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A benzimidazole-based single molecular multianalyte fluorescent probe for the simultaneous analysis of Cu^{2+} and Fe^{3+}

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This Letter is dedicated to the late Professor Chi Sun Hahn

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ABSTRACT

We synthesized a novel receptor with benzimidazole moieties in a tripodal framework. The receptor displays rarely observed metal specific fluorescence enhancement at two different wavelengths. The receptor was investigated for the simultaneous analysis of Cu^{2+} and Fe^{3+} and successfully quantified the ions without interference over a wide concentration range.

Development of sensitive and selective chemosensors that are capable of assaying cations and anions is of great importance in the areas of biology and environmental science.[1] Fluorescent probes are widely used for the detection of neutral molecules, cations, or anions because of their high sensitivity. Selective detection of transition metal ions is particularly critical owing to their biological and environmental implications.[2] Several methods for the selective and sensitive analysis of metal ions have been developed that include an array of detection methods[3] and mathematical analyses[4] such as PCA (principal components analysis), ANN (artificial neutral networks), and PLS (partial least squares regression). However, the development of single molecular sensors capable of detecting multiple metal analytes with different spectral responses is still a great challenge.[5]

Copper and iron are the most abundant transition metal ions in biological systems that play important roles in metabolic processes at low concentrations.[6] Fet3p, a yeast glycoprotein localized to the plasma membrane, contains a multicopper structure and catalyzes the oxidation of Fe^{2+} to Fe^{3+}.[7] For normal physiological functions, homeostasis is required between these two ions.[8] Thus, the content of these metal ions in bodily fluids is frequently analyzed. Several research efforts have been devoted to finding methods for the simultaneous determination of Cu^{2+} and Fe^{3+} concentrations that can be used for the clinical study of metabolic processes.[9] These methods mainly rely on the use of signal processing and multivariate techniques because of overlapping optical signals. These techniques suffer from either difficulty in their operational use or Cu^{2+} interference in Fe^{3+} analysis and vice versa. Moreover, these reported methods have sensitivity and selectivity limitations. In this context, developing a single molecular sensor for the simultaneous estimation of Cu^{2+} and Fe^{3+} through different optical signals is in great demand.

For a simultaneous analysis, a receptor should be semi-selective and should have moderate binding affinity for the metal ions in question. These properties will ensure real-time determination of metal ions without any cross interference. Herein, we report a fluorescent benzimidazole-based tripodal receptor for the real-time simultaneous analysis of Cu^{2+} and Fe^{3+}. The selection of the benzimidazole unit is based on imidazole having a good binding affinity for both Fe^{3+} and Cu^{2+} as well as being present as a proficient binding unit in numerous biomolecules.[10]

Compound **3** was synthesized by a condensation reaction of tripodal aldehyde **1** with amine **2** in the presence of a catalytic amount of $Zn(ClO_4)_2$, followed by reduction with $NaBH_4$ (Scheme 1). Compound **3** was fully characterized by spectroscopic methods,[11] and the ^1H NMR spectrum of the compound showed three unambiguous signals (without any splitting) for the three methylene groups, indicating that the compound adopts a symmetrical conformation in solution.

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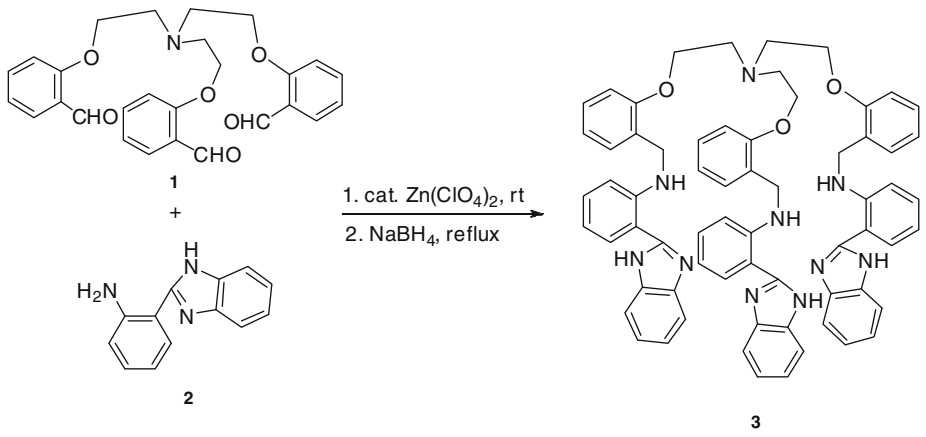

Scheme 1.

