ACS OMEGA

Article

Cite This: ACS Omega 2018, 3, 17863−17870

http://pubs.acs.org/journal/acsodf

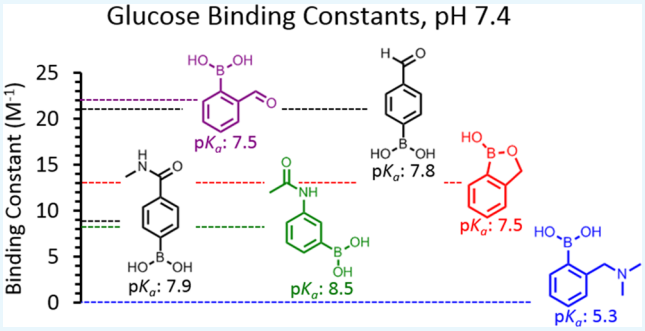
Structure−Reactivity Relationships in Boronic Acid−Diol Complexation

William L. A. Brooks, Christopher C. Deng, and Brent S. Sumerlin*

George & Josephine Butler Polymer Research Laboratory, Center for Macromolecular Science & Engineering, Department of Chemistry, University of Florida, Gainesville, Florida 32611-7200, United States

Supporting Information

ABSTRACT: Boronic acids have found widespread use in the field of biomaterials, primarily through their ability to bind with biologically relevant 1,2- and 1,3-diols, including saccharides and peptidoglycans, or with polyols to prepare hydrogels with dynamic covalent or responsive behavior. Despite a wide range of boronic acid architectures that have been previously considered, there is a need for greater understanding of the structure−reactivity relationships that govern binding affinity to diols. In this study, various boronic acids and other organoboron compounds were investigated to determine their pK_a and their binding constants with the biologically relevant diols including sorbitol, fructose, and glucose. Boronic acid pK_a values were determined through spectroscopic titration, whereas binding constants were determined by fluorescence spectroscopy during competitive binding studies. Key structure−reactivity relationships clearly indicated that both boronic acid structure and solution pH must be carefully considered. By considering a variety of boronic acids with systematically varied electronics and sterics, these results provide guidance during selection of organoboron compounds in sensing, delivery, and materials chemistry.



INTRODUCTION

Boronic acids and boronic acid analogs have been employed for a number of biomedical applications, for example, in sensors and drug delivery.[1] Sensors provide rapid, quantitative resolution of analytes and are important in the field of diagnostic medicine.[2,3] Boronic acids have been shown to be effective sensors for various saccharides, polysaccharides, glycoproteins, glycated proteins, and dopamine. Sensing occurs primarily through the formation of boronate esters with 1,2- or 1,3-diols present on the substrate.[4,5] Careful design of macromolecules containing boronic acids can also allow for the controlled release of encapsulated pharmaceuticals.[6,7] Release profiles are generally dictated by formation of boronate esters with the macromolecules, resulting in a concomitant adjustment in material properties or morphology. Mixing of boronic acid and diol functional polymers also allows access to self-healing materials[8,9] or hydrogels.[10−12] In boronic acid-containing thermoresponsive hydrogels, the binding of glucose or fructose with boronic acid units along the hydrogel backbone causes an increase in the hydrophilicity of the hydrogel, resulting in gel swelling,[13,14] increased pore size, and more rapid diffusion of encapsulated therapeutics through the gel. Similar effects were seen for sugar binding of thermoresponsive boronic acid co-polymers.[15,16] In boronic acid-modified nanoassemblies or in boronate ester-stabilized nanoparticles, the binding of saccharides often results in an increase of material solubility, causing a disruption in nanoassemblies and a release of encapsulated therapeutics.

Understanding the interactions of boronic acids with various substrates is important for the design of sensors and release devices. Diol-binding constants can be determined directly for boronic acids having absorbance or fluorescence characteristics that depend strongly on ester formation. However, for boronic acids that do not exhibit readily observable shifts in spectroscopic properties upon ester formation, determination of binding constants can be less straightforward and often relies on pH-depression methods which require high boronic acid concentrations and assumes that esterification always results in anionic boronate ester formation with a negligible concentration of neutral boronic ester.[17,18] The pH-depression method also assumes that the boronic esters have a lower pK_a than the free boronic acid, which may not always be the case.[19] Wang and Springsteen examined the nature of boronate ester formation and diol binding through the use of Alizarin Red S (ARS), a hydrophilic dye. In solution, ARS exhibits a blue shift in absorbance upon binding with a boronic acid.[20,21] Displacement of ARS by a diol results in a red shift back to the characteristic absorbance of unmodified ARS.

Received: October 29, 2018
Accepted: December 6, 2018
Published: December 19, 2018

DOI: 10.1021/acsomega.8b02999
ACS Omega 2018, 3, 17863−17870

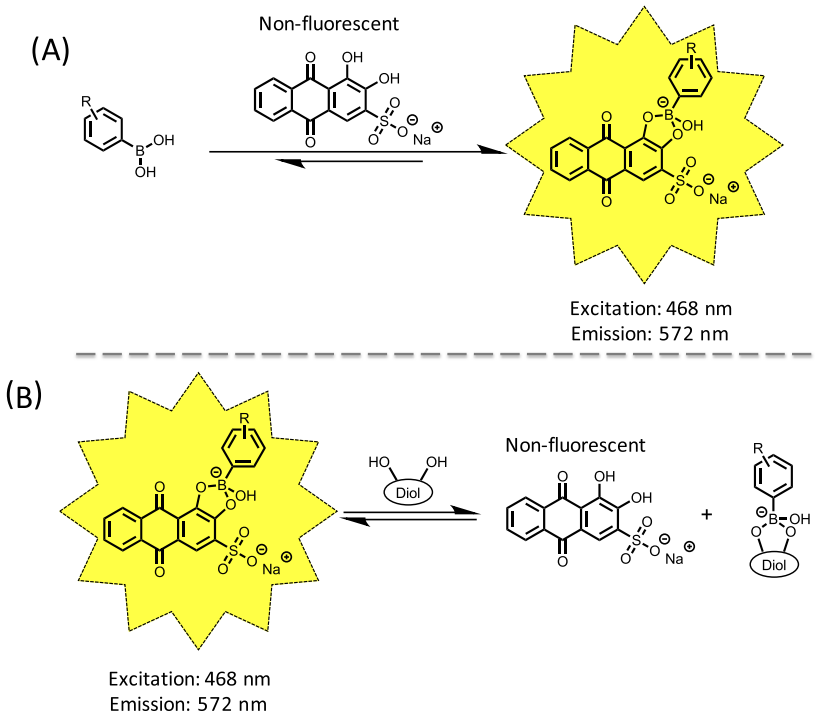


Figure 1. ARS binding and transesterification scheme. (A) Upon ester formation with the boronic acid, the ARS adduct becomes fluorescent. (B) The fluorescence intensity decreases upon displacement with a competing diol.

