Glucose selective bis-boronic acid click-fluor†‡

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Four novel bis-boronic acid compounds were synthesised via copper catalysed azide–alkyne cycloaddition (CuAAC) reactions. Glucose selectivity was observed for a particular structural motif. Moreover, a new glucose selective fluorescent sensor was designed and synthesised as a result.

In the past few decades, synthetic probes have shown significant promise for real-time and accurate detection of biomolecules. 1 Much attention has been devoted to boronic acid derivatives for saccharide detection. 2 Although boronic acid-mediated saccharide sensing showed encouraging results, a lack of selectivity for higher order saccharides needs to be addressed, and remains challenging. Phenylboronic acid derivatives have greater affinity for fructose over glucose, under physiological conditions. 3 For the purpose of increasing the binding affinity to glucose, and other saccharides, a more sophisticated receptor structure is required.

James et al. were the first to report that two appropriately positioned boronic acids could modulate selectivity by two-point binding interactions with glucose (sensor 1, Fig. 1). 4 Further studies conducted by James et al. demonstrated that design of the spacer unit between the borons, of a bis-boronic acid, was crucial for glucose recognition (sensor 2, Fig. 1). 5 Drueckhammer et al. showed that the distance between two p-tolylboronic acids could be optimised, for glucose selectivity, through a computational study, and a rigid four-fused ring scaffold was produced (sensor 3, Fig. 1). 6 Thus, appropriate positioning of boronic acids can give glucose selectivity. However, application of a universally simple methodology to selective saccharide receptor design remains a challenge. 7

Copper catalysed azide–alkyne cycloaddition (CuAAC) reaction, often referred to as the ‘‘click reaction,’’ 8 has been employed to synthesise novel boronic acid derivatives for various applications. In order to explore the advantages of the CuAAC reaction in the area of saccharide sensing, 9 Scrafton et al. employed the CuAAC reaction for a five-step synthesis of a boronic acid-based sensor molecule (Scheme 1). 10 The term ‘‘clickfluor’’ was used to refer to this class of molecular sensors. Recently, the scope has been elaborated to include incorporation of fluorophores. 11 Although these studies demonstrated the possibility of rapid synthesis of boronic acids via the CuAAC reaction, the reported mono-boronic acids cannot serve as glucose-selective sensors due to their relatively weak glucose binding. In order to engineer selectivity, CuAAC may be employed to construct bis-boronic acids. Wang and co-workers reported two triazole-linked bis-α-amidoboronic acids. 12 Their result showed significantly enhanced binding affinity for oligosaccharides. Zhao et al. also designed and synthesised three bis-boronic acid sensors through triazole formation. 13 However, in their studies, the receptors preferentially recognise α-fructose over α-glucose.

In this report, three bis-boronic acid molecules were designed such that the CuAAC reaction may be used to rapidly construct a series. Their synthesis is studied and saccharide
binding evaluated, for the purpose of developing a novel multi-
boronic acid “click” platform. As such, we built upon the
knowledge that glucose selectivity may be achieved by correctly
spacing two boronic acids in one molecule, to demonstrate
“click-compatibility” for selectivity, and pave the way for future
exploration in higher order saccharide sensor design.

