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New Advances in Fluorogenic Anion Chemosensors

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The development of anion chemosensors is an area of recent interest. We make here a comprehensive review of new advances on anion chemosensing, reported in the literature during the year 2004. The review follows a classification of the sensing systems based on design principles. It comprises: the binding site-signalling subunit approach, the displacement approach and the use of fluoro-chemodosimeters. The first two approximations are based on the use of a suitable anion coordination site coupled with a signalling unit which signals the anion coordination process via changes in its fluorescence behaviour. The two basic subunits are covalently linked in the binding site-signalling subunit approach and not in the displacement approach. In both approaches the fluorescence variation is reversible. The third way to the development of fluorogenic chemosensors is the use of chemodosimeters (also called reagents or reactands) that work usually through irreversible chemical reactions coupled with drastic changes in the fluorescence emission behaviour.

KEY WORDS: Anion; sensors; fluorescence.

INTRODUCTION

Supramolecular chemistry of anions has been of growing interest in the last few years and there are a number of appealing reviews on the chemistry of anion recognition and the development of host receptors for anions [1–5]. In addition, in anion recognition chemistry, when the coordination event is coupled to a suitable signal (optical, electrochemical, etc) a molecular chemosensor is obtained [6–11]. A number of studies have been done in which the anion–chemosensor interaction is transduced in simple signals such as changes in colour, fluorescence or oxidation potential shifts, thus opening the door to the sensing/determination (qualitative or quantitative) of target anions. We have published recently a comprehensive review of anion chromogenic and fluorogenic chemosensors and reagents for anions [12]. The present review highlights new advances in the area of fluorogenic chemosensors for anions covering the papers published in the last year.

A general approximation to the development of fluorogenic anion chemosensors is the coupling of two subunits exhibiting differentiable functions: the binding site and the signalling subunit. The former does the role of coordiantion to certain anion, whereas the task of the latter consists of signal the coordination event via changes in its fluorescence behaviour. The two subunits can be either covalently linked (binding site-signalling subunit approach) or not (displacement approach). In the above approaches the fluorescence alteration is reversible as the anion–host interaction is based on coordination events. The alternative or the third way to develop fluorogenic chemosensors for anions is the use of chemodosimeters (also called reagents or reactands) that work through chemical reactions (different to coordination) coupled with emission changes.

The use of fluorescent-signalling-subunits in the development of chemosensors for anions has been extensively used and the mechanisms of signal transduction are well known [13]. In the examples shown below there are basically two kind of systems, (i) those in which the signalling and coordination units are electronically not

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connected (for instance, using spacers with at least one carbon in sp^3 hybridation) that usually work via changes in photoelectron transfer (PET) or energy transfer (EET) processes induced by the coordination of the anion and (ii) those in which the binding site is part of the fluorophore (also called intrinsic chemosensors) that usually work via changes in intramolecular charge transfer (CT) processes induced by anion coordination to the binding site. In this latter case, the modification of the fluorescence behaviour is also usually coupled with a variation in colour.

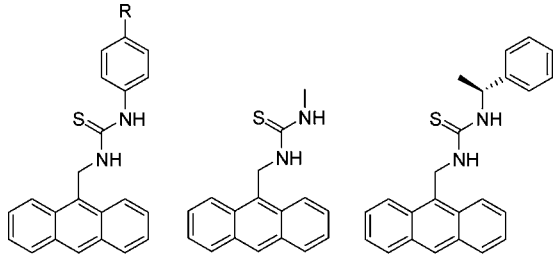
FOLLOWING THE BINDING SITE-SIGNALLING SUBUNIT APPROACH

This has been the most widely used approach in the development of fluorogenic anion chemosensors and consists of the attachment, through covalent bonds, of the two binding and signalling subunits in such a way that the fluorescent properties of the later are changed upon anion coordination to the former.

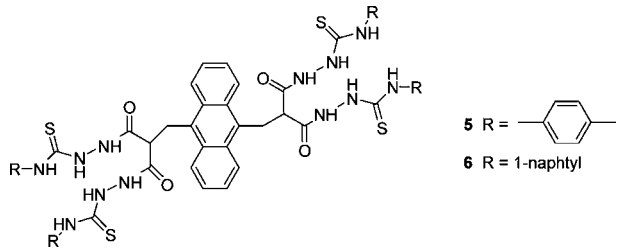
Fluorescent Chemosensors Based on Polycyclic Aromatic Hydrocarbons

Containing Anthracene

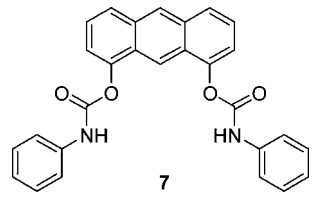
Anthracene is one of the most popular signalling subunits used in the development of fluorescent chemosensors for anion signalling. This is probably so because the photo-physics of anthacene derivatives are usually very well-known and because the anthacene functionalisation is relatively easy. Probably the main drawback in the use of anthracene and other similar polycyclic aromatic hydrocarbons is that they usually show emission near the UV region where serious matrix interferences can be found in real samples. Nevertheless, its use is widely extended and it still is an attractive group to test new sensing concepts.



Urea and thiourea groups are well-known anion binding sites and some examples of anion chemosensors based on anthryl-urea and thiourea derivatives have been recently reported. Those systems are usually fluorescent, however upon anion binding (via hydrogen bonding interactions with the urea or thiourea protons), the reduction potential of the thiourea moiety increases resulting in a quenching of the anthracene emission due to a photoinduced electron transfer (PET) process from the electron-rich urea–anion ensemble to the photo-excited anthracene subunit. This general effect was found in compounds **1–3** that showed typical structured anthracene emission bands that were quenched upon addition of an excess of the anions F^-, AcO^- and $H_2PO_4^-$ [14]. For instance, for receptor **2** the quenching observed upon addition of 32 equivalents of these anions were 90, 75 and 50%, respectively. In contrast, Cl^- and Br^- did not induce any noticeable change in the emission profiles of the three receptors. Receptor **4** with a chiral center was designed for the enantio-selective recognition of N-protected aminoacids with no success because (*S*)- and (*R*)-*N*-acetylalanine gave a similar response (60% quenching).

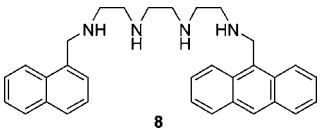


The two receptors **5** and **6** form 1:1 complexes with dicarboxylates through hydrogen bonding interactions with the amide/thiourea protons [15]. The association constants, determined by fluorescence titrations, showed the order adipate > suberate > sebacate > glutarate > succinate > malonate. Upon addition of the dicarboxylates malonate, succinate, glutarate, adipate, suberate and sebacate to DMSO solutions of **6** a remarkable emission quenching was observed (for instance 60% quenching upon addition of 2 equivalents of adipate anion). This quenching process is related in **6** with the presence of the electron-rich naphthalene subunit that induced a PET process from the receptor–anion unit to the photo-excited anthracene. In contrast the emission of **5**



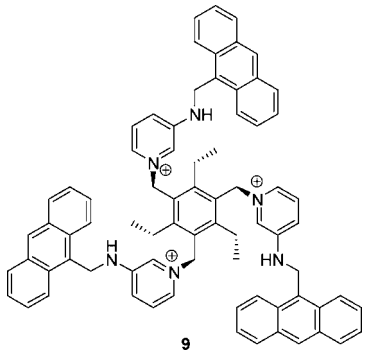
was enhanced upon addition of the same dicarboxylates cited above and attributed to an increase in rigidity upon complexation.

Anthracene containing receptor **7** exhibited selectivity towards F^- and AcO^- anions in acetonitrile-DMF (98:2 v/v) solutions [16]. **7** shows a typical structured anthracene emission centred at 420 nm (excitation at 364 nm) that decreased and was red-shifted to 445 nm as the concentration of F^- and AcO^- anions increased. These anions form 1:1 complexes with **7** through hydrogen bonding anion–amide interactions. The red-shifted emission was explained by the existence of hydrogen bonding interactions between the anions and the aromatic hydrogen placed near of the two amide groups. This $C_{aromatic}$–H hydrogen bonding was confirmed through NMR experiments. Addition of $H_2PO_4^-$ induced lower spectral changes and Cl^-, Br^- and HSO_4^- did not induce any remarkable emission variation.



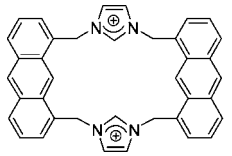
8

Polyamines have also been used as anion receptors due to their ability to form complexes with anions via both hydrogen bonding and electrostatic interactions in aqueous environments. When polyamines are anchored to anthracene via a spacer (usually a methylene) a quenching of the anthracene fluorescence is observed due to a favourable PET process from the lone pair of the benzilic nitrogen to the photo-excited anthracene unit. When, the amines are protonated or involved in hydrogen bonding interactions with anions the PET process is not operative and a revival of the fluorescence is observed. Several polyamines bearing anthracenyl groups have been reported recently. Additionally, **8** contains a naphthalene subunit [17]. Fluorimetric titrations of receptor **8** in aqueous solution in the presence of ATP (3-fold excess) showed a quenching of the emission at acidic and at basic pH. The quenching at basic pH was ascribed to photo-induced electron transfer processes from the lone pairs of the amino groups as stated above, whereas the observed quenching at acidic pH was attributed to the existence of an electron-transfer process from the photo-excited naphtalene or anthracene to the protonated adenine moiety of ATP. Additional time-correlated single-photon counting showed that, even in the presence of ATP, there was a significant energy transfer form naphtalene to anthracene in this bichromophoric receptor.



9

The 1,3,5-tripodal receptor **9** contains amines and pyridinium groups as anion binding sites [18]. The signalling product is not **9** but the highly fluorescent one obtained by UV irradiation (254 nm) of **9** that induced the formation of fluorescent species by a $4\pi + 4\pi$ cycloaddition of two anthracene units. This cycloadditon could occur in an intermolecular as well as intramolecular fashion. Thus, acetonitrile solutions of **9** are poorly fluorescent and addition of 20 equivalents of anions (Cl^-, Br^-, I^- and AcO^-) did not induce any significant change in the emission intensity profile. On addition of the same anions to acetonitrile solutions of the cycloadduct a quenching in the emission intensity was observed. The most important quenching was obtained in the presence of Cl^- (30%) and I^- (50%).



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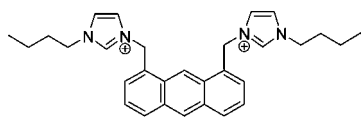
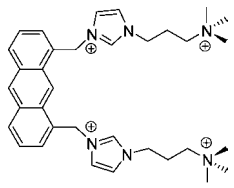


11

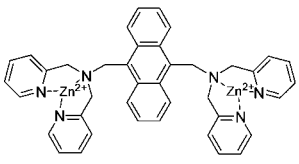
Compounds **10** and **11** are first examples of chemosensors containing positively charged imidazolium units as binding sites [19,20]. In these systems the charged nitrogen atom near the fluorophore was unable to induce PET processes. However upon anion binding there is an increase in electron density at the nitrogen resulting in an opening of the PET path from the imidazolium–guest ensemble to the photo-excited anthracene unit. Addition of $H_2PO_4^-$, Cl^-, Br^- and I^- anions to acetonitrile solutions of **11** resulted in different degrees of quenching, the most important being that observed in the presence of $H_2PO_4^-$. The selectivity observed with **11** for $H_2PO_4^-$ was around 200 times larger than that found for other anions tested. This selectivity was assigned to the preorganized binding site presented by receptor **11** that induced the formation of strong hydrogen bonds with the $H_2PO_4^-$ anion.

A similar selective quenching in the presence of $H_2PO_4^-$ was observed in acetonitrile-DMSO 9:1 (v/v) solutions of receptor **10**.



