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Boronic acid-appended bis-viologens as a new family of viologen quenchers for glucose sensing

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Abstract—A new family of boronic acid-appended viologen quenchers has been synthesized containing two viologen subunits in a single quencher moiety. Relative to the single viologen-based quenchers previously developed in our laboratory, the bis-viologen *ortho*-boronic acid (B*o*B) compounds, in combination with the fluorescent dye, HPTS, display greatly enhanced Stern–Volmer quenching constants and much greater signal modulation in response to glucose. The superior performance is realized at lower quencher-to-dye ratios than are required for the single-viologen systems.
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The application of supramolecular analytical chemistry to the development of indicator-displacement assays (IDAs) and differential analyte receptors is an important field of research.[1] A closely related area is the use of fluorescent chemosensors[2] for analyte detection; the measurement of blood glucose levels is a particularly important application for these techniques.[3,4] In many of these systems, a boronic acid is used as the saccharide receptor. Boronic acid-based fluorescent chemosensors take advantage of the ability of boronic acids to reversibly bind 1,2 and 1,3 diols.[5]

Many boronic acid-based fluorescent chemosensors are one-component systems; the boronic acid receptor is coupled to a fluorescent dye, causing a change in fluorescence upon glucose binding.[6] The present study uses a two-component system[7] comprising an anionic fluorescent dye as reporter and a boronic acid-appended viologen as a saccharide-responsive quenching component. In solution, because of ionic attraction, these components form a non-fluorescent ground-state complex. At or near neutral pH, reaction of the boronic acid with glucose converts it to the negatively charged boronate, thereby neutralizing the positive charge on the quencher. Consequently, the ground-state complex is weakened and an increase in fluorescence is observed. The change in fluorescence can be correlated directly with glucose concentration, making this system applicable to glucose detection.

In a two-component system, the ability to independently alter either component to optimize the sensing system provides a distinct advantage. For example, we have shown that the quencher can be synthetically modified to make it selective for glucose over other monosaccharides,[7h] and have also demonstrated that the dye can be changed to shift the excitation and emission wavelengths of the system.[7f] Many of our experiments have been carried out using 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS) as the anionic fluorescent dye and *N*,*N*′-4,4′-bis(benzyl-boronic acid)-bipyridinium dibromide (BBV) as the quencher.

The flexibility of our two-component system also allows for manipulation of the quencher-to-dye ratio (Q:D) to optimize glucose sensitivity in the physiological range. The BBV quenchers, with a single-viologen moiety in the structure, give optimum performance at Q:D = 125:1. The reason why a high Q:D ratio is needed has not been fully elucidated. It may be the result of weak interaction between the dye and quencher; or the quenching process may involve the cooperative action of multiple viologens.

It has been reported that bis-viologens have more positive reduction potentials than mono-viologens, indicating that they are better electron acceptors.[8] For the family of benzyl viologens (BV), we have shown that

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reduction potential correlates with quenching ability.[7h] We have also reported that increasing the number of cationic charges on the quenchers, as for example in the compound, *m*-BBVBP^{4+}, enhances quenching of HPTS.[7e] The linking of two viologen moieties into a single quencher molecule provides four positive charges, as opposed to only two on the BBV quenchers. In this study, we evaluate a family of glucose responsive bis-viologen quenchers. The purpose is to determine the effect of this structure modification on quenching and glucose modulation. Performance is investigated in a two-component saccharide-sensing system using HPTS as the reporter.

Four bis-viologens were synthesized by coupling two 4-(*N*-benzylboronic acid pyridinium) pyridine moieties with a xylylene linker. They include (**1**, *p*B*o*B), (**2**, *o*B*o*B), (**3**, *m*B*o*B), and (**4**, B*m*B*o*B). The mono-viologen quencher *o*BBV was used as a control compound (Fig. 1).

