ACCOUNTS
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Exploiting the Reversible Covalent Bonding of Boronic Acids: Recognition, Sensing, and Assembly

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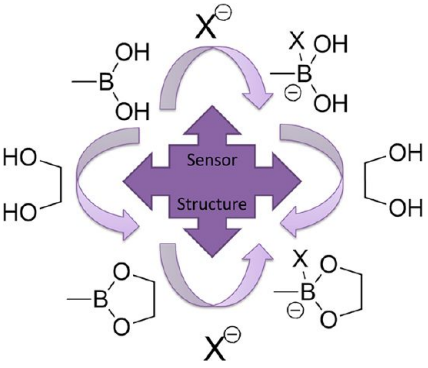
CONSPECTUS

Boronic acids can interact with Lewis bases to generate boronate anions, and they can also bind with diol units to form cyclic boronate esters. Boronic acid based receptor designs originated when Lorand and Edwards used the pH drop observed upon the addition of saccharides to boronic acids to determine their association constants. The inherent acidity of the boronic acid is enhanced when 1,2-, 1,3-, or 1,4-diols react with boronic acids to form cyclic boronic esters (5, 6, or 7 membered rings) in aqueous media, and these interactions form the cornerstone of diol-based receptors used in the construction of sensors and separation systems.



In addition, the recognition of saccharides through boronic acid complex (or boronic ester) formation often relies on an interaction between a Lewis acidic boronic acid and a Lewis base (proximal tertiary amine or anion). These properties of boronic acids have led to them being exploited in sensing and separation systems for anions (Lewis bases) and saccharides (diols).

The fast and stable bond formation between boronic acids and diols to form boronate esters can serve as the basis for forming reversible molecular assemblies. In spite of the stability of the boronate esters' covalent B−O bonds, their formation is reversible under certain conditions or under the action of certain external stimuli.

The reversibility of boronate ester formation and Lewis acid−base interactions has also resulted in the development and use of boronic acids within multicomponent systems. The dynamic covalent functionality of boronic acids with structure-directing potential has led researchers to develop a variety of self-organizing systems including macrocycles, cages, capsules, and polymers.

This Account gives an overview of research published about boronic acids over the last 5 years. We hope that this Account will inspire others to continue the work on boronic acids and reversible covalent chemistry.

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1. Introduction

The origins of boronic acid based receptor design can be traced back to the seminal work of Lorand and Edwards.[1] They used the pH drop observed on addition of saccharides to determine their association constants (Scheme 1). The acidity of the boronic acid is enhanced when 1,2-, 1,3-, or 1,4-diols react with boronic acids to form cyclic boronic esters (5, 6, or 7 membered rings) in aqueous media.[2,3]

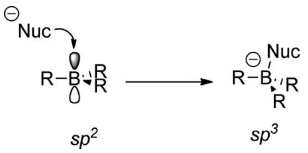
The recognition of saccharides through boronic acid complex (or boronic ester) formation often relies on an interaction between a Lewis acidic boronic acid and a proximal tertiary amine (Lewis base). The true nature of the nitrogen−boron (N−B) interaction has been much debated (especially in an aqueous environment), but it is clear that an interaction of some kind exists which offers two advantages.[4] First, this interaction enhances binding at neutral pH (by facilitating tetrahedral boronate formation), allowing the development of receptors with practical applicability. Second, saccharide binding enhances the N−B interaction (due an increase in Lewis acidity of the boron on saccharide binding) and modulates the fluorescence of nearby fluorophores (fluorescent photoinduced electron transfer (PET) from the nitrogen is controlled by the strength of the N−B interaction), which is extremely useful in the design and application of chemosensors.[2]

The Lewis acidic nature of boron has also lead to the development of anion receptors and sensors (Scheme 2).[2,5]

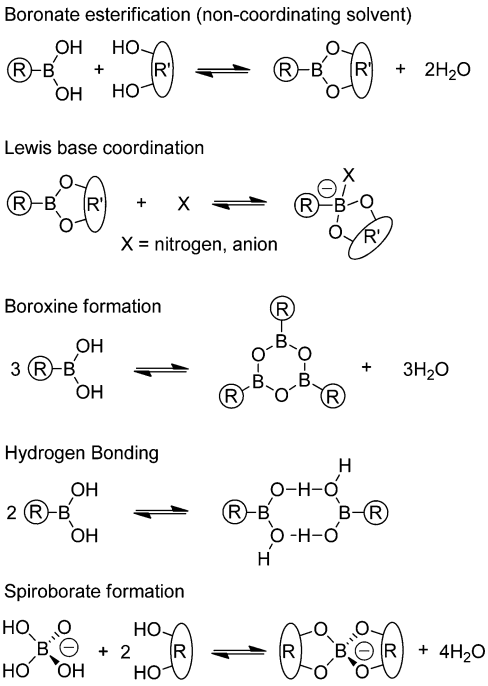
The fast and stable bond formation between boronic acids and diols to form boronate esters can also be utilized to build reversible molecular assemblies. In spite of the stability of the boronate esters' covalent B−O bonds, their formation is reversible under certain conditions or under the action of certain external stimuli (Scheme 3).[6,7]

SCHEME 1. Rapid and Reversible Formation of a Cyclic Boronate Ester

SCHEME 2. Diagram Illustrating the Change in Geometry at the Boron Centre on Interaction with a Nucleophile

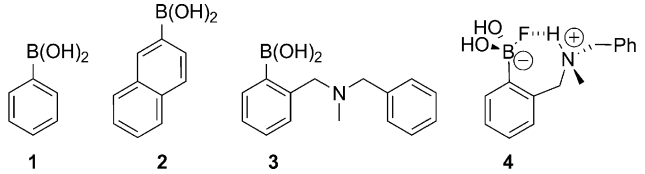


SCHEME 3. Some of the Key Interactions of Boron, With Respect to the Self-Assembly



2. Boronic Acids as Sensors

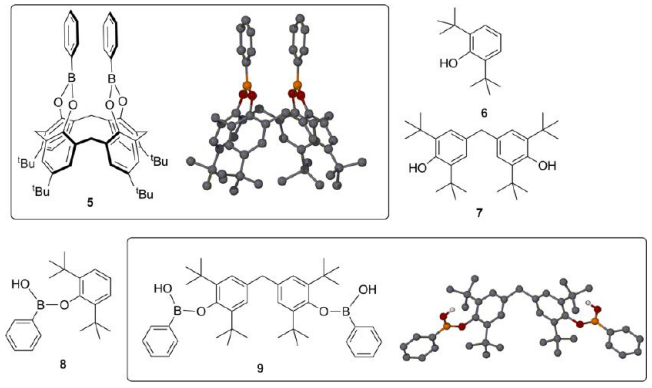
2.1. Anion Sensors. The first fluorescent sensor for fluoride was developed in 1998.[8] Fluorescence quenching of a series of simple aromatic boronic acids was observed in buffered aqueous methanol solution at pH 5.5 upon addition of KF. Tetrahedral boronate anions had already been shown to quench the fluorescence of directly attached fluorophores, and the same internal charge transfer (ICT) mechanism was shown to operate upon fluoride binding. The ^{11}B NMR spectroscopic observations of **1** and **2**, displayed shifts consistent with a change from an sp^2 to sp^3 boron center as the concentration of fluoride was increased.



The amine of **3** has a pK_a of 5.5, and so under the measurement conditions, the nitrogen is partially

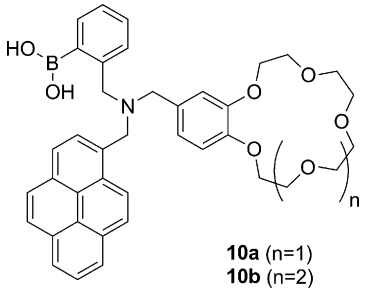
protonated, allowing a hydrogen bonding interaction with a bound fluoride (**4**). This family of chemosensors serves to demonstrate the importance of *tunability.* Since different environments require monitoring across varying concentration ranges, only simple modifications need to be made in order to enhance binding without changing the mode of action.

