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The mechanisms of boronate ester formation and fluorescent turn-on in *ortho*-aminomethylphenylboronic acids

Xiaolong Sun^{1,2}, Brette M. Chapin², Pedro Metola², Byron Collins², Binghe Wang^{3,*}, Tony D. James^{4,*}, Eric V. Anslyn^{2,*}

¹The Key Laboratory of Biomedical Information Engineering of Ministry of Education, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an, People's Republic of China

²Department of Chemistry, The University of Texas at Austin, Austin, TX, USA

³Department of Chemistry and Center for Diagnostics and Therapeutics, Georgia State University, Atlanta, GA, USA

⁴Department of Chemistry, University of Bath, Bath, UK

Abstract

ortho-Aminomethylphenylboronic acids are used in receptors for carbohydrates and various other compounds containing vicinal diols. 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 neighbouring 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 Review 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 phenomena and reactivity¹. 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 low-temperature (40 K) crystal structure². Another is the controversy surrounding interpretation

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*Correspondence should be addressed to B.W., T.D.J. or E.V.A. wang@gsu.edu; t.d.james@bath.ac.uk; anslyn@austin.utexas.edu. Author contributions

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Competing interests

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of data concerning a self-replicating system that occurred between Rebek and Menger, which was settled with extensive kinetic analysis by Reinhoudt³. 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, how receptors based on *o*-aminomethylphenylboronic acids turn-on emission and whether their structures possess N—B bonds when binding vicinal diols have 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 1860⁶. 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 polymers¹³, molecular self-assembled materials^{14,15}, tissue histology¹⁶ and fluorescence imaging of carbohydrates¹⁷. Further, they are among a small set of reactions that involve dynamic covalent bonding⁷. 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^{6,22–36}. The detection of carbohydrates is commonly pursued in disease diagnostics, such as diabetes^{16,37–41}. Carbohydrates present a challenge for molecular recognition because of their high solvation energies in water^{42–44} and their great structural diversity⁴⁵. 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)^{46–52}. Importantly, the incorporation of a boronic acid into almost any scaffold reliably imparts high-to-low millimolar 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^{53–55}. This is a structural feature that is often overlooked.

Early studies by Wulff^{55,56} as well as Lorand⁵⁷ 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 pH⁵⁸. 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^{13,41,59–66}. By exploiting *o*-aminomethylphenylboronic acids appended with different fluorophores, a series of turn-on fluorescence probes have been created for the sensing and imaging of carbohydrate and glycoproteins on cell surfaces^{17,61}. Most significant for practical applications, a modular sensory system of this type has been developed by Glysure into a fully functional fibre-optic sensor for the continuous monitoring of glucose with patients in intensive care units⁶⁷.

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 (especially in an aqueous environment) has been debated. Different mechanisms have been proposed for the emission turn-on of *o*-aminomethylphenylboronic acids appended with fluorophores in response to saccharide binding in aqueous media (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^{62,63}. However, as an alternative mechanism to the N—B dative bond, Wang put forth a pK_a switch theory involving the breaking of an N—B bond and insertion of solvent upon sugar binding^{64,65}. With the question ongoing, Anslyn and Larkin explored an unanticipated aggregation and disaggregation mechanism⁶⁶. However, none of the hypotheses were consistent with all the data for *o*-aminomethylphenylboronic-acid-based sensors. Thus, in 2018, Anslyn and James revealed that modulating the internal conversion induced by —B(OH)₂ (an example of a ‘loose-bolt’ effect) explains how potentially all *o*-aminomethylphenylboronic-acid-based fluorescence sensors signal the presence of sugars⁶⁸.

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 reanalysed; 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)^{62,63,69}. 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**, Fig. 1b)⁷⁰. By contrast, the boron in phenylboronic acid, PhB(OH)₂, is sp^2 at neutral pH (a fact that has been established using ¹¹B NMR)⁷¹. 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 -hybridized⁷². 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 neighbouring 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^{23,72}.

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 analogues^{62,63,69}. PET^{73,74} 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, Czarnik⁷⁵ and DeSilva⁷⁶, 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.^{77–79}

An alternative explanation

Wang put forth an alternative mechanism to the N—B dative bond theory^{64,65}. 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 modelling 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 behaviour 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 an N—H bond is far stronger than an N—B bond. In a subsequent analysis, Wang proposed what has come to be known as the 'p*K*_a switch' or 'hydrolysis/solvolytic' mechanism (Fig. 1d)⁶⁵. 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 p*K*_a of the boronate ester⁷⁹, even though the p*K*_a varies as a function of which sugar is used. It is well documented^{35,57} with phenylboronic acid that lowering of the p*K*_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 on the sugar⁷⁹, and Wang reconfirmed this experimentally with four different carbohydrates⁶⁵.

