Abstract

JUN YAN. Using Boronic Acid as the Recognition and Signaling Moiety for Sugar Sensing. (Under the direction of Dr. Binghe Wang.)

The interaction between a boronic acid and a diol is known to be one of the strongest single-pair reversible functional group interactions in an aqueous environment. During the last decade, much effort has been devoted to the development of boronic acid-based sensors for carbohydrates and other diol-containing compounds, and a great deal of progress has been made in this area. However, there are still several fundamental issues that have not been addressed, which hinder the development of such kind of sensors. For example, several factors have long been recognized as important in influencing the binding affinity of boronic acids to diol-containing compound, but there has never been a systematic examination of the relationship among those factors. To address these issues, in Chapter 2 we carefully examined a series of 25 arylboronic acids with various substituents and selected binding affinities with a series of diols at varying pH value. We have found that (1) the pKa of monosubstituted boronic acids can be predicted based on the substituent effect using a Hammet plot; (2) the common belief that boronic acids with lower pKa's have greater binding affinities at neutral pH is not always true; and (3) the optimal pH for binding is not always above the pKa of the boronic acid, instead it is related to the pKa values of both the boronic acid and the diol, although in a manner that cannot be readily predicted.

Second, critical to the construction of fluorescent sensors for carbohydrates is the availability of practical fluorescent reporters that respond to the binding event with significant fluorescence intensity changes under physiological conditions. So far a few boronic acid reporters have been described in the literature, but those reporters have undesirable properties such as low water solubility and poor photochemical stability. As described in chapter 2, we have discovered a novel type of fluorescent carbohydrate reporter, 8-quinolineboronic acid (8-QBA). It showed about 40 fold fluorescence increase upon binding of a carbohydrate compound and has very good water solubilty. All these properties make such a system ideal for the construction of fluorescent carbohydrate biosensors for biological applications. With this reporter compound in hand, we designed and syntesized a series of dibornic acids, developed an effective synthetic route for a different kind of 8-QBA-based diboronic acid compound. These results are described in Chapter 4.

Using Boronic Acid as the Recognition and Signaling Moiety for Sugar Sensing

by Jun Yan

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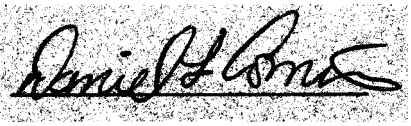
Binghe Wang

Chair of the Advisory Committee



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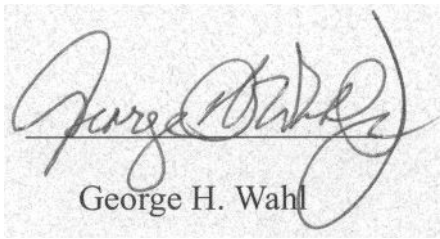

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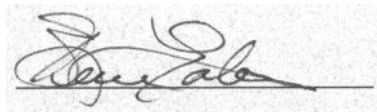

Bruce Eaton

Dedication

To my family:
Chengxian Yan
Shifen Guo
Li Yan
Yinghua Gao
and my lovely niece, Zhihan Gao.

I appreciate all of your love, encouragement and support.

Biography

The author was born on June 10, 1974 in Panzhihua, Sichuan Province, P.R.China. Upon graduating from high school, Jun attended Peking University in Beijing, China and received a bachelor's degree in Chemistry in 1997. In 1997 he began his graduate education in Guangzhou Institute of Geochemistry, Chinese Academy of Sciences in Guangzhou and earned his MS degree in 2000. He joined Professor Binghe Wang's group at North Carolina State University in the fall of 2000 and began working on boronic acid-based carbohydrate sensors.

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List of Abbreviations

Abs.: Absorbance
ARS: Alizarin Red S.
BOC: tert-butoxycarbonyl
DCC: 1,3-dicyclohexylcarbodiimide
DCM:Dichloromethane
D**MF**: *N,N*-dimetylformamide
DMSO: Dimethylsulfoxide
EDCI: 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride
HEPES: N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
ICT: internal Charge Transfer
$\mathbf{I_f}$: Fluorescence Intensity
$\mathbf{K_{a\text{-}acid}}$: Acid dissociation constant of the boronic acid
$\mathbf{K_{a\text{-}ester}}$: Acid dissociation constant of the boronic ester
$\mathbf{K_{eq\text{-}trig}}$: Equilibrium constant of the trigonal boronic acid with the diol
$\mathbf{K_{eq\text{-}tet}}$: Equilibrium constant of the tetrahedral boronic acid with the diol
NMR: nuclear magnetic resonance
PBA: Phenylboronic acid
PET: Photoinduced electron transfer
Ph: phenyl
QBA: quinolineboronic acid
rt: room temperature
s: singlet
t: triplet
TEA: triethylamine
TFA: trifluoroacetic acid
THF: tetrahydrofuran
TLC: thin layer chromatography
UV: ultralviolet

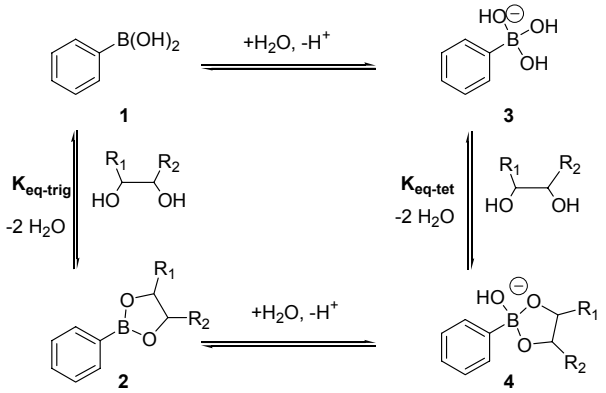
Chapter I. Introduction

It is well known that mammalian cell surfaces are coated with saccharides in the forms of glycoproteins and glycolipids**.** [1-9] These glycosylated biomolecules play very important roles in various physiological and pathological processes. [10,11] Critical to essentially all the saccharide-mediated events is the binding of a biomolecule to certain cell-surface saccharide or its conjugates. [12-14] For example, cell-cell adhesion in inflammatory processes involves lectin-carbohydrate interactions, which mediate leukocyte latching to inflamed tissues; [15,16] HIV infection is mediated by glycoprotein binding with cell surface receptors; embryonic development at early stages is known to rely on Lewis X for cell-cell adhesion; [17] and certain cancer metastasis also involves saccharide-mediated events. [18,19] Furthermore, binding of certain cell-surface carbohydrates by a class of carbohydrate-binding proteins, lectins, is known to trigger various biological events. [20] For example, galectin-3 binding to its target has been shown to trigger apoptosis. [21] Therefore, small molecule mimics of proteins that can bind cell-surface carbohydrates (artificial lectins) could have a variety of biological and biomedical applications ranging from diagnostics to medicinal agents. In designing such artificial lectins, boronic acids occupy a special place. This is due to the tight and reversible complexation of the boronic acid moiety with 1,2- or 1,3-diols commonly found on carbohydrates. [22-24] This dissertation work focuses on (1) the examination of various factors that affect the diol-boronic acid binding, (2) the development of a water-soluble fluorescent boronic acid reporter compound that responds to the diol binding by changing fluorescent properties, and (3) the development of new synthetic methods that allow for the synthesis of diboronic acid sensors using the new fluorescent reporter compound

discovered. Some background information on boronic acid-diol interactions, and previous effort on developing fluorescent boronic acid reporter compounds are described in this introductory chapter to put the dissertation work in perspective.

1.1. The Binding between a Boronic Acid and a Diol-the Chemistry Involved.

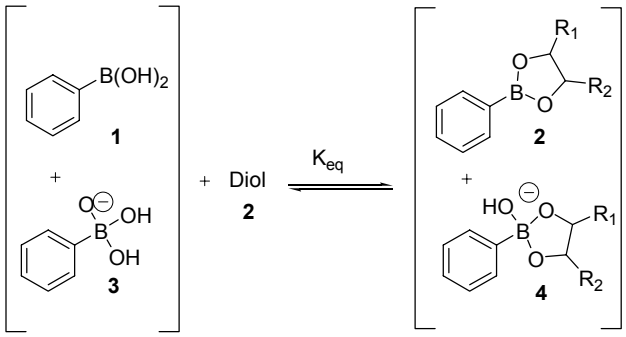
1.1.1. The Equilibriums in the Boronic Acid-Diol Aqueous Solution



Scheme 1.1. The binding process between phenylboronic acid and a diol.

The interaction between a boronic acid and a diol is known to be one of the strongest single-pair reversible functional group interactions in an aqueous environment. Critical to the effort of using boronic acid as the recognition motif is the understanding of the chemistry involved. However, before any further discussions of the binding, it is important to note that the acidity of the boronic acid is not reflected in its deprotonation as is commonly defined in carboxylic acids, rather its reaction with a water molecule that leads to the formation of the boronate species (**3**) with the release of a proton (Scheme 1.1). By the same token, the boronic ester (**2**) is also an acid and can react with water to release a proton. Because of this unique feature that both the boronic acid and boronic ester are acids, and both the complexed (**2** and **4**) and un-complexed (**1** and **3**) species

exist in two ionization states, there are several ways to describe the binding between a boronic acid and a diol. [23] These include (1) K_{trig} describing the equilibrium between the two boronic species (**1** and **2,** Scheme 1.1); (2) K_{tet} describing the equilibrium between the two boronate species (**3** and **4**); and (3) K_{eq} describing the overall equilibrium between the un-complexed (**1** and **3**) and the complexed species (**2** and **4**) (Scheme 1.2). [23]



Scheme 1.2. The overall binding constant K_{eq}.

The ability of boronic and boric acids (**1,** Scheme 1.1) to bind with diols was first recognized over a century ago. In 1842, it was reported that sugars increased the acidity of boric acid solutions. [25] Later publications included comparative studies of different diols and their binding abilities with boric and boronic acids. [26,27] In 1959, Lorand and coworkers published the first comprehensive study of the binding constants between various diol-containing compounds and phenylboronic acid (PBA) using the so-called pH-depression method (Table 1.1). [22] The study demonstrated that different diols have different affinities for the boronic acid group and lower the acidity of the boron species to different degrees. The numbers listed in Table 1.1, although referred to as binding constants, are very different from the binding constants of other monoboronic acids determined later using spectroscopic methods, sometimes by over 20-fold. A careful analysis of the situation indicates that this discrepancy is due to a lack of clear definition

of the meaning of the term "binding constants." The "binding constants" determined using the pH-depression method are K_{tet} instead of K_{eq}. [23] This was because the pH-depression method assumed that the boronic ester did not exist (or existed in negligible amount). Therefore, the K_{trig} part of the equation in Scheme 1.1 was omitted. Since the relationship between **1** and **3** is an acid-base equilibrium, then the only unknown that was determined was the K_{tet}. With this clarification, it became easy to understand why the discrepancy in the literature.

Table 1. 1. Association constants with PBA.

Diols	Association constants	
	a (K_{tet})	b (K_{eq})
1,3-propanediol	0.88	
ethylene glycol	2.8	
phenyl-1,2-ethanediol	9.90	
glucose	110	4.6
fructose	4370	160
catechol	17500	830
sorbitol		370
mannitol	2275	120
galactose	276	15

a. Measured by the pH-depression method. [22]
b. Measured by the ARS competition method at physiological pH. [23]

Springsteen and Wang in 2002 published the first systematic examination of the K_{eq} between various diol-containing compounds and phenylboronic acid (Table 1.1). These numbers are much lower than the K_{tet} determined using the pH-depression method. Springsteen and Wang also pointed out that buffer has an effect on the binding constants between a boronic acid and a diol. Therefore, the binding constants determined are really apparent binding constants under the specific experimental conditions described. The understanding of the buffer effect is very important for biological applications. James and co-workers have recently published a detailed study on the effect of buffer[28] which will be discussed later.

Springsteen and Wang also described the relations among K_{trig}, $K_{tet,}$ and K_{eq} (Eq 1-6). [23,29] Such relationship allows for the comparison of various "binding constants" determined using different methods.

(1)

$$K_{eq} = (([BD^-][H^+] / K_{a\text{-}ester}) + [BD^-]) / (([B^-][H^+] / K_{a\text{-}acid}) + [B^-]) ([D] + [D^-]))$$

$$K_{eq\text{-}tet} = [BD^-] / [H^+]([D^-]/[D]+[D^-])[B^-][Diol] \quad (2)$$

$$K_{eq\text{-}trig} = [BD] / [H^+]([D^-]/[D]+[D^-]) [B][Diol] \quad (3)$$

(4)

$$K_{eq\text{-}tet} = \frac{1 + \frac{[H^+]}{K_{a\text{-}acid}}}{1 + \frac{[H^+]}{K_{a\text{-}ester}}} \times K_{eq}$$

(5)

$$K_{eq\text{-}trig} = \frac{1 + \frac{[H^+]}{K_{a\text{-}acid}}}{1 + \frac{[H^+]}{K_{a\text{-}ester}}} \times K_{eq} \frac{K_{a\text{-}acid}}{K_{a\text{-}ester}}$$

$$K_{eq} = \%acid \times K_{eq-trig} + \%ester \times K_{eq-tet} \quad (6)$$

B = Trigonal Boronic Acid, **BD** = Complex of Trigonal Boronic Aicd and Diol
B$^-$ = Tetrahedral Boronic Acid **BD**$^-$ = Complex of Tetrahedral Boronic Acid and Diol

1.1.2. Methods for Binding Constants Determination

In order to examine factors that affect the binding between boronic acids and diols, there need to be sensitive methods for the determination of their binding constants. Early methods include the pH-depression method[22,30 31-34] and the ^{11}B-NMR method. [35-40] These methods, although served the purpose for the early determination of binding constants between boronic acids and diols, are insensitive, require a large amount of sample, and take long experiment times. Therefore, these two methods most of the time do not meet the need in modern day sensor and boronolectin (boronic acid-based artificial lectins) design. Recent effort focuses on spectroscopic methods, which are easy to use, sensitive,

rapid, and require very little sample. Below is a detailed description of the different methods used in the determination of the binding between boronic acids and diols.

1.1.2.1. The pH-Depression Method

The very first method used for the systematic determination of boronic acid-diol binding constants was the so-called pH-depression method. [22,30-34] This is based on the finding that most the time diol binding lowers the pKa of boronic acid. Therefore, addition of a diol to a boronic acid solution would lower its pH. The extent of the pH lowering effect of a particular diol can be correlated with the "binding constant" between these two species. In the mathematical derivation of the "binding constant," it was assumed that the trigonal boronic ester (**2**, Scheme 1.1) did not exist. This was based on the idea that the decreased pKa of the boron upon ester formation would ensure that it is converted to its tetrahedral anionic form (**4**). Therefore, the equation was simplified to K_{tet}. In addition to the fact that the "binding constants" determined using the pH-depression method is the K_{tet}, our own experiments later on have also shown that the assumption that the neutral boronic ester did not exist is incorrect. [23] For example, the apparent pKa of the phenylboronic ester of glucose is about 6.8. At physiological pH, about 20% is in the free ester form. Although the exact pH under which the pH-depression experiments were conducted is not known, it is reasonable to expect that the pH of a particular boronic ester be close to its pKa. Therefore, one would expect that the boronic ester exist in over 20-30% under some of the experimental conditions. In conclusion, the pH-depression method gives an approximate estimation of the K_{tet}, and this method requires a large amount of the boronic acid sample.

1.1.2.2. ^{11}B-NMR Method

Another method that had been used was that of ^{11}B-NMR. [35-40] This is based on the same concept that the boronic ester pKa is lower than that of the free boronic acid. Therefore, addition of a diol to a boronic acid solution would result in an increased portion of the boron being converted from the neutral trigonal form to the anionic tetrahedral form. This change can be detected using B-NMR because of the significant chemical shift differences (about 30 ppm for the neutral form and 7 ppm for the anionic form of boronic acids). The ^{11}B-NMR approach uses a similar mathematical derivation, and, therefore, also gives the K_{tet}. For example, the binding constants of boric acid with glucose and galactose were determined using NMR method. [36] They are generally in the range of 100 M^{-1}, which are in line with the K_{tet} numbers determined using the pH-depression method. When the distinct species can be directly detected, this offers a direct and excellent approach to the determination of the K_{tet}. However, the ^{11}B-NMR method suffers from low sensitivity, difficulties with peak resolution, and the requirement for high concentration of the sensor compound. Such restrictions make the ^{11}B-NMR method less useful in the development of boronic acid sensors. Therefore, if sample quantity is an issue, the utility of this method would be limited.

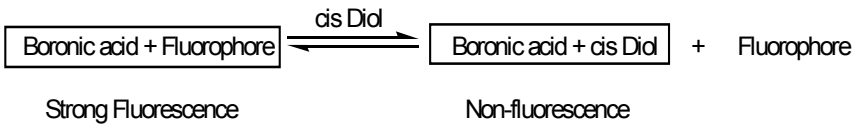
1.1.2.3. Spectroscopic Methods

It is well-known that spectroscopic methods are generally more sensitive than methods using NMR or pH determination, although not always. Therefore, it is easy to understand that there has been much effort in developing spectroscopic methods for the determination of the binding constants between boronic acids and diols. However, for this to happen, the binding event needs to trigger a change in the spectroscopic properties of the boronic acid component. There are certain boronic acids that show significant

fluorescent property changes upon diol binding. We term them as boronic acid spectroscopic/fluorescent reporter compounds. For those compounds, spectroscopic determination is a very sensitive and convenient way for determining the binding constants. In this regard, spectroscopic methods used for the binding constant determination include CD spectroscopy[4142-49] absorption spectroscopy, [50-55] and fluorescence spectroscopy. [23,56-67] For example, Yoon and Czarnik determined the dissociation constant of anthrylboronic acid with D-fructose at pH 7.4 using fluorescence spectroscopy. [68] Titration of anthrylboronic acid (0.75 μM) at pH 7.4 with a polyol gives a dissociation constant of about 3.7 mM with D-fructose. Similarly, UV absorption titration was used for the K_{eq} determination in nitrophenol-based boronic acid color sensors. The association constant was obtained for a couple of sugar boronic esters. [55] CD is another detection method for boronic acid based sugar sensors. The Shinkai group developed a porphyrin based boronic acid sensor system. Upon binding to sugars, changes in CD were observed, presumably due to the immobilization of two porphyrins by a saccharide bridge. From the plot of CD intensity vs saccharide concentration was obtained the association constant. [41-49] When the boronic acids possess the appropriate spectroscopic properties for direct measurement, such methods work very well. However, most boronic acids do not change their spectroscopic properties sufficiently and/or consistently enough for their binding constants to be determined this way. The section will discuss in detail how a fluorescent reporter compound can be used for the sensitive determination of boronic acid-diol binding constants in a competition assay.

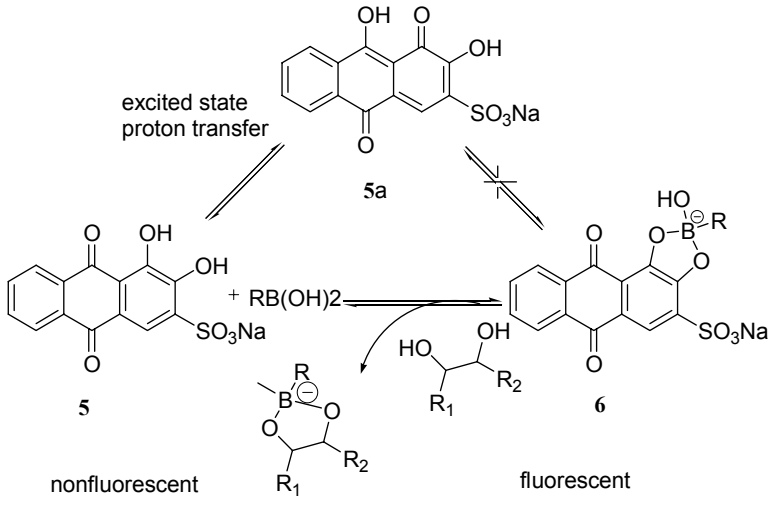
Because of the limitations of the various methods available, there was a need in the field for the development of a general and sensitive method for the determination of the

binding constants between boronic acids and diols, regardless of whether the boronic acids are fluorescent or not. To address this issue, our group developed a generally applicable, highly sensitive method for the determination of the binding constants between boronic acids and diols. This method uses Alizarin Red S. (ARS) as a reporter compound in a three-component competition assay (Scheme 1.3).



Scheme 1.3. Saccharide detection by using a competitive boronic acid receptor.

ARS is a dye commonly used in the textile industry. [69] It has been used as a reagent for the fluorometric detection of boric acid. [69] ARS is not fluorescent, because of the possible excited state proton transfer from one of the catechol dihydroxyl groups to the carbonyl oxygen (Scheme 1.4,) [70]



Scheme 1.4. Alizarin Red S. (ARS) binding with a Boronic Acid.

Conceivably, binding to a boronic acid would abolish the excited state proton

transfer process, which is responsible for the fluorescence quenching. This should result in significant fluorescent intensity increases upon boronic acid binding. Based on this assumption, we tested the binding of a series of 6 boronic/boric acids to ARS. All those tested caused a very significant fluorescent intensity change of ARS (30-90 fold, Table 1.2). [29] Even boric acid induced about 25 fold fluorescent intensity increase. It is also important to note that the most sensitive region is around physiological pH and ARS is very soluble in water. These are two important criteria for the study of binding under near physiological conditions. In addition, this method also allows for the determination of the binding constants at a given pH and in various buffers, both are factors that influence the apparent binding constants. One may argue that the "intrinsic" binding constants should be independent of external factors such as buffer. However, for what we and many other groups are interested, the application of boronolectins and boronic acid-based sensors in near physiological conditions, the apparent binding constants under certain designated conditions are more important than that of the "intrinsic" affinities. One can draw an analogy between this and enzyme-inhibitor binding, where the "intrinsic" binding affinity without the influence of external factors has little practical meaning because of the intended applications, although the theoretical studies of such "intrinsic" affinities may help understand the fundamental questions in binding and catalysis. Therefore, when one designs a study, it has to be very clear as to what the purpose is for such a study in order to remain focused on the problem one intends to address.

Using this method, we have determined the apparent binding constants of a series of diol-containing compounds with phenylboronic acid. This was carried out by first determining the binding constants between the boronic acid and ARS followed by the

addition of the interested diol (Scheme 1.4). The addition of a diol-containing molecule perturbs the ARS-boronic acid equilibrium, which provides a measure of the strength of the boronic acid-diol association. This method allows for the determination of the K_{eq} as well as K_{tet} and K_{trig} as defined in Scheme 1.1 (Equations 4 and 5). Again, we should note that these numbers are the apparent binding constants under these specific conditions.

The development of the ARS method allowed for the first time the sensitive determination of K_{eq} between a boronic acid and a diol regardless of the spectroscopic properties of the boronic acid portion. It is also easy to use and rapid. It allowed us to understand the relationship between K_{eq}, K_{tet}, and K_{trig}. Although this method is not free from interference by factors such as the nature of the buffer and its concentration, it allows for the determination of binding constants relevant to the intended applications, as long as one carefully designs the experiments.

