# Alteration of Selectivity in Rhodamine Based Probes for Fe(III) and Hg(II) Ion Induced Dual Mode Signaling Responses

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## Supplementary Information

*Synthesis*
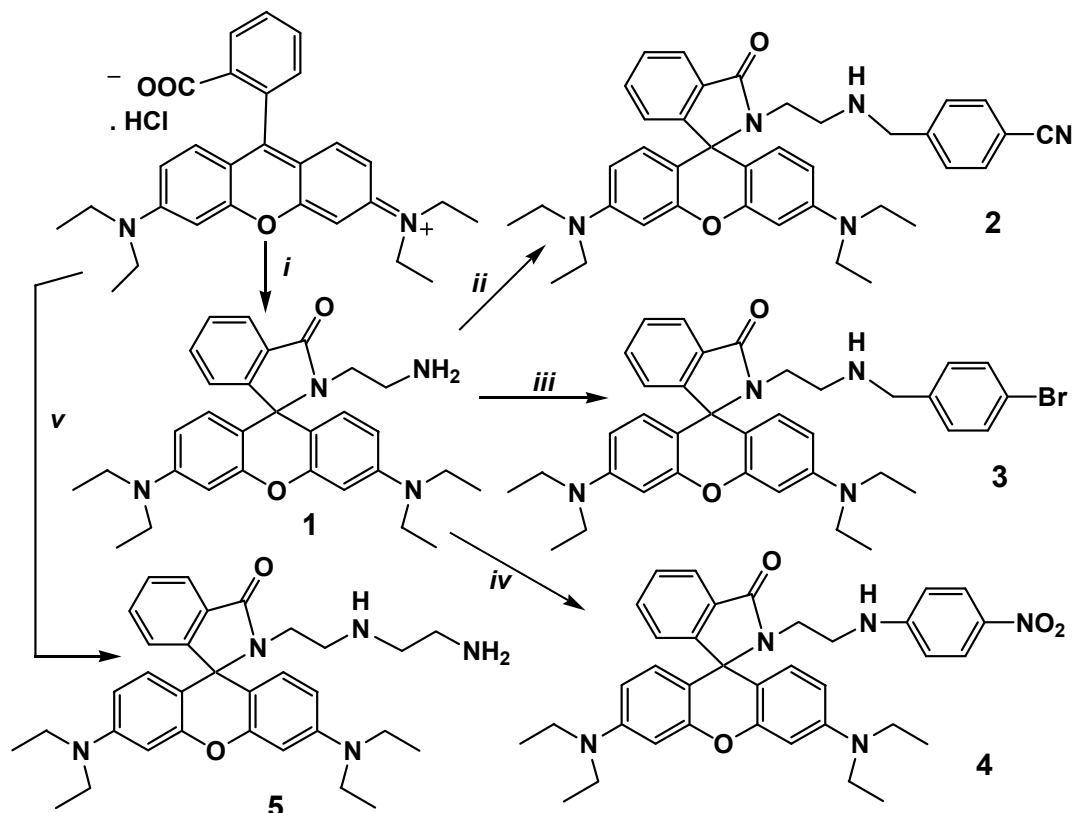


**Reaction conditions**: (i) ethylenediamine, EtOH, reflux, 18h; (ii) 4-cyanobenzaldehyde, EtOH, RT, 24h, NaBH$_4$; (iii) 4-bromo benzaldehyde, EtOH, RT, 24h, NaBH$_4$; (iv) 4-fluoro-1-nitrobenzene, Et$_3$N, DMSO, 70 °C, 12h; (v) diethylenetriamine, EtOH, reflux, 15h.

**Scheme S1**

*Compound* **1**

This compound was synthesized following the procedure reported[15,18] earlier.

*Compound* **2**

To a solution of **1** (0.97g, 2mmol) in ethanol(20 mL), an ethanolic (20mL) solution of 4-cyanobenzaldehyde (0.26g, 2mmol) was added and allowed to react at room temperature for 24h with constant stirring. The Schiff base thus formed was reduced with $NaBH_4$ stirring at room temperature for 6h and heated to reflux for 2h to ensure complete reduction followed by removal of the solvent under reduced pressure. The residual mass was added with water(30 mL) and extracted with chloroform(3×30 mL). The combined organic layers, after drying over anhydrous $Na_2SO_4$, were evaporated to dryness to obtain **2** as brown solid, which was further purified by passing through a column(alumina, neutral) with chloroform-methanol (98:2 v/v) mixture as eluent.

Yield:0.91g (76 %); mp: 94 °C; ESI-MS [$M_w$ ($C_{38}H_{41}N_5O_2$) = 599.76] m/z$^+$(%): 600.10 [**2**]$^+$ (100%); $^1$H-NMR(400MHz, $CDCl_3$, 25 °C, TMS): 7.789(dd, $J_1$ = 3.99Hz, $J_2$ = 7.19Hz, 1H), 7.390-7.528(m, 2H), 7.333(dd, $J_1$ = 3.99Hz, $J_2$ = 7.59Hz, 1H),7.190(t, J = 7.99Hz, 1H), 7.007(br s, 1H), 6.966(dd, $J_1$ = 3.99Hz, $J_2$ = 7.19Hz, 1H), 6.326(s, 1H), 6.285(t, $J$ = 5.59 Hz, 1H), 6.235(s, 2H), 6.192(d, $J$ = 3.59 Hz, 1H), 6.115(dd, $J_1$ = 3.19Hz, $J_2$ = 11.98 Hz, 2H), 4.652(s, 1H, $H_N$), 3.517(s, 2H), 3.197 (q, $J$ = 9.59Hz, 10H), 2.308(t, $J$ = 8.39 Hz, 2H), 1.065(t, $J$ = 9.59Hz, 12H);$^{13}$C-NMR(100MHz, $CDCl_3$, 25 °C, TMS): 168.87, 153.41(d), 148.89, 147.19, 146.07, 132.63, 132.08(d), 131.05, 128.63(d), 128.20, 127.08, 123.94, 122.81, 119.14, 110.45(d), 108.20, 105.51, 97.86, 65.19, 52.67, 47.64, 44.44, 40.08, 12.68; Anal. Calcd. for $C_{38}H_{41}N_5O_2$: C, 76.10, H, 6.89, N, 11.68; Found: C, 75.96; H, 6.93; N, 11.59.

*Compound* **3**

To a stirring ethanolic(20 mL) solution of **1** (0.97 g, 2 mmol), an ethanolic (20 mL) solution of 4-bromobenzaldehyde (0.37 g, 2 mmol) was added and allowed to react at room temperature for 24h with constant stirring. The Schiff base thus formed was reduced with $NaBH_4$ stirring at room temperature for 6h and heated to reflux for 2h to ensure complete reduction followed by removal of the solvent under reduced pressure. The residual mass was added with water(30 mL) and extracted with chloroform (3×30 mL). The combined organic layers, after drying over anhydrous

Na$_2$SO$_4$, were evaporated to dryness to get **3** as brown solid, which was further purified by passing through a column (alumina, neutral) with chloroform-methanol (99:1 v/v) mixture as eluent.

Yield:1.05 g (80 %); mp :86 °C; ESI-MS [M$_w$ (C$_{37}$H$_{41}$N$_4$O$_2$Br) = 653.65] m/z$^+$(%): 653.26 [**3**]$^+$ (98%), 655.27 [**3**+2H]$^+$ (100%); $^1$H-NMR (400MHz, CDCl$_3$, 25 °C, TMS): 7.799 (dd, $J_1$ = 2.39Hz, $J_2$ = 5.99Hz, 1H), 7.330 (dd, $J_1$ = 2.39Hz, $J_2$ = 5.59Hz, 2H), 7.247 (d, $J$ = 8.39Hz, 2H), 7.116 (d, $J$ = 7.59Hz, 1H), 6.975 (t, $J$ = 2.79Hz, 1H), 6.941 (d, $J$ = 7.99Hz, 1H), 6.301 (d, $J$ = 8.79Hz, 1H), 6.276 (d, $J$ = 2.40 Hz, 1H), 6.253 (s, 2H), 6.121 (dd, $J_1$ = 2.40Hz, $J_2$ = 8.79 Hz, 2H), 4.517 (s, 1H, H$_N$), 3.408 (s, 2H), 3.204 (q, $J$ = 6.79Hz, 10H), 2.308 (t, $J$ = 6.39 Hz, 2H), 1.047 (t, $J$ = 7.19Hz, 12H); $^{13}$C-NMR (100MHz, CDCl$_3$, 25 °C, TMS): 168.85, 153.76(d), 148.89, 140.61, 139.29, 132.59, 131.13(t), 130.65, 129.90, 128.65(d), 128.18, 123.94, 122.87, 121.13, 120.56, 108.22, 105.55, 65.18, 52.52, 47.61, 44.48, 40.15, 12.74. Anal. Calcd. for C$_{37}$H$_{41}$N$_4$O$_2$Br: C, 67.99, H, 6.32, N, 8.57; Found: C, 67.88; H, 6.39; N, 8.48.

*Compound* **4**

To a stirred solution of **1**(0.97 g, 2 mmol) in DMSO(15 mL), triethylamine (1.35 mL, 10 mmol) was added at room temperature followed by addition of 4-fluoro-1-nitrobenzene (0.28 g, 2 mmol) and allowed to react at 70 °C for 12h with constant stirring. The solution was then brought back to room temperature and poured to crushed ice where a yellowish precipitate crashed out, which was subsequently filtered. This solid was stirred for 30 min in water (100 mL) and extracted with CHCl$_3$ (3×30 mL). The combined organic layer, after drying over anhydrous Na$_2$SO$_4$, was evaporated to dryness to get **4** as pale yellow solid, which was further purified by passing through a column(100-200 mesh silica gel) with chloroform-methanol (98:2 v/v) mixture as eluent. It was further recrystallized from acetonitrile to obtain the desired product as pale yellow needle shaped crystals suitable for single crystal X-ray diffraction.

Yield:0.95g (78 %); Mixed mp:119-121 °C; ESI-MS [M$_w$ (C$_{36}$H$_{39}$N$_5$O$_4$) = 606.30] m/z$^+$(%): 606.31 [**4**]$^+$ (100%); $^1$H-NMR (400MHz, CDCl$_3$, 25 °C, TMS): 7.854 (d, $J$ = 9.19Hz, 2H), 7.816 (dd, $J_1$ = 3.18Hz, $J_2$ = 5.59Hz, 1H), 7.351 (dt, $J_1$ = 9.52Hz, $J_2$ = 4.39 Hz, 2H), 6.987 (dt, $J_1$ = 9.19 Hz, $J_2$ = 3.99Hz, 1H), 6.322 (d, $J$ = 5.20Hz, 1H), 6.314 (s, 2H), 6.286 (s, 1H), 6.125 (d, $J$ = 8,79 Hz, 3H), 6.103 (d, $J$ = 2.40 Hz, 1H), 5.408 (s, 1H, H$_N$), 3.337 (t, $J$ = 5.99Hz, 2H), 3.215 (dd, $J_1$ = 6.79Hz, $J_2$ = 13.98Hz, 8H), 2.876 (dd, $J_1$ = 5.59Hz, $J_2$ = 10.74Hz, 2H), 1.072 (t, $J$ = 6.79Hz,

12H); $^{13}$C-NMR (100MHz, CDCl$_3$, 25 °C, TMS): 169.60, 153.62(d), 149.03, 137.45, 132.98, 130.73, 128.40(d), 126.32, 124.05, 122.94, 110.86, 108.31, 104.88, 97.75, 65.42, 44.47, 42.98, 39.11, 12.68. Anal. Calcd. for C$_{36}$H$_{39}$N$_5$O$_4$: C, 71.38, H, 6.49, N, 11.56; Found: C, 71.22; H, 6.53; N, 11.47.

*Compound* **5**.

Although compound **5** has been reported[16] in literature, the following procedure was followed for its synthesis.

To a stirring solution of rhodamine B hydrochloride (0.478 g, 1 mmol) in EtOH (50 mL), diethylene triamine (0.55 mL, 5 mmol) in EtOH (20 mL) was added and allowed to react under reflux condition for 15h until the colour of the solution changes from pink to orange. The solution was then cooled to room temperature, filtered and the solvent was removed under reduced pressure. Water (50 mL) was added to the pale brown solid and extracted with CHCl$_3$ (3×30 mL). The combined organic layers, after drying over anhydrous Na$_2$SO$_4$, were evaporated under reduced pressure to obtain a brown semi-solid residue; which was further purified by passing through a column (neutral alumina) with chloroform and methanol mixture (98:2 v/v) as eluent to obtain **5** as a light brown solid.

