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Precise Tuning of Cationic Cyclophanes toward Highly Selective Fluorogenic Recognition of Specific Biophosphate Anions

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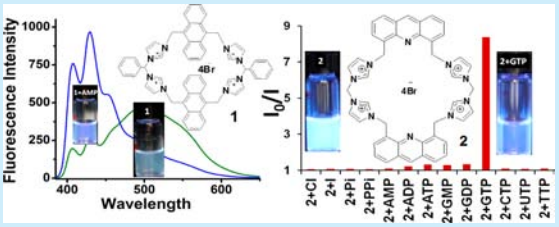
Supporting Information

ABSTRACT: Cationic cyclophanes with bridging and spacer groups possess well-organized semirigid cavities and are able to encapsulate and stabilize anionic species through diverse molecular interactions. We highlight the precise tuning of functionalized cyclophanes toward selective recognition of AMP, GTP, and pyrophosphate (PPi) using fluorescence, NMR spectroscopy, and density functional theory (DFT).



Molecular recognition, in its broadest sense, refers to the interaction between host and guest molecules and is generally governed by noncovalent forces.[1] Among various types of noncovalent interactions, H-bonding is a proven, effective way of binding, but other forces including $\pi-\pi$, cation$-\pi$, and $\pi^{+}-\pi$ interactions also have their own importance in molecular recognition.[2] The importance of molecular recognition in various biological processes has helped chemists to design molecular systems with fascinating properties.[3] Anions are ubiquitous in nature and play a significant role in numerous biological functions; thus considerable efforts have been devoted to develop detection methods for anions. Fluorescent sensors are powerful tools to monitor biologically relevant species because of the simplicity and high sensitivity of fluorescence.[4] The design of a suitable receptor for selective discrimination of a specific biomolecular anion in the presence of interfering analytes requires a complete understanding of all the structural features of the receptor and guest anion as well as the interactions favored by the anion of interest.[5] Though noncovalent interactions are weaker than covalent ones, the cooperative effect of such interactions aids to strengthen selective recognition. Thus, receptors having specific binding motifs, capable of specific noncovalent interactions, are expected to act as selective molecular probes.[6]

Fluorescent cyclophanes with defined cavities have proven to be a good choice for molecular recognition, drug delivery, and anion sensing for biologically important anionic moieties.[7] Fluorescent cyclophanes consist of fluorophore units linked together through suitable bridging and spacer groups.[8] They have unique geometries and give a quick response in fluorescence through bridges. The cavity size as well as the fluorogenic properties exhibited by these systems could be varied by altering the fluorophore moiety, the bridging unit, and the spacer groups. Then, such cyclic systems are capable of encapsulating and stabilizing guest molecules in defined cavities through noncovalent interactions.[9] Water-soluble cyclic systems having ionic H-bonding could be a good choice to develop chemosensors for selective discrimination of biologically important targets as compared to open systems.[10] However, cyclic systems are very hydrophobic and have low solubility in water.[11] Thus, it is difficult to synthesize cyclic systems that are soluble under physiological conditions and undergo specific interactions with anionic moieties.[12]

The molecular recognition by cyclic probes is generally based on neutral or charged H-bonds. Charged H-bond interactions for anions occur by amide, pyrrole, urea, ammonium, and guanidinium groups through the N$-$H$\cdots$A^{-} interaction. It has been observed that positively charged imidazolium functionalized receptors are fairly soluble in water and capable of binding with anions through ionic (C$-$H)$^{+}\cdots$A^{-} interactions.[8] Thus, utilization of the ionic H-bonding by the designed positively charged imidazolium based receptors with specific cavity sizes would enhance the binding affinity for selective recognition of anions.[13−15] There have been a number of reports regarding cyclophane systems that recognize biomolecular anions under physiological conditions.[11,12] However, to the best of our knowledge there is no report that describes the significance of different structural functionalities of cyclopane systems for selective recognition of biomolecular anionic species. In this study, we demonstrate that a slight variation of the fluorophore or spacer group created a great effect on the sensitivity and selectivity of receptors toward recognition of important biomolecular anions. Herein, we designed three different water-soluble imidazolium based probes (**1**−**3**) by careful tuning of fluorophores and spacer groups (Figure 1).

