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Hexaamide Molecule Annexed with Pyrenes for Selective Detection of Phosphate and Pyrophosphate Ions by Ratiometric Fluorescence Changes

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N,*N'*-bis{3,5-di[(1-pyrenylmethyl)carbamoyl]benzyl}pyridine-2,6-dicarbamide (compound **1**) provides a pseudo-tetrahedron cleft and multiple hydrogen bondings to form a 1:1 complex with phosphate (or pyrophosphate) ion in a highly selective manner, in comparison with other anions (F^-, Cl^-, Br^-, SCN^-, AcO^-, NO_3^-, and ClO_4^-). Compound **1** bears four pyrene rings as the sensitive fluorescent readout unit for phosphate and pyrophosphate ions. The binding strength can be inferred from the emission intensity ratio of the pyrene monomer (λ_{max} = 377 nm) to the excimer (λ_{max} ~ 480 nm). The enhanced multiple hydrogen bondings and ratiometric sensing mechanism render an opportunity for sensing pyrophosphate ion at micromolar concentration, even in water-containing solution. The binding model is supported by fluorescence and ^{1}H NMR studies.

Keywords: Phosphate ion; Pyrophosphate ion; Fluorescence; Pyrene; Sensor.

INTRODUCTION

Selective sensing of phosphate and pyrophosphate ions are of great importance in modern host-guest chemistry because these ions play essential roles in biological systems, such as in the metabolism of nucleoside triphosphates and farnesyl pyrophosphate.[1] In comparison with the anions of spherical (e.g. Br^-), linear (e.g. SCN^-) or planar (e.g. AcO^- and NO_3^-) geometries, the inherent tetrahedral structure of a phosphate center presents a challenging problem for the design of effective receptors.[2,3] Several synthetic receptors[2,3] bearing polyaza, urea, thiourea, amide and quanidinium moieties have been demonstrated to bind or transport phosphate ions. The neutral receptors of thiourea and amide types are particularly interesting, because the transport of phosphates through a cell membrane is also regulated by neutral binding proteins.

Although many artificial receptors have been designed for recognition of phosphate ions (PO_4^{3-} and $H_2PO_4^-$),[2,3] there are only a few reports on the binding study of pyrophosphate ion ($HP_2O_7^{3-}$, PP_i).[4] For example, macrocyclic polyamines and their metal complexes (e.g. with Cu^{2+}, Zn^{2+} and Cd^{2+} ions) are common PP_i receptors, which exert strong electrostatic interactions in host-guest recognition. The receptors bearing guanidine units also belong to this category. Some of these receptors are not selective to pyrophosphate ions and lack signal transduction units for real-time detection. To circumvent these drawbacks, some polyamine receptors are linked with anthrylmethyl moiety to render a chelation-enhanced fluorescence (CHEF) mechanism for direct readout.[4]

The neutral receptors of amide, urea and pyrrole types can also bind with pyrophosphate ions via multiple hydrogen bondings.[5] For example, calix[4]pyrroles linked with a fluorescent reporter via a thiourea or sulfonamide spacer have been shown to exhibit high affinity toward both phosphate and pyrophosphate ions, albeit with fluorescence quenching. The binding of anthryl bis-thiourea compounds with pyrophosphate ions also show decreased fluorescence due to a photoinduced electron transfer mechanism.[4] In comparison, detection of the binding event by fluorescence enhancement is superior. The ratiometric fluorescent detection is an alternative method that provides a self-calibration by the simultaneous changes of wavelengths and/or intensities of several emissions.[6]

Dedicated to the memory of the late Professor Ho Tong-Ing.

