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Equilibrium Studies of *ortho*-Iminylphenylboronic Acids

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Abstract
Structural studies of a three-component assembly – a host and two distinct guests – were carried out using a combination of ^{11}B and ^{1}H NMR. In aprotic solvent, the iminyl group that forms *ortho* to the boronic acid or boronate ester group can form a dative N-B bond. In protic solvent, a molecule of solvent inserts between the nitrogen and boron atoms, partially ionizing the solvent molecule. Additionally, ^{11}B NMR was used in combination with a seventh-order polynomial to calculate five binding constants for each of the individual steps in protic solvent. Comparison of these binding constants was used to establish positive cooperativity between the binding of the two guests.

Introduction

Host-guest equilibrium chemistry is typically described by one-to-one or two-to-one binding, and our group and others have previously published algebraic equations to describe the associated equilibria.[1,2] However, there could exist a system in which one host has two distinct guests, and a mathematical description of that complex equilibrium has not yet been described (Scheme 1).

$$H + G_1 + G_2 \rightleftharpoons HG_1 + G_2$$

$$HG_2 + G_1 \rightleftharpoons HG_1G_2$$
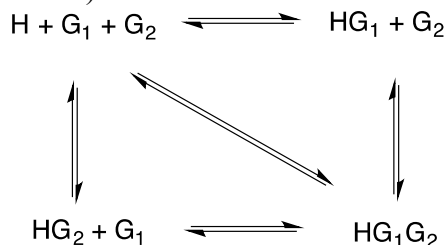
Scheme 1. Three-component assembly with one host and two distinct guests.

This three-component assembly allows for the possibilities of either guest binding the host first, or, potentially, an entropically unlikely simultaneous binding of both guests in a termolecular reaction. If, as expected, the two guests do not bind simultaneously, there is also a possibility for either positive or negative cooperativity – that is, binding of the first guest could effect a change in the binding constant of the second guest.

One paradigmatic example of a dual-guest equilibrium comes from the Bull and James groups, where they have developed a series of three-component assemblies using boronic acids with the goal of generating chiral shift reagents.[3–7] The enantio-discriminating unit is formed by the reaction of 2-formylphenylboronic acid (2-FPBA), enantiopure 1,1'-bi-2-naphthol (BINOL), and an α-chiral primary amine. The three-component assembly forms a Schiff base and a boronate ester (Scheme 2). The different diastereomers formed have distinct ^{1}H NMR spectra. The intensities thereof can be directly related to the enantiomeric composition of the original amine. Others have sought to exploit the reversibility and simplicity of the reaction to devise supramolecular architectures.[8–10] This reaction is fast, highly efficient, and relatively simple, requiring no purification steps.
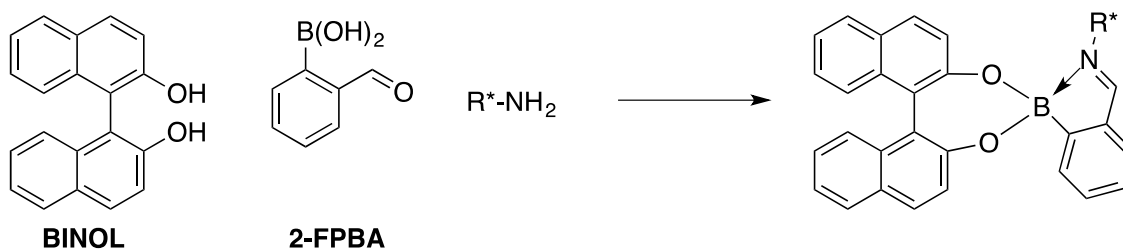
Scheme 2. Three-component assembly developed by the James and Bull groups.

While the Bull-James assembly employs an *ortho*-iminomethyl group, one of the most common design elements that facilitate the recognition of diols at neutral pH is an *ortho*-aminomethyl group on a phenylboronic acid. This functionality leads to an increase

in binding under physiological conditions.[11,12] One consequence of the interest in such systems was the discovery by our group of the prevalence of two different types of interactions between the boronic acid functionality and the amine.[13] Through the use of several coupled analytical techniques, such as ^{11}B NMR spectroscopy, X-ray diffraction, and computational modeling, it was determined that two different possibilities exist for these compounds. The first one involves a dative bond formed between the nitrogen and the boron, long envisioned as the main mode of N-B bond interaction (Scheme 3a). The other involves a single protic solvent molecule inserting between the nitrogen and boron (Scheme 3b). By characterizing crystal structures for each of the different interaction modes and then subjecting each type of species to ^{11}B NMR analysis, it was possible to assign distinct chemical shift values to each of the two possible variants of tetrahedral boron. One of the main conclusions of this work was that aprotic solvents favor the N-B bonded form while protic solvents promote near exclusive formation of the solvent-inserted mode of interaction. This was found for both boronic acids and boronate esters.

a)   b)
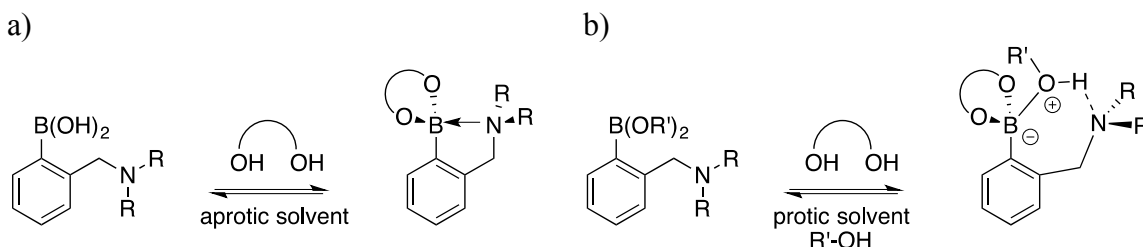
Scheme 3. a) N-B interaction in aprotic solvent. b) N-B interaction in protic solvent.

Thus, we reasoned that the same kind of analysis employed to structurally characterize *ortho*-aminomethylphenylboronic acid complexes could be used to characterize *ortho*-iminomethylphenyboronic acid complexes such as those that arise in the Bull-James assembly. Therefore, our aims were two-fold. First, an analysis of the James-Bull assembly could serve as a model system for a three-component assembly that binds two different guests, potentially displaying cooperativity, and second, we would be capable of

deciphering the extents of N-B bond or solvent insertion using our previously developed ^{11}B NMR spectroscopic methods.

The system we have elected to characterize is the three-component binding of *ortho*-formylphenylboronic acid (2-FPBA) as host (B), with catechol (D) and benzylamine (A) as the two distinct guests (Scheme 4).
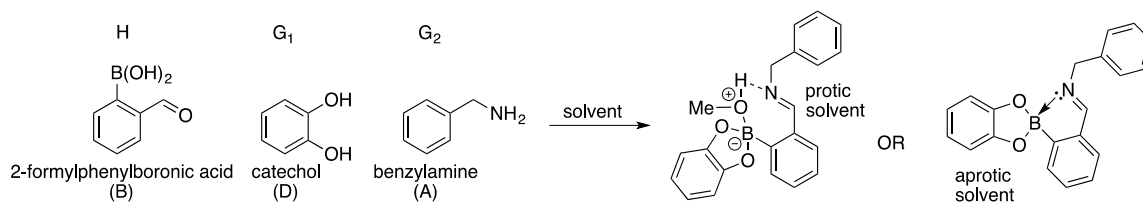
Scheme 4. *Three-component assembly with 2-FPBA, catechol, and benzylamine.*

Herein, we describe the use of our established ^{11}B NMR characterization methods in structural studies of in both aprotic and protic solvent. Further, we report an algebraic function for extracting the individual equilibrium constants involved in the study.

