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A Novel Phosphate Chemosensor Utilizing Anion-Induced Fluorescence Change

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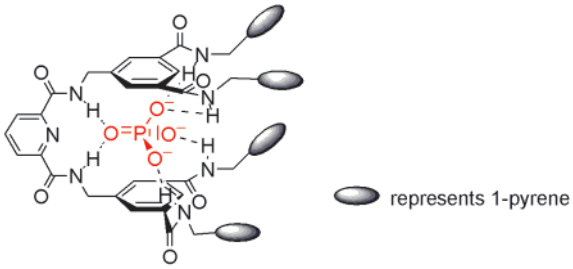
ABSTRACT



The neutral receptor *N*,*N*′-bis{3,5-di[(1-pyrenylmethyl)carbamoyl]benzyl} pyridine-2,6-dicarbamide (2) provides a pseudo-tetrahedron cleft and multiple hydrogen bondings to form a 1:1 complex with phosphate ion in a highly selective manner, by comparison with other anions (F^-, Cl^-, Br^-, SCN^-, AcO^-, NO_3^-, ClO_4^-, and HSO_4^-). The binding strength can be inferred from the emission intensity ratio of the pyrene monomer (λ_{max} 377 nm) to the excimer (λ_{max} 477 nm). Fluorescence titration, X-ray analysis, and NMR studies support a proposed complexation model.

As a result of the biological and environmental significance of phosphate ions, selective recognition and sensing of phosphates are of great importance in the modern host−guest chemistry.[1] However, the inherent tetrahedral structure of phosphate ion also disposes a challenging problem for the design of effective receptors.[2] Several synthetic receptors bearing polyaza,[1c,d,3] thiourea,[4a−c] urea,[4d,e] amide[1a,5] and quanidinium[1b,6] moieties have been demonstrated to bind or transport phosphates.[7] The neutral receptors of thiourea and amide types are particularly interesting, because the transport of phosphates through cell membrane is also regulated by neutral binding proteins. We report herein two artificial neutral phosphate receptors **1** and **2** with multiple amide

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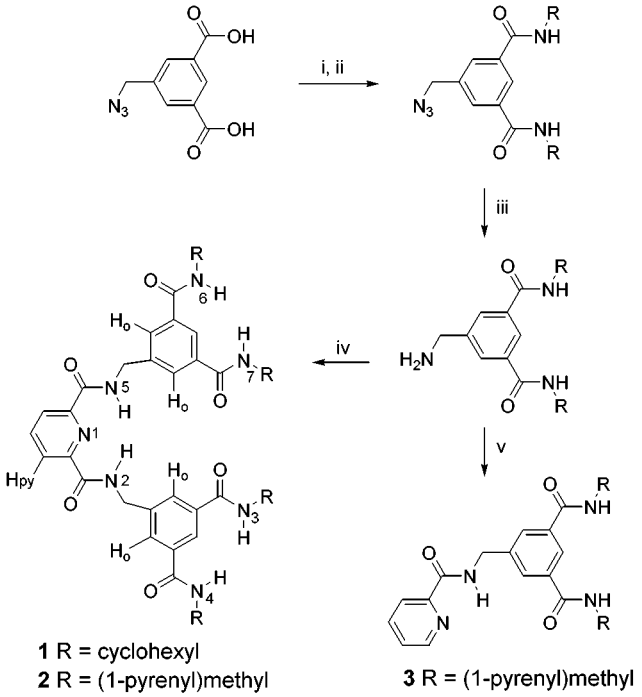
Scheme 1. Synthesis of Compounds **1**−**3**[a]



[a] (i) $SOCl_2$, CH_2Cl_2, rt; (ii) cyclohexylamine (for **1**) or (1-pyrenyl)methylamine hydrochloride (for **2**), Et_3N, CH_2Cl_2, rt; (iii) H_2, Pd/C, MeOH (for **1**); PPh_3/THF, then reflux in THF/H_2O (for **2** and **3**); (iv) pyridine-2,6-dicarboxyl dichloride, Et_3N, CH_2Cl_2, rt; overall yield: **1**, 75%; **2**, 66%; (v) pyridine-2-carboxyl chloride, Et_3N, CH_2Cl_2, rt; overall yield: **3**, 72%.

