Calix[4]arene with Lower-Rim β-Amino α,β-Unsaturated Ketones Containing Bis-Chelating Sites as a Highly Selective Fluorescence Turn-On Chemosensor for Two Copper(II) Ions

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We report herein the synthesis of a fluorescence turn-on chemosensor, 25,27-bis[N-1-4-[[4-amino-4-(1-naphthyl)-2-oxo-3-butenyl]oxy]phenyl]aminocarbonyl]methoxy]-26,28-dihydroxy-calix[4]arene (3b), which is highly selective toward Cu²⁺. The fluorescence intensity of 3b was enhanced upon adding [Cu(ClO₄)₂], which reached a maximum with approximately 4 equiv. of Cu²⁺ but then started to decrease in intensity at higher Cu²⁺ concentrations. Job plot experiments revealed a 1:2 binding stoichiometry of binding sites for Cu²⁺ in 3b. Based on ¹H NMR titration results, we infer that there are two possible binding sites for Cu²⁺ in 3b: one at the lower-rim phenolic-OH and amide groups, and the second at the β-amino α,β-unsaturated ketone groups. It is important to note that during the complexation of 3b with [Cu(ClO₄)₂], the Cu²⁺ ions were reduced to Cu⁺ by both the phenolic OH and the amines of the β-amino α,β-unsaturated ketones. Furthermore, control compounds 6 and 9b were synthesized to clarify the possible binding sites of Cu²⁺ in 3b. By comparing the binding constants of 3b, 6, and 9b with Cu²⁺, we found that 3b exhibited a positive allosteric behavior toward the coordination of two Cu²⁺ ions.

Introduction

Calix[4]arenes, which are obtained from the oligomerization of phenol and formaldehyde, offer a very useful molecular scaffold for the construction of multivalent binding sites.[1] Over the past few decades, much effort has been devoted to the development of appropriate calix[4]arene chemosensors for the selective and sensitive detection of heavy metal ions because of their essential or deleterious roles.[2,3] For instance, although Cu²⁺ is biologically important, its accumulation in the human body may induce hepatic cirrhosis or neurodegenerative diseases.[4] Accordingly, many fluorescent sensors for Cu²⁺ have been designed,[3,5] however, most of them undergo fluorescence quenching upon binding with Cu²⁺.[3a–3c,5a–5d] Sensors that give fluorescence enhancement upon binding with metal ions are preferred for biological imaging over those that respond to metal ions with a fluorescence quenching. Nevertheless, sensors that “turn on” its fluorescence when complexed with Cu²⁺ are still rare.[3d,3e,5e,5f] Thus, it is still a demanding task to develop new Cu²⁺ selective fluorescence turn-on sensors.

The design of most fluorescent chemosensors for metal ions are based on photophysical changes that involve, for example, photoinduced electron transfer (PET),[6] metal–ligand charge transfer (MLCT),[7] or excimer/exciplex formation.[8] Although excimer emission has been frequently observed in bis-fluorophore substituted chemosensors that change conformation upon adding metal ions, exciplex formation, in contrast, has rarely been reported as a signaling mechanism in ion-sensing systems.[8a,8b]

We have been using a strategy to construct a variety of functionalized isoxazole units on calix[4]arene skeletons through 1,3-dipolar cycloaddition of alkynes and various aryl nitrile oxides.[9] Subsequent N–O bond cleavage of the isoxazole units by [Mo(CO)₆]-mediated ring opening reaction leads to the formation of enamino derivatives efficiently.[10] Enaminones, also named β-amino α,β-unsaturated ketones, as one kind of 1,3-bifunctional compound, have been frequently used as metal ion chelating ligands.[11] For example, they have been used in the synthesis of metallo-mesogens by coordination with Ni²⁺ and Cu²⁺ in liquid crystal research.[12] Despite the useful properties of enamino one groups, to date, there have been few reports[13] on their use as metal ion binding ligands in calix[4]arene related ionophores. Our recent work showed that calix[4]arene with lower-rim distal bis-β-amino α,β-unsaturated ketones can function as a ditopic receptor for the simultaneous complexation of copper and acetate ions.[14] The redox properties of Cu²⁺/Cu⁺ with phenol seems to be a ubiquitous phenomenon, thus, the question arises: Can calix[4]arenes...
A Fluorescence Chemosensor for Copper(II) Ions

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which contain phenols) be used with fluorophores to generate useful Cu\(^{2+}\) sensors?

Herein, we report a copper(II)-induced fluorescence turn-on chemosensor, 25,27-bis\{N-\{(4-\{4-amino-4-(1-naphthyl)-2-oxo-3-butenyl\}oxy}phenyl\}aminocarbonyl\}methoxy\}-26,28-dihydroxycaix[4]arene (3b), which contains amide-linked \(\beta\)-amino \(\alpha,\beta\)-unsaturated ketones as the Cu\(^{2+}\) recognition sites and naphthyl pendants as the fluorophore units. On the lower rim of the conical calix[4]arene framework, host 3b possesses two potential recognition sites for Cu\(^{2+}\) ions: one is near the \(\beta\)-amino \(\alpha,\beta\)-unsaturated ketone groups and the second is situated near the cavity formed by the phenolic-OH and amide groups. The binding ability of 3b and control compounds 6, 9a, and 9b toward Cu\(^{2+}\) has been studied by UV/Vis, fluorescence, and \(^1\)H NMR titrations.

