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2,2′-(Hydrazine-1,2-diylidenedimethylylidene) bis(6-isopropyl-3-methylphenol) based selective dual-channel chemosensor for Cu^{2+} in semi-aqueous media†

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A novel receptor based on a substituted salicylaldehyde derivative, 2,2′-(hydrazine-1,2-diylidenedimethylylidene)bis(6-isopropyl-3-methylphenol) (**1**), was synthesized and characterized using various spectroscopic techniques. The UV-visible spectroscopic studies of the receptor showed selectivity for Cu^{2+} in the presence of other metal ions by a change in colour from colourless to yellow. A red shift was observed with the appearance of a new absorption band at 450 nm in $CH_3OH–H_2O$ (60 : 40 v/v). Fluorescence studies of the receptor showed "turn-off" recognition properties for the Cu^{2+} ion with a 1 : 1 binding stoichiometry and the detection limit was 50 nM.

Introduction

The design and synthesis of chemosensors with high selectivity and sensitivity for transition metal ions such as copper are receiving great interest in the field of supramolecular chemistry due to the importance of these metals in the material sciences and biology.[1–3] Copper is an essential trace nutrient in human metabolism and plays a vital part in the physiological processes of organisms, including connective tissue development and the formation of bone and blood. However, free Cu^{2+} is potentially both acutely and chronically toxic to aquatic life and micro-organisms are affected at even micromolar concentrations. In humans, the brain concentrates heavy metal ions such as copper for use in metabolic processes. Copper is important for the normal development and working of the brain and, as a cofactor for many enzymes, it actively participates in many physiological pathways in the brain. Any disturbance in the uptake of copper may lead to neurodegenerative disorders. Gene mutations are responsible for the two major genetic disorders of copper metabolism in humans, Menkes' disease and Wilson's disease, and it has been observed that these diseases are caused by excessive intracellular copper transport.[4–9] Cu^{2+} can also react with molecular oxygen to form reactive oxygen species, which can damage lipids, nucleic acids and proteins. The significant physiological relevance of Cu^{2+} and its associated biomedical implications has resulted in considerable interest in the design of highly selective and sensitive copper chemosensors.

Non-cyclic compounds containing multiple coordination sites have received considerable attention because of their ability to complex various ionic and/or neutral molecules.[10–16] Such non-cyclic derivatives of hydrazide show interesting coordination properties towards transition metal ions due to the presence of several potential coordination sites.[17] The proton of the –NH group becomes more labile when a condensation reaction takes place between the terminal NH_2 group and an aldehyde or ketone; the resulting acyl hydrazones react with metal ions in the enol form.[18–20] These receptors coordinate strongly with metal ions, making them excellent candidates for cation sensing. In the investigation reported here, we detected Cu^{2+} ions *via* UV-visible and fluorescence spectroscopic techniques using a chemosensor based on a derivatized salicyaldehyde and hydrazine hydrate. The substituted salicyaldehyde was selected based on the natural occurrence of the parent compound thymol (2-hydroxy-3-isopropyl-6-methyl benzene) and its broad spectrum of activity.[21,22]

Experimental

All laboratory reagents used for synthesis were of 99% pure grade and were used without further purification. ^1H- and

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^{13}C-NMR spectra were recorded on a Varian NMR Mercury System 300 spectrometer operating at 300 and 75 MHz, respectively, in $CDCl_3$ using Me_4Si as an internal standard. The fluorescence and UV-visible spectra were recorded on a Fluoromax-4 spectrofluorimeter and a Shimadzu UV-24500 spectrophotometer in the range 200–600 nm at 28 °C using a 1 cm cell. All the spectral experiments were performed in a mixed solvent system of CH_3OH–H_2O (60 : 40 v/v). This solvent mixture was used because it resolved the solubility issues of the ligand and the chosen receptor showed the best performance in this solvent system.