The synthetic route to the B*o*B compounds makes use of our standard preparative procedures for BBV compounds (Scheme 1). 2-Bromomethylphenylboronic acid was reacted with an excess of 4,4′-dipyridyl in acetone to afford the mono-substituted 4,4′-dipyridyl adduct (**5**). Combining an excess of **5** with the appropriate bis-bromomethylbenzene in DMF yielded *p*B*o*B, *m*B*o*B, and *o*B*o*B. Using 3,5-bis(bromomethyl)phenylboronic acid in this reaction yielded B*m*B*o*B. The products were precipitated from the reaction mixture by addition of acetone.

It has been previously demonstrated that boronic acid-appended mono-viologens quench the fluorescence of the anionic dye HPTS.[7] In the present study, we compare the quenching efficacy of the bis-viologen B*o*B quenchers with that of the mono-viologen quencher, *o*BBV. The quenching efficacies were determined using fluorescence spectroscopy to generate Stern–Volmer plots. Data collected from titrations of HPTS (4×10^{-6} M) with different B*o*B quenchers in phosphate buffer (pH 7.4, 39 mM) at ambient temperatures were used to generate a Stern–Volmer plot in the concentration range of 0–6×10^{-5} M (Fig. 2).

The best fit to the Stern–Volmer plots for the B*o*B compounds was obtained using the static quenching model (Eq. 1) with no contribution from dynamic quenching.[2a] The R^2 value for all fittings was higher than 0.99. In contrast, the 'sphere of action' model (Eq. 2) gave the best fit for *o*BBV and *m*-BBVBP^{4+} (Table 1).[9]

$$F_0/F = 1 + K_s[Q] \qquad (1)$$

$$F_0/F = (1 + K_s[Q])e^{V[Q]} \qquad (2)$$

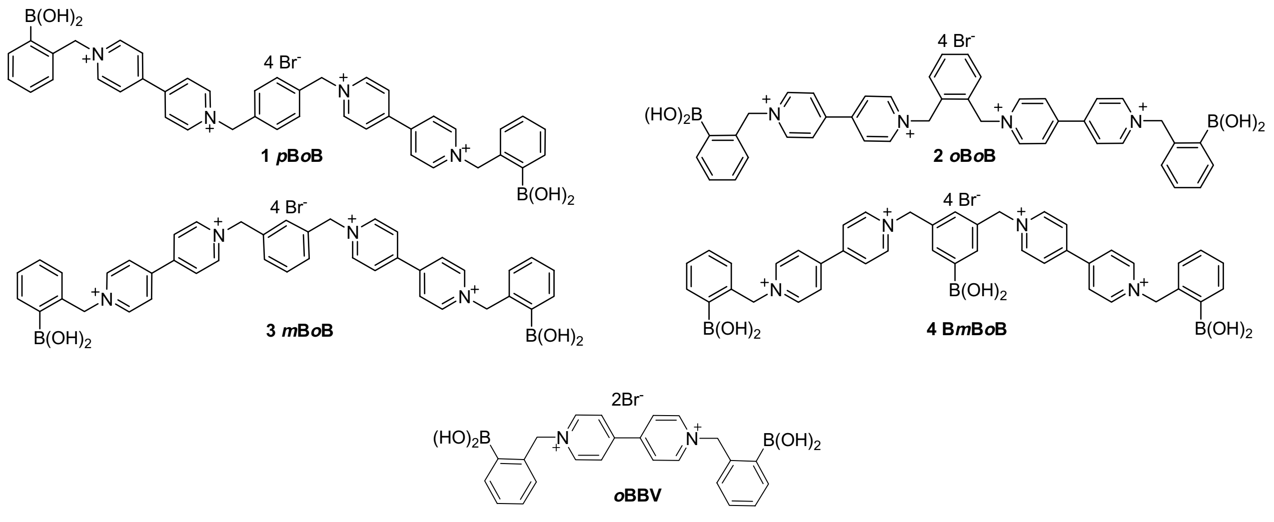
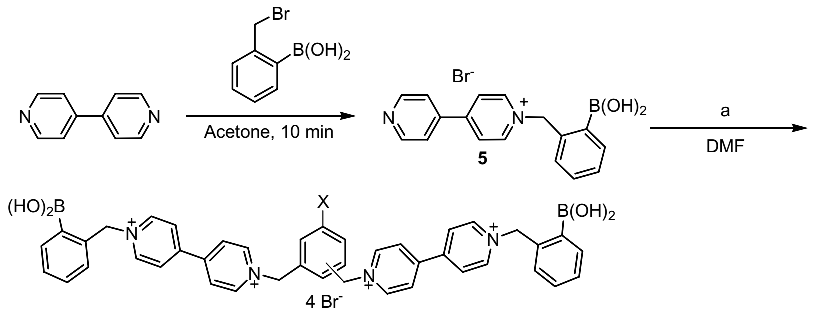