Yield: 0.42g (80 %); mixed mp: 103-105 °C; ESI-MS[M$_w$ (C$_{32}$H$_{41}$N$_5$O$_2$) = 527.33], m/z$^+$ (%): 528.41 [**5**+H]$^+$ (100%); $^1$H-NMR (400MHz, CDCl$_3$, 25 °C, TMS, δ): 7.873 (br s, 1H), 7.419 (dd, $J_1$ = 3.99 Hz, $J_2$ = 6.79 Hz, 2H), 7.068 (t, $J_1$ = 3.59 Hz, $J_{1a}$ = 6.79 Hz, 1H), 6.446 (s, 1H), 6.417 (br s, 2H), 6.377 (s, 1H), 6.256 (dd, $J_1$ = 2.39 Hz, $J_2$ = 11.58 Hz, 2H), 3.071-3.368 (m, 10 H), 2.653 ( t, $J$ = 7.19 Hz, 2H), 2.471 (br s, 5H), 2.364 (t, $J_1$ = 7.59 Hz, $J_{1a}$ = 7.99 Hz, 2H), 1.162 (t, $J$ = 8.79 Hz, 12H); $^{13}$C-NMR (100 MHz, CDCl$_3$, 25 °C, TMS, δ):168.65, 153.27(t), 148.70(d), 132.43, 131.09, 128.95, 128.65(d), 128.40, 123.79, 122.74, 108.06(d), 106.46(d), 97.66, 65.05, 51.41, 47.72, 44.33, 43.78, 41.36, 40.10, 12.55; Anal. Calcd. for C$_{32}$H$_{41}$N$_5$O$_2$: C, 72.83, H, 7.83, N, 13.27. Found: C, 72.66, H, 7.91, N 13.19 %.
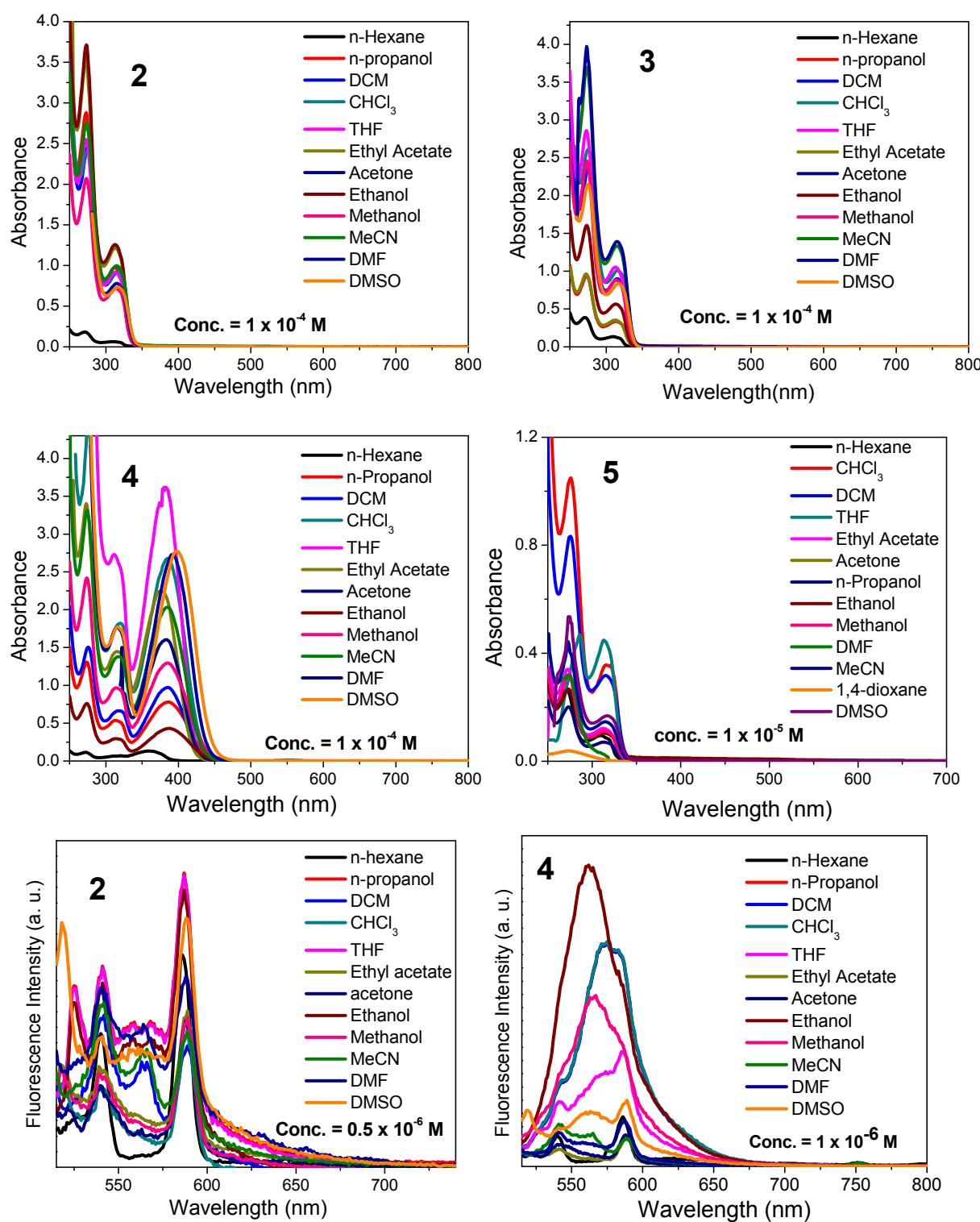
**Fig. S1**: Absorption spectra of **2 - 5** and emission spectra of **2 & 4** ($\lambda_{ex}$ = 500nm, ex. & em. b. p. = 5nm) in various solvents.
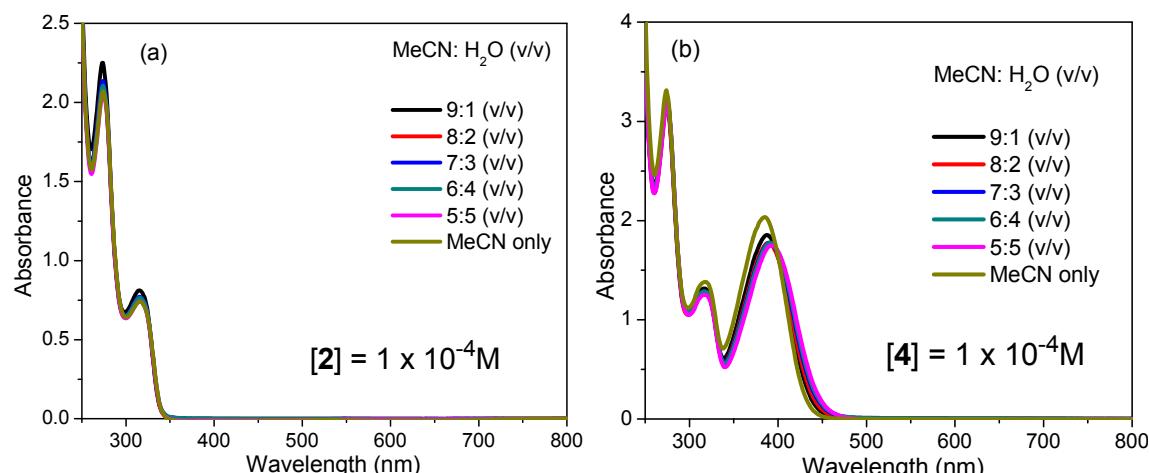
**Fig. S2**: Absorption spectra of (a) **2** and (b) **4** in varied proportions of MeCN-H$_2$O (v/v) mixture.
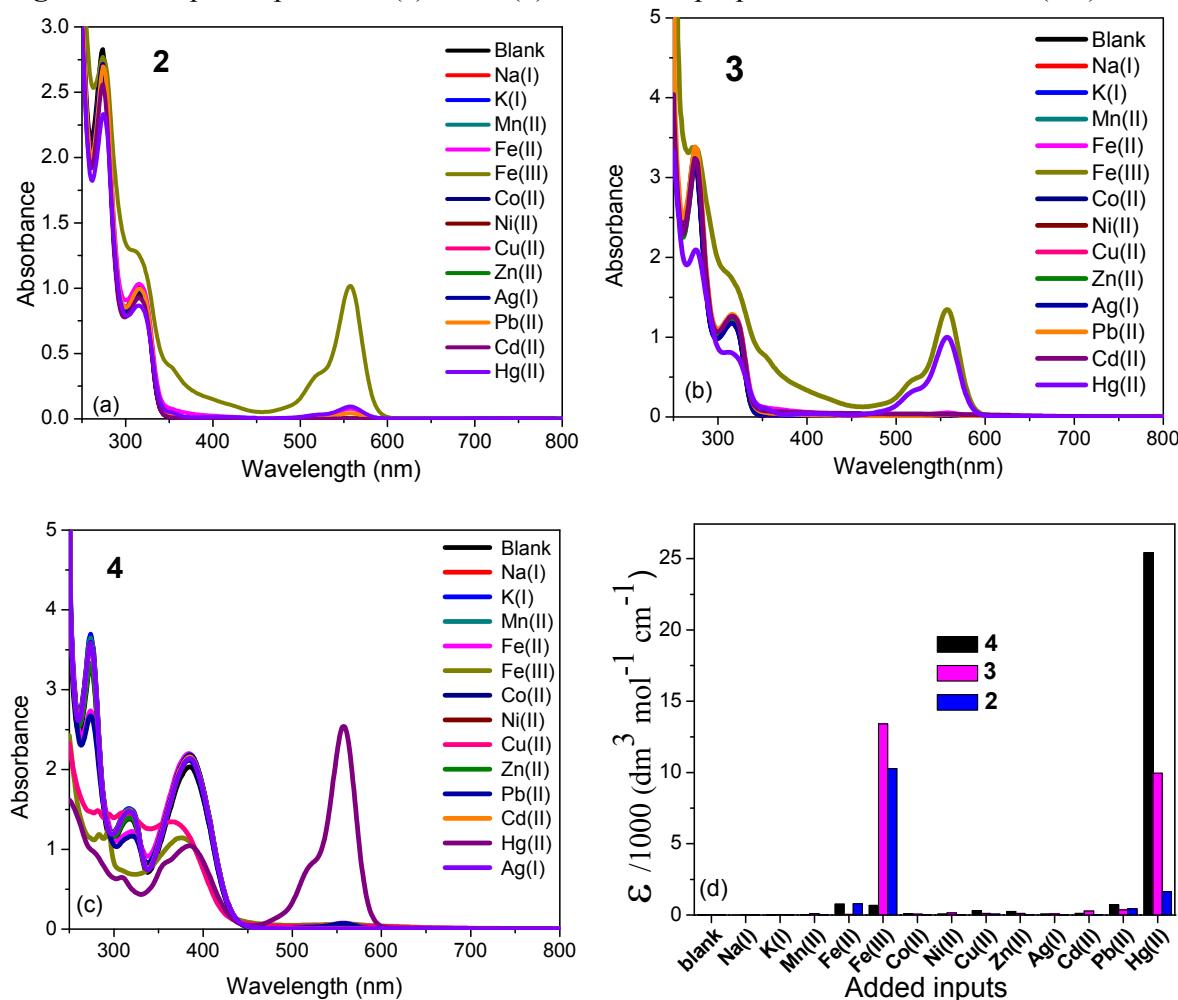


**Fig. S3**: Absorption spectra of (a) **2**, (b) **3** and (c) **4** alone and in presence of various metal ions in MeCN; [conc. of the probes] = $1 \times 10^{-4}$ M, [**M(II/I)**] = $1 \times 10^{-3}$ M. (d) The comparative absorption enhancement factors of these probes in presence of various metal ions.
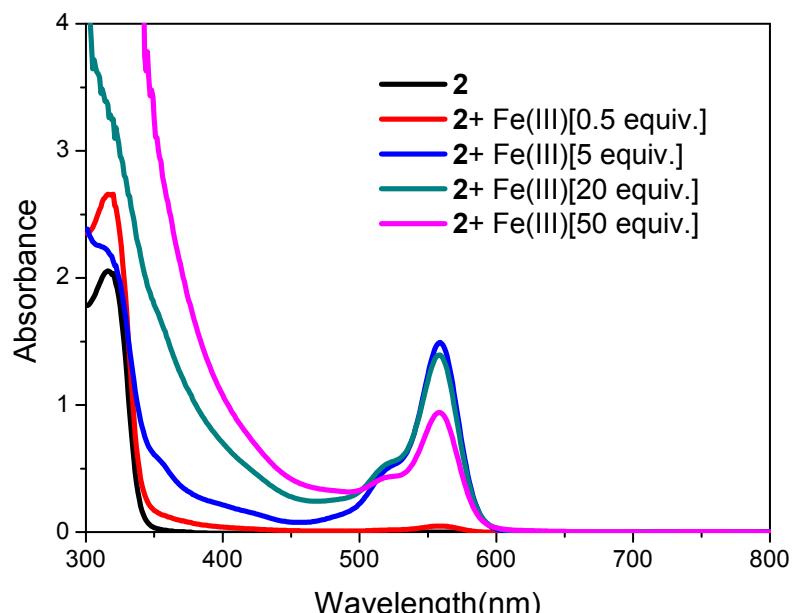
**Fig. S4**: Absorption spectral pattern of **2** (conc. $1 \times 10^{-4}$ M) alone in presence of various equivalents of added Fe(III) in MeCN showing the decrease in absorption($A_{557}$) with excess of metal ions.
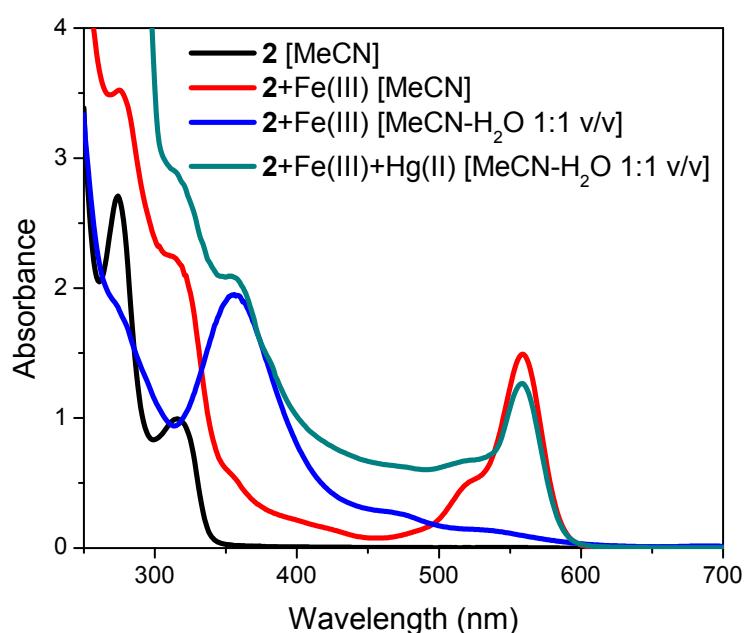


**Fig. S5**: Absorption spectral pattern of **2** alone in MeCN, **2**+Fe(III) in MeCN, **2**+Fe(III) in MeCN-H$_2$O(1:1 v/v), {**2**+Fe(III)}+ Hg(II) in MeCN-H$_2$O(1:1 v/v) exhibiting its change in coordination preferences as a function of solvent medium ([**2**] = $1 \times 10^{-4}$M).

SP8
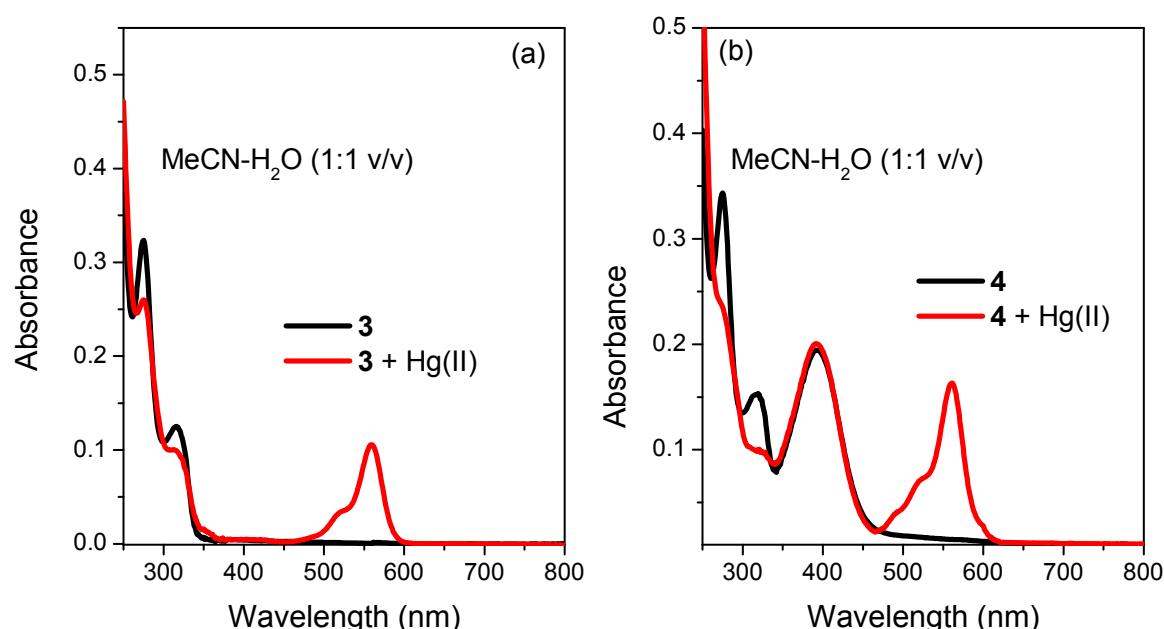
**Fig. S6**: Absorption spectra of (a) **3** and (b) **4** alone and in presence of Hg(II) in MeCN-H$_2$O(1:1 v/v) medium, conc. of the probes = $1 \times 10^{-5}$ M, [Hg(II)] = $2 \times 10^{-4}$ M.
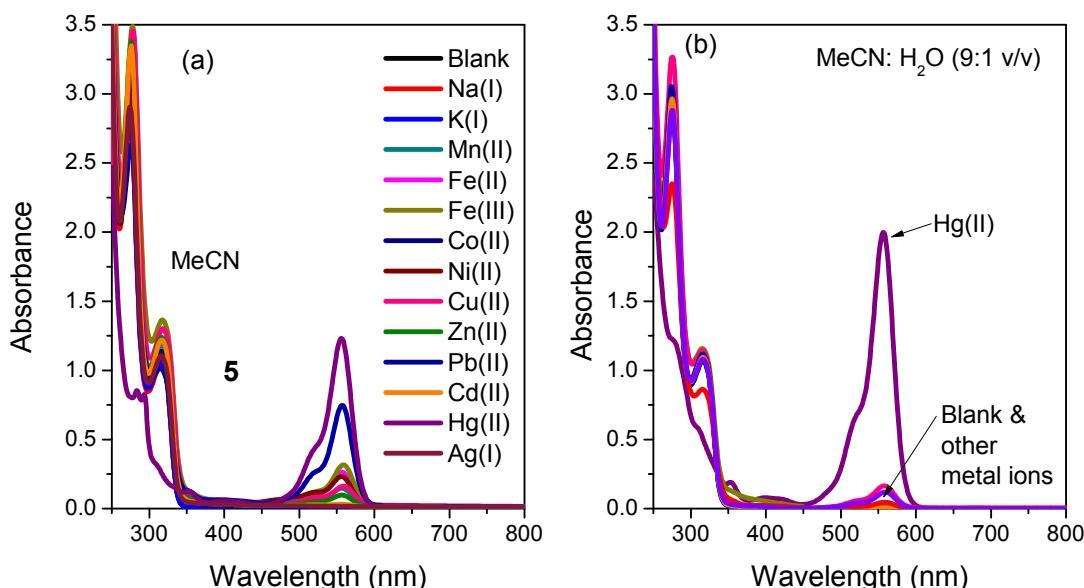


