

# A fluorescence-active 1,3-bis(isothiuronium)-derived naphthalene exhibiting versatile binding modes toward oxoanions in aqueous MeCN solution: new methodology for sensing oxoanions<sup>☆</sup>

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**Abstract**—A unique binding property of the PET (photoinduced electron transfer) sensor **1** is described, in which a naphthalene moiety at the 1,3-positions is attached by isothiuronium units that serve as anion-binding units. Fluorescence emission ( $\lambda_{\text{max}} = 339 \text{ nm}$ ) was significantly switched on at an excitation wavelength of 279 nm upon recognition of anion in 6% (v/v) H<sub>2</sub>O/MeCN solution at 25 °C. Titration curves with HPO<sub>4</sub><sup>2-</sup> and AcO<sup>-</sup> differ greatly; that with HPO<sub>4</sub><sup>2-</sup> shows a stoichiometric 2:1 host-to-guest binding, whereas that with AcO<sup>-</sup> shows a biphasic 1:1 and 1:2 host-to-guest binding, reflecting principally the charged shapes of the oxoanions. This finding is attributable to the plural built-in isothiuronium units with a high anion-binding ability, and suggests a new way to develop fluorescent sensors for oxoanion recognition.

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Much work has been directed to the design and synthesis of anion receptors<sup>1</sup> in molecular recognition chemistry, since anions play significant roles in biology.<sup>2</sup> For example, the switching activity of enzymes involves phosphorylation and dephosphorylation on a protein surface.<sup>3</sup> Application in material science as sensors<sup>4</sup> is desirable in analytical chemistry as well as in biomedical and environmental research. The need to detect anions has led us to explore a suitable anion-binding unit that can be built in the receptor. It is from this perspective that we have currently focused our attention on the estimation of isothiuronium group, which has several advantages that serves as an anion-binding site: (1) it enhances the NH acidity compared to the corresponding thiourea;<sup>5</sup> (2) the chemical modification is readily varied using synthetic methods to make several types of functional molecular systems.<sup>6</sup> Accordingly, we have synthesized fluorescence-active sensors comprising a naphthalene moiety attached covalently, where the electron-deficient property of the isothiuronium unit allows for a photoinduced electron transfer (PET) quenching process from

the excited naphthalene to the isothiuronium unit. Anion sensing properties then take place by PET regulation, based on interactions between the anion and the isothiuronium unit.<sup>7</sup> The sensing function of mono(isothiuronium)-naphthalene conjugate has been fully characterized, but the binding mode of bis(isothiuronium)-binaphthalene derivative with anions remains unclear.<sup>8</sup> Further investigation of the binding characteristics of plural isothiuronium-inserted molecules is needed. In this letter, we report a naphthalene derivative **1** with two isothiuronium units at the 1,3 positions. As described below in detail, it was interestingly found that this molecule shows diagnostic binding characteristics according to the basicity of the anions and also their charged shapes.<sup>1b,9</sup> The sensing properties of this molecule have been studied in aqueous medium, as the approach is important in analytical chemistry.<sup>5f,i,10</sup>

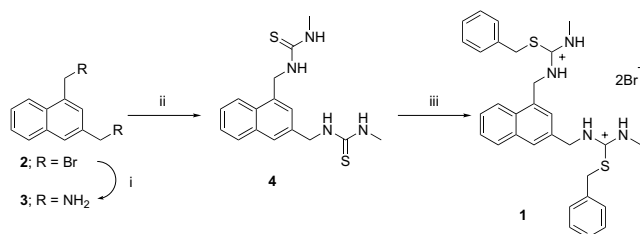
The desired **1** was synthesized as shown in Scheme 1. The starting materials, 1,3-dibromomethylnaphthalene **2**<sup>11</sup> was converted to the corresponding diamine derivative **3** via Gabriel synthesis in 83% yield, followed by the reaction with MeNCS to give bis(thiourea) **4** in 34% yield. Finally, **4** was allowed to react with 2 equiv of benzyl bromide to give **1**<sup>12</sup> in 66% yield.

