A lower rim triazole linked calix[4]arene conjugate as fluorescence switch on sensor for Zn^{2+} in blood serum milieu

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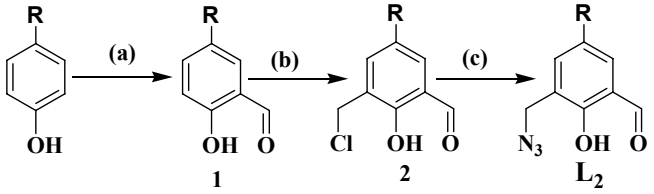
Contents

Materials and methods:

All the solvents used were dried and distilled by usual procedures immediately before use. Distilled and deionized water was used in the studies. ^{1}H and ^{13}C NMR spectra were measured on a Varian Mercury NMR spectrometer working at 400 MHz. The mass spectra were recorded on Q-TOF micromass (YA-105) using electrospray ionization method. The elemental analyses were performed on Thermo Quest microanalysis. Metal salts used for the titrations were their perchlorate salts (Caution: Since perchlorate salts are known to explode under certain conditions, these are to be handled carefully!) with formula, $M(ClO_4)_2.xH_2O$. All the solvents used were of analytical grade and were purified and dried by routine procedures immediately before use. Fluorescence emission spectra were measured on Perkin-Elmer LS55 by exciting the solutions at 380 nm and the emission spectra were recorded in 390 – 600 nm range. The fluorescence studies performed in aqueous methanolic HEPES buffer, pH = 7.5 solution used always a 50 µl of bulk solution of L prepared at 6 x 10^{-4} M concentration in methanol. A 50 mM HEPES buffer stock solution was prepared with deionized water and 600 µL of this bulk solution is used for each titration of 3 ml solution. All the measurements were made in 1 cm quartz cell and maintained a final L concentration of 10 µM. During the titration, the concentration of metal perchlorate was varied accordingly in order to result in requisite mole ratios of metal ion to L and the total volume of the solution was maintained constant at 3mL in each case by adding appropriate solvent or solvent mixtures. The absorption spectra were measured on Shimadzu JASCO V-570. Titrations were carried out with a final concentration of 100 µM prepared using bulk solution of ligands at 6 x 10^{-3} M.

Synthesis and characterization data for L, L_2, L_3, L_4

Starting from p-t-butyl phenol the azide partner 5-tert-butyl-3-(azidomethyl)-2-hydroxybenzaldehyde was prepared as shown in scheme **1.1**.



Schemes: Synthesis of 5-tert-butyl-3-(azidomethyl)-2-hydroxybenzaldehyde (a) $SnCl_4$, Bu_3N, $(CH_2O)_n$, dry toluene, reflux; (b) 37 % formaldehyde, conc. HCl, rt for 24 hr, and (c) NaN_3, DMF, rt, 12 h.

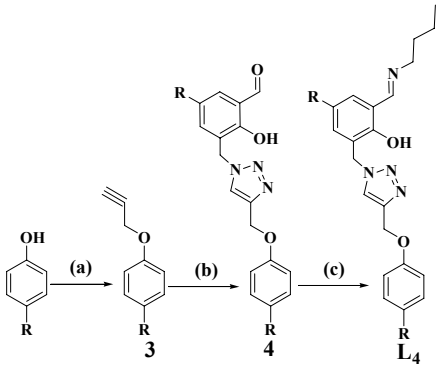
L_2: compound 2 (2.65 g, 11.89 mmol) was added to a solution of sodium azide (1.519 g, 23.37 mmol) in 30 mL dimethylformamide under stirring for 12 hr. After completion of reaction mixture was diluted with 100 mL of water and ethylacetate. The organic layer was separated and washed with water and brine. A yellow liquid was obtained upon evaporation of organic solvent. Yield 89 % 1H NMR ($CDCl_3$, 400 Hz) 11.2 (broad s, H, Sal-OH), 9.99 (s, H, CHO-H), 7.55 (dd, 1H, Sal-H), 7.59 (s, 1H, Ar-H), 4.48 (s, 2H, Sal-CH_2), 1.35 (s, 9 H, Ar-$(CH_3)_3$). IR:ν_{max} = 3471, 2961, 2686, 2104, 1676.