**Fig. S7**: Absorption spectra of **5** alone and in presence of various metal ions in (a) MeCN ([**5**] = $5 \times 10^{-5}$ M) and (b) MeCN-H$_2$O (9:1 v/v), [**5**] = $1 \times 10^{-4}$ M; [**M(II/I)**] = $1 \times 10^{-4}$ M in all cases.
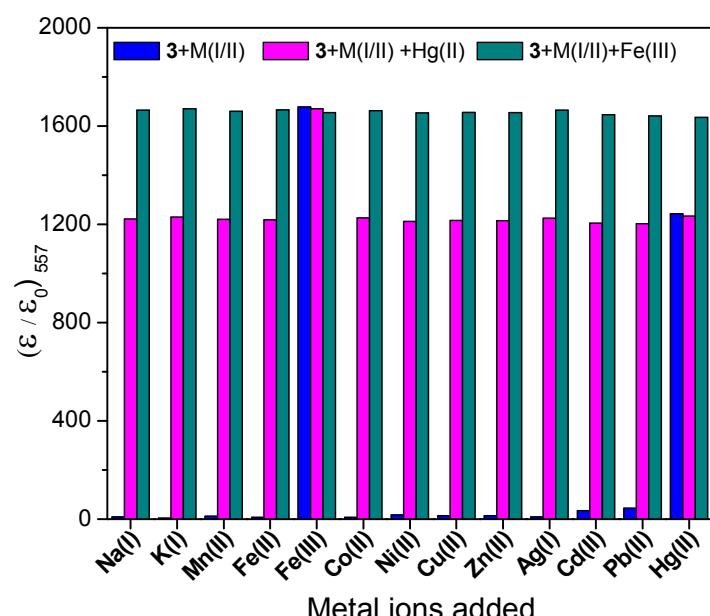
**Fig. S8**: Extent of absorption enhancement of **3** in MeCN in presence of other metal ions prior and after the addition of Fe(III) and Hg(II) ions. [**3**] = $1 \times 10^{-4}$ M, [M] = $1 \times 10^{-3}$ M.
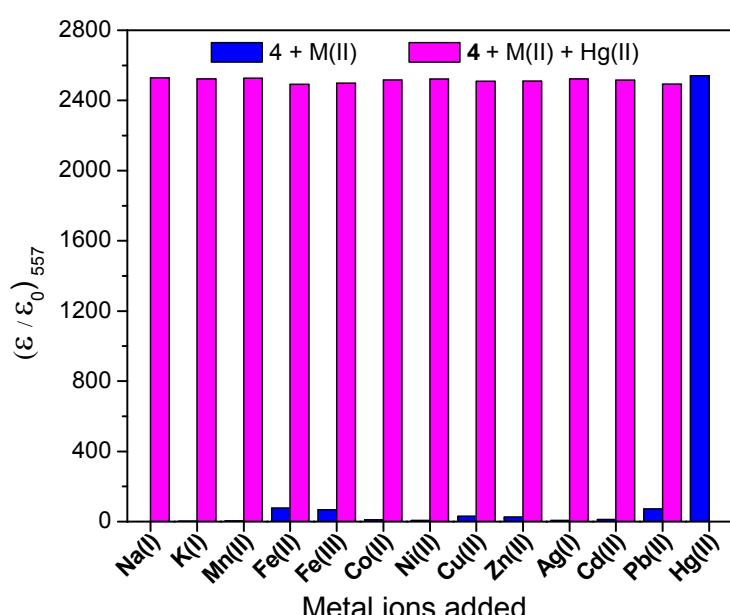


**Fig. S9**: Extent of absorption enhancement of **4** in MeCN in presence of other metal ions prior and after the addition of Hg(II) ion. [**4**] = $1 \times 10^{-4}$ M, conc. of metal ions = $1 \times 10^{-3}$ M.
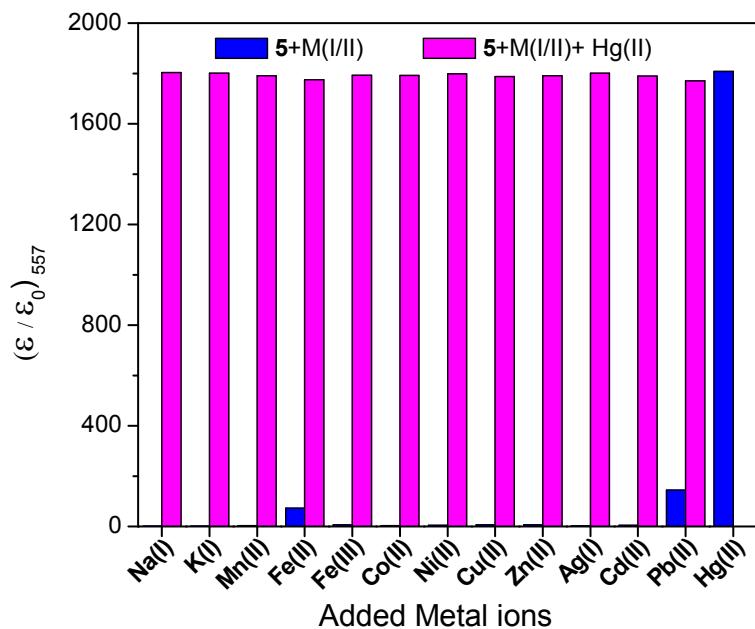
**Fig. S10**: Extent of absorption enhancement of **5** in presence of other metal ions prior and after the addition of Hg(II). [**5**] = $1 \times 10^{-4}$ M, [M(I/II)] = $1 \times 10^{-3}$M, MeCN-H$_2$O(1:1 v/v).
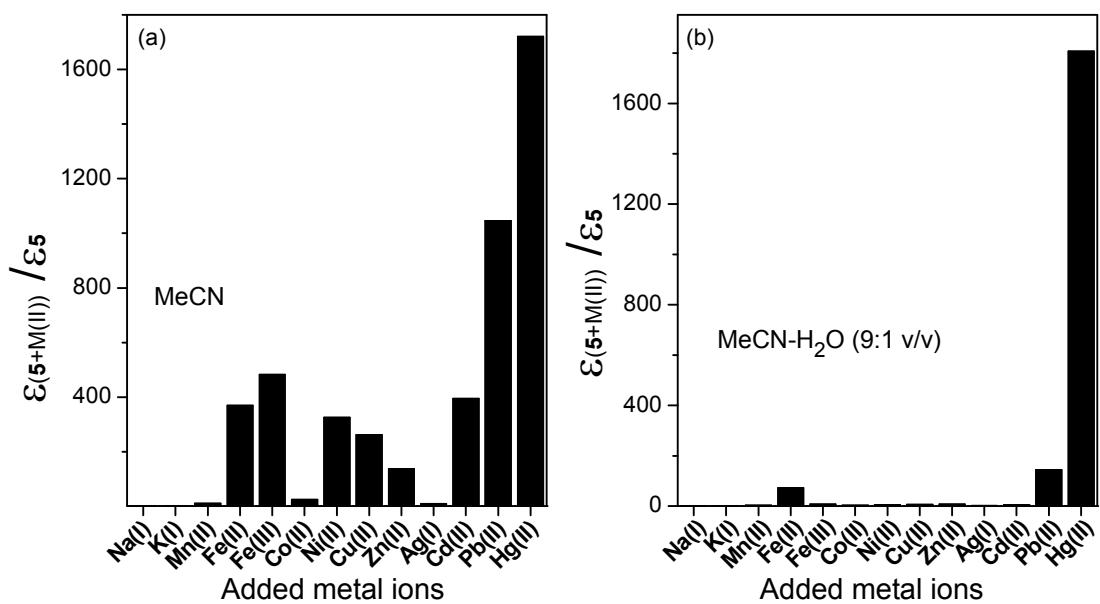


**Fig. 11**: Absorption enhancement factors of **5** in presence of various metal ions in (a) MeCN and (b) MeCN-H$_2$O (1:1 v/v) {[**5**] = $1 \times 10^{-4}$ M, [**M(II/I)**] = $1 \times 10^{-3}$ M}showing aqueous promoted Hg(II) selectivity.
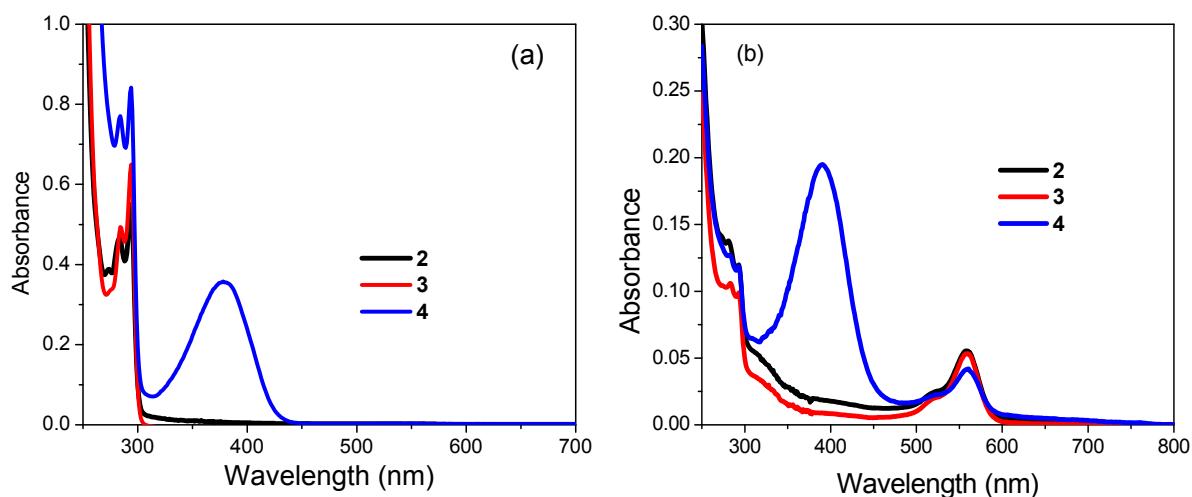
**Fig. S12**: Absorption spectral pattern of **2-4** (a) at pH = 4.0(conc. ~ $1\times10^{-4}$ M) and (b) pH = 1.0 (conc. ~ $1\times10^{-5}$ M) showing ring-opening of rhodamine under more acidic conditions.
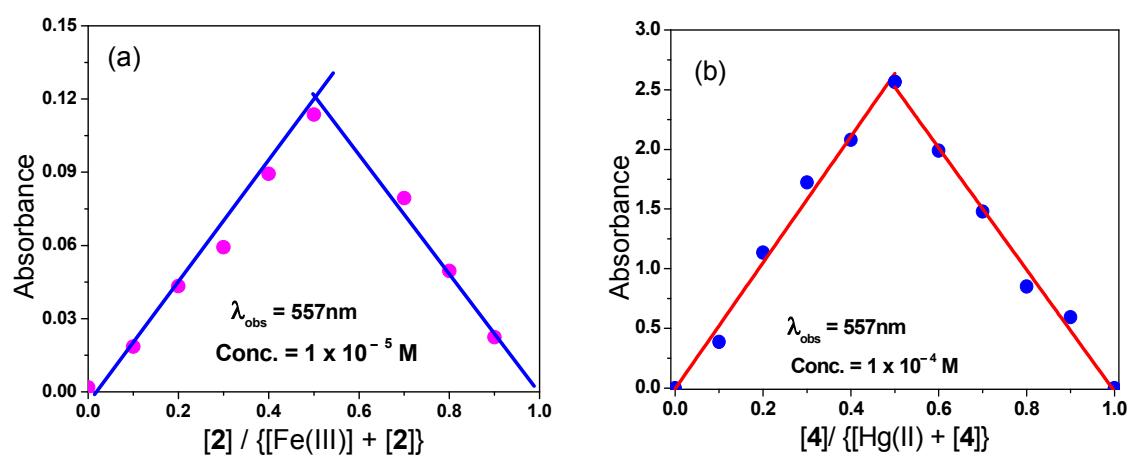


**Fig. S13**: Plot of absorption of (a) **2** and (b) **4** against mole fraction of metal ions added (Jobs plot) in MeCN and observed at 557 nm.

*Determination of association constants*:

The association constants($k_a$) were determined from the change in fluorescence resulted from the titration of dilute solutions ($\sim 10^{-6}$ M) of the probes in MeCN or MeCN-$H_2O$ (9:1 v/v) against metal ion solutions following the equation (eqn. 1),

$$I_0/(I_F - I_0) = [a/(b - a)][(1 / k_a[M]) + 1] \qquad \ldots\ldots eqn.\ 1$$

where $I_0$ and $I_F$ are the fluorescence intensity of the probe in absence and presence of added metal ions respectively, a and b are the constants which are dependent upon gain of the spectrofluorometer and [M] is the concentration of metal ion added. The intercept/slope ratio of the linear regression of the plot of $I_0/(I_F - I_0)$ against the reciprocal of the concentration of metal ion added determines the association constant($k_a$).

The association constants were also determined from the change in absorption ($A_{557}$) resulted from the titration of the probes against metal ion solutions following in MeCN or MeCN-$H_2O$ (9:1 v/v) Benesi-Hildebrand equation (eqn. 2), *i. e.*

$$1/(A - A_0) = [\{1 / k_a[M]\ (A_{max}-A_0)\} + \{1/(A_{max}-A_0)\}] \qquad \ldots\ldots eqn.\ 2$$

where $A_0$ is the absorbance of the probes at 557 nm, A is absorbance attained with added [M], $A_{max}$ is the absorbance obtained with excess amount of metal ion added and [M] is the concentration of metal ion added. The plot of absorption spectral change $(A_{max}-A_0)/(A-A_0)$ as a function of added metal ion concentration (1/ [M]) results in a linear regression and its slope determines the $K_a(M^{-1})$.

**Table ST1**: Association constants determined through fluorescence and absorption titrations of the complexes of these probes and their correlation factors.

| Complexes | $k_a$ ($M^{-1}$) (Fluorescence) | $k_a$ ($M^{-1}$) (Absorption) | Correlation factor log [$k_a$(fluorescence)/$k_a$(absorption)] |
|---|---|---|---|
| 2⊂Fe(III) | $5.810\times10^5$ | $1.451\times10^4$ | 1.60 |
| 3⊂Fe(III) | $3.829\times10^5$ | $8.932\times10^4$ | 0.63 |
| 3⊂Hg(II) | $1.226\times10^5$ | $9.930\times10^4$ | 0.09 |
| 4⊂Hg(II) | $8.746\times10^4$ | $7.310\times10^4$ | 0.08 |
| 5⊂Hg(II) | $8.527\times10^4$ | $1.132\times10^4$ | 0.87 |
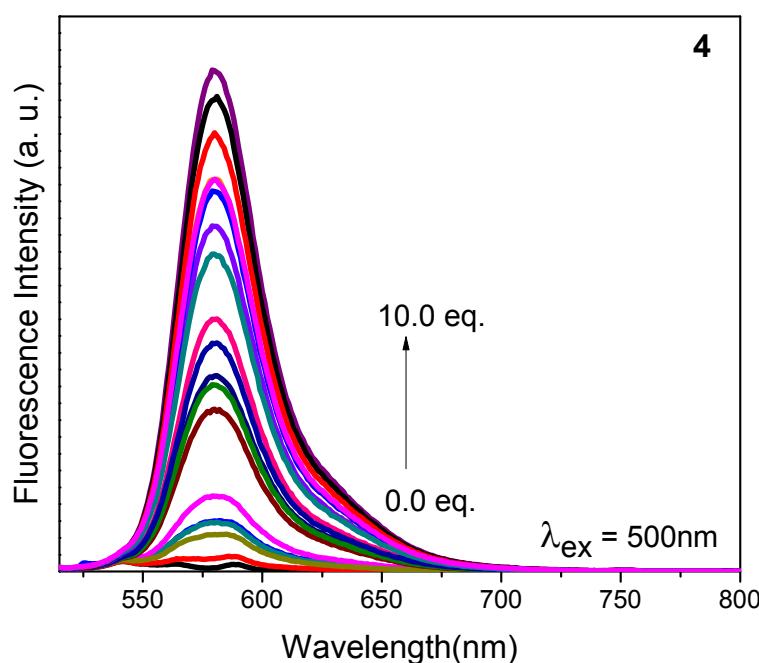
**Fig. S14**: Fluorescence spectral pattern of **4** as a function of added Hg(II) ions in MeCN-$H_2O$(9:1 v/v). Concentration of the probes $1 \times 10^{-6}$M, $\lambda_{ex}$= 500nm, RT, excitation and emission b. p. = 5nm. Each spectrum was taken after 1 min of Hg(II) addition. The error in fluorescence intensity ~ 10 % is due to lower concentration of the complex.
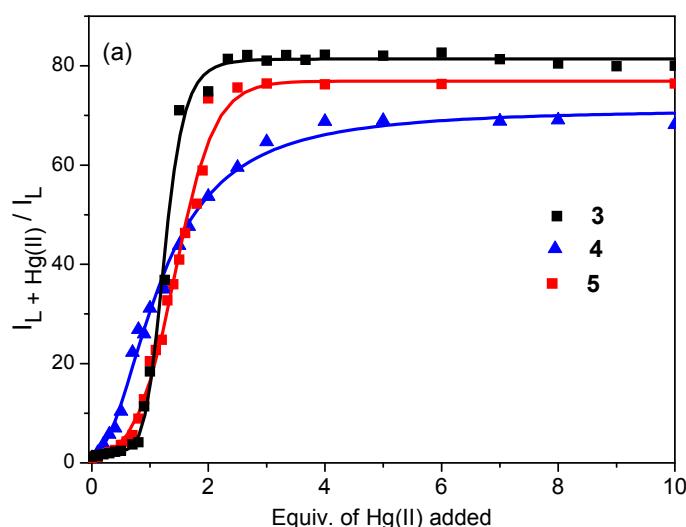


**Fig. S15**: (a) Change in fluorescence intensity ($I_{F(L+Hg(II))}/I_{0(L)}$) of **3** ($0.6 \times 10^{-6}$ M), **4** ($0.6 \times 10^{-6}$ M) and **5** ($1 \times 10^{-6}$ M) respectively as a function of concentration of Hg(II) added in MeCN-$H_2O$(1:1 v/v) (normalized with the emission of free probes). Experimental condition for fluorescence: $\lambda_{ex}$=500nm, RT, emission/excitation b. p. = 5nm, error in $I_F$ < 10 % in each cases.

