Synthesis of Boronic Acid based Scaffolds for Saccharide Sensing

A Thesis Submitted

In partial fulfilment for the award of the degree of

MASTER OF SCIENCE

IN

CHEMISTRY

Submitted by
Shally
Reg. No. 301102014

Under the Supervision of
Dr. Vijay Luxami
Assistant Professor

SCHOOL OF CHEMISTRY AND BIOCHEMISTRY

THAPAR UNIVERSITY, PATIALA

JULY, 2013

Dedicated to My Parents and Guide,

God, my guide, my parents, my brother who taught me the basics of life. Thank you all for being with me in all odd and pleasing times.

CERTIFICATE

This is to certify that the project entitled **"Synthesis of Boronic Acid based scaffolds for saccharide sensing"** being submitted by Shally, Roll No. 301102014 in partial fulfillment of the requirements for the award of degree of **Master of Science**, in School of Chemistry and Biochemistry, Thapar University, Patiala, is a bonifide work carried out under my supervision and guidance. The report has not been submitted for the award of any otherdegree or certificate in this or any other university.

Vijay Luxami 16-07-12

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CANDIDATE'S DECLARATION

I hereby declare that the work which is being presented in the dissertation entitled **"Synthesis of Boronic Acid based scaffolds for saccharide sensing"** in partial fulfillment of the requirements for the award of the degree of **Master of Science** in Chemistry, School of Chemistry and Biochemistry, Thapar University, Patiala is an authentic record of my own work during a period of six months from January 2013 to July 2013, under the supervision of **Dr. Vijay Luxami ,** Assistant Professor, School of Chemistry and Biochemistry, Thapar University, Patiala. The report has not been submitted for the award of any other degree or certificate in this or any other university.

Shally

SHALLY

Place: Patiala

Date:

This is to certify that the above statement given by the candidate is correct and true to the best of our knowledge.

Vijay Luxami 16-07-13

Dr. Vijay Luxami
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SCBC, TU, Patiala.

ACKNOWLEDGEMENTS

In pursuit of this academic endeavor, I feel that I have been singularly fortunate because inspiration, guidance, direction, co-operation, love and care all come in my way in abundance and it seems almost an impossible task for me to acknowledge the same in adequate term.

My wholehearted indebtness goes to my erudite guide, **Dr. Vijay Luxami**, Assistant Professor, School of Chemistry and Biochemistry, Thapar University, Patiala, for her guidness, support and patience. Their invaluable assistance and precious guidance helped me in executing this arduous task from its conception to its completion.

I also thankful to **Dr. Satnaam Singh** head of Department for his support and providing facilities.

I thank **Ms. Meenakshi Verma,** research scholar for their kind cooperation during the project work.

Life at Thapar University would be unforgettable for me throughout my life because I was blessed to spend it with my friends. I thank them all for their great company.

Words fail me to express my thanks to my family for their selfless sacrifice, encouragement and heart full blessings that continue to enlighten my life.

Above all I thank almighty God for blessing me with strength and wisdom to complete this project successfully.

shally

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Date:

Place: Patiala.

List of contents

Introduction & Review of Literature

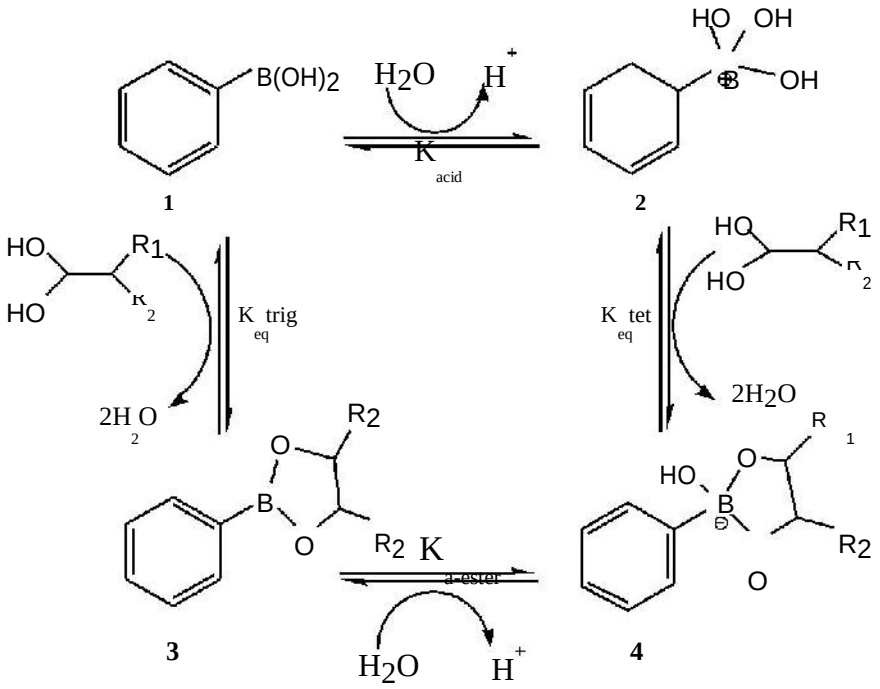
‘‘A sensor is a device that interacts with matter or energy and yields a measurable signal in response’’.[1] This definition bears witness to the extensive range of applications possible with sensors. We can distinguish biosensors, which utilise a biological element for analyte recognition, from chemosensors, in which the analyte interacts with a synthetically prepared entity. In keeping with convention the term saccharide is used to refer broadly to polyhydroxylated carbohydrates.[2] The product of photosynthesis, carbohydrates single-handedly account for the most prolific class of organic compounds that can be found on the surface of the Earth. Within biology they are of fundamental significance. In their most ubiquitous roles they endow nature with structural rigidity, in the form of cellulose, and function as the energy store that sustains life, in the forms of starch and glycogen.[3] Not only are these compounds abundant they are also incredibly versatile. Oligo-saccharides are involved in protein targeting and folding, as well as controlling the cell recognition events for infection, inflammation and immunity.[4] From a medicinal perspective the monitoring of D-glucose has proved of particular importance. D-Glucose provides the metabolic energy for most cells of higher organisms. In humans a breakdown in the transport pathways of D-glucose has been linked to conditions such as cancer,[5] cystic fibrosis[6] and renal glycosuria,[7,8] but by far the most prevalent condition resulting from ineffective D-glucose transport is diabetes mellitus.[9] Diabetes presents one of the largest health challenges to face us in the 21st century. Current reports indicate that diabetes affects 5% of the global population.[10] In the UK the increase in obesity, population age and a progressively more sedentary lifestyle has seen the prevalence of Type 1 diabetes double every 20 years since 1945.[11] Diabetes is associated with chronic ill health, disability and premature mortality. From a physiological perspective the debilitating long-term complications include heart disease,[12] blindness,[13] kidney failure,[14] stroke[15] and nerve damage leading to amputation.[16] At an economic level the repercussions are also serious. Within the UK 5% of the National Health Service’s budget is spent on treating diabetes and its complications.[17] This equates to d3.5 billion per year or d9.6 million per day. Following extensive and widespread trials, unequivocal evidence exists that monitoring and adjusting diabetic bloodsugar levels to maintain them within tight boundaries dramatically reduces the health risks faced by diabetics.[18–20] Since continuous and noninvasive systems are critical for the control of the disease status. Glucose chemosensors have become the focus of intense research, the ultimate aim is to provide diabetics simple more robust and less invasive methods of measuring blood glucose levels important for the long-term management of the disease. Towards that end one area of research of particular focus has been the development of boronic acid based saccharide receptors.[21] Present research proposal has been set out to construct modular boronic acid based fluorescent sensors for saccharides. The recognition of saccharides using the esterification with boronic acids is facilitated by the interaction with a proximal

