Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

A bifunctional colorimetric fluorescent probe for Hg^{2+} and Cu^{2+} based on carbazole-pyrimidine conjugate: Chromogenic and fluorogenic recognition on TLC, silica-gel and filter paper

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General information

Materials and instruments

All solvents were of analytical grade and were dried according to standard procedures if necessary. All reactions were magnetically stirred and monitored by thin-layer chromatography (TLC) using Spectrochem GF254 silica gel-coated plates. Column chromatography was performed using 100-200 mesh silica gel. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury NMR spectrometer at 300 and 400 MHz respectively, using deuterated chloroform purchased from Cambridge Isotope Laboratories; chemical shifts are represented in ppm with tetramethylsilane (TMS) as the internal reference. Elemental analyses were performed using an EA1112 (Thermo Electron Corp.) elemental analyzer. Mass spectra were recorded on a Shimadzu LCMS-2020 (Liquid Chromatograph Mass Spectrometer). UV-vis absorption spectra of the solution (conc=1×10^{-5}) were obtained using a UV-vis spectrometer (HP 8453, photodiode array type) in the wavelength range 190-1100 nm. Fluorescence spectra were recorded with a Hitachi F-7000 fluorescence spectrophotometer with a slit width of 5 nm used for excitation and emission.

Determination of binding constant and detection limit

The binding constants were calculated using the Benesi–Hildebrand equation.[1]

$$1/(A_f - A_{obs}) = 1/(A_f - A_{fc}) + 1/\mathrm{K}(A_f - A_{fc})\ [\mathrm{Ligand}]$$

Where A_f is the absorbance of the free host, A_{obs} is the observed absorbance, A_{fc} is the absorbance at saturation, and K is the binding constant.

The detection limit (DL) was determined from the calibration curve of the fluorescence intensity versus the metal ion concentration. Using this plot, the DL was determined by

multiplying the concentration at which there was a sharp change in the fluorescence intensity by the concentration of probe **3**.

The equation used for the calculating detection limit (DL) is as follows:

DL=CL× CT

Where CL = is the concentration of the ligand; CT = is the concentration of the titrant at which the change was observed.

Absorption coefficient

Absorption coefficient has been calculated by measuring the absorbance of probe **3** at 5 different concentrations and was calculated using the following equation:

$A = \varepsilon.Cd = (\varepsilon.d)C;$

Where, A is absorbance, ε molar extinction coefficient or absorption coefficient, C is concentration and d= light path length in centimetres

In a graph of A vs C,

the slope is $\varepsilon.d = y_2\text{-}y_1/x_2\text{-}x_1$ (y = absorbance values and x = concentration values) and d = 1cm

Fluorescence Quantum yield

Fluorescence quantum yield ϕ_{fs} for probe **3** and its complexes with Hg^{2+} and Cu^{2+} were determined in analytical grade THF using optically matching solutions of fluorescein ϕ_{fr} = 0.79 in ethanol as the standard at an excitation wavelength of 290 nm and the quantum yield was calculated using following equation:

$$\phi_{fs} = \phi_{fr} \times \frac{1-10^{-\mathrm{ArLr}}}{1-10^{-\mathrm{AsLs}}} \times \frac{N_s^2}{N_r^2} \times \frac{D_s}{D_r}$$

Where, ϕ_{fs} and ϕ_{fr} are the radiative quantum yields and the subscripts s and r denote sample and reference, respectively. A_s and A_r are the absorbance of the sample and the reference respectively, D_s and D_r are the respective areas of emission for sample and reference respectively. L_s and L_r are the lengths of the absorption cells respectively. N_s and N_r are the index of refraction of the sample and reference solutions (THF = 1.4040 at 25 °C).

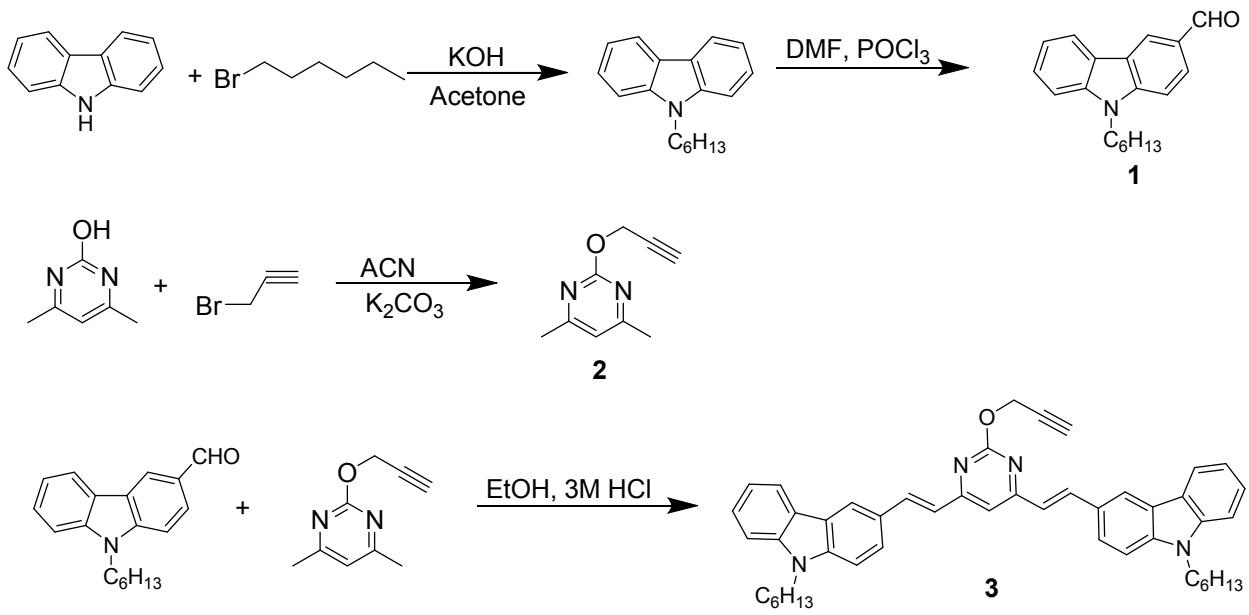
Preparation of stock solutions for UV-vis and fluorescence studies

UV-vis and fluorescence titrations were performed on 3.0 μM and 1.0 μM solutions of probe **3** in THF-HEPES buffer (v/v, 7:3, pH 7.4). Stock solutions of probe **3** (conc=10^{-4} M) and the metal ions (Li^+, Na^+, K^+, Ba^{2+}, Ca^{2+}, Cd^{2+}, Al^{3+}, Cr^{3+}, Zn^{2+}, Co^{2+}, Pd^{2+}, Fe^{3+}, Cu^{2+} and Hg^{2+} (10^{-3} M) were prepared in THF-HEPES buffer (v/v, 7:3, pH 7.4). In titration experiments, a 3 mL (total volume) solution of probe **3** was placed in a quartz cuvette (path length = 1 cm). UV-vis absorption and fluorescence spectra were recorded after incubation of the probe with the appropriate anions. For fluorescence spectrum measurements, excitation was provided at 450 nm, and emission was collected from 500 to 800 nm.

Preparation of stock solutions for optical detection of Hg^{2+} and Cu^{2+}

Stock solutions of probe **3** (conc=10^{-4} M) and of metal ions (Hg^{2+} and Cu^{2+}) were prepared in THF-HEPES buffer (v/v, 7:3, pH 7.4). Thin-layer chromatography (TLC) using Spectrochem GF254 silica gel-coated plates were employed for the experiment. For the free silica-gel experiments, we employed colorless silica-gel (2.50 g, 100–200 mesh).