Results and Discussion

In an effort to characterize the coordination modes and structures of the components involved in the Bull-James assembly, a number of ^{11}B NMR titrations were undertaken. The chemical shifts for the boron resonances were referenced to numbers obtained and established in prior work.[13,14] Peaks in the range of 25-35 ppm are assigned to a boron atom in an arylboronic acid that displays a trigonal planar geometry, while tetrahedral boron appears further upfield. For these species, previous examples show that signals attributed to a N-B bond are observed at approximately 10-15 ppm and those corresponding to solvent insertion are found at approximately 5-10 ppm. While such resonances present clear distinctions, several other resonances appear in the experiments discussed below, which are attributed to intermediates in the condensation mechanisms. Some of these assignments

are speculative, albeit quite logical based upon the mechanisms of Schiff-base formation, boronate ester formation, and the pK_a values of iminium and ammonium groups.

^{11}B and ^{1}H NMR Experiments Performed in Aprotic Solvent

Four titrations were carried out in acetonitrile-d3 in order to measure the four bimolecular binding constants shown in Scheme 1. Figure 1 shows all of the structures that have been assigned to the NMR peaks in the titrations that follow.
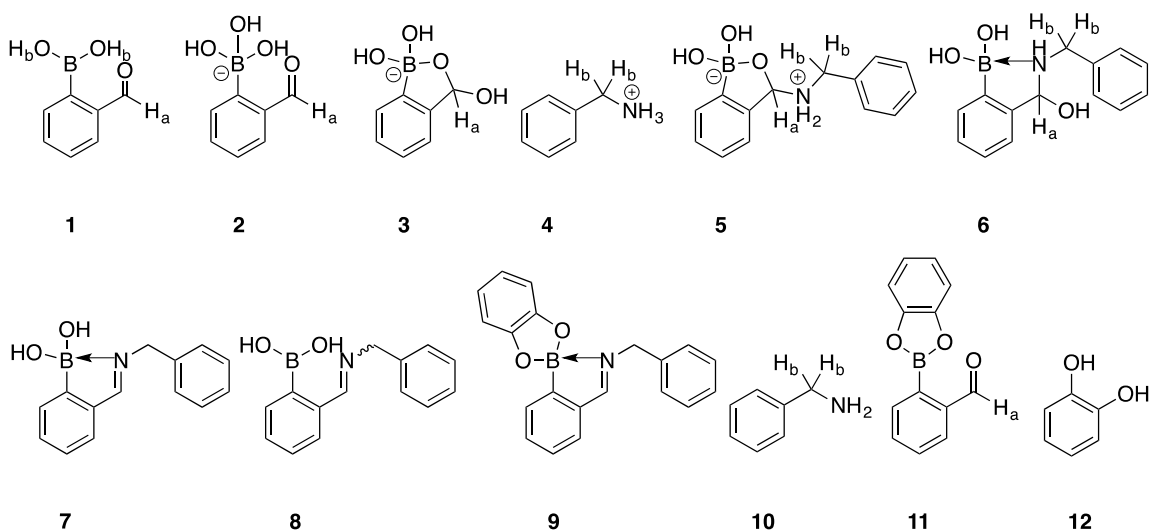
Figure 1. Structures assigned to the peaks in the ^{11}B and ^{1}H NMR titrations in aprotic solvent.

In the first study, benzylamine (A, 0-12 mM) was titrated into 2-FPBA (B, 10 mM) in acetonitrile, and an equivalent of catechol (D, 10 mM) was added at the end of the titration (Figure 2). The first ^{11}B spectrum (Figure 2a) shows 2-FPBA alone, with only one signal at 29.5 ppm, corresponding to the trigonal boron atom of 2-FPBA, **1**. When benzylamine is first added, a second signal at 7.5 ppm grows in. We attribute this peak to a structure with a hydroxylated boron atom, **2**, a result of the ionization of the small amount of water that is present in solution by the weakly basic amine. This supposition is supported by the presence of the downfield aldehyde peak at 10.4 ppm in the ^{1}H spectrum (Figure 2b). Compound **2** would be in equilibrium with **3**, a cyclic hemiacetal. Structure **3** is

consistent with the very small ^{1}H peak at 8.6 ppm. The hypothesis that structures **2** and/or **3** form upon ionization of water is supported by a control experiment in which triethylamine was added to 2-FPBA (**1**) in acetonitrile. A [11]B peak at 6.5 ppm in the presence of triethylamine is comparable to the peak at 7.5 ppm in Figure 2a. Since triethylamine is a tertiary amine and cannot form an imine, this result is consistent with formation of a tetrahedral, anionic boron species. The counterion for either **2** or **3** is benzylammonium, **4**. As additional amine is added, a third [11]B signal arises at 9 ppm. For this peak, we propose the hemiaminal species **5** and **6** as potential structures. These species would explain the presence of the ^{1}H peak at 8.25 ppm, corresponding to the methine proton, and the methylene peak is reasonably expected to overlap with the methylene peak of **4**, because the nitrogen atom either carries a positive formal charge or is the donor in a dative bond. In this interpretation of the ^{11}B NMR spectra, the first addition of amine acts to dehydrate the solution by being protonated and delivering an equivalent of hydroxide to the boron. As additional amine is added, it begins to incorporate into the assembly. Peaks corresponding to 2-FPBA (**1**) and the hydroxylated 2-FPBA species (**2** and **3**) both disappear as the **5**/**6** peak grows. With increasing concentration of amine, a signal at 15.7 ppm dominates the [11]B spectrum, while a minor peak at 26.6 ppm appears. The peak at 15.7 ppm is consistent with a N-B interaction, as shown in structure **7**, and the peak at 26.6 ppm is a new trigonal species. We attribute this peak to a small amount of an open-form structure **8**. That is, we believe this is an imine without a Schiff base interaction. It appears that the N-B bonded and open-form structures, **7** and **8**, respectively, are in equilibrium with one another, as the ratio between them remains constant. The open-form could be the *E*-imine without the N-B bond, but we suspect that it is more likely the *Z*-imine, which

