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PAPER

Studies on acedan-based mononuclear zinc complexes toward selective fluorescent probes for pyrophosphate†

Alla Sreenivasa Rao,[a] Subhankar Singha,[a] Wonyoung Choi[b] and Kyo Han Ahn*[a]

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We have demonstrated that mononuclear Zn(II)–dipicolylamine (DPA) complexes with an auxiliary ligand can fluorescently discriminate pyrophosphate over ATP with as high selectivity as the known fast responding dinuclear bis(ZnDPA) complexes.

Introduction

Fluorescent probes are indispensible tools in biochemical, medical, and environmental studies. Among various potential target analytes, biologically important anions have been extensively studied in recent years. Pyrophosphate (PPi), one of those target anions, plays an important role in bioenergetics and metabolic processes such as cellular signal transduction and protein synthesis.[1] It is also the hydrolysis product of nucleoside triphosphates such as ATP under cellular conditions.[2] The detection of PPi has an impact on real-time DNA sequencing and also cancer diagnosis.[3] Patients with calcium pyrophosphate dehydrate crystals and chondrocalcinosis have a high level of synovial fluid PPi.[4] Abnormal PPi levels can lead to vascular calcification, which results in severe medical conditions.[5] For these reasons, the monitoring of the PPi concentration level is thus important for the study of different cellular mechanisms and enzymatic processes. Fluorescent probes are powerful tools for monitoring of *in vitro* and *in vivo* PPi levels owing to the highly sensitive and easy-to-operate features of the fluorescent method.

Since Czarnik's pioneering work of a polyamine-appended anthracene as a fluorescent phosphate probe in aqueous solutions,[6] until now a handful of fluorescent probes for PPi in aqueous[7a–g] and non-aqueous media have been reported.[7h–m] In particular, zinc complexes based on dipicolylamine (DPA), represented as ZnDPA complexes, have proven to be effective for recognition of PPi in aqueous media. But the existing probes are not free from drawbacks such as low sensitivity,[8] low fluorescent enhancement factor,[9] interference from nucleoside tri- or diphosphates (ATP, ADP, and so on),[10] or slow response time.[11] With respect to the selectivity between PPi and ATP, a bis(ZnDPA) complex of Hong and co-workers shows a 9.5-fold fluorescent enhancement for PPi and a 2-fold enhancement for ATP.[12] This result points out that selective sensing of PPi over ATP or *vice versa* still remains a challenging task. Although a recently reported ratiometric system[13] shows complete selectivity for PPi over ATP, its response time seems to be very slow considering that a related phenoxy–Zn(DPA) system,[11] which also shows excellent selectivity, shows a sluggish response toward PPi. Recently, we reported a microarray chip system based on a Zn(DPA)-functionalized liposome,[14] which senses PPi over other competing phosphate compounds including ATP with an excellent selectivity and sensitivity. However, still there is a demand for the development of a homogeneous sensing system for PPi that is selective over di- and trinucleoside phosphates.

In our continuous efforts toward efficient fluorescent sensing systems for phosphate compounds of biological significance, we became interested in mononuclear Zn(DPA) complexes, which remain relatively unexplored in comparison with the widely studied bis(ZnDPA) complexes. Our approach is to introduce an auxiliary ligand to mononuclear Zn(DPA) complexes with two purposes: (1) to modulate fluorescence change as a consequence of de-coordination of the auxiliary ligand from the zinc complexes upon binding diphosphate anions, and (2) to provide additional stability to the mononuclear zinc complexes. Previously, we studied aryloxy–Zn(DPA) **1** and its copper derivative as fluorescent PPi probes (Fig. 1).[11]

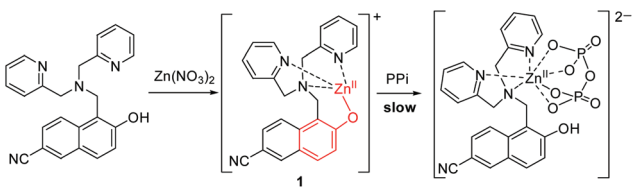


Fig. 1 Aryloxy–Zn(DPA) **1** and its binding mode with PPi.

[a]*Department of Chemistry and Center for Electro-Photo Behaviors in Advance Molecular Systems, San 31 Hyoja-dong, Pohang, 790-784, Republic of Korea. E-mail: ahn@postech.ac.kr; Fax: +82542795877; Tel: +82542792105*
[b]*School of Environmental Science and Engineering, POSTECH, San 31 Hyoja-dong, Pohang, 790-784, Republic of Korea*

†Electronic supplementary information (ESI) available: Characterization of all new compounds along with ^{1}H NMR, ITC and absorbance data of **3** with Zn^{2+}. Absorbance and fluorescence data of **4** with Zn^{2+} along with AMP, ADP, ATP, and PPi. CCDC 876211. For crystallographic data in CIF or other electronic format see DOI: 10.1039/c2ob26000j

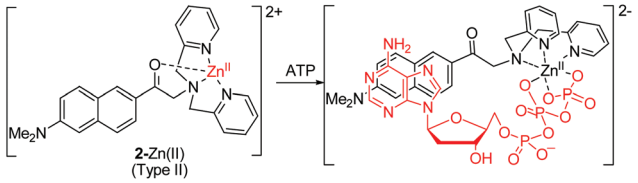


Fig. 2 Acedan derived keto–Zn(DPA) **2**–Zn(II) and its binding mode with ATP.

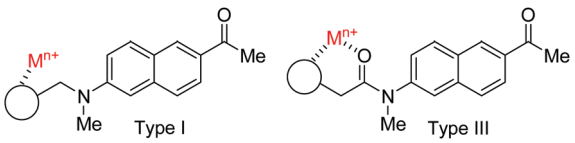


Fig. 3 Acedan derived type I and type III metal complexes.

