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A New Class of Fluorescent Boronic Acids That Have Extraordinarily High Affinities for Diols in Aqueous Solution at Physiological pH

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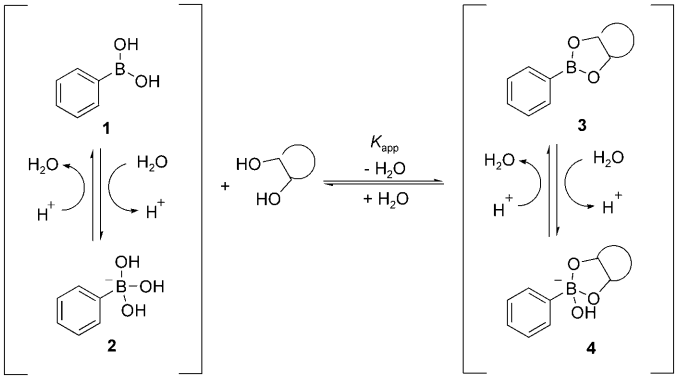
Abstract: The boronic acid group is an important recognition moiety for sensor design. Herein, we report a series of isoquinolinylboronic acids that have extraordinarily high affinities for diol-containing compounds at physiological pH. In addition, 5- and 8-isoquinolinylboronic acids also showed fairly high binding affinities towards D-glucose ($K_a = 42$ and $46\,M^{-1}$, respectively). For the first time, weak but encouraging binding of *cis*-cyclohexanediol was found for these boronic acids. Such binding was coupled with significant fluorescence changes. Furthermore, 4- and 6-isoquinolinylboronic acids also showed the ability to complex methyl α-D-glucopyranose ($K_a = 3$ and $2\,M^{-1}$, respectively).

Keywords: boronic acids · cyclohexanediol · fluorescent probes · methyl glucopyranose · sensors

Introduction

Boronic acids are commonly used in chemosensor design due to their intrinsic binding affinities to diols, amino alcohols, α-amino acids, α-hydroxyl acids, and alcohols as well as cyanide and fluoride.[1–4] The general interaction pathways are shown in Scheme 1. Since the rejuvenation of the boronic acid–diol recognition field by Czarnik et al.[5] and Shinkai et al.[6] in the early 1990s, research in this area has undergone some transformations, going from binding with simple monosaccharide to recognition of cell-surface carbohydrate biomarkers. Several recent reviews and research papers comprehensively summarized the use of boronic acids in sensor design for carbohydrates,[1,3,7–9] with in-depth discussions of factors[1,10,11] that should be considered in designing such sensors.



Scheme 1. Binding equilibrium of phenylboronic acid with a diol.

One critical need in the carbohydrate-sensing area is the availability of fluorescent boronic acid reporter compounds that 1) change fluorescent properties upon binding, 2) are water soluble, 3) have high intrinsic affinity for diols, and 4) are chemically and photochemically stable. There have also been quite a few reviews[3,12] and recent research papers[13–27] on boronic acids that change their fluorescent properties upon binding to a nucleophilic/Lewis base analyte.

Despite the impressive progress made in the design and synthesis of fluorescent boronic acid reporter compounds, several key issues remain. First, most fluorescent boronic acid reporter compounds have low to modest intrinsic affinities for diol-containing compounds. Second, boronic acids are known to have high affinity for *cis*-diols on five-membered rings and in linear structures. Binding to six-membered ring diols has commonly believed to be very hard unless the six-membered ring is constrained to give an ab-

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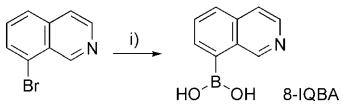
normally small dihedral angle.[3] This point is very important because biologically important cell-surface carbohydrate biomarkers only contain six-membered pyranose, but not five-membered furanose. Boronic acids that can bind to diols on pyranose sugars are very important for the design of sensors for carbohydrate biomarkers. It is our working hypothesis that because boronic acids are Lewis acids, they should interact with all nucleophiles/Lewis bases including diols on a six-membered ring under the appropriate conditions. Indeed, polystyrene-immobilized phenylboronic acid has been used for the separation of *cis*- and *trans*-cyclohexanediol,[28] indicating their interactions. Additional evidence comes from the binding studies with inositol.[29] Recently, Hall et al. reported a compound that can bind to a pyranose model (methyl α-D-glucopyranose).[20,30] The boronic acid binds to the 4,6-positions of a pyranose sugar. So far, to the best of our knowledge, there has been no report of monoboronic acid that can bind methyl α-D-glucopyranose with fluorescence intensity changes in an aqueous solution. Herein, we describe the very unique binding and fluorescent properties of a series of isoquinolinylboronic acids (Scheme 2), which show extraordinarily high binding affinities for carbohydrates. The 4- and 6-IQBA also showed detectable binding to methyl α-D-glucopyranose, with fluorescence changes upon binding. In addition, we also report the unique binding affinity of these boronic acids with *cis*-cyclohexanediol. These boronic acids represent the very first that can bind 1,2-diols on an unconstrained six-membered ring in aqueous solution at physiological pH with fluorescence intensity changes.

Results and Discussion

The 8-isoquinolinylboronic acid (8-IQBA) was synthesized through a one-step borylation reaction (Scheme 3) and all other isoquinolinylboronic acids were commercially available.



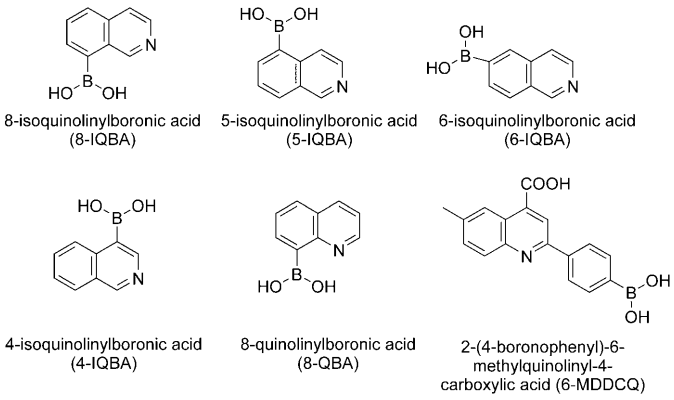
Scheme 3. i) *n*-Butyllithium, trimethyl borate, THF, −78 °C, 39 %.

Since a long-standing goal in our lab is the search for boronic acids that change their fluorescent properties upon binding, we examined whether these isoquinolinylboronic acids (IQBAs) would have such properties. Three representative sugars were used for this study: D-fructose, D-glucose, and D-sorbitol. It was found that all IQBAs changed their fluorescent properties upon sugar addition, though the direction and magnitude of the changes were different for the various boronic acids. For example, 8-IQBA showed a maximum of 35-fold increase in fluorescent intensity upon fructose addition at physiological pH in phosphate buffer (Figure 1 and Table 1). On the other hand, addition of sorbitol only induced a 1-fold fluorescent intensity increase and glucose addition induced a 60 % decrease in fluorescent intensity. The opposite direction of fluorescent intensity changes when fructose and glucose were added is something that had not been observed before, indicating the idiosyncratic nature of 8-IQBA in its fluorescent response upon binding. Interestingly, 5-IQBA showed similar properties in fluorescent responses upon sugar binding (Figure 2): fluorescent intensity increased with fructose or sorbitol addition and decreased with glucose addition. On the other hand, 4-IQBA only showed fluorescent intensity increases upon sugar additions (Figure 3), whereas 6-IQBA only showed fluorescent intensity decreases (Figure 4). The observed fluorescent changes upon sugar binding also allowed for the easy determination of the apparent binding constants of these boronic acids. Table 1 summarizes the results. The apparent association constants (K_a) with D-fructose, D-glucose, and D-sorbitol were 1493, 46, and 1588 M^{-1} for 8-IQBA; 1432, 42, and 2934 M^{-1} for 5-IQBA; 2170, 25, and 1001 M^{-1} for 4-IQBA; and 1353, 28, and 1620 M^{-1} for 6-IQBA, respectively. Several things are worth mentioning regarding these binding results. The first thing that stands out among all

Table 1. Apparent association constants (K_a) of isoquinolinylboronic acids with representative sugars.[a]

Isoquinolinyl boronic acids[a]	D-Fructose		D-Glucose		D-Sorbitol	
	K_a [M^{-1}]	$\Delta I_{max}/I_0$	K_a [M^{-1}]	$\Delta I_{max}/I_0$	K_a [M^{-1}]	$\Delta I_{max}/I_0$
8-IQBA	1493 ± 25	35	46 ± 12	−60 %	1588 ± 266	1
5-IQBA	1432 ± 242	9	42 ± 6	−60 %	2934 ± 61	1
4-IQBA	2170 ± 184	16	25 ± 7	2	1001 ± 10	2
6-IQBA	1353 ± 274	−60 %	28 ± 4	−82 %	1620 ± 247	−80 %
8-QBA	108[b]	47[b]	3 ± 2	11	616 ± 150	13

[a] Binding studies were conducted in phosphate buffer (0.1 M) at pH 7.4 (all experiments were duplicated). [b] Reference [31].



