Electronic Supplementary Information

Chemodosimeter approach for nanomolar detection of Cu^{2+} ions and its bio-imaging in PC3 cell lines

Shahi Imam Reja,[†] Vandana Bhalla,[†] Shaffi Manchanda,[‡] Gurcharan Kaur[‡] and Manoj Kumar[†]*

[†]Department of Chemistry, UGC Sponsored Centre for Advanced Studies-1and [‡]Department of Biotechnology, Guru Nanak Dev University, Amritsar, Punjab, India.
Fax: +91 (0)183 2258820; Tel: +91 (0)183 2258802 9x3205;
E-mail: mksharmaa@yahoo.co.in

Quantum Yield Calculation

Fluorescence quantum yields[1] were determined by using optically matching solutions of naphthalene (Φ_{fr} = 0.23 in ethanol) and rhodamine B (Φ_{fr} = 0.65 in ethanol) as standard at an excitation wavelength of 320 and 530 nm, respectively and quantum yield is calculated using the equation:

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

Φ_{fs} and Φ_{fr} are the radiative quantum yields of sample and the reference respectively, A_s and A_r are the absorbance of the sample and the reference respectively, D_s and D_r the respective areas of emission for sample and reference. L_s and L_r are the lengths of the absorption cells of sample and reference respectively. N_s and N_r are the refractive indices of the sample and reference solutions (pure solvents were assumed respectively)

General

All reagents were purchased from Aldrich and were used without further purification. Acetonitrile (AR grade) was used to perform analytical studies. UV-vis spectra were recorded on a SHIMADZU UV-2450 spectrophotometer, with a quartz cuvette (path length 1 cm). The cell holder was thermostatted at 25 °C. The fluorescence spectra were recorded with a SHIMADZU 5301 PC spectrofluorimeter. Mass spectra were recorded on a Bruker MicroTof QII mass spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL-FT NMR-AL 300 MHz spectrophotometer using DMSO-d_6 and $CDCl_3$ as a solvent and tetramethylsilane as the internal standard. Data are reported as follows: chemical shift in ppm (d), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad singlet), coupling constants *J* (Hz), integration and interpretation.