Synthesis and characterizaton



Scheme S1. Synthetic scheme for probe **3**.

9-Hexyl-*9H*-carbazole-3-carbaldehyde (**1**) was synthesized according to the literature procedure.[2]

Synthesis of 4,6-dimethyl-2-(prop-2-ynyloxy)pyrimidine (2):

A mixture of 4,6-dimethylpyrimidin-2-ol (0.5 g, 4.032 mmol), propargyl bromide (0.707 g, 6.042 mmol), and K_2CO_3 (0.834 mg, 6.034 mmol) in CH_3CN (15 mL) was placed in a 50 mL round-bottom flask (RBF). The reaction mixture was stirred overnight at room temperature and the solvent was removed *in vacuo*. The residue was purified *via* column chromatography using ethyl acetate : hexane (1:10) as the eluent to afford 0.420 g (84%) as a creamy solid.

Mp: 156 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm): 6.10 (s, 1H, ArH), 4.84 (d, J = 3.0 Hz, 2H, O-CH_2), 2.48 (s, 3H, CH_3), 2.32 (s, 3H, CH_3); ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm): 175.51, 156.52, 106.44, 73.15, 34.50, 25.29, 19.89; IR (cm^{-1}, KBr): 3168, 3062, 2993, 2105, 1675, 1537,

1321; Anal. Calcd for $C_9H_{10}N_2O$: C, 66.65; H, 6.21; N, 17.27. Found: C, 67.25; H, 6.19; N, 16.08; MS (ESI): *m/z* 163 ($M+H^+$).

Synthesis of 3,3'-(1*E*,1'*E*)-2,2'-(2-(prop-2-ynyloxy)pyrimidine-4,6-diyl)bis(ethene-2,1-diyl)bis(9-hexyl-9*H*-carbazole) (3):

A mixture of compound **1** (0.67 g, 2.46 mmol) and compound **2** (0.2 g, 1.23 mmol) in ethanol (15 ml) was placed in a 50 mL round bottom flask (RBF). After stirring the reaction mixture for 15 min, 3M HCl solution (~2 mL) was added. The reaction mixture was refluxed for overnight and then solvent was removed by evaporation. The residue was purified *via* column chromatography using CH_2Cl_2: ethyl acetate as the eluent to afford 0.360 g (74%) as a shiny yellow solid; Mp: 147 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm): 8.30 (d, 2H, *J* = 12 Hz, ArH), 8.10-8.19 (m, 3H, ArH), 7.74 (d, 2H, *J* = 9 Hz, ArH), 7.42-7.62 (m, 9H, ArH), 7.01-7.24 (m, 3H, ArH), 6.71 (s, 1H, C=C-), 4.99 (s, 2H, O-CH_2), 4.24-4.33 (m, 4H, *N*-CH_2), 2.42 (s, 1H, -C≡H), 1.86 (t, *J* = 9.0 Hz, 4H, 2 x CH_2), 1.30 (s, 12H, 6 × CH_2), 0.85-0.870 (m, 6H, 2 x CH_3); ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm): 168.86, 156.99, 156.09, 141.86, 141.70, 141.42, 141.29, 141.17, 141.09, 141.00, 126.71, 126.67, 126.38, 125.15, 126.09, 125.50, 123.58, 123.47, 122.96, 122.83, 122.75, 121.30, 121.06, 120.74, 119.95, 119.73, 115.15, 109.61, 109.48, 109.34, 109.30, 100.54, 73.61, 45.14, 43.52, 43.44, 35.04, 32.57, 31.75, 31.67, 29.14, 27.14, 22.76, 22.67, 14.24, 14.16; IR (cm^{-1}, KBr): 3580, 3526, 2926, 2853, 2013, 1733, 1613, 1546, 1160; Anal. Calcd for $C_{47}H_{48}N_4O$: C, 82.42; H, 7.06; N, 8.18. Found: C, 82.90; H, 7.22; N, 7.75; HRMS: *m/z* 685.39 $(M+H)^+$.

Spectroscopic data

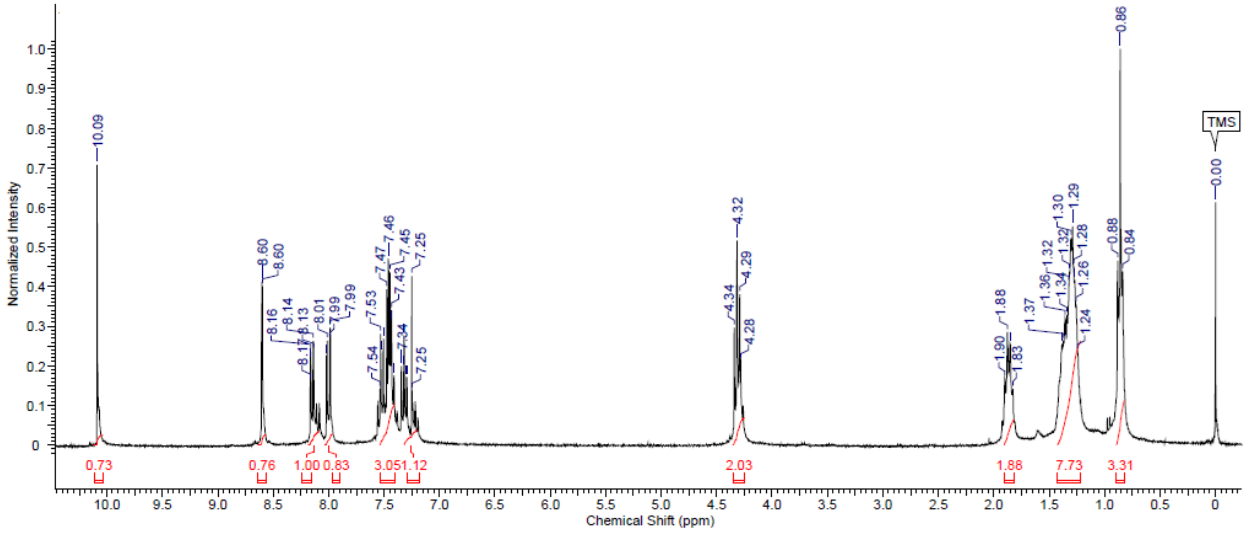


Figure S1 ^{1}H NMR spectrum of compound **1** in $CDCl_3$.

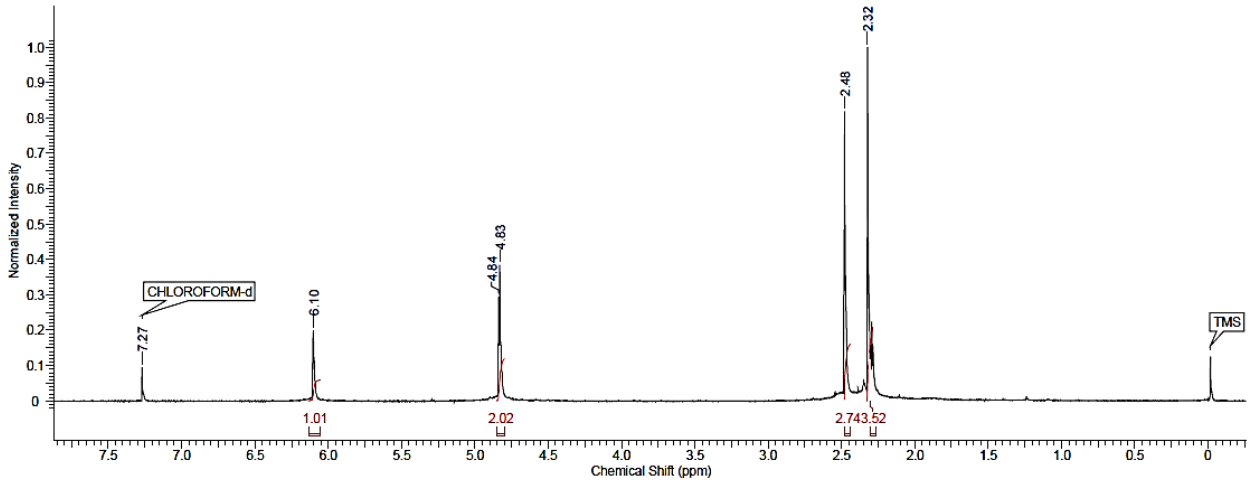


Figure S2 ^{1}H NMR spectrum of compound **2** in $CDCl_3$.

