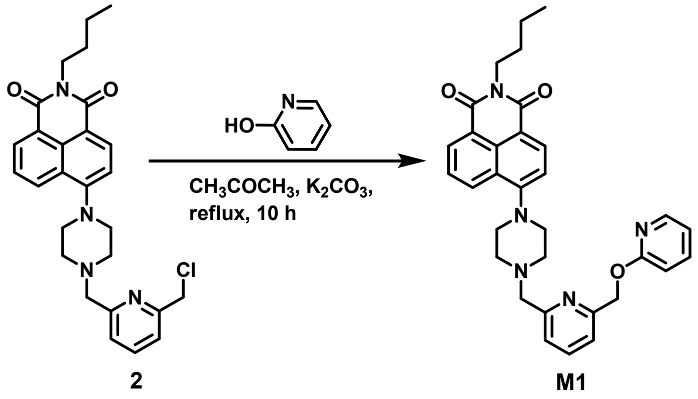
Hela Cells were grown in the exponential phase of growth on 35-mm glass-bottom culture dishes (Φ 20mm) for 1-2 days to reach 70-90% confluency. These cells were used for fluorescence imaging experiments. The cells were washed with DMEM for three times, and then incubated with 10 μM of probe in 2mL DMEM (containing 5% DMF) under an atmosphere of 5% CO_2 and 95% air for 30 min at 37°C. Cells were washed twice with 1mL PBS at room temperature, and then followed by addition of 1mL PBS and observed under microscopy (Olympus IX71, magnification 20 X), with excitation by 405 nm laser and 500-700 nm emission light was collected. For the Hg^{2+} treated samples, the cells were washed with DMEM for three times, and then incubated with 10 μM of probe in 2mL DMEM (containing 5% DMF)) under anatmosphere of 5% CO_2 and 95% air for 10 min at 37°C. Then followed by addition of 50 μMof Hg^{2+}, which was further incubated for another 20 min. After the cells were washed twice with 1mL PBSat room temperature,1mL PBSwas added and observed under microscopy (Olympus IX71, magnification 20 X), with excitation by 405 nm laser and 500-700 nm emission light was collected.

2. Synthesis of probe model compounds M1–2



Scheme S1 Synthesis of intermediates **2** and **3**

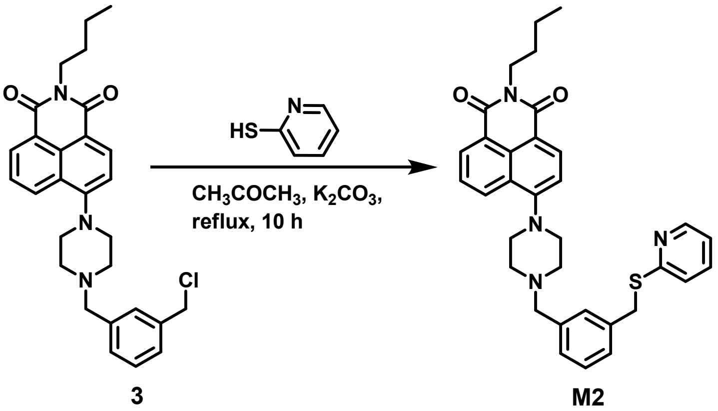
Compounds **1**, **2,** and **3** were prepared according to our previously reported methods (*Dalton Trans.*, 2014, **43**, 8102).



Scheme S2 Synthesis of model compound **M1**

Compound **M1**: Anhydrous potassium carbonate (174 mg, 1.26 mmol), compounds **2** (300 mg, 0.63 mmol) and 2-hydroxypyridine (144 mg, 1.51 mmol) were dissolved in acetone (12 mL), and the reaction mixture was refluxed for 10 h under argon atmosphere. The mixture was filtered, and the solvent was removed in a vacuum to give a yellow solid. The crude product was then chromatographed on silica gel using dichloromethane–methanol 20 : 1 (v/v) as eluant to afford **M1** as a yellow solid. Yield: 268 mg (80%). Mp: 70 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.57 (d, J = 8.0 Hz, 1H), 8.51 (d, J = 8.0 Hz, 1H), 8.39 (d, J = 8.0 Hz, 1H), 7.73–7.63 (m, 2H), 7.52 (dd, J = 8.0, 4.0 Hz, 1H), 7.41 (d, J = 7.6 Hz, 1H), 7.34 (ddd, J = 8.8, 6.6, 2.0 Hz, 1H), 7.21 (d, J = 8.0 Hz, 2H), 6.60 (d, J = 8.8 Hz, 1H), 6.19 (td, J = 6.8, 1.2 Hz, 1H), 5.26 (s, 2H), 4.16 (t, J = 8.0 Hz, 2H), 3.84 (s, 2H), 3.33 (s, 4H), 2.87 (s, 4H), 1.70 (dt, J = 15.2, 7.6

Hz, 2H), 1.50–1.38 (m, 2H), 0.96 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (CDCl$_3$, 100 MHz) δ: 164.41, 163.95, 162.54, 157.57, 155.75, 155.37, 139.77, 138.25, 137.57, 132.45, 131.01, 130.17, 129.79, 126.09, 125.62, 123.25, 122.43, 121.10, 121.05, 116.79, 114.92, 106.07, 64.05, 53.94, 53.20, 52.84, 40.04, 30.24, 20.38, 13.86. HR-ESI-MS calcd for $C_{32}H_{34}N_5O_3$ [(M + H)$^+$]: 536.2656, found: 536.2653.



