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Discrimination of Saccharides by a Simple Array

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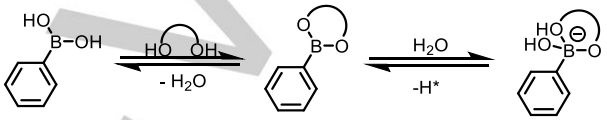
Abstract: We report the development of a two component probe system as fluorescence turn-on assay of simple saccharides. The quenching of an anionic conjugated water-soluble polymer by a cationic quencher has been reported previously. Three different boronic acid functionalized benzyl viologens and three conjugated polymers of the poly(aryleneethynylene) type form nine non-fluorescent complexes. This small library discriminates nine different simple saccharides in aqueous solutions by fluorescence turn-on in a displacememt assay. The saccharides can be discriminated and identified with this simple system.

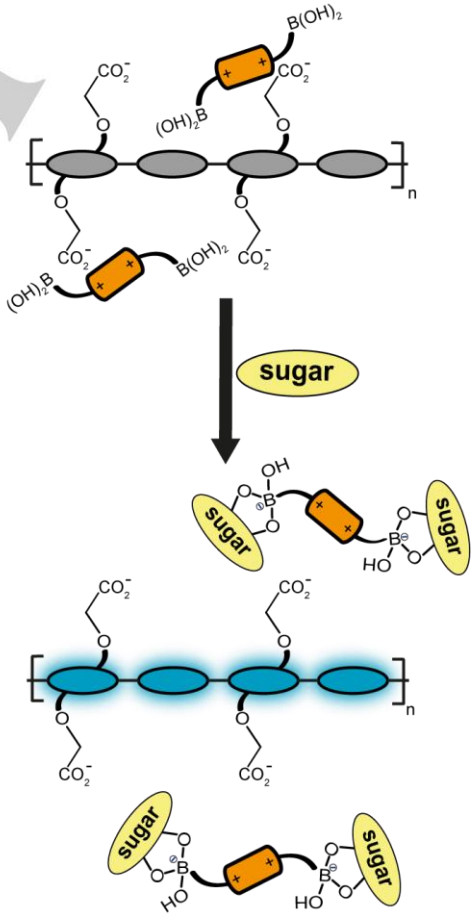
Introduction

We have prepared a small self-assembled library consisting of nine elements, consisting of pairwise combinations of three boronic acid quenchers and three conjugated polymers. The library discriminates simple saccharides in aqueous solution. Boronic acids,[1] "artificial lectins", are molecules that bind to saccharides, forming a boronic acid acetal (Scheme 1).[2] This binding is attractive for the detection and potentially also quantitation of sugars[3,4,5,6]: a significant number of glucose-sensors have successfully employed boronic acids. Such a task is obviously important both in biological systems, medicinal applications[7,8] but more so in quality control of food stuff and beverages, including wine and fruit juices. [9,10] While in biomedical applications the monitoring of glucose level is still interesting, issues in quality control of beverages allow and require potentially other approaches to discriminate saccharides in a specific environment. One example should be mentioned to explain our interest in discriminating saccharides. The continued demand for premium quality honey puts a strain on many bee colonies due to new, highly epidemic mites. Such a situation creates a lure to add artificial sugar-syrups to honey. Such artificial sugar syrups typically have a sugar composition that is different from that found in natural honey. Therefore, discrimination of simple sugars is a fundamental and attractive, but immediately application-relevant, yet difficult task, due to their chemical similarity.

Schanze et al. synthesized paraquat-based boronic acids.[11] They created a probe system in which poly(arylene-ethynylene) **(PAE 1)** is combined with the quencher **p-BV^{2+}** to give a non-fluorescent construct (Figures 1 and 2). Upon binding to fructose, galactose or glucose, the quencher is liberated from the anionic polyelectrolyte and the polymer solution experiences a turn-on in fluorescence. While sugars and saccharides are neutral, the corresponding boron-based chelates probably pick up water and turn into a negatively charged borate complex, counteracting the electrostatic interaction between the paraquat and the anionic polymer. Scheme 1 shows the simplified mechanism.



Scheme 1. Equilibria between free boronic acid and saccharide (diol).



Scheme 2. Turn-on sensing mechanism employed here.

