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A Zn(II) ion selective fluorescence sensor that is not affected by Cd(II)

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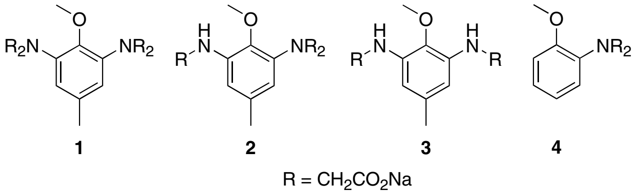
Abstract—Comparison of sensors sodium-2,6-diamino-(*N*,*N*,*N*′,*N*′-tetraacetate)-4-methylanisole **1** and sodium-2,6-diamino-(*N*,*N*,*N*′-triacetate)-4-methylanisole **2** reveal that the loss of an acetyl group in **2** leads to a more selective Zn(II) induced fluorescence enhancement and shows no response to any other metal ions including Cd(II). Structural modifications and AM1 calculations indicate that the sensor uses the three acetyl groups and the 3° amino nitrogen for binding the metal ion. AM1 calculations imply a trigonal bipyramidal coordination for Zn(II) with the solvent molecule occupying one of the axial positions.

Over 300 enzymes and proteins require zinc to carry out their biological functions.[1] For example, the zinc sites in protein domains play structural catalytic, and cocatalytic roles.[2] Zinc is essential for the regulation of DNA synthesis during the proliferation and differentiation of cells.[3] Zinc is also suspected to play a role in neurological disorders such as Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, and epileptic seizures.[4] Environmental impact of Zn ion in sea water due to human activity is being studied.[5] Zinc ion selective fluorescence sensors play an important role in studying these processes.

Several fluorescence sensors for Zn(II) ion have been reported in the literature.[5a,6] Unfortunately, sensors that can selectively detect Zn(II) ion without showing any response to Cd(II) are extremely rare. Selective detection of Zn(II) in the presence of Cd(II) becomes important in environmental matrix when both the ions are likely to be present.[5] Recently, we reported sodium-2,6-diamino-(*N*,*N*,*N*′,*N*′-tetraacetate)-4-methylanisole **1** (Chart 1) as a Zn(II) selective fluorescence sensor that can be synthesized in three simple steps.[7] When the sensor is excited at 300 nm, it has an emission maximum at 386 nm. In the presence of Zn(II) ion, the sensor shows a tenfold enhancement in emission intensity. This sensor also undergoes approximately fivefold increase in the fluorescence intensity in the presence of Cd(II).



1 **2** **3** **4**

R = CH_2CO_2Na

Chart 1. Structure of compounds **1**–**4**.

In this Letter, we present compound **2** as a Zn(II) ion selective sensor that itself has minimal fluorescence, but shows approximately twenty-fivefold enhancement of the fluorescence intensity in the presence of Zn(II), while showing no response to any other metal ions including Cd(II).

Semi-empirical AM1 calculations using SPARTAN 6 of **1**–Zn(II) complex indicates that only three acetate groups of the sensor are involved in binding the metal ion (Fig. 1). This was a surprising result given the geometric

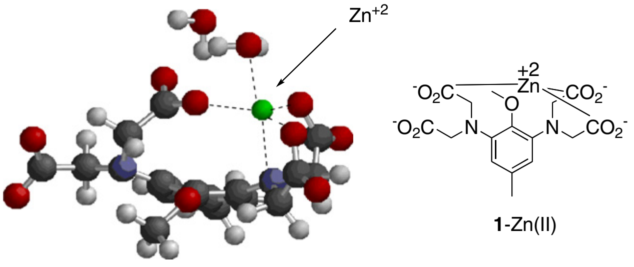


Figure 1. Energy minimized structure of **1**–Zn(II) complex as predicted by AM1 calculations.

Keywords: Zinc sensors; Fluorescence sensors.

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constraints of involving two groups in *meta* positions in binding the metal ion. The presence of the methoxy group between them also implies that the metal ion must be held above the aromatic ring. Also, only three of the acetyl groups interact with the metal, which lies more to the side that contributes two of the acetyl groups. It appears from the calculations that the Zn(II) ion is located roughly above the 3° amino nitrogen and is coplanar with the three carboxylate oxygen atoms. When a molecule of water was added, it occupied a position directly above the metal ion without affecting the positions of other atoms. Further addition of water molecules resulted in no change around the metal ion. The additional water molecules occupied positions close to the carboxylate C=O, implying hydrogen bonding. Based on this result, we propose distorted trigonal bipyramidal coordination for the metal ion with the carboxyl groups in the equatorial positions and the amino nitrogen and a solvent molecule occupying the two axial positions.

Based on the results of the AM1 calculations, we decided to investigate the effect of the number of acetyl groups on the sensor. We synthesized compounds **2** and **3** (Chart 1) by controlling the amount of ethyl bromoacetate using the same procedure as the one used for the synthesis of **1** (see Supplementary data for the detailed procedure). An alternative structure of **1**–Zn complex could involve one iminodiacetate group and the methoxy group. To test this possibility, we synthesized anisidine derivative **4**, which should be virtually indistinguishable from **1** as a sensor if indeed only one iminodiacetate group and the methoxy group were involved in binding the metal ion.

