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Benzoboroxoles as Efficient Glycopyranoside-Binding Agents in Physiological Conditions: Structure and Selectivity of Complex Formation

Marie Bérubé, Meenakshi Dowlut, and Dennis G. Hall*

Department of Chemistry, University of Alberta, Edmonton, Alberta T6G 2G2, Canada

dennis.hall@ualberta.ca

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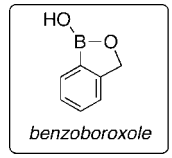

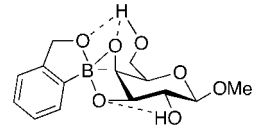

In contrast to normal boronic acids, *o*-hydroxymethyl phenylboronic acid (benzoboroxole) has the capability of complexing glycopyranosides efficiently in neutral water. The measurement of association constants with a panel of model hexopyranosides indicates that the preferred mode of binding is through a *cis*-3,4-diol, such as that found in galactopyranosides, and mass spectrometric studies support a 1:1 binding stoichiometry. The complexation of glucopyranosides is weaker, and they are bound through their 4,6-diol unit. Although several factors may explain the exceptional carbohydrate-binding behavior of this class of hemiboronic acids, the relatively high Lewis acidity of benzoboroxoles is a likely contributing factor along with subtle factors such as intramolecular hydrogen bonds with other hydroxyl groups in the resulting anionic complex. These results with hexopyranosides suggest that biologically relevant cell-surface oligosaccharides could be targeted in water using oligomeric benzoboroxole receptors.

Introduction

The selective recognition of natural biopolymers by small molecules has been captivating organic chemists for several decades. High levels of efficiency and selectivity have been attained in targeting two of the three major biopolymers: polypeptides and oligonucleic acids. There has been much less success in targeting oligosaccharides. Although a number of synthetic receptors have been described for the recognition of complex carbohydrates in organic solvents,[1] it is notoriously difficult to achieve the same success under physiological conditions (i.e., water, at neutral pH).[2] The essence of the problem lies in large part with the competition between the multiple hydroxyl groups on the carbohydrates and the overwhelming ones from the bulk solvent, water. The challenge of aqueous carbohydrate recognition presents several exciting opportunities in chemical biology and medicine. For example, the development of a selective and noninvasive molecular sensor for monitoring blood glucose has long been sought as a key component of insulin-releasing implants for diabetes patients.[3] Other potential applications include the sensing, transport, and purification of complex carbohydrates. Any approach to the recognition of carbohydrates in water should take advantage of the intrinsic geometrical orientation of the sugar's hydroxyl groups on the rigid oxacarbocyclic skeleton. In this regard, boronic acids have the ability to form boronic esters reversibly with polyols and sugars in water (eq 1, Figure 1).[4,5] While the

* To whom correspondence should be addressed. Tel: 780-492-3141. Fax: 780-492-8231.

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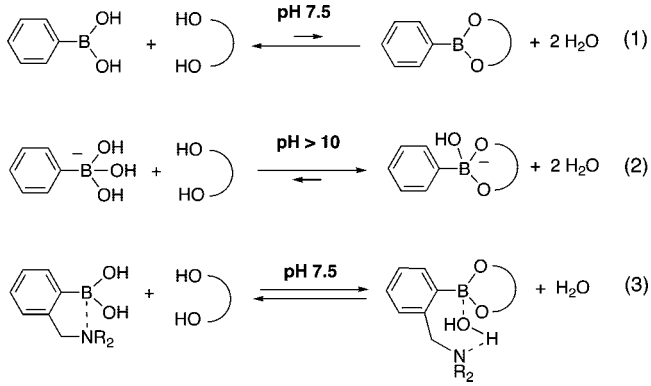

FIGURE 1. Complexation between arylboronic acids and diols in water.

use of boronic acids is regarded as one of the most promising approaches for the recognition of carbohydrate derivatives in water,[6] it is not without limitations. First and foremost, a high pH is generally required in order to favor the equilibrium toward the dialkoxyboronate anion (eq 2). By providing and stabilizing dialkoxyboronate anions at a lower pH, the "Wulff-type" *o*-dialkylaminomethyl arylboronic acids[7] (eq 3) have long stood as the established standard for the recognition of simple reducing sugars like glucose, fructose, etc. Their exact mode of complexation was recently corrected to that of a hydrolysis or "water-insertion" mechanism as opposed to a direct B−N coordination.[8] By virtue of the presence of basic and acidic functionalities, Wulff-type boronic acids are amphoteric, and as such, they tend to have a limited solubility in aqueous solutions.[9]

More importantly, aside from the binding of sialic acid,[10] no boronic acid unit has yet been demonstrated to bind effectively to nonreducing six-membered monosaccharides; hexopyranosides, which account for the large majority of biologically important oligosaccharides found in the form of cell-surface glycoconjugates. Although phenylboronic acid was shown to slowly transport glycosides from neutral water across a liquid dichloroethane membrane, no binding constants were measured.[11] With regards to reducing sugars, elegant studies by Norrild and co-workers have confirmed that glucose binds to boronic acids in water in its weakly populated furanose form, i.e., not in its pyranose form (Figure 2).[12,13] Studies from our

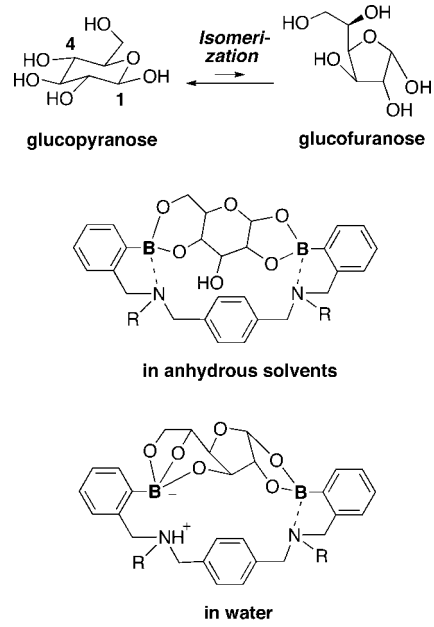

FIGURE 2. Complexation of glucose by diboronic acid receptors.[12]

group[14] and others[15] have emphasized the existence of similar requirements for disaccharides. This behavior is generally ascribed to geometrical preferences in the resulting boronate complexes. More precisely, rigid and coplanar vicinal diols such as the syn 1,2-diols of furanoses are strongly preferred because they minimize angle strain in the resulting boronic ester. The formation of a coplanar boronate with the noncoplanar (gauche, or trans) vicinal diols of a hexopyranose induces an unfavorable conformational change to the puckered sugar ring.[16] To occur, boronate formation with trans diols may even involve the formation of an expanded 7-membered boronic anhydride with two molecules of the boronic acid.[16b]