Results and Discussion

The syntheses of target molecules 3a and 3b and control compounds 6, 9a, and 9b are depicted in Scheme 1. 25,27-Bis(carboxymethoxy)calix[4]arene (1) was prepared according to literature procedures.\(^{[15]}\) Compounds 2a and 2b were synthesized in 75–80% yields by treatment of compound 1 with oxalyl chloride in anhydrous dichloromethane followed by coupling with phenylamines 4a and 4b (see the Supporting Information). Compounds 3a and 3b were then obtained through ring-opening reaction of the corresponding bis-isoxazoles 2a and 2b using [Mo(CO)]\(_6\) as a reagent.\(^{[16d]}\) Previously, the [Mo(CO)]\(_6\)-mediated ring-opening reactions were reported\(^{[16c]}\) to have 70–85% yields in molecular systems without calix[4]arenes, however, in calix[4]arene systems the isolated yields were in the range of 40–65%, despite the fact that the reactions were usually run to completion.\(^{[16b]}\) This result might be due to strong coordination of the \(\beta\)-amino ketone products with the pentacarbenylmolybdenum, thus, hampering its isolation. Gratifyingly, by extracting the reaction mixture successively with aqueous NH\(_4\)OH and ethylenediaminetetraacetic acid (EDTA), followed by column chromatography, we were able to improve the reaction yields to 60–80%. Control compounds 6, 9a, and 9b were also obtained using the same methodology. The control compound 6 was synthesized to test the metal ion binding ability of the calix[4]arene with two pendant \(N\)-arylamidomethyl groups (the upper component of compound 3b). Compounds 9a and 9b were synthesized to verify the requirement for a calix[4]arene scaffold for the recognition of two Cu\(^{2+}\) ions. The structures of all products were confirmed by \(^1\)H and \(^{13}\)C NMR spectroscopy, mass spectrometry, and HRMS analyses (see Exp. Sect.).

Using UV/Vis spectroscopy and fluorescence spectrometry, the binding properties of 3b toward 15 different perchlorate salts of metal ions (Li\(^+\), Na\(^+\), K\(^+\), Mg\(^{2+}\), Ca\(^{2+}\), Ba\(^{2+}\), Cr\(^{3+}\), Mn\(^{2+}\), Ni\(^{2+}\), Cu\(^{2+}\), Zn\(^{2+}\), Hg\(^{2+}\), Ag\(^{+}\), Cd\(^{2+}\), and

Scheme 1. Syntheses of target compounds 3a and 3b, control compounds 6, 9a, and 9b. Reagents and conditions: (a) (i) oxalyl chloride, CH\(_2\)Cl\(_2\), 40 °C, 3 h, (ii) 4-\{3-[4-(4-tert-butyl)phenyl]isoxazol-5-ylmethoxy\}phenylamine (4a), 4-\{3-(naphthalen-1-yl)isoxazol-5-yl\}methoxy\}phenylamine (4b), or 4-propoxyaniline (5), Et\(_3\)N, CH\(_2\)Cl\(_2\), room temp., 24 h; (b) [Mo(CO)]\(_6\), CH\(_3\)CN/H\(_2\)O, reflux, 2 h.
Pb\(^{2+}\)) were screened in CH\(_3\)CN, and the results are shown in Figure 1. In the absence of cations, calix[4]arene 3b exhibited an absorption band with \(\lambda_{\text{max}} = 308\) nm (Figure 1, a) and a very weak emission in the 300–600 nm region when excited at 308 nm (Figure 1, b). Among the 15 metal ions screened, only Cu\(^{2+}\) and Hg\(^{2+}\) led to some hypochromic and bathochromic shifts in the UV/Vis spectra of 3b; furthermore, a small hyperchromic and bathochromic shift was observed in the presence of Pb\(^{2+}\). In addition, only Cu\(^{2+}\) caused a dramatic enhancement of the fluorescence intensities of 3b at 341 and 452 nm by 4- and 40-fold, respectively (Figure 1, b). These observations indicated that calix[4]arene 3b has a high selectivity and sensitivity toward Cu\(^{2+}\).[16]

To gain further insight into the binding of Cu\(^{2+}\) to 3b, UV/Vis, fluorescence, and \(^1\)H NMR titration experiments were carried out. Upon titration of 3b with Cu\(^{2+}\), the absorption maximum at 308 nm gradually decreased in intensity with concurrent formation of a new absorption band near 438 nm (Figure 2, a). Two isosbestic points at 263 and 345 nm were observed, indicating the formation of a well-defined metal complex. The fluorescence intensity of the naphthyl monomer emission (341 nm) was enhanced and a new broad emission band at 452 nm emerged, which may be attributed to the emission of an excimer[17] or an exciplex[18] of the \(\beta\)-amino \(\beta\)-naphthyl groups of 3b. When more than 4 equiv. of Cu\(^{2+}\) was added, the emission band at 452 nm started to decrease (Figure 2, b), which might be

