Supplementary Information

Highly selective fluorescence turn-on chemodosimeter based on rhodamine for nanomolar detection of copper ions

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Instruments and experimental procedures

General information

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 fluorescence spectra were recorded with a SHIMADZU 5301 PC spectrofluorimeter (slit widths at excitation and emission of the spectrofluorimeter are 5-5). ^{1}H and ^{13}C NMR spectra were recorded on a JEOL-FT NMR-AL 300 MHz using $CDCl_3/CD_3CN/D_2O$ as solvent and tetramethylsilane $SiMe_4$ as internal standards. Data are reported as follows: chemical shifts in ppm (δ), multiplicity (s = singlet, d = doublet, q = quartet, br = broad singlet, m = multiplet, dd = doublet of doublet), coupling constants (Hz), integration, and interpretation. 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 380 and 540 nm, respectively and quantum yield is calculated using the equation:

$$\Phi_{fs} = \Phi_{fr} \times \frac{1-10^{-A_s L_s}}{1-10^{-A_r L_r}} \times \frac{N_s^2}{N_r^2} \times \frac{D_s}{D_r}$$

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, Ds and Dr the respective areas of emission for sample and reference. L_s and L_r are the lengths of the absorption cells of sample

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

and reference respectively. N_s and N_r are the refractive indices of the sample and reference solutions (pure solvents were assumed respectively).

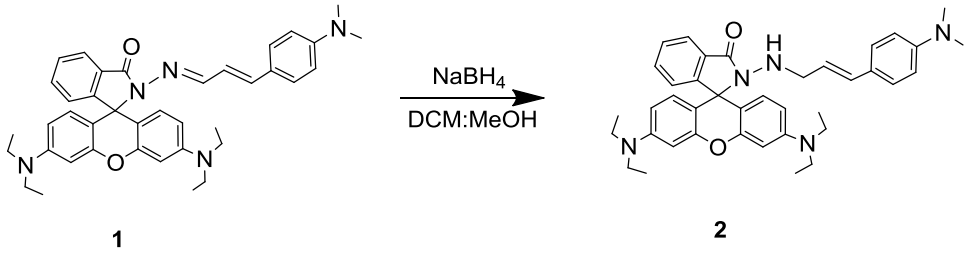
Procedure for metal ion sensing

UV-vis and fluorescence titrations were performed on 5.0 μM and 1.0 μM solutions of ligand in $CH_3CN:H_2O$ (8:2, v/v) mixture, respectively. Typically, aliquots of freshly prepared metal perchlorates (Hg^{2+}, Fe^{2+}, Fe^{3+}, Pb^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}, Ni^{2+}, Ag^{+}, Co^{2+}, Mg^{2+}, Ba^{2+}, Li^{+}, Na^{+}, and K^{+}) standard solutions (10^{-1} M to 10^{-3} M) in $CH_3CN:H_2O$ (8:2, v/v) were added to record the UV-vis and fluorescence spectra. In titration experiments, each time a 3 ml solution of **2** was filled in a quartz cuvette (path length, 1 cm) and metal ions were added into the quartz cuvette by using a micro-pippet.

Procedure for fluorescence imaging

The prostate cancer (PC3) cell lines were incubated with receptor **2** (5 μM in $CH_3CN:H_2O$ (8:2, v/v) buffered with HEPES, pH = 7.0) in RPMI-1640 medium for 20 min at 37ºC and washed with phosphate buffered saline (PBS) buffer (pH 7.4) to remove excess of receptor **2**. The cells pre-treated with **2** were then treated with copper perchlorate (30 μM) in RPMI-1640 medium and incubated for further 20 min at 37ºC and washed with PBS buffer. The cells were imaged by confocal fluorescence microscope OLYMPUS FLUO VIEW FV1000 with excitation wavelength 488 nm. Fluorescence images are recorded at both green (520 ± 20 nm) and red channels (570 ± 20 nm)

Synthetic routes and characteristic data



1 2

Synthesis of compound **2**

Compound **1**[2] was synthesized according to the literature procedure.

Synthesis of (2):

A mixture of compound **1** (0.20 g, 0.28 mmol) and $NaBH_4$ (0.06 g, 1.68 mmol) in a 1:1 mixture of dry dichloromethane and dry methanol was stirred for 12 h under ice cold conditions. After the completion of the reaction solvent was removed under vaccum. Water was added to the residue and the pH of the solution was maintained at 9 with aqueous ammonia. The solution was then extracted with chloroform and the organic layer was dried over anhydrous sodium sulfate and distilled under reduced pressure, the residue left was recrystallized from $CHCl_3$/CH_3OH to give compound **2** in 65 % yield; m.p. 170 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.16 (t, J = 6 Hz, 12 H, CH_3), 2.92 (s, 6 H, CH_3), 3.29-3.36 (m, 10 H, CH_2), 4.27 (br, 1 H, NH), 5.75-5.85 (m, 1 H, CH), 6.13-6.25 (m, 3 H, Ar-H and CH), 6.41-6.44 (m, 4 H, Ar-H), 6.60 (d, J = 9 Hz, 2 H, Ar-H), 7.08-7.15 (m, 3 H, Ar-H), 7.40-7.47 (m, 2 H, Ar-H), 7.88-7.91 (m, 1 H, Ar-H) ppm. ^{13}C NMR ($CDCl_3$, 300 MHz): δ = 12.67, 40.54, 44.40, 53.92 65.57, 97.26, 106.00, 107.79, 112.35, 122.13, 122.74, 124.01, 127.31, 128.00, 128.86, 131.57, 132.77, 148.71, 153.84, 166.72 ppm. MS ES+, *m/z*: = 616 $[M+H]^+$. $C_{39}H_{45}N_5O_2$: calcd. C 76.07, H 7.37, N 11.37. Found C 76.24, H 7.14, N 11.56.