As part of our efforts aimed at the challenging problem of aqueous carbohydrate recognition, we were concerned with the need for improved boronic acids that would be capable of binding to hexopyranosides under physiological conditions. In this context, we recently reported that *o*-hydroxyalkyl arylboronic acids such as the simple benzoboroxole **1m** (Figure 3) bind to monosaccharides like glucose and fructose with higher affinity than "Wulff-type" boronic acids in neutral water, and show a better solubility profile.[17] Moreover, we demonstrated the unprecedented ability of this "forgotten" class of boronic acids toward complexing nonreducing glycopyranosides.

Since the publication of our preliminary report, the potential usefulness of benzoboroxoles in the complexation of biological diols in physiological conditions has been documented by others. Benner and co-workers have employed benzoboroxole as a mass spectrometric reagent to analyze products from the formose reaction, a possible process for the prebiotic generation of small

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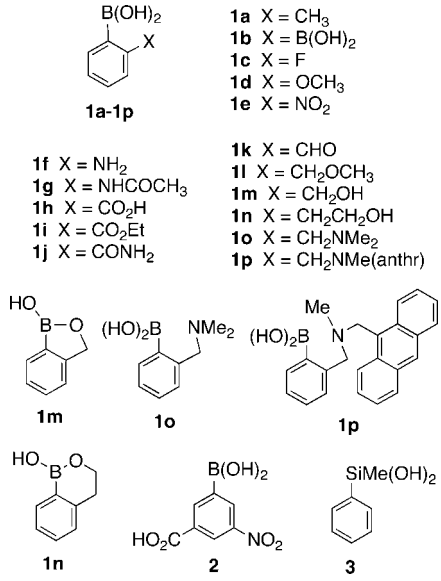

FIGURE 3. Selection of ortho-substituted arylboronic acids and other compounds evaluated for sugar complexation.

carbohydrates.[18] Hindsgaul and co-workers have used colored benzoboroxole conjugates as analytical agents in a novel approach to identify carbohydrates in the field of glycomics.[19] Tung and co-workers have employed benzoboroxole as a component of a promising sensor system for the physiological detection of glucose.[20] The mode of action of 4-fluorobenzoboroxole (AN2690),[21] an antifungi agent currently undergoing phase 3 clinical studies, has recently been shown to involve formation of a boronic ester with the terminal nucleotide in the repair site of the tRNA isoleucyl synthetase complex.[22] Herein, we present a comprehensive study aimed at understanding the nature and selectivity of the complexation between benzoboroxoles and glycopyranosides.

Results and Discussion

1. Qualitative Screening of Ortho-Substituted Arylboronic Acids. Our initial plan toward the discovery of glycopyranoside-binding boronic acids was based on taking advantage of bifunctional interactions, that is, covalent boronic ester formation complemented by secondary interactions from a suitable ortho substituent on the arylboronic acid template. Toward this end, a rapid means of screening ortho-substituted arylboronic acids for carbohydrate complexation was required. Because it is simple and was proven reliable by several laboratories,[9,23] we elected to use Wang's qualitative colorimetric assay based on the competitive displacement of alizarin red S (ARS).[24] From more than a dozen arylboronic acids tested at neutral pH in water, **1a**−**o** (Figure 3), *o*-hydroxymethylphenylboronic acid (benzoboroxole, **1m**)[25] stood out by showing strong binding to both glucose and fructose. To our greater satisfaction, modest but unequivocal binding of the model hexopyranoside methyl α-D-glucopyranoside was observed (we cautiously ensured by NMR that the samples of glucopyranoside contained no free glucose). Weak binding of the same glycopyranoside was also observed with the strongly acidic 3-carboxy-5-nitrophenylboronic acid (**2**).[26] All of the other boronic acids, including the "Wulff-type" *o*-dimethylaminomethylphenylboronic acid (**1o**), failed to provide any visible darkening of the solution even with a large excess of glycoside. The fact that the "open" methyl ether **1l** failed to show any signs of complexing to glycopyranosides is indicative that **1m** is active in its cyclic dehydrated form, a hemiboronic acid. For the purpose of comparison, 2-hydroxyethylphenylboronic acid, **1n**, was also submitted to the qualitative ARS assay with the model glycoside methyl α-D-glucopyranoside but failed to give any color change. In order to see if silanols could also act as a new class of sugar-binding agents, phenylmethylsilanediol (**3**) was prepared and submitted to the qualitative ARS assay. Unfortunately, no binding was observed, which was later corroborated by a qualitative NMR experiment with fructose (no peak broadening or significant chemical shift variations were seen). These results confirm that silanols most likely cannot be used as carbohydrate receptors under neutral aqueous conditions.

2. Measurements of Association Constants between Benzoboroxole (1m) and Carbohydrates. To investigate the scope and selectivity in the complexation between monosaccharides and benzoboroxole (**1m**), the initial colorimetric qualitative assay was followed up by the measurement of binding constants with glucose, fructose, and a panel of hexopyranosides using NMR spectroscopy and the quantitative three-component ARS assay by UV spectrophotometry. In the case of tighter binding carbohydrates like fructose, the bound and unbound forms were distinguishable by NMR spectroscopy at room temperature. Their proportions could be calculated by integration of selected peaks on the benzoboroxole, and the association constant was calculated based on a 1:1 binding stoichiometry (vide infra). For carbohydrates displaying a low binding affinity, like the glycopyranosides, it was not possible to observe both forms and there were no significant chemical shift variations allowing association constants to be easily extracted. Therefore, the ARS-based quantitative UV method described by Wang and co-workers was employed.[24] In this assay, high concentrations of both species were used, with excess carbohydrates, so as to make up for the weak complexation equilibrium. It should also be emphasized that although these methods for measuring association constants of boronic acid−sugar complexes are strongly dependent on the assay conditions, such as buffer components, all measurements were made under comparable conditions and were reproduced several times. The R^2 factors on the linear regressions leading to the