Scheme S3 Synthesis of model compound **M2**

Compound **M2**: Anhydrous potassium carbonate (174 mg, 1.26 mmol), compounds **3** (300 mg, 0.63 mmol) and 2-mercaptopyridine (168 mg, 1.51 mmol) were dissolved in acetone (12 mL), and the reaction mixture was refluxed for 10 h under argon atmosphere. The mixture was filtered, and the solvent was removed in a vacuum to give a yellow solid. The crude product was then chromatographed on silica gel using dichloromethane–methanol 30 : 1 (v/v) as eluant to afford **M2** as a yellow solid. Yield: 264 mg (76%). Mp: 130 °C. ^{1}H NMR (CDCl$_3$, 400 MHz) δ 8.60–8.54 (m, 1H), 8.50 (d, *J* = 8.0 Hz, 1H), 8.47–8.45 (m, 1H), 8.38 (d, *J* = 8.4 Hz, 1H), 7.67 (dd, *J* = 8.4, 7.4 Hz, 1H), 7.50–7.44 (m, 1H), 7.43 (s, 1H), 7.34 (d, *J* = 6.8 Hz, 1H), 7.32–7.26 (m, 2H), 7.18 (dd, *J* = 10.6, 8.0 Hz, 2H), 7.00–6.97 (m, 1H), 4.46 (s, 2H), 4.16 (t, *J* = 8.0 Hz, 2H), 3.65 (s, 2H), 3.28 (s, 4H), 2.76 (s, 4H), 1.70 (dq, *J* = 15.2, 7.6 Hz, 2H), 1.50–1.38 (m, 2H), 0.97 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (CDCl$_3$, 100 MHz) δ: 164.47, 164.01, 158.80, 155.92, 149.40, 138.09, 137.75, 135.97, 132.50, 131.01, 130.22, 129.85, 128.57, 128.05, 128.02, 126.14, 125.57, 123.31, 122.09, 119.61, 116.74, 114.91, 62.82, 53.06, 52.97, 40.07, 34.39, 30.28, 20.41, 13.87. HR-ESI-MS calcd for $C_{33}H_{35}N_4O_2S$ [(M + H)$^+$]: 551.2475, found: 551.2501.

3. The absorption and emissive properties of NPS on changing the pH

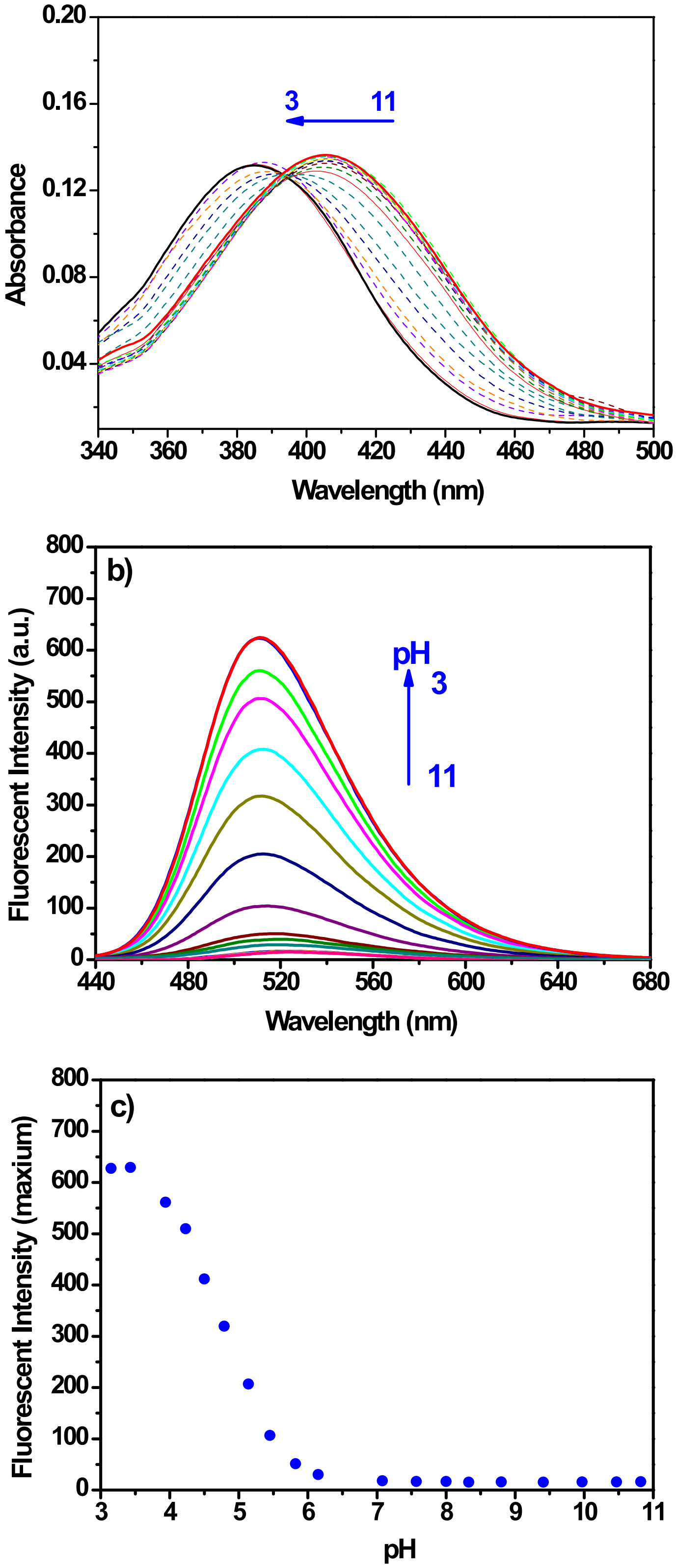


Fig. S1 The influence of pH on the absorption spectra (a) and fluorescence spectra (b) of **NPS** (10 μM) in THF/H_2O (1:1, v/v). (c) Curve of the maximum fluorescence intensity of **NPS** (10 μM) versus pH. (λex = 405 nm, slits: 2.5, 5 nm.).