Scheme 2. Structures of isoquinolinylboronic acids, 8-QBA, and 6-MDDCQ.

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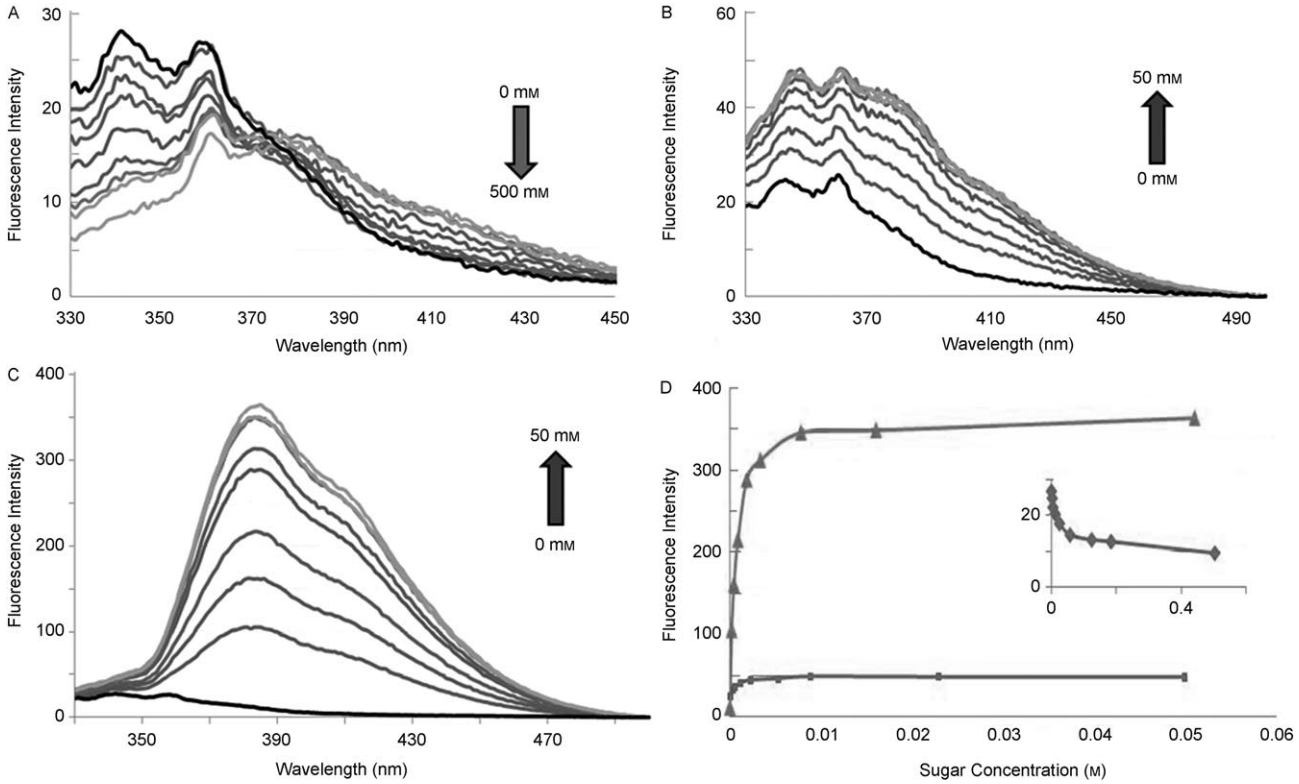

Figure 1. Fluorescent spectral changes of 8-IQBA upon addition of different diols in phosphate buffer (0.1 M) at pH 7.4: $\lambda_{ex}=322$ nm, $\lambda_{em}=361$ nm (for D-glucose and D-sorbitol) and $\lambda_{em}=383$ nm (for D-fructose). A) D-Glucose, B) D-sorbitol, C) D-fructose, and D) plots of the fluorescent intensity changes of 8-IQBA as a function of sugar concentration, [8-IQBA] $=1\times10^{-5}$ M (■ = D-sorbitol, ▲ = D-fructose, and ◆ = D-glucose). (Color version of Figures 2–10 are available in the Supporting Information).

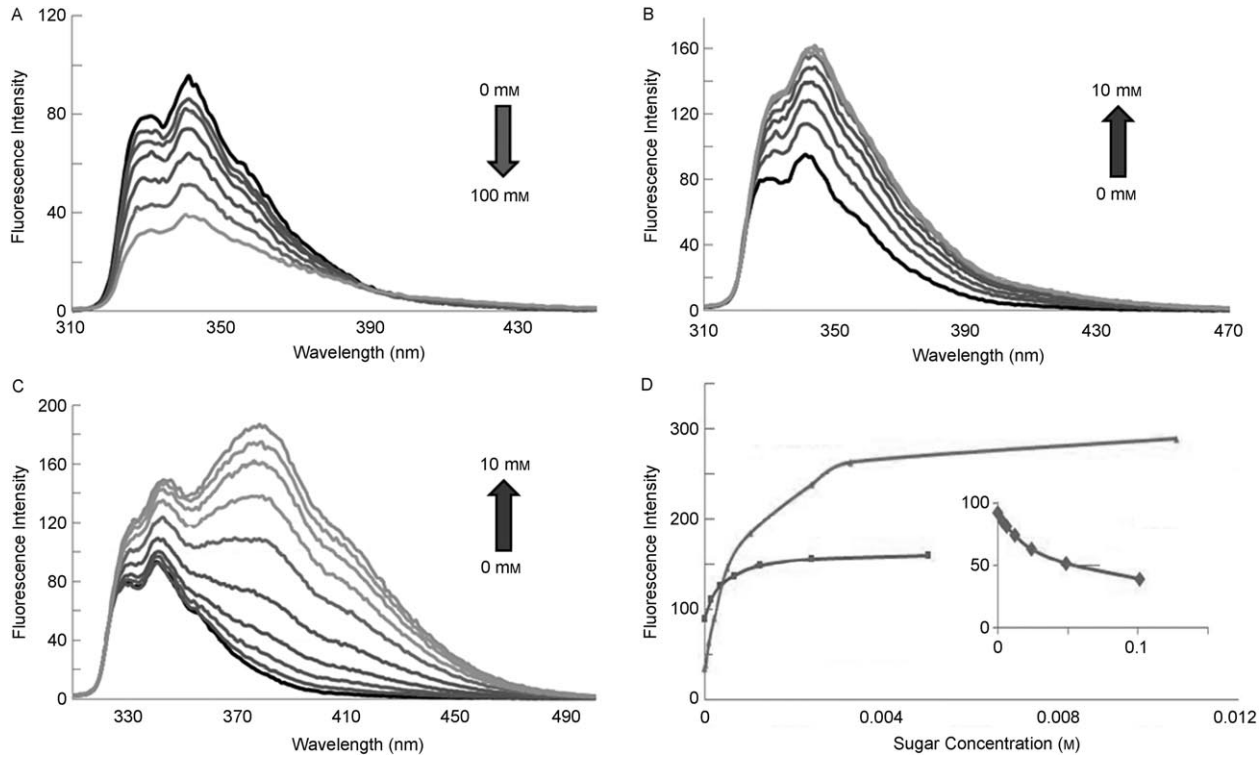

Figure 2. Fluorescent spectral changes of 5-IQBA upon addition of different diols in phosphate buffer (0.1 M) at pH 7.4: $\lambda_{ex}=272$ nm, $\lambda_{em}=342$ nm (for D-glucose), $\lambda_{em}=344$ nm (for D-sorbitol), and $\lambda_{em}=378$ nm (for D-fructose). A) D-Glucose, B) D-sorbitol, C) D-fructose, and D) plots of fluorescent intensity changes of 5-IQBA as a function of sugar concentration, [5-IQBA] $=1\times10^{-5}$ M (■ = D-sorbitol, ▲ = D-fructose, and ◆ = D-glucose).