Three regio-isomeric bis-boronic acids (8a–c), akin to the
mono-boronic acid click-fluors already reported, were designed
and their synthesis embarked upon (see Scheme 2). Following
pinacol protection of commercially available o-tolylboronic
acid, organic azide 5 was synthesised on a 10 gram scale, by
bromination and displacement of bromide by azide, according
to literature procedures (92% yield of compound 5).11 Three
bis-alkynes were required; fortunately 1,3- and 1,4-diethynyl-
benzene are commercially available and were used as purchased.
1,2-Diethynylbenzene was readily synthesised from 1,2-dibromo-
benzene via a palladium-catalysed Sonogashira coupling and
TMS removal following literature procedures.14 Initially, the
CuAAC reaction was conducted as per our previous reports,
but poor yields of the target bis-boronic esters (7a–c), as a result
of unwanted side reactions, plagued our experiments.15 For
example, during the synthesis of compound 7b, oxidation and
deborylation reactions occurred on one or both of the boronic
esters (confirmed by mass spectrometry). Therefore, the conditions
of the CuAAC reaction were further modified. More mild
conditions, use of TBTA as a ligand for copper and lessening
of catalyst loading helped improve the reaction outcomes and
minimize (copper-catalysed) de-borylation.16 Thus, the yields of
these three key intermediates were improved from 32% to 68%
(7a), 21% to 52% (7b), and 18% to 33% (7c), respectively. Next,
pinacol was removed by addition of compound 4 under acidic
conditions; note that this gives a by-product, pinacol protected-4,
which may be (and was) used in further syntheses.13a Bis-boronic
acids 8a–c were obtained after trituration and flash chromato-
graphy in 72–88% isolated yield.

The bis-pinacol esters, 7a–c, are crystalline solids, and crystals
suitable for single crystal XRD structure determination were
grown from mixtures of hexane and ethyl acetate. From the
obtained structures presented in Fig. 2, the distance between
Table 1  Binding constants of compounds 8a–c and fructose and glucose calculated according to ITC data

<table>
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<th>8a</th>
<th>8b</th>
<th>8c</th>
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<tbody>
<tr>
<td>Binding site (fructose)</td>
<td>1.89 ± 0.280</td>
<td>0.48 ± 0.005</td>
<td>1.30 ± 0.046</td>
</tr>
<tr>
<td>Binding constant (M⁻¹) (fructose)</td>
<td>1.90 × 10⁷ ± 280</td>
<td>1.30 × 10⁷ ± 1.10 × 10⁸</td>
<td>2.95 × 10⁷ ± 183</td>
</tr>
<tr>
<td>Binding site (glucose)</td>
<td>N/A</td>
<td>0.45 ± 0.081</td>
<td>1.33 ± 0.101</td>
</tr>
<tr>
<td>Binding constant (M⁻¹) (fructose)</td>
<td>N/A</td>
<td>5.03 × 10⁴ ± 479</td>
<td>6.19 × 10⁴ ± 731</td>
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In Fig. 4, the fluorescence response of sensor 14 towards fructose and glucose is shown, respectively. It was observed that the fluorescence intensity was quite weak for sensor 14, as the Raman scattering signal of the excitation source was also recorded on the spectrum. Upon addition of 5 mM fructose, the fluorescence signal was slightly enhanced (I/I₀ = 1.13, see Fig. 4a), which was similar to what we have observed with mono-boronic acid sensor binding with fructose in our previous study.¹¹

However, the fluorescence intensity gradually decreased upon addition of 5 mM glucose (I/I₀ = 0.79, see Fig. 4b and c). Presumably, the different fluorescence responses towards fructose and glucose were caused by the differing binding modes.
Meanwhile, it took no less than 30 minutes for the fluorescence signal to be stabilised after adding glucose, which agrees with the slow binding process in our ITC studies.

Three regioisomeric bis-boronic acids were synthesised using the CuAAC reaction. The binding constants of the synthesised compounds with fructose and glucose were measured by ITC experiment, respectively. It was found that selectivity for glucose binding is modulated by the distance between the two boronic acid groups.

To our delight, compound 8c presented higher binding affinity towards glucose over fructose. Moreover, a fluorescent receptor, 14, was synthesised showing divergent properties upon interaction with glucose versus fructose. It was shown that compound 14 can serve as an ‘on-off’ fluorescence sensor for selective glucose detection. More studies are required to better understand the fluorescence modulation mechanism, which is an ongoing work in our laboratory. We showed the utility of combining different functional components using the CuAAC reaction to construct selective molecular receptors for more challenging targets.

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Notes and references

17 See ESI for atom labels and further details and corresponding CIF files. CCDC deposition numbers 1510897–1510899. (i) Symmetry code for the generation of equivalent atoms: $x, -y, z$; (ii) symmetry code for the generation of equivalent atoms: $-x, y, 1/2 - z$.