As a dibromo salt, compound **1** is scarcely soluble in common organic solvents such as MeCN. However,

**Keywords:** Isothiuronium; Anion recognition; Sensor.

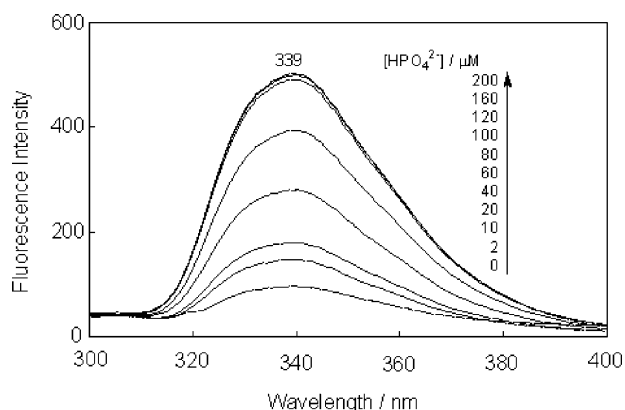
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**Scheme 1.** Reagents and conditions: (i) K<sup>+</sup> phthalimide<sup>-</sup>, dry DMF, and then NH<sub>2</sub>NH<sub>2</sub>·H<sub>2</sub>O, THF–EtOH; (ii) MeNCS, CHCl<sub>3</sub>; (iii) benzyl bromide, dry EtOH.

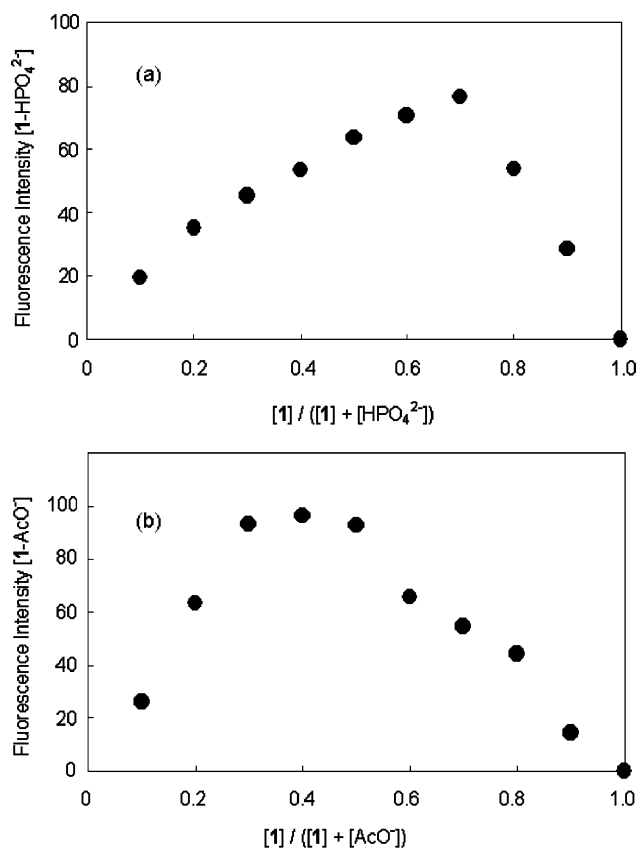
water-contained MeCN made fluorescence titrations possible with several anion species. On the other hand, it should be considered that the binding forces between **1** and anion species are mainly electrostatic interactions together with hydrogen bondings on the isothiuronium units, so that the binding strength ordinarily decreases with increasing in the polarity of the solvent. Indeed, some anion-binding experiments have been carried out in organic medium (e.g., MeCN) rather than water.<sup>1c</sup> As expected, the fluorescence response of **1** toward anions reduces as the ratio of water to MeCN increases, but **1** nevertheless significantly enhanced the fluorescent intensity when HPO<sub>4</sub><sup>2-</sup> as [K<sup>+</sup>-18-crown-6] salt was added to 6% (v/v) water containing MeCN solution of **1**, which corresponds to 3.5 M water concentration. This finding is useful for the development of a practical sensor because it is fully water miscible, making it possible to detect anions added in the form of aqueous solution. Figure 1 shows fluorescence response with HPO<sub>4</sub><sup>2-</sup>; there is almost no fluorescence spectra of **1** in aqueous MeCN based on a photoinduced electron transfer (PET) quenching process ('off switch' state), whereas the binding of HPO<sub>4</sub><sup>2-</sup> on the isothiuronium unit could regulate the efficiency of the PET process to induce HPO<sub>4</sub><sup>2-</sup>-induced fluorescence emission ( $\lambda_{em} = 339$  nm) at an excited wavelength of 279 nm, due to the fluorescent retrieval of the naphthalene unit. As a result, 4 equiv of HPO<sub>4</sub><sup>2-</sup> addition caused a remarkable enhancement in intensity up to ca. 520% in aqueous MeCN solution. The receptor-anion complexation process was assessed by <sup>1</sup>H NMR titrations; signals arising



