Electronic Supplementary Material (ESI) for Chemical Communications

Supporting information

A CN^- Specific *Turn-On* Phosphorescent Probe with Probable Application for Enzymatic Assay and as an Imaging Reagent

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Experimental Section.

Materials. 2-phenylpyridine (ppy), $IrCl_3.3H_2O$, di-(2-picolyl) amine (dpa), Sodium cyanoborohydrate ($NaBH_3CN$), 4,4'-dimethyl-2,2'-bipyridyl, mandelonitrile, hydroxynitrile lyase were obtained from Sigma Aldrich. Selenium dioxide was purchased from Across India. Glacial acetic acid, $Cu(ClO_4)_2.H_2O$, NaCl, NaBr, KI, KNO_3, NaCN, $CH_3COONa.3H_2O$, $NaIO_4$, Na_2SO_3, NaF, $Na_2HPO_4.2H_2O$, $NaH_2PO_4.2H_2O$ were purchased from SD Fine Chemicals in India.

Analytical Methods:

FTIR spectra were recorded as KBr pellets in a cell fitted with a KBr window, using a Perkin-Elmer Spectra GX 2000 spectrometer. ^{1}H NMR spectra were recorded on a Bruker 200 MHz FT NMR (Model: Avance-DPX 200) or on a Bruker 500 MHz FT NMR (Model: Avance-DPX 500) using CD_3CN, $CDCl_3$ and CD_3OD as the solvent and tetra methyl silane (TMS) as an internal standard. ESI-MS measurements were carried out on a Waters QTof-Micro instrument. Electronic spectra were recorded with a Shimadzu UV-3101 PC spectrophotometer; while fluorescence spectra were recorded using an Edinburgh instrument Xe 900 spectro fluorometer.

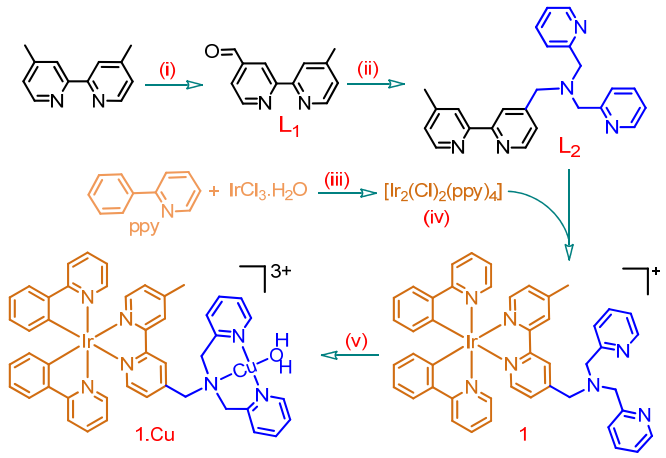
UV-Vis and Fluorescence study

1.0 x 10^{-4} M solution of the **1** and **1.Cu** in aq.-HEPES buffer-CH_3CN (98: 2; 99.6: 0.4(v/v); pH 7.6) medium was prepared and stored in dark. This solution was used for all spectroscopic studies after appropriate dilution. The effective ratio for the aq.-HEPES buffer-CH_3CN in the final solution was 99.6: 0.4. 1.0 x 10^{-3} M solutions of inorganic salt of respective anions were prepared in HEPES buffer (pH = 7.6). Solution of the compound **1** and **1.Cu** was further diluted for spectroscopic titrations, and the effective final concentration of the solution of compound **1** and **1.Cu** used for the fluorescence study was 2.0 x 10^{-5} M, while the final analyte concentration during emission spectral scanning was 8.0 x 10^{-4} M. For emission spectral titration effective [CN^-] was varied between (0 to 6.0 x 10^{-4} M), while maintaining [**1.Cu**] as 2.0 x 10^{-5} M. For the evaluation of the formation constant of **1.Cu**, emission spectral titration was carried out using effective [Cu^{2+}] between (0 to 1.4 x 10^{-4} M), while maintaining [**1**] unchanged as 2.0 x 10^{-5} M. All emission studies were performed in aq.-HEPES buffer-CH_3CN (99.6: 0.4(v/v); pH 7.6) medium using λ_{ext} = 380 nm, λ^{Mon} = 583 nm

and a slit width of 2.5 nm. The relative fluorescence quantum yields (ϕ_f) were estimated using equation 1 in aq.-HEPES buffer-CH_3CN (99.6 : 0.4(v/v); pH 7.6) medium by using the integrated emission intensity of $Ru(bpy)_3^{2+}$ ($\phi_f = 0.042$ in aqueous at RT) as a reference:[1]

$$\phi_f = \phi_f' \, (I_{sample}/I_{std})(A_{std}/A_{sample})(\eta^2_{sample}/\eta^2_{std}) \quad \text{Eq. 1}$$

where, ϕ_f' is the absolute quantum yield for the $Ru(bpy)_3^{2+}$, used as reference; I_{sample} and I_{std} are the integrated emission intensities; A_{sample} and A_{std} are the absorbances at the excitation wavelength, and η_{sample} and η_{std} are the respective refractive indices.



(i) SeO_2, Dioxane; (ii) Di-(2-picolyl) amine, Glacial acetic acid, Sodium cyano borohydrate; (iii) 2-Methoxy ethanol,water; (iv) CH_2Cl_2/MeOH, NH_4PF_6; (v) $Cu(ClO_4)_2.H_2O$, MeOH/Water.

Scheme 1: Methodologies that were adopted for synthesis of **L_1**, **L_2**, **$Ir_2(ppy)_4(Cl)_2$**, **1** and **1.Cu.**

Synthesis of $[Ir_2(ppy)_4Cl_2]$[2]

A mixture of 2-methoxy ethanol and water (3: 1, v/v) was added to round bottom flask containing $IrCl_3.H_2O$ (0.5 g, 1.58 mmol) and 2-phenyl pyridine (0.612 g, 3.95 mmol). The mixture was refluxed for 36 h. After cooling to room temperature, yellow solid precipitated. This was filtered to give crude cyclometallated Iridium(III) Chloro-bridged dimer ($(ppy)_2Ir(\mu\text{-}Cl)_2Ir(ppy)_2$), which was further washed with cold methanol and water for further purification and then dried in a desiccator. Yield: 0.6 gm, 33%. This was used for further reactions.

Synthesis of 4'-Methyl-2,2' bipyridyline-4-Carbaldehyde (L_1)[3]

The compound 4,4'-dimethyl-2,2'-bipyridyl (1.8 g, 9.78 mmol) and SeO_2 (1.1 g, 9.9 mmol) were added to 1,4-dioxane (50 mL) and the mixture was heated at reflux under an inert atmosphere for 24 h. The solution was filtered while hot to remove precipitated metallic selenium. Then this filtrate was cooled to room temperature and allowed to stand for 1 h,

while a yellow precipitate appeared. This was filtered again to remove this pale yellow precipitate. Then, the final filtrate was evaporated to dryness. The solid mass was redissolved in ~ 300 mL of ethyl acetate and was treated with aqueous Na_2CO_3 (0.1 M, 20 mL) to remove any carboxylic acid derivative that could have generated (during the oxidation reaction by SeO_2) and remained in this solution. The ethyl acetate layer was separated and further treated with 0.2 M aqueous solution of $NaHSO_3$ (3 x 30 mL). Three aqueous extracts were combined and pH was adjusted to ~ 9 by the adding aq. solution Na_2CO_3. Then the desired compound was extracted in to the dichloromethane (3 x 30 mL) layer. The dichloromethane extracts were combined and evaporated to give 4'-methyl-2,2'-bipyridyline-4-carbaldehyde. Yield: 0.6 g, (40%). ESI-Ms (*m/z*): Calculated for $C_{12}H_{10}N_2O$: 198.08, Observed: 199.10 $[M + H^+]$; ^1H NMR [200 MHz, $CDCl_3$: δ (ppm)] 10.19 (1H, s, -CHO); 8.90 (1H, d, J = 4.8 Hz, H^5 (bpy)); 8.83 (1H, s, H^3 (bpy)); 8.58 (1H, d, J = 4.6 Hz, H^6 (bpy)); 8.28 (1H, s, $H^{3'}$ (bpy)); 7.72 (1H, d, J = 4.2 Hz, $H^{6'}$ (bpy)); 7.20 (1H, d, J = 4.4 Hz, $H^{5'}$ (bpy)); 2.47 (3H, s, (bpy-CH_3)). IR (KBr) [γ/cm^{-1}]: 1705 (s, -CHO).

