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Highly selective fluorescent sensing of Cu^{2+} ion by an arylisoxazole modified calix[4]arene

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ABSTRACT

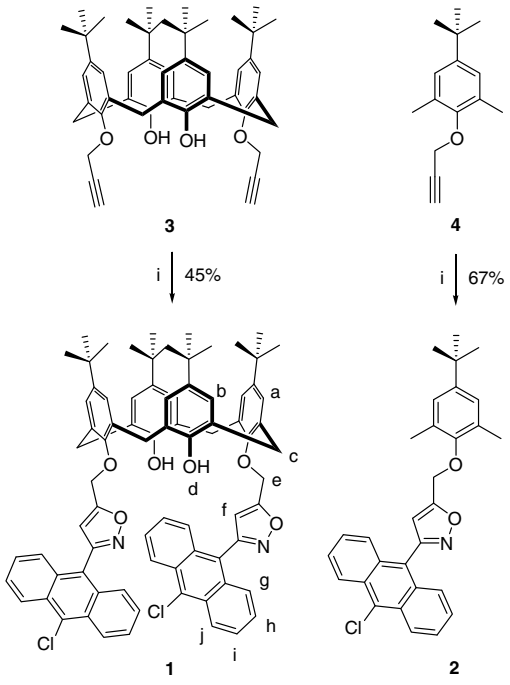
A novel fluorescent chemosensor **1** with two anthraceneisoxazolymethyl groups at the lower rim of calix[4]arene has been synthesized, which revealed a dual emission (monomer and excimer) when excited at 375 nm. This chemosensor displayed a selective fluorescence quenching only with Cu^{2+} ion over all other metal ions examined. When Cu^{2+} ion was bound to **1**, the fluorescence intensities of both monomer and excimer were quenched. Furthermore, the association constant for the 1:1 complex of **1**·Cu^{2+} was determined to be $(1.58 \pm 0.03) \times 10^4\ M^{-1}$.

The development of specific fluorescent chemosensors for the efficient detection of metal ion analytes is one of the most important areas in supramolecular chemistry due to their fundamental role in biological, environmental, and chemical processes.[1] In particular, chemosensors for the detection and measurements of Cu^{2+} ions are actively investigated as this metal ion is a significant environmental pollutant and an essential trace element in human body.[2,3]

Calix[4]arenes have been shown to be useful molecular scaffold in the development of fluorescent chemosensors especially for metal ion recognition.[4] Most calix[4]arene-based fluorescent sensors have been designed based on photophysical changes upon metal ion binding and their mechanisms include photoinduced electron transfer (PET),[5] photoinduced charge transfer (PCT),[6] formation of monomer/excimer,[7] and energy transfer.[8]

In continuation of our interests in the design and synthesis of chromogenic[9] and fluorogenic[10] chemosensors, we report here the synthesis of a novel fluorogenic calix[4]arene using the 1,3-dipolar cycloaddition of an alkyne and a nitrile oxide to form an isoxazole cationic binding site.

The synthetic routes of host **1** and control compound **2** are depicted in Scheme 1. The 25,27-bisfluoroionphores **1**[11] was synthesized in 45% yield starting from 25, 27-bis(*O*-propargyl)-*p*-*tert*-butylcalix[4]arene **3**[12] using a 1,3-dipolar cycloaddition reaction methodology recently reported by us in capping the lower and upper rims of calix[4]arenas.[13] In this work, 10-chloroanthracene (**CA**) instead of anthracene was chosen as the fluorophore because the preparation of parent anthracene hydroximoyl chloride requires an extremely careful control of the chlorination process, otherwise mixture of products is usually obtained. Therefore, excess chlorination reagents were used which produced pure **CA** related products in high yield. Similar procedure was employed in the synthesis of control compound **2**[14] from precursor **4**.[15] Fluorogenic calix[4]-arene **1** was found to be in cone conformation based on the information obtained from ^1H and ^{13}C NMR spectra data.



Scheme 1. Synthesis of fluorogenic calix[4]arene **1**. Reagents and conditions: (i) 10-chloroanthracene-9-hydroximoyl chloride, Et_3N, toluene, reflux, 24 h.

