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COMMUNICATION

Fluorescent chemosensors of carbohydrate triols exhibiting TICT emissions

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4-4'-Disubstituted biphenyl boronic acids (BBAs) are push-pull fluorophores with "turn-on" fluorescence properties. Upon carbohydrate triol binding, BBA boronate esters can participate in photon-induced electron transfer, giving a twisted intramolecular charge transfer (TICT) complex. The resulting TICT emissions distinguish between carbohydrates that bind to boron as a diol versus triol unit, thereby revealing stereochemical information about the carbohydrate.

The amount of information carried by polysaccharides is potentially greater than that of proteins and nucleic acids of equivalent molecular weights,¹ but decoding this information is especially difficult, since many saccharides differ only in the configuration of a single stereocenter.² One potential means to identify sub-categories of carbohydrates is based upon their ability to bind to boron as either a diol or triol unit. However, no fluorescent chemosensor has been previously reported to make this distinction.³

Boronic acids can bind to diols and triols under physiological conditions to give boronic acid diesters and boronate triesters (Figure 1).⁴ These groups act as electron withdrawing or electron donating groups, respectively, due to the differences in boron hybridization and charge. We reasoned that these differences could be reported by "push-pull" fluorophores capable of distinguishing between various carbohydrates based upon their propensity to bind boron as a diol or triol unit. Biphenyl was selected as a fluorescent scaffold to evaluate this concept, because biphenyl derivatives containing electron donating/withdrawing groups at the 4 and 4' positions constitute an important class of "push-pull" fluorophores.⁵ Upon photoexcitation, these compounds can undergo intramolecular electron transfer to give a twisted intramolecular charge transfer (TICT) excited state. In some cases, the back electron transfer gives fluorescent emissions at red-shifted wavelengths as compared to non-TICT, locally excited states.⁵

DFT calculations were used to predict the push-pull characteristics of 4-4' disubstituted biphenyl boronic acids (BBAs) based upon the π -molecular orbitals of BBAs in their neutral (sp^2) and hydroxide-bound (sp^3) forms (Figure 2, and Figures S1 – S2, Supporting Information). According to the highly dispersed HOMO and LUMO densities over both phenyl units, the cyano-substituted biphenylboronic acid "CBBA" (1) should lack push-pull

characteristics in its neutral form (Figure 2a). In contrast, the HOMO of hydroxide-bound CBBA is localized to the σ framework of the boronate group, and its LUMO on the cyanobenzene unit (Figure 2b). These results suggested that upon photoexcitation, CBBA should exhibit enhanced charge transfer and therefore red-shifted emission spectra when sp^3 hybridized. The differences in calculated HOMO-LUMO energy levels further support this conclusion (Table S1, SI). DFT calculations of the dimethylamino-substituted biphenylboronic acid "DBBA" (4) revealed trends that were contrary to the cyano derivative 1 (Figure S2, SI), suggesting that an inherent push-pull system in 4 would be lost upon triol binding, giving blue-shifts in its photoexcitation and emission wavelengths.

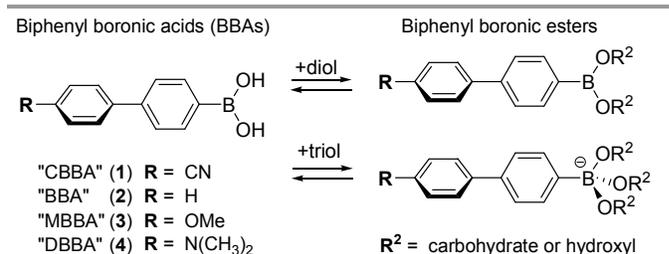


Figure 1: Structures of biphenyl boronic acids and their binding interactions with diols and triols. See Supporting Information for synthetic details of 1 – 4.

