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Fluorescent chemosensor for carbohydrates which shows large change in chelation-enhanced quenching

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Abstract

A new water-soluble saccharide receptor based on a naphthalic anhydride fluorophore was synthesized from 3-aminophenylboronic acid. The large change in fluorescence intensity (I/I_o=ca. 0.25) and measured pK_a (7.7) make this compound a useful chemosensor at neutral pH. © 1999 Elsevier Science Ltd. All rights reserved.

Within the last decade a considerable amount of effort has been directed towards the detection of saccharides by fluorescent chemosensors.[1] Such studies have shown that the response which signals an interaction between carbohydrate and receptor is frequently communicated by changes in fluorescence intensity either through chelation enhanced-quenching (CHEQ), or chelation-enhanced fluorescence (CHEF).[2,3] While significant advances continue in the areas of chemosensors for saccharides, invariably one or more of the requisite conditions necessary for biologists to measure these analytes often goes unmet. For carbohydrate measurements, conditions such as neutral pH as well as selectivity in an aqueous testing environment are essential. In addition to these physiological requirements, the signaling properties of the chemosensor must also meet certain criteria. Three critical prerequisites of the fluorescent sensor that must be satisfied for carbohydrate recognition have been outlined by Shinkai to include: strong fluorescence intensity, large pH dependent change in I_{max}, and shift of the pH–I_{max} profile to lower pH region in the presence of saccharides.[4]

Current designs in saccharide-sensors have relied almost exclusively upon a common anthracene or similar PAH-based fluorophore as the reporting unit, with synthetic modification of the phenylboronic acid or methylene spacer that binds these two groups together. The consequences of such unrelieved hydrophobicity often necessitate the addition of organic cosolvents to increase the solubility of the sugar sensor or synthesis of polar groups about the receptor component.[5] In response to limitations imposed by this system, we are developing saccharide receptors which utilize novel reporting groups based upon the naphthalic anhydride scaffold. Due to their high photostability and fluorescence quantum yields, this class of fluorophores has established a proven record as labels for spectroscopic measurements at

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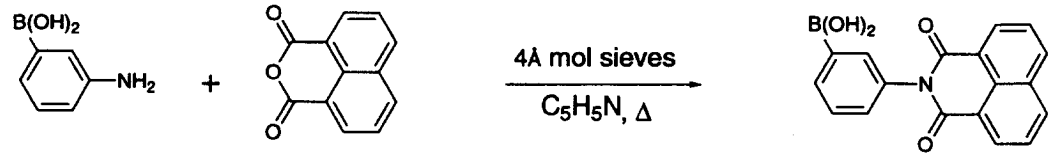
low concentrations.[6,7] In this Letter, we report on the synthesis and fluorescence properties of a simple monotopic saccharide sensor which satisfies several of the above listed requirements.

Receptor **1** was prepared in 50% yield from commercially available 3-aminophenylboronic acid via condensation with 1,8-naphthalic anhydride in refluxing pyridine (Scheme 1).[8]



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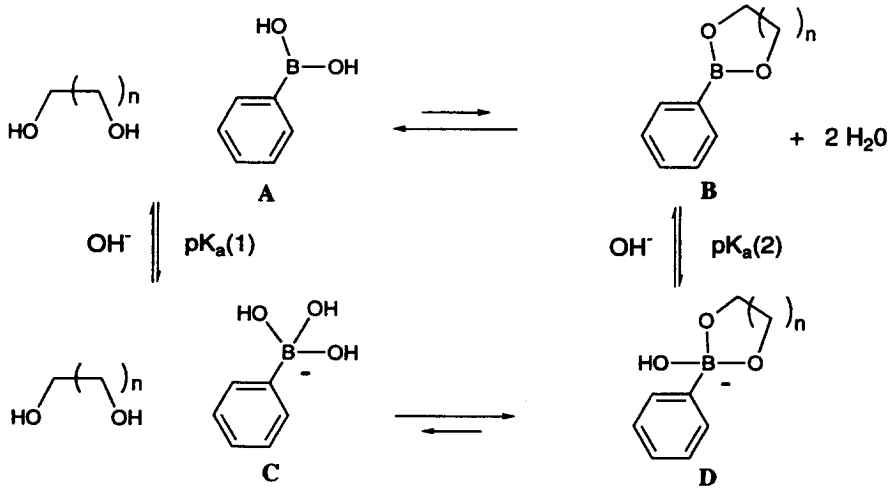
Scheme 1.

To compare fluorescence properties of the fluorophore with the saccharide sensor, quantum yield measurements were carried out on 1,8-naphthalic anhydride and **1** at neutral pH. Using a quinine standard, we obtained ϕ_F=0.34 for 1,8-naphthalic anhydride and ϕ_F=0.01 for the receptor.[9] This finding indicates that the phenylboronic acid group of **1** alters the electronic structure of the fluorophore and leads to fluorescence quenching.

Saccharide binding requires the assistance of OH- to generate hydroxyboronate anion **D** (Scheme 2) and the apparent pK_a for this complex is lower than $pK_a(1)$.[5] We observed a large pH-dependent change in I_{max} and a significant shift of the pH–I_{max} profile to lower pH region in the presence of fructose. The pH–fluorescence profile of **1** obtained in buffered solution is shown in Fig. 1, from which a pK_a of 7.7 is calculated. This value compares favorably to known monotopic receptors using anthracene-based fluorophores and enables the chemosensor to operate at physiological pH.[10] Ester formation between receptor **1** and fructose was observed as a function of pH to obtain a pK_a of hydroxyboronate nearly 2 pK_a units lower than unbound receptor. On the basis of this response to pH-dependence, we examined the selectivity of the sensor to different monosaccharides at neutral pH conditions.



