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# Probing disaccharide selectivity with modular fluorescent sensors

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**Abstract**—Six modular photoinduced electron transfer (PET) sensors bearing two phenylboronic acid receptors have been evaluated as fluorescent disaccharide sensors. The length of linker separating the two boronic acid moieties was varied and the sensors' interaction with disaccharides assessed via fluorescence spectroscopy. It was shown that saccharide selectivity was influenced by the choice of linker length. Diboronic acid sensors $\mathbf{3}_n$ also displayed significant specificity for the disaccharides linked to the carbon on the 3rd or 6th position (as numbered from the anomeric centre) over those linked at the 4th position.

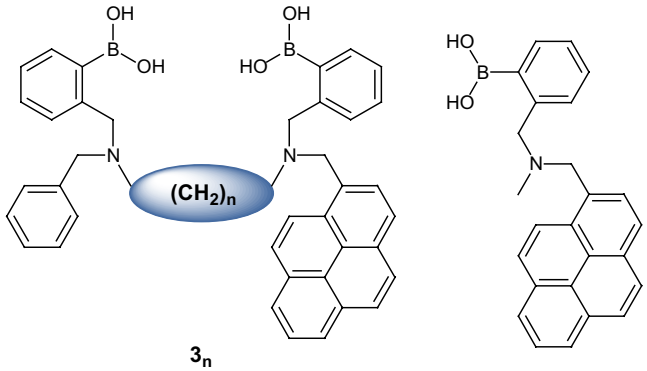

Saccharides are of great importance in biological systems.[1] It is therefore unsurprising that receptors with the capacity to detect chosen saccharides selectively and signal this presence by altering their optical signature have attracted considerable interest in recent years.[2–5]

While many synthetic receptors utilise hydrogen bonding interactions in aprotic media, this approach has not proved viable for saccharides in aqueous solvents. Boronic acids however, have been established as effective receptors for saccharides, circumventing the problem of solvent competition by utilising covalent interactions.[6] The introduction of a tertiary amine proximal to the boronic acid allows photoinduced electron transfer (PET) based systems to be developed, designing 'Off–On' functionality into the sensors.[2–5]

Over the last few years we have been involved in the development of sensors with increased selectivity for saccharides.[2] We have adopted a modular approach for the construction of our sensors. The sensors consist of three components; receptor units, linker units and 'read-out' units. This approach can be illustrated by describing the D-glucose selective fluorescent sensor $\mathbf{3}_6$, which contains two boronic acid units (receptors), a hexamethylene unit (linker) and a pyrene unit (fluorophore-'read-out'). Sensor $\mathbf{3}_6$ contains two boronic acid units because only through two point binding can saccharide selectivity be controlled. Sensor $\mathbf{3}_6$ with a hexamethylene linker unit displays enhanced D-glucose selectivity, whilst systems with longer linker units display enhanced selectivity for D-galactose.[7,8] The fluorescent pyrene 'read-out' unit has also been shown to enhance D-glucose selectivity whereas naphthalene fluorophores provide D-galactose selectivity.[9]

When compared to monosaccharide receptors only a small number of synthetic receptors for disaccharides[10–14] and oligosaccharides[15–22] currently exist. One reason is the problem associated with positioning the two binding sites to create a selective receptor for these larger and more flexible saccharides.

With this work we decided to probe the effect of the linker length on selected disaccharides. The synthesis of PET sensors $\mathbf{3}_n$ and the reference compound **4** have been previously reported.[8]




$\mathbf{3}_n$
n = 3 - 8

**4**

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The fluorescence titrations of $\mathbf{3_n}$ and **4** ($1.0\times10^{-7}$ mol dm$^{-3}$, $\lambda_{ex} = 342$ nm) with different saccharides, were carried out in aqueous methanolic buffer solution [52.1 wt% methanol at pH 8.21 (KCl, 0.01000 mol dm$^{-3}$; $KH_2PO_4$, 0.002752 mol dm$^{-3}$; $Na_2HPO_4$, 0.002757 mol dm$^{-3}$)].[23] The fluorescence intensity of $\mathbf{3_n}$ and **4** increased with increasing saccharide concentration. The stability constants ($K$) of PET sensors $\mathbf{3_n}$ and **4** were calculated by fitting the emission intensity at 397 nm versus concentration of saccharide curves. Fluorescence enhancements were calculated by the same method. Where the emission intensity at 397 nm versus concentration of saccharide curves failed to describe a distinct plateau the maximum observed fluorescence enhancements were reported. Stability constants $K$ and fluorescence enhancements are given in Tables 1 and 2. The stability constants $K$ of the diboronic acid sensors $\mathbf{3_n}$ and **4** with saccharides are displayed in Figures 1 and 3.

