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Fluorescent Chemosensor for Detection of PPi Through the Inhibition of Excimer Emission in Water†

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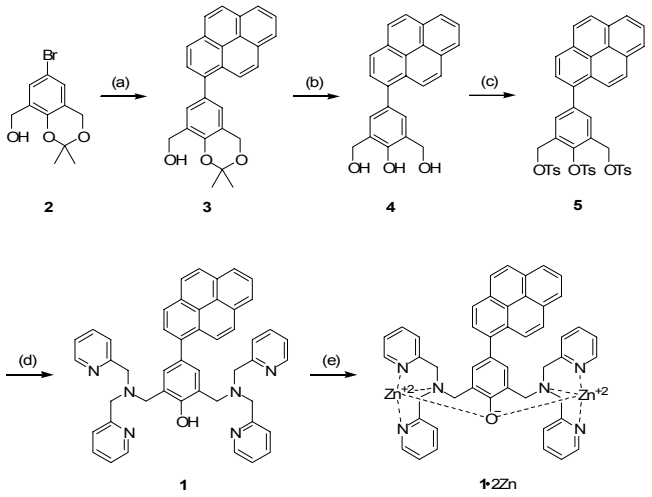
Pyrophosphate ($P_2O_7^{4-}$, PPi) is involved in energy transduction and metabolic processes within cells. For example, the concomitant release of PPi is indispensable to intracellular enzymatic reactions such as DNA polymerization catalyzed by DNA polymerase and the synthesis of cyclic adenosine monophosphate (cAMP), a second messenger used for signal transduction in many different organisms, catalyzed by adenylyl cyclase.[1] Also, pyrosequencing, a method of DNA sequencing based on the "sequencing by synthesis" principle, is realized through the detection of PPi released during DNA polymerization.[2] In this regard, several research groups have focused on the development of selective PPi chemosensors.

Over the last 10 years, only a few chemosensors for PPi have been developed that have shown selectivity and sensitivity through fluorescence change in water.[3] Due to the advantages of fluorescence detection methods, such as their high sensitivity, low cost, easy detection, versatility, and their possible applications in bioimaging, these techniques have gained a great deal of attention. Herein, we present a new fluorescent chemosensor based on a pyrenyl-Zn(II)·DPA conjugate (DPA = dipicolylamine) which shows a selective quenching effect of excimer emission for PPi relative to other anions through a PET (Photoinduced Electron Transfer) process in water.

Sensor **1**·2Zn was prepared as shown in Scheme 1.[3c] A pyrene group was attached to compound **2** by a Suzuki cross coupling reaction. Compound **1** was obtained in an overall 47% yield from compound **3**. Sensor **1**·2Zn was formed by the addition of an aqueous solution of 2 equiv $Zn(NO_3)_2$ to a DMSO solution of compound **1**.

First, we examined the fluorescence emission change of **1**·2Zn (5.56 μM) upon the addition of PPi (sodium salt) in 10 mM HEPES buffer (pH 7.4) at 25 °C (Fig. 1). In the absence of PPi, the fluorescence emission of **1**·2Zn displayed a broad band (from 380 nm to 620 nm) because of the pyrene excimer formation in water.[4,6] Increasing the PPi (sodium salt) concentration up to 0.24 equiv resulted in a gradual decrease in the excimer emission (λ_{max} = 473 nm) through the PET process. When 0.24 equiv PPi was added to a **1**·2Zn solution, the fluorescence intensity of **1**·2Zn showed a 12-fold emission decrease at 473 nm. However, further addition of PPi resulted in a slight enhancement of the fluorescence emission intensity at 509 nm and the excimer band of **1**·2Zn was bathochromic-shifted by 36 nm.

Also, we investigated the fluorescence emission changes of **1**·2Zn (5.56 μM) in the presence of various anions such as F^-,



(a) Pyrene-1-boronic acid, 10 mol % $Pd(PPh_3)_4$, aq. K_2CO_3, THF (b) 1 N HCl, THF (c) aq. NaOH, TsCl, THF (d) i. Cs_2CO_3, KI, dipicolylamine, CH_3CN ii. aq. KOH, MeOH (e) $Zn(NO_3)_2·6H_2O$, H_2O, MeOH