heteroatom. The precise nature of the Lewis acid–base interaction (heteroatom-B) has been the subject of some controversy.[22] However, the fact that the proximal heteroatom has a positive effect on the binding efficiency of boronic acids is not in debate. The interaction of the boron atom (Lewis acid) and neighbouring hetero atom (Lewis base) is strengthened on saccharide binding, thus the photo-physical processes from heteroatom to the attached fluorophore is operational[23]. The modular concept for the design of saccharide selective boronic acid sensors has been recently been reported by some groups.[24] A modular approach allows the linker and fluorophore units of a sensor to be varied independently. That way the dimensions of the binding pocket and emission wavelength could be altered in a controlled manner.

A more efficient approach is the use of fluorescent chemosensors that have an high affinity for glucose. Generally, selective sensors consist of three components: (i) proper functional groups that afford strong intermolecular interactions, (ii) a proper 'reporter'event/moiety, and c) the appropriate three-dimensional scaffold as the artificial receptor that provides the appropriate positioning and orientation for the appropriate functional groups in terms of size, shape, and functional group orientation (bridge) as shown in Figure 1.1. In designing such chemosensors for glucose, boronic acids occupy a special place because of their strong functional group interaction with the diols that exist on glucose and other sugars. Boronic acids have been used to develop fluorescent sensors.[25-28], color sensors,[29] carbohydrate transporters, and chromatographic stationary materials. Boronic acid compounds used as a chelating group for glucose sensing. Boronic acids and their esters are highly valuable compounds which have found extensive applications in organic medicinal chemistry. Boronic acid has a low toxicity and their degradation into the environmentally benign boric acid, they can be considered as "green" compounds.[30]

Design of boronic acid based molecules

Boronic acids covalently react with 1,2- or 1,3-diols to form five or six membered cyclic esters in aqueous solution. The *cis* diols of saccharides normally form stronger cyclic esters than the *trans* or acyclic diols. Boronic acids are Lewis acids and can react with water to form the neutral trigonal form (**1**) to the anionic tetrahedral form (**2**) (Scheme 1). The same is true for the diol – boronic complex or the boronic ester (**3**).



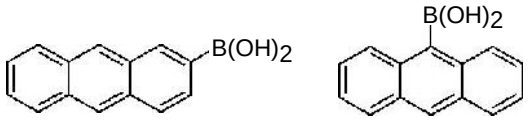
Scheme1: Binding process between phenylbronic acid and diol

Because boronic acids and their esters can exist in two different ionization states, there are three different "binding constants" to consider. The first one elates to the conversion of the trigonal boronic acid **1** to the trigonal ester **3**, termed K_{trig}. The second one refers to the conversion of tetrahedral boronate **2** to its ester counterpart **4**, termed K_{tet}. However, neither of these two truly represents the overall binding constant between a diol and boronic acid for the purpose of sensor design.[31] The third binding constant describes the overall binding strength, K_{eq} (**Scheme 1**).[32] Another important factor to consider is that, boronic acids with lower p*K*a's tend to have higher affinities for diols,[33] the optimal binding also depends on the p*K*a's of the boronic acid and diol and the pH .

Boronic acid based fluorescent sensors

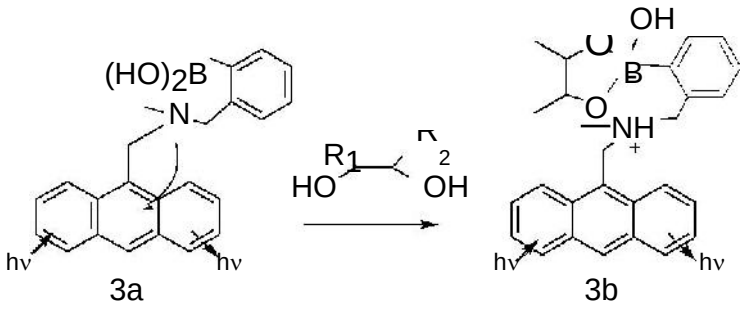
The first fluorescence PET sensors for saccharides was anthrylboronic acids **1** and **2**, showed significant fluorescence intensity changes upon binding with saccharide. The fluorescence intensity change was due to the change in the hybridization state of the ester, which has lower pK_a than the boronic acid (p*K*a about 8.8). Specifically boronic acid should exist mostly in the neutral trigonal state at the physiological pH and at such a state PET from open shell of the boron in the excited state leading to fluorescence quenching. However, upon ester formation, the boron functionality would exist in the anionic tetrahedral because of a

decreased p*K*a[32] and such a hybridization change eliminatees the excited PET and therefore removes fluorescence quenching mechanism and increased fluorescence.