In order to enhance fluoride binding, the use of a rigid framework as a scaffold for boronic acid based anion sensors was investigated. It was found that the bis(bora)calix[4]arene **5** acts as a sensor for tetra-*n*-butylammonium fluoride (Bu_4NF) in chloroform. Subsequently, in order to probe the factors affecting fluoride binding, related boronates **8** and **9** were prepared.[9]



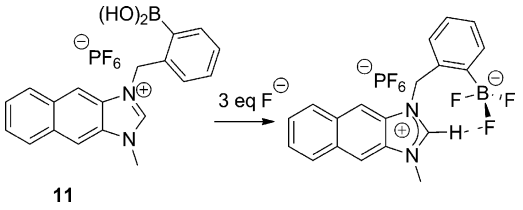
While fluoride caused deboronation of compound **9** and dramatic color changes, Bu_4NCl and Bu_4NBr produced no color change. However, Bu_4NCl caused fluorescence quenching of compound **9** but did not quench **8**, or alcohols **6** and **7**. Bu_4NBr did not cause a significant change in the fluorescence spectra of compounds **6**–**9**. The fluorescence quenching by chloride has been attributed to bidentate binding through two BOH hydrogen bonds, with the conformational change in the fluorophore causing the fluorescence quenching.

Ditopic receptors **10a** and **10b** function as AND logic gates; the boronic acid interacts strongly with a fluoride anion while a potassium cation is held partly by the crown ether and by an electrostatic interaction with bound fluoride anion. This cooperative complexation allows the cationic and anionic guests to be bound to the host as an ion pair, while allowing the host to discriminate between potassium fluoride and other similar ion pairs such as potassium chloride and potassium bromide.

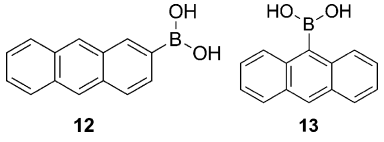


The bidentate fluoride receptor **11** was designed and synthesized (Scheme 4) that employs a boronic acid site and an imidazolium group.[10] The *ortho* derivative (shown) was crucial, since only this isomer gave the desired selectivity. The C–H hydrogen bond donor stabilizes the binding, allowing recognition to occur in competitive media (Scheme 4).

SCHEME 4. Sensor **11** Combines a Boronic Acid Receptor with CH–Anion Interaction

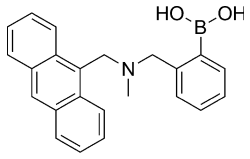


2.2. Saccharide Sensors. The ICT sensor **12** reported by Czarnik and Yoon in 1992 consisted of a boronic acid fragment directly attached to anthracene.[11] On addition of saccharide, it was noted that the intensity of the fluorescence emission for the 2-anthrylboronic acid **12** was reduced by ~30%. This change in fluorescence emission intensity is ascribed to the change in electronic properties that accompany rehybridization at boron. The 9-anthrylboronic isomer, **13**, was also examined but displayed smaller changes in fluorescence emission, a feature attributed to the unfavorable *peri*-interactions that would be expected at the 9-position from the ancillary hydrogens.



These ICT sensors have one drawback for potential *real world* sensor development, which is pH sensitivity. In the

case of PET sensors, the interaction between *o*-methylphenylboronic acids (Lewis acids) and proximal tertiary amines (Lewis bases) has been exploited.



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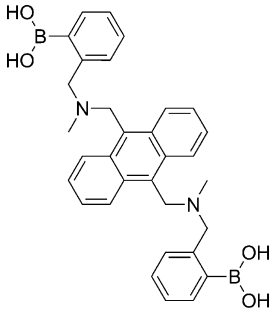
The first fluorescent PET sensor **14** for saccharides was developed in 1994, and it employs an *N*-methyl-*o*-(aminomethyl)phenylboronic acid receptor unit. The amino base–boronic acid (N–B) interaction found in sensor **14** allows the system to function as an "off–on" sensor, producing a large fluorescence enhancement on addition of saccharides, and functions over a broad pH range.

The simple fructose selective monoboronic acid based sensor **14** was improved in 1995 with the introduction of a second boronic acid group to form the diboronic acid sensor **15**.[12] This Receptor–Spacer–Fluorophore–Spacer–Receptor system retained the advantage of utilizing PET to modulate an "off–on" response to saccharides while introducing an advanced recognition site. The modification proved successful, and, fortuitously, the spacing of the two boronic acid groups provided an effective binding pocket for glucose.



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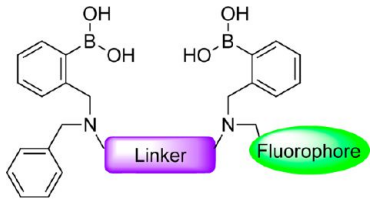
2.3. Modular Fluorescent Sensors. While retaining the same dual boronic acid recognition units throughout, modular systems, in which the linker and fluorophore units of these sensors could be modified independently, have been developed. The design **16** includes two boronic acid groups required for selectively but allows the separation between them to be varied by altering the linker. It also permits the fluorophore to be varied independently and by using only one fluorophore overcomes the problems that may arise from excimer emission, insolubility, excessive hydrophobicity, and steric crowding at the binding pocket.



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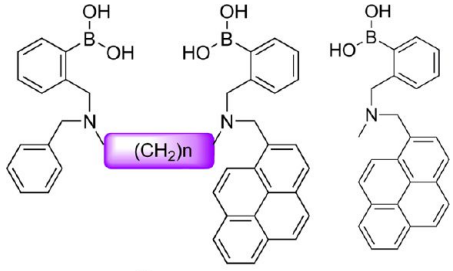
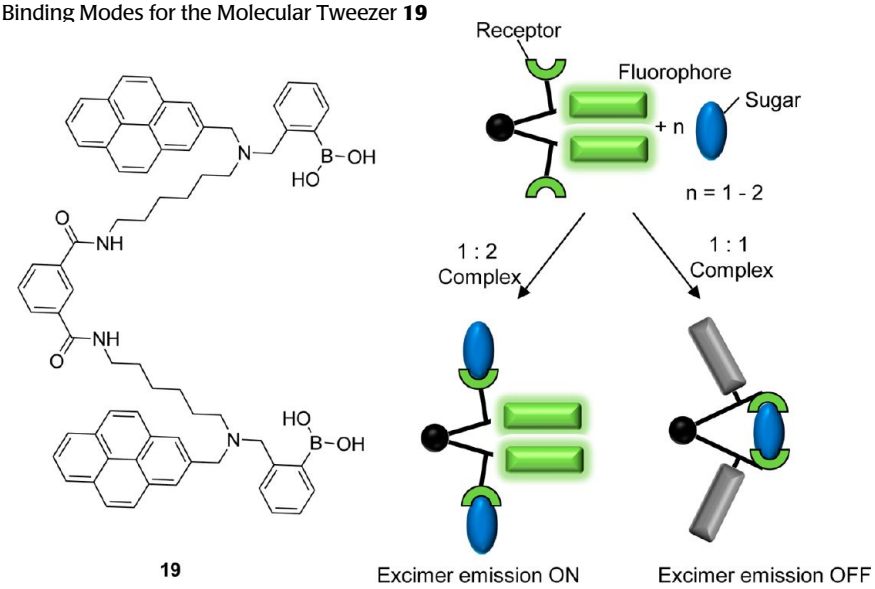
A modular PET sensor **17** was prepared, containing two phenylboronic acid groups, a pyrene fluorophore and a variable linker. The linker was varied from *n*-propylene ($n = 3$) to *n*-octylene ($n = 8$). In most cases, the observed stability constants (K_{obs}) with diboronic acid sensor **17** are higher than those for monoboronic acid sensor **18**. D-Glucose and D-galactose bind to diboronic acids readily using two sets of diols, thus forming stable, cyclic 1:1 complexes. The six carbon linker provided the optimal selectivity for glucose over other monosaccharides. However, there is an inversion in this selectivity on increasing the linker length to *n*-heptylene ($n = 7$) and *n*-octylene ($n = 8$) with the larger spacing between the two boronic acid groups producing a galactose selective system.



