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# A Click-Generated Triazole Tethered Ferrocene−Pyrene Dyad for Dual-Mode Recognition of the Pyrophosphate Anion

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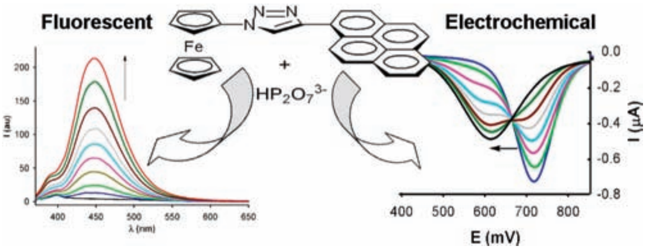
## ABSTRACT



**The ferrocene-pyrene dyad 3 is able to selectively sense the pyrophosphate anion. The anion recognition was evaluated using electrochemistry, $^1$H NMR, as well as fluorescence spectroscopy. The binding event can be inferred from either the redox-shift ($\Delta E_{1/2} = -100$ mV) or the emission intensity ratio of the pyrene monomer to the excimer emission bands in both the neutral and oxidized forms of the receptor upon complexation.**

The independent discoveries of the Cu(I)-catalyzed Huisgen's 1,3-dipolar cycloaddition (CuAAC)[1] by Sharpless and Medal[2] have led to thousands of new examples of this reaction subclass. The excellent regioselectivity for the 1,4-isomer of the 1,2,3-triazole and the enhanced reactivity of the Cu(I)-catalyzed process, along with the versatility of the reaction conditions, have made it the flagship reaction of the Sharpless "click" chemistry.[3] As such, it has been widely applied of late, particularly in bioconjugation and drug discovery and also in advanced material sciences toward dendrimers and surface modification.[4] However, much less effort has been devoted to the preparation of organometallic bioconjugates.[5] The role of the 1,2,3-triazole in the formation of stable metal complexes has also been realized, and accordingly, some triazole-based receptors for the recognition of metals have been reported.[6]

Several communications making use of a 1,2,3-triazolium cation for phosphate anion recognition,[7] a flexible triazolophane,[8] a triazole-based cyclic peptide,[9] and a preorganized and rigid triazole-based macrocyclic,[10] showing self-assembly as well as the anion binding properties of foldamers,[11] all demonstrate 1,2,3-triazoles participating in noncovalent interactions. Interestingly, 1,2,3-triazole-linked dendrimers

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also showed the ability of binding oxo anions through the 1,2,3-triazole ring localized inside the dendrimers.[12]

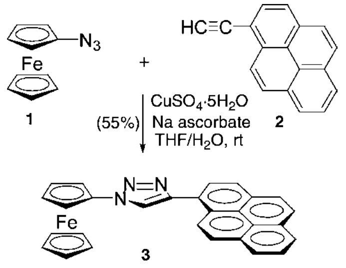
It has been recently reported[13] that there are two sources that contribute to the triazole's unexpected anion binding affinity. First, the electronegativities of the three nitrogen atoms combine to polarize the C—H bond. Second, the lone pairs of electron on the nitrogen atoms act to establish and orient along the C—H bond a large 5 D dipole, with its positive end directed almost in line with the C$^5$—H bond. These combined effects make them interesting candidates for amide bond surrogates.[14]

Pyrene is one of the most useful fluorogenic units because of its high detection sensitivity. Formation of the self-assembled complex results in a remarkable change in the fluorescence emission intensities of the pyrene excimer and monomer.[15] Two informative parameters associated with the pyrene excimer are the intensity ratio of the excimer to the monomer emission ($I_E/I_M$) and the wavelength corresponding to the maximum of the excimer emission ($\lambda_E$). Although the $I_E/I_M$ parameter is sensitive to the structure of the pyrene-labeled systems, the corresponding pyrene $\lambda_E$ is much less variable and generally locates at 475—485 nm.