Synthesis of (L_2):

L_1 (0.4 g, 2.08 mmol) and di-(2-picolyl) amine (0.413 g, 2.08 mmol) were dissolved in fresh methanol (35 mL). A catalytic amount of (two drops) of glacial acetic acid was added to the solution, which was refluxed for half an hour. The reaction mixture was allowed to cool to room temperature using an ice bath, and then to this cold solution $NaBH_3CN$ (0.262 g, 416 mmol) was slowly added with rapid stirring. The ice bath was then removed and the reaction mixture was stirred overnight at room temperature. After that, the reaction mixture was treated with saturated aqueous sodium carbonate solution and subsequent extraction using dichloromethane was performed. The organic layer was recovered, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was subjected to neutral alumina gel gravity chromatography using ethyl acetate: hexane (2: 3, v/v) as eluent. Major fraction was collected and dried under vacuum, which afforded a sticky oil solid. Yield: 0.396 g (50%). ESI-Ms (*m/z*): Calculated for $C_{24}H_{23}N_5$: 381, Observed: 382 $[M + H]^+$; ^1H NMR [200 MHz, CD_3OD: δ (ppm)]. 8.43 (1H, d, J = 5.2 Hz, H^6 (bpy)); 8.39 (1H, d, J = 5.2 Hz, $H^{6'}$ (bpy)); 8.32 (2H, d, J = 4.8 Hz, H^6, $H^{6'}$ (pyridyl rings of dpa)); 8.19 (1H, s, H^3 (bpy)); 7.98 (1H, s, $H^{3'}$ (bpy)); 7.73-7.58 (4H, m, H^4, $H^{4'}$, H^5 and $H^{5'}$ (pyridyl rings of dpa)); 7.40 (1H, d, J = 5.0 Hz, H^5 (bpy)); 7.18-7.12 (3H, m, H^3, $H^{3'}$ (pyridyl rings of dpa) and $H^{5'}$ (bpy)); 3.74 (4H, s, -CH_2 (dpa)); 3.70 (2H, s,-CH_2 (bpy)); 2.34 (3H, s, -CH_3 (bpy)).

Synthesis of the $[Ir(ppy)_2L_2]PF_6$ (1):

A mixture of $[Ir_2(ppy)_4Cl_2]$ (0.3 g, 0.28 mmol) and **L_2** (0.245 g, 0.644 mmol) in methanol-dichloromethane (60 mL; 1: 3 (v/v)) was refluxed in dark for 4 h. After cooling to ambient temperature, solution volume was reduced to ~ 45 mL under vacuum. To this concentrated solution a methanol solution (5 mL) of NH_4PF_6 (0.2 gm) was added with stirring. The solution was stirred for 30 min. A precipitate appeared and the solution was filtered. Solid was dissolved in dichloromethane and was purified by column chromatography using neutral alumina gel column. Eventually, the desired product was eluted with dichloromethane-methanol (9:1, v/v). Yield: 0.238 gm, (94 %). ESI-Ms (*m/z*): Calculated for $C_{46}H_{39}IrN_7P\ F_6$: 1027, Observed 882 $[M- PF_6]^+$; ^1H NMR [500 MHz, CD_3CN, δ (ppm)]: 8.66 (1H, s, H^3 (bpy)); 8.48 (2H, d, J = 4.0 Hz, (H^6, $H^{6'}$ (pyridyl rings of dpa); 8.40 (1H, s, $H^{3'}$ (bpy)); 8.06 (2H, t, J = 7.5 Hz, (H^4 , $H^{4'}$ (pyridyl rings of dpa)); 7.86-7.77 (6H, m, (H^5, $H^{5'}$ (pyridyl rings of dpa), H^6, $H^{6'}$ (pyridine of ppy), H^3, $H^{3'}$ (phenyl of ppy)); 7.65 (2H, t, J = 7.5 Hz, H^3, $H^{3'}$ (pyridine of ppy)); 7.60 (1H, d, J = 5.5 Hz, H^6 (bpy)); 7.55-7.51 (3H, m, H^6, $H^{6'}$ (phenyl of ppy), 1H, H^5 (bpy)); 7.48 (1H, d, J = 5.0 Hz, $H^{6'}$ (bpy)); 7.33 (1H, d, J = 5.0 Hz, $H^{5'}$ (bpy)); 7.16 (2H, t, J = 6 Hz, H^5, $H^{5'}$ pyridine of ppy)); 7.06-7.00 (4H, m, H^4, $H^{4'}$ (pyridine of ppy), H^4, $H^{4'}$ (phenyl of ppy)); 6.91 (2H, t, J = 7.5 Hz, H^5, $H^{5'}$ (phenyl of ppy)); 6.27 (2H, d, J = 7.5 Hz, H^3, $H^{3'}$ (pyridyl rings of dpa)); 3.95(2H, s, $-CH_2$ (bpy)); 3.88 (4H, s, $-CH_2$ (dpa)); 2.59 (3H, s, $-CH_3$ (bpy)); IR (KBr, γ/cm^{-1}): 840(s, PF_6^-)**.**

Synthesis of 1.Cu

The compound **1** (0.1 g, 0.097 mmol) was dissolved in 15 mL methanol, to this $Cu(ClO_4)_2$. H_2O.was added. Solution colour was changed immediately .The reaction mixture was stirred for 4 h and then transferred into a beaker. The solution was allowed to evaporate slowly and to reduce its volume to half. A precipitate was formed at the bottom of beaker. This was collected by filtration and this residue was washed thoroughly with cold water. This was then dried in a dissector. Dry solid was pure enough for further studies. Yield 0.095 g. ESI-Ms (m/z) calculated for $C_{46}H_{39}CuIrN_7PF_6.H_2O$: 963, observed: 963 $[M^{3+}]$.

