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Chalcone-analogue fluorescent probes for saccharides signaling using the boronic acid group

Nicolas DiCesare, Joseph R. Lakowicz*

Center for Fluorescence Spectroscopy, University of Maryland, School of Medicine, 725 W. Lombard St., Baltimore, MD 21201, USA

Abstract

Two new fluorescent probes based on 1,3-diphenylprop-2-en-1-one and on 1,5-diphenylpenta-2,4-dien-1-one structures are presented. Both probes possess one electron-donating dimethylamino group and one boronic acid group (electron-withdrawing group). The change between the neutral and the anionic form of the boronic acid group induced at high pH and/or in presence of sugar, induces optical changes for both probes. Spectroscopic data, pK_a and dissociation constants for different monosaccharides are presented and discussed in terms of sugar detection.

For almost a decade, the development of synthetic chemosensors for saccharides using the boronic acid group has been the focus of many research groups.¹⁻⁷ The boronic acids have the advantage of a reversible and fast equilibrium interaction with monosaccharides. In addition, the boronic acid group can be incorporated in many different systems giving large possibilities for the development of analytic devices for the recognition and detection of sugars. The development of these devices could find important applications, especially for diabetics. The actual technology of using enzymes for the sensing of glucose shows some limitations.⁸ For example, this technology is hardly applicable to implantable devices for continuous glucose monitoring in blood and/or in interstitial fluids.

The most promising probes developed to date are based on the boronic groups ability to increase its electrophilicity (acidity) in the presence of sugars. Probes using the photoinduced electron transfer based on the acid:base interaction between the boron group and an amino group has previously been developed.^{9,10} Most recently, we investigated the use of the electron-withdrawing property of the boronic group for the development of fluorescent probes based on the intramolecular charge transfer (ICT) mechanism.¹¹⁻¹³ The change of charge between the neutral $[R-B(OH)_2]$ and the anionic $[R-B(OH)_3^-]$ forms, at high pH and/or in presence of sugar, altered the electron-withdrawing property of the boron group and thus the spectral properties of the ICT of the excited state. Probes showing large intensity changes and/or wavelength shifts have been developed using the ICT mechanism.¹¹⁻¹³

In this paper we report the incorporation and the subsequent influence of the boronic acid group on a fluorophore showing ICT between a dimethylamino (electron-donor) and a

*Corresponding author. Fax: (410) 706-8408; lakowicz@umbi.umd.edu.

carbonyl (electron-withdrawing) group, Scheme 1. In these molecules, the boronic acid group does not directly participate in the ICT, Scheme 2. On the other hand, the boron group is in resonance with the electron-withdrawing group (the carbonyl in this case). Since the boronic acid group becomes an electron-donor under its anionic form,¹¹⁻¹³ the competition between the boronate and the dimethylamino groups for the ICT is expected to produce spectral changes due to the perturbation of the ICT nature of the excited state.

Scheme 1 shows the one-step synthetic pathway for both chalcone derivatives. Chalcone derivatives were prepared by the condensation reaction from the corresponding aldehydes and acetylphenylboronic acid, both commercially available. Reactions were carried at room temperature in ethanol with small amount of aqueous NaOH 10%. Spectroscopic data are in agreement with the molecular structure of both derivatives,¹⁴

Spectroscopic and photophysical data for **Chalc1** and **Chalc2** are shown in Table 1. The absorption spectrum of **Chalc2** shows only a slight red shift in comparison with that obtained for **Chalc1**. On the other hand, the conjugation length shows more pronounced effect on the emission spectrum. **Chalc1** shows very similar absorption and emission bands as compared with its analogue, that does not possess the boronic acid group, 3-[4'-(dimethylamino)phenyl]-1-phenyl-prop-2-en-1-one (**Chalc**),¹⁵ **Chalc** shows absorption and emission maxima at 418 and 552 nm in ethanol, respectively, with a ϕ_F of 0.011, in comparison to 414 and 545 nm with a ϕ_F of ~0.014 for **Chalc1** in MeOH. This suggests that the presence of the boronic acid group, under its neutral form, has no major effect on the spectroscopic parameters of the probes.

