

Supporting Information

Highly Selective Colorimetric Chemosensor for Co²⁺

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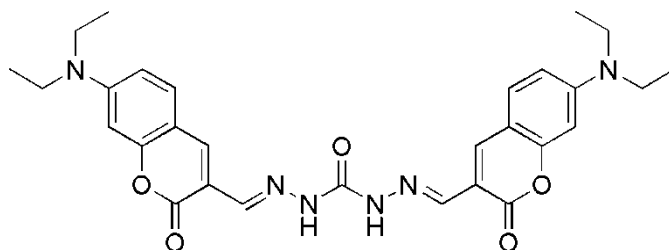
1. General experimental procedure

All the solvents and reagents (analytical grade and spectroscopic grade) were obtained from Sigma-Aldrich and used as received unless otherwise mentioned. The solutions of metal ions were prepared from $\text{Al}(\text{ClO}_4)_3 \cdot 9\text{H}_2\text{O}$, $\text{LiClO}_4 \cdot 3\text{H}_2\text{O}$, NaClO_4 , KClO_4 , $\text{Mg}(\text{ClO}_4)_2$, $\text{Sr}(\text{NO}_3)_2$, $\text{Ba}(\text{ClO}_4)_2$, $\text{Mn}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$, $\text{Fe}(\text{ClO}_4)_2$, $\text{Co}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$, $\text{Ni}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$, $\text{Cu}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$, $\text{Zn}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$, $\text{Cd}(\text{ClO}_4)_2 \cdot \text{H}_2\text{O}$, $\text{Ag}(\text{ClO}_4)_2$, $\text{Hg}(\text{ClO}_4)_2$ and $\text{Pb}(\text{ClO}_4)_2$ respectively in CH_3CN . ^1H and ^{13}C NMR were measured on a Bruker AV-400 spectrometer with chemical shifts reported as ppm (in $\text{DMSO}-d_6$, tetramethylsilane as internal standard). Mass spectra were measured on Shimadzu 2020 LC-MS and Bruker Ultraflex II MALDI/TOF spectrometers. Elemental analysis was carried out on ThermoScientific FLASH 2000 Organic Element Analyzer. UV/Vis spectra were recorded on a Perkin Elmer Model Lambda 900 spectrophotometer.

2. General synthetic Procedure of CC and CTC:

7-Diethylamino-2-oxo-2H-chromene-3-carbaldehyde was synthesized according to the literature method.¹ A solution of 7-diethylamino-2-oxo-2H-chromene-3-carbaldehyde (0.40 g, 1.63 mmol) in ethanol (20 mL) was added slowly to a solution of carbohydrazide or thiocarbohydrazide (0.81 mmol) in water (20 mL). Initially the solution turn turbid and after complete addition the solution became clear. The reaction mixture was refluxed with stirring for 24 h. A precipitated formed which was cooled and filtered. The precipitate was washed with ethanol and dried under vacuo to obtain biscarbonohydrazone CC or bithiocarbonohydrazone CTC in quantitative yield.

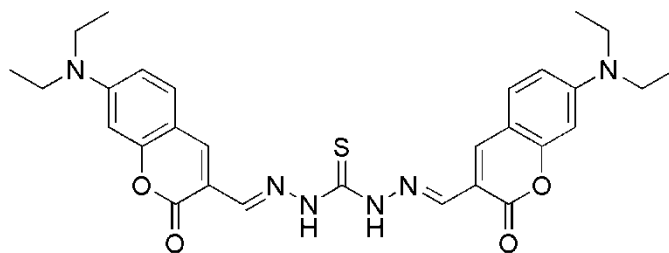
Bis[[7-(diethylamino)-2-oxo-2H-chromene]methylene]-carbonic dihydrazide (CC)



CC

Yield 92% (orange powder). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ_{ppm} 1.14 (12H, t, $J = 6.8$ Hz), 3.46 (8H, dd, $J = 6.8$ Hz, 7.2 Hz), 6.57 (2H, d, $J = 2.4$ Hz), 6.77 (2H, dd, $J = 2.4$ Hz, 6.8 Hz), 7.57 (2H, d, $J = 8.8$ Hz), 8.22 (2H, s), 8.45 (2H, s), 10.78 (2H, s). ^{13}C NMR (400 MHz, $\text{DMSO}-d_6$) δ_{ppm} 12.3, 44.2, 96.5, 108.1, 109.7, 113.2, 130.3, 137.2, 137.9, 150.9, 151.7, 156.2, 160.8. MS (EI): $m/z = 545.3$ $[\text{M}+\text{H}]^+$, calcd. 544.2 for $\text{C}_{29}\text{H}_{32}\text{N}_6\text{O}_5$. Elemental analysis: Found C, 63.92; H, 5.93; N, 15.44; Calcd. C, 63.96; H, 5.92; N, 15.43 for $\text{C}_{29}\text{H}_{32}\text{N}_6\text{O}_5$.