12

Additionally to the imidazolium units, **12** also contains two quaternary ammonium groups as anion coordination sites [21]. The chemosensing properties of **12** were studied in water at pH 7.4 (50 mM HEPES) in the presence of certain anions. The most remarkable effects were obtained upon addition of GTP which induced an important quenching of the emission intensity (11-fold with 200 equivalents of GTP), whereas ATP induced a relatively small emission enhancement (1.5-fold with 300 equivalents of ATP). In both cases 1:1 complexes were formed. The presence of ADP, AMP, pyrophosphate, $H_2PO_4^-$, F^- and Cl^- did not induce any significant effect. Computed geometries of the **12**-GTP and **12**-ATP showed the existence of anthracene–guanine or anthracene–adenine, π–H interactions. These interactions coupled with the different dipole moments of the guanine and adenine subunits perpendicular to the anthracene ring would explain the different fluorescent behaviour (quenching/enhancement) experimentally observed.



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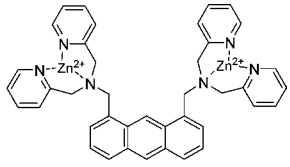


14

Finally, the dipicolylamine zinc(II) receptors **13** and **14** were designed as probes for phosphorylated derivatives and peptides in aqueous solutions (buffered at pH 7.2) [22,23]. Addition of phosphorylated tyrosine to aqueous solutions of **13** resulted in an increase in the emission intensity of the anthracene moiety. Addition of phosphate, phenyl phosphate, methyl phosphate, ATP and ADP induced a similar enhancement; bicarbonate also enhanced the emission but at larger concentrations and the azide anion induced a more moderate quenching in the same concentration range than bicarbonate. Addition of tyrosine, dimethyl phosphate, cAMP, sulfonate, nitrate, acetate and chloride gave no response. The emission behaviour of receptor **14** upon addition of the anionic species was the same than that observed with **13**. One important feature was that both receptors were able to distinguish phosphate and phosphate monoesters from phosphate diesters. Both receptors form 1:1 complexes with phosphorylated species with the two zinc–dipicolylamine sites contributing equally to the binding of the phosphate group. Emission enhancement was attributed to complexation-induced conformational rigidification of the receptor. Finally, addition of negatively charged phosphorylated peptides induced emission enhancements (4- or 5-fold magnitude), whereas the addition of positively charged phosphorylated or nonphosphorylated peptides scarcely affected the emission profile of the two receptors.

Containing Naphthalene and Naphthalene Derivatives

Naphthalene is another polycyclic aromatic hydrocarbon extensively used as fluorescent signalling unit and some examples containing naphthalene or naphthalene derivatives have recently been reported. For instance compounds **15**–**23** contain naphthalene units anchored to urea or thiourea groups without spacer. In those receptors an emission enhancement is usually observed upon anion binding. This behaviour has been attributed to the suppression of PET processes (from the electron-rich urea or thiourea to the photo-excited naphthalene) upon anion coordination via hydrogen bonding interaction of the anion with protons of the urea or thiourea groups.

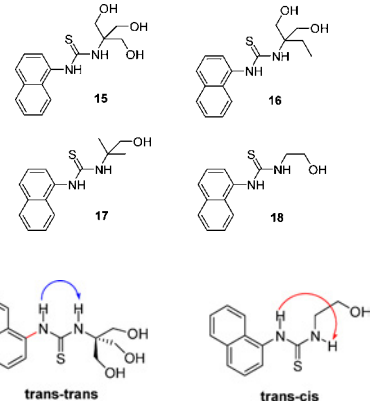
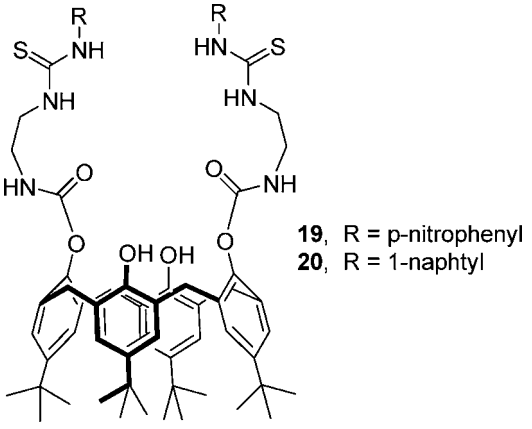


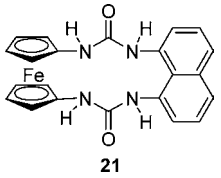
Fig. 1. Description of the different conformations presented by receptors **15** and **18**.

For instance, CO_3^{2-} selective sensing in methanol-water 4:1 using **15** was achieved via 30.6-fold enhancement of the naphthalene emission [24]. A lower enhancement (6.5-fold) was observed with HPO_4^{2-} or with the anions HCO_3^-, AcO^-, $H_2PO_4^-$, NO_3^- and HSO_4^-

(3.8–1.5-fold). The degree of the emission enhancement was related with the basicity of the anion. This enhancement of the emission upon anion binding was weaker for **16**, being negligible with receptors **17** and **18**. PC-MODEL calculations showed that receptors **15** and **16** displayed a trans–trans conformation (see Fig. 1) with an ideal fitting distance between the two thiourea protons for coordination, whereas **17** and **18** showed a trans–cis conformation.

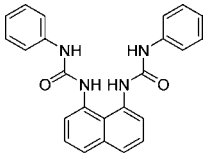


Calix[4]arenes containing thiourea and amide moieties **19** and **20** were used for the recognition of dicarboxylates with the observed sensitivities depending on the length chain [25]. Both **19** and **20** form 1:1 complexes with the studied dicarboxylates via formation of multiple hydrogen bonds. For **20** the presence of malonate, succinate, glutarate and adipate in DMSO induced an enhancement of the naphthalene emission ascribed to a suppression of a PET mechanism upon anion binding. **19** contains a p-nitrophenyl group that additionally acts as chromogenic signalling unit, and the addition of malonate, succinate, glutarate and adipate to DMSO solutions of **19** resulted in a colour change from yellow to red. **19** and **20** remained silent in the presence of acetate, dihydrogen phosphate, Cl^-, Br^- and I^-.



21

The ferrocene–naphtalene dyad **21** showed in DMF a weak naphthalene emission [26]. Addition of 2 equivalents of F^- induced a 12-fold enhancement in the emission intensity whereas $H_2PO_4^-$ induced only a 1.7-fold enhancement. The anions Cl^-, Br^- and HSO_4^- in large excess induced negligible changes in the emission profiles. ^{1}H-NMR titrations showed that the F^- anion forms a 1:2 (receptor–anion) complex whereas $H_2PO_4^-$ forms a 1:1 complex, both through hydrogen bonding interactions with the urea protons. These hydrogen bonding interactions inhibited the PET process as explained above with the subsequent emission enhancement.



22

The receptor **22** was studied independently by two different groups. Tarr *et al*. found a fluorescence enhancement upon addition of fluoride to DMSO–acetonitrile 2:3 v/v solutions of the receptor, whereas the anions Cl^-, Br^- and I^- induced a really small quenching of **22** [27]. ^{1}H-NMR studies revealed the formation of 1:1 species through hydrogen bonding interactions. Computing modelling indicated that coordination of F^- anion with the two urea moieties induced an increase in the planarity of the complex that the authors suggested might contribute to the fluorescence enhancement. The other halides did not induced in **22** the same degree of planarity and have weaker binding constants. The same product **22** was also studied by Lee *et al* in a somewhat different medium (acetonitrile–DMSO 9:1 v/v) [28,29]. In this solvent mixture, the fluorescence behaviour of **22** was somewhat different to that observed by Tarr. Thus, additionally to the emission enhancement of the 379 nm band upon F^- addition, a new intense emission band at 445 nm was also observed. Also here the anions Cl^-, Br^- and I^- induced no significant emission changes.

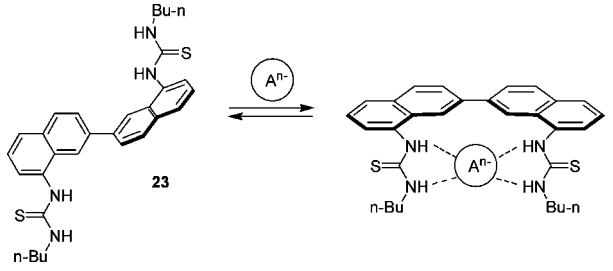
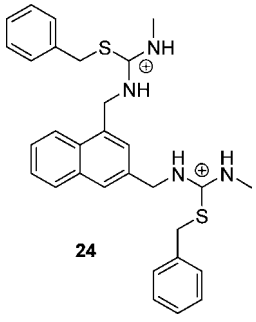


Fig. 2. Structure of the planar conformer formed upon coordination of receptor **23** with an anionic guest.

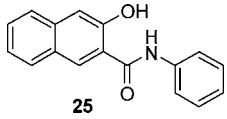
Titrations experiments in acetonitrile solutions of **23** showed a bathochromic shift of the band centred at 314.5 nm passing through isosbestic points at 333 and 295 nm upon addition of AcO^-, F^- and $H_2PO_4^-$ [30]. 1:1

(receptor–anion) complexes were formed with stability constants following the order $F^- > AcO^- > H_2PO_4^- > Cl^-$. Addition of Cl^-, Br^-, I^-, HSO_4^- and NO_3^- induced no change in the UV–visible profiles. In receptor **23** a relatively low quenching of the emission, concomitant with the apparition of a weak emission broad band at 650 nm, was observed upon addition of F^-, AcO^- and $H_2PO_4^-$. This thioureido–naphthalene derivative does not show an enhancement of the fluorescence emission (but a quenching) upon anion binding as it was found for instance in receptor such as **15**–**18** or **20** probably due to conformational changes and the formation of planar species with restricted rotation upon complexation (Fig. 2).



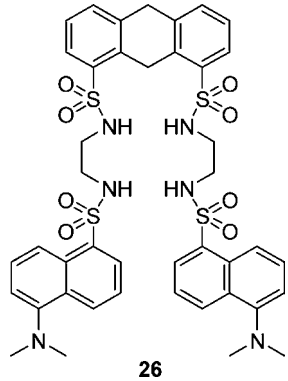
24

Receptor **24** contains two isothiouronium groups as binding sites [31]. These groups are prepared from the corresponding thiourea derivative by reaction with benzyl bromide. **24** is almost no fluorescent in 6% water-acetonitrile (v/v) mixtures due to a PET process from the photo-excited naphthalene to the electron-deficient isothiouronium moieties. Upon addition of HPO_4^{2-} (4 equiv.) a remarkable emission enhancement (520%) was observed. Moderate enhancements were obtained upon addition of AcO^- and no response was observed with $H_2PO_4^-$ and Cl^-. Emission development was attributed to suppression of the PET process upon coordination of HPO_4^{2-} and AcO^- through electrostatic and hydrogen bonding interactions with **24**. ^{1}H NMR studies suggested the formation of 2:1 (receptor–anion) complexes with HPO_4^{2-} and 1:2 (receptor–anion) complexes with AcO^-.



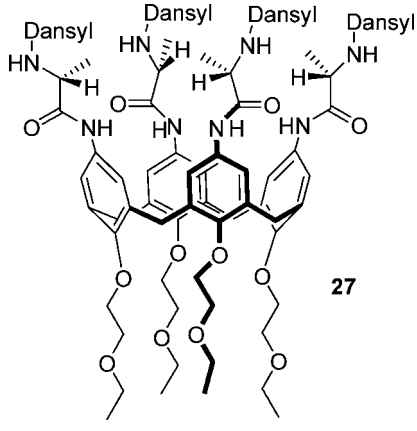
25

The commercially available 3-hydroxyl-2-naphthanilide **25** shows a fluorescence emission centred at 392 nm with a very low quantum yield [32]. Upon addition of F^- the intensity of the abpsortion bands centered at 236 and 360 nm decreases with the concomitant appearence of two new abpsortion peaks at 264 and 422 nm (the solution changed from colourless to yellow). In the fluorescence spectra, the addition of F^- resulted in new red-shifted emission band centered at 515 nm (upon excitation at 377 nm). AcO^-, $H_2PO_4^-$ and Cl^- induced similar variations in both abpsortion and fluorescence spectra to extents that depend on the anions basicity, whereas Br^- and ClO_4^- did not induce any change. In the ground state receptor **25** binds anions through hydrogen bonding interactions with the amide and the phenolic protons. The new red-shifted emission at 515 nm was assigned to an excited state proton transfer between the phenol group and the anion.



