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Benzimidazole and thiourea conjugated fluorescent hybrid receptor for selective recognition of PO_4^{3-}

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Abstract

We synthesized a novel hybrid receptor bearing an array of hydrogen bond donors from benzimidazole and thiourea moieties. The recognition behavior of the receptor toward various anions has been evaluated in a DMSO/H_2O (8:2, v/v) solvent system. The receptor showed changes in fluorescent intensity only with PO_4^{3-}, and it showed no such significant response to any of the other anions such as F^-, Cl^-, Br^-, I^-, CN^-, ClO_4^-, $H_2PO_4^-$, HPO_4^{2-}, AcO^-, NO_2^-, CO_3^{2-}, HCO_3^-, SO_4^{2-}, and HSO_4^-. The receptor is highly selective in recognizing PO_4^{3-}even in the presence of other anions in aqueous DMSO.

Keywords: Hybrid receptor; Fluorescent receptor; Benzimidazole; Thiourea; Phosphate

Supramolecular chemistry is defined as the chemistry of non-covalent interactions, encompassing the chemistry of multicomponent molecular assemblies.[1,2] Over the last few decades, chemists have learnt to manipulate receptor molecules by using synthetic skills in attempts to mimic natural systems.[3–6] In this context, selective recognition of molecules is an important challenge in the field of supramolecular chemistry. To achieve this goal in abiotic receptors, the alignment of the receptor binding sites on a platform must complement the size, shape, and electronic properties of a targeted guest.[7–9]

Phosphates are biologically relevant anions that commonly occur, and phosphorylated species play critical roles in a variety of fundamental processes including genetic information storage, energy transduction, signal processing, and membrane transport.[10] In addition, many chemotherapeutic and antiviral drugs contain phosphates.[11–13] Thus, the development of selective receptors for phosphate ions and phosphorylated biomolecules has received considerable attention.[14–19]

Many receptors show high binding affinities in organic solvents but the binding strength is often greatly diminished in an aqueous solution. This is due to solvent competition with hydrogen bonding to the receptor in aqueous medium.[20] Natural receptors bind anions, using convergent arrays of neutral hydrogen bond donors that match the sizes and the shapes of their targets. Thus, the construction of simple synthetic receptor capable of phosphate recognition by hydrogen bonding in aqueous solution can be achieved by taking advantage of the tetrahedral geometry of phosphates. We designed a receptor system that provides an array of hydrogen bonding with a tetrahedral environment in the pseudocavity of the receptor. Both thiourea[21] and benzimidazole-based[22–24] receptors have individually been reported to recognize anions. But to the best of our knowledge, no receptors have been reported so far that can provide hydrogen bonding array from the combination of both thiourea and benzimidazole. Development of such efficient hybrid receptor systems capable of binding a specific guest selectively can be recognized as a

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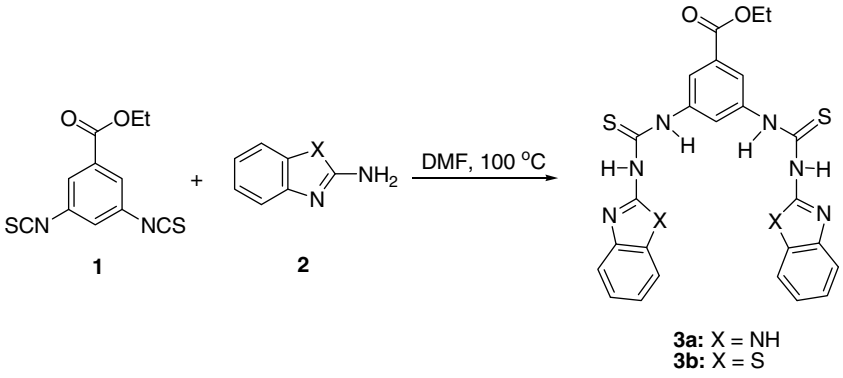
key predicate solving the fundamental problems of anion recognition in aqueous medium.

The design of receptor **3a** comprises two pods bearing mixed hydrogen bond donors of thiourea and benzimidazole. The third pod is an electron-withdrawing group to facilitate hydrogen bond donor from aromatic platform at its para position. Receptors **3a** and **3b** were prepared by the reaction of ethyl 3,5-diisothiocyanatobenzoate with 2-aminobenzimidazole and 2-aminobenzthiazole, respectively (Scheme 1).[25,26] Ethyl 3,5-diisothiocyanatobenzoate was prepared by the literature method.[27] Receptor **3a** in a DMSO/H_2O (8:2, v/v) solvent system exhibited the maximum at 457 nm in its fluorescence spectrum that was recorded with its 10 μM concentration when excited at 325 nm.

