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The effect of boronic acid acidity on performance of viologen-based boronic acids in a two-component optical glucose-sensing system

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Abstract—A two-component saccharide sensing system using the fluorescent dye, hydroxypyrene trisulfonic acid, combined with a boronic acid functional viologen as a receptor/quencher in pH 7.4 buffer solution has been further investigated. The effect of substituents on the acidity of the boronic acid was measured. The boronic acid pK_a changed in the expected manner when electron donating or withdrawing groups were present. The glucose binding constants were dependent on pK_a, but no simple correlation was observed for the Stern–Volmer quenching constants and the fluorescence signal modulation.
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The detection of glucose using boronic acid-based fluorescent chemosensors has been a major area of research in recent years.[1] This is driven in part by the need for better techniques for monitoring blood sugar concentrations in diabetics[2] and critically ill patients.[3] Boronic acid-based sensors can be utilized for this application because of their ability to bind reversibly to 1,2- and 1,3-diols in aqueous media.[4] In most of these systems, the boronic acid receptor is attached directly to a fluorophore, and the saccharide recognition event causes a change in fluorescence emission.[5]

In contrast to systems in which the boronic acid and fluorophore are incorporated into a single molecule, the study described in this Letter uses a two-component system, developed earlier,[6] comprising the anionic fluorescent dye 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS), and a boronic acid-appended viologen. The latter serves both as a fluorescence quencher and a glucose receptor.

Though the detailed mechanism of glucose modulation in the two-component system has not been completely elucidated, it has been established that the quenching step involves ground-state charge-transfer complex formation between anionic dye and cationic quencher. The existence of complexes has been shown both in solution[6b,e] and in the solid state.[6h] The complex exhibits diminished fluorescence relative to that of the free dye. We propose that a signal is generated when the complex dissociates as a consequence of the reaction of the quencher component with glucose, resulting in an increase in fluorescence intensity related to glucose concentration.

The first step in the dissociation process involves reaction of the boronic acid receptor on the viologen with glucose. The conversion of the neutral boronic acid (trigonal boron) to a negatively charged boronate ester (tetrahedral boron) alters the properties of the viologen leading to a reduction in quenching efficacy. Though electronic and steric effects may play a role, at least part of the process is simple charge neutralization, which reduces the electrostatic attraction of dye for quencher and leads to weaker binding and therefore enhanced fluorescence.

The purpose of the present study was to explore the effect of boronic acid acidity on quenching and glucose response. This knowledge should lead to a better understanding of the sensing mechanism. The system studied combines benzyl viologens as the quenching component with HPTS as the fluorescent dye in aqueous media at physiological pH. The approach taken is to manipulate the pK_a of the boronic acid by adding

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electron donating and withdrawing groups in the *para* position and observing how such substituents affect the quenching ability and glucose response of the viologens. It has been shown that the pK_a values for arylboronic acids are dependent upon the substitution pattern and nature of the substituents on the aromatic ring.[7]

Three quencher/receptors, *N*,*N*′-4,4′-bis(benzyl-3-boronic acid)-bipyridinium dibromide (BBV), *N*,*N*′-4,4′-bis-(benzyl-5-fluoro-3-boronic acid)-bipyridinium dibromide (F-BBV), and *N*,*N*′-4,4′-bis(benzyl-5-methoxy-3-boronic acid)-bipyridinium dibromide (OMe-BBV), were prepared and used in this study (Fig. 1). The effect of an electron donating substituent (methoxy) and electron withdrawing substituent (fluoro) on the pK_a of the boronic acid, as well as on the quenching ability and glucose response of these compounds was investigated. Non-boronic acid analogs, benzyl viologen (BV), fluorobenzyl viologen (F-BV), and methoxy-benzyl viologen (OMe-BV), were used as controls. The six compounds were synthesized as outlined in Scheme 1.

