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A new imidazolium acridine derivative as fluorescent chemosensor for pyrophosphate and dihydrogen phosphate

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ABSTRACT

A new acridine derivative bearing two imidazolium groups has been synthesized. This chemosensor displayed a large fluorescent quenching effect with pyrophosphate and a unique fluorescent enhancement with $H_2PO_4^-$. The strong $(C–H)^+\cdots X^-$ hydrogen bonding between the imidazolium moieties and anions is the key interaction for the recognition. The association constant of **1** with pyrophosphate was calculated as $4.9\times10^7\ M^{-1}$.

1. Introduction

Sensors based on the anion-induced changes in fluorescence appear to be particularly attractive due to the simplicity and high detection limit of the fluorescence.[1,2] Phosphate ions and their derivatives play important roles in signal transduction and energy storage in biological systems.[3] For example, pyrophosphate (PP) can be a biologically important target because it is the product of ATP hydrolysis under cellular conditions.[4]

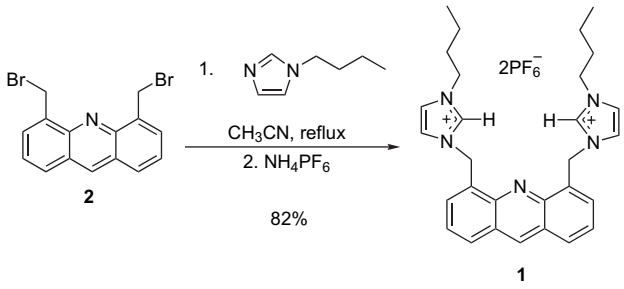
Accordingly, the fluorescent chemosensors, which can detect these phosphate derivatives have been actively studied for recent few years. Various approaches utilizing hydrogen bonding, metal ions, π–π interaction, or combination of these interactions have been adapted to the development of fluorescent chemosensors for pyrophosphate,[2b,5] phosphate ions,[2b,6] ATP/GTP,[2b,5l,7] or phosphorylated peptide.[2b,8]

Imidazolium group can make a strong interaction with anions through $(C–H)^+\cdots X^-$ type ionic hydrogen bond because the charge–charge electrostatic interaction dominates. In recent few years, various positively charged imidazolium derivatives have been synthesized and studied as selective anion receptors.[1a]

We report herein, the synthesis and binding property of a new imidazolium acridine derivative (**1**), which shows a selective fluorescent quenching effect with pyrophosphate and a selective fluorescent enhancement with $H_2PO_4^-$.

2. Results and discussion

4,5-Bis-bromomethylacridine **2** was prepared by following the published procedure.[9] This intermediate **2** was reacted with *n*-butyl imidazole followed by anion exchange with NH_4PF_6, which gave bisimidazolium acridine **1** in 82% yield (Scheme 1). Compound **1** was fully characterized by ^1H NMR, ^{13}C NMR (see Supplementary data), and high resolution FAB mass spectroscopy.



Scheme 1. Synthesis of compound **1**.

Figure 1 shows the fluorescence emission changes of compound **1** (6 μM) upon the addition of pyrophosphate, $H_2PO_4^-$, HSO_4^-, $CH_3CO_2^-$, I^-, Br^-, Cl^-, and F^- (10 equiv, tetrabutyl ammonium salts) in CH_3CN. As shown in Figure 1 (excitation and emission slit: 3 nm), there were unique changes in its emission spectrum upon the addition of pyrophosphate and $H_2PO_4^-$. A large fluorescence quenching effect was observed upon the addition of pyrophosphate, on the other hand, a selective CHEF (chelation enhanced fluorescence) effect with a slight bathochromic shift (~8 nm) was observed with