Scheme 1. Synthesis of Sensor **1**·2Zn

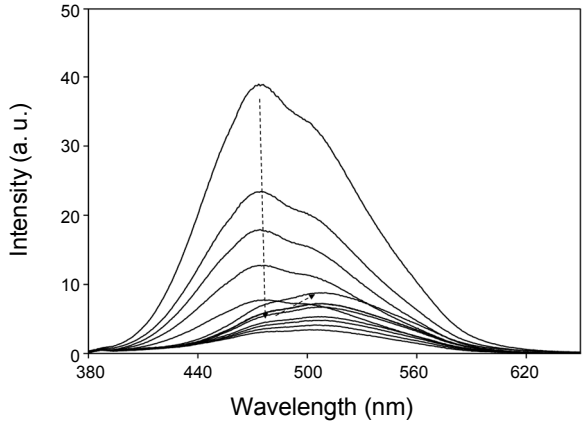


Figure 1. Fluorescence emission change (λ_{ex} = 355 nm) of **1**·2Zn (5.56 μM) upon the addition of PPi (sodium salt) in 10 mM HEPES buffer (pH 7.4) at 25 °C: PPi (equiv) = 0, 0.03, 0.05, 0.11, 0.16, 0.24, 0.31, 0.43, 0.65, 1.04, 1.79, 2.58, 3.28.

†This paper is dedicated to Professor Sunggak Kim on the occasion of his honorable retirement.

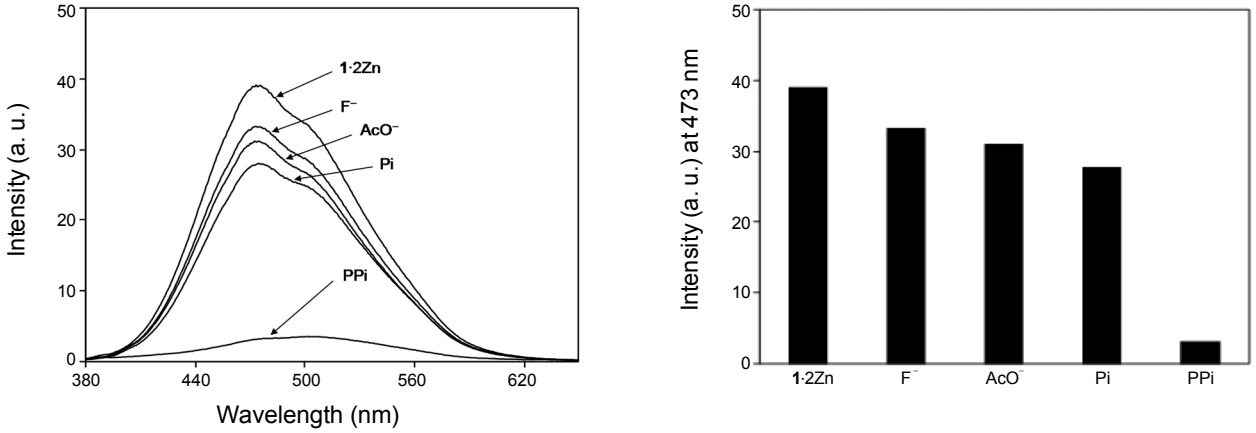


Figure 2. (Left) Fluorescence emission changes (λ_{ex} = 355 nm) of **1**·2Zn (5.56 μM) for various anions such as F^-, AcO^-, $H_2PO_4^-$, and PPi (sodium salts) in 10 mM HEPES buffer (pH 7.4) at 25 °C: F^- = 5.43 equiv (gray line), AcO^- = 5.43 equiv (orange line), $H_2PO_4^-$ = 0.54 equiv (sky blue line), PPi = 0.24 equiv (red line). (Right) Comparison to fluorescence emission intensities (λ_{em} = 473 nm) of **1**·2Zn (5.56 μM) for various anions such as F^-, AcO^-, $H_2PO_4^-$, and PPi (sodium salts) in 10 mM HEPES buffer (pH 7.4) at 25 °C.