SP14
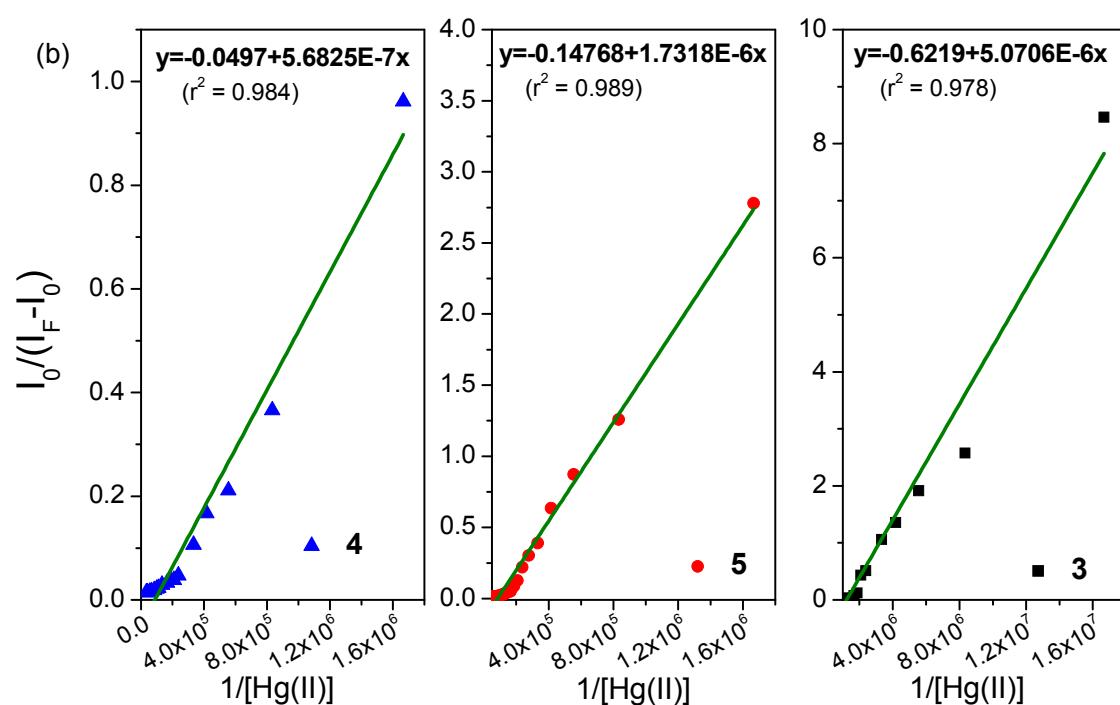
**Fig. S16**: Corresponding linear regression plot of $I_0/(I_F - I_0)$ vs. $1/[Hg(II)]$ M$^{-1}$ of **3**, **4** and **5** in Fig. S15. The complex stability constant ($K_a$) is determined as the intercept/ slope ratio of the linear regression. The data points at higher Hg(II) concentration (lower $1/[Hg(II)]$ values) are also shown only to illustrate that the $\Delta I_F$ remains constant after saturation of complexation, however, data points up to saturation of complexation were taken for the determination of linear regression.
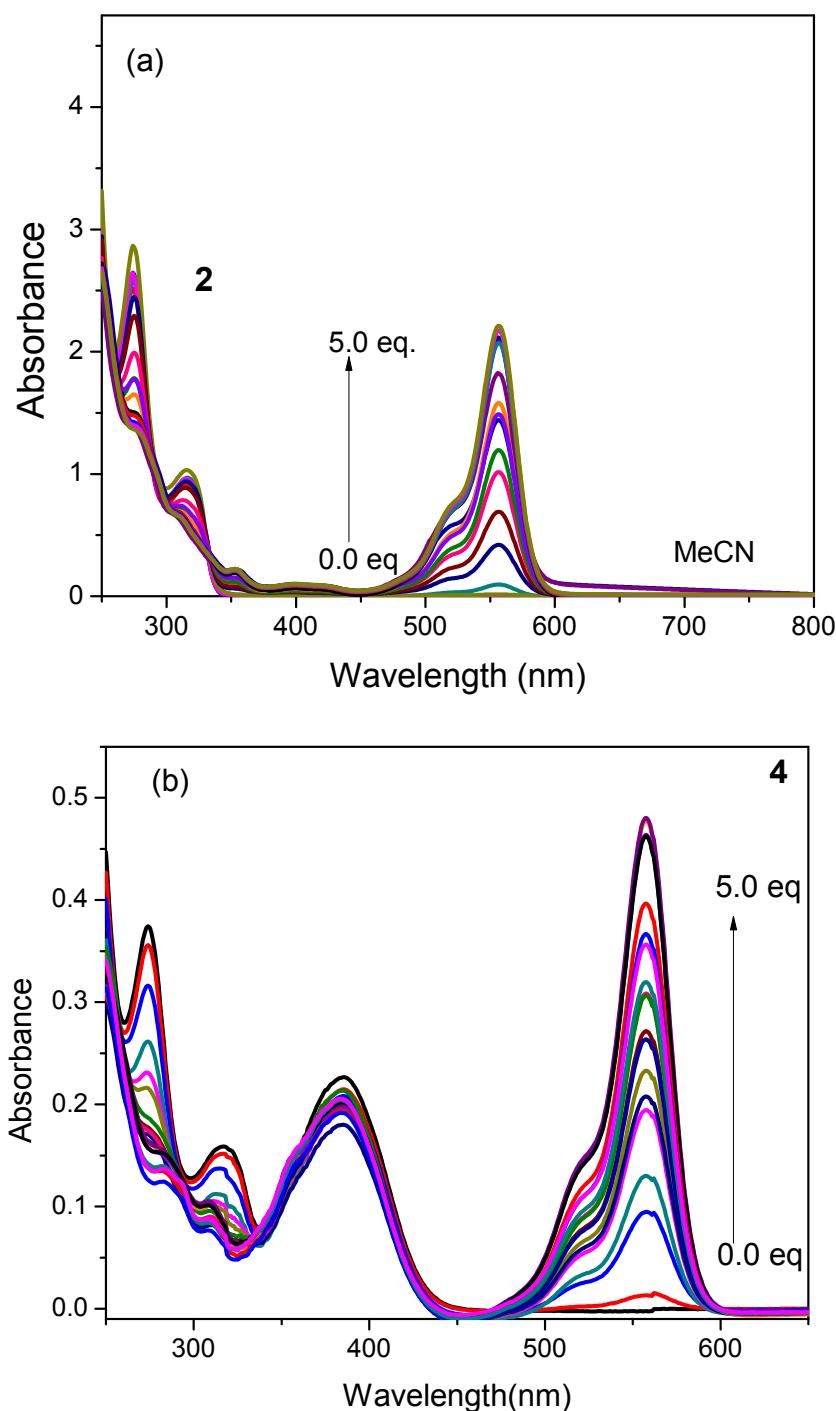
**Fig. S17**: Absorbance spectral pattern of (a) **2** (conc. $= 2.5 \times 10^{-4}$M) in presence of different equivalents of Fe(III) added in MeCN, (b) **4** (conc. $= 2.5 \times 10^{-5}$M) with different equivalents of Hg(II) ions added in MeCN-H$_2$O(1:1 v/v).
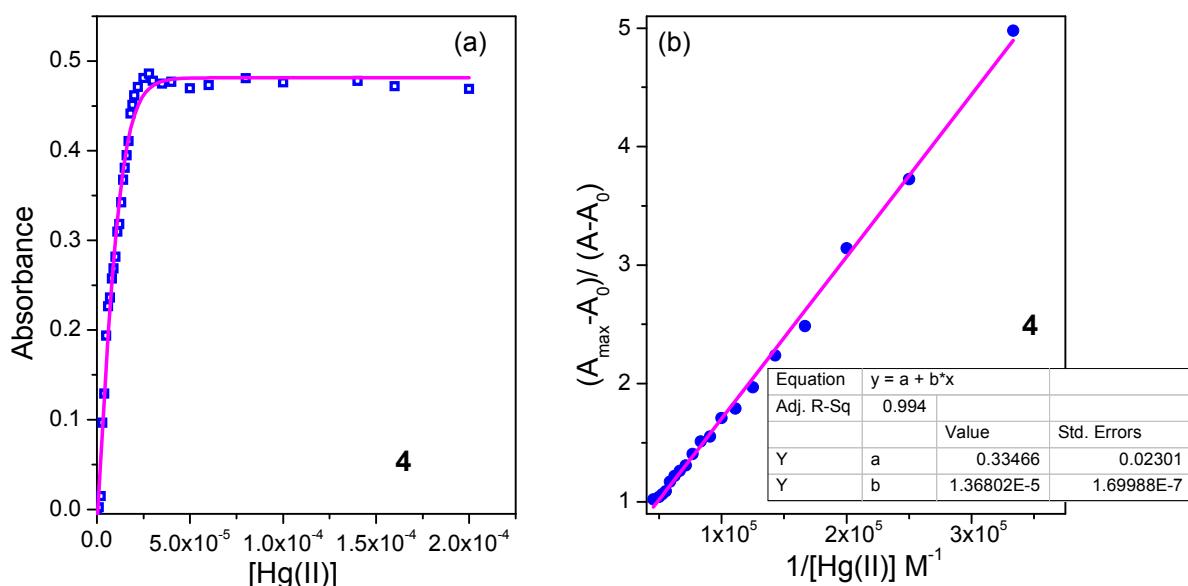
**Fig. S18**: (a) Plot of absorption ($A_{557}$) of **4** (conc. = $2.5 \times 10^{-5}$M) as a function of concentration of Hg(II) ion added in MeCN-$H_2O$(1:1 v/v), (b) linear regression plot of change in absorption [($A_{max}$-$A_0$) /($A$-$A_0$)] against reciprocal of concentration of Hg(II) added (1/[Hg(II)] $M^{-1}$).
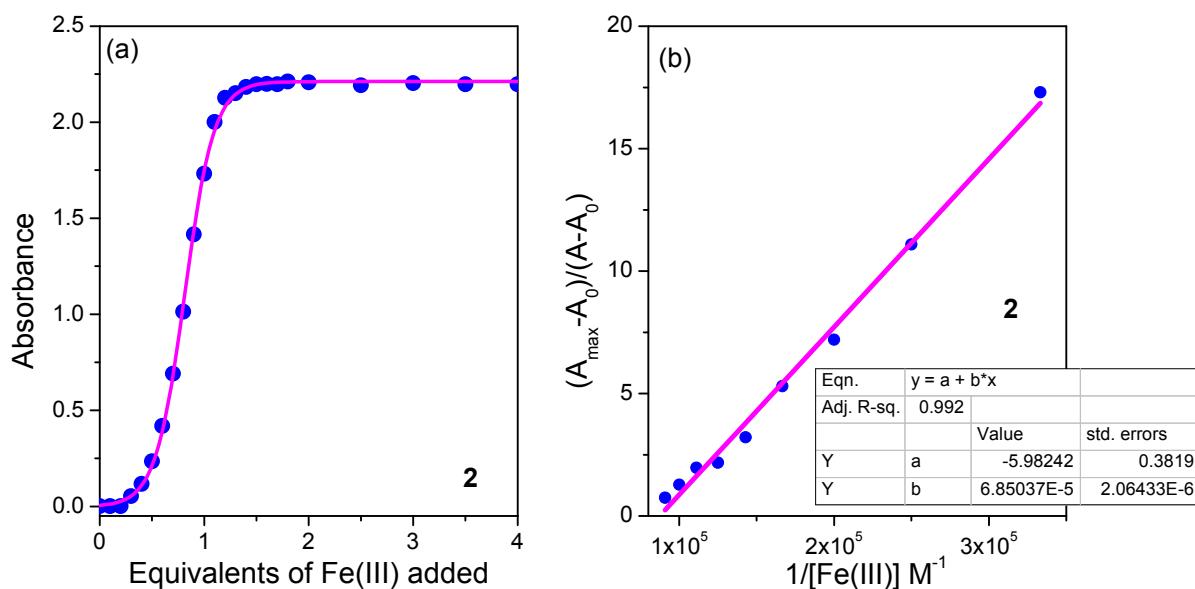


**Fig. S19**: (a) Plot of absorption ($A_{557}$) of **2** (conc. = $2.5 \times 10^{-4}$M) as a function of concentration of Fe(III) ion added in MeCN, (b) linear regression plot of change in absorption [($A_{max}$-$A_0$) /($A$-$A_0$)] against reciprocal of concentration of Fe(III) added (1/[Fe(III)] $M^{-1}$).
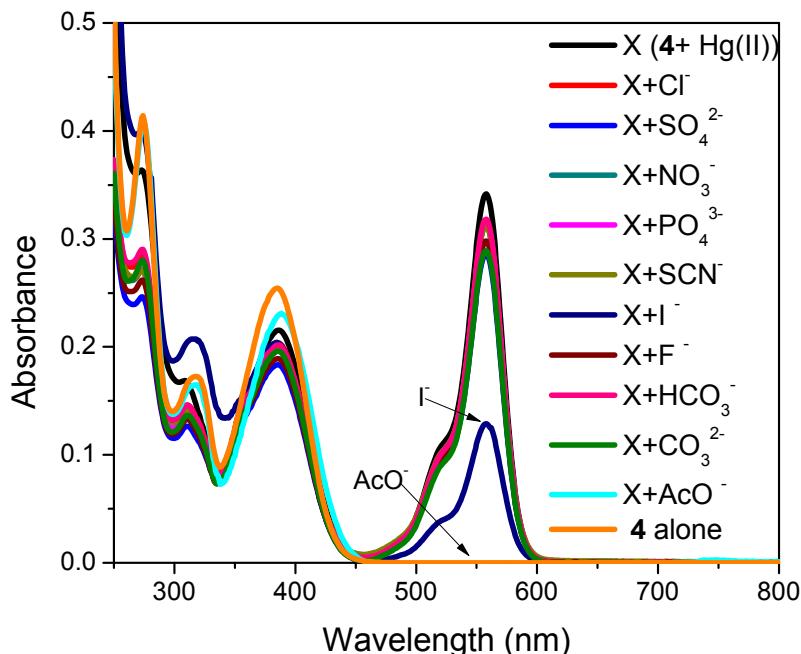
**Fig. S20**: Change in absorption spectra of the solution containing **4** (1 equiv.) and $Hg^{II}$ (5 equiv.) upon addition of various anions (10 equiv.). [**4**] = $2.0 \times 10^{-5}$ M, $MeCN-H_2O$ (9:1 v/v).
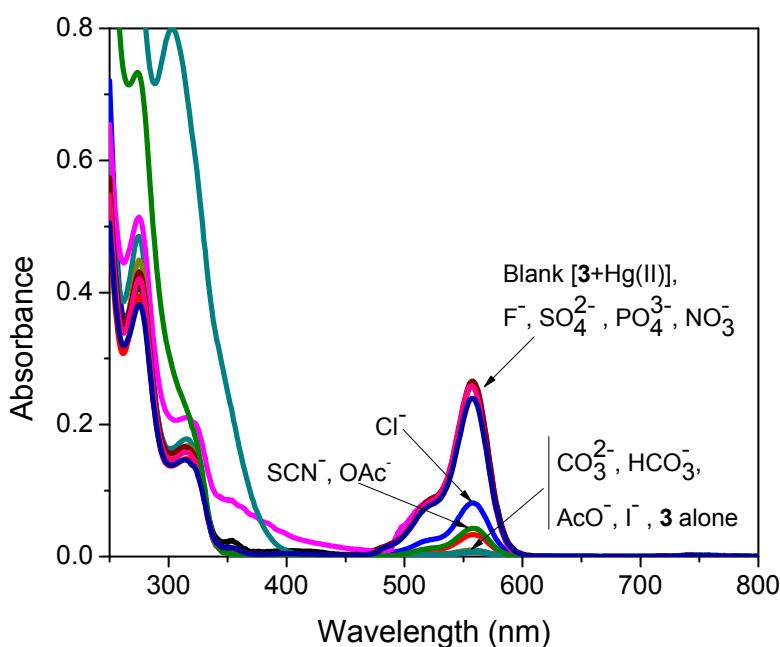