L_3: Compound **L_1** (3.0g, 4.14 mmol) was added to the solution of **L_2** (2.12g, 9.53 mmol) in 100mL of dichloromethane and water (50:50) mixture. To this solution, $CuSO_4.5H_2O$ (124.04 g, 0.50 mmol) and sodium ascorbate (328.0 mg, 1.70 mmol) were added. The resulting solution was stirred for 12 hrs at room temperature. Upon completion of the reaction as checked based on TLC, the organic layer was separated and the aqueous layer was extracted with dichloromethane (2 × 50 mL). The combined organic layer was washed water and with brine (2 x 100 mL), dried over anhydrous. Na_2SO_4, and the solvent were removed under *vacuo*. The crude product was purified by triturating with hexane followed by filtering the precipitate. Yield, 89.91%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ(ppm): 11.30 (s, 2H, Sal-OH), 9.83 (s, 2H, sal-CHO), 8.08 (s, 2H, triazole-H), 7.62 (s, 2H, Sal-H) 7.49 (d, 2H, Sal-H), 7.15 (s, 2H, Ar-OH), 6.98 (s, 4H, Ar-H), 6.77 (s, 4H, Ar-H), 5.56 (s, 2H, Sal-CH_2), 5.18 (s, 2H, Ar-O-CH_2), 4.14 (d, *J* = 13.0 Hz, 4H, ArCH_2Ar), 3.17 (d, *J* = 13.0 Hz, 4H, ArCH_2Ar), 1.27 (s, 18H, Ar-$(CH_3)_3$), 1.26 (s, 18H, Ar-$(CH_3)_3$), 0.96 (Sal-$(CH_3)_3$).^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 196.6, 157.1, 150.4, 149.6, 147.2, 144.2, 143.2, 141.5, 135.3, 132.6, 130.7, 127.8, 125.6, 125.0, 124.2, 123.1, 120.2, 69.8, 48.2, 34.2, 33.9, 33.8,, 31.7, 31.2, 31.1, 31.02. IR:ν = 3463, 2959, 1656, 1483, cm^{-1}. EA calcd. for $C_{74}H_{90}N_6O_8$: C, 74.59; H, 7.61; N, 7.05;. Found: C, 73.47; H, 7.24; N, 7.12 m/z (ES/MS) 1199.50 ([M]$^+$100%)

L: The mixture of **L_3** (200mg, 0.167 mmol) and the butylamine (24.56mg, 0.34 mmol) in methanol was stirred for 4 hrs. Then the reaction mixture was heated at 60°C for 1 hr to give clear yellow solution. The solvent was removed under vacuum to get yellow solid product, which was recrystalized using methanol. Yield 95% . ^{1}H NMR ($CDCl_3$, 400 Hz) 14.11 (broad s, 2H, Sal-OH), 8.27 (s, 2H, imine-H), 8.07 (s, 2H, triazole-H), 7.40 (d, Sal-H) 7.20 (d, Sal-H) 7.10 (s, 2H, Ar-OH), 6.96 (s, 4H, Ar-H), 6.74 (s, 4H, Ar-H), 5.58 (s, 4H, Sal-CH_2), 5.1 (s, 4H, Ar-OCH_2), 4.14 (d, 4H, Ar-CH_2-Ar), 3.52 (t, 4H, N-CH_2), 3.28 (d, 4H, Ar-CH_2-Ar), 1.62 (quintet, N-CH_2-CH_2), 1.36 (Sextet, 4H, N-CH_2CH_2*CH_2CH_3), 1.26 (s, 18H, $(CH_3)_3$), 1.25 (s, 18H, $(CH_3)_3$), 0.94 (s, 18H, Sal-$(CH_3)_3$)0.93 (t, 6H, N-CH2-CH_2-CH_2-CH_3). ^{13}C NMR ($CDCl_3$, 200 Hz) δ (ppm): 164.7, 158.1, 150.6, 149.8, 147.0, 144.0, 141.5, 141.2, 133.0, 130.6, 128.5, 128.0, 126.0, 125.0, 124.2, 123.0, 118.2, 69.8, 58.9, 48.9, 34.1, 34.0, 33.9, 32.9, 31.9, 31.5,

31.1, 29.8, 20.4, 13.9. IR:ν = 3442, 2958, 1635, 1482, cm^{-1}. EA calcd. for $C_{82}H_{108}N_8O_6$: C, 75.66; H, 8.36; N, 8.61; O, 7.37;. Found: C, 74.33; H, 8.22; N, 8.83 m/z (ES/MS) 1301.80$[M]^+$100%)

Control L_4:



Schemes: Synthesis control molecule L_4: (a) Propargyl bromide, K_2CO_3, acetone; (b) 5-tert-butyl-3-(azidomethyl)-2-hydroxybenzaldehyde, $CuSO_4.5H_2O$ and Sodium ascorbate in dichloromethane: water (1:1) rt, 12 hr (c) n-butylamine, Methanol/Ethanol, rt, 4 hr. R = tert-butyl.

L_4: Compound 4 was added to a solution of n-butylamine in 5 mL methanol under stirring for 2 hrs. After completion of reaction, the solvent was evaporated to dryness to get gummy liquid in quantitative yield. ^{1}H NMR ($CDCl_3$, 400 Hz) 14.2 (broad s, H, Sal-OH), 8.35 (s, H, imine-H), 7.82 (s, H, triazole-H), 7.38 (s, H, Ar-H), 7.31 (d, Sal-H), 7.25 (s, Ar-H), 6.89 (d, Sal-H), 5.59 (s, 2H, Sal-CH_2), 5.18 (s, H, Ar-OCH_2), 3.6 (t, 2H, N-CH_2), 1.7 (quintet, N-CH_2-CH_2), 1.4 (Sextet, 2H, N-CH_2CH_2*CH_2CH_3), 1.23 (s, 18H, $(CH_3)_3$), 0.98 (t, 3H, N-CH_2-CH_2-CH_2-CH_3). ^{13}C NMR ($CDCl_3$, 200 Hz) δ (ppm): 164.8, 158.5, 156.2, 144.2, 143.8, 141.0, 130.8, 128.6, 126.3, 123.5, 122.1, 118.0, 114.2, 62.2, 58.64, 49.09, 34.02, 32.9, 31.56, 31.42, 20.33, 13.9.

