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Selective Detection of Dihydrogen Phosphate Anion by Fluorescence Change with Tetraamide-Based Receptors Bearing Isoquinolyl and Quinolyl Moieties

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ABSTRACT

Novel fluororeceptors 2 and 3 based on tetraamide bearing 1-isoquinolyl and 2-quinolyl moieties were designed and synthesized. Highly selective and significant changes in the UV−vis and fluorescence spectra of the receptors upon the addition of $H_2PO_4^-$ were found. In particular, receptor 3 bearing 2-quinolyl groups shows selective and nearly perfect quenching by $H_2PO_4^-$, whereas 3 shows small or no fluorescence changes by another anion.

Anions play a significant role in life processes and in the environment; therefore, the synthesis of anion receptors showing a high selectivity has been focused upon in recent years.[1] Fluorescence detection of anions by an anion receptor is becoming crucial due to its simplicity and sensitivity.[2] Among anions, the phosphate anion is a fundamental component in living organisms. Therefore, the construction of new fluorescent indicators with selective recognition of phosphate is an emerging field of anion recognition chemistry.[3,4] We have reported that a tetraamide-based receptor **1** possessing 2-pyridyl groups into terminal groups as hydrogen bond acceptors showed selective recognition of

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(1) Antonisse, M. M. G.; Reinhoudt, D. N. *Chem. Commun.* **1998**, 443. Beer, P. D.; Gale, P. A. *Angew. Chem., Int. Ed.* **2001**, *40*, 486. Bianchi, A.; Bowman-James, K.; Garcia-España, E. *Supramolecular chemistry of anions*; Wiley-VCH: New York, 1997. Schmidtchen, F. P.; Berger, M. *Chem. Rev.* **1997**, *97*, 1609. Suksai, C.; Tuntulani, T. *Top. Curr. Chem.* **2005**, *255*, 163.

(2) Fabbrizzi, L.; Licchelli, M.; Rabaioli, G.; Taglietti, A. *Coord. Chem. Rev.* **2000**, *205*, 85. Martínez-Máñez, R.; Sancenón, F. *Chem. Rev.* **2003**, *103*, 4419. Martínez-Máñez, R.; Sancenón, F. *J. Fluorescnece* **2005**, *15*, 267. Callan, J. F.; de Silva, A. P.; Magri, D. C. *Tetrahedron* **2005**, *61*, 8551. Curiel, D.; Hayes, E. J.; Beer, P. D. In *Topics in Fluorescence Spectroscopy. Advanced Concepts in Fluorescence Sensing Part A: Small Molecule Sensing*; Geddes, C. D., Lakowicz, J. R., Eds.; Springer US: New York, 2005; p 59; Gunnlaugsson, T.; Glynn, M.; Tocci, G. M.; Kruger, P. E.; Pfeffer, F. M. *Coord. Chem. Rev.* **2006**, *250*, 3094.

(3) Xie, H.; Yi, S.; Yang, X.; Wu, S. *New J. Chem.* **1999**, *23*, 1105. Anzenbacher, P., Jr.; Jursikova, K.; Sessler, J. L. *J. Am. Chem. Soc.* **2000**, *122*, 9350. Sasaki, S.-i.; Citterio, D.; Ozawa, S.; Suzuki, K. *J. Chem. Soc., Perkin Trans. 2* **2001**, 2309. Liao, J.-H.; Chen, C.-T.; Fang, J.-M. *Org. Lett.* **2002**, *4*, 561. Kuo, L.-J.; Liao, J.-H.; Chen, C.-T.; Huang, C.-H.; Chen, C.-S.; Fang, J.-M. *Org. Lett.* **2003**, *5*, 1821. Tong, H.; Zhou, G.; Wang, L.; Jing, X.; Wang, F.; Zhang, J. *Tetrahedron Lett.* **2003**, *44*, 131. Yoon, J.; Kim, S. K.; Singh, N. J.; Lee, J. W.; Yang, Y. J.; Chellappan, K.; Kim, K. S. *J. Org. Chem.* **2004**, *69*, 581. Goswami, S.; Sen, D.; Das, N. K. *Tetrahedron Lett.* **2010**, *51*, 6707. Ghosh, K.; Kar, D.; Chowdhury, P. R. *Tetrahedron Lett.* **2011**, *52*, 5098. Gong, W.-t.; Bao, S.; Wang, F.-r.; Ye, J.-w.; Ning, G.-l.; Hiratani, K. *Tetrahedron Lett.* **2011**, *52*, 630. Ghosh, K.; Ali, S. S.; Joardar, S. *Tetrahedron Lett.* **2012**, *53*, 2054. Martí-Centelles, V.; Burguete, M. I.; Galindo, F.; Izquierdo, M. A.; Kumar, D. K.; White, A. J. P.; Luis, S. V.; Vilar, R. *J. Org. Chem.* **2012**, *77*, 490.