\[ y = a + bx \]
\[ a = 3.59 \]
\[ b = 1.78 \times 10^{-9} \]
\[ R^2 = 0.9993 \]
due to an “inner filter effect”[18] of the absorption band at 438 nm. Alternatively, the increased metal complex concentration may contribute to the static quenching of the emission at 452 nm. Interestingly, a Job plot experiment, using the fluorescence spectra of 3b and Cu2+ with a total concentration of 20 μM, revealed that the complexation between 3b and Cu2+ adopted a 1:2 binding ratio (Figure 2, c). The association constant (K_a) of the 1:2 complex 3b·(Cu2+)2 was calculated to be 2.02 × 10^9 M⁻² by a Banési–Hildebrand plot.[19]

In contrast to the 1:2 binding ratio of 3b with Cu2+, its binding ratios with Hg2+ and Pb2+ were both determined to be 1:1 by Job plot experiments (see Figures S-35 and S-36, see the Supporting Information). Moreover, using Banési–Hildebrand plots, the association constants (K_a) of 3b with Hg2+ and Pb2+ ions in CH3CN were calculated to be 6.66 × 10^7 and 1.47 × 10^4 M⁻¹, respectively (see Table 1).[19]

![Excitation spectrum (normalized) of 3b (20 μM) in the presence of 5 equiv. of [Cu(ClO4)2] in CH3CN monitored at 341 nm for the monomer (solid line) and at 452 nm for the excimer (dashed line).](Image)

As described above, the lower rim cavity around the phenolic-OH and amide groups of 3b may constitute the second possible binding site for Cu2+. The new absorption band of the complex at 438 nm is characteristic of an MLCT band between Cu⁺ and the lower rim phenolic-OH.[12b,13] This means that Cu2⁺ was reduced to Cu⁺ by the phenolic OH groups of calix[4]arene 3b, and that the oxidized phenols help to trap the reduced Cu⁺.[20] The auto-reduction of Cu2⁺ by phenol is well-documented,[21] and the reduction of Cu2⁺ by 3b was verified by EPR experiments (Figure 4). The EPR signals of the paramagnetic Cu2⁺ in CH3CN at 77 K were diminished by adding different amounts of 3b. The results are consistent with our previous observations in other calix[4]arene systems containing pendant β-amino α,β-unsaturated ketones.[14] The addition of 0.5 equiv. of 3b reduced the EPR intensity of Cu2⁺ to about 12%, which implied that both of the Cu2⁺ binding sites of 3b have the potential to reduce Cu2⁺. However, the two binding sites cannot effectively convert Cu2⁺ into Cu⁺ at once, therefore, 12% of EPR signal remained. To clarify whether 3b can bind Cu⁺ directly, we also studied its binding ability toward [Cu(CH3CN)4]PF6 by using UV/Vis spectroscopy; however, no change was observed (see Figure S-38 in the Supporting Information).

![EPR spectra of [Cu(ClO4)2] (5 mM) after adding different amounts (a) 0, (b) 0.2, (c) 0.5, and (d) 1.0 equiv. of 3b at 77 K, recorded at X-band with microwave power of 20 mW and microwave frequency of 9.5 GHz.](Image)

To further confirm the proposed double binding sites for Cu2⁺ in 3b, we carried out 1H NMR titration experiments.
with tert-butylphenyl-substituted calix[4]arene 3a by adding [Cu(ClO₄)₂]. We used 3a (R = tert-butylphenyl) instead of 3b (R = 1-naphthyl) to investigate the effect of added [Cu(ClO₄)₂] on the ¹H NMR chemical shift changes because the former has a simpler proton splitting pattern than the latter. Detailed assignment of the ¹H NMR signals of 3a was based on HMQC, HMBC, and 2D H,H-COSY experiments (Figures S-19 to S-22 in the Supporting Information). The ¹H NMR spectra of ligand 3a in CD₃CN in the presence of different amounts of Cu²⁺ are depicted in Figure 5 (for proton labeling of 3a, see Scheme 1). The methylene protons (Hₑ) near the α,β-unsaturated ketones were downfield shifted from δ = 5.26 ppm to δ = 5.64 ppm (Δδ = +0.38 ppm) in the presence of 2 equiv. of Cu²⁺. Such a downfield shift of Hₑ is expected when metal ions form a complex with the carbonyl groups of the α,β-unsaturated ketones. In addition, the signals of the amino protons (Hₕ and Hₗ) disappeared and this was accompanied by the appearance of a new 1:1:1 triplet signal at δ = 6.82 (J = 53 Hz); the unique triplet pattern was probably due to the splitting of a metal-bound amino group by the nitrogen nucleus (I = 1).[14,22] Furthermore, the signals in the aromatic region (δ = 7.5 to 8.8 ppm) became more complicated and was accompanied by the appearance of some new signals. Signals of the phenolic-OH and amide-Hₑ were first broadened and then disappeared during the addition of 0–3.0 equiv. of Cu²⁺ ions (Figure 5, a–e). Based on these observations, we believe that the lower rim phenol hydroxyl groups and the amide groups might assist each other in the coordination of Cu⁺.

