A treatise concerning the mechanisms of boronate ester formation and fluorescent turn-on in *ortho*-aminomethylphenylboronic acids†

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Abstract

ortho-Aminomethylphenylboronic acids are used in receptors for carbohydrates and various other vicinal diol-containing compounds. The presence of the *o*-aminomethyl group enhances the affinity towards diols at neutral pH, and the manner in which this group plays this role has been a topic of debate. Further, the aminomethyl group is believed to be involved in the turn-on of the emission properties of appended fluorophores upon diol binding. In this treatise, a uniform picture emerges for the role of this group; it primarily acts as an electron-withdrawing group that lowers the pK_a of the neighboring boronic acid thereby facilitating diol binding at neutral pH. The amine appears to play no role in the modulation of the fluorescence of appended fluorophores in the protic solvent-inserted form of the boronic acid/boronate ester. Instead, fluorescence turn-on can be consistently tied to vibrational-coupled excited-state relaxation (a loose-bolt effect). Overall, this treatise unifies and discusses the existing data as of 2019 whilst also highlighting why *o*-aminomethyl groups are so widely used, and the role they play in carbohydrate sensing using phenylboronic acids.

Physical organic chemistry is a discipline in which experimental and theoretical approaches are used to delineate reaction mechanisms, uncovering Mother Nature's chemical steps, physical phenomenon, and reactivity.[1] Many postulates, and sometimes heated debates, have been investigated and settled using the tools of this discipline. For example, the classic debate surrounding the norbornyl carbocation has only recently been settled with a 40 K temperature crystal structure.[2] Another is the controversy concerning the interpretation of data concerning a self-replicating system that occurred between Rebek and Menger, which was settled with extensive kinetic analysis by Reinhoudt.[3] As a last example, the theoretical and experimental examination of low-barrier hydrogen bonds has far-reaching implications from supramolecular chemistry to enzyme catalysis.[4,5] In the realm of chemosensors, the possibilities for how *o*-aminomethylphenylboronic acid-based receptors turn-on emission, and whether their structures possess N−B bonds when binding vicinal diols, has seen a variety of postulates. Once again, the studies described below demonstrate how exploration using physical organic chemistry ultimately results in a clear picture of mechanisms.

Boronic acids, both alkyl and aryl, have been accessible for around 150 years, since 1860.[6] The interaction of boronic acids with diols or anions has been intensively investigated,[6-10] and boronic acids have been exploited in a range of applications as diverse as NMR shift reagents,[11,12] functional polymers,[13] molecular self-assembled materials,[14,15] tissue histological work,[16] and fluorescence imaging of carbohydrates.[17] Further, they are among a small set of reactions that involve dynamic covalent bonding.[7] In addition, sensors for reactive oxygen and reactive nitrogen species have been developed based on oxidative removal of the boronic acid group.[18-21]

Significantly, boronic acids are extensively utilized in synthetic receptors for the molecular recognition and sensing of carbohydrates, as well as various other vicinal diol-containing compounds, and various anions.[22-37] The detection of carbohydrates is commonly pursued in disease diagnostics, such as diabetes.[38-43] Carbohydrates present a challenge for molecular recognition due to their high solvation energies in water[44-46] and their great structural diversity.[47] Importantly, boronic acids are able to overcome this solvation limitation because the binding event does not replace the solvent as with non-covalent binding, but rather interchanges covalent bonds. Thus, in the field of host-guest chemistry, boronate ester formation is recognized as a very unique reaction (Fig. 1a).[48-54] Importantly, the incorporation of a boronic acid into almost any scaffold reliably imparts high to low mM binding affinities to vicinal diols, catechols, and α-hydroxycarboxylate-containing guests in competitive media without further structural manipulation. However, phenylboronic acid itself is limited in its utility because it only shows significant binding a few pH units above physiological pH.[55-57] This is a structural feature that is often overlooked.

Early studies by Wulff,[57,58] as well as Lorand,[59] delineated substituent effects for phenylboronic acids binding various diol species. The most significant was placement of an aminomethyl group on the *ortho* position to the boronic acid, which is a structural motif that led to significantly improved binding at neutral pH.[60] Since the inception of this exploration, many groups have incorporated *o*-aminomethylphenylboronic acids into a variety of chemical receptors and/or sensors to improve affinity, tune selectivity, change quantum yield, and/or modulate the wavelength of emission.[13,43,61-68] By exploiting *o*-aminomethylphenylboronic acids appended with different fluorophores, scientists have created a series of turn-on fluorescence probes for the sensing and imaging of carbohydrate and glycoproteins on cell surfaces.[17,63] Most significant for practical applications, a modular sensory system of this type has been developed

by Glysure Ltd into a fully functional fiber optic sensor for the continuous monitoring of glucose with patients in intensive care units (ICU).[69]

Summarizing the debates

The recognition of saccharides via boronic acids often relies on an interaction between a Lewis acidic boronic acid and a proximal amine. However, the true nature of the nitrogen−boron interaction has been debated (especially in an aqueous environment). Different mechanisms for the emission turn-on of *o*-aminomethylphenylboronic acids appended with fluorophores in response to saccharide binding in aqueous media have been proposed (Table 1). Shinkai and James originally postulated a photo-induced electron transfer (PET) mechanism for saccharide sensing based upon a change of N−B bonding strength upon sugar binding.[64,65] However, Wang put forth a pK_a switch theory as an alternative mechanism to the N−B dative bond theory involving the breaking of an N−B bond and insertion of solvent upon sugar binding.[66,67] With the question ongoing, Anslyn and Larkin explored an unanticipated aggregation and disaggregation mechanism.[68] However, none of the hypotheses was consistent with all the data for *o*-aminomethylphenylboronic acid-based sensors. Thus, in 2018, Anslyn and James revealed that modulating the $-B(OH)_2$-induced internal conversion (an example of a 'loose bolt' effect) explains how potentially all *o*-aminomethylphenylboronic acid-based fluorescence sensors signal the presence of sugars.[70]

Thus, the goal of this treatise is to put forth a unifying theory that explains all the data and addresses several issues: the structural manner in which the boron and nitrogen interact, the role of the *o*-aminomethyl group in both thermodynamics and kinetics, assignment of pK_a values, the mechanism of boronate ester formation, and the manner in which an *o*-aminomethyl group influences the fluorescence. In order to do so, we give a historical synopsis of the use of an aminomethyl group in phenylboronic acids; the mechanistic postulates are compared side by side; structural studies and their mechanistic implications are reanalyzed, various computational studies are highlighted, and finally the most up-to-date understanding of the fluorescence response is discussed.