^{1}H, ^{13}C NMR and Mass spectral data

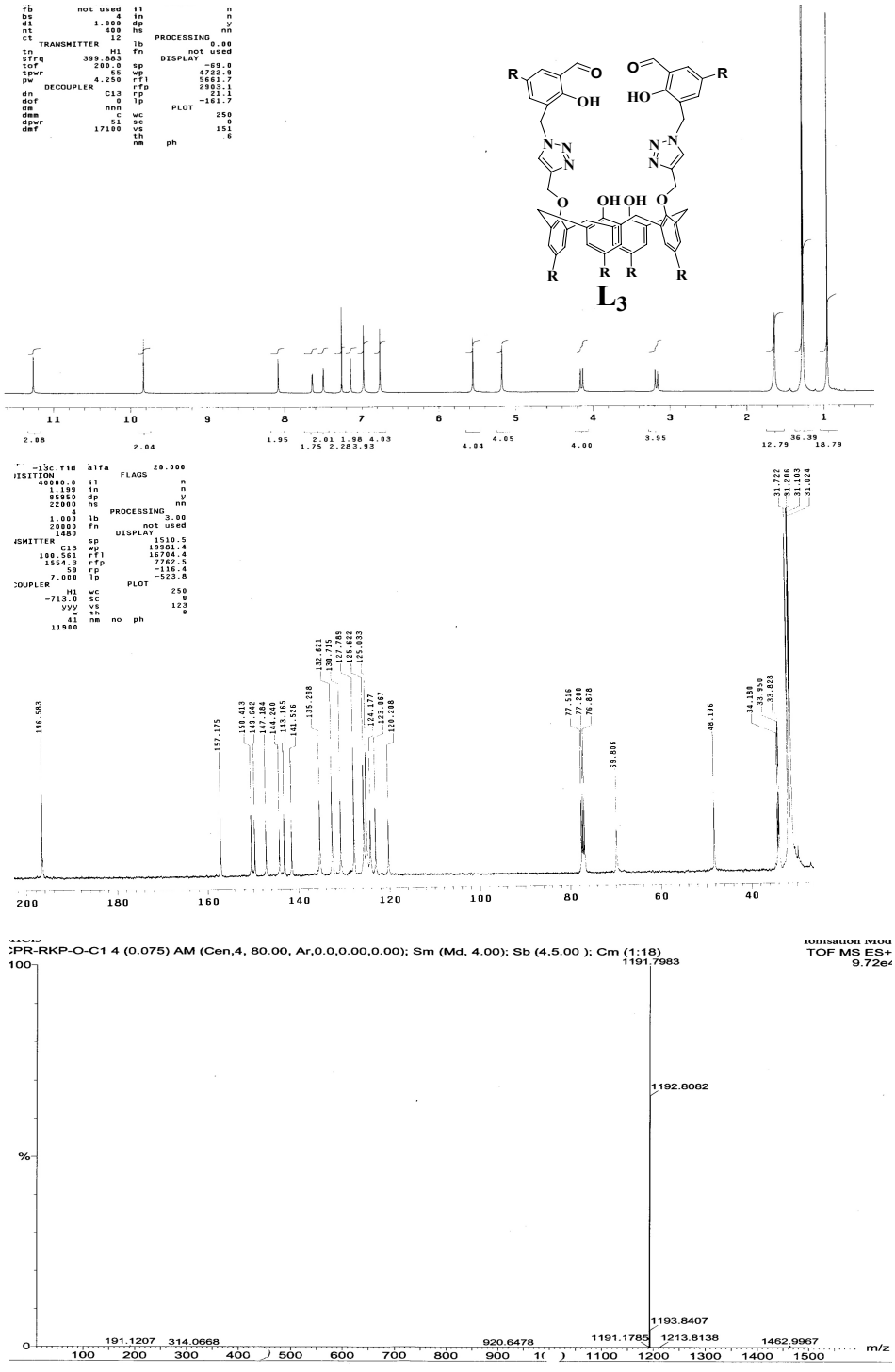


Figure: Spectra for $\mathbf{L_3}$: (a) ^{1}H NMR; (b) ^{13}C NMR and (c) ESI-MS.

