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Highly Selective Fluorescence Detection of Cu^{2+} in Water by Chiral Dimeric Zn^{2+} Complexes through Direct Displacement

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Fluorescent dinuclear chiral zinc complexes were synthesized in a "one-pot" method in which the lysine-based Schiff base ligand was generated in situ. This complex acts as a highly sensitive and selective fluorescent ON−OFF probe for Cu^{2+} in water at physiological pH. Other metal ions such as Hg^{2+}, Cd^{2+}, and Pb^{2+} gave little fluorescence change.

The design and synthesis of fluorescent chemosensors for the selective detection of metal ions and anions is one of the most important research areas in chemistry due to the fundamental and necessary role of these species in biological processes.[1] Chemosensors for the detection of various biologically important cations such as Cu^{2+}, Zn^{2+}, Ca^{2+}, Mg^{2+}, and Na^{+} are of continued interest in this age of biotechnology.[2] Particularly, rationally designed sensors for the detection and measurement of Cu^{2+} have received significant attention recently. Cu^{2+} is an essential *d*-block metal ion in human health[3] but is also harmful to biological systems in excessive amounts. Alterations in Cu^{2+} cellular homeostasis are connected to serious neurodegenerative diseases such as Wilson's disease, Alzheimer's disease, and prion-induced diseases.[4] Accordingly, the development of Cu^{2+}-specific molecular probes has been of considerable interest in the biological and analytical chemical sciences.[5]

An ideal small molecule metal ion probe should possess, (*i*) good water solubility, (*ii*) high stability, and (*iii*) the capability to quantitatively determine metal ion concentration with (*iv*) high selectivity and (*v*) sensitivity toward the specified metal ion when it is present in ion mixtures. If the sensor has a (*vi*) straightforward and high-yielding preparative route, this is an additional practical advantage.[6] While there are various reports that explore Cu^{2+}-selective probes, very few reports involved studies in pure aqueous solution.[7] Herein, we report the synthesis and characterization of the fluorescent dinuclear chiral compound $[\mathrm{Zn_2}(\textit{slys})_2(\mathrm{NO_3})_2]$ [$\mathrm{H_2}\textit{slys}$ = 6-amino-2-{(2-hydroxybenzylidene)amino}hexanoic acid] and also $[\mathrm{Zn_2}(\textit{slys})_2\mathrm{Cl_2}]$,[6a] which are isolated with solvents of crystallization: $\mathbf{2}\cdot(\mathrm{H_2O})_3$ and $\mathbf{1}\cdot(\mathrm{CH_3OH})_2\cdot(\mathrm{H_2O})_3$, respectively. These complexes engage in selective detection of Cu^{2+} over other metal ions (Hg^{2+}, Cd^{2+}, and Pb^{2+}) in water by direct displacement. This alternative direct-displacement approach of metal ion sensing is very much effective, in which the organic ligand solely shows poor sensing ability to the metal ion. Recently, Kim et al. reported

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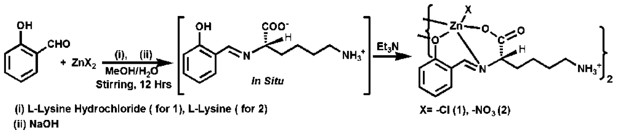
10.1021/ic802314u CCC: $40.75

Scheme 1



a fluorescent Zn^{2+} complex which is a highly effective "turn-off" sensor for Hg^{2+}. Herein, the Zn^{2+}-free organic ligand moiety is a less effective sensor than the complex.[8]

Complexes **1** and **2** have been synthesized by a typical "one-pot" method in which the L-lysine-based Schiff base ligand is generated in situ (Scheme 1; see Supporting Information). This simplified synthetic strategy is very effective in such cases in which a rational, stepwise ligand synthesis is difficult, labor-intensive, and low-yielding. Compounds **1** and **2** have been fully spectroscopically characterized as well as studied structurally by single-crystal X-ray diffraction. When considering the involvement of the H_2*slys* ligand in potential sensing studies, it was expected that the Zn^{2+} and Cu^{2+} complexes might adopt very similar coordination geometries. Once we verified the exact binding and dinuclear nature of Zn^{2+}, we then pursued a displacement reaction that would allow for a retention of this interesting dimeric geometry. We have recently reported the synthesis and characterization of compound **1** and the sensing of phosphate ions by **1** in water.[6a] Herein, we will detail our findings on the role of the nitrate analogue of compound **1** in Cu^{2+} sensing.

