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Triple Channel Sensing of Pb(II) Ions by a Simple Multiresponsive Ferrocene Receptor Having a 1-Deazapurine Backbone

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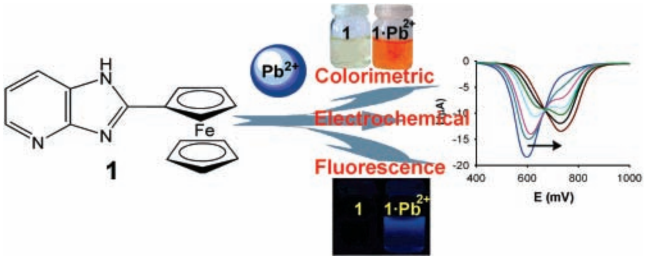
ABSTRACT



A new redox, chromogenic, and fluorescent chemosensor molecule based on a deazapurine ring selectively senses aqueous Pb^{2+} in acetonitrile over other metal ions examined: redox shift ($\Delta E_{1/2} = 0.15$ V of the Fe(II)/Fe(III) redox couple), the colorless to orange color change, and an emission change of 620-fold, with an unprecedented detection limit of 2.7 μg L^{-1}. The signal transduction occurs via a reversible CHEF with this inherent quenching metal ion.

Lead pollution is an ongoing danger to the environment[1] and human health, particularly, in children (memory loss, irritability, anemia, muscle paralysis, and mental retardation).[2] Thus, the level of this detrimental ion, which is present in tap water as a result of dissolution from household plumbing systems, is the object of several official norms. The World Health Organization established in 1996 guidelines for drinking water quality,[3] which included a lead maximal value of 10 mg L^{-1}. Recently, considerable efforts have been undertaken to develop fluorescent chemosensors for Pb^{2+} ions based on peptide,[4] protein,[5] DNAzyme,[6] polymer,[7] and small-molecule[8] scaffolds. There is, however, a paucity of use of multichannel receptors as potential guest reporters via multiple signaling patterns. Specifically, the development of triple chromogenic, fluorogenic, and electrochemical Pb^{2+} selective chemosensors is, as far as we know, an unexplored subject, and only two dual chromogenic and redox receptors have been recently described.[9]

Among the various artificial receptors reported in the literature, those employing benzimidazole derivatives as an anion-binding motif have attracted considerable attention very recently.[10] In the context of this work, it has been found

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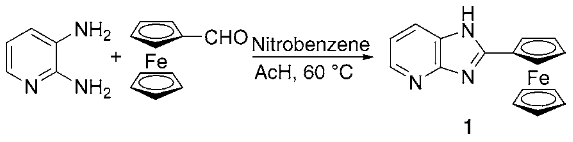
that the introduction of an additional heteroatom (N) in the benzimidazole ring, with similar basicity to that of the imidazole nitrogen, imparts an interesting behavior as the nitrogen atom of the pyridine ring cooperates with the basic nitrogen of the imidazole ring and consequently enhances the binding affinity toward metal cations. The 2-ferrocenylimidazo[4.5-*b*]pyridine **1** was chosen to develop a triple channel system, where the fluorescent reporter is integrated with and the redox unit is linked to the guest cation binding site. Despite their easy synthesis,[11] photophysical properties,[12] and biological applications,[13] imidazo[4.5-*b*]pyridines (1-deazapurines) have never been used for sensing purposes, to the best of our knowledge. As depicted in Scheme 1, receptor **1** was easily synthesized in 60% yield from the reaction of ferrocene carboxaldehyde with 2,3-diaminopyridine in nitrobenzene in the presence of acetic acid (see the Supporting Information).

Scheme 1. Preparation of 2-Ferrocenylimidazo[4,5-*b*]pyridine **1**



The metal cation complexing properties of **1** have been investigated by electrochemistry, spectroscopic measurements, and ^{1}H NMR spectroscopy. Previous studies on ferrocene-based ligands have shown that not only the characteristic LE band in the absorption spectra is perturbed upon complexation but also a positive shift of the Fe(II)/Fe(III) redox couple is observed.[14] The results show that the univalent metal ions do not cause significant changes in either redox potential, absorption, or the fluorescence emission spectrum, whereas redox shift and red-shift absorptions are observed upon the addition of Pb^{2+}, Zn^{2+}, and Hg^{2+} metal cations.