The zinc complex **1** selectively senses PPi in an aqueous medium with large fluorescence enhancement (17-fold), and, furthermore, with little interference from ATP and other phosphate analogues. Unfortunately, the zinc complex elicited slow response, probably owing to a slow dissociation of the aryloxy–Zn(II) bond. Also, the corresponding copper complex, in which the hydroxyl group binds the copper in its neutral form, exhibited lower sensitivity toward PPi, requiring a much higher concentration level compared with the zinc complex. This low sensitivity toward the phosphate anions seems to be a general property of such copper complexes, as they bind the anions less strongly than the corresponding zinc complexes. Also, the slow response behaviour toward diphosphate anions seems to be an inherent property of such aryloxy–Zn(DPA) systems. Therefore, we sought a new auxiliary ligand for related mononuclear Zn-(DPA) complexes. Recently, we have found that a carbonyl group can act as an effective auxiliary ligand to mononuclear Zn(DPA) complexes.[15] Thus, an acedane-derived keto–Zn(DPA) complex, **2**–Zn(II) (Fig. 2), responds fast to phosphate anions (ATP, ADP, and PPi), alleviating the slow response problem observed in the case of the aryloxy–zinc complexes. Acedan, (6-dimethylaminonaphthalen-2-yl)ethan-1-one, is a well-known "donor-π-acceptor" type fluorophore.

Furthermore, **2**–Zn(II) shows turn-on fluorescence change toward ATP (and ADP), presumably owing to the π–π stacking interactions between the adenine base and the naphthalene moiety (Fig. 2). Although **2**–Zn(II) shows a similar level of binding affinities toward ATP and PPi (6.2×10^6 M^{-1} and 4.0×10^6 M^{-1} respectively), significant fluorescence change takes place only in the case of ATP (and ADP) and not in the case of PPi where such π–π stacking interactions are absent. These results provide us with an important clue on how to discriminate ATP over PPi.

The valuable results observed with the aryloxy–Zn(DPA) **1** and **2**–Zn(II) led us to further investigate related acedan derivatives as fluorescent probes for the phosphate anions. Specifically, we were interested in the Zn(DPA) complexes of 6-*N*-acyl-acedan derivatives (type III, Fig. 3). Such complexes are differentiated from the type I acedan derivatives, which have been widely explored as two-photon probes for metal cations[16] (Fig. 3). The type III and type II probes have a common feature:

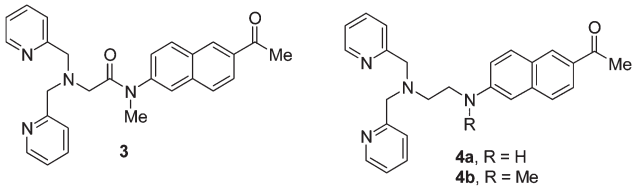
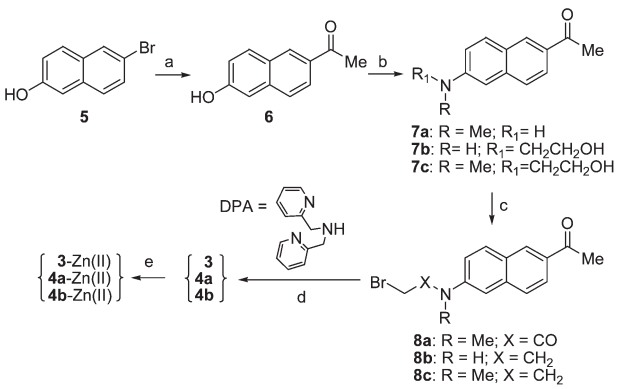


Fig. 4 Acedan derived Zn(DPA) complexes, **3**, **4a** and **4b**, evaluated in this study.



Scheme 1 Synthetic route to **3**–Zn(II). Reagents and conditions: (a) $Pd(OAc)_2$, $Ph_2P(CH_2)_3PPh_2$, ethylene glycol, 2-hydroxyethyl vinyl ether, Et_3N, 145 °C, 3–4 h; 5% aqueous HCl, room temp., 1 h (overall 80%). (b) Amine (**7a**: $MeNH_2$; **7b**: $NH_2CH_2CH_2OH$; **7c**: $NH(CH_3)$-(CH_2CH_2OH)), $Na_2S_2O_5$, H_2O, 145 °C, 72 h (**7a**: 85%; **7b**: 70%; **7c**: 70%). (c) For **8a**: Et_3N, $BrCH_2COCl$, CH_2Cl_2, 0 °C to room temp., (75%); for **8b** and **8c**: PPh_3, NBS, THF, 0 °C to room temp., (75%). (d) For **3**: DPA, K_2CO_3, CH_3CN, 80 °C, 5 h (70%); for **4a** and **4b**: DPA, NaI, Na_2CO_3, DMF/CH_3CN (1/1), 50 °C, 72 h (30%). (e) $Zn(ClO_4)_2 \cdot 6H_2O$, CH_3CN, room temp., 10 h (98%).

in both cases, anion binding to the metal complexes can induce an increase in the intramolecular charge transfer (ICT) in the acedan fluorophore, as the donor (6-acylamido group) or the acceptor (2-acyl group) ability changes.

DPA **3** is thus prepared and compared with **4a** and **4b** in which the naphthyl nitrogen may participate in the metal coordination (Fig. 4).

Results and discussion

Synthesis and characterization of 3–Zn(II) complex

Compounds **3**, **4a** and **4b** can be synthesized according to Scheme 1, starting from 6-bromo-2-naphthol.

6-Acetyl-2-naphthol (**6**) was efficiently prepared from 6-bromo-2-naphthol (**5**) through a Heck reaction using 2-hydroxyethyl vinyl ether.[17] This route greatly improves the existing synthesis that involves the Friedel–Craft acylation, which causes low conversion and also purification problems.[18] Substitution of the hydroxyl group of **6** with a primary or a secondary amino group was also efficiently carried out through the Bucherer reaction[19] to yield the corresponding acedan analogues (**7a**, **7b** and **7c**) in good yields. Then, *N*-acylation of **7a** with bromoacetyl chloride yielded the bromo compound **8a**, which was reacted

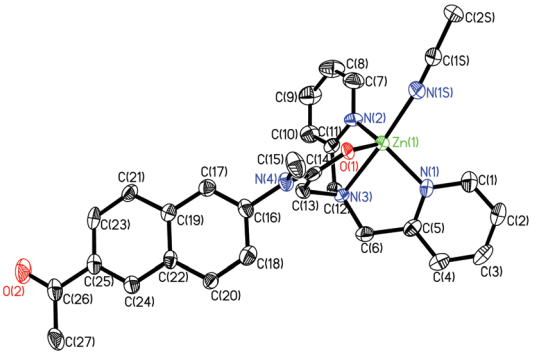
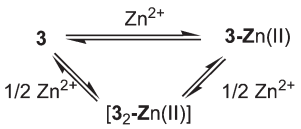


Fig. 5 Crystal structure of **3**–Zn(II). Counter anions ($2ClO_4^-$) were omitted for clarity.