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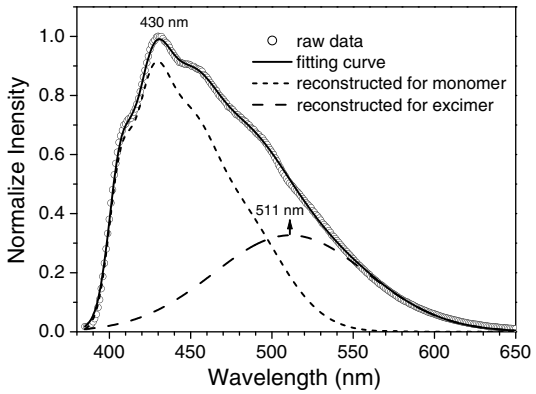

Figure 1. Normalize fluorescence spectra of **1** (solid line). The solid line is fitted by multi-Gaussian functions and deconvoluted into two components (short-dash curve: monomer band; dash curve: excimer band). Excitation wavelength was at 375 nm.

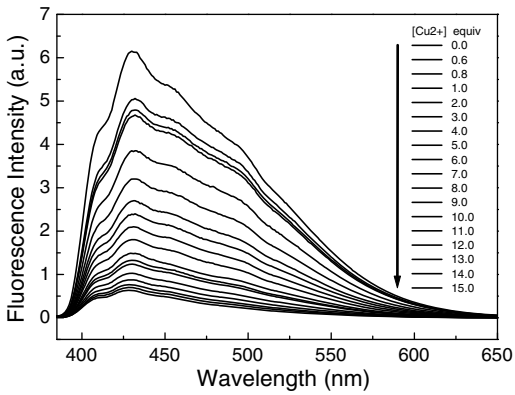

Figure 3. Fluorescence emission spectra of **1** (20 μM) upon addition of various equivalents of $Cu(ClO_4)_2$ in $CH_3CN/CHCl_3$ (v/v = 1000:4).

The UV/vis spectra of the anthracene group(s) **1** and **2** showed four absorption bands at 338, 357, 375, and 396 nm in $CH_3CN/CHCl_3$ (v/v = 1000:4) (see Fig. S7). The fluorescence spectrum of **1** (20 μM) in $CH_3CN/CHCl_3$ (v/v = 1000:4) exhibits a dual emission (i.e., monomer and excimer) when excited at 375 nm (Fig. 1). The emission spectrum of **1** is composed of a structured feature, culminating at 430 nm and assigned to the locally excited **CA** referred to as monomer (similar in shape to the emission spectrum of monofluorophore **2**) and a red-shifted, broad and structureless band with λ_{max} at 511 nm, ascribable to the emission from an intramolecular excimer $(\mathbf{CA}\cdot\mathbf{CA})^*$.[16] The excimer formation comes from the interaction of one **CA** unit in the singlet excited state with the other **CA** in the ground state.

Excess perchlorate salts (10 equiv) of Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Cr^{3+}, Pb^{2+}, Cu^{2+}, Hg^{2+}, Cd^{2+}, Ag^+, Ni^{2+}, Mn^{2+}, and Zn^{2+} (total 15 metal ions) were tested to evaluate the metal ion binding properties of **1** and **2**. The results are shown in Figures 2 and S8. Ligand concentration in all titration experiments was fixed at 20 μM in $CH_3CN/CHCl_3$ (v/v = 1000:4). The fluorogenic chemosensor **1**, having two isoxazoles as the metal ligating groups, is found to exhibit remarkable selectivity toward Cu^{2+} ion over all other metal ions. The fluorescence of **1** (20 μM) was strongly quenched by Cu^{2+} ion; however, no such quenching effect was found for the control compound **2** by any of the fifteen metal perchlorates used above. Moreover, the UV/vis spectra of **1** did not show any new absorption band after adding 10 equiv of Cu^{2+} ion (see Fig. S9). We have also carried out fluorescence quenching experiments on **1** using more polar solvents such as DMSO and $MeOH/CHCl_3$ (v/v = 1000:4) instead of $CH_3CN/CHCl_3$ (v/v = 1000:4) (see Figs. S10 and S11) and found that $CH_3CN/CHCl_3$ is by far the most efficient solvent system in sensing Cu^{2+} ion.