scaffolds (Scheme 1). These compounds provide a delicate structural cleft with complementary amido groups that selectively hydrogen bonded with PO_4^{3-} and $H_2PO_4^-$ ions. Compound **2** bearing the pyrene fluorophores also exhibits a unique sensing property when it binds with phosphate ion.[8]

The structural cleft is established by pyridine 2,6-biscarboxamide, which has a rigid conformation through the intramolecular hydrogen bondings.[9] It is known that the two amido protons can serve as hydrogen-bond donors for acetate,[10] nitrate,[10] and fluoride[11] ions. Thus, modification of pyridine-2,6-biscarboxamide by incorporating four additional amido groups would likely provide a pseudo-tetrahedral cleft to hold phosphates. As shown in the scheme, 5-(azidomethyl)isophthalic acid[12] was elaborated to *N*,*N*′-dicyclohexyl-5-(aminomethyl)benzene-1,3-dicarboxamide, which coupled with pyridine-2,6-dicarboxyl dichloride to give the host molecule **1** in 75% overall yield. Compound **2** was similarly prepared in 66% yield by replacing cyclohexylamine with (1-pyrenyl)methylamine.

The X-ray analysis of **1** revealed that the conformation of pyridine 2,6-biscarboxamide was indeed confined by intramolecular hydrogen bondings with N(1)···H−N(2,5) distances of 2.3 Å. The two phenyl rings were 6.73 Å apart and almost in a parallel disposition, with a dihedral angle of 5.6° (Figure 1). The pyridyl ring was perpendicular to the phenyl rings. This confined conformation thus constructed a preorganized structural motif for accommodation of the incoming phosphate ion.

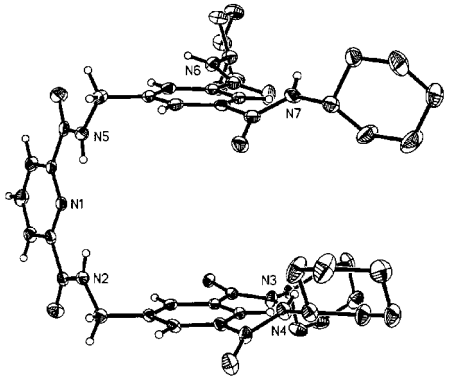


Figure 1. The crystal structure of compound **1** (side view). Incorporation of MeOH molecules and partial intercalation among molecules **1** was shown in the crystal packing (see Supporting Information).

The binding properties of **1** with a variety of anions were evaluated by the ^1H NMR studies in DMSO-d_6 solutions. Pertinent protons of **1** showed significant chemical-shift changes upon addition of tetrabutylammonium dihydrogen phosphate, for example, a 0.98 ppm downfield shift of N_2,N_5-H, 0.27 ppm downfield shift of N_3,N_4,N_6,N_7-H, and 0.16 ppm downfield shift of phenyl protons H_o were observed in the presence of 4 equiv of $(Bu_4N)^+H_2PO_4^-$. The 1:1 complexation stoichiometry of receptor **1** with $H_2PO_4^-$ was confirmed by a continuous variation method (Job plot).[13] The similar NMR changes also occurred when **1** was treated with tris(tetrabutylammonium)phosphate $(Bu_4N^+)_3PO_4^{3-}$. These experiments indicated that the complexes **1**·$H_2PO_4^-$ and **1**·PO_4^{3-} might be formed via hydrogen bondings with the six amido protons. Compound **1** also formed a 1:1 complex with $CH_3CO_2^-$ in a relatively weak binding. The association constants K_a and free energy data for acetate and phosphate ions were collected in Table 1.