1 **2**

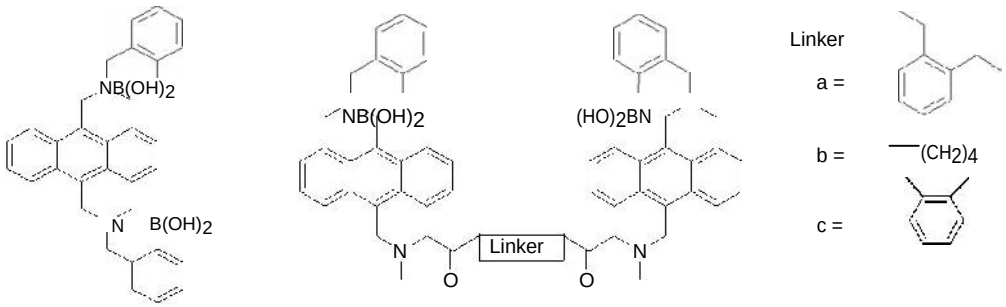
Another PET system **3** was developed by Shinkai,[33] where the amino group was positioned in a 1,5-relationship with the boronic acid. Such an arrangement promoted dative B-N bond formation. Such bonding lowered the pK_a of boronic acid and increase binding of diols[32-34]. It was further proposed that the B-N bond strengthens upon sugar binding[35], which "ties up" the lone pair electrons, eliminate the PET, and resulted in a fluorescence intensity increase.



Bronic acid weakly based fluorescent Bronate ester strong fluorescent

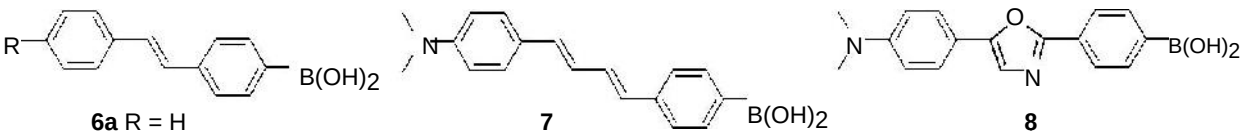
Scheme 3: Mechanism of an anthracene-based photoinduced electron transfer system

Shinkai and coworkers synthesized compound **4**, which has two appropriately spaced boronic acid moieties. This compound shows a maximum fluorescence intensity change of about 7-fold upon binding with glucose. Shinkai group also synthesized series of diboronic acids **5** which have different amide linkers **5a**[36], which has two acetamides attached to phenyl ring in an ortho relationship offers the proper diboronic acid orientation and distance for selective binding with glucose. It showed high affinity (*K*eq = 1472 M^{-1}) and 43-fold selectivity for glucose over fructose. Although neither **5b** nor **5c** have same number of carbons in the linker, showed the kind of selectivity and affinity for the glucose as **5a.** Thus, rigidity of the linker plays a important role in determining saccharide selectivety .



4 **5**

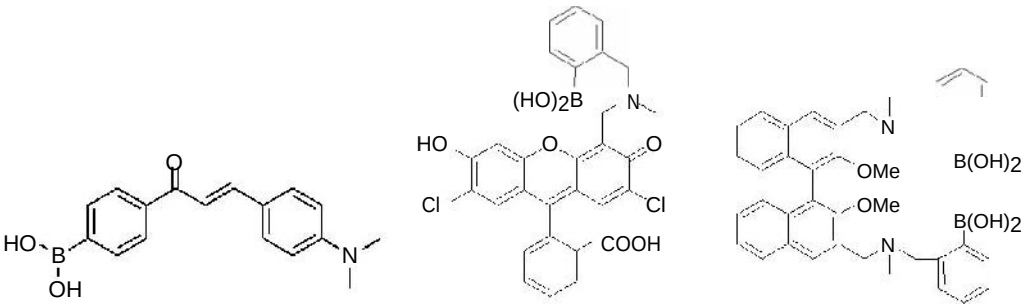
Four stillbene boronic acid analogs **6a-d** have a electron-donating group and **6c** have a electron-withdrawing cyano group. With pH change from low to high induced a blue shift in the emission spectrum of **6b** and **6d** and an increase in intensity by about one fold in the presence of sugar. Similarly, the polyene derivatives **7**[37], diphenyloxazole derivatives **8**[38-39]and chalcone derivatives **9**[40] were prepared and tested for binding with sugars. The fluorescence intensity changed by a maximum of five fold in these ICT systems. These mono-boronic acids showed preference for fructose over glucose as would have been expected based on the results of phenylboronic acid.



6a R = H
6b R = OCH_3
6c R = CN
6d R = $N(CH_3)_2$

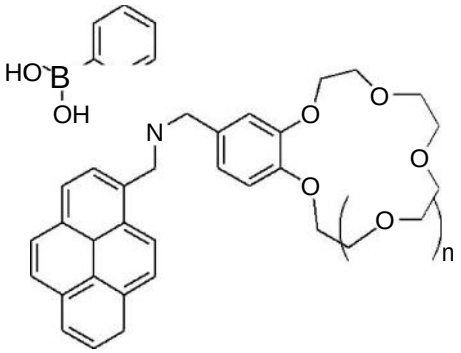
7 **8**

A new fluorescein derivative **10** bearing a boronic acid group was investigated as a fluorescent chemosensor for F^- . OFF-ON type fluorescence enhancement was observed by the blocking of the photoinduced electron transfer mechanism, which was induced by the interaction between fluoride and boronic acid moiety. Fluorescein derivative bears a boronic acid group as a binding site. A unique boronate formation between the boronic acid and adjacent phenolic oxygen as well as the interaction between the boron and nitrogen were confirmed by X-ray crystallography. The fluorescein moiety was used as the fluorescent source, the emission changes can be monitored over 500 nm. OFF-ON type fluorescence enhancement was observed by the blocking of the photoinduced electron transfer (PET) mechanism, which was induced by the interaction between fluoride and the boronic acid. Fluoride ion displayed a selective fluorescent enhancement among the halide ions. Fluorescence spectra were obtained by exciting of the fluorescein fluorophore at 483 nm. The overall emission change upon the addition of fluoride ion was more than 3-fold. On the other hand, the addition of chloride ion induced only about 2-fold enhancement in its fluorescence emission.[41]



