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Introducing Ligand-Based Hydrogen Bond Donors to a Receptor: Both Selectivity and Binding Affinity for Anion Recognition in Water Can Be Improved

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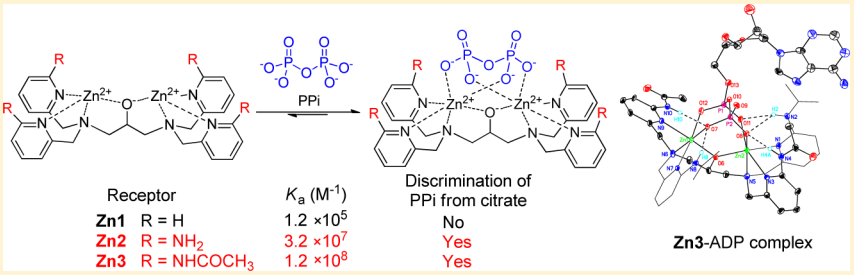
Supporting Information



ABSTRACT: Introducing hydrogen bond donors to a receptor was found to be an effective approach to improve both its selectivity and binding affinity for pyrophosphate in water. The crystal structure of **Zn3**−ADP complex showed the improvements come from the combination of H-bonding and metal coordination in a manner similar to many metalloenzymes.

Considerable attention has been given to the development of synthetic receptors for recognition and sensing of anions because anions play not only important roles in a wide range of chemical and biological processes but also crucial roles in both health and the environment.[1,2] In this regard, the creation of effective receptors for anions with high selectivity and high binding affinity in water is a major goal of current molecular recognition pursuits.[2] Although the differences in size, shape, and charge of anions are often used to design the needed selectivity for receptors, achieving high selectivity is not always easy because of the diversity of anions.[2d] In addition, the strong hydration of anions in water makes it a difficult and challenging task for synthetic receptors to achieve strong binding affinities for anions in pure aqueous solution.[3] For example, the use of electrostatic interactions and H-bonding-based organic host molecules are the most common strategies in the creation of anion receptors, but in general, these strategies are able to achieve high-binding affinities for anions only in organic solutions.[1,2] Therefore, the development of an effective method to improve selectivity or binding affinity, ideally to improve both for synthetic receptors in water, is of great significance.

Recent studies showed introducing ligand-based hydrogen bond donors to metal complex has many advantages for anion receptors.[5] For example, this strategy has been used to create receptors with improved binding affinities for phosphate and pyrophosphate anions.[6] Despite these findings, to the best of our knowledge, this strategy is rarely found to improve both selectivity and binding affinity for anion receptors.[6f]

We have used the structurally homologous dinuclear Zn(II) complexes **Zn1**, **Zn2**,[7] and **Zn3** to examine the effect of introducing ligand-based hydrogen bond donors (NH_2 for **Zn2** and $NHCOCH_3$ for **Zn3**) to the selectivity and binding affinity of synthetic receptors for PPi (Figure 1). PPi is one of the most popular sensing targets in anion recognitions because of its important roles in many biological processes.[4,8] Although significant progress has been made in the development of PPi receptors in recent years,[8,9] however, considering the complexity and the low concentration level of PPi in biological systems, receptors that can detect PPi with improved selectivity and binding affinity in water are always better choices in practical applications. Herein we report our new findings that introducing ligand-based hydrogen bond donors to **Zn1** provides a simple but effective approach to improve both its selectivity and binding affinity for PPi recognition in water.

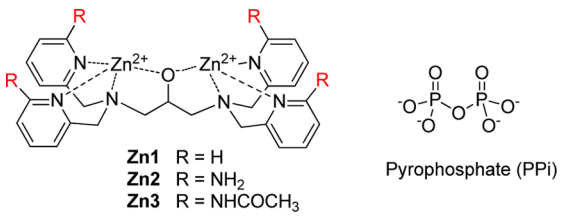


Figure 1. Complexes **Zn1**, **Zn2**, and **Zn3** and the sensing target PPi.

