Isomer-sensitive deboronation in reductive aminations of aryl boronic acids

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ABSTRACT

Deboronation is observed during the reductive amination of formylphenylboronic acid (FPBA) to the amine termini and side chains of peptides. This deboronation is sensitive to the isomerism of the boronic acid (BA), with *ortho*-FPBA yielding complete deboronation in the preparation of an *N*-terminally-modified dipeptide. The observed behavior is also clearly mediated by the chemical identity of the amine substrate. These results reveal a previously undocumented subtlety of BA functionalization and highlight the importance of thorough spectroscopic characterization in the preparation of peptide and small molecule BAs.

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Boronic acids (BAs) are useful functionalities for programmable stimuli-responsive chemistry, due to their Lewis acid character with pK_a values tunable across physiological pH and their reversible binding to saccharides, catechols, and other compounds bearing 1,2-diol moieties.¹ In addition, BAs have emerged as valuable reagents and intermediates in important synthetic pathways, such as Suzuki cross-coupling reactions,¹ and as selective enzyme inhibitors, exemplified by the commercial success of the anti-cancer drug Bortezomib.² Over the last decade, researchers have studied a variety of BA-modified peptides, focusing on the inhibitory properties of the BA or the use of the BA as a functional element in peptide self-assembly.³ For example, our group has recently reported the reversible self-assembly behavior of a BA dipeptide, controllable by pH, ionic strength, and the addition of polyols.^{3s}

BAs are frequently attached to peptides, and more generally a variety of small molecules, via conventional acid-amine coupling chemistry or reductive aminations. More specifically, formylphenylboronic acid (FPBA) has been used in solid phase peptide synthesis to graft the phenylboronic acid functionality through lysine side chains of the peptide via reductive amination.^{3g,3k,4} In addition, a variety of small molecule BAs have been derived from reductive aminations involving FPBA.⁵ Although several pathways are known by which deboronation of BA compounds can occur, most notably by strong oxidants such as peroxides,¹ deboronation has not been reported in reductive aminations. However, some groups have noted instability of

ortho-FPBAs to the borohydride reducing agents typical of such aminations, resulting in the formation of benzoxaborole species.^{5j,5l,5m,5o,6}

Based on the past literature, then, we were surprised to discover that reductive amination of *ortho*-FPBA to the *N*-terminus of a simple dipeptide resulted in a product (**2**) completely devoid of boron. Briefly, the dipeptide of phenylalanine (Phe-Phe), resin-bound at its *C*-terminus and bearing a deprotected *N*-terminus was shaken with an excess of *ortho*-FPBA and sodium cyanoborohydride, NaCNBH₃, predissolved in dimethylformamide (DMF). Additional synthetic details are provided in the Supplementary Information. Extensive deboronation was also similarly observed in the reductive amination of *ortho*-FPBA to the lysine side chain of a linear nonapeptide (**3**, Figures S1-S3). In contrast, the reductive amination of *para*-FPBA to the same dipeptide gives a product (**1**) that is more consistent with the expected outcome of amination, *i.e.*, there was little apparent deboronation.

Figure 1 shows the result of electrospray ionization-mass spectrometry (ESI-MS) performed on the *N*-terminally modified peptides obtained using the *para* and *ortho* isomers of FPBA. The spectrum for the *para* product **1** (Figure 1a) shows the expected [M-H]⁺ ion indicating the successful attachment of FPBA, as well as a small signature of the unmodified dipeptide, [M-H-FPBA]⁺. Furthermore, the [M-H]⁺ ion exhibits isotopic splitting characteristic of boron; namely, the principal peak is preceded by a peak less one Da, reflecting the natural occurrence

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of ^{10}B . The spectrum for the *ortho* product **2** (Figure 1b) instead shows a product that is 18 Da less than the expected $[\text{M}-\text{H}]^-$ ion. Indeed, this product no longer exhibits the isotopic splitting characteristic of boron, suggesting the elimination of $\text{B}(\text{OH})$ from the product. ^1H , ^{11}B , and ^{13}C nuclear magnetic resonance (NMR) spectroscopy (Figures S4, S5, and S7) confirms these ESI-MS results. Specifically, the *para* product shows a single ^{11}B resonance with a chemical shift consistent with typical BAs, whereas the *ortho* product shows no ^{11}B signal whatsoever. In addition, the *ortho* product exhibits a distinct ^{13}C resonance at 156 ppm that is not found in the *para* product, consistent with an aromatic carbon adjacent to a hydroxyl substituent.⁷ On the basis of these results, we postulate the reactions drawn in Scheme 1.

