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Pyrophosphate-Selective Fluorescent Chemosensor Based on 1, 8-Naphthalimide–DPA–Zn(II) Complex and Its Application for Cell Imaging

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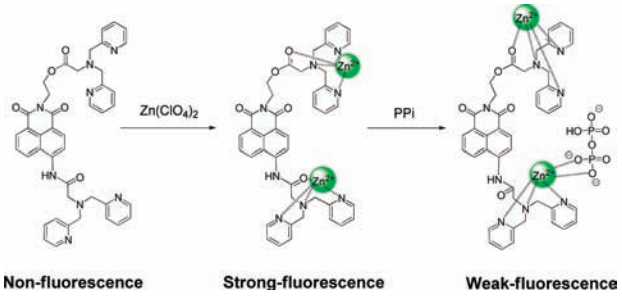
ABSTRACT



A new zinc(II) complex with a two-dipicolylamine-substituted 1,8-naphthalimide for recognition of pyrophosphate with ratiometrical fluorescence changes in aqueous solution has been synthesized and characterized. Its biological application to monitor the intracellular pyrophosphate (PPi) was successfully demonstrated by the observation that the fluorescence of 1 was enhanced by the presence of the Zn^{2+} ion and was quenched by addition of PPi.

The development of molecular-recognition and sensing systems for anions has received considerable attention in recent years.[1] Phosphates are among the most important anions in biological systems, as they play significant roles in many biological processes, such as cellular ATP hydrolysis, DNA and RNA polymerizations, and many enzymatic reactions.[2] Similar to the sensing of nucleosides and nucleotides,[3] the development of artificial pyrophosphate (PPi) anion receptors for use under physiological conditions is of continuous interest.[4] Such sensors are

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(1) (a) Martínez-Máñez, R.; Sancenón, F. *Chem. Rev.* **2003**, *103*, 4419. (b) Xu, Z.; Kim, S. K.; Yoon, J. *Chem. Soc. Rev.* **2010**, *39*, 1457. (c) Gale, P. A. *Chem. Soc. Rev.* **2010**, *39*, 3746. (d) Kim, S. K.; Sessler, J. L. *Chem. Soc. Rev.* **2010**, *39*, 3784. (e) Li, A.-F.; Wang, J.-H.; Wang, F.; Jiang, Y.-B. *Chem. Soc. Rev.* **2010**, *39*, 3729. (f) Lee, M. H.; Cao, Q.-Y.; Kim, S. K.; Sessler, J. L.; Kim, J. S. *J. Org. Chem.* **2011**, *76*, 870. (g) Zhang, J. F.; Lim, C. S.; Bhuniya, S.; Cho, B. R.; Kim, J. S. *Org. Lett.* **2011**, *13*, 1190. (h) Kim, H. J.; Ko, K. C.; Lee, J. H.; Lee, J. Y.; Kim, J. S. *Chem. Commun.* **2011**, *47*, 2886. (i) Cao, Q.-Y.; Pradhan, T.; Kim, S.; Kim, J. S. *Org. Lett.* **2011**, *13*, 4386.

(2) (a) Lipscomb, W. N.; Sträter, N. *Chem. Rev.* **1996**, *96*, 2375. (b) Ronaghi, M.; Karamohamed, S.; Pettersson, B.; Uhlen, M.; Nyren, P. *Anal. Biochem.* **1996**, *242*, 84. (c) Tabary, T.; Ju, L. J. *Immunol. Meth.* **1992**, *156*, 55. (d) Kim, S. K.; Lee, D. H.; Hong, J.-I.; Yoon, J. *Acc. Chem. Res.* **2009**, *42*, 23.

(3) (a) Zhou, Y.; Xu, Z.; Yoon, J. *Chem. Soc. Rev.* **2011**, *40*, 2222. (b) Kwon, T.-H.; Kim, H. J.; Hong, J.-I. *Chem.—Eur. J.* **2008**, *14*, 9613. (c) Kurishita, Y.; Kohira, T.; Ojida, A.; Hamachi, I. *J. Am. Chem. Soc.* **2010**, *132*, 13290.