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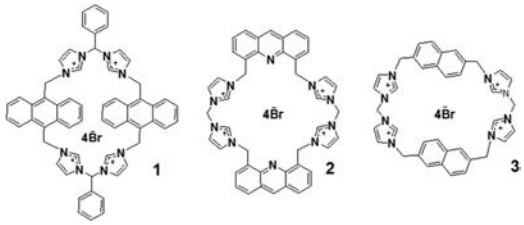

Figure 1. Probes **1**, **2**, and **3**.

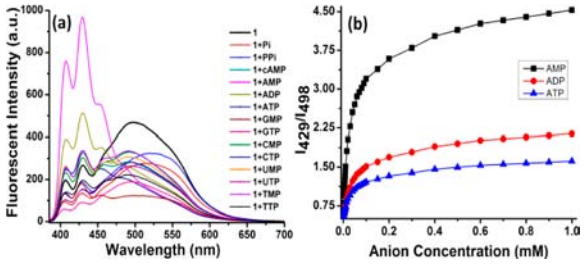

Figure 2. (a) Fluorescent emission changes of **1** (10 μM) upon addition of *n*-tetrabutylammomonium salts (100 equiv) of $H_2PO_4^-$, PPi, and sodium salts (10 equiv) of AMP, cAMP, ADP, ATP, GMP, GTP, CMP, CTP, UMP, UTP, TMP, and TTP (slit width = 5 nm; λ_{exc} = 365 nm). (b) Ratiometric calibration curve I_{429}/I_{498} as a function of the concentration of AMP, ADP, and ATP.

It remained a challenging task to design and synthesize a particular receptor which selectively recognizes a monophosphate nucleotide in comparison to di- or triphosphate analogues. We have designed anthracene based probe **1** having imidazolium units with bridging benzyl moieties, for fluorogenic sensing of AMP. Probe **1** gives a selective fluorescence enhancement response to AMP with subsequent quenching in the excimer in comparison to other nucleosides including ADP and ATP under physiological conditions. The adenine base in AMP acts as a fluorescence enhancer, resulting in fluorescence enhancement of probe **1**. The bridging benzyl group of probe **1** plays a major role in sensing of AMP by providing an additional $\pi-\pi$ interaction to the adenine moiety of AMP. To the best of our knowledge probe **1** is the first fluorogenic probe for selective sensing of AMP in aqueous media at physiological pH. Probe **2** has the acridine moiety as a fluorophore unit, and the imidazolium moiety as a binding site for GTP recognition. The electron-withdrawing nitrogen heteroatom in acridine assists in strengthening H-bonding with the guanine base of GTP and gave selective fluorescence quenching at physiological pH. Probe **2** shows ~87% fluorescent quenching with GTP, which makes this probe better than those in previous reports.[13,16] Probe **3** has a naphthalene–imidazolium based cyclic structure which senses selectively pyrophosphate (PPi) in the presence of other inorganic or biomolecular anions via excimer formation due to $\pi-\pi$ interactions[17] between fluorophore moieties.[18]

Initially, 1-(1*H*-imidazol-1-ylmethyl)-1*H*-imidazol,[19] 4,5-bis-(bromomethyl)acridine,[20] and 1,1′-(phenylmethylene)-bis(1*H*-imidazole) were synthesized. The synthesis of probes **1**–**3** was carried out with some modifications of previous procedures,[14b,15c,16d] and the details are described in the Supporting Information (SI). Fluorescence studies were performed in aqueous solution at pH 7.4 (0.01 M HEPES buffer). First, probe **1** was monitored for anions recognition studies. Visual features show blue fluorescence enhancement upon the addition of AMP (Figure S20). Probe **1** displayed monomer (430 nm)