The intramolecular charge transfer (ICT) properties of the excited state were investigated by the effect of the polarity of the solvent on the absorption and emission bands. Both probes, **Chalc1** and **Chalc2**, show bathochromic shifts in their absorption and emission bands with the increase of the polarity of the solvent. The shifts are more pronounced in the emission spectra than in the absorption spectra. The emission band of **Chalc1** shows a red shift of $\sim 3700\text{ cm}^{-1}$ from cyclohexane to water/methanol 2:1 (v/v), whilst a red shift of $\sim 4260\text{ cm}^{-1}$ was typically observed for **Chalc2**. It is interesting to note that large Stokes' shifts are desirable to maximize the wavelength split between the excitation and the detection emission wavelength to reduce as much as possible scattering and Raman scattering effects. Combined with the long wavelength of emission of the chalcones derivatives, both probes, **Chalc1** and **Chalc2**, possess good spectral properties for a chemosensor. Only the low quantum yields of fluorescence of this family of dye could limit their usefulness.

Fig. 1 shows the effect of the pH on the emission band of **Chalc1**. Similar effects were observed for **Chalc2** (not shown) and no effect of the pH was observed in the absorption bands, for both compounds. As the pH increased, we observe an increase of the fluorescence intensity and a slight blue shift of the emission band. These spectral changes are thought to be induced by the formation of the anionic form of the boronic acid group as the pH increases.¹⁶ Similar effects have also been observed on electron donor/electron withdrawing derivatives of stilbenes,¹¹ diphenylbutadiene,¹² diphenylhexatriene¹² and diphenyloxazole¹³ where the boronic acid group was directly involved in the ICT. For the later, the blue shift observed was much larger, ranging some $2000\text{ to }2600\text{ cm}^{-1}$, in comparison with the blue

shift observed for **Chalc1**, $\sim 660\text{ cm}^{-1}$, and for **Chalc2**, $\sim 560\text{ cm}^{-1}$. On the other hand, the increase of the intensity is larger for the chalcone derivatives than for the other derivatives, except for the diphenyloxazole derivative. For the four derivatives described above, the boronic acid functional group was the electron-withdrawing group, and given the charge change between the neutral and anionic forms of the boron group, one can clearly attribute this to the removal of the ICT property of the excited state. For the **Chalc1** and **Chalc2** derivatives, we interpret the blue shift and the increase of the intensity by a perturbation of the ICT. Since, the anionic form of the boronic acid group can act as an electron-donor group,¹¹ at high pH, the electron withdrawing property of the carbonyl group should be decreased due to charge transfer and/or partial charge transfer from the boronate group to the carbonyl group, Scheme 2. The increase of the electronic density on the carbonyl group would decrease the ICT from the dimethylamino group and this is thought to be the origin of the spectral changes observed.

Titration curves as function of pH, for **Chalc1**, are shown in Fig. 1. Both derivatives show similar pK_a values of 7.5 in the absence of sugar and 5.4 and 5.2 in the presence of fructose for **Chalc1** and **Chalc2**, respectively. The pK_a values for both chalcone derivatives are smaller in comparison with typical values of 8.8–9.2 in the absence of sugar and 6.0–6.6 in the presence of sugar.^{11,12,17,18} These lower values are attributed to the electron deficient phenyl ring which possesses two electron withdrawing groups (boronic acid and carbonyl groups) causing an increase of the electrophilicity of the boron group. It is for this reason that the maximum changes between the titration curves without and with sugar were observed for pH 6.5, for both derivatives. The low pK_a values allow sugar titration in the range of pH of 6 to 7 instead of the conventional pH range of 7 to 8. Such results are encouraging and this may well lead to downstream chemosensors for sugars.