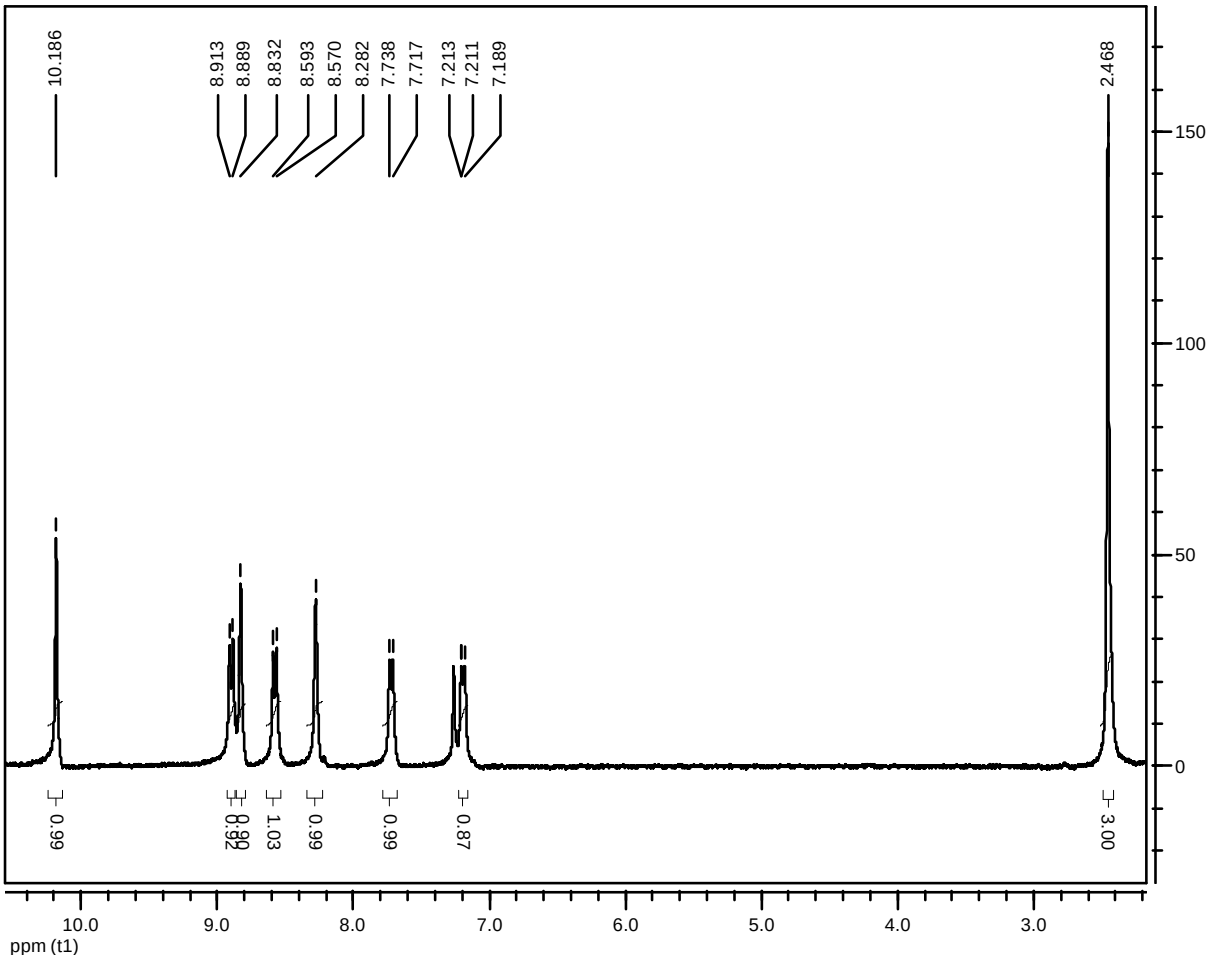
References ;

1. D. Bruce and M. M. Richter, *Anal. Chem*., 2002, **74**, 1340.

2. Q. Zhao, F. Li, S. Liu, M. Yu, Z. Liu, T. Yi and C. Huang. *Inorganic Chemistry.,* 2008, **47**, 9257.

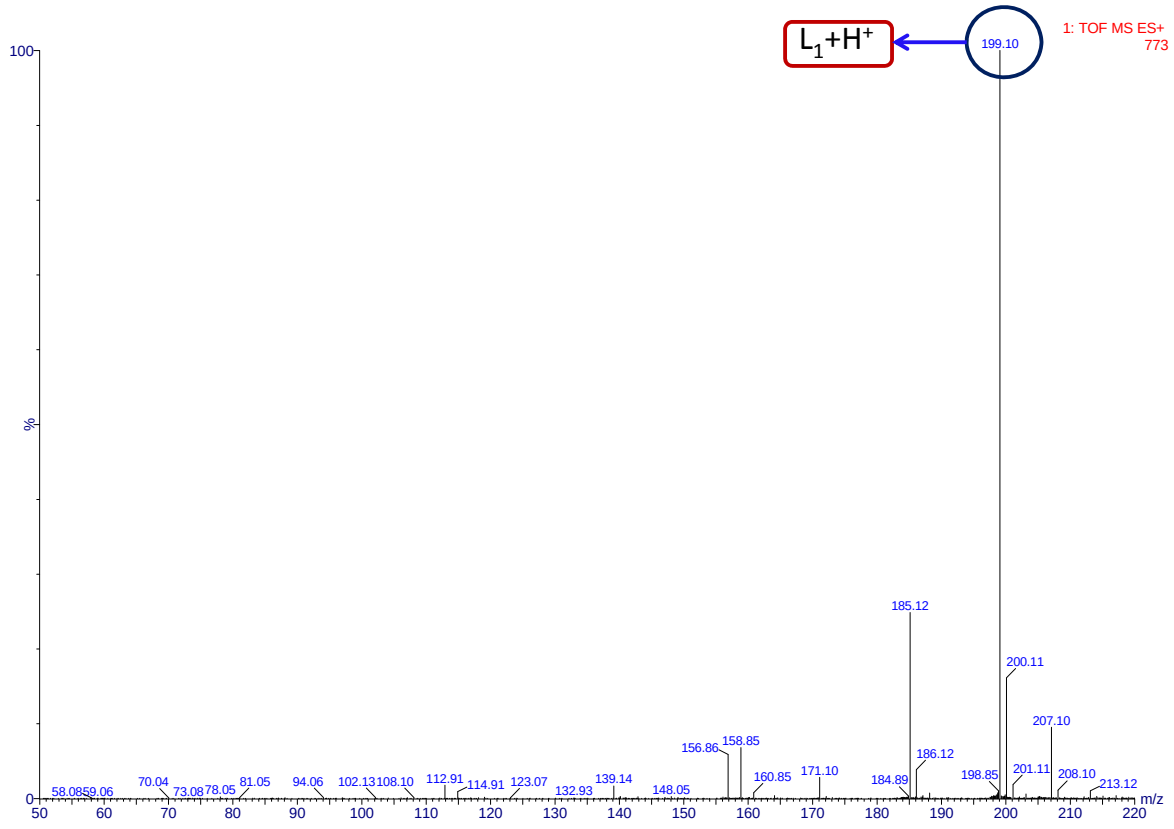
3. F. S. Geoffrey, J. R. Schoonover, R. Duesing, S. Boyde, Wayne E. Jr. Jones and J. M Thomas, *Inorg. Chem.,* 1995, ***34***, 473.

