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An Improved Class of Sugar-Binding Boronic Acids, Soluble and Capable of Complexing Glycosides in Neutral Water

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The selective recognition of carbohydrates under physiological conditions stands as one of the biggest challenges of chemical biology.[1] 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.[2] While the use of boronic acids is regarded as one of the most promising approaches for the recognition of carbohydrates in water,[3] it is not without severe limitations. No boronic acid unit has yet been demonstrated to bind to nonreducing sugars and glycosides,[4] which account for the large majority of biologically important oligosaccharides found in the form of cell-surface glycoconjugates. Moreover, the "Wulff-type" *ortho*-dialkylaminomethyl arylboronic acids,[5] currently the established standard for the recognition of simple reducing sugars, tend to have limited solubility in aqueous solutions.[6] Herein, we report that *ortho*-hydroxyalkyl arylboronic acids bind to monosaccharides, such as glucose and fructose, with higher affinity than do Wulff-type boronic acids in neutral water, and show a better solubility profile. Moreover, exciting preliminary evidence reveals the unprecedented capability of this new class of boronic acids to complex nonreducing glycopyranosides.

Elegant studies by Norrild and co-workers[7] have confirmed that glucose binds to boronic acids in water in its weakly populated furanose form.[8] The work of our group[9] and others[10] has emphasized the existence of similar requirements for disaccharides. This behavior is generally ascribed to geometrical preferences in boronate formation. Rigid and coplanar vicinal diols, such as the syn 1,2-pair of furanoses, are strongly preferred to minimize angle strain in the resulting boronic ester. The formation of a coplanar boronate with the non-coplanar diols of a glycopyranoside would induce a highly unfavorable conformational change to the puckered sugar ring.[11] It is clear that, if the use of oligoboronic acid receptors is to mature into a general approach for oligosaccharide recognition, new boronic acids with pyranoside-binding capability are required. Our initial approach envisioned the possible formation of a hemiarylboronic ester with cooperative covalent or noncovalent interactions from an ortho-substituent (Figure 1). To this end, we screened a panel of ortho-substituted arylboronic acids using Wang's qualitative colorimetric assay based on the competitive release of alizarin red S (ARS).[12] From more than a dozen candidates, **1a**−**1m**, *ortho*-hydroxymethyl phenylboronic acid (**1m**)[13] stood out by showing strong binding to both glucose and fructose. To our greater satisfaction, weak but encouraging binding of the glycosides methyl α-D-glucopyranoside and trehalose (a 1,1′-glucopyranose dimer) was observed. All of the other boronic acids, including Wulff-type *ortho*-dimethylaminomethyl phenylboronic acid (**1n**) and the highly acidic **2**,[14] failed to provide any visible darkening of the solution, even with a large excess of glycosides.[15,16] The complexed and uncomplexed forms of **1m** with methyl glucopyranoside were not distinguishable by ^{1}H NMR. Peak broadening of the arylboronate protons, however, was observed only in the case of **1m**, which

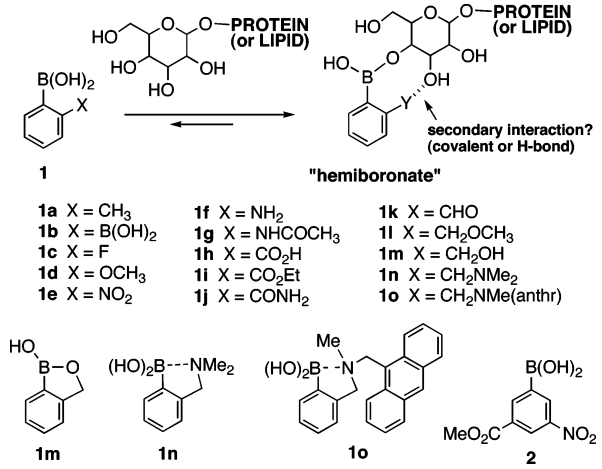
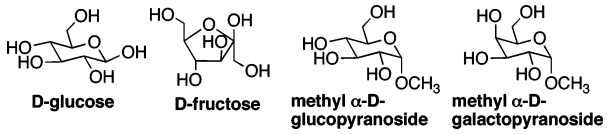


Figure 1. Ortho-substituted arylboronic acids tested for glycoside binding.

