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Recent Development of Anion Selective Fluorescent Chemosensors

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Sensing anions via fluorescence changes has been an active research target in recent years due to its simplicity and the high detection limits this approach permits. In this mini review, we cover the recent development of anion selective fluorescent chemosensors reported from our group. We classify the anion selective fluorescent chemosensors according to their topology and structure, which includes urea or thiourea derivatives, imidazolium derivatives, Zn-DPA derivatives and boronic acid modified systems.

Keywords: Anion receptor; Fluorescent chemosensors; Pyrophosphate sensing; Fluoride sensing; Molecular recognition

INTRODUCTION

Anions play an important role in various chemical and biological processes. Accordingly, there has been a great deal of effort devoted to the development of abiotic receptors for anionic species [1–7]. Sensors based on the anion-induced changes in fluorescence are particularly attractive on account of their simplicity and the high detection limit inherent in the method [1–13]. For example, pyrophosphate (PPi) can be a biologically important target because it is the product of ATP hydrolysis under cellular conditions [14]. On the other hand, many common enzymes, such as kinases and phosphatases, produce or consume inorganic phosphate (Pi), which is also related to protein phosphorylation [15]. The detection of an increase or decrease in phosphate concentration in the environment of these enzymes is a common way to monitor the enzyme activity or protein phosphorylation process [16,17]. Also, fluoride ions are biologically important anions because of their role in dental care [18] and the treatment of osteoporosis [19], etc. Accordingly, sensing anions via fluorescence changes has been an active research target in recent years [8–13]. Although there have been a few review papers regarding anion selective receptors [1–7] as well as fluorescent chemosensors for anions [9], a more focused presentation on our recent work involving anion selective fluorescent chemosensors was deemed appropriate in the context of this special issue. With this goal in mind, we have classified our fluorescent chemosensors according to their topological and structural characteristics, which include urea or thiourea derivatives, imidazolium derivatives, Zn-DPA derivatives and boronic acid functionalized systems.

RECEPTORS BEARING UREA OR THIOUREA GROUPS

Our group started to work on anion selective fluorescent sensors in 2002 and reported a relatively simple anthracene derivative bearing two urea groups at the 1,8-position of anthracene (**1**) (Scheme 1) [20]. Receptor **1** displayed a selective fluorescent quenching effect with F^- in acetonitrile-DMSO (9:1, v/v). Selectivity was observed among the halide anions. From fluorescent titrations with fluoride, chloride, bromide and iodide ions, the association constants were calculated to be 71,300, 610, 120 and $30\,M^{-1}$, respectively. The selectivity displayed for fluoride ions was almost 120 fold relative to that seen for chloride ions. Since a photoinduced electron transfer (PET) mechanism involving the benzylic

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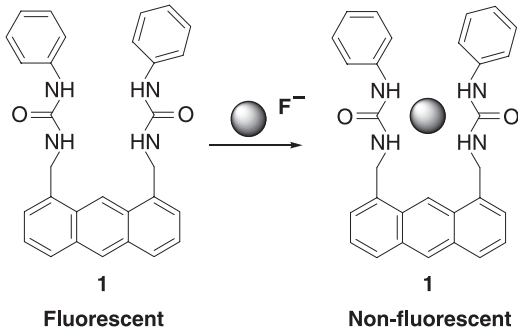

SCHEME 1 Proposed PET mechanism of **1** with fluoride ion.

amide and the anthracene fluorophore was used to monitor this binding, it is further notable that a fluorescent emission change of up to 20 fold was observed in the case of fluoride ions.

