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Fluorescent probe for monosaccharides based on a functionalized boron-dipyrromethene with a boronic acid group

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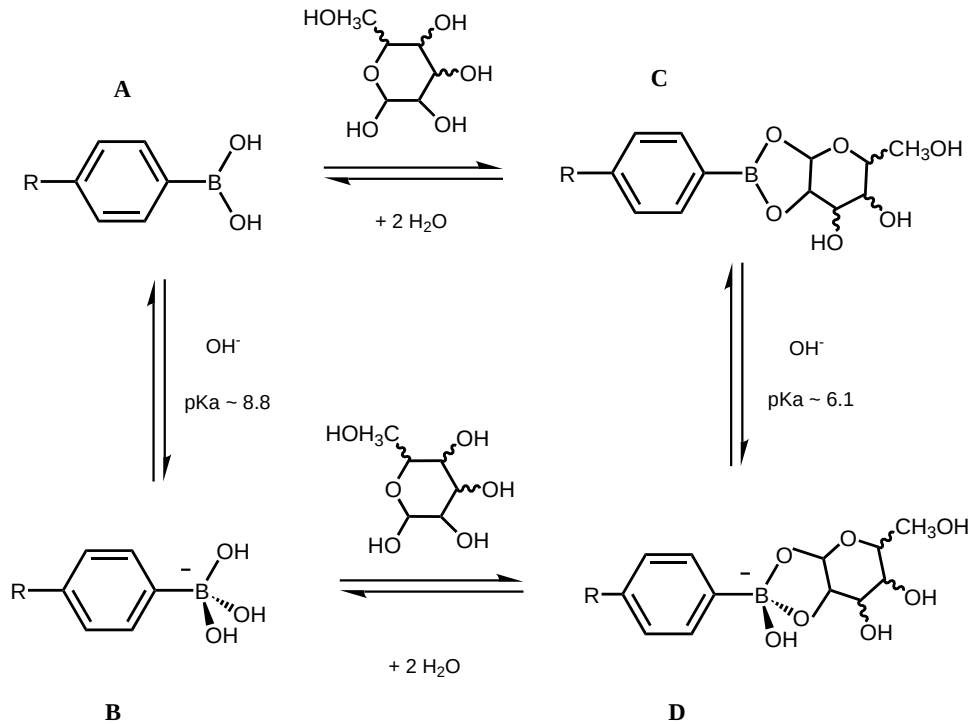
Abstract—A new highly fluorescent probe based on a boron-dipyrromethene functionalized with a phenylboronic acid group was synthesized from 2,4-dimethylpyrrol and 4-formylphenylboronic acid. Spectral changes in both absorption and emission spectra were observed in the presence of sugars.

Detection of sugar by fluorescence spectroscopy has been a long-standing goal for many research groups. Today, enzymatic assay based on the selective oxidation of glucose using enzymes (typically glucose oxydase) is the most reliable technique.[1] The consuming aspect and the generation of reactive species resulting from the oxidation have limited the usefulness of this technique for implantable devices and continuing glucose monitoring in blood and/or in interstitial tissues.[2,3] To overcome these limitations, fluorescent biosensors and chemosensors have been developed. Non-consuming enzymatic assay based on fluorescence resonance energy transfer and conformational changes have been investigated.[4–6]



Scheme 1. Equilibrium involved between the boronic acid group and the pH and/or sugar.

