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A new fluorescent chemosensor for transition metal cations and on/off molecular switch controlled by pH

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Abstract—A new fluorescent chemosensor with imidazole as ionophore was synthesized by the selective derivation of calixarene, which can effectively recognize Cu^{2+} and Zn^{2+} leading to different fluoroscopic behaviors in CH_3OH–H_2O. This system could be considered as a molecular switch. By modulating the pH of the solution, on–off–on fluorescent switching is carried out upon combinatory addition of acid, base and Cu^{2+}. © 2003 Elsevier Science Ltd. All rights reserved.

During the last decades, extensive research has been carried out to study and mimic biological systems such as enzymes, antibodies, DNA by designing novel receptors.[1] Molecular recognition and supramolecular chemistry have attracted considerable interest for the understanding of biological phenomena and development of new materials. Fluorescent chemosensors, which combine two fundamental functional units: a fluorophore and an ionophore, play an important role in supramolecular chemistry.[2] The part of ionophore can selectively bind the substrate, and the part of fluorophore is attached to the vicinity of the binding site for signal detection and transduction. The fluorescent chemosensors not only provide a means of detection to investigate the process of molecular recognition, but also can be used as the architons for assembling the molecular device. For example, on/off fluorescent switching based on molecular recognition has potential for information processing.[3]

The chemistry of calixarenes, cyclic oligomers composed of phenolic and methylene moieties, has been extensively studied in recent years.[4] The preorganized binding sites, easy derivatization and flexible three-dimensional steric structures make them perfect construction platforms for molecular design to generate fluorescent receptors. A number of fluorescent chemosensors based on calixarene have been synthesized and displayed remarkable selectivity, especially towards alkali metal cations,[5] and several transition metal cations have been investigated based on calixarene framework.[6] Nevertheless it is still worthwhile to synthesize new calixarene systems for selective recognition of transition metal ions for environmental or biological applications. We have reported a fluorescent chemosensor based on the calix[4]arene framework and transduction of 3-hydroxy-2-naphthoic acid, which showed selectivity in recognition for Cu^{2+} and Fe^{3+}.[7] Here, we report a new calixarene structure, which not only could selectively discriminate copper(II) and zinc(II), but also acted as on/off fluorescent switching simply by modulating the pH in a methanol–water solution.

The imidazole unit is an essential metal binding site in metalloproteins. One or more imidazole units are bound to metal ions in almost all copper and zinc metalloproteins to bring about profound effects on their biological actions.[8] In these metalloproteins the three-dimensional structures of the macromolecules facilitate the coordination of metal ions by independent side-chain residues. Therefore, ligands containing two or more imidazole rings can potentially mimic the binding sites and catalytic activities of these enzymes.[9] Because relatively few studies have been carried out on the fluorescent chemosensory properties of imidazole-containing systems based on calixarenes, we choose imidazoles as the preorganized ionophores to selectively recognize transition metal ions on calixarenes together with a fluorophore of naphthyl ring in order to transduce the recognition signal.

Keywords: calixarene; chemosensor; molecular switch.

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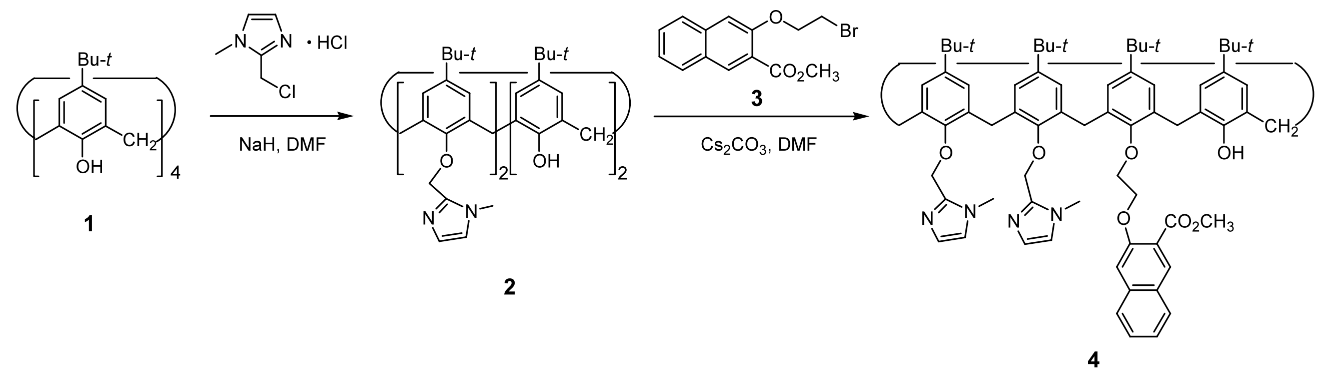

Scheme 1.

