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2-Aza-1,3-butadiene Derivatives Featuring an Anthracene or Pyrene Unit: Highly Selective Colorimetric and Fluorescent Signaling of Cu^{2+} Cation

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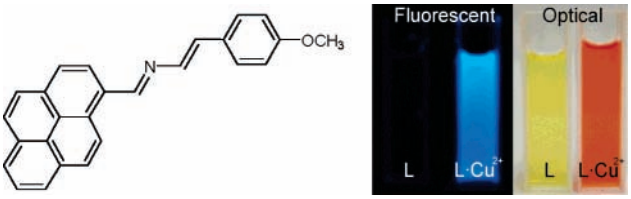
ABSTRACT



A new probe based on an anthryl derivative bearing an azadiene side chain selectively senses Cu^{2+} in acetonitrile through two different channels: the yellow-to-orange color change and a remarkable enhancement of the fluorescence, whereas the pyrenyl analogous behaves as a fluorescent sensor for Cu^{2+} and Hg^{2+} in aqueous environment.

The development of fluorescent molecular sensors for metal ions, especially for cations with biological interest, has always been of particular importance and usually involves the design and synthesis of molecules containing binding sites and a signaling subunit able to display selective changes in fluorescence emission intensity upon guest binding.[1] More specifically, sensors directed toward the detection and measurement of divalent copper have enjoyed particular attention. The soft transition metal ion Cu^{2+} is third in abundance (after Fe^{2+} and Zn^{2+}) among the essential heavy metal ions in the human body and plays a pivotal role in a variety of fundamental physiological processes in organisms ranging from bacteria to mammals.[2] On the other hand, Cu^{2+} can be toxic to biological systems when levels of Cu^{2+} ions exceed cellular needs, and it is also capable of displacing other metal ions which act as cofactor in enzyme-catalyzed reactions.[3] Thus, copper, on one hand, is important for life but, on the other hand, is highly toxic to organisms. For these reasons, the past few years have witnessed a number of reports on the design and synthesis of fluorescent sensors for the detection of Cu^{2+} ions. For most of the reported Cu^{2+} fluorescent sensors, the binding of the metal ion causes a quenching of the fluorescence emission,[4] due to its paramagnetic nature,[5] although a few sensors in which the binding of a Cu^{2+} ion causes an increase in the fluorescence have also been reported.[6] However, the low sensitivity and high order of interference by chemically closely related metal

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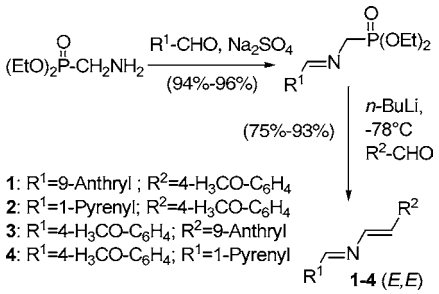
ions has necessitated the development of new highly selective and sensitive Cu^{2+} fluoroionophores.[7]

Fluorescene quenching is not only disadvantageous for a high signal output upon recognition but also hampers temporal separation of spectrally similar complexes with time-resolved fluorometry.[8] Thus, it is of interest that the recognition of Cu^{2+} by the sensor does not quench the fluorescence. To improve the fluorescence intensity enhancement of the receptor upon binding of Cu^{2+}, one needs to carefully design the receptor molecule containing a fluorophore so that the photoinduced intramolecular electron transfer (PET) responsible for fluorescence quenching is maximized in the receptor, whereas the PET is minimized in the Cu^{2+}-bound state of the receptor. Anthracene and pyrene are two of the fluorescent groups most widely used in the development of fluorescent chemosensors.[9]

On the basis of this body of work, we have designed and studied the metal ion binding properties of ligands **1** and **2** which are composed of two structural subunits: a ionophore for selective recognition of metal ions, constituted by a 2-aza-1,3-diene moiety as a putative cation-binding site,[10] and a fluorophore for signal transduction (anthracene or pyrene) linked to the 1-position of the azadiene bridge. These two components are intramolecularly correlated together such that the binding of the target metal ion causes significant changes to the photophysical properties of the fluorophore linked to the azadiene bridge.