The anion recognition properties of receptor **3a** were evaluated by analyzing the changes in fluorescence intensity of **3a** upon the addition of 5.0 equiv of sodium salt of a particular anion. The fluorescence ratio $(I_0 - I)/I_0$ is displayed in Figure 1. It is clear that there is a marked quenching upon the addition of PO_4^{3-}. No such significant responses were observed in any of the other anions such as F^-, Cl^-, Br^-, I^-, CN^-, ClO_4^-, $H_2PO_4^-$, HPO_4^{2-}, AcO^-, NO_2^-, CO_3^{2-}, HCO_3^-, SO_4^{2-}, and HSO_4^-. The preference for PO_4^{3-} suggests that the pseudocavity of receptor **3a** is complimentary to the size and tetrahedral geometry of the anion.[28,29] The high affinity of receptor **3a** for PO_4^{3-} among other tetrahedral anions seems to be due to highly negative charges because the receptor does not recognize its protonated forms like HPO_4^{2-} and $H_2PO_4^-$. This property of some tetrahedral anions has been used to achieve the binding selectivity by governing their propensity to undergo protonation.[14] The anion protonation changes the electron density present in the oxygen atoms, and this ultimately affects the anion receptor recognition process. To verify our idea of better organization of the receptor



Scheme 1.

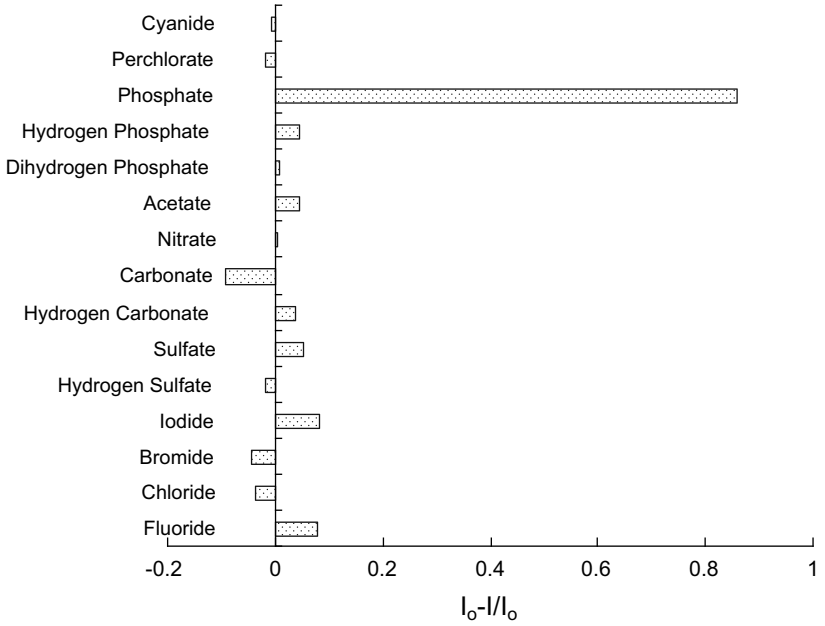


Fig. 1. Fluorescence ratio $(I_0 - I/I_0)$ of receptor **3a** (10 μM) at 457 nm upon the addition of 5.0 equiv sodium salt of a particular anion in DMSO/H_2O (8:2, v/v).

pseudocavity for the tetrahedral anion, we synthesized receptor **3b**. This compound is an assembly of thiourea and benzthiazole having homology with the structure of receptor **3a**. The same conditions were selected for the evaluation of recognition properties of compound **3b** as in the case of **3a**. The results showed that upon the addition of PO_4^{3-} to the solution of receptor **3b**, the relative changes in the fluorescence intensity of receptor **3b** are lesser than that in receptor **3a**. And the selectivity for phosphate is lost with receptor **3b** (Fig. S2 in Supplementary data).