It has been previously shown from NMR and fluorescence studies that phenylboronic acids have a strong preference to bind with the hydroxyls of saccharides in their furanose forms.[13, 24–27]

Monosaccharides and many disaccharides can isomerise between their pyranose and furanose forms in aqueous solvents. The aqueous methanolic buffer used for the fluorescence runs allows for this isomerisation. As shown in Figures 2 and 4, D-melibiose (α-D-Gal*p*-(1→6)-D-Glc) and D-lactulose (β-D-Gal*p*-(1→4)-D-Fru) can interconvert between their pyranose and furanose forms. By comparison D-maltose (α-D-Glc*p*-

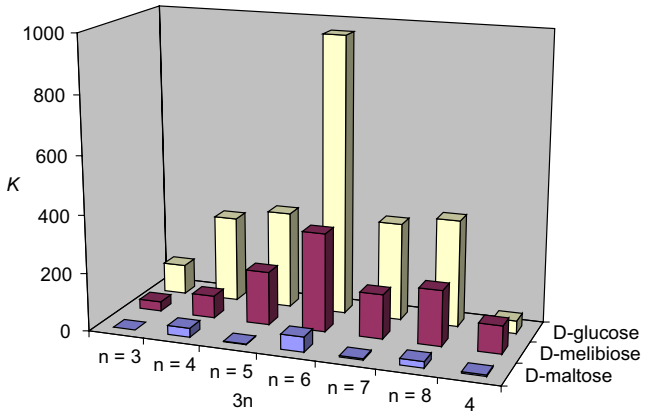


**Figure 1.** Stability constants $K$ of $\mathbf{3_n}$ and **4** with D-glucose and its derivatives.

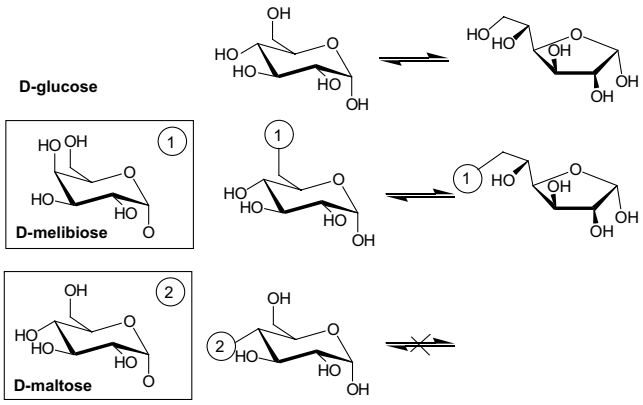


**Figure 2.** Structure of D-glucose and its derivatives.

**Table 1.** Stability constant $K$ (determination of coefficient; $r^2$) and fluorescence enhancement for the monosaccharide complexes of molecular sensors $\mathbf{3_n}$ and **4**. The $K$ valves were analysed in KaleidaGraph using nonlinear (Levenberg–Marquardt algorithm) curve fitting. The errors reported are the standard errors obtained from the best fit.