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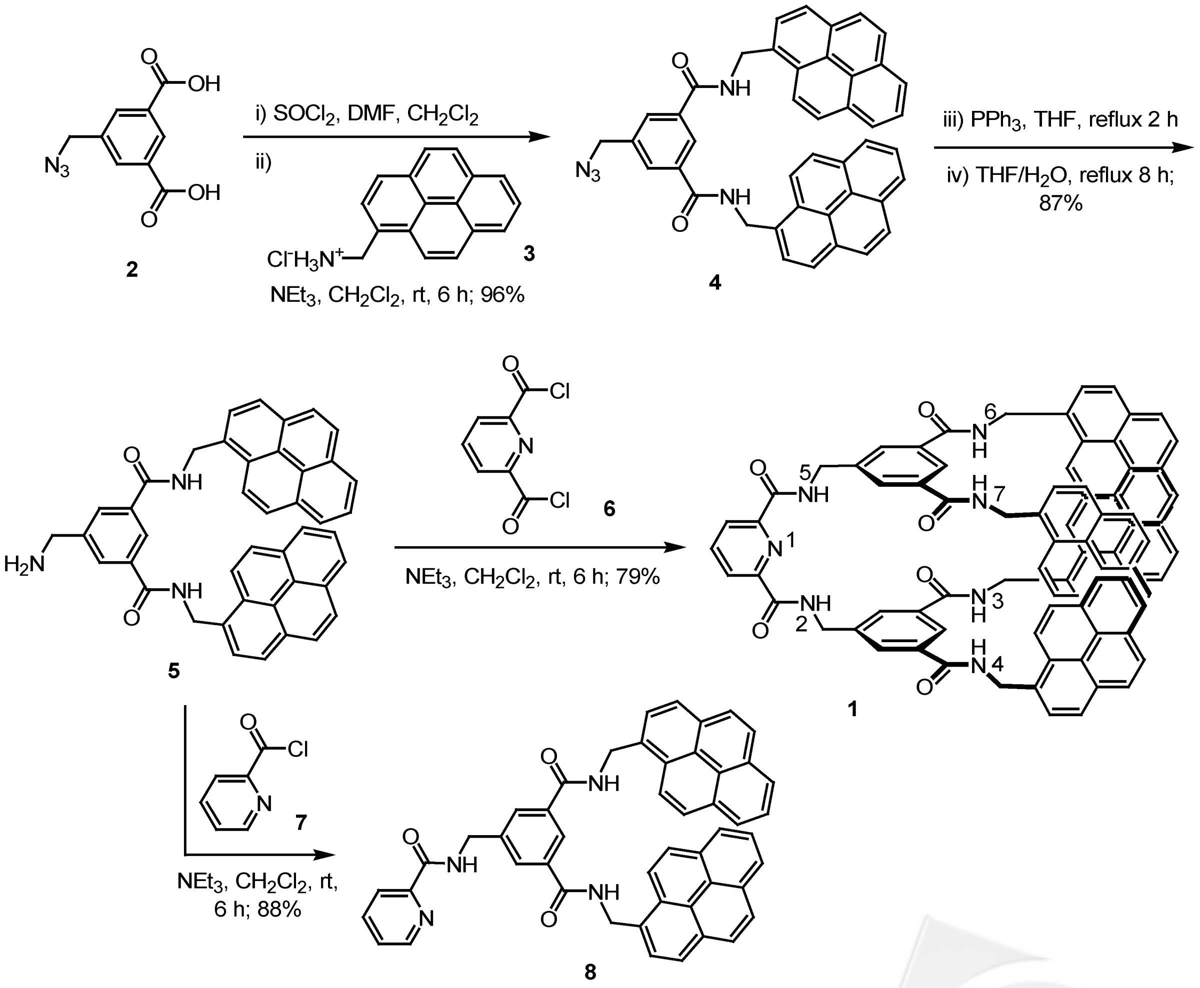
RESULTS AND DISCUSSION

In a previous communication,[2a] we reported a ratiometric fluorescence molecule **1** for sensing PO_4^{3-} and $H_2PO_4^-$ ions. The synthesis of **1** was straightforward (Scheme I). Activation of 5-(azidomethyl)isophthalic acid (**2**) to acid chloride, followed by a coupling reaction with (1-pyrenyl)methylamine (**3**), gave the corresponding diamide **4** in 96% yield. The azido group in **4** was reduced by triphenylphosphine (Staudinger reaction) to give amine **5**, which reacted with 2,6-pyridinedicarboxyl dichloride (**6**) in appropriate equivalents to afford the desired sensor molecule **1** and the one-arm analog **8**, respectively, in 79% and 88% yields.

Compound **1** contained a moiety of pyridine 2,6-bis-carboxamide to make a rigid conformation through intramolecular hydrogen bondings.[7] Four additional amido groups were incorporated into the scaffold to provide a preorganized motif to hold the incoming phosphate ion with multiple hydrogen bondings. The structural complementarity apparently played an important role to render effective complexation, i.e., a phosphate ion of tetrahedral geometry fitted the pseudo-tetrahedron cleft of **1** to form a 1:1 complex. By the ^{1}H NMR titration in DMSO-d_6 solution, the association constant (K_{ass}) of $\mathbf{1}\cdot H_2PO_4^-$ complex was determined to be 1374 M^{-1} using the chemical-shift changes of N_2,N_5-H of **1** as a function of anion concentrations (Fig. 1). In comparison, the one-arm triamide compound **8** did not hold the proper cleft, and thus showed a 7-fold inferior affinity (K_{ass} = 198 M^{-1}) than **1** in binding with phosphate ion.

The four pyrene rings in **1** functioned as a sensitive

Scheme I Synthesis of the two-arm hexaamide molecule **1** and the one-arm triamide molecule **8**



and direct readout unit for the ion complex via ratiometric fluorescence changes (Fig. 2).[6] The UV-vis spectrum of **1** in THF solution displayed the absorption maxima at 277 and 345 nm. By excitation at 345 nm, compound **1** in THF solution showed the typical monomer emissions at λ_{max} 377 and 397 nm, as well as the excimer emission at λ_{max} 477 nm. As the emission intensity ratio of monomer-to-excimer remained constant in various concentrations (10^{-7} to 10^{-5} M), the excimer emission should occur via intramolecular interactions of pyrenes, but not intermolecularly in this concentration range. This assertion was supported by the lack of excimer emission in the one-arm analog of triamide receptor **8** at 10^{-5} M in THF solution.

