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Highly sensitive chemosensor for detection of PPi with improved detection limit

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ABSTRACT

We have developed a new chemosensor **1**·2**Zn** for the detection of PPi in an aqueous solution by photo-induced electron transfer. Two coumarin units were introduced to enhance the fluorescent signals. Consequently, this chemosensor showed an improved detection limit as compared to the previously reported chemosensor **2**·2**Zn**.

The development of chemosensors for the detection of biologically significant anions is a growing research topic.[1] Chemosensor-based detection of pyrophosphate ($P_2O_7^{4-}$, PPi) is particularly important as PPi is involved in a range of bioenergetic and metabolic processes, such as gene duplication, gene transcription, and signal transduction.[2] Additionally, PPi is closely related to diseases: Accumulations of calcium pyrophosphate dihydrate crystals are frequently found in patients with osteoarthropathy or psuedogout.[3]

Approximately, 3 and 6 μM of PPi is normally present in human plasma and serum, respectively.[4] Thus, the PPi in biological samples can be detected using the known PPi chemosensors because these sensors respond to micromolar concentrations of PPi.[5–7]

However, there is a need to develop highly sensitive PPi sensors with improved detection limits because enzymatic reactions that involve the release of this anion often result in subtle changes in the PPi concentration. Further, the improvement of the detection limit results in efficient pyrosequencing, which is one of the most promising methods of DNA sequencing.[8] In this communication, we propose a fluorescent chemosensor (**1**·2**Zn**) for the detection of PPi; the chemosensor has an improved detection limit as compared to that of the existing PPi sensors and also shows selectivity for PPi over other anions including phosphate anions in aqueous media.

Generally, anion sensors consist of two basic elements.[1] The first one is the binding moiety, which overcomes strong hydration in an aqueous medium. The other element converts these binding-induced changes into optical signals via chromophores or fluorophores. Previously, we were able to improve the binding affinity of this sensor to PPi by more than hundred times that of **2**·2**Zn** to PPi by introducing additional hydrogen bonds to this sensor.[7d] Although the introduction of additional hydrogen bonds results in a notable increase in the binding affinity, introducing a high quantum yield signaling motif is expected to increase the sensitivity of PPi chemosensors, leading to an improved detection limit. Therefore, two coumarin units were introduced in place of the naphthyl group used in **2**·2**Zn** to amplify the fluorescent response caused by the addition of PPi (Scheme 1).[7b]

The synthesis of compound **1** is outlined in Scheme 2. The conversion of 1,3-phenylenediacetic acid into its acyl chloride facilitated α-bromination. The resulting halogenated acyl chloride was then hydrolyzed to the corresponding carboxylic acid, which was converted into its methyl ester product (compound **4**). Compound **3** was obtained through the reaction between compound **4** and di-(2-picolyl)amine in the presence of potassium carbonate and potassium iodide. Hydrolysis of compound **3** followed by amide bond formation between the resulting diacid and 2 equiv of 3-amino-7-diethylaminocoumarin furnished compound **1**.

The effect of metal coordination on the emission spectra was measured through fluorescence spectroscopy (Supplementary data): By blocking the photo-induced electron transfer (PET), complexes with zinc and cadmium cations showed strong fluorescence emissions compared to the fluorescence emissions of other metal complexes.[9] Blocking of the PET process through coordination of a d^{10}-metal with an amine (reductive quencher) is commonly observed in fluorescence chemosensor studies (Fig. 1).[10] We expected that the addition of PPi would weaken the metal coordination with the reductive quencher and lead to a significant change in the fluorescence intensity of **1**·2**Zn** and **1**·2**Cd** by restoring the PET pathway (Fig. 1).