Sample preparation

All stock and working solutions were prepared in ultrapure water and spectroscopic grade methanol. A stock solution of 2,2′-(hydrazine-1,2-diylidenedimethylylidene)bis(6-isopropyl-3-methylphenol) (receptor **1**) ($c = 5 \times 10^{-3}$ M) was prepared in CH_3OH–H_2O (60 : 40 v/v) solution and the corresponding working solutions ($c = 5 \times 10^{-6}$ M) were prepared simply by diluting with CH_3OH–H_2O (60 : 40 v/v). Similarly, the stock solutions of all the metal ions ($c = 5 \times 10^{-3}$ M) were prepared in CH_3OH–H_2O (60 : 40 v/v) and the corresponding working solutions ($c = 5 \times 10^{-5}$ M) were prepared by diluting with CH_3OH–H_2O (60 : 40 v/v).

Photophysical studies

The cation binding studies were performed on a UV-visible spectrophotometer using different metal ions (Cr^{3+}, Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, Na^{+}, K^{+}, Ba^{2+} and Al^{3+}) with receptor **1** in CH_3OH–H_2O (60 : 40 v/v) at room temperature. The ability of receptor **1** to bind selectively to a particular metal ion was investigated by performing titrations. The titrations confirmed the linear relationship with the selective metal ion and the change in absorbance intensity was used to calculation the linearity range and the correlation coefficient. These titrations were carried out through the addition of a metal salt solution in small aliquots ($c = 5 \times 10^{-5}$ M) to a solution of receptor **1** ($c = 5 \times 10^{-6}$ M) in CH_3OH–H_2O (60 : 40 v/v) in a 10 mL volumetric flask. The absorbance intensity was recorded in the range 200–600 nm together with a reagent blank.

The metal binding test was carried out on a Fluoromax-4 spectrofluorimeter in CH_3OH–H_2O (60 : 40 v/v) at 28 °C. The fluorescence intensity was recorded at $\lambda_{ex}/\lambda_{em}$ = 405/585 nm alongside a reagent blank. The excitation and emission slits were both set to 5.0 nm. Titrations between receptor **1** and Cu^{2+} were used to evaluate the association constant (K_a) and the limit of detection. These titrations were carried out by the successive addition of metal salt solutions ($c = 5 \times 10^{-5}$ M) to a solution of receptor **1** ($c = 5 \times 10^{-6}$ M) in a 10 mL volumetric flask.

The stoichiometry of the complex formed was determined by preparing solutions of receptor **1** and Cu^{2+} in the ratios 1 : 9, 2 : 8, 3 : 7, 4 : 6, 5 : 5, 6 : 4, 7 : 3, 8 : 2 and 9 : 1. These solutions were shaken and then the fluorescence spectra were recorded. The plot of $[HG]$ *versus* $[H]/([H] + [G])$ was used to determine the stoichiometry of the complex formed, where $[HG]$ is the concentration of the complex, $[H]$ is the host concentration and $[G]$ is the Cu^{2+} concentration. The fluorescence intensity at 485 nm was used for the calculation. The concentration of $[HG]$ was calculated by the equation $[HG] = \Delta I/I_o \times [H]$.

Synthesis of receptor 1

Hydrazine hydrate (0.05 g, 1.0 mmol) was added to a solution 2-hydroxyl-3-isopropyl-6-methylbenzaldehyde (0.35 g, 2.0 mmol) in ethanol (50 mL) and the mixture was refluxed for 8 h at 80 °C. The yellow solid obtained at room temperature was filtered, dried and further purified by recrystallization (83% yield).[30] IR (KBr, cm^{-1}): 3420 (–OH), 1600 (–C=N–). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.26–1.29 (d, $J = 7.2$ Hz, 12H, $4CH_3$), 2.37 (s, 6H, Ar-CH_3), 3.33 (heptet, $J = 7.2$ Hz, 2H, 2CH–Me_2), 6.68–7.11 (d, $J = 7.3$ Hz, 4H, Ar-H), 9.10 (s, 2H, CH=N). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ 17.3, 24.6, 27.4,24.3, 117.4, 120.8, 129.3, 136.3, 137.9, 149.2, 156.3.