(4) (a) Kim, M. J.; Swamy, K. M. K.; Lee, K. M.; Jagdale, A. R.; Kim, Y.; Kim, S.-J.; Yoo, K. H.; Yoon, J. *Chem. Commun.* **2009**, 7215. (b) Lee, H. N.; Xu, Z.; Kim, S. K.; Swamy, K. M. K.; Kim, Y.; Kim, S.-J.; Yoon, J. *J. Am. Chem. Soc.* **2007**, *129*, 3828. (c) Lee, J. H.; Jeong, A. R.; Jung, J.-H.; Park, C.-M.; Hong., J.-I. *J. Org. Chem.* **2011**, *76*, 417. (d) Zhao, X.; Schanze, K. S. *Chem. Commun.* **2010**, *46*, 6075.

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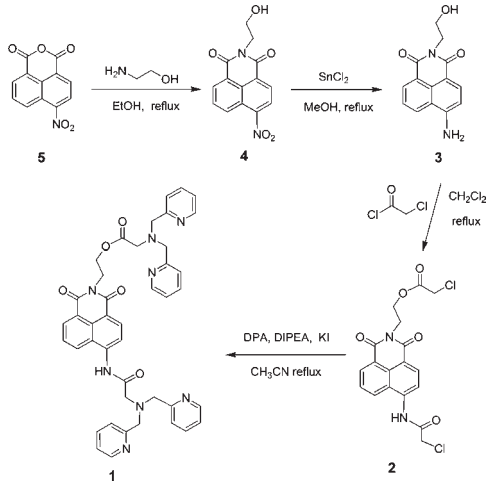
convenient tools for the detection of biologically important phosphates, with applications in molecular biology and life and environmental sciences.

To date, these studies frequently have adopted the fluorescent complexes containing a transition-metal (such as Zn^{2+}) chelator coupled with a variety of chromophores,[5] and one of the most common chelating groups used for this purpose is the dipicolylamine (DPA) unit.[6] The main difficulties in studying the intracellular PPi by way of fluorescent sensor are sensing in an aqueous solution and selectivity for PPi over a related analogue, such as ATP and inorganic phosphate (Pi).

With this in mind, herein we report a novel fluorescence PPi sensor based on 1,8-naphthalimide, bearing two dipicolylamino arms. Large fluorescent enhancements (FE of 59-fold) and a 29 nm red-shift were observed upon addition of Zn^{2+} to the solution of **1**. A selective 23 nm blue-shift and a 52% fluorescence quenching were only found with PPi over the related analogues, such as ATP and Pi, in 95% aqueous solutions. As a biological application, sensor **1** is successfully applied to monitor the intracellular Zn^{2+} ions and PPi staining experiments in C2C12 cells. Since the discrimination between PPi and Pi is of crucial importance to assays for detecting many enzymes,[7] **1** provides a facile platform for investigations of PPi-relevant biological processes.

Scheme 1 explains the synthetic route of compound **1**. Starting with 4-nitro-1,8-naphthalic anhydride (**5**), **4** and **3** were synthesized with improved yield (81% yield for **4**; 87% yield for **3**) by a modification of the reported procedure.[8] Reaction of **3** with 2-chloroacetyl chloride gave an intermediate **2** in 77% yield. The target **1**, which was prepared by the condensation of di(2-picolyl)amine (DPA) in the presence of *N,N*-diisopropylethylamine (DIPEA) and KI in acetonitrile, was purified by column chromatography in 69% yield.[9] The detailed procedures and characterization of the new compounds are described in the Supporting Information.

Scheme 1. Synthetic Route of Compound **1**



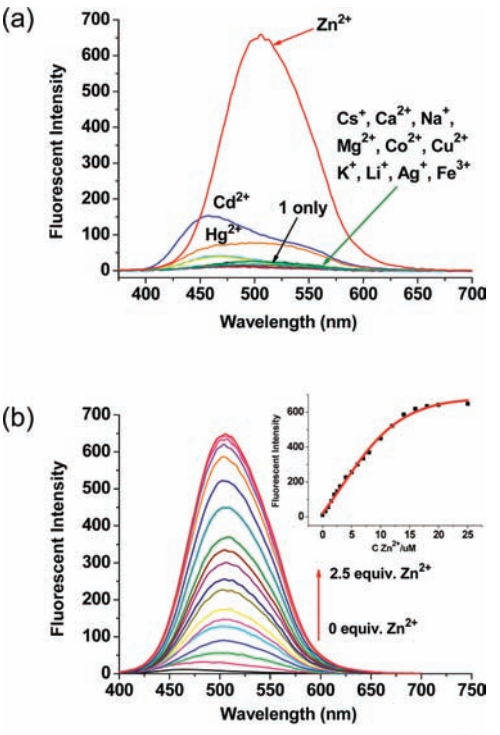


Figure 1. (a) Fluorescence changes of **1** (1×10^{-5} M, CH_3CN–HEPES buffer (0.02 M, pH 7.4) (5:95, v/v)) upon the addition of 5 equiv of various metal ions. (b) Fluorescent emission spectra of **1** (1×10^{-5} M) in the presence of different concentrations of Zn^{2+} in CH_3CN–HEPES buffer (0.02 M, pH 7.4) (5:95, v/v). Excitation wavelength was 360 nm. Inset: fluorescent intensity as a function of $[Zn^{2+}]$.