9 **10** **11**

The glucose receptor **10** and **11** developed by Shinkai was assessed and compared to the corresponding bis (diol), erythritol, as well as the corresponding mono(R-hydoxycarboxylate), malate. Bisboronate/bis(R-hydoxycarboxylate) interactions are stronger than the corresponding bis boronate/bis(diol) interactions. Results are in an order of magnitude more selective for tartrate than malate. Boronic acids have a much higher affinity for R-hydroxycarboxylic acids than 1,2-alkanediols. It was confirmed that the chiral, fluorescent compound **11** can bind D-glucose selectively over its enantiomer and has a binding constant 2 orders of magnitude higher than that of a monoboronate/glucose complex. Compound **11** offered an opportunity for direct comparison of bis boronate/bis(diol) versus bisboronate/ bis(R-hydroxycarboxylate) interactions. The strong binding of the receptor to tartrate and experiments performed with erythritol and malate has several implications. Binding constant of the receptor to tartrate is very similar to both literature values for the binding of the receptor to glucose. This was the first bisboronate receptor shown to bind adjacent R-hydroxycarboxylates.[42]

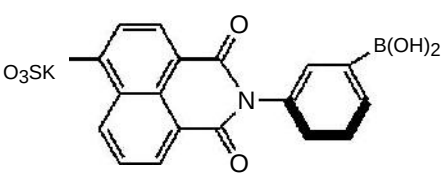


12a = n =1
12b = n = 2

A ditopic receptor reminiscent of Reetz's crown ether boronic acid system has been developed **12a** and **12b**. Compounds **12a** and **12b** function as AND logic gates via their selectivity for potassium fluoride. The sp^2 hybridised boronic acid, which is a hard Lewis acid, interacts strongly with a fluoride anion, which is a hard Lewis base, and becomes sp^3 hybridised. The potassium cation is held partly by the crown ether and partly by the electrostatic interaction with the fluoride anion. This co-operative complexation allows the

cationic and anionic guests to be bound to the host as an ion pair, whilst allowing the host to discriminate between potassium fluoride and other similar ion pairs such as potassium chloride and potassium bromide.[43]

Naphthalic anhydrides and their derivatives **13** have been generally used for fluorescent tags and receptor antagonists[44-45]. Introduction of a nitro group into the naphthalic anhydride ring, two emission bands (430 nm / 550 nm, respectively) of the dye molecule were observed.[46] 4-sulfo potassium salt group of 1,8-naphthalic anhydride substituted $–B(OH)_2$ positions on the phenyl ring was investigated. Steric effect and structural configuration were the key factors for saccharide binding. Highly water soluble monoboronic acid probes showed large fluorescence increases in the presence of monosaccharides and show remarkable sensitivity for glucose rather than fructose and galactose. This was the first highly water soluble monoboronic acid probe to display the more desirable OFF-ON fluorescence response. By changing position of the boronic acid group from *ortho* to *meta* positions of the phenyl ring, there was no significant spectroscopic and photophysical changes.[47]



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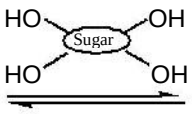
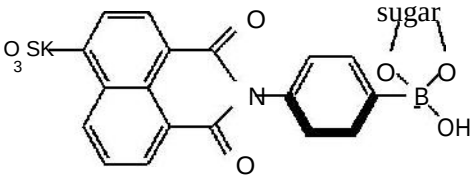
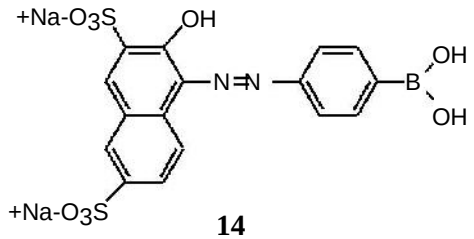


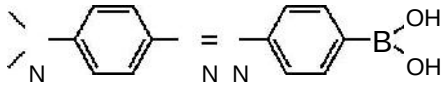


13.sugar complex

In water, dye **14** shows an orange solution with a maximum in the absorption spectrum of 495 nm (ε = 22 950 $M^{-1}cm^{-1}$). As the pH increases from 3 to 12, color change was observed from orange to purple associated with a red shifting of the absorption band with an isobestic point at 515 nm. In the absence of sugar, the probe showed an orange solution, while in the presence of sugar the solution showed a purple reddish color. Probe **15** showed a yellow solution in water at neutral pH. The absorption spectrum of **15** showed a maximum at 460 nm (ε = 21 800 $M^{-1}cm^{-1}$) which shifted to shorter wavelength at high pH. Boronic acid group in resonance with an azo dye lead to a color change of the dye. The color change was due to the conformational change of the boron atom between its neutral and anionic forms. Despite the change between the electron-withdrawing and electron-donating properties of the boronic group, the effect on the intramolecular charge transfer of the dye was weak. In the case of dye **15**, the electronic properties of the dye were governed by the presence of the dimethylamino group and independent of the presence of the electron acceptor boronic group. For example, upon protonation of the dimethylamino group at pH < 2, dye **15** showed a drastic colour change from pale yellow to deep red in solution.[48]

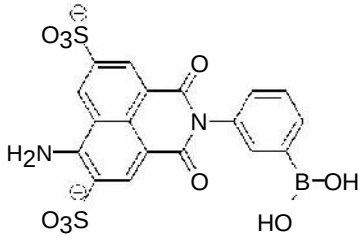


14

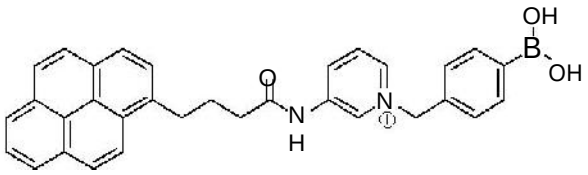


15

A phenylboronic acid derivative **16** behaved as well-known dye (Lucifer yellow) recognizes L-DOPA through a combination of reversible esterification charge transfer, and electrostatic interactions. In designing a fluorescent chemosensor for L-DOPA, dyes complementary charged groups and electron-deficient π-systems were present in dye. Lucifer dyes showed electrostatic interactions between the sensor and analyte due to its additive nature of these interactions. Compound **16** showed absorption spectrum in pH 7.2 buffer (MOPS, 0.1 M) centered at 425 nm (ε = 11,000 $M^{-1}cm^{-1}$), and emission spectrum in optically dilute solutions at 535 nm. Increasing concentrations of L-DOPA quenched the fluorescence emission, decreasing the intensity to 1/5 of the emission intensity of the free chemosensor **16**. NMR titration of 1 mM of the chemosensor **16** in buffered D_2O with L-DOPA showed that only the aromatic protons of phenylboronic acid moiety are affected.[49]