UV-vis and fluorescence titrations

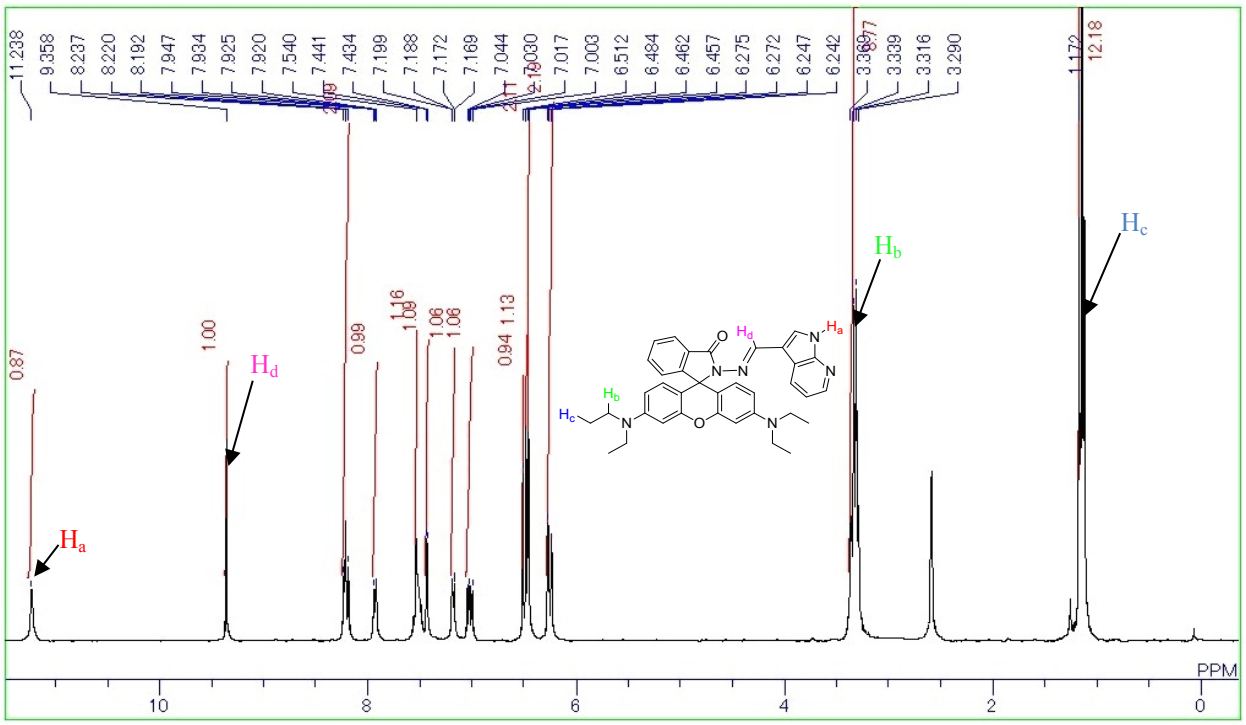
UV-vis and fluorescence titrations were performed with 5.0 & 1.0 μM solutions of ligand in CH_3CN and CH_3CN/H_2O (9.5:0.5, 9:1, 8:2 & 7:3, v/v) buffered with HEPES, pH = 7.0. Typically, aliquots of freshly prepared standard solutions (10^{-1} M to 10^{-3} M) of $M(ClO_4)_n$ (M = Hg^{2+}, Pb^{2+}, Ba^{2+}, Cd^{2+}, Ag^{+}, Zn^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Fe^{3+}, Fe^{2+}, K^{+}, Mg^{2+}, Na^{+} and