Boronate ester formation and N−B bonding

In a series of landmark studies by Shinkai and James, anthracene-based receptors such as **1** were used to signal the presence of various carbohydrates in neutral aqueous media *via* a fluorescence turn-on response (Fig. 1b).[64,65,71] It was clear that the *o*-aminomethyl group in **1** improved the thermodynamics of binding at neutral pH, but it was also proposed to be involved in modulating the fluorescence of the receptor in the presence and absence of sugars. Their postulate involved a weak dative bond to the boron atom from the amine nitrogen, resulting in near-sp^3 hybridization of the boron atom at neutral pH (dashed N−B line shown in **1**).[72] In contrast, the boron in phenylboronic acid, $PhB(OH)_2$, is sp^2 at neutral pH (a fact that has been established using ^{11}B NMR).[73] Upon boronate ester formation in either structure **1** or phenylboronic acid, a five-membered ring involving the boron atom is formed. Shinkai and James reasoned this ring would be more strained if the boron atom was sp^2–hybridized than if it was sp^3-hybridized.[74] Hence, the N−B bond was postulated to stabilize the boronate adduct because pyramidalization of the boron would be induced by the Lewis acid-base coordination with the neighboring nitrogen atom. In essence, the two interactions reinforce each other; N−B bonds involving a pyramidalized boron atom are

stronger, while boronate ester formation is stabilized by N−B bonding.[23,74] For these reasons, the strength of the N−B bond in the ester was proposed to increase relative to the acid (solid N−B line in Fig. 1b).

The postulated modulation of the N−B bond strength was derived because it nicely explained the turn-on of fluorescence found for structure **1** and its analogs.[64,65,71] Photo-induced electron transfer (PET)[75,76] from the nitrogen atom's lone-pair of electrons in **1** was proposed to quench the emission of anthracene because this lone pair was not strongly coordinated to boron. However, upon boronate ester formation, the energy of the nitrogen donor's electron pair drops due to stronger coordination, therefore lowering the extent of PET. Hence, PET quenching is decreased upon ester formation, which results in a turn-on of fluorescence (Fig. 1c). This was a very logical postulate in the 1990s because other pioneers in the chemosensing field, Czarnik[77] and DeSilva,[78] were reporting the use of sensors in which nitrogen lone pairs were involved in PET quenching. Irrespective of the actual role of the *o*-aminomethyl group, the discovery of the general class of compounds analogous to compound **1**, and the associated proposed mechanism, remains a landmark study in the history of chemosensors.[79,80]

An alternative explanation

Wang put forth an alternative mechanism to the N−B dative bond theory.[66,67] He noted that crystal structures of boronic acids actually have shorter N−B bond lengths than those in the corresponding boronate esters, meaning that the bond is stronger in the acid than the ester (this is the opposite of what is shown in Fig. 1b). He and Franzen used density functional theory (DFT) for computational modeling and found that the relative changes in the N−B bond strength upon ester formation were not significant enough to produce the large changes in PET quenching necessary to explain the behavior of **1** upon binding sugars. In this paper, they noted that protonation of the amine was a more likely explanation for the arresting of the PET quenching found with sugars, citing the fact that a N−H bond is far stronger than a N−B bond. In a subsequent analysis, Wang proposed what has come to be known as the 'pK_a switch' or 'hydrolysis/solvolysis' mechanism (Fig. 1d).[67] The notion is that N−B bonding exists in the boronic acid, while the boronate ester chelates a solvent between the boron and the amine (called 'hydrolysis'), thereby protonating the amine. For the purposes of this treatise, we note that the product in Fig. 1d has a solvent molecule inserted between the amine nitrogen and boron, which has turned out to be an important structural postulate first introduced by Wang.

Wang also pointed out other issues that were inconsistent with the N−B bond strength modulation postulate. First, the emission intensity of **1** is independent of the pK_a of the boronate ester,[81] even though the pK_a varies as a function of which sugar is used. It is well documented[36,59] with phenylboronic acid that lowering of the pK_a of the boronic acid depends upon which sugar is added to the solution. Since the strength of the N−B bond should modulate the extent of PET, and because each sugar has a different electron-withdrawing ability, the electrophilicity of different boronate esters should vary, and therefore, the fluorescence of **1** should also vary with different sugars. However, the earlier work by James and Cooper showed that the change in fluorescence is not dependent upon the sugar,[81] and Wang reconfirmed this experimentally with four different carbohydrates.[67] Wang further noted that sugars which form trivalent binding of the boron should not modulate the fluorescence if a N−B bond occurs, since the third valency would break the N−B bond, while the

hydrolysis mechanism would still show a fluorescence change, because the third valency would simply take the place of the hydroxyl group in the boronate ester. Experimentally, trivalent sugars give very large fluorescence changes.[67]

Evidence for alternative perspectives

pH titrations: A large amount of the experimental data revealing emission turn-on in the presence of sugars for boronic acids such as **1** comes from pH titrations (as seen in Fig. 1c). These titrations can be rationalized with either the N−B bond or hydrolysis mechanisms, and thus these experiments cannot differentiate between these mechanisms.[66,67] There are two types of pH titrations often performed: fluorescent and ^{11}B NMR. In fluorescence titrations of **1**, upon changing the pH from low to high, large emission decreases between pHs of 6 to 7 are observed, and another smaller decrease above pH 11 occurs. In contrast, in the presence of a sugar, the first fluorescence decrease is minor, while a large decrease occurs at the higher pH.[65]

Let us examine how to interpret the pH titrations in light of the two mechanistic postulates, and which chemical species the pK_a steps interconvert. First, with the notion of a N−B bond formation in mind, it is logical to assign pK_{a1} (6–7) to the deprotonation of the ammonium ion, which is lowered in comparison to the pK_a of a standard alkyl ammonium (9–11) due to the resulting coordination with boron (Fig. 2a). The lowering of the pK_a is analogous to that observed for ammonium ions in the presence of metals due to metal-amine coordination.[82] Therefore, pK_{a2} would correspond to hydroxylation of the boron with subsequent breaking of the N−B bond. This pK_a is in the standard range of that for boronic acids (9-10). Note that hydroxylation of a boronic acid is accompanied by the release of a proton (not shown), and hence is a Brønsted acid dissociation reaction with an associated pK_a value (Fig. 2a).

Continuing with the postulate of N−B bonding, deprotonation of the ammonium group leads to liberation of the nitrogen lone-pair (pK_{a1}), which is then weakly coordinated to the boron atom (Fig. 2b), and would therefore be predicted to quench the anthracene emission. Hydroxylation of the boron in the boronic acid only slightly increases quenching (pK_{a2}). On the other hand, for this theory to operate, addition of a sugar leads to boronate ester formation (Fig. 2b) and deprotonation of the ammonium (pK_{a1}) replaces a N-H bond with a strong dative N−B bond, essentially not affecting the ability of the nitrogen to be involved in PET (intermediate pH range of Fig. 1c). Hydroxylation of the boron in the boronate ester, however, does free up the amine's lone-pair leading to quenching of the anthracene emission (high pH range of Fig. 1c). Figs 2a, 2b show equilibrium arrows connecting individual species, while the red arrows depict the major structures formed when raising the pH in the presence of a sugar. Note that the structures on the upper right and lower left are presumed not to be present to a significant extent under neutral pH conditions.

Let us now examine the pH titrations with the assumption that the pK_a switch mechanism is operable (Figs 2c, 2d). At low pH the amine is protonated, and in the presence of a diol there is little binding. As the pH is raised in the absence of a sugar, pK_{a1} would correspond to deprotonation of the amine to make a N−B bond, while pK_{a2} is hydroxylation of the boron. This is exactly the same as Fig. 2a. However, in the presence of a sugar, the proposal is very different. As the pH is raised, the sugar binds and the N−B bond is hydrolyzed. Logically, it must follow that the second pK_a would be deprotonation of the ammonium ion, with a slightly raised value relative to a normal ammonium because of its proximity to the negative boron center. Note that in the absence of the diol, the first and second pK_as

are deprotonation of the ammonium ion and hydroxylation of the N−B bond, respectively, while in the presence of a diol it is reversed; the first and second pK_as are N−B bond hydroxylation and ammonium ion deprotonation, respectively. This mechanistic postulate is therefore called a 'pK_a switch'.

