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Monoboronic Acid Sensor That Displays Anomalous Fluorescence Sensitivity to Glucose

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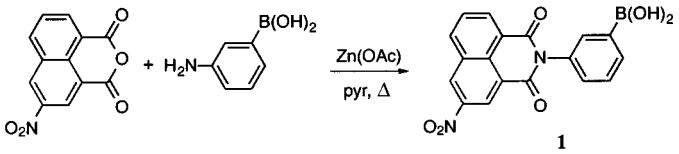
ABSTRACT



A monoboronic acid fluorescent sensor was conveniently synthesized from 3-nitronaphthalic anhydride and 3-aminophenylboronic acid. This novel saccharide probe exhibits dual emission suitable for ratiometric sensing and displays a remarkable sensitivity for glucose relative to fructose and galactose.

Among detection methods for saccharides, fluorescent chemosensors offer the advantages of analyte selectivity along with high sensitivity and real-time reporting. To signal recognition between analyte and receptor, fluorophores that exhibit $\pi-\pi^*$, internal charge transfer (ICT)[1] and metal-to-ligand charge transfer (MLCT)[2] excited states have been successfully employed as reporter components. Because saccharide complexation alters the oxidation state of phenylboronic acid, the fluorescence response for such chemosensory devices has been primarily communicated by photoinduced electron transfer (PET).[3] For use as biological probes, however, few saccharide sensors are currently eligible since large Stokes shifts are required to observe signals that are spectrally well separated from scattered excitation light and autofluorescence.[4]

A probe that features emission wavelengths greater than blue fluorescence along with a second band for ratiometric quantitation would hold distinct advantages over current sensor designs.[5] Recently, *N*-phenylnaphthalimides have been found to exhibit dual fluorescence when appropriately substituted at both the *N*-phenyl ring and naphthalene π-system.[6] These compounds display dual luminescence with two clearly resolved emission bands in the visible region from the locally excited (LE) state and a strongly red-shifted long-wavelength emitted by the ICT state, which is solvent dependent.[7] In this report, we introduce the first saccharide

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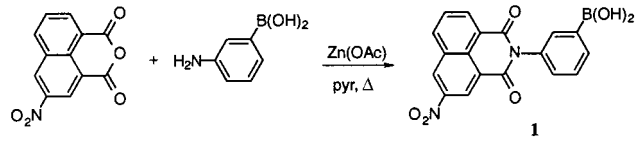
sensor that displays such dual fluorescence and a remarkable pH-dependent sensitivity to glucose over fructose.

To design a dual-fluorescent assembly that promotes charge-transfer excited states, 3-nitro-1,8-naphthalic anhydride was coupled to 3-aminophenylboronic acid in refluxing pyridine, giving sensor **1** (Scheme 1). In a manner similar

Scheme 1



1

to that of related *N*-phenylnaphthalimides, **1** displays two emission bands from a single excitation wavelength while increasing the solvent polarity causes the longer wavelength (LW) band to migrate toward the red. Table 1 summarizes

Table 1. Photophysical Properties of Sensor **1**

solvent	λ_F, nm SW[a]	λ_F, nm LW[a]	SE Stokes shift, cm^{-1}	LE Stokes shift, cm^{-1}	Φ_F[b]
CH_2Cl_2	389	506	4410	10370	0.010
acetone	385	506	4240	10450	0.008
ACN	367	510	2950	10510	0.009
EtOAc	365	505	3070	10590	0.008
EtOH	389	532	4410	11350	0.006
H_2O, pH 7	436	552	6790	11650	0.003

[a] SW, short-wavelength emission; LW, long-wavelength emission. [b] Quantum yield of LW band relative to quinine sulfate reference.

the trends in solvatochromic behavior and quantum yield as a function of solvent polarity.[8]

According to Figure 1, sensor **1** displays fluorescence quenching in basic pH conditions. The intensity changes recorded at 430 nm correlate with a mono-acid titration curve and pK_a of 8. The acidity of the boronic acid group increases in the presence of glucose, giving a pK_a value of 6.6. Contrary to the parent sensor sans nitro-substituent,[9] derivative **1** shows an unusual response to fructose and galactose as the decrease in fluorescence reaches a plateau between pH 7.5−8 followed by a second decrease at higher pH. On the basis of this fluorescence response to pH-dependence, the sensitivity of the sensor to fructose and glucose was further examined at pH 8.

Figure 2 shows the fluorescence ratio (430/550 nm) as a function of carbohydrate concentration. Sensor **1** displays a

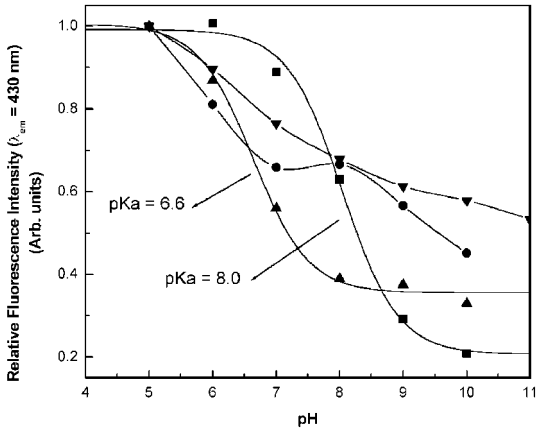


Figure 1. Titration curves vs pH for **1** (3.0×10^{-5} M, λ_{exc} = 337 nm, at 25 °C) in absence of sugar (■) and in the presence of fructose (●), glucose (▲), and galactose (▼).

decrease in the ratio of intensities for three common monosaccharides, showing the largest decrease in fluorescence for glucose. In the presence of sugar, the fluorescence spectrum of **1** shows a decrease in emission intensity at 430 nm while a small increase in intensity occurs at 550 nm. An isobestic point was observed between these two bands (Figure 3), indicating an equilibrium between two states.