On the other hand, in ferrocene derivatives cation (anion) binding at an adjacent receptor site induces a positive (negative) shift in the redox potential of the ferrocene/ferrocenium redox couple, and the complexation ability of the ligand can be switched on and off by varying the applied electrochemical potential. The magnitude of the electrochemical shift ($\Delta E_{1/2}$) upon complexation represents a quantitative measure of the perturbation of the redox center induced by complexation to the receptor unit.[16]

On the basis of such precedents, a suitable dual-signaling chemical probe can be built by combining the redox activity of the ferrocene, the photoactivity of the pyrene, and the recently proved anion binding ability of the 1,2,3-triazole ring. To this end, 1-ferrocenyl-4-pyrenyl-1,2,3-triazole **3** has been chosen to develop a new multichannel sensing system. Scheme 1 summarizes the synthesis of this motif, which starts with an improved preparation of azidoferrocene **1**, by initial metalation of ferrocene with the system $^t$BuLi/K$^t$BuO followed by azidation with trisyl azide (2,4,6-triisopropylbenzenesulfonyl azide).[17] Compound **1** undergoes the "click reaction" with 1-ethynylpyrene **2** to afford the dyad **3** in 55% yield. Cu(I) and oxidized Cu(II) species in the reaction mixture can be easily separated by treating with aqueous ammonia during the workup and subsequent silica column chromatography.

**Scheme 1.** Preparation of Dyad **3**



The formation of the 1,2,3-triazole ring was apparent from the $^1$H NMR by the appearance of the triazolo-*H* signal as a singlet at 9.28 ppm. In addition, the ferrocene signals are characteristic with the free Cp being found at 4.35 ppm, whereas the protons of the substituted cyclopentadienyl moiety are located downfield at 5.27 ppm for the α protons and 4.46 ppm for the $\beta$ protons.

The binding and recognition ability of receptor **3** toward various anions ($F^-$, $Cl^-$, $Br^-$, $AcO^-$, $NO_3^-$, $HSO_4^-$, $H_2PO_4^-$, and $HP_2O_7^{3-}$), in the form of their corresponding tetrabutylammonium salts ($TBA^+$), were evaluated by cyclic (CV) and Osteryoung square-wave voltammetry (OSWV). The electrochemical response of **3** in $CH_2Cl_2$, also containing 0.1

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M $TBAPF_6$ as supporting electrolyte, showed a one-electron oxidation peak at $E_{1/2} = 0.71$ V vs decamethylferrocene (DMFc) redox couple. Electrochemical anion-sensing experiments demonstrate that only the stepwise addition of $HP_2O_7^{3-}$ anions promotes a cathodic shift of the ferrocene/ferrocenium redox couple. It is notable that, upon the stepwise addition of the anion, a typical shifting behavior with the appearance of a new negatively shifted peak, at $E_{1/2} = 0.61$ V, was observed ($\Delta E_{1/2} = -100$ mV) (Figure 1).

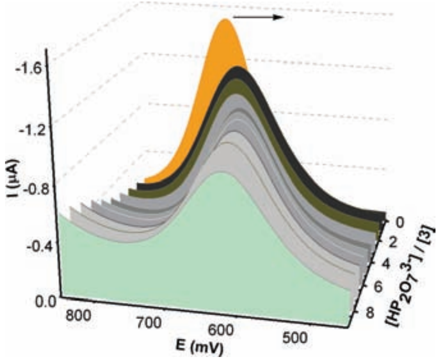


**Figure 1.** Evolution of the OSWV of **3** (orange) (1 mM) in $CH_2Cl_2$/$[(n\text{-}Bu)_4]ClO_4$ scanned at 0.1 V $s^{-1}$ in the presence of increasing amounts of $HP_2O_7^{-3}$ (green).

Binding assays using the method of continuous variations (Job's plot) are consistent with the formation of a complex with a 2:1 receptor/anion stoichiometry (see the Supporting Information). The negative shifts in potential indicate that the receptor−anion assemblies are easier to oxidize than the free receptor, probably due to the electron density donation from the anion to the redox center. Receptor **3** showed a similar perturbation of the oxidation peak in DMF solution. In contrast, the presence of $F^-$, $Cl^-$, $Br^-$, $AcO^-$, $NO_3^-$, $HSO_4^-$, and $H_2PO_4^-$ anions had no effect on the OSWV, even when present in large excess.

The binding properties of receptor **3** with $HP_2O_7^{3-}$ anions were also evaluated by $^1H$ NMR studies in deuterated DMF solutions (Figure 2). Remarkably, the hydrogen atom within the triazole ring of **3** showed a significant downfield shift by 0.11 ppm in the presence of $HP_2O_7^{3-}$ anions, demonstrating that this atom is involved in the ligand−anion binding event. Similar downfield shifts, although of smaller magnitude (0.06 ppm), were also observed for the pyrene ring hydrogen atoms H-2 and H-10, which would also contact with the $HP_2O_7^{3-}$ anion in the binding process. The cyclopentadieneyl ring protons were almost unchanged (0.03 ppm), whereas the remaining pyrenyl hydrogen atoms were unaffected (see the Supporting Information) during the titration. Addition of either $AcO^-$ or $F^-$ anions does not induce any noticeable change in the spectrum of the free receptor.

