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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 isoquinolinyboronic acids that have extraordinarily high affinities for diol-containing compounds at physiological pH. In addition, 5- and 8-isoquinolinyboronic acids also showed fairly high binding affinities with D-glucose ($K_a = 42$ and 46 M^{-1} , respectively). For the very first time, weak but encouraging binding with *cis*-cyclohexanediol was found for these boronic acids. Such binding was coupled with significant fluorescence changes. Furthermore, 4- and 6-isoquinolinyboronic acids also showed ability to complex methyl- α -D-glucopyranose ($K_a = 3$ and 2 M^{-1} , respectively).

Keywords

isoquinolinyboronic acids; fluorescence change; methyl- α -D-glucopyranose; *cis*-cyclohexanediol

Introduction

Boronic acids are commonly used in chemosensor design due to their intrinsic binding affinities with diols (Scheme 1), aminoalcohols, α -aminoacids, 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 Czarnik⁵ and Shinkai⁶ in the early 1990s, research in this area has undergone some transformations, going from binding with simple monosaccharides 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.

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^{12,3} and recent research papers^{13-14,15-27} on boronic acids that change fluorescent properties upon binding to a nucleophilic/Lewis base analyte.

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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 abnormally 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 since boronic acids are Lewis acids, under the appropriate conditions they should interact with all nucleophiles/Lewis bases including diols on a six-membered-ring. 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 inositols.²⁹ Recently, the Hall lab 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 our best 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 series of isoquinolinyboronic acids (Figure 1), which show extraordinarily high binding affinities for carbohydrates. 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

8-Isoquinolinyboronic acid (8-IQBA) was synthesized through a one-step borylation reaction (Scheme 2) and all other isoquinolinyboronic acids were commercially available.

Since a long-standing goal in our lab is the search for boronic acids that change fluorescent properties upon binding, we examined whether these isoquinolinyboronic 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 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 2 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 directions of fluorescent intensity changes when fructose and glucose were added were something that had not been observed before, indicating the idiosyncratic nature of 8-IQBA in its fluorescent response to binding. Interestingly, 5-IQBA showed similar properties in fluorescent responses upon sugar binding (Figure 3): 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 4), while 6-IQBA only showed fluorescent intensity decreases (Figure 5). 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

these binding constants is the extraordinarily high affinity of these isoquinolinyboronic acids for the monosaccharides studied. For example, the binding constants with fructose for all isoquinolinyboronic acids studied were in the range of $1353\text{--}2170\text{ M}^{-1}$. In contrast, the binding constant between 8-QBA and fructose was 108 M^{-1} .³¹ The difference is over 13-fold. Second, it is also interesting to note that these isoquinolinyboronic 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 between phenylboronic acid and glucose was about 5 M^{-1} . Third, the apparent association constants trend with 4-IQBA followed the order of D-fructose > D-sorbitol > D-glucose. This is different from that of other arylboronic acids,^{32,10,33} which has the order of 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 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 the ability for them to bind with 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 isoquinolinyboronic 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 1-position hydroxyl group is no longer available for binding. *cis*-Cyclohexanediol was selected as a representative of *cis*-diols on a six-membered ring. The summary of the binding results are shown in Table 2, and Figures 6 and 7. 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 acid solutions. Second, these isoquinolinyboronic 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 and 2 M^{-1} , respectively. It should be noted that the Hall lab has reported *ortho*-hydroxymethyl phenylboronic acid as binders for methyl- α -D-glucopyranose.^{20,30} However, they do not change fluorescence upon binding. To our best knowledge, these 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 isoquinolinyboronic acids with cyclohexanol in order 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 both *cis*-cyclohexanediol ($K_a = 1\text{ M}^{-1}$) and cyclohexanol ($K_a = 4\text{ M}^{-1}$) and the others 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 as to exactly which 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 with 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 (Figure 1) with *cis*-cyclohexanediol. 8-QBA was selected since it is a quinolinylboronic acid. 6-MDDCQ was selected because the boronic acid is attached to phenyl ring but the compound also contains a quinoline moiety. Both boronic acids also showed binding affinities with *cis*-cyclohexanediol (Figure 8). For 8-QBA, a 23-fold fluorescence change was found upon the addition of 1.5 M *cis*-cyclohexanediol at physiological pH in phosphate buffer. These results indicated that binding with *cis*-cyclohexanediol is not a unique binding affinity of isoquinolinylboronic acids.