The synthesis of fluorescent chemosensor **4** was very convenient through the selective alkylation of *p-tert*-butylcalix[4]arene (**1**) with 2-chloromethyl-1-methyl-1*H*-imidazole hydrochloride and methyl 3-(2-bromoethoxy)naphthalene-2-carboxylate (**3**) in succession (Scheme 1). We firstly synthesized *1,2-proximal* disubstituted calixarene **2** in 51% yield by the reaction of **1** and 2-chloromethyl-1-methyl-1*H*-imidazole hydrochloride in the presence of NaH according to the literature.[10] In its ^{1}H NMR spectrum, the signals of methylene protons between phenol units show three pairs of doublets (ratio 1:1:2). After further monoalkylation of **2** with methyl 3-(2-bromoethoxy)naphthalene-2-carboxylate (**3**)[7] in the presence of Cs_2CO_3 as base, the fluorescent ligand **4** could be obtained in 56% yield. In its ^{1}H NMR spectrum, the signals of methylene protons between phenol units show four pairs of doublets (ratio 1:1:1:1). The structures of **2** and **4** were identified by IR, ^{1}H NMR, ^{13}C NMR, MALDI-TOF MS and elemental analyses. [11] The spectroscopic data indicated the calixarene backbone of these two compounds adopt the cone conformation.

In the UV absorption spectrum for **4**, maximum bands were observed at 231, 278 and 340 nm. Upon the addition of $Cu(ClO_4)_2$ and $Zn(ClO_4)_2$, such absorption bands did not show any noticeable variation. However, their fluorescent spectra showed different behaviors. In order to avoid signal interference by the phenyl ring, we chose $\lambda = 340$ nm as the excitation wavelength to study the fluorescent properties of **4** toward Cu^{2+} and Zn^{2+}.

In the fluorescent spectra of **4**, maximum excitation and emission wavelength were observed at 340 and 408 nm, respectively. The fluorescent intensity of **4** (50 μM) was measured at pH 10 with $(CH_3)_4N^+OH^-$ as buffer in a mixed solvent of CH_3OH–H_2O (9:1, v/v). Relative changes are shown in Figure 1 in the presence of various concentrations of metal ions of perchlorate. Figure 1 shows that the intensity of the emission maximum of **4** decreases continually upon addition of Cu^{2+}. When the concentration of Cu^{2+} increased to 6 equiv., it changed to 18% of initial intensity. In contrast, a slow increase in the intensity of the emission maximum occurs upon addition of Zn^{2+}. Since $Cu(ClO_4)_2$ and $Zn(ClO_4)_2$ have little absorption at this excitation wavelength and such dilute guest concentration, the spectral changes of **4** are not due to the absorption of transition metal ions.

