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Review

Anion recognition and sensing in organic and aqueous media using luminescent and colorimetric sensors

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Contents

Abstract

This review article focuses primarily on the work carried in our laboratories over the last few years using luminescent and colorimetric sensors, where the anion recognition occurs through hydrogen bonding in organic or aqueous solvents. This review begins with the story of the discovery of fluorescent photoinduced electron transfer (PET) sensors for anions using charged neutral urea or thiourea receptors where both fluorescent and NMR spectroscopic methods monitored anion recognition. This work led to the development of dual luminescent and colorimetric anion sensors based on the use of the ICT based naphthalimide chromophore, where ions such as fluoride gave rise to changes in both the fluorescence and the absorption spectra of the sensors, but at different concentrations. Here, the former changes were due to hydrogen bonding interactions, whereas the latter was due to the deprotonation of acidic protons, giving rise to the formation of the bifluoride anion (HF_2^-). Modification of the 4-amino-l,8-naphthalimide moiety has facilitated the formation of colorimetric anion sensors that work both in organic or aqueous solutions. Such charge neutral receptor motifs have also been incorporated into organic scaffolds with norbomyl and calixarene backbones, which have enabled us to produce anion directed self-assembled structures.

Keywords: Anion sensing; Acetate; Fluoride; Dihydrogenphosphate; Urea; Thiourea; Deprotonation; Bifluoride; HF_2^-; Fluorescence sensors; Colorimetric sensors; Supramolecular chemistry; PET; Photoinduced electron transfer; Lanthanide luminescence; Self-assembly; NMR

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1. Introduction

The development of luminescent signaling systems and devices, such as switches, sensors and 'nano-machines' is an active area of research in organic and inorganic supramolecular photochemistry [1,2]. Examples of such signaling molecules or arrays of molecules, have been constructed from purely organic chromophores/lumophores, inorganic metal ion frameworks, coordination frameworks, or by the suitable combination of any of these. This gives rise to rich varieties of structural-chemical motifs where the luminescence signaling can be stimulated or generated, by light energy, *e.g.* by direct excitation, or *via* energy or electron transfer processes in donor–acceptor arrays, electrochemically or by chemical inputs, such as ions or neutral molecules. The discovery of the latter paved the way for the development of chemical sensors [3,4], which have been found to be of major importance in: (i) industry (for monitoring chemical processes, pollution, *etc.*); (ii) diagnostic and therapeutic medicine (for monitoring electrolytes, in critical care analysis and as therapeutics in photodynamic therapy, *etc.*); and (iii) various kinds of environmental monitoring.

In general, the criteria behind chemical sensing have involved the design of small single molecules that specifically recognize a single ion or a molecular species in a competitive media in a reversible manner and in a given concentration range. For continuous monitoring the need for reversibility is an essential requirement. However, in the case of once-off measurements, such as the analysis of blood and serum samples or glucose monitoring, such reversibility is not always necessary [5]. Moreover, single analyte sensing is possibly giving way to a new class of sensors that detect classes or mixtures of chemicals in a similar manner to which nature has developed human taste buds or other receptors such as those for detecting smells [6]. Nevertheless, single analyte detection is still of significant importance, particularly for understanding various physiological processes. Hence, Zn(II) is believed to play a pivotal role in many extra- and intracellular physiological functions which can only be monitored or observed *in vitro* by using single-ion selective sensors [7]. The same may be said for many organic and inorganic anions, whose role in nature is often well understood, but has been difficult to explore by real-time monitoring using non-invasive methods. Anions are essential to life, as many biological processes depend on the presence or transport of anions, or use anions to carry out chemical transformations. They are also important for many industrial processes and are often found as harmful pollutants [8]. Consequently, the need for developing anion sensors for complex media such as in blood, serum, cells, soil, freshwater, *etc*. is of utmost importance. For such sensing, single molecular sensors are ideal, provided that a clear selectivity and sensitivity can be achieved. For such, the same design strategies can be employed as have been used in the development of sensors for cations, an area that has been well explored over the last two decades [9]. Examples of such strategies include the use of ion or molecular receptor moieties as an integrated part of a signaling unit (either luminescent or colorimetric) or where a short spacer, with the aim of minimizing any ground state interactions, separates the two units. The latter is an example of the so called photoinduced electron transfer (PET) sensors, which were originally developed independently by de Silva and Czarnik, and have been employed in many excellent examples since [10,11]. Other alternative design strategies such as the displacement assays, as developed by Anslyn and coworkers, has also been developed [12]. Irrespective of the design strategy, the recognition event involves the binding of the analyte to the receptor and in all cases induces some changes in the physical properties of the receptor. These in turn give rise to concomitant changes in photophysical properties such as absorption or emission wavelength, intensity, quantum yield, lifetimes, polarization, *etc*., all of which can be employed to quantify the analyte concentration, or monitor its movement in real-time [13,14].

In the past 8 years, we have focused a facet of our research effort on the development of sensors for cations, using colorimetric or fluorescent PET or lanthanide luminescent sensors [15]. At the same time our interest in developing sensors for anions capable of detecting selectively a single ion using the changes in the various aforementioned photophysical properties also began [16]. Unlike the sensing of cationic ions and molecules, and even though the sensing of anions dates back to the late 1960s, anion recognition has been generally more difficult to achieve and only in the last decade or so has it become a 'popular' area of research, with large numbers of new publications and reviews having been published in this field in the last few years [17–20].

Our interest in anion sensing derived from the fact that, to the best of our knowledge, no anion PET sensors had been developed using charge neutral receptors where the anion recognition occurred through hydrogen bonding. Even though many elegant PET sensors had been developed for Groups I and II cations, transition metal ions, ammonium ions and even for anions, such as phosphates and pyrophosphate using charged ammonium moieties as receptors, the luminescent sensing of anions using charge neutral receptors had been largely unexplored [21]. This can in some ways been explained by the complex nature of the anions, which by definition: (i) have lone pairs of electrons (usually); (ii) possess high Lewis basicity; (iii) exhibit a wide range of geometries that can often be highly pH dependent (therefore the construction of complementary receptors necessitates a higher measure of design); and (iv) high solvation energies. Nevertheless, examples of luminescent anion sensors have emerged from the laboratories of Sessler and coworkers [22], Anslyn and coworkers [23], Gale [24], Parker and coworkers [25], Fabbrizzi [26], Beer [27] and many others. Inspired by their work, we set out to develop sensors that: (i) were designed on the PET principle; (ii) would be highly luminescent in either the presence or absence of anions; (iii) would emit at long wavelengths; (iv) could be made in a facile manner using few step synthesis; and (v) would bind the anion in a reversible manner using hydrogen-bond donor receptors. The work by Schmidtchen [27], Davis and Joos [28], Schneider [29], Umezawa [30], Hamilton [31], to name just a few, had established that functional groups such as urea, thiourea and guanidinium all bind anions through linear hydrogen bond-

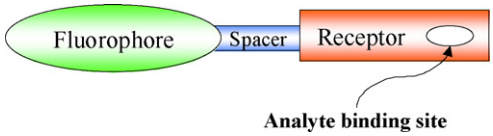


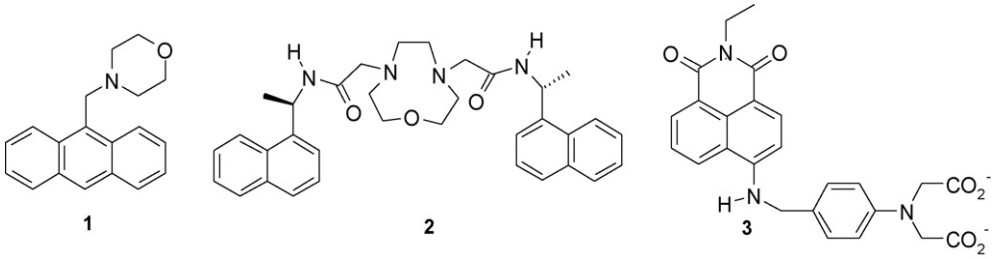
Fig. 1. A cartoon representing the fluorophore–spacer–receptor model use in PET sensors.

ing, and that in many cases good selectivity and sensitivity could be achieved using structurally simple hosts. However, for these, the anion recognition was usually monitored by ^{1}H NMR spectroscopy, in organic solvents such as $CDCl_3$ or DMSO-d_6, rather than monitoring the changes in the ground or the excited states of the receptors by UV/vis. or emission spectroscopy. We therefore set out to develop luminescent anion sensors using some of these charge neutral receptors. This review describes the way we achieved our initial goal of developing the first charge neutral PET sensor for anions and how the results from that work initiated other research projects in our laboratories centered on anion recognition, and how many other researchers have since employed similar strategies for anion sensing.

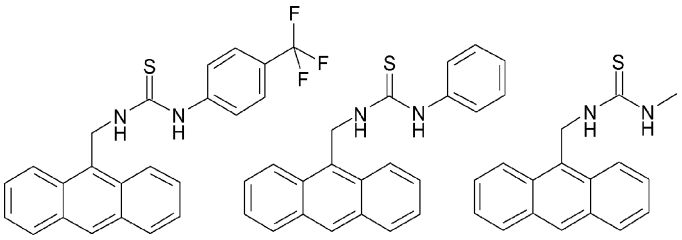
2. Photoinduced electron transfer sensors for anions

2.1. PET sensors based on the anthracene and naphthalene structures

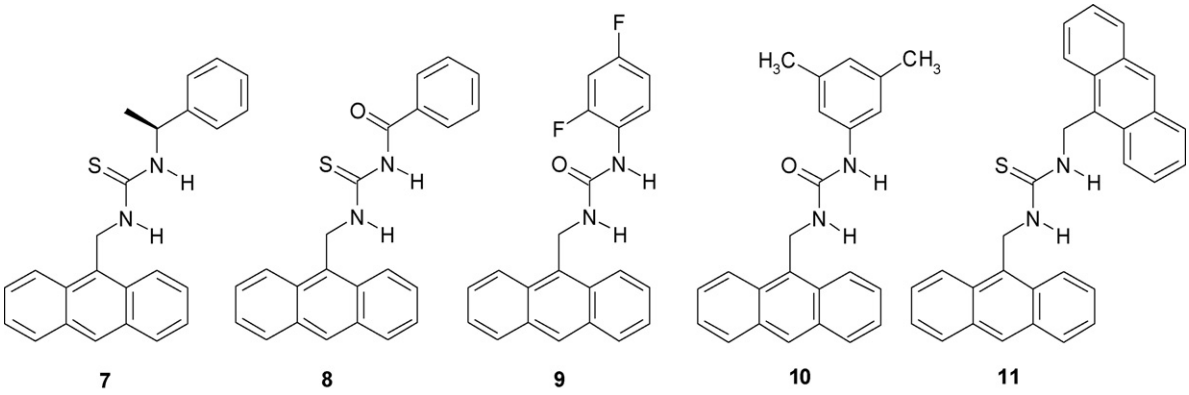
Our initial goal was to develop charge neutral anion sensors based on the use of PET mechanism. Ideally, PET sensors should only show changes in quantum yield or intensity upon recognition of analytes. Such sensors are based on the design principles developed independently by de Silva and Czarnik, who used a short covalent non-conjugated spacer to connect the two main components of the sensor, the ion or molecular receptor, and the fluorophore, Fig. 1. As no $n \rightarrow \pi$ or $\pi \rightarrow \pi$ ground state interactions would be possible between the two components, the absorption spectra of the fluorophore should be independent of the analyte recognition. However, by choosing the right components the excited state of the fluorophore should be modulated upon analyte recognition. Whether the emission is switched 'ON–OFF' or 'OFF–ON' will chiefly depend on the changes that occur in the oxidation or the reduction potential of the receptor, in comparison to that of the fluorophore, upon analyte recognition.



Many excellent examples of PET sensors for cations have been developed and reviewed. Generally, these systems, such as **1** developed by de Silva for monitoring pH [32], and **2** [33] and **3** [34] developed in our laboratory for Li(I) and Zn(II) sensing, respectively, show large enhancements in their fluorescence emission upon recognition of these ions. With this in mind we set out to prove the principle of anion sensing using charge-neutral receptors for anions. Our original aim was to combine the anthracene fluorophore as found in **1** with anion binding receptors based on the thiourea structure. We anticipated stronger hydrogen bonding interactions between acetate or phosphate anions and the receptor, as they each can participate in complementary linear 'Y-type' H-bonding, than with the spherical anions such as the halides, which would require more sophisticated designs. The thiourea-based sensors **4**, **5** and **6** are examples of this simple design [35,36].



The three molecules were made in a single step from anthracene-9-methylamine and an equimolar amount of 4-(trifluoromethyl)phenyl-, phenyl- and methyl-isothiocyanate, respectively. The aim of using different isothiocyanates was to modulate the acidity of the thiourea receptor moiety and thereby achieve different receptor-analyte stability and different binding constants. The analytically pure products precipitated in up to 80% yield either within 10 min or after stirring overnight. Many other analogues were also made following this simple approach, such as the chiral thiourea **7**, the carbazole **8** [36], the urea analogues **9** and **10** [37] and the bis-anthracene analogue of **6**, **11**. Even though most of these were made using commercially available isocyanates or isothiocyanates, compounds **7** and **11** were made from 9-(isothiocyanatomethyl) anthracene, which was formed in a single step from anthracene-9-methylamine by reacting it with thiophosgene in a biphasic mixture of saturated $NaHCO_3$ and THF, followed by the reaction of the appropriate amine in $CHCl_3$. Characteristic for all these compounds was the presence of the urea or the thiourea proton resonances in the ^{1}H NMR spectra, changes in which could be used to monitor their interaction with anions.



The absorption spectra of these compounds in DMSO or $CHCl_3$ showed typical bands for the anthracene moiety. In the case of those compounds carrying an aryl based urea or thiourea receptor, the absorption spectra showed the presence of these at lower wavelength. Upon titration of **4** with acetate (as its tetrabutylammonium salt solution) those transitions within the absorption spectra due to the anthracene moiety did not change, indicating that there was no ground state interaction between the fluorophore and the receptor, Fig. 2a. However, those compounds having an aryl-based receptor all showed some changes at lower wavelengths indicating that the ground state was affected to some degree by the anions, *e.g.* Fig. 2a. This can only be due to the formation of a *receptor:onion complex*, brought about through hydrogen bonding, Fig. 3a. In contrast however, the changes in the fluorescence emission spectra were dramatic, as can be seen for **4** upon titration with acetate, Fig. 2b. Here the fluorescence emission was quenched upon anion recognition with no significant changes in the structure of the emission bands. Similar results were observed for phosphate and indeed fluoride. However, other halides such as chloride and bromide did not give rise to strong enough binding to give rise to changes in either the absorption or the emission spectra.