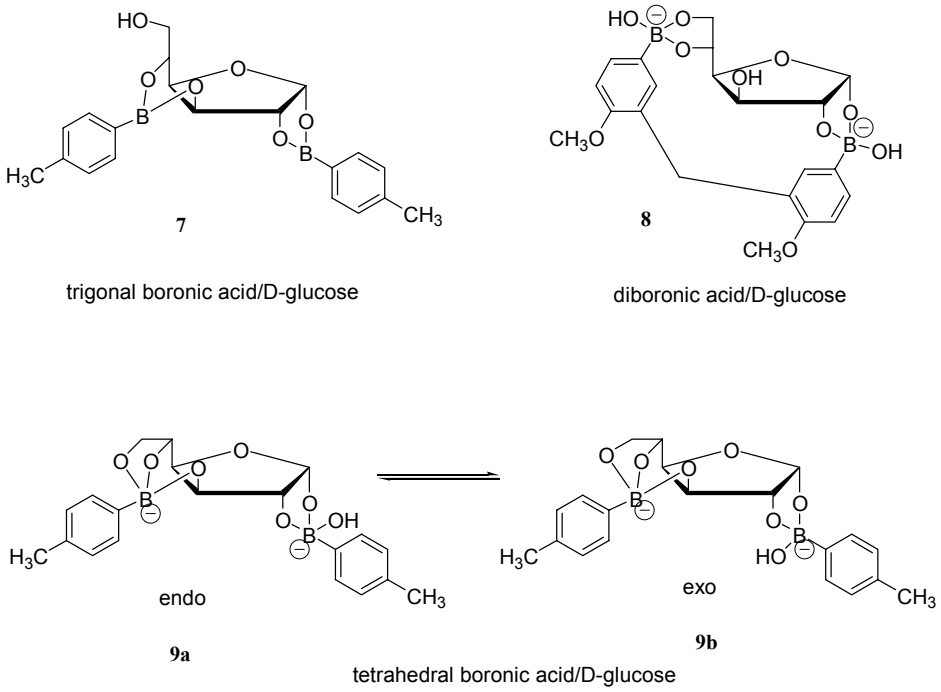
Table 1.2. Fluorescent intensity change of ARS in the presence of a series of aryl boronic acids. [29]

Boronic acids	Fluorescence Intensity Increase (fold)
methylpyridinium-3-boronic acid	30
phenylboronic acid	59
boric acid	26
2,4,6-trifluoroboronic acid	88
Pyridine-3-boronic acid	63
4-fluoro-2-methylphenyl boronic acid	53

1.1.3. The Structures of Boronic Acid-Diol Complex

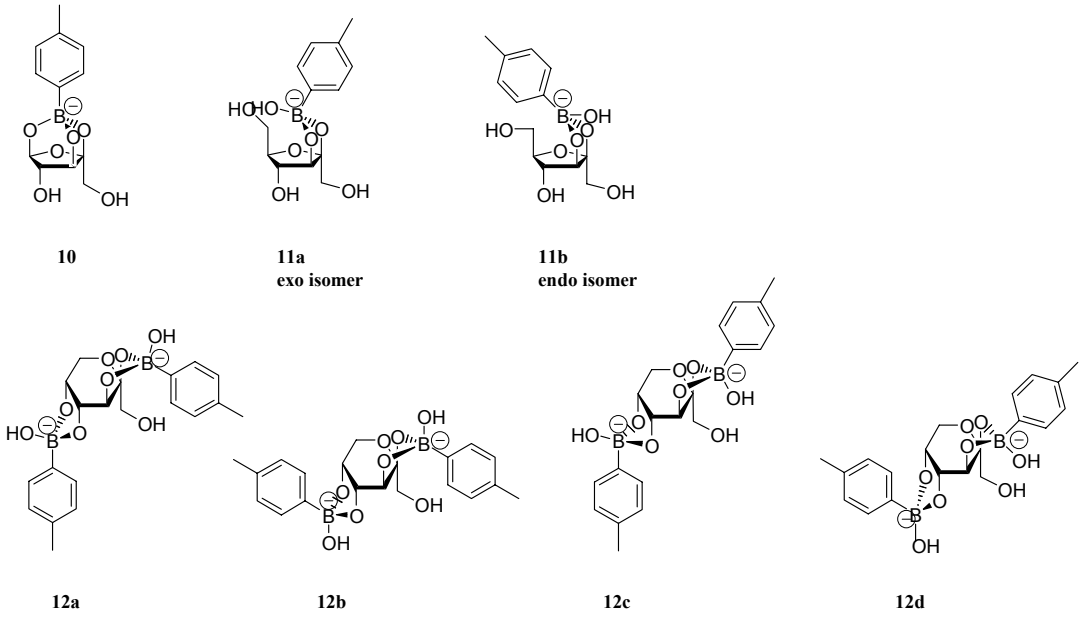
Although the high affinity of boronic acid for sugars was recognized in the 1950's, [22,26,71] the structural studies of the sugar-boronic acid complexes came at a later time with the availability of NMR tools. The first series of such studies were performed

in 1960's and 1970's using ^{1}H and ^{13}C NMR spectroscopy. [72-74] However, with the continuous wavelength instrument at relatively low field strength, the studies could only be carried out in organic solvents that provide the high solubility needed in order to see the signal. For carbohydrates sensor development, it is more important to understand the structural features under aqueous conditions. With the availability of high-field FT-NMR instrument, studies in aqueous environments became feasible. Most of the studies focused on using either mono- or diboronic acids and glucose or fructose as the model sugar. Along this line, several groups have devoted much effort. [75-80] The conformational and structural studies were mostly based on the determination of C-H and H-H coupling constants which are different depending on the ring size and conformation of the sugar in the complex. For a while, there was some debate as to whether the complex between glucose and phenylboronic acid exists in the pyranose and furanose form. [79,81,82] In 1995, Eggert and Norrild studied the complex formation between boronic acid and D-glucose using ^{1}H and ^{13}C NMR spectroscopy under both neutral non-aqueous and alkaline aqueous conditions. A furanose complex structure was deduced by comparing the chemical shifts and coupling constants of the boronic acid-glucose complex with those of the boronic acid-modified glucofuranose complex. [79] Their studies showed that in both neutral non-aqueous and alkaline aqueous conditions**,** the first binding site was position (1, 2) of the furanose form of D-glucose. Under neutral non-aqueous conditions the second boronic acid was bound to positions (3, 5) (**7,** Scheme 1.5). Under alkaline aqueous conditions the second monoboronic acid was bound to positions (3, 5, 6) (**9a** and **9b** scheme 1.5) in a tris coordinated manner, and a diboronic acid sensor (**8)** binds its second boronic acid group only to positions (5, 6) (Scheme 1.5).



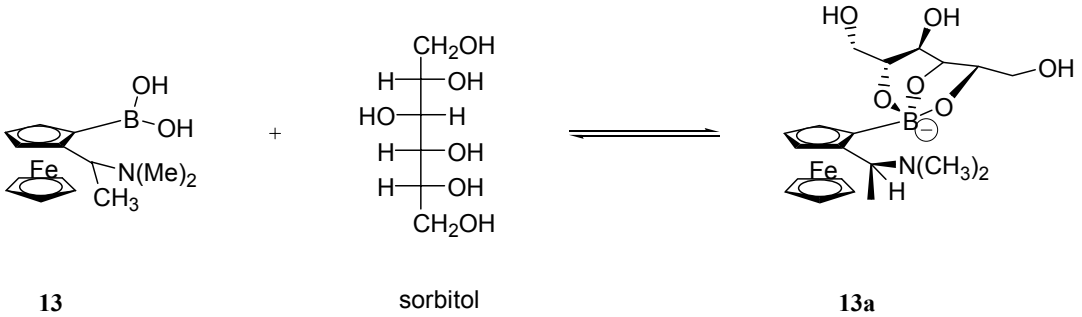
Scheme 1.5. Structures of D-glucose complexes formed with trigonal p-tolylboronic acid, tetrahedral p-tolylboronic acid and a diboronic acid. [79]

In 1996, Norrild reported the structural studies of the fructose complex with phenylboronic acid by using 2D NMR. [80] In a ratio of 1:1, boronic acid and fructose, under alkaline aqueous condition, form a 2, 3, 6 tridentate complex as the major product (**10**, Scheme 1.6, 82%). A small amount of 2, 3 exo and endo isomers (**11a** and **11b**, Scheme 1.6) were present while fructose was in furanose form in all cases. Four new complexes were observed at high boronic acid/fructose ratios, such as 2:1 and 4:1 (Scheme 1.6). Two boronic acids can bind to positions (2, 3) and (4, 5) of the fructopyranose, respectively, result in four endo/exo diastereomers as shown in Scheme 1.6.



Scheme 1.6. Structural assignments of additional D-fructose boronate esters formed at higher boronic acid: carbohydrate ratios. [80]

Sorbitol is another sugar that has been reported to form a trivalent complex with a boronic acid. (Scheme 1.7**)** In 2001, Norrild studied the complex structure between D-sorbitol and (S, S)-2-(*N,N*-dimetyl-1-amoniethyl) ferroceneboronic acid using ^{1}H, ^{13}C and ^{11}B NMR. A 2,3,5-bound sorbitol complex (**13a**, Scheme 1.7) was deduced. [83]Since sorbitol and fructose both bind to a sugar, when possible, in a tridentate fashion, it becomes easy to understand why they normally have higher affinities to boronic acids than other commonly seen sugars such as glucose, mannose, etc. [23]



Scheme 1.7. Complex of sorbitol and (S, S)-2-(N,N-dimetyl-1-amoniethyl) ferroceneboronic Acid.

1.1.4. Factors that Influence the Binding

One of the most important things in designing boronic acid-based sensors and boronolectins is the understanding of how various factors influence the binding between a simple boronic acid and a diol. It is generally believed that pH, the nature and concentration of the buffer, the pKa of the boronic acid, the pKa of the boronic ester, the dihedral angle of the diol, and of course temperature. Below is a summary discussion of all these issues.

1.1.4.1. The Apparent pKa Values of the Boronic Esters and the Stability of the Boronic Acid-Diol Complex.

It is well known that the pKa values of boronic esters are generally lower than that of the boronic acids. As discussed earlier, different diols have different affinity for a given boronic acid. It seems that the binding constant is generally correlated with the apparent pKa value of the boronic ester, with the exception of those engaged in trivalent binding such as sorbitol. [23] The lower the apparent pKa of the boronic ester, the higher the binding constant. Therefore, any factor that increases the boron acidity should increase the binding constants as well. Theoretically, the change in pKa values from boronic acid to boronic esters could be the result of either electronic or steric factors. However, the added alkyl groups in boronic esters, compared with the free boronic acids (**2**, Scheme 1.1), are electron-donating, and would be expected to increase the electron density on the boron atom, which should result in a decrease in the acidity of the ester. [29] This is apparently contrary to experimental results. Consequently, electronic factor is probably not the dominant factor. On the other hand, the different affinity among various diols can be easily explained based on steric (geometric) factors, i.e., their ability to force

the boron to go from trigonal to the tetrahedral form can be directly related to the boronic ester pKa and the diol-boronic acid binding constants. A key factor in affecting the conversion from trigonal to tetrahedral form is the dihedral angle of the diol. Smaller dihedral angles correlate with a smaller distance between the two diol oxygen, which in turn favors a smaller O-B-O bond angle that closely resembles the sp^3 form (109 degree) and moves away from the 120 degree needed for the boron to stay in the trigonal form. This is the reason why diols with small and constrained dihedral angles tend to bind to boronic acids with higher affinities. [29]

1.1.4.2 Buffer Effects

The use of buffer is very common in evaluating boronolectins and boronic acid-based sensors. For biological applications, phosphate is a commonly used buffer in simulating physiological conditions at pH 7.4. In order to get a good sense of how results obtained using certain buffer can be extrapolated to near physiological conditions, one needs to have a good understanding of the buffer effect on the binding constants between a boronic acid and a diol. Although studies in this area are limited, one thing is sure, i.e., buffer does play an important role in influencing the binding constants determined. This is understandable since boronic acid is a Lewis acid. The presence and concentration of Lewis bases such as phosphate, chloride, fluoride, and bromide are expected to affect the complexation state of the boron atom, and therefore its ability to interact with other Lewis bases such as water.

Our lab has shown that the nature and concentration of the buffer do affect the binding affinity. [23] For example, when determined in phosphate buffer, the binding constants between ARS and phenylboronic acid changed by over three folds in the buffer

concentration range of 0.05 – 1 M. On the other hand, the binding constants between fructose and phenylboronic acid did not change as much. It is not clear why this difference in binding constants between these two diols. Compared with phosphate buffer, the concentration of HEPES buffer, within the range tested, seemed to have very little effect on the binding constants. There are two possible reasons that could explain the lack of any significant effect of buffer concentration when HEPES was used. In the first scenario, HEPES buffer may not interact with boronic acid as a Lewis base. Under such a situation, one could envision a lack of buffer effect on the binding. A more likely explanation is with the second scenario, when at the lowest concentration tested, the buffer could have reached its maximal effect. This is very logical since HEPES has a 1,2-aminoalcohol structure, which could have a high affinity with a boronic acid. Furthermore, the binding constants determined in HEPES buffer are lower than that determined at the highest concentration of phosphate buffer, which is consistent with the higher effect of the HEPES. Clearly, there is much more to do in order to understand the effect of buffer on the binding constants between a boronic acid and a diol. In a very recent study, James and co-workers have studied the buffer (phosphate) effect in a quantitative fashion. [28] The same approach can be very useful for the quantitative de-convolution of the effect of other Lewis bases (buffer components) on the binding constants between a boronic acid and a diol.

1.1.4.3. pH and pKa of Boronic Acids

It's well known that pH affects the affinities of boronic acids toward diols, the higher the pH, the higher the binding constants between boronic acid and diol. It is also assumed that boronic acids with lower pKa's have higher affinities at neutral pH. [37,39,84-86]

Our group's study on the relationships between pKa, pH and binding constant indicates that this is not always true. [23] It's clear that pKa and pH play important role in the binding process. However, there has not been a systematic examination on the relationships among all those factors so far. Any information on this subject would undoubtedly assist the efforts to optimize the binding affinity and specificity of potential fluorescent sensors. Chapter 2 discusses in detail on the finding from our studies on this subject.

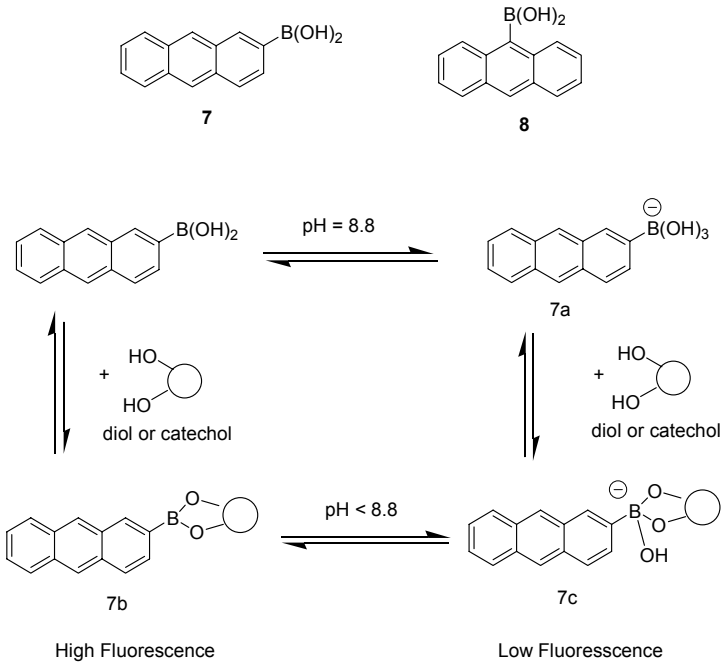
1.2. The Design of Fluorescent Boronic Acid Reporter Compounds

A key component in the design of fluorescent sensor is the fluorescent reporter unit that changes fluroescent properties upon binding. Therefore, it is not surprising that there has been much effort in the development of such reporter boronic acids. In this area, several groups have made very important contributions using various mechanisms. [75,79,81,87-89] In those fluorescent reporter systems, the binding event between a boronic acid and diols acts as an on-off or off-on switch to generate detectable fluorescence changes. In rare exceptional cases, the fluorescent properties change at more than one wavelength and therefore give a ratiometric sensing mechanism. [90] This section will give a brief description of literature fluorescent reporter compounds and their important properties. Because the work in this area cannot be easily categorized based on either mechanism, they are grouped based on the principal investigators.

1.2.1. Work of the Czarnik Group

In 1992, Czarnik and co-workers reported the first boronic acid system that showed significant fluorescent intensity changes upon sugar binding. Specifically, 2- and 9-anthrylboronic acids (**7** and **8,** Scheme 1.8) were prepared and examined. Addition of a

sugar or a catechol resulted in decreased fluorescence intensity. [68,91] As discussed earlier, since the boronic ester has a lower pKa, the addition of the sugar converted the boron from trigonal neutral form to the anionic tetrahedral form. For example, the fructose-**7** complex has an apparent pKa of about 5.9, which means that it exists in the anionic tetrahedral form at physiological pH. Although the mechanism through which the observed fluorescence intensity change happens was not extensively examined, it was thought that boronate anion (**7a**) quenches the anthracene fluorescence. This was a seminal study that stimulated much more work later on in using boronic acid for the preparation of fluorescent sensors for carbohydrates.



Scheme 1.8. Boronic acid reporters from the Czarnik group.

1.2.2. Work of the Shinkai Group

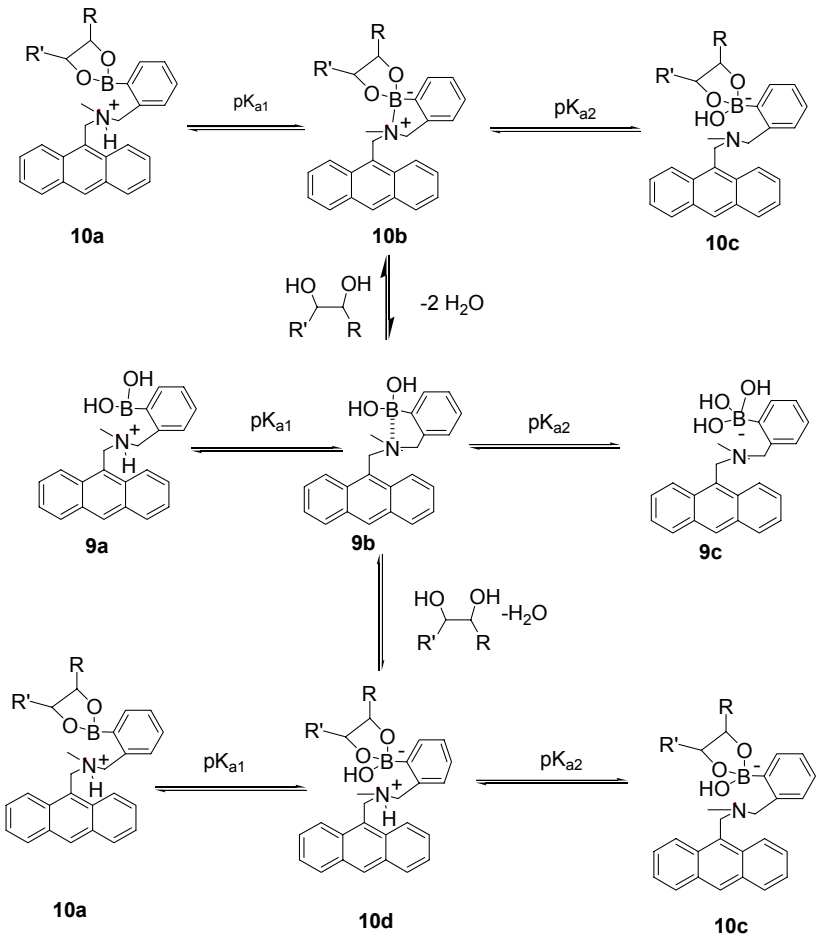
During the last decade, the Shinkai lab played a very prominent role in the field of using boronic acid as the recognition moiety for the preparation of sensors for saccharides. The work involves the preparation of multiboronic acid selective sensors for

various saccharides. Of course, at the center of such research activities is the availability of reporter compounds that change spectroscopic properties upon saccharide binding.

1.2.2.1. The Anthracence-based Systems

Shinkai's group also used the anthracene system in designing fluorescent reporter **9** (Scheme 1.9). In this system, an amino group is positioned in a 1,5-relationship with the boronic acid. It had previously been demonstrated by Wulff[92] that such an arrangement promotes B-N bond formation under certain conditions. When this happens, it helps to lower the apparent pKa of the boronic acid, which was presummed to help increase binding as well as B-N strength. Indeed, **9** showed much higher binding affinity to fructose and glucose compared with phenylboronic acid[24,60,93] This system (**9**) also exhibits a very significant fluorescence intensity change upon sugar binding. This system and its analogs have been used by many labs, [61,62,94] including our own, [59,63,95] for the synthesis of fluorescent sensors for saccharides.

Initially, the mechanism through which the fluorescence intensity changes was thought to be by the modulation of the excited state photoinduced electron transfer (PET) through the formation or strengthening of the B-N bond (Scheme 1.9). Specifically, the lone pair electrons on the benzylic amine moiety is known to quench the anthracene fluorescence. Consequently, **9** in the absence of any saccharide is only weakly fluorescent. Upon ester formation, due to the increased acidity of the boron, [29] it was proposed that B-N bond forms or strengthens, which helps to "tie up" the lone pair electrons, and therfore prevent fluorescence quenching through PET. The end result is an increased fluorescent intensity upon sugar binding. Although this mechanism seems to conform with the observed fluorescent intensity changes upon sugar binding, there are



Scheme 1.9. Possible mechanisms for the fluorescence intensity changes for the Shinkai anthracene system.

telltale signs that the mechanism through which such a system works may not have to involve B-N bond formation as originally proposed. Because of the enormous role that compound **9a** has played in the field of boronic acid-based sensor design, our lab undertook an effort to examined the mechanistic detail of the fluorescent intensity changes of this system upon sugar binding. Theoretically, one can write two reasonable mechanisms through which fluorescence intensity changes can happen upon binding to a sugar (Scheme 1.9). In addition to the B-N bond mechanism, which is shown in the top portion of Scheme 1.9, one can also write a hydrolysis mechanism (lower portion of Scheme 1.9) in which addition of a diol essentially leads to the hydrolysis of the weak B-

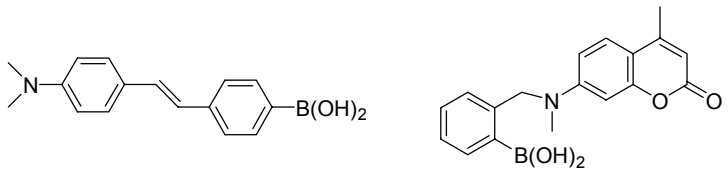
N bond alredy exsiting in the free acid form (**9b**). In the B-N bond mechanism, the strengthening of the B-N bond could help to "tie up" the amine nitrogen lone pair electrons and prevent PET. The diminished PET should result in increased fluorescence intensity. In the hydrolysis mechanism, the protonation of the nitrogen could "tie up" the nitrogen lone pair electrons and result in the same fluorescence intensity increase. We have analyzed these two possible mechanisms and designed a series tests to see which is the more likely mechanism. Along this line, we (1) studied the pH profile of the reporter system in the presence and absence of a sugar, (2) examined the maximal fluorescence intensity changes induced by various sugars to see whether there would be any difference or not, (3) tested the effect of sugars capable of trivalent binding to boronic acid, and (4) calculated the B-N bond strength. [55] All such anslyses are consistent with the hydrolysis mechanism, and inconsistent with the B-N bond formation mechanism. Specifically, the pH profiles of the free boronic acid (**9a**) shows a singificant fluorescence intensity decrease assoicated with the first pKa, but not the second. With the ester (**10a**), the situation is just opposite. This is only consistent with the hydrolysis mechanism because the B-N mechanism would give the ester the same pH profile as the free acid itself. The studies with different sugars also led to the same conclusion. It is well known that the pKa values of the boronic ester of different sugars are different. This would mean that the the B-N bond strength would be different for **10b**, which should in turn affect the quenching efficience by the lone pair electrons and consequently the maximal fluorescent intensity for a paricular ester. However, all sugars gave the same maximal fluorescence intensity, which is inconsistent with the B-N bond mechanism, but consistent with the hydrolysis mechanism because the fluorescent species (**10d**) have the same strucutral

featurs regardless of which sugar is used. In addition, if the B-N bond is the reason for the fluorescence intensity changes, sugars capable of trivalent binding to a boronic acid should not be able to induce fluorescent intensity changes. The same fluorescent intensity changes observed with sorbitol and fructose, both of which are capable of binding in a trivalent fashion, [80,83] are again inconsistent with the B-N bond mechansim, but are consistent with the hydrolysis mechanism. Lastly, the B-N bond strength has been estimated to be around 3 kcal/mol, [96] far smaller than what is needed to "tie up" the lone pair electrons to prevent PET. With all this evidence, it is reasonable to say that the Shinkai system most likely undergoes fluorescent intensitiy changes upon sugar binding through a hydrolysis mechanism (Scheme 1.9, **10d**). Specifically, in the free form the sensor can exist as **9b**. Upon sugar addition, because of the lowered intrinsic pKa of the boron, the first pKa becomes the reaction of the boron with a water molecule to give **10d**. The existence of the anionic boron next to the amine also helps to stabilize the protonated form, which increase its pKa. The conversion of the weak B-N bond form in **9b** to the amine-protonated form (**10d**) allows for the masking of the lone pair electrons, which abolishes the PET quenching of the anthracene fluorescence and results in an increase fluorescence intensity. [55]

1.2.2.2. ICT sensor

The Shinkai group also reported the use of stilllen boronoic acid **11** as fluorescent carbohydrate sensor in 1995. [97] Lackwoicz group later on characterized this system as an ICT (internal charge transfer) sensor (Figure 1.1). Therefore, this part will be discussed in detail in a later section focused on work from the Lackwoicz lab. In 1995,

the Shinkai group synthesized another boronic acid **12** with the coumarin chromophore as an ICT sensor. [98] However, compound **12** only showed a very small shift in fluorescence intensity and wavelength upon addition of sugars.

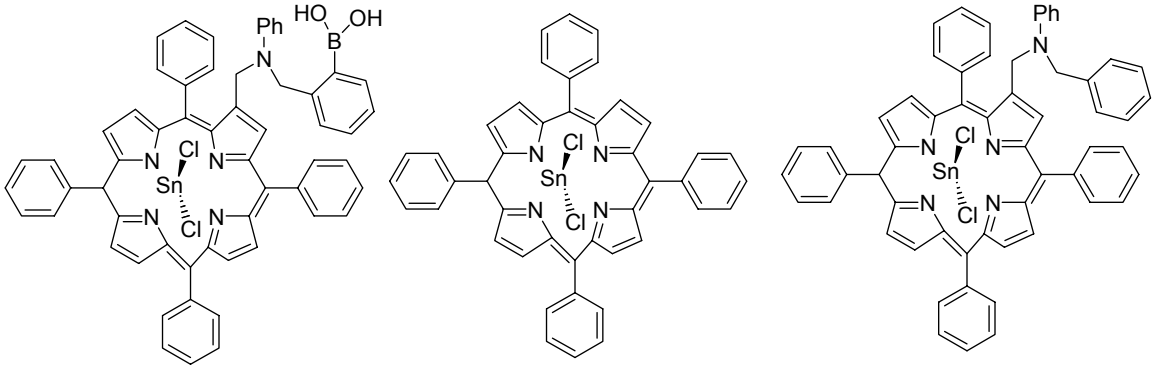


11 **12**

Figure 1.1. ICT fluorescent reporters from the Shinkai group.