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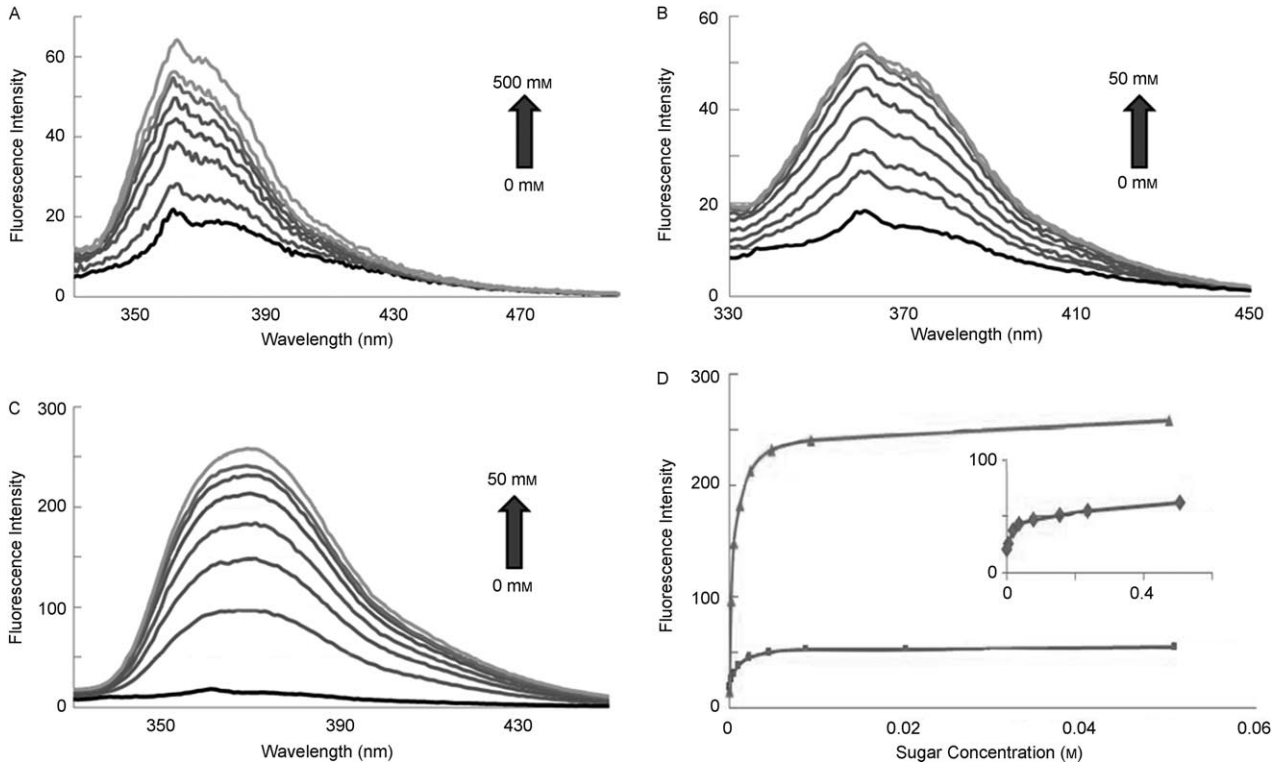

Figure 3. Fluorescent spectral changes of 4-IQBA upon addition of different diols in phosphate buffer (0.1 M) at pH 7.4: $\lambda_{ex}=322$ nm, $\lambda_{em}=361$ nm (for D-glucose, D-sorbitol,) and $\lambda_{em}=370$ nm (for D-fructose). A) D-Glucose, B) D-sorbitol, C) D-fructose, and D) plots of fluorescent intensity changes of 4-IQBA as a function of sugar concentration, [4-IQBA] $=1\times10^{-5}$ M (■ = D-sorbitol, ▲ = D-fructose, and ◆ = D-glucose).

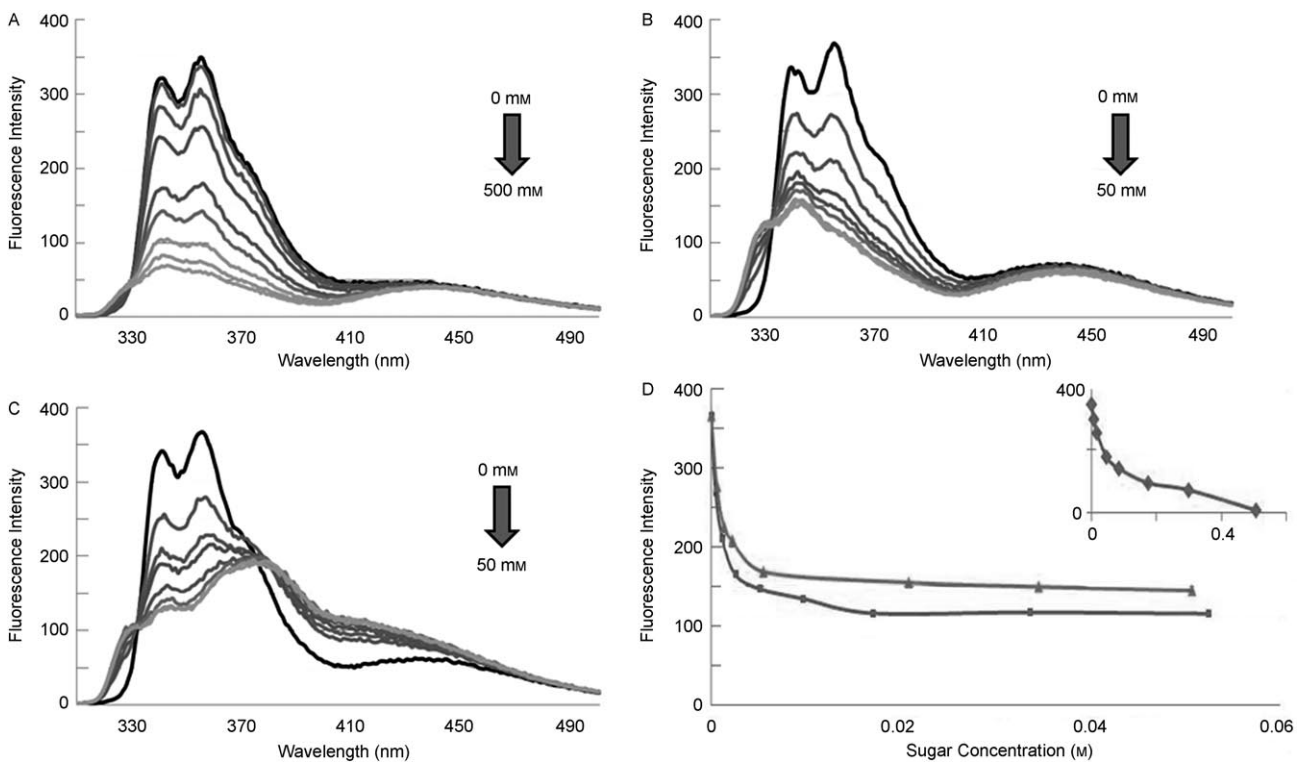

Figure 4. Fluorescent spectral changes of 6-IQBA upon addition of different diols in phosphate buffer (0.1 M) at pH 7.4: $\lambda_{ex}=272$ nm, $\lambda_{em}=356$ nm. A) D-Glucose, B) D-sorbitol, C) D-fructose, and D) plots of fluorescent intensity changes of 6-IQBA as a function of sugar concentration, [6-IQBA] $=1\times10^{-5}$ M (■ = D-sorbitol, ▲ = D-fructose, and ◆ = D-glucose).