The absorption and fluorescence properties of **1** were tested in a CH_3CN–HEPES buffer (20 mM, pH 7.4) (5: 95, v/v). Compound **1** exhibited a major absorption band centered at 372 nm. As 5.0 equiv of various metal ions was added to the solution of **1**, no obvious change of the peaks was found in the UV spectra (Figure S8, Supporting Information). The fluorescence spectrum of **1** in CH_3CN–HEPES buffer exhibited a characteristic emission band of 1,8-naphthalimide at 476 nm with a weak green color emission. To obtain insight into the sensing properties of **1** toward metal ions, the emission changes were examined with different ions such as Li^+, Na^+, K^+, Ag^+, Ca^{2+}, Cd^{2+}, Cu^{2+}, Fe^{3+}, Hg^{2+}, Mg^{2+}, Co^{2+}, Cs^+, and Zn^{2+}. As shown in Figure 1a, selective and large fluorescent enhancements

(5) (a) Cho, H. K.; Lee, D. H.; Hong, J.-I. *Chem. Commun.* **2005**, 1690. (b) McDonough, M. J.; Reynolds, A. J.; Lee, W. Y. G.; Jolliffe, K. A. *Chem. Commun.* **2006**, 2971. (c) Peng, X.; Xu, Y.; Sun, S.; Wu, Y.; Fan, J. *Org. Biomol. Chem.* **2007**, *5*, 226.

(6) (a) Sakamoto, T.; Ojida, A.; Hamachi, I. *Chem. Commun.* **2009**, 141. (b) Huang, X.; Guo, Z.; Zhu, W.; Xie, Y.; Tian, H. *Chem. Commun.* **2008**, 5143.

(7) Liu, Y.; Schanze, K. S. *Anal. Chem.* **2009**, *81*, 231.

(8) (a) Yuan, D.; Brown, R. G.; Hepworth, J. D.; Alexiou, M. S.; Tyman, J. H. P. *J. Heterocycl. Chem.* **2008**, *45*, 397. (b) Liu, B.; Tian, H. *Chem. Commun.* **2005**, 3156. (c) Zhao, P.; Jiang, J.; Leng, B.; Tian, H. *Macromol. Rapid Commun.* **2009**, *30*, 1715.

(9) Xu, Z.; Baek, K.-H.; Kim, H. N.; Cui, J.; Qian, X.; Spring, D. R.; Shin, I.; Yoon, J. *J. Am. Chem. Soc.* **2010**, *132*, 601.

(FE of 59 fold) and a 29 nm red-shift (form 476 to 505 nm) were observed upon the addition of Zn^{2+} to the solution of **1**. Notably, Cd^{2+} induced a blue-shift in the emission (from 476 to 456 nm) with a FE of 13-fold, while Hg^{2+} caused FE of 7-fold without an obvious shift. In contrast, other metal ions did not induce a significant fluorescence enhancement of **1**. The reason for the addition of Zn^{2+} to solution **1** resulting in fluorescence enhancement is due to suppression of photoinduced electron transfer effect (PET) from the lone pair electron of the tertiary amine (DPA group) used for metal binding. These results are consistent with other groups' works reported elsewhere.[10] Upon titration of Zn^{2+}, a 1:2 stoichiometry between **1** and Zn^{2+} was confirmed by a Job plot analysis (Figure S9, Supporting Information). A proposed binding mode of **1** and Zn^{2+} (complex **6**) is shown in Figure 3.

