

Fluorescence Sensing of Dihydrogen Phosphate and Pyrophosphate using Imidazolium Anthracene Derivatives

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Since anions play an important role in a wide range of chemical and biological processes, the development of anion selective receptors¹ and chemosensors based on the anion-induced changes in fluorescence² have been actively investigated. Specifically, phosphate-containing anions and their derivatives play important roles in signal transduction and energy storage in biological systems.³ For example, many of common enzymes, such as kinases and phosphatases, produce or consume inorganic phosphate (Pi), which is also related to the protein phosphorylation.⁴ Accordingly, the detection and discrimination of phosphate ions⁵ and pyrophosphate (PPi)⁶ have been the main focus of the efforts of several research groups.

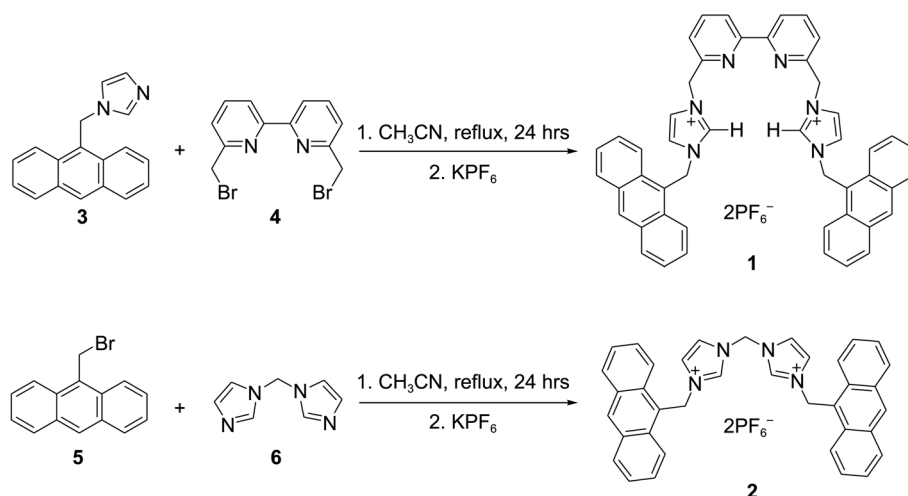
On the other hand, imidazolium group has been actively studied for the recognition of anions,⁷ where imidazolium group can interact strongly with anions through a (C-H)⁺⋯X⁻ type ionic hydrogen bond.

Herein, we report the synthesis and binding properties of new imidazolium-based fluorescent receptors (**1** and **2**) bearing two anthracene moieties and two imidazolium groups (Scheme 1), which show selectivity for H₂PO₄⁻ over other anions, such as CH₃CO₂⁻, HSO₄⁻, F⁻, Cl⁻, Br⁻ and I⁻.

Especially, fluorescent chemosensor **1** displayed a highly selective fluorescence quenching effect and a unique excimer emission peak upon the addition of H₂PO₄⁻.

For the synthesis of compound **1**, precursors **3**^{5g} and **4**⁸ were prepared by the reported procedures. Compounds **3** and **4** were then refluxed in acetonitrile for 24 hours. After washing the precipitate several times with cold CH₂Cl₂, aqueous KPF₆ was added to the bromide salt of compound **1** in DMF to give compound **1** as a light yellow solid in a yield of 81% (Scheme 1). Using a similar procedure, compound **2** was obtained as a light yellow solid in 89% yield from **5**⁹ and **6**¹⁰. Compound **1** and **2** were fully characterized by ¹H NMR, ¹³C NMR (see supporting information) and high resolution FAB mass spectroscopy.

Fluorescence emission changes of compound **1** (6 μM) with anions were examined upon the addition of PPi, H₂PO₄⁻, HSO₄⁻, CH₃CO₂⁻, I⁻, Br⁻, Cl⁻, and F⁻ (10 eq., tetrabutylammonium salts) (Fig. 1). The fluorescence spectra were obtained by the excitation of the anthracene fluorophore at 368 nm. Among these anions, H₂PO₄⁻ and PPi displayed highly selective fluorescence quenching effects associated with a unique excimer peak around 480 nm. Especially, **1**



Scheme 1. Syntheses of **1** and **2**.