Schanze et al. observed that the sugars bind differently to

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the PAE-viologen complexes and noted that fructose was the most successful saccharide in displacing the viologen from the PAEs. Strong binding of fructose to boronic acids is due to the presence of a five-membered syn-diol, long known and discussed by Peters in a recent review.[4] We thought that the system boronic acid-viologen/PAEs was attractive-not only to determine but perhaps also to *discriminate* different sugars. This type of approach has also recently been employed by different groups[3c,d] employing either boronic acid treated filter papers in a dye displacement assay or, more powerful, viologen-substituted cationic phenylboronic acids that form electrostatic complexes with pyrene sulfonic acids. In the latter case, upon decomplexation by polyols ("sugar alcohols") differential fluorescence turn on is observed, and PCA or LDA discriminate the observed patterns. This method is fairly sensitive for the sugar alcohols and allows their discrimination down to 0.4 mM, but the authors have not attempted to discriminate regular mono- and disaccharides.

Results and Discussion

We first investigated the binding of the three boronic acid substituted viologens (**o-, m-,p-BV^{2+})** to the PAEs **(PAE 1-3)**, employing the Stern-Volmer formalisms. Figures 1 and 2 display the chemical structures of the quenchers and the conjugated polymers, all of which are literature known, except for **PAE 5**.

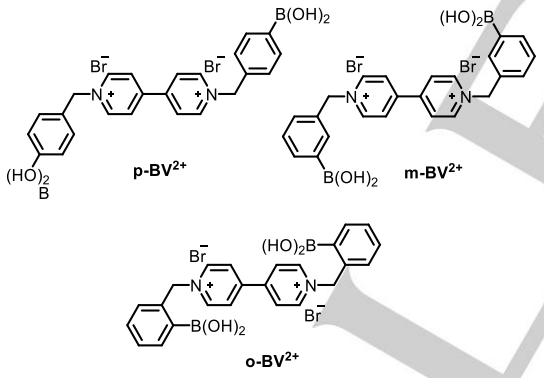

Figure 1. Chemical structures of reported glucose-selective benzyl viologens (BV^{2+}).[11-13]

Schanze`s scheme consists of a cationic viologen and an anionic PAE to create a neutral complex with quenched fluorescence (neutral PAEs show no evidence of complex formation). We expanded this concept and investigated three water-soluble and highly fluorescent PAEs (**PAE 1-3**) that were left after screening of **PAE 1-6**. **PAE 4-6** were discarded as their fluorescence is too weak. Titration curves were analyzed and **PAE 1-3** show a continuous increase in quenching upon adding **p-BV^{2+}**. A continuous increase in the fluorescence intensity suggests that upon adding of saccharides this intensity increase should be proportional to the quantity of added analyte.

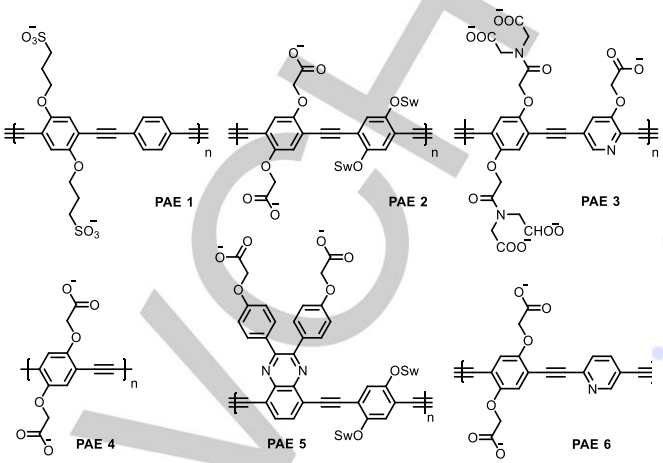

Figure 2. Selected conjugated polymers (**PAE 1 – PAE 3**)[14-16] and discarded polymers (**PAE 4 – PAE 6**).[17,18]

Table 1: Proposed sensor system and formed complexes.

complex	PAE 1	PAE 2	PAE 3
p-BV^{2+}	**P1**	**P2**	**P3**
m-BV^{2+}	**P4**	**P5**	**P6**
o-BV^{2+}	**P7**	**P8**	**P9**

Determination of binding constants for the viologens with PAEs.