By monitoring the changes in any of the anthracene emission bands the sensitivity of the anion recognition could be determined. This can be seen in Fig. 2c for the normalized intensity changes in the 419 nm transition, as a function of −log [anion]. Fitting these changes using least squares non-linear regression analysis showed that **4** bound fluoride ($\log \beta = 3.35 \pm 0.05$) more strongly than acetate ($\log \beta = 2.55 \pm 0.05$) and phosphate ($\log \beta = 2.05 \pm 0.05$). In all cases the changes were due to 1:1 binding stoichiometry. The fact that these anions gave rise to significant quenching indicates that the resulting receptor:anion complexes are participating in photoinduced electron transfer quenching (PET) phenomena. This view is further supported by that fact that no significant changes were observed in the ground state of the anthracene chromophore. This quenching is the result of the formation of an electron rich receptor:anion complex, which in turn enhances the free energy of PET (ΔG_{PET}) quenching from the receptor to the anthracene excited state and the emission is '*switched off*'. That this process occurs *via* hydrogen bonding recognition is further supported by the fact that upon addition of more competitive hydrogen bonding solvents such as ethanol or water DMSO solutions of the complex causes the fluorescence emission to be re-established, or 'switched on'. These systems operate in reverse to the classical PET cation sensing, of compounds such as **1–3** where the oxidation potential of the receptor is increased causing the emission to be '*switched on*', as the thermodynamic pathway for PET is removed.

The anion recognition was also monitored by observing the changes in the ^{1}H NMR spectra of **4** upon addition of these anions. On all occasions the thiourea protons were significantly shifted ($\Delta\delta \sim 3$ ppm) upon anion recognition. Plotting

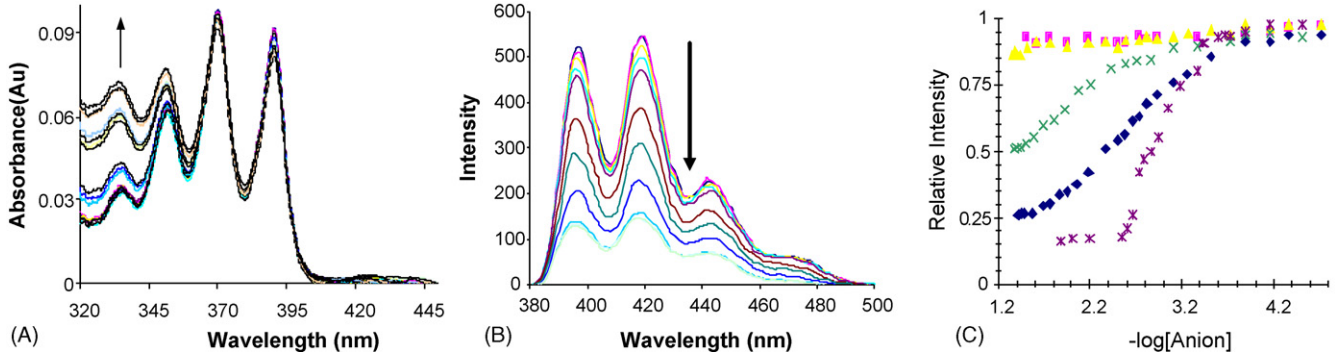


Fig. 2. (a) The changes in the absorption spectra of **4** upon titration with acetate. (b) The concomitant changes in the fluorescence emission spectra. (c) The changes in the relative intensity of the 429 nm wavelength of **4** using TBAOAc (blue); TBA H_2PO_4 (green); TBAF (purple); TBAC1 (yellow); TBABr (pink) in DMSO. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

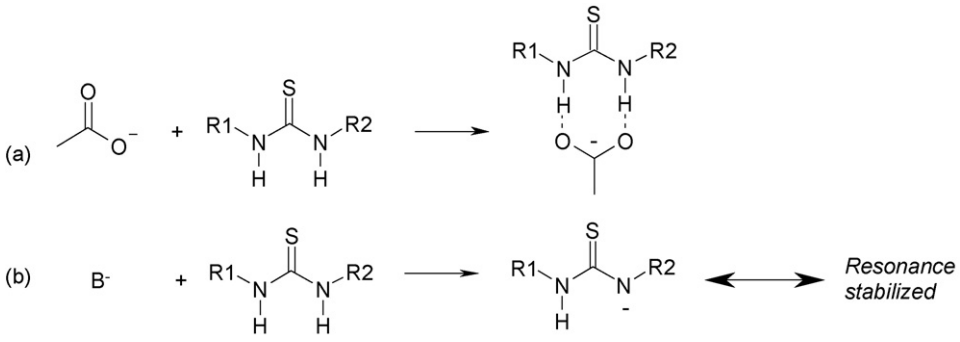


Fig. 3. (a) The formation of liner-Y type hydrogen boning receptor:anion complex between acetate and the thiourea receptor. (b) The possible deprotonation of urea based receptor by bases such as F^-, giving eventually HF_2^-. The resulting anion can be then being resonance stabilised. R_1 and R_2 are aryl or aliphatic functional groups.

the changes in these resonances as a function of added equivalents of anion also showed that that the recognition was in 1:1 stoichiometry. However, in the case of fluoride the resonances broadened, so tracking changes in the aryl proton resonances was employed. Again the 1:1 stoichiometry was established. The broadening of the ^{1}H NMR spectra could also be due to the fluoride induced deprotonation of the thiourea moiety giving rise to the formation of the resonance stabilised thiourea anion, Fig. 3b. Such deprotonation processes have been further investigated in our laboratory, and in those of Gale and coworkers [38] and Fabrizzi and coworkers [39].

In an analogous fashion, we evaluated the anion binding abilities of compounds **7**–**11**. The chiral compound **7** was designed to detect *N*-protected amino acids. However, no significant differences were observed between *S* and *R* isomers of amino acids such as Ala, Luc and Phe. The introduction of a second stereogenic centre into the structure of **7** has not yet been successful, while compound **8** did not give rise to any changes in the fluorescence within the anthracene moiety upon anion recognition. The urea analogues **9** and **10** both showed high degree of fluorescence quenching upon anion recognition with 97% quenching with fluoride, Fig. 4A, while the UV–vis spectra was not significantly affected [37]. The difluoro-substituted analogues **9** was also highly fluorescent prior to anion binding with a quantum yield of fluorescence of 0.53, while the quantum yield of **10** was much less at 0.06. Surprisingly, the affinity of these sensors for acetate was almost identical, with $\log \beta$ values of 2.14 (±0.1) and 2.07 (±0.1) for **9** and **10**, respectively. We anticipated the more electron deficient urea moiety **9** to be more acidic and hence to bind acetate more strongly. However, recent analysis of these and related bis-urea receptors (see later) has shown that highly electron withdrawing substituted aryl ureas and thioureas indeed cause the hydrogen bonding moieties to become substantially deconjugated from the aryl rings and hence, less delocalised, which makes them less effective anion receptors [38]. Stern–Volmer kinetic analysis was also carried out on these compounds. In the case of **9**, at low concentration of the anion both static and dynamic quenching were observed, while upon increasing concentration of the anion, *i.e.* fluoride as shown in Fig. 4B, the quenching became predominantly dynamic in nature with K_{SV} of 246.68 M^{-1}. Closer analysis of the initial changes ($[F^-] = 0 \rightarrow 0.0017$ M), showed, however, that there was significant ground state interaction between the anion and the fluorophore in this concentration range.

With the aim of achieving better selectivity in anion recognition we developed the bis-anthracene sensor **11**. Interestingly, this sensor showed higher selectivity for fluoride over acetate and phosphate, although recognition only occurred at very high concentration, which we attribute to the increased steric congestion induced by the presence of two anthracene moieties [40]. Because of this we developed the two naphthalene-based sensors **12** and **13**, which are less sterically congested. Indeed, these compounds showed significant changes in their luminescence spectra upon anion binding. However, the most interesting results of these studies are that anions such as $H_2PO_4^-$, F^- or AcO^- give rise to the induced CD changes in the circular

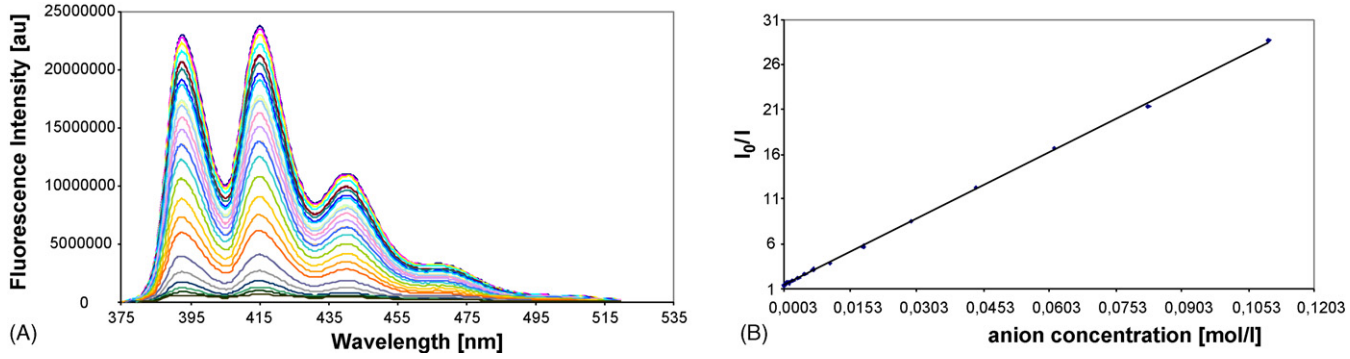


Fig. 4. (A) Changes in the fluorescence emission of **9** upon titration with F^- in DMSO. (B) Stern–Volmer plot of **9** in the presence of fluoride.

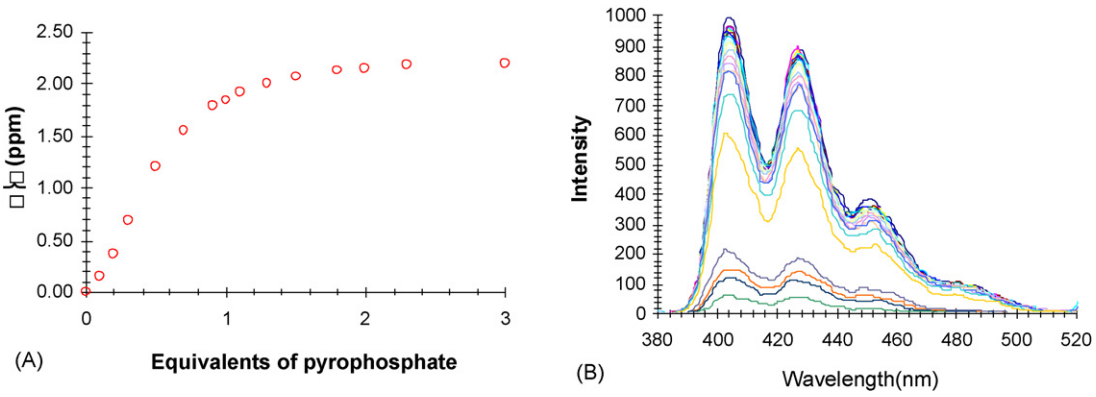
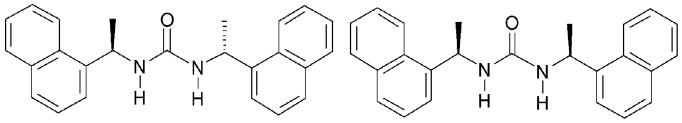


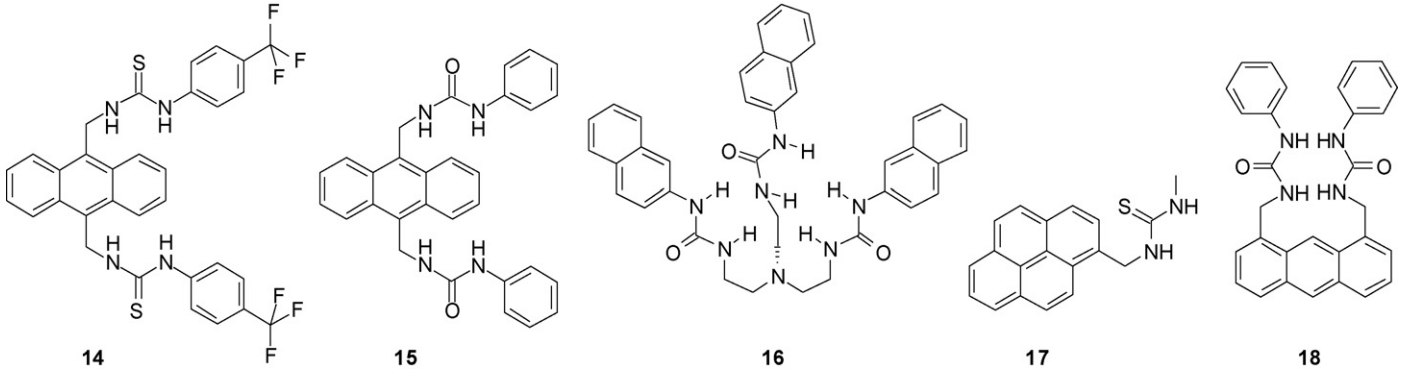
Fig. 5. (A) The changes in the aromatic N–H resonance of **14** upon addition of dihydrogenphosphate and pyrophosphate. (B) The changes in the anthracene emission of **14** upon titration with pyrophosphate.

dichromism spectra, which have, to the best of our knowledge not previously been used as a monitoring method for anion sensing in such urea based sensors.



12 **13**

For both **12** and **13**, large changes were observed in their fluorescence emission spectra upon addition of phosphate, but unlike that seen for the anthracene-based sensors above, the monomer emission was enhanced and a new band appeared at longer wavelengths and was assigned to an anion induced excimer emission. Binding to these sensors was analysed by tracking the changes in the monomer and the excimer emission and showed that dihydrogenphosphate bound to **13** more strongly than to **12** by an order of magnitude. Single crystal X-ray diffraction analysis of these compounds showed that the urea moiety in the meso compound places the two naphthalene moieties adjacent to each other, which might allow it to participate in stronger π–π interactions upon anion binding. Interestingly, these sensors did not significantly bind acetate.



