Supporting information

A Colorimetric and Fluorometric Investigation of Cu(II) ion in Aqueous Medium with a Fluorescein-based Chemosensor

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General comments: Reagent grade fluorescein, NaOH, chloroform, hydrazine hydrate and all metal perchlorate salts were purchased from commercial suppliers (Sigma-Aldrich and Rankem, India) and used as received without further purification. All the solvents were bought from commercial suppliers and were purified prior to use following standard literature methods. Melting points were determined on the Stuart SMP40 Automatic melting point apparatus and are uncorrected. IR spectra were determined on KBr pellets with a Perkin-Elmer's Spectrum 65 FT-IR Spectrometer. Mass was analyzed using Q-TOF MicroTM LC-MS instrument. Analytical thin layer chromatography was performed on aluminium plates pre-coated with silica gel 60 F_{254}. Visualization was performed by naked eyes under visible light. Chromatographic separations were performed on a silica gel column by flash chromatography. Yields are given after purification, unless differently stated. When reactions were performed under anhydrous conditions, the mixtures were maintained under nitrogen. NMR spectra were recorded on 500 MHz spectrometer (^{1}H NMR and ^{13}C NMR). Compounds were named following IUPAC rules as applied by Beilstein-Institute AutoNom software for systematic names in organic chemistry.

Caution! *Particularly in dry state, perchlorate salts are potentially explosive and should be handled with extreme care. We have not encountered any problem during this work since we used it in small quantities at a time.*

Synthesis of fluorescein monoaldehyde (2): To fluorescein (1.0 g, 3 mmol) in MeOH (2 mL), 50% NaOH solution (5g) was added in a 50 mL two-neck round-bottom flask. After reaching the temperature at 55 °C, 3 mL of chloroform was added drop wise and then the reaction was allowed to reflux for overnight. Completion of reaction was confirmed with silica coated thin layer chromatography. All the volatile impurities were removed under reduced pressure and the crude reaction mixture was purified by column chromatography using dichloromethane and methanol (20:1) to get fluorescein monoaldehyde (**2**) (160 mg, 15% yield) as yellow solid.

Characterization data: R_f = 0.65 (20:1 DCM: MeOH); **^{1}HNMR** (500 MHz, DMSO-d_6): δ 11.88 (s, 1 H), 10.63 (s, 1 H), 10.26 (s, 1 H), 8.02 (d, 1 H , *J* = 6.6 Hz), 7.81 (t, 1 H, *J* =1.6 Hz), 7.73 (t, 1 H, *J* = 7.6 Hz),7.32 (d,1 H, *J* =7.7 Hz) 6.95 (d, 1 H , *J* = 8.9 Hz), 6.85 (s, 1 H), 6.71 (d, 1 H, *J* = 9.1 Hz,), 6.61 (s, 2 H); **^{13}CNMR** (500 MHz, DMSO-d_6): 168.5, 162.8, 159.5, 152.3, 152.1, 150.8, 136.4, 135.8, 130.3, 128.9, 125.8, 124.7, 123.9, 113.5, 113.3, 109.6, 109.1, 109.1, 102.6, 81.7; **FTIR**: IR (KBr): λ_{max}/cm^{-1} 3326.2 (O-H stretching), 2928, 2853, (aldehyde C-H stretching) 1745, 1727 (C=O stretching of aldehyde and lactone); Elemental analysis Calcd for C21H12O6: C, 70.00; H, 3.36 %. Found: C, 69.90; H, 3.29 %; **Mass:** 361.54 $[M + H]^+$.

Synthesis of fluorescent chemosensor, 1,4-bis(1-fluorescein)-2,3-diaza-1,3-butadiene (**3**): To a solution of fluorescein monoaldehyde (**2**) (100 mg, 0.28 mmol) in ethanol (5 mL), hydrazine hydrate (5.2 µL) was added and refluxed for 22 hrs. The completion of reaction was confirmed using thin layer chromatography. The reaction mixture obtained was cooled to room temperature and centrifuged at 10,000 rpm for 10 min yielding