(4) Kameta, N.; Hiratani, K. *Chem. Commun.* **2005**, 725.

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the dihydrogen phosphate anion against structurally related acetate anions by additional hydrogen bonds between the pyridyl groups of the receptor and the hydroxy groups of the dihydrogen phosphate anion as shown in Figure 1.[5] However, no fluorescence detection can be achieved by receptor **1** due to the lack of a fluorophore. It is well-known that quinoline and isoquinoline derivatives show fluorescence emission; these have widely been applied to fluorescence sensors.[4,6] The information prompted us to design tetraamide-based receptors **2** and **3** bearing 1-isoquinolyl and 2-quinolyl groups, respectively, as selective fluorescence sensors for the dihydrogen phosphate anion.

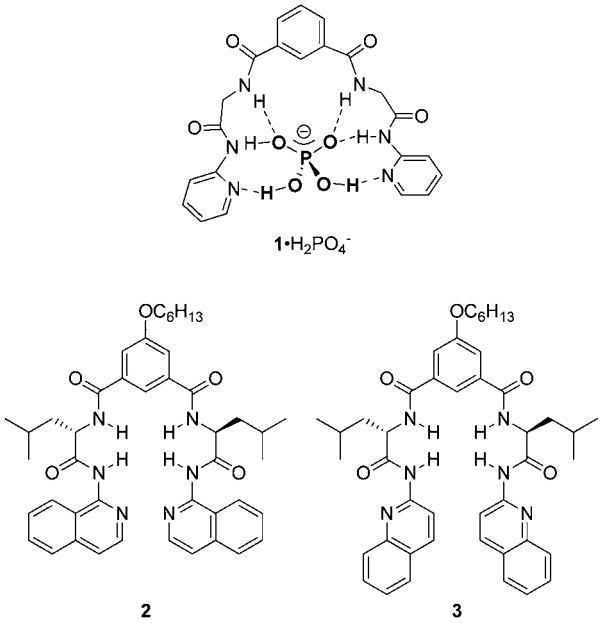
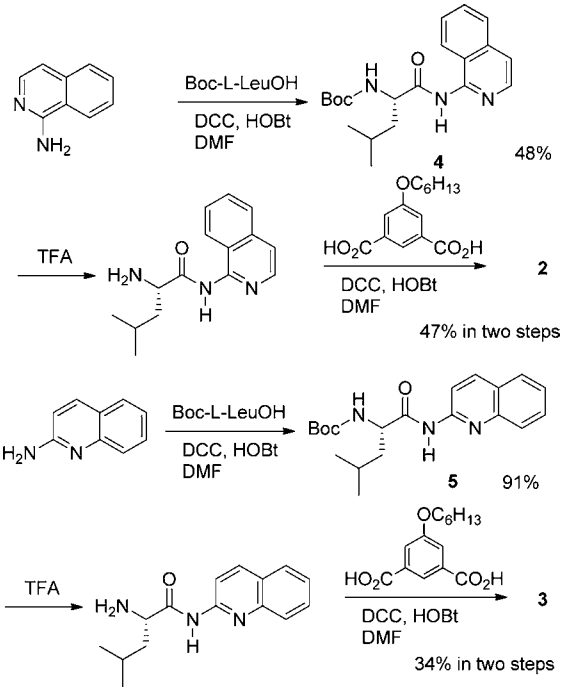


Figure 1. Structure of receptors **1**–**3**.