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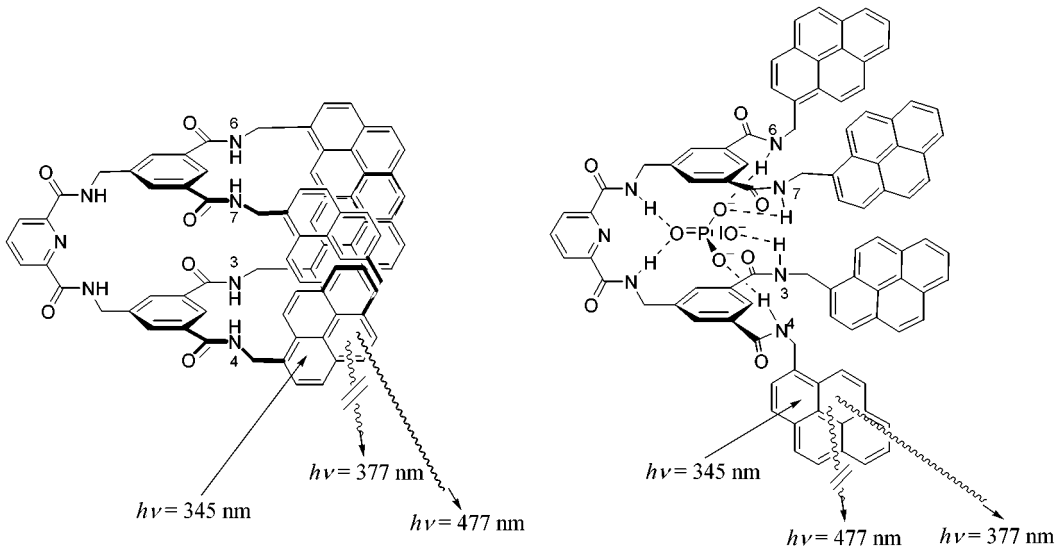

Figure 2. The proposed models for fluorescence change before and after complexation of compound **2** with PO_4^{3-}.

Unlike the previous report[10] on the binding of pyridine 2,6-biscarboxamide with NO_3^-, compound **1** did not bind effectively with this anion. Neither Br^-, I^-, SCN^-, ClO_4^-, nor HSO_4^- ions showed appreciable binding with **1**. The neutral amido groups of **1** might not interact effectively with the anions of low basicity such as Br^-, I^-, SCN^-, NO_3^-, ClO_4^-, and HSO_4^-. The structural complementarity was another important factor to account for the effective hydrogen bondings. Thus, molecule **1** with a pseudo-tetrahedron cleft would bind preferably with $H_2PO_4^-$ and PO_4^{3-} of tetrahedral geometry but not with spherical (e.g., Br^-), linear (e.g., SCN^-), or planar (e.g., NO_3^-) ions.

By replacing the cyclohexyl groups in **1** with 1-pyrenylmethyl groups, compound **2** served as a selective chemosensor for phosphates.[14] The pyrene rings were annexed to the terminals to avoid changing the cleft shape. The association constant of **2**·$H_2PO_4^-$ was determined to be 1374 M^{-1} in DMSO-d_6 by ^1H NMR titration method (Table 1). This value was even higher than that for **1**·$H_2PO_4^-$ (K_a = 549 M^{-1}). Receptor **2** also had a better $H_2PO_4^-/CH_3CO_2^-$ selectivity [K_a ($H_2PO_4^-$)/K_a ($CH_3CO_2^-$) = 5.75] by comparison with that for receptor **1** [K_a ($H_2PO_4^-$)/K_a ($CH_3CO_2^-$) = 3.45].

The UV−vis spectrum of **2** in anhydrous THF showed absorptions at λ_{max} = 277 and 345 nm at a concentration of 1×10^{-6} M. In the fluorescence spectrum, the monomer emission appeared at λ_{max} = 377 and 397 nm, whereas the excimer emission appeared at λ_{max} = 477 nm. The excimer emission was resulted from the intramolecular excimer, rather than intermolecularly, as indicated by the dilution experiments at different concentrations ($5 \times 10^-$,[6] $1 \times 10^-$,[6] 5×10^{-7}, and 1×10^{-7} M), in which the intensities of fluorescence of monomer and excimer decreased simultaneously on dilution.