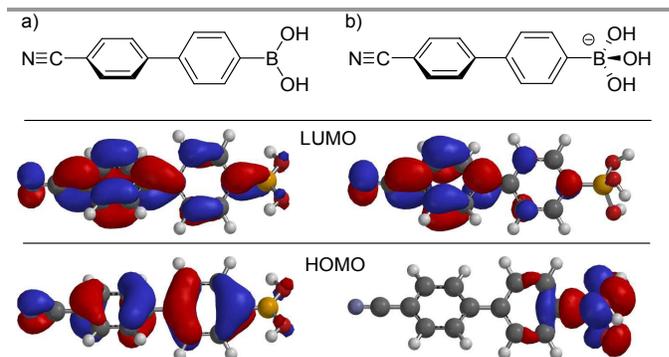


Figure 2. Structures and molecular orbitals of CBBA (a) and its hydroxide complex (b) calculated from DFT-optimized geometries using B3LYP/6-31G* in vacuum.

Encouraged by these results, compounds **1** – **4** were synthesized by cross-coupling chemistry (see SI), and characterized by NMR, MS and IR. The molar extinction coefficients (ϵ) of compounds **1** – **4** were $\epsilon_{272\text{nm}} = 35,600 \text{ cm}^{-1} \text{ M}^{-1}$ (**1**), $\epsilon_{258\text{nm}} = 23,000 \text{ cm}^{-1} \text{ M}^{-1}$ (**2**), $\epsilon_{271\text{nm}} = 17,700 \text{ cm}^{-1} \text{ M}^{-1}$ (**3**), and $\epsilon_{295\text{nm}} = 10,800 \text{ cm}^{-1} \text{ M}^{-1}$ (**4**). The quantum yields of compounds **1**, **3** and **4** ($\Phi = 0.30 - 0.81$, Table S2, SI) were significantly higher than that of biphenyl ($\Phi = 0.18$, $\epsilon_{248\text{nm}} = 15,000 \text{ cm}^{-1} \text{ M}^{-1}$).⁶

Excited-state intramolecular charge transfer (EICT) states are known to be stabilized by increasing solvent polarity, giving red-shifted emission maxima.⁵ To experimentally evaluate the presence of EICT under neutral conditions, the absorbance and emission spectra of boronic acids **1** – **4** were measured in solutions containing variable ratios of 1,4-dioxane and water (Figure 3, and Figure S3 Supporting Information). CBBA (**1**) exhibited almost no solvatochromic response, whereas DBBA (**4**) exhibited red shifting of its emission maxima with increasing solvent polarity. These results are consistent with DFT calculations suggesting that DBBA would act like a push-pull fluorophore under neutral conditions. To quantify these effects, the solvent polarity index E_T^{30} of each dioxane/water mixture was measured using *Reichardt's dye*.⁷ The Stoke's shift of each sample was plotted against E_T^{30} values to quantify solvatochromism.⁸ The methoxy (**3**) and dimethylamino (**4**) derivatives exhibited large slopes of $186 \text{ cm}^{-1}/\text{kcal}\cdot\text{mol}^{-1}$ and $342 \text{ cm}^{-1}/\text{kcal}\cdot\text{mol}^{-1}$, respectively. In contrast, the mono-substituted biphenyl (**2**) and cyano boronic acid (**1**) exhibited little or no push-pull characteristics, according to the slopes of $4 \text{ cm}^{-1}/\text{kcal}\cdot\text{mol}^{-1}$ and $55 \text{ cm}^{-1}/\text{kcal}\cdot\text{mol}^{-1}$, respectively (Figure S4, SI).

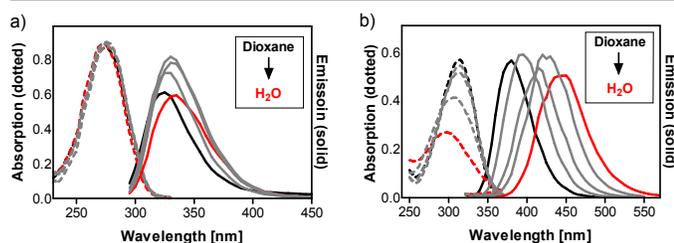


Figure 3: Absorption and normalized emission spectra of CBBA (a) or DBBA (b) in dioxane-water mixtures. Samples contained $25 \mu\text{M}$ of **1** or **4**, respectively. Emission spectra were recorded using excitation at the absorption maximum of each sample (Table S3, SI).