The infrared spectra of 3b and 3b(Cu⁺)₂ provided further structural information on the complex (see Figure S-39 in the Supporting Information). For compound 3b, the 1604 cm⁻¹ band is characteristic of the conjugated C=O group of an enaminone, in which intramolecular hydrogen bonding substantially lowers the C=O stretching frequency.[12b,23] Compound 3b also shows a typical C=O band of an amide group at 1684 cm⁻¹. The infrared spectrum of the complex 3b(Cu⁺)₂ showed a significant shift of the amide C=O frequency from 1684 to 1664 cm⁻¹, which might be due to the binding of Cu⁺ with the nitrogen of the amide group.[24] A literature survey also supported the idea that Cu²⁺/Cu⁺ ions preferentially coordinate with the nitrogen atoms of amide groups.[19b,25] Furthermore, the IR signals of enaminones (C=O at 1604 cm⁻¹ and C=C at 1531 cm⁻¹) were found to be broadened and slightly shifted to higher frequency when 3b complexed with Cu⁺. The formation of complex 3b(Cu⁺)₂ was also confirmed by the FAB mass spectrum (see Figure S-40 in the Supporting Information), which showed a peak at m/z 633.

Based on the information obtained from UV/Vis, fluorescence, IR, ¹H NMR, and EPR spectroscopic studies upon adding Cu²⁺ to 3b, we propose a possible binding mode for the 1:2 complex of 3b with two Cu⁺ as shown in Scheme 2.

Figure 5. ¹H NMR spectra of 3a (5 mM) in CD₃CN in the presence of different amounts of Cu[(ClO₄)₂]: (a) 0, (b) 0.2, (c) 0.4, (d) 0.6, (e) 0.8, (f) 1.0, (g) 1.5, (h) 2.0 and (i) 3.0 equiv. Where * denotes external standard CHCl₃. The intensity of spectra f–i was doubled for observing the weak new signals. For proton labeling of 3a, see Scheme 1.
In the lower part of the complex 3b·(Cu+)2, two β-amino \(\alpha,\beta\)-unsaturated ketone moieties collaborate to coordinate to Cu\(^+\) with a tetrahedral geometry.\(^{[26]}\) Concurrently, the phenolic hydroxyl group on the lower rim of calix[4]arene 3b also chelates with a Cu\(^+\) ion, and the amide groups nearby should assist in this coordination.

Control compounds 6, 9a, and 9b were also synthesized to provide further evidence for the proposed binding modes. The recognition of Cu\(^{2+}\) by calix[4]arene 6, which possessed only the pendant N-arylaminomethyl groups (the upper component of compound 3b) without the β-amino \(\alpha,\beta\)-unsaturated ketones, was investigated by UV/Vis spectroscopy, \(^1\)H NMR, ESI-MS, and EPR analyses. The UV/Vis spectrum of calix[4]arene 6 with Cu\(^{2+}\) in CH\(_3\)CN was similar to that observed in 3b, where a new broad absorption band at 438 nm appeared (see Figure S-41 in the Supporting Information). The association constants (\(K_a\)) of 6 with Cu\(^{2+}\) was calculated to be \(6.82 \times 10^3\) M\(^{-1}\) from a Benesi–Hildebrand plot.\(^{[19a]}\) The EPR signal of Cu\(^{2+}\) disappeared after adding 1 equiv. of calix[4]arene 6 (Figure S-42), which supported the proposal that the autoreduction of Cu\(^{2+}\) to Cu\(^+\) occurred in the presence of 6. In addition, \(^1\)H NMR signals of the hydroxyl protons of 6 disappeared after adding 1 equiv. of Cu\(^{2+}\), implying the participation of phenol hydroxyl groups in the complexation (see Figure S-43 in the Supporting Information). It is worth noting that all the proton signals, except the hydroxyl protons, were little shifted, which implied that the binding interaction of 6 with Cu\(^+\) was weak. Furthermore, the ESI mass spectrum (see Figure S-44 in the Supporting Information) showed two peaks at \(m/z\) 869.4 and 871.4, which corresponded to the calculated mass of 6\(^{4+}\)Cu\(^{+}\) and 6\(^{6+}\)Cu\(^{+}\), respectively. These results support the proposal that the lower rim of 6 alone can recognize Cu\(^{2+}\) and create a UV/Vis band at 438 nm, which is similar to the spectra observed in the complexation of 3b with Cu\(^{2+}\) (see above).