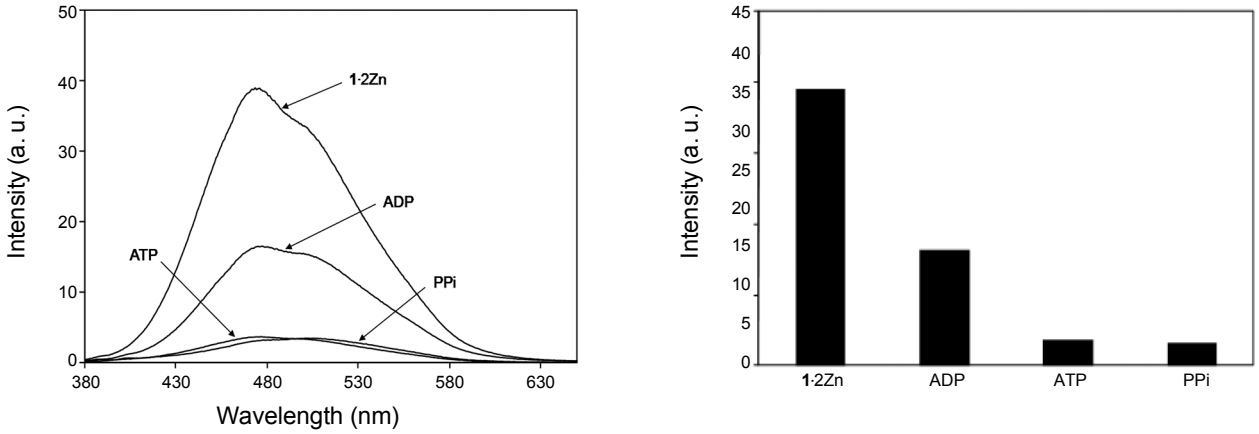


Figure 3. (Left) Fluorescence emission changes (λ_{ex} = 355 nm) of **1**·2Zn (5.56 μM) in the presence of ADP (0.54 equiv), ATP (0.24 equiv), and PPi (0.24 equiv) (sodium salts) in 10 mM HEPES buffer (pH 7.4) at 25 °C. (Right) Relative fluorescence intensities at 476 nm of **1**·2Zn (5.56 μM) and its complexes with ADP, ATP, and PPi (sodium salts) in 10 mM HEPES buffer (pH 7.4) at 25 °C.

Table 1. Fluorescence intensities, quenching ratios, and quenching efficiencies of each anion at λ_{max} = 473 nm upon the addition of anions (sodium salts) in 10 mM HEPES buffer (pH 7.4) at 25 °C

λ_{max} (473 nm)	F.I. (a.u.)[a]	Q.R. (fold)[b]	Q.E. (%)[c]
Host	39.08		
F^-	33.20	1.1	15.1
AcO^-	30.98	1.2	20.7
$H_2PO_4^-$	27.81	1.4	28.8
PPi	3.15	12.4	92.0

[a]F.I. (a.u.): Fluorescence Intensity, [b]Q.R. (fold): Quenching Ratio, [c]Q.E. (%): Quenching Efficiency.

AcO^-, and $H_2PO_4^-$ (sodium salts) in 10 mM HEPES buffer (pH 7.4) at 25 °C (Fig. 2). While the addition of 0.24 equiv PPi resulted in a 12-fold decrease in the emission intensity of **1**·2Zn, however, the addition of 5.4 equiv of F^- and AcO^- resulted in 1.1-, and 1.2-fold decreases, respectively, in the emission intensity at 473 nm, and the addition of 0.54 equiv $H_2PO_4^-$ resulted in a 1.4-fold decrease in the emission intensity. Based on the above data, Table 1 shows the values of fluorescence intensity (F.I.), quenching ratio (Q.R.), and quenching efficiency (Q.E.) for each anion at λ_{max} = 473 nm. Therefore, sensor **1**·2Zn can sense selectively PPi relative to other anions such as F^-, AcO^-, and $H_2PO_4^-$ in 10 mM HEPES buffer (pH 7.4) at 25 °C.

We also investigated emission changes of **1**·2Zn (5.56 μM) in the presence of ADP and ATP (sodium salts) in 10 mM HEPES buffer (pH 7.4) at 25 °C (Fig. 3). Addition of 0.54 equiv ADP to **1**·2Zn resulted in approximately 2-fold decrease in the emission intensity at 476 nm. The fluorescence intensity at 476 nm of **1**·2Zn after the addition of 0.24 equiv ATP was similar to that of the complex between **1**·2Zn and PPi. However, the fluorescence intensity at 505 nm of the 1:1 mixture of **1**·2Zn and PPi turned out to be 2.2-fold larger than that of **1**·2Zn and ATP. Interestingly, the fluorescence titration curves for ATP (0 ~ 3.2 equiv) revealed that the emission intensity continuously decreased at 476 nm and was almost saturated at 3.2 equiv while those

for PPi (0 ~ 3.2 equiv) displayed ratiometric behavior as shown in Fig. 1.