Wang further noted that sugars which form trivalent binding of the boron should not modulate the fluorescence if an 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 changes⁶⁵.

pH titration evidence for different perspectives

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^{64,65}. There are two types of pH titrations often performed: fluorescent and ¹¹B 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. By contrast, in the presence of a sugar, the first fluorescence decrease is minor, while a large decrease occurs at the higher pH⁶³.

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 an 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 coordination⁸⁰. 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 an 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). Figure 2a,b shows 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 (Fig. 2c,d). 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 an 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 hydrolysed. 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 centre. Note that in the absence of the diol, the first and second pK_a s 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_a s 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 (Fig. 2c,d) 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 Anslyn⁸¹. These experiments were the pH titrations of compounds **2** and **3** (Fig. 3), which were followed by ^{11}B NMR spectroscopy, which is very sensitive to the hybridization of the boron atom⁷¹. The goal of the Anslyn experiments was to determine if the pK_a s 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 interaction⁸¹. 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_a s 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 values⁶². 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 acids⁸², suggesting a role of the neighbouring 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 experiments⁸¹. 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 pyramidalization of the boron because N—B bonding was the prevailing picture⁸¹. But there is now a different interpretation that involves solvent insertion in both the boronic acids and boronate esters, as discussed immediately below.

Structural and ^{11}B NMR evidence for different perspectives

To distinguish between the N—B bond and $\text{p}K_{\text{a}}$ 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 examined⁶². 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 conclusion⁸³. 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 $\text{p}K_{\text{a}}$ switch mechanism can be operative. Thus, while logically sound, the postulates illustrated in Fig. 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^{83,84}. In these cases, the inserted solvent is replaced by an OH group from the sugar itself. This is well accepted for fructose⁸³, forming structures such as **5** (Fig. 3). In fact, Norrild found that glucose rearranges to its furanose form when binding with a bisboronic acid receptor **6** (Fig. 3), as revealed by a series of coupling constant measurements⁸⁴. The postulate is that the driving force is to exploit the energetically favourable trivalent interaction with glucose. The most recent computational studies by Larkin confirm the lower energy of trivalent sugar geometries⁸⁵.

The *o*-aminomethyl group in the assignment of the $\text{p}K_{\text{a}}$ s

As discussed above, the first $\text{p}K_{\text{a}}$ 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 $\text{p}K_{\text{a}}$ corresponds to hydroxylation of the boronic acid/ester giving directly a solvent-inserted structure (Fig. 2e,f, vide infra why this is called 'loose bolt'). Hence, the proximity of the positively charged ammonium group depresses the $\text{p}K_{\text{a}}$ 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 $\text{p}K_{\text{a}}$ values do not significantly vary between these two species (discussed above). With this conclusion in mind, the second $\text{p}K_{\text{a}}$ 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 $\text{p}K_{\text{a}}$ 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_a s are now clearly assigned.

The α -aminomethyl group in boronate ester formation

If the ammonium group perturbs the pK_a s 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 centre, 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 α -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 analysed 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 mechanism⁸⁶. Alternatively, a mechanism in which the solvent-inserted α -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^{78,87}. 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 kinetics⁸⁷. Yet, at high fructose concentrations saturation kinetics were found (that is, zero-order in fructose). The kinetic data show 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, which 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 mechanisms⁸⁷, but with boronic acids the ‘common ion’ is the solvent. By fitting the kinetic data, Anslyn estimated that fructose is around 1,000 times better as a 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-catalysed 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 solvent¹. Consistent with this finding, Anslyn uncovered an isotope effect of 1.42 for the reaction of **1** with fructose⁸⁷. This value is smaller than what may be expected but is clearly a substantial effect. The isotope effect is evidence that the general acid-catalysed pathway for expulsion of the inserted solvent occurs. It should be noted that if the expulsion is general acid-catalysed, then the reverse reaction, solvent insertion, would be general base-catalysed (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**⁶⁷. The original pH titration data from Shinkai and James⁶² revealed quite a large emission turn-on (around 30-fold) in a pH titration with fructose in a solution of 2:1 water/methanol with 50 mM NaCl. 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 analyse the emission spectra in more detail. First, they confirmed by ¹¹B NMR spectroscopy in a water/methanol

mixture⁶⁷ 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^{88–90}. 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 classic⁹¹ 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 ¹¹B 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 a near-identical manner as **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 hypothesis⁶⁷.