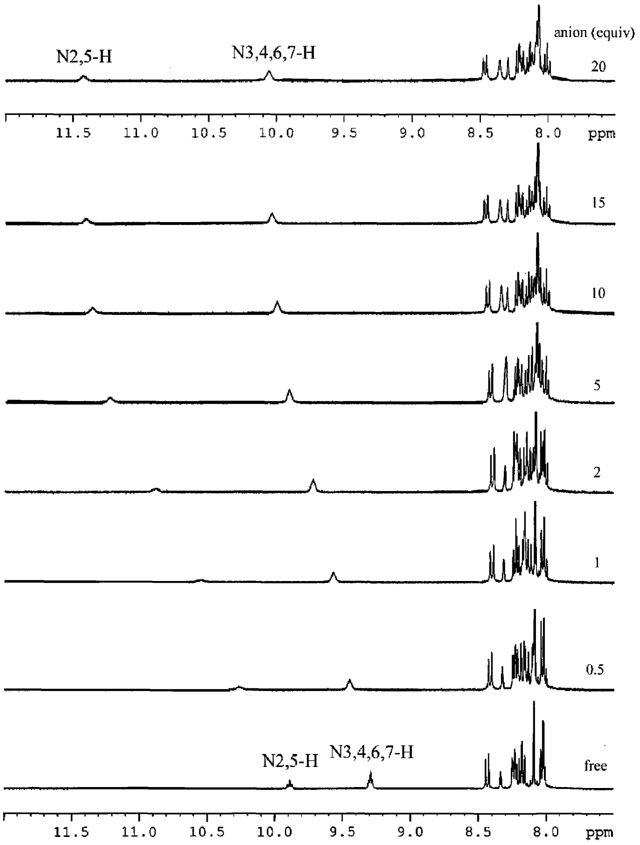


Fig. 1. ^{1}H NMR titration of compound **1** (1×10^{-3} M) with $H_2PO_4^-$ (0.125 M as the tetrabutylammonium salt) in DMSO-d_6 solution.

We also concluded that the excimer emission in the two-arm hexaamide receptor **1** was derived from the stacking dispositions of the pairs of N_3/N_6- and N_4/N_7-CH_2-pyrenes.[2] On complexation with a phosphate ion, the N_3/N_6 and N_4/N_7 pyrene pairs were likely pushed apart to favor the segregated monomers. The fluorescence changes, i.e. simultaneous increase of the monomer emissions and decrease of the excimer emission, were responsive to the segregation of pyrene rings on complex formation.[6]

An isoemissive point occurring at 430 nm indicated that the 1:1 complex ($\mathbf{1}\cdot H_2PO_4^-$ or $\mathbf{1}\cdot PO_4^{3-}$) existed in equilibrium with the free receptor. The binding constant of complex and 1:1 stoichiometry were determined by curve fitting using the nonlinear least-squares method, and the association constant (K_a) is derived from the following equation.[8]

$$F = F_0 + (\Delta F_{max}/2C_0) \times \{1/K_a + C_0 + C - [(1/K_a + C_0 + C)^2 - 4C_0C]^{1/2}\}$$

where F (at which wavelength) is the emission intensity, F_0 (at which wavelength) is the original emission intensity of the free receptor, and ΔF_{max} is the largest change of emis-

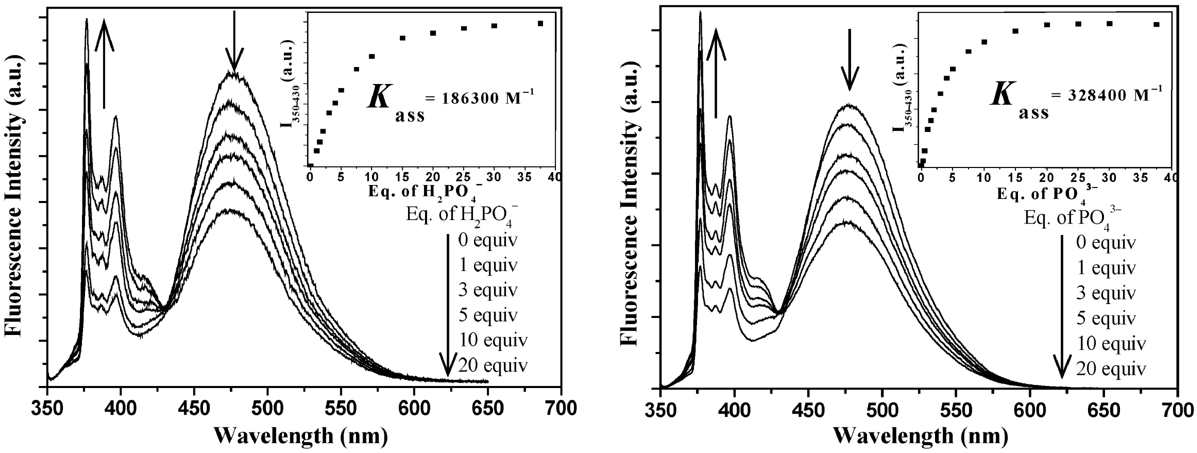


Fig. 2. Fluorescence titration of compound **1** (1×10^{-6} M) with $Bu_4N^+H_2PO_4^-$ (left panel) and $(Bu_4N^+)_3PO_4^{3-}$ in THF solution (right panel).