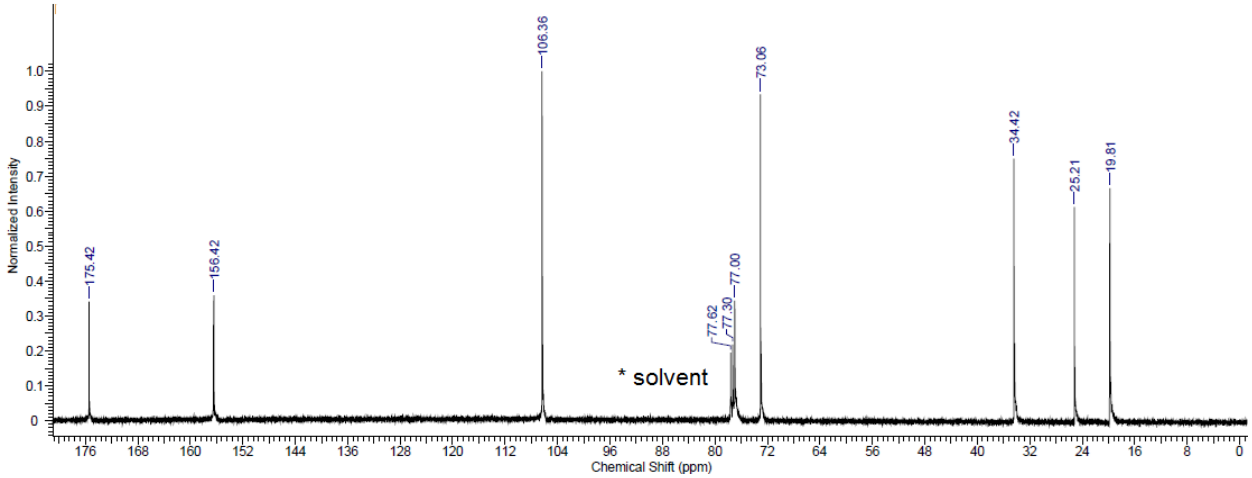


Figure S3 ^{13}C NMR spectrum of compound **2** in $CDCl_3$.

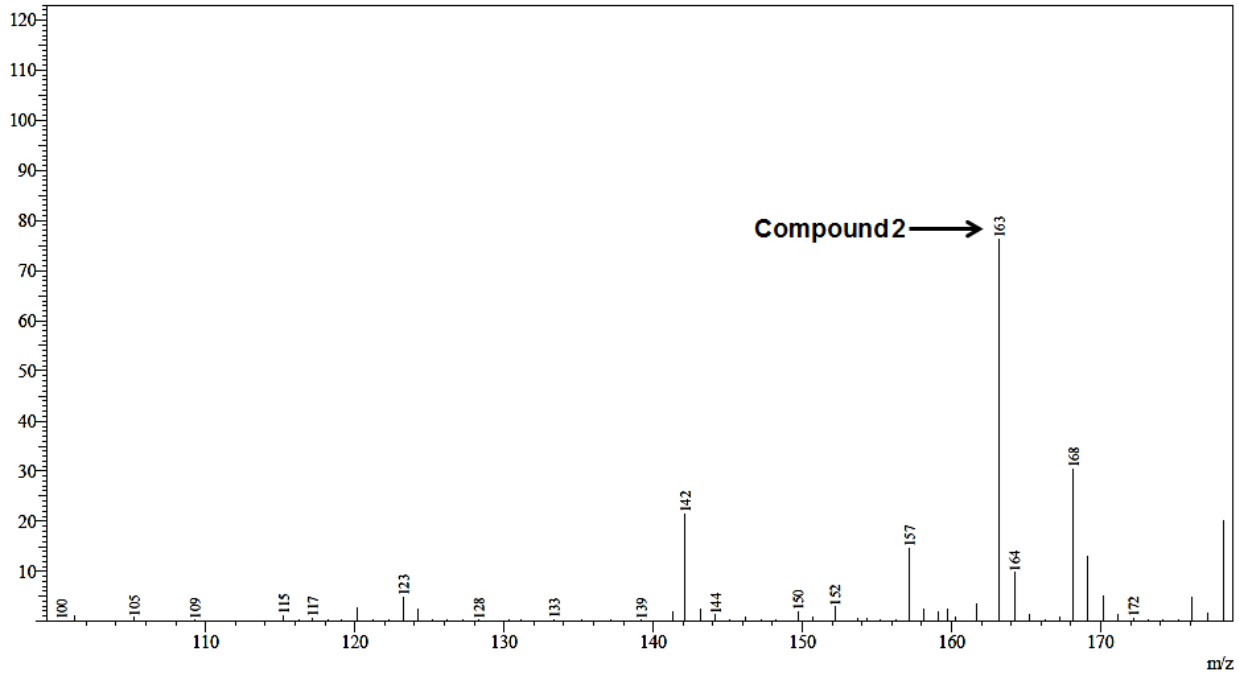


Figure S4 ESI-MS spectrum of compound **2**.

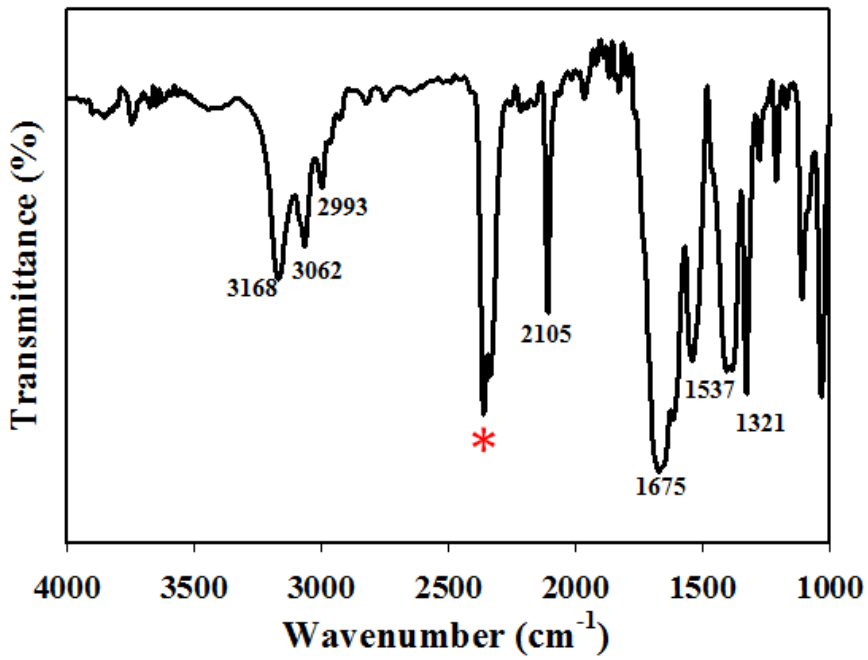


Figure S5 FTIR spectrum of compound **2**.