To study the influence of the relative position of the fluorescence unit, with respect to the nitrogen atom within the bridge, on the recognition properties of these kind of ligands, the corresponding regioisomers **3** and **4** were also prepared and studied (Scheme 1).

Scheme 1. Synthesis of the Fluoroionophores **1**−**4** Studied



Preparation of these ligands was achieved following the recently described method for the synthesis of 1,4-disubstituted 2-aza-1,3-butadienes.[11] Thus, diethyl aminomethylphosphonate[12] was first condensed with the appropriate aldehyde to give the corresponding *N*-substituted diethyl aminomethylphosphonate in almost quantitative yield. Generation of metalloenamine by reaction with *n*-BuLi at −78 °C and subsequent reaction with the adequate aldehyde provided the ligands **1**−**4**, characterized using conventional methods.

The chemosensor behavior of ligands **1** and **2** with several metal cations (Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, Sm^{3+}, Eu^{3+}, Yb^{3+}, and Lu^{3+}),[13] in CH_3CN or CH_3CN/H_2O (70/30), was investigated by UV−vis and fluorescence measurements. All titration studies carried out in CH_3CN/H_2O (70/30) were conducted at pH 7 (0.1 M HEPES), and the titration experiments were analyzed using a computer program.[14]

The UV−vis spectrum of **1** in CH_3CN exhibits the typical anthracene absorption bands at $\lambda = 256$ and 331 nm along with a low energy (LE) band centered at 413 nm attributed to the aza bridge. Titration experiments carried out by using the above-mentioned set of metal cations ($c = 2.5 \times 10^{-3}$ M in CH_3CN) demonstrate that only Cu^{2+} promotes remark-

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able responses. Thus, the bands at $\lambda = 413$ and 331 nm progressively disappear, and at the same time, new bands at $\lambda = 493$ and 372 continuously increase in intensity, reaching a maximum when 1 equiv of this metal cation was added. The red shift observed for the new low-energy absorption band ($\Delta\lambda = 80$ nm) resulted in a naked eye color change from yellowish to orange. Two well-defined isosbestic points at $\lambda = 352$ and 455 nm were found, indicating the presence of a unique complex in equilibrium with the neutral ligand (Figure 1b). The resulting titration isotherm fit nicely a 1:1

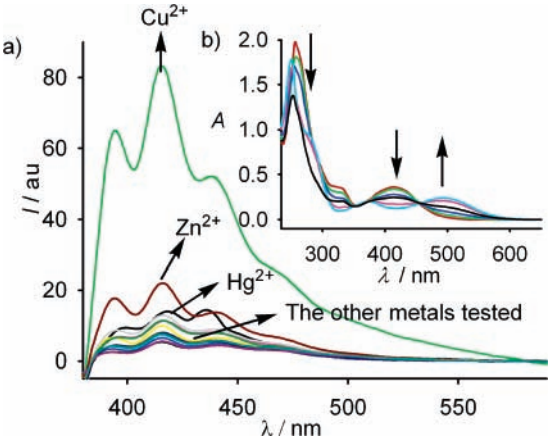

Figure 1. (a) Fluorescence spectra of **1** ($c = 2.5 \times 10^{-5}$ M) in CH_3CN in the presence of Cu^{2+} and other metal ions ($\lambda_{exc} = 370$ nm). (b) Evolution of the UV/vis spectrum of **1** ($1 \cdot 10^{-4}$ M in CH_3CN) with increasing amounts of Cu^{2+}. Arrows indicate the absorptions that increase or decrease during the experiment.

binding model, and the association constant was 3.6×10^6 M^{-1} (error < 10%).

