ORGANIC LETTERS

2004
Vol. 6, No. 10
1557−1560

Highly Selective Colorimetric Naked-Eye Cu(II) Detection Using an Azobenzene Chemosensor

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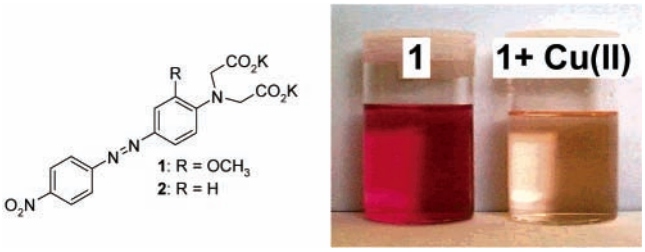
Received January 16, 2004

ABSTRACT



Colorimetric azobenzene based chemosensors 1 and 2 were designed for detection of transition-metal ions such as Cu(II) under physiological pH conditions. The internal charge transfer (ICT) sensors are highly colored, absorbing in the green. For 1, the Cu(II) recognition gives rise to red-to-yellow color changes that are visible to the naked-eye and reversible upon addition of EDTA, whereas for 2, which lacks the aromatic *o*-methoxy chelating group, no such changes were observed.

The recognition of ions and molecules is an essential part of supramolecular chemistry.[1] Over the years, many excellent examples of luminescent-based devices, such as chemosensors for various analytes, have been prepared and studied for application in physiology and medical diagnostics.[1−3] Such devices can give rise to real time, noninvasive, and on-line monitoring in vitro or in vivo. We are interested in this field and have developed fluorescent[4] and lanthanide luminescent Eu(II) and Tb(III) cyclen complexes as chemosensors,[5] switches,[6] and logic gate mimics.[7] Recently, we have also focused our efforts on developing novel colorimetric sensors for ions,[8] nucleic acids,[9] and oligonucleotides,[9] where the aim is to develop simple-to-use, naked-eye diagnostic tools (such as dipsticks), for the recognition of essential electrolytes and molecules in serum for critical care analysis.[10] Herein we describe the synthesis and the spec-

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10.1021/ol0498951 CCC: $27.50
Published on Web 04/20/2004

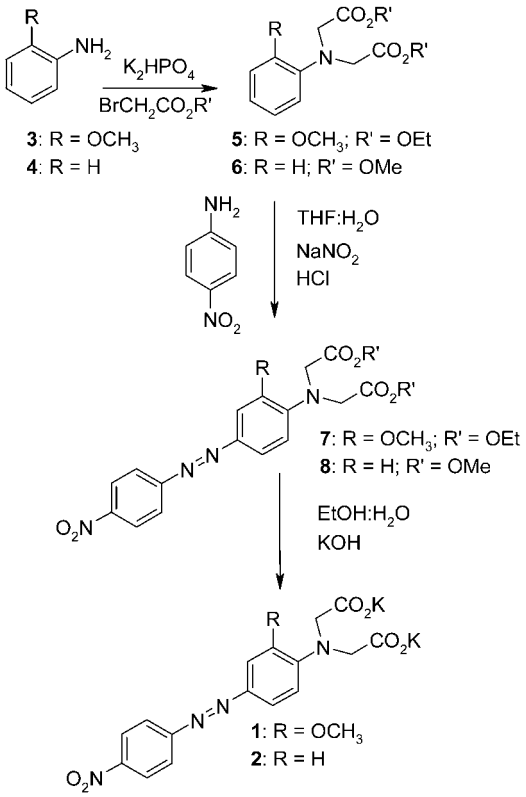
troscopic evaluation of the two colorimetric chemosensors **1** and **2**, which were developed for the detection of transition-metal ions such as Cu(II) in competitive physiological pH conditions.

Both sensors are based on the well-known azobenzene structure, where one of the aromatic rings is an integrated part of the ion receptor. In these cases, simple phenyl iminodiacetate receptors were chosen as the electron donors and a nitro benzene moiety as the electron acceptor. In the case of **1**, the use of *o*-methoxy functionality gives rise to an extra chelation site. The use of the *o*-methoxy group[11] in **1**, gives rise to high selectivity and sensitivity of this sensor for Cu(II) under physiological pH conditions. Here, the Cu(II) detection gives rise to large changes in the absorption spectra (form red to yellow), which are clearly visible to the naked eye. However, for **2**, no transition-metal ion sensing was observed. To the best of our knowledge, **1** is the first example of such a highly selective and sensitive Cu(II) colorimetric azobenzene-based chemosensor.

