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Abstract:

Normally the detection of biologically important molecules such as saccharides is difficult. Few chemical sensing mechanisms have been developed for saccharides as they are uncharged, neither fluoresce nor quench fluorescence, and are normally present in aqueous media, which presents competitive hydrogen bonding. The recognition and measurement of saccharides has been achieved recently by certain synthetic molecular receptors or "probes," containing a boronic acid receptor moiety, which circumnavigates the normal difficulties associated with the detection of saccharides. The synthesis of two novel bidentate boronic acid molecular probes was attempted with the intent of testing their relative binding selectivity in the molecular recognition of simple monosaccharides such as glucose, fructose, and galactose.

Introduction

The need to measure the concentration of selected ions and small organic molecules in nature both *in vivo* and *in vitro* is apparent. Examples include monitoring Ca^{2+} ions involved in muscle fatigue, measuring CO levels in city microclimates to ensure safe air for breathing, and measuring the disappearance of glucose in fermentation reactions to ensure quality alcoholic beverages. [1] The continuous monitoring of the concentration of these chemical species is accomplished with sensors. Sensors come in two forms: biosensors and chemosensors. Recently, the recognition of biologically important molecular species by synthetic molecular receptors (chemosensors) has gained momentum.[2]

"Molecular recognition," as it has been called, involves the design and synthesis of a specific molecule (chemosensor) that will bind to the target molecule (analyte) and, through some mechanism of signal transduction, relay information about its concentration. These chemosensors can employ a variety of signal transduction mechanisms including UV, visible, NMR, electrochemical, and fluorescent systems.[1] Fluorescence spectroscopy is one of the most useful systems for optical readout and there exists a plethora of mechanisms by which fluorescence signal transduction may be produced. One of the most frequently used mechanisms to vary fluorescence intensity is the photoinduced electron transfer (PET) mechanism.

A typical PET sensor is composed of three main components: a fluorophore, a spacer, and a receptor as illustrated in Figure 1. In a typical PET sensor, the fluorophore consists of some kind of conjugated π system such as naphthalene that will fluoresce when bombarded with UV-Visable light. The spacer consists of an electron rich group, such as nitrogen, that will readily transfer an electron to the fluorophore quenching fluorescence. The receptor is the segment of the sensor that directly binds to the target analyte simultaneously altering PET by some measurable magnitude.

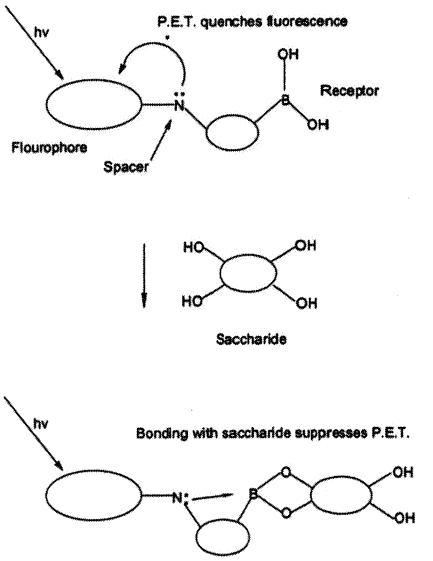


Figure 1.
General machanism of PET modulation upon saccharide binding.