4. UV-Vis absorption spectra of NPS in the presence of various metal ions

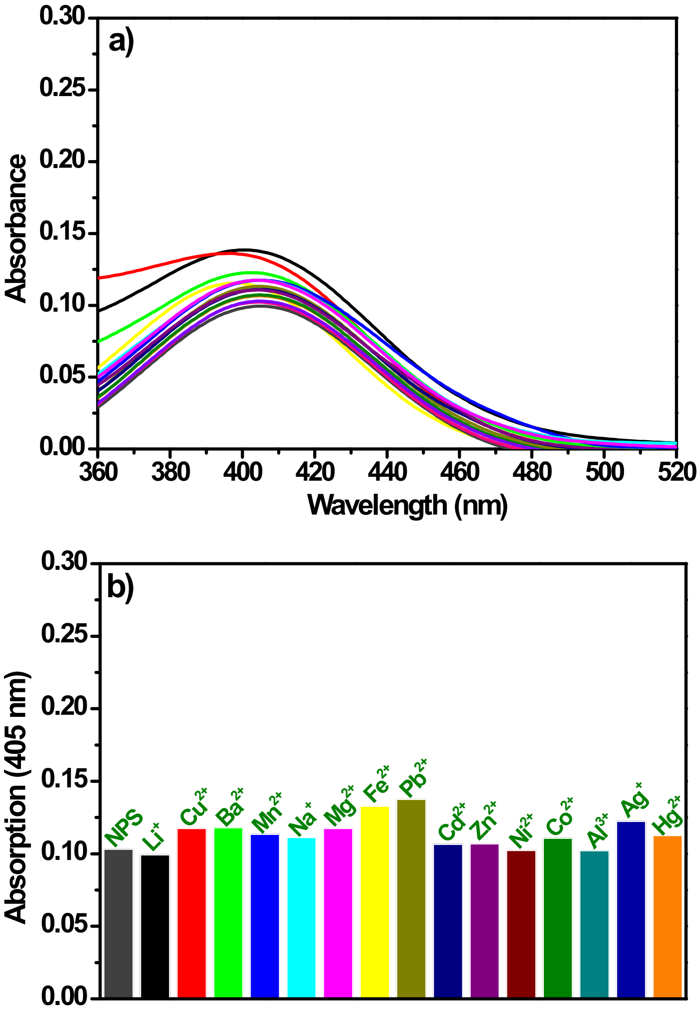


Fig. S2 UV-Vis absorption spectra (a) and absorption at 405 nm (b) of **NPS** (10 μM) in the presence of various metal ions (80 μM) in aqueous solution (THF/H_2O, v/v, 1:1, 10 mM Tris-HCl, pH 7.4).

5. UV-Vis absorption titration spectra of NPS with Hg^{2+}

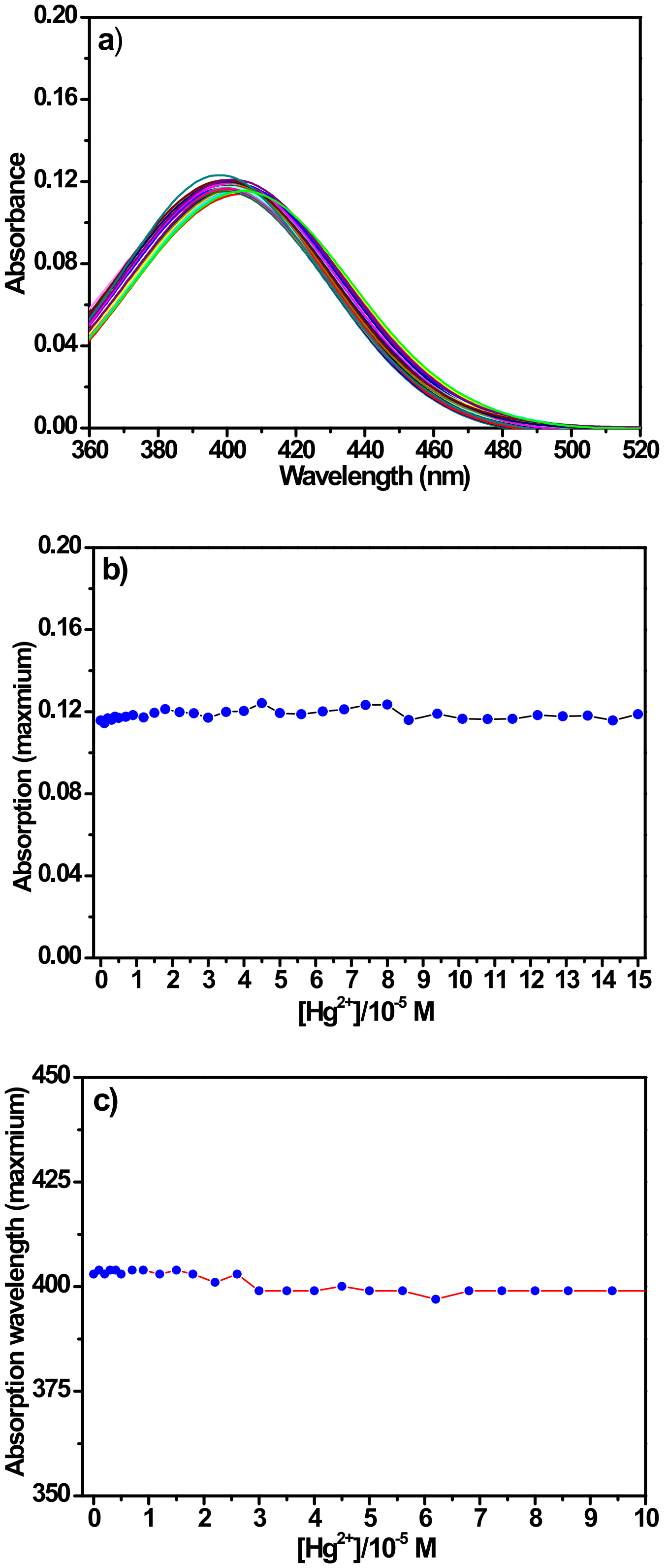


Fig. S3 (a) UV-Vis absorption spectra of **NPS** (10 μM) upon addition of Hg^{2+} (1–150 μM) in aqueous solution (THF/H_2O, v/v, 1:1, 10 mM Tris-HCl, pH 7.4). The curves of maximum absorption (b) and the wavelength of maximum absorbance (c) of **NPS** (10 μM) versus increasing concentrations of Hg^{2+} (1–150 μM).

