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FEATURE ARTICLE

Fluorescent saccharide receptors: a sweet solution to the design, assembly and evaluation of boronic acid derived PET sensors

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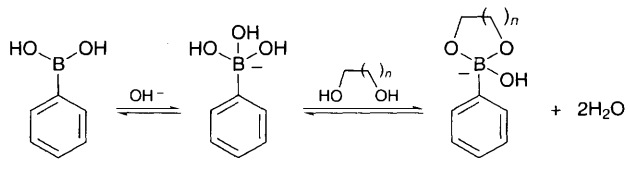
This review article briefly introduces the applications of photoinduced electron transfer receptors (PET) and then progresses from the design, assembly and evaluation of a simple monofunctional monoboronic acid through to a variety of bifunctional diboronic acid saccharide receptors.

Ions and molecules are abundant in nature and the need to measure the concentration of selected ions and small organic molecules both *in vivo*[1–3] and *in vitro*[4–6] processes can be critical. For example, the monitoring of calcium ions,[7,8] in the body to determine muscle fatigue, monitoring levels of carbon monoxide[9] in cities to ensure the air we breathe is safe and monitoring the dissappearance of glucose[10,11] in a typical fermentation process to ensure that the beer we drink is quite satisfactory. Historically batch processes carried out by conventional techniques were used to monitor the levels of ions and small molecules.[12] However, there is a significant time lag between the sampling and reporting of results in batch processes. For example, if patients undergoing open heart surgery incur a time lag in the measuring of their K^+ levels, this could be fatal if there is a sudden surge in K^+ levels indicating that the patients may be going into shock.[3] This time-delay could be avoided by continuous monitoring with a sensor.

Sensors come in two forms biosensors and chemosensors, both yield a measurable response in the presence of matter or energy. Biosensors often have limited stabilities that make their transition from the bench to practical applications difficult and are sometimes not amenable to large scale production making them expensive. However, the effort to synthesise molecules to bind desired analytes (chemosensors) is often substantial and time consuming. Chemosensors can possess various signal transduction systems such as CD,[13,14] UV, visible, NMR, electrochemical and fluorescent systems.[4–6] One of the most useful response systems for optical readout is fluorescence. Fluorescence spectroscopy can be enormously more sensitive than absorbance spectroscopy with the detection of single molecules possible. While synthetic schemes may be long and products may be obtained in low yields, the synthesis of only a few milligrams of fluorescent chemosensor will suffice for the measurement of a thousand analytes. Foremost, fluorescent chemosensors can be immobilised on optical fibres for continous readout[15] and this effectly removes the time-lag between sampling and readout. There exists a plethora of mechanisms by which fluorescent signal transduction may be produced. One of the most frequently used systems to vary fluorescence intensity is the photoinduced electron transfer (PET) mechanism.[5–6,13,14,16,17] Typical PET sensors are composed of three major components; a fluorophore, a spacer and a receptor. Nearly 20 years ago, Sousa described the synthesis of a naphthalene (fluorophore) crown ether (receptor) probe **1** in which the fluorophore π system is insulated from the donor atoms by at least one methylene group (spacer).[18] These compounds demonstrated fluorescence changes upon the bind ing of alkali metal salts. Subsequent reports by various groups have built on this original concept, in which the binding of metals and ammonium cations to crowns or aza-crowns has been coupled to changes in covalently linked fluorophores.[19–28] A representive example is deSilva's aza-crown ether anthracene cation sensor **2**.[25]