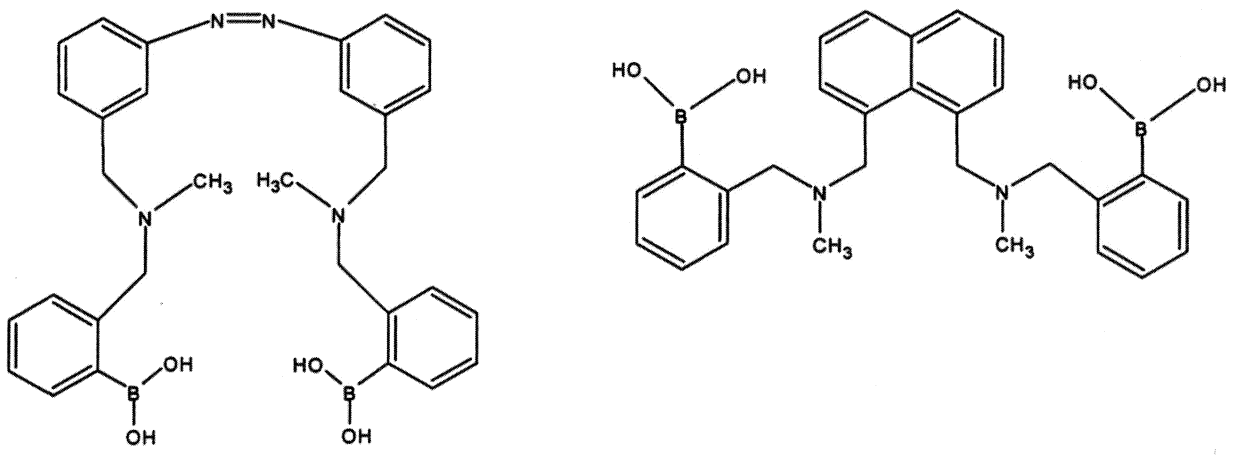
Since the chemistry of saccharides is very significant in the metabolic pathways of living organisms, detecting their presence and concentration in aqueous media is very important for a variety of medicinal and industrial applications. Most synthetic sensor molecules function on the basis of hydrogen bonding, and therefore competitive hydrogen bonding presented by the aqueous media surrounding target saccharides presents a serious drawback. To circumvent this problem, molecular sensors with a boronic acid receptor moiety have been developed. Covalent interactions readily form between the boronic acid receptor and the saccharide in aqueous media.[1] This represents a viable alternative binding force to be employed in the recognition of saccharides and related molecules.

The interaction between the boronic acid receptor and the amine spacer has been exploited in the creation of these PET sensors. When saccharides form cyclic

boronate esters with boronic acids, the acidity of the boronic acid is enhanced strengthening the Lewis acid-base interaction between the boronic acid and the amine.[2] The strength of this acid-base interaction modulates the transfer of an electron from the nitrogen to the fluorophore as depicted in Figure 1. These sensor compounds will demonstrate increased fluorescence at neutral pH through this suppression of the photoinduced electron transfer from nitrogen to the fluorophore upon saccharide binding.[3]

Many monosaccharides possess at least two binding sites, which are distinct from other monosaccharides, due to their particular stereochemistry.[1] Therefore, by employing two boronic acid receptor groups and controlling their spatial array, it should be possible to create probes that are selective for specific monosaccharides. It has also been demonstrated that different fluorophores can make a sensor more or less selective. In a study by Cooper and James, a probe with an anthracene fluorophore produced the same level of fluorescence recovery upon binding with four different saccharides, whereas a probe with a biphenyl fluorophore produced varying levels of fluorescence recovery with each of the same four saccharides.[4] Therefore, in the hope of achieving saccharide selectivity, we decided to design and synthesize two bidentate or di-boronic acid receptor molecules, each with a different fluorophore as depicted in Figure 2.

