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Fluorescent Photoinduced Electron Transfer (PET) Sensors for Anions; From Design to Potential Application

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This mini review highlights the synthesis and photophysical evaluation of anion sensors, for nonaqueous solutions, that have been developed in our laboratories over the last few years. We have focused our research mainly on developing fluorescent photoinduced electron transfer (PET) sensors based on the *fluorophore-spacer-anion receptor* principle using several anthracene (emitting in the blue) and 1,8-naphthalimide (emitting in the green) fluorophores, with the aim of targeting biologically and industrially relevant anions such as acetates, phosphate and amino acids, as well as halides such as fluoride. The receptors and the fluorophore are separated by a short methyl or ethyl spacer, where the charge neutral anion receptors are either aliphatic or aromatic urea (or thiourea) moieties. For these, the anion recognition is through hydrogen bonding, yielding anion:receptor complexes. Such bonding gives rise to enhanced reduction potential in the receptor moieties which causes enhancement in the rate of PET quenching of the fluorophore excited state from the anion:receptor moiety. This design can be further elaborated on by incorporating either two fluorophores, or urea/thiourea receptors into the sensor structures, using anthracene as a fluorophore. For the latter design, the sensors were designed to achieve sensing of bis-anions, such as di-carboxylates or pyrophosphate, where the anion bridged the anthracene moiety. In the case of the naphthalimide based mono-receptor based PET sensors, it was discovered that in DMSO the sensors were also susceptible to deprotonation by anions such as F^- at high concentrations. This led to substantial changes in the absorption spectra of these sensors, where the solution changed colour from yellow/green to deep blue, which was clearly visible to the naked eye. Hence, some of the examples presented can act as dual fluorescent-colorimetric sensors for anions. Further investigations into this phenomenon led to the development of simple colorimetric sensors for fluorides, which upon exposure to air, were shown to fix carbon dioxide as bicarbonate.

KEY WORDS: Anions; sensing; chemosensors; PET; fluorescence; acetate; phosphate; halides; fluoride.

INTRODUCTION

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 [1]. Anions are also increasingly important for many industrial processes as well as in agriculture, and consequently have become important pollutants [2]. It has thus become evident that there is significant need for developing novel molecules that can interact with anions, and report their presence, and ideally at the concentrations of anions in complex media such as in blood or serum, cells, soil, freshwater, *etc*. Despite the enormous advances that have taken place in the field of chemistry, particularly due to the development of supramolecular chemistry, the field of anion recognition and anion luminescence sensing has been relatively unexplored, and indeed it is only in the last a few years that it has become a fast growing field

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of research [3,4]. The reason for this, without a doubt, is the complex nature of the anions (in comparison to many cations) that has to be accounted for when designing anion-selective and sensitive luminescent probes. By definition, except for the anions AlH_4^-, B^- $(C_6H_5)_4B^-$ and *closo*-$B_{12}H_{12}^{2-}$, all anions have lone pairs of electrons. This Lewis basicity is the second most important feature of anions to be exploited in the construction of molecular hosts and sensors. It may add directionality to the system and therefore render it sensitive to the spatial arrangement and orientation of binding groups. This is an indispensable screen to differentiate between anions of similar size, structure and charge, e.g. phosphate and sulphate anions. The shape of the anion can thus be used advantageously in the design of a potent yet selective receptor. This is not just because of the size of the anion, as anions exhibit a wide range of geometries, which challenge the molecular designer to create a complementary binding site. For instance, the halides are spherical, their lone electron pairs do not introduce directionality to the system and are thus difficult to exploit in receptor design. However, the cavity size of a halide receptor may instead be manipulated, to introduce selectivity. The halides are all monatomic and the receptors discussed in this paper indeed fulfil the criteria discussed above, where large spherical anions such as chloride and bromide are not recognised/sensed, while fluoride is. Competition between the anion and the competitive solvent for binding sites makes sensing of anions more difficult than for cations of similar size, as anions are usually highly solvated. A vigorous analysis of solvent effects on the thermodynamics of the binding process for these systems was recently carried out, and it was determined that in less polar solvents than water, i.e. DMSO, complexation/recognition is enthalpically driven [5]. However, in methanol and methanol/water mixtures association becomes endothermic with favourable entropy providing the driving force for association. Thus, it is not surprising that most anion sensors today have been designed to sense anions in less competitive environments such as in DMSO and $CHCl_3$ [6].

Considerable effort has been recently directed toward the development of synthetic receptors that depend solely on hydrogen bond arrays [7]. The main virtue of incorporating H-bond donor groups into a receptor is to conserve electroneutrality. The range of hydrogen bond donor groups available (e.g. carbamates, ureas, thioureas) offers extreme versatility of construction. This versatility offers limitless options in receptor design, but the effectiveness largely depends on the extent of solvation. Anion recognition in biological systems is achieved *via* hydrogen bonding by highly preorganised proteins containing sterically well-defined complexation sites in the interior of the protein. The main challenge in the field of anion complexation has been the design of receptors with a high selectivity for biologically important anions for example; phosphates, (poly) carboxylates, and halides, especially chloride [8]. While few molecular details are known about anion specific ion pumps, a passive antiport system HCO_3^-/Cl^- important for respiration (CO_2 disposal) was identified in erythrocytes. The relatively common hereditary disease, cystic fibrosis, is known to stem from a genetically caused mis-regulation of chloride channels. This example illustrates the medicinal incentives for developing a sensor selective for chloride. Moreover, the oxoanions (carboxylates, phosphates, and sulphates) are of particular biological interest, while di- and tri-carboxylates are critical components of numerous metabolic processes including for instance, the citric acid and glyoxylate cycles [9]. They also play an important role in the generation of "high energy" phosphate bonds, as nucleotide polyphosphates are the basic components in the bioenergetics of all living organisms. From this account it is obvious that the challenge of developing sensors for anions is not trivial. However, this was in fact the main reason of our interest in the field of anion recognition and we asked ourselves, "can we develop anion selective and sensitive sensors using structurally simple receptors?" and secondly, "can we incorporate such anion receptors into luminescent sensors?"

We had previously developed both luminescent sensors for anions where the recognition occurred at a lanthanide metal ion centre [10], or by quinoxaline-based anion sensor [11]. Our choice was to further extend this field and develop fluorescent anion sensors, and the choice of method was PET [12], but PET anion sensing had not, prior to our work, been established using charge neutral anion receptors. However, examples of charged receptor moieties such as metal ions or protonated amines had been developed, for use in PET sensors [12,13].