17 18

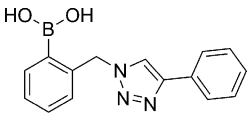
Molecular *tweezer* **19** was developed that selectively opens for certain saccharides.[13] The fluorescence intensity at 377 nm of *tweezer* **19** increases with increasing concentration of D-glucose, D-fructose, D-galactose, and D-mannose, and fluorescence intensity changes at 470 nm differ among the four carbohydrates. The 470 nm band decreases with increasing D-glucose and D-mannose concentration. The intensity of the 470 nm band is invariant with added D-fructose. Finally, D-galactose shows an initial quenching of the 470 nm band at low concentrations followed by fluorescence recovery as the concentration increases. In the case of D-glucose, D-galactose, and D-mannose, the complex formed is a cyclic 1:1 structure (Scheme 5). This explains the quenching of the intramolecular excimer

SCHEME 5. Two Possible Binding Modes for the Molecular Tweezer **19**



emission at 470 nm, since the binding of the saccharide separates the pyrene units. The more complex behavior of the sensor in the presence of D-galactose indicates initial formation of a 1:1 complex at low concentration and subsequent formation of a 1:2 complex at higher concentration. For molecular tweezer **19** with D-fructose, only the noncyclic 1:2 complex forms even at low saccharide concentration. (Scheme 5)

A convenient syntheses of fluorescent boronic acids using a Huisgen [3 + 2] cycloaddition to generate a *"click fluor"* **20** was developed,[14] an approach well suited to modular syntheses. The so-called "click reaction" was used to form a 1,2,3-triazole from an azide and a terminal alkyne, this created a fluorescent sensor from nonfluorescent constituent units. The phrase *"click-fluor"* describes the generation of a fluorophore from nonfluorescent units via the so-called "click reaction". Two of the most attractive features of *"click-fluor"* are that a fluorophore is generated when the triazole is formed and the wide availability of acetylene units facilitating potential diversity. Therefore, we believe that *"click-fluor"* will be particularly amenable to sensor array development.[15]



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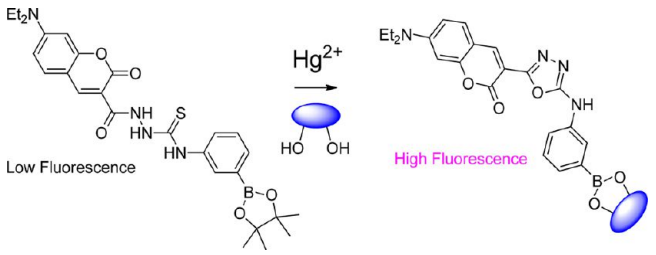
More recently a ditopic fluorescence sensor for saccharides and mercury has been developed based on a boronic acid receptor and desulfurisation reaction (Scheme 6).[16] The

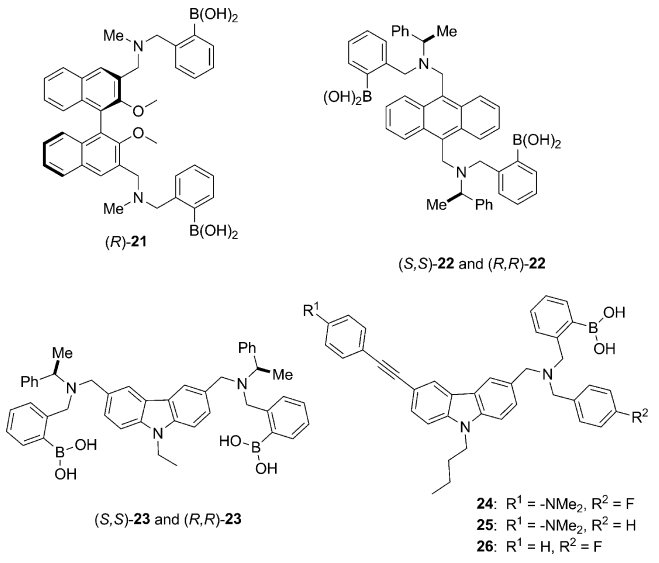
SCHEME 6. Fluorescence Dosimeter for Saccharides and Mercury



fluorescent output at 478 nm was significantly enhanced (>5-fold) in the presence of both Hg^{2+} and D-fructose in pH 8.21 buffer. While a less intense enhancement (~3-fold) was obtained on the addition of only Hg^{2+}, an even lower enhancement (<2-fold) was observed for the addition of D-fructose alone. The system can be construed as a dosimeter with AND logic functionality, in that it reports a HIGH output when two inputs are simultaneously applied.

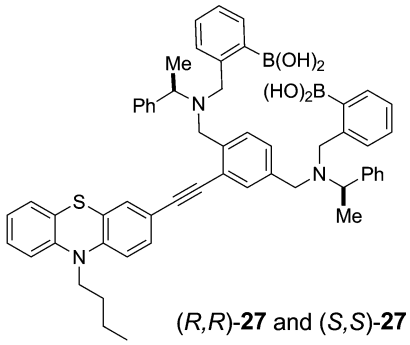
2.4. Chiral Sensors. Chiral fluorescent boronic acid sensors have attracted much interest for a number of years.[17−22] The first fluorescent chiral boronic acid sensor for glucose was prepared in 1995 (sensor **21**). With sensor **21**, the BINOL moiety performs the roles of fluorophore, scaffold, and the stereogenic center. More recently, the same BINOL-based chiral boronic acid sensors were used for enantioselective recognition of tartaric acids. However, the BINOL fluorophore emits UV light, whereas emission in the visible region is desired for routine applications. Furthermore, the fluorophore and the stereogenic centers of the BINOL-based chiral sensors are one and the same (the same is true for the chiral sensors **21**−**23**), making it difficult to vary the fluorophores

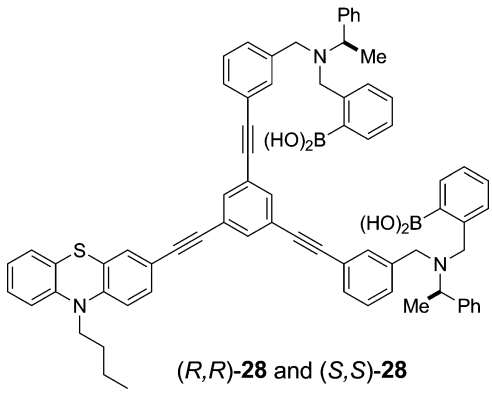
and binding sites to other units in order to optimize the molecular sensing performance of the sensors. To tackle the limitations of the BINOL system, anthracene based chiral boronic acid sensors, which produced visible emission and good chiral selectivity toward tartaric acid and sugar acids or sugar alcohols (sensor **22**), were developed. More recently, the carbazole based boronic acids were devised **23**–**26** with the fluorophore as the electron donor of the photoinduced electron transfer (d-PET) and protonated amine/boronic acid moiety as the acceptor of the PET; the background emission for the sensor at acidic pH was much lower than that with normal PET sensors where the fluorophore is the acceptor (a-PET).[17,23] However, the integrated carbazole fluorophores used in these sensors also restrict variation of the molecular scaffold.



The drawbacks of the BINOL, anthracene, and carbazole systems are that the fluorophore, the scaffold, and the chirogenic centers are integrated (sensors **21**–**23**). Therefore, the selection of the fluorophore for chiral sensors is limited. Also, the PET efficiency of the d-PET boronic acid sensors using carbazole as the fluorophore is low; the emission enhancement (or the contrast ratio) is about 2.0-fold on switching the pH from acidic pH to neutral pH, while the a-PET sensor **22** has a much larger contrast ratio of around 10-fold.