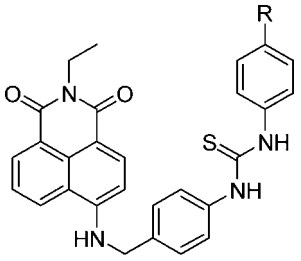
26

The receptors **26** and **27** contain a highly fluorescent dansyl groups as signalling units. Acetonitrile solutions of receptor **26** shows typical dansyl emission at 518 nm [33]. The addition of Cl^-, Br^- and I^- (2 equiv.) did not induce any noticeable spectral change whereas addition of F^- anion induced a blue shift of the emission band (10 nm) and an enhancement of the emission intensity (20%). ^{1}H NMR measurements indicates the formation of 1:1 complexes in which the F^- anion coordinates, through hydrogen-bonding interactions, with the four protons of the sulfonamide sites.



27

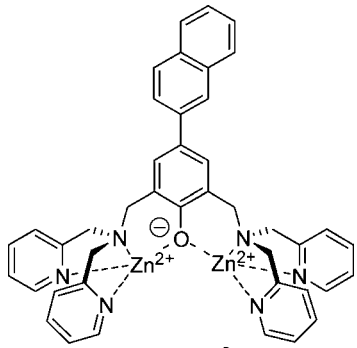
Acetonitrile solutions of calix[4]arene functionalised with four L-alanine and four dansyl groups, **27**, showed broad unstructured dansyl emission bands [34]. Upon addition of anions three different behaviours were be observed. A moderate enhancement (15%) and a blue shift (6 nm) of the emission intensity with $H_2PO_4^-$, a quenching of the emission intensity with both F^- (30%) and AcO^- (15%) anions, and no variation with Cl^-, Br^-, I^- and HSO_4^-. ^1H NMR studies carried out with receptor **27** and F^-, AcO^- and $H_2PO_4^-$ revealed the formation of 1:1 complexes through hydrogen bonding interactions between the anions and the SO_2NH moieties.



28, R = H
29, R = CF_3

The receptors **28** and **29**, bearing the ICT naphthalimide chromophore, display emission at 525 nm in DMSO upon excitation at 444 nm [35]. Addition of F^-, AcO^- and $H_2PO_4^-$ resulted in a quenching of the emission, whereas addition of Cl^- and Br^- induced no change. This quenching was assigned to a PET process from the highly charged anion–receptor hydrogen-bonding complex to the photo-excited fluorophore. The quenching effect was more severe for F^- and, at high F^- concentrations, was accompanied by the apparition of a new band centered at 536 nm that induced a colour change from light yellow to deep purple that was assigned to deprotonation of the 4-amino moiety (enhancement of the push–pull character of the ICT chromophore). AcO^- and $H_2PO_4^-$ formed 1:1 (anion:receptor) species, whereas F^- gave 2:1 complexes.

The $[Zn_2(\mathbf{30})]^{3+}$ complex showed an emission band centered at 436 nm (aqueous solutions buffered at pH 7.4, 0.01 mol dm^{-3} HEPES, $\lambda_{ex} = 305$ nm) [36]. Addition of ATP induced a 12 nm red shift and a 2-fold increase in the emission intensity (ADP, AMP and HPO_4^{2-} induced no variation). Also large changes were achieved upon addition of 1 equivalent of pyrophosphate (20 nm red shift and 9.5-fold emission intensity enhancement). The apparent association constant for the formation of the 1:1 adduct between $[Zn_2(\mathbf{30})]^{3+}$ and ATP was 40-fold lower than

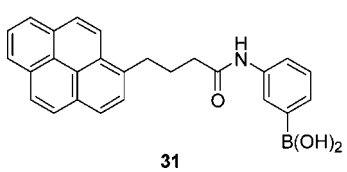


$[Zn_2(\mathbf{30})]^{3+}$

that for pyrophosphate. The structure of the 1:1 adduct formed between $[Zn_2(\mathbf{30})]^{3+}$ and pyrophosphate showed that the two sets of oxygen anions on each P atom of pyrophosphate bind to the two metal ions of the dinuclear complex giving rise to two haxacoordinated Zn(II) ions in the final adduct. The complex formation induced a weakening of the bond between the phenolate oxygen atom and the Zn(II) that increases the negative charge on the former leading to an emission enhancement. The authors suggested that the selectivity for pyrophosphate over ATP arises from the fact that the total anionic charge density of the four O–P oxygen atoms involved in the complexation of ATP with the complex $[Zn_2(\mathbf{30})]^{3+}$ is relatively smaller than that of the same oxygen atoms in pyrophosphate.

Containing Pyrene

Pyrene is also a highly fluorescent polycyclic aromatic hydrocarbon widely used as signalling subunit. Additionally, pyrene derivatives might form excimers and display dual monomer–excimer emission, a sensing protocol that has also been widely used in the design of anion chemosensors. For instance, excimer formation has been used as sensing principle in the receptors **31**, **33**–**37** and **39**.



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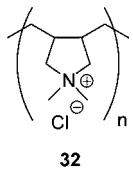
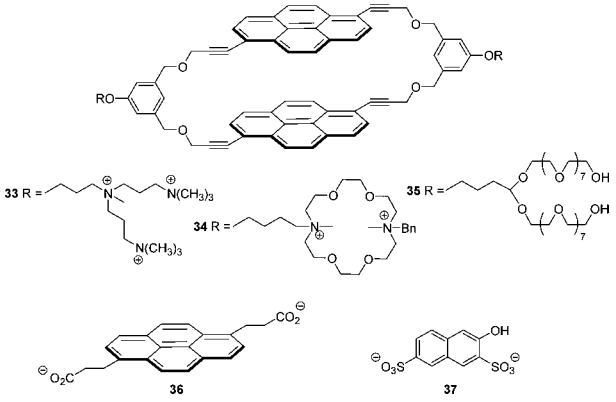


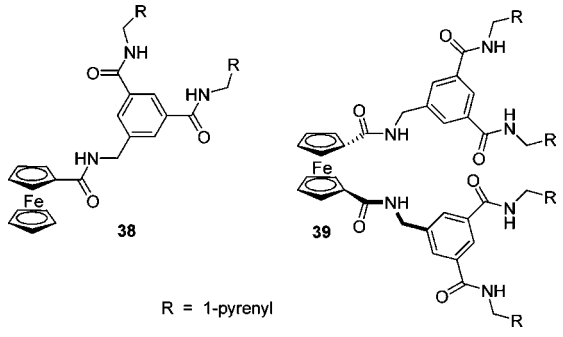
32

Thus, **31** and the polication **32** were used to selectively detect ATP through fluorescence excimer emission [37]. Aqueous solutions of receptor **31** (pH 10.2, $Na_2CO_3/NaHCO_3$) containing **32** showed typical pyrene

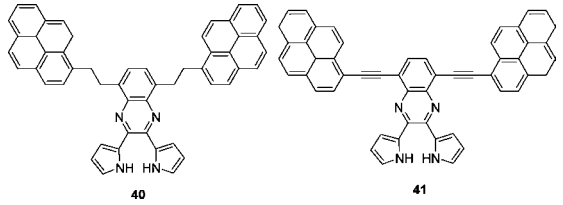
monomer emission bands. Upon addition of increasing quantities of ATP a new broad band centred at 482 nm was observed and attributed to excimer emission. The boronate group of receptor **31** forms an ester with the diol groups of ATP and the boronate ester formed was coordinated by the polycation **32** through electrostatic interactions. In this **31**-ATP-**32** aggregate form two pyrene moieties are placed in near proximity and induced the apparition of the broad excimer band. Monitoring the changes in the intensity ratios at 482 nm to 377 nm (I_{482}/I_{377}) upon nucleotide addition the larger spectral changes were obtained with ATP and ADP, whereas low or no changes were found with AMP and dATP. The observed selectivity correlates nicely with the total charge of the nucleotides (ATP, ADP and AMP are tetravalent, trivalent and divalent anions, respectively).



The water soluble pyrenophanes **33**–**35** recognize fluorimetrically anionic arenes (**36**, **37**) and nucleotides [38]. For instance aqueous solutions of receptor **34** showed typical pyrene excimer emission due to intramolecular π-stacking of the two pyrenyl groups. Upon addition of **37**, excimer emission quenching was observed as consequence of the formation of 1:1 aducts in which the anion was inserted between the two sandwich-like pyrene units. Similar results were observed with **33** and **37** whereas addition of **37** to water solutions of the neutral receptor **35** induced no changes. A similar excimer quenching in **35** was observed upon addition of the dianion **36**. Again the formation of a 1:1 "sandwich-type" species between **35** and **36** was the cause of the quenching. For **34**, built-up with two azoniacrown sites and the pyrenophane moiety, the excimer emission band centred at 512 nm was quenched upon addition of increasing quantities of ATP. This effect was attributed to coordination of the phosphate group of the ATP with the azoniacrown whereas the nucleobase was inserted between the two flat pyrene subunits. The other triphosphate nucleotides such as GTP, CTP and UTP showed comparable association constants than that obtained with ATP, whereas di- and monophosphate nucleotides (ADP, GDP, CDP, UDP, AMP, GMP, CMP and UMP) gave weaker complexes. It was suggested that the distance between the cavity of the pyrenophane and the diazoniacrown in **34** may fit well with triphosphate rather than with di- or monophosphate nucleotides.



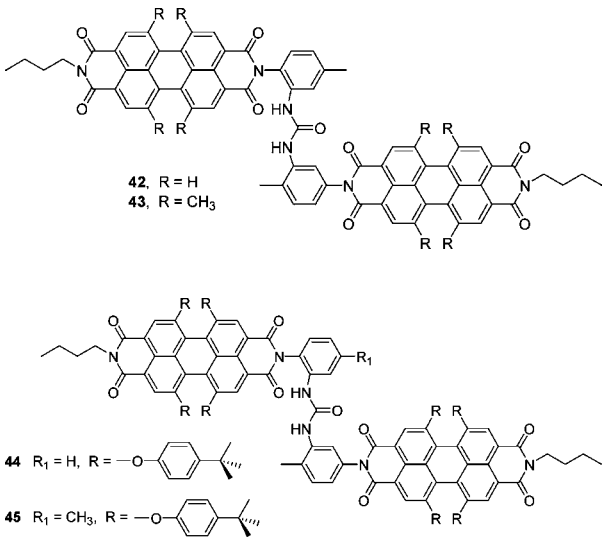
The ferrocene containing compounds **38** and **39** selectively bind $H_2PO_4^-$ over other anions via hydrogen bonding interactions [39]. **38** froms 1:1 complexes with $H_2PO_4^-$, whereas the two-arm receptor **39**, can accommodate two $H_2PO_4^-$ anions. THF solutions of receptor **38** showed pyrene monomer bands but no excimer emission. Upon addition of $H_2PO_4^-$ the monomer emission intensity was somewhat enhanced. The addition of AcO^-, Br^-, NO_3^-, ClO_4^- and HSO_4^- induced negligible changes. This complexation induced a conformational rigidification that was suggested to account for the observed emission increase. The receptor **39** in THF solutions showed dual monomer–excimer emission because of favourable intermolecular interactions between pyrenes. The addition of $H_2PO_4^-$ induced a large emission enhancement in the monomer band.