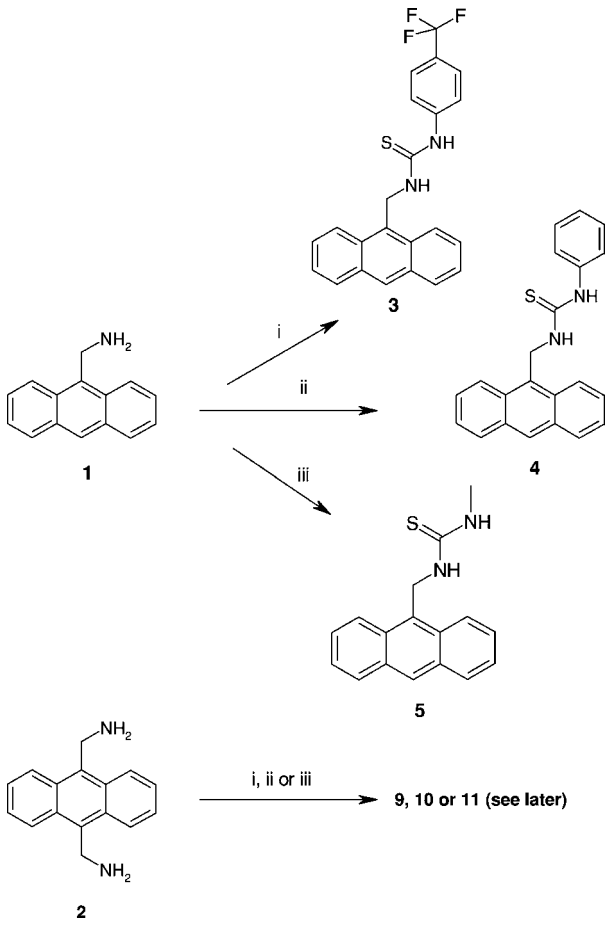
This mini review discusses our attempts to develop such sensors in chronological order, beginning with the formation of simple PET sensors, based on the use of anthracene, followed by our attempts to develop PET sensors that absorb and emit in the visible region and finally the formation of colorimetric anion sensors where anion sensing is visible to the naked eye.

FLUORESCENT PET SENSORS BASED ON ANTHRACENE

Over the last few years, many excellent examples of anion sensing have been demonstrated. Some of this work

has been reviewed in several articles that generally give good account of the advantages that have occurred within this field in a relatively short time [3,4]. However, when we set out to develop anion sensors, only a few examples of such luminescent anion sensors had indeed been reported, and most of these were designed using charge receptors, or as in the work of Beer *et al*., the receptors were charge neutral but were part of cationic metal complexes and as such often highly influenced by the presence of the metal ion, e.g. through inductive effects, etc. [14]. As stated above, we set out to use structurally simple receptors, our target being urea and thiourea, similar to those developed by Hamilton *et al*. [15], Umezawa *et al*. [16] and Kelly *et al*. [17] who individually developed several receptors for the detection of anions such as acetate, using NMR to observe the recognition process. The use of PET in chemical sensing was established by de Silva and Czarnick less than 20 years ago [12]. These researchers showed that by incorporating amines into the anthracene structure *via* a methyl spacer, the fluorescence of the fluorophore could be significantly enhanced, or '*switched on*', in the presence of a proton, without any significant changes in the wavelength of the emission, e.g. only the quantum yield and emission lifetimes were effected. This was simply due to the prevention of photoinduced electron quenching of the anthracene excited state by the electron rich amine upon protonation of the same, but upon protonation, the oxidation potential of the amine increased, making PET thermodynamically unfavourable. This was shown by simply employing the Rehm-Weller equation for determining the free energy of electron transfer [18]. Furthermore, for such ideal PET behaviour, the absorption spectra of the fluorophore should be independent of the recognition process, as the receptor was not an integrated part of the fluorophore.

In a similar manner, we foresaw that the reduction potential of the receptor would be modulated upon anion recognition, provided that the binding was strong. Our strategy shown schematically below was quite simple. We designed two sets of fluorescent anion sensors, based on the *fluorophore-spacer-anion receptor* principle, where the receptor is now a hydrogen-bonding acceptor. This was achieved by simply using anthracene and incorporating thiourea receptors at the 9th position on the anthracene ring (**3–5**) or at the 9th and 10th positions (**9–11**), *via* methylene spacers [19,20]. The advantage of this design became clear as by using 9-aminomethylanthracene **1**, or 9,10-diaminomethylanthracene **2** the amino moieties could be reacted with a wide range of commercially available isothiocyanates to form the respective thiourea receptor.



Our first examples of this design were the three mono-thioureas **3–5**. By simply reacting **1**, in dry CH_2Cl_2 at room temperature, under inert atmosphere with an equimolar amount of 4-(trifluoromethyl)phenyl (i), phenyl (ii) and methyl isothiocyanate (iii) respectively, compounds **3–5** were formed as off-white solids that were purified by recrystallisation from $CHCl_3$. The three different isothiocyanates were chosen with the aim of being able to modulate, or tune, the acidity of the thiourea receptor moiety, which would lead to different receptor-analyte complex stability and hence different binding constants. The synthesis of the starting material **1**, was achieved by several different methods, including conventional Gabriel amine synthesis, reduction of 9-cyanoanthracene, by sodium borohydride, lithium aluminium hydride and diborane, or by the conversion of 9-bromomethylanthracene to **1**, involving the use of hexamethylenetetramine. This latter method was superior to any of the former and produced the desired amine in good yield (80–90%) as the hydrochloride salt. The three sensors all exhibit simple ^{1}H NMR spectra in various solvents and the thiourea protons were easily identified due to their broad appearance

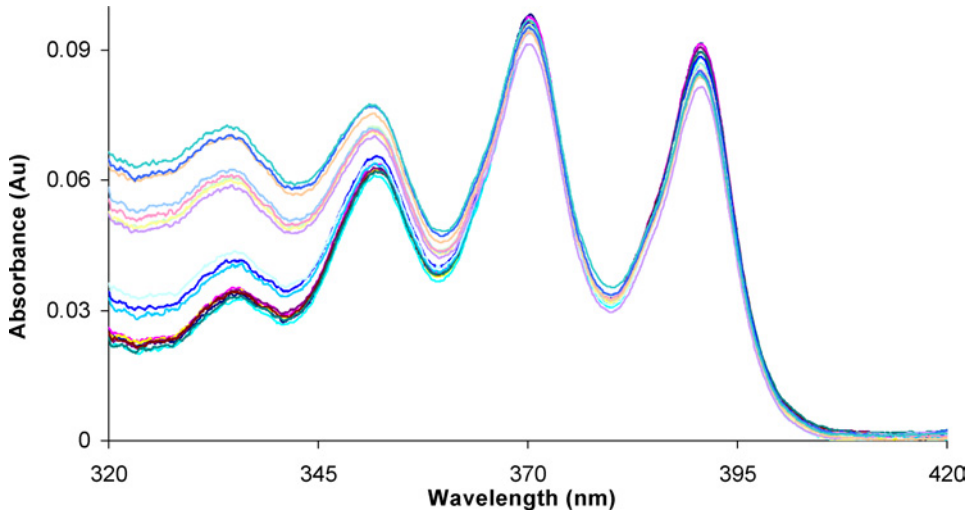


Fig. 1. Changes in the absorption spectra of **3** upon addition of acetate in DMSO.

and their downfield positions. Consequently, this also allowed for the use of ^{1}H NMR techniques to evaluate the stoichiometry of the binding interactions and for the determination of the binding constants. This will be discussed later.

