Sensitive Thin-Layer Chromatography Detection of Boronic Acids Using Alizarin

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Abstract: A new method for the selective and sensitive detection of boronic acids on thin-layer chromatography plates is described. The plate is briefly dipped in an alizarin solution, allowed to dry in ambient air, and observed under 366 nm light. Alizarin emits a bright yellow fluorescence only in the presence of a boronic acid.

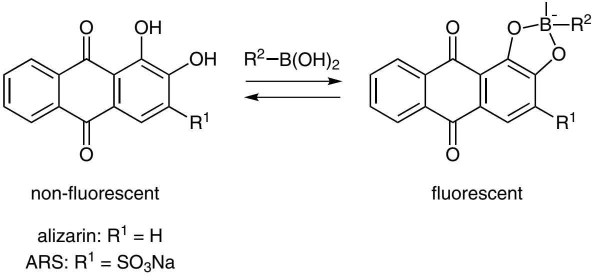
Key words: boronic acid, TLC, staining reagent, dyes/pigments, fluorescence

Boronic acids are key intermediates in organic synthesis that are involved in, for example, Suzuki[1] and Chan-Lam[2] coupling reactions. They have also been used as probes for saccharides and as potential drugs for diverse therapeutic applications,[3] implying the synthesis of target compounds that contain boronic acid groups. Easy detection of boronic acids during reactions, however, remains a challenge.

The conversion of boronic acids has been monitored in Suzuki–Miyaura reaction mixtures to which dihydroxycoumarins were added. Fluorescent complexes resulting from binding of these compounds with boronic acids were visualized under a hand-held UV lamp (366 nm).[4] TLC, however, is a more generally applicable and easier way to analyze a reaction mixture. The detection of boronic acids on TLC plates would enable the synthetic chemist to monitor not only the conversion of a boronic acid, but also the integrity of a boronic acid group during a reaction involving other functional groups. Moreover, it would not complicate the purification of the product because there would be no sensing molecule in the reaction mixture.

The natural anthraquinone alizarin, a non-fluorescent compound in its free form, becomes fluorescent when bound to a boronic acid via its 1,2-diol (Scheme 1).[5] This property has been more commonly exploited with the water-soluble derivative alizarin red S (ARS; Scheme 1), and the mechanism of the involved reaction has recently been studied in detail.[6] In particular, ARS has been used for the direct visualization of a boronic acid group on a cellulose surface[7] and on glass slides[8] by confocal microscopy.

During our synthetic work involving boronic acids, we were interested to see whether the commercially available and inexpensive alizarin would be of help in visualizing boronic acids on TLC plates. Alizarin has been used in combination with concentrated H_2SO_4 for the fluorescent detection of boric acid on spot plates.[9] Alizarin is also known as a TLC staining reagent for the detection of cations (e.g., Al^{III}), which requires subsequent treatment with ammonia.[10]



Scheme 1 Structures of alizarin and alizarin red S (ARS) and their fluorescent complexes with boronic acids

For the detection of boronic acids, preliminary tests looked promising: after brief immersion of a TLC plate in a saturated methanolic alizarin solution and spontaneous evaporation of the solvent, spots that contained phenylboronic acid (PBA) turned yellow, whereas the rest of the plate turned pink. Under 366 nm light, the spots showed an intense yellow fluorescence on a dark background, even though it is mentioned in the article of Barder and Buchwald that alizarin would require a significantly higher excitation wavelength (ca. 550 nm).[4]

The detection of 2 μL spots of PBA solutions was optimized. A 50 mM solution of Na_2CO_3 in 50% aqueous methanol has previously been used to measure the affinity of alizarin for boronic acids attached to solid supports.[11] Solubility tests, moreover, showed that 1 mM was near the saturation concentration of alizarin in methanol. The following solutions were therefore tested for the detection of PBA spots on a TLC plate: 1 mM alizarin in (1) Na_2CO_3 (20 mM in H_2O–MeOH, 20:80), (2) methanol, and (3) HCl (20 mM in H_2O–MeOH, 20:80). The most intense fluorescence was observed when pure methanol was used. Ethanol, ethyl acetate, dichloromethane, tetrahydrofuran and acetone were also tested, and all solvents gave similar results in terms of sensitivity: 2 μL spots of PBA solutions at concentrations down to 0.1 mM (24 ng) could be easily detected (trace fluorescence at 0.01 mM). Acetone was selected as the preferred solvent because it is in-