the orange colored crude solid mixture as pellet. To obtain pure product, the mixture was washed with DCM: hexane (20:1) for 3 times followed by DCM: EtOH (20:1) for another 3 times to get compound **3** in 35% (70 mg) yield. **Characterization data:** R_f = 0.37 (30:1 DCM: MeOH); ^{1}H NMR (500 MHz, acetone-d_6): δ 12.37 (s, 1 H), 9.78 (s, 1 H), 9.11 (s, 1 H), 8.03 (d, 2 H, *J* = 7.9 Hz), 7.85 (t, 2 H, *J* = 7.6), 7.77 (t, 2 H, *J* = 7.4 Hz,), 7.38 (t, 2 H, *J* = 6.8 Hz) 7.01 (s, 2H) 6.96(d, 1 H, *J* = 9.0 Hz), 6.79 (d, 2 H, *J* = 9.0), 6.71 (s, 4 H, *J* = 6.7 Hz); ^{13}CNMR (125 MHz, DMSO-d_6): δ 206.54, 168.60, 161.17, 160.27, 159.58, 151.21, 150.68, 135.76, 130.29, 125.98, 124.75, 124.05, 113.27, 109.72, 109.25, 105.43, 102.83; Elemental analysis Calcd for C42H24N2O10: C, 70.39; H, 3.38; N, 3.91%. Found: C, 70.33; H, 3.28; N, 3.88 %; FTIR (cm^{-1}): 3546 (O-H stretching), 1755 (C=O, lactone); Mass: 717.1558 $[M + H]^+$.

Preparation and characterization of L-Cu^{2+} complex: The acetonitrile solution of $Cu(ClO_4)_2$• $6H_2O$ (19 mg, 0.05 mmol) was added to the DMSO and ethyl acetate solution of ligand (71 mg, 0.1 mmol), and a dark brown precipitate was produced. Then the mixture was stirred at room temperature for 5 hours. The dark brown solid that had formed was filtered, washed with water 5 times, and dried under vacuum. TLC: mobile phase = MeOH:DCM (1:20) ESI-TOF-MS: m/z = 779.20 [L-Cu^{2+}].

Solution preparation for the solubility study by UV-Vis spectroscopy: 0.358 mg of chemosensor was dissolved in 10 mL of various solvents such as DMSO, DMF, acetone, acetonitrile, pyridine, chloroform, methanol, ethanol, dichloromethane, water, hexane to prepare a series of 5 x 10^{-5} M solution. Resulting mixture was agitated well for the proper mixing (Figure SI 10**)**.

Absorbance study by UV-Vis spectroscopy: 2 mL solution of 5 x10^{-5} M receptor is diluted with 2 mL of same solvent to prepare the series of 2.5 x10^{-5} M solution of ligand to study the effect of solvent. During the UV-Vis spectroscopic study, base line correction was done with the respective solution against the receptor in the range of 200-800 nm. The concentration of receptor was found to be 2.5 x10^{-5} M from the optimization of λ_{max} value of receptor as their characteristic absorbance is found to be suitable for its study of solvent effect.

pH solution preparation: 12.11 mg Tris base was dissolved in 100 mL (1 % DMSO –Tris medium) to prepare 1 mmol as Tris base as a stock solution. 121.14 mg Tris base dissolved in 100 mL (1% DMSO in aqueous medium) to prepare 10 mmol Tris base stock solution. A series of various solutions (25 mL of each) with pH 7.4, 8.0, 9.0, 10.0 adjusted with HCl solution was prepared.

Preparation of ligand solution for study of pH impact on absorbance by UV-Vis: 3.58 mg of cationic receptor was dissolved in 5 ml DMSO to prepare 1 mmol stock solution of cationic receptor. 10 mL of 2.5 x10^{-5} M solution was prepared using 250 μL of stock solution of receptor and then diluted upto 10 mL solution with 1 mmol and 10 mmol Tris base possessing 7.4 pH (water: DMSO). Similarly, various other pH solutions (pH 8.0, 9.0, and 10.0) were prepared (Figure SI 11).

Absorbance study by UV-Vis spectroscopy in buffer solution: 3 mL of 2.5×10^{-5} M of receptor solution in various pH (250 μL of stock solution of receptor in 1 mmol as well as 1 mmol Tris base) was used after 3 hrs of

solution preparation to determine the Absorbance pattern within the range of 200-800 nm. The base line correction was done with the same blank solution.

Fluorescence study by fluorescence spectroscopy in buffer solution: Screening of metal ions was performed using ten-fold higher concentration to the receptor and effective concentration of receptor was 15 μM in 1 % DMSO-Tris buffer at pH 7.4. The excitation of the fluorescein fluorophore was made at 491 nm and slit width was 2 nm.

