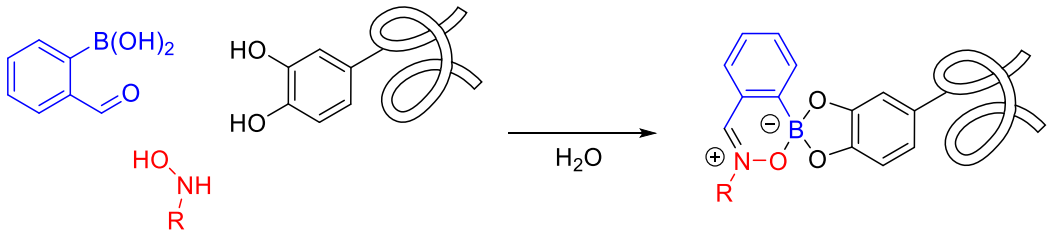
Boronic Acid-Mediated Coupling of Catechols and *N*-Hydroxylamines: a Bioorthogonal Reaction to Label Peptides

Margaret K. Meadows,[a,†] Emily K. Roesner,[a] Vincent M. Lynch,[a] Tony D. James,[b*] and Eric V. Anslyn[a*]

[a] Department of Chemistry, The University of Texas at Austin, Austin, Texas 78712, United States

[b] Department of Chemistry, University of Bath, Bath BA2 7AY, U.K.

Supporting Information Placeholder

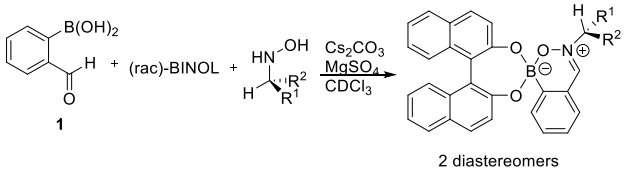


ABSTRACT: An irreversible, three-component assembly with 2-formylphenylboronic acid, catechol and *N*-hydroxylamines was achieved in aqueous media. The boronate ester product was formed with substituted catechols including L-DOPA. Assembly was found to be orthogonal to common biological functional groups and both copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) and aminoether/carbonyl condensations. Boronate ester formation and aminoether condensation were achieved in one-pot with a hexameric peptide.

There are currently a wide variety of coupling reactions being explored for applications in materials chemistry,[1–5] organometallic catalysis,[6–10] and chemical biology.[11,12] When these methods proceed under mild conditions, are high yielding, and do not generate noxious byproducts, they have been termed "click" reactions.[13] Those that do not involve or react with common biological functional groups act in a bioorthogonal fashion.[14,15] Developing bioorthogonal reactions that label peptides necessarily entails the incorporation of an unnatural amino acid or chemical modification of one of the common 20 amino acids.[16–18] Further, the popular coupling methods, except for those that are metal-catalyzed, such as the Cu(I)-catalyzed Sharpless-Huisgen cycloaddition,[19] involve reactions that commence immediately upon mixing of the components. Thus, overall common bioorthogonal reactions cannot be easily controlled in a spatial or temporal manner, and chemical synthesis is required prior to their utilization *in vitro* or *in vivo*.

While not bioorthogonal, we have recently described a method for a chemically-triggered reversible click reaction that yields the original coupling partners without modification.[20] In our continued pursuit of methods to trigger chemical coupling and decoupling upon demand, we explored the use of 2-formylphenylboronic acid (**1**) as a reagent to combine two otherwise unreactive components. This work was inspired by the recent Bull and James report that **1** can be used to form an assembly that acts as a ^{1}H-NMR chiral shift reagent to measure the enantiomeric excess values of chiral hydroxylamines when in the presence of BINOL in aprotic, organic media (Scheme 1).[21]

Scheme 1. Boronate ester assembly as published by James *et al.*[21]