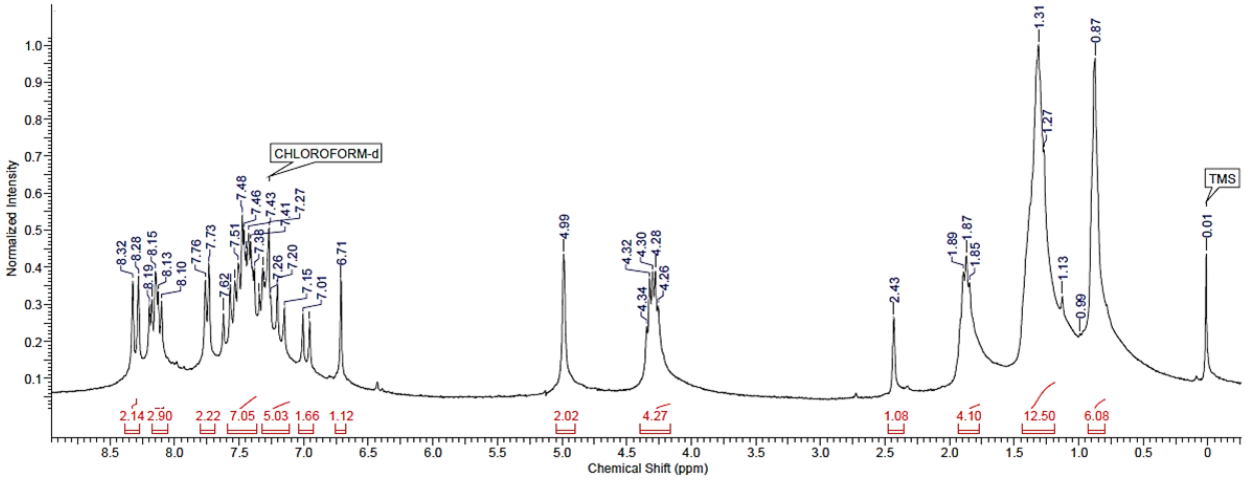


Figure S6 ^{1}H NMR spectrum of probe **3** in $CDCl_3$.

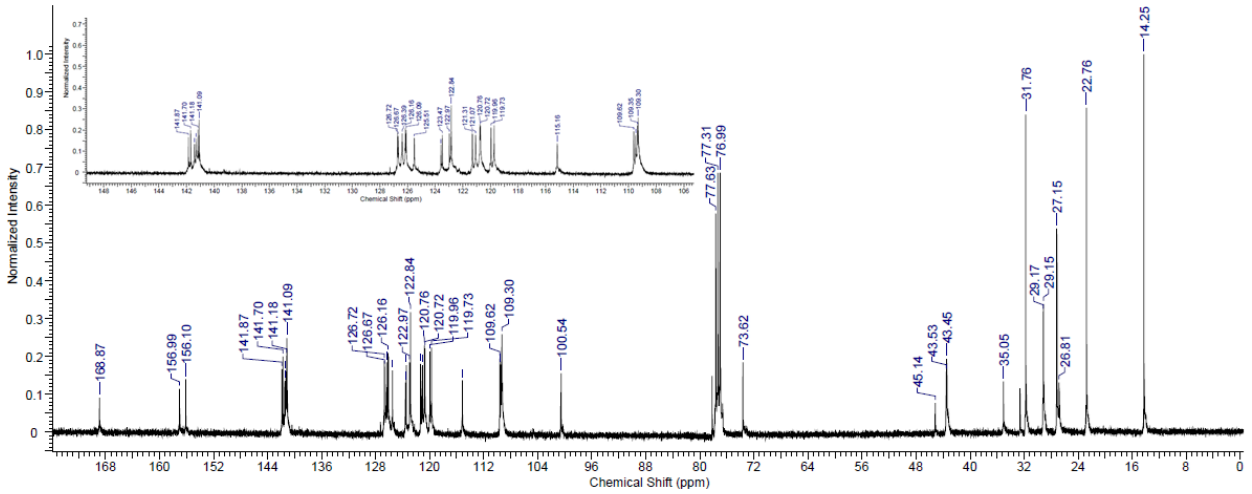


Figure S7 ^{13}C NMR spectrum of probe **3** in $CDCl_3$. Inset: shows the partial ^{13}C NMR spectrum (region 108-144 ppm).

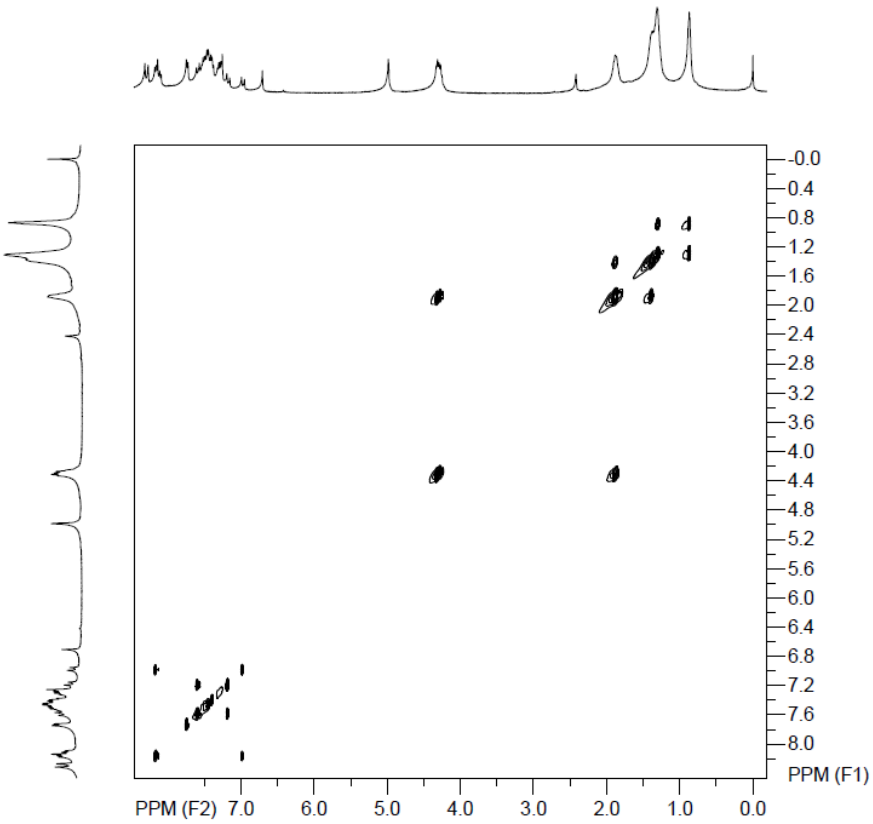


Figure S8 COSY spectrum of probe **3** in $CDCl_3$.