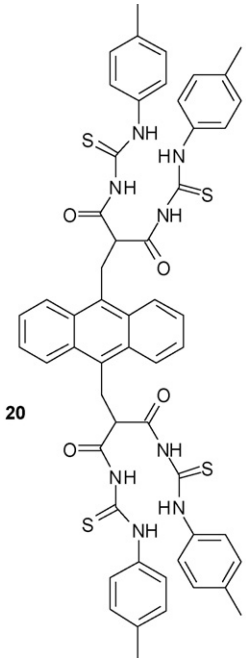
14 **15** **16** **17** **18**

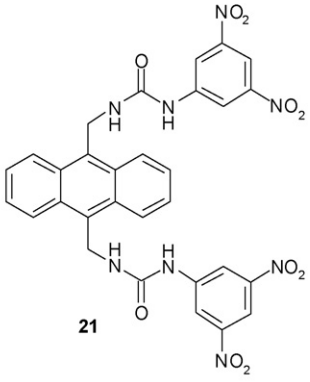
In each of the above examples the sensors have been flanked with a single anion recognition site. With the aim of developing fluorescent sensors for bis-anions such as pyrophosphate and bis-carboxylates, we developed **14** and **15**, where the anthracene moiety was functionalised at the 9 and 10 position with two thiourea and urea moieties, respectively [41]. Titration of these sensors with anions such as acetate and dihydrogenphosphate gave rise to significant quenching in the anthracene emission, while the absorption spectra were not significantly affected. Analysis of these changes showed that each sensor bound these anions in 2:1 anion:host stoichiometry. These interactions were also monitored by ^{1}H NMR spectroscopy, which demonstrated the same stoichiometry, Fig. 5A. The titration of bis anions such as biscarboxylates and pyrophosphate, in DMSO, also gave rise to significant quenching in the anthracene emission, as can be seen in Fig. 5B for **14**. Analysis of these changes revealed that the pyrophosphate was bound in 1:1 stoichiometry, which was also seen in the ^{1}H NMR spectrum, Fig. 5A, where after one equivalent of the anion the thiourea proton resonances were not significantly shifted further. These sensors both bind a series of bis-carboxylates in 1:1 stoichiometry, with the strongest binding observed for malonate. For such binding to be possible, the anion would have to bind across the anthracene moiety. Such binding would be expected to have some effect on the ground state and indeed the absorption spectra were affected upon titrating these sensors with such bis-anions. In the case of **14**, upon titration with pyrophosphate isosbestic points were observed in the anthracene transitions, which were absent when **14** was titrated with acetate.

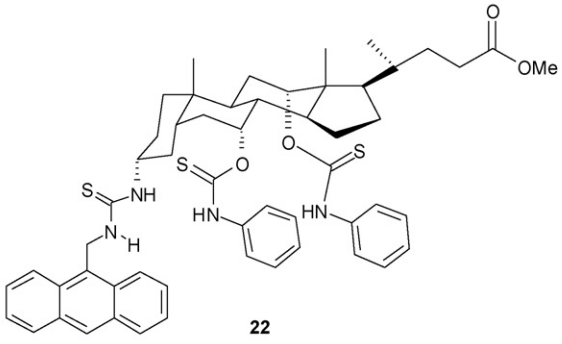
In a similar way, several examples of such PET and other fluorescent sensors for anions have recently been developed based on

the use of simple hydrocarbon based fluorophores such as naphthalene, anthracene and pyrene, by employing ureas, thioureas and amide receptors. Many of these examples have recently been reviewed. Examples of such designs include **16**, which was developed by Wu as a PET sensor, with the quaternary nitrogen atom acting as the donor, the receptor acting as a spacer and the fluorophore being the naphthalene unit [42]. However, **16** sensed anions *via* a combination of PET and an Energy Transfer mechanism. Binding of the anion to the NH group directly attached to the naphthalene unit allows energy transfer to occur between the receptor and the fluorophore therefore affecting the emission properties of the compound. In addition, the absorption spectra significantly changed upon addition of the anion, again breaking one of the criteria specified in order to be classified as an ideal PET sensor. Sensor **16** exhibited highly selective complexation of tetrahedral oxo-anions, especially dihydrogenphosphate, whereas poor binding of the spherical halide or trigonally disposed acetate anions was observed.

Teramae recently reported the synthesis of a thio-urea based anion receptor linked to a pyrene moiety via a methylene spacer, **17** [43]. Fluorescent and UV/vis binding studies showed that upon addition of various anions the monomer emission reduced dramatically with little change in the absorption spectra being observed. The association constant for acetate was determined to be $7.0 \times 10^3\ M^{-1}$, whereas K_a for dihydrogenphosphate and chloride was $5.2 \times 10^3\ M^{-1}$ and $1.0 \times 10^3\ M^{-1}$, respectively. This compound does not exhibit *ideal* PET behaviour since the monomer emission quenching was followed by the formation of an intramolecular exciplex emission. Teramae has also reported another particularly simple self-assembly system for sensing anions, with a pyrene functionalised monoguanidinium receptor [44]. Yoon reported a new anthracene derivative bearing two phenylurea groups at the 1,8 position of anthracene, **18** [45]. This sensor shows a selective fluorescence quenching effect with fluoride *via* a PET mechanism ($K_a = 71270\ M^{-1}$ in CH_3CN:DMSO 9:1 with K_a (Cl^-) $= 614\ M^{-1}$, K_a (Br^-) $= 121\ M^{-1}$, and K_a (I^-) $= 30\ M^{-1}$). This work was extended to use of a naphthalene unit as the fluorophore [45]. Similarly, Xu and Tarr [46a] have also developed such luminescent naphthalene bis-aryl urea sensor for anions, while Sasaki has incorporated three anthracene or pyrene derived urea moieties into a functionalised phenyl scaffold [46b]. Hong reported the conformationally flexible thiourea-based sensor **19** [47], Fig. 6, as a potential fluoride chemosensor using a biaryl fluorophore and showed that upon addition of fluoride the fluorescence of **19** was enhanced. The effect of various anions (as their TBA salts) on the fluorescence of **19** was investigated in chloroform. The addition of fluoride resulted in an enhancement of the fluorescence at 356 nm, induced by the formation of a 1:1 host–guest complex ($K = 1.08 \times 10^4\ M^{-1}$). However, the addition of more than 2.5 equivalents of fluoride to **19** caused a decrease in the fluorescence intensity. It was suggested that this was due to a loss of conformational restriction as a 1:2 host–guest complex was formed ($K = 2.28 \times 10^7\ M^1$). There were no spectral changes at 356 nm on the addition of $H_2PO_4^-$, Cl^-, AcO^-, HSO_4^- and Br to **19**. He and coworkers have recently developed the anthracene-based sensor **20**, which showed significant enhancement in the fluorescence of the anthracene moiety upon complexation with dicarboxylate anions such as adipate, sebacate and glutarate [48]. Even though the sensor bound these anions in 1:1 stoichiometry, the selectivity for their sensing was not exclusive. Related work has included the bis-urea based sensor **21**, developed by Yoon and coworkers [45c]. This compound is structurally related to **15** developed in our laboratory and was found to bind anions such as hydrogenpyrophosphate and adipate anions in 9:1 CH_3CN:DMSO solution, with large changes observed in the fluorescence emission of the anthracene moiety.







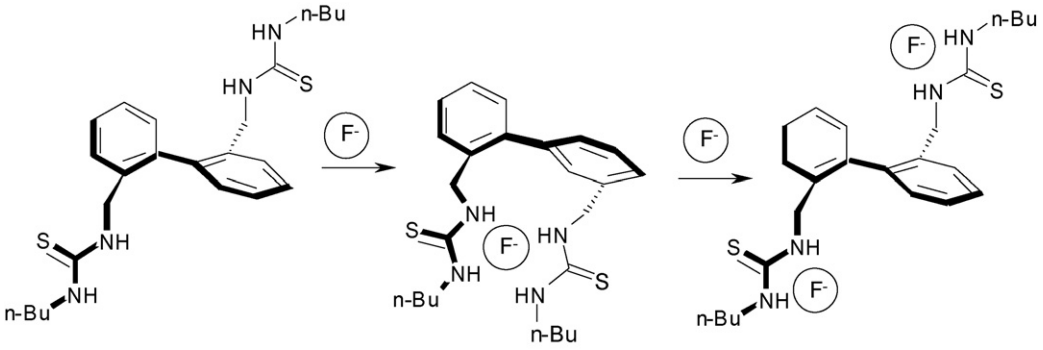


Fig. 6. The conformational changes occurring in **19** upon coordination to fluoride.

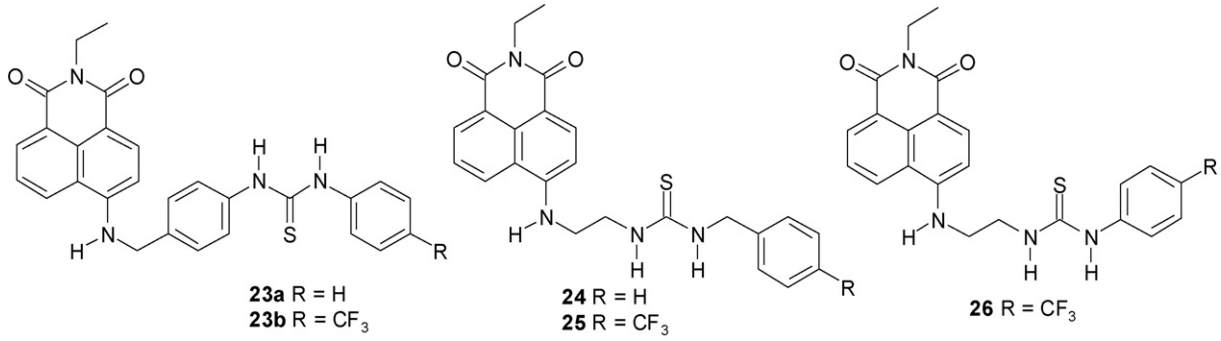
Chan et al. have also incorporated the anthracence urea based fluorophore into cholic acid **22**, as a sensor for dihydrogen phosphate, propionate and benzoate, and more recently for dicarboxylates [49]. For these anions the emission was quenched in a similar manner to that discussed for **4**–**11** above. Other recent examples include those of Qian and Liu [50a] and Mei and Wu [50b] who developed several fluorescent naphthylthiourea derivatives, respectively. Similarly, Ramamurthy and coworkers [51a] have developed a combined PET and ICT based sensors for anions, while He and coworkers [51b] have incorporated naphythl-based urea moieties into calix[4]arene (see later discussion) which gave rise to binding of dicarboxylates with sensitivity depending on the length of these ions. Other examples of the use of urea based sensors are those of Martínez-Máñez, who developed anthraquinone based colorimetric sensors [52]. These examples clearly show the attractiveness of the use of the simple urea-based PET sensing as developed in our laboratory.

The above examples have all, however, consisted on the use of simple fluorophores that absorb and emit at a short wavelengths. For potential biological application such sensors suffer from the affects of background auto-fluorescence and light scattering. Because of these diadvantages we set out to modify our design principles as discussed below.

2.2. Naphthalimide based fluorescent sensors

Having established the principle of PET quenching using structurally simple and charge neutral receptors we set out to design new PET chemosensors that emit at longer wavelengths and chose the 4-amino-l,8-naphthalimide fluorophore. We have previously used this in fluorescent PET sensors for Zn(II) and for pH sensing in water permeable hydrogels [53]. The naphthalimide fluorophore offers several advantages over others and these include: high photo-stability; strong absorption in the visible region due to an internal charge transfer excited state with λ_{max} at *ca*. 450 nm, and; high quantum yields of emission (Φ_F) in the green part of the electromagnetic spectrum with $\lambda_{max} \sim 540$–550 nm). Modifications of the basic fluorophore are also straight forward as a urea (or thiourea) receptor can be introduced *via* the 4-amino moiety. De Silva had demonstrated that such modification gave rise to PET quenching in receptors incorporating secondary and tertiary amines, while the same modification at the imide group does not result in PET quenching due to electron repulsion, which prevents electron transfer to the ICT excited state [54].

We synthesised compounds **23a**, **23b**, **24**, **25** and **26**, in syntheses requiring only a few straight-forward steps starting from 4-chloro or 4-bromo-l,8-naphthalimides. These sensors were designed on the classic *fluorophore–spacer–receptor* model discussed in Section 2.1. The synthesis of **23a** and **23b** was achieved from 4-bromo-l,8-naphthalic anhydride by first converting the anhydride to an imide using ethylamine in 1,4-dioxane. The nucleophilic displacement of the 4-bromo moiety was accomplished using an excess of 4-aminobenzylamine, and the resultant benzylamine was reacted, at room temperature, with either phenyl- or 4-(trifluoromethyl)phenyl isothiocyanate, to give **23a** and **23b** in 60% and 58% yields, respectively [55]. Compounds **24**–**26** were synthesised by reacting the 4-bromo-l,8-naphthalimide with excess ethylenediamine, followed by reaction with either phenyl- or 4-(trifluoromethyl)phenyl-isothiocyanate, or 4-(trifluoromethyl)benzyl isothiocyanate, respectively [56,57].



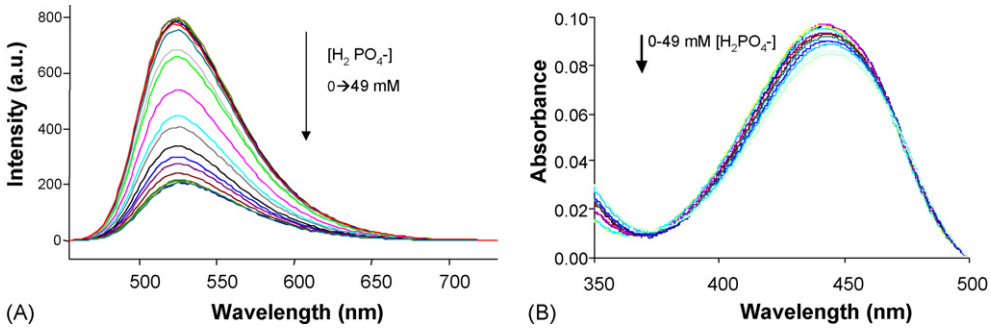


Fig. 7. (A) The changes in the fluorescence emission of **23b** upon addition of hydrogen phosphate.

The introduction of an aryl thiourea in **23a** and **23b** separated by a single methylene spacer would ensure that PET would be feasible from the receptor to the naphthalimide excited state. When the absorption spectra for **23a** and **23b** were recorded in DMSO, a broad absorption centred at *ca*. 450 nm due to the ICT was observed. Excitation of these at their respective maxima, gave rise to strong emission between 450 and 700 nm (green to the naked eye) with $\Phi_F = 0.60$ for **23a** and 0.71 for **23b**, respectively.