As our proposed assay is a dye displacement, we extract the quencher-dye binding constant forming **P1-9** from the Stern-Volmer quenching constants (Figure 3). The binding of PAEs to **p-BV^{2+}** is by far the strongest, while for **m-BV^{2+}** and **o-BV^{2+}** the binding to the polymers is a factor of 5 or so lower. Also, **PAE 1** binds strongest to the quencher, while the two other PAEs show a lesser affinity to the quenchers. The signalling mechanism is then the sugar-promoted decomposition of these complexes, freeing the anionic polymers under strong fluorescence turn-on. We expect a useful response range of the constructs with the saccharides. We would expect that the weakest bound complexes will give the greatest fluorescence turn-on responses.

Discrimination and binding of sugars.

Figure 4 shows the fluorescence response patterns of all complexes **P1-9** towards different sugars at a sugar concentration of 100 mM. As expected, the weaker bound complexes, particularly **P7**, show larger turn-on efficiencies, yet, the overall patterns are different for each sugar.

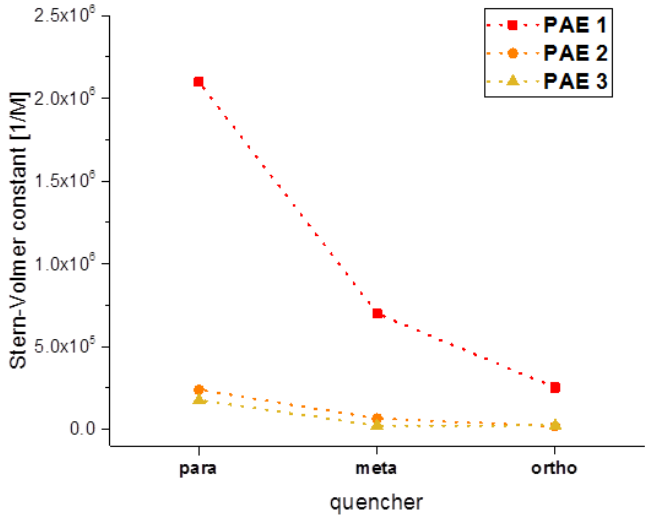

Figure 3 Stern-Volmer constants of **PAE 1-3** upon binding to **p-**, **m-** and **o-BV²⁺**.

Previous to that (Figure 5) we screened for the complexes **P1-3** the concentration range of the saccharides (glucose, fructose, galactose and sucrose) that would give the best responses; we found that 100 mM of sugar is optimal, even though 25 mM solutions could also be investigated. At higher concentrations, the solutions became too syrupy to allow precise analysis. Also, at around 200 mM of the saccharides we observe saturation and discrimination becomes less effective (Figure 5b).

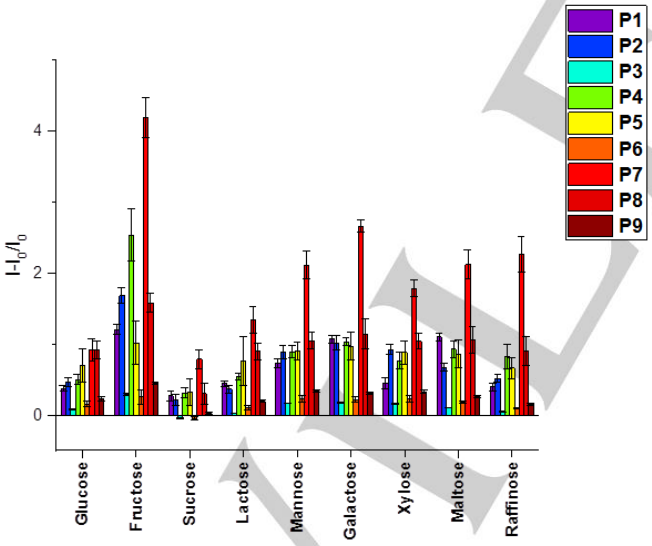

Figure 4. Fluorescence-response pattern ($(I-I_0)/I_0$) with **P1-9** (2.5 µM **PAE 1-3** and 25 µM **o-, m- and p-BV²⁺**, buffered at pH 7) treated with sugars (100 mM). Each value is the average of five independent measurements with standard error.

A 0.1 M solution of sucrose contains around 34 g sugar/L, while typical soft drinks like cola-types have a sugar content of around 110 g/L as a mix of sucrose, glucose and fructose. Similar amounts of sugars are hidden in energy drinks, suggesting that our system should be perfect for their investigation; our assay is not sufficiently sensitive to determine glucose levels in blood (4-8 mM). The most useful results are obtained at a concentration of the conjugated polymer of 2.5 µM, the paraquats at 25 µM and the saccharides at concentrations around 70-100 mM. With the results shown in Figure 4 we performed statistical analysis to discriminate.