Li^{+}; n = 1, 2 or 3) in CH_3CN were added to record the UV-vis and fluorescence spectra.

Binding constant of complex

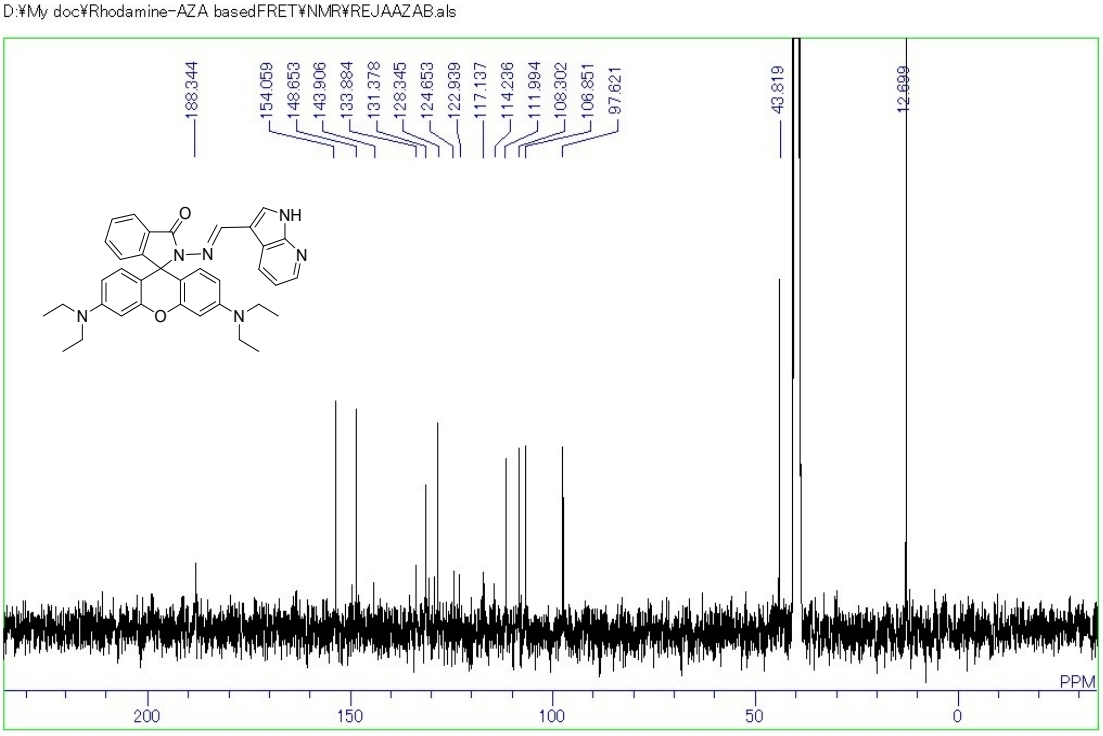
The binding constants (log β) of receptors **4** with copper ion was calculated from UV-vis/fluorescence titration experiments by means of SPECFIT programme (global analysis system V3.0 for 32-bit Window system), which uses singular value decomposition and nonlinear regression modeling by the Leverberg–Marquardt method.