The reversibility and the relative oxidation potential of the ferrocene/ferrocenium redox couple in receptor **1** was determined by cyclic voltammetry (CV) and Osteryoung square wave voltammetry (OSWV) in CH$_3$−CN solutions containing 0.1 M [(*n*-Bu)$_4$N]ClO$_4$ as supporting electrolyte. As expected, compound **1** displays a reversible one-electron oxidation process at $E_{1/2} = 0.59$ V versus decamethylferrocene, due to the ferrocene/ferrocenium redox couple. On stepwise addition of Pb^{2+} ions, a clear evolution of the oxidation wave to $E_{1/2} = 0.74$ V ($\Delta E_{1/2} = 150$ mV) was observed, and maximum perturbation of the CV was obtained with 1 equiv of added Pb^{2+} ions. Receptor **1** also showed a perturbation of the oxidation wave in the presence of Zn^{2+} and Hg^{2+} ions. Upon addition of small amounts of these metal cations, a new oxidation wave at $E_{1/2} = 0.68$ V ($\Delta E_{1/2} = 90$ mV) and $E_{1/2} = 0.71$ V ($\Delta E_{1/2} = 120$ mV) anodically shifted appeared. Remarkably, the presence of Li$^+$, Na$^+$, K$^+$, Ca^{2+}, Mg^{2+}, Cu^{2+}, Ni^{2+}, and Cd^{2+} metal cations had no effect on neither OSWV nor CV, even when present in large excess (Figure 1).

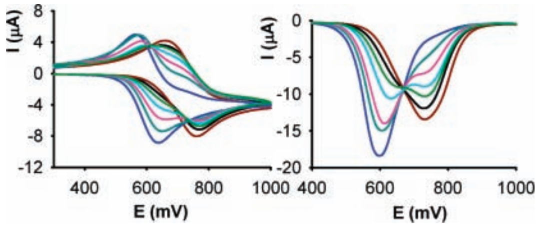


Figure 1. Evolution of the CV (left) and OSWV (right) of **1** (1×10^{-3} M) in CH$_3$CN using [(*n*-Bu)$_4$N]ClO$_4$ as supporting electrolyte when Pb(ClO$_4$)$_2$ is added: from 0 (blue) to 1 equiv (deep red).

The metal recognition properties of the receptor **1** toward the above-mentioned set of metal cations were also evaluated by UV/vis spectroscopy. Titration experiments for CH$_3$CN solutions of receptor **1** ($c = 1 \times 10^{-4}$ M) and the corresponding *aqueous* metal cation solutions ($c = 2.5 \times 10^{-2}$ M) were performed and analyzed quantitatively.[15] The

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(15) Association constants were obtained using the computer program Specfit/32 Global Analysis System, 1994−2004 Spectrum Software Associates (SpecSoft@compuserve.com). The Specfit program was acquired from Bio-logic SA (www.bio-logic.info) in January 2005.

spectrum of the receptor **1**, which displays a strong band at $\lambda = 309$ nm (HE) ($\epsilon = 24\,620$ M^{-1} cm^{-1}) and a broad band (LE) of lower intensity at $\lambda = 448$ nm ($\epsilon = 1014$ M^{-1} cm^{-1}), was also substantially affected by the Pb^{2+} recognition. Figure 2 shows the changes induced in the UV/vis spectrum of **1** upon addition of successive substoichiometric amounts of aqueous Pb^{2+} introduced as perchlorate. The most prominent features observed during the complexation process are a progressive red shift of the HE band ($\Delta\lambda = 11$ nm) and LE band ($\Delta\lambda = 44$ nm). These changes occur around one isosbestic point at 315 nm, which suggest interconversion between two species. These facts are responsible for a perceptible change of color from colorless to orange, which can be used for the "naked eye" detection of this divalent cation. Binding assays using the method of continuous variations (Job's plot) are consistent with a 1:1 receptor **1**/Pb^{2+} complex, and the association constant was calculated: $K_a = 3.3 \times 10^5$ M^{-1}.

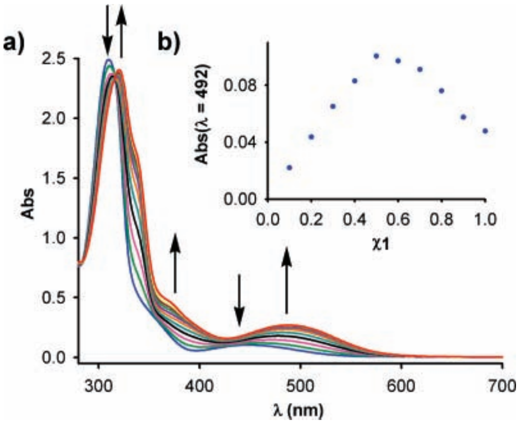

Figure 2. (a) Changes in the absorption spectra of **1** (1×10^{-4} M) in CH_3CN upon addition of increasing amounts of Pb^{2+} (2.5×10^{-2} M in H_2O). (b) Job's plot for **1** and Pb^{2+}, indicating the formation of 1:1 complexes. The total $[\mathbf{1}] + [Pb^{2+}] = 1 \times 10^{-4}$ M.