Figure 1. Negative mode ESI-MS of Phe-Phe *N*-terminally modified by reductive amination of (a) *para*-FPBA and (b) *ortho*-FPBA. M indicates 446.2 Da, the mass of the desired, BA-modified dipeptide. The insets show the isotopic splitting of the highest intensity peak in each spectrum.

We subsequently found that FPBA further exhibits an isomer-sensitive stability of the aldehyde functionality when exposed to the reducing agent NaCNBH_3 . When *para*-FPBA is exposed to NaCNBH_3 in d_7 -DMF, ^1H NMR spectroscopy shows no apparent change in the identity of the FPBA (Figure 2a). In contrast, when *ortho*-FPBA is exposed to NaCNBH_3 , the aldehyde functionality is clearly lost, with the appearance of a new proton signal well upfield of the original aldehyde proton (Figure 2b). In addition, the corresponding ^{11}B NMR spectra clearly show that the BA functionality is unstable only in the case of the *ortho* isomer (Figure S9). These data collectively suggest the reduction of *ortho*-FPBA to the corresponding benzoxaborole **4** by NaCNBH_3 , in agreement with previously reported data.^{5j,5l,5m,5o,6} Indeed, the NMR spectra of a commercially available sample of the hemiemer of 2-(hydroxymethyl)phenylboronic acid (Figure

S10) were consistent with the NMR spectra of the reduced *ortho*-FPBA. Similar results were obtained when CD_3OD was used as the solvent (Figure S11 and Figure S12), although reduction of *para*-FPBA was also evident over longer time scales (Figure S13). **Scheme 1.** Isomer-sensitive deboronation in *N*-terminal modification of Phe-Phe by reductive amination of FPBA.

Figure 2. ^1H NMR spectra showing isomer-dependent sensitivity of (a) *para*-FPBA and (b) *ortho*-FPBA to reducing agent NaCNBH_3 . In both panels, the bottom spectrum shows the neat FPBA in d_7 -DMF, while the top spectrum shows the FPBA in d_7 -DMF after addition of NaCNBH_3 . The proton signals indicating the stability of *para*-FPBA to NaCNBH_3 and the reduction of *ortho*-FPBA by NaCNBH_3 to the corresponding benzoxaborole **4** are denoted by the red asterisks.

These data led us to consider the possibility that isomer-dependent deboronation was associated in some manner with the instability of *ortho*-FPBA to reducing agents. Therefore, we repeated the reductive amination as a two-step process, first reacting the *N*-terminus of the peptide with *ortho*-FPBA to give the usual imine species, and then reducing the imine via NaCNBH_3 . However, in this case, the identical deboronated

product **2** was obtained (Figure S14). Thus, both the reduction of *ortho*-FPBA to the benzoxaborole followed by exposure to an amine, and the formation of an imine between *ortho*-FPBA and an amine followed by exposure to NaCNBH₃, leads to installation of the phenyl ring as a deborated substituent (Scheme 2).

Scheme 2. A deborated product is observed independent of the order of amination and reduction.

To provide further insight into the origin of deboronation in these reactions, as well as to explore its generality, we conducted NMR experiments tracking a solution-based reductive amination of FPBA to different primary amines. Figure 3 shows the result of ¹H and ¹¹B NMR spectroscopy performed on a mixture of *ortho*-FPBA and H-Phe-Phe-NH₂ in d₇-DMF, to which NaCNBH₃ is subsequently added (3:3:1 [FPBA]:[NaCNBH₃]:[H-Phe-Phe-NH₂]). H-Phe-Phe-NH₂ was used in this experiment instead of H-Phe-Phe-OH (Scheme 2) because of the poor solubility of the latter in DMF and MeOH. As expected, the ¹H spectrum in Figure 3a indicates attachment of a substituent to the *N*-terminus of the peptide, along with the reduction of excess *ortho*-FPBA to benzoxaborole. In addition, a prominent resonance is found at 6.8 ppm, this resonance being absent in the spectra of the benzoxaborole (Figure 2b and Figure S10), H-Phe-Phe-NH₂ (Figure S15), and a mixture of *ortho*-FPBA and H-Phe-Phe-NH₂ (Figure S17). In oxidative deboronation reactions, a distinct upfield shift of aromatic resonances has been previously shown to accompany the conversion of phenylboronic acids to phenols.⁸ The ¹¹B spectrum in Figure 3b, as expected, indicates reduction of excess *ortho*-FPBA to the benzoxaborole. However, there is also a distinct resonance at 20 ppm, consistent with the formation of boric acid as the byproduct of deboronation (see Figure S18 for the NMR spectra of neat boric acid).