1.2.2.3. Porphyrin systems and others

Using a similar concept to the anthracene system, the Shinkai group reported another PET sensor, tetraphenyl porphyrinato)tin(iv) **13** (Figure 1.2), which is an "on-off" reporter upon binding with carbohydrates. [99] Molecular dynamic simulation results suggested that the tertiary nitrogen and boron atom were close to each other in the free receptor form. However, in the complexed form, it was suggested that the distance between the boron and nitrogen atom was longer than its acid form due to steric effect. Such an effect was proposed to decrease the B-N interaction. Consequently, lone pair electrons on nitrogen were free to quench the fluorescence. The fluorescence profile of the compound **13** and reference compound (**14**, **15**) showed that the fluorescence intensity of **13** in the free reporter form is 30% of **14**, but is nearly nine fold of **15**. This implied that the B-N interaction in free receptor form prevented the tertiary amine from quenching the fluorescence to some extent. In light of the new mechanism described in the previous section, it is not clear as to what the exact mechanism is for the fluorescent intensity changes.



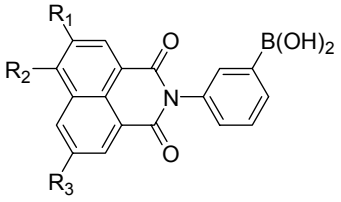
13 14 15

Figure 1.2. Porphyrin boronic acid reporter from Shinkai group.

1.2.3. Work of the Heagy group

Aimed at improving water solubility of the fluorescent saccharide reporter moiety, the Heagy group synthesized and evaluated the *N*-phenylnaphthalimide sensors **16a-c,** which were soluble in aqueous solution at least up to micromolar concentrations. [100-102] The fluorescence intensity of the **16a** and **16b** decreased with the addition of saccharides due to chelation enhanced quenching. Molecular modeling and quantum mechanics calculations suggested that the naphthalimide group (fluorophore) and phenylboronic acid (reporter) are not in same plane. After sensor (**16a-b)** binding to a carbohydrate, boron hybridization changed to the sp^3 tetrahedral form and the orbital energy of the HOMO for the phenyl boronic π-system was altered via occupation of the next highest molecular orbital. Compound **16a** showed the greatest fluorescence intensity changes in the presence of fructose with a dissociation constant of 1 mM. With the attachment of nitro group to the naphathalic anhydride ring, **16b** displayed dual fluorescence emission bands (430/550nm) and a remarkable pH-dependent sensitivity to

glucose over fructose. After reducing the nitro to an amino group, **16c** exhibited the ability to enhance fluorescence intensity upon addition of galactose at low pH.



16

16a R_1=H; R_2=H; R_3=H
16b R_1=H; R_2=H; R_3=NO_2
16c R_1=H; R_2=H; R_3=NH_2

Figure 1.3. Fluorescent reporters from Heagy group.

Table 1.3. Fluorescence properties of sensor **16**.

Sensor	λ_{ex} (nm)	λ_{em} (nm)	ϕ
16a	345	400	0.01
16b	337	430/550	0.006
16c	347	581	0.017

1.2.4. Work from the Lackowicz group

The fluorescent boronic acid carbohydrate sensors based on ICT (internal charge transfer) have been studied in the last ten years since Shinkai's group reported stillbene boronic acid **11** in 1995. [97] Generally, an ICT system contains an electron donor group and an electron acceptor group in the same fluorophore. The boronic acid acts as electron acceptor in the neutral form. When the boronic acid group turns into its anionic form (tetrahedral form) at certain pH upon binding with a sugar, it is no longer an electron acceptor. This leads to the spectral changes due to the perturbation of the charge transfer nature of the excited state and trigger the change of the fluorescence spectrum. [103] Four stillbene boronic acid analogs **17a-c** and **11** were synthesized and evaluated by the Lackowicz group. Compounds **17b** and **11** has an electron-donating group and **17c** has an electron-withdrawing cyano group. Changing the pH from low to high induced a

blue shift in the emission spectrum of **17b** and **11**, and an increased intensity by about one fold in the presence of sugar. This was thought to be the result of converting the electron-withdrawing properties of neutral boron at low pH to the anionic form upon sugar addition. Similarly, the polyene derivatives **18**, [104] diphenyloxazole derivatives **19**[105,106] and chalcone derivatives **20**[107] were prepared and tested for binding with sugars. The fluorescence intensity changed only by a maximum of five fold in these ICT systems. Furthermore, these compounds are not very water-soluble, which limit their applications in certain situations.

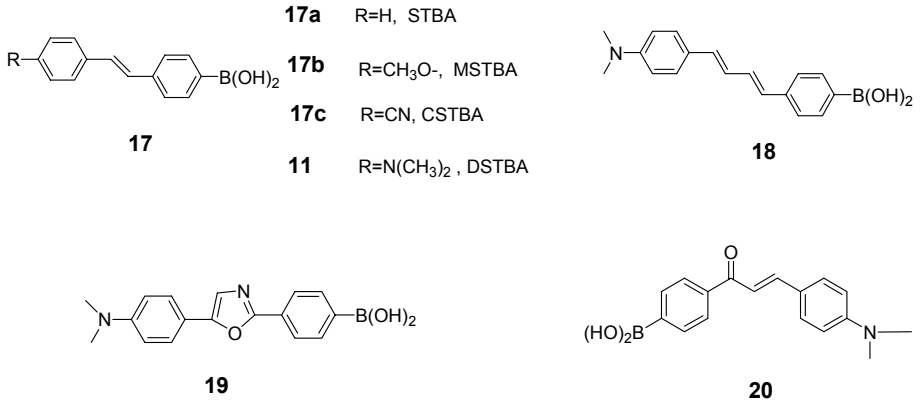
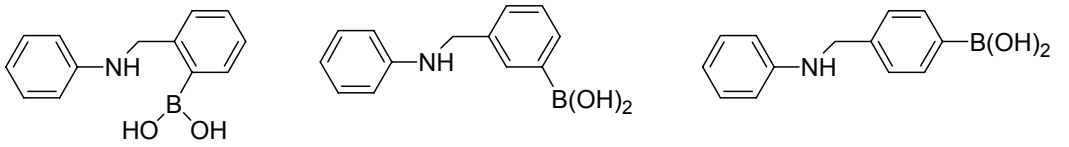


Figure 1.4. ICT reporter from Lackowicz group.

1.2.5. Work of the James group

In 2001, James and co-workers prepared monoboronic acid **21a** as a fluorescent reporter. [108] It was found that the emission λ_{max} shifted 40 nm from 404 to 365 nm with the addition of a saccharide. As expected based on the intrinsic binding affinity of monoboronic acids, D-fructose has higher binding affinities with **21a** than D-galactose and D-glucose. More recently, the James group published new results of **21a** compared with reference compound **21b** and **21c**. [28] The authors proposed that **21a** contains an intramolecular B-N bond that displays fluorescence based on both LE (locally excited)

and TICT (twisted internal charge transfer) states. The other two systems (**21b** and **21c**) were said to have no B-N bond and only showed fluorescence based on the LE state and did not show λ_{max} shift upon binding with sugar. Again, the mechanism of such systems may need to be re-examined in light of the new mechanism proposed for the original anthracene system (Section 1.2.2.1).



21a. **21b** **21c**

Figure 1.5. Fluorescent reporters from the James group.

1.2.6. Work of the Wang group

Our group has been working on the development of new water-soluble boronic acid reporter compounds with much success. We have developed seven such compounds that have superior properties compared with the literature compounds. One such example will be the subject of Chapter 3 in this dissertation.

1.3. Conclusions

Boronic acids occupy a special place in the design of fluorescent sensors for carbohydrates and boronolectins. There has been much progress in this area, and yet there are still many un-answered questions. This dissertation will address the questions of (1) the relationship among pH, the pKa of the boronic acid, and the binding constants, (2) the development of water-soluble fluorescent boronic acid reporter compounds, and

(3) the development of methods for the synthesis of di- or multi-boronic acids using the new fluorescent reporter compound developed.

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Chapter II. The Relationship among pKa, pH, and Binding Constants in the Interactions between Boronic Acids and Diols

2.1. Introduction

The formation of a boronic ester from a boronic acid and a diol is likely the strongest single-pair reversible functional group interaction in an aqueous environment among organic compounds that can be readily used for the construction of molecular receptors. The binding of phenylboronic acid with catechol, for example, has an equilibrium constant of about 800 M^{-1}.[1,2] Consequently, boronic acids have been used as the recognition moiety in the construction of sensors for saccharides,[3-13] as nucleotide and carbohydrate transporters,[14-21] and as affinity ligands for the separation of carbohydrates and glycoproteins. [22-29] Appropriately designed boronic acid compounds also have shown potential as antibody mimics targeted on cell-surface carbohydrates.[30-32] Critical to furthering these efforts is an understanding of the effect of various factors on the relative stability of boronate esters.[33] However, people put a lot of efforts on the development of boronic acid sensor molecules but only a little were dedicated to the elucidation of the boronic acid-diol complex structures and how various factors affect the binding affinity. The reason is that the chemistry involved is so complicated and there is not a universal and accurate method to determine the binding constant. Now we are able to accurately determine boronic acid/diol binding

affinities through a recently developed fluorescent method that is not limited by the requirement for a fluorescent boronic acid.[1,2] This was accomplished with the addition of a fluorescent reporter compound, Alizarin Red S. This three-component, competitive method allowed us to determine the binding constants for a series of diols with phenylboronic acid and corrected several literature mistakes regarding the strength of boronic acid-diol interactions.

In our efforts to design boronic acid-based fluorescent sensors that recognize cell-surface carbohydrates as biomarkers, we are interested in examining various factors that affect the binding affinity between a boronic acid and a diol. Such information will undoubtedly assist our efforts to optimize the binding affinity and specificity of potential fluorescent sensors.

It has been commonly believed that the higher the pH, the higher the binding constant between a boronic acid and a diol. It is also assumed that boronic acids with lower pKa's have higher affinities. Although some of our earlier work has indicated that this is probably not true,[1] there has never been a systematic examination of the relationship among the binding constants, boronic acid and diol pKa's, and the pH of the solution. Herein we report our findings with a series of 25 arylboronic acids with various substituents and selected binding affinities with a series of diols at varying pH's. We have found that (1) the pKa of monosubstituted boronic acids can be predicted based on the substituent effect using a Hammet plot; (2) the common belief that boronic acids with lower pKa's have greater binding affinities at neutral pH is not always true;

and (3) the optimal pH for binding is not always above the pKa of the boronic acid, instead it is related to both the pKa's of the boronic acid and the diol, although in an imprecise manner that cannot be directly predicted.

It would be very helpful to our sensor design if we can find some qualitative or even quantitative relationships between the affinity and some apparent parameters such as pKa of the boronic acid. It has been known that there is some correlation between the pKa of boronic acid and biding affinity. A series of 25 arylboronic acids with various substituents were chosen. We tried to reduce the structural factors and only focus on the substituent effect. The pKa's and the biding constants of 6 boronic acids with several diols were determined.

2.2. Results and Discussions

2.2.1. Effect of Substituents on the Apparent pKa of Boronic Acids

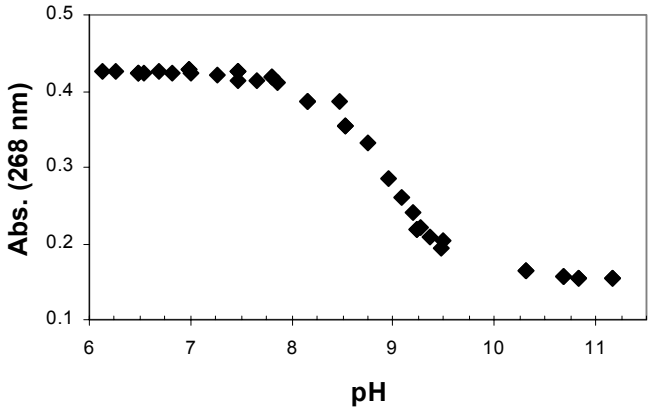


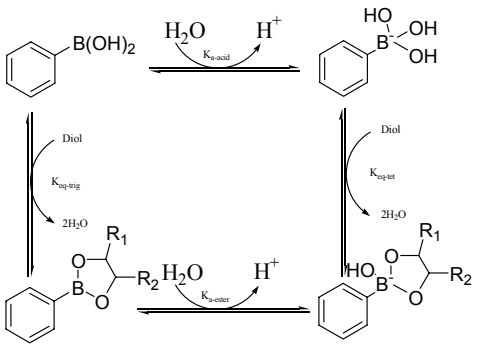
Figure 2.1. The pK_a of phenylboronic acid (PBA) can be determined by the absorbance change at 268 nm that occurs upon conversion from the trigonal form (low pH) to the tetrahedral form (high pH). ◆ - PBA (1.0×10^{-3} M) in 0.10 M phosphate buffer.

To determine the effect of different boronic acid acidities upon binding, we chose to examine a series of arylboronic acids with different substituents and two *N*-alkylated pyridinium boronic acids. At the extremes of this series were the electron rich 2-methoxyphenylboronic acid with the highest apparent pKa at 9.0, and the cationic *N*-benzylpyridinium boronic acid with the lowest apparent pKa at 4.2 (Table 2.1). With the pKa's of these boronic acids spanning about 5 pKa units, its effect on the binding constants can be examined over a wide range. As expected, electron-withdrawing groups decreased the pKa and electron-donating groups increased their pKa. However, it does seem that the effect of electron donating groups is marginal compared with the electron withdrawing groups. For example, the pKa of 2-methoxyphenyl boronic acid is only 0.2 pKa units higher than that of phenylboronic acid (8.8), while the pKa of 3-nitrophenyl boronic acid is 1.7 pKa units lower than that of phenylboronic acid (Table 2.1). In an effort to gain a quantitative appreciation of the effect of different substituents, the Hammet values for different substituents[34] were plotted against the experimentally determined apparent pKa's. A linear correlation was observed, and the measured slope of 2.1 is consistent with the formation of an anionic species as the final product (Figure 2.2). Although the pKa's of many boronic acids have been reported in the literature,[12,35] there has never been a systematic study examining such a large number of analogues. The availability of these pKa data will serve as a baseline reference for the future design of boronic acid sensors with optimal affinity and specificity at physiological pH.

Table 2.1. The pKa's of a series of substituted phenylboronic acid compounds.

Arylboronic acid	**pK_a**
2-methoxyphenylboronic acid	9.0
3-aminophenylboronic acid	8.9
phenylboronic acid	8.8
4-fluorophenylboronic acid	8.6
2,4-dichlorophenylboronic acid	8.5
4-bromophenylboronic acid	8.8
4-aminomethylphenylboronic acid	8.3
3-pyridinylboronic acid	8.1
4-pyridinylboronic acid	8.0
4-carboxyphenylboronic acid	8.0
3-acetylphenylboronic acid	8.0
3-chloro-4-fluorophenylboronic acid	7.8
3-formylphenylboronic acid	7.8
4-acetylphenylboronic acid	7.7
4-formylphenylboronic acid	7.6
2,4-difluorophenylboronic acid	7.6
3-nitrophenylboronic acid	7.1
2,5-difluorophenylboronic acid	7.0
3,4,5-triflurophenylboronic acid	6.8
2,3,4-trifluorophenylboronic acid	6.8
2,4,5-trifluorophenylboronic acid	6.7
2-dimethylaminomethylphenylboronic acid	6.7
2-fluoro-5-nitrophenylboronic acid	6.0
N-methyl-3-pyridiniumboronic acid	4.4
N-benzyl-3-pyridiniumboronic acid	4.2

2.2.2. Binding Affinities between Arylboronic Acids and Sugars



Scheme 2.1. The relationships between phenylboronic acid and its diol ester.$K_{eq\text{-}tert}$ and $K_{eq\text{-}trig}$ are the equilibrium constants of tetrahedral and trigonal forms of the boronic acid

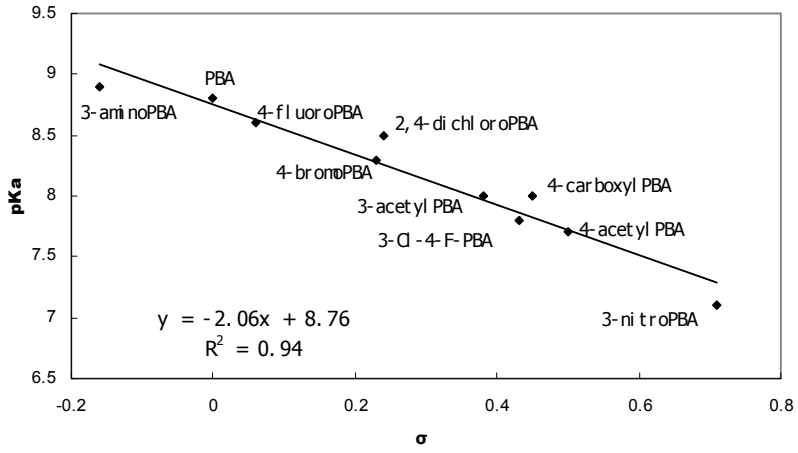


Figure 2.2. pKa vs σ for substituted arylboronic acid compounds.

As mentioned earlier, it has been generally believed that[36-39] (1) higher pH favors the binding between boronic acid and a diol, (2) the pH needs to be above the pKa of the boronic acid to see meaningful binding, and (3) more acidic boronic acids bind more tightly with diols. However, in our earlier studies the pH binding profile of phenylboronic acid with Alizarin Red S. showed an optimum at pH ~7 (the binding constant is 1500 M^{-1} at pH 7, 1100 M^{-1} at pH 7.5, and 450 M^{-1} at pH 8.5).[1] It is clear

that at a pH above the pKa of phenylboronic acid (8.8, Table 1) the binding constant is expected to be much lower than the optimal observed.[1] To further explore the theoretical basis for the pH dependence of the binding constants, we were interested in the further understanding of the relationship between proton concentration and the different equilibria involved. There are three acids in this mixture of boronic acid and diol. The first one is, of course, boronic acid itself (Scheme 1). The second one is the ester, which is often much more acidic than the acid, and the third is the diol. Although simple alcohols have pKa's in the range of 16, vicinal diols are often much more acidic. For example, the pKa of glucose and fructose is about 12. [40] For catechol, the pKa is about 9.3. [40]The lower pKa of vicinal diols is presumably due to a combination of the hydrogen-bonding stabilization of the oxyanion in the deprotonated form by the neighboring hydroxyl group and the inductive electron-withdrawing effect of a neighboring oxygen. Based on these acid-based equilibria, two independent laboratories[36,39] have proposed that the optimal pH should be between the pKa values of the boronic acid and diol (equation 2.1).

(equation 2.1) $$pH_{optimal} = (pKa_{-acid} + pKa_{-diol})/2$$

The prediction from eq 2.1 for the optimal binding of Alizarin Red S. with phenylboronic acid (6.4, the pKa of Alizarin Red S. is 4 and the pKa of PBA is 8.8) is consistent with the experimental data which reaches a maximum at between pH 6.6-7. [1]To further test this idea, the optimal pH for the binding of several boronic acids and

Alizarin Red S. has been studied. The results shown in Table 2.2 demonstrate two points. First, the optimal pH does not necessarily need to be above the boronic acid pKa. As a matter of fact, the optimal pKa in the binding of all boronic acids tested with Alizarin Red S. was below the pKa of the boronic acids due to the low pKa of Alizarin Red S. Second, equation 1 does not accurately predict the numerical values of the optimal pH. This is understandable since the equation does not take into consideration of the effect of the solvent, buffer, and other intermolecular interactions such as sterics, hydrogen bonding, etc. Therefore, equation 1 can only be used as the basis to make an approximate estimation of the optimal pH. However, it is not suitable to be used for the quantitative prediction of the optimal pH.

Table 2.2. Optimal pH for binding between some henylboronic acids and Alizarin Red S

Boronic acid	pKa	Optimal pH	Predicted Optimal pH
3,4,5-trifluorophenylboronic acid	6.8	6	5.4
2-fluoro-5-nitrophenylboronic acid	6	6	5.0
2-methoxyphenylboronic acid	9	7.5	6.5
N-benzyl-3-pyridiniumphenylboronic acid	4.2	4	4.1
o-dimethylaminomethylphenylboronic acid	6.7	6	5.4
3-chloro-4-fluorophenylboronic acid	7.8	6.5	5.9
4-bromophenylboronic acid	8.3	6	6.2

In an effort to examine the relationship among pKa, pH, and binding constants in a broader sense, the binding constants were determined for 6 substituted phenylboronic acids with glucose, fructose, and catechol at different pH, and the

results are summarized in Table 2.3. Figure 2.3 also shows a typical set of plots for the determination of the binding constants. These results show even more variation from the commonly believed situations stated above and that predicted by equation 2.1. First, the ranking of the binding constants among different boronic acids with a given diol at physiological pH does not always follow the trend of the boronic acid pKa. For example, although 2,5-difluorophenylboronic acid has a higher apparent pKa (7.6) than 3,4,5-trifluorophenylboronic acid (6.8), it also has a higher binding constant with glucose at pH 7.5, which goes against the conventional thinking. On the other hand, the ranking of the binding constants for these two boronic acids with fructose is reversed compared with binding with glucose. It is also interesting to note that the binding constants of 2,5-difluorophenylboronic acid and 3,4,5-trifluorophenylboronic acid with fructose at pH 7.5 are higher than that of 2-fluoro-5-nitrophenylboronic acid even though the latter has the lowest pKa among all six boronic acids tested.

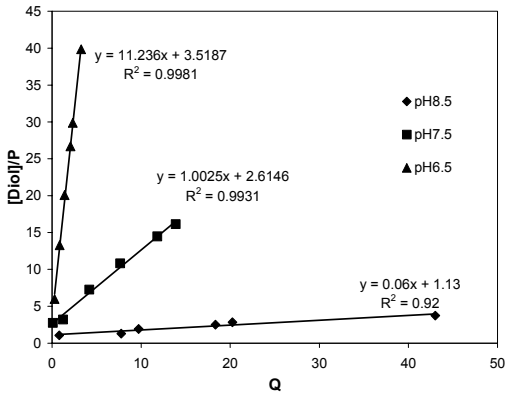


Figure 2.3. Binding constant determination for 3,4,5-trifluoroPBA with fructose at different pH's. $P=[L_0]-1/QK_{eq1}-[I_0]/(Q+1)$, $Q=[I]/[IL]$; [diol]=total diol concentration, I=indicator(ARS), $[I_0]$=total indicator concentration, L=ligand(PBA), K_{eq1}=association constant of ARS-PBA complex. The Keq of boronic acid-diol complex can be calculated by dividing K_{eq1} by the slope of the plot.

Second, the ranking of the binding constants among different boronic acids with a given diol depends on the pH of the solution. For example, the binding constant between 2,5-difluorophenylboronic acid and fructose is higher than that of 3-chloro-4-fluorophenylboronic acid at pH 7.5, but lower at pH 8.5. The binding of these two boronic acids with catechol also exhibits the same kind of pH-dependent reversal of ranking orders. Such results are partially attributable to the different optimal pH for these two boronic acids.

Table 2.3. Binding Constants [a] of Boronic Acids and Sugars.

Boronic acid (pKa)	PBA (8.8)[b]	4-Br-PBA (8.3)	3-Cl-4-F-PBA (7.8)	2,5-difluoro PBA (7.6)	3,4,5-tri-fluoroPBA (6.8)	2-F-5-nitroPBA (6.0)
Glucose (12.3)[b]						
pH6.5	0.84	5.6	7.6	33	17	25
pH7.5	4.6	20	26	47	41	47
pH8.5	11	2.4[c]	48	7.3[c]	52	56
Predicted Optimal pH	10.6	10.3	10.1	10.0	9.6	9.2
Fructose (12)						
pH6.5	29	123	562	237	545	1398
pH7.5	210	495	1003	2136	2523	2062
pH8.5	560	1194	1853	120	4443	378[c]
Predicted Optimal pH	10.4	10.2	9.9	9.8	9.4	9.0
Catechol (9.2)						
pH6.5	150	715	1646	8841	2137	3027
pH7.5	830	1557	6100	4703	2896	4359
pH8.5	3300	1966	7487	418[c]	5376	3792
Predicted Optimal pH	9.0	8.8	8.5	8.4	8.0	7.6

[a] Binding constants (M^{-1}) were determined in 0.10 M phosphate buffer (see experimental)

[b] The number in parenthesis is the pKa of the boronic acid.

[c] The experimental errors are in range of 20% to 40%. Others are below 10%.

Therefore, when one discusses the relative binding constants of various boronic acids with a particular diol, one has to consider the specific conditions, particularly the pH of the solution under which the binding constants were determined.

Among the six boronic acids tested for their binding to various diols, 2,5-difluorophenylboronic acid and 2-fluoro-5-nitrophenylboronic acid showed especially large "deviation" from that predicted by equation 1. For example, the predicted optimal pH for the binding between 2,5-difluorophenylboronic acid and glucose is 9.8. However, the highest binding constant was observed at pH 7.5. A similar situation with the binding to fructose was observed. In the latter case, the predicted optimal pH is 9.8 and the highest binding constant was observed at pH 7.5. Such results further indicate that the pKa's of the boronic acid and the sugars are not the only major determinants of the binding constants and optimal pH. Buffer[1] and steric factors[33] are all known to affect the binding constants. It is conceivable that conformational changes, interactions such as binding by a third hydroxyl, [4,41] and sterics are all idiosyncratic factors that affect binding.

2.3. Conclusions

Understanding the factors that affect the binding affinity between boronic acid and diol moieties is important to the future design of boronic acid based sensors. The results reported indicate: (1) as expected, electron-withdrawing groups can

significantly lower the pKa of boronic acids and a linear correlation can be used to predict the pKa of monosubstituted phenylboronic acids using the Hammet values of the substituents; (2) the general belief that boronic acids with lower pKa's show greater binding affinities is not always true and many other factors affect the binding constant; and (3) the optimal binding pH is not necessarily above the pKa of the boronic acid.