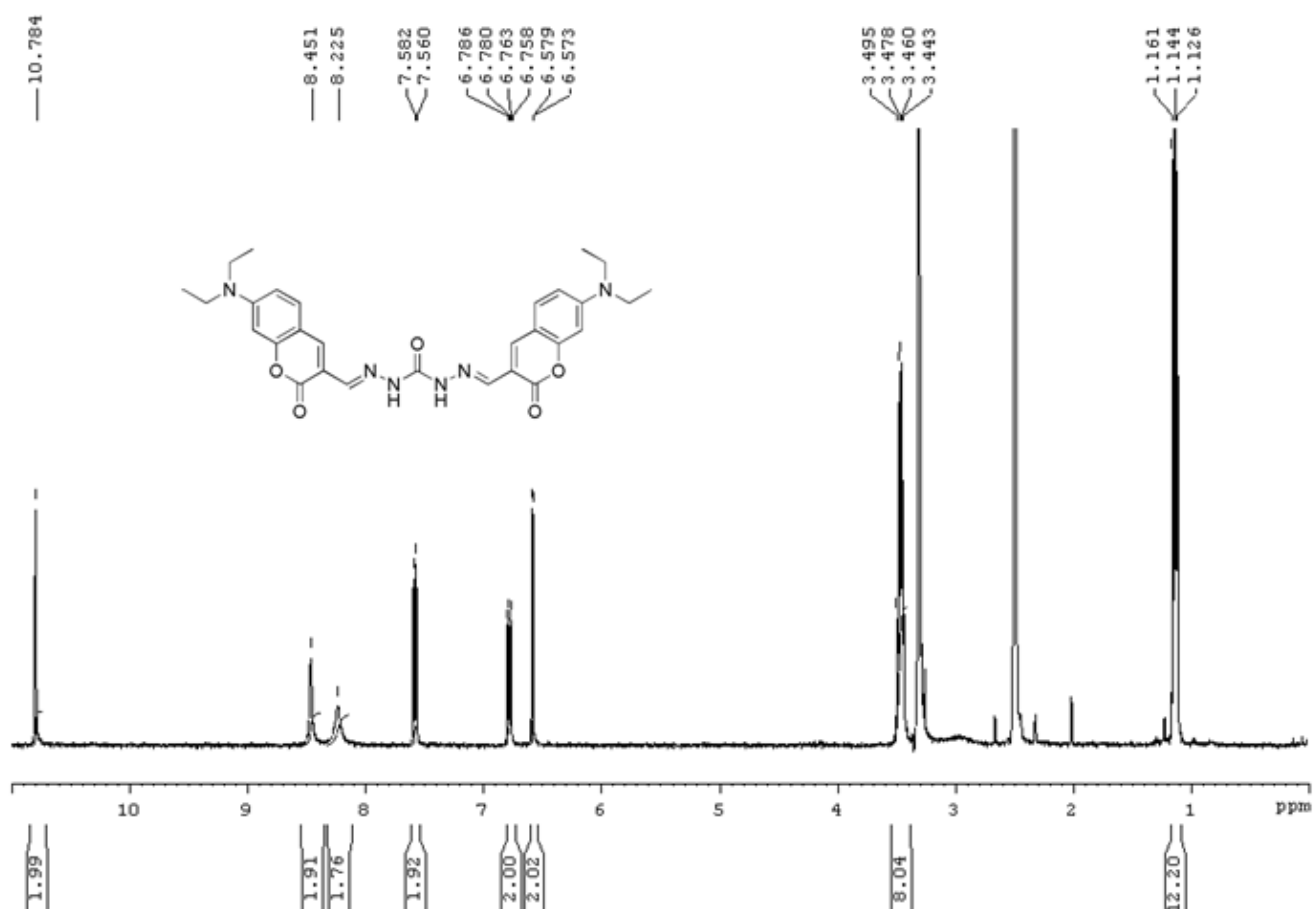
Bis[[7-(diethylamino)-2-oxo-2H-chromene]methylene]-carbonothioic dihydrazide (CTC)