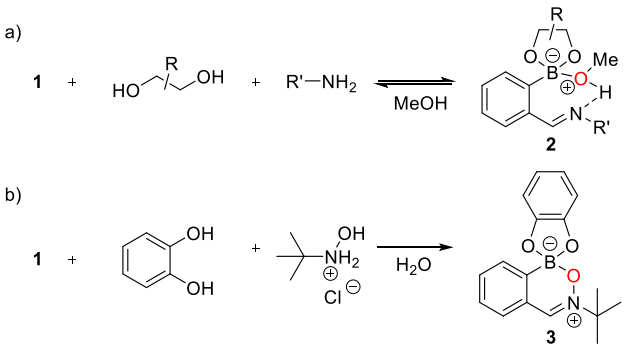
Phenyl boronic acids have been briefly studied in bioorthogonal labeling via boronate ester formation with dopamine[22] and Pd-catalyzed Suzuki couplings.[15,23] Because boronic acids generally have even greater affinities to catechols than alkyl diols, and because they bind catechols relatively well in water, we realized that the method had the potential to "click" together catechol-functionalized biological compounds, such as the amino acid L-DOPA, with virtually any moiety that carried a hydroxylamine. Although L-DOPA is not one of the common 20 amino acids, it has been incorporated into

both peptides and proteins,[24] and therefore provides a readily available coupling partner in a semi-bioorthogonal fashion.

Another avenue we were interested in exploring was the difference in reactivity between *N*-functionalized hydroxylamines (RNHOH) and *O*-functionalized hydroxylamines, hereafter referred to as aminoethers ($RONH_2$). Aminoethers have been previously reported in bioorthogonal couplings,[25] but have different reactivity than hydroxylamines; we hypothesized that these two isomeric functional groups could potentially be paired together while retaining their orthogonal reactivity. We demonstrate herein that the boronic acid-triggered coupling of catechols and hydroxylamines indeed works well in water, has broad catechol substrate scope, can be used to fluorescently label L-DOPA-containing peptides *in vitro*, and is compatible with couplings involving aminoethers.

Previous assemblies with **1** and standard amines have been shown to form reversibly and are capable of exchanging both diol and amine under equilibrium conditions.[26,27] These assemblies were also shown to be stabilized by solvent insertion in protic media (**2**).[28] We hypothesized that the hydroxyl group of the hydroxylamine would occupy the same geometric position as the oxygen of an inserted solvent, and increase the stability of these complexes in water (Scheme 2). Since we began work on this system, other groups have similarly found that these complexes are water stable.[29,30] We found that the assembly with catechol (**3**) formed in water under a variety of conditions: neutral water, pH 7 phosphate buffer, pH 5.5 acetate buffer, and pH 9 bicarbonate buffer. Although the addition of Na_2CO_3 or a similar base is not necessary, it does speed assembly formation considerably. A crystal of **3** grown from slow evaporation of CH_2Cl_2 shows the coordination of the oxygen from the hydroxylamine to the boron, as predicted (Figure 1).

Scheme 2. a) Formation of a three-component assembly with amine and solvent insertion (2) and b) *N*-hydroxylamine (3) in water with the stabilizing oxygen atoms highlighted in red.



Additionally, we found that assembly **3** is not nearly as reversible as the previously reported amine assembly (**2**). Once formed and isolated, **3** is stable to neutral methanol/water mixtures for more than 72 hours with no change by ^{1}H-NMR spectroscopy. Although the catechol is slowly replaced with hydroxyl groups under strongly basic conditions (pH 13), the hydroxylamine addition is irreversible and stable to acid (pH 1), base (pH 13), and heat (50 °C) over 24 hrs.