The ground and the excited state properties of the three sensors were evaluated in various solvents, including DMSO, CH_2Cl_2 and CH_3CN. The results from DMSO will be the focus of the discussion herein. The absorption spectra of all the sensors were similar to the reference compound 9-methylanthracene, with absorption bands at 390, 370, 352 and 336 nm respectively for **3** (Fig. 1). When the 370 nm transition was excited, a typical anthracene emission was observed with peaks at 443, 419 and 397 nm, and a shoulder at 473 nm, and a quantum yield of 0.1. For **4** and **5**, similar results were observed with quantum yields of 0.187 and 0.304, in comparison to 9-methylanthracence, which was recorded to have quantum yields of 0.284. Upon titrating a solution of any of these sensors with anions such as Cl^- or Br^- no changes occurred in either the absorption or the fluorescence emission spectra. However, using anions such as acetate, phosphate or fluoride, the emission was dramatically affected, being '*switched off*' by up to 90%, upon anion addition, e.g. Fig. 2, for phosphate, with ~70% quenching. In retrospect the changes in the absorption spectra were only

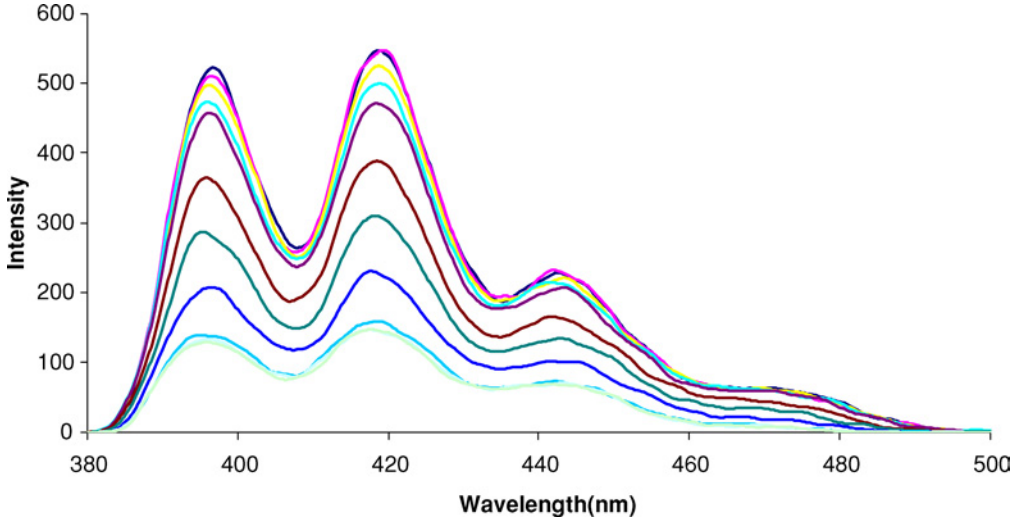


Fig. 2. Changes in the fluorescence emission of **3** upon addition of phosphate.

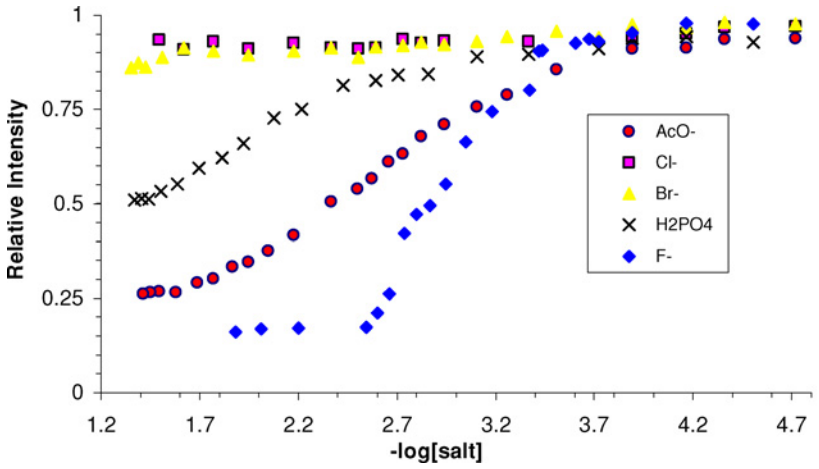
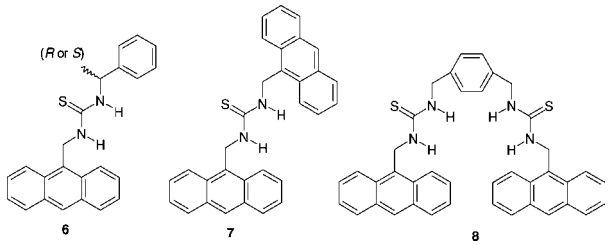


Fig. 3. The changes in the fluorescence emission at 420 nm *vs.* –log[anion].

minor, (Fig. 1.) This is in agreement with PET theory, as the only communication pathway between the two parts, the receptor and the fluorophore is through space and hence, the absorption spectra would generally not be expected to be significantly affected by the recognition at the receptor site. However, at lower wavelength there were some changes which were assigned to the structural changes occurring in the aromatic thiourea receptor itself upon anion recognition, which would be through hydrogen bonding between the anion and the thiourea protons, yielding an electron rich anion:receptor complex. This interaction would significantly enhance the reduction potential of the receptor, which would enhance the rate of electron transfer from the receptor to the fluorophore and hence enhance the quenching of the excited state. Unfortunately, we were unable to determine the oxidation-reduction potential of the receptors by cyclic voltammetry as they were irreversibly oxidised. However, the effect of this was indeed found as is shown above, where the emission was reduced due to the enhanced PET rate. In a similar manner compounds **4** and **5** showed that upon addition of the same anions the emission was quenched and the absorption spectra was not significantly affected, except at low wavelengths for **4**, whereas for **5**, no such changes were observed as the sensor lacks the aromatic receptor. We also evaluated the changes in the receptors themselves by synthesising the thiourea receptors in a single step. As expected, significant changes were observed in the absorption spectra of these (as well as in the NMR) upon anion titration, confirming the changes seen above in the absorption spectra where the major changes occurred at short wavelength.