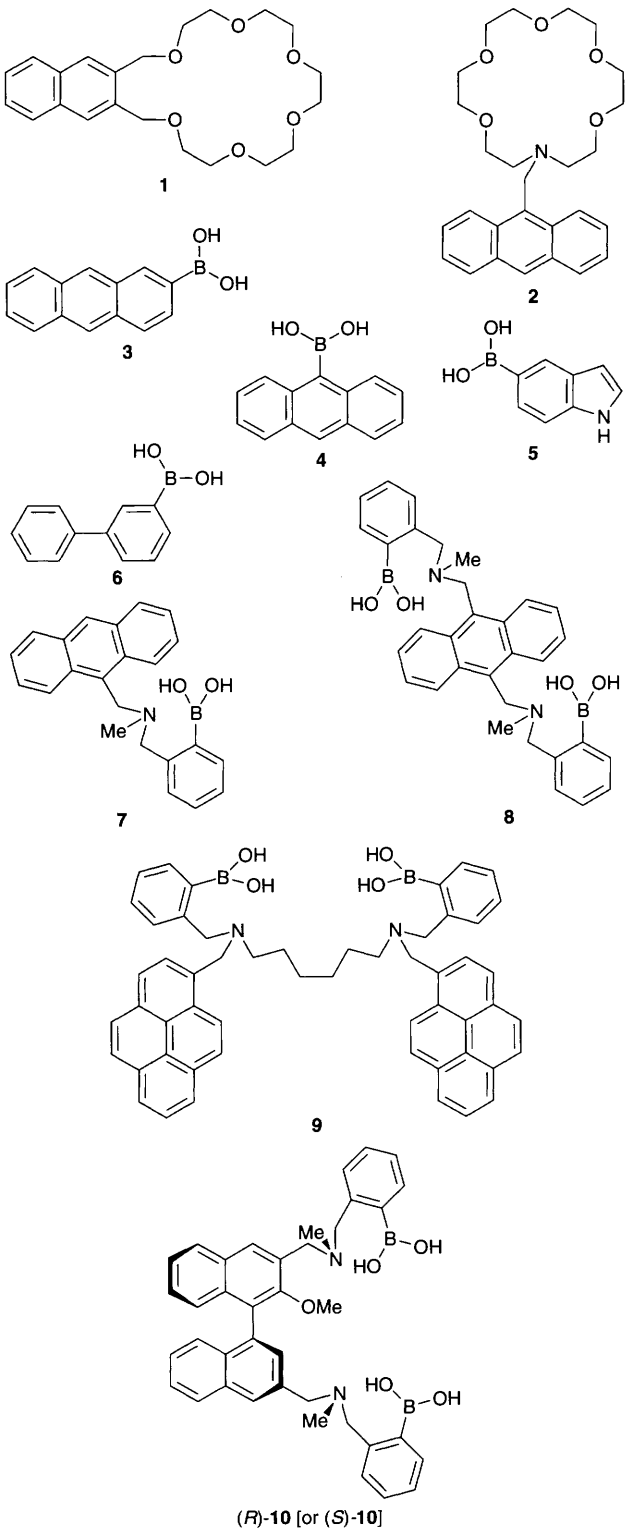
Recently the recognition of biologically important molecular species by synthetic molecular receptors has gained momentum. As the chemistry of saccharides plays a significant role in the metabolic pathways of living organisms, detecting the presence and concentration of biologically important sugars (glucose, fructose, galactose *etc.*) in aqueous solution, is necessary in a variety of medicinal[29–34] and industrial contexts. Applications range from the monitoring of fermenting processes to establishing the enantiomeric purity of synthetic drugs. Current enzymatic detection methods of sugars offer specificity for only a few saccharides; additionally, enzyme based sensors are unstable in harsh conditions. Many synthetic receptors are based on hydrogen bonding interactions.[35–50] The efficiency of such interactions has been well demonstrated in non-aqueous systems, but in aqueous media competitive hydrogen bonding by the solvent is a serious drawback. Few chemical sensing mechanisms have been described for saccharides as they are uncharged and neither fluorescent nor fluorescent quenchers. To date, only small fluorescent changes have been achieved when saccharides form complexes with boronic acids. With the aid of better fluorophores, receptors and novel transduction mechanisms it will be possible to develop selective and sensitive PET sensors to detect saccharides.

Boronic acid–saccharide covalent interactions readily form in aqueous media and represent an important alternative binding force in the recognition of saccharides and related molecular species. Stable boronic acid based saccharide receptors offer the possibility of creating saccharide sensors which are selective and sensitive for a specific saccharide. The fluorescence of sensors **3–6** were quenched by saccharide binding to the boronic acid moiety.[51–53] The pK_a of the boronic acid was shifted by the saccharide present in the medium. The PET from the boronate anion is believed to be the source of the fluorescence quenching. However, PET was only efficient for **6** despite the fact that the boronate anion is directly bound to the chromophore due to a subtle balance in the HOMO level between the aryl and boronic acid moiety.[54] Facile boronic acid saccharide complexation occurs only at high pH conditions where the assistance of OH^- is required to create a boronate anion of **3–6** (Scheme 1). This limits the usefulness of **3–6** as saccharide sensors and the lack of sufficient electronic changes upon complexation in either the boronic acid or the saccharide moiety makes PET inefficient. To



Scheme 1

overcome these disadvantages we decided to utilise the previously proposed boron–nitrogen interaction.[55,56] The neighbouring group participation of the amine group can lower the working pH of the sensor molecule and provides an electron rich centre for PET. Much progress in the construction and design of sensors based on boronic acids has been made but it is still in its infancy. From the knowledge gained from other groups and the fundamental principles available on the design of PET sensors we optimistically set out to design a saccharide receptor with a sensitive taste.



Design of a mono-boronic acid saccharide receptor

Mono-saccharides of biological importance possess a variety of stereocentres around each carbon atom creating individual stereoisomers with unique physical and chemical properties. Covalent interactions between boronic acids and the proximal OH groups of saccharides readily occur in water. The small differences in conformation around each stereocentre may lead to selective recognition of an individual saccharide. We assumed on the basis of previous results[4,17] that the criteria required for a mono-boronic acid saccharide receptor PET sensor should consist of the following points; (i) a short and inexpensive synthesis; (ii) adequate hydrophilicity; (iii) efficient transduction mechanism at physiological pH; (iv) adequate fluorescence enhancement on binding and finally (v) selective recognition. Our modular design strategy is depicted in Fig. 1. Scheme 2 shows the synthetic pathway of **7**, which was short and high yielding.