cannot form the N-B bond. Finally, when catechol is added, the ^{11}B spectrum shows only one peak at 13.5 ppm. This chemical shift is consistent with an N-B interaction, and since it is distinct from the shift of the boronic acid, **7**, we attribute this peak to the full assembly, **9**.
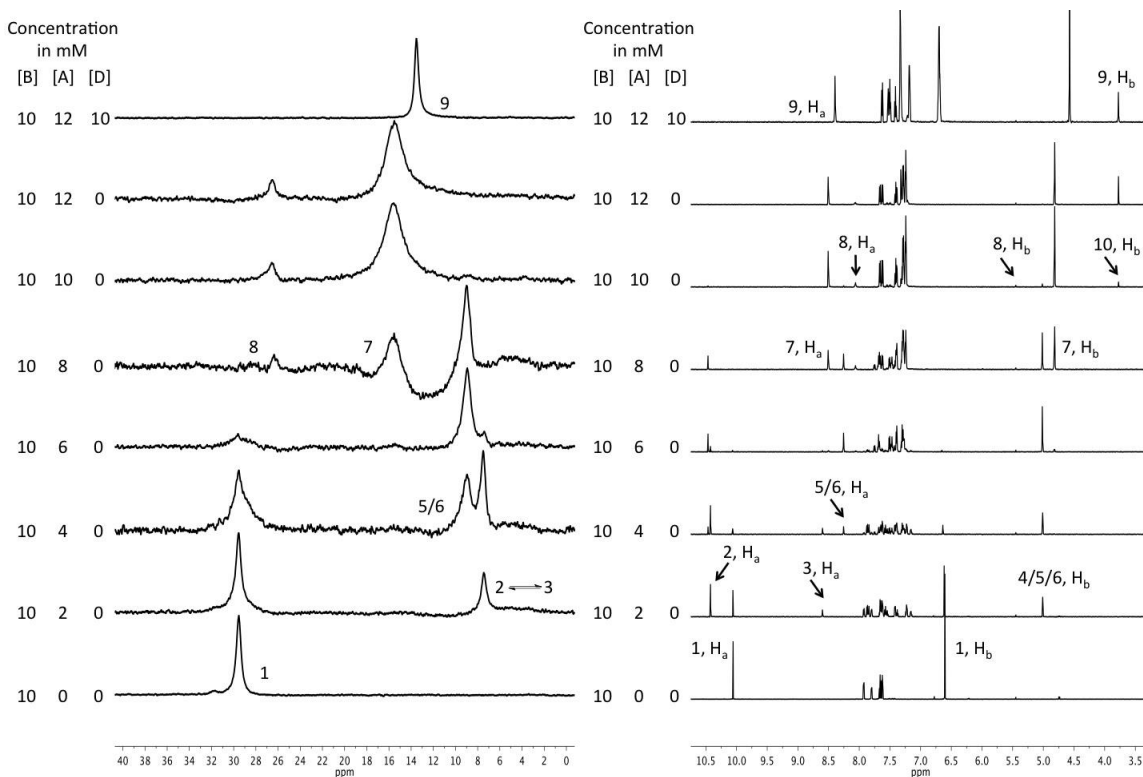
Figure 2. ^{11}B (a) and ^{1}H (b) spectra showing the addition of benzylamine (0-12 mM) into 2-FPBA (10 mM) in CD$_3$CN with the addition of one equivalent (10 mM) of catechol at the end of the titration. The bottom spectrum is 2-FPBA alone.

In the second titration, catechol (D, 0-12 mM) was titrated into 2-FPBA (B, 10 mM) and benzylamine (A, 10 mM) (Figure 3) in acetonitrile. The first ^{11}B spectrum (Figure 3a) shows 2-FPBA alone, with one signal at 29.5 ppm, corresponding to trigonal boron in 2-FPBA (**1**). The 1:1 mixture of 2-FPBA and benzylamine gives a signal at 15.7 ppm, which can be attributed to the N-B bonded imine **7**. As well, the peak attributed to structure **8** is present and still appears to be at equilibrium with **7**. As expected, this spectrum is identical to the spectrum with one equivalent of amine in Figure 2. As catechol is added, the ^{11}B

signal for the imine disappears and is replaced by a signal at 13.5 ppm, which represents the full three-component assembly, **9**. The imine presumably has a weaker N-B bond than the full assembly, as its signal is further downfield. This change suggests positive cooperativity in the sense that the binding of the diol to boronic acid strengthens the binding (increase the binding constant) of amine binding to the boronic acid.
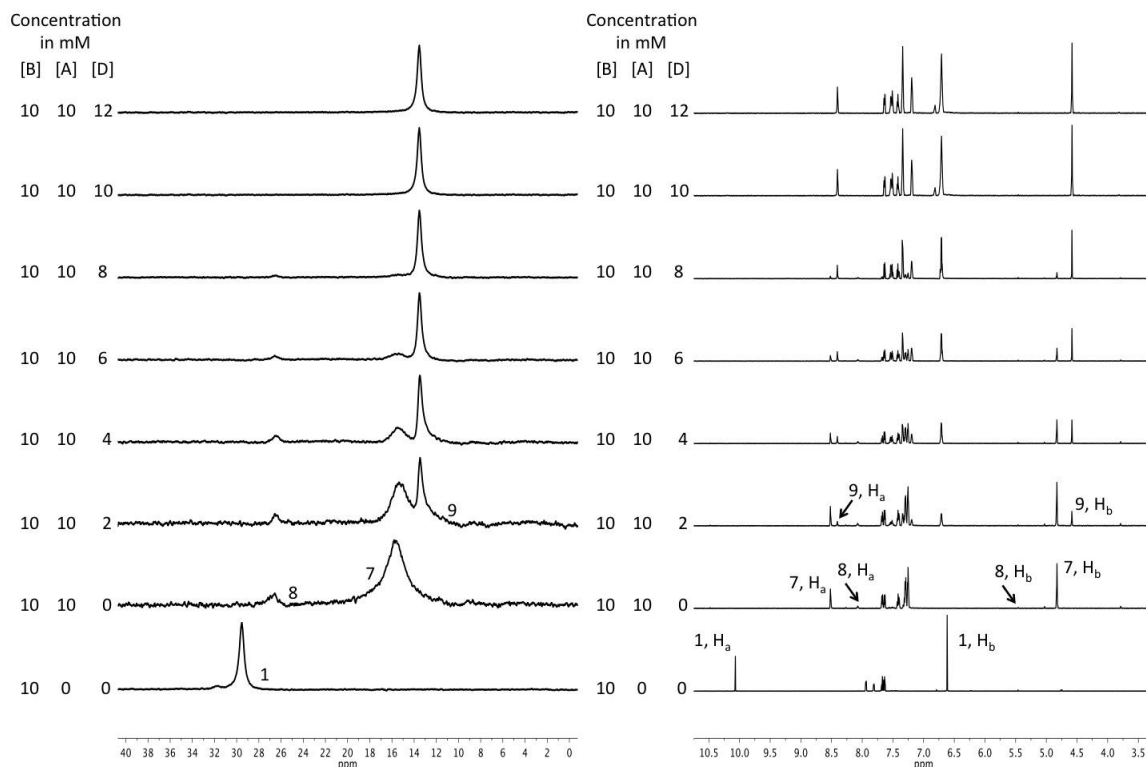
Figure 3. ^{11}B (a) and ^{1}H (b) spectra showing the addition of catechol (0-12 mM) into 2-FPBA (10 mM) and benzylamine (10 mM) in CD$_3$CN. The bottom spectrum is 2-FPBA alone.