2.4 Experimental

2.4.1. General Methods

Alizarin Red S. and arylboronic acids were purchased from Acros, Asymchem, Frontier Scientific and Aldrich, and were used as received. Sugars, buffers, and diols were bought from Aldrich and Acros, and were used as received. Water used for the binding studies was doubly distilled and further purified with a Milli-Q filtration system.

A Shimadzu RF-5301PC fluorometer was used for all fluorescent studies. A Shimadzu UV-1601 UV-visible spectrophotometer was used for the pKa determination. Quartz cuvettes were used in all studies.

2.4.2. Apparent pKa

The apparent pKa's of 8-QBA and esters were determined by observing the UV absorption changes that occur upon the hybridization change from the acidic trigonal form to the basic tetrahedral form (Figure 2.1).[42]

2.4.3. Binding Constant Determination

A typical procedure for the binding constant determination was illustrated as below. To a solution of 9.0×10^{-6} M ARS, enough boronic acid was added to keep about 20% of the ARS in free form as measured by fluorescence,[1] and the solution was brought to the correct pH in 0.10 M phosphate buffer (Solution A). A sugar was then added to a portion of solution A so that about 80% of the ARS was in free form (Solution B). Solution B was then titrated into solution A to make mixtures with a constant concentration of ARS and PBA and a range of concentrations of diol. In general, eight different concentrations were made in order to cover as much of the binding curve as possible. Each mixture was allowed to stand for at least 5 min. Then 3.5 mL of the mixture was transferred into a cuvette for fluorescence measurement.

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Chapter III. 8-Quinolineboronic Acid-a Novel Type of Fluorescent Carbohydrate Reporter.

3.1. Introduction

As discussed in the introductory chapter, critical to the construction of fluorescent sensors for carbohydrates is the availability of practical fluorescent reporters that respond to the binding event with significant fluorescence intensity changes under physiological conditions. During the last decade, there has been a great deal of progress made in the construction of boronic acid-based sensors for carbohydrates and other diol-containing compounds.[1-3] Among the most important discoveries is an anthracene-based fluorescent reporter system developed [4,5] by Shinkai and co-workers, which has been widely used because of its large change in fluorescence upon ester formation due to the switching of a photoelectron transfer process.[6,7] However, the anthracene-based fluorescent reporter has many undesirable properties such as low water solubility and poor photochemical stability. Furthermore, the fluorescence intensity of the anthracene fluorophore can be affected by minor changes in the environment such as temperature and oxygen concentration. All these affect the reproducibility and application both *in vitro* and *in vivo*. Therefore, there has been much interest in the search for new reporter compounds that change spectroscopic properties upon boronic acid binding with a carbohydrate. The ideal monoboronic acid reporter compound should (1) respond to the binding of a carbohydrate with fluorescence changes; (2) is soluble in aqueous solution; (3) shows

optimal fluorescence intensity changes upon binding at physiological pH; and (4) has stable fluorescence readings that are not affected by minor environmental changes. In this chapter, we describe a novel fluorescent reporter compound, 8-quinoline boronic acid that meets all of the requirements mentioned above and shows about 40 fold fluorescence increasing upon binding of a carbohydrate compound. All these properties make such a system ideal for the construction of fluorescent biosensors for carbohydrates. This chapter describes the combined contributions of Dr. Wenqian Yang and Jun Yan.

3.2. Design

The fluorescence properties of nitrogen heteroaromatic compounds are generally different from the parent hydrocarbons because of the presence of low-lying (n, π^*) excited states.[8] Since the energies of the (n, π^*) and (π, π^*) excited singlets in nitrogen heteroaromatics are a function of polarity and hydrogen-bonding ability of the solvent, the fluorescence of those compounds is quite dependent on the molecular environment (Table 3.1). The fluorescence spectroscopy of quinoline and its relatives have been studied before, and it was found that the luminescence properties of quinolines[9] can be significantly modified by introduction of substituents capable of hydrogen bonding with either the nitrogen or the solvent. Goldman and Wehry studied the environmental effects upon the photoluminescence of quinoline, 8-hydroxyquinoline, and 8-quinolineboronic acid (8-QBA). They found that different substituents on the quinoline ring change the photoluminescence property

tremendously and solvents affect their fluorescence behavior. Those results interest us in its potential on diol sensor reporter.

Table 3.1. The lowest excited singlet of quinoline derivatives in different solvents.

Compounds	Hydrocarbon Solvents	Alcoholic Solvent
Quinoline	(n, π^*)	(π, π^*)
8-QBA	(n, π^*)	(n, π^*)
8-Hydroxyquinoline	(π, π^*)	(π, π^*)

Molecular orbital calculations[10] indicate that, in the ground sate of 8-QBA, appreciable transfer of π electron density occurs from the oxygen atoms to the electron-deficient boron. Molecular orbital calculations also indicate that, upon photoexcitation, appreciable transfer of electron charges from the aromatic ring to the $-B(OH)_2$ group should occur. Both of these processes lead to fluorescence quenching. One might therefore anticipate that upon binding to a carbohydrate in aqueous solution both electron transfer processes will be abolished since the boron would be transformed from the electron-deficient trigonal form to the electron rich tetrahedral form. This might cause the photoluminescence properties **to** change in such a molecule.

On the other hand, Goldman and Wehry's experimental results also indicate interactions between the boronic acid hydroxyl group and the alcoholic and hydrogen bond acceptor solvent. By freezing the solution to get ride of the solvent relaxation, an

intense fluorescence appeared. Therefore, one can postulate that upon binding to a sugar compound this kind of solvent relaxation would be reduced and the fluorescence intensity should increase tremendously. Furthermore, 8-QBA possesses two ionizable functional groups and may exist as zwitterions in neutral aqueous media, which will increase its water solubility. For all these reasons, we were interested in examining the effect of carbohydrates on the fluorescent properties of 8-QBA.

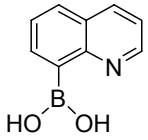

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Figure 3.1. 8-quinolineboronic acid.

3.3. Results and Discussions

3.3.1. Fluorescence Studies upon Binding to Sugars.

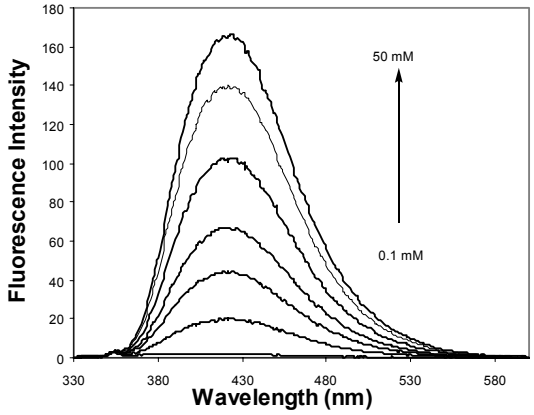


Figure 3.2. Fluorescence response of 8-QBA (6.3×10^{-5} M) in 0.10 M phosphate buffer at pH 7.4 in the presence of D-fructose (0.1, 1.0, 2.5, 5.0, 10, 25, 50 mM): λ_{ex} = 314 nm.

Figure 3.2 shows the fluorescence spectra of 8-QBA and the corresponding fructose esters. 8-QBA itself is essentially non-fluorescent at pH above 5 and weakly fluorescent at lower pH in aqueous solution. However, as we expected, the fluorescence intensity increased dramatically in a concentration-dependent manner upon addition of D-fructose. In an effort to examine the generality of this phenomenon, a few other sugars were tested. Figure 3.3 shows the concentration profiles of D-fructose, D-tagatose, D-galactose, and L-arabinose with binding constants of 108 M^{-1}, 62 M^{-1}, 7.5 M^{-1}, and 1.1 M^{-1}, respectively. It is interesting to point out that (1) D-glucose only showed a maximum of 3-fold increase in fluorescence intensity and (2) the fluorescence intensity increases of 8-QBA in the presence of various sugars at the lower concentration range (< 25 mM) seem to correlate with the intrinsic binding affinity of different sugars for 8-QBA (Figure 3.3).

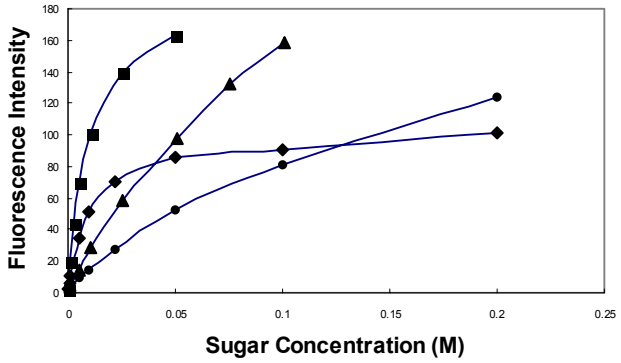


Figure 3.3. Fluorescence intensity of 8-QBA (6.3×10^{-5} M) in 0.10 M phosphate buffer at pH 7.4 in the presence of D-fructose (■), D-galactose (▲), D-tagatose (♦), and L-arabinose (●): λ_{ex} = 314 nm, λ_{em} = 417 nm.

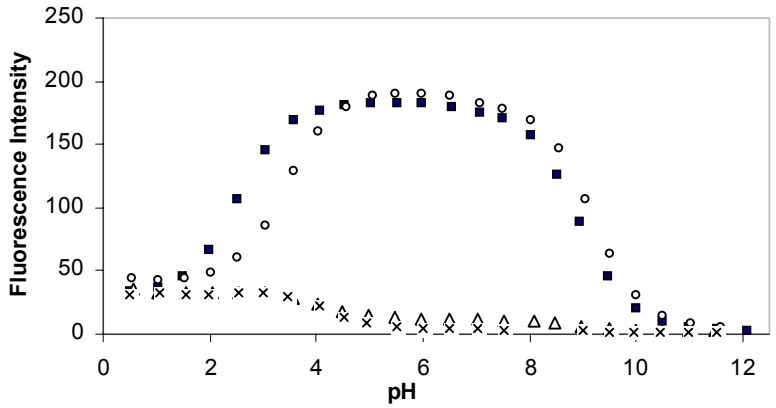


Figure 3.4. Fluorescence intensity pH profile of 8-QBA (6.3×10^{-5} M) in 0.10 M phosphate buffer: [saccharide] = 0.5 M, λ_{ex} = 314 nm, λ_{em} = 417 nm. × blank, ■ D-fructose, ▲ D-glucose, ○ D-galactose.

Since the fluorescence intensity increase upon binding with a sugar seems to be a general phenomenon, next we were interested in examining the pH profile of the fluorescence intensity changes. For this, we chose a fixed sugar concentration of 0.5 M. The fluorescence intensity increase with the addition of a sugar is observable between pH 2-10 with the maximum between pH 4.5-7.5 (Figure 3.4). This is true with D-fructuse and D-galactose while D-glucose only shows minor fluorescence change, which may be due to the weak binding affinity. The fluorescence intensity change at pH 7 and 7.5 (phosphate buffer) were about 42 and 47-fold, respectively, in the presence of 0.5 M fructose. Just as important is the feature that this sensor is functional at physiological pH without the need to add an organic co-solvent, which is required by the anthracene-based boronic acid sensors.

3.3.2. pKa Assignments

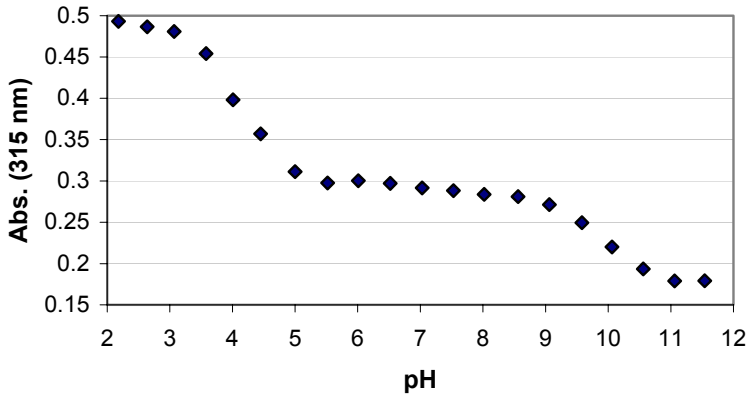


Figure 3.5. Absorbance intensity pH profile of 8-QBA (6.3×10^{-5} M) in 0.10 M phosphate buffer.

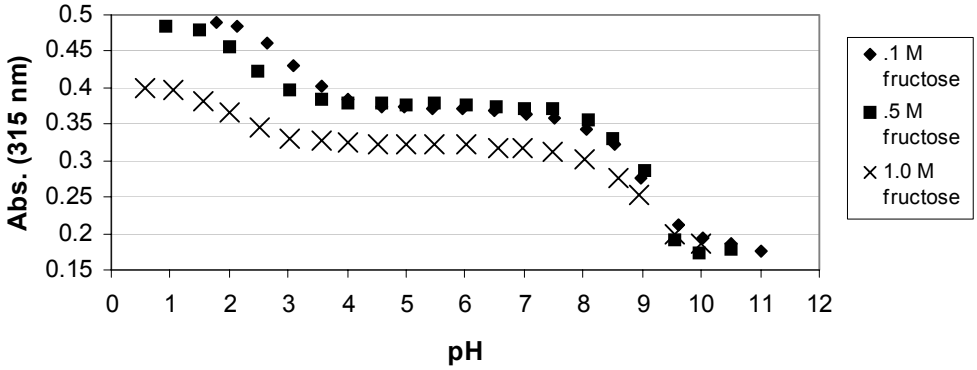


Figure 3.6. pKa of fructose ester

Aimed at examining how 8-QBA functions as a fluorescent probe for diols, we studied the UV pH profiles of both 8-QBA alone and 8-QBA in the presence of D-fructose (1.0 M). 8-QBA was found to have two pKa's (Figure 3.5), one at about 4 and the other at about 10. In order to be able to assign each pKa, we compared those numbers to literature reported pKa of some pyridine and quinoline derivatives (Table 3.2). It's reasonable to assign the first pKa at 4 to the quinolinium nitrogen and the second pKa at 10 to boronic acid, since the pKa of phenylboronic aicd is about 8.8.

As we know, the boron atom of boronic acid and boronate has different hybridization state, sp^2 for boronic acid and sp^3 for boronate. The direct evidence to assign the pKa is ^{11}B NMR. Therefore, we recorded the ^{11}B NMR spectra of 8-QBA in a mixed deuterated methanol-water (1:1) solvent at different pH. Methanol was used so that the concentration of 8-QBA can be increased to 44 mM so as to allow for a meaningful NMR determination within a reasonable period of time. The pH titration studies of 8-QBA in a mixed methanol-water (1:1) solution showed that both the fluorescence

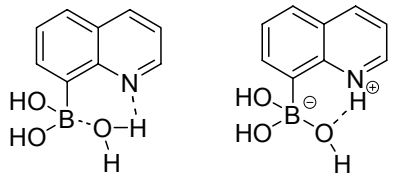
Table 3.2. pKa of pyridine and quinoline derivatives.[11]

Compounds	pKa	Compounds	pKa
Pyridine	5.23	Quinoline	4.9
2-Chloropyridine	0.49	8-Nitroquinoline	2.55
3-Chloropyridine	2.84		
2-Bromopyridine	0.71		
3-Bromopyridine	2.85		
3-Nitropyridine	1.18		

and UV profiles were nearly identical as that in 100% water solution (data not shown), and it is known that the addition of 50% methanol to water solution results in minimal changes of the solution pH.[12] Therefore, we measured the ^{11}B NMR in the mixed aqueous solution to investigate the boron hybridization state. The boron signal of 8-QBA appeared at 24.3 ppm at pH 1.5 and shifted to 8.3 ppm at pH 6.5 and 6.5 ppm at pH 11.5, respectively. These results indicated that the boron changed hybridization from sp^2 to sp^3 between pH 1.5 to 6.5.[13] This means that 8-QBA exists at pH 7.4 predominantly as the zwitterionic quinolinium boronate form (**22b**) (Scheme 3.1.[14,15] This allowed us to assign the first pKa at 4 of 8-QBA to the boronic acid group and

the second pKa at 10 to the quinolinium nitrogen. Since the formation of a boronic ester with a diol further lowers the pKa of the boron,[16] the same assignments for 8-QBA esters should also be correct.

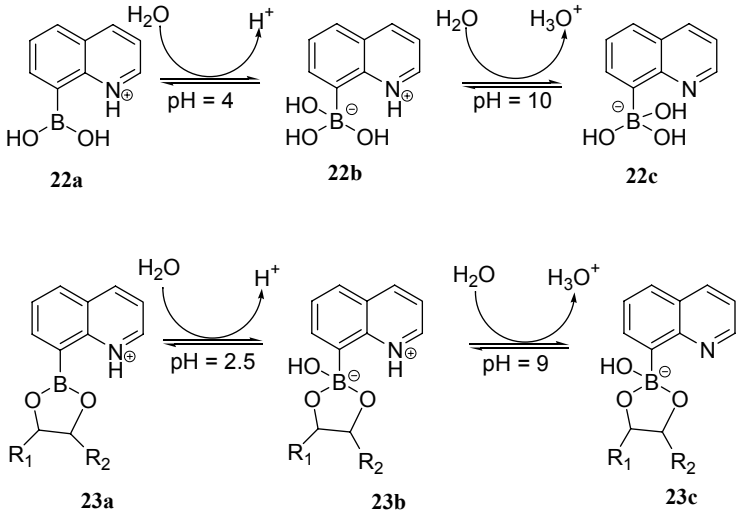
Since $-B(OH)_2$ is an electron-withdrawing group, one should expect a pKa of the quinolinium nitrogen in 8-QBA to be lower than 4.9, which is the pKa of quinoline nitrogen. (Table 3.2). However, it's very interesting to note that a pKa around 10 was observed for 8-QBA. There are two reasons that contribute to the pKa change. The transformation of boron atom from trigonal form to tetrahedral form makes the boron species no longer an electron-withdrawing group. The negatively charged boron helped to increase the pKa of the quinolinium nitrogen by ionically stabilizing the protonated form. On the other hand, the hydrogen bond between boronic acid and protonated nitrogen also helped to increase the pKa of the quinolinium nitrogen. Morrison and Letsinger[17] studied the IR spectrum of 8-QBA in the presence of water and postulated that, in aqueous media, simultaneous interaction of water with boron atom and the nitrogen occurs. The hydrogen bond stabilized the protonated nitrogen thus could also increase the pKa of the quinolinium nitrogen (Figure 3.7).



a **b**

Figure 3.7. Hydrogen bond between boron hydroxyl group and protonated nitrogen.

3.3.3. Fluorescence Mechanism



Scheme 3.1. The ionization steps of 8-QBA and its esters.

Scheme 3.1 shows the ionization steps of 8-QBA and its ester in aqueous media. This indicates that the fructose ester (**23b**) is the fluorescence species. According to the triplet-state fluorescence theory,[18] intersystem crossing can happen through spin-orbital coupling. This process can be enhanced by the presence of heavy and paramagnetic nuclei, and by the proximity of the n-electrons of hetero-atoms to the electric fields of their nuclei. The intersystem crossing may enhance the phosphorescence thus reduce the fluorescence. By tying up the lone pair electrons the fluorescence would be tremendously increased. Since the lone pair electron on the nitrogen atom is one of the fluorescence quenching sources, tying up the electrons through protonation could help minimize quenching and increase fluorescence intensity. It's easy to understand why 8-QBA and its esters are non-fluorescent above pH 10.

Furthermore, the boron atom in both 8-QBA and its esters is in the tetrahedral forms at pH 7.4 indicating that the fluorescence intensity changes are not due to the change in hybridization states of the boron. This is further substantiated by the fact that with the pH increasing from 2 to 7 the fluorescence intensity of 8-QBA showed a decrease, and that of 8-QBA esters showed an increase (Figure 3.3). As mentioned above in the design section, interactions between the boronic acid hydroxyl group and the solvent, either alcoholic or other hydrogen bond acceptor molecules, can occur. By freezing the solution at 77 K 8-QBA shows quite strong fluorescence in the alcoholic solvent through the inhibition of the solvent relaxation by which the first excited singlet state of 8-QBA is deactivated in fluid media. Goldman and Wehry[8] found that 8-QBA is neither fluorescent nor phosphorescent in liquid aqueous solution. That indicates a highly efficient $S_1^* \rightarrow S_0$ internal conversion which can be promoted by a solvent-solute interaction in the excited singlet state. One may postulate that upon binding to a sugar compound this kind of solvent relaxation would be reduced and the fluorescence intensity may increase tremendously, although more experiments need to be done to understand the detailed mechanism.

3.3.4. Evaluation of 8-QBA as a Potential Diol Sensor

Past work in searching for new boronic acid-based fluorescent reporter compounds has been focused on exploring the inductive effect of boronic ester formation on a conjugated π chromophoric system[19-21] and/or the utilization of B-N bond formation.[20,22-24] In this study, there is no B-N bond formation expected due to

the large angle strain if such a B-N bond were to form. The availability of 8-QBA-based fluorescent reporter compounds will be very useful to the effort of making fluorescent sensor for cell-surface carbohydrates for *in vivo* applications.[25] In our earlier efforts of making such cell-surface carbohydrate sensors using the anthracene-based fluorophore, it was always necessary to add some organic co-solvent (commonly methanol) for the cell-labeling studies due to their poor water solubility. The need for organic co-solvent can be tolerated in an *in vitro* experiment, but not in an *in vivo* experiment. The availability of water-soluble fluorescent reporter compounds such as 8-QBA will significantly help the effort of making biocompatible fluorescent sensors for cell-surface carbohydrates as biomarkers. Due to the zwitterions form, 8-QBA shows good water solubility. In our fluorescence study we used a 6.3×10^{-5} M solution of 8-QBA, which was much better than the previously reported reporters.

Table 3.3 lists the association constants of sugars with 8-QBA and phenylboronic acid (PBA). They have almost the same preference to various sugars while 8-QBA has somewhat lower affinity than PBA. However, the affinity is not a major problem with a monoboronic acid since the association constant can be tremendously increased with appropriate special alignment of the bis-boronic acid.[4,26-28]

Table 3.3. Association constants (M^{-1}) of 8-QBA and PBA with various sugars.

Boronic acids	D-fructose	D-tagatose	L-arabinose	D-galactose	D-glucose
PBA	160	130	23	15	4.6
8-QBA	108	62	1.1	7.5	NA

Based on the studies of the relationship between the pKa values of the boronic acids and the association constants,[29] we anticipated the optimal binding pH for 8-QBA be around neutral pH. Table 3.4 list the association constants of 8-QBA with fructose at various pH. 8-QBA shows high enough binding affinity to D-fructose at neutral pH comparing to phenylboronic acid. That's ideal for the construction of carbohydrate sensors.

Table 3.4. Association constants (K_{eq}) of the fructose ester with PBA and 8-QBA at various pH, in 0.10 M phosphate buffer.

pH	PBA	8-QBA
5.8	4.6	177[a]
6.5	29	180
7.4	160	108
8.0	310	NA
8.5	560	NA

a. Determined at pH 5.5.

The quantum yield of fructose ester at pH 7.4 in phosphate buffer was determined to be 0.28, as illustrated in the experimental section. The quantum yield of 8-QBA in 12 M sulfuric acid was used as standard for the calculations.[8]

Table 3.5. Quantum yield determinations.

Sample	Absorbance		Fluorescence	
	λ_{max}	ε	Integration of emission spectrum (334-570 nm)	Φ_F
8-Quinolineboronic acid in 12 M sulfuric acid.	314 nm	966	1.516×10^8	.58 (lit)
8-Quinolineboronic acid[a], and fructose, in pH 7.4, 0.10 M phosphate buffer	314 nm	411	3.065×10^7	.28

The chemical and photochemical stability of 8-QBA is another aspect we need to consider. It is known that the fluorescence intensity of the anthracene fluorophore can be affected by minor changes in the environment such as temperature and oxygen concentration.[30] Such fluctuations affect the reproducibility and application for the anthracene system both *in vitro* and *in vivo*. The fluorescence spectrum of the aqueous solution of 8-QBA stored on the bench at room temperature for one week was compared to that of a freshly made sample and a sample store at –40 ^oC away from light. No significant difference was observed among those spectra. Such results give a clear indication that 8-QBA is a stable fluorescent reporter under normal operating conditions. .

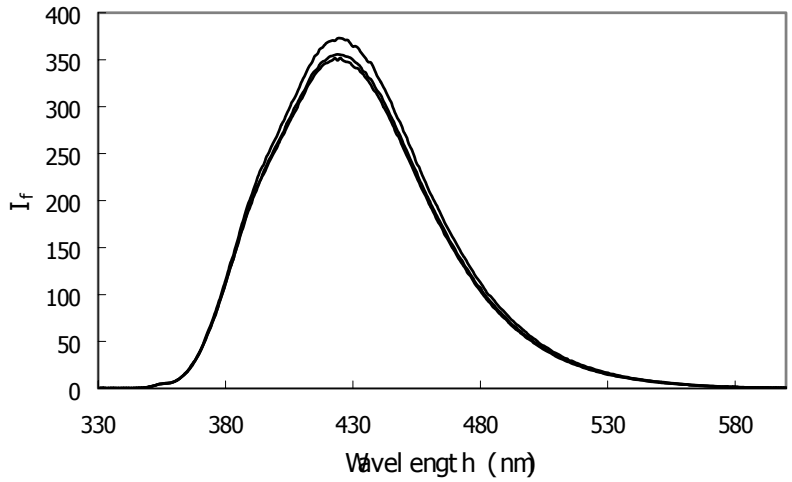


Figure 3.8. The fluorescent spectra of 8-QBA (6.0×10^{-5} M) in the presence of D-fructose (100 mM) under different storage conditions. From top to bottom: freshly made 8-QBA solution as a control; after storage under –40 ℃ for one week; after storage at room temperature for one week.