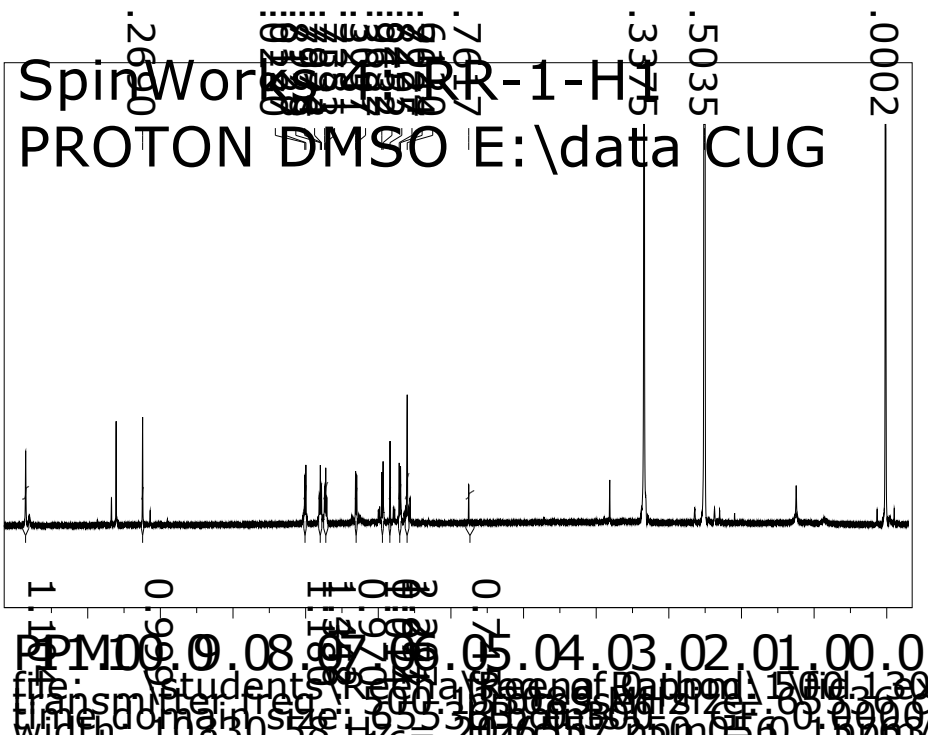


Figure SI 1. ^{1}H NMR spectrum of fluorescein monoaldehyde (**2**) in DMSO-d_6 in 500 MHz

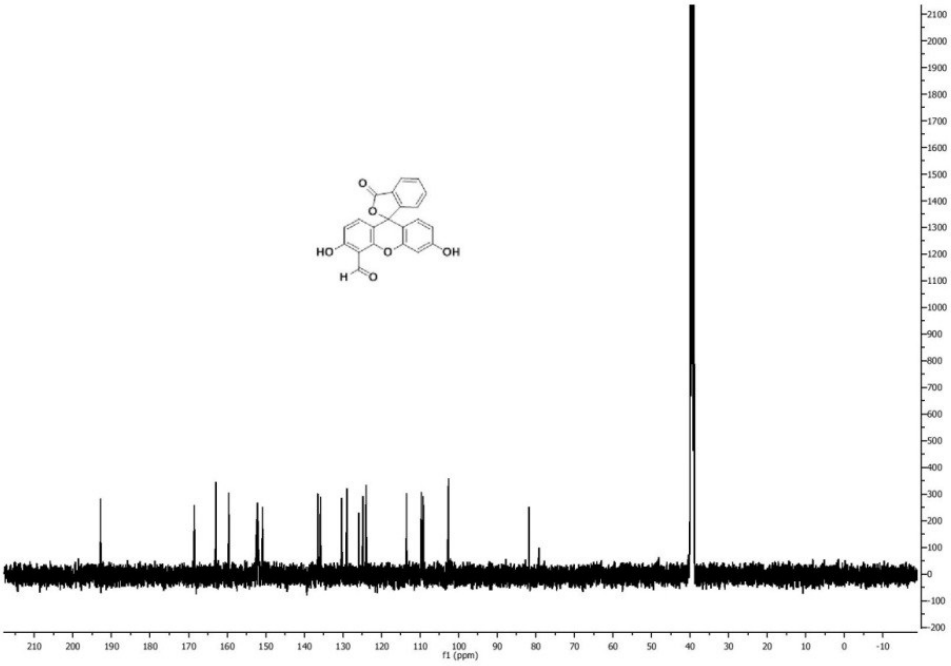


Figure SI 2. ^{13}C NMR spectrum of fluorescein monoaldehyde (**2**) in DMSO-d_6 in 500 MHz