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 PET, 'p*K*_a switch' and disaggregation. However, none of the hypotheses are 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 twofold to threefold 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 forces⁶⁸. They noted that most all *o*-aminomethylphenylboronic acid sensors for sugars typically show an emission turn-on in the range of twofold to fivefold^{61,92–96}. 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 fructose⁹⁷.

As originally done by Shinkai and James with **1**, the first step was to analyse 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 threefold (within a twofold to fivefold range) fluorescence turn-on response in the presence of fructose, which confirms fructose binding. As the first pK_a of the boronic acid (that is, hydroxylation of the boron) is approached by raising the pH, an anionic boronate ($R-B(OH_3)^-$) is formed without fructose (recall Fig. 2e). In the presence of fructose, a **10**–fructose complex is formed within the pH range 6–10. Above pH 10, the fluorescence of both **10** and the **10**–fructose complex drop, owing to 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. These 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 twofold to fivefold 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?⁶⁸

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 (Fig. 6b,c). 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 water⁹⁸. Further, intramolecularly attached alcohols, or carboxylic acids, can also take up this excited electronic state energy^{99,100}. This is a form of internal conversion that lowers the

fluorescence quantum yield. The classic^{98,101,102} test for this is to convert the –OH groups to –OD, which, due to their lower frequencies, are less efficient energy acceptors. The Anslyn–James study revealed that in D₂O, 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^{103–107}. 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 energy¹⁰⁸. In our case, the proposal is that the loose bolt enhances internal conversion because electronic energy ‘leaks out’ through –B(OH)₃[–] vibrations¹⁰⁹. 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 mechanism¹¹⁰.

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 p*K*_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 ¹¹B NMR spectroscopy from Anslyn, arguments concerning p*K*_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 p*K*_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 centre.

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 p*K*_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 twofold to fivefold.

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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References

- Anslyn EV & Dougherty DA Modern Physical Organic Chemistry (University Science Books, 2006).
- Scholz F et al. Crystal structure determination of the nonclassical 2-norbornyl cation. *Science* 341, 62–64 (2013). [PubMed: 23828938]
- Reinhoudt DN, Rudkevich DM & de Jong F Kinetic Analysis of the Rebek self-replicating system: is there a controversy? *J. Am. Chem. Soc.* 118, 6880–6889 (1996).
- Cleland W Low-barrier hydrogen bonds and enzymatic catalysis. *Arch. Biochem. Biophys.* 382, 1–5 (2000). [PubMed: 11051090]
- Schutz CN & Warshel A The low barrier hydrogen bond (LBHB) proposal revisited: the case of the Asp¹⁰²–His pair in serine proteases. *Proteins* 55, 711–723 (2004). [PubMed: 15103633]
- Hall DG Boronic Acids: Preparation, Applications in Organic Synthesis and Medicine (John Wiley and Sons, 2006).
- Bull SD et al. Exploiting the reversible covalent bonding of boronic acids: recognition, sensing, and assembly. *Acc. Chem. Res.* 46, 312–326 (2013). [PubMed: 23148559]
- Guo Z, Shin I & Yoon J Recognition and sensing of various species using boronic acid derivatives. *Chem. Commun.* 48, 5956–5967 (2012).
- Fang H, Kaur G & Wang B Progress in boronic acid-based fluorescent glucose sensors. *J. Fluoresc.* 14, 481–489 (2004). [PubMed: 15617256]
- Heagy MD & Meka RK in *Comprehensive Supramolecular Chemistry II* (ed. Atwood JL) Ch. 4.19, 615–647 (Elsevier, 2017).
- Kelly AM, Perez-Fuertes Y, Arimori S, Bull SD & James TD Simple protocol for NMR analysis of the enantiomeric purity of diols. *Org. Lett.* 8, 1971–1974 (2006). [PubMed: 16671759]
- Pérez-Fuertes Y et al. Simple protocol for NMR analysis of the enantiomeric purity of primary amines. *Org. Lett.* 8, 609–612 (2006). [PubMed: 16468723]
- Cambre JN & Sumerlin BS Biomedical applications of boronic acid polymers. *Polymer* 52, 4631–4643 (2011).
- Fujita N, Shinkai S & James TD Boronic acids in molecular self-assembly. *Chem. Eur. J.* 14, 1076–1091 (2008).
- Nishiyabu R, Kubo Y, James TD & Fossey JS Boronic acid building blocks: tools for self assembly. *Chem. Commun.* 47, 1124–1150 (2011).