The receptors **40** and **41** were designed with the aim to increase the sensing ability of the 2,3-di(1*H*-2-pyrrolyl)quinoxaline core which is know to display modest fluorescence quenching in the presence of F^- [40]. Pyrene moieties attached in receptor **40** induced a signal amplificaction by resonance energy transfer (RET)

from the pyrene subunit to the pyrrolylquinoxaline framework, whereas acetylene bridges on receptor **41** allowed excited state delocalization. CH_2Cl_2 solutions of both receptors showed increasing fluorescence emission intensity, when compared with the parent 2,3-di(1*H*-2-pyrrolyl)quinoxaline. Addition of F^- and $HP_2O_7^{3-}$ anions induced significant quenching of the emission intensity, whereas the presence of Cl^-, CN^- and $H_2PO_4^-$ did not induce any significant variation of the emission profile.

Containing Other Aromatic Hydrocarbons



Receptors **42** and **43** based on perylene derivatives linked through an urea moiety showed an emission band centered at 605 nm upon excitation at 450 nm in THF solutions [41]. The addition of 32 equivalents of F^- induced a near complete quenching. The parent perylene derivatives **44** and **45** also showed fluorescence quenching in the presence of fluoride anion in THF solutions [42]. Addition of other anions did not induce any noticeable change in the fluorescence behaviour. Fluoride forms 1:1 complexes through hydrogen bonding interactions with the urea moiety. As in other cases the F^- selectivity is attributed to the high charge density and small size of this anion which makes it a strong hydrogen bonding acceptor in non-aqueous solvents. The quenching of the emission intensity was attributed to a PET process between the electron-rich urea-bound F^- and the electron deficient perylene moiety without discharging posible intramolecular energy transfer paths. This coordination enhances the reduction potential of the urea opening a PET channel that is the responsible of the emission quenchig observed.

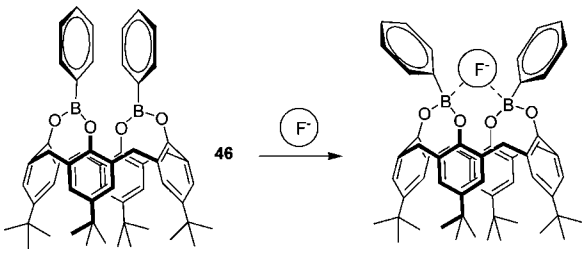
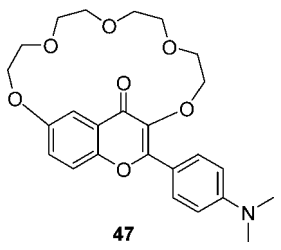
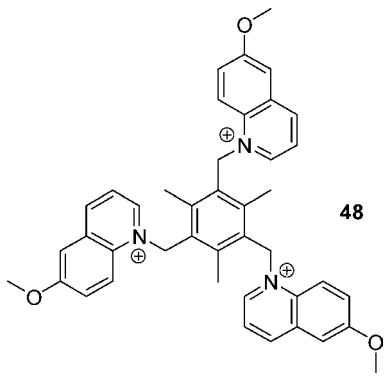


Fig. 3. Structure of the "endo" adduct formed between receptor **46** and the F^- anion.

Bis(bora)calixarene **46** shows fluorescence of the phenyl groups attached to boron atoms at 395 nm in $CHCl_3$ solution [43]. Among certain anions tested only F^- induced a significant emission quenching. The F^- anion binds with receptor **46** in a bidentate "endo" binding mode forming a 1:1 complex (Fig. 3).



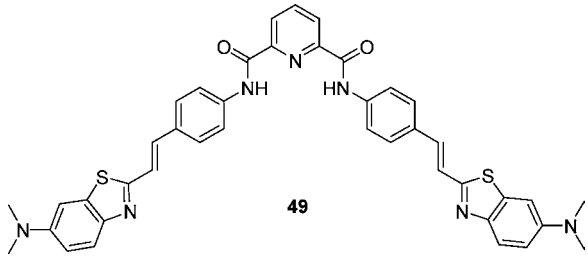
The $[Mg(\mathbf{47})]^{2+}$ and $[Ca(\mathbf{47})]^{2+}$ complexes selectively bind tetrahedral anions HSO_4^- and $H_2PO_4^-$ in acetonitrile solutions giving 1:1 aducts through hydrogen bonding interactions involving the anions, the metal center and the carbonyl group in **47** [44]. Acetonitrile solutions of complex $[Mg(\mathbf{47})]^{2+}$ showed an absorbance band centered at 402 nm (greenish yellow colour) that upon addition of an excess of HSO_4^- or $H_2PO_4^-$ shifted to 502 nm changing the colour of the solutions to pink. In contrast, in the presence of F^-, Cl^-, Br^-, I^-, HCO_3^-, AcO^- and NO_3^- the $[Mg(\mathbf{47})]^{2+}$ and $[Ca(\mathbf{47})]^{2+}$ complexes remained silent. Identical selectivity was obtained by fluorescence measurements; acetonitrile solutions of the $[Mg(\mathbf{47})]^{2+}$ or $[Ca(\mathbf{47})]^{2+}$ complexes showed a broad emission band at 500 nm that was quenched upon addition of HSO_4^- and $H_2PO_4^-$ anions, whereas other anions did not induce any noticeable change in the emission profile. The observed changes in the electronic transitions and the fluorescence quenching can be explained by the enhancement in the charge density in the oxygen atom of the carbonyl group upon anion coordination.



48

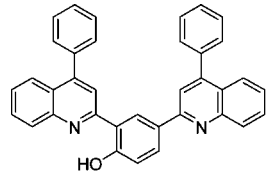
Fluorescent Sensors Based on Aromatic Heterocycles

Receptor **48** containing a 1,3,5-trimethylbenzene scaffold functionalised with three 6-methoxy-1-methylquinolinium contains three symmetrically placed positive charges in a cavity suitable for anion inclusion to form 1:1 aducts [45]. Acetonitrile solutions of receptor **48** showed an emission band centered at 440 nm ($\lambda_{exc} = 330$ nm). The addition of inorganic anions induced different degrees of emission quenching in the order $Br^- \gg I^- > NCS^- \gg Cl^- > NO_3^- > HSO_4^-$. The quenching is due to an electron transfer process from the anion bounded in the cavity to the photo-excited fluorophore. Emisión intensity versus anion concentrations fitted to Stern–Volmer type equations.



49

The UV-visible spectra of receptor **49** in DMSO-water (95:5 v/v) consists of an intense CT band centred at 415 nm that upon excitation gives emission at 551 nm with a low quantum yield (0.004) that was attributed to the opening of radiationless deactivation channel upon formation of hydrogen bonding interactions between the hydrogen of the amido groups and both a solvent molecule and the pyrido nitrogen atom [46]. Addition of AcO^- and $H_2PO_4^-$ anions induced a 3.5- and 6.5-fold enhancement of the emission intensity respectively that was due to the formation of strong hydrogen bonds between the anions and the receptor thus replacing the solvent molecule weakly bond to **49**. This replacement induced more planar conformations leading to the blocking of the quenching channel. Both anions form 1:1 complexes with **49**.



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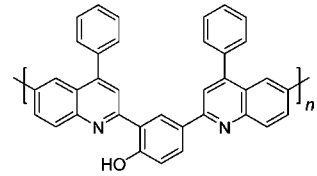
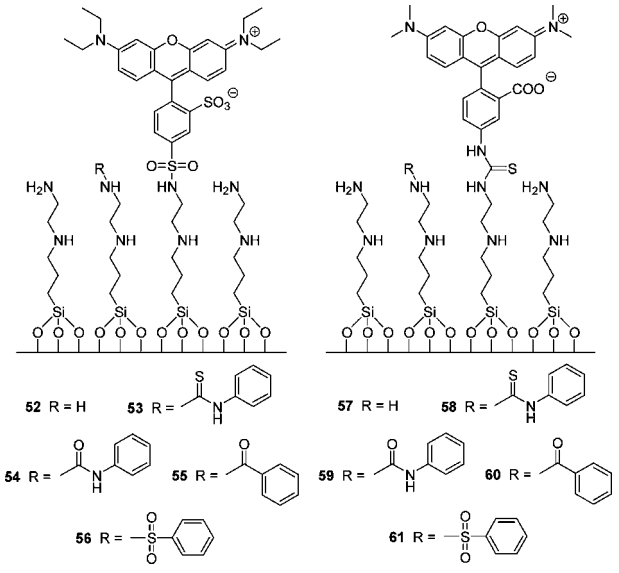


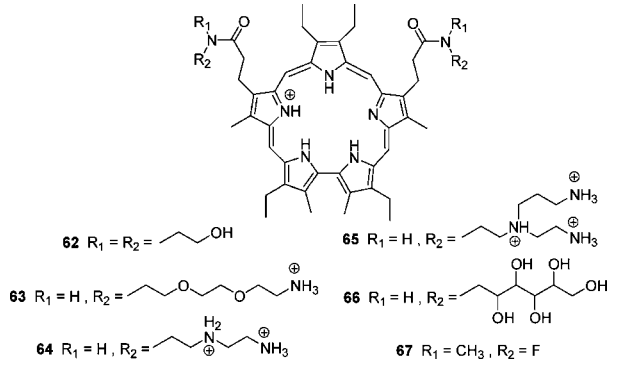
51

The receptor **50** and the polymer **51** act as chromogenic and fluorogenic fluoride chemosensors [47]. Upon addition of 100 equivalents of F^- to DMSO solutions of **50** a new band centered at 460 nm (colour change from colourless to yellow) was observed. That change in colour was ascribed to deprotonation of the phenol group upon addition of the basic F^- anion as could be observed by ^{1}H-RMN and by addition of OH^- that induced the same spectral changes than F^-. Addition of $H_2PO_4^-$, Cl^- and Br^- did not induce any variation. Upon excitation of **50** at 460 nm an emission band centered at 590 nm was observed. Addition of F^- induced a 17-fold enhancement of the emission intensity band centered at 590 nm, whereas $H_2PO_4^-$ induced a lower enhancement. The long wavelength emission has an ICT nature. The analogous polymer **51** enhances the response observed with fluoride. Thus, DMSO solutions of **51** changed from colourless to red ($\lambda_{max} = 500$ nm) upon addition of 100 equivalents of F^-, with an emission enhancement of 147-fold (emission band centered at 620 nm upon excitation at 500 nm). In this case no significant response was observed with other anions. The higher selectivity presented by polymer **51** was adscribed to the chain conformation that difficult the access to the phenol group of larger anions such as $H_2PO_4^-$.

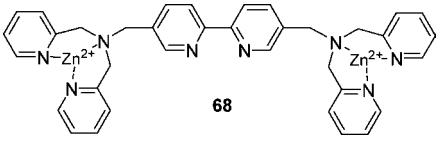
Self assembled monolayers (SAM) with different anion complexing functionalities (amino, amide, sulfonamide, urea and thiourea) and fluorophores (TRITC and lissamine) were fabricated and tested with certain anions (HSO_4^-, NO_3^-, $H_2PO_4^-$ and AcO^-) in acetonitrile solutions [48]. The direccional preorganization as well as random distribution brings the binding groups and the fluorophores in close proximity resulting in a change in the emission intensity upon anion complexation. As conclusions the SAM's functionalised with TRICT (**57–61**) presents a larger response than those functionalised with lissamine (**52–56**). Within the lissamine series the most remarkable response was found with **53** that sufers a 35% quenching in the presence of AcO^-, whereas the presence of HSO_4^- induced a moderate emission enhancement



(20%). The presence of HSO_4^- induced a 72% enhancement in the emission intensity of **57**, AcO^- induced a 34% quenching while NO_3^- and $H_2PO_4^-$ gave no response. In general TRITC-functionalised layers (**58–60**) presentes intensity enhancements (24 to 87%) with the addition of HSO_4^- and quenching in the presence of $H_2PO_4^-$ (35 to 56%).