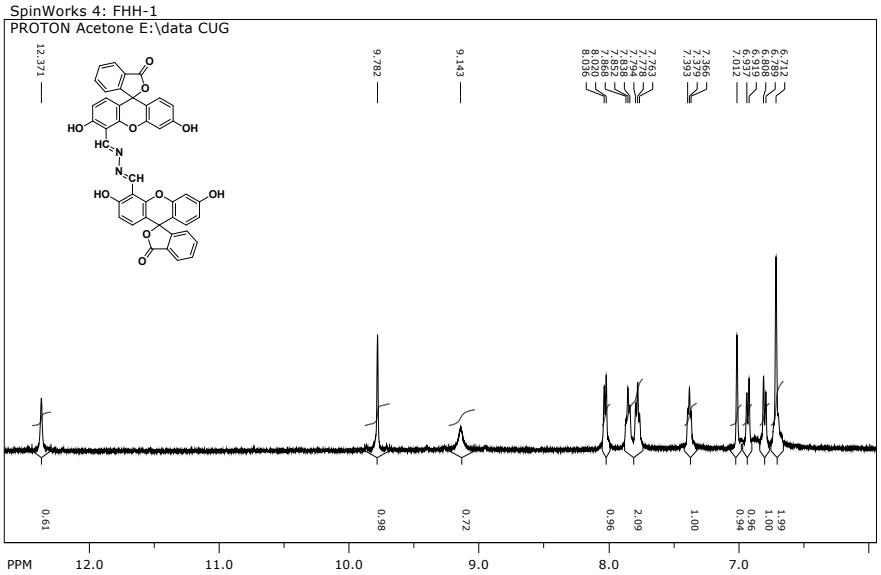


Figure SI 3. ^{1}H NMR spectrum of 1,4-bis(fluorescein)-2,3-diaza-1,3-butadiene (**3**) in acetone-d_6 in 500 MHz

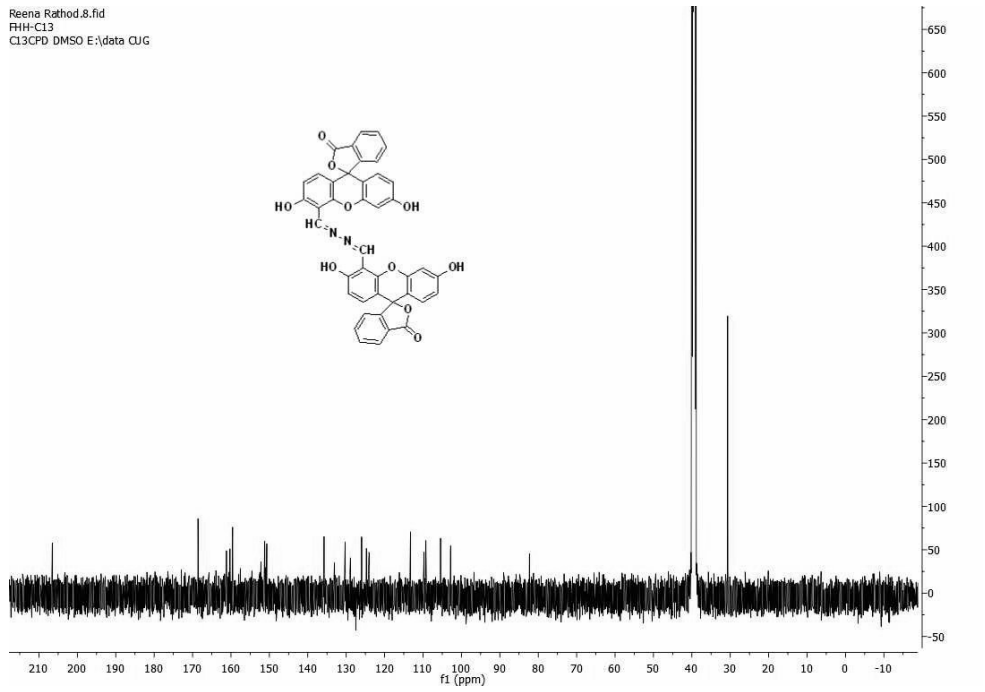


Figure SI 4. ^{13}C NMR spectrum of 1,4-bis(fluorescein)-2,3-diaza-1,3-butadiene (**3**) in DMSO-d_6 in 500 MHz