These sensors were titrated against various anions (as their TBA salts) and the changes in the emission spectra of **23b** can be seen in Fig. 7A, where the fluorescence emission is effectively quenched or completely '*switched off*' after the addition of 49 mM of $H_2PO_4^-$. As expected there were only minor changes in the absorption spectra of **23b** at this anion concentration, Fig. 7B. Moreover, the addition of methanol to this solution reversed this process, as the emission was restored. The emission in this case was '*switched off*' by approximately 74%, with a quantum yield Φ_F of 0.056 at $[H_2PO_4^-] = 49$ mM. The lack of any significant concurrent changes in the absorption spectrum verify the insulating role played by the spacer which minimises any ground state interactions between the receptor and the fluorophore as is inherent with this design strategy. However, some small changes were observed and can be assigned to through space charge interaction between the partial positive charge developing on the amino moiety in **23b** (due to the ICT) and the anion bound to the receptor. This would suggest that the changes in fluorescence emission are only due to PET. The binding constant for the interaction between **23b** and $H_2PO_4^-$, determined using least squares non-linear regression analysis against the intensity changes at 527 nm, gave $\log \beta = 3.7$ (± 0.1), where the quenching occurred over two log concentration units. This indicates that the stoichiometry of binding is 1:1 and is due to a simple equilibrium.

For sensor **23a**, similar behaviour was observed, where the anion induced a 67% quenching of fluorescence. However, here the binding constant was significantly smaller with $\log \beta = 2.9$ (± 0.1), indicating the effect that the electron withdrawing group had on the acidity of the thiourea protons in **23a**. Again, no changes were observed in the absorption spectra and the quenching was reversed on addition of methanol. In a similar manner, acetate gave rise to significant changes in the emission spectra of both sensors, while chloride and bromide were not detected using fluorescence. In the case of acetate, the manner of quenching initially followed that observed above, however, at an anion concentration of about 0.2 mM the fluorescence levelled-off briefly before increasing in intensity (0.4 mM → 44 mM). Obviously, two processes were occurring—the first probably indicating a normal mode of binding of the anion to the thiourea moiety, whilst the second indicated deprotonation. Both processes occurred over approximately two log units each, and so could each be considered as 1:1 binding. By treating them separately, two binding constants were obtained. The first process has a $\log \beta$ of 4.76 (± 0.1) (± 0.05), while the second process was determined to have $\log \beta$ of 2.48 (± 0.05). There was also a slight shift in the absorption spectrum, associated with the latter changes. In a similar manner, the introduction of a two-carbon spacer between the fluorophore and the receptors for analogues of **24** and **25** still gave rise to significant quenching of the excited state of the naphthalimide upon anion recognition. Similar results were observed for **24** to that observed to **25**. However, on all occasions, the binding affinity of the sensors was lower, indicating that the phenyl receptors were less able to form strong hydrogen bonds to these anions in comparison to that of **23b**.

For these systems, however, the most interesting results were observed when they were tested for the detection of fluoride. As in the case of dihydrogenphosphate, the emission was quenched upon addition of fluoride up to 40 mM concentration; although, here the emission was fully quenched indicating the enhanced ability of the receptor to participate in PET quenching of the fluorophore. Concomitantly, the absorption spectra were only slightly affected, mirroring the changes observed previously, Fig. 8A. However, at higher concentrations, the absorption spectra changed dramatically for both **23a** and **23b**, Fig. 8B. Here, the ICT band centred at *ca*. 450 nm was reduced in intensity with formation of a new band at longer wavelength, and a second band appearing at lower wavelength at *ca.* 350 nm. Analysis of these changes revealed a binding constant of $\log \beta = 3.8$ (± 0.1) and 4.4 (± 0.1) for **23a** and **23b**, respectively, and that these changes were perhaps due to the deprotonation of a thiourea proton, giving rise to a resonance stabilised structure as shown in Fig. 3b. However, this would not be expected to give such changes at long wavelength as the receptor separates the two components and hence no significant π–π interactions would exist in the ground state. It is more likely that these changes are due to the deprotonation of the 4-amino moiety of the naphthalimide fluorophore, which is expected to be relatively more acidic. This would give

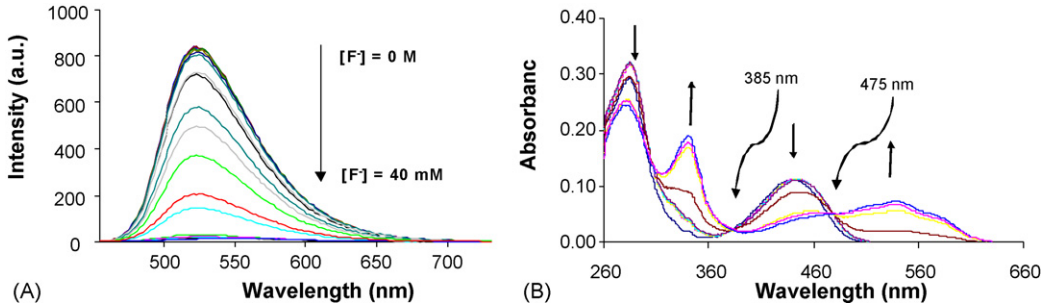
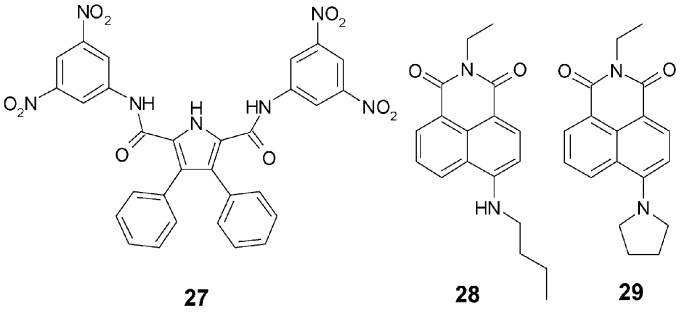


Fig. 8. (A) The changes in the fluorescence emission of **23b** upon addition of F^- (0 → 40 mM). (B) The changes in the absorption spectra of **23b** at concentration higher than 40 mM of F^-.

rise to a stronger ICT character and hence, the absorption would be shifted to longer wavelengths. While we were investigating this phenomenon, Gale reported similar deprotonation affects in the pyrrole based anion receptor **27**, where the addition of excess fluoride to an acetonitrile solution of **27** gave rise to a colour change to intense blue. Gale noted similar affects upon the addition of TBAOH and postulated that deprotonation of the receptor was responsible for the colour changes, as addition of less basic anions such as AcO^-, $H_2PO_4^-$, Cl^- and Br^- did not result in this colour change.



27 **28** **29**

In a similar way, the addition of base to solutions of **23a** or **23b** in DMSO or CH_3CN gave rise to the identical colour changes as seen for fluoride. Because of this we developed the model compounds **28** and **29** to investigate the feasibility of mimicking this deprotonation phenomenon in aprotic solvents [58]. As in the case of **23a** and **23b**, the absorption spectra of **28** were significantly affected upon titration with F^- but not with any of the other aforementioned anions, Fig. 9A. However, using TBAOH, identical changes were observed. Moreover, as these occur at long wavelength these changes, as in the case of **23a** and **23b**, gave rise to striking colour changes, Fig. 9B. In comparison, no significant changes were observed for **29**, which lacks the 4-amino proton, indicating that the colour changes were indeed due to the deprotonation of this moiety.

These changes were also reversible, as the addition of water shifted the absorption spectra back to that observed for the 'free receptor' in Fig. 9A. Several attempts were made to crystallise **28** in the presence of TBAF to gain better insight into the deprotonation phenomenon, but without any success. However, we noticed that allowing the solutions to stand exposed to air following the above titration experiments also resulted in a reversal of the colour change. Initially, we assumed that this was due to uptake of water from the air and the protonation of the amido anion. Because of this we attempted to grow crystals under anaerobic conditions. This was possible, and purple-green crystals were obtained, however, upon isolation, their colour changed to green. These new crystals showed that protonation had indeed occurred for the anionic form of **28**, but also that CO_2 had been 'fixed', as bicarbonate, Fig. 10A. Because of this, we reinvestigated **28** in solution. As expected, upon addition of TBAF to a solution of **28** in DMSO, the aforementioned colour changes occurred, however, upon addition of CO_2(g), the colour changes were instantly reversed, Fig. 10B. Moreover, the addition of TBAF to this solution did not regenerate the expected purple colour changes. Hence, our system was not only a colorimetric sensor for fluoride, but also able to fix CO_2 with irreversible concomitant colour changes.

It is apparent from the above results that the 4-amino moiety could be deprotonated by bases such as F^-. Hence, it should

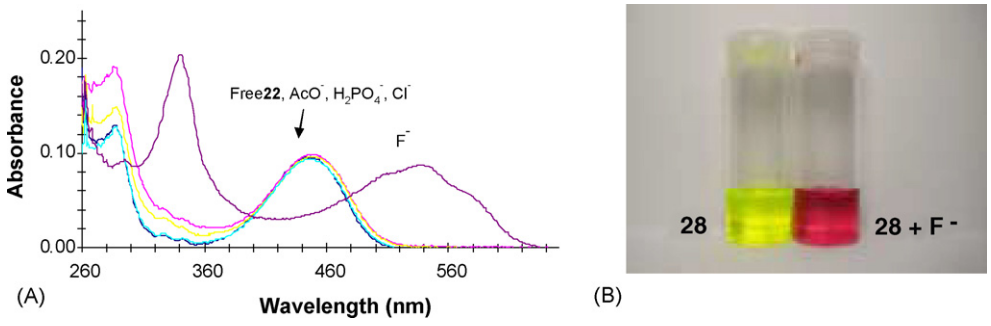


Fig. 9. (A) The changes in the absorption spectra of **28** upon addition of several anions. (B) The striking colour changes observed upon deprotonation of the 4-amino proton of **28**.

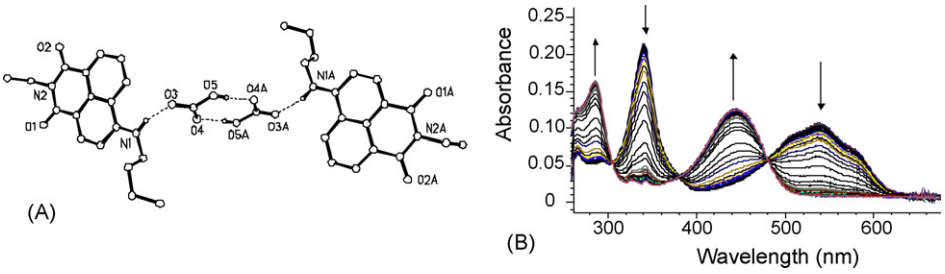


Fig. 10. (A) The X-ray crystal structure of **28**, showing the fixation (1:1 adduct) of CO_2 as HCO_3^-. (B) The changes in the absorption spectra of **28**, after deprotonation of the 4-amiono moiety using TBAF in DMSO, upon addition of CO_2; the long wavelength transition was reduced in intensity, generating the naphthalimde ICT band at *ca*. 450 nm.

also be possible to use the acidity of this proton donor to achieve stronger binding of certain anions, such as $H_2PO_4^-$, by employing it to bind in concert with the thiourea receptor. With this in mind we revisited the design of **23a** and **23b** and developed **24** and **25**. Both of these possess an ethyl spacer between the fluorophore and the receptor. The receptors for both **24** and **25** are derived from benzylamine (not phenylamine as previous). Taking these two design features into the account, we hoped that with this more flexible host, both the thiourea N–H units *together* with the naphthalimide N–H would *cooperatively* bind anions of appropriate size and geometry. However, the longer, more flexible, spacer would also reduce the rate of electron transfer between the two parts of the sensors. Moreover, the use of benzylic receptor further reduced the acidity of the thiourea protons and hence their ability to participate in PET. Indeed, the changes in the fluorescence emission spectra when a series of anions were added were only minor in comparison to those observed for **23a** and **23b** and this is most likely due to reduced PET efficiency. Because of this we used ^{1}H NMR to determine the binding affinity of these sensors in anion sensing. The titration of **24** with AcO^- in DMSO-d_6 solutions resulted in significant changes in the chemical shifts of several protons. Of these the largest changes were seen for the two thiourea proton resonances with $\Delta\delta$ of *ca*. 2.5 ppm which is indicative of strong hydrogen bonding between the anion receptor and the acetate anion. From these changes a 1:1 host–guest stoichiometry was determined with a binding constant, log β, of 3.6 ($\pm$0.1) (*cf*. log β of 4.7 ($\pm$0.1) for **23a**).

We anticipated that if the 4-amino moiety was able to participate in binding the N–H resonance should also be significantly affected as well, Fig. 11A. However, only small shifts were observed in the N–H resonance upon binding acetate and we concluded that it was not involved to any great extent despite the increased flexibility of the host. This is not totally unexpected as acetate would be expected to bind with 'Y' shape geometry as demonstrated in Fig. 3a. However, in contrast to these results, the use of $H_2PO_4^-$ gave rise to changes in the thiourea protons *and* also to large changes in the 4-amino N–H resonance, Fig. 11B. This demonstrates that cooperative binding was indeed observed; as such changes would imply hydrogen-bond interactions between $H_2PO_4^-$ and the N–H. Binding of $H_2PO_4^-$ was in 1:1 stoichiometry and fitting these changes using non-linear regression analysis gave log β = 3.4 ($\pm$0.1). The comparison of these results to that observed for **23a** in binding $H_2PO_4^-$ (log β = 2.9 ($\pm$0.1)) clearly demonstrates that the binding is significantly enhanced, which can only be due to the participation of the 4-amino moiety in cooperative binding. This was further supported by molecular modelling of **24** upon binding to both AcO^- and $H_2PO_4^-$. These showed that for acetate the interactions of the anion with the N–H proton was only minor, with no significant hydrogen bonding contribution, whereas for $H_2PO_4^-$, the anion was able to fully participate in hydrogen bonding to this moiety. In an attempt to achieve more significant changes in the luminescence in our improved design we made **26**. Preliminary investigation of the binding of $H_2PO_4^-$ clearly shows that the binding is strong with log $\beta \sim 3$, but the changes in the intensity are only *ca*. 20%.