**Fig. S21**: Change in absorption spectra of the solution containing **3** (1 equiv.) and $Hg^{II}$ (5 equiv.) upon addition of various anions (10 equiv.). [**3**] = $1.0 \times 10^{-5}$ M, $MeCN-H_2O$ (9:1 v/v).
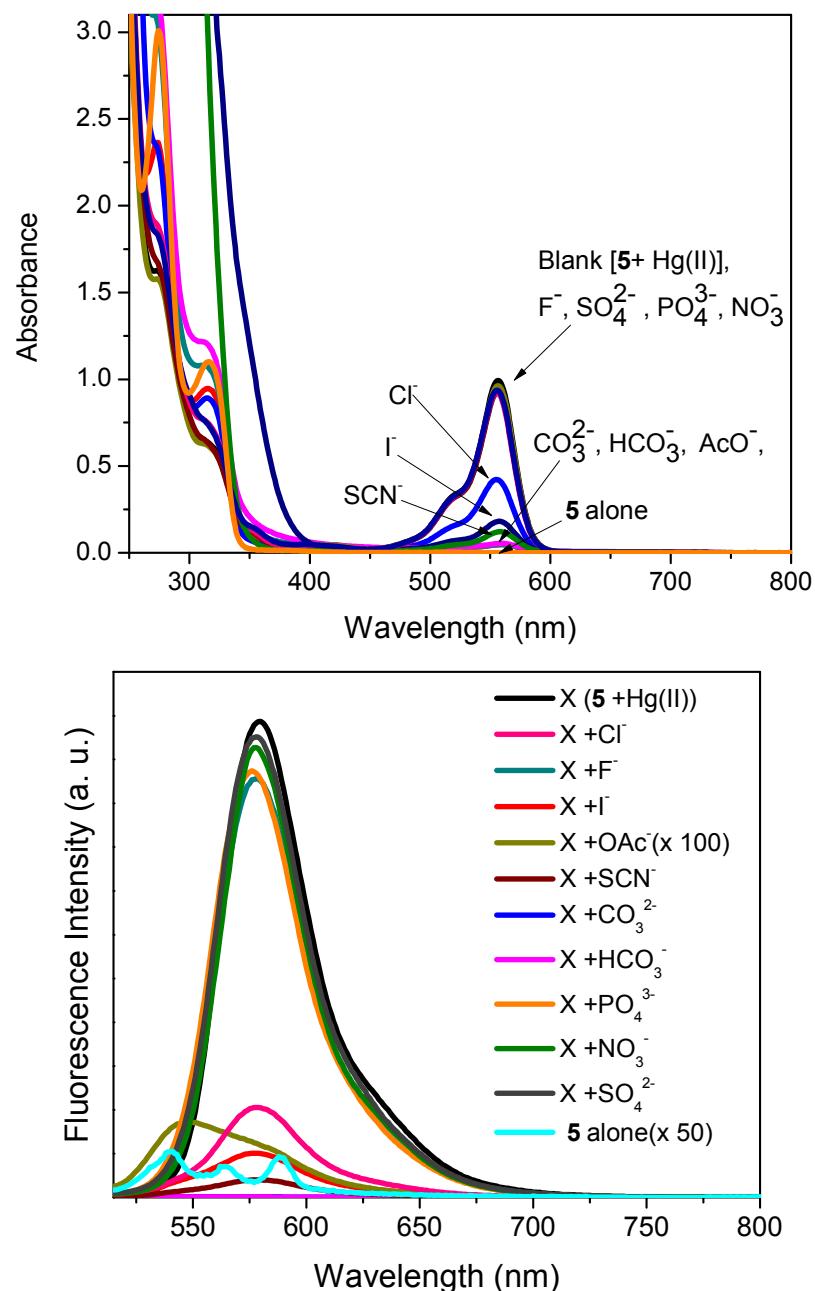
**Fig. S22**: Change in (a) absorption and (b) fluorescence intensity upon addition of various anions (10 equiv.) to the solution containing **5** (1 equiv.) and $Hg^{II}$ (5 equiv.). Conc. of **5** = $0.5 \times 10^{-4}$ M (abs) and $1.0 \times 10^{-6}$ M (em), $\lambda_{ex}$ = 500nm, ex./em. b. p. = 5nm, MeCN-$H_2O$(9:1 v/v).
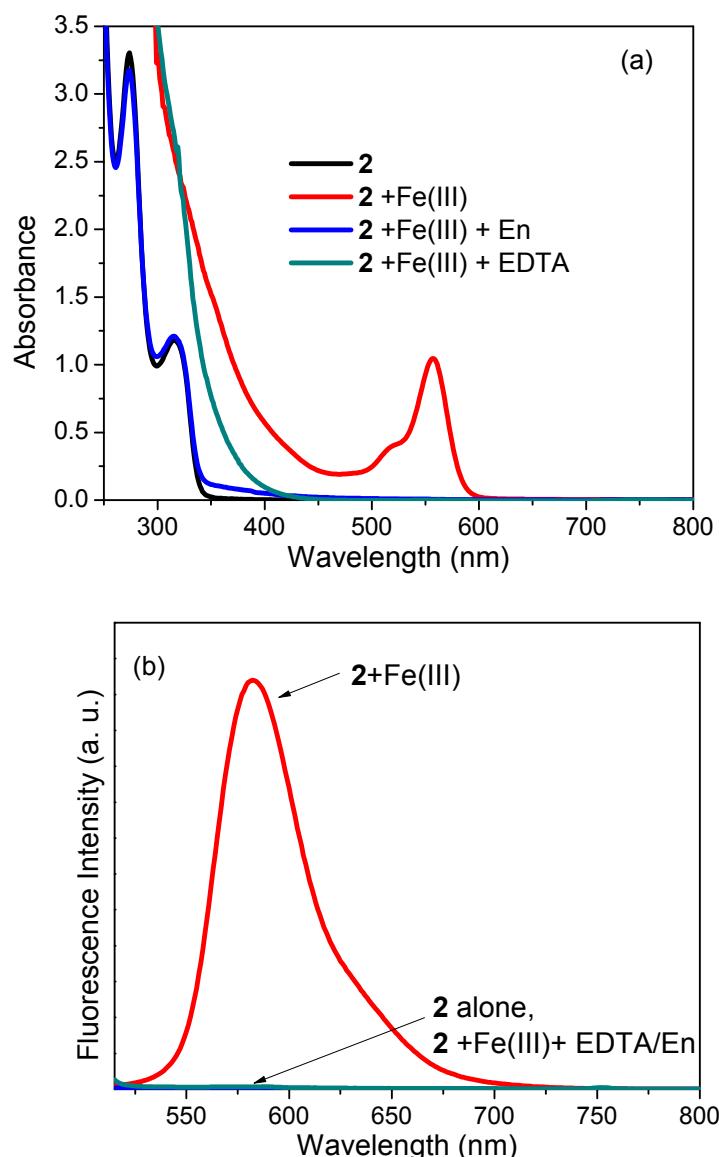
**Fig. S23**: (a) Absorbance and (b) fluorescence spectral responses of **2** alone, in presence of Fe(III) and on subsequent addition of chelating agents (EDTA or Ethylenediamine, En). [**2**] = 1.0 ×$10^{-4}$ M (abs) and 1.0 ×$10^{-6}$ M (em), $\lambda_{ex}$ = 500nm, ex. & em. b. p. = 5nm, MeCN.

*Geometrical optimization of the probes:*
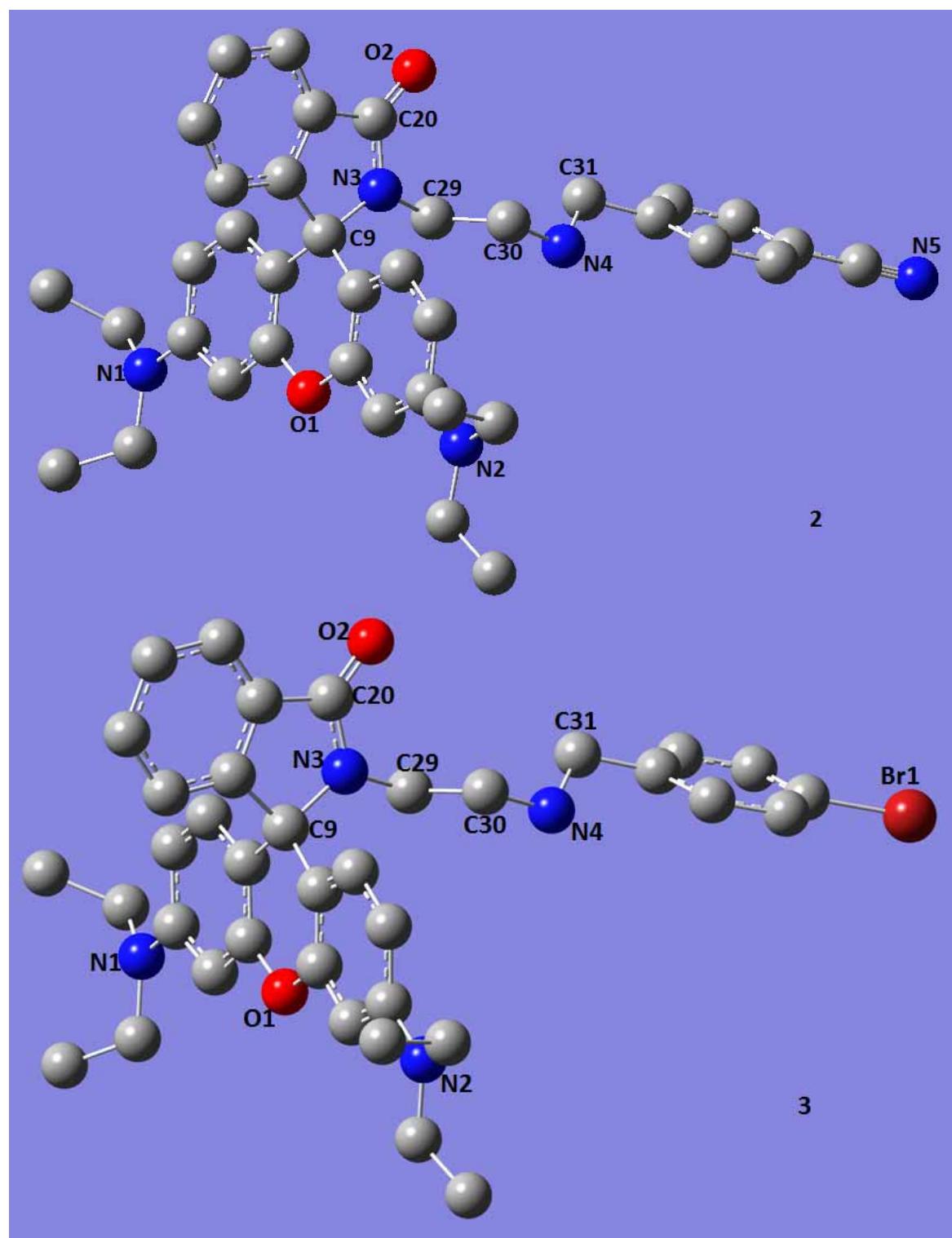


**Fig. S24**: Optimized geometries of **2** and **3**.
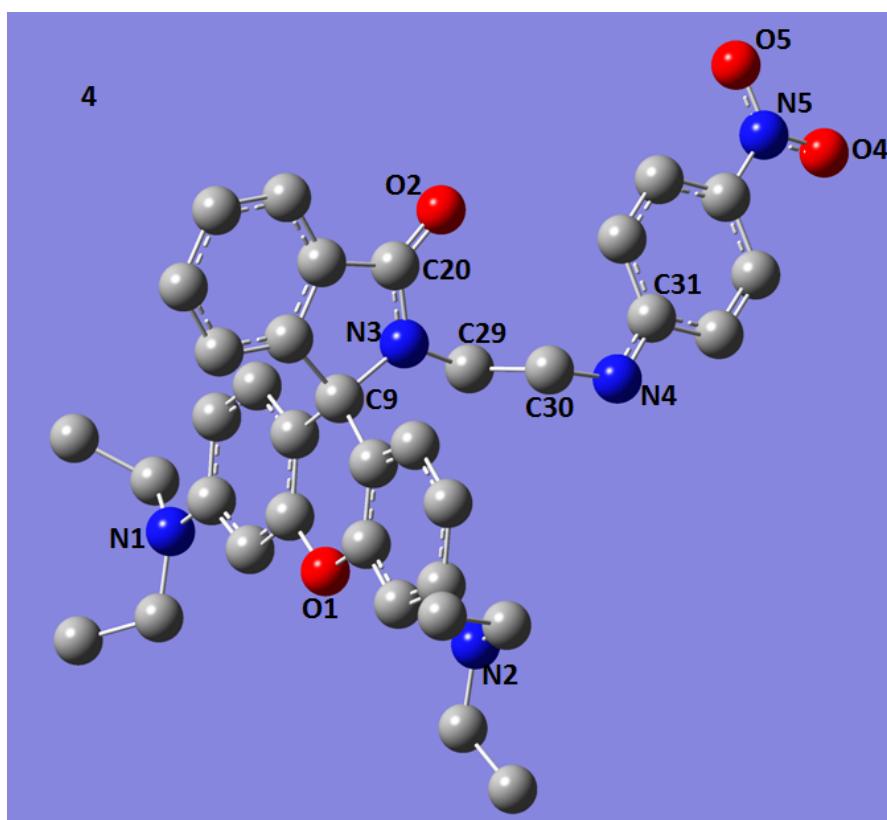
**Fig. S25**: Optimized geometry of **4**.