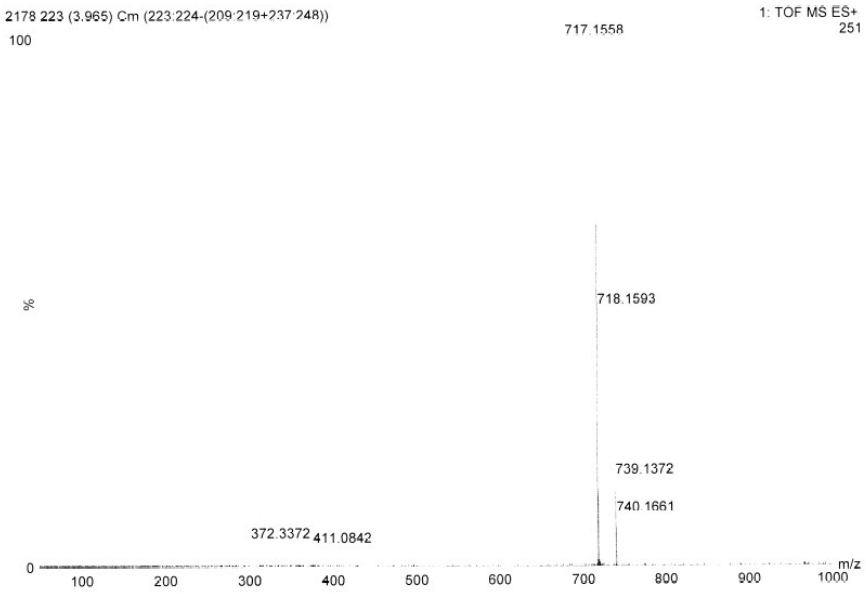


Figure SI 5. Mass spectrum of 1,4-bis(fluorescein)-2,3-diaza-1,3-butadiene (**3**)

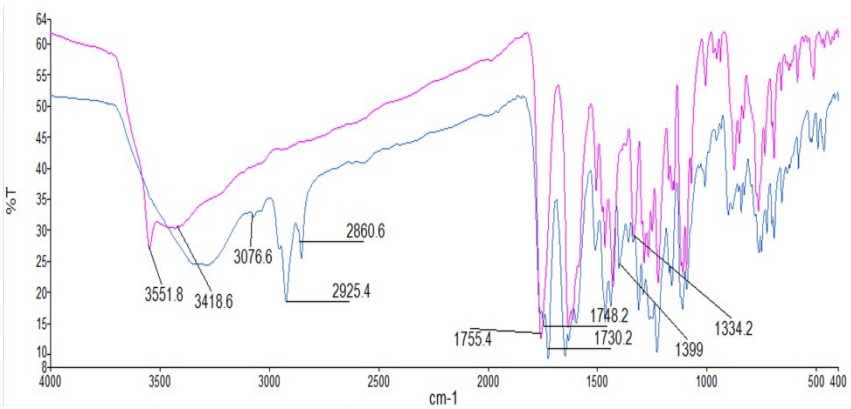


Figure SI 6. FTIR spectra of compound fluorescein monoaldehyde **2** (blue color) and ligand **3** (pink color)