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TABLE 1. Association Constants (K_a) by ^{1}H NMR at Neutral pH

entry	boronic acid	conditions[a]	K_a (M^{-1})[b] glucose	fructose
1	$PhB(OH)_2$	D_2O	0	79
2	**1m**	D_2O	17	606
3	**1m**	D_2O (pH 7.8)	[c]	339
4	**1m**	D_2O (pH 7.0)	[c]	172
5	**1m**	80% CD_3OD/D_2O	[c]	7
6	**1o**	33% CD_3OD/D_2O	[c]	115
7	**1o**	80% CD_3OD/D_2O	[c]	308
8	**1p**	80% CD_3OD/H_2O	[c]	1960

[a] In pH 7.4 sodium phosphate monobasic buffer. [b] Average of at least two measurements. [c] Not measured. Likely below 5 M^{-1} according to the ARS qualitative assay.

TABLE 2. Association Constants (K_a) between 1m and Different Hexopyranosides with the ARS-Based UV Method at Neutral pH

entry	hexopyranoside[a]	K_a (M^{-1})[b]
1	α-D-glucose	31
2	methyl α-D-glucopyranoside	22
3	methyl β-D-glucopyranoside	9
4	methyl α-D-galactopyranoside	29
5	methyl β-D-galactopyranoside	23
6	methyl α-D-mannopyranoside	24
7	methyl α-D-fucopyranoside	25
8	methyl 6-deoxy-α-D-glucopyranoside	0[c]

[a] See structures in Figure 4. [b] In pH 7.4 sodium phosphate monobasic buffer. Average of at least two reproducible measurements. [c] Likely below 5 M^{-1} according to ARS qualitative assay.

reported K_a's are all excellent (>0.98). Prior to measuring the model glycopyranosides using the ARS-based UV assay, known literature values for ARS and fructose[24] were carefully reproduced to ensure the validity of our technique. In this context, the values of Tables 1 and 2 can be deemed particularly useful in a comparative purpose.

We first compared benzoboroxole (**1m**) to phenylboronic acid and the two Wulff-type *o*-aminomethylarylboronic acids **1o** and **1p** in the complexation of glucose and fructose by NMR titrations in neutral aqueous conditions (Table 1). From these results, it is clear that benzoboroxole (**1m**) is a superior complexing agent for the reducing monosaccharides. Moreover, in contrast to **1o**, **1m** does not need an organic cosolvent for solubilization. These results led us to question the contribution of the covalent boronate interaction in the binding of "Wulff-type" polyaromatic boronic acid sensors to monosaccharides. The influence of hydrophobic interactions in the recognition of carbohydrates by natural (i.e., lectins) and unnatural receptors is well-known.[27] In particular, aromatic groups on receptor molecules are known to increase binding affinities and are thought to interact with the hydrophobic α face of carbohydrates.[28] Here, compared to **1o**, we found that the hydrophobic nature of the sensing unit of **1p** (e.g., the anthracene group)[6a] significantly increases the K_a values (compare entries 7 and 8). This result suggests for the first time that the saccharide-binding affinity of previously reported "Wulff-type" boronic acid receptors is probably significantly amplified by hydrophobic interactions.

To analyze the effect of pH, the binding strengths between benzoboroxole (**1m**) and fructose were determined under different pH conditions: pH 7.0, 7.4, and 7.8 (Table 1, entries 2−4). The optimal pH was deduced to be around 7.4 as the binding constant of **1m** with fructose is the greatest (606 M^{-1}), whereas lower binding constants of 172 and 339 M^{-1} were obtained at pH 7.0 and 7.8, respectively. Although it is unusual to observe a decreased binding affinity at higher pH, there is precedent for this phenomenon[29] and a possible explanation regarding benzoboroxole−diol complexes is depicted in Figure 5. We assume that the bimolecular **1m**−diol complex exists in its stable ionized form I at near neutral pH. At a lower pH, the less stable neutral complex II may compete and lead to an overall lower association constant. At a higher pH, the hydroxyboronate complex III typical of normal arylboronic acids may dominate and show decreased stability compared to I because of its trimolecular nature. The use of organic cosolvents (e.g., methanol) also has an important impact on the binding constants. A higher concentration of CD_3OD in the complexation of "Wulff-type" boronic acid **1o** led to an increase of the association constant (Table 1, entries 6−7), which may be explained by the reduced solvent polarity that favors solvation of the neutral complex (i.e., complex of eq 3, Figure 1). In contrast, an increased concentration of CD_3OD to 80% with **1m** results in a much lower binding constant of 7 M^{-1} with fructose at pH 7.4 (entry 5). This surprising observation may in fact be explained by the existence of anionic complex I (Figure 5), which would be destabilized in a less polar solvent mixture. Altogether, these results are supportive of the formation of a fully ionized complex of type I at neutral pH, which is in contrast with most normal arylboronic acids that are ionized only at a higher pH.[5]

The complexation of a panel of different hexopyranosides (Table 2) was examined using the ARS three-component assay with UV measurements in neutral water (buffered to pH 7.4).[24] To validate this method, the complexation of glucose was measured as a control. The resulting K_a of 31 M^{-1} is slightly higher than that measured by the NMR method (K_a = 17 M^{-1}, see Table 1), but it is likely that a small isotopic effect (D_2O was used in the NMR measurements) and the intrinsic differences between the two methods explain this small difference. In agreement with the qualitative ARS assay, complex formation with methyl α-D-glucopyranoside was found to be slightly weaker than with glucose (K_a = 22 vs 31 M^{-1}) (Table 2, entries 1 and 2). Interestingly, these affinities are comparable or superior to recently reported macrocyclic receptors,[30] however, with a much simpler and smaller compound. The complexation of **1m** to methyl α-D-galactopyranoside is even more favorable (K_a = 29 M^{-1}) than the corresponding glucopyranoside (entry 4). We looked at the effect of the anomeric configuration of these two hexopyranosides by comparing the α- and β-anomers. Only in the case of the glucopyranoside was a significant, unexplained difference observed (entries 2 and 3), with the β-anomer being a much weaker ligand. Both methyl α-D-mannopyranoside and methyl α-D-fucopyranoside bind to benzoboroxole (**1m**) with affinities comparable to methyl α-D-galactopyranoside (entries 6 and 7). On the other hand, the 6-deoxy derivative of methyl α-D-glucopyranoside was found not to bind to **1m** (entry 8). The large amount of sample required for the quantitative ARS