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these binding constants is the extraordinarily high affinity of these isoquinolinylboronic acids for the monosaccharide studied. For example, the binding constants with D-fructose for all isoquinolinylboronic acids studied were in the range of 1353–2170 M^{-1}. In contrast, the binding constant for 8-QBA and D-fructose was 108 M^{-1}.[31] The difference is over 13-fold. Second, it is also interesting to note that these isoquinolinylboronic acids showed much higher affinity for glucose than phenylboronic acid.[21,22] For example, the apparent binding constants of 8-IQBA and 5-IQBA with glucose were 46 and 42 M^{-1}, respectively. In contrast, the binding constant of phenylboronic acid and glucose was about 5 M^{-1}. Third, the apparent association-constant trend for 4-IQBA followed the order D-fructose > D-sorbitol > D-glucose. This is different from the trend observed for other arylboronic acids,[10,32,33] which has the order D-sorbitol > D-fructose > D-glucose. Even in the case of 8-IQBA, its binding constants with sorbitol and fructose were essentially the same, which was unexpected. Fourth, the binding constants are not directly correlated with the intensity of fluorescent changes. This is also different from most of the fluorescent boronic acids that we have been reported, which show higher magnitude changes for tight binders.

Encouraged by the high affinity of these IQBAs, especially with glucose, we were interested in probing their ability to bind the pyranose form of a sugar. This interest stems from the fact that cell-surface carbohydrates only contain sugars in the pyranose form and one of the important goals in our carbohydrate sensor effort is the design and synthesis of probes for cell-surface carbohydrate biomarkers. However, the "general consensus" seems to be that arylboronic acids do not bind to vicinal diols on six-membered ring, and thus, application of boronic acids in recognizing glycans in mammalian systems would be difficult. To address this fundamental question, we also tested the binding affinities of the isoquinolinylboronic acids with methyl α-D-glucopyranose and *cis*-cyclohexanediol. The selection of methyl α-D-glucopyranose is to ensure that the sugar is in its cyclic form. However, the disadvantage is that the hydroxyl group at the 1 position is no longer available for binding. *cis*-Cyclohexanediol was selected as a representative six-membered *cis*-diol. The summary of the binding results is shown in Table 2, and Figures 5 and 6. Several special binding properties were observed. First, weak but encouraging binding with *cis*-cyclohexanediol was observed for 8-IQBA, 5-IQBA, and 4-IQBA, with the apparent association constants (K_a) being 0.4 (15-fold fluorescence intensity change), 1.1 (66% fluorescence intensity change), and 0.8 M^{-1} (6-fold fluorescence intensity change), respectively. In control experiments, cyclohexanol did not show appreciable binding or fluorescence changes when added to the same boronic acids. Second, these isoquinolinylboronic acids showed binding with a model glycoside, methyl α-D-glucopyranose, under physiologically relevant conditions. The apparent binding constants (K_a) for 4-IQBA and 6-IQBA were 3.3 and 2.1 M^{-1}, respectively. It should be noted that Hall et al. reported *ortho*-hydroxymethyl phenylboronic acid as binder for methyl α-D-glucopyranose.[20,30] However, this boronic acid does not change fluorescence upon binding. To the best of our knowledge, 4-IQBA and 6-IQBA are the very first examples of boronic acid derivatives that can bind to methyl α-D-glucopyranose with fluorescence changes.

As a control, we also studied the binding of these isoquinolinylboronic acids with cyclohexanol to see whether their apparent binding was due to interactions with a single hydroxyl group (boronic acid interactions with single hydroxyl groups do have precedents).[3] As can be seen from Table 2, 6-IQBA was found to bind *cis*-cyclohexanediol ($K_a = 1\ M^{-1}$) and cyclohexanol ($K_a = 4\ M^{-1}$) and the other IQBAs did not show any binding with cyclohexanol. Such results indicate that single-hydroxyl-group binding might play an important role in the binding of 6-IQBA with methyl α-D-glucopyranose and cyclohexanediol. At this time, it is not clear in which exactly way 6-IQBA binds to these six-membered-ring diols.

With the weak but encouraging binding with *cis*-cyclohexanediol for all the isoquinolinylboronic acids discussed above, it becomes important to address the question of whether the ability to bind *cis*-diols on a six-membered ring is unique to isoquinolinylboronic acids. As a control, phenylboronic acid should be considered first. Binding between phenylboronic acid and cyclohexanediol had been studied before and no binding was observed.[34] Such results indicate that the ability to bind to *cis*-diols on a six-membered ring is not universal to all boronic acids. Next, we studied the binding of 8-QBA and 6-MDDCQ (Scheme 2) with *cis*-cyclohexanediol. 8-QBA was selected because it is a quinolinylboronic acid, 6-MDDCQ was chosen because the boronic acid is attached to a phenyl ring but the compound also contains a quinoline moiety. Both boronic acids also showed binding affinities towards *cis*-cyclohexanediol (Figure 7). For 8-QBA, a 23-fold fluorescence intensity change was found upon the addition of 1.5 M *cis*-cylcohexanediol at physiological pH in phosphate buffer. These results indicated that binding of *cis*-cyclohexanediol is not a unique property of isoquinolinylboronic acids.

Table 2. Apparent association constants (K_a) of isoquinolinylboronic acids with representative carbohydrates.[a]

Isoquinolinyl boronic acids[a]	Methyl α-D-glucopyranose		*cis*-Cyclohexanediol		Cyclohexanol	
	K_a [M^{-1}]	$\Delta I_{max}/I_0$	K_a [M^{-1}]	$\Delta I_{max}/I_0$	K_a ([M^{-1}]	$\Delta I_{max}/I_0$
8-IQBA	not observed	–	0.4 ± 0.0	15	not observed	–
5-IQBA	not observed	–	1.1 ± 0.8	−66%	not observed	–
4-IQBA	3.3 ± 0.9	1	0.8 ± 0.2	6	not observed	–
6-IQBA	2.1 ± 1.4	−30%	1.0 ± 0.2	−71%	4 ± 1	3
8-QBA	not observed	–	1.2 ± 0.7	23	not observed	–
6-MDDCQ	not observed	–	2.0 ± 0.2	−75%	not observed	–

[a] Binding studies were conducted in phosphate buffer (0.1 M) at pH 7.4 (all experiments were duplicated).