To characterize the changes in push-pull characteristics of BBAs upon hydroxide binding, the absorbance and emission spectra of compounds **1** – **4** were monitored as a function of pH (Figure 4 and Figure S5, SI). With increasing pH, red-shifted absorbance and emission maxima were observed for the cyano derivative CBBA (Figure 4a), whereas blue-shifted maxima were observed for the dimethylamino derivative DBBA (Figure 4b). These results are consistent with the conversion of boronic acid from an electron withdrawing group to a donating group upon hydroxide binding. According to the isosbestic points presented in these spectra, two-state transitions for hydroxide binding were observed (Figure 4). These data fit very well ($R^2 = 0.99$) to pK_a values of 8.5, 8.8, 8.9 and 8.9 for compounds **1** – **4**, respectively (Figure S6 and Table S3, SI). These values are similar to that of phenylboronic acid ($pK_a = 8.8$).⁹

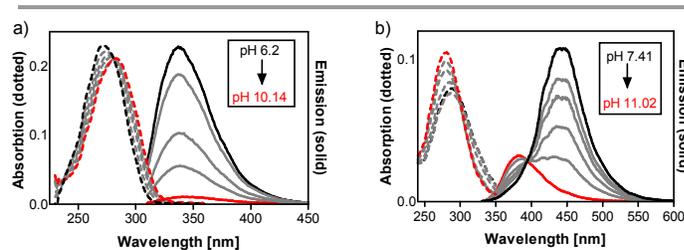


Figure 4: Absorption and emission spectra of CBBA (a) and DBBA (b) at different pH values measured in aqueous sodium phosphate buffer (100 mM). Samples contained $10 \mu\text{M}$ of **1** or $6.25 \mu\text{M}$ **4**. Excitation at 275 nm for **1** and 300 nm for **4**

D-Fructose and D-sorbitol are known to bind monoboronic acids as triol units, giving sp^3 -hybridized boronate esters.^{3b,4b} To evaluate the ability of BBAs to report carbohydrate triol binding, the absorbance/emission spectra of compound **1** – **4** were monitored upon addition of D-fructose or D-sorbitol (Figure 5, and Figure S7-S8, SI). With increasing fructose concentration, red-shifted absorbance and emission maxima were observed for the cyano derivative CBBA (Figure 5a), whereas blue-shifted maxima appeared for the dimethylamino derivative DBBA (Figure 5b). These results are consistent with the conversion of boronic acid from an electron-withdrawing group to an electron-donating group upon carbohydrate triol binding. Similar results were obtained with sorbitol (Figure S8, SI). According to the isosbestic points presented in these spectra, two-state binding interactions were observed, allowing for equilibrium dissociation constants (K_d) to be calculated for compounds **1** – **4** upon binding fructose and sorbitol (Figure S9 and Table S4, SI). With a $K_d = 4.5 - 4.8 \text{ mM}$, CBBA (**1**) exhibited slightly a higher binding affinity than the other BBA's (**2** – **4**, $K_d = 5.3 - 8.2 \text{ mM}$). Similar values were reported for phenylboronic acid.^{4c}

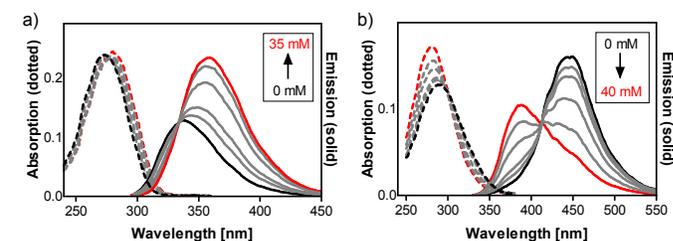


Figure 5: Absorption and emission spectra of CBBA (a) or DBBA (b) upon addition of D-fructose in an aqueous sodium phosphate buffer (100 mM, pH = 7.4). Samples contained $10 \mu\text{M}$ of **1** or $6.25 \mu\text{M}$ **4**, respectively. Emission spectra were recorded using the isosbestic wavelength of absorbance for excitation of each sample.