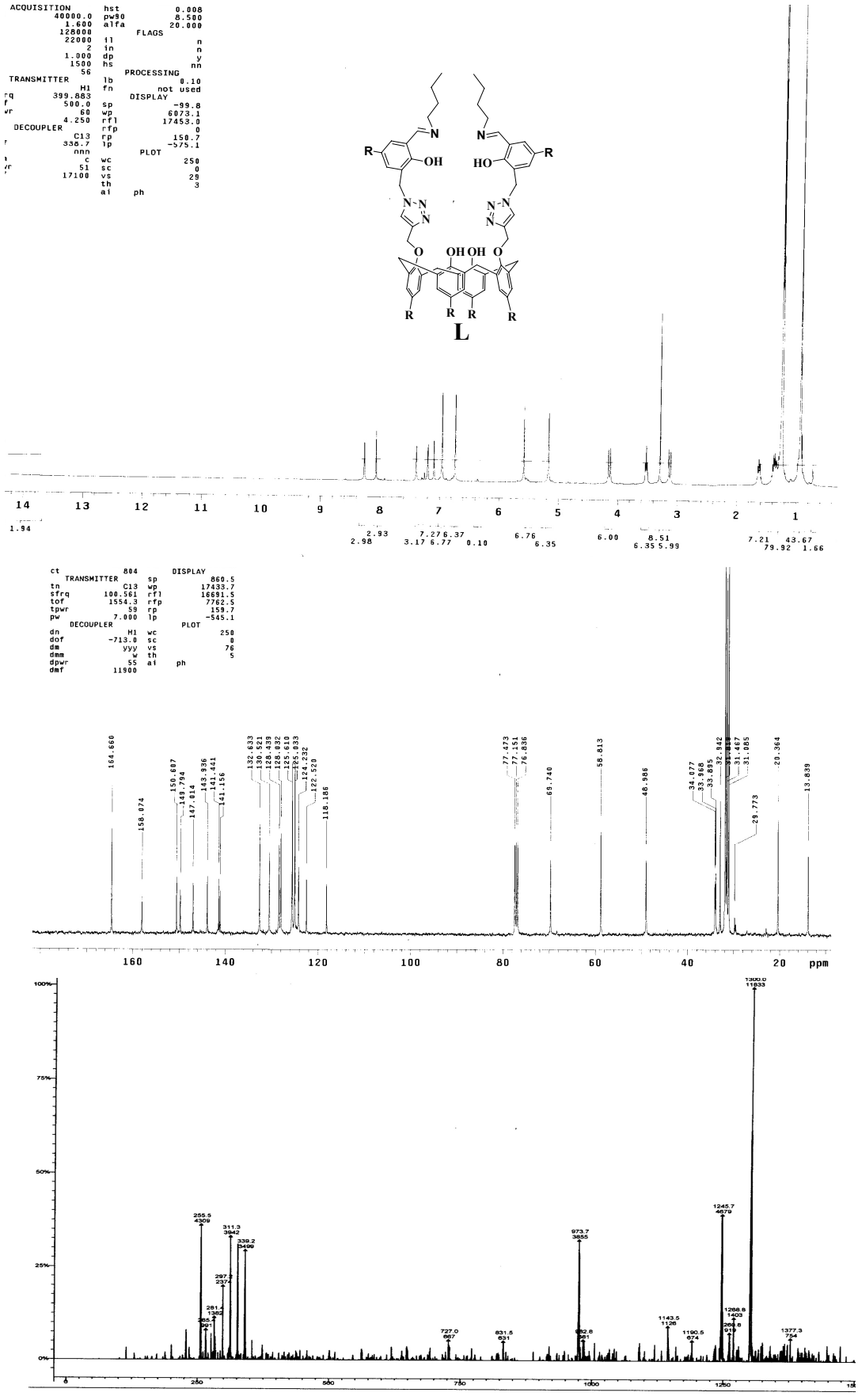


Figure: Spectra for **L**: (a) ^{1}H NMR; (b) ^{13}C NMR and (c) ESI-MS.