[1] J. N. Deams and G. A. Grosby, *J. Phys. Chem.,* 1971, **75**, 991.

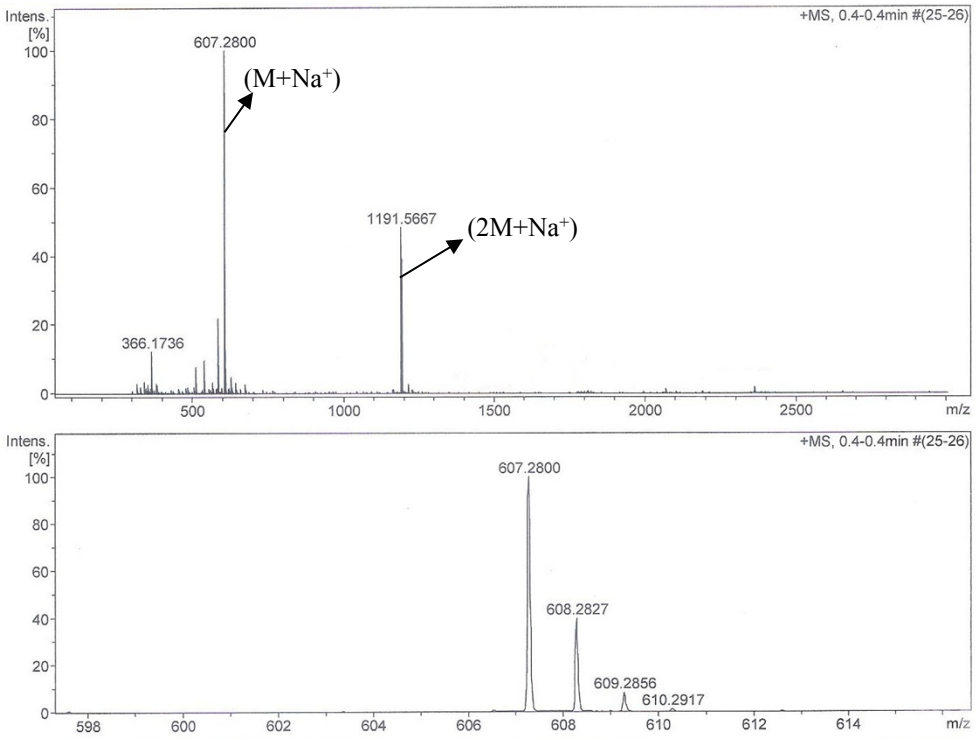
^{1}H NMR of compound **4**



^{13}C NMR of compound **4**



Mass spectra of compound **4**



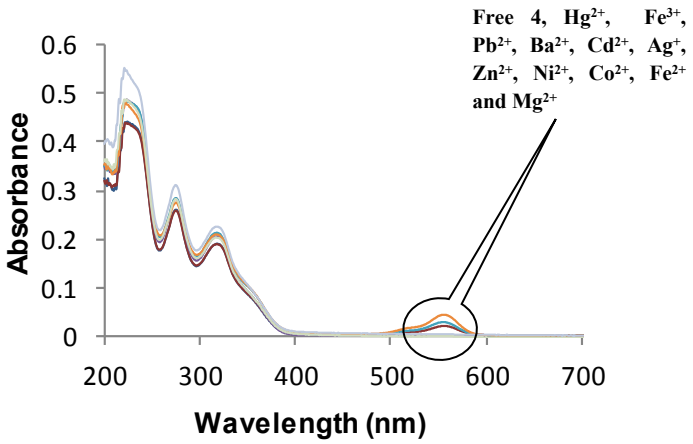


Figure S7. UV-vis spectra of **4** (5.0 μM) in the presence of various metal ions (35.0 μM each); in CH_3CN

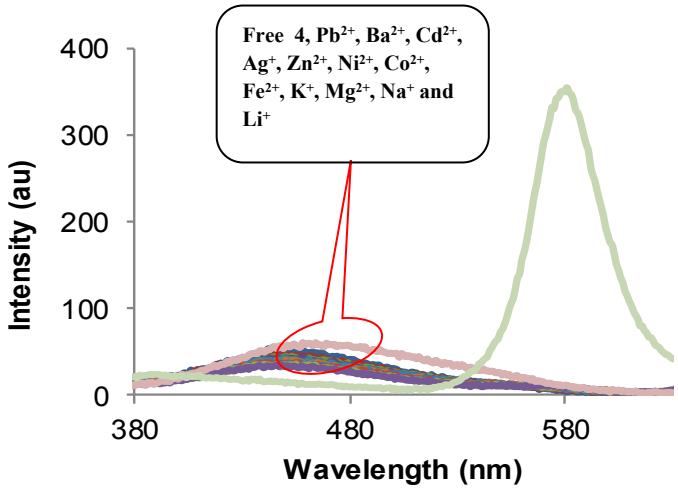


Figure S8. Fluorescence spectra of compound **4** (1.0 μM) in the presence of various metal ions (20 μM each); in CH_3CN; λ_{ex} = 320 nm.

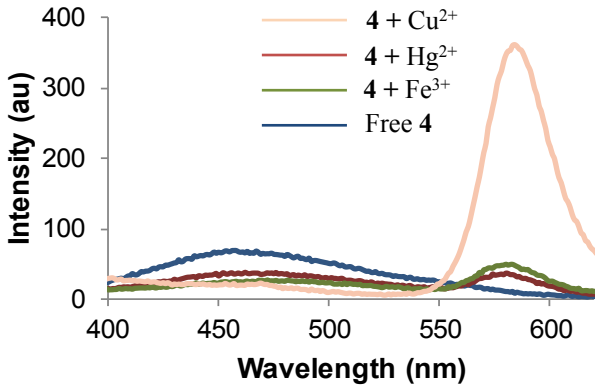


Figure S9. Fluorescence spectra of **4** (1.0 μM) in CH_3CN; blue line, free **4**, green line, **4** + Fe^{3+} (20 μM), red line, **4** + Hg^{2+} (20 μM), pink line, **4** + Cu^{2+} (20 μM).