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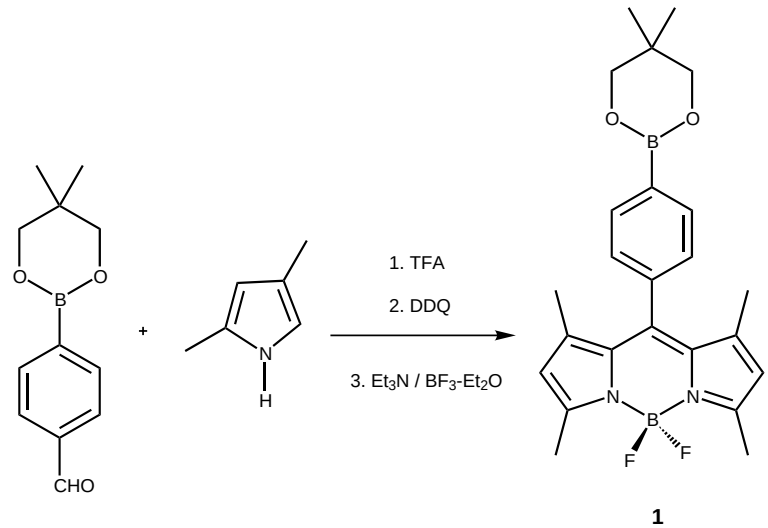
Chemosensors for sugar signaling involving fluorescent dyes and chelator groups have attracted important interest in the last decade, see Ref. 7 for a recent review. Synthetic probes for glucose are expected to overcome some problems associated with enzymatic assay and to provide new technologies for diabetes health care. To date, the most reliable chelator groups for sugar recognition are the boronic acids. The boronic acids are electron deficient Lewis acids having an sp^2-hybridized boron atom with a planar trigonal conformation under its neutral form,[8] structure **A** of Scheme 1. Boronic acid groups formed fast and reversible covalent interactions with carbohydrates. The complexation leads to a decrease of the pK_a and induces the formation of the anionic form of the boronic group at specific pH, structure **D** of Scheme 1. The anionic form of the boron group is electron rich and possesses an sp^3-hybridized boron atom with a tetrahedral conformation. When the boronic acids are conjugated with a fluorophore, the neutral form of the boron group acts as an electron-withdrawing group while the anionic form acts as an electron-donating group.[9] This change in the electronic properties of the boron group leads to spectral changes of the fluorophore and has been used for the development of wavelength-ratiometric and long-wavelength probes.[9–11]

In this letter, we report on the synthesis and spectroscopic characterization of a boron-pyrromethene derivative having a phenylboronic acid group. Probe **1** was prepared following a standard method from the protected 4-formylphenylboronic acid and the 2,4-dimethylpyrrol (Scheme 2).[12] Probe **1** was used directly without deprotection since the hydrolysis is expected to be complete in water and the same effect of sugar is observed with and without the protecting group.[9,13] Boron-dipyrromethene dyes (better known as BODIPY™[14]) present many advantages as fluorescence probes. They possess high extinction coefficients, high fluorescence quantum yields, good photostability, narrow emission band and their building block synthesis allows the development of many different analogues showing emission from 500 to 700 nm. Long-wavelength fluorescent probes for glucose are highly desirable for transdermal glucose monitoring and/or for measurements in whole blood. In addition, narrow emission bands are desirable for high signal to noise ratio.

Probe **1** shows a very narrow absorption and emission bands with maxima at 495 nm ($\varepsilon = 35\,000\ M^{-1}\ cm^{-1}$) and 510 nm ($\phi_F = 0.41$), respectively, in phosphate buffer, pH 7.5. As the pH increased from 4.0 to 12.0, we observed a blue shift of the absorption band with an increase of the absorption coefficient. An isobestic point was observed at 500 nm demonstrating the equilibrium between the neutral and anionic form of the boronic acid group. Fluorescence spectrum shows similar effects than observed in the absorption spectrum. Fig. 1 shows the titration curves of **1** in the absence and in the presence of D-fructose obtained from the absorption and emission spectra. The pK_a of **1** (8.3–8.8) is similar to the general pK_a obtained for phenylboronic acid derivatives.[9,13] In the presence of D-fructose (D-fructose was chosen because it shows a higher affinity for monoboronic acids in comparison with others sugars), the pH effect gives similar spectral changes in the absorption and emission spectra. The presence of D-fructose increases the spectral changes observed in the emission spectrum, while it decreases those in the absorption spectrum. As mentioned above, the complex probe:sugar shows a smaller pK_a (5.9–6.1). At pH 7–7.5, probe **1** exists under its neutral form, while, in the presence of sugar, it exists under its anionic form. This change allows the detection of sugars at neutral pH.

Fig. 2 shows the effect of D-fructose on the absorption and emission spectra of **1**. As observed for the pH, a



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Scheme 2. Synthetic scheme for the investigated probe.

