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Selective detection of epimeric pentose saccharides at physiological pH using a fluorescent receptor

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ABSTRACT

Epimerisation between ribofuranose and arabinofuranose sugars is crucial in several biosynthetic pathways, but is typically challenging to monitor. Here, we have screened for fluorescent boronic acids that can be used as molecular probes for the specific detection of ribofuranose over arabinofuranose sugars in solution. We show excellent specificity of the fluorescent response of 3-biphenylboronic acid to ribofuranose at physiological pH. This provides a tool for in situ monitoring of carbohydrate modifying enzymes and provides a viable alternative to traditional radiolabelled assays.

Carbohydrate containing molecules have vital roles in many biological processes[1,2] ranging from cell signalling,[3–5] host–pathogen interactions[6–9] and fertilization.[10,11] The biochemical modification of carbohydrates by many different enzyme classes, including isomerases, epimerases, phosphatases, hydrolases, mutases, transferases and phosphorylases are fundamental biological processes. Despite the biological importance of carbohydrate containing compounds, molecular recognition and chemosensory detection methods for them is difficult given the lack of chromophore or fluorophore moieties, the presence of multiple stereocentres and the isomerisation between furanose and pyranose ring forms. Consequently direct detection of specific mono- or oligosaccharides in complex milieu has proven to be challenging. This has led to the development of a range of molecular probes for improved sugar detection.[12–14] Fluorescent boronic acid probes bind to 1,2- and 1,3-diol containing molecules with high affinity.[15] For monosaccharides boronic acids, in general, have greater affinity for *cis*-1,2-diols > 1,3-diol ≫ *trans*-1,2-diol with rigid *cis*-diols forming stronger cyclic esters than acyclic diols such as ethylene glycol. However, fluorescent boronic acids are usually able to bind to a range of sugar substrates with different relative binding affinities.[12,16,17] Due to the medical relevance and importance of monitoring levels of D-glucose in the blood of diabetic patients, focus has been on the development of fluorescent boronic acid probes for the specific detection of this hexose sugar with less focus on the specific detection of pentose sugars. There is only one report of a boronic acid-functionalised rhodamine derivative with selectivity for ribose and ribose derivatives.[18] However, pentose sugars conformationally locked in the furanose form are important components in a number of compounds with biological activity, including DNA, RNA and ribonucelotide cofactors such as NAD and NADP. Furthermore furanose sugars are key to the cell wall integrity and pathogenicity of many mycobacteria. Polymers of D-arabinofuranosyl residues are prevalent in the cell wall complex of mycobacteria,[19] including the human pathogen *Mycobacterium tuberculosis* the causative agent of tuberculosis (TB).[20,21] In *M. tuberculosis* D-arabinofuranose is synthesized through the epimerization of the furanose sugar D-ribose (Scheme 1).

In *M. tuberculosis* the oxidoreductase enzymes DprE1 and DprE2 catalyse this epimerization reaction and convert decaprenyl-phosphoribose (DPR) to decaprenyl-phosphoarabinose (DPA).[22] The DprE1 enzyme is the focus of current research in the TB field since it has been validated genetically and chemically as an important anti-tubercular target.[23–25] BTZ043, a 1,3-benzothiazin-4-one derivative currently in late-stage preclinical development, targets DprE1 and is extremely potent against *M. tuberculosis*.[24] However, techniques that are currently available to monitor the epimeric conversion of the *cis*-1,2-diol of DPR to the *trans*-1,2-diol present in DPA

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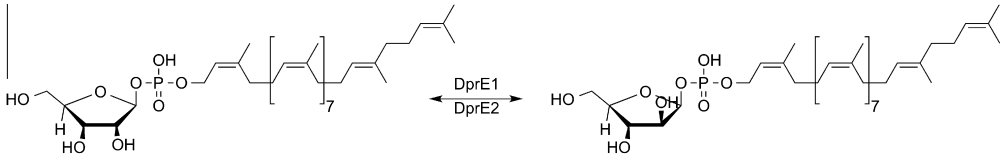


Scheme 1. Epimerisation of decaprenyl-phosphorylribose to decaprenyl-phosphorylarabinose. Conversion of decaprenyl-phosphorylribose (DPR) to decaprenyl-phosphorylarabinose (DPA) by the enzymes DprE1 and DprE2.

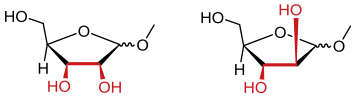
rely on chromatographic separation techniques along with the use radioactive substrates which are non-quantitative and time consuming and do not enable detailed studies of the enzymes to be undertaken.[22,24] Here, we communicate our initial efforts towards the development of a molecular probe that selectively binds and detects the *cis*-1,2-diol of ribofuranose and not the corresponding *trans*-1,2-diol epimer of arabinofuranose in a binary two-sugar system using an on–off boronic acid reporter system. The system has been developed such that the boronic acid is fluorescent: 'on', when it is not bound to the carbohydrate and the boronic acid fluorescence is quenched: 'off', only when it is bound to the *cis*-1,2-diol and not the corresponding *trans*-1,2-diol.