sion intensity after saturation with the substrate. C is the concentration of substrate; C_0 is the initial concentration of the receptor. Accordingly, the association constants for the **1**·$H_2PO_4^-$ and **1**·PO_4^{3-} were 186300 ± 16200 M^{-1} and 328400 ± 19200 M^{-1}, respectively. The stronger binding of **1**·PO_4^{3-} than **1**·$H_2PO_4^-$ might be attributable to the higher basicity of PO_4^{3-} ion resulting in stronger multiple hydrogen bondings with the hexaamide motif in receptor **1**.

The UV-vis spectrum of **1** in DMSO solution was similar to that in THF, showing the absorption maxima at 277 and 345 nm. The excimer emission of **1** in DMSO (λ_{max} = 482 nm) exhibited a smaller red-shift than that in THF (λ_{max} = 477 nm), whereas the monomer emissions (λ_{max} at 377 and 397 nm) remained the same in both solutions. Though the binding of **1**·$H_2PO_4^-$ in THF was reasonably strong ($K_a = 1.9 \times 10^5$ M^{-1}), the association of **1**·$H_2PO_4^-$ in DMSO was rather weak (K_a = 1500 ± 350 M^{-1}). Nonetheless, **1** still exhibited strong binding with pyrophosphate ion in DMSO solution (vide infra). Such high affinity provided a sensitive detection of pyrophosphate ion in a highly hydrogen-bonding competitive solution, and even in water-containing medium (e.g., H_2O/DMSO = 1:9, v/v).

In a typical fluorescence titration experiment (Fig. 3), a DMSO solution of $(Bu_4N)_3HP_2O_7$ (1×10^{-3} M) was added in small portions (1-400 equiv) to a DMSO solution of **1** (1×10^{-6} M) at 298 K. The fluorescence spectra showed that the monomer emissions of pyrene increased at the expense of the excimer emission (Fig. 2).

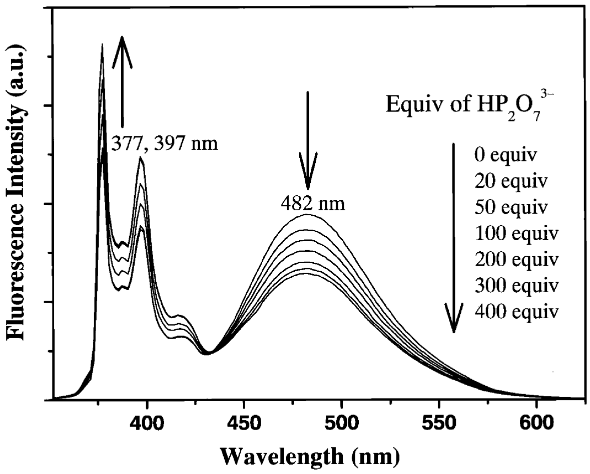


Fig. 3. The ratiometric fluorescence changes on addition of $(Bu_4N^+)_3HP_2O_7^{3-}$ (1×10^{-3} M) to a solution of compound **1** in DMSO (1×10^{-6} M). λ_{ex} = 347 nm.

An isoemissive point occurring at 428 nm suggested that only one type of complex (**1**·PP_i) existed in equilibrium with the free receptor molecule. The 1:1 stoichiometry of complexation was unambiguously deduced by the fluorescence changes using a continuous variation method (Job's plot).[8] The conclusion of 1:1 stoichiometry was in agreement with the ^{1}H NMR titration by monitoring the chemical-shift changes of N_2/N_5- and $N_3/N_4/N_6/N_7$-protons. The association constant of the **1**·PP_i complex in DMSO solution was determined to be 11100 ± 1050 M^{-1} by nonlinear curve fittings of the fluorescence intensity at 482 nm. Substantial ratiometric fluorescence changes were also observed when $(Bu_4N)_3HP_2O_7$ was added to the receptor **1** in 1:9 H_2O/DMSO solution. By comparison with the association constant of the **1**·$H_2PO_4^-$ complex in DMSO solution (K_a = 1500 ± 350 M^{-1} from the fluorescence titration), the binding strength of **1** with $HP_2O_7^{3-}$ ion was enhanced more than 7 fold. The higher affinity of $HP_2O_7^{3-}$ over $H_2PO_4^-$ might be attributed to the higher basicity and more accurate spatial layout of the triple charged $HP_2O_7^{3-}$ ion to render the enhanced interactions with the hexaamide receptor **1** via multiple hydrogen bondings. On the basis of the fluorescence and ^{1}H NMR studies, a binding model (Fig. 4) was proposed for the **1**·PP_i complex with the segregated pyrene rings to give the monomer emissions.