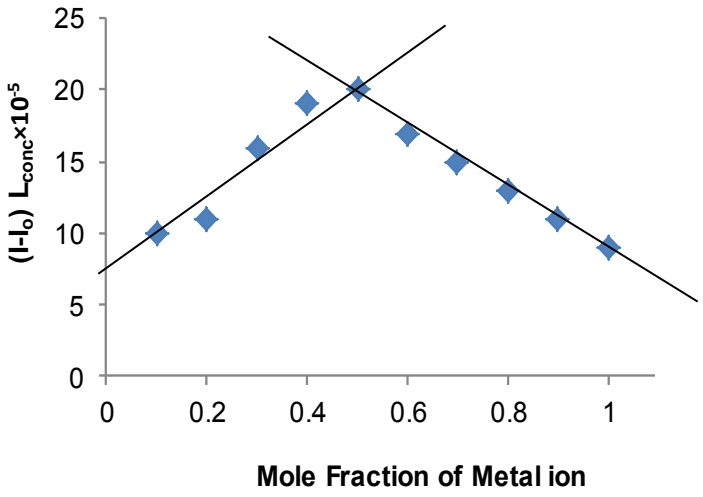
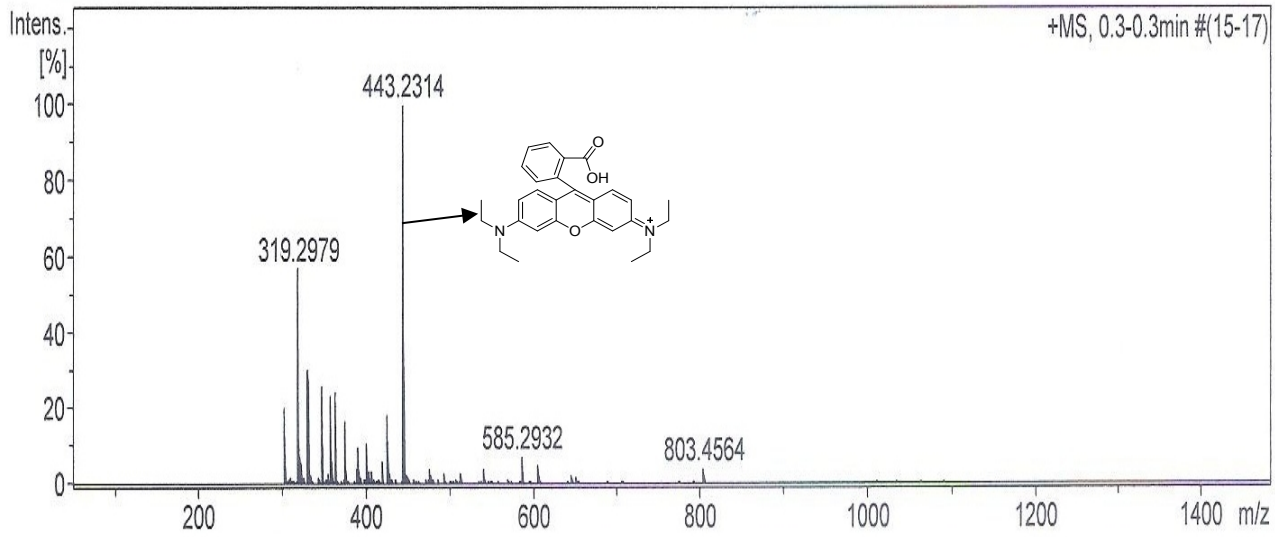


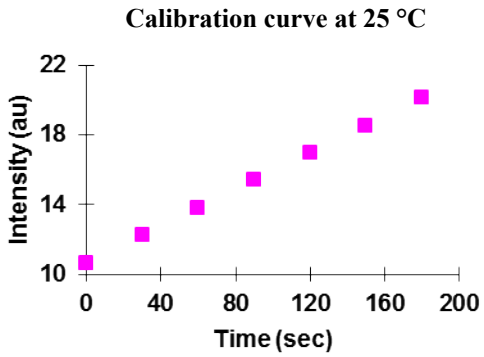
Figure S10. Job's plot for determining the stoichiometry (1:1) of receptor **4** and Cu^{2+} ions in CH_3CN; λ_{ex} = 320 nm

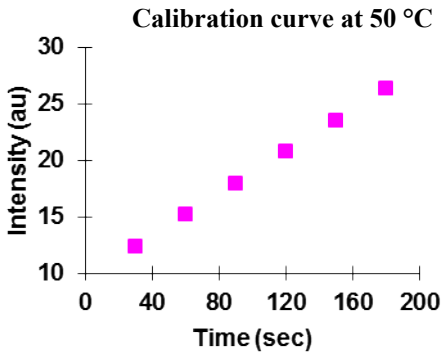
Mass spectra of hydrolysis product of rhodamine



Rate constant calculation

Fluorescence intensity *vs* time plot at fixed wavelength (582 nm) using first order rate equation, we get the rate constant at two temperature.