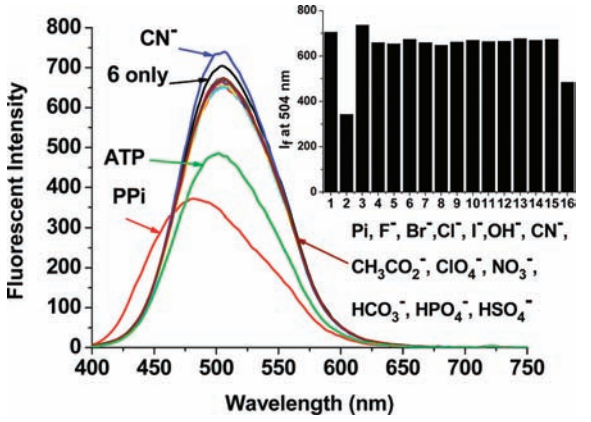


Figure 2. Fluorescence responses of **1**−Zn^{2+} complex (**6**) (1×10^{-5} M) to 10 equiv of various anions in CH_3CN−HEPES buffer (20 mM, pH 7.4) (5:95, v/v), excitation at 360 nm: (1) **6** only, (2) PPi, (3) CN^-, (4) F^-, (5) Br^-, (6) Cl^-, (7) I^-, (8) Pi, (9) OH^-, (10) $CH_3CO_2^-$, (11) ClO_4^-, (12) NO_3^-, (13) HCO_3^-, (14) HPO_4^-, (15) HSO_4^-, (16) ATP.

For the purpose of anion sensing, the fluorescence changes of **1**−Zn^{2+} complex (**6**) were further examined using PPi, CN^-, F^-, Br^-, Cl^-, I^-, Pi, OH^-, $CH_3CO_2^-$, ClO_4^-, NO_3^-, HCO_3^-, HPO_4^-, HSO_4^-,and ATP. Figure 2 shows the changes in fluorescence emission of complex **6** upon the addition of a range of anions (10 equiv) at CH_3CN−HEPES buffer (0.02 M, pH 7.4) (5:95, v/v). As shown in Figure 2, among these various anions, there was a selective 23 nm blue-shift (from 505 to 481 nm) and an obvious fluorescence quenching with PPi. Although ATP molecules also caused relatively weak fluorescence quenching, they did not induce any noticable blue-shift. Approximately 52% of the fluorescence of complex **6** was quenched upon the addition of 10 equiv of PPi. This system could detect the concentration of PPi in the range of 5.0 μM to 1.0 mM with a detection limit of 1.5 μM. In the case of ATP, only 31% of the fluorescence of complex **6** was quenched, and no obvious change was found for Pi addition. Therefore, it shows complex **6** has selectivity for PPi over related analogues such as ATP and Pi.

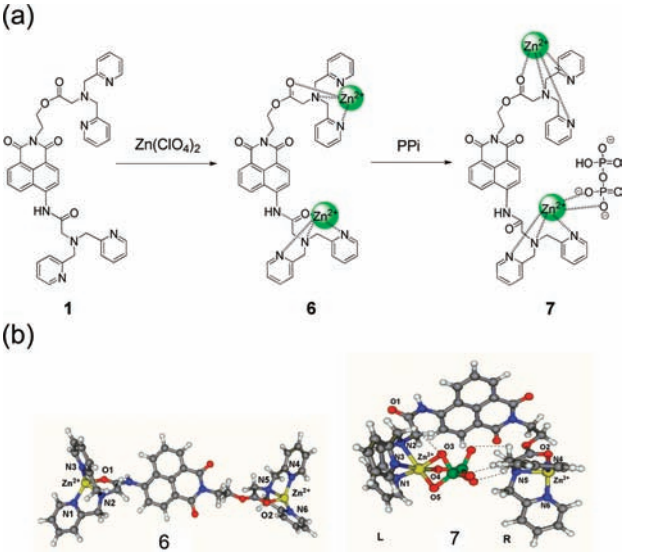


Figure 3. (a) Proposed binding modes of **1** and Zn^{2+} (complex **6**) with PPi (complex **7**). (b) Optimized structure of complexes **6** and **7** calculated at the B3LYP/6-31G(d) level of theory. The dotted line represents the hydrogen bond.

For a deeper understanding of the optical properties displayed by **1**, **6**, and **7**, the electronic excitation energies and oscillator strengths were calculated by using time-dependent density functional theory (TDDFT) at the B3LYP/6-31G (d) level of theory. All calculations were performed using a Gaussian 09 W program package.[11] The optimized structures of **6** and **7** are given in Figure 3b. After introduction of one PPi molecule into the optimized system of **6**, compound **1** with flexible chelating moieties changes its conformation. The strong bonds between the Zn^{2+} ion and nitrogen, and oxygen as well, become weaker in one of the chelating moieties of **1**. In **7**, one Zn^{2+} ion makes bonds with PPi, while the other Zn^{2+} ion bound to another chelating moiety does not make bonds with PPi. Instead, four H-bonds (Table S1, Supporting Information) are formed with the oxygen molecules of PPi and CH hydrogen atoms in the latter chelating moiety.