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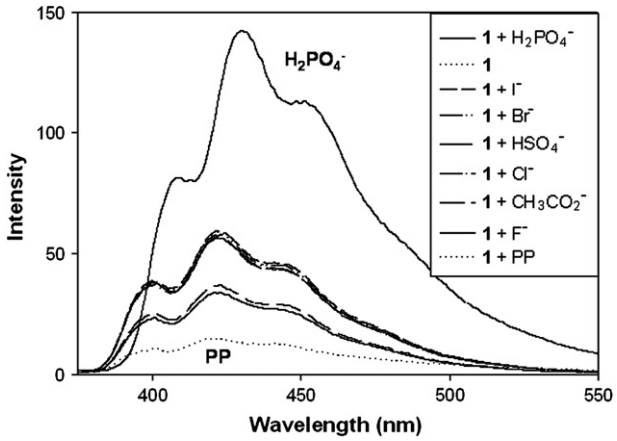

Figure 1. Fluorescent emission changes of **1** (6 μM) upon addition of tetrabutyl ammonium salts of pyrophosphate (PP), $H_2PO_4^-$, HSO_4^-, $CH_3CO_2^-$, F^-, Cl^-, Br^-, and I^- (10 equiv) in CH_3CN (excitation at 356 nm) (excitation and emission slit: 3 nm).

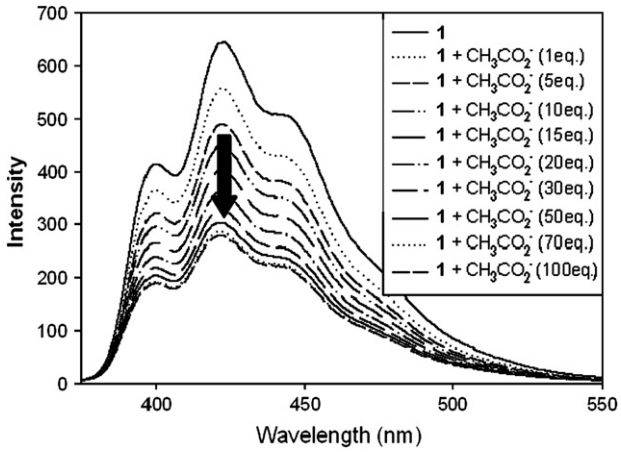

Figure 3. Fluorescent titrations of **1** (6 μM) with $CH_3CO_2^-$ in CH_3CN (excitation at 356 nm) (excitation and emission slit: 5 nm).

$H_2PO_4^-$. The addition of $CH_3CO_2^-$ or F^- induced relatively smaller CHEQ (chelation enhanced fluorescence quenching) effects compared to that of pyrophosphate. No significant change was observed upon the addition of other anions.

The strong $(C–H)^+\cdots X^-$ hydrogen bonding between the imidazolium moieties of our acridine based receptor (**1**) and anions should in principle induce a photo-induced electron transfer (PET) behavior upon anion recognition. As shown in Figure 1, fluorescence of **1** was quenched the emission effectively with pyrophosphate, F^- and $CH_3CO_2^-$, on the other hand, no other spectral changes were observed in the emission spectra, i.e., there was no evidence of either exciplex or excimer emission. Furthermore, the changes in the absorption spectra of acridine moiety were negligible. Since the binding sites of these receptors are separated from the fluorophore via methylene spacer, the observations are consistent with the typical PET behavior. Similar quenching effects due to a photo-induced electron transfer (PET) mechanism were also explained in the precedent reports.[2c,5e,n,6g] CV experiments with F^- and acetate were tried to get further evidence for PET mechanism, however, we could not get the meaningful results due to the precipitation problems.