One last piece of evidence that Wang used to support the hydrolysis mechanism (Figs 2c, 2d) was stated as, "there are ample literature precedents proving that the first pK_a is the deprotonation of the [ammonium] with concomitant formation of a N−B bond". This literature precedent was work done by Anslyn.[83] These experiments were the pH titrations of compounds **2** and **3** (Fig. 3), which were followed by ^{11}B NMR. ^{11}B NMR spectroscopy is very sensitive to the hybridization of the boron atom.[73] The goal of the Anslyn experiments was to determine if the pK_as of secondary and tertiary amines proximal to the boronic acids were similar or not, due to the possibility that with a secondary amine deprotonation could lead to a standard covalent N−B bond rather than a dative N−B bond interaction.[83] He found nearly identical pK_a values for *o*-aminomethylphenylboronic acids involving tertiary and secondary amines, which thereby rules out a standard covalent N−B bond.

To explore if the pK_as will switch between boronic acids and boronate esters, Anslyn examined the ^{11}B NMR spectra of **4** in the presence of saturating amounts of different diols, and found at most one-half of a pK_a unit difference between **4** and boronate esters formed from various diols for both the first and second pK_a values.[84] Further, he found that the pK_a of **4** does not change significantly with different sugars. These observations suggest that the acid/base reactions of the *o*-aminomethylphenylboronate esters are not significantly different from those of their corresponding boronic acids. Such results are different from literature publications for simple phenylboronic acids,[85] suggesting a role of the neighboring amino group in affecting the pK_a of the boron species through electrostatic effect due to the 1,5-relationship.

With the clarity of hindsight, it is obvious that there is a problem with Anslyn's interpretation of his ^{11}B NMR experiments.[83] The data really only reveal whether the boron atom is trigonal planar versus tetrahedral, and therefore the experiments cannot distinguish N−B bonds from other structures containing an sp^3-hybridized boron species. Anslyn had interpreted the deprotonation associated with the first pK_a to result in N−B bond formation upon pyramidilization of the boron because N−B bonding was the prevailing picture.[83] But there is now a different interpretation that involves solvent insertion in both the boronic acids and boronate esters, as discussed immediately below.

Structural studies and ^{11}B-NMR studies: To distinguish between the N−B bond and pK_a switch mechanisms, Anslyn's group performed a series of X-ray crystallographic and ^{11}B NMR studies. The ^{11}B NMR spectra of titrations of **4** (Fig. 3) with catechol, hydrobenzoin, and α-hydroxyisobutyric acid were examined.[84] The chemical shifts of the products formed during titration were correlated with those found for purified boronate esters for which crystal structures were obtained. A trigonal planar boron has a resonance in the range of 28-30 ppm, while signals for N−B bonds appear around 14-15 ppm, and solvent insertion resonances are found between 8 and 10 ppm. The conclusion was that solvent insertion is observable in protic media for boronic acid **4** and all the boronate esters created. There was one exception — a small extent of N−B bond formation (estimated as 5%) was found when using catechol, meaning that N−B bonds and solvent insertion can co-exist in equilibrium.

Computational results from Larkin and James led to a similar conclusion.[86] They found that the solvent-inserted species are lower in energy than N−B dative bonded species. Importantly, all evidence points to solvent insertion being the dominant species in protic media for both boronic acids and boronate esters. This being the case, neither the N−B bond mechanism nor the pK_a switch mechanism can be operative. Thus, while logically sound, the postulates illustrated in Figs 2a-d were shown to be incorrect in protic media.

Further support for solvent insertion

Solvent insertion is also supported by crystal structures of boronic acids with sugars that can act as trivalent ligands, supplying three oxygen atoms to the boron.[86,87] In these cases, the inserted solvent is replaced by an OH group from the sugar itself. This is well accepted for fructose,[86] forming structures such as **5** (Fig. 3). In fact, Norrild found that glucose rearranges to its furanose form when binding with a bis-boronic acid receptor **6** (Fig. 3), as revealed by a series of coupling constant measurements.[87] The postulate is that the driving force is to exploit the energetically favorable trivalent interaction with glucose. The most recent computational studies by Larkin confirm the lower energy of trivalent sugar geometries.[88]

The role of the *ortho*-aminomethyl group

In the assignment of the pK_a values As discussed above, the first pK_a of *o*-aminomethylphenylboronic acids and esters is between 5 and 7 and leads directly to solvent insertion. Thus, we must now conclude that the first pK_a corresponds to hydroxylation of the boronic acid/ester giving directly a solvent-inserted structure (Fig. 2e, 2f, *vida infra* why this is called "Loose Bolt"). Hence, the proximity of the positively charged ammonium group depresses the pK_a of the boronic acid to around 5–7 from the common values of 9–10. This occurs irrespective of whether the boron atom is part of a boronic acid or a boronate ester, because the first pK_a values do not significantly vary between these two species (discussed above). With this conclusion in mind, the second pK_a value of *o*-aminomethylphenylboronic acids or boronate esters must correspond to the deprotonation of the ammonium ion, occurring at pH values of around 11–12. This second pK_a is raised 1–2 units above that of normal ammonium groups due to the proximity of the negatively charged boronate group. Thus, the first and second pK_as are now clearly assigned.

In the mechanism of boronate ester formation If the ammonium group perturbs the pK_as of the boronic acids and boronate esters, it is logical that it could also play a key role in the mechanism of boronate ester formation. For example, consider what steps need to be involved when starting with a solvent-inserted boronic acid and transitioning to a solvent-inserted boronate ester (Fig. 4a). The inserted solvent first needs to be expelled, then replaced by an alcohol of the diol or saccharide, followed by bond rotations and further stepwise replacements of inserted solvent(s), leading to a fully bound guest. Because the first replacement of the inserted solvent by the guest is intermolecular, it is likely slow relative to the subsequent steps that are intramolecular and chelate the diol or saccharide to the boron atom.

The release of an inserted solvent would lead to species **7** in Fig. 4. This could happen in two steps: ammonium deprotonation and loss of hydroxide/alkoxide from the boron center, in any order. In either case, this would require loss of a very poor leaving group at neutral pH. Alternatively, the solvent could be lost in one step by simple decomplexation from the boron (see the 'general acid-catalysis' pathway). This would involve forming an *o*-aminomethylphenylboronic acid in a high-energy state (**7**) because it does not possess the preferred boronic acid and amine protonation states at the operating pH. Interestingly, the question of stepwise or single-step loss of the inserted solvent is dependent on the ionization state of the inserted solvent. If the solvent is fully deprotonated when inserted, thereby forming a zwitterionic boronate anion and ammonium cation, its single step departure as a neutral species requires a proton transfer to occur simultaneously with departure (as shown in the pathway that is shown with red arrows in Fig. 4a). If the solvent is not ionized when inserted, it can simply depart with no proton transfer (Fig. 4b). The former possibility, concerted protonation and leaving group departure, is an example of intramolecular general acid-catalysis, and can be analyzed by classic experiments such as isotope effects.