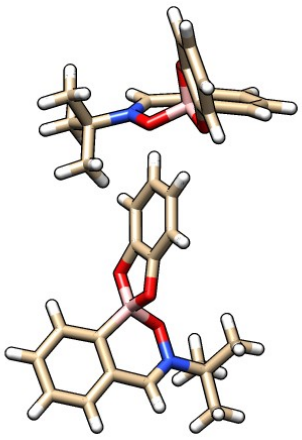
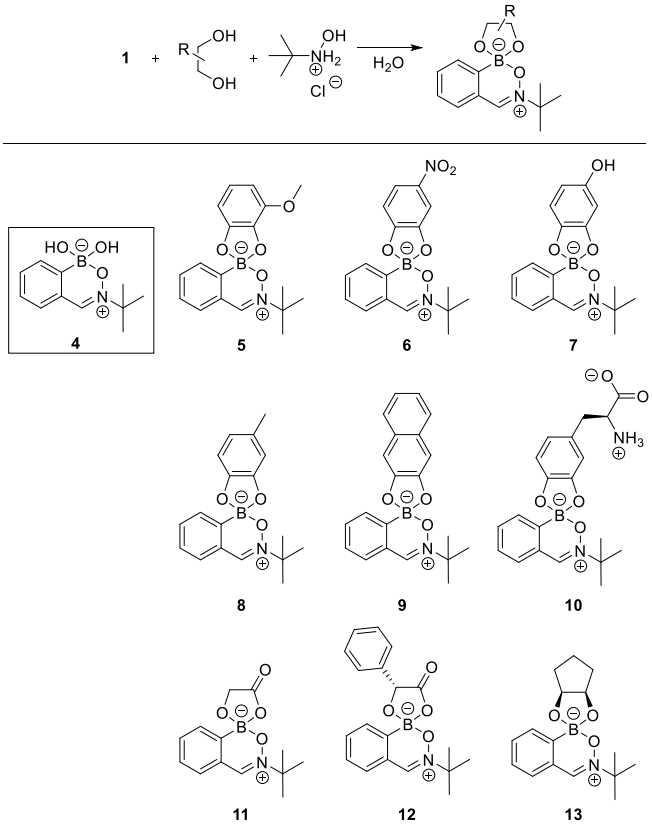


Figure 1. Crystal structure showing of **3**, grown from CH_2Cl_2.

Varying the substitution on the catechol provided a range of assemblies; we were also able to complex α-hydroxy acids and *cis*-1,2-cyclopentanediol (Scheme 3). The determining factors in assembly appear to be solubility of the diol and strength of its binding to **1**. Those that were less soluble or bound poorly were unable to outcompete assembly formation with water, yielding **4**.

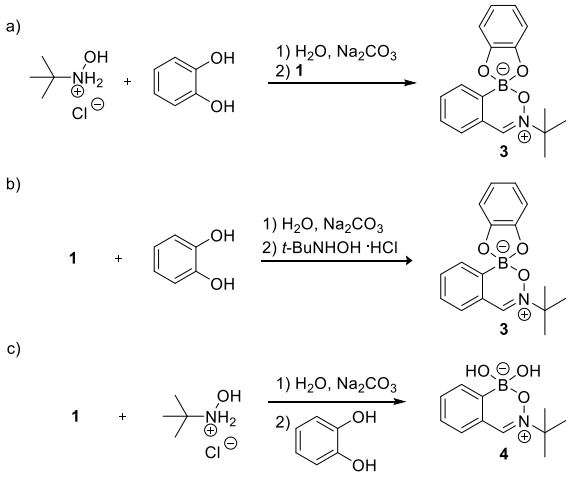
Scheme 3. Substrate scope of the three-component assembly with *N*-hydroxylamine in water.



Once compound **4** forms, it does not equilibrate with any added catechols to give a different assembly. This gives insight into the sequence of steps. To achieve the

desired product, the catechol must condense with the boronic acid prior to the addition of the hydroxylamine into the aldehyde. To support this hypothesis, we examined the order of addition of the three components to this assembly. When **1** and catechol or *t*-BuNHOH and catechol are combined prior to the addition of the third component, the assembly with catechol is formed (Schemes 4a and 4b). When **1** and *t*-BuNHOH are combined, the condensation occurs prior to catechol addition and is unreactive to the catechol (Scheme 4c).

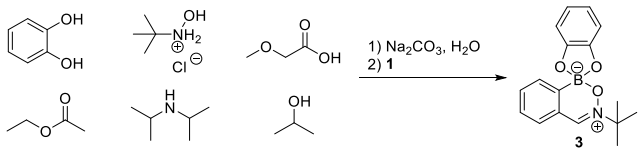
Scheme 4. Order of addition studies showing a) combination of *t*-BuNHOH and catechol prior to addition of 1, b) combination of 1 and catechol prior to addition of *t*-BuNHOH, and c) combination of 1 and *t*-BuNHOH prior to addition of catechol.