**Table ST 2**: Selected geometrical parameters of optimized structures of the probes (**2-4**).

| Parameters / Probe | 2 | 3 | 4 |
|---|---|---|---|
| substituent | (ethyl-phenyl-CN) | (ethyl-phenyl-Br) | (methyl-phenyl-NO$_2$) |
| C20 = O2 (Å) | 1.252 | 1.252 | 1.255 |
| C20—N3 (Å) | 1.380 | 1.380 | 1.379 |
| N4—C31 (Å) | 1.462 | 1.464 | 1.378 |
| O2……N4 (Å) | 4.462 | 4.491 | 4.557 |
| N3……N4 (Å) | 3.820 | 3.822 | 4.533 |
| $\angle \sum$N4 (°) | 346.56 | 346.53 | 359.99 |
| Φ (O2-C20-N4-C31, °) | -31.624 | -29.597 | -20.131 |
| Φ (C20-N3-N4-C31, °) | -53.19 | -50.728 | -40.295 |
| $\mu_{GS}$ (D) | 10.46 | 8.13 | 15.74 |

**Table ST3**: The Cartesian coordinates (in Å) for optimized geometries of the probes (**2-4**):

**Probe 2 (**$C_{38}H_{41}N_5O_2$**)**

| Atomic Type | X | Y | Z | Atomic Type | X | Y | Z |
|---|---|---|---|---|---|---|---|
| C | 3.211118 | -2.981250 | 0.061934 | H | 5.919316 | -3.772370 | -5.029535 |
| C | 2.588710 | -2.028297 | 0.855876 | H | 6.561142 | -3.692527 | -3.375915 |
| C | 2.153241 | -0.791133 | 0.349124 | H | 4.487702 | -2.351771 | -3.648734 |
| C | 2.373836 | -0.574268 | -1.014829 | H | 3.587742 | -3.769437 | -4.191030 |
| C | 2.987196 | -1.514913 | -1.844146 | C | 4.116143 | -5.113803 | -1.675648 |
| C | 3.436379 | -2.746438 | -1.324733 | H | 3.227810 | -5.318484 | -1.066023 |
| C | 1.393592 | 1.627895 | -0.908976 | C | -0.234506 | 6.271153 | -1.021523 |
| C | 1.143756 | 1.505195 | 0.460971 | H | -0.783082 | 6.903734 | -1.720932 |
| C | 0.570861 | 2.627797 | 1.086140 | H | -0.953822 | 6.009598 | -0.238850 |
| H | 0.366841 | 2.582936 | 2.151252 | C | 0.507843 | 5.089394 | -3.163829 |
| C | 0.257549 | 3.789541 | 0.395026 | H | 1.603528 | 5.067161 | -3.290237 |
| C | 0.498853 | 3.895474 | -1.005751 | H | 0.123303 | 4.172879 | -3.628828 |
| C | 1.094010 | 2.781693 | -1.635944 | C | 0.943652 | 7.059746 | -0.421969 |
| H | 3.546462 | -3.899915 | 0.523179 | H | 0.581283 | 7.949562 | 0.106910 |
| H | 2.439382 | -2.241996 | 1.909481 | H | 1.509032 | 6.445905 | 0.286802 |
| H | 3.067838 | -1.258381 | -2.890275 | H | 1.633644 | 7.388949 | -1.207512 |
| H | -0.173825 | 4.613772 | 0.945598 | C | -0.061115 | 6.281776 | -3.937349 |
| H | 1.347901 | 2.774010 | -2.685144 | H | -1.153476 | 6.323602 | -3.870586 |
| O | 1.981351 | 0.603695 | -1.659997 | H | 0.347206 | 7.239406 | -3.598638 |
| N | 4.068511 | -3.707683 | -2.119360 | H | 0.203182 | 6.168905 | -4.994478 |
| N | 0.161144 | 5.042273 | -1.727622 | N | -3.317495 | -0.442515 | 0.325298 |
| C | 4.402919 | -3.432817 | -3.526925 | H | -3.190133 | -0.476035 | -0.681825 |
| C | 1.450912 | 0.230169 | 1.240221 | C | 5.384062 | -5.502525 | -0.895921 |
| C | 2.239659 | 0.511730 | 2.529301 | H | 5.324070 | -6.549264 | -0.572744 |
| C | 1.507196 | 0.107329 | 3.650385 | H | 6.279776 | -5.386062 | -1.513092 |
| C | 3.504141 | 1.077133 | 2.677764 | H | 5.509488 | -4.876630 | -0.006869 |
| C | 2.006606 | 0.250703 | 4.944126 | H | 4.024571 | -5.748060 | -2.565735 |
| C | 4.016493 | 1.226514 | 3.975109 | C | -4.039124 | -1.593917 | 0.866765 |
| H | 4.080259 | 1.393691 | 1.814516 | H | -4.093062 | -1.450298 | 1.955927 |
| C | 3.277075 | 0.818035 | 5.098758 | H | -3.525805 | -2.562027 | 0.717775 |
| H | 1.417309 | -0.072165 | 5.795696 | C | -5.449043 | -1.706870 | 0.309532 |
| H | 5.000172 | 1.664226 | 4.113650 | C | -6.052927 | -2.966034 | 0.156564 |
| H | 3.696703 | 0.944711 | 6.091509 | C | -6.182063 | -0.558494 | -0.033471 |
| C | 0.209126 | -0.457405 | 3.207511 | C | -7.359525 | -3.083700 | -0.316315 |
| O | -0.708994 | -0.938023 | 3.910933 | H | -5.492696 | -3.862104 | 0.407909 |
| N | 0.192938 | -0.362009 | 1.830307 | C | -7.487843 | -0.661924 | -0.510705 |
| C | -0.921244 | -0.866596 | 1.032260 | H | -5.704598 | 0.408710 | 0.072402 |
| H | -1.201724 | -1.851106 | 1.425636 | C | -8.090630 | -1.928111 | -0.655857 |
| H | -0.570681 | -0.999907 | 0.003208 | H | -7.816752 | -4.060219 | -0.431855 |
| C | -2.159080 | 0.066159 | 1.061836 | H | -8.048309 | 0.228880 | -0.772838 |
| H | -2.453422 | 0.215108 | 2.106571 | C | -9.429270 | -2.039472 | -1.149616 |
| H | -1.886216 | 1.044003 | 0.651932 | N | -10.527906 | -2.130379 | -1.555864 |
| C | 5.728933 | -4.055338 | -3.987795 | | | | |
| H | 5.717790 | -5.148958 | -3.942478 | | | | |

**Probe 3** ($C_{37}H_{41}N_4O_2Br$)

| Atomic Type | Coordinates (Angstroms) | | |
|---|---|---|---|
| | X | Y | Z |
| C | 3.441347 | -3.267250 | 0.047809 |
| C | 3.023260 | -2.209479 | 0.842981 |
| C | 2.701765 | -0.947464 | 0.312520 |
| C | 2.820548 | -0.816250 | -1.074861 |
| C | 3.227826 | -1.862201 | -1.905001 |
| C | 3.560687 | -3.120496 | -1.363652 |
| C | 2.124117 | 1.492596 | -1.001185 |
| C | 1.987027 | 1.458570 | 0.389299 |
| C | 1.605065 | 2.666680 | 1.000857 |
| H | 1.493882 | 2.690565 | 2.080233 |
| C | 1.364593 | 3.826799 | 0.278536 |
| C | 1.485396 | 3.842907 | -1.141398 |
| C | 1.892406 | 2.641643 | -1.759988 |
| H | 3.700774 | -4.202879 | 0.523995 |
| H | 2.948018 | -2.359315 | 1.915300 |
| H | 3.240772 | -1.661570 | -2.966295 |
| H | 1.081563 | 4.719008 | 0.819343 |
| H | 2.049251 | 2.559681 | -2.824886 |
| O | 2.522483 | 0.375756 | -1.744255 |
| N | 3.982082 | -4.189895 | -2.160530 |
| N | 1.209736 | 4.988838 | -1.892029 |
| C | 4.218683 | -4.013600 | -3.603454 |
| C | 2.215644 | 0.190798 | 1.206671 |
| C | 3.154197 | 0.429803 | 2.400403 |
| C | 2.492573 | 0.164085 | 3.603876 |
| C | 4.483746 | 0.845653 | 2.402118 |
| C | 3.128615 | 0.301165 | 4.836894 |
| C | 5.133399 | 0.988030 | 3.637309 |
| H | 5.006226 | 1.054645 | 1.474350 |
| C | 4.464814 | 0.718644 | 4.844174 |
| H | 2.591518 | 0.087279 | 5.754779 |
| H | 6.169593 | 1.310805 | 3.661437 |
| H | 4.990697 | 0.836257 | 5.786158 |
| C | 1.100092 | -0.257637 | 3.312942 |
| O | 0.207302 | -0.595691 | 4.123499 |
| N | 0.960052 | -0.216137 | 1.940180 |
| C | -0.275470 | -0.616049 | 1.270583 |
| H | -0.621238 | -1.553028 | 1.723495 |
| H | -0.041547 | -0.814657 | 0.219178 |
| C | -1.396035 | 0.450521 | 1.377022 |
| H | -1.596984 | 0.634337 | 2.438304 |
| H | -1.041072 | 1.389345 | 0.939019 |
| C | 5.386982 | -4.845258 | -4.151977 |

| Atomic Type | Coordinates (Angstroms) | | |
|---|---|---|---|
| | X | Y | Z |
| H | 5.215487 | -5.922453 | -4.060019 |
| H | 5.520083 | -4.624468 | -5.217402 |
| H | 6.317554 | -4.597569 | -3.631146 |
| H | 4.451606 | -2.962131 | -3.780026 |
| H | 3.303301 | -4.242108 | -4.177210 |
| C | 3.862293 | -5.572138 | -1.658833 |
| H | 3.019698 | -5.617460 | -0.958516 |
| C | 1.056154 | 6.291378 | -1.224467 |
| H | 0.514903 | 6.957026 | -1.898522 |
| H | 0.405088 | 6.165529 | -0.353767 |
| C | 1.408092 | 4.929203 | -3.355305 |
| H | 2.477685 | 4.795087 | -3.591223 |
| H | 0.893805 | 4.032372 | -3.721725 |
| C | 2.386387 | 6.943101 | -0.806252 |
| H | 2.204531 | 7.898219 | -0.298813 |
| H | 2.945448 | 6.293648 | -0.124671 |
| H | 3.018015 | 7.137818 | -1.680746 |
| C | 0.868794 | 6.129642 | -4.138065 |
| H | -0.198864 | 6.286203 | -3.950682 |
| H | 1.403670 | 7.057873 | -3.912721 |
| H | 0.993748 | 5.932327 | -5.208413 |
| N | -2.652654 | 0.077509 | 0.727607 |
| H | -2.600876 | 0.025762 | -0.285506 |
| C | 5.128694 | -6.128649 | -0.986371 |
| H | 4.944788 | -7.144511 | -0.614935 |
| H | 5.965832 | -6.167986 | -1.689445 |
| H | 5.435013 | -5.505524 | -0.140079 |
| H | 3.585720 | -6.212253 | -2.505767 |
| C | -3.464262 | -0.982965 | 1.328364 |
| H | -3.471657 | -0.798666 | 2.412714 |
| H | -3.048026 | -1.999575 | 1.199168 |
| C | -4.890085 | -0.975816 | 0.802069 |
| C | -5.592425 | -2.178467 | 0.627785 |
| C | -5.539346 | 0.233930 | 0.505354 |
| C | -6.917525 | -2.182146 | 0.177593 |
| H | -5.100493 | -3.123298 | 0.842348 |
| C | -6.862065 | 0.245160 | 0.051885 |
| H | -4.989260 | 1.160724 | 0.624269 |
| C | -7.532680 | -0.965860 | -0.103859 |
| H | -7.455907 | -3.112671 | 0.042979 |
| H | -7.362095 | 1.179100 | -0.176142 |
| Br | -9.377511 | -0.958163 | -0.740663 |

**Probe 4** ($C_{36}H_{39}N_5O_4$):

| Atomic Type | Coordinates (Angstroms) | | |
|---|---|---|---|
| | X | Y | Z |
| C | -1.361748 | 3.808570 | 0.259138 |
| C | -0.963087 | 2.640275 | 0.892098 |
| C | -1.393281 | 1.369340 | 0.470747 |
| C | -2.245776 | 1.345544 | -0.637735 |
| C | -2.660550 | 2.500691 | -1.302226 |
| C | -2.237794 | 3.772797 | -0.863783 |
| C | -2.389295 | -1.062445 | -0.582359 |
| C | -1.556792 | -1.135693 | 0.538664 |
| C | -1.315413 | -2.426033 | 1.045224 |
| H | -0.686338 | -2.531564 | 1.923648 |
| C | -1.852455 | -3.568048 | 0.468434 |
| C | -2.682871 | -3.483058 | -0.687115 |
| C | -2.950284 | -2.189926 | -1.185779 |
| H | -1.014047 | 4.754057 | 0.651442 |
| H | -0.300716 | 2.710031 | 1.749129 |
| H | -3.284835 | 2.361082 | -2.172545 |
| H | -1.628606 | -4.524474 | 0.919715 |
| H | -3.595626 | -2.016171 | -2.033634 |
| O | -2.738508 | 0.152449 | -1.180138 |
| N | -2.639898 | 4.953618 | -1.492683 |
| N | -3.204266 | -4.621653 | -1.304799 |
| C | -3.633796 | 4.939284 | -2.580092 |
| C | -0.928297 | 0.099278 | 1.176566 |
| C | -1.163767 | 0.157340 | 2.694872 |
| C | 0.053777 | 0.113738 | 3.383847 |
| C | -2.366486 | 0.242848 | 3.392159 |
| C | 0.112046 | 0.155668 | 4.776324 |
| C | -2.319646 | 0.285358 | 4.793490 |
| H | -3.316084 | 0.276868 | 2.868392 |
| C | -1.094236 | 0.242810 | 5.481046 |
| H | 1.069476 | 0.121209 | 5.284886 |
| H | -3.245193 | 0.352953 | 5.356545 |
| H | -1.086525 | 0.277794 | 6.565510 |
| C | 1.161571 | 0.020777 | 2.405054 |
| O | 2.394872 | -0.037053 | 2.629123 |
| N | 0.579541 | 0.005359 | 1.155165 |
| C | 1.378104 | -0.035879 | -0.064330 |
| H | 2.178638 | 0.708100 | 0.012922 |
| H | 0.740208 | 0.243999 | -0.907018 |
| C | 2.009470 | -1.430639 | -0.296747 |
| H | 2.458600 | -1.775016 | 0.640005 |
| H | 1.231757 | -2.148805 | -0.570987 |