16. Dai C et al. Using boronolactin in MALDI-MS imaging for the histological analysis of cancer tissue expressing the sialyl Lewis X antigen. *Chem. Commun* 47, 10338–10340 (2011).
17. Sun X, Zhai W, Fossey JS & James TD Boronic acids for fluorescence imaging of carbohydrates. *Chem. Commun* 52, 3456–3469 (2016).
18. Sun X et al. Reaction-based Indicator displacement Assay (RIA) for the selective colorimetric and fluorometric detection of peroxynitrite. *Chem. Sci* 6, 2963–2967 (2015). [PubMed: 28706677]
19. Sun X et al. “Integrated” and “insulated” boronate-based fluorescent probes for the detection of hydrogen peroxide. *Chem. Commun* 49, 8311–8313 (2013).
20. Chan J, Dodani SC & Chang CJ Reaction-based small-molecule fluorescent probes for chemoselective bioimaging. *Nat. Chem* 4, 973–984 (2012). [PubMed: 23174976]
21. Lippert AR, Van de Bittner GC & Chang CJ Boronate oxidation as a bioorthogonal reaction approach for studying the chemistry of hydrogen peroxide in living systems. *Acc. Chem. Res* 44, 793–804 (2011). [PubMed: 21834525]
22. James T & Shinkai S in *Topics in Current Chemistry* (ed. Penadés S) Vol. 218, Ch. 6, 159–200 (Springer, 2002).
23. James TD, Phillips MD & Shinkai S *Boronic Acids in Saccharide Recognition* (Royal Society of Chemistry, 2006).
24. James TD, Sandanayake KRAS & Shinkai S Saccharide sensing with molecular receptors based on boronic acid. *Angew. Chem. Int. Ed. Engl* 35, 1910–1922 (1996).
25. Jin S, Cheng Y, Reid S, Li M & Wang B Carbohydrate recognition by boronolactins, small molecules, and lectins. *Med. Res. Rev* 30, 171–257 (2010). [PubMed: 19291708]
26. Wright AT et al. Differential receptors create patterns that distinguish various proteins. *Angew. Chem. Int. Ed* 44, 6375–6378 (2005).
27. Bicker KL et al. Synthetic lectin arrays for the detection and discrimination of cancer associated glycans and cell lines. *Chem. Sci* 3, 1147–1156 (2012). [PubMed: 29051806]
28. Gray CW & Houston TA Boronic acid receptors for α -hydroxycarboxylates: high affinity of Shinkai’s glucose receptor for tartrate. *J. Org. Chem* 67, 5426–5428 (2002). [PubMed: 12126445]
29. Wang W, Gao X & Wang B Boronic acid-based sensors. *Curr. Org. Chem* 6, 1285–1317 (2002).
30. Lee JW, Lee J-S & Chang Y-T Colorimetric Identification of carbohydrates by a pH indicator/pH change inducer ensemble. *Angew. Chem. Int. Ed* 45, 6485–6487 (2006).
31. Schiller A, Wessling RA & Singaram B A Fluorescent sensor array for saccharides based on boronic acid appended bipyridinium salts. *Angew. Chem. Int. Ed* 46, 6457–6459 (2007).
32. Edwards NY, Sager TW, McDevitt JT & Anslyn EV Boronic acid based peptidic receptors for pattern-based saccharide sensing in neutral aqueous media, an application in real-life samples. *J. Am. Chem. Soc* 129, 13575–13583 (2007). [PubMed: 17927178]
33. Musto CJ, Lim SH & Suslick KS Colorimetric detection and identification of natural and artificial sweeteners. *Anal. Chem* 81, 6526–6533 (2009). [PubMed: 20337402]
34. Zhang X, You L, Anslyn EV & Qian X Discrimination and classification of ginsenosides and ginsengs using bis-boronic acid receptors in dynamic multicomponent indicator displacement sensor arrays. *Chem. Eur. J* 18, 1102–1110 (2012). [PubMed: 22213109]
35. Springsteen G & Wang B A detailed examination of boronic acid–diol complexation. *Tetrahedron* 58, 5291–5300 (2002).
36. Zaubitzer F, Buryak A & Severin K Cp*Rh-based indicator-displacement assays for the identification of amino sugars and aminoglycosides. *Chem. Eur. J* 12, 3928–3934 (2006). [PubMed: 16521137]
37. Yasuda H, Kurokawa T, Fujii Y, Yamashita A & Ishibashi S Decreased d-glucose transport across renal brush-border membrane vesicles from streptozotocin-induced diabetic rats. *Biochim. Biophys. Acta Biomembr* 1021, 114–118 (1990).
38. Fedorak RN, Gershon MD & Field M Induction of intestinal glucose carriers in streptozotocin-treated chronically diabetic rats. *Gastroenterology* 96, 37–44 (1989). [PubMed: 2521211]
39. Mallia AK, Hermanson GT, Krohn RI, Fujimoto EK & Smith PK Preparation and use of a boronic acid affinity support for separation and quantitation of glycosylated hemoglobins. *Anal. Lett* 14, 649–661 (1981).