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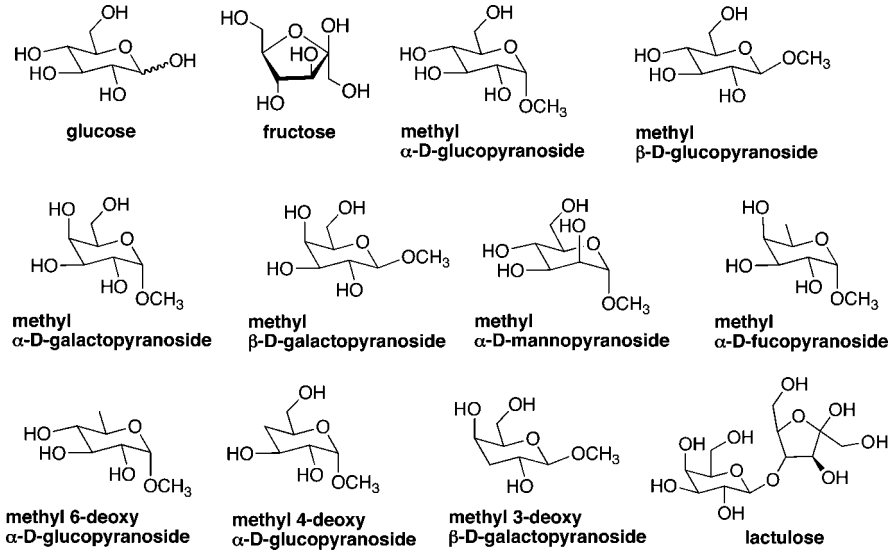

FIGURE 4. Model carbohydrates used in this study of binding affinity and selectivity.

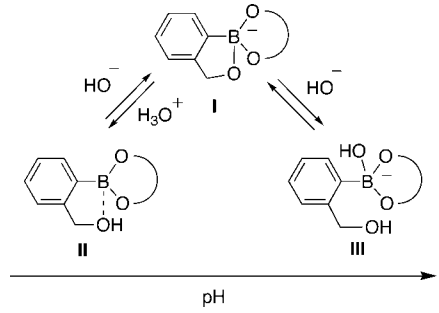

FIGURE 5. Effect of pH on the putative structures of the benzoboroxole−diol complex.

UV assay made it impossible to obtain K_a values for a number of expensive sugars. Nonetheless, qualitative ARS assays were performed with methyl 3-deoxy-β-D-galactopyranoside and methyl 4-deoxy-α-D-glucopyranoside. A slight darkening of the two solutions was observed but they were not as intense as with methyl α-D-galactopyranoside and methyl α-D-fucopyranoside, which is indicative of a lower affinity between benzoboroxole (**1m**) and those deoxy sugars. Differences of binding affinity with respect to the detailed structure of the hexopyranosides will be discussed in section 4.

3. Determination of Binding Stoichiometry in the Complexation of Benzoboroxole (1m) to Glycopyranosides. It was necessary to address the binding stoichiometry to understand the nature of the complexation between benzoboroxole (**1m**) and the different carbohydrates tested and to identify the structural determinants for the diol (sugar) component. Unfortunately, it was not possible to obtain a Job's plot because there was no significant change in the UV spectrum of **1m** upon complexation with methyl α-D-galactopyranoside in solution. Therefore, we tried to examine this issue by mass spectrometry using electrospray ionization in the negative mode. Insofar as gas-phase data relates to dilute solutions in this system, the results were conclusive (see the Supporting Information for spectra). Thus, upon injecting a 10:1 solution of benzoboroxole (**1m**) in the presence of methyl α-D-galactopyranoside in a 1:1 mixture of acetonitrile and water, a large peak (M = 309.1) corresponding to a 1:1 complex was observed along with the peaks of individual components. This signal corresponds, as expected, to the adduct between **1m** and the glycoside with the loss of one water molecule. No traces of a 2:1 **1m**/glycoside complex were observed. A similar result was obtained with methyl α-D-glucopyranoside under the same conditions. To ascertain the validity of these results, we needed a control experiment with a carbohydrate derivative known to form a 2:1 boronic acid/sugar complex. Because mannitol is known to form a complex in solution with several molecules of a boronic acid,[16a] we analyzed its complexation with benzoboroxole (**1m**) and phenylboronic acid under the same analytical conditions described above. For benzoboroxole (**1m**), a large peak (M = 297.1) corresponding to a 1:1 complex was observed as well as another large peak (M = 413.2) associated to a 2:1 complex corresponding to the adduct of two molecules of **1m** with one molecule of mannitol accompanied with the loss of two molecules of water. The same pattern was observed with phenylboronic acid. It is also noteworthy that an ESMS analysis of benzoboroxole (**1m**) and lactulose injected as a 10:1 mixture gave predominantly the signal of the single adduct (M = 457.1), but also a small peak corresponding to the expected complex of two molecules of **1m** binding to one molecule of lactulose (M = 573.2). These results make it highly improbable that a 2:1 **1m**/carbohydrate complex is involved in the case of monoglycopyranosides. The 1:1 binding model is also strongly supported by the observed complexation selectivity (cf., section 2 and 4).