One cautionary note related to all the binding constants of six-membered diols is their small magnitude, which could severely affect the accuracy of determination. The other issue to consider is the change of solvent bulk properties 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 boronic acid-concentration (6-IQBA, 10–30 μ M) dependent fluorescent changes in the presence and absence of 0.75 M *cis*-cyclohexanediol. From Figure 9, it can be seen clearly that the fluorescence intensity increases have linear relationships with 6-IQBA concentrations in the absence of added *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.

p*K*_a and quantum yields

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 10). As can be seen in Figure 10, 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 associated with the p*K*_a of individual species. Since each boronic acid has two ionizable functional groups, the boronic acid group and the isoquinolinyl nitrogen, there is the question of which p*K*_a each fluorescent change corresponds to.

As shown in Schemes 3 and 4, there are two possible routes for the ionization steps of IQBAs and their esters. For example, in route A, p*K*_{a1} was assigned to the deprotonation of isoquinolinium nitrogen and p*K*_{a2} was assigned to the hybridization state change of the boronic acid group, while in route B, p*K*_{a3} was assigned to the hybridization state change of the boronic acid group and p*K*_{a4} was assigned to the deprotonation of isoquinolinium nitrogen. In order to assign each p*K*_a, we recorded the ¹¹B NMR spectra of IQBAs and their esters in a mixed deuterated methanol-water (1:1) solvent at different pH values. Since it has been reported that 50% methanol 50% 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 of 8-IQBA appeared at 28.9

ppm at pH 1.3 and 29.5 at pH 7.3 respectively, consistent with the neutral trigonal boron (**5** or **6**). At pH 11.9, the boron signal of 3.2 ppm was observed 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 5.7 was assigned to pK_{a1} and pK_{a2} was assigned to be >7 , which was consistent with route A (Scheme 3). 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 glucose ester and sorbitol ester only decreases slightly, and then increases. So it is reasonable to assign ^{11}B the pK_a values for all the esters of 8-IQBA in the same way. NMR spectra of the fructose ester of 8-IQBA were studied as a model case. Single peaks of 29.3 ppm at pH 1.3 and 8.2 ppm at pH 7.2 were observed respectively. These chemical shifts clearly indicated that the boron atom in the ester form changes hybridization state between pH 1.3 and 7.2. Based on this, 4.1 was assigned to pK_{a7} and 7.2 was assigned to pK_{a8} , consistent with route B' (Scheme 4). 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 pK_a -switch seems to correspond to the highest fluorescence intensity change at pH 6 (Figure 10A), and suggests that the zwitterionic specie **11** (Scheme 4) 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 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 increase is due to diol binding as that of 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 they have similar pK_a assignments as 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 of 8-QBA³¹ and consistent with those of 5-QBA described in previous reports.³⁶

In the case of 5-IQBA, at pH 7.4, 5-IQBA fructose ester exists predominantly in a mixture of zwitterionic quinolinium boronate form **11** and boronate form **12**. 5-IQBA itself also exists in the neutral trigonal boron form **6**. It should be pointed out that the boronate form **8** of 5-IQBA itself is non-fluorescent, while the boronate form **12** of 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 neutral trigonal boron form **6** and boronate form **8**. Both **6** and **8** are non-fluorescent, and yet the zwitterionic quinolinium boronate form **11** is fluorescent. So it also seems that the fluorescence increase is due to diol binding as is that of 8-QBA. Finally, at pH 7.4, 6-IQBA fructose ester exists predominantly in the boronate form **12**, while 6-IQBA itself exists in the neutral trigonal boron form **6**. Although both **6** and **12** are fluorescent, the neutral trigonal boron form **6** seems to have higher fluorescence intensity than **12**. This might explain the fluorescence intensity decrease after binding with fructose. One thing needs to be mentioned here is that in some cases two peaks were found for IQBA or its ester. For example, the ^{11}B NMR spectrum of 4-IQBA shows two peaks at 28 and 19 ppm. It is possible that the second peak might be the methyl ester of boronic acid. This is reasonable since a mixed deuterated methanol-water (1:1) solvent was used to increase the boronic acid solubility. Another possibility is the formation of a cyclic dimeric boronic anhydride -O-B-O-B-O- via 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 compound³⁷ and using Eq. 1,³⁸ where Q represents quantum yield, I is the integrated intensity, OD is the optical density,

and subscript R denotes reference compound. The results are shown in Table 5 (see Support Information for general calculation).

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

The quantum yields 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%, while 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 isoquinolinyboronic 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 isoquinolinyboronic acids have different trends and are not directly correlated with the apparent pK_a of each compound. This is understandable since many other factors such as flexibility, solvation, and excited state electron density distribution are expected to affect the quantum yields of these compounds as well.