Any of the alternatives for loss of solvent all generate high-energy intermediates with which the diol-containing guest subsequently reacts. This means there is a step that forms an intermediate prior to reaction with the guest, which is a mechanism that should show saturation kinetics, as with an S_N1 mechanism.[89] Alternatively, a mechanism in which the solvent-inserted *o*-aminomethylphenylboronic acid reacts directly with the guest would consistently show second-order kinetics (as with an S_N2 reaction).

Anslyn studied the kinetics of the reaction of **1** with fructose, both at low and high fructose concentrations.[80,90] At low concentrations of fructose, the kinetics appeared second-order, and the y-intercept of the kinetic plots revealed ratios of k_1 and k_{-1}. Hence, the reaction appeared analogous to an S_N2 mechanism, except for the fact that there was a non-zero y-intercept, which is indicative of equilibrium kinetics.[90] Yet, at high fructose concentrations saturation kinetics were found (i.e. zero-order in fructose). The kinetic data shows that a mechanism involving a rate-determining step prior to reaction with the guest is operative. It was proposed that this first step is loss of the inserted solvent to generate **7** (Fig. 4a). Such a mechanism is analogous to an S_N1 reaction, in which leaving group departure leads to a reactive intermediate that takes on a nucleophile. However, unlike S_N1 chemistry that loses kinetic dependence at low concentrations of nucleophile, the boronic acid mechanism requires hundreds of equivalents of fructose to reach saturation. This makes perfect sense, given that the reverse step that competes with the first insertion of guest is insertion of a solvent molecule. This competing re-insertion of solvent is analogous to the common ion effect in S_N1 mechanisms,[90] but with boronic acids the 'common ion' is the solvent. By fitting the kinetic data, Anslyn estimated that fructose is around a 1000 times better nucleophile when adding to **7** than the solvent.

The Anslyn group also addressed the issue of whether the inserted solvent is significantly ionized or retains substantial O–H bonding (**8**, Fig. 4b). General acid-catalyzed loss of the solvent involves the movement of a proton, and hence should have an isotope effect, while the other possibilities would have almost no isotope effect. A crystal structure of solvent-inserted species **8** (Fig. 4) with the proper resolution to find the position of the hydrogen between the O and N atoms shows a shorter H–N bond than O–H bond, supporting a significant extent of ionization of the inserted solvent.[1] Consistent with this finding, Anslyn uncovered an isotope effect of 1.42 for the reaction of **1** with fructose.[90] This value is smaller than what may be expected but is clearly a substantial effect. The isotope effect is evidence that the general acid-catalyzed pathway for expulsion of the inserted solvent occurs. It should be noted that if the expulsion is general acid-catalyzed, then the reverse reaction, solvent insertion, would be general base-catalyzed (as noted in Fig. 4a).

In summary, the *o*-aminomethyl group plays the roles of: 1) increasing the thermodynamics of binding diol species at neutral pH by lowering the pK_a of the boronic acids to the physiological range, and 2) speeding up the sugar binding by an intramolecular general acid-catalysis of leaving group departure from the boronic acid.

Aggregation and disaggregation

Having settled the assignment of pK_a values, whether solvent insertion or N−B bonding occurs in protic media, and how the *o*-aminomethyl group affects the thermodynamics and kinetics, the only major remaining issue concerns the role of the *o*-aminomethyl group on the fluorescence modulation upon sugar binding. We have already covered that the original PET postulate is not consistent with structural data. Further, the structural evidence also disagrees with the pK_a switch notion because solvent insertion is dominant for both boronic acids and boronate esters. Therefore — what is the mechanism of the fluorescence turn-on?

To probe the role(s) of the *o*-aminomethyl group in modulating the emission of boronic acid-based sensors that incorporate this group, Anslyn initiated a detailed photophysical study of compound **1**.[69] The original pH titration data from Shinkai and James revealed an emission turn-on in a pH titration with fructose that was quite large in a solution of 2:1 water/methanol with 50 mM NaCl, i.e. around 30-fold.[64] Anslyn found that the emission would gradually turn on with sonication without addition of fructose, or just by sitting in the cuvette with continued irradiation. These surprising results led his group to analyze the emission spectra in more detail. First, they confirmed by ^{11}B-NMR spectroscopy in a water/methanol mixture[69] and X-ray crystallography, that solvent insertion dominates. Second, when increasing the wavelength of the emission spectra of **1** out to 600 nm, a broad structureless emission from 460 to 600 nm was found. Admittedly, this peak could sometimes be quite small and easily missed, or with other preparations, this peak could be very large. Upon irradiation or sonication, this peak diminished even without the addition of a sugar, while the highly structured emission of anthracene would increase at the same time. Upon delving into the literature, it became clear that this emission peak was associated with a known excimer of anthracene.[91-93] Thus, it seemed that **1** was aggregated in the condition used for the original pH titrations (2:1 water/methanol with 50 mM NaCl) (Fig. 5a).

To confirm the presence of an excimer, Anslyn performed the classic[94] analysis of measuring an excitation spectrum at a wavelength that the excimer absorbs, but that the anthracene monomer does not. This gave an excitation spectrum in the region of the anthracene monomer that was also broad and structureless (Fig. 5b). This confirmed that the broad emission at long wavelengths was indeed indicative of an aggregated species of **1**. Apparently, the aggregate breaks up with sonication and/or irradiation, but also upon addition of fructose.

As just stated, the emission turn-on of **1** is complicated by a disaggregation phenomenon, but potentially the emission turn-on was additionally due to some electronic factors resulting from fructose binding. To decipher the extent that disaggregation and fructose binding influence the emission, compound **1** was titrated with fructose in pure methanol. Albeit ^{11}B NMR spectroscopy confirmed the binding of fructose, there was no emission turn-on in this solvent, and no excimer was observed in the absence of sugar. In addition, a

compound lacking a boronic acid (**9**, Fig. 3) turned on fluorescence upon the addition of fructose in nearly an identical manner as did **1** in the NaCl methanol/water solution. This revealed that a vast majority of the emission turn-on was not related to the binding of fructose, but rather a disaggregation phenomenon that occurs as a result of the addition of fructose into the solution. Molecular dynamics from the Larkin group showed that, indeed, fructose causes a solvent effect that breaks up aggregates of **1**, supporting this hypothesis.

So far, different mechanisms for the emission turn-on of *o*-aminomethylphenylboronic acids with appended fluorophores in response to saccharide binding in aqueous media have been postulated, such as photo-induced electron transfer (PET), 'pK_a switch', and disaggregation. However, none of the hypotheses is consistent with all the data for boronic acid-based sensors. For example, there was a curious feature to the Anslyn studies. In the NaCl methanol/water mixture there was consistently an additional 2- to 3-fold increase in fluorescence of **1** upon fructose binding that was never achieved by sonication or irradiation of **1** alone. This meant that of the nearly 30-fold increase, about a 10- to 15-fold increase was due to disaggregation, but the additional 2- to 3-fold increase was, indeed, due to fructose binding.