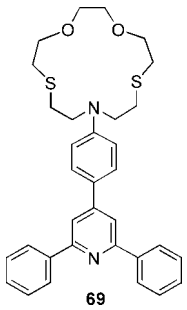


Anion-induced aggregation–deaggregation processes coupled with colour or fluorescence variation is an interesting sensing protocol that allows target anion detection in water media. This approach has been mainly used with sapphyrin derivatives. In this new example, the water-soluble sapphyrins **62–67** were characterised by the presence of a broad band centerd around 410 nm assigned to the presence of aggregates [49]. By addition of increasing amounts of sodium dodecyl sulfate (SDS) this band was substituted for an abpsortion centered at 420 nm as consequence of the rupture of the aggregates and formation of dimers. Upon increasing amounts of SDS the band at 420 nm was replaced by a strong, sharp Soret-like band centered at 450 due to the monomeric form of the sapphyrins which is higly fluorescent when compared with the dimeric or the aggregate forms. Addition of phosphate anion to water solutions buffered at pH 7.0 induced the shift of the aggregation equilibrium towards the monomeric form of the sapphyrins inducing an enhancement in the emission intensity band centerd at 690 nm (excitation at 450 nm) due to the formation of the highly fluorescent 1:1 complex between the monomeric cationic form of the sapphyrins and the phosphate. Additon of Cl^- induced deaggregation but only to the dimeric form (no emission response was observed). The authors suggested that similar systems could open the door towards a possible application for phosphate sensing in biological milieus.



Aqueous solutions of receptor **68** buffered at pH 8.0 showed two emission bands centered at 330 and 389 nm upon excitation at 297 nm [50]. Upon addition of model peptides with two phosphorylated serines the emission intensity at 389 nm gradually decreases. Addition of mono-phosphorylated peptides induced less important emission intensity variation. Emission changes were used to calculate binding constants (formation of 1:1 adducts). These constants showed that the selectivity towards di-phosphorylated was more than 10-fold over mono-phosphrylated peptides. The emission behaviour of receptor **68** was also tested in the presence of natural peptides such as IRK (insulin receptor kinase) containing two (IRK-2P), one (IRK-1P) and none (IRK-0P) phosphorylated segment. IRK-2P induced a decrease of the emission at 389 nm (formation of a 1:1 adduct) with a binding constant 20-fold greater than that presented by IRK-1P, whereas IRK-0P did not induce any remarkable change in the emission spectrum profile.

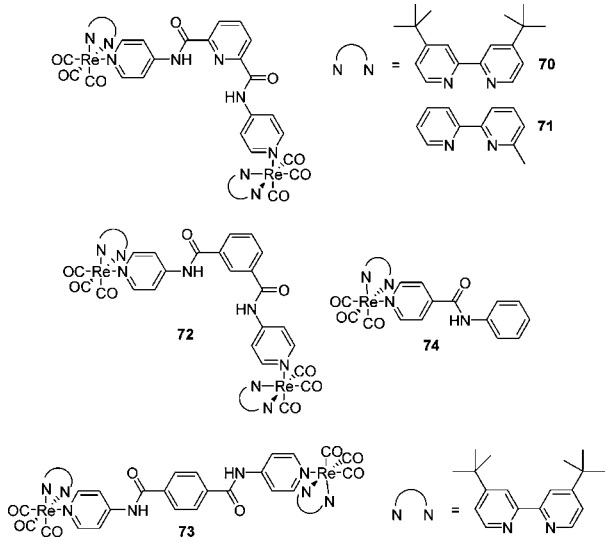
Acetonitrile solutions of the anilinopyridine dye **69** showed a band centered at 338 nm in its UV–visible spectrum [51]. This band has a CT nature and was shifted to longer wavelenghts (ca. 432 nm change of the colour solution form colourless to yellow) by the addition of Fe^{3+}, Cu^{2+}, Zn^{2+} and Pb^{2+} cations as consequence of metal coordination to the 2,6-diphenylpyridine acceptor unit. Upon addition of certain anions to solutions of the metal complexes a progressive decoloration was observed due



69

to the formation of the correspondent ternary complexes **69**-M-X. The formation of this ternary complexes was confirmed through fluorescence lifetime measurements, thus **69** was highly emissive with a $\tau_f = 2.86$ ns and, upon formation of the Pb^{2+} complex the emission is strongly quenched ($\tau_f = 32$ ps). Measured lifetime in the presence of AcO^- anion was 2.66 ns a value which was significantly different from that of the free **69**. These **69**-M ensembles were used to identify certain anions by PCA (Principal Component Analysis) analysis. In the case of **69**-Pb(II) a selective acetate recognition was achieved.

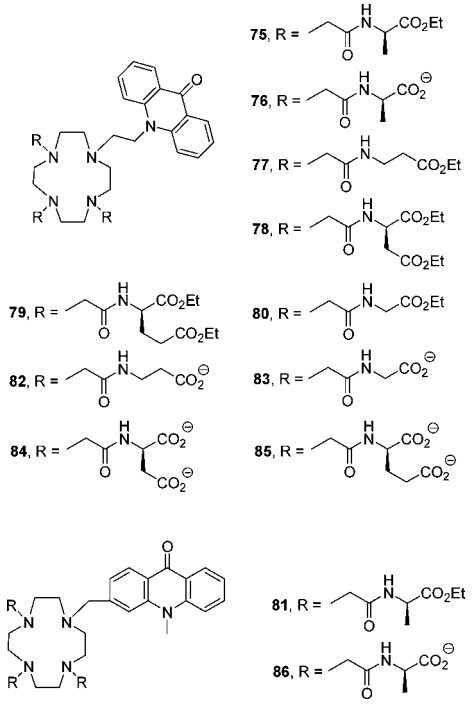
Fluorescent Chemosensors Based on Rhenium and Lanthanide Complexes



70 71 72 73 74

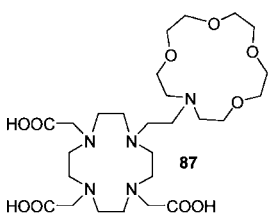
Dichloromethane solutions of **70–74** showed typical ^{3}MLCT emission bands in the range 524–544 nm which were significantly quenched upon addition of F^- and CN^-; moderately quenched in the presence of I^-, Br^-, Cl^- and AcO^- and very weakly quenched with $H_2PO_4^-$, NO_3^- and ClO_4^- [52]. All the receptors formed 1:1 complexes with the corresponding anion through hydrogen bonding interactions with the amide hydrogens. Receptors **70** and **71** showed the stronger association constants. The authors suggested that the observed quenching of the emission intensity was due to a change of the lowest excited state from 4,4′-tBu$_2$bpy-based ^{3}MLCT state to the amide–pyridine-based ^{3}MLCT state upon hydrogen-bonding anion coordination, that enhanced nonradiative decay. An alternative explanation of the quenching was an anion-enhanced reductive quenching of the MLCT excited state that might take place upon complexation.

Eu and Tb complexes are smart examples of chemosensors that allow fluorogenic anion sensing in aqueous environments. Recent examples in the last year involve the use of the receptors **75–89**. In these complexes the direct metal excitation is very inefficient and it is usual the use of antennas for indirect excitation of the metal centre. Two general mechanisms have been reported for explaining emission variation upon anion interactions in these complexes; (i) one involves the perturbation upon anion coordination of the energy transfer from the excited antenna to the metal centre that results in an emission quenching; (ii) the other mechanism is related with the displacement of water molecules from the coordination sphere of the metal upon anion coordination that usually results in an enhancement of the fluorescence emission [53]. In some examples shown below the recognised anion is used as antenna.



75, R = 76, R = 77, R = 78, R = 79, R = 80, R = 81, R = 82, R = 83, R = 84, R = 85, R = 86, R =

The europium cyclen complexes Eu(**75**)$^{3+}$-Eu(**86**), functionalised with acridone chromophores and several amino acids moieties, were used to selectively detect the hydrogencarbonate anion in aqueous solutions at physiological pH [54,55]. The europium emission was sensitized upon excitation at 410 nm (acridone chromophore) in aqueous solutions (pH 7.4, MOPS buffer). Addition of HCO_3^- induced an enhancement of the europium luminescence that was assigned to the displacement of metal bound water molecule by the carbonate anion. Changes in the intensity ratios 618 nm/588 nm and 618 nm/702 nm were used to obtain calibration curves for HCO_3^- in the presence of competing anions (Cl^-, HPO_4^{2-} and lactate) with fine results. The larger stability constants for the formation of 1:1 adducts with the HCO_3^- anion were obtained with the Eu(**80**)$^{3+}$, Eu(**77**)$^{3+}$and Eu(**75**)$^{3+}$ complexes. The affinity of the different complexes for hydrogencarbonate followed the trend cationic > neutral > anionic. Preliminary studies revealed that the complexes were non-toxic and opened possible uses in viable ratiometric imaging protocols.



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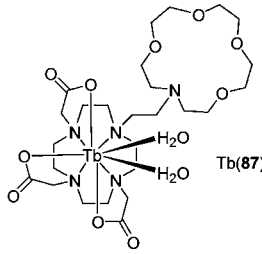
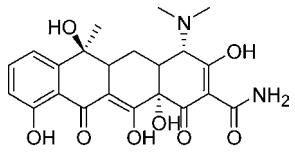


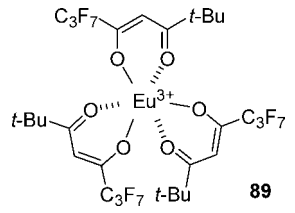
Tb(**87**)·2H$_2$O

The Tb(**87**)·2H$_2$O complex was used for the recognition of biologically significant anions lactate and salicylate via enhancements in luminescence lifetime and luminescence intensity [56]. Upon excitation in the range 270–350 nm (aqueous solutions buffered at pH 7.4) the Tb(**87**)·2H$_2$O complex was non-emissive. Salicylate addition induced the substitution of two water molecules coordinated with the metal centre by one salicylate anion forming a six-membered ring chelate. Salicylate acted as a suitable antenna and promoted the enhancement of the luminescence at 545 nm ($^5D_4 \rightarrow ^7F_5$ transition) upon excitation at the salicylate band. Additionally, the luminescence lifetime of complex Tb(**87**)·2H$_2$O increased by 48% upon lactate addition in water. This increase in luminescent lifetime was assigned to the substitution of two water molecules in the coordiantion shell of the metal centre by one molecule of lactate. The addition of HCO_3^-, $H_2PO_4^-$ and AcO^- (14 equivalents) induced an 19%, 12% and 6% increase in the luminescence lifetime respectively, whereas the anion Cl^- did not lead to any significant change.



88

Tetracycline (**88**) forms an europium complex that was able to sense citrate in aqueous solutions buffered at pH 8.0. Upon addition of citrate to aqueous solutions of the Eu-**88** complex, a 22-fold enhancement in the emission band centered at 615 nm ($^5D_0 \rightarrow ^7F_2$ electronic transition) was observed ($\lambda_{ex} = 405$ nm) [57]. Citrate forms a 1:1:2 Eu^{3+}-**88**-citrate adduct in which citrate coordinates the Eu^{3+} ion through the oxygen atoms of the carboxy and hydroxy groups. The emission enhancement was assigned to coordination of the citrate and displacement of water molecules that were quenching the Eu^{3+} luminescence. This complex was used in the fluorescent imaging of citrate and other intermediantes in the citric acid cycle.



89

Addition of 3 equivalents of Cl^- to acetonitrile solutions of **89** induced an enhancement (2.0-fold) in the europium luminescence at 611 nm (excitation at 294 nm) [58]. Addition of Br^-, I^- and ClO_4^- slightly enhanced the luminescence intensity (<1.3-fold). As an opposite behaviour addition of F^- anion induced remarkable changes in the UV–visible spectrum of **89** and a suppression of the luminescence intensity. This receptor forms 1:1 complexes with the anions tested by coordination with Eu^{3+} vacant coordination site.