^{1}H NMR spectra of L_1



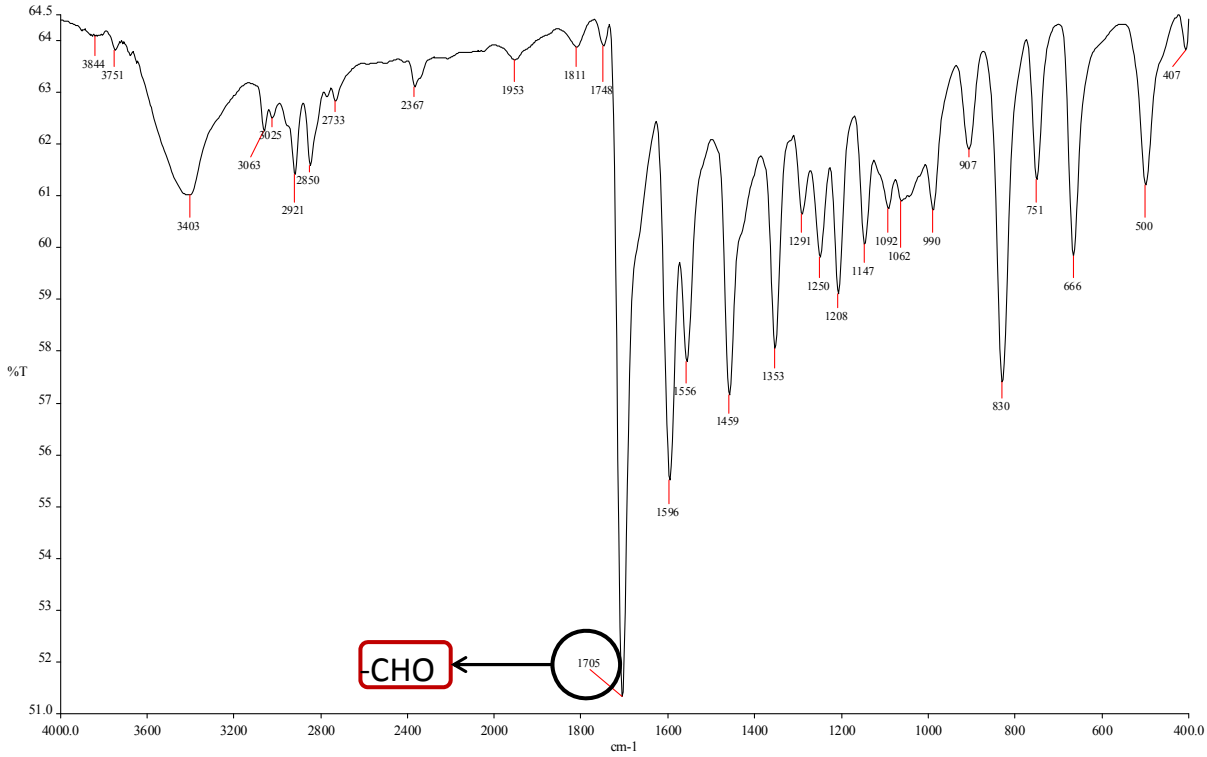
SI Figure 1: ^{1}H NMR spectra of **L_1** in $CDCl_3$ medium.

Mass spectra of L_1



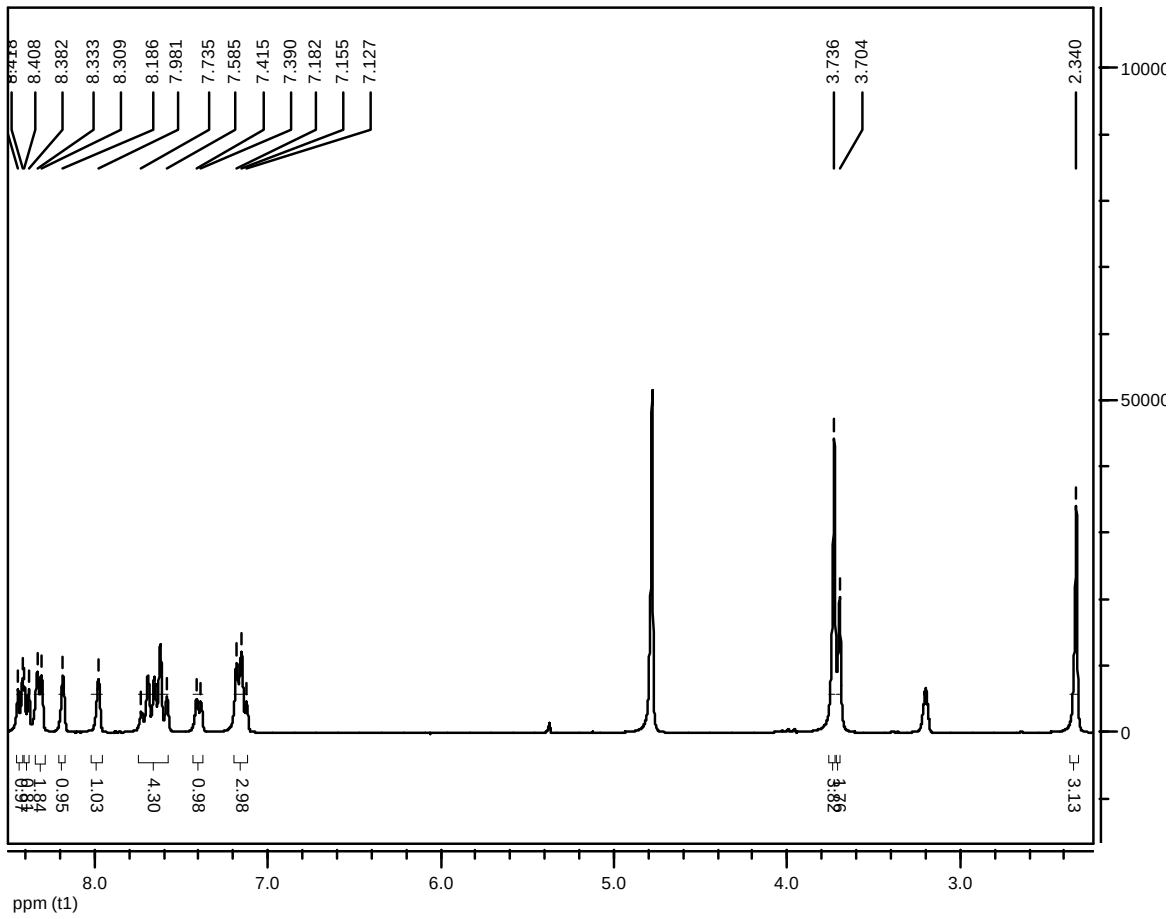
SI Figure 2: ESI- mass spectra of $\mathbf{L_1}$.