[2] Kumar, M.; Kumar, N.; Bhalla, V. *Tetrahedron Lett*. **2011**, *52*, 4333.

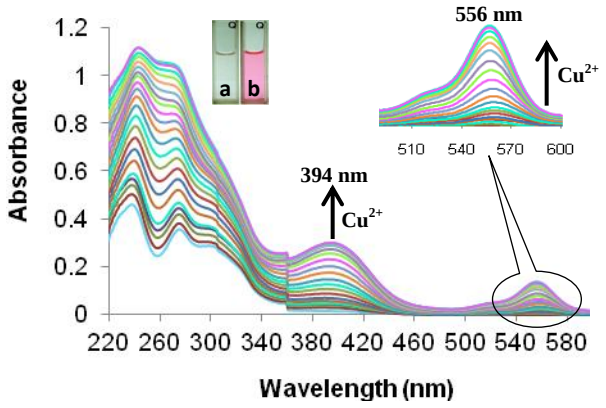


Figure S1. UV-vis spectra of **2** (5 µM) in the presence of Cu^{2+} ions (0-36 equiv) in $CH_3CN:H_2O$ (8:2, v/v) buffered with HEPES, pH = 7.0; Inset showing the color change (a) before and (b) after the addition of Cu^{2+} ions.

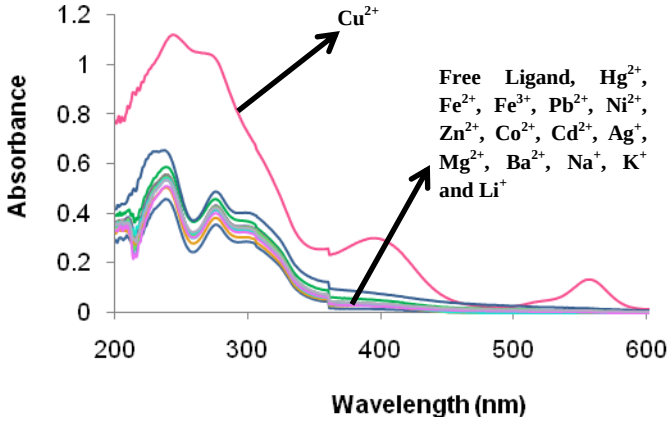


Figure S2. UV-vis spectra of **2** (5 µM) in the presence of various metal ions (36 equiv each) in $CH_3CN:H_2O$ (8:2, v/v) buffered with HEPES, pH = 7.0.

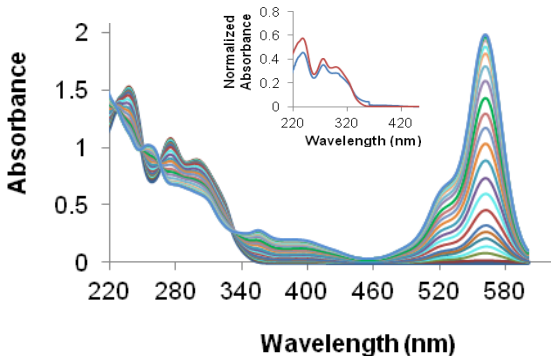


Figure S3. UV-vis spectra of **2** (5 μM) in the presence of Cu^{2+} ions (0-16 equiv) in CH_3CN; Inset showing the normalized absorbance spectra of **2**; blue, in aqueous media; red, in acetonitrile.

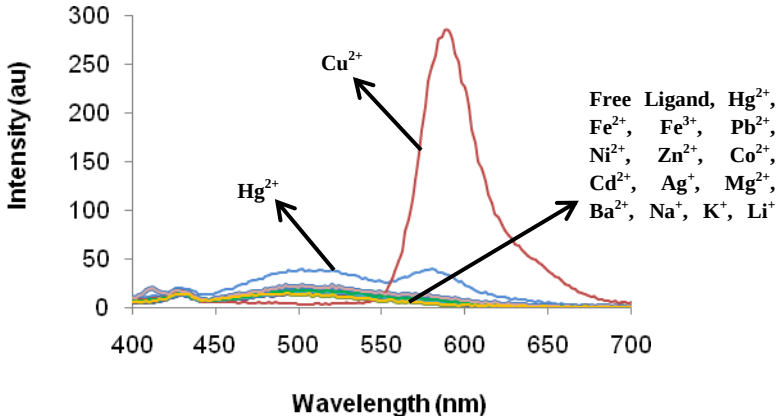


Figure S4. Fluorescence spectra of **2** (1.0 μM) in the presence of various metal ions (24 μM each) in CH_3CN; λ_{ex} = 380 nm.