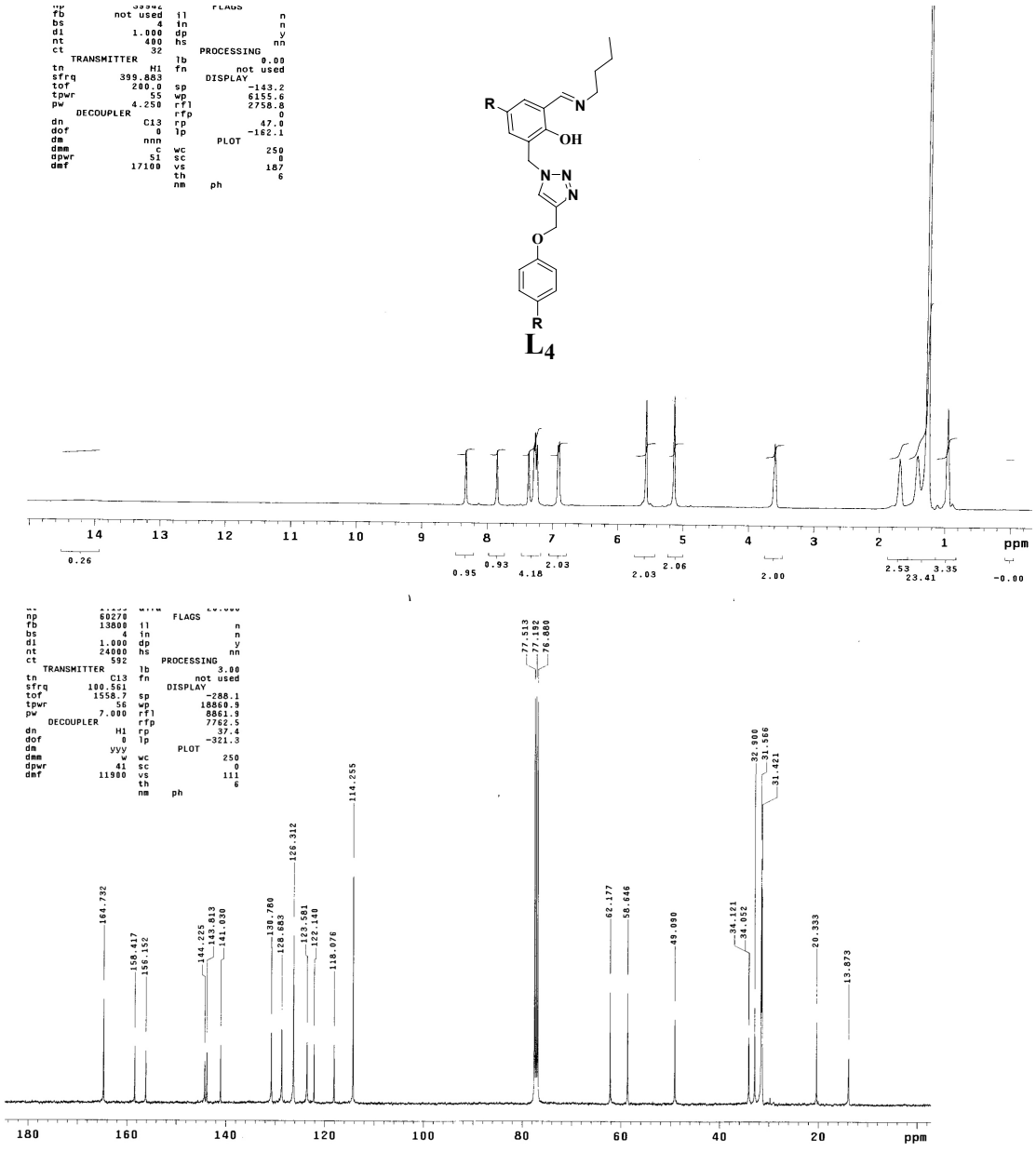


Figure: Spectra for $\mathbf{L_4}$: (a) ^{1}H NMR; (b) ^{13}C NMR

Mass, UV-visible titration and visual color change experiment

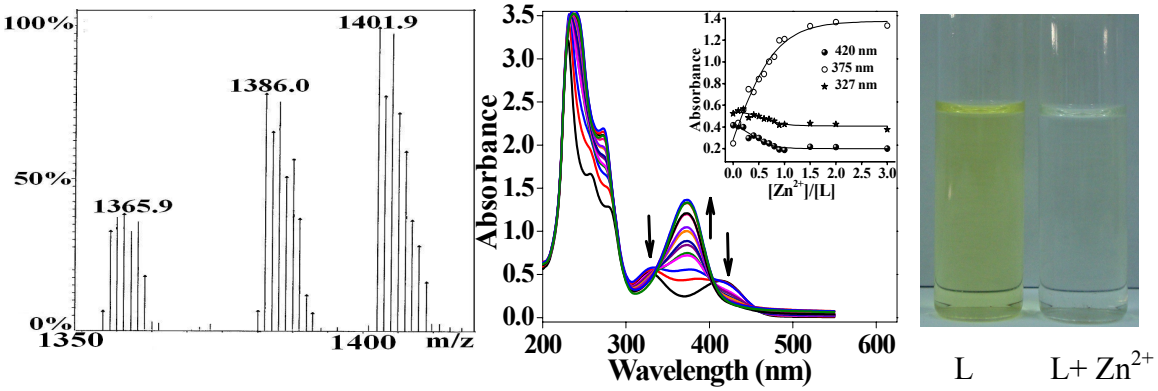


Figure: (a) ESI MS spectrum obtained during the titration of **L** with Zn^{2+} (only a part of the spectrum is shown). (b) absorption spectra obtained during the titration L with Zn^{2+} as measured under the same conditions given in Figure 1. Plot of absorbance vs. $[Zn^{2+}]/[L]$ for different bands was given in the inset. (c) visual color change shown upon addition of Zn^{2+}.