Therefore, sensors **27** and **28** were devised, in which the *fluorophore* (phenothiazine) and the *chirogenic centers* (*R*- or *S*-α-benzylamine) are joined to a *scaffold* of 2-iodo-1,4-benzenedicarboxaldehyde.[21] The rigid linker between the fluorophore and the boronic acid binding sites ensures that unwanted interactions between the binding sites and the fluorophore are avoided. Phenothiazine was selected as the fluorophore for d-PET because the chromophore is a strong electron donor. The contrast ratio obtained with the new sensors of 8.0 is significantly better than the carbazole-based d-PET fluorescent sensors.[17–19] Enantioselective discrimination of D- and L-tartaric acid was achieved with these sensors, and the recognition of the analytes is dependent on the size of the binding pocket of the boronic acid sensors. The sensors were also used for the chemoselective discrimination of disaccharides (sucrose, lactose, and maltose) and glycosylated steroids (ginsenosides).


(*R*,*R*)-**27** and (*S*,*S*)-**27**


(*R*,*R*)-**28** and (*S*,*S*)-**28**

2.5. Dye Displacement Assay. Boronic acids in combination with (1,2-diol containing) alizarin red-S (ARS) have been used in competitive binding assays. Of particular note is the anion mediated enhancement of alizarin red binding to boronic acids used to determine the presence and amount of analyte anions in a given system.[24] For example, when a boronic acid and a zinc complex are combined in one molecule to create a pyrophosphate sensor that binds the fluorescent molecule ARS in the absence of phosphate (and displays reduced fluorescence as a result). Upon exposure to PPi, ARS becomes part of a now highly fluorogenic unit, and the fluorescence increases (Scheme 7).[24]

Hydrogel spheres, 5 mm in diameter, incorporating phenylboronic acid functionality were exposed to ARS dye, and

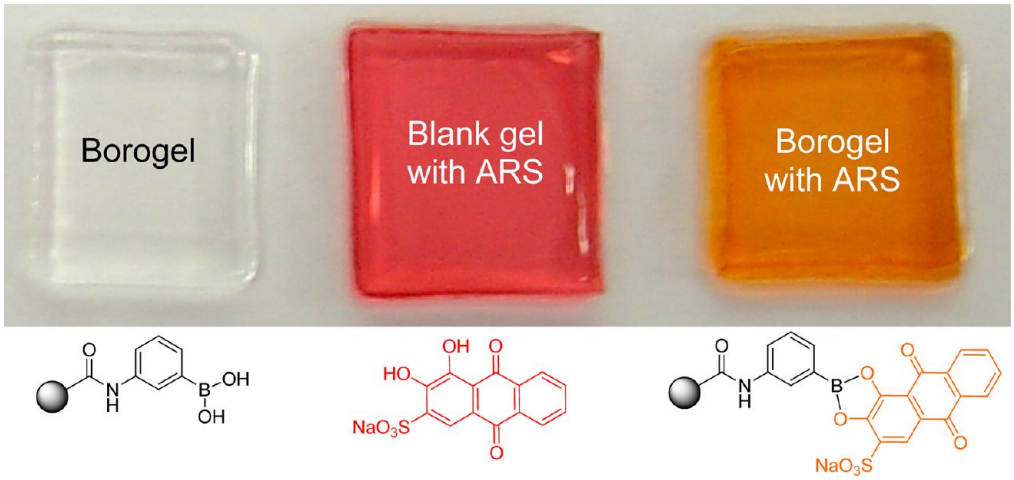


FIGURE 1. Gel slabs: Borogel (left), blank gel plus alizarin red-S (middle), and borogel plus alizarin red-S (right).

the corresponding boronic ester was formed. Once excess dye had been removed by washing, the spheres were exposed to an analyte diol (fructose, for example), releasing the dye into solution (as represented in Scheme 8). To demonstrate the hydrogel displacement assay, the relative amounts of boron binding species (saccharides) in samples of fruit juices were determined.[25]

A hypsochromic shift is indicative of ARS binding to boron; ARS develops an orange color on binding to boron. Comparing $10 \times 10 \times 1.5$ mm^3 gel slabs with and without boron, Figure 1 right and center, respectively (a boron containing gel prior to exposure to ARS is shown for comparison, left), it is possible to observe the color difference.

The dye displacement assay has also been used to evaluate biocompatible polymers as potential drug delivery conjugates.[26] Boronic acid terminated PLA (BA-PLA) was prepared and the reversible formation of a well-defined fluorescent ARS-PLA conjugate was used to follow the binding of a range of hydroxyl-containing species to the BA-PLA polymer. Displacement of the ARS and selectivity for diols over simple alcohols was observed. The potential for formation of strong complexes between BA-PLA and hydroxyl-containing therapeutic agents suggests a versatile and highly specific route to quantitative polymer–drug conjugates and nanoconjugates, which have become a key target as drug delivery vehicles.

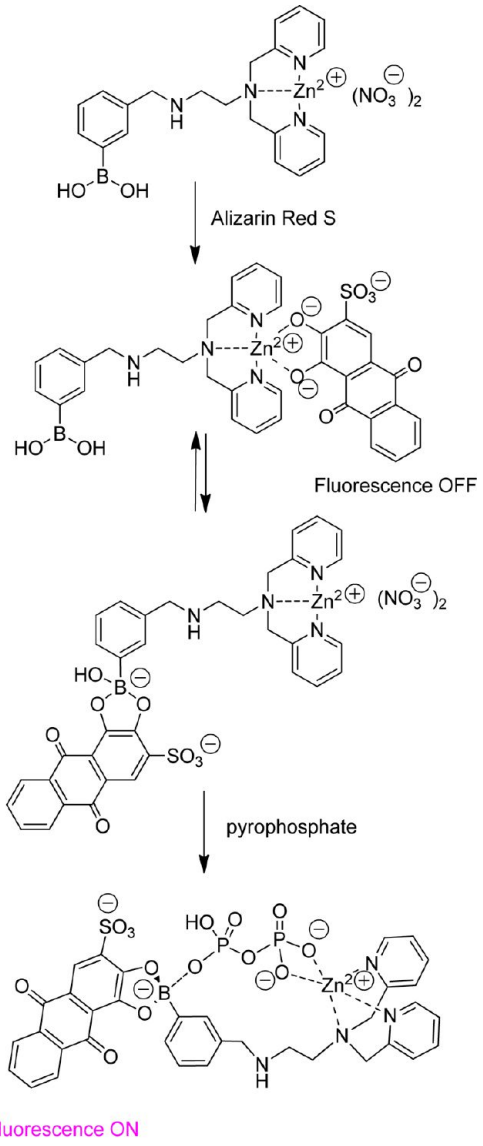
2.6. Cation–π Interactions. Recently, it has been demonstrated that pyridium cation–π interactions can be evaluated using fluorescence spectroscopy.[27–29] Since pyridinium boronic acids have already been used to prepare a saccharide sensor,[30] the next logical step was to combine cation–π interactions and pyridinum boronic acids into one construct for diol detection. A simple propylene (Leonard Linker) linked phenyl alkyl pyridinium boronic acid showed characteristic cation–π stacking exciplex fluorescence.[31] Of the anions investigated, the most intense fluorescence response came from the boronic acid ion pair with the most diffuse negative charge (e.g., *boronic acid*, Scheme 9, $X^- = PF_6$). The relative fluorescence intensity ($PF_6^- > Br^- > Cl^- > F^-$) may be interpreted as the tighter the ion pair the weaker the fluorescence, so for a "turn on" sensor it is better to start with a low fluorescence (tight pair) situation that has the potential to undergo fluorescence enhancement upon interaction with a diol analyte.

Indeed, for the sensing regime depicted in Scheme 9 when $X = PF_6$, no fluorescence change was observed; essentially, the starting position was already at maximum fluorescence. The best "fluorescence on" response for pinacol was observed when chloride was used as the counteranion (X = Cl).