6. The normalized emission spectra of NPS upon the addition of Hg^{2+} and at various pH

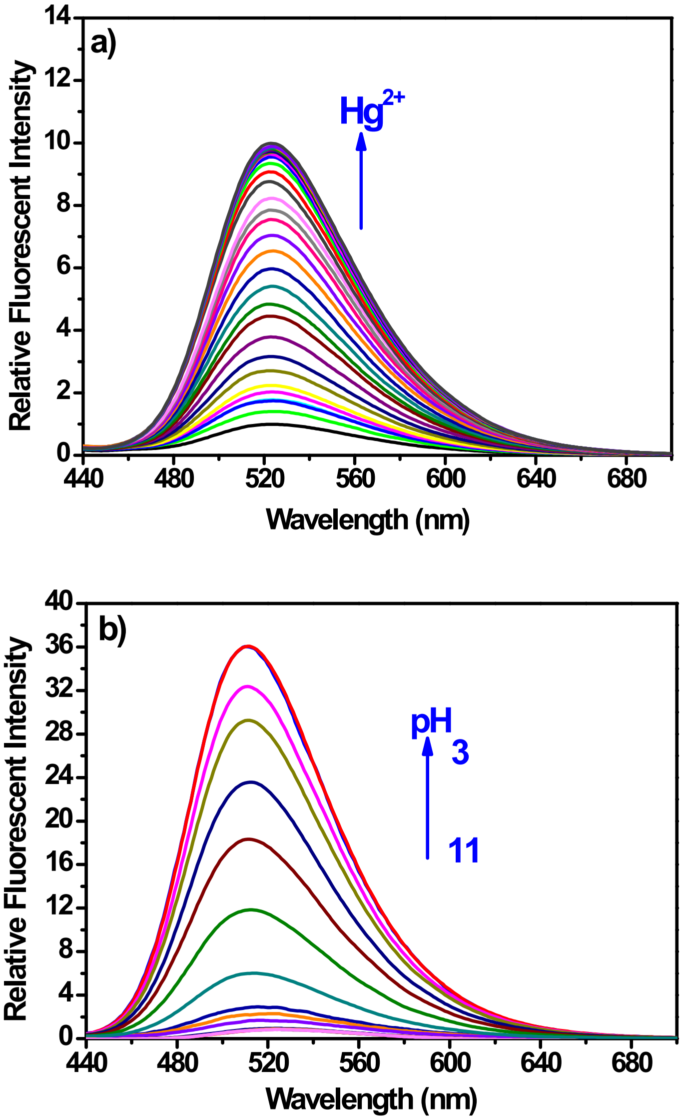


Fig. S4 (a) The normalized emission spectra of **NPS** (10 μM) upon the addition of Hg^{2+} (0.1–15 equivalents) in aqueous solution (THF/H_2O, 1:1, v/v, 10 mM Tris-HCl, pH 7.4) and (b) The normalized emission spectra of **NPS** (10 μM) at various pH in THF/H_2O (1:1, v/v).

7. The response of NPS to Hg^{2+} at different water-THF ratios

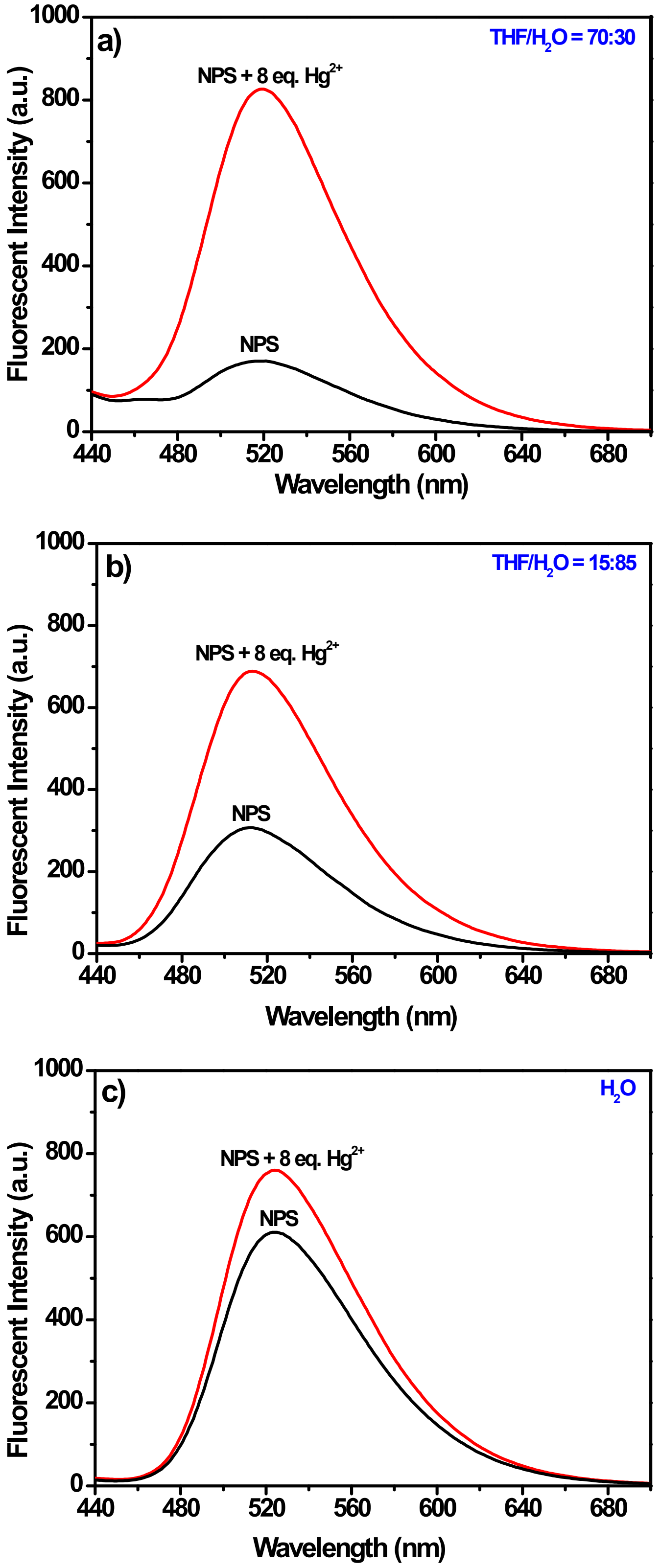


Fig. S5 The fluorescence spectra of **NPS** (10 μM) in the absence and presence of 8 equivalents of Hg^{2+} in aqueous solution (THF/H_2O, 10 mM Tris-HCl, pH 7.4) with different water-THF ratios (a, THF/H_2O, 70/30; b, THF/H_2O, 15/85; c, 100% H_2O).

8. Fluorescence spectra of NPS and M1–2 in the absence and presence of Hg^{2+}

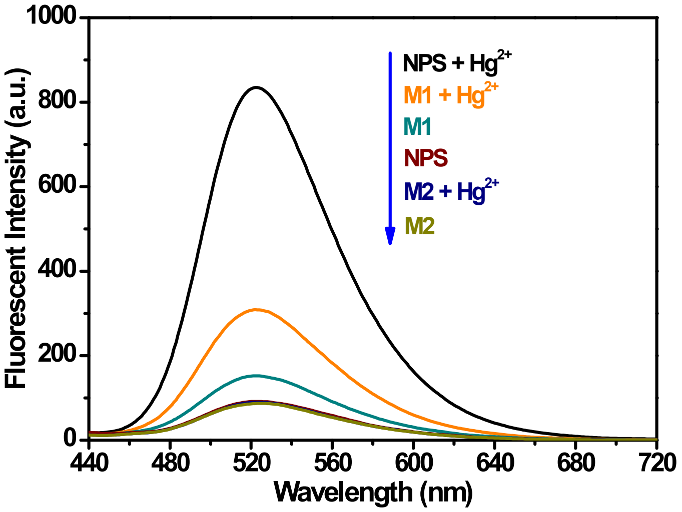


Fig. S6 Fluorescence spectra of **NPS** and **M1–2** (all compounds were 10 μM) in the absence and presence of Hg^{2+} (80 μM) in aqueous solution (THF/H_2O, v/v, 1:1, 10 mM Tris-HCl, pH 7.4). λ_{ex} = 405 nm, slits: 5, 5 nm.