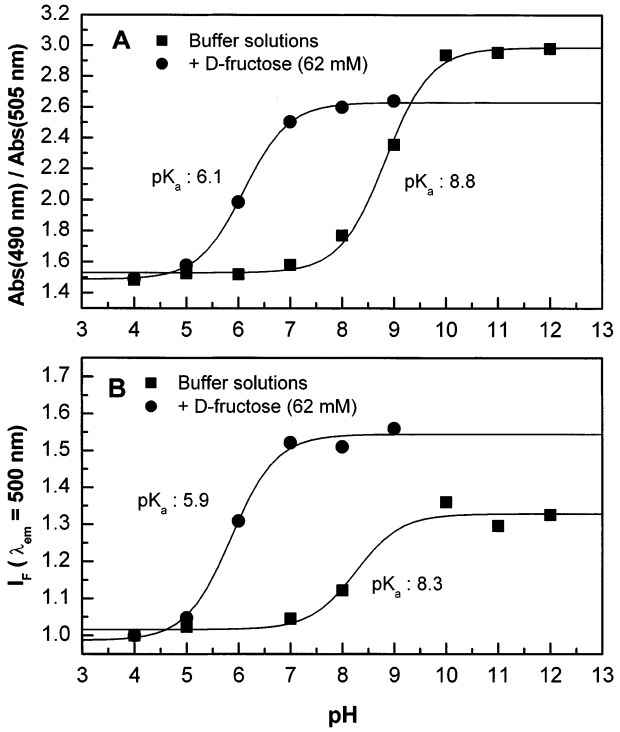

Figure 1. Titration curves against the pH obtained from the spectral changes observed in the absorption (**A**) and fluorescence (**B**) spectra for **1** (2.9×10^{-6} M). Measured in buffer solutions at room temperature. Fluorescence intensities have been corrected for the absorption changes ($\lambda_{ex}=475$ nm).

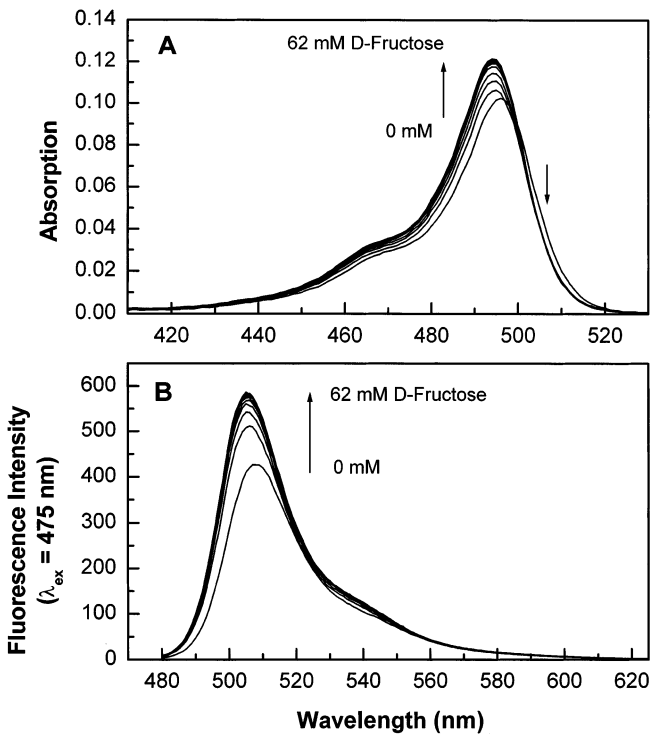

Figure 2. Effect of D-fructose on the absorption (**A**) and fluorescence (**B**) spectra of **1** (2.9×10^{-6} M). Measured in phosphate buffer, pH 7.5, at room temperature ($\lambda_{ex}=475$ nm).

blue shift and an increase of the absorption coefficient were observed as the concentration of sugar increased. A small blue shift and an increase of the emission were also observed in the emission spectrum. Titration curves against D-fructose, D-glucose and D-galactose are reported in Fig. 3. As generally observed for monoboronic acids, the affinity decrease from D-fructose to D-galactose and D-glucose, respectively. Dissociation constants are listed in Table 1.