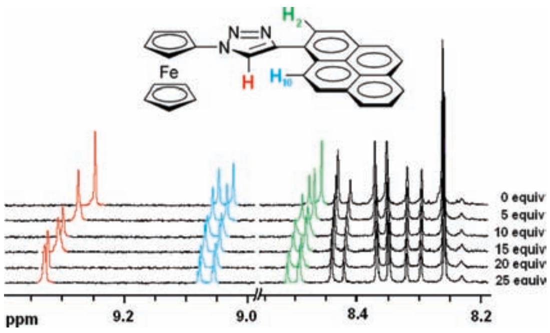


**Figure 2.** Evolution of the $^1H$ NMR spectra of **3** in DMF-$d^7$ upon addition of increasing amounts of $HP_2O_7^{3-}$(blue).

From the combined electrochemical (strong cathodic shift) and spectroscopic (small downfield shift) data, we believe that the $HP_2O_7^{3-}$ anion is essentially recognized through the negatively charged oxygen atoms which interact strongly with the Fe center. Nevertheless, this $Fe^{\delta+}\cdots O^{\delta-}$ electrostatic perturbation of the redox center is presumably facilitated by chelation of the oxoanion group through hydrogen bonding involving the triazole and H-2 and H-10 pyrenyl hydrogen atoms.

The anion binding and sensing properties of receptor **3** have also been studied by using absorption and emission techniques. The UV−vis spectrum of receptor **3** in $CH_2Cl_2$ ($c = 1 \times 10^{-4}$ M) exhibits two strong bands at $\lambda = 281$ nm ($\varepsilon = 42\,740$ $M^{-1}$ $cm^{-1}$) and 349 nm ($\varepsilon = 28\,290$ $M^{-1}$ $cm^{-1}$). As expected, receptor **3** showed a weak fluorescence. The emission spectrum ($\lambda_{exc} = 355$ nm) displays typical emission bands at 387 and 406 nm, which are attributed to the pyrene monomeric emission, and a broad and structureless band at 448 nm due to the excimer emission band, with rather low quantum yield ($\Phi = 6 \times 10^{-3}$).[15a] The UV−vis spectrum of **3** in $CH_2Cl_2$ was not substantially affected by addition of $HP_2O_7^{3-}$ anions. However, stepwise addition of this anion to the solution of the receptor **3** in $CH_2Cl_2$ ($c = 1 \times 10^{-6}$ M) induces an evident perturbation of the fluorescence emission (Figure 3). The fluorescene intensity of the excimer band at $\lambda = 448$ nm gradually grew upon addition of $HP_2O_7^{3-}$ anions. As a consequence, a final fluorescence

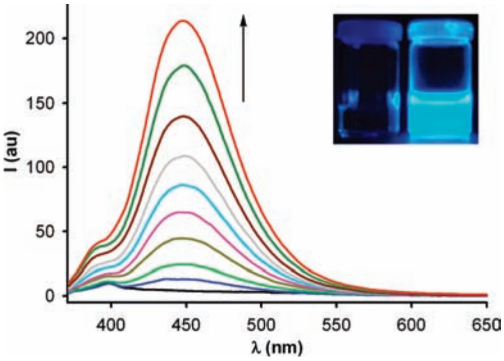


**Figure 3.** Changes in the fluorescence emission spectrum ($\lambda_{exc} = 355$ nm) of **3** (black) ($c = 1 \times 10^{-6}$ M in $CH_2Cl_2$) upon titration with $HP_2O_7^{3-}$ (red). Inset: visual changes in the fluorescence of **3** (left) after addition of the $HP_2O_7^{3-}$ anion (right).

enhancement factor (FEF) of 53 and a 19-fold quantum yield increase ($\Phi = 0.111$) were observed, with regard to the free receptor **3**. The stoichiometry of the complex system was determined by the changes in the fluorogenic response of **3** in the presence of varying concentrations of $HP_2O_7^{3-}$, and the results obtained indicate the formation of a 1:2 complex giving an apparent association constant of log $\beta = 8.74 \pm 0.30$.

To test that the recognition event is mainly due to an electrostatic interaction between the iron center of the ferrocene unit and the oxoanion, titration experiments with the electrochemically generated oxidized form, $\mathbf{3}^{\bullet+}$, were carried out.