| Atomic Type | Coordinates (Angstroms) | | |
|---|---|---|---|
| | X | Y | Z |
| C | 4.328174 | -1.013725 | -1.182323 |
| C | 4.812769 | -0.520497 | 0.058453 |
| C | 5.223411 | -1.093174 | -2.282832 |
| C | 6.141381 | -0.136062 | 0.181071 |
| H | 4.153910 | -0.422896 | 0.914686 |
| C | 6.546024 | -0.706293 | -2.153901 |
| H | 4.862186 | -1.467948 | -3.236324 |
| C | 7.006523 | -0.228250 | -0.917806 |
| H | 6.521992 | 0.242204 | 1.120939 |
| H | 7.234576 | -0.766243 | -2.986509 |
| N | 8.385454 | 0.176402 | -0.782027 |
| O | 9.146138 | 0.084697 | -1.798049 |
| O | 8.783815 | 0.607476 | 0.345490 |
| C | -4.587980 | 6.142109 | -2.569957 |
| H | -4.066688 | 7.092670 | -2.720848 |
| H | -5.315367 | 6.034788 | -3.382947 |
| H | -5.136221 | 6.195098 | -1.623747 |
| H | -4.238955 | 4.037638 | -2.472283 |
| H | -3.131752 | 4.875977 | -3.561180 |
| C | -1.850817 | 6.188105 | -1.314078 |
| H | -0.801483 | 5.911962 | -1.155745 |
| C | -3.131319 | -5.928109 | -0.630060 |
| H | -3.256581 | -6.706600 | -1.383845 |
| H | -2.119069 | -6.064012 | -0.236085 |
| C | -4.103182 | -4.437595 | -2.463980 |
| H | -5.032871 | -3.934124 | -2.148876 |
| H | -3.603633 | -3.757417 | -3.164897 |
| C | -4.169461 | -6.115814 | 0.490358 |
| H | -4.052300 | -7.098749 | 0.962334 |
| H | -4.057986 | -5.348935 | 1.263899 |
| H | -5.189021 | -6.048934 | 0.093627 |
| C | -4.459153 | -5.716584 | -3.226685 |
| H | -3.563721 | -6.236018 | -3.584382 |
| H | -5.053881 | -6.415036 | -2.629241 |
| H | -5.058045 | -5.444325 | -4.102649 |
| N | 3.021967 | -1.420840 | -1.348052 |
| H | 2.748698 | -1.739060 | -2.265181 |
| C | -2.328402 | 7.101750 | -0.172686 |
| H | -1.670554 | 7.975502 | -0.087497 |
| H | -3.347534 | 7.457125 | -0.350101 |
| H | -2.322944 | 6.575140 | 0.786855 |
| H | -1.877763 | 6.740639 | -2.261086 |
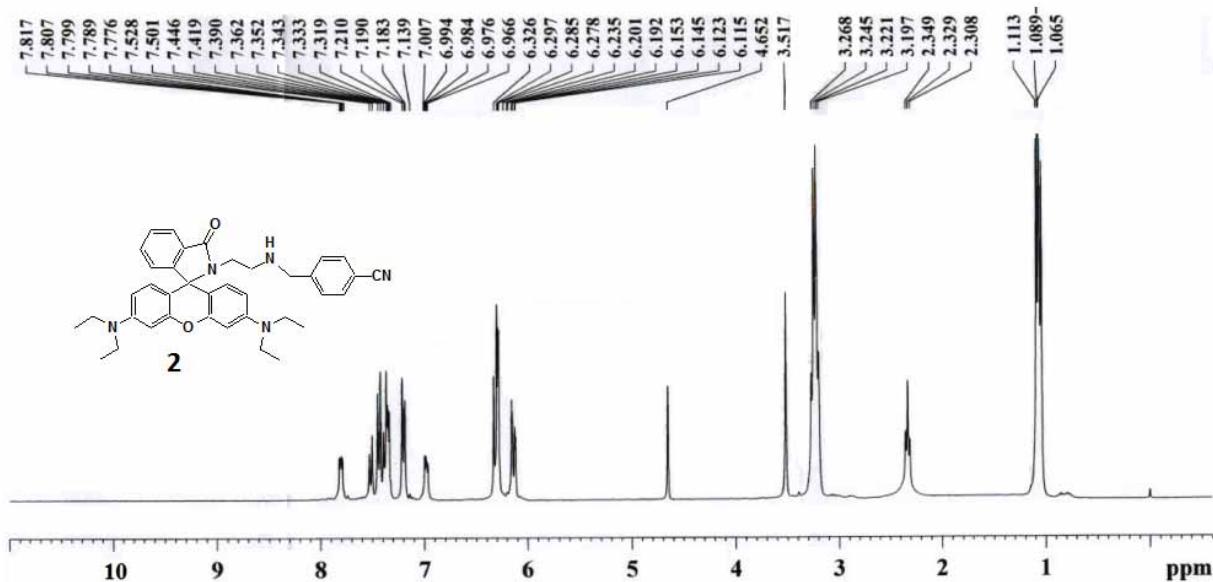
**Fig. S26**: $^{1}$H-NMR spectrum of **2** (CDCl$_3$)
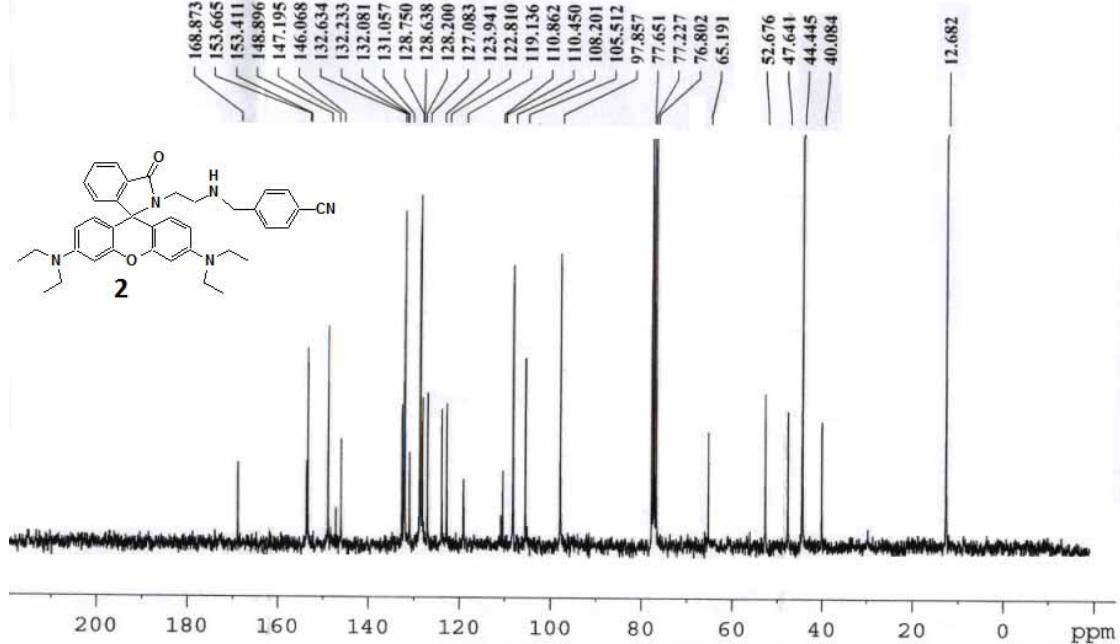


**Fig. S27**: $^{13}$C-NMR spectrum of **2** (CDCl$_3$)

SP26
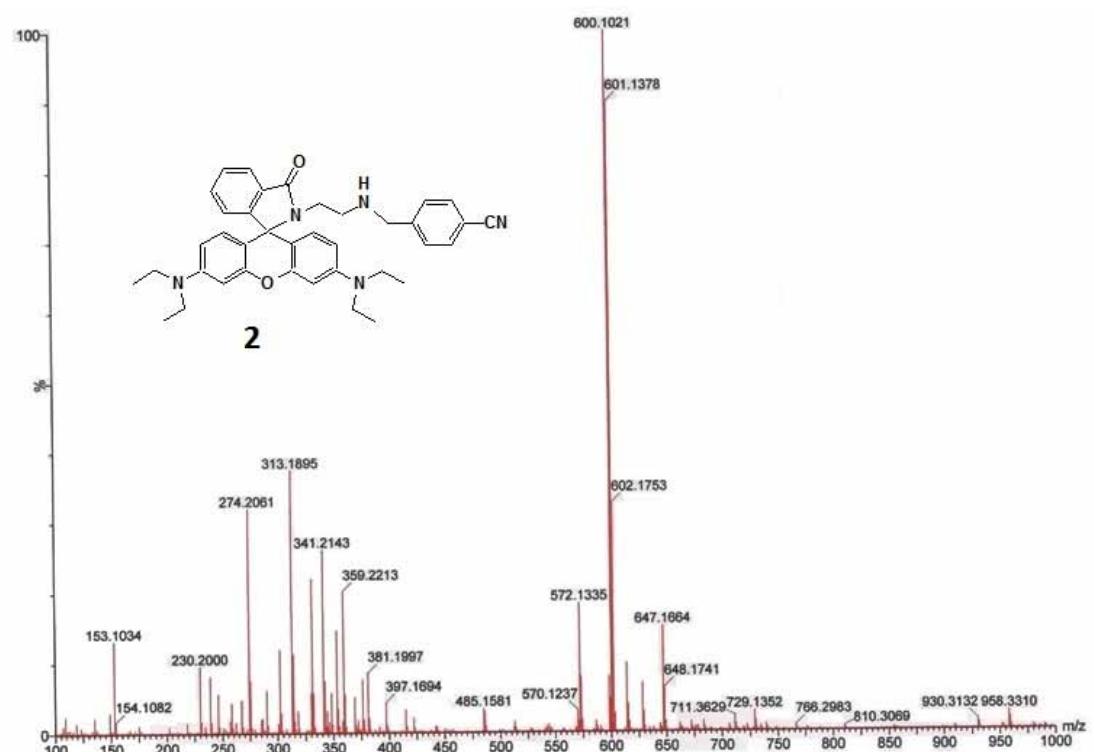
**Fig. S28**: ESI-MS spectrum of **2**
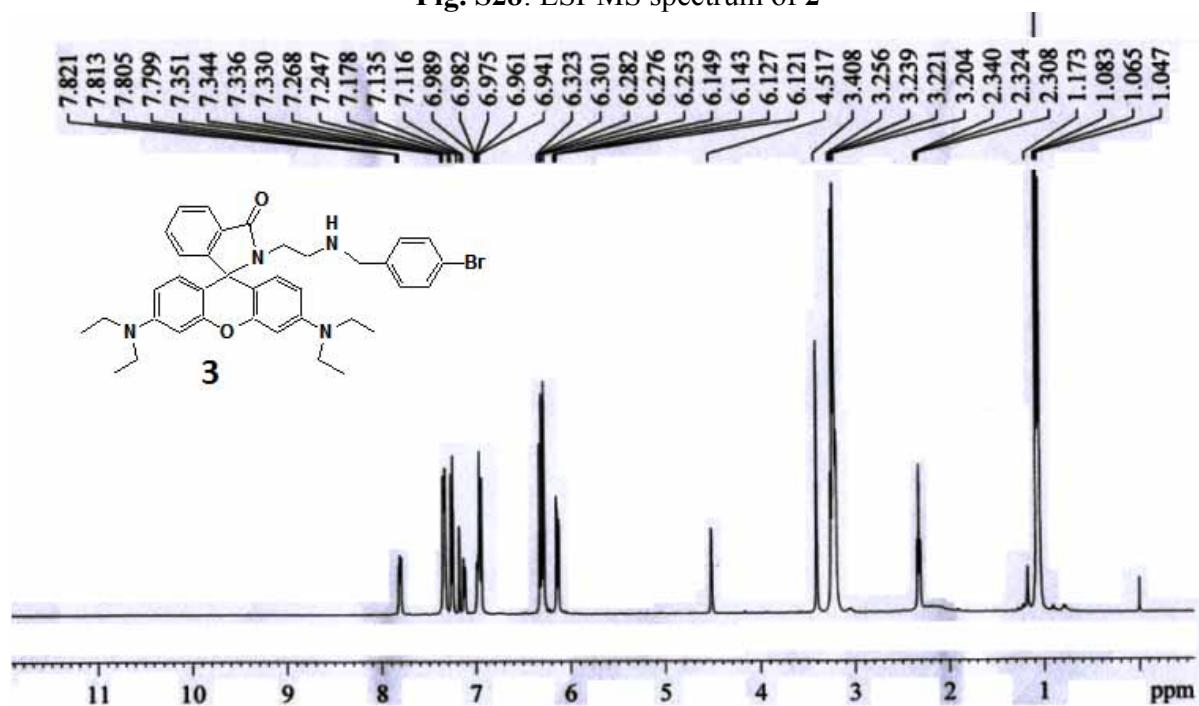


**Fig. S29**: $^1$H-NMR spectrum of **3** (CDCl$_3$)
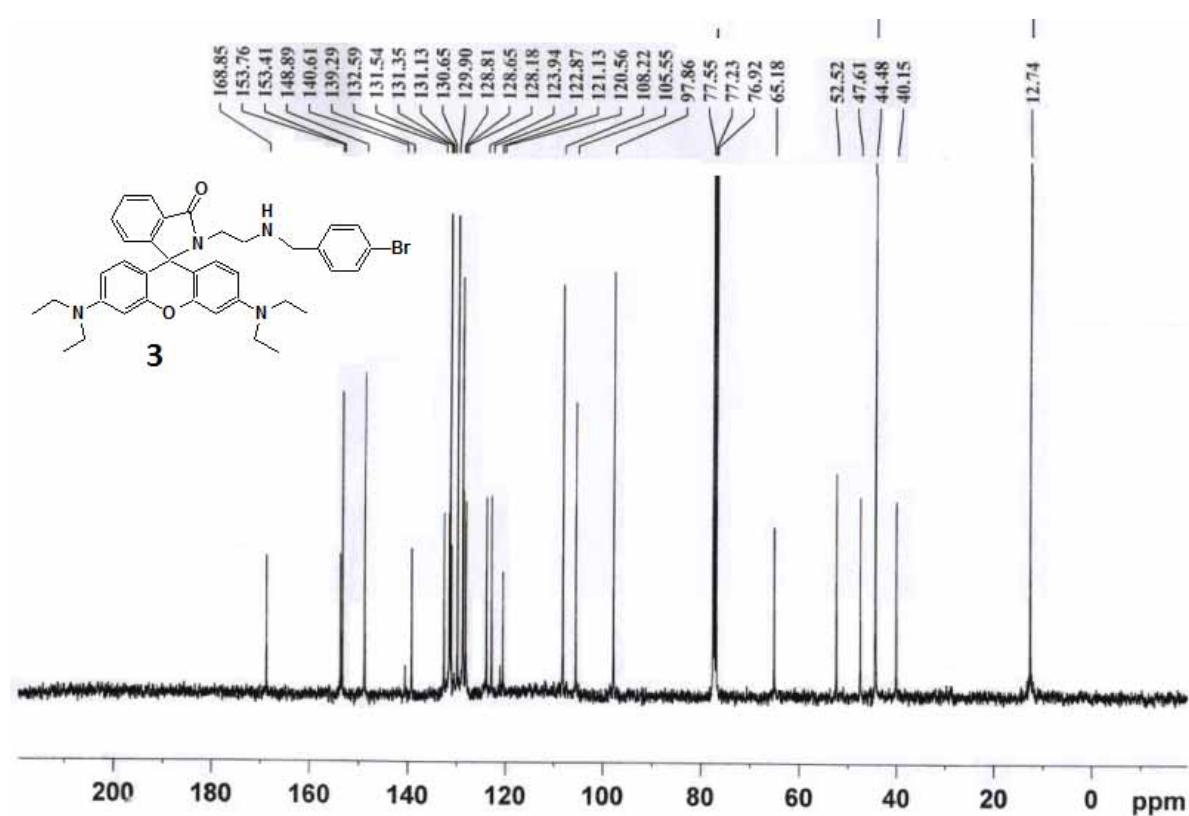
**Fig. S30**: $^{13}$C-NMR spectrum of **3** (CDCl$_3$)
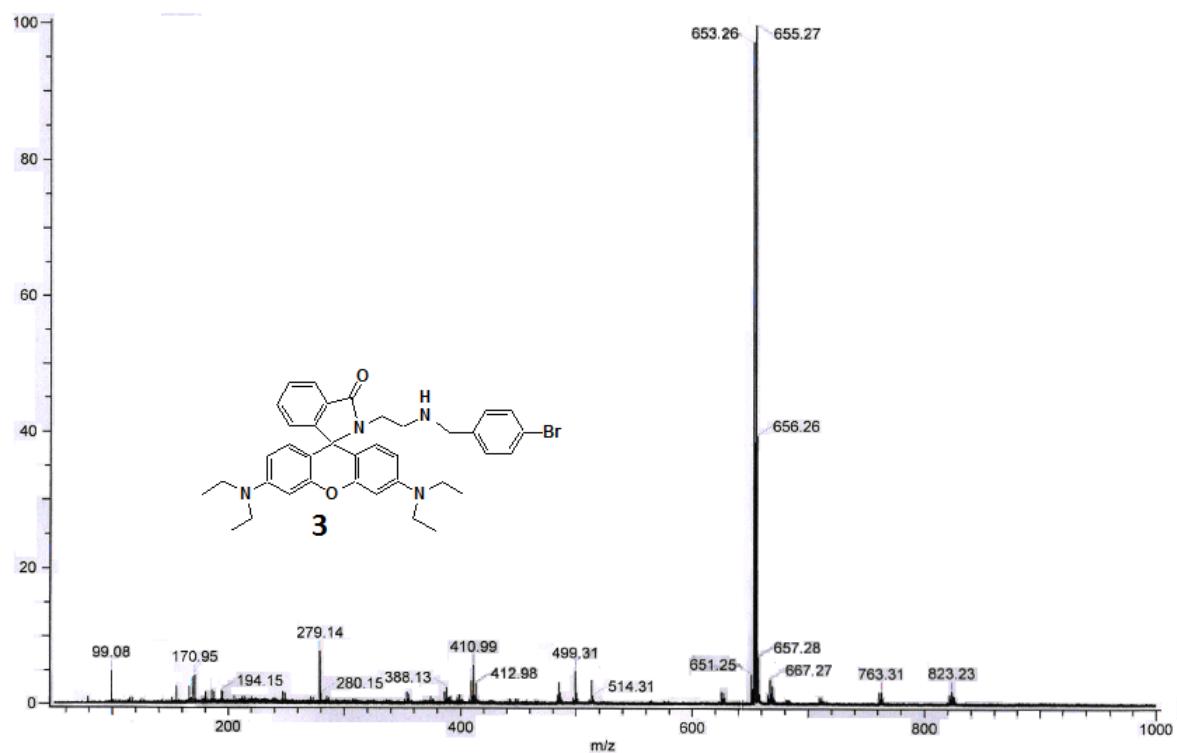