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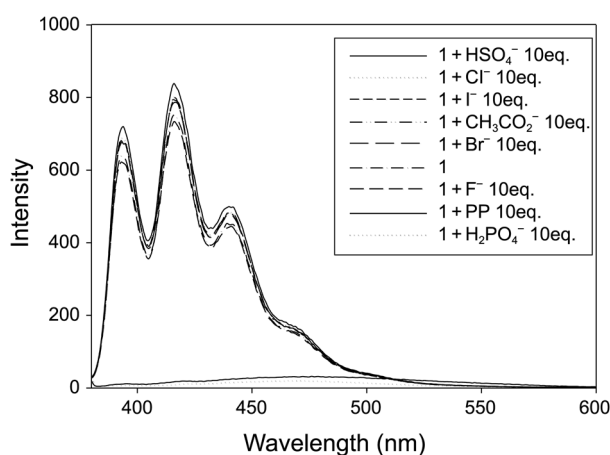


Figure 1. Fluorescent changes of **1** (6 μM) upon the addition of various anions (10 equiv.) in CH_3CN (excitation at 368 nm).

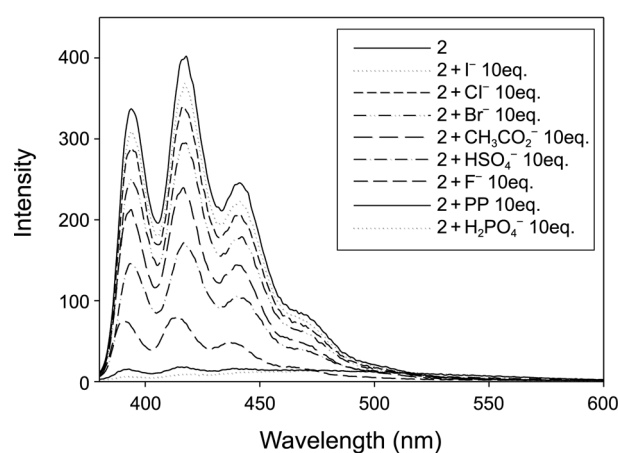


Figure 3. Fluorescent changes of **2** (6 μM) upon the addition of various anions (10 equiv.) in CH_3CN (excitation at 368 nm).

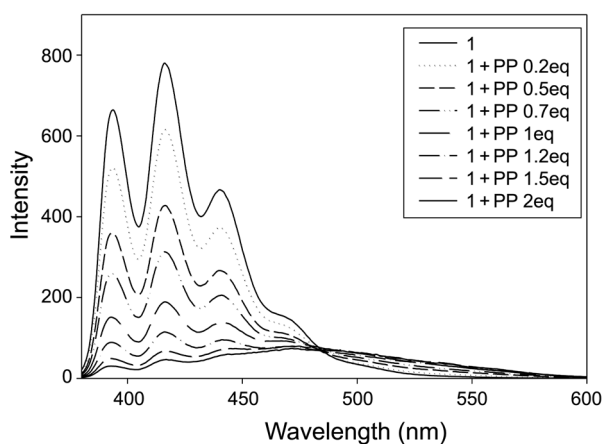


Figure 2. Fluorescent titrations of **1** (6 μM) with PPI in CH_3CN (excitation at 368 nm).

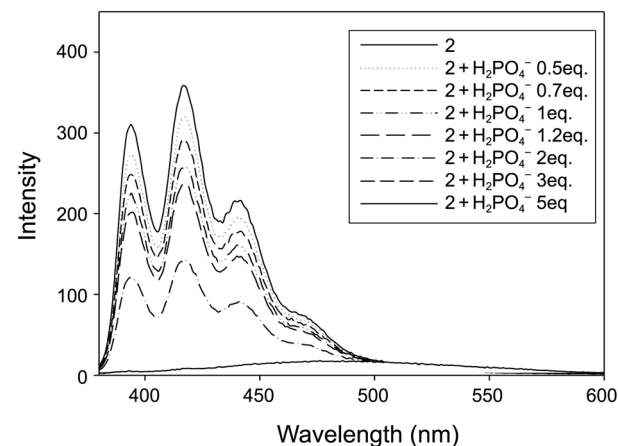


Figure 4. Fluorescent titrations of **2** (6 μM) with H_2PO_4^- in CH_3CN (excitation at 368 nm).

showed highly selective fluorescent changes with H_2PO_4^- and PPI compared with those of other anions, particularly F^- or CH_3CO_2^- . Figure 2 shows the fluorescent titration data for compound **1** with PPI. Ratiometric changes at 420 nm and 425 nm were clearly observed. From the fluorescence titrations (Figure 2, S-Figure 5, S-Figure 7 and S-Figure 8) the association constant of **1** with PPI and H_2PO_4^- were observed to be $6.19 \times 10^6 \text{ M}^{-1}$ and $4.68 \times 10^5 \text{ M}^{-1}$ (errors < 15%), respectively.¹¹

The fluorescent quenching effect with PPI and H_2PO_4^- can be attributed to a photo-induced electron transfer (PET) mechanism as explained in the precedent reports.^{5,c,d} On the other hand, the new peaks at 485 nm correspond to the excimer peak of anthracenes. This suggests that the two anthracene groups are located closer due to the hydrogen bonding between PPI/ H_2PO_4^- and the two imidazolium moieties, which can induce excimer formation.