In the third titration, catechol (D, 0-12 mM) was titrated into 2-FPBA (B, 10 mM) and an equivalent of benzylamine (A, 10 mM) was added at the end of the titration. (Figure 4). The first ^{11}B spectrum (Figure 4a) shows 2-FPBA alone. The 1:1 mixture of 2-FPBA and catechol shows signals for the boronic acid **1** as well as the boronate ester **11**, at 32.4 ppm. The addition of catechol to form the boronate ester results in a downfield shift, suggesting that catechol is more electron-withdrawing than the hydroxyl groups. The ^{1}H

spectrum (Figure 4b) is consistent with this assignment, as the catechol peaks centered at 6.7 ppm (**12**) shift to 7.3 ppm (**11**). Unlike the addition of amine that leads to full condensation on the aldehyde at one or slightly more than one equivalent, the combination of catechol is far from complete with the boronic acid at one equivalent. Yet, addition of an equivalent of amine leads to full complexation of the catechol. Thus, it appears that the amine cooperatively assists the condensation of catechol.
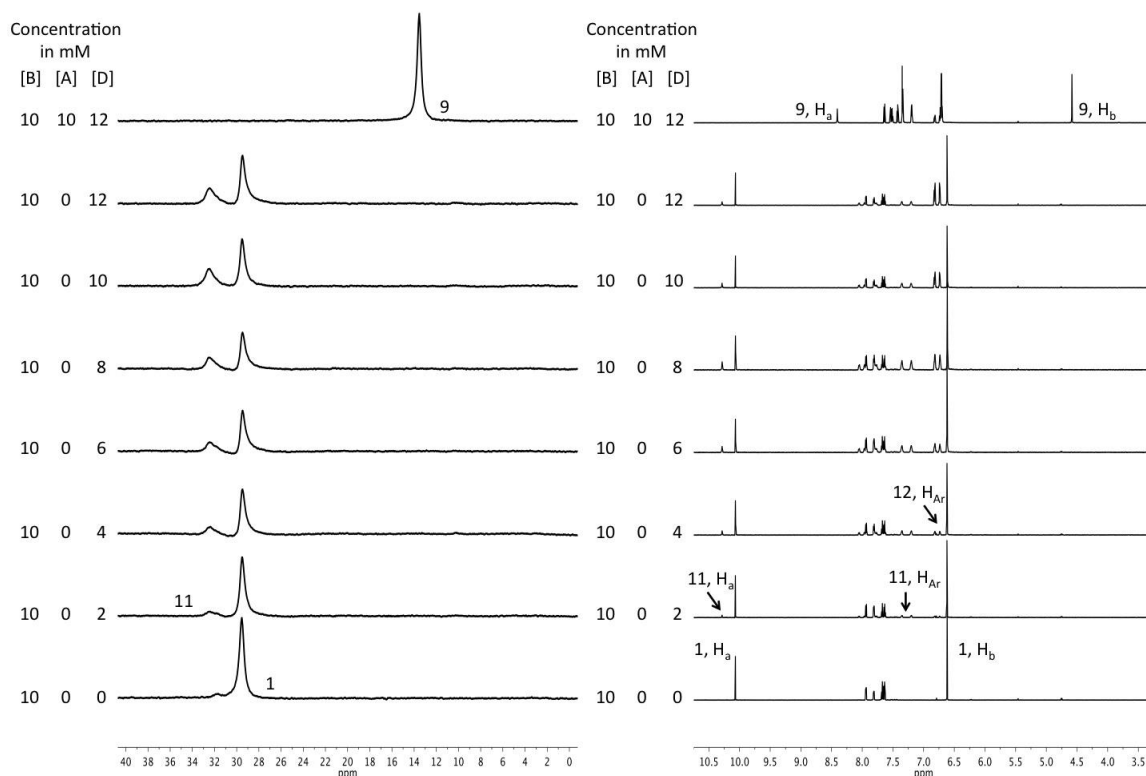
Figure 4. ^{11}B (a) and ^{1}H (b) spectra showing the addition of catechol (0-12 mM) into 2-FPBA (10 mM) in CD$_3$CN with the addition of one equivalent (10 mM) of benzylamine at the end of the titration. The bottom spectrum is 2-FPBA alone.

In the fourth titration, benzylamine (A, 0-12 mM) was titrated into 2-FPBA (B, 10 mM) and catechol (D, 10 mM) (Figure 5). The first ^{11}B spectrum (Figure 5a) shows 2-FPBA alone. The 1:1 mixture of 2-FPBA and catechol again shows signals for the boronic acid **1** and the boronate ester **11**. The ^{1}H shift of the catechol peaks in Figure 4b is also

evident in Figure 5b. As benzylamine is added, the signal for the three-component assembly begins to dominate and then becomes the sole signal when [A] = 10 mM.

Figure 5. ^{11}B (a) and ^{1}H (b) spectra showing the addition of benzylamine (0-14 mM) into 2-FPBA (10 mM) and catechol (10 mM) in CD$_3$CN. The bottom spectrum is 2-FPBA alone.

Throughout these four titrations, various hemiaminal and N-B bonded species were assigned using a combination of ^{11}B and ^{1}H peaks. A crystal grown from acetonitrile confirms our assignment of the N-B bond in the 1:1:1 complex (Figure 6). This N-B bond measures 1.68 Å, which is consistent with a dative bond. The imine bond is 1.25 Å, which is a typical bond length for a C=N bond.
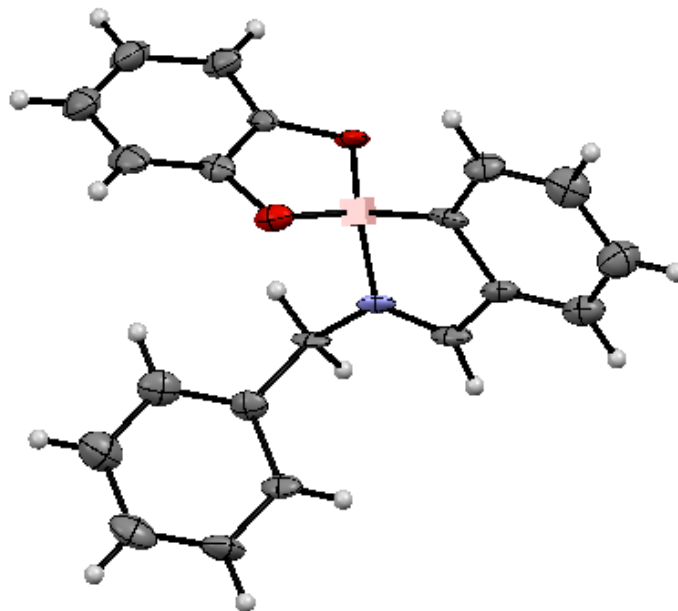
Figure 6. Crystal structure of 2-formylphenylboronic acid, catechol, and benzylamine; the crystal was grown in acetonitrile.