Given its promising “turn on” fluorescent properties, the photophysical specificity of CBBA (**1**) for reporting carbohydrate triols was evaluated in detail. Similar to the results obtained with fructose and sorbitol (Figure 5a), addition of xylitol, D-mannose, and D-mannitol to solutions of CBBA elicited the emergence of strong, red-shifted fluorescent emissions, suggesting that these carbohydrates bind to CBBA as triol units (Figure S10, SI). Surprisingly, little or no changes in CBBA fluorescence were observed upon addition of non-carbohydrate triols including 1,2,4-butanetriol, 1,2,5-pentanetriol, 1,2,6-hexanetriol, and 1,3,5 pentane triol. The only non-carbohydrate diol/triol that elicited a (small) fluorescence increase was *cis,cis*-1,3,5-cyclohexanetriol (Figures S11–S12, SI). This compound is known to bind boronic acids as a “tripod” boronate ester.¹⁰ Since fructose is also known to bind to phenyl boronic acid in a similar way,^{3b} these results suggest that

carbohydrate triols have a higher propensity for forming kinetically stable tripod boronate esters as compared to less structured, non-carbohydrate triols.

D-Ribose and D-glucose are known to bind to monoboronic acids as diol units and cause pK_a depression that results in an equivalent of hydroxide bound to the complex.^{4d,11} Upon addition of D-ribose or D-glucose to solutions of CBBA (**1**), fluorescence quenching was observed (Figure 6a, and Figure S10, SI). These results are similar to those obtained by addition of hydroxide (Figure 4a), suggesting that the binding of carbohydrate diols can facilitate reversible binding of hydroxide ions that quench CBBA fluorescence. The addition of non-carbohydrate diols including ethylene glycol, 1,1-bis(hydroxymethyl)cyclopropane, and 1,2-cyclohexanediol caused no significant changes in CBBA fluorescence (Figure S11, SI). These results are consistent with the minimal impact that simple diols have on the pK_a of aryl boronic acids in water.^{4c, 12}

In contrast to the results obtained with D-glucose (Figure 6a), the addition of D-galactose to CBBA caused an increase in red-shifted fluorescence (Figure 6b). Taken together, these results suggest that D-galactose can bind to CBBA as a triol unit, whereas glucose binds as a diol unit.^{4d,11} This is a remarkable outcome since these carbohydrates differ only in the configuration of a single stereocenter. In all cases where concentration-dependent fluorescence changes were observed, equilibrium dissociation constants (K_d) values were calculated from the binding isotherms (Figure S13 and Table S5, SI). The trends in boronic acid-carbohydrate were found to be: D-fructose > D-ribose > D-galactose > D-mannose > D-glucose. The same trend was also obtained using a dye-displacement assay.^{4c} Taken together, these results indicate that there is no strict correlation between carbohydrate binding affinity and binding mode (diol versus triol) in water.

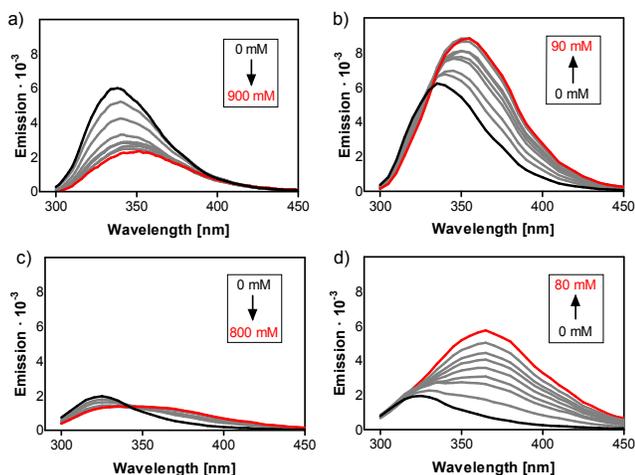


Figure 6: Emission spectra of 2 μ M CBBA (**1**) upon addition of D-glucose (a) or D-galactose (b). Emission spectra of 50 μ M DM-CBBA (**5**) upon addition of D-glucose (c) or D-galactose (d). All samples were prepared in aqueous sodium phosphate buffer (100 mM, pH = 7.4) and excited at 275 nm.