40. Yang W et al. Diboronic acids as fluorescent probes for cells expressing sialyl Lewis X. *Biorg. Med. Chem. Lett* 12, 2175–2177 (2002).
41. Sun X & James TD Glucose sensing in supramolecular chemistry. *Chem. Rev* 115, 8001–8037 (2015). [PubMed: 25974371]
42. Goldberg RN & Tewari YB Thermodynamic and transport properties of carbohydrates and their monophosphates: the pentoses and hexoses. *J. Phys. Chem. Ref. Data* 18, 809–880 (1989).
43. Cesàro A in *Thermodynamic Data for Biochemistry and Biotechnology* 177–207 (Springer, 1986).
44. Franks F Physical chemistry of small carbohydrates-equilibrium solution properties. *Pure Appl. Chem* 59, 1189–1202 (1987).
45. *Essentials of Glycobiology* 2nd edn (eds. Varki A et al.) 23–36 (Cold Spring Harbor Laboratory Press, 2009).
46. Moulin E, Cormos G & Giuseppone N Dynamic combinatorial chemistry as a tool for the design of functional materials and devices. *Chem. Soc. Rev* 41, 1031–1049 (2012). [PubMed: 21909573]
47. Lehn J-M From supramolecular chemistry towards constitutional dynamic chemistry and adaptive chemistry. *Chem. Soc. Rev* 36, 151–160 (2007). [PubMed: 17264919]
48. Lehn J-M *Supramolecular Chemistry: Concepts and Perspectives* (Vch, 1995).
49. Rowan SJ, Cantrill SJ, Cousins GR, Sanders JK & Stoddart JF Dynamic covalent chemistry. *Angew. Chem. Int. Ed* 41, 898–952 (2002).
50. Corbett PT et al. Dynamic combinatorial chemistry. *Chem. Rev* 106, 3652–3711 (2006). [PubMed: 16967917]
51. Cougnon FBL & Sanders JKM Evolution of dynamic combinatorial chemistry. *Acc. Chem. Res* 45, 2211–2221 (2012). [PubMed: 22206218]
52. Wilson A, Gasparini G & Matile S Functional systems with orthogonal dynamic covalent bonds. *Chem. Soc. Rev* 43, 1948–1962 (2014). [PubMed: 24287608]
53. Weith HL, Wiebers JL & Gilham PT Synthesis of cellulose derivatives containing the dihydroxyboryl group and a study of their capacity to form specific complexes with sugars and nucleic acid components. *Biochemistry* 9, 4396–4401 (1970). [PubMed: 5472711]
54. Hirata O, Kubo Y, Takeuchi M & Shinkai S Mono- and oligosaccharide sensing by phenylboronic acid-appended 5,15-bis(diarylethynyl)porphyrin complexes. *Tetrahedron* 60, 11211–11218 (2004).
55. Wulff G Selective binding to polymers via covalent bonds. The construction of chiral cavities as specific receptor sites. *Pure Appl. Chem* 54, 2093–2102 (1982).
56. Wulff G, Dederichs W, Grotstollen R & Jupe C in *Affinity Chromatography and Related Techniques* 207–216 (Elsevier, 1982).
57. Lorand JP & Edwards JO Polyol complexes and structure of the benzenboronate ion. *J. Org. Chem* 24, 769–774 (1959).
58. Wulff G, Lauer M & Bohnke H Rapid proton transfer as cause of an unusually large neighboring group effect. *Angew. Chem. Int. Ed. Engl* 23, 741–742 (1984).
59. Jin S, Wang J, Li M & Wang B Synthesis, evaluation, and computational studies of naphthalimide-based long-wavelength fluorescent boronic acid reporters. *Chem. Eur. J* 14, 2795–2804 (2008). [PubMed: 18228545]
60. Zhu L et al. A structural investigation of the N—B Interaction in an α -(*N,N*-dialkylaminomethyl)arylboronate system. *J. Am. Chem. Soc* 128, 1222–1232 (2006). [PubMed: 16433539]
61. Zhai W, Sun X, James TD & Fossey JS Boronic acid-based carbohydrate sensing. *Chem. Asian J* 10, 1836–1848 (2015). [PubMed: 26177967]
62. James TD, Sandanayake KRAS & Shinkai S Novel photoinduced electron-transfer sensor for saccharides based on the interaction of boronic acid and amine. *J. Chem. Soc. Chem. Commun* 1994, 477–478 (1994).
63. James TD, Sandanayake KRAS & Shinkai S A glucose-selective molecular fluorescence sensor. *Angew. Chem. Int. Ed. Engl* 33, 2207–2209 (1994).
64. Franzen S, Ni W & Wang B Study of the mechanism of electron-transfer quenching by boron—nitrogen adducts in fluorescent sensors. *J. Phys. Chem. B* 107, 12942–12948 (2003).