$R^2 = 0.909$

Slope = 0.052452

Rate constant at 25 °C (K_{25}) = slope × 2.303

= 0.05242 × 2.303

= 12.07 × 10^{-2} Sec^{-1}

$R^2 = 0.90$

Slope = 0.092929

Rate constant at 50 °C (K_{50}) = slope × 2.303

= 0.092929 × 2.303

= 0.214015 Sec^{-1}

Ratio of rate constant = K_{50}/K_{25}

= 1.7731

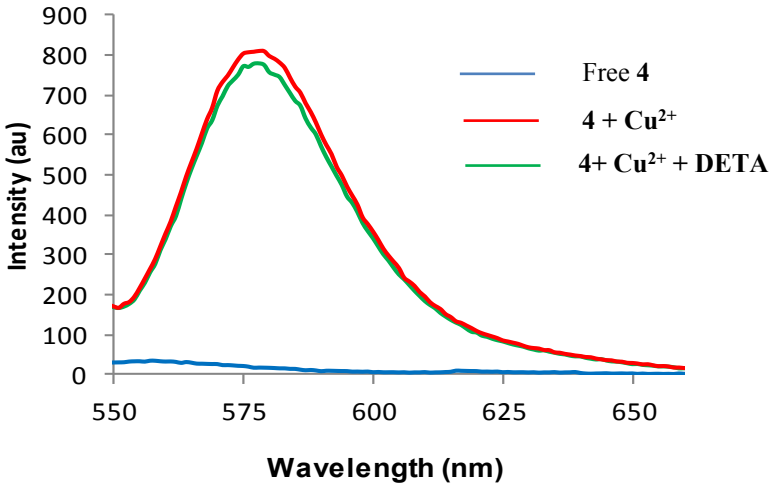
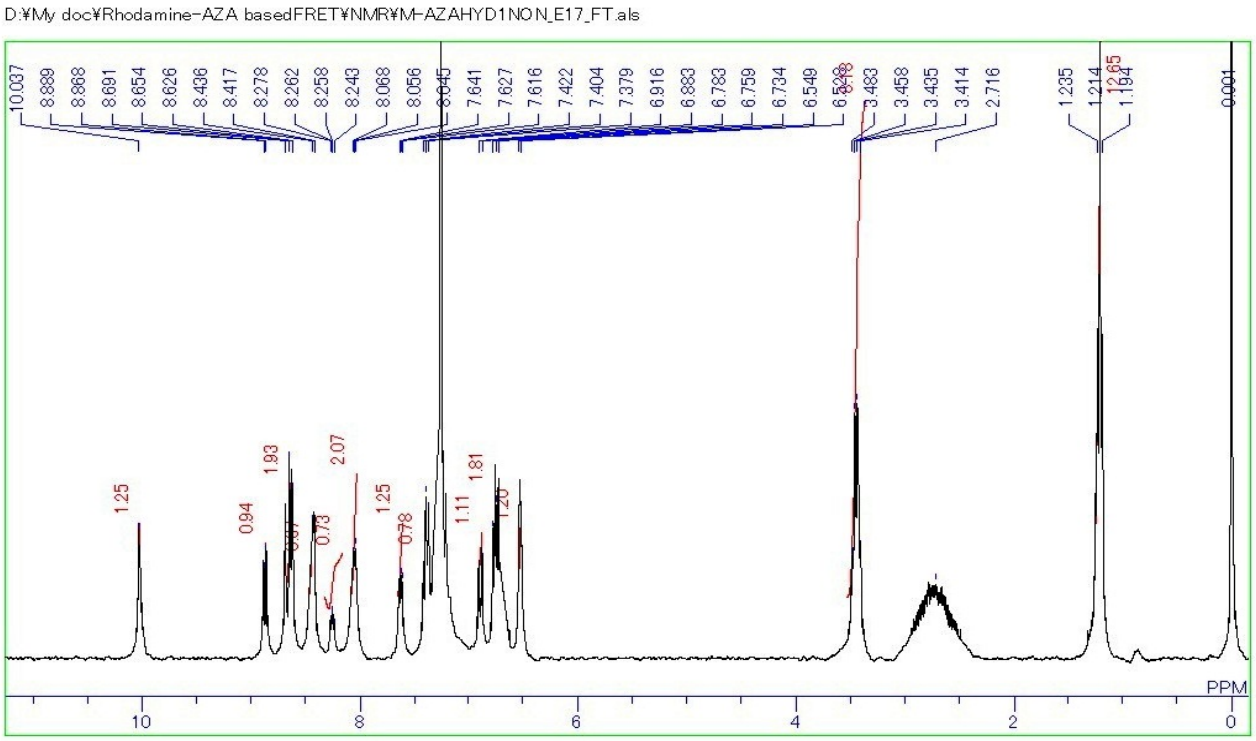