The fluorescent spectral properties of ligand **1** ($c = 2.5 \times 10^{-5}$ M) were determined in both CH_3CN and CH_3CN/H_2O (70/30) as solvents ($\lambda_{exc} = 370$ nm) showing a very weak fluorescence and absence of the typical structured pattern of the parent anthracene. However, when ligand **1** (Figure 1a and Supporting Information) was titrated with Cu^{2+} in both solvents, the anthracene-like spectrum was clearly observable with three maxima at $\lambda = 394$, 416, and 438 nm. After addition of 1 equiv of Cu^{2+} to a solution of the ligand **1**, in CH_3CN ($\phi = 0.0006$) or CH_3CN/H_2O (70/30) ($\phi = 0.002$), the fluorescence quantum yield increased by a factor of 87 ($\phi = 0.056$) and 3 ($\phi = 0.006$), respectively. These data suggest that the coordination of the metal ion with the N atom in the aza-bridge is taking place so that the responsible mechanism for fluorescence quenching in the free ligand is minimized in its metal-bound state. Moreover, ligand **1** was found to have a detection limit[15] of 2.55×10^{-6} and 4.58×10^{-6} M as fluorogenic sensor for the analysis of Cu^{2+} in CH_3CN and CH_3CN/H_2O (70/30), respectively (see the Supporting Information). While Zn^{2+}, Hg^{2+}, and Pb^{2+} show a very slight increase in the fluorescent emission of ligand **1**, the other tested alkali, alkaline earth, transition, and lanthanide metal ions showed almost insignificant changes. In the aqueous solvent, the stoichiometry of the complex system was also determined by the changes in the fluorogenic response of **1** in the presence of varying concentrations of Cu^{2+}, the results indicating the formation of a 1:1 complex with an association constant of 6.3×10^5 M^{-1} and a detection limit of 4.66×10^{-6} M (see the Supporting Information).

The absorption spectrum of compound **2**, with a pyrene subunit conjugated to a 2-aza-1,3-butadiene group, displays the typical pyrene absorption bands[16] along with a LE broad band centered at $\lambda = 404$ nm, attributed to the aza-bridge, which is responsible for its pale yellow color. Among the above-mentioned set of cations tested, only Cu^{2+} and Hg^{2+} interact with **2** in CH_3CN ($c = 2.5 \times 10^{-5}$ M) causing a red shift in its LE band by 92 and 96 nm, respectively (see the Supporting Information): the absorption band at 404 nm of **2** gradually decreases with concomitant increase of a new band at 496 and 500 nm, respectively, which is responsible for the change of color from yellowish to deep orange. In both cases, two clear isosbestic points at 275 and 440 nm were observed during the spectral titration, indicating the formation of a well-defined **2**−metal cation complex (Figure 2a). From these experiments, a 1:1 (**2**/Cu^{2+} or **2**/Hg^{2+})

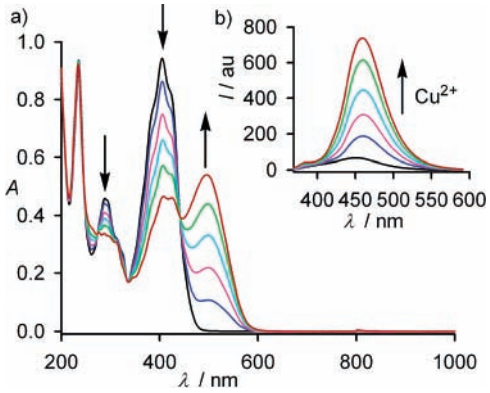

Figure 2. Variation of the UV/vis in CH_3CN (a) and fluorescence spectra in CH_3CN/H_2O (b) of ligand **2** ($c = 2.5 \times 10^{-5}$ M) upon addition of increasing amounts of $Cu(OTf)_2$; arrows indicate the absorptions (or emissions) that increase (up) and decrease (down) during the experiments.

binding model was confirmed and the association constants (K_a) of **2** with Cu^{2+} and Hg^{2+} were calculated to be 8.55×10^5 and 2.36×10^5 M^{-1}, respectively, which indicate that Cu^{2+} and Hg^{2+} bind to a similar extent to **2** in the ground state. The cation binding by receptor **2** was also detected by changes in the ^1H NMR spectrum; upon addition of variable amounts of Hg^{2+}, the downfield shift of the CH═N protons and the upfield shift showed the protons within the *p*-

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methoxyphenyl group being the most significant. Moreover, after the cation concentration has reached the ligand concentration, further addition of Hg^{2+} is ineffective (see the Supporting Information). This observation is also in agreement with the above-mentioned 1:1 binding model.