Compound **2** was crystallized in the monoclinic chiral space group *C*2.[9] Its structure reveals that both zinc centers adopt a distorted square-pyramidal geometry through tridentate coordination of the *slys* ligand (Figure 1), bridging of the phenolate oxygen, and the presence of the axial monodentate nitrate ion.

Compounds **1** and **2** are highly blue-fluorescent in solution ($\Phi_F = 0.17$ and 0.21, respectively; reference, fluorescein) and in the solid state. The metal-free Schiff base ligand, H_2*slys*, isolated in a slightly impure form, is weakly green-fluorescent in aqueous solution. In the presence of Zn^{2+}, the C=N conjugation with the phenyl ring is inhibited due to the formation of strong chelation with Zn^{2+} giving rise to intense fluorescence. Heavy metal ions such as Hg^{2+}, Pb^{2+}, and Cd^{2+} and *d*-block metals such as Cu^{2+} are known generally as quenchers. As the Schiff base ligand H_2*slys* is weakly fluorescent, it is a less effective *turn-off* sensor for these ions. But compound **2** is highly fluorescent, so a significant quenching effect is expected from the aforementioned metal ions via direct displacement.

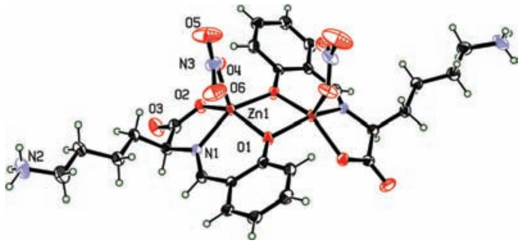


Figure 1. Crystal structure of compound **2**. Thermal ellipsoids at 30% probability. Solvent waters are removed for clarity. Selected bond lengths: Zn(1)−N(1), 2.057(4) Å; Zn(1)−O(1), 2.043(3) Å; Zn(1)−O(4), 2.044(4)Å.

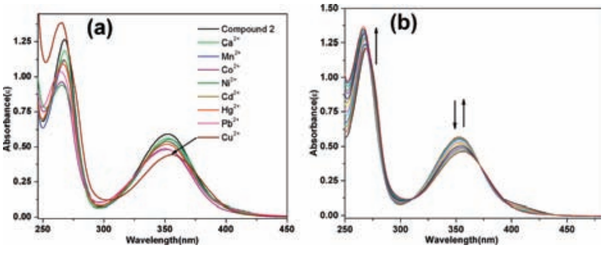


Figure 2. (a) UV−vis spectra of **2** (50 μM) with different cations (100 μM). (b) Absorbance titration of **2** (50 μM) in an aqueous buffer (pH 7.4, 0.01 M HEPES) with increasing amount of Cu^{2+} solution (100 μM).

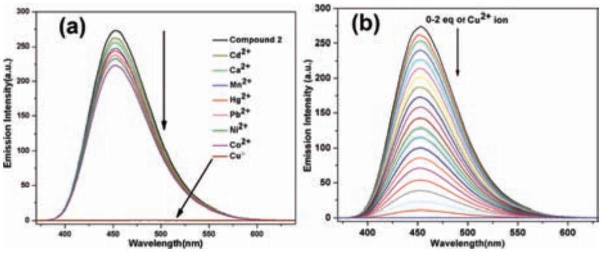


Figure 3. (a) Emission spectra of **2** (50 μM, $\lambda_{ex} = 352$ nm, $\lambda_{em} = 452$ nm) with different cations (100 μM). (b) Emission titration of **2** (50 μM) in an aqueous buffer solution (pH 7.4, 0.01 M HEPES) with increasing amount of Cu^{2+} solution (100 μM).

The sensing behavior of compounds **1** and **2** with various metal cations (Ca^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, and Pb^{2+}) in water (pH 7.4, 0.01 M HEPES buffer) was investigated using UV−vis and fluorescence spectroscopy. The absorption spectrum of compound **2** (50 μM) in water reveals two bands at 267 and at 352 nm. These bands may be assigned to intraligand charge transfer transitions. Upon the addition of 2.0 equiv of Cu^{2+} ions (100 μM), the absorbance decreased slightly while the λ_{max} was red-shifted to 357 nm. Under these same conditions, other metal ions, such as Ca^{2+}, Pb^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Hg^{2+}, and Cd^{2+}, showed very minor and unremarkable changes in the UV−vis spectrum (Figure 2a). During the absorbance titration of **2** (50 μM) with increasing amounts of Cu^{2+} (100 μM), the band at 352 nm slowly red-shifted to 357 nm; no more shifting occurred after the addition of more than 2.0 equiv of Cu^{2+} ions (Figure 2b).