Table 1 Selected bond angles and bond distances of **3**–Zn(II)

	Bond angle		Bond distance
N_1–Zn–N_2	123.42°	N_1–Zn	2.01 (4) Å
N_1–Zn–N_3	81.47°	N_2–Zn	2.03 (4) Å
N_2–Zn–N_3	80.74°	N_3–Zn	2.18 (3) Å
N_1–Zn–O	109.07°	O–Zn	2.03 (3) Å
N_2–Zn–O	119.16°	N(1S)–Zn	2.05 (4) Å
N_3–Zn–O	78.71°		



Scheme 2 Formation of a **3**–Zn(II) complex from **3** and Zn^{2+}.

with DPA to yield **3**. Compounds **4a** and **4b** were synthesized similarly from the respective bromo compounds **8b** and **8c**, which were in turn obtained by brominating alcohols **7b** and **7c** respectively using *N*-bromosuccinimide (NBS) and triphenylphosphine. To synthesize **3**–Zn(II), **3** was treated with zinc perchlorate hexahydrate in acetonitrile. Similarly, **4a**–Zn(II) and **4b**–Zn(II) were synthesized.

The solid structure of **3**–Zn(II) was determined by single crystal X-ray crystallography (Fig. 5).[20] The solid structure shows that the carboxamido oxygen (O1) coordinates to zinc, together with the nitrogens in DPA. One acetonitrile molecule (Zn–N(1S) = 2.046(4) Å)[21] completes the fifth coordination site. Selected bond angles and distance data are listed in Table 1.

Further studies on the complex formation between **3** and Zn^{2+} by ^{1}H NMR (Fig. S1†) and Isothermal Titration Calorimetry (ITC) (Fig. S2†) indicate that, in the early stage of titration, the [$\mathbf{3}_2$–Zn(II)] complex forms but eventually it is converted to the more stable **3**–Zn(II) (Scheme 2). Two DPA ligands seem to bite zinc ions in a crossover form in the case of the [$\mathbf{3}_2$–Zn(II)] complex, which is converted to the more stable **3**–Zn(II) complex. Through a nonlinear least squares fit for the ITC binding isotherm, stepwise association constants were obtained: $K_1 = 5.98 \times 10^6$ and $K_2 = 2.51 \times 10^8$ in acetonitrile at 303 K. A similar behaviour was previously observed in the case of **2**–Zn(II) (Fig. 2).[15] HRMS FAB analysis in the positive mode for

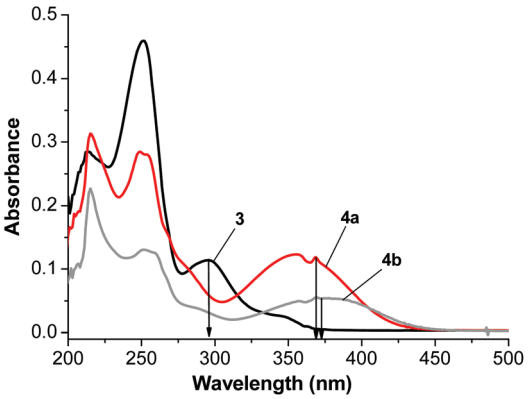


Fig. 6 Absorption data of **3**, **4a** and **4b** (10 μM) in pH 7.4 buffer (10 mM HEPES containing 1% CH_3CN).

a solution of the **3**–Zn(II) complex exhibited its perchlorate salt peak at *m*/*z* 603.08 ($C_{27}H_{26}O_6N_4ClZn$), which also supports the formation of a 1 : 1 complex in solution (Fig. S3†).

Optical and luminescent properties of 3 and 4, and their zinc complexes

UV absorption spectra of **3**, **4a** and **4b** are collectively represented in Fig. 6. The absorption band of **3** in the longer wavelength region indicates that acyl substitution at the 6-amino group causes hypsochromic shifts of 85–87 nm in comparison with **4a** (368 nm) and **4b** (370 nm), owing to the reduced electron donating ability of the 6-acylamino group to the (naphthalen-2-yl)ethan-1-one moiety.

When **3** were treated with an equimolar amount of zinc perchlorate, the absorption maximum of **3** at 296 nm underwent a hypsochromic shift of 10 nm, plausibly owing to reduced electron density on the nitrogen adjacent to the naphthalene moiety as zinc complex formation (Fig. S4a†). Little enhancement was observed in emission spectra (Fig. S5†). In contrast to **3**, the band of **4a** at 368 nm underwent a significant hypsochromic shift ($\Delta\lambda$ = 52 nm, λ_{max} = 316 nm) (Fig. S4b†) upon coordination of the zinc ion, implying that the 6-amino group participates in the metal coordination. In the case of **4b**, the broad band near 380 nm underwent a small hypsochromic shift ($\Delta\lambda$ = ~25 nm) with a small decrease in the intensity (Fig. S4c†), which suggests that the 6-methylamino group weakly participates in the zinc coordination, plausibly owing to steric strain. Absorption and emission data are summarized in Table 2.