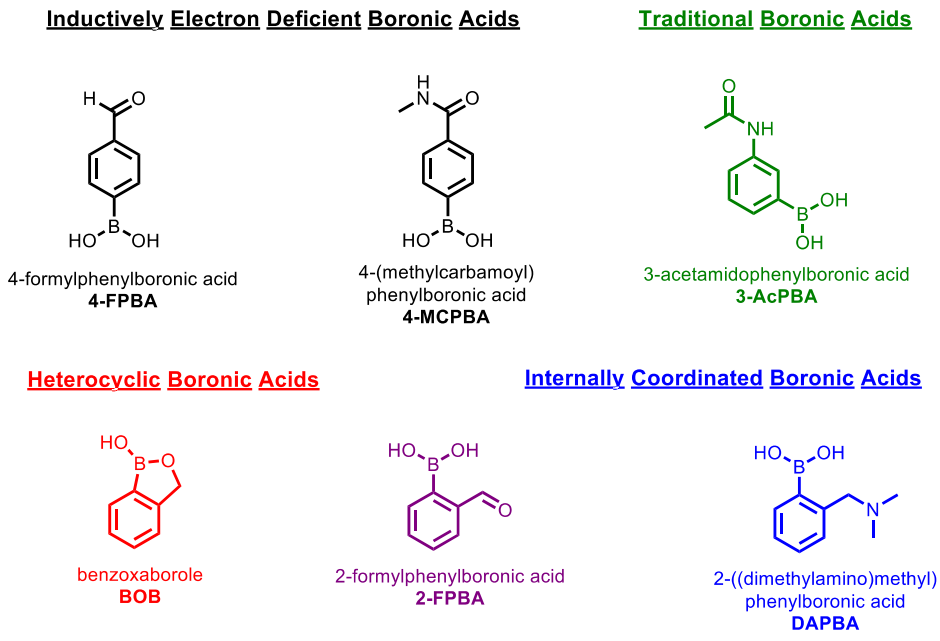


Figure 2. Structures of various boronic acids and boronic acid analogs investigated.

The binding of ARS with boronic acids also results in an increase in fluorescence intensity. In solution, excited-state proton transfer between the catechol hydroxyl groups and the adjacent ketone results in quenching of the ARS fluorescence.[22] Upon ester formation, this proton transfer is no longer possible, and fluorescence quenching is not observed. To examine the binding of diol substrates with various boronic acids, the displacement of the ARS−boronate ester with the substrate disrupts the ARS-ester equilibrium, resulting in reduced fluorescence intensity (Figure 1). This three-component system allows for determination of diol association constants.

ARS−boronate esters have been employed for both the examination of diol association constants[23−26] and the development of diol sensors.[27−29] Previous studies have investigated the effects of substituents on boronic acid−diol binding but have not broadly compared various boronic acid classes. Furthermore, apparent diol-binding constants as determined by the ARS competitive binding assay have been shown to be dependent on a number of factors, including solution pH, boronic acid structure, and most importantly,

DOI: 10.1021/acsomega.8b02999
ACS Omega 2018, 3, 17863−17870

buffer composition. As such, it is important to ensure that apparent association constants are determined under identical conditions to allow comparative evaluations of boronic acid species. This study evaluates six boronic acids and boronic acid analogs across a wide pH range and under identical solution conditions to provide a true comparison between boronic acid classes in terms of diol binding.

RESULTS AND DISCUSSION

Six boronic acids and analogs were chosen (Figure 2). Both 3-acetamidophenylboronic acid (3-AcPBA) and 4-methylcarbamoylphenylboronic acid (4-MCPBA) were chosen as analogs of boronic acids used previously in our research[30−32] and by other groups.[14,33−35] Benzoxaboroles (benzoboroxoles, BOB) have also seen limited use in self-assembled polymeric nanoassemblies.[36−39] Wulff-type boronic acids, similar in structure to 2-dimethylaminomethylphenylboronic acid (DAPBA), are of great interest in biomedical applications.[40−44] However, there has been very limited investigation of glucose binding with Wulff-type boronic acids, which is vital in development of glucose-responsive materials.[25] The monomers are divided into classes, with 4-formylphenylboronic acid (4-FPBA) and 4-MCPBA being electron-deficient acids and 3-AcPMA representing more traditional boronic acids as an analog of the most widely studied motif. DAPBMA is an internally coordinated boronic acid, and BOB is a heterocyclic boronic acid analog. Interestingly, as discussed later, 2-formylphenylboronic acid (2-FPBA) exists somewhere between a heterocyclic boronic acid and an internally coordinated boronic acid.

The determination of boronic acid−diol-binding constants involves a three-component competitive binding assay.[20,21] The association constant between each boronic acid and ARS (K_{ARS}) must first be determined. To do this, each boronic acid was titrated into a constant concentration of ARS. Formation of the boronic acid−ARS adduct resulted in an increase in solution fluorescence intensity. To derive the relationship between the association constant (K_{ARS}) and fluorescence intensity, we first considered the relationship between the association constant and the concentration of the solution components, given by eq 1, where [BA·ARS] is the concentration of the boronic acid−ARS conjugate, [BA] is the free boronic acid concentration, and [ARS] is the free ARS concentration. As the boronic acid is in large excess relative to ARS, the concentration of the boronic acid is considered constant within each sample.

$$K_{ARS} = \frac{[BA\cdot ARS]}{[BA][ARS]} \quad (1)$$

Equation 1 can be rewritten as eq 2.