3.4. Conclusions

In conclusion, 8-QBA was found to be a fluorescent reporter compound with many desirable properties for biosensor preparation. Such properties include (1) large fluorescence intensity changes upon binding, (2) water solubility, (3) being functional in aqueous solution at physiological pH, (4) chemical and photochemically stability, and (5) stable fluorescent properties which are not affect by factors commonly encountered under normal experimental conditions.. The relatively high water solubility and high fluorescence stability make it an excellent monoboronic acid based fluorescent reporter compound. Work is underway to better understand the fluorescence change mechanism and use this new type of fluorescent boronic acid compounds for the synthesis of diboronic acid compounds for high selectivity and affinity recognition of carbohydrates of biological interest.

3.5. Experimental

3.5.1. General Methods

All chemicals were purchased from Acros, Asymchem, Frontier Scientific and Aldrich, and used as received. Water used for the binding studies was doubly distilled and further purified with a Milli-Q filtration system.

A Shimadzu RF-5301PC fluorometer was used for all fluorescent studies. A Shimadzu UV-1601 UV-visible spectrophotometer was used for the pKa determination. Quartz cuvettes were used in all studies. All pH values were measured with an Accumet portable pH meter.

3.5.2. pKa Determination

The apparent pKa's of 8-QBA and esters were determined by observing the UV absorption changes that occur upon the hybridization change from the acidic trigonal form to the basic tetrahedral form (Figure 2.1).[31]

3.5.3. Binding Constant

For a typical sugar-boronic acid binding constant measurement, a 6.3×10^{-5} M solution of 8-QBA in 0.10 M sodium phosphate monobasic buffer was brought to the appropriate pH (pH 7.4, within 0.01 units,) with 4 N NaOH (solution A). Sugar was added to a portion of solution A to make a solution B which contains 6.3×10^{-5} M 8-QBA and 0.02 M sugar. The pH was again checked and adjusted if necessary. Solution B was titrated into solution A in order to make mixtures with a constant concentration of 8-QBA and a range of concentrations of sugar. In general, eight

different concentrations were made in order to cover as much of the binding curve as solubility allowed. Each mixture was allowed to stand for at least five minutes. Then 3.5 mL of the mixture was transferred into a quartz cuvette for fluorescence measurements. The excitation wavelength was set as 314 nm and emission wavelenth was set as 417 nm. A sample experimental sheet follows.

Benesi-Hildebrand association constant calculation

IF	*If - Ifo*	sugar (M)	1/sugar	1/If	Ka	slope
101.51	98.5270	0.004264	234.50	0.01015	179.960	2.4E-05
112.63	109.6470	0.004877	205.04	0.00912		
123.86	120.8770	0.005886	169.90	0.008273		**intercept**
132.8	129.8170	0.00682	146.71	0.007703		0.00431
152.43	149.4470	0.00983	101.72	0.006691		
166.65	163.6670	0.01400	71.42	0.00611	**8qba If**	
182.2	179.2170	0.020000	50.00	0.00558	2.983	

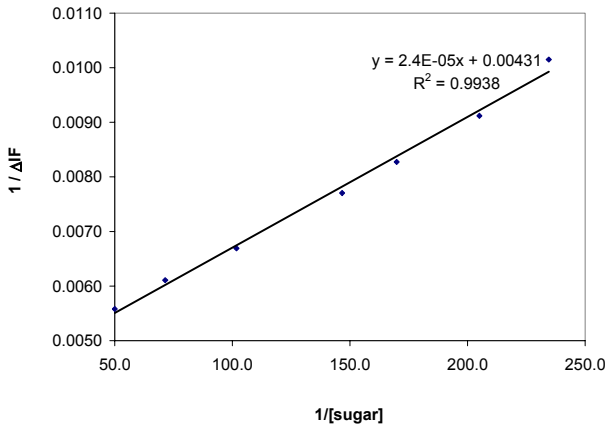


Figure 3.9. Plot for binding constant determination.
1/**If** was plot vs. **1**/**sugar**. (as shown below)
$\mathbf{K_a}$ = intercept / slope
IF: fluorescence intensity of samples (8-QBA + sugars)
$\mathbf{If_0}$ = **8 QBA If** : fluorescence intensity of 8-QBA itself

3.5.4. Quantum Yield Determination

The fluorescence quantum yield of the sample was calculated based on the following equation.

$$\Phi_{\text{f-sample}} = \Phi_{\text{f-standard}} \frac{F_{\text{int-sample}} \cdot Abs_{\text{standard}}}{F_{\text{int-standard}} \cdot Abs_{\text{sample}}}$$

$\Phi_{\text{f-sample}}$: the fluorescence quantum yield of the sample
$\Phi_{\text{f-standard}}$: the fluorescence quantum yield of the standard
$F_{\text{int-sample}}$: the integration of the emission spectra of the sample
$F_{\text{int-standard}}$: the integration of the emission spectra of the standard
Abs_{sample} : the UV absorbance of the sample
Abs_{standard} : the UV absorbance of the standard

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Chapter IV. The Design and Synthesis of 8-QBA-based Diboronic Carbohydrate Sensors

4.1 Introduction

It is well known that cell surface carbohydrates, as part of glycoproteins and glycolipids, often form characteristic signatures of different cell type. The transformations of normal to cancerous cells are also often associated with the alteration of cell surface carbohydrates.[1,2] The expression or over-expression of certain carbohydrates, such as sialyl Lewis X, sialyl Lewis a, Lewis X and Lewis Y, has been correlated with the development of certain cancers, particularly carcinomas.[3-7] Therefore, these cell surface carbohydrates can be used for the cell specific identification and targeting of certain cancers. Sensors capable of recognizing specific cell surface biomarkers are useful diagnostics. Our lab has been particularly interested in the development of fluorescent sensors for the cell surface carbohydrates mentioned above and a successful sensor has been synthesized.[8,9]

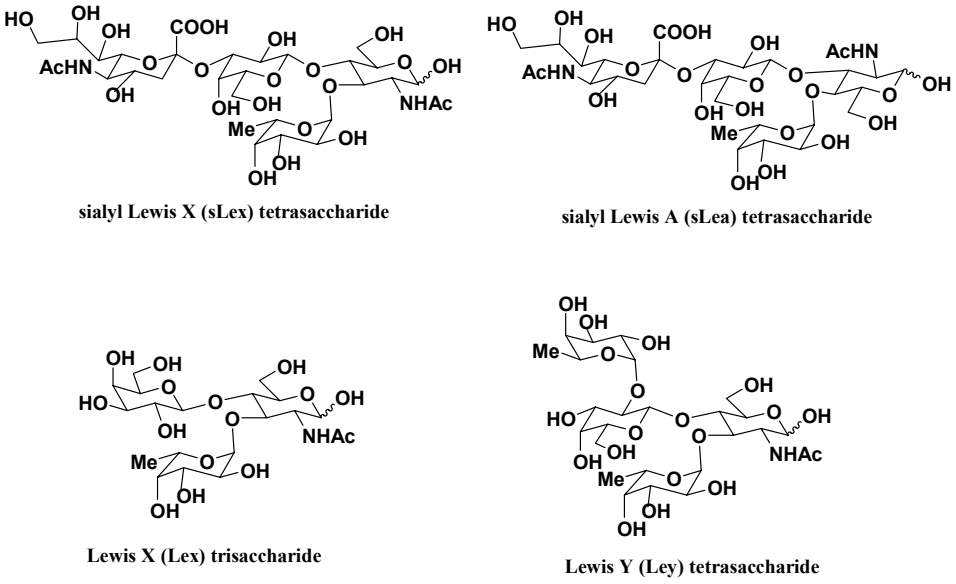


Figure 4.1. Structures of some cell surface carbohydrates.

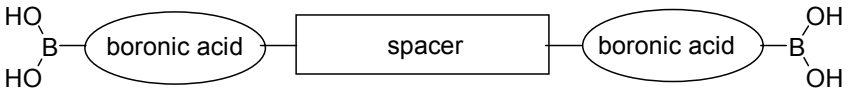
There are three critical issues to the development of high affinity and high specificity sensors. Firstly, a binding motif that could recognize the unique structural features of the target molecules is required. Second, a detectable signal should be generated upon binding. Third, an appropriate 3-dimentional scaffold is needed to yield selective binding. In this chapter, boronic acid is chosen as the binding motif and a fluorescence change is the reporting event for the binding. It is well known that monoboronic acids have certain intrinsic preference of various sugars, which are not necessarily in line with the desired selectivity of the target carbohydrates.[10] The affinity depends on the intrinsic structural features of the sugars as described in Chapter 1. In order to increase the binding affinity and selectivity to specific carbohydrates, at least one more binding site should be built into the system.[11] It could be another boronic acid or other recognition motifs. As is well known, most of the biological important oligosaccharides have more than one set of vicinal diols. Therefore in most of the cases, an additional boronic acid can be used as the

secondary binding motif. In this chapter we are interested in the development of efficient synthetic strategies that allow for the preparation of various diboronic acids using a new water-soluble fluorescent boronic acid, 8-QBA (Chaper 3), as the reporter unit. The specific chemistry used for the coupling is the Huisgen cycloaddition, which is commonly used in click chemistry,[12,13] and amidation chemistry.

4.2. Results and Discussion

4.2.1 The Design of Diboronic Acid Compounds for Carbohydrate Sensing

A common strategy for boronic acid-based sensors construction is shown in Scheme 4.1. Two monoboronic acids can be linked with a spacer to give a proper special arrangement that make the sensors selectively bind to the target carbohydrates. For this study, we are especially interested in developing a strategy to link 8-QBA (Chapter 3) to another boronic acid for sensor synthesis. 8-QBA is chosen because of its high water solubility, large fluorescent intensity changes upon sugar binding, and chemical and photochemical stability as described in Chapter 3..

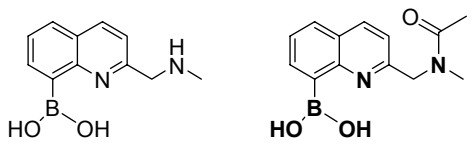


Scheme 4.1. Conceptual design of bisboronic acid-based sensors.

4.2.1.1. The monoboronic acid building block.

For the construction of 8-QBA based bisboronic acid sensors, we need to

introduce a functional group to 8-QBA, which could be tethered to other functional moieties without significantly affecting the fluorescence and binding properties of QBA. On one hand, the structure of the diboronic acids should have certain rigidity in order for it to adopt a well-defined conformation for the selective recognition of the target carbohydrate. One approach is to link the monoboronic acid to a spacer through amide bond formation. Therefore, an aminomethyl group was introduced into the 8-QBA system at the 2-position. Specifically, compound **24** was designed as the basic building block. A methylaminomethyl side chain was incorporated to the QBA ring. Amide bond was chosen as the linkage because of its rigidity, and also the amide bond formation is often a very clean reaction so that the purification of the final diboronic acid compounds would not present a major problem. The amino group is separated from the quinoline ring by one carbon in order to avoid the potentially drastic effect on the electronic and fluorescent properties of 8-QBA by directly attaching such a function to the quinoline ring. Another reason for choosing the amidation chemistry as a way to link two boronic acid moieties is to minimize the potential negative effect of the free amino group on the fluorescent property of the quinoline fluorophore. It is known that the lone pair electrons on amino nitrogen quite often quench the fluorescence of various fluorophores.[14,15] However, converting the amino group to an amide quite often abolishes the fluorescent quenching ability of the amino group. Model compound **25** was designed to study the feasibility of this design.

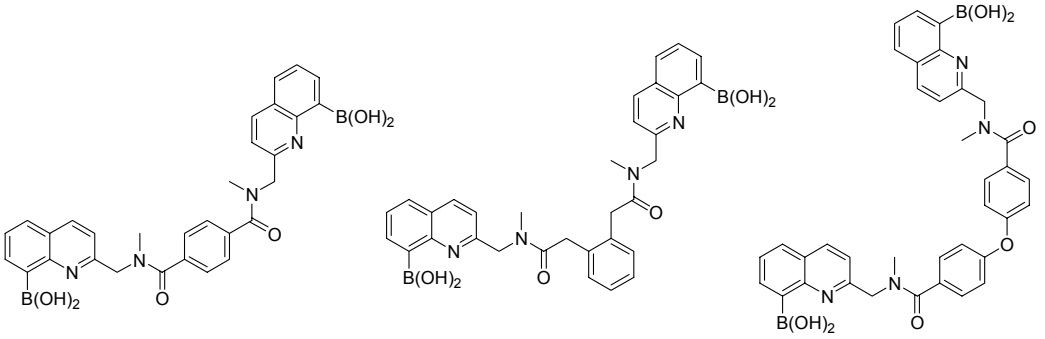


24 **25**

Figure 4.2. Monoboronic acid building block.

4.2.1.2. 8-Quinolineboronic Acid-based Cell Surface Carbohydrate Sensors.

Dicarboxylic acids were chosen as one type of spacer used to link two boronic acid units. Our group has made a series of diboronic acid using a similar strategy. We sampled three dicarboxylic acids, and the sensors were designed as below.



26 **27** **28**

Figure 4.3. Symmetric diboronic acid **26**, **27** and **28**.

Since the affinity of 8-QBA to sugars is relatively low compared with phenylboronic acid, another type of bisboronic acid using a second boronic acid unit was design as below (Figure 4.4). It is well-know that an electron-withdrawing group on an arylboronic acid will tremendously increase the binding affinity (Chapter 2). The 4-carboxylphenylboronic acid and 3-carboxylphenylboronic acid should have much higher intrinsic affinity for a diol-containing compound and will act as the main binding site in this system. The 8-QBA unit acts as both a recognition and a signaling (fluorescence) moiety.

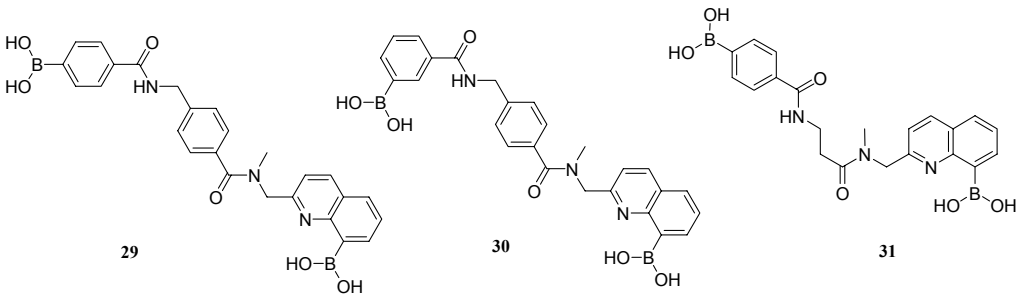


Figure 4.4. Unsymmetric diboronic acid **29**, **30** and **31**.

A third type of design is shown below. In this case, a triazole ring is used as the spacer through Huisgen 1,3-dipolar cycloaddition reaction.[12,13] This design has several unique features. First, the Huisgen cycloaddition is of high yield and mild conditions. Second, it gives a triazole ring, which might help to increase the water solubility of the final compound. Third, the triazole ring is very rigid, which helps to control the conformation of the sensor compound. Fourth, the triazole ring might also be able to engage in hydrogen-bond interactions in augmenting both the binding and affinity of the sensor.

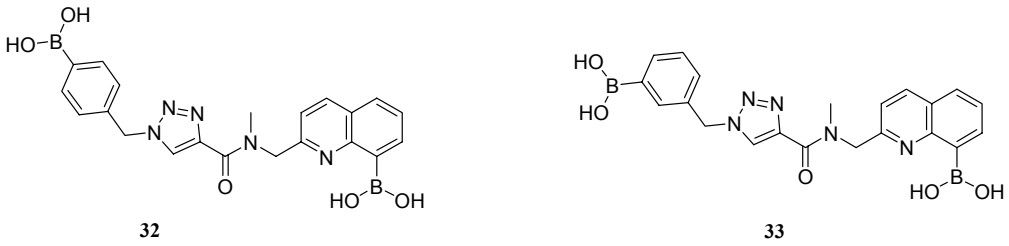


Figure 4.5. Unsymmetric diboronic acid **32** and **33**.

Computer aided modeling study was also performed to examine the feasibility of those designs. The distances between the two binding sites (the cis-diols in the carbohydrates, Figure 4.1) in the target carbohydrates are generally in the range of 12

Å. Therefore, it is important that the linkers selected fall within that range. Conformation search of the compounds designed has been done using Spartan. The distance of the two boronic acids in the design sensors fall in the range of 11 to 16 Å, which indicate a reasonable starting point. A few typical conformations are shown in Figure 4.6.

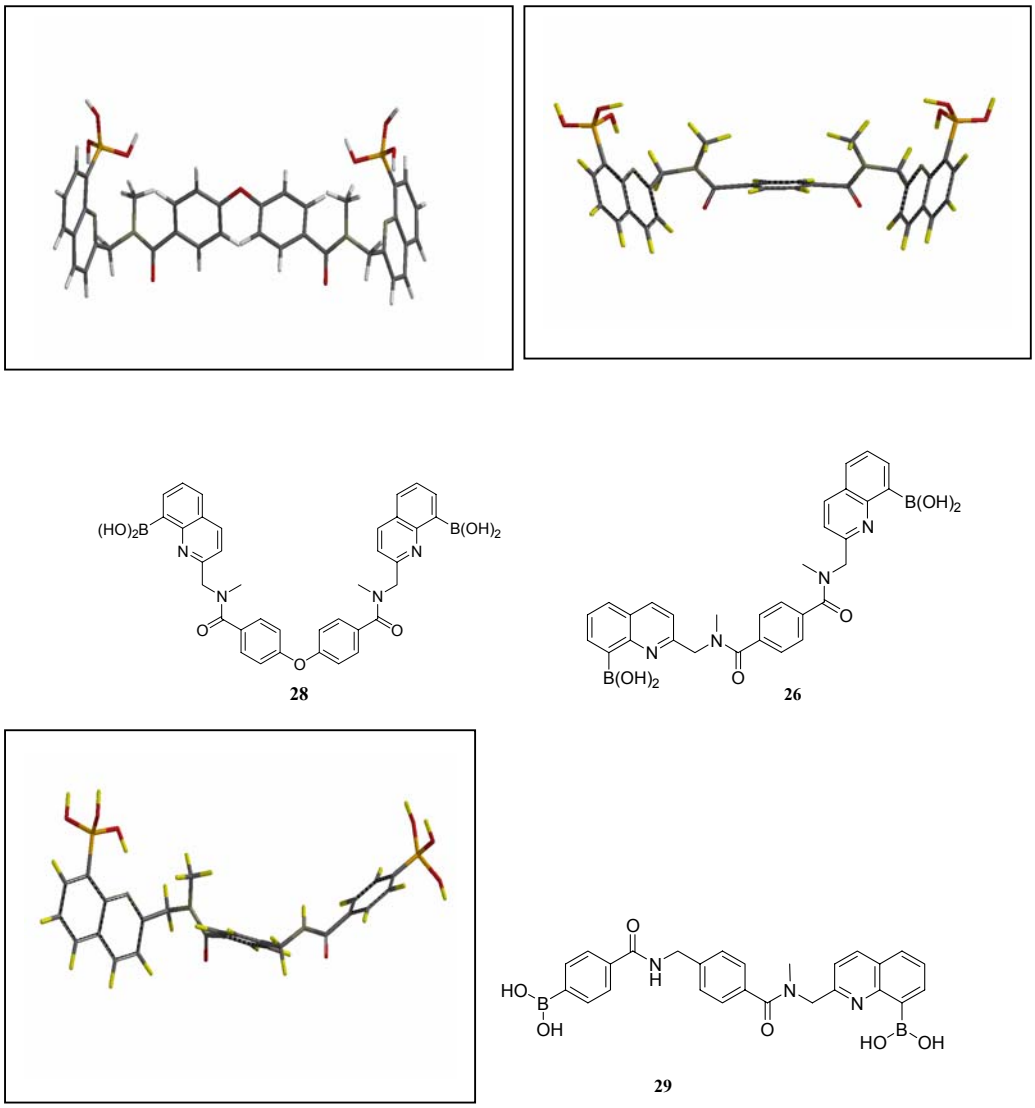


Figure 4.6. Favorable conformations of **26, 28** and **29**.

4.2.1.3. 8-Quinolineboronic Acid Based Glucose Sensors.

Glucose is another carbohydrate that attracted extensive attention. Several boronic acid-based glucose sensors have been developed by a few research groups.[16-20] However, the poor water solubility of these literature compounds limit their applications. With the water soluble 8-QBA in hand, we are interested in the design and synthesis of 8-QBA-based diboronic acids as potential glucose sensors.

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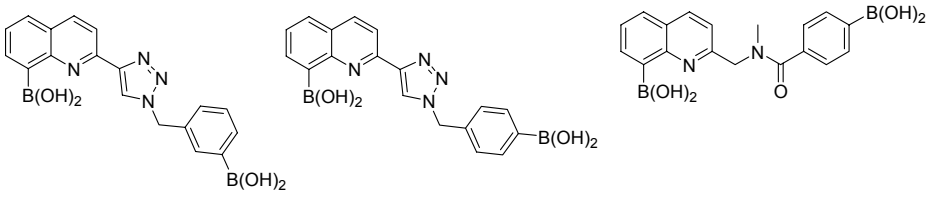
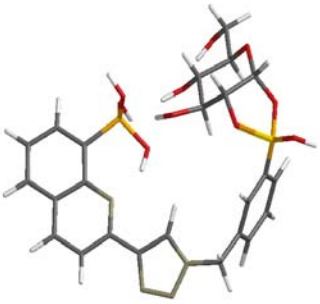


34 **35** **36**

Figure 4.7. 8-QBA-based glucose sensor.

Therefore, compounds **34-36** (Figure 4.7) have been designed. In this design, two different monoboronic acids are used. Phenylboronic acid is used as a recognition unit because of its relatively high affinity to diols. 8-QBA is used as both a recognition and a fluorescent signaling unit. It has been well-established that normally the boronic acids bind to glucoryranose in aqueous condition even though the major species is glucopyranose (See Chapter 1).[21,22] This is because the five-member furanose form gives a smaller dihedral angles which allow for high intrinsic affinity for boronic acids. However, under well-designed conditions, high affinity binding to the pyranose is also possible. Yang and Drueckhammer used the CAVEAT program to

develop a phenylboronic acid based glucopyranose receptor.[20] Therefore, for our computer modeling studies, we examined the binding with both the glucopyronose and the glucofuranose forms. Figure 4.8, 4.9 and 4.10 show a few diboronic acids that shows favorable conformation change in the boronic acid-sugar complex (Spartan, Molecular mechanics, MMFF). It should be noted that when doing the computer modeling studies, we first formed the sugar-sensor complex by "reacting" the phenylboronic acid moiety with glucose. This was done because of the known higher intrinsic affinity of phenylboronic acid to diols than the modified 8-QBA analog (**25**).



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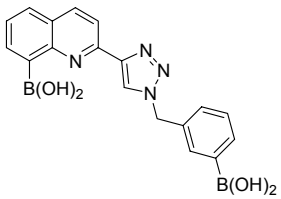
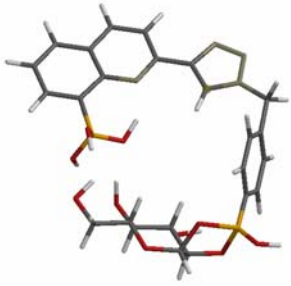
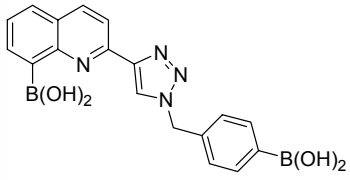


34

Figure 4.8. Conformation of the complex of D-glucose and **34** after the first boronic acid moiety binds to D-glucopyranose. Spartan (molecular mechanics, MMFF). The distance between boronic acid and sugar hydroxyl groups is 3.0 -3.2 Å.

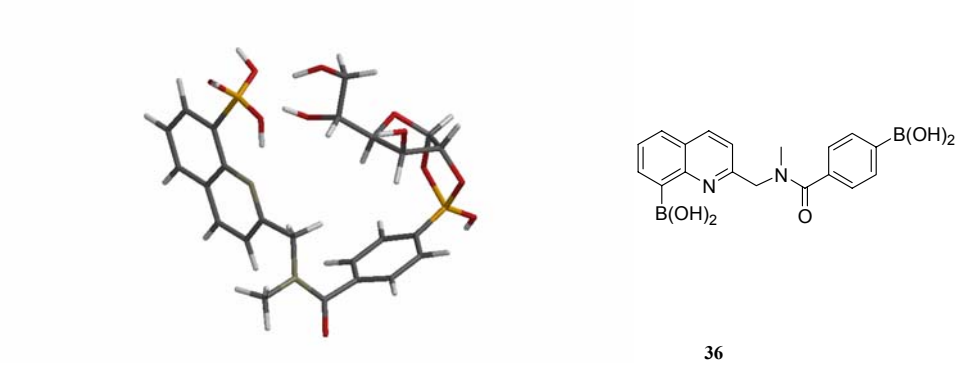




35

Figure 4.9. Conformation of the complex of D-glucose and **35** after the first boronic acid moiety

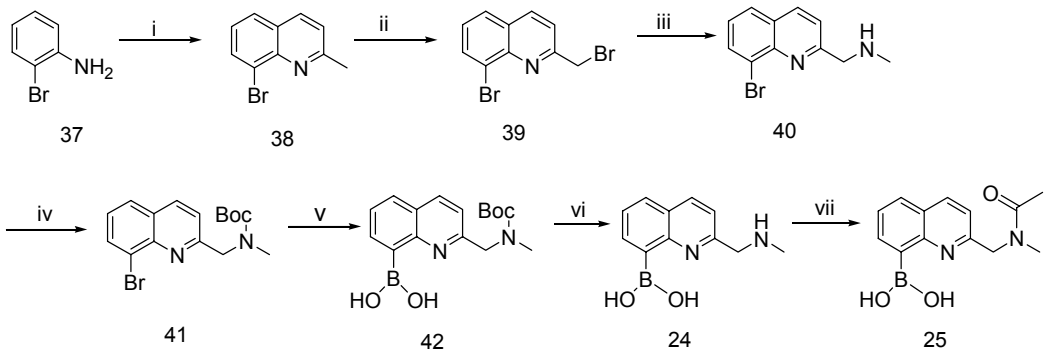
binds to D-glucopyranose. Spartan (molecular mechanics, MMFF). The distance between boronic acid and sugar hydroxyl groups is 2.8 -3.0 Å.