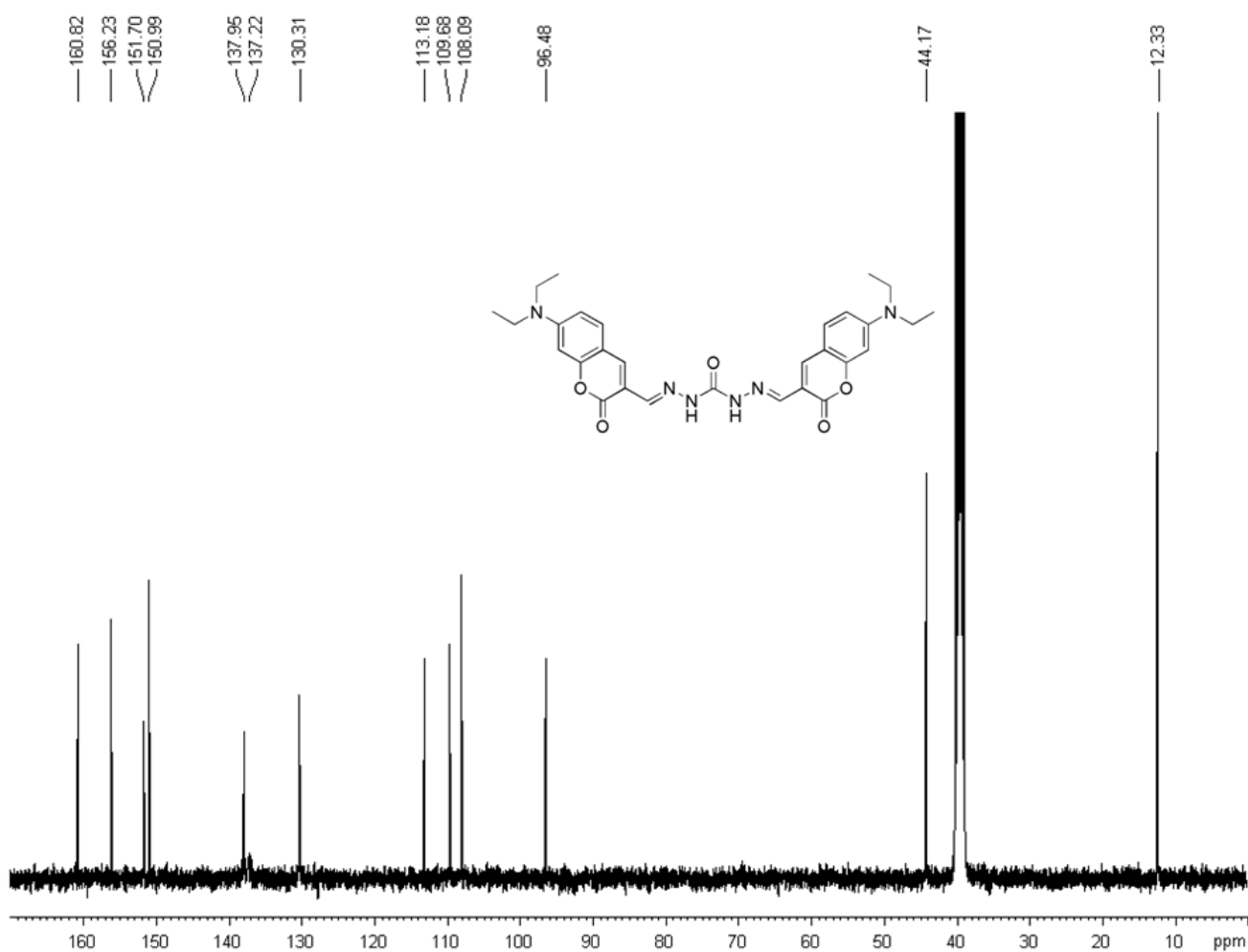
CTC

Yield 92% (brick red powder). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ_{ppm} 1.12-1.16 (12H, m), 3.45-3.50 (8H, m), 6.56-6.59 (2H, dd, $J = 2$ Hz, 6.4 Hz), 6.77-6.79 (2H, d, $J = 8.4$ Hz), 7.57-7.59 (2H, d, $J = 6.4$ Hz), 8.18 (1H, br), 8.30 (1H, br), 8.67 (2H, br), 11.67 (1H, br), 11.94 (1H, br). ^{13}C NMR (400 MHz, $\text{DMSO}-d_6$) δ_{ppm} 12.3, 44.2, 96.5, 108.1, 109.8, 112.5, 130.6, 136.3, 138.8, 151.3, 156.5, 160.7, 173.9. MS (EI): $m/z = 561.2$ $[\text{M}+\text{H}]^+$, calcd. For 560.2 $\text{C}_{29}\text{H}_{32}\text{N}_6\text{O}_4\text{S}$. Elemental analysis: Found C, 62.08; H, 5.76; N, 15.02; S, 5.71; Calcd. C, 62.12; H, 5.75; N, 14.99; S, 5.72 for $\text{C}_{29}\text{H}_{32}\text{N}_6\text{O}_4\text{S}$.