FOLLOWING THE DISPLACEMENT APPROACH

This approach, somehow inspired in displacement reactions in immunoassay protocols [59], is relatively new in the field of anion chemosensing but despite its novelty it has been used for the development of new elegant examples for the sensing of target anion. This approach also involves, as in the above cases, the use of a binding site and a signalling reporter although here both subunits are

not covalently attached but forming a molecular ensemble [60]. In these systems the addition of a certain guest to solutions containing the sensing ensemble results in a displacement reaction: the binding site coordinates to the guest whereas the signalling subunit is released. This approach has several advantages; for instance, the non-covalent anchoring of binding sites and indicator groups allows to test with a minimum effort a large number of combinations in order to obtain tuned sensing systems. Additionally, these sensing ensembles usually work in pure water or water mixtures allowing the design of realistic sensing protocols. Despite these interesting features the number of examples reported following this protocol is by far less abundant that those using the binding site-signalling subunit approach. Only two examples have been recently reported using the systems **90** and **91**.

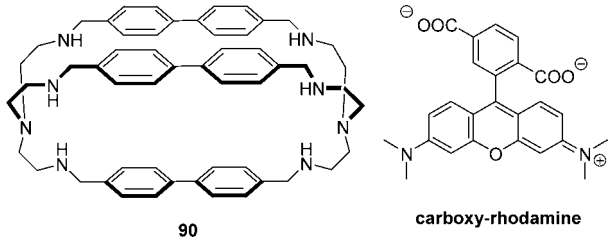
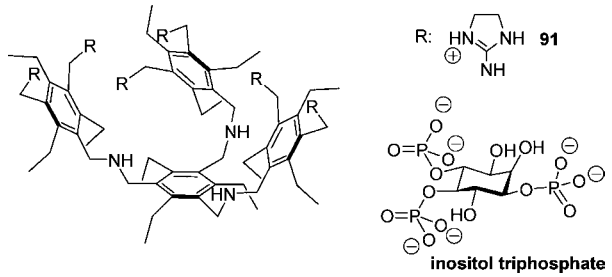


Fig. 4. The carboxy-rhodamine dye employed in a displacement assay with receptor **90** for the sensing of different carboxylates.

The receptor **90** composed by two tren subunits linked by three 4,4′-ditolyl spacers forms a dicopper complex with a long ellipsoidal cavity (Cu–Cu separation of 11.305(6) Å) suitable for the inclusion of dicarboxylate anions and the $[Cu_2(\mathbf{90})]^{4+}$ complex was used together with carboxy–rhodamine (Fig. 4) as indicator (in a displacement assay) for the fluorescence discrimination of different carboxylates in aqueous solutions buffered at pH 7.0 [61]. Titration of a solution of the indicator with increasing quantities of $[Cu_2(\mathbf{90})]^{4+}$ induced the gradual decrease of the rhodamine emission band due to indicator inclusion in the long ellipsoidal cavity. In this situation the metal centers could quench the emission of the fluorophore through either an electron transfer or an electronic energy transfer process. This non-fluorescent adduct was tested against two families of dicarboxylates. For instance, terephthalate induced full revival of the rhodamine emission due to the formation of a more stable adduct between the $[Cu_2(\mathbf{90})]^{4+}$ complex and terephthalate with the consequent release of the carboxy–rhodamine to the solution, whereas the addition of phthalate and isophthalate gave minor changes. A family of linear dicarboxylates was also tested and only glutarate and adipate were capable of release the dye to the solution with the consequent restoration of the emission intensity. The presence of succinate and pimelate did not induce any retrieval of the emission intensity. Oxalate dianion was able to induce certain recovery of the fluorescence emission due to the inclusion of two oxalate molecules into the complex cavity.



Receptor **91** was developed to sense IP_3 (a second messenger in living cells) through a competition assay using 5-carboxyfluorescein as indicator in methanol solutions [62]. To overcome the fact that the affinity of receptor **91** to IP_3 was drastically reduced in water solutions the authors suggested the use of surfactants. In water solutions containing 2% of Triton X-100 (buffered at pH 4) the 5-carboxyfluorescein was incorporated into the interior of the micelles in its cyclised form (colorless and non-fluorescent, see Fig. 5). Upon addition of the receptor **91** the yellow colour and the fluorescence was recovered due to the shift of the cyclised form of the 5-carboxyfluorescein equilibrium to the open one (yellow and fluorescent, see Fig. 5). This was because the more anionic open form interacts better with the highly charged cavity presented by **91**. Addition of IP_3 to the carboxyfluorescein-**91** ensemble displaced the dye into the surfactant environment leading to remarkable changes in absorbance/emission. The authors suggested that the binding between **91** and IP_3 occurs in the lower dielectric environment of the micelles whereas the complex between **91** and the dye would prefer the aqueous environment.

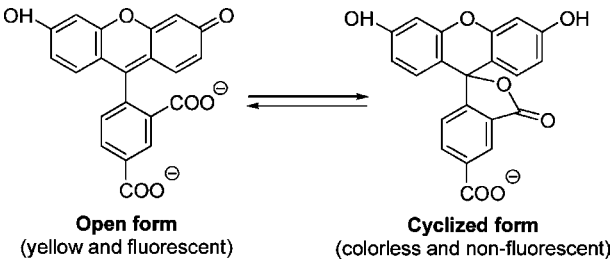


Fig. 5. Open and closed form of 5-carboxyfluorescein employed in a displacement assay with receptor **91**.

FOLLOWING THE CHEMODOSIMETER APPROACH

The chemodosimeter approach involves the use of specific and selective (very often irreversible) reactions which are coupled with remarkable changes in the emission (or colour) behaviour. Following this approach it is possible to design highly selective anion-induced reactions that might work at room temperature and in aqueous environments for feasible sensing protocols. Despite the attractiveness of this approach there are still relatively few examples when compared with the more common binding site-signalling subunit approach.

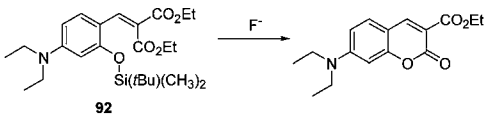
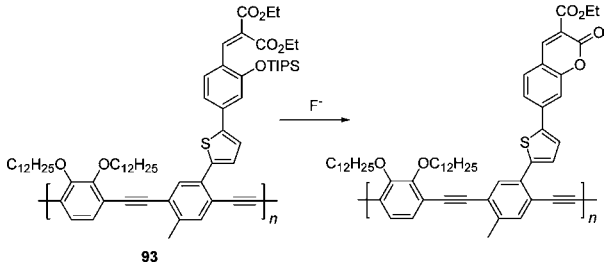


Fig. 6. F^- anion induced the cleavage of the Si—O bond presented in receptor **92** that suffers a cyclization process to give the highly fluorescent coumarin.



Receptor **92** was used as a chemodosimeter for F^- anion [63]. Addition of F^- anion to THF solutions of receptor **92** induced the Si–O bond cleavage and cyclization to give the correspondent highly fluorescent coumarin (Fig. 6). Addition of 10-fold excess of F^- induced the saturation of the emission intensity in two hours. In order to enhance the sensitivity of the chemodosimeter the authors synthesized the semiconducting organic polymer **93**. In **93** a few cyclisation events induced by F^- produces a local minima in the band gap that trap mobile excitons to give a new emission. Without F^- anion **93** presents an emission band centred at 482 nm (excitation at 378 nm) that was red-shifted to 517 nm upon F^- addition. Complete saturation was again achieved in two hour but the quantity of F^- needed was 100 times lower.

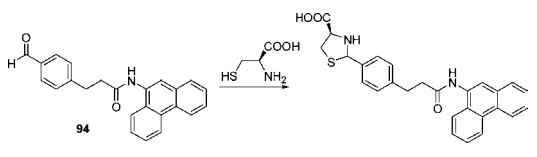
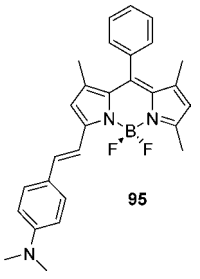


Fig. 7. Reaction of fluorogenic receptor **94** and cysteine to afford the highly fluorescent thiazolidine.

94 was used as chemodosimeter for the determination of cysteine in aqueous solutions buffered at pH 7 [64]. Among all the aminoacids tested (cysteine, methionine, serine, lysine, proline, glycine and histidine) only the presence of cysteine induced the formation of the highly fluorescent thiazolidine derivative ($\lambda_{exc} = 250$ nm, $\lambda_{em} = 380$ nm, see Fig. 7). Addition of glutation and glucose did not induce the apparition of the emission band. Concentrations of cysteine in the range of 100 μM–5 mM were detected by using **94**.



Difluorobora-s-diazaindacene dye **95** shows an absorption maximum at 599 nm in acetone solutions [65]. Upon excitation a CT emission band centered at 686 nm was developed. Upon F^- addition the blue colour of acetone solutions turned colourless. The emission spectrum also showed significant changes with the disappearance of the emission peak at 686 nm with a concomitant growth of two new emission peaks centered at 452 and 482 nm. The authors suggested two possible explanations: (i) a nucleophylic displacement by fluoride, breaking a B—N bond and forming a B—F bond and (ii) fluoride abstraction of acidic methyl protons at 7 position of the boradiazaindacene system that initiates a degradation reaction. In the presence of Cl^-, Br^-, I^-, SO_4^{2-}, NO_3^-, $H_2PO_4^-$ and SCN^- the chemodosimeter **95** remained silent.

Boronic acid groups display chemical reactions with nucleophiles such as cyanide to form the electron-donating group R-B^--$(CN)_3$ (Fig. 8). This particularity was used to design the fluorogenic cyanide chemosensors **96–99** [66]. Stilbene derivatives **96**, **97** and the chalcone **98** acts through a charge transfer (CT) mechanism, whereas anthracene derivative **99** acts through a photoinduced electron transfer (PET) mechanism. Receptor **96** present an emission band centred at 490 nm that, upon addition of CN^-, suffers a 40 nm hypsochromic shift together with an increase in the emission intensity. This changes were assigned by the loss of electron-withdrawing character of the boronic acid group by formation of the R-B^--$(CN)_3$ group. Receptor **97** (with two electro-withdrawing groups and $\lambda_{em} = 388$ nm) presented a bathochromic shift of 35 nm accompanied by a decrease in intensity upon reaction with cyanide. The chalcone **98**

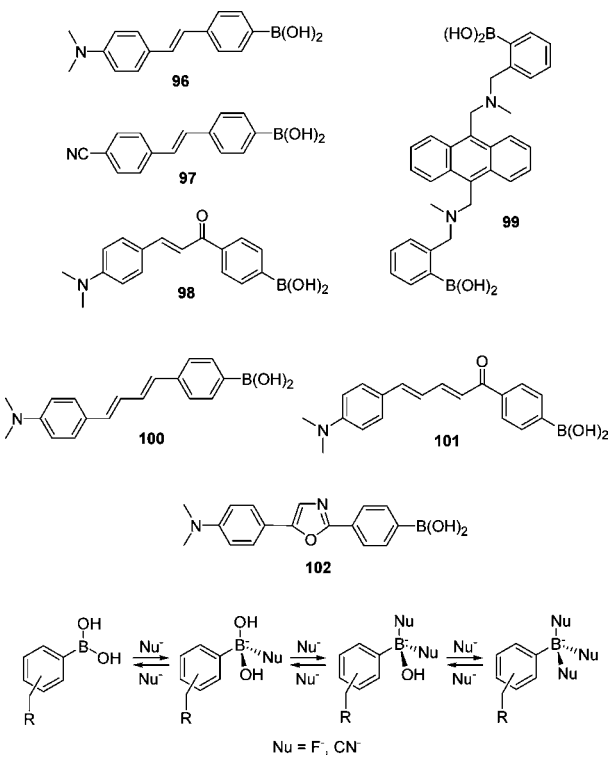
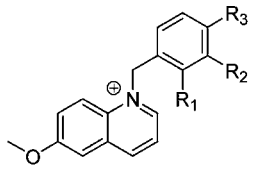


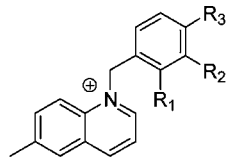
Fig. 8. Equilibrium involved in the interaction between the boronic acid group and nucleophiles (CN^- and F^-).

acts in a similar manner as **96** and addition of cyanide induced a hypsochromic shift (from 580 to 570 nm) with an enhancement in the emission intensity. The fluorescence of the anthracene derivative **99** was quenched upon additon of an excess of cyanide. This quenching was assigned to the formation of electron rich R-B^--$(CN)_3$ group that deactivated the emission via a PET path.