4. Discussion of Structural Determinants in the Complexation and Selectivity Profile of Benzoboroxole (1m) for Glycopyranosides. As shown in section 2, NMR titrations were successfully employed for measuring binding constants between **1m** and reducing sugars. In contrast, proton NMR spectroscopy did not distinguish the bound and unbound forms of benzoboroxole (**1m**) to allow the measurement of binding constants with glycopyranosides. However, a significant peak broadening effect was observed on the aromatic signals of benzoboroxole in the presence of a large excess of carbohydrate. The qualitative extent of peak broadening, or absence thereof, correlated very well with the results of binding constant measurements of Table 2 obtained with the quantitative ARS-based UV assays. This observation was useful in the case of hexopyranosides for which there were insufficient amounts available to obtain a K_a value using the quantitative ARS assay. Thus, as illustrated in Figure 6, benzoboroxole (**1m**) shows sharp peaks in the aromatic part

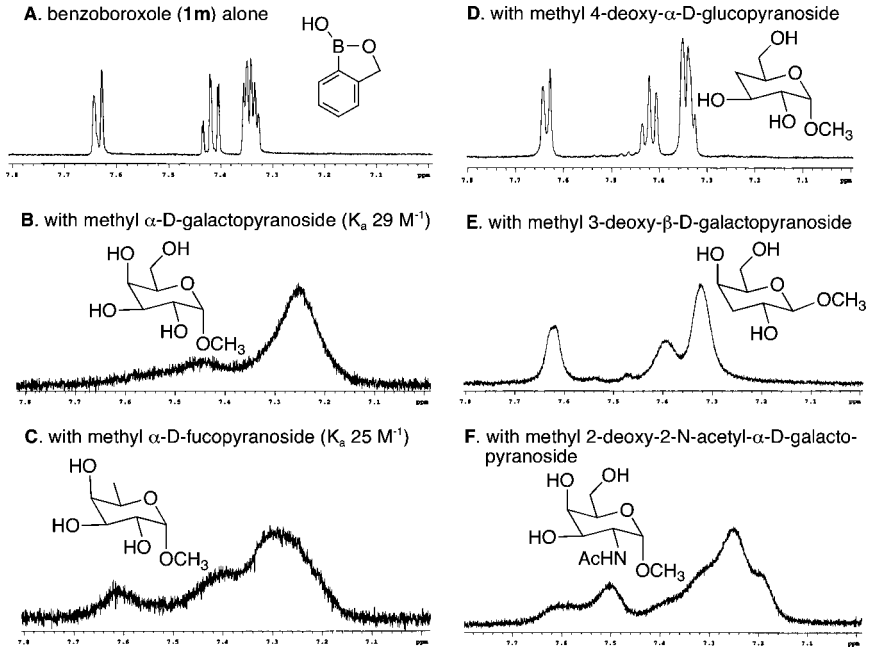

FIGURE 6. ^{1}H NMR spectra for the aromatic region (7.0−7.8 ppm) of 0.01 M benzoboroxole (**1m**) in 0.1 M phosphate-buffered D_2O at pH 7.4, alone (A) or with 0.25 M of different hexopyranosides (B−F).

of its ^{1}H NMR spectrum (Figure 6A). The addition of 25 equiv of Me-α-D-Gal to benzoboroxole (**1m**) provides significant peak broadening in the aromatic region of the ^{1}H NMR spectrum (Figure 6B). Expectedly, the addition of Me-α-D-Glc led to substantial peak broadening as well (spectrum not shown) but not to the same extent as Me-α-D-Gal. The same peak broadening phenomenon was also observed with the addition of Me-α-D-Fuc (methyl 6-deoxy-α-D-galactopyranoside) (Figure 6C). It is noteworthy that both glycopyranosides gave similar K_a values in the three-component ARS UV assays (cf. Table 2). Extensive broadening of peaks was observed in those spectra most likely as a result of a complexation equilibrium slower than the NMR time scale or, alternatively, by the presence of multiple bound conformations. On the other hand, in the presence of 25 equiv of methyl 4-deoxy-α-D-glucopyranoside or methyl 3-deoxy-β-D-galactopyranoside (Figure 6D,E), none or only marginal broadening was observed for the aromatic signals of benzoboroxole. In agreement with the outcome of the qualitative ARS assays, these results show that the 3-hydroxy and especially the 4-hydroxy groups are very important in the binding of benzoboroxole (**1m**) to the sugar ring in the galactopyranoside series. Moderate peak broadening of methyl 3-deoxy-β-D-galactopyranoside (Figure 6E) indicates that complexation of the 4,6-diol unit is also possible but much less favorable than the 3,4-diol unit in the galactopyranosides. To complete the series, methyl 2-deoxy-2-*N*-acetyl-α-D-galactopyranoside was also submitted to this experiment (Figure 6F). Significant broadening of peaks for the aromatic signals of **1m** suggests that the 2-hydroxy group of galactopyranosides is not important for the complexation. On the other hand, if we look at the glucopyranoside series, no ^{1}H NMR peak broadening in the aromatic signals of **1m** and no darkening of the solution in the qualitative ARS assay were observed with methyl 6-deoxy-α-D-glucopyranoside (see the Supporting Information). These observations are consistent with the K_a values of Table 2 and suggest that the 6-hydroxy group is very important for the complexation of benzoboroxole (**1m**) in the glucopyranoside series. As discussed above, the 6-hydroxy group is not crucial for the complexation of **1m** to galactopyranosides.

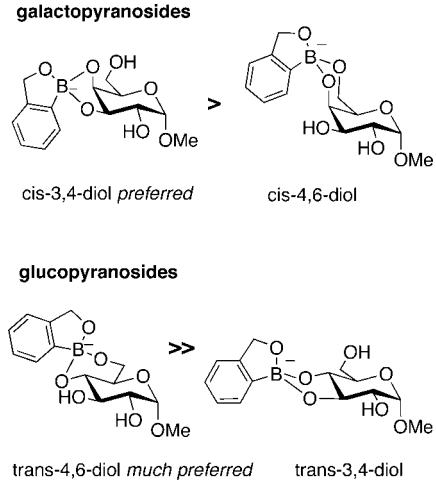

FIGURE 7. Favored diol binding modes between benzoboroxole (**1m**) and glycopyranosides.

With all of these results in hand, we believe that benzoboroxole (**1m**) binds hexopyranosides in a 1:1 stoichiometry to form a boronate complex that is anionic at neutral pH. In our initial communication, we proposed 4,6-diol complexation based on a limited data set of glycopyranosides. It is now clear that **1m** binds preferentially to the 3,4-*cis*-diol (equatorial−axial) over the 4,6-diol as suggested by a stronger complexation with the galactopyranosides and mannopyranosides compared to the glucopyranosides (Figure 7).[31] It should be noted that the boron center is stereogenic in the respective complexes and that only one form is depicted in Figure 7. This issue of selectivity will be addressed in the next section.