In a similar way, the binding ability of control compound 9b toward Cu\(^{2+}\) was also investigated. Compound 9b, which resembles the structure of 3b but without a calix[4]arene scaffold, also showed selective binding toward Cu\(^{2+}\) (see Figure S-45 in the Supporting Information) and its absorption spectra showed a hypochromic effect and a small bathochromic shift with isosbestic points at 243 and 349 nm (Figure 6, a). It should be noted that there is a major difference between the absorption spectra of 9b and 3b when these compounds were titrated with Cu\(^{2+}\); the former did not show an MLCT band around 438 nm (cf. Figure 2, a). Despite this difference in absorption spectra, the emission phenomena of both compounds were quite similar: both the monomer emission (\(\lambda_{em} = 341\) nm) and the longer emission bands (\(\lambda_{em} = 446\) nm) of 9b were gradually enhanced upon adding Cu\(^{2+}\) (Figure 6, b). The new, broad emission band at 446 nm is unlikely to arise from the emission from a naphthyl excimer because the possibility of forming an intermolecular complex at such a low concentration (20 µM) is slim.\(^{[28]}\) Thus, the new emission band may be attributed to exciplex formation between Cu\(^+\) and the β-amino \(\beta\)-naphthyl \(\alpha,\beta\)-unsaturated ketone of 9b. Exciplex formation between Ag\(^+\) and several aromatic moieties has been reported;\(^{[8a,27]}\) however, to the best of our knowledge, there has been no report of such an exciplex formation between a β-amino \(\beta\)-naphthyl \(\alpha,\beta\)-unsaturated ketone and Cu\(^{2+}\). To fully understand the mechanism of exciplex formation, additional photophysical measurements, such as time-resolved fluorescence or laser flash photolysis experiments, would be needed in the future. We also found that Cu\(^{2+}\) was reduced by 9b, as evidenced by the silent EPR signal of Cu\(^{2+}\) by added 9b (see Figure S-42c in the Supporting Information). In our previous work,\(^{[14]}\) we found that the β-amino \(\alpha,\beta\)-unsaturated ketone could participate in the reduction of Cu\(^{2+}\) in a lower-rim propyl ether protected calixarene. Here,
the autoreduction of Cu\textsuperscript{2+} to Cu\textsuperscript{+} by a β-amino α,β-unsaturated ketone was again proven to be efficient in the control compound 9b, therefore, the need for a calixarene scaffold can be excluded for the autoreduction of Cu\textsuperscript{2+} to Cu\textsuperscript{+}. From the titration experiment, the association constant\textsuperscript{[19a]} of 9b with Cu\textsuperscript{2+} was calculated to be 2.52 × 10\textsuperscript{4} M\textsuperscript{-1}, and the binding ratio determined by the Job plot was 1:1 (see Figure S-47 in the Supporting Information). The binding of Cu\textsuperscript{+} with 9a was also supported by \textsuperscript{1}H NMR titration experiments (see Figure S-48 in the Supporting Information). By comparing the binding constants of 3b, 6, and 9b with Cu\textsuperscript{2+} (see Table 1), we found that 3b exhibited positive allosteric\textsuperscript{[29]} behavior toward the coordination of two Cu\textsuperscript{2+} ions. In other words, when one of the two binding sites of 3b chelates the first Cu\textsuperscript{2+}, the conformation of the second binding site changes and helps the complexation of the second Cu\textsuperscript{2+} ion.

Conclusions

We have reported the syntheses of a novel fluorescence turn-on chemosensor 3b, which showed high selectivity and sensitivity toward Cu\textsuperscript{2+} among 15 different metal ions screened. Calix\textsuperscript{4}arene 3b was found to coordinate with two equivalents of [CuCl\textsubscript{2}]\textsuperscript{2+} through two binding sites: (1) the lower-rim phenolic-OH and amide groups, and (2) the pendant β-amino α,β-unsaturated ketones. It should be noted that the autoreduction of Cu\textsuperscript{2+} to Cu\textsuperscript{+} was observed during complexation of 3b. The association constant (K\textsubscript{a}) of the 1:2 complex Cu\textsuperscript{2+} 3b was calculated to be 2.02 × 10\textsuperscript{4} M\textsuperscript{-2} by the Benesi–Hildebrandt plot. Finally, an exciplex emission from the complexation of 9b with Cu\textsuperscript{2+} is unprecedented, and the system deserves further study with time-resolved fluorescence lifetime measurements to understand the dynamics of such novel exciplex formation.

Experimental Section

General: \textsuperscript{1}H NMR spectra were measured with either a 300 or 500 MHz spectrometer. Natural abundance \textsuperscript{13}C NMR spectra were measured using pulse Fourier transform techniques, with a 300 or 500 MHz NMR spectrometer operating at 75.4 and 125.7 MHz, respectively. Broad-band decoupling, DEPT, H,H-COSY, H,C-COSY, HMQC, and HMBC were carried out to simplify spectra and aid peak identification. All reported yields are quoted as an average of three runs and are based on recovered starting materials.

General Procedure for the Synthesis of 2a and 2b: To a well-stirred solution of I (100 mg, 0.19 mmol) and 2–3 drops of N,N-dimethylformamide (DMF) in anhydrous CH\textsubscript{2}Cl\textsubscript{2} (10 mL), was added oxalyl chloride (0.18 mL, 2.05 mmol) under nitrogen at room temperature. The reaction mixture was heated at reflux temperature for 3 h. Solvent and excess oxalyl chloride were removed under reduced pressure and the residue was further dried in vacuo. The residue was dissolved in anhydrous CH\textsubscript{2}Cl\textsubscript{2} (10 mL) followed by addition of a solution of amines 4a–b (4a: 123 mg, 0.38 mmol; 4b: 120 mg, 0.38 mmol) and triethylamine (8–10 drops) in anhydrous CH\textsubscript{2}Cl\textsubscript{2} (10 mL). The reaction mixture was heated to 40 °C for 24 h. After evaporation of solvent, the crude product was purified by column chromatography to afford 2a–b.