First of all, confirmation of the ferroceniun state in $\mathbf{3}^{\bullet+}$ was achieved by rotating disk voltammetry (see the Supporting Information). It is important to note that the UV−vis spectrum of $\mathbf{3}^{\bullet+}$ is almost identical to that of the neutral receptor. However, the emission spectrum displays two strong emission bands at 387 and 406 nm and a weak broad excimer emission band at 448 nm, the quantum yield ($\Phi = 0.372$) being 62-fold higher than that of the neutral receptor **3** (Figure 4b). Addition of $HP_2O_7^{3-}$ anions to a solution of $\mathbf{3}^{\bullet+}$ induced a remarkable ratiometry since the excimer emission increases as its monomer emission declines. The final ratio of the fluorescence intensity ($\lambda_{448}/\lambda_{397} = 0.5$) was lower than that calculated for the free receptor ($\lambda_{448}/\lambda_{397} = 5$), and the quantum yield ($\Phi = 0.616$) resulted in a 2-fold increase compared to that of $\mathbf{3}^{\bullet+}$ (Figure 4a). From the fluorescence titration, the apparent association constant, log $\beta = 10.65 \pm 0.39$, was calculated, which is in good agrement with that derived from the equation $\Delta E° = nF/RT \ln(K_{ox}/K_{red})$.

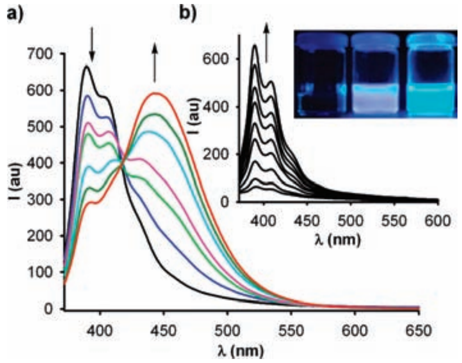
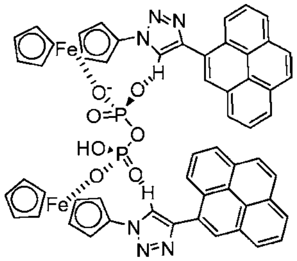


**Figure 4.** (a) Changes in the fluorescence emission spectrum of $\mathbf{3}^{\bullet+}$ (black) ($c = 5 \times 10^{-7}$ M in $CH_2Cl_2$) upon titration with $HP_2O_7^{3-}$ until complete complexation (red). (b) Evolution of fluorescence emission spectrum ($\lambda_{exc} = 355$ nm) during the course of the oxidation of **3** at 0.8 V. Inset: visual changes in the fluorescence of **3** (left) after its oxidation $\mathbf{3}^{\bullet+}$ (center) and the subsequent addition of $HP_2O_7^{3-}$ anion (right).

Perturbations in the emission spectrum of **3** after complexation with $HP_2O_7^{3-}$ anions could be rationalized as follows: (i) the weak emission spectrum of **3** may be explained by a quenching of the pyrene's subunit fluorescence by the ferrocene moiety;[18] (ii) upon complexation with the $HP_2O_7^{3-}$ anion, the direct interaction between the Fe(II) center and the negative oxygen atoms within the oxoanion induces a lessening of the electron-donating ability in the ferrocene subunit, and as a result, the electron transfer is arrested leading to a fluorescence enhancement; (iii) concomitantly, the presence of two pyrene subunits in the complex (2:1 stoichiometry) promotes excimer emission (Scheme 2).

**Scheme 2.** Schematic Binding Interactions in the Complex



This plausible explanation is supported by two experimental facts: (i) the electrochemically generated especies $\mathbf{3}^{\bullet+}$ displays a strong fluorescence, basically due to the monomer emission and (ii) after complexation with the $HP_2O_7^{3-}$ anion, not only is the excimer emission the salient feature but also is the notable increase in its quantum yield. These studies show that a combination of metal coordination (electrochemical and emission spectral data) and hydrogen bonding (NMR data) interactions can provide substantial synergistic effects which contribute to the recognition of the $HP_2O_7^{3-}$ anions by a triazole tethered ferrocene−pyrene dyad.

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**Supporting Information Available:** Synthesis and spectral data, Job's plot, LSW during the monooxidation of **3**, changes in the UV−vis of $\mathbf{3}^{\bullet+}$ upon addition of $HP_2O_7^{3-}$, and changes in the fluorescence spectra of **3** and $\mathbf{3}^{\bullet+}$ upon addition of anions. This material is available free of charge via the Internet at http://pubs.acs.org.

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