Fluorescence titration of **7** in aqueous media shows a very high pK_a shift together with a very high fluorescence 'switch-on' factor on saccharide binding. The increased acidity of the boronic acid moiety strengthens the boron–nitrogen bond and effectively suppresses the PET process. Fig. 2 shows the pH titration of **7** in aqueous media with and without glucose. The very large pK_a shift found upon glucose binding provides a wide pH range for saccharide sensing. Having proven the validity of our design strategy, the selectivity and sensitivity of our saccharide PET sensors was determined. Aqueous solution at physiological pH is the ideal testing ground for any saccharide

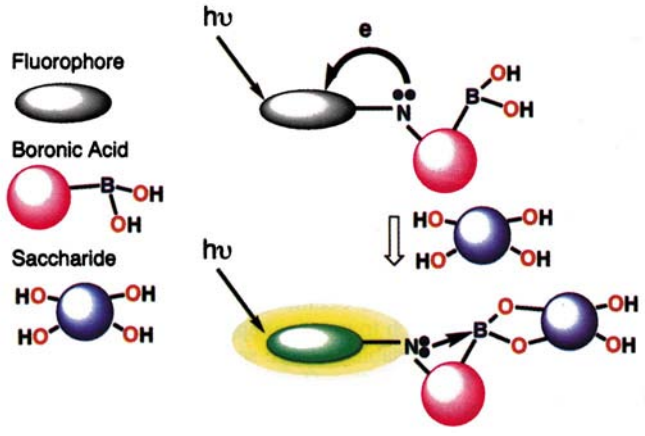
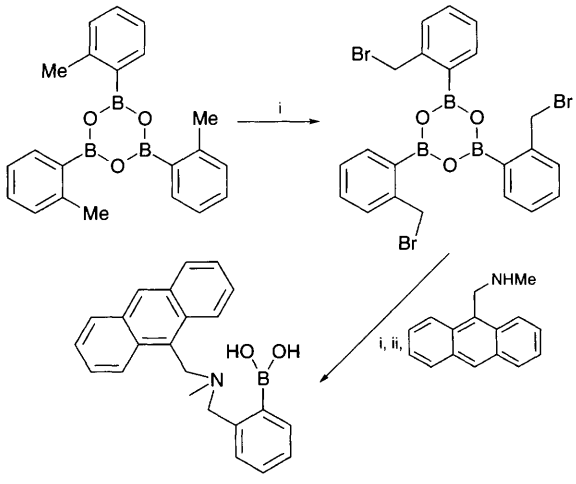


Fig. 1 Modular design strategy



Scheme 2 Synthesis of boronic acid derivative **7**. *Reagents and conditions*: i, *N*-bromosuccininide, AIBN, CCl_4, heat (60%); ii, 2.1 equiv. of amine, heat, $CHCl_3$ (33%); iii, OH^-/H_2O (quant).

sensor. Therefore, selectivity studies were carried out in 33% methanol–water buffered at pH 7.77. A water only buffer was suitable at low saccharide concentrations, but at higher concentrations precipitation becomes a problem. A mixed solvent was therefore chosen to avoid any complications arising from precipitation. The data plots from which the stability constants were calculated are shown in Fig. 3. The selectivity of **7** is in line with that observed for other phenylboronic acids.[6]

Design of di-boronic acid saccharide selective receptors

(*a*) *A glucose sensor*

Many monosaccharides possess at least two binding sites, which differ from other monosaccharides. Thus, by controlling the spatial disposition of two boronic acids, it should be possible to construct saccharide selective receptors. Our molecular design strategy is depicted in Fig. 4. Glucose selectivity has been achieved in the cleft like strategy[57] of **8**[59] (Table 1). Also, the 'switch-on' factor (ratio of maximum to minimum fluorescence intensity) for **8** (7) is greater than that for **7** (3). The formation of the large macrocyclic structure upon 1 : 1 binding of glucose to **8** holds glucose close to the anthracene aromatic face (Fig. 5). The C-3 proton of D-glucose, in particular, points towards the π-electrons of the anthracene moiety giving a very large paramagnetic shift in the ^{1}H NMR spectrum ($\delta_{H\text{-}3}$ = −0.3). The coupling constant $J_{2,3}$ = 7.5 Hz implies that the pyranose form of glucose is complexed in the cleft of **8**.[58] The existence of a 1 : 1 complex of **8** and D-glucose was further confirmed by mass spectral data of the complex.

Such cooperative binding of saccharides, specifically glucose, occurs at very low saccharide concentrations. Owing to the PET design, non-cyclic 1 : 1 bound species could not be detected by fluorescence spectroscopy; only the 1 : 1 cyclic and 1 : 2 complexes give fluorescent signals. The most important species involved in the equilibrium process are shown in Scheme 3. In human blood three main monosaccharides are present: D-glucose (0.3–1.0 mmol dm^{-3}), D-fructose (≤0.1 mmol dm^{-3}) and D-galactose (≤0.1 mmol dm^{-3}). Competitive binding studies show that **8** is suitable for the detection of glucose at physiological levels.

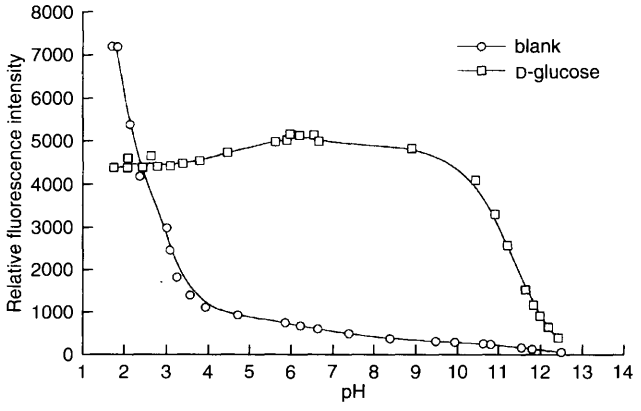


Fig. 2 Fluorescence intensity *vs.* pH profile of **7** at 25 °C; 1.2 × 10^{-5} mol dm^{-3} of **7** in 0.05 mol dm^{-3} sodium chloride solution, [glucose] = 0.05 mol dm^{-3}