Results and discussion

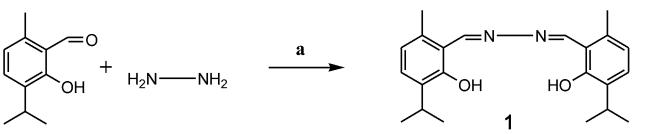
Synthesis and characteristics of receptor 1

Receptor **1** was synthesized by the Schiff base condensation of hydrazine hydrate with two moles of 2-hydroxyl-3-isopropyl-6-methylbenzaldehyde in ethanol (Scheme 1). The compound was characterized using various techniques.[30]

Recognition studies of receptor 1

The absorption behaviour of receptor **1** ($c = 5 \times 10^{-6}$ M) with various metal ions was studied in CH_3OH–H_2O (60 : 40 v/v). The absorption spectrum of receptor **1** showed two maxima at 325 and 375 nm (Fig. 1). With the addition of Cu^{2+}, the band at 375 nm disappeared and a new peak developed at 450 nm. The reason behind the development of the new peak at 450 nm may be the possible charge transfer between receptor **1** and Cu^{2+} (Fig. 1). The spectral shift also clearly delineated that the core functionality provided by receptor **1** was suitable for the selective encapsulation of Cu^{2+}. The effect of Cu^{2+} was so substantial that it could also be detected by the naked eye as a distinct colour change of the solution from colourless to yellow. In general, cations such as Fe^{3+}, Ni^{2+} and Co^{2+} are known to interfere in Cu^{2+} ion detection. However, in this study, no significant change in the colour of the solution was observed with the addition of these potentially interfering cations.[23]

For an in-depth study of the sensing ability of the Cu^{2+} ion, titrations were performed by the addition of small amounts of Cu^{2+} to a solution of receptor **1** ($c = 5 \times 10^{-6}$ M). With successive additions of Cu^{2+}, there was a decrease in the absorbance at 375 nm and an increase in the absorbance at 450 nm, with two isosbestic points at 295 and 405 nm (Fig. 2). To



Scheme 1 Synthesis of receptor **1** (a = ethanol, 8 h reflux).

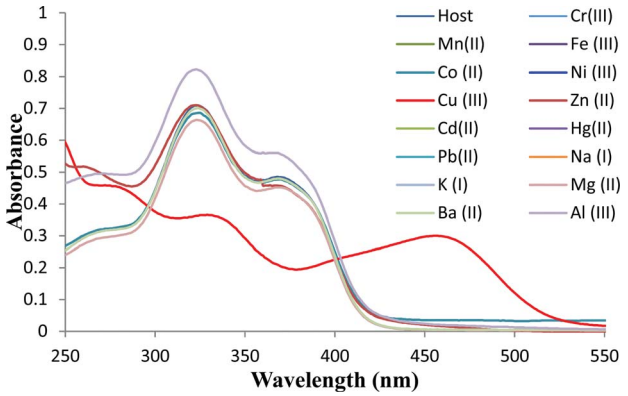


Fig. 1 Changes in the absorbance spectrum of receptor **1** ($c = 5 \times 10^{-6}$ M) in the presence of various metal ions ($c = 5 \times 10^{-5}$ M) in $CH_3OH–H_2O$ (60 : 40 v/v).

confirm the relationship between the absorbance intensity and the concentration of Cu^{2+}, a graph was plotted of A_{450}/A_{375} *vs.* [Cu(II)] (Fig. 2 inset). The linear dependence of the concentration of Cu^{2+} confirmed that receptor **1** could be used for the quantitative determination of Cu^{2+}.

The charge transfer process during the encapsulation of Cu^{2+} by receptor **1** was investigated by density functional theory calculations applying the B3LYP functional and the basis sets 6-31G** (for C, H, N and O atoms) and LANL2DZ (for the Cu atom), available in the computational code Gaussian 09W.[24] The optimized structure of receptor **1** and its complex with Cu^{2+} is shown in Fig. 3a. With the complexation of **1** with Cu^{2+}, a lowering of the interaction energy by −106.67 kcal mol^{-1} was observed, which indicated the formation of a stable complex with the calculated bond length for Cu–N and an average Cu–O of 2.044 Å and 1.876 Å, respectively. The highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) of **1** were distributed uniformly over the entire molecule (Fig. 3b and c). However, analysis of the frontier molecular orbital plots of the **1**:Cu^{2+} complex indicated that intramolecular charge transfer occurred between receptor **1** and Cu^{2+}. The band gap between the HOMO and LUMO of **1** was also lowered on complexation, which confirmed the observed red shift in the absorption band.