The results above have shown that luminescent urea and thiourea based sensors can be formed where the emission is switched '*on–off*' upon anion recognition and that such recognition can be extended to yield colorimetric (first generation) sensors that give rise to naked-eye detection of anions. In the above cases the recognition process was also monitored by ^{1}H NMR spectroscopy, which gave information about the nature

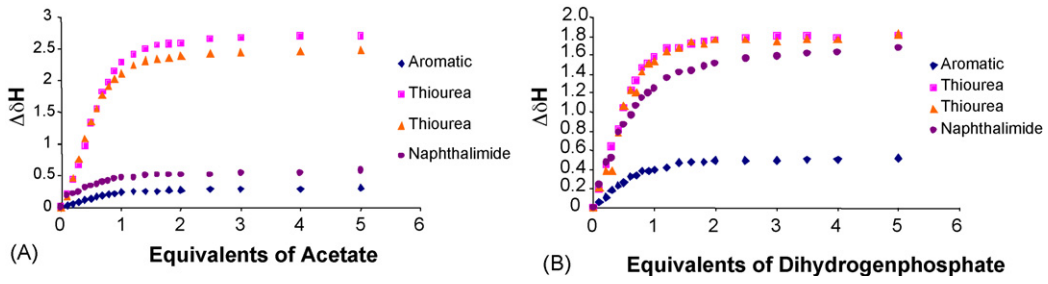


Fig. 11. (A) The changes in various resonances in **24** upon addition of acetate. (B) The changes in the same resonances upon addition of $H_2PO_4^-$. The aromatic proton is the one in position 3.

of the anion sensing, as demonstrated using compound **24**, **25** and **28**. The next section deals with these results in more detail.

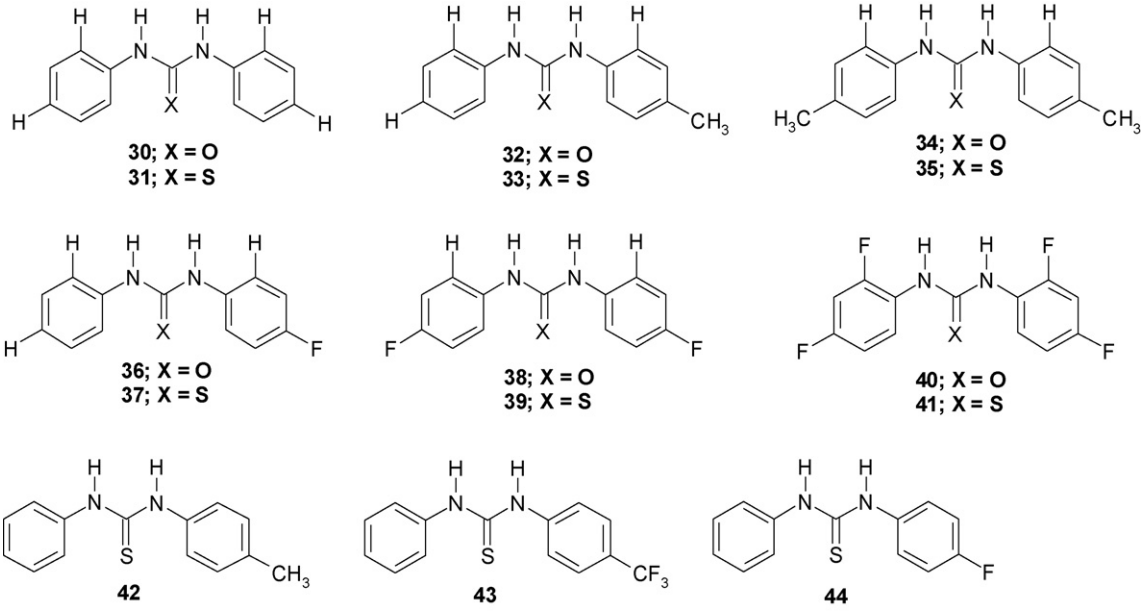
3. Monitoring anion recognition by ^{1}H NMR spectroscopy

^{1}H NMR spectroscopy was used to monitor anion binding to compounds **24**, **25** and **28**. Such monitoring of host–guest interactions is well known and has been the subject of many articles and reviews. In general it has been postulated that: (i) aromatic based receptors bind anions more strongly than aliphatic ones; and (ii) electron withdrawing substituents on the aromatic receptor give rise to stronger binding than electron donating ones. It is thought that this trend is due to enhanced acidity of the receptors, making the urea/thiourea stronger hydrogen bonding donors, hence, the more electron deficient these moieties the better. Moreover, the thiourea-based receptors have been considered to be stronger hydrogen bonding donors than the urea ones for the same reason.

In ongoing research we set out to: (a) firmly establish the stoichiometry of the anion recognition; (b) gain insight into the mechanism of host–guest interactions; and (c) investigate the effect of the electron donating and electron withdrawing substituents on the anion recognition. To achieve this we have carried out detailed ^{1}H NMR spectroscopic investigations upon all our sensors, discussed above, and on a large number of simple substituted bis-phenyl urea and thiourea compounds [59]. The overall results of these investigations have shown generally that bis-phenyl based receptors, such as **30**–**41**, bind anions more strongly than those based on a single phenyl group. However, we have also demonstrated that stronger electron withdrawing groups do not necessarily give rise to enhanced anion binding over those containing electron donating groups and that the choice of such electron withdrawing groups can have significant effects on anion binding.

Receptor **30** binds AcO^- with $\log\beta = 3.06$ (± 0.05), while **34** and **38** bind acetate with $\log\beta$ of 3.34 and 3.70, respectively, clearly demonstrating that *para* substitution gives rise to enhanced anion binding with stronger electron withdrawing groups. The thiourea analogues **31**, **35** and **39** showed a similar trend for AcO^-, except here the binding affinities of **31** and **35** were only marginally different while **39** ($\log\beta = 3.68$ (± 0.05)) had, within experimental error, the same affinity as its urea analogue **38**. However, **38** and **39** both showed an order of magnitude stronger binding of AcO^- when compared to **40** and **41** ($\log\beta = 2.30$ (± 0.05) and 2.37 (± 0.05, respectively), which have electron withdrawing *ortho*- and *para*-fluoride substitution.

This somewhat counter-intuitive observation could possibly be due to steric hindrance of the fluoride substituents in these latter compounds, but more likely these are due to repulsive electrostatic interactions. Furthermore, solid-state data from X-ray crystallography has shown that more electron withdrawing groups often give rise to significant changes in the dihedral angle between the urea/thiourea and the aryl groups, *e.g.* the urea/thiourea moieties become 'deconjugated'. This suggests that the electron withdrawing nature of the substituents may not influence binding at the receptor in a predictable fashion. To illustrate this point further, receptors **42**–**44** were found to bind acetate with $\log\beta$ values of 3.63 (± 0.05), 2.59 (± 0.05) and 3.61 (± 0.05), respectively. This clearly shows that the nature of the electron withdrawing group has a significant influence on the receptor's anion affinity, and sometimes affects it in an apparent counter-intuitive manner. Further investigation involving other anions has also shown that 1:1 binding stoichiometry does not necessarily occur. In the case of fluoride, the most dominant stoichiometry is 1:2, with the concomitant formation of bi fluoride (HF_2^-) and the chemical transformation of the receptors into their anionic form. The structural characterisation of HF_2^- within an anion–host has recently been published [60a], although we have, along with others [60b], observed its formation in DMSO-d_6 However, it is not only the urea/thiourea moiety that is susceptible to deprotonation, as can be seen for the



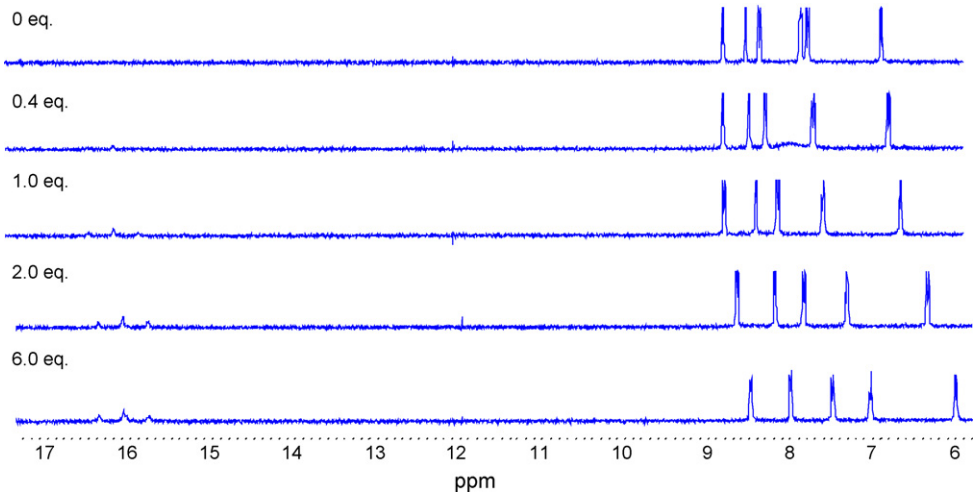
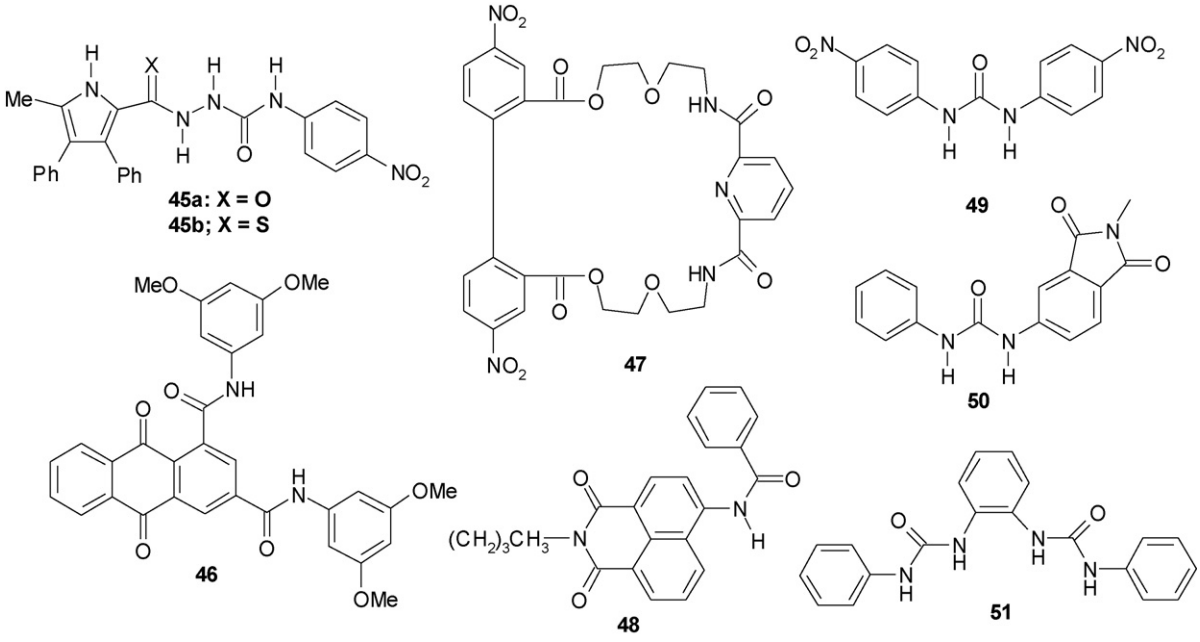


Fig. 12. ^{1}H NMR stack plot of **28** after addition of various quantities of F^- (DMSO-d_6, 400 MHz) showing formation of downfield triplet characteristic for the formation of HF_2^-.

changes in the ^{1}H NMR spectrum of compound **28** following the addition of fluoride anions, which clearly shows the formation of the characteristic bi fluoride signal at *ca*. 16 ppm, Fig. 12. In this particular case the only available deprotonation site is that of the 4-amino moiety.

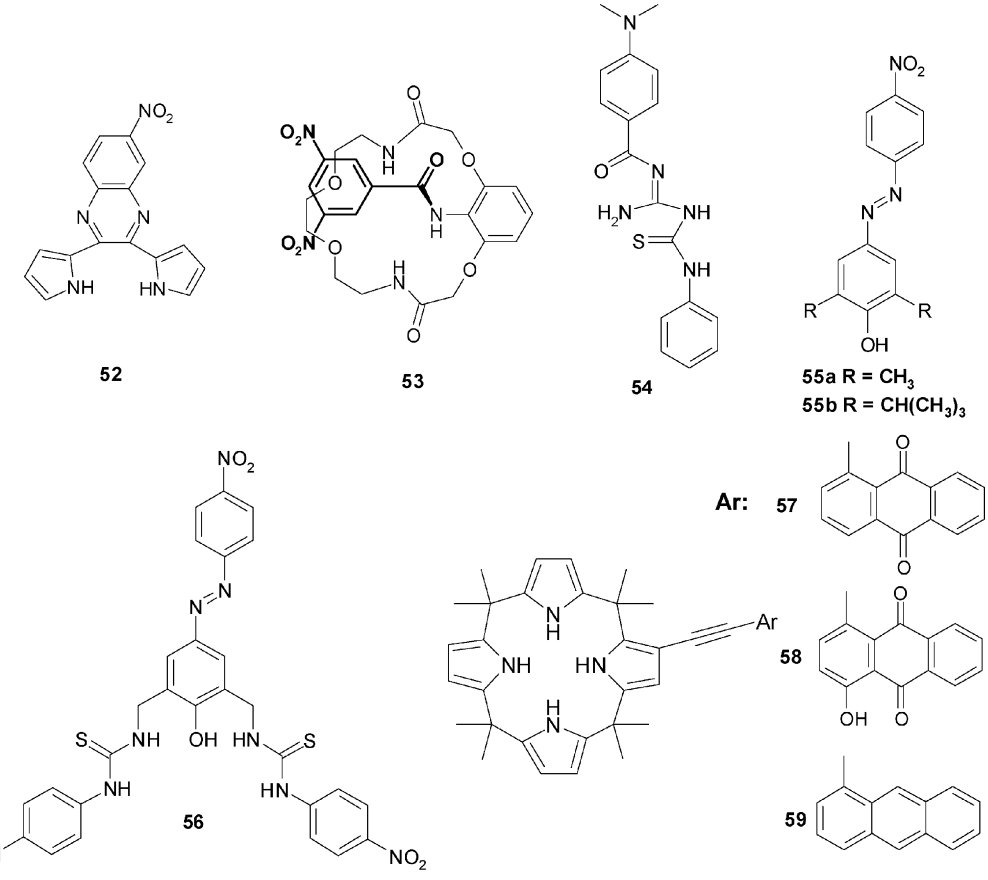
4. Anion recognition involving deprotonation as monitored by ^{1}H NMR spectroscopic and colorimetric methods

It is clear from the discussion above that deprotonation is an important method for achieving fluoride recognition. We have shown that deprotonation of the receptor site (urea or thiourea) and sites remote to the receptor (4-amino moieties) can lead to drastic colour changes that allow the molecules to act as potential naked-eye sensors. In a similar fashion, other researchers have seen such colorimetric changes and/or changes in the ^{1}H NMR spectrum upon deprotonation by fluoride. Some of these examples will be discussed below, but these are only some of a number of such examples recently published. We have previously mentioned the work of Gale who demonstrated that deprotonation could occur in compound **27**. Recently, Gale extended this work by developing the pyrrolylamidothiourea based colorimetric sensors **45a** and **45b**, each of which showed large changes in both their absorption and ^{1}H NMR spectra upon anion recognition [61]. In the latter case, several anions led to the formation of the deprotonated urea or thiourea moiety in solution. Moreover, the authors were able to grow crystals suitable for structure analysis from a solution containing TBAF and **45**, which showed the formation of the deprotonated thiourea moiety.