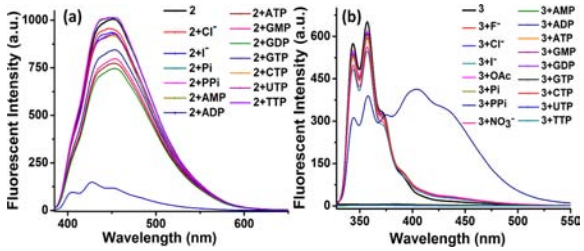

Figure 3. (a) Fluorescent emission changes of **2** (10 μM) upon addition of TBA salts (10 equiv) of Cl^-, I^-, Pi, PPi, and sodium salts (10 equiv) of AMP, ADP, ATP, GMP, GDP, GTP, CTP, UTP, TTP (slit width = 5 nm; λ_{exc} = 365 nm). (b) Fluorescence spectra of **3** (10 μM) upon addition of the sodium salts of AMP, ADP, ATP, GMP, GDP, GTP, CTP, TTP, UTP and TBA salts of F^-, Cl^-, I^-, $CH_3CO_2^-$, Pi, PPi, NO_3^-(10 equiv) (slit width = 5 nm; λ_{exc} = 290 nm).

and excimer (495 nm) peaks.[21] The concentration independent changes in the monomer-to-excimer (I_{429}/I_{498}) ratio indicate intramolecular excimer formation (Figure S21). The fluorescence spectrum of **1** with anions is in Figure 2a which shows the maximum fluorescence enhancement at the monomer upon the addition of AMP. Other anions gave more or less fluorescence quenching in monomer and excimer peaks (Figure 2a). This shows unique fluorescence discrimination of AMP from the structurally similar nucleoside phosphates (Figure S25). Figures S22–S24 show the fluorescence titrations of **1** with AMP, ADP, and ATP (0–100 equiv). There was a significant fluorescence enhancement in monomer and quenching in excimer upon the addition of AMP to **1**, a moderate change in the case of ADP, and a very small change in ATP. The ratio for AMP (I_{429}/I_{498}) is large enough to discriminate ADP and ATP (Figure S25), and most importantly it can serve as the calibration curve for the detection of AMP (Figure 2b).

Visual inspection of color changes in the aqueous solution of **2** shows selective sensing of GTP through fluorescence quenching (Figure S30). The emission spectra of **2** in aqueous solution show broad fluorescence (Figure S32) which indicates strong H-bonding with water molecules.[22] Probe **2** displayed minor changes in fluorescence upon the addition of anions except GTP (Figure 3a). However, GTP displayed the most significant fluorescence quenching with probe **2** (Figure S31). Fluorescence spectra of probe **2** illustrates the quenching effect upon the addition of GTP, which might be due to strong interactions with probe **2** in the competitive solvent (Figures 3a and S33).

The fluorescence spectra of probe **3** show the structured naphthalene monomer emission centered at 356 nm (Figure 3b). Probe **3** displayed decreased fluorescence with inorganic anions F^-, Cl^-, I^-, $CH_3CO_2^-$, $H_2PO_4^-$, and NO_3^-. Probe **3** showed significant fluorescence quenching with nucleobases AMP, ADP, ATP, GMP, GDP, GTP, CTP, TTP, and UTP because of the enhanced photoinduced electron transfer (PET) by the introduction of the electron-rich anionic moieties. On the other hand, a unique change was observed in the emission spectrum with PPi. The addition of PPi induced not only the quenching effect but also a structureless band with an emission maximum at 407 nm. Figures 3b shows the fluorescence quenching at 356 nm and the formation of a unique structureless band with increasing concentration of PPi at 407 nm. The new peak at 407 nm can be attributed to excimer formation because no significant changes were observed in UV absorption (Figure S46) even by adding a large amount of PPi, excluding a charge-transfer mechanism.

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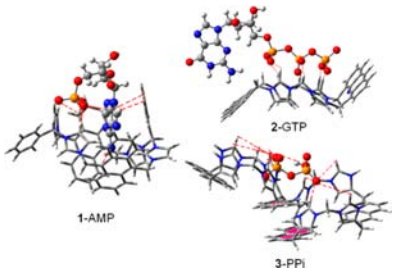

Figure 4. Optimized structures of **1**-AMP and **3**-PPi using DFT(B97-D3/TZVPP) and **2**-GTP using DFTB.