In a continuation of this work, we prepared four new urea derivatives and examined the binding properties of these anthracene bearing systems as well as compound **1** using various anions. In this case, the binding processes were monitored using fluorescence, ^{1}H NMR spectroscopy, and *ab initio* calculations [21]. The 1,8-bisurea anthracene derivative (**1**) showed a slightly better binding for dicarboxylates with 1:1 complexation stoichiometry than the 9,10-bisurea anthracene derivative (**4**). Among the dicarboxylates, both 1,8 and 9,10 derivatives showed particularly strong binding to adipate anion. On the other hand, for pyrophosphate, only 1,8-bisurea receptor was found to be particularly selective (i.e., ten times more selective than the 9,10-bisurea receptor). Both 1,8 and 9,10 derivatives were confirmed to form a 1:2 complexation with $H_2PO_4^-$, based on ^{1}H NMR spectroscopic analyses and theoretical investigations. It was also demonstrated that H-bonding, the strain on the connecting chain of the two carboxyl groups, and the ionic solvation energies of the anions in the solvent were three main factors regulating the binding

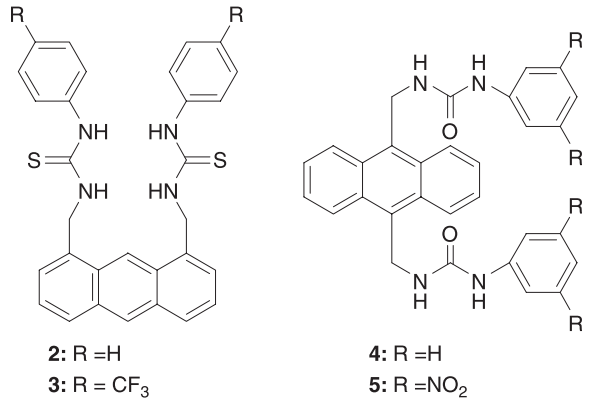

FIGURE 1 Structures of 1,8-bisthiourea and 9,10-bisurea anthracenes **2**–**5**.

behaviour of the different dicarboxylates studied using this serious of anthracene-based bisurea receptors (Fig. 1).

Recently, Lee *et al.* reported [22] a strapped calix[4]pyrrole in which not only four pyrrole hydrogens but also an aromatic hydrogen in a strap form hydrogen bonds with fluoride and chloride ions. These hydrogen bond interactions between aromatic hydrogens and the anions were confirmed by the large downfield shifts of the aromatic hydrogens observed in the ^{1}H NMR spectrum ($\Delta\delta = 0.41$ for F^-, 0.96 for Cl^-) [22]. Also, other examples of receptors in which aromatic hydrogens participate in hydrogen bonding with anions have been reported [22–25]. These reports have led us to pose an interesting question: when urea groups are directly attached to the 1,8-positions of anthracene, will the 9-H proton of anthracene participate in anion binding? With such thought in mind, we developed colorimetric and fluorescent sensors for fluoride and pyrophosphate ions in which two *p*-nitrophenylurea groups (**6**) or two phenylurea groups (**7**) are attached to the 1 and 8-positions of anthracene (Fig. 2) [26]. The resulting colorimetric sensors display unique color changes upon the addition of fluoride ions in DMSO. Furthermore, as expected hydrogen bonds between the 9-H of anthracene and both fluoride and pyrophosphate ions are inferred based on ^{1}H NMR spectroscopic experiments. When tetrabutylammonium chloride was added to a solution of **7**, large downfield shifts in the amide N—H bonds as well as 9-H of anthracene moiety were observed. On the other hand, when tris (tetrabutylammonium)hydrogenpyrophosphate or tetrabutylammonium fluoride were added to the solution of **7** in DMSO-d_6, four amide N—H signals as well as the 9-H resonance in the anthracene moiety, became severely broadened. From the temperature dependent ^{1}H NMR spectroscopic studies in DMF-d_6, it was found that the amide peaks as well as the 9-H signal of the anthracene moiety of host **7** begin to appear concurrently at -5°C in the presence of 7 equivalents of pyrophosphate.

We also produced a system wherein the thiourea group directly on the 9-position of an anthracene moiety (Fig. 3) [27]. X-ray crystal structures of these anthracene derivatives (**8**–**10**) were also reported. These chemosensors display selective ratiometric changes in their fluorescence spectra upon the addition of fluoride ion. Upon the addition of fluoride ion, a new charge transfer peak at 568 nm was observed (Fig. 3). The blue fluorescence changed to an orange fluorescence upon the addition of fluoride ion. This new peak can also be attributed to the intramolecular charge transfer (ICT) process. We also observed new peaks with a large red-shift upon the addition of fluoride in the corresponding UV spectra.