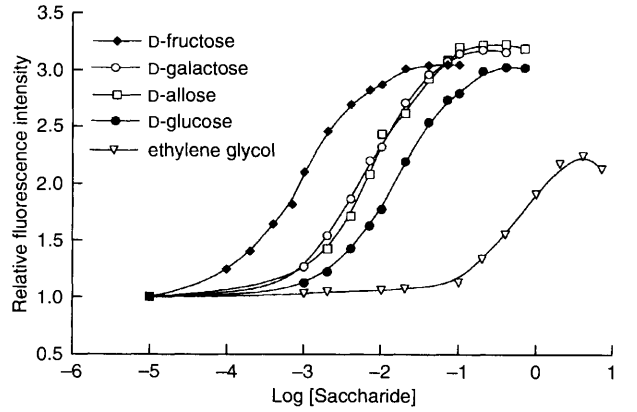


Fig. 3 Fluorescence intensity *vs.* log [saccharide or ethylene glycol] profile of **7** at 25 °C; 1.0 × 10^{-5} mol dm^{-3} of **7** in 33.3% (*m*/*m*) MeOH/H_2O buffer at pH 7.77, λ_{ex} 370 nm, λ_{em} 423 nm

(*b*) *A bimodal sensor*

Diboronic acid derivative **9**, which has a flexible spacer between the two boronic acids, behaves similarly to **8**.[59] Pyrene fluorophores which are capable of forming excimers give 'bimodal' information on both the saccharide concentration and the structure of the complex. The 1 : 1 binding of a saccharide to **9** leads to an increase in the monomer fluorescence intensity.

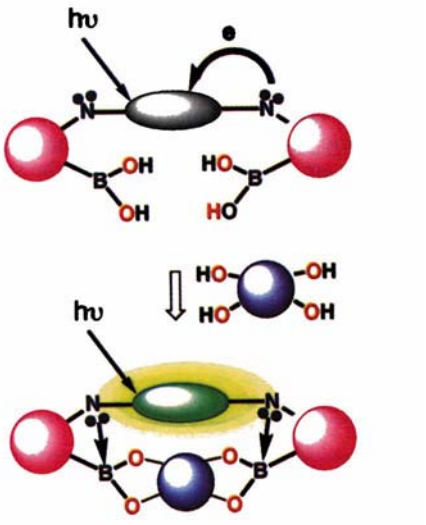


Fig. 4 Modular design strategy

Table 1 Stability constants (log K_a) for monosaccharide and ethylene glycol complex with boronic acid **7**, **8** and **9**. pH 7.77 (0.05 mol dm^{-3} phosphate buffer)

Saccharide	Boronic acid **7** log K^a	Boronic acid **8** log K^a	Boronic acid **9** log K^b
D-Glucose	1.8	3.6	3.3
D-Fructose	3.0	2.5	—[c]
D-Allose	2.5	2.8	2.9
D-Galactose	2.2	2.2	2.9
(Ethylene glycol)	0.4	0.2	−0.5

[a] Measurements were done in 33% methanolic aqueous solutions. [b] Measurements were done in 67% methanolic aqueous solutions. [c] The plot of relative fluorescence intensity *vs.* saccharide concentration could not be analysed precisely by a simple Benesi–Hildebrand type equation assuming the formation of a 1 : 1 complex.

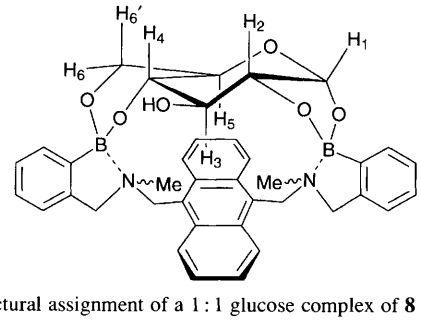
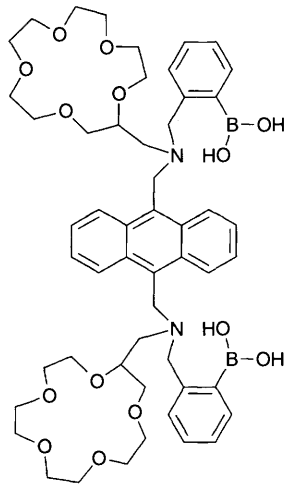


Fig. 5 Structural assignment of a 1 : 1 glucose complex of **8** by ^{1}H NMR

Monomer fluorescence increase was partially produced by the decrease of excimer formation and partially by the increased overall fluorescence quantum yield *via* the suppression of the PET process. The 1 : 2 binding of **9** to saccharides, on the other hand, increased the excimer : monomer fluorescence intensity ratio. In all cases the formation of a 1 : 1 complex was observed at low saccharide concentrations while the predominant complex changed to 1 : 2 as the saccharide concentration increased. The selectivity of **9** was found to be similar to that of **8** as shown in Table 1. However, the higher concentration of methanol used in the measurements leads to somewhat lower stability constants with glucose than those found for **8**.