Sensing studies for phosphate anions

The absorption and emission responses of the three zinc complexes (**3**, **4a** and **4b**) were evaluated in the presence of various anions including PPi, ATP, ADP and AMP. According to our rationale in its design, absorption/emission spectra of the zinc complexes are expected to change upon interaction with phosphate derivatives. All the sensing experiments were carried out in an aqueous buffer solution of pH 7.4 (10 mM HEPES containing 1% CH_3CN). Upon addition of di- and tri-phosphate anions (ATP, ADP and PPi), the absorption spectrum of **3**–Zn(II)

Table 2 Summary of absorption and emission data of **3** and **4** in pH 7.4 buffer (10 mM HEPES containing 1% CH_3CN)

Ligand	**3** λ_{max}; λ_{em} [a]	**4a** λ_{max}; λ_{em} [b]	**4b** λ_{max}; λ_{em} [c]
Ligand alone	295; 437 nm	368; 498 nm	370; 513 nm
Ligand + Zn^{2+}	285; 421 nm	316; 488 nm	370; 505 nm
+ATP[d]	293; 434 nm	368; 491 nm	370; 504 nm
+ADP[d]	293; 431 nm	368; 489 nm	370; 504 nm
+AMP[d]	293;[e] 424 nm	316; 489 nm	370; 505 nm
+PPi[d]	293; 442 nm	368; 494 nm	370; 505 nm
Fluorescence enhancement	10-fold for PPi	1.5-fold for ATP and ADP	2.7-fold for ATP and ADP

[a] λ_{ex} = 295 nm. [b] λ_{ex} = 330 nm. [c] λ_{ex} = 370 nm. [d] The phosphate analyte was added to an equimolar mixture of the ligand and the probe in the buffer solution. [e] In the case of **3**, the absorption value of AMP is not accurate due to interference from that of adenosine.

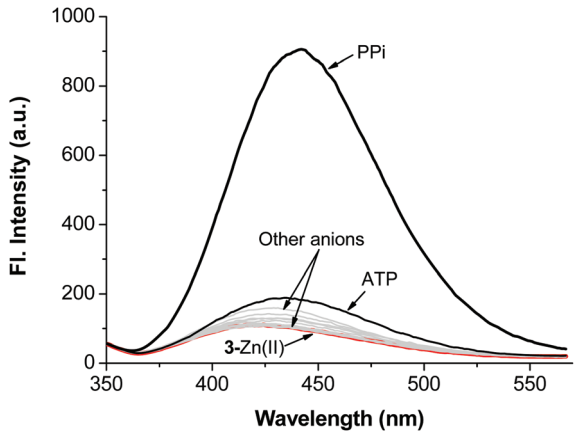


Fig. 7 Fluorescence spectral change of **3**–Zn(II) (10 μM) (the bottom red line) upon addition of each of various anions (5 equiv.; F^-, Cl^-, OH^-, CN^-, CH_3COO^-, HSO_4^-, HPO_4^-, PO_4^-, AMP, ADP, ATP and PPi; as sodium salts, except KF) in pH 7.4 buffer (10 mM HEPES containing 1% CH_3CN). λ_{ex} = 295 nm.

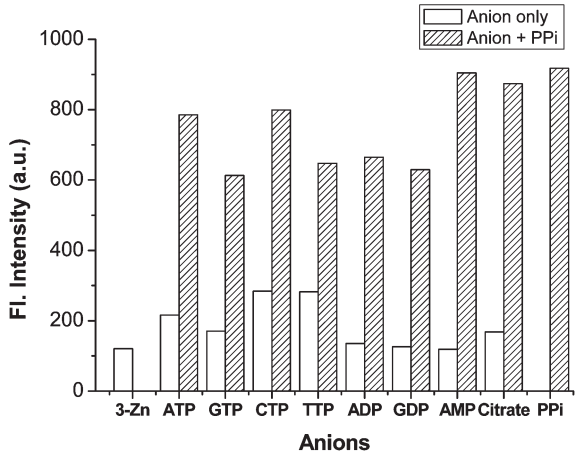


Fig. 8 Fluorescence spectral change of **3**–Zn(II) (10 μM) upon addition of each of various anions (50 μM, empty bars) followed by PPi (50 μM, filled bars) as sodium salts; in pH 7.4 buffer (10 mM HEPES containing 1% CH_3CN). λ_{ex} = 295 nm.

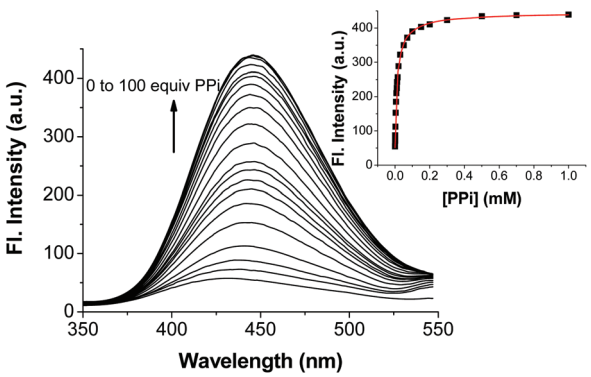


Fig. 9 Fluorescence spectral change of **3**–Zn(II) (10 μM) upon gradual addition of PPi (0–1 mM) in pH 7.4 buffer (10 mM HEPES containing 1% CH_3CN). λ_{ex} = 295 nm. Inset: plot of fluorescence intensity *vs.* [PPi]. Due to the second-order diffraction interference at 570 nm, the fluorescence data were cut down at 550 nm.

in the buffer overlapped with that of **3**, resulting in a bathochromic shift of 10 nm (Fig. S4a†); this result suggests that the zinc coordination by the carboxamido group becomes weak or broken on binding the anions, as we intended in its design. The emission spectrum obtained for an equimolar mixture of **3**–Zn(II) and PPi showed significant enhancement in the intensity (10.1-fold). Other anions (F^-, Cl^-, OH^-, CN^-, CH_3COO^-, HSO_4^-, HPO_4^-, PO_4^-, AMP, and ADP) induced slight changes in the fluorescence intensity except ATP, which caused a small enhancement (1.86-fold) (Fig. 7). Thus, **3**–Zn(II) selectively senses PPi over ATP with a relative fluorescence enhancement factor of >5 (10.1/1.86), which is comparable to the best selectivity obtained with a fast-reacting dinuclear bis(ZnDPA) probe by Hong and co-workers.[12] A competitive anion screening also confirmed the selective response of **3**–Zn(II) toward the competing anions (Fig. 8). The detection limit of **3**–Zn(II) from the fluorescence enhancement data (up to 20 μM) was estimated to be 0.1 μM (Fig. S6†).