36

Figure 4.10. Conformation of the complex of D-glucose and **36** after the first boronic acid moiety binds D-glucofuranose. Spartan (molecular mechanics, MMFF). The distance between boronic acid and sugar hydroxyl groups is 3.1 -3.2 Å.

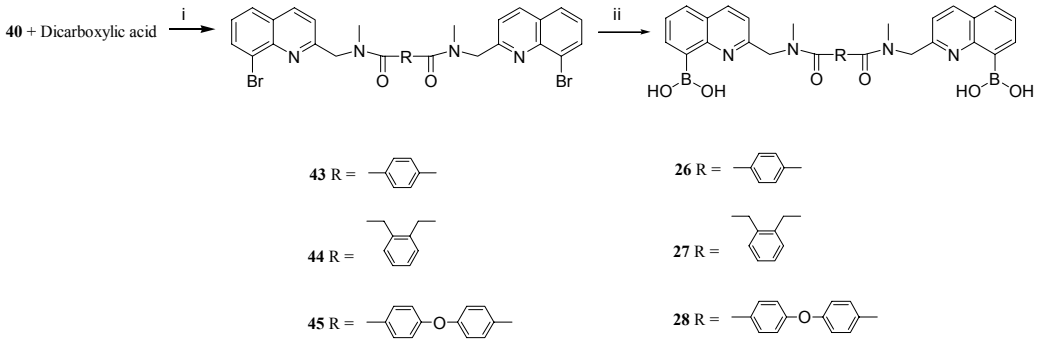
4.2.2. Synthesis



Scheme 4.2. Synthesis of the monoboronic acid building block.
Reagents and conditions. (i). Crotonaldehyde, 6N HCl, reflux, 56%; (ii). NBS, AIBN, CCl_4, 39%; (iii). $MeNH_2$ (40%, wt), THF, 96%; (iv). $(Boc)_2O$, TEA, MeOH, 96%; (v). $Pd(dppf)Cl_2$, bis(neopentyl glycolato)diboron, KOAc,DMSO, 70%; (vi). TFA, DCM; (vii). acetic anhydride, TEA, DCM, 87%.

The synthesis of the monoboronic acid building block stared with commercial available 2-bromoaniline (**37**). It was converted to 2-methylquinoline (**38**) by

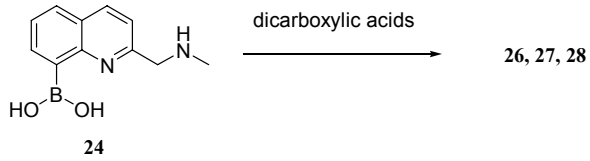
refluxing with crotonaldehyde in 6N HCl. Bromination at methyl group gives compound **39,** which was reacted with 40% methylamine aqueous solution in THF to yield **40.** The amino group was first protected with Boc before borylation under the catalysis of $Pd(dppf)Cl_2$ to give compound **42**. Deprotection by TFA gives the final monoboronic acid (**24**). However, compound **24** is not stable under neutral condition. Compound **42** was used directly for the synthesis of compound **25** and diboronic acids after deprotection without further purification.



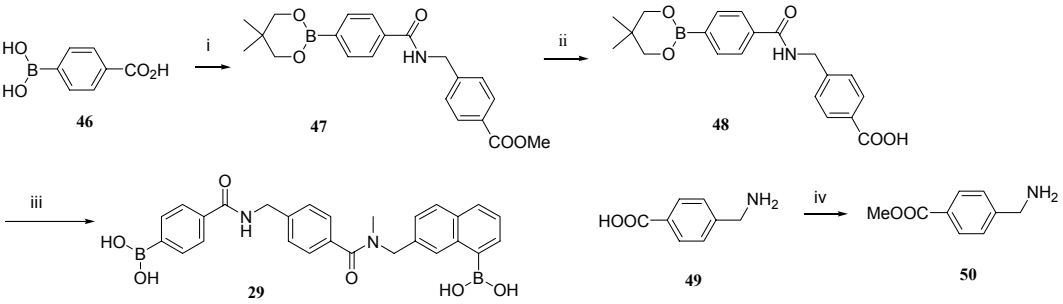
Scheme 4.3. Synthesis of symmetric 8-quinolineboronic acid **26**, **27** and **28**.
Reagents and conditions: (i). EDC, TEA, DCM, 80%; (ii). $Pd(dppf)Cl_2$, bis(neopentyl glycolato)diboron, KOAc, DMSO.

The synthesis of the symmetric diboronic acids proceeded as illustrated in Scheme 4.3. Compound **40** was coupled with dicarboxylic acids in the presence of EDCI to give the dibromo compounds **43**, **44** and **45**. Palladium catalyzed borylation gives the final diboronic acids **26**, **27** and **28,** respectively. An alternative approach has been tested as shown in Scheme 4.4. However, purification in this approach is much more difficult due to the existence of mono-coupling product and unreacted starting material **24**. The purification of boronic acid compounds is very hard due to

the Lewis acidity of the boron atom, and it is even harder for quinolineboronic acid since it exists in a zwitterionic form at neutral pH condition. In the previous approach, diboronic acid is the only species containing boron atom. Thus it is relatively easier for purification.



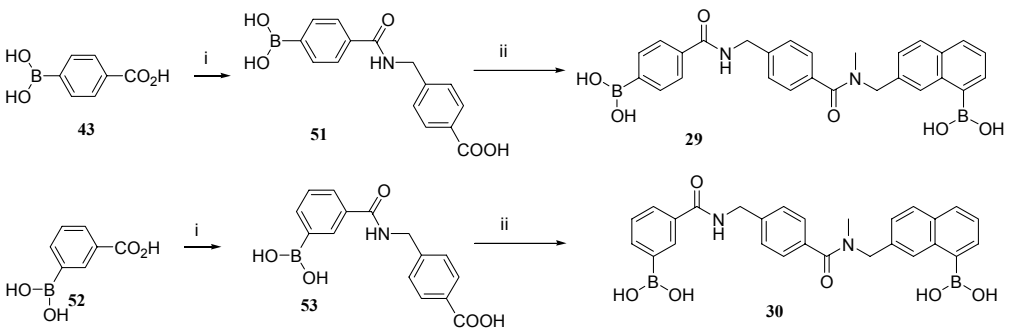
Scheme 4.4. An alternative approach to symmetric diboronic acids **26**, **27** and **28**.



Scheme 4.5. Synthesis of **29**.
Reagents and conditions: (i). (a.) neopentyl chloride, benzene, reflux, (b). $SOCl_2$, benzene, reflux, (c). **50**, TEA, THF/H_2O, 75%; (ii). NaOH, H_2O/MeOH, (iii). (a). $SOCl_2$, benzene, reflux, (b). **24**, TEA, DCM; (c). HCl, acetone/water; (iv). MeOH, H_2SO_4 (conc.), reflux.

Synthesis of **29** started with commercial available 4-carboxyphenylboronic acid (**46**). Refluxing with neopentylglycol in benzene with a Dean-Stark trap for 2 h followed by addition of thionyl chloride converted the carboxylic acid to the acid chloride. Compound **47** was generated by coupling reaction of the acid chloride with compound **50** followed by deprotection in NaOH alcoholic solution. It was expected

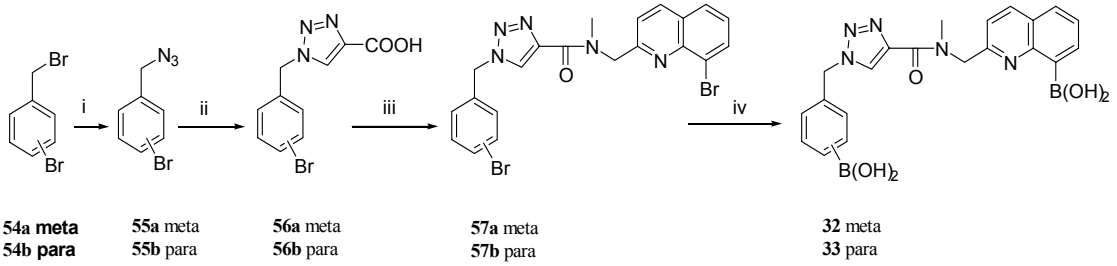
that compound **29** could be prepared by a coupling reaction with the monoboronic acid **24** followed by a simple deprotection in HCl. However, it was found that decomposition occurred during the deprotection step. Similar approach was applied to the synthesis of compound **31**. Decomposition was also observed and compound **49** was re-generated.



Scheme 4.6. Alternative approach to **29** and **30.**
Reagents and conditions: (i). (a) $SOCl_2$, benzene, cat. DMF, reflux; (b) **49**, TEA, THF/H_2O; (ii). (a).$SOCl_2$, benzene, reflux; (b). **24**, TEA, THF.

An alternative approach was tested as illustrated in Scheme 4.6. The preparation of compound **29** was executed through two coupling reaction starting with unprotected carboxyphenylboronic acid. Similar condition was applied to the synthesis of compound **30**. We took advantage of the solubility difference of the monoboronic acid and diboronic acid for the purification. It was found that the diboronic acid had very poor solubility in THF while the mono ones dissolved very well. The diboronic acids would precipitate out during the reaction, and filtration followed by column chromatography would give a pure product. Unfortunately, attempts at the synthesis of compound **31** using the same synthetic route failed again

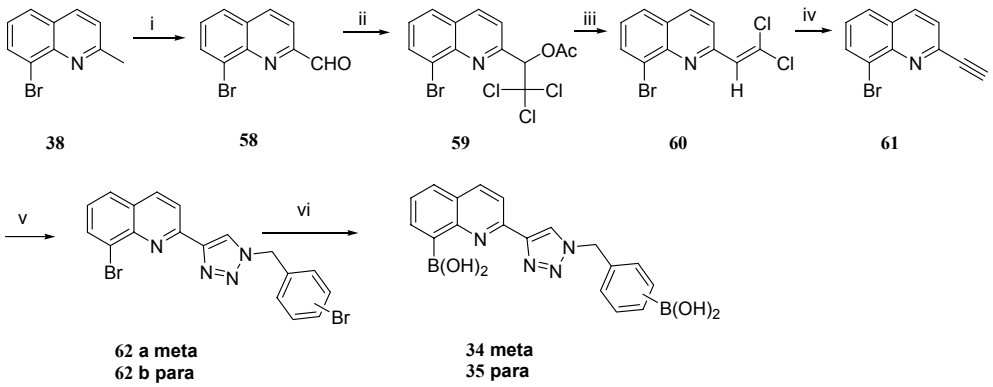
as decomposition was observed.



Scheme 4.7. Synthesis of compound **32** and **33**.

Reagents and conditions: (i). NaN_3, THF/H_2O, 99%; (ii) propynoic acid, sodium ascorbate, $CuSO_4.5H_2O$, H_2O/t-BuOH, 96%; (iii) (a) $SOCl_2$, DMF, reflux; (b) **40**, TEA, 95%; (iii) bis(neopentyl glycolato)diboron, $Pd(dppf)Cl_2$, KOAc, DMSO, 80℃.

Scheme 4.7 shows the synthetic route for compound **32** and **33**. Following the literature reported procedure, [12,13] compound **56** was prepared almost quantitatively. Coupling with **40** and followed by a palladium catalyzed borylation gave the target diboronic acids.



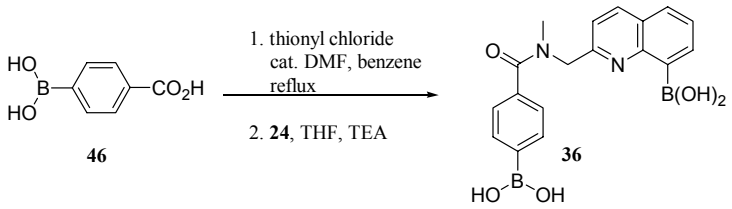
Scheme 4.8. Syhthesis of **34** and **35**.

Reagents and conditions: (i) SeO_2, dioxane, reflux, 90%; (ii) (a) trichloacetic acid, sodium trichloroacetate, DMF, 0 ℃; (b) acetic anhydride, DMAP, 95%; (iii) SmI_2, THF, 52%; (iv) MeLi,

THF, -78 ℃,71%; (v) **55**, sodium ascorbate, $CuSO_4$, H_2O/t-BuOH, 91%; (vi) bis(neopentyl glycolato)diboron, Pd(dppf)Cl_2, KOAc, DMSO, 80℃.

Synthesis of **34** and **35** proceeded as illustrated in Scheme 4.8. The key intermediate is compound **60**. Traditionally, vinyl dichlorides were prepared via Wittig reaction. Corey has reported a new method which involves reacting the aldehyde with trichloacetic acid and its sodium salt in DMF to prepare α-trichloromethyl carbinols, [23] which could be converted to the vinyl dichloride by a reductive elimination reaction in almost quantitative yield. Following the literature reported procedure[23,24] with minor modification compound **59** was prepared. However, attempts to synthesize **60** failed, and no reaction occurred under the literature reported conditions. Therefore, SmI_2 was selected as an alternative reducing agent to convert the α-trichloromethyl carbinols to the vinyl dichloride.[25] Due to the strong electron withdrawing ability of the quinoline ring and the terminal dichloro substituent, compound **60** is so reactive and it quickly polymerized during workup. Careful examination of the reaction conditions indicates that most likely residual Sm reagent catalyzed the polymerization. Diethyl ether, in which the Sm reagent residue has poor solubility, was then used to extract the solution right after the addition of HCl to destroy the Sm reagent. The organic layer was separated and filtered through a silica gel plug. This workup turned out to be very successful and a yield of 52% was obtained. A Huisgen 1,3-dipolar cycloaddition reaction[12,13] was carried out to make compound **62**, and the target boronic acid compounds were prepared through a palladium catalyzed borylation reaction.

Preparation of compound **36** proceeded as illustrated in Scheme 4.9.



Scheme 4.9. Preparation of **36**.

4.2.3 Binding Studies with the Monoboronic Acid Building Blocks

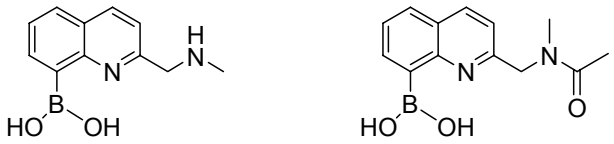


Figure 4.11. 8-QBA building blocks.

In order to understand how modified 8-QBA would behave after the introduction of a "handle" for tethering to other functional groups, compound **25** was synthesized and carefully studied. It was screened with a series of sugars including D-fructose, D-glucose, D-galactose, D-Sorbitol and D-Tagatose. The association constants were obtained and listed in Table 4.1. It is very interesting to find that the binding affinity to D-fructose and D-sorbitol, which are known to bind with boronic acids in a trivalent fashion,[26,27] decreased significantly, while the affinity to other sugars remain the same or slightly enhanced compared with boronic acid.

Table 4.1 The binding constants of 8-QBA and **25** with sugars.

	D-fructose	D-sorbitol	D-galactose	D-tagatose	L-arabinose
QBA	108	222	7.5	62	1.1
25	49	47	7.1	87	3.9

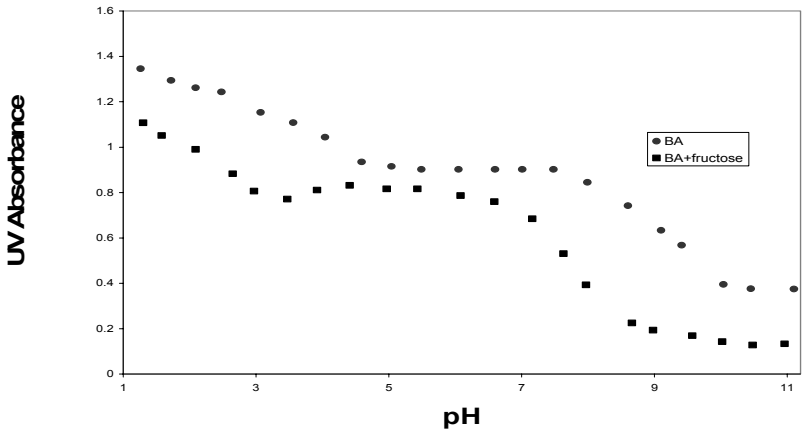
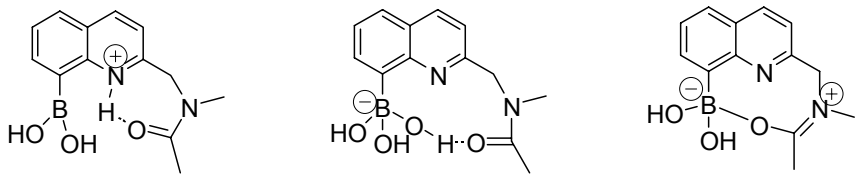


Figure 4.12. UV absorbance of compound **25** and its fructose complex vs. pH. at 314 nm in 0.10 M sodium phosphate buffer.

Table 4.2. pKa's of 8-QBA and compound **25**.

	pKa of the boronic acids		pKa of the fructose esters	
	pKa_1	pKa_2	pKa_1	pKa_2
QBA	4.2	9.6	2.0	9.0
25	3.5	8.8	2.3	7.7

To further understand the affinity changes, a few more experiments were carried out. The pKa of **25** and its fructose ester were obtained and compared with 8-QBA. A significant drop of both pKa_1 and pKa_2 was observed in both cases. Since the side chain in **25** is neither a strong electron-withdrawing group nor an electron-donating group. It is reasonable to conclude that the pKa change is not due to the inductive effect of the side chain. There must be some other factors. Possible interactions among the functional groups within this molecule are shown below.



25a **25b** **25c**

Figure 4.13. Interactions between the functional groups in **25**.

A hydrogen bond between the carbonyl group and protonated quinoline nitrogen as shown in **25a** is unlikely to happen since it would increase the pKa of the protonated quinoline nitrogen while a pKa decrease was observed. The existence of

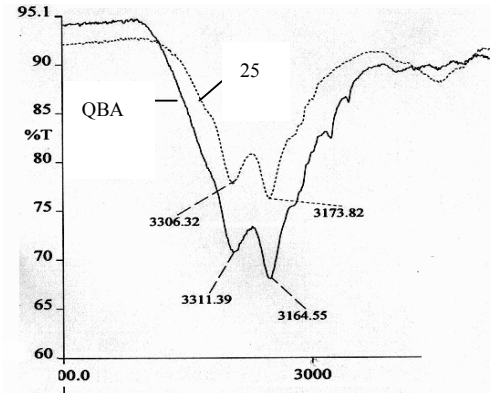


Figure 4.14. IR Spectrum of 8-QBA and **25** (trigonal).

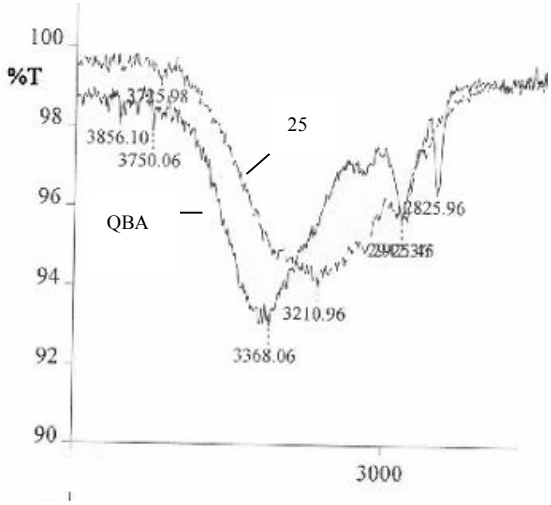


Figure 4.15. IR Spectrum of 8-QBA and **25** (tetrahedral).

25b and **25c** would be consistent with the observed pKa decrease of the boron species. However, **25c** is not favorable because the ring would be twisted. The ^{11}B NMR of 8-QBA and **25** in DMSO shows that the boron species are in trigonal form in both cases, which exclude the possibility of **25c**. Figure 4.14 and 4.15 shows the IR spectra of 8-QBA and **25** with boron atoms in trigonal form and tetrahedral form (see Experimental Part for details). In Figure 4.15 (tetrahedral boron) the OH stretch peak of **25** shifted about 160 cm^{-1} to lower wavenumbers and the peak becomes a somewhat broad comparing to that of 8-QBA while they have almost the same OH stretch peak in Figure 4.14 (trigonal boron). It indicates the formation of hydrogen bond between the OH group of the boronic acid and the carbonyl oxygen, which stabilized the tetrahedral boron species thus decreased the pKa.

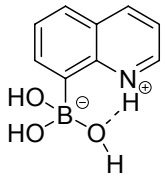


Figure 4.16. Hydrogen bond between boronic acid moiety and quinolinium nitrogen.

On the other hand, the hydrogen bond between the protonated quinoline nitrogen and the boronic acid OH group (as shown in Figure 4.16) no longer exists in compound **25**. Therefore, the pKa of the protonated quinoline nitrogen decreased. This kind of structure is similar to o-aminomethylarylboronic acid, in which the lone pair electron in the amino group goes to the open shell orbital of trigonal boron to thus facilitate the transformation to the tetrahedral boron and increase the binding affinity.

Since the tetrahedral boron species is stabilized by the hydrogen bond, the binding affinity should increase comparing to 8-QBA. Sorbitol and fructose were known to bind with boronic acid in a trivalent fashion. There is no stabilization for the boronate esters of those two sugars and the steric effect from the side chain could decrease the affinity. It should be noted that in our design, the building block shows decreased affinity to fructose so that higher selectivity to glucose or other sugars would be expected.

4.2.4. Binding Study of Diboronic Acids

Compounds **26** through **33** were screened with sialyl Lewis X, sialyl Lewis a and Lewis Y. Unfortunately, no significant fluorescence changes were observed. Since the concentration of the cell surface carbohydrates is low (60 μM) in the binding study, a very high binding affinity is required to see the fluorescence change. Further study with higher carbohydrate concentration will be done in the future.

Compounds **34** through **36** were tested with D-glucose. A weak fluorescence enhancement was observed. That might be due to very low affinity of 8-QBA to D-glucose. More study need to be done in the future to optimize the spatial alignment for higher binding affinity.

4.3. Conclusions

Synthetic routes for different designs of 8-QBA based diboronic acids have developed, and several diboronic acids have been synthesized. Enhanced binding

affinity was observed for the monoboronic acid building block, although no desired binding results were found for the diboronic acids. More diboronic acid compounds need to be synthesized and screened in search for potential carbohydrate sensors.

4.4. Experimental

4.4.1. General Methods

All chemicals were purchased from Acros, Asymchem, Frontier Scientific and Aldrich, and used as received. Dichloromethane was distilled from calcium hydride under nitrogen. THF was distilled from sodium and benzophenone. Water used for the binding studies was doubly distilled and further purified with a Milli-Q filtration system.

Analytical thin layer chromatography (TLC) was performed with Scientific Adsorbents plastic-backed TLC silica gel 60F hard layer plates. TLC plates were visualized with a 5% (w/v) solution of phosphomolybdic acid in ethanol or UV light (254 nm). TLC for boronic acid compounds was visualized with a 5% (w/v) solution of diphenylcarbazone in ethanol. Column chromatography was performed using silica gel (200-400 mesh size).

Mass spectrometry (MS) analyses were performed by the Mass Spectrometry Laboratories of North Carolina State University and Georgia State University. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded with a Varian Mercury 300, Varian Mercury 400 and Bruker 400 NMR spectrometry. ^{11}B NMR spectra were recorded with EtO·BF3 in deuterated chloroform as external standard. Chemical shift

(δ) for ^{1}H spectra were given in ppm relative to TMS and relative to residue solvent for ^{13}C spectra.

A Shimadzu RF-5301PC fluorometer was used for all fluorescent studies. A Shimadzu UV-1601 UV-visible spectrophotometer was used for the pKa determination. Quartz cuvettes were used in all studies. A Perkin Elmer Spectrum One FT-IR Spectrometer was used for IR studies.

4.4.2. pKa Determination

The apparent pKa's of 8-QBA and esters were determined by observing the UV absorption changes that occur upon the hybridization change from the acidic trigonal form to the basic tetrahedral form (Figure 2.1).[28]

4.4.3. Binding Constant Determination

For a typical sugar-boronic acid binding constant measurement, a 6.0×10^{-5} M solution of boronic acid compound in 0.10 M sodium phosphate monobasic buffer was brought to the appropriate pH (pH 7.4, within 0.01 units, pH was measured with an Accumet portable pH meter) with 4 N NaOH (solution A). Sugar was added to a portion of solution A to make a 6.0×10^{-5} M 8-QBA, 0.02 M sugar solution (Solution B). The pH was again checked and corrected if necessary. Solution B was titrated into solution A in order to make mixtures with a constant concentration of 8-QBA and a range of concentrations of sugar. In general, eight different concentrations were made in order to cover as much of the binding curve as solubility allowed. Each mixture was allowed to stand for at least five minutes. Then 3.5 mL of the mixture

was transferred into a cuvette for fluorescence measurement. The excitation wavelength was set as 314 nm. A sample experimental sheet was shown in experimental section of chapter III, Figure 3.9.