Fluorescence titrations were performed to investigate the comparative affinity of binding PO_4^{3-} in the coordination sphere of receptors **3a** and **3b**. A 10 μM solution of **3a** was selected for the titration, and with the addition of every equivalent of PO_4^{3-}, the fluorescence intensity decreased without changing the wavelength as shown in Figure 2. It indicates that the changes in fluorescent intensity upon the recognition of the guest anion are due to a photo-induced electron transfer (PET) process. Receptor **3a** exhibited a high sensitivity toward PO_4^{3-} quenching 80% of its fluorescence intensity with 5.0 equiv of PO_4^{3-}. The association constants K_a were calculated on the basis of Benesi–Hildebrand plots (Figs. S3 and S6 in Supplementary data).[30] Receptor **3a** showed stronger binding affinity for PO_4^{3-} than receptor **3b**. The binding constants of **3a** and **3b** for PO_4^{3-} were found to be $(1.0 \pm 0.15) \times 10^4\ M^{-1}$ and $(1.8 \pm 0.1) \times 10^3\ M^{-1}$, respectively. The lower binding constant with receptor **3b** reflects the importance of the hydrogen bond donor from benzimidazole moiety. Thus, if the receptor system is devoid of hydrogen bond donors from benzimidazole moiety, the receptor recognition capacity suffers appreciably. A number of species of the host–guest complex were examined with Stern–Volmer plot (Fig. S4 in Supplementary data). The straight line plot of **3a** for PO_4^{3-} confirms the formation of one type of complex between **3a** and PO_4^{3-}.[31] The stoichiometries of the PO_4^{3-} complexes formed with both **3a** and **3b** were determined by Job's plot (Fig. 3),[32] and they were found to be 1:1 for both.

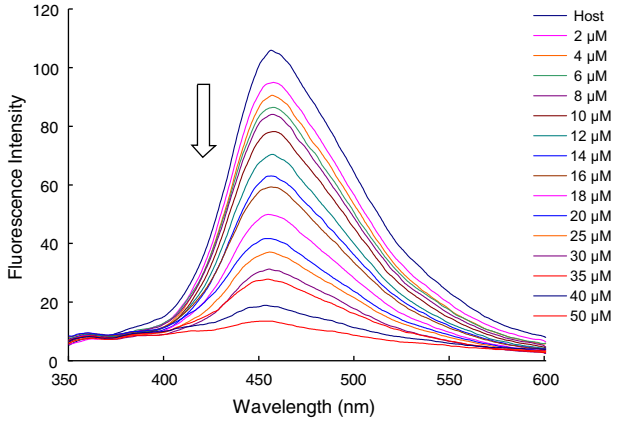


Fig. 2. Fluorescence spectra changes of receptor **3a** (10 μM) upon the addition of sodium phosphate (0–50 μM) in DMSO/H_2O (8:2, v/v).

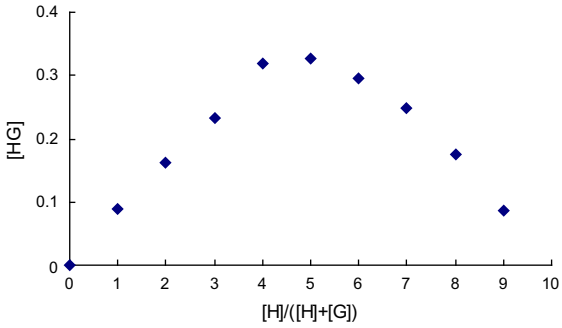


Fig. 3. Job's plot between receptor **3a** and phosphate. The concentration of [HG] was calculated by the equation $[HG] = \Delta I / I_0 \times [H]$.

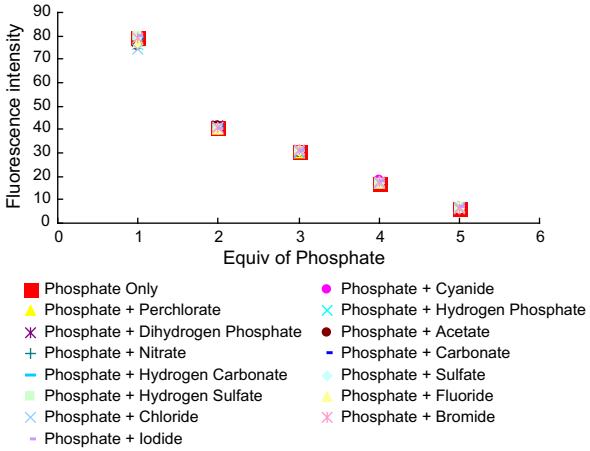


Fig. 4. Estimation of phosphate in the presence of other anions in DMSO/H_2O (8:2, v/v).