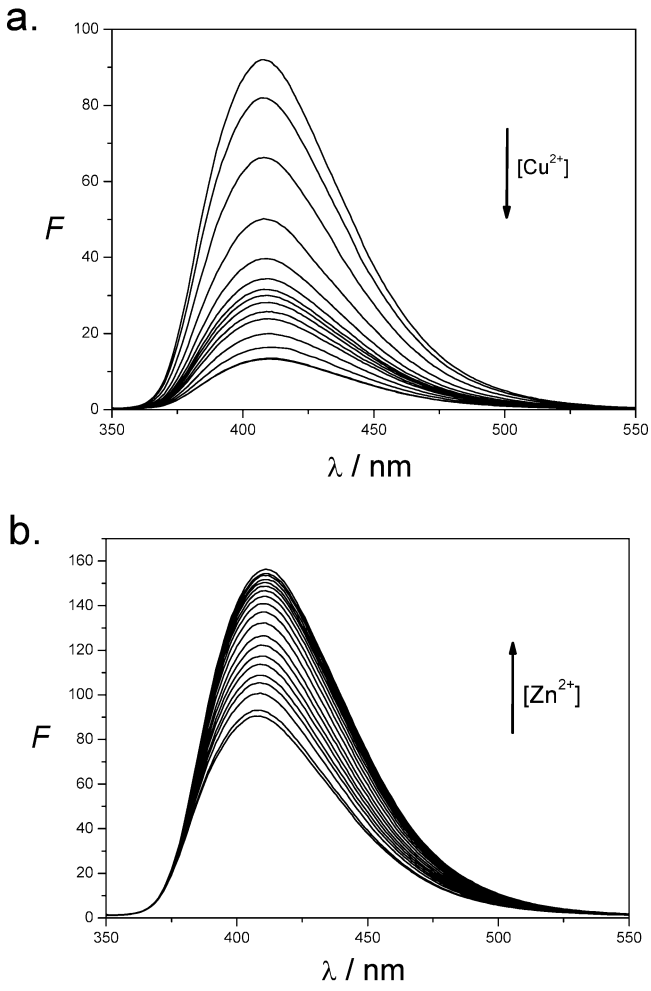

Figure 1. Fluorescence emission spectra of **4** (5×10^{-5} M) in the presence of (a) $Cu(ClO_4)_2$ (b) $Zn(ClO_4)_2$ in CH_3OH–H_2O (9:1 v/v, pH 10). The concentration of $Cu(ClO_4)_2$: 0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5, 30, 32.5, 35×10^{-5} M; The concentration of $Zn(ClO_4)_2$: 0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5, 30, 32.5, 35, 37.5, 40, 42.5, 45, 47.5, 50×10^{-5} M; $\lambda_{ex} = 340$ nm.

According to the Stern–Volmer plot for fluorescent quenching,[12] we could deduce that the binding constant between **4** and Cu^{2+} under this condition was about 9000 M^{-1}. Because to the $\Phi_{4\text{-}Zn^{2+}} \neq 0$, the Scatchard-type equation was used to calculate the binding constant between **4** and Zn^{2+}, which was about 2500 M^{-1}. Similar to Cu^{2+}, Cu^{+} also caused a similar fluorescence quenching phenomenon. However, no noticeable spectral changes were observed upon addition of other metal ions such as Li^{+}, Na^{+}, K^{+}, Ba^{2+}, Mn^{2+}, Co^{2+} and Ni^{2+} under similar conditions. For Fe^{3+}, due to the additional absorption at $\lambda = 340$ nm, the decreasing of fluorescent intensity could not be solely attributed to complexation. Therefore the ligand **4** may be conceived as a new fluorescent chemosensor based on calixarene, which could selectively recognize Cu^{2+} and Zn^{2+} as manifested by the aforementioned fluorescene responses. Such selectivity is likely to originate from the recognition ability of the imidazole units together with the interaction between fluorophore and ionophore.

In the course of the present work, we found that the recognition ability of fluorescent chemosensor **4** to Cu^{2+} can be modulated effectively by the pH of the solution. In order to investigate the influence of pH on fluorescence quenching, we measured the variation of fluorescence intensity of ligand **4** with additions of different amounts of acid and base (Fig. 2, curve (a)). Along with the adding of the acid, the fluorescence intensity began to increase and reached maximum after addition of 2 equiv. of $HClO_4$ to the solution. Curve (a) shows that the intensity remains constant under alkaline condition. So we chose such condition for measurements.

The effect of pH on the fluorescence of **4**-Cu^{2+} solution exhibited a quite different feature from that of free ligand **4** (Fig. 2, curve (b)). Under neutral solution, the fluorescence intensity of **4** was quenched maximally. Evidently, the binding between imidazole and Cu^{2+} caused this pronounced decrease, which also demonstrated the recognition ability of imidazole toward Cu^{2+}. On the other hand, the distance between the two preorgnized imidazoles made possible the selective detection of Cu^{2+} in the presence of other metal ions. When acid was added, the fluorescence appeared to increase, presumably due to the dissociation of the **4**-Cu^{2+} complex. The intensity returned to the original value of **4** with decreasing pH, which indicated that there was a complete dissociation, and thus Cu^{2+} had no influence on the fluorescence of the ligand. (Fig. 3) The dissociation is likely to be caused by the protonation of the imidazoles. On increasing the pH to higher than 7, the fluorescence also increased slowly, this may be explained by the complexed Cu^{2+} being partly displaced from the recognition sites under alkaline condition. Moreover, the on/off process of this system is reversible by adding acid or base just similar to other known fluorescent switch controlled by pH.[13] In the solution of **4**-Cu^{2+}, the system rested at the off state under neutral condition and can be turned on by adding either acid or base.