The two isoxazole moieties of **1** are proven to form an efficient metal ion binding site, whereas compound **2** is lack of such an efficient metal ion binding site. Furthermore, the geometry of the binding site of the host **1**, comprising the two oxygen atoms of isoxazole units and two hydroxyls of the phenol units, seems to be ideal in terms of size and arrangement for recognition of Cu^{2+} ion. The fluorescence quenching of **1** may be explained by either a reverse PET[17] or a heavy atom effect.[18] In the former case, when the Cu^{2+} ion is bound by two isoxazole oxygen atoms and two phenol moieties, the **CA** units probably behave as PET donors whereas the metal ion bound isoxazole groups behave as electron acceptors.

The fluorescence spectra of **1** (20 μM) at various concentrations of $Cu(ClO_4)_2$ are depicted in Figure 3, as can be seen, no shift in the fluorescence emission maximum was observed. However, the fluorescence intensities of both the monomer and excimer emission of **1** gradually decreased as the $Cu(ClO_4)_2$ concentration increased from 12 μM to 300 μM. Based on the fluorescence intensity of **1** as a function of $[Cu^{2+}]$, the association constant for complex $\mathbf{1}\cdot Cu^{2+}$ in $CH_3CN/CHCl_3$ (v/v = 1000:4) was calculated to be $(1.58 \pm 0.03) \times 10^4\ M^{-1}$ by Stern–Volmer plot.[19] (Fig. S12) In the Job plot,[20] the maximum fluorescence change was observed when the molar fraction of ionophore **1** versus Cu^{2+} was 0.5, indicative of a 1:1 complex (Fig. 4).

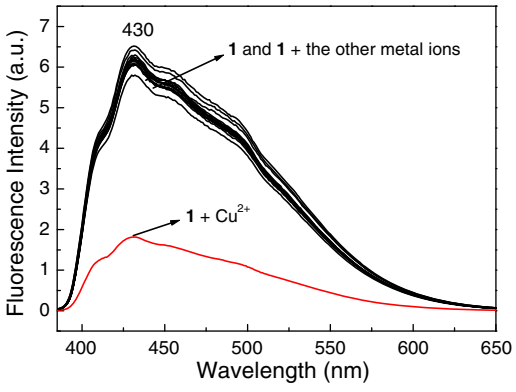

Figure 2. Changes in fluorescence emission spectra of **1** (20 μM) before and after adding 200 μM concentration of various metal perchlorates in $CH_3CN/CHCl_3$ (v/v = 1000:4).

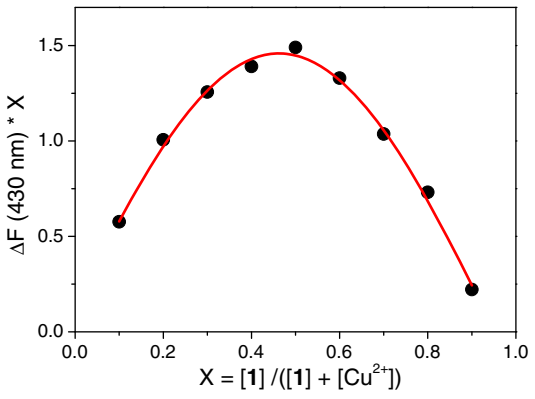

Figure 4. Job plot of a 1:1 complex of **1** and Cu^{2+} ion, where the emission at 430 nm was plotted against the mole fraction of **1** at an invariant total concentration of 20 μM in $MeCN/CHCl_3$ (1000:4, v/v).