Gale et al. have also recently developed **46**, which was described as 'twisted' isophthalamide analogues [62]. This amide was shown to bind benzoate and dihydrogenphosphate in DMSO/0.5%H_2O solution with the latter giving rise to distinct colour changes. However, the most significant findings of this study was that the changes observed in the ^{1}H NMR spectra for the titration of **46** with fluoride could not be fit to simple 1:1 or 1:2 binding models. Instead the fluoride was found to bind to **46** with 2:2 stoichiometry, which gave rise to the aforementioned 'twisted' geometry as confirmed by crystallography. The structure revealed that amide deprotonation had not occurred and that the anion binding was purely through hydrogen bonding.

Amide deprotonation has also been reported by Costero et al. who showed the selective detection of fluoride by **47** occurred through deprotonation of the amide moieties [63]. Liu and Tian have also observed such amide deprotonation in **48** [64]. They made several examples of naphthalimide-based sensors that are structurally similar to **28** and indeed showed significant colour changes upon deprotonation of the amide in CH_3CN. Moreover, as in the case of **23a**, **23b** and **28** the emission from the deprotonated molecule occurred in the red. Teramae and coworkers have also developed colorimetric sensors for anions using structurally simple urea and thiourea receptors that displayed significant changes in their absorption spectra upon binding acetate [65a,b]. Teramae and coworkers have developed this work further and demonstrated anion recognition in thiourea-based chromo-ionophores occurred *via* hydrogen bonding in aqueous vesicle solutions [65c]. Similarly, Fabbrizzi and coworkers have employed structurally similar urea based receptors such as **49** and **50** to demonstrate fluoride facilitated deprotonation and colorimetric changes [65d,e]. While the simple anion receptor **51** was developed by Gale and coworkers, and was shown to bind acetate selectively over dihydrogenphosphate and benzoate [66], the binding fluoride to **51** was not reported. Other examples of the use of such aryl thiourea moieties for anion sensing in organic chromophores, includes that of Das who used anthraquinones to achieve such sensing of fluoride, which most likely occurs through the formation of HF_2^- in solution [67].



Several other researchers have developed such colorimetric based anion sensors based upon similar deprotonation phenomenon. The pyrole-based receptor **52** was developed by Sessler and coworkers reported to undergo a yellow to purple colour change upon addition of fluoride. Whilst initially attributed to the binding of the anion to the receptor *via* the pyrrolic NH bonds [68a], in the view of the above discussion it is perhaps likely that this molecule is undergoing deprotonation. This work was recently elegantly extended by Sessler and coworkers by making fused dipyrrolylquinoxaline phenanthroline derivatives which allowed the incorporation of Ru(II) and Co(III) ions into these structures which concomitantly gave rise to long wavelength metal based (MLCT) detection of F^- [68b]. Similarly, Jurczak and coworkers have developed several amide containing macrocycles such as **53**, which contains two aromatic rings connected by an amide [69]. The association of **53** with various anionic guests was investigated by the ^{1}H NMR spectroscopic titra-

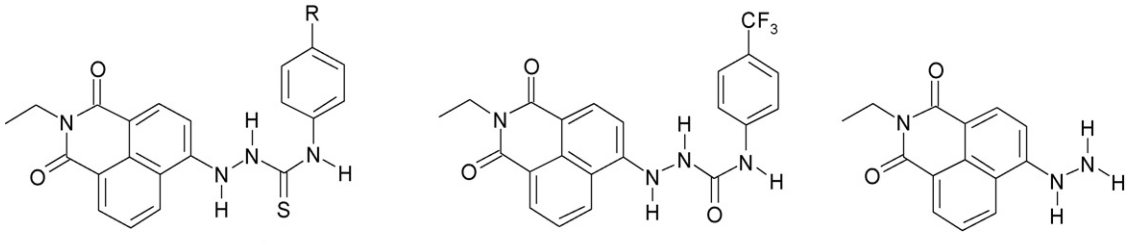
tion in DMSO-d_6 and the following selectivity trend established: $F^- \gg AcO^- > H_2PO_4^- > HSO_4^- > Cl^- \sim Br^-$. These data were fit to 1:1 binding models except in the case of fluoride, which appeared to have a 1:2 binding stoichiometry due to the deprotonation of the amide proton. Jiang and coworkers have developed several elegant examples of colorimetric and radiometric dual fluorescence anion sensors, *e.g.* **54**, based on the use of benzamide with conjugated thioureas [70]. These compounds generally give rise to long wavelength emissions which are modulated upon anion recognition. Hong and coworkers recently described the use of azophenol-based dyes as a chromophoric unit for the selective colorimetric detection of fluoride [71]. Although selective complexation and colouration of azophenol hosts with cations and amines had been reported before, the recognition of anions had not been seen previously. The azophenols **55a** and **55b** were prepared and their binding abilities with various anions were investigated by ground state spectroscopy. From the derived binding constants the following selectivity trend was obtained for **55a** in chloroform: $F^- \gg H_2PO_4^- > AcO^- > N_3^- > HSO_4^- > Cl^- > Br > I^-$. The binding constant for **55a** with F^- was found to be $1 \times 10^6\ M^{-1}$, and by using ^{19}F NMR spectroscopy, it was established that the phenolic proton had been deprotonated by F^-. The addition of one equivalent of F^- to **55a** and **55b** led to an increase in absorbance of the 615 nm and 632 nm bands, respectively. However, once an equivalent had been added no further increases were observed. In a related study, anion recognition was investigated using **56** [72]. This system can be considered as having two types of chromophoric units, where now the *p*-nitrophenyl group appended to the thiourea moiety acts as an additional chromophore enabling colour differentiation of anions in a cooperative manner along with the azophenol group. This modification enables colorimetric discrimination between $H_2PO_4^-$, F^- and AcO^-. Hong et al. have also developed a colorimetric sensor by making *para*-nitro phenyl aza derivatives of porphyrin-based ureas [73]. These compounds showed dramatic colour changes upon addition of F^- due to the increased interaction with the nitrophenylazo-phenolic OH group.

The pioneering work of Sessler demonstrated that it was possible to generate fluorescent calix[4]pyrroles as anion sensors [74a]. The fluorescent moieties used were dansyl, Lissamine-rhodamine B and fluorescein all of which were tethered into the calyx[4]pyrroles *via* a spacer. These labels show significant fluorescence intensity in aqueous solutions even at very low concentrations, but the anion sensing was carried out in acetonitrile (0.01%, v/v water) or in acetonitrile–water mixture (96:4). However, they were initially unable to prepare inherently coloured derivatives that could work as naked-eye anion sensors. They have since then discovered that attaching a chromophore to the calix[4]pyrrole skeleton in a conjugated manner allowed the colorimetric detection of certain anions to be facilitated [74b]. With this design in mind compounds **57**, **58** and **59** were prepared, differing only in the chromophore unit. The absorption spectrum of **57** in dichloromethane had two characteristic absorption peaks with λ_{max} at 357 and 467 nm. Upon the addition of F^- the peak at 467 nm decreased while a new peak at 518 nm appeared, resulting in a colour change from yellow to red. This large spectral shift indicates that the fluoride ion is most likely deprotonating the highly conjugated pyrrole unit. In comparison, the addition of Cl or $H_2PO_4^-$ also caused a colour change from yellow to reddish-orange, but required a greater addition of anion than in the case of F^-. Similar changes were also seen for **58** and the binding constants obtained yielded the following selectivity trend: $F^- > Cl^- > H_2PO_4^- \gg Br^- \sim I^- > HSO_4^-$. However, even though **59** was found to be an efficient receptor for F^-, Cl^- and $H_2PO_4^-$, from ^1H NMR and fluorescence spectroscopic analyses, it did not act as a naked-eye anion sensor. This work has continued in collaboration with Gale who has made several fluorescent analogues of such calix[4]pyrrole derivatives [75]. Recently, extension of this work by Nishiyabu and Anzenbacher Jr. through conjugation of a 1,3-indane chromophore into a calixpyrroles has given rise to push-pull colorimetric based sensing of anions [76].

The above examples clearly show that sensing of fluoride ions, where the recognition is achieved by initial hydrogen bonding to the receptor, can then lead to deprotonation. However, in the examples discussed the bonding only ever occurred in non-aqueous media such as DMSO. Moreover, in most cases the observed colorimetric changes were due usually to enhanced efficiency for internal charge transfer following deprotonation and were difficult to achieve for other anions. With the aim of achieving such colour changes for other anions as well as fluoride, and to achieve this in more competitive media, we set out to further modify our naphthalimide systems and the next section summarises these results.

5. Colorimetric naphthalimide anion sensors for use in organic and aqueous solutions

As detailed in Section 3, we had discovered that deprotonation of the 4-amino moiety of the naphthalimide-based molecules **23a**, **23b** and **28** gave rise to colorimetric changes in the ICT based chromophore. We then set out to modify these basic structures to enable colorimetric sensing of anions by incorporating a receptor directly at the four-position. Compounds **60**, **61** and **62** are examples of such colorimetric anion sensors [77,78]. These easily prepared compounds were synthesised in a few steps by reacting *N*-ethyl-4-bromo-1,8-naphthalimde with hydrazine mono-hydrate to give **63** which when reacted with either 4-(trifluoro-methyl)phenyl- or 4-(methyl)phenyl-isothiocyanate or 4-(trifluoromethyl)phenyl-isocyanate gave **60**, **61** and **62**, respectively. Iso-structural single crystals of **61**, suitable for an X-ray diffraction study, were grown from methanol, ethanol or propanol, and the resulting structures are shown in Fig. 13. The thiourea protons are in the *anti*-conformation and adjacent molecules participate in numerous intermolecular interactions such as π–π interactions and hydrogen bonding. If the *anti*-conformation was to be maintained in solution then the binding of anions at the thiourea moiety might be perturbed.



In a similar way to that described for **28** and related structures we carried out detailed spectroscopic analyses of **60–62** when titrated with various anions. All the compounds gave rise to strong absorption bands in the visible region, centred at *ca.* 414 nm with a smaller shoulder at *ca.* 560 nm, which was more pronounced for the thiourea derivatives. In the case of **61**, upon addition of anions such as AcO^- or $H_2PO_4^-$ significant changes were observed in the absorption spectra. The absorption band at 414 nm reduced in intensity with concomitant enhancement in the 560 nm transition and the formation of a new band at 350 nm with two clear isosbestic points being observed at 465 and 380 nm, Fig. 14A. In a similar manner large changes were seen in the absorption spectra for the titration using F^-, as can be seen for **62** in Fig. 14B. These changes were also clearly visible to the naked eye as the solution changes from green/yellow to purple, as can be seen for **68** in DMSO upon addition of various anions, Fig. 15. Moreover, excitation of the newly formed long wavelength absorption band gave rise to red fluorescence emission, indicating that the changes could also be observed by fluorescence. In related work, Liu and Tian have recently described fluorescent sensors for anions that show identical behaviour in CH_3CN [79]. Here, the fluorescence emission was quenched upon anion recognition and a new band was observed at longer wavelengths in a similar manner to that described above. Related work has also been carried out by Fabbrizzi et al. [80].

Analysis of these titration data using non-linear regression analysis showed that the observed changes were due to 1:1

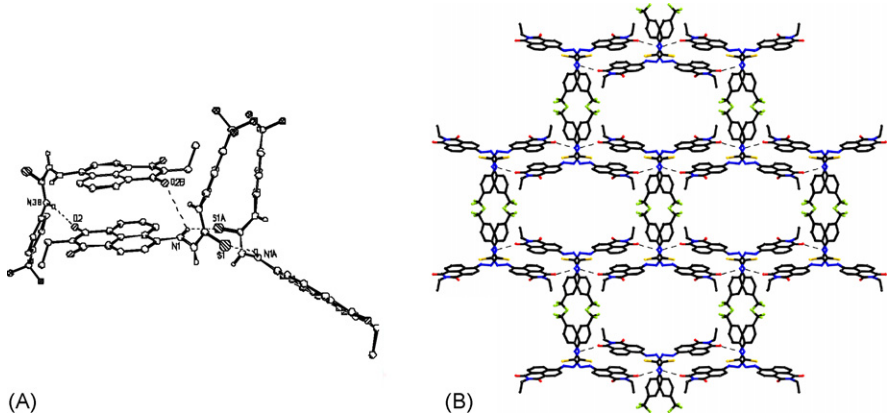


Fig. 13. (A) Hydrogen bonding and π stacking between molecules of **61**. (B) 3D crystal network of **61** looking down the *z*-axis. Ethanol molecules located inside the cavities have been omitted for clarity.

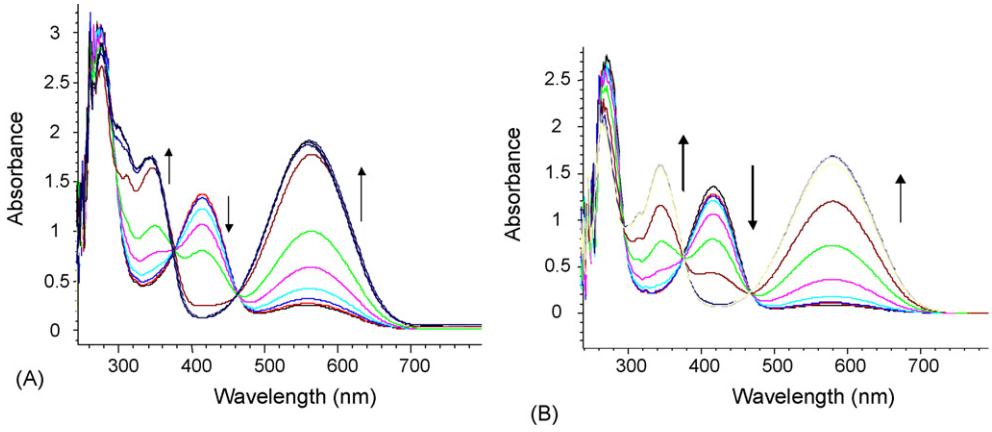


Fig. 14. (A) The changes in the absorption spectra of **61** upon titration with acetate in DMSO. (B) The changes in the absorption spectra of **62** upon titration with fluoride in DMSO.