Figure S13. Fluorescence spectra showing no reversibility of Cu^{2+} ions to receptor **4** by diethylenetriamine (DETA); blue line, free **4** (1.0 μM), red line, **4** + 20 μM Cu^{2+}, green line, **4** + 20 μM Cu^{2+}+ 40 μL DETA, CH_3CN:H_2O (7:3, v/v) buffered with HEPES, pH = 7.0; λ_{ex} = 530 nm in 3ml solution.

^{1}H NMR of hydrolysis products



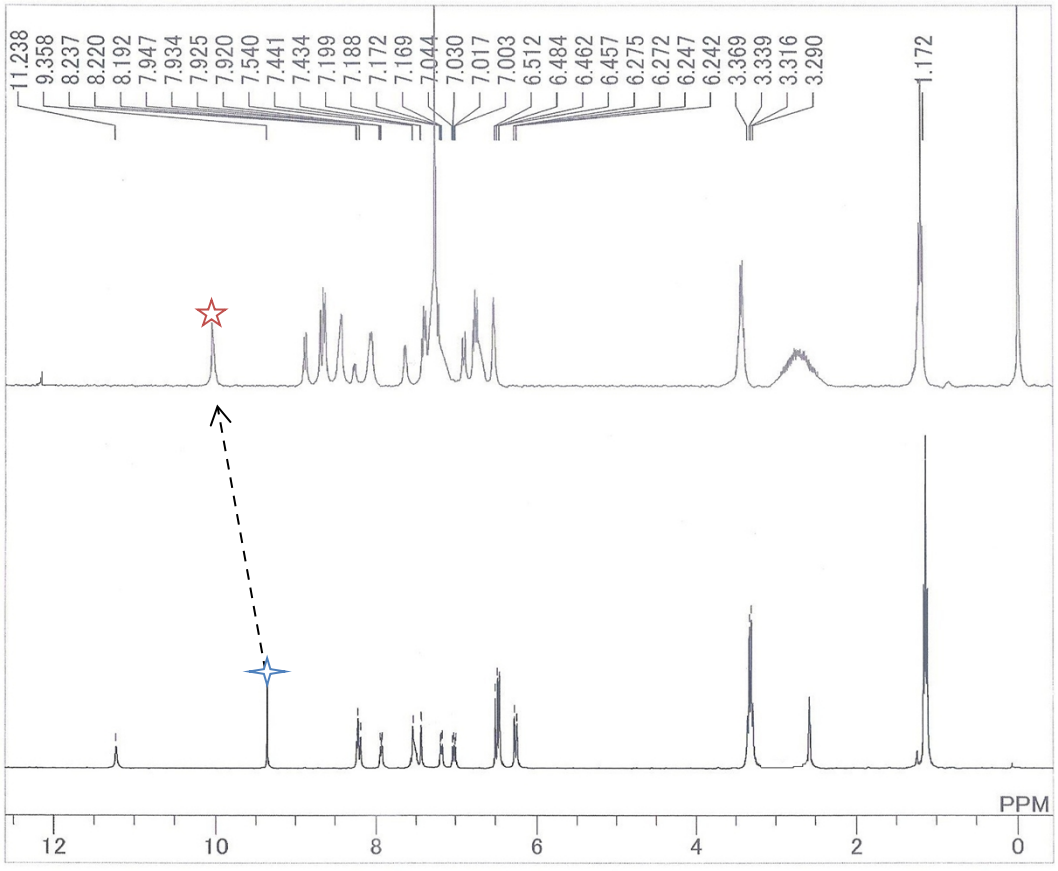
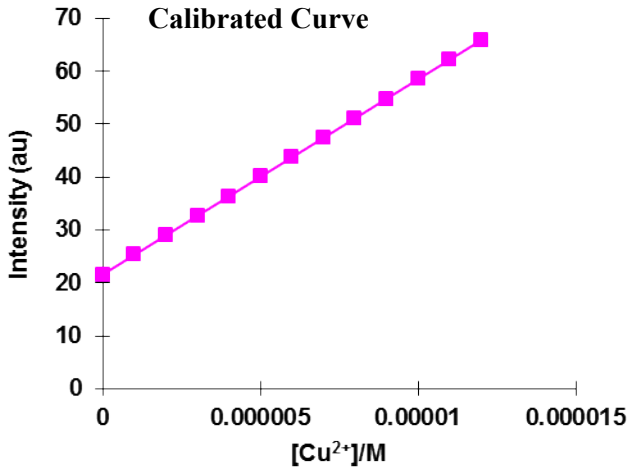