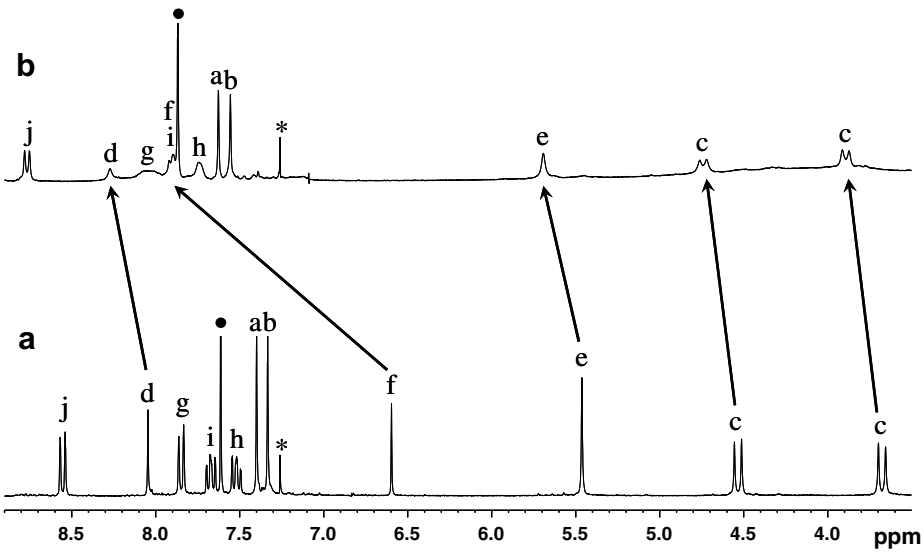

Figure 5. ^{1}H NMR spectra of **1** (5.0 mM) in $CDCl_3/CD_3CN$ (3:1) (a) and in the presence of 25 mM (5.0 equiv) of $Cu(ClO_4)_2$ (b), where $*$ denotes an external standard $CHCl_3$ and the descriptors are shown in Scheme 1.

Metal ion-induced chemical shift changes in the ^{1}H NMR spectra support that Cu^{2+} is bound to the two oxygen atoms of the isoxazole units and the two hydroxyl phenol groups of **1** (see Fig. 5). In the presence of 5.0 equiv of Cu^{2+}, the peaks of H_a–H_c were downfield shifted by 0.23, 0.22, 0.21, and 0.22 ppm, respectively, and the peak of H_d in the hydroxyls of the phenol units was also downfield shifted by 0.22 nm with a reduced intensity. Furthermore, the peak of H_e on the OCH_2-isoxazole unit was downfield shifted by 0.24 ppm; however, the peak of H_f on the isoxazole group was significantly downfield shifted by 1.30 ppm, suggesting that the two isoxazole groups were involved in the complexation of Cu^{2+}. In contrast, the chemical shifts of protons H_g–H_j on the anthracene groups were modestly downfield shifted and the peak shapes were slightly broadened. However, upon adding 5.0 equiv of Cu^{2+} to the solution of **2**, the peak of H_f on the isoxazole group was downfield shifted by only 0.26 ppm (Fig. S13). Its downfield shift is much smaller than that for **1** (Fig. 5), suggesting that the complexation of Cu^{2+} with the mono-isoxazole group of **2** is weaker than its complexation with the two isoxazole groups of **1**. In principle, the ^{1}H NMR spectrum of **1** should become broadened in the presence of Cu^{2+}; however, it appeared to be quite sharp, implying that the Cu^{2+} might have been reduced to Cu^+. The auto-reduction of Cu^{2+} by a phenol is well documented,[21] which is probably the source for the redox observed in the addition of Cu^{2+} to **1**. We have also carried out experiments using $[(CH_3CN)_4Cu]PF_6$ to affect the fluorescence of **1**; however, no change in fluorescence emission spectra of **1** was observed in the presence of excess Cu^+ ion (Fig. S14). The above results suggest that Cu^{2+} might be reduced by the phenols of the host **1** and the oxidized phenols then help to trap the reduced Cu^+ ions (Fig. S15).[22] Thus, the compound **1** is a highly selective fluorogenic chemodosimeter for Cu^{2+} ion.

In conclusion, we have synthesized a new bisisoxazolyl-methylcalix[4]arene **1** bearing two anthracene groups, which displayed a dual fluorescent emission (monomer and excimer) and showed an extreme selectivity toward Cu^{2+} ion over all other metal ions examined.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.06.060.

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11. *Compound* **1**: A mixture of **3** (0.200 g, 0.276 mmol) and 10-chloroanthracene-9-hydroximoyl chloride (10.0 equiv) in toluene (50 mL) was stirred. Then, Et_3N (20.0 equiv) was added dropwise and the mixture was refluxed for 24 h. The solvent was removed under vacuum, and the residue was dissolved in chloroform (30 mL), washed with water, and dried over $MgSO_4$. The residue