$$K_{ARS} = \frac{[BA\cdot ARS]}{[BA]([ARS]_o - [BA\cdot ARS])} \quad (2)$$

Equation 2 can be further rearranged to give eq 3

$$\frac{[ARS]_o}{[BA\cdot ARS]} = \frac{1}{[BA]K_{ARS}} + 1 \quad (3)$$

The fluorescence intensity for any given concentration of BA·ARS is given by eq 4, where I_f is the fluorescence intensity, k is a constant related to instrument parameters such as monochromator throughput, ϕ is the quantum yield, I_o is the incident light intensity, ε is the molar absorptivity, and b is the path length.

$$I_f = k\phi I_o \varepsilon b[BA\cdot ARS] \quad (4)$$

Rearrangement of eq 4 allows for determination of BA·ARS concentration, as given by eq 5.

$$[BA\cdot ARS] = \frac{I_f}{k\phi I_o \varepsilon b} \quad (5)$$

Substitution of eq 5 into eq 3 gives eq 6.

$$\frac{k\phi I_o \varepsilon b[ARS]_o}{I_f} = \frac{1}{[BA]K_{ARS}} + 1 \quad (6)$$

Equation 6 can be rearranged to give eq 7, the Benesi−Hildebrand equation.

$$\frac{1}{I_f} = \frac{1}{k\phi I_o \varepsilon b[ARS]_o K_{ARS}[BA]} + \frac{1}{k\phi I_o \varepsilon b[ARS]_o} \quad (7)$$

Given eq 7, the ARS association constant, K_{ARS}, can be found from the plot of $1/I_f$ vs $1/[BA]$ for a titration of a boronic acid with a solution of constant ARS concentration. The association constant was determined by dividing the plot intercept by the slope. Examples of these plots can be found in the Supporting Information.

Once K_{ARS} was known, the apparent boronic acid−diol association constant (K_{eq}) was determined through a competitive binding assay. Each diol was titrated into a solution with constant boronic acid and ARS concentrations. Increasing the diol concentration displaced a fraction of the ARS from the boronic acid−ARS adduct, resulting in a decrease in fluorescence intensity. The equilibrium for the displacement of the boronic acid−ARS adduct with a diol is shown in Scheme 1.

$$BA\cdot ARS + diol \rightleftharpoons BA\cdot diol + ARS$$

Scheme 1. Equilibrium for the Displacement of the Boronic Acid−ARS Adduct with a Diol

$$BA\cdot ARS + Diol \rightleftharpoons BA\cdot Diol + ARS$$

The mass-balance expressions for the individual components are given by eqs 8−10.

$$[diol]_o = [diol] + [BA\cdot diol] \quad (8)$$

$$[ARS]_o = [ARS] + [BA\cdot ARS] \quad (9)$$

$$[BA]_o = [BA] + [BA\cdot diol] + [BA\cdot ARS] \quad (10)$$

The association constant for each diol, K_{eq}, is given by eq 11.

$$K_{eq} = \frac{[BA\cdot diol]}{[diol][BA]} \quad (11)$$

Combining the mass-balance equations with eqs 1 and 11 gives eq 12.

$$[BA]_o = [BA] + \frac{K_{eq}[BA][diol]_o}{1 + K_{eq}[BA]} + \frac{K_{ARS}[BA][ARS]_o}{1 + K_{ARS}[BA]} \quad (12)$$

If we define the indicator ratio, Q, by eq 13, then eq 12 can be rewritten as eq 14.

DOI: 10.1021/acsomega.8b02999
ACS Omega 2018, 3, 17863−17870

$$Q = \frac{[\mathrm{ARS}]}{[\mathrm{BA{\cdot}ARS}]} \tag{13}$$

$$[\mathrm{BA}]_o = \frac{1}{QK_{\mathrm{ARS}}} + \frac{K_{eq}[\mathrm{diol}]_o}{QK_{\mathrm{ARS}} + K_{eq}} + \frac{[\mathrm{ARS}]_o}{1+Q} \tag{14}$$

If we define a quantity, P, as given by eq 15, then eq 14 can be rewritten as eq 16.

$$P = [\mathrm{BA}]_o - \frac{1}{QK_{\mathrm{ARS}}} - \frac{[\mathrm{ARS}]_o}{1+Q} \tag{15}$$

$$P = \frac{K_{eq}[\mathrm{diol}]_o}{QK_{\mathrm{ARS}} + K_{eq}} \tag{16}$$

Equation 16 can be rewritten as eq 17.

$$\frac{[\mathrm{diol}]_o}{P} = \frac{K_{\mathrm{ARS}}}{K_{eq}}Q + 1 \tag{17}$$

Given eq 17, the boronic acid−diol association constant, K_{eq}, can be found from a plot of $[\mathrm{diol}]_o/P$ vs Q. The association constant can be determined by dividing K_{ARS} by the slope of the plot. The value Q can be obtained from measured fluorescence intensities as given by eq 18, where $I_{f,\mathrm{BA{\cdot}ARS}}$ is the fluorescence intensity of the boronic acid−ARS adduct in the absence of competitive diol, $I_{f,\mathrm{ARS}}$ is the fluorescence intensity of ARS, and I_f is the measured fluorescence intensity in the presence of competitive diol.[16,36]

$$Q = \frac{I_{f,\mathrm{BA{\cdot}ARS}} - I_f}{I_f - I_{\mathrm{ARS}}} \tag{18}$$

Examples of these plots can be found in the Supporting Information.

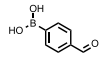
A number of studies have examined the substitution (or heterocyclic ring structure in the case of benzoxaboroles) of boronic acids to determine the effect of these modifications of boronic acid pK_a and binding affinity.[19,20,25,45,46] However, these studies have been performed under a number of different conditions, leading to determinations of association constants that can vary between studies.[47] In many of these investigations, the binding affinity was found in aqueous/organic co-solvent systems, which was necessary to solubilize the boronic acids in question.[25,48] As such, there is a need for a study that examines the association constants of a variety of boronic acid families under identical conditions to better identify the most viable candidates for future applications.