65. Ni W, Kaur G, Springsteen G, Wang B & Franzen S Regulating the fluorescence intensity of an anthracene boronic acid system: a B–N bond or a hydrolysis mechanism? *Bioorg. Chem* 32, 571–581 (2004). [PubMed: 15530997]
66. Chapin BM et al. Disaggregation is a mechanism for emission turn-on of *ortho*-aminomethylphenylboronic acid-based saccharide sensors. *J. Am. Chem. Soc* 139, 5568–5578 (2017). [PubMed: 28358506]
67. Crane BC et al. The development of a continuous intravascular glucose monitoring sensor. *J. Diabetes Sci. Technol* 9, 751–761 (2015). [PubMed: 26033921]
68. Sun X, James TD & Anslyn EV Arresting “loose bolt” internal conversion from —B(OH)₂ groups is the mechanism for emission turn-on in *ortho*-aminomethylphenylboronic acid-based saccharide sensors. *J. Am. Chem. Soc* 140, 2348–2354 (2018). [PubMed: 29360350]
69. James TD, Samankumara Sandanayake KRA & Shinkai S Chiral discrimination of monosaccharides using a fluorescent molecular sensor. *Nature* 374, 345–347 (1995).
70. James TD, Linnane P & Shinkai S Fluorescent saccharide receptors: a sweet solution to the design, assembly and evaluation of boronic acid derived PET sensors. *Chem. Commun* 1996, 281–288 (1996).
71. Nöth H & Wrackmeyer B in *Nuclear Magnetic Resonance Spectroscopy of Boron Compounds* (eds Diehl P, Fluck E & Kosfeld R) (NMR Basic Principles and Progress 14, Springer, 1978).
72. James TD in *Creative Chemical Sensor Systems* 107–152 (Springer, 2007).
73. Silva AP Recent evolution of luminescent photoinduced electron transfer sensors. A review. *Analyst* 121, 1759–1762 (1996).
74. Bissell RA et al. in *Photoinduced Electron Transfer* V 223–264 (Springer, 1993).
75. Beeson JC, Huston ME, Pollard DA, Venkatachalam TK & Czarnik AW Structural requirements for efficient photoinduced electron transfer (PET) quenching in 9-aminoalkylanthracenes. *J. Fluoresc* 3, 65–68 (1993). [PubMed: 24234769]
76. De Silva AP et al. Signaling recognition events with fluorescent sensors and switches. *Chem. Rev* 97, 1515–1566 (1997). [PubMed: 11851458]
77. Lehn JM *Supramolecular chemistry—scope and perspectives molecules, supermolecules, and molecular devices* (Nobel Lecture). *Angew. Chem. Int. Ed. Engl* 27, 89–112 (1988).
78. Matsumura T, Iwatsuki S & Ishihara K Direct kinetic measurements for the fast interconversion process between trigonal boronic acid and tetragonal boronate ion at low temperatures. *Inorg. Chem. Commun* 8, 713–716 (2005).
79. Cooper CR & James TD Selective fluorescence signalling of saccharides in their furanose form. *Chem. Lett* 27, 883–884 (1998).
80. Chaberek S, Courtney RC & Martell AE Stability of metal chelates. II. β -Hydroxyethyliminodiacetic acid. *J. Am. Chem. Soc* 74, 5057–5060 (1952).
81. Wiskur SL et al. pK_a values and geometries of secondary and tertiary amines complexed to boronic acids—implications for sensor design. *Org. Lett* 3, 1311–1314 (2001). [PubMed: 11348222]
82. Yoon J & Czarnik AW Fluorescent chemosensors of carbohydrates. A means of chemically communicating the binding of polyols in water based on chelation-enhanced quenching. *J. Am. Chem. Soc* 114, 5874–5875 (1992).
83. Larkin JD, Fossey JS, James TD, Brooks BR & Bock CW A computational investigation of the nitrogen—boron interaction in α -(*N,N*-dialkylaminomethyl)arylboronate systems. *J. Phys. Chem. A* 114, 12531–12539 (2010). [PubMed: 21050022]
84. Norrild JC & Eggert H Boronic acids as fructose sensors. Structure determination of the complexes involved using $^1J_{CC}$ coupling constants. *J. Chem. Soc. Perkin Trans. 2* 1996, 2583–2588 (1996).
85. Kearns FL et al. Modeling boronic acid based fluorescent saccharide sensors: computational investigation of d-fructose binding to dimethylaminomethylphenylboronic acid. *J. Chem. Inf. Model* 59, 2150–2158 (2019). [PubMed: 30908030]
86. Norrild J A fluorescent glucose sensor binding covalently to all five hydroxy groups of α -D-glucofuranose. A reinvestigation. *J. Chem. Soc., Perkin Trans. 2* 1999, 449–456 (1999).
87. Collins BE, Metola P & Anslyn EV On the rate of boronate ester formation in *ortho*-aminomethyl-functionalised phenyl boronic acids. *Supramol. Chem* 25, 79–86 (2013). [PubMed: 23441105]