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expensive, relatively safe, and the solubility of alizarin was higher in acetone than in the other solvents. Alizarin solutions at different concentrations in acetone were subsequently tested (10, 1, 0.1, and 0.01 mM). The most intense fluorescence was observed with the initial concentration of 1 mM. Staining the TLC plate using a piece of cotton, or dipping it in the alizarin solution for 1, 5 or 10 seconds resulted in the same sensitivity. Spraying the TLC plate with the alizarin solution did not result in any color change. The following method was therefore chosen for subsequent experiments:

- Briefly dip the TLC plate in a 1 mM alizarin solution in acetone;
- Let dry and wait until the TLC plate becomes pink;
- Observe under 366 nm light.

The ability of this method to detect diverse boronic acids and derivatives was tested (Figure 1). All compounds appeared yellow-orange. Electron-deficient boronic acids produced a slightly more intense color than electron-rich boronic acids, which is probably due to the pK_a dependence of the boronic acid–diol complexation.[12] PBA pinacol ester was revealed with the same fluorescence intensity as PBA, which can be explained by the higher affinity of PBA for alizarin than for aliphatic diols.[12] Trifluoroborates were revealed with the same fluorescence intensity as the corresponding boronic acids. PBA *N*-methyliminodiacetate (MIDA) ester could also be detected, although it gave a much weaker fluorescence than PBA itself. The flavonoid reagent diphenylborinic acid 2-aminoethyl ester (DPBA) gave a distinct orange fluorescence upon contact with alizarin. Under neutral conditions the catechol group of alizarin, like the catechol group of some flavonoids (e.g., rutin), probably gives a negatively charged tetrahedral complex with DPBA after loss of ethanolamine.[13] The difference in fluorescence suggests that alizarin can be used to distinguish between boronic and borinic esters.

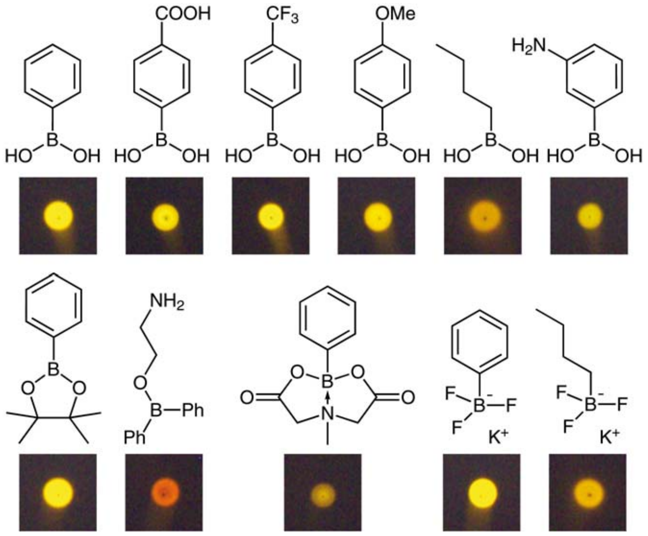


Figure 1 Use of alizarin to detect diverse boronic acids and derivatives thereof. Selections from the original photograph of a TLC plate spotted with diverse boron compounds (10 mM in MeOH*, 2 µL; *MIDA ester in anhydrous THF), briefly immersed in 1 mM alizarin solution in acetone and placed under 366 nm light.

The selectivity of this method towards boronic acids was tested by spotting compounds with various functional groups on a TLC plate (Figure 2). Most compounds gave some fluorescence, but PBA at a 10-fold lower concentration gave a much more intense fluorescence than any of the other compounds.