This result suggests that mononuclear ZnDPA complexes, which have been much less explored than bis(ZnDPA) complexes, can be as good as or even better (with respect to synthesis) candidates compared to bis(ZnDPA) complexes for the development of more selective fluorescent probes for PPi over ATP, which remains a challenging issue in the case of homogeneous fluorescent probes. The fluorescence response is fast and thus can be immediately followed at room temperature.

The data of fluorescent titration of **3**–Zn(II) with varying concentrations of PPi (Fig. 9) show a gradual increase in the intensity and saturation at 20 equiv. of PPi in the pH 7.4 buffer.

From the titration data, an association constant of **3**–Zn(II) toward PPi was estimated to be 2×10^4 M^{-1}: a similar level of association constant ($K_{ass} = 1 \times 10^4$ M^{-1}) was observed for ATP, demonstrating again that the fluorescent behaviour does not directly correlate to the association constant.[15,22] The quantum yield (Φ_F) of **3**–Zn(II) complexed with PPi was determined to be 4.8% (quinine sulfate as reference: Φ_F = 54.6% in 1.0 N H_2SO_4 at λ_{ex} = 305 nm), which is six times larger than that of **3**–Zn(II) itself (Φ_F = 0.8%).

Even though ATP binds **3**–Zn(II) as strongly as PPi does, its influence on the fluorescence change is moderate. When 1 equiv.

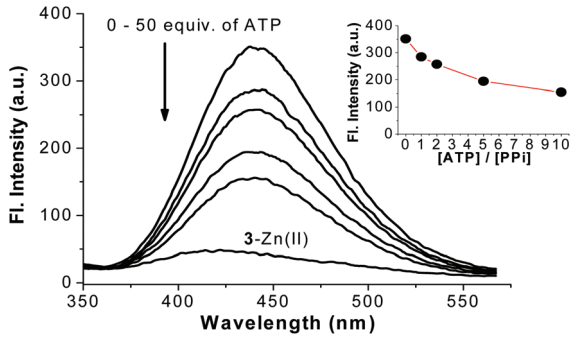


Fig. 10 Influence of ATP on the fluorescence of **3**–Zn(II) (10 μM) and PPi (50 μM) in pH 7.4 buffer (10 mM HEPES containing 1% CH_3CN): 0, 1, 2, 5, 10 equiv. of ATP with respect to PPi was added. λ_{ex} = 295 nm. Inset: plot of the fluorescence intensity with respect to the molar ratio of [ATP]/[PPi].

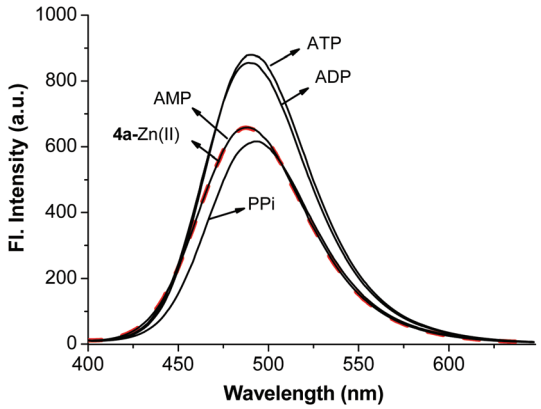


Fig. 11 Fluorescence spectral change of **4a**–Zn(II) (10 μM) in HEPES buffer (10 mM, pH 7.4; containing 1% CH_3CN. λ_{ex} = 330 nm) upon addition of anions (1 equiv.). PPi, ATP, ADP, and AMP were used as corresponding sodium salts.

of ATP with respect to PPi was added to a solution of **3**–Zn(II) (10 μM) and PPi (50 μM), the emission intensity decreased only by 20%; upon addition of 10 equiv. of ATP, the intensity became roughly a half of that without interference (Fig. 10).

In contrast to **3**–Zn(II), **4a**–Zn(II) shows slight or little changes in the emission intensity upon addition of PPi or AMP, whereas it shows small increase in the fluorescence intensity toward ATP or ADP (0.5-fold) as the metal complex itself emits sizeable fluorescence (Fig. 11). Similarly, **4b**–Zn(II) shows little changes in the fluorescence upon treatment with PPi or AMP, whereas it shows a 2.7-fold enhancement in the fluorescence intensity (Fig. 12). Absorption and emission data are summarized in Table 2.

The fluorescent response of **4a**–Zn(II) and **4b**–Zn(II) toward ATP and ADP may be explained by evoking π–π stacking interactions between the adenine base and the naphthalene moiety, as in the case of the type II complex, **2a**–Zn(II). In the absence of such π–π stacking interactions, however, mononuclear ZnDPA complexes seem to selectively sense PPi over ATP and ADP, as observed in the case of **3**–Zn(II) in which the rigid carboxamido link seems to hinder the stacking interactions. These results,

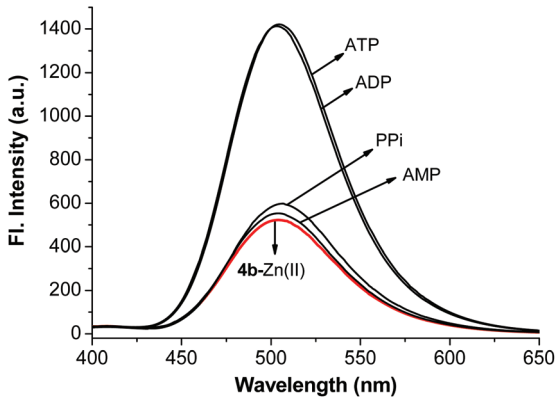


Fig. 12 Fluorescence spectral change of **4b**–Zn(II) (10 μM) in pH 7.4 buffer (10 mM HEPES containing 1% CH_3CN) upon addition of anions (1 equiv.). λ_{ex} = 370 nm. PPi, ATP, ADP, and MP were used as corresponding sodium salts.

together with the previous results observed by **2a**–Zn(II),[15] suggest that the π–π stacking interactions between such Zn(DPA) complexes and ATP (and also ADP) play an important role in fluorescently discriminating PPi over ATP or *vice versa*.