(c) A chiral discriminating sensor

Work by Irie *et al.* on the control of intermolecular chiral 1,1′-binapthyl fluorescence quenching by chiral amines[60] and the use of 1,1′-binaphthyl in the recognition of chiral amines by Cram *et al.*[61] inspired the design of **10**.[62] Chiral recognition of saccharides by **10** utilizes both steric and electronic factors. The asymmetric immobilization of the amine groups relative to the binaphthyl moiety upon 1 : 1 complexation of saccharides by D- or L-isomers creates a difference in PET. This difference is manifested in the maximum fluorescence intensity of the complex. Steric factors arising from the chiral binaphthyl building block are chiefly represented by the stability constant of the complex. However, the interdependency of electronic and steric factors upon each other is not excluded. Fig. 6 shows titrations of *R*- and *S*-isomers of **10** with D- and L-saccharides. This new molecular cleft, with a longer spacer unit compared to the anthracene based diboronic acid **8**, gave the best recognition for fructose. D-Fructose was best bound by (*R*)-**10** with a large fluorescence increase. Table 2 shows the binding constants for some D- and L-monosaccharides. In this system steric factors and electronic factors bimodally discriminate the chirality of the saccharide. Competitive studies with D- and L-monosaccharides show the possibility of selective detection of saccharide isomers. The availability of both *R*- and *S*-isomers of this particular molecular sensor is an important advantage, since concomitant detection by two probes is possible.



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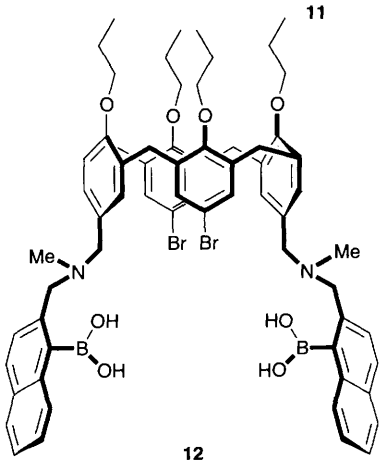

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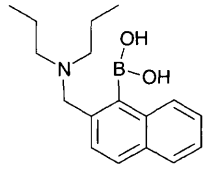

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(d) An allosteric saccharide sensor

Nature relies on allosteric interactions to modulate modes of action and message transduction.[63] Simple synthetic models should allow for a greater understanding of the more complex allosteric interactions occurring in nature. In the design of an allosteric system binding at the first or main site should either activate (positive allostericity) or deactivate (negative allostericity) binding at the second site. To facilitate activation or deactivation, binding at the first site should induce a major conformational change in the molecules. Our design strategy is depicted in Fig. 7.

On examination of the CPK models for compound **11** we found that when the two 15-crown-5 rings form a metal ion sandwich, the distance between the two boronic acid moieties is lengthened making the formation of the 1 : 1 fluorescent saccharide complex[62,64] very unlikely. This is an example of a negative allosteric device. The stability of the 1 : 1 intramolecular complex between **11** and D-glucose was determined from the titration curve of D-glucose with **11** to give a stability constant log K_S of 1.73 for D-glucose. Fig. 8 shows the normalized metal ion titration curves for compound **11** in the presence of 0.03 mol dm^{-3} D-glucose. Table 3 contains the stability constants (log *K*) for the metal complexes in the presence of 0.03 mol dm^{-3} D-glucose and the ionic diameter of the metal ions involved. From Table 3 metal ions with a diameter similar to potassium have the greatest affect on the 1 : 1 glucose complex. These metal ions are believed to have the largest contribution of a sandwich structure to metal ion binding.[65] Scheme 4 is an indicator of the main species and reasonably explains the observed metal binding events.

Further confirmation that the 1 : 1 complex is the important fluorescent species involved in these measurements was given by circular dichroism (CD) spectroscopy. The CD spectra of compound **11** with 0.06 mol dm^{-3} D-glucose and 0.1 mol dm^{-3} of sodium, potassium, strontium and barium are given in Fig. 9. The decrease in CD intensity at 258 nm for added metal ion is proportional to the change in fluorescence intensity at 0.1 mol dm^{-3} metal ion. The following decrease in CD intensity: sodium (35%), potassium (69%), strontium (65%) and barium (96%); corresponds with a decrease in fluorescence intensity: sodium (29%), potassium (65%), strontium (60%) and barium (100%). Clearly, decomposition of the 1 : 1 complex is the cause of the decrease in the fluorescence intensity. This is a novel allosteric system which mimics the action of the Na^+/D-glucose co-transport protein in nature. D-Glucose binds in the 'cleft' of **11** as a 1 : 1 complex in the presence of 0.03 mol dm^{-3} sodium and released from the 'cleft' at the same concentration of potassium.

(e) A bowl shaped sensor

Our aim was to develop a fluorescent diboronic acid built on a 3-dimensional platform capable of binding sugars at neutral pH in aqueous media. This was achieved by attaching two 2-aminomethylnaphthalene boronic acids to the upper rim of a tetra-alkylated calix[4]arene. The aminomethylnaphthalene boronic acid moieties act as a PET sensor on binding to saccharides. To our knowledge this is the first fluorescent saccharide sensing calixarene 'sugar bowl.' The elegance of using calixarenes[66,67] as building blocks for saccharide sensors stems from the multitude of latent functionality that can be attached to the calixarene platform. Compound **12** possesses four propyl groups necessary to keep the calixarene in the cone

conformation and two Ar–Br units used to block the 1,3-positions. These groups can all be elaborated into more complex functionalities.