Receptors **1**–**3** consist of three parts, that is, an isophthaloyl spacer, amino acids, and terminal groups.[5,7] The L-leucyl and 5-hexyloxyisophthaloyl groups were employed for the design of receptors **2** and **3** as amino acids and a spacer group to increase the solubility in common organic solvents. 1-Isoquinoyl and 2-quinoyl groups in receptors **2** and **3** would provide dual roles, as a fluorophore and a hydrogen bond acceptor for $H_2PO_4^-$ with 2-pyrdyl groups of receptor **1** mentioned above. The synthetic route to **2** is illustrated in Scheme 1. *N*-Boc-L-leucine was condensed with 1-aminoisoqunoline by *N*,*N*′-dicyclohexylcarbodiimide (DCC) in the presence of a stoichiometric amount of 1-hydroxybenzotriazole (HOBt) in DMF to give **4** in 48% yield. After deprotection of boc group of **4** by trifluoroacetic acid, the produced amine was immediately condensed with 0.5 equiv of 5-hexyloxyisophthalic acid by DCC in the presence of HOBt in DMF to give receptor **2** in 47% yield in two steps. Receptor **3** was also prepared by the same procedure from 2-aminoquinoline as shown in Scheme 1. The structures of receptors **2** and **3** were fully confirmed by ^{1}H NMR, ^{13}C NMR, DQF-COSY, HMQC, HMBC, and elemental analysis.

Scheme 1. Synthesis of Receptors **2** and **3**



Anion binding properties of **2** and **3** were studied by UV–vis and fluorescence spectroscopies in MeCN. UV–vis spectroscopic titration of **2** upon the addition of $H_2PO_4^-$ as a tetrabutylammonium salt is shown in Figure 2. The characteristic absorbance peaks at 276.5, 349, and 365.5 nm were decreased along with a small increase of bands at 277.5, 288.5, and 314 nm through isosbestic points at 259.5, 310, and 332 nm. Similar but less significant changes in the case of **2** were observed upon the addition of AcO^- and Cl^-; however, smaller changes were found upon the addition of HSO_4^-, Br^-, and NO_3^- (Figure S5). Figure 2b shows absorbance changes for **2** at 350 nm upon the addition of anions. The prominent change for $H_2PO_4^-$ and AcO^- were clearly found suggesting strong complexation with these anions. Receptor **3** showed drastic and selective changes as shown in Figure 3. The absorbance peaks at 315.5 and 329.5 nm were decreased concomitant with an increase of peaks at 321 and 334 nm through clear isosbestic points at 318, 328.5, and 330 nm. A smaller spectral change was observed for AcO^-, and negligible spectral changes were found upon the addition of other anions (Figure S6).

(5) Kondo, S.; Hiraoka, Y.; Kurumatani, N.; Yano, Y. *Chem. Commun*. **2005**, 1720.

(6) Lee, S. k.; Han, Y.; Choi, Y.; Kang, J. *J. Incl. Phenom. Macrocycl. Chem*. **2012**, *74*, 177. Hu, H.-Y.; Chen, C.-F. *Tetrahedron Lett*. **2006**, *47*, 175. Pramanik, A.; Das, G. *Tetrahedron* **2009**, *65*, 2196. Kang, J.; Jang, S. P.; Kim, Y.-H.; Lee, J. H.; Park, E. B.; Lee, H. G.; Kim, J. H.; Kim, Y.; Kim, S.-J.; Kim, C. *Tetrahedron Lett*. **2010**, *51*, 6658.

(7) Kondo, S.; Nakajima, S.; Unno, M. *Bull. Chem. Soc. Jpn*. **2012**, *85*, 698.