The competitive binding experiments were conducted for probe **1** in 100 equiv of Pi, PPi, cAMP, ADP, ATP, GMP, GTP, CMP, CTP, UMP, UTP, TMP, or TTP, and the fluorescence intensity for AMP was found to be unaffected (Figure S26). The presence of 10 equiv of GTP was unaffected by the addition of 10 equiv of competing anions for probe **2** (Figure S35). In the case of probe **3** it was found that the fluorescence intensity of **3** in the presence of 10 equiv of PPi was unaffected by the addition of 10 equiv of competing anions (Figure S37). The calculated binding stoichiometries, binding constants,[23] and detection limits[24] for probes **1**−**3** with selected anions are summarized in Table S1. The absorption spectra of **1** (Figure S44) show the characteristic absorption maximum at 376 nm due to the anthracene moiety. The maximum absorption increase was observed with AMP (100 equiv), and the maximum absorption decrease was found with GTP (100 equiv). The absorption spectra of **2** (Figure S45) show the characteristic absorption maximum at 356 nm due to the acridine moiety. The absorption spectra of **2** with GTP (10 equiv) show a slight increase in absorption with a distinguished red shift by 3 nm. Moreover quantum yields of probes **1**, **2**, and **3** are calculated to be 0.27, 0.13, and 0.24, respectively (see SI).

Probe **1** shows fluorescence quenching in the excimer and enhancement in the monomer during complexation with AMP (1:1 (**1**-AMP)) through strong ionic interactions. In the complex the $\pi-\pi$ interaction between anthracene moieties of probe **1** might become weak and develop strong interactions with the adenine moiety of AMP through the H$-\pi$ interaction which might result in fluorescence enhancement in the monomer and quenching in the excimer (Figure 4). A remarkable fluorescence quenching in probe **2** was observed during complexation with GTP (1:1 (**2**-GTP)) through ionic interactions. We propose that such strong fluorescence quenching might be due to strong H-bonding between the $-NH_2$ group of the guanine moiety and heteroatom (N) in the acridine moiety of probe **2** in a perpendicular edge-to-face orientation (Figure 4). While the binding mode of probe **3** is 2:1 for PPi, the characteristic fluorescence response indicates different types of interaction. As in Figure 4, we postulate that probe **3** bound PPi through the ionic interaction of the imidazolium protons $(C-H)^+$ with the O atoms of phosphate groups. The excimer emission exhibited by **3**-PPi originates from the intermolecular $\pi-\pi$ interaction between fluorophore moieties of two molecules of receptor **3**.

To monitor physical interactions of probes (**1**−**3**) with AMP, GTP, and PPi, ^{1}H NMR titration experiments were conducted (Figures S7, S14, S19). 2D NOESY of **1** and **2** with AMP and GTP were also recorded (Figures S6 and S13, respectively) in order to investigate the proposed binding pattern. The addition of AMP to **1** (Figure S7) caused the upfield shift (<0.2 ppm) of imidazolium protons $(C-\mathbf{H}a)^+$ with the quenching effect, upfield shifts of the remaining imidazolium protons $H_{d,g}$ (0.2−0.3 ppm), and peak broadening and upfield shifts of bridged methylene protons $H_{c,h}$ (0.2−0.3 ppm), due to strong ionic binding of the phosphate group of AMP. A slight broadening and upfield shift of anthracene ring protons $H_{b,e}$ (0.1−0.2 ppm) were also observed. There was an upfield shift of protons $H_{i,j}$ (0.2−0.3 ppm) of the adenine moiety. The relatively weak NOE correlation between the H_j proton of adenine and the H_b proton of the anthracene moiety indicate the presence of the adenine moiety close enough to the anthracene moiety to have the H$-\pi$ interaction (Figure S6). The peak splitting and broadening accompanied by upfield shifts of benzene protons H_f (<0.2 ppm) was observed with a subsequent weak NOE signal between benzene protons H_f and the acridine protons $H_{i,j}$, indicating the presence of the acridine moiety of AMP in the vicinity of the benzene ring (Figure S6).