'Loose bolt' internal conversion

To reveal the mechanism of this additional emission turn-on, the Anslyn and James groups joined forces.[70] They noted that most all *o*-aminomethylphenylboronic acid sensors for sugars typically show an emission turn-on in the range of 2- to 5-fold.[95-100] Thus, they set out to study a series of sensors with this functionality, but using those that are freely soluble in water and methanol (**10**, **11**, and **12**, Fig. 3), thereby removing any complications from aggregation. This study allowed them to focus on the roles of the boronic acid, boronate ester, and *o*-aminomethyl group on the photophysics, revealing the mechanism for the fluorescence turn-on. This study primarily focused on the use of **10**, which James had shown could be used as a sensor for peroxynitrite after binding fructose.[101]

As originally done by Shinkai and James with **1**, the first step was to analyze the pH titration of **10** with and without fructose and assign the structures of the *o*-aminomethylphenylboronic acid and fructose ester thereof in different pH ranges (Fig. 6a). In the pH range between 7 and 10, there was approximately a 3.0-fold (within 2.0 ~ 5.0-fold range) fluorescence turn-on response in the presence of fructose, which confirms fructose binding. As the first pK_a of the boronic acid (i.e., hydroxylation of the boron) is approached by raising the pH, an anionic boronate (R-B$(OH_3)^-$) is formed without fructose (recall Fig. 2d). In the presence of fructose, a **10**-fructose complex is formed within the range of pHs 6–10. At pHs above 10.0, the fluorescence of both **10** and the **10**-fructose complex drop, owing to photoinduced electron transfer (PET) from an amine lone-pair through deprotonation of the ammonium (pK_{a2}). ^{11}B NMR spectroscopy revealed that compound **10** was solvent-inserted in protic media with and without fructose binding. An analogous compound to **10** without the boronic acid group did not show any response towards fructose in aqueous conditions. Further, no excimer was observed. This data supported the design criteria that there was no aggregation of this boronic acid species.

To further probe the emission turn-on with water-soluble entities, compounds **11** and **12** were prepared. Compound **11** is meant to mimic all the features of **1** except with a solubilizing ammonium group, while **12** carries a pyrene fluorophore. Compounds **10**, **11**, and **12** showed a 2- to 5-fold turn-on of emission in water upon binding fructose, but no turn-on of emission when binding fructose in methanol.

Yet ^{11}B NMR spectroscopy showed fructose was binding. In other words, upon converting –$B(OMe)_2$ groups to esters with a sugar, there is no emission response — but upon replacing –$B(OH)_2$ groups with esters of either methanol or a sugar, the emission does turn on.

These results prompted Anslyn and James to ask a simple question: "What is fundamentally different about the structure of such groups in water or methanol?"[70]

The answer was obvious: the –$B(OH)_2$ groups in water possess B–OH bonds, which are converted to B–OR bonds in either methanol or when binding a sugar (Figs 6b, 6c). The fact that the replacement of B–OMe groups with B–OR groups does not change the fluorescence, yet the replacement of B–OH groups with B–OR groups in water does turn on the emission, is key to the puzzle. It must be that the –$B(OH)_2$ groups quench the fluorescence of the anthracene, and upon conversion to any form of a boronate ester, the fluorescence increases.

It is well known that the O–H bond vibrations in water quench the fluorescence of fluorophores by accepting the electronic excitation energy into excited vibrational states of the water.[102] Further, intramolecularly attached alcohols, or carboxylic acids, can also take up this excited electronic state energy.[103,104] This is a form of internal conversion that lowers the fluorescence quantum yield. The classic[102,105,106] test for this is to convert the –OH groups to –OD groups, which, due to their lower frequencies, are less efficient energy acceptors. The Anslyn/James study revealed that in D_2O, in which all the –$B(OH)_2$ groups are converted to –$B(OD)_2$ groups, the emissions of the sensors alone are just as high as the emission in methanol. Further, upon addition of fructose, no additional emission turn-on was found (Fig. 6d).

Such an internal conversion mechanism is commonly referred to as the 'Loose Bolt Effect'.[107-111] Just as a loose bolt can absorb energy from a running motor via vibrating and further loosening (or tightening), a high-frequency rotor in resonance with an electronic excited state can absorb energy.[112] In our case, the proposal is that the 'loose bolt' enhances internal conversion because electronic energy 'leaks out' through –$B(OH)_3^-$ vibrations.[113] In methanol the B–OH groups are B–OMe groups, and the quenching from the $B(OH)_3^-$ vibrations is arrested, thereby turning on fluorescence, and the same occurs when a sugar binds. Even studies that postulate PET from the boronate anion could alternatively quench from this loose-bolt mechanism.[114]

Conclusions

This treatise has delineated a historical account of the ideas and concepts put forth by Shinkai/James and Wang relating to the role of the *o*-aminomethyl group in phenylboronic acids upon sugar binding, covering both the N−B bond and the pK_a switch postulates. Both concepts were able to explain how the emission properties of boronic acids and boronate esters would change. However, with evidence from ^{11}B NMR spectroscopy from Anslyn, arguments concerning pK_a values, as well as crystal structures from Norrild, it was clear that alternative explanations were needed for the mechanism of fluorescence turn-on.

In terms of chemical structures, solvent-inserted species consistently dominant for both boronic acids and boronate esters in protic media. In turn, the *o*-aminomethyl group lowers the pK_a of the proximal boronic acid due to its electron-withdrawing nature and a field effect from the ammonium ion. Consequently, the pyramidalization of the boron atom required for boronate ester formation is facilitated at a lower pH. Further, the intramolecular hydrogen bond between the ammonium cation and the boronate anion formed upon solvent insertion facilitates leaving group departure because the ammonium group acts as a general acid-catalyst for liberating the inserted solvent. This conclusion is supported by both kinetics and isotope effect studies. Thus, the *o*-aminomethyl group both improves the thermodynamics of boronate ester formation, but also improves the kinetics of exchange at the boron center.

As to the mechanism of fluorescence changes upon sugar binding, the evidence points to the *o*-aminomethyl group having no role. Neither a PET nor a pK_a switch mechanism is operative. Instead, with poorly soluble sensors, there is the possibility of disaggregation of the hosts upon addition of sugar, and this can turn on the fluorescence. However, emission enhancement seems to generally occur for boronic acids by arresting a form of internal conversion, commonly referred to as a loose bolt effect. Importantly, by converting $-B(OH)_3^-$ groups to $-B(OR)_3^-$ groups (or even $-B(OD)_3^-$ groups), emission turns on commonly by factors of 2- to 5-fold.

Thus, after 25 years since the 1994 landmark study from Shinkai and James that spawned an explosion of work into the use of boronic acids as sugar sensors and receptors, and of excitement and enthusiasm in the chemosensing community, a series of studies from various researchers can be combined to paint a consistent picture of the role that *o*-aminomethyl groups play in the molecular recognition properties of phenylboronic acids. This body of work reveals how consistent and coordinated physical organic studies from numerous groups can lead to a unified mechanistic picture, even when the mechanisms only differ by subtle changes in positions of protons, or even just vibrational changes between –OH, –OD and –OR groups.

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Author contributions

Although the writing of the paper was spearheaded by EVA, the contents are a collaboration with TDJ and BW. XS, BMC, PM and BC all contributed with experimental data and writing.

Competing interests

The authors declare no competing interests.