^{1}H NMR spectra of L and its Zn complex

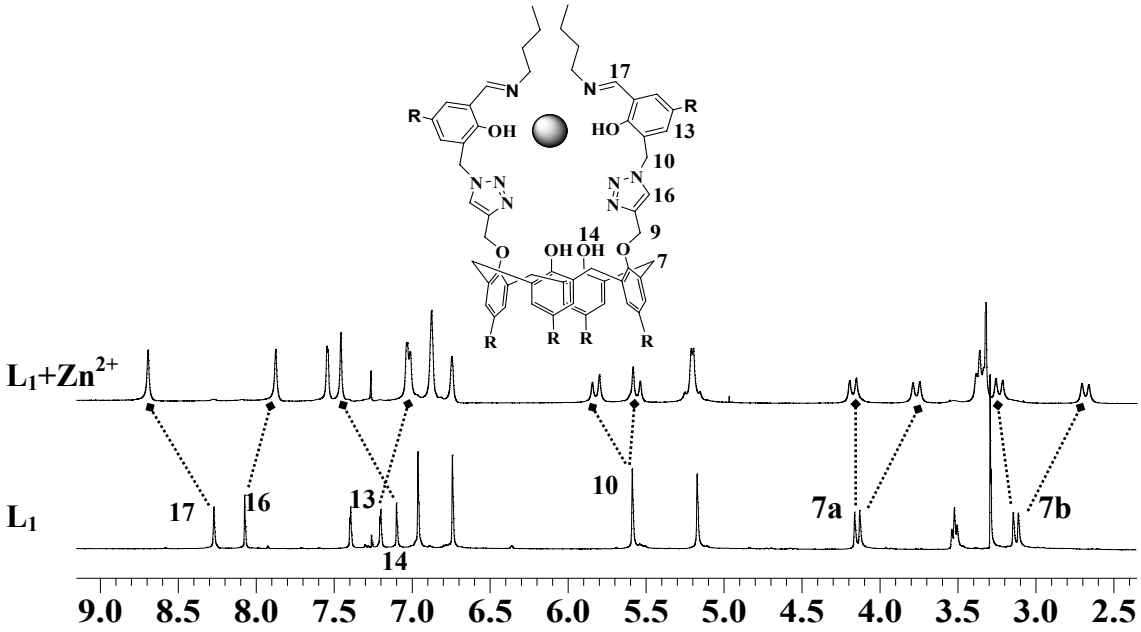


Figure: ^{1}H NMR spectra of **L** and **L** +Zn^{2+}

Job's plot of L with Zn^{2+}

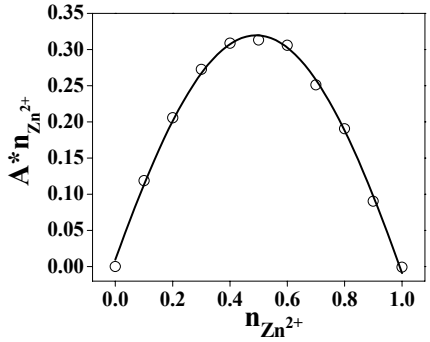


Figure: Job's plot of n_m verses A^* n_m where n_m is mole fraction of the metal ion added and A is absorbance.

Fluorescence response of L with all 17 metal ions and competitive metal ion titration

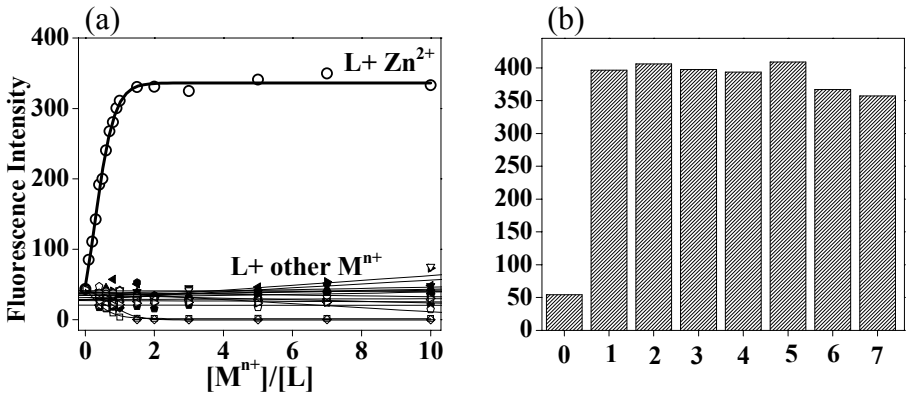


Figure: (a) Fluorescence intensity as a function of $[M^{n+}]/[L]$ mole ratio for different metal ions. The symbols corresponds to, ○ = Zn^{2+}, ● = Li^{+}, ▲ = Na^{+}, ▼ = K^{+}, ◀ =Cs^{+}, ▶ = Mg^{2+}, ◆ = Ca^{2+}, ⬟ =Sr^{2+}, ⬢ = Ba^{2+}, ★ = Mn^{2+}, □ = Fe^{2+}, ⬠ = Co^{2+}, ⬡ = Ni^{2+}, ◈ = Cu^{2+}, ▽ = Cd^{2+}, ◁ = Hg^{2+}, ▷ = Ag^{+}. (b) Histogram showing the fluorescence response of various metal ions on the [ZnL] complex. (0 = L; 1 = [ZnL]; 2 = [ZnL] + Na^{+}; 3 = [ZnL] + K^{+}; 4 = [ZnL] + Ca^{2+}; 5 = [ZnL] + Mg^{2+}; 6 = [ZnL] + Cd^{2+}; 7 = [ZnL] + Hg^{2+}). Thirty equivalents of alkali and alkaline earth metal ion and 10 equivalents of Cd^{2+} and Hg^{2+}were used.

