Fluoride protects boronic acids in the copper(I)-mediated click reaction†

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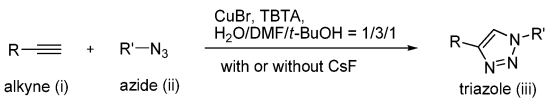
Fluoride has been found to protect boronic acids from copper(I)-mediated decomposition; such findings should be very useful for the preparation of boronic acid-based carbohydrate sensors and boronic acid conjugates using the copper(I)-mediated click reaction.

It is well known that boronic acids are very important in synthetic organic chemistry, medicinal chemistry and molecular recognition. Specifically, in synthetic chemistry, boronic acids are also very important intermediates in reactions such as the Suzuki coupling;[1,2] boronic acid compounds have shown diverse biological activities and could be useful pharmaceutical agents,[3] and in molecular recognition, boronic acids are known to form tight complexes with compounds containing the diol moiety, which can be used for chemosensor design.[2,4–6] Along this line, the copper(I)-mediated Huisgen cycloaddition[7,8] (click reaction, Scheme 1) could be a very powerful method for the preparation of boronic acids with diverse structural features for synthetic and medicinal chemistry applications, and bisboronic acids and boronic acid conjugates for biosensing applications. The ability to modify boronic acids using click chemistry should allow for a drastic increase in the structural diversity of available boronic acids. Recently, our lab has prepared boronic acid–nucleic acid conjugates using the copper-mediated click reaction for the selection of DNA aptamers for glycoproteins.[9] In expanding our work to include a large set of boronic acids for conjugation with nucleotides, we found that copper(I)-mediated degradation was a problem with some boronic acids. This is understandable since copper is known to insert between the carbon–boron bond, which leads to useful coupling reactions when desired.[10,11] However, in applying this click reaction to the preparation of boronic acid conjugates, we desired to find a way to minimize copper(I)-mediated decompositions. Herein, we report our findings that fluoride can protect boronic acids from copper(I)-mediated decomposition either alone or in a cycloaddition reaction mixture. Such results should be very useful for the future development of boronic acid-based carbohydrate sensors and boronic acid conjugates for various other applications such as aptamer selections.



Scheme 1 The click reaction between alkyne (**i**) and azide (**ii**) in the presence of copper(I) leading to triazole (**iii**).

Again, our initial interest was for the preparation of boronic acid–nucleotide conjugates for incorporation into DNA aptamers.[9] The very first boronic acid used was 8-quinolineboronic acid (8-QBA, **1**, Fig. 1), which led to the relatively uneventful synthesis of the desired conjugates. This turned out to be fortuitous because later efforts in incorporating other boronic acids in a similar fashion led to decomposition, presumably due to copper insertion. In looking for a way to minimize this side reaction, we thought about the ability for fluoride to convert the boron atom in a boronic acid to its anionic tetrahedral form.[12] We reasoned that the presence of a negative charge on boron would change the overall electronic properties of the B–C bond and negatively affect copper insertion, thus leading to a reduction in side reactions. Therefore, we first studied the stabilities of a series of eight boronic acids (Fig. 1) for a period of 5 h in the presence of Cu(I). This time course was chosen because that was the typical length of time for our click reaction for conjugation. In doing so, HPLC was used to monitor the progression of the decomposition reaction. The conditions for the stability studies were based on the optimized conditions in our lab (Fig. 2).

Among the eight boronic acid studied, very different stabilities in the presence of Cu(I) were observed. For example, 8-QBA (**1**) was essentially stable with 95% remaining after being exposed to 100 mM of Cu(I) for 5 h. On the other hand, when boronic acid **3** was subjected to the same conditions, 35% degradation was observed. Fig. 2 shows a set of representative HPLC chromatograms using boronic acids **1**, **3** and **5** as examples. One can see that at the 5 h point (Fig. 2c), multiple peaks appeared for compounds **3** and **5**. However, the

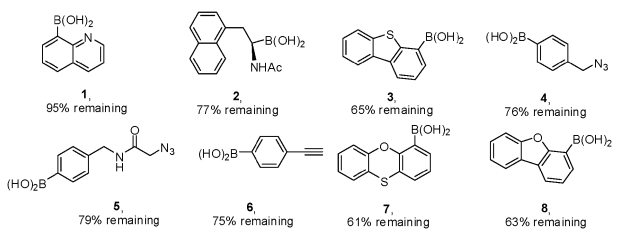


Fig. 1 Structures of compounds **1–8** for the HPLC stability studies and the percentage of remaining boronic acids after being treated with Cu(I) for 5 h without any protection.