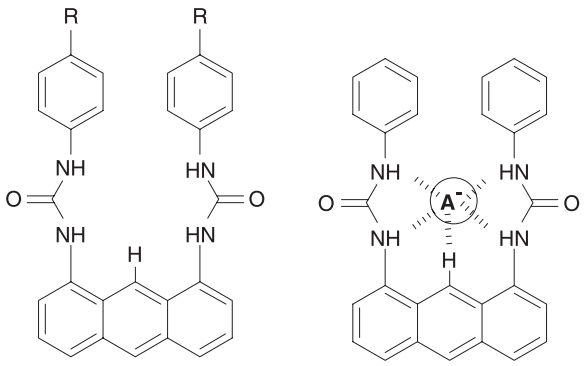

FIGURE 2 Structures of **6** and **7** and a proposed general binding mode for the interaction of an anion with receptor **7**.

IMIDAZOLIUM RECEPTORS

The imidazolium group can interact strongly with anions through $(C{-}H)^{+}{-}X^{-}$ type ionic hydrogen bonding as well as likely dominant charge–charge electrostatic interactions. Such binding modes stand in contrast to well-known neutral anion receptors that rely on hydrogen bonding for anion recognition, including amide, pyrrole, urea, etc [1].

The first examples of imidazolium units as molecular recognition motifs for anions were reported by Alcalde *et al.* [28], Sato *et al.* [29] and Kim *et al.* [30] a few years ago. Inspired by these reports, we started to utilize imidazolium moieties to generate anion selective fluorescent receptors. Two imidazolium moeities were first immobilized on the 1,8-positions of the chemosensor (**11**) (Fig. 4), and a unique feature of the binding mode was predicted based on *ab initio* calculations [31]. 1,8-Bis-imidazolium anthracene **11** effectively and selectively recognizes the biologically important $H_2PO_4^-$ ion over other anions, such as I^-, Br^- and Cl^- in acetonitrile. In contradiction to other previous imidazolium receptors, with these new systems anion binding can be easily monitored *via* fluorescence quenching effects.

We further demonstrated that the selectivity of these imidazolium receptors towards anions can be controlled by changing the topology of the binding site (e.g., enhancement of rigidity). Compared to host **11**, the greater rigidity in host **12** enhances the binding selectivity for $H_2PO_4^-$ over F^- [32]. Competitive binding studies involving $H_2PO_4^-$ and F^- with **12** monitoring the fluorescent changes demonstrated that fluoride did not interfere with $H_2PO_4^-$ and binding of $H_2PO_4^-$ up to 1.5 molar equivalents of F^-. This stands in contrast with the extremely strong interference seen in the case of host **11**.

9,10-Anthracene and binaphthyl derivatives (**13** and **14**) bearing two imidazolium moieties were also studied as fluorescent chemosensors for anions [33]. The anthracene and binaphthalene based receptors display particularly selective binding for pyrophosphate and phosphate among the various anions studied. Anthracene and binaphthalene based receptors (**11**–**14**) showed PET behaviour upon anion recognition, with $H_2PO_4^-$ and $HP_2O_7^{3-}$ acting to quench the emission effectively (~95%). Among the series of hosts (**11**–**14**) examined, a dimer host (**12**) displays a largest binding constant with $HP_2O_7^{3-}$, a finding that we interpret in terms of the preorganized rigid binding pocket playing an important role in the binding of $HP_2O_7^{3-}$. The binding constants of **11**–**14** with $HP_2O_7^{3-}$ are on the order of **12** > **14** ≅ **11** > **13**, which is consistent with our assumption. From these results, we conclude that the selectivity of these imidazolium receptors against anions can be controlled by the topology of the binding site.