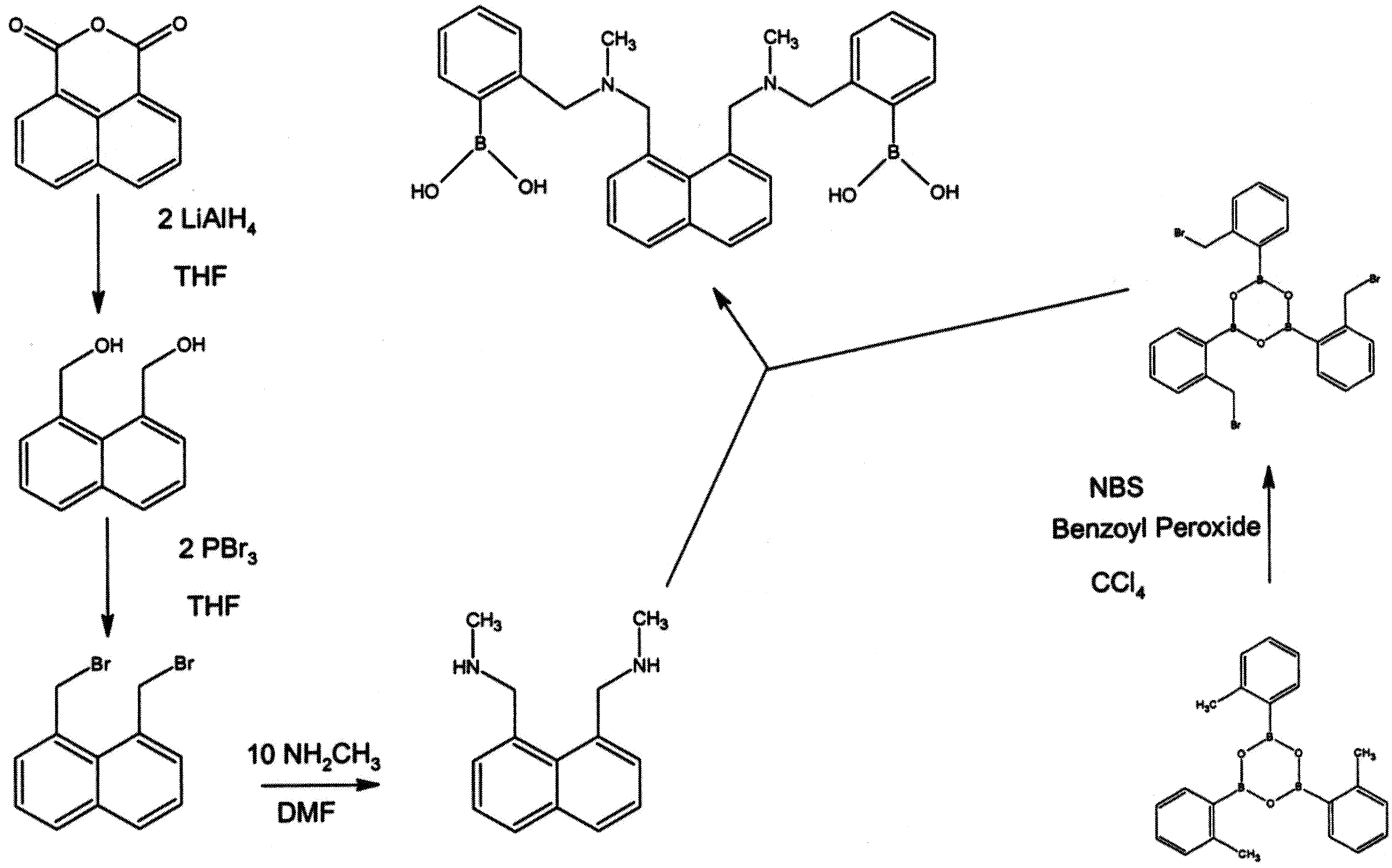
Figure 2.
Two novel bidentate boronic acid probes. The structure of the spacer and receptor segments are identical while the fluorophore is varied.



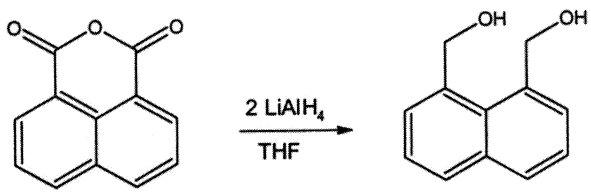
Methods

All melting points were taken on a Mel Temp II apparatus and are uncorrected. All ^{1}H NMR spectra were obtained and recorded with a Nicolet 360 MHz and associated computer software. All chemicals were purchased from Aldrich Chemical Company in high purity and were used without further purification.

Proposed Synthesis I



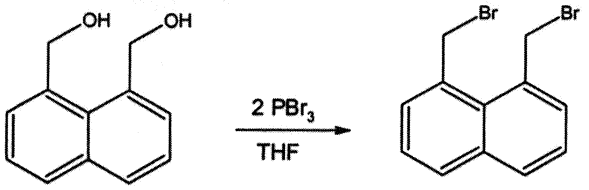
Reduction of 1,8-Napthalic Anhydride
Reference: Ohkawa, Journal of Medicinal Chemistry, 1997.



1,8-Napthalic Anhydride (25 mmol, 5.02g) was reduced to 1,8-Bis(hydroxymethyl)-napthalene using 1.0 M $LiAlH_4$ (50 mmol, 50 mL) in THF. The 1,8-Napthalic Anhydride was dissolved in 20 mL of THF and the $LiAlH_4$ solution was added via syringe. The reaction system was held under N_2 gas to eliminate oxidative interference from the ambient air. The mixture was refluxed for 18 hours.