The effect of fructose on the emission spectrum of **Chalc2** is shown in Fig. 2. An increase in the fluorescence intensity combined with a small blue shift is observed. Similar spectral changes were observed for **Chalc1**. At pH 6.5, a predominant neutral form of the boronic acid is present in solution, whilst in the presence of sugar, a predominant anionic form is present due to the decrease of the pK_a of the boronic acid:sugar complex. As previously discussed for the pH effect, the change in charge of the boronic acid is thought to result in the spectral changes observed. As observed for the majority of monoboronic acid probes, **Chalc1** and **Chalc2** show a higher affinity for D-fructose ($K_D = 2.5$ for **Chalc1** and 2.1 for **Chalc2**) and this affinity decreases for D-galactose ($K_D = 16$ for **Chalc1** and 14 for **Chalc2**) and D-glucose ($K_D = 34$ for **Chalc1** and 30 for **Chalc2**), respectively.^{7,9,11,16}

In summary, two new fluorescent probes have been presented for sugar signaling based on the ICT mechanism. To date, they are the longest wavelength probes for sugar involving ICT presented in the literature. Long-wavelength probes are highly desirable for biochemical analysis to decrease the interference of the autofluorescence background of biological samples. In addition, we demonstrated that the boronic acid group does not have to be directly involved in the charge transfer, as previously described for ICT probes for saccharides, to induce interesting spectral changes after sugar binding. This paves the way for the development of new synthetic probes for saccharides, especially the use of near-

infrared fluorophores that are not based on a direct ICT between an electron-donor and electron-withdrawing groups.

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14. **Chalc1**: orange solid (57%), mp 157–158°C. ¹H NMR (CD₃OD) δ (ppm) 3.01 (s, 6H) 6.79–8.05 (m, 10H). FAB-MS (glycerol matrix), *m/z* 352.2, [**Chalc1**⁺+glycerol-2H₂O]. Anal. calcd for C₁₇H₁₈BNO₃: C, 69.18; H, 6.15; N, 4.75. Found: C, 68.47; H, 6.38; N, 4.53. **Chalc2**: dark red solid (40%), m.p. 266–267°C. ¹H NMR (CD₃OD) δ (ppm) 3.05 (s, 6H), 6.78–7.92 (m, 12H). FAB-MS (glycerol matrix), *m/z* 378.2, [**Chalc2**⁺+glycerol-2H₂O]. The elementary analysis of **Chalc2** differ slightly from the expected values, but only one spot was observed on TLC and the sample shows to be spectroscopically pure.
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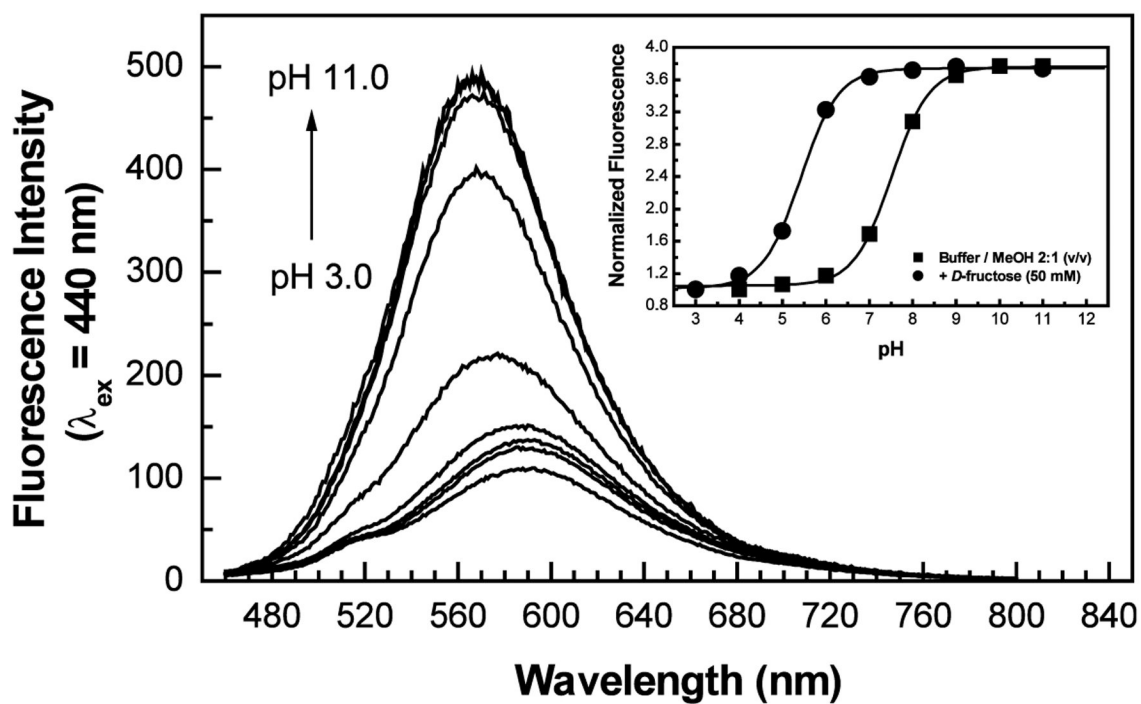