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† Electronic supplementary information (ESI) available: HPLC conditions for analytical studies, procedures for model click reactions, and all the NMR and MS spectra, and HPLC chromatograms of the final products. See DOI: 10.1039/b909575f

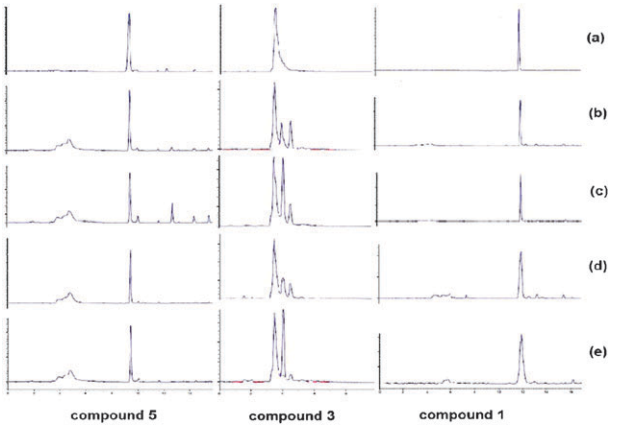


Fig. 2 HPLC chromatograms of compounds **5**, **3**, and **1** (50 mM for each) in the presence of 20 mM Cu(I) in (H_2O–DMF–*t*-BuOH = 1 : 3 : 1) solution, monitored at 260 nm. (a) Pure boronic acids; (b) immediately after addition of Cu(I); (c) 5 h after mixing with Cu(I); (d) immediately after addition of Cu(I) and fluoride; (e) 5 h after mixing with Cu(I) and fluoride.

8-QBA (**1**) solution gave essentially one peak (starting material) indicating minimal degradation.

To test the ability for fluoride to protect boronic acids from Cu(I)-mediated degradation, we studied the stabilities of boronic acids **2–8** in the presence of 100 mM of fluoride (CsF). Fig. 2d–e shows a set of representative chromatograms using boronic acid **5** as an example. As can be seen, **5** underwent extensive degradation at the 5 h point after mixing with Cu(I), giving four additional peaks (Fig. 2c). In the meantime, the intensity of the boronic acid peak decreased by 21%. In contrast, if fluoride was added together with Cu(I), degradation diminished to a negligible level. Similar results were observed with boronic acids **5–8**. To our surprise, the degradation of boronic acids **2–4** was not prevented with the addition of fluoride. It is not readily clear as to what structural features led to the variation in fluoride protection.

Though experimental results from boronic acids alone seem to suggest that fluoride is able to protect at least some boronic acids from Cu(I)-mediated degradations, there is more than one way to interpret these results. For example, if the observed protective effect was due to interactions between fluoride and Cu(I), then fluoride addition may not help in increasing the yield of the desired cycloaddition reaction, which is our ultimate goal. To further examine this, we also studied the effect of added fluoride on the copper(I)-mediated click reaction of several pairs of boronic acid-containing alkynes/azides. All studies clearly indicated that fluoride addition indeed increases the reaction yields. Fig. 3 shows an example set of chromatograms (reaction C, Table 1) of these HPLC studies. The yield studies were conducted on an analytical column using the same conditions as the stability studies (see the ESI† for details). In this case, both starting materials (Fig. 3; **C-i** and **C-ii**) disappeared after 5 h of reaction with a concomitant increase in product peak. In the absence of fluoride, multiple peaks appeared after 5 h of reaction (Fig. 3d). On the other hand, in the presence of fluoride, the desired product is the predominant component (Fig. 3e). Such