Conclusion

In conclusion, we have described a series of water-soluble isoquinolinyboronic acids that change fluorescent properties significantly upon binding. These isoquinolinyboronic 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 isoquinolinyboronic 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. All isoquinolinyboronic acids also showed weak but encourage binding affinity with *cis*-cyclohexanediol with significant fluorescence changes. These are the very first examples of the binding of boronic acids with a six-membered vicinal diol with fluorescence intensity change. 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. The above findings are especially important because carbohydrates found in glycoproteins, glycolipids, and lipopolysaccharides are 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 ring with a boronic acid for boronolactin design.

Experimental Section

Synthesis of 8-isoquinolinyboronic acid (8-IQBA)

To a flask charged with 8-bromoisoquinoline (20 mg, 0.096 mmol, 1 equiv) in a nitrogen atmosphere was added anhydrous THF (0.5 mL). The mixture was stirred at -78°C . *n*-Butyllithium (2.0 M solution in pentane, 0.2 mL, 0.4 mmole, 4 equiv) was added, then the solution was stirred at -78°C for 45 min. After adding trimethyl borate (0.05 mL, 0.45 mmol, 4.7 equiv), the reaction mixture was stirred at -78°C for another 5 min. Then the reaction was allowed to room temperature and left to react for an additional hour. H_2O (0.5 mL) and saturated NaHCO_3 (1.0 mL) was added to quench the reaction. The mixture was extracted with ethyl acetate ($2 \times 30 \text{ mL}$), washed with H_2O ($2 \times 5 \text{ mL}$), and brine ($2 \times 5 \text{ mL}$), and dried over anhydrous Na_2SO_4 . The residue was purified by chromatography ($\text{MeOH}/\text{CH}_2\text{Cl}_2 = 5/1$) to yield a brown solid (6.5 mg, 39%). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 89.7 (1H, s), 8.6 (2H, s), 8.5 (1H, d, $J = 5.6 \text{ Hz}$), 8.0 (1H, d, $J = 8.0 \text{ Hz}$), 7.9 (1H, d, $J =$

6.4 Hz), 7.8 (1H, d, $J = 5.6$ Hz), 7.7 (1H, dd, $J = 6.4, 8.0$ Hz); ^{13}C NMR (100 Hz, DMSO- d_6) δ 153.0, 142.2, 135.1, 133.7, 130.8, 129.6, 127.8, 120.8; ESI-MS, m/z 174, $M+1$; HRMS, 174.0735.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Structures of isoquinolinyboronic acids, 8-QBA and 6-MDDCQ.

Figure 2.

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); 383 nm (for D-fructose). (A) D-Glucose; (B) D-Sorbitol; (C) D-Fructose; (D) plots of fluorescent intensity changes of 8-IQBA as a function of sugar concentration, $[8\text{-IQBA}] = 1 \times 10^{-5}$ M. (Color version of Figures 2 – 10 are available in the Supporting Information)

Figure 3.

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); 344 nm (for D-sorbitol); 378 nm (for D-fructose). (A) D-Glucose; (B) D-Sorbitol; (C) D-Fructose; (D) plots of fluorescent intensity changes of 5-IQBA as a function of sugar concentration, $[5\text{-IQBA}] = 1 \times 10^{-5}$ M.

Figure 4.

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); 370 nm (for D-fructose). (A) D-Glucose; (B) D-Sorbitol; (C) D-Fructose; (D) plots of fluorescent intensity changes of 4-IQBA as a function of sugar concentration, $[4-IQBA] = 1 \times 10^{-5}$ M.

Figure 5.

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; (D) plots of fluorescent intensity changes of 6-IQBA as a function of sugar concentration, $[6\text{-IQBA}] = 1 \times 10^{-5}$ M.

Figure 6.

Fluorescent spectral changes of isoquinolinyboronic acids upon addition of *cis*-1,2-cyclohexanediol in phosphate buffer (0.1 M) at pH 7.4: (A) 8-IQBA, $\lambda_{\text{ex}} = 332 \text{ nm}$, $\lambda_{\text{em}} = 442 \text{ nm}$; (B) 5-IQBA, $\lambda_{\text{ex}} = 272 \text{ nm}$, $\lambda_{\text{em}} = 341 \text{ nm}$; (C) 4-IQBA, $\lambda_{\text{ex}} = 322 \text{ nm}$, $\lambda_{\text{em}} = 440 \text{ nm}$; (D) 6-IQBA, $\lambda_{\text{ex}} = 272 \text{ nm}$, $\lambda_{\text{em}} = 355 \text{ nm}$; Insets: plots of fluorescent intensity changes of IQBAs as a function of sugar concentration, $[\text{IQBAs}] = 1 \times 10^{-5} \text{ M}$.