Since the binding properties of previous imidazolium receptors were examined in organic solvents, ammonium groups were introduced to the receptor to enhance the solubility in aqueous solution (Fig. 5). A novel water-soluble imidazolium anthracene derivative (**15**) not only differentiates between the structurally similar compounds GTP and ATP, but

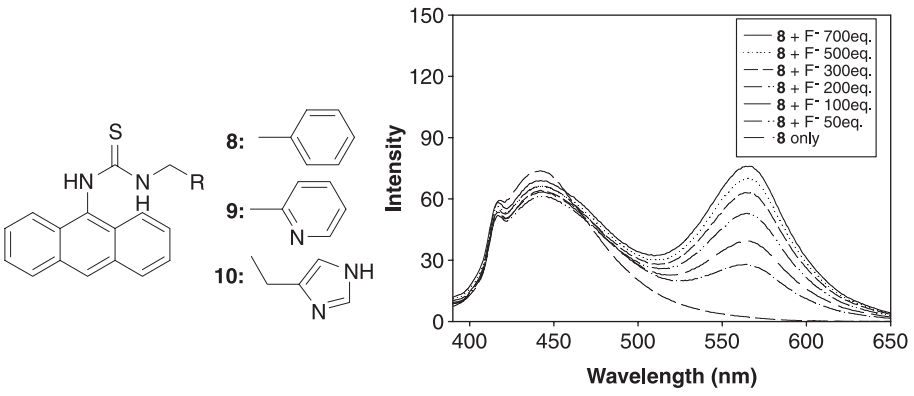

FIGURE 3 Structures of compounds **8**–**10** and the fluorescent changes of **8** (3 μM) seen upon the addition of tetrabutylammonium fluoride in DMSO.

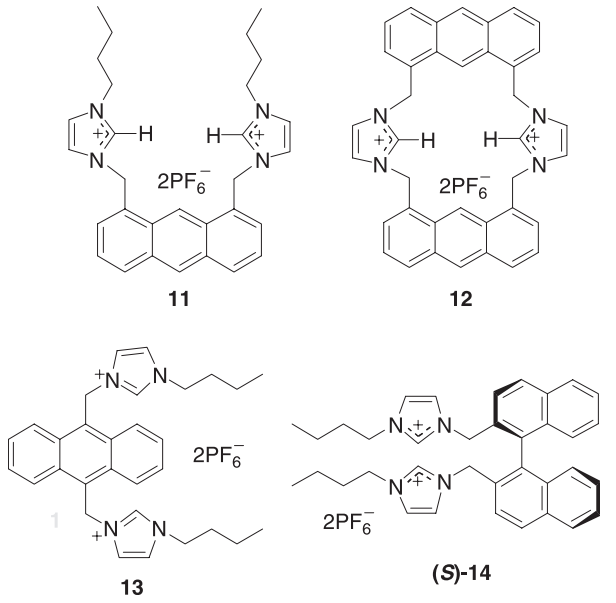

FIGURE 4 Structures of imidazolium receptors **11**–**14**.

also acts as a potential fluorescent chemosensor for GTP in 100% aqueous solution (pH = 7.4, 10 mM HEPES) [34]. This new fluorescent chemosensor senses GTP by chelation-enhanced fluorescence quenching (CHEQ), whereas it displayed a chelation-enhanced fluorescence (CHEF) effect for ATP, ADP and AMP. This was the first example of fluorescent chemosensor which can sense GTP in 100% aqueous solution. From the fluorescent titrations the association constants for GTP, ATP, ADP and AMP were determined to be 87000, 15000, 610 and 120 M^{-1}, respectively.

Recently, we attached imidazolium groups to various supramolecular systems, such as a cavitand moiety (Fig. 6). The binding properties of resulting tetraimidazolium cavitand (**16**) towards various anions, including dicarboxylates, were investigated using the ^{1}H NMR spectroscopic experiments

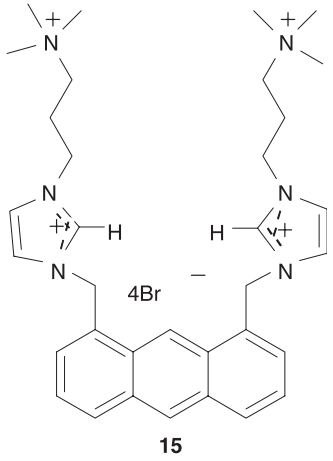

FIGURE 5 Structure of the water-soluble imidazolium receptor **15**.

