

Detection of Boronic Acids through Excited-State Intramolecular Proton-Transfer Fluorescence

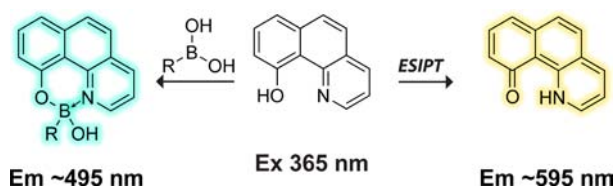
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ABSTRACT



Boronic acids are versatile reagents for the chemical synthesis of organic molecules. They and other boron-containing compounds can be detected readily by the interruption of the excited-state intramolecular proton transfer (ESIPT) of 10-hydroxybenzo[*h*]quinolone. This method is highly sensitive and selective, and useful for monitoring synthetic reactions and detecting boron-containing compounds on a solid support.

Boronic acids are among the most useful reagents in modern synthetic organic chemistry.¹ Boronic acids also have notable utility in carbohydrate sensing² and medicinal chemistry.³ These applications and underlying synthetic transformations could benefit from facile means to detect boronic acid moieties, a task that is now problematic.⁴ Neither UV absorption nor common staining reagents (e.g., KMnO₄, ceric ammonium molybdate, or vanillin) identify boronic acid containing synthetic targets selectively.^{3,5} Buchwald and co-workers reported the *in situ* detection of boronic acid consumption using dihydroxycoumarins.^{4a} This method does not, however, extend to the

detection of boronic acids during thin-layer chromatography (TLC). Alizarin (ARS) has also been put forth as a boron-selective TLC stain,⁶ but is not especially sensitive (*vide infra*). Here, we present a new approach for the selective and sensitive detection of boronic acids based on the photo-physical process known as excited-state intramolecular proton transfer (ESIPT).⁷

We were aware that the absorbance of phenols can be modulated by their complexation to boronic acids.^{8,4c} We also knew that protic solvents interrupt the ESIPT of 10-hydroxybenzo[*h*]quinolone (HBQ)⁹ by disrupting the intramolecular hydrogen bond.¹⁰ Accordingly, we envisioned that boronic acids could disrupt the ESIPT of HBQ

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In its ground state, the HBQ chromophore exists as an enol with an intramolecular hydrogen bond (**A**; Figure 1). At its absorbance maximum (365 nm), singlet excitation of HBQ occurs without geometry relaxation, in accord with the Franck–Condon principle (**B**). There are two fates for this excited state: (i) relaxation back to the ground state (**A**) through fluorescence (~500 nm) or (ii) ultrafast ESIPT (~100 fs) to the keto tautomer in its singlet excited state (**C**). The geometry-relaxed keto form **C** is distinct from the enol form **B**, leading to a large Stokes shift upon emissive relaxation (~600 nm) to **D**, where ground-state reverse proton transfer returns the enol form **A**. ESIPT (**B**→**C**) is typically faster than fluorescence relaxation (**B**→**A**), and the emission from ESIPT tends to dominate.

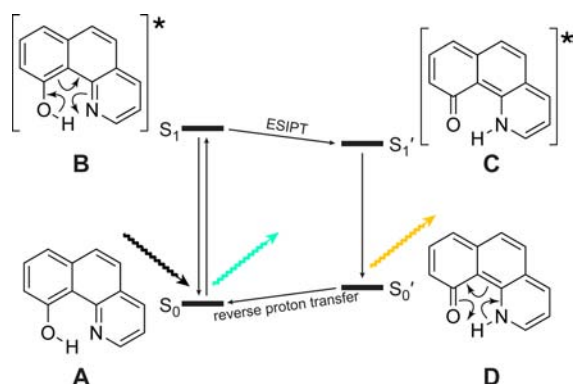


Figure 1. Excited-State Intramolecular Proton Transfer (ESIPT) cycle of 10-hydroxybenzo[*h*]quinoline (HBQ). A Lewis acidic boronic acid or other boron-containing compound can coordinate to **A** and **B**, which interrupts the cycle by shutting down long wavelength emission ($S_1' \rightarrow S_0'$) and activating short wavelength emission ($S_1 \rightarrow S_0$).

In initial experiments, we compared the sensitivity of HBQ and ARS as a TLC-stain for phenylboronic acid. We found that the 365-nm absorbance maximum of HBQ (which, conveniently, is the output wavelength of most common bench lamps) and the large Stokes shift provided by ESIPT lead HBQ to have $\sim 10^3$ -fold greater sensitivity than ARS (Figure 2).