According to the linear Benesi–Hilderand expression, the measured emission $[1/(F–F_0)]$ at 422 nm varied as a function of pyrophosphate in linear relationship ($R\cong 0.9926$), indicating the ~1:1 stoichiometry between pyrophosphate and compound **1** (S-Fig. 1 in Supplementary data). The 1:1 stoichiometry was further confirmed by Job plot (Fig. 4). From the fluorescent titrations, the association constant of complex **1** with pyrophosphate (Fig. 2, excitation and emission slit: 5 nm), F^- (S-Fig. 2 in Supplementary data), and $CH_3CO_2^-$ (Fig. 3) was observed to be 4.9×10^7, 1.3×10^5, and 2.1×10^4 M^{-1}, respectively (errors <15%).[10] The expected strong $(C–H)^+\cdots X^-$ hydrogen bonding between the imidazolium moieties and pyrophosphate was further confirmed by ^1H NMR (Fig. 5). As shown in Figure 3, the imidazolium C–H displayed a large downfield shift (δ 9.29–10.15) upon the addition of pyrophosphate (0.6 equiv) in DMSO-d_6. The ^1H NMR titration of pyrophosphate in DMSO-d_6 is shown in S-Figure 3 in Supplementary data.

The unique CHEF effect, which was induced by $H_2PO_4^-$, can be attributed to the additional hydrogen bonding between the hydrogen of OH in the $H_2PO_4^-$ and nitrogen on the acridine moiety. Similar hydrogen bondings between ammonium ion or metal ion and nitrogen on the acridine were recently reported.[11] We previously reported that a 1,8-bisimidazolium anthracene derivative displayed a large fluorescent quenching effect with $H_2PO_4^-$ in acetonitrile.[6g] The only difference between the acridine derivative (**1**) and previously reported anthracene derivative is the central nitrogen on the acridine moiety, which can induce opposite fluorescent changes with same anion. This difference can be again attributed to the additional hydrogen bonding interaction between nitrogen on acridine moiety and $H_2PO_4^-$. Further effort for ^1H NMR experiment with $H_2PO_4^-$ failed due to the solubility problem. When

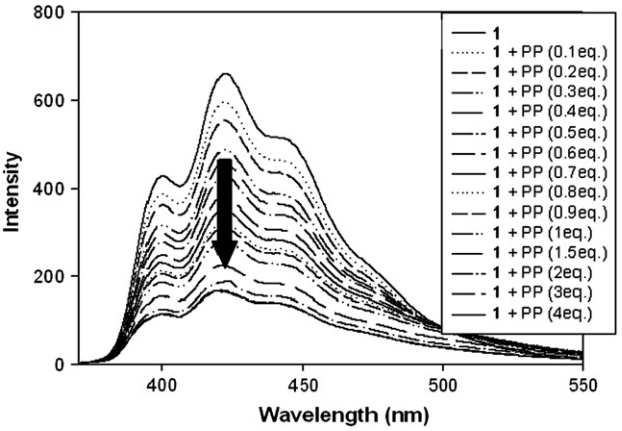

Figure 2. Fluorescent titrations of **1** (6 μM) with pyrophosphate (PP) in CH_3CN (excitation at 356 nm) (excitation and emission slit: 5 nm).

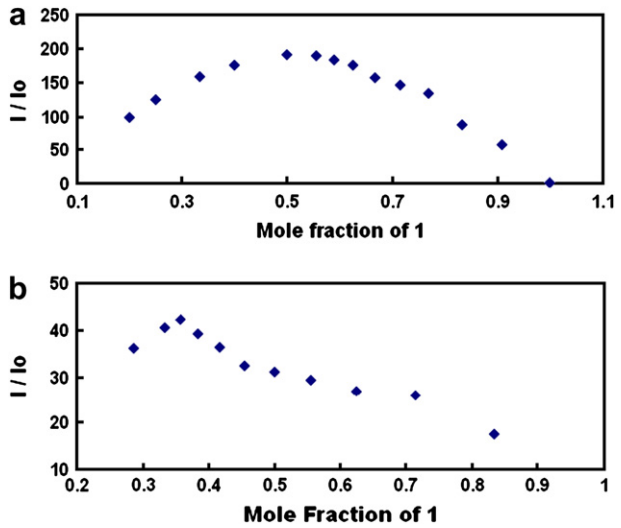

Figure 4. (a) Job's plot of **1** with pyrophosphate in CH_3CN. (b) Job's plot of **1** with $H_2PO_4^-$ in CH_3CN.