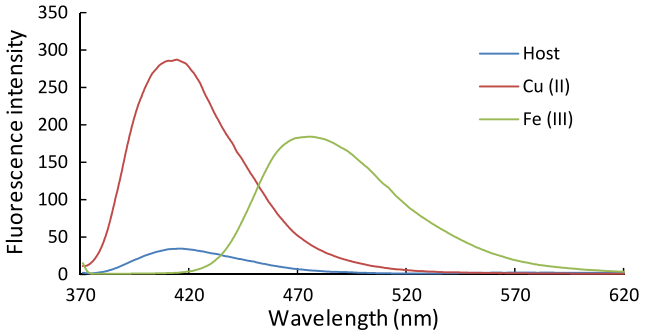

Figure 1. Changes in fluorescence intensity of receptor **3** (10 μM) upon addition of a particular metal salt (200 μM) in acetonitrile (λ_{ex} = 361 nm).

A 10 μM solution of receptor **3** in acetonitrile upon excitation at 361 nm exhibited a fluorescence spectrum with an emission band at 415 nm and a Stokes shift of 54 nm (Fig. 1). The cation recognition behavior of receptor **3** was evaluated from changes in the fluorescence intensity upon addition of a particular metal salt. Upon addition of a solution of Cu^{2+} to a solution of receptor **3**, a significant enhancement in the intensity was observed in the emission band at 415 nm. On the other hand, addition of a solution of Fe^{3+} to a solution of receptor **3** showed significant quenching in the intensity of the emission band at 415 nm with the concomitant formation of a new red-shifted emission band at 475 nm. Other metal ions such as Ca^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, and Ag^{+} showed no such significant changes in the fluorescence spectrum under the same conditions. A comparison of the fluorescence intensity of receptor **3** upon addition of various metal ions reveals remarkable selectivity of Cu^{2+} binding at 415 nm and Fe^{3+} binding at 475 nm (Fig. 2). These phenomena allow receptor **3** to be used for the real-time simultaneous analysis of Cu^{2+} and Fe^{3+} by directly correlating the fluorescent intensity at 415 or 475 nm with the concentration of Cu^{2+} or Fe^{3+} ions, respectively.

The enhancement of fluorescence intensity upon addition of Cu^{2+} reflects a change from the flexible conformation of receptor **3** to a rigid form upon metal complexation, making non-irradiative decay less probable.[12] Another factor affecting the enhancement of fluorescence intensity can be attributed to quenching due to PET from the nitrogen donor lone pairs to the fluorophores in the free receptor being obviated by metal complexation engaging the nitrogen donor lone pairs, resulting in quenching via PET (photoinduced electron transfer).[13] Only a few examples have been reported on receptors which show enhancement with complexation of transition metal ions.[14] Interestingly, Fe^{3+} complexation induce enhancement with a blue shift in λ_{max} by 55 nm. The blue shift in fluorescence is due to ICT (internal charge transfer) processes; but on the other hand, the complexation leads to the enhancement of fluorescence intensity, which is most likely due to the cancelation of PET from nitrogen donors. Thus, the blue shift along with fluorescence enhancement is the result of two operation, that is, the combination of PET and ICT processes.[15]

The fluorescence intensity increased continuously at 415 nm with the titration of receptor **3** with Cu^{2+}, while during the course of titration with Fe^{3+}, the intensity of the emission band decreased at 415 nm with a continuous increase in the intensity of band at 470 nm (Figs. 3 and 4). These continuous and stepwise changes