Minimum detection limit experiment for L and L_4 in deferment medium

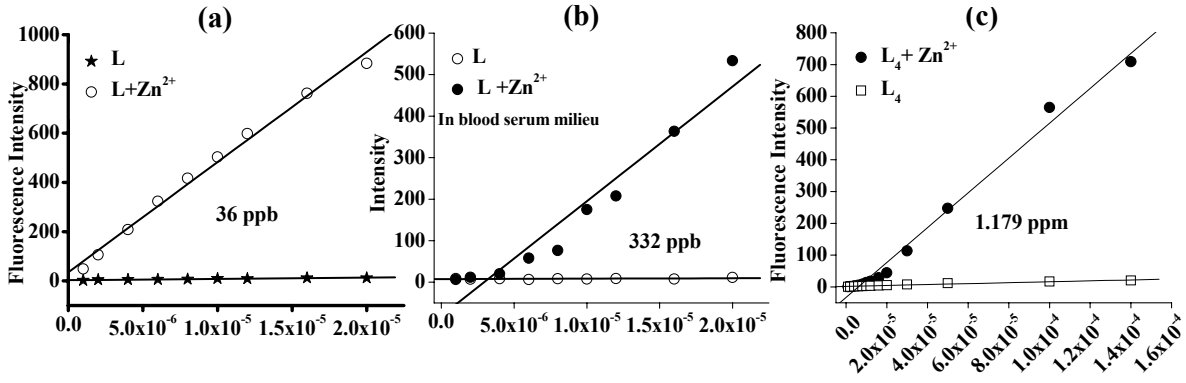


Figure: Minimum detection limit experiments (a) L with Zn^{2+} in aqueous methanolic HEPES buffer (pH=7.5). (b) L with Zn^{2+} in blood serum dissolved in aqueous methanolic HEPES buffer (pH=7.5). (c) L_4 with Zn^{2+} keeping the M^{2+} to L ratio as 1:1 in order to identify the lowest detectable M^{2+} concentration by L.

Effect of pH on sensitivity of L towards Zn^{2+}

The fluorescence spectral change of **L** during the titration of Zn^{2+} as a function of pH was examined in aqueous methanolic solution as shown in figure 1**,** and found no significant change in the intensity in the pH range 6 to 10 suggesting its utility for the biological samples.

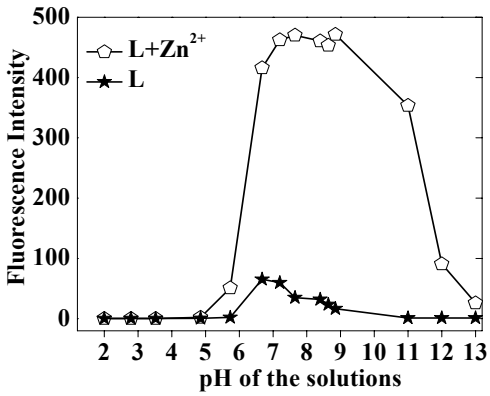


Figure: Fluorescence intensity for **L** and **L** + Zn^{2+} as a function of pH.

Fluorescence studies of L_3 and L_4 towards Zn^{2+}

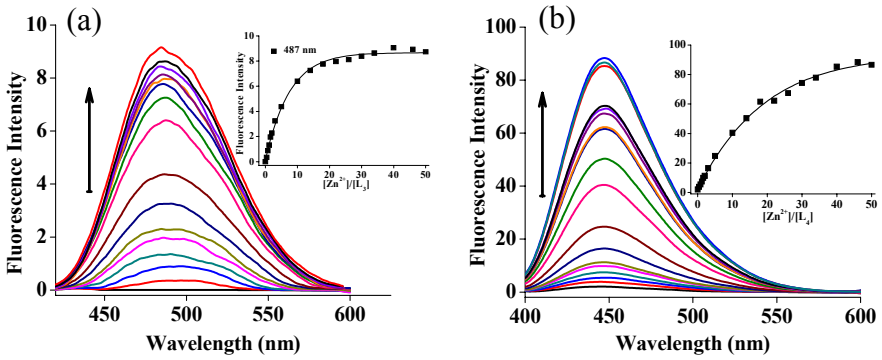


Figure: Fluorescence spectra obtained during the titration of (a) L_3 with Zn^{2+} (b) L_4 with Zn^{2+} in aqueous Methanolic HEPES buffer (4:1, v/v pH =7.4) at an excitation wavelength of 380 nm. The inset shows the fluorescence intensity as a function of $[Zn^{2+}]/[\mathbf{L}]$ mole ratio.