^{11}B and ^{1}H NMR Experiments Performed in Protic Solvent

Four titrations were carried out in methanol-d4 in order to measure the four bimolecular binding constants shown in Scheme 1. Figure 7 shows all of the structures that have been assigned to the NMR peaks in the titrations that follow.
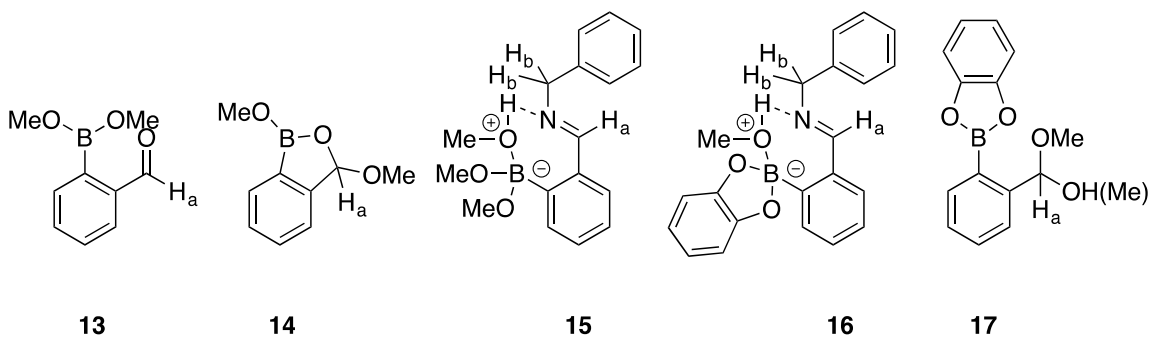

Figure 7. Structures assigned to the peaks in the ^{11}B and ^{1}H NMR titrations in protic solvent.

For the first study in protic media, benzylamine (A, 0-14 mM) was titrated into 2-FPBA (B, 10 mM) in methanol, and an equivalent of catechol (D, 10 mM) was added at the end of the titration (Figure 8). The first ^{11}B spectrum (Figure 8a) shows 2-FPBA alone,

with two signals at 30 and 31 ppm, corresponding to trigonal boron. We attribute one signal to the boronate ester formed with the solvent (**13**), and the other signal to a cyclic boronate acetal (**14**). This is consistent with the ^{1}H spectrum (Figure 8b), which shows a singlet at 6 ppm for the acetal proton. As amine is added, a [11]B signal at 10.6 ppm emerges. This signal corresponds to the solvent-inserted imine, **15**. When an equivalent of catechol is added, no new peaks emerge, and the signal remains at 10.6 ppm. Presumably, the imine and the full assembly, **16**, coincidentally have the same chemical shift. We have previously seen the same coincidental overlap in our study of *ortho*-aminophenylboronic acid condensation with diols in methanol.[13]

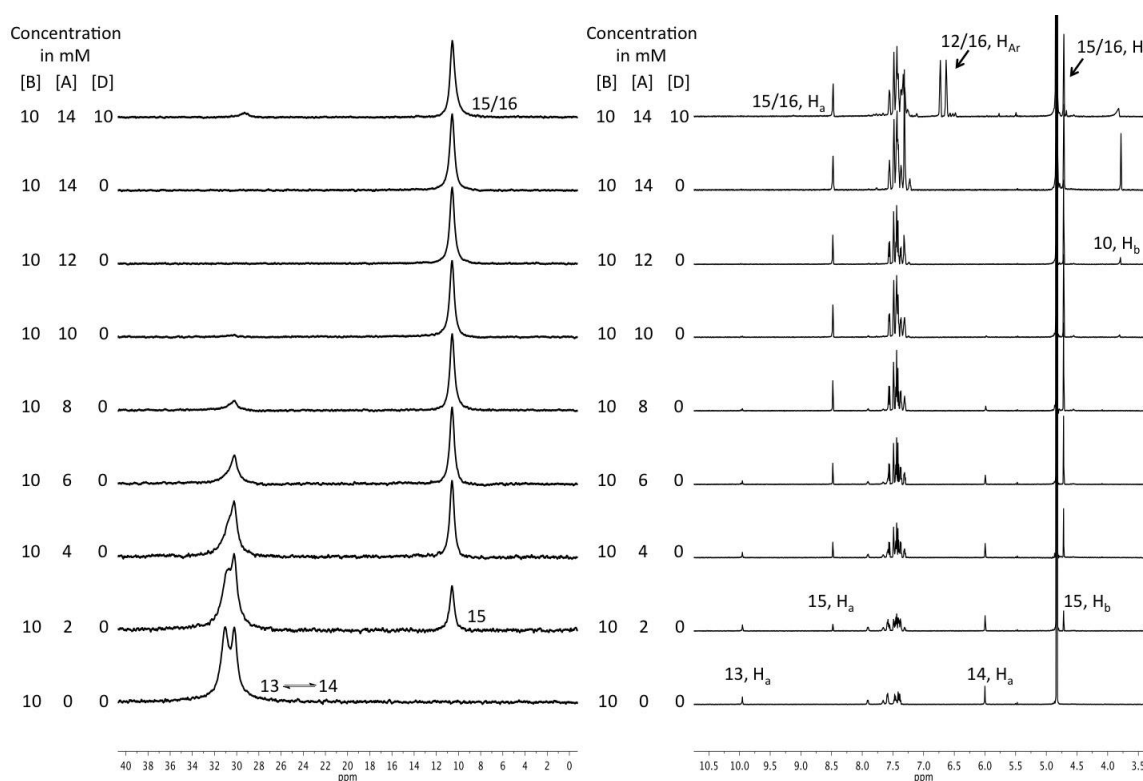*Figure 8. [11]B (a) and [1]H (b) spectra showing the addition of benzylamine (0-14 mM) into 2-FPBA (10 mM) in CD₃OD with the addition of one equivalent (10 mM) of catechol at the end of the titration. The bottom spectrum is 2-FPBA alone.*

In the second titration, catechol (D, 0-12 mM) was titrated into 2-FPBA (B, 10 mM) and benzylamine (A, 10 mM) (Figure 9). The first [11]B spectrum (Figure 9a) shows 2-FPBA