A ^{1}H NMR titration experiment of probe **2** upon interaction with GTP (Figure S14) caused quenching with an upfield shift (<0.2 ppm) of imidazolium protons $(C-\mathbf{H}a)^+$, upfield shifts (<0.1 ppm) of $H_{e,f}$, and broadening with slight upfield shifts of methylene protons $H_{h,i}$ (<0.2 ppm), indicating strong binding of the phosphate groups of GTP with probe **2** (Figure S14). Significant quenching with the upfield shift of acridine proton H_b (<0.2 ppm) was noted which is at the *para* position to nitrogen, indicating H-bonding with the nucleobase of GTP. Also the upfield shifts (<0.2 ppm) of H_j and H_k protons of GTP were observed. Owing to different chemical shifts accompanied by broadening of the H_b proton compared to other acridine protons, the splitting of acridine protons and the absence of an NOE correlation between H_j and acridine (Figure S13) suggest some additional interactions of the nucleic base of GTP with the acridine moiety of **2** in the T-shape. Here, the amino group of the guanine moiety interacts with the heteroatom of fluorophore through H-bonding and, hence, causes effective fluorescence quenching.[13]

A ^{1}H NMR titration experiment of probe **3** upon the addition of PPi (Figure S19) caused quenching, an upfield shift (<0.4 ppm) of the imidazolium protons $(C-\mathbf{H}a)^+$, upfield shifts (<0.2 ppm) of $H_{c,d}$, and peak broadening and splitting of $H_{g,h}$ protons of the methylene groups accompanied by an upfield shift (<0.2 ppm), due to strong binding of the phosphate group of PPi (Figure S19). Splitting and upfield shifts (<0.2 ppm) of the aromatic protons of naphthalene could be attributed to the $\pi-\pi$ interaction between two receptor naphthalene moieties which would be responsible for excimer formation.

Conformational analysis of probes **1**−**3** was carried out to theoretically determine the lowest energy conformers and their binding modes with specific anions. The computational details are in the SI. The strong H$-\pi$ interactions in **1**-AMP and **2**-GTP would cause fluorescence enhancement and quenching at the monomer, respectively. In the binding mode of **1**-AMP, the H2 and $-NH_2$ of the adenine moieties of AMP interact with the anthracene moieties of probe **1** at distances of ∼2.7 and ∼2.8 Å via H$-\pi$ interaction, respectively[13,16] (Figure 4). On the other hand, **2**-GTP shows H$-\pi$ interaction at a distance of ∼2.7 Å between the fluorophore (acridine moiety) and quencher ($-NH_2$), which indicates more conspicuous fluorescence quenching compared with **2**-GDP showing H$-\pi$ at a distance of ∼3.3 Å in **2**-GDP (Figure S48h).[13,16] Moreover, the longer phosphate in GTP makes it bind tighter to **2** (Figure 4). In the binding mode of **3**-PPi, the imidazolium protons in the proximity of PPi interact with the O atoms of the phosphate group at a distance of ∼2.0−2.5 Å. The interactions between PPi and two molecules of **3** via imidazolium protons bring the fluorophore moieties close to each other at a distance of ∼3.0−3.6 Å (Figure

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4) where $\pi-\pi$ interactions[18] become effective, consistent with the experimental ^{1}H NMR data (Figure S19) which show splitting of aromatic protons of cyclophane **3**. Such $\pi-\pi$ interaction between naphthalene moieties would cause excimer formation.

The experiments and calculations reported here provide new insights into the selectivities of novel fluorescent chemosensors which sense AMP, GTP, and PPi through fluorescence enhancement/quenching over other biologically relevant anions in an aqueous solution of physiological pH 7.4. The bridging benzyl moiety of **1** plays a major role in attaining selective fluorescence enhancement/quenching (monomer/excimer) toward AMP by developing additional $\pi-\pi$ interactions along with ionic interactions. Significant and selective fluorescence quenching of **2** for GTP arises from the strengthened H-bonding developed between the $-NH_2$ group of the guanine moiety and N of the acridine moiety in a perpendicular manner, which was assisted by the phosphate−imidazolium ionic H-bonding. In the case of **3**, the intermolecular $\pi-\pi$ interaction develops along with ionic interactions upon complexation with PPi in aqueous solution which results in excimer formation at 407 nm. The H−π, $\pi-\pi$, charged H-bond, and ionic H-bond interactions were confirmed by DFT calculations and NMR spectroscopy.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

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Notes

The authors declare no competing financial interest.

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