The fluorescence titration experiments revealed that the binding of compound **1** with phosphate ions (PO_4^{3-} and HPO_4^{2-}) was highly selective over other anions (F^-, Cl^-, Br^-, SCN^-, AcO^-, NO_3^-, ClO_4^- and HSO_4^-) in THF solution (Fig. 5). The binding of **1** with $HP_2O_7^{3-}$ was relatively strong in DMSO, in comparison with the weak affinity of HPO_4^{2-} and no apparent binding with Cl^- and AcO^- ions. The geometric complementarity appeared to be the main cause, associated with the auxiliary factor of basicity, to render the binding preference of **1** with phosphate and pyrophosphate ions.

CONCLUSION

We have demonstrated a new type of fluorescence sensor for phosphate and pyrophosphate ions. The receptor molecule **1** has a well-defined structure with pseudo-tetrahedral cleft to accommodate phosphate and pyrophosphate ions, forming 1:1 complexes. In comparison with other anions, the selective binding of **1** with phosphate and pyro-

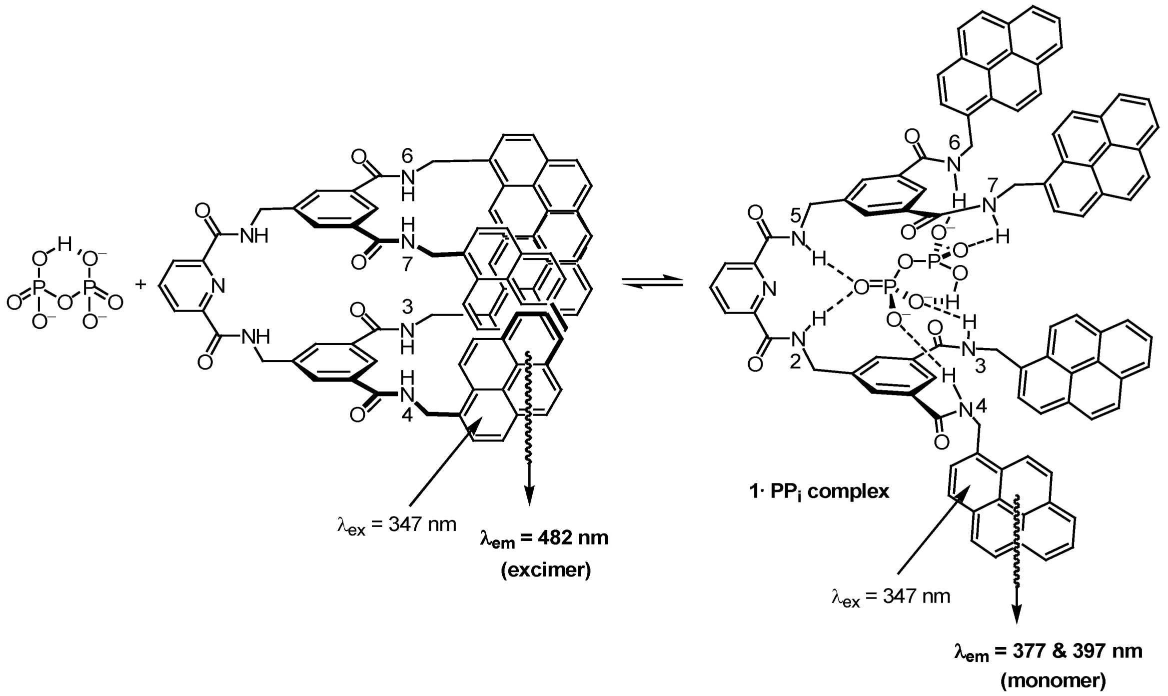


Fig. 4. Proposed binding model for receptor **1** with pyrophosphate ion.