To discern which pair of pyrene rings were responsible for the excimer emission, the one-arm analogue **3** was prepared as a control molecule by condensation of pyridine-2-carboxyl chloride with *N*,*N*′-di(1-pyrenylmethyl)-5-aminomethyl-1,3-benzenedicarboxamide (Scheme 1). Compound **3** in THF solution at 1×10^{-6} M concentration showed only monomer emissions but no excimer emission. Upon addition of $H_2PO_4^-$ to **3**, the chemical-shift change of N_3,N_4,N_6,N_7-H was much more prominent than that of N_2,N_5-H, indicating

Table 1. Association Constants K_a (M^{-1}) of Receptors **1** and **2** with Anions in DMSO-d_6

	receptor **1**		receptor **2**	
anion[a]	K_a (M^{-1})[b]	$-\Delta G_{300}$ (kJ/mol)[c]	K_a (M^{-1})[b]	$-\Delta G_{300}$ (kJ/mol)[c]
AcO^-	159	12.6	239	13.7
$H_2PO_4^-$	549	15.7	1374	18.0

[a] Bu_4N^+ as the counterion. [b] The average value of two independent titrations at 300 K. [c] $-\Delta G_{300} = RT \ln K_a$.

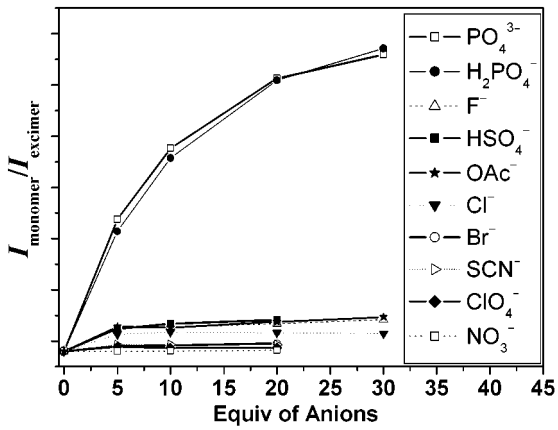

Figure 3. Fluorescence titration of **2** with various anions in THF. The binding strength can be inferred from the intensity ratio of the pyrene monomer to the excimer emission.

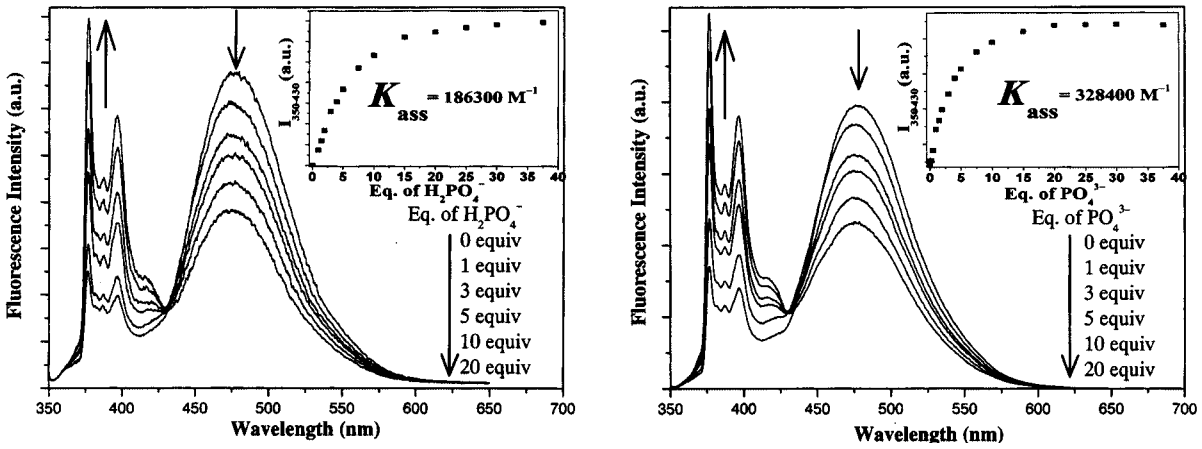

Figure 4. Fluorescence spectra of **2** with $Bu_4N^+H_2PO_4^-$ (left) and $(Bu_4N^+)_3PO_4^{3-}$ (right). $[R_0] = 1 \times 10^{-6}$ M in THF (2 mL).