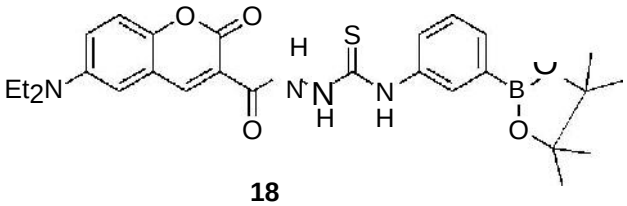
16



17

Ratiometric fluorescent chemosensor **17** based on an amphiphilic monoboronic acid, proved to be highly selective and sensitive for glucose and also resulted in a very large modulation to changes in glucose concentration in aqueous solution. The presence of glucose leads to pyrene excimer emission, while its monomer emission remains more or less unchanged, whereas fructose results in a modest enhancement of the monomer emission. The positively charged sensor molecule containing a pyridinium moiety becomes zwitterionic at high pH and exists in aggregates. Glucose binding lead to more ordered aggregates of **17**, and since one glucose molecule could bind with two boronic acid groups, a more hydrophobic unit is formed, resulting in the pyrene fluorophores being brought into closer proximity and enabling the pyrene excimer emission. With fructose, however, the 1:1 binding stoichiometry resulted in a neutral zwitterionic boronate of higher hydrophilicity, destabilizing the aggregates of **17** and producing monomeric fructose

boronates. Thus, use the aggregates of the monoboronic acid receptors to develop new, highly selective and sensitive receptors for the sensing of glucose in aqueous solutions. Compound **17** demonstrated for the first time the concept of competitive "knock-out" of the fructose interference by adding phenylboronic acid to the sensing ensemble.



18

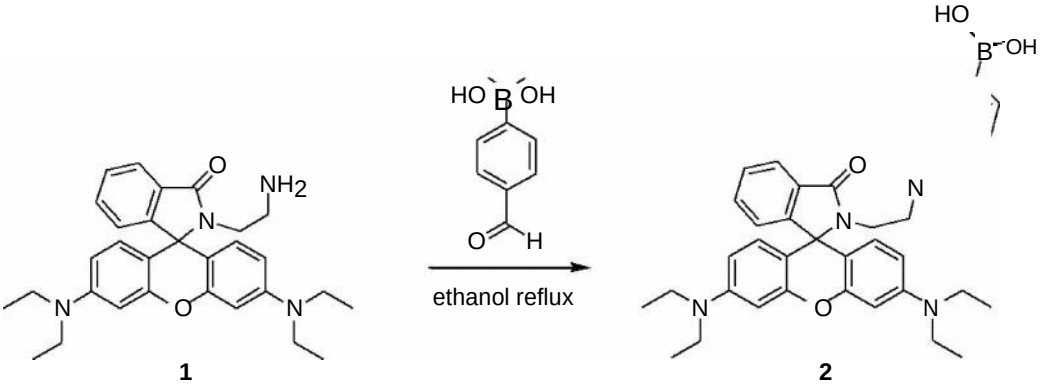
Ditopic fluorescence sensor **18** for saccharides and mercury has been developed based on a boronic acid receptor and desulfurisation reaction. The fluorescent output at 478 nm was significantly enhanced (>5-fold) in the presence of both Hg^{2+} and D-fructose in pH 8.21 buffer. While a less intense enhancement (~3-fold) was obtained on the addition of only Hg^{2+}, an even lower enhancement (<2-fold) was observed for the addition of D-fructose alone. The system can be construed as a dosimeter with AND logic functionality, in that it reports a HIGH output when two inputs are simultaneously applied.

Results and Discussion

Synthesis of compounds 1-7

1. **Synthesis of N-(Rhodamine B) lactam–ethylenediamine:** The N-(Rhodamine B) lactam–ethyenediamine was synthesized as reported in literature.[52] Rhodamine B (1 mmol, 0.479 gm) was dissolved in 20 ml ethanol, followed by addition of

   ethylenediamine (1 mmol, 0.61 gm). The reaction mixture was refluxed for 24 hours till the fluorescence of the solution quenched. The reaction mixture was cooled to room temperature and solid was filtered and washed with absolute ethanol for three times to obtain the pure product **1** with 97.8% yield.
2. **Synthesis of compound 2**

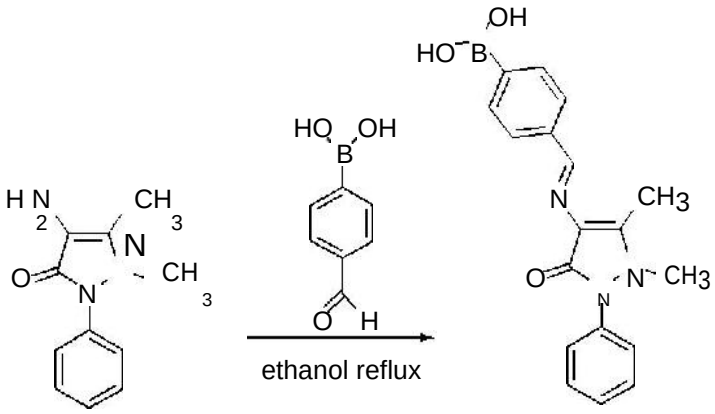


1 **2**

N-(Rhodamine B) lactam–ethylenediamine (1 mmol, 0.484 gm) was dissolved in 25 mL ethanol followed by the addition of 4-phenylboronic acid (1 mmol, 0.149 gm). The reaction mixture was reflux for 24 hours. After the completion of reaction (monitored by TLC), reaction mixture was cooled to room temperature. The solvent was evaporated and product was filter and solid was washed with ether. The pure product **2** was obtained with 81.2% yield; m.pt: 180 decomposition at 230 ^{0}C; ^{1}H NMR spectrum of compound **2** showed 1H singlet at 9.02 due to N=CH, 3H multiplet at δ 8.04 for aromatic-H, 2H multiplet at δ 7.56 for aromatic-H, 1H multiplet at δ 7.07 for aromatic-H, 2H multiplet at δ 6.44 for aromatic-H, 2H multiplet at δ 6.29 for aromatic-H, 8 H multiplet at δ 3.45 for CH_2, 6H multiplet at δ 1.28 for aliphatic-CH_3. ^{13}C NMR (100MHz, $CDCl_3$): δ 193.01, 170.04, 168.48, 163.34, 153.90, 153.75, 153.39, 153.24,

