1. Introduction

Ionic recognition has drawn great attention in the past decades due to its crucial role in biological, environmental, and chemical fields. Although many selective chemosensors have been reported, multi-functional chemosensors that can accomplish cascade recognition by detecting a second analyte based on a coordination complex of a host molecule and the first analyte are relatively rare. Therefore, the development of recyclable, cost effective, and high performance sensors for cascade detection is of great interest.

Among the various anions, phosphates, including inorganic species (H$_2$PO$_4^-$, HPO$_4^{2-}$, PO$_4^{3-}$, PP$_3^-$) and their derivatives, such as nucleoside polyphosphates (ATP, ADP, and AMP), play a key role in life processes, such as energy storage, effects of excess phosphate in blood also cause some diseases, such as hyperphosphatemia and cardiovascular complications, is known to cause severe deleterious effects. However, an imbalanced iron concentration is known to cause severe deleterious effects. For example, an excess accumulation of iron can cause clinical deterioration and often death in patients with severe forms of thalassemia.

Though several studies on PO$_4^{3-}$ and Fe$^{3+}$ ion recognition have been reported, there has been no report of a cascade recognition system that can detect PO$_4^{3-}$ ions first, followed by Fe$^{3+}$ ions. Herein, we exploited a fluorescent probe, 1, containing an amide H-bonding donor and benzimidazole with better fluorescence properties. Binding with PO$_4^{3-}$ led to the fluorescence of probe 1 switching on. Due to the high binding ability of Fe$^{3+}$ toward PO$_4^{3-}$, Fe$^{3+}$ can snatch PO$_4^{3-}$ from the [1–PO$_4^{3-}$] complex to form a stable FePO$_4$ complex and fully restore the emission, which can be applied to the further detection of Fe$^{3+}$. Interestingly, using PO$_4^{3-}$ and Fe$^{3+}$ as chemical inputs and the fluorescence intensity at 409 nm as
output, probe 1 can be used to mimic an INHIBIT (INH) logic gate.

2. Experimental

2.1 Materials and general methods

Deionized water was used throughout the experiment. All reagents and solvents were obtained from commercial sources and were of analytical grade. Solvents were dried according to standard procedures. The salts and solvents were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China) or Alfa-Aesar Chemical Co. (USA). NMR spectra of 1,4-benzenedi-[3-(1-pentylbenzimidazole)]-acetylaniline dichloride (1) were recorded in DMSO-d6 solution on a Bruker Avance 600 MHz spectrometer. 1H chemical shifts were reported in ppm downfield from tetramethylsilane (TMS), with the solvent resonances used as internal standards, and coupling constants (J) are given in Hz. Mass spectra were recorded on a Waters Xevo UPLC/G2-QTof MS instrument equipped with an electrospray (ESI) ion source. Infrared spectra were performed on a Nicolet AVATAR-750 FTIR spectrometer and samples were prepared as KBr pellets. Melting points were determined using an MPA100 Optimelt Automated Melting Point System and uncorrected. All pH measurements were prepared with a Sartorius basic pH-meter PB-10. UV absorption spectra were determined in 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) solution with a TU-1901 UV-vis spectrophotometer.

2.2 General procedure for spectroscopic measurements

All metal ion and anion detections were operated at pH 7.4, maintained with HEPES buffer (10 mM). The 10 µM stock solution of probe 1 was prepared in HEPES. The working solutions of probe 1 were freshly prepared by diluting the concentrated stock solution to the desired concentration prior to spectroscopic measurements. Anion solutions (0.1 M) were obtained from the tetrabutylammonium salt of F−, Cl−, Br−, I−, AcO−, H2PO4−, NO3−, CO32−, HCO3−, SO42−, NO2−, HPO42−, BrO3−, SO32−, HSO3−, SCN−, and PO43−. Metal ion solutions (0.1 M) were obtained from NaCl, KBr, Mg(NO3)2, CaCl2, Cr(NO3)3, FeSO4, Co(NO3)2, NiCl2, Cu(NO3)2, Zn(NO3)2, AgNO3, Cd(NO3)2, HgCl2, Pb(NO3)2, Ba(NO3)2, Fe(NO3)3, and Al(NO3)3. Compounds were stored in a vacuum desiccator containing self-indicating silica, and dried fully before use. All fluorescence data were recorded with an excitation of 269 nm and the slit widths of excitation and emission were 10 nm. Fluorescence measurements used titration experiments and the volume added did not exceed 3% of the total. After the solution mixture was shaken for 1 min, new spectra were measured. All experiments were performed at room temperature and conducted in triplicate at least.