4.4.4. IR study

The sample for the IR study in Figure 4.15 (tetrahedral boron) was prepared as illustrated below. 8-QBA and compound **25** was dissolved in methanol and then evaporated to remove the solvent. One molecule of methanol will bind to the boron atom and change the boron species from trigonal form to tetrahedral form. ^{11}B NMR indicates the existence of tetrahedral boron atom.

4.4.5. Synthesis

8-bromo-2-methyl-quinoline (38). To a refluxed solution of 2-bromoaniline (**37**) (5.0 g, 29.1 mmol) in 6 N hydrochloric acid (15 mL) was added crotonaldehyde (2.2409 g, 32.0 mmol) drop wise. After heating under reflux for 8 h, the reaction mixture was cooled down and washed with 20 mL of ether, followed by addition of zinc chloride (3.95 g). The reaction mixture was stirred for 30 min at room temperature and another 15 min at 0 ℃ to give a yellow solid. The solid was collected and washed with 3 N cold hydrochloric acid, and then suspended in 2-propanol (20 mL) and stirred for 5 min at room temperature. The solid was filtered and washed with 2-propanol until the washing became colorless. This was followed by washing with 20 mL of ether and drying in air. The solid was re-suspended in 15 mL of cold water followed by the addition of 5 mL of concentrated ammonium hydroxide. The solution was shaken and extracted with 3 × 20 mL of ether. After drying over magnesium sulfate, the solvent

was evaporated to give a dark solid. Purification by chromatography (EtOAC/hexanes 10:90) yielded a white solid (3.62g, 56%). ^{1}H NMR (400 MHz $CDCl_3$) δ 8.02 (2H, t, *J* = 8.4 Hz), 7.73 (1H, d, *J* = 8 Hz), 7.33 (2H, t, *J* = 8), 2.82 (3H, s); ^{13}C NMR (300 MHz $CDCl_3$) δ 160.2, 144.7, 136.4, 132.8, 127.6, 127.3, 125.9, 124.0, 122.7, 25.6; EIMS, m/z 221/223 (M/(M+2)); Anal. Calcd for $C_{10}H_8BrN$: C, 54.08; H, 3.63; N, 6.31. Found: C, 54.25; H, 3.41; N, 5.89.

8-bromo-2-bromomethyl-quinoline (39). To a solution of **38** (2.5477 g, 11.47 mmol) in carbon tetrachloride (40 mL) was added NBS (2.2461 g, 12.62 mmol) and AIBN (0.094 g, 0.57 mmol) followed by refluxing overnight under UV light. The mixture was filtered to remove the precipitate and concentrated to give a yellow solid. Purification by chromatography (hexanes/DCM 80:20) yielded a white solid (1.33 g, 39%). ^{1}H NMR (400 MHz $CDCl_3$) δ 8.16 (1H, d, *J* = 8.4 Hz), 8.05 (1H, d, *J* = 7.2 Hz), 7.78 (1H, d, *J* = 7.6 Hz), 7.65 (1H, d, *J* = 8.4 Hz), 7.41 (1H, t, *J* = 7.6 Hz), 4.78 (3H, s); ^{13}C NMR (300 MHz $CDCl_3$) δ 158.3, 144.7, 138.0, 133.9, 128.9, 127.7, 127.6, 125.1, 122.4, 34.6; EIMS, m/z 299/300/301 (M/M+1/M+2); Anal. Calcd for $C_{10}H_7Br_2N$: C, 39.91; H, 2.34; N, 4.65. Found: C, 40.13; H, 2.281; N, 4.34.

(8-bromoquinolin-2-yl-methyl)methylamine (40). To a solution of **39** (1g, 3.32 mmol) in THF (5 mL) was added methylamine (10.5 mL of 40% water solution). The solution was stirred for 30 min and then extracted with EtOAc (30 mL). The organic phase was washed with DI water (2 × 20 mL), dried over $MgSO_4$, and concentrated to

give a red oil. Purification by chromatography (MeOH/DCM 1:99) yielded a yellow solid (0.8 g, 96%). ^{1}H NMR (400 MHz $CDCl_3$) δ 8.09 (1H, d, *J* = 8.4 Hz), 8.02 (1H, d, *J* = 7.2 Hz), 7.77 (1H, d, *J* = 8 Hz), 7.49 (1H, d, *J* = 8.4 Hz), 7.36 (1H, t, *J* = 8.0 Hz), 4.12 (2H, s), 2.58 (3H, s); ^{13}C NMR (400 MHz $CDCl_3$) δ 161.3, 144.7, 136.8, 133.1, 128.5, 127.5, 126.4, 124.7, 121.2, 57.6, 36.5; FABMS, m/z 251/253 (M+H/M+2+H); Anal. Calcd for $C_{11}H_{11}BrN_2$: C, 52.61; H, 4.42; N, 11.16. Found: C, 52.17; H, 4.46; N, 11.10.

(8-bromoquinolin-2-yl-methyl)methylcarbamic acid tert-butyl ester (41). To a solution of **40** (0.7501 g, 2.99 mmol) in methanol was added $(Boc)_2O$ (1.4992 g, 6.87 mmol) and triethylamine (2.1 mL, 14.9 mmol). The mixture was stirred at rt for 2 h and then concentrated in vacuum to remove all of the solvent. The residue was dissolved in DCM (20 mL) and then washed with DI water (2 × 10 mL) and brine (10 mL). The organic solution was dried over $MgSO_4$ and concentrated to give yellow oil. Purification by chromatography (hexanes/EtOAc 10:90) yielded a light-yellow oil. ^{1}H NMR (400 MHz $CDCl_3$) δ 8.16 (1H, t, *J* = 8.4 Hz), 8.06 (1H, d, *J* = 6.9 Hz), 7.80 (1H, d, *J* = 7.8 Hz), 7.41 (2H, m), 4.81 (2H, s), 3.03 (3H, d, *J* = 11.7 Hz), 1.4-1.6 (9H); ^{13}C NMR (400 MHz $CDCl_3$) δ 160.0, 144.9, 137.5, 133.4, 128.7, 127.7, 126.9, 124.9, 120.7, 119.8, 80.1, 55.5, 35.2, 28.7; ESIMS, m/z 351/353, M/(M+2); Anal Calcd for $C_{16}H_{19}BrN_2O_2$: C, 54.71; H, 5.45; N, 7.98. Found: C, 54.97; H, 5.62; N, 7.75.

2-[(tert-butoxycarbonylmethylamino)methyl]-quinoline-8-boronic acid (42). To a flask charged with **41** (0.4440 g, 1.26 mmol), bis(neopentyl glycolato)diboron (0.3427 g, 1.52 mmol), Pd(dppf)Cl_2 (0.0310g, 0.038 mmol) and KOAc (0.3722 g, 3.79 mmol) under nitrogen atmosphere was added anhydrous DMSO (10 mL). The mixture was stirred at 80 ℃ overnight, then poured into DCM (20 mL) and washed with DI water (4 × 30 mL). The organic solution was dried over $MgSO_4$ and concentrated to give a dark oil. Purification by chromatography (MeOH/DCM, 1:99) yielded a yellow oil (0.3313 g, 82%). ^{1}H NMR (400 MHz $CDCl_3$) δ 8.45 (1H, d, *J* = 5.4 Hz), 8.14 (1H, d, *J* = 6.6 Hz), 7.97 (1H, d, *J* = 8.1 Hz), 7.623 (1H, t, *J* = 7.5 Hz), 7.49 (1H, d, *J* = 8.4 Hz), 4.80 (2H , d, *J* = 6.0 Hz), 3.09 (3H, d, *J* = 4.2 Hz), 1.3-1.5 (9H); ^{13}C NMR (400 MHz $CDCl_3$) δ 157.3, 156.8, 156.2, 150.3, 139.4, 137.4, 129.7, 127.2, 126.6, 119.1, 118.9, 80.5, 74.7, 34.7, 27.4, 23.9; ESIMS, m/z 317, M+1.

2-methylaminomethyl-quinoline-8-boronic acid (24). To a solution of **42** (0.2501 g, 0.79 mmol) in DCM (20 mL) was added TFA (5 mL). The solution was stirred for 2 h, and then concentrated under vacuum for 4 h to give a yellow oil. This compound is not stable under neutral conditions, so no further purification was preformed. ^{1}H NMR (300 MHz $CDCl_3$) δ 8.35 (1H, d, *J* = 8.4 Hz), 8.12 (1H, d, *J* = 6.0 Hz), 7.87 (1H, d, *J* = 6.0 Hz), 7.60 (1H, t, *J* = 9.0 Hz), 7.44 (1H, d, *J* = 8.4 Hz), 4.47 (2H , s), 2.94 (3H, s); ESIMS, m/z 217, M+1.

2-[(acetylmethylamino)methyl]-quinoline-8-boronic acid (25). To a solution of **42**

(0.2429 g, 0.69 mmol) in DCM (20 mL) was added TFA (5 mL). The solution was stirred for 2 h, and then concentrated under vacuum for 4 h to give a yellow oil. The oil was dissolved in DCM (10 mL), and then acetic anhydride (0.1 mL, 1.06 mmol) and TEA (0.5 mL, 3.56 mmol) were added at 0 ^{o}C. The solution was stirred at room temperature for 3 h and then washed with water (2 × 10 mL) and brine (10 mL). The organic solution was dried over $MgSO_4$ and concentrated to give a red oil. Purification by chromatography (MeOH/DCM, 5:95) yielded a yellow solid (0.1550 g, 87%). ^{1}H NMR (400 MHz CD_3OD) δ 8.35 (1H, m), 8.19 (1H, d, *J* = 6 Hz), 7.98 (1H, d, *J* = 8.0 Hz), 7.60 (1H, m), 7.49 (1H, m), (4.98, 4.95) (2H , rotamer), (3.24, 3.07) (3H, rotamer), (2.30, 2.18) (3H, rotamer); ^{13}C NMR (300 MHz CD_3OD) δ 173.1, 156.2, 151.7, 138.9, 137.4, 130.1, 127.1, 126.4, 119.1, 118.9, 55.5, 52.3, 36.4, 33.9, 20.5, 20.3; ESIMS, m/z 259, M+1.

_N,N'_-bis-(8-bromoquinolin-2-yl-methyl)-_N,N'_-dimethylterephthalamide (43).

To a solution of **40** (1.0331 g, 4.11 mmol) in dry DCM (15 mL) was added EDCI (1.1830 g, 6.17 mmol), terephthalic acid (0.3417 g, 2.06 mmol) and TEA (0.64 mL, 4.5 mmol). The reaction mixture was stirred overnight at room temperature, and then washed with DI water (2 × 20 mL) and brine (10 mL). The organic solution was dried over $MgSO_4$ and concentrated to give a yellow solid. Purification by chromatography (MeOH/DCM 1:99) yielded a white solid (0.94 g, 72%). ^{1}H NMR (400 MHz $CDCl_3$) δ 8.15 (2H, m), 8.05 (2H, m), 7.79 (2H, m), 7.72 (2H, m), 7.56 (3H, m), 7.40 (2H, m), 7.29 (1H, m), (5.11, 5.07, 4.81, 4.78) (4H, rotamer, -CH_2-), (3.21, 3.18, 3.16) (6H, rotamer, -CH_3); ^{13}C NMR (400 MHz $CDCl_3$) δ 172.0, 171.11, 158.2, 157.3, 145.0,

144.7, 137.6, 137.5, 137.4, 133.6, 133.3, 128.7, 127.5, 127.3, 127.2, 127.1, 126.8, 125.1, 124.9, 121.0, 120.0, 57.0, 53.1, 38.7, 34.0; FABMS, 631/633/635, (M+H)/(M+2+H)/(M+4+H); Anal. Calcd for $C_{30}H_{24}Br_2N4O_2$: C, 56.98; H, 3.83; N, 8.86. Found: C, 56.73; H, 4.05; N, 8.29.

***N*-(8-bromoquinolin-2-yl-methyl)-2-(2-{[(8-bromo-quinolin-2-ylmethyl)-methyl-carbamoyl]-methyl}-phenyl)-*N*-methylacetamide (44).** To a solution a **40** (1.0 g, 3.98 mmol) in dry DCM (15 mL) was added EDCI (1.1451 g, 5.97 mmol), 1,2-phenylenediacetic acid (0.3866 g, 1.99 mmol) and TEA (0.62 mL, 4.4 mmol). The reaction mixture was stirred overnight at room temperature and then washed with DI water (2 × 20 mL) and brine (10 mL). The organic solution was dried over $MgSO_4$ and concentrated to give a yellow solid. Purification by chromatography (MeOH/DCM 1:99) yielded a white solid (1.0802 g, 82%). ^{1}H NMR (400 MHz $CDCl_3$) δ 8.0 (4H, m), 7.72 (2H, t, *J* = 8.4 Hz), 7.35 (2H, d, *J* = 7.2 Hz), 7.30-7.10 (6H, m), 4.82-5.04 (4H, rotamer), 3.10-3.20(6H, rotamer). EIMS, m/z, 658/660/662, M/(M+2)/(M+4).

***N,N*'-bis-(8-bromo-quinolin-2-ylmethyl)-*N,N*'-dimethyl-4,4'-oxybisbenzamide (45).** To a solution of **40** (0.3163 g, 1.26 mmol) in dry DCM (10 mL) was added EDCI (0.3622 g, 1.89 mmol), 4,4'-oxybis(benzoic acid) (0.1464 g, 0.57 mmol) and TEA (0.20 mL, 1.4 mmol). The reaction mixture was stirred overnight at room temperature and then washed with DI water (2 × 20 mL) and brine (10 mL). The

organic solution was dried over $MgSO_4$ and concentrated to give a yellow solid. Purification by chromatography (MeOH/DCM 1:99) yielded a white solid (0.3580 g, 87%). ^{1}H NMR (300 MHz $CDCl_3$) δ 8.16 (1H, d, *J* = 8.4 Hz), 8.05 (1H, d, *J* = 7.2 H), 7.79 (1H, d, *J* = 8.1 H), 7.63 (4H, m), 7.39 (3H, m), 7.08 (4H, s, broad), (5.31, 5.10, 4.89, 4.70) (4H, rotamer), 3.21 (6H, m, rotamer); ESIMS, m/z, 723/725/727, (M+H/(M+2+H)/(M+4+H);

***N,N'*-bis-(8-quinolinboronic acid-2-ylmethyl)-*N,N'*-dimethylterephthalamide (26).** A flask charged with **58** (0.500 g, 0.79 mmol), KOAc (0.4656 g, 4.74 mmol), bis(neopentyl glycolato)diboron (0.4465 g, 1.98 mmol) and $Pd(dppf)Cl_2$ (0.0388 g, 0.048 mmol) was flushed with nitrogen. Anhydrous DMSO was added and the mixture was stirred at 80 ℃ overnight. The mixture was poured into EtOAc (20 ml) and washed with DI water (2 × 30 ml). The organic phase was separated, dried over $MgSO_4$ and concentrated to give a brown oil. Purification by chromatography (MeOH/DCM 10:90) yielded a yellow oil (1.0802 g, 82%). ^{1}H NMR (300 MHz DMSO) δ 8.18 (2H, m), 8.10 (2H, m), 7.83 (2H, m), 7.69 (2H, m), 7.60 (3H, m), 7.43 (2H, m), 7.29 (2H, m), (5.13, 5.09, 4.84, 4.80) (4H, rotamer, $-CH_2-$), (3.23, 3.20, 3.18) (6H, rotamer, $-CH_3$); ESIMS, m/z 544, M-18.

***N*-(8-quinolinboronic acid-2-ylmethyl)-2-(2-{[(8-quinolinboronic acid-2-ylmethyl)methylcarbamoyl]methyl}phenyl)-*N*-methylacetamide (27).** To a flask charged with **59** (1.0686 g, 1.62 mmol), KOAc (0.9529 mg, 9.7 mmol), bis(neopentyl

glycolato)diboron (0.9140 g, 4.05 mmol) and $Pd(dppf)_2CH_2Cl_2$ (0.080 g, 0.098 mmol) was added anhydrous DMSO (20 mL). The mixture was stirred at 80 ℃ overnight, and then poured into DCM (20 mL), washed with DI water (2 × 30 mL). The organic solution was separated, dried over $MgSO_4$ and concentrated to give a grey solid. Recrystallization in $EtOH/H_2O$ yielded a white solid (0.675 g, 45%). ^1H NMR (400 MHz $CDCl_3$, 363 K) δ 8.35-8.20 (4H, m), 8.03 (2H, s), 7.62 (2H, s), 7.45 (2H, s), 7.16 (4H, s), 4.90 (4H, s), 3.85 (4H,s), 3.10 (6H, s); HRMS, Calcd for $C_{32}H_{32}B_2N_4O_6$-H_2O: m/z 572.24023. Found. m/z 572.24298.

***N,N'*-bis-(8-quinolinboronic acid-2-ylmethyl)-*N,N'*-dimethyl-4,4'-oxybis benzamide (28).** To a flask charged with **60** (0.3564 g, 0.49 mmol), KOAc (0.2897 mg, 2.95 mmol), bis(neopentyl glycolato)diboron (0.2778 g, 1.23 mmol) and $Pd(dppf)_2CH_2Cl_2$ (0.0241 g, 0.029 mmol) was added anhydrous DMSO (10 mL). The mixture was stirred at 80 ℃ overnight, and then poured into DCM (20 mL), and washed with DI water (2 × 30 mL). The organic solution was separated, dried over $MgSO_4$ and concentrated to give a red oil. Purification by chromatography (MeOH/DCM, 5:95) yielded a yellow oil (0.190 g, 59%). ^1H NMR (400 MHz DMSO) δ 8.28 (2H, m), 8.28 (2H, m), 8.11 (2H, m), 7.85 (2H, m), 7.56 (6H, s), 7.25 (4H, m), (4.78, 4.91, 5.50) (4H, rotamers, -CH_2-), (3.07-3.19) (6H, rotamers, -CH_3); HRMS, calcd for $C_{36}H_{32}B_2N_4O_7$-H_2O: m/z 636.2531. Found: m/z 636.2542

4-[(4-neopentylboronic ester-benzoylamino)-methyl]-benzoic acid methyl ester (47). To a solution of **46** (0.9413 g, 5.67 mmol) in benzene (70 mL) was added

neopentyl glycol (0.6510 g, 6.25 mmol). The solution was refluxed with a Dean Stark trap for 2 h and then $SOCl_2$ (2.4 ml, 32.98 mmol) was added in. After stirring under reflux for another 4 h, the solution was cooled room temperature and the solvent was evaporated out to give a white solid residue. The residue was re-dissolved in dry DCM (20 mL) and **50** (0.9368 g, 5.67 mmol) was added in at 0 ℃. After stirring at room temperature for 4 h, the solution was washed with 5% $NaHCO_3$ aqueous solution (20 mL) and DI water (2 × 20 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated to give white solid. Purification by chromatography (MeOH/DCM, 1:99) yielded a white solid (1.83 g, 85%). ^{1}H NMR (300 MHz $CDCl_3$) δ 8.02 (2H, d, *J* = 8.4 Hz), 7.91 (2H, d, *J* = 8.4 Hz), 7.81 (2H, d, *J* = 8.4 Hz), 7.40 (2H, d, *J* = 8.4 Hz), 4.72 (2H, d, *J* = 8.1 Hz), 3.94 (3H, s), 3.81 (4H, s), 1.06 (6H, s); ^{13}C NMR (300 MHz $CDCl_3$) δ 142.5, 138.0, 134.4, 133.9, 132.1, 130.4, 130.3, 127.9, 126.2, 72.6, 59.1, 53.3, 32.2, 22.1; ESIMS, m/z 382, M+1. Anal. Calcd for $C_{21}H_{24}BNO_4$: C, 66.16; H, 6.35; N, 3.67. Found: C, 65.91; H, 6.20; N, 3.76.

4-[(4-neopentylboronic ester-benzoylamino)-methyl]-benzoic acid (48). To a solution of **47** (0.83 g, 2.18 mmol) in MeOH (10 mL) was added 2N NaOH aqueous solution (1 mL). The solution was stirred for 8 h and then acidified with 4N HCl aqueous solution to bring the pH down to pH 3 with a white solid precipitated out. The solid was collected and washed with DI water (10 mL) followed by drying in vacuum overnight. No further purification needed (0.7765 g, 97%). ^{1}H NMR (300 MHz $CDCl_3$) δ 8.12 (2H, d, *J* = 8.1 Hz), 7.91 (2H, d, *J* = 8.4 Hz), 7.81 (2H, d, *J* =

8.4 Hz), 7.49 (2H, d, *J* = 8.4 Hz), 4.77 (2H, d, *J* = 6.0 Hz), 3.82 (4H, s), 1.07 (6H, s); ESIMS, *m/z* 217 (M+1); Anal. Calcd for $C_{20}H_{22}BNO_5$: C, 65.42; H, 6.04; N, 3.81. Found: C, 65.34; H, 5.80; N, 3.76.

4-aminomethyl-benzoic acid methyl ester (50). To a solution of **49** (1.0 g, 6.62 mmol) in MeOH (30 mL) was added concentrated sulfuric acid (1 mL). After stirring under reflux for 6 h, the solution was cooled and the solvent was removed in vacuum. The residue was dissolved in DCM (20 mL) followed by washing with 10% ammonium hydroxide solution and DI water (2 × 20 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated to give a clear oil. Purification by chromatography (MeOH/DCM, 1:99) yielded a white solid (0.607 g, 56%). ^{1}H NMR (300 MHz $CDCl_3$) δ 7.90 (2H, d, *J* = 8.4 Hz), 7.36 (2H, d, *J* = 8.1 Hz), 4.78 (2H, s), 3.81 (3H, s); ^{13}C NMR (500 MHz $CDCl_3$) δ 167.3, 148.8, 129.7, 128.9, 128.1, 127.4, 126.9, 51.5, 45.0; EIMS, *m/z* 165/164, M/M-1.

4-[(4-boronic acid-benzoylamino)-methyl]-benzoic acid (51). To a flask charged with **46** (0.300 g, 1.82 mmol) was added $SOCl_2$ (4 mL) and DMF (1 drop). The mixture was stirred under reflux for 2 h and then cooled down to room temperature. All of the liquid was removed under vacuum to give a white solid. It was dissolved in THF (10 mL) and then added to an aqueous solution of **49** (0.315 g, 1.90 mmol) and NaOH (0.157g, 3.92 mmol). The solution was stirred for 2 h and then the pH was adjusted with 4N HCl aqueous solution to pH 3 to give a white precipitate. The solid was collected and dried in vacuum overnight. No further purification needed (0.507 g,

93%). ^{1}H NMR (300 MHz CD_3OD) δ 8.03 (2H, d, *J* = 8.1 Hz), 7.88 (2H, d, *J* = 7.2 Hz), 7.77 (2H, s), 7.49 (2H, d, *J* = 8.4 Hz), 4.68 (2H, s),) ^{13}C NMR (300 MHz CD_3OD) δ 176.1, 168.6, 168.5, 157.7, 144.5, 141.2, 133.6, 129.9, 127.2, 126.2, 43.1; ESIMS, m/z 298, M-1.

4-[(3-boronic acid-benzoylamino)-methyl]-benzoic acid (53). To a flask charged with **52** (0.539 g, 3.25 mmol) was added $SOCl_2$ (6 mL) and DMF (2 drop). The mixture was stirred under reflux for 2 h and then cooled down to room temperature. All of the liquid was removed under vacuum to give a white solid. It was dissolved in THF (15 mL) and then added to an aqueous solution of **49** (0.6331 g, 3.83 mmol) and NaOH (0.2555g, 6.39 mmol). The solution was stiired for 2 h and then the pH was adjusted with 4N HCl aqueous solution to pH 3 to give a white precipitate. The solid was collected and dried in vacuum overnight. No further purification needed (0.869 g, 91%). ^{1}H NMR (300 MHz CD_3OD) δ 8.22 (1H, broad), 8.02 (2H, d, *J* = 8.4 Hz), 7.92 (2H, m), 7.50 (3H, m), 4.68 (2H, s); ^{13}C NMR (300 MHz CD_3OD) δ 169.4, 168.5, 144.3, 136.6, 133.5, 132.3, 129.9, 129.6, 128.6, 127.7, 127.2, 43.1; ESIMS, m/z 298, M-1.

2-[({4-[(4-boronic acid-benzoylamino)methyl]benzoyl}methylamino)methyl]-quinoline-8-boronic acid (29). To a flask charged with **51** (0.1386 g, 0.46 mmol) was added added $SOCl_2$ (3 mL) and DMF (1 drop). The mixture was stirred under reflux for 2 h and then cooled down to room temperature. All of the liquid was removed

under vacuum to give a white solid. The residue was then dissolved in dry THF (5 mL) (**solution A**).

To a solution of **42** (0.1626 g, 0.46 mmol) in DCM (10 mL) was added TFA (2mL). The solution was stirred for 6 h and concentrated in vacuum for another 4 h. The residue was then dissolved in dry THF (5 mL) and added into solution A at 0 ℃ followed by stirring at room temperature for 2 h with a yellow solid precipitated out. The yellow solid was then collected. Purification by chromatography (MeOH/DCM, 10:90) yielded a yellow solid (0.078 g, 34%). ^{1}H NMR (300 MHz DMSO) δ 8.9 (1H, broad), 8.51 (1H, d, *J* = 6.3 Hz), 8.33 (1H, m), 8.15 (1H, d, *J* = 6.3 Hz), 7.81 (2H, broad), 7.64 (1H, m), 7.41 (4H, broad), 8.45 (2H, d, *J* = 6.3 Hz), 5.0 (2H, m, rotamer), 4.50, (2H, broad, rotamer), 2.52 (3H, s); ESIMS, m/z 499, M+2.