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As a control, the recognition ability of **Zn1** for PPi was first tested. Colorimetric sensing by naked eye is the simplest way to investigate the selectivity of **Zn1** for PPi over other anions. Since **Zn1** itself cannot be used for colorimetric sensing of PPi, indicator displacement assay (IDA)[10] is used, as this assay is simple and convenient. Addition of 1 equiv of **Zn1** to a pyrocatechol violet (PV) solution in HEPES buffer (50 mM, pH = 7.4, HEPES = 2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid) can cause distinct color changes (from yellow to blue, Figure S1, Supporting Information), so PV was selected as an indicator in this study (the same to **Zn2** and **Zn3**). Figure 2a shows the color changes of the blue **Zn1**-PV

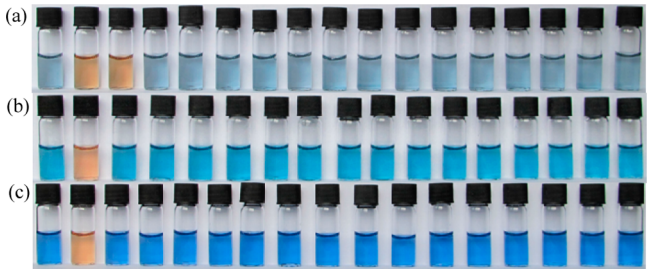

Figure 2. Color changes of ensemble **Zn1**-PV (a), **Zn2**-PV (b), and **Zn3**-PV (c) in 50 mM aqueous HEPES buffer solution (pH 7.4), [ensemble] = 25 μM, [anions] = 125 μM. Anions from left to right: none, PPi, citrate, PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$, F^-, Cl^-, Br^-, I^-, NO_3^-, SO_4^{2-}, HCO_3^-, $CH_3CO_2^-$, N_3^-, ClO_4^-, $S_2O_7^{2-}$.

ensemble solution upon addition of various anions (sodium salts) in HEPES buffer (50 mM, pH = 7.4). Clearly, although this ensemble system shows good selectivity for PPi over other anions including inorganic phosphate (Pi), halides ions, nitrate, sulfate, etc., it cannot discriminate PPi from citrate, as addition of PPi and citrate caused the same color changes (blue to yellow). Citrate as a strong competitor for PPi was also found in the previously reported sensing systems.[11] This result indicates the selectivity of **Zn1** for PPi is not good enough and needs to be improved.

Interestingly, under the same conditions, as shown in Figure 2b, while addition of various anions to the ensemble of **Zn2**–PV, only the addition of PPi caused obvious color changes (blue to yellow) of the ensemble solution. In this case, citrate is not a competitor anymore for PPi. This indicates the selectivity of receptor **Zn2** for PPi is improved compared to that of **Zn1**. To shed light on this improvement, the effect of anions on the absorption spectrum of ensemble **Zn1**–PV and **Zn2**–PV were examined in HEPES buffer (50 mM, pH 7.4) at 25 °C. As shown in Figure 3a, addition of 5 equiv of PPi and citrate caused almost the same changes to the UV–vis spectrum of **Zn1**–PV ensemble, which is consistent with the color changes shown in Figure 2a. In contrast, the effect of other anions is almost negligible, so no color changes can be observed in these systems. As for ensemble **Zn2**–PV, from Figure 3b, we can clearly see that only the addition of PPi caused obvious change to the UV–vis spectrum of the ensemble, which is also consistent with the color changes shown in Figure 2b. These experiments clearly show that **Zn2** is more selective than **Zn1** for recognition of PPi, in other words, the selectivity of **Zn1** for PPi can be effectively improved simply by introducing NH_2 as hydrogen bond donors group to its pyridyl ligand.

In order to investigate the effect of introducing ligand-based hydrogen bond donors to the binding affinity, the apparent