By analysis of the changes in the fluorescence emission, as a function of the anion concentration, we established two major principles; firstly, the fluorescence of the fluorophore can be modulated by the recognition of a charge neutral receptor of an anion, *via* hydrogen bonding, and secondly, even the use of simple mono-aromatic thiourea receptors can lead to selective anion recognition. The latter is evident from Fig. 3, where the changes in the recognition of bromide, chloride, acetate, phosphate and fluoride are shown as changes in the relative intensity vs. the concentration of the anions (as –log[anion]).

It is also important to note in Fig. 3, that the changes occur over two logarithmic units, which is an indication of a 1:1 binding. From the above figure the selectivity and the sensitivity of the recognition is also evident, and the same trend was seen for all the sensors; the emission was most significantly modulated, '*switched off*,' by fluoride, followed by acetate and finally phosphate, whereas the larger spherical anions did not give rise to such quenching. In a similar manner we have also recently made the urea analogues of the above complexes and observed that the changes in the ground and the excited state properties of these were also affected by the anion recognition, demonstrating that the active PET mechanism is also operative in these systems.



With the aim of elaborating further on this simple design principle we have developed other structurally

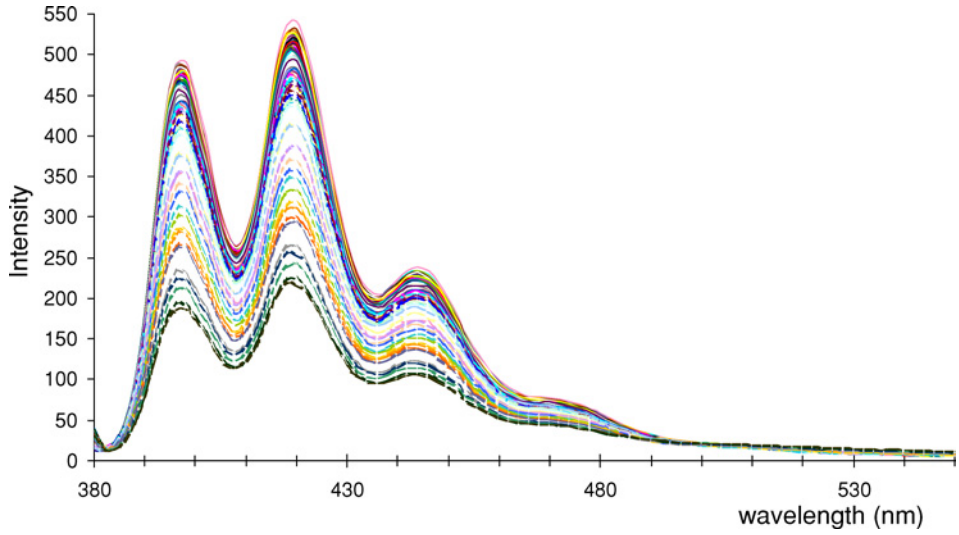
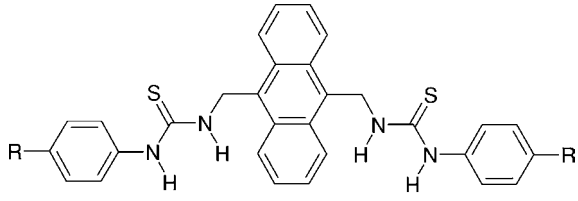


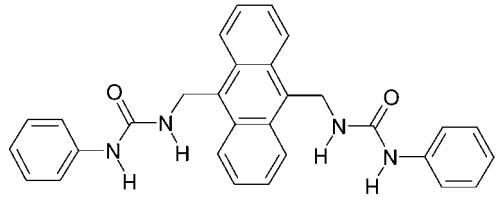
Fig. 4. Changes in the fluorescence of **8** in the presence of fluoride ions, where the emission is quenched upon anion recognition. Notice the absence of any long wavelength emission bands indicating the lack of excimer emission upon anion sensing.

similar sensors [20,21]. Compounds **6**, **7** and **8** are examples of our design, where for instance an asymmetric centre has been incorporated adjacent to the receptor moiety in **6** (both *R* and *S* were made) and two fluorophores have been attached to a single thiourea moiety in **7** and **8**. For the former, we aimed to achieve chiral discrimination of anions such as *N*-acetal or *N*-Boc protected amino acids. However, we quickly demonstrated that a single stereogenic centre was not enough to achieve such enantiomeric discrimination. Attempts to incorporate a second stereogenic centre at the methylene spacer have, unfortunately, not been successful in our laboratory to date, but we are currently involved in such synthesis [20]. For **7**, the addition of two anthracene moieties into the structure gave some interesting results, e.g. the selectivity of the recognition was greatly improved, with fluoride being detected strongest. At the same time, the two fluorophores were shown to give rise to significant steric strain, which resulted in the decrease in the sensitivity of the anion recognition. Compound **8** was designed to give strongest binding to phosphate, which is often difficult to achieve using a single urea/thiourea binding site. However, the response to phosphate was not as significant as for fluoride, Fig. 4, where it was almost 70% quenched in comparison to about 5% for acetate and 20% for phosphate. Most interestingly, both sensors gave rise to 1:1 binding between the sensor and the anion.

In related work we have used the same structural motifs and developed sensors such as **9**, **10** and **11**, all of which have two binding sites [21,22]. For **9** and **10**, the basic design principle employed above was used, e.g. the



9; R = H
10; R = CF_3



11

anthracene fluorophore was separated from the receptor by a methyl spacer, but on these occasions, two receptors were incorporated into the design, and as before, the choice of aromatic thioureas gave rise to tuning of the anion sensitivity.