On the other hand, **2** displayed fluorescence quenching effects with anions examined (Fig. 3). However, the quenching effects were obtained to different extents according to different anions ($\text{PPI} \approx \text{H}_2\text{PO}_4^- > \text{F}^- > \text{HSO}_4^- > \text{CH}_3\text{CO}_2^- > \text{Br}^- > \text{Cl}^- > \text{I}^-$). From the fluorescence titrations (Figure 4,

S-Figure 6, S-Figure 9 and S-Figure 10), the association constant of **2** with PPI and H_2PO_4^- were calculated to be 1.38×10^6 and $3.49 \times 10^4 \text{ M}^{-1}$ (errors < 15%), respectively.¹¹

The selective fluorescence quenching effects of compound **1** for PPI and H_2PO_4^- over other anions can be attributed to the bipyridine moiety in compound **1**, which may act as a template and an adequate linker.

In current study, new imidazolium receptors **1** and **2** containing two anthracene moieties were synthesized as a fluorescent chemosensor for PPI and H_2PO_4^- . Especially, compared to compound **2**, compound **1** displayed effective fluorescence quenching effects with PPI and H_2PO_4^- over other anions. Upon the addition of PPI and H_2PO_4^- , compound **1** and **2** displayed selective PET quenching effects and unique excimer peaks at 485 nm. The association constants of **1** and **2** with PPI and H_2PO_4^- were in the range of $10^4 \sim 10^6 \text{ M}^{-1}$. This selectivity and affinity for PPI and H_2PO_4^- can be attributed to the strong $(\text{C-H})^+ \cdots \text{X}^-$ hydrogen bonding between the imidazolium moieties and phosphate groups and an adequate spacer group between two imidazolium groups.

Experimentals

Synthesis of Compound 1. A mixture of **3** (300 mg, 1.16 mmol) and **4** (160 mg, 0.47 mmol) in acetonitrile (50 mL) was refluxed for 24 h under N₂. After cooling to the room temperature, the precipitate was filtered and washed with cold CH₂Cl₂. The bromide salt was dissolved in 5 mL methanol. During the dropwise addition of saturated aqueous KPF₆ solution, light yellow precipitate was formed. After washing the precipitate several times with water and methanol, desired product was obtained as a light yellow solid (374 mg, 81%); mp 247.6 °C; ¹H-NMR (DMSO, 250 MHz) δ 9.15 (s, 2H), 8.90 (s, 2H), 8.53 (m, 4H), 8.25 (m, 4H), 7.84 (m, 8H), 7.63 (m, 8H), 7.48 (m, 2H), 6.61 (s, 4H), 5.59 (s, 4H); ¹³C-NMR (DMSO, 62.5 MHz) δ 154.55, 153.83, 139.00, 136.93, 131.53, 131.08, 130.63, 129.88, 128.20, 126.04, 124.00, 123.80, 123.15, 120.07, 53.15, 45.45; HRMS (FAB) calcd for C₄₈H₃₈F₆N₆P [M-PF₆]⁺ 843.2800, found 843.2796.

Synthesis of Compound 2. A mixture of **5** (165 mg, 0.6 mmol) and **6** (30 mg, 0.2 mmol) in acetonitrile (20 mL) was refluxed for 24 h under N₂. After cooling to the room temperature, the precipitate was filtered and washed with cold CH₂Cl₂. The bromide salt was dissolved in 5 mL methanol. During the dropwise addition of saturated aqueous KPF₆ solution, white precipitate was formed. After washing the precipitate several times with water and methanol, desired product was obtained as a light yellow solid (148 mg, 89%); mp 228.6 °C; ¹H-NMR (CD₃CN, 250 MHz) δ 8.82 (s, 2H), 8.23-8.15 (m, 10H), 7.66-7.60 (m, 8H), 7.52 (s, 2H), 7.45 (d, 2H, *J* = 1.72 Hz), 6.32 (s, 2H), 5.93 (s, 2H); ¹³C-NMR (CD₃CN, 62.5 MHz) δ 138.67, 136.75, 136.46, 131.48, 130.99, 129.57, 128.56, 128.11, 127.94, 125.76, 124.32, 123.87, 123.69, 122.84, 122.76, 122.34, 122.19, 121.26, 120.80, 58.55, 46.26; HRMS (FAB) calcd for C₃₇H₃₀F₆N₄P [M-PF₆]⁺ 675.2112, found 675.2110.

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