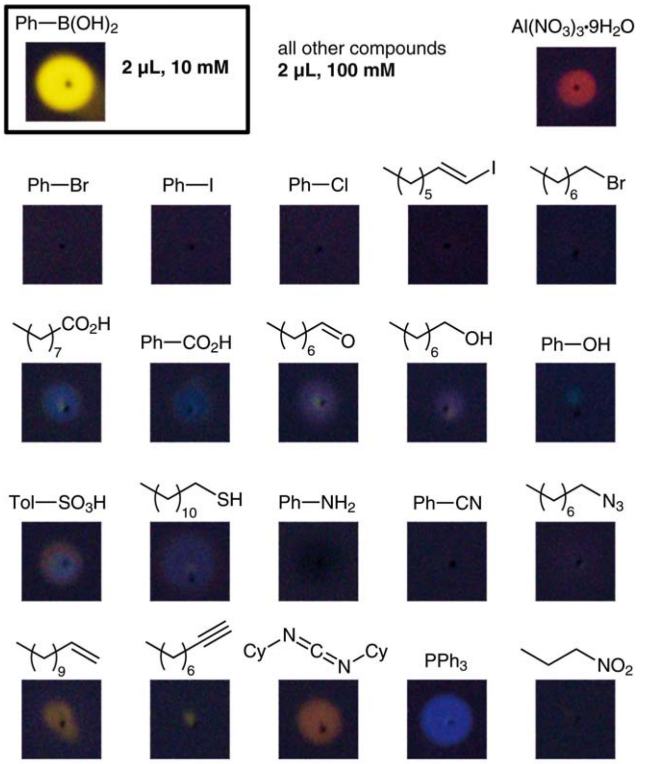
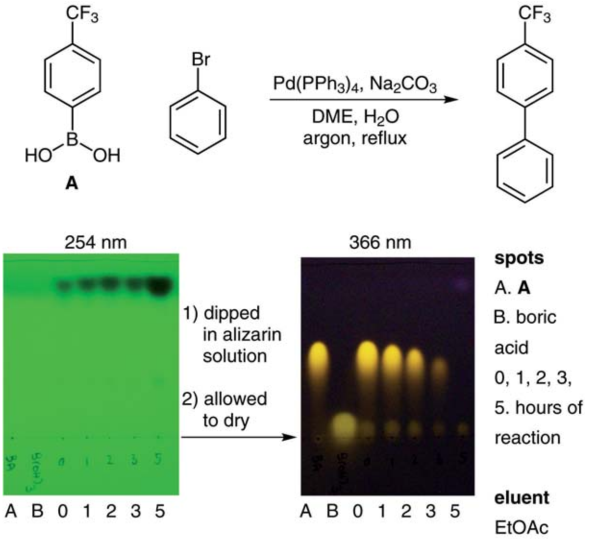


Figure 2 Selectivity of alizarin towards boronic acids. Selections from the original photographs of TLC plates spotted with phenylboronic acid (10 mM in MeOH, 2 µL) and diverse compounds (100 mM in CH_2Cl_2, 2 µL), briefly immersed in 1 mM alizarin solution in acetone and placed under 366 nm light.

The applicability of this method to the analysis of reactions involving boronic acids was tested in 3 examples.

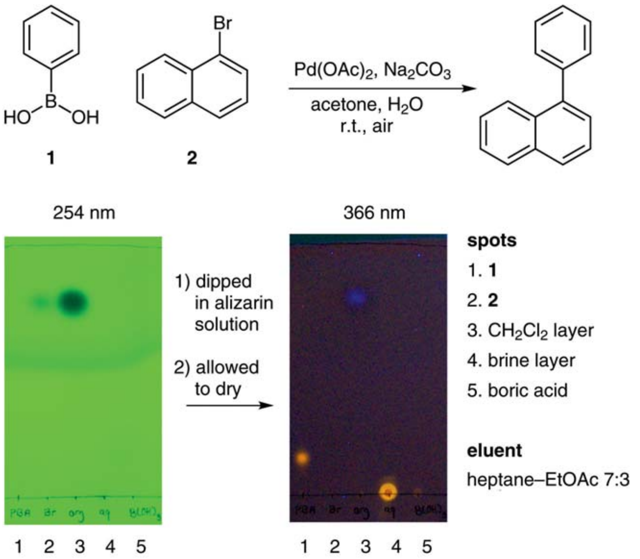
Example 1: Monitoring the consumption of a boronic acid during a classical Suzuki reaction (Scheme 2). The reaction between 4-(trifluoromethyl)phenylboronic acid and bromobenzene was carried out, based on the procedure described by Yang et al.[14,15] A sample was spotted every hour on a TLC plate. TLC analysis using 254 nm light did not give any indication of the progress of the reaction. TLC analysis using the proposed method, however, showed a clear decrease in the concentration of boronic acid over time. After five hours, the boronic acid was no longer detected, which suggested that full conversion had been achieved.