As expected, a Job's plot for binding between **1**·2Zn and PPi showed a 1:1 binding stoichiometry. The binding mode for PPi-**1**·2Zn was presented in a previous paper from our team involving a similar sensor molecule and its X-ray crystal structure.[5] When PPi is coordinated to **1**·2Zn, the negative charge density of the phenolate oxygen atom in **1**·2Zn increases to more than that before binding to PPi. In the previous naphthyl-Zn(II)·DPA system, an increased charge characteristic on the phenolate oxygen atom induced a fluorescence enhancement through induced charge transfer.[3c] However, in the case of pyrenyl-Zn(II)·DPA system (**1**·2Zn), the PET process is expected to take place from the nitrogen atoms of the DPA moiety to pyrene because of the orthogonal conformation (dihedral angle = −83.60°, Spartan ´08 program) between the phenolate and pyrene (Fig. 4) which prohibits extended π-conjugation through the π system involving phenyl and pyrenyl groups. Calculation (Spartan ´08 program) shows that excimer formation is possible in **1**·2Zn (Fig. 5). Therefore, the PET process induces quenching in the excimer emission of **1**·2Zn.[6,7]

In conclusion, we have developed a new fluorescent chemosensor based on the pyrenyl-DPA system which shows the selective quenching effect of excimer emission for PPi relative to other anions through the PET process in water. The quenching effect of the pyrene excimer emission is expected to take place through the PET process from the nitrogen lone pair electrons of the DPA moiety to pyrene.

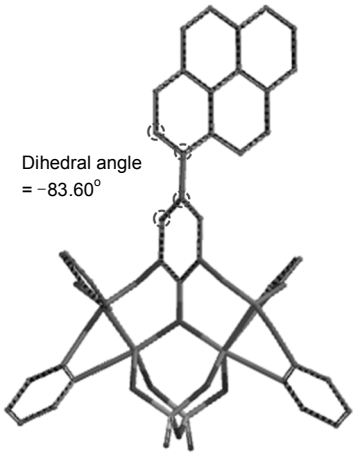


Figure 4. Energy-minimized structure of the complex between **1**·2Zn and PPi (Spartan ´08 program).

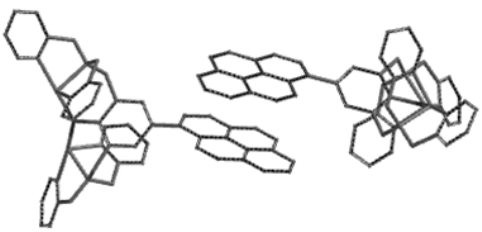


Figure 5. Energy-minimized structure of the excimer formed by two **1**·2Zn complexes (Spartan ´08 program).

Synthesis and Characterization

Compound 3. To a solution of compound **2** (220 mg, 1 equiv), pyrene-1-boronic acid (239 mg, 1.2 equiv), and 10 mL of 2 N aq. K_2CO_3 in 5 mL of distilled THF under N_2 gas was added slowly tetrakis(triphenylphosphine)palladium(0) (101 mg, 0.1 equiv). The mixture was heated at reflux temperature for 12 h, which was then filtrated by Celite 545 (washed with THF). The combined organic extracts were evaporated. The residue was chromatographed on silica gel using hexane:EtOAc = 100:1 - 5:1 to afford compound **4** (120 mg, 38% yield). ^{1}H NMR (300 MHz, acetone-d_6) δ 1.63 (s, 6H), 4.77 (s, 2H), 5.04 (s, 2H), 7.27 (s, 1H), 7.65 (s, 1H), 8.01-8.35 (m, 9H). ^{13}C NMR (75 MHz, acetone-d_6) δ 24.30, 58.48, 60.57, 99.77, 119.23, 124.79, 125.13, 125.27, 126.18, 127.21, 127.33, 127.45, 127.76, 127.96, 130.17, 131.06, 131.59, 132.44. HRMS (FAB): *m*/*z* calcd. for $C_{27}H_{22}O_3$ $[M+H]^+$ 394.1569, found 394.1572.