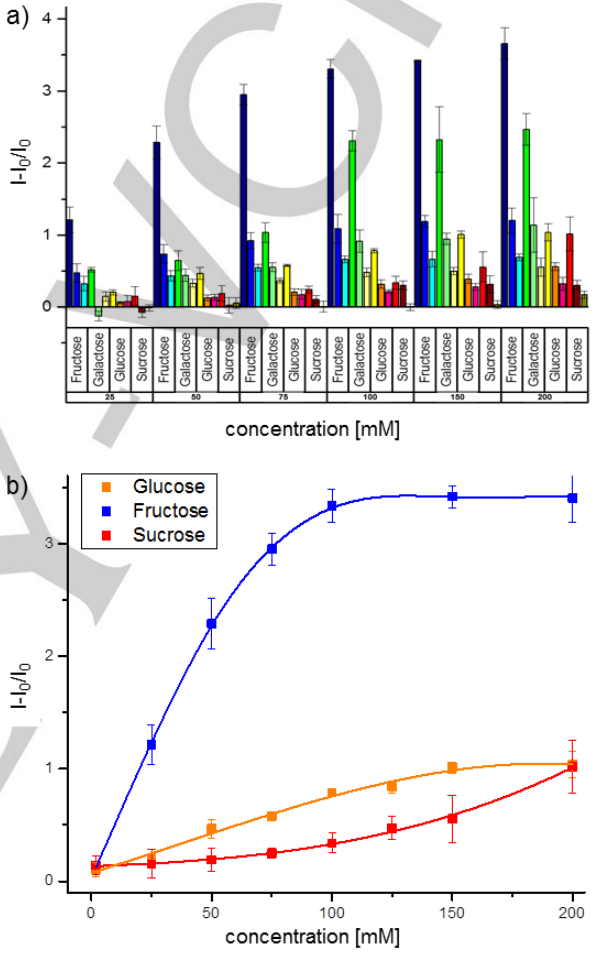

Figure 5. a) Fluorescence response pattern ($(I-I_0)/I_0$) obtained with **P1**, **P2** and **P3** (2.5 µM PAE and 25 µM **p-BV²⁺**, buffered at pH 7) for four different saccharides (fructose, galactose, glucose and sucrose) at five different concentrations (25 – 200 mM). Each value is the average of five independent measurements with standard error. b) Detailed concentration course of the fluorescence response ($(I-I_0)/I_0$) of **P1** applied to glucose, fructose and sucrose.

Mathematical processing and interpretation of the turn-on data

We first analyzed responses of the complexes **P1-P3** (Figure 6a); some of the saccharides are immediately discriminated, viz. fructose, maltose, and sucrose. There is a second, unresolved cluster of lactose, raffinose and glucose, and a third cluster with partial resolution, which contains mannose, galactose and xylose. While xylose is discriminated from galactose, the data for mannose overlap with those for both of the other sugars. If we