alone. The 1:1 mixture of 2-FPBA and benzylamine gives a signal at 10.6 ppm, and titrating in catechol does not effect any change due to the chemical shift overlap of **15** and **16**. Importantly, in this titration there is only one set of aromatic peaks for catechol in the ^{1}H spectrum (Figure 9a). It is possible that catechol is thus only in one form (all bound or none bound) or that in the ^{1}H spectrum, as well as the ^{11}B spectrum, there is chemical shift overlap between species.
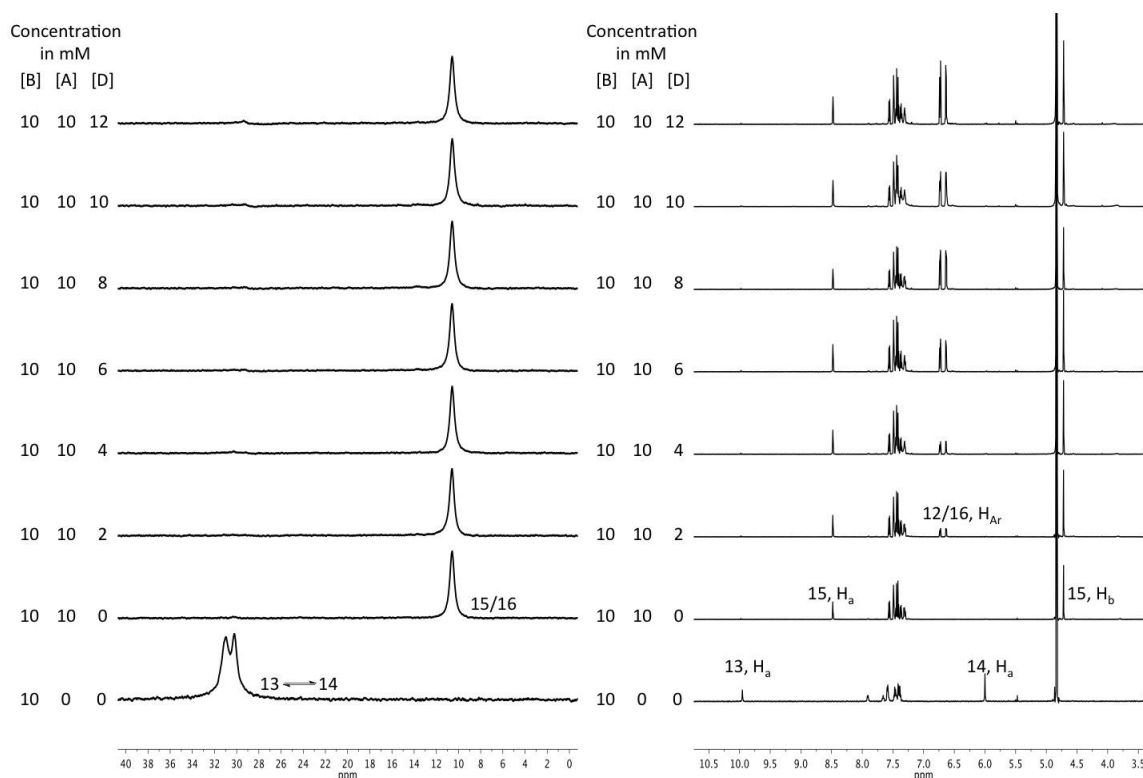
Figure 9. ^{11}B (a) and ^{1}H (b) spectra showing the addition of catechol (0-12 mM) into 2-FPBA (10 mM) and benzylamine (10 mM) in CD$_3$OD. The bottom spectrum is 2-FPBA alone.

In the third titration, catechol (D, 0-60 mM) was titrated into 2-FPBA (B, 10 mM) and an equivalent of benzylamine (A, 10 mM) was added at the end of the titration (Figure 10). The first ^{11}B spectrum (Figure 10a) shows 2-FPBA alone. As catechol was added, a peak grew in at 29.3 ppm. This peak in the trigonal boron region was thought to be the boronate ester formed with catechol, **11**. However, the only aldehyde peak in the ^{1}H

spectrum (Figure 10b) is for the methanol ester, **13**. For this reason, we propose structure **17**, which still contains a boronate ester, but no longer contains an aldehyde due to attack of the solvent. This is reasonable because the acetal **14** is observed, so the acetal or hemiacetal **17** can be expected to form under the same conditions. After one equivalent of benzylamine was added, the signal at 10.6 ppm demonstrated formation of the three-component assembly, **16**. Once again, as in acetonitrile, catechol does not bind strongly enough to fully convert the boronic acid (in this case, methyl boronate ester) to catechol boronate ester until an equivalent of amine is added. Note here that formation of the catechol boronate ester doesn't go to completion even in the presence of six equivalents of catechol. This titration shows that there is indeed chemical shift overlap between species; even as **13**, **14**, and **17** can be seen to form **15** and **16** in the [11]B spectrum as amine is added, the peaks for catechol in the ^{1}H spectrum still remain unchanged.

In the fourth titration, benzylamine (A, 0-14 mM) was titrated into 2-FPBA (B, 10 mM) and catechol (D, 10 mM) (Figure 11). The first ^{11}B spectrum (Figure 11a) shows 2-FPBA alone. The 1:1 mixture of 2-FPBA and catechol shows signals for the methanolic boronate ester **13** and the catechol boronate ester **16**, just as in Figure 10. As benzylamine is added, the signal for the three-component assembly begins to dominate.
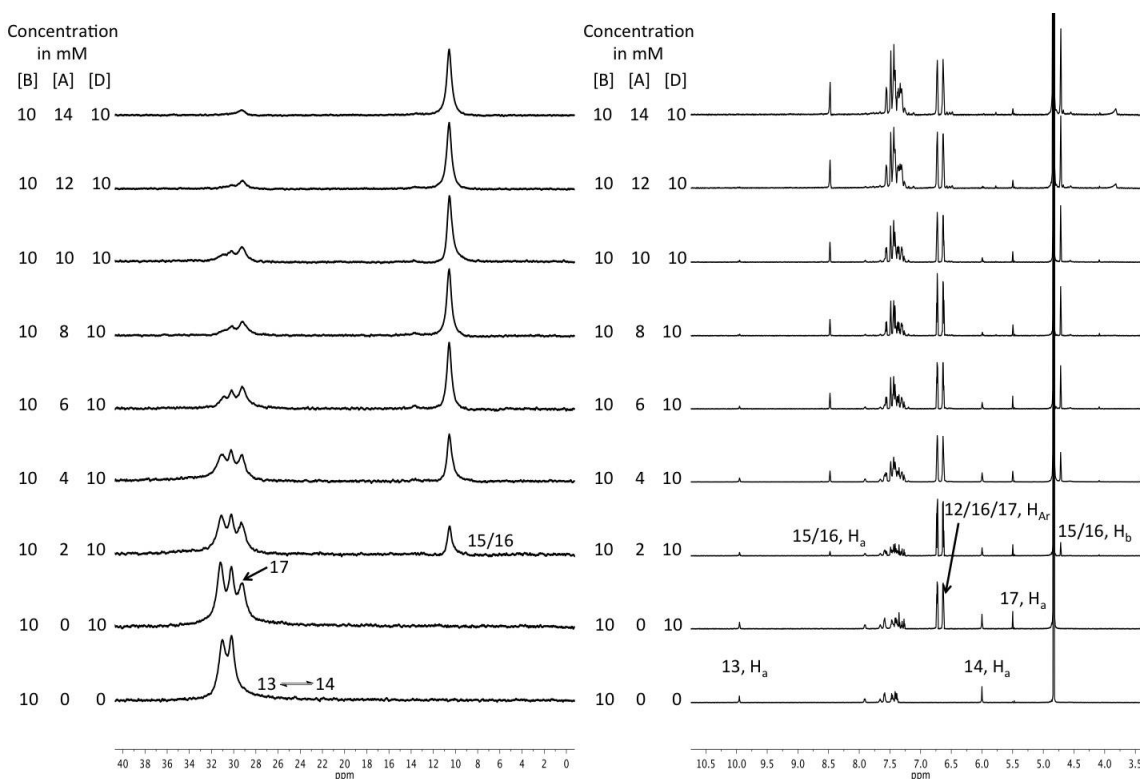

Figure 11. ^{11}B (a) and ^{1}H (b) spectra showing the addition of benzylamine (0-14 mM) into 2-FPBA (10 mM) and catechol (10 mM) in CD₃OD. The bottom spectrum is 2-FPBA alone.