IR spectra of L_1



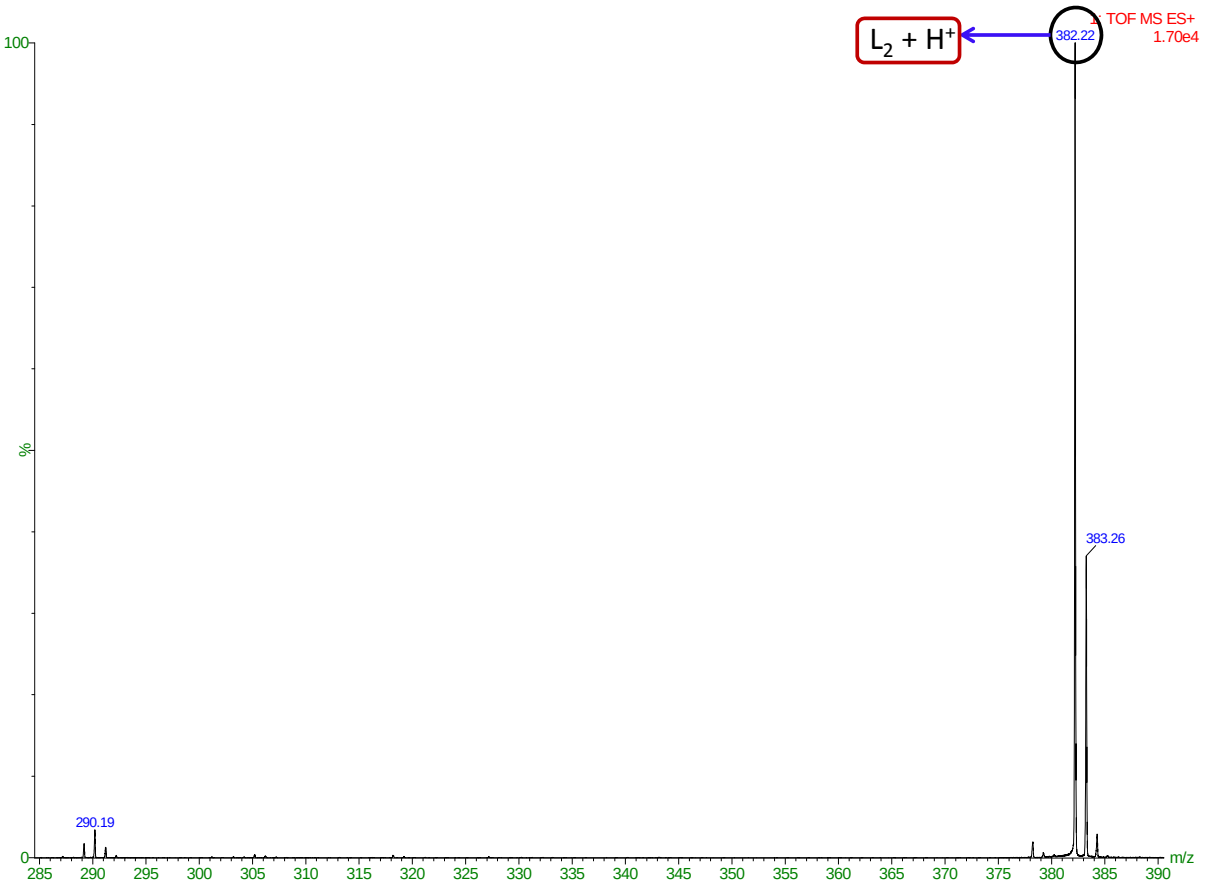
SI Figure 3: FTIR spectra of **L_1** as KBR pellet.

^{1}H NMR spectra of L_2



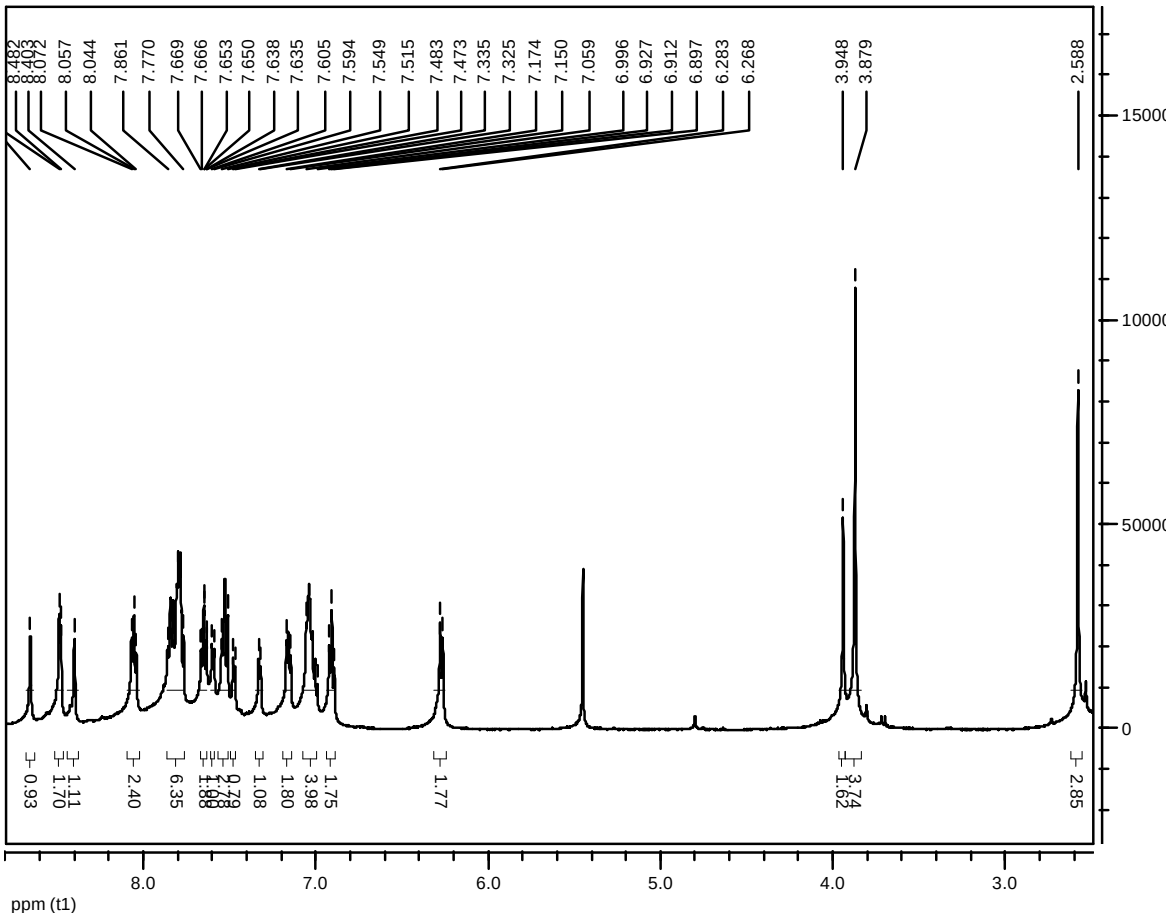
SI Figure 4: ^{1}H NMR spectra of **L_2** in CD_3OD medium.