The fluorescence spectra for compounds **2**–**4** were measured in 0.1 M HEPES buffer at pH 7 in the presence of 0.135 M NaCl using Fluorolog-3 spectrofluorometer. Compound **4** has an emission maximum at 370 nm, which is quenched in the presence of Zn(II) ion (Supplementary data). This result shows that the binding behavior of **4** is different from sensor **1**. Based on this observation, we conclude that the geometry optimization of the AM1 calculations is at least partially correct in predicting the involvement of the acetyl groups from both the amino groups. Compound **3** did not fluoresce either in the presence or absence of the metal ions. While this does not rule out Zn(II) binding of **3**, this compound cannot act as a fluorescence sensor for the metal ion. The fluorescence spectrum of compound **2** ($\lambda_{ex} =$ 300 nm, pH 7) was very interesting (Fig. 2). As expected, similar to compound **1**, Co(II), Ni(II), and Cu(II) quenched the fluorescence of sensor **2**. Also, Ca(II) and Mg(II) had no effect on the fluorescence of the sensor. Surprisingly, Cd(II) enhanced the fluorescence intensity of the sensor marginally, while Zn(II) addition resulted in nearly twenty-fivefold enhancement of the fluorescence intensity. These results do not rule out the binding of the metal ions to the sensors, but the fluorescence enhancement is observed only for Zn(II). The fluorescence enhancement of the sensor in the presence of Zn(II) was independent of pH between 6 and 7.6. However, at higher pH the fluorescence intensity dropped due to the precipitation of $Zn(OH)_2$. UV spectrum of sensor **1** or **2** did not show any detectable change in the presence of the metal ions.

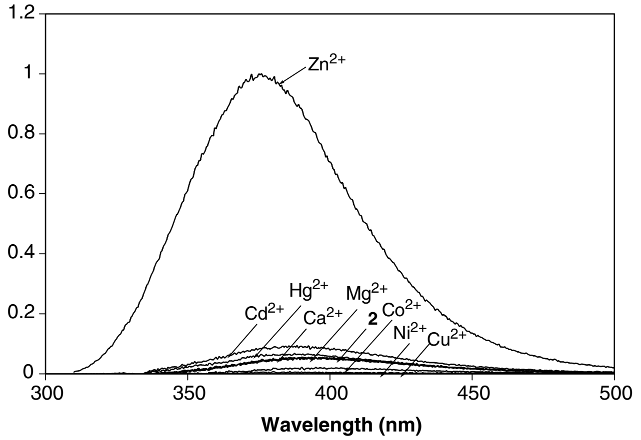

Figure 2. Fluorescence spectrum of **2** in the presence of various metal ions in 0.1 M HEPES buffer at pH 7 ($\lambda_{ex} = 300$ nm).

Sensors **1** and **2** act as sensors due to enhanced fluorescence intensity rather than a change in the emission wavelengths. The emission profile of the sensors as well as their metal bound complexes have the same emission profile. This implies that the fluorescence quantum yield of the sensor is increased only in the presence of Zn(II) ions. While the reason for this enhancement is still not clear, it is possible that Zn(II) binding provides rigidity to the molecule thereby blocking some of the nonradiative decay pathways leading to an enhanced fluorescence intensity.

Figure 3 compares the fluorescence intensity of sensors **1** and **2** in the presence of Zn(II) and Cd(II) metal ions. When excited at 300 nm in 0.1 M HEPES buffer at pH 7 in the presence of 0.135 M NaCl, both the sensors emit with approximately the same intensity. The emission maxima for **2** and its complexes with the metal ions are blue shifted by about 10 nm. While addition of Cd(II) marginally affects the emission spectrum of **2**, a fourfold enhancement of intensity was observed for **1**.

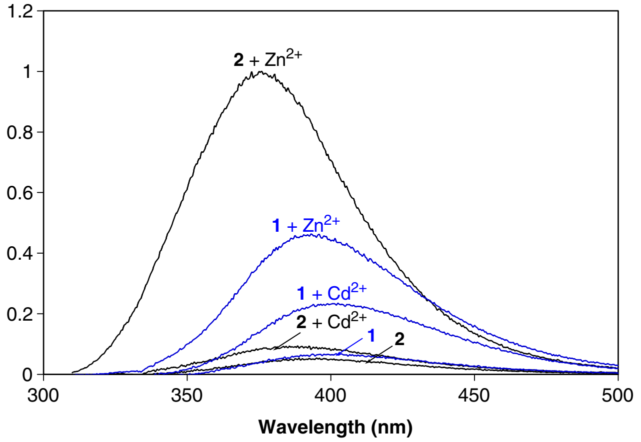

Figure 3. Comparison of the fluorescence spectra of **1** and **2** in the presence of Zn(II) and Cd(II) ions in 0.1 M HEPES buffer at pH 7 ($\lambda_{ex} = 300$ nm).