25,27-Bis(N-[1-[4-[[3-[4-(tert-butylyphenyl]-5-isoxazolyl]methoxy]phenyl]aminocarbonyl]methoxy]-26,28-dihydroxy-calix[4]arene (2a): Yield 80% (175 mg); white solid; m.p. >270 °C (decomp.); R\textsubscript{f} = 0.35 (n-hexane/ethyl acetate, 1:1). \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}): δ = 1.32 (s, 18 H, tBu), 3.58 (d, J = 13.5 Hz, 4 H, ArCH\textsubscript{2}Ar), 4.22 (d, J = 13.5 Hz, 4 H, ArCH\textsubscript{2}Ar), 4.62 (s, 4 H, H\textsubscript{a}), 5.16 (s, 4 H, H\textsubscript{b}), 6.70 (s, 2 H, H\textsubscript{c}), 6.76–6.82 (m, 6 H, Ar-H), 6.90 (t, J = 7.5 Hz, 2 H, Ar-H), 7.03 (d, J = 7.5 Hz, 4 H, Ar-H), 7.15 (d, J = 7.4 Hz, 4 H, Ar-H), 7.34 (d, J = 9.0 Hz, 4 H, Ar-H), 7.45 (d, J = 8.4 Hz, 4 H, tBu-Ph), 7.78 (d, J = 8.4 Hz, 4 H, tBu-Ph), 8.36 (s, 2 H, OH), 10.19 (s, 2 H, H\textsubscript{o}), ppm. \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}): δ = 31.5 (CH\textsubscript{a}), 31.8 (CH\textsubscript{b}), 34.8 (CH\textsubscript{c}), 61.9 (CH\textsubscript{d}), 74.8 (CH\textsubscript{e}), 101.4 (CH), 115.1 (CH), 120.4 (CH), 121.1 (CH), 125.8 (CH), 125.9 (Cq), 126.6 (CH), 127.1 (CH), 127.5 (Cq), 129.2 (CH), 129.9 (CH), 131.6 (Cq), 132.6 (Cq), 150.3 (Cq), 151.7 (Cq), 153.3 (Cq), 154.3 (Cq), 162.4 (Cq), 164.7 (Cq), 168.4 (Cq) ppm. MS (FAB): m/z = 1149 [M + H\textsuperscript{+}]. HRMS: calcd. for C\textsubscript{72}H\textsubscript{69}N\textsubscript{4}O\textsubscript{10} 1149.5013; found 1149.5032.

25,27-Bis(N-[1-[[4-[[3-(1-naphthyl)-5-isoxazolyl]methoxy]phenyl]aminocarbonyl]methoxy]-26,28-dihydroxy-calix[4]arene (2b): Yield 75% (162 mg); yellowish-brown solid; m.p. 150–151 °C; R\textsubscript{f} = 0.44 (n-hexane/ethyl acetate, 1:1). \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}): δ = 3.59 (d, J = 13.5 Hz, 4 H, ArCH\textsubscript{2}Ar), 4.23 (d, J = 13.5 Hz, 4 H, ArCH\textsubscript{2}Ar), 4.64 (s, 4 H, H\textsubscript{a}), 5.22 (s, 4 H, H\textsubscript{b}), 6.71 (s, 2 H, H\textsubscript{c}), 6.77–6.94 (m, 4 H, Ar-H), 7.05 (d, J = 7.5 Hz, 4 H, Ar-H), 7.16 (d, J = 7.5 Hz, 4 H, Ar-H), 7.38 (d, J = 9.1 Hz, 4 H, Ar-H), 7.49–7.57 (m, 2 H, naphthyl-H), 7.74 (dd, J = 7.2, 1.2 Hz, 2 H, naphthyl-H), 7.87–7.95 (m, 4 H, naphthyl-H), 8.35 (s, 2 H, OH), 8.41–8.45 (m, 2 H, naphthyl-H), 10.20 (s, 2 H, H\textsubscript{o}), ppm. \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}): δ = 31.8 (CH\textsubscript{a}), 61.9 (CH\textsubscript{b}), 74.8 (CH\textsubscript{c}), 104.8 (CH), 115.2 (CH), 120.5 (CH), 121.1 (CH), 125.2 (CH), 125.7 (CH), 126.3 (CH), 126.5 (Cq), 127.1 (CH), 127.1 (CH), 127.5 (Cq), 128.0 (CH), 128.4 (CH), 129.2 (CH), 129.9 (CH), 130.3 (CH), 130.9 (Cq), 131.7 (Cq), 132.6 (Cq), 133.8 (Cq), 150.3 (Cq), 151.7 (Cq), 154.5 (Cq), 162.7 (Cq), 164.7 (Cq), 167.9 (Cq) ppm. MS (FAB): m/z = 1137 [M + H\textsuperscript{+}]. HRMS: calcd. for C\textsubscript{72}H\textsubscript{69}N\textsubscript{4}O\textsubscript{10} 1136.3999; found 1136.3983.
A Fluorescence Chemosensor for Copper(II) Ions