148.96, 148.80, 131.11, 129.00, 128.88, 128.46, 128.26, 127.26, 123.96, 123.87, 123.08, 122.82, 108.36, 108.06, 105.50, 103.90, 97.83, 97.71, 66.00, 65.13, 59.26, 41.37. NMR spectra confirmed the structure of compound

3. **Synthesis of 4-((1,5-Dimthyl-3-oxo-2-phenyl-2,3dihydro-1H-pyrazole-4-ylimino)methyl) phenyl boronic acid (3)**



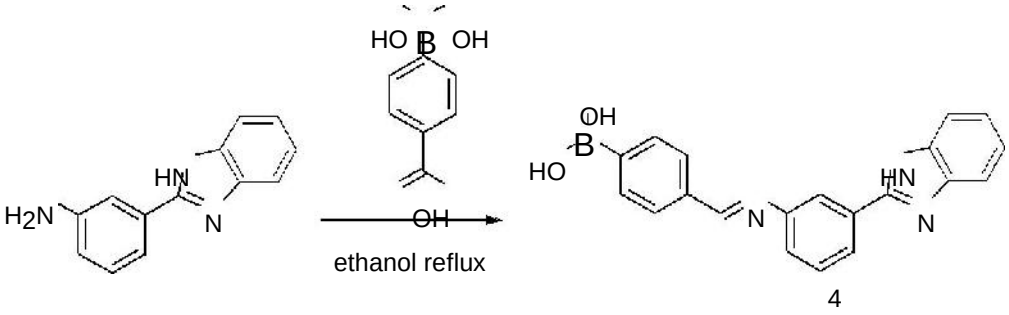
3

Aminoantipyrine (1 mmol, 0.203 gm) was dissolved in 20 mL dry ethanol followed by the addition of 4-phenylboronic acid (1 mmol, 0.149 gm). The reaction mixture was stirrer 16 hours. After the completion of reaction mixture (monitored by TLC) and cooled the reaction mixture at room temperature. The product was filtered and washed with ether. The pure product **3** was obtained 79.4%.; m.pt-210-220 ^{0}C;

^{1}H NMR spectrum of compound **3** shows1H singlet at 9.12 due to N=CH, 2H doublet at δ 7.82 for aromatic-H, 2H singlet at δ 7.76 for aromatic-H, 2H doublet at δ 7.69 for aromatic-H, 2H triplet at δ 7.45 for aromatic-H, 2H multiplet at δ7.32 for aromatic-H, 2H singlet at δ 3.27 for , 3H singlet at δ 3.13, 3H singlet at δ 2.43 for. ^{13}C NMR (100 MHz, $CDCl_3$): δ 134.70, 129.33, 127.14, 126.14, 124.76, 35.83,

22.81, 10.21. NMR spectra confirmed the structure of 4-((1,5-Dimthyl-3-oxo-2-phenyl-2,3dihydro-1H-pyrazole-4-ylimino)methyl)phenylboronic acid **3**.

4. Synthesis of 4-((3-(1H-benzo[d]imidazole-2-y)phenylimino)methyl)phenylboronic acid



4

Benzimidazole amine (1 mmole, 0.209 gm) was dissolved in 20 mL dry ethanol followed by the addition of phenylboronic acid (1 mmol, 0.149 gm). The reaction mixture was stirrer 12 hours. After completion of reaction mixture (monitored by TLC) and reaction mixture cooled at room temperature. The product was filtered by sodium carbonate and washed with ether. The pure

product 4 was obtained 80.3%; m.pt- 180-190^{0}c; ^{1}H NMR spectrum shows singlet at δ 8.74 for aromatic-H, 1H singlet at δ 8.26 for aromatic-H, 1H doublet at δ 8.07 for aromatic-H, 2H doublet at δ 7.98 for aromatic-H, 2H multiplet at δ 7.89 for aromatic-H, 2H multiplet at δ 7.74 for aromatic-H, 1H multiplet at δ 7.48 for aromatic-H, 2H multiplet at δ

7.30 for aromatic-H, 1H singlet at 6.56 for aromatic-H. NMR spectra confirmed the structure of 4-((3-(1H-benzo[d]imidazole-2-y)phenylimino)methyl)phenylboronic acid **4**.

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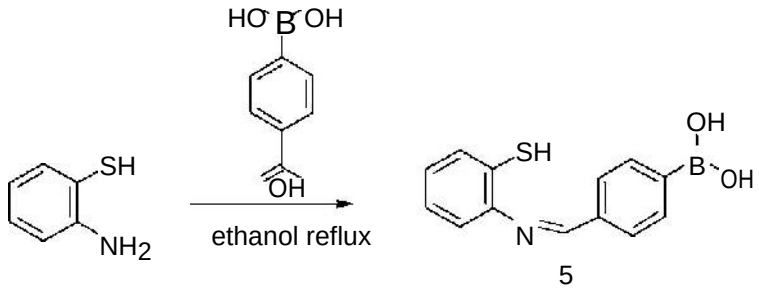
5. Synthesis of 4-((2- mercaptophenylimino)methyl) phenylboronic acid