Experiments were carried out to estimate PO_4^{3-} in the competitive medium of other anions, which may interfere in estimation (Fig. 4). For these studies, a number of solutions were prepared containing receptor **3a**, different amounts of PO_4^{3-}, and other anions having a concentration five times greater than the concentration of PO_4^{3-} in DMSO/H_2O (8:2, v/v). The anions such as F^-, Cl^-, Br^-, I^-, CN^-, ClO_4^-, $H_2PO_4^-$, HPO_4^{2-}, AcO^-, NO_2^-, CO_3^{2-}, HCO_3^-, SO_4^{2-}, and HSO_4^- were selected as the competitor anions. The fluorescence intensity of each solution was measured at 457 nm, and the results showed that the intensity of fluorescence was almost identical to that obtained in the absence of anions with the exception of some anions, which caused a small interference when the sample contained a small amount of PO_4^{3-}.

To understand the character of the receptor–anion interactions, the possible intramolecular hydrogen bonding of receptors **3a**–**b** and intermolecular bonding between **3a** and PO_4^{3-} were determined from MacroModel calculations (Fig. 5).[33] In both receptors **3a**–**b**, one NH of thiourea moiety undergoes intramolecular hydrogen bonding with the nitrogen of benzimidazole/benzthiazole by adopting a six membered ring structure. This intramolecular hydrogen bonding was further confirmed by comparing the position

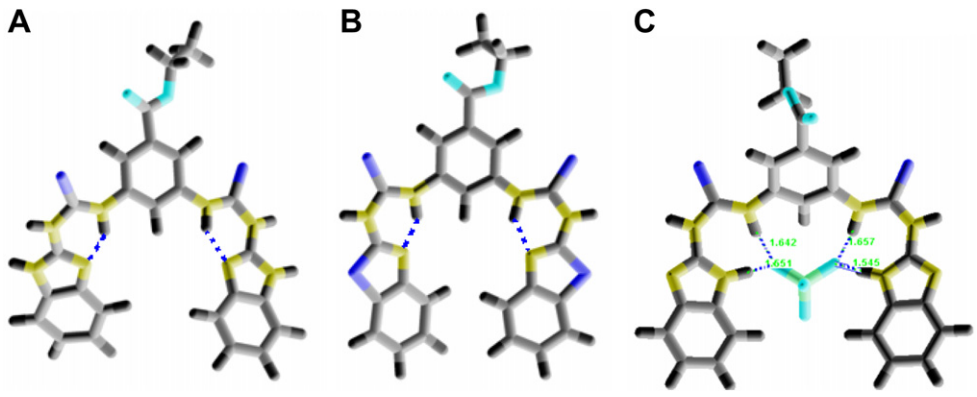


Fig. 5. Energy minimized structure of (**A**) receptor **3a**, (**B**) **3b** and (**C**) the complex formed between receptor **3a** and PO_4^{3-} obtained by MacroModel calculation.

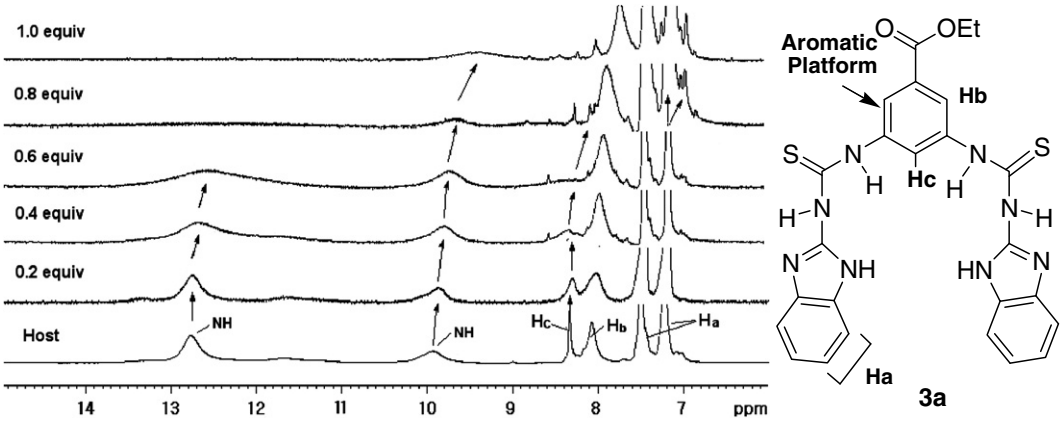


Fig. 6. Family of ^{1}H NMR spectra of receptor **3a** on the addition of tri(tetrabutyl-ammonium)phosphate in DMSO-d_6.