Using a similar sensing principle, the compounds **96–98** and **100–102** were used to signalling the presence of F^- anions through changes in the emission intensity in water–methanol (2:1, v/v) mixtures [67]. The probes are based on the ability of the boronic acid to interact with F^- to form a trifluoroboronate anion (R-B^--F_3). Again the interpretation of the observed effect is related with the perturbation of the CT state via formation of the R-B^--F_3 group. Probes **96** and **97** gave a similar response to that shown in the presence of cyanide. **100** also showed a similar response to that observed with **96**. Probe **102** showed a 14-fold enhancement in the emission intensity concomitant with a hypsochromic shift upon F^- addition in accordance with the mechanism cited above. The chalcones **98** and **101** display a CT between the dimethylamino and the carbonyl group. Addition of F^- anion induced hypsochromic shift of the emission band and an enhancement in the emission intensity. These changes were ascribed to the formation of the boronate anion than acts as an electron donating group to the carbonyl moiety. This increase in the electronic density of the carbonyl decreases the importance of the CT.

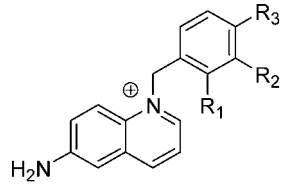


103, $R_1 = B(OH)_2$, $R_2 = R_3 = H$
104, $R_1 = R_3 = H$, $R_2 = B(OH)_2$
105, $R_1 = R_2 = H$, $R_3 = B(OH)_2$



106, $R_1 = B(OH)_2$, $R_2 = R_3 = H$
107, $R_1 = R_3 = H$, $R_2 = B(OH)_2$
108, $R_1 = R_2 = H$, $R_3 = B(OH)_2$

The compounds **103–108** based on quinolinium derivatives bearing a boronic acid funcitionality have also been used for cyanide sensing in aqueous solutions [68]. The sensing mechanism was the same than that proposed above for the probes **96–99**. The anionic R-B^--$(CN)_3$ group formed interacts with an electron deficient quaternary heterocyclic nitrogen centre providing the spectral changes observed. Probes **103–105** showed a broad emission band centred at 450 nm that was quenched upon addition of increasing quantities of cyanide anion. The most remarkable response was obtained with **104** with a 10-fold intensity change upon the addition of 20 μM of cyanide. The response of **106–108** upon cyanide addition was quite similar being the most significant response obtained with **107** (12-fold intensity decrease with the addition of 30 μM cyanide). The lifetimes of the cyanide bound probes were shorter than the free forms also allowing cyanide sensing.



109, $R_1 = B(OH)_2$, $R_2 = R_3 = H$
110, $R_1 = R_3 = H$, $R_2 = B(OH)_2$
111, $R_1 = R_2 = H$, $R_3 = B(OH)_2$

Fluorescent probes **109–111** are quite similar to **103–108** and were used to sense cyanide in aqueous solutions through fluorescence and colorimetric measurements [69]. For instance, UV–Visible spectra of **109** showed a band centred at 388 nm (yellow colour) that decreases in intensity upon cyanide addition concomitant with the apparition of a new band centred at 340 nm (colorless). This change in colour allows visual detection of amounts of 10 μM cyanide. Upon excitation at 358 nm, **109** presented an unstrutured emission band centred at

546 nm. Addition of increasing quantities of cyanide induced a decrease in the emission centred at 546 nm with the apparition of a new band at 450 nm which was attributed to the emission of the cyanide-bound complex form. **110** and **111** probes gave identical responses. Again the changes in absorption and emission were assigned to the formation of tricyanide anion R-B^--$(CN)_3$ (donor group) that interacts with the quaternary heterocyclic nitrogen (acceptor group) presented in the quinolinium backbone. Cyanide determinations in nearly physiological conditions (presence of glucose, fructose and chloride) were carried out with these probes with very fine results.

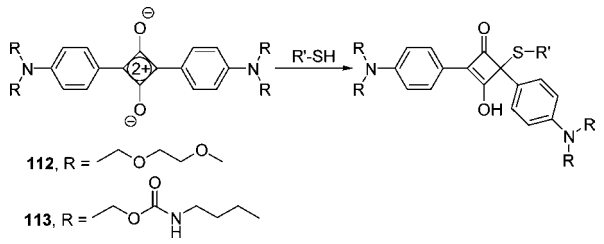


Fig. 9. Reaction between squarine derivatives **112** and **113** with thiol-containing compounds.

Squaraine based receptors **112** and **113** acts as chemodosimeters again thiols and has been used to chromogenic and fluorogenic sensing of cysteine in aqueous environments [70]. Acetonitrile-water 20:80 v/v (buffered at pH 6.0) solutions of both receptors showed a very intense typical-squaraine absorption band at 640 nm responsible of the blue colour observed. Cysteine addition resulted in a remarkable bleaching due to the nucleophilic attack of the thiol moiety in cysteine to the highly electrophilic four membered ring presented in the structure of the receptors (Fig. 9). Quenching of the emission band centered at 670 nm was also observed. Those squaraine derivatives were successfully applied to the determination of low-molecular mass amino thiols in a complex multicomponent mixture such as human plasma.

The typical method for the detection of ATP is via bioluminescence using the ATP-dependent luciferase–luciferin reaction [71]. However this method shows the disadvantage that, when used in solution, luciferase is unstable and cannot be reused. In order to minimize those effects luciferase was immobilized in a sugar-modified sol–gel silica. D-gluconolactone or D-maltonolactone were covalently linked to (aminopropyl)triethoxysilane to form *N*-(3-triethoxysilylpropyl)-gluconamide or *N*-(3-triethoxysilylpropyl)-maltonamide. These two intermediates were hydrolized and condensed with diglycerylsilane leading to a material with nonhydrolyzable sugar moieties covalently bonded into the silica network. The entrapment of luciferase in those solid produced an appreciable enzymatic activity and allows reusing the materials (the kinetic parameters of the entrapped enzyme were highly stable after five cyles of the catalytic reaction).

REFERENCES

1. J. Scheerder, J. F. J. Engbersen, and D. N. Reinhoudt (1996). Synthetic receptors for anion complexation. *Recl. Trav. Chim. Pays-Bas* **115**, 307–320.
2. F. P. Schmidtchen and M. Berger (1997). Artificial organic host molecules for anions. *Chem. Rev.* **97**, 1609–1646.
3. P. D. Beer and D. K. Smith (1997). Anion binding and recognition by inorganic based receptors. *Prog. Inorg. Chem.* **46**, 1–96.
4. M. M. G. Antonisse and D. N. Reinhoudt (1998). Neutral anion receptors: Design and application. *Chem. Commun.* 443–448.
5. P. D. Beer and P. A. Gale (2001). Anion recognition and sensing: The state of the art and future perspectives. *Angew. Chem. Int. Ed.* **40**, 486–516.
6. P. A. Gale (2000). Anion coordination and anion-diercted assembly: Highlights from 1997 and 1998. *Coord. Chem. Rev.* **199**, 181–233.
7. P. A. Gale (2001). Anion receptor chemistry: Highlights from 1999. *Coord. Chem. Rev.* **213**, 79–128.
8. P. A. Gale (2003). Anion and ion-pair receptor chemistry: Highlights from 2000 and 2001. *Coord. Chem. Rev.* **240**, 191–221.
9. L. Fabbrizzi, M. Licchelli, G. Rabaioli, and A. Taglietti (2000). The design of luminescent sensors for anions and ionisable analytes. *Coord. Chem. Rev.* **205**, 85–108.
10. P. D. Beer (1998). Transition-metal receptor systems for the selective recognition and sensing of anionic guest species. *Acc. Chem. Res.* **31**, 71–80.
11. P. D. Beer and J. Cadman, (2000). Electrochemical and optical sensing of anions by transition metal based receptors. *Coord. Chem. Rev.* **205**, 131–155.
12. R. Martínez-Máñez and F. Sancenón (2003). Fluorogenic and chromogenic chemosensors and reagents for anions. *Chem. Rev.* **103**, 4419–4476.
13. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher, and T. E. Rice (1997). Signaling recognition events with fluorescent sensors and switches. *Chem. Rev.* **97**, 1515–1566.
14. T. Gunnlaugsson, A. P. Davis, G. M. Hussey, J. Tierney, and M. Glynn (2004). Design, synthesis and photophysical studies of simple fluorescent anion PET sensors using charge neutral thiourea receptors. *Org. Biomol. Chem.* **2**, 1856–1863.
15. Z.-Y. Zeng, Y.-B. He, J.-L. Wu, L.-H. Wei, X. Liu, L.-Z. Meng, and X. Yang (2004). Synthesis of two branched fluorescent receptors and their binding properties for dicarboxylate anions. *Eur. J. Org. Chem.* 2888–2893.
16. Q.-Y. Chen and C. F. Chen (2004). A new fluorescent as well as chromogenic chemosensor for anions based on an anthracene carbamate derivative. *Tetrahedron Lett.* **45**, 6493–6496.
17. M. T. Albelda, J. Aguilar, S. Alves, R. Aucejo, P. Díaz, C. Lodeiro, J. C. Lima, E. García-España, F. Pina, and C. Soriano (2003). Potentiometric, NMR, and fluorescence-emission studies on the binding of adenosine 5′-triphosphate (ATP) by open-chain polyamine receptors containing naphthylmethyl and/or anthrylmethyl groups. *Helv. Chim. Acta* **86**, 3118–3135.
18. K. J. Wallace, W. J. Belcher, D. R. Turner, K. F. Syed, and J. W. Steed (2003). Slow anion exchange, conformational equilibria, and fluorescent sensing in venus flytrap aminopyridinium-based anion hosts. *J. Am. Chem. Soc.* **125**, 9699–9715.
19. S. K. Kim, N. J. Singh, S. J. Kim, H. G. Kim, J. K. Kim, J. W. Lee, K. S. Kim, and J. Yoon (2003). New fluorescent photoinduced electron transfer chemosensor for the recognition of $H_2PO_4^-$. *Org. Lett.* **5**, 2083–2086.