This extended design yielded an overall *receptor-spacer-fluorophore-spacer-receptor* motif, and allowed the sensing of bis-anions such as dicarboxylates and pyrophosphate in DMSO. For **9** and **10**, the fluorescence was '*switched off*' upon recognition of both mono-dentate anions such as acetate and by dicarboxylates such as glutarate and malonate. The changes in the fluorescence of the former were shown to occur over 4 logarithmic units, indicating that the binding was in the form of 2:1

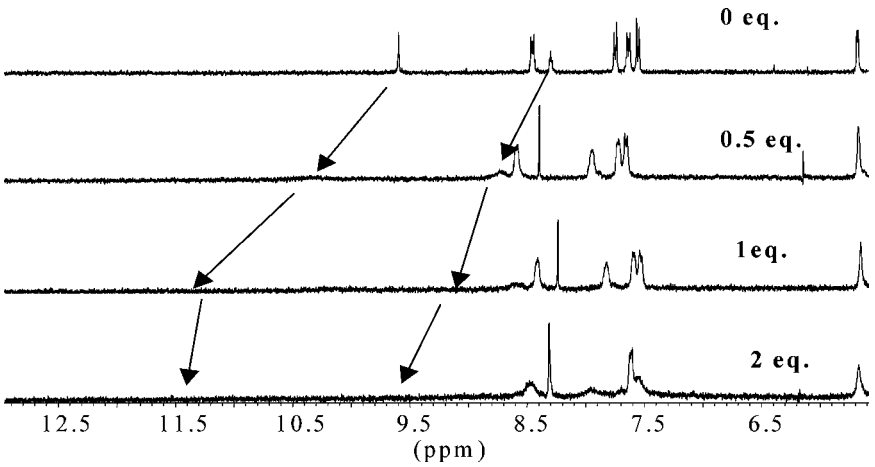
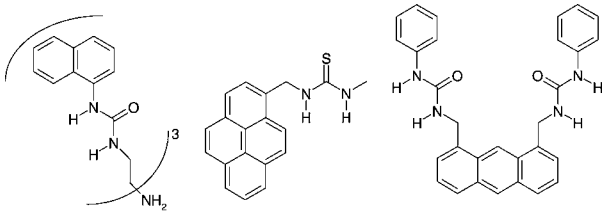


Fig. 5. Changes in the ^{1}H NMR of **10**, upon addition of pyrophosphate in DMSO-d_6. Notice the broadening in the aromatic signals which was found to be absent in the titration of **10** with phosphate, which was determined to be in 2:1 ratio.

(anion:sensor), whereas for the bis-carboxylates the sensing was determined to be 1:1. These latter results would imply that the anion would have to bridge the anthracene unit. Similarly for **11**, having a urea-binding motif, the emission was also quenched upon ion sensing, but at different concentration ranges in comparison to **9**. To evaluate the possibility of the bis-anions bridging the fluorophore, we investigated the changes in the ground state of the interaction of pyrophosphate with **9**. Whereas no isosbestic points were seen for **3**, **4** and **5** above upon titration with mono-dentate anions, there were measurable changes in the absorption spectra upon addition of pyrophosphate which suggested that the anion was in someway affecting the electronic structure of the fluorophore, e.g. by bridging it. However, in the absence of X-ray crystallographic results, this was not a significant enough change to determine the 2:1 binding accurately. We thus carried out a series of ^{1}H NMR titrations of both the mono- and the bis-receptor based sensors in DMSO-d_6. These investigations revealed that for **3**, **4** and **5** the binding occurred in the stoichiometry of 1:1, where the changes in the thiourea protons were monitored upon addition of anions such as acetate and phosphate (as their tetrabutylammonium salts). This was in good agreement with work carried out by Hamilton *et al.* and Kelly *et al.* on structurally related receptors, which also showed the binding to be 1:1 in their cases [15,17]. For **9** and **10**, however, similar titrations showed that the binding of acetate and phosphate (see Fig. 5, for **10**), was indeed in 2:1 binding, whereas using the bis-anion pyrophosphate, the titration showed 1:1 binding. Moreover, unlike that seen in the titration of **10** with acetate and phosphate, the fine structure of the resonance for **10** upon titration with pyrophosphate was also significantly affected, being broadened after one equivalent. We assigned this to be due to substantial electronic effects inflicted by the phosphorus moiety in the pyrophosphate, which would be central over the anthracene fluorophore. Hence, from the above results we are confident to say that the binding is 1:1 and for this to occur the anion has to bridge the fluorophore. Indeed the use of different bis-anions with different spacers, showed that the larger anions bound in 2:1 and the smaller ones such as malonate and glutarate in 1:1 ratio.

The above account has so far focused on the results from our work on anion sensing using anthracene as the fluorophore. Several other examples of similar designs have also emerged in the last a few years. For instant, Wu *et al.* developed a thiourea-based receptor **12**, containing three naphthalene units [23]. The bis and mono equivalents of this sensor were also examined and the mechanism for the sensing of **12** was described as a PET sensor with the quaternary nitrogen acting as the donor, the receptor acting as a spacer and the fluorophore being the naphthalene unit. However, **12** senses anions *via* a combination of PET and energy transfer. Binding of the anion to the NH 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 changes upon addition of the anion to the system, again breaking one of the criteria specified in order to be classified as an ideal PET sensor. Teramae *et al.* recently synthesised a thiourea-based anion receptor linked to a pyrene moiety via a methylene spacer, **13** [24]. Binding studies of 13 with TBA acetate were conducted in CH_3CN. ^{1}H NMR could prove that binding was occurring

via the N–H bonds however the signal was too broad to quantify the binding constant. A concurrent analysis of the fluorescent and UV/vis properties showed that upon addition of various anions to the system the monomer emission reduced dramatically with little change in the absorption spectra being observed. This compound does not exhibit *ideal* PET behaviour since the monomer emission quenching was followed by the formation of an intramolecular exciplex emission. Recently, Yoon *et al*. synthesised a new anthracene derivative bearing two phenylurea groups at the 1,8 position of anthracene, **14** [25]. This sensor shows a selective fluorescence quenching effect with F^- *via* a PET mechanism.

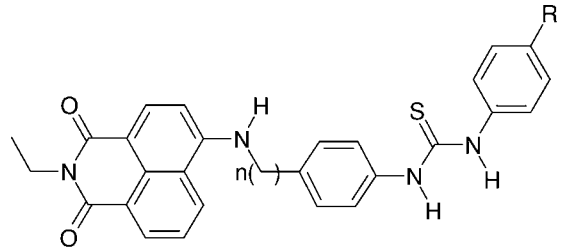


12 13 14

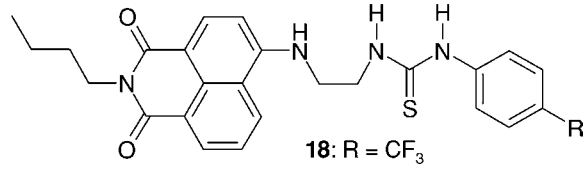
Having demonstrated PET anion sensing using charge neutral receptors, we decided to explore this method of sensing using other fluorophores, some of that work is described in the next section. The main reason for extending this line of research was to develop novel luminescent anion sensors that could be used to determine anion concentrations in aqueous solutions. Recently we have indeed managed to achieve such sensing in buffered water, however, that work will be the focus of our future articles. What follows is the account of the work leading to such real anion sensors for competitive media.