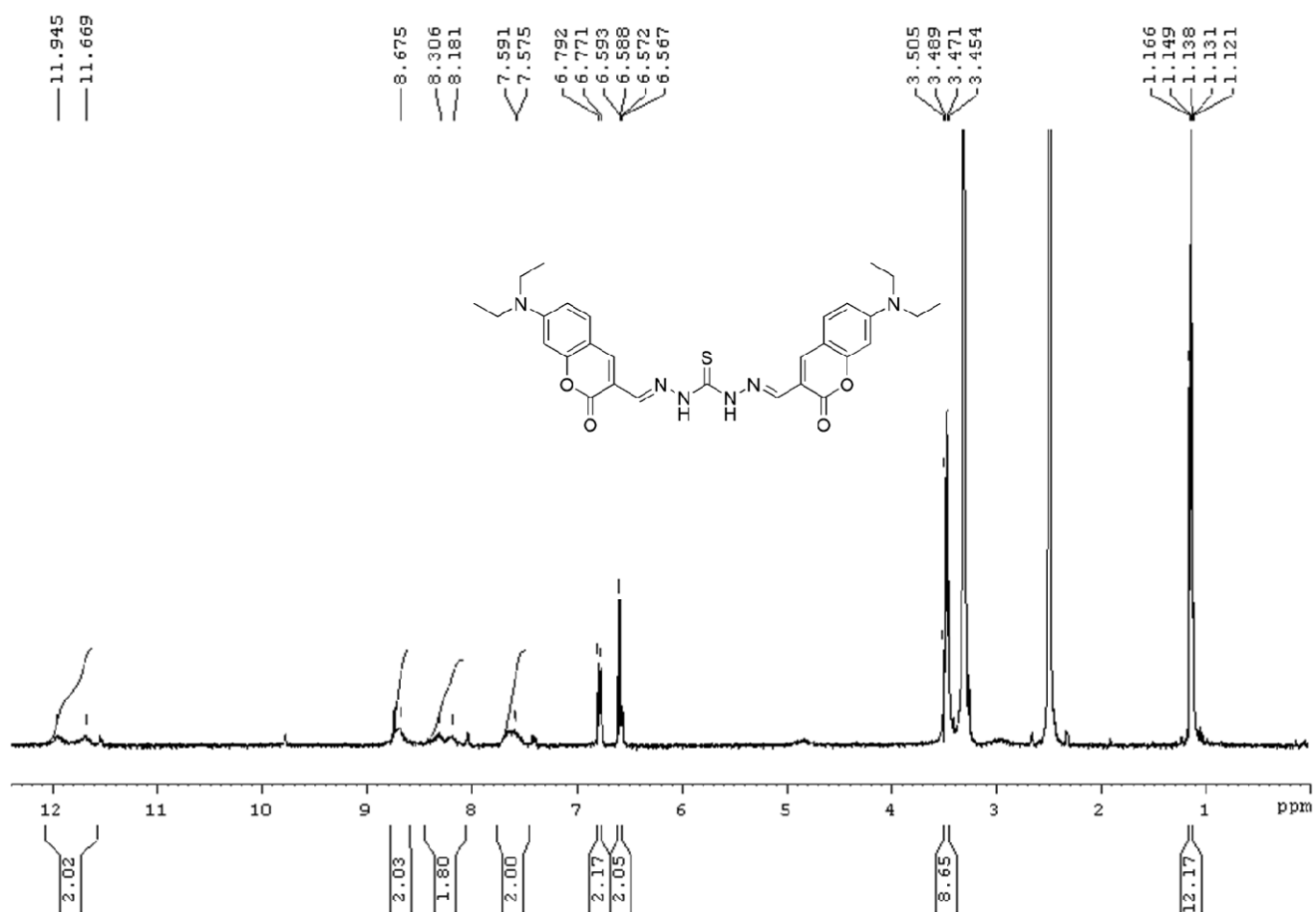
3. NMR Spectra



^1H NMR spectrum of bis[[7-(diethylamino)-2-oxo-2H-chromene]methylene]-carbonic dihydrazide (CC)