Scheme 2.

Fig. 2 shows the relative fluorescence at 400 nm as a function of carbohydrate concentration. The figure includes the CHEQ responses to the three most abundant monosaccharides in human blood. The decrease in fluorescence intensity (I in the presence of saccharide/I_o in the absence of saccharide) for this

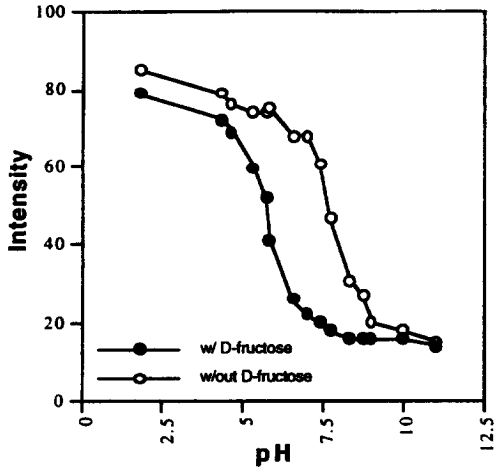


Figure 1. Fluorescence intensity (400 nm) versus pH profile of **1** at 25°C: 3.2×10^{-6} M of **1** in aqueous solution. [D-fructose]=3.30×10^{-2} M

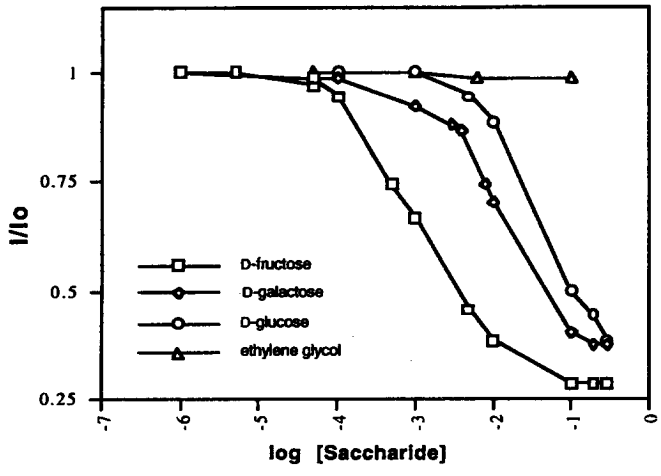


Figure 2. Relative fluorescence of **1** (3.2×10^{-6} M, aqueous phosphate buffer, pH 7.4) as a function of saccharide concentration. λ_{ex}=354 nm, λ_{em}=400 nm

series=ca. 0.25. These measured fluorescence levels remained unchanged after 12 h. To our knowledge this is the largest CHEQ response for a monotopic saccharide receptor in aqueous solution.

The selectivity of receptor **1** compares with other monoboronic acid sensors and shows the greatest association constant with D-fructose.[4] Assuming a 1:1 complex, the binding constant was found to be log K=3.0 for fructose and a lower binding constant of 1.6 was calculated for glucose. The large fluorescence response lies within detection requirements for fructose but remains less sensitive to physiological levels of glucose.[1]

In summary, a new water-soluble carbohydrate receptor is described which shows a large change in CHEQ signal response. This monotopic receptor is based on a naphthalic carboximide fluorophore which serves to lower the working pH of the sensor to near physiological conditions. In order to enhance selectivity between these sugars, we are currently investigating the bisdentate binding of sugars by two boronic acid components.[11] Further studies involving compounds that utilize this modular synthetic approach will be reported in due course.

Acknowledgements

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8. 3-Phenylboronic acid 1,8-naphthalenedicarboximide (**1**). A 25 mL round-bottom flask, equipped with a Dean–Stark receiver and condenser was charged with 3-aminophenylboronic acid (0.20 g, 1.30 mmol), 1,8-naphthalenedicarboxylic acid anyhydride (0.21 g, 1.09 mmol), 4 Å molecular sieves, and 15 mL of pyridine. The reaction mixture was allowed to reflux for 6 h. Pyridine was removed by distillation and replaced with dichloromethane. The dichloromethane solution was concentrated using a rotary evaporator and the crude solid purified over a plug of silica gel using 80:20 dichloromethane:acetone. The reaction afforded a tan powder (0.21 g, 50%): ^{1}H NMR (300 MHz, CD_3COCD_3) δ: 8.57 (d, *J*=7.5 Hz, 2H), 8.48 (d, *J*=8.5 Hz, 2H), 7.95 (d, *J*=7.0 Hz, 1H), 7.91 (t, *J*=7.2 Hz, 2H), 7.82 (s, 1H), 7.53 (t, *J*=7.4 Hz, 1H), 7.47 (d, *J*=8.0 Hz, 1H), 7.28 (s, 2H); ^{13}C NMR (75 MHz, CD_3SOCD_3) δ: 164.3, 136.5, 135.8, 135.0, 134.4, 132.2, 131.3, 129.7, 128.8, 128.6, 128.2, 127.8, 123.1. Anal. calcd for $C_{18}H_{12}BNO_4$: C, 69.29; H, 3.85; N, 4.48. Found: C, 68.82; H, 4.14; N, 4.38.
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