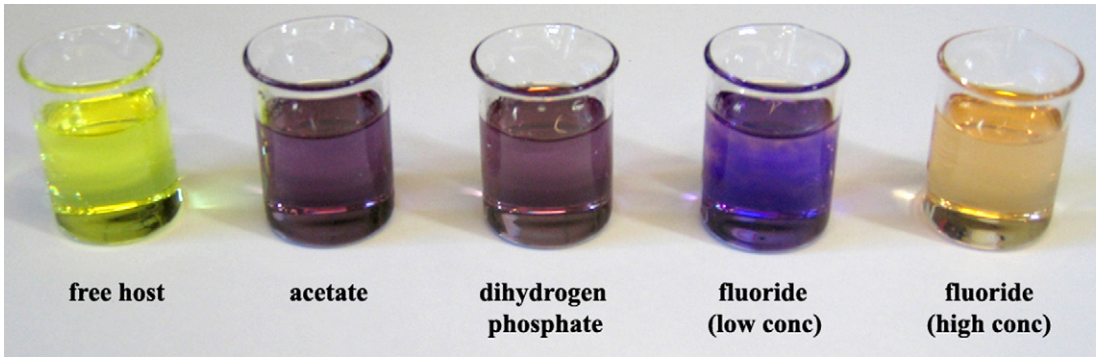


Fig. 15. The changes in colour of **68** in DMSO upon addition of several anions, clearly showing the ability of these to function as naked eye sensors for anions.

binding for AcO^-, $H_2PO_4^-$ or F^- to **60–62**. Receptor **61** bound acetate and fluoride with binding constants of log $\beta = 4.95$ (± 0.1) and log $\beta = 4.4$ (± 0.1), respectively, whereas **62** bound fluoride with log $\beta = 4.3$ (± 0.1). This indicates that, firstly the ICT nature of naphthalimide unit possibly has an 'overriding effect' over the phenyl part and secondly, that the changes observed in the absorption spectra were due to hydrogen bonding interactions between the urea or thiourea moiety of the sensor and the anions. In the case of **63** no such spectral changes were observed upon titration with AcO^- or $H_2PO_4^-$ and these results clearly support our theory. These measurements were all carried out in the concentration of $\sim 1 \times 10^{-4}$ M of the sensor as at lower concentrations, below $\sim 1 \times 10^{-5}$ M, the long wavelength absorption band was more pronounced. However, in the case of **62** no such concentration dependence was observed and similar changes were indeed observed for **62** in the absorption spectra upon titration with acetate as seen previously seen for both **60** and **61** above. When other anions such as chloride or bromide were tested, no colour changes were observed.

The addition of large excess of anions such as AcO^- or $H_2PO_4^-$ to **60–63** did not give rise to any additional changes in the absorption spectra. However, when excess of F^- was used additional colour changes were observed that we attributed to the deprotonation of the 4-amino-naphthalimide moiety and the formation of bifluoride (HF_2^-), as previously discussed. To investigate these interactions further, we carried out ^{1}H NMR (DMSO-d_6) analysis of the anion recognition. As expected, by monitoring the changes associated with the thiourea protons that initially appear at *ca.* 10.0–10.1 ppm, and the aromatic N–H proton at *ca.* 9.9, the binding of anions was clearly evident. For titrations with AcO^- the aromatic N–H proton was also visible as a broad signal appearing at *ca.* 12 ppm, but after *ca.* 0.8 equivalent of AcO^- it had disappeared, Fig. 16. Integration of the newly formed signals confirmed the 1:1 binding seen in the absorption spectra. For F^-, similar changes were observed in that the N–H resonances disappeared with the formation of new resonances within the addition of one equivalent. Integration of these showed that the anion recognition was in 1:1 stoichiometry. However, upon further additions the ^{1}H NMR spectrum changed dramatically as can be seen in Fig. 17. After *ca.* two equivalents the N–H resonance had shifted downfield and the formation of a broad resonance located similarly to where the HF_2^- is observed becomes visible. After *ca.* four equivalents the characteristic triplet for HF_2^- is observed. This possibly indicates that two deprotonation phenomena have occurred.

The addition of excess anion also gives rise to large changes in the absorption spectra. The changes previously observed during the acetate titration in Fig. 16 are now worth reinvestigating. There the thiourea protons were dramatically affected. While one of the thiourea protons shifted downfield to 11.2 ppm in the bound form the other shifted upfield to 8.05 ppm. The amino proton, shifted upfield to 11.97 ppm, but disappeared approaching one equivalent of AcO^-. The former of these is also clearly visible in the titration of **61** with F^-, but gradually disappears upon addition of increasing equivalents of F^-. In fact, after *ca.* five equivalents of F the resonance is dramatically broadened, at the same time as the HF_2^- resonance becomes clear. Furthermore, the second thiourea proton has disappeared as well and the aromatic region is much simpler. We thus propose that in the case of **61**, two deprotonation steps occurred, where the first one is associated with the deprotonation of the naphthalimide N–H moiety, while the second one occurs at the thiourea moiety. These results show that ICT based colorimetric sensors can be developed based on the naphthalimide moiety, where the anion sensing occurs thorough hydrogen bonding, and is not necessarily dependant on the deprotonation of labile protons.

Having established that the anion recognition occurred by hydrogen bonding, we investigated the reversibility of the ion sensing by adding ethanol to the DMSO solutions. In the case of **62** the colour changes were immediately reversed. However, for both **60** and **61** the colour changes were not reversed. Because of this we carried out titrations of **60** and **61** in ethanol. The changes observed for **61** can be seen in Fig. 18A. These results mirror that observed in DMSO where the absorption band assigned to the ICT band was reduced in intensity with the formation of a new band centred at 404 and 540 nm, respectively, at high concentrations. At lower concentrations, the main bands appear at 340 and 540 nm, respectively. Two clear isosbestic points were also visible at 368 and 454 nm. Similar results were seen for both F^- and $H_2PO_4^-$. Of the anions tested in ethanol AcO^- yielded the strongest binding with a log $\beta = 4.47$ (± 0.1). Furthermore, excitation of the ICT band initially gave rise to a broad

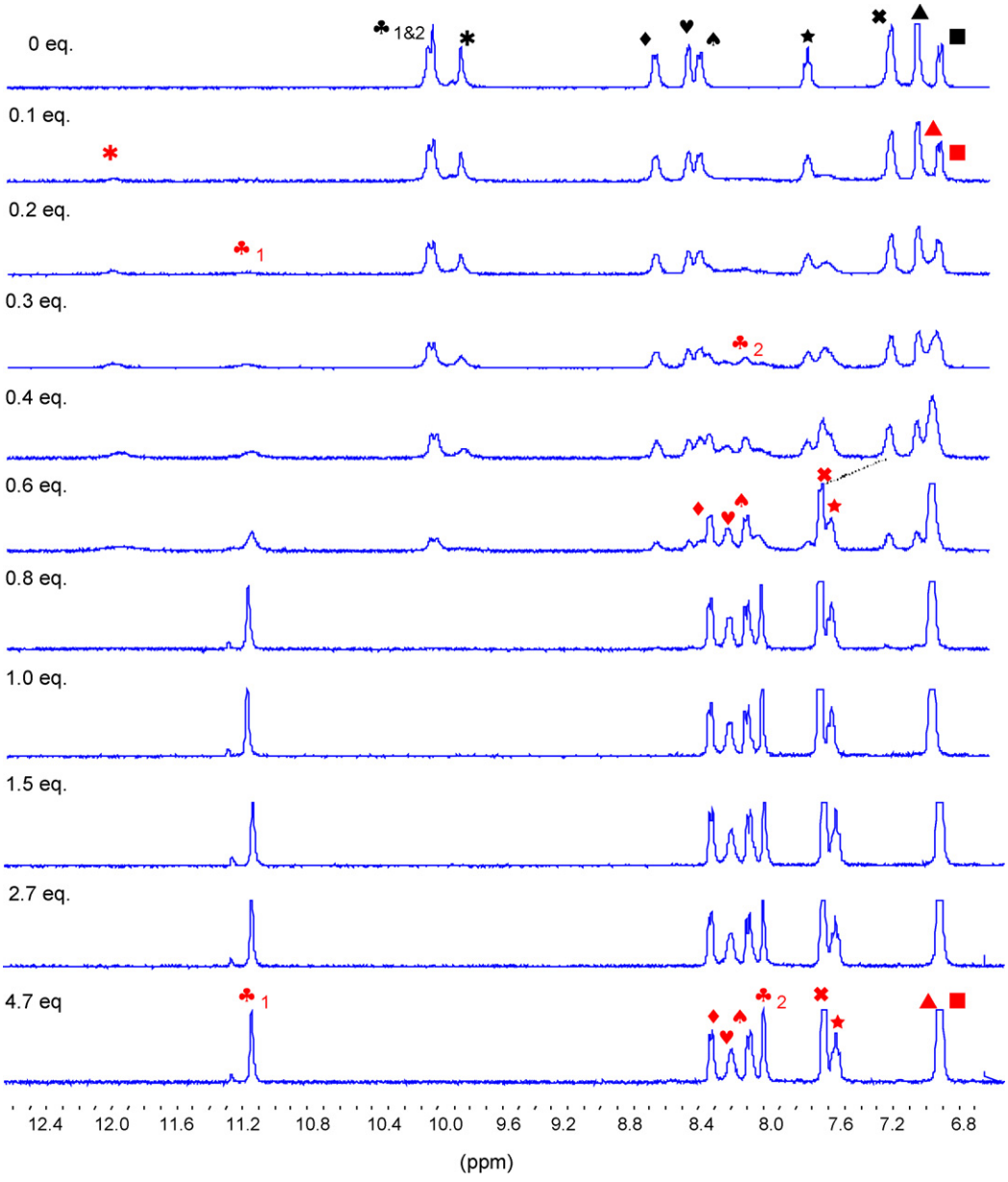


Fig. 16. Stack plot of ^{1}H NMRs of **61** on addition of AcO^- (DMSO-d_6, 400 MHz)—black symbols show position of resonance before addition of anion, red symbols show position of resonance upon binding.

band centred at 440 nm and this emission was quenched upon titration of acetate in a similar manner to that seen in DMSO, Fig. 18B. Moreover, the addition of F^- to a solution of **61** in ethanol showed the yellow–orange to purple colour change as previously described in DMSO. However, the second colour change, previously observed in DMSO upon addition of high concentrations of the anion was not observed, clearly indicating that no deprotonation occurred. These successful outcomes are extremely significant as they demonstrate that anion sensing is possible using charge neutral receptors in competitive media.

Unfortunately, these sensors were not soluble in pure water. However, in a 90:10 mixture of water/ethanol, colour changes were observed upon addition of acetate. Because of these results we turned our investigation to the use of ethanol/water 50:50 as solvent and the same general changes in colour and absorption spectra were seen as for the titrations in neat ethanol. Using this solvent system, the main absorption bands were seen at 407 and 548 nm at high concentrations of **61**, and at 339 and 548 nm at low concentrations. For the AcO^- titration isosbestic points at ~367 and 458 nm were observed, while the log β value obtained was 3.4 (±0.1). The titration of F^- with **61** in ethanol/water 50:50 yielded a log $\beta = 2.2$ (±0.1). The changes in absorption spectra are shown in Fig. 19A. The addition of $H_2PO_4^-$ to **61**, did begin to turn the solution purple, but the changes only began to occur at very high concentrations of anion and a binding constant could not be determined, however it can be deduced from the curve in Fig. 19B that it would be ~log $\beta = 1$. Fig. 19B also shows that in this mixed solution **61** shows a clear selectivity for acetate. Similar results were observed for **60**.

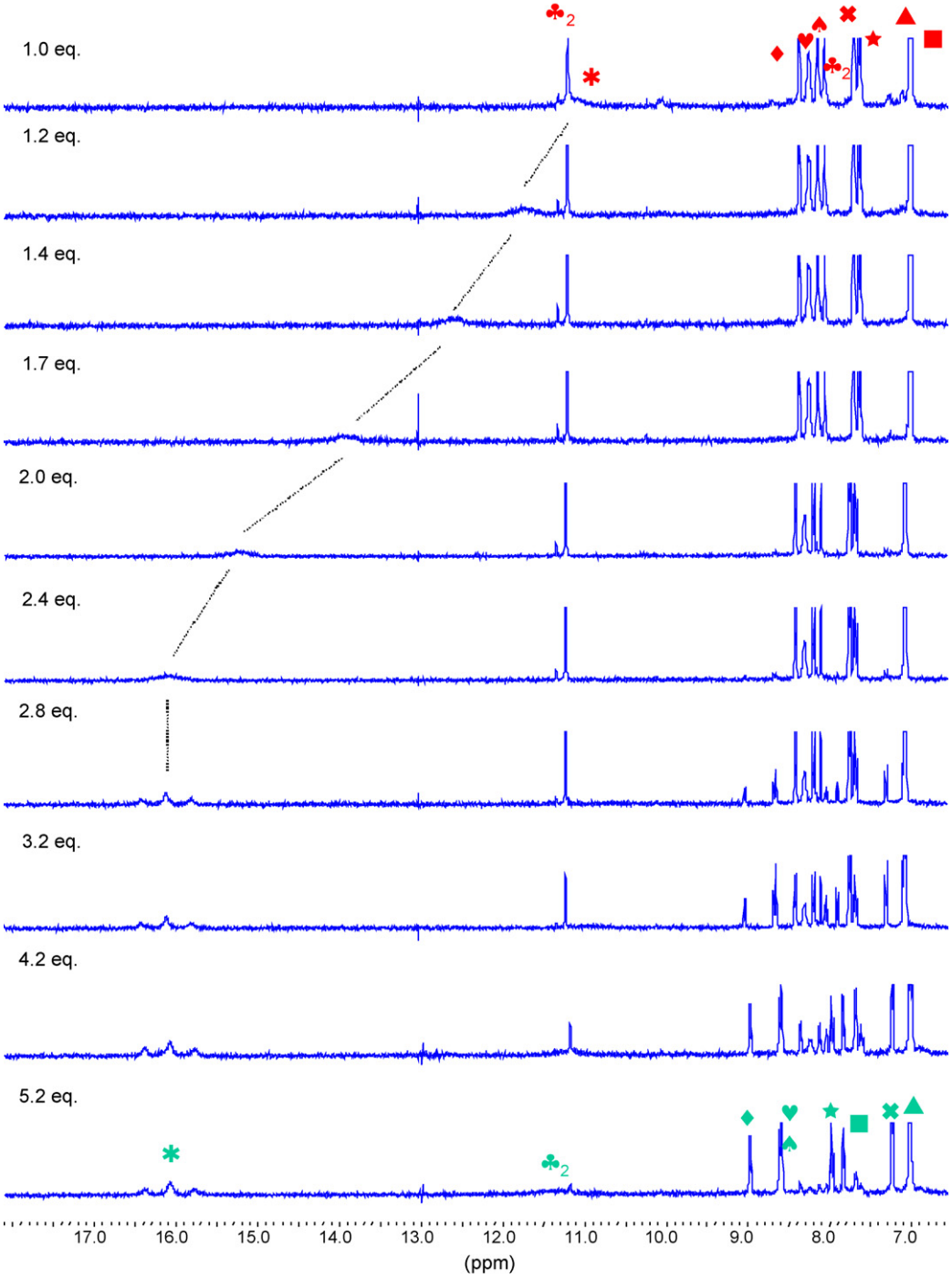


Fig. 17. Stack plot of ^{1}H NMRs of **61** with F^{-} (1–5.2 equivalents, DMSO-d_6, 400 MHz).