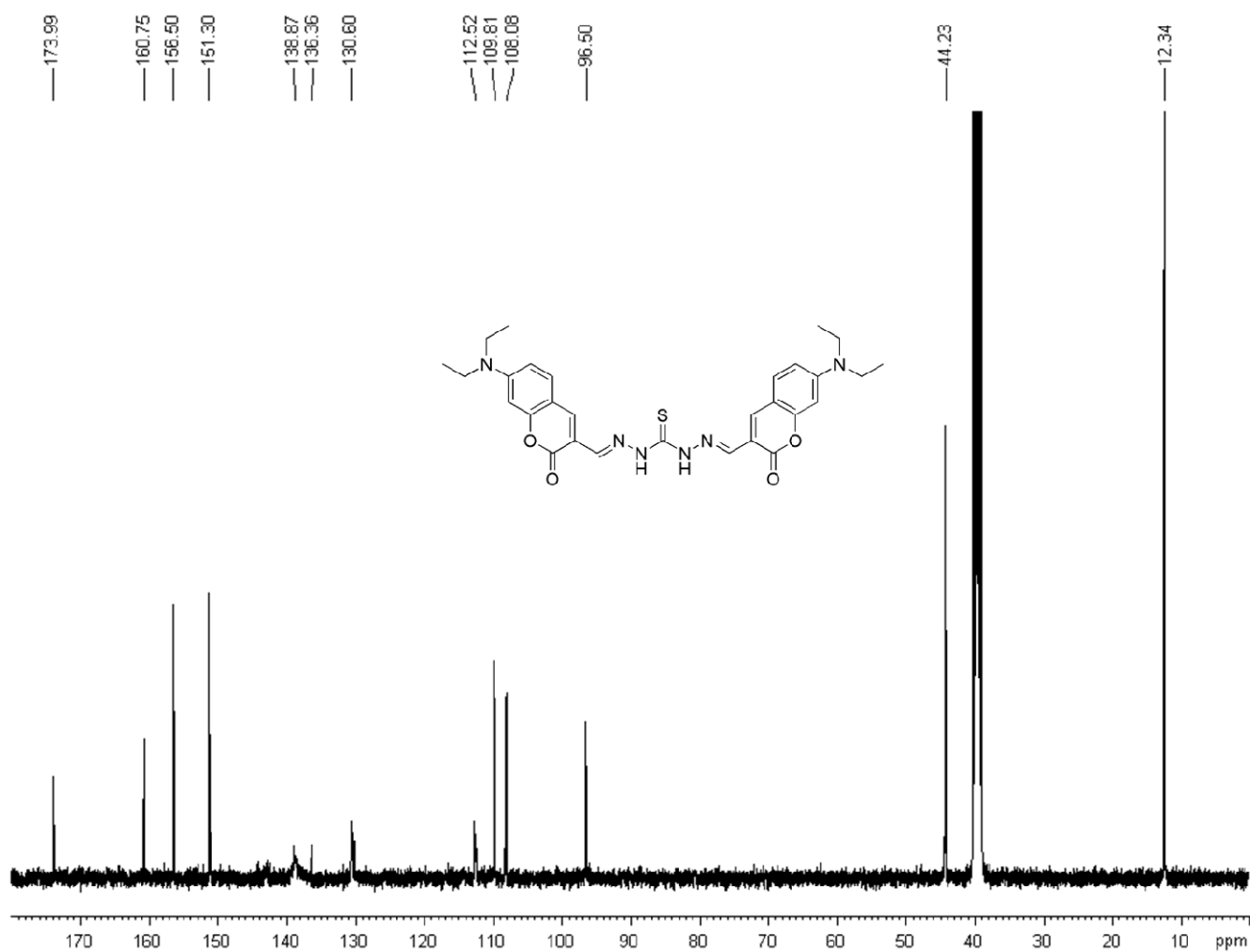


^{13}C NMR spectrum of bis[[7-(diethylamino)-2-oxo-2H-chromene]methylene]-carbonic dihydrazide (CC)



¹H NMR spectrum of bis[[7-(diethylamino)-2-oxo-2H-chromene]methylene]-carbonothioic dihydrazide (CTC)

[Note: Two thiourea –NH and two imine proton signals arise due to tautomeric forms arising from –NH-C=S group.]



^{13}C NMR spectrum of bis[[7-(diethylamino)-2-oxo-2H-chromene]methylene]-carbonothioic dihydrazide (CTC)

4. General method of UV-vis titration

UV/vis spectra were recorded on a Perkin Elmer Model Lambda 900 spectrophotometer. 1 cm cell was used for titration. For UV/vis titrations, stock solutions of **CC** and **CTC** were prepared ($c = 2000 \mu\text{M}$) in acetonitrile. The solutions of guest cations were prepared in acetonitrile/water in the order of 10^{-3} M .