PET ANION SENSING USING NAPHTHALIMIDE FLUOROPHORES

Over the last a few years we have developed various fluorescent PET sensors for cations using the 4-amino-1,8-napththalimide structure [26]. With the aim of extending the above PET anion sensing we developed several sensors based on the naphthalimide unit by simply incorporating a thiourea or urea moiety into the structure *via* a spacer in a similar manner as discussed above [27]. Compounds **15–18** are examples of this design where different receptors or spacer lengths were used. The advantage of using the naphthalimide fluorophore is twofold; firstly, it has an internal charge transfer excited state (ICT) due to the electron donating amine and the electron withdrawing imide. This makes the fluorophore absorb in the visible region and the colour of the above compounds is yellow or orange. Consequently they also emit in the visible spectrum, having a strong emission band centred around 530–550 nm. Secondly, these compounds are usually found to be highly emissive, with quantum yields of *ca*. 80–90%.



15: n = 1, R = CF_3
16: n = 2, R = CF_3
17: n = 1, R = H



18: R = CF_3

The synthesis of the above compounds was achieved in good yields from the 4-chloro or the 4-bromo-1,8-naphthalic anhydride by first condensation with either *n*-aminoethane or *n*-aminobutane, to yield the corresponding naphthalimides. This was then treated with either diethyl amine or 4-aminobenzylamine to yield the two free amines, which then were susceptible to reaction with various isothiocyanates. The synthesis of **15** is shown in Fig. 6.

The sensing of several anions was demonstrated in DMSO. For **15** and **17**, which have di-aromatic thiourea receptors, the fluorescence emission was highly dependent on the anion, where it was almost fully quenched by fluoride. The changes for **15** are shown in Fig. 7. From this it can be seen that the emission occurs in the visible region, being centred at 525 nm, which was highly fluorescent green to the naked eye. Titration with other spherical anions such as chloride or bromide did not lead to any quenching in the naphthalimide emission. However, both acetate and phosphate gave rise to significant changes as seen in the insert graph in Fig. 7. In the same way, the changes for **17** showed that the emission was highly sensitive to fluoride, phosphate and acetate, but as in previous examples the sensitivity was somewhat shifted to higher concentrations due to the lack of the electron withdrawing CF_3 group. For **16**, an analogue of **15**, except for the presence of the two methyl spacer, the emission was also reduced upon addition of these ions. However, the quenching efficiency was significantly reduced as the electron transfer quenching is dependent on the distance between the receptor and the fluorophore, where the rate

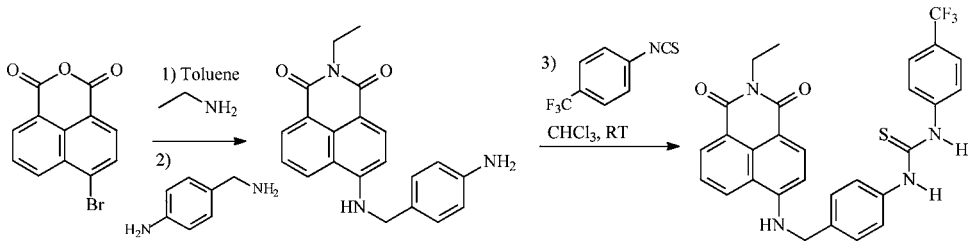


Fig. 6. Synthesis of **15** from 4-bromo-1,8-naphthalic anhydride.

of electron transfer is a function of $1/r^6$. Interestingly, for **18**, which is a mono-aromatic thiourea, the emission was only slightly quenched confirming that the efficiency of the PET quenching is also dependent on the structure of the receptor. Furthermore, and as expected, this quenching only occurred at relatively high concentrations. For all the naphthalimide sensors the absorption spectra were not significantly affected upon titration of any of the above anions, being only slightly red-shifted upon anion recognition. This is evident from Fig. 8, upon titration of **15** with phosphate.

As in the case of the anthracene examples discussed above, the changes in the ^{1}H NMR were also evaluated in DMSO-d_6. For the same concentration range (Fig. 7), the changes in the thiourea protons or the aromatic signals of the receptors can be used to evaluate binding strength and the stoichiometry of the recognition. On all occasions it was shown that the binding was 1:1 and that the thiourea protons were shifted by up to ~3 ppm. To establish an accurate endpoint we titrated the solutions beyond two equivalents of anions. For acetate and phosphate, only saturation was observed in the NMR, e.g. the spectra were unchanged at higher equivalents. However, for fluoride it was noticed that at higher equivalents, the colour of the solution changed from slight yellow to deep blue. These changes were clearly visible to the naked eye. At the same time, the NMR also changed significantly and a new species was clearly observed in the NMR. Because of these changes, we reinvestigate the absorption spectra of these compounds at higher concentrations. Figure 9 shows the changes observed in the absorption spectra of **15**, upon titration with fluoride in DMSO. From this titration two significant results emerged. Firstly, the absorption spectra did not change significantly in the concentration range where the fluorescence was quenched, e.g. the molecule showed 'ideal' PET behaviour. Secondly, the colour changes observed in the NMR was also clearly observed in the absorption spectra, where a new band was formed, centred at *ca*. 550 nm and a second band at 338 nm, and with the presence of two isosbestic points at 385 and 475 nm, respectively, upon exceeding two equivalents of fluoride, at the same time that the 440 nm band

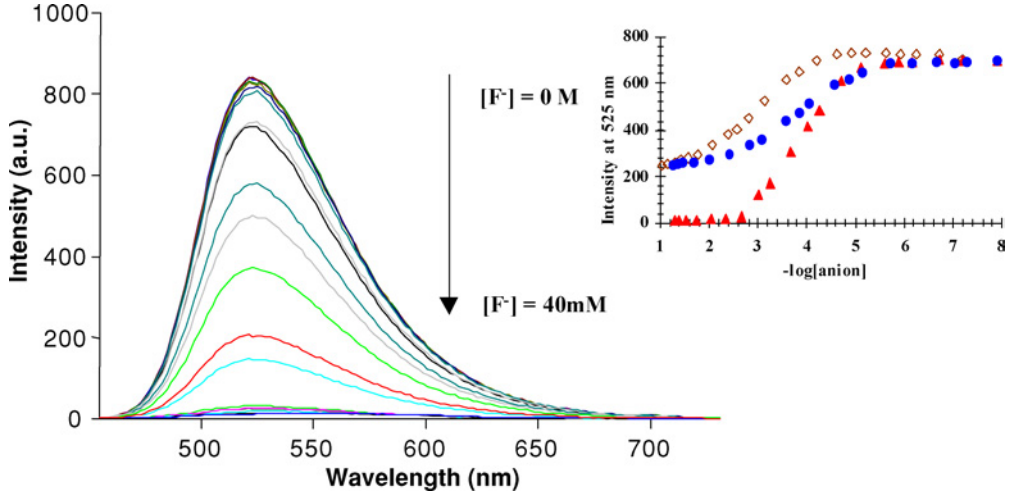


Fig. 7. Changes in the fluorescence emission of **15** upon titration with fluoride in DMSO. Insert are the changes in the emission at 525 nm upon titration with fluoride (red), acetate (blue) and phosphate (brown), as a function of their concentrations (as the –log[anion]).