Figure 1. Structures of bis-viologen boronic acid substituted quenchers, *p*B*o*B, *m*B*o*B, *o*B*o*B, B*m*B*o*B, and their mono-viologen counterpart, *o*BBV.



Scheme 1. Synthesis of B*o*B quenchers. *p*B*o*B, a = 1,4-bis(bromomethyl)benzene, X = H; *m*B*o*B, a = 1,3-bis(bromomethyl)benzene, X = H; *o*B*o*B, a = 1,2-bis(bromomethyl)benzene, X = H; B*m*B*o*B, a = 3,5-bis(bromomethyl)benzene boronic acid, X = $B(OH)_2$.

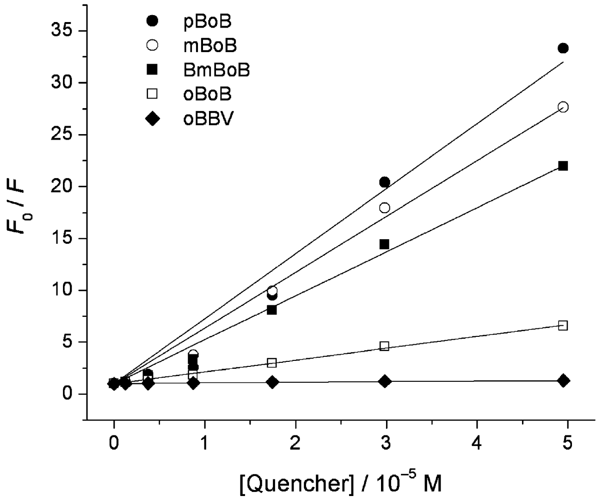


Figure 2. Stern–Volmer Plot for the quenching of HPTS (4×10^{-6} M) with the different quenchers ($0–6 \times 10^{-5}$ M). The data was fit using Eqs. 1 and 2.

Table 1. Quenching constants for B*o*B quenchers and *o*BBV

	K_s (M^{-1})	V (M^{-1})
*o*B*o*B	$1.2 \pm 0.2 \times 10^5$	—
*m*B*o*B	$5.6 \pm 0.4 \times 10^5$	—
*p*B*o*B	$6.8 \pm 0.8 \times 10^5$	—
B*m*B*o*B	$4.4 \pm 0.2 \times 10^5$	—
*o*BBV	$8.9 \pm 0.2 \times 10^3$[a]	$2.9 \pm 0.2 \times 10^3$[a]
m-BBVBP^{4+}	$4.7 \pm 0.4 \times 10^4$[b]	$4.5 \pm 0.7 \times 10^3$[b]

[a] See Ref. 7g.

[b] See Ref. 7e.

The B*o*B compounds are clearly superior quenchers compared to either *o*BBV or *m*-BBVBP^{4+}. This was expected in the case of *o*BBV because the B*o*B quenchers have four positive charges compared to only two on the former. However, when comparing the B*o*B quenchers to *m*-BBVBP^{4+},[7e] which also has four positive charges but only one viologen unit, the B*o*B compounds are still better quenchers (Table 1). This indicates that it is primarily the bis-viologen core that enhances quenching, not the high positive charge. Among the three B*o*B positional isomers, that with the lowest quenching constant is *o*B*o*B, followed by *m*B*o*B, then *p*B*o*B. We speculate that the difference may be related to the electron affinity of the different B*o*B structures and also possibly to pimer formation. It has been reported that bis-viologen systems in which the viologens are in close proximity can stack to form intramolecular pimers.[10] Assuming pimerization reduces the ability of the bis-viologen to accept electrons,[10] this could explain the aforementioned trend in quenching efficacy.