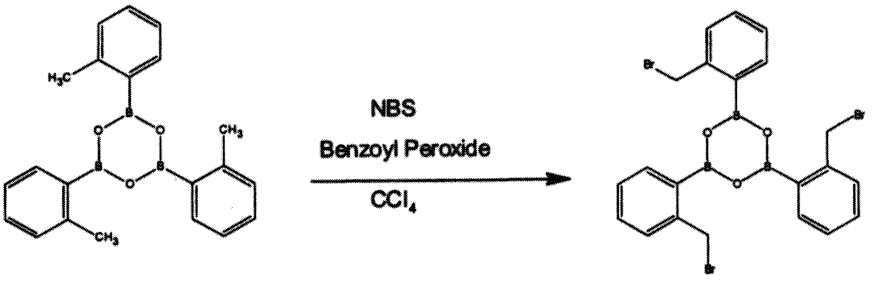
Cool distilled water was added to react with excess $LiAlH_4$. The cooled mixture was then neutralized with 1.0 M HCl. The mixture was heated and hot filtration was employed to remove Lithium and Aluminum salt byproducts. The crude product was extracted with EtOAc providing a crude yield of 2.45g (53.2%). Recrystallization in 50:50 Hexane/Ethyl Acetate afforded 1.45g (31.5%) of pure product with a melting point of 157.2–159.0°C (Lit. 160–161°C). A Proton Nuclear Magnetic Resonance (^{1}H NMR) spectra of the product in deuterated Chloroform was used to confirm the correct structure of the product.

Bromination of 1,8-Bis (hydroxymethyl)-naphthalene
Reference: Ohkawa, Journal of Medicinal Chemistry, 1997.



1,8-Bis(hydroxymethyl)–naphthalene (38 mmol, 0.70g) was brominated to 1,8-Bis(bromomethyl)–naphthalene using 1.0 M PBr_3 in CH_2Cl_2 (72 mmol, .0072 L). The 1,8-Bis(hydroxymethyl)–naphthalene was dissolved in 10 mL THF and the PBr_3 solution was added via syringe. The reaction system was held under N_2 gas due to the highly reactive nature of PBr_3. The reaction was allowed to reflux until completion (1.5 hr), its progress monitored via Thin Layer Chromatography (TLC). At completion the mixture was diluted with water and cooled on ice. The product was extracted with EtOAc providing a crude yield of 0.56g (48.4% yield). Recrystallization in 50:50 Chloroform/Hexane afforded 0.44g (37.4% yield) of pure product with a melting point of 149.6–152.3°C (Lit. 147–148°C). A Proton Nuclear Magnetic Resonance (^{1}H NMR) spectra of the product in deuterated Chloroform confirmed the correct structure of the product.

Bromination of o-toluene boronic anhydride
Reference: Snyder, Journal of American Chemistry, 1958.



o-toluene boronic acid (37 mmol, 0.50g) was brominated to *w*-bromo-*o*-toluene boronic acid using N-bromosuccinimide (0.75g) and Benzoyl Peroxide (1 mg) in CCl_4 (25 mL). The reaction involved a radical mechanism in which N-bromosuccinimide was the source of Bromine and Benzoyl Peroxide the radical initiator. A 200-Watt incandescent light bulb was placed adjacent to the reaction flask and functioned in initiating the reaction. The reaction was allowed to reflux for 2.5 hours. Rapid hot filtration removed the succinimide and the product crystallized in the filtrate as it cooled. The crude product (0.36g, 72.0% yield) was collected via vacuum filtration. Recrystallization in CCl_4 afforded 0.28g (56.0% yield) of pure product possessing a melting point of 156.7–159.4°C (Lit. 165–168°C).