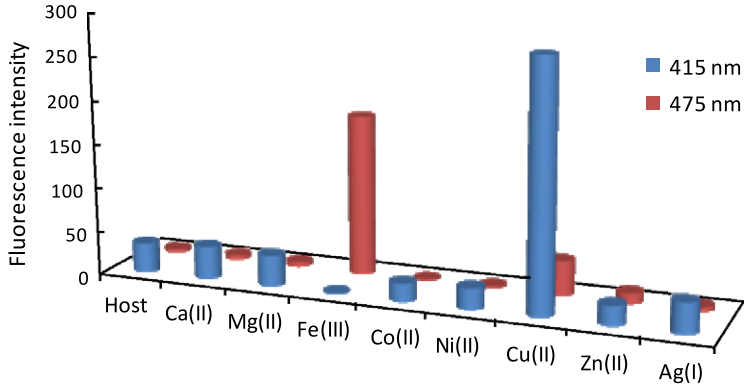

Figure 2. Fluorescence intensity of receptor **3** (10 μM) upon addition of a particular metal salt (200 μM) in acetonitrile at 415 nm and 470 nm (λ_{ex} = 361 nm).

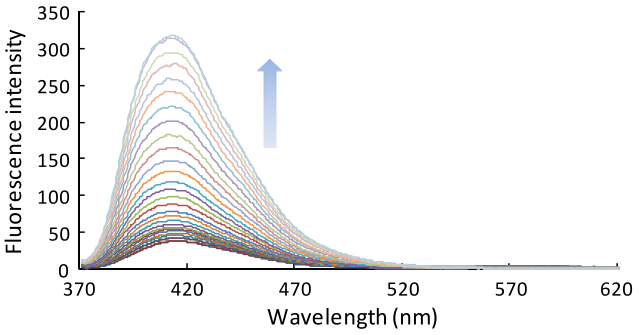

Figure 3. Changes in the fluorescence spectra of receptor **3** (10 μM) upon successive addition of $Cu(NO_3)_2$ (0–200 μM) in CH_3CN (λ_{ex} = 361 nm).

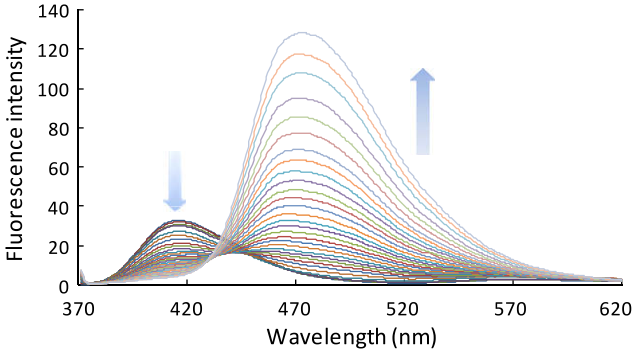

Figure 4. Changes in the fluorescence spectra of receptor **3** (10 μM) upon successive addition of $Fe(NO_3)_3$ (0–150 μM) in CH_3CN (λ_{ex} = 361 nm).

in fluorescence intensities ensure the use of receptor **3** for analytical applications of metal analysis over a wide range of metal concentration. Receptor **3** can detect a minimum concentration of 2.3×10^{-5} M for Cu^{2+} and 2.1×10^{-5} M for Fe^{3+}, respectively.[16] Binding constants of receptor **3** with metal ions were calculated by using Benesi–Hildebrand plots and were found to be $(5.9 \pm 0.1) \times 10^3$ M^{-1} for Cu^{2+} and $(1.3 \pm 0.1) \times 10^3$ M^{-1} for Fe^{3+}, respectively.[17] The Job plots reveal that Cu^{2+} and Fe^{3+} ions each form a 1:1 complex with receptor **3**.[18]

For the simultaneous analysis of metal ions, the receptor must exhibit the same optical behavior for a particular metal ion in the presence of other competing ions. To examine this point, an experiment was designed in which the Cu^{2+} concentration was measured in the presence of an equivalent amount of Fe^{3+} and in the absence of any metal ions. The same experiment was performed with Fe^{3+} using Cu^{2+} as the interfering ion. The results are shown in Figure 5. The precision in the analysis of Cu^{2+} and Fe^{3+} ions in the presence of the interfering ion unambiguously demonstrates that receptor **3** can be used for the real time analysis of Cu^{2+} and Fe^{3+}.