| Sensor | D-Glucose | | D-Galactose | | D-Fructose | | D-Mannose | |
|---|---|---|---|---|---|---|---|---|
| | $K$ (dm$^3$ mol$^{-1}$) | Fluorescence enhancement | $K$ (dm$^3$ mol$^{-1}$) | Fluorescence enhancement | $K$ (dm$^3$ mol$^{-1}$) | Fluorescence enhancement | $K$ (dm$^3$ mol$^{-1}$) | Fluorescence enhancement |
| $\mathbf{3_3}$ | 103 ± 3 (1.00) | 3.9 | 119 ± 5 (1.00) | 3.5 | 95 ± 9 (0.99) | 3.6 | 45 ± 4 (1.00) | 2.7 |
| $\mathbf{3_4}$ | 295 ± 11 (1.00) | 3.3 | 222 ± 17 (1.00) | 3.7 | 266 ± 28 (0.99) | 4.2 | 39 ± 1 (1.00) | 3.4 |
| $\mathbf{3_5}$ | 333 ± 27 (1.00) | 3.4 | 177 ± 15 (1.00) | 3.0 | 433 ± 19 (1.00) | 3.4 | 48 ± 2 (1.00) | 3.0 |
| $\mathbf{3_6}$ | 962 ± 70 (0.99) | 2.8 | 657 ± 39 (1.00) | 3.1 | 784 ± 44 (1.00) | 3.2 | 74 ± 3 (1.00) | 2.8 |
| $\mathbf{3_7}$ | 336 ± 30 (0.98) | 3.0 | 542 ± 41 (0.99) | 2.9 | 722 ± 37 (1.00) | 3.3 | 70 ± 5 (1.00) | 2.7 |
| $\mathbf{3_8}$ | 368 ± 21 (1.00) | 2.3 | 562 ± 56 (0.99) | 2.3 | 594 ± 56 (0.99) | 2.3 | 82 ± 3 (1.00) | 2.2 |
| **4** | 44 ± 3 (1.00) | 4.5 | 51 ± 2 (1.00) | 4.2 | 395 ± 11 (1.00) | 3.6 | 36 ± 1 (1.00) | 3.7 |

**Table 2.** Stability constant $K$ (determination of coefficient; $r^2$) and fluorescence enhancement for the disaccharide complexes of molecular sensors $\mathbf{3_n}$ and **4**. The $K$ valves were analysed in KaleidaGraph using nonlinear (Levenberg–Marquardt algorithm) curve fitting. The errors reported are the standard errors obtained from the best fit.

| Sensor | D-Melibiose | | D-Maltose | | D-Lactulose | | D-Leucrose | |
|---|---|---|---|---|---|---|---|---|
| | $K$ (dm$^3$ mol$^{-1}$) | Fluorescence enhancement | $K$ (dm$^3$ mol$^{-1}$) | Fluorescence enhancement | $K$ (dm$^3$ mol$^{-1}$) | Fluorescence enhancement | $K$ (dm$^3$ mol$^{-1}$) | Fluorescence enhancement |
| $\mathbf{3_3}$ | 33 ± 7 (0.98) | 2.4 | 0 ± 2 (0.96) | 1.5[a] | 126 ± 14 (0.99) | 3.5 | 29 ± 3 (0.99) | 2.5[a] |
| $\mathbf{3_4}$ | 77 ± 9 (0.99) | 4.9[a] | 31 ± 7 (0.98) | 3.5[a] | 477 ± 92 (0.95) | 4.5 | 35 ± 2 (1.00) | 3.4[a] |
| $\mathbf{3_5}$ | 184 ± 11 (1.00) | 3.8 | 2 ± 1 (0.99) | 2.1[a] | 616 ± 114 (0.97) | 5.0 | 41 ± 2 (1.00) | 3.5[a] |
| $\mathbf{3_6}$ | 339 ± 17 (1.00) | 2.9 | 52 ± 14 (0.98) | 2.3 | 595 ± 30 (1.00) | 3.2 | 69 ± 2 (1.00) | 2.9[a] |
| $\mathbf{3_7}$ | 153 ± 4 (1.00) | 3.3 | 5 ± 1 (1.00) | 2.0[a] | 493 ± 28 (1.00) | 3.3 | 21 ± 5 (0.97) | 3.9[a] |
| $\mathbf{3_8}$ | 192 ± 30 (0.98) | 2.9 | 22 ± 4 (0.98) | 2.4[a] | 528 ± 29 (1.00) | 2.6 | 72 ± 5 (1.00) | 2.7[a] |
| **4** | 96 ± 5 (1.00) | 3.9 | 5 ± 1 (0.99) | 2.1[a] | 473 ± 10 (1.00) | 3.7 | 58 ± 3 (1.00) | 3.7 |

[a] Maximum observed fluorescence enhancement.