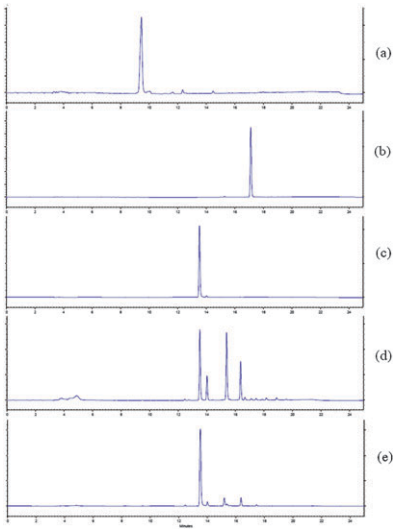
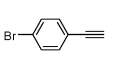
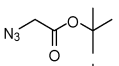
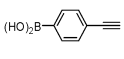
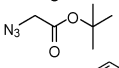
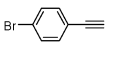
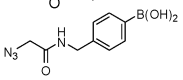
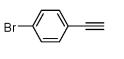
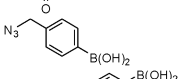
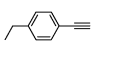
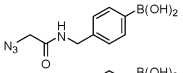
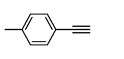
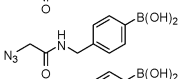
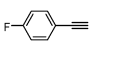
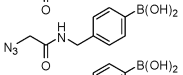
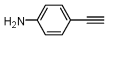
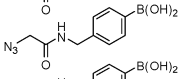
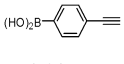
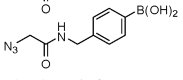


Fig. 3 HPLC chromatograms of model reaction (**C**) in the presence of 20 mM Cu(I) in (H_2O–DMF–*t*-BuOH = 1 : 3 : 1) solution, monitored at 260 nm. (a) pure compound **C-ii**, (namely compound **5**); (b) pure compound **C-i**, namely the alkyne (**i**) in the reaction (**C**); (c) pure compound **C-iii**, namely the triazole product (**iii**) in the reaction (**C**); (d) 5 h reaction mixture with Cu(I); (e) 5 h reaction mixture with Cu(I) and fluoride.

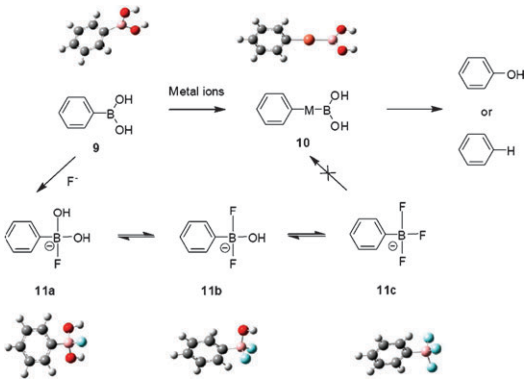
results clearly indicate that fluoride played a protective role and can help increase the yield of such cycloaddition reactions.

In an effort to achieve a quantitative understanding of the beneficial role of fluoride addition, reaction yields were calculated from the ratio of the product concentrations to starting material concentrations. Standard curves were used for the quantitative studies. First, we studied the reaction (**A**, Table 1) that did not involve a boronic acid. Essentially the same yields (80%) were obtained with and without fluoride

Table 1 Reaction yields of model click reactions[a]

Entry	Alkyne (**i**)	Azide (**ii**)	Yield (%)[a] without F^-	Yield (%)[a] with F^-
(**A**)			79 ± 6	81 ± 3
(**B**)			57 ± 5	96 ± 1
(**C**)			49 ± 1	89 ± 1
(**D**)			60 ± 5	71 ± 2
(**E**)			79 ± 1	94 ± 2
(**F**)			43 ± 3	77 ± 9
(**G**)			47 ± 6	83 ± 4
(**H**)			57 ± 4	86 ± 11
(**I**)			43 ± 5	86 ± 11

[a] All the yields were calculated from standard curves (HPLC peak integrals over concentrations).



