

Communications to the Editor

Redox Switchable Fluorescent Probe Selective for Either Hg(II) or Cd(II) and Zn(II)

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During the past few years considerable attention has been devoted to the design and synthesis of functional molecules that could serve as molecular devices for sensors, switchable and signal transducing.¹ The enormous potential of fluorometric methods for the analytical detection of environmentally and biologically relevant heavy metal cations requires the development of highly selective and highly sensitive fluorescent probes.² Due to sensitivity reasons, probes showing fluorescence enhancement as a result of the binding event are to be favored over those which exhibit fluorescence quenching upon cation complexation.³ Especially for the mercury (II) ion, a widely recognized fluorescence quencher, only very few systems have been described which combine sufficient selectivity⁴ with a high signal output, i.e., fluorescence enhancement.⁵

For the design of a redox switchable fluorescent probe system we chose the thiadiazole/iminoylthiourea system as a redox active building block. This system offers the possibility of external control via reversible redox switching. The high affinity of nitrogen and sulfur heteroatoms for heavy metal ions makes it a useful complexation unit.⁶ A simple anthryl group functions as the fluorescent reporter unit. The oxidized form of the fluorescent probe system **1** was synthesized by a Boulton–Katritzki type of rearrangement,⁷ reacting 3,4-diphenyl-5-cyanimino-1,2,4-thiadiazoline⁸ with 9-(methylaminomethyl)-anthracene in dioxane under alkaline conditions. The resulting 1,2,4-thiadiazole could be reduced by zinc in glacial acetic acid to yield **2**. Using iodine in chloroform, **2** was reconverted almost quantitatively into the system's oxidized form **1** again (Figure 1). Hence, the thiadiazole **1** and the iminoylthiourea derivative **2** can be considered as the two components of a redox switchable system.

Both forms contain distinctively different binding sites, regarding the steric features as well as the electron-donating capacity of the heteroatoms. Therefore, we expected different selectivities of **1** and **2** for the metal cations studied. Concerning the system's

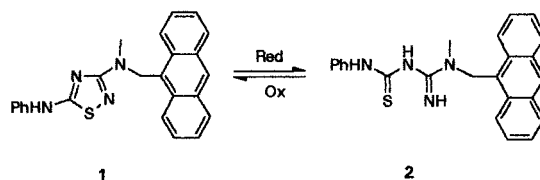


Figure 1. Redox switchable fluorescent probe, oxidized (**1**) and reduced form (**2**).

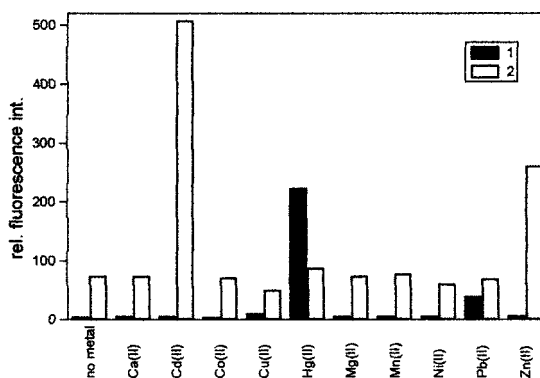


Figure 2. Relative fluorescence intensities of **1** (2.74×10^{-5} M) and **2** (2.75×10^{-5} M) in the presence of various metal cations ($c = 2.8 \times 10^{-3}$ M, only for Cd²⁺ $c = 4.5 \times 10^{-3}$ M), complete complexation.

fluorescence properties, the effect of intramolecular quenching of the fluorophore emission by amines is well-known.⁹ In the case of compound **1**, fluorescence quenching most likely occurs via photoinduced electron transfer (PET) from the alkylated nitrogen atom to the excited state of the anthracene fluorophore. Binding of certain metal cations as well as of protons to the lone electron pair of the nitrogen atom should then lead to fluorescence enhancement without a spectral shift in the emission spectrum. For the reduced form **2** a bathochromic shift (4 nm) of the fluorescence spectrum was found upon addition of protons without a significant change in the emission intensity. Therefore, a simple chelation effect is to be taken into consideration rather than a PET effect to explain the increase in fluorescence upon addition of certain metal cations as described below.

We investigated the influence of various metal perchlorates on the fluorescence behavior of **1** and **2** in acetonitrile solution (Figure 2).

Upon addition of Ca²⁺, Cd²⁺, Co²⁺, Cu²⁺, Hg²⁺, Mg²⁺, Mn²⁺, Ni²⁺, Pb²⁺, and Zn²⁺ to a solution of **1**, a tremendous increase in fluorescence intensity was observed only for Hg²⁺ (44-fold; Figure 3). The quantum yield ϕ_f of fully complexed **1** ($c = 2.74 \times 10^{-5}$ M, $c_{\text{Hg(II)}} = 2.8 \times 10^{-3}$ M) is 0.12, for the free ligand **1** $\phi_f = 0.0027$. Addition of Cu²⁺ and Pb²⁺ also resulted in a modest emission enhancement (2-fold and 7.7-fold, respectively). All of the other cations yielded no significant change in emission intensity under identical conditions. The enhanced fluorescence of **1** after addition of Hg²⁺ was not influenced by subsequent addition (100-fold excess) of the other metal ions apart from Cu²⁺, where almost complete bimolecular quenching occurred.

