Specific Recognition of Disaccharides by *trans*-3,3′-Stilbenediboronic Acid: Rigidification and Fluorescence Enhancement of the Stilbene Skeleton upon Formation of a Sugar–Stilbene Macrocycle

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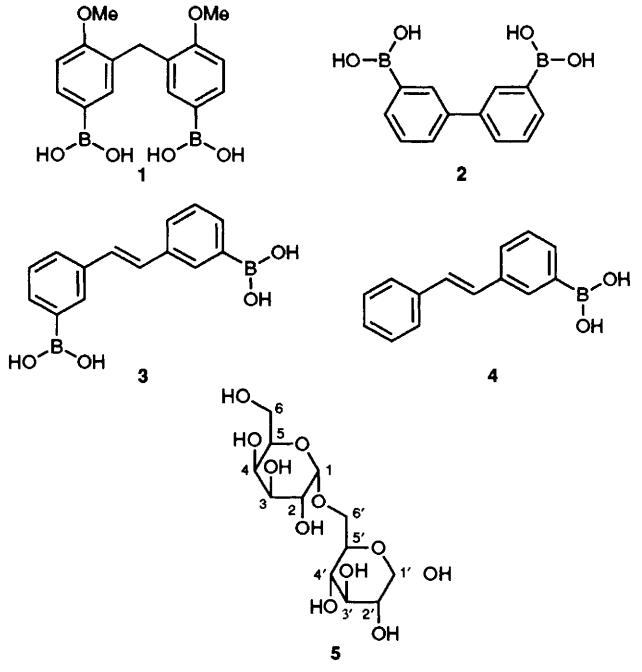
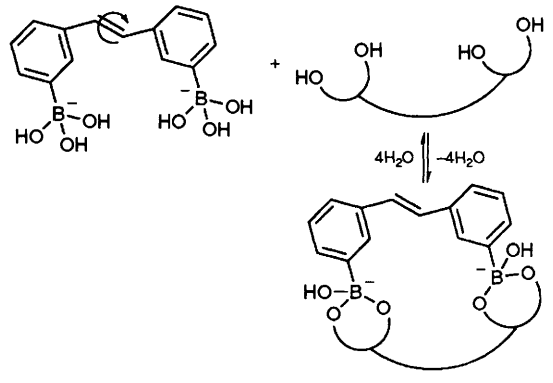
The molecular sensor *trans*-3,3′-stilbenediboronic acid **3** gave enhanced fluorescence in the presence of disaccharides whereas no response was found for monosaccharides.

Molecular recognition of neutral and ionic species by synthetic receptors has been the facination of many chemists for the last few decades. In most reported synthetic receptors hydrogen-bonding interactions play a central role.[1] It is shown, however, that the hydrogen-bonding interactions are effective in aprotic solvents and less effective for recognition of guests soluble only in aqueous solutions. We are currently investigating the recognition of saccharides which are only soluble in aqueous media. Covalent interactions between saccharide and boronic acid have been utilized for sugar recognition in affinity chromatography by Wulff *et al.*[2] Recently, Shinkai and coworkers.[3] reported the formation of rigid, cyclic complexes of diboronic acids **1** and **2** with mono- and di-saccharides. The induced chirality upon formation of rigid, chiral complexes was monitored by CD spectroscopy. Yoon and Czarnic[4] also reported the fluorescence suppression of anthrylboronic acid in the presence of saccharides.

Rigidification of the diboronic acid skeleton upon formation of macrocycles with either mono- or di-saccharides[3] with induced chirality has been detected by CD spectroscopy. This rigidification process could be utilized in the design of spectroscopic sensors. The main path of nonradiative deactivation of the lowest excited singlet state of stilbene is known to be *via* rotation of the ethylenic double bond.[5] Inhibited bond rotation, followed by enhanced fluorescence emission has been reported for stilbenes in solid matrices,[5] viscous solvents[6] and cyclodextrin inclusion complexes.[7] We now have rationally designed and studied the first known fluorescence sensor for disaccharides based on molecular rigidification of the stilbene skeleton upon saccharide binding.