Since the ease of pimerization between the two viologen units is related to their proximity to each other, it makes sense that *o*B*o*B would pimerize most readily since the viologens are closest in this structure. Pimerization has been reported for non-functionalized bis-viologens linked by an *o*-xylylene group.[10] The *meta*-xylene linker in *m*B*o*B places the viologens further apart relative to their positioning in *o*B*o*B. And, since the *para*-xylylene linker in *p*B*o*B positions the viologens farthest apart, intramolecular pimer formation would be least likely to occur in this molecule.

In the case of B*m*B*o*B, where the viologens are connected with a *meta*-xylylene linker substituted with an additional boronic acid on the benzene ring, the static quenching constant falls between *m*B*o*B and *o*B*o*B. We anticipated that the quenching of B*m*B*o*B should be similar to *m*B*o*B, but somewhat diminished because of the presence of the extra boronic acid. At pH 7.4, the boronic acid is partially present in the negative boronate form.[7g] Since the ground-state complex formation involves electrostatic interaction, reducing the net positive charge on the bis-viologen would weaken the complex resulting in the reduced static quenching seen in B*m*B*o*B.

As stated earlier, the ability to adjust the quencher-to-dye ratio is a unique advantage of our two-component sensing system. For the BBV quenchers, a Q:D ratio of 125:1 gives the optimum glucose modulation in the physiological range. At this ratio, *o*BBV gives a fluorescence increase of 160% ($F/F_0 = 2.6$) within the range of 0–20 mM glucose. Because the B*o*B quenchers are much stronger quenchers than their BBV counterparts, high Q:D ratios are not necessary for efficient quenching of HPTS. We were therefore able to use far lower Q:D ratios in the glucose sensing studies. The results of a Q:D optimization study for B*m*B*o*B are shown below to illustrate the trend (Fig. 3).

The binding curves displayed in Figure 3 show that the increase in intensity obtained upon glucose addition is greater at higher Q:D ratios. At ratios of 4:1 and 5:1, however, the glucose binding curves are very similar indicating that the response is approaching a maximum. The other quenchers showed similar trends. Therefore, a comparison of the glucose response for the set of B*o*B quenchers was made at Q:D = 4:1 (Fig. 4).

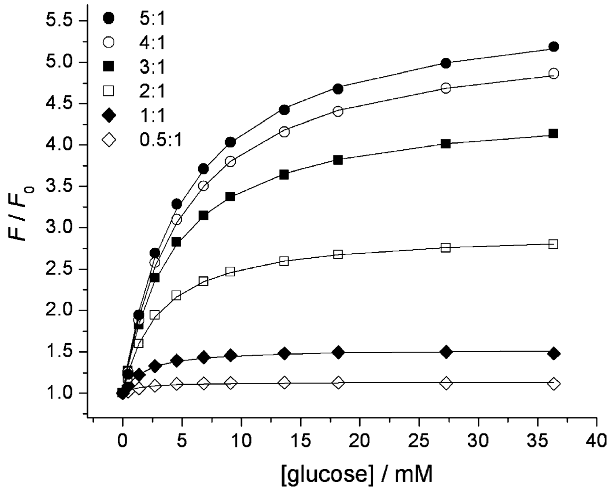


Figure 3. Comparison of glucose binding curves for B*m*B*o*B-HPTS at different Q:D ratios. The data were fit with a 1:1 binding algorithm. The concentration of HPTS was 4×10^{-6} M. The Q:D ratios used are seen in the legend.

