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A Highly Selective Fluorescent Chemosensor for Lead Ions

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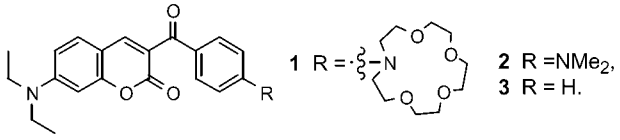
Pb^{2+} is a detrimental metal ion causing adverse environmental and health problems. A wide variety of symptoms (which include memory loss, irritability, anemia, muscle paralysis, and mental retardation) have been attributed to lead poisoning, suggesting that Pb^{2+} affects multiple targets in vivo.[1] Development of fluorescent sensors for Pb^{2+} would help to clarify the cellular role of lead ions in vivo as well as to monitor Pb^{2+} concentration temporally in the Pb^{2+} contaminated sources. While many effective fluorescent sensors[2] have been successfully developed for alkali, alkaline earth, and Zn^{2+},[3] few have been explored for Pb^{2+}. As many of heavy metals are known as fluorescence quenchers via enhanced spin−orbital coupling,[4] energy, or electron transfer,[5] development of selective as well as sensitive fluorescent sensors for Pb^{2+} presents a challenge.

A selective, ratiometric fluorescent sensor based on polypeptide scaffolds equipped with a microenvironment-sensitive fluorophore for Pb^{2+} has been described.[6] In addition, some of fluorophore-appended macrocycles were shown to respond to Pb^{2+} via photoinduced charge transfer,[7a] inhibition of photoinduced electron transfer[7b−d] or self-assembling fluorescence enhancement.[7e] However, discrimination between chemically closely related metal ions is not very high. And yet, there is still plenty of room for improvements in terms of fluorescence intensity enhancement, selectivity, as well as suitable fluorophores with visible light excitation.

Herein we report a new chemosensor **1**, which can signal Pb^{2+} selectively and display large fluorescence enhancement.



Ketoaminocoumarin is selected as a signal-transducing unit owing to its high photostability and visible excitation wavelength. Moreover, it has been established that the quantum yield (Φ) of 7-diethylamino-3-(4-dimethylaminobenzoyl)coumarin (**2**, $\Phi = 0.001$) is approximately 350-fold lower than that of 3-benzoyl-7-diethylaminocoumarin (**3**, $\Phi = 0.35$).[8] Thus, judicious incorporation of **2** in the fluorescent chemosensor design as the signal transduction unit would provide low basal fluorescence and give fluorescence enhancement upon the substrate binding, only if the lone pair of the nitrogen atom from benzoyl moiety is involved in the binding event.

Chemosensor **1** is easily prepared by the condensation of 4-(*N*,*N*-diethylamino)sailicylaldehyde with β-ketoester appended with 15-monoazacrown-5 ether in the presence of piperidine.[9] To address the role of the monoazacrown ether played in the complexation, **2** is also prepared by the similar procedure to serve as a control. Absorption and fluorescence emission titrations of 1×10^{-5} M of **1** and **2** with group I (Li^+, Na^+, and K^+), group II (Mg^{2+}, Ca^{2+}, and Ba^{2+}), and heavy metal ions (Fe^{2+}, Mn^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Ag^+, and Pb^{2+})[10] are performed in acetonitrile at 25 °C.

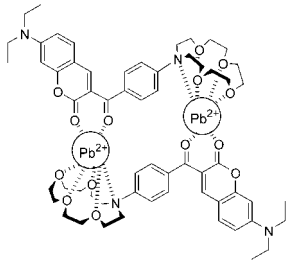


Figure 1. A proposed 2:2 complex formed between **1** and Pb^{2+}.

The results show that the univalent metal ions do not cause significant changes in either absorption or fluorescence emission spectra of **1** or **2**, whereas red-shift absorptions are observed upon the addition of divalent metal ions. It is worth noting that the complex **1**·Pb^{2+} exhibits only 15 nm red-shift and **2**·Pb^{2+} displays 63 nm shift, reflecting the binding modes are different in these two complexes. The large bathochromic shift for **2**·Pb^{2+} can be attributed to the strong stabilization of internal charge-transfer (ICT) state by the complexation of metal ions with the two carbonyl groups of the fluorophore via chelation.[2c,11] In fluorescence titration studies, **1** displays 40-, 12-, and 18-fold fluorescence enhancements for Pb^{2+} (10 equiv), Ba^{2+} (100 equiv), and Cu^{2+} (100 equiv), respectively, and **2** only responds to Cu^{2+} (50 equiv) with 26-fold fluorescence enhancement among 15 metals investigated.

Disappearance of the characteristic carbonyl absorption of **1**·Pb^{2+} complex in the infrared spectra and downfield shifts experienced by the proton or carbon nuclei in the carbonyl proximity support that the relatively strong binding and the high preference for Pb^{2+} are due to the lariat carbonyl participation. While Job plot[12] shows that the stoichiometry of **1**·Pb^{2+} complex is 1:1, the observed sigmoidal binding curves and large Hill coefficient suggest that the binding of Pb^{2+} and **1** might be cooperative. A 2:2 complex of Pb^{2+} and **1** is thus proposed (Figure 1)[7e,13,14] The structure is also consistent with the small red-shift in the absorption since one of the coordination sites is situated close to the negative, and the other, to the positive end of molecular dipole, the opposite effects counterbalancing each other to some extent.[2c,11a] The pronounced fluorescence enhancement can be rationalized in terms of photoinduced charge transfer (PCT)[2c] and metal binding-induced conformational restriction upon complexation.[15] The magnitude of enhancement in emission and selectivity from **1** are remarkable and can be detected visually.