The stability constants for **12** at neutral pH (methanol/water 33% *m/m*) are log K_s = 1.38 for D-glucose and log K_s = 2.06 for D-fructose. With **12** four main species exist under the experimental conditions: among them the fluorescent species are a non-cyclic 2 : 1 complex and a cyclic 1 : 1 complex (*cf.* Scheme 3). Confirmation of the stoichiometry was first obtained by mass spectroscopy. The mass (SIMS positive) spectra of a 1 : 1 mixture of **12** with D-glucose contained the M^+ ion of the cyclic 1 : 1 complex and D-fructose contained the M^+ ion of the non-cyclic 2 : 1 complex. The results establish that the fluorescent active species are the non-cyclic 2 : 1 complex for D-fructose and the cyclic 1 : 1 complex for D-glucose. Further evidence for the stoichiometry of binding of D-glucose to **12** was obtained by compairing the stability constants for complexes of both D-glucose and D-glucose monophosphate with both **12** and the monoboronic reference compounds **13**. One would expect the stability constants to decrease in both cases, because monoboronic acid **13** can only form a 1 : 1 complex or a 2 : 1 intermolecular complex, neither of which should be as strong as the suspected 1 : 1 intramolecular complex with **12**. What we actually see is a lower stability constant (log K_s = 0.06) of D-glucose with **13**, indicating that the stronger binding of D-glucose to **12** is due to the intramolecular 1 : 1 complex. The stability constant of D-glucose-1-monophosphate with **12** should be smaller than that of D-glucose with **12** because it can only form a 2 : 1 complex since one of its primary binding sites is blocked. Experimentally we could not detect binding of D-glucose-1-monophosphate to **12** therefore a 2 : 1 complex can be ruled out for D-glucose. The stoichiometry of binding of D-fructose to **12** was verified by determining the stability constant of D-fructose to mono-boronic acid **13**. We expected to see no change in the stability constant of D-fructose with **13** relative to **12** if a 2 : 1 complex were to exist. Experimentally we found this to be true: both stability constants are similar (log K_s = 1.56 with **13** and log K_s = 2.06 with **12**) indicating the intermolecular complex is the major complex formed while the intramolecular complex may be formed but only to a minor extent.

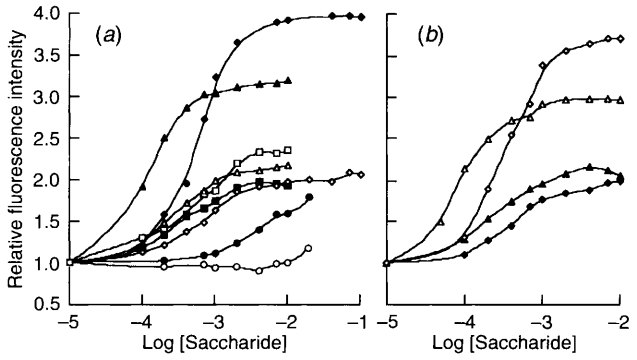
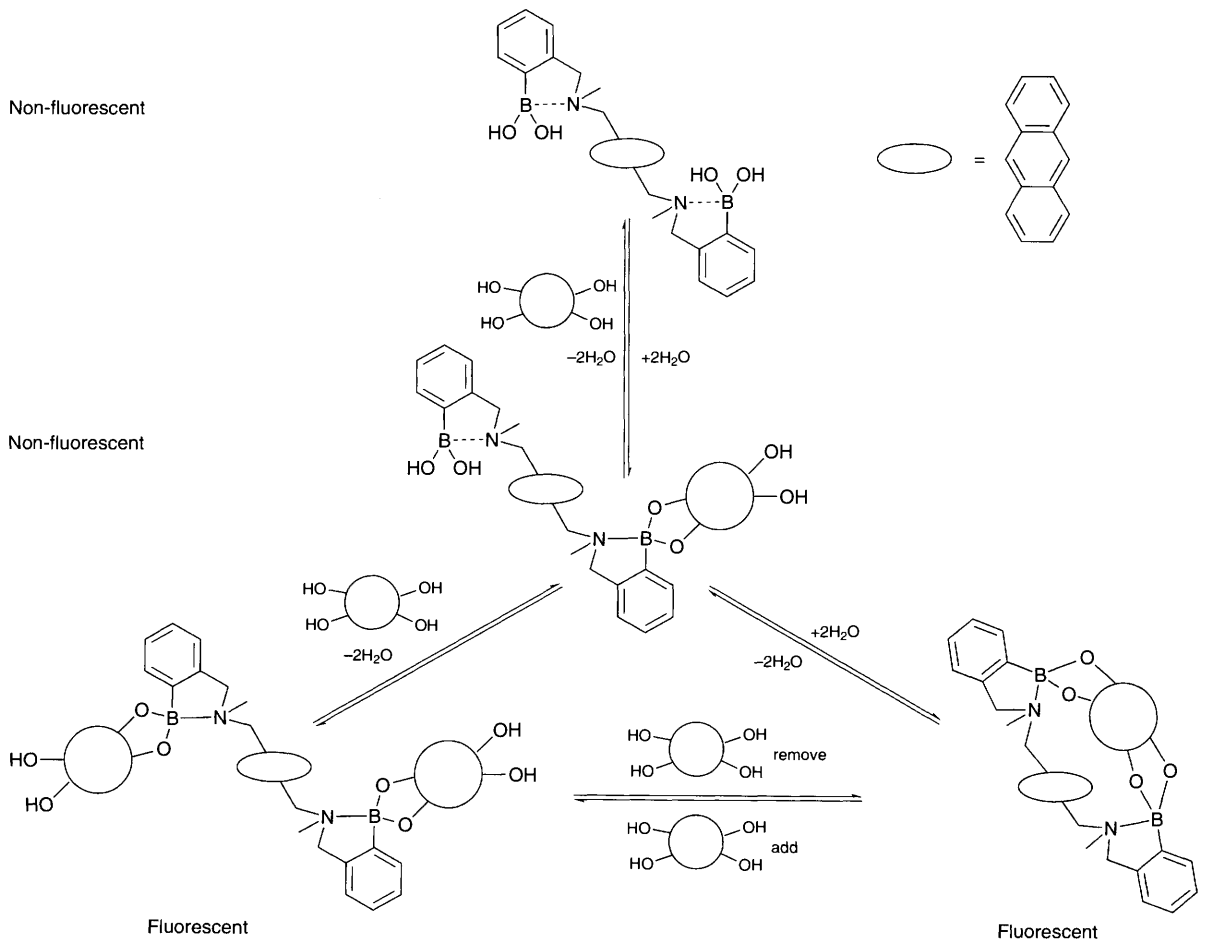