Absorbance changes for receptor **3** at 336 nm upon the addition of anions suggest strong and selective complexation with $H_2PO_4^-$ as shown in Figure 3b. The existence of isosbestic points for UV−vis titrations of receptors **2** and **3** suggests a 1:1 complexation, and this was also confirmed by Job's plot analysis (Figure S7). The association constants of **2** and **3** for anionic species were calculated by nonlinear curve fitting analysis of the UV−vis titrations, and the results are collected in Table 1. The association constants of **2** and **3** for $H_2PO_4^-$ are $(1.94 \pm 0.24) \times 10^6$ and $(5.41 \pm 0.40) \times 10^6$ mol^{-1} dm^3, respectively, and these value are 1 order of magnitude larger than those for AcO^-. The selectivity of receptors **2** and **3** between $H_2PO_4^-$ and AcO^- are calculated to be 13.7 and 46.6, respectively. It should be noted that the presence of 1 equiv of competitive anions such as AcO^- and Cl^- caused no influence on the UV−vis change for **3** upon the addition of $H_2PO_4^-$ (Figure S8).

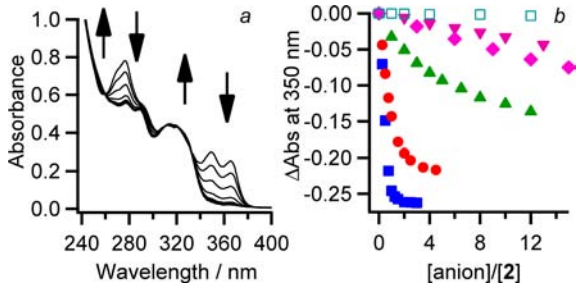


Figure 2. (a) UV−vis spectral titrations of **2** upon the addition of $H_2PO_4^-$. (b) UV−vis spectral changes of **2** at 350 nm upon the addition of $H_2PO_4^-$ (■), AcO^- (●), HSO_4^- (◆), NO_3^- (□), Cl^- (▲), and Br^- (▼). Measured in MeCN at 298 K. [**2**] = 5.0×10^{-5} mol dm^{-3}.

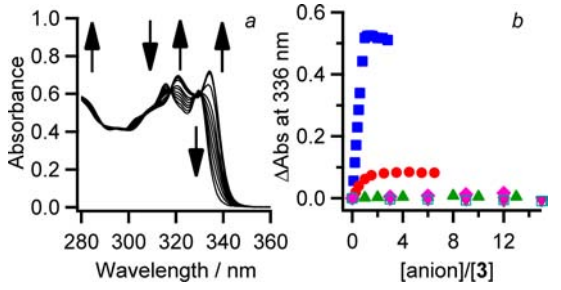


Figure 3. (a) UV−vis spectral titrations of **3** upon the addition of $H_2PO_4^-$. (b) UV−vis spectral changes of **3** at 336 nm upon the addition of $H_2PO_4^-$ (■), AcO^- (●), HSO_4^- (◆), NO_3^- (□), Cl^- (▲), and Br^- (▼). Measured in MeCN at 298 K. [**3**] = 5.0×10^{-5} mol dm^{-3}.

Both receptors **2** and **3** show strong fluorescence emission as expected. Fluorescence emission of receptor **2** was observed at 395 nm excited at 332 nm which is one of the isosbestic points during the UV−vis titration experiment. Receptor **3** also showed fluorescence emission at 336 and 350 nm excited at 318 nm (one of the isosbestic points of **3**) in MeCN. The quantum yields of receptors **2** and **3** in the absence of any anions were determined to be 0.222 and 0.339, respectively, in MeCN by comparing with quinine sulfate in 0.5 mol dm^{-3} sulfuric acid as a standard.[8] A gradual decrease of the emission of **2** was observed upon the addition of $H_2PO_4^-$ and AcO^- (Figures 4a and S9). Fluorescence quenching of **2**·$H_2PO_4^-$ (I_{max}/I_0 at 395 nm was calculated to be 0.47 from the curve-fitting analysis to a 1:1 complexation model) is smaller than that of **2**·AcO^- (0.33); however, the association constant for $H_2PO_4^-$ is significantly larger than that for AcO^- as observed by the UV−vis titration of **2**.