The treatment of compound **2** with 2.0 equiv of various metal ions revealed the specific behavior of Cu^{2+} (Figure 3a). All metal ions except Cu^{2+} quenched the fluorescence partially (Figure 3a), whereas Cu^{2+} quenched the fluorescence of **2** fully, giving an emission intensity decrease of ~1290-fold after the addition of 2.0 equiv (Figure 3b). The apparent association constant (K_a) of $\sim(1.5 \pm 0.14) \times 10^8$ M^{-1} [$(1.8 \pm 0.19) \times 10^8$ M^{-1} for compound **1**] is determined

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(9) Crystal data for **2**: $C_{26}H_{40}N_6O_{15}Zn_2$, mw = 807.38, $T = 293(2)$ K, monoclinic, *C*2, $a = 19.6098(14)$ Å, $b = 7.9168(6)$ Å, $c = 10.8343(8)$ Å, $\beta = 98.633(6)°$, $V = 1662.8(2)$ Å^3, $Z = 2$, $\rho_{calcd} = 1.613$ Mg/m^3, $\mu = 1.521$ mm^{-1}, $\lambda = 0.71073$ Å, $2\theta_{max} = 51.34$, reflns collected/unique = 7387/3015, $R_{int} = 0.0495$, R1 = 0.0382, wR2 = 0.0708 for 2397 reflections [$I > 2\sigma(I)$], goodness-of-fit on $F^2 = 0.989$, Flack (*x*) parameter = 0.038(16), CCDC 698239.

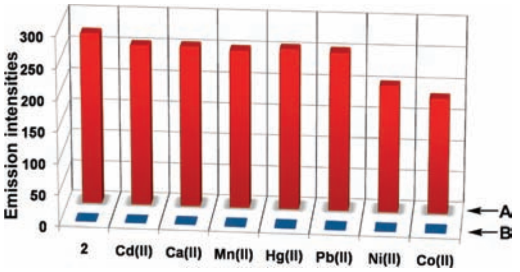

Figure 4. (Row A) Emission spectra of **2** (50 μM) in an aqueous buffer solution (pH 7.4, 0.01 M HEPES) with various M^{2+} species (500 μM). The far left bar is only compound **2**. (Row B) Emission spectra of a mixture of **2** (50 μM) with other M^{2+} species (500 μM) followed by addition of Cu^{2+} solution (100 μM).

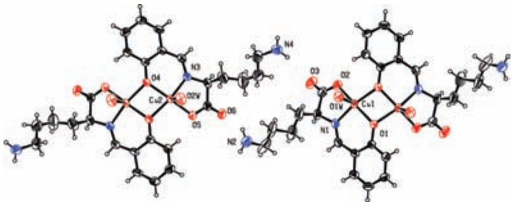

Figure 5. ORTEP of complex **3**. The Na^+ and ClO_4^- counterions are removed for clarity. Selected bond lengths: Cu(1)−O(1), 1.927(5)Å; Cu(1)−O(2), 1.945(5) Å; Cu(1)−N(1), 1.946(6) Å; Cu(1)−O(1W), 2.326(7) Å.

from the emission titration signifying the strong binding affinity of the Cu^{2+} ion for the ligand.[10] As the Cu^{2+} detection occurs through the zinc displacement reaction, we checked the possible reversibility of the reaction by adding an excess of Zn^{2+} ions to the compound **2**/$2Cu^{2+}$ mixture. Fluorescence did not return, even after the addition of ~40 equiv of Zn^{2+} ions. This result implies that Cu^{2+} has a very strong binding affinity to the H_2*slys* ligand compared to that of Zn^{2+}. It is notable that the addition of Fe^{2+} to compound **2** in a buffered solution (water, 7.4 pH) fully quenches the fluorescence. Here, Fe^{2+} is rapidly oxidized to Fe^{3+}, and the Schiff base ligand is hydrolyzed (see the Supporting Information).[11] As with compound **2**, compound **1** also shows similar spectral features with Cu^{2+} addition (Supporting Information).