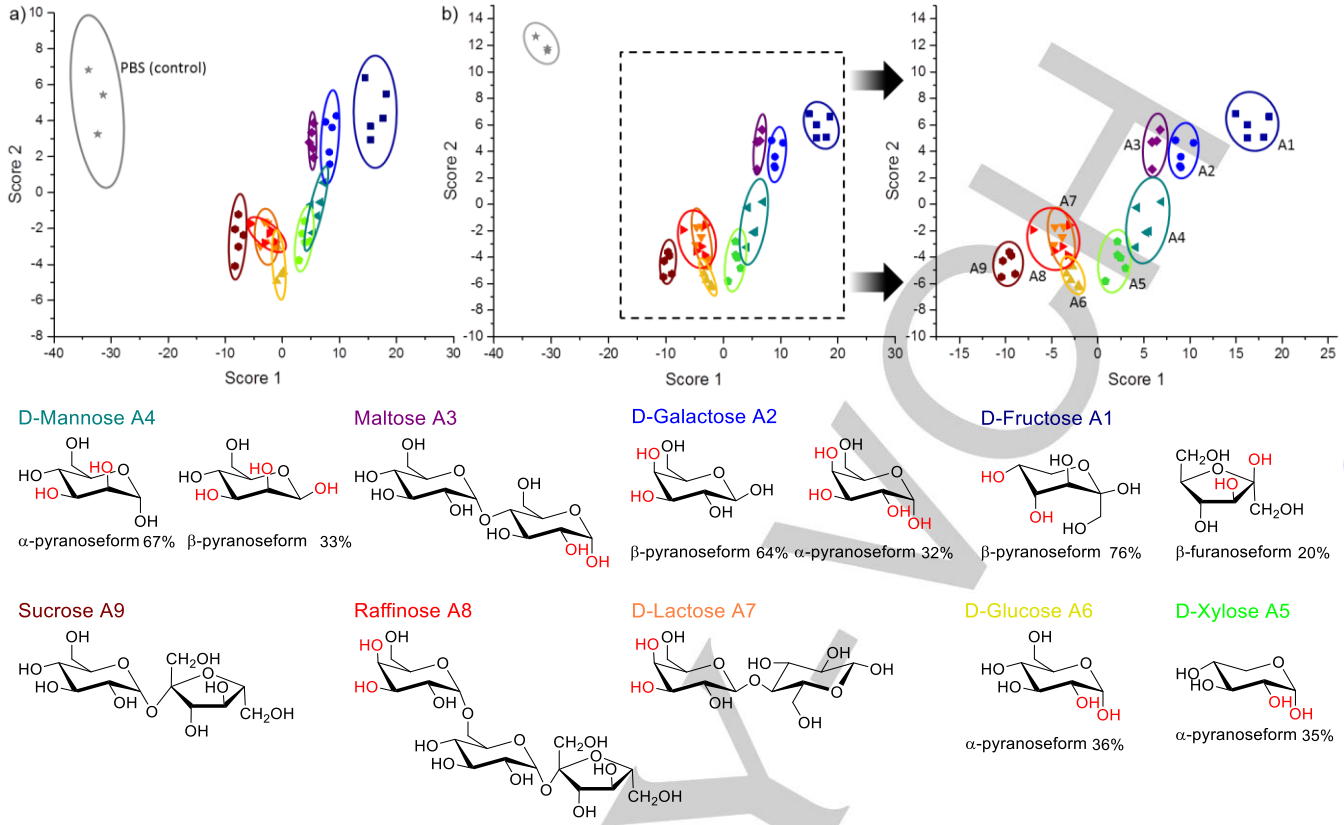

Figure 6. a) Canonical score plot for the first two factors of simplified fluorescence response patterns obtained with an array of **P1-3** (2.5 µM PAE 1-3 and 25 µM ***p*-BV^{2+}** buffered at pH 7) treated with nine different sugars. **b)** Canonical score plot for the first two factors of simplified fluorescence response patterns obtained with an array of **P1-6** (2.5 µM **PAE 1-3** and 25 µM ***p*-BV^{2+}** und ***m*-BV^{2+}** buffered at pH 7) treated with nine different sugars.

increase the library to include **P1-P6**, the resolution increases somewhat and the group galactose, mannose and xylose is better resolved (Figure 6b).

Upon going to the full array **P1-P9**, the resolution of our assay increases again and now, to our surprise, even A7 (lactose) and A8 (raffinose) are resolved, as they are most similar in structure. This is not gleaned from the first two factors, but if score 1 and score 3 are plotted, the discrimination is clear (inset Figure 7).

An important question is what and if the two axes depicted in the LDA plots are correlated with any physical property. As usual, score 1 is connected to the change in emission intensity, here emission turn on. While in comparison to water all of the added sugar solutions increase the fluorescence, there is a significant differentiation between the sugars. Fructose, due to the presence of the furanose form, featuring a *cis*-diol, in equilibrium with its pyranose form, is the sugar giving the highest turn-on. This tight binding to boronic acids is literature known and discussed.[4] On the other hand, sucrose, without any *cis*-diol present is the weakest binding sugar. Table 2 shows the identification of blind samples. With exception of mannose and sucrose we could classify all of the tested sugars correctly. Even lactose and raffinose, similar in their structure and hardly separated using Score 1 and 2 in a canonical plot, are discriminated.

The concentration dependence for the first three elements **P1-3** of the turn-on fluorescence pattern (Figure 5), shown in a standard plot, determines quantitatively the concentration of one saccharides, similar to experiments published by Schanze et al. And we can easily quantify solutions of glucose, fructose or sucrose.