Then, after evaporation of solvent, the crude product was purified by column chromatography to afford 8a-b. N-[4-S-[3-(tert-Butylphenyl)isoxazol-5-ylmethoxy]phenyl]-2,6-dimethylphenoxacylacetamide (8a): Yield 82% (218 mg); grayish white solid; mp: 142–144 °C; Rf = 0.13 (n-hexane/ethyl acetate, 2:1). 1H NMR (300 MHz, CDCl3); δ = 1.34 (s, 9 H, TBu); 2.28 (s, 6 H, Ar-CH2-), 4.38 (s, 2 H, H2), 5.16 (s, 2 H, H2), 6.62 (s, 1 H, Hb), 6.97–7.05 (m, 5 H, Ar-H), 7.46 (d, J = 8.3 Hz, 2 H, Ar-H), 7.60 (d, J = 8.9 Hz, 2 H, Ar-H), 7.73 (d, J = 8.3 Hz, 2 H, Ar-H), 8.64 (s, 1 H, Hb) ppm. 13C NMR (75 MHz, CDCl3); δ = 16.2 (CH2), 31.1 (CH), 34.7 (Cq), 61.6 (CH2), 70.3 (Cq), 101.3 (Cq), 115.2 (Cq), 121.6 (Cq), 124.8 (Cq), 125.8 (Cq), 126.5 (Cq), 129.1 (Cq), 130.2 (Cq), 131.2 (Cq), 153.3 (Cq), 154.0 (Cq), 154.7 (Cq), 162.3 (Cq), 166.4 (Cq), 168.0 (Cq) ppm. MS (FAB); m/z = 485 [M + H]+. HRMS: calculated for C30H26N2O4 484.2362; found 484.2367.

2-(2,6-Dimethylphenox)-N-[4-[(naphthalen-1-yl)-isoxazol-5-ylmethoxy]phenyl]acetamide (8b): Yield 77% (203 mg); pale-yellow solid; mp: 129–130 °C; Rf = 0.65 (n-hexane/ethyl acetate, 1:1). 1H NMR (300 MHz, CDCl3); δ = 2.26 (s, 6 H, Ar-CH2-), 4.36 (s, 2 H, H2), 5.17 (s, 2 H, H2), 6.63 (s, 1 H, Hb), 6.97–7.03 (m, 5 H, Ar-H), 7.45–7.53 (m, 3 H, naphthyl-H), 7.59 (d, J = 8.9 Hz, 2 H, Ar-H), 7.66 (d, J = 6.6 Hz, 1 H, naphthyl-H), 7.85–7.91 (m, 2 H, naphthyl-H), 8.34–8.39 (m, 1 H, naphthyl-H), 8.64 (s, 1 H, Hb) ppm. 13C NMR (75 MHz, CDCl3); δ = 16.2 (CH2), 61.5 (CH2), 70.3 (Cq), 104.7 (Cq), 115.2 (Cq), 121.5 (Cq), 124.8 (Cq), 125.0 (Cq), 125.4 (Cq), 126.1 (Cq), 126.3 (Cq), 127.0 (Cq), 127.7 (Cq), 128.3 (Cq), 129.1 (Cq), 130.2 (Cq), 131.0 (Cq), 131.2 (Cq), 133.6 (Cq), 154.0 (Cq), 154.6 (Cq), 165.2 (Cq), 166.4 (Cq), 167.5 (Cq) ppm. MS (EI); m/z = 478 [M]+. HRMS: calculated for C30H26N2O4 478.1884; found 478.1884.

General Procedure for the Synthesis of 9a and 9b: To a well-stirred solution of compound 8a-b (8a: 100 mg, 0.21 mmol; 8b: 200 mg, 0.42 mmol) and [Mo(CO)6] (1.3 equiv.) in CH3CN (15 mL), was added 2–3 drops of water, then the reaction mixture was heated to reflux temperature for 2 h. The solvent was removed and the residue was dissolved in CH2Cl2 (10 mL). The solution was treated with aq NH4OH (10 mL) and the organic layer was washed with water (100 mL) and aq. 1 M EDTA (50 mL), dried with MgSO4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography to afford pure 9a-b in 65–80% yields.

N-[4-[4-Amino-4-(tert-butylphenyl)-2-oxobut-3-enyloxy]phenyl]-2,6-dimethylphenoxacylacetamide (9a): Yield 65% (65.0 mg, 0.13 mmol); pale-yellow solid; mp: 70–72 °C; Rf = 0.43 (n-hexane/ethyl acetate, 1:1). 1H NMR (300 MHz, CDCl3); δ = 1.33 (s, 9 H, TBu); 2.29 (s, 6 H, Ar-CH2-), 4.38 (s, 2 H, H2), 4.57 (s, 2 H, H2), 5.03 (s, 1 H, Hb), 5.81 (s, 1 H, Hb), 6.93–7.06 (m, 5 H, Ar-H), 7.43–7.57 (m, 6 H, Ar-H), 8.58 (s, 1 H, Hb) ppm. 13C NMR (75 MHz, CDCl3); δ = 16.3 (CH2), 31.1 (CH), 34.8 (Cq), 70.3 (Cq), 102.5 (Cq), 115.1 (Cq), 121.6 (Cq), 124.9 (Cq), 125.9 (Cq), 126.0 (Cq), 129.2 (Cq), 130.3 (Cq), 130.5 (Cq), 153.3 (Cq), 154.0 (Cq), 154.7 (Cq), 162.3 (Cq), 166.4 (Cq), 168.0 (Cq) ppm. MS (FAB); m/z = 487 [M + H]+. HRMS: calculated for C30H22N2O4 486.2529; found 486.2520.