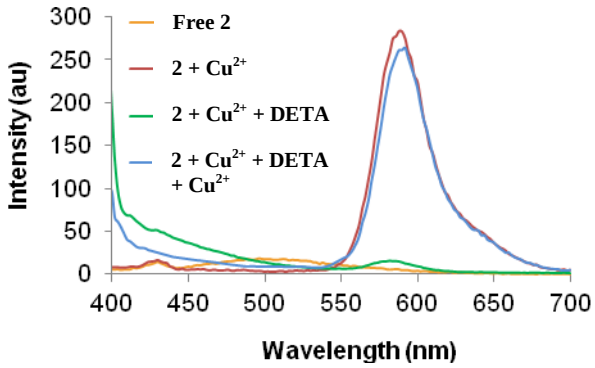


Figure S5. Fluorescence spectra showing reversibility of Cu^{2+} coordination to receptor **2** by diethylenetriamine (DETA); orange line, free **2** (1.0 µM), red line, **2** + 24 µM Cu^{2+}, green line, **2 +** 24 µM Cu^{2+} + 10 µL DETA, blue line, **2 +** 24 µM Cu^{2+} + 10 µL DETA + 70 µM Cu^{2+} in CH_3CN; λ_{ex} = 380 nm.

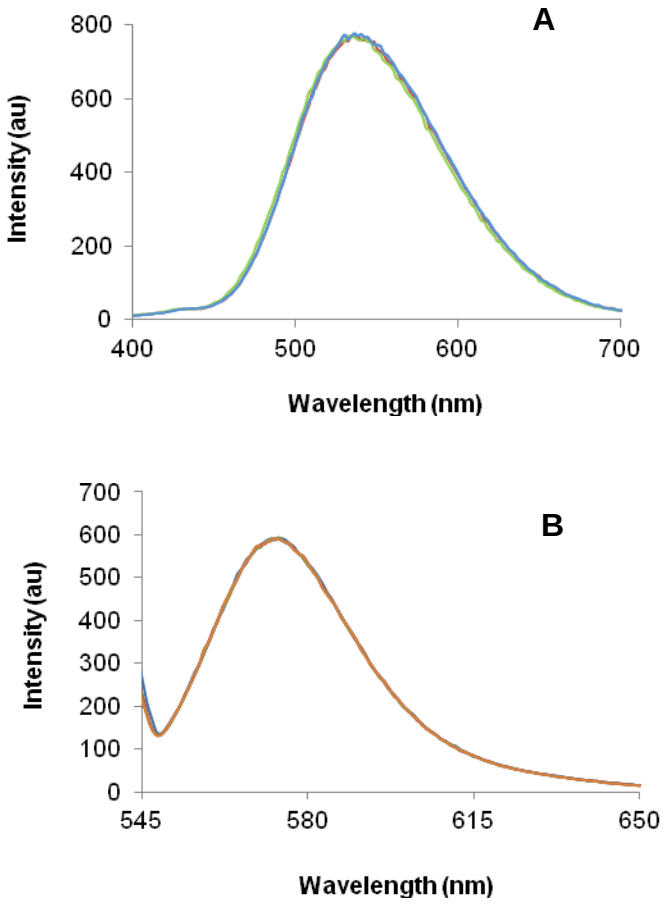


Figure S6. Fluorescence spectra of **2**-Cu^{2+} in the presence of diethylenetriamine (DETA) in CH_3CN:H_2O (8:2, v/v) buffered with HEPES, pH = 7.0; (**A**) **2** (1.0 μM) + Cu^{2+} (22 μM) + DETA (50 μL) at λ_{ex} = 380 nm; (**B**) **2** (1.0 μM) + Cu^{2+} (40 μM) + DETA (50 μL) λ_{ex} = 540 nm.