Plausible binding mode between 3–Zn(II) and PPi

The anion binding process was studied by following ^{1}H NMR spectral change for **3** as it forms the zinc complex and then binds to PPi in CD_3CN (Fig. 13). Protons of **3**, upon complexation with Zn^{2+}, show significant shifts to downfield owing to the inductive effect of the metal ion: for example, $\Delta\delta$ = 0.2, 0.12, 0.3, 0.28, and 1.15 ppm for H-4, H-8, H-9, H-10 and H-13 protons, respectively.

Significant downfield shifts of H-4, H-5 and H-8 can be explained by the coordination of the carboxamido oxygen to the Zn^{2+}. Upon addition of PPi to the zinc complex of **3**, significant upfield-shifts were observed for these protons, supporting that the coordination between the carboxamido oxygen and the zinc became weak or broken. Thus, both the NMR, absorption and emission data support the participation of the carboxamido group in the metal complexation in solution. On the basis of the optical, luminescent, and NMR data, a plausible binding process in the case of PPi is depicted in Fig. 14. In the case of ATP, possible π–π stacking interactions between the acedan moiety and the adenine base of ATP seem to be absent, in contrast to the previous case of **2a**–Zn(II).

Conclusions

We have evaluated a new type of acedan-derived mononuclear zinc–dipicolylamine complexes as fluorescent sensing systems for phosphate-containing anions. Among them, a zinc complex coordinated by a carboxamido group as an auxiliary ligand, which is characterized by single crystal X-ray crystallography, is found to selectively sense pyrophosphate anions over other phosphate containing anions including ATP, showing a 10.1-fold fluorescence enhancement. The fluorescence response is fast, overcoming the slow response problem previously observed by

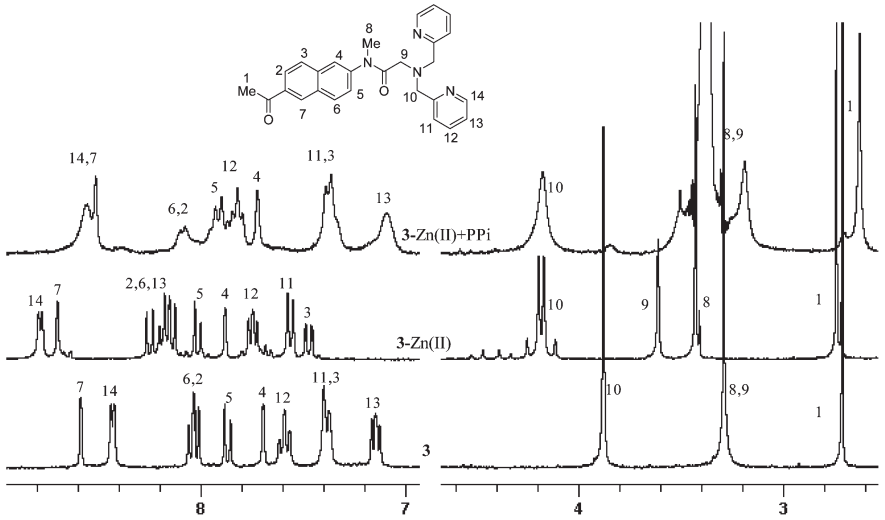


Fig. 13 ^{1}H NMR data of **3**, **3**–Zn(II), **3**–Zn(II)/PPi (1 : 1 ratio), taken in CD_3CN (D_2O used for PPi).

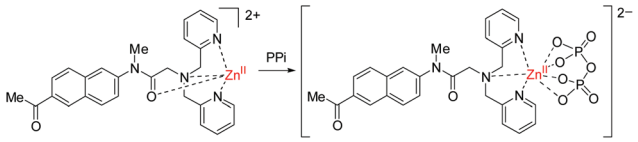


Fig. 14 Plausible binding mode between PPi and **3**–Zn(II).

mononuclear aryloxy–zinc complexes. The fluorescent enhancement is caused by binding induced weakening or breaking of the metal coordination by the carboxamido ligand, which causes change in the intramolecular charge transfer in the acedan moiety. The relative fluorescence enhancement of the probe toward PPi over ATP is comparable to the best selectivity obtained by a fast-responding dinuclear Zn(II)–dipicolylamine complex known so far, demonstrating effectiveness of such an approach of mononuclear complexes for that purpose. The results shown here, together with the previous works, provide valuable information on how to selectively sense pyrophosphate over ATP and *vice versa*. A further structure elaboration of the related mononuclear zinc complexes is under study toward fluorescent probes with desirable two-photon absorption property and improved selectivity between the phosphate anions.

Experimental

Materials and methods

All chemicals were of reagent grade, purchased from Sigma-Aldrich and used without further purification. All the reactions were performed under an argon atmosphere unless otherwise stated. Compounds purified by column chromatography were carried out on silica gel 60 (230–400 mesh). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX-300 spectrometer. NMR chemical shifts are reported in units of δ (ppm). UV/Vis spectra were recorded on an HP 8453 spectrophotometer. Fluorescence spectra were recorded on a Photon Technology International fluorimeter with a 10 mm cuvette. Melting point was measured on an Electrochemical MEL-TEMP @3.0. Mass spectral analysis was recorded with a Jeol JMS 700 and was reported in units of mass to charge (m/z). HRMS was performed at the Korea Basic Science Center, Kyungpook National University.

Synthesis

Compounds **3**, **4a**, and **4b**, and the corresponding zinc complexes were synthesized according to Scheme 1. Compounds **7c** and **8c** were synthesized according to the literature procedure[23] from compounds **6** and **7c**, respectively.