In the case of **2**, a 6-fold fluorescence enhancement was observed upon addition of Cd²⁺ (Figure 4) and, to a minor extent

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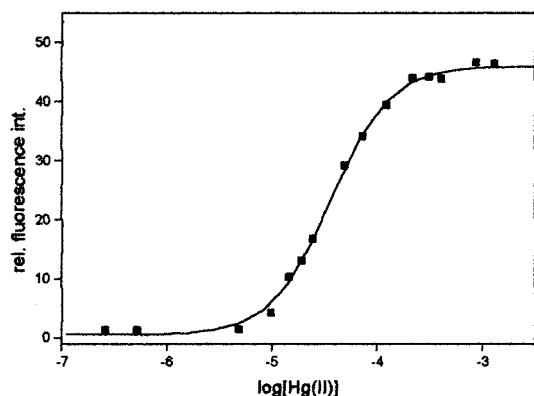


Figure 3. Fluorescence enhancement of **1** (2.74×10^{-5} M) in CH_3CN upon addition of Hg^{2+} ; $\lambda(\text{ex}) = 350$ nm, $\lambda_{\text{max}}(\text{em}) = 393, 413, 437, 466$ nm.

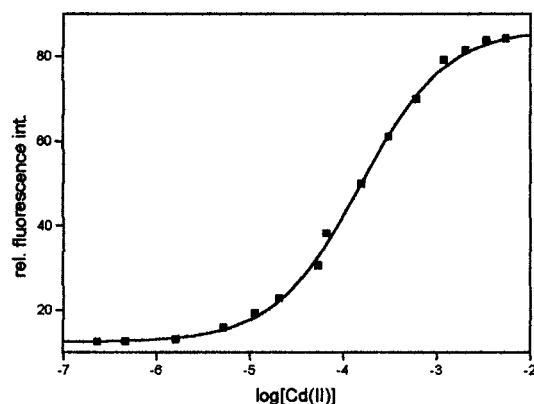


Figure 4. Fluorescence enhancement of **2** (2.75×10^{-5} M) in CH_3CN upon addition of Cd^{2+} ; $\lambda(\text{ex}) = 350$ nm, $\lambda_{\text{max}}(\text{em}) = 393, 413, 437, 466$ nm.

(3.6-fold) upon addition of Zn^{2+} . The uncomplexed ligand **2** had $\phi_f = 0.039$, while after complete complexation of Cd^{2+} ($c = 2.74 \times 10^{-5}$ M, $c_{\text{Cd(II)}} = 4.5 \times 10^{-3}$ M) it showed $\phi_f = 0.22$. In the

presence of Cu^{2+} , Ni^{2+} and Pb^{2+} , compound **2** displayed fluorescence quenching (Figure 2). For both compound **1** and **2**, the detection limit for Hg^{2+} or Cd^{2+} , respectively, where a significant increase in the emission intensity can be observed, is in the range of $10 \mu\text{mol}$.

A simple cuvette experiment proved in principle the system's behavior as a fluorescence switch.¹⁰ The fluorescence of **2** was switched ON upon addition of Cd^{2+} , OFF by adding iodine to the obtained Cd^{2+} -**2**-complex yielding **1**. The fluorescence was switched ON again upon addition of Hg^{2+} .

For both forms **1** and **2** of the redox switchable fluorescent probe cation complexation led to neglectable changes in absorption and only to very small shifts in the emission spectra. All of the effects described occurred immediately after cation addition. The only exception was observed for compound **2** in the presence of Hg^{2+} . While the emission spectrum of **2** remained unaffected directly after addition of Hg^{2+} , after 1 h the absorption changed in the region between 240 and 290 nm, and the fluorescence intensity increased by a factor of 5. These effects are attributed to a desulfurization of the iminoylthiourea as has been described for a similar system.¹¹

In conclusion, the redox switchable fluorescent system presented shows distinctly different cation selectivities, depending on its redox state. Especially the oxidized form **1** proved to be a highly selective fluorescent probe for Hg^{2+} , showing chelation-enhanced fluorescence over a wide concentration range.

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Supporting Information Available: Experimental details, ^1H and ^{13}C NMR spectra, HRMS data, and absorption and emission spectra of **1** and **2** in acetonitrile (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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(10) To a solution of **2** ($c = 2.75 \times 10^{-5}$ M) in basic acetonitrile ($100 \mu\text{l}$ of saturated NaOH in acetonitrile in total 1.5 mL) acetonitrile solutions of Cd^{2+} (100-fold excess), iodine (1 equiv), and Hg^{2+} (100-fold excess) were added subsequently under stirring. The fluorescence measurement were performed immediately after addition of the corresponding solutions.

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