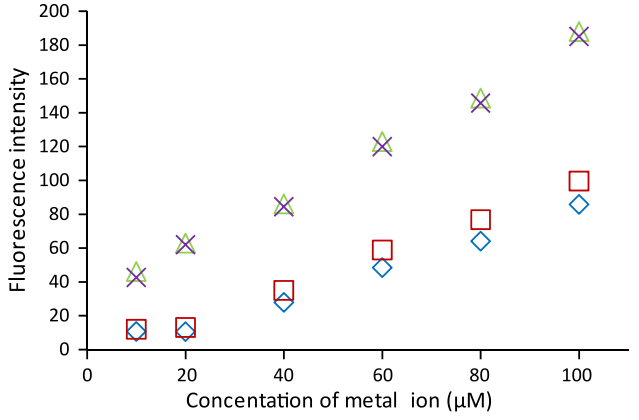

Figure 5. Plot of fluorescence intensity of receptor **3** against equivalents of metal ion: (△) Cu^{2+} at 415 nm, (×) Cu^{2+} in the presence of equimolar Fe^{3+} at 415 nm, (◇) Fe^{3+} at 475 nm, and (□) Fe^{3+} in the presence of equimolar Cu^{2+} at 475 nm.

In conclusion, we have developed an easily engineered single molecular multianalyte fluorescent probe for the simultaneous determination of Cu^{2+} and Fe^{3+} in a wide concentration range. The receptor was successfully used for the real time analysis of Cu^{2+} and Fe^{3+} without any cross interference.

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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.2009.12.085.

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11. *Synthesis of compound* **3**: A solution of tripodal aldehyde **1** (300 mg, 0.650 mmol) and 2-(2-aminophenyl)-1*H*-benzimidazole (**2**) (476 mg, 2.28 mmol) in MeOH (30 mL)/THF (30 mL) was stirred in the presence of a catalytical amount of $Zn(ClO_4)_2$ at room temperature. Upon completion of the

reaction, $NaBH_4$ (241 mg, 6.50 mmol) in THF (50 mL) was added and stirred at reflux for 14 h. After evaporation of the solvent, the crude product was dissolved in CH_2Cl_2, washed with water, and dried over anhydrous $MgSO_4$. After evaporation, the residue was purified by column chromatography on silica gel eluting with hexane: EtOAc (7:3) to give the compound **3** as a light yellow solid (528 mg, 78%): ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.04 (t, 6H, $-CH_2$, J = 5.6 Hz), 3.97 (t, 6H, $-CH_2$, J = 5.6 Hz), 4.31 (s, 6H, $-CH_2$), 6.54–6.60 (m, 6H, Ar), 6.71–6.73 (m, 3H, Ar), 6.77–6.81 (m, 3H, Ar), 7.05–7.12 (m, 6H, Ar), 7.16–7.20 (m, 6H, Ar), 7.24–7.26 (m, 3H, Ar), 7.33 (br, 3H, Ar), 7.47–7.49 (m, 3H, Ar), 7.67 (br, 3H, Ar), 8.84 (br, 3H, –NH), 9.57 (br, 3H, –NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 41.6, 59.8, 67.0, 110.8, 111.2, 114.6, 118.1, 120.1, 121.4, 122.6, 127.0, 127.5, 127.9, 130.8, 133.6, 142.7, 147.6, 152.5, 156.4, 170.3. HR-MS (FAB) calcd for $C_{66}H_{61}N_{10}O_3$ $[M+H]^+$: 1041.4928; found 1041.4930.

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