The synthesis of **1** and **2**, Scheme 1, began with receptors **5** and **6**, formed form *o*-anisidine (**3**) and aniline (**4**), respectively, by alkylation using 2 equivalent of either ethyl bromoacetate (for **5**) or methyl boromoacetate (for **6**) in the presence of potassium dihydrogen phosphate in CH_3CN or DMF. Both were formed in ca. 90% yield. The azobenzene esters **7** and **8** were made using standard diazonium chemistry. For **1**, this was achieved by using *p*-nitroaniline that was diazonated using $NaNO_2$ in a 1:1 mixture of THF/H_2O (20 mL), in the presence of (1 mL) HCl (12 M) at 0 °C. This mixture was then added at 0 °C to a 1:1 mixture of THF/H_2O and **5** and stirred for 2 h, followed by stirring at room temperature for 12 h. The resulting dark-red aqueous solution was reduced in volume and washed with $CHCl_3$. The organic solution was then further washed with water and dried over $MgSO_4$ and the solvent removed under reduced pressure to give **7** as red oil. This crude material was purified using flash silica column chromatography (70:30 hexane/EtOAc) to produce **7** in a pure form as a red solid in 15% yield. The final chemosensor **1** was then formed by hydrolysis of **7** under alkaline conditions in a refluxing mixture of aqueous KOH in EtOH in 95% yield. Similarly, **8** was formed in 10% yield and **2** in 90% yield using identical conditions.

Both chemosensors and intermediates were characterized using conventional methods (see the Supporting Information). Figure 1 shows the ^{1}H NMR of **1** in D_2O, where the *o*-methoxy group appeared as a singlet at 3.69 ppm, the methylene spacer of the iminodiacetate appeared as a singlet at 3.89 ppm, and five sets of the aromatic protons appeared at 8.19, 7.67, 7.42, 7.27, and 6.56 ppm, respectively. Similar results were observed for **2**.

Scheme 1. Synthesis of **1** and **2** from the Amines Receptors **5** and **6**, Respectively



Both compounds were highly soluble in water. Initially the pK_a of **1** and **2** were determined in water in the presence of 0.1 M tetramethylammonium chloride (to maintain constant ionic strength), by observing the changes in the UV−Vis spectra upon titration of an alkaline solution of either **1** or **2** with diluted HCl solution. Figure 2 shows the changes for **1**. In alkaline solution (at pH 11.7), **1** had a strong absorption band centered (λ_{max}) at 509 nm (log ϵ = 4.35 cm^{-1} M^{-1}) and a second band at ca. 289 nm. The former was assigned to the internal charge-transfer character, ICT, of the chromophore due to the *push−pull* effect of the electron-donating amine and the electron-withdrawing nitro group. As can be seen from these changes the absorption

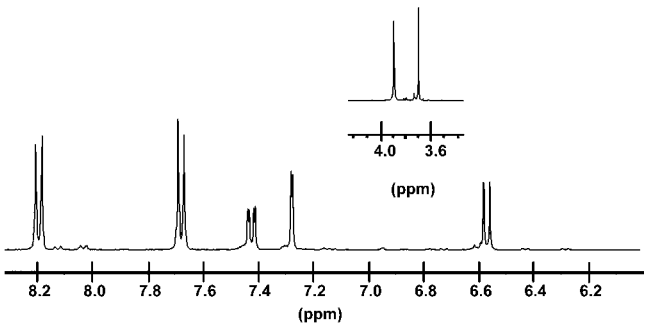


Figure 1. Partial ^{1}H NMR of **1** in D_2O (400 MHz).

(10) Fluorescent sensors for Na^+ and K^+ have been developed recently for critical care analysis: (a) He, H.; Mortellaro, M. A.; Leiner, M. J. P.; Fraatz, R. J.; Tusa, J. K. *J. Am. Chem. Soc.* **2003**, *125*, 1468. (b) He, H.; Mortellaro, M. A.; Leiner, M. J. P.; Young, S. T.; Fraatz, R. J.; Tusa, J. K. *Anal. Chem.* **2003**, *75*, 549.

(11) (a) An example of Cd(II) sensing: Gunnlaugsson, T.; Lee, C. T.; Parkesh, R. *Org. Lett.* **2003**, *5*, 4065. (b) Example of Zn(II) sensing: Gunnlaugsson, T.; Lee, C. T.; Parkesh, R. *Org. Biomol. Chem.* **2003**, *1*, 3265.