Determination of Boronic Acid pK_a Values. The pK_a of a boronic acid is defined as the pH at which 50% of the boronic acid exists as the hydroxyboronate anion species. Many applications of boronic acids rely on a shift in solubility or solute−solvent favorability, which is often dictated by the transition from hydrophobic neutral boronic acid to the anionic hydroxyboronate or boronate ester. As such, employing a boronic acid with a pK_a much higher than physiological pH may not yield a material or device with an optimum response profile under physiological conditions. It has been shown that the binding affinity of a boronic acid is often related to the pK_a of the boronic acid, although the most effective binding is not always near or above the pK_a of the boronic acid.[19] Several studies have proposed that optimal binding should occur at a pH between the pK_a of the boronic acid and of the diol, as given by eq 19, although this may not account for secondary effects, such as buffer composition, steric hindrance, or solvent composition, which can affect the ability to form boronic esters.[19]

$$\mathrm{pH}_{\mathrm{optimal}} = (\mathrm{p}K_{\mathrm{a\text{-}acid}} + \mathrm{p}K_{\mathrm{a\text{-}diol}})/2 \tag{19}$$

In this study, the pK_a of each boronic acid was determined by monitoring the UV absorption of each molecule as a function of pH. As the boronic acid transitions from the neutral trigonal planar species to the tetrahedral boronate center, the absorbance at wavelengths ranging from approximately 200 to 260 nm decreases, allowing for facile pK_a determinations. The measured pK_a's are given in Table 1, and examples of titration data can be found in the Supporting Information.

Table 1. Measured pK_a Values for Various Boronic Acids and Analogs

Boronic Acid	Abbreviation	pK_a
	3-AcPBA	8.5
	4-MCPBA	7.9
	4-FPBA	7.8
	BOB	7.5
	2-FPBA	7.5
	DAPBA	5.3

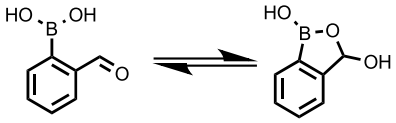
Unmodified phenylboronic acid has previously been shown to have a pK_a of approximately 9.0.[49] To adjust the pK_a, the aromatic ring can be modified with electron-withdrawing or -donating substituents. Of the boronic acids investigated, 3-acetamidophenylboronic acid (3-AcPBA) was found to have the highest pK_a, whereas the electron-withdrawing inductive and resonance effects for both 4-methylcarbamoylphenylboronic acid (4-MCPBA) and 4-formylphenylboronic acid (4-FPBA) result in a decrease of pK_a for both boronic acids.[50]

Benzoxaborole (BOB) is a heterocycle-modified boronic acid, with a pK_a significantly lower than 3-AcPBA, 4-MCPBA, or 4-FPBA. The lower pK_a is driven by the release of ring strain in the five-membered ring as the boron center transitions from a trigonal planar geometry in the neutral form to a tetrahedral geometry in the boronate anion form, with the heterocycle being retained.[46] Interestingly, the pK_a of 2-formylphenylboronic acid (2-FPBA) is similar to that of BOB. This is likely due to intramolecular interaction between the carbonyl oxygen and the boron center. It has been shown that in aqueous solution, 2-FPBA can exist as an isomer of similar structure to BOB, forming a benzoxaborole heterocycle with a hydroxyl substituent on the methylene unit between the aromatic ring

DOI: 10.1021/acsomega.8b02999
ACS Omega 2018, 3, 17863−17870

and the oxygen derived from the carbonyl. This observation is due to tautomerization of the carbonyl, followed by a ring closing cyclization (Scheme 2).[51] The formation of this heterocycle is more favorable as the pH of the solution increases, resulting in 2-FPBA having a pK_a similar to that of BOB.

Scheme 2. Tautomeric Equilibrium of 2-FPBA



The boronic acid with the lowest measured pK_a was 2-dimethylaminomethylphenylboronic acid (DAPBA). This family of boronic acids, containing an adjacent coordinating amine center, is often referred to as a "Wulff-type" boronic acid[52] and is characterized by the nitrogen center coordinating with boron to form a tetrahedral boronate. As such, the pK_a of this boronic acid is formally the pK_a of the amine center, as deprotonation of the amine results in the B−N coordinative interaction. It has been previously shown that both secondary and tertiary amines have similar effects on boronic acid activity.[53] Further increase in solution pH results in formation of the hydroxyboronate anion and release of the adjacent amine (Scheme 3). Recently, there have been some evidences of a solvent insertion mechanism also taking place with Wulff-type boronic acids, whereby a polar protic solvent such as water or methanol can donate electron density to the boron center. In this mechanism, the solvent insertion results in the formation of an anionic boronate center and a cationic ammonium center.[54,55]

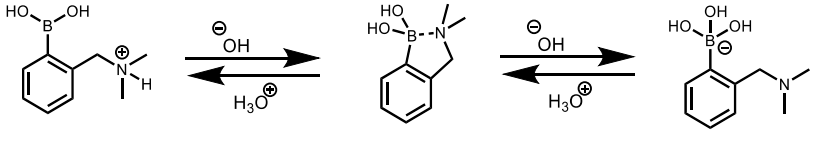
Apparent Association Constants for Various Boronic Acid Families. The association between a boronic acid and a diol is affected by a number of factors, including boronic acid pK_a, diol pK_a, dihedral angle of the diol, steric hindrance, and stabilization of the boronate center. Given the acidity of aromatic hydroxyl groups and the planar nature of the diol, it is common for catechol-functionalized molecules to have high binding constants at relatively low pH. As such, Alizarin Red S, a catechol functional molecule, demonstrates a high affinity for boronic acids is often used as a fluorescent reporter, as it becomes fluorescent upon binding with a boronic acid. This provides a direct method for determination of binding constants with ARS (Table 2). The binding of ARS was found to follow a similar pattern for most of the boronic acids in question, with the highest association constant found near physiological pH. The exception was DAPBA, where the association constant was significantly higher for DAPBA under acidic conditions, potentially due to an electrostatic attraction between the protonated ammonium center below the amine pK_a and the anionic sulfonate group on ARS. For the boronic acids with functional groups directly attached or adjacent to boron (BOB, 2-FPBA, and DAPBA), the association constants were much lower than those for the other boronic acids, likely due to steric hindrance at the boron center. For measurements where the apparent association constant was too low to be reliably determined, the value is reported as n/d (not determined).