Encouraged by the high sensitivity of HBQ, we sought to explore the generality of the HBQ stain by testing a series of structurally diverse boronic acids. High concentrations of aliphatic boronic acids were not visible under a standard short-wave UV hand-held lamp (Figure 3). Nonetheless, by immersing the TLC plate in a 1 mM solution of HBQ and drying, all spots became brightly fluorescent, with differences in emission wavelength related to the substituents

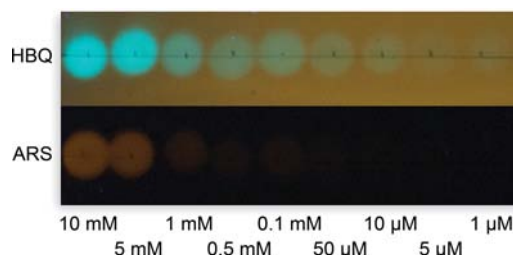


Figure 2. Comparison of the sensitivity of HBQ and ARS for the detection of a boronic acid. Serial dilutions of phenylboronic acid (PBA) were spotted on a silica gel thin-layer chromatography plate, stained with HBQ or ARS, and illuminated at 365 nm with a standard hand-held lamp.

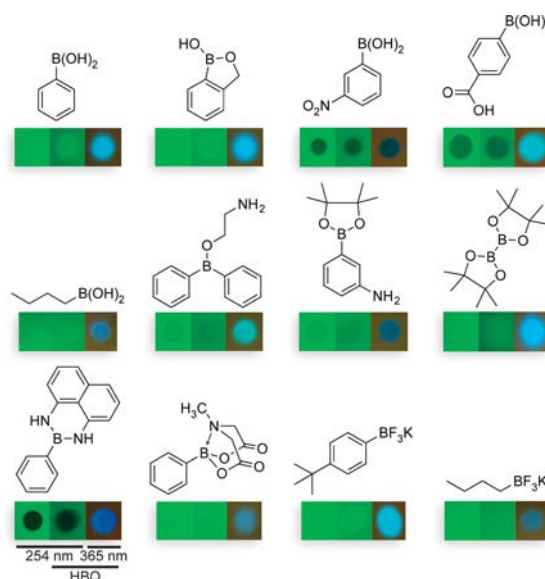


Figure 3. Detection of boronic acids and other boron-containing compounds with HBQ. Whereas most compounds at 10 mM concentrations were not visible upon illumination at 254 nm, all produced a brilliant blue ESIPT-off fluorescence after staining with HBQ.

on the boronic acid.¹³ The spots appear as bright blue-green (emission from **B**) against a yellow-orange background (emission from **C**). Both pinacol- and diammononaphthalene-protected boronic acids possess a vacant *p*-orbital, allowing efficient staining with HBQ according to our proposed mechanism. Even a boronic acid protected with *N*-methyliminodiacetic acid (MIDA) is detectable by the (presumably) small amount of boron with a vacant *p*-orbital. Trifluoroborates likely suffer hydrolysis on the TLC plate¹⁴ to form a detectable boronic acid.

Next, we assessed the selectivity of HBQ for boronic acids. Compounds with a wide variety of functional groups

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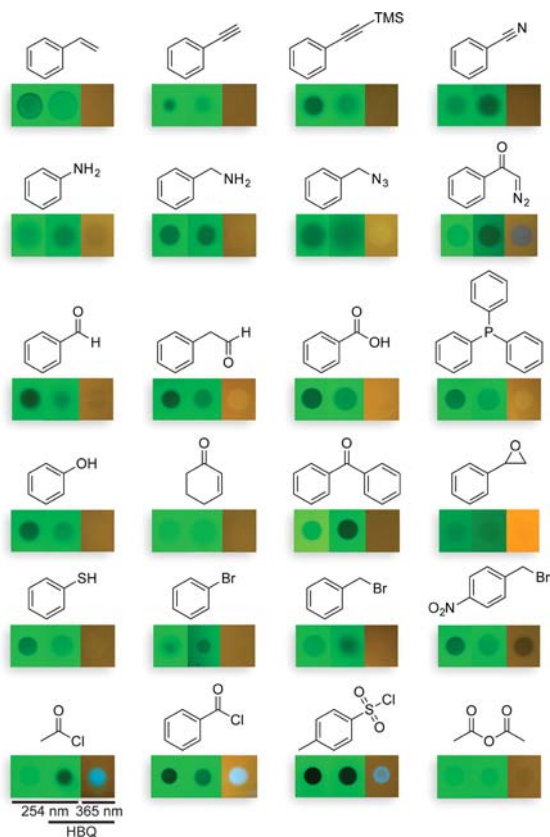


Figure 4. Selectivity of HBQ for functional groups. Compounds visible upon illumination at 254 nm retained this quality following staining with HBQ.