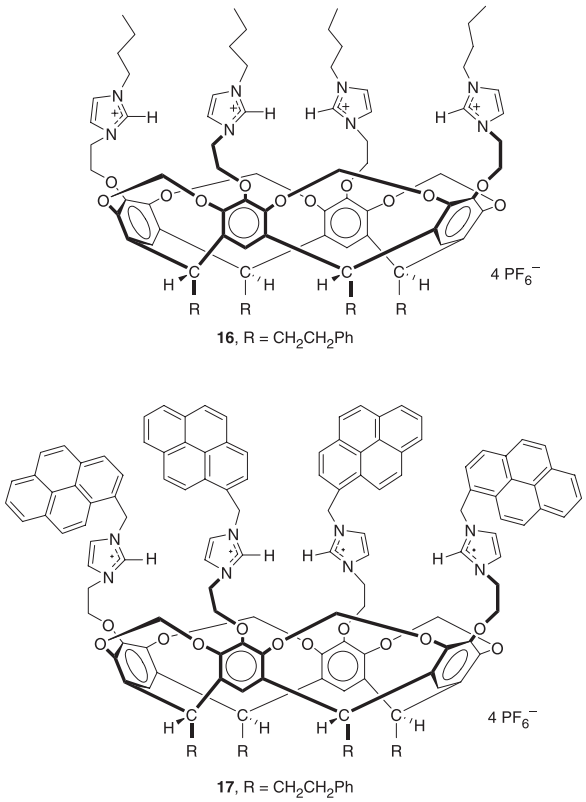

FIGURE 6 Structures of imidazolium cavitands **16** and **17**.

carried out in acetonitrile-d_3 [35]. The tetrabutylammonium salts of various anions, such as 1,3-adamantanedicarboxylate, adipate, terephthalate, 1,4-phenylenediacetate, succinate, acetate, Cl^-, Br^- and I^- were used in this binding study. As expected, cavitand **16** displayed 1:1 binding stoichiometries with dicarboxylates, while 1:2 complex formation was confirmed in the case of acetate, Cl^-, and Br^-. In particular, among the anions examined, cavitand **16** displayed a highly selective binding for bis(tetrabutylammonium) 1,4-phenylenediacetate.

A fluorescent cavitand derivative bearing four imidazolium groups as well as four pyrene groups was synthesized and studied as a fluorescent receptor for GTP (Fig. 6) [36]. Since the host in question, **17**, contains pyrene groups, the binding properties towards various anions were investigated using the fluorescence experiments. Compound **17** displayed a large CHEQ (chelation enhanced fluorescence quenching) effect with GTP in DMSO/20 mM aqueous HEPES buffer at pH 7.4 (6:4, v/v), even though **17** also displayed relatively small CHEF effects for ATP, CTP, and ADP. There were almost no fluorescent changes even when 100 equivalents of pyrophosphate and $H_2PO_4^-$ were added. From associated fluorescent titrations, the association constants for GTP, ATP and CTP were calculated as 73800, 14040, and 7700 M^{-1}, respectively.