The effect of pH on the emission spectrum of **1** does not show this equilibrium (result not shown) and suggests that conformational restriction in the **1**:sugar complex could play an important role in the observed optical change. Stoichiometric analysis using Job's method indicates a 1:1 sensor/saccharide complex.[10] Despite this unusual fluorescence response to glucose, the dissociation constants reflect the expected trend for boronic acid:saccharide complexes.[3] For the conditional dissociation constant of **1** with glucose, K_d = 26 mM was measured at pH 8. A lower dissociation value

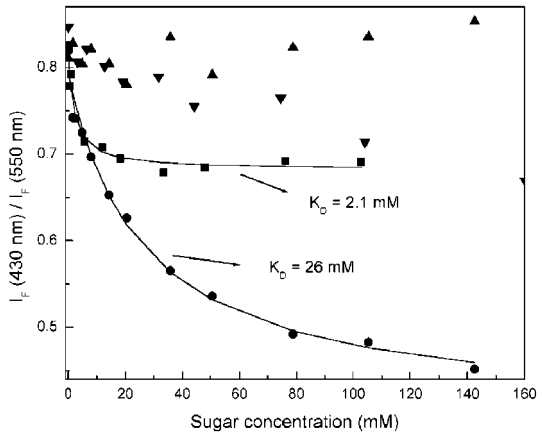


Figure 2. Titration curves against fructose (■), glucose (●), galactose (▲), and ethylene glycol (▼) for **1**, measured in phosphate buffer (100 mM), pH 8.0 at 25 °C.

(7) For *N*-phenylnaphthalimides related to **1**, an "excited state with extended conjugation" mechanism is proposed. For example, see: Demeter, A.; Berces, T.; Biczok, L.; Wintgens, V.; Valta, P. Kossanyi, J. *J. Phys. Chem.* **1996**, *100*, 2001−2011.

(8) Excitation wavelength varied between 329 and 336 nm on the basis of λ_{max} absorption for each solution. Solvatochromic studies were limited to polar solvents as aggregation resulted in less polar media.

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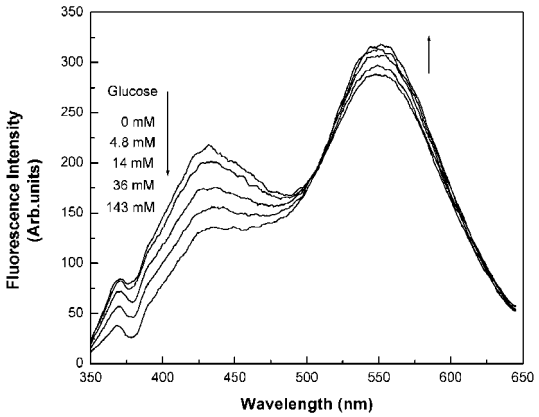

Figure 3. Fluorescence response for **1** (3.0×10^{-5} M) with D-glucose, measured in phosphate buffer (100 mM), pH 8.0 at 25 °C.

for fructose was obtained at $K_d = 2.1$ mM. Whereas several bisboronic acid sensors have been developed to provide a greater fluorescence response of glucose over fructose,[3] to our knowledge, this system represents the first monoboronic acid sensor to display higher sensitivity for glucose over fructose. In addition, because bis-boronic acid sensors tend to complex glucose in the micromolar range,[11] such sensing devices are often unsuitable for blood glucose levels which occur in the millimolar range. Sensor **1** provides an advantage at pH 8 by selectively sensing glucose while maintaining an affinity within physiological limits.

This anomalous chelation-enhanced quenching from a monoboronic acid sensor opposes the expected signal response involving monosaccharides.[12] In this case, the fluorescence data show that the optical change which results from fructose complexation is weak relative to glucose. Therefore, it appears that the binding affinity is not proportional to the observed optical change for this saccharide sensor. These findings suggest that other competing factors may be simultaneously operating, such as conformational restriction between the phenylhydroxy-boronate:saccharide complex and its influence on the excited state.[13] Synthetic modifications involving various substituent groups on the 1,8-naphthalic anhydride component are currently underway to help uncover the role that nitro-substitution plays in this glucose-sensitive/fructose-insensitive response.

In conclusion, a monoboronic acid fluorescent sensor that exhibits dual emission has been shown to display a remarkable sensitivity for glucose relative to fructose and galactose through subtle changes in pH. It is anticipated that this simple design which utilizes a nitro group to alter the fluorescence response will promote further investigations into substituent effects of chemosensory devices.

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Supporting Information Available: Synthetic procedures, spectral data, NMR, UV−vis, and fluorescence spectra for **1** as well as Job plot. This material is available free of charge via the Internet at http://pubs.acs.org.

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