To explore further the utility of **1** as an ion-selective fluorescent chemosensor for Pb^{2+}, the competition experiments are conducted

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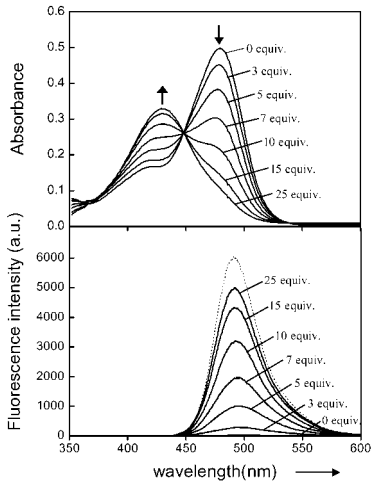

Figure 2. Absorption and fluorescence spectra of **1** (1×10^{-5} M in acetonitrile) in the presence of 1 mM of Mg^{2+} upon addition of increasing amounts of Pb^{2+} (Dashed line corresponds to the **1**·Pb^{2+} complex without Mg^{2+}). Excitation is at 411 nm.

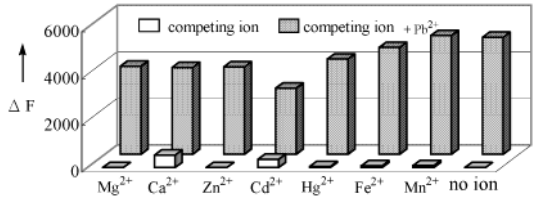

Figure 3. The fluorescence intensity change profile of **1** (1×10^{-5} M in acetonitrile) in the presence of selected metal ions. Excitation is at 411 nm, and emission is monitored at 491 nm.

in which **1** is first incubated with various metal ions at their saturation concentrations, and Pb^{2+} is added until the total concentration of Pb^{2+} reaches saturation concentration (100 μM). Although absorption bands provide the most structurally relevant information about the binding, the absorption as well as fluorescence spectra are exploited to monitor the competition events. Figure 2 illustrates the changes of these spectra upon the addition of increasing amounts of Pb^{2+} in the presence of 1 mM of Mg^{2+}. The absorption spectrum of **1** · Mg^{2+} is consistent with the two carbonyl groups of the fluorophore taking part in the binding, regardless of the presence of the monoazacrown moiety. As the Pb^{2+} concentration increases, the characteristic absorption at 478 nm for **1**·Mg^{2+} complex gradually decreases, and a new characteristic absorption at 428 nm, responsible for **1**·Pb^{2+} complex, starts to appear with a concomitant fluorescence increase. Notably, Pb^{2+} ions do not simply replace Mg^{2+} in the same binding sites but form a new fluorescent complex.

The selectivity of **1** for Pb^{2+} over Ca^{2+}, Zn^{2+}, Cd^{2+}, Fe^{2+}, Mn^{2+}, and Hg^{2+} is particularly important because Pb^{2+} targets both Ca^{2+}- and Zn^{2+}-binding sites in vivo[16] and Cd^{2+}, Hg^{2+}, Fe^{2+}, and Mn^{2+} are metal ions that frequently interfere with Pb^{2+} analysis. The competition-based fluorescence intensity profiles for these metal ions are shown in Figure 3. On the basis of the photospectroscopic data, it seems that Zn^{2+} and Mn^{2+} behave like Mg^{2+}, whereas Cd^{2+}, Hg^{2+}, and Fe^{2+} act differently. Cd^{2+}, Hg^{2+}, and Pb^{2+} are probably bound in the same binding site with different affinities, and the replacement occurs once the higher-affinity metal ion is added in the solution. No interference is observed while performing titrations with Pb^{2+} in a complex matrix containing 50-fold excess of univalent metal ions.

We also tested the possibility of using fluorescent chemosensor **1** for determining Pb^{2+} concentration in aqueous solution. When the aqueous solution of $Pb(ClO_4)_2$ is added to **1** in acetonitrile, neither the binding strength nor the magnitude of fluorescence enhancement is affected, whereas the stability of **1**·Pb^{2+} complex is attenuated (2 orders of magnitude weaker) when 5% of water−acetonitrile is used as the solvent for both of $Pb(ClO_4)_2$ and **1**. It is worth noting that the fluorescence enhancement is still quite pronounced ($I/I_0 = 6$) in this organic/aqueous mixture.

In summary, the new fluorescent chemosensor **1** exhibits a high affinity and selectivity for Pb^{2+}. The remarkable fluorescent response to Pb^{2+} binding is unprecedented. Our future efforts will be focused on developing fluorescent chemosensors, which can function in aqueous systems with high affinities for Pb^{2+}. In addition, a fluorescent chemosensor library[17] for other metal ions will be established by appending versatile molecular recognition units, for example, different crown ethers, to the current molecular design.

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Supporting Information Available: Selected synthetic procedures and structural characterization for **1**, metal binding profiles, binding isotherms, as well as photospectroscopic data of titrations (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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