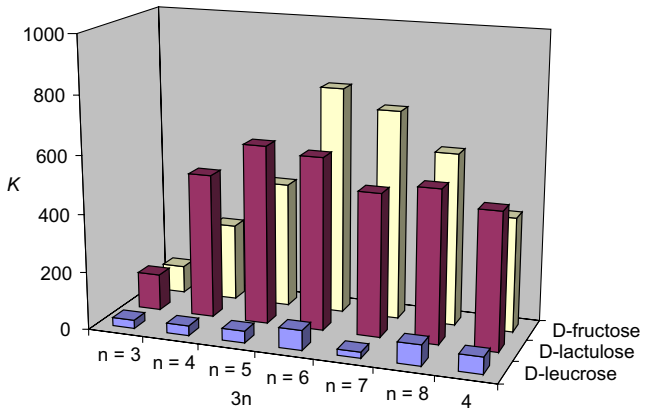


**Figure 3.** Stability constants $K$ of $\mathbf{3_n}$ and **4** with D-fructose and its derivatives.

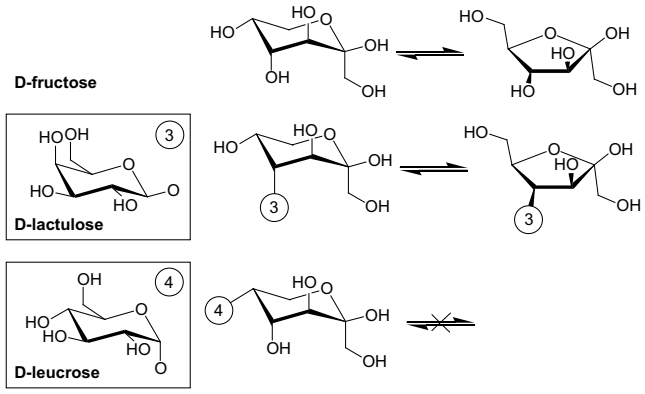


**Figure 4.** Structure of D-fructose and its derivatives.

(1 → 4)-D-Glc) and D-leucrose (α-D-Glc*p*-(1 → 5)-D-Fru) are disaccharides linked to the 4th carbon (as numbered from the anomeric centre) and as such they are conformationally fixed as pyranosides.[28]

The stability constants $K$ for $\mathbf{3_n}$ with D-fructose and D-lactulose are at most double that of the reference compound **4**, indicating that the saccharides are binding to $\mathbf{3_n}$ in a 2:1 ratio, forming an acyclic structure.

The relative increase in stability constant $K$ for $\mathbf{3_n}$ on binding to D-glucose and D-melibiose are larger (increases in stability of up to 22 times and 3.5 times that of the reference compound were observed for D-glucose and D-melibiose, respectively). This increase is due to binding occurring in a 1:1 ratio and the formation of a stable cyclic structure.[2]

The selectivity trend displayed by $\mathbf{3_n}$ towards D-melibiose mirrors that of D-glucose, which implies that $\mathbf{3_n}$ is forming stable cyclic structures with the furanose ring segment of these saccharides. This phenomenon is well known for D-glucose[24–27] but this is the first time this binding motif has been documented for disaccharides.

It is also apparent in Figures 1 and 3 that whilst effective binding can be observed for the disaccharides linked to the carbon on the 6th or 3rd position (as numbered from the anomeric centre), D-melibiose and D-lactulose, respectively, the binding constants for the disaccharides linked to the carbon on the 4th position, D-maltose and D-leucrose, are very small indeed.

Our results indicate that binding with diboronic acid receptors occurs preferentially with disaccharides, that can isomerise between their pyranose and furanose conformations. D-Melibiose, for instance, can isomerise into a glucofuranose form and displays enhanced binding with the D-glucose selective sensor $\mathbf{3_6}$.

The ability not only to tune for specific terminal monosaccharides appended to larger carbohydrates but also to distinguish between them by their linker position could prove immensely useful in the field of glycobiology.[1]

Boronic acids provide us with the potential to further develop receptors of low molecular mass for biological systems by taking advantage of the covalent bond formation between receptors and their guests. The results above indicate that when designing diboronic acid receptors for oligosaccharides one must consider not just the oligosaccharide as a whole but specifically the individual saccharide fragments within it and the connectivity between them.

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