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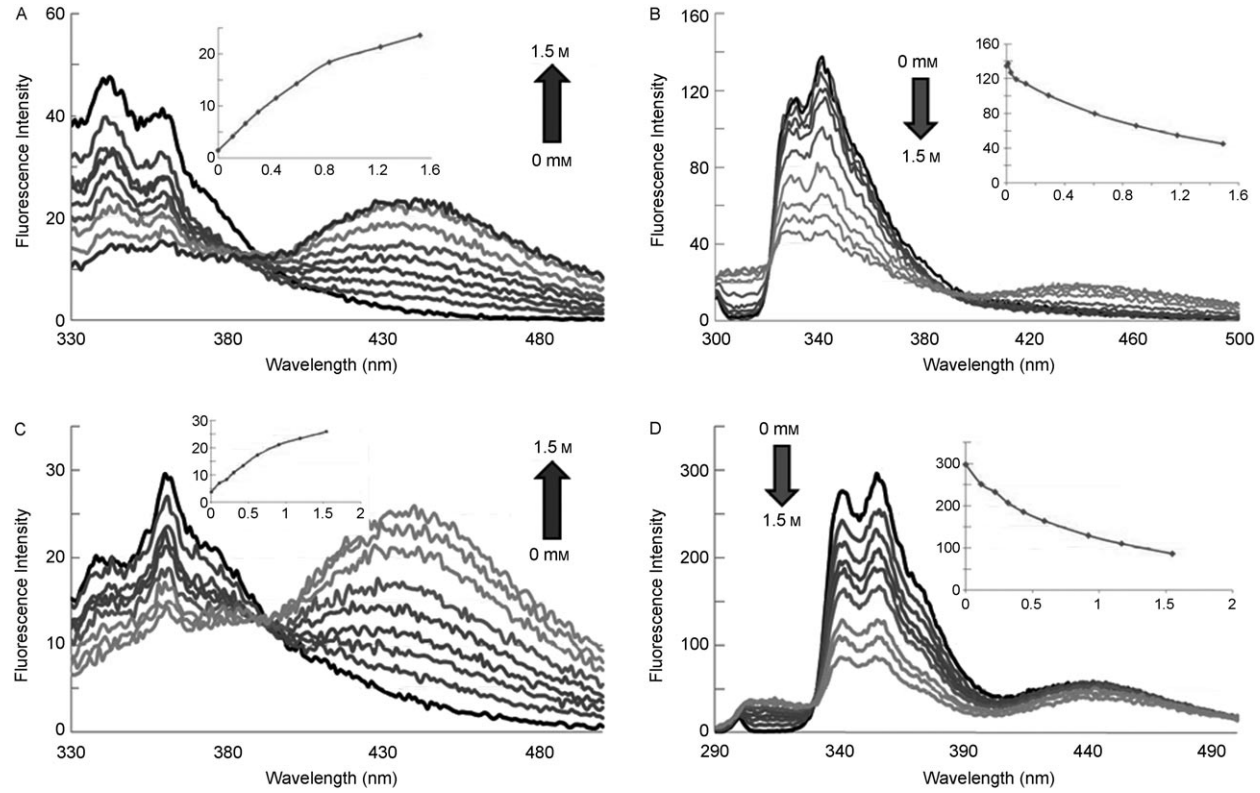

Figure 5. Fluorescent spectral changes of isoquinolinylboronic acids upon addition of *cis*-cyclohexanediol in phosphate buffer (0.1 M) at pH 7.4: A) 8-IQBA, $\lambda_{ex}=332$ nm, $\lambda_{em}=442$ nm, B) 5-IQBA, $\lambda_{ex}=272$ nm, $\lambda_{em}=341$ nm, C) 4-IQBA, $\lambda_{ex}=322$ nm, $\lambda_{em}=440$ nm, and D) 6-IQBA, $\lambda_{ex}=272$ nm, $\lambda_{em}=355$ nm. Insets: plots of fluorescent intensity changes of IQBAs as a function of sugar concentration, $[IQBAs]=1\times10^{-5}$ M.

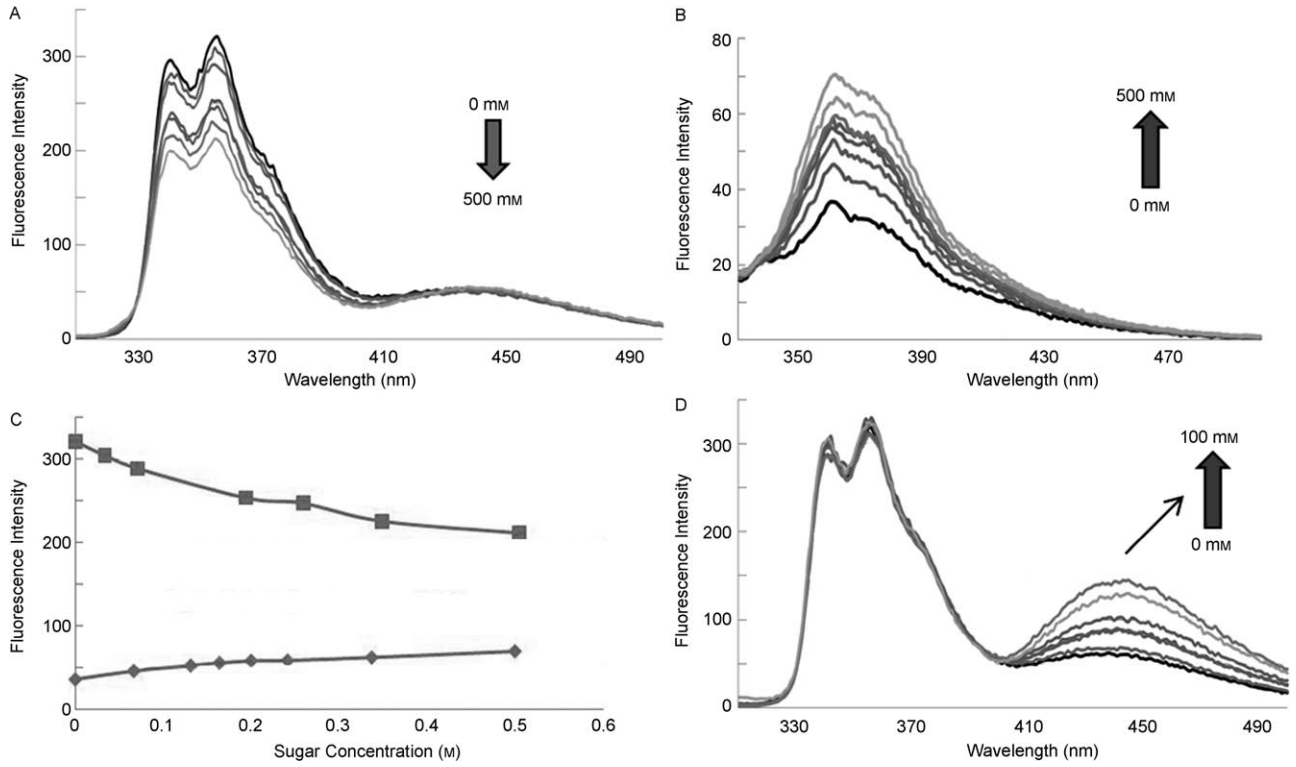

Figure 6. Fluorescent spectral changes of isoquinolinylboronic acids upon addition of methyl α-D-glucopyranose and cyclohexanol in phosphate buffer (0.1 M) at pH 7.4: A) 6-IQBA with methyl α-D-glucopyranose, $\lambda_{ex}=272$ nm, $\lambda_{em}=356$ nm, B) 4-IQBA with methyl α-D-glucopyranose, $\lambda_{ex}=322$ nm, $\lambda_{em}=361$ nm, C) plots of fluorescent intensity changes of 4-IQBA (◆) and 6-IQBA (■) as a function of methyl α-D-glucopyranose concentration, $[IQBAs]=1\times10^{-5}$ M, and D) 6-IQBA with cyclohexanol, $\lambda_{ex}=272$ nm, $\lambda_{em}=455$ nm.