The specificity of receptor **1** for the detection of Cu^{2+} in the presence of other interfering metal ions was determined by the addition of 1 equiv. of Cu^{2+} to receptor **1** in the presence of 2 equiv. of all other metal ions. As shown in Fig. 4, the results showed that the miscellaneous competitive cations did not lead to any significant spectral change. The data clearly suggest that there is no interference from other metal ions in the sensing of Cu^{2+}.

The fluorescence properties of receptor **1** were studied with addition of various metal nitrates ($c = 5 \times 10^{-5}$ M) at an excitation wavelength of 405 nm in $CH_3OH–H_2O$ (60 : 40 v/v). No significant change was observed with the addition of different metal nitrate salts, but on adding Cu^{2+} there was quenching in the broad spectrum peak at 585 nm (Fig. S1†). To investigate the sensing capability of the receptor for Cu^{2+}, a titration was carried out with incremental additions of Cu^{2+} to receptor **1**. Fig. 5 shows that the fluorescence intensity is quenched with the successive addition of Cu^{2+}. Receptor **1** contains an intramolecular hydrogen bond between the phenolic –OH moiety and the nitrogen of the imine group that undergoes excited state intramolecular proton transfer and yields a normal emission at 585 nm from the proton transfer tautomer.[25] Quenching

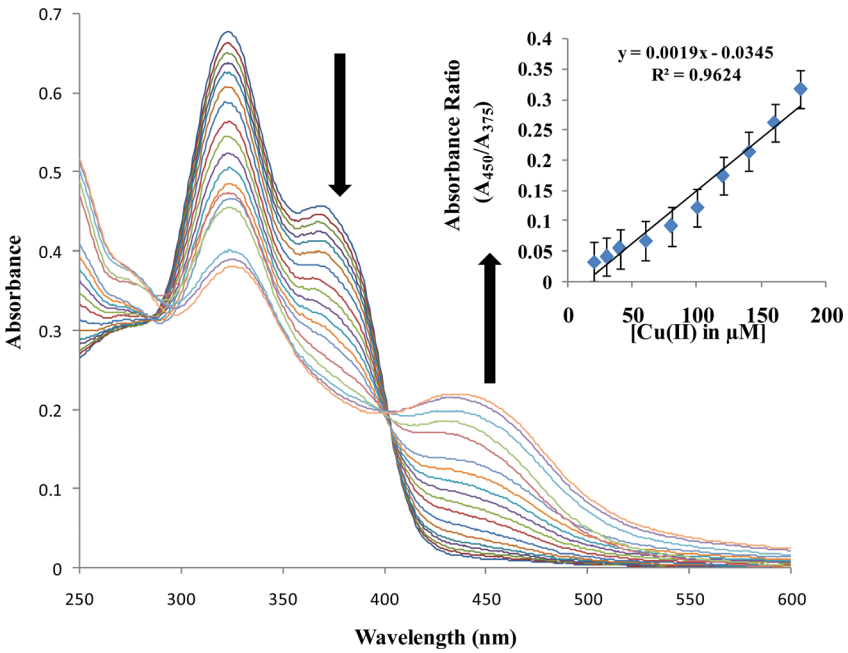


Fig. 2 Change in absorption profile of receptor **1** ($c = 5 \times 10^{-6}$ M) upon gradual addition of Cu^{2+} ($c = 5 \times 10^{-5}$ M) in $CH_3OH–H_2O$ (60 : 40 v/v). Inset shows the normalized response of absorbance signal with a regression of 0.9624.