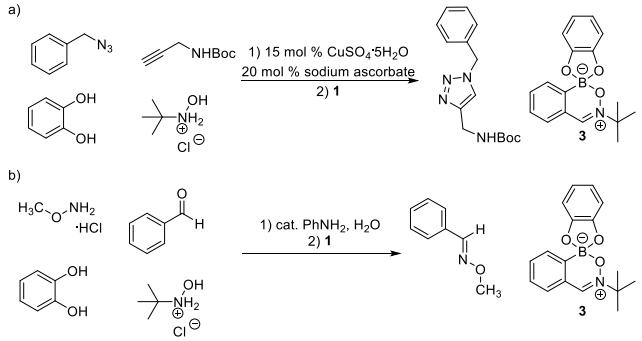
Boronic acid complexes are well known for their ability to reversibly bind sugars;[31] however, the hydroxylamine assembly using **1** did not show the same affinity. If fructose or glucose was combined with **1** and *t*-BuNHOH, the only product was **4**. We hypothesize that this is due to the low binding constants of boronic acids with sugars without the assistance of an *o*-aminomethyl group to increase binding,[32] and once **4** is formed, it will not then exchange with an added diol.

With our desire to transition this assembly to a biocompatible platform, we tested the robustness of our complex to the presence of common biological functional groups with no interferences found (Scheme 5). Additionally, the assembly can be run in parallel with other common bioorthogonal coupling reactions (Scheme 6); CuAAC chemistry can be used to label azidolysine residues, and aldehyde and ketone residues can be labeled using aminoethers. The latter is of particular interest due to the similarity between hydroxylamine and aminoether structures, as discussed above. In order to further explore this opportunity for orthogonal reactivity, we moved to a peptide scaffold.

Scheme 5. Assembly formation in the presence of various functional groups.

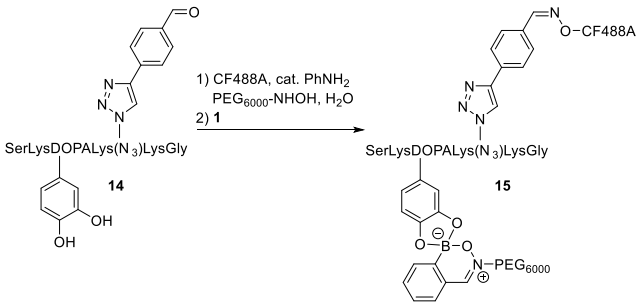


Scheme 6. Assembly formation alongside a) CuAAC and b) aminoether condensation.



Peptide **14** was synthesized using automated solid-phase synthesis. The benzaldehyde moiety was incorporated to allow for conjugation of a commercially available aminoether fluorescent dye, CF488A. PEG ethers have been used in pharmaceuticals to increase hydrophilicity and label proteins to increase water solubility and stability.[33–36] The dual-labeling of **14** was accomplished in a one-pot fashion (Scheme 7); **14**, CF488A, catalytic aniline, and the PEGylated hydroxylamine were dissolved in water at room temperature. After 1 hour, **1** was added. The dual-labeled peptide **15** was isolated after 4 hours, and characterized by fluorescence and ^{11}B-NMR spectroscopies, and by MS.

Scheme 7. Dual, one-pot labeling of peptide 14.



In summary, we extended the utility of a three-component assembly to the purpose of labeling peptides containing L-DOPA. We further expanded the substrate scope, as well as uncovering the sequence of formation in aqueous conditions, and irreversibility of the coupling. The three-component assembly discussed herein is thus a semi-bioorthogonal coupling reaction, showing no cross-reactivity with other common "click" reactions when simultaneously performed in the same pot.

ASSOCIATED CONTENT

SUPPORTING INFORMATION

The Supporting Information is available free of charge on the ACS Publications website.

Synthesis and characterization of compounds **3**-**15** and reversibility, order of addition, and bioorthogonal studies. (PDF)

Single-crystal X-ray data for **3**. (CIF)

AUTHOR INFORMATION

Corresponding Authors

*t.d.james@bath.ac.uk, anslyn@austin.utexas.edu

Present Addresses

†Current address: Department of Chemistry, Northwestern University, Evanston, IL, 60208, United States.

Notes

The authors declare no competing financial interest.

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