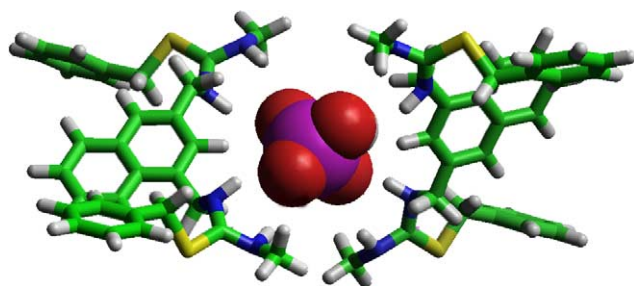
**Figure 1.** Emission spectra of **1** (20 μM) in 6% (v/v) H<sub>2</sub>O–MeCN at 25 °C excited at 279 nm upon addition of HPO<sub>4</sub><sup>2-</sup> as a [K<sup>+</sup>-18-crown-6] salt.

from two types of methylene (NaphCH<sub>2</sub>NH– and PhCH<sub>2</sub>S–) and methyl (–NHCH<sub>3</sub>) protons, which are located in the periphery of the anion-binding sites, shifted significantly upfield ( $\Delta\delta = 0.2 - 0.25$  ppm) of the resonances upon complexation with HPO<sub>4</sub><sup>2-</sup>. The understanding of binding profile was taken from a job plot<sup>13</sup> using mixtures of **1** and HPO<sub>4</sub><sup>2-</sup> in such a solution. Interestingly, this approach reveals that the relative **1**-HPO<sub>4</sub><sup>2-</sup> complex concentration clearly approaches a maximum when the molar function of **1** ( $[1]/([1] + [HPO_4^{2-}]) = 0.7$  (Fig. 2a), suggesting the formation of a 2:1 stoichiometric complex between **1** and HPO<sub>4</sub><sup>2-</sup>. This intrigued finding is a consequence of the tetrahedral shape of the phosphate, in which four phosphate oxygens can bind to the isothiuronium units as depicted in Figure 3.<sup>14</sup>

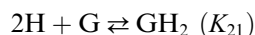
We estimated the association constant based on the titration result. The Job plot (vide supra) suggest that a 2:1 stoichiometry complex between **1** and HPO<sub>4</sub><sup>2-</sup> forms immediately in the fluorescence titration condition. A mathematical treatment based on Eq. (1)<sup>15</sup> below was then applied for the estimation:  $K_{21}$  [ $H_{tot}$ ] and [ $G_{tot}$ ], respectively, denote the observed association constant for GH<sub>2</sub> complex formation and the total concentrations of **1** and anion, and  $a$  is the fluorescent intensity ratio,  $a = (I - I_0)/(I_i - I_0)$ , where  $I$  is the concentration-dependent intensity, and  $I_0$  and  $I_i$  are the intensity at zero and infinite anion concentrations.



**Figure 2.** Job plots for (a) **1**-HPO<sub>4</sub><sup>2-</sup> and (b) **1**-AcO<sup>-</sup> complexes in 6% (v/v) H<sub>2</sub>O–MeCN;  $[1] + [HPO_4^{2-}] = [1] + [AcO^-] = 20$  μM.