5. Discussion of Benzoboroxole's Ability to Complex Hexopyranosides. Several possible factors could explain the special capability of benzoboroxole (**1m**) to complex glycopyranosides under physiological conditions (i.e., water at pH 7.4). One of the most important factors in carbohydrate recognition

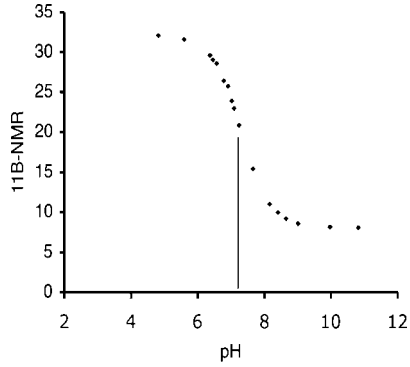

FIGURE 8. ^{11}B NMR chemical shifts of **1m** with increasing pH (10% D_2O in H_2O, 16 mM in 0.10 M phosphate buffer). Referenced to Et_2O-BF_3 in deuterated chloroform as 0.

with boronic acids concerns the actual Lewis acidity of the boronic acid.[5,29] Although there are exceptions to this trend,[29] the most acidic boronic acids usually provide tighter complexation. To address the role of Lewis acidity, we measured the pK_a of benzoboroxole (**1m**) using a ^{11}B NMR titration to find a pK_a of approximately 7.2 (Figure 8). Such a relatively low value is most likely the result of the strained nature of the boroxole unit of **1m** and the favorable rehybridization that accompanies the formation of a tetrahedral hydroxyboronate complex. The pK_a of its one-carbon homologue (**1n**) and the electron-poor 3-carboxy-5-nitrophenylboronic acid **2** were also found to be, respectively, 8.4 and 7.0 (see the Supporting Information). All these boronic acid derivatives are more acidic than phenylboronic acid (pK_a 8.8).[29]

Both the homologue **1n** and $PhB(OH)_2$ have a similar, relatively high pK_a and fail to show any binding with methyl α-D-glucopyranoside as supported by the absence of ^1H NMR peak broadening or any color change in the qualitative ARS assay in the presence of over 25 equiv of the glycopyranoside. On the other hand, both benzoboroxole (**1m**) and the very acidic boronic acid **2** bind to glycopyranosides. Boronic acid **2** binds methyl α-D-galactopyranoside with a K_a of 29 M^{-1} very similar to benzoboroxole (**1m**) according to the same ARS three-component assay. However, **2** led to a lower K_a with methyl α-D-fucopyranoside (16 vs 25 M^{-1} for **1m**). Furthermore, no ^1H NMR peak broadening was observed in the aromatic region of **2** when mixed with 25 equiv of Me-α-D-Gal or Me-α-D-Fuc. These results indicate that boronic acid **2** also binds to glycopyranosides but with a different selectivity and probably with a different geometry and/or dynamics. Although more studies would be necessary to determine the structural determinants for complexation of **2** with hexopyranosides, these preliminary results comparing **1m**, **1n**, and **2** indicate that the low pK_a of benzoboroxole (**1m**) plays an important role in its hexopyranoside-binding ability. Other factors than Lewis acidity must be involved, however, because the very acidic Wulff-type boronic acid **1o** (pK_a ~6.7)[29] does not appear to complex glycopyranosides. It is well established that conformational distortions of hexopyranosides are accompanied with significant enthalpy costs.[32] Therefore, the complexation of hexopyranosides with boronic acids must minimize such conformational changes in the carbohydrate component. Although this factor is less problematic in the complexation of the more flexible 4,6-diol, it is of particular concern in the complexation of the 3,4-diol unit. Given its unusual, strained cyclic structure, we wondered whether geometrical factors could play a role and provide benzoboroxole (**1m**) with structural features that could better accommodate, compared to normal boronic acids, gauche vicinal diols such as the cis-3,4-diol unit found in galactopyranosides. For instance, it was envisaged that the small internal C−B−O angle of 110.5° for **1m** (confirmed in its X-ray crystal structure[25b]) might open up the external cone angle (O−B−O) in the resulting **1m**−diol complex I (Figure 9) and allow it to better reach the O atoms of a gauche 1,2-diol, which are more distant than the essentially coplanar 1,2-diols found in furanoses. To this end, the structures of simplified acyclic complexes I and II were calculated at the DFT B3LYP 6-31G* level of theory (Figure 9). To our surprise, no noticeable differences were found in the B−O bond lengths and the external $CH_3O-B-OCH_3$ angle. Thus, the peculiar structure and geometry of **1m** does not appear to be important in the complexation of galactopyranosides.

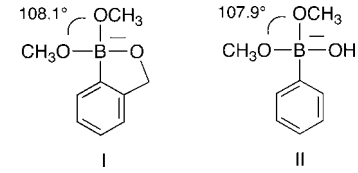

FIGURE 9. Ground-state equilibrium geometry optimization of acyclic dimethoxy complexes I and II using density functional theory (B3LYP method with 6-31G* basis set).

The presence of a ring to provide a hemiboronic ester as in **1m** could be thought important especially to decrease the entropic penalty in the formation of a complex. Indeed, normal boronic acids require the hydroxide anion as fourth ligand (cf. Figure 1, eqs 2 and 3) as opposed to the internal alkoxide anion in the case of **1m**. However, the poor apparent binding ability of homologue **1n** would tend to rule out a determining influence for the internal alkoxy unit (other than the much higher Lewis acidity it lends to **1m**). A possible explanation for the binding behavior and selectivity of benzoboroxoles was provided from semiempirical calculations of **1m** and its resulting complexes with methyl hexopyranosides (Figure 10).[33] Ground-state semiempirical energy minimizations (AM1, MacSpartan 06) were performed for the 3,4 and 4,6 complexes between benzoboroxole (**1m**) and both methyl β-D-galactopyranoside and methyl β-D-glucopyranoside.[34] Because the boron atom in the complex is stereogenic, both epimers were calculated for each complex (denoted α-aryl and β-aryl). For methyl β-D-galactopyranoside, the 3,4 complexes (Figure 10A,B) were found to be much favored over the 4,6 complexes (Figure 10C,D), which is in agreement with the above-described experimental results. The highly preferred α-aryl complex (A) features three H-bonds between the accessible 2-hydroxyl and 6-hydroxyl protons and the basic oxygens of the boronate anion, including the internal oxygen of the boroxole ring.[35] Thus, the high probability that the complex is anionic at neutral pH goes a long way in explaining the binding affinity and selectivity of benzoboroxole

(31) Similar conclusions were reached with borate esters, see: van der Berg, R.; Peters, J. A.; van Bekkum, H. *Carbohydr. Res.* **1994**, *253*, 1–12.