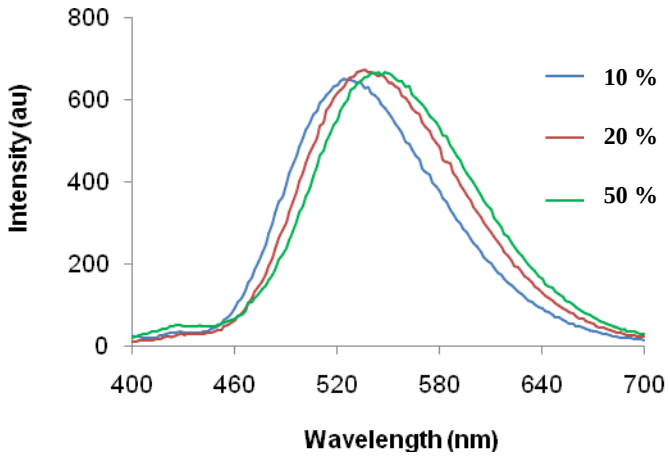


Figure S7. Fluorescence spectra of **2** (1.0 μM) in the presence of Cu^{2+} ions in varying concentrations of CH_3CN:H_2O; 10 % = 9:1, v/v; 20 % = 8:2, v/v; 50 % = 1:1 v/v; buffered with HEPES, pH = 7.0; λ_{ex} = 380 nm. As we increase the water content of solvent system, there is a continuous red shift of the emission from 525 nm to 548 nm confirming that twisted intramolecular charge transfer (TICT) state is responsible for this emission. While, locally excited band (LE) appears as a shoulder (at 430 nm) on the main emission band and any increase in polarity of the solvent did not affect the position of excited band (LE) band.

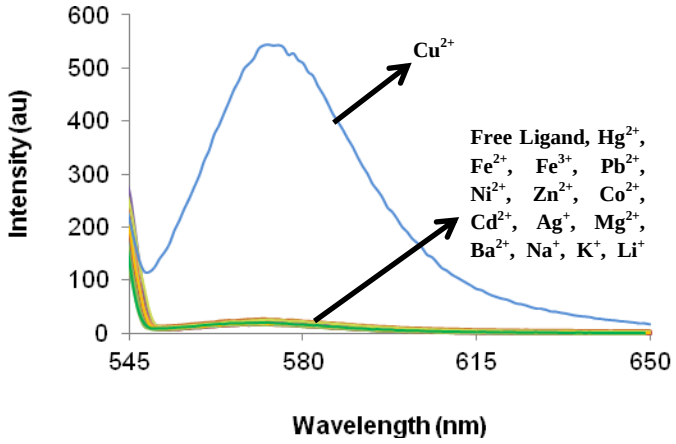


Figure S8. Fluorescence spectra of **2** (1.0 μM) in the presence of various metal ions (40 μM each) in $CH_3CN:H_2O$ (8:2, v/v) buffered with HEPES, pH = 7.0; λ_{ex} = 540 nm.

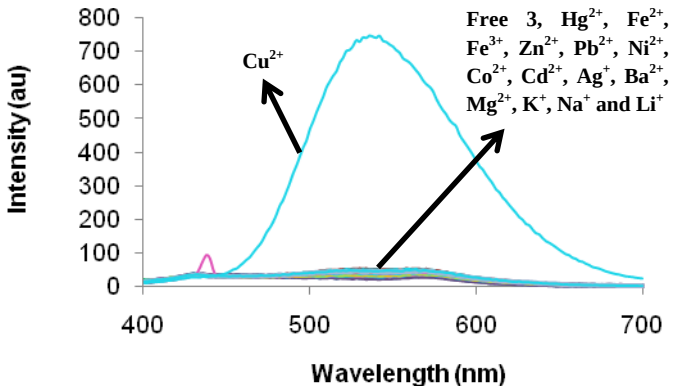


Figure S9. Fluorescence spectra of **2** (1.0 μM) in the presence of different metal ions (22 μM each) in $CH_3CN:H_2O$ (8:2, v/v) buffered with HEPES, pH = 7.0; λ_{ex} = 380 nm.

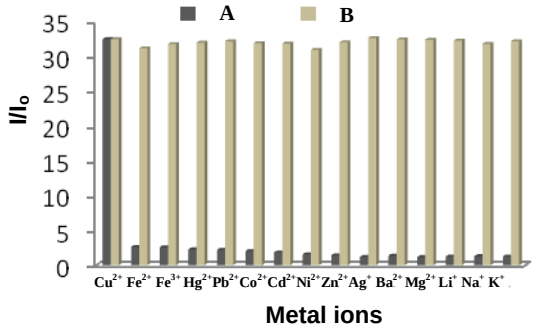


Figure S10. (A) Black bars represent selectivity (I/I_o; I_o = initial fluorescence intensity at 534 nm; I = fluorescence intensity after the addition of metal ions at 534 nm) of **2** (1.0 μM) upon addition of different metal ions (22 μM each). (B) Grey bars represent competitive selectivity of receptor **2** (1.0 μM) towards Cu^{2+} ions (22 μM) in the presence of other metal ions (22 μM) in CH_3CN/H_2O (8:2, v/v) buffered with HEPES, pH = 7.0; λ_{ex} = 380 nm.