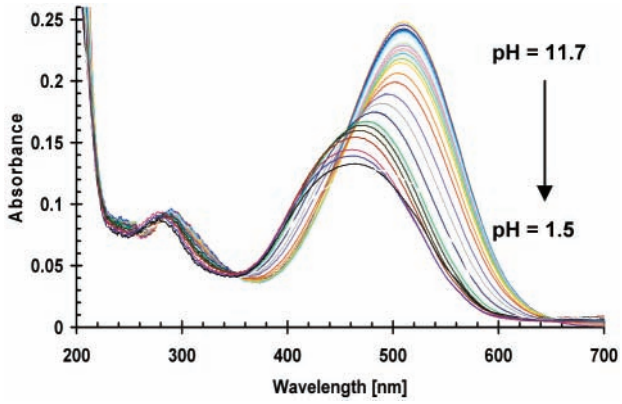


Figure 2. Changes in the UV−vis spectra of **1** upon pH titration of an alkaline solution of **1** with acid.

maxima gradually decreased in intensity upon addition of acid. These changes correspond to the protonation of the amino moiety, reducing the ICT character of the molecule.

By plotting the changes at 509 nm as a function of pH a sigmoidal curve was observed where the major changes occurred over ca. two pH units. These changes were fully reversible, as the addition of strong base to this acidic solution reversed these effects. As can be seen from Figure 3, only minor changes occurred above ca. pH 5.5. From these

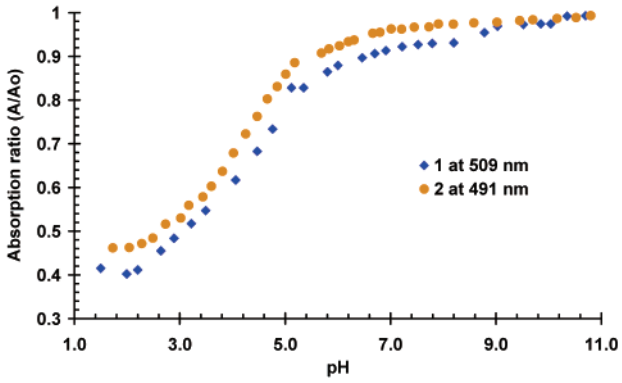


Figure 3. Changes in the UV−vis spectra of **1** and **2** at 509 and 491 nm, respectively, upon titration of an alkaline solution of these sensors with diluted HCl.

changes a pK_a of 4.2 (±0.1) was determined. This clearly demonstrates that **1** can be used in physiological environment where the pH >5. Similar results were observed for **2**, Figure 3.

We next investigated the affinity of both **1** and **2** for a series of transition and group II metal ions, at pH 7.4 (20 mM HEPES) and in the presence of 135 mM NaCl to maintain constant ionic strength. For the latter, only minor changes were seen at very high concentrations for Mg(II) or Ca(II). We have previously demonstrated the use of simple based aryl iminodiacetate receptors in fluorescent PET sensors, where we were able to selectively detect ions such as Zn(II) and Cd(II) over other competitive ions such as Ni(II), Fe(II), and Cu(II) under physiological pH conditions.[11]

However, we also established that this selectivity was highly dependent on the nature of the fluorophore used.[11]

The changes in the absorption spectra of **1** in buffered water at pH 7.4 are shown in Figure 4, where it is clear that

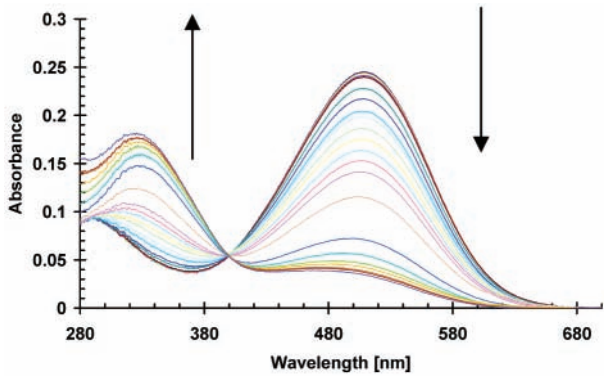


Figure 4. Changes in the UV−vis spectra of **1** at pH 7.4 (20 mM HEPES, 135 mM NaCl) upon titration with $CuCl_2$.

the absorption of **1** is highly affected by the presence of Cu(II). Here, the absorption maxima at 509 nm gradually reduced in intensity with the formation of a new absorption band at ca. 325 nm and with the formation of an isosbestic point at 401 nm. We propose that this is due to the coordination of the Cu(II) to the two carboxylates, the amino moiety and the *o*-methoxy group. This reduces the ability of the amino moiety to participate in ICT in the same manner as for the protonation of the amino moiety discussed earlier. However, the role of the *o*-methoxy group is vital here, as upon its coordination to the metal ion, it forces the amine of the receptor to become deconjugated from the aromatic receptor, inhibiting the ICT. Even though we do not have direct ^{1}H NMR or X-ray crystal structure evidence for this, we have previously observed such phenomena in related 15-crown-5 and 18-crown-6 based receptor, for the sensing of Na^+ and K^+ respectively.[12] Unlike that seen for the protonation event (Figure 2), a clear isosbestic point is observed in Figure 4.