Fluorescence changes induced by the presence of sugars are also corroborated by fluorescence decay measurements. Fluorescence decay profiles did not show changes as the pH changes from 4.0 to 12.0. Both measurements at pH 4.0 and 12.0 show single exponential decay profiles with a fluorescence lifetime of 3.5 ns. On the other hand, significant changes were observed in the fluorescence decay profile in the presence of D-fructose (62 mM) in phosphate buffer at pH 7.5. In the presence of D-fructose, the intensity decay remains monoexponential but with a longer fluorescence lifetime of 4.1 ns. This seems correlated by the larger fluorescence intensity change observed in the presence of D-fructose (55%) than without sugar (30%) as a func-

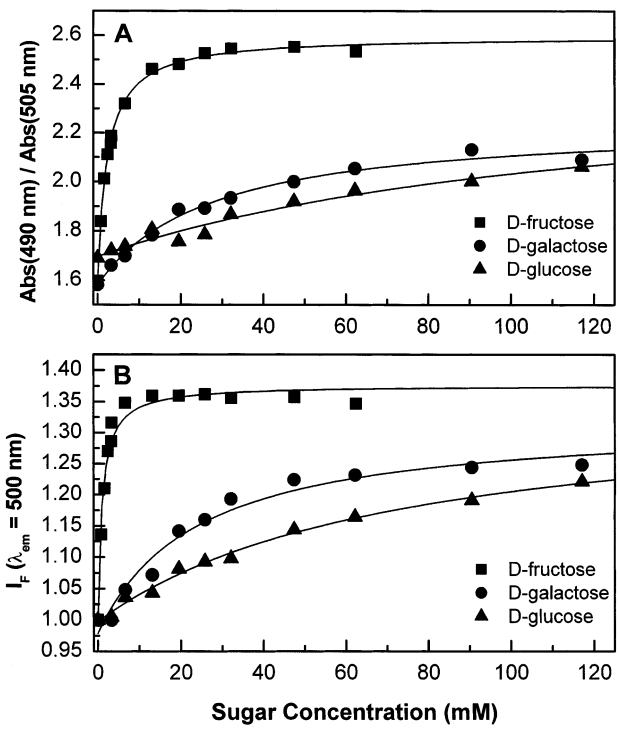

Figure 3. Titration curves against sugars for **1** (2.9×10^{-6} M). Measured in phosphate buffer, pH 7.5, at room temperature. Fluorescence intensities have been corrected for the absorption changes ($\lambda_{ex}=475$ nm).

Table 1. Dissociation constants (K_D) of **1** against sugars, measured in phosphate buffer, pH 7.5, at room temperature

	D-Fructose	D-Galactose	D-Glucose
Absorption (mM)	2.4	27	130
Emission (mM)	1.0	24	73

tion of the pH changes (Fig. 1). Both absorption and fluorescence decay measurements seem to demonstrate that the formation of the anionic form of the boronic acid group in the presence of sugar is not the only origin of the spectral change but conformational changes and/or restriction should be included. Deeper spectroscopic measurements would be needed to investigate the origin of the spectral changes observed.

In summary, we showed the synthesis of a new family of fluorescence probes for sugars based on the boron-dipyrromethene fluorophore. The building block synthesis of the probe and the numerous descriptions of substituted pyrroles available in the literature could easily lead to long-wavelength and conjugable fluorescence probes for sugars. The probe described here showed spectral changes in both absorption and emission spectra. Works are in progress to maximize the spectral response and shift the absorption and emission to the near-infrared region.

Acknowledgements

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12. To a solution of protected 4-formylphenylboronic acid (333 mg, 1.53 mmol) and 2,4-dimethylpyrrol (300 mg, 3.15 mmol) in 100 ml of N_2-saturated CH_2Cl_2 was added 10 μl of trifluoroacetic acid. The solution was stirred for 3 h at rt under a nitrogen atmosphere. DDQ (715 mg, 3.15 mmol) was then added, and the solution was stirred for an additional 30 min. To the mixture, 3 ml of Et_3N was added followed by the addition of 3 ml of $BF_3{\cdot}Et_2O$. The resulting mixture was stirred for 30 min and then washed with water. The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The desire product was purified by silica gel column chromatography (CH_2Cl_2). The yield was 71 mg (11%) of orange crystals: ^1H NMR ($CDCl_3$, 300 MHz) δ 1.06 (s, 6H), 1.36 (s, 6H), 2.55 (s, 6H), 3.80 (s, 4H), 5.97 (s, 2H), 7.27 (s, 2H), 7.90 (s, 2H); MS (FAB): found: 436.1, calcd for **1**: 436.2. Anal. calcd for $C_{24}H_{28}B_2F_2N_2O_2$: C, 66.02; H, 6.42; N, 6.42. Found: C, 66.34; H, 6.86; N, 6.36.
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