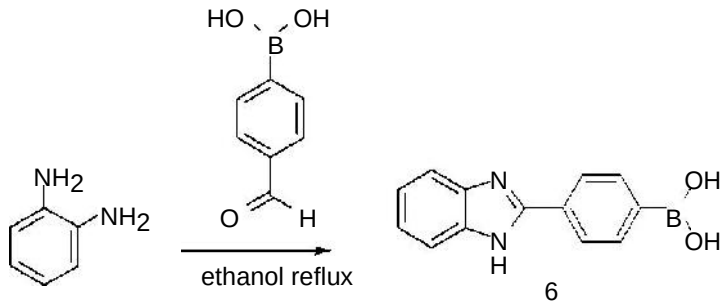


5

2-aminothioamine (1 mmol, 0.125 gm) was dissolved in 20 mL ethanol followed by the addition of phenylboronic acid (1 mmol, 0.149 gm). The reaction mixture was stirrer 16h. After completion of reaction mixture (monitored by TLC) and reaction mixture cooled at room temperature. The product was filtered by the sodium carbonate and washed with ether. The pure product 5 was obtained 80.4%; m.pt- 200-210^{0}c; ^{1}H NMR spectrum shows 1H singlet at 9.02 due to N=CH, 3H triplet at δ 7.37 for aromatic-H, 3H doublet at δ 7.32 for aromatic-H, 1H triplet at δ 6.83 for aromatic-H, 1H triplet at δ 6.75 for aromatic-H. NMR spectrum confirmed the structure of (E)-4-((2-mercaptophenylimino)methyl)phenylboronic acid **5**.

6. Synthesis of 4((2-aminophenylimino)methyl)phenylboronic acid

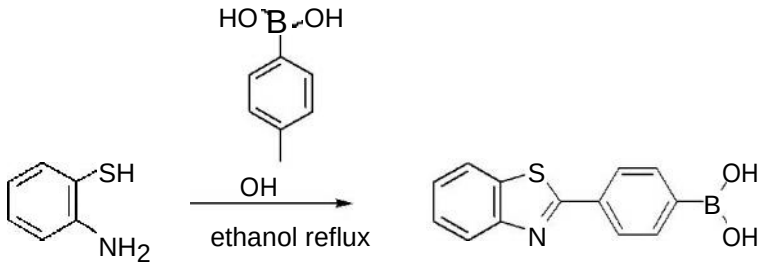
Orthophenyldiamine (1 mmol, 0.106 gm) was dissolved in 20 mL ethanol followed by the addition of phenylboronic acid (1 mmol, 0.149 gm). The reaction mixture was stirrer 24 hours. After completion of reaction mixture (monitored by TLC) and reaction mixture cooled at room temperature. The product was filtered by the sodium carbonate and washed with ether. The pure product 6 was obtained 82.4%; m.pt -230-240^{0}c; ^{1}H NMR spectrum shows 1H doublet at δ 8.21 for aromatic-H, 1H doublet at δ 8.21 for aromatic-H, 2H multiplet at δ 7.93 for aromatic-H, 3H multiplet at δ 7.69 for aromatic-H,



6

1H multiplet at δ 7.22 for aromatic-H. NMR spectrum confirmed the structure of (Z)-4((2-aminophenylimino)methyl)phenylboronic acid **6**.

7. Synthesis of 4-[benzo[d]thiazol-2-yl]phenylboronic acid



7

2-Aminothiophenol (1 mmol, 0.125 gm) was dissolved in nitrobenzene (2-3 ml) followed by the addition of phenylboronic acid (1 mmol, 0.149 gm). The reaction mixture was stirred 7h. The reaction mixture was stirrer 24 hours. After completion of reaction mixture (monitored by TLC) and reaction mixture cooled at room temperature. The product was filtered by Buchner funnel and washed with ether. The product **7** was obtained 84.6%; m.pt-140-150^{0}c; ^{1}H NMR spectrum shows 1H multiplet at δ 8.18 for aromatic-H, 2H multiplet at δ 8.04 for aromatic-H, 2H multipet at δ 7.92 for aromatic-H.

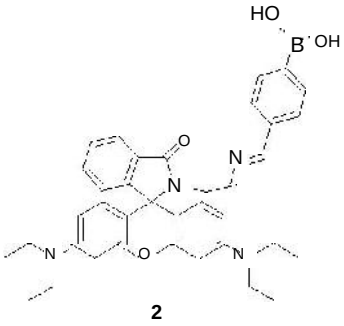
1H triplet at δ 7.76 for aromatic-H, 1H triplet at δ 7.69 for aromatic-H, 1H triplet at δ 7.62 for aromatic-H. NMR spectrum confirmed the structure of 4-[benzo[d]thiazol-2-yl]phenylboronic acid **7**.

Experimental

Melting points were obtained with Perfit India melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was performed on glass plates coated with silica gel purchased from Merck Inc. Purification was carried out by column chromatography using chloroform:methanol (80:20) as eluents and 60-120 mesh silica gel. ^{1}H NMR and ^{13}C NMR were obtained on a Bruker AC-400 (400 MHz) spectrometer with use of chloroform-*d* and dimethylsulfoxide-d_6 as solvents. Chemicals shifts were recorded in parts per million (ppm, δ) and were reported relative to the solvent peak or TMS. Multiplicities are recorded with the following abbreviations: s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet; *J*, coupling constant (hertz). All the chemicals viz Rhodamine B, Ethylenediamine, 4-formyl-phenylboronic acid, 8-Aminoquinoline, Aminoantipyrine, Benzimidazole amine, 2-Aminothiophenol, 1,2-diaminobenzene were purchased from Aldrich, Lobachem and Spectrochem were used without further purification.

General Procedure for synthesis of compounds 1-7

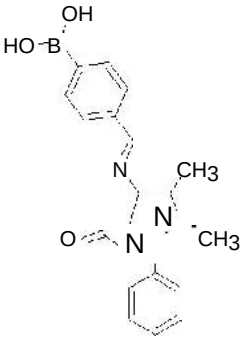
4-Formyl-phenylboronic acid (1 mmol) was refluxed with different amines (1 mmol) in ethanol. Progress of reaction was monitored through TLC and after completion, reaction mixture was concentrated under vacuo and residue purified through column chromatography CHCl3/MeOH, Ethylacetate / hexane as eluting system. Thier ^{1}H and ^{13}C NMR spectra are consistent with proposed structure.



2

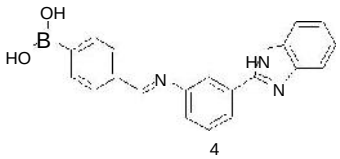
N-(Rhodamine B) lactam–ethylenediamine (1 mmol, 0.484 g) was dissolved in 25 mL ethanol followed by the addition of 4-phenylboronic acid (1 mmol, 0.149 gm). The reaction mixture was reflux for 24 hours.