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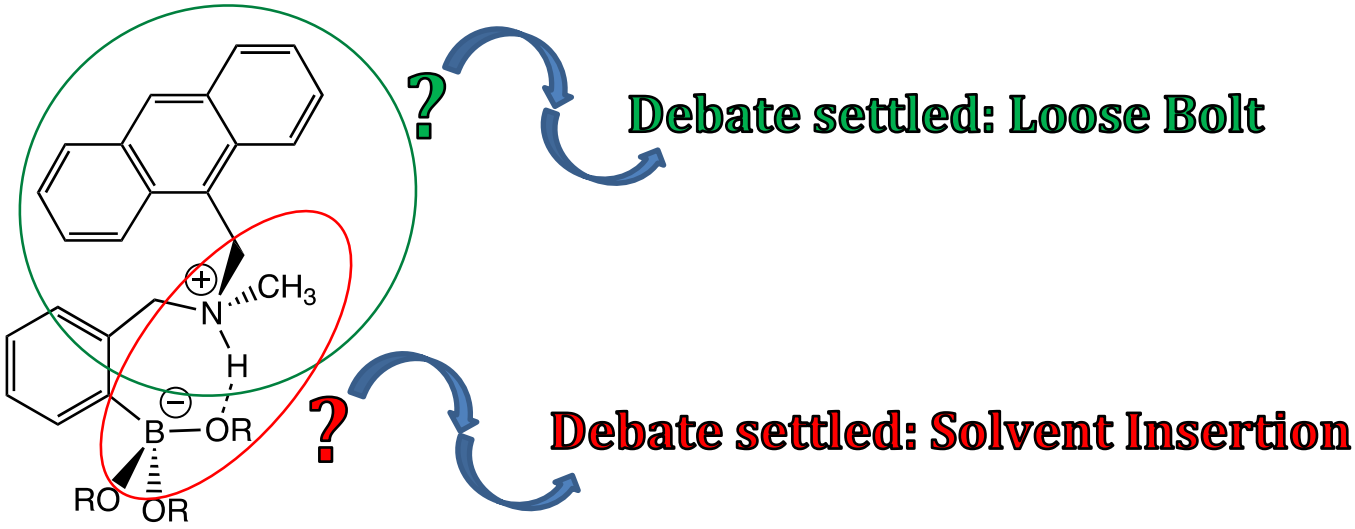
Table of contents summary

ortho-Aminomethylphenylboronic acids are routinely used in sensors for carbohydrates, where the function of the *o*-aminomethyl group in enhancing binding affinity and modulating the emission of appended fluorophores heretofore was unclear. This treatise presents a unified picture of structural features, mechanisms of sugar complexation, and photophysics of these kinds of sensors.

Table of contents graphic



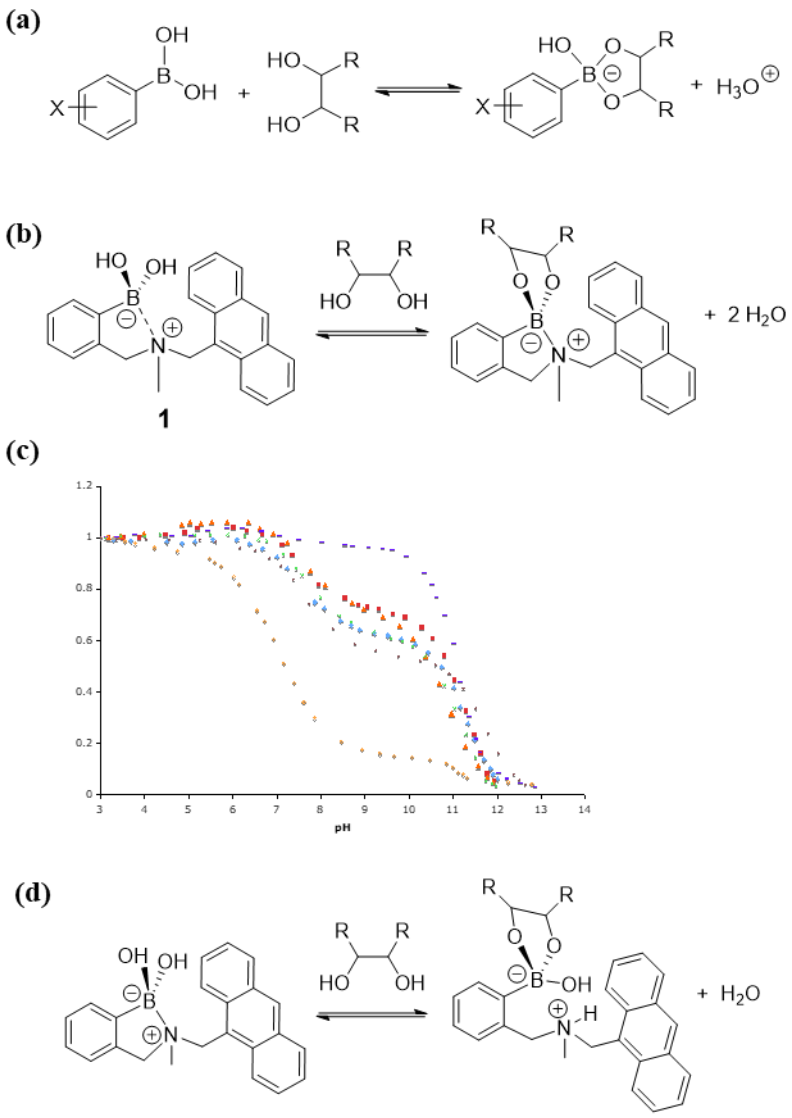
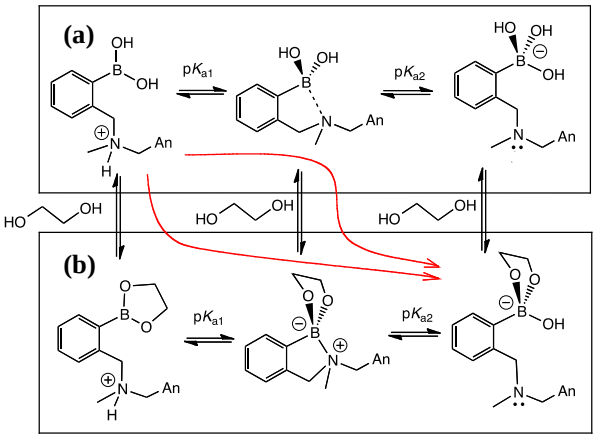
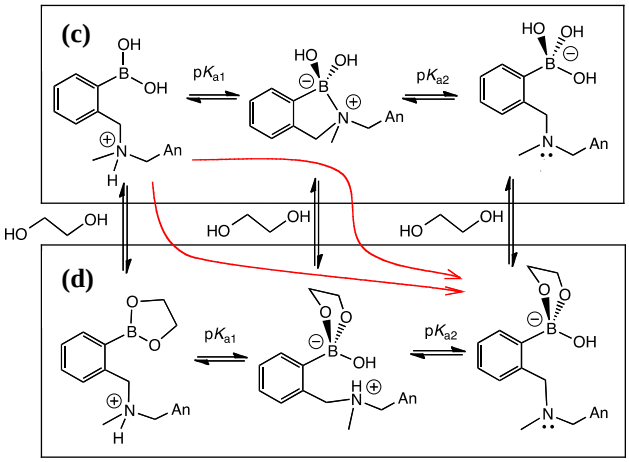


Figure 1. Chemical reactions and primary data relevant to discussions presented herein. **(a)** Reversible binding motifs for boronate ester formation. **(b)** Boronate ester formation and N−B bonding mechanism in carbohydrate fluorescence sensing. **(c)** pH titrations of **1** (1 μM) in 2:1 H_2O:CH_3OH and 50 mM NaCl alone (●) and in the presence of 50 mM D-mannose (◆), D-galactose (■), L-sorbose (▲), D-glucose (×), inositol (+), and D-fructose (–). **(d)** "pK_a switch" or "hydrolysis/solvolysis" mechanism in carbohydrate fluorescence sensing.