Mass spectra of L_2



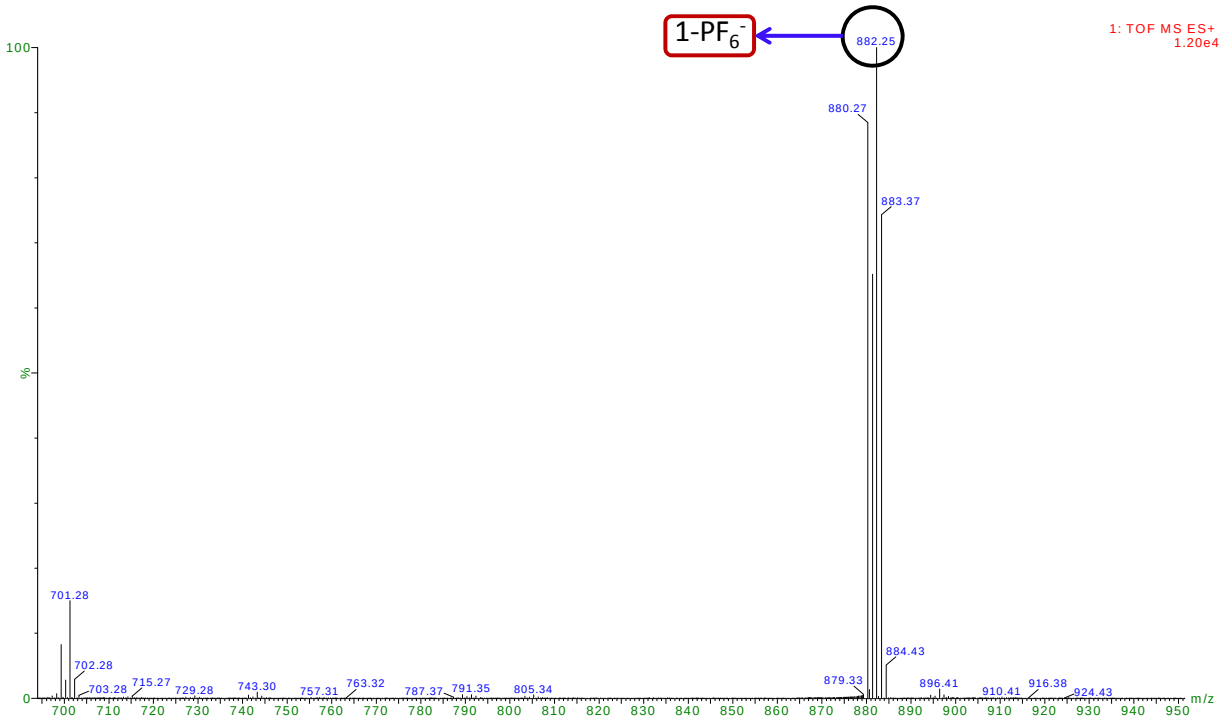
SI Figure 5: ESI- mass spectra of **L_2**.

^{1}H NMR spectra of 1



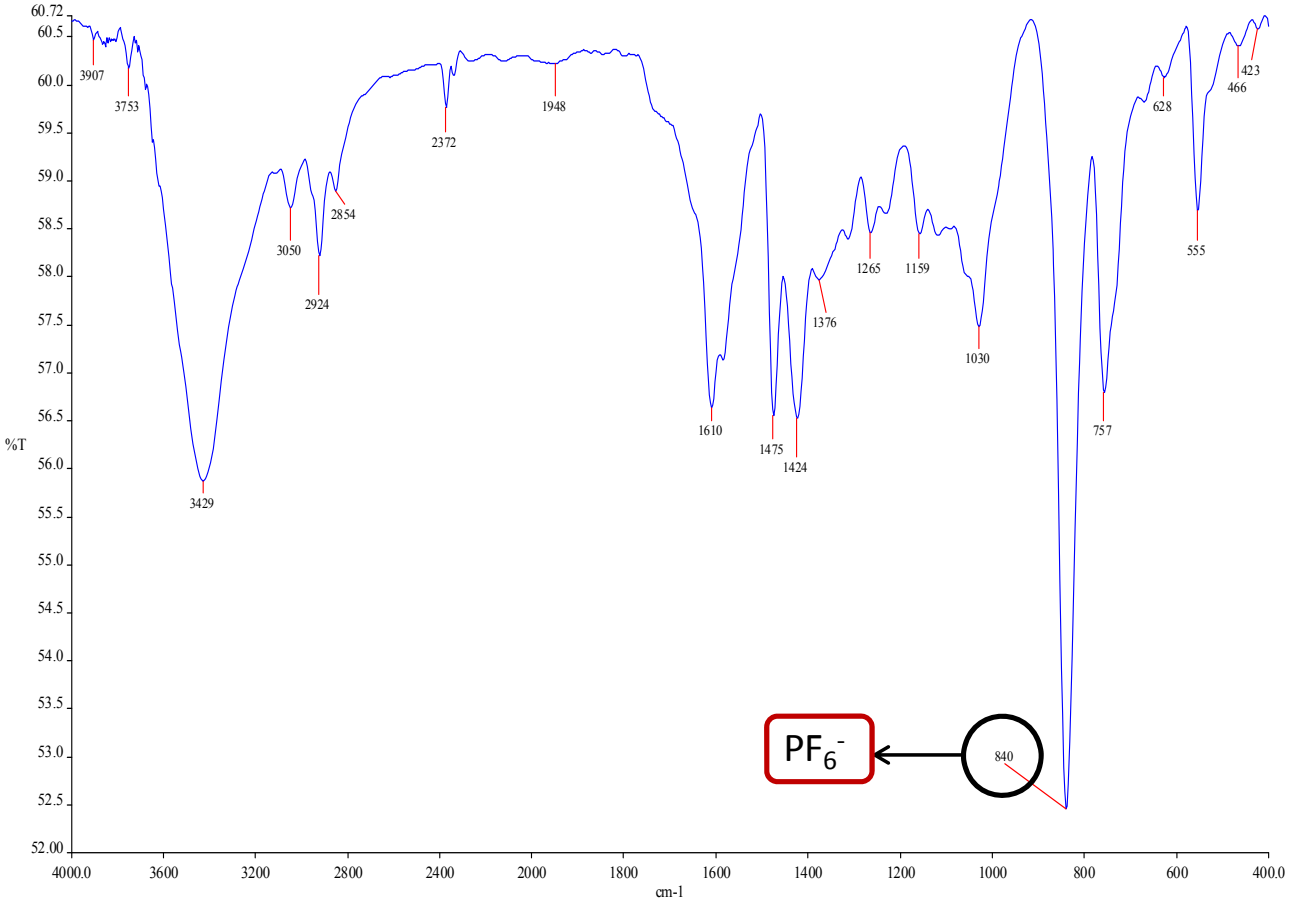
SI Figure 6: ^{1}H NMR spectra of **1** in CD_3CN medium.

Mass spectra of 1



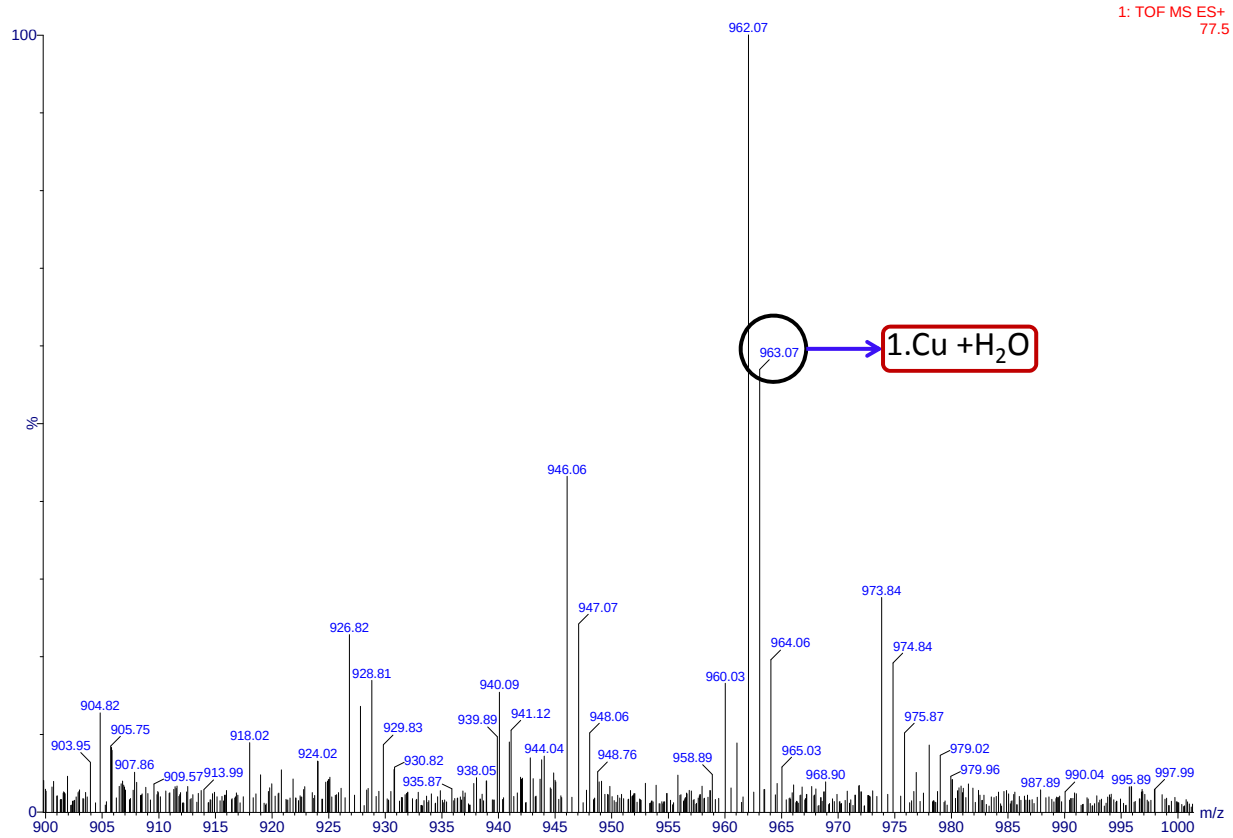
SI Figure 7: ESI- mass spectra of **1.**