Synthesis and characterization of the [ZnL]

[ZnL] To a solution of L (0.167 mmol) in CH_3CN (6 mL) was added a methanolic solution of $Zn(CH_3COO)_2.2H_2O$ (0.175 mmol) and refluxed for 5 hrs.. After concentrating this solution, a light yellow precipitate started to form and the solid formed was then filtered, washed with cold MeOH, and dried in vacuo to give the desired product. ν_{max} (KBr)/cm^{-1}: 2958, 1621, 1547, 1483, 1461. ^{1}H NMR ($CDCl_3$, 400 MHz): 8.69 (s, 2H, imine-H), 7.87 (s, 2H, triazole-H), 7.53 (d, Sal-H) 7.45 (s, 2H, Ar-OH) 7.02 (d, Sal-H), 7.02-6.74 (t, 8H, Ar-H), 5.84-5.80 (dd, 4H, Sal-CH_2), 5.20 (s, 4H, Ar-OCH_2), 4.19-3.76-3.25-2.67 (4d, 4H, Ar-CH_2-Ar), 3.34 (t, 4H, N-CH_2), 1.46 (quintet, N-CH_2-CH_2), 1.36 (Sextet, 4H, N-CH_2CH_2*CH_2CH_3), 1.30 (s, 18H, $(CH_3)_3$), 1.26 (s, 18H, $(CH_3)_3$), 0.99 (s, 18H, Sal-$(CH_3)_3$)0.76 (t, 6H, N-CH2-CH_2-CH_2-CH_3). ^{13}C NMR ($CDCl_3$, 200 Hz) δ (ppm): 171.5, 166.3, 150.4, 149.2, 148.0, 144.2, 142.5, 137.4, 134.0, 133.5, 133.2, 133.0, 128.5, 128.3, 128.17, 126.1, 126.0, 125.4, 117.7, 70.3, 61.2, , 50.5, 34.4, 34.3, 34.2, 32.8, 32.1, 31.8, 31.4, 20.4, 14.0, (ES/MS) 1403$[M]^+$+ K^+ (100). EA calcd. for $C_{74}H_{92}N_8O_{10}$: C, 70.90; H, 7.40; N, 8.94;. Found: C, 66.88; H, 7.35; N, 8.87

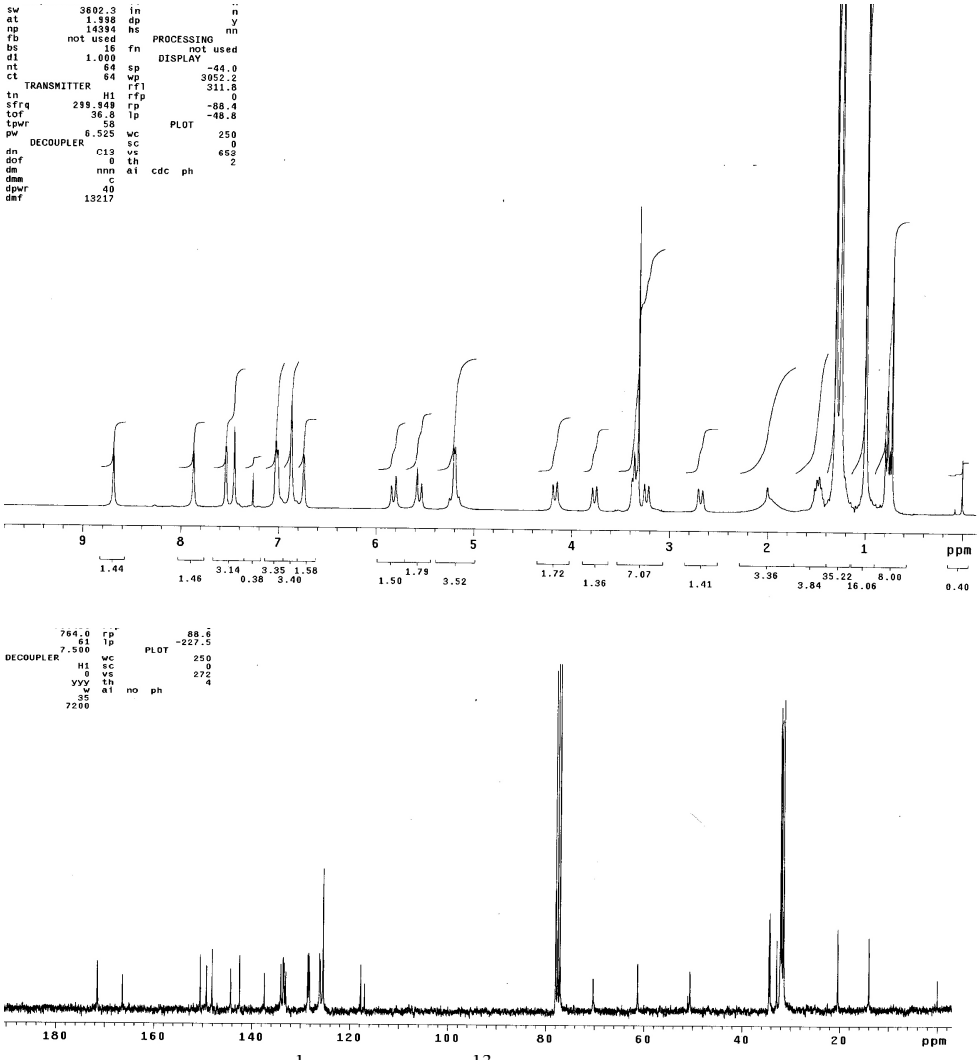


Figure: Spectra for [ZnL]: (a) ^{1}H NMR; (b) ^{13}C NMR