9. The ^1H NMR titration experiments of Hg^{2+}

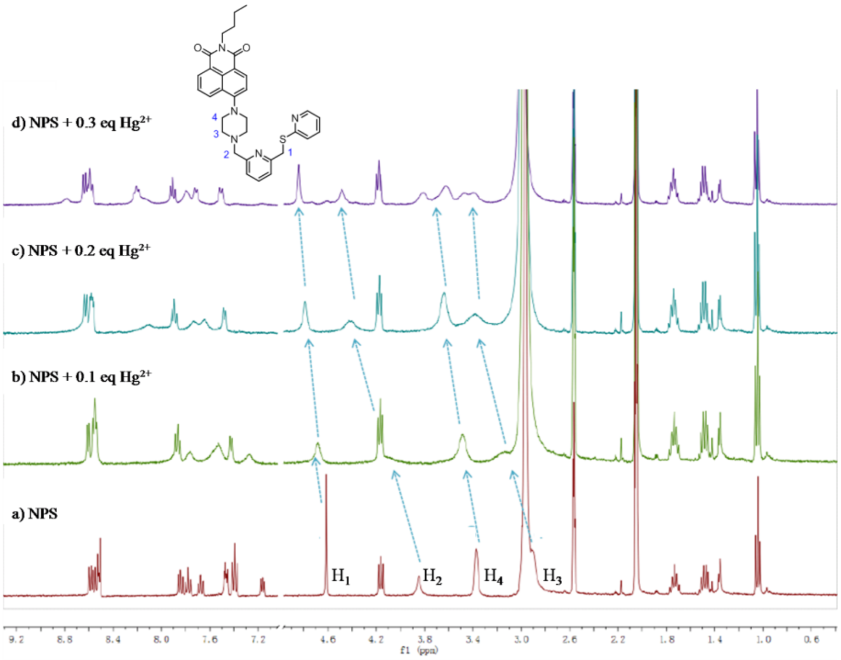
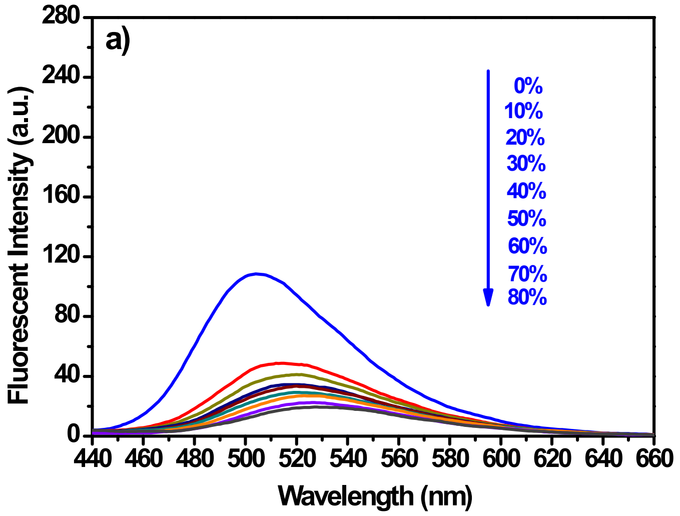
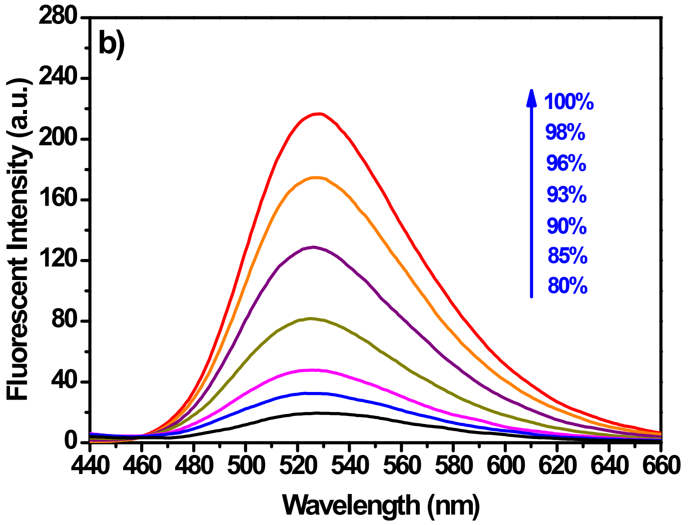
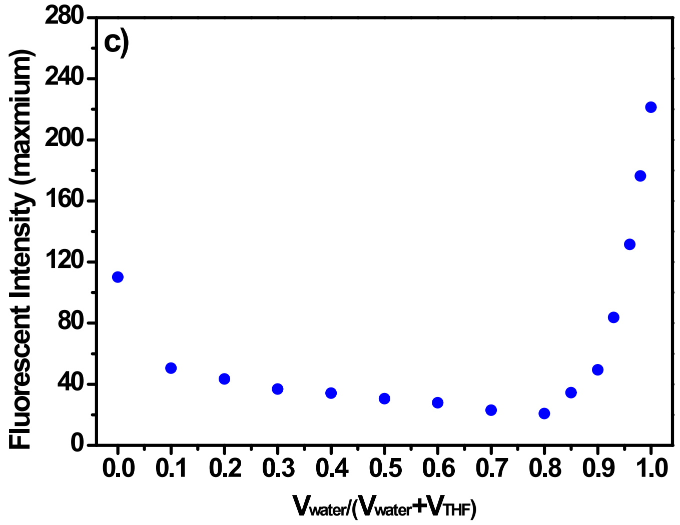


Fig. S7 The ^1H NMR spectra changes of **NPS** with the addition of Hg^{2+} in the mixture solution of CD_3CN and DMSO-d_6.