RECEPTORS BEARING ZN-DPA MOIETIES AS BINDING SITES

So far, the utilization of metal ion complex as a binding site for PPi has proved to be one of the most successful strategies. This is because the strong binding affinity between metal ion and PPi makes it possible to detect PPi in 100% aqueous solution [37–42]. Recently, we reported that complex **18**·2Zn acts as a fluorescent and colorimetric sensor for pyrophosphate at pH 7.4 (Fig. 7) [43]. Complex **18**·2Zn displays unique fluorescent changes only with PPi under conditions where HSO_4^-, CH_3COO^-, I^-, Br^-, Cl^-, F^-, Pi, and PPi were added. The emission maximum of complex **18**·2Zn gradually shifted from 523 nm to 534 nm upon the addition of PPi, while a fluorescence enhancement (~150%) was observed (Fig. 7). From fluorescence titrations, the association constant of **18**·2Zn complex was observed to be 98400 M^{-1}. However, there was almost no change in both the λ_{max} and the fluorescence intensity when excess Pi was added to **18**·2Zn. The addition of pyrophosphate induced ring opening of the lactone moiety through an interaction between Zn^{2+} and pyrophosphate that may interrupt the interaction between Zn^{2+} and the phenolate moiety. A pink color change was also observed upon the addition of pyrophosphate, which a characteristic change associated with lactone ring opening.

We also reported a new acridine-DAP-Zn complex (**19**·2Zn), which displays different complex responses when challenged with pyrophosphate or phosphate in 100% aqueous solution [44]. Complex **19**·2Zn displays a selective CHEF (chelation enhanced fluorescence) effect with Pi and a selective CHEQ (chelation enhanced fluorescence quenching) effect with PPi in 100% aqueous solution, at least among the various anions examined (Fig. 8). From

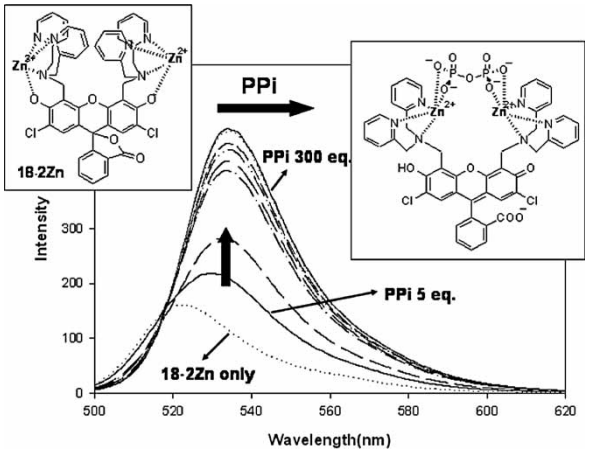

FIGURE 7 Fluorescent changes observed for complex **18**·2Zn (1 μM) upon the addition of PPi at pH 7.4 (20 mM HEPES) and a proposed binding mode for the complex formed between **18**·2Zn and PPi.

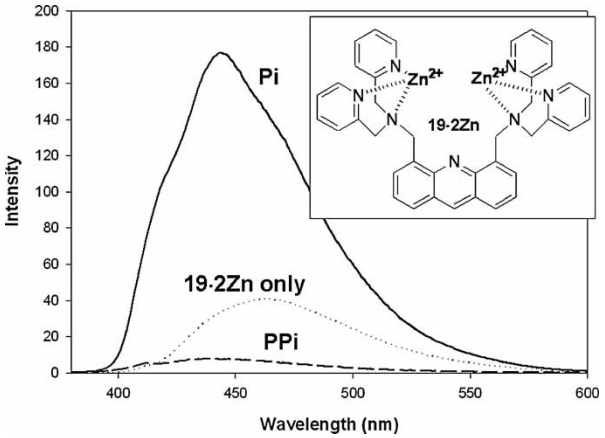

FIGURE 8 Fluorescent changes seen for complex **19**·2Zn (3 μM) upon the addition of PPi and Pi (100 eq.) at pH 7.4 (10 mM HEPES).

fluorescent titrations, association constants for PPi and Pi were calculated as 4.85×10^7 and 9.36×10^4 M^{-1}, respectively. The large CHEF effect with Pi can be attributed to the additional hydrogen bonding between nitrogen atom on the acridine ring and hydrogen atoms of Pi.