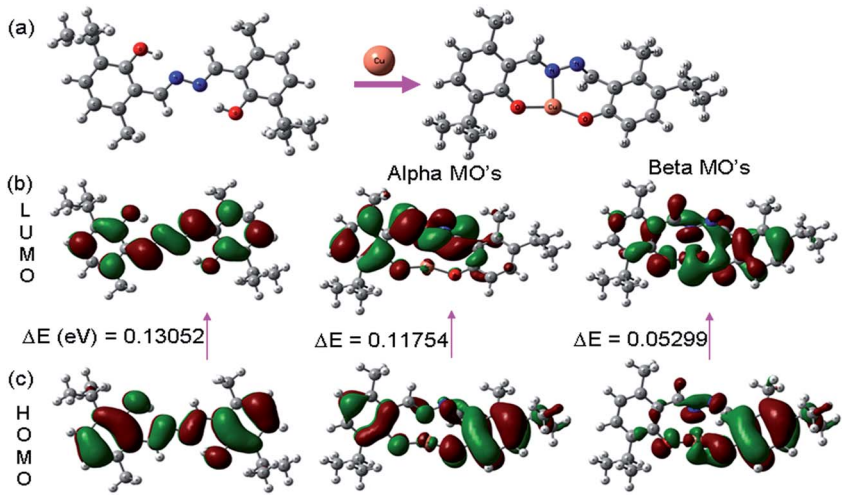


Fig. 3 (a) Density functional theory computed optimized structure of receptor **1** and its complex with Cu^{2+}. (b) LUMO and (c) HOMO diagrams of **1** and the **1**–Cu^{2+} complex.

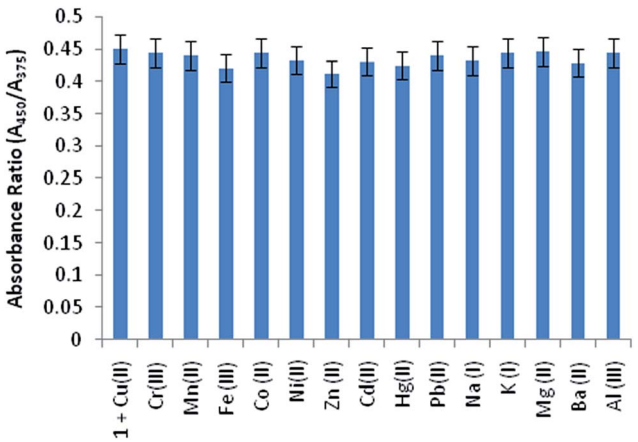


Fig. 4 Absorbance ratio (A_{450}/A_{375}) of receptor **1** ($c = 5 \times 10^{-6}$ M) with 1 equiv. of Cu^{2+} and 2 equiv. of the competitive metal ions.

of the emission at 585 nm was observed due to the involvement of the phenolic –OH groups of receptor **1** in complex formation with Cu^{2+}, which inhibited the excited state intramolecular proton transfer phenomenon.

The emission intensity of receptor **1** was linearly proportional to the Cu^{2+} concentration (Fig. 5, inset). The detection limit was calculated using $3*S/M$ following the IUPAC criterion, where S is the standard deviation of a blank signal and M is the slope of the regression line. The detection limit was 50 nM, which is comparable with other reported Cu^{2+} sensors (Table S1†).

The stoichiometry of the complexation of receptor **1** with Cu^{2+} was studied using the continuous variation method (Job's plot).[26] Job's plot and the normalized plot obtained from fluorescence measurements showed the formation of the receptor **1** and Cu^{2+} complex in a 1 : 1 ligand to metal ratio (Fig. S2†). These data were further confirmed by mass spectrometry analysis. The ESI MS data showed the formation of a 1 : 1 complex between the deprotonated ligand (receptor **1**) and a metal ion: [**1**–Cu^{2+} + $2Na^{+}$], MW = 458.046; calculated [$C_{22}H_{26}N_2O_2$ Cu^{2+} + 2Na], 458.20. The association constant (K_a) value of the **1**–Cu^{2+} complex calculated from the fluorescence titration by the Benesi–Hildebrand[27] (Fig. S3†) methodology was 666667 M^{-1}. The quenching can be expressed mathematically by the Stern–Volmer equation (eqn (1)), which allows the calculation of quenching constants:[28]

$$F_0/F = 1 + k_q\tau_0[Q] = 1 + K_{sv}[Q] \quad (1)$$

where F_0 and F are the fluorescence intensities in the absence and presence of the quencher, k_q is the bimolecular quenching constant, τ_0 is the lifetime of the fluorescence in the absence of the quencher, $[Q]$ is the concentration of the quencher and K_{sv} is the Stern–Volmer quenching constant. In the presence of a quencher, the fluorescence intensity is reduced from F_0 to F. The ratio (F_0/F) is directly proportional to the quencher concentration $[Q]$. Evidently:

$$K_{sv} = k_q\tau_0 \quad (2)$$

$$F_0/F = 1 + K_{sv}[Q] \quad (3)$$

According to eqn (3), a plot of F_0/F *versus* $[Q]$ shows a linear graph (Fig. S4†) with an intercept of 1 and a slope of K_{sv}. The linearity observed in Fig. S4† cannot confirm whether the quenching is static or dynamic. This can be explained as a dynamic process affects the excited fluorophore, but not the ground state, which results in no change in the absorption spectra during dynamic quenching. To reveal static or dynamic quenching without measurement of the fluorescence lifetime, the absorption spectrum was measured carefully to distinguish between static and dynamic quenching. We observed the change in the absorption spectrum that is the criterion for static quenching[29] and thus our quenching process is static in nature.

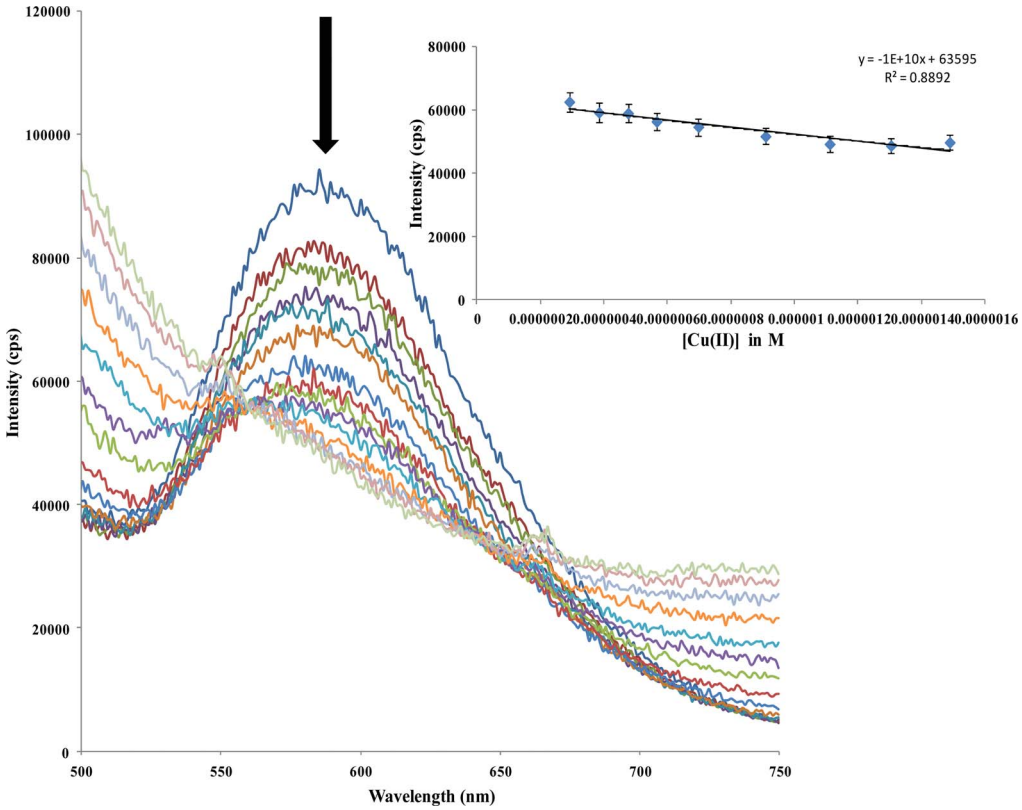


Fig. 5 Changes in fluorescent intensity of receptor **1** upon gradual addition of Cu^{2+} ($\lambda_{ex}/\lambda_{em}$ = 405/585). Inset shows the response of fluorescence signal with a regression of 0.96.

Conclusion

We have designed and developed a selective and sensitive chemosensor **1** for the detection of Cu^{2+} in aqueous medium. The detection of Cu^{2+} gave rise to significant UV-visible absorption and a colour change from colourless to yellow that could be seen with the naked eye. Receptor **1** was not affected by the presence of other interfering metal ions. The 1 : 1 stoichiometry of the host–guest complex formation was confirmed from Job's plot and a mass spectrometric method. Chemosensor **1** showed a fluorescence "turn-off" response for the selective detection of Cu^{2+} with a detection limit as low as 50 nM.

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