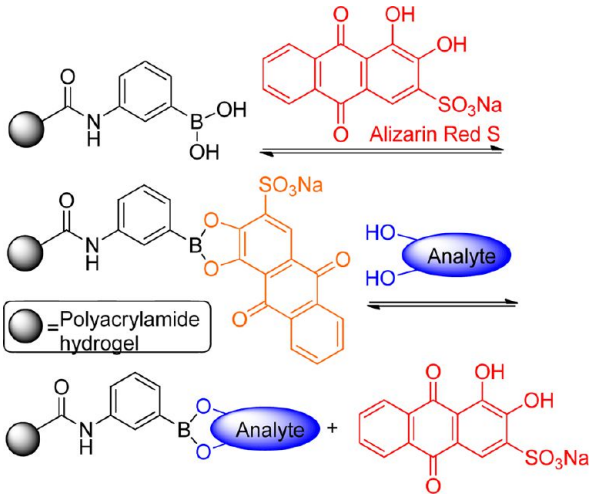
2.7. Electrochemical Methods. Boronic acid functionality has been employed in electrochemical sensing systems predominantly based on direct effects of analyte binding on current and/or potential responses in voltammetric experiments. In a recent review, the role of phenylboronic acids, in particular, for electrochemical sugar sensing, was highlighted.[32] Broadly, electrochemical assays employing boronic acids can be divided into *solution phase processes* and *surface immobilized processes*.

2.7.1. Solution Processes. Most widely studied are soluble ferrocenylboronic acid redox probes which were first synthesized by Nesmayanov and have been shown to allow direct electrochemical saccharide sensing in aqueous media. A typical differential pulse voltammetry solution essay for sorbitol binding is shown in Figure 2. Ferrocenylboronic acids suitable for sensing glucose have been prepared, and

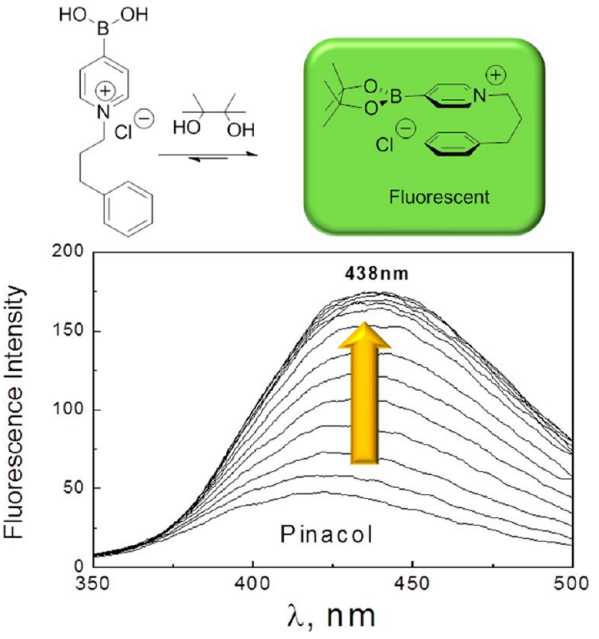
SCHEME 7. Pyrophosphate-Induced Reorganization of a Reporter–Receptor Assembly



SCHEME 8. Binding and Analyte-Mediated Release of Alizarin Red-S with Hydrogel-Bound Boronic Acid (Reproduced with permission from ref 25. Copyright 2009 The Royal Society of Chemistry)



SCHEME 9. Phenyl Propylpyridium Boronic Acid Receptor for Diols



chiral ferrocenylboronic acids have been reported for chiral electroanalysis.[33]

2.7.2. Surface Immobilized Processes. Due to electrochemical processes fundamentally being heterogeneous in nature, much more sensitive and probably more selective sensing processes can occur directly at the electrode/solution interface. Amphiphilic *N*-hexadecyl-pyridinium-4-boronic acid cations have been self-assembled into a monolayer at graphite electrodes.[34] Figure 3 shows the effect of binding *ortho*-quinols to the modified graphite surface. The area under process P2 is consistent with the amount of immobilized boronic acid, and typical binding constants for a range of *ortho*-quinols suggest selectivity for the hydrophobic alizarin red-S. The self-assembly of boronic acid dendrimers with nanocellulose whiskers has also been investigated.[35]

Finally, in recent studies on biphasic redox systems, microdroplet deposits of water-insoluble organic liquids at electrode surfaces have been employed with dissolved boronic acids. In this liquid–liquid system, highly hydrophobic naphtalenyl and anthracenyl derivatives of boronic acids are dissolved in microdroplets of 4-(3-phenylpropyl)-pyridine solvent (see Figure 4).

An additional redox system such as tetraphenylporphyrinatomanganese(II/III) then allows anions to be actively transferred from aqueous to organic phase with

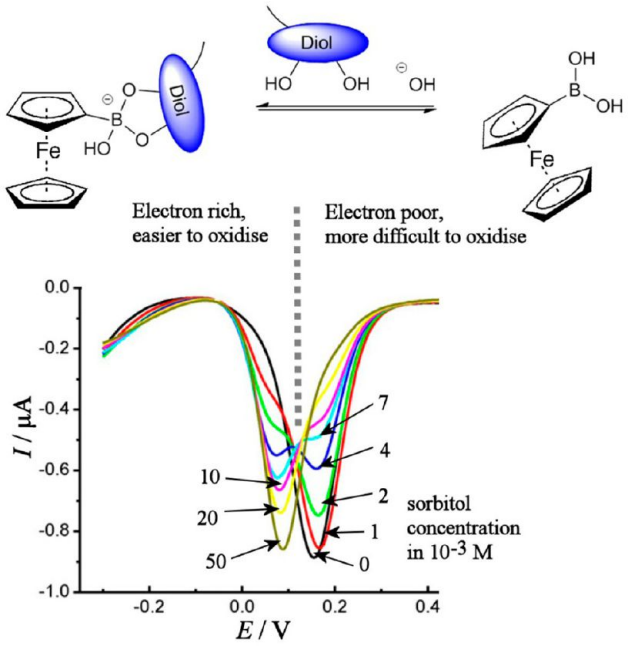


FIGURE 2. Binding of a diol to ferrocene boronic acid and differential pulse voltammetry data set for sorbitol binding associated with a shift in reversible potential.

selectivity for anions with affinity for boronic acids. The transfer of carbonate and bicarbonate[36] as well as the transfer of α-hydroxycarboxylates have been reported.[30] Here, the selectivity of the boronic acid is combined with the selectivity effect introduced by the Gibbs energy of anion transfer from the aqueous into the organic phase. In future, these biphasic redox systems with boronic acids could potentially be incorporated into hydrophobic membranes for analytical and also for separation purposes.

2.8. Fluorophore Quencher Interactions. A new signaling regime was conceived whereby a fluorescent boronic acid, that when exposed to an analyte diol, appended with a quencher, would reduce the fluorescence output of the system due to the formation of a *static* fluorphore−quencher pair. Thus, signaling the presence of the diol appended quencher (Figure 5).