(32) Angyal, S. J. *Angew. Chem., Int. Ed.* **1969**, *8*, 157–226.

(33) For similar semiempirical calculations between *m*-nitrophenylboronic acid and furanoses, see: Nicholls, M. P.; Paul, P. K. C. *Org. Biomol. Chem.* **2004**, *2*, 1434–1441.

(34) Similar trends are observed with the α anomers.

(35) Intramolecular H-bonds between sugar hydroxyls and basic oxygen atoms of tetrahedral boronates were also proposed to explain the selectivity and efficacy of diboronic acid transporters for fructose: Draffin, S. P.; Duggan, P. J.; Duggan, S. A. M.; Norrild, J. C. *Tetrahedron* **2003**, *59*, 9075–9082.

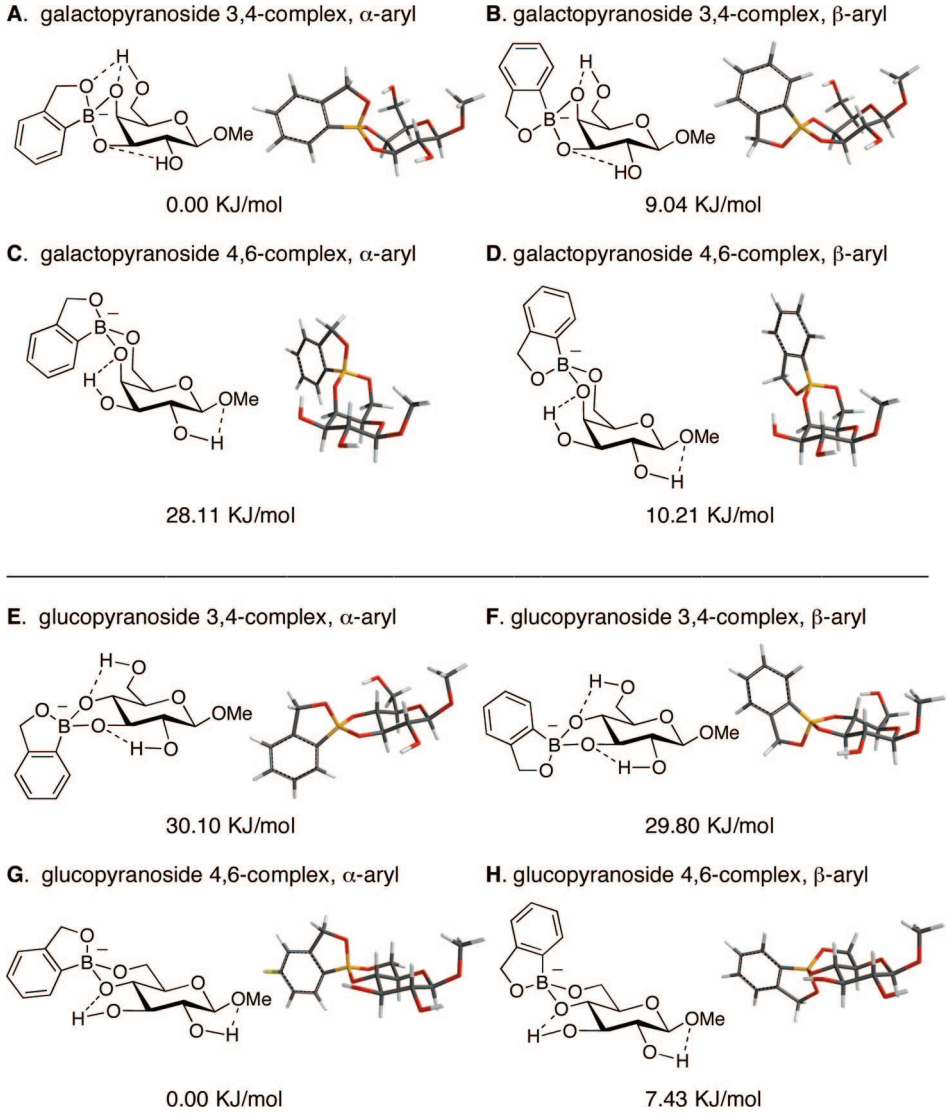

FIGURE 10. Semiempirical (AM1, MacSpartan 06) geometrical minimization of the possible complexes between benzoboroxole (**1m**) and methyl β-D-galactopyranoside (A−D) and methyl β-D-glucopyranoside (E−H). Note: The indicated values of energy are relative ones, with the minima set at 0 kJ/mol for the respective lowest energy structures A and G.

(**1m**). The less stable β-aryl complex (B) features one less geometrically viable H-bond. Although they do not feature any significant distortion of the pyran ring, the 4,6-complexes (C and D) allow only one H-bond with the basic boronate moiety. In agreement with the experimental results, the 4,6 complexes of methyl β-D-glucopyranoside (Figure 10G,H) were found to be highly favored over the corresponding 3,4 complexes (E and F). The latter show energy-costly pyran ring distortions through a closing of the diol's dihedral angle. Moreover, the 4,6 complexes of glucopyranosides are particularly favorable due to their *trans*-decalin like geometry. Between the two epimeric complexes, G (α-aryl) is favored over H (β-aryl) because the aryl unit lies in a pseudoequatorial position.