Table 2. Discrimination of Blind Samples of Different Sugars.

	Number of samples	Correctly identified	Accuracy [%]
Fructose	5	5	100
Galactose	5	4	80
Glucose	5	5	100
Lactose	5	3	60
Maltose	5	5	100
Mannose	5	3	60
Raffinose	5	4	80
Sucrose	5	5	100
Xylose	5	5	100
Total	**45**	**39**	**87**

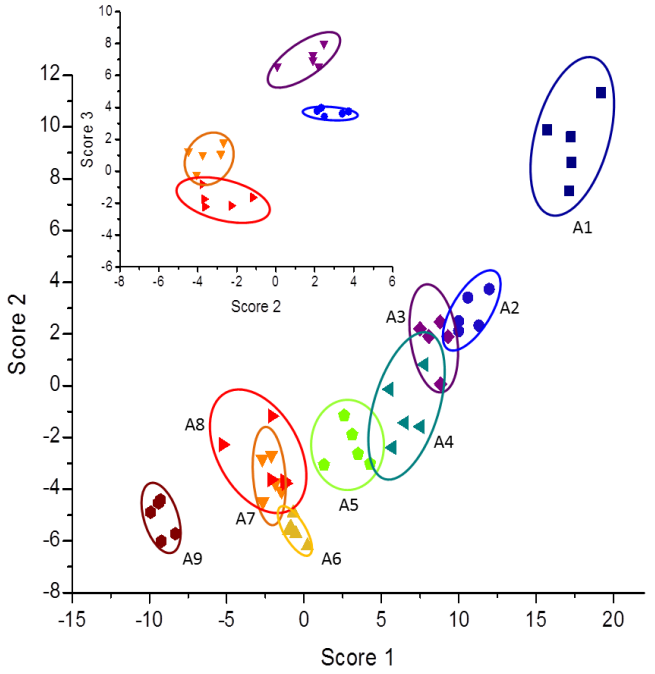

Figure 7. Canonical score plot for the first two factors of simplified fluorescence response patterns obtained with an array of **P1-9** (2.5 μM **PAE 1-3** and 25 μM **p-BV^{2+}**, **m-BV^{2+}** and **o-BV^{2+}** buffered at pH 7) treated with nine different sugars and the first and third factors factors of simplified fluorescence response patterns (inset, A7 and A8 are only represented).

But fundamentally, the discrimination of tightly related analytes by such ad-hoc sensor arrays is encouraging and attractive in its simplicity. The lack of sample preparation and the use of a simple plate reader do not hurt either to further investigate these attractive systems.

Conclusions

We have expanded Schanze's elegant saccharide assay, employing a focused nine-element library, consisting of three anionic conjugated polymers and three different boronic-acid substituted, cationic paraquat-based quencher molecules. Their combination allows for a fluorophore displacement assay, in which the differential fluorescence turn-on of the conjugated polymers upon release from the quencher gives a unique pattern that identifies and discriminates the investigated sugars. This power of discrimination is surprising for such a simple, focused system, based only on the formation of fairly similar adducts. And while the sensitivity of this assay is not as great as the dye displacement assay published by Singaram et al. for sugar polyols, the flexibility in both the viologen but also the conjugated polymer in our case should make our system attractive where limit of detection and sensitivity do not play a critical role. Therefore, in the future, this approach will be exploited to determine sugar concentrations in beverages and discriminate types of honey.

Experimental Section

We prepared the poly(*para*-aryleneethynylene)s **PAE1–PAE6** through standard Sonogashira reactions (see the Supporting Information). The benzyl viologens (BV2+) were prepared according to literature. Analytical data, UV-measurements and canonical score plots are detailed in the Supporting Information.

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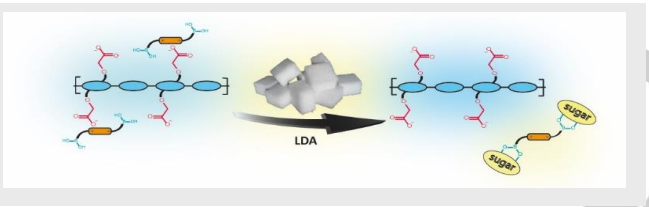
FULL PAPER

A poly(aryleneethynylene) tongue that discriminates different saccharides works through complex formation with different boronic acid substituted viologens.



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