Here we describe and critically evaluate four commercially available fluorescent boronic acids for their ability to selectively act as molecular probes for ribofuranose sugars (*cis*-1,2-diol) in a system comprising ribofuranose and its *trans*-isomer arabinofuranose. For this purpose, we used as models for these two sugars the respective 1-methylated forms: 1-methyl β-D-ribofuranoside and 1-methyl α/β-D-arabinofuranoside (Scheme 2). This approach provides a novel method that is relevant for the direct detection of ribofuranose in the presence of its epimer arabinofuranose.

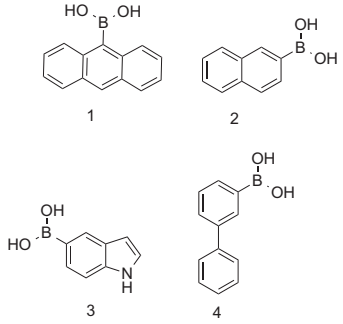
As proof of principle for our hypothesis, four fluorescent boronic acids were selected: 9-anthracene boronic acid **1**,[17] 2-napthylboronic acid **2**,[26] 5-indolylboronic acid **3**[27] and 3-biphenylboronic acid **4**[26] (Scheme 3).

The selected boronic acids have previously been reported as good fluorescent sensors with strong fluorescent intensity (*I*), a pH-dependent shift in the I_{max} and also have a significant shift of the pH-I_{max} profile to a lower pH region in the presence of the hexose sugar fructose. These probes have not been tested previously for the specific detection of the *cis*-1,2-diol of ribofuranose over a *trans*-1,2-diol in the case of arabinofuranose. In order to evaluate our hypothesis ribose and arabinose were locked as furanose conformers, by methylation of the anomeric hydroxyl group. 1-Methyl-D-arabinofuranoside (arabinofuranose) was synthesized via a standard Fischer glycosidation in acidic methanol in which the reaction was allowed to proceed under kinetic control[28] (Scheme S1, Supporting information). The resulting α/β anomers of the arabinofuranose product were not separated since the alternative configurations at this position will not be involved in the binding arabinofuranose to the fluorescent probes. 1-methyl-β-D-ribofuranoside (ribofuranose) was commercially available.

To assess the ability of the selected boronic acid probes **1**–**4** to act as fluorescent probes in biological buffer systems each boronic acid probe was examined in 0.2 M potassium phosphate buffer in the range pH 2–12 (5% v/v DMSO). Previously the pH-fluorescence intensity profile boronic acid of probes **1**–**4** had been tested in water.[17,26,27] The fluorescent excitation and emission wavelengths used, λ_{ex} and λ_{em} respectively, were taken from the reported



Scheme 2. Model ribofuranose and arabinofuranose sugars used in this study.



Scheme 3. Fluorescent boronic acids: 9-anthracence boronic acid **1**, 2-napthylboronic acid **2**, 5-indolyl boronic **3** acid, 3-biphenylboronic acid **4**.

literature values and are detailed in the experimental section.[17,26,27] In agreement with previous observations for the pH-fluorescence intensity (I_{max}) profile for the boronic acids in water, probes **2**–**4** displayed a large pH-dependent change in I_{max} in potassium phosphate buffer with fluorescence quenching occurring with an increase in pH, with little fluorescence observed at high pH (Fig. 1).

Boronic acid **1** did not display pH-fluorescence dependent quenching in phosphate buffer at high pH and was not evaluated further.

Subsequently the three remaining boronic acids **2**–**4** were evaluated for their ability to specifically detect *cis*-1,2-diols over *trans*-1,2-diols by the addition of either 250 mM ribofuranose or 250 mM arabinofuranose to the boronic acid in potassium phosphate buffer pH 2–12 and the fluorescent intensity versus pH profile measured. The pH-I_{max} profile in the presence of sugars must shift to a lower pH region in order for the sensitive detection of sugars at constant pH.

Boronic acid **2** displayed slight specificity for the *cis*-1,2-diol of ribofuranose compared to the *trans*-1,2-diol of arabinofuranose (Fig. 2).

A greater pK_a shift of the fluorescence-pH profile of **2** is observed when 250 mM ribofuranose is added to probe **2** compared to the

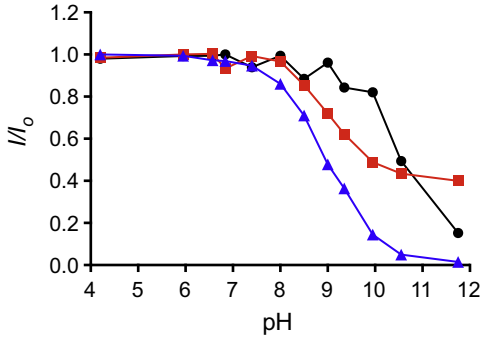


Figure 1. Fluorescent intensity versus pH of **2** (red ■), **3** (black ●), **4** (blue ▲) at varying pH. (**2**, 5 μM, λ_{ex} = 268 nm, λ_{em} = 344 nm; **3**, 50 μM, λ_{ex} = 290 nm, λ_{em} = 361 nm; **4**, 50 μM, λ_{ex} = 230 nm, λ_{em} = 340 nm; 50 mM potassium phosphate buffer). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)