N−B Bonding Postulate



p*K*$_a$ Switch Postulate



"Loose Bolt" Postulate

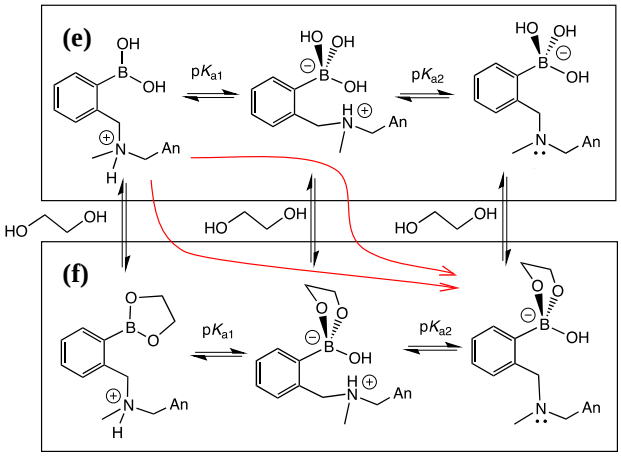


Figure 2. The assignment of p*K*$_a$ values that would be associated with the three mechanistic postulates described herein. Each scheme represents the structures involved with *o*-aminomethylphenylboronic acids as a function of pH (low to high pH given from left to right). The red arrows depict the dominate structures formed when raising the pH in the presence of a sugar. **N−B Bonding Postulate –** In the absence **(a)** and presence **(b)** of a diol, such as a sugar, hydroxycarboxylate, or catechol. **p*K*$_a$ Switch Postulate** (i.e. hydrolysis, or solvent-insertion) - In the absence **(c)** and presence **(d)** of a diol, such as a sugar, hydroxycarboxylate or catechol. **"Loose Bolt Postulate"** - In the absence **(e)** and presence **(f)** of a diol, such as a sugar, hydroxycarboxylate, or catechol. Given the discussion presented in this treatise, this last postulate shows the proper assignments of the p*K*$_a$ values.

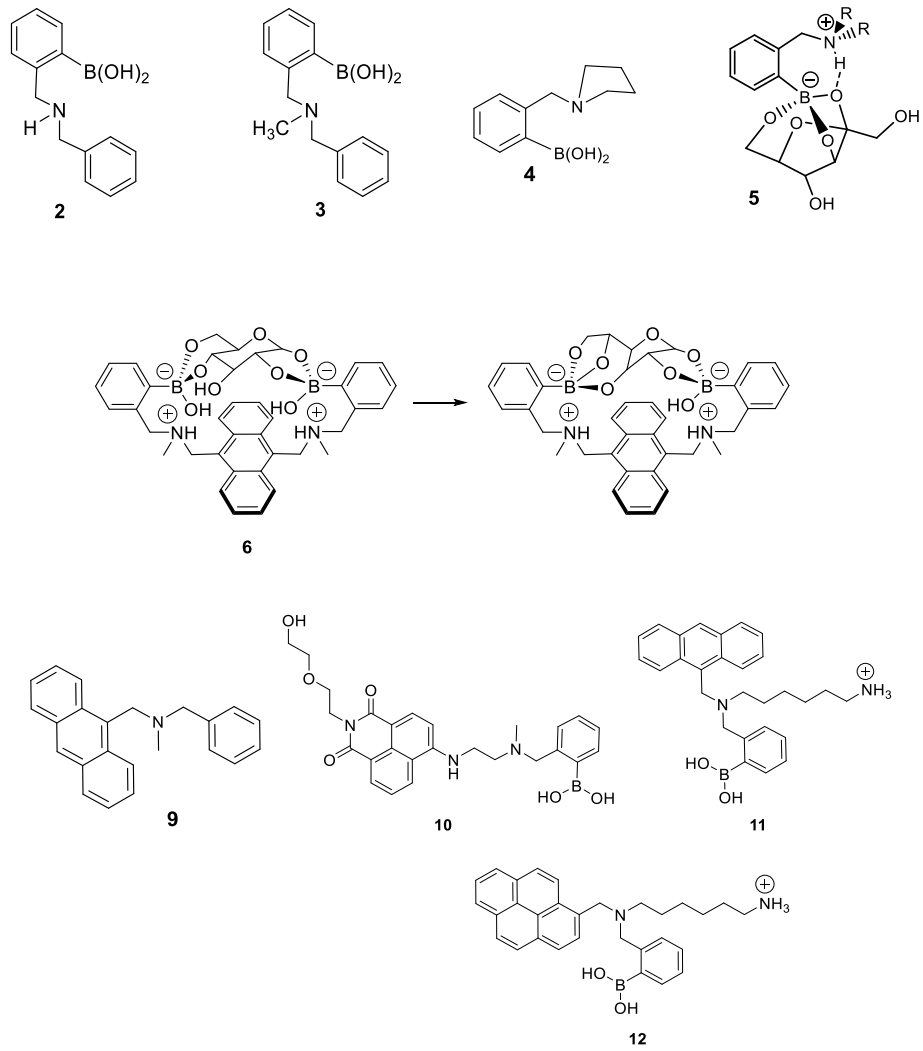


Figure 3. Molecular structures discussed in this treatise.

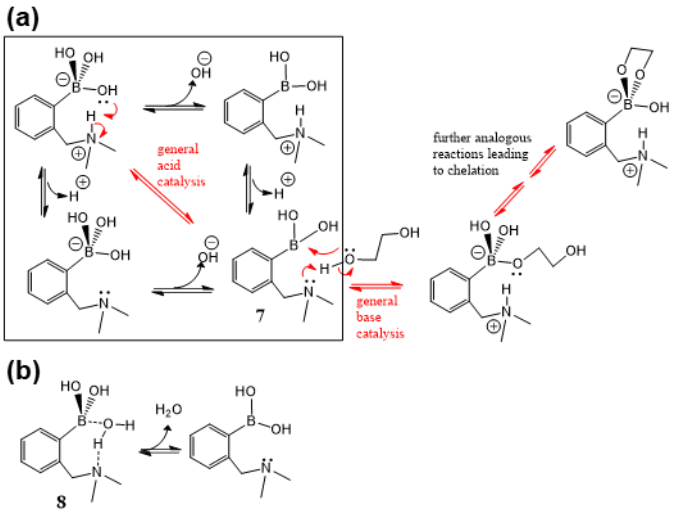


Figure 4. Mechanistic considerations for the role of the *o*-aminomethyl group. **(a)** The proposed mechanism for boronate ester formation at pH's between the first and second pK_a's, based upon kinetics, crystal structures, and isotope effects. The red equilibrium arrows show the dominant pathway, involving general acid-catalyzed expulsion of an inserted solvent with a general base-catalyzed delivery of the guest. **(b)** The possibility of losing the inserted solvent in a single step if the solvent is not highly ionized between the N and B.