Interestingly, a drastic fluorescence change for receptor **3** was found upon the addition of $H_2PO_4^-$ and the I_{max}/I_0 at 355 nm reached ~0 indicating perfect quenching of **3**; however, less significant fluorescence changes for AcO^- (I_{max}/I_0 = 0.46) and smaller or negligible spectral changes for other anions suggest a weak interaction of **3** with these anions as shown in Figure 5. Indeed, the association constant of **3** for $H_2PO_4^-$ and AcO^- can be elucidated to be $(2.76 \pm 0.10) \times 10^6$ and $(9.94 \pm 0.53) \times 10^4$ mol^{-1} dm^3, respectively, which are in fairly good agreement with those calculated from UV−vis titrations as shown in Table 1.

Table 1. Association Constants of Receptors **2** and **3** with Anions in MeCN

	K_{11}/mol^{-1} dm^3			
	2		**3**	
anion	UV−vis[a]	fluorescence[b]	UV−vis[a]	fluorescence[b]
AcO^-	$(1.42 \pm 0.15) \times 10^5$	$(1.41 \pm 0.14) \times 10^5$	$(1.16 \pm 0.06) \times 10^5$	$(9.94 \pm 0.53) \times 10^4$
$H_2PO_4^-$	$(1.94 \pm 0.24) \times 10^6$	$(2.51 \pm 0.10) \times 10^6$	$(5.41 \pm 0.40) \times 10^6$	$(2.76 \pm 0.10) \times 10^6$
HSO_4^-	ND[c]	ND[c]	ND[c]	$(3.17 \pm 0.20) \times 10^3$
NO_3^-	ND[c]	ND[c]	ND[c]	ND[c]
Cl^-	$(4.02 \pm 0.31) \times 10^3$	$(7.04 \pm 0.76) \times 10^3$	ND[c]	$(3.28 \pm 0.10) \times 10^3$
Br^-	ND[c]	ND[c]	ND[c]	ND[c]
selectivity[d]	13.7	17.8	46.6	27.8

[a] Determined by UV−vis spectroscopy. [receptor] = 5.0×10^{-5} mol dm^{-3}. [b] Determined by fluorescence spectroscopy. [receptor] = 1.0×10^{-5} mol dm^{-3}. [c] The association constant was not determined due to small spectral changes. [d] $K_{11}(H_2PO_4^-)/K_{11}(AcO^-)$.

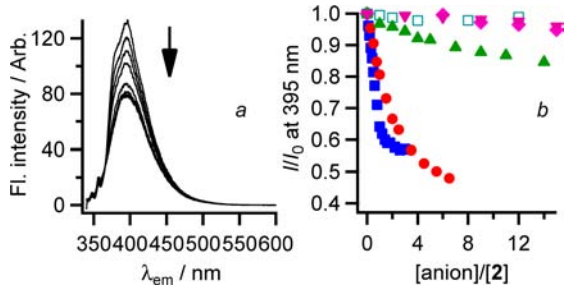


Figure 4. (a) Fluorescence spectral titrations of **2** upon the addition of $H_2PO_4^-$. (b) Fluorescence spectral changes of **2** at 395 nm upon the addition of $H_2PO_4^-$ (■), AcO^- (●), HSO_4^- (◆), NO_3^- (□), Cl^- (▲), and Br^- (▼). Measured in MeCN at 298 K. λ_{ex} = 332 nm and [**2**] = 1.0×10^{-5} mol dm^{-3}.