As shown in Figure S10, (Supporting Information), the electron density of HOMO-1 and HOMO resides on either of the two chelating moieties of **1**, whereas LUMO electron density resides only on the fluorophore moiety. This predicts that available electron density in either of the chelating moieties may participate in photoinduced electron transfer (PET), which causes fluorescence quenching in **1**. Binding of Zn^{2+} with chelating moieties eliminates the

(10) Moro, A. J.; Cywinski, P. J.; Körsten, S.; Mohr, G. J. *Chem. Commun*. **2010**, *46*, 1085.

(11) Frisch, M. J. et al. *Gaussian 09*, Revision B.01; Gaussian, Inc.: Wallingford, CT, 2010. (See the full reference in the Supporting Information.)

available electron density from the chelating moieties in **6**. Therefore, PET is prohibited in complex **6**, which is the reason for fluorescence turn-on after adding Zn^{2+} into the solution of **1**. In **7**, HOMO-1 and HOMO are located in PPi, while LUMO and LUMO+1 are located either in the chelating moiety or in fluorophore moiety. This suggests that upon excitation the photoinduced charge transfer occurs from a nonfluorophore moiety (negatively charged PPi) to either a nonfluorophore moiety (chelating unit) or a fluorophore moiety. Both kinds of charge transfer diminish fluorescence intensity or inhibit the process fully, which is in accordance with the results of spectral studies.

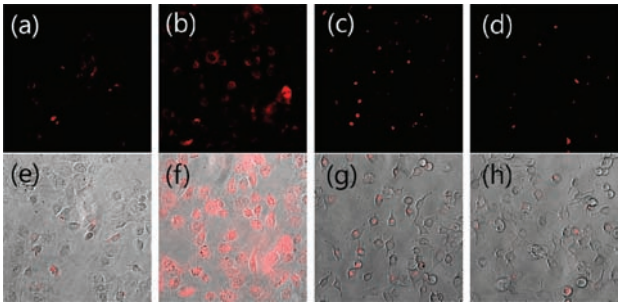


Figure 4. (a) Fluorescence image of C2C12 cells treated with **1** (1.0 μM). (b) Fluorescence images of C2C12 cells treated with **1** (1.0 μM) and Zn^{2+} (5.0 μM). (c) Fluorescence images of C2C12 cells treated with **1** (1.0 μM), Zn^{2+} (5.0 μM) and PPi (0.5 mM). (d) Fluorescence images of C2C12 cells treated with **1** (1.0 μM), Zn^{2+} (5.0 μM) and PPi (1.0 mM). (e) Bright field images of (a). (f) Bright field images of (b). (g) Bright field images of (c). (h) Bright field images of (d).

Taking advantage of the excellent selectivity of compound **6** to detect PPi, a biological application was attempted to monitor intracellular Zn^{2+} ions and PPi..

Little fluorescence was observed by addition of **1** to C2C12 cells without any exogenous Zn^{2+} ions; however, it was dramatically enhanced with the addition of **1** to the cells preloaded with 5.0 μM zinc acetate (Figure 4a,b). These results indicate that the probe emits fluorescence in the presence of Zn^{2+}. With the subsequent addition of PPi to the cells, the fluorescence intensity from the C2C12 cell decreased accordingly in a dose-dependent manner (Figure 4c,d). Some portion of the fluorescence intensty in the cells might be quenched by the intracellular ATP, and the remaining intensity was shown in Figure 4b. However, it is also clear that the intensity was quenched by the presence of PPi, and **1** can detect the change of PPi concentration.

In conclusion, a new 1,8-naphthalimide-based receptor bearing two Zn^{2+} center was investigated as selective fluorescent chemosensor for PPi over other anions in a 95% aqueous solution. Large fluorescent enhancements (FE of 59 fold) and a 29 nm red-shift were observed upon addition of Zn^{2+} to the solution of **1**. A selective 23 nm blue-shift and a fluorescence quench were only found with PPi over the related analogue, such as inorganic phosphate (Pi), in 95% aqueous solutions. As the biological application, sensor **1** is successfully applied to monitor the intracellular zinc(II) ions and PPi staining experiments in C2C12 cells. The cell study also confirmed that the fluorescence of sensor **1** enhances with Zn^{2+} ion followed by quenching in the presence of PPi.

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Supporting Information Available. Experimental procedures and characterization data of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.