A fluorescein boronic acid derivative was simply prepared from commercially available materials in order to function as the fluorescent partner, and a series of methyl red inspired diols were synthesized as potential boronate

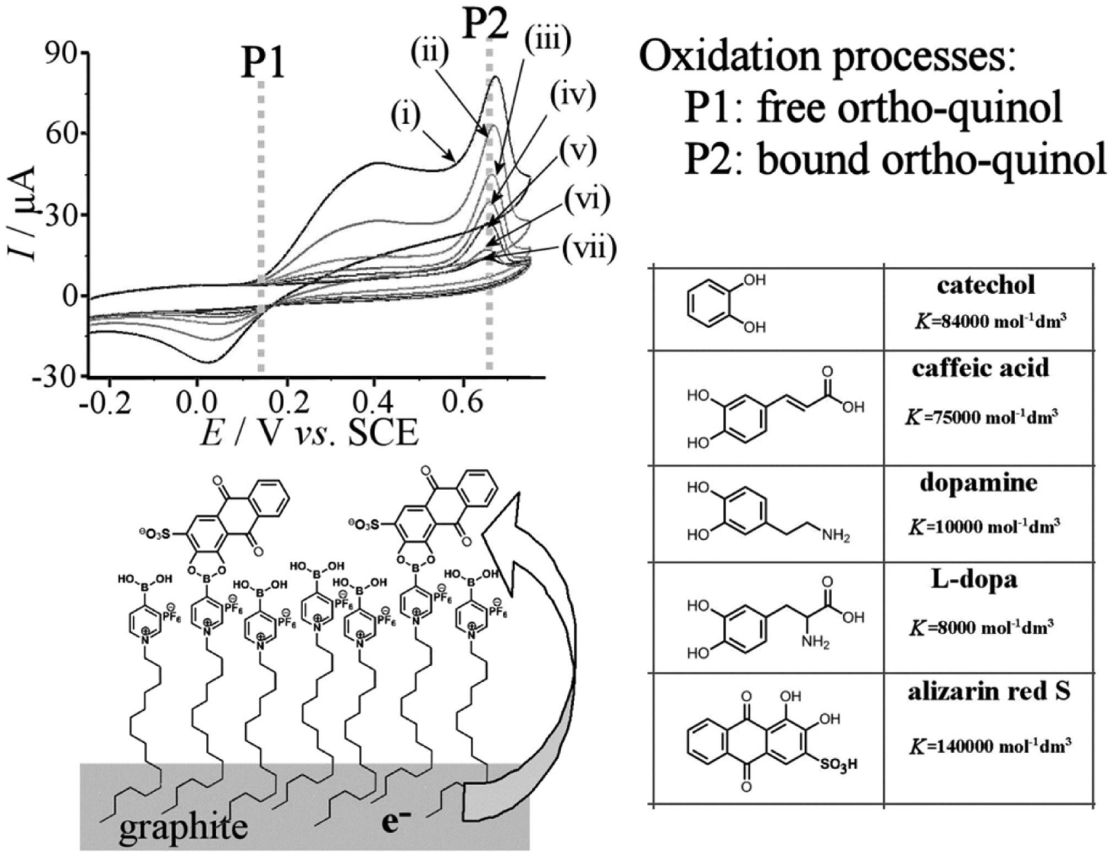


FIGURE 3. Voltammetric responses (scan rate 0.1 V s^{-1}) for the oxidation of dopamine at a 4.9 mm diameter graphite electrode with a 1 nmol *N*-hexadecyl-pyridinium-4-boronic acid hexafluorophosphate deposit immersed in aqueous 0.1 M phosphate buffer containing (i) 1×10^{-5}, (ii) 2×10^{-5}, (iii) 5×10^{-5}, (iv) 1×10^{-4}, (v) 2×10^{-4}, (vi) 5×10^{-4}, and (vii) 1×10^{-3} mol dm^{-3} dopamine. The schematic drawing shows the binding of alizarin red-S, and the table summarizes binding constants.

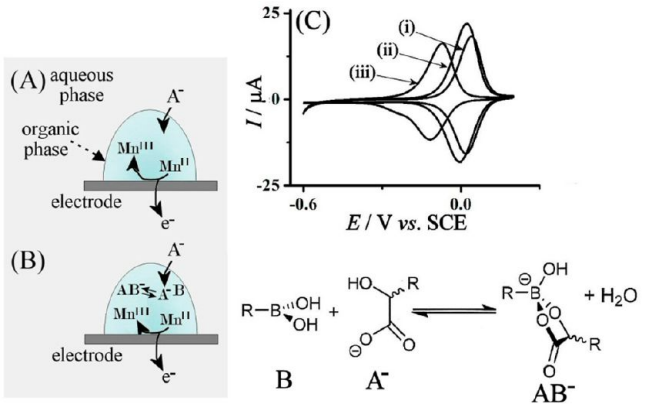


FIGURE 4. (A) Schematic drawing of the electrochemically driven anion transfer at an oil microdroplet/aqueous electrolyte interface. (B) Schematic drawing of the process with boronic acid B present (see equation). (C) Typical cyclic voltammograms (scan rate 10 mV s^{-1}) for the oxidation and re-reduction of 75 mM MnTPP dissolved in 4-(3-phenylpropyl)-pyridine (75 nL) and immobilized onto a 4.9 mm diameter graphite electrode immersed in aqueous 0.1 M sodium lactate pH 7.34. The presence of (i) 0 mM, (ii) 114 mM, and (iii) 973 mM naphtalene-2-boronic acid is shown to cause a shift in the voltammetric response.

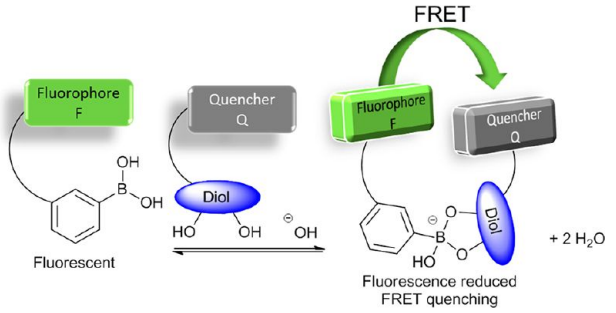


FIGURE 5. Fluorophore appended boronic acid interacting with a diol-appended quencher.

ester forming quencher partners to probe a FRET quenching/sensing regime based on boronate ester formation (see Figure 6).[37]

A detailed study of the combination of fluorescein boronic acid with diol appended quenchers **29a**–**c** and comparison with the fluorescence outputs of nonboron or nondiol containing systems (i.e., fluorescein or methyl red were employed directly) revealed boronate ester formation does indeed result in a quenching enhancement in each case, and that compound **29c** was the best overall quencher based on the ratiomentric quenching enhancement between the nonbinding *dynamic* systems versus the boronate forming *static* systems.[37] Utilizing a boronic acid receptor to catch quencher analytes is a generic sensing format, schematically illustrated in Figure 7.

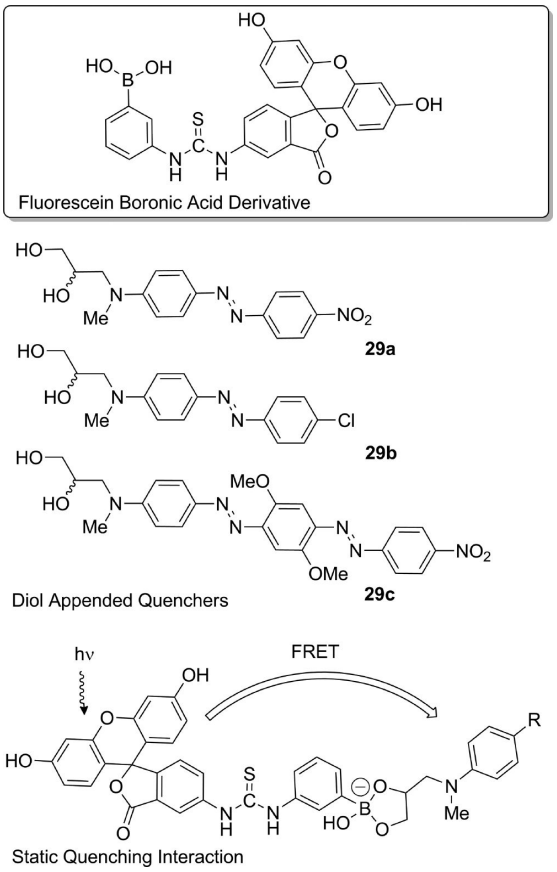
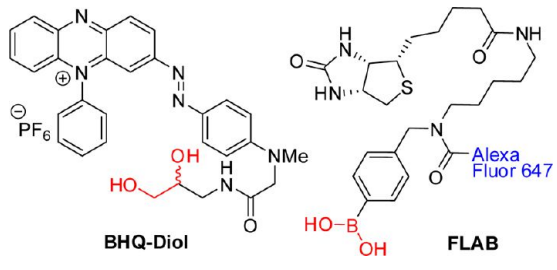


FIGURE 6. Fluorescein boronic acid derivative, three diol-appended quenchers, and a representation of the FRET quenching interaction.