Fig. 6 (*a*) Fluorescence intensity *vs.* log [saccharide] profile of (*R*)-**10** at 25 °C; 1.0 × 10^{-5} mol dm^{-3} of (*R*)-**10** in 33.3% (*m/m*) MeOH/H_2O buffer at pH 7.77, λ_{ex} 289 nm, λ_{em} 358 nm. (*b*) Fluorescence intensity *vs.* log [saccharide] profile of (*S*)-**10** at 25 °C; 1.0 × 10^{-5} mol dm^{-3} of (*S*)-**10** in 33.3% (*m/m*) MeOH/H_2O buffer at pH 7.77, λ_{ex} 289 nm, λ_{em} 358 nm. ◆ D-glucose; ◇ L-glucose; ■ D-galactose; □ L-galactose; ▲ D-fructose; △ L-fructose; ● D-mannose; ○ L-mannose.



Scheme 3

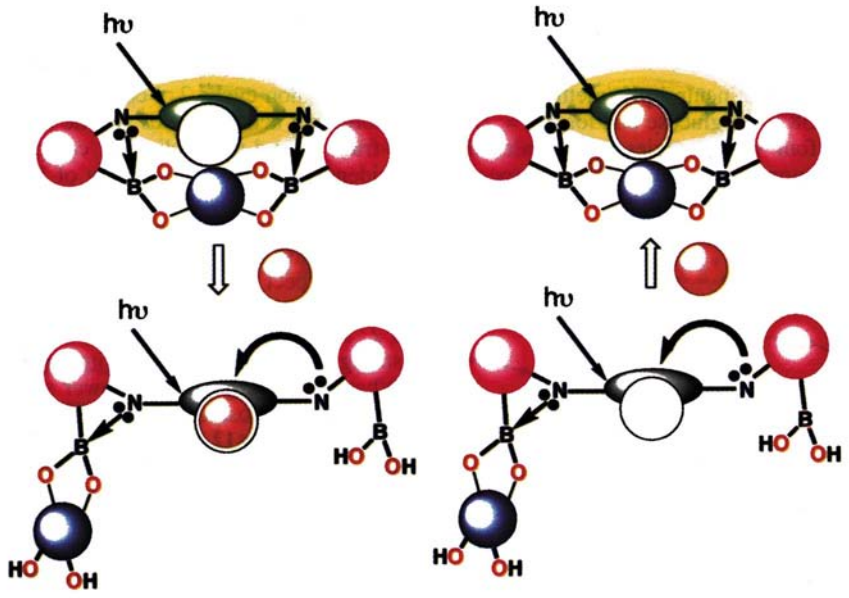


Fig. 7 Modular design strategy

Table 2 Stability constants and fluorescence enhancements for saccharides with *R*-**9** (or *S*-**9**)

Saccharide	D log*K* (±0.05)	L log*K* (±0.05)	D/L Fluorescence intensity ratio
Fructose	4.0 (3.7)	3.5 (4.0)	1.47 (0.69)
Glucose	3.3 (3.4)	3.1 (3.5)	1.93 (0.53)
Galactose	3.1	3.3	0.82
Mannose	< 2.4	—	—

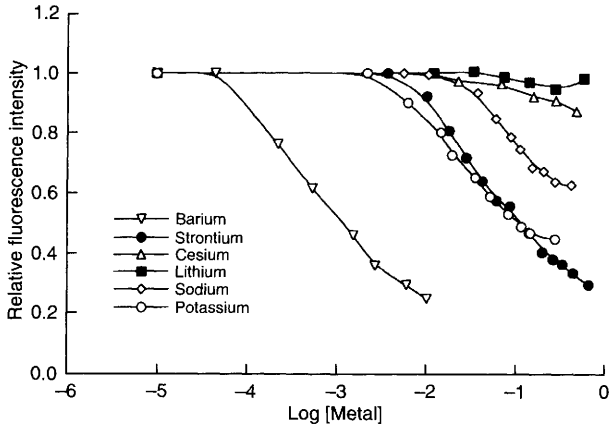


Fig. 8 Fluorescence intensity *vs.* log [metal ion] profile of **11** at 25 °C; 1.0 × 10^{-5} mol dm^{-3} of **10** in 33.3% MeOH/H_2O and 0.03 mol dm^{-3} of D-glucose λ_{ex} 370 nm, λ_{em} 423 nm

Considering the plethora of shapes and sizes of functionalised calixarenes available, it will not be long before precise saccharide sensors are developed employing calixarenes as building blocks.