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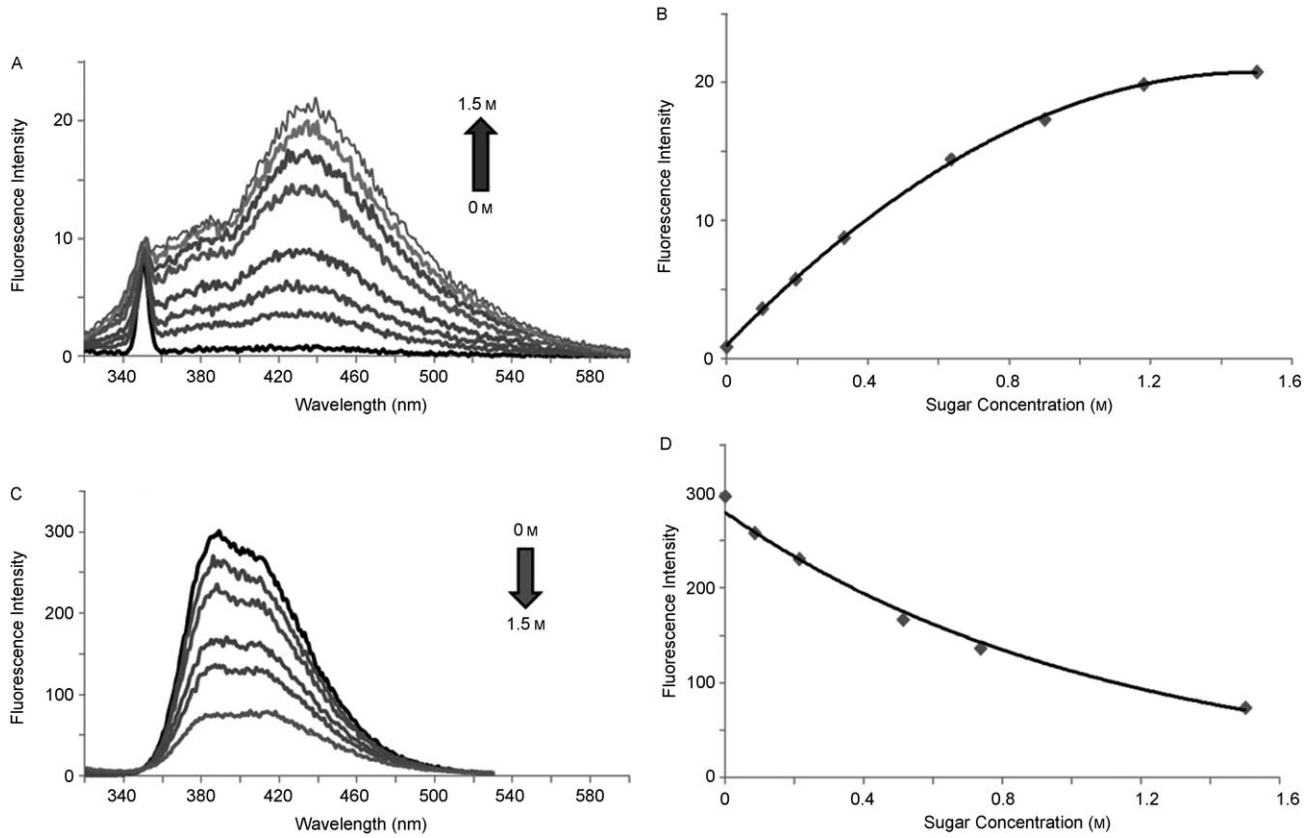

Figure 7. Fluorescent spectral changes of 8-QBA and 6-MDDCQ upon addition of *cis*-cylcohexanediol in phosphate buffer (0.1 M) at pH 7.4: A) 8-QBA: $\lambda_{ex}=270$ nm, $\lambda_{em}=314$ nm, B) plot of fluorescent intensity change of 8-QBA as a function of sugar concentration, [8-QBA] $=1\times10^{-5}$ M, C) 6-MDDCQ: $\lambda_{ex}=270$ nm, $\lambda_{em}=387$ nm, and D) plot of fluorescent intensity change of 6-MDDCQ as a function of sugar concentration, [6-MDDCQ] $=2\times10^{-5}$ M.

One cautionary note related to all the binding constants of diols on six-membered rings is their small magnitude, which could severely affect the accuracy of the determination. The other issue to consider is the change of the bulk properties of the solvent due to diol addition at high concentrations, which could affect fluorescence. This aspect is especially important for 6-IQBA because it showed tighter "binding" with cyclohexanol than with *cis*-cyclohexanediol. To specifically differentiate the effect of bulk properties and binding on fluorescence and to probe whether the observed fluorescent changes were due to specific binding, we conducted additional experiments to study the dependence of the fluorescent changes of the concentration of the boronic acid (6-IQBA, 10-30 μM) in the presence and absence of *cis*-cyclohexanediol (0.75 M). Figure 8 clearly shows that the fluorescence intensity increases have linear relationships to the concentration of 6-IQBA in the absence of *cis*-cyclohexanediol. On the other hand, significant curvature was observed in the presence of 0.75 M *cis*-cyclohexanediol. Such results cannot be explained by the bulk effect of the added sugar and are consistent with specific binding interactions, which are concentration dependent. Bulk effect of the added sugar on fluorescent properties should only result in two parallel lines with different intercepts.

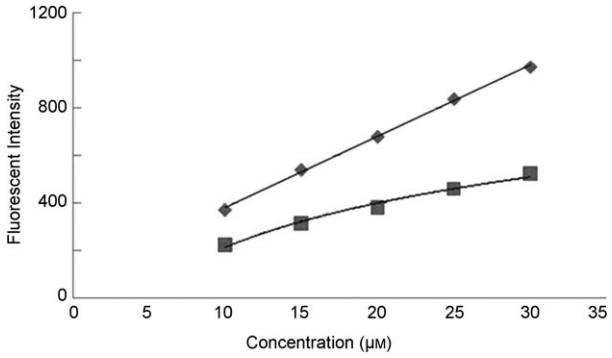

Figure 8. Relationship between the fluorescent intensity change and the concentration of 6-IQBA in the presence (■) and absence (◆) of *cis*-cyclohexanediol (0.75 M) in phosphate buffer (0.1 M) at pH 7.4: $\lambda_{ex}=272$ nm, $\lambda_{em}=354$ nm.

Quantum yields and pK_a: Aimed at understanding the basic mechanism through which fluorescent intensity changes occur, we also studied the pH profiles of the fluorescence intensity in the absence and presence of sugars (Table 3 and Figure 9). As can be seen in Figure 9, fluorescence intensities change with pH for both the boronic acids and their presumed esters with various sugars. Numerous previous reports have demonstrated that such fluorescent changes are

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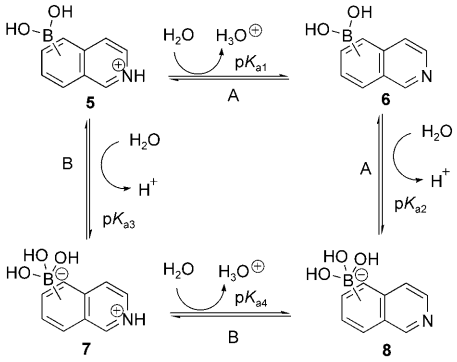
Table 3. Apparent pK_a values of the isoquinolinylboronic acids in the absence and presence of sugars.[a]

	p*K*a				
	No diol	D-Fructose	D-Glucose	D-Sorbitol	Methyl α-Dglucopyranose
8-IQBA	5.7	4.1, 7.2	4.8, 7.5	4.1; 7.3	–
5-IQBA	5.9, 8.5	6.9	4.9, 6.8	6.8	–
4-IQBA	5.0	3.4, 7.6	4.4	6.0	5.7
6-IQBA	5.4, 7.7	4.2, 6.8	4.8, 7.0	3.8, 6.6	5.1, 7.4
8-QBA	4,[b] 10[b]	2.5,[b] 9[b]	ND[c]	ND[c]	ND[c]

[a] All experiments were duplicated. [b] Reference [31]. [c] Not determined.

associated with the pK_a of the individual species. Because each boronic acid has two ionizable functional groups, the boronic acid group and the isoquinolinyl nitrogen, there is the question which pK_a corresponds to each fluorescent change.

As shown in Schemes 4 and 5, there are two possible routes for the ionization steps of IQBAs and their esters. For example, in route A (Scheme 4), pK_{a1} is assigned to the deprotonation of the isoquinolinium nitrogen and pK_{a2} is assigned to the hybridization-state change of the boronic acid group, whereas in route B, pK_{a3} is assigned to the hybridization-state change of the boronic acid group and pK_{a4} is assigned to the deprotonation of the isoquinolinium nitrogen. In order to assign each pK_a, we recorded the ^{11}B NMR spectra of the IQBAs and their esters in a mixed deuterated methanol/water (1:1) mixture at different pH values. Because it has been reported that a 1:1 methanol/water solution resulted in minimal changes of the solution pH, methanol was used to increase the boronic acid solubility.[31,35,36] The results are shown in Table 4. In the case of 8-IQBA, the boron signal ap-



Scheme 4. Proposed ionization steps of the IQBAs.