Figure S15: Change in chemical shift from imino proton (✧) to aldehyde proton (☆) of azaindole moiety after Cu^{2+} induced hydrolysis of receptor **4**.

Calculations for detection limit



Multiple R = 0.988647

R^2 = 0.977423

Standard deviation = 2.27902

Observation = 13

Intercept = 21.61725

Slope = 3686484

The detection limit was calculated based on the fluorescence titration. To determine the S/N ratio, the emission intensity of receptor **4** without Cu^{2+} was measured by 13 times and the standard deviation of blank measurements was determined. The detection limit is then calculated with the following equation:

$DL = 3 \times SD/S$

Where SD is the standard deviation of the blank solution measured by 13 times; S is the slope of the calibration curve.

From the graph we get slope (S) = 3686484, and SD value is 0.024671

Thus using the formula we get the Detection Limit (DL) = 2.00768×10^{-8} M i.e. probe **4** can detect Cu^{2+} in this minimum concentration through fluorescence method.

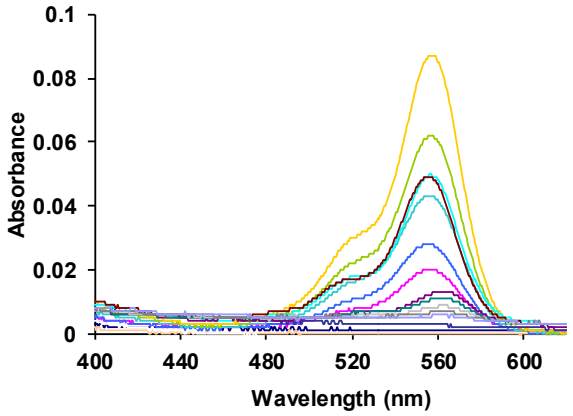


Figure S17. UV–vis spectra of compound **4** (0.5 μM) in the presence of Cu^{2+} ions (0-3.3 equiv) in CH_3CN/H_2O (7:3, v/v); buffered with HEPES, pH = 7.0.