Compound 4. To a stirred solution of compound **3** (120 mg, 1 equiv) in 4 mL THF was added 4 mL of 1 N HCl. The reaction mixture was stirred at room temperature for 2 days, and then all the volatile components were evaporated and the residue was neutralized with aq. $NaHCO_3$, then partitioned between ethyl acetate and brine. The organic phase was washed with water (×3), and then dried in anhydrous Na_2SO_4. This solution was evaporated. Flash chromatographic purification (Hexane: EtOAc = 50:1, v/v) afforded **4** (50 mg, 46% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.99 (s, 4H), 7.38 (s, 2H), 7.93-8.23 (m, 9H). ^{13}C NMR (75 MHz, acetone-d_6) δ 61.33, 124.76, 124.80, 125.07, 125.33, 126.18, 127.14, 127.26, 127.47, 127.78, 128.19, 128.36, 130.27, 131.06, 131.59, 131.70, 138.14, 153.30. HRMS (FAB): *m*/*z* calcd. for $C_{24}H_{18}O_3$ $[M+H]^+$ 354.1256, found 354.1257.

Compound 5. To a stirred solution of compound **4** (50 mg, 1 equiv) in 10 mL of THF was added aq. NaOH (45 mg, 8 equiv) at 0 °C. To this solution was added *p*-toluenesulfonyl chloride (214 mg, 8 equiv) dissolved in 10 mL of THF. The reaction mixture was stirred at 0 °C for 4 h, and then all the volatile components were evaporated and the residue was neutralized with 1 N HCl and was partitioned between ethyl acetate and brine. The organic phase was washed with water (×3), and then dried in anhydrous Na_2SO_4. Flash chromatographic purification (Hexane:EtOAc = 10:1 - 3:1, v/v) afforded **5** (12 mg, 10% yield). ^{1}H NMR (300 MHz, acetone-d_6) δ 2.36 (s, 6H), 2.57 (s, 3H), 5.20 (s, 4H), 7.40 (d, J = 8.2 Hz, 4H), 7.62-7.65 (m, 4H), 7.90 (d, J = 8.3 Hz, 4H), 8.00 (d, J = 8.2 Hz, 2H), 8.13-8.39 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.57, 21.91, 66.41, 124.35, 124.63, 125.21, 125.55, 126.27, 127.32, 127.90, 128.16, 128.26, 128.42, 129.18, 129.86, 130.38, 131.43, 132.77, 132.96, 140.90, 142.91, 145.02. HRMS (FAB): *m*/*z* calcd. for $C_{45}H_{36}O_9S_3$ $[M+H]^+$ 816.1521, found 816.1517.

Compound 1. To a solution of **5** (12 mg, 1 equiv) in acetonitrile were added 3 equiv of KI (7.5 mg), 3 equiv of Cs_2CO_3 (15 mg) and 2.1 equiv of dipicolylamine (6.3 mg). The reaction mixture was stirred at 40 °C overnight, and then all the volatile components were evaporated. The resulting residue was extracted with ethylacetate/brine. The organic phase was washed with

water (×3), then dried in anhydrous Na_2SO_4, and evaporated. To the resulting solution was added aq. KOH (20 equiv) for hydrolysis. The reaction mixture was stirred at room temperature for 24 h. The mixture was then neutralized with 1 N HCl, and partitioned between ethyl acetate and water. The organic phase was washed with water (×3), and then dried in anhydrous Na_2SO_4. Flash chromatographic purification (CH_2Cl_2:MeOH = 100:1 - 20:1) afforded **1** (5 mg, 47% yield). ^{1}H NMR (300 MHz, acetone-d_6) δ 3.96 (s, 4H), 3.98 (s, 8H), 7.22 (t, 4H, J = 11.5 Hz), 7.58-7.59 (m, 6H), 7.68 (t, 4H, J = 9.1 Hz), 7.99-8.28 (m, 9H), 8.54 (d, 4H, J = 4.2 Hz). HRMS (FAB): m/z calcd. for $C_{48}H_{40}N_6O$ $[M+H]^+$ 717.3342, found 717.3355.

Sensor 1·2Zn. To a solution of 1 (1.1 mg) in 1 mL of MeOH, was added an aqueous solution of $Zn(NO_3)_2·6H_2O$ (2.1 equiv), and the mixture was stirred for 30 min at room temperature. **1**·2Zn was then dissolved in 10 mM HEPES buffer (pH 7.4) and used as a stock solution for complexation studies. HRMS (FAB): m/z calcd. for $C_{48}H_{40}N_6O·2Zn·2NO_3$ $[M]^+$ 967.1525, found 967.1524.

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