Conclusion

We have described the unique capability of *o*-hydroxymethyl phenylboronic acid (benzoboroxole, **1m**) to complex glycopyranosides in neutral water. The behavior of this class of hemiboronic acids contrasts with that of normal arylboronic acids, which can bind to reducing saccharides but generally fail to complex nonreducing ones like hexopyranosides. The measurement of association constants with a panel of model glycopyranosides indicates that the preferred mode of binding of benzoboroxole is through a *cis*-3,4-diol, such as that found in galactopyranosides, and mass spectrometric studies support a 1:1 binding stoichiometry. Several factors may explain the exceptional saccharide-binding behavior of benzoboroxoles. Control experiments and pK_a measurements with other arylboronic acids as well as calculations were performed to address the nature of the complexation. The relatively high Lewis acidity of benzoboroxoles is a likely contributing factor along with subtle factors such as intramolecular hydrogen bonding with other pyranoside hydroxyl groups in the resulting anionic complex. The binding affinities observed between benzoboroxole and monopyranosides are still too weak for most practical applications targeting oligosaccharides. It is known, however, that diboronic acid receptors that possess the right spacers between the boronate units can lead to a significant increase of binding affinities in two-point complexation of mono- and oligosaccharides.[6] The nature of the linker separating the two arylboronic acid units can even greatly affect the binding

selectivity. Thus, it is anticipated that the application of the same concept of multipoint recognition with benzoboroxoles could lead to potent receptors of nonreducing oligosaccharides including biologically important ones found as cell surface conjugates. Work in this direction is currently in progress.

Experimental Section

Methodology for screening of Ortho-Substituted Arylboronic Acids (Qualitative ARS Assay[24]). Two separate solutions were prepared. Solution A: 50 mL of 10^{-3} M stock solution of alizarin red S (ARS) solution in 0.10 M sodium phosphate monobasic buffer was diluted 10-fold with 0.10 M sodium phosphate monobasic buffer in a 500 mL volumetric flask. The pH of the solution was adjusted to 7.4 with 4 M NaOH (a portable pH meter was used which gave pH values within 0.01 units). The resultant solution containing 10^{-4} M solution of ARS in 0.10 M phosphate buffer at pH 7.4 was referred to as solution A. Solution B: The controls were prepared by dissolving the boronic acids (0.10 mmol) in solution A in a 5 mL volumetric flask to give 0.02 M solution with respect to the boronic acid. The pH was adjusted to 7.4 with 4 M NaOH before diluting to the 5 mL mark with ARS solution A.

Colorimetric assays were attempted with these solutions except for the less soluble boronic acids that required 10−33% methanol as solvent. The carbohydrate solutions (0.5 M) were prepared by adding 0.5 mmol of sugar to the control solution B in 1 mL volumetric flasks. The pH was adjusted to 7.4 with 4 M NaOH.

Methodology for K_a Measurements by the Three-Component ARS Method.[5,24] K_a of 1m and ARS (K_{ARS}). Following the procedure of Wang and co-workers[5,24] a 0.144 mM ARS solution was prepared in 0.1 M phosphate buffer at pH 7.4. The solution needs to be sonicated for 2−3 h to obtain complete dissolution of the ARS in the phosphate buffer. A solution of **1m** (15 mM) in the ARS solution was prepared in a volumetric flask and adjusted to pH 7.4. By mixing this boronic acid solution with the ARS solution together in the UV cuvette, a range of boronic acid concentration (1.2−4.0 mM) was obtained. The UV absorbance of each solution was taken at 450 nm and plotted to determine the K_{ARS}. At least three experiments were carried out to determine an average value of K_{ARS}. (Average value of K_{ARS} of **1m** used in glycoside measurements: 1180 M^{-1}. See the Supporting Information for graph.)

Example: K_a of 1m and α-D-Glucose (Table 2, Entry 1). A solution of **1m** (3.1 mM) in ARS solution (0.144 mM in 0.1 M phosphate buffer) was prepared in a volumetric flask and adjusted to pH 7.4. Then, a part of this solution was used to make a 2.0 M α-D-glucose solution at pH 7.4. By mixing the two solutions together in the UV cuvette, a range of sugar concentrations (0.1−0.4 M) was obtained. The UV absorbance of each solution was taken at 454 nm and plotted as described by Wang and co-workers[5,24] to determine the K_a (see the Supporting Information for a graph). A plot of [S]/P versus Q is constructed where $Q = [RI]/[I] = (A_{RI} - A)/(A - A_I)$, where R is the receptor (**1m**), A is measured absorbance, A_{RI} is absorbance of the receptor−indicator complex, and A_I is absorbance of free indicator (ARS). $P = [R] - 1/(QK_{ARS}) - [I_0]/(Q + 1)$, where $[I_0]$ is total indicator concentration (ARS). The K_a is given by $[S]/P = K_{ARS}/K_aQ + 1$, where [S] is sugar concentration.

Example: K_a of 1m and Methyl α-D-Glucopyranoside (Table 2, Entry 2). A solution of **1m** (3.1 mM) in ARS solution (0.144 mM in 0.1 M phosphate buffer) was prepared in a volumetric flask and adjusted to pH 7.4. Then, a part of this solution was used to make a 2.0 M methyl α-D-glucopyranoside solution at pH 7.4. By mixing the two solutions together in the UV cuvette, a range of sugar concentrations (0.8−1.1 M) was obtained. The UV absorbance of each solution was taken at 454 nm and plotted as described above to determine the K_a (see the Supporting Information for a graph).

Molecular Modeling Calculations. All calculations were performed using MacSpartan 06. Density functional theory (DFT) calculations (SCF model) of Figure 9 used the B3LYP method with the 6-31G* basis set. Heats of formation of **1m**-glycoside complexes of Figure 10 used the semiempirical AM1 method on the monoanionic species.

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Supporting Information Available: Full experimental conditions, detailed methodology and graphs for K_a measurements of Table 1 and 2, ESMS spectra for study of binding stoichiometry, preparation of the unknown methyl 3-deoxy-β-D-galactopyranoside including NMR spectral reproductions, additional NMR qualitative experiments for complex formation, details of pK_a measurements, and atom coordinates from molecular modeling calculations of Figures 9 and 10. This material is available free of charge via the Internet at http://pubs.acs.org.

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