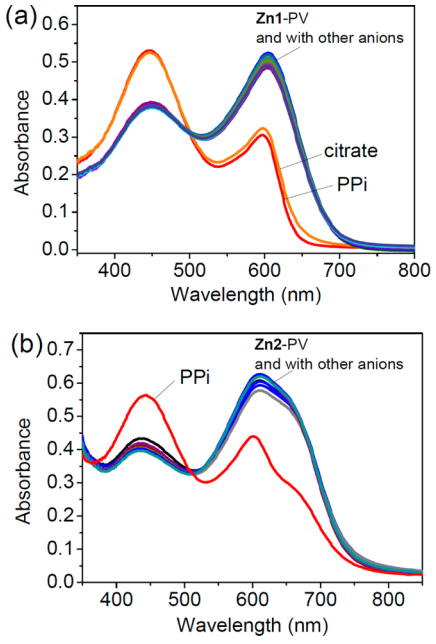

Figure 3. UV–vis spectra of ensemble **Zn1**-PV (50 μM, a) and **Zn2**-PV (50 μM, b) in the presence of various anions (5 equiv). All spectra were measured in pure aqueous solution of 50 mM HEPES buffer (pH 7.4) at 25 °C. Anions: PPi, citrate, PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$, F^-, Cl^-, Br^-, I^-, NO_3^-, SO_4^{2-}, HCO_3^-, $CH_3CO_2^-$, N_3^-, ClO_4^-, $S_2O_7^{2-}$.

association constants (K_a) of **Zn1** and **Zn2** for PPi were examined by the method of competitive UV–vis titration. Titration of complex **Zn1** and **Zn2** to PV resulted in the UV–vis spectra of PV gradually decreasing at 442 nm and increasing at 600 nm until saturation occurred (Figures S1 and S2, Supporting Information). The association constants K_a between PV and complexes **Zn1** and **Zn2** were estimated to be $(1.4 \pm 0.1) \times 10^4$ M^{-1} and $(2.5 \pm 0.7) \times 10^6$ M^{-1} in 50 mM HEPES buffer (pH 7.4) at 25 °C, respectively. Under the same conditions, titration of PPi to the ensemble solution of **Zn1**–PV and **Zn2**–PV resulted in the revival of PV's absorption, which indicates the successful displacement of the indicator PV from the ensemble by PPi in each case. A typical indicator displacement assay for receptor **Zn2** is illustrated in Figure 4. Job's plot for the binding between **Zn2** and PV, and between **Zn2** and PPi, shows a 1:1 stoichiometry, respectively (Figure S3, Supporting Information). From the gradual revival of the PV's absorption curves, the association constant K_a between PPi and complex **Zn1** and **Zn2** were estimated to be $(1.2 \pm 0.1) \times 10^5$ M^{-1} and $(3.2 \pm 0.3) \times 10^7$ M^{-1}, respectively, by fitting the titration data with a competitive binding equilibria model (Figures S4 and S5, Supporting Information).[12−14] Clearly, receptor **Zn2** equipped with a hydrogen bond donor group binds PPi more tightly than **Zn1**. Based on this, we tested receptor **Zn3** (Figure 1), which was introduced with four carboxyamido groups as better hydrogen bond donors than the aminopyridyl group in **Zn2**;, therefore, high selectivity and even better binding affinity for PPi are expected. Indeed, like **Zn2**, colorimetric sensing assays and UV–vis studies of **Zn3** showed that it has high selectivity for PPi (Figure 2c and Figure S6, Supporting Information). By the competitive UV–vis titration assays, the K_a between PPi and complex **Zn3** was estimated to be $(1.2 \pm 0.2) \times 10^8$ M^{-1} (Figures S7 and S8, Supporting Information), which is about 1000-fold and 4-fold higher than that of **Zn1** and **Zn2** binding to PPi, respectively.

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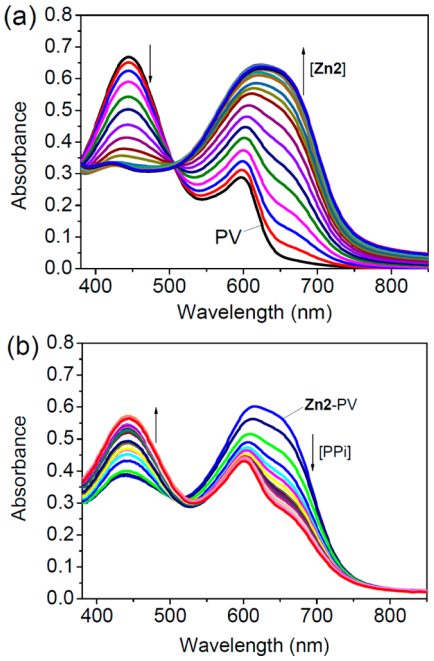

Figure 4. (a) UV−vis spectra changes of PV (50 μM) upon addition of **Zn2** (0−150 μM). (b) UV−vis spectra changes of **Zn2**−PV (50 μM) while titration of PPi (0−450 μM). All the spectra were measured in pure aqueous solution of 50 mM HEPES buffer (pH 7.4) at 25 °C.