Conclusion

The recognition of saccharides by boronic acid based molecular receptors has shown tremendous growth during the last few years: from inherent saccharide selectivity with monoboronic acids and controlled selectivity with simple diboronic acids through to the chiral recognition of saccharides. A bifunctional saccharide receptor was designed which could control saccharide binding allosterically and finally a chiral bowl shaped saccharide detector was assembled from a calixarene. We believe that such sensors will find many applications in biological systems for both the monitoring and mapping of biologically important saccharides. This relatively new field will attract many scientists' attention in the years to come.

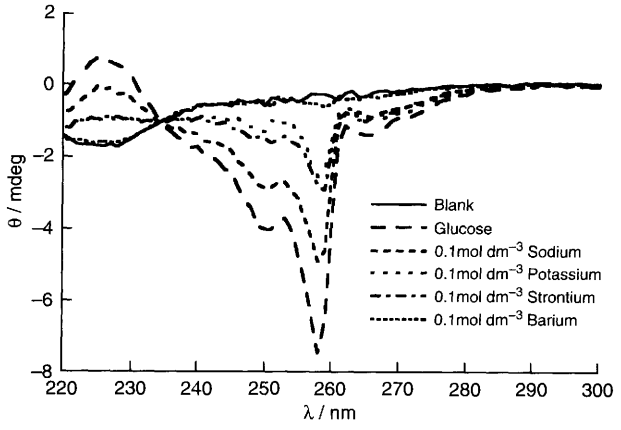


Fig. 9 Circular dichroism (CD) spectra of **11** at 25 °C; 1.4 × 10^{-3} mol dm^{-3} of **11** in 33.3% MeOH/H_2O

Acknowledgements

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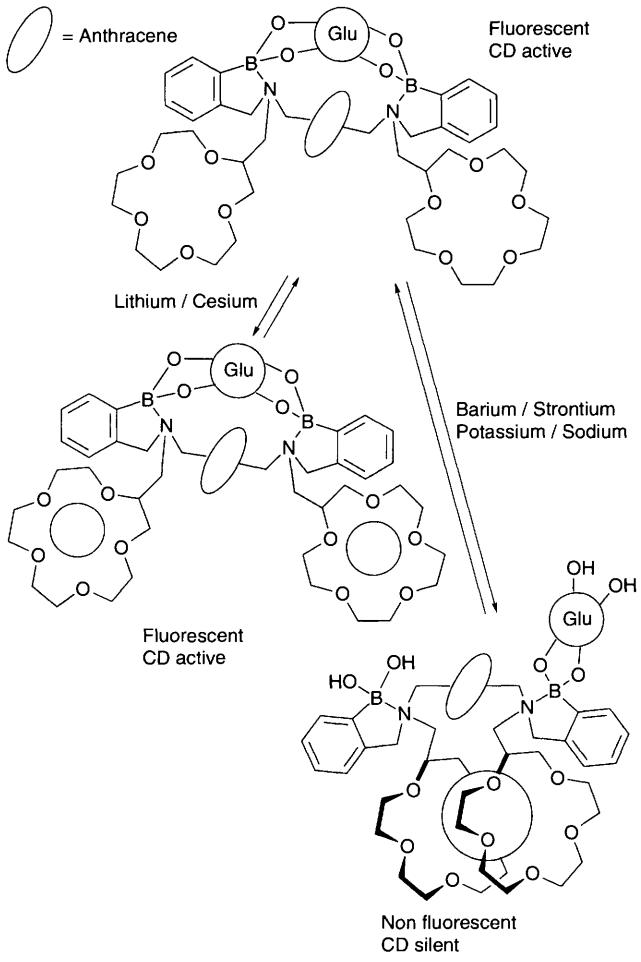
Patrick Linnane was born in Limerick, Ireland in 1966. He received his BSc and PhD from the University of

Table 3 Metal ion stability constants in the presence of 0.03 mol dm^{-3} D-glucose and ionic diameter of metal cations

Metal cation	Stability constant log K (±0.05)	Ionic diameter/Å	Metal cation	Stability constant log K (±0.05)	Ionic diameter/Å
Li^+	—	1.52			
Na^+	1.28	2.04	Sr^{2+}	1.54	2.36
K^+	1.80	2.76	Ba^{2+}	3.39	2.70
Cs^+	—	3.40			



Scheme 4 Possible complexes of **11** with glucose and metal ions

Manchester Institute of Science and Technology under the supervision of D. A. Leigh on the investigation and applications of the Mannich Reaction in 1993. After postdoctoral work at the Chemirecognics Project in Kyushu, Japan from 1993–1995 with S. Shinkai he joined P. Magnus at the University of Texas at Austin in October 1995 as a postdoctoral fellow. His interests include molecular recognition, synthetic methodology and adventure sports.

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