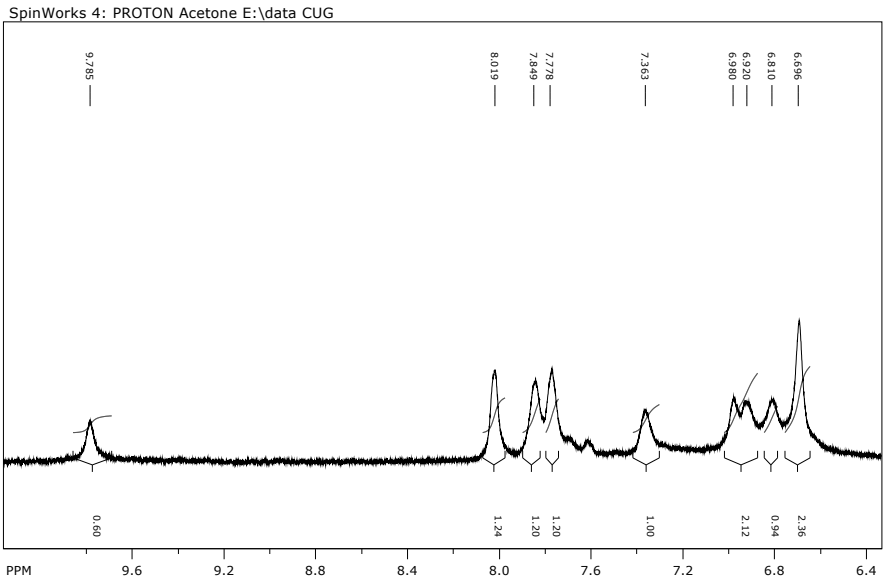
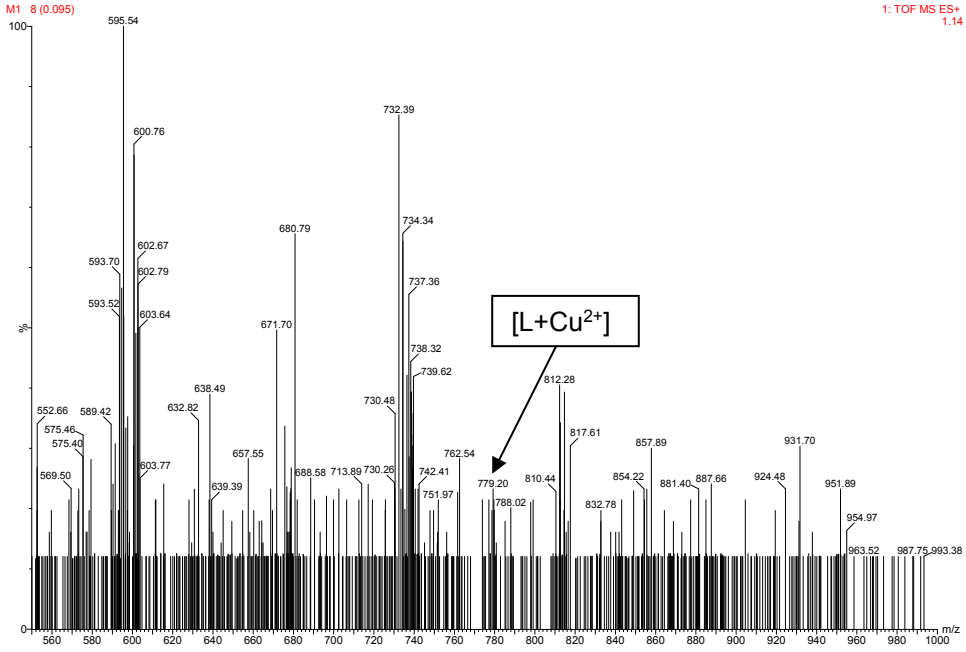