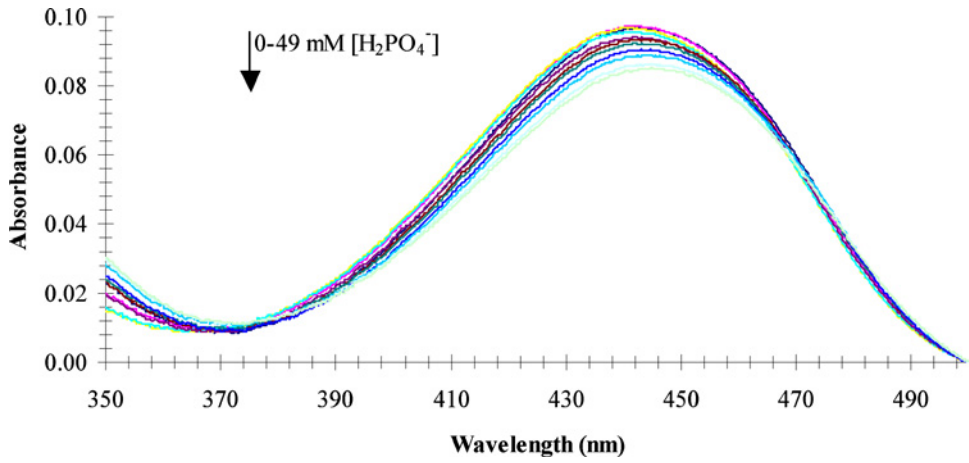


Fig. 8. The changes in the absorption spectra of **15** upon titration with phosphate. These changes were too small to allow for accurate binding constant determination.

was significantly reduced in intensity. In a similar way, **16**, **17** and **18** all showed this behaviour at higher fluoride concentrations. We assigned these changes to the deprotonation of the 4-amino moiety in the fluorophore and the enhancement in the ICT of the excited state, which shifts the absorption spectra to the red. In fact upon excitation of the 550 nm band, a weak emission was observed at long wavelength, centred at *ca*. 650 nm.

These compounds can thus be described as dual fluorescent-colorimetric anion sensors. With the aim of further investigating these effects; we designed several other naphthalimide 'sensors' where the thiourea moiety was absent. Compounds **19** and **20** are examples of this design [28]. As expected only fluoride gave rise to any significant changes in the absorption spectrum of **19** upon addition of several anions. A full titration of **19** with TBA-F led to a reduction in the intensity of the bands at 446 and 287 nm, while two new bands centred at 535 and 341 nm respectively, were formed. At low concentrations, three clear isosbestic points at 480, 380 and 300 nm were visible. At higher concentrations, the bands broadened, as observed with sensors **15–18** on addition of F^-. A simultaneous visible colour change from green to red/purple was observed as the absorption maximum was bathochromically shifted from 446 nm to 535 nm. These spectral changes are most likely due to deprotonation of the amino moiety by F^-, or strong hydrogen bonding that could eventually result in deprotonation. In both cases, deprotonation leads to the formation of a negatively charged naphthalimide, with concurrent enhancement of

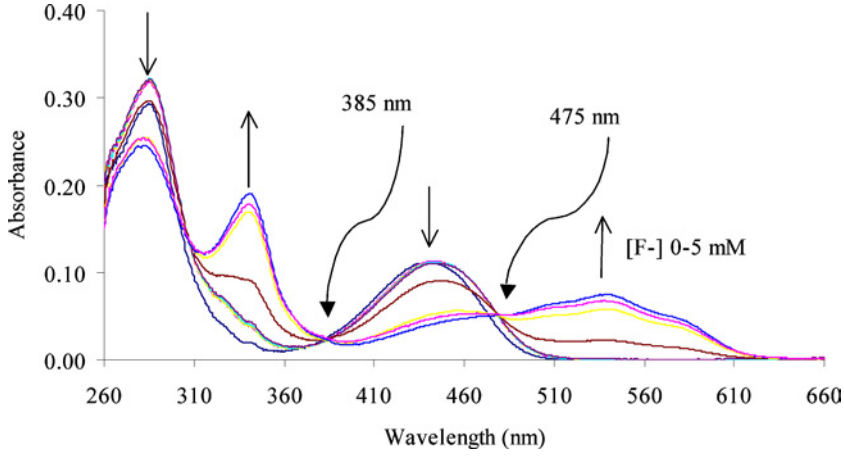


Fig. 9. The changes in the absorption spectra of **15**, upon titration with fluoride. Only at high concentrations, where the fluorescence had stopped changing, did the absorption band centred at 550 nm begin to appear.

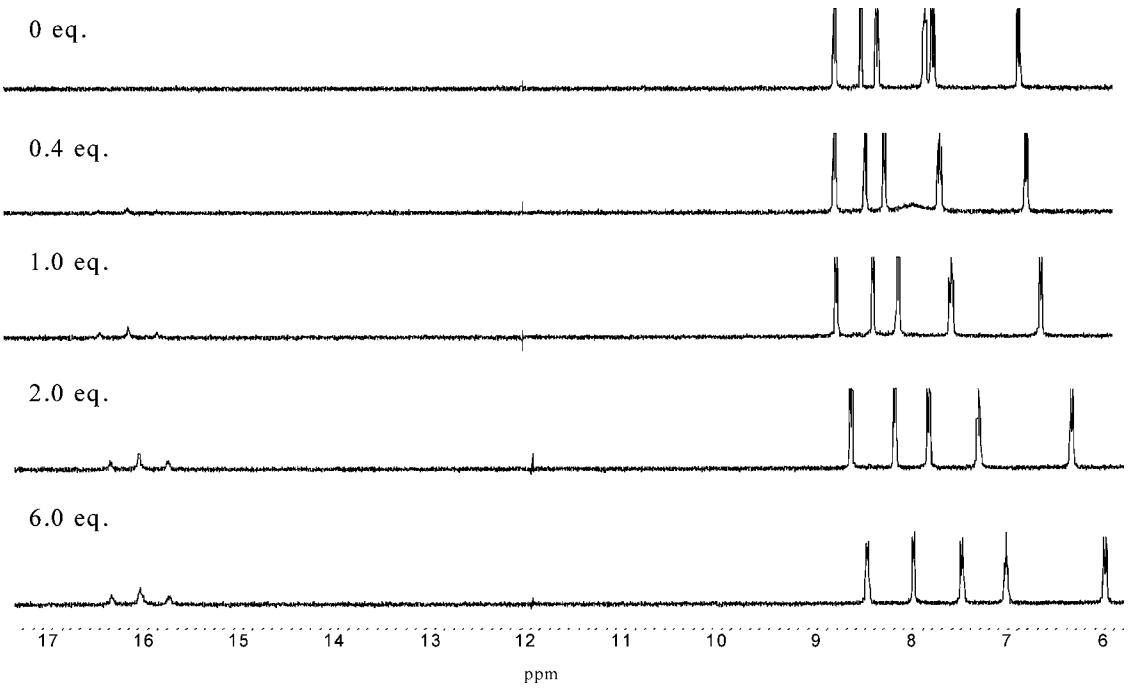
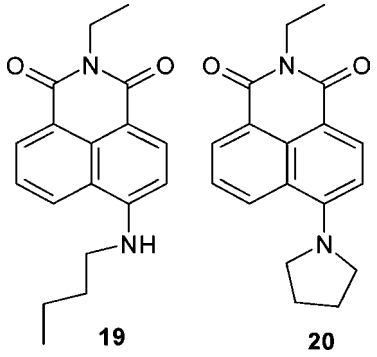