10. Fluorescence spectra of M1 and M2 in THF-H_2O mixture







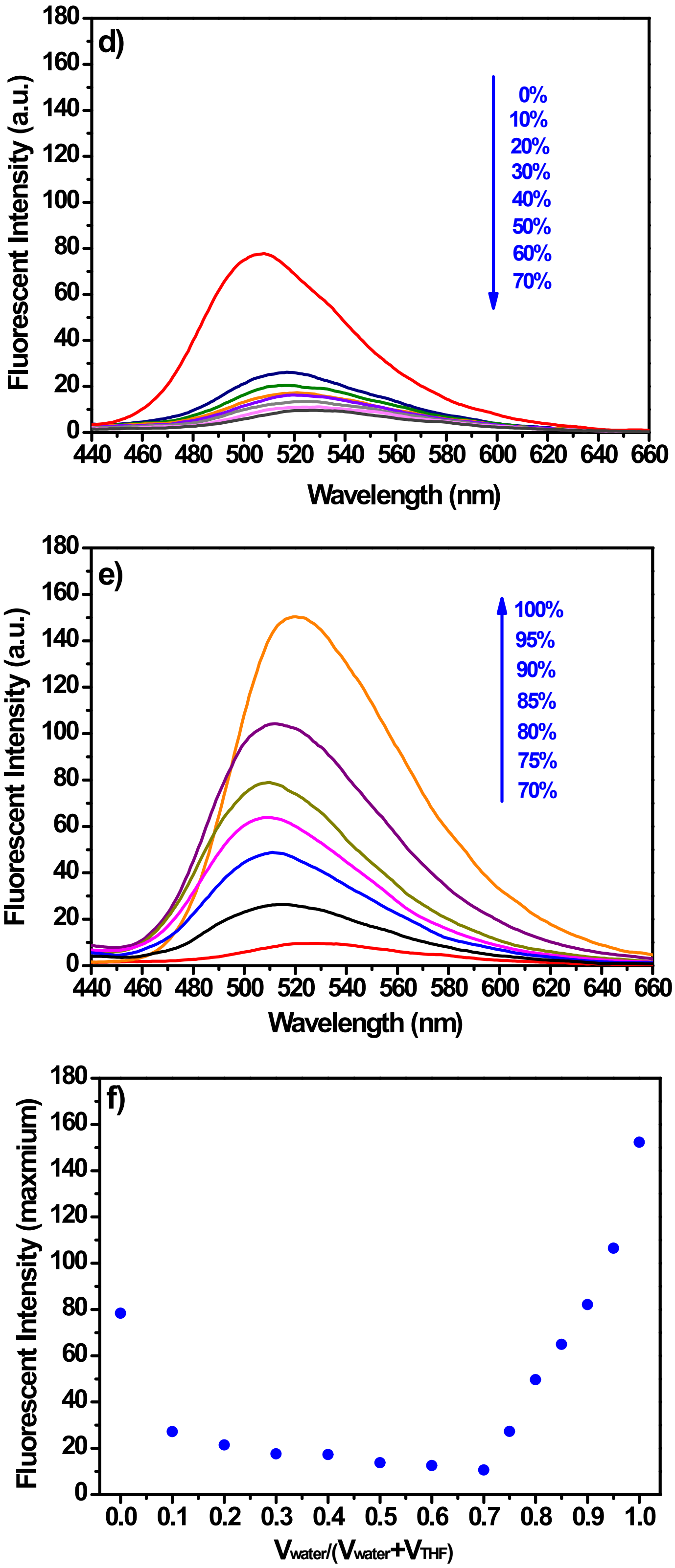


Fig. S8 The fluorescence spectra of **M1** (a, b) and **M2** (d, e) in THF-H_2O mixture with different fraction (a, 0–80%; b, 80–100%; d, 0–70%; e, 70–100%); Plots of the maximum fluorescent intensity of **M1** (c) and **M2** (f) versus water fraction in THF-H_2O mixture. (λex = 405 nm, slits: 2.5, 5 nm.)

11. ^{1}H NMR and ^{13}C NMR spectra of compounds NPS and M1–2

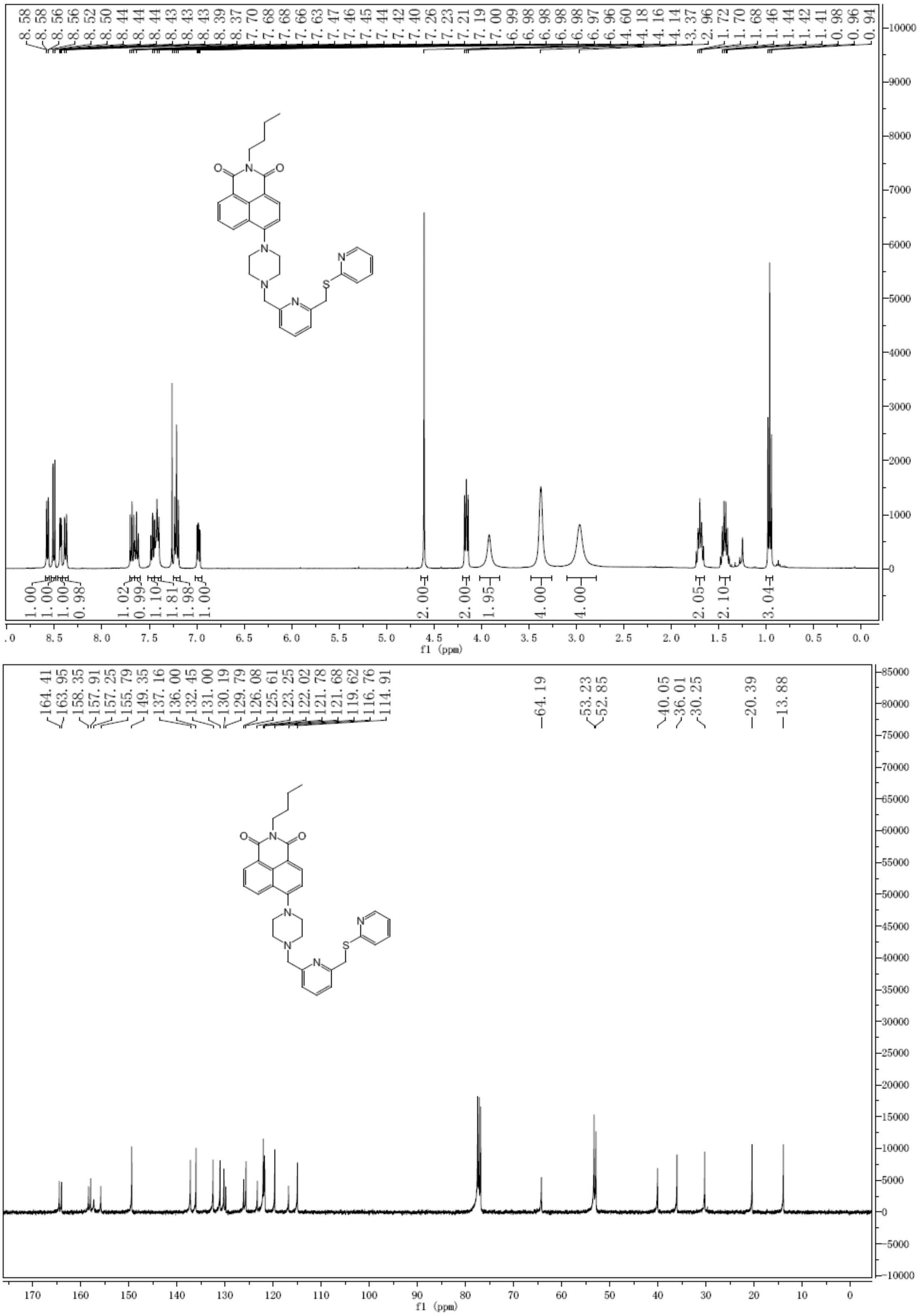


Fig. S9 ^{1}H NMR (top) and ^{13}C NMR (bottom) spectra of **NPS** in $CDCl_3$.