88. Chandross EA Photolytic dissociation of dianthracene. *J. Chem. Phys* 43, 4175–4176 (1965).
89. Chandross EA, Ferguson J & McRae E Absorption and emission spectra of anthracene dimers. *J. Chem. Phys* 45, 3546–3553 (1966).
90. McVey JK, Shold DM & Yang N Direct observation and characterization of anthracene excimer in solution. *J. Chem. Phys* 65, 3375–3376 (1976).
91. Momiji I, Yoza C & Matsui K Fluorescence spectra of 9-anthracenecarboxylic acid in heterogeneous environments. *J. Phy. Chem. B* 104, 1552–1555 (2000).
92. Arimori S, Bell ML, Oh CS, Frimat KA & James TD Modular fluorescence sensors for saccharides. *J. Chem. Soc. Perkin Trans. 1* 2001, 803–808 (2002).
93. Camara JN, Suri JT, Cappuccio FE, Wessling RA & Singaram B Boronic acid substituted viologen based optical sugar sensors: modulated quenching with viologen as a method for monosaccharide detection. *Tetrahedron Lett.* 43, 1139–1141 (2002).
94. Arimori S, Phillips MD & James TD Probing disaccharide selectivity with modular fluorescent sensors. *Tetrahedron Lett.* 45, 1539–1542 (2004).
95. Phillips M & James T Boronic acid based modular fluorescent sensors for glucose. *J. Fluoresc* 14, 549–559 (2004). [PubMed: 15617262]
96. Xing Z et al. Selective saccharide recognition using modular diboronic acid fluorescent sensors. *Eur. J. Org. Chem* 2012, 1223–1229 (2012).
97. Sun X et al. A water-soluble boronate-based fluorescent probe for the selective detection of peroxynitrite and imaging in living cells. *Chem. Sci* 5, 3368–3373 (2014).
98. Dereka B & Vauthey E Direct local solvent probing by transient infrared spectroscopy reveals the mechanism of hydrogen-bond induced nonradiative deactivation. *Chem. Sci* 8, 5057–5066 (2017). [PubMed: 28970892]
99. Harris CM & Selinger BK Proton-induced fluorescence quenching of 2-naphthol. *J. Phy. Chem* 84, 891–898 (1980).
100. Sun Z-N, Liu F-Q, Chen Y, Tam PKH & Yang D A highly specific BODIPY-based fluorescent probe for the detection of hypochlorous acid. *Org. Lett* 10, 2171–2174 (2008). [PubMed: 18447382]
101. Mirbach MJ, Mirbach MF, Cherry WR, Turro NJ & Engel P Solvent isotope effect on the fluorescence of azoalkanes. *Chem. Phys. Lett* 53, 266–269 (1978).
102. Shizuka H & Tobita S Proton-induced quenching and hydrogen-deuterium isotope-exchange reactions of methoxynaphthalenes. *J. Am. Chem. Soc* 104, 6919–6927 (1982).
103. Lewis GN, Magel TT & Lipkin D The absorption and re-emission of light by cis- and trans-stilbenes and the efficiency of their photochemical isomerization. *J. Am. Chem. Soc* 62, 2973–2980 (1940).
104. Kortüm G & Dreesen G Über die Konstitutionsabhängigkeit der Schwingungsstruktur im Absorptionsspektrum von aromatischen Kohlenwasserstoffen. *Chem. Ber* 84, 182–203 (1951).
105. Guesten H, Mintas M & Klasinc L Deactivation of the fluorescent state of 9-*tert*-butylanthracene. 9-*tert*-butyl-9,10(Dewar anthracene). *J. Am. Chem. Soc* 102, 7936–7937 (1980).
106. Lewis GN & Calvin M The color of organic substances. *Chem. Rev* 25, 273–328 (1939).
107. Hofer LJE, Grabenstetter RJ & Wiig EO The fluorescence of cyanine and related dyes in the monomeric state I. *J. Am. Chem. Soc* 72, 203–209 (1950).
108. Rurack K, Dekhtyar ML, Bricks JL, Resch-Genger U & Rettig W Quantum yield switching of fluorescence by selectively bridging single and double bonds in chalcones: involvement of two different types of conical intersections. *J. Phy. Chem. A* 103, 9626–9635 (1999).
109. Ogunsipe A, Chen J-Y & Nyokong T Photophysical and photochemical studies of zinc(ii) phthalocyanine derivatives-effects of substituents and solvents. *New J. Chem* 28, 822–827 (2004).
110. DiCesare N, Adhikari DP, Heynekamp JJ, Heagy MD & Lakowicz JR Spectroscopic and photophysical characterization of fluorescent chemosensors for monosaccharides based on *N*-phenylboronic acid derivatives of 1,8-naphthalimide. *J. Fluoresc* 12, 147–154 (2002). [PubMed: 32132808]