2.3 Synthesis of probe 1

The synthetic route to probe 1 is shown in Scheme 1. 1,4-Bis(chloroethylamido)benzene (A) and N-pentyl benzimidazole (B) were synthesized using reported methods.27-28 Characterization results for 1 are given in the ESL†.

A mixture of A (0.783 g, 3.0 mmol), B (1.412 g, 7.5 mmol), and N,N-dimethylformamide (30 mL) in a 100 mL round bottom flask was placed in an oil bath. The reaction mixture was heated until a homogeneous solution was formed. The solution was stirred for 4.5 h at 120 °C under a nitrogen atmosphere. The mixture was cooled to room temperature and a yellow solid was separated and filtered. The resultant solid was recrystallized from methanol–ethyl acetate (1 : 1, v/v) to afford the pure product, 1.528 g (80.1%) of probe 1, as a light yellow powder. Mp: 244.4–245.9 °C. 1H NMR (DMSO-d6, 600 MHz, ppm): 11.08 (s, 1H), 9.88 (s, 1H), 8.13–8.14 (m, 2H), 8.06–8.08 (m, 2H), 7.68–7.72 (m, 4H), 7.60 (s, 4H), 5.57 (t, J = 7.2 Hz, 4H), 1.90–1.95 (m, 4H), 1.30–1.37 (m, 8H), 0.87 (t, J = 6.9 Hz, 6H). 13C NMR (DMSO, 150 MHz, ppm): 163.73, 143.89, 134.79, 132.31, 131.27, 127.28, 127.06, 120.23, 114.32, 114.20, 79.68, 79.46, 79.24, 49.53, 47.22, 28.72, 28.27, 22.03, 14.23. Mass (ESI-MS): [M + 2Cl]2+, calcd 283.3676, found 283.3718. FTIR (KBr) ν (cm−1): 3406, 3142, 3035, 2959, 2934, 2862, 1687, 1588, 1566, 1513, 1486, 1309, and 751.

3. Results and discussion

3.1 Anion sensing studies using probe 1

The availability of probe 1 as a highly selective probe for PO43− was investigated using UV-vis absorbance spectra and fluorescence spectra of probe 1 after the addition of other representative

![Scheme 1](image-url)
anions (I\(^-\), Cl\(^-\), Br\(^-\), CO\(_3^{2-}\), HCO\(_3^-\), SO\(_3^{2-}\), NO\(_2^-\), AcO\(^-\), H\(_2\)PO\(_4^-\), HPO\(_4^{2-}\), BrO\(_3^-\), NO\(_3^-\), SO\(_4^{2-}\), HSO\(_3^-\), F\(^-\), and SCN\(^-\)). The UV-vis absorbance spectra (Fig. S5\(^\ddagger\)) showed a decrease in absorption intensity at 269 nm soon after PO\(_4^{3-}\) addition, while other anions did not induce significant changes. Furthermore, as shown in Fig. 1, only PO\(_4^{3-}\) led to an approximate three-fold increase in intensity at 409 nm in the fluorescence spectrum, with a noticeable red shift in emission wavelength observed. Fluorescence emission around 373 nm was not apparent, and only slight quenching was detected, for other anions, indicating the selective sensing behavior of probe 1 toward PO\(_4^{3-}\) ions. These results indicated that probe 1 was highly selective toward PO\(_4^{3-}\) and might be a potential fluorescent probe for PO\(_4^{3-}\) in aqueous solution. Moreover, when L-tryptophan was dissolved in aqueous solution as a reference standard, the fluorescence quantum yield of the [1–PO\(_4^{3-}\)] complex was 0.2%, which was higher than that of probe 1 (0.03%). This indicated that the binding of probe 1 and PO\(_4^{3-}\) could improve the fluorescence quantum yield.