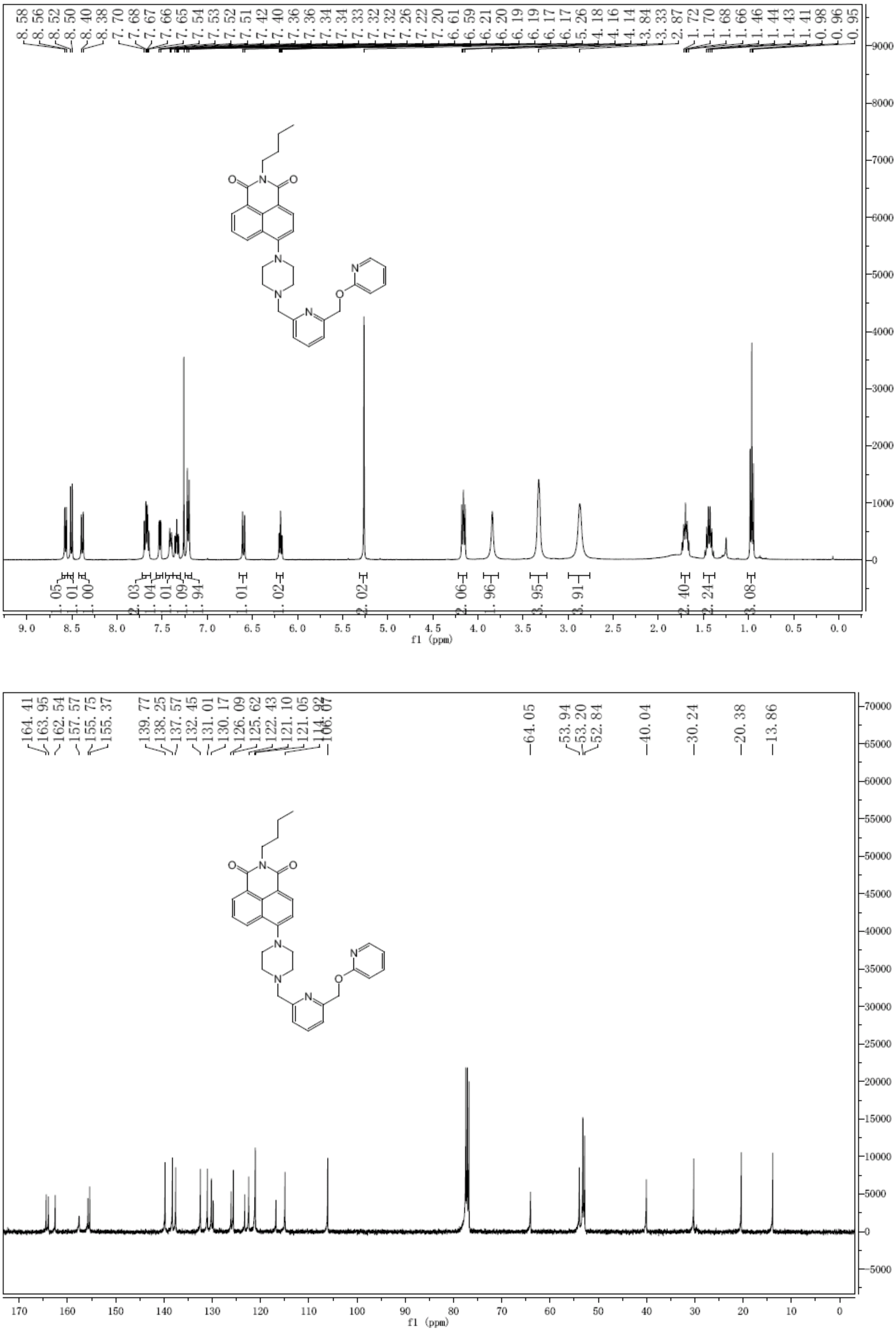


Fig. S10 ^{1}H NMR (top) and ^{13}C NMR (bottom) spectra of **M1** in $CDCl_3$.