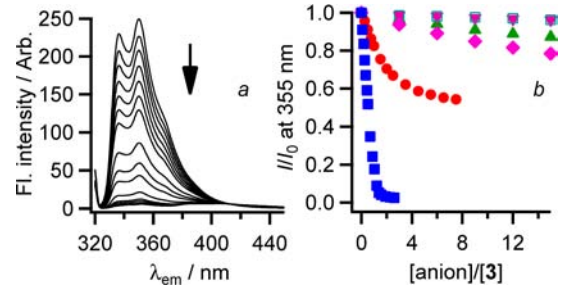


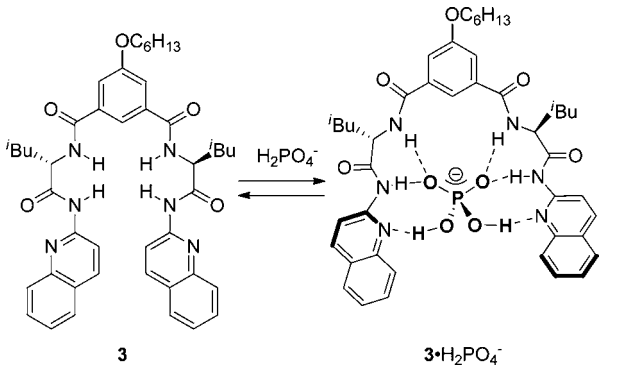
Figure 5. (a) Fluorescence spectral titrations of **3** upon the addition of $H_2PO_4^-$. (b) Fluorescence spectral changes of **3** at 355 nm upon the addition of $H_2PO_4^-$ (■), AcO^- (●), HSO_4^- (◆), NO_3^- (□), Cl^- (▲), and Br^- (▼). Measured in MeCN at 298 K. λ_{ex} = 318 nm and [**3**] = 1.0×10^{-5} mol dm^{-3}.

The selectivities ($K_{11}(H_2PO_4^-)/K_{11}(AcO^-)$) of **2** and **3** from fluorescence titrations are also calculated to be 17.8 and 27.8, respectively. The association constants of receptors **2** and **3** for other anions were significantly smaller or negligible. These results indicate that receptors **2** and **3** showed a high selectivity for $H_2PO_4^-$ over other anions.

A plausible equilibrium between receptor **3** and $H_2PO_4^-$ is illustrated in Scheme 2. The results of the UV−vis and fluorescence spectral titrations of the receptors **2** and **3** suggest that the nitrogen atoms of the isoquinolyl and quinolyl groups play an important role in the discrimination between $H_2PO_4^-$ and AcO^- as observed for receptor **1**.[5]

(8) Meech, S. R.; Phillips, D. *J. Photochem.* **1983**, *23*, 193.

Scheme 2. A Plausible Equilibrium between Receptor **3** and $H_2PO_4^-$



Four amide NH groups act as hydrogen bond donors to recognize anionic oxygen atoms of $H_2PO_4^-$ and AcO^-. In addition, isoquinolyl and quinolyl groups act as hydrogen bond acceptors for hydroxy groups of $H_2PO_4^-$. However, AcO^- cannot form such hydrogen bonds due to the lack of a hydroxy group in AcO^-. Therefore, the high selectivity of $H_2PO_4^-$ over AcO^- was achieved. The fluorescence quenching induced by the association with $H_2PO_4^-$ over AcO^- could be attributed to photoinduced electron transfer (PET).

In conclusion, we have thus achieved fluorescence receptors **2** and **3** based on a tetraamide scaffold bearing 1-isoquinolyl and 2-quinolyl groups into the terminal positions. The receptors show UV−vis spectral change and fluorescence quenching upon the addition of anions, in particular biologically relevant oxoanions such as $H_2PO_4^-$ and AcO^-. Meanwhile, these receptors exhibit a high degree of selectivity for $H_2PO_4^-$ over AcO^-, which may be due to the additional hydrogen bonds between the hydroxy groups of $H_2PO_4^-$ and the nitrogen atoms of isoquinolyl and quinolyl groups. From the viewpoints of selectivity and sensitivity, receptor **3** bearing 2-quinolyl groups was found to be suitable for fluorescence sensors for $H_2PO_4^-$ rather than receptor **2**.

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Supporting Information Available. Experimental details including synthesis, NMR spectra of receptors, UV−vis and fluorescence titrations. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.