Interestingly, none of the aforementioned features are observed in the corresponding NMR spectra when a simple aliphatic amine is substituted for the dipeptide in these reductive aminations. Instead, both the ¹H and ¹¹B spectra (Figure S19) suggest successful installation of the phenylboronic acid with no conspicuous loss of the boronic acid functionality, *i.e.*, liberation of boric acid. This observation is consistent with the relatively widespread and apparently successful use of *ortho*-FPBA in reductive aminations for the preparation of aminomethylphenylboronic acids.^{3g,3k,4-5,9}

Based on this collective information, it is therefore apparent that the deboronation phenomenon observed is influenced by not only the isomerism of the boronic acid, but also the chemical identity of the amine substrate. Related phenomena have been reported recently for several chemical reactions involving boronic acids.^{8,10} For example, Fu, *et al.*, reported that secondary amines catalyze air oxidation of aryl boronic acids and boronate esters to the corresponding phenols, while primary and tertiary amines do not, due to the relative basicity of amines: 2° > 1° > 3°.^{8b} Their work could implicate the secondary amine resulting from imine reduction as having a critical role in deboronation; however, *ortho*-substituted products, unlike *para*-substituted

ones, can form a B-N dative bond accompanied by decreased basicity of the amine,^{5a} which is contrary to the poorer stability of the *ortho*-substituted product we have observed. Other work has shown oxidation of aryl boronic acids to be dramatically accelerated by the presence of electron-withdrawing substituents on the phenyl ring, yet the aminomethyl substituents at present are electron-donating in character.^{10b} Thus, the mechanism of deboronation at present is decidedly more subtle. Cammidge and coworkers,^{8a} and Zhu and coworkers,^{10a} have found that catalytic amounts of certain quinones and *N*-oxides, respectively, can effect deboronation of various aryl boronic acids. We suspect a similar process is occurring here, wherein the peptide catalyzes deboronation to which the *ortho*-substituted boronic acid is particularly susceptible.

Figure 3. (a) ¹H and (b) ¹¹B NMR spectra of reductive amination of *ortho*-FPBA to H-Phe-Phe-NH₂ with NaCNBH₃ as reducing agent. The solvent is d₇-DMF. Deboronation is suggested by the appearance of a new aromatic resonance in the ¹H spectrum, marked by the red asterisk, as well as the appearance of a new resonance in the ¹¹B spectrum corresponding to liberation of boric acid.

Although additional work is required to elucidate further the mechanism and details of deboronation that we have observed, we feel the present results serve as an important cautionary account, particularly in light of increasing interest in peptide and small molecule BAs. ESI-MS is regularly used to confirm the outcome of solid phase peptide synthesis, and deboronation has been reported as an artifact of in-source fragmentation of aryl BAs.¹¹ However, our combined MS and NMR characterizations have clearly shown that extensive deboronation can occur during

the reductive amination of *ortho*-FPBA to peptide substrates. Therefore, the signatures of deboronation in MS must be given careful consideration when determining the outcome of peptide BA syntheses. If isomerism of the boronic acid is irrelevant, the improved stability of *para*-FPBA to NaCNBH₃ appears to advocate its use in reductive amination schemes. Alternatively, we recommend the use of carboxyphenylboronic acids, which can be used to install BAs onto peptides via standard acid-amine coupling chemistry (exemplified by compound **6**, Figures S20-S22) with no apparent deboronation.

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Supplementary Material

Supplementary material (details of peptide syntheses and characterization techniques, additional NMR, HPLC, and MS data) associated with this article can be found, in the online version, at.