5. UV/vis absorption spectral data

UV/vis absorption spectra of **CC** with different metal ions

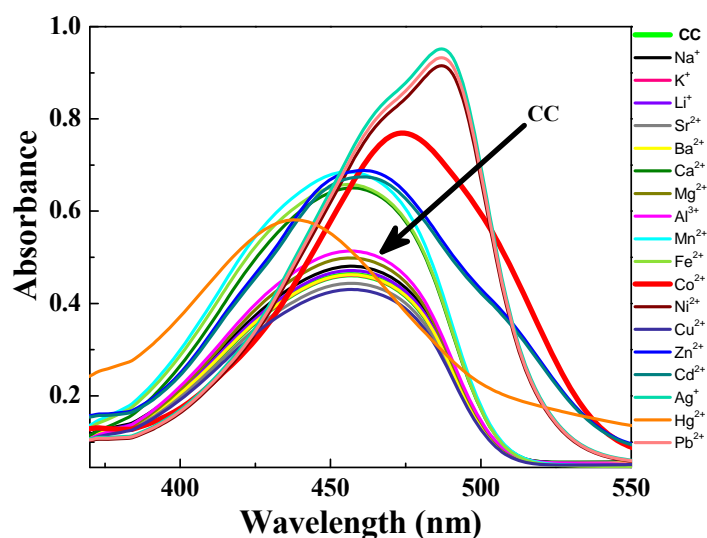


Figure S1. Absorbance spectra of **CC** ($10.0 \mu\text{M}$) and on addition of salts of Li^+ , Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Al^{3+} , Ba^{2+} , Sr^{2+} , Mn^{2+} , Fe^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} , Ag^+ , Hg^{2+} , Pb^{2+} , and Co^{2+} (50.0 equiv) in aqueous medium (0.05 mM HEPES, $\text{pH}=7.2/\text{MeCN} = 60:40$).

UV/vis absorption spectra of CTC with different metal ions

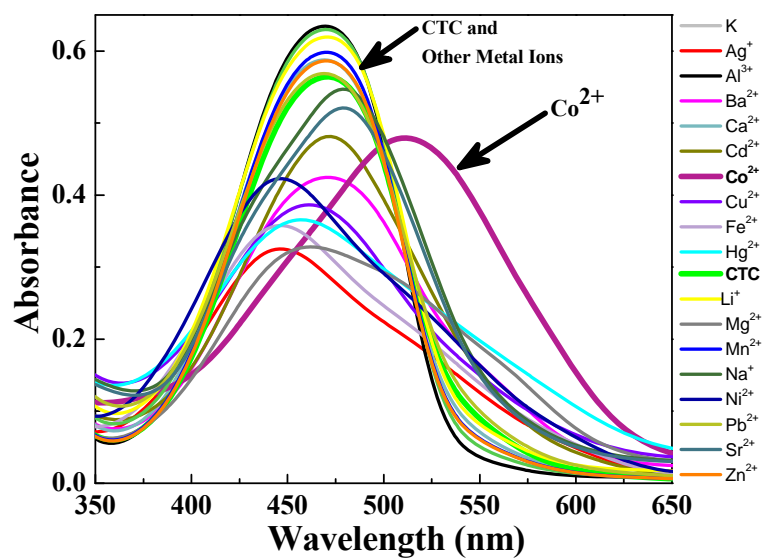


Figure S2. UV/vis absorption spectra of CTC (10.0 μM) and on addition of salts of Li⁺, Na⁺, K⁺, Ca²⁺, Mg²⁺, Al³⁺, Ba²⁺, Sr²⁺, Mn²⁺, Fe²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Cd²⁺, Ag⁺, Hg²⁺, Pb²⁺, and Co²⁺ (50.0 equiv) in aqueous medium (0.05 mM HEPES, pH=7.2/MeCN = 60:40).

6. Job Plot by UV/Vis method

A series of solutions containing **CTC** and $\text{Co}(\text{ClO}_4)_2$ were prepared such that the sum of the total concentration of metal ion and **CTC** remained constant ($10\ \mu\text{M}$). The mole fraction (X) of Co^{2+} was varied from 0.1 to 1.0. The corrected absorbance ($[A-A_0] / [A_0]$) at 510 nm was plotted against the molar fraction of the Co^{2+} solution.