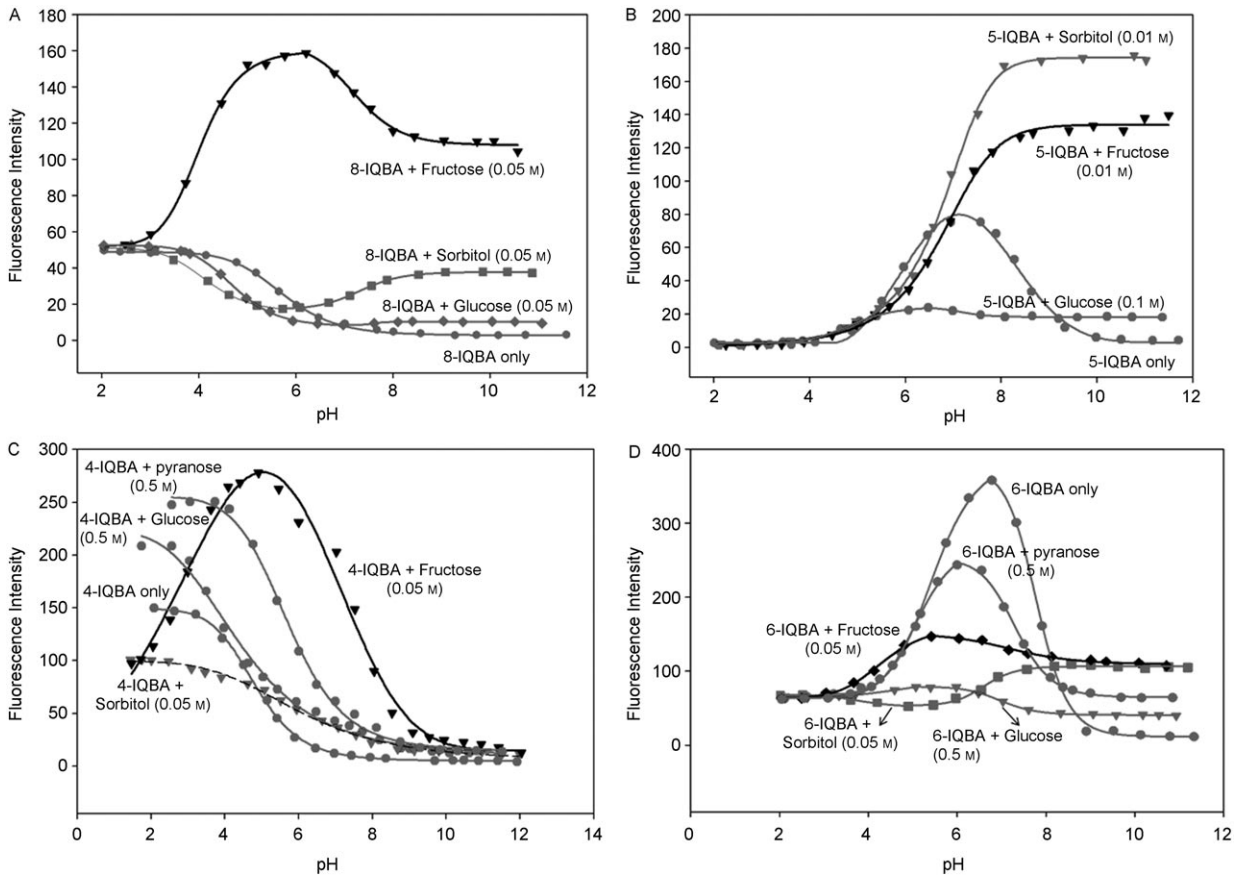


Figure 9. pH profiles of the fluorescence intensities of isoquinolinyl boronic acids in the absence and presence of sugars in aqueous phosphate buffer (0.1 M): A) [8-IQBA] = 1×10^{-5} M, B) [5-IQBA] = 1×10^{-5} M, C) [4-IQBA] = 1×10^{-5} M, and D) [6-IQBA] = 1×10^{-5} M.

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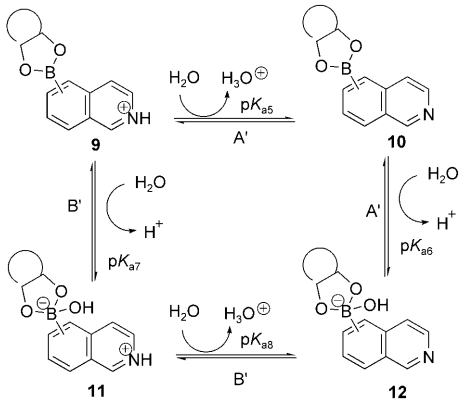

Scheme 5. Proposed ionization steps of the esters of the IQBAs.

Table 4. ^{11}B NMR shifts of the isoquinolinyl boronic acids in the absence and the presence of fructose.

Entry	δ [ppm]/pH-1	δ [ppm]/pH-2	δ [ppm]/pH-3
8-IQBA[a]	28.9/1.3	29.5/7.3	3.2/11.9
8-IQBA and fructose[b]	29.3/1.3	8.2/7.2	7.6/11.1
5-IQBA[a]	30.1 and 20.3/1.6	29.3 and 19.3/7.3	4.6/12.8
5-IQBA and fructose[b]	29.7/2.1	7.3/7.6	10.2/11.7
4-IQBA[a]	28.1 and 19.1/1.5	24.6 and 19.2/5.7	3.5 and 2.3/12.2
4-IQBA and fructose[b]	20.4/2.0	7.6 and 11.1/6.6	7.6/12.0
6-IQBA[a]	27.8 and 18.9/1.6	28.3 and 18.9/7.6	2.6/12.6
6-IQBA and fructose[b]	28.8 and 18.8/1.5	20.7 and 9.0/5.7	8.0/12.1

[a] [IQBAs] = 30 mM. [b] [fructose] = 50 mM.

peared at $\delta = 28.9$ and 29.5 ppm at pH 1.3 and pH 7.3, respectively, consistent with the neutral trigonal-coordinated boron (**5** or **6**). At pH 11.9, the boron signal was observed at $\delta = 3.2$ ppm, indicating the presence of the anionic tetrahedral state **8**. These results indicated that the boron atom of the free acid changed hybridization from sp^2 to sp^3 between pH 7.3 and 11.9. So pK_{a1} was assigned to 5.7 and pK_{a2} was assigned to >7, which was consistent with route A (Scheme 4). The esters of 8-IQBA all have two pK_a values based on the fluorescent data. The only difference is that the fluorescence of the fructose ester increases first, and then decreases. On the other hand, the fluorescence of the glucose and sorbitol ester only decreases slightly, and then increases. So it is reasonable to assign the pK_a values for all the esters of 8-IQBA in the same way. ^{11}B NMR spectra of the fructose ester of 8-IQBA were studied as a model case. Single peaks at $\delta = 29.3$ and 8.2 ppm at pH 1.3 and 7.2, respectively, were observed. These chemical shifts clearly indicated that the boron atom in the ester form changes its hybridization state between pH 1.3 and 7.2. Based on this, pK_{a7} was assigned to 4.1 and pK_{a8} was assigned to 7.2, consistent with route B′ (Scheme 5). Such results indicate that the pK_a of the boronic acid group is higher in the absence of a sugar, but lower in the presence of a sugar than that of the protonated quinolinium group. Such a switch of the pK_a seems to correspond to the highest fluorescence intensity change at pH 6 (Figure 9A), and suggests that the zwitterionic specie **11** (Scheme 5) is the more fluorescent one. At pH 7.4, the 8-IQBA fructose ester exists predominantly in the boronate form **12** and 8-IQBA itself exists as a mixture of the neutral trigonal-coordinated boron form **6** and the boronate form **8**. Both, **6** and **8**, are almost non-fluorescent, and yet the boronate ester form **12** is fluorescent. It seems that the fluorescence increases due to diol binding, which was also observed for 8-QBA.[31] All the pK_a values of other IQBAs in the absence and presence of a sugar were assigned by the same method. The results showed that the pK_a values are similar to those of 8-IQBA, going through route A with IQBA only and route B′ in the presence of a sugar. It should be noted that these pK_a assignments are opposite to those found for 8-QBA[31] and consistent with those found for 5-QBA.[36]