To exclude the effect of pH upon these changes, we also carried out these measurements in buffered solution at pH 7.3 and 7.6 for **61** and **62**, respectively. Again the same trend as was seen in non-buffered solution was observed and no significant changes (less than 0.1) were observed in the pH during the titration, excluding the possibility that the changes observed to the naked-eye were due to modulation of pH. To further verify this, we also carried out a pH titration upon both **60** and **61**. Even though both showed pH dependent changes in the absorption spectra, that were of similar nature to that seen for the anion sensing, these occurred at more alkaline pH, and as such support our suggestion that the spectral changes seen in both DMSO and EtOH as well as in the mixed solvent systems are due to hydrogen bonding recognition of these anions and not due to 'environmental' pH changes. The results presented in this section clearly demonstrate the feasibility of the use of charge neutral anion receptors in mixed aqueous media, which is a significant steppingstone towards real

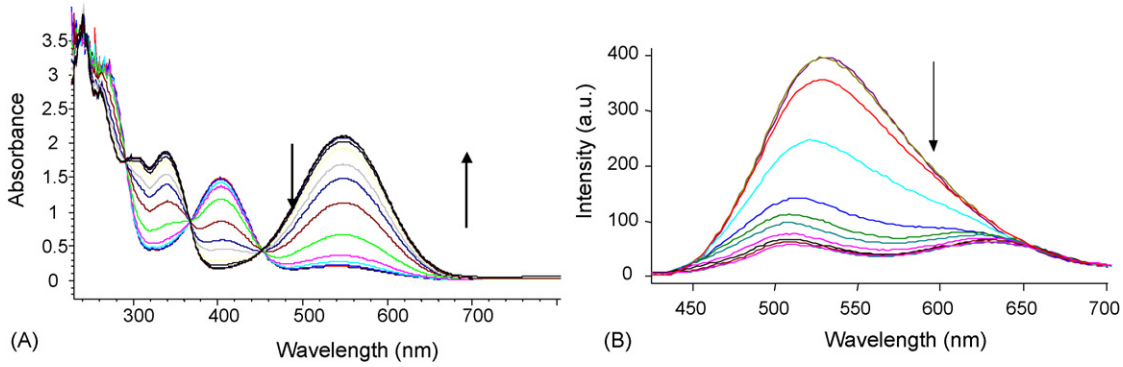


Fig. 18. (A) The changes in the absorption spectra of **61** in ethanol upon titration with AcO^-. (B) The changes in the fluorescence emission spectra of the ICT exited state transition in ethanol.

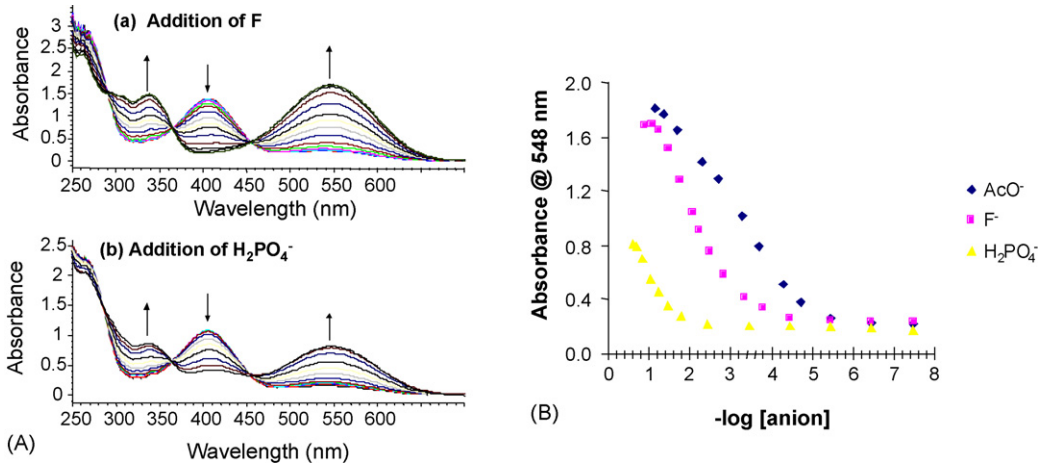


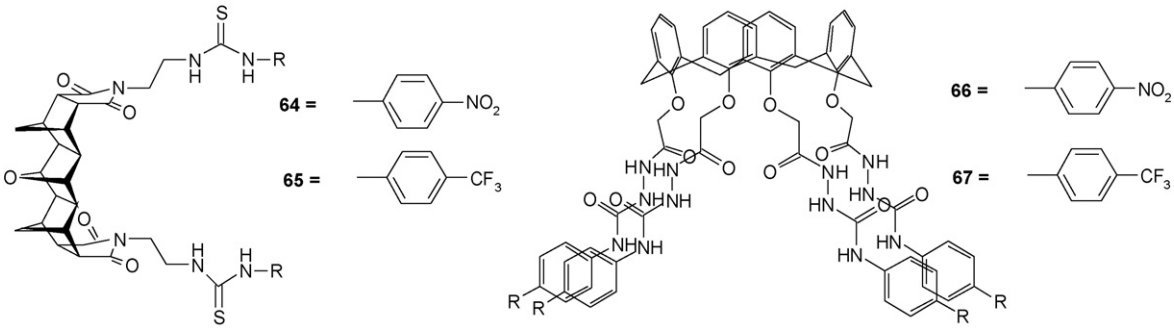
Fig. 19. (A) The changes in the absorption spectra of **61** in 1:1 water:ethanol mixture for (top) F^- and (bottom) $H_2PO_4^-$. (B) The changes in the absorption spectra at 548 nm, in the same solvent mixture for F^-, AcO^- and $H_2PO_4^-$.

application, work that is currently underway in our laboratory.

6. Formation of anion directed self-assemblies

The previous sections have dealt with the formation of either luminescent or colorimetric sensors for anions. In most cases the binding of the anion to the host was in 1:1 stoichiometry. Also the selectivity has usually been in the order of $F^- > AcO^- > H_2PO_4^-$. We were interested in expanding our design principle to selective detection of $H_2PO_4^-$ over AcO^-. In a way this was partly achieved for compounds **24** and **25**, through cooperative binding of the 4-amino-l,8-naphthalimide moiety. Moreover, the binding of pyrophosphate had also been achieved in 1:1 binding for **14**, **15** and **21**. With this in mind we set out to incorporate our aryl based thiourea moieties into rigid preorganised structures with the aim of achieving selectivity binding of $H_2PO_4^-$ and pyrophosphate. Molecules **64** and **65** are examples of our design, based on the use of the [*n*]polynorbornane framework [81], while compounds **66** and **67** (and other related compounds from our laboratory) are examples of the use of calixarine scaffolds, [82] but such urea or thiourea based molecules have previously been used by several researchers such as Reinhoudt and coworkers and [83] Bohmer and coworkers [84] to name just a few, for the formation of anion or hydrogen bonding based supramolecular self-assemblies and sensors [85]. The hydrogen bonding moieties have recently also been used very elegantly by Steed and coworkers in such acyclic analogues [86].

The synthesis of **64** and **65** was accomplished in several steps beginning with the Diels–Alder cycloaddition of cyclopentadiene with maleic anhydride to afford the corresponding *endo*-norborn-5-ene-2,3-dicarboxylic anhydride, which upon heating in neat 1,2-diaminoethane and treatment with di-*tert*-butyldicarbonate gave the boc-protected amine. The use of the Mitsudo reaction followed by a modified Weitz–Scheffer epoxidation gave the fused epoxide flanked by two methyl ester groups. This arrangement is crucial as it predisposes the scaffold so that subsequent cycloaddition occurs from 'below', which ensures the formation of the preorganised receptor. Subsequent modification of this structure, using a 1,3 dipolar cycloaddition reaction gave the desired [3]polynorbornane framework. Deprotection of the amines using TFA and reaction with 4-trifluoromethyl-phenylisothiocyanate and 4-nitrophenylisothiocyanate gave receptors **64** and **65**, respectively.



The anion binding of these receptors was monitored using ^{1}H NMR spectroscopy in DMSO-d_6, where each was titrated with AcO^-, F^-, $H_2PO_4^-$, $HP_2O_7^{3-}$. Significant changes were seen for the thiourea protons in addition to changes observed in the aromatic protons upon anion addition that could also be used to monitor the anion recognition. In general, the spectral changes indicated that the anion recognition was in fast exchange where the thiourea N–H's protons were shifted up to 3 ppm. These observations are consistent with the anion interacting with the thiourea protons through hydrogen bonding, as we have previously discussed. As expected, the binding of AcO^- was in 1:2 (host–guest) with log β_1 and log β_2 of 3.5 (±0.1) and 2.4 (±0.1), respectively, for **64** and 3.5 (±0.1) and 3.0 (±0.1), respectively for **65**. For $H_2PO_4^-$, however, the binding was determined to be of a 1:1 stoichiometry, with log β values of 3.9 (±0.1) and 3.6 (±0.1), respectively, for these receptors. These indicate that both of the thiourea receptors partake in the binding which is also significantly stronger than that of AcO^-, clearly indicating that the design principles employed here, worked. However, the most interesting results were that of the binding of these cleft like molecules to pyrophosphate. As can be seen in Fig. 20A, the changes in the ^{1}H NMR spectrum for the thiourea protons clearly indicated that the binding of $HP_2O_7^{3-}$, was in 2:1 stoichiometry (host–guest). Analysis of the titration data showed also that the resulting structure was formed with high stability constants of log β = 7.9 (±0.1) and 7.8 (±0.1), for **64** and **65**, respectively. To achieve such binding, *two* molecules of each of the caged ligands must bind a *single* $HP_2O_7^{3-}$ anion in a way that might be viewed to give a sandwich complex as shown schematically in Fig. 20B. Interestingly, these complexes are so stable that the addition of large equivalents of pyrophosphate did not shift the equilibrium of this self-assembly towards the 1:1 stoichiometry. Both receptors were also tested for their affinity towards fluoride. In both cases the formation of the bifluoride was observed in the ^{1}H NMR spectrum. These changes were also clearly visible to the naked eye, where a yellow to red colour changes occurred.

With the aim of extending this kind of work towards larger anion directed self-assemblies, we synthesised **66** and **67**, and also the 1,3-disubstituted calix[4]arene analogue of **66**. These were formed in high yields in two step synthesis from the corresponding tetra or di-ethyl esters of calix[4]arene. Both compounds have shown to be able to complex anions through hydrogen bonding as described above. Compound **66** is based on a receptor employed by Teramae as a colorimetric sensor for acetate. The ICT based sensor gave rise to significant changes in the absorption spectra upon anion titration, being shifted to longer wavelengths upon anion coordination. In the case of AcO^-, the binding was in found to be in 1:2 stoichiometry (host–guest), indicating a cooperative binding of acetate, by neighbouring thiourea receptors. This binding was also quite strong with log β values of 5.11 (±0.1) and 4.4 (±0.1), for **66**. For $H_2PO_4^-$, again the 1:2 stoichiometry was observed, with log β values of 5.5 (±0.2) and 4.9 (±0.2). This is currently under further investigation.

7. Conclusion and future work

This review gives account of the work carried in our laboratories in Dublin and Geelong, but also that of other researchers that have contributed to this fast growing field of research. Our objective throughout our work has been to establish the proof of principle for anion sensing using luminescence and colorimetric techniques. The examples discussed above have demonstrated our successes in these areas. We have shown that anion sensing can be achieved using charge neutral PET sensors and that the

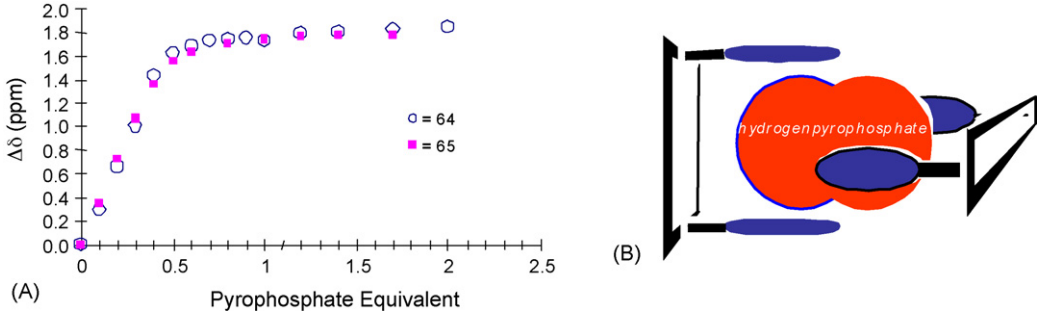


Fig. 20. (A) The change in one of the thiourea resonances for **64** and **65**, respectively, upon titration with pyrophosphate, clearly showing the formation of the 2:1 stoichiometry. (B) Cartoon of the formation of the resulting 2:1 self-assembly complex.

incorporation of such urea/thiourea into different chromophores or fluorophores can give rise to either luminescent or colorimetric sensing of anions in the visible region. Often these changes are clearly visible to the naked eye, which can give rise to the formation of potential 'test-kits' for 'on-the-ground' or 'at-scene' anion sensing. This work has in more recent time, directed us towards using anions to construct/direct the formation of supramolecular self-assemblies, which can be analysed using various spectroscopic techniques. From this work and that of our colleagues and friends, it is obvious that the future of anion recognition/sensing is bright and many new structures, structural motives and applications are still yet to be fully explored. We hope to be able to participate in those future endeavours.

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