Fluorescence of stilbene-3,3′-diboronic acid **3**† increases upon binding to disaccharides in basic aqueous media. Fig. 1 shows the fluorescence intensity and the saccharide concentration profiles for **3**. Large fluorescence increases were observed specifically for the disaccharide D-(+)-melibiose in basic aqueous media compared to small increases observed for monosaccharides [D-(+)-glucose, D-(+)-mannose and D-(−)-arabinose] (Fig. 1). This fluorescence increase was attributed to the formation of a cyclic complex of diboronic acid with the disaccharide and subsequent freezing of ethylenic bond rotation in the excited state (Scheme 1).‡ Existence of such a cyclic complex was supported by the CD (circular dichroism) activity of the complex under the same conditions used for fluorescence measurements. This was further corroborated by mass spectroscopy: the molecular ion of the cyclic complex was detected by SIMS (secondary ionisation mass spectroscopy) (M^+, 538). The monoboronic acid analogue of **3**, **4** gave neither CD activity with any saccharides nor fluorescence enhancements supporting the suggested cyclic structure of complexation.





Scheme 1

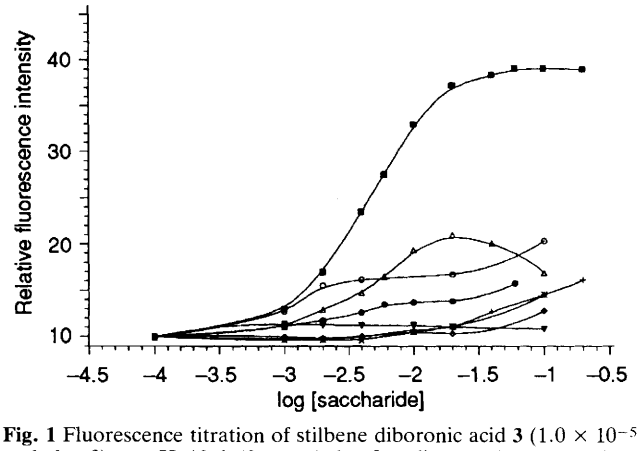
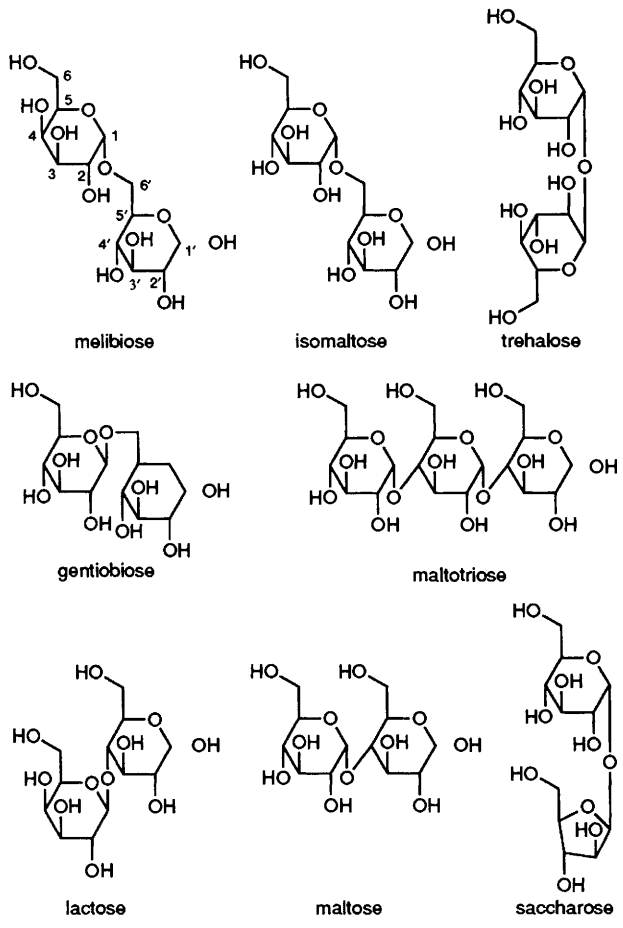