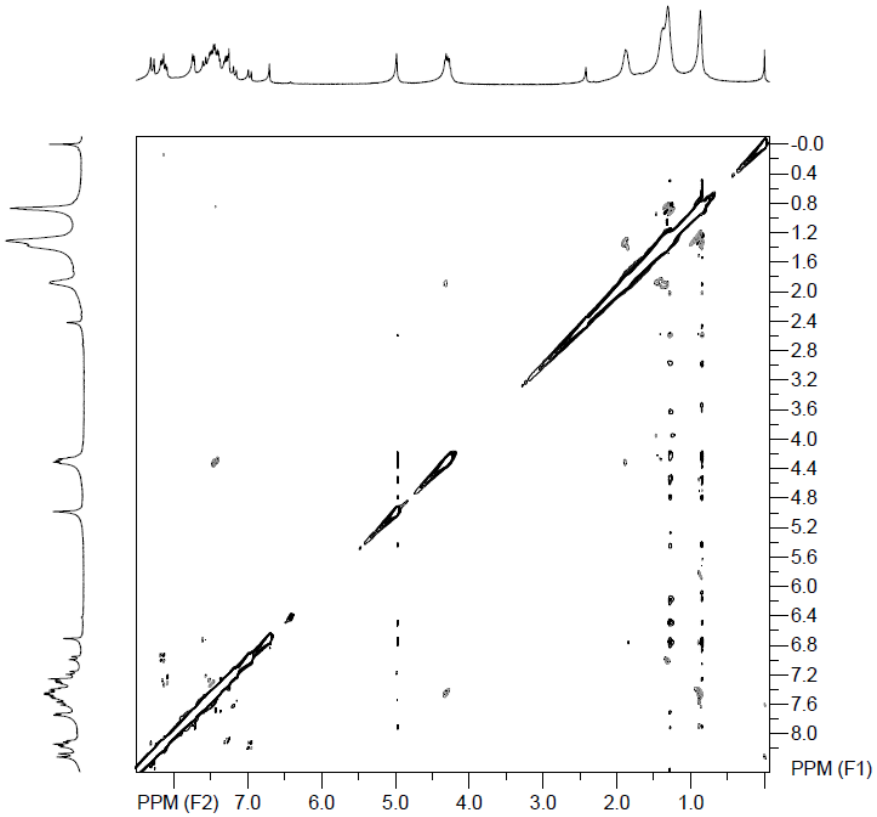


Figure S9 NOESY spectrum of probe **3** in $CDCl_3$.

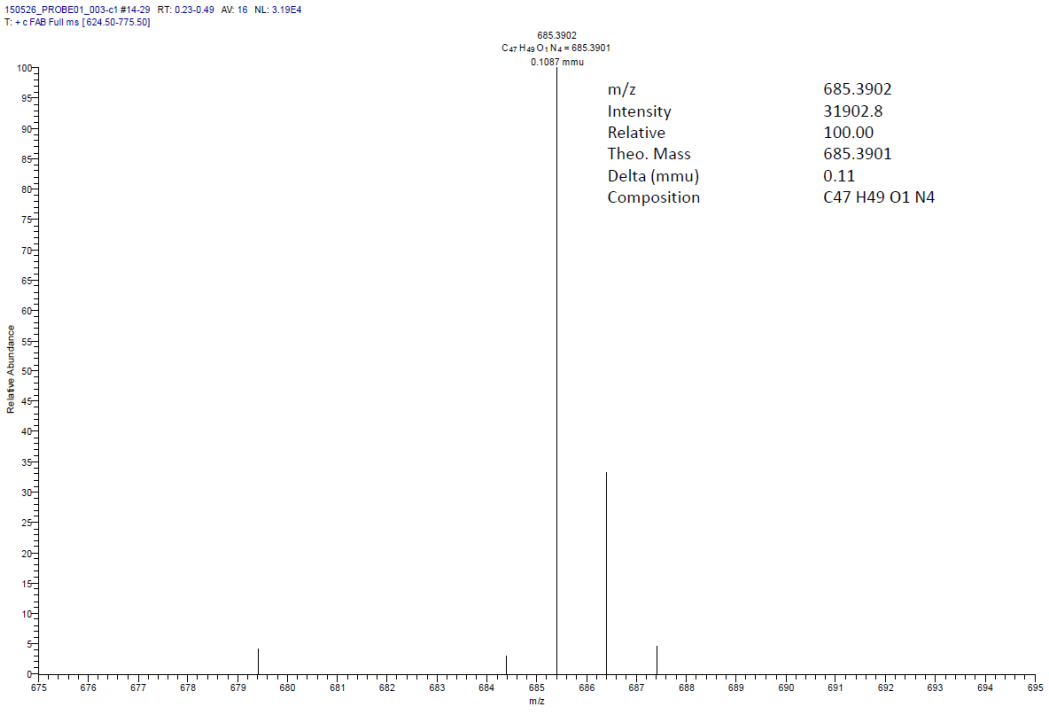


Figure S10 HRMS spectrum of probe **3**.

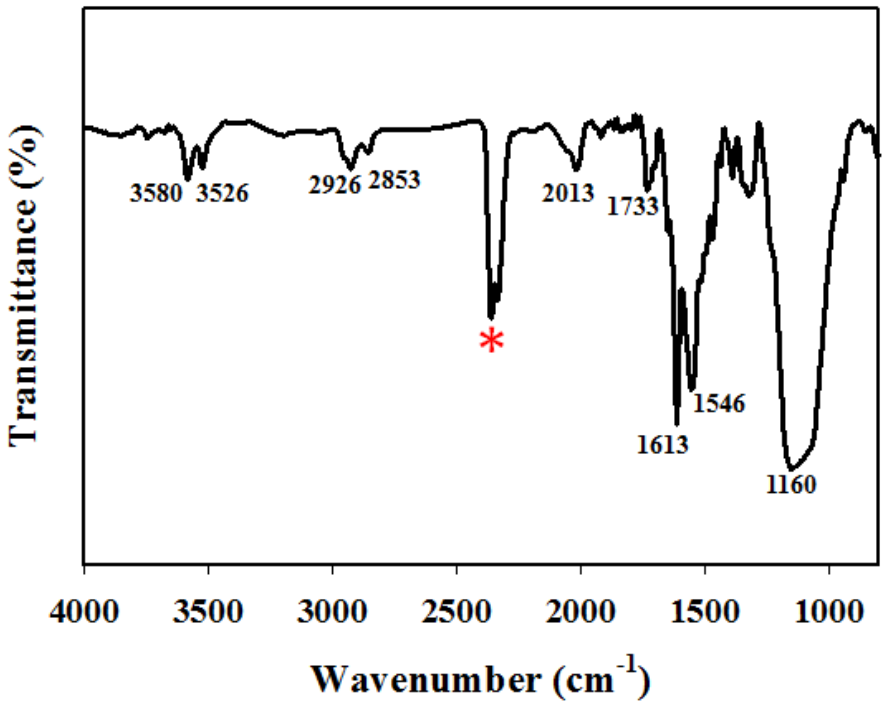


Figure S11 FTIR spectrum of probe **3.**

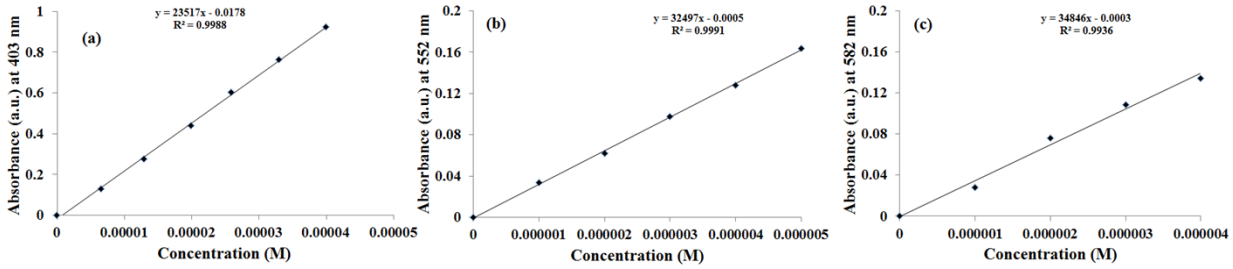


Figure S12 Linear correlation between the absorbance and metal ion concentration, (a) probe **3**, probe **3** in the presence of various concentrations of (b) Hg^{2+} and (c) Cu^{2+}.