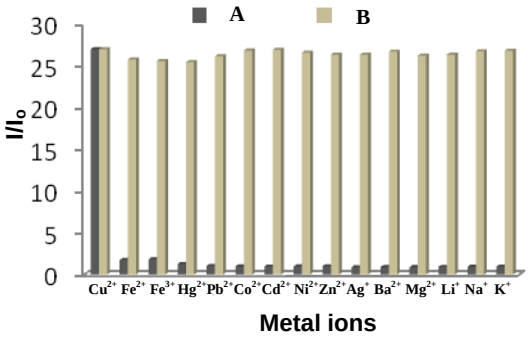


Figure S11. (A) Black bars represent selectivity (I/I_o; I_o = initial fluorescence intensity at 575 nm; I = fluorescence intensity after the addition of metal ions at 575 nm) of **2** (1.0 μM) upon addition of different metal ions (40 μM each). (B) Grey bars represent competitive selectivity of receptor **2** (1.0 μM) towards Cu^{2+} ions (40 μM) in the presence of other metal ions (40 μM) in CH_3CN/H_2O (8:2, v/v) buffered with HEPES, pH = 7.0; λ_{ex} = 540 nm.

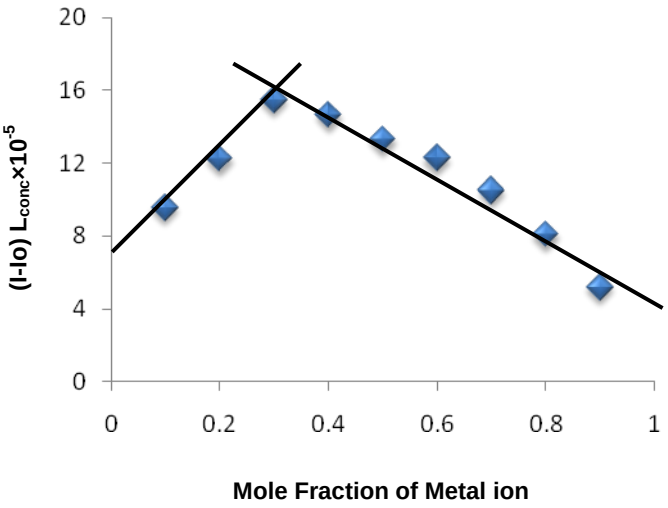


Figure S12. Job's plot for determining the stoichiometry (2:1) of receptor **2** and Cu^{2+} ions in CH_3CN; λ_{ex} = 380 nm.

Calculations for detection limit:

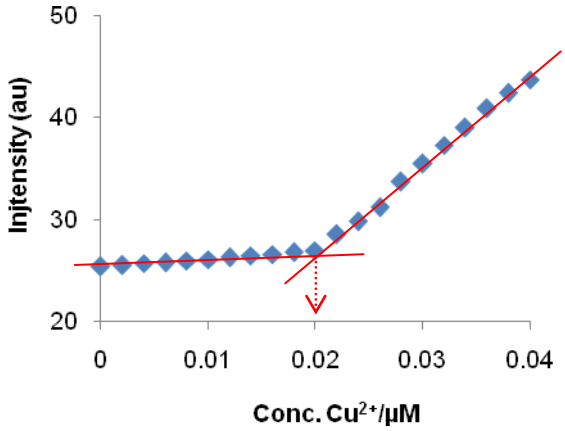


Figure S13. Figure showing the fluorescence intensity at 534 nm as a function of Cu^{2+} ions concentration.

To determine the detection limit, fluorescence titration of compound **2** with copper ions was carried out by adding aliquots of copper solution of micromolar concentration and the fluorescence intensity as a function of Cu^{2+} ions added was then plotted. From this graph the concentration at which there was a sharp change in the fluorescence intensity multiplied with the concentration of receptor **2** gave the detection limit.[3]

Equation used for calculating detection limit (DL):

$DL = C_L \times C_T$

C_L = Conc. of Ligand; C_T = Conc. of Titrant at which change observed.