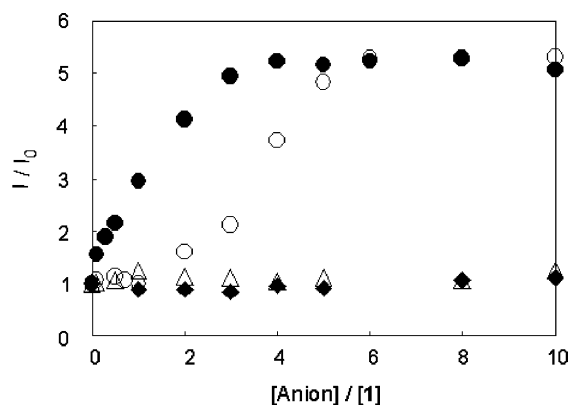
**Figure 3.** A plausible binding mode for a 2:1 host-to-guest complexation of **1** with  $\text{HPO}_4^{2-}$ .



$$[\text{G}]_{\text{tot}} = \frac{a}{2K_{21}(1-a)^2[\text{H}]_{\text{tot}}} + \frac{a[\text{H}]_{\text{tot}}}{2} \quad (1)$$

The analysis was carried out in KaleidaGraph using nonlinear curve fitting based on the Levenberg–Marquardt algorithm (see supporting information), giving  $K_{21} = 1.6 \times 10^{11} \text{ M}^{-2}$ ,<sup>16</sup> and revealing strong complexation with  $\text{HPO}_4^{2-}$ .

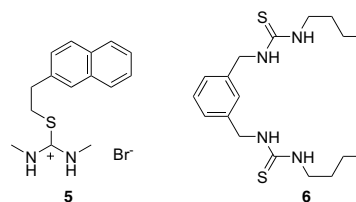
Response ability toward several anions of physiological importance regarding fluorescence spectra was examined in 6% (v/v)  $\text{H}_2\text{O}/\text{MeCN}$  at 25 °C; Figure 4 summarizes the resulting titration curves. Sensor **1** shows significant response with  $\text{HPO}_4^{2-}$ . In contrast, no response was observed upon addition of  $\text{H}_2\text{PO}_4^-$ , since the Lewis basicity is lower than that of  $\text{HPO}_4^{2-}$  [ $\text{p}K_{\text{a}}$ : 7.21( $\text{HPO}_4^{2-}$ ), 2.16( $\text{H}_2\text{PO}_4^-$ )].<sup>17</sup> The low basicity of  $\text{Cl}^-$  also caused no response in the fluorescence spectra, whereas the addition of  $\text{AcO}^-$  as an oxoanion causes moderate enhancement in the water-contained MeCN solution. Taken together, **1** shows selectivity in the order of  $\text{HPO}_4^{2-} > \text{AcO}^- \gg \text{H}_2\text{PO}_4^- \approx \text{Cl}^-$ , the trend being consistent with that of mono(isothiouonium)-naphthalene conjugate **5** in MeOH (as reported by Teramae and co-workers<sup>7b</sup>). Our present attention, however, is focused on the binding profiles with oxoanions. It is interesting to note that the profile with  $\text{AcO}^-$  is quite different from that of  $\text{HPO}_4^{2-}$ ; the  $\text{AcO}^-$ -induced sigmoidal curve in



**Figure 4.** Fluorescence intensity at 339 nm of **1** (20  $\mu\text{M}$ ) in 6% (v/v)  $\text{H}_2\text{O}-\text{MeCN}$  at 25 °C excited at 279 nm as a function of anion concentration: (●)  $\text{HPO}_4^{2-}$ , (○)  $\text{AcO}^-$ , (◆)  $\text{H}_2\text{PO}_4^-$ , (△)  $\text{Cl}^-$  as [K<sup>+</sup>-18-crown-6].