Compound **2** exhibits a very weak fluorescence in CH_3CN when excited at $\lambda_{exc} = 350$ nm. The emission spectrum shows typical bands at 387 and 410 nm, attributed to the pyrene monomeric emission, and a red-shifted structureless maximum at 450 nm, typical of pyrene excimer fluorescence,[9] with low quantum yield ($\phi = 0.003$). The fluorescence behavior of **2** in CH_3CN ($c = 2.5 \times 10^{-5}$ M) and in the presence of the previously mentioned metal ions was also examined, and a sizable fluorescence intensity enhancement was only observed upon addition of Cu^{2+} ($I_{complex}/I_{free\ ligand}$ = 22-fold) with a simultaneous blue shift of the pyrene excimer emission. An intensity maximum is reached at [**2**]/[Cu^{2+}] = 1, where the excimer emission (λ_E) shifts 21 nm (from 450 to 429 nm) (see the Supporting Information). The stoichiometry of the complex system was also determined by the changes in the fluorogenic response of **2** in the presence of varying concentrations of Cu^{2+} and the results obtained indicate the formation of a 1:1 complex giving an association constant of 5.71×10^5 M^{-1}.

The response of the fluorescence of **2** toward such set of metal ions was also studied in CH_3CN/H_2O (70/30). Under these conditions, **2** emits a very weak fluorescence (λ_{exc} = 350 nm) showing the characteristic emission bands for pyrene and a total fluorescence quantum yield of $\phi = 0.005$. Titration experiments demonstrate that only Cu^{2+} (Figure 2b) and Hg^{2+} (see the Supporting Information) ions yielded progressively an intensity enhancement of the pyrene excimer emission along with a slight red shift (10 nm) of this emission band. Moreover, the increase in quantum yield of **2** induced by Cu^{2+} ($\phi = 0.05$) and Hg^{2+} ($\phi = 0.066$) ions was 10- and 13-fold, respectively. The fluorescence titration data indicate an empirical 1:1 stoichiometry for the complexes formed being the estimated association constants of 1.05×10^6 and 8.45×10^5 when Cu^{2+} or Hg^{2+} were added. On the other hand, the calculated detection limits were 3.91×10^{-6} and 4.29×10^{-6} M for Cu^{2+} and Hg^{2+}, respectively. The other metal ions studied revealed relatively insignificant responses in the spectrum.

The interference in the selective responses of **1** and **2** in the presence of Cu^{2+} and Hg^{2+}, from the other metal cations tested, was also studied by using cross-selectivity experiments (see the Supporting Information).

Similar studies were carried out using the regioisomers **3** and **4**, in which the fluorescent unit is linked to the 4 position of the aza-bridge. However, the results obtained, both in CH_3CN and CH_3CN/H_2O (70/30), show neither optical nor fluorescence selectivity for any of the metal ions tested (see the Supporting Information).

DFT calculations (see the Supporting Information) showed a preference for a 2:2 complex [**1**·$Cu(OTf)_2$]$_2$ having overall C_i symmetry in which every Cu atom lies in a rather unusual trigonal pyramidal environment, characteristic of the active redox site T1Cu in blue copper proteins,[17] made up by one N atom, two triflate O atoms, and one long contact with the anthryl C-1 atom of the same ligand. In principle, this 2:2 binding model seems to be also confirmed by the ESI-MS spectra of an acetonitrile solution of equimolar amounts of $Cu(CF_3SO_3)_2$ and the corresponding ligand **1** or **2**. These spectra show a base peak corresponding to a complex [L·Cu]$^{2+}$ and a peak of lower intensity due to the adduct [L_2·Cu]$^{2+}$, formed from the [L_2·Cu_2]$^{4+}$ complex, although the peak derived from a 2:2 stoichiometry was silent (see the Supporting Information).

In conclusion, we have demonstrated that ligand **1**, with an efficient signaling anthracene unit directly linked to a cation-binding moiety of 2-aza-1,3-butadiene, behaves as a new class of Cu^{2+}-responsive chromo- and fluorogenic chemosensor, whereas the fluorescent behavior of **2**, bearing a pyrene unit, has shown its ability for sensing both Cu^{2+} and Hg^{2+} in an aqueous environment.

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Supporting Information Available: Characterization data of the free ligands. UV−vis and fluorescence spectra upon titration with metal ions. Titration profiles. Semilogarithmic plot for determining the detection limits. Figure with the calculated structure for the **1**·$Cu(OTf)_2$ 1:1 complex and Cartesian coordinates for both 1:1 and 2:2 complexes. This material is available free of charge via the Internet at http://pubs.acs.org.

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