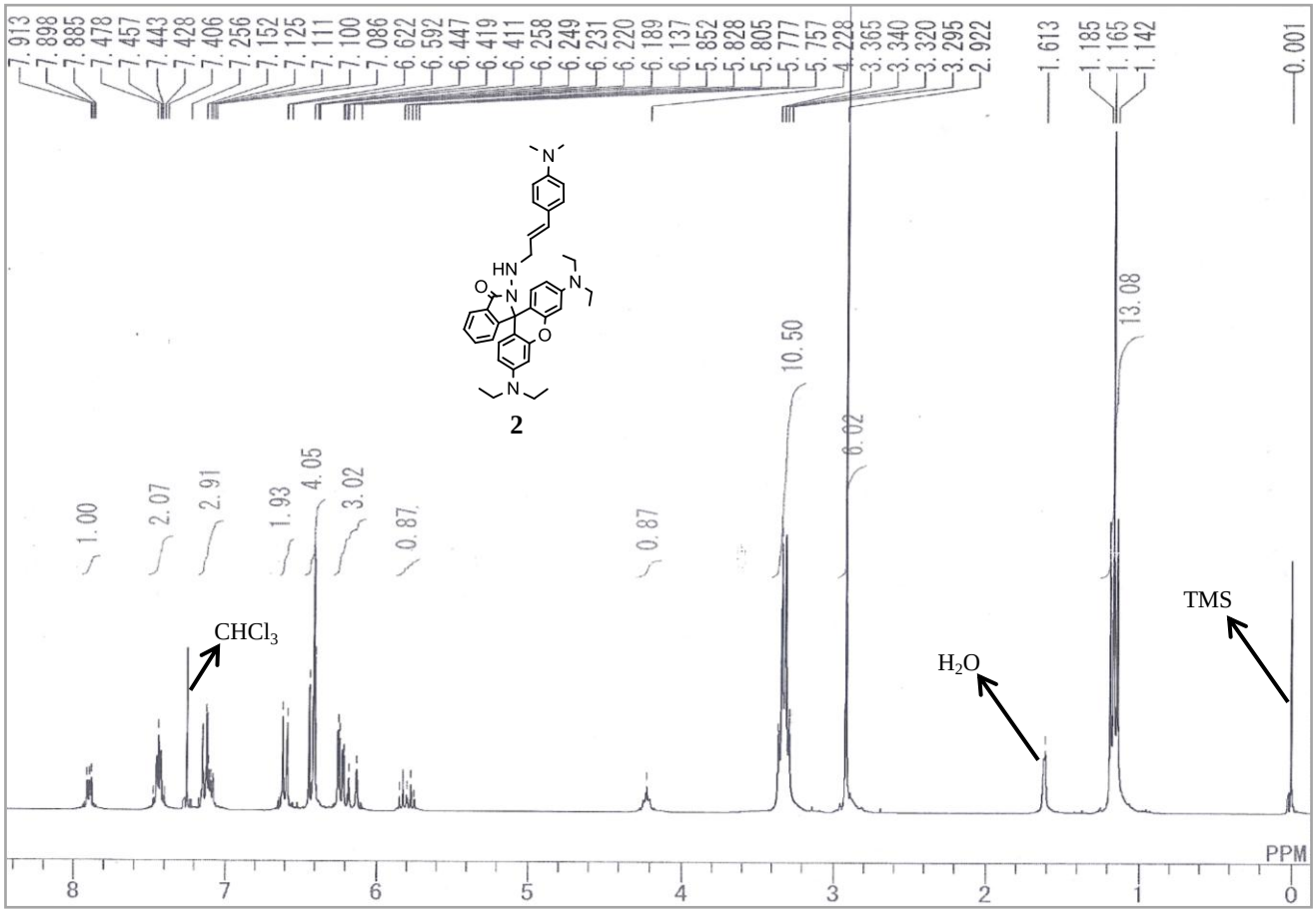
Thus;

$DL = 1 \times 10^{-6} \times 0.02 \times 10^{-6} = 0.02 \times 10^{-6} = 2 \times 10^{-8}$