N-[4-[4-Amino-4-(naphthalen-1-yl)-2-oxobut-3-enyloxy]phenyl]-2,6-dimethylphenoxacylacetamide (9b): Yield 80% (160 mg, 0.33 mmol); pale-yellow solid; mp: 79–81 °C; Rf = 0.2 (n-hexane/ethyl acetate, 2:1). 1H NMR (300 MHz, CDCl3); δ = 2.31 (s, 6 H, Ar-CH2-), 4.35 (s, 2 H, H2), 4.60 (s, 2 H, H2), 5.74 (s, 1 H, Hb), 5.84 (br, s, 1 H, Hb), 6.91 (d, J = 8.8 Hz, 2 H, Ar-H), 6.99–7.09 (m, 2 H, Ar-H), 7.46–7.57 (m, 6 H, Ar-H and naphthyl-H), 7.66–7.92 (m, 2 H, naphthyl-H), 8.09–8.12 (m, 1 H, naphthyl-H), 8.61 (s, 1 H, Ar-CH2-), 4.38 (s, 2 H, H2), 5.16 (s, 2 H, H2), 6.62 (s, 1 H, Hb), 6.97–7.05 (m, 5 H, Ar-H), 7.46 (d, J = 8.3 Hz, 2 H, Ar-H), 7.60 (d, J = 8.9 Hz, 2 H, Ar-H), 7.73 (d, J = 8.3 Hz, 2 H, Ar-H), 8.64 (s, 1 H, Hb) ppm. 13C NMR (75 MHz, CDCl3); δ = 16.2 (CH2), 31.1 (CH), 34.7 (Cq), 61.6 (CH2), 70.3 (Cq), 101.3 (Cq), 115.2 (Cq), 121.6 (Cq), 124.8 (Cq), 125.8 (Cq), 126.5 (Cq), 129.1 (Cq), 130.2 (Cq), 131.2 (Cq), 153.3 (Cq), 154.0 (Cq), 154.7 (Cq), 162.3 (Cq), 166.4 (Cq), 168.0 (Cq) ppm. MS (FAB); m/z = 485 [M + H]+. HRMS: calculated for C30H26N2O4 484.2362; found 484.2367.
H2), 10.25 (br. s, 1 H, H2) ppm. 13C NMR (75 MHz, CDCl3): δ = 16.2 (CH3), 70.3 (CH3), 71.8 (CH2), 93.8 (CH), 115.0 (CH), 121.5 (CH), 124.8 (CH), 124.9 (CH), 124.9 (CH), 125.6 (CH), 126.4 (CH), 126.9 (CH), 128.4 (CH), 129.1 (CH3), 130.1 (CH), 130.3 (Cq), 130.5 (Cq), 133.5 (Cq), 135.0 (Cq), 154.0 (Cq), 155.3 (Cq), 163.5 (Cq), 166.4 (Cq). 191 (Cq) ppm. MS (FAB): m/z = 481 [M + H+]. HRMS: calcd. for C30H28N2O4 480.2049; found 480.2049.

General Procedures for the UV/Vis and Fluorescence Spectroscopic Experiments: UV/Vis spectra were recorded with an HP-5435 spectrophotometer equipped with a diode array detector; the resolution was set at 1 nm. Fluorescence spectra were recorded with an Amic-noc Bowman Series 2 type spectrofluorimeter. For all measurements of fluorescence spectra, excitation was at 308 nm with the excitation and emission slit width at 4.0 nm. UV/Vis and fluorescence titration experiments were performed with 20 μM solutions of 3b and 9b in CH3CN and varying concentrations of metal perchlorate in CH3CN. During the measurements, the temperature of the quartz sample cell and chamber was kept at 25 °C.

General Procedures for the 1H NMR Titration Experiments: 1H NMR titration spectra were recorded at 300 MHz with tetramethylsilane (TMS) in a coaxial capillary tube as an external standard. Experiments were performed with 5 mM solutions of 3b, 6, and 9b in CH3CN and varying concentrations of [Cu(ClO4)2] in CH3CN at 25 °C.

Supporting Information (see footnote on the first page of this article): Additional experimental procedures, 1D and 2D NMR spectroscopic information, and X-ray crystallographic information.

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We thank one of the reviewers for providing an alternative explanation for the longer emission band of 9b with Cu^{2+}. For exciplex formation between a metal ion and the aromatic moiety, see ref. [8] and a) H. Dreeskamp, A. Laufer, M. Zander, Chem. Phys. Lett. 1985, 121, 523–528.

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