Fig. 10. ^{1}H NMR stack plot of **19** after addition of various quantities of F^- (DMSO-d_6, 400 MHz) showing formation of downfield triplet.

the push-pull character of the ICT transition. When the same titrations were done using compound **20**, which lacks the 4-amino moiety, no such colour changes were observed. This clearly indicated that these changes were due to deprotonation of the 4-amino moiety. As before we investigated these changes by ^{1}H NMR for **19**. These results confirmed what was observed in the NMR titrations for **15–18**. Furthermore, we had observed the formation of a new signal resonating at *ca*. 16 ppm, which we assigned to the formation of bifluoride (HF_2^-), as is evident from Fig. 10 after *ca*. one equivalent of fluoride. From these results it was thus clear that the colour change was due to the formation of the deprotonated form of the naphthalimide moiety with concomitant enhancement in the ICT character of the fluorophore.



However, we also observed that if the DMSO solution of this deprotonated form was left standing open to air, the colour was reversed (from blue to green-yellow) over some time. When samples of **15–18** were also dissolved in DMSO, treated with excess fluoride and left standing exposed to air, the colour was also reversed. Interestingly, the addition of fluoride did not reverse this colour change to red/purple. With the aim of investigating this phenomenon further, we attempted to grow crystals of the adduct formed upon addition of two equivalents of fluoride under anaerobic conditions. Nonetheless, even though red crystals were formed, these melted upon exposure to air. From this newly 'coloured' solution we were however able to grow crystals suitable for X-ray crystallography. The structure is shown in Fig. 11, and clearly shows that the resulting solution had produced crystals that had bicarbonate hydrogen bonded to the amino moieties of the sensor. We thus concluded that the uptake of carbon dioxide from air was being converted to bicarbonate in the presence of the bifluoride and the deprotonated sensor.

To evaluate this, we observed the changes in the absorption spectra upon adding carbon dioxide to the 'blue' coloured solution of **19**. This showed that the absorption spectra were reversed, giving rise to the colour previously assigned to the free form of **19**. This supported thus our finding that the deprotonated form of **19** was able to fix

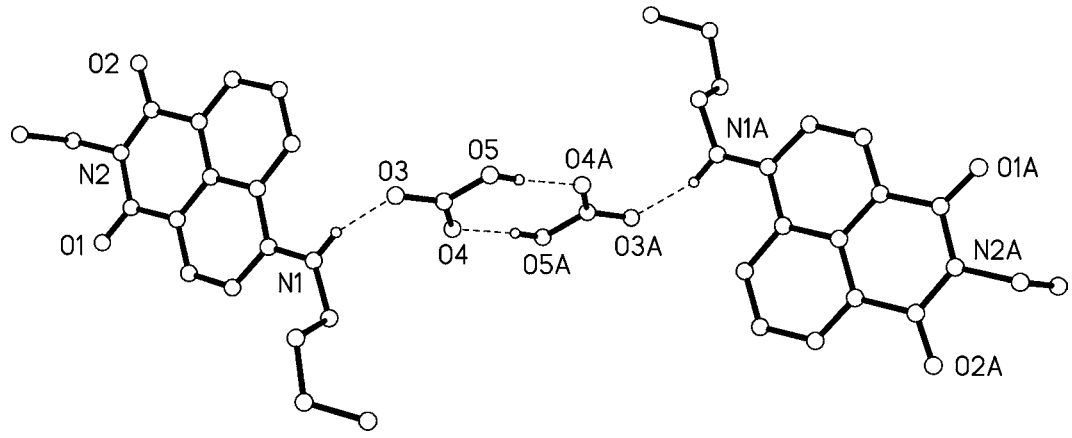


Fig. 11. Crystal structure of the 1:1 adduct formed between **19** and HCO_3^- showing H-bonded pairs.

carbon dioxide as bicarbonate. We propose that this process involves the formation of carbamide that is broken down to give the resulting amine and bicarbonate in the presence of water. Furthermore, further additions of fluoride did not reverse this process. Importantly, the fixation of carbon dioxide is not possible in the absence of the deprotonation step. Hence, no changes are observed in the absorption spectra of **19** upon passing CO_2 into the solution prior to the deprotonation step.

From the above we have demonstrated that PET anion sensing is highly feasible using charge neutral receptors. Additionally, we have demonstrated that for our particular systems the 4-amino moiety can be deprotonated, which provides the platform for carbon dioxide fixation, which is common in many nonmetal-containing enzymes, as well as being of significant application.

CONCLUSIONS

This mini-review has focused on the work carried out in our laboratories in Dublin over the last a few years using charge neutral receptors for anion sensing. We have demonstrated that PET anion sensing is possible using charge neutral receptors, where the anion recognition and consequent sensing comes about *via* hydrogen bonding between the receptor and the anion. We have demonstrated that even by using simple urea or thiourea receptors both the sensitivity and the selectivity of the sensing process can be tuned. We have also demonstrated that by simply changing the fluorophore from anthracene to naphthalimide gave rise to new families of sensors where the detection of most anions gave rise to changes in the fluorescence. We also demonstrated that in the case of fluoride, both the fluorescence and the absorption spectra were modulated, albeit at different concentrations, yielding a combined fluorescence-colorimetric sensor for such anions. For the latter, the colour could be reversed in the presence of carbon dioxide giving rise to the formation of new adduct, where carbon dioxide was fixated as bicarbonate.

We are currently working on this kind of systems and we have recently shown that anion recognition in buffered pH 7.0 water solution is feasible through hydrogen bonding interactions. These results will be the subject of our future publications.

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