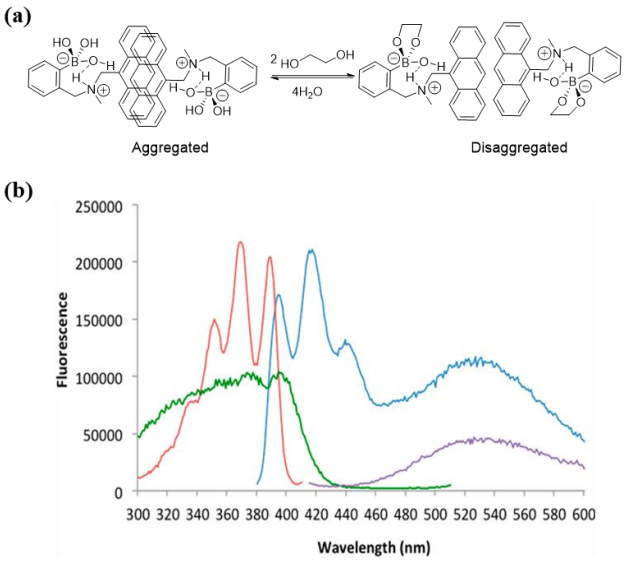


Figure 5. Reactions and data relevant to the discussion of photophysics. **(a)** Aggregation and disaggregation before and after binding with diols. **(b)** Fluorescence spectra of a saturated solution of **1** in 2:1 water/methanol with 50 mM NaCl. Emission scan with λ_{ex} = 368 nm (blue). Excitation scan with λ_{em} = 417 nm (red). Excitation scan with λ_{em} = 520 nm (green). Emission scan with λ_{ex} = 408 nm (purple).[67]

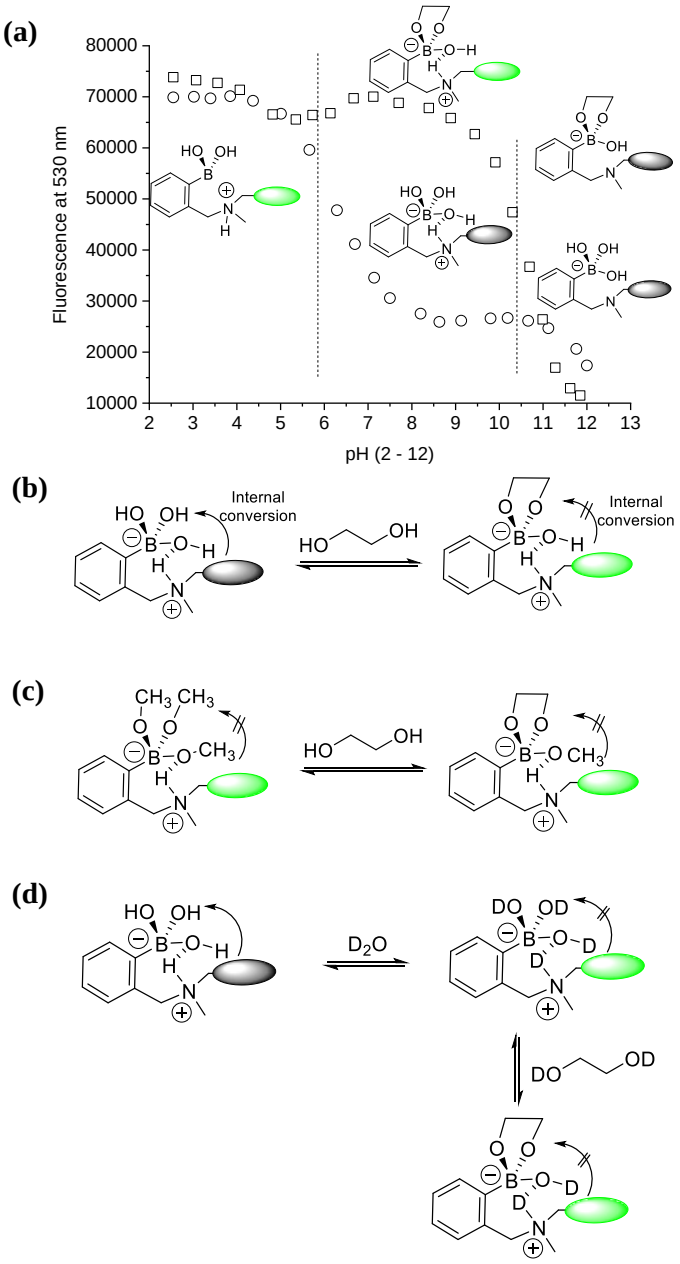
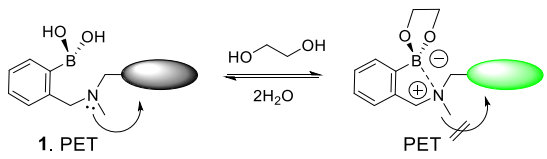
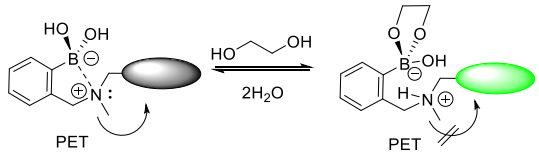
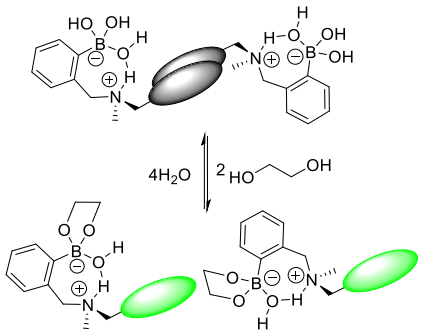
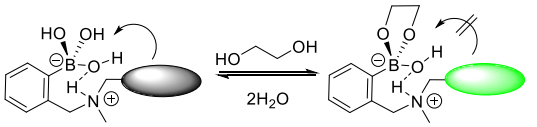


Figure 6. Further data and reactions related to the photophysics. **(a)** Fluorescence pH titration for boronic acid probe **10** (4 µM) (circles) and **10** with fructose complex (**10**: 4 µM; fructose: 100 mM) (squares) in water. E_x = 450 nm, slit/slit: 2 nm/2 nm and fluorescence changes in the replacement of B-$(OH)_2$ to B-OR groups in water **(b)** and B-OMe to B-OR groups in methanol **(c)**. Arrow denotes internal conversion quenching, which is blocked with any form of a boronate ester. **(d)** Isotopic effect for fluorescence changes when the -$B(OH)_2$ groups are converted to -$B(OD)_2$.

Table 1. Different theory and proponents for saccharide sensing

Structure	Theory	Assertor	Year
	B-N bond	Seiji Shinkai Tony D. James	1994
	pK_a switch	Binghe Wang	2003 2004
	Aggregation and Disaggregation	Eric V. Anslyn Joseph D. Larkin	2017
	Loose Bolt Internal Conversion	Eric V. Anslyn Tony D. James	2018

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