**Fig. S31**: ESI-MS spectrum of **3**
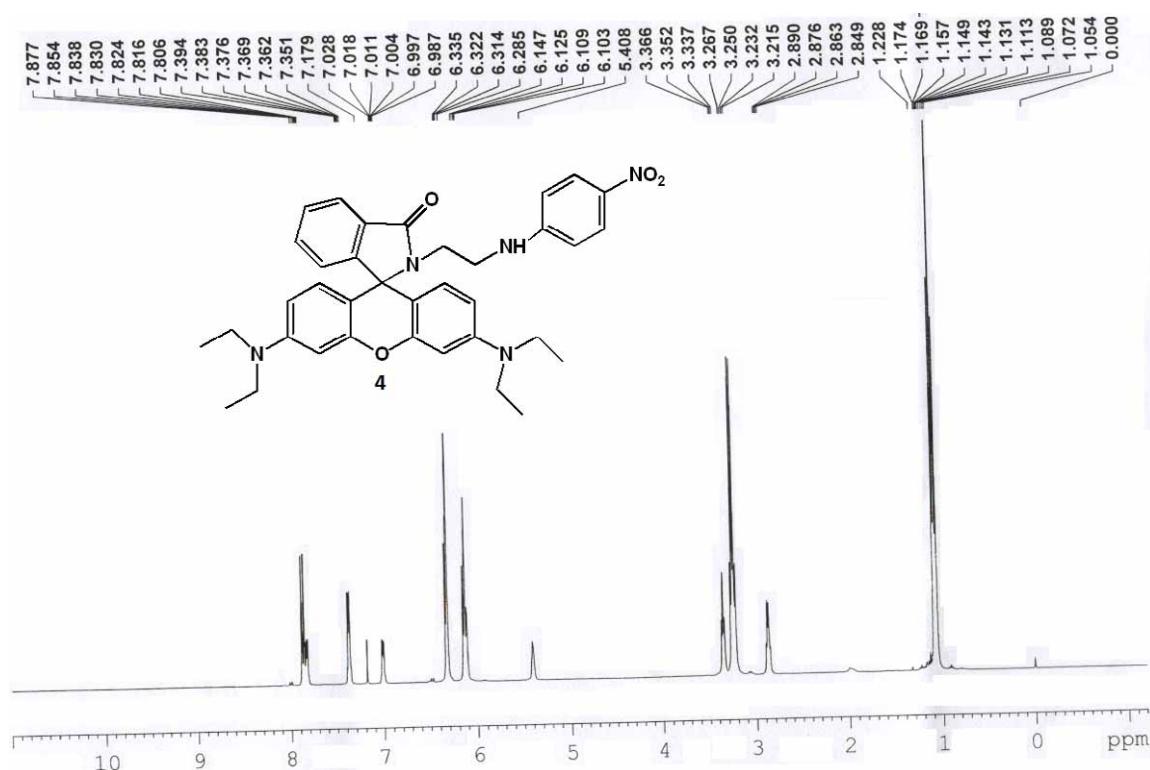
**Fig. S32**: $^1$H-NMR spectrum of **4** (CDCl$_3$)
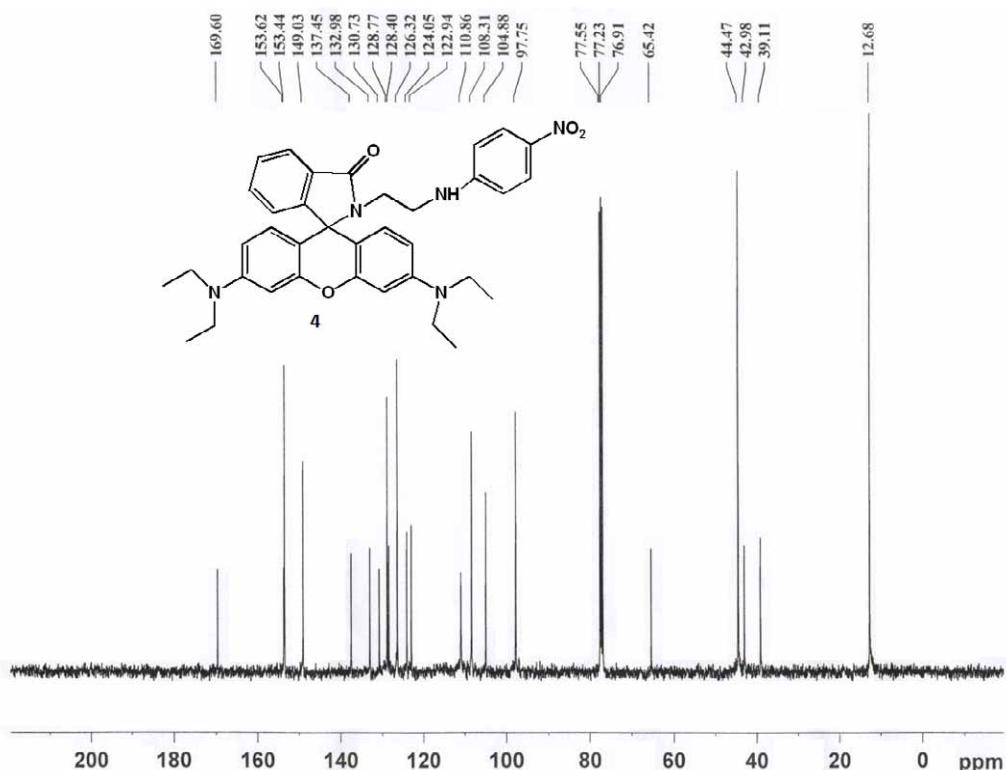


**Fig. S33**: $^{13}$C-NMR spectrum of **4** (CDCl$_3$)
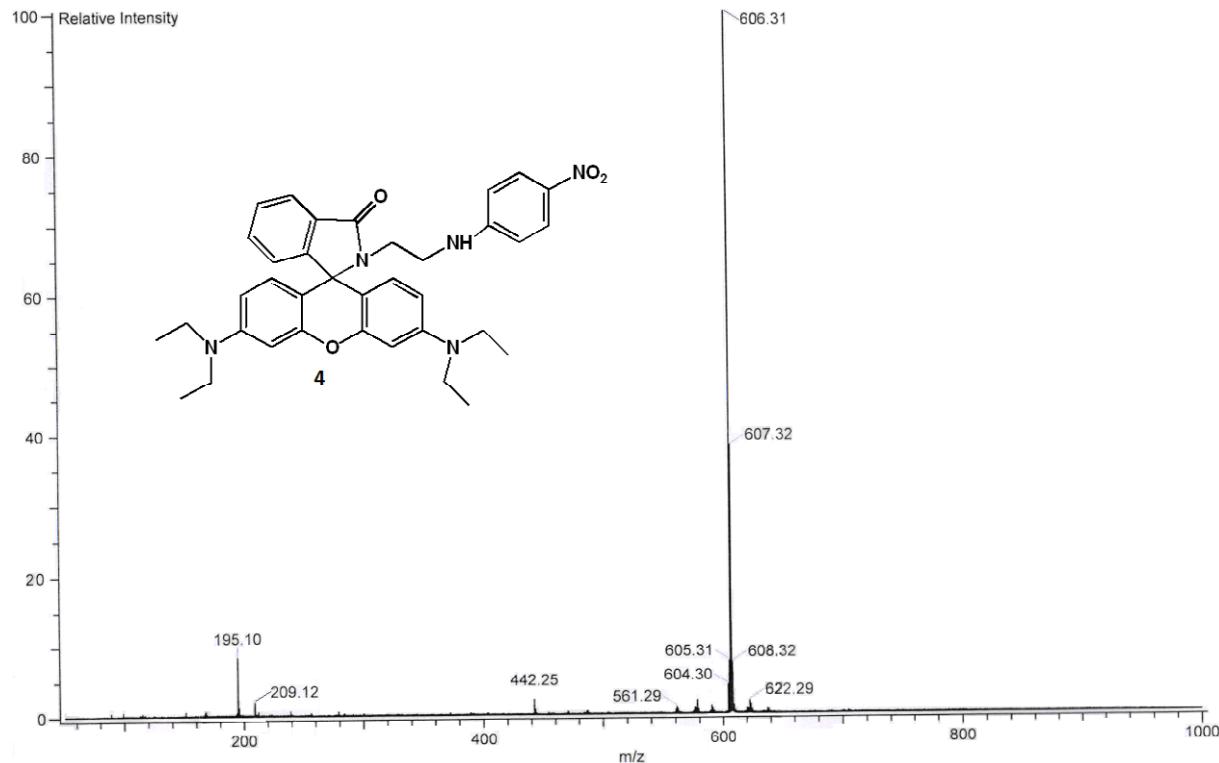
**Fig. S34**: ESI-MS spectrum of **4**
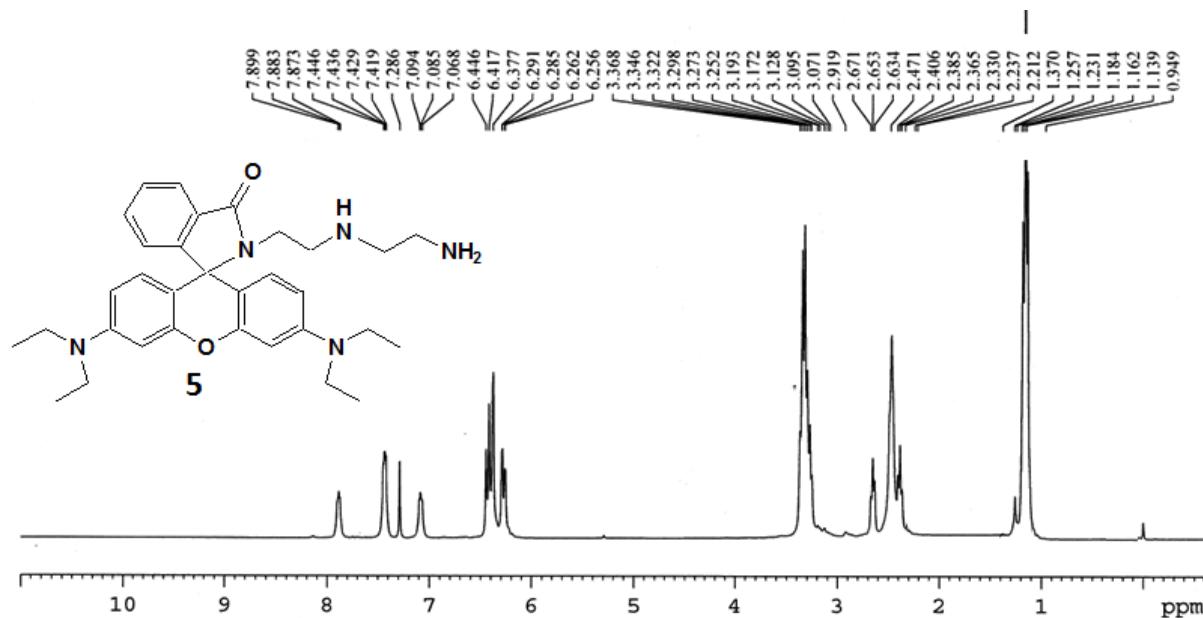


**Fig. S35**: $^1$H-NMR spectrum of **5** (CDCl$_3$)
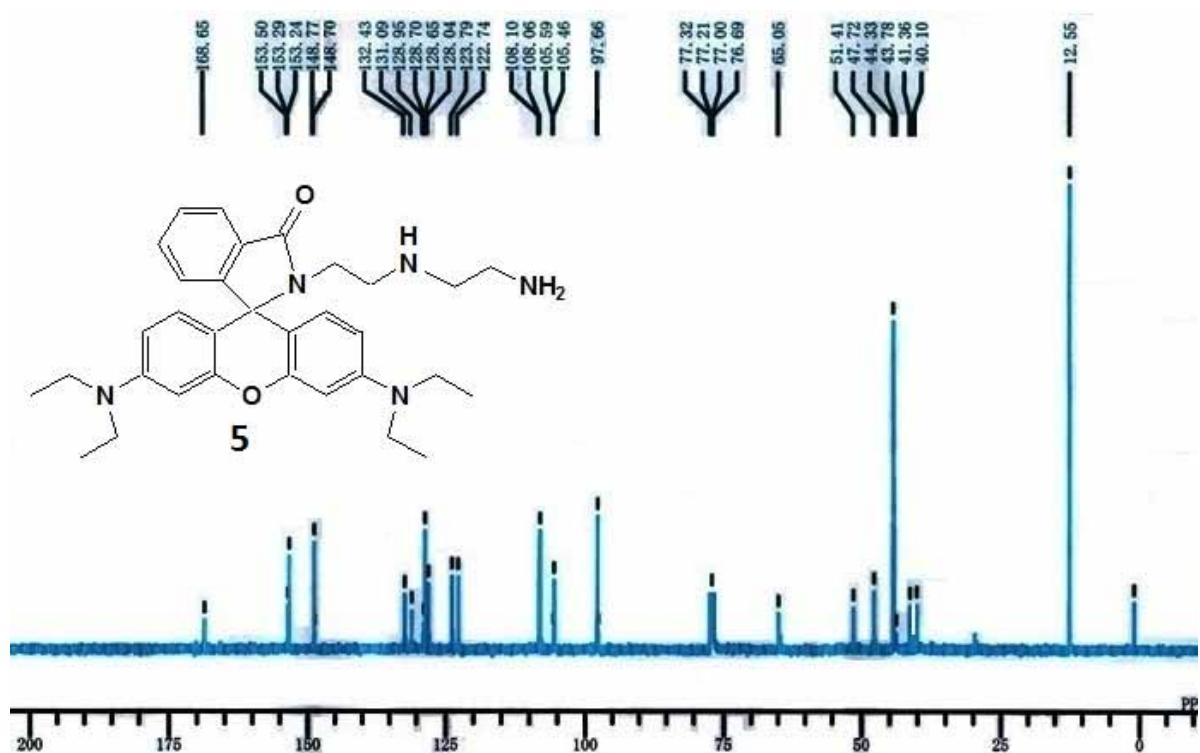
**Fig. S36**: $^{13}$C-NMR spectrum of **5** (CDCl$_3$)
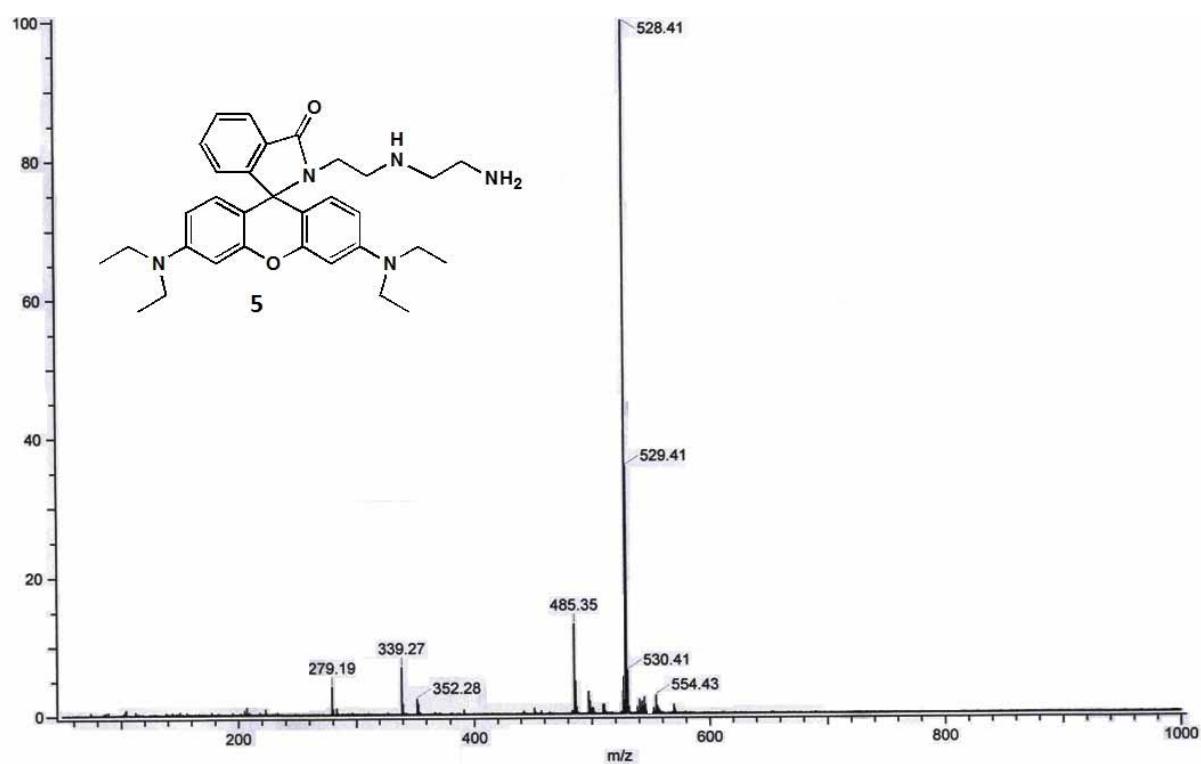


**Fig. S37**: ESI-MS spectrum of **5**.

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