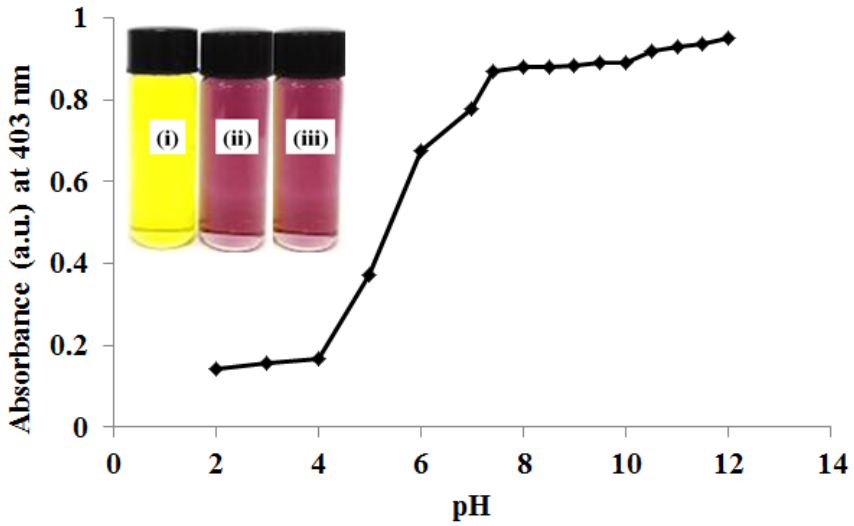


Figure S13 Absorbance of probe **3** with the pH value monitored at 403 nm. *Inset: Images showing the solution colors of (i) probe **3**, (ii) probe **3** with Hg^{2+} and (iii) probe **3** at acidic pH (>3). pH is adjusted by using aqueous solutions of 1 M HCl or 1 M NaOH.

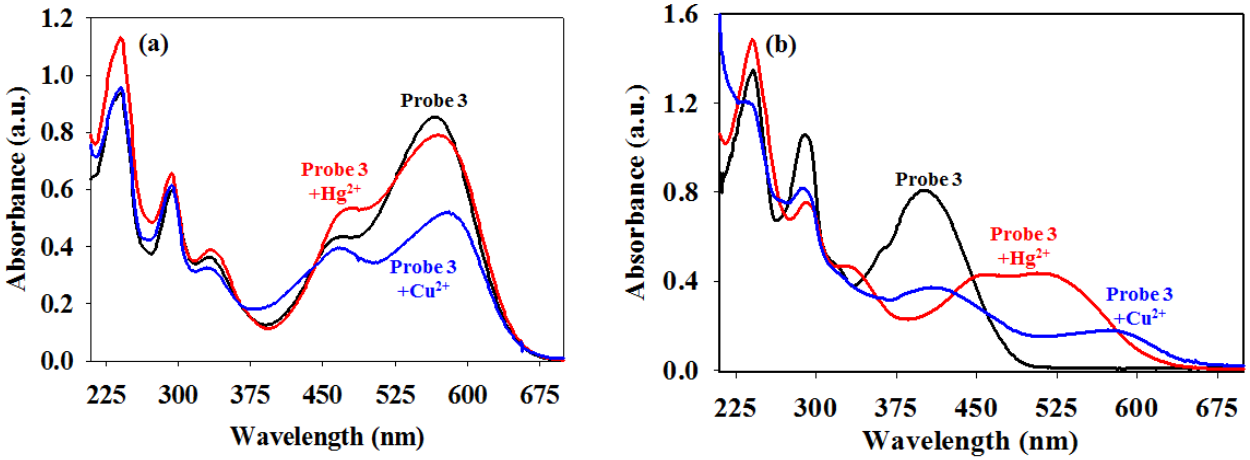


Figure S14 UV-vis absorption spectra of probe **3** with Hg^{2+} and Cu^{2+} (20 equiv. each) at (a) acidic pH (~4) and basic pH (~9).

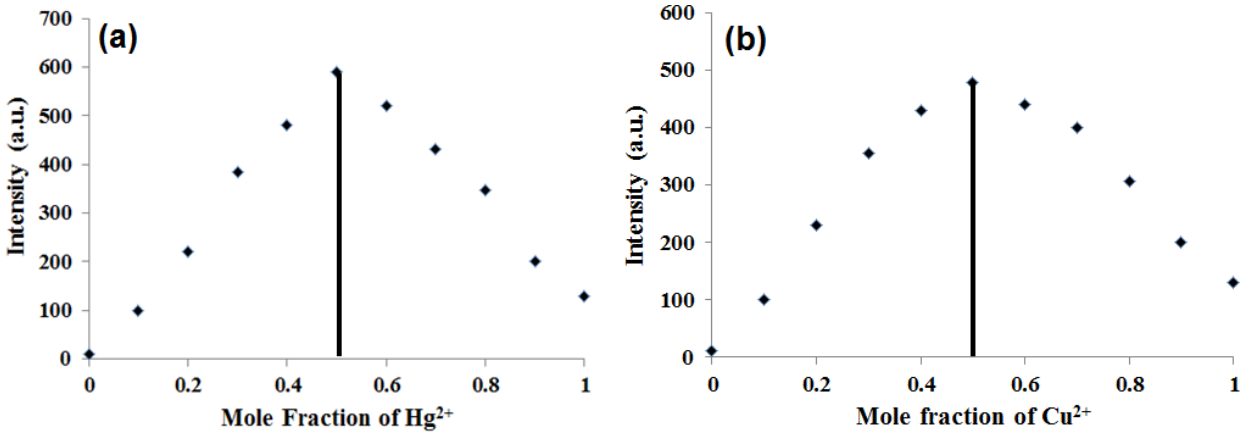


Figure S15 Job's plot for probe **3** with (a) Hg^{2+} ions and (b) Cu^{2+} ions.