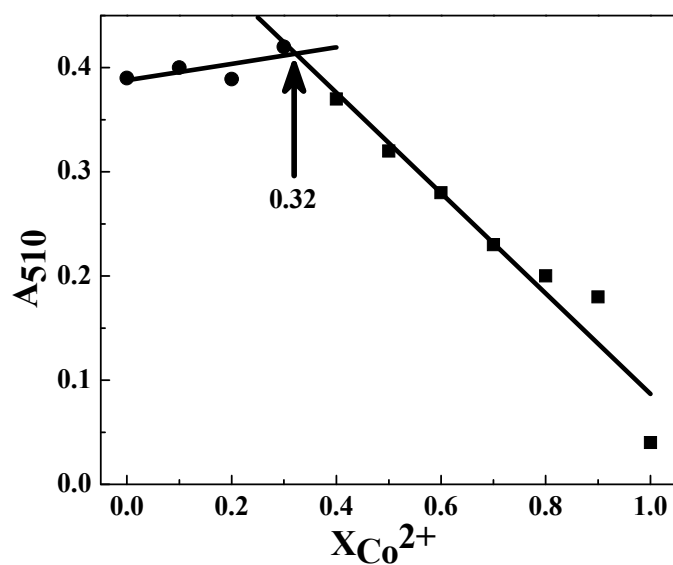


Figure S3. Job plot for the determination of the stoichiometry of **CTC** and Co^{2+} complex formation.

7. Mass spectral analysis of 2CTC:Co²⁺ complex

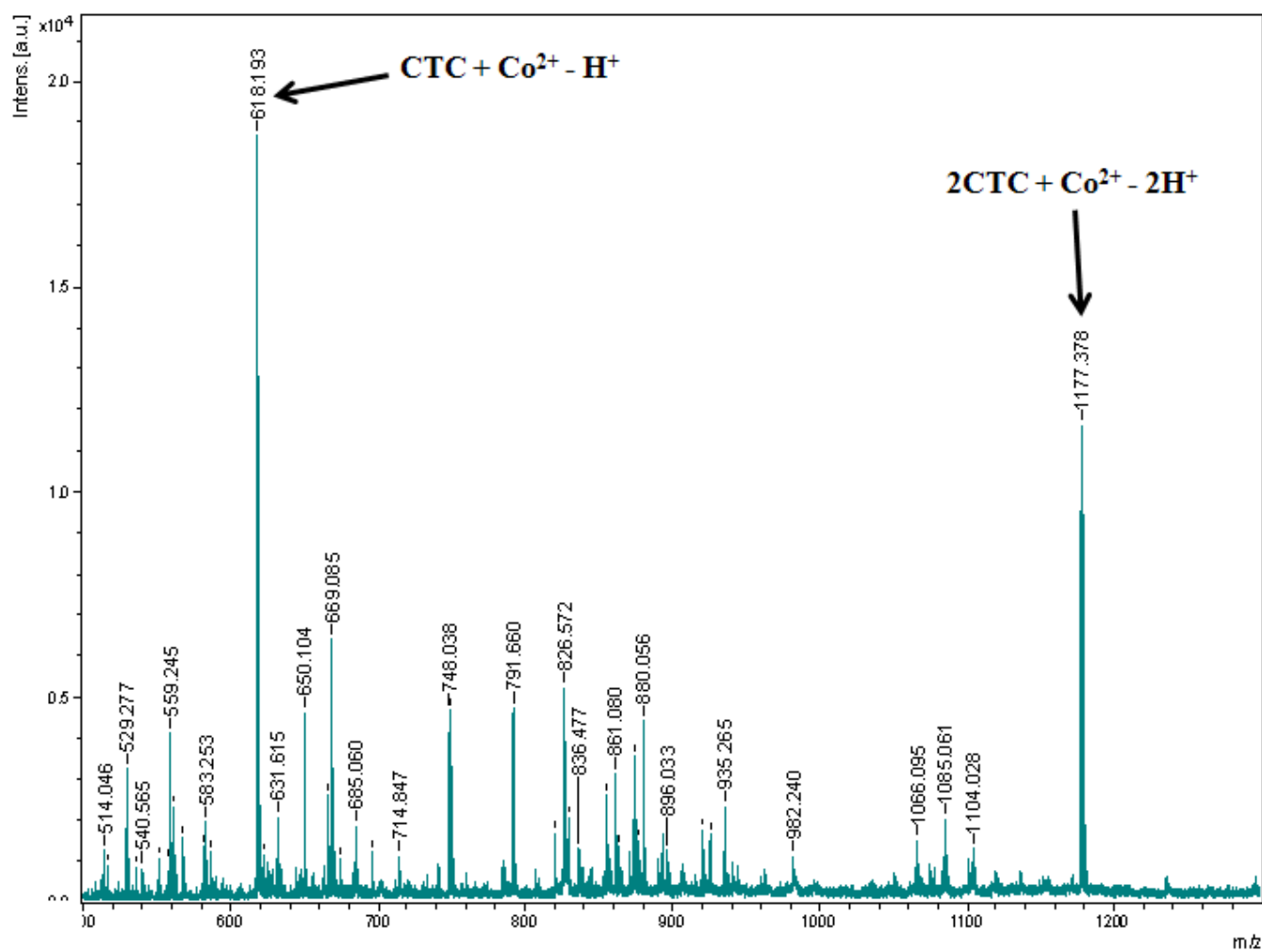


Figure S4. MALDI-MS spectrum of 2CTC:Co²⁺ complex

8. Determination of Binding Constant

Li *et al.* derived the Tsein equation² to the following equations that can be used in any stoichiometric ratio between the ligand and analyte.