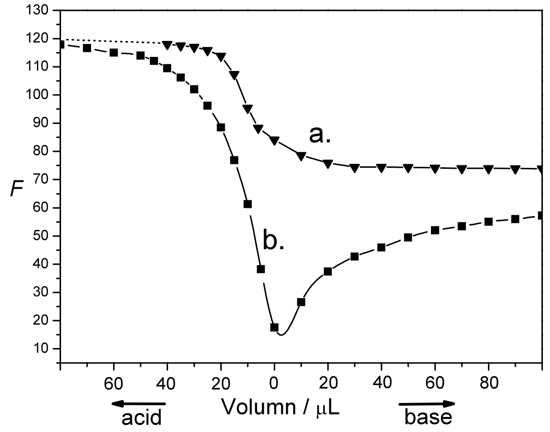

Figure 2. Fluorescence intensity of **4** (2 mL) upon adding acid or base (a) free **4**, (b) in the presence of 1 equiv. of Cu^{2+} ([**4**] = 5×10^{-5} M, in CH_3OH:H_2O = 9:1 v/v; acid: aqueous $HClO_4$, 0.01 M; base: aqueous $(CH_3)_4NOH$, 0.01 M.)

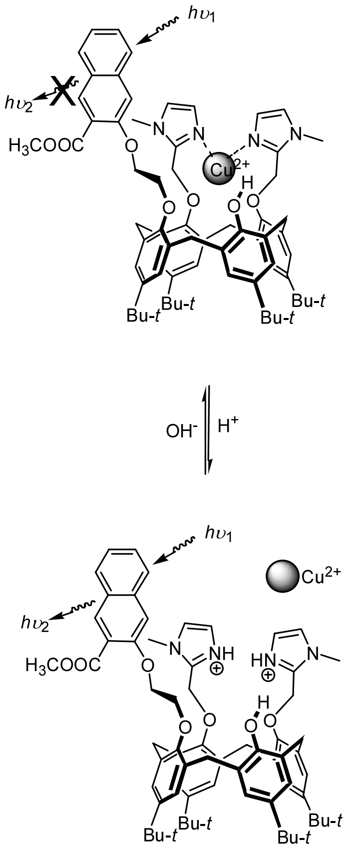

Figure 3. On/off molecular switching behavior modulated by pH.

We chose methyl 3-(2-alkoxy)naphthalene-2-carboxylate as fluorophore because it includes an electron-donating substituent (alkoxy) conjugated to an electron-withdrawing substituent (carbomethoxy), which may undergo environmentally sensitive photoinduced charge transfer (PCT) upon excitation.[14] However, in our system, PCT is not the key factor to affect the fluorescent change. We propose that there is a weak photoinduced electron transfer (PET) between the imidazoles and fluorophore. Protonation of nitrogen atoms prevents the PET process and the fluorescence intensity increased. (Fig. 2, curve (a)). Although PET would also be prevented by Cu^{2+} or Cu^{+} complexed, electron transfer (ET) between copper and the fluorophore would become the major factor to affect the emission intensity, and fluorescence would be quenched. Of course, Cu^{2+} and Cu^{+} undergo different ET process.[15] Protonation decreases the complexation and fluorescence intensity is increased.[13] Under basic condition, copper may be bound by the deprotonated oxygens of the two phenol groups and shifted away from the fluorophore in a manner similar to our previously reported system.[7] Therefore, the fluorescent intensity is increased slightly. Due to its closed shell electronic configuration Zn^{2+} is resistant to redox activity.[15] Under neutral or basic conditions, PET would also be prevented by the complextion of imidazoles with zinc ions, and the emission intensity would increase. Other cations do not affect the fluorescent spectra because of their weaker complexation.

In conclusion, we have synthesized a new fluorescent chemosensor with imidazoles as ionophore based on calixarene framework, which can effectively recognize Cu^{2+} and Zn^{2+} leading to different fluoroscopic behaviors. More interestingly this chemosensor could be considered as a molecular switch. By modulating pH, on–off–on fluorescent switching is realized by addition of acid, base and Cu^{2+}.