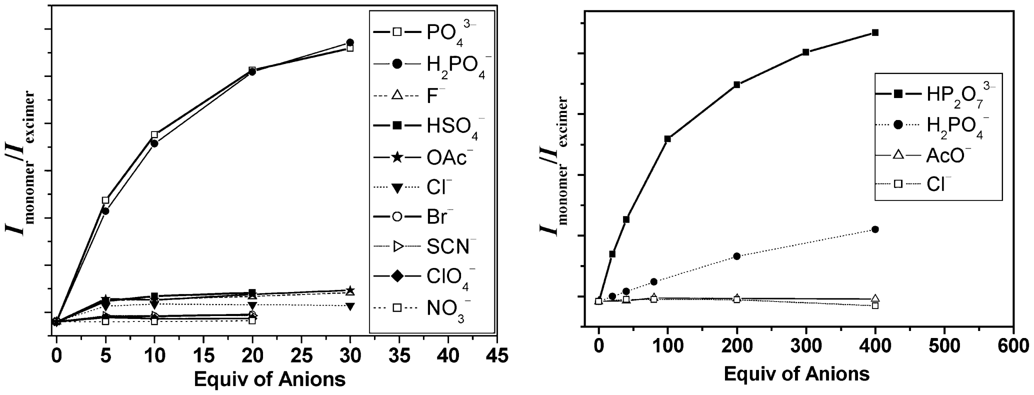


Fig. 5. Left: Fluorescence titration of compound **1** with various anions in THF solution (as tetrabutylammonium salts). Right: Fluorescence titration of compound **1** with various anions in DMSO solution. I_{monomer} represents the integrated fluorescence intensity in the range of 350-430 nm, whereas I_{excimer} represents the integrated fluorescence intensity in the range of 430-650 nm. The binding strength can be inferred from the intensity ratio of the pyrene monomer to the excimer emissions.

phosphate ions is attributable to the geometry complementarity. The annexed pyrene rings in **1** render sensitive and real-time detection with ratiometric changes of the monomer and excimer fluorescence emissions. The enhanced multiple hydrogen bondings and ratiometric sensing mechanism provide an opportunity for sensing pyrophosphate ion at low concentration, 10^{-6} M in this study, even in water-containing solutions.

EXPERIMENTAL

General Method

All reactions requiring anhydrous conditions were conducted in flame-dried apparatus under an inert atmosphere using standard techniques. Syringes and needles for the transfer of reagents were dried at 100 °C and allowed to cool in a desiccator over P_2O_5 before use. All the reagents and solvents were reagent grade and were used without further purification unless otherwise specified. Ethers were distilled from sodium benzophenone ketyl; (chlorinated) hydrocarbons, and amines were from CaH_2. Reactions were monitored by TLC using aluminum plates pre-coated with a 0.25 mm layer of silica gel containing a fluorescent indicator (Merck Art. 5544); compounds were visualized by UV lamp and ninhydrin spray. Kieselgel 60 (40-63 μm) and neutral aluminum oxide (Acros, 50-200 μm) were used for column chromatography. Melting points are uncorrected. Chemical shifts (δ) are given in parts per million (ppm) relative to δ_H 7.24/δ_C 77.0 (central line of t) for $CHCl_3/CDCl_3$ and δ_H 2.49 (m)/δ_C 39.5 (m) for DMSO-d_6. The splitting patterns are reported as s (singlet), d (doublet), t (triplet) and multiplet (m). Coupling constants (J) are given in Hz. Distortionless enhancement polarization transfer (DEPT) spectra were taken to determine the types of carbon signals. UV spectra were recorded on a Hewlett Packard-8453 spectrophotometer. Fluorescence spectra were taken on an Hitachi F-4500 spectrophotometer.

N,N′-Di(1-pyrenylmethyl)-5-(azidomethyl)isophthalamide (3)

To a suspension of 5-(azidomethyl)isophthalic acid[9] (110.5 mg, 0.5 mmol) in CH_2Cl_2 (5 mL) was added 2 drops of DMF, followed by addition of $SOCl_2$ (1 mL). The resulting mixture was allowed to stir at room temperature for 2 h until the solution became clear. After evaporation and subsequent azeotropic distillation with toluene, the residue was dried in vacuo for an additional 1 h and used without further purification. The above-prepared acyl chloride solution in CH_2Cl_2 (10 mL) was added dropwise into a flask containing (1-pyrenyl)methylamine hydrochloride (268 mg, 1 mmol) and Et_3N (0.5 mL) in CH_2Cl_2 (10 mL). The mixture was stirred at ambient temperature for 6 h. After evaporation under reduced pressure, the residue was further dried in vacuo for 30 min. The resulting yellow solid was dissolved in THF/EtOAc (1:1) and passed through a short plug of silica gel. The collected filtrates were concentrated to dryness, and then washed with CH_2Cl_2/Et_2O (1:4) to afford the title compound (310 mg, 96%). Pale yellow solid, mp = 181.5-183 °C; TLC ($MeOH/CH_2Cl_2$ (9:1)) R_f = 0.75; IR (KBr) 3281, 3045, 2103, 1643 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 9.39 (2H, t, J = 5.6 Hz), 8.49 (2H, d, J = 9.3 Hz), 8.47 (1H, s), 8.30-8.22 (8H, m), 8.14 (4H, s), 8.11-8.04 (6H, m), 5.23 (4H, d, J = 5.6 Hz), 4.59 (2H, s); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 165.5 (C), 136.4 (C), 132.7 (C), 130.8 (C), 130.3 (C), 130.1 (C), 129.8 (CH), 128.1 (C), 127.6 (CH), 127.3 (CH), 127.0 (CH), 126.8 (CH), 126.2 (CH), 126.1 (CH), 125.2 (CH), 125.1 (CH), 124.7 (CH), 124.0 (C), 123.9 (C), 123.2 (CH), 53.1 (CH_2), 41.1 (CH_2); FAB-MS m/z (rel intensity) 648 (M^+ + H, 10%), 647 (M^+, 12); HRMS calcd for $C_{43}H_{30}N_5O_2$ (M^+ + H) 648.2399, found 648.2396.