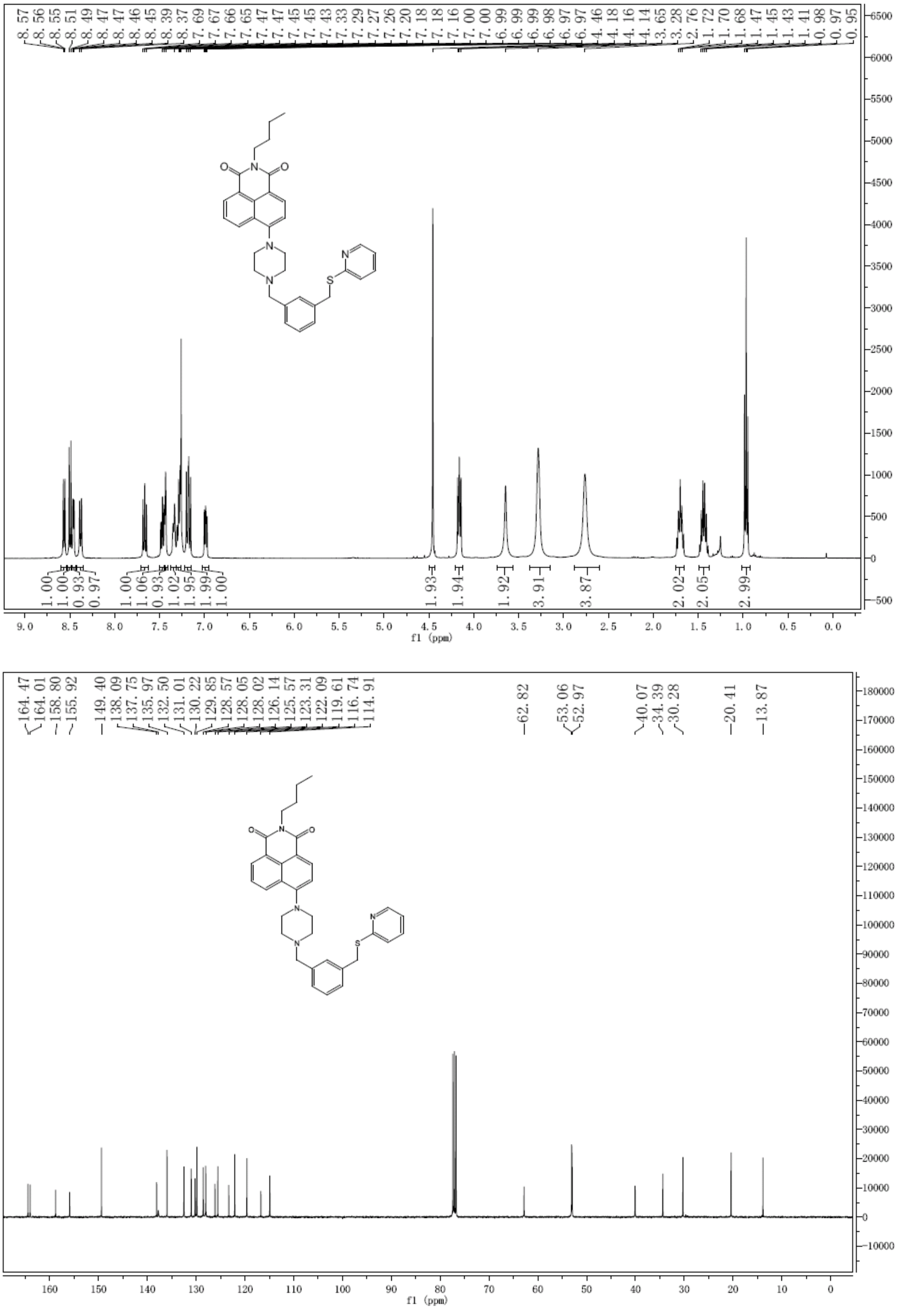
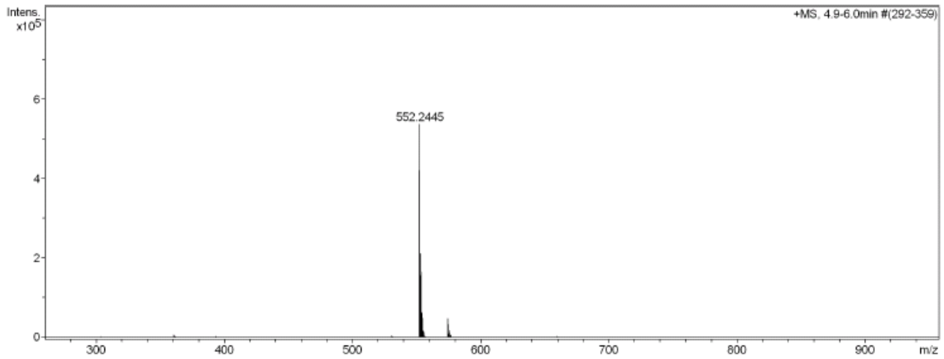
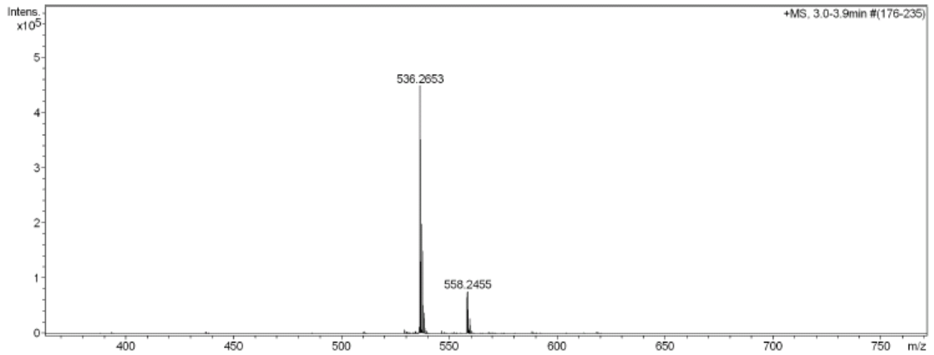


Fig. S11 ^{1}H NMR (top) and ^{13}C NMR (bottom) spectra of **M2** in $CDCl_3$.

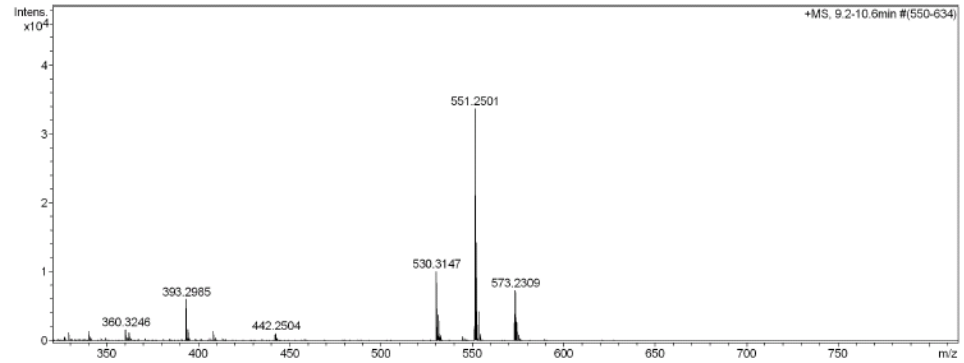
12. The HR-ESI-MS spectra of compounds NPS and M1–2



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
552.2445	1	C 32 H 34 N 5 O 2 S	552.2428	-3.2	-2.0	18.5	ok	even	8.40	0.0107	0.0020	0.0039	0.0044	0.5167



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
536.2653	1	C 32 H 34 N 5 O 3	536.2656	0.6	1.1	18.5	ok	even	37.27	0.0513	0.0010	0.0159	0.0020	0.5326



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
551.2501	1	C 33 H 35 N 4 O 2 S	551.2475	-4.8	-6.6	18.5	ok	even	20.73	0.0273	0.0040	0.0091	0.0030	0.6530

Fig. S12 The HR-ESI-MS spectra of compounds **NPS** and **M1**–**2** (from top to bottom, **NPS**, **M1**, and **M2**).