This result has also been confirmed by ESI-MS, where a peak at *m*/*z* 510 corresponding to the 1:1 complex is observed. The relative abundance of the isotopic cluster was in good agreement with the simulated spectrum of the $[\mathbf{1}\cdot Pb^{2+}]$ complex. In addition, a peak at *m*/*z* 609 corresponding to $[\mathbf{1}\cdot Pb^{2+} + (ClO_4)]^+$ also appeared (see the Supporting Information). The HE and LE bands were also red shifted (6−11 and 29−34 nm, respectively) although without any change of color upon addition of Zn^{2+} ($K_a = 1.4 \times 10^4$ M^{-1}) and Hg^{2+} ions, and a well-defined isosbestic point at $\lambda = 318$ nm was also observed.

Receptor **1** exhibits a very weak fluorescence in CH_3CN ($c = 10^{-5}$ M), revealing that the excitation spectrum at $\lambda_{exc} = 330$ nm is an ideal excitation wavelength (see the Supporting Information). The emission spectrum shows two broad and structureless bands at 364 and 377 nm due to the 1-deazapurine ring, with rather low quantum yield[12a] ($\Phi = 0.0004$). Upon addition of the above-mentioned set of metal cations, receptor **1** showed a large CHEF (chelation-enhanced fluorescence) effect only with aqueous Pb^{2+} cations, even though there was a relatively small CHEF effect with Zn^{2+} (Figure 3). From the fluorescence titrations, the association constants for Pb^{2+} and Zn^{2+} were calculated to be $K_a = 6.1 \times 10^5$ and 2.7×10^4 M^{-1}, respectively. The selectivity for Pb^{2+} was more than 20 times greater than that for Zn^{2+}. Overall emission changes of 620- and 150-fold were observed for Pb^{2+} and Zn^{2+}. Moreover, the increases in quantum yield of **1** induced by Pb^{2+} ($\Phi = 0.044$) and Zn^{2+} ($\Phi = 0.014$) ions were 110- and 35-fold, respectively.

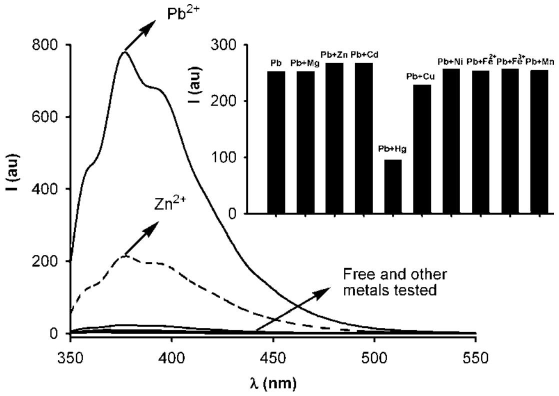

Figure 3. Changes in the fluorescence spectra of **1** (1×10^{-5} M) in CH_3CN upon addition of the several cations tested. Inset: fluorescence emission intensity of **1** upon addition of 0.5 equiv of Pb^{2+} in the presence of 0.5 equiv of background metal ions in H_2O.

In addition, it should be noted that high sensitivity for Pb^{2+} is achieved by the receptor **1** in the present study. The detection limit, calculated as 3 times the standard deviation of the background noise, is unprecedented, and it was found to be 1.32×10^{-8} M (2.7 μg L^{-1}), which is lower than the maximum permitted amount of Pb^{2+} in drinking water defined by the World Health Organization.

The selectivity of **1** for Pb^{2+} over Ca^{2+}, Cd^{2+}, and Hg^{2+} is particularly important because Pb^{2+} targets Ca^{2+}-binding sites in vivo[16] and Cd^{2+} and Hg^{2+} are metal cations that frequently interfere with Pb^{2+} analysis. Therefore, the selective binding ability of **1** for Pb^{2+} over other metal ions such as Ca^{2+}, Cd^{2+}, Zn^{2+}, and Hg^{2+} was investigated by the competition-based fluorescence experiments. Profiles for these metal cations are shown in Figure 3, and it is obvious that receptor **1** has a highly selective response to Pb^{2+}.