After the completion of reaction (monitored by TLC), cooled the reaction mixture at room temperature. The solvent was evaporated and product was filter and solid was washed with ether. Colour- light brown; 81 % yield; m.pt: 180 ^{O}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.02 (s. 1H, N=CH), 8.04 (m, 3H), 7.57 (m, 2H), 7.07 (m, 1H), 6.44 (m, 2H), 6.29 (m, 2H), 3.45 (m, 10H), 1.28 (m, 10H). ^{13}C NMR ($CDCl_3$): δ 193.01, 170.04, 168.48, 163.34, 153.90, 153.75, 153.39, 153.24, 148.96, 148.80, 131.11, 129.00, 128.88, 128.46, 128.26, 127.26, 123.96, 123.87, 123.08, 122.82, 108.36, 108.06, 105.50, 103.90, 97.83, 97.71, 66.00, 65.13, 59.26, 41.37.



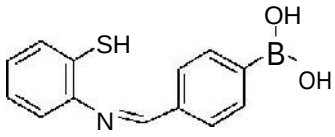
3

Aminoantipyrine (1 mmol, 0.203 gm) was dissolved in 20 mL dry ethanol followed by the addition of 4- phenylboronic acid(1 mmol, 0.149 gm). The reaction mixture was stirrer 16 hours. After the completion of reaction mixture (monitored by TLC) and cooled the reaction mixture at room temperature. The product was filtered by sodium carbonate and washed with ether. The pure product 3 was obtained. Colour- Yellow; 79.4%; yield; m.pt 210-220^{0}c; ^{1}H NMR (400 MHz; DMSO): δ 9.12 (s. 1H, N=CH), 7.82 (d, 2H, J = 8.28), 7.76 (s, 2H), 7.69 (d, 2H, J =7.8), 7.45 (t, 2H, ^{1}J =7.32, ^{2}J = 14.2), 7.32 (m, 2H), 3.27 (s, 2H), 3.13 (s, 3H), 2.43 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 134.70, 129.33, 127.14, 126.14, 124.76, 35.83, 22.81, 10.21.



4

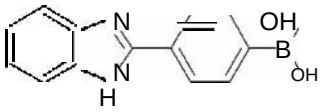
Benzimidazole amine (1 mmol, 0.209 g) was dissolved in 20 mL dry ethanol followed by the addition of phenylboronic acid (1 mmol, 0.149 gm). The rection mixture was stirrer 12 hours. After the completion of reaction mixture (monitored by TLC) and cooled the reaction mixture at room temperature. The product was filtered by sodium carbonate and washed with ether. The pure product **4** was obtained. Colour- Brown; 80.3% yield; m.pt 180-190 ^{0}C; ^{1}H NMR (400 MHz; $CDCl_3$): δ9.02 (s. 1H, N=CH), 8.74 (s, 1H), 8.26 (s, 1H), 8.07 (d, 1H, J = 5.96), 7.98 (d, 2H, J = 14.64), 7.89 (m, 2H), 7.74 (m, 2H), 7.48 (m, 1H), 7.30 (m, 2H), 6.56 (s,1H).



5

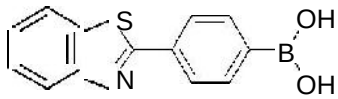
2-aminothioamine (1 mmol, 0.125 g) was dissolved in 20 mL ethanol followed by the addition of phenylboronic acid (1 mmol, 0.149 gm). The reaction mixture was stirrer 16 hours. The product was filtered by the sodium carbonate and washed with ether. After

the completion of reaction mixture (monitored by TLC) and cooled the reaction mixture at room temperature. The pure product 5 was obtained. Colour- Pink; 80.4%; m.pt 200-210^{0}c; ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.37 (t, 3H, ^{1}J = 7.8, ^{2}J =14.24), 7.32 (d, 3H, J =8.24), 6.83 (t, 1H, ^{1}J =7.32,^{2}J =15.12), 6.75 (t, 1H, ^{1}J =7.32, ^{2}J =15.12). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.36, 153.60, 134.61, 134.21, 126.04, 125.84, 124.99, 122.63, 121.51, 14.02, 3.55.



6

Orthophenyldiamine (1 mmol, 0.106 g) was dissolved in 20 mL ethanol followed by the addition of phenylboronic acid (1 mmol, 0.149 gm). The reaction mixture was stirrer 24 hours. After the completion of reaction mixture (monitored by TLC) and cooled the reaction mixture at room temperature. The product was filtered by the sodium carbonate and washed with ether. The pure product 6 was obtained. Colour- Rust; 82.4% yield; m.pt 230-240^{0}c; ^{1}H NMR (300MHz, DMSO): δ 8.21 (d, 1H, J =8.24), 8.21 (d, 1H, J =12.36), 7.93 (m, 2H), 7.69 (m, 3H), 7.22 (m, 1H).



7

2-aminothioamine (1 mmol, 0.125 g) was dissolved in nitrobenzene (2-3ml) followed by the addition of phenylboronic acid (1 mmol, 0.149 gm). The reaction mixture was stirrer 7 hours. After the completion of reaction mixture (monitored by TLC) and cooled the reaction mixture at room temperature. The product was filtered by Buchner funnel and washed with ether. The product **7** was obtained Colour- Brown; 84.6% yield; m.pt-140-150^{0}c; ^{1}H NMR (400 MHz, DMSO) : δ 8.18 (m, 1H), 8.04 (m, 2H), 7.92 (m, 2H), 7.76 (t, 1H, ^{1}J =6.4, ^{2}J=14.6), 7.69 (t, 1H, ^{1}J =7.76, ^{2}J =14.64), 7.62 (t, 1H, ^{1}J =8.68, ^{2}J =16.48).

Conclusions

1. Boronic acid based Schiff bases has been synthesised by using different amines viz., amino-antipyrine, rhodamine ethylenediamine, aminothiophenols etc
2. Boronic acid based benzimidazoles and benzthiazoles has been synthesised by using o-diaminobenzene, o-aminothiophenol etc.
3. New molecules have been characterized by using ^{1}H NMR and ^{13}C NMR.
4. These compounds will be useful as template for selective and or semi-selective saccharide sensing.

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