IR spectra of 1



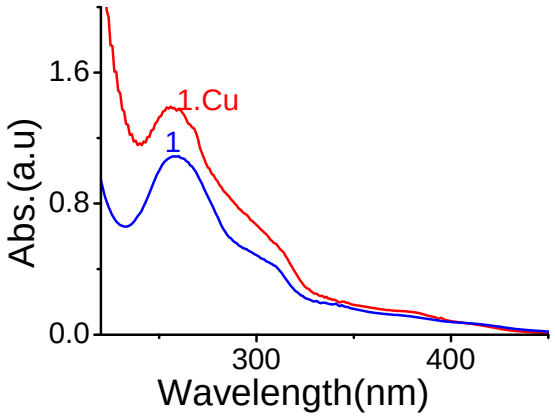
SI Figure 8: FTIR spectra of **1** as KBR pellet.

Mass spectra of 1.Cu



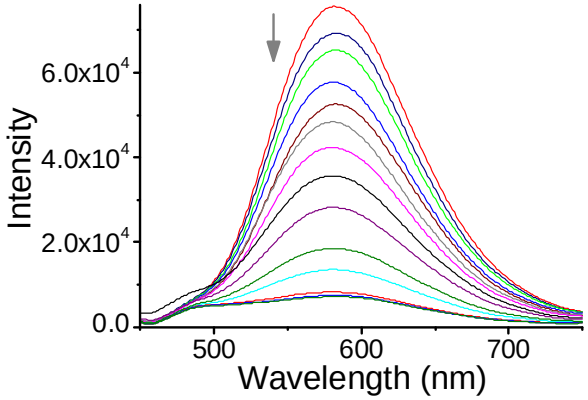
SI Figure 9: ESI- mass spectra of **1.Cu.**

Absorbance spectra of 1 and 1.Cu



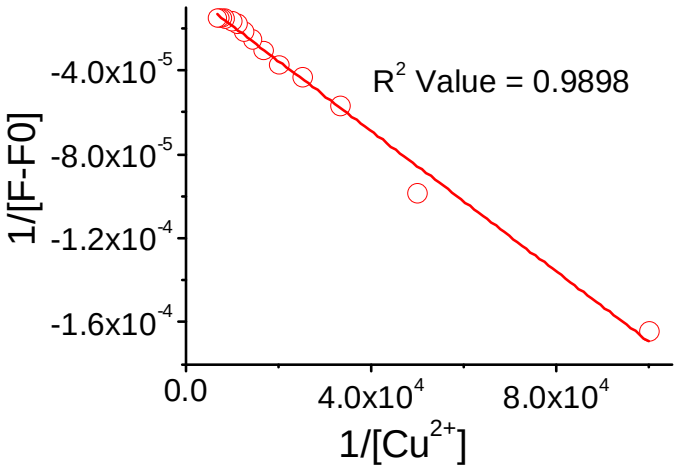
SI Figure 10. Absorbance spectra of **1** (2.0 x 10^{-5} M) and **1.Cu** (2 x 10^{-5} M) were performed in 10 Mm HEPES buffer (pH 7.6) medium.

Fluorescence response of 1 with different Concentrations of Cu.



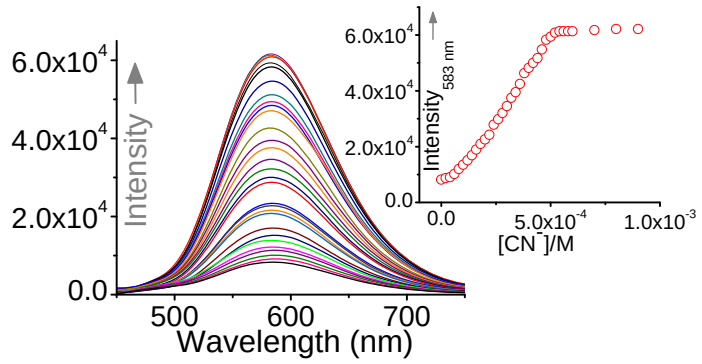
SI Figure 11. Emission spectral responses of **1** (2.0 x 10^{-5} M) towards varying [$Cu(ClO_4)_2$] (0 to 1.4 x 10^{-4} M) in aq.-HEPES buffer-CH_3CN (99.6 : 0.4(v/v); pH 7.6) medium using λ_{ext} = 380 nm and λ_{Mon} = 583 nm. Slit width 2.5 nm. 10 mM HEPES buffer solution was use for maintaining solution pH.

Benesi-Hildebrand plot for binding studies of Cu^{2+} towards 1:



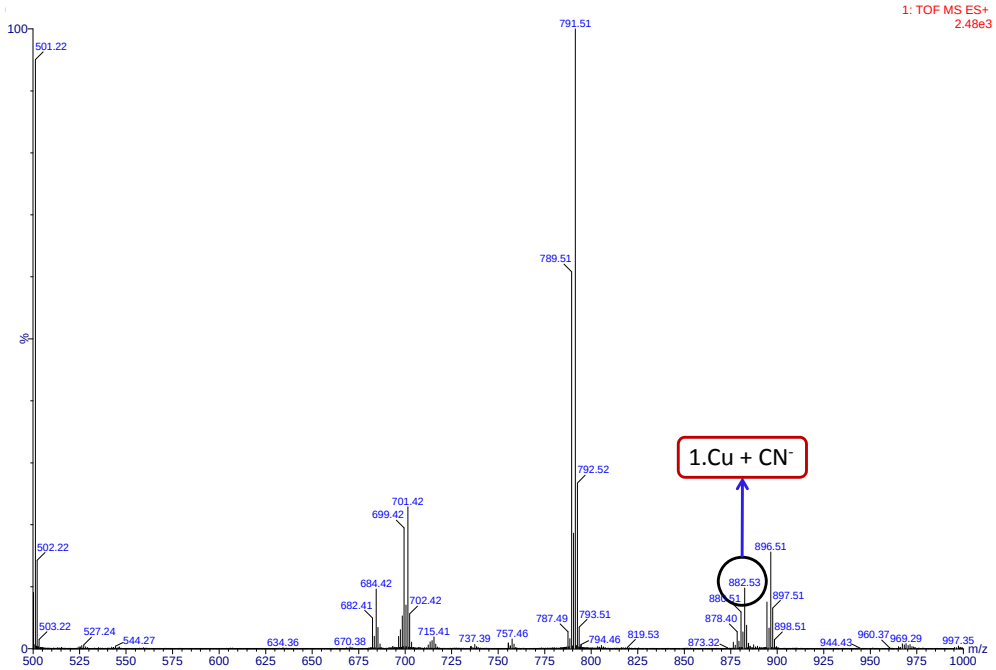
SI Figure 12. Benesi-Hildebrand plot of **1** (2 x 10^{-5} M) for varying [Cu(II)] (0 to 1.4 x 10^{-4} M) λ_{ext} = 380 and λ_{Mon} = 583 nm. Good linear fit confirms the 1: 1 binding stoichiometry. in aq.-HEPES buffer-CH_3CN (99.6: 0.4(v/v); pH 7.6) medium using λ_{ext} = 380 nm and λ_{Mon} = 583 nm. Slit width 2.5 nm. 10 mM HEPES buffer solution was use for maintaining solution pH.