In the four titrations in methanol, tetrahedral boron was always in the range of what has been assigned to solvent-inserted species in *ortho*-(aminomethyl)phenylboronic acids, and thus the *ortho*-iminophenylboronic acids and boronate esters were assigned to solvent-inserted species as well.

Deriving a Polynomial for the Three-Component Assembly

For the purposes of deriving the mathematical equations that describe the complex equilibrium of the three-component assembly, the binding constants and species involved will be defined as shown in Scheme 5.
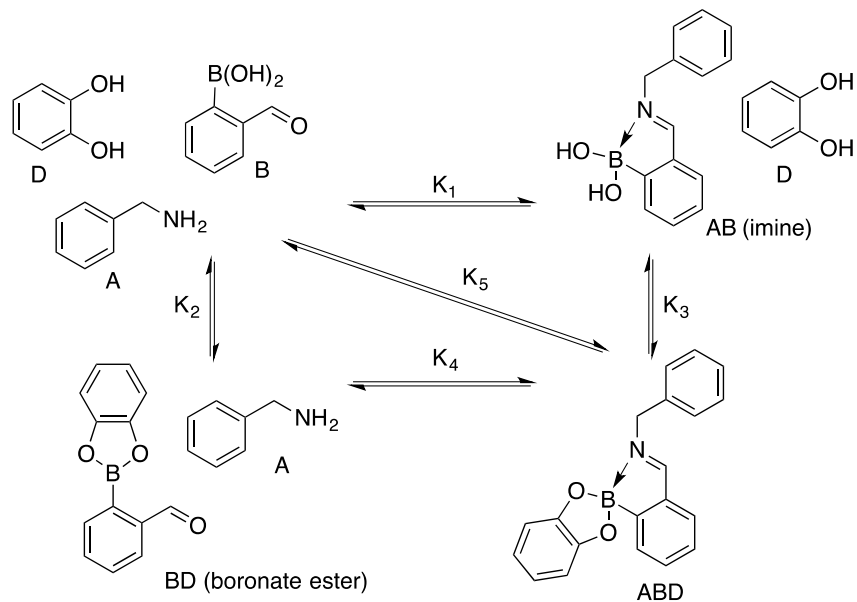
Scheme 5. Binding constants and species involved in the formation of the three-component assembly. The analogous structures for AB and ABD in methanol would have inserted solvent.

We begin the process by outlining the system of equations as defined by the association constant expressions and mass balances

$$K_1[A][B] = [AB] \qquad \text{Equation 1}$$
$$K_2[B][D] = [BD] \qquad \text{Equation 2}$$
$$K_3[AB][D] = [ABD] \qquad \text{Equation 3}$$
$$K_4[BD][A] = [ABD] \qquad \text{Equation 4}$$
$$K_5[A][B][D] = [ABD] \qquad \text{Equation 5}$$

$$[A]_T = [A] + [AB] + [ABD] \qquad \text{Equation 6}$$
$$[B]_T = [B] + [AB] + [BD] + [ABD] \qquad \text{Equation 7}$$
$$[D]_T = [D] + [BD] + [ABD] \qquad \text{Equation 8}$$

Our objective is to express [ABD] as a function of the constants K_i, $[A]_T$, $[B]_T$, $[D]_T$ and one variable, which we choose to be [B]. Note that $[X]_T$ denotes the initial

concentration of X, and the total amount of all species that contain X at equilibrium.

Substitutions give

$$[B]_T = [B] + K_1[B]\left(\frac{[A]_T-[ABD]}{1+K_1[B]}\right) + K_2[B]\left(\frac{[D]_T-[ABD]}{1+K_2[B]}\right) + [ABD] \quad \text{Equation 9}$$

which can be rearranged and simplified to

$$[ABD] = \frac{(1+K_1[B])(1+K_2[B])([B]_T-[A]_T-[D]_T-[B])+(1+K_2[B])[A]_T+(1+K_1[B])[D]_T}{1-K_1K_2[B]^2} \quad \text{Equation 10}$$

Another series of substitutions give

$$[ABD] = K_5\frac{[B]([A]_T-[ABD])([D]_T-[ABD])}{(1+K_1[B])(1+K_2[B])} \quad \text{Equation 11}$$

in which Equation 10 can be substituted for [ABD] in Equation 11 to give a seventh-order polynomial with respect to [B] (Equation 12). (See supplementary information for the full explanation of how to arrive at this solution.) Then Wolfram Mathematica can be used to solve for the coefficients of each ordered term.

$$Polynomial([B]) = \sum_{k=0}^{7}[B]^k(K_5\rho_k - \lambda_k) \quad \text{Equation 12}$$

where the ρ_k terms and the λ_k terms are as follows.

$\rho_0 = 0$
$\rho_1 = -([A]_T - [B]_T)([B]_T - [D]_T)$
$\rho_2 = ([A]_T - 2[B]_T + [D]_T)(1 + K_1[A]_T + K_2[D]_T - (K_1 + K_2)[B]_T)$
$\rho_3 = 1 + K_1[D]_T + 3K_2[D]_T + K_1K_2[D]_T^2 + K_2^2[D]_T^2 + K_1(K_1 + K_2)[A]_T^2$
$\qquad + (K_1^2 + 4K_1K_2 + K_2^2)[B]_T^2$
$\qquad - 2(2K_1(1 + K_2[D]_T) + K_2(2 + K_2[D]_T))[B]_T + (-2K_1^2[B]_T + K_2$
$\qquad + K_1(3 - 4K_2[B]_T + 2K_2[D]_T))[A]_T$
$\rho_4 = K_1^2([A]_T - [B]_T)(2 + K_2[A]_T - 2K_2[B]_T + K_2[D]_T)$
$\qquad + 2K_2(1 - K_2[B]_T + K_2[D]_T) + K_1(2 + 4K_2[D]_T + 2K_2^2[B]_T^2 + K_2^2[D]_T^2$
$\qquad + K_2(4 - K_2[B]_T + K_2[[D]]_T)[A]_T - K_2(8 + 3K_2[D]_T)[B]_T)$
$\rho_5 = K_2^2 + K_1K_2(4 + K_2[A]_T - 4K_2[B]_T + 3K_2[D]_T) + K_1^2(1 + K_2[D]_T + K_2^2[B]_T^2$
$\qquad - K_2(4 + K_2[D]_T)[B]_T + K_2(3 - K_2[B]_T + K_2[D]_T)[A]_T)$
$\rho_6 = K_1K_2(2K_2 + K_1(2 + K_2[A]_T - 2K_2[B]_T + K_2[D]_T))$
$\rho_7 = K_1^2K_2^2$