Fig. 1 Fluorescence titration of stilbene diboronic acid **3** (1.0×10^{-5} mol dm^{-3}) at pH 10.6 (0.1 mol dm^{-3} sodium carbonate–sodium hydrogencarbonate buffer) as a function of log of disaccharide concentration (excitation 310 nm, emission 358 nm): (○) maltotriose, (▼) trehalose, (◇) saccharose, (X) maltose, (l) lactose, (△) gentiobiose, (●) isomaltose, (■) melibiose



It has been established that phenylboronic acid binding to monosaccharides in basic aqueous media occurs primarily at 1,2-diols and secondary binding at 4,6-diols creating five- and six-membered rings respectively.[3] The hydroxy group at the 3 position does not participate in complexation as proved by complexation of boronic acid with glucose derivatives.[3] Diboronic acids **1** and **2** formed cyclic, CD active complexes with saccharides. It was shown that the cyclic complex formed in aqueous media with saccharides employs the same binding sites as the 1 : 2 complex formed with monoboronic acid[3] *i.e.* 1,2- and 4,6-diols participate in complexation creating a cyclic complex and the 3-hydroxy group plays no part in the complexation process. D-(+)-melibiose **5**, which has 4,6-*cis*- and possible 1′,2′-*cis*-diols in aqueous media, gave the best fluorescence enhancement whereas disacharide gentiobiose, which has 4,6-*trans* and 1′,2′-*cis*-diols, gave somewhat less fluorescence enhancements. Gentiobiose suppressed the fluorescence at high concentrations possibly due to competitive 1 : 2 complex formation. Isomaltose which has a 1,6′-ether link between sugar monomers, as do melibiose and gentiobiose gave small fluorescence enhancements. It seems that among these three disaccharides, which have 1,6′-ether links between sugar monomers, D-(+)-melibiose has the best fit for the diboronic acid receptor. Maltose, which has a 1,4′-ether link between saccharide monomers did not give any fluorescence enhancement although, interestingly, maltotriose which has an extended monomer unit gave some fluorescence enhancement. Other disaccharides studied which have shorter distances between prospective diol groups gave no fluorescence enhancements. This suggests the importance of the length of the disaccharide. The small fluorescence enhancements observed for **3** with monosaccharides and **4** with mono- and di-saccharides at high concentrations are believed to be due to the formation of 1 : 2 complexes with saccharides and restricted rotation induced by solvent interactions. It is known that increase in viscosity of the solvent and bulky substituents on the phenyl rings enhance the fluorescence quantum yield of stilbene.[6]

In summary, we report a new class of artificial receptors which respond to sugar substrates with two binding sites. We believe that spectroscopic changes stemming from molecular rigidification and upon specific saccharide binding are useful in the sensing of saccharides and in the prediction of their absolute configuration.

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Footnotes

† Compounds **3** and **4** were synthesized from *trans*-3,3′-dibromostilbene and *trans*-3-bromostilbene which were synthesized using standard Wittig reaction conditions. *trans*-3,3′-Dibromostilbene and *trans*-3-bromostilbene were lithiated using *n*-butylithium in tetrahydrofuran followed by reaction with excess of trimethoxyborane and hydrolysis in dilute HCl. All new compounds gave satisfactory NMR, microanalysis and mass spectal data.

‡ The plot of relative fluorescence intensity *vs.* saccharide concentration could not be analysed precisely by a simple Benesi–Hilderbrand type equation assuming the formation of a 1 : 1 complex.

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