Figure 1. Effect of pH on the emission spectrum of **Chalc1**. Insert, titration curves for **Chalc1** as function of pH in absence and presence of fructose.

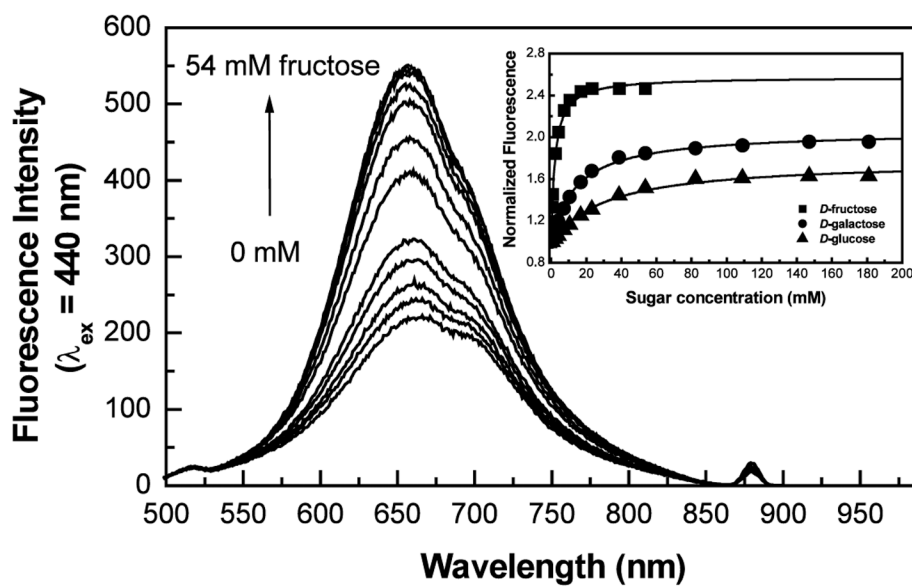
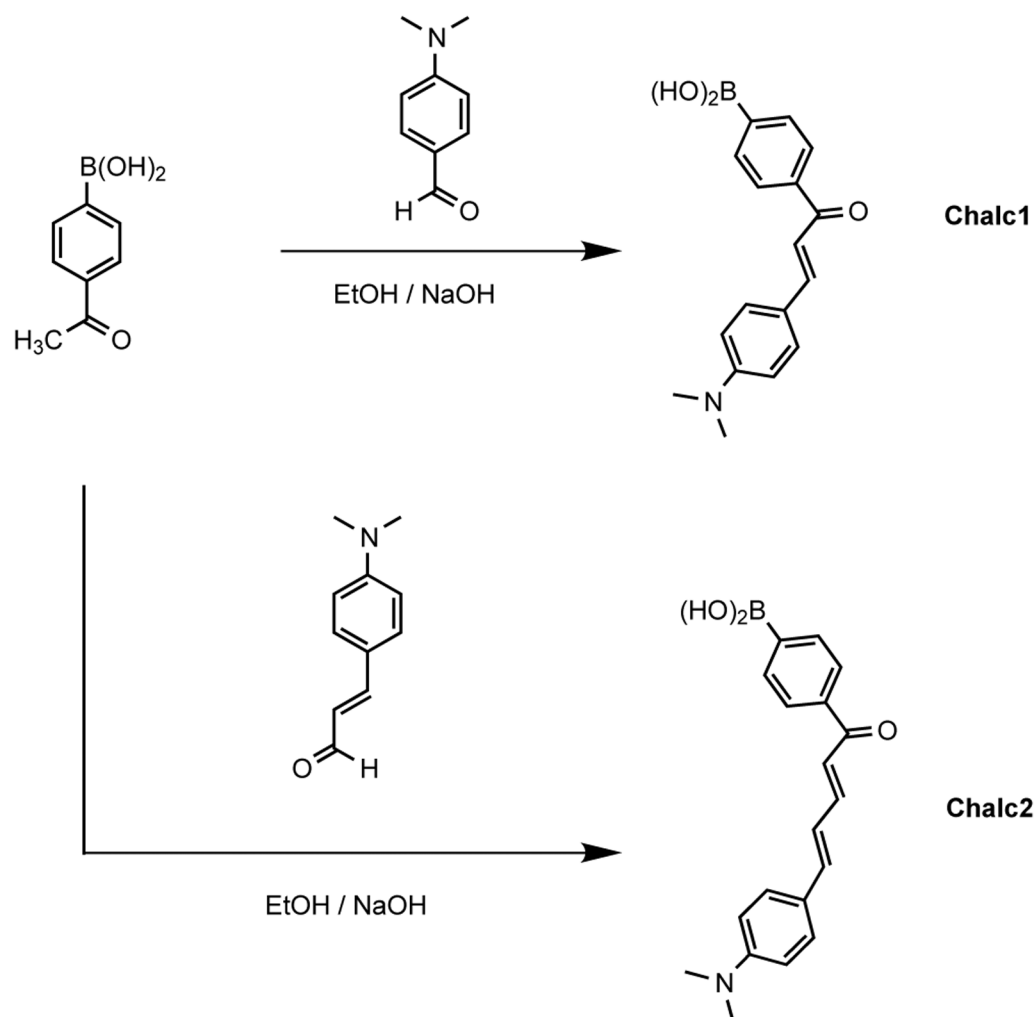
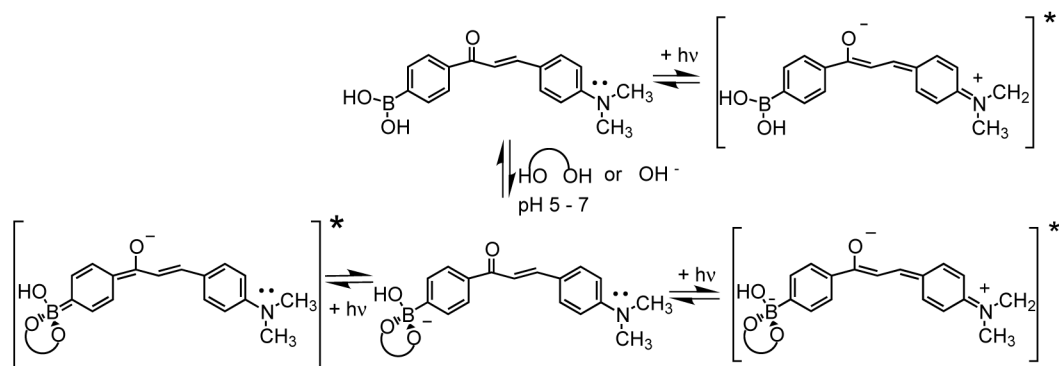


Figure 2. Effect of fructose on the fluorescence emission spectrum of **Chalc2**. Insert, titration curves of **Chalc2** for various sugars, measured in phosphate buffer pH 6.5/MeOH 2:1 (v/v).



Scheme 1.
Molecular structure of the investigated probes.

**Scheme 2.**

Ground and excited state electronic distributions involved in the neutral and anionic forms of the boronic acid group for **Chalc1**. For the case of interaction with OH^- , the diol should be replaced by two $-\text{OH}$ groups.

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Table 1.Spectroscopic and photophysical parameters of **Chalc1** and **Chalc2** measured in phosphate buffer pH 7.0/MeOH 2:1 (v/v) at room temperature.

Probe	λ_{Abs} (nm)	ϵ ($\text{M}^{-1} \text{cm}^{-1}$)	λ_{F} (nm)	τ (ns)	ϕ_{F}
Chalc1	438	21800	577	5500	0.007
Chalc2	445	19050	663	7390	0.008

: Stokes' shift.