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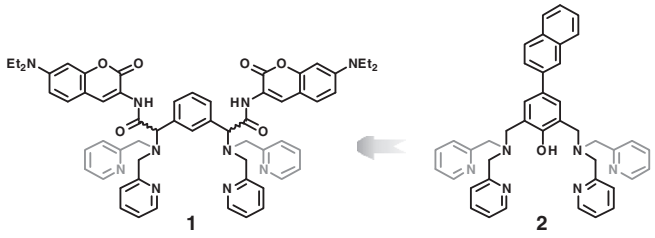
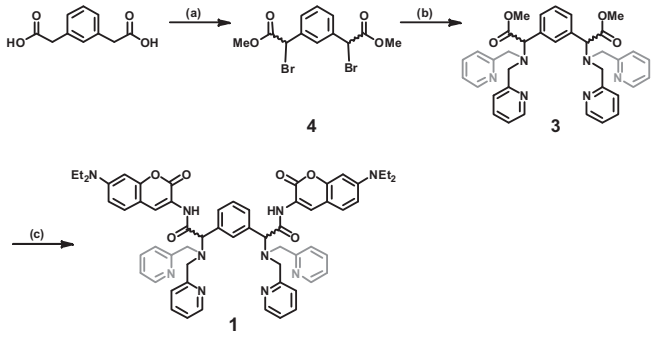


Scheme 1. Introduction of two coumarin units to improve the detection limit of PPi.



Scheme 2. Reagents and conditions: (a) (i) $(COCl)_2$, DMF, CH_2Cl_2, (ii) Br_2, benzene, (iii) H_2O, (iv) cat. H_2SO_4, MeOH; (b) di-(2-picolyl)amine, K_2CO_3, KI, MeCN; (c) (i) NaOH, MeOH/H_2O, (ii) 3-amino-7-diethylaminocoumarin, EDC–HCl, pyridine/CH_2Cl_2.

As expected, we found that the fluorescence of the complex (**1**·2**Zn**) between **1** and zinc cations was significantly reduced on the addition of PPi (Fig. 2). The effect of other anions on the emission spectra of **1**·2**Zn** was examined by adding various anions to **1**·2**Zn** in an aqueous 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) buffer solution (0.01 M, pH 7.4) at 25 °C.[11] However, other anions, including those of phosphate, showed no detectable changes in the fluorescence of **1**·2**Zn** (Fig. 2). These results suggest that **1**·2**Zn** has a high selectivity for PPi over other anions.

To measure the change in the fluorescence of **1**·2**Zn** upon the addition of PPi, fluorescence titration was carried out in an aqueous HEPES buffer solution (10 mM, pH 7.4, 25 °C). On the addition of PPi, fluorescence decreased by a factor of 9.8 and was saturated at 3.3 equiv (Fig. 3). 1:1 Complexation between **1**·2**Zn** and PPi was confirmed by Job's plot (Fig. 3 inset, refer to Supplementary data for the Job's plot for adenosine triphosphate)

The emission spectra of **1**·2**Zn** containing coumarin dye were expected to vary significantly upon the addition of PPi, resulting in an improved detection limit. As depicted in Figure 4, the change in fluorescence intensity of **1**·2**Zn** was 4.7 times greater than that

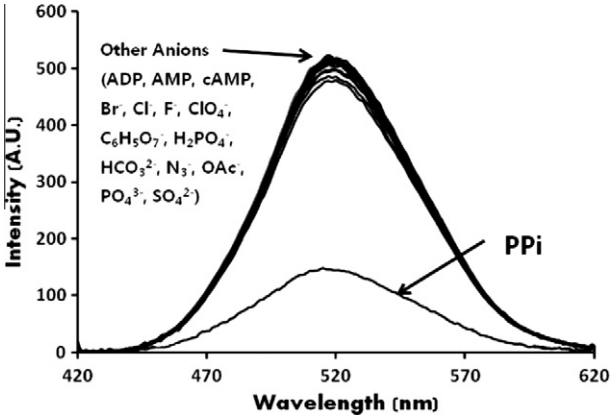


Figure 2. Fluorescence emission spectra of **1**·2**Zn** (10 μM) on addition of various anions (20 μM, sodium salts) in an aqueous HEPES buffer solution (10 mM, pH 7.4).