^1H NMR experiments were performed to explore the coordination mechanism of receptor **1** and Pb^{2+} ions (Figure 4). The complexation process induces not only noticeable downfield changes in the chemical shifts in the pyridine ring protons ($\Delta\delta = 0.13{-}0.39$ ppm) but also relatively large downfield shifts in the ferrocene protons ($\Delta\delta = 0.12{-}0.27$

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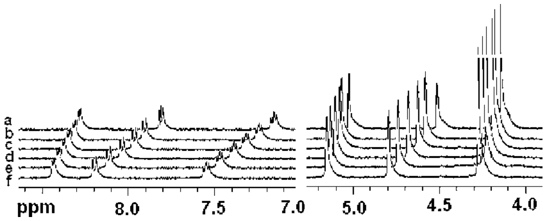

Figure 4. Evolution of the ^{1}H NMR spectra of **1** in CD_3CN upon addition of 0 (a), 0.2 (b), 0.4 (c), 0,6 (d), 0.8 (e), and 1 equiv (f) of Pb^{2+}.

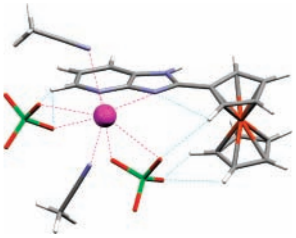

Figure 5. Calculated (B3LYP/6-31G*/SDD-ecp) structure for complex **1**$\cdot Pb(ClO_4)_2(CH_3CN)_2$.

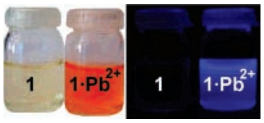

Figure 6. Visual features observed in CH_3CN solutions of **1** and **1**$\cdot Pb^{2+}$. Left, absorptions; right, emissions.

ppm). Addition of 1 equiv of Zn^{2+}, however, induces only downfield shifts in the pyridine ring protons (see the Supporting Information), whereas Hg^{2+} metal ions caused a broadening of all peaks in the ^{1}H NMR spectrum, from which accurate chemical shifts could not be obtained.

Upon addition of Pb^{2+} metal cations to a colorless solution of **1** in CH_3CN, both an orange color and a strong fluorescence appear. The turn-on response is reversible because both signals disappear upon the addition of an excess of cyclen or ethylenediamine. Furthermore, a CH_2Cl_2 solution of the complex **1**$\cdot Pb^{2+}$ was treated with an ethylenediamine aqueous solution, and the absorption, ^{1}H NMR spectra, and CV of the extracted product were identical to those of free receptor **1**.

Formation of the complex **1**$\cdot Pb^{2+}$ and the subsequent extraction of the metal cation with ethylenediamine were carried out over several cycles. The optical and emission spectra were recorded after each step and were found to be fully recovered on completion of the step, thus demonstrating the high degree of reversibility of the sensing process.

DFT-based quantum chemical calculations (see the Supporting Information) have shown that **1** is the most stable of the two possible tautomers (by $\Delta G°_{MeCN} = -0.36$ kcal$\cdot$mol^{-1}), and that this ligand forms a rather stable complex with $Pb(ClO_4)_2$ ($\Delta G°_{MeCN} = -16.24$ kcal$\cdot$mol^{-1}). Two additional weakly coordinating acetonitrile ligands ($d_{Pb-N} = 2.871$ and 3.006 Å) are explicitly added in order to complete an eight-coordination sphere around the large Pb^{2+} metal cation (Figure 5). The azapurine ligand **1** provides only one strong linkage to the Pb^{2+} ion through the pyridine-like N atom ($d_{Pb-N} = 2.557$ Å), whereas the other adjacent N atom is weakly bound ($d_{Pb-N} = 3.157$ Å). Finally, four coordination positions are occupied by one strongly bound ($d_{Pb-O} = 2.441$ Å) and other weakly interacting O atoms ($d_{Pb-O} = 3.021$ and 3.200 Å) in every perchlorate unit. We assume that the obtained structure agrees with the obtained ^{1}H NMR data because it explains that the pyridine H-7 and the ferrocene Cp_1-Hα and Cp_2-H atoms are less deshielded due to the formation of hydrogen bonding with perchlorate O-atoms or the imidazole N-atom (Figure 5).

In conclusion, the structurally simple and easily synthesized receptor **1** not only displays a strong interaction between the azapurine ring and Pb^{2+} ions but also distinguishes this metal cation from other metal ions, including the strong competitors Hg^{2+} and Zn^{2+}. Receptor **1** represents the first example of a Pb^{2+} chemosensor molecule, which combines redox detection, and the sensitivity of fluorescence (2.7 μg L^{-1}) with the convenience of a colorimetric assay (Figure 6). Another advantage afforded by **1** is that it closely mimics the interaction of natural purines with metal ions.

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Supporting Information Available: General comments, computational details, synthesis, characterization, electrochemical, UV/vis, and fluorescence data, detection limits and reversibility experiments, energy and Cartesian coordinates for all calculated compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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