Acknowledgements

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11. Compound **2**: Yield: 51%; mp: 230–232°C; ^{1}H NMR: δ 7.06 (s, 2H, OH), 6.90–7.01 (m, 12H, ArH, PyH), 5.14 (d, $J=12.6$ Hz, 2H, ArOCH$_2$), 4.92 (d, $J=12.6$ Hz, 2H, ArOCH$_2$), 4.50 (d, $J=12.9$ Hz, 1H, ArCH$_2$Ar), 4.28 (d, $J=13.6$ Hz, 1H, ArCH$_2$Ar), 4.21 (d, $J=12.9$ Hz, 2H, ArCH$_2$Ar), 3.67 (s, 6H, NCH$_3$), 3.40 ($J=12.9$ Hz, 1H, ArCH$_2$Ar), 3.34 (d, $J=13.6$ Hz, 1H, ArCH$_2$Ar), 3.22 (d, $J=12.9$ Hz, 2H, ArCH$_2$Ar), 1.20 (s, 18H, C(CH$_3$)$_3$), 1.13 (s, 18H, C(CH$_3$)$_3$); ^{13}C NMR: δ 150.4, 147.8, 145.5, 143.4, 140.7, 132.3, 132.1, 126.8, 126.4, 126.2, 124.8, 124.5, 123.9, 123.9, 120.8, 67.0, 66.6, 32.7, 32.4, 31.5, 31.2, 30.9, 30.6, 30.5, 30.3, 30.1, 29.9, 29.4, 29.2, 29.0, 24.5; IR (KBr): ν 3365, 2957, 1595, 1484 cm^{-1}; MALDI-TOF MS: m/z 837 ([M+H]$^+$). Anal. calcd for C$_{54}$H$_{68}$N$_4$O$_4$: C, 77.48; H, 8.19; N, 6.69. Found: C, 77.19; H, 8.22; N, 6.41. Compound **4**: Yield: 56%; mp: 166–168°C; ^{1}H NMR: δ 8.26 (s, 1H, naph-H), 7.84 (d, $J=8.1$ Hz, 1H, naph-H), 7.78 (d, $J=8.2$ Hz, 1H, naph-H), 7.56 (t, $J=7.2$ Hz, 1H, naph-H), 7.41 (t, $J=7.5$ Hz, 1H, naph-H), 7.30 (s, 1H, naph-H), 7.11 (s, 1H, Im-H), 7.09 (s, 1H, Im-H), 7.03 (s, 1H, Im-H), 7.00 (s, 2H, ArH, Im-H), 6.94 (s, 1H, ArH), 6.80 (s, 1H, ArH), 6.63 (s, 1H, ArH), 6.59 (d, $J=2$ Hz, 1H, ArH), 6.56 (s, 2H, ArH), 6.45 (d, $J=2$ Hz, 1H, ArH), 6.18 (s, 1H, OH), 5.13 (d, $J=13.1$ Hz, 1H, ArOCH$_2$), 4.92 (d, $J=13.1$ Hz, 1H, ArOCH$_2$), 4.76 (d, $J=12.4$ Hz, 1H, ArOCH$_2$), 4.67 (d, $J=12.4$ Hz, 1H, ArOCH$_2$), 4.64 (t, $J=7.6$ Hz, 1H, ArOCH$_2$), 4.42 (t, $J=7.6$ Hz, 1H, ArOCH$_2$), 4.42 (d, $J=13.1$ Hz, 1H, ArCH$_2$Ar), 4.28–4.18 (m, 2H, ArOCH$_2$), 4.23 (d, $J=12.5$ Hz, 1H, ArCH$_2$Ar), 4.18 (d, $J=13.3$ Hz, 1H, ArCH$_2$Ar), 4.09 (d, $J=13.0$ Hz, 1H, ArCH$_2$Ar), 3.59 (s, 3H, NCH$_3$), 3.48 (s, 3H, NCH$_3$), 3.28 (s, 3H, OCH$_3$), 3.27 (d, $J=12.5$ Hz, 1H, ArCH$_2$Ar), 3.16 (d, $J=12.7$ Hz,

1H, ArCH$_2$Ar), 3.06 (d, J = 12.7 Hz, 1H, ArCH$_2$Ar), 2.94 (d, J = 13.2 Hz, 1H, ArCH$_2$Ar), 1.32 (s, 9H, C(CH$_3$)$_3$), 1.31 (s, 9H, C(CH$_3$)$_3$), 0.85 (s, 9H, C(CH$_3$)$_3$), 0.80 (s, 9H, C(CH$_3$)$_3$); ^{13}C NMR: δ 166.4, 154.7, 154.4, 150.6, 150.5, 150.3, 146.0, 145.9, 144.3, 141.3, 135.9, 135.5, 135.4, 132.7, 132.5, 132.4, 132.3, 132.1, 128.7, 128.6, 128.4, 127.9, 127.6, 127.5, 127.4, 126.6, 125.6, 125.5, 125.2, 125.0, 124.9, 124.8, 124.5, 122.2, 121.9, 121.7, 107.5, 73.6, 68.1, 67.5, 66.2, 52.0, 34.1, 33.8, 33.7, 32.6, 32.6, 31.7, 31.6, 31.4, 31.3, 31.2, 31.0, 30.7, 29.7; IR (KBr): ν 3432, 2957, 1731 (CO), 1632, 1480 cm^{-1}; MALDI-TOF MS: m/z 1065.95 ([M+H]$^+$). Anal. calcd for $C_{68}H_{80}N_4O_7{\cdot}2H_2O$: C, 74.15; H, 7.69; N, 5.09. Found: C, 73.93; H, 7.70; N, 5.04.

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