Although efforts failed to achieve the crystal of **Zn3**-PPi, crystallization of **Zn3** in the presence of adenosine diphosphate (ADP) revealed that the two sets of oxygen anions on each P of the PPi unit of ADP bind to the dinuclear zinc complex by bridging the two metal ions to give rise to the two hexacoordinated Zn^{2+} ions in the **Zn3**−ADP complex (Figure 5). This places the phosphoryl oxygens in hydrogen bond distance to the carboxyamido groups so that they can assist the binding of PPi to the Zn^{2+} ion centers. The hydrogen bond distances (N−O) and angles (N−H···O) shown in Figure 5

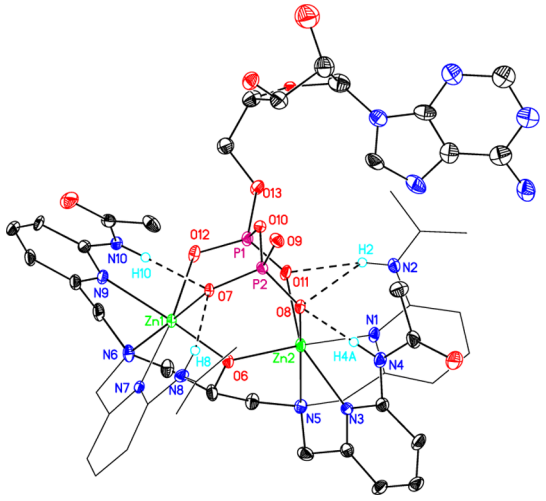

Figure 5. Crystal structure of the **Zn3**−ADP complex. Only selected N-bound hydrogen atoms are shown for clarity. The dashed lines are hydrogen bondings. Distance of the main hydrogen bondings (Å): H4A···O8, 1.928; H8···O7, 1.982; H10···O7, 1.976; N4···O8, 2.761; N8···O7, 2.824; N10···O7, 2.829. Angle of N−H···O: N4−H···O8, 162.57; N8−H···O7, 165.93; N10−H···O7, 171.42.

range between 2.76 and 2.83 Å and between 163° and 171°, respectively, which are well within the range of normal hydrogen bond distances and angles. In addition, the steric effect of the preorganized carboxyamido groups together with the metal binding center provide a more suitable cavity size to host PPi, by which the selectivity of **Zn3** for PPi is improved compared to that of **Zn1**. This provides useful insight to understand why and how enzymes can selectively recognize and tightly bind their substrates, as in nature, many metalloenzymes use amino acid side chains to provide hydrogen bondings that help the enzyme to selectively recognize and bind phosphate ester before catalyze the cleavage reaction.[15]

In summary, we have demonstrated that both the selectivity and binding affinity of a synthetic receptor for biologically important pyrophosphate can be significantly improved by an approach of introducing hydrogen bond donors group. The crystal structure of the **Zn3**−ADP complex showed that exceptional improvements by this approach come from the combination of hydrogen bonding and metal coordination. It is worth noting that this approach to improve a receptor's selectivity and binding affinity is significant because it works in aqueous solution and there are many hydrogen bond donor groups available from which to choose. We believe this approach should be applicable to a variety of host molecules to achieve better selectivity and affinity for their guest molecules.

EXPERIMENTAL SECTION

General experimental details and the method for determination of the apparent association constants (K_a) can be found in the Supporting Information. Zinc complexes **Zn1**, **Zn2**, and **Zn3** were prepared according to the published procedures.[7] The preparation and characterization of their corresponding ligands **1**, **2**, and **3** are shown as below:

Synthesis of 1. Ligand **1** was prepared as a pale yellow oil (82% yield) according to a published procedure:[16] ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.49 (dd, J = 10.8, 3.9 Hz, 4H), 7.58 (td, J = 7.7, 1.8 Hz, 4H), 7.37 (d, J = 7.8 Hz, 4H), 7.18−7.03 (m, 4H), 3.99 (dd, J = 7.8, 3.9 Hz, 1H), 3.95−3.79 (m, 8H), 2.70 (dd, J = 13.3, 3.9 Hz, 2H), 2.61 (dd, J = 13.3, 7.9 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 159.0 (4C, Py-C), 148.8 (4C, Py-C), 136.4 (4C, Py-C), 123.1 (4C, Py-C), 122.0 (4C, Py-C), 66.9 (1C, CH), 60.6 (4C, CH_2), 58.9 (2C, CH_2); IR (KBr, cm^{-1}) 3423 (br), 2924, 2853, 1663, 1592, 1571, 1474, 1434, 1382, 1263, 1150, 1125, 1094, 1049, 999, 979, 764; MS (EI+) m/z 454 (M^+, 2), 362 (M^+ − $PyCH_2$, 15), 344 (M^+ − $PyCH_2$ − H_2O, 83), 93 ($PyCH_2^+$, 100); HR-MS (ESI+) calcd for $C_{27}H_{31}N_6O^+$ (M + H^+) 455.2554, found 455.2534.

Synthesis of 3. To a mixture of 1,3-diaminopropane-2-ol (270 mg, 3 mmol) and *N*-(6-bromomethyl-2-pyridinyl)-2-acetylamide[17] (2.82 g, 12.3 mmol) in acetonitrile (60 mL) was added K_2CO_3 (1.242 g, 12 mmol) with stirring, and then the mixture was heated to reflux for 24 h under N_2. After the mixture was cooled to room temperature, the solvent was removed under reduced pressure. Cold water (50 mL) was added to the residue, and the solution was extracted with dichloromethane (4 × 50 mL). The organic extract was dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. The crude product was purified by a short column (CH_2Cl_2/CH_3OH 3:1) to afford a faint yellow foamlike solid, 1.2 g (60%): mp 118−120 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.01 (s, 4H), 8.03 (d, J = 8.0 Hz, 4H), 7.56 (t, J = 7.9 Hz, 4H), 6.93 (d, J = 7.4 Hz, 4H), 3.86 (s, 1H), 3.73 (d, J = 14.3 Hz, 4H), 3.62 (d, J = 14.2 Hz, 4H), 2.68−2.40 (m, 4H), 2.17 (s, 13H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.3 (C═O), 156.6, 151.1, 138.7, 118.8, 112.6, 77.3, 77.0, 76.7, 66.2, 59.9, 58.5, 24.4 (CH_3); IR (KBr, cm^{-1}) 3490, 3269, 1680, 1579, 1540, 1455, 1409, 1371, 1304, 1158, 1035, 997, 802; MS (EI+) m/z 682.77 (M^+, 1.8); HR-MS calcd for $C_{35}H_{43}N_{10}O_5^+$ (M + H^+) 683.3412, found 683.3419.

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Synthesis of 2.[7a] To compound **3** (0.528 mmol) was added 25 mL of 4 M HCl, and then the mixture was heated to reflux for 24 h. After being cooled to room temperature, the reaction mixture was washed with dichloromethane (3 × 20 mL). The acidic aqueous solution was then basified to pH about 12 by dropwise adding aqueous NaOH (2 M) and was extracted with dichloromethane (4 × 30 mL), dried over Na_2SO_4, and concentrated in vacuo to yield a yellow foamlike solid: 233 mg (86%); mp 67−68.5 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.45−7.28 (m, 4H), 6.67 (d, J = 7.3 Hz, 4H), 6.41 (d, J = 8.0 Hz, 4H), 3.90 (m, 1H), 3.56 (d, J = 11.0 Hz, 8H), 2.61 (dd, J = 13.2, 4.4 Hz, 2H), 2.47 (dd, J = 13.2, 7.6 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 160.6 (4C), 158.0 (4C), 139.6 (4C), 113.3 (4C), 108.5 (4C), 68.0 (1C, CHOH), 61.6 (8C, $4CH_2$), 60.1 (2C, $2CH_2N$); IR (KBr, cm^{-1}) 3323, 3193, 2963, 2819, 1622, 1573, 1465, 1337, 1262, 1163, 1098, 1029, 990, 800; TOF MS (ES+) 537 (M + Na^+), 515 (100, M + H^+); HR-MS calcd for $C_{27}H_{35}N_{10}O^+$ (M + H^+) 515.2990, found 515.2989.

■ ASSOCIATED CONTENT

Supporting Information

General experimental procedures, method for determination of the apparent association constants, Job's plot, and additional spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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