Figure 7.

Fluorescent spectral changes of isoquinolinyboronic 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, $\lambda_{\text{ex}} = 272$ nm, $\lambda_{\text{em}} = 356$ nm; (B) 4-IQBA with methyl- α -D-glucopyranose, $\lambda_{\text{ex}} = 322$ nm, $\lambda_{\text{em}} = 361$ nm; (C) plots of fluorescent intensity changes of 4-IQBA and 6-IQBA as a function of methyl- α -D-glucopyranose concentration, (D) 6-IQBA with cyclohexanol, $\lambda_{\text{ex}} = 272$ nm, $\lambda_{\text{em}} = 455$ nm; [IQBAs] = 1×10^{-5} M.

Figure 8.

Fluorescent spectral changes of 8-QBA and 6-MDDCQ upon addition of *cis*-cyclohexanediol in phosphate buffer (0.1 M) at pH 7.4: (A) 8-QBA: $\lambda_{\text{ex}} = 270 \text{ nm}$, $\lambda_{\text{em}} = 314 \text{ nm}$, $[8\text{-QBA}] = 1 \times 10^{-5} \text{ M}$; (B) plot of fluorescent intensity change of 8-QBA as a function of sugar concentration, $[8\text{-QBA}] = 1 \times 10^{-5} \text{ M}$; (C) 6-MDDCQ: $\lambda_{\text{ex}} = 270 \text{ nm}$, $\lambda_{\text{em}} = 387 \text{ nm}$, $[6\text{-MDDCQ}] = 2 \times 10^{-5} \text{ M}$; (D) plot of fluorescent intensity change of 6-MDDCQ as a function of sugar concentration, $[6\text{-MDDCQ}] = 2 \times 10^{-5} \text{ M}$.

Figure 9.

Fluorescent intensity change-6-IQBA concentration relationships in the presence and absence of 0.75 M *cis*-cyclohexanediol in phosphate buffer (0.1 M) at pH 7.4: $\lambda_{\text{ex}} = 272$ nm, $\lambda_{\text{em}} = 354$ nm. : 6-IQBA only; $\blacklozenge$: 6-IQBA with $\blacksquare$ 0.75 M *cis*-cyclohexanediol.

Figure 10.

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

Scheme 1.

Overall binding equilibrium of phenylboronic acid with a diol.

Scheme 2.

Reagents and condition: i). *n*-butyllithium, trimethyl borate, THF, -78 °C, 39%.

Scheme 3.
Proposed ionization steps of IQBAs.

Scheme 4.

The proposed ionization steps of esters of IQBAs.

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 the experiments were duplicated);

^[b] reference number.³¹

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 the experiments were duplicated).

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

pK_a	In the absence of a diol	In the presence of D-fructose	In the presence of D-glucose	In the presence of D-sorbitol	In the presence of methyl- α -D-glucopyranose
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	ND	ND

^[a] All the results were duplicated;

^[b] reference number. 31

Table 4¹¹B NMR of isoquinolinyl boronic acids alone and in 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&20.3/1.6	29.3&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&19.1/1.5	24.6&19.2/5.7	3.5&2.3/12.2
4-IQBA and Fructose ^[b]	20.4/2.0	7.6&11.1/6.6	7.6/12.0
6-IQBA ^[a]	27.8&18.9/1.6	28.3&18.9/7.6	2.6/12.6
6-IQBA and Fructose ^[b]	28.8&18.8/1.5	20.7&9.0/5.7	8.0/12.1

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

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

FQY (%)	Isoquinoliny boronic acid alone	In the presence of D-Fructose	In the presence of D-Glucose	In the presence of D-Sorbitol	In the presence of Methyl- α -D-glucopyranose
8-IQBA	2.2 $\pm$ 0.02	24 $\pm$ 0.8	2.1 $\pm$ 0.2	6.9 $\pm$ 0.6	$\times$
5-IQBA	2.5 $\pm$ 0.02	19 $\pm$ 0.01	3.7 $\pm$ 0.4	7.7 $\pm$ 2.0	$\times$
4-IQBA	1.0 $\pm$ 0.08	17 $\pm$ 0.01	1.7 $\pm$ 0.2	2.5 $\pm$ 0.7	3.4 $\pm$ 0.02
6-IQBA	13.2 $\pm$ 3.2	12 $\pm$ 0.3	4.8 $\pm$ 0.9	10.1 $\pm$ 0.7	1.9 $\pm$ 0.2
8-QBA	ND	28 ^[b]	ND	ND	ND

^[a] All the results were duplicated;

^[b] reference number.³¹