The B*m*B*o*B quencher gives the highest glucose modulation. This is probably the result of an additional boronic acid receptor in this compound. When reacted with glucose, three negative charges are formed on B*m*B*o*B versus two negative charges on the other B*o*B quenchers. This favors a more effective dissociation of the ground-state complex. The quenchers, *p*B*o*B and *m*B*o*B, were only slightly less effective than B*m*B*o*B. Apparently, the difference between *para*- and *meta*-substitution on the xylene core is not a significant factor in determining glucose response. In contrast, the system using *o*B*o*B as the quencher had much lower modulation. This may be the result of the quenching process being affected by apparent pimer formation, favored by the proximity of the viologen subunits in *o*B*o*B.[10]

The glucose sensing abilities of the *meta*- and *para*-substituted B*o*B compounds are substantially improved relative to the mono-viologen quencher, *o*BBV. To illustrate, the performance of *p*B*o*B at Q:D = 4:1 is compared to that of *o*BBV at two Q:D ratios (Fig. 5). The appropriate ratios for making a direct comparison between *o*BBV and *p*B*o*B are 8:1 and 4:1, respectively, because the comparison, to be valid, has to be made on the basis of viologen equivalents.

At an 8:1 Q:D ratio, the glucose response of the *o*BBV system is relatively low. Even at a 125:1 Q:D, the glucose-sensing performance of *o*BBV is still substantially poorer as compared to *p*B*o*B at a 4:1 Q:D ratio.

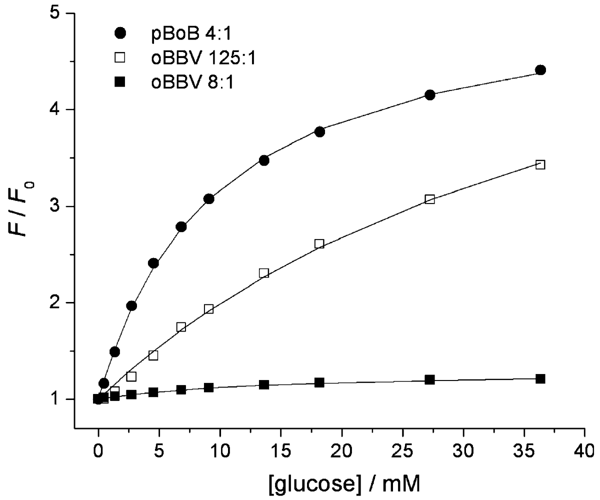


Figure 5. Comparison of *p*B*o*B and *o*BBV at different quencher–dye ratios. The data were fit with a 1:1 binding algorithm. The concentration of HPTS was 4×10^{-6} M. The Q:D ratios used are seen in the legend.

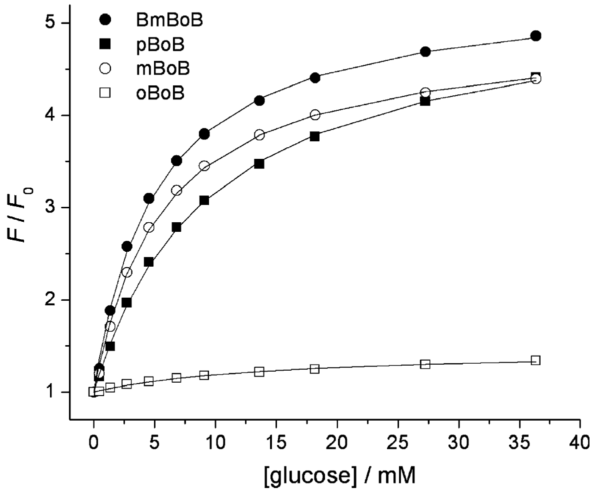


Figure 4. Comparison of glucose modulation obtained with B*o*B quenchers at 4:1 quencher-to-dye ratios. The data were fit with a 1:1 binding algorithm. The concentration of HPTS was 4×10^{-6} M.

In summary, we have synthesized a new family of glucose responsive bis-viologens for use in a two-component saccharide sensing system. These compounds quench HPTS much more effectively than previously used BBV quenchers, and show much higher signal modulation in the physiological glucose range. Also, the B*o*B quenchers are most effective at a Q:D ratio of only 4:1 as opposed to 125:1 for *o*BBV. Within this family, *o*B*o*B is the weakest quencher and also gives the lowest glucose modulation. B*m*B*o*B, with an additional boronic acid receptor, gives the highest glucose modulation. Studies are underway to further exploit these findings to make improved glucose sensing systems.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.11.053.

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