obtained after evaporation of the solvent was subjected to a silica gel column chromatography using a gradient polarity (eluent: ethyl acetate/*n*-hexane, 1:14 to 1:6) to afford 0.153 g (45%) of **1** as a white solid; mp 172–174 °C; R_f = 0.93 (ethyl acetate/*n*-hexane = 1:3); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.49 (d, J = 8.7 Hz, 4H), 7.83 (d, J = 8.7 Hz, 4H), 7.60–7.49 (m, 4H), 7.47–7.36 (m, 4H), 7.22 (s, 4H), 6.94 (s, 4H), 6.91 (s, 2H), 6.55 (s, 2H), 5.34 (s, 4H), 4.41 (d, J = 13.2 Hz., 4H), 3.49 (d, J = 13.2 Hz, 4H), 1.43 (s, 18H), 1.07 (s, 18H); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 168.0 (Cq), 160.5 (Cq), 150.3 (Cq), 149.5 (Cq), 147.7 (Cq), 141.9 (Cq), 132.4 (Cq), 131.1 (Cq), 130.7 (Cq), 128.1 (Cq), 127.7 (Cq), 126.6 (CH), 126.5 (CH), 125.8 (CH), 125.8 (CH), 125.2 (CH), 124.8 (CH), 122.4 (Cq), 108.0 (CH), 67.6 (CH_2), 33.9 (Cq), 33.8 (Cq), 31.8 (CH_2), 31.7 (CH_3), 30.9 (CH_3); FABMS m/z 1231 (M^+); HR FABMS calcd for $C_{80}H_{76}{}^{35}Cl_2N_2O_6$, 1230.5080; found, 1230.5098.

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14. *Compound* **2**: A mixture of **4** (0.200 g, 0.926 mmol) and 10-chloroanthracene-9-hydroximoyl chloride (2.50 equiv) in toluene (50 mL) was stirred. Then, Et_3N (15.0 equiv) was added dropwise and the mixture was refluxed for 24 h. The solvent was removed under vacuum, and the residue was dissolved in chloroform (30 mL), washed with water, and dried over $MgSO_4$. The solid residue was purified by column chromatography with ethyl acetate/*n*-hexane (v/v = 1:10) to give 0.292 g (67%) of **2** as a yellowish green powder; mp 175–177 °C; R_f = 0.91 (ethyl acetate/*n*-hexane = 1:3); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.61 (d, J = 8.8 Hz, 2H), 7.88 (d, J = 8.8 Hz, 2H) 7.70–7.58 (m, 2H), 7.57–7.47 (m, 2H), 7.09 (s, 2H), 6.62 (s, 1H), 5.13 (s, 2H), 2.40 (s, 6H), 1.32 (s, 9H); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 169.2 (Cq), 160.7 (Cq), 152.9 (Cq), 147.4 (Cq), 131.3 (Cq), 131.0 (Cq), 130.0 (Cq), 128.4 (Cq), 126.8 (CH), 126.7 (CH), 126.0 (CH), 125.1 (CH), 122.8 (Cq), 107.0 (CH), 64.7 (CH_2), 34.2 (Cq), 31.5 (CH_3), 16.6 (CH_3); EIMS m/z 469 (M^+, 34), 237 (100), 177 (54); HRMS calcd for $C_{30}H_{28}{}^{35}ClNO_2$, 469.1809; found, 469.1795.
15. *Compound* **4**: A solution of 4-*tert*-butyl-2,6-dimethylphenol (0.200 g, 1.12 mmol), potassium carbonate (2.00 equiv) and propargyl bromide (1.50 equiv) in acetonitrile (50 mL) was stirred and heated at reflux for 4 h. The solvent was removed under vacuum and the residue was purified by column chromatography with ethyl acetate/*n*-hexane (v/v = 1:6) to give 0.206 g (85%) of **4** as a colorless oil; R_f = 0.83 (ethyl acetate/*n*-hexane = 1:3); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.05 (s, 2H), 4.50 (d, J = 2.1 Hz, 2H), 2.53 (t, J = 2.1 Hz, 1H), 2.35 (s, 6H), 1.32 (s, 9H); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 153.1 (Cq), 146.9 (Cq), 130.2 (Cq), 125.7 (CH), 79.3 (Cq), 74.7 (CH), 59.8 (CH_2), 34.1 (Cq), 31.4 (CH_3), 16.7 (CH_3); EIMS m/z 216 (M^+, 39), 177 (100), 119 (81); HRMS calcd for $C_{15}H_{20}O$, 216.1514; found, 216.1515.
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22. It was found that if compound **1** in $CH_3CN/CHCl_3$ (v/v = 1000:4) was pretreated with 3 equiv of Cu^{2+}, then, any added Cu^+ (ca. 10–30 equiv) showed significant quenching of the fluorescence of **1**; however, no such quenching effect was found if compound **1** was directly titrated with 30 equiv of Cu^+ (see Fig. S15).