6-Acetyl-2-hydroxynaphthalene (6)[17]

A mixture of 6-bromo-2-naphthol (2.0 g, 8.97 mmol) and $Pd(OAc)_2$ (100 mg, 0.45 mmol), DPPP (370 mg, 0.9 mmol) in ethylene glycol (15 mL) under argon at room temperature was degassed three times, and then it was treated with 2-hydroxyethyl vinyl ether (2.37 g, 27 mmol) and triethylamine (3.12 mL, 22.4 mmol). The reaction mixture was heated to 145 °C and the progress of the reaction was monitored by thin layer chromatography. After completion of the reaction (3–4 h), the mixture was cooled to room temperature, diluted with CH_2Cl_2 (15 mL) and aqueous HCl (5%, 30 mL), and then it was further stirred for 60 min at room temperature. The organic layer was separated and the aqueous layer was extracted twice with CH_2Cl_2 (2 × 30 mL), and the combined organic layer was washed with water, dried over sodium sulfate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2 as eluent) to yield **6** (1.33 g, 80%); mp 172 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.41 (1H, s), 7.98 (1H, dd, J = 8.7, J = 1.6 Hz), 7.87 (1H, d, J = 8.7 Hz), 7.70 (1H, d, J = 8.7 Hz), 7.16 (1H, dd, J = 8.7, J = 1.6 Hz), 5.4 (1H, s), 2.71 (3H, s).

6-Acetyl-2-methylaminonaphthalene (7a)

A mixture of methyl amine (50% in H_2O) (4 mL, 53.75 mmol), compound **6** (2.0 g, 10.75 mmol), $Na_2S_2O_5$ (3.4 g, 21.5 mmol),

and H_2O (20 mL) in a sealed tube was stirred at 145 °C for 48 h. The product was collected by filtration, washed with water, and the combined filtrate was extracted with CH_2Cl_2. The extract was condensed *in vacuo* and then purified by flash column chromatography (CH_2Cl_2/MeOH = 50/1 as eluent) to afford **7a** (1.82 g, 85%): mp 182 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.3 (1H, s), 7.91 (1H, dd, J = 8.7, J = 1.6 Hz), 7.70 (1H, d, J = 8.7 Hz), 7.62 (1H, d, J = 8.7 Hz), 6.89 (1H, dd, J = 8.8, J = 2.2 Hz), 6.77 (1H, s), 4.17 (1H, br. s), 2.97 (3H, s), 2.67 (3H, s).

[6-(2-Hydroxyethylamino)naphthalen-2-yl]ethan-1-one (7b)

A mixture of compound **6** (1.0 g, 5.37 mmol), 2-aminoethanol (1.64 g, 26.85 mmol), $Na_2S_2O_5$ (2.0 g, 10.74 mmol), and H_2O (15 mL) in a sealed tube was stirred at 145 °C for 48 h. The product was collected by filtration, washed with water, and the combined filtrate was extracted with CH_2Cl_2. The extract was then purified by flash column chromatography (CH_2Cl_2/MeOH = 50/1 as eluent) to afford **7b** (0.86 g, 70%): ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.31 (1H, s), 7.91 (1H, dd, J = 9.0, J = 3.0 Hz, s), 7.72 (1H, d, J = 9.0 Hz), 7.60 (1H, d, J = 9.0 Hz), 6.94 (1H, dd, J = 9.0 Hz), 6.84 (1H, s), 4.46 (1H, br. s), 3.94 (2H, t), 3.44 (2H, t), 2.67 (3H, s), 1.66 (1H, br. s). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6) δ 197.74, 148.56, 138.05, 130.68, 130.63, 130.34, 125.87, 125.82, 124.60, 118.83, 103.45, 60.49, 45.75, 26.39. HRMS-EI (+): m/z calcd for $C_{14}H_{15}NO_2$: 229.28, found 229.11.

N-(6-Acetylnaphthalen-2-yl)-2-bromo-*N*-methylacetamide (8a)

A solution of compound **7a** (0.25 g, 1.26 mmol) and Et_3N (350 µL, 1.26 mmol) in CH_2Cl_2 (10 mL) at 0 °C was treated with bromoacetyl chloride (210 µL, 1.26 mmol) under an argon atmosphere. The mixture was stirred at 0 °C and then allowed to attain room temperature. Progress of the reaction was monitored by TLC (100% CH_2Cl_2). The reaction mixture was diluted with cold water after completion of the reaction. After usual work-up, the crude product was purified by silica gel column chromatography (CH_2Cl_2 as eluent) to yield **8a** (0.3 g, 75%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.51 (1H, s), 8.06 (2H, m), 7.91 (1H, d, J = 9.0 Hz), 7.83 (1H, s), 7.45 (1H, dd, J = 9.0, J = 3.0 Hz), 3.72 (1H, s), 3.42 (3H, s), 2.75 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.53, 168.43, 144.46, 137.66, 137.30, 133.74, 131.63, 130.38, 127.52, 127.13, 40.00, 31.55, 28.61, 28.59. HRMS-EI (+): m/z calcd for $C_{14}H_{15}NO_2$: 319.02, found 319.02.

[6-(2-Bromoethylamino)naphthalen-2-yl]ethan-1-one (8b)[23]

To a stirred solution of **7b** (0.5 g, 2.18 mmol) and triphenylphosphine (1.72 g, 6.55 mmol) in dry THF (50 mL) in an ice bath was added NBS (1.17 g, 6.55 mmol) in small portions over a period of 10 min. After being stirred for 2 h at 0 °C, it was allowed to attain room temperature. Progress of the reaction was monitored by TLC. The reaction mixture was quenched by $Na_2S_2O_3$ (10% w/v, 15 mL) after completion of the reaction. The organic layer was washed with water, dried over anhydrous $NaSO_4$ and evaporated. The residue was purified by column chromatography on silica gel (hexane/ethyl acetate) to afford a pale yellow solid **8b** (478 mg, 75%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.31 (1H, s), 7.92 (1H, dd, J = 9.0, J = 3.0 Hz, s), 7.74 (1H, d, J = 9.0 Hz), 7.61 (1H, d, J = 9.0 Hz), 6.93 (1H, dd, J = 9.0, J = 3.0 Hz), 6.81 (1H, d, J = 3.0 Hz), 4.48 (1H, br, s), 3.68 (2H, m), 3.62 (2H, m), 2.67 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6) δ 197.91, 147.10, 137.95, 131.51, 131.28, 130.46, 126.57, 126.27, 125.08, 118.67, 104.39, 45.11, 31.62, 26.61. HRMS-EI (+): m/z calcd for $C_{14}H_{14}BrNO$: 292.18, found 291.03.