### 3.2 Anti-interference of 1 in PO\(_4^{3-}\) detection

To further gauge the increased antidisturbance performance of probe 1 toward PO\(_4^{3-}\) compared with other anions, competition experiments were carried out by adding 1.0 equiv. PO\(_4^{3-}\) to solutions of probe 1 (10 \(\mu\)M) in the presence of 5.0 equiv. of other anions (I\(^-\), Cl\(^-\), Br\(^-\), CO\(_3^{2-}\), HCO\(_3^-\), SO\(_3^{2-}\), NO\(_2^-\), AcO\(^-\), H\(_2\)PO\(_4^-\), HPO\(_4^{2-}\), BrO\(_3^-\), NO\(_3^-\), SO\(_4^{2-}\), HSO\(_3^-\), F\(^-\), and SCN\(^-\)). Fluorescence spectra were recorded, as shown in Fig. 2. The added competing ions had a negligible influence on the fluorescence maxima of the [1–PO\(_4^{3-}\)] solutions, which indicated the excellent antidisturbance performance of probe 1 in monitoring PO\(_4^{3-}\).

### 3.3 Fluorescent titration of PO\(_4^{3-}\)

To investigate the PO\(_4^{3-}\) ion binding ability of probe 1, fluorescent titration of probe 1 with varying PO\(_4^{3-}\) concentration (0–1.5 equiv.) was carried out. As shown in Fig. 3, as the PO\(_4^{3-}\) concentration increased, at \(\lambda_{ex} = 269\ nm\) the fluorescence intensity increased gradually, accompanied by a red shift and narrowing. Using 1.0 equiv. of PO\(_4^{3-}\) enhanced the emission of probe 1 significantly in the fluorescence titration data due to formation of the [1–PO\(_4^{3-}\)] complex. Fluorescence intensity has a linear response toward PO\(_4^{3-}\) in the PO\(_4^{3-}\) concentration range of 0–1.0 \(\mu\)M, with the linear equation found to be \(Y = 161.8002 + 62.6713X\) (linearly dependent coefficient: \(R^2 = 0.9989\)), as shown in Fig. 4. The detection limit derived from the fluorescence titration was 0.60 nM with 3σ/slope.\(^{29}\) The results suggested that probe 1 detected PO\(_4^{3-}\) qualitatively with high sensitivity. Furthermore, the binding constant of probe 1 with PO\(_4^{3-}\), which was obtained from fluorescence titration data using the Benesi–Hildebrand equation,\(^{30,31}\) was \(2.3 \times 10^5\ M^{-1}\), as shown in Fig. 5.

### 3.4 Effect of pH

To eliminate disturbance from protonation or deprotonation of the fluorophore during detection, the effect of pH on the fluorescence signal of probe 1 in the absence and presence of PO\(_4^{3-}\) was studied. As shown in Fig. 6, with increasing pH from 2.0 to
results were very different, showing a rapid increase in the fluorescence emission of probe 1 in the presence of 1.0 equiv. PO$_4^{3-}$. However, strong fluorescence was observed from the solution of probe 1 with PO$_4^{3-}$ in the pH range 6.5–10.1, which implied the potential application of probe 1 at environmental and physiological pH values.

3.5 Time-dependence of detection

Time is an important parameter for reaction-based probes. The time-dependent fluorescence of probe 1 (10 μM) using 1.0 equiv. of PO$_4^{3-}$ was studied by monitoring the fluorescence intensity vs. time, as shown in Fig. 7. Upon addition of PO$_4^{3-}$ to the solution of probe 1, the fluorescence intensity increased rapidly, reaching a maximum within 10 s, and then approximately leveling off, while that of the probe 1 solution alone was stable at a very low level. These results indicated that probe 1 was sensitive and reliable, and that PO$_4^{3-}$ detection can be completed within minutes.