We were interested in investigating the association constants of the boronic acids and sorbitol, as the sugar alcohol can potentially behave as a multifunctional diol for crosslinking between boronic acids or as a competitive binding agent. The latter is of great importance in heterogeneous separation devices for release of captured diol functional molecules in affinity chromatography.[56] Of the sugar and sugar alcohols investigated, sorbitol had the highest association constants with each boronic acid as compared to fructose and glucose, even though sorbitol, fructose, and glucose all have a pK_a close to 12.5. This increased affinity is likely due to the availability of the diols in solution, as both fructose and glucose can exist as various linear and cyclic isomers. Of the possible fructose isomers, β-D-fructofuranose has been shown to have the highest binding constant and an abundance of around 25% in aqueous solution, whereas the glucose isomer with the highest binding constant has been shown to be α-D-glucofuranose, which has an abundance of approximately 0.14%.[57] For the unhindered boronic acids, the association constant with sorbitol increased with decreasing pK_a. This pattern remained true for both fructose and glucose.

Despite the fact that the sterically hindered boronic acids have significantly lower pK_a values than the unhindered boronic acids, it was observed that the association constants for the bulky boronic acids were considerably lower for sorbitol and fructose, with similar binding constants for glucose. This counterintuitive observation highlights the need for an understanding of the effect of structure on binding affinity and to not assume that a lower pK_a will consistently result in a higher association constant. The glucose association constants for BOB and 2-FPBA were among the highest values of the various boronic acids, with 2-FPBA having a slightly higher association constant than BOB at neutral pH. At higher pH, where intramolecular cyclization of 2-FPBA is increasingly favored, the association constants for 2-FPBA and BOB are very similar. The slightly higher glucose association constants of BOB and 2-FPBA, coupled with the lower pK_a of the boronic acids, make these classes of materials fit for glucose-responsive systems.

DAPBA, the Wulff-type boronic acid, which has been applied for self-healing hydrogels[31] and glucose detection devices,[2] had the lowest association constants at neutral and basic pH of all of the boronic acids investigated. Other groups have observed the lower binding affinity of Wulff-type boronic acids at neutral and basic pH.[16] Interestingly, the association constants at acidic pH were notably higher than those for all other boronic acids, likely due to the electrostatic stabilization of the boronate species with the cationic ammonium ion. The ability to stabilize esters at relatively low pH has been utilized to prepare hydrogels at acidic pH.[32]

Scheme 3. pH-Dependent Coordination of an Amine to a Boron Center in Wulff-Type Boronic Acids



DOI: 10.1021/acsomega.8b02999
ACS Omega 2018, 3, 17863−17870

Table 2. Measured Apparent Association Constants (M^{-1}) for Various Boronic Acids with ARS, Sorbitol, Fructose, and Glucose at pH 5.2, 7.4, and 8.7[a]

boronic acid—pK_a	3-AcPBA 8.5	4-MCPBA 7.9	4-FPBA 7.8	BOB 7.5	2-FPBA 7.5	DAPBA 5.3
			diol			
			ARS			
pH 5.2	1220 (110)	1720 (140)	2580 (130)	620 (32)	820 (55)	17 400 (680)
pH 7.4	2200 (73)	2490 (82)	4850 (85)	940 (54)	760 (5)	600 (34)
pH 8.7	490 (38)	470 (30)	720 (17)	100 (1.6)	89 (4)	43 (1.2)
			sorbitol			
pH 5.2	6.5 (0.2)	13 (0.1)	22 (1.4)	5.5 (0.2)	9.7 (0.2)	130 (5.6)
pH 7.4	610 (13)	980 (8)	2100 (90)	420 (16)	440 (20)	380 (20)
pH 8.7	3200 (190)	4200 (320)	3000 (220)	1200 (7)	1200 (50)	230 (1.4)
			fructose			
pH 5.2	n/d	n/d	3.1 (0.1)	n/d	1.9 (0.1)	4 (0.2)
pH 7.4	350 (11)	470 (17)	1260 (31)	290 (21)	360 (30)	64 (2.0)
pH 8.7	1400 (50)	1600 (98)	2000 (120)	770 (14)	790 (60)	9.6 (0.3)
			glucose			
pH 5.2	n/d	n/d	n/d	n/d	n/d	n/d
pH 7.4	8.1 (0.7)	8.8 (0.6)	21 (0.8)	13 (0.6)	22 (0.2)	n/d
pH 8.7	30 (0.9)	38 (2)	52 (3)	42 (2)	42 (1)	n/d

[a]Under some conditions, the binding constant was too low to accurately measure and was thus not determined (n/d). Standard deviation in parenthesis.

CONCLUSIONS

Even with the increased association constants of BOB and 2-FPBA, glucose binding is still much lower than the binding of other biologically relevant diols, such as fructose. This binding preference may be overcome through the use of specially designed diboronic acid ligands, in which the three-dimensional space between the boronic acids is designed to favor binding of one type of diol over others.[58] Furthermore, there is a need for an investigation of binding constants with polymeric boronic acids as a function of boronic acid structure, comonomer composition, and polymer architecture (block copolymers, star polymers, hyperbranched polymers, etc.) It is likely that for a given boronic acid structure, polymeric boronic acids likely have a higher pK_a and lower binding affinity than their small molecule analogs. This is largely a result of neighboring effects, where steric hindrance between neighboring units would limit available binding sites, and electrostatic effects would make ionization of neighboring boronic acid units more energetically unfavorable. However, sterics and molecular geometry in polymeric systems can be used to gain selectivity, such as in molecularly imprinted networks, where the targeted substrate is incorporated during network formation, precluding the binding of other diols for purely steric reasons.[59] However, these approaches are often more synthetically challenging and may not be applicable in all scenarios. As such, to enhance the efficiency and specificity of binding between boronic acid−diol pairs, it is necessary to have knowledge of the characteristics of each boronic acid family.