More importantly, the Cu(II) sensing and the concomitant absorption changes were clearly visible to the naked eye, as can be seen in the graphical abstract, where the red solution of **1** became yellow upon titration with Cu(II). Moreover, these changes are also fully reversible as the addition of EDTA reversed the color changes, Figure 5. When these titrations were repeated using Cd(II) or Zn(II), the absorption spectra was also affected in a manner similar to that shown above. By plotting the changes at λ_{max} as a function of pM,

(12) Gunnlaugsson, T.; Leonard, J. P. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1954.

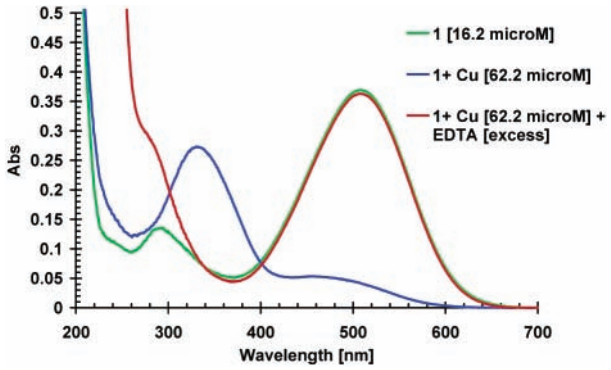


Figure 5. UV−vis spectra of **1** before and after addition of Cu(II) and reversed changes upon addition EDTA.

where pM = −log[M] where M is the cations used, the sensitivity and the selectivity of these ion recognitions can be evaluated. From Figure 6, it is apparent that both Zn(II) and Cd(II) modulate the absorption spectra.

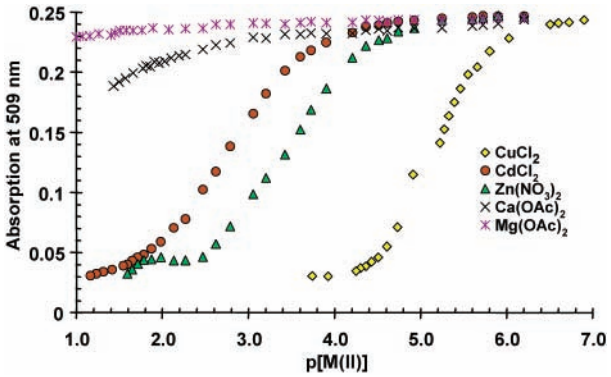


Figure 6. Changes in the UV−vis spectra of **1** at 509 nm in water at pH 7.4 (20 mM HEPES, 135 mM NaCl) upon titration of several cations.

However, these changes occur at much higher concentrations than that of Cu(II). Studies using different anions had no affect upon either the sensitivity or the selectivity of these titration's (see the Supporting Information). Importantly, all three transition ions modulate the absorption over 2 pM units, which is indicative of a simple equilibrium and one to one binding. From the binding isotherms in Figure 6, we were able to determine the binding constants (log β) as log β = 5.0 (±0.1), 3.4 (±0.1), and 3.0 (±0.1) for Cu(II), Zn(II), and Cd(II), respectively, for **1**. From these changes it can be concluded that **1** is a good naked-eye sensor for the selective detection of Cu(II) in competitive media. In comparison to these result the changes in the absorption spectra of **2** (λ_{max} = 491 nm) were very different.

As stated above, the absorption spectrum was blue shifted upon pH titration. However, when **2** was titrated at pH 7.4 in HEPES buffer and in the presence of 135 mM of NaCl, using the above ions, no significant spectral changes were observed. However, at relatively high concentrations of Zn(II) and Cu(II) precipitation occurred (See SI). This is quite extraordinary, as the only difference between the two sensors is the presence of the *o*-methoxy group. Hence, the selectivity and the sensitivity of **1** is dependent on the presence of the *o*-methoxy group.

In summary, we have developed a highly selective azobenzene based colorimetric chemosensor **1**, for the detection of Cu(II) under physiological conditions. The recognition of the ion gave rise to major color changes from red to yellow that was clearly visible to the naked eye. Such Cu(II) selective colorimetric chemosensors could be of great importance in medical diagnostics such as serum studies. We are currently working toward the development of such a device by incorporating **1** into soft material such as hydrogels.

Acknowledgment. We thank TCD and Enterprise Ireland for financial support. We also thank Dr. John E. O'Brien for NMR and Donal F. O'Shea (University College Dublin) for helpful discussions.

Supporting Information Available: Synthesis of **1**, **2**, **7**, and **8**; the UV−Vis spectral changes upon titration of **1** and **2** with solutions of Zn(II), Cd(II), Ca(II), and Mg(II)l the absorption vs pH profile for **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0498951