3.6 Mechanism for PO$_4^{3-}$ sensing using 1

To confirm the stoichiometry of binding between probe 1 and PO$_4^{3-}$ ions, Job's plots analysis was carried out. As shown in Fig. 8, the maximal increment of fluorescence intensity between 1 and [1–PO$_4^{3-}$] appeared at a PO$_4^{3-}$ molar fraction of 0.5. According to the equation $n = x/(1 - x)$, in which $x$ is the molar fraction of PO$_4^{3-}$, corresponding to the maximal fluorescence change and $n$ is the binding stoichiometry, 0.5 indicated a binding stoichiometry for 1 and PO$_4^{3-}$ of 1 : 1.

This 1 : 1 stoichiometry was further confirmed by ESI-MS results. A series of peaks at $m/z$ 679.5204 corresponding to [1$^2+$ + PO$_4^{3-}$ + 4H$^+$ + H$_2$O]$^{3+}$, 701.4925 corresponding to [1$^2+$ + PO$_4^{3-}$ + 3H$^+$ + H$_2$O + Na$^+$]$^{3+}$, 715.3033 corresponding to [1$^2+$ + PO$_4^{3-}$ + 4H$^+$ + H$_2$O + Cl$^-$]$^{3+}$, 737.2858 corresponding to [1$^2+$ + PO$_4^{3-}$ + 4H$^+$ + H$_2$O + Cl$^-$ + Na$^+$]$^{3+}$, 759.2678 corresponding to [1$^2+$ + PO$_4^{3-}$ + 3H$^+$ + H$_2$O + Cl$^-$ + 2Na$^+$]$^{3+}$, and 781.2493 corresponding to [1$^2+$ + PO$_4^{3-}$ + 2H$^+$ + H$_2$O + Cl$^-$ + 3Na$^+$]$^{3+}$ in ESI-MS corresponded with a strong 1 : 1 complex formed between probe 1 and PO$_4^{3-}$ anion (Fig. S6). Therefore, the significant fluorescence intensity enhancement could be attributed to...
hydrogen bonding interactions between PO₄³⁻ and probe 1 in which oxygen atoms of PO₄³⁻ coordinate to the NH proton of amide and CH proton of imidazole. Moreover, hydrogen bonding interactions between amide NH protons and imidazole CH proton in the probe with oxygen atoms in PO₄³⁻ was pre-cedented¹⁴,²⁰,³⁵ and PO₄³⁻ formed three-coordinate complexes.¹⁹

The binding ability of probe 1 toward PO₄³⁻ was investigated using ¹H NMR titration experiments. Fig. 9 shows the partial ¹H NMR spectra of the interaction of probe 1 and PO₄³⁻. Free probe 1 exhibited two singlet peaks at 11.08 and 9.88 ppm in DMSO-d₆ in the far downfield region, attributed to amide H₄ and imidazole H₆ protons in probe 1, respectively. With increasing of PO₄³⁻ concentrations, the singlet of H₄ disappeared gradually and the signal of H₆ broadened significantly through strong intramolecular hydrogen-bonding interactions in the probe and anion-ligand coordination between probe 1 and PO₄³⁻. The disappearance of H₄ was also caused by the acidity of NH, which resulted in H-D exchange in D₂O. Meanwhile, a small and definite upfield shift (0.12 ppm) for the imidazole H₆ signal was observed. A small yet definite upfield shift for the H₆, H₇, and H₈ signals from the aromatic units in the benzimidazolium cation occurred, from 7.72–8.14 to 7.70–8.11 ppm. This evidence showed that the high affinity of probe 1 for phosphate ions among other anions was attributed to high negative charges and the oxyacid tetrahedron that binds the coordinate complex, intermolecular hydrogen bonding interactions with amide Ha and imidazole Hc, and the bis-cation was going to interact with the tris-anion through ion pairing with the anion between the two benzimidazolium units to form new host–guest complexes.