As the data above demonstrate, the design of boronic acid-functional materials requires an understanding of fundamental structure−property and structure−reactivity relationships, including pK_a, binding affinity, and relative selectivity. This study provides a direct comparison of various boronic acid families, allowing for the informed selection of boronic acid moieties for each application. Many materials intended for physiological applications have relied on boronic acids that are similar in structure to 3-AcPBA or 4-MCPBA, whereas those boronic acids have been shown to have a pK_a much higher than physiological pH and glucose association constants lower than those of other boronic acid families. Wulff-type boronic acids have seen relatively widespread use, even though the bulky nature of the amine results in very low diol-binding constants at neutral and basic pH. The results of this research indicate that heterocyclic boronic acids, like benzoxaboroles, or boronic acids with less hindered electron-donating species, like 2-formylphenylboronic acid, may provide the best response profile under physiological conditions.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b02999.

Synthetic methods, methods for pK_a and association constant measurements; ^{1}H NMR spectra of synthesized compounds; plots of data from pK_a titrations and association constant determinations (PDF)

AUTHOR INFORMATION

Corresponding Author

*E-mail: sumerlin@chem.ufl.edu.

ORCID

Brent S. Sumerlin: 0000-0001-5749-5444

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Brooks, W. L. A.; Sumerlin, B. S. Synthesis and Applications of Boronic Acid-Containing Polymers: From Materials to Medicine. *Chem. Rev.* **2015**, *116*, 1375−1397.

(2) Crane, B. C.; Barwell, N. P.; Gopal, P.; Gopichand, M.; Higgs, T.; James, T. D.; Jones, C. M.; Mackenzie, A.; Mulavisala, K. P.; Paterson, W. The Development of a Continuous Intravascular Glucose Monitoring Sensor. *J. Diabetes Sci. Technol.* **2015**, *9*, 751−761.

(3) Sun, X.; James, T. D. Glucose Sensing in Supramolecular Chemistry. *Chem. Rev.* **2015**, *115*, 8001−8037.