In order to assemble a sensor construct at a gold–strepatividin surface, the molecule FLAB (Fluorophore Linker boronic Acid Biotin) was prepared. The design incorporated a terminal biotin for attachment to surface bound streptavidin, a boronic acid receptor and a fluorophore (Alexa Fluor 647, Invitrogen, ex_{max} 647 nm). The quencher–diol conjugate was prepared utilizing a quencher for Alexa Fluor 647, BHQ-3 (Biosearch Tech).[38] Attachment of FLAB to a streptavidin-appended gold surface was confirmed by both SPR and concomitant fluorescence (f-SPR). Exposure of the surface prepared to BHQ-diol gave rise to both fluorescence quenching and an SPR response, demonstrating the potential for the dual techniques of SPR and fluorecence to work in unison in a sensor regime under the guise of f-SPR.



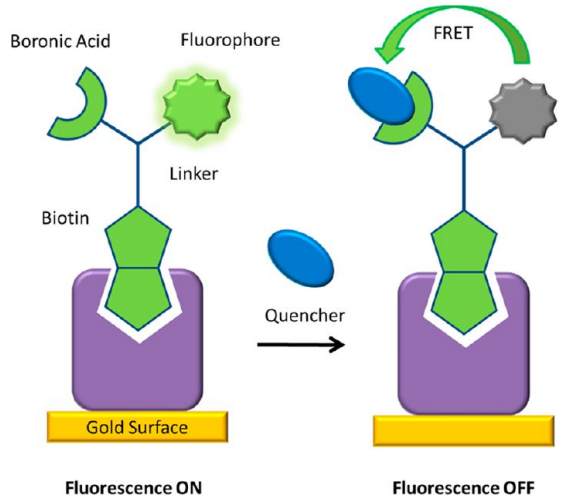


FIGURE 7. Surface appended FLAB (Fluorophore Linker boronic Acid Biotin) for use in a fluorescence surface plasmon resonance (f-SPR).

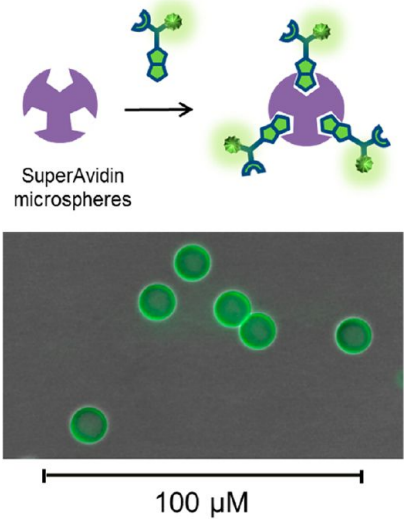


FIGURE 8. Upper: Schematic of SuperAvidin microsphere functionalization with fluorescein-FLAB. Lower: Overlaid fluorescence and bright field images of FLAB functionalized microspheres.

Taking advantage of biotin appended sensors in other scenarios directly led to another model system, using microscale avidin appended polystyrene microspheres (Bang Laboratories) (Figure 8). These microspheres could be functionalized with FLAB fluorescein, as demonstrated by fluorescence microscopy.[39]

3. Electrophoresis

Polyacrylamide gel electrophoresis exploits hydrogel polymers to separate molecules on a size and charge basis. Among the useful separations of biomolecules that electrophoresis is commonly employed for is a technique for the separation of carbohydrates called FACE (Fluorophore Assisted Carbohydrate Electrophoresis). However, the FACE technique requires the labeling of analytes with a fluorophore, does not separate saccharides of similar size and charge particularly well, and is limited to reducing sugars.

In order to address the need to provide a separation tool for similar mass saccharides, Boron Affinity Saccharide Electrophoresis (BASE) was developed.[40] Hydrogels could be prepared that contained *ortho*, *meta*, and *para* boronic acids although solubility allowed up to about 3% reliable incorporation, but this was more than enough to obtain excellent results in electrophoresis. For the majority of investigations, the *meta* derivative was preferred due to its overall higher synthetic yield. In comparison to the *para* derivative, no differences were observed in terms of electrophoresis applications; however, the *ortho* derivative gave less effective separation (analyte mobility was more facile) and was prone to degradation.

While FACE performs poorly in separating a series of 2-AMAC labeled saccharides, the BASE system induces dramatic mobility differences among the saccharides investigated. In the BASE gel, previously inseparable saccharides are now clearly resolved, even though in some cases resolution is not perfect the modulated mobility achieved as a function of reversible boron diol interactions within a hydrogel domain opened the door to a range of new applications, not only in electrophoresis,[41] but also for applications such as hydrogel sensors mentioned earlier.[25]

BASE was next employed in the detection of a gluconolactone modification of a protein that had been shown by van den Elsen to inhibit the innate immune system and is under development as a therapy for complement mediated acute inflammatory diseases. Purified protein was exposed to gluconolactone, and its electrophoretic analysis was performed at various time intervals by standard polyacrylamide gel electrophoresis (PAGE) and protein BASE (coined Pro-BASE or mP-AGE); see Figure 9. For protein incubation with gluconolactone, Pro-BASE reveals a new band which was almost indistinguishable from the main band by a normal electrophoresis experiment.

4. Boronic Acids as Building Blocks for Self Assembly

Despite the stability of boronate ester covalent B–O bonds, their formation is reversible under certain conditions or

under the action of certain external chemical stimuli. The reversible nature of boronate formation enables reversible molecular assembly. The reaction of 2-formyl-aryl-boronic acids with 1,2-amino alcohols results in dynamic covalent self-assembly to quantitatively afford macrocyclic Schiff base boracycles containing bridging boron−oxygen−boron functionality (Scheme 10).[42]

A similar self-assembly protocol with chiral constituents provided a robust probe for enantiomeric excess of either chiral amines or chiral diols by NMR spectroscopy, electrochemical, and circular dichroism (CD) methods (Scheme 11).[33,43−45]