Based on the Job’s plot, ESI-MS spectra, and ¹H NMR titration experiment results, a plausible mechanism for anion sensing is shown in Scheme 2. The energy minimized structure also shows that the amide NH proton and imidazole CH proton in [1−PO₄³⁻] bind to phosphate through strong hydrogen bonding interactions between PO₄³⁻ and probe 1 in which oxygen atoms of PO₄³⁻ coordinate to the NH proton of amide and CH proton of imidazole. Moreover, hydrogen bonding interactions between amide NH protons and imidazole CH proton in the probe with oxygen atoms in PO₄³⁻ was pre-cedented¹⁴,²⁰,³⁵ and PO₄³⁻ formed three-coordinate complexes.¹⁹

Scheme 2 Proposed binding mechanism between probe 1 and PO₄³⁻.

Fig. 9 Partial ¹H NMR spectra of probe 1 (10 µM) in DMSO-d₆ after addition of PO₄³⁻. (a) 1; (b) 1 + 0.5 equiv.; (c) 1 + 1.0 equiv.
bonds. From the optimized geometry of the complex of probe 1 in Fig. 10, it was evident that the guest was strongly bound in the cleft through several hydrogen bonding interactions with both oxygen atoms of PO₄³⁻ and amide and imidazole protons. Both the amide and imidazole protons form hydrogen bonds through C–H···O and N–H···O bonds.

3.7 Cascade recognition of [1–PO₄³⁻] toward Fe³⁺

Iron is one of the most abundant and versatile transition metals, and plays various important roles in many biochemical processes. There is increasing evidence that neither iron deficiencies nor excess iron induce biological disorders in the human body. Therefore, the development Fe³⁺-selective probes with short response time, low detection limit, and low cytotoxicity is highly desirable. After selective recognition with a turn-on response, 17 metal ions (Al³⁺, Zn²⁺, Ag⁺, Ca²⁺, Mg²⁺, Fe³⁺, Fe²⁺, Hg²⁺, Pb²⁺, Na⁺, Ba²⁺, Ni²⁺, K⁺, Cu²⁺, Cr³⁺, Cd²⁺, and Co²⁺) were utilized to study the cascade recognition abilities of [1–PO₄³⁻] using UV-vis absorbance spectra and fluorescence spectroscopy in HEPES buffer (pH 7.4) solution. Complex [1–PO₄³⁻] (10 μM) was treated with 1.0 equiv. of each of the 17 metal ions, as shown in Fig. 11. Of the metal ions, only Fe³⁺ led to a noticeable increase in absorbance, a color change from colorless to light yellow, and precipitate formation after standing for 3 min, as observed by the naked eye (inset of Fig. 11). The other metal ions did not result in significant absorption changes. The selectivity of [1–PO₄³⁻] toward Fe³⁺ was further confirmed by fluorescence spectra. As shown in Fig. 12, only 1.0 equiv. Fe³⁺ restored emission completely. In comparison, the addition of 1.0 equiv. of the other metal ions resulted in small changes. Fe³⁺ was believed to snatch PO₄³⁻ to form a stable FePO₄ complex due to strong electrostatic forces, which resulted in the release of free probe 1. Apparently, the [1–PO₄³⁻] complex can serve as a fluorescent probe in the cascade recognition, enabling its potential use in bioanalysis.

3.8 Anti-interference of [1–PO₄³⁻] in Fe³⁺ detection

The sensing properties of fluorescence probe [1–PO₄³⁻] toward Fe³⁺ detection were verified using competitive experiments, which were conducted by adding 1.0 equiv. Fe³⁺ to [1–PO₄³⁻] solution in the presence of 5.0 equiv. of other metal ions (Al³⁺, Zn²⁺, Ag⁺, Ca²⁺, Mg²⁺, Fe³⁺, Hg²⁺, Pb²⁺, Na⁺, Ba²⁺, Ni²⁺, K⁺, Cu²⁺, Cr³⁺, Cd²⁺, and Co²⁺). As shown in Fig. 13, the added competing ions had a negligible influence on the fluorescence maxima of [1–PO₄³⁻] solutions in Fe³⁺ solution, which indicated the excellent anti-disturbance performance of [1–PO₄³⁻] for monitoring Fe³⁺.