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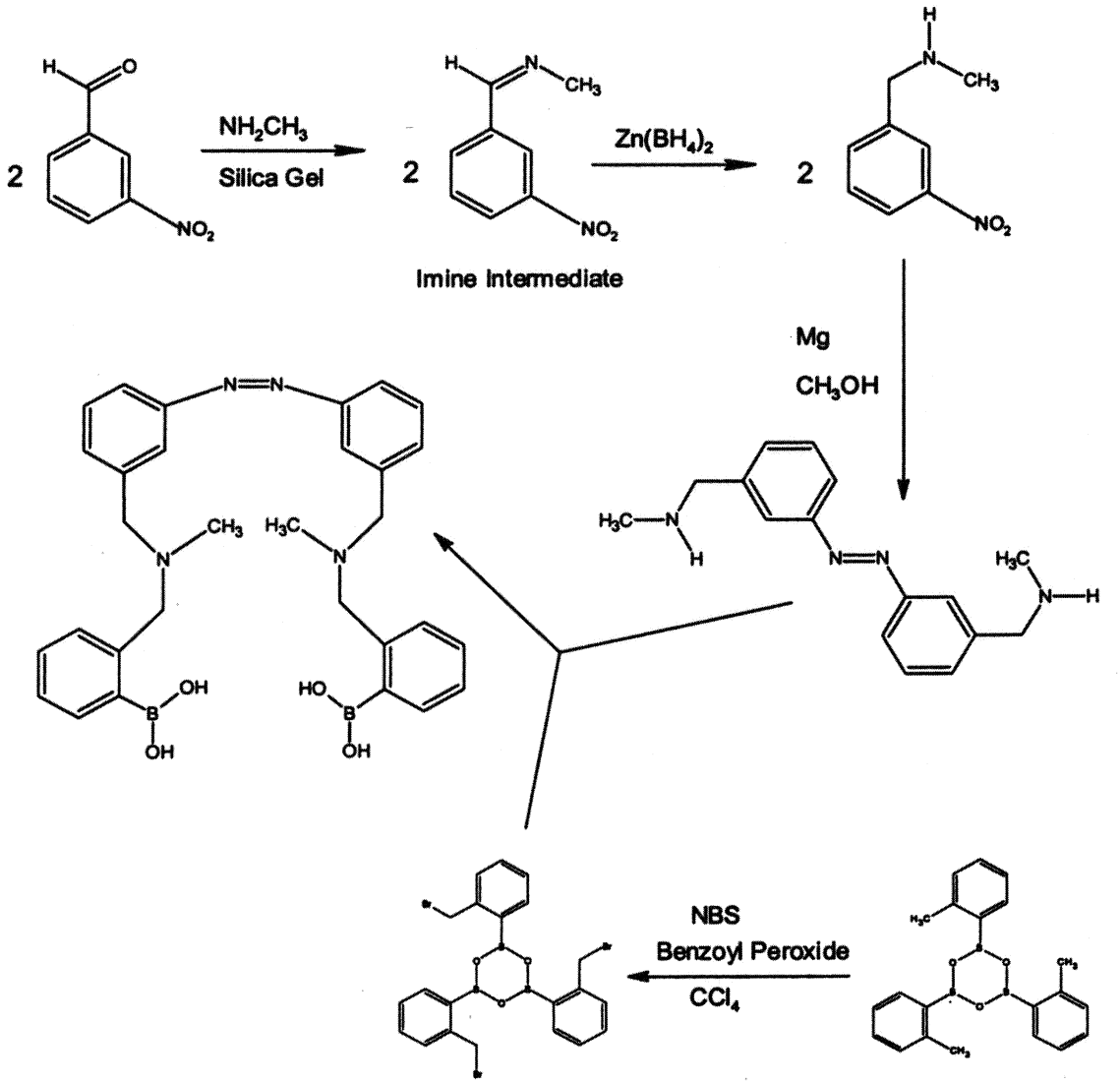

Results and Discussion

The synthesis of the first probe molecule is near completion. Currently, the primary obstacle is the third step of the synthesis. This step involves the substitution of methylamino groups for each of the bromines on 1,8-Bis(bromomethyl)–naphthalene. Thus far, the reaction has proven to be unsuccessful, despite several attempts. Research into various journals will hopefully yield some new successful methodology for this reaction.

Despite consisting of only three steps, the synthesis of the second probe molecule has been ridden with many setbacks. The proposed synthesis II displayed in the methods section above is not the original plan for synthesis, but an alternate and current plan. Originally, the chemoselective reductive coupling reaction was the first step involving the coupling of two *m*-nitrobenzaldehyde molecules into an azoarene. After several trials, each involving an arduous separation of byproducts, the best yield achieved was a mere eight percent. This was unexpected, as the yield in the literature was ninety-seven percent for a similar compound. As no variations in our methodology could produce a satisfactory yield, this reaction was aborted and an alternate route of synthesis was proposed.

The alternate route attempts the reductive amination of *m*-nitrobenzaldehyde to *m*-N-methyl-methanamine-nitrobenzene as the first step, followed by the chemoselective reductive coupling of this product into the di-amino azoarene; the precursor to the final probe. It is believed that the chemoselective reductive coupling reaction may work better with a methylamine substituent than it did with an aldehyde substituent. Unfortunately, no success in the reductive amination reaction thus far has proven frustrating and prompted our search for possibly yet another route of synthesis.

Conclusion

At this time, this research should be considered a work in progress. Future plans include finishing the synthesis of both of the proposed boronic acid receptor molecules. Following the completion of these syntheses, fluorescence spectroscopy will be employed to test the binding affinity and specificity of both probe molecules on a number of selected saccharides. Hopefully, this testing will reveal probe specificity and a set of standards will be developed for the relationship between the fluorescence of the sensor and the concentration of the analyte sugar. This would allow these molecules to be put into practical use as well as the publication of my research in a chemistry journal.

Acknowledgements

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