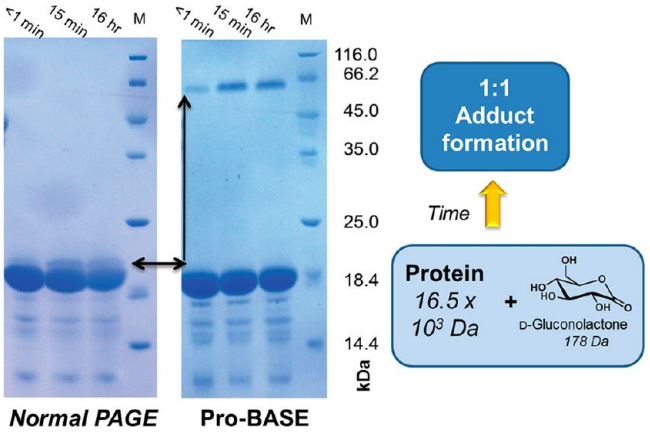
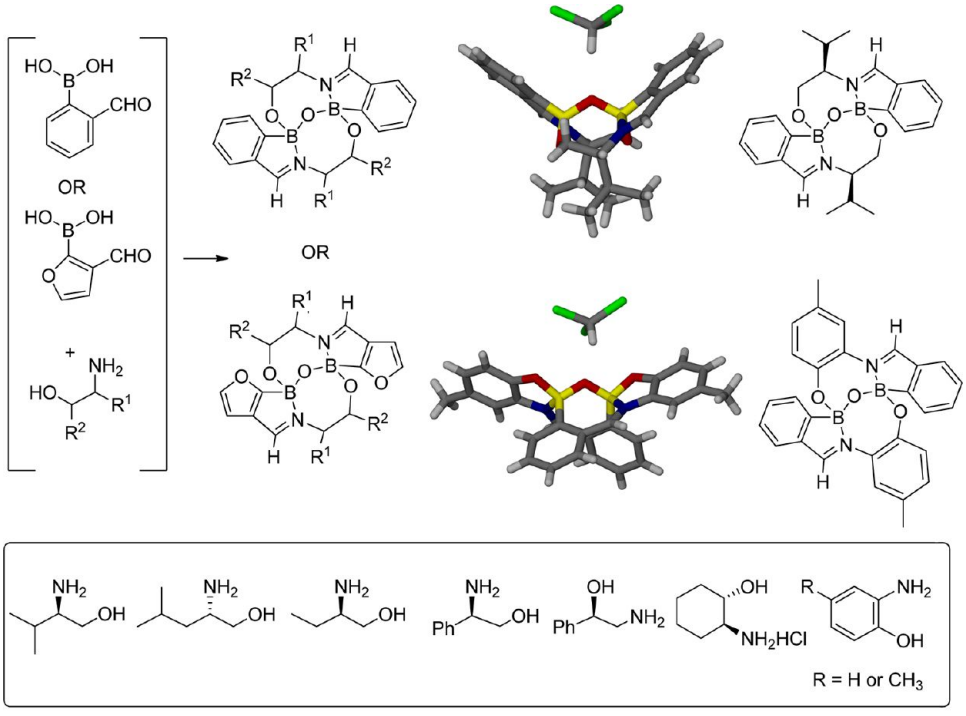


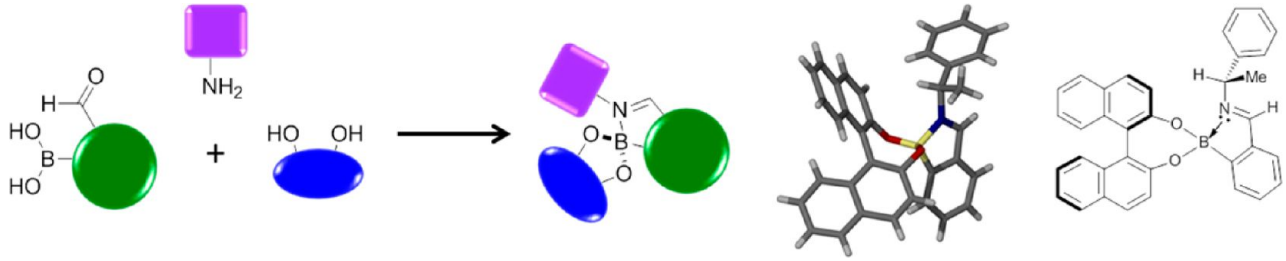
FIGURE 9. Separation by protein-Boron Assisted Saccharide Electrophoresis (pro-BASE or mP-AGE) of a glyconoylated protein utilizing stationary phases that do not (left) and do (right) contain boronic acid.

Boronate esterification can also be utilized in the construction of capsule structures which display three-dimensional cavities, such as the recently reported ion-pair-driven heterodimeric capsule formation.[46,47] The system consists of cyclotricatechylene and a boronic-acid-appended hexahomotrioxacalix[3]arene. The two components do not interact with each other until Et_4NOAc is added to the solution. On addition of Et_4NOAc, quantitative capsule formation by boronate esterification is observed. The self-assembly process is a direct result of anion directed boronate ester formation and the presence of the Et_4N^+ template. A similar capsule was also formed when Et_3N was added to a methanolic solution containing cyclotricatechylene and the boronic acid-appended hexahomotrioxacalix[3]arene. The reaction involves the initial formation of a capsule consisting of cyclotricatechylene, the boronic acid and three amines; subsequent solvolysis results in encapsulation of Et_3NH^+ within the capsule. When the larger Bu_3N was used to trigger capsule formation, solvolysis in the presence of guests such as Et_4N^+, Me_4N^+, Me_4P^+, and Cs^+ results in encapsulation of these guests rather than the bulky Bu_3NH^+ (Scheme 12).

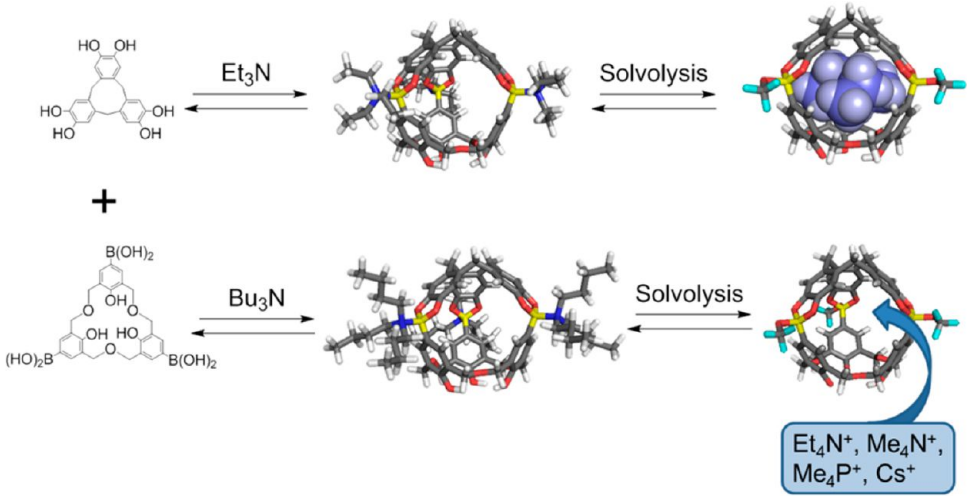
SCHEME 10. Synthesis of Macrocyclic Schiff Base Boracycles Containing Bridging Boron−Oxygen−Boron Bond from [2 + 2]-type Condensation of 2-Formyl-aryl-boronic Acids and 1,2-Amino Alcohols



SCHEME 11. Three Component Reaction for Boron Mediated Enantiomeric Excess Determination



SCHEME 12. Amine-Triggered Molecular Capsules via Dynamic Boronate Esterification



5. Conclusions

This is a personal perspective on the use of boronic acid based receptors and focuses on papers and reviews published over the last 5 years. While assembling this Account, it was pleasing to note that while some areas of research have faded, many new and exciting areas have developed. More importantly, from a personal perspective, the science of boronic acid receptors is as exciting now as it has ever been. It is hoped that this Account will inspire others to explore some of the yet uncharted regions of reversible covalent chemistry of boronic acids to even more exciting discoveries.

While this is a personal account of research into Boronic Based Receptors, the 10 coauthors have contributed to the construction of this Account and the research that underpins it and are the people without whose friendship and collaboration the research concepts contained in this Account would not be possible. The contribution of Postdoctoral Researchers, Ph.D. Students, and Undergraduate Students is also acknowledged, since it was their work that provides the results discussed in this Account (see references). The research presented in this Account has been supported by many funding sources, but the EPSRC, The Royal Society (including International Joint Projects with Y.K., K.S., and J.Z.), The Leverhulme Trust, The Japan Society for the Promotion of Science (JSPS), the GB Sasakawa Foundation, the Daiwa Foundation, the University of Birmingham and the University of Bath are all thanked for providing funding and support. The core of the research presented has been enhanced by collaboration both within the University of Bath, the U.K., and Internationally. S.D.B., J.S.F., Y.-B.J., J.Z., and T.D.J. would also like to thank The Catalysis and Sensing for our Environment (CASE) network for networking opportunities.

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FOOTNOTES

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The authors declare no competing financial interest.

This Account is dedicated to J. Grant Buchanan, a Great Scholar, Educator, and above all Friend. Your endless enthusiasm and quest for knowledge will be sadly missed.

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