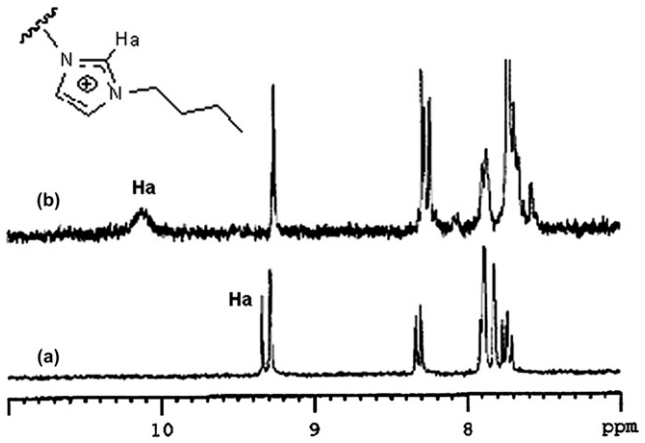

Figure 5. Partial ^{1}H NMR (250 MHz) spectra of **1** (2 mM) in DMSO-d_6: (a) **1** only and (b) **1**+0.6 equiv of pyrophosphate.

excess $H_2PO_4^-$ was added (>5 equiv), there was a small fluorescent decrease. From the fluorescent titrations (Fig. 6), the association constant of complex **1** with $H_2PO_4^-$ was observed to be $>10^8$ M^{-2},[10] which means the binding was quantitative. As shown in Figure 4, Job's plots indicate that pyrophosphate binds compound **1** by 1:1 stoichiometry, on the other hand, the stoichiometry between **1** and

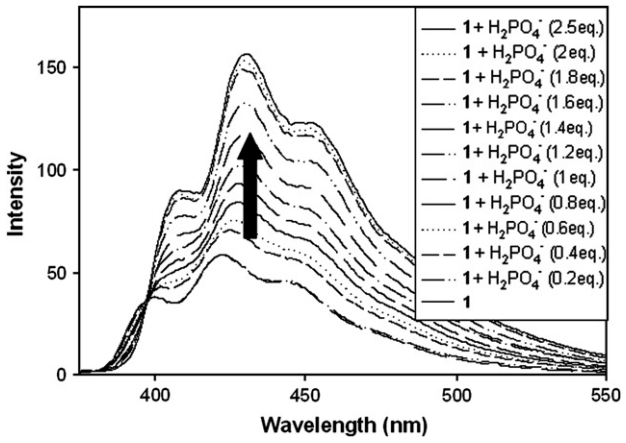

Figure 6. Fluorescent titrations of **1** (6 μM) with pyrophosphate (PP) in CH_3CN (excitation at 356 nm) (excitation and emission slit: 3 nm).

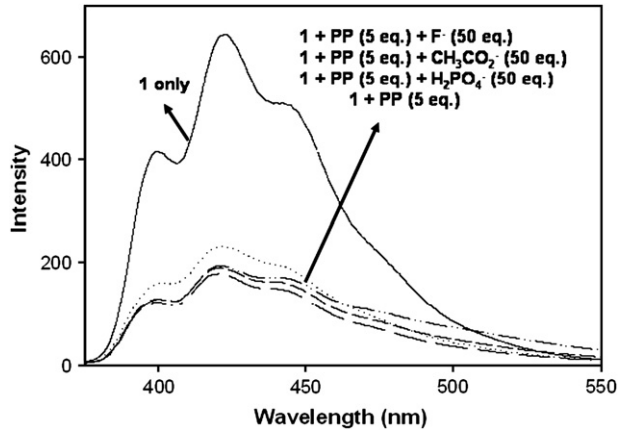

Figure 7. Fluorescent changes of **1** (6 μM) with pyrophosphate (PP) (5 equiv) in the presence of 50 equiv of various anions in CH_3CN (excitation at 356 nm) (excitation and emission slit: 5 nm).