Scheme 2 A possible mechanism of boronic acid protection by fluoride from copper-mediated degradation.

addition. Such results indicate that fluoride has no intrinsic effect on the click reaction yield (Table 1). For the other reactions that do involve boronic acids, the reaction yields increased by 11–43% with the addition of fluoride (Table 1). For example, in the two cases (**B** and **I**) where the boronic acid moiety is part of an arylalkyne, yield improvements from 57 to 96% (about 70% increase) (**B**) and 43 to 86% (one-fold increase) (**I**) were observed, respectively. In cases where the boronic acid compounds contain an azido group, 20–75% improvements in yield were observed.

It is well known that metals can insert into the C–B bond of boronic acids in general coupling reactions.[13,14] It is reasonable to suspect that this insertion is a prerequisite step in the Cu(I)-mediated decomposition of boronic acids (Scheme 2). To understand how fluoride addition could affect the relative stabilities of the different species involved, we have also conducted DFT calculations based on the BP86 functional using the standard 6-31G* basis.[15] The PCM solvation model[16] was used in single-point energy calculations (PCM(sp)), and for geometry optimizations and frequency calculations (PCM(opt)). All the calculations using the PCM solvent model employed the UAHF atomic radii when constructing the solvent cavity, as recommended in the Gaussian 03 user's reference. All geometries were fully optimized, and the characters of the stationary points found were confirmed by a harmonic frequency calculation at the same level of theory to ensure a minimum was located. As shown in Fig. 4 of the relative energy diagram, the insertion of Cu(I) into phenylboronic acid (**9**) without fluoride protection would give compound **10d** with release of 13.2 kcal mol^{-1} of energy. The addition of fluoride would generate tetrahedral anionic species **11**, which would decrease the energy level by 5.4 kcal mol^{-1} for monofluoride form **11a**, 9.5 kcal mol^{-1} for difluoride form **11b**, and 14.3 kcal mol^{-1} for the trifluoride form **11c**. The energy of **11c** is even lower than that of the copper insertion forms **10d,c**. Based on the calculated energies of **10a–c**, Cu(I) insertion would release less energy from fluoride-protected tetrahedral forms **11a–c** to **10a–c** than from phenylboronic acid **9** to **10d**. The transition of the trifluoride form **11c** (−14.3 kcal mol^{-1}) to **10c** (−11.2 kcal mol^{-1}) is an energy gaining process. Therefore, fluoride addition decreases (or sometimes reverses) the energy drives for copper insertion into a B–C bond.

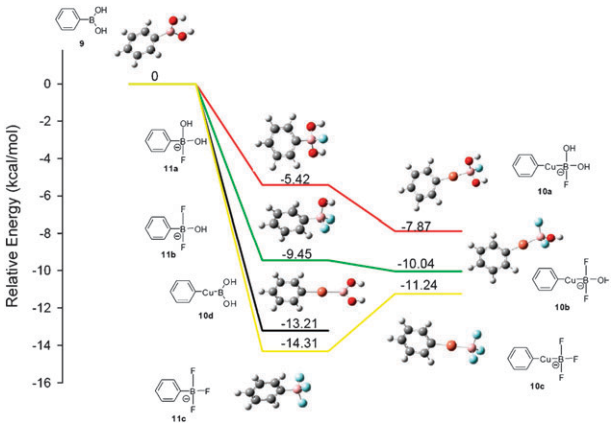


Fig. 4 Energy profile for the fluoride-mediated stability studies of boronic acid.

In conclusion, fluoride can protect some boronic acids from copper(I)-mediated degradation and thus increase the yields of click reactions. Reaction yield improvements of 20–100% were observed. The mechanism of fluoride protection is proposed to be the prevention of copper insertion by the formation of anionic tetrahedral form of boron. This work should be very useful for the preparation of boronic acid-based carbohydrate sensors, boronic acid-modified aptamers, and structurally diverse boronic acids for synthetic and medicinal chemistry applications.

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