DOI: 10.1021/acsomega.8b02999
ACS Omega 2018, 3, 17863−17870

(4) Wang, L.; Zhang, J.; Kim, B.; Peng, J.; Berry, S. N.; Ni, Y.; Su, D.; Lee, J.; Yuan, L.; Chang, Y.-T. Boronic Acid: A Bio-Inspired Strategy To Increase the Sensitivity and Selectivity of Fluorescent NADH Probe. *J. Am. Chem. Soc.* **2016**, *138*, 10394−10397.
(5) Liu, J.; Yang, K.; Shao, W.; Qu, Y.; Li, S.; Wu, Q.; Zhang, L.; Zhang, Y. Boronic Acid-Functionalized Particles with Flexible Three-Dimensional Polymer Branch for Highly Specific Recognition of Glycoproteins. *ACS Appl. Mater. Interfaces* **2016**, *8*, 9552−9556.
(6) Zhao, Z.; Yao, X.; Zhang, Z.; Chen, L.; He, C.; Chen, X. Boronic Acid Shell-Crosslinked Dextran-b-PLA Micelles for Acid-Responsive Drug Delivery. *Macromol. Biosci.* **2014**, *14*, 1609−1618.
(7) Yang, H.; Zhang, C.; Li, C.; Liu, Y.; An, Y.; Ma, R.; Shi, L. Glucose-Responsive Polymer Vesicles Templated by α-CD/PEG Inclusion Complex. *Biomacromolecules* **2015**, *16*, 1372−1381.
(8) Cash, J. J.; Kubo, T.; Bapat, A. P.; Sumerlin, B. S. Room-Temperature Self-Healing Polymers Based on Dynamic-Covalent Boronic Esters. *Macromolecules* **2015**, *48*, 2098−2106.
(9) Cash, J. J.; Kubo, T.; Dobbins, D. J.; Sumerlin, B. S. Maximizing the Symbiosis of Static and Dynamic Bonds in Self-Healing Boronic Ester Networks. *Polym. Chem.* **2018**, *9*, 2011−2020.
(10) Deng, C. C.; Brooks, W. L. A.; Abboud, K. A.; Sumerlin, B. S. Boronic Acid-Based Hydrogels Undergo Self-Healing at Neutral and Acidic pH. *ACS Macro Lett.* **2015**, *4*, 220−224.
(11) Pettignano, A.; Grijalvo, S.; Häring, M.; Eritja, R.; Tanchoux, N.; Quignard, F.; Díaz, D. D. Boronic Acid-Modified Alginate Enables Direct Formation of Injectable, Self-Healing and Multistimuli-Responsive Hydrogels. *Chem. Commun.* **2017**, *53*, 3350−3353.
(12) Dong, Y.; Wang, W.; Veiseh, O.; Appel, E. A.; Xue, K.; Webber, M. J.; Tang, B. C.; Yang, X.-W.; Weir, G. C.; Langer, R.; et al. Injectable and Glucose-Responsive Hydrogels Based on Boronic Acid−Glucose Complexation. *Langmuir* **2016**, *32*, 8743−8747.
(13) Matsumoto, A.; Yoshida, R.; Kataoka, K. Glucose-Responsive Polymer Gel Bearing Phenylborate Derivative as a Glucose-Sensing Moiety Operating at the Physiological pH. *Biomacromolecules* **2004**, *5*, 1038−1045.
(14) Matsumoto, A.; Ikeda, S.; Harada, A.; Kataoka, K. Glucose-Responsive Polymer Bearing a Novel Phenylborate Derivative as a Glucose-Sensing Moiety Operating at Physiological pH Conditions. *Biomacromolecules* **2003**, *4*, 1410−1416.
(15) Vancoillie, G.; Brooks, W. L. A.; Mees, M. A.; Sumerlin, B. S.; Hoogenboom, R. Synthesis of Novel Boronic Acid-Decorated Poly(2-Oxazoline)s Showing Triple-Stimuli Responsive Behavior. *Polym. Chem.* **2016**, *7*, 6725−6734.
(16) Brooks, W. L. A.; Vancoillie, G.; Kabb, C. P.; Hoogenboom, R.; Sumerlin, B. S. Triple Responsive Block Copolymers Combining pH-Responsive, Thermoresponsive, and Glucose-Responsive Behaviors. *J. Polym. Sci., Part A: Polym. Chem.* **2017**, *55*, 2309−2317.
(17) Lorand, J. P.; Edwards, J. O. Polyol Complexes and Structure of the Benzeneboronate Ion. *J. Org. Chem.* **1959**, *24*, 769−774.
(18) Conner, J. M.; Bulgrin, V. C. Equilibria between Borate Ion and Some Polyols in Aqueous Solution. *J. Inorg. Nucl. Chem.* **1967**, *29*, 1953−1961.
(19) Yan, J.; Springsteen, G.; Deeter, S.; Wang, B. The Relationship among pKa, pH, and Binding Constants in the Interactions between Boronic Acids and Diols - It Is Not as Simple as It Appears. *Tetrahedron* **2004**, *60*, 11205−11209.
(20) Springsteen, G.; Wang, B. A Detailed Examination of Boronic Acid-Diol Complexation. *Tetrahedron* **2002**, *58*, 5291−5300.
(21) Springsteen, G.; Wang, B. Alizarin Red S. as a General Optical Reporter for Studying the Binding of Boronic Acids with Carbohydrates. *Chem. Commun.* **2001**, 1608−1609.
(22) Palit, D. K.; Pal, H.; Mukherjee, T.; Mittal, J. P. Photodynamics of the S1 State of Some Hydroxy- and Amino-Substituted Naphthoquinones and Anthraquinones. *J. Chem. Soc., Faraday Trans.* **1990**, *86*, 3861.
(23) Yang, B.; Lv, Y.; Zhu, J. Y.; Han, Y. T.; Jia, H. Z.; Chen, W. H.; Feng, J.; Zhang, X. Z.; Zhuo, R. X. A pH-Responsive Drug Nanovehicle Constructed by Reversible Attachment of Cholesterol to PEGylated Poly(l-Lysine) via Catechol-Boronic Acid Ester Formation. *Acta Biomater.* **2014**, *10*, 3686−3695.
(24) Liu, J.; Yang, K.; Qu, Y.; Li, S.; Wu, Q.; Liang, Z.; Zhang, L.; Zhang, Y. An Efficient Approach to Prepare Boronate Core−shell Polymer Nanoparticles for Glycoprotein Recognition via Combined Distillation Precipitation Polymerization and RAFT Media Precipitation Polymerization. *Chem. Commun.* **2015**, *51*, 3896−3898.
(25) Dowlut, M.; Hall, D. G. An Improved Class of Sugar-Binding Boronic Acids, Soluble and Capable of Complexing Glycosides in Neutral Water. *J. Am. Chem. Soc.* **2006**, *128*, 4226−4227.
(26) Scarano, W.; Lu, H.; Stenzel, M. H. Boronic Acid Ester with Dopamine as a Tool for Bioconjugation and for Visualization of Cell Apoptosis. *Chem. Commun.* **2014**, *50*, 6390−6393.
(27) Elmas, B.; Senel, S.; Tuncel, A. A New Thermosensitive Fluorescent Probe for Diol Sensing: Poly(N-Isopropylacrylamide-Co-Vinylphenylboronic Acid)-Alizarin Red S Complex. *React. Funct. Polym.* **2007**, *67*, 87−96.
(28) Cannizzo, C.; Amigoni-Gerbier, S.; Larpent, C. Boronic Acid-Functionalized Nanoparticles: Synthesis by Microemulsion Polymerization and Application as a Re-Usable Optical Nanosensor for Carbohydrates. *Polymer* **2005**, *46*, 1269−1276.
(29) Zhang, K.-D.; Matile, S. Complex Functional Systems with Three Different Types of Dynamic Covalent Bonds. *Angew. Chem., Int. Ed.* **2015**, *54*, 8980−8983.
(30) Roy, D.; Cambre, J. N.; Sumerlin, B. S. Triply-Responsive Boronic Acid Block Copolymers: Solution Self-Assembly Induced by Changes in Temperature, pH, or Sugar Concentration. *Chem. Commun.* **2009**, 2106−2108.
(31) Cambre, J. N.; Roy, D.; Sumerlin, B. S. Tuning the Sugar-Response of Boronic Acid Block Copolymers. *J. Polym. Sci., Part A: Polym. Chem.* **2012**, *50*, 3373−3382.
(32) Roy, D.; Sumerlin, B. S. Glucose-Sensitivity of Boronic Acid Block Copolymers at Physiological pH. *ACS Macro Lett.* **2012**, *1*, 529−532.
(33) Hasegawa, U.; Nishida, T.; Van Der Vlies, A. J. Dual Stimuli-Responsive Phenylboronic Acid-Containing Framboidal Nanoparticles by One-Step Aqueous Dispersion Polymerization. *Macromolecules* **2015**, *48*, 4388−4393.
(34) Meng, H.; Zheng, J.; Wen, X. F.; Cai, Z. Q.; Zhang, J. W.; Chen, T. pH- and Sugar-Induced Shape Memory Hydrogel Based on Reversible Phenylboronic Acid-Diol Ester Bonds. *Macromol. Rapid Commun.* **2015**, *36*, 533−537.
(35) Tarus, D.; Hachet, E.; Messager, L.; Catargi, B.; Ravaine, V.; Auzély-Velty, R. Readily Prepared Dynamic Hydrogels by Combining Phenyl Boronic Acid-and Maltose-Modifi Ed Anionic Polysaccharides at Neutral pH. *Macromol. Rapid Commun.* **2014**, *35*, 2089−2095.
(36) Jay, J. I.; Lai, B. E.; Myszka, D. G.; Mahalingam, A.; Langheinrich, K.; Katz, D. F.; Kiser, P. F. Multivalent Benzoboroxole Functionalized Polymers as Gp120 Glycan Targeted Microbicide Entry Inhibitors. *Mol. Pharmaceutics* **2010**, *7*, 116−129.
(37) Kim, H.; Kang, Y. J.; Jeong, E. S.; Kang, S.; Kim, K. T. Glucose-Responsive Disassembly of Polymersomes of Sequence-Specific Boroxole-Containing Block Copolymers under Physiologically Relevant Conditions. *ACS Macro Lett.* **2012**, *1*, 1194−1198.
(38) Jeong, E. S.; Park, C.; Kim, K. T. Doubly Responsive Polymersomes towards Monosaccharides and Temperature under Physiologically Relevant Conditions. *Polym. Chem.* **2015**, *6*, 4080−4088.
(39) Kim, H.; Kang, Y. J.; Kang, S.; Kim, K. T. Monosaccharide-Responsive Release of Insulin from Polymersomes of Polyboroxole Block Copolymers at Neutral pH. *J. Am. Chem. Soc.* **2012**, *134*, 4030−4033.
(40) Cromwell, O. R.; Chung, J.; Guan, Z. Malleable and Self-Healing Covalent Polymer Networks through Tunable Dynamic Boronic Ester Bonds. *J. Am. Chem. Soc.* **2015**, *137*, 6492−6495.
(41) Piest, M.; Zhang, X.; Trinidad, J.; Engbersen, J. F. J. pH-Responsive, Dynamically Restructuring Hydrogels Formed by Reversible Crosslinking of PVA with Phenylboronic Acid Functional-