$\lambda_0 = [B]_T$
$\lambda_1 = -1 - K_1[A]_T - K_2[D]_T + 2(K_1 + K_2)[B]_T$
$\lambda_2 = K_1^2(-[A]_T + [B]_T) + K_2(-2 + K_2[B]_T - K_2[D]_T) - K_1(2 + 2K_2[A]_T - 3K_2[B]_T$
$\qquad + 2K_2[D]_T)$

$$\lambda_3 = -K_2^2 - K_1^2(1 + K_2[A]_T + K_2[D]_T) - K_1K_2(3 + K_2[A]_T + K_2[D]_T)$$
$$\lambda_4 = K_1K_2(K_1(K_1 + K_2)[A]_T + K_2(K_1 + K_2)[D]_T - (K_1^2 + 3K_1K_2 + K_2^2)[B]_T)$$
$$\lambda_5 = K_1K_2(K_2^2 + K_1^2(1 + 2K_2[A]_T - 2K_2[B]_T + K_2[D]_T)$$
$$+ K_1K_2(3 + K_2[A]_T - 2K_2[B]_T + 2K_2[D]_T))$$
$$\lambda_6 = K_1^2K_2^2(2K_2 + K_1(2 + K_2[A]_T - K_2[B]_T + K_2[D]_T))$$
$$\lambda_7 = K_1^3K_2^3$$

Application of the Polynomial to the Three-Component Assembly

Now we turn to the application of the polynomial derived in the section above. In theory, K_1 could be determined using the integrations from Figure 2 (aprotic media) and Figure 8 (protic media), K_3 could be determined using Figures 3 and 9, K_2 could be determined using Figures 4 and 10, and K_4 could be determined using Figures 5 and 11. However, some of these theoretically possible calculations have complications that render this approach impossible. In acetonitrile, K_1 cannot be determined because formation of the imine (AB) is not represented by a single step, and doesn't even generate a single form of AB. In acetonitrile, K_3 cannot be determined because the imine is formed quantitatively. Thus, the binding constant is too large to calculate due to quantitative formation of ABD from AB and D. In methanol, K_3 cannot be calculated because the chemical shift of AB is indistinguishable from the chemical shift of ABD. In both solvents, K_4 cannot be calculated because the first step, formation of the boronate ester (BD) is not complete, and thus adding amine would conflate the two steps whose individual binding constants we wish to measure.

This means that the only individual steps we can measure by integration of the ^{11}B NMR spectra is the formation of BD in both solvents, which is represented by the binding constant K_2, and the formation of AB in methanol, which is represented by the binding constant K_1. K_2 was thus calculated in methanol by integrating the B and BD signals for

three different concentrations of [D], as shown in Figure 12. The three spectra shown were chosen because the overlapping B and BD peaks were similar enough in size to make a vertical division in their integrations and still reasonably estimate their areas, as shown in the figure. The resulting calculated concentrations of B, BD, and D (calculated as [D] = $[D]_T - [BD]$) are shown in Table 1. Then the values of K_2 were computed and averaged over the three measurements to give $K_2 = 112$ M^{-1}. Importantly, the three values are consistent and thus a credible estimation of the value of K_2.
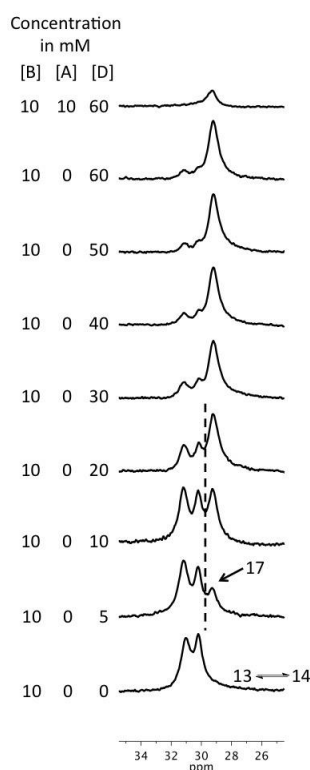

Figure 12. Portion of Figure 11a showing the integrations of the B (structures 13 and 14) and BD (structure 17) peaks.

Table 1. Calculated values of [B], [BD], and [D] from the integrations of ^{11}B NMR peaks corresponding to B and BD.

$[D]_0$	[B]	[BD]	[D]	K_2
5 mM	7.61 mM	2.39 mM	2.61 mM	121 M^{-1}
10 mM	6.04 mM	3.96 mM	6.04 mM	109 M^{-1}
20 mM	4.03 mM	5.97 mM	14.0 mM	106 M^{-1}
			Average	**112 M^{-1}**

For the binding constant in acetonitrile, the peaks in Figure 4 were (more simply) integrated, concentrations were calculated, and four values of K_2 between 94 and 101 gave an average of $K_2 = 98$ M^{-1}. Likewise, the precision of the calculated binding constants lends credibility to the value. The value of K_1 in methanol was calculated to be 1100 M^{-1} using integrations from Figure 8.

Only K_1 and K_2 were able to be calculated directly and empirically in methanol, but the other values can be calculated using the polynomial([B]). With K_1 and K_2 given, K_5 is the only unknown in the polynomial and it can be calculated using K_1 and K_2. A termolecular reaction is unlikely, and we will treat K_5 as only a theoretical possibility. However, given the fact that $K_5 = K_1 \times K_3 = K_2 \times K_4$, and with K_1, K_2, and K_5 known, we can calculate K_3 and K_4. To execute this approach, Figure 11 was reexamined. K_4 could not be calculated from this titration because the reaction corresponding to K_2 was incomplete, but this titration can still be used because it contains A, B, and D, all simultaneously. For a given concentration of $[A]_T = 4$ mM, [B] was calculated by integrating all peak areas to give [B] = 5.417 mM. With a constant $[A]_T$ and measured variable [B], K_5 was calculated to be 2.69 x 10^6 M^{-1} using Wolfram Mathematica. The

calculated values of K_1, K_2, and K_5 were then used to determine K_3 and K_4. The summary of binding constants is shown in Table 2. The same process in acetonitrile could not be carried out due to the fact that K_1 could not be calculated in acetonitrile.

Table 2. Summary of binding constants in methanol.

Binding Constant	Value
K_1	1100 M^{-1}
K_2	112 M^{-1}
K_3	$2.45 \times 10^3 \text{ M}^{-1}$
K_4	$2.40 \times 10^4 \text{ M}^{-1}$
K_5	$2.69 \times 10^6 \text{ M}^{-1}$

To evaluate cooperativity, the values of K_1 and K_4 should be compared (since these binding constants correspond to addition of amine) and the values of K_2 and K_3 should be compared (since these binding constants correspond to the addition of diol). Since K_4 is greater than K_1 and K_3 is greater than K_2, it can be concluded that both guests experience positive cooperativity. In other words, the binding of a guest is improved when the other guest has already bound, and the two binding events reinforce one another. In this way, the numerical analysis mirrors the structural interpretation of binding throughout the titrations.

Conclusions

We have described the binding of a three-component *ortho*-iminophenylboronate ester assembly. Like *ortho*-(aminomethyl)phenylboronic acids and boronate esters, these assemblies form N-B bonds in aprotic solvent and solvent-inserted species in protic solvent. We have also demonstrated that the equilibrium between one host and two distinct guests can be described by a seventh-order polynomial, and that this polynomial can be used along with ^{11}B NMR data to calculate the five equilibrium constants involved in this complex equilibrium in methanol. Finally, the comparison of these five equilibrium

constants leads to the conclusion that guest binding is cooperative, in that binding one guest strengthens the binding of the second guest.

Acknowledgements

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