2-[({4-[(3-boronic acid-benzoylamino)methyl]benzoyl}methylamino)methyl]-quinoline-8-boronic acid (30). To a flask charged with **53** (0.2799 g, 0.94 mmol) was added added $SOCl_2$ (4 mL) and DMF (1 drop). The mixture was stirred under reflux for 2 h and then cooled down to room temperature. All of the liquid was removed under vacuum to give a white solid. The residue was then dissolved in dry THF (5 mL) (**solution A**).

To a solution of **42** (0.3287 g, 0.94 mmol) in DCM (10 mL) was added TFA (2mL). The solution was stirred for 6 h and concentrated in vacuum for another 4 h. The residue was then dissolved in dry THF (5 mL) and added into solution A at 0 ℃ followed by stirring at room temperature for 2 h with a brown solid precipitated out.

The yellow solid was then collected. Purification by chromatography (MeOH/DCM, 10:90) yielded a yellow solid (0.140 g, 30%). ^{1}H NMR (300 MHz DMSO) δ 8.9 (1H, broad), 8.51 (1H, d, *J* = 6.3 Hz), 8.33 (1H, m), 8.15 (1H, d, *J* = 6.3 Hz), 7.81 (2H, broad), 7.64 (1H, m), 7.41 (4H, broad), 8.45 (2H, d, *J* = 6.3 Hz), 5.0 (2H, m, rotamer), 4.50, (2H, broad, rotamer), 2.52 (3H, s); ESIMS, m/z 499, M+2.

1-azidomethyl-3-bromobenzene (55a). To a solution of **54a** (1.9210 g, 7.69 mmol) in THF (40 mL) was added a solution of sodium azide (0.9993 g, 15.4 mmol) in water (20 mL) followed by stirring for 6 h at room temperature. The solution was saturated with sodium chloride and then the organic layer was separated. The organic solution was dried over $MgSO_4$ and concentrated to give a clear oil. Further purification by chromatography (hexanes/DCM, 80:30) yielded a clear oil (1.58 g, 97%); ^{1}H NMR (400 MHz $CDCl_3$) δ 7.50 (2H, m), 7.28 (2H, m), 4.35 (2H, s); ^{13}C NMR (400 MHz CD_3OD) δ 133.5, 132.1, 129.8, 128.0, 122.8, 53.0; EIMS, *m/z* 211/213 (M/M+2).

1-azidomethyl-4-bromobenzene (55b). To a solution of **54b** (0.5011 g, 2.00 mmol) in THF (20 mL) was added a solution of sodium azide (0.2701 g, 4.15 mmol) in water (10 mL) followed by stirring for 6 h at room temperature. The solution was saturated with sodium chloride and then the organic layer was separated. The organic solution was dried over $MgSO_4$ and concentrated to give a clear oil. Further purification by chromatography (hexanes/DCM, 80:30) yielded a clear oil (0.34 g, 81%); ^{1}H NMR (300 MHz $CDCl_3$) δ 7.51 (2H, d, *J* = 4.2 Hz), 7.20 (2H, d, *J* = 3.6 Hz), 5.31 (2H, s);

^{13}C NMR (400 MHz $CDCl_3$) δ 134.4, 132.0, 129.8, 122.4, 54.1; EIMS, *m/z* 211/213 (M/M+2).

1-(3-bromobenzyl)-1H-[1,2,3]triazole-4-carboxylic acid (56a). To a flask charged with **55a** (0.6074 g, 2.86 mmol) and propynoic acid (0.2011 g, 2.87 mmol) was added t-BuOH/H_2O (1:1, 20 mL) followed by addition of $CuSO_4$•$5H_2O$ (0.0071 g, 0.029 mmol) and a solution of sodium ascorbate in water (0.1 M, 2.86 mL). After stirring at room temperature for 20 hrs, a solution of NaOH (1N, 10 mL) was added, and the aqueous layer was washed with EtOAc (2 × 10 mL) followed by neutralization with hydrochloric acid (1N, 10 mL) to give a white solid. The solid was collected and dried in Vacuum (0.7963 g, 99%). No further purification required. ^{1}H NMR 300 MHz, $CDCl_3$) δ 10.316 (1H, d, *J* = 0.6 Hz), 8.34 (1H, d, *J* = 8.1 Hz), 8.17 (1H, dd, *J* = 7.5, 1.2 Hz), 8.1 (1H, d, *J* = 8.4 Hz), 7.89 (1H, dd, *J* = 8.1, 1.2 Hz), 7.55 (1H, dd, *J* = 8.1, 7.5 Hz); EIMS, *m/z* 280/282 (M-1/M+2-1)

1-(4-bromobenzyl)-1H-[1,2,3]triazole-4-carboxylic acid (56b). To a flask charged with **55b** (0.6057 g, 2.86 mmol) and propynoic acid (0.2003 g, 2.86 mmol) was added t-BuOH/H_2O (1:1, 20 mL) followed by addition of $CuSO_4$•$5H_2O$ (0.0071 g, 0.029 mmol) and a solution of sodium ascorbate in water (0.1 M, 2.86 mL). After stirring at room temperature for 20 hrs, a solution of NaOH (1N, 10 mL) was added, and the aqueous layer was washed with EtOAc (2 × 10 mL) followed by neutralization with hydrochloric acid (1 N, 10 mL) to give a white solid. The solid was collected and

dried in Vacuum (0.8011 g, 99%). No further purification required. ^{1}H NMR (300 MHz, CD_3OD) δ 8.44 (1H, s), 7.55 (2H, dd, *J* = 6.6, 2.1 Hz), 7.28 (2H, dd, *J* = 6.6, 2.1 Hz), 5.63 (2H, s); EIMS, *m/z* 211/213 (M/M+2);EIMS, *m/z* 280/282 (M-1)/（M+2-1）. Anal. Calcd for $C_{10}H_8BrN_3O_2$: C, 42.58; H, 2.86; N, 14.90. Found: C, 42.61; H, 2.83; N, 14.19.

1-(3-bromobenzyl)-1H-[1,2,3]triazole-4-carboxylic acid (8-bromoquinolin-2-ylmethyl)methylamide (57a). A mixture of **56a** (0.3380 g, 1.2 mmol) in $SOCl_2$ (5 mL) with DMF (1 drop) was heated under reflux for 6 hrs. $SOCl_2$ was removed in vacuum for 4 h, and the residue was dissolved in dry DCM (10 mL) followed by addition of **40** (0.3013 g, 1.2 mmol) in DCM (5 mL) and TEA (0.8 mL) at 0 ℃ in a nitrogen atmosphere . The solution was stirred for another 4 h at room temperature, and then washed with DI water (2 × 10 mL), brine (10 mL). The organic layer was separated, dried over $MgSO_4$ and concentrated to give a brown oil. Purification by chromatography (MeOH/DCM, 1:99) yielded a yellow solid. (0.5864 g, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.316 (1H, d, *J* = 0.6 Hz), 8.34 (1H, d, *J* = 8.1 Hz), 8.17 (1H, dd, *J* = 7.5, 1.2 Hz), 8.1 (1H, d, *J* = 8.4 Hz), 7.89 (1H, dd, *J* = 8.1, 1.2 Hz), 7.55 (1H, dd, *J* = 8.1, 7.5 Hz); ESIMS, *m/z* 514/515/516/517/518/519 (M-1)/M/M+2-1)/(M+2)/(M+4-1)/(M+4).

1-(4-bromobenzyl)-1H-[1,2,3]triazole-4-carboxylic acid (8-bromoquinolin-2-ylmethyl)methylamide (57b). A mixture of **56b** (0.3122 g, 1.11 mmol) in $SOCl_2$ (5

mL) with DMF (1 drop) was heated under reflux for 6 hrs. $SOCl_2$ was removed in vacuum for 4 h, and the residue was dissolved in dry DCM (10 mL) followed by addition of **40** (0.3899 g, 1.11 mmol) in DCM (5 mL) and TEA (0.7 mL) at 0 ℃ in a nitrogen atmosphere . The solution was stirred for another 4 h at room temperature, and then washed with DI water (2 × 10 mL), brine (10 mL). The organic layer was separated, dried over $MgSO_4$ and concentrated to give a brown oil. Purification by chromatography (MeOH/DCM, 1:99) yielded a yellow solid. (0.4246 g, 75%) ^{1}H NMR (300 MHz, DMSO) δ 8.73 (1H, d, *J* = 6.3 Hz), 8.45 (1H, dd, *J* = 8.4, 3.6 Hz), 8.15 (1H, dd, *J* = 7.2, 3.6 Hz), 8.04 (1H, d, *J* = 8.1 Hz), 7.50-7.65 (4H, m), 7.37 (1H, d, *J* = 8.4 Hz), 7.26 (1H, d, *J* = 8.4 Hz), 5.70, 5.62 (2H, rotamer), 5.49, 5.01 (2H, rotamer), 3.59, 3.15 (2H, rotamer); ^{13}C NMR (300 MHz, DMSO) δ 161.9, 161.8, 159.7, 159.5, 144.3, 144.2, 143.8, 138.1, 135.6, 135.4, 133.7, 132.2, 132.1, 130.8, 130.7, 129.4, 129.3, 128.8, 128.6, 127.5, 124.1, 122.0, 121.1, 121.0, 56.0, 54.1, 52.7, 52.6, 38.1, 35.4; ESIMS, *m/z* 514/515/516/517/518/519, (M-1)/M/M+2-1)/ (M+2)/(M+4-1)/(M+4).

2-({[1-(4-boronic acid-benzyl)-1H-[1,2,3]triazole-4-carbonyl]methylamino}-methyl)quinoline-8-boronic acid (32). To a flask charged with **72a** (0.4246 g, 0.82 mmol), bis(neopentyl glycolato)diboron (0.5272 g, 2.06 mmol), $Pd(dppf)Cl_2$ (0.0404 g, 0.049 mmol) and KOAc (0.4853 mg, 4.94 mmol) was added anhydrous DMSO (20 mL). The mixture was stirred at 80 ℃ for 12 hr. The mixture was poured into ice water (10 mL) and extracted with EtOAc (20 mL). The organic layer was washed with

DI water (4 × 10 mL) and brine (10 mL), and then dried over $MgSO_4$ and concentrated to give a grey solid. Recrystallization in MeOH/DCM yielded a grey solid (0.197 g, 54%). ^{1}H NMR (300 MHz, CD_3OD) δ 8.44-8.38 (2H, m), 8.21 (1H, d, *J* = 6.6 Hz), 7.98 (1H, d, *J* = 8.1 Hz), 7.71 (2H, m), 7.62-7.39 (3H, m), 7.30 (1H, s, broad), (5.70, 5.58) (2H, rotamer), (3.34, 3.22) (3H, rotamer); EIMS, *m/z* 445, M+1.

2-({[1-(3-boronic acid-benzyl)-1H-[1,2,3]triazole-4-carbonyl]methylamino}-methyl)quinoline-8-boronic acid (33). To a flask charged with **72b** (0.2396 g, 0.465 mmol), bis(neopentyl glycolato)diboron (0.2975 g, 1.16 mmol), $Pd(dppf)Cl_2$ (0.0256 g, 0.0313 mmol) and KOAc (0.2739 mg, 2.79 mmol) was added anhydrous DMSO (20 mL). The mixture was stirred at 80 ℃ for 12 h. Then the mixture was poured into ice water (10 mL) and extracted with EtOAc (20 mL). The organic layer was washed with DI water (4 x 10 mL) and brine (10 mL), and then dried over $MgSO_4$ and concentrated to give a grey solid. Recrystallization in MeOH/DCM yielded a grey solid (0.125 g, 60%). ^{1}H NMR (300 MHz, CD_3OD) δ 8.69-8.45 (2H, m), 8.19 (1H, d, *J* = 6.0 Hz), 8.06 (1H, d, *J* = 8.0 Hz), 7.79 (2H, m), 7.58 (3H, m), 7.19 (1H, d, *J* = 8.0 Hz), (5.64, 5.50) (2H, rotamer), (3.18, 3.08) (3H, rotamer); EIMS, *m/z* 445. M+1.

8-bromoquinoline-2-carbaldehyde (58) To a solution of **38** (0.431 g, 1.94 mmol) in dioxane (10 mL) was added selenuim dioxide (10 mL). The mixture was stirred under reflux for 6 h and then poured into DI water (10 mL). The mixture was extracted with EtOAc (40 mL) and the organic layer was washed with water (2 × 10 mL) and brine

(5 mL). The solution was dried over $MgSO_4$ and concentrated to give a dark oil. Purification by chromatography (Hexanes/DCM, 50:50) yielded a yellow solid (0.3930 g, 86%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.316 (1H, d, *J* = 0.6 Hz), 8.34 (1H, d, *J* = 8.1 Hz), 8.17 (1H, dd, *J* = 7.5, 1.2 Hz), 8.1 (1H, d, *J* = 8.4 Hz), 7.89 (1H, dd, *J* = 8.1, 1.2 Hz), 7.55 (1H, dd, *J* = 8.1, 7.5 Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ 193.4, 153.1, 145.1, 138.1, 134.3, 131.4, 129.5, 127.7, 126.2, 118.1; EIMS, *m/z* 235/237 (M/M+2). Anal Calcd for $C_{10}H_6BrNO$: C, 50.88; H, 2.56; N, 5.93. Found: C, 50.69; H, 2.33; N, 5.57.

acetic acid 1-(8-bromoquinolin-2-yl)-2,2,2-trichloroethyl ester (59) To a flask charged with **58** (0.9990 g, 4.23 mmol), tricholoroacetic acid (1.3829 g, 8.46 mmol) and sodium trichloroacetate (1.5690 g, 8.46 mmol) was added DMF (15 mL) at 0 ℃ under N_2 atmosphere. After stirring at 0 ℃ for 8 h, acetic anhydride (0.6 mL, 8.45 mmol) and DMAP (0.1034 g, 0.85 mmol) was added into the reaction mixture. The solution was stirred for another 30 min and then quenched with ice-water (10 mL). The mixture was extracted with EtOAc (30 mL), and the organic layer was washed with DI water (3 × 10 mL) and brine (10 mL). The solution was dried over $MgSO_4$ and concentrated to give a yellow solid. Purification by chromatography (hexanes/DCM, 60:40) yielded a yellow solid (1.5976 g, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.21 (1H, d, *J* = 8.4 Hz), 8.09 (1H, d, *J* = 7.5 Hz), 7.81 (1H, d, *J* = 8.4 Hz), 7.44 (1H, t, *J* = 8.4 Hz), 7.26 (1H, s), 6.74 (1H, s), 2.30 (3H, s); ^{13}C NMR (300 MHz, $CDCl_3$) δ 168.7, 153.6, 144.4, 136.9, 133.6, 129.2, 127.9, 127.3, 125.8, 121.4, 97.8,

83.4, 20.9; EIMS, *m/z* 395/397/399 (M/M+2/M+4). Anal Calcd for $C_{13}H_9BrCl_3NO_2$: C, 39.28; H, 2.28; N, 3.52. Found: C, 39.35; H, 2.15; N, 3.06.

8-bromo-2-(2,2-dichlorovinyl)quinoline (60) To a solution of **59** (1.008 g, 2.54 mmol) in anhydrous THF (10 mL) was added a solution of samarium iodide in THF (0.1M, 76.1 mL) under Ar atmosphere. The solution was stirred for 30 min and then quenched with HCl (0.1 N, 5 mL). The reaction mixture was extracted with ether (15 mL), and the organic layer was washed with DI water (2 × 10 mL), dried over $MgSO_4$ and concentrated to give a yellow oil. Purification by chromatography (hexanes/DCM, 60:40) yielded a clear oil (0.396 g, 52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (1H, d, *J* = 8.8 Hz), 8.05 (1H, Dd, *J* = 7.2, 1.2 Hz), 7.83 (1H, d, *J* = 8.8 Hz), 7.76 (1H, dd, *J* = 8.4, 1.2 Hz), 7.39 (1H, t, *J* = 7.6 Hz), 5.30(1H, s); ^{13}C NMR (400 MHz, $CDCl_3$) δ 153.4, 145.0, 136.9, 133.6, 129.2, 128.4, 127.4, 127.3, 127.0, 125.1, 122.0; EIMS, *m/z* 301/303/305 (M/M+2/M+4); Anal Calcd for $C_{11}H_6BrCl_2N$: C, 43.61; H, 2.00; N, 4.62. Found: C, 43.88; H, 2.09; N, 4.17.

8-bromo-2-ethynylquinoline (61) To a solution of **60** (0.396 g, 1.31 mmol) in THF (5 mL) was added a solution of methyllithium in ether (1.0 M, 1.64 mL) at -78 ℃. The mixture was slowly warmed up to room temperature for about 30 min and then quenched with water (5 mL) followed by extraction with ether (10 mL). The organic solution was dried over $MgSO_4$ and concentrated to give a dark oil. Purification by chromatography (hexanes/DCM, 70:30) yielded a light yellow solid (0.216 g, 71%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 (1H, d, *J* = 8.4 Hz), 8.08 (1H, dd, *J* = 7.5, 1.2 Hz), 7.78 (1H, dd, *J* = 8.1, 1.2 Hz), 7.60 (1H, d, *J* = 8.4), 7.41 (1H, dd, *J* = 8.1, 7.2 Hz), 3.30 (1H, s); ^{13}C NMR (300 MHz, $CDCl_3$) δ 145.8, 143.9, 137.1, 134.1, 128.9, 128.0, 127.7, 125.5, 125.0, 83.6, 79.0; EIMS, *m/z* 231/2337 (M/M+2); Anal Calcd for $C_{11}H_6BrN$: C, 56.93; H, 2.61; N, 6.04. Found: C, 57.64; H, 2.72; N, 5.60.

8-bromo-2-[1-(3-bromobenzyl)-1H-[1,2,3]triazol-4-yl]-quinoline (62a) To a suspension of **61** (0.210 g, 0.90 mmol) and **55a** (0.1919 g, 0.90 mmol) in H_2O/t-BuOH (2 mL, 1:1) was added a solution of sodium ascorbate in water (0.1 M, 0.9 mL) and a solution of $CuSO_4$ in water (0.1 M, 0.1 mL). The mixture was stirred vigorously for 10 h, and then extracted with DCM (10 mL). The extract was washed with DI water (10 mL) and brine (5 mL), dried over $MgSO_4$ and concentrated to give a yellow solid. Purification by chromatography (MeOH/DCM, 5:95) yielded a yellow solid (0.3665 g, 91%). ^{1}H NMR (300 MHz $CDCl_3$) δ 8.45 (2H, d, *J* = 9 Hz), 8.28 (1H, d, *J* = 8.7 Hz), 8.07 (1H, dd, *J* = 7.5, 1.2 Hz), 7.83 (1H, dd, *J* = 8.1, 1.2 Hz), 7.55 (2H, m), 7.40 (1H, t, *J* = 7.5 Hz), 7.31 (2H, m), 5.65 (2H, s); ^{13}C NMR (300 MHz $CDCl_3$) δ 151.1, 149.1, 145.0, 137.5, 136.7, 133.5, 132.1, 131.1, 130.7, 129.1, 127.7, 126.8, 126.6, 124.6, 123.5, 123.2, 119.3, 53.6; EIMS, *m/z* 442/444/446 (M/M+2/M+4).

8-bromo-2-[1-(4-bromobenzyl)-1H-[1,2,3]triazol-4-yl]quinoline (62b) To a suspension of **61** (0.384 g, 1.65 mmol) and **55b** (0.4211 g, 1.99 mmol) in

H_2O/t-BuOH (4 mL, 1:1) was added a solution of sodium ascorbate in water (0.1 M, 1.5 mL) and a solution of $CuSO_4$ in water (0.1 M, 0.17 mL). The mixture was stirred vigorously for 10 h, and then extracted with DCM (20 mL). The extract was washed with DI water (10 mL) and brine (5 mL), dried over $MgSO_4$ and concentrated to give a yellow solid. Purification by chromatography (MeOH/DCM, 5:95) yielded a yellow solid (0.6801 g, 93%). ^{1}H NMR (300 MHz $CDCl_3$) δ8.42 (2H, m), 8.26 (1H, d, *J* = 8.0 Hz), 8.05 (1H, d, *J* = 7.6 Hz), 7.81 (1H, d, *J* = 8.0 Hz), 7.56 (2H, d, *J* = 8.4 Hz), 7.39 (1H, t, *J* = 8.4 Hz), 7.26 (2H, d, *J* = 8.4 Hz), 5.62 (2H, s); ^{13}C NMR (300 MHz CDCl3) δ 151.3, 149.2, 145.1, 137.8, 135.9, 133.7, 132.3, 131.3, 131.0, 129.2, 127.9, 127.1, 126.9, 124.9, 123.7, 123.5, 119.6, 53.9; EIMS, *m/z* 442/444/446 (M/M+2/M+4)

2-[1-(3-boronic acid-benzyl)-1H-[1,2,3]triazol-4-yl]-quinoline-8-boronic acid (34). To a flask charged with **62a** (0.1602 g, 0.36 mmol), bis(neopentyl glycolato)diboron (0.2308 g, 0.90 mmol), Pd(dppf)Cl_2 (0.0183 g, 0.022 mmol) and KOAc (0.2129 mg, 2.17 mmol) was added anhydrous DMSO (20 mL). The mixture was stirred at 80 □ for 12 h. Then the mixture was poured into ice water (10 mL) and extracted with EtOAc (20 mL). The organic layer was washed with DI water (4 x 10 mL) and brine (10 mL), and then dried over $MgSO_4$ and concentrated to give a grey solid. Purification by chromatography (MeOH/DCM, 10:90) yielded a grey solid (0.125 g, 63%). ^{1}H NMR (300 MHz CD_3OD) δ 8.29 (1H, S), 8.06 (3H, m), 7.74 (3H, m), 7.40 (3H, m), 5.64 (2H, s), 3.72 (4H, s), 0.96 (6H, s); ^{13}C NMR (400 MHz CD_3OD) δ 151.1, 148.1, 147.4, 138.4, 137.6, 134.1, 133.9, 133.4, 130.2, 128.1, 127.1, 126.3,

123.0, 117.7, 72.0, 54.3, 31.5, 29.8, 21.0; EIMS, *m/z* 442, M.

2-[1-(4-boronic acid-benzyl)-1H-[1,2,3]triazol-4-yl]-quinoline-8-boronic acid (35). To a flask charged with **62b** (0.2000 g, 0.45 mmol), bis(neopentyl glycolato)diboron (0.2880 g, 1.13 mmol), $Pd(dppf)Cl_2$ (0.021 g, 0.026 mmol) and KOAc (0.2901 mg, 2.96 mmol) was added anhydrous DMSO (20 mL). The mixture was stirred at 80 □ for 12 h. Then the mixture was poured into ice water (10 mL) and extracted with EtOAc (20 mL). The organic layer was washed with DI water (4 × 10 mL) and brine (10 mL), and then dried over $MgSO_4$ and concentrated to give a grey solid. Purification by chromatography (MeOH/DCM, 10:90) yielded a grey solid (0.125 g, 63%). ^{1}H NMR (400 MHz CD_3OD) δ 8.30 (4H, m), 7.93 (1H, dd, *J* = 8.0, 1.6 Hz), 7.84 (1H, d, *J* = 8.0 Hz), 7.59 (1H, dd, *J* = 8.0, 6.8 Hz), 7.35 (3H, m), 5.69 (2H, s), 3.78 (4H, s), 1.03 (6H, s); ^{13}C NMR (400 MHz CD_3OD) δ 151.7, 148.7, 148.2, 138.8, 138.2, 136.8, 134.8, 130.7, 127.5, 127.2, 126.8, 122.9, 118.4, 72.5, 70.4, 54.7, 32.0, 21.9; EIMS, *m/z* 442, M.

2-{[(4-boronic acid-benzoyl)-methyl-amino]-methyl}-quinoline-8-boronic acid (36). To a flask charged with **43** (0.2799 g, 0.94 mmol) was added added $SOCl_2$ (4 mL) and DMF (1 drop). The mixture was stirred under reflux for 2 h and then cooled down to room temperature. All liquid was removed under vacuum to give a white solid. The residue was then dissolved in dry THF (5 mL) (**solution A**).

To a solution of **42** (0.3287 g, 0.94 mmol) in DCM (10 mL) was added TFA (2mL).

The solution was stirred for 6 h and concentrated under vacuum for another 4 h. The residue was then dissolved in dry THF (5 mL) and added into solution A at 0 ℃ followed by stirring at room temperature for 2 h. A brown solid precipitated out. The solid was collected and dried in vacuum. Purification by chromatography (MeOH/DCM, 10:90) yielded a yellow solid (0.140 g, 30%). ^{1}H NMR (300 MHz CD_3OD) δ 8.48 (1H, m), 8.31 (1H, d, J = 6.0 Hz), 8.07 (1H, s, broad), 7.89 (2H, s, broad), 7.64 (3H, m), 7.43 (1H, broad), 5.16 (2H, s), (3.23, 3.18) (3H, rotamer); ^{13}C NMR (300 MHz CD_3OD) δ 179.5, 156.0, 138.5, 136.9, 133.9, 130.7, 127.4, 126.9, 126.2, 125.4, 124.3, 120.0.

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