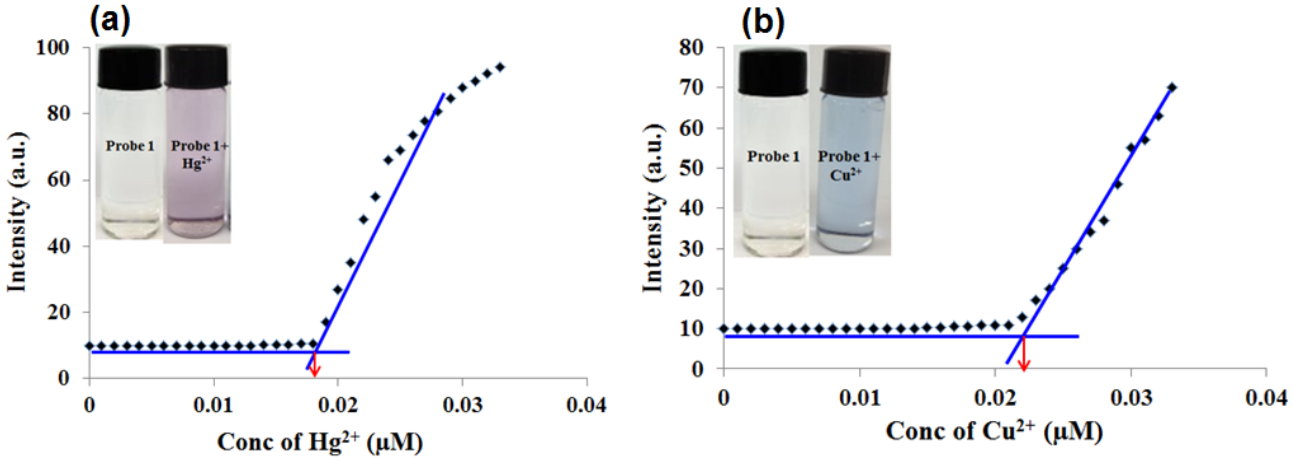


Figure S16 Fluorescence intensity of probe **3** (a) monitored at 657 nm as a function of Hg^{2+} concentration and (b) monitored at 663 nm as a function of Cu^{2+} concentration.

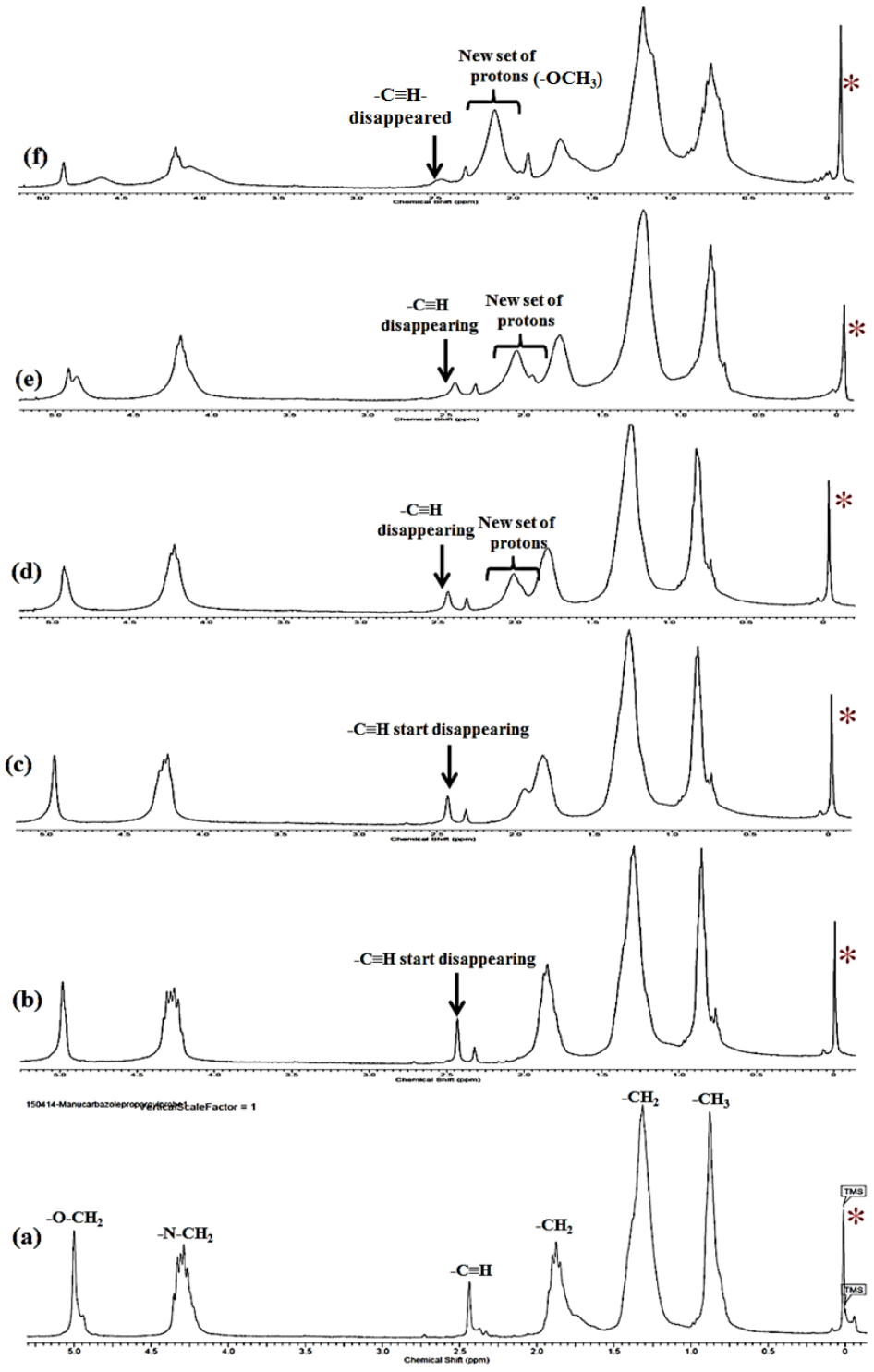


Figure S17 ^{1}H NMR spectra (0 ~ 5 ppm) of probe **3** (20 mM in $CDCl_3/CD_3CN$) upon the addition of $Hg(ClO_4)_2$ (0-1.0 equivalent) at 25 °C; (a) probe **3**, (b) immediately after mixing , (c) after 30 sec, (d) after 1 min, (e) after 2 min and (f) after 3min.

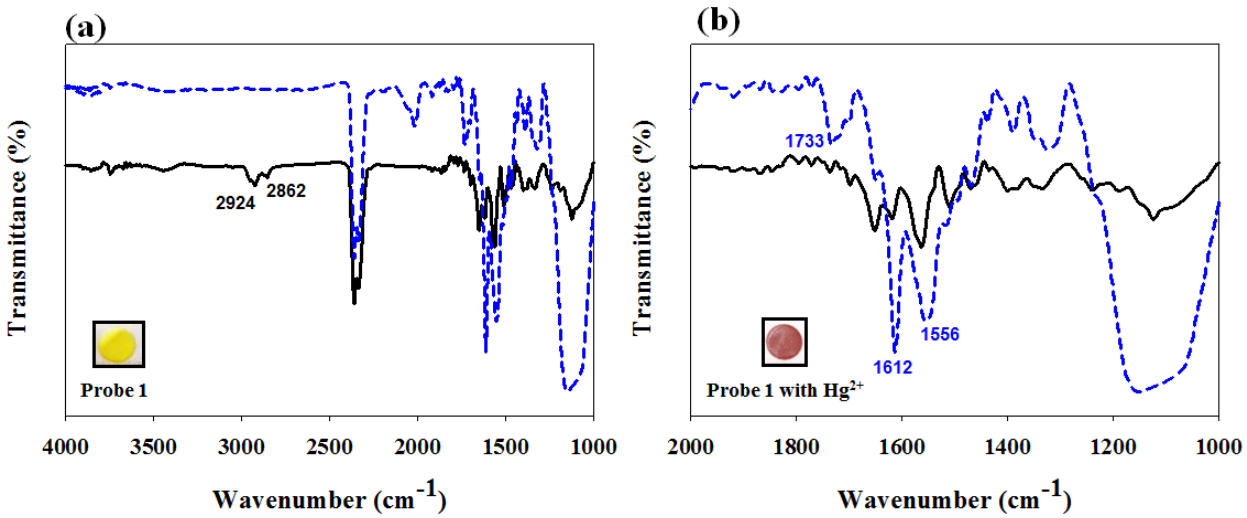


Figure S18 FTIR spectra of probe **3** (solid line) with Hg^{2+} (dashed line) over (a) 4000-1000 cm^{-1}, (b) 2000-1000 cm^{-1}. Insets: change in color of KBr pellets of probe **3** with Hg^{2+}.