the **3**·$H_3PO_4^-$ complex was formed with a binding mode different from that of **1**·$H_2PO_4^-$ or **2**·$H_2PO_4^-$ complexes. By ^{1}H NMR titration, the association constant of **3**·$H_2PO_4^-$ was determined to be 198 M^{-1} in DMSO-d_6, much smaller than that of **2**·$H_2PO_4^-$ (K_a = 1374 M^{-1}).

Thus, the excimer emission of compound **2** could be attributable to the excimer formed by the pair of N_3- and N_6-CH_2-pyrenes (or N_4- and N_7-CH_2-pyrenes), rather than the pyrene rings on the same sidearm. This uncommon organized conformation of **2** might be partly due to the $\pi-\pi$ stacking of pyrene rings (Figure 2).

The sensing properties of compound **2** with various anions (F^-, Cl^-, Br^-, SCN^-, AcO^-, NO_3^-, ClO_4^-, HSO_4^-, $H_2PO_4^-$, and PO_4^{3-}) were investigated by fluorescence titration studies (Figure 3). It appeared that the conformation of **2** only changed substantially on binding with phosphate ions.

A typical fluorescence titration curve of sensor **2** (in THF) with phosphates (Figure 4) was derived from the experiments with portionwise additions of $H_2PO_4^-$ or PO_4^{3-} [1−37.5 equiv of $Bu_4NH_2PO_4$ or $(Bu_4N)_3PO_4$ in THF/$CHCl_3$ (1:1)]. In agreement to our prediction, the ratio of excimer emission to monomer emissions decreased as the amounts of anion increased. An isoemissive point occurring at 430 nm that supported only one type of complexation (**2**·phosphate) involved. The association constants in THF were deduced to be 186300 ± 16200 and 328400 ± 19200 M^{-1} for **2**·$H_2PO_4^-$ and **2**·PO_4^{3-}, respectively. The sensing mechanism appeared to have phosphate ion encapsulated into the core of the cleft via six hydrogen bonds (Figure 2).[15] The binding of one-arm compound **3** with $H_2PO_4^-$ is relatively weak as it cannot provide six hydrogen bondings. Formation of the intramolecular excimer of **2** requires an orientation of pyrene rings in close proximity. The pyrene rings would be pushed apart on complexation with phosphate ion to disfavor the excimer formation. The N_3/N_6 and N_4/N_7 pyrene pairs were thus segregated to reduce the excimer emission, accompanied by increase of the monomer emissions.[16]

In summary, we have designed a new type of phosphate receptor with unique recognition and sensing properties. Receptors **1** and **2** have well-defined structures for efficient and selective complexation with phosphates in 1:1 binding stoichiometry. Although the binding of **1** and **2** with phosphates is not extremely strong, the binding selectivity is very high by comparison with other anions (e.g., halide, thiocyanate, acetate, nitrate, perchlorate, and sulfate ions). Addition of phosphate causes a simultaneous change of the monomer and excimer emissions of **2**. This fluorescence change can possibly be utilized in diagnostic devices for the measurement of phosphate concentrations, even in the presence of other anions. Indeed, our preliminary experiments indicated that receptor **2**, in the presence of excess F^- or AcO^- ions (50 equiv), showed a significant fluorescence change upon addition of $(Bu_4N^+)_3PO_4^{3-}$.

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Supporting Information Available: The detailed experimental procedures, spectral data, NMR spectra of selected compounds, and NMR and fluorescence titration curves, as well as the crystal data, bond distances and bond angles of compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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