N-(6-Acetylnaphthalen-2-yl)-2-[bis(pyridin-2-ylmethyl)-amino]-*N*-methylacetamide (3)[15]

A solution of compound **8a** (0.16 g, 0.5 mmol) in CH_3CN (10 mL) was treated with 2,2′-dipicolylamine (90 µL, 0.5 mmol) and K_2CO_3 (69 mg, 0.5 mmol) under an argon atmosphere. The mixture was heated to reflux for 5 h, and then cooled to room temperature; it was diluted with CH_2Cl_2 (20 mL), the inorganic salts were filtered off, and the filtrate was concentrated. The residue was purified by column chromatography using MeOH and CH_2Cl_2 to afford **3** as a sticky solid (0.15 g, 70%): ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.45 (1H, s), 8.07 (1H, d, J = 3.0 Hz), 8.04 (1H, d, J = 3.0 Hz), 7.91 (1H, d, J = 9.0 Hz), 7.47 (5H, m), 7.25 (1H, m), 7.07 (2H, m), 3.98 (4H, s), 3.35 (5H, s), 2.73 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.61, 170.32, 159.24, 148.81, 142.91, 136.34, 135.77, 134.99, 131.34, 131.22, 129.67, 128.29, 126.07, 125.24, 124.76, 123.19, 121.92, 60.16, 55.04, 37.36, 26.66. HRMS-EI (+): m/z calcd for $C_{27}H_{26}N_4O_2$: 438.21, found 438.21.

(6-{2-[Bis(pyridin-2-ylmethyl)amino]ethylamino}naphthalen-2-yl)ethan-1-one (4a)

A solution of compound **8b** (190 mg, 0.65 mmol) in DMF–CH_3CN (7 : 7 mL) was treated with 2,2′-dipicolylamine (117 µL, 0.65 mmol), NaI (16 mg, 0.78 mmol) and Na_2CO_3 (83 mg, 0.78 mmol) under an argon atmosphere at 50 °C. The mixture was heated to reflux for 72 h, and then cooled to room temperature; solvent was removed under vacuum and the residue was purified by silica gel column chromatography using 5% MeOH in CH_2Cl_2 to afford **4a** as a sticky solid (80 mg, 30%): ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.53 (2H, d, J = 3.0 Hz), 8.24 (1H, s), 7.86 (1H, dd, J = 9.0, J = 3.0 Hz), 7.64 (1H, d, J = 9.0 Hz), 7.50 (3H, m), 7.35 (2H, d, J = 6.0 Hz), 7.06 (2H, m), 6.98 (1H, dd, J = 9.0, J = 3.0 Hz), 6.63 (1H, s), 5.77 (1H, br, s), 3.88 (4H, s), 3.21 (2H, t, J = 6.0 Hz), 2.86 (2H, t, J = 6.0 Hz), 2.60 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.54, 158.70, 148.85, 148.63, 138.02, 136.36, 130.40, 130.25, 130.10, 125.55, 125.39, 124.34, 123.11, 122.02, 118.75, 102.81, 59.96, 52.13, 40.76, 26.18. HRMS-EI (+): m/z calcd for $C_{26}H_{26}N_4O$: 410.52, found 410.21.

(6-{2-[Bis(pyridin-2-ylmethyl)amino]ethylmethylamino}-naphthalen-2-yl)-ethan-1-one (4b)

A solution of compound **8c** (280 mg, 0.92 mmol) in DMF–CH_3CN (7 : 7 mL) was treated with 2,2′-dipicolylamine (165 µL,

0.92 mmol), NaI (163 mg, 1.1 mmol) and Na_2CO_3 (117 mg, 1.1 mmol) under an argon atmosphere at 50 °C. The mixture was heated to reflux for 72 h, and then cooled to room temperature; solvent was removed under vacuum and the residue was purified by silica gel column chromatography using 5% MeOH in CH_2Cl_2 to afford **4b** as a sticky solid (116 mg, 30%): ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.52 (2H, m), 8.27 (1H, s), 7.88 (1H, dd, J = 9.0, J = 3.0 Hz), 7.65 (1H, d, J = 9.0 Hz), 7.57 (1H, dd, J = 9.0, J = 3.0 Hz), 7.52 (2H, m), 7.43 (2H, d, J = 6.0 Hz), 7.11 (2H, m), 6.99 (1H, dd, J = 9.0, J = 3.0 Hz), 6.73 (1H, d, J = 3.0 Hz), 3.92 (4H, s), 3.58 (2H, t, J = 6.0 Hz), 2.99 (3H, s), 2.80 (3H, t, J = 6.0 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.89, 159.27, 149.11, 149.09, 137.88, 136.60, 130.81, 130.67, 130.44, 126.12, 124.89, 124.65, 123.10, 122.29, 116.01, 104.88, 61.08, 51.06, 50.69, 38.90, 26.52. HRMS-EI (+): m/z calcd for $C_{27}H_{28}N_4O$: 424.55, found 424.23.

3–Zn(II)[15]

A mixture of **3** (20 mg, 45.6 mmol) and zinc perchlorate hexahydrate (16.98 mg, 45.6 mmol) in CH_3CN was stirred at room temperature for 10 h. The reaction mixture was condensed by evaporation of the solvent under vacuum below 30 °C, and the residue, after titration with CH_2Cl_2 and hexane, was filtered to give the zinc complex **3**–Zn(II) as an off-white solid (hygroscopic) (27 mg, 98%): ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.75 (2H, d, J = 6.0 Hz), 8.67 (1H, s), 8.21 (1H, d, J = 9.0 Hz), 8.10 (3H, m), 7.97 (1H, d, J = 9.0 Hz), 7.86 (1H, d, J = 3.0 Hz), 7.70 (2H, t, J = 6.0 Hz), 7.52 (2H, d, J = 9.0 Hz), 7.43 (1H, dd, J = 9.0 Hz), 4.09 (4H, m), 3.59 (2H, s), 3.41 (3H, s), 2.72 (3H, s). HRMS FAB-(+): m/z calcd for $C_{27}H_{26}ClN_4O_6Zn$: 603.37, found 603.08.

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