RECEPTORS BEARING BORONIC ACID GROUPS

During last decade, boronic acid derivatives have been actively investigated as fluorescent receptors for sugars, such as glucose, fructose, etc [45]. On the other hand, there have been relatively few reports regarding fluoride ion detection based on what is regarded as being a unique fluoride-boron interaction [46–49]. Recently, we prepared a new fluorescein derivative (**20**) bearing a boronic acid group (Fig. 9) [50]. The title compound, the first of its class, displayed a selective fluorescent enhancement when treated with fluoride ion, but not other halide ions in acetonitrile. Compound **20** is characterized by a unique boronate species that is derived from the boronic acid and the phenolate of the fluorescein moiety, a motif that was confirmed by a single crystal X-ray crystal structure. In addition, the interaction between boron and nitrogen was confirmed by this X-ray crystal structure. The relatively weak interaction between the benzylic nitrogen atom and the boron centre is attributed to the moderate fluorescence emission of **18** seen prior to adding anions. Upon the addition of fluoride ion, a fluoride adduct of compound **18** can be stabilized by additional hydrogen bonding interaction involving the proton in the phenol moiety. The phenolic hydrogen can make a strong hydrogen bond with fluoride as can the proton of the benzylic amine. This blocks any PET process, resulting in fluorescence enhancement as proposed in Fig. 8. The chemical shift as well as a

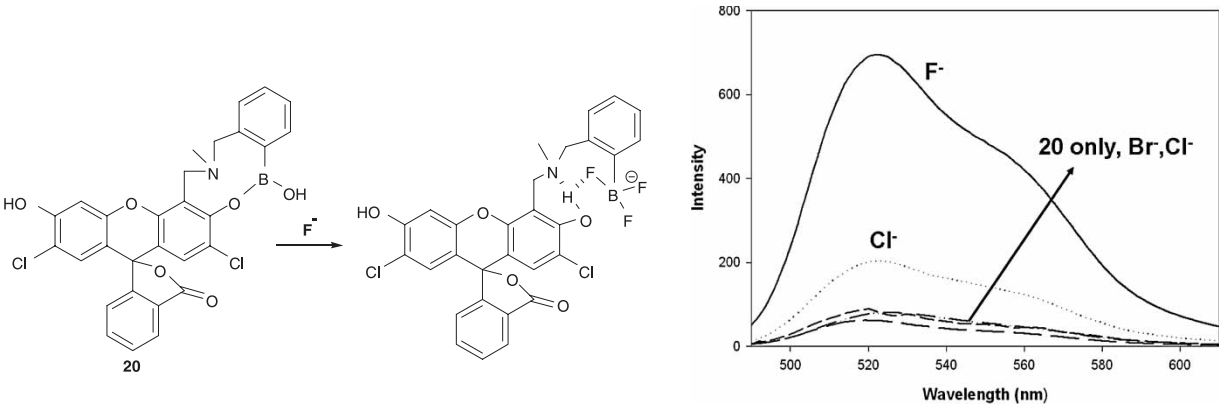

FIGURE 9 Proposed binding mode for the complex formed between compound **20** and fluoride ion. Also shown are the fluorescent changes of **20** (3 μM) upon the addition of halide anions (100 equiv.) in acetonitrile-MeOH (9:1, v/v).

clear quartet in the ^{11}B NMR spectrum of this species supports the formation of a ternary complex, as well as the presence of a sp^3-hybrdized boronate complex.

CONCLUSIONS

In this review, we have covered the recent development of anion selective fluorescent chemosensors developed in our group. Two very important binding motifs in anion binding, namely, hydrogen bonding and electrostatic interactions, have been utilized, along with various mechanisms that result in fluorescence changes. Probably the most interesting features of this combined approach is that it highlights the benefits of rational design and a study of various sensing mechanisms, and shows that such approaches allow one to build a wide variety of molecular sensors. Recent advances in the area of anion coordination chemistry, ligand engineering, supramolecular chemistry, environmental and biological processes, as well as mechanisms, analytical chemistry, etc. have recently established the basis for what we predict will be a further flowering of the anion chemosensing field. We believe that the biological, medical and clinical importance of various anions can guarantee that relatively simple optical methods can be used to detect specific anions and will continue to gain importance.

Acknowledgements

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