Table 1. pK_a values determined by potentiometric titration for the BBVs (4 mM) in the absence and presence of glucose (0.1 M)

	pK_a without glucose	pK_a with 0.1 M glucose	ΔpK_a
BBV	7.7	6.8	0.9
F-BBV	7.5	6.7	0.8
OMe-BBV	8.2	7.4	0.8

The apparent pK_a values of the boronic acid-containing compounds were determined using potentiometric titration and are listed in Table 1. As expected, the presence of an electron-donating methoxy group *para* to the boronic acid in OMe-BBV raised the apparent pK_a relative to that of the unsubstituted compound (BBV). Similarly, the apparent pK_a of F-BBV was lower than that of the unsubstituted compound, BBV, due to the electron-withdrawing fluoro group *para* to the boronic acid. As observed earlier,[6g] the value of BBV is substantially lower than that of phenyl boronic acid (pK_a = 8.8) and this is attributed to the effect of the positively charged nitrogen atom in the pyridinium ring. The change in pK_a observed by adding a second substituent to the aromatic ring is therefore, the net effect of both groups. The F-atom adds to the electron withdrawing effect of N^+; the OMe-group counteracts it.

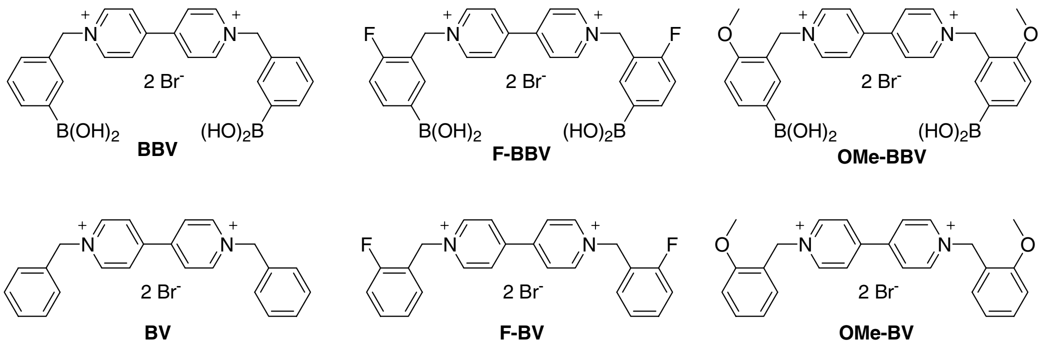
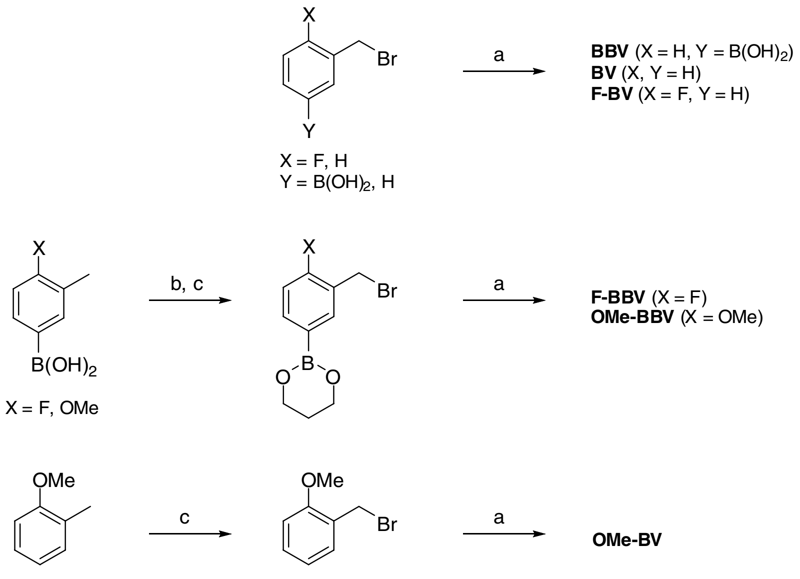


Figure 1. Structures of boronic acid-substituted benzyl viologens and benzyl viologens.



Scheme 1. Synthesis of viologen-based quenchers. Reagents and conditions: (a) 4,4′-dipyridyl, DMF, 60 °C, 48 h; (b) 1,3-propanediol, CaH_2, DCE, 88 °C, 1 h; (c) *N*-bromo succinimide, 2,2′-azobisisobutyronitrile, DCE, 88 °C, 3 h.