In the case of the fructose ester 5-IQBA at pH 7.4, it was found that it predominantly exists in a mixture of the zwitterionic quinolinium boronate form **11** and the boronate form **12**. 5-IQBA itself also exists in the neutral trigonal-coordinated boron form **6**. It should be pointed out that the boronate form **8** of 5-IQBA is non-fluorescent, whereas the boronate form **12** of the 5-IQBA fructose ester is the most fluorescent species. In the case of 4-IQBA at pH 7.4, its fructose ester exists predominantly in the zwitterionic quinolinium boronate form **11**, and 4-IQBA itself exists in a mixture of the neutral trigonal-coordinated boron form **6** and the boronate form **8**. Both, **6** and **8**, are non-fluorescent, and yet the zwitterionic quinolinium boronate form **11** is fluorescent. Therefore, it appears that the fluorescence increase is due to diol binding as found for 8-QBA. Finally, at pH 7.4, the fructose ester of 6-IQBA exits predominantly in the boronate form **12**, whereas 6-IQBA itself exits in the neutral trigonal-coordinated boron form **6**. Although **6** and **12** are fluorescent, the neutral trigonal-coordinated boron form **6** seems to have higher fluorescence intensity than **12**. This might explain the fluorescence intensity decrease after binding of fructose. It should be mentioned that in some cases two peaks were found for the IQBA or its ester. For example, the ^{11}B NMR spectrum of 4-IQBA shows two peaks at $\delta = 28$ and 19 ppm. It is possible that the second peak corresponds to the methyl ester group of the boronic acid. This is reasonable because a solvent mixture of deuterated methanol/water (1:1) was used to increase the solubility of the boronic acid. Another possibility is the formation of a cyclic dimeric boronic anhydride (-O-B-O-B-O-) through a 2:1 boronic acid/diol binding mode. However, the likelihood of this cyclic structure is probably high only in an organic solvent, but not in aqueous solution.

The fluorescent quantum yields for these boronic acids and their sugar esters were determined with 4-indolylboronic acid as the reference compound[37] and by using Equation (1),[38] where Q represents the quantum yield, I is the integrated intensity, OD is the optical density, and a subscript R denotes the reference compound. The results are

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Table 5. Fluorescence quantum yields of the isoquinolinylboronic acids alone and in the presence of various sugars.[a]

Entry	Q [%]				
	Isoquinolinylboronic acid	D-Fructose	D-Glucose	D-Sorbitol	Methyl α-D-glucopyranose
8-IQBA	2.2±0.02	24±0.8	2.1±0.2	6.9±0.6	–
5-IQBA	2.5±0.02	19±0.01	3.7±0.4	7.7±2.0	–
4-IQBA	1.0±0.08	17±0.01	1.7±0.2	2.5±0.7	3.4±0.02
6-IQBA	13.2±3.2	12±0.3	4.8±0.9	10.1±0.7	1.9±0.2
8-QBA	ND	28[b]	ND[c]	ND[c]	ND[c]

[a] All experiments were duplicated. [b] Reference [31]. [c] Not determined.

shown in Table 5 (see the Supporting Information for a general calculation).

$$Q = Q_R(I/I_R)(OD_R/OD) \quad (1)$$

The quantum-yield trend of 8-IQBA and its esters followed the order of D-fructose ester > D-sorbitol ester > 8-IQBA alone > D-glucose ester. For example, the quantum yield of the D-fructose ester of 8-IQBA is 24%, whereas that of 8-IQBA alone and its D-glucose ester is only about 2%, giving about a 12-fold difference. In the case of other isoquinolinylboronic acids, the following orders were observed for the respective apparent quantum yield: D-fructose ester > D-sorbitol ester > D-glucose ester > 5-IQBA alone, D-fructose ester > methyl α-D-glucopyranose ester > D-sorbitol ester > D-glucose ester > 4-IQBA alone, and 6-IQBA alone > D-fructose ester > D-sorbitol ester > D-glucose ester > methyl α-D-glucopyranose. All the isoquinolinylboronic acids show different trends that are not directly correlated with the apparent pK_a of each compound. This is understandable because many other factors such as flexibility, solvation, and excited-state electron density distribution are expected to affect the quantum yields of these compounds.

Conclusion

In conclusion, we have described a series of water-soluble isoquinolinylboronic acids that change their fluorescent properties significantly upon binding of diol-containing compounds. These isoquinolinylboronic acids bind to three representative sugars, D-fructose, D-glucose, and D-sorbitol, much more tightly than 8-QBA and most other simple arylboronic acids. Besides, all the isoquinolinylboronic acids, especially 5-IQBA and 8-IQBA, showed modest binding affinities with D-glucose (K_a = 42 and 46 M^{-1}, respectively). These numbers are much higher than that observed with phenylboronic acid.[21,22] All isoquinolinylboronic acids also showed weak but encourage binding affinity with *cis*-cyclohexanediol indicated by significant fluorescence changes. These are the very first examples for the binding of boronic acids with a vicinal diol on a six-membered ring observed by fluorescence intensity changes. Also very significant are the findings that 4-IQBA and 6-IQBA can complex methyl α-D-glucopyranose (K_a = 3 and 2 M^{-1}, respectively) under physiologically relevant conditions. These findings are especially important because carbohydrates in glycoproteins, glycolipids, and lipopolysaccharides contain almost universally six-membered ring sugars and linear diols, and one area that has not been widely recognized is the potential to take advantage of the interactions between hydroxyl groups on six-membered rings with a boronic acid for boronolectin design.

Experimental Section

^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer in deuterated DMSO with tetramethylsilane (TMS) (0.00 ppm) or as the internal reference unless otherwise specified. All boronic acids were provided by Frontier Scientific except 8-IQBA, which was prepared from reagent grade starting material as purchased from Aldrich or Acros unless otherwise noted. Absorption spectra were recorded on a Shimadzu UV-1700 UV/Vis spectrophotometer. Fluorescence spectra were recorded on a Shimadzu RF-5301 PC spectrofluorometer, and quartz cuvettes were used in all fluorescent and UV studies. All pH values were determined by a UB-10 Ultra Basic Benchtop pH meter (Denver Instrument). Sugars, buffer ingredients and chemicals were bought from Aldrich or Acros, unless noted otherwise and were used as received. Water used for the binding studies was doubly distilled and further purified with a Milli-Q filtration system.

Synthesis of 8-isoquinolinylboronic acid (8-IQBA): Anhydrous THF (0.5 mL) was added to a flask charged with 8-bromoisoquinoline (20 mg, 0.096 mmol, 1 equiv) under a nitrogen atmosphere. The mixture was cooled to −78°C and *n*-butyllithium (2.0 M solution in pentane, 0.2 mL, 0.4 mmole, 4 equiv) was added. The solution was stirred at −78°C for 45 min. After trimethyl borate (0.05 mL, 0.45 mmol, 4.7 equiv) was added, the reaction mixture was stirred at −78°C for another 5 min. Then the reaction mixture was allowed to warm to room temperature and was stirred an additional hour. Water (0.5 mL) and a saturated aqueous solution of $NaHCO_3$ (1.0 mL) were added to quench the reaction. The mixture was extracted with ethyl acetate (2×30 mL), washed with water (2×5 mL) and brine (2×5 mL), and dried over anhydrous Na_2SO_4. The residue was purified by column chromatography ($MeOH/CH_2Cl_2$ = 5:1) to yield a brown solid (6.5 mg, 39%). ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 9.7 (s, 1H), 8.6 (s, 2H), 8.5 (d, J = 5.6 Hz, 1H), 8.0 (d, J = 8.0 Hz, 1H), 7.9 (d, J = 6.4 Hz, 1H), 7.8 (d, J = 5.6 Hz, 1H), 7.7 ppm (dd, J = 6.4, 8.0 Hz, 1H); ^{13}C NMR (100 Hz, [D_6]DMSO): δ = 153.0, 142.2, 135.1, 133.7, 130.8, 129.6, 127.8, 120.8 ppm; MS (ESI): m/z (%): 174 [M+1]; HRMS: m/z: calcd for $C_9H_9BNO_2$: 174.0726; found: 174.0735.

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