of NH signal in the ^{1}H NMR spectra of pure **3a** and **3b**. These NH signals are at 12.74 ppm and 13.09 ppm for **3a** and **3b**, respectively. The downfield shift of NH in **3b** is due to higher electron density on the benzthiazole N, and hence exhibits stronger intramolecular hydrogen bonding in **3b**. The structure of the complex formed between **3a** and PO_4^{3-} revealed the intermolecular hydrogen bonding through one NH of benzimidazole and one NH of thiourea moiety. To clarify these types of hydrogen bonding, a ^{1}H NMR titration experiment was performed. The family of ^{1}H NMR spectra of receptor **3a** upon the addition of increasing amounts of $(Bu_4N)_3PO_4$ in DMSO-d_6 is shown in Figure 6. The NH signal of pure receptor **3a** at 9.87 ppm shifted to 9.43 ppm upon the addition of 1.0 equiv of PO_4^{3-} salt. The signals of aromatic platform were also shifted drastically. Especially, the signal of proton (H_c) hanging into the pseudocavity of receptor **3a** at 8.29 ppm shifted to 7.75 ppm. The aromatic signals of benzimidazole were split into two signals upon the addition of 0.8 equiv of PO_4^{3-}. This splitting shows that the migration of hydrogen, which normally occurs easily with free benzimidazole tautomers, is hindered by the phosphate that bounded with benzimidazole. The NH signals at 12.74 ppm specify that these protons are in the tone of strong hydrogen bonding. Upon phosphate complexation, the signals of these protons underwent an upfield shift. These upfield shifts confirm that the magnitude of hydrogen bonding in the complex is lesser than the one prevailed in the pure host. These signals also get broadened during the course of titration. These concurrent shifts in the proton signals of benzimidazole and of aromatic platform lead us to conclude that PO_4^{3-} binds in the tetrahedral environment of the receptor pseudocavity.

In conclusion, we synthesized a benzimidazole and thiourea conjugated fluorescent anion receptor. The receptor employs the hydrogen bond donor array for anion with tetrahedral geometry. The receptor is sensitive for 2.0 μM concentration of phosphate,[34] and the receptor acts as a selective sensor for PO_4^{3-} even in the presence of other anions in 20% aqueous DMSO.

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Supplementary data

Supplementary data (Spectroscopic measurements) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.01.107.

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25. *Synthesis of ethyl 3,5-bis(3-(1H-benzo[d]imidazol-2-yl)thioureido)-benzoate* (**3a**): A mixture of ethyl 3,5-diisothiocyanatobenzoate (300 mg, 1.14 mmol) and 2-aminobenzimidazole (340 mg, 2.56 mmol) in dry DMF was maintained at 100 °C for 24 h under inert atmosphere of argon. Upon the completion of reaction, the content of reaction mixture was cooled to room temperature. Solid material was separated out. The solid material was washed with dichloromethane and ether. The recrystallization from methanol afforded a light yellow product (420 mg, 70%): mp 217–218 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.74 (s, 4H, NH), 9.87 (s, 2H, NH), 8.29 (s, 1H, Ar), 8.04 (s, 2H, Ar) 7.49–7.46 (m, 4H, Ar), 7.23–7.20 (m, 4H, Ar), 4.43–4.29 (m, 2H, CH_2), 1.33 (t, 3H, CH_3, $J = 7.2$ Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 165.4, 155.1, 139.8, 138.5, 129.9, 126.7, 123.5, 122.4, 119.04, 111.5, 60.9, 14.1. HRMS (FAB): $m/z = 531.1385$ (calcd $m/z = 531.1385$ for $M+H^+$).
26. *Synthesis of ethyl 3,5-bis(3-(benzo[d]thiazol-2-yl)thioureido)benzoate* (**3b**): Compound **3b** was synthesized by the same procedure as was adopted for the synthesis of compound **3a** except that 2-aminobenzthiazole (375 mg, 2.5 mmol) was used instead of 2-aminobenzimidazole. The pure lemon yellow product **3b** was obtained (430 mg, 67%): mp 208–209 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 13.09 (s, 2H, NH), 10.79 (s, 2H, NH), 8.30 (s, 1H, Ar), 8.07 (s, 2H, Ar) 7.85 (d, 2H, Ar, $J = 7.2$ Hz), 7.42–7.40 (m, 4H, Ar), 7.29–7.25 (m, 2H, Ar), 4.36–4.30 (m, 2H, CH_2), 1.33 (t, 3H, CH_3, $J = 6.8$ Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 166.2, 165.4, 152.8, 139.8, 130.9, 129.9, 126.7, 125.4, 123.5, 122.4, 120.8, 120.7, 117.7, 60.9, 14.12. HRMS (FAB): $m/z = 565.0609$ (calcd $m/z = 565.0609$ for $M+H^+$).
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