Table 1. K_a Measurements by ^{1}H NMR at Neutral pH[16,18]

			K_a (M^{-1})[b]	
entry	boronic acid	conditions[a]	glucose	fructose
1	$PhB(OH)_2$	D_2O	0	79
2	**1m**	D_2O	17	606
3	**1n**	33% CD_3OD/D_2O	*c*	115
4	**1n**	80% CD_3OD/D_2O	*c*	308
5	**1o**	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 ARS qualitative assay.



further supports binding of the model glycosides only with this boronic acid.[16] The association constant of **1m** to methyl α-D-glucopyranoside in water (pH 7.4) was best measured by the ARS method.[12] In agreement with the qualitative assay, complex formation with methyl α-D-glucopyranoside was found to be slightly weaker than with glucose (K_a = 22 vs 36 M^{-1}). These affinities are comparable or superior to recently reported macrocyclic receptors,[17] however, with a much simpler compound. A comparison of the binding of phenylboronic acid, **1m**, and **1n** to glucose and fructose was done by NMR titrations in neutral aqueous conditions (Table 1).[18] Although K_a measurements are sensitive to the method and conditions employed,[19] these values are useful for comparative purposes. The data of Table 1 confirm that boronic acid **1m** is superior to the Wulff-type analogue **1n**. Moreover, in contrast to **1n**, **1m** does not need an organic cosolvent for solubilization

Table 2. K_a Measurements[a] of Glycopyranosides Using the ARS UV Assay at Neutral pH[12,16]

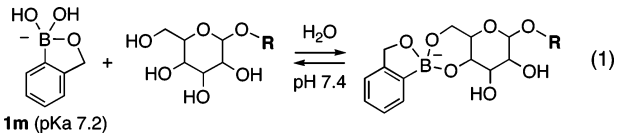
pyranoside	K_a (M^{-1})[b]
methyl α-D-glucopyranoside	22
methyl α-D-galactopyranoside	34
methyl β-D-glucopyranoside	22
methyl β-D-galactopyranoside	34

[a] Conditions: pH 7.4 sodium phosphate monobasic buffer (in 100% H_2O). [b] Average of at least two measurements.

These results led us to question the contribution of the covalent boronate interaction in the binding of Wulff-type boronic acids to monosaccharides. The influence of hydrophobic interactions in the recognition of carbohydrates by natural (i.e., lectins) and unnatural receptors is well-known.[20] Here, compared to **1n**, we found that the hydrophobic nature of the sensing unit of **1o**,[3a] specifically, the anthracene group, significantly increases the K_a values (compare entries 4 and 5). Thus, when measured in a minimum percentage of methanol, the simple Wulff-type boronic acid **1n**, which is devoid of a hydrophobic unit, is clearly inferior to **1m**. This suggests for the first time that the saccharide-binding affinity of previously reported Wulff-type boronic acid receptors is significantly amplified by hydrophobic interactions.

We looked at the binding requirements of **1m** by comparing methyl α-D-glucopyranoside with its 6-deoxy derivative.[16] The latter was found not to bind significantly to **1m**, which suggests a key role for the 4,6-diol unit in the complexation of **1m** to the glucopyranoside. Although more work is warranted to examine the binding selectivity, the ARS assay revealed that complexation of **1m** to methyl α-D-galactopyranoside is even more favorable, and the β-glycosides display a similar selectivity (Table 2).[16]

The precise binding mode of boronic acid **1m** to hexopyranosides is currently unclear. Certainly, the pK_a of **1m** is quite low (ca. 7.2) as measured by potentiometric and ^{11}B NMR methods,[16] but as shown with the inefficiency of **1n** and **2**, acidity alone can hardly explain the surprising ability of **1m** to bind hexopyranosides. The ether derivative **1l** is ineffective, so the presence of a coordinating oxygen is not sufficient. Boronic acid **1m** is believed to exist in its cyclic, dehydrated boronophthalide form.[13] It is possible that the unusually small C−B−O dihedral angle of **1m**, as observed by X-ray crystallography,[21] opens up the cone angle in the resulting tetrahedral diol−boronate complex. This distorted geometry may better accommodate the 4,6-diol of hexopyranosides compared to the usual boronic acids, leading to the proposed complexation model of eq 1. This complex may also benefit entropically from the internal alkoxy arm of **1m** (as compared to a hydroxy ligand with the usual boronic acids).



1m (pKa 7.2) (1)

Dimerization has been shown to be a very effective strategy in the development of boronic acid based receptors and sensors, leading to large increases in binding affinity and often drastic changes in selectivity profiles.[3] In the current case, the development of synthetic routes to oligomeric derivatives of **1m** would allow multivalency effects to be exploited in the recognition of cell-surface glycoconjugates.

In conclusion, a new class of carbohydrate-binding boronic acids was characterized. *ortho*-Hydroxymethyl phenylboronic acid was shown to be superior to the well-established dialkylamino (Wulff-type) analogues. The most significant finding is the ability of *ortho*-hydroxyalkyl arylboronic acids to complex model glycosides under physiologically relevant conditions. This unique boronic acid unit appears to complex hexopyranosides mainly using their 4,6-diol, and we note that a majority of cell-surface glycoconjugates present free 4,6-diols. Conjugatable forms of these boronic acids could be used in the design of oligomeric receptors and sensors to exploit multivalency effects. Such receptors could dramatically expand the potential of boronic acids toward the selective recognition of cell-surface glycoconjugates.

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Supporting Information Available: Full experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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