the titrations suggests that a 1:2 host-to-guest complex would form via the corresponding a 1:1 complex, as inferred from the observation that the job plot (Fig. 2b) is not symmetric and shows a broad peak with an approximate maximum of 0.4. In this case the binding mode should be estimated on the basis of biphasic equilibria ( $K_{11}$  and  $K_{12}$ ), as follows:  $\text{H} + \text{G} \rightleftharpoons \text{HG}$  ( $K_{11}$ ),  $\text{HG} + \text{G} \rightleftharpoons \text{HG}_2$  ( $K_{12}$ ). Curve fitting is possible using the SPANA program.<sup>18</sup> As a result, the analysis could fully reproduce the experimental data (supporting information) to estimate each association constant ( $K_{11} = 5.4 \times 10^3 \text{ M}^{-1}$  and  $K_{12} = 7.3 \times 10^5 \text{ M}^{-1}$ ).<sup>16</sup> The noteworthy feature is that the value of  $K_{12}$  is ca. 140 times larger than  $K_{11}$  possibly because the first  $\text{AcO}^-$  binding to **1** involves with dehydration of the isothiouonium units in the aqueous MeCN solution, so that  $K_{11}$  decreases.

In this way, not only the anion's basicity but also the negatively charged shapes, tetrahedral ( $\text{HPO}_4^{2-}$ ) and planar ( $\text{AcO}^-$ ), gave rise to clear binding curves on the fluorescence titrations, distinguishing the oxoanions. This finding uncovers a new function of isothiouonium-derived sensors, since the anion binding characters for the related 1,3-bis[(thio)urea]-benzene derivatives **6** shows only a 1:1 host-to-guest complex with oxoanions.<sup>19</sup>



In conclusion, as part of our continuing program to develop isothiouonium-derived molecular sensors to recognize biologically important anion species, we have synthesized the 'concave'-shaped bis(isothiouonium)-naphthalene conjugate **1**. Based on the PET process from the excited naphthalene to the isothiouonium units, the fluorescent response of **1** depends on the basicity of the anions in 6% (v/v)  $\text{H}_2\text{O}-\text{MeCN}$ . But the most striking finding in this research is that **1** binds to  $\text{HPO}_4^{2-}$  in a 2:1 host-to-guest complex, whereas in a similar condition the binding curve with  $\text{AcO}^-$  fits biphasic 1:1 and 1:2 host-to-guest bindings.<sup>20,21</sup> This indicates that the binding modes of **1** with oxoanions differs according to the shapes of the negatively charged anion mainly. From the shape of binding curve on the fluorescence titrations it is therefore possible to read-out the oxoanions. This provides a promising methodology for designing optical sensors to recognize oxoanion species.

#### Acknowledgements

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- For obtaining further insight into the host-guest binding modes we conducted electrospray ionization mass spectrometry (ESI-MS) as a direct method for examining noncovalent associations. After several trials in the negative ion mode using a mixture of 20 μM of **1** and 5 equiv of the oxoanion as a [K<sup>+</sup>-18-crown-6] salt in 6% (v/v) H<sub>2</sub>O-MeCN, we detected a molecular peak corresponding to the formation of a 1:1 complex with oxoanion (*m/z* = 609 for [bis(isothiuronium)-H + HPO<sub>4</sub>]<sup>-</sup> and *m/z* = 571 for [bis(isothiuronium)-2H + AcO]<sup>-</sup>) in each spectrum at a nozzle temperature of 70 °C. However, expected peak based on a 2:1 or 1:2 host-to-guest complex in each spectrum could not be obtained. Although further decrease

of the temperature might assist the detection, we discontinued the trial because measurement at lower temperature can pollute the electrospray chamber. The employed electrospray ionization process could not fully reproduce complexation in the bulk solution environment in this study, possibly because the electron-deficient property of the isothiuronium group may be because the remarkable heating on the ionization may make it difficult to detect the 2:1 or 1:2 host-to-guest complex with oxoanions in the negative ion mode of ESI-MS. An alternative approach involving X-ray studies toward this end is now underway.

21. During the preparation of this manuscript Ahn et al. reported that a benzene-based tripodal isothiuronium receptor shows mixed binding modes of phosphate ( $\text{PO}_4^{3-}$ ) by means of isothermal titration calorimetry. See: Seong, H. R.; Kim, D.-S.; Kim, S.-G.; Choi, H.-J.; Ahn, K. H. *Tetrahedron Lett.* **2004**, *45*, 723–727. However, our compound **1** shows individual binding modes toward each oxoanion ( $\text{HPO}_4^{2-}$  versus  $\text{AcO}^-$ ) in the fluorescence titrations. The corresponding binding curves allow us to distinguish the oxoanions easily.