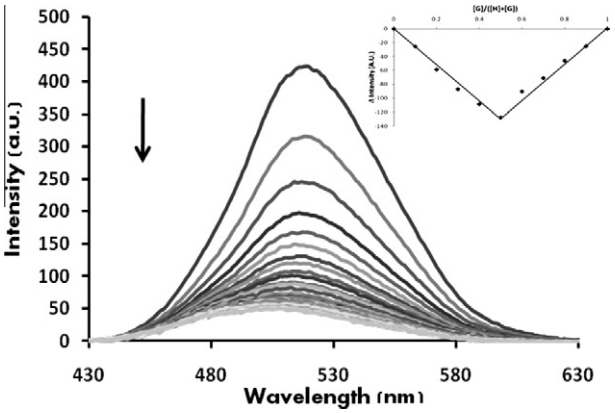


Figure 3. Change in the fluorescence of **1**·2Zn (10 μM) upon addition of PPi (0–33 μM) in aqueous HEPES buffer solutions (10 mM, pH 7.4).

of **2**·2**Zn** upon the addition of PPi under the same conditions. The detection limit was calculated to be three times that of the standard deviation of the background noise. As expected, the detection limit of **1**·2**Zn** for PPi (49 nM; refer to Supplementary data) was better than that of **2**·2**Zn** (83 nM).[12] Considering the reduced binding affinity of **1**·2**Zn** compared to that of **2**·2**Zn**, the improved detection limit of **1**·2**Zn** indicates that high quantum yield fluorophores may have a dominant effect on the determination of the detection limit.

In summary, we developed a new fluorescent chemosensor (**1**·2**Zn**) for the detection of PPi in an aqueous solution. The sensor system shows an improved detection limit for PPi compared to that of the naphthyl-based PPi sensor by the introduction of coumarin dye. In addition, it shows good selectivity for PPi over other anions.

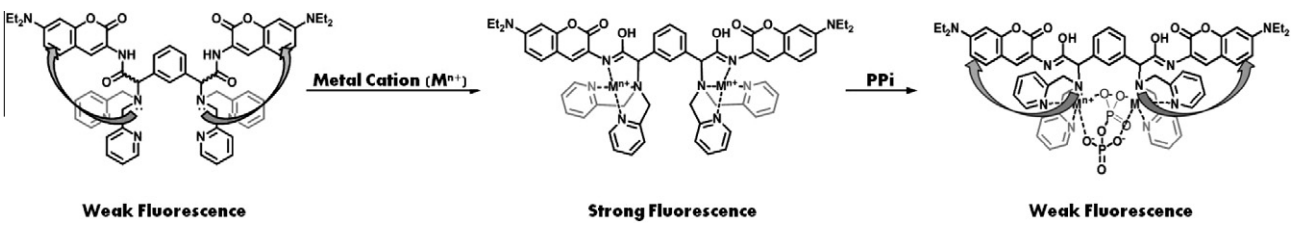


Figure 1. Proposed mechanism for controlling the fluorescence by blocking and restoring PET.

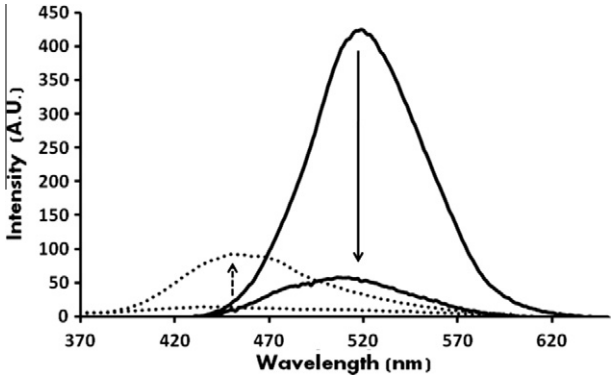


Figure 4. Fluorescence spectra of sensor **1**·2**Zn** (10 μM, solid lines) and **2**·2**Zn** (10 μM, dotted lines) before and after the addition of PPis (20 μM) in an aqueous buffer solution (10 mM HEPES, pH 7.4).

Acknowledgments

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Supplementary data

Supplementary data (spectral data for the new compounds, effect of metal cations and anions on the emission spectra, determination of the detection limit, and Job's plot for ATP) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.07.067.

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