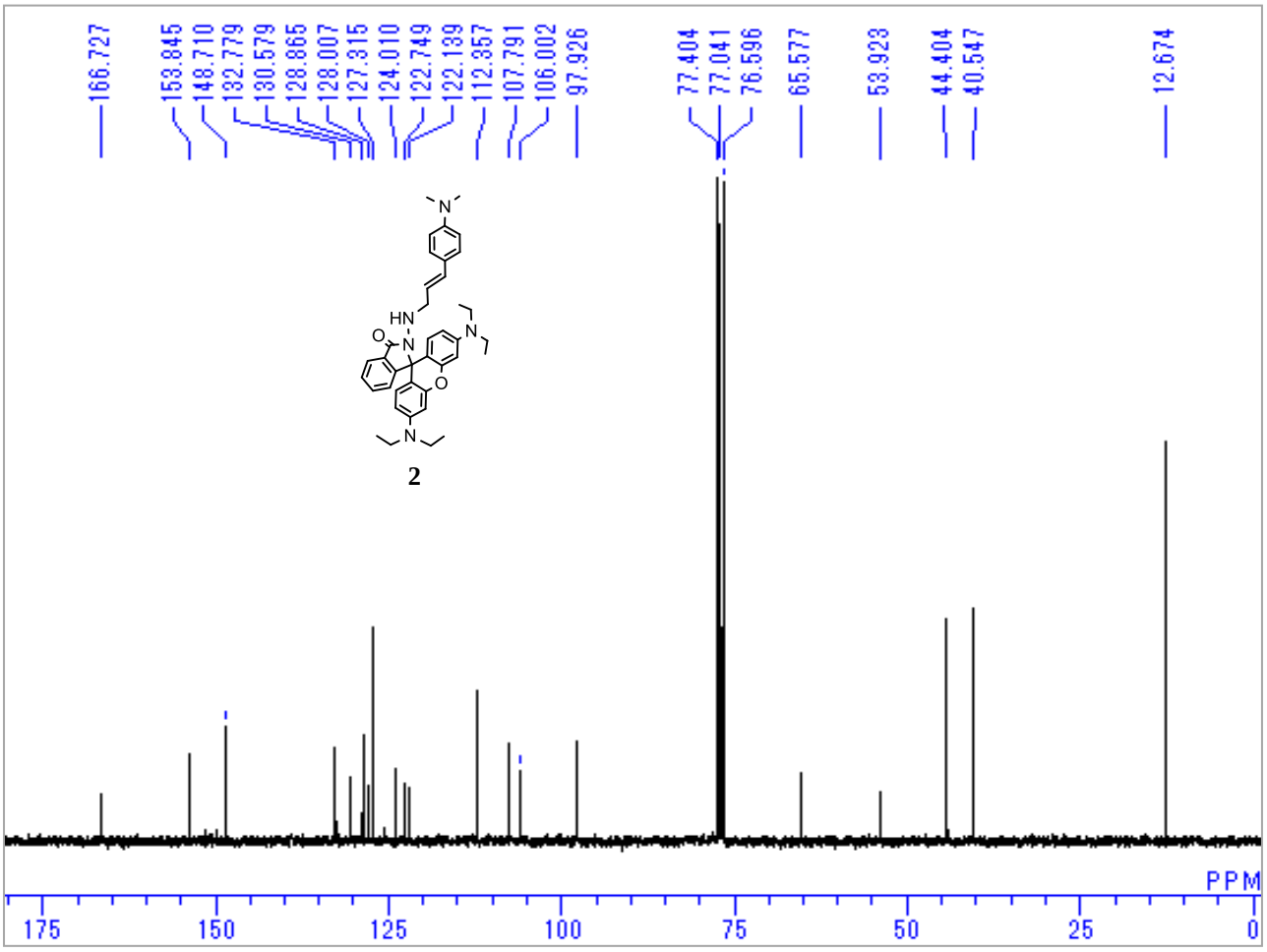
$= 20 \times 10^{-9}$

[3] G. L. Long and J. D. Winefordner, *Anal. Chem.*, 1983, **55**, 712A.

^{1}H NMR spectrum of compound **2**.

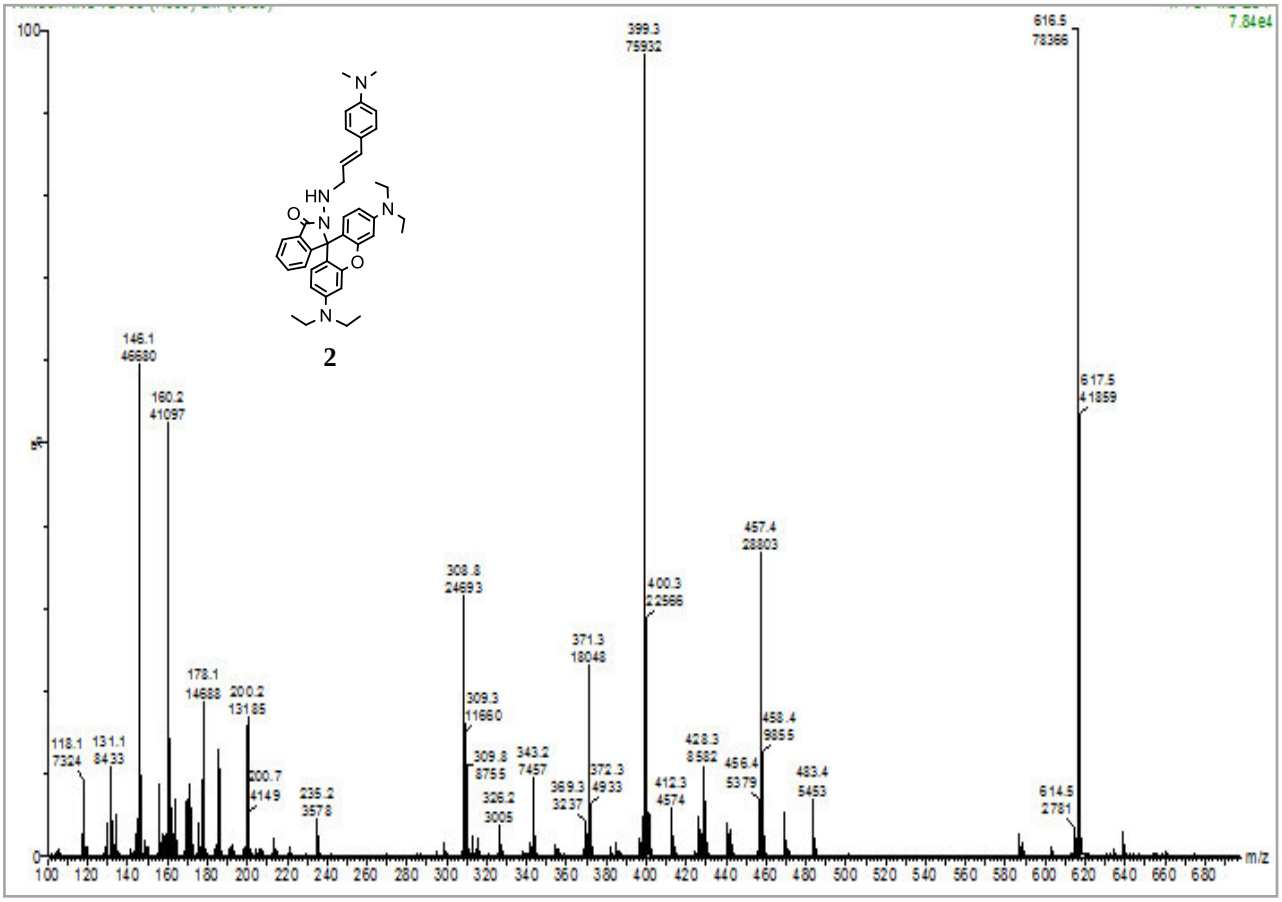


^{13}C NMR spectrum of compound **2**.