To evaluate the origin of the red-shifted fluorescence emissions from CBBA (**1**) upon carbohydrate triol binding, a CBBA derivative containing two methyl groups “DM-CBBA” (**5**) was synthesized and evaluated (Figure 7). According to DFT calculations, the ground-state biphenyl twisting in **5** is 90°, in contrast to the 35 – 37° angles calculated for compounds **1** – **4** (Figures S1 – S2, SI). Accordingly, compound **5** exhibits properties consistent with a non-conjugated biaryl system such as blue-shifted absorbance/emission maxima (λ_{abs}

= 239 nm, λ_{em} = 319 nm) and a 30-fold lower quantum yield (Φ = 0.01) as compared to the CBBA (**1**). Remarkably, compounds **1** and **5** exhibited the emergence of a red-shifted fluorescence emission peak (λ_{em} = 360 nm) upon addition carbohydrate triols such as D-fructose, D-sorbitol, xylitol, D-mannose, D-mannitol, and D-galactose (Figure 6b,d and Figures S14–S15, SI). Taken together, these results demonstrate that the increases in fluorescence intensity at 360 nm upon carbohydrate triol binding are the result of emissive TICT states in compounds **1** and **5**.

The type of “turn on” fluorescence response exhibited by CBBA and DM-CBBA is an important aspect to numerous applications including sensor construction, microarray analyses and cellular imaging.¹³ Due to its lower initial fluorescence intensity, DM-CBBA (**5**) gives a much larger (up to 20-fold) increase in fluorescence intensity as compared to CBBA upon addition of carbohydrate triols (Figure 6 b,d). When used at a concentration of 50 μ M in a standard fluorimeter, solutions of DM-CBBA have a limit of detection of 5 μ M of D-fructose (0.54 ppm), with a linear range of 0.005 – 2 mM (Figure S16). The sensitivity and dynamic range of DM-CBBA is therefore about 20-fold higher than reported fluorescent boronic acids exhibiting PET,¹⁴ and about 2-fold higher than fluorescent boronic acids that disaggregate upon addition of carbohydrates.¹⁵ The sensitivity of DM-CBBA is comparable to the best electrochemical and enzymatic carbohydrate sensors reported to date.¹⁶ Boronic-acid based TICT probes with higher extinction coefficients, red-shifted excitation/emission maxima, and higher quantum yields should exhibit $\sim 10^4$ -fold higher sensitivity than DM-CBBA. Such compounds will find important new applications in cellular imaging and microarray analyses.

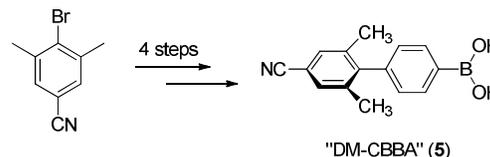


Figure 7: Synthesis and structure of “DM-CBBA” (**5**). See Supporting Information for synthetic details.

Conclusions

To the best of our knowledge, CBBA (**1**) and DM-CBBA (**5**) provide the first examples of carbohydrate sensors that exhibit TICT luminescence. The emergence of TICT emissions from **1** and **5** required the addition of carbohydrates that can bind to boronic acid as triol a unit.^{3b,4b} Non-carbohydrate diols, triols, and carbohydrates that bind to boron as a diol unit,^{4d,11} failed to elicit TICT emissions. The results reveal a new strategy for distinguishing subcategories of carbohydrates by their ability to bind to boron as a diol versus triol unit. To the best of our knowledge, CBBA and DM-CBBA provide the first fluorescent probes capable of making such a distinction.

Notes and references

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† **Electronic Supporting Information (ESI) available:** HOMO-LUMO calculations, absorption and emission spectra, and synthetic details and characterization of compounds **1** – **5**.

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Graphical Abstract