DOI: 10.1021/acsomega.8b02999
ACS Omega 2018, 3, 17863−17870

ised PPO−PEO−PPO Spacers (Jeffamines). *Soft Matter* **2011**, *7*, 11111.
(42) Li, H.; Liu, Y.; Liu, J.; Liu, Z. A Wulff-Type Boronate for Boronate Affinity Capture of Cis-Diol Compounds at Medium Acidic pH Condition. *Chem. Commun.* **2011**, *47*, 8169.
(43) Kim, K. T.; Cornelissen, J. J. L. M.; Nolte, R. J. M.; van Hest, J. C. M. Polymeric Monosaccharide Receptors Responsive at Neutral pH. *J. Am. Chem. Soc.* **2009**, *131*, 13908−13909.
(44) Appleton, B.; Gibson, T. D. Detection of Total Sugar Concentration Using Photoinduced Electron Transfer Materials: Development of Operationally Stable, Reusable Optical Sensors. *Sens. Actuators, B* **2000**, *65*, 302−304.
(45) Bérubé, M.; Dowlut, M.; Hall, D. G. Benzoboroxoles as Efficient Glycopyranoside-Binding Agents in Physiological Conditions: Structure and Selectivity of Complex Formation Benzoboroxoles as Efficient Glycopyranoside-Binding Agents in Physiological Conditions: Structure and Selectivity of C. *J. Org. Chem.* **2008**, *73*, 6471−6479.
(46) Tomsho, J. W.; Pal, A.; Hall, D. G.; Benkovic, S. J. Ring Structure and Aromatic Substituent Effects on the pKa of the Benzoxaborole Pharmacophore. *ACS Med. Chem. Lett.* **2012**, *3*, 48−52.
(47) Adamczyk-Woźniak, A.; Borys, K. M.; Sporzyński, A. Recent Developments in the Chemistry and Biological Applications of Benzoxaboroles. *Chem. Rev.* **2015**, *115*, 5224−5247.
(48) Adamczyk-Woźniak, A.; Brzózka, Z.; Cyrański, M. K.; Filipowicz-Szymańska, A.; Klimentowska, P.; Żubrowska, A.; Żukowski, K.; Sporzyński, A. Ortho-(Aminomethyl)Phenylboronic Acids—synthesis, Structure and Sugar Receptor Activity. *Appl. Organomet. Chem.* **2008**, *22*, 427−432.
(49) *Boronic Acids: Preparation and Applications in Organic Synthesis, Medicine and Materials*; Hall, D. G., Ed.; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2011; Vol. *1*−*2*.
(50) Soundararajan, S.; Badawi, M.; Kohlrust, C. M.; Hageman, J. H. Boronic Acids for Affinity Chromatography: Spectral Methods for Determinations of Ionization and Diol-Binding Constants. *Anal. Biochem.* **1989**, *178*, 125−134.
(51) Luliński, S.; Madura, I.; Serwatowski, J.; Szatyłowicz, H.; Zachara, J. A Tautomeric Equilibrium between Functionalized 2-Formylphenylboronic Acids and Corresponding 1,3-Dihydro-1,3-Dihydroxybenzo[c][2,1]Oxaboroles. *New J. Chem.* **2007**, *31*, 144.
(52) Wulff, G.; Lauer, M.; Böhnke, H. Rapid Proton Transfer as Cause of an Unusually Large Neighboring Group Effect. *Angew. Chem., Int. Ed.* **1984**, *23*, 741−742.
(53) Wiskur, S. L.; Lavigne, J. J.; Ait-Haddou, H.; Lynch, V.; Chiu, Y. H.; Canary, J. W.; Anslyn, E. V. pK(a) Values and Geometries of Secondary and Tertiary Amines Complexed to Boronic Acids-Implications for Sensor Design. *Org. Lett.* **2001**, *3*, 1311−1314.
(54) Zhu, L.; Shabbir, S. H.; Gray, M.; Lynch, V. M.; Sorey, S.; Anslyn, E. V. A Structural Investigation of the NB Interaction in an O-(N, N-Dialkylaminomethyl) Arylboronate System. *J. Am. Chem. Soc.* **2006**, *128*, 1222−1232.
(55) Collins, B. E.; Sorey, S.; Hargrove, A. E.; Shabbir, S. H.; Lynch, V. M.; Anslyn, E. V. Probing Intramolecular B-N Interactions in Ortho-Aminomethyl Arylboronic Acids. *J. Org. Chem.* **2009**, *74*, 4055−4060.
(56) Borlido, L.; Azevedo, A. M.; Roque, A. C. A.; Aires-Barros, M. R. Potential of Boronic Acid Functionalized Magnetic Particles in the Adsorption of Human Antibodies under Mammalian Cell Culture Conditions. *J. Chromatogr. A* **2011**, *1218*, 7821−7827.
(57) Egawa, Y.; Miki, R.; Seki, T. Colorimetric Sugar Sensing Using Boronic Acid-Substituted Azobenzenes. *Materials* **2014**, *7*, 1201−1220.
(58) Draffin, S. P.; Duggan, P. J.; Duggan, S. A. M. Highly Fructose Selective Transport Promoted by Boronic Acids Based on a Pentaerythritol Core. *Org. Lett.* **2001**, *3*, 917−920.
(59) Awino, J. K.; Gunasekara, R. W.; Zhao, Y. Selective Recognition of d-Aldohexoses in Water by Boronic Acid-Functionalized, Molecularly Imprinted Cross-Linked Micelles. *J. Am. Chem. Soc.* **2016**, *138*, 9759−9762.

DOI: 10.1021/acsomega.8b02999
ACS Omega 2018, 3, 17863−17870