Figure SI 7. ^{1}H NMR of ligand-Cu^{2+} complex in acetone-d_6 in 500MHz



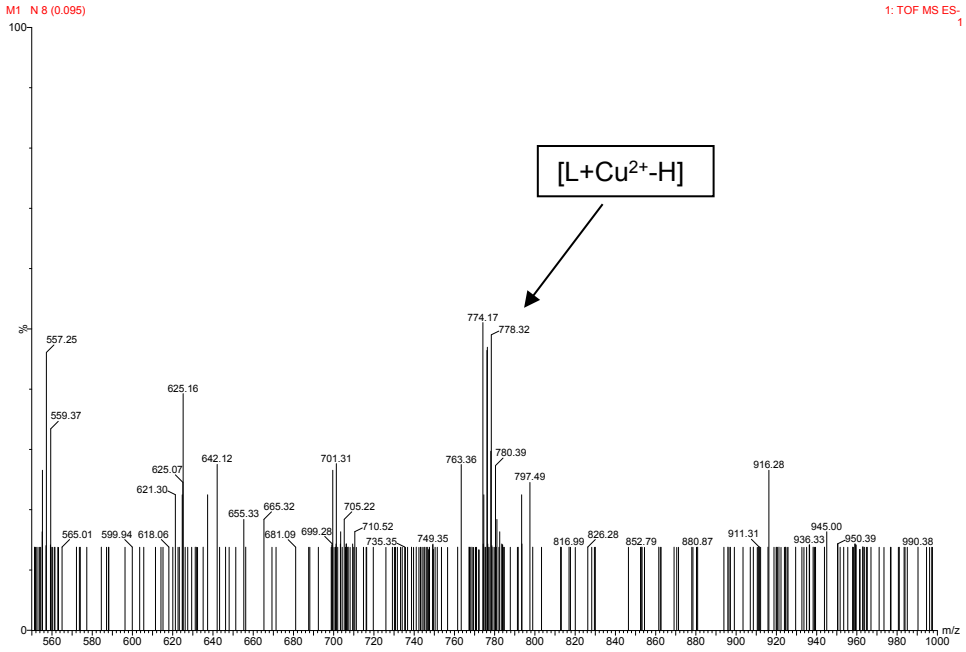


Figure SI 8. ESI mass spectrum of ligand-Cu^{2+} complex in DMSO

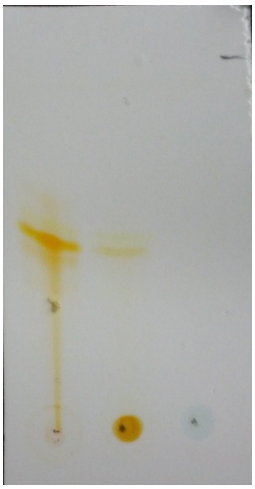


Figure SI 9. TLC of ligand (R_f = 0.50) and ligand-Cu^{2+} complex (R_f = 0.0) in 1:20 MeOH:DCM mixture

Sr. No.	Solvents	Polarity of solvents	Solubility
1.	Water	9.0	Insoluble
2.	Hexane	0.0	Insoluble
3.	Dichloromethane	3.1	Insoluble
4.	Chloroform	4.1	Partially insoluble
5.	Methanol	5.1	Partially insoluble
6.	Ethanol	5.2	Partially insoluble
7.	Acetone	5.1	Soluble
8.	Acetonitrile	5.8	Soluble
9.	Pyridine	5.3	Highly soluble

10.	Dimethylformamide	6.4	Highly soluble
11.	Dimethyl sulfoxide	7.2	Highly soluble

Figure SI 10. Solubility of receptor **3** in different solvents

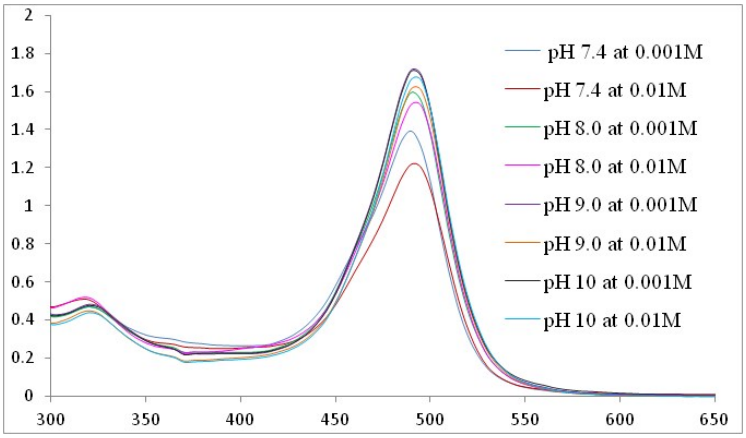


Figure SI 11. Absorbance of ligand **3** (2.5×10^{-5} M) in 1%DMSO-Tris buffer at different pH

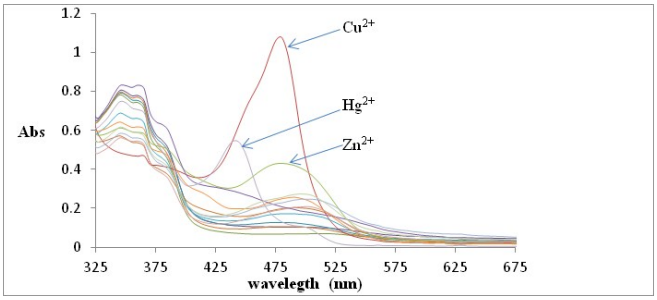


Figure SI 12. Absorbance spectra of ligand **3** with 10 times more different metal ion concentration