$$[M^{n+}]^m = \frac{1}{n \cdot K} \cdot \frac{1}{[L]_T^{n-1}} \cdot \frac{1-\alpha}{\alpha^n}$$

Where K is complex equilibrium constant, M_mL_n is metal-ligand, L is ligand, [L], $[M^{n+}]$, and $[M_mL_n]$ are the concentrations of respective species. α is the ratio between free ligand concentration, [L], and the initial concentration of ligand, $[L]_T$. In our case, the stoichiometric ratio of the CTC and Co^{2+} is 2:1. So, this equation can be written as

$$[Co^{2+}]^{0.5} = \frac{1}{2KL_T} \cdot \frac{1-\alpha}{\alpha^2}$$

The curve fitting experimental data points were calculated from this equation with $\log K_a = 7.95 M^{-2}$.

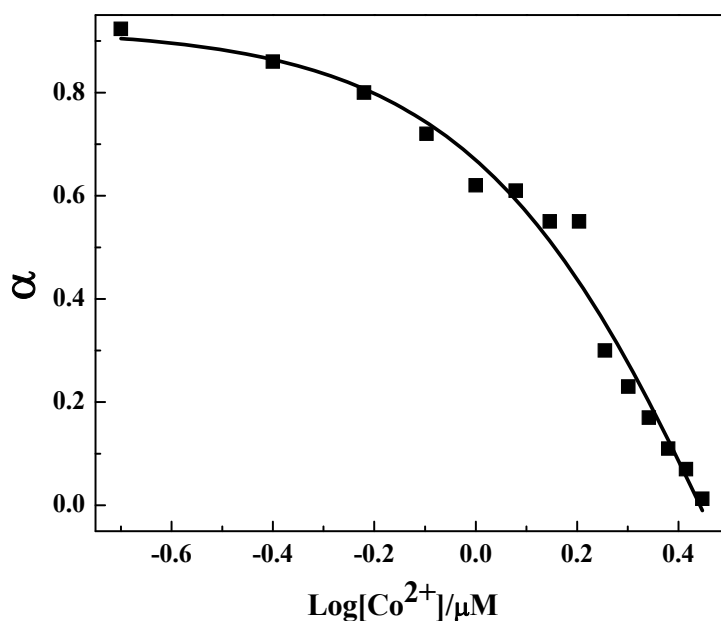


Figure S5. Response parameter values (α) as a function of the logarithm of Co^{2+} concentration. α is defined as the ratio between the free ligand concentration and the initial concentration of ligand.

9. pH Dependent Study

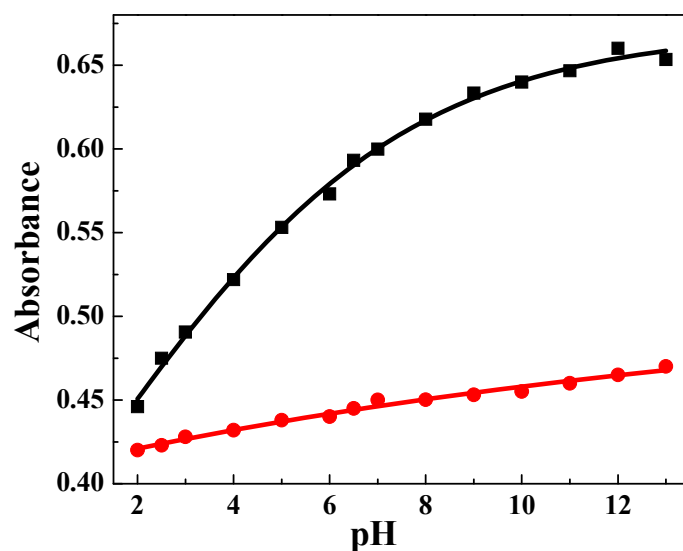


Figure S6. Absorbance at 510 nm of CTC as function of pH. Red trace: absorbance intensity of a solution of CTC and black trace: absorbance intensity of a solution of CTC and Co²⁺ (1 equiv) are plotted against pH in aqueous medium (50 mM HEPES, 40% CH₃CN).

10. References

- (1) Ray, D.; Bharadwaj, P. K. *Inorg. Chem.* **2008**, 47, 2252.
- (2) Gryniewicz, G.; Poenie, M.; Tsein, R. Y. *J. Biol. Chem.* **1985**, 260, 3440.