3.9 Fluorescent titration of Fe³⁺

The sensing ability of [1–PO₄³⁻] toward Fe³⁺ was further determined by fluorescence titration. As shown in Fig. 14, the emission intensity of [1–PO₄³⁻] at 409 nm decreased gradually until 1.0 equiv. of Fe³⁺ was added. The linear response of fluorescence intensity toward Fe³⁺ occurred in the Fe³⁺ concentration range 0–1.0 μM, and the linear equation was found to be $Y = 569.3283 - 103.8911X$ (linearly dependent coefficient: $R^2 = 0.9905$), as shown in Fig. 15. The detection limit, evaluated using fluorescent titration, was 0.36 nM, which was lower than the maximum level of Fe³⁺ (5.4 μM) ions permitted in drinking water by the US Environmental
Protection Agency, implying that probe [1–PO₄³⁻] has great potential for use in the development of probe materials for Fe³⁺.

3.10 Molecular logic circuits

Molecular logic gates, which perform logical operations by converting inputs into detectable outputs at the molecular level, have become an active research field in supramolecular chemistry and material science because of their promising applications in molecular-scale information technology.⁴⁸,⁴⁹ These systems contain binary switches to determine the output state by controlling input conditions. Due to the cascade recognition abilities of probe 1, the logic gate properties of probe 1 were studied using PO₄³⁻ ions (IN 1) and Fe³⁺ ions (IN 2) as input signals, and the fluorescence intensity at 409 nm as the output signal. The presence of PO₄³⁻ and Fe³⁺ was defined as "1" for input, and the absence of these species was defined as "0". Similarly, the "turn-on" and "turn-off" of fluorescence were defined as "1" and "0" for the output, respectively. From the perspective of logic functions, the turn-on of 1 (output "1") can be observed only in the presence of PO₄³⁻ ions (IN 1 = 1) and in the absence of Fe³⁺ ions (IN 2 = 0), as shown in Fig. 16. Under other circumstances, output "0" would be afforded due to the weak emission of 1. Therefore, probe 1 can be used as an INHIBIT (INH) logic gate toward PO₄³⁻ ions in the absence of Fe³⁺ ions by observing emission at 409 nm. The pictorial representation and truth table for the corresponding INH logic gate are shown in Fig. 16b and c, respectively.

4. Conclusions

In summary, we designed and synthesized a fluorescent probe (1) for PO₄³⁻ with excellent overall performance, such as 100%...
aqueous solution working medium, high selectivity and sensitivity, low detection limits (0.60 nM), strong interference immunity, favorable working pH span, and a rapid response time (10 s). A Job’s plot and MS analysis showed only the formation of a \(1/\text{PO}_4^{3-}\) complex with \(1:1\) stoichiometry. The binding constant of \(1/\text{PO}_4^{3-}\) complex was calculated to be \(2.3 \times 10^3\ \text{M}^{-1}\). Interestingly, the resulting \([1–\text{PO}_4^{3-}]\) complex exhibited turn-off response toward \(\text{Fe}^{3+}\) ions in 100% aqueous solution, with a detection limit of 0.36 nM. The cascade recognition in the present study was used to construct an INH logic gate, which could inspire a wide range of multi-functional chemosensors based on natural products.

**Acknowledgements**

This work was financially supported by the Graduate Student Innovation Projects of Qiqihar University of China (No. YJSCX2016-ZD08 & 2015-ZD13), the Overseas Scholars Foundation of Scientific and Technical Agency of Heilongjiang province of China (No. LC2011C17), and the National Natural Science Foundation of China (No. 21506106 & 81403067).

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