Compound **2** is highly selective toward Cu^{2+} over other competitive metal ions (Figure 4). The emission intensity of compound **2** in the presence of 10.0 equiv of other metal ions only slightly decreases. Upon the addition of only 2.0 equiv of Cu^{2+} to the 1:10 mixture of **2** and other metal ions, the fluorescence is instantly and entirely quenched.

Compound **2** is chiral due to the incorporation of L-lysine moieties. In an aqueous buffer solution, it shows a negative Cotton effect at 354 nm. The addition of 2 equiv of Cu^{2+} effected the immediate appearance of a band at 359 nm, which signifies the formation of a new complex through metal ion displacement (Supporting Information). The ^{1}H NMR spectrum (D_2O) of **2** in the presence of 2.0 equiv of Cu^{2+} shows an immediate broadening and downfield shifting of the imine and α-proton peaks of ~0.1 ppm (Supporting Information). Both the circular dichroism and ^{1}H NMR spectra clearly indicate the direct displacement of Zn^{2+} by Cu^{2+}, followed by the formation of a new species. The absorbance titration of **2** (100 μM) with a Cu^{2+} solution (200 μM) shows the formation of a characteristic Cu^{2+} complex *d*−*d* transition band (500−800 nm), further confirming Zn^{2+} displacement (Supporting Information).

To obtain a clearer idea of the type of binding and the stoichiometry of complex **2** with Cu^{2+}, we performed a reaction in which compound **2**, $Cu(ClO_4)_2 \cdot 6H_2O$, and $Na[ClO_4]$[12] were combined in a water−methanol (1:1) mixture. After the reaction mixture changed from yellow to green, crystals were obtained from an ethanol solution and confirmed to be compound **3** (Figure 5) by single-crystal X-ray diffraction.[13] The unit cell of compound **3** contains two independent neutral dicopper complexes. The copper centers, as in the $[Zn^{2+}]_2$ analog, adopt a distorted square-pyramidal geometry bearing water molecules in the axial positions. Interestingly, an entire $Na[ClO_4]$ is present in the unit cell of **3**, which likely assisted in the crystallization. From the crystal structure, it is clear that the stoichiometry of the binding of complex **2** with Cu^{2+} ions is 1:2. The Job plot analysis further supports that the complex **2**/Cu^{2+} stoichiometry in solution converts nearly to 1:2 via direct displacement (see the Supporting Information).[14]

In conclusion, we have synthesized fluorescent dimeric chiral zinc(II) complexes with an easy "one-pot" method in which the L-lysine-based Schiff base ligand is generated in situ. These dizinc complexes act as highly selective probes for Cu^{2+} ions in aqueous media under physiological pH via direct M^{n+} displacement, as monitored by spectroscopy. The binding of Cu^{2+} is highly selective, allowing for selective detection in the presence of competitive nontransition and transition-metal ions alike. The presence of molecular chirality in these complexes is suggested to be of future interest in these systems in targeting detection of chiral anions and biomolecules.

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Supporting Information Available: A variety of NMR spectra related to this work, UV−vis and emission spectra, crystal data collection and refinements in PDF and CIF formats. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) Crystal data for **3**: $C_{52}H_{72}N_8O_{24}Cl_2Cu_4Na_2$, mw = 1564.22, $T = 293(2)$ K, monoclinic, *C*2, $a = 17.1452(13)$ Å, $b = 8.7748(4)$ Å, $c = 23.3633(10)$ Å, $\beta = 94.655(2)°$, $V = 3503.3(3)$ Å^3, $Z = 2$, $\rho_{calcd} = 1.483$ Mg/m^3, $\mu = 1.364$ mm^{-1}, $\lambda = 0.71073$ Å, $2\theta_{max} = 55.52$, reflns collected/unique = 21229/4390, $R_{int} = 0.0447$, R1 = 0.0650, wR2 = 0.1805 for 3690 reflections [$I > 2\sigma(I)$], GOF on $F^2 = 1.076$, Flack (*x*) parameter = 0.03(3), CCDC 706470.

(14) The value is ~0.60, although for a 1:2 stoichiometry, the ideal value is 0.66. In solution, an equilibrium may exist between 1:1 and 1:2 coordination. Lee, C.-H.; Miyaji, H.; Yoon, D.-W.; Sessler, J. L. *Chem. Commun.* **2008**, 24.