Example 2: Checking the completion of a ligand-free Suzuki reaction (Scheme 3). The reaction between PBA and 1-bromonaphthalene was carried out using a modified procedure of Liu et al.[16,17] After four hours of reaction, the



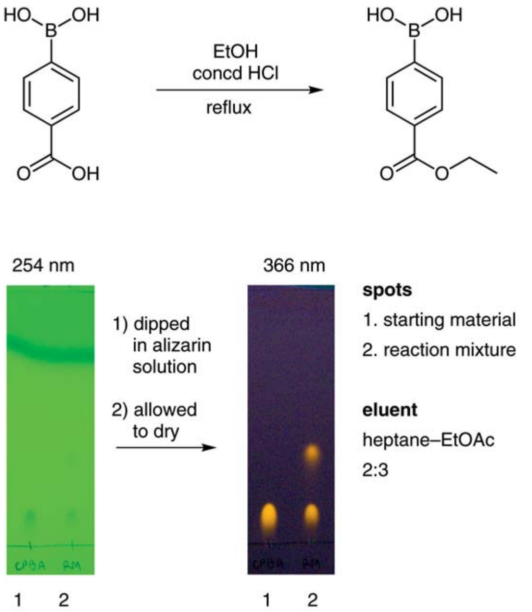
Scheme 2 Monitoring the consumption of a boronic acid during a classical Suzuki reaction using alizarin. Reaction scheme and corresponding TLC, selections from original photographs

reaction mixture was partitioned between dichloromethane and brine, and both layers were analyzed by TLC. TLC analysis using 254 nm light (Scheme 3, left) was inconclusive. TLC analysis using the proposed method, however, showed that neither the dichloromethane layer nor the brine layer contained any significant amount of PBA, and that full conversion had therefore been obtained (Scheme 3, right).



Scheme 3 Checking the completion of a ligand-free Suzuki reaction using alizarin. Reaction scheme and corresponding TLC, selections from original photographs.

Example 3: Visualizing the transformation of a boronic acid into another boronic acid (Scheme 4). The esterification of 4-carboxyphenylboronic acid in acidic ethanol was carried out by using the procedure of Davison et al.[18,19] After one hour of reaction, the reaction mixture was analyzed by TLC. TLC analysis using 254 nm light showed only weak spots that corresponded to starting material and a new compound (Scheme 4, left). TLC analysis using the proposed method, however, showed the same spots with an intense yellow fluorescence. Apart from much clearer staining, this method also indicated directly that the new compound contained a boronic acid moiety (Scheme 4, right).



Scheme 4 Visualizing the transformation of a boronic acid into another boronic acid using alizarin. Reaction scheme and corresponding TLC, selections from original photographs.

In conclusion, the difficult monitoring of boronic acids during organic reactions can be simply overcome by briefly dipping a TLC plate in a 1 mM alizarin solution, to reveal boronic acids on TLC plates in a selective and sensitive manner.

Acknowledgment

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(14) **Experimental Procedure for Example 1:** A mixture of 4-(trifluoromethyl)phenylboronic acid (190 mg, 1 mmol), bromobenzene (0.15 mL, 1.4 mmol) and Na_2CO_3 (318 mg, 3 mmol) in DME (6 mL) and H_2O (1.6 mL) was degassed with argon for 10 min and heated to reflux. $[Pd(PPh_3)_4]$ (58 mg, 0.05 mmol) was added and the reaction mixture was stirred under reflux. Immediately after adding the catalyst, and then after 1, 2, 3 and 5 h, aliquots (ca. 0.2 mL, except ca. 0.5 mL after 5 h) of the reaction mixture were partitioned between Et_2O (1 mL) and 0.1 M HCl (2 mL), and the Et_2O layer was spotted on a TLC plate.

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(16) **Experimental Procedure for Example 2:** A mixture of phenylboronic acid (122 mg, 1 mmol), 1-bromonaphthalene (0.15 mL, 1.1 mmol), Na_2CO_3 (212 mg, 2 mmol) and $Pd(OAc)_2$ (ca. 1 mg) in acetone (3 mL) and H_2O (3.5 mL) was stirred at r.t. in air.

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(18) **Experimental Procedure for Example 3:** 4-Carboxyphenylboronic acid (92 mg, 0.55 mmol) was dissolved in absolute EtOH (2.5 mL). A mixture of absolute EtOH (2.5 mL) and concd HCl (2 drops) was added, and the solution was heated to reflux.

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