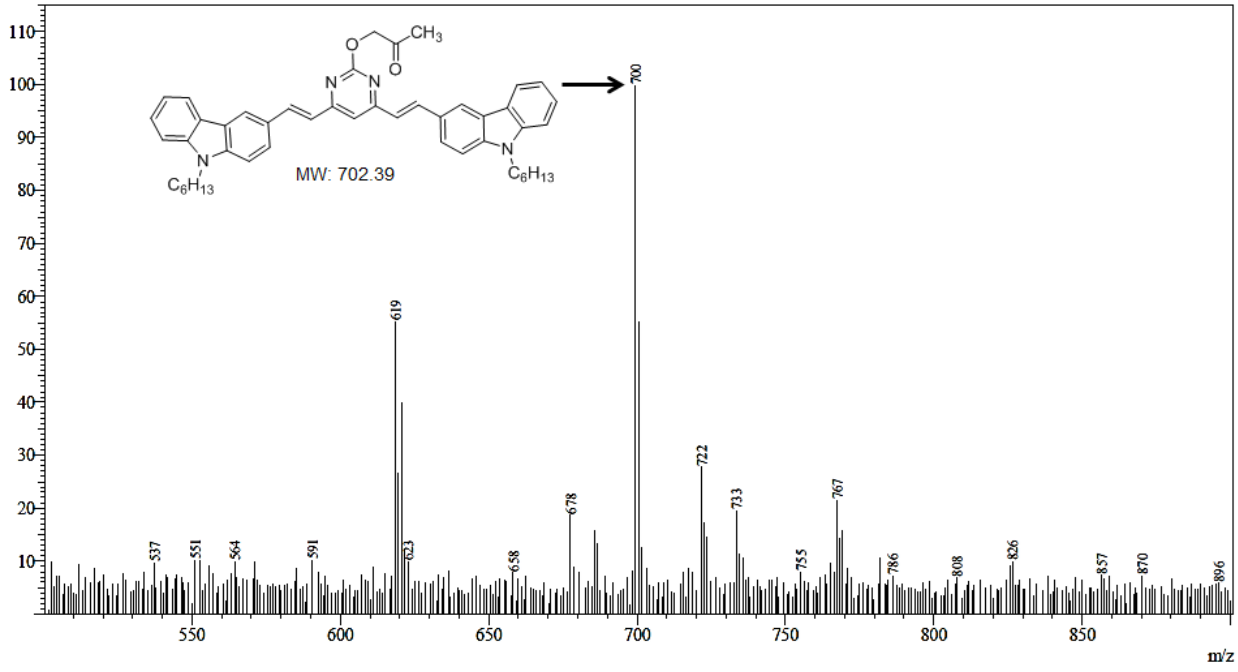


Figure S19 Mass spectrum of probe **3** with Hg^{2+}.

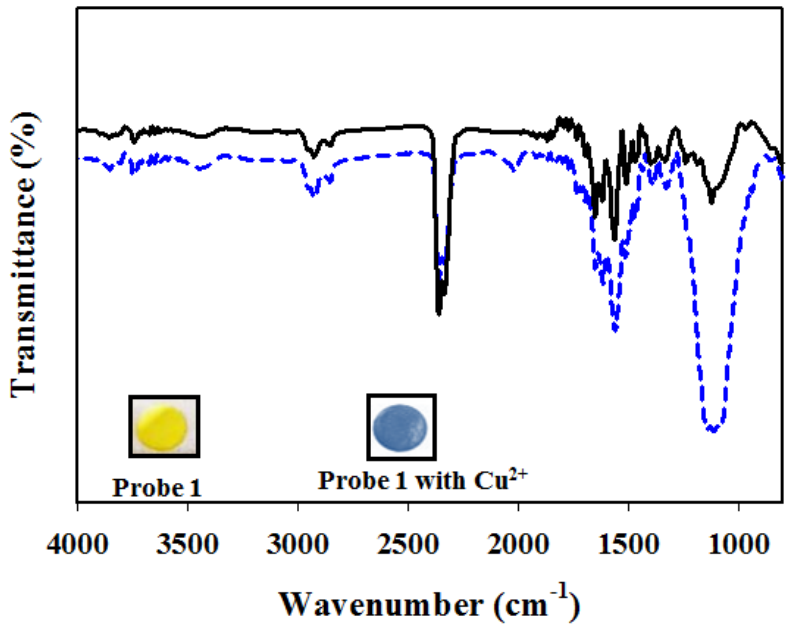


Figure S20 FTIR spectra of probe **3** (solid line) with Cu^{2+} (dashed line). Insets: change in color of KBr pellets of probe **3** with Cu^{2+}.

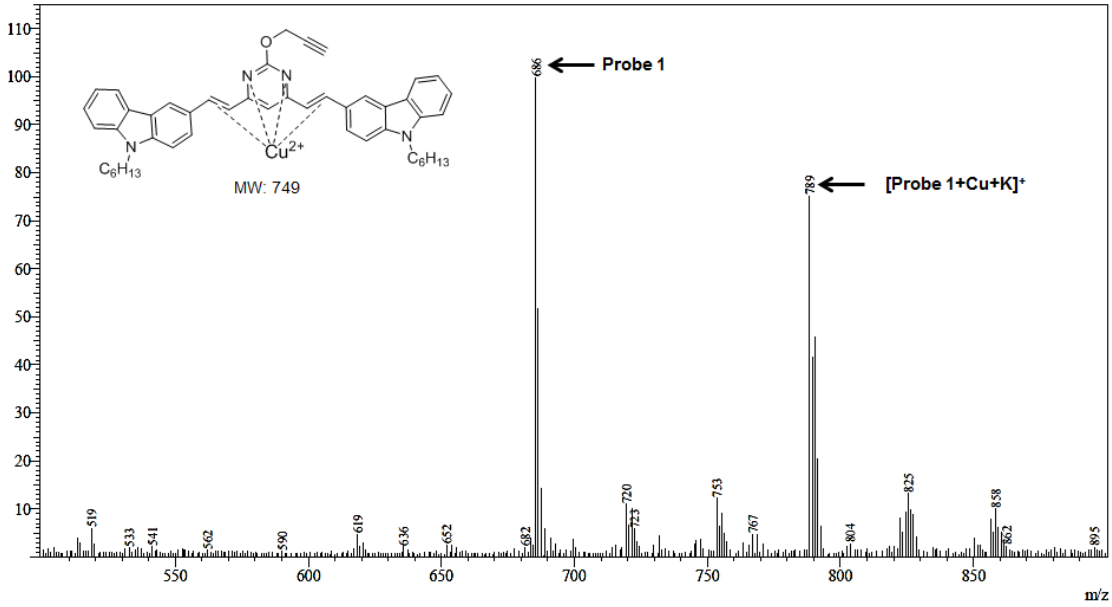


Figure S21 Mass spectrum of probe **3** with Cu^{2+}.

References:

1. J. H. Hildebrand, H. A. Benesi, *J. Am. Chem. Soc.,* 1949, **71**, 2703.

2. C.-S. Wu, S.-W. Fang and Y. Chen, *Phys. Chem. Chem. Phys.,* 2013, **15**, 15121.