N,N′-Di(1-pyrenylmethyl)-5-(aminomethyl)isophthalamide (5)

To a solution of 5-azidomethyl-*N,N′*-di(1-pyrenylmethyl)isophthalamide (259 mg, 0.4 mmol) in anhydrous THF (20 mL) was added PPh_3 (147 mg, 0.56 mmol). The resulting mixture was heated at reflux for 2 h and then cooled a little. After addition of a mixture of THF (5 mL) and H_2O (58 mg), refluxing was continued for an additional 8 h. After evaporation under reduced pressure, the crude product was washed with CH_2Cl_2/Et_2O (1:9) to afford the desired product (177 mg). Evaporation of filtrate and washing with CH_2Cl_2/Et_2O (1:9) gave the second crop (37 mg). The total yield is 87%. Pale yellow solid, mp = 205-207 °C; TLC ($MeOH/CH_2Cl_2$ (9:1)) R_f = 0.22; IR (KBr) 3369, 3298, 3046, 1657 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 9.27 (2H, t, J =5.7 Hz), 8.50 (2H, t, J = 9.2 Hz), 8.30-8.23 (8H, m), 8.15 (4H, s), 8.10-8.05 (4H, m), 8.03 (2H, s), 5.22 (4H, d, J = 5.5 Hz), 3.78 (2H, s); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 166.0 (C), 134.4 (C), 132.8 (C), 130.8 (C), 130.3 (C), 130.1 (C), 128.8 (CH), 128.1 (C), 127.5 (CH), 127.4 (CH), 127.0 (CH), 126.8 (CH), 126.2 (CH), 125.2 (CH), 125.1 (CH), 124.7 (CH), 124.5 (CH), 124.0 (C), 123.9 (C), 123.2 (CH), 45.2 (CH_2), 41.1 (CH_2); FAB-MS m/z 622 (M^+ + H); HRMS calcd for $C_{43}H_{32}N_3O_2$ (M^+ + H) 622.2495, found 622.2501.

N,N′-Bis{3,5-di[(1-pyrenylmethyl)carbamoyl]benzyl}-pyridine-2,6-dicarbamide (1)

To a solution of 5-aminomethyl-*N,N′*-di(1-pyrenylmethyl)isophthalamide (40 mg, 0.064 mmol) and Et_3N (0.03 mL) in THF (5 mL) was added 2,6-pyridinedicarboxyl dichloride (6.6 mg, 0.032 mmol) in THF (5 mL) dropwise at room temperature. The resulting mixture was allowed to stir at ambient temperature for 6 h. After evaporation under reduced pressure, the residue was further dried in vacuo for 30 min. The resulting yellow solid was dissolved in THF/EtOAc (1:1) and passed through a short plug of silica gel. The collected filtrates were evaporated to dryness and then washed with Et_2O to give the desired product

(35 mg, 79%). Pale yellow solid, mp = 210-211.5 °C; TLC (THF/EtOAc (3:1)) $R_f = 0.55$; IR (KBr) 3089, 3045, 1657 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.90 (2H, t, $J = 6.5$ Hz), 9.31 (4H, t, $J = 5.9$ Hz), 8.44 (4H, d, $J = 9.3$ Hz), 8.34 (2H, s), 8.25-8.14 (19H, m), 8.12-8.09 (8H, m), 8.04-8.01 (12H, m), 5.18 (8H, d, $J = 5.4$ Hz), 4.66 (4H, d, $J = 5.6$ Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 165.8 (C), 163.5 (C), 148.5 (C), 140 (C), 139.6 (CH), 134.7 (C), 132.7 (C), 130.7 (C), 130.2 (C), 130.0 (C), 128.8 (CH), 128.0 (C), 127.5 (CH), 127.3 (CH), 127.0 (CH), 126.6 (CH), 126.2 (CH), 125.2 (CH), 125.1 (CH), 124.9 (CH), 124.6 (CH), 124.0 (C), 123.9 (C), 123.1 (CH), 113.8 (CH), 42.1 (CH_2), 41.0 (CH_2); FAB-MS *m*/*z* 1376 ($M^+ + H$), 1375 (M^+).