Luminescence response of 1.Cu towards varying[CN^-]



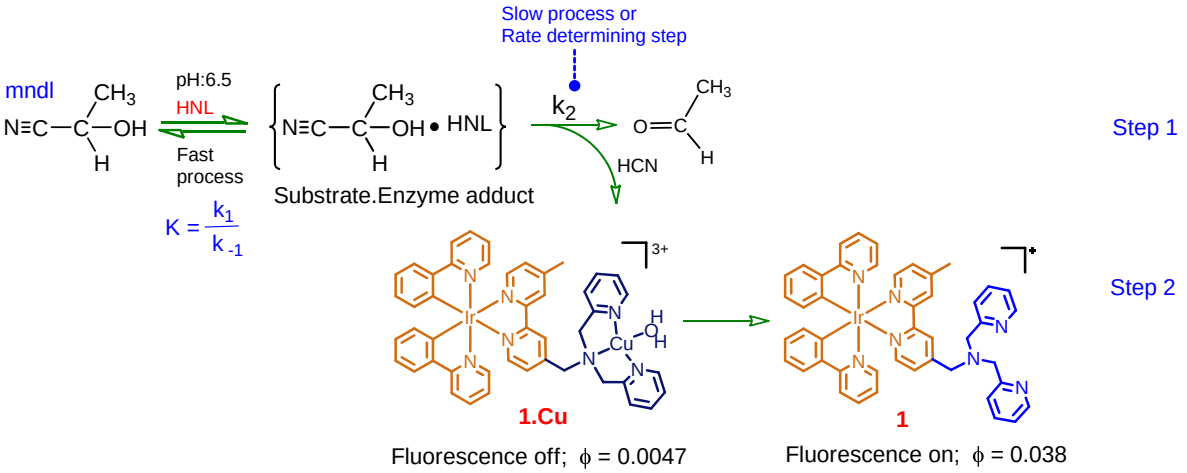
SI Figure 13. Luminescence response of **1.Cu** (2.0×10^{-5} M) towards varying [CN^-] (0 to 6.0×10^{-4} M). Studies were performed in aq.-HEPES buffer-CH_3CN (99.6: 0.4(v/v); pH 7.6) using λ_{ext} = 380 nm and λ_{Mon} = 583 nm. Slit width 2.5 nm. 10 mM HEPES buffer solution was use for maintaining solution pH.

Mass spectra of TBACN addition to 1.Cu



SI Figure 14. ESI mass spectra of **1.Cu** in presence of TBACN.

Evaluation of Michaelis Constant of the enzymatic reaction for the generation of cyanide from the mandelonitrile (mdnl) using hydroxynitrile lyase (HNL).



Liberated cyanide could demetalate **1.Cu** to generate **1** with subsequent enhancement in emission at ~ 583 nm, i.e., the switch on emission response. Please note that relative quantum yield for **1.Cu** was much lower than that for **1**.

Our studies reveal that the displacement of Cu(II) from **1.Cu** for the generation of **1** and $Cu(CN)_x^{2-X}$ is an instantaneous process and thus the reaction rate is fast. In step 1, generation of HCN at pH 6.5 is the slow step or rate determining step.

As mentioned in the above scheme decomposition of the {**mndl.HNL**} adducts into corresponding acetaldehyde and HCN in Step-1 is the rate determining step with overall rate constant of k.

For the above reaction shown in step 1, the rate of the reaction (v) is as follows:

$$v = k_2[mndl.HNL] = \frac{k_2[HNL]_0[mndl]}{K_m + [mndl]}$$

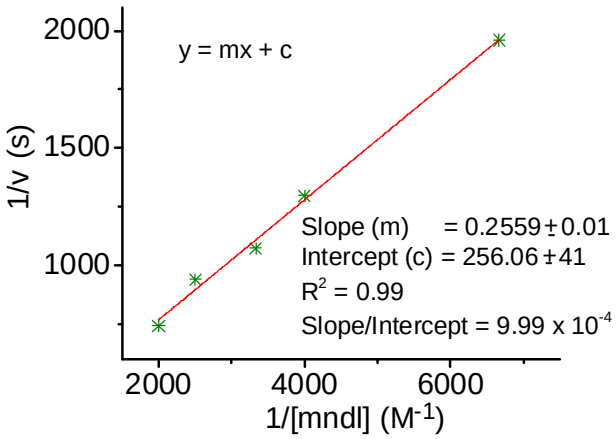
where $K_m = (k_{-1} + k_2) / k_1$

$$\frac{1}{v} = \frac{K_m}{k_2[HNL]_0[mndl]} + \frac{1}{1/k_2[HNL]_0}$$

Overall rate constant (v) for a certain [mndl] with [HNL] = 30 μL, [**1.Cu**] = 2.0 x 10^{-5} M and pH = 6.5 (10 mM HEPES buffer-CH_3CN (99.6: 0.4(v/v)) solution) was evaluated from the plot of Log $[F_t-F_0]$ v_s time (in sec), where F_t is the luminescence intensity for **1** at 583 nm (λ_{ext} = 380 nm) at time t and F_0 is the initial luminescence intensity of **1.Cu** at 570 nm. Kinetics of the individual reaction was studied for initial 60 % of the total reaction (based on the change in luminescence intensity at 570 nm). A reasonably linear plot was obtained for v vs. [mndl] for [mndl] varying from 1.5 x 10^{-4} M to 5 x 10^{-4} M. Beyond the concentration of

5.0 x 10^{-4} M, a deviation from linearity was observed due to the saturation effect and thus, were not considered for the 1/v vs. 1/[mndl] for the evaluation of K_m.

Thus, a plot of 1/v v_s. 1/[mndl] would give intercept of $1/k_2[HNL]_0$ and slope of $K_m/k_2[HNL]_0$. Thus, {slope/intercept} would result K_m (9.99 x 10^{-4}).



SI Figure 15: 1/v v_s. 1/[mndl] plot, while [HNL] = 30 μL, [**1.Cu**] = 2.0 x 10^{-5} M; pH was maintained at 6.5 with 10 mM HEPES buffer-CH_3CN (99.6: 0.4(v/v)) solution having temperature of 22°C.