(but not a boronic acid) were spotted onto silica plates at concentrations visible with a standard short-wave UV hand-held lamp and treated with HBQ stain. In general, there was no fluorescence with the functional groups (Figure 4). Notably, the dark spots remained visible upon illumination at 254 nm following treatment with the HBQ stain. Thus, information from short-wave illumination is retained after the stain develops, in marked contrast to other staining methods. Whereas sensitive functional groups (e.g., aldehydes, diazos, anhydrides, and epoxides) resisted staining, highly electrophilic functional groups (e.g., acyl chlorides and sulfonyl chlorides) gave false-positive results, producing a blue fluorescence upon illumination at 365 nm that is similar to that observed from boronic acids. These data validate our mechanism, as these electrophilic functional groups can react with the phenolic hydroxyl group of HBQ and interrupt the ESIPT cycle. Moreover, these false-positive results do not compromise selectivity in practice, as TLC is used only rarely to monitor such reactive functional groups.

We reasoned that the detection method could apply to boronic acids bound to a solid support. Immobilized boronic acids have found application in glycan-affinity

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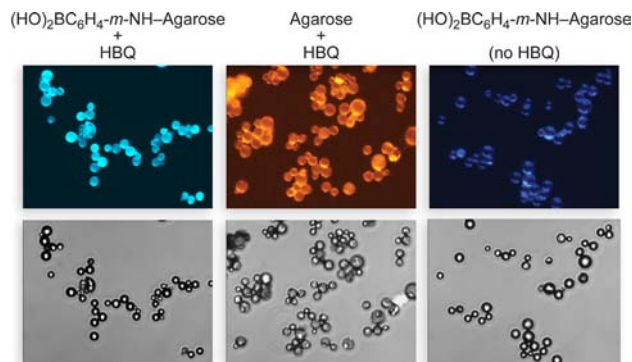


Figure 5. Detection of a boronic acid on a solid support. Agarose beads (6% cross-linking) were modified covalently with *m*-aminophenylboronic acid and visualized under a microscope upon excitation at 365 nm (top) and in a bright field (bottom).

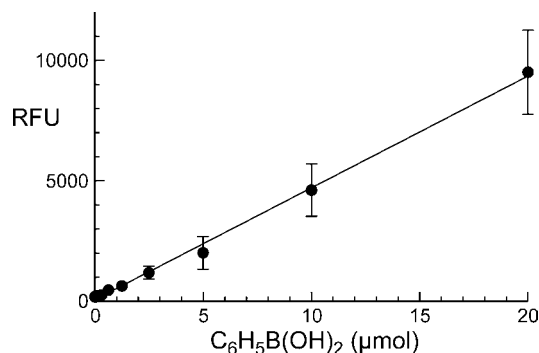


Figure 6. Relationship between phenylboronic acid concentration and emitted fluorescence following staining with HBQ. Fluorescence was detected using a standard plate reader and expressed as relative fluorescence units (RFU). Slope = (462 ± 7) RFU/ μmol by linear regression analysis ($R^2 = 0.9973$).

chromatography because of their ability to bind to diols.^{15,8b} Similarly, boronated solid supports are used widely for the immobilization of biomolecules,¹⁶ lithography,¹⁷ and various glycan-sensing schemes.¹⁸ Using a boronated agarose as a model, we observed ESIPT-off fluorescence upon treatment with 10 mM HBQ in EtOH. In contrast, only ESIPT-on fluorescence was observed for unconjugated agarose, and no native fluorescence was

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ascribed to conjugated agarose in the absence of HBQ (Figure 5). Such facile detection could be used to assess boron functionalization qualitatively during solid-support device fabrication.

Lastly, we investigated the potential of HBQ for the quantitative detection of boron-containing compounds. A simple plate reader enabled the detection of nanomoles of phenylboronic acid (Figure 6). Notably, fluorescence intensity correlated linearly with the amount of boron.

In summary, we present a novel method for the sensitive and selective detection of boronic acids and other boron-containing compounds. The method, which is based on the ability to turn off the ESIPT of HBQ, provides much greater sensitivity than extant methods. Moreover, the resultant HBQ–boron complexes remain fluorescent in

the solid state. Accordingly, this method could be beneficial to synthetic chemistry and materials science.

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Supporting Information Available. Additional images, camera, and microscopy settings. This material is available free of charge via the Internet at <http://pubs.acs.org>.

The authors declare no competing financial interest.