111. Collins BE A Kinetic Investigation of Boronic Acid/Diol Interactions and Pattern-Based α -Chiral Carboxylate Recognition. PhD Thesis, Univ. Texas at Austin (2005).

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Fig. 1 |. Chemical reactions and primary data relevant to the topics discussed in this Review.
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 $\text{H}_2\text{O}:\text{CH}_3\text{OH}$ and 50 mM NaCl alone (yellow circle) and in the presence of 50 mM of each of the following carbohydrates: D-mannose (blue diamond), D-galactose (red square), L-sorbose (orange triangle), D-glucose (green cross), inositol (brown plus) and D-fructose (purple dash); data taken from ref. ¹¹¹. The fluorescence responses are all normalized to 1 at

the initial values. **d**, The ' pK_a switch' or 'hydrolysis/solvolysis' mechanism in carbohydrate fluorescence sensing.

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Fig. 2 |. The assignment of pK_a values that would be associated with the three mechanistic postulates described in this Review.

Each scheme represents the structures involved with *o*-aminomethylphenylboronic acids as a function of pH (low to high pH given from left to right). In each case, the red arrows depict the dominant structures formed when raising the pH in the presence of a sugar. **a,b**, The N—B bonding postulate in the absence (**a**) and presence (**b**) of a diol, such as a sugar, hydroxycarboxylate or catechol. **c,d**, The pK_a switch postulate (that is, hydrolysis or solvent-insertion) in the absence (**c**) and presence (**d**) of a diol, such as a sugar,

hydroxycarboxylate or catechol. **e,f**, The 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 Review, this last postulate shows the proper assignments of the pK_a values.

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Fig. 3 |
The molecular structures of the key compounds discussed in this Review.

Fig. 4 |. Mechanistic considerations for the role of the *o*-aminomethyl group.

a, The proposed mechanism for boronate ester formation at pHs 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-catalysed expulsion of an inserted solvent with a general base-catalysed 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.

Fig. 5 |. Reactions and data relevant to the discussion of photophysics of the systems described in this Review.

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 $\lambda_{\text{ex}} = 368$ nm (blue). Excitation scan with $\lambda_{\text{em}} = 417$ nm (red). Excitation scan with $\lambda_{\text{em}} = 520$ nm (green). Emission scan with $\lambda_{\text{ex}} = 408$ nm (purple). Adapted from ref. ⁶⁶, American Chemical Society (**b**).

Fig. 6 |. Further data and reactions related to the photophysics of the systems described in this Review.

a, Fluorescence pH titration for boronic acid probe **10** (4 μ M; circles) and **10** with fructose complex (**10**: 4 μ M; fructose: 100 mM; squares) in water. $E_x = 450$ nm, slit/slit: 2 nm/2 nm; data taken from ref. ⁶⁸. **b,c**, 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 proposed mechanisms for emission turn-on

Structure	Theory	Assertor	Year
	N-B 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