$H_2PO_4^-$ was 1:2. Figure 7 shows the competition experiment of compound **1** between pyrophosphate and $H_2PO_4^-$, F^-, or $CH_3CO_2^-$. The presence of 10 times excess these anions (50 equiv) did not cause any significant changes in the emission of compound **1** with pyrophosphate (5 equiv). However, in the aqueous system (CH_3CN–H_2O, 9:1, v/v), compound **1** did not display any significant fluorescent changes with these anions.

3. Conclusion

In conclusion, a new imidazolium acridine receptor (**1**) synthesized as a fluorescent chemosensor for pyrophosphate and $H_2PO_4^-$. The title compound displays a large CHEQ effect with effective selectivity for pyrophosphate over other anions in acetonitrile. On the other hand, a unique CHEF effect was observed when $H_2PO_4^-$ was added. The association constant of **1** with pyrophosphate was calculated as 4.9×10^7 M^{-1}.

4. Experimental

4.1. General methods

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Flash chromatography was carried out on silica gel 60 (230–400 mesh ASTM, Merck). Thin layer chromatography (TLC) was carried out using Merck 60 F_{254} plates with a thickness of 0.25 mm. Preparative TLC was performed using Merck 60 F_{254} plates with the thickness of 1 mm.

Melting points were measured using a Büchi 530 melting point apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker 250 MHz or Varian 500 MHz. Chemical shifts were given in parts per million and coupling constants (J) in hertz. UV absorption spectra were obtained on UVIKON 933 Double Beam UV/VIS Spectrometer. Fluorescence emission spectra were obtained using RF-5301/PC Spectrofluorophotometer (Shimadzu).

*4.1.1. 4,5-Bis(N-butyl-imidazoliummethyl)acridine **1***

A mixture of 4,5-bis(bromomethyl)acridine **2** (50 mg, 0.137 mmol) and 1-butylimidazole (35 mg, 0.28 mmol) in acetonitrile (17 mL) was refluxed for 12 h under N_2. After the solvent was evaporated under vacuum, the resulting solid was dissolved in DMSO (2 mL). During the dropwise addition of saturated aqueous NH_4PF_6 solution (5 mL), light yellow precipitate was formed. After washing the precipitate several times with water, the desired product was obtained as a light yellow solid (266 mg, 82%). Mp: 136–138 °C; ^{1}H NMR (CD_3CN, 250 MHz) δ 9.16 (s, 1H), 8.45 (s, 2H), 8.28 (d, 2H, J=8.5 Hz), 7.90 (d, 2H, J=6.5 Hz), 7.69 (t, 2H, J=7.0 Hz), 7.49 (s, 2H), 7.37 (s, 2H), 6.00 (s, 4H), 4.05 (t, 4H, J=7.3 Hz), 1.69–1.81 (m, 4H), 1.19–1.29 (m, 4H), 0.86 (t, 6H, J=7.3 Hz); ^{13}C NMR (CD_3CN, 62.5 MHz) δ 147.3, 139.7, 136.8, 133.8, 132.6, 131.8, 128.2, 127.3, 124.3, 123.7, 50.9, 50.7, 32.8, 20.3, 13.9; HRMS (FAB) m/z=598.2537 $[M-PF_6]^+$, calcd for $[C_{29}H_{35}F_6N_5P-PF_6]$=598.2534.

4.2. Preparation of fluorometric anion titration solutions

Stock solutions (1 mM) of tetrabutyl ammonium salts of pyrophosphate (PP), $H_2PO_4^-$, HSO_4^-, $CH_3CO_2^-$, F^-, Cl^-, Br^-, and I^- were prepared in acetonitrile. Stock solutions of host (0.01 mM) were also prepared in acetonitrile. Test solutions were prepared by placing 4–40 μL of the probe stock solution into a test tube, adding an appropriate aliquot of each anion stock, and diluting the solution to 4 mL with acetonitrile.

For all measurements, excitation was at 356 nm. Both excitation and emission slit widths were 3 or 5 nm.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.04.085.

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