20. J. Yoon, S. K. Kim, K. N. Singh, J. W. Lee, Y. J. Yang, K. Chellappan, and K. S. Kim (2004). Highly effective fluorescent sensor for $H_2PO_4^-$. *J. Org. Chem.* **69**, 581–583.
21. J. Y. Kwon, N. J. Singh, H. N. Kim, S. K. Kim, K. S. Kim, and J. Yoon (2004). Fluorescent GTP-sensing in aqueous solution of physiological pH. *J. Am. Chem. Soc.* **126**, 8892–8893.
22. A. Ojida, Y. Mito-oka, M. Inoue, and I. Hamachi (2002). First artificial receptors and chemosensors toward phosphorylated peptide in aqueous solution. *J. Am. Chem. Soc.* **124**, 6256–6258.
23. A. Ojida, Y. Mito-oka, K. Sada, and I. Hamachi (2004). Molecular recognition and fluorescence sensing of monophosphorylated peptides in aqueous solution by bis(zinc(II)-dipicolylamine)-based artificial receptors. *J. Am. Chem. Soc.* **126**, 2454–2463.
24. X. Qian and F. Liu (2003). Promoting effects of the hydroxymethyl group on the fluorescent signalling recognition of anions by thioureas. *Tetrahedron Lett.* **44**, 795–799.
25. S.-Y. Liu, Y.-B. He, J.-L. Wu, L.-H. Wei, H.-J. Qin, L.-Z. Meng, and L. Hu (2004). Calix[4]arenes containing thiourea and amide moieties: Neutral receptors towards α,ω-dicarboxylate anions. *Org. Biomol. Chem.* **2**, 1582–1586.
26. F. Otón, F. Tárraga, M. D. Velasco, A. Espinosa, and P. Molina (2004). A new fluoride selective electrochemical and fluorescent chemosensor based on a ferrocene-naphthalene dyad. *Chem. Commun.* 1658–1659.
27. G. Xu and M. A. Tarr (2004). A novel fluoride sensor based on fluorescence enhancement. *Chem. Commun.* 1050–1051.
28. E. J. Cho, J. W. Moon, S. W. Ko, J. Y. Lee, S. K. Kim, J. Yoon, and K. C. Nam (2003). A new fluoride selective fluorescent as well as chromogenic chemosensor containing a naphthalene urea derivative. *J. Am. Chem. Soc.* **125**, 12376–12377.
29. J. Y. Lee, E. J. Cho, S. Mukamel, and K. C. Nam (2004). Efficient fluoride-selective fluorescent host: Experiment and theory. *J. Org. Chem.* **69**, 943–950.
30. S.-I. Kondo, M. Nagamine, and Y. Yano (2003). Synthesis and anion recognition properties of 8,8′-dithioureido-2,2′-binaphthalene. *Tetrahedron Lett.* **44**, 8801–8804.
31. Y. Kubo, M. Kato, Y. Misawa, and S. Tokita (2004). A fluorescence-active 1,3-bis(isothiouronium)-derived naphthalene exhibiting versatile binding modes toward oxoanions in aqueous MeCN solution: New methodology for sensing oxoanions. *Tetrahedron Lett.* **45**, 3769–3773.
32. X. Zhang, L. Guo, F.-Y. Wu, and Y.-B. Jiang (2003). Development of fluorescent sensing of anions under excited-state intermolecular proton transfer signaling mechanism. *Org. Lett.* **5**, 2667–2670.
33. C.-F. Chen and Q.-Y. Chen (2004). A tetra-sulfonamide derivative bearing two dansyl groups designed as a new fluoride selective fluorescent chemosensor. *Terahedron. Lett.* **45**, 3957–3960.
34. R. Miao, Q.-Y. Zheng, C.-F. Chen, and Z.-T. Huang (2004). A C-linked peptidocalix[4]arene bearing four dansyl groups: A highly selective fluorescence chemosensor for fluoride ions. *Tetrahedron Lett.* **45**, 4959–4962.
35. T. Gunnlaugsson, P. E. Kruger, T. C. Lee, R. Parkesh, F. M. Pfeffer, and G. M. Hussey (2003). Dual responsive chemosensors for anions: The combination of fluorescent PET (photoinduced electron transfer) and colorimetric chemosensors in a single molecule. *Tetrahedron Lett.* **44**, 6575–6578.
36. D. H. Lee, S. Y. Kim, and J.-I. Hong (2004). A fluorescent pyrophosphatesensor with high selectivity over ATP in water. *Angew. Chem. Int. Ed.* **43**, 4777–4780.
37. Y. Kaneyiko, R. Naganawa, and H. Tao (2004). Fluorescence detection of ATP based on the ATP-mediated aggregation of pyrene-appended boronic acid on a polycation. *Chem. Commun.* 1006–1007.
38. H. Abe, Y. Mawatari, H. Teraoka, K. Fujimoto, and M. Inouye (2004). Synthesis and molecular recognition of pyrenophanes with polycationic or amphiphilic functionalities: Artificial plate-shaped cavitant incorporating arenes and nucleotides in water. *J. Org. Chem.* **69**, 495–504.
39. L.-J. Kuo, J.-H. Liao, C.-T. Chen, C.-H. Huang, C.-S. Chen, and J.-M. Fang (2003). Two-arm ferrocene amide compounds: Synclinal conformations for selective sensing of dihydrogen phosphate ion. *Org. Lett.* **5**, 1821–1824.
40. R. Pohl, D. Aldakov, P. Kubát, K. Jursíková, M. Marquez, and P. Anzenbacher Jr. (2004). Strategies toward improving the performance of fluorescence-based sensors for inorganic anions. *Chem. Commun.* 1282–1283.
41. J. Ren, Q. Wang, D. Qu, X. Zhao, and H. Tian (2004). New fluoride-selective red fluorescent chemosensors based on perylene derivatives. *Chem. Lett.* **33**, 974–975.
42. J. Ren, X.-L. Zhao, Q.-C. Wang, C.-F. Ku, D.-H. Qu, C. P. Chang, and H. Tian (2005). New fluoride fluorescent chemosensors based on perylene derivatives linked by urea. *Dyes Pigments* **64**, 193–200.
43. S. Arimori, M. G. Davidson, T. M. Fyles, T. G. Hibbert, T. D. James, and G. I. Kociok-Köhn (2004). Synthesis and structural characterisation of the first bis(bora)calixarene: A selective, bidentate, fluorescent fluoride sensor. *Chem. Commun.* 1640–1641.
44. L.-L. Zhou, H. Sun, H.-P. Li, H. Wang, X.-H. Zhang, S.-K. Wu, and S.-T. Lee (2004). A novel colorimetric and fluorescent anion chemosensor based on the flavone quasi-crown ether-metal complex. *Org. Lett.* **6**, 1071–1074.
45. V. Amendola, L. Fabbrizzi, and E. Monzani (2004). A concave fluorescent sensor for anions based on 6-methoxy-1-methylquinolinium. *E. Chem. Eur. J.* **10**, 76–82.
46. A. Kovalchuk, J. L. Bricks, G. Reck, K. Rurack, B. Schulz, A. Szumna, and H. Weißhoff (2004). A charge transfer-type fluorescent molecular sensor that "lights up" in the visible upon hydrogen bond-assisted complexation of anions. *Chem. Commun.* 1946–1947.
47. H. Tong, L. Wang, X. Jing, and F. Wang (2003). "Turn-on" conjugated polymer fluorescent chemosensor for fluoride ion. *Macromolecules* **36**, 2584–2586.
48. L. Basabe-Desmonts, J. Beld, R. S. Zimmerman, J. Hernando, P. Mela, M. F. García Parajó, N. F. van Hulst, A. van den Bergh, D. N. Reinhoudt, and M. Crego-Calama (2004). A simple approach to sensor discovery and fabrication on self-assembled monolayers on glass. *J. Am. Chem. Soc.* **126**, 7293–7299.
49. J. L. Sessler, J. M. Davis, V. Král, T. Kimbrough, and V. Lynch (2003). Water soluble sapphyrins: Potential fluorescent phosphate anion sensors. *Org. Biomol. Chem.* **1**, 4113–4123.
50. A. Ojida, M. Inoue, Y. Mito-oka, and I. Hamachi (2003). Cross-linking strategy for molecular recognition nad fluorescent sensing of a multi-phosphorylated peptide in aqueous solution. *J. Am. Chem. Soc.* **125**, 10184–10185.
51. B. García-Acosta, X. Albiach-Martí, E. García, L. Gil, R. Martínez-Máñez, K. Rurack, F. Sancenón, and J. Soto (2004). Coordinative and electrostatic forces in action: From the design of differential chromogenic anion sensors to selective carboxylate recognition. *Chem. Commun.* 774–775.
52. S.-S. Sun, A. J. Lees, and P. Y. Zavalij (2003). Highly sensitive luminescent metal-complex receptors for anions through charge-assited amide hydrogen bonding. *Inorg. Chem.* **42**, 3445–3453.
53. D. Parker (2000). Luminescent lanthanide sensors for pH, pO_2 and selected anions. *Coord. Chem. Rev.* **205**, 109–130.
54. P. Atkinson, Y. Bretonniere, and D. Parker (2004). Chemoselective signalling of selected phospho-anions using lanthanide luminescence. *Chem. Commun.* 438–439.
55. Y. Bretonniere, M. J. Cann, D. Parker, and R. Slater (2004). Design, synthesis and evaluation of ratiometric probes for hydrogencarbonate based on europium emission. *Org. Biomol. Chem.* **2**, 1624–1632.
56. C. Li and W.-T. Wong (2004). Luminescent heptadentate Tb^{3+} complex with pendant aza-15-crown-5 showing recognition of lactate and salicylate in aqueous solution. *Tetrahedron Lett.* **45**, 6055–6058.
57. Z. Lin, M. Wu, M. Schäferling, and O. S. Wolfbeis (2004). Fluorescent imaging of citrate and other intermediates in the citric acid cycle. *Angew. Chem. Int. Ed.* **43**, 1735–1738.
58. R. K. Mahajan, I. Kaur, R. Kaur, S. Uchida, A. Onimaru, S. Shinoda, and H. Tsukube (2003). Anion receptor functions of lanthanide

tris(β-diketonate) complexes: Naked eye detection and ion-selective electrode determination of Cl^- anion. *Chem. Commun.* 2238–2239.
59. M. J. Perry (1995). In J. R. Birch and E. S. Lennox (Eds.), *Monoclonal Antibodies: Principles and Applications*, Wiley-Liss, New York, pp. 107–120.
60. S. L. Wiskur, H. Aït-Haddou, J. J. Lavigne, and E. V. Anslyn (2001). Teaching old indicators new tricks. *Acc. Chem. Res.* **34**, 963–972.
61. M. Boiocchi, M. Bonizzoni, L. Fabbrizzi, G. Piovani, and A. Taglietti (2004). A dimetallic cage with long ellipsoidal cavity for the fluorescent detection of dicarboxylate anions in water. *Angew. Chem. Int. Ed.* **43**, 3847–3852.
62. K. Niikura and E. V. Anslyn (2003). Triton X-100 enhances ion-pair-driven molecular recognition in aqueous media. Further work on a chemosensor for inositol triphosphate. *J. Org. Chem.* **68**, 10156–10157.
63. T.-H. Kim and T. M. Swager (2003). A fluorescent self-amplifying wavelenght-responsive sensory polymer for fluoride ion. *Angew. Chem. Int. Ed.* **42**, 4803–4806.
64. F. Tanaka, N. Mase, and C. F. Barbas, III (2004). Determination of cysteine concentration by fluorescence increase: Reaction of cysteine with a fluorogenic aldehyde. *Chem. Commun.* 1762–1763.
65. A. Coskun and E. U. Akkaya (2004). Difluorobora-s-diazaindacene dyes as highly selective dosimetric reagents for fluoride anions. *Tetrahedron Lett.* **45**, 4947–4949.
66. R. Badugu, J. R. Lakowicz, and C. D. Geddes (2005). Cyanide-sensitive fluorescent probes. *Dyes Pigments* **64**, 49–55.
67. N. DiCesare and J. R. Lakowicz (2002). New sensitive and selective fluorescent probes for fluoride using boronic acids. *Anal. Biochem.* **301**, 111–116.
68. R. Bagudu, J. R. Lakowicz, and C. D. Geddes (2004). Fluorescence intensity and lifetime-based cyanide sensitive probes for physiological safeguard. *Anal. Chim. Acta* **522**, 9–17.
69. R. Badugu, J. R. Lakowicz, and C. D. Geddes (2004). Excitation and emission wavelenght ratiometric cyanide-sensitive probes for physiological sensing. *Anal. Biochem.* **327**, 82–90.
70. J. V. Ros-Lis B. García, D. Jiménez, R. Martínez-Máñez, F. Sancenón, J. Soto, F. Gonzalvo, and M. C. Valldecabres (2004). Squaraines as fluoro-chromogenic probes for thiol-containing compounds and their application to the detection of biorelevant thiols. *J. Am. Chem. Soc.* **126**, 4064–4065.
71. J. A. Cruz-Aguado, Y. Chen, Z. Zhang, N. H. Elowe, M. A. Brook, and J. D. Brennan (2004). Ultrasensitive ATP detection using firefly luciferase entrapped in sugar-modified sol-gel-derived silica. *J. Am. Chem. Soc.*, **126**, 6878–6879.