3,5-Di[(1-pyrenylmethyl)carbamoyl]benzyl pyridine-2-carbamide (8)

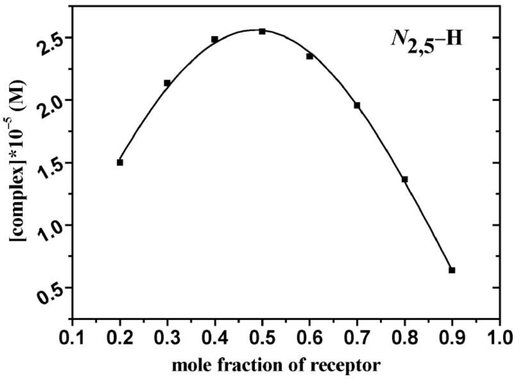
By a procedure similar to that for compound **1**, 2-picolic acid (34.8 mg, 0.2 mmol) was activated to the corresponding acyl chloride and treated with 5-aminomethyl-*N*,*N'*-di(1-pyrenylmethyl)isophthalamide (122.4 mg, 0.2 mmol) to give compound **8** (114 mg, 88%). White solid, mp = 159-160 °C; (MeOH/CH_2Cl_2 (9:1)) $R_f = 0.59$; UV-vis λ_{max} (THF): 277, 344 nm; ε = 88000; fluorescence λ_{max} (THF): 377, 477 nm; IR (KBr) 3331, 3045, 1658 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.44 (1H, t, $J = 6.3$ Hz), 9.30 (2H, t, $J = 4.5$ Hz), 8.62 (1H, d, $J = 4.5$ Hz), 8.47 (2H, d, $J = 9.1$ Hz), 8.32 (1H, s), 8.29-8.21 (8H, m), 8.14 (4H, s), 8.07-8.00 (7H, m), 7.97 (1H, t, $J = 7.8$ Hz), 7.59 (1H, t, $J = 6.3$ Hz), 5.21 (4H, d, $J = 4.5$ Hz), 4.57 (2H, d); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 165.9 (C), 164.1 (C), 148.5 (C), 140 (C), 137.8 (CH), 134.6 (C), 132.8 (C), 130.8 (C), 130.3 (C), 130.1 (C), 129.2 (CH), 128.1 (C), 127.6 (CH), 127.4 (CH), 127.0 (CH), 126.7 (CH), 126.2 (CH), 125.2 (CH), 125.1 (CH), 124.8 (CH), 124.7 (CH), 124.0 (C), 123.9 (C), 123.2 (CH), 122.0 (CH), 42.2 (CH_2), 41.1 (CH_2); FAB-MS *m*/*z* 727 ($M + H^+$); HRMS calcd for $C_{49}H_{35}N_4O_3$ ($M + H^+$) 727.2709, found 727.2699.

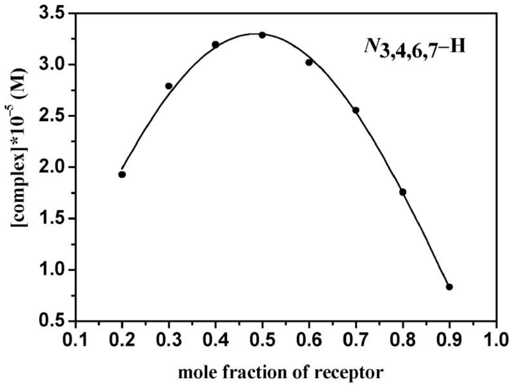
Fluorescence Titration Studies of 1 with Various Anions

A typical fluorescence titration experiment was carried out as follows. A solution of **1** in anhydrous THF (1×10^{-6} M) was prepared, and a 2-mL portion was transferred to a 1-cm fluorescence cell. To the solution was added a small aliquot of the stock solution of the examined anion, e.g. $(Bu_4N^+)_3HP_2O_7^{3-}$ in DMSO (5×10^{-4} M). The relative fluorescence change of **1** was recorded as a function of anion concentrations.

The Stoichiometry of Receptor 1·PP$_i$ Complexes (Job's Plots)

Stock solutions of **1** (1×10^{-3} M) and $(Bu_4N^+)_3HP_2O_7^{3-}$ (1×10^{-3} M) in DMSO-d_6 (99%) were prepared in separate volumetric flasks. Nine sample solutions containing the anion and receptor in different ratios (2/8 to 9/1) were made to maintain a total volume of 0.5 mL. The chemical shift of N_2,N_5-H (or N_3,N_4,N_6,N_7-H) of compound **1** was monitored as a function of mole fractions of receptor **1**. The complex concentration was calculated as follows: [complex] = $[\mathbf{1}]_{tot} \times (\Delta\delta/\Delta\delta_{max})$. Both Job's plots of **1** with $(Bu_4N^+)_3HP_2O_7^{3-}$ corresponded to 1:1 stoichiometry.





^{1}H NMR Titration Studies for the Binding of 1 with Anions

^{1}H NMR titrations were measured on Bruker Avance-400 NMR spectrometer. A typical experiment was performed as follows. A solution of receptor **1** in DMSO-d_6 (99%) was prepared (1×10^{-3} M), and a 0.5 mL portion was transferred to a 5-mm NMR tube. To the solution was added a small aliquot of the solution containing the examined anion, e.g. $(Bu_4N^+)_3HP_2O_7^{3-}$ in DMSO-d_6 (0.125 M). The chemical shift of N_2,N_5-H of compound **1** was monitored as a function of anion concentrations. Nonlinear regression analyses were used to determine the binding constants.

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