2

Mass spectrum of compound **2**.



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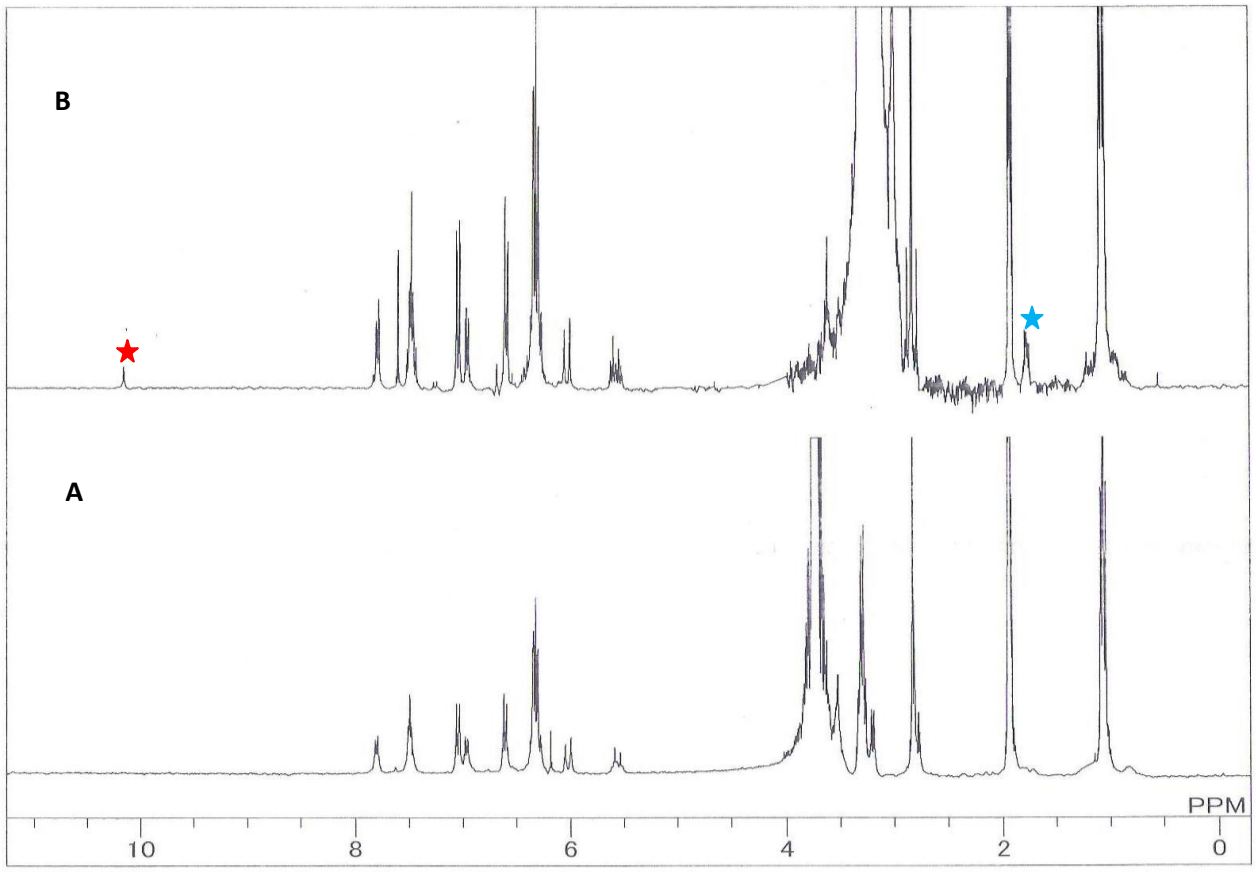

^{1}H NMR spectra of receptor **2** in CD_3CN– D_2O (9:1, v/v); (**A**) Free **2**; (**B**) reaction of **2** with Cu^{2+} was carried out in CD_3CN– D_2O (9:1, v/v) for 5 min and then ^{1}H NMR spectrum of the resulting solution was recorded after eliminating Cu^{2+} ions by using Chelex resin[4]

[4] (*a*) D. Y. Lee and H. C. Zheng, *Plant Soil*, 1994, **164**, 19; (*b*) M. H. Kim, H. H. Jang, S. Yi, S. K. Chang and M. S. Han, *Chem. Commun*., 2009, 4838.